Supplementary Tables

Supplementary Table S1. The primers for genotyping of mice

gene name	sequence
K5-cre-F	CAGGGTGTTATAAGCAATCCC
K5-cre-R	TGGTTGTCAATACTCCTGGTCCTG
Ncstn-F	AGCTCTTCACCAGGTAAGAAC
Ncstn-R	TTGGACAGTCCTTCCCTGAAG

Supplementary Table S2. Quantitative real-time PCR primers for mice

gene name	sequence
m-Il1f6-qF1	CAGTCACTATTACCTTGCTCCCA
m-Il1f6-qR1	TTCCCTTCCCCAAGCTGTAG
m-Actin-qF	TGGCATTGTTACCAACTGGGAC
m-Actin-qR	GAAGGTCTCAAACATGATCTGG
m-Tnf-qF	CCCAGACCCTCACACTCAGAT
m-Tnf-qR	CAAGGTACAACCCATCGGCTG
m-Tlr4-qF	TCAGAACTTCAGTGGCTGGATT
m-Tlr4-qR	GGGTTTCCTGTCAGTATCAAGT
m-Il23a-qF	CTCAAGGACAACAGCCAGTTCT
m-Il23a-qR	TAGTAGGGAGGTGTGAAGTTGC
m-Sprr2d-qF	CTGTGTGCCCACCCAAGAAG
m-Sprr2d-qR	GACATGGCTCAGGACAAGGC

m-Notch1-qF	TGTATGCCAGGTTATGAAGGT
m-Notch1-qR	TGTAACCTTCTGTACACACGC
m-Sprr2a-qF	TGTCTTACTACCAGCAGCAGTG
m-Sprr2a-qR	TGGAAATTGCACAGGAGGGC
m-Sprr2b-qF	TCTTACCACCAGCAGCAGTG
m-Sprr2b-qR	TCTGCTGGCATGGTGGAAAT
m-Sprr2f-qF	CAGAGCCTTGTTCTCCCTCG
m-Sprr2f-qR	CTCTTGGGTGGGCACTTCTG

Supplementary Table S3. Quantitative real-time PCR primers for HaCaT cells

gene name	sequence
H-ACTB-QF	CATGTACGTTGCTATCCAGGC
H-ACTB-QR	CTCCTTAATGTCACGCACGAT
H-IL36A-QF	ACCGTATGTCTCCAGTCACT
H-IL36A-QR	ATCCTTTTCCTTCAGCTGCA
H-SPRR2D-QF	CCCTGCCCATCACCAAAGT
H-SPRR2D-QR	CTTGCTCTTGGGTGGACACT
H-Notch1-QF	AATGACTTCCACTGCGAGTGC
H-Notch1-QR	CCGCAGGGCACTTGCAGATGA
H-SPRR2A-QF	TCAGCAGTGCCAGCAGAAAT
H-SPRR2A-QR	TACTTTGACTGGCAGGGTGG
H-SPRR2B-QF	TCAGCAGTGCCAGCAGAAAT

H-SPRR2B-QR	GATACTTTGGCTGGCAGGGT
H-SPRR2F-QF	GCCAGCAGAAATGTCCTCCT
H-SPRR2F-QR	CTTGCTCTTGGGTGGACACT

Supplementary Table S4. The sequences of the three Notch1-siRNAs

Gene name	Sense	antisense
NOTCH1-1	GATCCCCCACCAGTTTGAATGGTCAATTCAAGAGATTGACCATTCAAACTGGTGTTTTTGGAAA	AGCTTTTCCAAAAACACCAGTTTGAATGGTCAATCTCTTGAATTGACCATTCAAACTGGTGGGG
NOTCH1-2	GCAGCTGCACTTCATGTACGT	CGTCGACGTGAAGTACATGCA
NOTCH1-3	TCGCATTGACCATTCAAACTGGTGG	
NCSTN	UAGACUGGAACCAUGAGUUGUUGGC	GCCAACAACUCAUGGUUCAGUCUA

Supplementary Methods

DNA extraction and genotyping

Genomic DNA was extracted from mice toes using saturation sodium chloride method （5M NaCl）. Polymerase chain reaction (PCR) amplification was performed for genotyping under the following conditions: pre-denaturation, 98°C for 1 minute,

followed by 35 cycles of 98°C for 10 seconds, 60°C for 30 seconds, and 72°C for 1 minute. The PCR products were then separated on a 3% agarose gel. The K5-Cre specific primers can amplify fragments of K5-Cre recombinase gene about 1000 bp. And the *Ncstn* genotyping primers were designed on both sides of loxp, which is 40 bp in length. The heterozygous mice (ie, the heterozygous *Ncstn* knockout in *K5-Cre*–positive mice) carried one loxp site in one allele, and none in the other allele. So it had two bands with a difference of 40 bp. While, the homozygous mice (ie, the homozygous *Ncstn* knockout in *K5-Cre*–positive mice) carried loxp sites in both alleles, and their amplified product fragment size is 241 bp. DL2000 was used for DNA marker. The bands from top to bottom correspond to 2000, 1000, 750, 500, and 250 base pairs (bp) The primers for genotyping listed in Supplementary Table S1.

