SUPPLEMENTARY MATERIALS AND METHODS

Generation and genotyping of conditional knockout mice

The animal experiments were conducted in accordance with the guidelines of the Arbeitsgemeinschaft der Tierschutzbeauftragten in Baden-Württemberg (Officials for Animal Welfare) and were approved by the Regierungspräsidium Karlsruhe, Germany (Az. G-6/17).

Mice harboring a loxP-containing *Alox12b* allele were reported earlier (Epp et al., 2007). The Tk/neo resistance cassette of the targeted allele was removed by crossing with Cre-deleter mice to obtain *Alox12b*$^{+/fl}$ mice in which exon 8 of *Alox12b* is flanked by two loxP sites. Mice with a tamoxifen-inducible epidermis-specific inactivation of *Alox12b* (*Alox12b*$^{fl/fl}$/*K14-CreJ* mice) were generated by crossing *Alox12b*$^{fl/fl}$ animals with mice expressing a Cre recombinase fused to the human estrogen receptor under the control of a truncated keratin-14 promoter (*K14-Cre-ER*^{T2} knock-in, designated *K14-CreJ*; Amen et al., 2013). The *Alox12b*$^{fl/fl}$/*K14-CreJ* mice used in this study were backcrossed into the hairless SKH1 background for at least five generations. *Alox12b* inactivation was induced by repeated intraperitoneal injection of 1 mg tamoxifen (Merck, Darmstadt, Germany) in 100 µl sunflower seed oil (Merck, Darmstadt, Germany) as indicated in Supplementary Figure S1. Homozygous floxed littermates (*Alox12b*$^{fl/fl}$) treated in parallel served as controls. Genotyping was performed by PCR as described earlier (Epp et al., 2007). Primers are listed in Supplementary Table S1.

Determination of transepidermal water loss

Transepidermal water loss was quantified with the Tewameter ZM300 (Courage-Khazaga Electronics, Cologne, Germany) according to the manufacturer's instructions.

Bright field and immunofluorescence microscopy

For light microscopic examination, tissue samples fixed in 4% paraformaldehyde and dehydrated in 70% ethanol were embedded in paraffin. Sections of 5 µm thickness were mounted on slides, dewaxed, rehydrated, and stained with hematoxylin and eosin. Images were obtained using an Axio Scope.A1 microscope and AxioVision Software (Carl Zeiss, Oberkochen, Germany).

For immunofluorescence microscopy, 3 µm cryosections of the tissue samples were fixed in acetone for 10 minutes at −20 °C, then permeabilized with 0.5% Triton X-100 in phosphate buffered saline, blocked with 1% BSA in phosphate buffered saline for 20 minutes, and incubated with a mouse monoclonal anti-12R-LOX antibody, as described previously (Epp et al., 2007). Cell nuclei were counterstained with Hoechst 33258 dye. Immunofluorescence images were taken on a LSM 700 confocal microscope with an EC Plan-Neofluar 40x/1.30 Oil DIC objective lens (Carl Zeiss, Oberkochen, Germany) and the software ZEN 2009 (version 5.5.0.375; scan parameters 1024 × 1024 Pixel, speed 2, line average 2).

Electron microscopy

For ultrastructural analysis by transmission electron microscopy, frozen skin samples from mouse dorsal skin were thawed in absolute pyridine for 2 hours and then fixed in 2.0% glutaraldehyde and 2.0% paraformaldehyde in 0.1 M cacodylate buffer overnight. Tissues were then rinsed and fixed in 1.0% osmium tetroxide in 0.2 M cacodylate buffer for 1 hour, dehydrated through a graded series of alcohol, followed by two changes of propylene oxide, and embedded in epoxy resin. The samples were cut on a Leica Ultracut E microtome (Leica Microsystems, Wetzlar, Germany) and imaged on a JEOL 100CX transmission electron microscope (JEOL, Tokyo, Japan) using a Gatan digital camera. Quantification of the corneocyte lipid envelope was performed by measuring the length of both corneocyte lipid envelope and cornified envelope in five random high-powered electron micrographs of the mid stratum corneum. The observer recording these measurements was blinded to the experimental groups. Statistical significance was determined using Student *t* test.

