

Supporting Information

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SI Methods

SNP Selection for Genotyping. At the outset of this study, 2 nonsynonymous polymorphisms in the human *TRPV4* gene were listed in the public domain. One, rs11068298, coded for a variant allele of TRPV4 with an Ala-to-Thr substitution at amino acid residue 565; however, no data on minor allele frequency were available. The second, rs3742030, coded for a variant allele with a Pro-to-Ser substitution at residue 19. We screened a panel of anonymized genomic DNA samples of various ethnicities and were unable to detect the rs11068298 minor allele in any subjects. In contrast, the rs3742030 minor allele was detected in $\approx 5\%$ of these samples, consistent with data reported in the International HapMap Project. Therefore, we focused exclusively on this polymorphism in all subsequent genotyping studies.

Cell Transfection, Immunodetection, and Electrophysiological Recordings. HEK293 cells were transiently transfected with polyethylenimine ExGen500 (Fermentas MBI) using 8 equivalents of polyethylenimine together with 0.3 μg of pEGFPN1 and 3 μg of pcDNA3-TRPV4^{WT} or mutant TRPV4^{P19S}. Cells were used 12–48 h after transfection. Confocal immunofluorescence detection of TRPV4 (Figs. S1 and S3) was carried out as previously described (1, 2). Patch pipettes were filled with a solution containing (in mM): 20 CsCl, 100 Cs-acetate, 1 MgCl₂, 0.1 EGTA, 10 Hepes, 4 Na₂ATP and 0.1 NaGTP; 300 mosmol/L (pH

7.2–7.3). Pipette solutions containing 156 nM EET (Biomol) were also used where indicated. Cells were bathed in isotonic solutions containing (in mM): 100 NaCl, 6 CsCl, 1 MgCl₂, 1 EGTA, 5 glucose, 10 Hepes, and 80 D-mannitol (315 mOsmol/L [pH 7.35]). The 15% (270 mOsmol/L) and 30% hypotonic (220 mosmol/L) bathing solutions were prepared by reducing D-mannitol from the isotonic solution. Currents in response to 4 α PDD and EET were obtained using isotonic bathing solutions containing (in mM): 140 NaCl, 5 KCl, 1 MgCl₂, 10 Hepes, and 1 EGTA (≈ 310 mosmol/L [pH 7.3–7.4]). HEK293 were clamped at 0 mV, and ramps from -100 mV to $+100$ mV (400 ms) were applied at a frequency of 0.2 Hz. Ramp data were acquired at 10 KHz and low-pass filtered at 1 KHz. All experiments were carried out at room temperature. Only those cells that presented GFP fluorescence were recorded. All experiments were carried out at room temperature. Cell surface biotinylation experiments (Fig. S2) were performed as previously described (3). HEK293 cells were transiently transfected with full-length cDNA coding for wild-type human TRPV4 (hTRPV4) or for human TRPV4 point-mutated to incorporate either the A565T or the P19S nonsynonymous polymorphism. Whole-cell detergent lysates were resolved via SDS-PAGE and subjected to anti-TRPV4 immunoblotting as previously described (4). Cells transfected in parallel were also subjected to cell-surface biotinylation and avidin-affinity precipitation, followed by anti-TRPV4 immunoblotting (Fig. S2) as previously described (3).

1. Arniges M, Fernandez-Fernandez JM, Albrecht N, Schaefer M, Valverde MA (2006) Human TRPV4 channel splice variants revealed a key role of ankyrin domains in multimerization and trafficking. *J Biol Chem* 281:1580–1586.
2. Lorenzo IM, Liedtke W, Sanderson MJ, Valverde MA (2008) TRPV4 channel participates in receptor-operated calcium entry and ciliary beat frequency regulation in mouse airway epithelial cells. *Proc Natl Acad Sci USA* 105:12611–12616.
3. Xu H, Fu Y, Tian W, Cohen DM (2006) Glycosylation of the osmosensitive transient receptor potential channel TRPV4 on Asn-651 influences membrane trafficking. *Am J Physiol* 290:F1103–F1109.

4. Xu H, et al. (2003) Regulation of a transient receptor potential (TRP) channel by tyrosine phosphorylation. SRC family kinase-dependent tyrosine phosphorylation of TRPV4 on TYR-253 mediates its response to hypotonic stress. *J Biol Chem* 278:11520–11527.

