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der Rheinischen Friedrich – Wilhelms – Universität Bonn

Identification of candidate genes for boar taint
using RNA deep sequencing

I n a u g u r a l – D i s s e r t a t i o n

zur Erlangung des Grades

Doktor der Agrarwissenschaft

der

Landwirtschaftlichen Fakultät

der

Rheinischen Friedrich – Wilhelms – Universität

zu Bonn

vorgelegt am 17. Mai 2013

von

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aus

Bandung, Indonesia

Dedicated to my beloved parents, sisters, brothers and my beloved wife

Identification of candidate genes for boar taint using RNA deep sequencing

The aim of the present study was to identify candidate genes for boar taint using RNA deep sequencing. For this purpose, two experiments were performed. In the first experiment, transcriptome profiling was analyzed in the testis and liver tissues between boars with high and low androstenone levels in their backfat and polymorphisms that appeared on the differentially expressed genes. In the second experiment, differential gene expression analysis was performed in the liver tissues of boars with high skatole and low skatole in their backfat, differential exon expression and association analysis of gene variants that appeared on the selected differentially expressed genes. In the first experiment, the total number of reads produced for each testis and liver sample ranged from 13.2 to 33.2 million and 12.7 to 46.0, respectively. In testis samples, 46 genes were differentially regulated, whereas 25 genes showed differential expression in the liver. Differentially regulated genes in high androstenone testis and liver samples were enriched in metabolic processes such as lipid metabolism, small molecule biochemistry and molecular transport. Moreover, polymorphism and association analysis revealed mutations in IRG6, MX1, IFIT2, CYP7A1, FMO5 and KRT18 genes that could be potential candidate markers for androstenone levels in boars. In the second experiment, the total number of reads produced for each liver sample ranged from 11.8 to 39.0 million with a median of 22.8 million. Approximately 448 genes were differentially regulated at a strict false discovery rate (FDR) <0.05. Among them, 383 genes were significantly up-regulated in higher skatole group and 65 were significantly down-regulated (p<0.01, FC > 1.5). Differentially regulated genes in high skatole liver samples were enriched in metabolic processes such as small molecule biochemistry, protein synthesis, carbohydrate metabolism, energy production, lipid metabolism and amino acid metabolism. Gene expression analysis identified candidate genes in ATP binding, cytochrome P450, keratin, phosphoglucomutase, isocitrate dehydrogenase and solute carier family. Additionally, association analysis suggested that gene variants in ATP5B, KRT8, PGM1 and SLC22A7 might be potential candidate markers for skatole levels in boars. Furthermore, differential exon usages analysis of three genes (ATP5B, KRT8 and PGM1) revealed significant differential exons expression of these genes between the high and low skatole group. The results of these two experiments postulated several metabolic pathways, genes and their polymorphisms, and differential exon expressions that might be involved in the controlling of boar taint compounds levels with an aim to indetify potential candidate genes for boar taint related traits. However, further validation is required to confirm the effect of these genetic markers in other animal populations.

Identifizierung von Kandidatengenen für Ebergeruch mittels RNA Deep Sequenzierung

Das Ziel dieser Studie war es Kandidatengene für Ebergeruch mittels RNA Deep Sequenzierung zu identifizieren. Zu diesem Zweck wurden zwei Experimente durchgeführt. Im ersten Versuch wurden Transkriptionsprofile im Hoden- und Lebergewebe von Ebern mit hohem und niedrigem Androstenongehalt im Rückenspeck analysiert und Polymorphsimen, die bei unterschiedlich exprimierten Gene auftraten, wurden identifiziert. In dem zweiten Experiment wurde unterschiedlich exprimierten Gene im Lebergewebe von Ebern mit hohem und niedrigem Skatolgehalt im Rückenspeck analysiert. Ebenfalls wurden alternative exon transkripts identifiziert sowie eine Assoziationsanalyse der Genvarianten, die bei den ausgewählten unterschiedlich exprimierten Genen zu beobachten waren, durchgeführt. Im ersten Experiment rangierte die Gesamtzahl an „reads" für jede Hodenprobe zwischen 13,2 und 33,2 Million und für jede Leberprobe zwischen 12,7 und 46,0 Millionen. Bei den Hodenproben waren 46 Gene unterschiedlich reguliert, dagegen zeigten nur 25 Gene eine unterschiedliche Expression in der Leber. Die identifizierten Gene in den Hoden- und Leberproben mit hohem Androstenongehalt traten in metabolischen Prozessen wie dem Lipidmetabolismus, der Biochemie der kleinen Moleküle sowie dem molekularen Transport auf. Zudem zeigten die Polymorphismen und Assoziationsanalyse Mutationen in den Genen IRG6, MX1, IFIT2, CYP7A1, FMO5 und KRT18, die potentielle Kandidatenmarker für den Androstenongehalt beim Eber sein könnten. Im zweiten Versuch lag die Gesamtzahl an „reads" für jede Leberprobe zwischen 11,8 und 39,0 Millionen. Mit einem Signifikanzlevel von $p < 0,05$ waren etwa 448 Gene unterschiedlich reguliert. Von diesen Genen waren bei der Gruppe mit hohem Skatolgehalt 383 signifikant hoch-reguliert sowie 65 Gene signifikant runter-reguliert. Bei den Leberproben mit hohem Skatolgehalt traten die unterschiedlich regulierten Gene bei metabolischen Prozessen wie der Biochemie der kleinen Moleküle, der Proteinsynthese, der Energieproduktion, dem Metabolismus von Kohlenhydraten, Lipiden und Aminosäuren auf. Durch die Genexpressionsanalyse wurden Kandidatengene in der ATP Bindungs-, der Cytochrom P450, der Keratin, der Phosphoglucomutase, der Isocitrat Dehydrogenase sowie der Solute Carrier Familie identifiziert. Zusätzlich wurde durch die Assoziationsanaylse gezeigt, dass die Genvarianten von ATP5B, KRT8, PGM1 und SLC22A7 mögliche Kandidatenmarker für den Skatolgehalt von Ebern sein könnten. Des Weiteren wurde eine Analyse der alternative exon transkripts für drei Gene durchgeführt (ATP5B, KRT8 und PMG1) und signifikant unterschiedliche Expression der Exons dieser Gene zwischen der niedrigen und hohen Skatolgruppe gezeigt. Die Ergebnisse dieser zwei Untersuchungen ergaben mehrere Stoffwechselwege, Gene und ihre Polymorphismen sowie unterschiedliche Exonexpressionen, die an der Kontrolle des Ebergeruchs beteiligt sein könnten. Hierbei war das Ziel, potentielle Kandidatengene für die Ebergeruchsmerkmale zu identifizieren. Dennoch werden weitere Validierungen benötigt, um den Effekt dieser genetischen Marker in anderen Schweinepopulationen zu bestätigen.

Contents

List of abbreviations

A	:	Adenine
A260	:	Absorbance at 260 nm wavelength (UV light)
ANOVA		Analysis of variance
ATP	:	Adenosine triphosphate
ATP5B	:	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide
bp	:	Base pairs
BWA	:	Barrow whaller alligment
cDNA	:	Complementary DNA
cM	:	Centimorgan
COX5A	:	Cytochrome c oxydase subunit 5A
CYP4B24	:	Cytochrome family 4B member 24
ddH$_2$O	:	Distilled & deionized water
ddNTP	:	Dideoxyribonucleoside triphosphate
DEGs	:	Differential expressed genes
DESeq	:	Differntial expressed sequencing
DEXSeq	:	Differential exon sequencing
dH$_2$O	:	Deionized or distilled water
DHT	:	Dihydrotestosterone
DKK2	:	Dickkopf-related protein 2
DNA	:	Deoxynucleic acid
dNTP	:	Deoxyribonucleoside triphosphate
DTT	:	Dithiothreitol
EDTA	:	Ethylenediaminetetraacetic acid
EtBr	:	Ethidium bromide
ETOH	:	Ethanol
EST	:	Expressed sequence tags
FADH	:	Flavin adenine dinucleotide
FDR	:	False discovery rate
Fig	:	Figure
FMOs	:	Flavin-containing monooxygenases

FMO5	:	Flavin-containing monooxygenases member 5
g	:	Gram
G	:	Guanine
GAPDH	:	Glyceraldehyde-3-phosphate dehydrogenase
GATK	:	Genome analysis toolkit
GC-MS	:	Gas-chromatography/mass spectrometry
GEO	:	Gene expression Omnibus
GO	:	Gene ontology
GWAS	:	Genome wide association study
HSD17B2	:	Hydroxysteroid (17-beta) dehydrogenase 2
IDH1	:	Isocitrate dehydrogenases
IPA	:	Ingenuity pathway analysis
IP6KI1	:	Inositol hexakisphosphate kinase 1
IPTG	:	Isopropylthio-ß-D-galactoside
kb	:	Kilobases
KRT8		Keratin 8
mA	:	Milliamperes
Mb	:	Megabase
$MgCl_2$	:	Magnesium chloride
min	:	Minute
mRNA	:	Messenger RNA
MW	:	Molecular weight
NaCl	:	Sodium chloride
NGS	:	Next generation sequencing
OD260	:	Optical density at 260 nm wavelength (UVlight); = A260
PAGE	:	Polyacrylamide gel electrophoresis
PCR	:	Polymerase chain reaction
PGM1	:	Phosphoglucomutase 1
PPIA	:	Peptidylprolyl isomerase A
PRDX1	:	Peroxiredoxin 1
qRT-PCR	:	Quantitative Real Time PC
QTL	:	Quantitative trait loci
RACK1	:	Receptor of activated C kinase 1

RefSeq	:	Reference sequence
RFLP	:	Restriction fragment length polymorphism
RNA	:	Ribonucleic acid
RNA Seq	:	RNA sequencing
rpm	:	Revolutions per minute
RT-PCR	:	Reverse-trancription
SAGE	:	Serial analysis of gene expression
SLC	:	Solute carrier family
SLC22A7	:	Solute carrier family 22 (organic anion transporter), member 7
SMART	:	Switching mechanism at 5' end of RNA transcript
SNP	:	Single nucleotide polymorphism
SSC	:	Sus scrofa chromosome
T	:	Thymine
TAE	:	Tris-acetate buffer
TBE	:	Tris- borate buffer
TE	:	Tris- EDTA buffer
tRNA	:	Transfer RNA
UGT	:	Uridine 5'-diphospho-glucuronosyltransferase
UTR	:	Untranslated region
UV	:	Ultra-violet light
V	:	Volts
v/v	:	Volume per volume
W	:	Watts

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1.1 Introduction

Boar taint is the offensive odour or taste that can be evident during the cooking or eating of porcine meat derived from non-castrated male pigs which is primarily due to high levels of androstenone and skatole in fat (Wackers et al. 2011). The taint has been described as being similar to urine and manure and may occur in meat from uncastrated sexually mature male pigs (Grindflek et al. 2011). Androstenone and skatole are the two compounds thought to be responsible for boar taint that are deposited in the fat. Androstenone is synthesized in the testis from pregnenolone (Gower 1972; Kwan et al. 1985; Robic et al. 2008), in relation with sexual development. It is mainly degraded in liver and deposited in adipose tissue because of its lipophilic properties (Doran et al. 2004). Metabolism of androstenone is presented in two phases: phase I consists of metabolism by hydrogenation and phase II consists of metabolism by sulfoconjugation in testis liver or in liver (Doran et al. 2004; Sinclair and Squires 2005; Sinclair et al. 2005b). Therefore, high levels of androstenone in fat can be dedicated to a high intensity of testicular synthesis and/or a low intensity of liver degradation (Robic et al. 2008). The most common practice to prevent this smell is castration of male piglets (Haugen et al. 2012). But castration is undesirable due to ethical and economical concerns (Pauly et al. 2008) and castration of piglets is announced to be banned in the European Community by 2018 (Morlein et al. 2012a). Notably, it is well known that the high concentration of male sex steroids such as androstenone prevent the enzymes responsible for skatole metabolism resulting in the reduction of skatole metabolism in liver and accumulation in adipose tissues (Andresen 2006). Therefore, other than the castration, alternative methods needs to developed to prevent tainted meat. Skatole is a metabolite of tryptophan which is produced by intestinal bacteria such Clostridium and Bacteroides genera in gut and catabolised in liver (Wesoly and Weiler 2012). In pig, skatole is absorbed by the intestinal mucosa into the portal vein passes through the liver where it is efficiently metabolised. Three major metabolites isolated from pigs are 6-sulfatoxyskatole (MII), 3-hydroxy-3-methyloxindole (MIII) and 3-methyl indole (Baek et al., 1995). Among these skatole metabolites, MII that is secreted in plasma and urine as a sulphate conjugate, and MIII is found to be related to the skatole levels in fat (Baek et al. 1995). However, a proportion of skatole passes the liver without being metabolised and accumulates in adipose tissue that produced tainted meat in pigs (Baek

et al. 1995). A relationship between levels of androstenone and skatole has been established and it has been suggested that elevated levels of skatole is secondary to elevated androstenone (Robic et al. 2008; Squires and Lundstrom 1997). Genetic and breeding is proposed as one of the most realistic approaches for reducing levels of boar taint. Notably, androstenone and skatole are the well defined compounds describing the phenotypic trait (boar taint) which is possible to improve through genetic selection. The average heritability for androstenone levels is high (h^2=0.56), ranging from 0.25 to 0.88 (Sellier et al. 2000). Skatole levels show medium heritability ranging from 0.19 to 0.54 (Pedersen 1998; Tajet et al. 2006), whereas, Windig et al. (2012) estimated heritability for androstenone and skatole was 0.54 and 0.41, respectively.

Reduction of boar taint levels without castration is of utmost interest for pig breeders worldwide. Identification of genetic factors controlling boar taint may be implemented in breeding programmes to select animals that produce low levels of taint. Approaches to lower the level of boar taint focus to lower the boar taint compounds: androstenone and skatole. A number of candidate genes have been identified for reducing boar taint compounds in different populations (Grindflek et al. 2011; Moe et al. 2009; Robic et al. 2011a) with the final goal to utilize this genetic information in the breeding schemas. Androstenone synthesis is initiated by cleavage of cholesterol to produce pregnenolone. This reaction is catalysed by the enzyme CYP11A (Robic et al., 2008). Formation of 16-androstene steroids from pregnenolone is orchestrated by CYB5 which causes overproduction of 16-androstene steroids in testis (Davis et al. 1998; Katkov and Gower 1970). Two other cytochrome P450 enzymes CYP17 and CYP21 have also been investigated for the involvement in steroidogenesis (Robic et al. 2008). 3-β-hydroxysteroid dehydrogenase (3β-HSD) enzyme encoded by HSD3B gene (Nicolau-Solano et al. 2006) reduces androstenone to β-androstenol in pig liver microsomes (Doran et al. 2004). The 16-androstene steroids in the liver and testis are sulfoconjugated by hydroxysteroid sulfotransferase (SULT2A) (Sinclair et al. 2005a; Sinclair and Squires 2005). In case of skatole, a proportion of skatole, passes the liver without being metabolised and accumulates in adipose tissue (Baek et al. 1995). The reason must be related to testicular activity and especially to the action of sex steroids, androstenone included. In this regard, genes coding for enzyme of the cytochrome family received considerable interest, due to their role in the skatole metabolism. Such as hepatic cytochrome P4502E1 (CYP2E1) is the main hepatic enzyme involved in the

metabolism of skatole (Babol et al., 1999). Significant association between SNPs within the CYP2E1, CYP21 gene and reduced skatole levels are identified in pigs (Moe et al. 2009; Morlein et al. 2012b). A mutation in the coding region of CYP2A6, another member of the cytochrome family was found to be associated with higher level skatole in fat (Lin et al. 2005).

With the aim to identify candidate genes, a number of quantitative trait loci (QTL) affecting boar taint compound have been identified in pigs (Lee et al. 2005; Quintanilla et al. 2003). Several QTL for androstenone were identified in different pigs. QTL for androstenone in fat and plasma, testosterone, and estrogens were detected on SSC 3, 4, 13, and 15, and a QTL affecting solely androstenone in plasma was detected on SSC 6 (Grindflek et al. 2011). Quintanilla et al. (2003) reported major QTL for androstenone on pig chromosome SSC 3, 7 and 14. For skatole, several QTL were identified on different pig chromosomes such as on SSC6, SSC7, SSC12, SSC13, SSC14 and SSCX in different pig populations (Lee et al. 2005; Varona et al. 2005). Another approach to identify candidate genes was reported using transcriptome studies. Several candidate genes have been proposed for divergent androstenone levels in different pig populations by global transcriptome analysis in boar testis and liver samples (Moe et al. 2008; Moe et al. 2007; Robic et al. 2008). They confirm the involvement of CYP17 and CYB5 and detected a number of other genes involved in the steroid hormone pathway that seem to be essential for androstenone levels. Besides SULT2A1, other conjugation enzyme genes that might be important were identified including SULT2B1, AKR1C4, GSTO1, MGST1 and HSD17B4. Moreover, differential expression were also found for genes encoding 17beta-hydroxysteroid dehydrogenase (HSD17B2, HSD17B4, HSD17B11 and HSD17B13) and plasma proteins alpha-1-acid glycoprotein (AGP) and orosomucoid (ORM1) (Moe et al. 2008). The functional approach comprises of investigations to estimate gene expression patterns (Lin et al. 2005; Luo et al. 2006), pathway analysis (Whitehead et al. 2008) and of association studies between SNP within candidate genes boar taint compound levels (e.g. (Doran et al. 2002; Skinner et al. 2006). Special emphasis is laid on genes coding for enzymes that are involved in the liver metabolism of androstenone and skatole. A decreased boar taint compounds metabolism will finally lead to a higher accumulation of them in the adipose tissues (Diaz et al. 2009; Diaz et al. 1999). For androstenone, a genome wide association study (GWAS) was initiated using the SNP array to identify the chromosomal regions and

specific SNPs influencing boar taint levels in commercial breeding population (Duijvesteijn et al. 2010). The association analysis with 47,897 SNPs revealed that androstenone levels in fat tissue were significantly affected by 37 SNPs on pig chromosomes SSC1 and SSC6 (Duijvesteijn et al. 2010). CYP11A1 is reported to be involved in the androstenone metabolism in boars (Robic et al. 2011b). Another cytochrome sub family gene such as cytochrome P450A19 (CYP2A19) was reported to be a potential candidate gene for androstenone levels (Duijvesteijn et al. 2010). Additionally, genes belongs to sulfotransferases family (SULT2A1 and SULT2B1) and hydroxysteroid-dehydrogenases family (HSD17B14) were also reported to be involved in the androstenone metabolism (Duijvesteijn et al. 2010). For skatole, genome wide association studies using Porcine 60 K SNP bead chips were applied to map associations with skatole levels in fat on SSC6 (Ramos et al. 2011). The genome-wide association study revealed that 16 SNPs located on the proximal region of chromosome 6 were significantly associated with skatole levels (Ramos et al. 2011).

Transcriptome sequencing or RNA seq has emerged recently as a powerful tool to gain a holistic picture of the expression profile of an organism, tissue or cells (Nagalakshmi et al. 2009; Ozsolak et al. 2009). RNA-Seq also provides evidence for identification of splicing events, polymorphisms and different family isoforms of transcripts (Marguerat and Bahler 2010). Recent studies in livestock species have employed RNA seq to identify the transcriptome of animal cells, such as cow milk (Canovas et al. 2010), bovine embryos (Huang and Khatib 2010), sheep bone (Jager et al. 2011), bovine abomasum (Li et al. 2011) and tissues as pig gonads (Esteve-Codina et al. 2011), liver (Chen et al. 2011), muscle, abdominal fat (Jung et al. 2012) and porcine endometrium (Saborski et al. 2013). Although RNA-seq greatly advances our understanding of complex transcriptome landscapes, such as those found in mammals, complete RNA-seq studies in livestock and in particular in the pig are still lacking. In accordance with many studies done in this field as well as future perspectives, there is an inevitable need for using RNA deep sequencing for transcriptome profiling related boar taint compounds androstenone and skatole to elucidate the genes involved in androstenone and skatole metabolism and to select animals that produce low levels of taint. With this background, two experiments were conducted in this thesis to achieve the following aims:

1. In the first experiment, to identify the transcriptome profiling in the testis and liver tissues between boars with high and low androstenone levels in their backfat and to study polymorphisms and associations that appeared on the selected differentially expressed genes (Chapter 1).

2. In the second experiment, to investigate the differential expression of genes in the liver tissues of boars with high skatole and low skatole in their backfat, to perform differential exon expression and association analysis of gene variants that appeared on the selected differentially expressed genes (Chapter 2).

1.2 Materials and methods

To achieve the objectives of this study, several materials and methods were used. The details of materials and methods are described in the respective chapters of this thesis. The importance of the main methods and their description are briefly summarized here.

1.2.1 Animals and phenotype

Tissue samples and phenotypes were collected from the Duroc × F_2 cross animals. F_2 was created by crossing F_1 animals (Leicoma × German Landrace) with Large White pig breed. Duroc × F_2 boars were on average 116 days old and had on average 90 kg live weight when slaughtered. All the pigs were slaughtered in a commercial abattoir. Animals were bred and growth, carcass and meat quality data were collected according to guidelines of the German performance test (ZDS 2003). Tissue samples from testis and liver were frozen in liquid nitrogen immediately after slaughter and stored at -80°C until used for RNA extraction. Fat samples were collected from the neck and stored at -20°C until used for androstenone and skatole measurements. For the quantification of androstenone and skatole an in-house gas-chromatography/mass spectrometry (GC-MS) method was applied (Fischer et al. 2011). In the first experiment (androstenone study), pigs having a fat androstenone level less than 0.5 µg/g and greater than 1.0 µg/g were defined as low and high androstenone samples, respectively (Bonneau et al. 2000; Frieden et al. 2011). Ten boars were selected from a pool of 100 pigs and the average androstenone value for these selected animals was 1.36 ± 0.45 µg/g. RNA was isolated from testis and liver of 5 pigs with extreme high (2.48 ± 0.56 µg/g) and 5 pigs with extreme low levels of androstenone (0.24 ± 0.06 µg/g). In the second experiment (skatole compound), pigs having a fat skatole level less than 0.25 µg/g and greater than 0.25 µg/g were defined as low and high skatole samples, respectively (Andresen, 2006; Mortensen et al. 1986; Strathe et al. 2012). Six boars were selected from the same pool of 100 pigs and the average skatole value for these selected animals was 0.27 ± 0.20 µg/g. RNA was isolated from liver of 3 pigs with extreme high (0.45 ± 0.08 µg/g) and 3 pigs with extreme low levels of skatole (0.09 ± 0.02 µg/g). Notably, these six boars were among the ten boars used previously for androstenone study (Experiment 1) Among the ten pigs used in androstenone study, six pigs were found with extremely high and low skatole levels and were considered for this study. Notably, only liver

sample were considered for skatole study. Total RNA was extracted using RNeasy Mini Kit according to manufacturer's recommendations (Qiagen). Total RNA was treated using on-column RNase-Free DNase set (Promega) and quantified using spectrophotometer (NanoDrop, ND8000, Thermo Scientific). RNA quality was assessed using an Agilent 2100 Bioanalyser and RNA Nano 6000 Labchip kit (Agilent Technologies).

1.2.2 Library construction and sequencing

The ideal method for transcriptomics should be able to directly identify and quantify all RNAs, small or large. RNA-Seq provides evidence for transcriptome profiling, polymorphisms, identification of splicing events and different family isoforms of transcripts (Marguerat and Bahler 2010). In this thesis, the RNA deep sequencing technology was used to obtain differential expression, polymorphism and alternative splicing detection. For this purpose, full-length cDNA was obtained from 1 μg of RNA, with the SMART cDNA Library Construction Kit (Clontech, USA), according to the manufacturer's instructions. Libraries of amplified RNA for each sample were prepared following the Illumina mRNA-Seq protocol. The library preparations were sequenced on an Illumina HiSeq 2000 as single-reads to 100 bp using 1 lane per sample on the same flow-cell (first sequencing run) at GATC Biotech AG (Konstanz, Germany). All sequences were analysed using the CASAVA v1.7 (Illumina, USA). As described in Gunawan et al. (2013), the deep sequencing data have been deposited in NCBI SRA database and are accessible through GEO series accession number GSE44171 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE44171).

1.2.3 Differential gene expression analysis

The differential gene expression analysis was designed to contrast the difference in the expression of genes between two different conditions of samples. For differential gene expression analysis with raw count data the R package DESeq was used (Anders and Huber 2010). The normalization procedure in DESeq handles the differences in the number of reads in each sample. For this purpose, DESeq first generates a fictitious reference sample, with read counts defined as the geometric mean of all the samples. The read counts for each gene in each sample is divided by this geometric mean to obtain the normalized counts. To model the null distribution of the count data, DESeq

follows an error model that uses the negative binomial distribution, with variance and mean linked by local regression. The method controls type-I error and provides good detection power (Anders and Huber 2010). After analysis using DESeq, DEGs were filtered based on *p*-adjusted value (Benjamini and Hochberg 1995) 0.05 and fold change ≥ 1.5. Additionally, the gene expression data was also analyzed using a Generalized Linear Model (GLM) function implemented in DESeq to calculate both within and between group deviances. As sanity checking and filtration step, we cross matched the results from both analysis (*p*-adjusted ≤ 0.05 and fold change ≥ 1.5 criteria and GLM analysis) and only those genes which appeared to be significant in both of the tests (*p*-value ≤ 0.05), were selected for further analysis

1.2.4 Gene variant analysis

Gene variant or polymorphisms analysis have great potential for use in genetic-mapping studies, which locate and characterize genes that are important in biological functions. In this thesis, for gene variation analysis the mapping files were generated by aligning the raw reads to the reference sequence set. All the downstream analysis was performed using Genome Analysis Toolkit (GATK) (McKenna et al. 2010) and Picard Tools (http://picard.sourceforge.net/). SNPs were furthermore classified as synonymous or non-synonymous using the GeneWise software (http://www.ebi.ac.uk/Tools/psa/genewise/ by comparing between protein sequence and nucleotides incorporated SNP position (Birney et al. 2004). Covariate counting and base quality score recalibration were done using the default parameters suggested by GATK toolkit. The re-aligned and recalibrated mapping files were grouped according to tissue and phenotype categories. Variant calling was performed for each group using GATK UnifiedGenotyper (McKenna et al. 2010). To find out the differentially expressed genes that also harboured sequence polymorphisms, we filtered the results from UnifiedGenotyper with chromosomal positions of DEGs and retained only those which mapped to DEG chromosomal positions.

1.2.5 Pathways and networks analysis

A list of the DEGs was uploaded into the Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, www.ingenuity.com) to identify relationships between the genes of interest and to uncover common processes and pathways. The 'Functional Analysis'

tool of the IPA software was used to identify the biological functions that were most significant to the data set. Canonical pathway analysis was also utilized in case of second experiment to identify the pathways from the IPA library of canonical pathways that were most significant to the data set. The significance of the association between the data set and the canonical pathway was calculated as the ratio of the number of genes from the data set that mapped to the pathway divided by the total number of genes that mapped to the canonical pathway (www.ingenuity.com).

1.2.6 Quantitative Real Time PCR (qRT-PCR)

cDNA were synthesised by reverse transcription PCR using 2 μg of total RNA, SuperScript II reverse transcriptase (Invitrogen) and oligo(dT)12 primer (Invitrogen). Gene specific primers for the qRT-PCR were designed by using the Primer3 software (Rozen and Skaletsky 2000). In each run, the 96-well microtiter plate contained each cDNA sample and no-template control. The qRT-PCR was conducted with the following program: 95 °C for 3 min and 40 cycles 95 °C for 15 s/60 °C for 45 s on the StepOne Plus qPCR system (Applied Biosystem). For each PCR reaction 10 μl iTaqTM SYBR$^{®}$ Green Supermix with Rox PCR core reagents (Bio-Rad), 2 μl of cDNA (50 ng/μl) and an optimized amount of primers were mixed with ddH$_2$O to a final reaction volume of 20 μl per well. All samples were analysed twice (technical replication) and the geometric mean of the Ct values were further used for mRNA expression profiling. The geometric mean of two housekeeping genes GAPDH and PPIA were used for normalization of the target genes. The delta Ct (ΔCt) values were calculated as the difference between target gene and geometric mean of the reference genes: (ΔCt = Ct$_{target}$- Ct$_{housekeeping \, genes}$) as described in Silver et al.(2006). Final results were reported as fold change calculated from delta Ct-values.

1.2.7 Genotyping using PCR-RFLP

PCR-RFLP was used for genotyping SNPs validation. The restriction enzymes were selected according to the recognition (http://tools.neb.com/NEBcutter2/index.php) of the polymorphic sites. The fragments with the detected mutation were amplified using different annealing temperature to get the PCR products. An aliquot of the PCR product of each reaction was checked on 1.5% agarose gel (Fisher Scientific Ltd.) before digestion using different endonucleases. The digested products were separated using

3.0% agarose gel. The fragments were visualized under ultraviolet light, and the sizes and the number of fragments analysed using the molecular analyst software (BioRad Laboratories, Molecular Bioscience Group).

1.2.8 Statistical analysis

Statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, USA) for association study. Effects of slaughter age, husbandry system (pen) as well as genotype on boar taint compound androstenone (Chapter 1) and skatole (Chapter 2) were assessed with fixed effect model (ANOVA) using PROC GLM. For all models, fixed effects included genotype and pen (group, individual), and age of slaughter was fitted as a covariate for boar taint compound androstenone and skatole. Due to the skewed nature of boar taint compound androstenone (Chapter1) and skatole (Chapter 2), data were transformed with natural logarithm before ANOVA to achieve normality. Least square mean values for the loci genotypes were compared by t-test and p-values were adjusted by the Tukey–Kramer correction (Cinar et al. 2012; Kayan et al. 2011).

1.2.9 Differential exon usage analysis

Differential exon usage analysis was performed only in case of second experiment to understand the exon usages contributed to skatole levels. For this purpose, we used the R package DEXSeq (Anders et al. 2008). The mapped read count data were converted into exon "counting bins" as described by Anders et al. (2008) and the algorithm of normalized sequencing depths for all the samples were performed according to Anders and Huber (2010). Generalized Linear Models (GLMs) were employed by the algorithm for each counting bin to test for differential expression between phenotype samples. After the analysis, differentially used exons were filtered using the criteria p-adjusted value < 0.05.

1.3 Results

The main results in this thesis are briefly described here. The detailed results can be found in the respective chapters in this thesis.

1.3.1 Transcriptome profile and polymorphism detection in boar testis and liver with divergent androstenone levels.

In the first experiment (Chapter 1), we sequenced cDNA libraries from 10 samples per tissue using Illumina HiSeq 2000. Samples were obtained from testis and liver of 5 pigs with extreme high (2.48 $\pm$ 0.56 µg/g) and 5 pigs with extreme low levels of androstenone in their backfat(0.24 $\pm$ 0.06 µg/g). The total number of reads produced for each testis and liver sample ranged from 13.2 to 33.2 million and 12.7 to 46.0 million respectively. In testis samples, 46 genes were differentially regulated whereas 25 genes showed differential expression in the liver. The fold change values ranged from -4.68 to 2.90 in testis samples and -2.86 to 3.89 in liver samples. Differentially regulated genes in high androstenone testis and liver samples were enriched in metabolic processes such as lipid metabolism, small molecule biochemistry and molecular transport. This study provides evidence for transcriptome profile and gene polymorphisms of boars with divergent androstenone level using RNA-Seq technology. On the basis of number of DEGs, our results confirm that transcriptome activity in testis is higher in comparison to liver tissue for androstenone biosynthesis. Differential expression analysis identified candidate genes in cytochrome P450 family (CYPs), flavin monooxygenease family (FMOs) and hydroxysteroid dehydrogenase family (HSD). We propose additional functional candidate genes such as, DKK2 and CYP2B22 in testis and IP6K1 and HSD17B2 in liver for androstenone metabolism. Importantly, most of the DEGs are in QTL positions functionally related to pathways involved in boar taint. Furthermore, various gene polymorphisms were also detected in testis and liver DEGs and associations were validated with androstenone levels. Potential polymorphisms and association were identified in DEGs such as IRG6, MX1 and IFIT2 in testis and CYP7A1, FMO5 and KRT18 in liver.

1.3.2 Trancriptome profile, polymorphism and alternative splicing events in boar liver with divergent skatole levels

In the second experiment (Chapter 2), six boars were selected from a pool of 100 pigs and the average fat skatole value for these selected animals was 0.27 ± 0.20 µg/g. The sample was isolated from liver of 3 pigs with extreme high (0.45 ± 0.08 µg/g) and 3 pigs with extreme low levels of skatole (0.09 ± 0.02 µg/g). The total number of reads produced for each liver sample ranged from 11.8 to 39.0 million with a median of 22.8 million. Approximately 448 genes were differentially regulated at a strict false discovery rate (FDR) <0.05. The fold change values ranged from -6.79 to 5.82. Among them, 383 genes were significantly up-regulated in higher skatole group and 65 were significantly down-regulated (p<0.01, FC > 1.5). Differentially regulated genes in high skatole liver samples were enriched in metabolic processes such as small molecule biochemistry, protein synthesis, carbohydrate metabolism, energy production, lipid metabolism and amino acid metabolism. Morever, the pathways showing the highest level of significance were remodeling of epithelial adherens junction and TCA cycle which play an important regulatory role in metabolism of the high skatole groups. Differential gene expression analysis identified candidate genes in ATP binding, cytochrome P450, keratin, phosphoglucomutase, isocitrate dehydrogenase and solute carier family. Moreover, polymorphism and association analysis revealed mutations in ATP5B, KRT8, PGM1, SLC22A7 and IDH1; this genes could be potential candidate markers for skatole levels in boars. In addition, expression analysis of differential exon usage of three genes (ATP5B, KRT8 and PGM1) revealed significant differential expression of these genes in the different skatole level groups. These polymorphisms and differential exon expression may have an impact on the gene activity ultimately leading to skatole variation and could be used as genetic marker for boar taint related traits.

1.4 Conclusions

This thesis was devoted to identify the transcriptome of testis and liver for the boar taint compound androstenone and to the transcriptome of liver for skatole. The transcriptome profiling in boar testis and liver with divergent androstenone levels are described in Chapter 1 and the transcriptome profiling in boar liver with divergent skatole levels are described in Chapter 2.

In the case of transcriptome analysis related to androstenone levels (Chapter 1), 45% to 50% fragments of the transcriptome do not map to annotated exons. However, it was possible to identify genes associated with divergent androstenone levels. The percentage of annotated reads varies from 15.6% to 60.8% in similar porcine transcriptome studies using RNA deep sequencing (Bauer et al. 2010; Chen et al. 2011; Esteve-Codina et al. 2011). The differences for mapping percentages might be due to several factors such as primer biases, GC content, dinucleotide fragmentation sites, independent cell types and laboratory protocols (McIntyre et al. 2011; Sendler et al. 2011). Another factor is that the current reference transcriptome assembly (Sscrofa10.2) might not cover all transcribed mRNA and consequently low abundant transcripts or rare alternative splicing isoforms are less likely to be mapped to transcriptome assembly (Esteve-Codina et al. 2011).

The transcriptome analysis of testis tissues for androstenone revealed a number of differentially expressed genes that are similar to the differentially regulated genes reported previously in porcine testis using microarray (Leung et al. 2010; Moe et al. 2007). The differentially regulated genes are enriched with metabolic process such as lipid metabolism which is coinciding with a previous study (Moe et al. 2007). Cytochrome P450 superfamily genes are found to be differentially regulated in the investigated testis samples wihch is supported by other studies (Grindflek et al. 2010; Moe et al. 2007). This study found that CYP4B1, CYP4A11 and CYP2C33 genes were up regulated and CYP2B22 gene was down regulated in testis sample. CYP2C33 belongs to the same sub-family as CYP2C49, but its function has yet to be described in the literature. Metabolism of androstenone is presented in two phases: phase 1 consists metabolism by hydrogenation and phase 2 consists metabolism by sulfoconjugation in testis or in liver (Doran et al. 2004; Robic et al. 2008). Phase I includes the initial, mostly oxidative reactions usually performed by the membrane bound cytochrome P450

system (Guengerich 1991). Therefore, the differential expression of CYP4B1, CYP4A11, CYP2C33 and CYP2B2 might suggest that more genes are involved in phase 1 reaction of androstenone metabolism.

When the liver tissues are analysed for the androstenone levels, Gene Ontology categories molecular transport, small molecule biochemistry and lipid metabolism are the most dominant functional pathways involved. Similar GO categories are reported previously by Moe et al. (2008). In the liver sample, HSD17B2 was the highest down regulated gene in the higher androstenone boars. HSD17B2 regulates the availability of testosterone and androstenedione in tissues by catalysing interconvertion of active and inactive forms of steroids (Baker 2001). Another gene family found to be differentially expressed in this transcriptome analysis is the flavin-containing monooxygenases (FMOs) gene family. The FMO family of enzymes converts lipophilic compounds into more polar metabolites and decreases activity of the compounds (Cashman 2005). Using microarray analysis, FMO1 is reported to be up-regulated in higher androstenone pigs (Moe et al. 2008), but FMO5 was found to be down-regulated in high androstenone liver samples in this study. Since androstenone is a lipophilic compound, we speculate that androstenone level may be negatively correlated with FMO5 activity.

In addition to transcriptome quantification of liver and testis, RNA-Seq technology provides valuable information regarding gene polymorphisms which could be correlated with the relevant phenotype of androstenone levels. This study extends these observations by correlating differentially regulated genes with associated polymorphisms. Potential polymorphisms and association were identified in DEGs such as in IRG6, MX1 and IFIT2 in the testis, and in CYP7A1, FMO5 and KRT18 in the liver. An association study was performed for a SNP (g.494 A>G) in the FMO5 gene but no statistical relation could be detected with the off flavour score in the Berkshire x Yorkshire resource population (Glenn et al. 2007). Location of IFIT2 gene on SSC14 incorporated the QTL affecting androstenone in Yorkshire pig (Gregersen et al. 2012) and subjective pork flavour in Large White and Meishan pigs (Lee et al. 2005). MX1 is an interesting candidate gene for disease resistance in farm animals (Morozumi et al. 2001) but this study first identifies association with boar taint compounds. No study investigated the association of CYP7A1 with boar taint compounds. Association of this gene with plasma cholesterol is reported previously in pigs (Davis et al. 1998). Boar taint is related to the adipose tissues since lean pigs have low boar taint compounds

(Wesoly and Weiler 2012). Furthermore, KRT18 gene maps close to a region on SSC5 affecting androstenone levels in pigs (Grindflek et al. 2011). Although, the function of highly polymorphic KRT18 is relating to pathological processes in liver, its involvement in boar taint is not quite clear.

In case of the transcriptome profiling in the liver for the skatole level (Chapter 2), the average of the total number of reads was 22.85 million and about 65.5 % were categorized as mapped reads corresponding to exon reads. The proportion of reads mapped to exons of annotated genes was in accordance with several other studies (Chen et al. 2011; Jung et al. 2012; Ramayo-Caldas et al. 2012) in pig liver transcriptome (60.2-74.9%), but was higher than that reported in porcine male gonad of around 44.1% (Esteve-Codina et al. 2011). Notably, Illumina deep sequencing has been described as replicable with relatively little technical variation (Marioni et al. 2008). Therefore, the findings of this study clearly demonstrated the power of RNA-Seq and provide further insights into the transcriptome of liver at a finer resolution in skatole divergent boars.

When the enriched GO categorisation for DEGs in liver samples was performed, result showed small molecule biochemistry and energy production are to be the most dominant functional categories. Similar functional categories are reported previously in the liver tissues for meat quality (Ramayo-Caldas et al. 2012). Moreover, pathway analysis of DEGs showed remodelling epithelial of adherens junction as to be the most dominant pathways in this study. The intercellular adherens junctions are specialized sub-apical structures that function as principle mediators of cell-cell adhesion (D'Souza-Schorey 2005). Their assembly-disassembly is dynamic and stringently regulated during tissue morphogenesis and homeostasis (Gumbiner 1996). This deep sequencing result identified for the first time the remodelling epithelial of adherens junction to be possibly involved in the metabolism of skatole in porcine liver. The metabolism of skatole can be divided into two phases: an oxidative step (phase 1 metabolism) and a conjugative step (phase 2 metabolism). Phase 1 includes the oxidative reactions usually performed by the membrane bound cytochrome P450 system (Guengerich 1991). Notably, CYP4A24, CYP4A25 and CYB4B24 were found to be up-regulated in high skatole in Duroc × F2 population in this study which is in agreement with previous result for Landrace population (Grindflek et al. 2011). Cytochrome P450 isoenzymes are the main enzymes playing roles in phase 1 skatole metabolism where skatole is degraded to several intermediate products including such as indole-3-carbinol(I3C), 2-aminoacetophenone

(2AAP), 3-metyloxyindole (3MOI) etc (details reviewed by Deslandes et al. 2001; Robic et al. 2008; Wesoly and Weiler 2012). The main enzymes of phase 2 metabolism are UGT (uridine 5'-diphospho-glucuronosyltransferase) and SULT1A1 (sulfotrasferase) (Agergaard and Laue 1993). Different groups of transferases including glutation S transferase omega 2 (GSTO2) and glutathione S-transferase mu 2 (GSTM2) were found to be differentially regulated in this study. The GSTs, reported to transport different molecules (Litowsky et al. 1988), might indicate that the GSTO transports the skatole to the tissues. It could be speculated that GSTO2 might be involved in the excretion skatole from the porcine body. During phase 2 metabolism, the water solubility metabolism of skatole is increased to facilitate excretion via urine (Baek et al. 1995; Diaz et al. 1999).

Potential polymorphisms in genes ATP5B, KRT8, PGM1, SLC22A7 and IDH1 were found to be associated with the phenotype skatole levels in this study. The ATP5B gene encodes the catalytic subunit of mitochondrial ATP synthesis complex and catalyzes the rate-limiting step of ATP formation in eukaryotic cells (Izquierdo 2006). ATP5B probably plays a key role in porcine skeletal muscle development and may provide further insight into the molecular mechanisms responsible for breed-specific differences in meat quality (Xu et al. 2012). However, this study implies that in addition to the meat quality traits this gene could an important candidate for boar taint trait. The function of KRT8 gene is relating to pathological processes in liver but its involvement in boar taint is not quite clear. However, this gene maps close to a region on SSC5 affecting skatole and indole (Gregersen et al., 2012), warranting to study more about this gene and polymorphisms with regards to boar taint. PGM1 is involved in glucose metabolism pathway and Lefaucher (2010) reported the higher expression of genes of glycolytic pathways including this gene in the Large White. This agrees with the more glycolytic and less oxidative muscle metabolism. However, no study is available to unravel the involvement of this gene in boar taint compounds metabolism. The SLC22A7 gene is involved in the sodium-independent transport and excretion of organic anions and the substrate panel of SLC22As includes important endogenous compounds like tryptophan metabolites and sulphated steroids (Bahn et al. 2005). Skatole results from a multistep degradation of tryptophan by microbial activity, mainly in the hind gut of the pigs (reviewed by Wesoly and Weiler 2012). Therefore, the marker identified on SLC22A7 could be a valuable SNP for boar taint but needs to be validated in other porcine

populations. Additionally, this study extends these observations by identifying a number of genes with differential exon expression between high and low skatole level. Chen et al. (2011) reported that about 18.8% of the annotated genes showed differential exon usage events in pigs with divergent meat quality traits. This study revealed differential expression levels of differential exon expression for ATP5B, KRT8 and PGM1 genes in low skatole in comparison to high skatole group suggesting that differential processing of RNA could be associated with the regulation of skatole level.

From the results of this study, it could be concluded that metabolic processes might be important for the boar taint compounds androstenone and skatole levels. This study also highlighted several genes that are differentially regulated between boars with high and low boar taint compounds levels. Furthermore, the polymorphisms in several candidate genes confirmed to be associated with the phenotypes boar taint compounds could be valuable markers for the boar taint compounds androstenone and skatole.

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RNA Deep Sequencing Reveals Novel Candidate Genes and Polymorphisms in Boar Testis and Liver Tissues with Divergent Androstenone Levels

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Abstract

Boar taint is an unpleasant smell and taste of pork meat derived from some entire male pigs. The main causes of boar taint are the two compounds androstenone (5α-androst-16-en-3-one) and skatole (3-methylindole). It is crucial to understand the genetic mechanism of boar taint to select pigs for lower androstenone levels and thus reduce boar taint. The aim of the present study was to investigate transcriptome differences in boar testis and liver tissues with divergent androstenone levels using RNA deep sequencing (RNA-Seq). The total number of reads produced for each testis and liver sample ranged from 13,221,550 to 33,206,723 and 12,755,487 to 46,050,468, respectively. In testis samples 46 genes were differentially regulated whereas 25 genes showed differential expression in the liver. The fold change values ranged from -4.68 to 2.90 in testis samples and -2.86 to 3.89 in liver samples. Differentially regulated genes in high androstenone testis and liver samples were enriched in metabolic processes such as lipid metabolism, small molecule biochemistry and molecular transport. This study provides evidence for transcriptome profile and gene polymorphisms of boars with divergent androstenone level using RNA-Seq technology. Digital gene expression analysis identified candidate genes in flavin monooxygenease family, cytochrome P450 family and hydroxysteroid dehydrogenase family. MoreverMoreover, polymorphism and association analysis revealed mutation in *IRG6, MX1, IFIT2, CYP7A1, FMO5* and *KRT18* genes could be potential candidate markers for androstenone levels in boars. Further studies are required for proving the role of candidate genes to be used in genomic selection against boar taint in pig breeding programs.

Introduction

Boar taint is an off-odor and off-flavor meat trait, mainly caused by high levels of androstenone, skatole and/or indole in adipose tissue [1]. The taint has been described as being similar to urine and manure and may occur in meat from uncastrated sexually mature male pigs [2]. Consumers commonly show a strong aversion to tainted meat. Currently, surgical castration of male piglets is a common practice in many countries to produce taint-free porcine meat [3]. However, castration is undesirable due to ethical and economical concerns [4] and rearing entire males instead of castrates has a number of advantages including higher efficiency, leaner carcasses and lower faecal and urinary nitrogen losses [5]. By 2018, castration of piglets is going to be banned in

the European Community [6]. Consequently, there is an urgent need to develop alternative methods to prevent tainted meat. In literature, it has been mentioned that lowering the slaughter weight or choosing a definite breed can reduce the boar taint [7], however, these could lead to some economical drawbacks. Skatole is a derivative of tryptophan produced in the hindgut of pigs by intestinal bacteria. The level of intestinal skatole production is mainly dependent on nutritional factors and no genetic control has been demonstrated so far [8]. On the other hand, for androstenone high heritability estimates (h^2 = 0.25 to 0.87) and differences between sire lines have been reported[9; 10; 11]. Consequently molecular breeding seems to be a promising way to produce pigs without boar taint.

Androstenone is synthesized in the testis from pregnenolone [8; 12; 13], in relation with sexual development. It is mainly degraded in liver and deposited in adipose tissue because of its lipophilic properties [14]. Metabolism of androstenone is presented in two phases: phase I consists metabolism by hydrogenation and phase II consists metabolism by sulfoconjugation in testis or in liver [8; 14; 15; 16]. Therefore, in theory, high levels of androstenone in fat can be dedicated to a high intensity of testicular synthesis and/or a low intensity of liver degradation [8]. This phenomenon is mainly controlled by enzymes and regulatory proteins such as cytochrome P450 and hydroxysteroid sulfotransferase family. Cytochrome P450s (CYPs) act as mono-oxygenases, with functions ranging from the synthesis to the degradation of endogenous steroid hormones [17]. Androstenone synthesis is initiated by cleavage of cholesterol to produce pregnenolone. This reaction is catalysed by the enzyme *CYP11A* [8]. Formation of 16-androstene steroids from pregnenolone is orchestrated by *CYB5* which causes overproduction of 16-androstene steroids in testis [18; 19]. Two other cytochrome P450 enzymes *CYP17* and *CYP21* have also been investigated for the involvement in steroidogenesis [8]. 3-β-hydroxysteroid dehydrogenase (3β-HSD) enzyme encoded by *HSD3B* gene [20] reduces androstenone to β-androstenol in pig liver microsomes [14]. The 16-androstene steroids in the liver and testis are sulfoconjugated by hydroxysteroid sulfotransferase (*SULT2A*) [16; 21].

A number of quantitative trait loci (QTL) and genome-wide association analysis have been conducted for androstenone in the purebred and crossbred pig populations [2; 22; 23; 24; 25; 26]. Gene expression analysis has been used to identify candidate genes related to the trait of interest. Several candidate genes have been proposed for divergent

androstenone levels in different pig populations by global transcriptome analysis in boar testis and liver samples [27; 28; 29]. Functional genomics provides an insight into the molecular processes underlying phenotypic differences [30] such as androstenone levels. RNA-Seq is a recently developed next generation sequencing technology for transcriptome profiling that boosts identification of novel and low abundant transcripts [31]. RNA-Seq also provides evidence for identification of splicing events, polymorphisms, and different family isoforms of transcripts [32]. The major aim of this study was to elucidate the genes involved in androstenone metabolism in testis and liver tissues using RNA-Seq technology. For this purpose, we analyzed differential expression of genes between high and low androstenone sample groups and polymorphisms that appear on the differentially expressed genes.

Results

Analysis of RNA-Seq Data

We sequenced cDNA libraries from 10 samples per tissue using Illumina HiSeq 2000. The sequencing produced clusters of sequence reads with maximum 100 base-pair (bp) length. After quality filtering the total number of reads for testis and liver samples ranged from 13.2 million (M) to 33.2 M and 12.1 M to 46.0 M, respectively. There was no significant difference in the number of reads from low and high androstenone samples ($p = 0.68$). Total number of reads for each tissue group and the number of reads mapped to reference sequences are shown in Table 1 and 2. In case of testis 42.20% to 50.34% of total reads were aligned to reference sequence whereas, in case of liver 40.8% to 56.63% were aligned.

Differential Gene Expression Analysis

Differential gene expression for testis and liver with divergent androstenone levels were calculated from the raw reads using the R package DESeq [33]. The significance scores were corrected for multiple testing using Benjamini-Hochberg correction. We used a negative binomial distribution based method implemented in DESeq to identify differentially expressed genes (DEGs) in testis and liver with divergent androstenone levels. The smear plots for differential expression between high and low androstenone levels in testis and liver are given in Supplementary Figure S1. A

GLM analysis (implemented in DESeq package) was also done on the same data set to identify genes with a significant difference between within group deviance and between group deviances. Finally DEGs were selected based on criteria $p_{\text{adjusted}} < 0.05$ and fold change ≥ 1.5 from first analysis and $p_{\text{adjusted}} < 0.05$ in GLM analysis (Supplementary Table 1). A total of 46 and 25 DEGs were selected from the differential expression analysis for testis and liver tissues respectively (Table 3 and 4). In testis tissues, 14 genes were found to be highly expressed in high androstenone group whereas, 32 genes were found to be highly expressed in low androstenone group. In the liver tissue, 9 genes were found to be highly expressed in high androstenone group whereas, 16 genes were found to be highly expressed in low androstenone group (Table 3 and 4). The range of log fold change values for DEGs was from -4.68 to 2.90 for testis and from -2.86 to 3.89 for liver. Heat maps (Figures 1A and 1B) illustrate the DEGs identified in high and low androstenone testis and liver tissues.

Biological Function Analysis for DEGs

To investigate gene functions and to reveal the common processes and pathways among the selected DEGs, Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, www.ingenuity.com) was used. In testis samples, out of 46 DEGs 39 were assigned to a specific functional group based on the information from IPA (Figure 2). A large proportion (84.7%) of the DEGs from testis high androstenone group fell into Gene Ontology (GO) categories such as molecular transport, small molecule biochemistry, amino acid metabolism, embryonic development, carbohydrate metabolism, lipid metabolism and reproductive system development and function (Figure 2). The genes classified into each functional group are listed in the Table 5.

For the liver androstenone samples, out of 25 DEGs, 22 could be assigned to a specific functional group based on the information from IPA (Figure 3). A large proportion (88.0%) of the DEGs from liver high androstenone group was enriched with GO functional categories such as amino acid metabolism, small molecule biochemistry, cellular development, lipid metabolism, molecular transport, cellular function and maintenance and cellular growth and proliferation (Figure 3). The genes classified into each functional group are listed in Table 6.

Validation of Selected DEGs with Quantitative Real Time PCR (qRT-PCR)

In order to validate the RNA-Seq results, on the basis of differential expressions and functions related to androstenone, a total of 10 genes were selected and quantified using qRT-PCR. *ARG2, CYP2C33, MSMO1*, *EDN1* and *CYP2B22* genes from testis samples and *IP6K1, BTG3, CYP7A1, FMO5* and *HSD17B2* genes from liver samples were selected. For this purpose, the same samples used in the deep sequencing were used. Comparison of qRT-PCR data for 10 selected genes showed complete concordance of expression with the RNA-Seq results (Figure 4A and 4B). To further validate the expression of selected genes more robustly, new grouping of independently high (n=5) and low (n=5) androstenone are done among the remaining 90 pigs. The mRNA expressions of selected genes showed similar pattern of expression in this new groups (Figure 4C and D). Gene expression values for qRT-PCR were normalized using housekeeping genes *PPIA* and *GAPDH* [34].

Gene Variant Analysis

In total 222,225 and 202,249 potential polymorphism were identified in high and low androstenone testis groups. Among these identified polymorphisms, 8,818 in high androstenone group and 8,621 in low androstenone group were global polymorphisms with reference and accession identifiers in dbSNP database. Similarly in liver high and low androstenone samples 169,181 and 164,417 potential polymorphisms were identified. There were 6,851 global polymorphisms in high androstenone liver sample and 6,436 global polymorphisms in low androstenone liver sample.