Quantitative real-time reverse transcriptase–PCR

Skin of killed animals was extended on paper and immediately frozen in liquid nitrogen. The epidermis was separated from the dermis by scratching. Total RNA samples were extracted from epidermal sheets using Precellys Lysing kits (VWR, Radnor, PA) and RNeasy Mini kits (Qiagen, Hilden, Germany). cDNA was synthesized with the QuantiTect Reverse Transcription kit (Qiagen), followed by real-time RT-PCR using the QuantiFast SYBR Green PCR kit (Qiagen) with a total reaction volume of 10 µl per well in the LightCycler 480 Multiwell Plate 384 (Roche Diagnostics, Risch, Switzerland) under the following conditions: 45 cycles of 95 °C for 10 seconds, 60 °C for 10 seconds, and 72 °C for 10 seconds. Primer sequences are given in Supplementary Table S2. Amplification was normalized to the housekeeping gene *Ppia*, and differences between samples were quantified based on the $2^{-\Delta\Delta Ct}$ calculation. Data were obtained from triplicate measurements and depicted as -fold induction of gene expression versus controls. Statistical analysis was conducted using PRISM6 software (GraphPad Software, La Jolla, CA) and Student *t* test.

Microarray and data mining

Biotin-labeled single-stranded cRNA was generated from 100 ng of total RNA isolated from epidermal sheets by using the Affymetrix Gene Chip 3′IVT PLUS Reagent kit. A total of 11 ng of fragmented and labeled cRNA were hybridized for 17 hours at 45 °C on Affymetrix GeneChip Mouse Genome U430 2.0 arrays. Gene expression microarrays were scanned using the Affymetrix GeneChip Scanner 3000 according to the GeneChip Expression Wash, Stain and Scan Manual for Cartridge Arrays (Affymetrix, Santa Clara, CA). Raw intensity data were normalized by quantile normalization with R using the function normalize.quantiles from Bioconductor package "preprocessCore." Expression values were robust multi-array average–normalized, followed by $\log_2$ transformation. Differentially expressed probesets were identified using the empirical Bayes approach (Smyth, 2004) based on moderated *t* statistics as implemented in

the Bioconductor package limma (Smyth, 2005). Gene set enrichment analysis was performed using the camera test (Wu and Smyth, 2012). In case of a gene represented by multiple probesets, the probeset with the strongest effect was selected for pathway analysis. The Reactome database (Fabregat et al., 2018) and Gene Ontology (The Gene Ontology consortium, 2017) were used for pathway analysis. *P*-values were adjusted to control the false discovery rate using the Benjamini-Hochberg correction. All analyses were conducted with statistical software R 3.5 (https://www.R-project.org/).

Lipid analysis

Skin pieces were scratched on ice to separate epidermis from dermal and subcutaneous tissue. Lipid extraction from the epidermis was performed as described earlier (Krieg et al., 2013). In brief, the tissue was treated with chloroform-methanol mixtures (1:1 and 2:1 v/v) several times to extract free lipids. Covalently bound omega-hydroxyceramides were released by mild alkaline hydrolysis with 1 M KOH in methanol/water (19:1 v/v) overnight at room temperature and add-on neutralization with an equal volume of 1 M HCl. After treatment with 0.88% aqueous KCl, lipid extracts in organic layers were collected, dried under nitrogen stream, and resolved in liquid chromatography solvents for ceramide analysis.