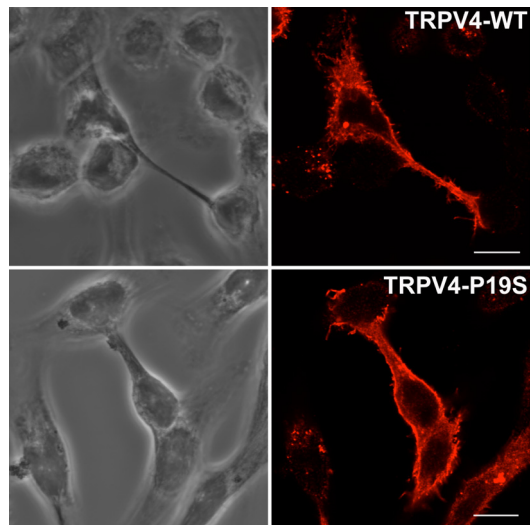


Fig. S1. TRPV4^{WT} and TRPV4^{P19S} are expressed to an equivalent degree at the plasma membrane in transfected HEK cells. HEK cells transfected with the wild-type TRPV4 (*Upper*) or the P19S variant (*Lower*) as in Fig. 2 were subjected to confocal immunofluorescence microscopy (*Right*) or brightfield microscopy (*Left*) following immunolabeling for TRPV4. Abundant staining of the plasma membrane is noted in both transfectants. (Scale bar: 10 μm .)

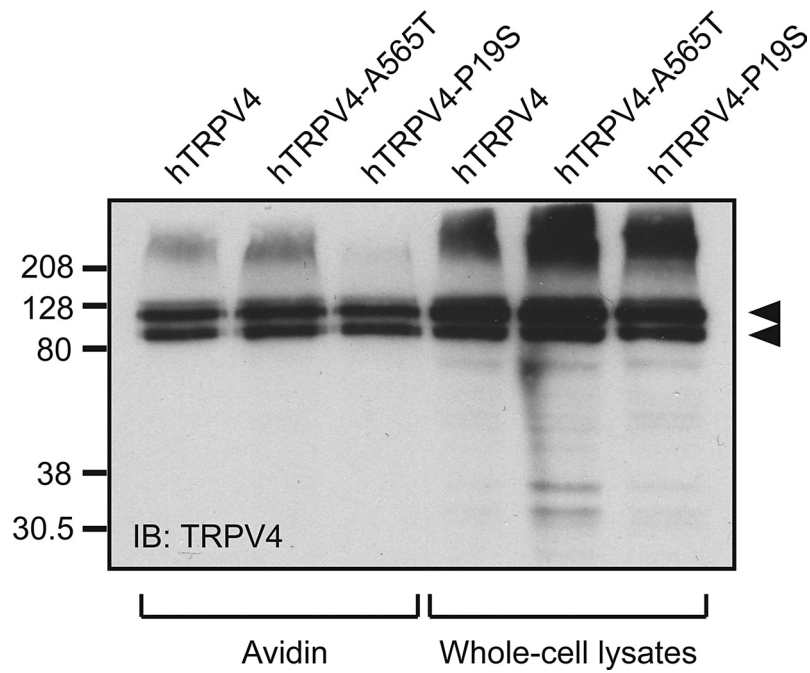


Fig. S2. Equivalent cell-surface expression of TRPV4 and variant TRPV4 following transient transfection in HEK293 cells. Cells were transiently transfected with full-length cDNA coding for wild-type human TRPV4 (hTRPV4) or for human TRPV4 point-mutated to incorporate either the A565T or the P19S nonsynonymous polymorphisms. TRPV4 expression in whole-cell lysates was equivalent in the 3 transfectants (right half of the figure) in the depicted anti-TRPV4 immunoblot. Cell surface expression (left half of the figure) was similarly equivalent, as demonstrated via avidin-affinity precipitates following cell surface biotinylation (see [SI Methods](#)). Molecular mass markers (in kDa) are shown on the left; arrowheads indicate the TRPV4.

