PNAS

Supporting Information for

Dual amplification strategy turns TRPM2 channels into supersensitive central heat detectors

Ádám Bartók[1,2,3] and László Csanády[1,2,3]

[1]HCEMM-SE Molecular Channelopathies Research Group,
[2]MTA-SE Ion Channel Research Group,
[3]Department of Biochemistry
Semmelweis University, Tuzolto u. 37-47, Budapest, H-1094, Hungary

Corresponding author: László Csanády
Email: csanady.laszlo@med.semmelweis-univ.hu

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Movie S1

Supporting Information Text

SI Materials and methods

Measuring temperature dependence of Ca-gluconate K_d, calibration of free [Ca^{2+}] at each temperature. Free [Ca^{2+}] in the micromolar range was determined as described (12). Ca-Green 5N (Invitrogen) fluorescence (excitation, 505 nm; emission, 535 nm) was measured in a cuvette-based fluorimeter (DeltaRAM, Photon Technology Industries). Apparent binding parameters (K_d, *n*) of the dye at 25, 30, 37 and 40°C and pH 7.1 were first obtained by stepwise titration with $CaCl_2$ in a solution initially containing 100 mM KCl, 2 mM $MgCl_2$, 10 mM HEPES and 1 μM Ca-Green 5N at each test temperature, followed by fitting plots of $(F-F_{min})/(F_{max}-F)$ vs. [Ca^{2+}] with the function $y=(x/K_d)^n$ (Fig. S5A-C). Using the determined dye parameters free [Ca^{2+}] could be accurately predicted in the range of 2-100 μM (Fig. S5D).

In electrophysiological experiments Ca^{2+} concentrations in the micromolar range were buffered using 144 mM gluconate. Temperature dependence of K_d of Ca-gluconate was determined as described (1) by stepwise titration, at each temperature, of our standard bath solution (144 mM Na-gluconate, 2 mM Mg-$gluconate_2$, 10 mM HEPES, pH=7.1) with $CaCl_2$; plots of free [Ca^{2+}] vs. added [Ca^{2+}] (Fig. S5F) were fitted to a simple binding equation with K_d and total background [Ca^{2+}] as the free parameters. The obtained, temperature-adjusted K_d of Ca-gluconate (Fig. S5F) and the obtained total background Ca^{2+} (~17 μM) were used to calculate, at each temperature, free [Ca^{2+}] following addition of various concentrations of Ca-$gluconate_2$ to the standard bath solution (summarized in Table S1).

