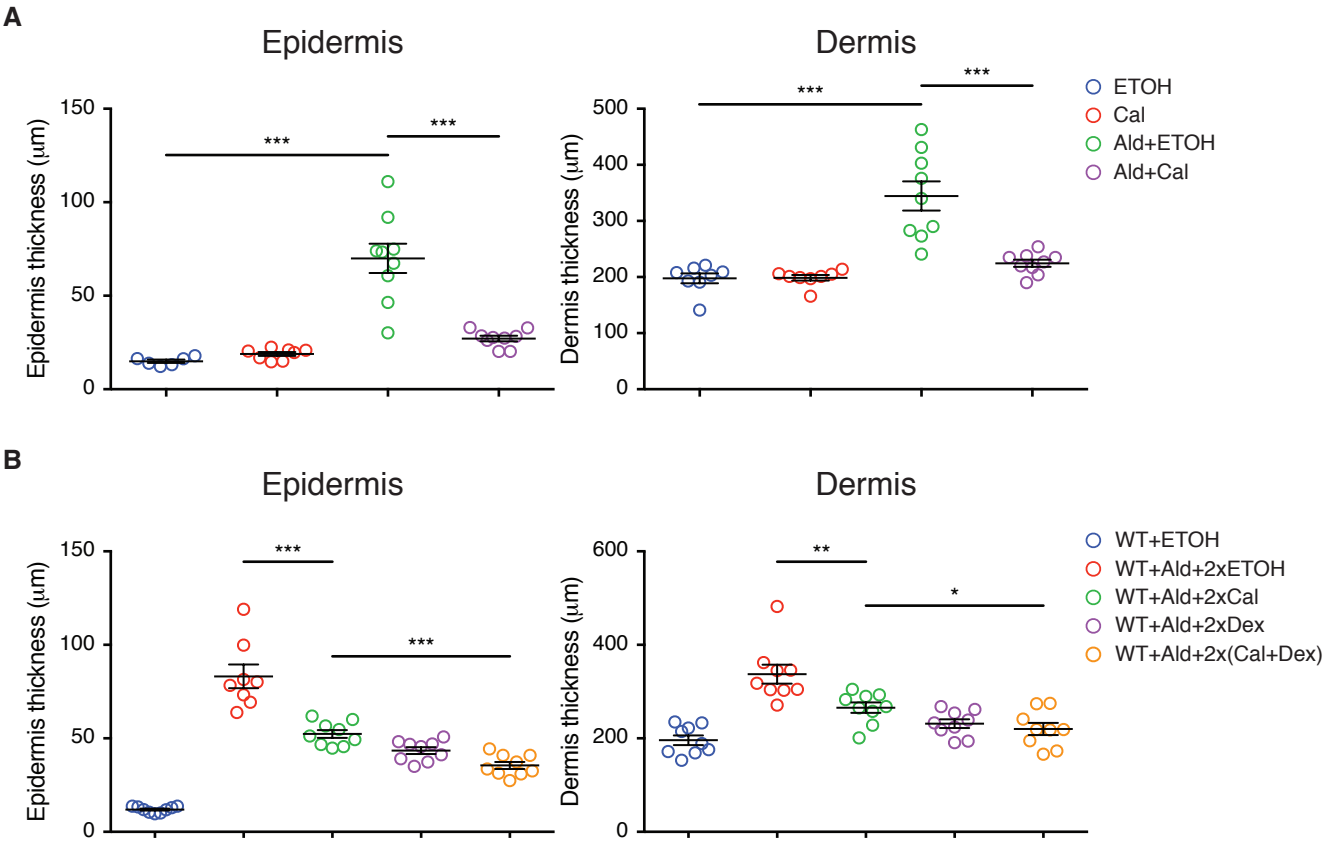
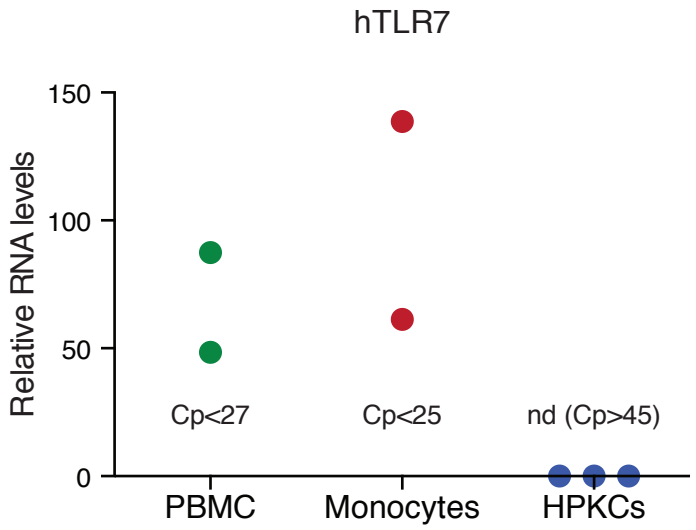
