Biophysical Journal, Volume 97

Supporting Material

An inter-subunit salt bridge near the selectivity filter stabilizes the active state of Kir1.1

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Suppl Fig S1. Pore Helix and selectivity filter alignment of Kir1.1b with some representative K channels. Number at the far right of each sequence refers to the numbering of the last residue shown for that channel. Other residues of particular importance are designated by superscripts. The solid horizontal bar shows the inter-intra subunit link: E118-R128-E132 in Kir1.1b.

Suppl Fig S2. Wild-type Kir1.1 inactivation can also be produced by K removal after acidification, as evidenced by failure to recover channel activity following realkalization to pHi=8. Subsequent return of 100mM K to the bath produced a rapid, 4.7 ± 0.6 fold increase in whole-cell conductance, consistent with a K-dependent increase in single channel conductance from 6.4pS to 34pS; followed by a slower recovery from inactivation (cyan section of curve).

Suppl Fig S3. E132N inactivated in 100mM K (green), but was rescued from inactivation by 500nM TPNQ (magenta), similar to E132Q. However, TPNQ could not rescue E132N from inactivation in 1mM K (brown). The double mutant E132Q-Q133E inactivated in 1mM K (blue) but not in 100mM external K (orange). TPNQ could not rescue E132Q-Q133E from inactivation in 1mM K (red). Oocyte pH was controlled with permeant acetate buffers. External K was maintained constant at either 1mM or 100mM, and whole-cell conductances were normalized to their initial values.

Suppl Fig S4. The R128Y mutant inactivated during acidification in 100mM external K (green), similar to the E132Q mutant. TPNQ (500nM) protected R128Y from inactivation in 100mM external K (magenta), but not from inactivation in 1mM external K (brown). Protection of wt-Kir1.1 from inactivation by 100mM external K is shown for comparison (cyan). Oocyte pH was controlled with permeant acetate buffers. External K was maintained constant at either 1mM or 100mM, and whole-cell conductances were normalized to their initial values.

Suppl Fig S5A. Putative H-bonding between K61-Kir1.1b on the outer transmembrane helix and the carbonyl oxygen of A158 on the inner transmembrane helix near the bundle crossing stabilizes the closed state (shown here) of Kir1.1b. Closed-state H-bonding occurs within each of 4 subunits.

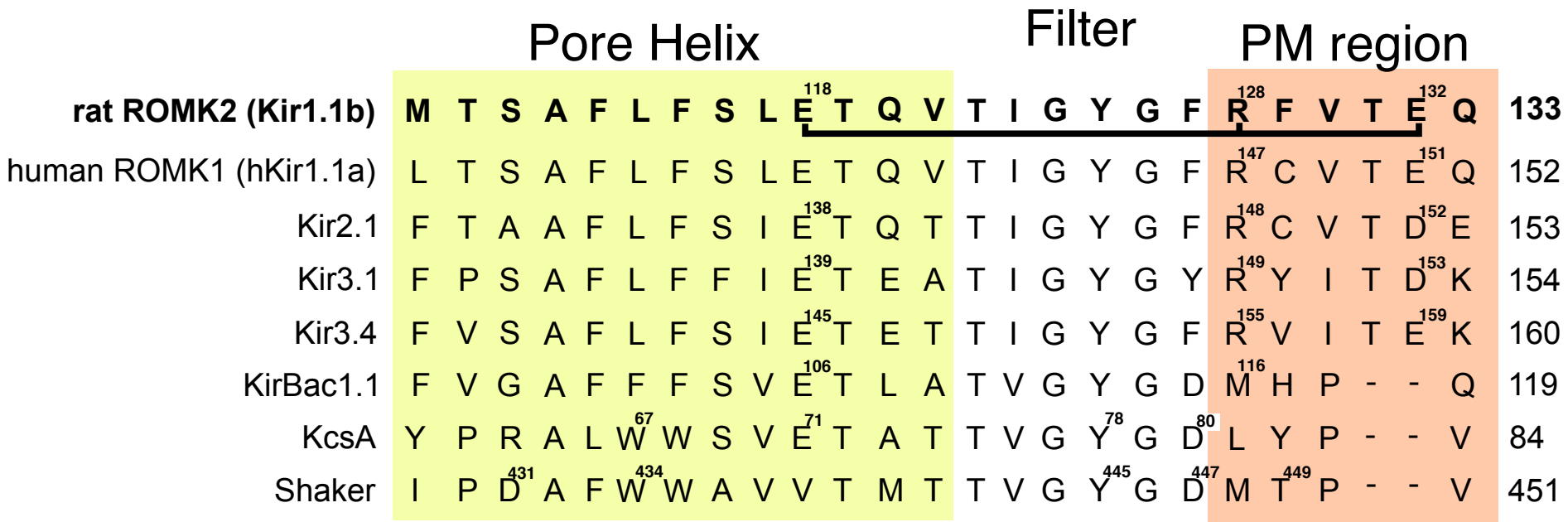
Suppl Fig S5B. Absence of H-bonding in K61M-Kir1.1b may prevent Kir1.1 inactivation in low external K.

