Supporting Information

Zhang et al. 10.1073/pnas.1717192115

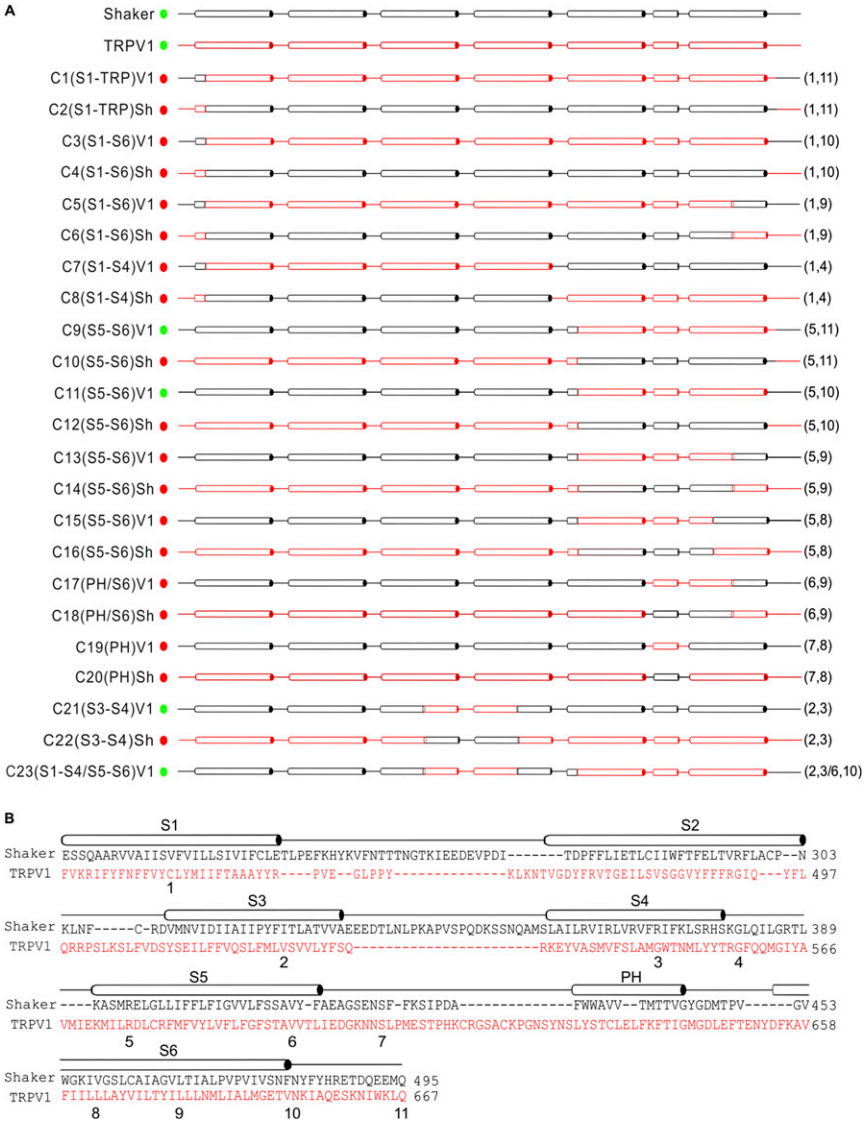

Fig. S1. (*A*) Summary of chimeras constructed between TRPV1 and the Shaker Kv channel. Functional (green circles) and nonfunctional (red circles) constructs are named based on the region being transferred. Nomenclature on the *Left* of each construct is CX [region transferred] donor channel, where x is a number assigned to each chimera. Splice site codes for each construct are shown to the *Right* of the chimera and the code is depicted on the alignment of Shaker and TRPV1 (*B*). Transferred regions include the residue in the TRPV1 sequence directly above the code number.

