

Figure S1. Voltage-dependence of enzymatic activity of VSD mutants as reported by Kir currents

A, representative traces of Kir current from oocytes expressing GIRK2 (Kir3.2) channel and G-protein β and γ subunits with Ci-VSP D151N (red) or D164N/D186N (blue), respectively. Inset shows a pulse protocol. GIRK2 current was recorded by a short hyperpolarizing step to -100 mV with intervals to varied voltages from -90 mV (D164N/D186N) or -80 mV (wild-type and D151N) by 10 mV increment. For clarity, only traces with four voltages during intervals are shown for D151N and D164N/D186N, respectively. Interval duration was 60 seconds.

B, the maximum gating charges (Q-off) integrated from OFF-gating currents. These were obtained from oocytes that were microinjected with distinct doses of cRNAs. Data were obtained from the oocytes of the same batch of preparation.

C, comparison of voltage-dependency of Kir-reporting phosphatase activity among wild-type (Ci-VSP), D151N and D164N/D186N with different doses of injection. Data sets were obtained from the sister oocytes of the same microinjection as the cells used for recording of data shown in **B**. Current amplitudes of GIRK2 currents were normalized as previously reported and plotted against the interval voltages. The curve was fitted with the Boltzmann equation: Enzyme Activity = $a+b/[1+\exp\{(V-V_{1/2})/k\}]$. $V_{1/2}$ (mV) = -29.3 (Ci-VSP), -3.79 (D151N 1x), -23.5 (D151N 3x), -28.4 (D151N 10x), -43.6 (D164N/D186N 1/3), -47.9 (D164N/D186N 1/10). k = 9.77 (wild -type), 8.62 (D151N 1x), 7.69 (D151N 3x), 8.46 (D151N 10x), 10.7 (D164N/D186N 1/3), 8.62 (D164N/D186N 1/10).

D, $V_{1/2}$ s and maximum gating charges were X-Y plotted for each batch of oocytes that were injected with distinct doses of cRNAs for each Ci-VSP construct. Note that $V_{1/2}$ s

of D151N are slightly shifted in a negative direction as the magnitude of gating charges increases. $V_{1/2}$ of D151N is significantly less negative than D164/D186N within a fixed range of gating charges (indicated as box).

