

Supporting Information

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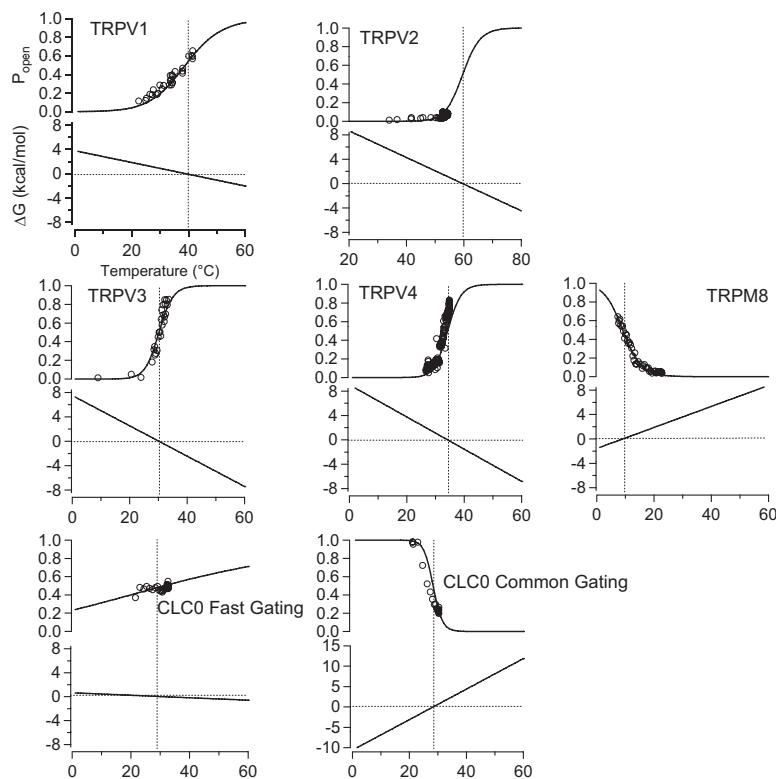


Fig. S1. The channel open probability and free energy associated with temperature-sensitive activation for each thermoTRP channel type and CLC-0. Raw data points represent channel open probabilities recorded from inside-out patches. Superimposed are P_{open} -Temperature curves calculated from thermodynamic measurements of ΔH and ΔS . Vertical dotted lines indicate $T_{0.5}$.

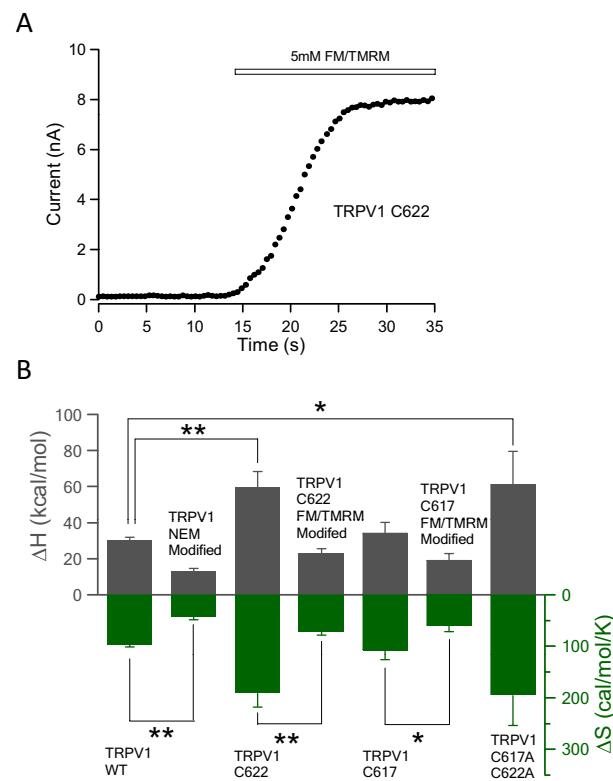


Fig. S2. Cysteines at the pore turret of TRPV1 are used for fluorophore attachment. (A) Time course of fluorophore labeling of C622 with a mixture of FM and TMRM. (B) Thermodynamic measurements from WT TRPV1 and pore cysteine mutants before and after modification with either fluorophores or N-ethylmaleimide (NEM). ** $P < 0.01$; * $P < 0.05$. $n = 3$ –14 patches.

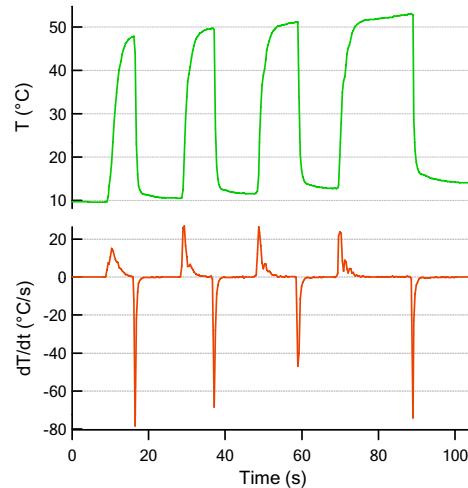


Fig. S3. Characterization of the temperature control method. The time course (Upper) and speed (Lower) of temperature change are shown.

