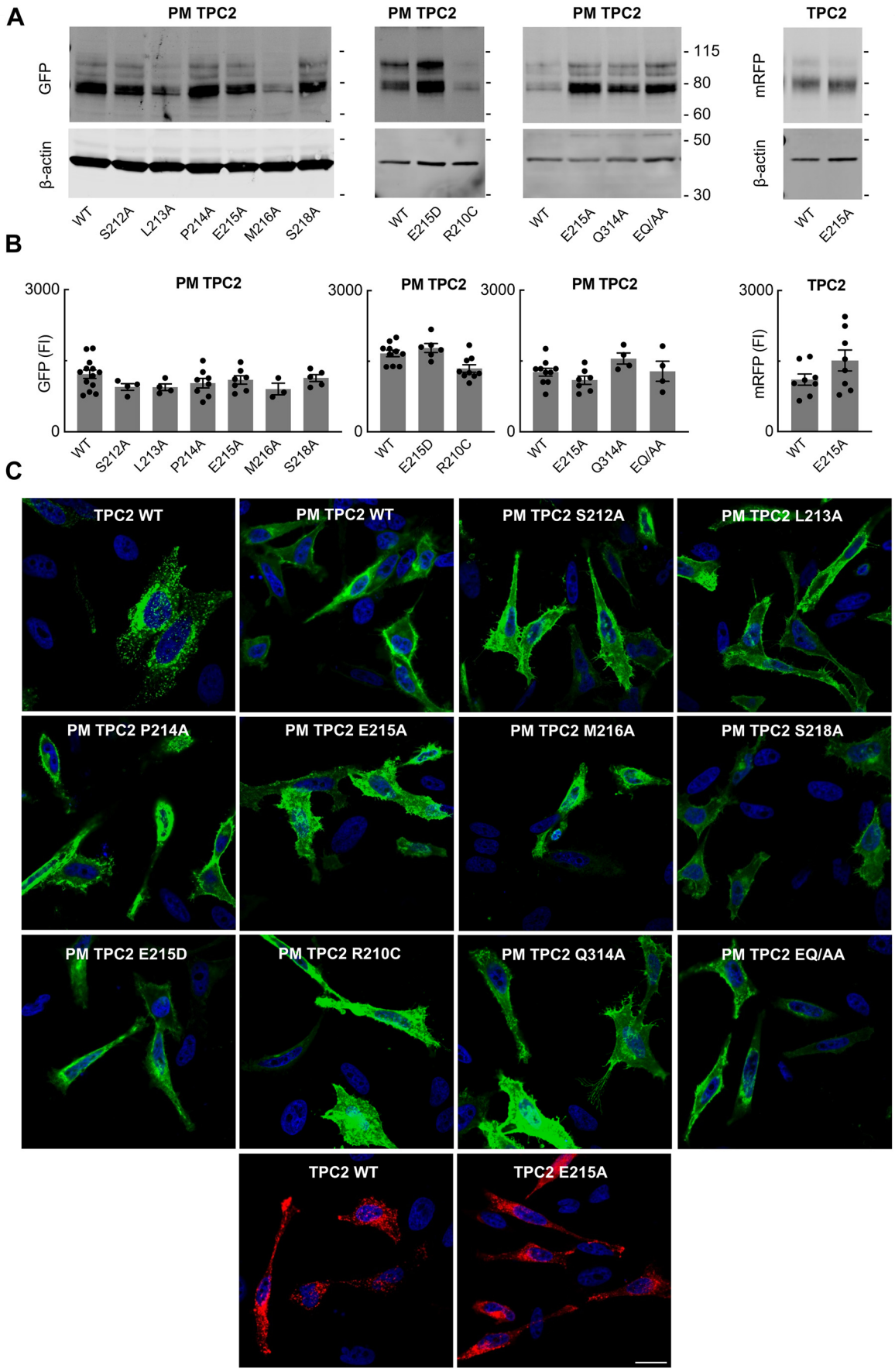
Expanded View Figures

Figure EV1. Expression analysis of recombinant TPC2.

(**A–C**) HeLa cells were transiently transfected with GFP-tagged TPC2$^{L11A/L12A}$ (PM TPC2) or mRFP-tagged TPC2 (TPC2) with and without (WT) the additional indicated mutation. Western blot analyses of cell lysates expressing TPC2 using an antibody to GFP, mRFP or β-actin (**A**). Data are representative of three experiments. Fluorescence intensity (FI) analysis of individual cells expressing TPC2 (**B**). Pooled data (mean ± s.e.m.) quantifying GFP or mRFP fluorescence from 3 to 23 independent experiments where each point represents the mean intensity from an individual population. Confocal analyses of PM (green) and lysosomal (red)-targeted TPC2 constructs (**C**). Nuclei were stained with DAPI (blue). Scale bar 20 μm. Data are representative of three experiments.


