Supplementary materials and methods

Generation of animal models

All animal study protocols were approved by the NIA Institutional Review Board (Animal Care and Use Committee).

Generation of Stim2 KO mice

Stim2 KO mice were purchased from Jackson Laboratories [B6N(Cg)-Stim2tm1.1(KOMP)Vlcg/J, #026638]. A LacZ cassette was inserted into the exon I of the Stim2 gene to replace the coding sequences in this strain. Heterozygous mice were crossed with each other to have homozygous mice. Genotyping was done by PCR. Two sets of primers were used for detecting mutant and wild-type alleles. Primers for detecting Stim2 mutant: Forward, 5'-CGGTCGCTACCATTACCAGT-3'; Reverse, 5'-AGAAACCACTGCATTGCTCA-3'. PCR conditions: 94 ºC for 20", 60 ºC for 30", 72 ºC for 30", for 35 cycles. A band at 648 bp represents the homo or hetero allele; no band, the WT allele. Primers for detecting WT: Forward, 5'-AAGACGCCGTACCACTGC-3'; Reverse, 5'-CTGACCCGCTCCCTTCAC-3'. PCR conditions: 94 ºC for 20", 61 ºC for 30", 72 ºC for 30", for 35 cycles. A band at 120 bp represents the hetero or WT allele; no band, the homo allele.

Generation of skin-specific Stim1 cKO (Stim1cKO) mice

Skin-specific Stim1 cKO mice were generated by crossing *Stim1*$^{loxP/loxP}$ mice with *K14Cre* mice (#023350 for Stim1, and #004782 for K14Cre mice, Jackson Laboratory). The resulting *Stim1*$^{loxP/+}$/*K14*$^{Cre+}$ progeny was crossed to obtain homozygous skin specific *Stim1*$^{loxP/loxP}$/*K14*$^{Cre+}$ mutant mice (Stim1cKO). Genotyping was done by PCR. Primers for *Stim1* genotyping: Forward, 5'-CGATGGTCTCACGGTCTCTA-3'; Reverse, 5'-GCTCTGCTGACCTGGAACTA-3'. PCR conditions: 94 ºC for 20", 61 ºC for 30", 72 ºC for 30", for 35 cycles. A band at 347 bp represents the WT allele; a band at 397 bp, the KO allele. Primers for K14Cre genotyping: Forward, 5'-TTCCTCAGGAGTGTCTTCGC-3'; Reverse, 5'-GTCCATGTCCTTCCTGAAGC-3'. PCR conditions: 94 ºC for 30", 61 ºC for 1', 72 ºC for 1', for 35 cycles. A band at 494 bp represents the K14Cre transgene.

Sweat test

An Iodine-starch sweat test was done with wild-type, Stim1cKO, and Stim2KO mice. Briefly, iodine/alcohol solution (1 g iodine/50 mL ethanol) was applied to mouse ventral hind paw. Once dry, the surface was painted with starch-oil (10 g starch/10 mL castor oil). Dark sweating spots appear in a minute and peak at around 10 min.

To quantify the surface area of the sweat drops, we carried out sweat tests and took photos with a scale bar (1 mm) for normalization. We calculated the surface area for the whole ventral paw skin, and subtracted the non-sweat area determined by changing the color threshold with an ImageJ program (https://imagej.nih.gov/ij/). Statistical significance between wild-type and Stim1cKO mice was analyzed by Student T-Test.

Western blotting

Ventral digit tip skin, containing the epidermis, sweat glands and dermis, was collected from wild-type (WT), Stim1cKO and Stim2KO mice. Denatured total proteins were extracted by a Total Protein Extraction Kit (101Bio, P502) according to the manufacturer's recommendation. Protein lysates were separated by electrophoresis in SDS-containing polyacrylamide gels (SDS-PAGE), and transferred onto nitrocellulose membranes (Invitrogen iBlot Stack). Western blot analysis was performed using standard methods with the primary antibodies recognizing Stim1 (abcam, ab106531, 1:1000) and Stim2 (LSBio, LS-B1262, 1:1000). Membranes were stained with Ponceau S solution (Sigma, P7170) to visualize protein loading.

X-gal staining

X-gal staining was done with Stim2KO footpad skin using a "Beta-Gal staining kit" (Invitrogen, K1465-01). Briefly, 12-μm thick frozen sections from adult mouse footpad skin were fixed in a 2% formaldehyde + 0.2% glutaraldehyde mixture for 10 min at room temperature, and then incubated with X-gal staining solution overnight at room temperature. Positive signals appear after washing sections in PBS.

Histology

For histological analyses, ventral paw skin was taken from adult mice after euthanization under a dissection microscope, fixed in 10% formaldehyde solution (Ricca Chemical), dehydrated with alcohol, and embedded in paraffin. Five-μm paraffin sections were cut and deparaffinized. H&E staining was performed following the manufacturer's standard protocol (Sigma).

Immunofluorescence staining

The paraffin sections were also used for immunostaining. Antibodies recognizing STIM1 (abcam, ab106531, 1:1000), STIM2 (LS Bio, LS-B1262, 1:1000), and CD31 (abcam, ab9498, 1:25) were incubated with deparaffinized, “antigen-unmasked” (antigen unmasking solution from Vector Laboratories, H3300) paw skin sections overnight at 4 °C. AlexaFluor conjugated secondary antibodies were used to detect the primary antibodies (Invitrogen), and images were obtained with a Deltavision Confocal Microscope.

Calcium imaging

Sweat gland spheres were isolated from the digit tips of wild-type, Stim1cKO and Stim2KO mice, and were put into HBSSMGCa solution (HBSS 500 ml + 1 ml 1 M $CaCl_2$ + 0.5 ml 1 M $MgCl_2$ + 4.5 ml 200 g/L glucose). Isolated sweat glands were then digested in 0.25% collagenase I solution for 30 min at 37 ºC. After washing with HBSSMGCa solution, digested sweat glands were incubated with 5 μM Fluo4-AM (Molecular Probes, F14201) in HBSSMGCa solution containing 0.1% of Pluronic F-127 (Molecular Probes, P3000MP) for 1 hour. After washing, sweat glands were transferred to a 35-mm glass bottom dish (MatTek Corp, P35GC-1.5-14-C) with 200 μl of HBSSMGCa solution. The HBSSMGCa solution was replaced with HBSSMGCa +1 μM acetylcholine (Sigma, A2661) solution. Photos were taken before and 1, 2, 3, 4, and 5 min after the solution replacement under a DeltaVision confocal microscope (wavelength at 488, with 4x lens).