Polymorphisms identified in DEGs for testis and liver samples are given in table 7 and table 8. In the testis samples 12 gene polymorphisms were identified in 8 DEGs (Table 7). Additionally our results of deep sequencing in limited number of animals revealed that mutations for the genes *CD244* and *ARG2* were specific for high androstenone testis tissues, whereas mutations in genes *IFIT2, DSP* and *IRG6* were specific for low androstenone testis samples. Furthermore, we have selected SNPs in *IRG6, DSP, MX1* and *IFIT2* genes to validate their segregation and association in our population (Supplementary Table S3 and Table S4). Polymorphisms in *IRG6* (g.118838598G>A), *MX1* (g.144420441C>T) and *IFIT2* (g.106102335 G>T) were associated with androstenone level (Table 10).

Thirty six mutations were identified in 11 DEGs in liver samples (Table 8).

Variation in *HAL* gene was specific for high androstenone liver samples whereas *FMO5*, *HIST1H4K* and *TSKU* gene variations were specific for low androstenone liver samples (Table 8). Read counts for individual samples for identified polymorphisms in testis and liver tissues are given in Supplementary Table S2. Additionally, we have validated SNPs in highly polymorphic genes *CYP7A1, KRT18* and *FMO5* and their association in our population (Supplementary Table S3 and Table S4). The SNP in *CYP7A1* (g.77201533 A>G), *KRT18* (g.16788495 G>A) and *FMO5* (g.104473018 G>A) were found to be associated with the phenotype androstenone level (Table 10).

Discussion

Analysis of RNA-Seq data

The present study describes the transcriptome profiles of testis and liver for androstenone by using RNA-Seq. To the best of our knowledge this study provides the first comprehensive insight into the transcriptome of androstenone metabolism in testis and liver tissue by using RNA-Seq. Using the whole transcriptome sequencing technique, we were able to identify the levels of differentially expressed genes and associate these genes with divergent androstenone levels in terms of boar taint. Our findings clearly demonstrated the power of RNA-Seq and provide further insights into the transcriptome of testis and liver in androstenone at a finer resolution. Illumina sequencing data have been described as replicable with relatively little technical variation[35].

Although 45% to 50% (Table 1 and 2) of the fragments do not map to annotated exons in our study, we were able to identify genes associated with divergent androstenone levels. Porcine annotation is incomplete, as evidenced by read mapping annotation. The percentage of annotated reads varies from 15.6% to 60.8% in similar porcine transcriptome studies [36; 37; 38]. The differences between mapping percentages might be due to several factors such as primer biases, GC content, dinucleotide fragmentation sites, independent cell types and laboratory protocols [39; 40]. Another factor is that the current reference transcriptome assembly might not cover all transcribed mRNA and consequently low abundant transcripts or rare alternative splicing isoforms are less likely to be mapped to transcriptome assembly[38].

Differential Gene Expression and Gene Polymorphism Analysis in Testis

In this study, 46 genes were differentially regulated in testis with divergent androstenone levels (Table 3). Our findings of differential gene expression are in accordance with the current understanding of androstenone metabolism as well as the previous findings in functional studies. In our study, the most up and down regulated genes *DKK2* and *KRT82* were found to be novel genes related to androstenone metabolism. Dickkopf-related protein 2 is encoded by the *DKK2* gene which was identified as the highest up regulated gene in our study (Table 3). *DKK2* can act as either an agonist or antagonist of Wnt/beta-catenin signalling [41]. WNT signalling in the testis has not been well understood, however it has been shown to play an important role in proliferation and self-renewal of mouse and human spermatogonia [42]. Mutation in β-catenin leads to the over activity of β-catenin in Sertoli cells caused testicular cord disruption, germ cell depletion, and inhibition of Müllerian duct regression suggesting that inhibition of β-catenin signalling is essential for Sertoli cell and testicular cord maintenance and germ cell survival [43]. Baes et al.[44] found that breeding against androstenone may have slightly adverse effects on semen quality. In the light of these external references it could be speculated that up regulation of *DKK2* gene in this study may have antagonistic effect on Wnt/beta-catenin signalling pathway which has shown to cause negative effects on sperm production. *KRT82* was the highest down regulated gene in high androstenone testis tissues in our study (Table 3). The protein encoded by this gene is a member of the keratin gene family which contains at least 54 functional keratin genes in humans [45]. Keratin-related genes are known to be affected by androgen exposure, especially by Dihydrotestosterone (DHT) exposure [46]. Relation of DHT with androsterone has been shown by Rizner et al. [47]. These literature evidences show that down-regulation of *KRT82* gene is the end result of high androgen metabolism in testis and not directly involved in androsterone synthesis.

There are similarities between gene expression differences found with RNA-Seq and those reported in previous microarray studies in porcine testis and liver tissues [27; 28; 29]. Grindflek et al. [48] and Moe et al. [29] reported cytochrome P450 superfamily genes to be differentially regulated in their investigated testis samples. In our study, other members of cytochrome P450 family genes were found to be differentially regulated in addition to genes reported by these previous studies. Our findings showed that genes *CYP4B1*, *CYP4A11* and *CYP2C33* were up regulated and gene *CYP2B22* was

down regulated (Table 3). Among these genes, *CYP4A11* was enriched in Gene Ontology categories molecular transport, small molecule biochemistry and lipid metabolism and gene *CYP4B1* was revealed in small molecule biochemistry (Table 5). In accordance with our results, Moe et al. [29] also showed lipid metabolism to be one of the enriched GO categories for DEGs in testis samples.

In addition to transcriptome quantification, RNA-Seq technology provides valuable information regarding gene polymorphisms which could be directly correlated with the relevant phenotype. Several holistic gene expression analyses have been performed for boar taint compounds by using microarray or Real-Time PCR technology [27; 28; 29]. Our study extends these observations by correlating differentially regulated genes with associated polymorphisms. Gene polymorphisms in the exonic regions might have direct effect on the expression of transcripts and connecting our identified polymorphisms from RNA deep sequencing with GWAS studies may give additional insight to variation in the androstenone levels. Results in our study revealed 12 mutations in androstenone testis samples (Table 7). On SSC3 four polymorphism were identified, two at 35 Mb (insertion) on gene *HBA2*, one at 48 Mb (SNP) on gene *LOC100516362* and one at 118 Mb (SNP) on gene *IRG6* (Table 7). Grindflek et al. [2] found two QTL regions on the same chromosome for androstenone at 38 to 40 Mb in Duroc breed by using SNP chip genotyping which is in agreement with our results. Similar to our detected SNP at 118 Mb, an androstenone QTL on SSC3 was identified between 113 Mb and 122 Mb regions in Duroc, Landrace and Yorkshire breeds [23]. We identified a SNP on SSC4 at position 93 Mb on gene *CD244*, however, no previous QTL region associated with boar taint related traits was reported before. Three polymorphisms were identified on SSC7, two SNPs on gene *DSP* at 4.9 Mb and an insertion polymorphism at 99 Mb on gene *ARG2*. SNP genotyping study by Grindflek et al. identified an androstenone related QTL region on chromosome 7 between region 80.8 Mb and 88.3 Mb [2] which is in close proximity to the polymorphism detected on gene *ARG2* in this study. Additionally, Ren et al.[49] identified a male reproductive trait (testosterone level) related QTL on the same chromosome at 77.2 cM region. In our study, two SNPs were identified on gene *MX1* at position 144 Mb on SSC13, yet to the best of our knowledge no QTL regions related with boar taint or male reproductive traits has been reported in this region. On SSC14 at position 106 Mb, two SNPs were identified on gene *IFIT2*. Cross matching the chromosomal positions of these SNPs

with data from dbSNP database showed that one of the SNPs (at position 106,102,335) has already been annotated in the SNP database (dbSNP ID: rs80925743). A QTL region for androstenone was identified on the same chromosome between 87.9 cM and 108.7 cM by Lee et al. [24] and the SNPs identified in our study fit into this previously identified androstenone QTL region.

Differential Gene Expression and Gene Polymorphism Analysis in Liver

Twenty five genes were found to be differentially regulated in liver tissue with divergent androstenone levels (Table 4). The top two up regulated genes in our liver sample were *LOC100512122* with log fold change 3.89 and *LOC100511195* with log fold change 3.57. However, we were not able to identify either the gene names or function through orthologue databases or BLAST sequence similarity searches. As a result, the functions of these genes cannot be discussed in detail here.

IP6K1 was the third highest up regulated in our liver samples. Inositol hexakisphosphate kinase 1 (*IP6K1*) is a member of the inositol phosphokinase family which encodes protein responsible for the conversion of inositol hexakisphosphate (InsP6) to diphosphoinositol pentakisphosphate (InsP7/PP-InsP5) [50]. Chakraborty et al. [51] have shown that targeted deletion of *IP6K1* in mice liver has increased Akt and mTOR signalling and decreased GSK3β signalling. Since this gene is highly expressed in liver, several factors including the diet of the sample population might have a larger impact on the expression of this gene. At this point, we are not able to pinpoint the effect of this gene (*IP6K1*) on androstenone metabolism in liver.

In our liver sample, *HSD17B2* was the highest down regulated gene with fold change -2.86 (Table 4). Hydroxysteroid (17-beta) dehydrogenase 2 (*HSD17B2*) regulate the availability of testosterone and androstenedione in tissues by catalysing interconvertion of active and inactive forms of steroids [52]. Gene expression studies by Moe et al. [28] have also shown the down regulation of *HSD17B2* gene in liver sample. Moreover, different members of the HSD enzyme family (*HSD17B4*, *HSD17B11* and *HSD17B13*) were found to be differentially regulated in Duroc and Norwegian Landrace populations [28].

Our results showed that cytochrome P450 family gene *CYP7A1* is differentially regulated in liver samples. *CYP7A1* is the rate-limiting enzyme in the synthesis of bile acid from cholesterol. The conversion of cholesterol to bile acid is the major pathway

for cholesterol metabolism [30]. *CYP7A1* is a cytochrome P450 heme enzyme that oxidizes cholesterol using molecular oxygen. Down-regulation of *CYP7A1* causes reduced fat catabolism in liver which may lead to higher fat accumulation and androstenone level due to the dynamic relationship between androstenone in plasma and adipose tissue [53]. Gene expression profiles by Moe et al. [28] have also shown cytochrome P450 family genes to be differentially regulated in liver samples.

Another gene family found to be differentially expressed in our transcriptome analysis is flavin-containing monooxygenases (FMOs) gene family. The FMO family of enzymes converts lipophilic compounds into more polar metabolites and decreases activity of the compounds [54]. In the study conducted by Moe et al. [28], using microarray analysis, *FMO1* is reported to be up-regulated in higher androstenone pigs. In contrast, *FMO5* was found to be down-regulated in high androstenone liver samples in our study. Since androstenone is a lipophilic compound, we speculate that androstenone level was negatively correlated with *FMO5* activity. Since androstenone is a lipophilic compound, we speculate that androstenone level was negatively correlated with *FMO5* activity.

Among the differentially expressed genes in liver, the gene *CDKN1A* was enriched in GO categories amino acid metabolism, small molecule biochemistry, lipid metabolism and molecular transport. The differentially expressed gene *CYP7A1* was enriched in GO categories such as small molecule biochemistry, lipid metabolism and molecular transport. Gene Ontology functional analysis by Moe et al [28] has also shown that the GO categories lipid metabolism and amino acid metabolism were enriched.

Gene polymorphism analysis has shown that there were thirty six mutations in 11 DEGs in liver samples (Table 8). Eight SNPs were identified on SSC4 at position 77 Mb (Table 8) which were mapped to gene *CYP7A1*. In close adjacency to this region Quintanilla et al. [25] identified an androstenone related QTL at position 72 cM. An additional SNP was identified on SSC4 at position 104 Mb mapped to gene *FMO5* however, this position was not mapped as androstenone related QTL region by any previous studies. Our results have also shown that this SNP at position 104,473,018 has been already reported in dbSNP database (dbSNP ID: rs80837900) (Table 8). Six polymorphisms on SSC5 at position 16.7 Mb were mapped to gene *KRT8*. Out of these identified polymorphisms, four SNPs were previously mapped to dbSNP database

(Table 8). Another set of 6 polymorphisms on SSC 5 at position 16.7 Mb mapped to the gene *KRT18*. Three SNPs among these six polymorphisms were already identified and reported in dbSNP database (Table 8). Grindflek et al. [2] detailed an androstenone QTL region on SSC5 between 20.4 and 22.2 Mb in close proximity to our reported polymorphisms (Table 8). An insertion gene polymorphism at position 82 Mb on the same chromosome mapped to *HAL* gene was also indentified in our study. On SSC 7 we identified 3 SNPs, one at position 22 Mb on gene *HIST1H4K* and two at position 36 Mb mapped to gene *CDKN1A*. One of the SNP mapped to *CDKN1A* at position 36.9 Mb has already been reported in dbSNP database (dbSNP ID: rs80964639). An androstenone QTL region on SSC7 between position 33.6 and 88.3 Mb was already described by Grindflek et al. [2]. Two SNPs on *CDKN1A* identified in our study falls into this previously mentioned QTL region. Genome wide association study by Grindflek et al. [2] described androstenone related QTL region on SSC9 at position 7.5 to 8.0 Mb. We report an SNP on the same chromosome at position 10 Mb, close to the previously reported QTL region (Table 8). In addition, we obtained an insertion polymorphism mapped to *NNMT* gene at position 40 Mb. We identified two polymorphisms at 38 Mb on *SDS* gene in the vicinity of the androstenone QTL region on SSC14 at 37 cM [25]. Furthermore, our analysis revealed 7 additional SNPs on SSC14 at 101 Mb on *MBL2* gene. Lee et al. [24] described a QTL on SSC14 at position 87.9 to 108.7 cM for androstenone in the Large White × Meishan crossbred population

Selected polymorphisms in genes *IRG6, MX1, IFIT2, FMO5, CYP7A1* and *KRT18* were found to be associated with the phenotype androstenone level in this study (Table 10). An association study was performed for a SNP (g.494 A>G) in the *FMO5* gene but no statistical relation could be detected with the off flavour score in the Berkshire x Yorkshire resource population [55]. Location of *IFIT2* gene on SSC14 incorporated the QTL affecting androstenone in Yorkshire pig [56] and subjective pork flavour in Large White and Meishan pigs [24]. *MX1* is an interesting candidate gene for disease resistance in farm animals [57] but this study first identifies association with boar taint compounds. No study investigated association of *CYP7A1* with boar taint compounds. Some study reported association of this gene with plasma cholesterol in pigs [58]. Boar taint is related to the adipose tissues since lean pigs have low boar taint compounds [59]. The function of highly polymorphic *KRT18* is relating to pathological processes in liver but involvement in boar taint is not quite clear. However, this gene

maps close to a region on SSC5 affecting androstenone in pigs [2].

Conclusion

Here we showed whole genome expression differences for varying androstenone levels in testis and liver tissues. RNA-Seq provided high resolution map of transcriptional activities and genetic polymorphisms in these tissues. However, due to incomplete porcine annotations, only around 50% of the total reads could be mapped to annotated references. The improvements in pig genome annotations may lead to better coverage and detailed understanding of genetic and functional variants such as novel transcripts, isoforms, sequence polymorphisms and non-coding RNAs. Integration of high throughput genomic and genetic data (eQTL) with proteomic and metabolomic data can provide additional new insight into common biological processes and interaction networks responsible for boar taint related traits.

On the basis of number of DEGs, our results confirm that transcriptome activity in testis is higher in comparison to liver tissue for androstenone biosynthesis. These results also show that the entire functional pathway involved in androstenone metabolism is not completely understood and through this study, we propose additional functional candidate genes such as, *DKK2* and *CYP2B22* in testis and *IP6K1* and *HSD17B2* in liver for androstenone metabolism. Importantly, most of the DEGs are in QTL positions functionally related to pathways involved in boar taint. Furthermore, various gene polymorphisms were also detected in testis and liver DEGs and associations are validated with androstenone levels. Potential polymorphisms and association were identified in DEGs such as *IRG6, MX1,* and *IFIT2* in testis and *CYP7A1*, *FMO5* and *KRT18* in liver. This transcriptome and polymorphisms analysis using RNA deep sequencing combining with association analysis has revealed potential candidate genes affecting boar taint compound. It is speculated that these polymorphisms could be used as biomarkers for boar taint related traits. However, further validation is required to confirm the effect of these biomarkers in other animal populations.

Material and Methods

Animals and Phenotypes

Tissue samples and phenotypes were collected from the Duroc $\times$ F_2 cross animals. F_2 was created by crossing F_1 animals (Leicoma $\times$ German Landrace) with Large White pig breed. Duroc $\times$ F_2 boars were on average 116 days old and had on average 90 kg live weight when slaughtered. All pigs were slaughtered in commercial abattoir, called Landesanstalt für Schweinezucht – LSZ Boxberg. Slaughterhouse management gave the necessary permissions for the tissue and organ collections. Animals were bred and growth, carcass and meat quality data were collected according to guidelines of the German performance test [60]. Tissue samples from testis and liver were frozen in liquid nitrogen immediately after slaughter and stored at -80°C until used for RNA extraction. Fat samples were collected from the neck and stored at -20°C until used for androstenone measurements. For the quantification of androstenone an in-house gas-chromatography/mass spectrometry (GC-MS) method was applied as described previously [61]. Pigs having a fat androstenone level less than 0.5 µg/g and greater than 1.0 µg/g were defined as low and high androstenone samples, respectively. Ten boars were selected from a pool of 100 pigs and the average androstenone value for these selected animals was 1.36 ± 0.45 µg/g. Notably, these 100 boars were used for association study (Supplementary Table S3 and Table S4). RNA was isolated from testis and liver of 5 pigs with extreme high (2.48 ± 0.56 µg/g) and 5 pigs with extreme low levels of androstenone (0.24 ± 0.06 µg/g). Total RNA was extracted using RNeasy Mini Kit according to manufacturer's recommendations (Qiagen). Total RNA was treated using on-column RNase-Free DNase set (Promega) and quantified using spectrophotometer (NanoDrop, ND8000, Thermo Scientific). RNA quality was assessed using an Agilent 2100 Bioanalyser and RNA Nano 6000 Labchip kit (Agilent Technologies).

Library Construction and Sequencing

Full-length cDNA was obtained from 1 µg of RNA, with the SMART cDNA Library Construction Kit (Clontech, USA), according to the manufacturer's instructions. Libraries of amplified RNA for each sample were prepared following the Illumina mRNA-Seq protocol. The library preparations were sequenced on an Illumina HiSeq

2000 as single-reads to 100 bp using 1 lane per sample on the same flow-cell (first sequencing run) at GATC Biotech AG (Konstanz, Germany). All sequences were analysed using the CASAVA v1.7 (Illumina, USA). The deep sequencing data have been deposited in NCBI SRA database and are accessible through GEO series accession number GSE44171 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE44171).

Reference Sequences and Alignment

Two different reference sequence sets were generated from NCBI Sscrofa 9.2 assembly: (1) the reference sequence set generated for differential expression analysis comprised of RefSeq mRNA sequences (cDNA sequences) and candidate transcripts from NCBI UniGene database (Sscrofa). (2) For gene variation analysis a different reference sequence set, generated from whole genome sequence (chromosome assembly) was used. During sequencing experiment, Sscrofa NCBI 10.2 assembly was not released and Sscrofa 9.2 covered ~8.5 K unannotated SNPs (dbSNP database). The released Sscrofa 10.2 assembly consists of 566 K SNP http://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=9823 (Accession date 4/02/2013) with annotation information for 460 K SNP (dbSNP database). In order to make use of this enriched SNP information, we used NCBI Remap tool (http://www.ncbi.nlm.nih.gov/genome/tools/remap) to convert Sscrofa 10.2 SNP genomic positions to Sscrofa9.2 positions. Using the whole chromosomal assembly for read mapping in gene variation analysis step allowed us to match the polymorphisms identified in this analysis with polymorphisms available for porcine genome in dbSNP database based on chromosomal positions. Raw reads were mapped to reference sets using BWA algorithm (http://bio-bwa.sourceforge.net/) with the default parameters [62].

Differential Gene Expression Analysis

The differential gene expression analysis was designed to contrast the difference in the expression of genes between high and low androstenone samples. For differential gene expression analysis with raw count data a R package DESeq was used [33]. The normalization procedure in DESeq handles the differences in the number of reads in each sample. For this purpose, DESeq first generates a fictitious reference sample, with read counts defined as the geometric mean of all the samples. The read counts for each

gene in each sample is divided by this geometric mean to obtain the normalized counts. To model the null distribution of the count data, DESeq follows an error model that uses the negative binomial distribution, with variance and mean linked by local regression. The method controls type-I error and provides good detection power [33]. After analysis using DESeq, DEGs were filtered based on *p*-adjusted value [63] 0.05 and fold change ≥ 1.5. Additionally, the gene expression data was also analyzed using a Generalized Linear Model (GLM) function implemented in DESeq to calculate both within and between group deviances. As sanity checking and filtration step, we cross matched the results from both analysis (*p*-adjusted ≤ 0.05 and fold change ≥ 1.5 criteria and GLM analysis) and only those genes which appeared to be significant in both the tests (*p*-value ≤ 0.05), were selected for further analysis. The results of GLM analysis are given in Supplementary Table S2.

Gene Variation Analysis

For gene variation analysis the mapping files generated by aligning the raw reads to reference sequence set (2) were used. All the downstream analysis was performed using Genome Analysis Toolkit (GATK) [64] and Picard Tools (http://picard.sourceforge.net/). The Genome Analysis Toolkit (GATK) was used for local realignment incorporating Sscrofa 9.2 converted SNPs which was described in the previous section. SNPs were furthermore classified as synonymous or non-synonymous using the GeneWise software (http://www.ebi.ac.uk/Tools/psa/genewise/ last accessed 21.03.2013) by comparing between protein sequence and nucleotides incorporated SNP position [65]. Covariate counting and base quality score recalibration were done using the default parameters suggested by GATK toolkit. The re-aligned and recalibrated mapping files were grouped according to tissue and phenotype categories. Variant calling was performed for each group using GATK UnifiedGenotyper [64]. To find out the differentially expressed genes that also harboured sequence polymorphisms, we filtered the results from UnifiedGenotyper with chromosomal positions of DEGs and retained only those which mapped to DEG chromosomal positions. By this way, we were able to isolate a handful of mutations that mapped to DEGs from many thousands of identified potential sequence polymorphisms. Additionally to understand whether these identified polymorphisms segregate either in only one sample group (high androstenone or low androstenone group) or in both the groups (high and low

androstenone group) we calculated the read/coverage depth of these polymorphisms in all the samples. The results of this analysis are detailed in the results section and read coverage for individual samples are given in Supplementary Table S2.

Pathways and Networks Analysis

A list of the DEGs was uploaded into the Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, www.ingenuity.com) to identify relationships between the genes of interest and to uncover common processes and pathways. The 'Functional Analysis' tool of the IPA software was used to identify the biological functions that were most significant to the data set [5].

Quantitative Real-Time PCR (qRT-PCR) Analysis

For qRT-PCR experiment, total RNA from testis and liver samples were isolated from the 10 boars used for deep sequencing. Additionally, RNA was isolated from the similar tissues of 10 independent boars with divergent androstenone level among the remaining 90 boars. cDNA were synthesised by reverse transcription PCR using 2 μg of total RNA, SuperScript II reverse transcriptase (Invitrogen) and oligo(dT)12 primer (Invitrogen). Gene specific primers for the qRT-PCR were designed by using the Primer3 software [66]. Detailed information for primers used in this study was given in Table 9. In each run, the 96-well microtiter plate contained each cDNA sample and no-template control.. The qRT-PCR was conducted with the following program: 95 °C for 3 min and 40 cycles 95 °C for 15 s/60 °C for 45 s on the StepOne Plus qPCR system (Applied Biosystem). For each PCR reaction 10 μl iTaqTM SYBR® Green Supermix with Rox PCR core reagents (Bio-Rad), 2 μl of cDNA (50 ng/μl) and an optimized amount of primers were mixed with ddH$_2$O to a final reaction volume of 20 μl per well. All samples were analysed twice (technical replication) and the geometric mean of the Ct values were further used for mRNA expression profiling. The geometric mean of two housekeeping genes *GAPDH* and *PPIA* were used for normalization of the target genes. The delta Ct (ΔCt) values were calculated as the difference between target gene and geometric mean of the reference genes: (ΔCt = Ct$_{target}$- Ct$_{housekeeping\ genes}$) as described in Silver et al. [67]. Final results were reported as fold change calculated from delta Ct-values. Details of primers which were used for qRT-PCR study are shown in Table 9.

Validation of SNP and association study

Seven SNPs were selected covering both the testis and liver samples for further validation and association study (Table 10). Genotyping in 100 boars were performed by PCR-RFLP method. In brief, a working solution with a final concentration of 50 ng/µl DNA was prepared and stored at 4 °C for further analysis. Polymerase chain reactions (PCR) were performed in a 20 µl volume containing 2 µl of genomic DNA, 1 × PCR buffer (with 1.5 mM $MgCl_2$), 0.25 mM of dNTP, 5 pM of each primer and 0.1 U of Taq DNA polymerase (GeneCraft). The PCR product was checked on 1.5 % agarose gel (Fischer Scientific Ltd) and digested by using the restriction enzyme (Table 9). Digested PCR-RFLP products were resolved in 3% agarose gels. Details of GenBank accession numbers, primers sequences, annealing temperature and SNP position used in this study are listed in Table 9. Statistical analyses were performed using SAS 9.2 (SAS Institute Inc., Cary, USA). Effects of slaughter age, husbandry system (pen) as well as genotype on boar taint compound androstenone were assessed with fixed effect model (ANOVA) using PROC GLM. For all models, fixed effects included genotype and pen (group, individual), and age of slaughter was fitted as a covariate for boar taint compound androstenone. Due to the skewed nature of the androstenone, data were transformed with natural logarithm before ANOVA to achieve normality. Least square mean values for the loci genotypes were compared by t-test and p-values were adjusted by the Tukey–Kramer correction [68; 69; 70].

Supporting Information

Supplementary Figure S1 The smear plots for differential expression between high and low androstenone levels in testis and liver (TIF)

Supplementary Table S1 GLM analysis results for testis and liver DEGs (DOC)
Supplementary Table S2 Sample read counts for polymorphisms on testis and liver DEGs (DOC)

Supplementary Table S3 Selected SNP detected by RNA-seq that were validated using RFLP (DOC)

Supplementary Table S4 Genotype, allele frequencies and the chi-square test of selected

SNPs validated using RFLP (DOC)

Author Contributions

AGN performed the experiments and wrote the manuscript; SS analysed the RNA-Seq data and wrote manuscript; CN contributed to sampling; CG contributed in statistical analysis; AG performed IPA analysis; LF contributed in sampling and phenotyping; DT was responsible for kits and reagents; ET were responsible for the statistical analysis; CL revised the manuscript; MJU edited and criticized the results; KS edited manuscript and designed the experiment; MUC was responsible for the whole experiment and supervised the overall work. All authors read and approved the final manuscript.

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Tables

Table 1. Summary of sequence read alignments to reference genome in testis samples

Group	Sample	Total number of reads	Un-mapped reads	Mapped reads	Percentage of unmapped reads	Percentage of mapped reads
Low androstenone	A1	15,142,756	7,811,096	7,331,660	51.50	48.50
	A2	13,221,550	6,564,679	6,656,871	49.66	50.34
	A3	32,389,084	16,697,785	15,691,299	51.50	48.50
	A4	27,068,779	14,123,318	12,945,461	52.10	47.90
	A5	27,015,712	14,465,669	12,550,043	53.54	46.46
High androstenone	A6	32,691,057	18,919,738	13,771,319	57.80	42.20
	A7	33,206,723	17,271,473	15,935,250	51.20	48.80
	A8	15,111,453	7,764,418	7,347,035	51.38	48.62
	A9	14,330,069	8,070,092	6,259,977	56.31	43.69
	A10	15,605,400	8,276,052	7,329,348	53.30	46.70

Table 2. Summary of sequence read alignments to reference genome in liver samples

Group	Sample	Total number of reads	Un-mapped reads	Mapped reads	Percentage of unmapped reads	Percentage of mapped reads
Low androstenone	B1	29,549,267	15,632,809	13,916,458	53.50	46.50
	B2	46,050,468	25,270,695	20,779,773	54.87	45.13
	B3	16,420,055	7,659,515	8,760,540	46.64	53.36
	B4	13,323,763	6,989,584	6,334,179	52.46	47.54
	B5	27,085,837	11,747,225	15,338,612	43.37	56.63
High androstenone	B6	28,976,693	16,123,777	12,852,916	55.64	44.36
	B7	12,755,487	5,879,896	6,875,591	46.10	53.90
	B8	45,203,089	18,443,608	26,759,481	59.20	40.8
	B9	14,559,329	8,540,379	6,018,950	58.66	41.34
	B10	14,527,329	8,062,992	6,464,337	55.51	44.49

Table 3. Differentially expressed genes in testis androstenone samples

Gene	Orthologue gene description	Reference ID	Log fold change	*p*-adj.
DKK2	Dickkopf homolog 2	XM_003129269.1	2.89	4.46e-06
AMN	Amnionless homolog	XM_001925648.2	2.28	0.025
LOC100519550	LOC100519550	XM_003127761.1	2.22	9.67e-12
CYP4B1	Cytochrome P450 family 4 subfamily B. polypeptide 1	XM_003128017.1	2.20	8.55e-10
CD244	CD244 molecule natural killer cell receptor 2 B4	XM_001928325.2	2.15	5.35e-08
ADAMTS4	A disintegrin and metalloproteinase with thrombospondin motifs 4-like	XM_001927507.2	2.10	0.04
CYP4A11	Cytochrome P450 family 4 subfamily A polypeptide 11	XM_003128032.1	2.03	3.38e-06
HAL	Histidine ammonia-lyase	XM_001925061.1	2.03	0.014
CYP2C33	Cytochrome P450 2C33	NM_214414.1	1.91	0.04
AMY2B	Amylase, alpha 2B (pancreatic)	XM_003125887.1	1.85	0.002
ARG2	Arginase type II	XM_001928679.2	1.84	6.96e-06
LOC100516362	LOC100516362	XM_003124870.1	1.74	0.022
LOC100521272	LOC100521272	XM_003126855.1	1.71	4.46e-05
MSMO1	Methylsterol monooxygenasse 1	NM_213752.1	1.66	5.55e-08
KRT4	Keratin 4	XM_001927218.2	-1.52	0.02
MPP7	Membrane protein, palmitoylated 7	XM_003130762.1	-1.54	0.0004
DSP	Desmoplakin	XM_003128168.1	-1.55	6.03e-05
AMHR2	Anti-Mullerian hormone receptor, type II	XM_003126187.1	-1.58	0.025
SLA-3	MHC class I antigen 3	AB105388.1	-1.60	5.15e-07
HAAO	3-hydroxyanthranilate 3,4-dioxygenase	XM_003125193.1	-1.61	0.004
MX1	Myxovirus (influenza virus) resistance 1	NM_214061.1	-1.62	1.63e-09
MX2	Myxovirus (influenza virus) resistance 2	NM_001097416.1	-1.63	7.47e-06
IFIT2	Interferon-induced protein with tetratricopeptide repeats 2	XM_001928671.2	-1.64	0.0094
HBB	Hemoglobin, beta	NM_001144841.1	-1.69	2.12e-08
ARL4C	ADP-ribosylation factor-like 4C	XM_003133753.1	-1.72	0.04
EDN1	Endothelin 1	NM_213882.1	-1.73	0.004
HBM	Hemoglobin, mu	XM_003124683.1	-1.74	0.04

HBD	Hemoglobin, delta	XM_003129515.1	-1.83	1.93e-07
HBA2	Hemoglobin, Alpha 2	XM_003124688.1	-1.90	7.42e-11
HBA2	Hemoglobin, Alpha 2	XM_003124690.1	-1.90	2.17e-10
HBA2	Hemoglobin, Alpha 2	XM_003124687.1	-1.93	1.87e-11
HBA2	Hemoglobin, Alpha 2	XM_003124689.1	-1.95	2.62e-11
HBA2	Hemoglobin, Alpha 2	XM_003124685.1	-1.97	1.87e-11
HBA2	Hemoglobin, Alpha 2	XM_003124684.1	-1.97	1.87e-11
HBA2	Hemoglobin, Alpha 2	XM_003124686.1	-1.99	1.38e-11
FRK	Fyn-related kinase	XM_001925792.2	-2.12	0.002
IRG6	Inflammatory response protein 6	NM_213817.1	-2.17	7.24e-07
SYT10	Synaptotagmin 10	XM_001927016.2	-2.23	9.03e-05
S100A2	S100 calcium binding protein A2	XM_001929559.1	-2.35	0.0008
CD5	CD5 molecule	XM_003122679.1	-2.42	0.02
CYP2B22	Cytochrome P450 2B22	NM_214413.1	-2.48	0.02
CYTL1	Cytokine-like 1	XM_003128849.1	-2.82	0.002
S100A2	S100 calcium binding protein A2	XM_001929556.1	-2.83	1.42e-07
CHRNA3	Cholinergic receptor, nicotinic, alpha 3	XM_001925760.2	-3.45	5.07e-08
OLFRA03	Olfactory receptor 3A1	XM_001926523.1	-4.12	0.01
KRT82	Keratin 82	XM_003126157.1	-4.68	1.49e-09

Table 4. Differentially expressed genes in liver androstenone samples

Gene	Orthologue gene description	Reference ID	Log fold change	*p*-adj.
LOC100512122	LOC100512122	XM_003130359.1	3.89	1.10e-14
LOC100511195	LOC100511195	XR_115925.1	3.57	9.26e-15
IP6K1	Inositol hexakisphosphate kinase 1	XM_001925759.2	3.04	0.002
AMPD3	Adenosine monophosphate deaminase 3	XM_003135226.1	2.99	0.0004
LOC100521668	LOC100521668	XR_116002.1	2.52	7.77e-08
SDS	Serine dehydratase	XM_001928302.2	2.12	8.15e-05
BTG3	BTG family member 3	XM_003132741.1	2.12	1.51e-06
KRT78	Keratin 78	XM_001927194.2	2.09	7.80e-05
SMPDL3A	Sphingomyelin phosphodiesterase, acid-like 3A	XM_003121227.1	1.99	9.38e-05
KRT8	Keratin 8	NM_001159615.1	1.96	6.50e-05
LEAP2	Liver expressed antimicrobial peptide 2	NM_213788.1	1.94	3.84e-06
HAL	Histidine ammonia-lyase	XM_001925061.1	1.91	3.36e-06
NNMT	Nicotinamide N-methyltransferase	NM_001123146.1	1.86	3.83e-05
BTG3	BTG family member 3	NM_001097517.1	1.70	0.0007
KRT18	Keratin 18	XM_003126180.1	1.69	0.010
CDKN1A	Cyclin-dependent kinase inhibitor 1A	XM_001929558.1	1.67	6.50e-05
TSKU	Tsukushi small leucine rich proteoglycan homolog	XM_003129674.1	-1.72	0.0017
FMO5	Flavin containing monooxygenase 5	XM_001928594.1	-1.75	0.0043
TSKU	Tsukushi small leucine rich proteoglycan homolog	XM_003129672.1	-1.81	0.002
TSKU	Tsukushi small leucine rich proteoglycan homolog	XM_003129673.1	-1.81	0.002
CYP7A1	Cytochrome P450, family 7, subfamily A, polypeptide 1	NM_001005352.2	-1.87	3.32e-07
HIST1H4K	Histone cluster 1, H4k	XM_001928022.2	-2.60	0.001
MBL2	Mannose-binding lectin (protein C) 2	NM_214125.1	-2.79	0.0001
BCAM	Basal cell adhesion molecule	XM_003127227.1	-2.83	0.017
HSD17B2	Hydroxysteroid (17-beta) dehydrogenase 2	NM_001167649.1	-2.86	3.92e-09

Table 5. Functional categories and corresponding DEGs in high androstenone testis tissues

Function	Number of genes	p-value*		Genes
Molecular transport	9	1.00E-05 4.96E-02	to	HBB, HBD, HBA1/HBA2, CYP4A11, EDN1, MARCO, AMN, CD44, CD5
Small molecule biochemistry	12	1.00E-05 4.95E-02	to	HBB, HBD, ARG2, HBA1/HBA2,CYP4B1, MX1, CYTL1, CYP4A11, MARCO, MSMO1, DSP
Amino acid metabolism	4	3.80E-04 3.48E-02	to	ARG2, EDN1, HAL, FRK
Embryonic development	4	6.80E-04 4.40E-02	to	HBB, HBD, CYTL1, EDN1
Carbohydrate metabolism	3	7.54E-04 4.96E-02	to	CD244, EDN, CYTL1
Lipid metabolism	7	7.54E-04 4.96E-02	to	CD244, EDN1, CYP4A11, HBB, MARCO, MSMO1, DSP
Reproductive system development and function	2	1.95E-03 4.96E-02	to	NQO1, TNC
Protein synthesis	3	1.03E-02 2.70E-02	to	HBA1/HBA2, HBB, ADAMTS4
Energy production	2	1.64E-03 2.43E-02	to	EDN1, MARCO
Vitamin and mineral metabolism	3	1.50E-02 2.37E-02	to	EDN1, CD244, CD5

* Numbers in the *p*-value column showed a range of *p*-values for the genes from each category

Table 6. Functional categories and corresponding DEGs in high androstenone liver tissues

Function	Number of genes	p-value*		Genes
Amino acid metabolism	3	8.71E-06 3.49E-02	to	HAL, SDS, CDKN1A
Small molecule biochemistry	8	8.71E-06 2.51E-02	to	HAL, CYP7A1, MBL2, AMPD3, HSD17B2, IP6K1, SDS, CDKN1A
Cellular development	4	3.15E-04 2.49E-02	to	CDKN1A, KRT8, HIST1H4A, MBL2
Lipid metabolism	5	1.10E-03 2.41E-02	to	CYP7A1, MBL2, HSD17B2, IP6K1, CDKN1A, KRT8
Molecular transport	3	1.11E-03 4.41E-02	to	CYP7A1, MBL2, CDKN1A
Cell function and maintenance	4	1.20E-03 4.90E-02	to	CDKN1A, MBL2, KRT8, KRT18
Cell growth and proliferation	3	1.20E-03 2.90E-02	to	CDKN1A, MBL2, KRT8

* Numbers in the *p*-value column showed a range of *p*-values for the genes from each category

Table 7. Polymorphisms detected in testis samples

Refseq ID	Gene name	Chr	Position	dbSNP	Reference	Alternate	Quality	Root mean square phred score	SNP Classification	Sample group
XM_003124689	HBA2	3	35253219	0	G	GA	333.66	39.10	Insertion	High and Low
XM_003124689	HBA2	3	35253521	0	G	GCTC	617.84	39.89	Insertion	High and Low
NM_213817	IRG6*	3	118838598	0	G	A	181.62	37.00	Synonymous	Low androstenone
XM_003124870	LOC100516362	3	48107044	0	G	GT	260.38	38.59	Insertion	High androstenone
XM_001928325	CD244	4	93149337	0	T	A	84.61	36.72	Synonymous	High androstenone
XM_003128168	DSP	7	4940734	0	G	A	31.18	37.00	Synonymous	Low androstenone
XM_003128168	DSP*	7	4944881	0	C	T	69.47	37.00	Synonymous	Low androstenone
XM_001928679	ARG2	7	99786827	0	A	AT	106.28	39.51	Insertion	High androstenone
NM_214061	MX1	13	144402807	0	A	G	43.93	37.40	Non- synonymous	High androstenone
NM_214061	MX1*	13	144420441	0	C	T	59.51	37.00	Non- synonymous	Low androstenone
XM_001928671	IFIT2*	14	106102335	rs80925743	G	T	98.81	37.00	Synonymous	Low androstenone
XM_001928671	IFIT2	14	106102694	0	G	A	40.63	37.00	Synonymous	Low androstenone

*the SNP validated in boar population using RFLP (see Table 10)

Table 8. Polymorphisms detected in liver samples

Refseq ID	Gene name	Chr	Position	dbSNP	Reference	Alternate	Quality	Root mean square phred score	SNP Classification	Sample group
XM_001928594	FMO5*	4	104473018	rs80837900	G	A	112.94	37.02	Synonymous	Low androstenone
NM_001005352	CYP7A1	4	77195279	0	G	A	1373.52	37.15	Non-synonymous	Low androstenone
NM_001005352	CYP7A1	4	77195397	0	T	C	4026.2	36.93	Synonymous	High and Low
NM_001005352	CYP7A1	4	77197364	0	T	C	1624.62	37.00	Synonymous	High and Low
NM_001005352	CYP7A1	4	77199510	0	A	T	416.26	37.00	Synonymous	Low androstenone
NM_001005352	CYP7A1	4	77199576	0	T	G	242.3	36.07	Synonymous	Low androstenone
NM_001005352	CYP7A1	4	77200294	0	G	A	274.6	37.00	Synonymous	Low androstenone
NM_001005352	CYP7A1	4	77200408	rs80923210	A	G	494.32	36.44	Synonymous	Low androstenone
NM_001005352	CYP7A1*	4	77201533	rs196960866	A	G	1880.98	37.00	Synonymous	Low androstenone
NM_001159615	KRT8	5	16715238	rs80814240	C	T	2365.07	36.65	Synonymous	High and Low
NM_001159615	KRT8	5	16718099	rs80845521	C	T	2186.76	37.01	Synonymous	High and Low
NM_001159615	KRT8	5	16720725	0	G	GT	1160.84	39.98	Insertion	High and Low
NM_001159615	KRT8	5	16721108	0	GGT	G	355.1	38.63	Deletion	High and Low
NM_001159615	KRT8	5	16721708	rs55618932	T	C	2941.94	36.61	Synonymous	High and Low
NM_001159615	KRT8	5	16721831	rs80916149	A	G	9343.88	37.02	Synonymous	High and Low
XM_003126180	KRT18*	5	16788495	0	G	A	327.13	37.16	Synonymous	Low androstenone
XM_003126180	KRT18	5	16789240	0	G	GA	304.54	38.77	Insertion	High and Low

XM_003126180	KRT18	5	16789379	rs81211893	G	A	596.36	36.52	Synonymous	High and Low
XM_003126180	KRT18	5	16789412	rs81211894	A	G	2116.22	36.56	Synonymous	High and Low
XM_003126180	KRT18	5	16789808	rs81211895	G	C	2188.63	36.89	Synonymous	High and Low
XM_003126180	KRT18	5	16789954	0	A	ATC	926.56	35.84	Insertion	High Androstenone
XM_001925061	HAL	5	82556747	0	G	GT	80.57	40.20	Insertion	High Androstenone
XM_001928022	HIST1H4K	7	22186329	0	C	T	717.72	33.45	Synonymous	Low androstenone
XM_001929558	CDKN1A	7	36992673	rs80964639	A	G	544.05	37.00	Synonymous	High Androstenone
XM_001929558	CDKN1A	7	36992792	0	A	G	214.41	37.00	Synonymous	High and Low
XM_003129674	TSKU	9	10759263	0	G	A	127.72	36.56	Synonymous	Low androstenone
NM_001123146	NNMT	9	40584781	0	G	GA	460.61	42.34	Insertion	High and Low
NM_214125	MBL2	14	101464163	0	C	T	236.74	38.58	Synonymous	High and Low
NM_214125	MBL2	14	101464174	0	T	G	624.35	38.02	Synonymous	High and Low
NM_214125	MBL2	14	101464216	0	A	T	2136.83	37.43	Synonymous	High and Low
NM_214125	MBL2	14	101464268	0	A	G	2123.64	37.42	Synonymous	High and Low
NM_214125	MBL2	14	101464309	0	T	C	1038.03	37.83	Synonymous	High and Low
NM_214125	MBL2	14	101464842	0	A	ACT	1693.34	37.6	Insertion	High and Low
NM_214125	MBL2	14	101467788	0	A	G	4598.53	36.97	Synonymous	High and Low
XM_001928302	SDS	14	38865735	0	G	GT	194.2	39.81	Synonymous	High Androstenone
XM_001928302	SDS	14	38868514	0	C	T	51.13	37.00	Non- synonymous	Low androstenone

*the SNP validated in boar population using RFLP (see Table 10

Table 9. Details of primers used for qRT-PCR analysis and genotyping

Gene	Reference ID	Primer sequences (5'→3')	Application	Position*	Enzyme	Annealing temperature (°C)	Product size (bp)	RFLP-patterns
CYP2B22	NM_214413.1	F: CACCACCATCCTCCAGAACT R: GGCAGGAACTGGATCTGGTA	qRT-PCR	-	-	52	120	-
ARG2	XM_001928679.2	F: GGAAGCTGGCTTGATGAAAA R: CCACTGAGCGAGGATTCACT	qRT-PCR	-	-	55	128	-
MSMO1	NM_213752.1	F: CCTGGCACTATTTCCTGCAT R: TAGGGTTTCCAGAGGGTGTG	qRT-PCR	-	-	55	128	-
EDN1	NM_213882.1	F: TTCAGGGAGAAACACCCAAG R:CGAGACGGAAGAAAGCAAAG	qRT-PCR	-	-	55	121	-
CYP2C33	NM_214414.1	F: AGCTGTGCCTCATCCCTAGA R: GTGTTTCTGTCCCAGGCAAT	qRT-PCR	-	-	56	133	-
IP6K1	XM_001925759.2	F: CTGCCAGCCTGTGTCTGTAA R: ATGGCACCAGAATCAGAAGG	qRT-PCR	-	-	55	136	-
BTG3	XM_003132741.1	F: CCAGGAATGTACCGAGGAAA R: ACAATGCATTCCAGGAGGAG	qRT-PCR	-	-	55	138	-
CYP7A1	NM_001005352.2	F: TTCCCGATTCATGTGTTCAA R: ACCAGTTCCGAGATGTGGTC	qRT-PCR	-	-	54	104	-
FMO5	XM_001928594.1	F:GGCCTGAAGCCTAAACACAG	qRT-PCR	-	-	55	147	-

Gene	Accession	Primer	Application	Exon	Enzyme			Genotype
		R:CCTGGAGCCATCCTCAAATA						
HSD17B2	NM_001167649.1	F: TGCAGAACAGAGGACTGTGG	qRT-PCR	-	-	54	103	-
		R: GCCATGCATCGTTTGTATTG						
PPIA	NM_214353	F: CACAAACGGTTCCCAGTTT	qRT-PCR	-	-	58	171	-
		R: TGTCCACAGTCAGCAATGGT						
GAPDH	AF017079	F:ACCCAGAAGACTGTGGATGG	qRT-PCR	-	-	60	247	-
		R:ACGCCTGCTTCACCACCTTC						
IRG6	NM_213817	F: CTGGTACCTGTCACCTTTGC	Genotyping	Exon 3	HaeIII	60	232	GG: 154+ 78
		R: GGGTGAAGTGGTAATTGACG						AA: 232
DSP	XM_003128168	F: AACCTGATTGATCGGGAAAC	Genotyping	Exon 1	HpyCH4IV	55	207	CC: 109+98
		R: GCTGACCTTCTTTTTGGTGA						TT: 207
MX1	NM_214061	F:CACTTCCAAATGGAGCAGAT	Genotyping	Exon 2	AciI	55	204	CC: 125+79
		R:GACTCGCAGACTCACCTGAT						TT: 204
IFIT2	XM_001928671	F:AAGAAGTTTTCCAGCCCCTA	Genotyping	Exon 3	DrdI	55	188	GG: 145+107
		R:TTATCCAGACGGTAGCTTGC						TT: 252
FMO5	XM_001928594	F:AAAGGTTCGACCATGAAATG	Genotyping	Exon 3	HpyCH4III	55	223	GG: 134+89
		R: TATGGCAGCTGTCTCTGTGA						AA: 223
CYP7A1	NM_001005352	F:TGTCCAGGAAATCAAGCAAT	Genotyping	Exon 2	HpyCH4V	55	199	AA: 101+98
		R:CGTCATCAGCTGTCCTCTTT						GG: 199
KRT18	NM_001159615	F: GGGTTGAGAAGGTTCTGGAT	Genotyping	Exon 2	HpyCH4V	55	215	GG: 149+66
		R: CTCCTCGTGGTTCTTCTTCA						AA: 215