Suppl Fig S6A. In the V121T mutant, there are polar contacts between the carbonyl oxygen of V121T and the *Thr* side-chain of T122 as well as a link between the V121T side-chain and the carbonyl oxygen of L117. The V121T mutation prevents inactivation in 1mM external K. The location of the inter-subunit salt bridge, R128-E132 is indicated. Two (of 4) adjacent subunits in the region of the selectivity filter are depicted as cyan and green ribbons.

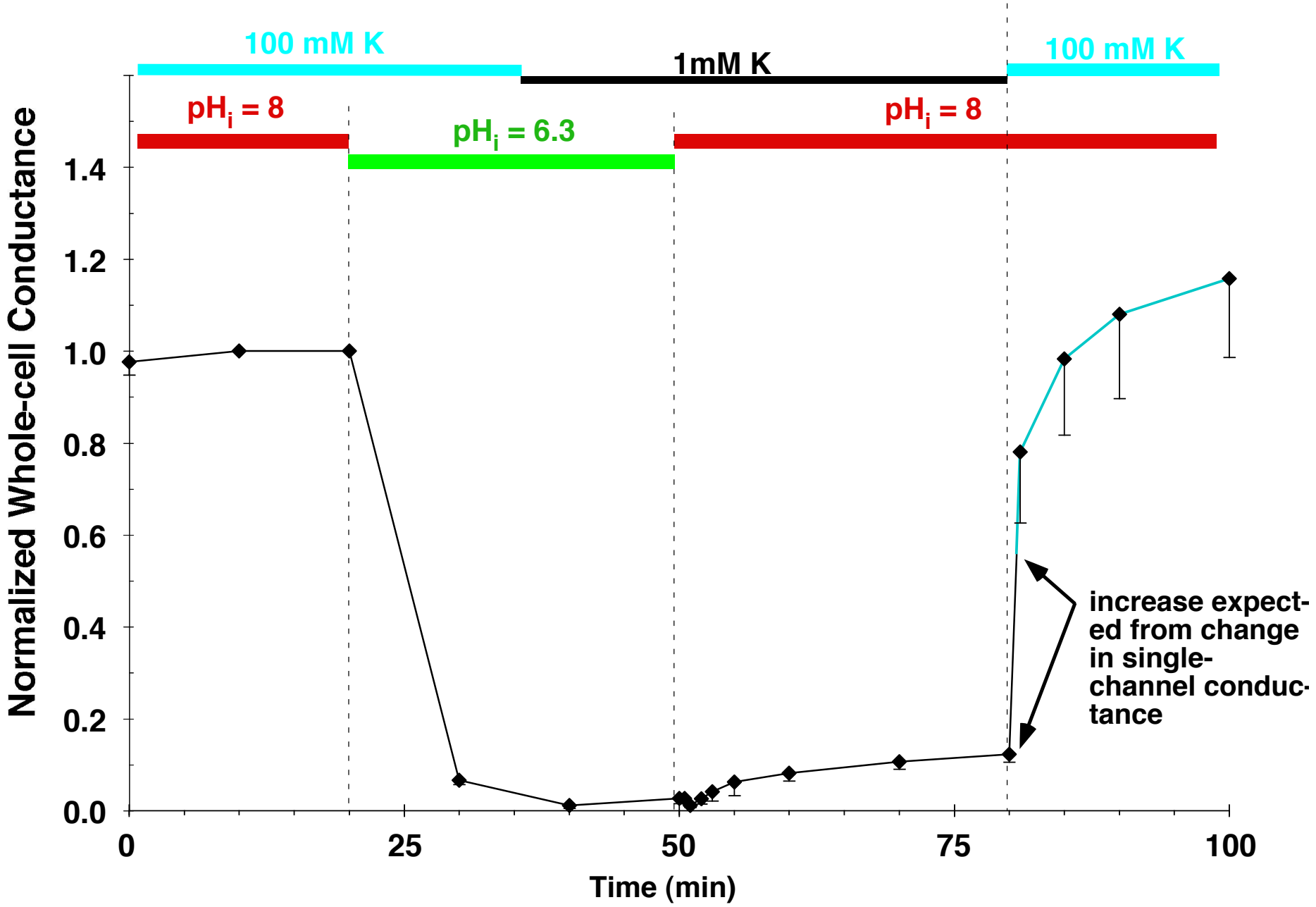
Suppl Fig S6B. Polar linkages between the *Ser* side-chain of V121S to T122 and L117, similar to V121T, also protect against inactivation. Two (of 4) adjacent subunits in the region of the selectivity filter are depicted as cyan and green ribbons.