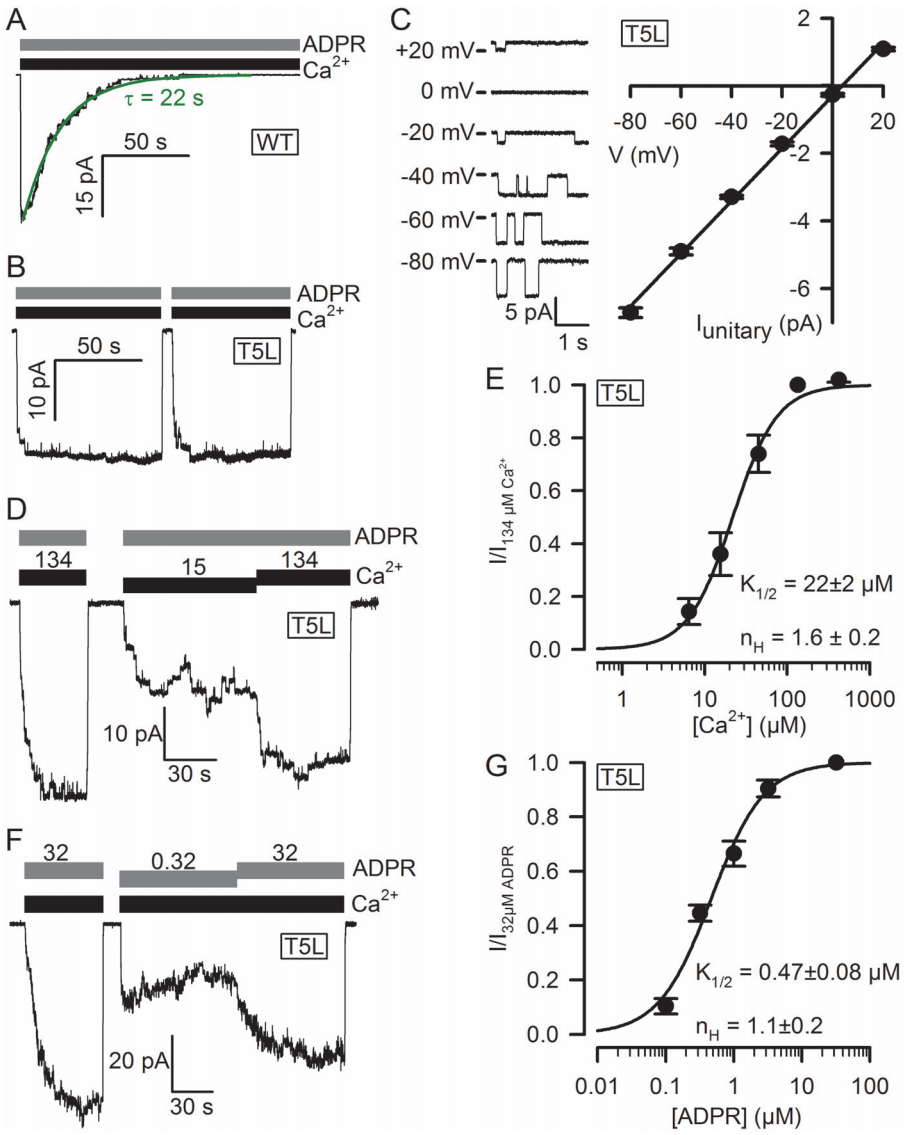

Fig. S1. Basic biophysical properties of TRPM2 channels in HEK-293 cell membranes resemble those reported for TRPM2 in *Xenopus laevis* oocyte membranes. *A*-*B*, Quasi-macroscopic inward currents of WT (*A*) and T5L (*B*) TRPM2 channels in inside-out patches at 25°C, activated by 134 μM Ca^{2+} (*black bars*) + 32 μM ADPR (*gray bars*). Membrane potential (V_m) was -20 mV. Inactivation time course in *A* is fitted by a single exponential (*green curve*). *C*, Unitary openings at various voltages (*left*) and unitary current-voltage relationship (*right*) of T5L TRPM2 in symmetrical 140 mM Na^+ at 25°C; the slope of the linear regression fit (*black line*) is 78±2 pS. *D*, *F*, T5L TRPM2 currents evoked by (*D*) 32 μM ADPR (*gray bars*) and various concentrations of Ca^{2+} (*black bars*, [Ca^{2+}] in μM), or (*F*) 134 μM Ca^{2+} (*black bars*) and various concentrations of ADPR (*gray bars*, [ADPR] in μM). V_m was -20 mV. *E*, *G*, Dose response curves of macroscopic T5L TRPM2 currents for (*E*) Ca^{2+} and (*G*) ADPR, with the other ligand kept saturated. Currents were normalized to the mean of the currents observed during bracketing exposures to 134 μM Ca^{2+} + 32 μM ADPR in the same patch. Data in *C*, *E*, *G* represent mean±SEM, n=3 (*C*), 6-7 (*E*), 15-21 (*G*). *Curves* are fits to the Hill equation with fit parameters plotted in the panels. Obtained parameters closely resemble those reported for T5L TRPM2 studied in *Xenopus laevis* oocyte membranes (2).