**Position according to coding region in Sus scrofa

Table 10. Genotype and association analysis of candidate genes with androstenone

Polymorphism	Boar taint compound (Ln)	Genotype ($\mu \pm$ S.E.)			Effect ($\mu \pm$ S.E.)	
		GG	GA	AA	Additive	Dominance
IRG6 G>A	Androstenone (μg/g)	4.93 ± 0.30^c	5.95 ± 0.21^d	5.82 ± 0.10^d	$-0.44 \pm 0.15^{**}$	$-0.44 \pm 0.15^*$
		CC	CT	TT		
DSP C>T	Androstenone (μg/g)	5.76 ± 0.21	6.06 ± 0.18	5.94 ± 0.20	-0.09 ± 0.08	-0.20 ± 0.13
		CC	CT	TT		
MX1 C>T	Androstenone (μg/g)	5.73 ± 0.12^a	5.92 ± 0.11^a	5.30 ± 0.18^b	0.21 ± 0.11	$-0.40 \pm 0.14^{**}$
		GG	GT	TT		
IFIT2 G>T	Androstenone (μg/g)	6.27 ± 0.18^a	5.63 ± 0.18^b	5.61 ± 0.10^b	$0.32 \pm 0.09^{**}$	$0.31 \pm 0.19^{**}$
		GG	GA	AA		
FMO5 G>A	Androstenone (μg/g)	5.96 ± 0.27^a	5.48 ± 0.27^b	5.97 ± 0.20^a	0.01 ± 0.15	$0.47 \pm 0.20^*$
		AA	AG	GG		
CYP7A1 A>G	Androstenone (μg/g)	4.63 ± 0.32^a	5.60 ± 0.22^b	4.73 ± 0.08^e	$-0.55 \pm 0.16^{**}$	-0.42 ± 0.26
		GG	GA	AA		
KRT18 G>A	Androstenone (μg/g)	5.70 ± 0.01^c	5.82 ± 0.23^c	4.59 ± 0.30^d	$-0.55 \pm 0.15^{**}$	$-0.66 \pm 0.25^*$

a,b < 0.05; c,d< 0.01 ; e,f < 0.001 ; * p <0.05 ; **p<0.01
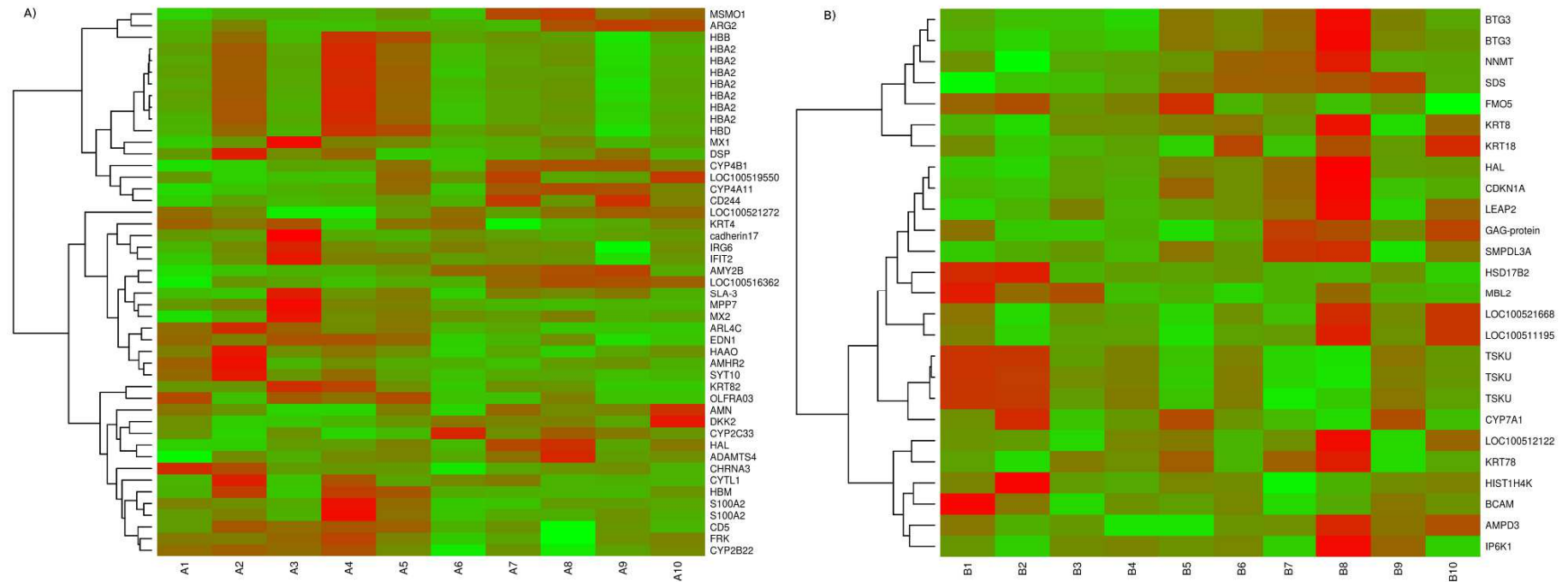
Figure 1. Heatmap showing differentially expressed genes in (A) testis and (B) liver samples. The red blocks represent over expressed genes, and the green blocks represent under expressed genes. Legend: A1-A5 testis with low androstenone and A6-A10 testis with high androstenone, B1-B5 liver with low androstenone and B6-B10 liver with high androstenone
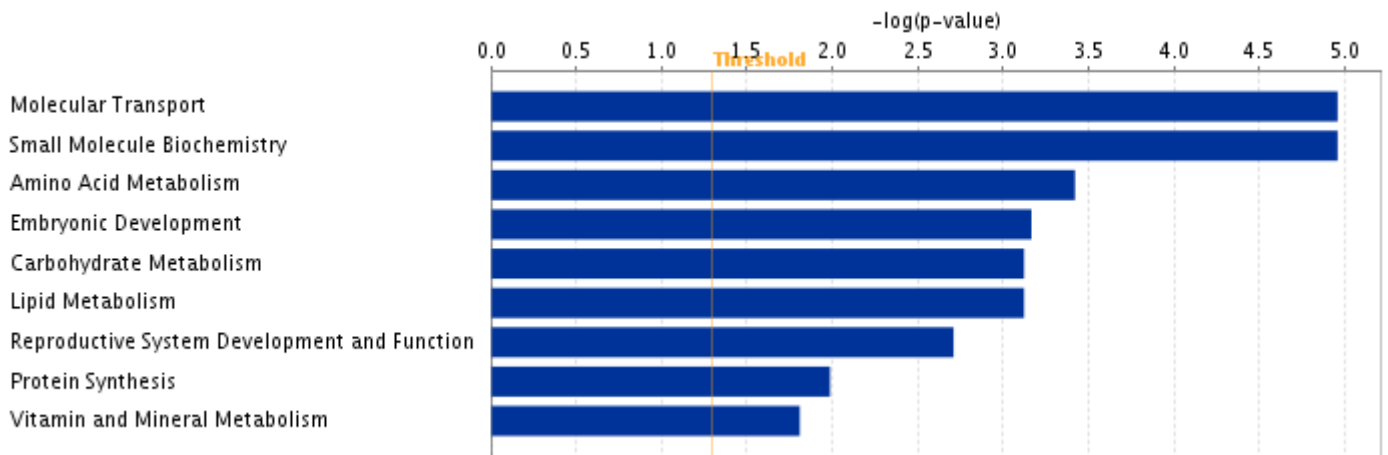
Figure 2. Functional grouping of DEGs in testis with high and low androstenone using Ingenuity Pathways Analysis (IPA) software. The most significant functional groups ($p < 0.05$) are presented graphically. The bars represent the p-value on a logarithmic scale for each functional group.
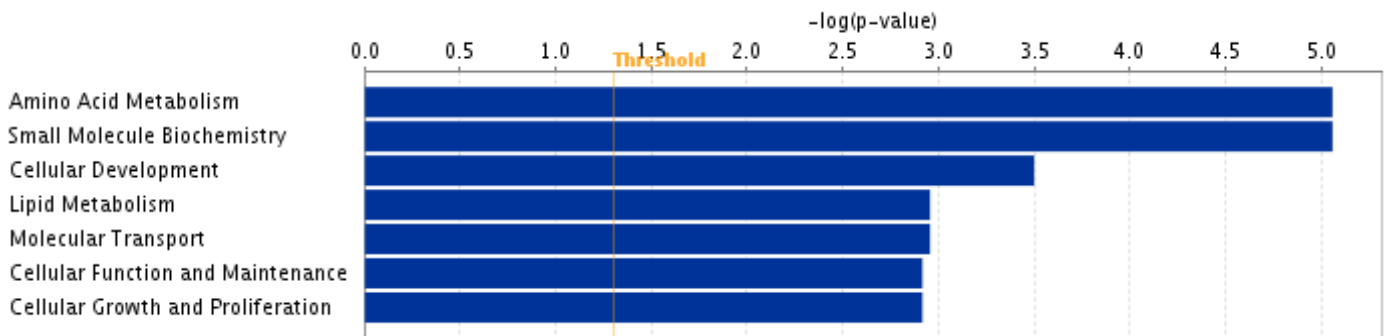
Figure 3. Functional grouping of DEGs in liver with high and low androstenone using Ingenuity Pathways Analysis software. The most significant functional groups ($p < 0.05$) are presented graphically. The bars represent the p-value on a logarithmic scale for each functional group
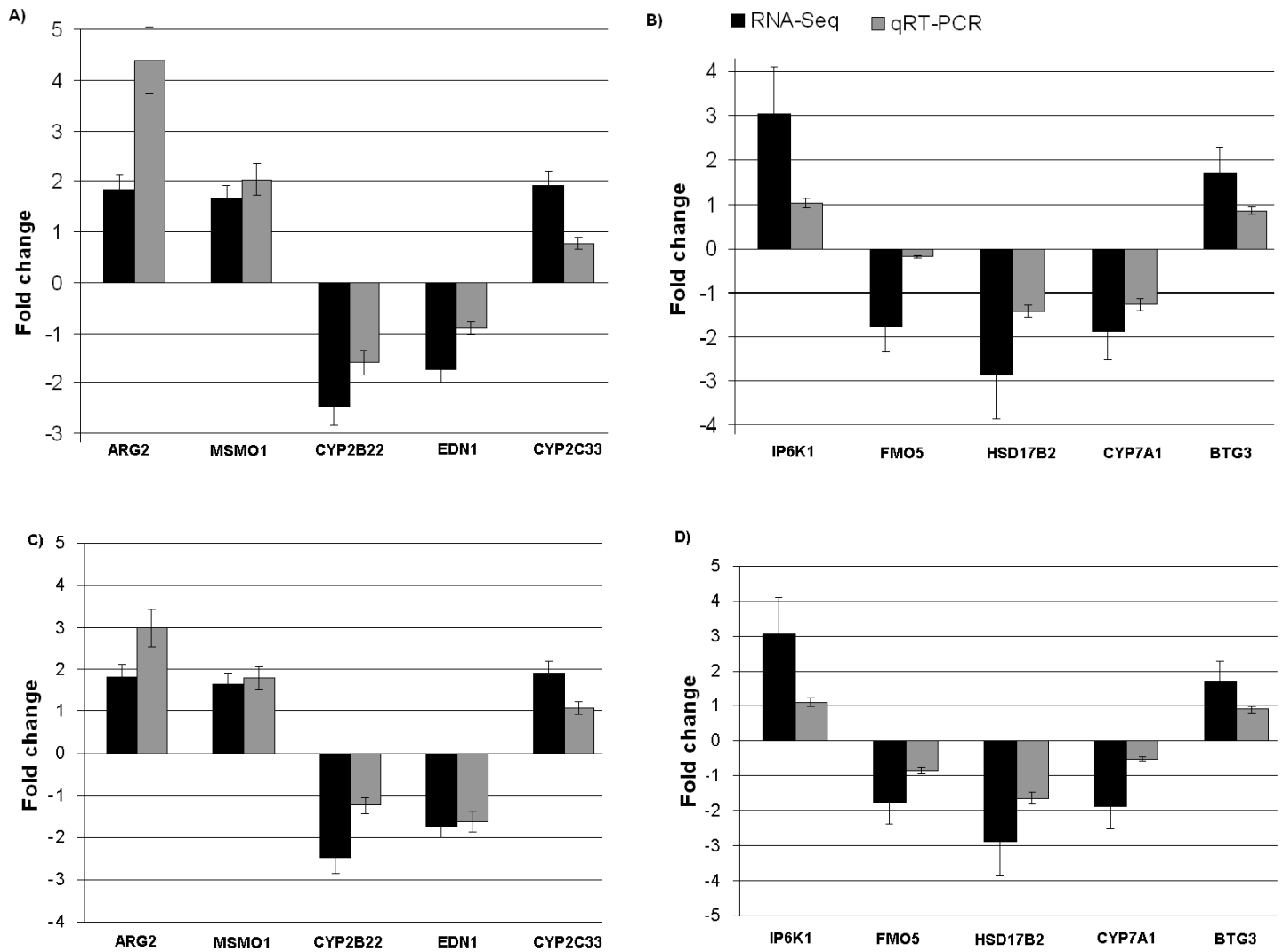
Figure 4. qRT-PCR validations for ten DEGs from divergent androstenone levels in (A and C) testis and (B and D) liver samples. Fold change determined via division of high androstenone group gene expression value by low androstenone group gene expression value
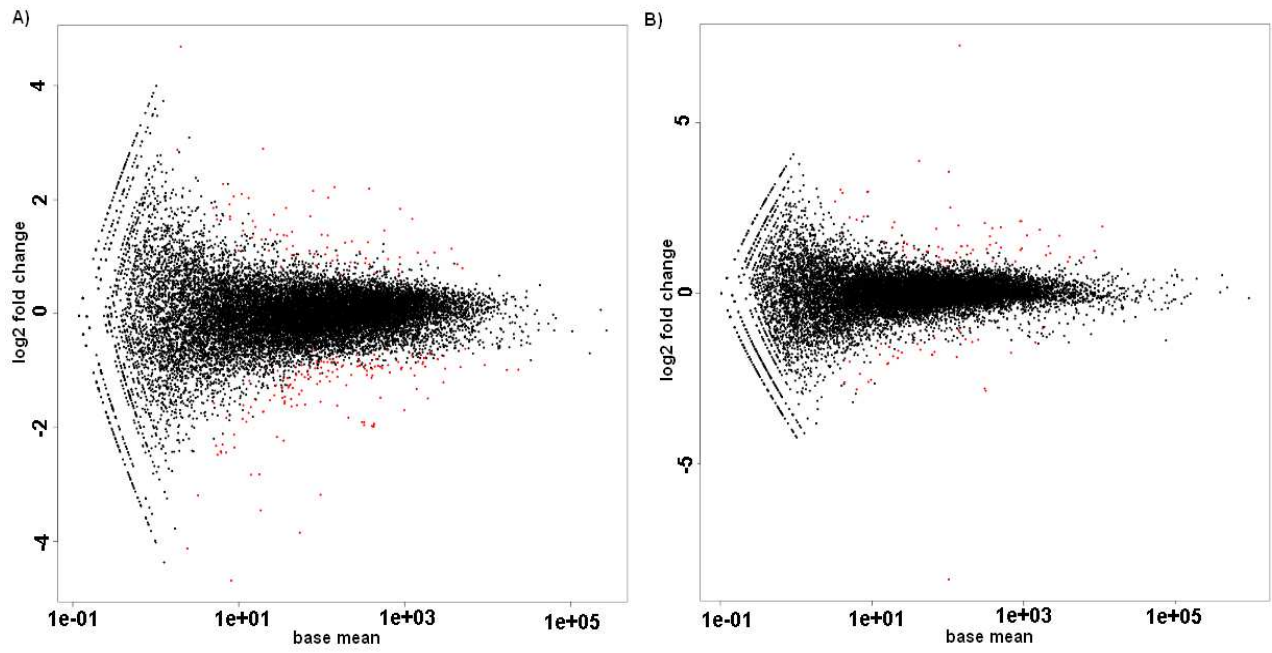
Supplementary Figure S1 The smear plots for differential expression between high and low androstenone levels in testis (A) and liver (B)

Supplementary Table S1 GLM analysis results for testis and liver DEGs

Gene	Reference ID	Total deviance	Within group deviance	Between group deviance	pvals.GLM	padj.GLM
DKK2	XM_003129269.1	94,57405344	49,01098891	45,56306453	1.48e-11	1.82e-08
AMN	XM_001925648.2	56,13687537	41,18519362	14,95168175	0,00011	0,0224
LOC100519550	XM_003127761.1	219,1547338	157,6625993	61,49213449	4.44e-15	2.19e-11
CYP4B1	XM_003128017.1	187,8129657	121,4060867	66,40687909	3.33e-16	2.19e-12
CD244	XM_001928325.2	99,5360863	48,82215609	50,71393021	1.07e-12	1.54e-09
ADAMTS4	XM_001927507.2	59,06631207	41,80910286	17,25720921	3.26e-05	0,00882
CYP4A11	XM_003128032.1	149,5756533	100,5911754	48,98447792	2.58e-12	3.39e-09
HAL	XM_001925061.1	49,77083672	31,15547537	18,61536134	1.6e-05	0,00538
CYP2C33	NM_214414.1	24,18405324	11,4387478	12,74530544	0,000357	0,0453
AMY2B	XM_003125887.1	41,72791373	12,91784401	28,81006972	7.98e-08	4.92e-05
ARG2	XM_001928679.2	107,5216802	56,85667722	50,66500299	1.1e-12	1.54e-09
LOC100516362	XM_003124870.1	35,68008104	13,78452286	21,89555818	2.88e-06	0,00124
LOC100521272	XM_003126855.1	166,7184634	131,6674609	35,05100243	3.21e-09	2.53e-06
MSMO1	NM_213752.1	71,2762284	29,07879541	42,19743299	8.25e-11	8.14e-08
KRT4	XM_001927218.2	92,01257247	75,96178014	16,05079233	6.17e-05	0,015
MPP7	XM_003130762.1	43,08332655	20,92364435	22,15968221	2.51e-06	0,00115
DSP	XM_003128168.1	103,5494219	74,14399114	29,40543073	5.87e-08	3.73e-05
AMHR2	XM_003126187.1	57,92658111	37,42076386	20,50581725	5.95e-06	0,00234
SLA-3	AB105388.1	142,0949217	111,8059984	30,28892327	3.72e-08	2.45e-05
HAAO	XM_003125193.1	51,35356658	26,37997478	24,97359181	5.81e-07	0,000327
MX1	NM_214061.1	107,1777724	71,41987495	35,75789748	2.23e-09	1.84e-06
MX2	NM_001097416.1	103,425223	76,19198866	27,2332343	1.8e-07	0,000105

IFIT2	XM_001928671.2	46,35404599	31,18693736	15,16710863	9.84e-05	0,0209
HBB	NM_001144841.1	101,7538105	58,47173398	43,28207657	4.74e-11	4.92e-08
ARL4C	XM_003133753.1	23,53224016	6,019485451	17,51275471	2.85e-05	0,00853
EDN1	NM_213882.1	24,61048373	4,989608498	19,62087523	9.44e-06	0,00365
HBM	XM_003124683.1	33,48467605	18,62044326	14,86423279	0,000116	0,0228
HBD	XM_003129515.1	102,6293463	57,88556504	44,74378126	2.25e-11	2.61e-08
HBA2	XM_003124688.1	104,7535393	53,57587865	51,17766061	8.44e-13	1.51e-09
HBA2	XM_003124690.1	97,49026851	46,61657353	50,87369498	9.85e-13	1.54e-09
HBA2	XM_003124687.1	106,5499909	52,87307029	53,67692061	2.36e-13	4.68e-10
HBA2	XM_003124689.1	104,3856804	50,71524294	53,67043745	2.37e-13	4.68e-10
HBA2	XM_003124685.1	104,3751932	49,03679511	55,33839812	1.01e-13	2.86e-10
HBA2	XM_003124684.1	110,7205225	55,3359343	55,38458821	9.91e-14	2.86e-10
HBA2	XM_003124686.1	104,4061617	48,13099392	56,27516781	6.31e-14	2.49e-10
FRK	XM_001925792.2	33,43199904	15,45709943	17,97489961	2.24e-05	0,0069
IRG6	NM_213817.1	128,465334	92,61463483	35,85069918	2.13e-09	1.83e-06
SYT10	XM_001927016.2	68,17520468	30,58017177	37,59503291	8.71e-10	7.8e-07
S100A2	XM_001929559.1	47,37447874	26,54715015	20,8273286	5.03e-06	0,00206
CD5	XM_003122679.1	22,08153823	7,920153303	14,16138493	0,000168	0,0282
CYP2B22	NM_214413.1	25,33892965	11,22154026	14,11738939	0,000172	0,0282
CYTL1	XM_003128849.1	120,4327297	87,45572125	32,97700847	9.33e-09	6.81e-06
S100A2	XM_001929556.1	78,45311566	39,61130608	38,84180959	4.6e-10	4.32e-07
CHRNA3	XM_001925760.2	122,9108731	68,39829968	54,51257342	1.54e-13	3.81e-10
OLFRA03	XM_001926523.1	23,41749837	9,255928537	14,16156983	0,000168	0,0282
KRT82	XM_003126157.1	84,08300443	39,50775683	44,57524759	2.45e-11	2.68e-08

Table S2 Read counts for individual samples for identified polymorphisms in testis and liver tissues

Table S2.1 Sample read counts for polymorphisms on testis DEGs**.**

Refseq Id	Gene name	Chr	position	A1.DP	A2.DP	A3.DP	A4.DP	A5.DP	A6.DP	A7.DP	A8.DP	A9.DP	A10.DP
NM_213817	IRG6	3	118838598	NIL	NIL	6	2	NIL	0	0	0	0	0
XM_003124689	HBA2	3	35253219	5	8	9	18	11	5	5	5	NIL	3
XM_003124689	HBA2	3	35253521	NIL	9	9	11	11	8	6	8	NIL	6
XM_003124870	LOC100516362	3	48107044	0	0	0	0	0	1	3	3	NIL	NIL
XM_001928325	CD244	4	93149337	0	0	0	0	0	4	21	1	12	3
XM_003128168	DSP	7	4940734	NIL	2	1	NIL	NIL	0	0	0	0	0
XM_003128168	DSP	7	4944881	NIL	6	1	2	1	0	0	0	0	0
XM_001928679	ARG2	7	99786827	0	0	0	0	0	2	1	4	8	3
NM_214061	MX1	13	144402807	NIL	NIL	18	5	5	0	0	0	0	0
NM_214061	MX1	13	144420441	0	0	0	0	0	3	4	11	3	4
XM_001928671	IFIT2	14	106102335	NIL	NIL	12	4	3	0	0	0	0	0
XM_001928671	IFIT2	14	106102694	NIL	NIL	5	NIL	2	0	0	0	0	0

A1.DP : individual read depth for polymorphism in sample A1. Read depth value 'NIL' indicates that the polymorphism is identified in the sample group, but not in the sample. Read depth value '0' indicates that polymorphism is not at all present in the sample group. A1.DP-A5.DP read depths for low androstenone testis sample. A6.DP-A10.DP read depths for high androstenone testis sample.

Table S2.2 Sample read counts for polymorphisms on liver DEGs.

Refseq Id	Gene name	Chr	position	B1.DP	B2.DP	B3.DP	B4.DP	B5.DP	B6.DP	B7.DP	B8.DP	B9.DP	B10.DP
XM_001928594	FMO5	4	104473018	15	26	6	5	5	0	0	0	0	0
NM_001005352	CYP7A1	4	77195279	2	48	NIL	NIL	NIL	0	0	0	0	0
NM_001005352	CYP7A1	4	77195397	6	140	1	1	6	7	NIL	NIL	15	NIL
NM_001005352	CYP7A1	4	77197364	7	50	1	2	2	6	NIL	1	9	1
NM_001005352	CYP7A1	4	77199510	1	13	NIL	NIL	NIL	0	0	0	0	0
NM_001005352	CYP7A1	4	77199576	NIL	11	NIL	NIL	NIL	0	0	0	0	0
NM_001005352	CYP7A1	4	77200294	NIL	11	NIL	NIL	NIL	0	0	0	0	0
NM_001005352	CYP7A1	4	77200408	1	14	NIL	NIL	3	0	0	0	0	0
NM_001005352	CYP7A1	4	77201533	NIL	76	NIL	1	5	0	0	0	0	0
NM_001159615	KRT8	5	16715238	29	18	47	37	39	77	52	174	6	28
NM_001159615	KRT8	5	16718099	26	10	42	31	36	61	23	117	4	21
NM_001159615	KRT8	5	16720725	5	7	14	12	8	23	7	36	2	9
NM_001159615	KRT8	5	16721108	6	4	10	6	5	14	7	22	NIL	10
NM_001159615	KRT8	5	16721708	48	45	53	52	38	65	39	74	17	55
NM_001159615	KRT8	5	16721831	124	116	136	127	91	173	95	179	31	152
XM_003126180	KRT18	5	16788495	52	32	30	27	7	0	0	0	0	0
XM_003126180	KRT18	5	16789240	10	6	3	4	NIL	16	4	15	3	7
XM_003126180	KRT18	5	16789379	60	40	34	32	2	85	21	89	17	70
XM_003126180	KRT18	5	16789412	69	48	34	32	2	102	21	102	17	70
XM_003126180	KRT18	5	16789808	147	120	83	70	22	167	51	166	64	159
XM_003126180	KRT18	5	16789954	0	0	0	0	0	17	2	22	3	9

XM_001925061	HAL	5	82556747	0	0	0	0	0	2	NIL	11	NIL	NIL
XM_001928022	HIST1H4K	7	22186329	NIL	28	NIL	NIL	NIL	0	0	0	0	0
XM_001929558	CDKN1A	7	36992673	0	0	0	0	0	5	6	93	1	2
XM_001929558	CDKN1A	7	36992792	1	2	4	3	3	3	3	57	NIL	1
XM_003129674	TSKU	9	10759263	11	10	2	NIL	NIL	0	0	0	0	0
NM_001123146	NNMT	9	40584781	10	3	7	5	NIL	14	9	18	NIL	3
NM_214125	MBL2	14	101464163	24	17	9	NIL	4	NIL	1	15	NIL	NIL
NM_214125	MBL2	14	101464174	42	26	12	NIL	4	1	2	17	NIL	NIL
NM_214125	MBL2	14	101464216	100	58	35	2	4	5	2	49	3	3
NM_214125	MBL2	14	101464268	112	53	42	5	3	3	1	48	3	5
NM_214125	MBL2	14	101464309	67	23	23	3	3	NIL	1	22	NIL	2
NM_214125	MBL2	14	101464842	34	19	8	2	1	NIL	2	9	1	1
NM_214125	MBL2	14	101467788	188	68	75	5	3	7	3	76	7	3
XM_001928302	SDS	14	38865735	0	0	0	0	0	4	NIL	9	NIL	1
XM_001928302	SDS	14	38868514	7	14	9	14	14	0	0	0	0	0

B1.DP : individual read depth for polymorphism in sample B1. Read depth value 'NIL' indicates that the polymorphism is identified in the sample group, but not in the sample. Read depth value '0' indicates that polymorphism is not at all present in the sample group. B1.DP-B5.DP read depths for low androstenone liver sample. B6.DP-B10.DP read depths for high androstenone liver sample

Supplementary Table S3 Selected SNP detected by RNA-seq that were validated using RFLP

Gene	Chromosome	SNP	Position	Frequency (%)	Confirmed*
IRG6	3	G/A	118838598	15/85	Yes
DSP	7	C/T	4944881	49/51	Yes
MX1	13	C/T	144420441	69/31	Yes
IFIT2	14	G/T	106102335	24/76	Yes
FMO5	4	G/A	104473018	47/53	Yes
CYP7A1	4	A/G	77201533	10/90	Yes
KRT18	5	G/A	16788495	89/11	Yes

*SNP confirmed by RFLP genotyping of DNA

Supplementary Table S4 Genotype, allele frequencies and the chi-square test of selected SNPs validated using RFLP

Polymorphism position	Number of boars	Genotype frequency			Allele frequency		p-value	Chi-square test
		GG	GA	AA	G	A		χ^2
IRG6 g.118838598G>A	100	0.06(6)	0.17(17)	0.77(77)	0.15	0.85	0.009	0.10
		CC	CT	TT	C	T		
DSP g.4944881 C>T	100	0.26(26)	0.45 (45)	0.29(29)	0.49	0.51	0.191	0.01
		CC(n)	CT(n)	TT(n)	A	G		
MX1 g.144402807 C>T	100	0.35(35)	0.52 (51)	0.14(13)	0.69	0.31	0.01	0.01
		GG	GT	TT	G	T		
IFIT2 g.106102335 G>T	100	0.16(16)	0.16(16)	0.68(68)	0.24	0.76	0.004	0.32
		GG	GA	AA	G	A		
FMO5 g.104473018 G>A	100	0.08 (8)	0.77(77)	0.15(15)	47	53	0.04	0.30
		AA	AG	GG	A	G		
CYP7A1 g.77201533 A>G	100	0.05(5)	0.10(10)	0.85(85)	0.10	0.90	0.004	0.20
		GG	GA	AA	G	A		
KRT18 g.16788495 G>A	100	0.85(85)	0.09(9)	0.06(6)	0.89	0.11	0.001	0.27

2.2   Chapter 2 (Submitted to *PLoS ONE:* under review)

Deciphering of Candidate Genes and Novel Variants in Boar with Divergent Skatole Levels using RNA Deep Sequencing

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Abstract

Boar taint is the unpleasant odour of meat derived from non-castrated male pigs, caused by the accumulation of androstenone and skatole in fat. Skatole is a tryptophan metabolite produced by intestinal bacteria in gut and catabolised in liver. Since boar taint affects consumer's preference, the aim of this study was to perform transcriptome profiling in liver of boars with divergent skatole levels in backfat by using RNA-Seq. The total number of reads produced for each liver sample ranged from 11.8 to 39.0 million. Approximately 448 genes were differentially regulated with significance level of p-adjusted value <0.05. Among them, 383 genes were up-regulated in higher skatole group and 65 were down-regulated (p<0.01. FC>1.5). Differentially regulated genes in high skatole liver samples were enriched in metabolic processes such as small molecule biochemistry, protein synthesis, lipid and amino acid metabolism. Pathway analysis identified the remodeling of epithelial adherens junction and TCA cycle as the most dominant pathways which may play important roles in skatole metabolism. Differential gene expression analysis identified candidate genes in ATP synthesis, cytochrome P450, keratin, phosphoglucomutase, isocitrate dehydrogenase and solute carrier family. Additionally, polymorphism and association analysis revealed that mutations in *ATP5B, KRT8, PGM1, SLC22A7* and *IDH1* genes could be potential markers for skatole levels in boars. Furthermore, expression analysis of exon usage of three genes (*ATP5B, KRT8* and *PGM1*) revealed significant differential expression of exons of these genes in different skatole levels. These polymorphisms and exon expression differences may have impacts on the gene activity ultimately leading to skatole variation and could be used as genetic marker for boar taint related traits. However, further validation is required to confirm the effect of these genetic markers in other pig populations in order to use in genomic selection against boar taint in pig breeding programs.

Introduction

Boar taint is the offensive odour or taste that can be evident during the cooking or eating of porcine meat derived from non-castrated male pigs. It is preliminary caused by the accumulation of androstenone and skatole in the adipose tissues. Skatole is a metabolite of tryptophan which is produced by intestinal bacteria such Clostridium and Bacteroides genera in gut and metabolised in the liver (reviewed by Wesoly and Weiler [1]). It has a fecal-like odor and unlike the smell of androstenone, the vast majority of people are able to detect the smell of skatole. It is well known that high concentrations of male sex steroids such as androstenone prevent the enzymes responsible for skatole metabolism resulting in the reduction of skatole metabolism in liver and accumulation in adipose tissue [2]. Notably, the most common practice to prevent this smell is castration of male piglets. Castration is undesirable due to ethical and economical concerns [3,4] and castration of piglets is announced to be banned in the European Community by 2018 [5] creating an urgent need to develop alternative methods to prevent tainted meat.

In pigs, skatole is absorbed by the intestinal mucosa into the portal vein and passes through the liver where it is efficiently metabolised. Three major metabolites of skatole isolated from pigs are 6-sulfatoxyskatole (MII), 3-hydroxy-3-methyloxindole (MIII) and 3-methyl indole [6]. Among these skatole metabolites, MII is secreted in plasma and urine as a sulphate conjugate, and MIII is found to be related to the skatole levels in fat [6]. It has been demonstrated that the liver has a potential capacity to extract skatole from blood in quantities that greatly exceed what is found under physiological conditions [7]. However, in boars a proportion of skatole passes the liver without being metabolised and accumulates in adipose tissue producing tainted meat [6]. Several studies are devoted to indentify the genes and pathways involved in the androstenone metabolism in liver [8,9,10] but no study performed transcriptome studies involved in the skatole metabolism in liver. In this regard, the genes coding for enzymes of the cytochrome family received considerable interest due to their role in skatole metabolism. Hepatic cytochrome P4502E1 (CYP2E1) which is the main hepatic enzyme involved in the metabolism of skatole [11]. Significant associations have been identified between SNPs within the CYP2E1 and CYP21 genes and reported to reduce skatole levels in pigs [7]. A mutation in the coding region of CYP2A6 was found to be associated with high level skatole in fat [12].

With the aim to identify candidate genes, a number of quantitative trait loci (QTL) and genome-wide association analysis have been conducted for skatole in purebred and crossbred pig populations [13,14]. Several QTL for skatole were identified on different pig chromosomes such as on SSC6, SSC7, SSC12, SSC13, SSC14 and SSCX in different pig populations [14,15]. Only a few studies performed polymorphism and association of selected genes [16,17] and a study was devoted to describe the genome wide association [18] for skatole in pigs. However, to the authors' knowledge, no study was devoted to unravel global transcriptome analysis for divergent skatole levels in boar fat as well as to identify pathways involved in skatole metabolism in liver. RNA-Seq is a recently developed next generation sequencing technology for transcriptome profiling that boosts identification of novel and low abundant transcripts [19]. It could be used to analyse changes in gene expression across the entire transcriptome [19,20]. RNA-Seq also provides evidence for identification of splicing events, polymorphisms and different family isoforms of transcripts [21]. Therefore, the major aim of this study was to elucidate the genes and pathways involved in skatole metabolism in liver tissue using RNA deep sequencing technology. For this purpose, we performed differential expression analysis of genes in liver samples from boars with high skatole (HS) and low skatole (LS) in their backfat. Additionally, gene polymorphism analysis and differential exon usage analysis were also performed for the differentially expressed genes.

Results

Analysis of RNA Deep Sequencing Data

We sequenced cDNA libraries from 6 samples from liver tissues (3 from HS in backfat and 3 from LS in backfat) using Illumina HiSeq 2000 as a part of our previous work [8]. The details of the sequencing are mentioned by Gunawan et al [8] and the raw sequencing data is deposited in GEO database and available under the accession id GSE44171. The sequencing produced clusters of sequence reads with maximum of 100 base-pair (bp). After quality control and filtering, the total number of reads for liver samples ranged from 11.8 to 39.0 million with a median of 22.8 million. Total number of reads for each group of liver sample and the number of reads mapped to reference sequences are shown in Table 1. In case of liver from LS group 43% to 74.4% of total reads were aligned to reference sequence whereas, in case of liver from the HS group

61.3 % to 84% were aligned.

Differential Gene Expression Analysis

Differential gene expression from livers of boars with HS and LS levels in backfat were calculated from the raw reads using the R package DESeq [22]. The significance scores were corrected for multiple testing using Benjamini-Hochberg correction. We used a negative binomial distribution based method implemented in DESeq to identify differentially expressed genes (DEGs) in liver with divergent (HS and LS in backfat) skatole levels. A total of 448 DEGs were selected from the differential expression analysis using the criteria $p_{adjusted} < 0.05$ and log2 fold change > 1.5 (Supplementary Table S1). In the liver tissues, 383 genes were found to be highly expressed in high skatole group whereas, 65 genes were found to be highly expressed in low skatole group (Supplementary Table S1). The range of log2 fold change values for DEGs was from -6.79 to 5.82. Heatmaps (Figure 1) illustrate the top 30 up and top 30 down regulated genes identified in the liver tissues from HS and LS boars. The top 30 up and down regulated genes the liver with different skatole levels along with log FC and p values are listed in Table 2. The differential expression analysis of our data revealed both novel transcripts and common genes which were previously identified in various gene expression studies. Novel transcripts from our analysis and commonly found genes are mentioned in detail in the discussion section.

Biological Function Analysis for DEGs

To investigate gene functions and to uncover the common processes and pathways among the selected DEGs, Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, www.ingenuity.com) was used. In liver samples, out of 448 DEGs 300 were assigned to a specific functional group based on the information from IPA (Figure 2). A large proportion (67.0%) of the DEGs from liver in the high skatole group fell into Gene Ontology (GO) category metabolic processes. The enriched GO metabolic process classes where: metabolic processes including small molecule biochemistry, protein synthesis, carbohydrate metabolism, DNA replication, recombinant and repair, energy production and lipid metabolism. Other enriched GO categories include post translation modification and amino acid metabolism. The genes classified into each functional group are listed in the Table 3. IPA assigned 68 DEGs between high and low skatole

group liver samples to six different canonical pathways. Canonical pathway analysis highlighted pathways involving remodelling of epithelial adherens junctions and TCA cycle as the dominant pathways which play important regulatory roles in metabolic pathways (Figure 3). Other pathway categories including super pathways of methionine degradation, mitochondrial dysfunction UDP-N-acetyl-D-galactosamine biosynthesis and cystein were also enriched (Figure 3). The genes assigned to these pathways in the liver with high and low skatole levels are presented in Table 4.

Validation of Selected DEGs with Quantitative Real Time PCR

In order to validate the RNA-Seq results, on the basis of differential expressions and functions related to skatole, a total of 10 genes (*ATP5B*, *DHRS4*, *GSTO2*, *IDH3B*, *HSD17B2*, *KRT8*, *PGM1*, *PRDX1*, *SDHD* and *SLC22A7*) were selected and quantified using qRT-PCR. For this purpose, the same samples used in the deep sequencing were used. Comparison of qRT-PCR data for 10 selected genes showed quantitative concordance of expression with the RNA-Seq results (Figure 4A). To further validate the expression of selected genes more robustly, new grouping of independently high (n=3) and low (n=3) skatole were done among the remaining 94 pigs. The mRNA expressions of selected genes showed similar pattern of expression in this new groups (Figure 4B). Gene expression values for qRT-PCR were normalized using housekeeping genes *PPIA* and *GAPDH* [23].

Differential Exon Usages Analysis

In order to explore the expression pattern of different exons of a gene between HS and LS boars, selected DEGs were analysed for differential exon expression [24]. Distribution of differential exon events and selected differential exon usage genes are shown in figure 6A and 6B, respectively. We identified 126 (P-adj<0.05) differential exon expressions in 66 DEGs (Supplementary Table S5) that showed differential usage of exons between high and low skatole. Selected differential exon usage identified in DEGs for liver samples are given in Table 7. It is important to note that some genes showed more than one variable splicing. We found that about 34.8 % of the alternative spliced genes underwent multiple differential exon usage events (Figure 6C), illustrating the complexity of porcine transcriptome. Figure 7A, 7B and 7C shows an example of differential exon expression for three genes (*ATP5B*, *KRT8* and *PGM1*) which were

shown above to be associated with the skatole level. The first and second exon of the ATP5B gene showed significantly higher expressions in the low skatole (Figure 7A) group. The first and fifth exon of the KRT8 gene showed significantly higher levels in the low skatole than in high skatole group (Fig 7B). Figure 7C showed that the 12th exon of the PGM1 gene was expressed significantly higher in the LS than in HS group of boars.

Gene variation analysis

In the liver samples 427 gene polymorphisms were identified in 107 DEGs (Supplementary Table 2). Selected polymorphisms identified in DEGs for liver samples are given in Table 5. The distribution of SNPs number and selected SNPs used for validation is shown in figure 5A and 5B, respectively. We found that about 68.4 % of genes had multiple polymorphisms (Figure 5A). Read counts for individual samples for identified polymorphisms in liver tissues are given in Supplementary Table S3. In order to validate the SNP results, on the basis of functional SNP and functions related to skatole, a total of 6 SNP were selected for association study (Figure 5B and supplementary table S4). We have selected SNPs in *ATP5B, KRT8, PGM1, CYP4A25, SLC22A7* and *IDH1* to validate their segregation (Supplementary Table S4) and association in our population (n=100). Out of 6 SNP, five SNPs were found to be associated with skatole levels in our population (Supplementary Table S4). Polymorphisms in *ATP5B* (g.23661024 T>C)*, KRT8* (g.18670859>A), *PGM1* (g.137174784C>A), *SLC22A7* (g.43833000 G>A) and *IDH1* (g.122862530 C>T) were associated with skatole level (Table 6).

Discussion

Analysis of RNA-Seq data

The present study describes the transcriptome profiles of liver from boars with high (HS) and low (LS) skatole content in the backfat by using RNA-Seq. To the best of our knowledge, this study provides the first insight into the transcriptome signature in liver tissues by using RNA-Seq that might be involved in the skatole metabolism. Using the whole transcriptome sequencing technique, we were able to identify the levels of differentially expressed genes and to associate these genes with divergent skatole levels

in terms of boar taint. According to the mapping results, the average number of reads was 22.85 million reads and on an average 65.5 % of the reads was categorized as mapped reads corresponding to exon reads (Table 1). The proportion of reads mapped to exons of annotated genes was in accordance the previous studies [25,26,27] in pig liver transcriptome (60.2-74.9%), but was higher than that reported by Esteva-Codina et al. [28] (44.1%) in porcine male gonad and Gunawan et al. [8] (40.8% - 56.63%) in boar with divergent androstenone levels. The percentage of annotated reads varies from 15.6% to 74.9% in porcine transcriptome studies [8,25,26,27,28,29], supporting our results. The differences between mapping percentages might be due to several factors such as primer biases, GC content, dinucleotide fragmentation sites, independent cell types, laboratory protocols and the selection of reference genome build for annotations [30]. Another factor is that the current reference transcriptome assembly might not cover all transcribed mRNA [31] and consequently low abundant transcripts or rare alternative splicing isoforms are less likely to be mapped to transcriptome assembly [28]. Illumina sequencing data have been described as replicable with relatively little technical variation [32]. Therefore, the findings of this study clearly demonstrated the power of RNA-Seq and provide further insights into the transcriptome of liver at a finer resolution in skatole divergent boars.

Differential Gene Expression Analysis

In this study, 448 genes were differentially regulated in liver with divergent skatole levels (Supplementary Table S1). The top two up regulated gene in the liver sample were *SERPINA12* with log2 fold change 5.81 and *KRT8* with log2 fold change 4.32 (Table 2). *SERPINA12* was identified from visceral adipose tissues of rats, is an animal model for obesity and type 2 diabetes [33]. It is reported that SERPINA12 plays an important role in the progression of obesity and insulin resistance [34]. Wada et al. [35] categorized the serine proteases as to be genes for small hormone-like molecules such as corticosteroid and thyroid hormones. SERPINF2, another member of the same family was involved in 2-aminoacetophenone metabolism which is an important intermediate product of phase 1 skatole metabolism. Notably, the skatole metabolism is divided into two phases: an oxidative phase 1 metabolism and a conjugative phase 2 metabolism. During phase I, skatole is degraded to several intermediate products. Keratins (Ks), the intermediate filaments (IFs) of epithelial cells, constitute the most diversified family of

IF proteins with its type I (K9-22) and type II (K1-K8) classes [36]. KRT8/KRT18 IFs can modulate the adhesion, size and cell-cycle progression of hepatic cells, in association with differential plectin/receptor of activated C kinase 1 (RACK1) [37]. The function of highly polymorphic *KRT8* in the skatole metabolism in liver is not quite clear. However, this gene mapped close to a region on SSC5 affecting skatole and indole [38]. The top two down regulated gene in higher skatole group were LOC100737759 with log2 fold change -6.79 and LOC100625674 with log2 fold change -5.88. Till now, it is difficult to identify either the actual gene name or function through orthologous database or BLAST sequence similarity searches. The NCBI database referred LOC100737759 as 'putative 2-oxo-4-hydroxy-4-carboxy-5-ureidoimidazoline decarboxylase-like' which might be involved in amino acid metabolism. Similarly, LOC100625674 is referred as 'cadherin-18-like' which is a calcium-dependent cell adhesion protein and preferentially contributes to in connecting cells. Cadherin-18 is a dominant gene belonging to the remodelling of epithelial adherens junction pathway [39] which is found to be a dominant pathway in our study.

There are similarities between gene expression differences found with RNA-Seq and those reported in previous transcriptome studies in pig [9,10,38,40]. Similar to the previous studies [41,42], cytochrome P450 superfamily genes were found to be differentially regulated in skatole catabolism. Phase I skatole metabolism includes mostly oxidative reactions usually performed by the membrane bound cytochrome P450 system [43]. Cytochrome P450 isoenzymes are the main enzymes playing roles in phase 1 skatole metabolism where skatole is degraded to several intermediate products including such as indole-3-carbinol(I3C), 2-aminoacetophenone (2AAP), 3-metyloxyindole (3MOI) etc (details reviewed by [1,44,45]). Notably, CYP4A24, CYP4A25 and CYB4B24 were found to be up-regulated in high skatole in Duroc × F2 population in this study which is in agreement with previous results for Landrace population [13]. Another cytochrome gene family found to be differentially expressed in our transcriptome analysis is cytochrome subunit 5A (COX5A) coding for cytochrome oxidases and previously reported to be associated with skatole levels [7,18,46]. Ramos et al. [18] reported that the cytochrome subunit family COX4I1 gene, coding for a subunit of a cytochrome oxidase, is involved in the regulation of porcine skatole metabolism. Additionally, cytochrome subunit 8C (COX8C) gene, coding for a subunit of cytochrome oxidase is also reported to be involved in the regulation of

porcine skatole metabolism [18,46]. The COX5A gene on SSC7 at position 63.3 Mb maps to an already identified QTL region between 61.5 and 69.6 Mb which is known to affect skatole levels in Duroc and Landrace population. The main enzymes of phase 2 skatole metabolism are UGT (uridine 5'-diphospho-glucuronosyltransferase) and SULT1A1 (sulfotrasferase) [47]. UGT is a family of conjugation enzymes and UGT1A3 is found to be differentially regulated in this study (Table 2). Members of the UGT enzyme family such as UGT1A5 and UGT2A1 are reported to be differentially regulated in porcine transcriptome analysis for androstenone [10]. Different groups of transferases including glutation S transferase omega 2 (GSTO2) and glutathione S-transferase mu 2 (GSTM2) were found to be differentially regulated in this study (Table 2). Glutahione S-transferases (GSTs) are functionally diverse enzymes mostly known to catalyse conjugation reactions of endogenous substances, haem, fatty acids, xenobiotics and products of oxidative processes [48]. In most cases, the effect of conjugation is a decreased biological activity and increased excretion of these metabolites from the body [49]. During phase 2 metabolism, the water solubility of the skatole metabolism is increased to facilitate excretion via urine [6,50]. The GSTs, reported to transport different molecules [48], might indicate that the GSTO transports the skatole to the tissues. It could be speculated that GSTO2 might be involved in the excretion of skatole from the porcine body.

Interestingly in this study, four members of SLC family genes such as SLC22A7, SLC25A1 and SLC25A25 were found to be up-regulated and SLC9A4 was found to be down regulated in the high skatole group (Table 2). The solute carrier family (SLC) are important proteins in the regulation of body iron homeostasis and skeletal muscle contains a large proportion of body iron implies the importance of SLC for meat quality traits [51]. The SLC25A1 gene encodes a transporter protein which is responsible for the movement of citrate across the mitochondrial inner membrane [52]. Two members of SLC family (SLC22A13 and SLC22A14) genes were reported to be candidates for taint compounds and sex steroids in pigs [13]. The SLC superfamily is comprised of transporter families involved in the cellular uptake and secretion of endogenous molecules. The substrate panel of SLC22As includes endogenous compounds like tryptophan metabolites and sulphated steroids [53]. Skatole results from a multistep degradation of tryptophan by microbial activity, mainly in the hind gut of the pigs [1,45]. High concentration of skatole requires a high amount of tryptophan for microbial

degradation [1]. Our results show that among the differentially expressed gene in liver, genes with roles in ATP binding (ATP5A1, ATP5B, ATP5D), isocitrate dehydrogenases (IDH1 and IDH3B) and acetyl-CoA (ACSL5, ACOX1) were enriched for functional categories such as in small molecule biochemistry, protein synthesis, carbohydrate metabolism and energy production (Table 3). In accordance with these results, Ramayo-Caldes et al. [27] also showed small molecule biochemistry and energy production to be members of the enriched GO categories for DEGs in liver samples.

Pathway analysis of DEGs (Table 4) seemed similar patterns with GO analysis and remodelling epithelial of adherens junction and tricarboxylic (TCA) pathway were found to be the most dominant pathways in this study. Additionally, the mithochondrial dysfunction and UDP-N-acetyl-D-galactosamine biosynthesis pathways also found to be enriched in our study (Figure 3). A total of eight genes (*ACTB*, *ARPC3*, *ARPC1A*, *TUBA1A1*, *TUBA1B*, *TUBA1C*, *TUBB2A* and *TUBB4B*) belonging to the remodelling epithelial of adherens junction pathway are identified in this study postulate that these genes may involve in the skatole metabolism in liver (Table 4). The intercellular adherens junctions (AJ) are specialized sub-apical structures that function as principle mediators of cell-cell adhesion [39]. Their assembly-disassembly is dynamic and stringently regulated during tissue morphogenesis and homeostasis [54]. The TCA cycle found to contain six genes (*DLST*, *IDH3B*, *MDH1*, *MDH2*, *OGDHL* and *SDHD*) involved in the most important metabolic steps in the mitochondria. The TCA cycle is a catabolic pathway of aerobic respiration and the main source of ATP used to maintain homeostasis, is produced by oxidation of pyruvate in the TCA cycle [55]. Another over represented canonical pathway in high skatole liver group was cystein biosynthesis pathway which was previously reported to be responsible for diet metabolism in mammalian liver [56]. This pathway includes adenosylhomocysteinase (ACHY), cystathionase (CTH) and FtsJ RNA methyltransferase homolog 1 (FTSJ1) genes (Table 4) which are involved in the amino acid metabolism. These deep sequencing results identified for the first time the TCA cycle and the cystein biosynthesis to be possibly involved in the metabolism of skatole in porcine liver.

Differential Exon Expression Analysis

Since an additional important advantage of RNA deep sequencing is detecting differential exon usage events [25], we used the RNA deep sequencing data to

characterize and compare the patterns of differential exon expressions variation in high and low skatole levels. RNA deep sequencing technology provide valuable information regarding alternative and novel splice variants reflecting more complex mechanism of RNA regulation. A previous study by Moe et al. [7] showed that number of genes involved in RNA processing and translation were differentially expressed between boar taint compounds. This study extends these observations by identifying a number of genes with differential exon expression between high and low skatole level. Chen et al. [25] reported that about 18.8% of the annotated genes showed differential exon usage events in pigs with divergent meat quality traits. This study revealed differential level of exon expression for *ATP5B*, *KRT8* and *PGM1* genes in low skatole in comparison to high skatole group suggesting that differential processing of RNA could be associated with the regulation of skatole level.

Gene Variation Analysis

In addition to transcriptome quantification, RNA-Seq technology provides valuable information regarding gene polymorphisms which could be directly correlated with the relevant phenotype. Several holistic gene expression analyses have been performed for boar taint compounds by using microarray or Real-Time PCR technology [9,10,40]. Our study extends these observations by correlating differentially regulated genes with associated polymorphisms. Gene polymorphisms in the exonic regions might have direct effect on the expression of transcripts and the identified polymorphisms from RNA deep sequencing may give additional insight to variation in the skatole levels. This study revealed 45 SNPs in 8 highly polymorphic DEGs from liver samples (Table 5). Two highly polymorphic genes *KRT8* and *ATP5B* containing five and three SNPs are mapped close to a region on SSC5 at 18.6 Mb and 23.6 Mb, respectively (Table 5). Several QTL incorporating the KRT8 location are reported to affect skatole levels in pigs [38]. On SSC6, we identified 13 polymorphisms on gene *PGM1* at position 137.1 Mb, six polymorphisms on gene *CYP4A25* at position 152.1 Mb and a set of 7 polymorphisms mapped to the gene *PRDX1* at position 153.2Mb. Several QTL have been identified previously for skatole on SSC6 [14,18,57]. On the region of interest, Ramos et al. [18] reported several SNP markers located close on the region on SSC6 that were significantly associated with skatole levels. Genes coding for cytochrome family have previously been shown to be associated with skatole levels [17,58] and are

mapped on SSC6. These genes are known to be involved in phase I metabolism of skatole [58,59] implying that these genes could be important positional and functional candidate for boar taint compounds.

Two polymorphism were identified on SSC7 at position 43.8 Mb, mapped to gene SLC22A7 and a SNP was identified on SSC7 at position 80.5 Mb on the gene DHRS4 (Table 5). A SNP genotyping study by Grindflek et al. [13] identified an androstenone related QTL region on SSC7 between region 33.6-41.9 Mb and 80.8-88.3 which is in close proximity to the polymorphisms on gene SLC22A7 and DHRS4, respectively. In addition, a suggestive QTL for skatole is identified on SSC7 in the Yorkhsire pigs [13], overlapping the region harbouring our genes indicating that these markers could be very important for skatole levels. In this study, eight SNPs were identified on *IDH1* gene at position 122.8 Mb on SSC15; yet to the best of our knowledge, no QTL regions related with skatole traits are been reported in this region. However, a highly significant QTL with wide a confidence interval from 42.5-70.7 Mb is located on SSC15 in Norwegian Landrace and Duroc affecting the boar taint compounds including androstenone and skatole [13]. Moreover, this region is very rich for genes including several genes involved in cytochrome P450 family and sulfotransferase family activity which are the key enzymes in both the phases of skatole degradation [1,13]. Therefore, fine mapping and robust study of the genes on this region could be interesting.

The selected polymorphisms in genes *ATP5B, KRT8, PGM1, SLC22A7* and *IDH1* were found to be associated with the phenotype skatole level in this study (Table 6). To the best of our knowledge, no study investigated association of the highly polymorphic *ATP5B, KRT8, PGM1, SLC22A7* and *IDH1* genes with boar taint compounds before. Xu et al. [60] reported an association for a SNP in exon 8 (g.75 G>A) in the ATP5B gene with the meat quality traits such as ratio lean to fat, fat meat percentage, intramuscular fat content and intramuscular water content. The ATP5B gene encodes the catalytic subunit of mitochondrial ATP synthesis complex and catalyzes the rate-limiting step of ATP formation in eukaryotic cells [61]. ATP5B probably plays a key role in porcine skeletal muscle development and may provide further insight into the molecular mechanisms responsible for breed-specific differences in meat quality [60]. However, this study implies that in addition to the meat quality traits this gene could an important candidate for boar taint trait. The function of highly polymorphic *KRT8* is associated to pathological processes in liver but involvement in boar taint is not quite

clear. Mutation in KRT8 is reported to be involved in human liver disease [62]. However, this gene maps close to a QTL region on SSC5 affecting skatole and indole levels [38] warranting more studies about this gene and polymorphisms with regards to boar taint. The gene *PGM1* is involved in glucose metabolism pathway and Lefaucheur et al. [63] reported the higher expression of genes in glycolytic pathways including this gene in the Large White which is in agreement with high glycolytic and low oxidative metabolism muscle tissues. However, no study devoted to unravel the involvement of this gene in boar taint compounds metabolism has been published so far. The gene *SLC22A7* is involved in the sodium-independent transport and excretion of organic anions and the substrate panel of SLC22As includes important endogenous compounds like tryptophan metabolites and sulphated steroids [53]. Skatole results from a multistep degradation of tryptophan by microbial activity, mainly in the hind gut of the pigs (reviewed by Wesoly and Weiler [1]). Therefore, the marker identified on *SLC22A7* could be a valuable SNP for boar taint. *IDH1* is the most important isocitryte dehydrogenase in the citrate and fatty acid synthesis that is related to energy metabolism and tissue morphology [46]. Energy metabolism is represented by glycolysis and glycogenolyis. It is well established that leaner pigs have a lower ability to synthesize fatty acids combined with greater mobilization, which results in adipose depots with more unsaturated lipids [64]. The pigs with higher metabolism rate such as fatter pigs like Large White and Duroc have higher androstenone and skatole levels than the lean breeds with lower energy metabolism like Pietrain [65]. It is worth to mention that the polymorphisms identified in this study are mostly synonymous and three SNPs from each synonymous and non-synonymous category are validated in this study (Table 5). However, these polymorphisms are suggested to be validated in other porcine populations before considering in selection breeding.

Conclusion

Here we showed the whole genome expression differences in liver tissues for varying skatole levels in backfat of boars. RNA-Seq provided a high resolution map of transcriptional activities and genetic polymorphisms in this tissue. However, due to incomplete porcine annotations, only around 65.5 % of the total reads could be mapped to annotated references. The improvements in pig genome annotations may lead to better coverage and detailed understanding of genetic and functional variants such as

novel transcripts, isoforms, sequence polymorphisms and non-coding RNAs. On the basis of number of the DEGs, our results confirm regulation of transcriptome activity in liver tissue for skatole degradation. This study proposed candidate genes such as *SERPINA12, KRT8, CYP4A25, COX5A, SLC22A7*, *PRDX1* and *HSD17B2* that might be involved in liver for skatole metabolism. Importantly, most of the DEGs are functionally related to pathways involved in boar taint and incorporated within published QTL positions affecting boar taint compounds. Furthermore, various gene polymorphisms were detected in liver DEGs and associations are validated with skatole levels. Potential polymorphisms and association were identified for selected mutations in selected DEGs such as *ATP5B, KRT8, PGM1, SLC22A7* and *IDH1*. In addition, differential exon usage analysis of three genes (*ATP5B, KRT8* and *PGM1*) revealed significant differential expression of exons of these genes in the pigs with divergent skatole levels. This transcriptome, polymorphisms and alternative splicing analysis using RNA deep sequencing combined with association analysis revealed potential candidate genes affecting boar taint compound. It is speculated that these polymorphisms could be used as biomarkers for boar taint related traits. However, further validation is required to confirm the effect of these genetic markers in other pig populations.