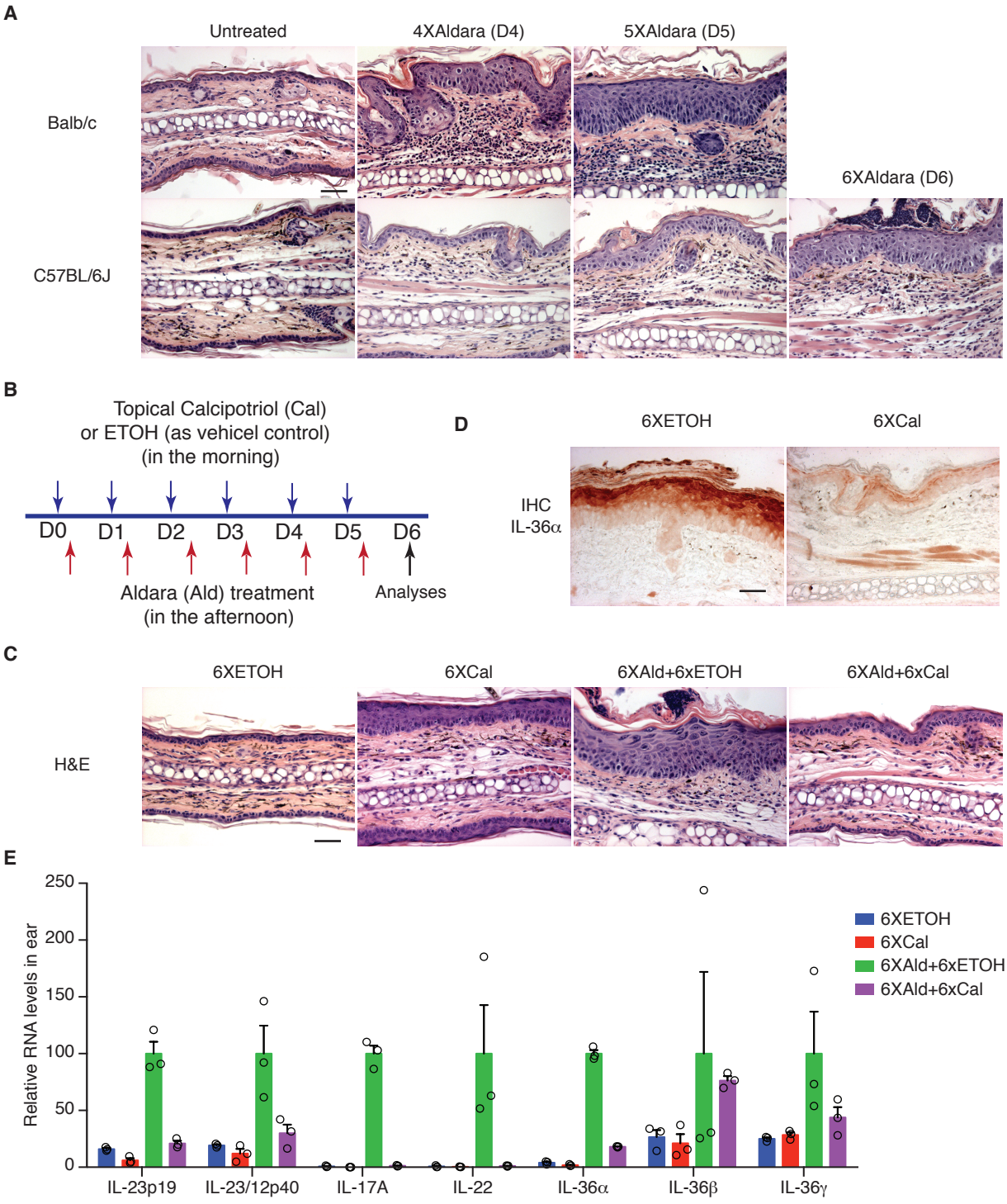


