Pharmacological properties of TRPM3 isoforms are determined by the length of the pore loop

SUPPLEMENARY INFORMATION

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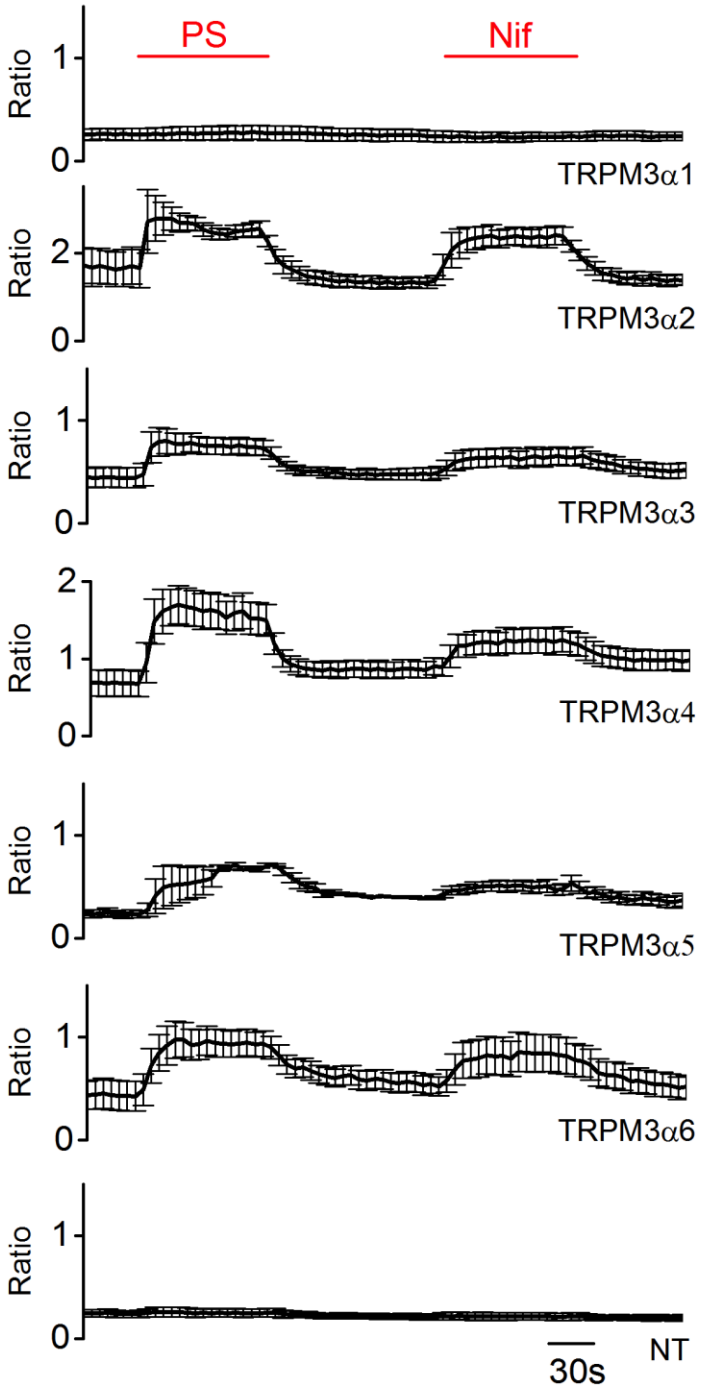

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22 **FIGURE S1**

23 Mean fluorescence ratio ($Fura_{340}/Fura_{380}$) of HEK293T cells transfected with TRPM3α1 - α6 or

24 of non-transfected cells during treatment with PS (40 µM) and Nif (50 µM) (N = 3 cover slips

25 for each experiment).

