A sodium-mediated structural switch that controls the sensitivity of Kir channels to PIP_2.

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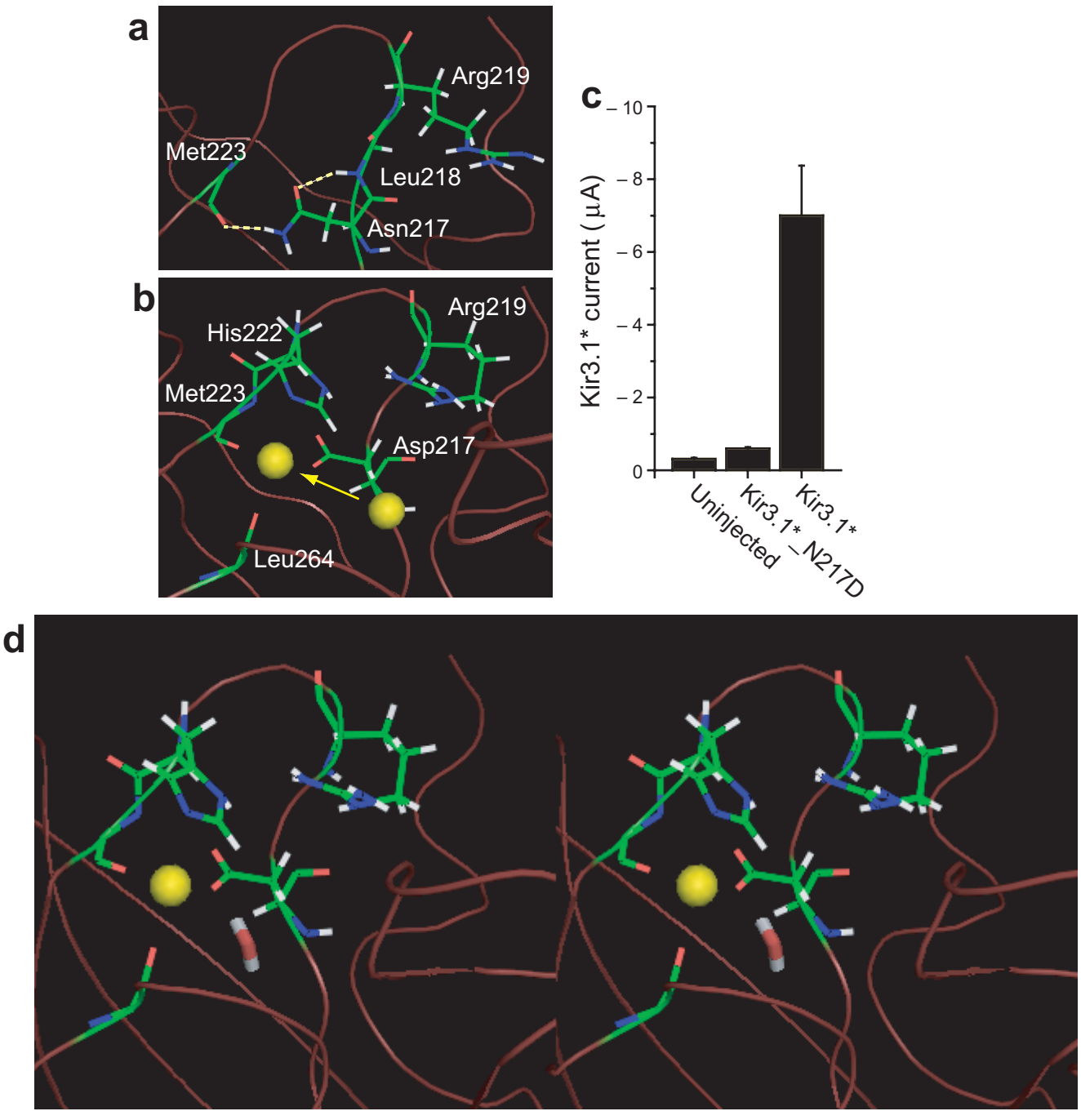
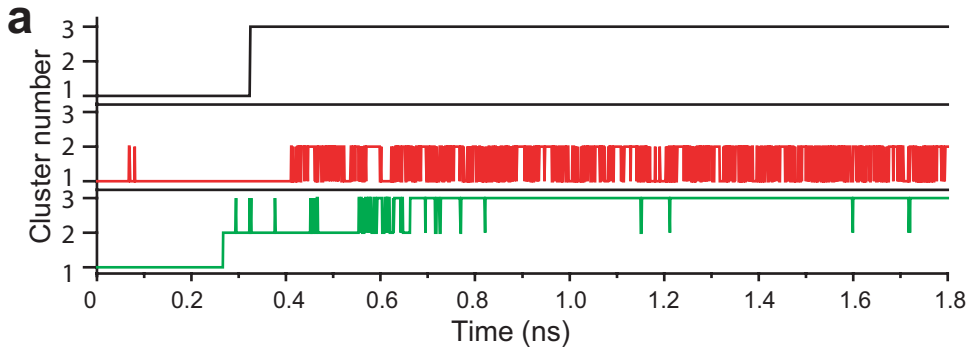
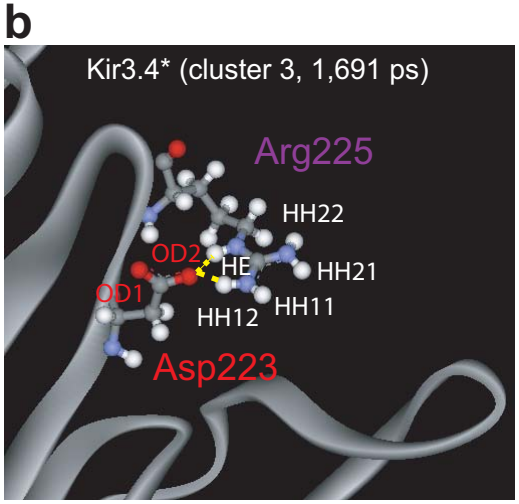
Running title:

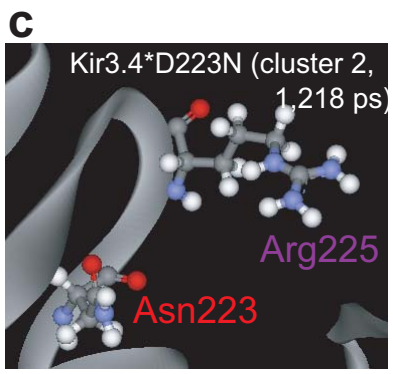
Sodium mediated structural switch

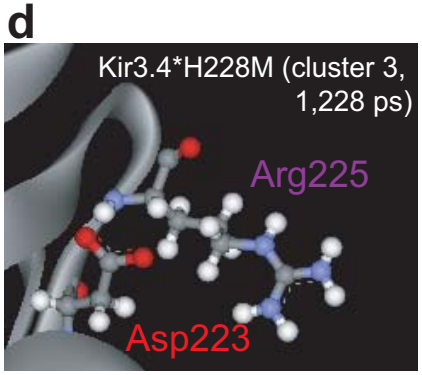

a
Arg219
Met223
Leu218
Asn217
b
Arg219
His222
Met223
Asp217
Leu264
c
Kir3.1* current (μA)
– 10
– 8
– 6
– 4
– 2
0
Uninjected
Kir3.1*
Kir3.1* – N217D
d