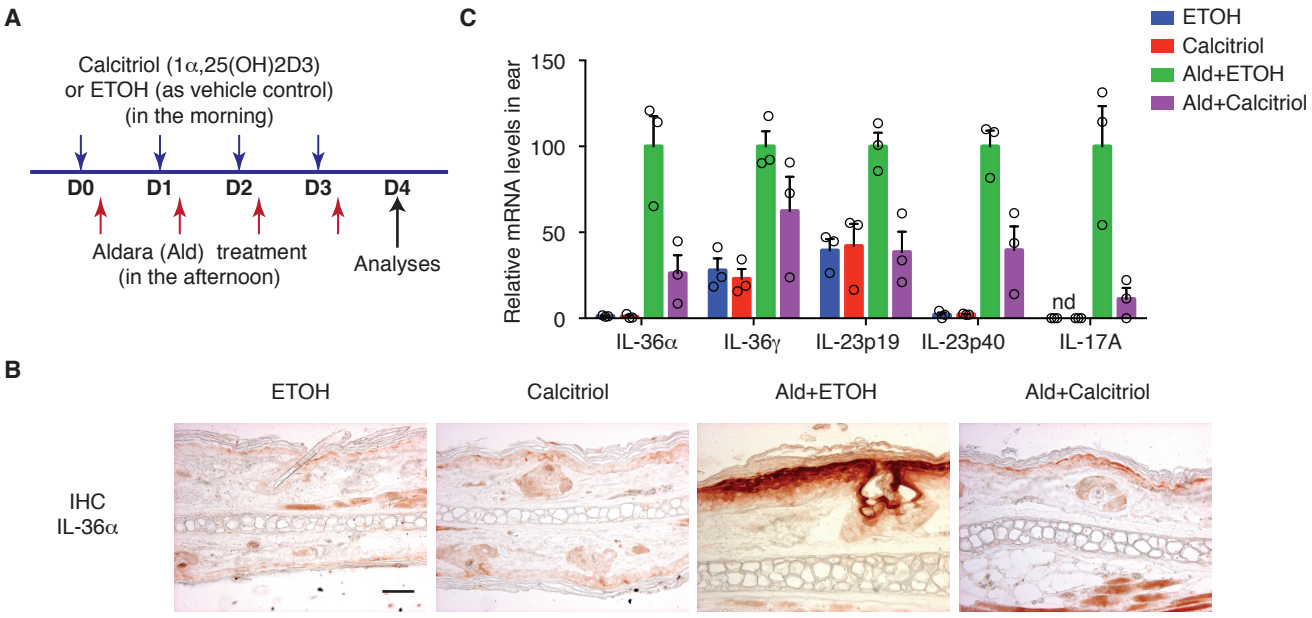
Supplemental Figure 1. Epidermal and dermal thicknesses of the mouse ears with topical treatments. H&E stained slides were digitally scanned using the 20x (0.46 μm/pixe) mode of the Hamamatsu NDP slide scanner (Hamamatsu Photonics, Nanozoomer 2.0HT). Three measurements of the thickness of the epidermis and the dermis were taken from the representative section of each ear using a digital ruler on the virtual slides using the NDP.viewer2 software. (**A**) Wildtype Balb/c mouse ears were topically treated with ETOH (vehicle control), calcipotriol (Cal), Aldara (Ald)+ETOH or Ald+Cal every day from day (D) 0 to D3, as shown in Fig. 1A. (**B**) Wildtype Balb/c mouse ears were topically treated with Aldara (Ald) from D0 to D3 to induce psoriatic inflammation, followed by two times of treatment with ETOH, calcipotriol (Cal), dexamethasone (Dex) or Cal+Dex; as shown in Fig. 7A.Values are mean ± SEM. *P<0.05; **P<0.01; ***P<0.001 (2-tailed student's *t* test).