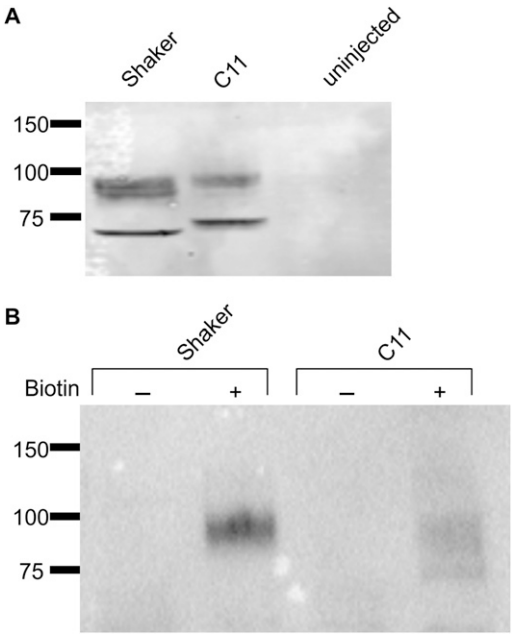

Fig. S2. Biochemical detection of Shaker and C11 surface expression. (*A*) Western blot from a SDS PAGE gel of the total protein fraction solubilized from cells expressing 1D4-tagged Shaker, the C11 chimera or no exogenous proteins. For Shaker, the lower molecular weight band (~70 kDa) is the core glycosylated species found in the ER, whereas the broad higher molecular weight band (~80–95 kDa) corresponds to a more heavily glycosylated species found in the Golgi and on the plasma membrane. For C11, the most abundant species migrates at a molecular weight slightly heavier than the core glycosylated species of Shaker, which is expected because the external pore domain of TRPV1 contains 24 additional residues compared with Shaker. A higher molecular weight band is also evident for C11, and this band is broader than the lower molecular weight species when viewed with higher exposure. (*B*) Western blot from a SDS/PAGE gel of the surface protein fraction obtained by biotinylating cells and then purifying biotinylated proteins using streptavidin beads. For Shaker, only the heavily glycosylated species is seen in the surface fraction, consistent with previous results (1, 2). For C11, both core and heavily glycosylated species are evident in the surface fraction. Glycosylation of C11 would be expected to be complex because this construct contains glycosylation sites from Shaker (at N259 and N263 within the S1–S2 loop) (1, 3) and TRPV1 (at N604 within the outer pore domain) (4). Similar results were obtained in three separate experiments.

1. Silberberg SD, Chang TH, Swartz KJ (2005) Secondary structure and gating rearrangements of transmembrane segments in rat P2X4 receptor channels. *J Gen Physiol* 125:347–359.
2. Hackos DH, Chang TH, Swartz KJ (2002) Scanning the intracellular S6 activation gate in the shaker K^+ channel. *J Gen Physiol* 119:521–532.
3. Santacruz-Toloza L, Huang Y, John SA, Papazian DM (1994) Glycosylation of shaker potassium channel protein in insect cell culture and in Xenopus oocytes. *Biochemistry* 33:5607–5613.
4. Veldhuis NA, et al. (2012) N-glycosylation determines ionic permeability and desensitization of the TRPV1 capsaicin receptor. *J Biol Chem* 287:21765–21772.

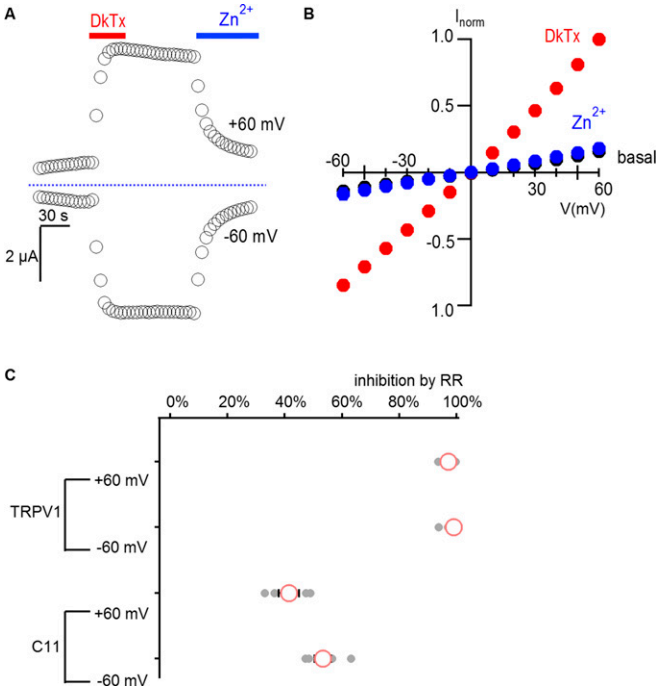

Fig. S3. The C11 chimera can be activated by DkTx. (*A*) Representative time course for membrane currents recorded at −60 and +60 mV from an oocyte injected with the C11 chimera. The cell was held at V_{hold} = −60 mV and voltage was stepped to +60 mV for 100 ms at 3-s intervals. Mean inward current at −60 mV and outward current at +60 mV are plotted as a function of time. DkTx (5 μM) and Zn^{2+} (2 mM) were applied as indicated by the horizontal bars. The dotted blue lines denote the zero-current level. The external recording solution contained (in millimoles): 100 KCl, 10 Hepes, 2 $MgCl_2$, pH 7.4. (*B*) Normalized I–V relations before (black symbols) and after DkTx application (5 μM, red symbols), followed by Zn^{2+} application (2 mM, blue symbols), obtained from experiments as in *A*. Currents were normalized to the amplitude of current in the presence of 5 μM DkTx at +60 mV (mean ± SEM for n = 4). (*C*) Inhibition of TRPV1 and the C11 chimera by RR (50 μM). K2K2-activated membrane currents at −60 and +60 mV were measured and values for individual cells are shown as gray circles and the mean ± SEM (n = 5–7) as red circles with black bars.