Suppl Fig S6C. Polar linkages between T122, V121Q and N152 may protect against inactivation in the V121Q mutant. The location of the inter-subunit salt bridge, R128-E132 is indicated. Two (of 4) adjacent subunits in the region of the selectivity filter are depicted as cyan and green ribbons.

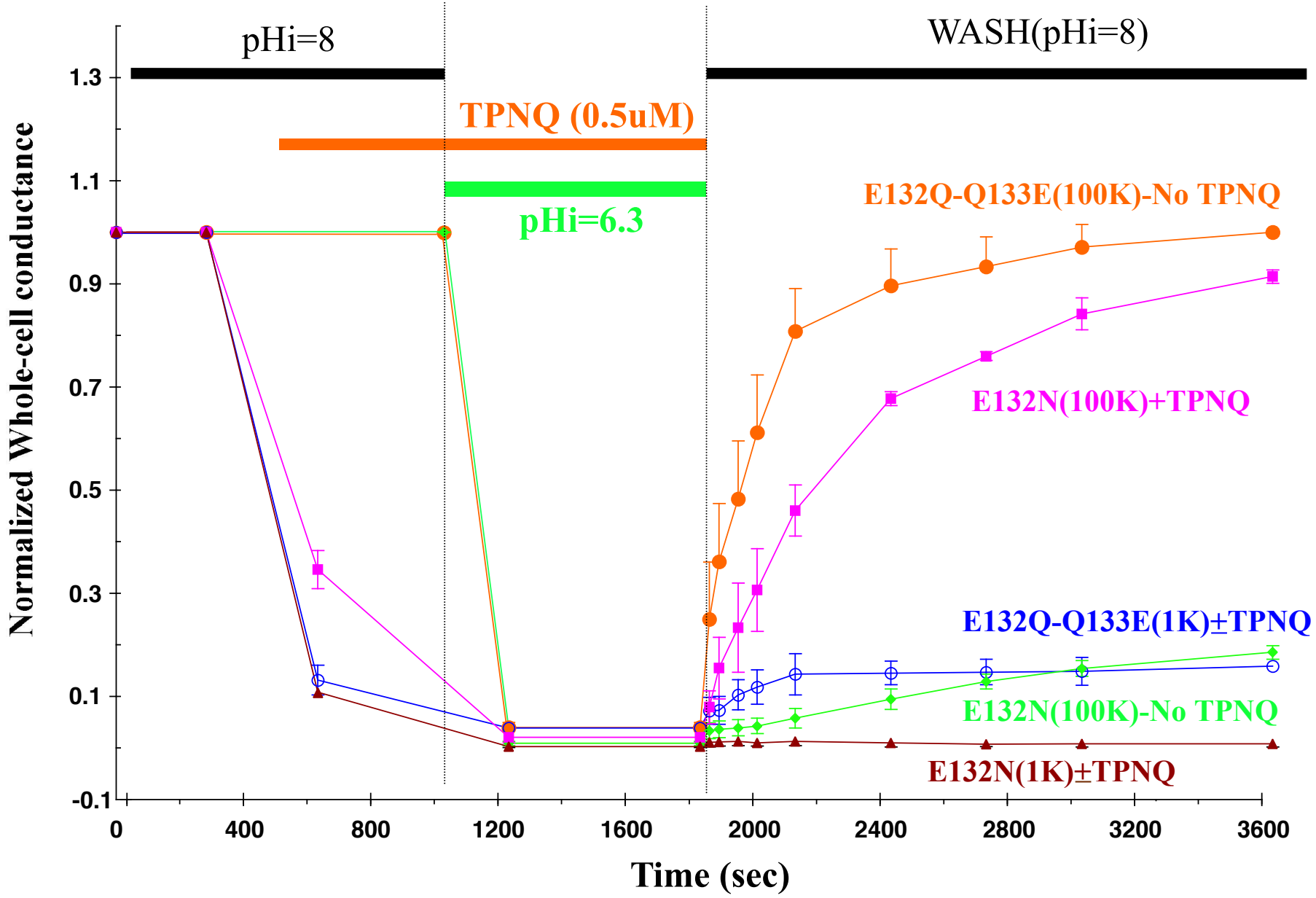
Suppl Fig S6D. Absence of polar linkages between V121N and T122, L117 or N152 allow inactivation under low K, low pH conditions, similar to wt-Kir1.1. Two (of 4) adjacent subunits in the region of the selectivity filter are depicted as cyan and green ribbons.