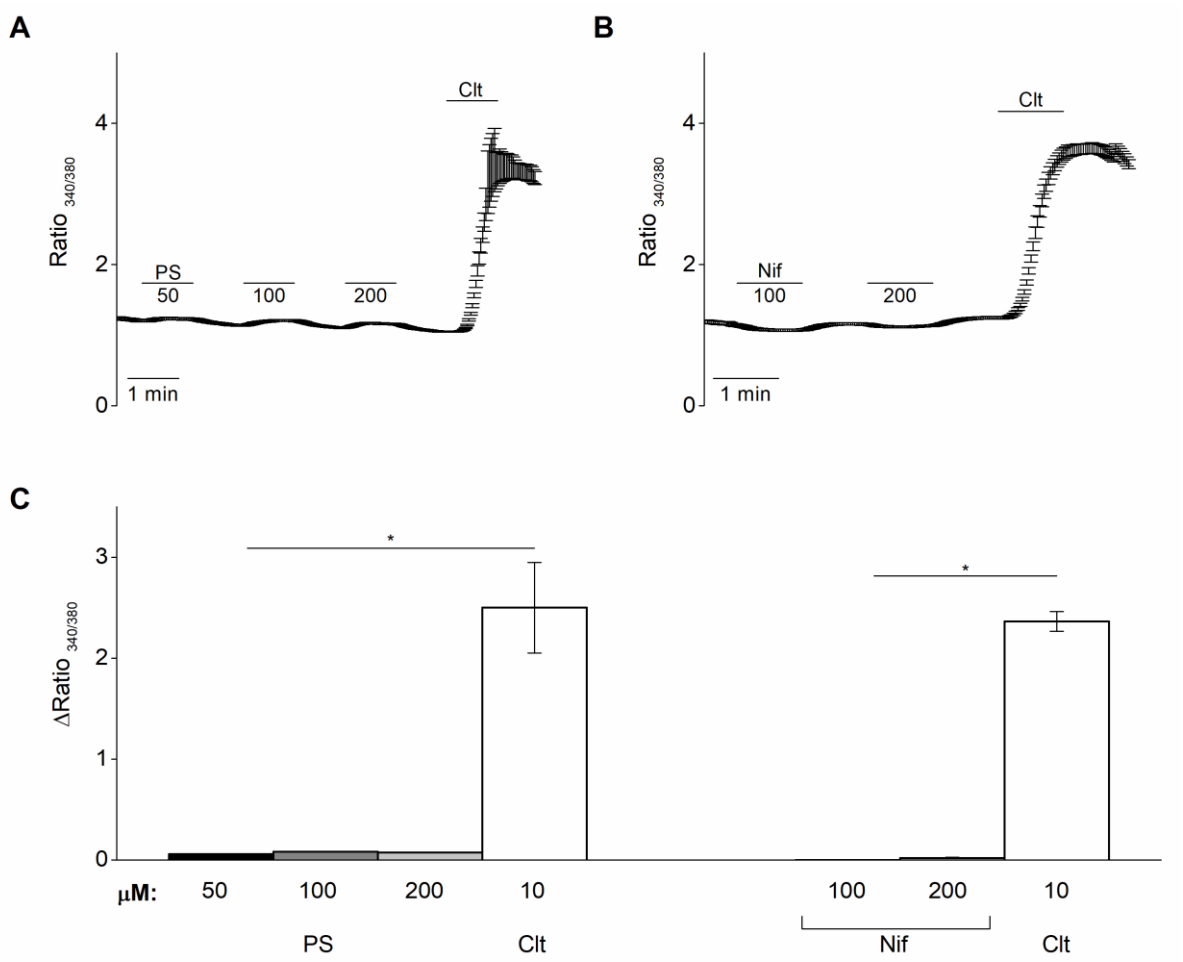


FIGURE S2

A) Time course of calcium fluorimetric measurements in HEK293T cells transiently expressing TRPM3α1 upon application of different concentrations of PS (50, 100 and 200 µM) and Clt (10 µM). **B)** As in *A)* during application of different concentrations of Nif (100 and 200 µM) and Clt (10 µM). **C)** Statistics of calcium increases that occur during the different treatments in *A)* and *B)* with n = 366 for PS treatment and n = 393 for Nif treatment with N = 3 cover slips for both experiments. * < 0.05 with Kruskal-Wallis ANOVA.

