

**Supplementary Figure 1.** Effects of internal Mg<sup>2+</sup> on TRPM7 and its mutants. Currents were elicited by a ramp protocol ranging from -120 to +100 mV with the pipette solutions containing 3 mM free Mg<sup>2+</sup>. Current amplitudes at +100 mV were measured at various time points and normalized to the value of peak current amplitude. Normalized currents were plotted as a function of time for TRPM7 (A), E1047Q (B), and E1029Q (C). Note the decline process of currents while Mg<sup>2+</sup> in the pipette was dialyzing into the cells. The speed of current decline was similar in TRPM7, E1047Q, and E1052Q. Similar results were obtained in another 4 cells for each condition. No statistical significance was observed, indicating that E1047 and E1052 residues are not the binding sites for internal Mg<sup>2+</sup>.

## Supplemental Data

**Supplementary Table 1.** Primers directed against mTRPM7 or hTRPM6 (targeted codons are marked in bold).

	Primers (5' to 3')
M7 D1035N (F)	cca tct tgg tct ctt gct aaa <b>aat</b> ata gtt ttt cat cca tac tgg
M7 D1035N (R)	cca gta tgg atg aaa aac tat <b>att</b> ttt agc aag aga cca aga tgg
M7 H1039E (F)	gg tct ctt gct aaa gat ata gtt ttt <b>gag</b> cca tac tgg atg att ttt ggt g
M7 H1039E (R)	c acc aaa aat cat cca gta tgg <b>etc</b> aaa aac tat atc ttt agc aag aga cc
M7 H1039M (F)	gg tct ctt gct aaa gat ata gtt ttt <b>atg</b> cca tac tgg atg att ttt ggt g
M7 H1039M (R)	c acc aaa aat cat cca gta tgg <b>cat</b> aaa aac tat atc ttt agc aag aga cc
M7 E1047K (F)	cat cca tac tgg atg att ttt ggt <b>aaa</b> gtt tat gca tat gaa att
M7 E1047K (R)	aat ttc ata tgc ata aac <b>ttt</b> acc aaa aat cat cca gta tgg atg
M7 E1047Q (F)	cat cca tac tgg atg att ttt ggt <b>caa</b> gtt tat gca tat gaa att
M7 E1047Q (R)	aat ttc ata tgc ata aac <b>ttg</b> acc aaa aat cat cca gta tgg atg
M7 E1052K (F)	ggt gaa gtt tat gca tat <b>aaa</b> att gat gtg tgt gca aat gac
M7 E1052K (R)	gtc att tgc aca cac atc aat <b>ttt</b> ata tgc ata aac ttc acc
M7 E1052Q (F)	ggt gaa gtt tat gca tat <b>caa</b> att gat gtg tgt gca aat gac
M7 E1052Q (R)	gtc att tgc aca cac atc aat <b>ttg</b> ata tgc ata aac ttc acc
M7 E1047-52Q (F)	ggt <b>caa</b> gtt tat gca tat <b>caa</b> att gat gtg tgt gca aat gac
M7 E1047-52Q (R)	gtc att tgc aca cac atc aat <b>ttg</b> ata tgc ata aac <b>ttg</b> acc
M7 E1054A (F)	ggt gaa gtt tat gca tat gaa att <b>gtt</b> gtg tgt gca aat gac tcc
M7 E1054A (R)	gga gtc att tgc aca cac <b>agc</b> aat ttc ata tgc ata aac ttc acc
M6 E1024Q (F)	gg atg ata tac gga <b>caa</b> gtc tat gct gga g
M6 E1024Q (R)	c tcc agc ata gac <b>ttg</b> tcc gta tat cat cc
M6 E1029Q (F)	gga gaa gtc tat gct gga <b>caa</b> ata gat gtt tg
M6 E1029Q (R)	ca aac atc tat <b>ttg</b> tcc agc ata gac ttc tcc

**Supplementary Table 2.** Analysis of dose-response and voltage-dependent block of WT TRPM7, E1047Q, and E1052Q by Mg<sup>2+</sup> and Ca<sup>2+</sup>.

	WT TRPM7		E1047Q		E1052Q	
	Mg <sup>2+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>	Mg <sup>2+</sup>	Ca <sup>2+</sup>
K <sub>1/2</sub> (-120 mV, μM)	3.6±0.4	4.1±0.2	442.9±53.6	593.6±69.9	154.7±23.4	202.2±14.3
k (depol) (mV)	15.2±0.8	15.6±1.7	35.9±2.4	38.7±3.6	24.5±2.7	22.8±2.3
δ <sub>out</sub>	0.84±0.1	0.81±0.2	0.36±0.05	0.33±0.05	0.52±0.04	0.56±0.03
k (hyperpol) (mV)	50.4±4.5	58±5.6	N.D	N.D	29.1±3.4	18.1±2.7
δ <sub>in</sub>	0.25±0.04	0.22±0.5	N.D	N.D	0.43±0.06	0.54±0.03

k (depol): Steepness of Boltzmann fit for voltage-dependent block (at depolarization voltage);

δ<sub>out</sub>: Electrical distance δ calculated from  $k=RT/z\delta F$  based on k (depol), representing the electrical distance from extracellular to the binding site (δ<sub>out</sub>).

k (hyperpol): Steepness of Boltzmann fit for voltage-dependent relief of block (at hyperpolarization voltage).

δ<sub>in</sub>: Electrical distance calculated from  $k=RT/z\delta F$  based on k (hyperpol), representing the electrical distance from intracellular to the binding site (δ<sub>in</sub>).

N.D: not determined, because there was no voltage-dependent relief of block at hyperpolarizing potential for E1047Q.

\*n=5 for analysis of k (depol), δ, and k (hyperpol) for each group.

## Supplementary Figure 1

