Chatelain *et al.* supplemental material

Supplemental Figure 1 T141 mutants do not have changes in monovalent cation permeability. Comparison of I_x/I_K ratios at –100 mV for Kir2.1 (black) (n=6), T141S (open bars) (n=6), T141R (dark blue) (n=6), T141K (blue) (n=7), T141M (green) (n=9), T141E (red) (n=5), T141H (orange) (n=5), T141Q (brown) (n=5). No significant changes are seen (compare with Bichet *et al.*(Bichet et al., 2004)). Error bars indicate SD.

Supplemental Figure 2 I-V relations in 90 mM KCl solutions for **a,** T141K/D172Q & T141K/D172E, **b,** T141R/D172Q & T141R/D172E, and **c,** S165D/D172N.

Supplemental Figure 3 a, Calculated changes in the free energy of transfer of a K^+ ion to KcsA site 4 and the KcsA cavity ion positions. , **b,** Calculated changes in the free energy of transfer of Ba^{2+} (filled bars) or K^+ (open bars) from solution to site 4 in MthK when site 2 is occupied by K^+. Energies are shown for conditions (left to right) with the uncharged protein, charges on pore helix residues 97-109 only, charges on the last turn of the pore helix only (residues A58,57,56, and 55), charges on the whole protein, and the mutants A58K, A58K/I84D, A58K/F87D, and A109K/V91D in the background of the fully charged protein. It is important to note that the transmembrane crystallographic model of MthK is polyalanine. Thus, in addition to the question of whether MthK is an accurate model for an open Kir channel pore, there are other uncertainties due to challenges in the accurate threading of Kir channel residues onto the MthK scaffold.

Supplemental Figure 1

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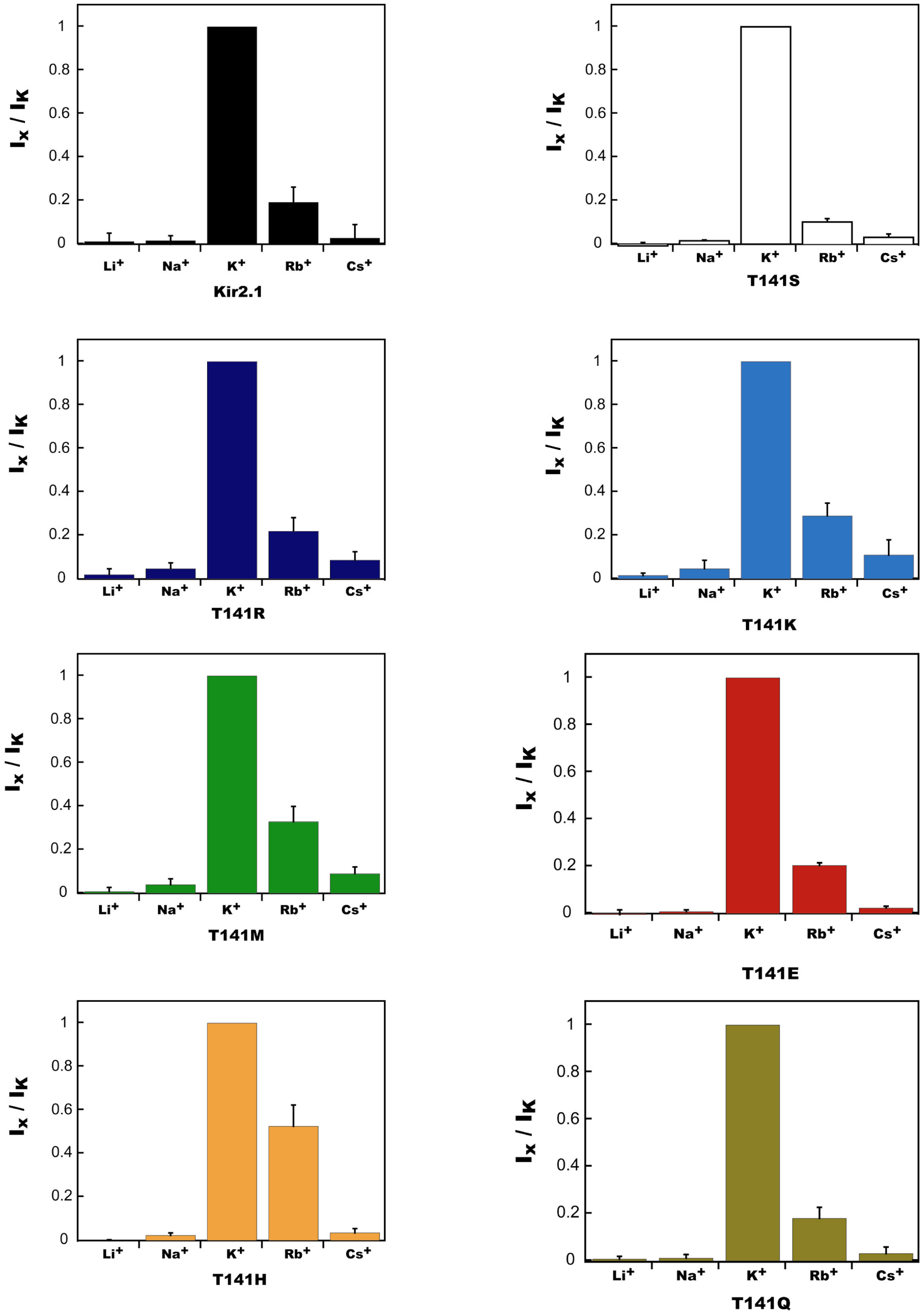