Liquid chromatography tandem mass spectrometry–based analysis of esterified omega-hydroxyacyl sphingosines was performed as reported previously (Ohno et al., 2017) with small modifications. In brief, reversed phase liquid chromatography separation was carried out using a Chromolith HPLC column (Performance RP-18e 100–2 mm; Merck, Darmstadt, Germany) and a binary gradient system (flow rate 0.7 ml/min; mobile phase A: acetonitrile/-water, 3:2 v/v, containing 10 mM ammonium formate; mobile phase B: 2-propanol/acetonitrile, 9:1 v/v). Liquid chromatography tandem mass spectrometry of omega-hydroxyceramides was conducted as described (Krieg et al., 2013). Lipid extracts were separated by normal phase HPLC on an Onyx Monolithic silica column (100–4.6 mm; Phenomenex, Aschaffenburg, Germany). The mobile phase consisted of acetonitrile, methanol, and acetic acid, 97:2:1 v/v/v, containing 5 mM ammonium acetate (flow rate 1 ml/min). For mass detection in positive ion mode, a 4000 QTrap triple quadrupole mass spectrometer (Sciex, Toronto, Canada) equipped with an atmospheric pressure chemical ionization source was used. The peak areas of omega-hydroxyceramides and omega-hydroxyacyl sphingosines were normalized to a C17:0 and an omega-hydroxyacyl sphingosines–D3-br-D3 internal standard (kindly provided by Prof. Dobner, Halle), respectively. Data analysis was performed with Analyst Software 1.6.3 (Sciex).