A
PM TPC2
PM TPC2
PM TPC2
TPC2
GFP
β-actin
mRFP
β-actin
115
80
60
50
30
WT
S212A
L213A
P214A
E215A
M216A
S218A
WT
E215D
R210C
WT
E215A
Q314A
EQ/AA
WT
E215A
B
PM TPC2
PM TPC2
PM TPC2
TPC2
GFP (FI)
mRFP (FI)
3000
0
WT
S212A
L213A
P214A
E215A
M216A
S218A
WT
E215D
R210C
WT
E215A
Q314A
EQ/AA
WT
E215A
C
TPC2 WT
PM TPC2 WT
PM TPC2 S212A
PM TPC2 L213A
PM TPC2 P214A
PM TPC2 E215A
PM TPC2 M216A
PM TPC2 S218A
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PM TPC2 R210C
PM TPC2 Q314A
PM TPC2 EQ/AA
TPC2 WT
TPC2 E215A

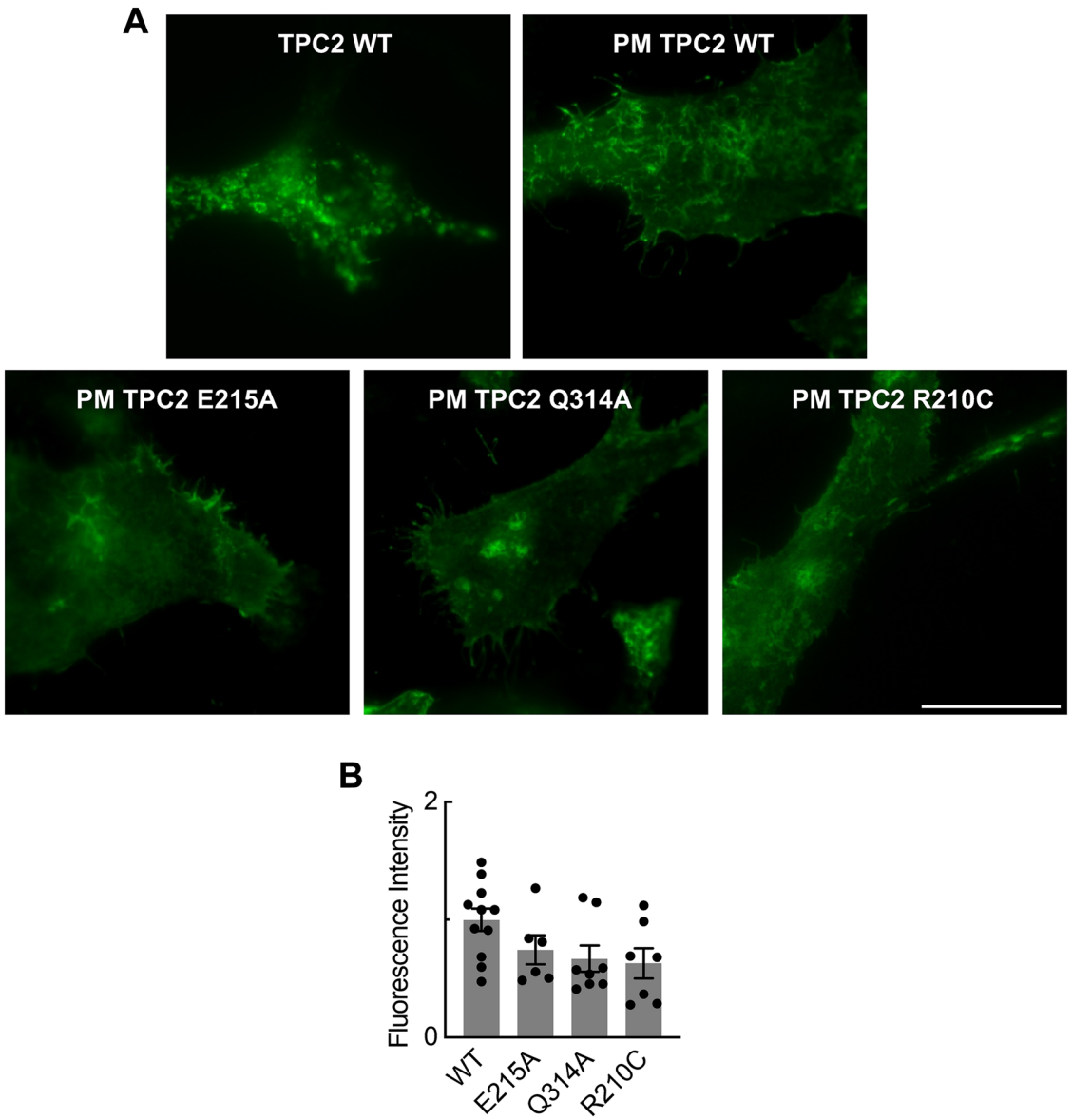

Figure EV2. Expression analysis of plasma membrane-targeted TPC2.

(**A**) Exemplar TIRF images of the indicated GFP-tagged TPC2 constructs expressed in HeLa cells. Scale bar 20 μm. (**B**) Pooled data (mean ± s.e.m.) quantifying fluorescence from two independent transfections where each point represents the intensity from an individual cell expressing the indicated plasma membrane-targeted mutant. Data are normalised to wild-type PM TPC2.

