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Oral tolerance modulates the skin transcriptome in mice with induced atopic dermatitis

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Abbreviations used

AD: Atopic dermatitis
OT: Oral tolerance
OVA: Ovalbumin
EC: Epicutaneous
RNA-seq: RNA sequencing
DEG: Differentially expressed gene
FPKM: Fragments per kilobase of exon per million fragments mapped
GO: Gene ontology
siRNA: Small interfering RNA
RT-PCR: real time- polymerase chain reaction

Abstract

Defective gut immune reactions have been implicated in the development of atopic dermatitis (AD), whereas oral tolerance (OT), i.e., the immune unresponsiveness induced by oral antigen administration, protects mice against AD. To investigate this protective role of OT, the transcriptomic profiles of skin were obtained by RNA sequencing from mice that were epicutaneously sensitized, orally tolerized prior to epicutaneous sensitization, or neither (control). OT inhibited the upregulation of keratin- and allergic inflammation-associated genes that occurred in the epicutaneously sensitized group. Compared to the controls, mice that were orally tolerized and epicutaneously sensitized showed an upregulation of genes that regulate inflammation or keratinocyte differentiation. Knocking

down two of those genes, *SCGB1A1* and *TSC22D3*, upregulated Th2 inflammatory mediators and downregulated a cornified cell envelope-related gene. Based on our findings, OT may protect skin against allergic inflammation by promoting the expression of genes that regulate Th2 inflammatory responses and skin barrier function.

Atopic dermatitis (AD) is a Th2 inflammatory disease of skin that usually begins in infancy or early childhood (1). Genetic susceptibility, a dysregulated immune response to environmental antigens, and aberrant barrier functions of the skin epithelium and gut mucosa contribute to the pathogenesis of AD (2, 3). An immature barrier function of the infant intestine may account for the development of AD, which is strongly associated with perturbations of immunological responses and the microbial composition in the gut (4). The intestinal immune system frequently encounters non-pathogenic antigens such as food proteins and commensal bacteria (5). Therefore, the oral administration of an antigen may induce local and systemic immunologic tolerance, which is known as oral tolerance (OT), to avoid wasteful inflammatory responses (6). A breakdown of OT might induce allergic food sensitization with AD (7), while NC/Nga mice, which have a defect in OT, show the spontaneous development of AD-like lesions (8). Although intestinal immune responses have been implicated in AD development, the underlying immunologic interactions between the skin and intestine have been incompletely understood. Repeated epicutaneous exposure of murine skin to ovalbumin (OVA) induces AD-like lesions and we have previously shown that OT induction prior to epicutaneous sensitization protects mice from Th2 inflammation (9). To further explore the effect of OT on AD-like skin responses, we compared the transcriptomic profiles of skin obtained from mice that were epicutaneously sensitized (EC), orally tolerized prior to epicutaneous sensitization (OT-EC), or neither (control) using high-throughput RNA sequencing (RNA-seq). We found that OT efficiently inhibited the transcriptional changes induced by epicutaneous sensitization. Compared to controls, OT-EC mice showed an upregulation of six genes. Five of those genes (*Scgb1a1*, *Tsc22d3*, *Pon1*, *Btc*, and *Per1*), which have human homologs, were significantly downregulated in skin specimens from AD patients. The knockdown of *SCGB1A1* or *TSC22D3* in HaCaT cells upregulated Th2 chemokines or cytokines and downregulated a cornified cell envelope-related gene. These findings suggest that

OT protects skin against allergic inflammation and promotes the expression of genes that regulate Th2 inflammatory responses and skin barrier function.

Materials and methods

OT induction and epicutaneous sensitization

Six- to eight-week-old female BALB/c wild-type mice (Orient Bio, Gapyeong, Korea) were housed in standard laboratory conditions of temperature and humidity at Gachon University. To induce OT to OVA (Sigma-Aldrich, St. Louis, MO), mice were provided with drinking water containing 1% OVA for 5 days, while a control group was given standard drinking water. Two days after the oral treatment, epicutaneous sensitization was performed as described previously (10).

RNA extraction, library construction, and sequencing

RNA was extracted from the skin of the mice using QIAzol[®] and purified using an RNeasy[®] mini kit (Qiagen, Hilden, Germany). The purified RNA was treated with DNase I (New England Biolabs, Ipswich, MA) to remove genomic DNA. The RNA concentration and integrity in each sample was measured on a 2100 Bioanalyzer (Agilent, Santa Clara, CA) with an RNA integrity value ≥ 8 . cDNA libraries were prepared using a TruSeq[™] RNA library kit (Illumina, San Diego, CA). The libraries were amplified by 15 cycles of polymerase chain reaction (PCR), and the amplified libraries were sequenced by Macrogen (Seoul, Korea) using a HiSeq[™] 2000 sequencing system (Illumina) with 100-bp paired-end reads.

Sequence annotation and identification of differentially expressed genes (DEGs)

Sequencing data were demultiplexed and reads were mapped to the mm10 mouse genome reference using TopHat v2.0.13. The total number of mapped reads for each transcript was determined and normalized to detect the number of fragments per kilobase of exon per million fragments mapped (FPKM) using Cufflinks. Among the analyzed samples, genes with FPKM values of zero in ≥ 1 of the samples were excluded. For DEG identification, the values of $\log_2(\text{FPKM} + 1)$ were calculated and

normalized by quantile normalization. Transcripts with fold change values ≥ 2 and $p \leq 0.05$ were included as DEGs. Data analysis of DEGs was conducted using R 3.2.2 (www.r-project.org).

Gene ontology (GO) and enrichment analysis

Functional groups and pathways encompassing the DEGs were identified based on GO analysis using the Database for Annotation, Visualization, and Integrated Discovery (DAVID v.6.8) software. The threshold was set as a modified Fisher's exact test p -value ≤ 0.05 .

Human samples

Specimens were collected from the lesional skin of six patients with AD and the normal skin of nine healthy controls with the approval of the Institutional Review Board at Gachon University Gil Medical Center (GBIRB2016-082). Patients with an acute exacerbation of AD (SCORAD between 20–70 and elevated IgE) who had not received treatment for > 4 weeks were included.

Cell culture and small interfering RNA (siRNA) transfection

HaCaT cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37 °C and 5% CO₂. To reduce *SCGB1A1* or *TSC22D3* expression, HaCaT cells were transfected with 50 pmol of the appropriate siRNA (Dharmacon, Lafayette, CO) using Lipofectamine[®] 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. Cells were harvested after 12 h for real time (RT)-PCR analysis.

RT-PCR validation

RT-PCR was performed for selected genes to validate the RNA-seq results and the gene knockdown following siRNA transfection. Total RNA was extracted and cDNA was synthesized using an iScript[™] cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). RT-PCR was performed using

iQ™ SYBR® Green SuperMix (Bio-Rad Laboratories) and a CFX Connect™ system (Bio-Rad Laboratories). The sequences of the primers used are shown in Table S1.