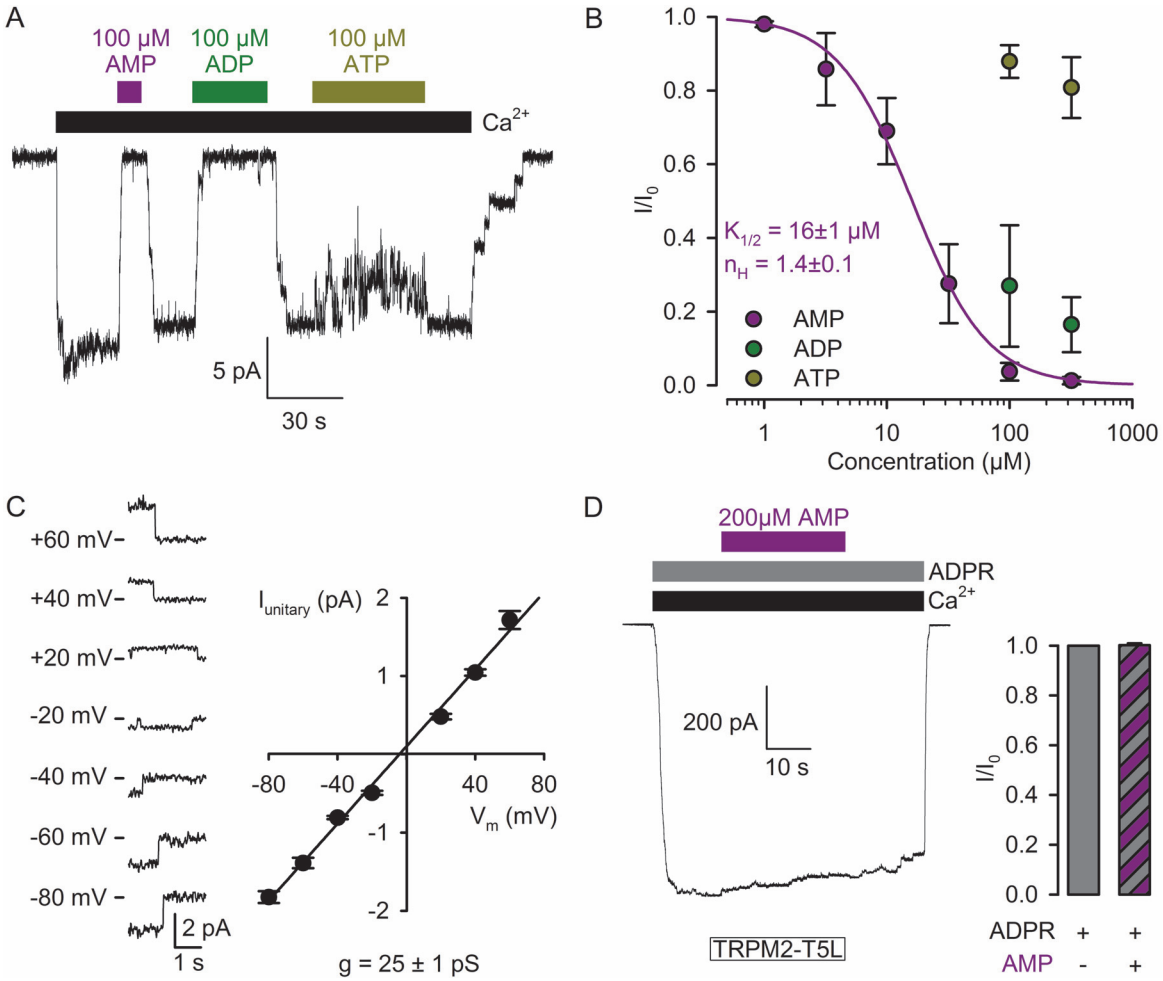

Fig. S2. Endogenous TRPM4-like channels, but not TRPM2, are blocked by cytosolic AMP. *A*, Exposure to cytosolic Ca^{2+} (134 μM) activates endogenous cation channels in an inside-out patch from an untransfected HEK-293T cell. The currents are inhibited by cytosolic exposure to AMP, ADP, and ATP (*colored bars*). *B*, Dose response curves of inhibition by AMP (*purple*), ADP (*green*) and ATP (*gold*) of the endogenous current. Currents were normalized to the mean of the currents observed during bracketing exposures to 134 μM Ca^{2+} alone in the same patch. *Purple curve* is a fit of the AMP data to the Hill equation with fit parameters plotted. *C*, Unitary openings at various voltages (*left*) and unitary current-voltage relationship (*right*) of the endogenous channels in symmetrical 140 mM Na^{+}; the slope of the linear regression fit (*black line*) is 25±1 pS. *D*, (*Left*) Large macroscopic T5L TRPM2 current in inside-out patch from a transfected HEK-293T cell, evoked by exposure to 134 μM Ca^{2+} + 32 μM ADPR with or without 200 μM AMP (*purple bar*). (*Right*) Fractional effect of 200 μM cytosolic AMP on TRPM2 currents (*striped bar*). In *A*, *D*, V_m=-20 mV. Temperature is 25°C. Data in *B*, *C*, *D* represent mean±SEM, n=4-8 (*B*, AMP), 3-5 (*B*, ADP), 4-5 (*B*, ATP), 3 (*C*), 7 (*D*).