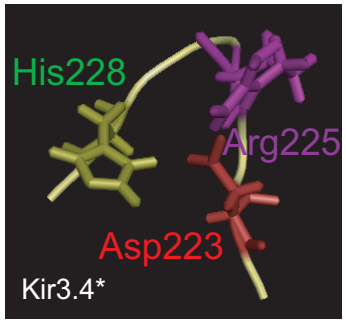
Supplemental Figure 1. H-bonding pattern between position 217 and residues in its vicinity in (**a**) Kir3.1 after minimization. The model was based on the crystallographic structure of Kir3.1 (PDB accession number 1N9P[19]). (**b**) 4.6Å displacement of a Na^+ ion during minimization to its coordination site. (**c**) Summary of whole-cell basal current from Kir3.1* and Kir3.4*_N217D expressed in *Xenopus* oocytes and recorded at -80mV. (**d**) Stereo view of the sodium coordination site in Kir3.1_N217D that shows a nearby water molecule. Figures (**a**), (**b**), and (**d**) were drawn using PyMol[48].

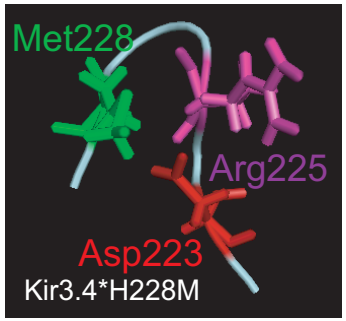

a
Cluster number
Time (ns)
0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
1.8


