SUPPLEMENTARY MATERIALS AND METHODS

Animals

The donor vector used to generate *Trpv3*$^{+/G568V}$ mice covers the intron between exon 12 and 13 and the intron between exon 17 and 18. It also contains a point mutation c.1703 G/T in exon 13 and a LoxP-flanked neomycin resistance cassette between exon 13 and 14. The targeting construct was electroporated into C57BL/6N ES cells. G418-resistant clones were picked for PCR, gel electrophoresis, sequencing and Southern Blot. 1 clone (2G7) were confirmed with correct homologous recombination. The 2G7 ES cell clone was injected into C57BL/6N mouse blastocysts and transferred into the uteri of pseudopregnant females, from which F0 chimeras were obtained. Tails of F0 chimeras were cut for PCR, gel electrophoresis and sequencing. Potentially positive F0 mice were bred with EIIa-Cre transgenic C57BL/6J mice to remove the neomycin resistance cassette from the targeted allele in F1 offspring. Tails of F1 pups were cut for PCR (forward primer: GAAGCCATCAATTATCATGGT; reverse primer: ATCATCTTCTAGACATGCCAG), gel electrophoresis and sequencing, and finally 8 pups were confirmed positive and carrying the correct allele. Thereafter, these *Trpv3*$^{+/G568V}$ mice were maintained on C57BL/6N and C57BL/6J mixed background.

The donor vector used to generate *Trpv3-Flag* mice covers the intron between exon 13 and 14 and the sequence of 3' UTR. It also contains a 3XFlag-IRES-CreERT2 cassette right before the stop codon of the last exon (exon 18). Donor vectors were purified and screened by restriction endonuclease digestion. The positive donor vector, Cas9 mRNA and gRNA (gRNA1: GAAACGTCGGTGTAGATGCCTGG, gRNA2: AGATGAATTCCCAGAAACGTCGG) were microinjected into C57BL/6J mice zygotes, which were then transferred into the uteri of pseudopregnant females. Tails of F0 pups were cut for PCR and gel electrophoresis, and 4

potentially positive pups were obtained. F0 mice were bred with C57BL/6J mice to get F1 offspring. 4 F1 pups were confirmed positive and carrying the correct allele by PCR (*Trpv3-Flag* forward primer: ACATTTCTGTCCAGCACCCT; wild type forward primer: TAACACAGGGACAGGACACA; common reverse primer: GTGCTTCCCTTTCAGATCCT), gel electrophoresis and sequencing. Thereafter, these *Trpv3-Flag* mice were maintained on a C57BL/6J background.

Trpv3 knockout (*Trpv3*$^{-/-}$) mice were obtained from Jackson Laboratory (Stock No: 010773).

Electrophysiology

Wild type and G568V mutant *TRPV3* cDNA were cloned into pCMV6-AC-GFP vector (ORIGENE, PS100010). 12 hours after transfection, HEK293T cells with green fluorescence on the membrane were chosen for patch-clamp recordings under an inverted fluorescence microscope (Nikon, Tokyo, Japan). Currents were recorded using a HEKA EPC10 amplifier with PatchMaster software (HEKA, Lambrecht, Germany) in whole-cell configuration. Patch pipettes were pulled from borosilicate glass and fire-polished to have resistance of 2-5 MΩ. Membrane potential was held at 0 mV. For voltage-dependent activation, patches were held at 0 mV, stepped from −160 to +160 mV with an increment of 20 mV for 300 ms, and then back to 0 mV for 100 ms. For 2-APB (Sigma, 100065) induced activation, currents were elicited by a protocol consisting of a 400 ms step to +80 mV, followed by a 400 ms step to −80 mV at 2 s intervals. 10 μM Ruthenium Red (Sigma, R2751) was used to assess leak currents. Both pipette and bath solutions contained 130 mM NaCl, 0.3 mM EGTA and 3 mM HEPES (buffered to pH 7.4 with NaOH). All experiments were conducted at room temperature (22-25 °C).

RNA isolation and qRT-PCR

Total RNA of mouse dorsal skin was isolated with GeneJET RNA Purification Kit (K0732, Thermo Scientific) according to the manufacturer's instruction. Specifically, complementary DNA was synthesized from 2 μg of total mRNA using a high-capacity cDNA reverse transcription kit (4374967, Applied Biosystems, Grand Island, NY) according to the manufacturer's instruction. Quantitative real-time PCR was performed with TaqMan™ Fast Advanced Master Mix (4369510, Applied Biosystems, Grand Island, NY) according to the manufacturer's instruction on ABI Prism 7500 with the following TaqMan probes: *Trpv3*, Mm00455003_m1; and *Actb (β-actin)*, Mm02619580_g1 (Life Technologies, Grand Island, NY). Results were analyzed using the ΔΔCt method. Relative expression levels of target genes were determined by comparing with wild type controls after normalizing with *β-actin*.

Histological analyses and immunofluorescence labeling

Specimens for histological analyses were examined with an Olympus BX40 (Olympus, Tokyo, Japan) or Nikon Eclipse 80i (Melville, NY) microscope. For immunofluorescence labeling, paraffin-embedded tissue sections were deparaffinized, rehydrated, and then microwaved in Tris-EDTA-Tween buffer (10 mM Tris Base, 1 mM EDTA, 0.05% Tween 20, pH 8.0) for 20 min for antigen retrieval. Sections were blocked in 10% BSA with 0.3% Triton-X 100 at room temperature for 30 min, and then incubated with primary antibodies diluted in 10% BSA with 0.3% Triton-X 100 at 4℃ overnight or at room temperature for 4 hours. The following primary antibodies were used: rabbit anti-FLAG (2368S, Cell Signaling, Danvers, MA), 1: 200; guinea pig anti-KRT71 (GP-K6irs1, Progen, Heidelberg, Germany), 1:200; guinea pig anti-KRT75 (GP-K6hf, Progen, Heidelberg, Germany), 1:200; rabbit anti-KRT75 (a gift from Dr. Dennis R. Roop),

