Supplementary Data

Methods

PCR cloning of TRPV3

For PCR cloning of mouse TRPV3, primers (5' TGACATGATCCTGCTGAGGAGTG 3' and 5'-ACGAGGCAGGCGAGGTATTCTT-3') were designed from the HMM sequences for TRPV3 as a result of blast hits to the ankyrin and transmembrane domains and used to amplify a 699-nucleotide fragment of TRPV3 from skin cDNA. From this initial fragment, Rapid Amplification of cDNA Ends (RACE) PCR (Clontech) was used to obtain the 5' and 3' ends of TRPV3 from mouse newborn skin cDNA.

Generation of stable TRPV3-expresing cell lines

Mouse TRPV3 and rat TRPV1 cDNA were subcloned into pcDNA5 (Invitrogen) and transfected into CHO-K1/FRT cells using Fugene 6 (Roche). The transfected cells were selected by growth in MEM medium containing 200μg/ml hygromycin (Gibco BRL). Populations were frozen at early passages and these stocks used for further studies. Stable clones that expressed the mRNAs were identified by Northern blot analysis as well as Southern blotting to confirm integration site. Long-term cultures were subsequently maintained at 33°C.

Electrophysiology

TRPV3 expressing CHO cells were assayed electrophysiologically using whole cell voltage clamped techniques. Currents were recorded via pCLAMP8 suite of software via an Axopatch 200A and filtered at 5kHz. Series resistance compensation for all experiments was 80% using 2-5MΩ resistance, fire-polished pipettes. Unless stated the holding potential for most experiments was –60mV, apart from the current-voltage relationship studies (2 second ramp from –100 to +80mV). Cells were normally bathed in a medium containing [(mM): NaCl, 140; KCl, 5; Glucose; 10, HEPES, 10; $CaCl_2$, 2; $MgCl_2$ 1; titrated to pH 7.4 with NaOH] apart from the monovalent permeability studies, when NaCl was replaced by equimolar KCl or CsCl with the omission of KCl 5mM. For

the divalent permeability studies the solutions either contained 1 mM Ca^{2+} or Mg^{2+} and (mM) NaCl, 100; Glucose, 10; Hepes, 10; sucrose, 80 or 30mM test ion in the above solution minus sucrose. The experiments in calcium free media had no added CaCl with the addition of 100μM EGTA. Pipette solution was always (mM) CsCl, 140; $CaCl_2$, 1; EGTA, 10; HEPES, 10; MgATP, 2; titrated to pH7.4 with CsOH. For the permeability rations for the monovalent cations relative to Na (P_X/P_{Na}) were calculated as follows: $P_X/P_{Na} = E_{shift} = \{RT/F\} \log (P_X/P_{Na} [X]_O /[Na]_O)$, where *F* is Faraday's constant, *R* is the universal gas constant, and *T* is absolute temperature. For the divalent ions P_{Ca} or P_{Mg} /P_{Na} was calculated as follows: $Eshift = \{RT/F\} \log \{[Na]_O + 4B' [X]_{O(2)}\} / \{ [Na]_O 4B' [X]_{0(1)}\}$, where $B' = P'_X / P_{Na}$ and $P'_X = P_X/ (1 + e^{EF/RT})$ and $[X]_{O(1)}$ and $[X]_{O(2)}$ refer to the two different concentrations of the divalent ion tested.

Northern blot analysis

For Northern blot analyses approximately 3 μg of polyA+ RNA extracted from adult and newborn rodent tissues were electrophoresed on 1% glyoxal gels, transferred and hybridized at high-stringency with a ^{32}P labeled probe representing the entire full-length TRPV3 sequence. Northern blots (Clontech) were hybridized with the same TRPV3 full-length probe. Human Northern blots were hybridized with a probe corresponding to the ankryin 1-TM2 region of the TRPV3 human sequence. For TRPV1 hybridizations, a probe corresponding to nucleotides 60–605, encoding the amino terminus of rat TRPV1 was used.