Material and Methods

Animals and Phenotype

Tissue samples and phenotypes were collected from the Duroc $\times$ F$_2$ cross animals. F$_2$ was created by crossing F$_1$ animals (Leicoma $\times$ German Landrace) with the Large White pig breed. Duroc $\times$ F$_2$ boars were on average 116 days old and had on average 90 kg live weight at slaughter. All pigs were slaughtered in a commercial abattoir called Landesanstalt für Schweinezucht - LSZ Boxberg. Slaughterhouse management gave the necessary permissions for the tissue and organ collection. Carcass and meat quality data were collected according to guidelines of the German performance test [66]. As described in Gunawan et al.[8], tissue samples from liver were frozen in liquid nitrogen immediately after slaughter and stored at -80°C until used for RNA extraction. Fat samples were collected from the neck and stored at -20°C until used for skatole measurements. For the quantification of skatole an in-house gas-chromatography/mass spectrometry (GC-MS) method was applied as described previously [67]. Pigs having a

fat skatole level less than 0.25 µg/g and greater than 0.25 µg/g were defined as low and high skatole samples, respectively [68,69]. Six boars were selected from a pool of 100 pigs and the average skatole value for these selected animals were 0.27 ± 0.20 µg/g. RNA was isolated from the liver tissues of 3 pigs with (HS, high skatole group) high (0.45 ± 0.08 µg/g) and 3 pigs with (LS, low skatole group) low levels of skatole (0.09 ± 0.02 µg/g). Notably, these six boars were among the ten boars which have been previously used for androstenone study [8]. Among the ten pigs used in androstenone study, six pigs were found with extremely high and low skatole levels and were considered for this study. Furthermore, these 100 boars were used for association study (Supplementary Table S4). Total RNA was extracted using RNeasy Mini Kit according to manufacturer's recommendations (Qiagen). Total RNA was treated using on-column RNase-Free DNase set (Promega) and quantified using a spectrophotometer (NanoDrop, ND8000, Thermo Scientific). RNA quality was assessed using an Agilent 2100 Bioanalyser and RNA Nano 6000 Labchip kit (Agilent Technologies).

Library Construction and Sequencing

Details of the library construction and sequencing procedures were described previously by Guanwan et al. [8]. The library preparations were sequenced on an Illumina HiSeq 2000 at GATC Biotech AG (Konstanz, Germany). All sequences were analysed using the CASAVA v1.7 (Illumina, USA). As described in Gunawan et al.[8], the deep sequencing data have been deposited in NCBI SRA database and are accessible through GEO series accession number GSE44171 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE44171).

Genome reference and mapping

In this study, the raw reads after quality control were mapped to NCBI Sscrofa10.2 genome build using RNA-seq read mapper TopHat [70]. TopHat is a "splice aware" mapper that uses Bowtie short read aligner [71] for aligning the raw reads to the genomes and further analyses these mapping results for splice junction discovery. After mapping the raw reads to the genome build BEDTools utilities [72] was used to compute the coverage of raw reads to Sscrofa10.2 gene positions for each sample. The expression table thus created was further used in the analysis of differentially expressed

Differential gene expression analysis

The differential gene expression analysis was designed to contrast the difference in the expression of genes between high and low skatole samples. For differential gene expression analysis with raw count data, the R package DESeq was used [73]. The normalization procedure in DESeq handles the differences in the number of reads in each sample. For this purpose, DESeq first generates a fictitious reference sample, with read counts defined as the geometric mean of all the samples. The reads count for each gene in each sample is divided by this geometric mean to obtain the normalized counts. To model the null distribution of the count data, DESeq follows an error model that uses the negative binomial distribution, with variance and mean linked by local regression. The method controls type-I error and provides good detection power [73]. After analysis using DESeq, DEGs were filtered based on p-adjusted value < 0.05 and fold change $\geq$ 1.5 [74]. Additionally, the gene expression data was also analyzed using a Generalized Linear Model (GLM) function implemented in DESeq to calculate both within and between group deviances. As a sanity checking and filtration step, we cross matched the results from both analysis (p-adjusted ≤ 0.05 and fold change ≥ 1.5 criteria and GLM analysis) and only those genes which appeared to be significant in both the tests (*p*-value ≤ 0.05), were selected for further analysis [8]. The results of GLM analysis are given in Supplementary Table S6.

Differential exon expression analysis

In addition to analyzing the genes that are differentially expressed between high and low skatole samples, the differential expression levels of exons of the same genes between the different phenotype samples were also analysed. For this purpose, we used the R package DEXSeq [24]. The mapped read count data were converted into exon "counting bins" as described in [24]. In the next step, the algorithm normalized sequencing depths for all the samples as described in [73]. In the final step, Generalized Linear Models (GLMs) were employed by the algorithm for each counting bin to test for differential expression between phenotype samples. After the analysis, differentially used exons were filtered using the criteria p-adjusted value < 0.05.

Pathways and Networks Analysis

A list of the DEGs was uploaded into the Ingenuity Pathway Analysis (IPA) software (Ingenuity Systems, www.ingenuity.com) to identify relationships between the genes of interest and to uncover common processes and pathways. Networks of the genes were then algorithmically generated based on their connectivity. The 'Functional Analysis' tool of the IPA software was used to identify the biological functions most significant to the data set. Canonical pathway analysis was also utilized to identify the pathways from the IPA library of canonical pathways that were most significant to the data set. We used "Benjamini-Hochberg" multiple testing correction to calculate a *p*-value determining the probability that each biological function or canonical pathway assigned to the data set. The significance levels of p-values obtained for every biological function or canonical pathways were corrected for multiple testing using "Benjamini-Hochberg" correction.

Gene Variation Analysis

In this analysis, SNP calling was performed on the mapping files generated by TopHat algorithm using samtools mpileup command and associated algorithms [75]. From the variants so generated, only those variants with a minimum Root Mean Square (RMS) mapping quality of 20 and a minimum read depth of 100 were selected for further analysis. In the final step, the selected variants were cross-checked against dbSNP database to identify mutations that are already studied. In order to find out the differentially expressed genes that also harboured sequence polymorphisms, we crosschecked and filtered with the chromosomal positions of these variants against those of DEGs and retained only those variants which mapped to DEG the chromosomal positions. By this way, we were able to isolate a handful of mutations that mapped to DEGs from many thousands of identified potential sequence polymorphisms. In the next step, to understand whether these identified polymorphisms segregate either in only one sample group (high skatole or low skatole group) or in both groups (high and low skatole group), we calculated the read/coverage depth of these polymorphisms in all the samples [8]. The identified SNPs were furthermore classified as synonymous or non-synonymous using the GeneWise software (http://www.ebi.ac.uk/Tools/psa/genewise/ last accessed 21.03.2013) by comparing between protein sequence and nucleotides incorporated SNP position [76]. The results

of this analysis are detailed in the results section and read coverage for individual samples are given in Supplementary Table S3.

Quantitative Real-Time PCR (qRT-PCR) Analysis

For qRT-PCR experiment, total RNA from liver samples were isolated from the 6 boars used for deep sequencing. Additionally, RNA was isolated from the similar tissues of 6 independent boars with divergent skatole level among the remaining 94 boars. cDNA were synthesised by reverse transcription PCR using 2 μg of total RNA, SuperScript II reverse transcriptase (Invitrogen) and oligo(dT)12 primer (Invitrogen). Gene specific primers for the qRT-PCR were designed by using the Primer3 software [77]. Detailed information for primers used in this study was given in Table 8. In each run, the 96-well microtiter plate contained each cDNA sample and no-template control. The qRT-PCR was conducted with the following program: 95 °C for 3 min and 40 cycles 95 °C for 15 s/60 °C for 45 s on the StepOne Plus qPCR system (Applied Biosystem). For each PCR reaction 10 μl iTaqTM SYBR$^®$ Green Supermix with Rox PCR core reagents (Bio-Rad), 2 μl of cDNA (50 ng/μl) and an optimized amount of primers were mixed with ddH$_2$O to a final reaction volume of 20 μl per well. All samples were analysed twice (technical replication) and the geometric mean of the Ct values were further used for mRNA expression profiling. The geometric mean of two housekeeping genes GAPDH and PPIA were used for normalization of the target genes. The delta Ct (ΔCt) values were calculated as the difference between target gene and geometric mean of the reference genes: ($\Delta Ct = Ct_{target} - Ct_{housekeeping\ genes}$) as described in Silver et al. [78]. Final results were reported as fold change calculated from delta Ct-values.

Validation of SNP and association study

For the validation of association, six SNPs from the highly polymorphic DEGs as well as the genes known to be involved in the skatole metabolism were selected (Table 7). Genotyping in 100 boars were performed by PCR-RFLP method. In brief, a working solution with a final concentration of 50 ng/μl DNA was prepared and stored at 4 °C for further analysis. Polymerase chain reactions (PCR) were performed in a 20 μl volume containing 2 μl of genomic DNA, 1 × PCR buffer (with 1.5 mM MgCl$_2$), 0.25 mM of dNTP, 5 pM of each primer and 0.1 U of Taq DNA polymerase (GeneCraft). The PCR

product was checked on 1.5 % agarose gel (Fischer Scientific Ltd) and digested by using the appropriate restriction enzyme (Table 8). Digested PCR-RFLP products were resolved in 3% agarose gels. Details of GenBank accession numbers, primers sequences, annealing temperature and SNP position used in this study are listed in Table 8. Statistical analyses were performed using SAS 9.2 (SAS Institute Inc, Cary, USA). Effects of slaughter age, husbandry system (pen) as well as genotype on boar taint compound skatole were assessed with a fixed effect model (ANOVA) using PROC GLM. For all models, fixed effects included genotype and pen (group. individual) and age of slaughter was fitted as a covariate for boar taint compound skatole. Due to the skewed nature of skatole, data were transformed with natural logarithm before ANOVA to achieve normality. Least square mean values for the loci genotypes were compared by t-test and p-values were adjusted by the Tukey-Kramer correction [79].

Supporting Information

Supplementary Table S1. Differentially expressed genes in liver from boars with high and low skatole in backfat

Supplementary Table S2. Polymorphisms in DEGs detected in liver from boars with high and low skatole in backfat

Supplementary Table S3. Sample read counts for polymorphisms in liver from boars with high and low skatole in backfat

Supplementary Table S4. Genotype, allele frequencies and the chi-square test of selected SNPs validated using RFLP

Supplementary Table S5. Differential exon expression in liver from boars with high and low skatole in backfat

Supplementary Table S6. GLM analysis results for liver DEGs

Acknowledgements

(measurement)   of   skatole.
This work was financially supported by the Federal Ministry of Food, Agriculture and Consumer Protection, Germany (BMELV), the Federal Institute of Agriculture and Food, Germany (BLE) and State of North Rhine Westphalia with the programme USL "Umweltverträgliche und Standortgerechte Landwirtschaft".

Author Contributions

AG performed the experiments and wrote the manuscript; SS analysed the RNA-Seq data and wrote manuscript; MUC supervised the works and criticised the experiment; CN contributed to sampling; CG contributed in statistical analysis; LF contributed in sampling and phenotyping; DT was responsible forkits and reagents; ET were responsible for the statistical analysis; CL revised the manuscript; DSW edited and criticized the results; KS edited manuscript and designed the experiment; MJU was responsible for the whole experiment and supervised the overall work. All authors read and approved the final manuscript. The authors declared of no competing interest.

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Table 1. Summary of sequence read alignments to reference genome in liver samples

Group	Sample*	Total number of reads (million)	Un-mapped reads (million)	Mapped reads (million)	Percentage of unmapped reads	Percentage of mapped reads
	LS1	23.4	6.0	17.4	25.6	74.4
Low skatole	LS2	35.6	10.0	25.6	28.0	72.0
	LS3	12.6	7.2	5.4	47.0	43.0
	HS1	14.7	5.7	9.0	38.7	61.3
High skatole	HS2	11.8	4.9	6.9	41.5	58.5
	HS3	39.0	6.3	32.7	16.0	84.0

*LS 1, 2, 3 indicate the low skatole sample; HS 1, 2, 3 indicate the high skatole samples.

Table 2. Top 30 up and down regulated genes in liver tissues collected from boars with high and low skatole levels in bacfat.

Gene	Orthologue gene description	Reference ID	log2Fold Change	p-adj.
SERPINA12	Serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 12	XM_003128704.2	5.82	4.73e-06
KRT8	Keratin 8	NM_001159615.1	4.33	6.03e-05
TNFRSF12A	Tumor necrosis factor receptor superfamily, member 12A	NM_001142839.1	3.75	2.05e-09
HSPA8	Heat shock 70kDa protein 8	NM_001243907.1	3.70	0.004
HSP71	Heat shock 70kDa protein 8	XM_003129961.3	3.70	0.004
GSTO2	Glutathione S-transferase omega 2	XM_001927288.3	3.56	2.55e-07
CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)	XM_001929558.1	3.44	1.95e-12
HSP90AA1	Heat shock protein 90kDa alpha (cytosolic), class A member 1	NM_213973.1	3.21	0.0027
ALDH3B1	Aldehyde dehydrogenase 3 family, member B1	XM_003480660.1	3.15	4.06e-08
DLST	Dihydrolipoamide S-succinyltransferase (E2 component of 2-oxo-glutarate complex)	NM_214397.1	3.11	3.30e-10
UGT1A3	UDP glucuronosyltransferase 1 family, polypeptide A3	XM_003133742.3	3.06	3.55e-07
DHRS4	Dehydrogenase/reductase (SDR family) member 4	NM_214019.1	3.02	0.0039
SLC5A6	Solute carrier family 5 (sodium-dependent vitamin	XM_003125293.3	2.97	0.0035

	transporter), member 6			
PMM1	Phosphomannomutase 1	NM_001184895.1	2.90	1.56e-09
NKIRAS2	NFKB inhibitor interacting Ras-like protein 2	XM_003358038.1	2.89	5.60e-05
FOS	FBJ murine osteosarcoma viral oncogene homolog	NM_001123113.1	2.86	3.21e-08
CYP4A25	Cytochrome P450 4A24	XM_003128016.3	2.81	2.81e-08
CYP4A24	Cytochrome P450 4A24	XM_003356476.2	2.76	2.03e-05
ATP5B	ATP synthase, H+ transporting, mitochondrial F1 complex, beta polypeptide	XM_001929410.2	2.74	0.00012
TUBA1A	Tubulin, alpha 1a	XM_003355375.1	2.71	1.50e-07
SLC25A5	Solute carrier family 25 (mitochondrial carrier; adenine nucleotide translocator), member 5	XM_001927440.2	2.71	0.00104
ATP5A1	ATP synthase, H+ transporting, mitochondrial F1 complex, alpha subunit 1, cardiac muscle	NM_001185142.1	2.66	2.88e-05
SDHD	Succinate dehydrogenase complex, subunit D, integral membrane protein	NM_001097516.1	2.66	3.34e-07
CRYAB	Crystallin, alpha B	XM_003357294.1	2.62	3.21e-06
UQCRFS1	Ubiquinol-cytochrome c reductase, Rieske iron-sulfur polypeptide 1	XM_003127002.1	2.63	4.62e-06
PGM1	Phosphoglucomutase 1	XM_003127945.2	2.60	6.22e-06
SLC22A7	Solute carrier family 22 (organic anion transporter), member 7	NM_001044617.1	2.60	2.81e-08
CYP4B24	Cytochrome P450 4B24	XM_003482090.1	2.58	2.35e-06
SLC25A25	Solute carrier family 25 (mitochondrial carrier; phosphate carrier), member 25	NM_001164510.1	2.53	1.351e-05
COX5A	Cytochrome c oxidase subunit Va	XM_003482239.1	2.47	0.00031
PRDX1	Peroxiredoxin 1	XM_003128040.1	2.47	8.22e-05
ACSL5	Acyl-CoA synthetase long-chain family member 5	XM_003359369.1	2.38	0.0001684
MDH2	Malate dehydrogenase 2, NAD (mitochondrial)	NM_001244153.1	2.34	5.91e-05
MDH1	Malate dehydrogenase 1, NAD (soluble)	NM_213874.1	2.30	5.89e-06
TUBA1B	Tubulin, alpha 1b	NM_001044544.1	2.16	0.00018
IDH1	Isocitrate dehydrogenase 1 (NADP+), soluble	XM_003483721.1	2.14	0.00043
DHRS1	Dehydrogenase/reductase (SDR family) member 1	XM_003128543.1	2.11	3.02e-05
HSPA5	Heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)	XM_001927795.4	2.11	0.0037
PGM3	Phosphoglucomutase 3	XM_001924419.2	2.05	9.56e-06
SLC25A1	Solute carrier family 25 (mitochondrial carrier;	NM_001190189.1	2.03	0.0022

	citrate transporter), member 1		
GSTM2	Glutathione S-transferase mu 2	NM_001078684.1 2.02	0.0017
TNFAIP1	Tumor necrosis factor, alpha-induced protein 1 (endothelial)	XM_003483067.1 1.91	0.00066
HSD3B7	Hydroxy-delta-5-steroid dehydrogenase, 3 beta- and steroid delta-isomerase 7	XM_003124487.1 1.84	0.0012
SEC13	SEC13 homolog (S, cerevisiae)	XM_003483983.1 -1.93	0.0022
ZNF238	Zinc finger protein 238	XM_003357648.1 -1.93	0.0029
HDAC9	Histone deacetylase 9	XM_003357464.1 -1.94	0.0017
CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha	XM_003127015.1 -1.94	0.0009
GUCY1A2	Guanylate cyclase 1, soluble, alpha 2	XM_003130093.3 -2.00	0.0036
LAMP1	Lysosomal-associated membrane protein 1	NM_001011507.1 -2.10	0.00029
SEMA3E	Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3E	XM_003130220.3 -2.37	0.0044
ZNF829	Zinc finger protein 829	XM_003127093.3 -2.40	0.00257
GABARAPL1	GABA(A) receptor-associated protein like 1	XM_003126479.3 -2.46	8.44e-05
UNC13D	Unc-13 homolog D (C, elegans)	XM_003131192.1 -2.54	0.00021
HSD17B2	Hydroxysteroid (17-beta) dehydrogenase 2	NM_001167649.1 -2.78	0.0032
CXCL9	Chemokine (C-X-C motif) ligand 9	NM_001114289.2 -2.80	0.0002
PNOC	Prepronociceptin	NM_001244476.1 -2.81	0.00085
CDK5	Cyclin-dependent kinase 5	XM_003480595.1 -2.82	0.00017
PHF7	PHD finger protein 7	XM_001928213.2 -2.83	0.00345
SOCS2	Suppressor of cytokine signaling 2	NM_001097461.1 -2.96	0.00054
PCLO	Piccolo presynaptic cytomatrix protein	XM_003357489.2 -2.97	0.0006
SLC9A4	Solute carrier family 9 (sodium/hydrogen exchanger), member 4	XM_003354711.1 -3.36	0.0006
GABRG2	Gamma-aminobutyric acid (GABA) A receptor, gamma 2	XM_003359825.1 -3.40	0.0006
LOC100737161	LOC100737161	XM_003482919.1 -3.50	0.0001
LOC100512296	LOC100512296	XM_003129119.2 -4.07	1.01e-07
LOC100155734	LOC100155734	XM_001927727.2 -4.27	0.002
LOC100739739	LOC100739739	XM_003482938.1 -5.02	0.0011
LOC100625674	LOC100625674	XM_003359731.2 -5.89	0.0012
LOC100737759	LOC100737759	XM_003482870.1 -6.80	7.76e-05

Table 3. Functional categories and corresponding genes those were over expressed in liver from high skatole boars

Function	Number of genes	B-H p-value	Genes
Small molecule biochemistry	53	7.41E-03-1.46E-01	DLST, IDH3B, MDH1, MDH2, ATP5A1, ATP5D, HSP90AA1, HSPA8, TAP1, CRYAB, GPI, NUDT15, AGXT, CTH, GNMT, GOT1, HAL, CTPS1, SLC25A5, CD1D, LBP, ASS1, BCKDHA, PEPD, GPX4, PRDX1, ACSL5, HAL, ABHD5, ARF1, FOS, MYD88, PISD, SERINC2, ANXA1, CIDEC, GOS2, PLA2G7, AP2M1, ACOX1, GPR39, GUCY1A2, GALE, CDKN1A, GUCY1A1, LT4N, PLOD1, POFUT1, PGM1, PMM1, PGM3, DNPEP, SLC22A7
Protein synthesis	11	7.41E-03-1.46E-01	CTH, GPX1, GPX4, GSTM2, IDH1, SOD2, CD1D, HSPA5, GNMT, DNPEP, DHRS4
Carbohydrate metabolism	24	2.21E-02-1.46E-01	NUDT15, TAP1, CEBPA, CDKN1A, HSP90AA1, HSPA8, IDH1, IDH3B, CRYAB, GALE, GPI, PGM1, PGM3, PMM1, AP2M1, CD1D, LBP, GOT1, ABHD5, PLA2G7, FOS, MYD88, GSTO2, GNPNAT1
DNA replication. recombination and repairs	9	4.10E-04-1.85E-02	ATP5A1, ATP5B, ATP5D, HSP90AA1, NUDT15, HSPA8, CEBPA, CDKN1A, HSPA5
Energy production	8	1.72E-03-2.52E-02	ATP5A1, ATP5B, ATP5D, HSP90AA1, HSPA8, TAP1, MDH1, MDH2
Lipid metabolism	18	9.59E-02-1.46E-02	MDH1, MDH2, CD1D, LBP, ABHD5, ACSL5, ARF1, FOS, GPX4, MYD88, PISD, CIDEC, PLA2G7, AP2M1, ACOX1, HSPA8, SAT1, SLC22A7
Post-tralslation modification	10	9.82E-02-1.46E-01	CRYAB, HSP90AA1, HSPA5, HSPA8, PDIA6, CTH, GNMT, SOD2, PLOD1, DHRS4
Amino acid metabolism	8	7.52E-02-9.59E-02	AGXT, CTH, GNMT, GOT1, ASS1, GNMT, GPX4, CDKN1A

Table 4: The canonical pathways from the IPA knowledge base that involve transcripts over expressed in liver with higher skatole

Category	-log (B-H p-value)	Number of genes	B-H- p-value	Genes
Remodeling of epithelial adherens junction	2.99	8	1.01E-03	ACTB, ARPC3, ARPC1A, TUBA1A1, TUBA1B, TUBA1C, TUBB2A, TUBB4B
TCA cycle	2.60	6	2.48E-03	DLST, IDH3B, MDH1, MDH2, OGDHL, SDHD
Superpathway of methionine degradation	1.78	6	1.66E-02	AHCY, CTH, FTSJ1, GOT1, MUT, PRMT1,
Mithochondria1 dysfunction	1.78	9	1.66E-02	ATP5A1, ATP5B, COX5A, GPX4, NDUFA9, PSEN2, SDHD, SOD2, UQCRFS1
UDP-N-acety1-D-ga1actosamine biosynthesis II	1.78	4	1.66E-02	GALE, GNPNAT1, GPI, PGM3
Cysteine biosynthesis	1.50	4	3.11E-02	AHCY, CTH, FTSJ1, PRMT1

Table 5. Polymorphisms detected in highly polymorphic DEGs

Refseq ID	Gene name	Chr	Position	db SNP	Ref	Alt	High skatole coverage	High skatole mean phred score	Low skatole coverage	Low skatole mean phred score	Sample group	SNP clasification
XM 001929410.2	ATP5B	5	23659117	0	A	G	175	49	182	50	High and Low	Non Synonymous
XM 001929410.2	ATP5B	5	23661024	0	T	C	532	47	366	47	High and Low	Non Synonymous
XM 001929410.2	ATP5B	5	23661627	rs80908111	C	T	481	48	253	47	High and Low	Non Synonymous
NM 214019.1	DHRS4	7	80515824	0	T	C	0	0	162	49	Low skatole	Non Synonymous
NM 001159615.1	KRT8	5	18663991	0	T	C	0	0	343	50	Low skatole	Synonymous
NM 001159615.1	KRT8	5	18664266	0	T	C	515	49	317	48	High and Low	Non Synonymous
NM 001159615.1	KRT8	5	18664389	0	A	G	513	49	286	49	High and Low	Synonymous
NM 001159615.1	KRT8	5	18667998	0	G	A	328	49	0	0	High skatole	Synonymous
NM 001159615.1	KRT8	5	18670859	0	G	A	303	50	0	0	High skatole	Synonymous
XM 003128016.3	CYP4A25	6	152197351	0	A	C	251	49	0	0	High skatole	Non Synonymous
XM 003128016.3	CYP4A25	6	152198727	0	T	C	210	48	0	0	High skatole	Synonymous
XM 003128016.3	CYP4A25	6	152206224	0	A	G	279	48	0	0	High skatole	Synonymous
XM 003128016.3	CYP4A25	6	152206341	0	G	C	308	46	188	49	High and Low	Synonymous
XM 003128016.3	CYP4A25	6	152206385	0	A	T	184	45	172	49	High and Low	Synonymous
XM 003128016.3	CYP4A25	6	152206818	0	C	T	0	0	253	49	Low skatole	Non Synonymous
XM 003127945.2	PGM1	6	137171304	0	G	T	134	50	0	0	High skatole	Non Synonymous
XM 003127945.3	PGM1	6	137171323	0	T	C	204	50	0	0	High skatole	Synonymous

XM 003127945.4	PGM1	6	137171408	0	T	C	435	50	254	49	High and Low	Non Synonymous
XM 003127945.5	PGM1	6	137171452	0	A	G	403	50	0	0	High skatole	Non Synonymous
XM 003127945.6	PGM1	6	137171481	0	C	A	0	0	200	49	Low skatole	Non Synonymous
XM 003127945.7	PGM1	6	137171535	0	T	C	389	50	175	49	High and Low	Synonymous
XM 003127945.8	PGM1	6	137171741	0	A	C	383	50	145	50	High and Low	Synonymous
XM 003127945.9	PGM1	6	137171813	0	C	T	383	49	0	0	High skatole	Non Synonymous
XM 003127945.10	PGM1	6	137171857	0	C	T	380	49	123	50	High and Low	Non Synonymous
XM 003127945.11	PGM1	6	137174682	0	A	G	332	47	0	0	High skatole	Synonymous
XM 003127945.12	PGM1	6	137174727	0	C	T	294	45	0	0	High skatole	Synonymous
XM 003127945.13	PGM1	6	137174784	0	C	A	327	47	0	0	High skatole	Synonymous
XM 003127945.14	PGM1	6	137195153	0	A	G	256	49	0	0	High skatole	Synonymous
XM 003128039.1	PRDX1	6	153255729	rs81215265	C	T	451	48	236	48	High and Low	Non Synonymous
XM 003128039.3	PRDX1	6	153257939	rs81215269	C	T	0	0	242	45	low skatole	Synonymous
XM 003128039.4	PRDX1	6	153257940	rs81215270	C	G	448	48	242	45	High and Low	Synonymous
XM 003128039.5	PRDX1	6	153265783	0	G	A	513	48	365	46	High and Low	Synonymous
XM 003128039.6	PRDX1	6	153265829	rs196949554	G	T	475	48	338	46	High and Low	Synonymous
XM 003128039.7	PRDX1	6	153265881	0	A	G	307	49	187	47	High and Low	Synonymous
XM 003128039.9	PRDX1	6	153265905	0	T	C	201	49	0	0	High skatole	Synonymous
XM 003128039.10	SLC22A7	7	43833000	0	G	A	182	48	0	0	High skatole	Non Synonymous
XM 003128039.11	SLC22A7	7	43833898	0	G	A	197	49	0	0	High skatole	Synonymous
XM 003483721	IDH1	15	122861718	0	T	G	225	49	124	48	High and Low	Synonymous
XM 003483722	IDH1	15	122861896	0	C	T	430	48	235	48	High and Low	Synonymous
XM 003483723	IDH1	15	122861903	0	CGGG	CGG	424	48	233	48	High and Low	Deletion
XM 003483724	IDH1	15	122861968	0	C	T	441	49	223	49	High and Low	Non Synonymous

XM 003483725	IDH1	15	122862291 0	T	C	374	49	177	49	High and Low	Synonymous
XM 003483727	IDH1	15	122862513 0	C	T	406	49	133	48	High and Low	Synonymous
XM 003483728	IDH1	15	122862530 0	C	T	397	48	118	48	High and Low	Synonymous
XM 003483729	IDH1	15	122876927 0	G	A	292	49	0	0	High skatole	Synonymous

Table 6. Genotype and association analysis selected candidate gens with skatole

Polymorphism	Boar taint compound (Ln)	Genotype ($\mu \pm$ S.E.)			Effect ($\mu \pm$ S.E.)	
		TT	TC	CC	Additive	Dominance
ATP5B T>C	Skatole (µg/g)	4.62±0.34 [e]	5.43±0.29 [f]	4.32±0.16 [e]	0.14±0.17	-0.95±0.31**
		GG	GA	AA		
KRT8 G>A	Skatole (µg/g)	5.25±0.24 [e]	4.71±0.30 [e]	4.20±0.16 [f]	0.52±0.13**	0.01±0.29
		CC	CA	AA		
PGM1 C>A	Skatole (µg/g)	4.95±0.20[a]	4.29±0.19[b]	4.09±0.28[b]	0.42±0.16**	0.23±0.22
		AA	AC	CC		
CYP4A25 A>C	Skatole (µg/g)	4.98±0.33	5.00±0.45	4.29±0.18	0.34±0.19	-0.36±0.46
		GG	GA	AA		
SLC22A7 G>A	Skatole (µg/g)	5.11±0.19 [e]	4.73±0.39 [e]	4.08±0.16 [f]	0.51±0.11**	-0.12±0.39
		CC	CT	TT		
IDH1 C>T	Skatole (µg/g)	5.02±0.22[c]	4.50±0.24[cd]	4.06±0.20[d]	0.48±0.12 **	0.03±0.27

a.b < 0.05; c.d< 0.01 ; e.f < 0.001 ; * p <0.05 ; **p<0.01

Table 7. Differential exon expression in selected DEGs in liver samples from boars with divergent skatole levels in backfat.

Chr	GeneID (+/- indicates strand)	Transcripts	Gene Name	Exon ID	Start	End	Log2fold (high/low)	*P*-adjust
1	407060-	XM_001927795.4	HSPA5	E001	299754033	299754610	-0.175	0.049
3	100525281+	XM_003124487.1	HSD3B7	E007	17843326	17844524	-0.689	3.89e-05
4	100156038+	XM_003355245.1	PGM3	E010	119097423	119098520	-0.341	0.013
5	100157156-	XM_001929410.2	ATP5B	E001	23659081	23659341	-0.321	0.026
5	100157156-	XM_001929410.2	ATP5B	E002	23660897	23661098	-0.224	0.039
5	100152077-	NM_001159615.1	KRT8	E001	18663339	18663700	-0.286	0.001
5	100151790+	NM_001184895.1	PMM1	E008	4347163	4347697	-0.354	0.013
5	100151790+	NM_001184895.1	PMM1	E008	4347163	4347697	-0.354	0.013
6	397566-	XM_003127946.1	PGM1	E010	137200412	137200574	-0.417	0.013
6	397566-	XM_003127945.2	PGM1	E010	137200412	137200574	-0.417	0.013
6	397566-	XM_003127945.2	PGM1	E012	137233155	137233581	-0.504	3.35e-05
6	100512476+	XM_003128039.1	PRDX1	E003	153249744	153249811	-2.125	0.001
6	100621392+	XM_003356202.1	GALE	E011	75420510	75420891	-0.393	0.001
12	100737417+	XM_003483067.1	TNFAIP1	E007	46536059	46537702	0.222	0.040

Table 8. Details of primers used for qRT-PCR analysis and genotyping

Gene	Reference ID	Primer sequences (5'→3')	Application	Position*	Enzymes	Annealing temp (°C)	Product size (bp)	RFLP pattern
ATP5B	XM_001929410.2	F:AATCCTTTGATGGTCTCCTT	qRT-PCR	-	-	55	201	
		R:AAGATATCATTGCCATCCTG						
DHRS4	NM_214019	F:TCCTGATGACAAAGGCAGTG	qRT-PCR	-	-	60	108	
		R:TGCCTTATCCATCCACAACA						
GSTO2	XM_001927288.3	F:CACCAGAGTTCCGTTGTCCT	qRT-PCR	-	-	55	211	
		R:GTCACGTTCTCCCGATGTTT						
IDH3B	NM_001044575.2	F:TGTCAGCTTCCAACATGCTA	qRT-PCR	-	-	55	205	
		R:TGTGAGGTTGGAGGGAATAA						
HSD17B2	NM_001167649.1	F:TGCAGAACAGAGGACTGTGG	qRT-PCR	-	-	54	103	
		R:GCCATGCATCGTTTGTATTG						
KRT8	NM_001159615.1	F:ACTTGGACAGGACATCAGAG	qRT-PCR	-	-	55	166	
		R:ACTCCAGGCTTCAACTACAG						
PGM1	XM_003127945.3	F:CCTCCTTCATGTAAAACCTG	qRT-PCR	-	-	55	190	
		R:GTTAAGACCAAGGCGTATCA						
PRDX1	XM_003128039	F:GTCCATGAGAACAACGTCTT	qRT-PCR	-	-	55	208	
		R:AAGTGAAACCCTGCTACTGA						
SDHD	NM_001097516.1	F:GGAGGCTCAGTGTTCTTTGC	qRT-PCR	-	-	54	148	
		R:CTGGGTGACAGGTGAATGTG						
SLC22A7	NM_001044617.1	F:TGGATGGAGTATGGCTGTCA	qRT-PCR	-	-	56	139	
		R:GCACTCTTCCTCTCCACGTC						
PPIA	NM_214353	F: CACAAACGGTTCCCAGTTT	qRT-PCR	-	-	58	171	
		R:TGTCCACAGTCAGCAATGGT						
GAPDH	AF017079	F:ACCCAGAAGACTGTGGATGG	qRT-PCR	-	-	60	247	

Gene	Accession	Primer	Purpose	Exon	Enzyme	Temp	Product	Genotype
		R:ACGCCTGCTTCACCACCTTC						
ATP5B	XM_001929410.2	F:GTAAAGACCTCAGCAACCTG	Genotyping	Exon 7	BciVI	58	167	TT: 113+ 54
		R:TGTTTACTCAGGCCTCTCAT						CC: 167
KRT8	NM_001159615.1	F:GGAGGCAAACTTATTGTTGA	Genotyping	Exon 9	BtsCI	55	170	GG:104+66
		R:TGAGTCTGGTTGGAGGTTAC						AA:170
PGM1	XM_003127945.3	F:TCCTTCTCATAGCTGTCGAT	Genotyping	Exon 3	AciI	55	172	CC:172
		R:CATAATTACCCAGGCTTCAG						AA:117+55
CYP4A25	XM_003128016.3	F:GCTGACAGATCCACACCTAT	Genotyping	Exon 1	HpyCH4V	55	230	AA:123+107
		R:ACCACCTTCATGTAGTCAGG						CC:230
SLC22A7	XM_003128039.9	F:AAAGGTTCGACCATGAAATG	Genotyping	Exon 8	BstNI	55	201	GG:201
		R: TATGGCAGCTGTCTCTGTGA						AA:110+81
IDH1	NM_001159615	F: GGGTTGAGAAGGTTCTGGAT	Genotyping	Exon 4	HhaI	55	177	CC:98+79
		R: CTCCTCGTGGTTCTTCTTCA						TT:177

Figure 1. Heatmap showing differentially expressed genes in liver samples. The red blocks represent over expressed genes, and the green blocks represent under expressed genes. Legend: LS1- LS3 boars liver with low skatole in backfat and HS1-HS3 boars liver with high skatole in backfat.
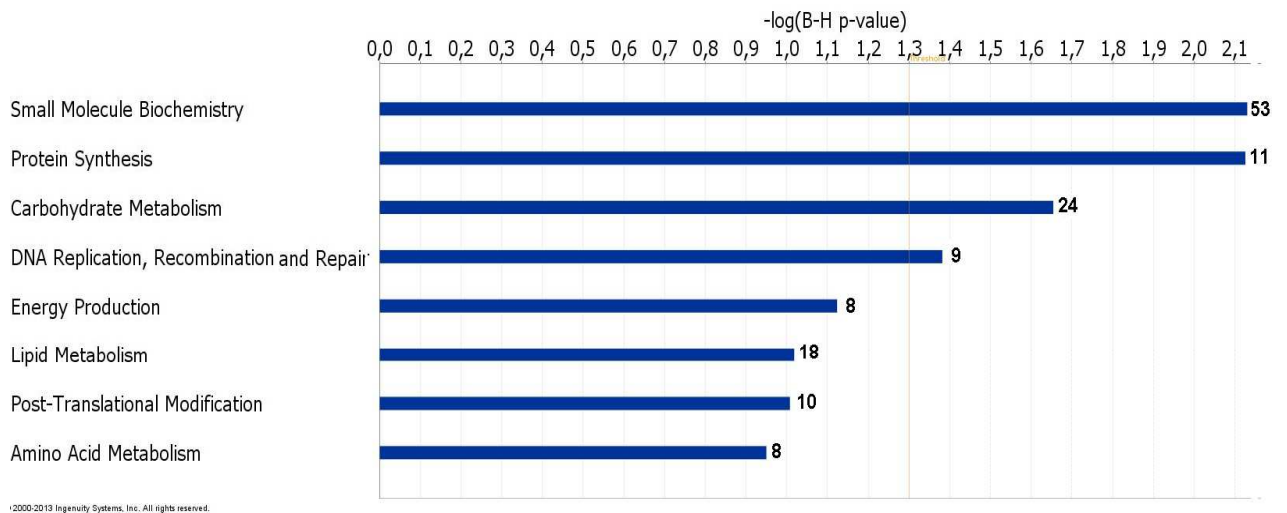
Figure 2. Functional grouping of DEGs in liver from boars with high and low skatole using Ingenuity Pathways Analysis (IPA) software. The most significant functional groups ($p < 0.05$) are presented graphically. The bars represent the p-value on a logarithmic scale for each functional group.
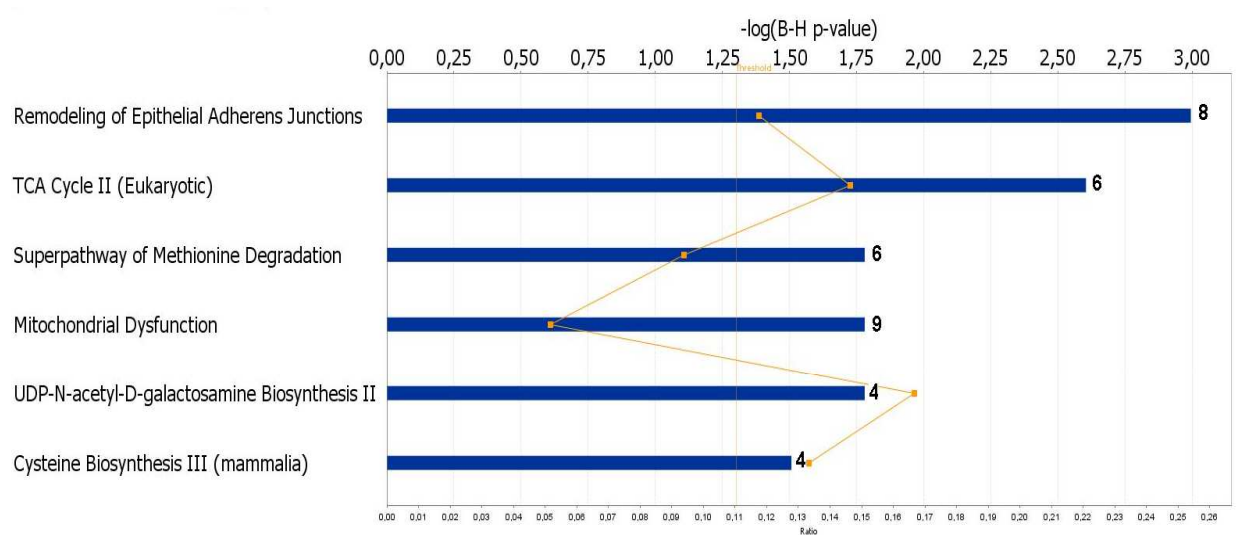
Figure 3. Canonical pathways of DEGs in liver from boars with high and low skatole using Ingenuity Pathways Analysis software. The most significant functional groups ($p < 0.05$) are presented graphically. The bars represent the p-value on a logarithmic scale for each functional group.
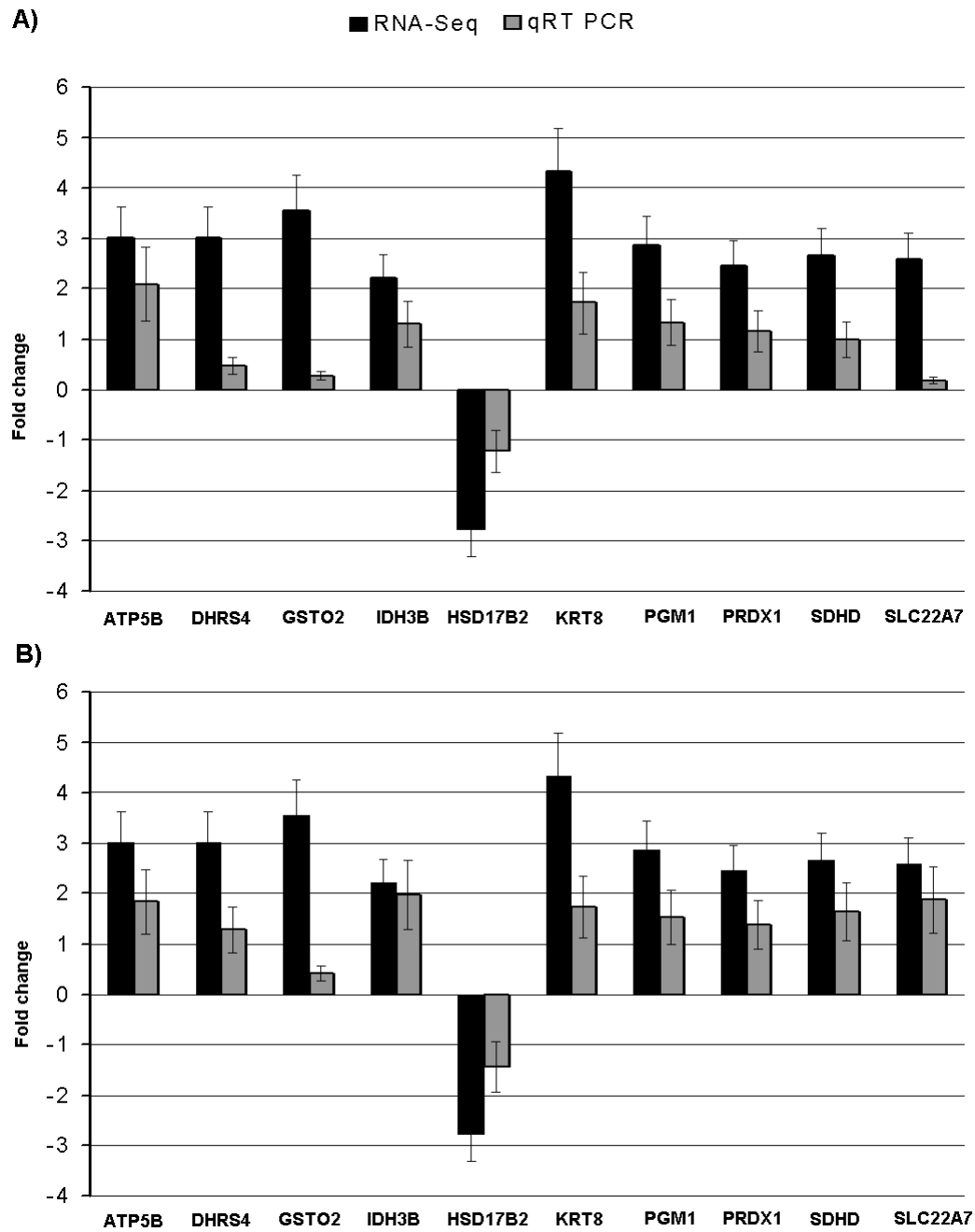
Figure 4. qRT-PCR validations for ten DEGs in liver from boars with divergent skatole levels. The validation was performed using the same RNA samples as used in the RNA deep sequencing (A); new group of boars with divergent skatole levels were created from the remaining 94 boars for the validation of the same DEGs using qRT-PCR (B). Fold change determined via division of high skatole group gene expression value by low skatole group gene expression value
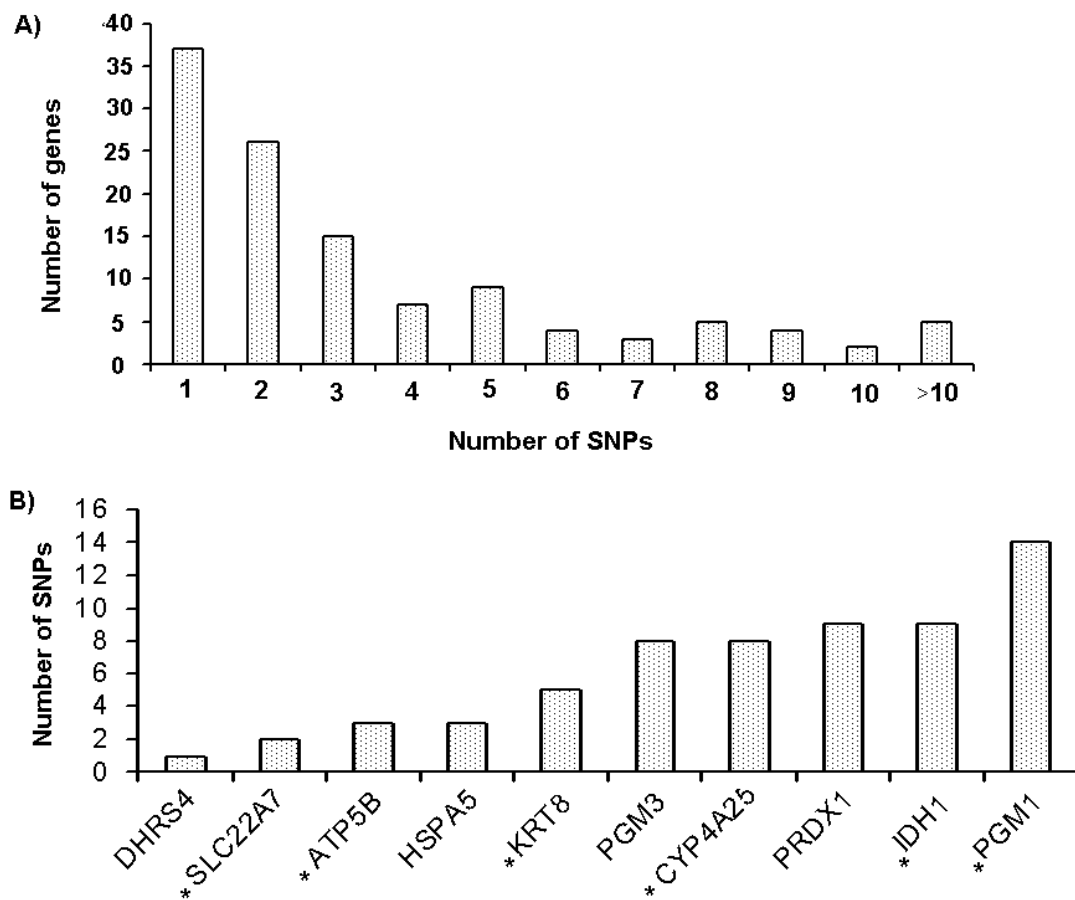
Figure 5. Distribution of the number of SNPs detected in the DEGs. The distribution of the number of SNPs occurred in each gene (A); numbers of SNPs in the genes selected for the association validation (B). *indicate the genes selected for the SNPs validation.

A)

B)
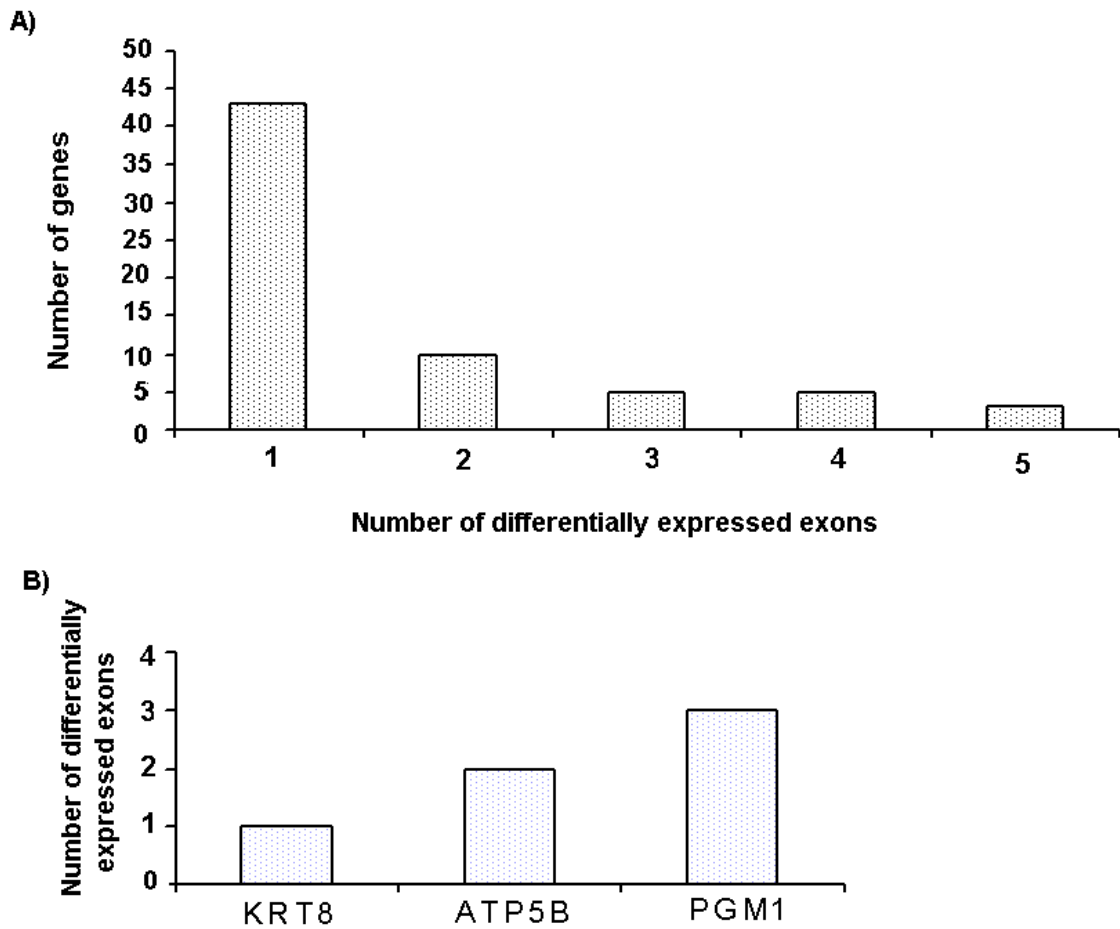
Figure 6. Distribution of the number of alternate splicing. The distribution of the number of alternate splicing the DEGs (A); number of alternate splicing in the selected genes (B).
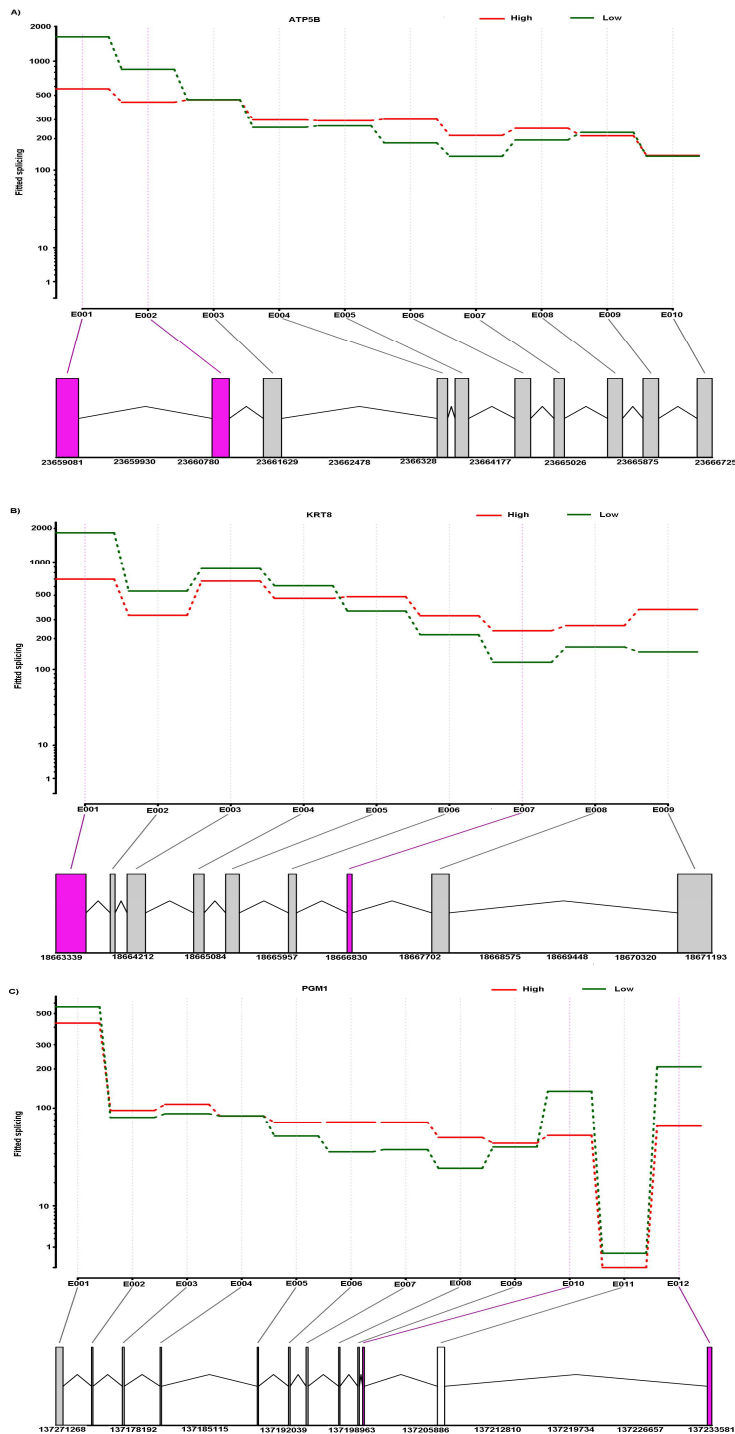
Figure 7. The schematic diagram of differential exon expression in selected genes. Differential exon expression in ATP5B (A). KRT8 (B) and PGM1(C). (Top panel) Fitted values according to the linear model; (middle panel) normalized counts for each sample; (bottom panel) flattened gene model. (Red) Data for high skatole samples; (green) low skatole.