Supplemental Figure 2

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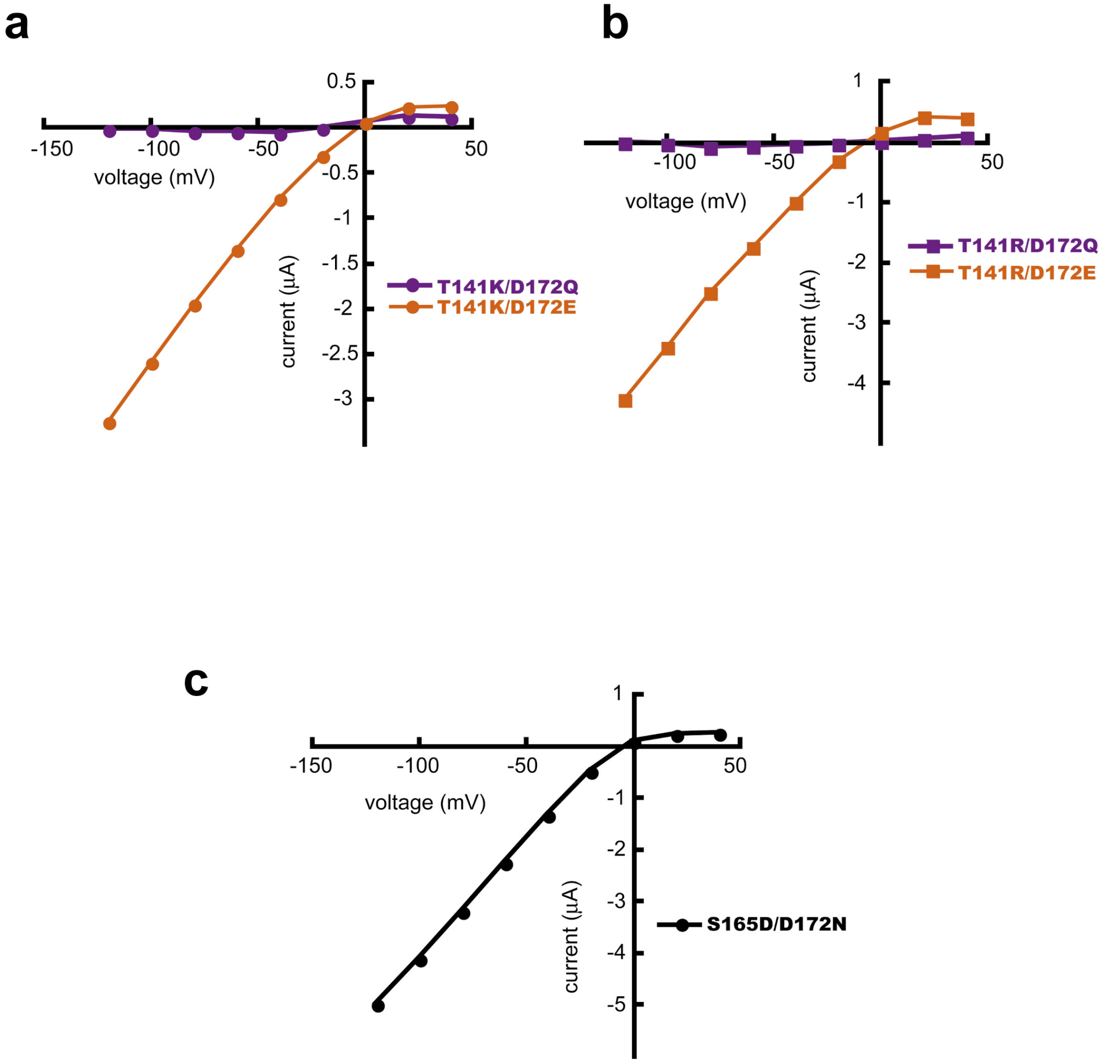

Supplemental Figure 3

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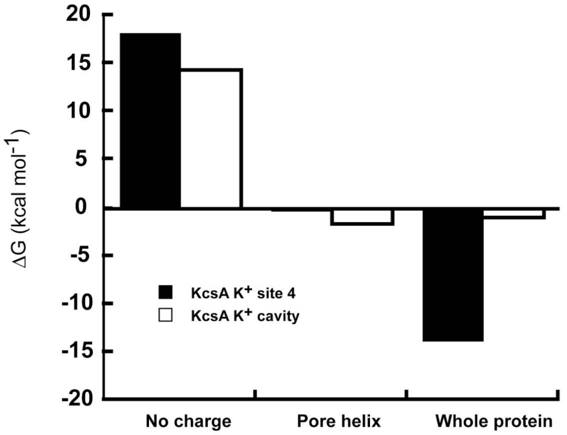
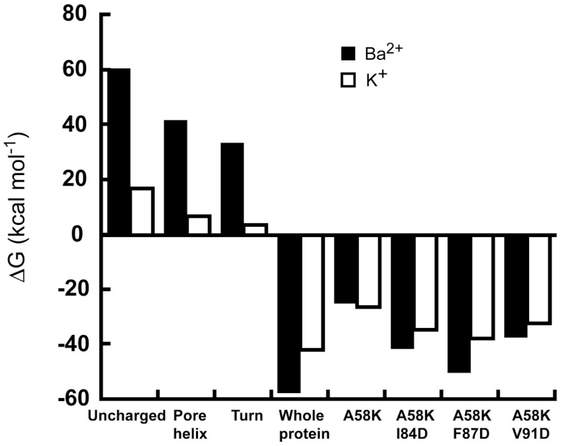
a



b



Supplemental Table 1

	A	B	C
KirBac	15.8	-25.3	4.9
A109K	18.2	11.1	6.7
A109K/M135D	18.4	-16.2	4.3
A109K/I138D	18.4	-14	4.1
A109K/T142D	18.4	-11.5	4.4