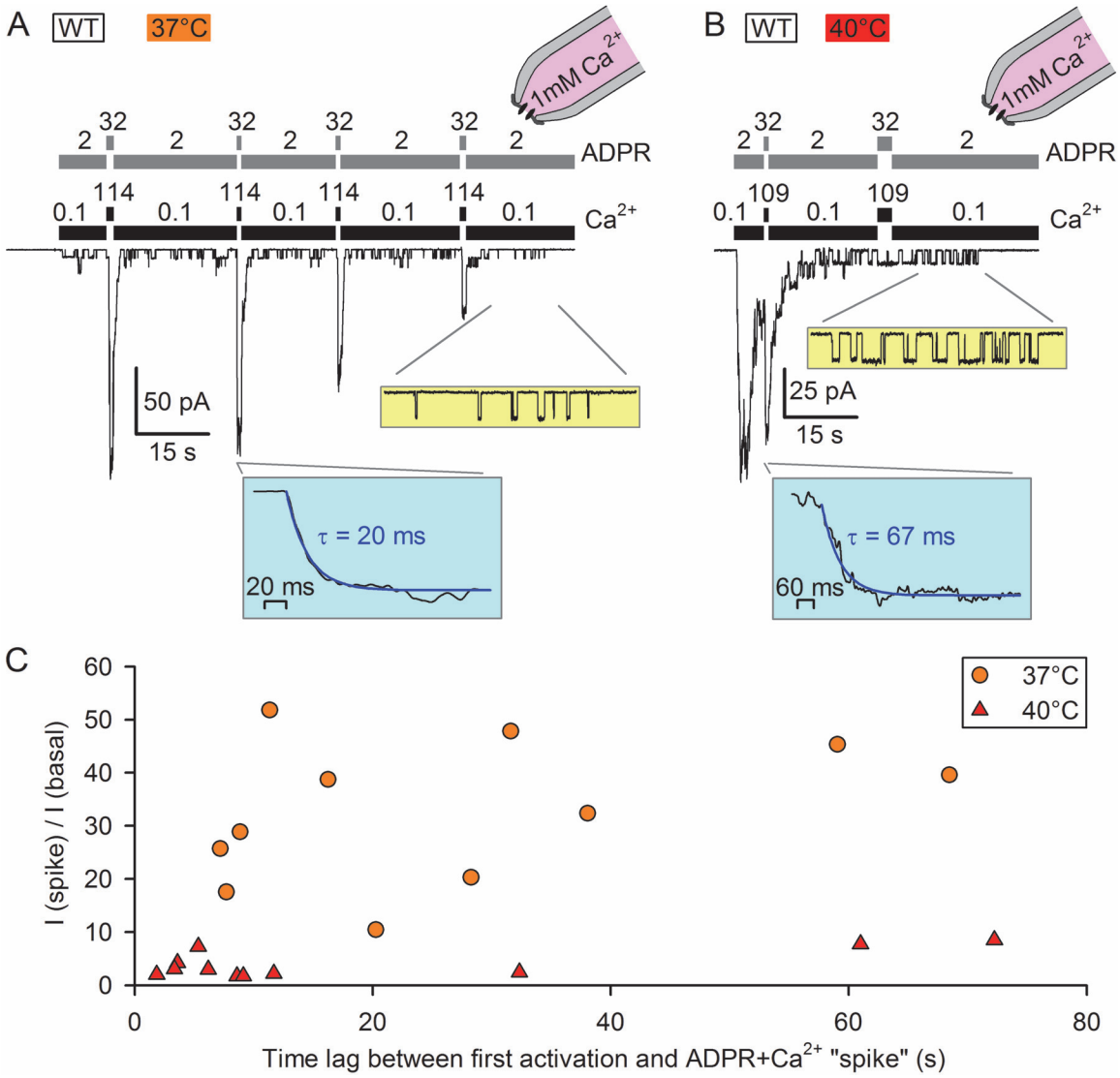

Fig. S3. Fractional stimulation of WT TRPM2 by agonist spiking is invariant to current rundown. *A-B*, Transient activation of macroscopic inside-out patch currents of WT TRPM2 channels at 37°C (*A*) and 40°C (*B*) by cytosolic superfusion with 0.1 μM Ca^{2+} + 2 μM ADPR, with ~1 mM free Ca^{2+} present in the extracellular (pipette) solution. Cytosolic agonist concentrations were repeatedly briefly (for ~1-2 s) raised to saturating levels (concentrations indicated above bars; in μM). V_m was -80 mV. *Cyan insets* illustrate relaxation time courses during agonist spiking, with time constants comparable to that of solution exchange. *Yellow insets* illustrate gating patterns of last surviving channels. *C*, Fractional current responses to brief agonist "spiking" at 37°C (*orange circles*) and 40°C (*red triangles*) plotted as a function of the time lag between initial channel activation (by exposure to 0.1 μM Ca^{2+} + 2 μM ADPR) and the subsequent high-agonist "spike".