1:250; guinea pig anti-KRT82 (GP-hHb2, Progen, Heidelberg, Germany), 1:200; mouse anti-NFATc1 (sc-7294, Santa Cruz, Dallas, TX), 1:50; rat anti-CD34 (13-0341-82, eBioscience, Carlsbad, CA), 1:50; mouse anti-AE13 (ab16113, Abcam, Cambridge, MA), 1:200; mouse anti-AE15 (a gift from Dr. Tung-Tien Sun), 1:200; rabbit anti-KRT1 (ab24643, Abcam, Cambridge, MA), 1:1000; chicken anti-KRT14 (906001, BioLegend, Dedham, MA), 1:800; rabbit anti-Loricrin (905101, BioLegend, Dedham, MA), 1:1000; AlexaFluor 594 conjugated mouse anti-BrdU (B35132, Invitrogen, Eugene, OR). Then AlexaFluor 488 or AlexaFluor 594 conjugated goat secondary antibodies (1:250, A-11034, A-11073, A-11029, A-11076, A-11032, A-11007, A-11042,Life Technologies, Eugene, OR) were incubated at room temperature for 2 h. Sections were sealed in mounting medium with 4',6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA). Images were acquired by Nikon 80i (Nikon, Melville, NY) fitted with a Nikon DS-Qi1Mc camera and processed with Photoshop, version 6.1 CS (Adobe, San Jose, CA).

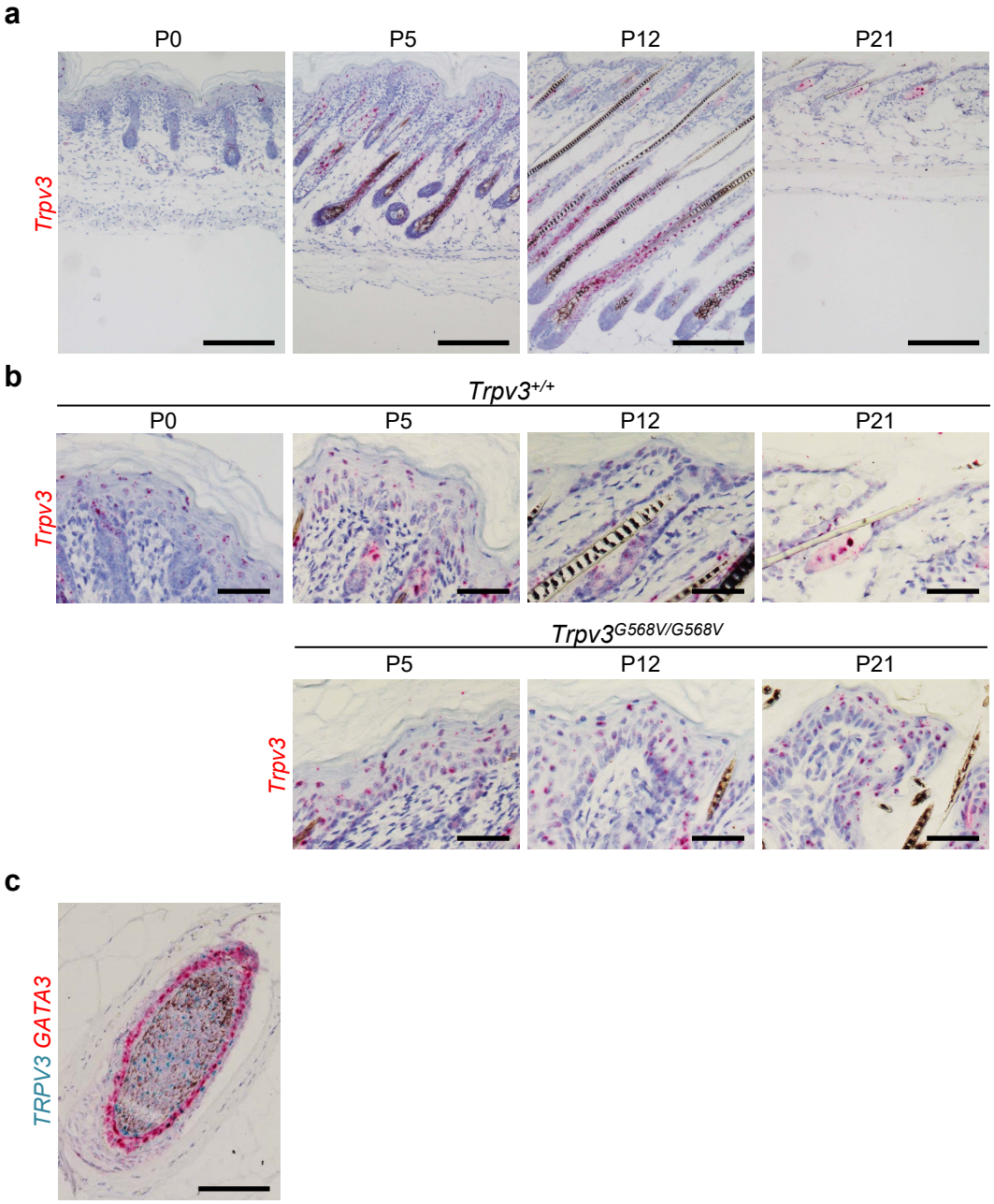
Scanning and transmission electron microscopy

To investigate the surface morphology of hair shafts, Scanning Electron Microscopy (Crossbeam 340, Carl Zeiss Microscopy, Germany) was used. Samples were first fixed in 2.5% glutaraldehyde and 2% paraformaldehyde in PBS then followed by postfix in 1% $Os(OH)_4$ for 1 hour. Samples were then dehydrated with series of concentration of ethanol (50%, 70%, 90%, then 3 times in 100%) for 10 minutes each. For drying, samples were immersed in 100% HMDS (MilliporeSigma, St. Louis, MO) for 3 minutes after ethanol dehydration, then transferred to desiccator for 30 minutes. For imaging, samples were coated with Au/Pd particles with the thickness of 8 nm to help electrons dissipate from the surface. Images were taken at the EHT of 3kV with a secondary electron detector.