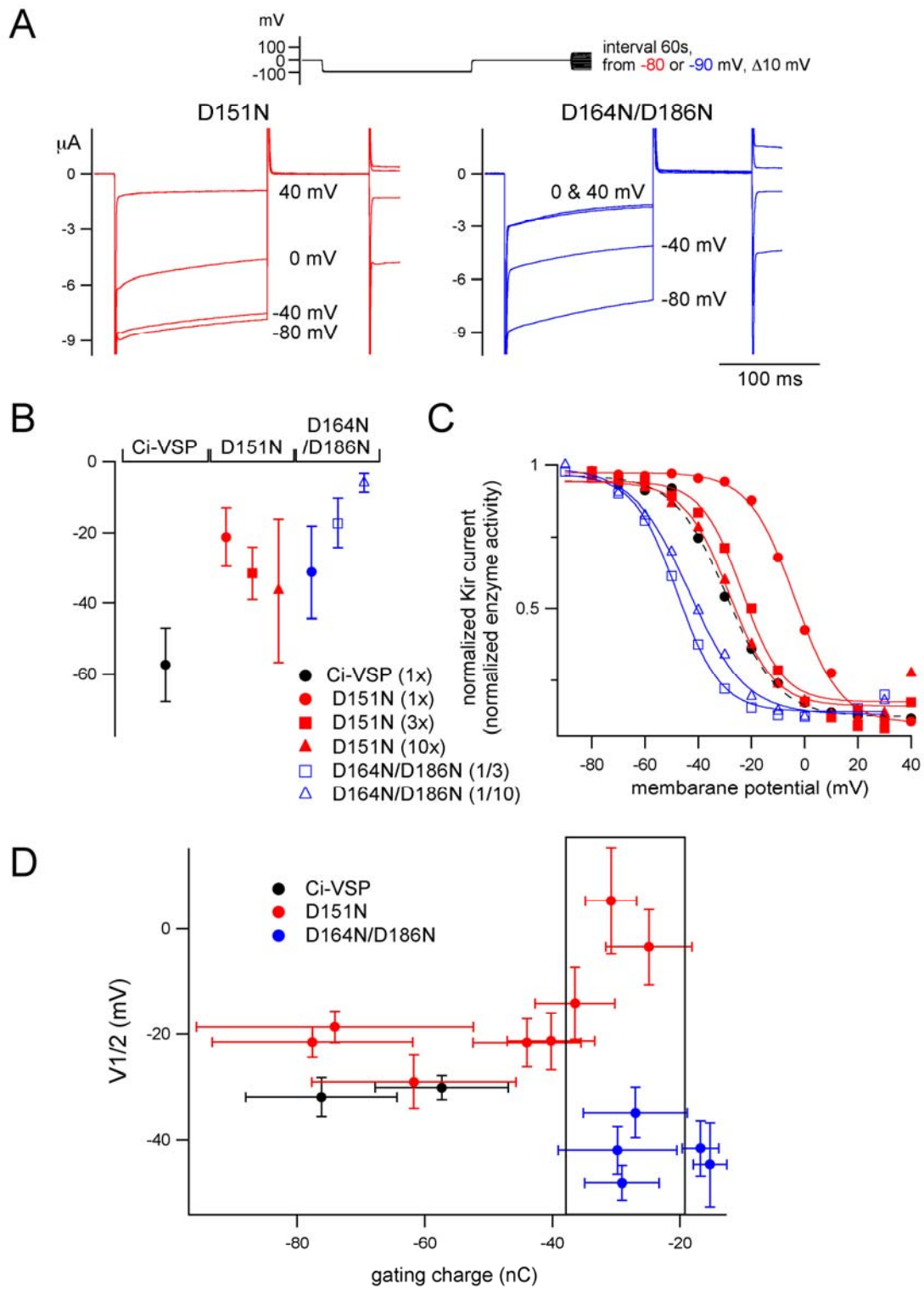


Fig.S1 Murata and Okamura

Fig. 2S Murata and Okamura

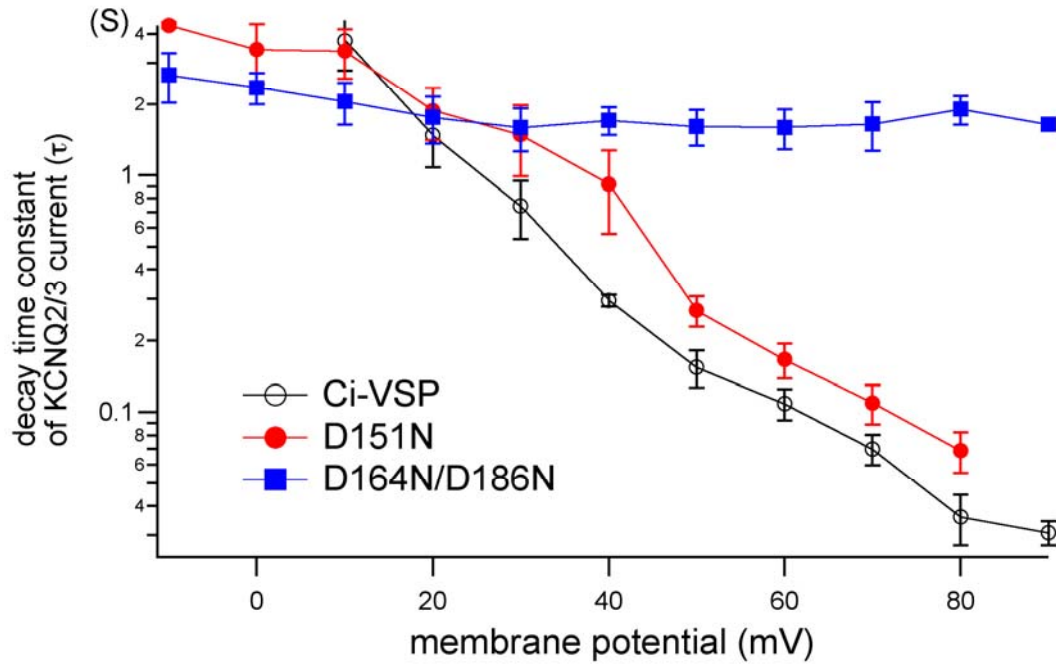


Figure S2. Decay time constants of KCNQ2/3 current coexpressed with wild-type and the VSD mutants

Rate constant for fitting decay of KCNQ2/3 currents in single exponentials was plotted in a semilogarithmic scale. The numbers of cells were 3, 3, 4 for Ci-VSP, D151N, and D164N/D186N, respectively.