Quantitative Real –time PCR

Total RNA was extracted from skin tissue using Trizol® reagent (ambion, Life Technologies, 144303), followed by removing genomic DNA by an additional DNase-treatment. Then, the RNA was reverse transcribed to cDNA using GoScript™ Reverse Transcription System （Promega, USA, A5003） according to the manufacturer's instructions. The cDNA was diluted in appropriate dilutions based on the cycle threshold of the reference gene. The cycling parameters were set under the following conditions: pre-denaturation, 95°C for 5 minutes, followed by 40 cycles of 95°C for 10 seconds, 60°C for 15 seconds, and 72°C for 20 seconds. The quantitative real-time PCR assay was carried out and analyzed by SYBR® Premix Ex Taq™ (TaKaRa, RR420A) and Rotor-Gene 6000 (Corbett, Australia). All results were performed with 3 biological

repeats. Exon-spanning primers were designed to avoid amplification of genomic DNA as showed in Supplementary Table S2-S3.

Western blot

Total protein was extracted from the skin of wild type mice and cKO/cKO mice separately, using lysis buffer (RIPA, Beyotime, P0013C) containing a soluble protease inhibitor cocktail (Roche, 4693132001). Protein concentration was determined using the BCA Protein Assay Kit (TIANGEN, PA115-01). In brief, equal amounts of protein samples were separated by 12% SDS-PAGE gels and transferred to polyvinylidene fluoride (PVDF) membranes （Millipore, IPVH00010） at 220 mA for 2 hours. Then, the nonspecific binding sites were blocked with 5% skimmed milk (BD, 232100) at room temperature for 1 hour. Membranes were incubated overnight at 4 ℃ with appropriate dilutions of primary antibodies against Ncstn (SANTA CRUZ, SC376379), Cleaved Notch1 (CST, #4147), Lipocalin-2 (abcam, ab216462), Mouse IL-36a (R&D systems, AF-2297) and washed with PBS and then incubated with HRP-conjugated secondary antibodies (ZSGB-Bio, ZB2301, ZB2305 and ZB2306) at room temperature for 1 hour. Finally, membranes were visualized by chemiluminescence (High-Sig ECL, Tanon, 180-5001). While, quantitative analysis chart of the bands was performed using Image J analysis software. All results were performed with 3 biological repeats.

Immunohistochemistry

Tissue sections of 3 μm from mice skin after formalin-fixed, paraffin-embedded were deparaffinized and rehydrated with xylene and gradient ethanol. Slides were then subjected to antigen retrieval (citrate retrieval buffer ZLI-9065, ZSGB-Bio) and rinsed

in phosphate buffered saline(PBS), followed by blocking with 5% bovine serum albumin (BSA) incubating at room temperature for 45 minutes. Subsequently, slides were incubated overnight at 4 °C with appropriate dilutions of primary antibodies against IL-36a (R&D systems, AF-2297), Cytokeratin 5 (abcam, ab52635), CD68 (abcam, ab31630), CD11c (proteintech, 60258-1-Ig), TNF-α (abcam, ab6671), RORγ t (abcam, ab207082), IL-17A (Novus, NBP176337). Then the sections were incubated with secondary antibodies of the corresponding genus (ZSGB-Bio, ZB2301, ZB2305 and ZB2306) and streptavidin-biotin complex (Boster, SA1022 and SA1021), respectively. The immunostaining was carried out with a diaminobenzidine tetrahydrochloride (DAB) substrate kit (Boster, AR1022).

Immunofluorescence staining

Formalin-fixed, paraffin-embedded tissue sections of 3 μm from mice skin were deparaffinized and rehydrated in xylene and gradient ethanol. Antigen retrieval was performed in citrate retrieval buffer by boiling and rinsed in tris-buffered saline and Tween 20 (TBST). Then, sections were blocked in 5% bovine serum albumin for 45 minutes. Appropriate dilutions of primary antibodies against Nicastrin (santa cruz, sc-376379) and Cytokeratin 5 (abcam, ab52635) were incubated for 2h followed by incubation in secondary antibodies for 30 minutes at room temperature. Sections were then stained with TSA-CY3 (NEL744E001KT, Perkinelmer), TSA-FITC (NEL741E001KT, Perkinelmer) and 4′,6-diamino-2-phenylindole (DAPI) for 10 minutes and mounted using antifade mounting media.