Results and discussion

The RNA-seq read numbers of the control, OT-EC, and EC groups varied between 39 million and 42 million and over 90% of these reads could be mapped as shown in Table S2. Genes with FPKM values of zero in ≥ 1 samples were excluded, and 17,140 transcripts were detected using this criterion. The transcripts detected with at least a two-fold difference among groups ($p \leq 0.05$) were classified as DEGs, and 62 upregulated and 10 downregulated genes were observed in the EC group compared to the control group (Table S3). In addition to several keratin-associated genes, the expression of *Arg1*, *Ear11*, *Car4*, and *Cd209e*, which have been implicated in allergic inflammation (11, 12), was increased in EC mice compared to controls (Table S3). One hundred and thirty DEGs were identified between the OT-EC and EC groups with 116 genes upregulated and 14 genes downregulated in EC (Table S4). A substantial overlap existed between the DEGs of the control vs. EC and OT-EC vs. EC groups, which shared 45 upregulated genes (Table S3 and Table S4). Accordingly, a scatter volume plot of DEGs for control vs. EC that shows the fold change of expression for each gene was highly similar to the corresponding plot for OT-EC vs. EC (Fig. 1A). In addition, the GO distribution of the DEGs between the control and EC groups highly coincided with that between the OT-EC and EC groups, except for the GO terms of “proteolysis” and “calcium binding” (Fig. S1). The visualization of a functionally grouped network using the ClueGO plugin (13) also showed the grouping of DEGs into similar pathways between the control vs. EC and OT-EC vs. EC groups (Fig. S2). We have previously shown that orally tolerized mice are protected from the Th2 inflammation induced by EC sensitization, including the inflammatory changes in skin of papillomatosis, epidermal hyperplasia, and dermal infiltration (9). Our findings presented here indicate that OT also efficiently abrogated the transcriptional changes induced by EC sensitization in skin. This conclusion is supported by the dramatically lower number of DEGs between the control and OT-EC groups than that between the control and EC groups (Table S5). A heat map of two-way hierarchical clustering was created to visualize the similarity between the transcriptomes of the control and OT-EC groups (Fig. 1B). To

validate the DEGs identified by RNA-seq, 13 genes of interest were selected for the confirmation of their differentially expressed status by RT-PCR (Fig. 1C). The fold changes determined by RT-PCR significantly correlated with the RNA-seq results with a correlation coefficient of 0.73 ($p = 0.0021$) (Fig S3).

Compared to the control group, OT-EC mice showed an upregulation of six genes. Five of those genes, which have human homologs and are known to regulate inflammation (*Scgb1a1*, *Tsc22d3*, and *Pon1*) (14-16) or keratinocyte differentiation (*Btc* and *Per1*) (17, 18), were significantly downregulated in the skin specimens from AD patients (Fig. 2A). The AD patients also showed a significant upregulation of Th2 cytokines and chemokines, and a downregulation of skin barrier molecules, corresponding with the typical pathologies of AD (Fig. S4) (1, 2). To investigate whether those upregulated genes have a functional involvement in AD-like inflammatory responses, we knocked down *SCGB1A1* or *TSC22D3* in HaCaT cells using siRNA. Transfection with siRNA against *SCGB1A1* upregulated the expression of genes encoding Th2 inflammatory mediators (*IL13* and *CCL22*) (Fig. 2B). Transfection with siRNA against *SCGB1A1* or *TSC22D3* decreased the expression of *LOR*, which encodes loricrin (Fig. 2B). This decrease in the expression of *LOR* corresponded with the decreased expression of *Lor*, *Lce1l*, and *Lce1m* in EC relative to OT-EC (Table S4).

Based on our findings, we propose that OT induction protects skin against allergic inflammation by promoting the expression of genes that regulate Th2 inflammatory responses and skin barrier function. Although the mechanisms by which the gut and skin communicate are poorly understood, the high lipid content of skin may allow it to function as a reservoir for molecules derived from the diet, gut microbiota, or intestinal immune reactions (19), which may modulate immune responses in the skin.

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Author contributions

J.Y.R. and Y.J. designed the study. J.O.B. and Y.J. performed the experiments and analyzed the data. J.R.L. performed RT-PCR of human samples. J.O.B., J.Y.R., and Y.J. drafted and revised the manuscript. All authors read and approved the final manuscript.

Conflicts of interest

The authors have no conflict of interest to declare.

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Figure legends

Figure 1. RNA-sequencing data analysis of skin specimens obtained from control, epicutaneously sensitized (EC), and ovalbumin-fed and epicutaneously-sensitized (OT-EC) mice. (A) Plot showing the relationship between the geometric average volume and fold change of each indicated treatment group. Positive $\log_2(\text{fold change})$ values represent upregulation, whereas negative values represent downregulation. Navy (fold change ≥ 2 , $P < 0.05$) and blue (fold change ≥ 2) dots above and below the dotted line represent differentially expressed genes (DEGs) between the analyzed groups. (B) Heat map of two-way hierarchical clustering based on 160 DEGs among the indicated treatment groups (fold change ≥ 2 , $p < 0.05$). Red indicates high expression and green indicates low expression. (C) Real-time polymerase chain reaction analysis of selected upregulated and downregulated genes between the indicated treatment groups. The data are representative of two independent experiments ($n = 5-8$ mice per group). Graphs show the mean $\pm$ standard error of the mean values. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (Student's *t*-test).

Figure 2. Functional characteristics of genes upregulated in ovalbumin-fed and epicutaneously-sensitized mice. (A) The mRNA expression of *SCGB1A1*, *TSC22D3*, *PON1*, *BTC*, and *PER1* was downregulated in the skin specimens of atopic dermatitis (AD) patients compared to healthy controls.

(B) Silencing *SCGB1A1* or *TSC22D3* by transfection with small interfering RNAs induced AD-like inflammatory changes in the HaCaT cells. The data are representative of two independent experiments. Graphs show the mean $\pm$ standard error of the mean values. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (Student's *t*-test).

Supplemental figure legends

Figure S1. Distribution of gene ontology (GO) terms of differentially expressed genes between the indicated treatment groups. The GO terms were classified into three categories: “Biological Process,” “Cellular Component,” and “Molecular Function.”

Figure S2. The functional interactions of differentially expressed genes between the indicated treatment groups. The enriched pathways were predicted using the ClueGO (<http://apps.cytoscape.org/apps/cluego>) plugin of Cytoscape.

Figure S3. Comparison of relative fold changes between RNA-sequencing and real-time polymerase chain reaction. Correlation coefficient $R = 0.73$; $p = 0.0021$.

Figure S4. mRNA expression levels of *IL5*, *IL13*, *CCL22*, *CCL17*, *LOR*, and *FLG* in skin specimens obtained from atopic dermatitis (AD) patients and healthy controls. Graphs show the mean $\pm$ SEM values. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ (Student's *t*-test).