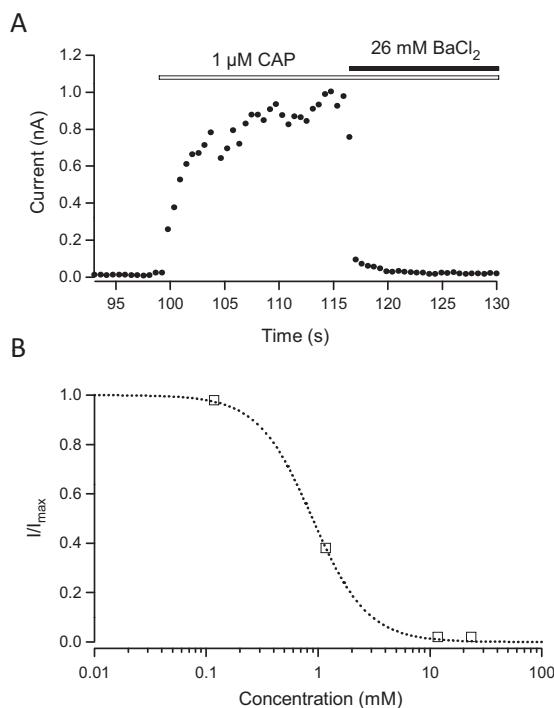


Fig. S4. Ba²⁺ block of TRPV1 current. (A) Macroscopic TRPV1 current induced by the application of 1 μ M capsaicin (CAP) is completely blocked by 26 mM Ba²⁺. (B) A representative dose–response curve of Ba²⁺ block of TRPV1. The estimated IC_{50} and Hill slope are 0.89 mM and 1.8, respectively.

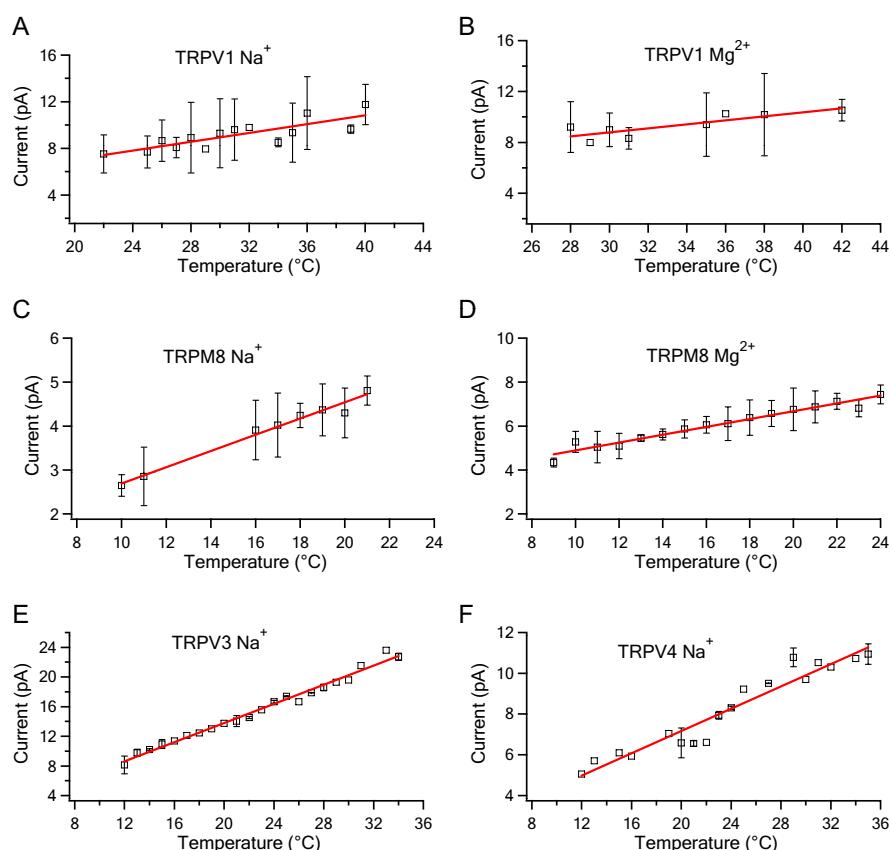


Fig. S5. Temperature dependence of single-channel current amplitudes. All single-channel currents were recorded at +80 mV with 130 mM permeant ion in the bath (intracellular) solution and 130 mM Na⁺ in the pipette (extracellular) solution. Red lines represent linear fits.

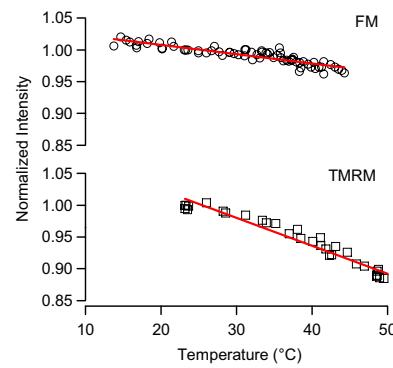


Fig. S6. Temperature dependence of fluorescence emission by FM and TMRM. Red lines represent linear fits. The slope factors for FM and TMRM are $-0.00146/\text{degree}$ and $-0.00439/\text{degree}$, respectively.