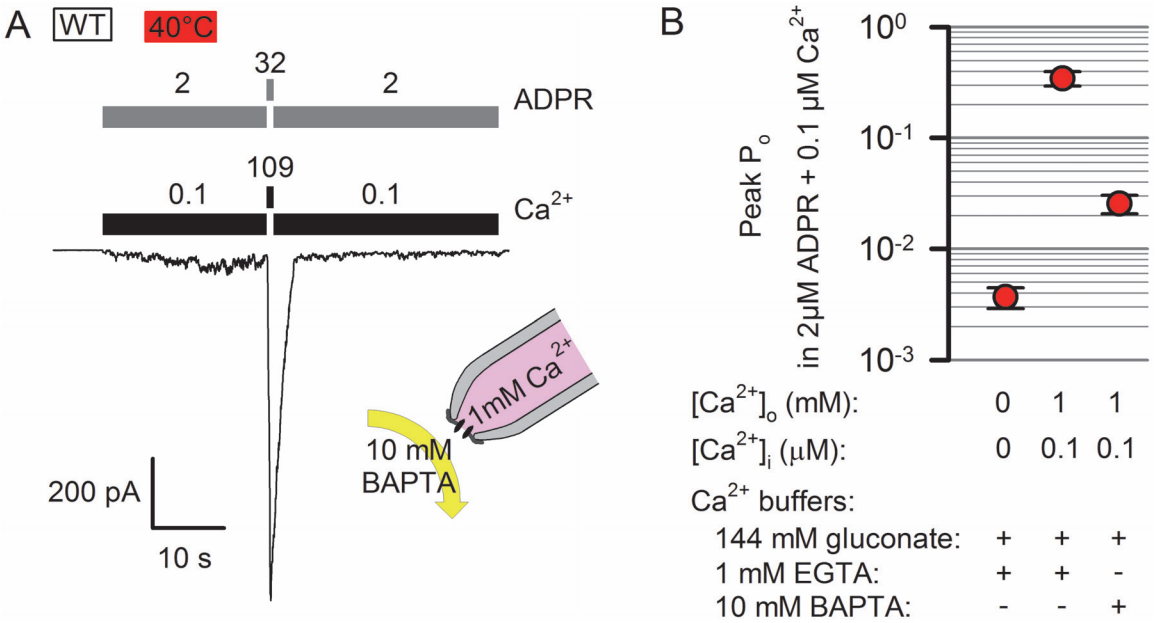

Fig. S4. Excessive cytosolic Ca^{2+} buffering blunts stimulation of WT TRPM2 channels by Ca^{2+} influx. *A*, Transient activation of macroscopic inside-out patch current of WT TRPM2 channels at 40°C by cytosolic superfusion with 0.1 μM Ca^{2+} + 2 μM ADPR, with ~1 mM free Ca^{2+} present in the extracellular (pipette) solution. Cytosolic [Ca^{2+}] was buffered using 10 mM BAPTA. Cytosolic agonist concentrations were briefly (for ~1.5 s) raised to saturating levels (concentrations indicated above bars; in μM). V_m was -80 mV. *B*, Comparison of estimated P_o (mean±SEM) of TRPM2 channels at 40°C under the following three conditions: (*Left*) 2 μM extracellular Ca^{2+}, cytosolic 4 nM Ca^{2+} (1 mM EGTA) + 32 μM ADPR (T5L, replotted from Fig. 1C); (*Center*) 1 mM extracellular Ca^{2+}, cytosolic 100 nM Ca^{2+} (1 mM EGTA) + 2 μM ADPR (WT, replotted from Fig. 5E); (*Right*) 1 mM extracellular Ca^{2+}, cytosolic 100 nM Ca^{2+} (10 mM BAPTA) + 2 μM ADPR (WT, from panel *A*, n=11). For the latter condition estimated (using Eq. 1) free [Ca^{2+}] around the activating sites is 1.2 μM.