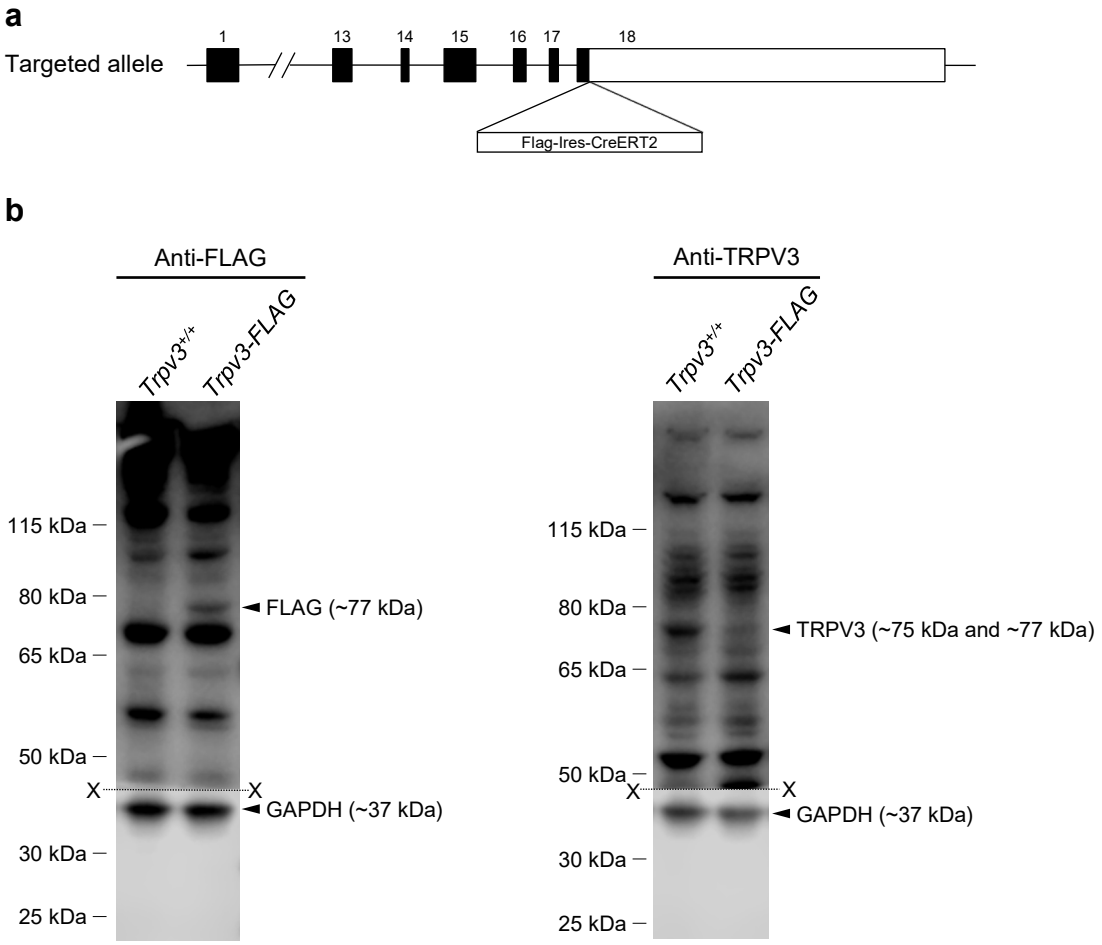
To investigate the structure of hair follicles, small pieces of the back skin of mice were fixed in 2.5% glutaraldehyde and 4% paraformaldehyde in 0.1 M sodium cacodylate (pH 7.4) at 4 ℃ overnight. After several washes in 0.1 M cacodylate buffer (pH 7.4), samples were post-fixed in 1% osmium tetroxide in 0.1 M sodium cacodylate buffer for 1 h at room temperature. Samples were then dehydrated through a graded ethanol series, and then embedded in a mixture of epon-araldite resin for overnight at 60 ℃. Finally, resin blocks were cut into 80 nm ultrathin sections with an ultramicrotome (PT-XL, RMC, USA), and sections were post-stained with lead citrate and uranyl acetate before being examined on a transmission electron microscope (JEM 1400, JEOL, USA).

Western blotting

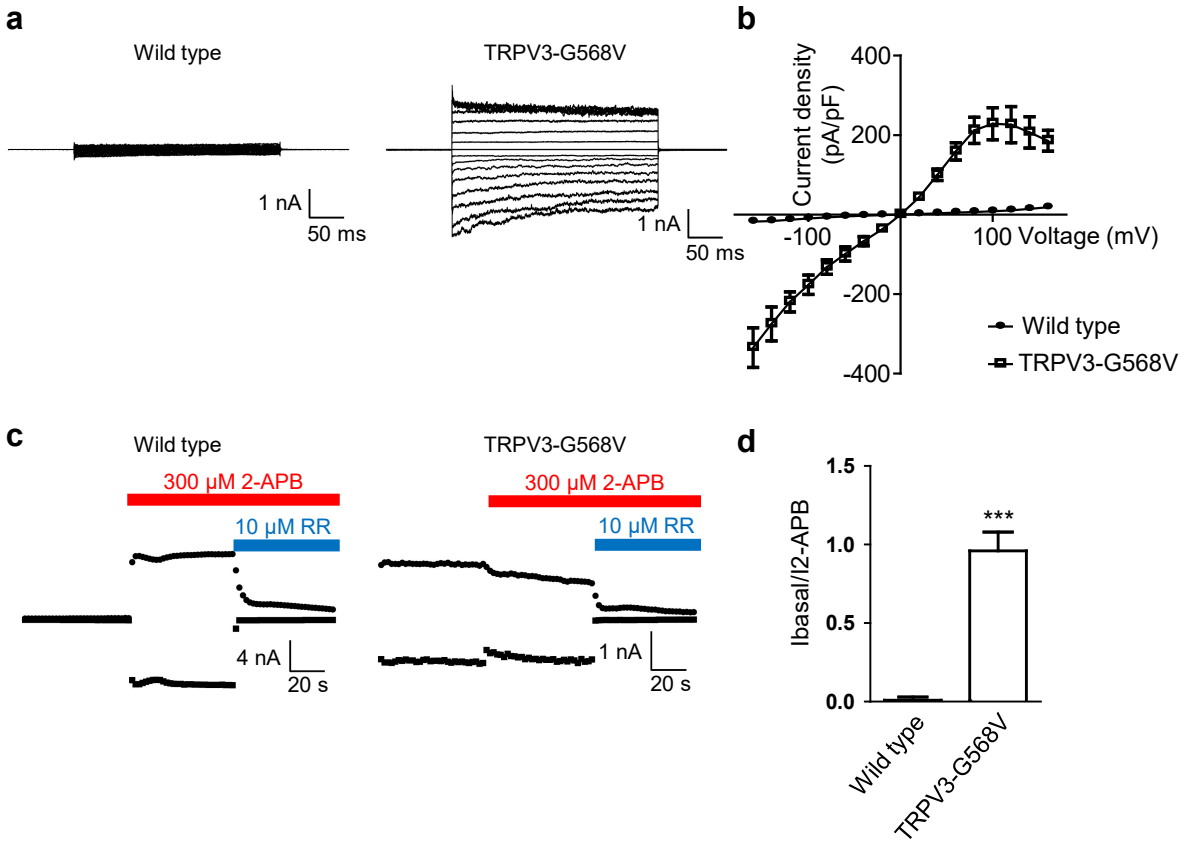
Protein was extracted from whole dorsal skin of mice in cold RIPA lysis buffer (50 mM Tris-HCl pH 7.4, 150 mM NaCl, 1% Triton X-100, 1% sodium deoxycholate, and 0.1% SDS) supplemented with proteinase inhibitors. Tissue or cell lysates were cleared and separated by 10% SDS-PAGE and transferred to polyvinylidene fluoride (PVDF, Millipore) or Hybond nitrocellulose (GE Healthcare) membranes, following standard procedures. Blots were probed with the primary antibodies which were then detected with HRP-conjugated secondary antibodies (BD biosciences) and SuperSignal substrates (Thermo Scientific, Waltham, MA). Enhanced chemiluminescent (ECL) substrate (Pierce, Rockford, IL USA) and CL-XPosure film (Thermo Scientific) was used for detection. GAPDH (2118S, Cell Signaling, Danvers, MA), 1:1000 was used as a loading control. Quantification was performed with densitometry and ImageJ software (1.43u, NIH, Bethesda, MD). The following primary antibodies were used for western blotting: TRPV3 (ab231150, Abcam), 1:1000; FLAG (2368S, Cell Signaling), 1:500; KRT71 (GP-K6irs1, Heidelberg, Germany), 1: 1000; KRT75 (GP-K6hf, Heidelberg, Germany), 1: 1000; AE13 (ab16113, Abcam, Cambridge, MA), 1: 1000.



