Supplemental Data

LEGENDS TO SUPPLEMENTAL FIGURES

Figure S1. Increased pH sensitivity of K^+ conductance in cells expressing heteromeric Kir4.1/Kir5.1 channels. Representative currents measured on cell, and on excised membrane patches exposed to pH 8.5, 7.5, 6.5 and 5.5, in cells transfected with WT Kir4.1 (***A***) or co-transfected with WT Kir4.1 and Kir5.1, at a 1:1 ratio (***B***). The pulse protocol is shown in the upper left. Pipette and external solution were K_{INT} buffer at pH 7.5. Dotted line indicates zero current.

Figure S2. Disruption of the inter-subunit E288–R297 salt bridge accounts for the effect of R297C. ***A–B***, Time-course of relative $^{86}Rb^+$ efflux in mock-transfected control cells and in cells expressing WT, E288C, R297C or the double mutant E288C-R297C, in homomeric Kir4.1 (***A***) and in heteromeric Kir4.1/Kir5.1 channels (***B***). ***C,*** Rate constants for Kir4.1-dependent (white bars) and Kir4.1/Kir5.1-dependent (gray bars) $^{86}Rb^+$ efflux k_2, as obtained by fitting flux data to equation (1). Results are means ± s.e.m. of 3–12 experiments, each in triplicate. Data for control, WT and R297C are the same as in Fig. 2 and Fig. 3.

Figure S3. The intra-subunit C108–C140 disulfide bond is essential for channel function, and cannot be replaced by a salt bridge. ***A–B***, Time-course of relative $^{86}Rb^+$ efflux in control cells and in cells expressing WT, C108E, C140R or the double mutant C108E-C140R, in homomeric Kir4.1 (***A***) and in heteromeric Kir4.1/Kir5.1 channels (***B***). ***C,*** Rate constants for Kir4.1-dependent (white bars) and Kir4.1/Kir5.1-dependent (gray bars) $^{86}Rb^+$ efflux k_2, as obtained by fitting flux data to equation (1). Results are means ± s.e.m. of 3–12 experiments, each in triplicate. Data for control, WT and C140R are the same as in Fig. 2 and Fig. 3.

Figure S1.

