Supplementary Methods

TRPM4 gene targeting and generation of mouse lines

For construction of the targeting vector, genomic DNA-fragments were amplified from a 129/SvJ murine BAC clone (RPCI 22 library, Roswell Park Cancer Institute, Buffalo, NY). A floxed *Trpm4* allele was constructed. 5' homology contained exons 13-16 of the *Trpm4* gene and exon 15 and 16 were flanked with two loxP sites. A lacZ-FRT-PGKneo-FRT cassette[5] with a third loxP site was cloned downstream of the flanked exon (Suppl. Figure 1A). 3' homology contained exon 17 and was followed by a HSV thymidine kinase cassette (HSV*tk*). R1 embryonic stem cells (1 x 10^7) were electroporated with the linearized targeting vector and plated on irradiated G418-resistant embryonic feeder cells isolated from *Trpc4*$^{+/-}$ embryos[6]. Recombinant clones were selected with G418 (0.25 mg ml^{-1}) and ganciclovir (2µM). Homologous recombination was confirmed using Southern Blot analysis in 25 of 309 double resistant ES cell clones. From 25 clones, 8 clones displayed a TRPM4 allele containing all 3 loxP sites (*Trpm4*^{L3F2}) whereas 17 clones were lacking the first loxP site (*Trpm4*^{L2F2}). Two independent *Trpm4*^{L3F2} cell clones clones were used to generate chimeric mice. Mating of mice heterozygous for the *Trpm4*^{L3F2} allele (*Trpm4*$^{+/L3F2}$) with the deleter mouse strain CMV-Cre[7] produced mouse lines carrying a null-allele (*Trpm4*$^{-}$). Mice were kept in essentially specific pathogen-free environment and all phenotypic analyses were performed using mice of a 129/SvJ genetic background, matched of gender and age. Mice were routinely genotyped using PCR.

Northern and Western Blotting

Northern blot analysis was performed as described[8]. Poly(A)+ RNA (10 µg) isolated from *Trpm4*$^{+/+}$ and *Trpm4*$^{-/-}$ BMMC and kidney were hybridized with a random labelled cDNA probe corresponding to nucleotides 2325–3156 of mouse *Trpm4* (GenBank accession nr. AJ575814) and a 239-bp PCR fragment from the human glyceraldehyde-3-phosphate dehydrogenase (GAPDH) cDNA. For Western blots a rabbit polyclonal antibody (578) directed against the amino-terminal end of mouse TRPM4 was generated and affinity purified. Specificity of antibodies was confirmed using microsomal membrane protein fractions (BMMCs, 150 µg per lane) from *Trpm4*$^{+/+}$ mice and mice deficient in *Trpm4* as well as from non-transfected, mouse *Trpm4*-transfected (pM4-26,[9]) and human *Trpm4*-transfected (TRPM4b, [9]) HEK293 cells. Anti-mouse GAPDH antibody served as controls for protein loading.

Electrophysiology and Ca^{2+} imaging

Currents were measured in the whole-cell or inside-out configuration using an EPC-7 or EPC-9 patch-clamp amplifier (HEKA Elektronik, Lambrecht, Germany). Patch electrodes had a DC resistance between 2 and 4MΩ. Currents were sampled at 3kHz and filtered at 1kHz. Step protocols consisted of a 400ms step to -100mV from a holding of 0mV, followed by a 250ms step to +100mV applied at 0.5Hz. Ramp protocol consisted of a 400ms ramp from -100 to +100mV from a holding potential of 0mV, also applied at 0.5Hz. Capacitance measurements were performed according to the Lindau-Neher technique[10]. A 1 kHz, 40mV peak-to-peak sinusoid stimulus was applied about a DC holding potential of 0mV. Data were acquired through a combination of the time resolution pulse software and

the lower time resolution X-chart plug-in to the Pulse software. Pipette solution contained 135KGlutamate, 20NaCl, 1$MgCl_2$, 10Hepes, 0.2Na_2ATP, 300µM GTP, 100µM GTPγS, pH7.2[11]. Amphotericine-B (250µg/ml) was utilized for perforated patches as in[12]. $[Ca^{2+}]_{cyt}$ was measured as described in[13]. Cells were loaded with 2 µM Fura-2 acetoxymethyl ester for 20min at 37°C in standard extracellular solution. For every condition, at least 20 cells in at least three independent experiments were assayed. Time-points after 3 and 6hrs of stimulation were gathered by loading 10^6 cells with Fura-2am as above, and measured in an Aminco-Bowman Luminescence Spectrometer. Electrophysiological experiments were performed at room temperature (22–25°C). Ca^{2+} imaging was performed at 37°C.