SUPPLEMENTARY REFERENCES

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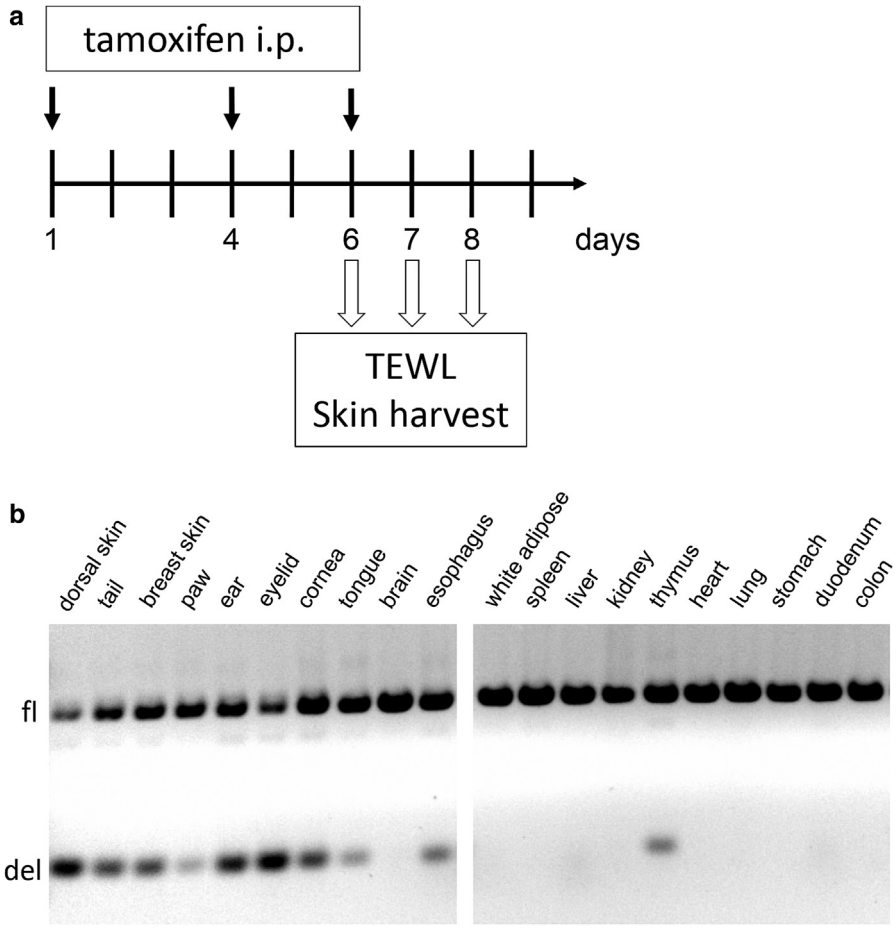
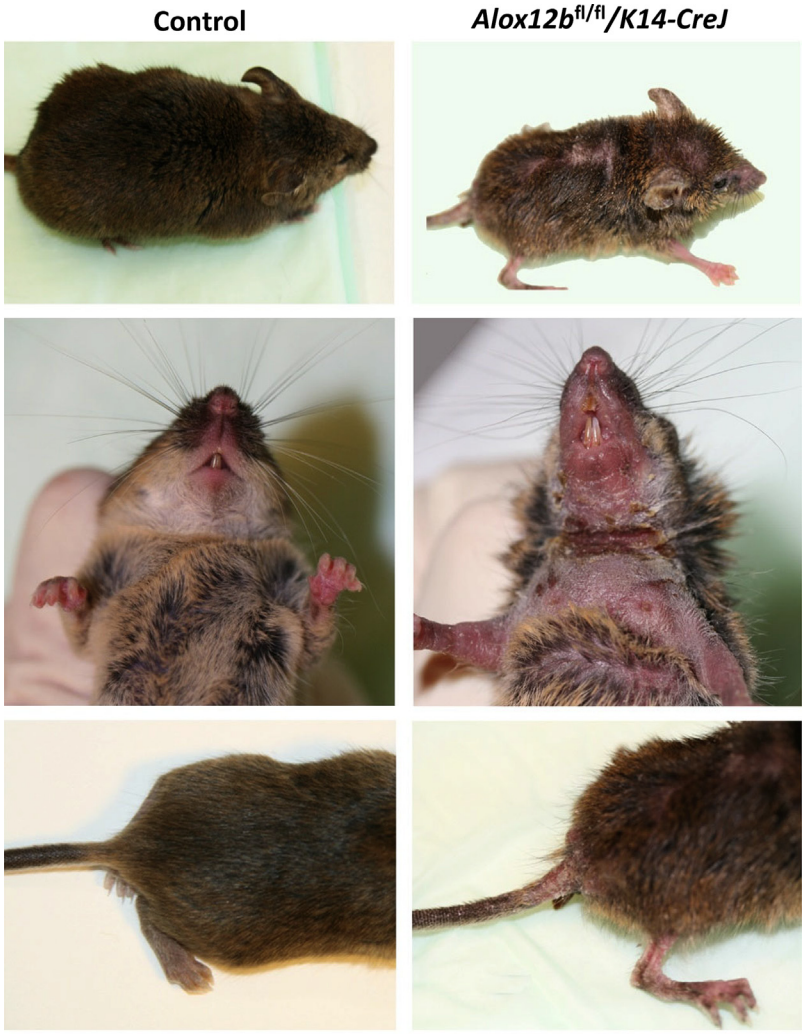
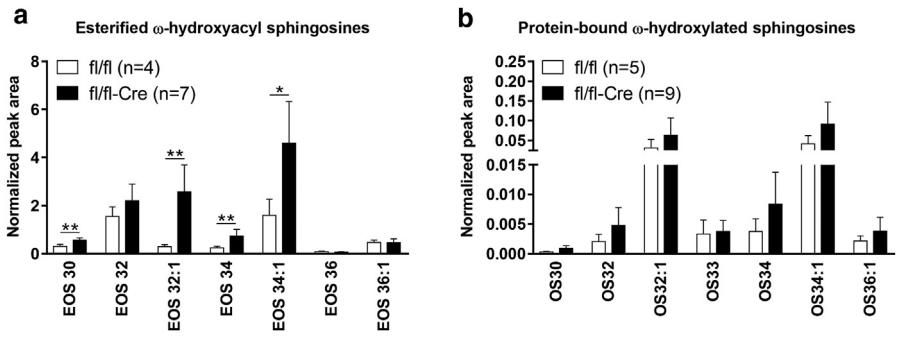