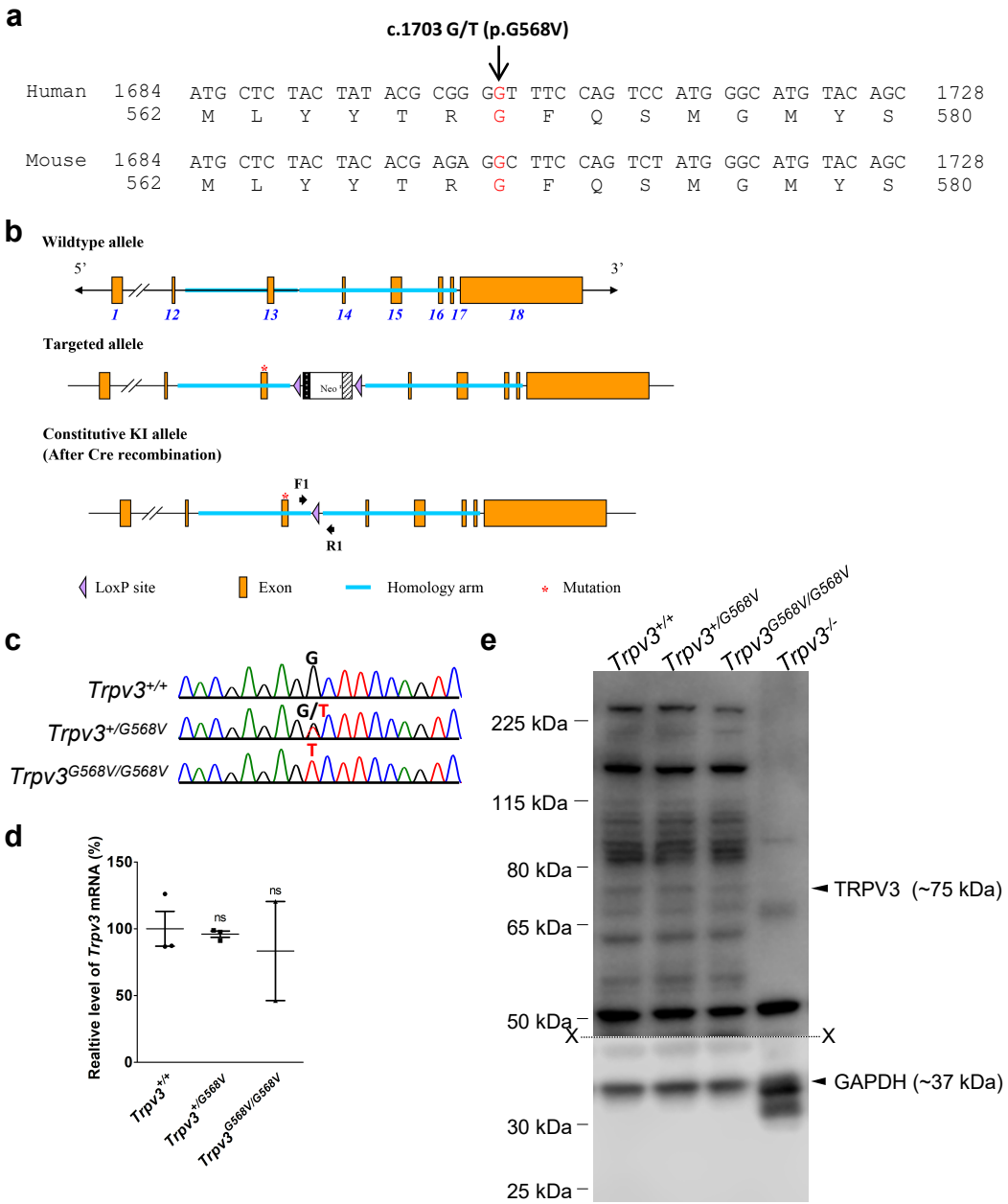
Supplementary Figure S1. *Trpv3* expression pattern in mouse and human HFs. (**a**) *In situ* hybridization of *Trpv3* (pink) in dorsal skins of wild type mice at indicated postnatal (P) days. At P21, hair follicles are in telogen. (**b**) *In situ* hybridization of *Trpv3* (pink) in dorsal epidermis of *Trpv3*$^{+/+}$ and *Trpv3*$^{G568V/G568V}$ mice at indicated postnatal days. (**c**) Duplex *in situ* hybridization of *TRPV3* (cyan) with *GATA3* (pink) in an obliquely transverse section of an adult human scalp hair follicle. Nuclei were stained with hematoxylin (blue). Scale bar = 200 μm (**a**), 100 μm (**b** - **c**) .