TRPM4-Immunostaining

Skin samples were fixed in 4% fresh paraformaldehyde in 0.1M PBS overnight, pH 7.2. After several steps of washing with 0.1M PBS the skin sample were transferred to 18% sucrose overnight and then frozen at -80°C. Thereafter 7µm cryosection was performed. For immunocytochemical stainings, 5000 BMMCs, $CD3^+CD4^+$, $CD3^+CD8^+$ or $CD19^+$ cells were fixed in acetone following cytospin on poly-lysine coated coverslips. Samples were incubated 1h at RT with the anti-TRPM4 antibody 587 (1:50) that was preabsorbed using microsomal membrane protein fractions from *Trpm4*$^{-/-}$ BMMCs. As biotinylated secondary antibody a goat-anti rabbit-IgG (E 0433 Dako, Denmark, 1:400) was used for 1h at RT. Thereafter cells were incubated with extravidin-horseradish-peroxidase-complex (PRN 1051, Amershan, 1:150) for 60min and visualized by incubation with a DAB/NiSo4 solution for 15min.

Bone Marrow Isolation and Mast Cell Differentiation

Isolation and differentiation of murine bone marrow cells (BMMC) were performed as in[14]. In brief, bone-marrow cells were centrifuged from excised femurs and cultured at 10^6 cells/ml in IMDM (Invitrogen) supplemented with 10% heat-inactivated fetal calf serum, 50 µM β-mercaptoethanol (Sigma), 10 U/ml of each penicillin and streptomycin, and 2 ng/ml recombinant murine IL-3 (R&D Systems) at 37°C, 5% CO_2. IL-3 was added twice weekly. The culture was weekly diluted to 0.5 x 10^6 cells/ml. After 4 weeks of differentiation, expression of FcεRI in BMMC was analysed after the blockage of Fcγ receptors with 2.4G2 rat anti-mouse FcγRII/RIII antibody (Pharmingen), followed by individual incubations with mouse IgE (Sigma) and FITC-labeled monoclonal rat anti-mouse IgE antibody (Pharmingen). For c-Kit expression, Fcγ receptors were blocked as above, and cells were subsequently incubated with PE-labeled rat anti-c-Kit monoclonal antibody (Pharmingen). Flow cytometry was carried out on a Galaxy Flow Cytometry system (DAKO). 96.5 – 99.5 % of cultured cells of different preparations from both genotypes expressed both FcεRI and c-Kit.

Electron microscopy

BMMC's were fixed with 2.5% glutaraldehyde, 1% freshly depolymerised paraformaldehyde in PBS (3hrs at 4°C). During the first 30min of fixation, mast cells were fixed in suspension, and after brief centrifugation further fixed as a cell pellet in the same fixative for additional 2.5hrs. Samples were osmicated with 2%OsO_4 in 100mM cacodylate buffer, pH 7.4 (1hr, 4°C), bloc-contrasted with 2% uranyl acetate in 50mM Na-maleate, pH 5.2 (3hrs, 4°C), dehydrated using an ascending ethanol

concentration series and using propyleneoxide, infiltrated with Epon 812 resin and polymerized at 60^{o}C for 24 hours. Ultrathin sections were cut with an ultramicrotome (Reichert) and analyzed with a Tecnai 12 Biotwin digital electron microscope (FEI) operated at 100kV. Skin samples were fixed in 4% fresh paraformaldehyde in 0.1M PBS overnight, pH 7.2, dehydrated in a graded alcohol series, and embedded in araldite. Sections of plastic-embedded specimens were cut with glass for thin sections and diamond knife for ultrathin sections on a Reichert ultramicrotome. Ultrathin 60 nm sections were examined using an electron microscope (902A, Leo, Oberkochen, Germany) after further contrasting with uranyl acetate-lead citrate.

Histology

For detection of mast cells in skin biopsies a May-Grünwald – Giemsa staining was performed. Paraffin slices were made, deparaffined, incubated with May Grünwald solution 5 min., rinsed with aqua dest. 5 min, incubated with Giemsa solution 15 min, rinsed with Aqua dest. and incubated with 7 drops Giemsa solution in 10ml Aqua dest. Mast cells were counted in 15 fields (320µm x 240µm) per biopsy using KS300 software (Zeiss). The number of mast cells is given as mean±S.D. The experimentator was blinded to the genotype of the biopsies.