b
Kir3.4* (cluster 3, 1,691 ps)
Arg225
HH22
OD2
HE
HH21
OD1
HH12
HH11
Asp223

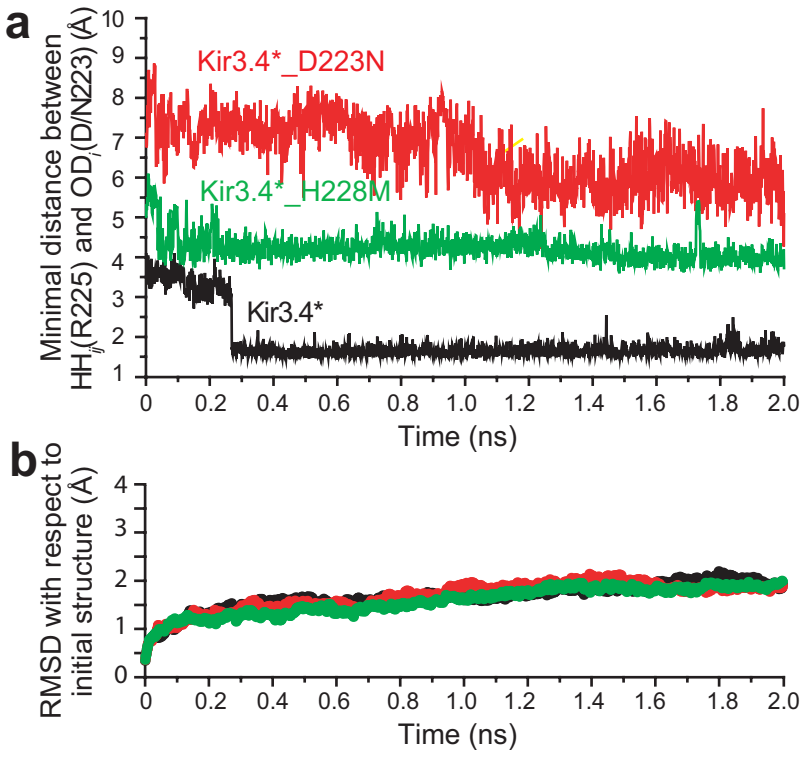

c
Kir3.4*D223N (cluster 2, 1,218 ps)
Arg225
Asn223

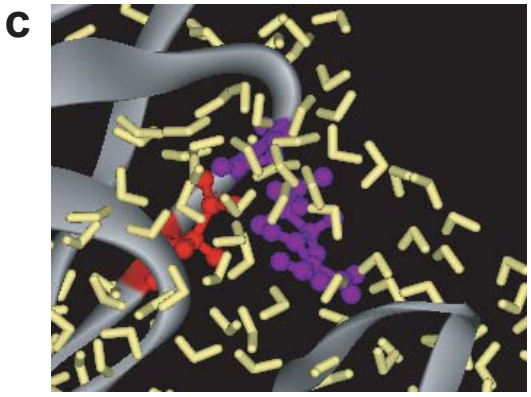

d
Kir3.4*H228M (cluster 3, 1,228 ps)
Arg225
Asp223


e
His228
Arg225
Asp223
Kir3.4*

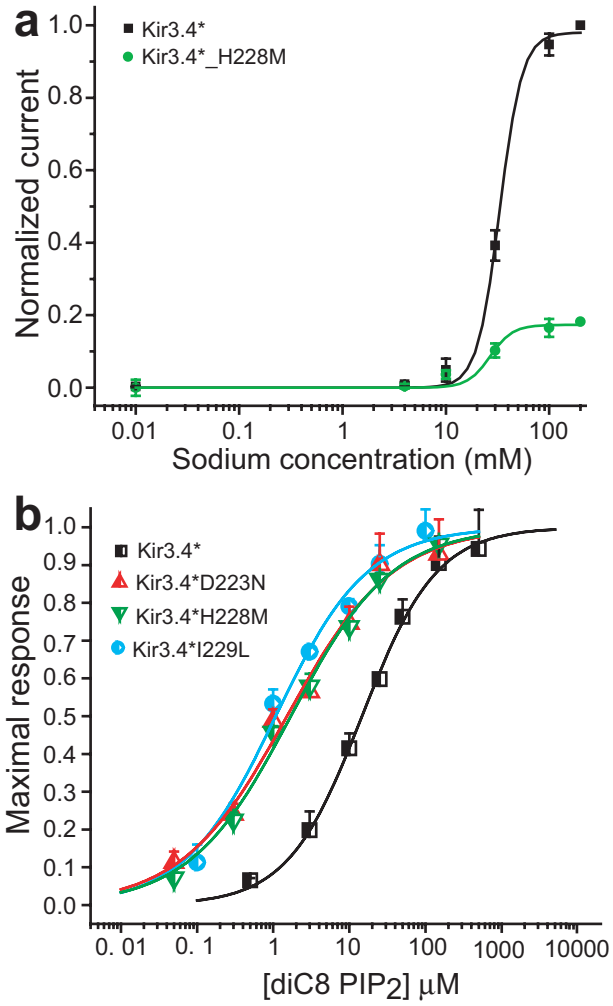

f
Met228
Arg225
Asp223
Kir3.4*H228M

Supplemental Figure 2. Conformational transitions revealed through clustering analysis. (**a**) Classification of the structures obtained during the MD simulations of Kir3.4, Kir3.4_D223N, and Kir3.4_H228M according to clustering analysis. (**b**)-(**d**) Representative conformations of the backbone atoms of the critical loop of dominant clusters obtained from the simulation of Kir3.4, Kir3.4_D223N and Kir3.4_H228M portraying the relative orientations of the side-chains of positions 223 and 225. (**e**)-(**f**) Orientations of the side-chains of position 223, 225 and 228 in the representative conformations of the dominant clusters of Kir3.4 and in Kir3.4_H228M.


a
Minimal distance between HH$_{ij}$(R225) and OD$_i$(D/N223) (Å)
Kir3.4*_D223N
Kir3.4*_H228M
Kir3.4*
Time (ns)
b
RMSD with respect to initial structure (Å)
Time (ns)