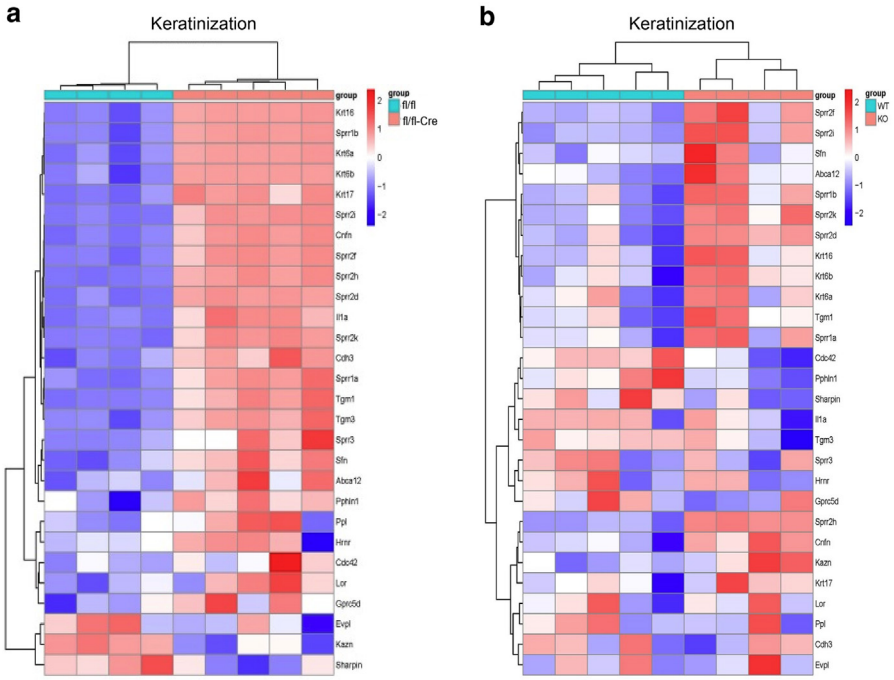
Supplementary Figure S1. Conditional *Alox12b* knockout. (**a**) Treatment scheme: *Alox12b* was inactivated in keratinocytes by three intraperitoneal injections of tamoxifen within 6 days. TEWL was measured and skin samples were harvested on three consecutive days. (**b**) Tissue specificity of *Alox12b* knockout was confirmed by PCR amplifying a 789-bp product of the floxed allele and a 189-bp product of the allele with the deletion. The deletion was detected not only in the skin but also in other *Krt14*-expressing tissues, such as cornea, tongue, esophagus, and thymus. Bp, base pair; TEWL, transepidermal water loss.