Pore Helix
Filter
PM region
rat ROMK2 (Kir1.1b) M T S A F L F S L E T Q V T I G Y G F R F V T E Q 133
human ROMK1 (hKir1.1a) L T S A F L F S L E T Q V T I G Y G F R C V T E Q 152
Kir2.1 F T A A F L F S I E T Q T T I G Y G F R C V T D E 153
Kir3.1 F P S A F L F F I E T E A T I G Y G Y R Y I T D K 154
Kir3.4 F V S A F L F S I E T E T T I G Y G F R V I T E K 160
KirBac1.1 F V G A F F F S V E T L A T V G Y G D M H P - - Q 119
KcsA Y P R A L W W S V E T A T T V G Y G D L Y P - - V 84
Shaker I P D A F W W A V V T M T T V G Y G D M T P - - V 451

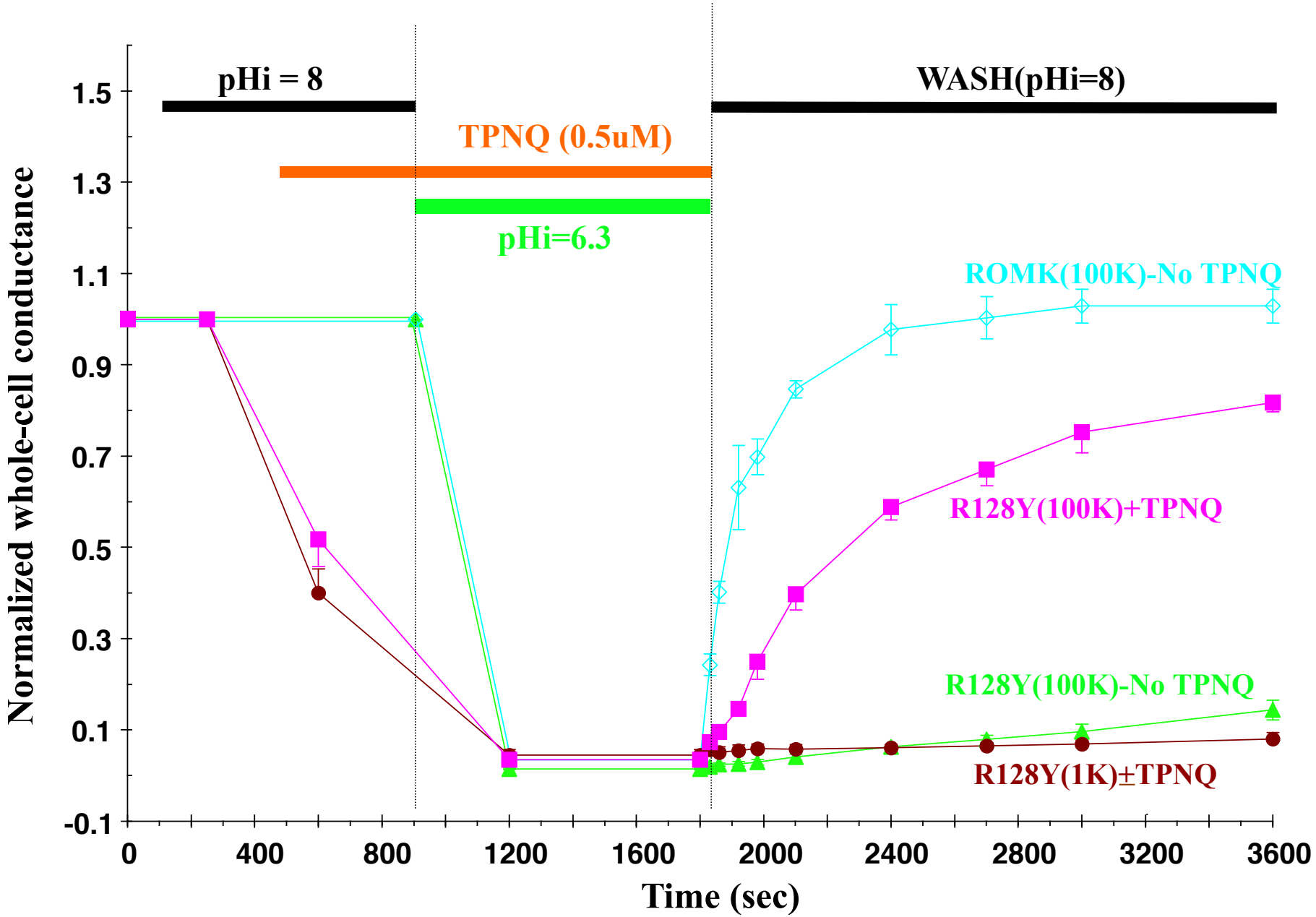
Suppl Fig S1.


100 mM K
1mM K
100 mM K
pH$_i$ = 8
pH$_i$ = 6.3
pH$_i$ = 8
Normalized Whole-cell Conductance
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
0
25
50
75
100
Time (min)
increase expect-
ed from change
in single-
channel conduc-
tance

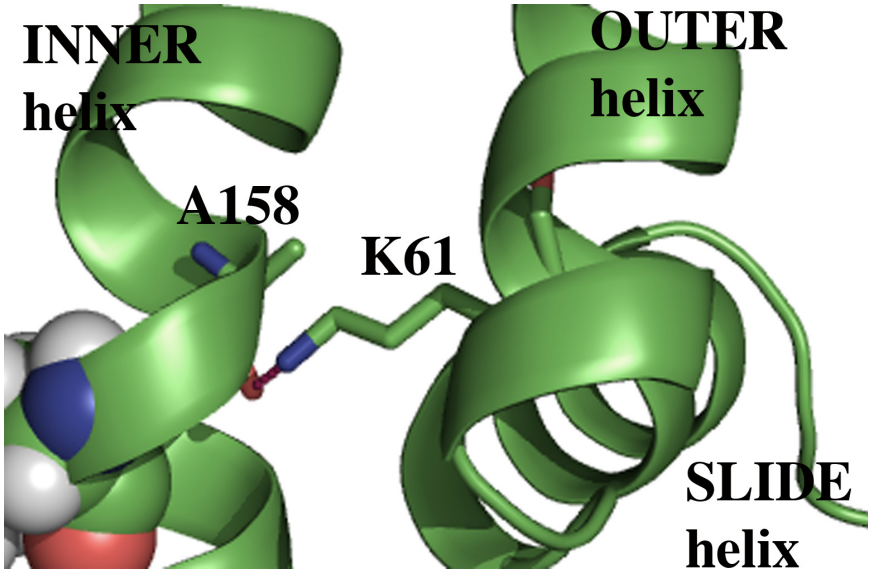
Suppl Fig S2.