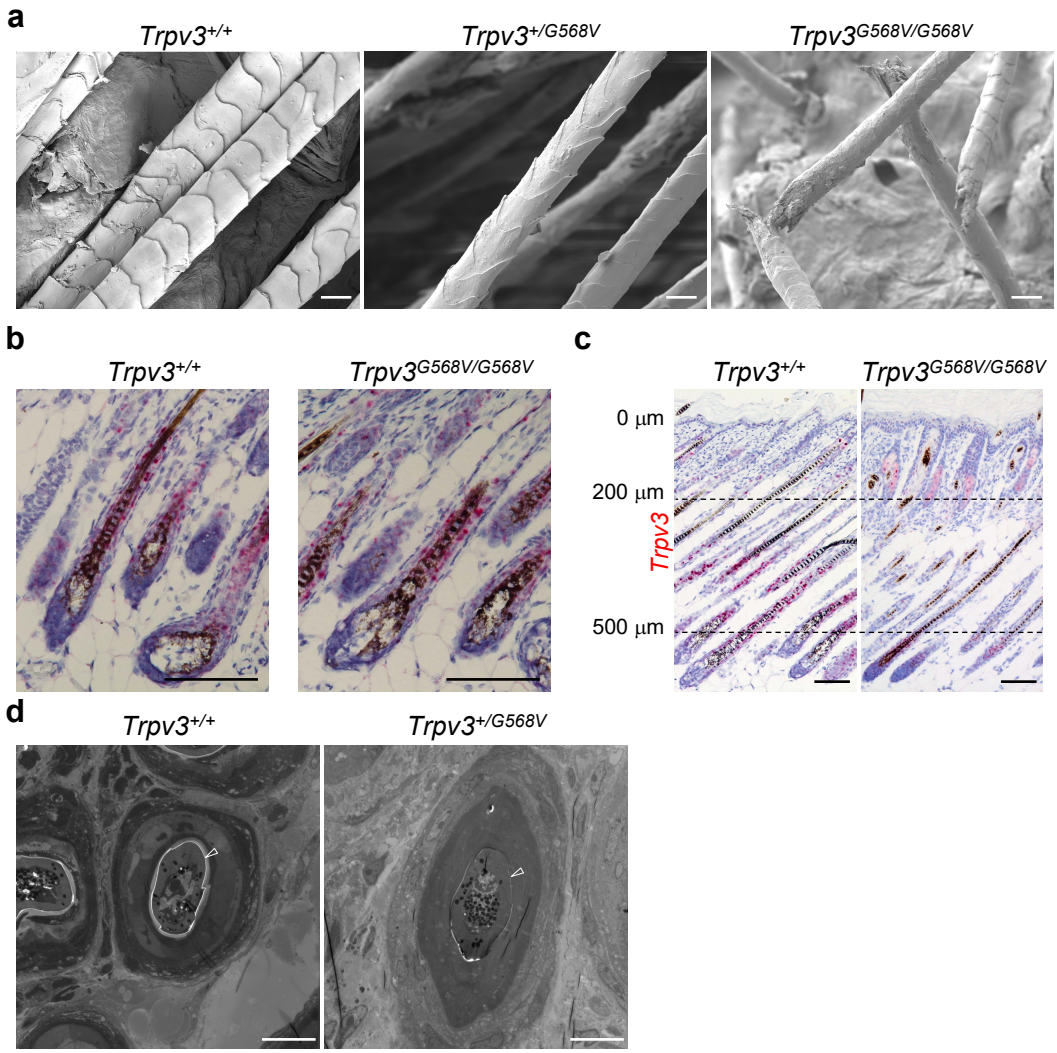
Supplementary Figure S2. Construction strategy and verification of *Trpv3-Flag* reporter mice. (**a**) Structure of the targeted *Trpv3* allele, in which *Flag* is inserted before the stop codon of *Trpv3*. (**b**) Verification of *Trpv3-Flag* knock-in mice by western blotting with FLAG antibody (left) or TRPV3 antibody (right). Heterozygous *Trpv3-Flag* mice were used. Arrowheads indicate FLAG-fused TRPV3 (~77 kDa), putative endogenous TRPV3 (~75 kDa), and GAPDH (~37 kDa). X indicates where the blot was cut prior to incubating with primary antibodies. Note that the TRPV3 antibody detected multiple bands. It is uncertain whether these bands are specific and represent isoforms, multimers, or posttranslationally modified forms of TRPV3.