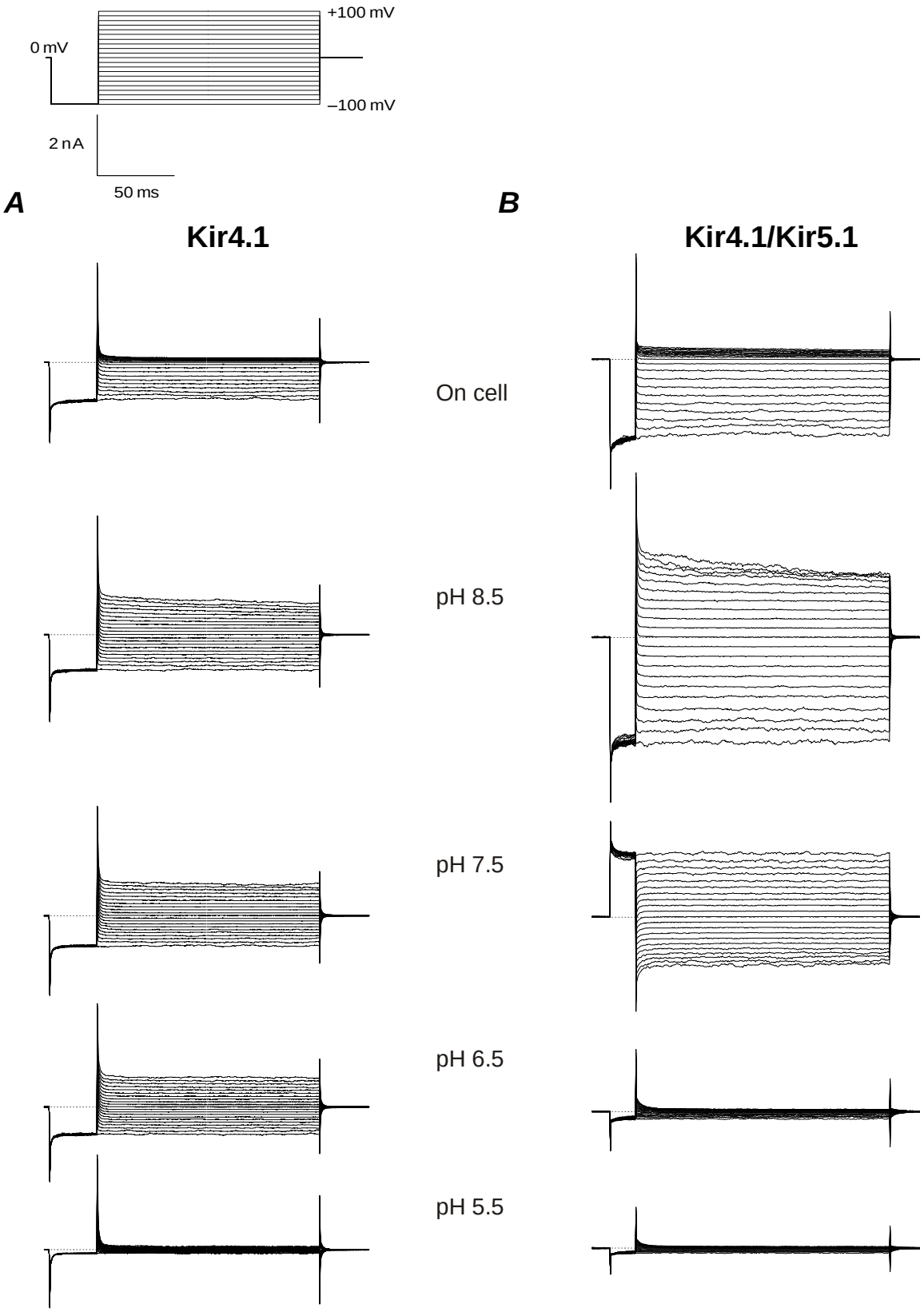

+100 mV
0 mV
–100 mV
2 nA
50 ms
A
Kir4.1
B
Kir4.1/Kir5.1
On cell
pH 8.5
pH 7.5
pH 6.5
pH 5.5

Figure S2.