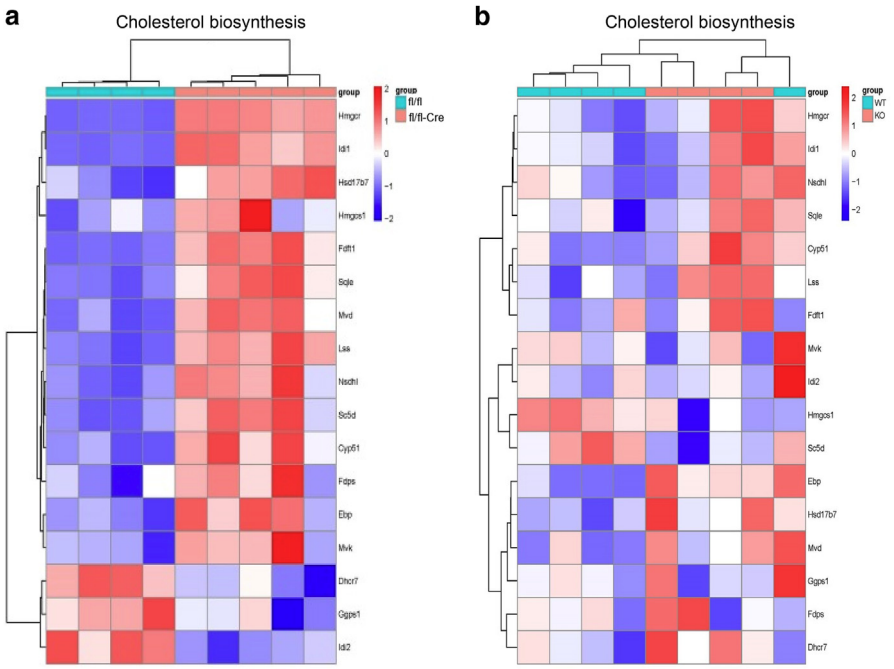
Supplementary Figure S2. Skin and hair phenotype of hairy *Alox12b*$^{fl/fl}$*/K14-CreJ* mice (on a 129S6-C57BL/6 background) upon tamoxifen treatment.



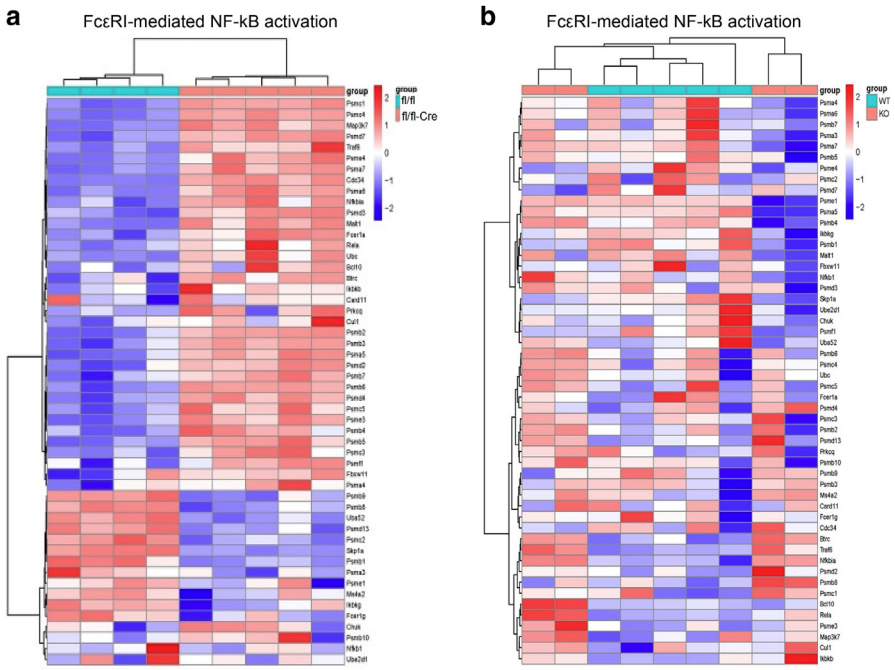
Supplementary Figure S3. Effects of tamoxifen-induced *Alox12b* inactivation on acylceramide processing and protein-bound products. Epidermal ceramides extracted from murine skin tissue of *Alox12b*$^{fl/fl}$*/K14-CreJ* mice and controls were investigated. Analysis of (**a**) Cer [EOS] and (**b**) protein-bound ω-OH-Cer was performed by liquid chromatography tandem mass spectrometry. The results were normalized to epidermal tissue weight and to an internal standard. Data are displayed ± SEM; $^{*}P < 0.05$, $^{**}P < 0.01$. Cer [EOS], esterified omega-hydroxyacyl sphingosines; SEM, standard error of the mean; ω-OH-Cer, omega-hydroxyceramides.