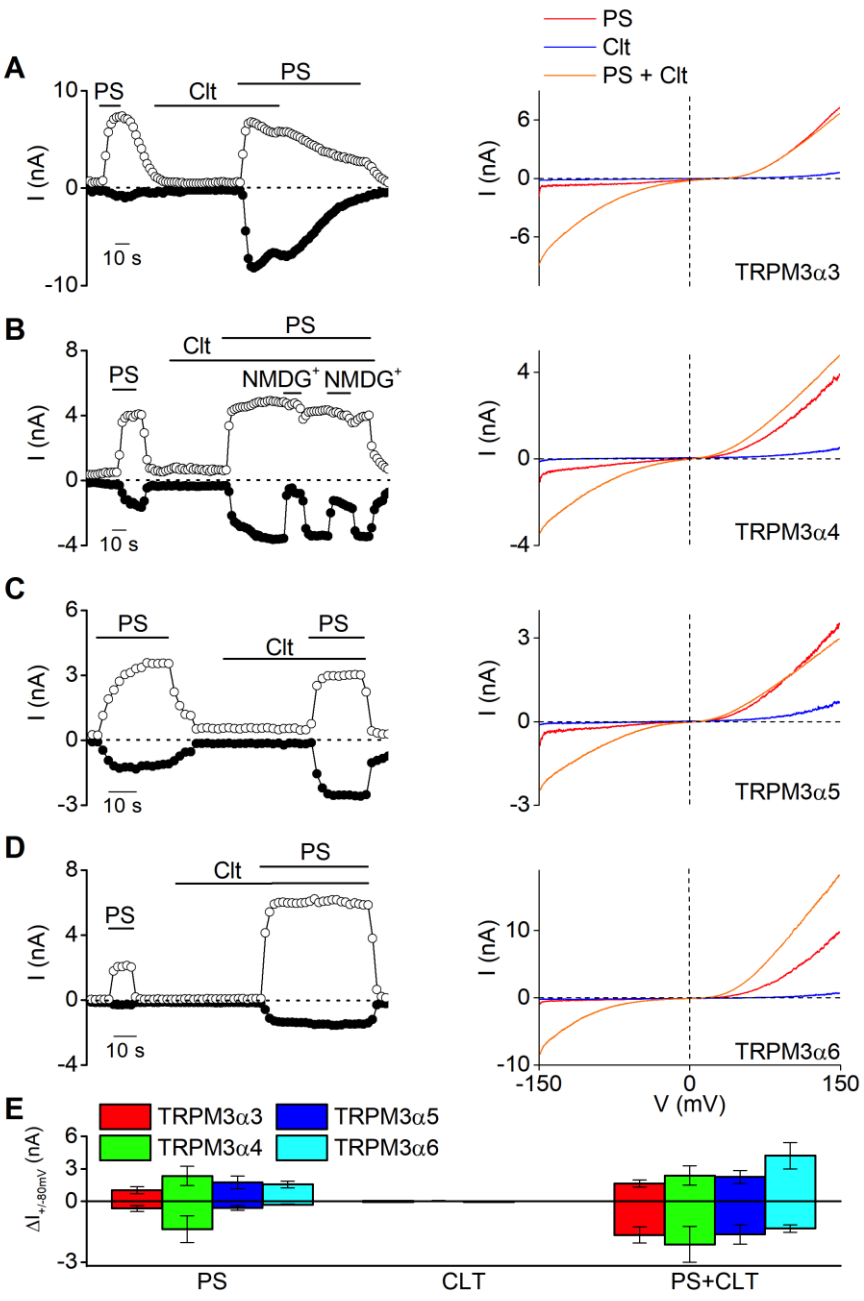
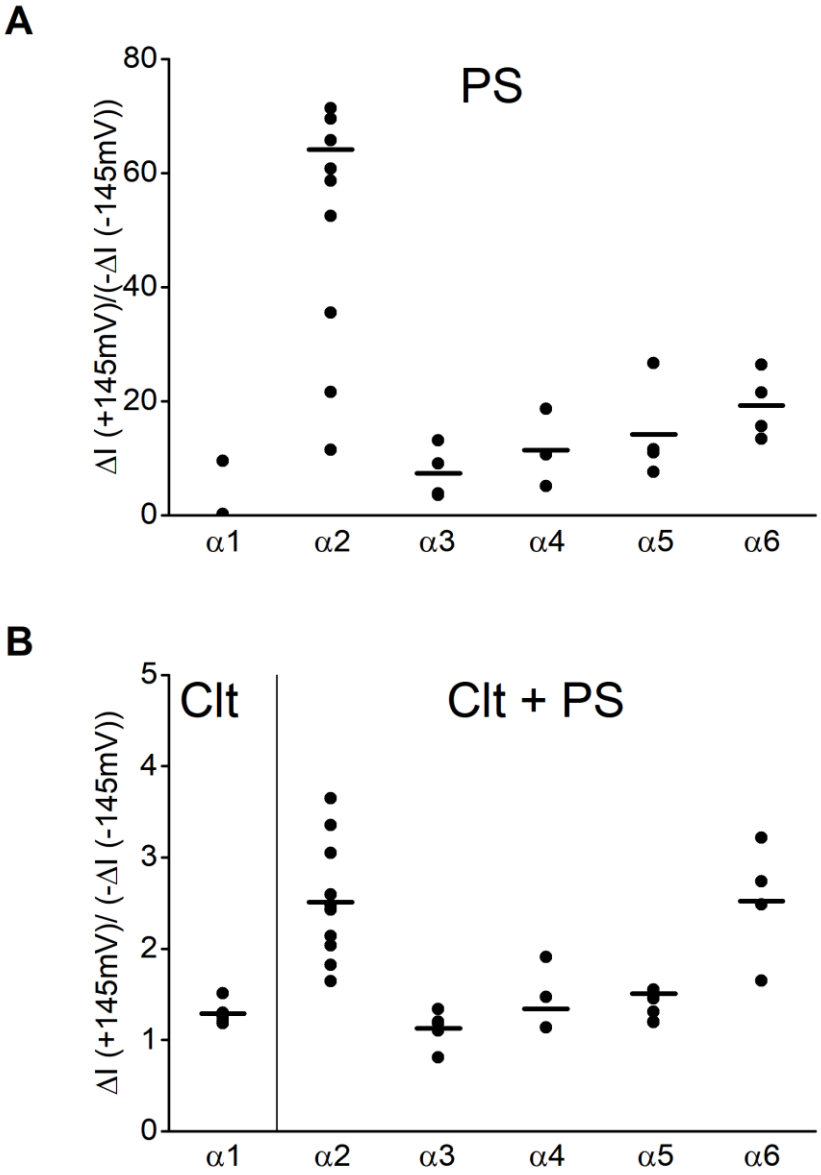


FIGURE S3

A - D) Time courses and corresponding *I-V* curves of whole-cell currents recorded in HEK293T cells transiently transfected with *A)* TRPMα3, *B)* TRPMα4, *C)* TRPMα5, *D)* TRPMα6 during treatment with PS and Clt. **E)** Current increases of the different TRPM3α variants at ± 80 mV upon chemical treatment as shown in *B) – E)* (n = 5 for TRPM3α3, n = 3 for TRPM3α4 and n = 5 for TRPM3α5 and n = 4 for TRPM3α6).