Supplemental Figure 2. The expression of TLR7 is not detected in human primary keratinocytes (HPKCs). Comparison of RT-qPCR analyses of hTLR7 expression in HPKCs from three independent healty donors and in isolated peripheral blood mononuclear cells (PBMC) and monocytes from two independent healty donors. Primers used to amplify hTLR7 (amplicon length 155bp) were: 5'-TTGGCACCTCTCATGCTCTG-3' (forward) and 5'-ACCATCTAGCCCCAAGGAGT-3' (reverse). Results showed that the expression of TLR7 was not detectable in HPKCs (with amplification cross point Cp>45), while it was well detected in PBMC and monocytes (Cp<27 and 25, respectively).



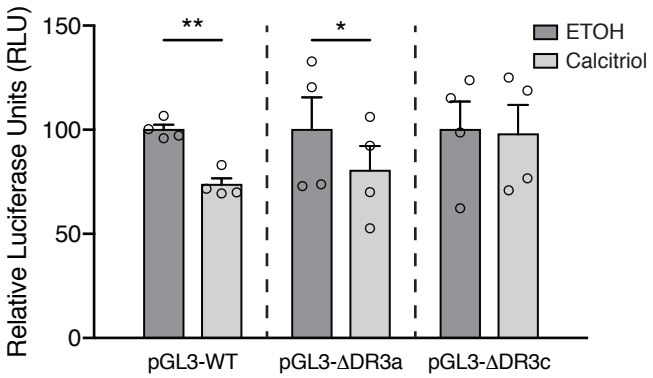
Supplemental Figure 3. Topical calcipotriol inhibits the psoriatic inflammation and the expression of IL-36α/γ expression in Aldara-treated wildtype C57BL/6J (B6) mouse skin. (**A**) Histological analyses of ears sections from wildtype Balb/c and B6 mice upon Aldara treatment (once per day from D0, and analyzed at D4, D5 or D6). Results showed that B6 mice at D6 exhibited comparable inflammation as Balb/c mice at D4. (**B**) Experimental protocol. Wildtype B6 mouse ears were topically treated with ETOH (vehicle control), calcipotriol (Cal), Aldara (Ald)+ETOH or Ald+Cal every day from day (D) 0 to D5, with ETOH or Cal treatment in the morning, and Ald treatment in the afternoon. Mouse ears were analyzed at D6. (**C**) Hematoxylin/eosin (H&E) staining of ear sections. (**D**) IHC staining with IL-36α antibody on ear sections. Bar = 50 μm for all pictures. (**E**) Quantitative RT-PCR analyses (RT-qPCR), showing that calcipotriol treatment inhibited the expression of IL-23, IL-17A, IL-22, as well as IL-36α and IL-36γ, in Aldara-treated B6 mouse ears. Data are representative of two independent experiments with similar results.



Supplemental Figure 4. Topical calcitriol (1α,25(OH)2D3, active vitamin D3) inhibits the psoriatic inflammation and IL-36/IL-23/IL-17 in Aldara-treated skin. (**A**) Experimental protocol. Wildtype Balb/c mouse ears were topically treated with ETOH (vehicle control), Calcitriol (2 nmol), Aldara (Ald)+ETOH or Ald+calcitriol every day from day (D) 0 to D3, with ETOH or calcitriol treatment in the morning, and Ald treatment in the afternoon. Mouse ears were analyzed at D4. (**B**) Immunohistochemical (IHC) staining with IL-36α antibody (in dark red) of ear sections, showing that Calcitriol treatment reduced IL-36α in Ald-treated ear skin. Arrows point to one of positive signals. Bar = 50 μm for all pictures. (**C**) Quantitative RT-PCR analyses (RT-qPCR). Relative RNA levels were calculated using HPRT as internal control. nd, not detected.

