Identification of pore residues engaged in determining divalent cationic permeation in transient receptor potential melastatin subtype channel 2

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Running title: **Divalent cationic permeation in TRPM2**

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Supplemental figure legends

Fig.1 Mutational effects on protein expression and membrane localization

Western blotting analysis using an anti-EE antibody of membrane (biotin-labelled) and total (whole cell lysate) TRPM2 protein expression in non-transfected cells (HEK) or cells transfected with the indicated plasmids for WT and the indicated mutant TRPM2 subunits. The arrowhead and star symbols denote the TRPM2 protein and non-specific protein respectively. Similar results were observed in two independent experiments.

Fig.2 Na^+ currents for WT and D987E mutant channels in the absence of extracellular calcium

Representative ADPR-evoked currents at -80 mV (denoted by circles), obtained from voltage ramps applied every 5 s, from cells expressing the WT or D987E mutant channel in extracellular 147 mM NaCl solutions containing no calcium and magnesium (divalent cation free solution) (A) or containing 2 mM $MgCl_2$ (B). The currents for both WT and D987E mutant channels were relative stable. Similar results were seen in more than three cells for each case.

Fig.3 Ca^{2+} currents for WT and D987E mutant channels

Left, representative ADPR-evoked currents at -80 mV (denoted by circles), obtained from voltage ramps applied every 5 s, from cells expressing the WT or D987E mutant channel in isotonic 110 mM $CaCl_2$ solution. Right, summary of the sustained currents, 1 min after reaching the peak in 110 mM $CaCl_2$ solution, expressed as percentage of the peak currents. The number of cells examined in each case is indicated. There is significant difference between WT and D987E mutant channels (***, $p<0.001$).

Fig.4 Protein expression of subunit concatemers

Summary of Western blotting results as those shown in Fig.4B. The protein expression level was normalized to WT in each parallel experiment. The number of independent experiments for each case is indicated. The expression of all the subunit concatemers was comparable, but was significantly lower compared to the WT or mutant subunit alone (*, $p<0.05$, compared to WT).

Fig.5 Ca^{2+} inhibition of Na^+ currents for functional subunit concatemers

A. Representative ADRP-evoked currents at -80 mV (denoted by circles), obtained by voltage ramps applied every 5 s, from cells expressing the WT-WT or WT-D987E concatemer in extracellular 147 mM NaCl solutions containing the indicated $CaCl_2$ concentrations.

B. Representative ADPR-evoked currents at -80 mV (denoted by circles), obtained by voltage ramps applied every 5 s, from cells expressing the WT-D987N or WT-E960D concatemer in extracellular 147 mM NaCl solution containing 10 mM $CaCl_2$.

C. Summary of the sustained currents expressed as a percentage of the peak currents. The smooth solid line for the WT-D987E concatemer represents the curve fit to the Hill equation (IC_{50} = 2.4 mM and n = 0.8). n = 3-8 cells for each datapoint. The dashed line is for the D987E mutant channel (from Fig.3D).