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42 **FIGURE S4**

43 **A)** Current ratios for HEK293 cells expressing the different TRPM3α isoforms calculated for

44 values at ±145 mV during application of PS (40 µM). **B)** Current ratios at ±145 mV for

45 TRPM3α1 and TRPM3α2 during Clt (10 µM) application and for TRPM3α2- α6 during PS (40

46 µM) + Clt (10 µM) co-application. For both graphs: n = 5 for TRPM3α1, n = 11 for TRPM3α2,

47 n = 5 for TRPM3α3, n = 3 for TRPM3α4 and n = 5 for TRPM3α5 and n = 4 for TRPM3α6)

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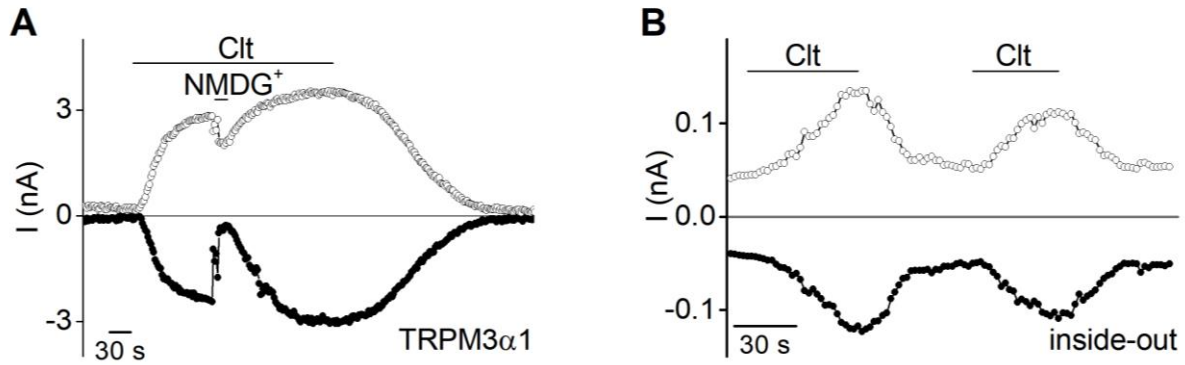


FIGURE S5

A) Time course of whole-cell patch clamp recording in HEK293T cells transiently transfected with TRPM3α1 upon application of Clt (10 µM). At the indicated time points all monovalent ions in the extracellular solution were replaced by $NMDG^+$. **B)** Time course of inside-out patch clamp recording in HEK293T expressing TRPM3α1 in presence of Clt (10 µM). Mean current increase in TRPM3α1 after stimulation by Clt (ΔI_{+80mV}: 67.3 ± 23.7 pA; ΔI_{-80mV}: -58.9 ± 20.3 pA) (n=8).