A

B



C

DR3 elements	p-value	Sequence	Start	End	Strand
DR3a	0.000225	GACTTCTTCCGGGTCA	-656	-671	-
DR3b	0.000726	AGGGTCACTGGAGTGA	-862	-847	+
DR3c	5.03e-05	GAGTCCATTGAGGTGG	-967	-952	+
DR3d	0.000513	GGGGGCATGGATGGCA	-1169	-1184	-
DR3e	1.28e-05	GGGTTCTGCAGGGTCA	-2152	-2137	+
DR3f	0.000716	CAGTGCTCTGAGCTCG	-2350	-2365	-
DR3g	0.000742	AGGTACTGCGAGCTCA	-2373	-2358	+
DR3h	0.000623	GAGCTCATTCAGGGGA	-2674	-2659	+
DR3i	0.000997	AAGGAGACAGGGTGTA	-3106	-3091	+
DR3j	9.55e-05	CAGTGCACAGGGTTGG	-4209	-4224	-
DR3k	0.000281	TGGTTCTTAGAGGTTG	-4284	-4269	+

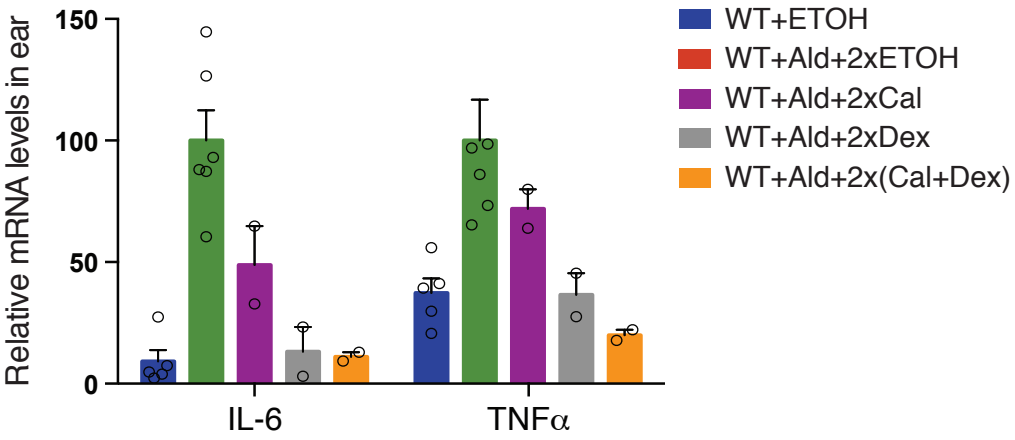
Supplemental Figure 5.

(**A**) Schematic showing of the 1085 bp fragment upstream of ATG (translational starting codon, set as +1) of hIL-36α gene, containing DR3 elements a, b and c. TSS, transcription start site.

(**B**) The 1085 bp fragment upstream of ATG of hIL-36α gene, in its wildtype (WT) form, or the form with the deletion of DR3a or DR3c element, was cloned into the pGL3-Basic vector (Promega) using In-Fusion HD cloning kit (Clontech), to obtain pGL3-WT, pGL3-ΔDR3a, or pGL3-ΔDR3b luciferase reporter constructs (see cloning methods described below). These constructs were transiently transfected into the HaCaT cells using PolyJet (SignaGen Laboratoires). A renilla luciferase plasmid (pRL-CMV) was co-transfected to control for transfection efficiency. Following transfections, the cells were treated with 10-5 M calcipotriol, or ETOH (vehicle control). Cells were lysed 24 h later and analyzed for luciferase activity using the Dual-Luciferase Reporter Assay System (Promega). Data are expressed as Relative Units of Firefly Luciferase normalized by the Renilla Luciferase (RLU). Values are mean ± SEM. *P<0.05; **P<0.01 (one-tailed student's t test).

(**C**) DR3 elements within a 5kb promoter region of hIL-36α gene. Red highlights the DR3 elements with the p-value less than 10^{-4}.