Supplementary Table S1. Differentially expressed genes in liver from boars with high and low skatole in backfat

mRNA ID	Gene	Base Mean	Base Mean A	Base Mean B	Fold Change	log2FoldChange	Padj
XM_003128704.2	SERPINA12	50.30409124	1.75358794	98.85459453	56.3727612	5.81692633	4.73796879706205e-06
XM_003132142.1	LOC100524900	23.66859076	0.82277588	46.51440563	56.5335063	5.811034272	4.97553325849938e-05
XM_003480851.1	LOC100737815	4.69631103	0.3789088	9.013713263	23.7886091	4.572199013	0.002722114
NM_001159615.1	KRT8	15440.76234	1466.02242	29415.50226	20.0648379	4.326597597	6.03742613029937e-05
XM_003131367.3	LOC100514791	10.3080425	1.19064522	19.42543979	16.315053	4.028131769	2.23472642192613e-05
XM_003133834.2	LOC100517616	1173.765058	137.610911	2209.919204	16.0591859	4.005326854	8.94527949952251e-08
XM_003360048.2	LOC100626091	11.44312817	1.35865994	21.52759641	15.8447275	3.985930939	0.000604887
NM_001099923.1	MYD88	55.70072413	6.78206168	104.6193866	15.4258972	3.9472825	0.002029297
NM_001142839.1	TNFRSF12A	70.47639345	9.76439299	131.1883939	13.4353865	3.747965921	2.05937388432579e-09
NM_001243907.1	HSPA8	14297.12289	2036.90013	26557.34564	13.0381187	3.704663808	0.004697468
XM_003129961.3	HSP71	13379.24115	1915.97508	24842.50723	12.9659866	3.696660081	0.004110936
XM_001927288.3	GSTO2	42.52246797	6.64110564	78.4038303	11.80584	3.561428784	2.55955869912397e-07
NM_213788.1	LEAP2	930.5066429	145.66963	1715.343656	11.775575	3.557725607	7.07975240298195e-05
XM_003481948.1	LOC100738870	2595.573741	417.368175	4773.779306	11.4378134	3.51573937	0.001914074
XM_003483155.1	CCBP2	18.87312861	3.05813053	34.6881267	11.3429189	3.503720041	3.78052729156085e-05
XM_001929262.4	MTFP1	303.4042489	49.762538	557.0459598	11.1940826	3.484664392	8.15123893579265e-13
XM_003359343.1	NPM3	104.1690742	17.2061956	191.1319528	11.1083215	3.473568937	6.2756082168337e-11
XM_001929558.1	CDKN1A	506.1766976	85.4736105	926.8797848	10.8440462	3.438831265	1.95846638540283e-12
XM_003354697.2	LOC100622812	175.320308	31.2360664	319.4045495	10.2255049	3.354100171	0.004495865
NM_001160427.1	ARF1	680.0606281	122.646049	1237.475207	10.089809	3.334826953	0.000108016
NM_214134.1	EPO	7.108407193	1.34762047	12.86919392	9.54956845	3.255435538	0.003607168
NM_001048072.1	ARF4	9625.655521	1834.49694	17416.8141	9.49405457	3.247024342	0.000792426
NM_213973.1	HSP90AA1	4119.238936	805.880265	7432.597607	9.22295524	3.205229096	0.002722347
XM_003131015.3	LOC100519729	94.14417579	18.5536175	169.7347341	9.14833638	3.193509414	2.05937388432579e-09
NM_214201.1	GPX1	11981.00124	2415.78628	21546.21619	8.91892479	3.156869799	7.75830699089813e-05
XM_003480660.1	ALDH3B1	62.20921065	12.6053712	111.8130501	8.87027034	3.148978074	4.06930678771622e-08
NM_213951.1	NST	64.50859842	13.1189778	115.8982191	8.83439405	3.143131183	5.45971093055574e-06
NM_214397.1	DLST	1138.740188	236.625446	2040.85493	8.6248329	3.108496505	3.30987326043021e-10
XM_003480842.1	LOC100738590	40.24051509	8.43737441	72.04365577	8.53863445	3.094005364	0.000144706

XM_003356844.1	LOC100621287	4410.621095	925.555903	7895.686288	8.53075029	3.092672634	3.13982785811148e-05
NM_001113013.1	PLA2G7	855.2440566	180.761388	1529.726726	8.46268524	3.081115508	5.19334683880729e-11
XM_003353686.1	ASS1	33926.28993	7213.53821	60639.04165	8.40628272	3.071467979	0.001045575
XM_003133742.3	UGT1A3	69.22216804	14.8409244	123.6034117	8.3285521	3.058065708	3.55535893730073e-07
NM_214019.1	DHRS4	2614.789642	574.182138	4655.397146	8.10787525	3.019323891	0.003910339
XM_003481158.1	LOC100736718	14.00504784	3.11224788	24.8978478	7.99995654	2.999992163	0.002411285
XM_003353565.2	LOC100516776	222.954015	50.3486396	395.5593905	7.85640672	2.973869618	0.000769641
XM_003125293.3	SLC5A6	6184.145492	1403.46076	10964.83022	7.81270878	2.965822839	0.003597708
XM_003357720.1	NOL6	34.50324374	7.96166743	61.04482006	7.66734112	2.938726366	2.81571838554174e-06
XM_003124444.1	LOC100517757	8853.075487	2043.31463	15662.83634	7.66540606	2.938362218	4.35638063218287e-05
XM_003124443.1	LOC100517757	8853.075487	2043.31463	15662.83634	7.66540606	2.938362218	4.35638063218287e-05
XM_003354001.2	LOC100623769	505.6851145	116.881282	894.4889473	7.65297004	2.936019752	1.55400052115556e-07
XM_003357928.1	ACTB	27966.56029	6470.84252	49462.27806	7.64386985	2.934303215	0.001649851
NM_001078666.1	PSEN2	986.3888291	228.414995	1744.362663	7.63681325	2.932970745	0.00229042
XM_001927658.3	SNCG	131.4941118	30.6607184	232.3275053	7.57736665	2.921696559	5.74439163913376e-09
NM_001184895.1	PMM1	917.2948967	216.635361	1617.954433	7.46856112	2.900830323	1.56430734397639e-09
XM_003126864.2	LDHD	148.6063996	35.1751885	262.0376106	7.44950125	2.897143839	5.05985965442840e-09
XM_003358038.1	NKIRAS2	17.4901144	4.14571931	30.83450949	7.43767419	2.894851551	5.60450652855208e-05
NM_001112689.1	CIDEC	277.4101058	66.1789648	488.6412467	7.38363388	2.884331018	0.000557335
XM_003122404.2	LOC100515296	339.760283	81.3981151	598.122451	7.34811181	2.87737358	2.59724882477752e-09
XM_003127946.1	PGM1	2889.091307	693.963572	5084.219041	7.3263486	2.873094348	5.98019541797452e-07
XM_003480765.1	LOC100739130	25.86000149	6.25115737	45.4688456	7.27366836	2.862683148	1.49806333030474e-05
NM_001123113.1	FOS	103.516474	25.0318867	182.0010613	7.27076882	2.862107924	3.21849850113168e-08
XM_003356482.2	LOC100621142	892.5206311	217.713155	1567.328107	7.19905101	2.84780674	0.000577849
XM_003360303.2	LOC100620167	147.221884	35.9603506	258.4834173	7.1880116	2.845592737	0.002094373
XM_003128105.1	CTPS	46.21159683	11.2960474	81.12714626	7.18190562	2.844366695	5.71216408250311e-06
XM_003123992.2	LOC100524488	120.0096817	29.3899771	210.6293862	7.16670806	2.841310588	4.66254468163532e-08
XM_003356202.1	GALE	1235.189132	302.778257	2167.600007	7.15903457	2.839765047	0.000881531
XM_003124919.3	LOC100511252	170.9948493	42.4005217	299.5891769	7.06569554	2.820831585	2.81571838554174e-06
XM_003128016.3	CYP4A25	612.5068439	152.451621	1072.562067	7.03542581	2.814637744	2.81342324407347e-08
XM_003482120.1	LOC100739771	655.7938802	166.222611	1145.36515	6.89054964	2.784619067	0.000207247
XM_003354500.1	TBL2	216.0569119	54.9584308	377.155393	6.86255753	2.778746338	5.64505869284559e-06
XM_001925349.4	SNRPA1	265.1415152	67.6242888	462.6587416	6.84160602	2.774335028	6.88418792773547e-09

XM_003481733.1	LOC100738422	3312.118096	847.890644	5776.345548	6.81260678	2.768206937	8.45535938048703e-07
XM_003482350.1	LOC100739772	1413.201295	361.91249	2464.4901	6.80962986	2.767576382	0.001108363
XM_003356476.2	CYP4A24	1130.956839	291.436185	1970.477493	6.76126575	2.757293352	2.03435015853509e-05
NM_001100193.1	EIF4A3	435.8003484	112.499198	759.1014989	6.74761699	2.754378085	1.59189414287879e-08
XM_003129868.2	HSPB2	1275.898733	330.06533	2221.732136	6.73118904	2.750861375	8.94527949952251e-08
XM_003354105.1	LOC100515361	160.1541806	41.4962839	278.8120772	6.71896495	2.748239005	2.81342324407347e-08
XM_003123370.2	LOC100515361	160.4799096	41.7182174	279.2416017	6.6935171	2.742764471	2.81342324407347e-08
XM_001929410.2	ATP5B	8050.69828	2099.23813	14002.15843	6.67011439	2.737711503	0.000127623
XM_003129127.1	LOC100514171	4433.292164	1160.95597	7705.62836	6.63731319	2.730599351	0.00015804
NM_001044578.1	GRN	3451.930942	905.032186	5998.829698	6.62830537	2.72864007	1.3651406967614e-06
NM_213785.1	F3	151.9372777	40.174928	263.6996275	6.56378594	2.714528191	8.91918065558024e-06
XM_003355375.1	TUBA1A	1917.031101	506.92995	3327.132252	6.56329785	2.714420905	1.50254556152261e-07
XM_001927440.2	SLC25A5	894.9173988	236.98885	1552.845947	6.55240086	2.71202362	0.00104925
NM_001112688.1	CIDE-B	5047.110374	1339.53654	8754.684206	6.53560686	2.708321202	0.001135041
NM_214130.1	CYSLT2	18.3614432	4.87667676	31.84620963	6.53030972	2.707151417	0.000604887
XM_003358419.1	C3orf75	314.5531224	83.9595397	545.1467051	6.4929692	2.698878366	7.37497481876936e-05
XM_003132411.3	LOC100518399	211.1749391	57.2909807	365.0588974	6.37201341	2.671749303	4.61973870154321e-08
XM_003359985.2	LOC100513730	183.9982382	50.2239013	317.7725751	6.3271185	2.661548616	0.000160569
NM_001185142.1	ATP5A1	4659.54597	1272.7484	8046.343543	6.32202214	2.660386087	2.88650937546321e-05
NM_001097516.1	SDHD	1108.217701	303.670645	1912.764756	6.29881349	2.655080093	3.34053913132158e-07
NM_001103211.1	SNCG	128.0294455	35.1250582	220.9338327	6.28992076	2.653041841	0.001246305
XM_003483218.1	SEC13	549.6223606	150.90401	948.3407109	6.284397	2.651774323	8.4292654438711e-05
NM_214439.1	HYAL3	13.01916719	3.59401458	22.44431979	6.24491618	2.642682207	0.001617854
XM_003127002.1	UQCRFS1	1281.382604	357.14991	2205.615297	6.17560087	2.626579514	4.62591913600158e-06
XM_003357294.1	CRYAB	3118.797186	871.619967	5365.974405	6.15632341	2.622069022	3.21422399781268e-06
NM_001243369.1	RANGRF	67.48357416	18.9329234	116.0342249	6.12870092	2.615581303	7.31721047063858e-06
XM_001925061.1	HAL	635.095771	179.236493	1090.955049	6.08667929	2.605655351	0.003607168
NM_001005728.1	HIG2	51.84642804	14.6519107	89.04094539	6.07708764	2.603380099	0.003042537
XM_003127945.2	PGM1	3228.575831	913.740796	5543.410866	6.06672143	2.600917067	6.22373541181366e-06
NM_001044617.1	SLC22A7	569.5841669	161.502267	977.6660672	6.0535749	2.597787369	2.81342324407347e-08
XM_003358202.1	TAX1BP3	387.7822338	110.044817	665.5196509	6.04771465	2.596390071	2.81342324407347e-08
XM_003131848.1	TAX1BP3	388.2613524	110.358767	666.1639375	6.03634812	2.593676009	2.81342324407347e-08
XM_003482090.1	CYP4B24	1163.704144	332.564115	1994.844173	5.9983747	2.584571646	2.35412495059425e-06

XM_003481570.1	TUBA1A	1267.983774	362.596845	2173.370703	5.99390407	2.583495993	6.4283983097165e-07
XM_003481571.1	TUBA1A	1262.463037	361.39516	2163.530913	5.98660732	2.581738643	6.4283983097165e-07
XM_003481798.1	LOC100738166	33.46933373	9.59617973	57.34248772	5.97555374	2.579072411	2.47465861280339e-05
NM_001243370.1	RANGRF	48.12554645	13.8722127	82.37888021	5.93840954	2.570076592	2.77982755151096e-05
XM_003131408.1	NKIRAS2	20.17885788	5.87224858	34.48546719	5.87261706	2.554003567	0.000292286
NM_001122986.2	C22orf28	781.8244564	227.563498	1336.085415	5.87126419	2.553671176	0.000320015
XM_003123337.1	FARSA	1003.745241	292.476853	1715.013629	5.86375849	2.551825686	0.000187814
NM_001184755.1	FTSJ1	157.5628746	46.0879429	269.0378063	5.83748783	2.545347638	1.39754741039536e-06
XM_003126320.1	LOC100518554	243.2711813	71.1636778	415.3786849	5.83694798	2.54521421	0.000182529
XM_003121089.1	BRP44L	3797.508447	1111.1086	6483.908289	5.83553062	2.544863846	0.000793387
XM_003482000.1	PAQR7	279.008698	82.0475636	475.9698324	5.80114523	2.536337737	0.000669352
NM_001243707.1	CNDP2	1726.022079	509.215038	2942.82912	5.7791481	2.530856841	1.3651406967614e-06
NM_001164510.1	SLC25A25	180.6075215	53.4205182	307.7945249	5.76172855	2.526501694	1.35125204900616e-05
NM_213739.1	PAQR7	286.0334203	85.2784901	486.7883506	5.70821962	2.513040843	0.000884867
XM_003132181.1	NME6	139.0810062	41.5302838	236.6317287	5.69781151	2.510407896	0.00013931
XM_003132179.1	NME6	139.5332604	41.687259	237.3792617	5.69428807	2.50951548	0.000114841
XM_003132178.1	NME6	139.5332604	41.687259	237.3792617	5.69428807	2.50951548	0.000114841
XM_003132177.1	NME6	139.4258792	41.687259	237.1644995	5.68913632	2.508209652	0.00011495
XM_003132180.1	NME6	138.32211	41.3733085	235.2709114	5.6865385	2.507550724	0.000171191
XM_003133679.3	FARSB	295.8768123	88.5494358	503.2041888	5.68274867	2.506588911	4.56577346335614e-07
XM_003480931.1	IK	306.5033582	91.870791	521.1359253	5.67248763	2.503981558	1.00533786117287e-06
XM_003359369.1	LOC100627133	3503.750532	1053.62488	5953.876183	5.65085003	2.498467901	2.99168119293791e-05
XM_003481099.1	LOC100737559	1598.386716	482.157991	2714.61544	5.63013678	2.493169973	5.64505869284559e-06
XM_003481098.1	LOC100737559	1598.386716	482.157991	2714.61544	5.63013678	2.493169973	5.64505869284559e-06
XM_003483237.1	LOC100739335	295.8643238	89.3551125	502.3735351	5.62221367	2.491138284	2.69952312971916e-07
XM_001928354.2	LOC100155360	31.47961907	9.50914251	53.45009564	5.62091646	2.490805373	3.18125201495179e-05
XM_003480518.1	LOC100738731	62.32214256	18.9281422	105.7161429	5.58513044	2.481590974	0.002263538
NM_001244149.1	COPS3	579.4489754	176.391137	982.5068134	5.57004637	2.477689337	3.55535893730073e-07
NM_214233.1	GLRX	4946.001856	1512.49652	8379.507195	5.5401828	2.46993358	0.002295275
XM_003482239.1	COX5A	1803.369	552.12023	3054.61777	5.53252282	2.467937497	0.000315384
XM_003482240.1	COX5A	1803.369	552.12023	3054.61777	5.53252282	2.467937497	0.000315384
XM_003128040.1	PRDX1	3591.147732	1101.11751	6081.177957	5.52273297	2.465382374	8.22523689135965e-05
XM_003128038.1	PRDX1	3591.226219	1101.27448	6081.177957	5.52194576	2.465176718	8.22523689135965e-05

XM_003128042.1	PRDX1	3597.071329	1104.80374	6089.338921	5.51169291	2.462495509	8.4292654438711e-05
XM_001926778.1	CRIP3	25.72228106	7.91272835	43.53183377	5.50149478	2.45982366	0.001207773
XM_003122500.3	BCAP31	2438.258714	750.418679	4126.098749	5.49839558	2.459010704	0.000415286
XM_003126114.3	LOC100513426	621.4054181	191.701446	1051.10939	5.48305405	2.454979694	6.24733800806721e-07
XM_003483502.1	SEC24C	501.9399824	155.91876	847.9612048	5.43848094	2.443203739	5.75617470433273e-07
XM_001928193.4	SEC24C	501.9399824	155.91876	847.9612048	5.43848094	2.443203739	5.75617470433273e-07
XM_003128041.1	PRDX1	3611.204787	1125.48606	6096.923513	5.41714706	2.437533257	0.000108016
XM_003128039.1	PRDX1	3611.204787	1125.48606	6096.923513	5.41714706	2.437533257	0.000108016
NM_001048070.1	HN1L	76.68748457	23.9455764	129.4293927	5.40514834	2.434334211	0.002230295
XM_003135207.2	LOC100518105	240.0380002	75.5115229	404.5644775	5.35765221	2.421600933	0.004685919
XM_003355743.1	KARS	2166.611028	681.859536	3651.36252	5.3550069	2.420888433	3.13982785811148e-05
XM_003126856.2	KARS	2166.611028	681.859536	3651.36252	5.3550069	2.420888433	3.13982785811148e-05
NM_001244377.1	PTPMT1	117.3451089	37.4305494	197.2596684	5.27001798	2.397807883	0.000819478
XM_003130678.1	NOL6	75.38433612	24.1831321	126.5855401	5.23445596	2.388039599	0.000441143
XM_003360301.2	LOC100628142	52.30401104	16.7893872	87.8186349	5.23060395	2.386977537	0.000181736
NM_213927.1	GOT1	2553.330077	819.84623	4286.813925	5.22880239	2.386480548	3.22190132255362e-06
NM_001195321.1	ACSL5	4443.208047	1428.77643	7457.639659	5.21959873	2.3839389	0.0001684
XM_003482162.1	LOC100738270	55.9328532	18.0453877	93.82031866	5.19913011	2.378270258	0.000404777
XM_003358153.1	NLE1	137.3012389	44.4965958	230.1058819	5.17131429	2.370530988	0.000351185
NM_001139476.1	DAZAP2	800.4222192	260.724963	1340.119476	5.13997379	2.361761003	4.79729788613173e-05
XM_003124533.3	LCMT1	482.3534748	157.48567	807.2212798	5.12568084	2.35774365	1.24416318541857e-06
XM_003481031.1	LCMT1	478.9183008	156.413902	801.4226999	5.12373064	2.357194633	1.17220987113849e-06
NM_001110419.1	GNMT	9849.933852	3219.01682	16480.85088	5.11983994	2.356098708	0.000520288
XM_003130748.2	LOC100513228	247.1371092	81.1018594	413.172359	5.09448689	2.348936847	0.000247172
XM_003124605.1	LOC100521260	252.7496297	82.9699403	422.5293192	5.09255904	2.3483908	0.000457126
XM_003124606.1	LOC100521260	252.7496297	82.9699403	422.5293192	5.09255904	2.3483908	0.000457126
NM_001144843.1	ADAMTS1	452.5259581	149.075155	755.9767608	5.07111167	2.342302045	4.73796879706205e-06
XM_003481750.1	LOC100737277	218.124114	71.9717369	364.2764911	5.06138252	2.339531513	0.000830893
NM_001244153.1	MDH2	2802.086973	925.230184	4678.943761	5.05705914	2.338298651	5.91012173182443e-05
NM_001128487.1	GPR39	113.5744564	37.5687969	189.5801159	5.04621206	2.335200834	0.000320015
XM_003354272.1	P4HA2	711.2337531	235.620113	1186.847393	5.03712259	2.332599841	1.28480020276829e-06
XM_001928658.2	PIGH	78.39704751	25.9748182	130.8192768	5.03638854	2.332389586	4.67674184811527e-05
NM_001123083.1	BCKDHA	1996.192434	661.452298	3330.93257	5.03578653	2.332217127	0.001189084

XM_001925833.4	IPO4	174.6437043	58.2309581	291.0564504	4.9983112	2.321440729	3.69436927041584e-07
XM_003122803.3	KBTBD4	58.33184	19.4748672	97.18881285	4.99047373	2.319176771	0.0040014
NM_001244246.1	NOP2	256.6347782	85.7134374	427.556119	4.98820409	2.318520494	4.73796879706205e-06
NM_001128435.1	LBP	4198.640183	1402.39016	6994.890208	4.98783464	2.318413636	0.001310993
XM_003131543.3	CDK5RAP3	379.65548	127.453225	631.8577345	4.95756567	2.309631882	5.64505869284559e-06
NM_001243644.1	AP2M1	1791.535422	603.560994	2979.50985	4.93655137	2.30350354	3.13982785811148e-05
NM_001244543.1	DDX56	904.0205031	305.35779	1502.683216	4.92105741	2.298968347	3.13982785811148e-05
NM_213874.1	MDH1	433.2941207	146.430132	720.1581093	4.91810052	2.298101223	5.89353192654019e-06
XM_003124042.3	KIAA0141	152.4938531	51.6608705	253.3268357	4.90365016	2.293856056	4.73796879706205e-06
XM_003358455.1	RRP9	236.0546362	80.0262557	392.0830167	4.89942973	2.292613837	2.25058339570664e-07
XM_003483938.1	LOC100739269	57.15327454	19.4082333	94.8983158	4.88959064	2.289713686	1.68470131276693e-05
XM_003131905.2	OMC	319.572903	109.052859	530.0929471	4.86088079	2.281217754	8.69142221986312e-07
NM_214364.1	VDAC1P5	456.8549141	156.035921	757.673907	4.85576591	2.279698873	0.000842303
NM_001100196.1	EIF4A1	2001.384479	686.90954	3315.859418	4.8272141	2.271190815	0.000361598
XM_001927577.2	ABHD4	74.93486534	25.7576659	124.1120648	4.81845154	2.268569595	3.19425978828833e-05
NM_001244123.1	DHDDS	49.98388431	17.2444923	82.72327635	4.79708391	2.262157675	0.000400787
XM_003481817.1	LOC100737013	3741.185008	1292.42914	6189.940878	4.78938511	2.259840447	0.000566164
XM_001927253.2	DRG1	925.4478418	319.730838	1531.164846	4.78891826	2.25969981	1.61640391496567e-05
NM_001134355.1	ARPC1A	674.7986268	234.24823	1115.349024	4.76139788	2.25138519	1.24138714464696e-05
NM_001243574.1	ACAD8	735.6511182	255.51591	1215.786326	4.75816291	2.250404668	0.001529127
XM_003135011.2	LOC100526026	111.4989805	38.8570344	184.1409266	4.73893413	2.244562607	2.52728970063072e-05
NM_001044581.1	TAP1	139.0455904	48.7020079	229.389173	4.71005577	2.235744142	1.1597246982457e-05
XM_003355245.1	LOC100156038	577.0299561	202.289584	951.7703285	4.70498931	2.234191444	0.001615501
XM_003483346.1	LOC100738012	481.2376234	169.051422	793.4238243	4.69338745	2.230629563	1.63464493380355e-05
XM_003355999.1	PRMT1	306.2813265	107.67428	504.8883731	4.68903412	2.229290778	4.73796879706205e-06
XM_003356001.1	PRMT1	306.2813265	107.67428	504.8883731	4.68903412	2.229290778	4.73796879706205e-06
XM_003481898.1	PRMT1	306.2813265	107.67428	504.8883731	4.68903412	2.229290778	4.73796879706205e-06
XM_003131396.3	LOC100521218	30.7716186	10.8251216	50.71811557	4.68522362	2.228117909	0.001050166
NM_001185130.1	PISD	73.26688679	25.7788634	120.7549102	4.68426045	2.227821294	8.59933726046657e-05
XM_003126035.3	LOC100525990	55.39080479	19.5177465	91.26386307	4.67594264	2.225257231	0.000367109
NM_001044575.2	IDH3B	885.2373531	312.527116	1457.947591	4.66502751	2.221885588	5.36756331317697e-06
XM_003123453.2	ILVBL	2810.478801	992.323062	4628.63454	4.66444318	2.22170487	6.17078904772899e-05
NM_001168419.1	ZFP36	323.031556	114.060024	532.0030882	4.66423792	2.22164138	3.12657070518041e-06

XM_003126946.1	LOC100525048	1347.682483	476.737442	2218.627524	4.65377235	2.218400639	5.37374807395186e-06
XM_003353957.1	WDR18	212.7054007	75.4230086	349.9877928	4.6403319	2.214227998	2.4514542434389e-06
XM_003133109.3	OGDHL	70.65272588	25.1467767	116.158675	4.6192272	2.207651507	0.001149292
XM_003355907.1	TTP	335.3342339	119.456566	551.2119023	4.61432907	2.206120892	4.48671254626965e-06
XM_003133369.1	PROSC	164.0144227	58.459547	269.5692984	4.61121087	2.205145641	3.03221985790242e-06
NM_001244509.1	FIBP	866.402193	308.908266	1423.89612	4.60944647	2.204593515	1.47716589377891e-06
XM_003482481.1	ATPCG2	115.5074485	41.1885917	189.8263054	4.60871076	2.204363227	1.40260331760628e-05
NM_001206441.1	TAP2	236.3797589	84.3420624	388.4174554	4.6052639	2.203283832	4.73796879706205e-06
XM_001925433.1	RAN	2083.892016	746.422589	3421.361443	4.58367886	2.19650597	0.000178753
XM_003133583.3	WBSCR22	44.74794187	16.042609	73.4532747	4.57863646	2.194918021	0.000669541
XM_003132358.1	CDK5RAP3	1203.382729	431.4614	1975.304057	4.57817097	2.194771341	0.004169094
XM_003123371.3	LOC100515528	87.52364932	31.504382	143.5429166	4.55628415	2.187857724	0.00052826
XM_003358591.1	LOC100514874	216.1935074	78.0872038	354.299811	4.53723266	2.181812638	2.71392907108177e-05
XM_003135480.2	BCAP31	2210.349531	798.505304	3622.193757	4.53621753	2.181489824	3.926213468607e-05
XM_003483181.1	LOC100620512	351.257323	127.249734	575.2649121	4.52075533	2.176563838	7.18849992270571e-07
XM_003128065.1	MED8	1625.868268	589.136013	2662.600522	4.51950053	2.176163343	1.94905371543923e-05
XM_003133935.1	PELO	262.7052434	95.1953227	430.2151641	4.51928889	2.176095782	2.19365497617756e-05
XM_003132226.1	LOC100517004	118.021396	42.7854029	193.2573891	4.51690007	2.175332996	2.18115712567669e-05
XM_003358715.1	ECE2	210.6474564	76.3929988	344.9019141	4.51483669	2.174673805	0.001408832
NM_001244382.1	GHITM	3510.794321	1274.97137	5746.61727	4.507252	2.172248113	0.000279606
XM_003356342.2	LOC100622916	383.3024494	139.577314	627.0275845	4.49233163	2.167464434	0.00147896
XM_003127972.1	PARS2	62.84026271	22.8833708	102.7971547	4.49222083	2.167428848	3.926213468607e-05
XM_003357966.1	ACO	1536.293005	561.034138	2511.551871	4.47664714	2.162418608	0.000540267
XM_003360390.1	LOC100155098	40.24206181	14.7489074	65.73521623	4.45695498	2.156058388	0.000872945
NM_001044544.1	TUBA1B	2077.428241	761.74949	3393.106993	4.4543607	2.155218389	0.000189992
XM_001927635.2	TRMT12	133.8023218	49.2214756	218.383168	4.43674565	2.14950185	2.71392907108177e-05
XM_003484101.1	LOC100737045	63.68538408	23.4421279	103.9286403	4.43341325	2.148417845	0.000604887
XM_003481568.1	LMBR1L	376.7593928	138.770511	614.748275	4.42996334	2.14729476	0.000344849
XM_003481569.1	LMBR1L	376.7593928	138.770511	614.748275	4.42996334	2.14729476	0.000344849
NM_214407.1	GPX4	1787.25817	659.13735	2915.37899	4.42302198	2.145032412	0.000239301
XM_003483466.1	LOC100738456	244.4168866	90.4223853	398.4113879	4.40611456	2.139507005	0.000400787
XM_003483721.1	IDH1	3907.283324	1447.10916	6367.457484	4.40012242	2.137543664	0.000431831
XM_003131728.2	TMEM98	1446.08842	536.075273	2356.101567	4.39509465	2.135894235	2.19365497617756e-05

XM_003482888.1	NUDT15	254.8457002	94.8112356	414.8801649	4.37585443	2.129564744	2.03435015853509e-05
XM_003123191.2	ECSIT	796.605177	296.364701	1296.845653	4.37584385	2.129561256	5.10260028562715e-06
XM_003481028.1	LOC100626661	447.5519186	166.553018	728.5508193	4.37428771	2.129048115	2.19365497617756e-05
XM_003354569.2	LOC100626661	447.5519186	166.553018	728.5508193	4.37428771	2.129048115	2.19365497617756e-05
XM_003127022.3	PEPD	515.8908433	192.374578	839.4071082	4.36339934	2.125452516	1.82073183727281e-06
NM_001195119.1	PDIA6	2783.979598	1039.00556	4528.953641	4.35893111	2.123974404	0.001483256
XM_003134232.2	LOC100526021	865.4834803	323.234598	1407.732362	4.35514134	2.122719541	0.001128181
XM_001929074.2	LOC100156930	4112.831442	1539.72338	6685.939501	4.34229912	2.118459111	0.000770416
XM_003128658.1	LOC100156930	4115.499485	1540.97899	6690.019983	4.34140896	2.118163331	0.000769641
XM_003128657.1	LOC100156930	4115.499485	1540.97899	6690.019983	4.34140896	2.118163331	0.000769641
XM_003128543.1	DHRS1	1815.820448	681.755322	2949.885574	4.32689776	2.113333032	3.0229928631562e-05
NM_001243528.1	MRPL15	1188.238579	446.136598	1930.340559	4.32679266	2.113297989	6.20989207061921e-05
XM_003354090.2	LOC100624460	114.6762415	43.0711035	186.2813795	4.32497346	2.11269128	0.001013675
XM_003124410.1	WBSCR22	575.7355598	216.578629	934.8924908	4.31664239	2.10990958	2.71972782716511e-05
XM_001927795.4	HSPA5	7891.9142	2973.1511	12810.6773	4.30878784	2.107282062	0.003700641
NM_001123110.1	EFNA1	642.6769159	243.036573	1042.317259	4.28872596	2.100549133	7.23179002276693e-06
XM_003126204.1	COPZ1	975.3178791	369.497521	1581.138237	4.2791579	2.097326916	0.00107871
XM_001927549.2	COPZ1	975.3178791	369.497521	1581.138237	4.2791579	2.097326916	0.00107871
NM_001244383.1	GHITM	2998.177305	1138.11386	4858.240745	4.26867724	2.093789081	0.000350168
XM_003357844.2	NUDT15	257.7160461	97.8695647	417.5625275	4.26652074	2.093060061	3.02812519892427e-05
XM_003128368.2	PPIL1	397.709233	151.212036	644.2064296	4.26028539	2.090950079	1.53567784208824e-05
NM_001101028.1	ACOX1	12244.62206	4656.06339	19833.18073	4.25964577	2.090733461	0.002459615
NM_001123220.1	CREB3	601.1780284	228.824192	973.5318647	4.25449711	2.088988613	5.86620004114608e-05
XM_003122549.2	ARL2	382.1950042	146.241007	618.1490012	4.22691975	2.079606722	1.74648914633058e-05
XM_003353781.1	ARL2	382.1950042	146.241007	618.1490012	4.22691975	2.079606722	1.74648914633058e-05
XM_003127795.1	SF3A3	547.5839189	209.708251	885.4595865	4.22234023	2.078042834	2.60539487001764e-05
XM_003131295.1	TACO1	405.6115212	155.677964	655.5450781	4.21090474	2.074130239	1.09539042649534e-05
XM_003122812.2	ACP2	758.261734	291.262904	1225.260564	4.20671684	2.07269471	0.000187291
XM_003353872.1	ACP2	758.0469718	291.262904	1224.831039	4.20524214	2.072188874	0.000187291
XM_003130436.3	LOC100515848	177.332382	68.1367042	286.5280598	4.205194	2.072172359	2.28553479532208e-05
XM_003356316.1	SF3A3	539.7420754	207.434799	872.0493522	4.20396847	2.071751848	2.50800585110157e-05
NM_001244418.1	DLL4	74.62288697	28.701899	120.5438749	4.19985711	2.070340246	0.000153743
XM_001926349.3	ORMDL1	446.8734034	172.156752	721.5900546	4.19147112	2.067456688	8.61668103605138e-05

XM_001926938.4	C20ORF4	654.4878355	252.250523	1056.725148	4.18918912	2.066671016	3.47253713768994e-05
XM_003132757.3	LOC100510984	588.349099	226.841245	949.8569529	4.18732031	2.066027282	2.06072933796958e-05
XM_003127502.2	SSU72	490.8277298	189.457276	792.1981833	4.18140807	2.063988844	8.24545841572935e-06
XM_001924268.2	EIF2B5	50.25070915	19.4467285	81.0546898	4.1680373	2.05936819	0.000692208
XM_001924196.4	FAM206A	90.78867971	35.1816453	146.3957141	4.16113894	2.05697846	0.000298965
NM_001123198.1	ST7	60.81183905	23.5922493	98.03142882	4.15523877	2.05493138	0.000144939
XM_003354567.2	LOC100626278	215.2765842	83.5180953	347.0350731	4.15520818	2.05492076	1.25352874613787e-05
NM_001243292.1	IMP3	528.1734984	205.185585	851.1614121	4.14825151	2.052503369	0.000249394
NM_001163408.1	SELT	470.0843108	182.660498	757.5081239	4.14708234	2.052096691	7.61865152049325e-05
XM_003481263.1	LOC100739228	55.7572195	21.6706466	89.84379239	4.14587502	2.051676625	0.000131312
XM_001924419.2	PGM3	466.8160816	181.732259	751.899904	4.1374047	2.048726083	9.56214752867952e-06
XM_003483719.1	IDH1	4013.842465	1563.20071	6464.484222	4.13541536	2.048032241	0.001819881
XM_003483720.1	IDH1	4013.842465	1563.20071	6464.484222	4.13541536	2.048032241	0.001819881
NM_001244121.1	DEGS1	494.7464666	192.922542	796.5703917	4.12896484	2.045780135	4.02356441348398e-05
XM_003482652.1	LOC100628005	606.5849005	236.718432	976.4513695	4.12494863	2.044376152	5.59151147663799e-05
NM_001044579.1	ATP6V0E1	718.2701676	280.306326	1156.23401	4.12489446	2.044357206	0.001335387
XM_003125702.1	LOC100519480	676.596903	264.135761	1089.058045	4.12309957	2.043729302	0.002212291
XM_003483555.1	XPNPEP1	647.1302769	252.810202	1041.450352	4.11949495	2.042467475	8.13381202394086e-06
XM_003133180.3	XPNPEP1	647.1302769	252.810202	1041.450352	4.11949495	2.042467475	8.13381202394086e-06
XM_003131141.3	LOC100526132	605.7741213	236.660422	974.8878206	4.11935301	2.042417763	0.000400113
XM_003127991.3	LOC100517534	1053.259701	411.768476	1694.750926	4.11578599	2.041167968	0.000146766
NM_001044607.1	ECH1	1066.706349	417.161635	1716.251064	4.11411529	2.040582225	0.001760363
NM_001001630.1	PDZD11	313.67985	122.720086	504.6396143	4.11211915	2.039882067	0.00097503
XM_003134336.1	LOC100512637	147.5053953	57.7194242	237.2913664	4.11111805	2.039530798	0.000181736
XM_001925378.2	TUBA1C	85.77294101	33.5692119	137.9766701	4.11021475	2.039213775	0.003430425
XM_003134862.1	MRPS24	726.7223856	284.70593	1168.738842	4.10507376	2.037408145	0.003331959
NM_001190189.1	SLC25A1	933.8163651	366.785483	1500.847247	4.09189381	2.032768705	0.002245518
NM_001078670.1	IRF9	222.5307169	87.5203485	357.5410853	4.08523379	2.030418644	4.99092111054383e-05
NM_214405.1	MUT	2266.280826	892.706144	3639.855508	4.07732772	2.02762392	8.4292654438711e-05
XM_003124033.3	INHBE	418.0799591	164.720057	671.4398612	4.07624835	2.027241953	6.75713111063823e-06
NM_001185135.1	ARPC3	412.5320999	162.592921	662.471279	4.0744165	2.026593466	0.000718183
XM_003359592.1	ORMDL1	453.1254276	178.695	727.5558552	4.07149532	2.025558742	0.000133063
XM_001928070.2	SNX12	163.1801725	64.3660178	261.9943273	4.07038273	2.025164454	0.00442418

XM_003481166.1	LOC100737616	63.44464022	25.0316882	101.8575923	4.06914594	2.024726023	6.81077965278831e-05
NM_001044585.1	CTH	1485.653303	586.54319	2384.763417	4.06579338	2.023536899	0.003910339
XM_003354180.1	NHP2	466.7678592	184.510597	749.0251211	4.05952358	2.021310426	0.004596846
XM_003130472.2	LOC100524641	53.39916753	21.1301799	85.66815518	4.05430317	2.019453972	0.000724835
NM_001001643.1	GDI2	5342.894736	2114.68447	8571.105001	4.05313659	2.019038793	0.000641189
NM_001078684.1	GSTM2	584.884318	231.905752	937.8628842	4.04415534	2.015838413	0.001766804
NM_001243468.1	CD5L	7229.526363	2870.60016	11588.45256	4.03694416	2.01326363	0.001427
NM_001244488.1	NUCB1	2917.163494	1158.70003	4675.626959	4.03523504	2.012652709	0.000415286
XM_003481476.1	SEC22B	950.0139355	377.379617	1522.648254	4.03479198	2.012494297	0.000298386
XM_003481815.1	LOC100736819	1102.917604	438.253621	1767.581588	4.03323898	2.011938893	0.000373362
NM_214127.2	SOD2	583.9380751	232.0731	935.8030501	4.03236329	2.011625624	6.20989207061921e-05
NM_001097416.1	MX2	139.8877338	55.62443	224.1510376	4.02972287	2.010680625	0.004036223
NM_001097412.1	HMBS	153.9389013	61.2216203	246.6561822	4.02890647	2.010388314	8.47941184602852e-05
NM_001123140.1	MRPS18B	689.1883668	274.419242	1103.957492	4.02288661	2.008231074	4.05238519983993e-05
XM_003480846.1	ATP5D	4531.244298	1805.30743	7257.181163	4.0199143	2.007164744	0.000553969
XM_003122991.3	ATP5D	4531.723417	1805.62138	7257.82545	4.01957216	2.007041951	0.000554038
XM_003353963.1	POLR2E	2221.952923	885.316368	3558.589477	4.01956815	2.007040511	0.000876102
XM_003128619.2	YIF1A	141.162103	56.3615584	225.9626476	4.00916252	2.003300903	0.000668251
NM_214330.1	GPI	2476.976123	989.194833	3964.757414	4.00806523	2.002905988	0.000246786
NM_001244354.1	SEC61A1	403.9395829	161.785433	646.0937325	3.99352228	1.997661763	8.59933726046657e-05
NM_001143708.1	GK	3615.411675	1449.71673	5781.106623	3.98774913	1.995574654	0.004194892
XM_003129258.1	LOC100517665	759.8357632	305.004057	1214.667469	3.98246332	1.993661074	0.002408926
XM_001925393.2	VPS52	222.2935114	89.4223823	355.1646405	3.97176447	1.98978007	5.64505869284559e-06
NM_001031788.1	RAB11A	483.0959005	194.833129	771.3586724	3.95907348	1.985162843	7.51082392382182e-05
XM_003123476.1	C2H19orf42	1283.768659	519.303816	2048.233501	3.94419112	1.979729462	0.003597708
NM_001243616.1	DPH3	817.577808	331.106846	1304.04877	3.93845306	1.97762908	0.001169107
NM_001123192.1	RPL7L1	915.772973	371.765645	1459.780301	3.92661431	1.973285897	0.003910339
XM_003127209.3	ETHE1	111.6291526	45.3773888	177.8809164	3.9200342	1.970866243	0.000160569
XM_003134980.3	LOC100525970	400.7342225	163.069025	638.3994203	3.91490304	1.968976576	0.003303994
NM_001122994.1	OAZ1	4755.216711	1935.66895	7574.764471	3.91325411	1.968368794	0.001649851
XM_003483189.1	MANF	873.1571037	355.814323	1390.499885	3.9079368	1.966407135	0.000535181
NM_214358.1	SAT1	1833.969968	748.465319	2919.474617	3.90061442	1.963701393	0.000139074
XM_003481340.1	LOC100736636	143.8566792	58.7637365	228.9496218	3.89610388	1.962032143	0.000133236

NM_001244556.1	AIMP2	240.8414825	98.390311	383.292654	3.89563414	1.961858191	0.004010719
NM_001244557.1	AIMP2	240.8414825	98.390311	383.292654	3.89563414	1.961858191	0.004010719
XM_003355598.1	COPS7A	575.2482315	235.413603	915.0828599	3.88712822	1.958704697	1.43658111204624e-05
XM_003126528.2	COPS7A	575.2482315	235.413603	915.0828599	3.88712822	1.958704697	1.43658111204624e-05
XM_003122352.2	TUBB4B	437.8430566	179.66858	696.017533	3.87389677	1.953785511	0.00017525
XM_003134450.3	LOC100157757	358.618652	147.168976	570.0683277	3.87356318	1.95366127	0.000559514
XM_003356648.1	FAH	4873.43792	2002.03973	7744.836114	3.86847275	1.95176411	0.000640324
NM_001243341.1	POLR2H	237.2185652	97.4633113	376.9738191	3.86785359	1.951533187	0.000217773
NM_001185186.1	DNPEP	378.1845179	155.410594	600.9584414	3.86690781	1.95118037	2.27192369372267e-05
NM_001243852.1	STT3A	638.9410154	262.877069	1015.004962	3.86113923	1.949026576	0.000176626
XM_001929375.3	FLAD1	520.6705538	214.337517	827.0035908	3.85841734	1.948009198	0.002245518
NM_001244459.1	SGK1	465.7068146	191.713828	739.6998008	3.85835392	1.947985484	0.000156789
XM_003353758.1	LOC100519350	387.3363791	159.50365	615.1691086	3.85677137	1.947393624	2.07397681446191e-05
XM_003480667.1	DPP3	390.0427099	160.629336	619.4560834	3.85643181	1.947266602	2.07397681446191e-05
XM_003353753.2	DPP3	390.0427099	160.629336	619.4560834	3.85643181	1.947266602	2.07397681446191e-05
XM_003355138.1	FLAD1	520.4557915	214.337517	826.5740663	3.85641338	1.947259705	0.002245518
XM_003481768.1	CSRP2	147.5298184	60.8035334	234.2561034	3.85267254	1.945859568	8.4292654438711e-05
XM_003482948.1	LOC100739290	387.7667076	159.87379	615.6596251	3.8509103	1.945199519	4.72253201732957e-05
XM_003480778.1	LOC100739748	419.5496485	173.140864	665.9584333	3.84633887	1.943485872	0.000442456
XM_003122683.1	LOC100514446	198.7985481	82.0806417	315.5164544	3.84398133	1.942601329	0.000181736
XM_003130612.1	LOC100511035	156.748064	64.7216565	248.7744715	3.84375934	1.94251801	0.000228998
XM_003126889.3	LOC100512637	150.215706	62.1259693	238.3054427	3.83584265	1.939543541	0.000962254
XM_003122940.1	SAAL1	99.72018573	41.3136397	158.1267318	3.82747037	1.936391209	0.001845033
XM_003481864.1	ATP5SL	227.8360638	94.4174988	361.2546288	3.82614063	1.935889903	0.000540014
XM_003481634.1	LOC100514459	824.6966749	342.114366	1307.278983	3.82117535	1.934016463	4.20235967437693e-05
XM_003126297.2	LOC100514459	824.6966749	342.114366	1307.278983	3.82117535	1.934016463	4.20235967437693e-05
XM_003481823.1	LOC100739300	411.2376344	171.309357	651.165912	3.80111118	1.926421225	5.17876842231626e-05
XM_003481415.1	LOC100154856	327.9501254	136.743254	519.1569965	3.79658214	1.924701221	2.52880216336344e-05
XM_001927335.3	LOC100154856	327.9501254	136.743254	519.1569965	3.79658214	1.924701221	2.52880216336344e-05
XM_001927348.1	LOC100154856	327.9501254	136.743254	519.1569965	3.79658214	1.924701221	2.52880216336344e-05
NM_001099932.1	PGK1	1060.202678	442.493517	1677.91184	3.79194672	1.922938693	0.000640324
NM_001011727.1	AHCY	2462.765724	1028.49789	3897.033555	3.78905351	1.921837514	0.000127473
XM_003126322.3	LOC100519091	1027.56719	429.588761	1625.545618	3.78395751	1.919895888	0.000194215

XM_003483067.1	TNFAIP1	280.4875586	117.657985	443.317132	3.76784568	1.913739879	0.000662674
XM_003123324.1	LOC100520362	188.7158714	79.253213	298.1785297	3.76235257	1.91163505	7.34131544578862e-05
XM_003482380.1	OCIAD2	619.7193239	260.387783	979.0508646	3.75997235	1.910722051	8.61668103605138e-05
XM_003482482.1	LOC100739483	119.4873857	50.2428276	188.7319438	3.75639574	1.909349062	0.000150761
XM_001929196.2	LOC100154911	205.7052968	86.6364029	324.7741907	3.74870354	1.906391739	3.926213468607e-05
NM_213966.1	LCTHIO	1097.732274	462.776483	1732.688066	3.74411434	1.904624494	0.004111855
XM_003127577.2	PLOD1	827.0004122	349.673885	1304.326939	3.73012396	1.899223576	0.000717671
NM_001129952.1	POFUT1	217.7978584	92.3056442	343.2900726	3.7190583	1.894937365	6.20989207061921e-05
XM_003128049.2	LOC100514239	522.3748972	221.890929	822.8588654	3.70839344	1.890794313	5.91012173182443e-05
XM_003134287.3	LOC100512320	563.6457726	239.794836	887.4967091	3.70106681	1.887941179	0.003389322
NM_001243376.1	GNPNAT1	824.4457005	350.873195	1298.018206	3.69939404	1.887288975	0.004616099
XM_003128155.1	LOC100522444	200.1997436	85.2898624	315.1096248	3.69457302	1.885407643	0.001214858
XM_003132296.3	PSMD6	347.0730449	148.021169	546.1249205	3.68950551	1.883427471	0.000171191
XM_003126264.3	PA2G4	315.0619259	134.600472	495.5233799	3.68143865	1.880269661	0.001032398
NM_001244404.1	PPP6C	611.2336916	261.180529	961.2868541	3.68054563	1.879919656	0.00039092
XM_003133041.3	LOC100511475	2600.881513	1112.41465	4089.348374	3.67610078	1.87817632	0.001288388
NM_001044612.1	TUBB2A	2338.873175	1000.76081	3676.985542	3.67419019	1.877426306	0.001292399
NM_001185167.1	SSR1	2154.618816	924.537247	3384.700386	3.66096704	1.872224784	0.000712734
XM_003483435.1	LOC100157055	390.8455074	168.020198	613.6708165	3.65236336	1.868830302	0.000187291
XM_003126560.2	NDUFA9	2035.013077	876.157514	3193.868639	3.6453133	1.866042814	0.000391074
NM_001185132.1	LTA4H	623.7957721	269.925	977.666544	3.62199331	1.85678388	0.000474907
NM_001144926.2	PSMB6	1532.327832	665.294354	2399.36131	3.60646576	1.850585728	0.001654512
XM_003353794.1	LOC100623923	827.7303879	360.067253	1295.393523	3.59764325	1.847052135	4.79729788613173e-05
XM_003124487.1	HSD3B7	2163.394617	943.47504	3383.314195	3.58601346	1.842380905	0.001238057
XM_003483983.1	SEC13	522.9735728	827.960442	217.9867034	0.26328154	-1.9253217	0.002245518
XM_003357648.1	ZNF238	111.6024915	176.87354	46.33144305	0.26194672	-1.93265472	0.002915349
XM_003357464.1	HDAC9	98.26029077	155.909175	40.61140702	0.26048119	-1.94074891	0.001785155
XM_003357462.1	HDAC9	98.26029077	155.909175	40.61140702	0.26048119	-1.94074891	0.001785155
XM_003127015.1	CEBPA	83.27639148	132.164866	34.38791746	0.26018955	-1.94236505	0.00095874
XM_003130093.3	GUCY1A2	247.8181834	396.539559	99.0968077	0.24990396	-2.00055431	0.003683824
NM_001243811.1	HOMER1	369.7120166	592.005354	147.418679	0.24901579	-2.00569089	0.004915775
XM_003357463.1	HDAC9	93.00628582	149.332829	36.67974302	0.24562411	-2.02547592	0.001395212
XM_003130194.2	HDAC9	93.00628582	149.332829	36.67974302	0.24562411	-2.02547592	0.001395212

XM_003481471.1	HIST2H2AC	256.9382007	414.46861	99.40779115	0.23984396	-2.05983201	0.000176451
XM_003482246.1	AKAP6	107.6544978	174.523962	40.78503387	0.23369303	-2.09731337	0.000748377
NM_001123104.1	CTSZ	429.2950089	696.00961	162.580408	0.23358931	-2.09795381	0.000871266
NM_001011507.1	LAMP-1	910.2149174	1476.28721	344.1426285	0.2331136	-2.10089491	0.000292573
XM_003354614.1	ATF7IP2	35.71977001	57.9739917	13.46554827	0.23226878	-2.10613285	0.001365778
XM_003483617.1	ODZ1	218.9330101	355.38102	82.48499975	0.232103	-2.10716295	0.000554038
XM_003133100.2	C10ORF116	88.08192539	143.198364	32.96548628	0.23020854	-2.11898675	0.003588321
XM_003133565.2	PLCL1	148.8429268	242.607225	55.07862866	0.22702798	-2.13905797	0.002893005
XM_003357604.1	G0S2	350.7008855	571.824733	129.5770384	0.22660272	-2.14176293	0.003428478
XM_003133075.3	TTC18	134.6693555	219.762817	49.57589366	0.22558818	-2.14823663	0.000457126
XM_003482319.1	LOC100739812	45.47048452	74.313738	16.62723108	0.22374371	-2.160081	0.00255711
XM_003482321.1	LOC100739812	45.47048452	74.313738	16.62723108	0.22374371	-2.160081	0.00255711
XM_003482320.1	LOC100739812	45.47048452	74.313738	16.62723108	0.22374371	-2.160081	0.00255711
XM_003128932.1	LOC100512294	42.36778908	69.3081373	15.42744089	0.22259206	-2.16752598	0.002182296
NM_001244227.1	RGC32	131.0802941	214.705538	47.45504989	0.22102387	-2.17772588	0.003879561
XM_003354284.1	LOC100627041	30.1164589	49.3877275	10.84519035	0.21959282	-2.18709721	0.002467029
XM_003484097.1	LOC100738608	63.3030719	103.812919	22.79322444	0.21956058	-2.18730906	0.004906071
XM_003355206.2	LOC100523785	220.4855936	362.275049	78.69613855	0.2172276	-2.20272069	8.05760234586599e-05
XM_001928557.2	LOC100157763	45.52063661	75.2918844	15.74938881	0.20917777	-2.25719852	0.003591751
XM_003483014.1	COL1A1	191.9950295	318.139991	65.85006822	0.20698457	-2.27240489	0.001845033
XM_003129821.2	GRIA4	34.61516491	57.4270682	11.8032616	0.20553481	-2.28254534	0.001845033
XM_003357269.1	GRIA4	34.61516491	57.4270682	11.8032616	0.20553481	-2.28254534	0.001845033
XM_003127682.1	LOC100516807	534.6807439	894.69482	174.666668	0.19522486	-2.35679134	8.05760234586599e-05
XM_003130220.3	SEMA3E	20.50638178	34.359465	6.65329856	0.19363801	-2.36856589	0.004457218
XM_003127093.3	ZNF829	131.3226068	220.778099	41.86711453	0.18963436	-2.39870767	0.002579961
XM_003359868.2	SFRP1	25.32693592	42.7697807	7.884091105	0.18433789	-2.43957546	0.001754704
XM_003126479.3	GABARAPL1	227.1758815	384.55367	69.79809305	0.18150417	-2.46192543	8.44545446951054e-05
XM_003131192.1	UNC13D	66.24569841	113.09105	19.40034651	0.17154626	-2.54333044	0.000212238
XM_003121294.1	LCMT1	58.29016658	99.6126453	16.96768784	0.17033669	-2.55353891	0.00147896
XM_003480521.1	LOC100621472	130.0359343	223.57381	36.49805909	0.16324837	-2.61485954	8.05760234586599e-05
XM_003131154.2	LOC100512659	27.11326458	47.1519757	7.074553423	0.15003726	-2.73660724	0.001149292
NM_001167649.1	HSD17B2	47.52885683	82.9694156	12.08829809	0.14569583	-2.77896848	0.003296467
XM_003480781.1	LOC100737135	193.8532831	338.607632	49.09893397	0.14500244	-2.78585089	0.000274717