Supplemental tables

Table S1. Primer sequences for real-time polymerase chain reaction.

Table S2. Summary alignment statistics in control, epicutaneously sensitized (EC), and ovalbumin-fed and epicutaneously-sensitized (OT-EC) group libraries.

Table S3. List of differentially expressed genes in the skin of epicutaneously sensitized mice compared to controls.

Table S4. List of differentially expressed genes in the skin of epicutaneously sensitized mice compared to ovalbumin-fed and epicutaneously-sensitized mice.

Table S5. List of differentially expressed genes in the skin of ovalbumin-fed and epicutaneously-sensitized mice compared to controls.

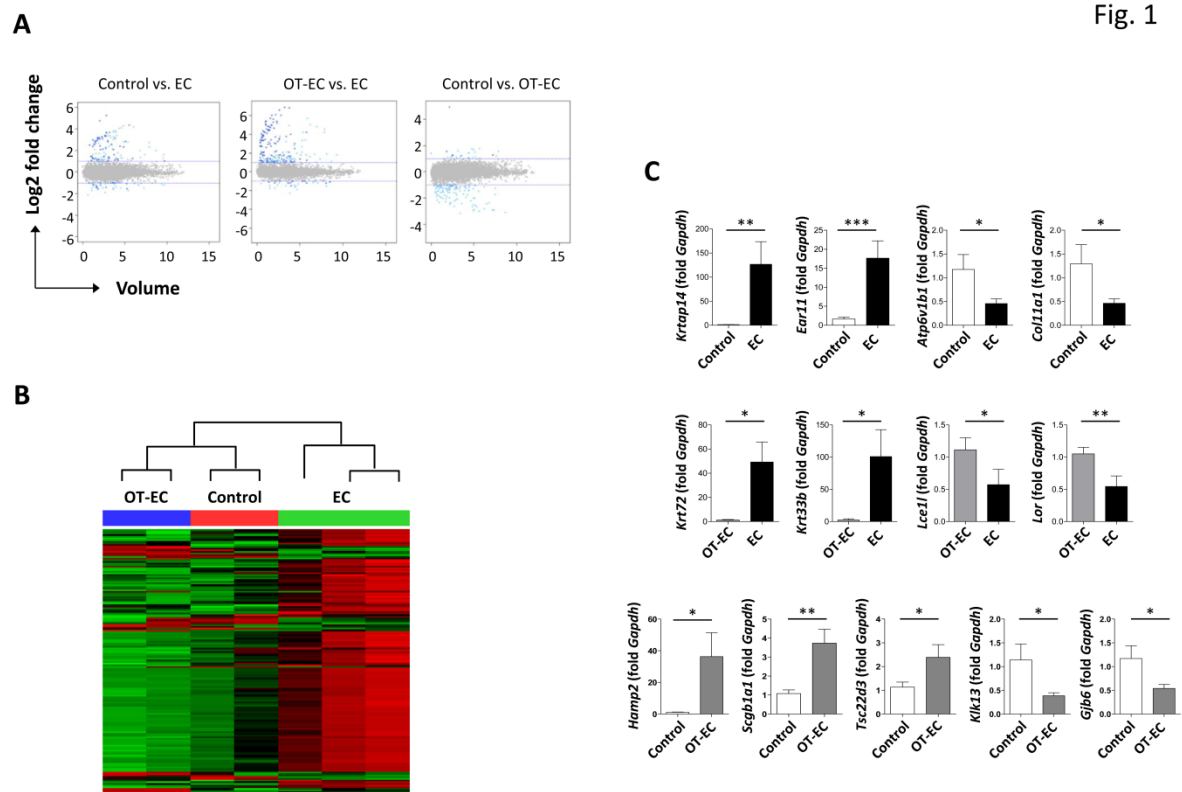
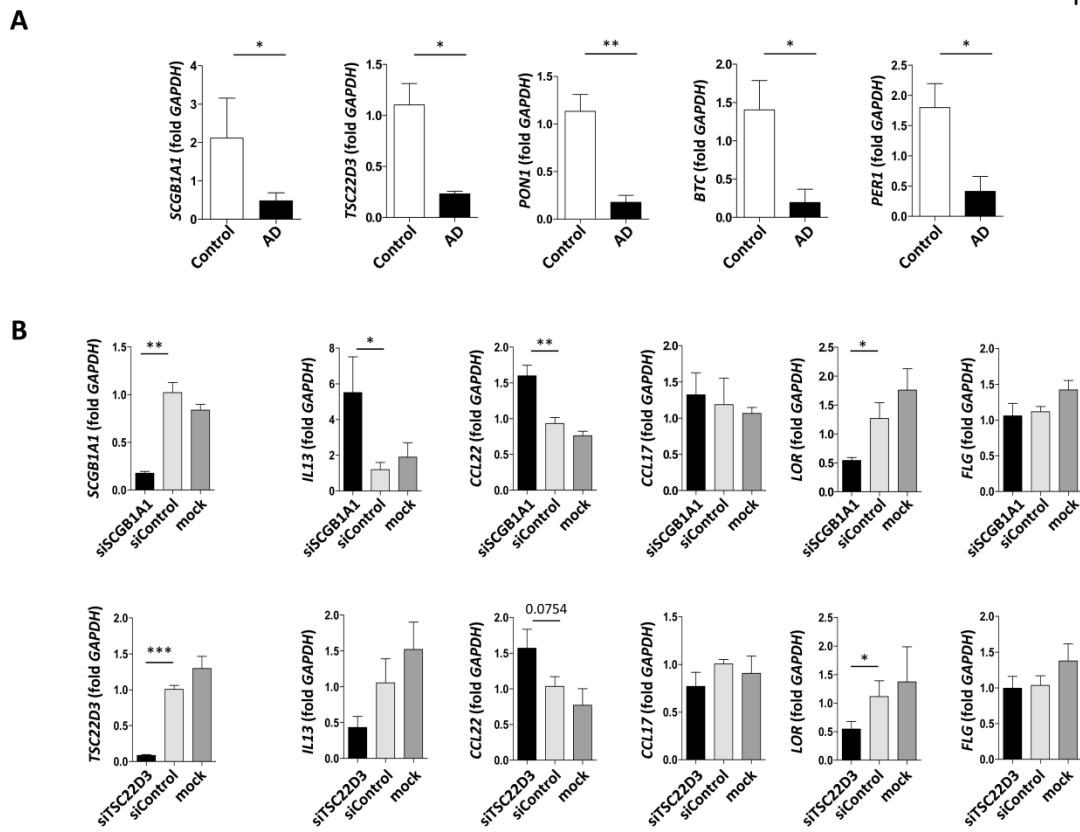
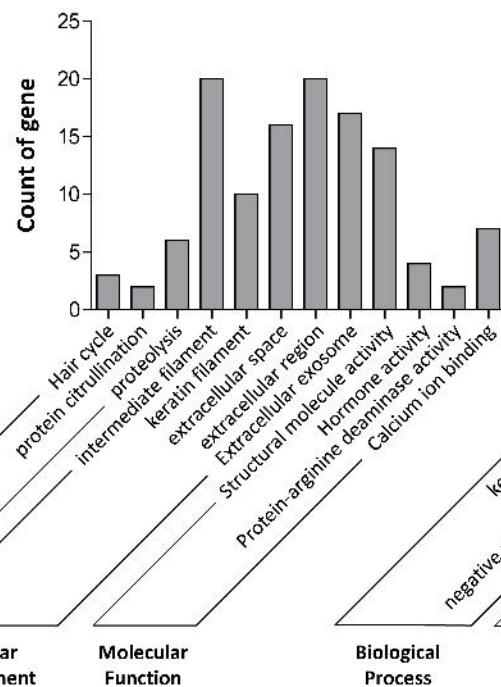