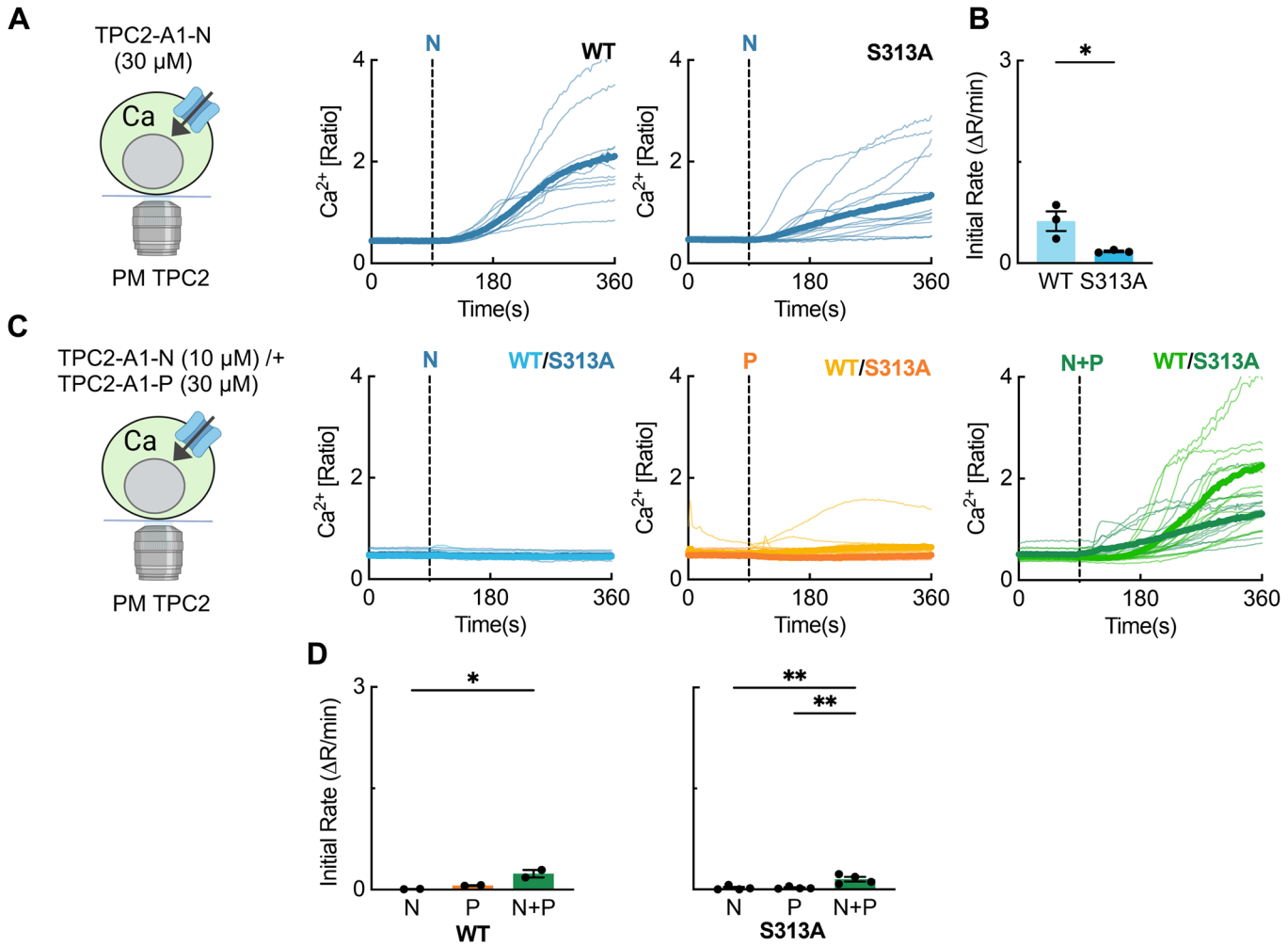

Figure EV3. Effect of the S213A TPC2 mutation on Ca^{2+} flux.

(**A**) HeLa cells expressing the indicated TPC2 construct were loaded with the ratiometric Ca^{2+} indicator, Fura-2 and stimulated with 30 μM TPC2-A1-N in the presence of 2 mM extracellular Ca^{2+}. Each trace is the fluorescence ratio imaged from a single GFP-positive cell from a typical field of view. The thicker trace is the average of the population. (**B**) Pooled data (mean ± s.e.m.) quantifying the initial rate of change of the Fura-2 ratio from three independent experiments where each point represents the mean response from an individual population. *$P = 0.04$ (unpaired *t* test). (**C**) HeLa cells expressing the indicated TPC2 construct were loaded with the ratiometric Ca^{2+} indicator, Fura-2 and stimulated with 10 μM TPC2-A1-N, 30 μM TPC2-A1-P or a combination of the two in the presence of 2 mM extracellular Ca^{2+}. Each trace is the fluorescence ratio imaged from a single GFP-positive cell from a typical field of view. The thicker trace is the average of the population. (**D**) Pooled data (mean ± s.e.m.) quantifying the initial rate of change of the Fura-2 ratio from four independent experiments where each point represents the mean response from an individual population. *$P = 0.03$, **$P = 0.006$ (one-way ANOVA followed by Dunnett's post hoc test).