pHi=8
WASH(pHi=8)
TPNQ (0.5uM)
pHi=6.3
E132Q-Q133E(100K)-No TPNQ
E132N(100K)+TPNQ
E132Q-Q133E(1K)±TPNQ
E132N(100K)-No TPNQ
E132N(1K)±TPNQ
Normalized Whole-cell conductance
Time (sec)
1.3
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
0
400
800
1200
1600
2000
2400
2800
3200
3600

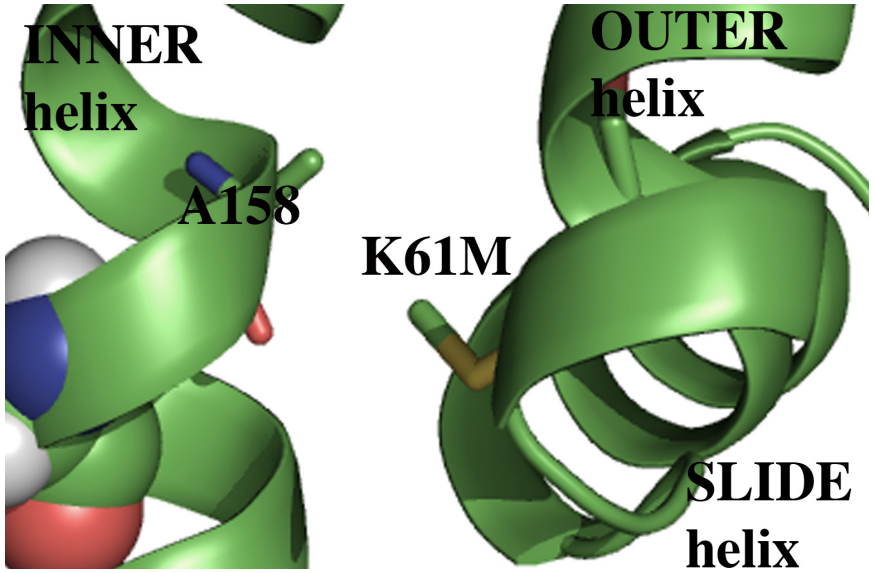
Suppl Fig S3.


pHi = 8
WASH(pHi=8)
TPNQ (0.5uM)
pHi=6.3
ROMK(100K)-No TPNQ
R128Y(100K)+TPNQ
R128Y(100K)-No TPNQ
R128Y(1K)±TPNQ
Normalized whole-cell conductance
Time (sec)
1.5
1.3
1.1
0.9
0.7
0.5
0.3
0.1
-0.1
0
400
800
1200
1600
2000
2400
2800
3200
3600

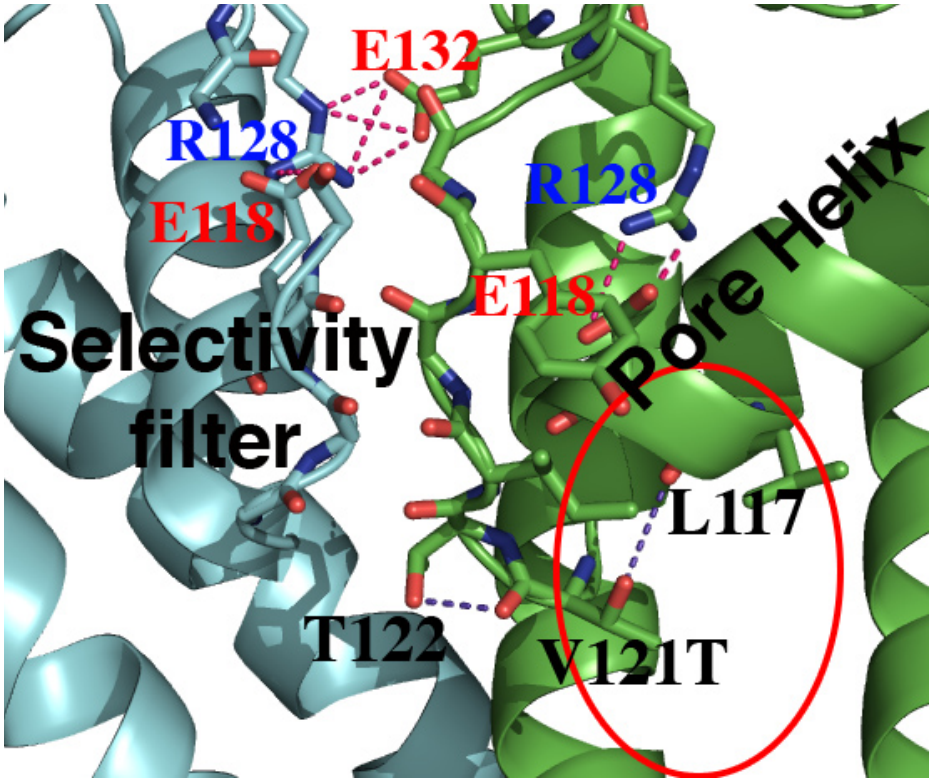
Suppl Fig S4.