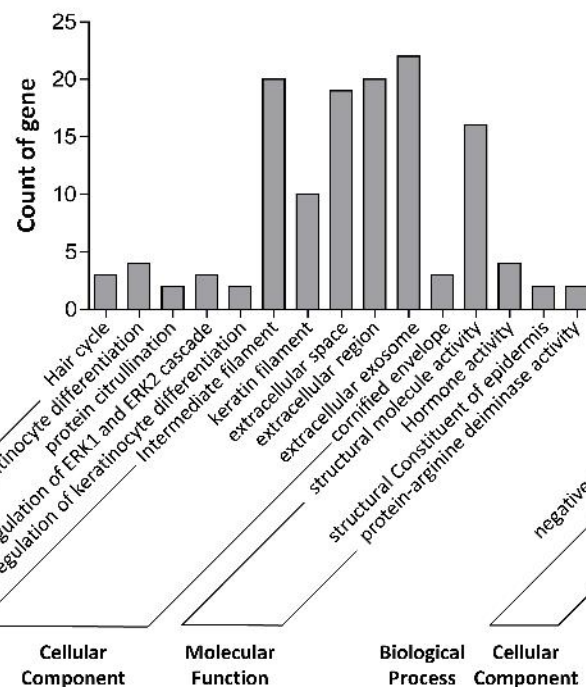
Fig. 2



Control vs. EC



OT-EC vs. EC



Control vs. OT-EC

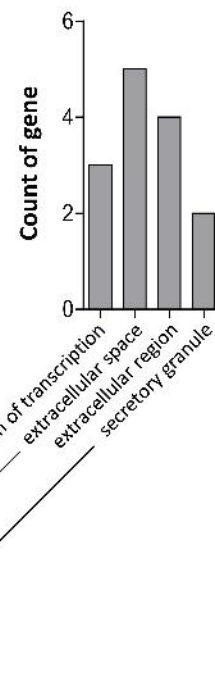


Fig. S2

Fig. S3

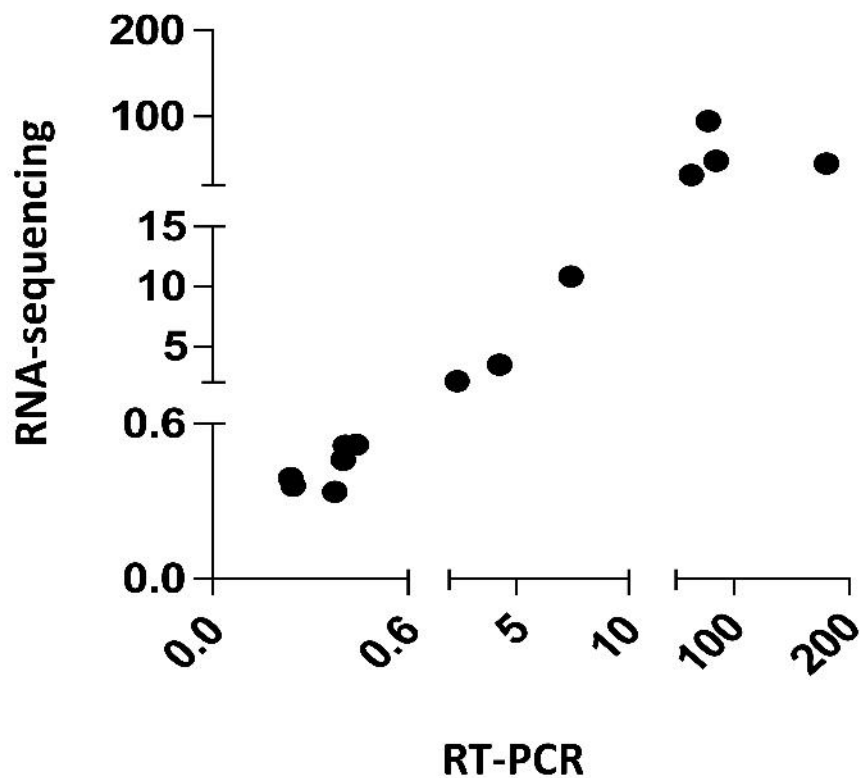


Fig. S4

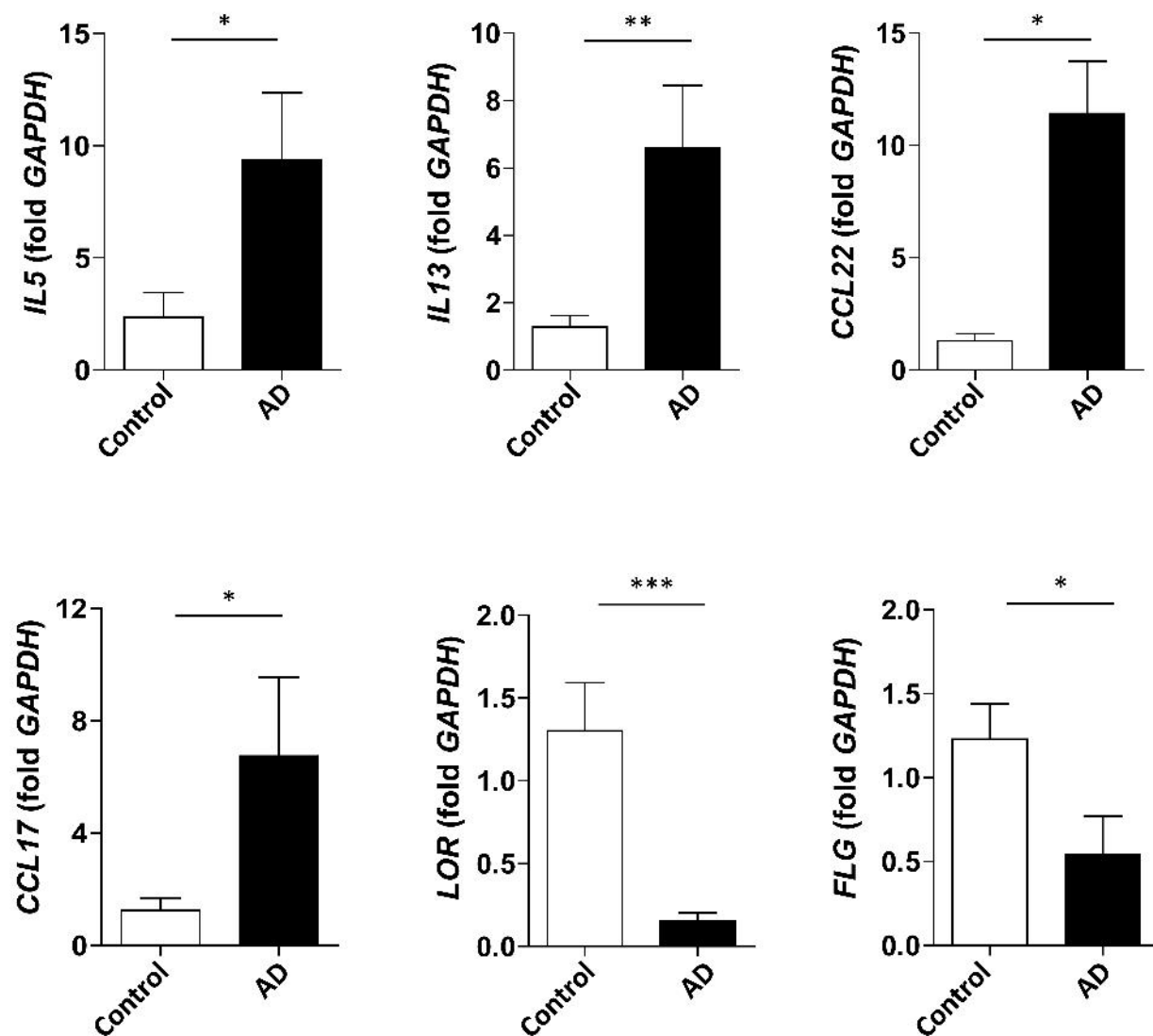


Table S1.

Gene	Primer sequence
<i>Krtap14</i>	Forward: 5'-TCC GAT CTC TTC ATG CGG TT-3' Reverse: 5'-GTG GGT GAT GGG AGT TTG GA-3'
<i>Ear11</i>	Forward: 5'-ACA TGG GTC TGG AGC AAC TT-3' Reverse: 5'-TCA TCA CAT CGG GGA TAG GC-3'
<i>Atp6v1b1</i>	Forward: 5'-GGC TGT GAC CCG AAA CTA CAT-3' Reverse: 5'-CTC AGC ATA CTG GGC AAA CTT-3'
<i>Col11a1</i>	Forward: 5'-AGG TGG AAA ACG AAA CGG TG-3' Reverse: 5'-GGA AGA GAA AAG TCA AGG CGA-3'
<i>Krt72</i>	Forward: 5'-CGC CAG TTG ACC CTC TAT CC-3' Reverse: 5'-AAA TGC TGC TCC CTT GAC TCC-3'
<i>Krt33b</i>	Forward: 5'-CAC CGT AGG GAT CAT GGT CTG-3' Reverse: 5'-CAA GCC CTG GAT GAA GAT GGT -3'
<i>Lce1l</i>	Forward: 5'-ACC TGT TCC TGC CTG CTA TT-3' Reverse: 5'-TGG AAT CAC AGA AGG AGA TGA G-3'
<i>Lor</i>	Forward: 5'-ACA TCA GCA TCA CCT CCT TC-3' Reverse: 5'-TCT TTC CAC AAC CCA CAG G-3'
<i>Hamp2</i>	Forward: 5'-CTC CTG TGG TAT CTG TTG TGA-3' Reverse: 5'-ATG AAT ACT GGG GTT GAG GGG-3'
<i>Scgb1a1</i>	Forward: 5'-GCT GAA GAG ACT GGT GGA TA-3' Reverse: 5'-CTT GCT TAC ACA GAG GAC TTG-3'
<i>Tsc22d3</i>	Forward: 5'-TGA GAT GTG ATG CCA GTG T-3' Reverse: 5'-GCT TCT TCA TAG GAA CAG TCA TT-3'