Supplemental Figures

Fig.1

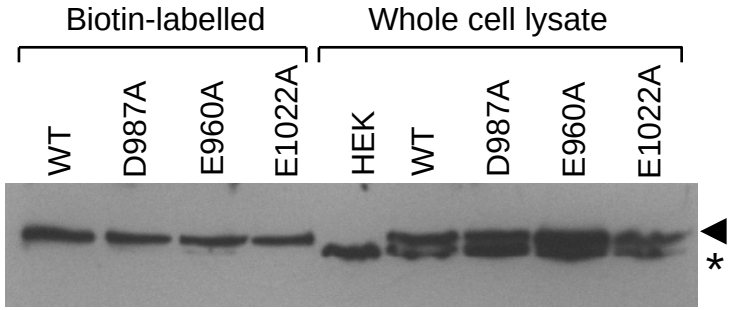


Fig.2

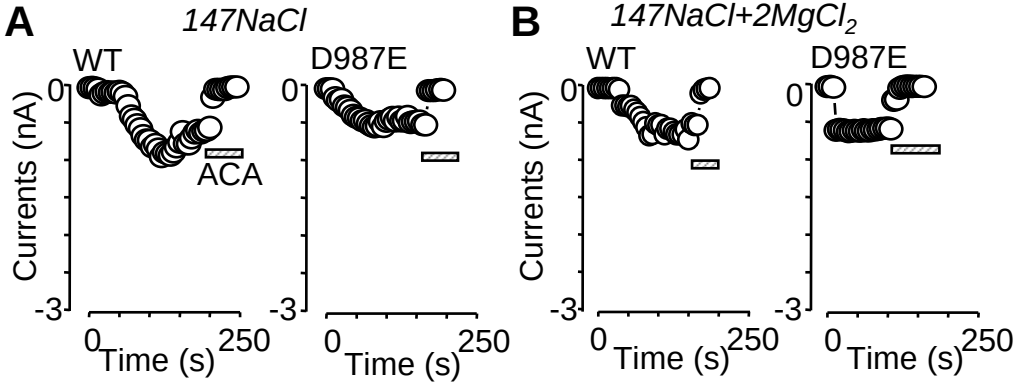


Fig.3

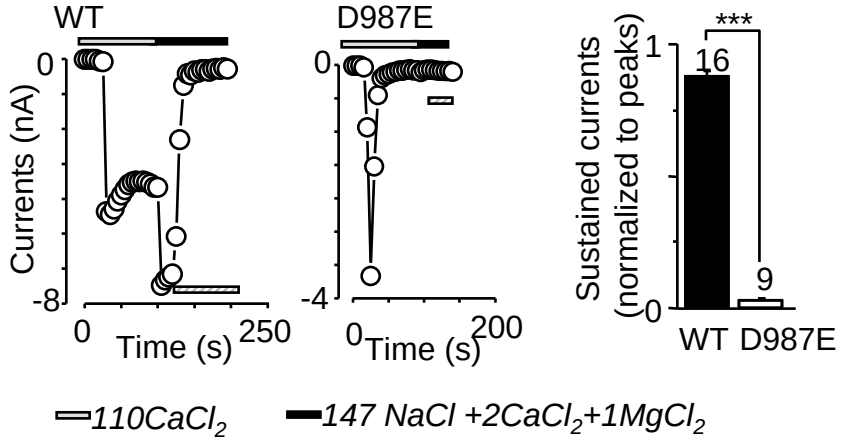


Fig.4

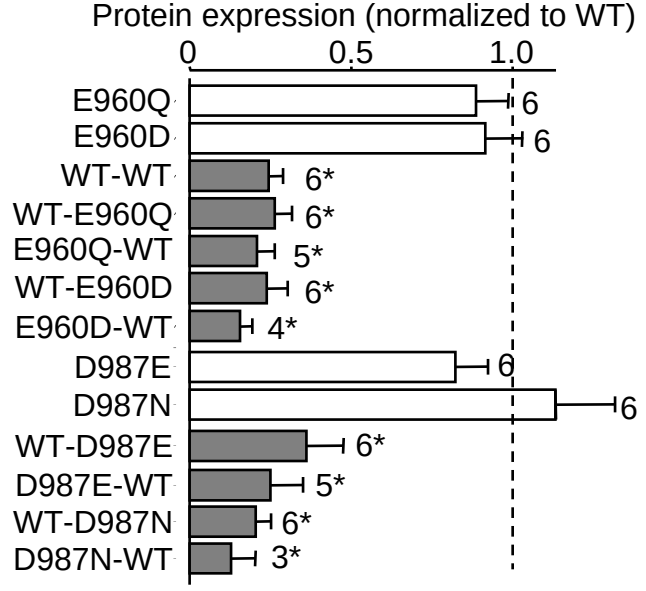