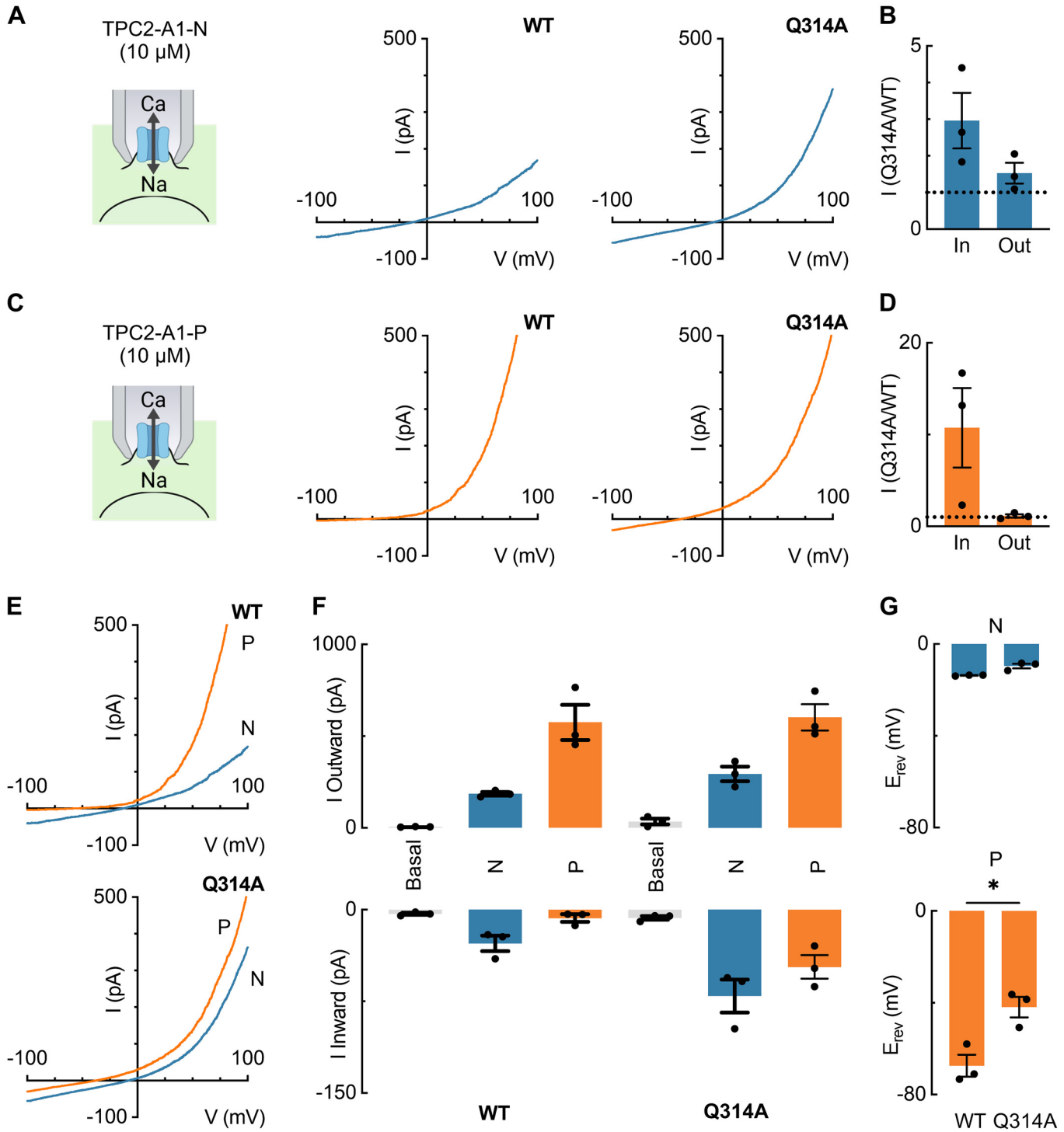

Figure EV4. Effect of the Q314A TPC2 mutation on Na^+ and Ca^{2+} currents.

(**A–D**) Macropatches from HEK cells expressing the indicated TPC2 construct were stimulated with 10 μM TPC2-A1-N (**A**) or 10 μM TPC2-A1-P (**C**) under bi-ionic condition using a pipette (luminal) solution containing 105 mM $CaCl_2$, 5 mM HEPES and 5 mM MES (pH 4.6) and a bath (cytosolic) solution containing 160 mM NaCl and 5 mM HEPES (pH 7.2). Exemplar recordings are shown. Pooled data (mean ± s.e.m.) quantifying the fold-increase in the inward Ca^{2+} currents at −100 mV or the outward Na^+ currents at +100 mV in cells expressing the mutant channel versus the wild-type in response to TPC2-A1-N (**B**) or TPC2-A1-P (**D**). Data are from three independent experiments where each point represents the response of an individual patch. (**E–G**) Comparison of the effects of TPC2 activators on currents from HEK cells expressing wild-type or the Q314A mutant at the plasma membrane. Exemplar recordings for TPC2-A1-N and TPC2-A1-P overlaid in e are the same as those shown in (**A**, **C**), respectively. Pooled data (mean ± s.e.m.) quantifying the amplitudes of the inward Ca^{2+} currents at −100 mV and the outward Na^+ currents at +100 mV (**F**) and the reversal potentials (**G**) in cells expressing the mutant channel versus the wild-type in response to the indicated agonist. Data are from three independent experiments where each point represents the response of an individual patch. *$P = 0.02$ (unpaired t test).

