Supporting Information

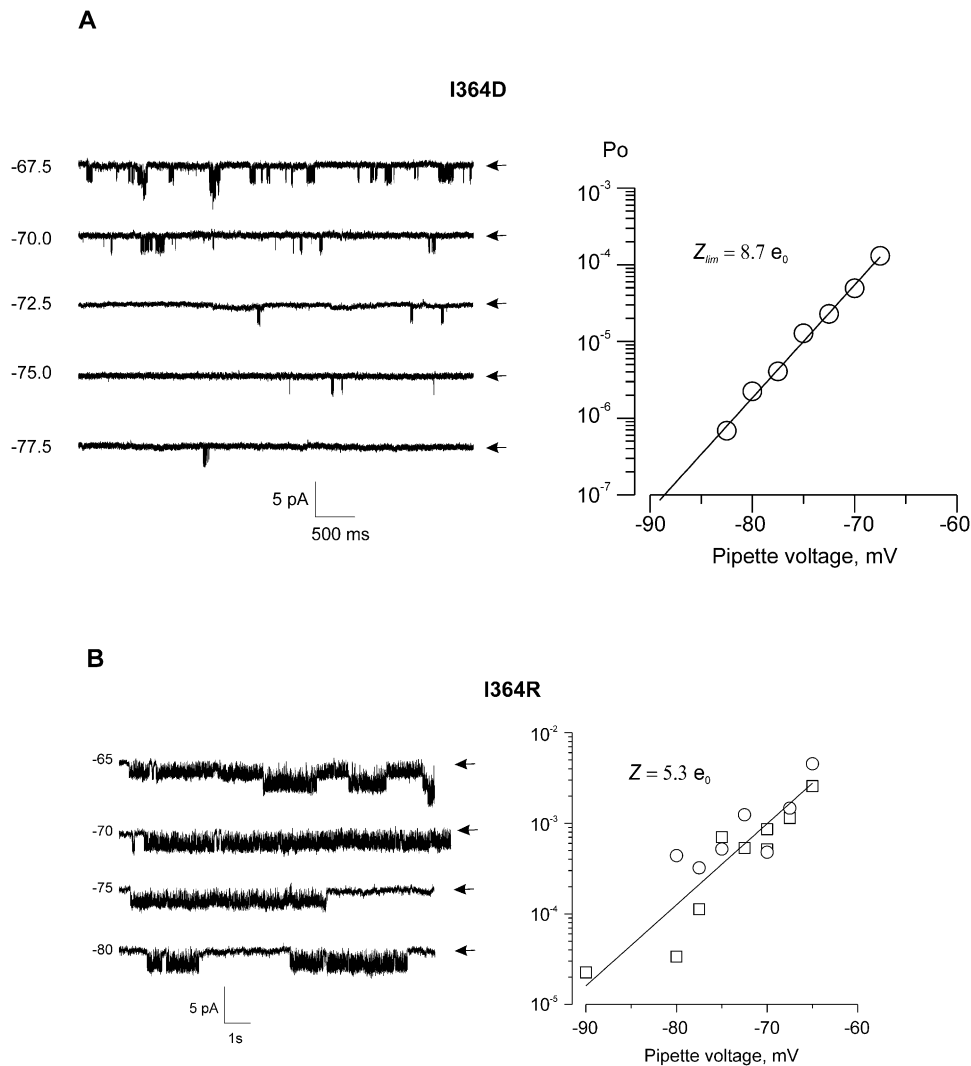## González-Pérez et al. 10.1073/pnas.1000963107

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Fig. S1. Limiting slope analysis of mutants I364D and I364R. (*A*) Five-second fragments representative of a 5-min recording (*Left*) and analysis of the I364D variant (*Right*). Voltages stepped every 2.5 mV are shown on the left of each trace. Records were acquired as described in the main text (*Materials and Methods*). Relative open probability (NPo) was estimated from the fractional time the current trace showed 0, 1, 2… single-channel open levels. *Right:* Absolute Po vs. V plot (Po calculated from NPo/N); N was estimated from the nonstationary fluctuation analysis as in Fig. 2 in the main text. Data points were fitted to the function Po(V) = exp(Z_{lim} V/kT), where Z_{lim} is the effective valence of opening. Average of data from three patches gave z = 9.0 ± 0.5 (mean ± SD). (*B*) *Left:* Segments extracted from 10-min traces recorded from membrane macropatches having hundreds of I364R channels. NPo and N were estimated as in Fig. 2 of the main text. Traces are not representatives for Po; instead, they were chosen to illustrate extreme long burst duration of this variant. *Right:* Absolute Po vs. V plot (Po calculated from NPo/N) containing data superimposed for three different patches. Each data point corresponds to a 10-min Po measurement. The best fit to the pooled data gave z = 5.3 ± 1.0.

Fig. S2. Gating currents measurements. (*A*) Cut-open oocyte voltage-clamp gating currents were recorded in 10-mV steps from a holding potential of −140 mV for V363D and WT and from −100 mV for V363R. Traces acquired at 20 kHz and Bessel filtered to 2 kHz (−3 dB). (*B*) Charge–voltage (Q-V) and conductance–voltage (G-V) curves. Measurements for V363R were very sensitive to the pulse subtraction protocol, suggesting the presence of omega currents in this mutant Tombola F, Pathak MM, Isacoff EY (2005) Voltage-sensing arginines in a potassium channel permeate and occlude cation-selective pores. *Neuron* 45:379–388. Results are means ± SE. Data points were fitted to a Boltzmann distribution function with fitting values as follows. V363R (Q-V): $V_{1/2}$ = −35.8 mV, z = 2.18 e_0; V363R (G-V): $V_{1/2}$ = 8.7 mV, z = 1.38 e_0. V363D (Q-V): $V_{1/2}$ = −62.2 mV, z = 1.93 e_0; V363D (G-V): $V_{1/2}$ = −37.5 mV, z = 2.48 e_0. *wt* (Q-V): $V_{1/2}$ = −39.6 mV, z = 2.65 e_0; WT (G-V): $V_{1/2}$ = −15.5 mV, z = 3.7 e_0.

Fig. S3. Charge addition at 363 may not disrupt R1-I241 structural proximity in the closed state and that of R1-A419 in the open state. (*A*) Scheme of the physical displacement of R1 during activation: going from the vicinity of I241, in S1, at rest (1) to nearby A419, in S5, at the activated state (2). (*B*) Shaker-R1C/ I241C and (*C*) Shaker-R1C/A419C with and without charged side chains at 363 were expressed in oocytes and recorded with the two-electrode voltage-clamp technique. Activity was evoked by depolarizing pulses incremented by 10 mV from a holding potential of −90 mV. In *B* and *C*, currents were measured before (*Left*), after 30 min in the presence of fresh DTT 5 mM added to recording solution (*Middle*), and after 30-min exposure to 100 μM CdCl₂ (*Right*).

1. Campos FV, Chanda B, Roux B, Bezanilla F (2007) Two atomic constraints unambiguously position the S4 segment relative to S1 and S2 segments in the closed state of Shaker K channel. *Proc Natl Acad Sci USA* 104:7904–7909.
2. Lainé M, et al. (2003) Atomic proximity between S4 segment and pore domain in Shaker potassium channels. *Neuron* 39:467–481.