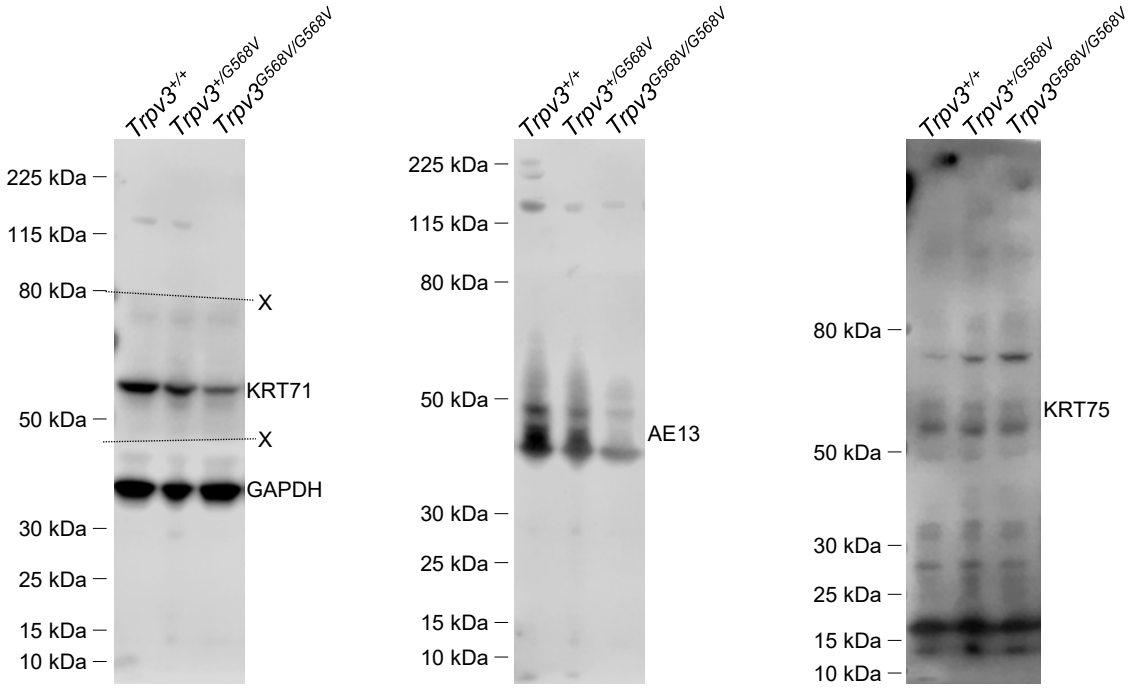
Supplementary Figure S3. Electrophysiological evaluation of HEK293T cells transfected with wild type and TRPV3-G568V constructs. (**a**) Representative whole-cell recordings of current. Patches were held at 0 mV, steps from -160 to +160 mV in 20 mV increments for 300 ms, and then back to 0 mV for 100 ms. (**b**) Respective current density-voltage plots of steady-state currents shown in (**a**). (**c**) Representative current traces at ± 80 mV in response to 300 µM 2-aminoethoxydiphenylborate (2-APB). 10 µM ruthenium red (RR) was used to assess leak currents. (**d**) Comparison of ratios of basal current amplitude over current amplitude evoked by 2-APB. Data are presented as mean ± SEM. n = 3 – 4 cells. *** $P < 0.001$ (Two-tailed Student's unpaired *t* test).



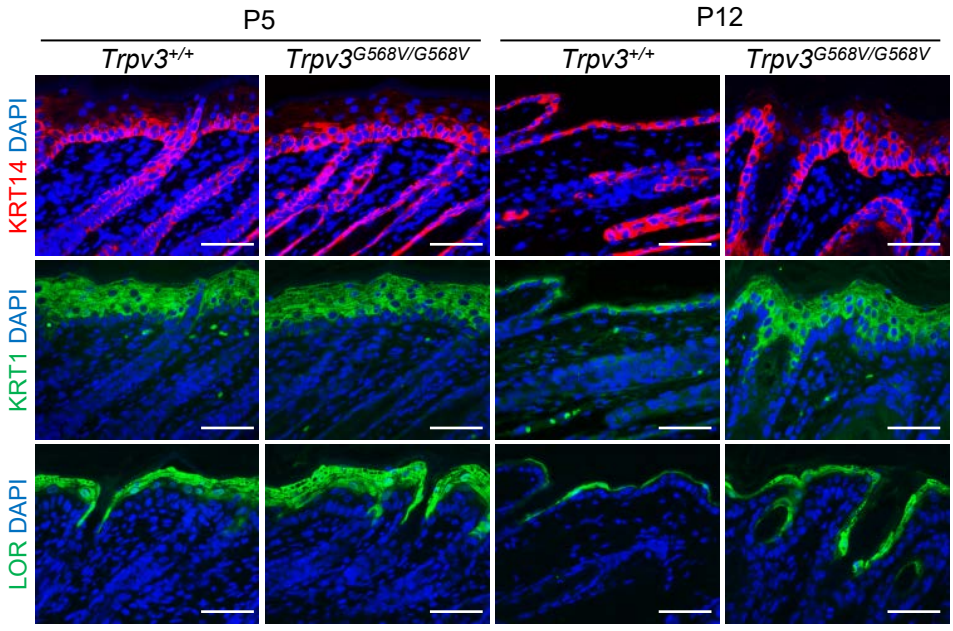
Supplementary Figure S4. Construction strategy and verification of *Trpv3 (G568V)* knock-in mouse model. (**a**) Alignment of TRPV3 genomic DNA and peptide sequences surrounding the G568V mutation found in Olmsted syndrome patients. (**b**) Targeting strategy of *Trpv3 (G568V)* knock-in mice. (**c – e**) Verification of *Trpv3 (G568V)* knock-in mouse models by sanger sequencing of genomic DNA (**c**), RT-qPCR (**d**), and western blotting (**e**) in P5 control (*Trpv3*$^{+/+}$), heterozygous mutant (*Trpv3*$^{+/G568V}$), and homozygous mutant (*Trpv3*$^{G568V/G568V}$) littermates. (**d**) Data are presented as mean ± SEM, and one-way ANOVA analysis was used. ns, not significant. (**e**) Arrowheads indicate putative TRPV3 (~75 kDa) and GAPDH (~37 kDa). X indicates where the blot was cut prior to incubating with primary antibodies. Note that the TRPV3 antibody detected multiple bands. It is uncertain whether these bands are specific and represent isoforms, multimers, or posttranslationally modified forms of TRPV3.