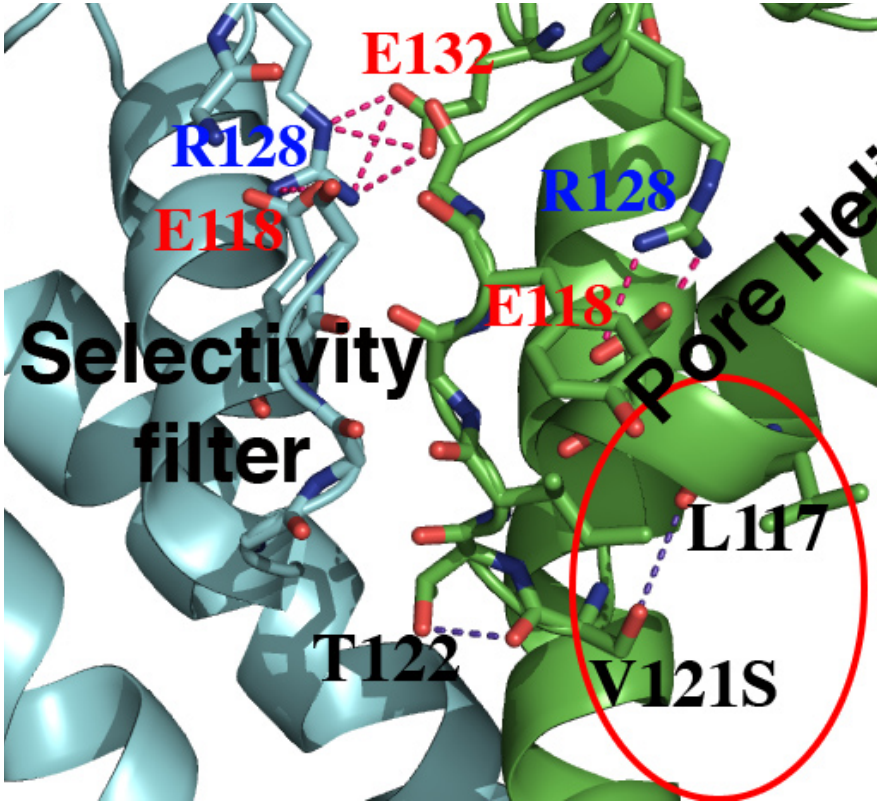
Suppl Fig S5A.