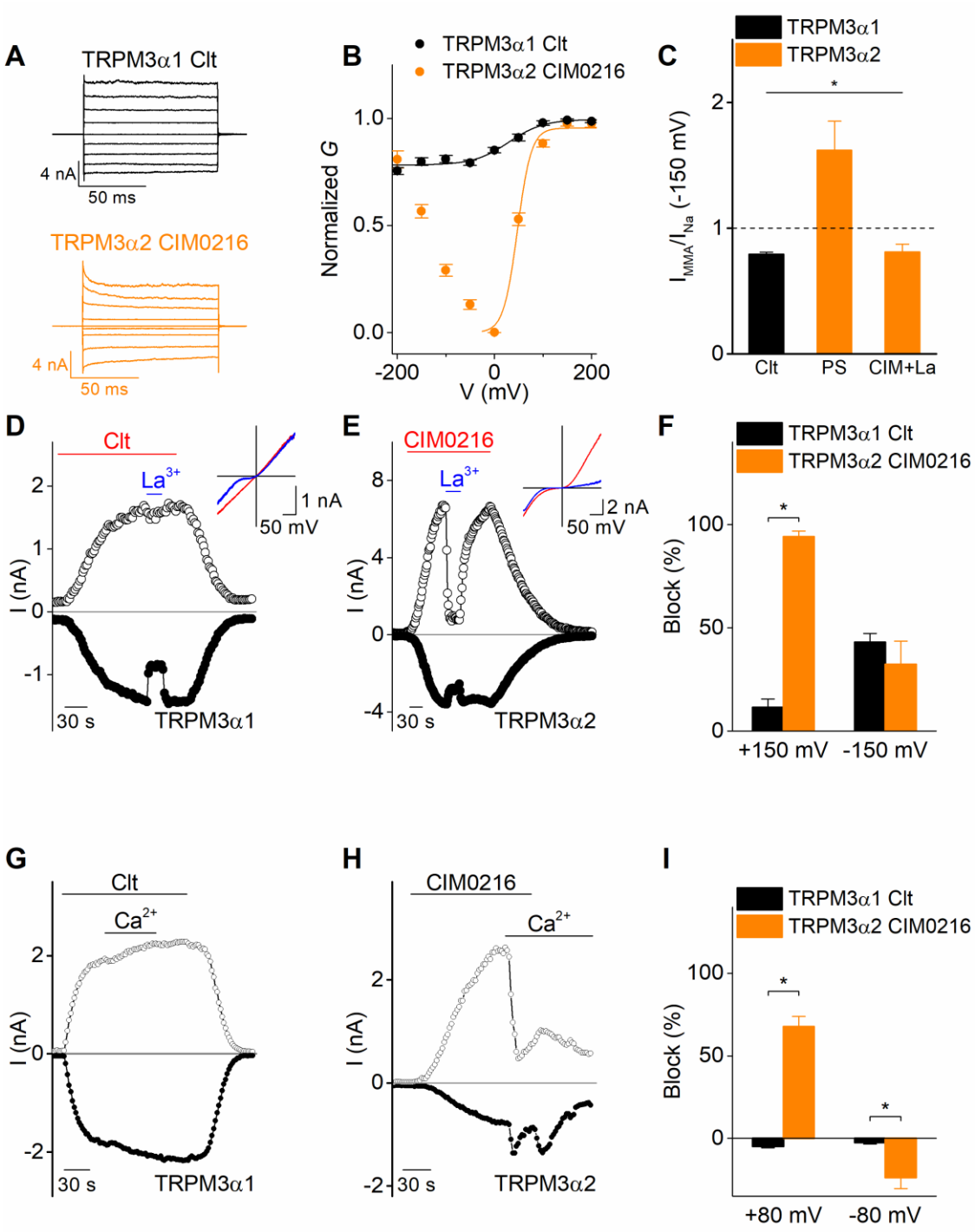


FIGURE S6

A) Current traces obtained with a step protocol ranging from -200 mV to +200 mV in +50 mV steps for HEK293T cells transiently transfected with TRPM3α1 (black, upper panel) or stably transfected with TRPM3α2 (orange, lower panel). **B)** Normalized *G-V* relation for TRPM3α1 Clt-induced and TRPM3α2 CIM0216-induced currents obtained from current traces as in *A)*,

n = 6 for both conditions. **C)** Relative currents at -150 mV carried by monomethylammonium (MMA^+) for TRPM3α1 Clt-induced currents (black) and TRPM3α2 PS-induced currents and CIM0216-induced currents in the presence of Lanthanum (La^{3+}, orange). MMA^+ currents were normalized to the currents carried by Na^+; PS (40 µM), Clt (10 µM), CIM0216 (1 µM) and La^{3+} (100 µM), n = 5 for all conditions. * < 0.05, One-way ANOVA. **D** and **E)** Time course of whole-cell patch clamp recordings showing the effect of La^{3+} on TRPM3α1 Clt-induced currents *D)* and TRPM3α2 CIM0216-induced currents *E)*. **F)** Percentage of La^{3+}-induced block for experiments as in *D)* and *E)* for n = 6 cells for each experiment. * < 0.05 with students two-tailed unpaired t-test. **G** and **H)** Time course of whole-cell patch clamp recordings showing the effect of calcium on TRPM3α1 Clt-induced currents *G)* and TRPM3α2 CIM0216-induced currents *H)*. **I)** Percentage (%) of calcium-induced block for experiments as in *G)* and *H)* for n = 5 cells. * < 0.05 with Mann-Whitney test.

