Finol-Urdaneta et al., http://www.jgp.org/cgi/content/full/jgp.201311037/DC1

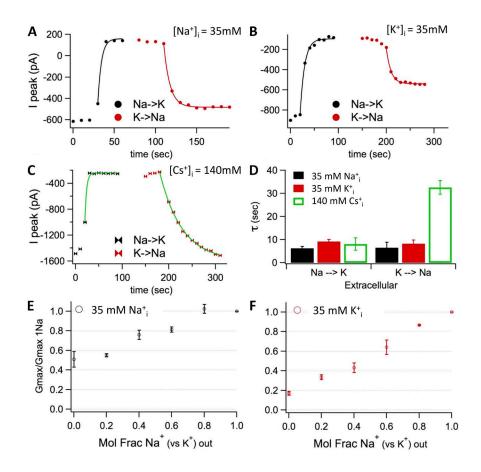


Figure S1. Wash-in and washout, as observed when a 142.5-mM Na $^+$  extracellular solution is fully replaced by 142.5 mM K $^+$  and vice versa. Intracellular conditions are as follows: (A) 35 mM Na $^+$ 105 mM Cs; (B) 35 mM K $^+$ 105 mM Cs; and (C) 140 mM Cs. The slow wash-out observed with 140 mM of internal Cs $^+$  is reminiscent of observations with 140 mM of internal Na $^+$  (see Fig. 4, A and B, in the main text). (D) Summary of wash-in and washout time constants ( $\tau$ ) for the three different internal solutions, specified in parts A–C. (E and F) Near-linear mole-fraction dependence observed with 105 Cs, plus 35 Na or K in the internal solution. These results contrast with the striking anomalous mole-fraction dependence observed when intracellular solutions contained only Na or only K (see Fig. 4 D in the main text).

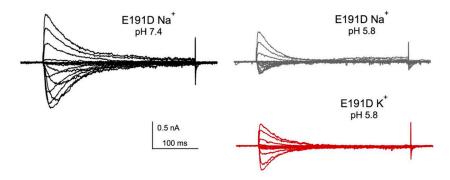
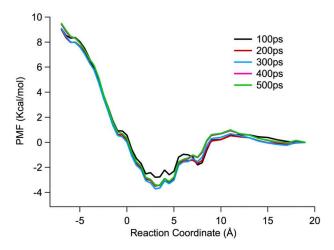


Figure S2. Effect of lowering extracellular pH on E191D currents. (Top left) Control voltage-clamp current records, taken at pH 7.4 with external Na $^+$ . (Right) Inward currents carried by Na $^+$  are preferentially reduced at pH 5.8. At pH 5.8, inward currents carried by K $^+$  are greater than for Na $^+$ , reflecting the higher PK/PNa measured at this pH (see Fig. 5 in the main text).



**Figure S3.** Convergence of the 1-D PMF profiles integrated from a set of 2-D PMF maps. The durations of simulation vary from 100–500 ps. Note that relatively little change occurs in the PMFs for simulations of 200 ps and longer.