<i>Klk13</i>	Forward: 5'-CCA GGG GCA CTT GAT ACA CTC-3' Reverse: 5'-TGT TCG TCA CCT CTT TCC CA-3'
<i>Gjb6</i>	Forward: 5'-GAC ATG AAA CTG CCC GAA AGT-3' Reverse: 5'-CGA AGA TGA TGC GGA AGA AAA TG-3'
<i>Gapdh</i>	Forward: 5'-CTG GTA TGA CAA TGA ATA CG-3' Reverse: 5'-GCA GCG AAC TTT ATT GAT GG-3'

<i>SCGB1A1</i>	Forward: 5'-CAG AGA CGG AAC CAG AGA C-3'
	Reverse: 5'-GAG AGC CAG TGT GAC CAG-3'
<i>TSC22D3</i>	Forward: 5'-CCA GTT TGC TCC AGA AAG T-3'
	Reverse: 5'-CAG TCG TTG TCA GGT GAA G-3'
<i>PON1</i>	Forward: 5'-TTT GGG TTT AGC GTG GTC GT-3'
	Reverse: 5'-TGC CAT CGG GTG AAA TGT TG-3'
<i>BTC</i>	Forward: 5'-CCT GGG TCT AGT GAT CCT TCA-3'
	Reverse: 5'-CTT TCC GCT TTG ATT GTG TGG-3'
<i>PER1</i>	Forward: 5'-CAC CCT GAT GAC CCA CTC TT-3'
	Reverse: 5'-GGT AAG GCT GGA CTG GAT GA-3'
<i>IL13</i>	Forward: 5'-TGT TTG TCA CCG TTG GGG AT-3'
	Reverse: 5'-TGA GTC TCT GAA CCC TTG GC-3'
<i>CCL22</i>	Forward: 5'-GAA GCC TGT GCC AAC TCT CT-3'
	Reverse: 5'-GGG AAT CGC TGA TGG GAA CA-3'
<i>CCL17</i>	Forward: 5'-CGG ACC CCA ACA ACA AGA GA-3'
	Reverse: 5'-CTC CCT CAC TGT GGC TCT TC-3'
<i>LOR</i>	Forward: 5'-GAG GTG TTT TCC AGG GGC A-3'
	Reverse: 5'-TGG GGT TGG GAG GTA GTT GTA-3'
<i>FLG</i>	Forward: 5'-TGA AGC CTA TGA CAC CAC TGA-3'
	Reverse: 5'-TCC CCT ACG CTT TCT TGT CCT-3'
<i>IL5</i>	Forward: 5'-CTG ATA GCC AAT GAG ACT CTG-3'
	Reverse: 5'-CTC CAG TGT GCC TAT TCC-3'
<i>GAPDH</i>	Forward: 5'-CTG GGC TAC ACT GAG CAC C-3'
	Reverse: 5'-AAG TGG TCG TTG AGG GCA ATG-3'

Table S2.