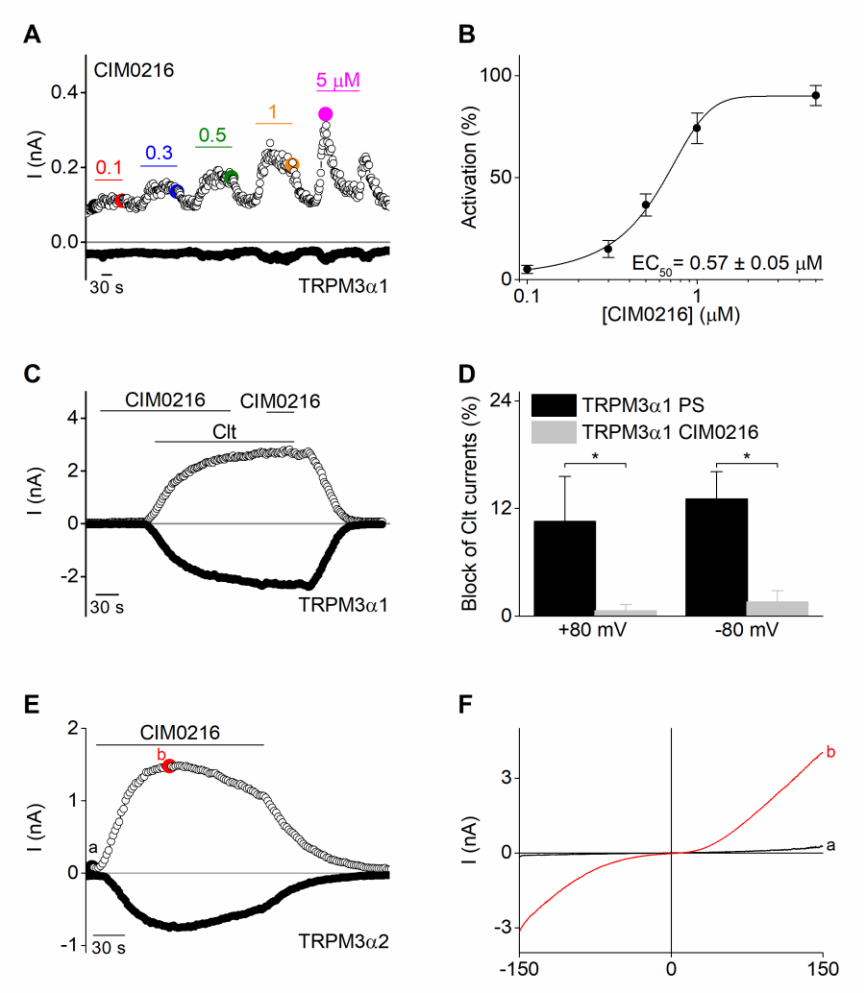


FIGURE S7

A) Time course of whole-cell patch clamp recording at ± 80 mV in HEK293T cells transiently expressing TRPM3α1 upon application of different concentrations of CIM0216 (in µM). **B)** Concentration-response curve for CIM0216 on TRPM3α1 extracted from experiments as in *A)* for n = 9 cells. **C)** Time course of whole-cell patch clamp recording at ± 80 mV in HEK293T cells transiently expressing TRPM3α1 upon co-application of CIM0216 and Clt. **D)** Statistics of the inhibiting effects of PS and CIM0216 on Clt-induced TRPM3α1 currents for n = 5 cells per treatment. **E)** Time course of whole-cell patch clamp recording at ± 80 mV in HEK293T cells stably expressing TRPM3α2 upon application of CIM0216 (1 µM). **F)** *I-V* traces for time points indicated in *E)*.