Isolation of lymphocytes by fluorescence activated cell sorting:

Spleen tissues were obtained from adult 129 SVJ-, C57B6l- or *Trpm4*$^{-/-}$ mice. Splenocytes cell suspensions were obtained by squeezing tissues through a wire screen. Erythrocytes were lysed by incubation for 10 min in 0.83% ammoniumchloride, Tris-Cl pH 7.5. After centrifugation (250g) cells were resuspended in phosphate-buffered saline and cell clumps were removed by filtration (Filcons 30 µm, Becton Dickinson). CD3 (CD3 ε chain), CD4 (L3T4) and CD8 (Ly-2) surface markers were stained using Pe-Cy7-, PE- or FITC-conjugated antibodies, respectively, present in the mouse T-lymphocytes subset antibody cocktail (Becton Dickinson). CD19 positive cells were labeled using a FITC-conjugated rat anti-mouse monoclonal antibody (clone 1D3, Becton Dickinson). Isotype control stainings were negative. Cells were analysed and sorted at highest purity at a MoFlo cell sorting instrument (DakoCytomation) using a 488nm argon laser beam for excitation. For PCR analysis 500 cells of $CD3^{+}CD4^{+}CD8^{-}$-, $CD3^{+}CD4^{-}CD8^{+}$- and $CD19^{+}$ populations as well as MIN6 cells and BMMCs were sorted directly into PCR tubes, frozen in liquid nitrogen and stored at -80°C until used for RT-PCR analysis.

RT-PCR analysis of mouse immune cells

A defined number of 500 mouse cells each were directly subjected to combined cDNA-synthesis/PCR reactions (40 amplification cycles) in single tubes using Superscript™ One-Step RT-PCR with Platinum® Taq (Invitrogen). The following primer pairs, deduced from cDNA sequences and flanking at least one intron were used: 5´GACCTGCTTATTTGGGCTCTG and 5´AGATGGGAGTTGTGCTGTCC (TRPM4); 5´AGTCACCTGTAGAATGGTGC and 5´GAATGTGTAGCTGAACATGGC (TRPM5); 5´GCTCGAGATGTCATGAAGG and 5´GGCTGTACTGCTTAACCAGG (HPRT1): No template control reactions were included for each primer pair in every set of experiments (not shown in Supplementary Fig.2).

Mast cell mediator release

Histamine release from mast cells was determined using the O-phthaldialdehyde assay[15]. 0.5 x 10^6 cells were washed twice and resuspended in KRH buffer (130NaCl, 4.75KCl, 1.2KH_2PO_4, 1.2$MgSO_4$, 11glucose, 10Hepes, 2.54$CaCl_2$, pH 7.4). DNP, adenosine and ionomycin were added to the suspension and cells were incubated for 5 to 90 min at 37°C. To determine total histamine content (F_{total}), one sample is resuspended in 1% Triton X-100 and incubated at 95°C for cell lysis. After incubation, cells were centrifuged and 350µl H_2O, 100µl 1N NaOH and 25µl 1% phthaldialdehyde (Sigma StLouis, MO) were added to 150 µl supernatant. After 4 min incubation at room temperature 50µl 3N HCl was supplemented. The amount of histamine derivative was measured fluorometrically in a 96-well reader (Tecan GENios), at 360nm (excitation) and 450nm (emission). The ratio F_{test}/F_{total} gives the percentage of total histamine content released. Data were collected from 4 independent BMMC preparations from each genotype. In each preparation results were similar to the overall average.

Release of IL-6 and TNFα was assayed using ELISA duokits (R&D Biosystems) whereas LTC4, LTD4 and LTE4 release was assayed using an enzyme-linked immunoassay (GE Healthcare) according to the manufacturers guidelines.

Insulin secretion and glucose tolerance test

Islets were hand-picked in groups of ten and washed twice in RPMI (in the absence of glucose). Islets were subsequently pre-incubated for 60 min at 37°C in Krebs-Ringer bicarbonate buffer, pH 7.4, supplemented with 0.05% BSA, and 1 mM glucose. Following pre-incubation, the supernatant was discarded and the islets were incubated in buffer solution containing 1 or 20 mM glucose for 60 min at 37°C. An aliquot of the supernatant was removed immediately after incubation and frozen for insulin assay. The pellets were resuspended in acid ethanol and frozen for insulin content measurement. The ELISA kit used for insulin determination was obtained from Mercodia (Sweden). Insulin secretion data are expressed as ng/islet/h. Student's *t*-tests were used to assess statistical significance between treatments: differences were considered statistically significant at $p<0.05$.

Seven to eight week old male *Trpm4*$^{+/+}$ and *Trpm4*$^{-/-}$ mice were fasted over night (14-16 h). Blood glucose levels were measured using an FreeStyle Blood Glucose Monitoring System (Disetronic) from tail bleedings before and 15, 30, 60 and 120 min after intraperitoneal injection of D-glucose (2 g/kg body weight) as described[16].

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