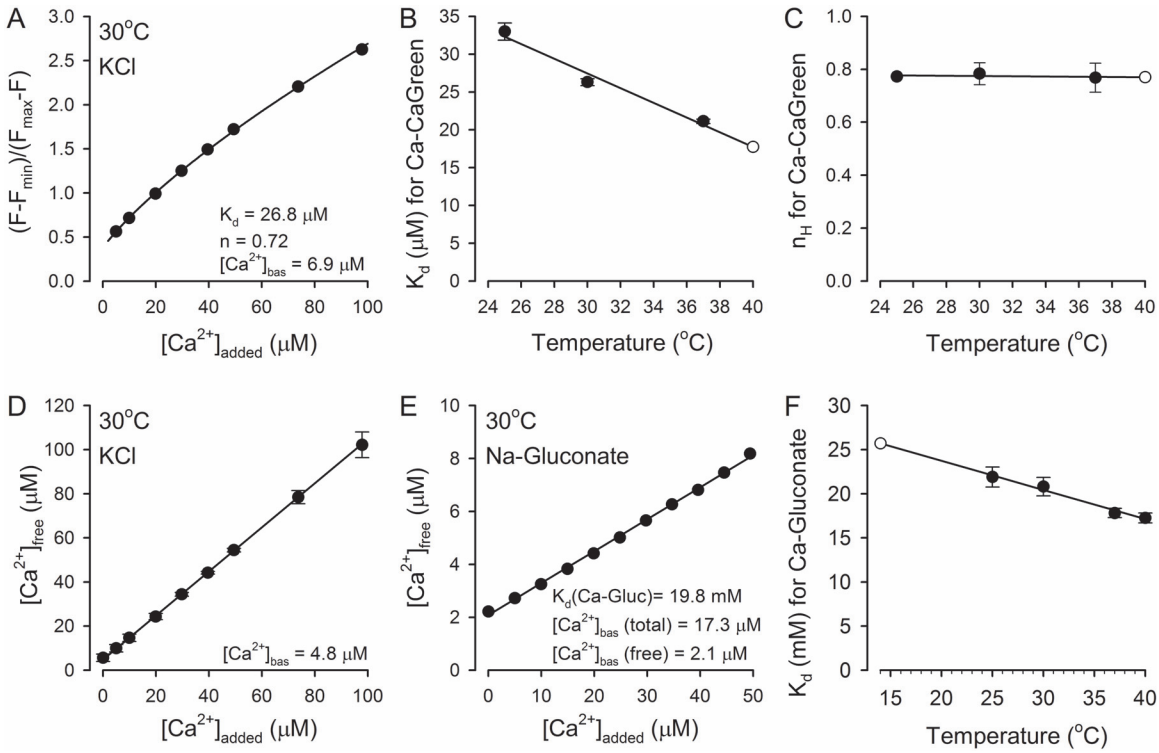

Fig. S5. Temperature dependence of the dissociation constant of Ca-gluconate. *A*, Calibration curve of the dye Ca-green-5N obtained by incremental addition of $CaCl_2$ to a 140 mM KCl-based solution at 30°C (*black symbols*) and a fit of the plot to $y=(x/K_d)^n$ (Materials and methods; *black curve*) to obtain the plotted dye parameters K_d and *n*. *B*, *C*, Temperature dependence of dye parameters (*B*) K_d and (*C*) *n* (*black symbols*), and linear regression fits (*black lines*). *Solid symbols* represent mean±SEM, n=2-4. *Open symbols* represent extrapolated dye parameters at 40°C. *D*, Control $[Ca^{2+}]$ prediction at 30°C, obtained by incremental addition of $CaCl_2$ to a 140 mM KCl-based solution. *Black symbols* plot predicted free Ca^{2+} (mean±SEM, n=4) using the dye parameters from *A*, *B*; *black line* is a fit of the function $y=x+[Ca^{2+}]_{bas}$. *E*, Gluconate titration with Ca^{2+} at 30°C, obtained by incremental addition of $CaCl_2$ to a 140 mM Na-gluconate based solution. *Black symbols* plot predicted free Ca^{2+} using the dye parameters from *A*, *B*; *black curve* is a fit of a simple binding equation (Materials and methods) yielding the plotted K_d of Ca-gluconate. *F*, Temperature dependence of the K_d of Ca-gluconate (*black symbols*) and linear regression fit (*black line*). *Solid symbols* represent mean±SEM, n=2-4. *Open symbol* represents extrapolated K_d at 14°C. For all titrand solutions pH was adjucted to 7.1 at all studied temperatures.

