Supplemental Information

Differential regulation of TRPV1, TRPV3 and TRPV4 sensitivity through a conserved binding site on the ankyrin repeat domain
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Supplementary Figure 1. (A) Alignment of the amino acid sequences of the ankyrin repeat domains of TRPV1 from chicken (Gg, NP_989903), human (Hs, NP_542436), and rat (Rn, NP_114188), TRPV3 from chicken (Gg, XP_001235155), human (Hs, NP_659505) and rat (Rn, NP_001020928), and TRPV4 from chicken (Gg, NP_990023), human (Hs, NP_067638) and rat (Rn, NP_076460). The alignment was colored with identical residues in black and similar residues in grey. Amino acids that contact ATP in the crystal structure of the TRPV1-ARD (PDB ID 2PNN) are indicated by purple arrows above the sequences. Colored bars above the sequence indicate the individual ankyrin repeats. An insertion and two deletions in the TRPV3-ARD compared to TRPV1 and TRPV4 are indicated by light green and teal boxes, respectively. (B) Unrooted phylogenetic tree showing the relationship between the ARD sequences in A. The alignment and phylogenetic analysis were performed with ClustalW and PHYLIP as part of the SDSC Biology Workbench. (C) Ribbon diagram the structure of the TRPV1-ARD (2PNN) with individual ankyrin repeats colored according to A. The ATP is shown as purple sticks. (D) The location of the insertion and two deletions in the TRPV3-ARD is mapped onto the structure of TRPV1-ARD. The ARD is shown as a ribbon diagram with the location of ATP-binding residues in purple, and the insertion and deletions in the TRPV3-ARD colored as in A. The ATP is shown as sticks with carbon atoms colored purple.
Supplementary Figure 2. Surface mutations on the TRPV2-ARD do not promote ATP or calmodulin binding. (A) Alignment of the rat TRPV1 and rat TRPV2 ARD sequences. Differing residues are highlighted green. Black arrowheads indicate residues within 4 Å of the ATP in the TRPV1-ARD structure; blue arrowheads point to other proximal residues. The two residues mutated in TRPV2 are highlighted pink. (B) Structure of the TRPV2-ARD (PDB code 2ETB) with the ATP molecule (sticks) bound to TRPV1-ARD shown for reference. The coloring follows (A), and the two mutated sidechains are shown as spheres on a ribbon diagram at the top, whereas the molecular surface is shown at the bottom. (C) Coomassie-stained gel of wildtype and mutant TRPV2-ARD loaded (left) and bound to ATP-agarose in the absence (middle) or presence (right) of competing free ATP. (D) Coomassie-stained gel shows wildtype and mutant TRPV2-ARD loaded (left) and bound to CaM-agarose in the presence of Ca^{2+} or EGTA. In (C) and (D), wildtype TRPV1-ARD was used as a positive control and the average percentage of protein recovered (+/- standard deviation) is plotted below, and the average % pulldown value is indicated under the histogram. In (C), the statistical significance of the difference in binding to ATP-agarose between TRPV1-ARD and TRPV2-ARD, and TRPV2-ARD and the DH-NQ TRPV2-ARD double mutant over three experiments are noted, with p<0.02 and p<0.01 indicated by * and **, respectively, using a one-tailed modified Student t-test.
Supplementary Figure 3. (A) Voltage step protocol and sample recordings from insect cells infected with empty virus (top right) or virus carrying TRPV4 in the absence (lower left) or presence (lower right) of intracellular ATP. (B) Voltage step protocol and sample recordings from HEK293 cells transfected with a TRPV4-expression plasmid and perfused with 5 μM 4αPDD. Recordings in the absence (middle right) or presence of intracellular ATP (left) or anti-CaM antibody (bottom right). A control cell transfected with a GFP-expression plasmid is included at the top right.
Supplementary Figure 4. TRPV3 response to extracellular agonists is decreased and inactivation is faster in NaGluconate extracellular solution. (A) Response of mock-infected Sf21 insect cells and insect cells expressing WT and K169A TRPV3 to 0.5 mM 2-APB in extracellular solutions were the primary anion is either gluconate (left) or chloride (right). For all recordings BAPTA was used as the intracellular calcium chelator. Shown are currents at +100 (grey circles) or -100 mV (black circles) extracted from linear voltage ramps from a control cell (top) and cells with intracellular ATP (bottom). Applications of 0.5 mM 2-APB are indicated by grey bars and zero current by black lines. (B) Sample dose response recordings for wildtype (top) and K169A (bottom). Cells were pulsed once with 2-APB to insure that the TRPV3 was pre-sensitized by BAPTA in the intracellular solutions. Recordings were carried out in NaGluconate extracellular solution to allow for timely inactivation from the test application of 2-APB. Application of increasing concentrations of 2-APB are indicated; for wildtype: 0.05 mM – white, 0.25 mM – light grey, 0.5 mM – grey, 2 mM – dark grey and 4 mM – black; due to K169A’s higher sensitivity the 2-APB concentrations are half of wildtype (0.025, 0.125, 0.25, 1 and 2 mM).
Supplementary Figure 5. Widtype, R188A and K169A TRPV3 are expressed to the same level in baculovirus-infected insect cells. Cells were harvested 48 hours after infection with baculovirus carrying FLAG-tagged TRPV3 wildtype, R188A and K169A or a no-insert control virus and samples were subjected to 10% SDS-PAGE/Western blot using the M2 anti-FLAG-alkaline phosphatase conjugated monoclonal antibody (Sigma). The position of molecular weight standards are indicated on the left.