Appendix S1.

Animal

Male Slc:ICR mice were used at 7–8 weeks of age, and in a separate series of experiments, male mast-cell deficient mice (WBB6F1-*W/W^v*) and normal littermates (WBB6F1-+/+) were used at 7 weeks of age. All animals were purchased from Japan SLC, Inc. (Hamamatsu, Japan). The animals were housed in a room under controlled temperature (21–23°C), humidity (45–65%), and light (lights on from 7:00 AM to 7:00 PM). Food and water were freely available. Procedures used in the animal experiments were approved by the committee for animal experiments of the University of Toyama.

Enzyme immunoassay of histamine

Mice were transcardially perfused with 0.1 M phosphate-buffered saline under sodium pentobarbital (80 mg/kg, intraperitoneal) anaesthesia. The skin was removed from the rostral part of the back 22–24 h after each SDS treatment, and skin specimens of 18 mm diameter were taken immediately using a punch. The epidermis and dermis were separated by heating, as described previously (14). Epidermis and dermis samples were homogenised in a mammalian cell lysis buffer (Sigma). After centrifugation (10,000 × g for 10 min at 4°C), the histamine content in the supernatant was determined using a histamine enzyme immunoassay kit (Immunotech, Marseilles, France) according to the manufacturer's instructions; values were normalised to the weight of tissues.

Western blot analysis

Skin isolation, epidermis–dermis separation, and extraction were performed as described above. Protein concentration was determined using a 2-D Quant kit (GE Healthcare Bio-Sciences Co., NJ, USA), which avoids interference from SDS. Protein extracts (5 μg) were electrophoresed on a NuPAGE® 4–12% Bis-Tris gel (Invitrogen, Carlsbad, CA, USA) and then transferred to a polyvinylidene difluoride membrane. After blocking with 0.1 M phosphate-buffered saline containing 0.1% Tween 20 and 1% skim milk, the membranes were incubated in Can Get Signal® solution I (Toyobo, Osaka, Japan) containing rabbit polyclonal anti-L-histidine decarboxylase (HDC; 1:1,000; Progen Biotechnik GmbH, Heidelberg, Germany) or anti-β-actin antibody (1:1000; Abcam, Cambridge, England) overnight at 4°C. After washing, the membranes were incubated in Can Get Signal® solution II (Toyobo) containing fluorophore labelled donkey anti-rabbit IgG (H+L) antibody (1:1,000; Invitrogen) for 2 h at room temperature. Fluorescence images were scanned with a biomolecular imager (Typhoon™ FLA 9500, GE Healthcare Life Sciences, Munich, Germany). The density of immunoreactive bands with molecular weights of HDC (53 and 74 kDa) and β-actin (42 kDa) was analysed using Scion Image software (Scion Corp., Frederick, MD, USA).

Reverse transcription PCR

Skin isolation and epidermis dermis separation were performed as described above. The epidermis samples were lysed with TRIzol® reagent (Invitrogen) for RNA preparation. Total RNA (0.4 or 0.8 μg/sample) was used for cDNA synthesis with oligo (dT)16 primer and reverse transcriptase (Reverscript III, Wako Pure Chemical Industries). cDNA was amplified using the following primers: HDC (sense), 5′-agcacaagctgtcgtccttt-3′; HDC (antisense), 5′-gtggatcacgaagaccctgt-3′; glyceraldehyde 3-phosphate dehydrogenase (sense) 5′-ccaaggtcatccatgacaac-3′; glyceraldehyde 3-phosphate dehydrogenase (antisense) 5′-ttactccttggaggccacgt-3′. The cycling conditions were 5 min at 94°C, 35 cycles of 30 s at 94°C, 30 s at 60°C and 30 s at 72°C, and 7 min at 72°C. After the separation of the PCR product with gel electrophoresis and ethidium bromide staining, the density of the band of predicted size was analysed using NIH Image software (National Institute of Health, Bethesda, MD, USA).