## **Supporting Information**

## Kim et al. 10.1073/pnas.1705802114



Fig. S1. PIP2 depletion produces a voltage-independent increase in Q3\*VCF fluorescence. (A) Exemplar current and fluorescence traces from oocytes expressing Q3\*VCF + CiVSP displaying variable kinetics of PIP2 rundown at +100 mV. (B) Time to half-maximal CiVSP-induced current rundown plotted against time to half-maximal increase in secondary fluorescence component  $\Delta F_2$  for several oocytes (n = 8).



**Fig. 52.** Functional characterization of Q3\*VCF charge neutralization mutants. (*A*) Summary of current expression levels 1 or 2 d after injection for all mutants normalized to Q3\*VCF (n = 4-10). (*B*) Activation V<sub>1/2</sub> values for all constructs in control or 100- $\mu$ M RTG conditions (n = 4). Constructs with  $\Delta V_{1/2}$  that were significantly different from Q3\*VCF ( $-60.3 \pm 3.4 \text{ mV}$ ) were S6-KRAA ( $-41.4 \pm 6.7 \text{ mV}$ ), R364A ( $-25.4 \pm 0.9 \text{ mV}$ ), S6-AARK ( $-20.3 \pm 2.7 \text{ mV}$ ), and K248A ( $-44 \pm 3 \text{ mV}$ ), (ANOVA and Dunnett's post hoc test, P < 0.05, n = 4-10 per construct).  $\Delta V_{1/2}$  for the S6-AAAA mutant was not measurable because of the lack of currents in control conditions. Other mutants were not statistically different from Q3\*VCF, with  $\Delta V_{1/2}$  values of  $-56 \pm 6 \text{ mV}$  (R183A),  $-61 \pm 3 \text{ mV}$  (K185A),  $-59 \pm 3 \text{ mV}$  (R188A),  $-57 \pm 2 \text{ mV}$  (R190A),  $-58 \pm 3 \text{ mV}$  (K192A),  $-70 \pm 2 \text{ mV}$  (R195A), and  $-61 \pm 3 \text{ mV}$  (K196A).



**Fig. S3.** Retigabine confers protection against CiVSP-mediated rundown of KCNQ3 currents. (*A*) From a holding potential of -100 mV, oocytes were stepped to 0 mV for 1.5 s to activate channels, followed by a step to +50 for 1.5 s to activate CiVSP leading to current rundown. Exemplar currents for various mutants illustrate the protection of a significant fraction of current in the presence of retigabine (red sweeps) in Q3\*VCF and other retigabine-responsive channels. Several C-terminal mutations abolish the protection of current by retigabine. (*B*) Time constants of current rundown were estimated by single exponential fits to the decaying phase of current in control (black) and 100- $\mu$ M retigabine conditions. Overall, retigabine has little consistent effect on the kinetics of decay, but leads to protection of a large fraction of current in certain mutants.