Library	Processed reads	Mapped reads	Suppressed reads by multiple mapping	Overall read mapping ratio (%)	Gene expression (FPKM)	
					Range	Mean
Control 1	40386764	37269778	2052118	91.8	3.10E-69 ~ 4565.67	33.43
Control 2	39154080	36379291	1542388	92.3	1.68E-60 ~ 4616.45	30.29
EC 1	41269878	38179959	1735012	92.0	2.63E-126 ~ 4033.43	30.71
EC 2	40706298	37626387	1740633	91.9	5.70E-122 ~ 4026.57	30.87
EC 3	42005452	38890647	1879544	91.9	3.02E-53 ~ 4018.39	31.52
OT EC1	39151455	36302141	1799524	92.2	6.19E-86 ~ 4289.51	30.32
OT EC2	40268013	36920971	1921891	91.2	3.12E-18 ~ 4168.21	33.46

Table S3.

Gene symbol	Gne name	NCBI Gene ID	Fold change	p-value
<i>Krtap15</i>	keratin associated protein 15	26560	37.79	0.02
<i>Krtap14</i>	keratin associated protein 14	23927	26.86	0.02
<i>Krtap7-1</i>	keratin associated protein 7-1	71363	20.49	0.05
<i>Krt34</i>	keratin 34	16672	12.57	0.02
<i>5430421N21Rik</i>	RIKEN cDNA 5430421N21 gene	100126226	12.53	0.03
<i>Krtap9-3</i>	keratin associated protein 9-3	75586	12.49	0.02
<i>Gprc5d</i>	G protein-coupled receptor, family C, group 5, member D	93746	10.50	0.01
<i>Krt81</i>	keratin 81	64818	10.40	0.04
<i>Krt86</i>	keratin 86	16679	9.96	0.04
<i>Krt33b</i>	keratin 33B	16671	9.87	0.04
<i>Krtap4-7</i>	keratin associated protein 4-7	76444	9.58	0.03
<i>Gm11567</i>	predicted gene 11567	670533	9.04	0.01
<i>Krtap26-1</i>	keratin associated protein 26-1	69533	8.96	0.01
<i>Gm11559</i>	predicted gene 11559	100415785	8.88	0.01
<i>Krtap3-3</i>	keratin associated protein 3-3	66380	8.82	0.04
<i>Gm11562</i>	predicted gene 11562	100502803	8.46	0.03
<i>Krtap1-3</i>	keratin associated protein 1-3	435273	8.19	0.01
<i>Krtap1-4</i>	keratin associated protein 1-4	629873	8.14	0.01
<i>Gm2696</i>	predicted gene 2696	100040299	7.84	0.02
<i>Krtap13-1</i>	keratin associated protein 13-1	268905	7.56	0.01
<i>Krtap4-2</i>	keratin associated protein 4-2	68673	7.56	0.02
<i>Krtap10-4</i>	keratin associated protein 10-4	100191037	7.52	0.02
<i>Gm3285</i>	predicted gene 3285	100041351	7.30	0.02

<i>Krtap9-1</i>	keratin associated protein 9-1	16705	7.19	0.02
<i>Gm11568</i>	predicted gene 11568	432600	7.10	0.01
<i>Ear11</i>	eosinophil-associated, ribonuclease A family, member 11	93726	7.07	<0.01
<i>Krtap17-1</i>	keratin associated protein 17-1	77914	6.97	0.03
<i>Krtap4-16</i>	keratin associated protein 4-16	435285	6.51	0.02
<i>Krtap10-10</i>	keratin associated protein 10-10	544710	6.04	0.02
<i>Krtap3-2</i>	keratin associated protein 3-2	66708	5.84	0.02
<i>Gm11595</i>	predicted gene 11595	100040276	5.74	0.01
<i>Krtap5-4</i>	keratin associated protein 5-4	50775	5.58	0.03
<i>Col6a5</i>	collagen, type VI, alpha 5	665033	5.36	<0.01
<i>Car6</i>	carbonic anhydrase 6	12353	5.28	0.03
<i>Gm11570</i>	predicted gene 11570	100504183	5.06	0.02
<i>Psors1c2</i>	psoriasis susceptibility 1 candidate 2 (human)	57390	4.97	0.03
<i>Krt83</i>	keratin 83	406219	4.67	0.04
<i>Gm13032</i>	predicted gene 13032	100049161	4.37	0.02
<i>Padi1</i>	peptidyl arginine deiminase, type I	18599	4.27	0.02
<i>Pyy</i>	peptide YY	217212	4.25	0.05
<i>2310007B03Rik</i>	RIKEN cDNA 2310007B03 gene	71874	3.00	0.02
<i>Otop2</i>	otopetrin 2	237987	2.75	0.03
<i>Car4</i>	carbonic anhydrase 4	12351	2.66	0.01
<i>Ctse</i>	cathepsin E	13034	2.62	0.01
<i>Fgf5</i>	fibroblast growth factor 5	14176	2.60	0.03
<i>Angptl7</i>	angiopoietin-like 7	654812	2.59	0.05
<i>Chac1</i>	ChaC, cation transport regulator 1	69065	2.46	0.01
<i>Ear2</i>	eosinophil-associated, ribonuclease A family, member 2	13587	2.40	0.04
<i>Alox8</i>	arachidonate 8-lipoxygenase	11688	2.40	0.04

<i>Cd209e</i>	CD209e antigen	170780	2.38	0.04
<i>Capn8</i>	calpain 8	170725	2.36	0.05
<i>Krt18</i>	keratin 18	16668	2.35	0.01
<i>Elf5</i>	E74-like factor 5	13711	2.29	0.04
<i>Fam46b</i>	family with sequence similarity 46, member B	100342	2.29	0.05
<i>Il31ra</i>	interleukin 31 receptor A	218624	2.23	0.03
<i>Ear10</i>	eosinophil-associated, ribonuclease A family, member 10	93725	2.20	0.03
<i>Zbtb16</i>	zinc finger and BTB domain containing 16	235320	2.15	0.01
<i>Arg1</i>	arginase, liver	11846	2.13	0.04
<i>Ppp1r3c</i>	protein phosphatase 1, regulatory (inhibitor) subunit 3C	53412	2.12	0.02
<i>Tagln3</i>	transgelin 3	56370	2.05	0.04
<i>Sprr1a</i>	small proline-rich protein 1A	20753	2.01	0.02
<i>Pla2g2e</i>	phospholipase A2, group IIE	26970	2.00	0.04
<i>Uhrf1</i>	ubiquitin-like, containing PHD and RING finger domains, 1	18140	-2.03	<0.01
<i>Naglu</i>	alpha-N-acetylglucosaminidase (Sanfilippo disease IIIB)	27419	-2.03	0.01
<i>Acan</i>	aggrecan	11595	-2.04	0.01
<i>Klk8</i>	kallikrein related-peptidase 8	259277	-2.12	0.05
<i>Prss35</i>	protease, serine 35	244954	-2.30	<0.01
<i>Shh</i>	sonic hedgehog	20423	-2.57	0.01
<i>Klk13</i>	kallikrein related-peptidase 13	626834	-2.67	0.03
<i>Col11a1</i>	collagen, type XI, alpha 1	12814	-2.76	0.01
<i>Casp14</i>	caspase 14	12365	-2.99	<0.01
<i>Atp6v1b1</i>	ATPase, H ⁺ transporting, lysosomal V1 subunit B1	110935	-3.01	0.02

Table S4.