Supplementary Figure S4. Upregulation of keratinization-associated gene products upon *Alox12b* inactivation. Heat maps represent expression profiles of the indicated genes (**a**) in fl/fl control (left panels, n = 4) and *Alox12b*$^{fl/fl}$*/K14-CreJ* mice (right panels, n = 5), and (**b**) in wild-type control (left panels, n = 5) and constitutive *Alox12b* knockout mice (right panels, n = 4), respectively.



Supplementary Figure S5. Upregulation of cholesterol biosynthesis upon *Alox12b* inactivation. Heat maps represent expression profiles of the indicated genes (**a**) in fl/fl control (left panels, n = 4) and *Alox12b*$^{fl/fl}$*/K14-CreJ* mice (right panels, n = 5), and (**b**) in wild-type control (n = 5) and constitutive *Alox12b* knockout mice (n = 4), respectively.



Supplementary Figure S6. Upregulation of FcεRI-mediated NF-kB activation upon *Alox12b* inactivation. Heat maps represent expression profiles of the indicated genes (**a**) in fl/fl control (left panels, n = 4) and *Alox12b*$^{fl/fl}$*/K14-Cre*J mice (right panels, n = 5), and (**b**) in wild-type control (n = 5) and constitutive *Alox12b* knockout mice (n = 4), respectively. FcεRI**,** Fc epsilon receptor.

Supplementary Table S1. Primers Used for Genotyping

Gene	Primer Sequence (5′→3′)	Product Size (bp)
Alox12b forward	TCTGAGTGGGACTGGCTGTTG G	wild-type (421) floxed (446)
Alox12b reverse	AGAGACCTCCCTTGTTGAGAAG	
Krt14-wt forward	AGGGATCTGATCGGGAGTTG	wild-type (442) Cre knock-in (357)
Krt14-wt reverse	ATCCATCAAATCGACCACCA	
Krt14-Cre forward	CGCCAATTAACCCTCACTAAAGG	
Alox12b forward	GACGTAAACTCCTCTTCAGACCT	floxed (789) deleted (189)
Alox12b reverse	CTATTGATCTGGACGTTGTACC	

Abbreviation: Bp, base pair.

Supplementary Table S2. Primers Used for QRT-PCR Analyses

Gene	Forward Primer (5′→3′)	Reverse Primer (3′→5′)	Product Size, bp
Ppia	AGCTCTGAGCACTGGAGAGA	GCCAGGACCTGTATGCTTTA	178
Krt16	GGTGGCCTCTAACAGTGATCT	TGCATACAGTATCTGCCTTTGG	159
Rptn	AGCATTCTCAACGTAAGCAAGG	TGGTCTCCGAAGGATGTCTCC	126
Tgm1	TCTGGGCTCGTTGTTGTGG	AACCAGCATTCCCTCTCGGA	194
Hmgcr	TGTGCTTGGGGCTTCTGTA	TCACTAAGGAACTTTGCACCTTT	144
Hsd17b7	CCAATCTCTTTGGCCACTTT	CCTTTGGAGTGCTGGATGTC	145
Nsdh1	CCAGGTCACAGGAACACATTT	GCCTTGGTGGATATCAAATACAT	148
Psmc1	GATAGGGGTGCTAATGGATGACA	GCTTTATCCCCATCTCCTCGTA	176
Map3k7	CGGATGAGCCGTTACAGTATC	ACTCCAAGCGTTTAATAGTGTCG	168
Cdc34	CCCCAACACCTACTATGAGGG	ACATCTTGGTGAGGAACCGGA	101
Traf6	AAAGCGAGAGATTCTTTCCCTG	ACTGGGGACAATTCACTAGAGC	125
JunB	TCACGACGACTCTTACGCAG	CCTTGAGACCCCGATAGGGA	125
FosB	TTTTCCCGGAGACTACGACTC	GTGATTGCGGTGACCGTTG	174

Abbreviations: Bp, base pair; QRT-PCR, quantitative real-time reverse transcriptase–PCR.