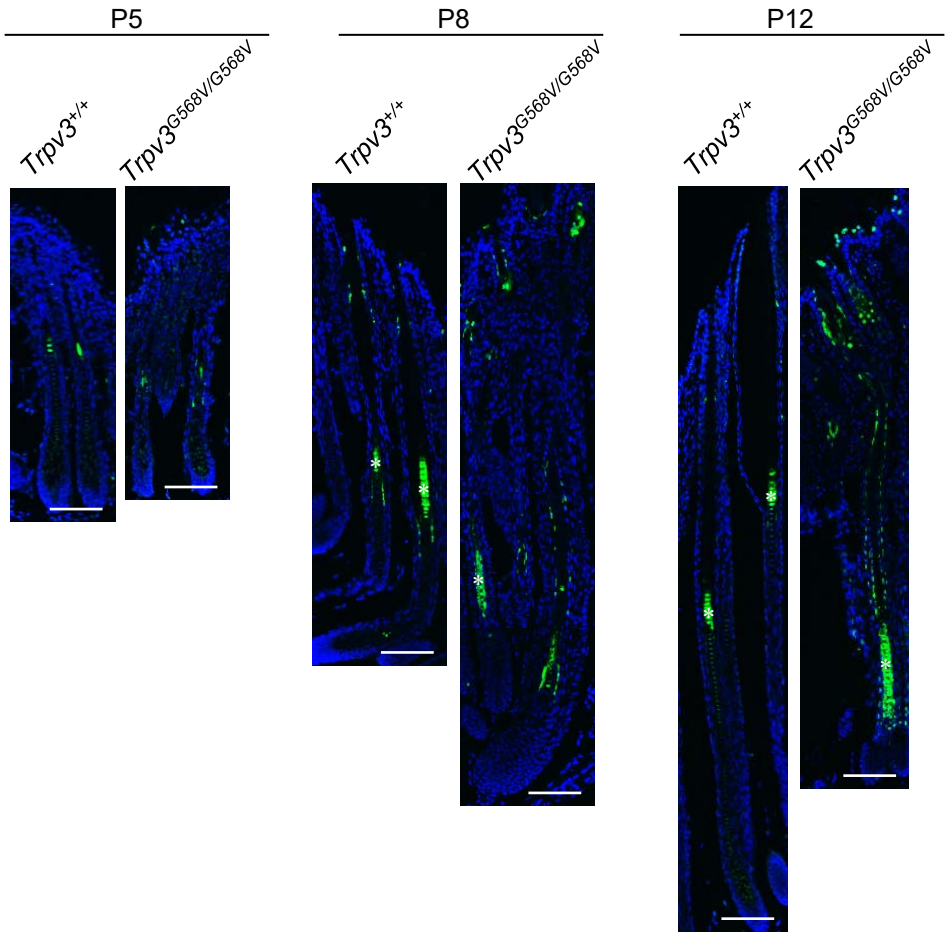
Supplementary Figure S5. Ultrastructure of hair shaft and hair follicle. (**a**) Representative scanning electron microscopy of hair shafts in P12 *Trpv3*$^{+/+}$, *Trpv3*$^{+/G568V}$, and *Trpv3*$^{G568V/G568V}$ littermates. (**b** - **c**) *In situ* hybridization of *Trpv3* (pink) in dorsal skins of P5 (**b**) and P12 (**c**) *Trpv3*$^{+/+}$ and *Trpv3*$^{G568V/G568V}$ littermates. Dotted lines indicate approximate depth from skin surface. (**d**) Transmission electron microscopy of distal region (approximately 200 μm below skin surface) of hair follicles in *Trpv3*$^{+/+}$ and *Trpv3*$^{+/G568V}$ littermates. Arrowheads point to the gap between IRS cuticle and hair shaft cuticle. Scale bar = 10 μm (**a, d**), 100 μm (**b** - **c**).



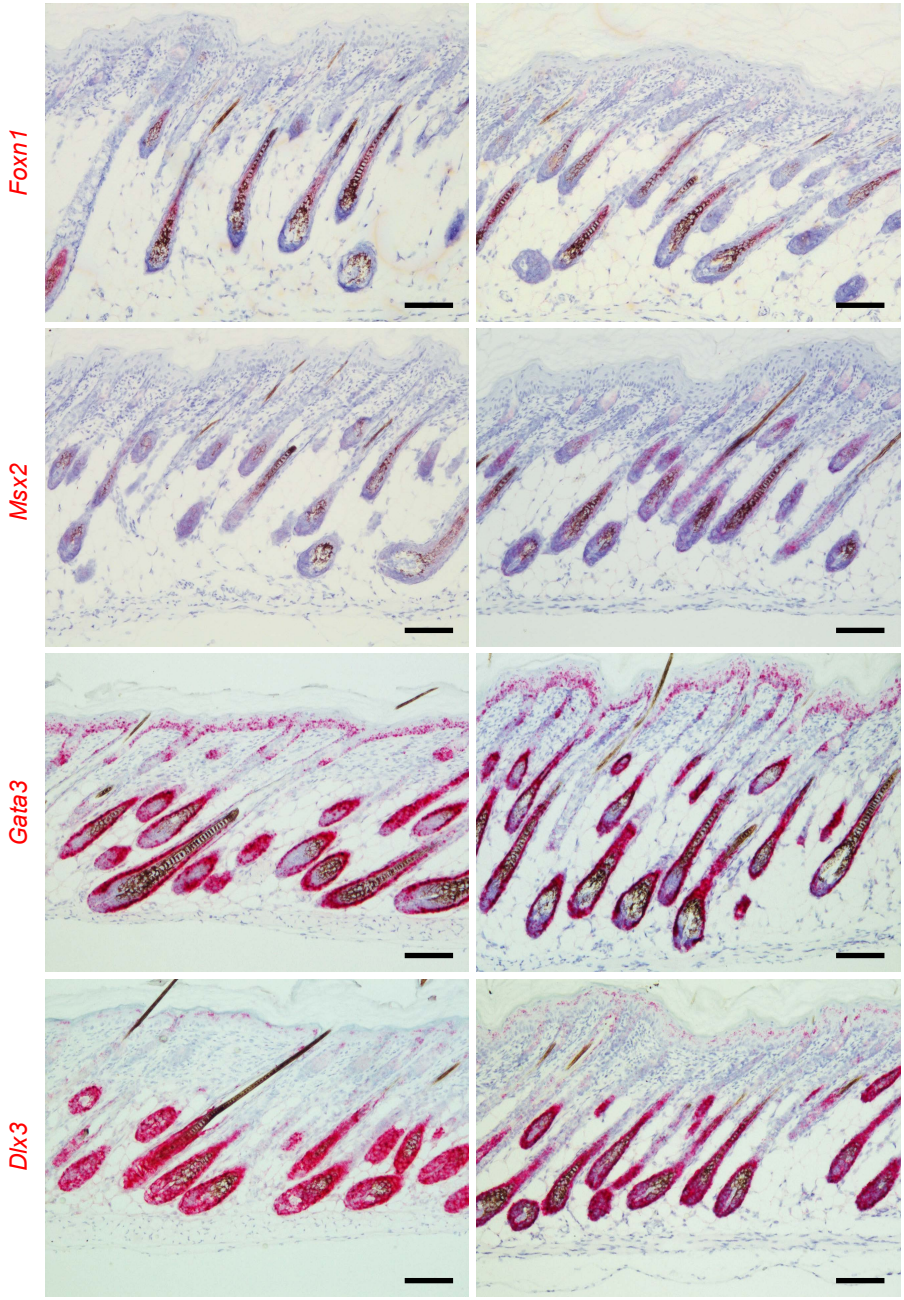
Supplementary Figure S6. Western blotting of hair follicle keratins. KRT71, KRT75 and GAPDH were observed around 57 kDa, 59 kDa and 37 kDa, respectively. Hair cortex keratins detected by the AE13 antibody were observed around 44 kDa and 46 kDa. X indicates where the blot was cut prior to incubating with primary antibodies.