Gene symbol	Gene name	NCBI Gene ID	Fold change	p-value
<i>Krtap7-1</i>	keratin associated protein 7-1	71363	112.94	0.01
<i>Krtap8-1</i>	keratin associated protein 8-1	16703	111.20	0.01
<i>Krt72</i>	keratin 72	105866	95.11	0.01
<i>Gm10229</i>	predicted gene 10229	100040201	67.47	<0.01
<i>Krtap15</i>	keratin associated protein 15	26560	63.52	0.01
<i>Krtap22-2</i>	keratin associated protein 22-2	68740	63.37	0.01
<i>Tchhl1</i>	trichohyalin-like 1	71325	50.84	<0.01
<i>Krtap3-1</i>	keratin associated protein 3-1	69473	48.02	0.01
<i>Gm10228</i>	predicted gene 10228	100040214	42.41	<0.01
<i>Krtap11-1</i>	keratin associated protein 11-1	16693	41.05	<0.01
<i>Krtap14</i>	keratin associated protein 14	23927	40.10	0.02
<i>Krt33a</i>	keratin 33A	71888	38.39	0.01
<i>Krt26</i>	keratin 26	320864	36.21	0.02
<i>Krtap6-1</i>	keratin associated protein 6-1	16700	33.92	<0.01
<i>1110025L11Rik</i>	RIKEN cDNA 1110025L11 gene	68637	31.22	0.01
<i>5430421N21Rik</i>	RIKEN cDNA 5430421N21 gene	100126226	31.11	0.01
<i>Krtap9-3</i>	keratin associated protein 9-3	75586	31.00	0.01
<i>Krtap1-5</i>	keratin associated protein 1-5	69664	28.28	0.01
<i>Krt34</i>	keratin 34	16672	27.51	0.01
<i>Gm11938</i>	predicted gene 11938	100041412	26.34	<0.01
<i>Krt31</i>	keratin 31	16660	24.50	0.01
<i>Krt81</i>	keratin 81	64818	23.66	0.01
<i>Gprc5d</i>	G protein-coupled receptor, family C, group 5, member D	93746	22.46	<0.01
<i>Spink12</i>	serine peptidase inhibitor, Kazal type 11	78242	21.76	<0.01

<i>Krt86</i>	keratin 86	16679	21.33	0.01
<i>Krtap13-1</i>	keratin associated protein 13-1	268905	21.13	0.01
<i>Krt33b</i>	keratin 33B	16671	20.57	0.01
<i>Krtap4-7</i>	keratin associated protein 4-7	76444	20.54	0.01
<i>Krtap4-1</i>	keratin associated protein 4-1	665891	18.93	0.01
<i>Krtap2-4</i>	keratin associated protein 2-4	71453	18.11	0.01
<i>Pinlyp</i>	phospholipase A2 inhibitor and LY6/PLAUR domain containing	641361	18.07	0.01
<i>Gm11562</i>	predicted gene 11562	100502803	16.94	0.01
<i>Krtap26-1</i>	keratin associated protein 26-1	69533	16.51	0.01
<i>Crym</i>	crystallin, mu	12971	15.96	0.01
<i>Krtap1-3</i>	keratin associated protein 1-3	435273	15.18	<0.01
<i>Gm11567</i>	predicted gene 11567	670533	14.31	0.01
<i>Gm11559</i>	predicted gene 11559	100415785	14.29	0.01
<i>Krtap1-4</i>	keratin associated protein 1-4	629873	13.39	0.01
<i>Krtap3-3</i>	keratin associated protein 3-3	66380	13.37	0.01
<i>Krtap17-1</i>	keratin associated protein 17-1	77914	13.18	0.01
<i>Krtap4-2</i>	keratin associated protein 4-2	68673	12.22	0.01
<i>Krtap10-4</i>	keratin associated protein 10-4	100191037	11.91	0.02
<i>Ly6g6d</i>	lymphocyte antigen 6 complex, locus G6D	114654	11.74	0.01
<i>Cryba4</i>	crystallin, beta A4	12959	10.87	0.02
<i>Lyg2</i>	lysozyme G-like 2	332427	10.72	0.01
<i>Krt83</i>	keratin 83	406219	10.40	0.03
<i>Gm11568</i>	predicted gene 11568	432600	10.33	0.01
<i>Krtap9-1</i>	keratin associated protein 9-1	16705	10.18	0.01
<i>Gm2696</i>	predicted gene 2696	100040299	10.14	0.02
<i>Gm3285</i>	predicted gene 3285	100041351	9.97	0.02
<i>Krtap4-16</i>	keratin associated protein 4-16	435285	9.79	0.01

<i>D730001G18Rik</i>	RIKEN cDNA D730001G18 gene	78725	9.42	0.03
<i>Gm13032</i>	predicted gene 13032	100049161	8.94	<0.01
<i>Krtap10-10</i>	keratin associated protein 10-10	544710	8.86	0.02
<i>Gm11595</i>	predicted gene 11595	100040276	8.45	0.01
<i>Vsig8</i>	V-set and immunoglobulin domain containing 8	240916	8.04	0.01
<i>Psors1c2</i>	psoriasis susceptibility 1 candidate 2 (human)	57390	8.02	0.01
<i>S100a7a</i>	S100 calcium binding protein A7A	381493	7.94	<0.01
<i>Sct</i>	secretin	20287	7.70	0.02
<i>Padi1</i>	peptidyl arginine deiminase, type I	18599	7.56	0.01
<i>Gm11570</i>	predicted gene 11570	100504183	6.79	0.03
<i>Gabrp</i>	gamma-aminobutyric acid (GABA) A receptor, pi	216643	6.50	0.02
<i>Krtap5-4</i>	keratin associated protein 5-4	50775	6.45	0.03
<i>Fam26d</i>	family with sequence similarity 26, member D	270711	6.40	0.03
<i>Tgm6</i>	protein-glutamine gamma-glutamyltransferase 6 isoform 2	241636	5.97	0.01
<i>Car6</i>	carbonic anhydrase 6	12353	5.50	0.02
<i>Col6a5</i>	collagen, type VI, alpha 5	665033	4.54	0.01
<i>Krt82</i>	keratin 82	114566	4.35	0.04
<i>Wfdc18</i>	WAP four-disulfide core domain 18	14038	4.32	0.02
<i>2310061N02Rik</i>	RIKEN cDNA 2310061N02 gene	69661	4.31	0.03
<i>Krtap3-2</i>	keratin associated protein 3-2	66708	4.18	0.04
<i>Krt84</i>	keratin 84	16680	4.07	<0.01
<i>AU018091</i>	expressed sequence AU018091	245128	4.02	0.01
<i>Kcne1</i>	potassium voltage-gated channel, Isk-related subfamily, member 1	16509	3.70	0.05
<i>Otop2</i>	otopetrin 2	237987	3.62	0.02
<i>Fgf5</i>	fibroblast growth factor 5	14176	3.44	0.03
<i>Krt32</i>	keratin 32	16670	3.44	0.03
<i>Gjb6</i>	gap junction protein, beta 6	14623	3.44	0.04