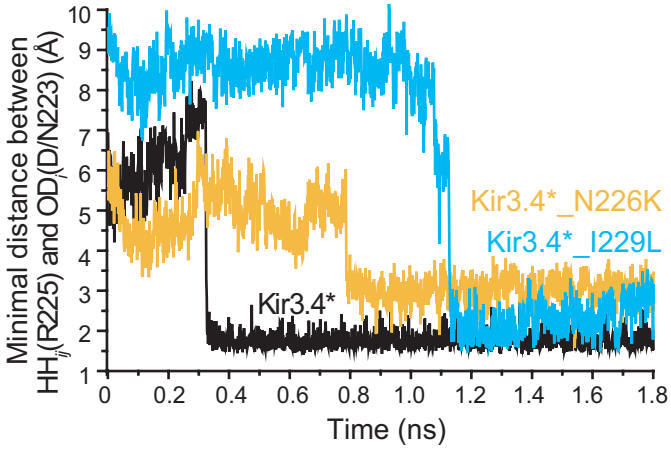

c

Supplemental Figure 3. Simulations in the presence of water. (**a**) Minimal distance between positions 223 and 225 as obtained from MD simulations of Kir3.4*, Kir3.4*_D223N, and Kir3.4*_H228M in the presence of the primary hydration shell (PHS - see methods). (**b**) RMSD of the backbone residues of the cytosolic domain relative to the starting minimized structure that corresponds to the MD simulations of Kir3.4, Kir3.4_D223N, and Kir3.4_H228M in the presence of the PHS. The calculations were performed using Simulaid: http://atlas.physbio.mssm.edu/~mezei/simulaid/simulaid.html. (**c**) Representative structure of the critical loop in Kir3.4 surrounded by the PHS.