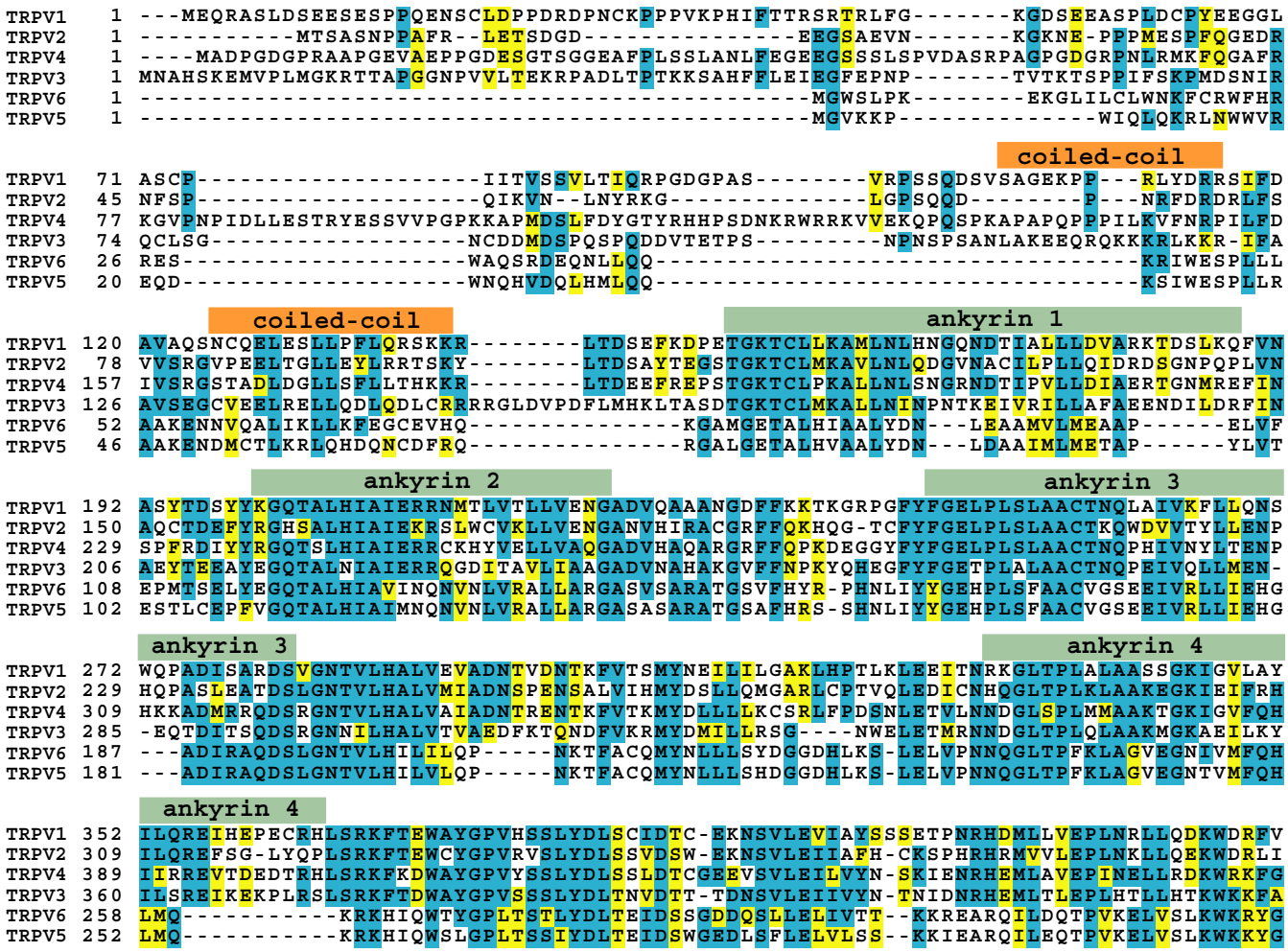
In situ hybridization

For *in situ* hybridizations, newborn tissues were dissected, fixed in 4% paraformaldehyde in PBS, cryoprotected, and frozen in liquid nitrogen in OCT mounting medium. Cryostat sections (10μm) were processed and probed with a ^{35}S-labeled probe generated by *in vitro* transcription essentially as described in (21). Two mouse TRPV3 specific anti-sense riboprobes were used, one corresponding to nucleotides 235-1020 encoding the amino terminus and the other spanning nucleotides 980-1675 corresponding to the region between the last ankyrin and fourth transmembrane domains. Both probes gave similar results.

Immunohistochemistry

For immunohistochemistry, rabbits were immunized (AnimalPharm Services, Healdsburg, Ca) with KLH conjugated peptide corresponding to either the N- terminus of mouse TRPV3 (CDDMDSPQSPQDDVTETPSN) or a C -terminus peptide (KIQDSSRSNSKTTL). Affinity purified antiserum recognized a band of relative molecular mass ~85 kDa in whole-cell extracts prepared from CHO cells stably transfected with mouse TRPV3 (not shown). Immunofluorescence was performed on fixed frozen and paraffin sections using rabbit anti-TRPV3, pan- cytokeratin (Abcam), Cytokeratin 10 (K8.60, Sigma), pan- basal Cytokeratin (Abcam), PGP9.5 (Abcam) followed by FITC-labeled goat anti-rabbit (10 μg/ml) and Cy-3-labeled donkey anti-mouse (Jackson Immunoresearch) antibodies.

Supplemental Figure 1. Comparison of mouse TRPV3 protein sequence to other TRPV channels (excluding C-terminal half containing transmembrane domains). Identical sequences are highlighted in blue; conserved residues, in yellow. Predicted coiled-coil and ankyrin domains are marked and correspond to regions for TRPV3 only. The protein alignment was generated with Megalign and Boxshade. The coiled-coil domains were predicted with the program Coils. The ankyrin domains were predicted with the PFAM protein search.



Supplemental Figure 2: TRP Channels in thermosensation.
Four TRP channels implicated in thermosensation cover most but not all physiologically relevant temperatures. Note: TRPV3 has not been tested at temperatures above 48°C.