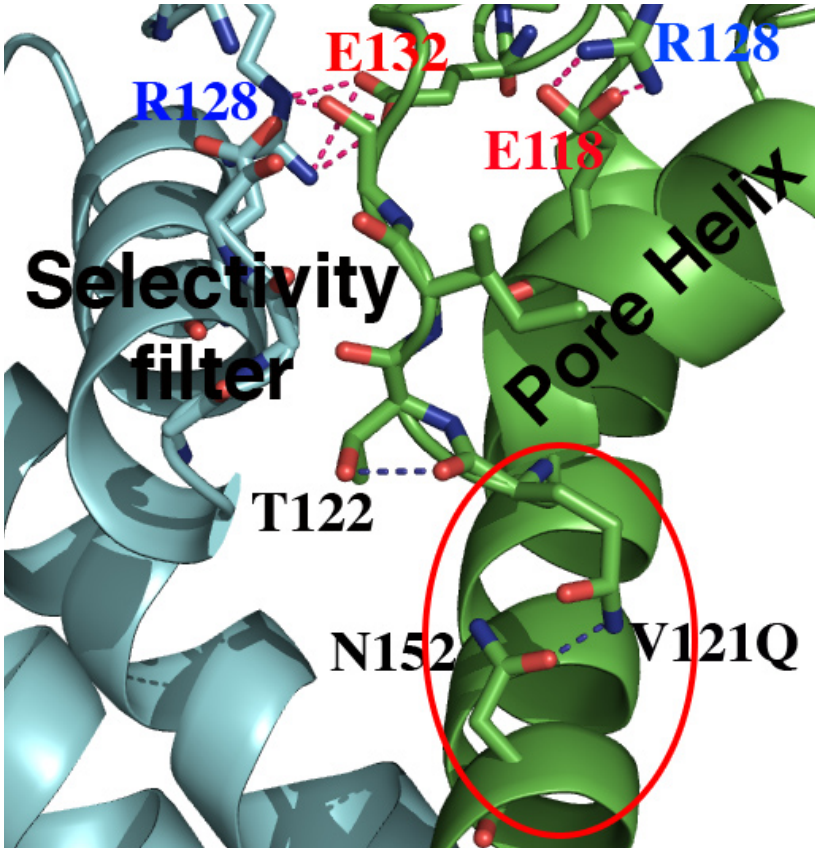
Suppl Fig S5B.



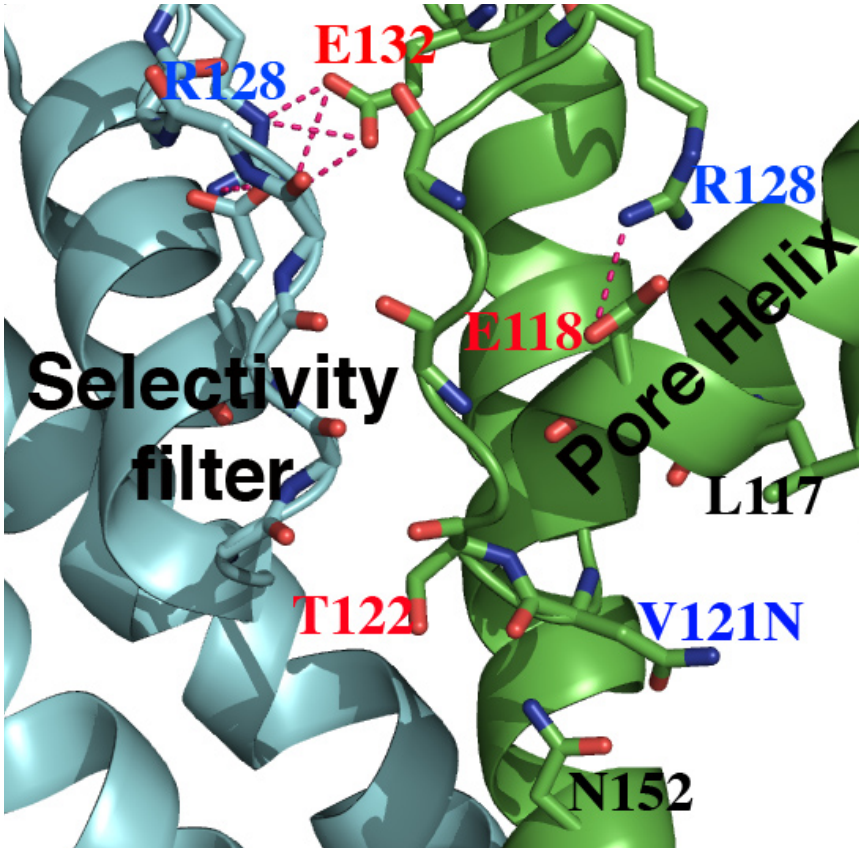
Suppl Fig S6A.



Suppl Fig S6B.



Suppl Fig S6C.



Suppl Fig S6D.