Methods for plasmid cloning: the 1085 bp wild type (WT) fragment upstream of ATG of hIL-36α gene was amplified with CloneAmp HiFi PCR premix (In-Fusion HD cloning kit), using HaCaT cell genomic DNA as template with the primer pair, 5'-CGAGATCTGCGATCTCACCCTCATAGACTTACCCCA-3', and 5'- CGGAATGCCAAGCTTTGTGGTGGTGTTGTTTCAGA-3'. The luciferase reporter vector pGL3-basic (Promega) was amplified using the primer pair (5'-AAGCTTGGCATTCCGGTACT-3' and 5'- AGATCGCAGATCTCGAGCC-3'). The two PCR products were used for in-fusion cloning to obtain pGL3-WT. The constructs pGL3-ΔDR3a and pGL3-ΔDR3c were obtained by amplifying the pGL3-WT with the following primer pairs flanking the DR3a or DR3c region and re-ligate each PCR product by In-Fusion cloning (Clontech). Primer pairs for DR3a deletion were: 5'- GTCATCCGTAATGTTTCTCAACTCAGTATCTG-3' and 5'- AACATTACGGATGACTCATCTCTGATGCCACAA-3'. Primer pairs for DR3c deletion: 5'- TAGTAGATCACTATCTCTAGTATAGGTAGCGTGAT-3' and 5'- GATAGTGATCTACTAGAGTGCTCAGGAGCTGGCT-3'.



Supplemental Figure 6. A synergy between calcipotriol and dexamethasone in inhibiting IL-6 and TNFa expression in the established psoriasis in mice. Wild-type Balb/c mouse ears were topically treated with Aldara (Ald) from D0 to D3 to induce psoriatic inflammation, followed by two times of treatment with ETOH, calcipotriol (Cal), dexamethasone (Dex) or Cal+Dex at D4 and D5, as shown in Fig. 7A. RT-qPCR analyses of the treated skin for the RNA levels of IL-6 and TNFα. Data are representative of two independent experiments with similar results.

Supplementary Table 1. Sequences of the primers used for quantitative RT- PCR analysis.

Gene name	Sequence (5' to 3')
HPRT (164 bp)	F TGGATACAGGCCAGACTTTG
	R GATTCAACTTGCGCTCATCTTA
mIL17A (239 bp)	F CCAGGGAGAGCTTCATCTGT
	R ACGTGGAACGGTTGAGGTAG
mIL22 (166 bp)	F CCGAGGAGTCAGTGCT AAGG
	R GCTGATGTGACAGGAGCTGA
mIL23p19 (213 bp)	F AATAATGTGCCCCGTATCCA
	R CTGGAGGAGTTGGCTGAGTC
mIL-23/12p40 (176 bp)	F CCTGAAGTGTGAAGCACCAA
	R AGTCCCTTTGGTCCAGTGTG
mIL36α (187bp)	F CCACGTACATGGGAGTGCAA
	R GGGAAGGCTGCAGACTCAAA
mIL36β (185bp)	F AGATGGTATGGGTCCTGACTGG
	R GCCCTCCATCTCAACACAGC
mIL36γ (200bp)	F GCAGGTGTGGATCTTTCGTAATCA
	R GCAGCAAAGTAGGGTGTCCA
mS100A7A (167 bp)	F CTTGTCCCTGGAGGAGTTGA
	R GCTTGCCCAAGATGTACAGG
mS100A8 (178 bp)	F GGAAATCACCATGCCCTCTA
	R GAGATGCCACACCCACTTTT
mTSLP (194 bp)	F AGCTTGTCTCCTGAAAATCGAG
	R AGGTTTGATTCAGGCAGATGTT

mCyp24 (187bp)	F	CCAGCGGCTAGAGATCAAAC
	R	CCCCATAAAATCAGCCAAGA
hGAPDH (158bp)	F	GTCAAGGCTGAGAACGGGAA
	R	AAATGAGCCCCAGCCTTCTC
hIL36α (225bp)	F	TGGGGTCGGTCTGCACATAA
	R	GGGTCTCCACATGTCGGCAT
hIL36β (185bp)	F	CGACAGATGGTGTGGGTCCT
	R	TCCCTTGATTCCCAGGTAAACCA
hIL36γ (200bp)	F	GAGCAAGGCAGAGGGGATCC
	R	GCCACAGACTCAAGGGTGGA
hCyp24 (249bp)	F	GGCAACAGTTCTGGGTGAAT
	R	TATTTGCGGACAATCCAACA
hTSLP (184bp)	F	CCAGGCTATTCGGAAACTCA
	R	TGGTGCTGTGAAATATGACCA