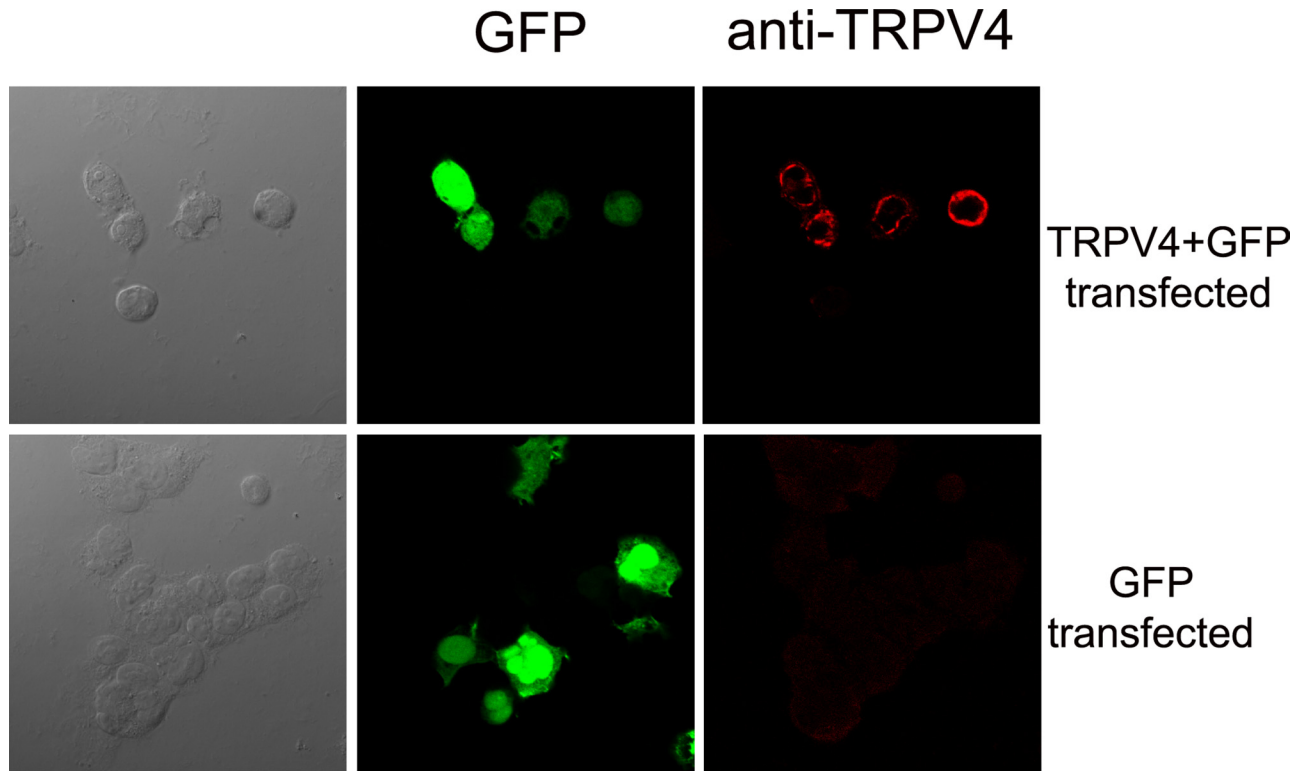


Fig. S3. Immunodetection of TRPV4 in transfected HEK293 cells. HEK293 cells transfected with TRPV4 + GFP (*Top*) or GFP alone (*Bottom*). (*Left*) Transmitted light images. (*Center*) GFP fluorescence (green). (*Right*) TRPV4 immunofluorescence signal (red), as described in [SI Methods](#).

HEK293 GFP

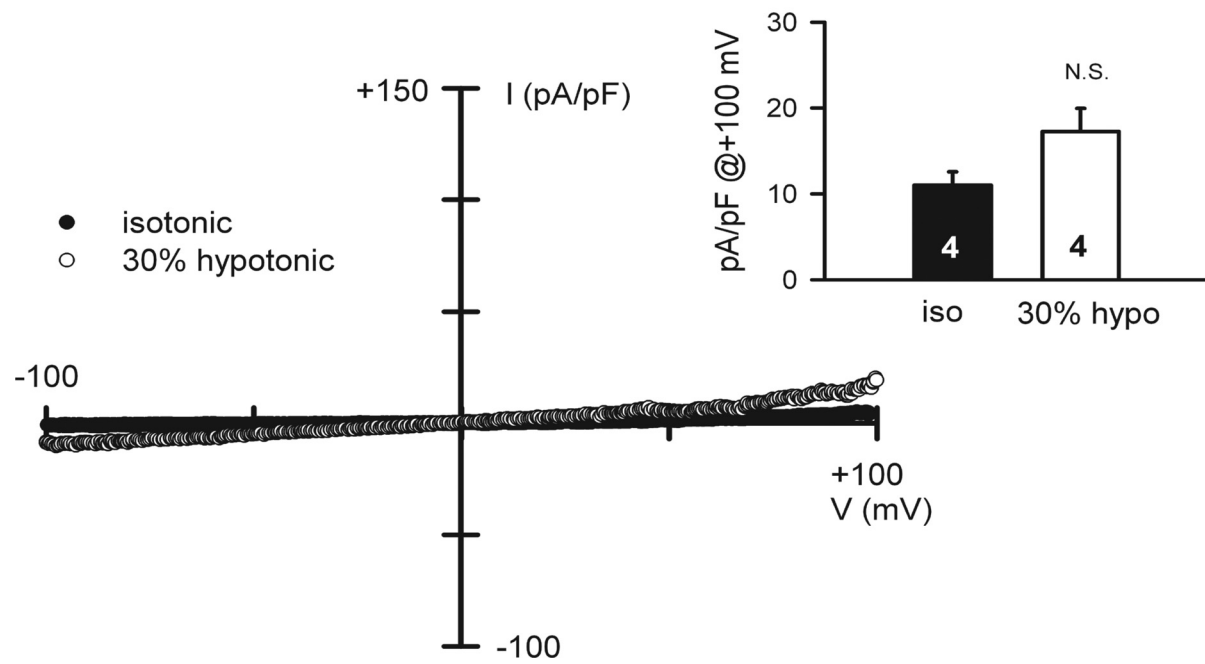


Fig. S4. Whole-cell currents recorded in HEK293 cells transfected with GFP and exposed to 30% hypotonic shock. Inset shows average current density. $P > 0.05$ Student's t test.