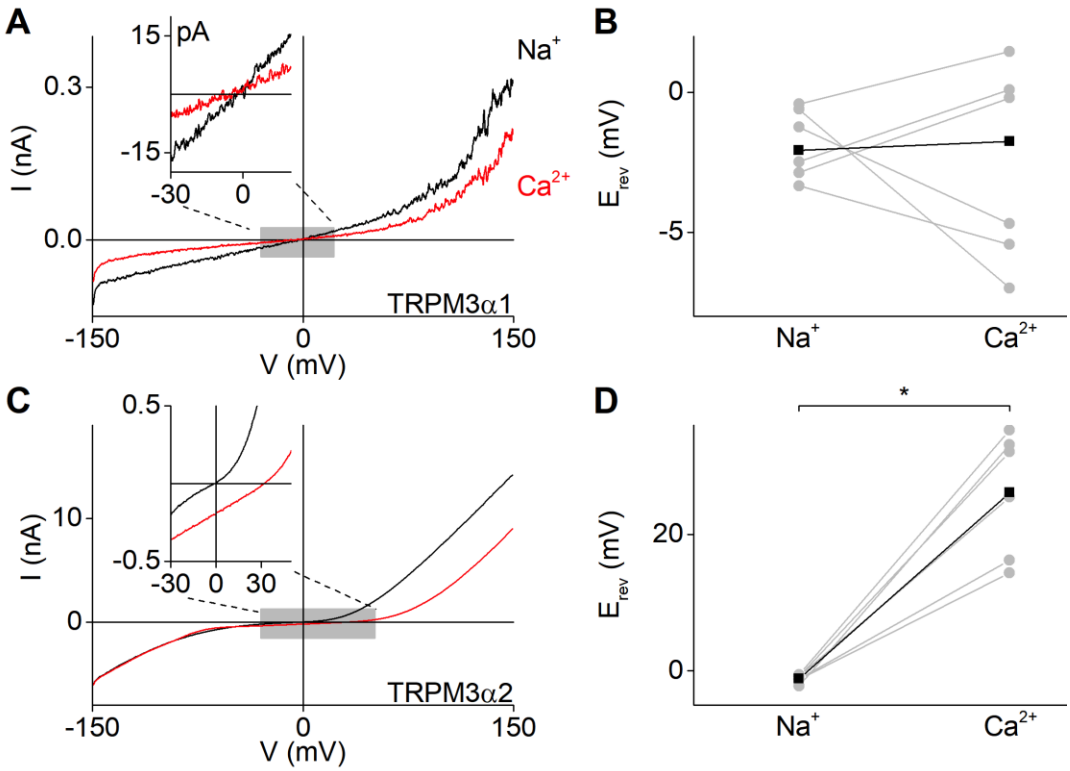


FIGURE S8

A) *I-V* Traces of whole-cell patch clamp recording in HEK293 cells transiently expressing TRPM3α1 upon application of CIM0216 (1 µM) in standard extracellular solution (black) and in 100 mM extracellular Ca^{2+} (red trace). **B)** Reversal potentials of n = 6 individual cells measured in *A)* in 150 mM extracellular Na^{+} and 100 mM extracellular Ca^{2+}, mean values represented in black squares. **C)** As in panel *A)* but for TRPM3α2. **D)** Reversal potentials of n = 6 individual cells (grey circles) measured in *C)* in 150 mM extracellular Na^{+} and 100 mM extracellular Ca^{2+}; mean values represented in black squares. * < 0.05 with student two-tailed unpaired t-test.

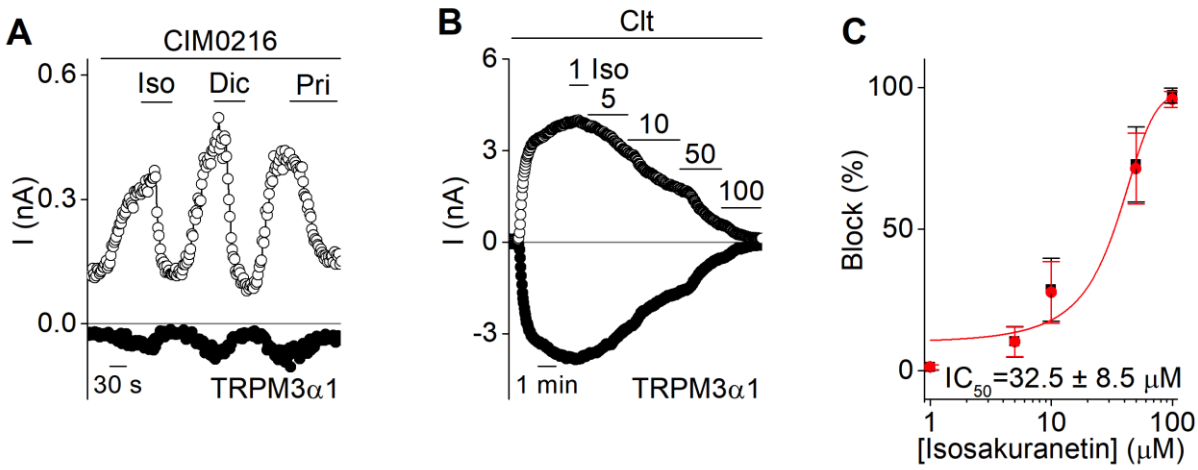


FIGURE S9

A) Time course of whole-cell patch clamp recording at ± 80 mV in HEK293T cells transiently expressing TRPM3α1 upon application of CIM0216 (1 µM) and the TRPM3 antagonists isosakuranetin (Iso; 5 µM), diclofenac (Dic; 100 µM) and primidone (Pri; 25 µM). **B)** Time course of whole-cell patch clamp recording at ± 80 mV in HEK293T cells transiently expressing TRPM3α1 upon application of different concentrations of Isosakuranetin (1 - 100 µM) during Clt-treatment (10 µM). **C)** Concentration-response curve for Isosakuranetin on TRPM3α1 at +80 mV (red) and -80 mV (black trace) extracted from experiments shown in *B)* (n = 4).