NM_001114289.2	CXCL9	265.4270817	464.049963	66.80420008	0.14395907	-2.79626943	0.000239961
NM_001244476.1	PNOC	70.35342618	123.169092	17.53776083	0.14238768	-2.8121038	0.000852375
XM_003480595.1	CDK5	832.3967089	1458.7003	206.093114	0.14128544	-2.8233153	0.00017525
XM_003122798.2	LOC100525679	417.2663591	731.745624	102.7870937	0.14046834	-2.83168309	0.004192461
XM_001928213.2	PHF7	10.15853861	17.8184745	2.498602762	0.1402254	-2.83418037	0.003453075
XM_003354990.2	LOC100621539	88.26808925	155.568522	20.96765644	0.13478084	-2.89131265	2.6769150660913e-05
NM_001097461.1	SOCS2	40.2911215	71.4303417	9.151901322	0.12812344	-2.96439362	0.000540983
XM_003357489.2	PCLO	34.77690551	61.6822134	7.871597589	0.12761536	-2.97012616	0.000602464
XM_001927912.2	TECTB	19.78586516	35.4563065	4.115423873	0.11607029	-3.10692932	0.004445783
XM_003484312.1	BRD3	59.03665297	105.817787	12.25551881	0.11581719	-3.11007869	0.00015642
XM_003121615.1	LOC100525797	42.59979849	76.6629496	8.53664733	0.11135297	-3.16678802	0.000955938
XM_003358262.2	TMEM102	11.72615855	21.1684766	2.283840545	0.10788875	-3.21238361	0.000814747
XM_003355209.1	HB2F2	534.4120067	964.752889	104.0711244	0.10787335	-3.21258963	5.03662052013401e-06
XM_003354711.1	SLC9A4	667.9329371	1217.60154	118.2643304	0.09712893	-3.36395518	0.000604887
XM_003360323.1	LOC100623679	17.84792016	32.5529509	3.142889415	0.09654699	-3.37262491	0.002061961
XM_003134087.1	GABRG2	679.9293961	1242.03845	117.8203427	0.09486046	-3.39804928	0.000664696
XM_003359825.1	GABRG2	679.9293961	1242.03845	117.8203427	0.09486046	-3.39804928	0.000664696
XM_003482919.1	LOC100737161	21.49578519	39.4894308	3.502139573	0.08868549	-3.49515809	0.00013931
XM_003129119.2	LOC100512296	180.1298457	340.018805	20.24088655	0.05952873	-4.07027015	1.01131728912424e-07
XM_001927727.2	LOC100155734	7.588512867	14.4294928	0.747532976	0.05180591	-4.27073955	0.002071791
XM_003482938.1	LOC100739739	192.7343983	373.981574	11.48722275	0.03071601	-5.02486524	0.001114554
XM_003359731.2	LOC100625674	6.46741245	12.7200627	0.214762218	0.01688374	-5.88822176	0.001258623
XM_003482870.1	LOC100737759	12.06195805	23.9091539	0.214762218	0.00898243	-6.79867906	7.7659254265902e-05

Supplementary Table S2. Polymorphisms in DEGs detected in liver from boars with high and low skatole in backfat

Chr	Position	Ref	Alt	RsId	mRNA_ID	High_skatole_coverage	High_skatole_mean_phred_score	low_skatole_coverage	low_skatole_mean_phred_score	Group
1	3691558	G	C	0	XM_003121089.1	329	48	165	48	low_skatole AND high_skatole
1	3691600	C	T	rs55618867	XM_003121089.1	309	48	185	48	low_skatole AND high_skatole
1	3692078	A	G	0	XM_003121089.1	523	49	311	49	low_skatole AND high_skatole
1	3692305	T	C	0	XM_003121089.1	0	0	342	48	low_skatole
1	3692310	G	A	0	XM_003121089.1	546	49	352	48	low_skatole AND high_skatole
1	3692383	A	G	rs81350031	XM_003121089.1	0	0	330	49	low_skatole
1	93275813	G	A	0	XM_001924419.2	103	50	0	0	high_skatole
1	93279053	T	C	0	XM_001924419.2	121	48	0	0	high_skatole
1	165539177	G	A	0	NM_001243707.1	145	48	0	0	high_skatole
1	165539206	A	G	rs81216666	NM_001243707.1	142	48	0	0	high_skatole
1	165547082	C	T	0	NM_001243707.1	268	49	0	0	high_skatole
1	202681574	ATCT	ATCTTCT	0	NM_001243376.1	167	49	0	0	high_skatole
1	202681690	A	G	0	NM_001243376.1	166	49	0	0	high_skatole
1	202684404	G	A	0	NM_001243376.1	168	48	0	0	high_skatole
1	263613893	T	G	0	XM_003353565.2	133	42	103	42	low_skatole AND high_skatole
1	263613928	TCCC	TCC	0	XM_003353565.2	173	42	146	42	low_skatole AND high_skatole
1	263613962	A	C	0	XM_003353565.2	174	42	147	42	low_skatole AND high_skatole
1	292669754	C	T	0	XM_003480595.1	0	0	124	49	low_skatole
1	299726599	G	A	0	NM_001244404.1	151	47	0	0	high_skatole
1	299755462	G	A	0	XM_001927795.4	513	49	0	0	high_skatole
1	299756546	A	G	0	XM_001927795.4	436	49	0	0	high_skatole
1	299756555	G	A	0	XM_001927795.4	432	49	0	0	high_skatole
1	304460787	C	T	rs81212139	XM_003353686.1	579	48	304	48	low_skatole AND high_skatole

1	304465878	A	C	rs81212141	XM_003353686.1	608	48	359	49	low_skatole AND high_skatole
1	304477489	A	G	0	XM_003353686.1	602	46	390	47	low_skatole AND high_skatole
1	304503924	G	GCAT	0	XM_003353686.1	602	50	0	0	high_skatole
1	314136085	A	G	0	XM_003122352.2	116	50	0	0	high_skatole
2	611111	G	A	0	XM_003122404.2	201	49	0	0	high_skatole
2	612590	T	C	0	XM_003122404.2	210	49	0	0	high_skatole
2	5174965	G	A	0	XM_003122500.3	391	48	133	49	low_skatole AND high_skatole
2	5177369	T	C	0	XM_003122500.3	442	48	255	49	low_skatole AND high_skatole
2	6290116	T	C	0	XM_003353781.1	124	47	0	0	high_skatole
2	6290130	G	A	0	XM_003353781.1	150	48	0	0	high_skatole
2	6290278	C	G	0	XM_003353781.1	163	49	0	0	high_skatole
2	6295073	C	T	0	XM_003353781.1	157	50	0	0	high_skatole
2	6978835	C	A	0	XM_003353794.1	203	48	0	0	high_skatole
2	6978918	C	T	0	XM_003353794.1	203	47	0	0	high_skatole
2	6979563	C	T	0	XM_003353794.1	150	47	0	0	high_skatole
2	11088926	C	T	0	XM_003122683.1	131	49	0	0	high_skatole
2	16565323	G	A	0	XM_003353872.1;XM_003122812.2	178	49	0	0	high_skatole
2	16565924	A	G	0	XM_003353872.1;XM_003122812.2	0	0	159	50	low_skatole
2	60557280	G	A	0	XM_003123476.1	0	0	177	50	low_skatole
2	61999361	GG	GGC	0	XM_003123453.2	249	50	0	0	high_skatole
2	66283929	A	G	0	XM_003123337.1	0	0	149	50	low_skatole
2	66287168	TCC	TCCC	0	XM_003123337.1	0	0	110	50	low_skatole
2	77656001	C	T	0	XM_003353963.1	117	48	0	0	high_skatole
2	77784393	G	T	0	XM_003122991.3	0	0	300	50	low_skatole
2	77784563	T	G	0	XM_003122991.3	496	49	0	0	high_skatole
2	77784722	C	T	0	XM_003122991.3	0	0	519	50	low_skatole
2	139853975	C	T	0	XM_003354272.1	222	48	0	0	high_skatole

2	139854040	A	G	0	XM_003354272.1	196	48	0	0	high_skatole
2	139856979	G	A	0	XM_003354272.1	159	47	0	0	high_skatole
2	139862136	A	G	0	XM_003354272.1	137	49	0	0	high_skatole
2	139865658	A	G	0	XM_003354272.1	134	48	0	0	high_skatole
2	139866532	T	C	0	XM_003354272.1	101	47	0	0	high_skatole
2	139868241	A	G	rs81365336	XM_003354272.1	119	48	0	0	high_skatole
2	139871844	C	T	0	XM_003354272.1	107	47	0	0	high_skatole low_skatole AND
2	142695214	A	G	0	XM_003354284.1	128	48	179	48	high_skatole
2	146849157	G	A	0	XM_003123992.2	128	47	0	0	high_skatole
3	10664883	G	A	0	XM_003124410.1	178	48	0	0	high_skatole
3	10668640	T	C	0	XM_003124410.1	189	48	0	0	high_skatole low_skatole AND
3	10669064	C	A	0	XM_003124410.1	206	48	144	49	high_skatole
3	10669149	TAAA	TAA	0	XM_003124410.1	131	50	0	0	high_skatole low_skatole AND
3	16894034	G	A	0	XM_003124443.1	487	49	105	50	high_skatole low_skatole AND
3	16895991	C	T	0	XM_003124443.1	573	49	231	50	high_skatole low_skatole AND
3	16896510	G	A	0	XM_003124443.1	556	49	232	49	high_skatole low_skatole AND
3	16897187	T	C	0	XM_003124443.1	570	49	318	50	high_skatole low_skatole AND
3	16897520	C	T	rs45433508	XM_003124443.1	567	48	355	49	high_skatole low_skatole AND
3	16897532	G	C	0	XM_003124443.1	565	48	353	49	high_skatole
3	16897845	T	C	0	XM_003124443.1	0	0	354	49	low_skatole low_skatole AND
3	16898996	A	G	0	XM_003124443.1	562	50	371	50	high_skatole
3	17843985	TC	T	0	XM_003124487.1	189	50	0	0	high_skatole
3	22164601	A	G	0	XM_003124533.3	111	47	0	0	high_skatole
3	34623372	C	T	rs196951274	XM_003124605.1	113	49	0	0	high_skatole
3	49194413	A	G	0	XM_003354697.2	102	49	0	0	high_skatole
3	118921141	C	T	0	XM_003125293.3	190	49	0	0	high_skatole
3	118921945	G	A	0	XM_003125293.3	139	49	0	0	high_skatole

3	118922095	T	C		0	XM_003125293.3	123	49	0	0	high_skatole	
3	118922235	C	T		0	XM_003125293.3	189	48	0	0	high_skatole	
3	118923104	G	T		0	XM_003125293.3	213	48	0	0	high_skatole	
3	118924059	T	C		0	XM_003125293.3	219	49	0	0	high_skatole	
3	118924763	T	C		0	XM_003125293.3	257	49	0	0	high_skatole	
3	118927460	T	G		0	XM_003125293.3	404	49	0	0	high_skatole	
3	118927840	G	A		0	XM_003125293.3	457	48	108	49	low_skatole high_skatole	AND
3	118928779	C	T		0	XM_003125293.3	534	49	293	49	high_skatole	
4	84142659	C	T		0	NM_001243528.1	0	0	215	48	low_skatole	
4	84142718	C	T		0	NM_001243528.1	317	49	215	48	low_skatole high_skatole	AND
4	97140658	A	G	rs55618226		XM_001927348.1	110	50	0	0	high_skatole	
4	100739907	G	A		0	NM_001243468.1	292	49	135	49	low_skatole high_skatole	AND
4	100739976	G	A		0	NM_001243468.1	312	47	109	47	low_skatole high_skatole	AND
4	100746277	C	G		0	NM_001243468.1	323	49	0	0	high_skatole	
4	100746367	A	C		0	NM_001243468.1	340	49	0	0	high_skatole	
4	100746403	T	C		0	NM_001243468.1	346	49	0	0	high_skatole	
4	100749464	G	T		0	NM_001243468.1	447	50	155	50	low_skatole high_skatole	AND
4	103565015	T	C		0	XM_001929375.3	136	48	0	0	high_skatole	
4	110850047	T	C		0	XM_003481476.1	182	49	0	0	high_skatole	
4	110850516	G	A		0	XM_003481476.1	176	49	0	0	high_skatole	
4	119097471	C	A		0	XM_003355245.1	128	48	0	0	high_skatole	
4	119097520	A	G	rs80833376		XM_003355245.1	129	48	0	0	high_skatole	
4	119097584	T	C		0	XM_003355245.1	150	48	0	0	high_skatole	
4	119097685	C	T		0	XM_003355245.1	158	48	0	0	high_skatole	
4	119097890	A	G		0	XM_003355245.1	100	49	0	0	high_skatole	
4	119097894	A	G		0	XM_003355245.1	100	49	0	0	high_skatole	
4	119098055	A	G		0	XM_003355245.1	121	48	0	0	high_skatole	
4	119098103	G	A		0	XM_003355245.1	134	48	0	0	high_skatole	
5	4337150	C	T		0	NM_001184895.1	113	48	0	0	high_skatole	

5	4346018	A	G		0	NM_001184895.1	257	47	0	0	high_skatole
5	4346746	T	C		0	NM_001184895.1	248	48	0	0	high_skatole
5	4347560	G	A		0	NM_001184895.1	0	0	150	50	low_skatole
5	15481037	C	T		0	XM_003481568.1	101	49	0	0	high_skatole
5	15481092	C	T		0	XM_003481568.1	103	49	0	0	high_skatole
5	15481160	T	G		0	XM_003481568.1	105	49	0	0	high_skatole
5	15672827	GG	GGCT		0	XM_003355375.1	117	36	0	0	high_skatole
5	18663991	T	C		0	NM_001159615.1	0	0	343	50	low_skatole
5	18664266	T	C		0	NM_001159615.1	515	49	317	48	low_skatole AND high_skatole
5	18664389	A	G		0	NM_001159615.1	513	49	286	49	low_skatole AND high_skatole
5	18667998	G	A		0	NM_001159615.1	328	49	0	0	high_skatole
5	18670859	G	A		0	NM_001159615.1	303	50	0	0	high_skatole
5	20398450	G	A		0	XM_001927549.2	172	49	0	0	high_skatole
5	23659117	A	G		0	XM_001929410.2	175	49	182	50	low_skatole AND high_skatole
5	23661024	T	C		0	XM_001929410.2	532	47	366	47	low_skatole AND high_skatole
5	23661627	C	T	rs80908111		XM_001929410.2	481	48	253	47	high_skatole
5	24761578	C	T		0	XM_003126322.3	105	50	0	0	high_skatole
5	67966033	C	G		0	XM_003126560.2	196	49	0	0	high_skatole
5	67969367	C	T		0	XM_003126560.2	389	49	0	0	high_skatole
5	67972024	C	T		0	XM_003126560.2	407	48	0	0	high_skatole
5	67972057	G	T		0	XM_003126560.2	401	47	264	49	low_skatole AND high_skatole
5	67981621	A	G		0	XM_003126560.2	318	49	0	0	high_skatole
5	67987994	T	C		0	XM_003126560.2	287	50	0	0	high_skatole
5	67988477	C	T		0	XM_003126560.2	249	49	0	0	high_skatole
5	67988489	A	T		0	XM_003126560.2	224	49	0	0	high_skatole
5	85046072	A	G		0	XM_003481733.1	520	50	317	49	low_skatole AND high_skatole
5	85046134	G	A		0	XM_003481733.1	532	49	352	49	low_skatole AND high_skatole
5	91736131	G	C		0	XM_001925061.1	107	47	0	0	high_skatole

Chr	Position	A1	A2	rsID		Transcript					Phenotype
5	91736161	C	T		0	XM_001925061.1	115	48	0	0	high_skatole
5	91746651	A	G		0	XM_001925061.1	184	47	0	0	high_skatole
5	91746654	C	T		0	XM_001925061.1	183	47	0	0	high_skatole
5	91749359	C	G		0	XM_001925061.1	188	50	0	0	high_skatole
6	11898507	G	C		0	XM_003126856.2	141	49	0	0	high_skatole
6	11903140	A	G	rs81214236		XM_003126856.2	248	49	124	49	low_skatole AND high_skatole
6	11903194	C	T	rs81214238		XM_003126856.2	224	49	0	0	high_skatole
6	11907376	A	T		0	XM_003126856.2	260	49	0	0	high_skatole
6	11907863	G	A		0	XM_003126856.2	251	49	0	0	high_skatole
6	11907915	G	A		0	XM_003126856.2	226	48	0	0	high_skatole
6	11907918	G	C		0	XM_003126856.2	232	49	0	0	high_skatole
6	11908169	G	A		0	XM_003126856.2	245	49	0	0	high_skatole
6	11908170	T	C		0	XM_003126856.2	243	49	0	0	high_skatole
6	11910088	C	T	rs81214246		XM_003126856.2	234	50	0	0	high_skatole
6	11911405	A	G	rs81214247		XM_003126856.2	290	48	0	0	high_skatole
6	11911429	C	T	rs81214249		XM_003126856.2	298	48	0	0	high_skatole
6	11911929	C	T		0	XM_003126856.2	301	50	0	0	high_skatole
6	11912333	C	T	rs81214253		XM_003126856.2	305	49	0	0	high_skatole
6	11912360	T	C		0	XM_003126856.2	322	49	0	0	high_skatole
6	11913057	C	T	rs81214255		XM_003126856.2	338	48	0	0	high_skatole
6	11913066	G	A		0	XM_003126856.2	343	48	156	49	low_skatole AND high_skatole
6	11913770	C	T	rs81214261		XM_003126856.2	355	49	170	49	low_skatole AND high_skatole
6	11913869	G	C		0	XM_003126856.2	288	49	0	0	high_skatole
6	11913872	T	C		0	XM_003126856.2	280	49	169	49	low_skatole AND high_skatole
6	11914431	A	G	rs81214264		XM_003126856.2	268	49	166	49	low_skatole AND high_skatole
6	11914497	G	A		0	XM_003126856.2	397	49	295	49	high_skatole
6	11914501	C	T		0	XM_003126856.2	396	49	0	0	high_skatole
6	11914573	C	T		0	XM_003126856.2;XM_003355743.1	235	50	163	49	low_skatole AND high_skatole

			GCAC								
6	25357672	A	GCA	0	XM_003126946.1	170	50	0	0	high_skatole
6	27371307	T	C	0	XM_003481817.1	0	0	123	48	low_skatole
6	27371336	C	T	0	XM_003481817.1	0	0	146	46	low_skatole
6	27371339	G	A	0	XM_003481817.1	225	47	149	47	low_skatole AND high_skatole
6	38934700	T	C	0	XM_003127022.3	114	32	0	0	high_skatole
6	46148740	A	G	0	XM_003127209.3	132	48	0	0	high_skatole
6	50119920	C	T	0	NM_001244488.1	342	50	0	0	high_skatole
6	50128184	A	G	0	NM_001244488.1	251	49	101	49	low_skatole AND high_skatole
6	58158742	C	T	0	XM_003127502.2	156	48	0	0	high_skatole
6	58158753	C	T	0	XM_003127502.2	138	48	0	0	high_skatole
6	63739697	T	C	0	XM_003481948.1	368	47	140	48	low_skatole AND high_skatole
6	63742814	G	A	0	XM_003481948.1	351	49	106	48	low_skatole AND high_skatole
6	63746674	C	T	rs80962342	XM_003481948.1	316	49	117	49	low_skatole AND high_skatole
6	63746779	T	C	0	XM_003481948.1	0	0	167	50	low_skatole
6	63748257	C	T	0	XM_003481948.1	0	0	137	50	low_skatole
6	63748275	A	G	rs81217714	XM_003481948.1	366	49	109	50	low_skatole AND high_skatole
6	63748320	T	C	0	XM_003481948.1	165	48	0	0	high_skatole
6	65926547	C	T	0	XM_003127577.2	112	48	0	0	high_skatole
6	65926580	T	C	0	XM_003127577.2	110	46	0	0	high_skatole
6	65926607	C	T	0	XM_003127577.2	111	47	0	0	high_skatole
6	75419371	T	C	0	XM_003356202.1	265	48	0	0	high_skatole
6	75419446	C	T	0	XM_003356202.1	295	49	0	0	high_skatole
6	75420230	G	A	0	XM_003356202.1	292	49	0	0	high_skatole
6	90304285	G	A	0	XM_003356342.2	173	48	0	0	high_skatole
6	90304417	T	G	0	XM_003356342.2	139	48	0	0	high_skatole
6	90305912	A	G	0	XM_003356342.2	156	48	0	0	high_skatole
6	90306237	A	G	0	XM_003356342.2	146	48	0	0	high_skatole
6	90315646	G	A	0	XM_003356342.2	132	46	0	0	high_skatole

6	90318567	G	T		0	XM_003356342.2	122	46	0	0	high_skatole
6	137171304	G	T		0	XM_003127945.2	134	50	0	0	high_skatole
6	137171323	T	C		0	XM_003127945.2	204	50	0	0	high_skatole
										low_skatole	AND
6	137171408	T	C		0	XM_003127945.2	435	50	254	49	high_skatole
6	137171452	A	G		0	XM_003127945.2	403	50	0	0	high_skatole
6	137171481	C	A		0	XM_003127945.2	0	0	200	49	low_skatole
										low_skatole	AND
6	137171535	T	C		0	XM_003127945.2	389	50	175	49	high_skatole
										low_skatole	AND
6	137171741	A	C		0	XM_003127945.2	383	50	145	50	high_skatole
6	137171813	C	T		0	XM_003127945.2	383	49	0	0	high_skatole
										low_skatole	AND
6	137171857	C	T		0	XM_003127945.2	380	49	123	50	high_skatole
6	137174682	A	G		0	XM_003127945.2	332	47	0	0	high_skatole
6	137174727	C	T		0	XM_003127945.2	294	45	0	0	high_skatole
6	137174784	C	A		0	XM_003127945.2	327	47	0	0	high_skatole
6	137195153	A	G		0	XM_003127945.2	256	49	0	0	high_skatole
6	147870177	T	G		0	XM_003127991.3	250	50	0	0	high_skatole
6	147870526	G	A		0	XM_003127991.3	205	49	0	0	high_skatole
6	152197351	A	C		0	XM_003128016.3	251	49	0	0	high_skatole
6	152198727	T	C		0	XM_003128016.3	210	48	0	0	high_skatole
6	152206224	A	G		0	XM_003128016.3	279	48	0	0	high_skatole
										low_skatole	AND
6	152206341	G	C		0	XM_003128016.3	308	46	188	49	high_skatole
										low_skatole	AND
6	152206385	A	T		0	XM_003128016.3	184	45	172	49	high_skatole
6	152206727	CTT	CT		0	XM_003128016.3	132	48	0	0	high_skatole
6	152206818	C	T		0	XM_003128016.3	0	0	253	49	low_skatole
										low_skatole	AND
6	153255729	C	T	rs81215265		XM_003128039.1	451	48	236	48	high_skatole
										low_skatole	AND
6	153257567	AT	ATT		0	XM_003128039.1	446	49	235	49	high_skatole
6	153257939	C	T	rs81215269		XM_003128039.1	0	0	242	45	low_skatole
										low_skatole	AND
6	153257940	C	G	rs81215270		XM_003128039.1	448	48	242	45	high_skatole

6	153265783	G	A	0	XM_003128039.1	513	48	365	46	low_skatole	AND high_skatole
6	153265829	G	T	rs196949554	XM_003128039.1	475	48	338	46	low_skatole	AND high_skatole
6	153265881	A	G	0	XM_003128039.1	307	49	187	47	low_skatole	AND high_skatole
6	153265900	GGGT	G	0	XM_003128039.1	0	0	126	47	low_skatole	
6	153265905	T	C	0	XM_003128039.1	201	49	0	0	high_skatole	
6	153912274	C	T	0	XM_003128049.2	160	49	0	0	high_skatole	
6	153913158	T	C	0	XM_003128049.2	167	48	0	0	high_skatole	
6	153913607	G	A	0	XM_003128049.2	130	50	0	0	high_skatole	
6	155359585	C	T	0	XM_003128065.1	219	48	145	49	low_skatole	AND high_skatole
7	1825765	T	C	0	XM_003482120.1	197	49	0	0	high_skatole	
7	1825782	T	C	0	XM_003482120.1	237	49	0	0	high_skatole	
7	26841460	G	A	0	NM_001123140.1	188	40	0	0	high_skatole	
7	37263243	A	G	0	XM_001929558.1	112	47	0	0	high_skatole	
7	37426438	C	A	0	XM_003128368.2	104	49	0	0	high_skatole	
7	37427047	A	C	0	XM_003128368.2	135	49	0	0	high_skatole	
7	37427122	G	A	0	XM_003128368.2	135	48	0	0	high_skatole	
7	37427142	A	G	0	XM_003128368.2	132	49	0	0	high_skatole	
7	37427238	T	C	0	XM_003128368.2	109	49	0	0	high_skatole	
7	43389880	A	C	rs80946507	NM_001123192.1	166	45	0	0	high_skatole	
7	43389886	G	A	0	NM_001123192.1	175	45	0	0	high_skatole	
7	43394230	C	T	rs80831622	NM_001123192.1	224	48	0	0	high_skatole	
7	43395437	C	G	0	NM_001123192.1	237	49	0	0	high_skatole	
7	43395573	T	C	0	NM_001123192.1	267	47	155	48	low_skatole	AND high_skatole
7	43486846	G	A	0	NM_001110419.1	463	49	182	48	low_skatole	AND high_skatole
7	43486876	A	G	0	NM_001110419.1	516	47	263	47	low_skatole	AND high_skatole
7	43486921	A	G	0	NM_001110419.1	558	46	362	47	low_skatole	AND high_skatole
7	43488849	A	C	0	NM_001110419.1	604	49	507	49	low_skatole	AND high_skatole

Chr	Position	Ref	Alt	ID	Filter	Transcript					Category
7	43489263	A	G		0	NM_001110419.1	608	48	508	49	low_skatole AND high_skatole
7	43489429	G	A		0	NM_001110419.1	589	47	471	48	low_skatole AND high_skatole
7	43489476	G	A		0	NM_001110419.1	555	46	377	47	low_skatole AND high_skatole
7	43833000	G	A		0	NM_001044617.1	182	48	0	0	high_skatole
7	43833898	G	A		0	NM_001044617.1	197	49	0	0	high_skatole
7	48164570	T	A		0	NM_001113013.1	192	47	0	0	high_skatole
7	48164591	T	G		0	NM_001113013.1	198	47	0	0	high_skatole
7	54563945	C	T		0	XM_003356648.1	0	0	138	48	low_skatole
7	54564644	G	T		0	XM_003356648.1	382	49	167	48	low_skatole AND high_skatole
7	54579630	C	T		0	XM_003356648.1	441	47	211	47	low_skatole AND high_skatole
7	54581678	G	A		0	XM_003356648.1	433	46	221	46	low_skatole AND high_skatole
7	54581683	G	A		0	XM_003356648.1	431	47	0	0	high_skatole
7	54582545	G	A		0	XM_003356648.1	470	48	270	48	low_skatole AND high_skatole
7	54582575	A	T	rs80837741		XM_003356648.1	466	48	0	0	high_skatole
7	54585108	C	T		0	XM_003356648.1	430	48	220	44	low_skatole AND high_skatole
7	54585144	C	T		0	XM_003356648.1	450	48	274	45	low_skatole AND high_skatole
7	54590695	G	T		0	XM_003356648.1	497	49	0	0	high_skatole
7	54596139	T	C		0	XM_003356648.1	0	0	464	50	low_skatole
7	54596190	G	T		0	XM_003356648.1	511	49	474	49	low_skatole AND high_skatole
7	54596259	G	A		0	XM_003356648.1	338	48	320	49	low_skatole AND high_skatole
7	54596291	A	G		0	XM_003356648.1	0	0	182	49	low_skatole
7	54596310	G	A		0	XM_003356648.1	119	48	113	48	low_skatole AND high_skatole
7	63332410	TA	TAA		0	XM_003482240.1	0	0	104	50	low_skatole
7	80515824	T	C		0	NM_214019.1	0	0	162	49	low_skatole
7	102941889	G	T	rs55619011		XM_003128658.1	360	47	167	48	low_skatole AND high_skatole

7	102941936	T	C		0	XM_003128658.1	0	0	134	47	low_skatole	
7	102941942	T	C		0	XM_003128658.1	0	0	124	47	low_skatole	
7	102943214	T	C		0	XM_003128658.1	0	0	282	49	low_skatole	
7	102949897	TA	TAA		0	XM_003128658.1	0	0	334	49	low_skatole	
7	102949951	G	T	rs80859223		XM_003128658.1	0	0	327	49	low_skatole	
7	102950048	T	C		0	XM_003128658.1	0	0	443	49	low_skatole	
7	102950069	AAA	AAAC		0	XM_003128658.1	0	0	423	49	low_skatole	
8	3118494	T	C		0	XM_003356844.1	416	50	0	0	high_skatole	
8	3118494	TC	TCC		0	XM_003356844.1	0	0	118	50	low_skatole	
8	3118581	G	A		0	XM_003356844.1	416	47	158	47	low_skatole high_skatole	AND
8	3118628	A	T	rs55618970		XM_003356844.1	0	0	171	47	low_skatole	
8	3118784	C	T	rs55618971		XM_003356844.1	437	45	180	46	low_skatole high_skatole	AND
8	3118827	A	T	rs55618972		XM_003356844.1	420	46	187	46	low_skatole high_skatole	AND
8	3118871	G	A		0	XM_003356844.1	0	0	223	49	low_skatole	
8	3119149	C	T	rs55618968		XM_003356844.1	444	48	217	49	low_skatole high_skatole	AND
8	3119286	A	G		0	XM_003356844.1	469	49	317	50	low_skatole high_skatole	AND
8	12403657	A	G		0	XM_003482350.1	0	0	130	48	low_skatole	
8	40885091	T	C		0	XM_003482380.1	261	48	113	48	low_skatole high_skatole	AND
8	121675034	C	G		0	XM_003129258.1	145	49	0	0	high_skatole	
8	121675139	T	C		0	XM_003129258.1	199	49	129	48	low_skatole high_skatole	AND
9	44432121	A	T	rs55618913		XM_003357294.1	406	49	165	49	low_skatole high_skatole	AND
9	44432191	T	C		0	XM_003357294.1	357	49	118	49	low_skatole high_skatole	AND
9	44434535	T	A		0	XM_003129868.2	134	47	122	47	high_skatole	
9	67435267	G	A		0	XM_003482652.1	251	48	0	0	high_skatole	
9	67435294	A	G	rs81212178		XM_003482652.1	263	48	0	0	high_skatole	
9	146494183	A	C		0	XM_003357604.1	0	0	138	48	low_skatole	
9	146494231	A	T		0	XM_003357604.1	0	0	182	48	low_skatole	

9	146494262	G	T	rs81418687	XM_003357604.1	0	0	260	48	low_skatole
9	146494391	T	G	0	XM_003357604.1	0	0	228	49	low_skatole
		GAAA								
11	20026042	A	GAA	0	XM_003357844.2	111	50	0	0	high_skatole
11	20030234	A	G	0	XM_003357844.2	140	48	0	0	high_skatole
11	20030396	T	C	0	XM_003357844.2	115	50	0	0	high_skatole
12	1048782	C	T	0	XM_003482938.1	0	0	209	47	low_skatole
12	1163230	T	TGG	0	XM_003357928.1	100	50	0	0	high_skatole
12	1163704	G	A	0	XM_003357928.1	449	49	0	0	high_skatole
12	1163716	G	C	0	XM_003357928.1	468	49	0	0	high_skatole
12	1163923	C	T	0	XM_003357928.1	562	49	0	0	high_skatole
										low_skatole AND
12	1164781	T	C	0	XM_003357928.1	591	47	433	47	high_skatole
12	1165434	TGTA	TGT	0	XM_003357928.1	602	50	0	0	high_skatole
12	1165572	G	C	0	XM_003357928.1	603	50	0	0	high_skatole
12	1165992	AC	ACGC	0	XM_003357928.1	284	49	0	0	high_skatole
12	1166023	G	GCAA	0	XM_003357928.1	101	50	0	0	high_skatole
12	2298517	G	A	0	XM_003131141.3	220	49	0	0	high_skatole
12	2388039	AGG	AGGAG	0	XM_003131141.3	135	49	0	0	high_skatole
12	2388058	G	A	0	XM_003131141.3	121	47	0	0	high_skatole
12	2388062	G	A	0	XM_003131141.3	119	47	0	0	high_skatole
12	2388543	C	T	0	XM_003131141.3	129	50	0	0	high_skatole
12	5357348	G	A	0	XM_003482948.1	118	49	0	0	high_skatole
12	5357410	T	C	0	XM_003482948.1	109	49	0	0	high_skatole
12	5357451	C	T	0	XM_003482948.1	107	49	0	0	high_skatole
12	5357565	G	A	0	XM_003482948.1	161	48	0	0	high_skatole
12	5357598	G	A	0	XM_003482948.1	203	49	0	0	high_skatole
12	5407136	AA	AACTT	0	XM_003357966.1	415	49	0	0	high_skatole
12	15295333	A	G	0	XM_003131295.1	123	47	0	0	high_skatole
12	15299116	G	A	0	XM_003131295.1	114	49	0	0	high_skatole
12	24050251	C	T	0	XM_003131543.3	112	50	0	0	high_skatole
12	24050285	G	A	0	XM_003131543.3	127	50	0	0	high_skatole
12	43758652	G	T	0	XM_003131728.2	0	0	320	49	low_skatole
12	43758772	A	G	0	XM_003131728.2	0	0	185	50	low_skatole

Chr	Position	Ref	Alt	ID	Transcript					Phenotype
12	43758774	G	A	0	XM_003131728.2	0	0	188	50	low_skatole
12	43758879	AC	ACC	0	XM_003131728.2	0	0	126	50	low_skatole
12	43758886	A	G	0	XM_003131728.2	0	0	123	50	low_skatole
12	43761467	C	T	0	XM_003131728.2	262	48	0	0	high_skatole
12	43767008	G	A	0	XM_003131728.2	229	47	0	0	high_skatole
12	46537688	C	T	0	XM_003483067.1	103	47	0	0	high_skatole
12	51614480	G	A	0	XM_003131848.1	131	47	0	0	high_skatole
12	51614658	G	A	0	XM_003131848.1	137	45	0	0	high_skatole
12	54042323	G	A	0	XM_003131905.2	129	50	0	0	high_skatole
12	54317095	G	A	0	NM_001144926.2	153	50	0	0	high_skatole
12	54317130	T	C	0	NM_001144926.2	281	50	206	49	low_skatole AND high_skatole
12	54318953	T	G	0	NM_001144926.2	182	48	0	0	high_skatole
13	3583461	A	C	0	NM_001243616.1	105	47	0	0	high_skatole
13	3583490	C	T	0	NM_001243616.1	134	47	0	0	high_skatole
13	3583545	C	T	0	NM_001243616.1	192	47	102	46	low_skatole AND high_skatole
13	3583558	C	T	0	NM_001243616.1	191	46	0	0	high_skatole
13	3583691	T	A	0	NM_001243616.1	176	48	0	0	high_skatole
13	3583721	C	T	0	NM_001243616.1	200	48	0	0	high_skatole
13	3583749	A	G	0	NM_001243616.1	205	47	101	48	low_skatole AND high_skatole
13	3583781	C	T	0	NM_001243616.1	217	48	0	0	high_skatole
13	3583882	A	C	0	NM_001243616.1	246	50	0	0	high_skatole
13	3583964	A	C	0	NM_001243616.1	230	47	0	0	high_skatole
13	3583967	C	T	0	NM_001243616.1	230	47	0	0	high_skatole
13	3584177	T	A	0	NM_001243616.1	144	49	0	0	high_skatole
13	3584300	G	A	0	NM_001243616.1	191	49	0	0	high_skatole
13	33563223	C	A	0	XM_003358419.1	102	48	0	0	high_skatole
13	36895404	C	A	0	XM_003483189.1	234	50	162	50	low_skatole AND high_skatole
13	36895572	C	G	0	XM_003483189.1	276	49	145	48	low_skatole AND high_skatole
13	49976969	A	G	0	XM_003132296.3	142	47	0	0	high_skatole
13	73233058	A	G	rs196956369	XM_003132358.1	305	50	120	49	low_skatole AND

										high_skatole	
13	73233143	CAG	CA	0	XM_003132358.1	0	0	134	50	low_skatole	
										low_skatole	AND
13	73233329	T	A	0	XM_003132358.1	250	50	203	50	high_skatole	
13	73548157	A	G	0	XM_003483218.1	259	50	0	0	high_skatole	
13	73562335	G	A	0	XM_003483218.1	210	49	0	0	high_skatole	
										low_skatole	AND
13	79727769	A	G	0	XM_003483237.1	254	50	112	49	high_skatole	
13	79737479	C	T	0	XM_003483237.1	166	49	0	0	high_skatole	
13	79737516	G	A	0	XM_003483237.1	140	49	0	0	high_skatole	
14	42952441	G	A	0	XM_003483435.1	104	47	0	0	high_skatole	
14	42952626	A	G	0	XM_003483435.1	120	47	0	0	high_skatole	
										low_skatole	AND
14	42956704	G	C	0	XM_003483435.1	186	47	101	48	high_skatole	
14	50478245	T	A	0	XM_001929262.4	120	48	0	0	high_skatole	
14	50478689	C	T	0	XM_001929262.4	121	49	0	0	high_skatole	
14	82624759	C	A	0	XM_001928193.4	100	49	0	0	high_skatole	
14	131109160	TAA	TAAAA	0	XM_003133180.3	143	50	0	0	high_skatole	
14	131109271	C	G	0	XM_003133180.3	133	49	0	0	high_skatole	
14	131109445	A	G	0	XM_003133180.3	160	48	0	0	high_skatole	
14	131113925	G	A	0	XM_003133180.3	123	48	0	0	high_skatole	
14	131120227	G	A	0	XM_003133180.3	107	49	0	0	high_skatole	
14	133739004	C	T	0	XM_001927912.2	211	43	0	0	high_skatole	
14	133740424	C	T	0	XM_001927912.2	215	41	0	0	high_skatole	
										low_skatole	AND
14	133751226	T	A	0	XM_001927912.2	292	26	173	29	high_skatole	
										low_skatole	AND
14	133751272	TCC	TC	0	XM_001927912.2	436	49	295	49	high_skatole	
										low_skatole	AND
15	122861718	T	G	0	XM_003483721.1	225	49	124	48	high_skatole	
										low_skatole	AND
15	122861896	C	T	0	XM_003483721.1	430	48	235	48	high_skatole	
										low_skatole	AND
15	122861903	CGGG	CGG	0	XM_003483721.1	424	48	233	48	high_skatole	
										low_skatole	AND
15	122861968	C	T	0	XM_003483721.1	441	49	223	49	high_skatole	

15	122862291	T	C		0	XM_003483721.1	374	49	177	49	low_skatole AND high_skatole
15	122862295	GAA	GAAAT		0	XM_003483721.1	375	49	181	49	low_skatole AND high_skatole
15	122862513	C	T		0	XM_003483721.1	406	49	133	48	low_skatole AND high_skatole
15	122862530	C	T		0	XM_003483721.1	397	48	118	48	low_skatole AND high_skatole
15	122876927	G	A		0	XM_003483721.1	292	49	0	0	high_skatole
15	137967693	A	G	rs81211172		XM_003133679.3	127	49	0	0	high_skatole
15	137979323	G	A		0	XM_003133679.3	142	50	0	0	high_skatole
15	157617314	T	C		0	XM_003133834.2	125	48	0	0	high_skatole
15	157617534	C	T		0	XM_003133834.2	139	46	0	0	high_skatole
15	157617616	A	G		0	XM_003133834.2	201	48	0	0	high_skatole
15	157621921	A	G		0	XM_003133834.2	191	43	0	0	high_skatole
15	157621933	A	G		0	XM_003133834.2	194	42	0	0	high_skatole
15	157621986	C	T		0	XM_003133834.2	208	44	0	0	high_skatole
15	157624384	G	A		0	XM_003133834.2	297	44	0	0	high_skatole
15	157625354	T	C		0	XM_003133834.2	158	45	0	0	high_skatole
15	157625392	T	C		0	XM_003133834.2	185	44	0	0	high_skatole
15	157626216	G	A		0	XM_003133834.2	351	41	0	0	high_skatole
15	157626237	C	T		0	XM_003133834.2	369	43	0	0	high_skatole
16	33954843	C	G	rs81216839		XM_003133935.1	107	50	0	0	high_skatole
17	13109675	C	G		0	XM_003134232.2	121	50	0	0	high_skatole
17	13129640	A	G		0	XM_003134232.2	290	47	119	47	low_skatole AND high_skatole
17	31800979	C	T		0	XM_003134287.3	176	49	0	0	high_skatole
17	31801702	T	A		0	XM_003134287.3	173	49	0	0	high_skatole
17	31806249	C	T		0	XM_003134287.3	212	49	0	0	high_skatole
17	31807010	CTA	CTATA		0	XM_003134287.3	212	50	116	50	low_skatole AND high_skatole
17	31807099	C	T		0	XM_003134287.3	235	48	166	49	low_skatole AND high_skatole
17	31807104	G	A		0	XM_003134287.3	233	48	169	49	low_skatole AND high_skatole
17	37464407	A	G		0	XM_003134336.1	140	49	0	0	high_skatole

17	37466400	G	A	0	XM_003134336.1	152	50	0	0	high_skatole
17	37466457	A	G	0	XM_003134336.1	102	48	0	0	high_skatole
17	44907666	G	A	0	XM_001926938.4	127	49	0	0	high_skatole
17	44907733	A	C	0	XM_001926938.4	117	49	0	0	high_skatole
17	44907761	G	A	0	XM_001926938.4	130	50	0	0	high_skatole
17	44907801	A	G	0	XM_001926938.4	137	50	0	0	high_skatole
17	44907863	A	G	0	XM_001926938.4	132	50	0	0	high_skatole
17	45782027	G	A	0	XM_003359985.2	116	48	0	0	high_skatole
17	51649343	G	A	0	XM_003134450.3	119	49	0	0	high_skatole
17	51649560	C	T	0	XM_003134450.3	108	50	0	0	high_skatole
17	66511969	ACC	AC	0	NM_001123104.1	0	0	227	50	low_skatole
18	53538831	T	C	0	XM_003134862.1	236	49	0	0	high_skatole
18	53541435	G	A	0	XM_003134862.1	0	0	215	49	low_skatole
18	53541504	C	G	0	XM_003134862.1	266	49	147	49	low_skatole AND high_skatole
18	53541513	G	A	0	XM_003134862.1	237	49	125	49	low_skatole AND high_skatole

Supplementary Table S3. Sample read counts for polymorphisms in liver from boars with high and low skatole in backfat

Chr	Pos	Ref	Alt	RsId	mRNA_ID	Gene_name	HS 1	HS 2	HS3	LS 1	LS 2	LS 3
1	3691558	G	C	0	XM_003121089.1	BRP44L	121	65	138	78	67	16
1	3691600	C	T	rs55618867	XM_003121089.1	BRP44L	110	73	124	82	83	17
1	3692078	A	G	0	XM_003121089.1	BRP44L	192	124	199	134	141	31
1	3692305	T	C	0	XM_003121089.1	BRP44L	NIL	NIL	NIL	155	147	38
1	3692310	G	A	0	XM_003121089.1	BRP44L	193	145	200	159	151	39
1	3692383	A	G	rs81350031	XM_003121089.1	BRP44L	NIL	NIL	NIL	140	146	44
1	93275813	G	A	0	XM_001924419.2	LOC100156015	23	13	66	NIL	NIL	NIL
1	93279053	T	C	0	XM_001924419.2	LOC100156015	21	11	86	NIL	NIL	NIL
1	165539177	G	A	0	NM_001243707.1	CNDP2	23	9	111	NIL	NIL	NIL
1	165539206	A	G	rs81216666	NM_001243707.1	CNDP2	20	12	109	NIL	NIL	NIL
1	165547082	C	T	0	NM_001243707.1	CNDP2	61	43	162	NIL	NIL	NIL
1	202681574	ATCT	ATCTTCT	0	NM_001243376.1	GNPNAT1	35	20	106	NIL	NIL	NIL
1	202681690	A	G	0	NM_001243376.1	GNPNAT1	31	16	115	NIL	NIL	NIL
1	202684404	G	A	0	NM_001243376.1	GNPNAT1	36	22	108	NIL	NIL	NIL
1	263613893	T	G	0	XM_003353565.2	LOC100516776	42	33	53	37	45	15
1	263613928	TCCC	TCC	0	XM_003353565.2	LOC100516776	58	43	72	54	64	28
1	263613962	A	C	0	XM_003353565.2	LOC100516776	59	42	71	53	64	27
1	292669754	C	T	0	XM_003480595.1	LOC100737979	NIL	NIL	NIL	36	66	18
1	299726599	G	A	0	NM_001244404.1	PPP6C	32	17	99	NIL	NIL	NIL
1	299755462	G	A	0	XM_001927795.4	HSPA5	171	135	204	NIL	NIL	NIL
1	299756546	A	G	0	XM_001927795.4	HSPA5	135	97	200	NIL	NIL	NIL
1	299756555	G	A	0	XM_001927795.4	HSPA5	130	95	200	NIL	NIL	NIL
1	304460787	C	T	rs81212139	XM_003353686.1	ASS1	193	177	207	114	133	54
1	304465878	A	C	rs81212141	XM_003353686.1	ASS1	199	194	208	143	142	67
1	304477489	A	G	0	XM_003353686.1	ASS1	202	192	206	152	150	82
1	304503924	G	GCA	0	XM_003353686.1	ASS1	4	1	1	NIL	NIL	NIL
1	314136085	A	G	0	XM_003122352.2	LOC100516352	11	15	85	NIL	NIL	NIL
2	611111	G	A	0	XM_003122404.2	LOC100515296	34	12	152	NIL	NIL	NIL
2	612590	T	C	0	XM_003122404.2	LOC100515296	41	23	141	NIL	NIL	NIL

2	5174965	G	A	0	XM_003122500.3	LOC100521064	125	75	182	65	52	12
2	5177369	T	C	0	XM_003122500.3	LOC100521064	139	101	194	113	102	35
2	6290116	T	C	0	XM_003353781.1; XM_003122549.2	LOC100515705	20	19	82	NIL	NIL	NIL
2	6290130	G	A	0	XM_003353781.1; XM_003122549.2	LOC100515705	26	21	96	NIL	NIL	NIL
2	6290278	C	G	0	XM_003353781.1; XM_003122549.2	LOC100515705	28	21	104	NIL	NIL	NIL
2	6295073	C	T	0	XM_003353781.1; XM_003122549.2	LOC100515705	26	20	105	NIL	NIL	NIL
2	6978835	C	A	0	XM_003353794.1	LOC100623923	44	42	112	NIL	NIL	NIL
2	6978918	C	T	0	XM_003353794.1	LOC100623923	45	37	117	NIL	NIL	NIL
2	6979563	C	T	0	XM_003353794.1	LOC100623923	31	23	92	NIL	NIL	NIL
2	11088926	C	T	0	XM_003122683.1	LOC100514446	37	14	79	NIL	NIL	NIL
2	16565323	G	A	0	XM_003353872.1; XM_003122812.2	ACP2	35	36	104	NIL	NIL	NIL
2	16565924	A	G	0	XM_003353872.1; XM_003122812.2	ACP2	NIL	NIL	NIL	55	89	12
2	60557280	G	A	0	XM_003123476.1	C2H19orf42	NIL	NIL	NIL	61	97	18
2	61999361	GG	GGCCCAGTG	0	XM_003123453.2	ILVBL	0	0	3	NIL	NIL	NIL
2	66283929	A	G	0	XM_003123337.1	LOC100524304	NIL	NIL	NIL	57	79	10
2	66287168	TCCCCC	TCCCCCC	0	XM_003123337.1	LOC100524304	NIL	NIL	NIL	28	42	10
2	77656001	C	T	0	XM_003353963.1	LOC100623333	25	10	79	NIL	NIL	NIL
2	77784393	G	T	0	XM_003122991.3; XM_003480846.1	LOC100523423	NIL	NIL	NIL	120	124	49
2	77784563	T	G	0	XM_003122991.3; XM_003480846.1	LOC100523423	159	133	194	NIL	NIL	NIL
2	77784722	C	T	0	XM_003122991.3; XM_003480846.1	LOC100523423	NIL	NIL	NIL	202	198	110
2	139853975	C	T	0	XM_003354272.1	P4HA2	61	37	121	NIL	NIL	NIL
2	139854040	A	G	0	XM_003354272.1	P4HA2	42	27	119	NIL	NIL	NIL
2	139856979	G	A	0	XM_003354272.1	P4HA2	25	15	114	NIL	NIL	NIL
2	139862136	A	G	0	XM_003354272.1	P4HA2	20	14	101	NIL	NIL	NIL
2	139865658	A	G	0	XM_003354272.1	P4HA2	13	17	100	NIL	NIL	NIL
2	139866532	T	C	0	XM_003354272.1	P4HA2	7	9	77	NIL	NIL	NIL
2	139868241	A	G	rs81365336	XM_003354272.1	P4HA2	17	15	84	NIL	NIL	NIL

2	139871844	C	T	0	XM_003354272.1	P4HA2	24	17	62	NIL	NIL	NIL
2	142695214	A	G	0	XM_003354284.1	LOC100627041	37	31	57	62	86	26
2	146849157	G	A	0	XM_003123992.2	LOC100524488	31	19	76	NIL	NIL	NIL
3	10664883	G	A	0	XM_003124410.1	LOC100525688	52	20	101	NIL	NIL	NIL
3	10668640	T	C	0	XM_003124410.1	LOC100525688	47	21	119	NIL	NIL	NIL
3	10669064	C	A	0	XM_003124410.1	LOC100525688	68	29	103	64	60	16
3	10669149	TAAAAA	TAAAAAA	0	XM_003124410.1	LOC100525688	22	20	64	NIL	NIL	NIL
3	16894034	G	A	0	XM_003124443.1; XM_003124444.1	LOC100517757	160	117	201	57	28	18
3	16895991	C	T	0	XM_003124443.1; XM_003124444.1	LOC100517757	185	172	210	111	69	44
3	16896510	G	A	0	XM_003124443.1; XM_003124444.1	LOC100517757	186	170	197	102	78	50
3	16897187	T	C	0	XM_003124443.1; XM_003124444.1	LOC100517757	191	172	202	149	95	70
3	16897520	C	T	rs45433508	XM_003124443.1; XM_003124444.1	LOC100517757	194	161	205	144	113	91
3	16897532	G	C	0	XM_003124443.1; XM_003124444.1	LOC100517757	194	164	204	141	115	89
3	16897845	T	C	0	XM_003124443.1; XM_003124444.1	LOC100517757	NIL	NIL	NIL	147	125	75
3	16898996	A	G	0	XM_003124443.1	LOC100517757	192	165	199	153	120	92
3	17843985	TC	T	0	XM_003124487.1	HSD3B7	28	10	62	NIL	NIL	NIL
3	22164601	A	G	0	XM_003124533.3	LOC100520426	18	9	84	NIL	NIL	NIL
3	34623372	C	T	rs196951274	XM_003124605.1; XM_003124606.1	LOC100521260	41	13	57	NIL	NIL	NIL
3	49194413	A	G	0	XM_003354697.2	LOC100622812	4	0	96	NIL	NIL	NIL
3	118921141	C	T	0	XM_003125293.3	LOC100522014	17	15	151	NIL	NIL	NIL
3	118921945	G	A	0	XM_003125293.3	LOC100522014	7	9	107	NIL	NIL	NIL
3	118922095	T	C	0	XM_003125293.3	LOC100522014	7	6	109	NIL	NIL	NIL
3	118922235	C	T	0	XM_003125293.3	LOC100522014	16	9	158	NIL	NIL	NIL
3	118923104	G	T	0	XM_003125293.3	LOC100522014	18	18	175	NIL	NIL	NIL
3	118924059	T	C	0	XM_003125293.3	LOC100522014	27	19	170	NIL	NIL	NIL
3	118924763	T	C	0	XM_003125293.3	LOC100522014	34	28	194	NIL	NIL	NIL
3	118927460	T	G	0	XM_003125293.3	LOC100522014	106	90	201	NIL	NIL	NIL
3	118927840	G	A	0	XM_003125293.3	LOC100522014	112	113	217	67	21	20