<i>Dsg4</i>	desmoglein 4	16769	3.37	0.03
<i>Slc7a8</i>	solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	50934	3.32	0.01
<i>Capn8</i>	calpain 8	170725	3.24	0.02
<i>Scrg1</i>	scrapie responsive gene 1	20284	3.22	<0.01
<i>2310007B03Rik</i>	RIKEN cDNA 2310007B03 gene	71874	3.18	0.03
<i>Gm5424</i>	predicted gene 5424	432466	3.07	0.01
<i>Npy</i>	neuropeptide Y	109648	2.99	0.01
<i>Cpm</i>	carboxypeptidase M	70574	2.98	0.04
<i>Dusp2</i>	dual specificity phosphatase 2	13537	2.87	<0.01
<i>Sptssb</i>	serine palmitoyltransferase, small subunit B	66183	2.86	0.05
<i>Prss53</i>	protease, serine 53	330657	2.81	0.02
<i>Krt18</i>	keratin 18	16668	2.68	0.01
<i>Nppc</i>	natriuretic peptide type C	18159	2.62	0.02
<i>Padi4</i>	peptidyl arginine deiminase, type IV	18602	2.55	0.01
<i>Alox8</i>	arachidonate 8-lipoxygenase	11688	2.53	0.03
<i>Tm4sf1</i>	transmembrane 4 superfamily member 1	17112	2.53	0.01
<i>Plb1</i>	phospholipase B1	665270	2.44	0.04
<i>Ctse</i>	cathepsin E	13034	2.37	0.01
<i>Cxcl14</i>	chemokine (C-X-C motif) ligand 14	57266	2.34	0.02
<i>Slc5a5</i>	solute carrier family 5 (sodium iodide symporter), member 5	114479	2.32	0.01
<i>Dio3</i>	deiodinase, iodothyronine type III	107585	2.29	0.02
<i>Bhlha9</i>	basic helix-loop-helix family, member a9	320522	2.24	0.02
<i>Tnni1</i>	troponin I, skeletal, slow 1	21952	2.24	0.02
<i>Tmem229b</i>	transmembrane protein 229B	268567	2.22	0.03
<i>Csdc2</i>	cold shock domain containing C2, RNA binding	105859	2.21	0.01
<i>Ace2</i>	angiotensin I converting enzyme (peptidyl-dipeptidase A) 2	70008	2.20	0.04

<i>Ntrk1</i>	neurotrophic tyrosine kinase, receptor, type 1	18211	2.17	0.01
<i>Trpv6</i>	transient receptor potential cation channel, subfamily V, member 6	64177	2.17	0.03
<i>Rab15</i>	RAB15, member RAS oncogene family	104886	2.15	0.01
<i>Pthlh</i>	parathyroid hormone-like peptide	19227	2.15	0.03
<i>Tmprss11e</i>	transmembrane protease, serine 11e	243084	2.14	0.03
<i>Fads3</i>	fatty acid desaturase 3	60527	2.09	<0.01
<i>Ero1l</i>	ERO1-like (<i>S. cerevisiae</i>)	50527	2.09	<0.01
<i>4933409K07Rik</i>	RIKEN cDNA 4933409K07 gene	108816	2.07	0.05
<i>Foxn1</i>	forkhead box N1	15218	2.04	0.04
<i>Slc25a37</i>	solute carrier family 25, member 37	67712	2.04	0.04
<i>Lrat</i>	lecithin-retinol acyltransferase (phosphatidylcholine-retinol-O-acyltransferase)	79235	2.00	0.03
<i>4732456N10Rik</i>	RIKEN cDNA 4732456N10 gene	239673	2.00	0.01
<i>Btc</i>	betacellulin, epidermal growth factor family member	12223	-2.00	0.02
<i>Cyp2e1</i>	cytochrome P450, family 2, subfamily e, polypeptide 1	13106	-2.00	0.05
<i>Klf2</i>	Kruppel-like factor 2 (lung)	16598	-2.00	0.01
<i>Errfi1</i>	ERBB receptor feedback inhibitor 1	74155	-2.01	<0.01
<i>Dusp1</i>	dual specificity phosphatase 1	19252	-2.01	0.01
<i>Serpina12</i>	serine (or cysteine) peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 12	68054	-2.04	0.02
<i>Gm6537</i>	predicted gene 6537	100504112	-2.15	0.01
<i>Lor</i>	loricrin	16939	-2.17	0.02
<i>Scgb1a1</i>	secretoglobin, family 1A, member 1 (uteroglobin)	22287	-2.25	0.04
<i>Pdk4</i>	pyruvate dehydrogenase kinase, isoenzyme 4	27273	-2.25	0.04
<i>Lce1m</i>	late cornified envelope 1M	66203	-2.35	0.01
<i>Naglu</i>	alpha-N-acetylglucosaminidase (Sanfilippo disease IIIB)	27419	-2.35	0.01
<i>Lce1l</i>	late cornified envelope 1L	73730	-2.36	0.03
<i>Fos</i>	FBJ osteosarcoma oncogene	14281	-2.80	0.03

Table S5.

Gene symbol	Gene name	NCBI Gne ID	Fold change	p-value
<i>Hamp2</i>	hepcidin antimicrobial peptide 2	66438	30.10	0.03
<i>Scgb1a1</i>	secretoglobin, family 1A, member 1 (uteroglobin)	22287	2.87	0.04
<i>Tsc22d3</i>	TSC22 domain family, member 3	14605	2.40	<0.01
<i>Btc</i>	betacellulin, epidermal growth factor family member	12223	2.35	<0.01
<i>Pon1</i>	paraoxonase 1	18979	2.31	0.04
<i>Per1</i>	period circadian clock 1	18626	2.00	0.02
<i>Gjb6</i>	gap junction protein, beta 6	14623	-2.05	0.01
<i>Klk13</i>	kallikrein related-peptidase 13	626834	-2.29	0.04