Table S1. Calculated free [Ca^{2+}] in our Na-gluconate based bath solution with various amounts of added Ca-gluconate$_2$, at the temperatures employed in this study.

T(^{o}C)	$[Ca^{2+}]_{added}$ (μM)	$[Ca^{2+}]_{free}$ (μM)
	0	2.47
	32	7.31
	100	17.6
14	320	50.8
	1000	153
	3200	477
	10000	1422
	0	2.16
	32	6.42
25	100	15.4
	320	44.6
	1000	134
	3200	418
37	1000	113
	0	1.73
	10	2.79
40	32	5.13
	100	12.4
	1000	107

Movie S1 (separate file). Morphs created (USCF Chimera) by linear interpolation between the structures of (*Top left*) apo (6BPQ) vs. WS-12+PIP2-bound (6NR2) TRPM8 from *Ficedula albicollis*, (*Bottom left*) apo (6O6A) vs. Ca^{2+}-bound (6O77) TRPM8 from *Parus major*, (*Top right*) apo (6PUO) vs. Ca^{2+}+ADPR-bound (6PUS) human TRPM2, and (*Bottom right*) apo (6DRK) vs. Ca^{2+}+ADPR-bound (6DRJ) TRPM2 from *Danio rerio*. Conserved TRPM domains are in *light pink*, the NUDT9H domains of the TRPM2 orthologs are in *pale cyan*. *Yellow lines* indicate approximate membrane boundaries.

SI References

1. L. Csanády, B. Torocsik, Four Ca2+ ions activate TRPM2 channels by binding in deep crevices near the pore but intracellularly of the gate. *J. Gen. Physiol* 133, 189-203 (2009).
2. B. Tóth, L. Csanády, Pore collapse underlies irreversible inactivation of TRPM2 cation channel currents. *Proc. Natl. Acad. Sci. U. S. A* 109, 13440-13445 (2012).