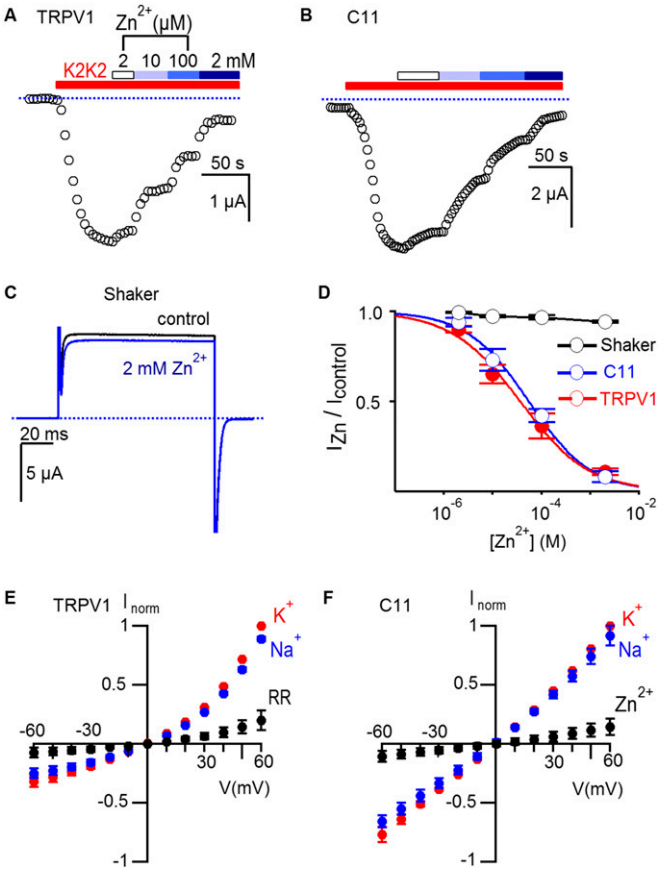

Fig. S4. Zinc sensitivity of TRPV1, Shaker, and the C11 chimera. (*A*) Representative time course showing the concentration dependence for Zn^{2+} inhibition of K2K2-activated (5 μM) currents at −60 mV for cells expressing TRPV1. (*B*) Representative time course showing the concentration dependence for Zn^{2+} inhibition of K2K2-activated (5 μM) currents at −60 mV for cells expressing the C11 chimera. (*C*) Representative current traces for a cell expressing Shaker in the absence (black) or presence (blue) of 2 mM Zn^{2+}. The cells were held at −60 mV, activated by stepping to +60 mV for 100 ms before stepping to −60 mV. (*D*) Normalized dose–response relations for Zn^{2+} inhibition of Shaker, TRPV1, and the C11 chimera obtained from experiments as in *A*–*C* at −60 mV. Data points are the mean ± SEM (n = 4–6). The continuous curves for TRPV1 and C11 are fits to the Hill equation with K_d = 36 ± 7 μM and n = 0.6 for TRPV1 and K_d = 55 ± 9 μM and n = 0.7 for C11. (*E* and *F*) I–V relations for (*E*) TRPV1- or (*F*) C11-expressing cells after application of either 10 μM capsaicin for TRPV1 or 5 μM K2K2 for C11 in 100 mM K^+ (red), 100 mM Na^+ (blue), or after application of 50 μM RR or 2 mM Zn^{2+} in 100 mM K^+ (black). Symbols are mean ± SEM for n = 4 for both TRPV1 and the C11 chimera.

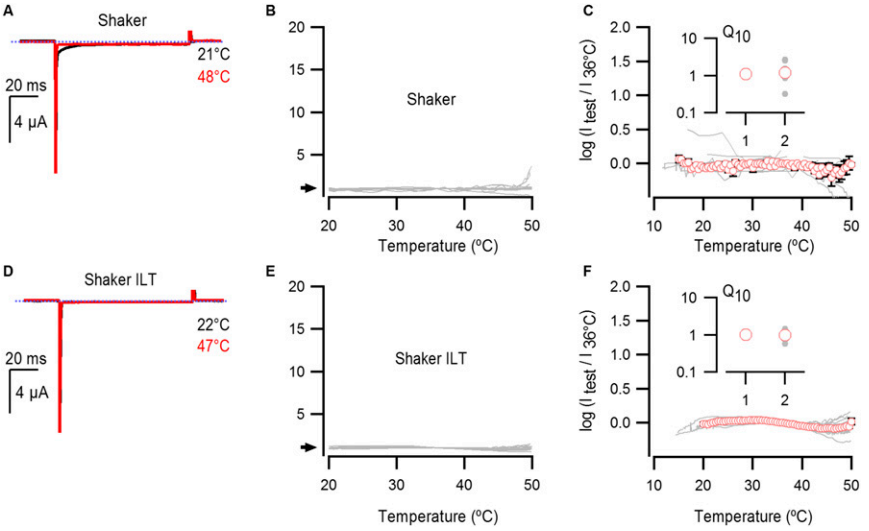

Fig. S5. Shaker- and Shaker ILT-expressing cells exhibit no temperature-activated currents. Representative current traces, temperature-activation relations, and Q_{10} determinations for cells expressing (*A*–*C*) Shaker (n = 12) or (*D*–*F*) the Shaker ILT mutant (n = 19).