3	118928779	C	T	0	XM_003125293.3	LOC100522014	173	154	197	151	79	60
4	84142659	C	T	0	NM_001243528.1	MRPL15	NIL	NIL	NIL	77	85	47
4	84142718	C	T	0	NM_001243528.1	MRPL15	85	66	164	87	89	39
4	97140658	A	G	rs55618226	XM_001927348.1; XM_003481415.1; XM_001927335.3	LOC100154856	26	23	60	NIL	NIL	NIL
4	100739907	G	A	0	NM_001243468.1	CD5L	66	42	178	65	44	23
4	100739976	G	A	0	NM_001243468.1	CD5L	72	50	188	58	30	17
4	100746277	C	G	0	NM_001243468.1	CD5L	81	45	192	NIL	NIL	NIL
4	100746367	A	C	0	NM_001243468.1	CD5L	87	61	183	NIL	NIL	NIL
4	100746403	T	C	0	NM_001243468.1	CD5L	81	66	192	NIL	NIL	NIL
4	100749464	G	T	0	NM_001243468.1	CD5L	148	95	196	64	72	15
4	103565015	T	C	0	XM_001929375.3; XM_003355138.1	FLAD1	35	20	76	NIL	NIL	NIL
4	110850047	T	C	0	XM_003481476.1	SEC22B	52	26	96	NIL	NIL	NIL
4	110850516	G	A	0	XM_003481476.1	SEC22B	50	27	97	NIL	NIL	NIL
4	119097471	C	A	0	XM_003355245.1	LOC100156038	21	10	95	NIL	NIL	NIL
4	119097520	A	G	rs80833376	XM_003355245.1	LOC100156038	26	12	86	NIL	NIL	NIL
4	119097584	T	C	0	XM_003355245.1	LOC100156038	31	18	97	NIL	NIL	NIL
4	119097685	C	T	0	XM_003355245.1	LOC100156038	32	20	103	NIL	NIL	NIL
4	119097890	A	G	0	XM_003355245.1	LOC100156038	17	12	68	NIL	NIL	NIL
4	119097894	A	G	0	XM_003355245.1	LOC100156038	16	13	66	NIL	NIL	NIL
4	119098055	A	G	0	XM_003355245.1	LOC100156038	27	9	82	NIL	NIL	NIL
4	119098103	G	A	0	XM_003355245.1	LOC100156038	28	14	89	NIL	NIL	NIL
5	4337150	C	T	0	NM_001184895.1	PMM1	44	3	64	NIL	NIL	NIL
5	4346018	A	G	0	NM_001184895.1	PMM1	110	22	122	NIL	NIL	NIL
5	4346746	T	C	0	NM_001184895.1	PMM1	110	25	108	NIL	NIL	NIL
5	4347560	G	A	0	NM_001184895.1	PMM1	NIL	NIL	NIL	50	60	37
5	15481037	C	T	0	XM_003481568.1; XM_003481569.1	LMBR1L	9	14	76	NIL	NIL	NIL
5	15481092	C	T	0	XM_003481568.1; XM_003481569.1	LMBR1L	10	14	75	NIL	NIL	NIL
5	15481160	T	G	0	XM_003481568.1; XM_003481569.1	LMBR1L	18	12	75	NIL	NIL	NIL
5	15672827	GG	GGCTG	0	XM_003355375.1	LOC100127131	1	0	3	NIL	NIL	NIL

5	18663991	T	C	0	NM_001159615.1	KRT8	NIL	NIL	NIL	153	139	46
5	18664266	T	C	0	NM_001159615.1	KRT8	156	143	203	151	129	30
5	18664389	A	G	0	NM_001159615.1	KRT8	155	141	213	133	116	36
5	18667998	G	A	0	NM_001159615.1	KRT8	74	47	202	NIL	NIL	NIL
5	18670859	G	A	0	NM_001159615.1	KRT8	54	43	203	NIL	NIL	NIL
5	20398450	G	A	0	XM_001927549.2; XM_003126204.1	COPZ1	35	14	116	NIL	NIL	NIL
5	23659117	A	G	0	XM_001929410.2	ATP5B	65	25	83	69	78	34
5	23661024	T	C	0	XM_001929410.2	ATP5B	187	135	202	149	160	52
5	23661627	C	T	rs80908111	XM_001929410.2	ATP5B	179	97	202	113	113	23
5	24761578	C	T	0	XM_003126322.3	LOC100519091	19	18	67	NIL	NIL	NIL
5	67966033	C	G	0	XM_003126560.2	LOC100524622	53	32	110	NIL	NIL	NIL
5	67969367	C	T	0	XM_003126560.2	LOC100524622	118	78	187	NIL	NIL	NIL
5	67972024	C	T	0	XM_003126560.2	LOC100524622	128	89	187	NIL	NIL	NIL
5	67972057	G	T	0	XM_003126560.2	LOC100524622	124	88	184	98	124	42
5	67981621	A	G	0	XM_003126560.2	LOC100524622	95	41	175	NIL	NIL	NIL
5	67987994	T	C	0	XM_003126560.2	LOC100524622	74	39	169	NIL	NIL	NIL
5	67988477	C	T	0	XM_003126560.2	LOC100524622	58	36	155	NIL	NIL	NIL
5	67988489	A	T	0	XM_003126560.2	LOC100524622	51	27	141	NIL	NIL	NIL
5	85046072	A	G	0	XM_003481733.1	LOC100738422	195	122	200	127	134	52
5	85046134	G	A	0	XM_003481733.1	LOC100738422	197	131	203	129	154	66
5	91736131	G	C	0	XM_001925061.1	LOC100154617	1	1	101	NIL	NIL	NIL
5	91736161	C	T	0	XM_001925061.1	LOC100154617	5	3	106	NIL	NIL	NIL
5	91746651	A	G	0	XM_001925061.1	LOC100154617	17	3	164	NIL	NIL	NIL
5	91746654	C	T	0	XM_001925061.1	LOC100154617	16	3	163	NIL	NIL	NIL
5	91749359	C	G	0	XM_001925061.1	LOC100154617	22	6	155	NIL	NIL	NIL
6	11898507	G	C	0	XM_003126856.2; XM_003355743.1	KARS	15	9	112	NIL	NIL	NIL
6	11903140	A	G	rs81214236	XM_003126856.2; XM_003355743.1	KARS	40	25	175	39	71	12
6	11903194	C	T	rs81214238	XM_003126856.2; XM_003355743.1	KARS	45	27	149	NIL	NIL	NIL
6	11907376	A	T	0	XM_003126856.2; XM_003355743.1	KARS	50	34	173	NIL	NIL	NIL
6	11907863	G	A	0	XM_003126856.2;	KARS	44	29	176	NIL	NIL	NIL

6	11907915	G	A	0	XM_003355743.1 XM_003126856.2; XM_003355743.1	KARS	40	14	171	NIL	NIL	NIL
6	11907918	G	C	0	XM_003126856.2; XM_003355743.1	KARS	44	15	172	NIL	NIL	NIL
6	11908169	G	A	0	XM_003126856.2; XM_003355743.1	KARS	44	27	170	NIL	NIL	NIL
6	11908170	T	C	0	XM_003126856.2; XM_003355743.1	KARS	42	28	172	NIL	NIL	NIL
6	11910088	C	T	rs81214246	XM_003126856.2; XM_003355743.1	KARS	35	26	170	NIL	NIL	NIL
6	11911405	A	G	rs81214247	XM_003126856.2; XM_003355743.1	KARS	67	35	185	NIL	NIL	NIL
6	11911429	C	T	rs81214249	XM_003126856.2; XM_003355743.1	KARS	65	43	183	NIL	NIL	NIL
6	11911929	C	T	0	XM_003126856.2; XM_003355743.1	KARS	68	50	183	NIL	NIL	NIL
6	11912333	C	T	rs81214253	XM_003126856.2; XM_003355743.1	KARS	75	45	184	NIL	NIL	NIL
6	11912360	T	C	0	XM_003126856.2; XM_003355743.1	KARS	81	49	187	NIL	NIL	NIL
6	11913057	C	T	rs81214255	XM_003126856.2; XM_003355743.1	KARS	82	62	188	NIL	NIL	NIL
6	11913066	G	A	0	XM_003126856.2; XM_003355743.1	KARS	89	65	188	61	79	13
6	11913770	C	T	rs81214261	XM_003126856.2; XM_003355743.1	KARS	92	69	192	63	88	14
6	11913869	G	C	0	XM_003126856.2; XM_003355743.1	KARS	56	58	166	NIL	NIL	NIL
6	11913872	T	C	0	XM_003126856.2; XM_003355743.1	KARS	54	54	160	63	81	17
6	11914431	A	G	rs81214264	XM_003126856.2; XM_003355743.1	KARS	74	48	143	63	79	23
6	11914497	G	A	0	XM_003126856.2; XM_003355743.1	KARS	110	93	189	112	134	42
6	11914501	C	T	0	XM_003126856.2; XM_003355743.1	KARS	110	92	189	NIL	NIL	NIL
6	11914573	C	T	0	XM_003126856.2; XM_003355743.1	KARS	61	34	140	53	76	28
6	25357672	GCACAC	GCAC	0	XM_003126946.1	LOC100525048	31	17	77	NIL	NIL	NIL

6	27371307	T	C	0		XM_003481817.1	LOC100737013	NIL	NIL	NIL	29	82	11
6	27371336	C	T	0		XM_003481817.1	LOC100737013	NIL	NIL	NIL	44	85	14
6	27371339	G	A	0		XM_003481817.1	LOC100737013	53	38	133	45	88	15
6	38934700	T	C	0		XM_003127022.3	PEPD	23	12	78	NIL	NIL	NIL
6	46148740	A	G	0		XM_003127209.3	LOC100523003	34	12	84	NIL	NIL	NIL
6	50119920	C	T	0		NM_001244488.1	NUCB1	82	63	192	NIL	NIL	NIL
6	50128184	A	G	0		NM_001244488.1	NUCB1	53	34	162	50	33	14
6	58158742	C	T	0		XM_003127502.2	LOC100525522	42	31	79	NIL	NIL	NIL
6	58158753	C	T	0		XM_003127502.2	LOC100525522	31	24	77	NIL	NIL	NIL
6	63739697	T	C	0		XM_003481948.1	LOC100738870	101	69	197	70	62	7
6	63742814	G	A	0		XM_003481948.1	LOC100738870	90	65	191	53	45	8
6	63746674	C	T	rs80962342		XM_003481948.1	LOC100738870	96	68	148	56	46	12
6	63746779	T	C	0		XM_003481948.1	LOC100738870	NIL	NIL	NIL	87	60	17
6	63748257	C	T	0		XM_003481948.1	LOC100738870	NIL	NIL	NIL	102	18	13
6	63748275	A	G	rs81217714		XM_003481948.1	LOC100738870	90	90	177	90	5	12
6	63748320	T	C	0		XM_003481948.1	LOC100738870	28	40	95	NIL	NIL	NIL
6	65926547	C	T	0		XM_003127577.2	LOC100525583	32	22	55	NIL	NIL	NIL
6	65926580	T	C	0		XM_003127577.2	LOC100525583	32	24	54	NIL	NIL	NIL
6	65926607	C	T	0		XM_003127577.2	LOC100525583	35	27	48	NIL	NIL	NIL
6	75419371	T	C	0		XM_003356202.1	GALE	57	45	161	NIL	NIL	NIL
6	75419446	C	T	0		XM_003356202.1	GALE	74	49	171	NIL	NIL	NIL
6	75420230	G	A	0		XM_003356202.1	GALE	73	58	158	NIL	NIL	NIL
6	90304285	G	A	0		XM_003356342.2	LOC100622916	55	14	101	NIL	NIL	NIL
6	90304417	T	G	0		XM_003356342.2	LOC100622916	28	8	100	NIL	NIL	NIL
6	90305912	A	G	0		XM_003356342.2	LOC100622916	37	9	110	NIL	NIL	NIL
6	90306237	A	G	0		XM_003356342.2	LOC100622916	41	9	93	NIL	NIL	NIL
6	90315646	G	A	0		XM_003356342.2	LOC100622916	23	9	98	NIL	NIL	NIL
6	90318567	G	T	0		XM_003356342.2	LOC100622916	19	8	94	NIL	NIL	NIL
6	137171304	G	T	0		XM_003127945.2; XM_003127946.1	PGM1	33	20	77	NIL	NIL	NIL
6	137171323	T	C	0		XM_003127945.2; XM_003127946.1	PGM1	54	33	113	NIL	NIL	NIL
6	137171408	T	C	0		XM_003127945.2; XM_003127946.1	PGM1	138	101	192	91	134	23

6	137171452	A	G	0	XM_003127945.2; XM_003127946.1	PGM1	115	94	189	NIL	NIL	NIL
6	137171481	C	A	0	XM_003127945.2; XM_003127946.1	PGM1	NIL	NIL	NIL	69	118	10
6	137171535	T	C	0	XM_003127945.2; XM_003127946.1	PGM1	110	83	194	55	102	16
6	137171741	A	C	0	XM_003127945.2; XM_003127946.1	PGM1	112	76	191	49	82	9
6	137171813	C	T	0	XM_003127945.2; XM_003127946.1	PGM1	111	77	193	NIL	NIL	NIL
6	137171857	C	T	0	XM_003127945.2; XM_003127946.1	PGM1	115	68	191	47	63	12
6	137174682	A	G	0	XM_003127945.2; XM_003127946.1	PGM1	91	48	189	NIL	NIL	NIL
6	137174727	C	T	0	XM_003127945.2; XM_003127946.1	PGM1	81	35	175	NIL	NIL	NIL
6	137174784	C	A	0	XM_003127945.2; XM_003127946.1	PGM1	98	44	178	NIL	NIL	NIL
6	137195153	A	G	0	XM_003127945.2; XM_003127946.1	PGM1	49	28	173	NIL	NIL	NIL
6	147870177	T	G	0	XM_003127991.3	LOC100517534	55	21	172	NIL	NIL	NIL
6	147870526	G	A	0	XM_003127991.3	LOC100517534	45	15	141	NIL	NIL	NIL
6	152197351	A	C	0	XM_003128016.3	LOC100523742	74	20	149	NIL	NIL	NIL
6	152198727	T	C	0	XM_003128016.3	LOC100523742	18	16	174	NIL	NIL	NIL
6	152206224	A	G	0	XM_003128016.3	LOC100523742	55	30	192	NIL	NIL	NIL
6	152206341	G	C	0	XM_003128016.3	LOC100523742	129	32	144	81	88	14
6	152206385	A	T	0	XM_003128016.3	LOC100523742	88	30	60	67	92	10
6	152206727	CTT	CT	0	XM_003128016.3	LOC100523742	95	23	8	NIL	NIL	NIL
6	152206818	C	T	0	XM_003128016.3	LOC100523742	NIL	NIL	NIL	88	144	11
6	153255729	C	T	rs81215265	XM_003128039.1	PRDX1	171	79	199	84	120	31
6	153257567	AT	ATT	0	XM_003128039.1	PRDX1	38	26	50	22	27	3
6	153257939	C	T	rs81215269	XM_003128039.1	PRDX1	NIL	NIL	NIL	91	125	20
6	153257940	C	G	rs81215270	XM_003128039.1 XM_003128041.1 XM_003128040.1 XM_003128038.1 XM_003128042.1	PRDX1	176	69	199	90	126	20
6	153265783	G	A	0	XM_003128039.1	PRDX1	190	120	194	140	166	50

6	153265829	G	T	rs196949554	XM_003128039.1	PRDX1	178	101	194	141	147	48
6	153265881	A	G	0	XM_003128039.1	PRDX1	120	42	141	65	97	22
6	153265900	GGGTTT	G	0	XM_003128039.1	PRDX1	NIL	NIL	NIL	40	66	14
6	153265905	T	C	0	XM_003128039.1	PRDX1	81	23	94	NIL	NIL	NIL
6	153912274	C	T	0	XM_003128049.2	LOC100514239	38	27	93	NIL	NIL	NIL
6	153913158	T	C	0	XM_003128049.2	LOC100514239	34	34	97	NIL	NIL	NIL
6	153913607	G	A	0	XM_003128049.2	LOC100514239	27	23	77	NIL	NIL	NIL
6	155359585	C	T	0	XM_003128065.1	LOC100517766	69	30	115	54	65	23
7	1825765	T	C	0	XM_003482120.1	LOC100739771	49	18	113	NIL	NIL	NIL
7	1825782	T	C	0	XM_003482120.1	LOC100739771	67	25	138	NIL	NIL	NIL
7	26841460	G	A	0	NM_001123140.1	MRPS18B	52	32	102	NIL	NIL	NIL
7	37263243	A	G	0	XM_001929558.1	CDKN1A	6	4	98	NIL	NIL	NIL
7	37426438	C	A	0	XM_003128368.2	LOC100524445	21	15	66	NIL	NIL	NIL
7	37427047	A	C	0	XM_003128368.2	LOC100524445	26	8	100	NIL	NIL	NIL
7	37427122	G	A	0	XM_003128368.2	LOC100524445	25	11	95	NIL	NIL	NIL
7	37427142	A	G	0	XM_003128368.2	LOC100524445	24	15	91	NIL	NIL	NIL
7	37427238	T	C	0	XM_003128368.2	LOC100524445	24	9	76	NIL	NIL	NIL
7	43389880	A	C	rs80946507	NM_001123192.1	RPL7L1	46	14	106	NIL	NIL	NIL
7	43389886	G	A	0	NM_001123192.1	RPL7L1	48	15	111	NIL	NIL	NIL
7	43394230	C	T	rs80831622	NM_001123192.1	RPL7L1	74	30	120	NIL	NIL	NIL
7	43395437	C	G	0	NM_001123192.1	RPL7L1	63	39	129	NIL	NIL	NIL
7	43395573	T	C	0	NM_001123192.1	RPL7L1	77	49	139	54	80	13
7	43486846	G	A	0	NM_001110419.1	GNMT	151	123	184	79	71	31
7	43486876	A	G	0	NM_001110419.1	GNMT	164	138	184	105	95	48
7	43486921	A	G	0	NM_001110419.1	GNMT	178	164	185	140	125	79
7	43488849	A	C	0	NM_001110419.1	GNMT	201	198	204	183	169	147
7	43489263	A	G	0	NM_001110419.1	GNMT	200	199	205	184	172	145
7	43489429	G	A	0	NM_001110419.1	GNMT	198	187	199	175	167	123
7	43489476	G	A	0	NM_001110419.1	GNMT	180	164	197	144	134	90
7	43833000	G	A	0	NM_001044617.1	SLC22A7	39	32	108	NIL	NIL	NIL
7	43833898	G	A	0	NM_001044617.1	SLC22A7	51	31	108	NIL	NIL	NIL
7	48164570	T	A	0	NM_001113013.1	PLA2G7	51	13	127	NIL	NIL	NIL
7	48164591	T	G	0	NM_001113013.1	PLA2G7	49	17	131	NIL	NIL	NIL

7	54563945	C	T	0	XM_003356648.1	LOC100623036	NIL	NIL	NIL	65	55	14
7	54564644	G	T	0	XM_003356648.1	LOC100623036	117	58	197	76	67	21
7	54579630	C	T	0	XM_003356648.1	LOC100623036	148	89	200	98	81	31
7	54581678	G	A	0	XM_003356648.1	LOC100623036	141	87	202	99	81	36
7	54581683	G	A	0	XM_003356648.1	LOC100623036	142	87	201	NIL	NIL	NIL
7	54582545	G	A	0	XM_003356648.1	LOC100623036	147	115	203	132	96	41
7	54582575	A	T	rs80837741	XM_003356648.1	LOC100623036	147	116	198	NIL	NIL	NIL
7	54585108	C	T	0	XM_003356648.1	LOC100623036	139	93	196	96	80	39
7	54585144	C	T	0	XM_003356648.1	LOC100623036	148	102	197	118	107	46
7	54590695	G	T	0	XM_003356648.1	LOC100623036	166	125	203	NIL	NIL	NIL
7	54596139	T	C	0	XM_003356648.1	LOC100623036	NIL	NIL	NIL	165	174	116
7	54596190	G	T	0	XM_003356648.1	LOC100623036	163	145	197	163	174	131
7	54596259	G	A	0	XM_003356648.1	LOC100623036	104	76	154	102	114	94
7	54596291	A	G	0	XM_003356648.1	LOC100623036	NIL	NIL	NIL	61	70	51
7	54596310	G	A	0	XM_003356648.1	LOC100623036	36	26	55	33	41	34
7	63332410	TA	TAA	0	XM_003482240.1	LOC100156967	NIL	NIL	NIL	35	51	12
7	80515824	T	C	0	NM_214019.1	DHRS4	NIL	NIL	NIL	93	41	26
7	102941889	G	T	rs55619011	XM_003128658.1	LOC100156930	112	58	185	83	63	20
7	102941936	T	C	0	XM_003128658.1	LOC100156930	NIL	NIL	NIL	63	50	20
7	102941942	T	C	0	XM_003128658.1	LOC100156930	NIL	NIL	NIL	57	45	19
7	102943214	T	C	0	XM_003128658.1	LOC100156930	NIL	NIL	NIL	123	112	43
7	102949897	TA	TAA	0	XM_003128658.1	LOC100156930	NIL	NIL	NIL	149	125	48
7	102949951	G	T	rs80859223	XM_003128658.1	LOC100156930	NIL	NIL	NIL	131	128	60
7	102950048	T	C	0	XM_003128658.1	LOC100156930	NIL	NIL	NIL	167	163	109
7	102950069	AAA	AAACTAA	0	XM_003128658.1	LOC100156930	NIL	NIL	NIL	158	151	95
8	3118494	T	C	0	XM_003356844.1	LOC100621287	117	79	185	NIL	NIL	NIL
8	3118494	TC	TCC	0	XM_003356844.1	LOC100621287	NIL	NIL	NIL	54	35	7
8	3118581	G	A	0	XM_003356844.1	LOC100621287	127	85	196	79	63	13
8	3118628	A	T	rs55618970	XM_003356844.1	LOC100621287	NIL	NIL	NIL	92	61	15
8	3118784	C	T	rs55618971	XM_003356844.1	LOC100621287	143	88	198	89	76	14
8	3118827	A	T	rs55618972	XM_003356844.1	LOC100621287	130	81	196	88	85	8
8	3118871	G	A	0	XM_003356844.1	LOC100621287	NIL	NIL	NIL	103	100	17
8	3119149	C	T	rs55618968	XM_003356844.1	LOC100621287	141	95	195	90	98	24

8	3119286	A	G	0	XM_003356844.1	LOC100621287	156	109	197	116	143	56
8	12403657	A	G	0	XM_003482350.1	LOC100739772	NIL	NIL	NIL	83	35	11
8	40885091	T	C	0	XM_003482380.1	LOC100738638	63	32	162	33	66	14
8	121675034	C	G	0	XM_003129258.1	LOC100517665	31	9	103	NIL	NIL	NIL
8	121675139	T	C	0	XM_003129258.1	LOC100517665	54	21	123	47	73	8
9	44432121	A	T	rs55618913	XM_003357294.1	CRYAB	128	75	199	52	87	24
9	44432191	T	C	0	XM_003357294.1	CRYAB	101	55	194	48	52	16
9	44434535	T	A	0	XM_003129868.2	LOC100520143	44	27	58	49	37	30
9	67435267	G	A	0	XM_003482652.1	LOC100628005	56	38	151	NIL	NIL	NIL
9	67435294	A	G	rs81212178	XM_003482652.1	LOC100628005	63	38	159	NIL	NIL	NIL
9	146494183	A	C	0	XM_003357604.1	LOC100624161	NIL	NIL	NIL	77	44	16
9	146494231	A	T	0	XM_003357604.1	LOC100624161	NIL	NIL	NIL	99	53	24
9	146494262	G	T	rs81418687	XM_003357604.1	LOC100624161	NIL	NIL	NIL	134	80	36
9	146494391	T	G	0	XM_003357604.1	LOC100624161	NIL	NIL	NIL	93	96	37
11	20026042	GAAAAA	GAAAA	0	XM_003357844.2; XM_003482888.1	LOC100621855	19	13	61	NIL	NIL	NIL
11	20030234	A	G	0	XM_003357844.2; XM_003482888.1	LOC100621855	36	10	93	NIL	NIL	NIL
11	20030396	T	C	0	XM_003357844.2; XM_003482888.1	LOC100621855	19	16	78	NIL	NIL	NIL
12	1048782	C	T	0	XM_003482938.1	LOC100739739	NIL	NIL	NIL	38	141	20
12	1163230	T	TGGGCT	0	XM_003357928.1	ACTB	1	1	4	NIL	NIL	NIL
12	1163704	G	A	0	XM_003357928.1	ACTB	146	94	201	NIL	NIL	NIL
12	1163716	G	C	0	XM_003357928.1	ACTB	158	103	203	NIL	NIL	NIL
12	1163923	C	T	0	XM_003357928.1	ACTB	193	159	203	NIL	NIL	NIL
12	1164781	T	C	0	XM_003357928.1	ACTB	197	181	200	174	178	72
12	1165434	TGTATC	TGTATC	0	XM_003357928.1	ACTB	189	187	190	NIL	NIL	NIL
12	1165572	G	C	0	XM_003357928.1	ACTB	201	198	197	NIL	NIL	NIL
12	1165992	ACGCG	ACGCGC	0	XM_003357928.1	ACTB	73	67	73	NIL	NIL	NIL
12	1166023	G	GCAAAAA	0	XM_003357928.1	ACTB	18	10	19	NIL	NIL	NIL
12	2298517	G	A	0	XM_003131141.3	LOC100526132	51	27	138	NIL	NIL	NIL
12	2388039	AGGAG	AGGAGA	0	XM_003131141.3	LOC100526132	7	4	33	NIL	NIL	NIL
12	2388058	G	A	0	XM_003131141.3	LOC100526132	21	17	80	NIL	NIL	NIL
12	2388062	G	A	0	XM_003131141.3	LOC100526132	19	15	78	NIL	NIL	NIL

12	2388543	C	T	0	XM_003131141.3	LOC100526132	23	13	92	NIL	NIL	NIL
12	5357348	G	A	0	XM_003482948.1	LOC100739290	30	28	55	NIL	NIL	NIL
12	5357410	T	C	0	XM_003482948.1	LOC100739290	35	20	51	NIL	NIL	NIL
12	5357451	C	T	0	XM_003482948.1	LOC100739290	25	18	55	NIL	NIL	NIL
12	5357565	G	A	0	XM_003482948.1	LOC100739290	37	45	74	NIL	NIL	NIL
12	5357598	G	A	0	XM_003482948.1	LOC100739290	57	61	82	NIL	NIL	NIL
12	5407136	AA	AACTTTG	0	XM_003357966.1	ACO	8	21	10	NIL	NIL	NIL
12	15295333	A	G	0	XM_003131295.1	LOC100514364	31	30	54	NIL	NIL	NIL
12	15299116	G	A	0	XM_003131295.1	LOC100514364	31	18	65	NIL	NIL	NIL
12	24050251	C	T	0	XM_003131543.3	LOC100521291	15	31	60	NIL	NIL	NIL
12	24050285	G	A	0	XM_003131543.3	LOC100521291	23	35	66	NIL	NIL	NIL
12	43758652	G	T	0	XM_003131728.2	LOC100520269	NIL	NIL	NIL	103	149	63
12	43758772	A	G	0	XM_003131728.2	LOC100520269	NIL	NIL	NIL	55	93	32
12	43758774	G	A	0	XM_003131728.2	LOC100520269	NIL	NIL	NIL	56	94	35
12	43758879	AC	ACC	0	XM_003131728.2	LOC100520269	NIL	NIL	NIL	42	55	23
12	43758886	A	G	0	XM_003131728.2	LOC100520269	NIL	NIL	NIL	36	57	21
12	43761467	C	T	0	XM_003131728.2	LOC100520269	66	27	165	NIL	NIL	NIL
12	43767008	G	A	0	XM_003131728.2	LOC100520269	52	20	155	NIL	NIL	NIL
12	46537688	C	T	0	XM_003483067.1	TNFAIP1	18	15	68	NIL	NIL	NIL
12	51614480	G	A	0	XM_003131848.1; XM_003358202.1	LOC100515576	21	22	88	NIL	NIL	NIL
12	51614658	G	A	0	XM_003131848.1; XM_003358202.1	LOC100515576	23	18	93	NIL	NIL	NIL
12	54042323	G	A	0	XM_003131905.2	OMC	43	27	56	NIL	NIL	NIL
12	54317095	G	A	0	NM_001144926.2	PSMB6	46	20	86	NIL	NIL	NIL
12	54317130	T	C	0	NM_001144926.2	PSMB6	84	44	145	73	92	39
12	54318953	T	G	0	NM_001144926.2	PSMB6	37	22	121	NIL	NIL	NIL
13	3583461	A	C	0	NM_001243616.1	DPH3	28	6	59	NIL	NIL	NIL
13	3583490	C	T	0	NM_001243616.1	DPH3	39	9	84	NIL	NIL	NIL
13	3583545	C	T	0	NM_001243616.1	DPH3	56	27	107	63	21	18
13	3583558	C	T	0	NM_001243616.1	DPH3	54	29	104	NIL	NIL	NIL
13	3583691	T	A	0	NM_001243616.1	DPH3	51	23	100	NIL	NIL	NIL
13	3583721	C	T	0	NM_001243616.1	DPH3	56	24	116	NIL	NIL	NIL
13	3583749	A	G	0	NM_001243616.1	DPH3	60	21	120	58	30	11

13	3583781	C	T	0	NM_001243616.1	DPH3	63	26	123	NIL	NIL	NIL
13	3583882	A	C	0	NM_001243616.1	DPH3	76	35	133	NIL	NIL	NIL
13	3583964	A	C	0	NM_001243616.1	DPH3	73	32	120	NIL	NIL	NIL
13	3583967	C	T	0	NM_001243616.1	DPH3	74	32	119	NIL	NIL	NIL
13	3584177	T	A	0	NM_001243616.1	DPH3	32	22	85	NIL	NIL	NIL
13	3584300	G	A	0	NM_001243616.1	DPH3	48	20	116	NIL	NIL	NIL
13	33563223	C	A	0	XM_003358419.1	C3orf75	16	19	66	NIL	NIL	NIL
13	36895404	C	A	0	XM_003483189.1	MANF	52	31	147	52	81	25
13	36895572	C	G	0	XM_003483189.1	MANF	60	36	173	46	80	12
13	49976969	A	G	0	XM_003132296.3	PSMD6	31	10	96	NIL	NIL	NIL
13	73233058	A	G	rs196956369	XM_003132358.1	LOC100521991	81	39	178	63	47	9
13	73233143	CAGCTG	CA	0	XM_003132358.1	LOC100521991	NIL	NIL	NIL	57	59	13
13	73233329	T	A	0	XM_003132358.1	LOC100521991	69	38	139	78	95	28
13	73548157	A	G	0	XM_003483218.1	LOC100739864	59	40	155	NIL	NIL	NIL
13	73562335	G	A	0	XM_003483218.1	LOC100739864	45	30	135	NIL	NIL	NIL
13	79727769	A	G	0	XM_003483237.1	LOC100739335	69	49	132	43	52	12
13	79737479	C	T	0	XM_003483237.1	LOC100739335	37	11	113	NIL	NIL	NIL
13	79737516	G	A	0	XM_003483237.1	LOC100739335	29	7	102	NIL	NIL	NIL
14	42952441	G	A	0	XM_003483435.1	LOC100157055	13	6	85	NIL	NIL	NIL
14	42952626	A	G	0	XM_003483435.1	LOC100157055	19	15	84	NIL	NIL	NIL
14	42956704	G	C	0	XM_003483435.1	LOC100157055	46	25	107	28	63	9
14	50478245	T	A	0	XM_001929262.4	LOC100157687	34	5	76	NIL	NIL	NIL
14	50478689	C	T	0	XM_001929262.4	LOC100157687	18	11	85	NIL	NIL	NIL
14	82624759	C	A	0	XM_001928193.4	SEC24C	13	13	73	NIL	NIL	NIL
14	131109160	TAAAAA	TAAAAAA	0	XM_003133180.3	XPNPEP1	31	17	70	NIL	NIL	NIL
14	131109271	C	G	0	XM_003133180.3	XPNPEP1	25	25	72	NIL	NIL	NIL
14	131109445	A	G	0	XM_003133180.3	XPNPEP1	29	31	97	NIL	NIL	NIL
14	131113925	G	A	0	XM_003133180.3	XPNPEP1	28	14	79	NIL	NIL	NIL
14	131120227	G	A	0	XM_003133180.3	XPNPEP1	16	11	79	NIL	NIL	NIL
14	133739004	C	T	0	XM_001927912.2	TECTB	64	25	120	NIL	NIL	NIL
14	133740424	C	T	0	XM_001927912.2	TECTB	83	24	105	NIL	NIL	NIL
14	133751226	T	A	0	XM_001927912.2	TECTB	121	61	106	66	84	20
14	133751272	TCC	TC	0	XM_001927912.2	TECTB	112	100	112	96	98	44

15	122861718	T	G	0	XM_003483721.1	IDH1	81	42	100	49	60	14
15	122861896	C	T	0	XM_003483721.1	IDH1	148	95	179	91	118	20
15	122861903	CGGG	CGG	0	XM_003483721.1	IDH1	142	60	169	86	112	1
15	122861968	C	T	0	XM_003483721.1	IDH1	150	98	190	89	106	26
15	122862291	T	C	0	XM_003483721.1	IDH1	122	62	177	72	79	19
15	122862295	GAAAAT	GAAAATA	0	XM_003483721.1	IDH1	109	59	162	60	73	16
15	122862513	C	T	0	XM_003483721.1	IDH1	138	78	187	64	58	9
15	122862530	C	T	0	XM_003483721.1	IDH1	129	73	187	55	53	7
15	122876927	G	A	0	XM_003483721.1	IDH1	88	33	162	NIL	NIL	NIL
15	137967693	A	G	rs81211172	XM_003133679.3	LOC100524650	26	9	90	NIL	NIL	NIL
15	137979323	G	A	0	XM_003133679.3	LOC100524650	34	10	94	NIL	NIL	NIL
15	157617314	T	C	0	XM_003133834.2	LOC100517616	19	9	91	NIL	NIL	NIL
15	157617534	C	T	0	XM_003133834.2	LOC100517616	29	14	93	NIL	NIL	NIL
15	157617616	A	G	0	XM_003133834.2	LOC100517616	47	29	116	NIL	NIL	NIL
15	157621921	A	G	0	XM_003133834.2	LOC100517616	46	40	103	NIL	NIL	NIL
15	157621933	A	G	0	XM_003133834.2	LOC100517616	45	42	105	NIL	NIL	NIL
15	157621986	C	T	0	XM_003133834.2	LOC100517616	48	58	99	NIL	NIL	NIL
15	157624384	G	A	0	XM_003133834.2	LOC100517616	75	80	141	NIL	NIL	NIL
15	157625354	T	C	0	XM_003133834.2	LOC100517616	39	42	75	NIL	NIL	NIL
15	157625392	T	C	0	XM_003133834.2	LOC100517616	42	47	93	NIL	NIL	NIL
15	157626216	G	A	0	XM_003133834.2	LOC100517616	100	103	144	NIL	NIL	NIL
15	157626237	C	T	0	XM_003133834.2	LOC100517616	111	114	138	NIL	NIL	NIL
16	33954843	C	G	rs81216839	XM_003133935.1	LOC100511788	15	15	70	NIL	NIL	NIL
17	13109675	C	G	0	XM_003134232.2	LOC100526021	12	11	96	NIL	NIL	NIL
17	13129640	A	G	0	XM_003134232.2	LOC100526021	67	40	180	52	58	9
17	31800979	C	T	0	XM_003134287.3	LOC100512320	33	18	121	NIL	NIL	NIL
17	31801702	T	A	0	XM_003134287.3	LOC100512320	40	8	121	NIL	NIL	NIL
17	31806249	C	T	0	XM_003134287.3	LOC100512320	51	22	138	NIL	NIL	NIL
17	31807010	CTATAA	CTATAAT	0	XM_003134287.3	LOC100512320	54	26	117	38	55	11
17	31807099	C	T	0	XM_003134287.3	LOC100512320	52	43	135	64	89	11
17	31807104	G	A	0	XM_003134287.3	LOC100512320	51	41	137	66	90	11
17	37464407	A	G	0	XM_003134336.1	LOC100512637	26	10	100	NIL	NIL	NIL
17	37466400	G	A	0	XM_003134336.1	LOC100512637	38	10	102	NIL	NIL	NIL

17	37466457	A	G	0	XM_003134336.1	LOC100512637	19	5	75	NIL	NIL	NIL
17	44907666	G	A	0	XM_001926938.4	LOC100152525	24	12	86	NIL	NIL	NIL
17	44907733	A	C	0	XM_001926938.4	LOC100152525	19	13	82	NIL	NIL	NIL
17	44907761	G	A	0	XM_001926938.4	LOC100152525	20	15	93	NIL	NIL	NIL
17	44907801	A	G	0	XM_001926938.4	LOC100152525	19	17	100	NIL	NIL	NIL
17	44907863	A	G	0	XM_001926938.4	LOC100152525	18	18	95	NIL	NIL	NIL
17	45782027	G	A	0	XM_003359985.2	LOC100513892	13	11	90	NIL	NIL	NIL
17	51649343	G	A	0	XM_003134450.3	LOC100157757	44	41	31	NIL	NIL	NIL
17	51649560	C	T	0	XM_003134450.3	LOC100157757	36	36	31	NIL	NIL	NIL
17	66511969	ACCCCC	ACCCC	0	NM_001123104.1	CTSZ	NIL	NIL	NIL	70	105	49
18	53538831	T	C	0	XM_003134862.1	LOC100524778	70	20	137	NIL	NIL	NIL
18	53541435	G	A	0	XM_003134862.1	LOC100524778	NIL	NIL	NIL	74	114	26
18	53541504	C	G	0	XM_003134862.1	LOC100524778	62	45	153	59	52	32
18	53541513	G	A	0	XM_003134862.1	LOC100524778	52	37	140	51	48	25

Supplementary Table S4. Genotype, allele frequencies and the chi-square test of selected

Polymorphism position	Number of boars	Genotype frequency			Allele frequency		p-value	Chi-square test
		TT	TC	CC	T	C		χ^2
ATP5B g. 23661024 T>C	100	0.11(11)	0.17 (17)	0.72	0.20	0.80		0.21
		GG	GA	AA	G	A		
KRT8 g.18670859>A	100	0.22(22)	0.16(16)	0.77(77)	0.30	0.70	0.004	0.38
		CC	CA	AA	C	A		
PGM1 g.137174784C>A	100	0.36(36)	0.48 (48)	0.16(16)	0.60	0.40	0.012	0.04
		AA(n)	AC(n)	CC(n)	A	C		
CYP4A25 g.152197351 A>C	100	0.13(13)	0.07 (7)	0.80(80)	0.16	0.84	0.08	0.56
		GG	GA	AA	G	A		
SLC22A7 g.43833000 G>A	100	0.47(47)	0.08(8)	0.45(68)	0.51	0.49	0.001	0.71
		CC	CT	TT	C	T		
IDH1 g.122862530 C>T	100	0.38(38)	0.21 (21)	0.41(41)	0.49	0.51	0.0001	0.34

Supplementary Table S5. Differential exon expression in liver from boars with high and low skatole in backfat

Chr	GeneID(+ or – indicates strand)	Transcripts	Gene_Name	Exon ID	Start	End	Dispersion	log2fold(high/low)	P-adjust
12	396771+	XM_003357966.1	ACO	E001	5406195	5406691	0.07083967	-0.24623623	0.005927867
12	100113422-	NM_001101028.1	ACOX1	E001	5472406	5472472	0.10583768	-0.3393849	0.002366488
12	100113422-	NM_001101028.1	ACOX1	E002	5475167	5475373	0.05575147	-0.30200343	2.342e-05
12	100113422-	NM_001101028.1	ACOX1	E003	5475523	5475666	0.05610964	-0.18512347	0.043068285
12	100113422-	NM_001101028.1	ACOX1	E009	5479984	5480099	0.05775449	0.28322446	0.032033703
12	100113422-	NM_001101028.1	ACOX1	E011	5482509	5482616	0.06583003	0.35193959	0.028556943
2	100513002+	XM_003122812.2	ACP2	E012	16565165	16566578	0.05976333	-0.32491668	0.004280893
2	100513002+	XM_003353872.1	ACP2	E012	16565165	16566578	0.05976333	-0.32491668	0.004280893
14	100157521+	NM_001195321.1	ACSL5	E022	133751213	133751489	0.22921177	-0.60749238	0.011760709
17	497050-	NM_001011727.1	AHCY	E001	42693955	42694874	0.10249708	-0.49678662	0.00010207
17	497050-	NM_001011727.1	AHCY	E006	42700880	42700992	0.10367865	1.52728657	0.0140869
13	100626761+	NM_001243644.1	AP2M1	E012	131542569	131543179	0.05809582	-0.41707213	1.503e-05
1	414411+	XM_003353686.1	ASS1	E015	304508699	304508988	0.10612214	-0.27125445	0.0113442
1	100157880+	NM_001185142.1	ATP5A1	E001	105944306	105944384	0.08124919	-0.49257032	0.027967034
1	100157880+	NM_001185142.1	ATP5A1	E004	105950282	105950448	0.05879777	-0.34693165	0.001040802
1	100157880+	NM_001185142.1	ATP5A1	E006	105951342	105951493	0.06150912	0.37007263	0.043811123
1	100157880+	NM_001185142.1	ATP5A1	E007	105951609	105951833	0.05908307	0.37798072	0.005425927
5	100157156-	XM_001929410.2	ATP5B	E001	23659081	23659341	0.0960272	-0.32064166	0.02598853
5	100157156-	XM_001929410.2	ATP5B	E002	23660897	23661098	0.0565894	-0.22437269	0.038629196
4	100154019+	NM_001243468.1	CD5L	E002	100736206	100736232	0.07455165	-0.45219819	0.026168356
4	100154019+	NM_001243468.1	CD5L	E003	100739717	100740037	0.06017357	-0.30247239	0.02067958
4	100154019+	NM_001243468.1	CD5L	E006	100746207	100746521	0.05945015	0.41147619	0.001711705
4	100154019+	NM_001243468.1	CD5L	E007	100747595	100747912	0.05833982	0.29280523	0.015807883
1	100158127+	NM_001243707.1	CNDP2	E012	165547878	165548510	0.0576216	-0.37167198	0.000840925
17	100141405-	NM_001123104.1	CTSZ	E001	66511905	66512391	0.0593009	-0.35370613	0.000876761
18	100513388+	NM_001244543.1	DDX56	E014	55618007	55618273	0.064711	-0.41093837	0.010479405
14	100157976+	XM_001927253.2	DRG1	E009	51313505	51314337	0.0620438	-0.40198767	0.004269813
4	100153896-	XM_003355138.1	FLAD1	E006	103566707	103567451	0.0702215	0.45165564	0.025089627

4	100153896-	XM_001929375.3	FLAD1	E006	103566707	103567451	0.0702215	0.45165564	0.025089627
X	100431100-	NM_001184755.1	FTSJ1	E001	47678181	47678753	0.08534662	-0.49822484	0.009287598
6	100621392+	XM_003356202.1	GALE	E011	75420510	75420891	0.0601952	-0.3930611	0.000930637
14	100156458+	NM_001244382.1	GHITM	E004	92191925	92192089	0.06059495	-0.2890803	0.013171572
14	100156458+	NM_001244383.1	GHITM	E004	92191925	92192089	0.06059495	-0.2890803	0.013171572
6	397602-	NM_214330.1	GPI	E001	39516820	39517221	0.06717298	-0.26159866	0.031442442
12	733645+	NM_001044578.1	GRN	E006	19213755	19213849	0.07093604	0.66634219	0.015836725
12	733645+	NM_001044578.1	GRN	E007	19214091	19214217	0.06560387	0.73730055	0.00049566
12	733645+	NM_001044578.1	GRN	E012	19215821	19216283	0.0687953	-0.39041894	0.002114675
3	100525281+	XM_003124487.1	HSD3B7	E007	17843326	17844524	0.14350157	-0.68858754	3.897e-05
7	397028+	NM_213973.1	HSP90AA1	E001	129758754	129758816	0.07416684	-0.63273134	0.000993046
7	397028+	NM_213973.1	HSP90AA1	E003	129759579	129759912	0.05674035	0.26476933	0.01279063
1	407060-	XM_001927795.4	HSPA5	E001	299754033	299754610	0.05577796	-0.17463422	0.048765534
15	100170146-	XM_003483721.1	IDH1	E002	122865752	122865914	0.06164664	0.42922793	0.002294225
15	100170146-	XM_003483721.1	IDH1	E003	122866809	122866949	0.06327674	0.39982343	0.012646525
15	100170146-	XM_003483721.1	IDH1	E004	122869162	122869313	0.06496363	0.50723259	0.00226593
15	100170146-	XM_003483721.1	IDH1	E008	122879689	122879826	0.06162227	-0.40683036	0.001191433
15	100170146-	XM_003483721.1	IDH1	E012	122882651	122882707	0.09080814	-0.48064864	0.013515885
15	100170146-	XM_003483719.1	IDH1	E002	122865752	122865914	0.06164664	0.42922793	0.002294225
15	100170146-	XM_003483719.1	IDH1	E003	122866809	122866949	0.06327674	0.39982343	0.012646525
15	100170146-	XM_003483719.1	IDH1	E004	122869162	122869313	0.06496363	0.50723259	0.00226593
15	100170146-	XM_003483719.1	IDH1	E008	122879689	122879826	0.06162227	-0.40683036	0.001191433
15	100170146-	XM_003483719.1	IDH1	E011	122882642	122882650	0.08448137	-0.49260264	0.016527363
15	100170146-	XM_003483719.1	IDH1	E012	122882651	122882707	0.09080814	-0.48064864	0.013515885
15	100170146-	XM_003483720.1	IDH1	E002	122865752	122865914	0.06164664	0.42922793	0.002294225
15	100170146-	XM_003483720.1	IDH1	E003	122866809	122866949	0.06327674	0.39982343	0.012646525
15	100170146-	XM_003483720.1	IDH1	E004	122869162	122869313	0.06496363	0.50723259	0.00226593
15	100170146-	XM_003483720.1	IDH1	E008	122879689	122879826	0.06162227	-0.40683036	0.001191433
15	100170146-	XM_003483720.1	IDH1	E010	122882364	122882446	0.12486085	-0.73775251	0.033879574
15	100170146-	XM_003483720.1	IDH1	E011	122882642	122882650	0.08448137	-0.49260264	0.016527363
15	100170146-	XM_003483720.1	IDH1	E012	122882651	122882707	0.09080814	-0.48064864	0.013515885
17	733641-	NM_001044575.2	IDH3B	E001	37446330	37446752	0.06119268	-0.41066915	0.002023062
2	100514155+	XM_003480931.1	IK	E007	148328163	148329086	0.06842618	-0.55361328	0.000410654

2	100523720+	XM_003123453.2	ILVBL	E015	62007363	62007468	0.06025485	-0.34582457	0.005927848
2	100523720+	XM_003123453.2	ILVBL	E016	62007590	62008056	0.11324812	-0.42026035	0.000615851
5	100152077-	NM_001159615.1	KRT8	E001	18663339	18663700	0.05568801	-0.28639226	0.000789995
11	494565-	NM_001011507.1	LAMP-1	E001	86117963	86118806	0.05685183	-0.20179228	0.002469475
5	100525991-	XM_003481568.1	LMBR1L	E001	15480567	15481168	0.07380557	-0.82427147	5.098e-06
5	100525991-	XM_003481569.1	LMBR1L	E001	15480567	15481168	0.07380557	-0.82427147	5.098e-06
5	100154617+	XM_001925061.1	LOC100154617	E002	91726887	91726947	0.17676369	-1.38143363	0.025498317
5	100154617+	XM_001925061.1	LOC100154617	E003	91727058	91727085	0.16502849	-1.32831921	0.00992027
5	100154617+	XM_001925061.1	LOC100154617	E004	91727420	91727494	0.15895467	-1.15353971	0.033868507
5	100154617+	XM_001925061.1	LOC100154617	E014	91737714	91737794	0.13475225	1.56217239	0.018301554
4	100156038+	XM_003355245.1	LOC100156038	E010	119097423	119098520	0.06148838	-0.34055297	0.012922305
7	100156930+	XM_003128658.1	LOC100156930	E013	102949752	102950202	0.07135793	-0.25264583	0.043246094
7	100156930+	XM_001929074.2	LOC100156930	E013	102949752	102950202	0.07135793	-0.25264583	0.043246094
7	100156930+	XM_003128657.1	LOC100156930	E013	102949752	102950202	0.07135793	-0.25264583	0.043246094
14	100157055+	XM_003483435.1	LOC100157055	E001	42936398	42936645	0.14836031	-0.69447394	0.028556943
6	100514239-	XM_003128049.2	LOC100514239	E001	153911648	153911885	0.06657991	-0.36473713	0.019367104
3	100517757+	XM_003124444.1	LOC100517757	E003	16893486	16893680	0.05816717	0.33245209	0.043170927
3	100517757+	XM_003124444.1	LOC100517757	E004	16894013	16894096	0.05854952	0.32238398	0.042334209
3	100517757+	XM_003124443.1	LOC100517757	E003	16893486	16893680	0.05816717	0.33245209	0.043170927
3	100517757+	XM_003124443.1	LOC100517757	E004	16894013	16894096	0.05854952	0.32238398	0.042334209
6	100517766-	XM_003128065.1	LOC100517766	E001	155358248	155359858	0.07482855	-0.24211013	0.038393152
5	100519091-	XM_003126322.3	LOC100519091	E001	24761529	24761985	0.0599894	-0.31219652	0.011263
2	100519412-	XM_003123191.2	LOC100519412	E002	70508009	70508351	0.07998717	-0.46140239	0.004626242
12	100520269-	XM_003131728.2	LOC100520269	E001	43758499	43759377	0.05728032	-0.26258323	0.017548635
2	100524304-	XM_003123337.1	LOC100524304	E001	66271546	66271930	0.06123001	-0.47314555	0.000371058
6	100525048-	XM_003126946.1	LOC100525048	E001	25357266	25357888	0.07736487	-0.40462762	0.000976954
3	100525688+	XM_003124410.1	LOC100525688	E012	10668837	10669259	0.06346639	-0.37816934	0.004957424
12	100526132-	XM_003131141.3	LOC100526132	E021	2406599	2407162	0.23984203	-1.50061062	0.003225578
3	100622812+	XM_003354697.2	LOC100622812	E006	49193756	49193975	0.17637502	13.4173298	0.043068285
3	100622812+	XM_003354697.2	LOC100622812	E007	49194094	49195003	0.07425184	-0.03689041	0.034301566
X	100628142+	XM_003360301.2	LOC100628142	E011	47582591	47583961	0.48219643	-2.49154439	0.002569742
3	100737559+	XM_003481099.1	LOC100737559	E002	42509767	42509827	0.42489197	-0.87911985	0.033774899
3	100737559+	XM_003481099.1	LOC100737559	E004	42512962	42513149	0.06865541	-0.59984562	3.260e-06