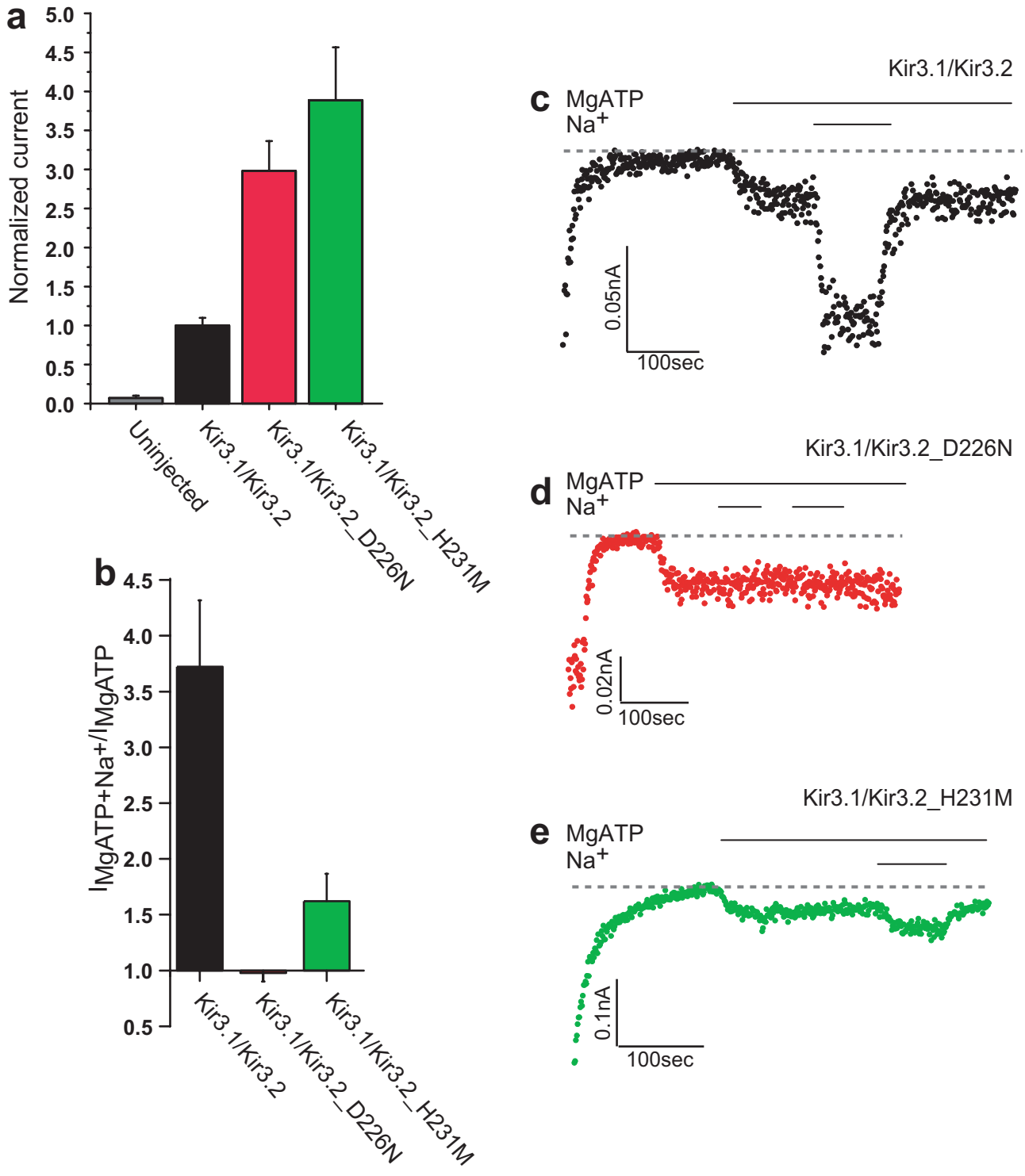

a
Kir3.4*
Kir3.4*_H228M
Normalized current
1.0
0.8
0.6
0.4
0.2
0.0
0.01
0.1
1
10
100
Sodium concentration (mM)
b
Kir3.4*
Kir3.4*D223N
Kir3.4*H228M
Kir3.4*I229L
Maximal response
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0. 01
0. 1
1
10
100
1000
10000
[diC8 PIP2] μM

Supplemental Figure 4. Effects of mutations of Kir3.4* residues in the loop where Na^+ is coordinated on Na^+ and PIP_2 sensitivity (**a**) Na^+ dose-response curve of Kir3.4* and Kir3.4*_H228M obtained in the presence of 3μM PIP_2. (**b**) PIP_2 dose-response curves of Kir3.4*, Kir3.4*_D223N, Kir3.4*_H228M, and Kir3.4*I229L obtained using diC8 in the presence of 30mM Na^+.

Rosenhouse-Dantsker et al., Supplemental Fig. 5

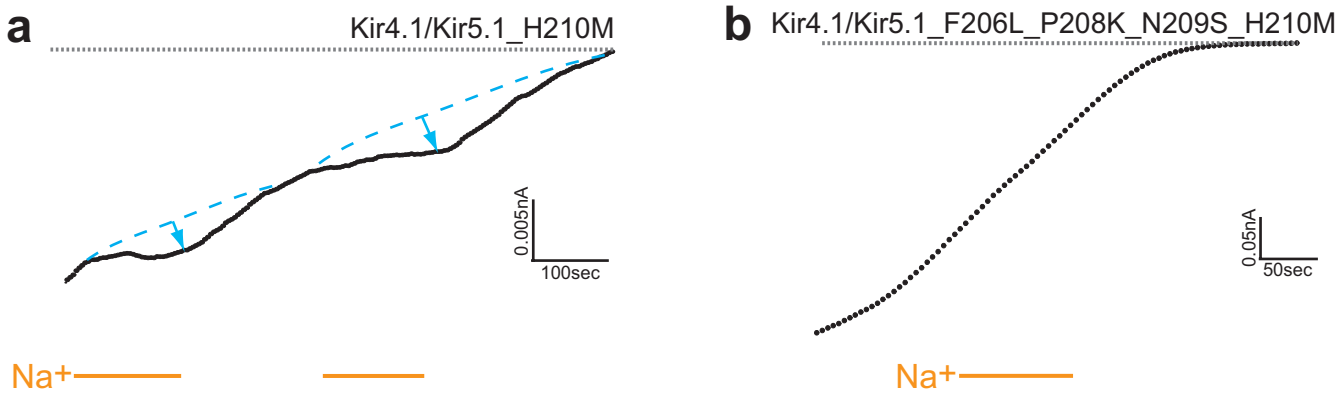


Supplemental Figure 5. MD simulations monitoring distance of Na^+-insensitive neighboring residues to Kir3.4*_R225. Minimal distance between positions 223 and 225 as obtained from MD simulations of Kir3.4, Kir3.4_N226K, and Kir3.4_I229L.