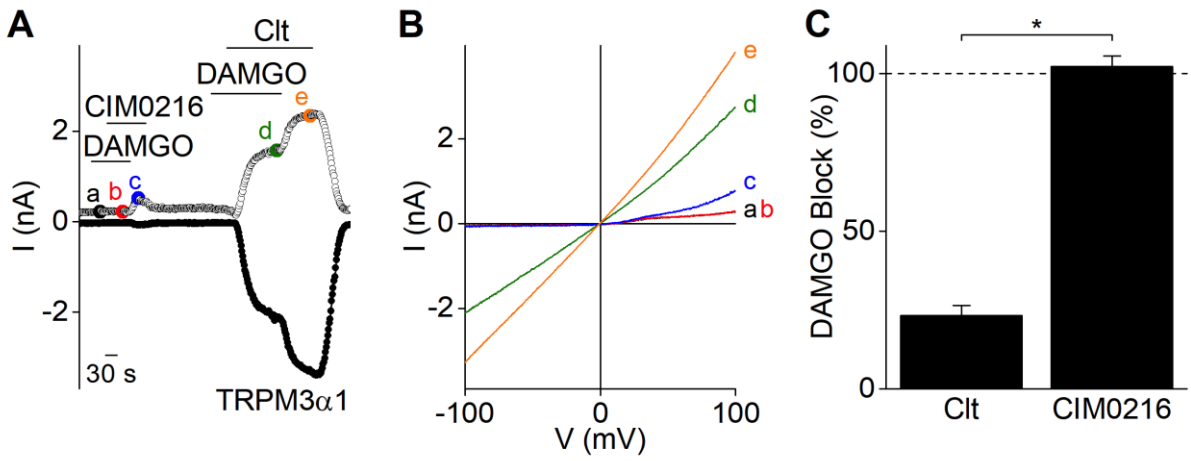
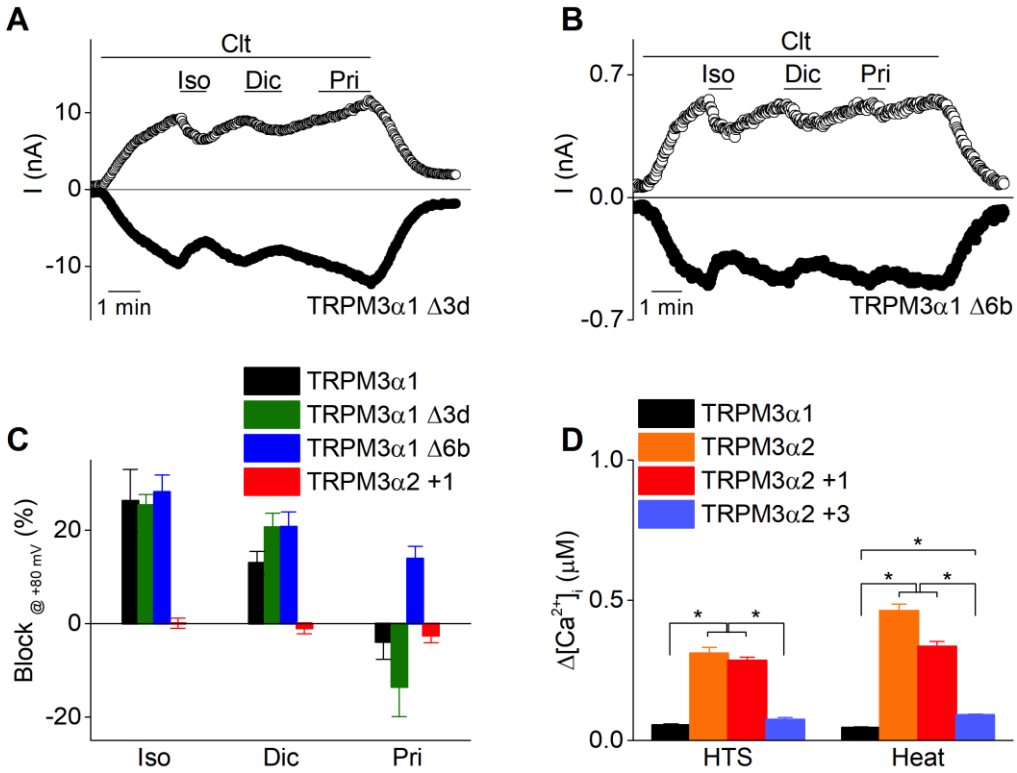


FIGURE S10

A) Time course of whole-cell patch clamp recording at ± 80 mV in HEK293T cells co-expressing TRPM3α1 and μ-opioid receptors upon application of DAMGO (1 μM), CIM0216 (5 μM) and Clt (10 μM). **B)** *I-V* Traces for time points indicated in *A)*. **C)** Percentage of block induced by DAMGO in experimental conditions of panel *A)* (n = 8). * < 0.05 with student two-tailed unpaired t-test.



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119 **FIGURE S11**

120 **A)** Time course of a whole-cell patch clamp recording at ± 80 mV in HEK293T cells transiently
121 expressing deletion mutant TRPM3α1 Δ3d upon application of Clt (10 μM) and different
122 TRPM3 antagonists (isosakuranetin (Iso; 5 μM), diclofenac (Dic; 100 μM), primidone (Pri; 25
123 μM)). **B)** As in *A)* but for the deletion mutant TRPM3α1 Δ6b. **C)** Percentage of block calculated
124 from experiments in *A)* and *B)* and compared to results of TRPM3α1 and insertion mutant
125 TRPM3α2 +1; n = 5 for TRPM3α1, n = 3 for TRPM3α1 Δ3d, n = 4 for TRPM3α1 Δ6b and n = 6
126 for TRPM3α2 +1. **D)** Statistics of calcium increases induced by hypotonicity (HTS) and heat in
127 HEK293T cells transfected with TRPM3α1, TRPM3α2 or the mutant channels TRPM3α2 +1
128 and TRPM3α2 +3. For HTS: n = 678 for TRPM3α1, n = 547 for TRPM3α2, n = 539 for TRPM3α2
129 +1 and n = 788 for TRPM3α2 +3. For heat: n = 484 for TRPM3α1, n = 651 for TRPM3α2, n =
130 751 for TRPM3α2 +1, n = 487 for TRPM3α2 +3. N = 3 independent recordings for each
131 experiment. * < *0.05*, Kruskal-Wallis ANOVA with Dunn's posthoc test.