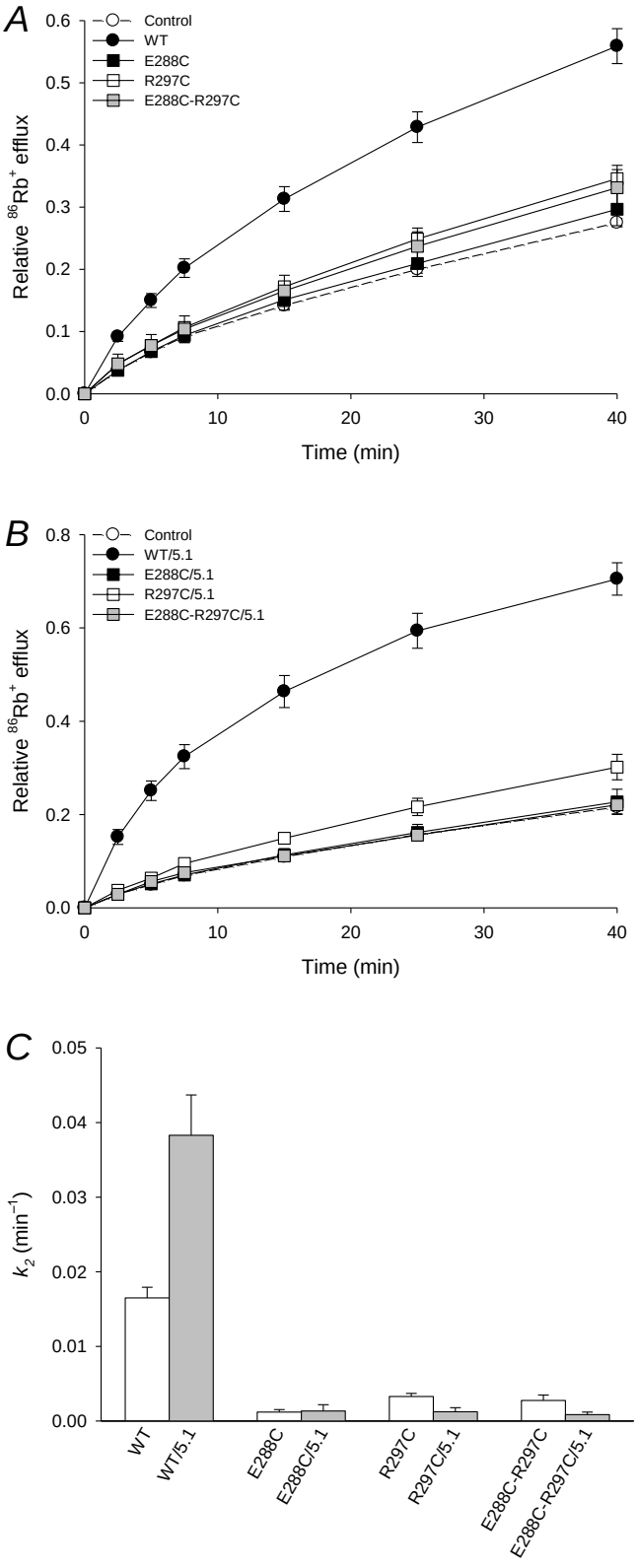
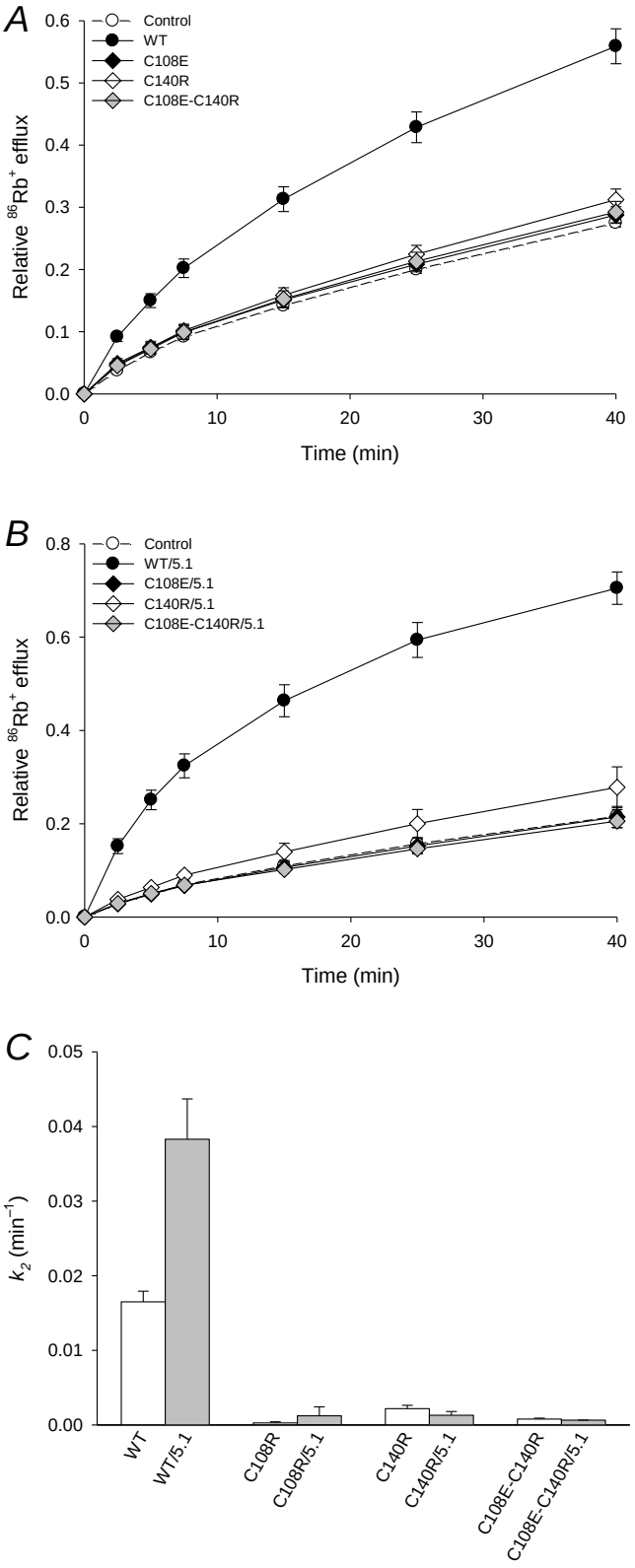

A
Control
WT
E288C
R297C
E288C-R297C
Relative $^{86}Rb^+$ efflux
Time (min)
B
Control
WT/5.1
E288C/5.1
R297C/5.1
E288C-R297C/5.1
Relative $^{86}Rb^+$ efflux
Time (min)
C
k_2 (min^{-1})
WT
WT/5.1
E288C
E288C/5.1
R297C
R297C/5.1
E288C-R297C
E288C-R297C/5.1

Figure S3.


A
Control
WT
C108E
C140R
C108E-C140R
Relative $^{86}Rb^+$ efflux
Time (min)
B
Control
WT/5.1
C108E/5.1
C140R/5.1
C108E-C140R/5.1
Relative $^{86}Rb^+$ efflux
Time (min)
C
k_2 (min^{-1})
WT
WT/5.1
C108R
C108R/5.1
C140R
C140R/5.1
C108E-C140R
C108E-C140R/5.1