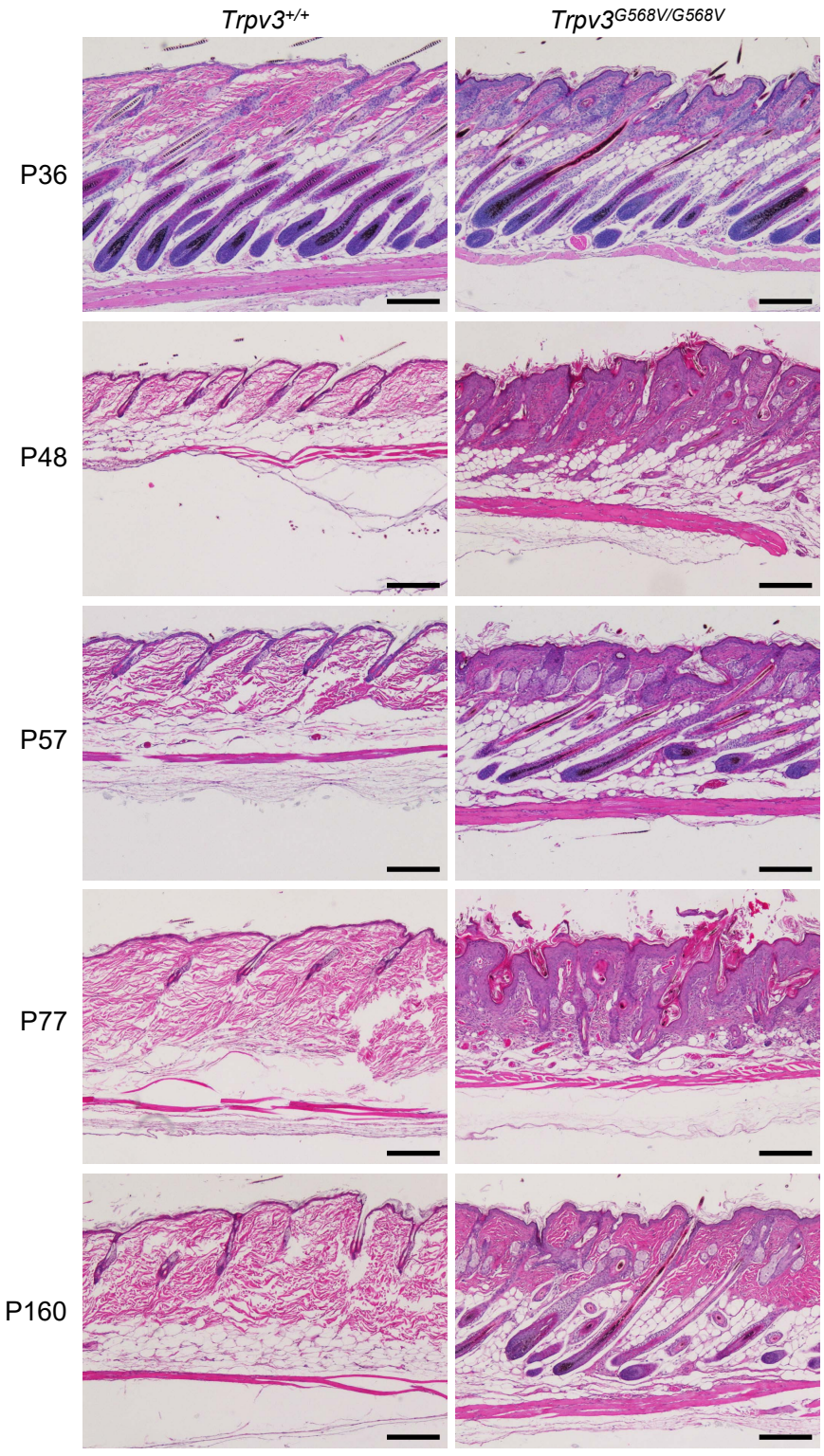
Supplementary Figure S7. Differentiation makers of epidermis. Immunofluorescence labeling of keratin 14 (KRT14), keratin 1 (KRT1), and loricrin (LOR) in dorsal skins of P12 *Trpv3*$^{+/+}$ and *Trpv3*$^{G568V/G568V}$ littermates. Nuclei were stained with DAPI (blue). Scale bar = 50 μm.



Supplementary Figure S8. Apoptosis in hair follicles. TUNEL staining in dorsal skin at indicated postnatal (P) days in *Trpv3*$^{+/+}$ and *Trpv3*$^{G568V/G568V}$ littermates. Asterisks (∗) indicate nonspecific signal caused by autofluorescence in hair medulla. Note that hair follicles were imaged with 20 X objectives before individual images were jointed manually to show details of hair follicles in their entirety. Scale bar = 100 μm.



Supplementary Figure S9. Examination of regulators for hair follicle differentiation at P5. *In situ* hybridization of *Fxon1, Msx2, Gata3 and Dlx3* (pink) in dorsal skins of wild type and *Trpv3*$^{G568V/G568V}$ mice at P5. Scale bar = 100 μm.



Supplementary Figure S10. Hair follicle regeneration in dorsal skin of adult mice. Age of mice were indicated by postnatal (P) days. Scale bar = 200 μm.