a
Normalized current
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
Uninjected
Kir3.1/Kir3.2
Kir3.1/Kir3.2_D226N
Kir3.1/Kir3.2_H231M
b
I MgATP+Na+/I MgATP
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Kir3.1/Kir3.2
Kir3.1/Kir3.2_D226N
Kir3.1/Kir3.2_H231M
c
Kir3.1/Kir3.2
MgATP
Na+
0.05nA
100sec
d
Kir3.1/Kir3.2_D226N
MgATP
Na+
0.02nA
100sec
e
Kir3.1/Kir3.2_H231M
MgATP
Na+
0.1nA
100sec

Supplemental Figure 6. Experimental evidence for Kir3.2 residues involved in Na^+ sensitivity (**a**) Whole-cell basal currents of Kir3.1/Kir3.2, Kir3.1/Kir3.2_D226N, and Kir3.1/Kir3.2_H231M recorded in *Xenopus* oocytes at –80mV. (**b**)-(**d**) Representative traces of inside-out macropatch recordings of *Xenopus* oocytes. Na^+ (30mM) and MgATP (5mM) were applied as indicated by the bars in the control solution (ND96K+EGTA with 2mM Mg^{++}) (**b**) Kir3.1/Kir3.2 (**c**) Kir3.1/Kir3.2_D226N (**d**) Kir3.1/Kir3.2_H231M. (**e**) Summary data showing the effect of perfusion of Na^+ (30mM) on currents obtained from inside-out patches of *Xenopus* oocytes after a short application of MgATP (5mM).

Rosenhouse-Dantsker et al., Supplemental Fig. 7



Supplemental Figure 7. Experimental evidence of a role for His210 in Na^+ sensitivity of Kir5.1. (**a**) The rundown of Kir4.1/Kir5.1_H210M is still slowed down following application of sodium. (**b**) The rundown of Kir4.1/Kir5.1_F206L_P208K_N209S_H210M becomes Na^+ insensitive.

Supplemental Table 1: Summary of the cluster analysis of the trajectories obtained from the molecular dynamics simulations of Kir3.4, Kir3.4_D223N, and Kir3.4_H228M according to the distances between the atoms in the side-chains of the residues at positions 223 and 225 that could form hydrogen bonding.

		Average distance (Å)									
Cluster number	number of members	OD1(223)-HH11(225)	OD1(223)-HH12(225)	OD1(223)-HH21(225)	OD1(223)-HH22(225)	OD1(223)-HE(225)	OD2(223)-HH11(225)	OD2(223)-HH12(225)	OD2(223)-HH21(225)	OD2(223)-HH22(225)	OD2(223)-HE(225)
Kir3.4											
1	323	8.138	7.454	7.502	6.286	5.675	9.858	9.163	9.14	7.843	7.31
2	1	8.22	6.856	8.581	7.972	5.18	6.236	4.748	7.02	6.75	3.544
3	1476	5.466	3.74	7.006	7.034	3.666	3.574	1.78	4.934	4.958	1.688
Kir3.4_N216D											
1	991	8.186	6.663	9.757	9.699	6.548					
2	809	8.988	7.409	10.595	10.502	7.206					
Kir3.4_H228M											
1	266	8.729	8.033	7.991	6.704	6.125	10.722	10.097	9.816	8.46	8.18
2	342	10.671	10.153	9.585	7.963	8.106	8.834	8.328	7.786	6.17	6.297
3	1192	9.262	8.926	8.033	6.373	7.01	7.175	6.931	5.964	4.349	5.182