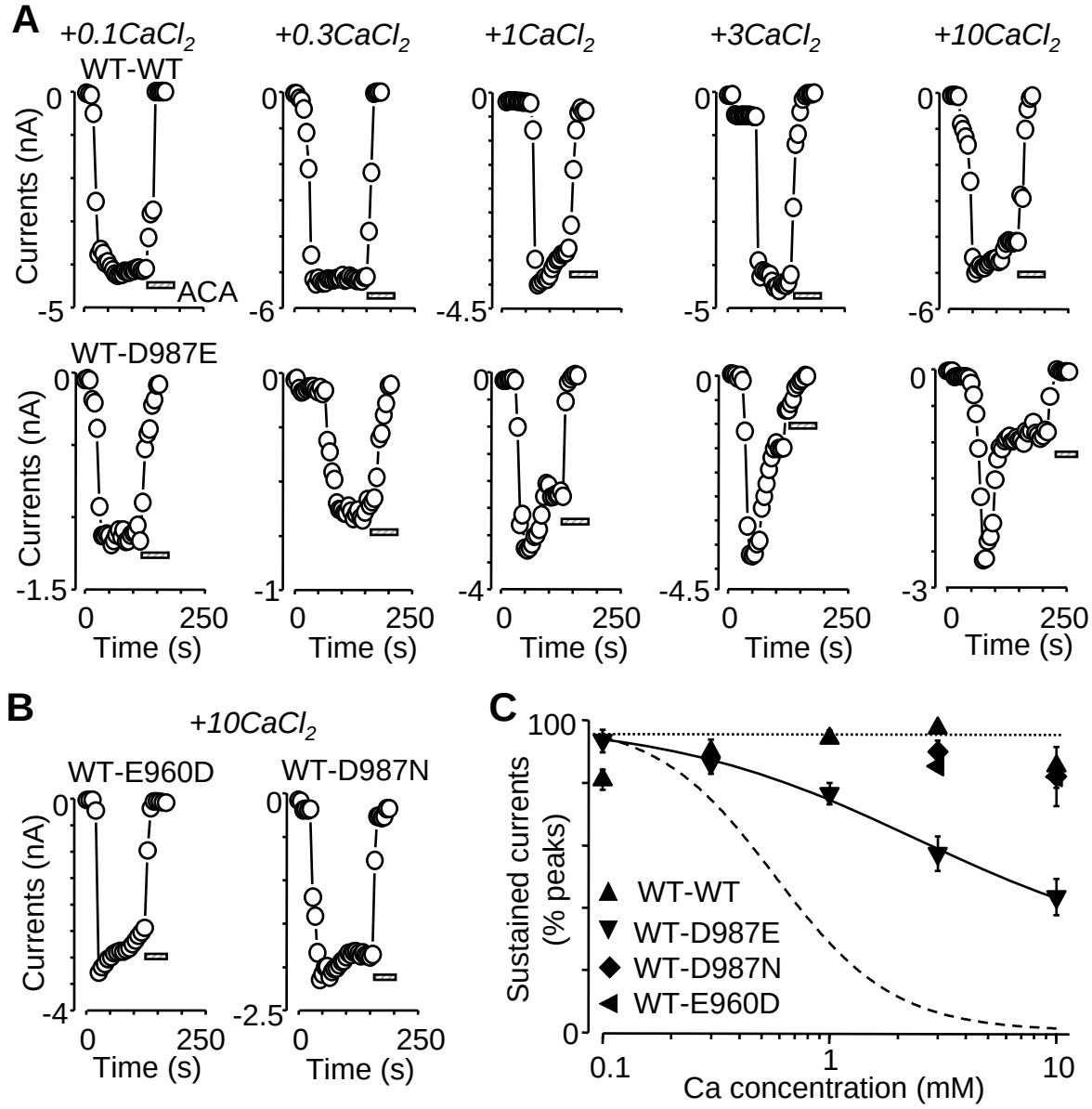


Fig.5


A
+0.1CaCl2
+0.3CaCl2
+1CaCl2
+3CaCl2
+10CaCl2
WT-WT
WT-D987E
Currents (nA)
ACA
Time (s)
B
+10CaCl2
WT-E960D
WT-D987N
C
Sustained currents (% peaks)
WT-WT
WT-D987E
WT-D987N
WT-E960D
Ca concentration (mM)