3	100737559+	XM_003481099.1	LOC100737559	E008	42515534	42515687	0.06976174	0.56870122	0.006978856
3	100737559+	XM_003481098.1	LOC100737559	E002	42509767	42509827	0.42489197	-0.87911985	0.033774899
3	100737559+	XM_003481098.1	LOC100737559	E004	42512962	42513149	0.06865541	-0.59984562	3.260e-06
3	100737559+	XM_003481098.1	LOC100737559	E008	42515534	42515687	0.06976174	0.56870122	0.006978856
12	100739290+	XM_003482948.1	LOC100739290	E008	5356976	5357763	0.06288562	-0.28998559	0.02299141
5	100154219+	NM_001185132.1	LTA4H	E019	91720702	91720819	0.0690949	-0.45044624	0.038997559
3	397039-	NM_001244153.1	MDH2	E002	9955158	9955309	0.06607456	0.3804048	0.049023852
3	397039-	NM_001244153.1	MDH2	E003	9955580	9955679	0.07089347	0.5904684	0.00756318
3	397039-	NM_001244153.1	MDH2	E004	9956661	9956738	0.07294498	0.56138893	0.018894217
12	100626208+	XM_003358153.1	NLE1	E013	41555176	41555536	0.13020012	-0.90421101	0.007308336
5	100516080-	NM_001244246.1	NOP2	E007	66467312	66467399	0.26859637	13.1727205	0.043954742
6	100512538-	NM_001244488.1	NUCB1	E001	50119224	50120107	0.0709113	-0.23842451	0.047158503
6	100512538-	NM_001244488.1	NUCB1	E005	50121468	50121560	0.07133939	0.53775896	0.016194086
12	397390+	XM_003131905.2	OMC	E009	54042114	54042778	0.07148148	-0.57249748	0.000739471
2	100623801-	XM_003354272.1	P4HA2	E008	139865550	139865726	0.0989376	0.94009448	0.018552536
2	100623801-	XM_003354272.1	P4HA2	E010	139868199	139868438	0.08000281	-0.52463233	0.048765534
3	100499564-	NM_001195119.1	PDIA6	E003	134585976	134586134	0.06008581	0.30011674	0.029418625
X	407608-	NM_001099932.1	PGK1	E001	71339754	71340216	0.06017897	-0.37981099	0.000920641
6	397566-	XM_003127946.1	PGM1	E010	137200412	137200574	0.06618332	-0.41737011	0.012832243
6	397566-	XM_003127945.2	PGM1	E010	137200412	137200574	0.06618332	-0.41737011	0.012832243
6	397566-	XM_003127945.2	PGM1	E012	137233155	137233581	0.06378838	-0.50447402	3.359e-05
5	100151790+	NM_001184895.1	PMM1	E008	4347163	4347697	0.06017695	-0.35418136	0.013402972
6	100512476+	XM_003128039.1	PRDX1	E003	153249744	153249811	0.25609262	-2.12485111	0.000840925
13	100519870-	XM_003132296.3	PSMD6	E006	49977915	49978158	0.08053395	-0.59770726	0.00054636
1	100156347+	NM_001164510.1	SLC25A25	E002	302558673	302558799	0.14715188	-0.97100638	0.009119443
12	100737417+	XM_003483067.1	TNFAIP1	E007	46536059	46537702	0.06917051	0.2221774	0.04015592
7	733686+	NM_001044612.1	TUBB2A	E004	26972199	26974207	0.05802181	-0.20851928	0.007780342
7	100152596-	XM_001925393.2	VPS52	E008	34136466	34136584	0.25711284	13.0486522	0.01460417
14	100152688-	XM_003483555.1	XPNPEP1	E001	131108926	131109477	0.07998717	-0.5861998	0.000506204
14	100152688-	XM_003483555.1	XPNPEP1	E012	131124284	131124494	0.11968621	1.17051858	0.031575962
14	100152688-	XM_003133180.3	XPNPEP1	E001	131108926	131109477	0.07998717	-0.5861998	0.000506204
14	100152688-	XM_003133180.3	XPNPEP1	E012	131124284	131124494	0.11968621	1.17051858	0.031575962

Supplementary Table S6. GLM analysis results for liver DEGs

mRNA ID	Gene	Total Deviance	Within Group	Between Group	p-Value	p-Adj
XM_003353686.1	ASS1	30.88536428	5.946443182	24.9389211	5.91756083467843e-07	0.000269108
NM_001185130.1	PISD	18.89159852	6.391672872	12.49992564	0.000406968	0.021334045
NM_001044579.1	LOC733646	16.72804384	4.931972946	11.79607089	0.000593559	0.025942721
XM_003355598.1	COPS7A	12.67170831	1.796757152	10.87495116	0.000974736	0.034205815
XM_003124410.1	LOC100525688	15.34602253	2.891430617	12.45459192	0.000416965	0.021351315
NM_001195321.1	ACSL5	18.08294781	2.224909636	15.85803818	6.82759056553905e-05	0.006299854
XM_003128105.1	LOC100525292	19.47090427	2.206113956	17.26479031	3.25156546927552e-05	0.004054524
NM_001243644.1	AP2M1	19.30648222	4.46483801	14.84164421	0.000116925	0.009543872
XM_003129821.2	GRIA4	12.74789938	1.646628424	11.10127096	0.000862686	0.031505351
XM_003481568.1	LMBR1L	18.44708293	5.610737162	12.83634576	0.000339951	0.019267245
XM_003482481.1	LOC100519855	14.62439198	1.989066461	12.63532552	0.000378525	0.020320268
XM_003482919.1	LOC100737161	23.37722463	3.715616094	19.66160854	9.24400121604663e-06	0.001590634
NM_001243369.1	RANGRF	19.16021803	3.138778038	16.02143999	6.26292235305081e-05	0.005921872
XM_003360303.2	LOC100620167	27.50250574	7.204963424	20.29754231	6.62856742039697e-06	0.001361351
XM_001925378.2	LOC100151786	13.83968568	3.376456569	10.46322911	0.001217742	0.038894444
XM_003135207.2	LOC100518105	23.18411709	7.581581397	15.6025357	7.81497178614643e-05	0.007107903
NM_213951.1	NST	29.02300512	6.477968002	22.54503711	2.05274151166002e-06	0.000633111
XM_003355245.1	LOC100156038	19.29546134	5.444923518	13.85053782	0.00019794	0.013631661
XM_003128155.1	LOC100522444	13.65434715	3.837901205	9.81644595	0.001729582	0.048580918
XM_003354711.1	SLC9A4	36.28251707	7.772113215	28.51040385	9.31963574091199e-08	6.59277935745996e-05

XM_003133041.3	LOC100511475	17.43399405	7.240697728	10.19329632	0.001409521	0.043144
XM_003127015.1	CEBPA	12.6432771	2.817806998	9.825470106	0.001721117	0.048552411
NM_001123110.1	EFNA1	19.16194714	6.731621528	12.43032561	0.000422418	0.021571659
XM_003130220.3	SEMA3E	12.15084648	2.043645257	10.10720123	0.001476911	0.044442143
NM_001044607.1	ECH1	14.36991128	2.537055195	11.83285608	0.000581947	0.025670163
XM_003124042.3	LOC100520236	17.61881612	3.679837341	13.93897877	0.000188842	0.013163773
XM_003482938.1	LOC100739739	59.23417072	9.095782153	50.13838857	1.43274281327876e-12	9.64980687948961e-09
NM_001048070.1	HN1L	17.06380822	2.876212105	14.18759612	0.000165458	0.011880604
XM_003126560.2	LOC100524622	12.56100224	2.502898916	10.05810332	0.001516793	0.045125773
XM_003481340.1	LOC100736636	12.80225383	2.471150133	10.33110369	0.00130807	0.040890556
XM_003483466.1	LOC100738456	15.50186442	3.045909152	12.45595527	0.000416661	0.021351315
XM_003126204.1	COPZ1	16.55090415	4.132434816	12.41846934	0.000425109	0.021571659
XM_003129119.2	LOC100512296	52.55556281	15.8525869	36.70297591	1.37569278191307e-09	2.09842503971449e-06
XM_003356342.2	LOC100622916	19.7633284	6.765584698	12.9977437	0.000311867	0.018384725
XM_003133935.1	LOC100511788	17.73047196	4.802261027	12.92821094	0.000323667	0.018847681
XM_003480595.1	LOC100737979	24.95969202	3.756909371	21.20278265	4.131642206473e-06	0.001052192
XM_003481031.1	LOC100520426	18.59229147	3.358414033	15.23387744	9.49841405692986e-05	0.008126935
XM_003126035.3	LOC100525990	12.4323617	0.878952177	11.55340952	0.000676251	0.027099077
XM_003128049.2	LOC100514239	11.35577708	1.207314029	10.14846305	0.001444216	0.043715572
NM_001044575.2	IDH3B	17.03587331	3.222555216	13.81331809	0.0002019	0.013688469
XM_003359868.2	LOC100621622	12.34682256	0.792585427	11.55423713	0.00067595	0.027099077
XM_003132296.3	PSMD6	12.9381618	2.960068463	9.978093337	0.001584136	0.04598329
XM_003481028.1	LOC100626661	15.89817396	3.281014104	12.61715986	0.000382222	0.020392272
NM_001190189.1	SLC25A1	13.88625841	2.177756151	11.70850225	0.000622152	0.026174226

NM_001243574.1	ACAD8	17.7457219	3.650288147	14.09543376	0.000173765	0.012337968
XM_003481569.1	LMBR1L	18.44708293	5.610737162	12.83634576	0.000339951	0.019267245
NM_001122986.2	LOC733658	22.78968663	5.041043281	17.74864335	2.52097203026214e-05	0.003297984
NM_214201.1	GPX1	35.54245684	9.414077757	26.12837909	3.19454138586472e-07	0.000156451
XM_003130472.2	LOC100524641	12.29223647	2.411897405	9.880339067	0.001670542	0.047784839
XM_003357464.1	HDAC9	12.13044155	2.250135641	9.880305905	0.001670572	0.047784839
XM_001929410.2	ATP5B	28.25234372	7.894937141	20.35740658	6.42439411491225e-06	0.00133376
XM_003480667.1	LOC100626654	13.20205972	2.524044732	10.67801498	0.001084163	0.036232753
XM_003133565.2	PLCL1	13.38458678	1.335685921	12.04890086	0.000518229	0.024430002
NM_001101028.1	ACOX1	15.40744454	2.921049233	12.48639531	0.000409926	0.021334045
XM_003354284.1	LOC100627041	12.54922807	2.373755511	10.17547256	0.001423212	0.043285593
XM_003122404.2	LOC100515296	30.39037231	8.857770492	21.53260181	3.47864246874607e-06	0.000959576
XM_003481570.1	LOC100125553	21.66785217	3.457807964	18.2100442	1.97832766013484e-05	0.002791414
XM_003124033.3	LOC100518652	13.88495359	2.35172832	11.53322527	0.000683633	0.027258308
XM_003133109.3	OGDHL	12.80120316	1.021974265	11.7792289	0.000598953	0.025965505
NM_214439.1	HYAL3	12.39647299	2.153965222	10.24250777	0.001372409	0.042279045
XM_003354569.2	LOC100626661	15.89817396	3.281014104	12.61715986	0.000382222	0.020392272
XM_003122991.3	LOC100523423	14.12594048	2.569124412	11.55681607	0.000675013	0.027099077
NM_214397.1	DLST	31.6444191	6.439079945	25.20533915	5.15393183242452e-07	0.000244847
XM_003125702.1	LOC100519480	14.14799605	2.383935233	11.76406082	0.000603853	0.02596903
XM_003127972.1	PARS2	13.68812882	2.092595646	11.59553317	0.000661104	0.026980961
XM_003122803.3	KBTBD4	15.79151232	3.304774642	12.48673768	0.000409851	0.021334045
NM_001185167.1	SSR1	13.30989557	3.188120405	10.12177516	0.001465278	0.044212971
XM_001928354.2	LOC100155360	16.711747	3.219653413	13.49209358	0.000239571	0.015458788

NM_001114289.2	CXCL9	28.58237144	8.3204912	20.26188024	6.75327957755378e-06	0.001366583
NM_001159615.1	KRT8	67.62776297	23.58632656	44.0414364	3.21497273247928e-11	1.22811958380709e-07
XM_001927912.2	TECTB	18.97681998	3.660473524	15.31634645	9.09262953994183e-05	0.008040242
XM_003131192.1	UNC13D	15.85913783	0.778841678	15.08029615	0.000103033	0.008655783
XM_003135480.2	LOC100520774	15.70160178	2.252162279	13.4494395	0.000245079	0.015696806
XM_003126946.1	LOC100525048	16.81090722	2.976280075	13.83462715	0.000199623	0.013631661
NM_001243852.1	STT3A	14.2714185	3.489893267	10.78152524	0.001025182	0.035597702
NM_001044617.1	SLC22A7	19.42987595	1.241075548	18.1888004	2.00051787014877e-05	0.002791414
XM_003131367.3	LOC100514791	21.28490107	2.643057976	18.64184309	1.57720611633216e-05	0.002372019
XM_003134287.3	LOC100512320	14.94436161	4.81800894	10.12635267	0.001461644	0.044173087
XM_001927795.4	HSPA5	18.5710966	5.908516085	12.66258051	0.000373047	0.020184694
XM_003122812.2	ACP2	14.01923104	1.912419129	12.10681191	0.00050238	0.023988624
XM_003124533.3	LOC100520426	18.68067768	3.43852901	15.24214867	9.45690677275346e-05	0.008126935
XM_003132226.1	LOC100517004	12.81389517	0.508599113	12.30529606	0.000451675	0.022428252
XM_003131905.2	OMC	15.95911576	1.754498317	14.20461744	0.000163968	0.011818047
NM_001244227.1	RGC32	13.53149916	1.202477007	12.32902216	0.00044597	0.022298498
NM_001243907.1	HSPA8	45.08128725	10.84005993	34.24122731	4.8686522591268e-09	6.61093708368791e-06
XM_001926349.3	ORMDL1	18.224058	6.274605696	11.9494523	0.000546634	0.024682549
XM_001927440.2	LOC100158185	26.05145233	6.295031565	19.75642077	8.79656726981981e-06	0.001555689
XM_003122683.1	LOC100514446	12.84738948	2.520262513	10.32712696	0.001310891	0.040911795
XM_003355138.1	FLAD1	13.85224374	3.110974514	10.74126923	0.001047725	0.035927357
XM_003355999.1	PRMT1	18.77626343	5.154983147	13.62128028	0.000223636	0.014693627
XM_003124444.1	LOC100517757	27.80562313	4.736227247	23.06939588	1.56258181738256e-06	0.000514574
XM_003357720.1	NOL6	19.15007626	1.73023939	17.41983687	2.99682057584016e-05	0.003765742

XM_003356648.1	LOC100623036	14.81767658	3.843201365	10.97447521	0.000923754	0.032978885
NM_213788.1	LEAP2	53.81891119	22.27864687	31.54026432	1.95347326092943e-08	2.3319587052345e-05
XM_003482319.1	LOC100739812	11.56478737	0.882426926	10.68236045	0.00108162	0.036232753
XM_003481823.1	LOC100739300	12.39125399	1.890954388	10.5002996	0.001193552	0.038257675
XM_003132757.3	LOC100510984	15.57029327	3.557980408	12.01231286	0.000528502	0.024430002
XM_003126864.2	LDHD	23.8291107	2.940944462	20.88816624	4.86893270179589e-06	0.001148106
NM_213739.1	PAQR7	19.66382787	2.730870181	16.93295769	3.87233938267117e-05	0.004482526
XM_003480846.1	LOC100523423	14.12726929	2.569137414	11.55813188	0.000674536	0.027099077
XM_003122549.2	LOC100515705	14.69289012	2.66667571	12.02621441	0.000524575	0.024430002
XM_003354567.2	LOC100626278	12.70140718	1.153310053	11.54809713	0.000678186	0.027099077
XM_003133679.3	LOC100524650	20.18816318	3.453779788	16.73438339	4.29946933971914e-05	0.004654191
XM_003358455.1	LOC100620746	15.41691493	1.208249448	14.20866548	0.000163615	0.011818047
XM_003482888.1	LOC100621855	15.6751184	3.284759329	12.39035907	0.000431556	0.021748614
XM_003131015.3	LOC100519729	29.46896179	5.688871843	23.78008995	1.07993794085104e-06	0.000400409
XM_003483067.1	TNFAIP1	14.69763223	4.484172487	10.21345975	0.001394194	0.042831059
XM_003481166.1	LOC100737616	12.32630338	1.992081934	10.33422145	0.001305862	0.040888464
XM_003353753.2	LOC100626654	13.20205972	2.524044732	10.67801498	0.001084163	0.036232753
XM_003131396.3	LOC100521218	11.45749076	1.16240267	10.29508809	0.001333848	0.041357945
XM_003127209.3	LOC100523003	13.86919705	3.555355649	10.3138414	0.001320362	0.041140142
XM_003357648.1	LOC100623289	10.96541856	1.133333572	9.832084985	0.001714938	0.048526406
XM_001927288.3	GSTO2	48.7101984	24.62537357	24.08482483	9.21839053424911e-07	0.000352143
XM_003131154.2	LOC100513228	14.47751681	0.486055515	13.9914613	0.000183643	0.012884137
XM_003483502.1	SEC24C	19.72330806	3.474054386	16.24925367	5.55314648996719e-05	0.005579945
XM_003129868.2	LOC100520143	31.96625807	11.57650136	20.38975671	6.31670033424125e-06	0.001325813

XM_003481750.1	LOC100737277	22.09753369	7.518659337	14.57887436	0.000134413	0.010474859
XM_003484312.1	BRD3	22.48570693	1.78295799	20.70274894	5.36389417016281e-06	0.001234342
XM_003356482.2	LOC100621142	27.56570648	6.054009063	21.51169742	3.51676937559997e-06	0.000959576
NM_001244382.1	GHITM	18.35203847	4.980527872	13.37151059	0.000255476	0.015965762
NM_001097461.1	SOCS2	19.36183152	1.822496167	17.53933535	2.81424797419216e-05	0.003631901
NM_001112689.1	CIDEC	25.49358397	4.09873742	21.39484655	3.73774499118351e-06	0.000991541
NM_001112688.1	CIDE-B	24.20130913	4.250089096	19.95122003	7.94432461348471e-06	0.001473171
XM_003132358.1	LOC100521991	20.32180106	6.784297015	13.53750404	0.000233843	0.015140355
NM_001048072.1	ARF4	32.6393513	5.227901887	27.41144941	1.64454684981052e-07	9.77232699944326e-05
XM_003128932.1	LOC100512294	11.27021692	0.698072548	10.57214437	0.001148045	0.037355459
XM_003127022.3	PEPD	15.01274951	2.357523141	12.65522637	0.000374517	0.020207004
XM_003357966.1	ACO	17.84372873	4.635849494	13.20787924	0.000278775	0.016984987
XM_003358038.1	LOC100524706	18.36777129	3.700315233	14.66745605	0.000128241	0.010121537
XM_003131848.1	LOC100515576	22.54686587	4.526419324	18.02044654	2.18545023049632e-05	0.002939584
XM_003359731.2	LOC100625674	19.84740821	3.154222383	16.69318583	4.39385382365876e-05	0.004688414
XM_003482321.1	LOC100739812	11.56478737	0.882426926	10.68236045	0.00108162	0.036232753
XM_003482000.1	LOC100737469	19.87862459	2.682865601	17.19575899	3.37188270751465e-05	0.004083475
NM_001243616.1	DPH3	14.98333625	3.868015877	11.11532038	0.000856175	0.031455049
XM_003133834.2	LOC100517616	40.93702928	2.415269376	38.5217599	5.41466094183818e-10	1.29275029986387e-06
XM_003357463.1	HDAC9	13.00161724	2.370174447	10.63144279	0.001111809	0.036803396
NM_001129952.1	POFUT1	12.22266857	2.211190671	10.0114779	0.001555676	0.045642732
NM_001243468.1	CD5L	13.74542874	2.11211127	11.63331746	0.000647809	0.02678172
XM_003126479.3	LOC100518837	16.09383285	0.121191836	15.97264102	6.42645331058178e-05	0.006016924
XM_003127577.2	LOC100525583	12.03253348	1.728211058	10.30432242	0.00132719	0.041285544

XM_003355209.1	LOC100155404	30.40742508	4.113824242	26.29360084	2.93258748507164e-07	0.000147401
NM_001044578.1	GRN	24.16723566	3.961883812	20.20535185	6.95580615739377e-06	0.001369648
NM_001243707.1	CNDP2	20.99147367	3.388537157	17.60293651	2.72167758597064e-05	0.003536329
NM_001113013.1	PLA2G7	28.52830792	3.788591237	24.73971668	6.56179602320783e-07	0.000280115
NM_001044585.1	CTH	14.06335763	2.36921335	11.69414428	0.000626971	0.026319001
XM_003359592.1	ORMDL1	17.78018664	6.270693724	11.50949291	0.000692417	0.027438087
NM_001184755.1	FTSJ1	20.07667131	3.438894294	16.63777702	4.52409022121403e-05	0.004800562
XM_003131295.1	LOC100514364	12.54136197	0.550589113	11.99077286	0.000534646	0.024430002
XM_003353565.2	LOC100516776	30.1307028	7.871839382	22.25886342	2.38256446238871e-06	0.000711047
XM_003481948.1	LOC100738870	41.011806	9.774197174	31.23760883	2.28300049975871e-08	2.56501820855244e-05
NM_001163408.1	SELT	16.91490954	5.112574092	11.80233545	0.000591565	0.025914874
NM_001244556.1	AIMP2	13.34678836	2.749328843	10.59745952	0.001132432	0.037163991
NM_001185132.1	LTA4H	16.44491849	6.585038439	9.859880047	0.001689221	0.04801209
XM_003483983.1	LOC100739855	11.01013273	0.522304201	10.48782853	0.001201635	0.038444274
XM_003480521.1	LOC100621472	19.24446631	2.068756372	17.17570994	3.40765831023937e-05	0.004093476
XM_003127002.1	LOC100522678	23.94497895	5.190379253	18.7545997	1.48664351855565e-05	0.002253563
NM_001244476.1	PNOC	24.24677981	5.962189785	18.28459002	1.90239870956344e-05	0.002736875
XM_003483555.1	XPNPEP1	13.04635681	1.273098684	11.77325813	0.000600877	0.025965505
XM_003481768.1	CSRP2	11.69083331	1.478191765	10.21264155	0.001394812	0.042831059
XM_003134232.2	LOC100526021	18.30158631	5.614646538	12.68693977	0.000368218	0.020036956
XM_003128042.1	PRDX1	23.2165552	6.410810035	16.80574517	4.14077508783528e-05	0.004625076
XM_003123370.2	LOC100515361	25.04793617	5.829449366	19.21848681	1.16578943576418e-05	0.001919533
XM_001927549.2	COPZ1	16.55090415	4.132434816	12.41846934	0.000425109	0.021571659
XM_001929375.3	FLAD1	13.86426195	3.115177065	10.74908488	0.001043309	0.035904867

XM_003123371.3	LOC100515528	14.88046859	2.523762895	12.3567057	0.000439405	0.022027932
XM_003355375.1	LOC100127131	22.48439606	2.525767992	19.95862807	7.91360389640339e-06	0.001473171
NM_001244149.1	COPS3	21.86142852	5.127579198	16.73384932	4.30067976896487e-05	0.004654191
XM_003130678.1	NOL6	15.58833453	1.737261297	13.85107323	0.000197884	0.013631661
NM_213785.1	LOC396677	34.61239507	15.63759186	18.9748032	1.32456110322199e-05	0.002056839
XM_003124605.1	LOC100521260	17.23864259	2.509689858	14.72895273	0.000124125	0.009886764
XM_003481798.1	LOC100738166	16.79133552	2.763881498	14.02745402	0.000180161	0.012697688
NM_001244557.1	AIMP2	13.34678836	2.749328843	10.59745952	0.001132432	0.037163991
XM_003480518.1	LOC100738731	28.58805847	14.12824129	14.45981718	0.000143182	0.01081732
XM_003131408.1	LOC100524706	16.50564673	4.019241335	12.48640539	0.000409924	0.021334045
XM_003127682.1	LOC100516807	17.37749367	2.115092722	15.26240094	9.35604439908921e-05	0.008085993
XM_001927658.3	LOC100154755	22.66407362	1.650545855	21.01352776	4.56051927399947e-06	0.001107935
XM_003121615.1	LOC100525797	29.07663736	9.031304364	20.04533299	7.56278730207338e-06	0.001459083
XM_003126889.3	LOC100512659	11.69221331	1.631073292	10.06114002	0.001514295	0.045121737
NM_214127.2	SOD2	17.92860913	6.48366425	11.44494488	0.000716888	0.027819393
XM_003482948.1	LOC100739290	11.32513431	0.701906334	10.62322798	0.001116759	0.036851164
XM_003132411.3	LOC100518399	23.50235346	4.976207435	18.52614602	1.67589480010166e-05	0.002481364
XM_001926778.1	LOC100154285	13.99507285	2.112689185	11.88238367	0.000566674	0.025288476
NM_001139476.1	DAZAP2	19.36295163	3.961867699	15.40108393	8.69384100353754e-05	0.007759456
NM_213973.1	HSP90AA1	36.22836709	9.462931616	26.76543547	2.29709508303699e-07	0.00011858
NM_214233.1	GLRX	22.82242279	5.905907649	16.91651514	3.90602619498859e-05	0.004494283
XM_003132180.1	LOC100520587	19.57521899	3.488771916	16.08644708	6.05155784252354e-05	0.005779238
XM_003482652.1	LOC100628005	14.42290688	2.635409123	11.78749775	0.000596298	0.025965505
XM_003481817.1	LOC100737013	20.41426652	6.035810678	14.37845585	0.000149503	0.01102513

NM_001123140.1	MRPS18B	13.93496464	2.522964007	11.41200063	0.000729712	0.028043261
XM_003127795.1	SF3A3	14.47867489	2.368833341	12.10984155	0.000501564	0.023988624
XM_003128658.1	LOC100156930	18.04043769	5.271312907	12.76912478	0.000352388	0.019396555
XM_003134980.3	LOC100525970	17.3522621	6.473272143	10.87898996	0.000972613	0.034205815
XM_003483938.1	LOC100739269	15.96583768	3.172915918	12.79292176	0.000347933	0.019396555
XM_003354001.2	LOC100623769	25.87915327	3.336344888	22.54280838	2.05512438966604e-06	0.000633111
XM_003128038.1	PRDX1	23.22676414	6.388002566	16.83876158	4.06935876382519e-05	0.004625076
XM_003481099.1	LOC100737559	22.33421287	5.216835558	17.11737731	3.51393206571293e-05	0.004142969
NM_001244383.1	GHITM	17.66621031	5.17888665	12.48732366	0.000409723	0.021334045
NM_001206441.1	TAP2	13.93136954	0.783696299	13.14767325	0.000287877	0.01736856
XM_003354105.1	LOC100515361	25.0965595	5.811612467	19.28494704	1.12590511684996e-05	0.001886385
XM_003357294.1	CRYAB	31.13946305	12.31638667	18.82307639	1.43421323881565e-05	0.002191478
XM_003127502.2	LOC100525522	13.5305999	1.591128381	11.93947152	0.000549571	0.024756603
XM_003481263.1	LOC100739228	13.41024479	2.99062845	10.41961634	0.001246838	0.039570237
XM_003356202.1	GALE	26.55941265	5.023023879	21.53638878	3.47178004789228e-06	0.000959576
XM_001928658.2	LOC100151845	16.29863285	2.92988293	13.36874992	0.000255852	0.015965762
XM_003357462.1	HDAC9	12.13044155	2.250135641	9.880305905	0.001670572	0.047784839
NM_001184895.1	PMM1	29.82613499	7.516535866	22.30959912	2.3204377529451e-06	0.000703498
XM_003483721.1	IDH1	16.17999814	3.200252652	12.97974549	0.000314879	0.018505183
XM_003121294.1	LOC100520526	20.07895018	4.791958682	15.2869915	9.23502999605752e-05	0.008054296
XM_001927253.2	DRG1	18.97294037	4.727951609	14.24498876	0.000160488	0.011655181
XM_003358262.2	LOC100623827	15.5466241	1.835944023	13.71068007	0.000213238	0.014240747
XM_003353872.1	ACP2	14.0111363	1.90986047	12.10127583	0.000503873	0.023999952
NM_214407.1	GPX4	18.7159348	5.694907881	13.02102692	0.000308013	0.018213764

XM_003357928.1	ACTB	30.04318584	7.014267413	23.02891843	1.59582808734182e-06	0.000516616
XM_003483155.1	LOC100737352	33.91359218	14.67741798	19.2361742	1.1550383042791e-05	0.001918368
XM_003355743.1	KARS	22.26825611	6.004232473	16.26402363	5.5100252746354e-05	0.005579945
NM_213874.1	MDH1	19.74108127	5.238024865	14.50305641	0.000139932	0.010772409
NM_001097516.1	SDHD	24.56941872	5.472665899	19.09675282	1.24256118229082e-05	0.001977743
NM_001078684.1	LOC780435	15.37953876	3.923355807	11.45618295	0.000712565	0.027819393
XM_003131728.2	LOC100520269	16.68107801	3.767551597	12.91352642	0.000326216	0.018938383
XM_003355206.2	LOC100523785	13.9307152	0.829244638	13.10147057	0.000295064	0.017666845
NM_001185135.1	ARPC3	15.43199801	3.953742405	11.4782556	0.000704152	0.027616635
NM_001128435.1	LBP	22.00080692	6.926158554	15.07464837	0.000103342	0.008655783
XM_003125293.3	LOC100522014	30.35275253	6.922618712	23.43013382	1.29533487802647e-06	0.000466809
XM_003354180.1	LOC100526157	15.44802275	3.996534476	11.45148828	0.000714368	0.027819393
XM_003482090.1	LOC100737897	21.6176197	3.421927819	18.19569188	1.99329211678645e-05	0.002791414
XM_003483719.1	IDH1	15.52101153	3.529643659	11.99136787	0.000534475	0.024430002
NM_001005728.1	HIG2	23.06391742	7.788551789	15.27536563	9.29204538206996e-05	0.008067185
XM_003483237.1	LOC100739335	18.99017701	2.435219904	16.5549571	4.72602825342117e-05	0.004987135
NM_213966.1	LCTHIO	14.44989197	4.053947612	10.39594436	0.001262924	0.039780006
XM_003132179.1	LOC100520587	19.52613924	3.410293951	16.11584529	5.95834246379656e-05	0.005756828
XM_001925349.4	SNRPA1	21.80140354	1.876473044	19.9249305	8.05431552020064e-06	0.001479206
XM_001925393.2	VPS52	12.20628471	1.274221141	10.93206357	0.000945142	0.033679488
XM_001924268.2	EIF2B5	10.51972022	0.464642664	10.05507755	0.001519286	0.045129653
XM_003123191.2	LOC100519412	16.05426448	3.281296786	12.77296769	0.000351664	0.019396555
XM_003122500.3	LOC100521064	20.23217393	3.492426704	16.73974723	4.28733157810113e-05	0.004654191
XM_003482162.1	LOC100738270	14.35104788	1.391083424	12.95996446	0.000318223	0.018644367

XM_001925433.1	RAN	19.45287208	5.840143721	13.61272835	0.000224657	0.014693627
XM_003133180.3	XPNPEP1	13.04635681	1.273098684	11.77325813	0.000600877	0.025965505
XM_003483218.1	LOC100739864	22.50918494	3.627211285	18.88197365	1.3906054437518e-05	0.002141981
XM_003135011.2	LOC100526026	14.77691023	1.909972277	12.86693795	0.000334438	0.01923246
NM_001143708.1	GK	17.54967503	6.114820789	11.43485424	0.000720791	0.027819393
XM_003128041.1	PRDX1	22.91927109	6.420295527	16.49897556	4.8676367257805e-05	0.00508043
XM_003360390.1	LOC100155098	15.40990817	4.820316808	10.58959136	0.001137261	0.03719467
NM_001244377.1	PTPMT1	24.37033579	9.39788366	14.97245213	0.000109092	0.009020178
XM_003122798.2	LOC100525679	39.33963129	18.34785928	20.991772	4.61260061379587e-06	0.001107935
NM_001244404.1	PPP6C	14.14495349	4.069814457	10.07513904	0.001502833	0.044972751
NM_001164510.1	SLC25A25	23.55021988	6.839181328	16.71103855	4.35269974999875e-05	0.004670594
XM_003134087.1	GABRG2	36.41452964	7.414370712	29.00015893	7.23723609930005e-08	5.52924837986524e-05
XM_003484101.1	LOC100737045	13.15221835	2.091955436	11.06026292	0.000881976	0.031844515
XM_003128065.1	LOC100517766	15.27371838	1.910512928	13.36320545	0.000256609	0.015965762
XM_003480842.1	LOC100738590	27.68941871	7.033638723	20.65577999	5.49709876307425e-06	0.001249936
XM_001924419.2	LOC100156015	15.24206054	3.458589891	11.78347065	0.00059759	0.025965505
XM_003481471.1	LOC100738895	12.33254435	0.668297706	11.66424665	0.000637127	0.026570159
XM_001929074.2	LOC100156930	18.03812865	5.265740253	12.7723884	0.000351773	0.019396555
XM_001928557.2	LOC100157763	18.41554917	6.395267603	12.02028157	0.000526248	0.024430002
XM_003131141.3	LOC100526132	14.4255582	2.688687274	11.73687092	0.00061274	0.025996335
XM_003483617.1	ODZ1	12.84503233	0.789514016	12.05551831	0.000516393	0.024413624
NM_001244121.1	DEGS1	18.23761283	6.476579963	11.76103287	0.000604837	0.02596903
XM_003127991.3	LOC100517534	18.07495988	6.241651079	11.8333088	0.000581805	0.025670163
XM_003354272.1	P4HA2	18.87785886	3.819397388	15.05846147	0.000104232	0.008655783

XM_001927577.2	ABHD4	16.10592534	3.288367848	12.81755749	0.000343382	0.019358185
XM_001928213.2	PHF7	12.0354073	1.444725035	10.59068227	0.00113659	0.03719467
XM_003360301.2	LOC100628142	24.19273883	10.75449786	13.43824097	0.000246547	0.015696806
NM_001078670.1	LOC780415	13.73707823	2.472041721	11.26503651	0.000789806	0.029812845
XM_003134336.1	LOC100512637	16.70888352	5.593980055	11.11490347	0.000856368	0.031455049
XM_003484097.1	LOC100738608	15.08015108	3.530033896	11.55011719	0.00067745	0.027099077
XM_003121089.1	BRP44L	22.69318065	4.858156476	17.83502417	2.40907790334255e-05	0.003195374
XM_003358419.1	C3orf75	21.93095152	2.799314174	19.13163734	1.22005632746669e-05	0.001977743
XM_003130612.1	LOC100511035	14.45045923	4.196447538	10.25401169	0.001363877	0.042188797
XM_003124487.1	HSD3B7	16.43690723	6.613964296	9.822942931	0.001723484	0.048552411
NM_001099923.1	MYD88	40.72583763	10.52168159	30.20415603	3.88875261991828e-08	3.71375875202196e-05
NM_001243292.1	IMP3	15.40176552	3.580757566	11.82100796	0.000585661	0.025774502
XM_003481634.1	LOC100514459	12.37957867	1.702511052	10.67706762	0.001084719	0.036232753
XM_003123992.2	LOC100524488	22.79422141	3.019240997	19.77498041	8.71155961457148e-06	0.001555054
XM_003481098.1	LOC100737559	22.33421287	5.216835558	17.11737731	3.51393206571293e-05	0.004142969
XM_003480765.1	LOC100739130	16.68040435	0.927279544	15.75312481	7.21686325190207e-05	0.006595315
XM_003130194.2	HDAC9	13.00161724	2.370174447	10.63144279	0.001111809	0.036803396
XM_003128543.1	LOC100519262	13.89543993	1.223720933	12.671719	0.000371228	0.020143341
XM_001928070.2	LOC100155023	15.11098935	4.103484497	11.00750485	0.000907437	0.032517918
XM_003356316.1	SF3A3	14.26776718	2.228790764	12.03897641	0.000520996	0.024430002
NM_214330.1	GPI	13.63483638	2.148779619	11.48605676	0.000701202	0.027557545
NM_001142839.1	TNFRSF12A	49.87876216	20.72209335	29.15666881	6.67555740685444e-08	5.52924837986524e-05
XM_003357844.2	LOC100621855	15.25313972	3.250510421	12.0026293	0.000531255	0.024430002
NM_001011727.1	AHCY	13.02308796	2.380881394	10.64220656	0.001105357	0.036781047

NM_001123198.1	ST7	12.90537781	2.405461772	10.49991604	0.0011938	0.038257675
XM_003480778.1	LOC100739748	11.42994227	0.807597723	10.62234455	0.001117293	0.036851164
NM_001244509.1	FIBP	15.19483441	1.579539444	13.61529496	0.00022435	0.014693627
XM_003126322.3	LOC100519091	12.71297046	2.169057471	10.54391299	0.001165714	0.037737508
XM_003481864.1	LOC100738209	12.14692144	1.851217744	10.29570369	0.001333403	0.041357945
XM_003354500.1	LOC100626099	25.18503997	5.317224787	19.86781518	8.29857398521039e-06	0.00150955
NM_001001630.1	PDZD11	15.62906269	4.131654801	11.49740789	0.000696933	0.02750294
XM_003126264.3	PA2G4	12.40800366	2.53063074	9.877372917	0.001673237	0.047784839
XM_003356476.2	LOC100627844	26.15381549	5.751597761	20.40221773	6.27570262468069e-06	0.001325813
NM_001100196.1	EIF4A1	19.21446198	4.746395563	14.46806642	0.000142556	0.01081732
XM_001928193.4	SEC24C	19.72330806	3.474054386	16.24925367	5.55314648996719e-05	0.005579945
XM_003356844.1	LOC100621287	32.51925665	7.351690146	25.1675665	5.2558712759776e-07	0.000244847
NM_213927.1	GOT1	16.68868202	0.821206509	15.86747551	6.79362674715911e-05	0.006298945
XM_003130093.3	GUCY1A2	13.54613419	2.500120626	11.04601357	0.000888781	0.032029655
XM_003122352.2	LOC100516352	16.54680422	5.782583382	10.76422083	0.001034811	0.035676704
XM_003359369.1	LOC100627133	19.58595921	2.335125975	17.25083323	3.27553870482777e-05	0.004054524
XM_003354990.2	LOC100621539	20.95184803	1.247198886	19.70464914	9.03812150132577e-06	0.001579243
XM_003133075.3	TTC18	12.84627036	0.726813778	12.11945658	0.000498984	0.023946238
NM_001097416.1	MX2	21.211285	10.53484283	10.67644216	0.001085086	0.036232753
NM_214134.1	EPO	23.07784801	11.2619888	11.81585921	0.000587283	0.02578646
NM_001044612.1	TUBB2A	13.5032827	3.32665434	10.17662836	0.00142232	0.043285593
NM_214358.1	SAT1	14.02813619	2.96613104	11.06200515	0.000881148	0.031844515
XM_003133100.2	LOC100517243	12.83842011	1.589843394	11.24857672	0.000796841	0.029901104
NM_214130.1	CYSLT2	13.07164955	0.769844848	12.3018047	0.000452521	0.022428252

XM_003481415.1	LOC100154856	12.83563544	2.422662113	10.41297333	0.001251331	0.039570237
NM_001134355.1	ARPC1A	19.24924203	5.132895478	14.11634656	0.000171844	0.012247085
XM_003132181.1	LOC100520587	19.55736308	3.433224523	16.12413856	5.93230839311953e-05	0.005756828
NM_001243811.1	HOMER1	15.55564187	4.286361312	11.26928056	0.000788003	0.029803664
XM_001927335.3	LOC100154856	12.83563544	2.422662113	10.41297333	0.001251331	0.039570237
NM_001123220.1	CREB3	15.29591974	3.036824009	12.25909573	0.000462996	0.022674923
XM_003128619.2	LOC100521100	12.05194449	1.417465676	10.63447881	0.001109986	0.036803396
NM_001103211.1	SNCG	24.88431719	6.885110971	17.99920622	2.20997102654552e-05	0.002951779
XM_003353794.1	LOC100623923	11.30349719	1.507817238	9.795679952	0.001749223	0.048916783
XM_003360323.1	LOC100623679	22.92379595	5.731453943	17.19234201	3.37795319179657e-05	0.004083475
NM_001044544.1	TUBA1B	17.25531382	4.110157999	13.14515582	0.000288264	0.01736856
XM_003480851.1	LOC100737815	15.75549938	3.52705282	12.22844656	0.000470664	0.022874495
XM_003359343.1	LOC100627227	30.78376684	3.480948285	27.30281856	1.73957653393231e-07	9.77232699944326e-05
XM_003127946.1	PGM1	26.36478194	4.258594757	22.10618719	2.57976924344927e-06	0.000758055
XM_003128016.3	LOC100523742	26.03269046	5.052498804	20.98019166	4.64056592852646e-06	0.001107935
NM_001167649.1	HSD17B2	29.46787344	12.73947193	16.72840151	4.31304641969055e-05	0.004654191
XM_003353758.1	LOC100519350	13.18009198	2.503349472	10.67674251	0.001084909	0.036232753
XM_003482239.1	LOC100156967	22.04014078	5.224801274	16.81533951	4.11989309230254e-05	0.004625076
XM_003480781.1	LOC100737135	28.47923674	8.66572309	19.81351365	8.53769251329961e-06	0.001538396
XM_001929558.1	CDKN1A	52.07335428	22.55239446	29.52095983	5.53152623705699e-08	4.80237050580857e-05
XM_003123453.2	ILVBL	15.99664651	2.071379981	13.92526653	0.000190224	0.013211931
NM_001243370.1	RANGRF	17.5524	2.821907347	14.73049266	0.000124024	0.009886764
XM_003124606.1	LOC100521260	17.23864259	2.509689858	14.72895273	0.000124125	0.009886764
XM_003481815.1	LOC100736819	19.81403688	8.286928093	11.52710879	0.000685886	0.027258308

XM_003480931.1	IK	20.55049393	3.762189705	16.78830422	4.17900804212668e-05	0.004640643
XM_003133742.3	UGT1A3	36.95001502	15.62593809	21.32407693	3.8782974780549e-06	0.00100102
XM_003482240.1	LOC100156967	22.04014078	5.224801274	16.81533951	4.11989309230254e-05	0.004625076
XM_003123476.1	C2H19orf42	13.82450699	2.641503079	11.18300391	0.0008255	0.0306752
XM_003131543.3	LOC100521291	15.52836371	0.956468712	14.571895	0.000134912	0.010474859
XM_003354697.2	LOC100622812	59.84057199	32.78912817	27.05144382	1.98112198313005e-07	0.000108113
NM_001144926.2	PSMB6	13.37256201	3.495570598	9.876991412	0.001673584	0.047784839
NM_214364.1	VDAC1P5	17.37034734	3.111900249	14.25844709	0.000159344	0.011616302
XM_003483189.1	MANF	17.97503572	6.959601454	11.01543427	0.000903564	0.03243997
XM_003129258.1	LOC100517665	18.07160113	6.79978917	11.27181196	0.000786929	0.029803664
XM_003483181.1	LOC100620512	14.03299689	0.948469938	13.08452695	0.000297745	0.017771656
XM_003358591.1	LOC100514874	16.95764414	4.120697637	12.83694651	0.000339842	0.019267245
XM_003359825.1	GABRG2	36.41452964	7.414370712	29.00015893	7.23723609930005e-08	5.52924837986524e-05
XM_003483720.1	IDH1	15.52101153	3.529643659	11.99136787	0.000534475	0.024430002
NM_001168419.1	ZFP36	30.36043376	16.75394317	13.60649059	0.000225405	0.014693627
XM_003480660.1	LOC100739347	26.43133512	4.355966095	22.07536902	2.62152001317961e-06	0.000758652
XM_003128657.1	LOC100156930	18.04043769	5.271312907	12.76912478	0.000352388	0.019396555
XM_003358202.1	LOC100515576	22.58585371	4.531804788	18.05404893	2.14721513717153e-05	0.002908639
XM_003360048.2	LOC100626091	23.4082622	5.913658749	17.49460345	2.88124361319531e-05	0.003644487
XM_003483346.1	LOC100738012	27.06559414	13.26080597	13.80478817	0.000202819	0.013688469
NM_214019.1	DHRS4	36.19371486	12.07756121	24.11615366	9.06962875468764e-07	0.000352143
XM_003126856.2	KARS	22.26825611	6.004232473	16.26402363	5.5100252746354e-05	0.005579945
NM_001044581.1	TAP1	14.58728926	1.534477907	13.05281135	0.00030283	0.017962868
XM_001925061.1	LOC100154617	41.4386205	22.98325345	18.45536705	1.73930300869651e-05	0.002552121

XM_003128039.1	PRDX1	22.91927109	6.420295527	16.49897556	4.8676367257805e-05	0.00508043
NM_001243528.1	MRPL15	18.51472985	5.880485459	12.63424439	0.000378744	0.020320268
XM_003129127.1	LOC100514171	28.61319305	8.363698504	20.24949454	6.79714183660085e-06	0.001366583
XM_003358715.1	ECE2	14.62127476	1.908987103	12.71228765	0.000363261	0.019880461
XM_003130436.3	LOC100515848	15.24779616	3.592675851	11.65512031	0.000640261	0.026642654
XM_003358153.1	NLE1	17.00816422	2.511877791	14.49628643	0.000140436	0.010772409
NM_001244543.1	DDX56	17.49465499	2.781022005	14.71363299	0.000125138	0.009917593
XM_001926938.4	LOC100152525	14.86659451	2.838961612	12.0276329	0.000524176	0.024430002
NM_001123083.1	BCKDHA	18.18115086	2.969016029	15.21213483	9.60840071531077e-05	0.008156465
NM_001031788.1	RAB11A	14.44325041	3.341709042	11.10154137	0.00086256	0.031505351
XM_003132177.1	LOC100520587	19.50288458	3.403009214	16.09987536	6.00879906665908e-05	0.005767239
XM_003481476.1	SEC22B	13.15788556	1.658064287	11.49982127	0.000696029	0.02750294
NM_001244153.1	MDH2	18.95420931	3.667066859	15.28714245	9.23429201037385e-05	0.008054296
XM_003127945.2	PGM1	22.37030236	3.828090681	18.54221168	1.6618285812342e-05	0.00247976
NM_001244488.1	NUCB1	14.70416941	3.100555046	11.60361436	0.000658238	0.026921499
XM_003357604.1	LOC100624161	18.33302413	5.635122397	12.69790173	0.000366066	0.01997675
XM_003133583.3	LOC100525788	11.19559053	0.272902207	10.92268832	0.000949937	0.033787317
XM_003482120.1	LOC100739771	24.6790128	4.101564096	20.57744871	5.72667468656807e-06	0.001256625
XM_003481733.1	LOC100738422	27.04055345	6.314666964	20.72588649	5.29947164396027e-06	0.001234342
XM_003353781.1	LOC100515705	14.69289012	2.66667571	12.02621441	0.000524575	0.024430002
XM_003356001.1	PRMT1	18.77626343	5.154983147	13.62128028	0.000223636	0.014693627
XM_003128040.1	PRDX1	23.22872061	6.387419755	16.84130086	4.06391770777503e-05	0.004625076
NM_001244459.1	SGK1	21.68055902	10.93649931	10.74405971	0.001046146	0.035927357
XM_003123324.1	LOC100520362	12.24095453	2.177611186	10.06334334	0.001512485	0.045121737

NM_001099932.1	PGK1	16.68046076	6.094873353	10.5855874	0.001139727	0.037211592
NM_001243376.1	GNPNAT1	13.97762382	3.798638566	10.17898525	0.001420503	0.043285593
XM_001925833.4	IPO4	15.19605746	0.872123321	14.32393413	0.000153896	0.011305418
XM_003359985.2	LOC100513892	24.53584553	6.380028847	18.15581669	2.03546757109319e-05	0.002817205
XM_003355907.1	LOC100623625	30.6151021	17.17304295	13.44205915	0.000246045	0.015696806
XM_003126320.1	LOC100518554	19.91147309	2.830038451	17.08143464	3.58106687305959e-05	0.004196219
NM_001144843.1	ADAMTS1	27.66443866	12.6018133	15.06262536	0.000104002	0.008655783
NM_001123104.1	CTSZ	14.52641309	2.265864924	12.26054817	0.000462636	0.022674923
XM_003132178.1	LOC100520587	19.52613924	3.410293951	16.11584529	5.95834246379656e-05	0.005756828
XM_003483014.1	COL1A1	19.73935921	5.895327264	13.84403195	0.000198627	0.013631661
NM_001011507.1	LAMP-1	13.59671308	1.133783817	12.46292926	0.000415108	0.021351315
NM_214405.1	MUT	14.72500198	2.972934386	11.75206759	0.000607757	0.02596903
NM_001123113.1	FOS	25.89146801	5.866786421	20.02468159	7.6449019190683e-06	0.001460176
NM_001185186.1	DNPEP	11.70994711	1.009201666	10.70074544	0.001070924	0.036232753
XM_001929196.2	LOC100154911	10.94911072	0.92382082	10.0252899	0.001544054	0.045604712
XM_003481158.1	LOC100736718	24.65820856	11.26442323	13.39378533	0.000252459	0.015914106
XM_003126528.2	COPS7A	12.67170831	1.796757152	10.87495116	0.000974736	0.034205815
XM_003481571.1	LOC100125553	21.6079837	3.420801792	18.18718191	2.00221868679185e-05	0.002791414
NM_001128487.1	GPR39	15.64850055	1.815874248	13.8326263	0.000199836	0.013631661
NM_001110419.1	GNMT	17.62114338	2.070144231	15.55099915	8.03091938382794e-05	0.007235404
XM_003124443.1	LOC100517757	27.80562313	4.736227247	23.06939588	1.56258181738256e-06	0.000514574
XM_003132142.1	LOC100524900	50.18575089	12.7622486	37.42350229	9.50695744350583e-10	1.81582887170961e-06
XM_001927635.2	LOC100155406	14.72684809	2.571993571	12.15485452	0.000489603	0.023555189
XM_003483435.1	LOC100157055	12.60133178	2.738589678	9.862742104	0.001686595	0.04801209

NM_001078666.1	PSEN2	28.70303665	5.869046033	22.83399062	1.76614345392956e-06	0.000562222
XM_003354090.2	LOC100624460	18.59626131	6.742819509	11.8534418	0.000575549	0.025565072
XM_003354614.1	LOC100520369	12.24199491	2.23957721	10.0024177	0.001563348	0.045797479
XM_003134450.3	LOC100157757	14.81961599	4.158296224	10.66131977	0.001093993	0.036466435
XM_001929262.4	LOC100157687	34.14186543	4.548573195	29.59329224	5.32890316318202e-08	4.80237050580857e-05
XM_003130748.2	LOC100513426	23.6027361	8.786630431	14.81610567	0.000118519	0.009592003
XM_003482350.1	LOC100739772	23.43659427	2.847975731	20.58861854	5.6933586116692e-06	0.001256625
XM_003124919.3	LOC100511252	24.6956792	4.476118874	20.21956033	6.90433426409065e-06	0.001369648
XM_003482482.1	LOC100739483	12.68276425	2.880174687	9.802589562	0.001742663	0.048804786
XM_003127093.3	ZNF829	21.15361623	6.336553333	14.81706289	0.000118459	0.009592003
NM_001185142.1	ATP5A1	25.11724719	5.792668541	19.32457865	1.10277640087419e-05	0.001873928
XM_003122940.1	SAAL1	12.47008604	2.71839306	9.751692979	0.001791576	0.049882085
XM_003482246.1	AKAP6	12.10455478	0.74122818	11.3633266	0.000749085	0.028729981
XM_003128704.2	LOC100523620	93.05251184	44.1168644	48.93564744	2.64499533386697e-12	1.26298527192148e-08
NM_001100193.1	EIF4A3	24.93584639	4.876330874	20.05951552	7.50690778783536e-06	0.001459083
XM_003357269.1	GRIA4	12.74789938	1.646628424	11.10127096	0.000862686	0.031505351
NM_001122994.1	OAZ1	14.71245267	3.568577349	11.14387532	0.000843096	0.031087121
NM_001244246.1	NOP2	17.33108134	2.874912318	14.45616902	0.000143459	0.01081732
NM_001243341.1	POLR2H	14.90249498	4.397263849	10.50523113	0.001190371	0.038257675
XM_003133369.1	LOC100524716	14.10539565	1.153296618	12.95209903	0.000319563	0.018665606
XM_003134862.1	LOC100524778	16.23731782	4.513135242	11.72418258	0.000616931	0.026054069
XM_003353963.1	LOC100623333	16.63623631	5.110662747	11.52557356	0.000686453	0.027258308
XM_003482320.1	LOC100739812	11.56478737	0.882426926	10.68236045	0.00108162	0.036232753
NM_001123192.1	RPL7L1	14.39491811	3.320227571	11.07469054	0.00087514	0.031838435

XM_003126297.2	LOC100514459	12.37957867	1.702511052	10.67706762	0.001084719	0.036232753
NM_001001643.1	GDI2	14.13810167	2.448809513	11.68929216	0.000628608	0.026329856
XM_001927727.2	LOC100155734	17.89564441	3.276157001	14.61948741	0.000131547	0.010303368
XM_003126114.3	LOC100513730	19.92452724	3.467230843	16.45729639	4.97583048890693e-05	0.005165128
XM_003482380.1	LOC100738638	14.81021175	4.404065094	10.40614665	0.001255965	0.039651142
NM_001244418.1	DLL4	13.75284044	2.88034596	10.87249447	0.00097603	0.034205815
XM_003482870.1	LOC100737759	39.6074946	12.23830066	27.36919394	1.68087726404309e-07	9.77232699944326e-05
XM_003357489.2	LOC100524640	20.44919066	3.206912016	17.24227864	3.29032013260955e-05	0.004054524
XM_001927348.1	LOC100154856	12.83563544	2.422662113	10.41297333	0.001251331	0.039570237
NM_001097412.1	HMBS	12.26994932	1.46267828	10.80727104	0.001011022	0.035307359
NM_001244123.1	DHDDS	12.77673657	1.104836815	11.67189976	0.000634512	0.026518986
XM_003128368.2	LOC100524445	15.32876254	3.146864498	12.18189804	0.000482555	0.023274757
XM_003353957.1	LOC100622895	14.51764717	1.312824234	13.20482293	0.00027923	0.016984987
NM_001160427.1	ARF1	34.40877412	6.29407673	28.11469739	1.14334277601102e-07	7.53029207648641e-05
NM_001195119.1	PDIA6	17.40398266	4.587518571	12.81646409	0.000343582	0.019358185
XM_003481898.1	PRMT1	18.77626343	5.154983147	13.62128028	0.000223636	0.014693627
NM_001244354.1	SEC61A1	18.39219753	7.191028046	11.20116948	0.000817458	0.030495014
XM_003129961.3	LOC100524210	44.76181718	10.64565901	34.11615817	5.19183540603763e-09	6.61093708368791e-06
XM_003123337.1	LOC100524304	22.42660109	4.633984319	17.79261677	2.46337296525567e-05	0.003244857
XM_001924196.4	LOC100156819	13.74240214	2.953056313	10.78934583	0.00102086	0.035516242