

Mutations in the S6 Gate Isolate a Late Step in the Activation Pathway and Reduce 4-AP Sensitivity in *Shaker* K_v Channel

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Supporting Material

Figure S1: Voltage-dependence of activating gating charge movement (QV curves) of P475A. **A**, Scaled up view of the I_{Qdeac} recordings at -90 mV from the activation protocol shown in Figure 2B. The green, blue and red colored recordings were obtained upon depolarization to 0, +50 and +130 mV, respectively. Note the reduction in I_{Qdeac} amplitude upon stronger depolarizations, which indicates a slowing in the decay times of I_{Qdeac} due to BC gate opening. **B**, Red symbols and line (which represent average fit by a sum of two Boltzmann distributions) represent the QV curve of P475A obtained from integrating the I_{Qdeac} recordings of the activation protocol as shown in panel A. Integrating these I_{Qdeac} currents yielded a split QV curve, similar to the QV curve obtained from integrating the I_{Qac} recordings (blue symbols and line), with two components. The first carried $87 \pm 3\%$ of the charges and displayed a $V_{1/2}$ of -40.5 ± 1.2 mV with a slope factor of 13.4 ± 0.9 mV ($n = 6$). The second component carried the remaining 13% of charges and had a $V_{1/2}$ of 32.5 ± 2.9 mV with a slope factor of 7.1 ± 2.8 mV. For comparison, the GV curve is represented in dotted lines. The reduction in charge at more depolarized potentials, noted in the QV curve obtained from the I_{Qdeac} recordings (red symbols), is due to the slowing in the decay time of I_{Qdeac} , which manifest itself by a reduction in I_{Qdeac} amplitude (panel A).