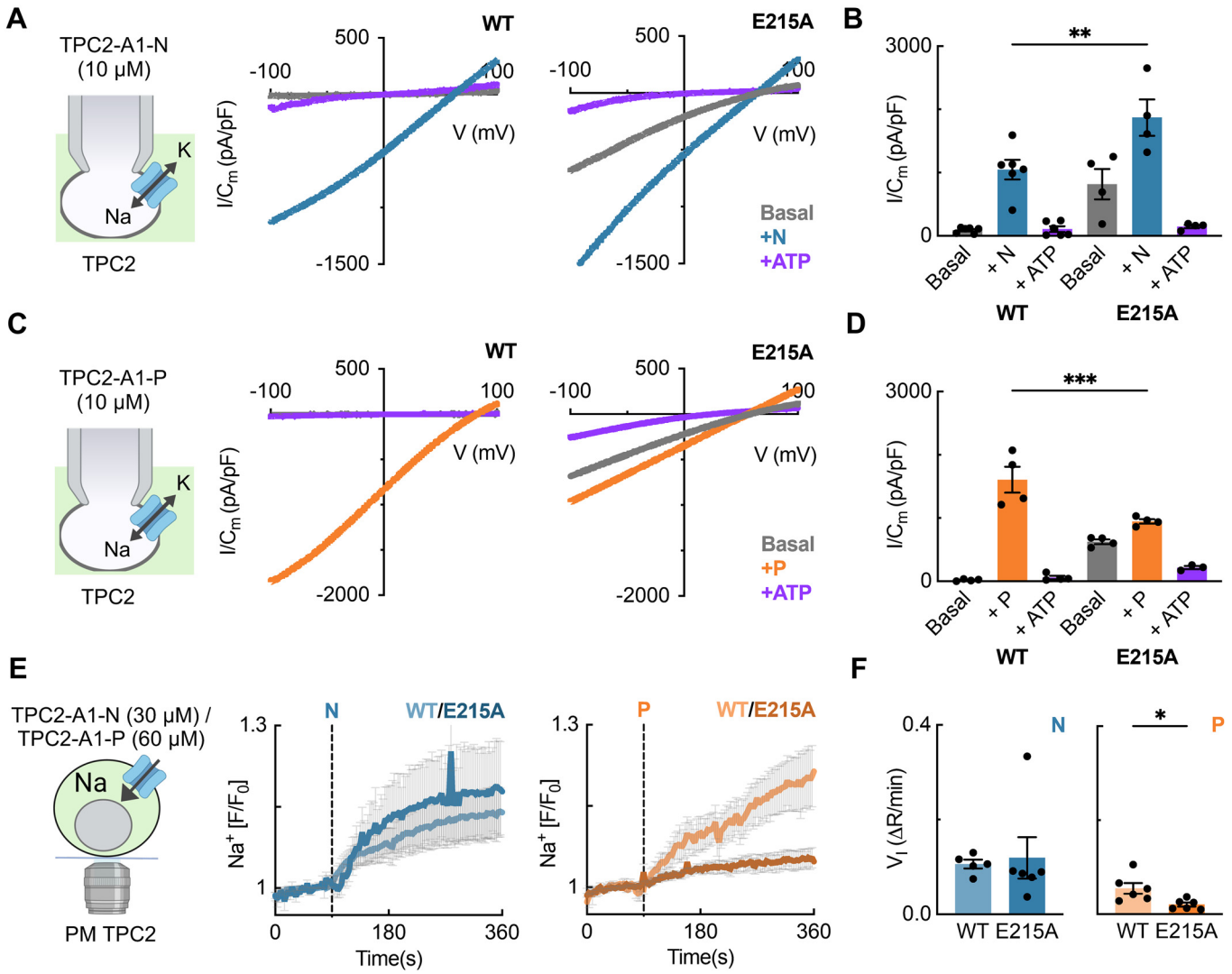

Figure EV5. Effect of the E215A TPC2 mutation on Na^+ currents and Na^+ flux.

(**A–D**) Vacuolar currents to TPC2 agonists recorded from HEK cells expressing wild-type or the E215A mutant in the lysosome using a Na^+-rich luminal solution. Enlarged vacuoles were stimulated with TPC2-A1-N (**A**) or 10 μM TPC2-A1-P (**C**) followed by ATP (1 mM) using a pipette (luminal) solution containing 140 mM Na-MSA, 5 mM K-MSA, 2 mM Ca-MSA, 1 mM $CaCl_2$, 10 mM HEPES and 10 mM MES (pH 4.6), and a bath (cytosolic) solution containing 140 mM K-MSA, 5 mM KOH, 4 NaCl mM, 0.39 mM $CaCl_2$, 1 mM EGTA and 10 mM HEPES (pH 7.2). Exemplar recordings are shown. Pooled data (mean ± s.e.m.) quantifying the inward currents at −100 mV in response to TPC2-A1-N (b) or TPC2-A1-P (**D**). Data are from four to six independent experiments where each point represents the response of an individual patch. $^{**}P = 0.007$, $^{***}P = 0.001$ (one-way ANOVA followed by Dunnett's post hoc test). (**E**, **F**) HeLa cells expressing the indicated TPC2 construct were loaded with the Na^+ indicator, ING-2 and stimulated with 30 μM TPC2-A1-N or 60 μM TPC2-A1-P in the presence of 2 mM extracellular Ca^{2+}. Traces in e are pooled time course data (mean ± s.e.m.) where each point combines averages from five to six independent cell populations. Pooled data (mean ± s.e.m.) quantifying the initial rate of change of the ING-2 ratio from five to six independent experiments where each point represents the mean response from an individual population is shown in (**E**). $^{*}P = 0.02$ (unpaired *t* test).

