Supplementary Information for

Molecular Structure of an Open Human K_{ATP} Channel

Chen Zhao and Roderick MacKinnon

*Roderick MacKinnon

[a]Laboratory of Molecular Neurobiology and Biophysics, Howard Hughes Medical Institute, The Rockefeller University, New York, NY 10065

Email: mackinn@rockefeller.edu

This PDF file includes:

Figures S1 to S6

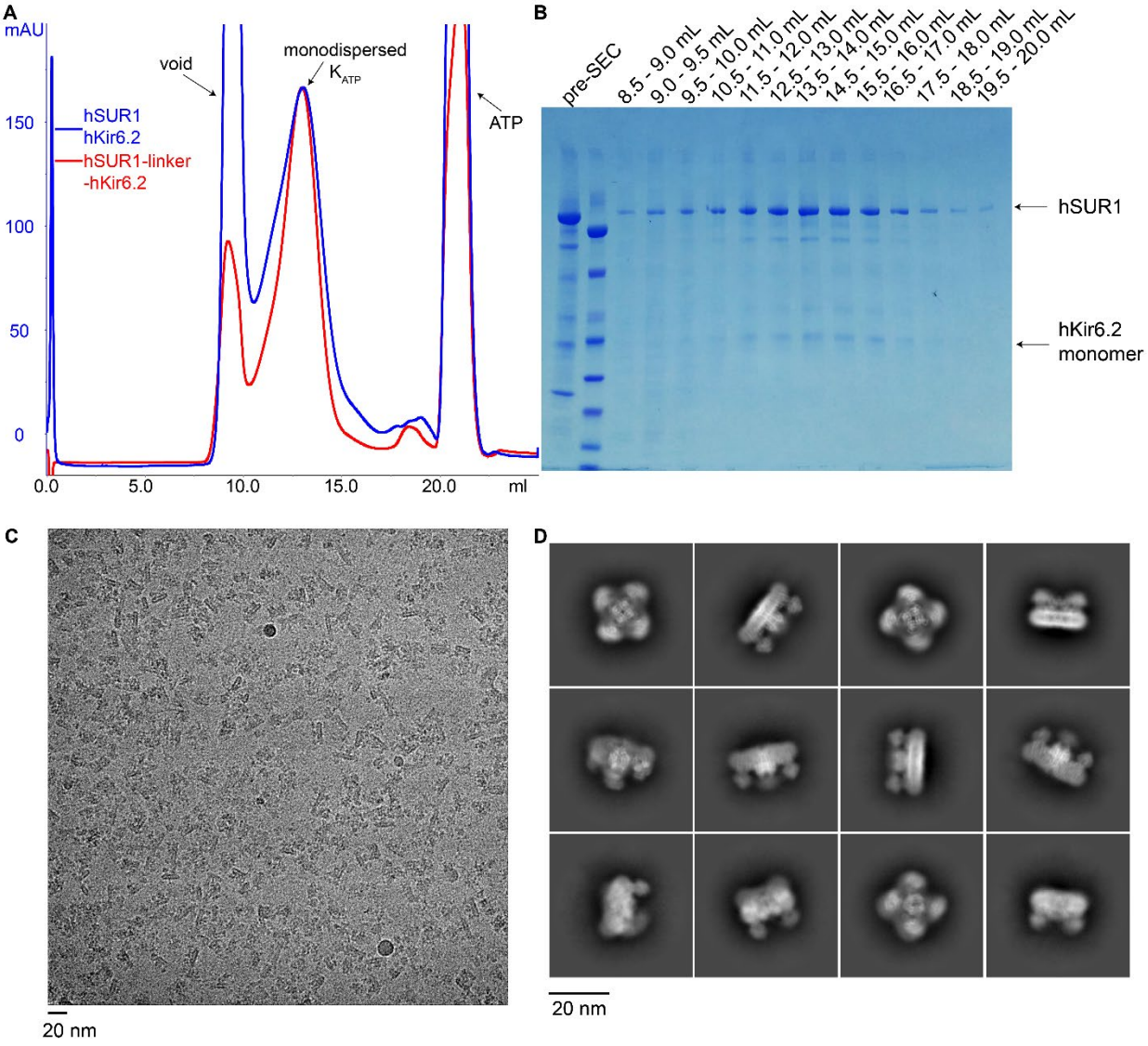
A
mAU
150
100
50
0
void
monodispersed
K_{ATP}
ATP
hSUR1
hKir6.2
hSUR1-linker
-hKir6.2
0.0
5.0
10.0
15.0
20.0
ml
B
pre-SEC
8.5 - 9.0 mL
9.0 - 9.5 mL
9.5 - 10.0 mL
10.5 - 11.0 mL
11.5 - 12.0 mL
12.5 - 13.0 mL
13.5 - 14.0 mL
14.5 - 15.0 mL
15.5 - 16.0 mL
16.5 - 17.0 mL
17.5 - 18.0 mL
18.5 - 19.0 mL
19.5 - 20.0 mL
hSUR1
hKir6.2
monomer
C
20 nm
D
20 nm

Fig. S1. Sample preparation and cryo-EM analysis of hK_{ATP}. (A) Comparison of gel-filtration profile of different WT hK_{ATP} constructs. Samples were separated by a superpose 6 increase column. Trace for hK_{ATP} expressed as independent polypeptides is shown in blue and trace for hK_{ATP} expressed with a covalent linker between hSUR and hKir is shown in red. (B) SDS-PAGE of fractions collected from gel-filtration in (A). Pre-SEC: sample before size-exclusion (gel-filtration) chromatography. Retention volumes for fractions are labeled on top of corresponding gel lanes. (C) Representative micrograph of hK_{ATP} ($C166S_{Kir}$, $G334D_{Kir}$) in GDN in the presence of 10 mM Mg^{2+}-ATP and 0.1 mM Mg^{2+}-ADP. This specific micrograph was collected at a defocus value of -2 μm with a total dose of 56 $e^-/Å^2$. The image was lowpass-filtered to 15 Å. (D) Selected 2D class averages.

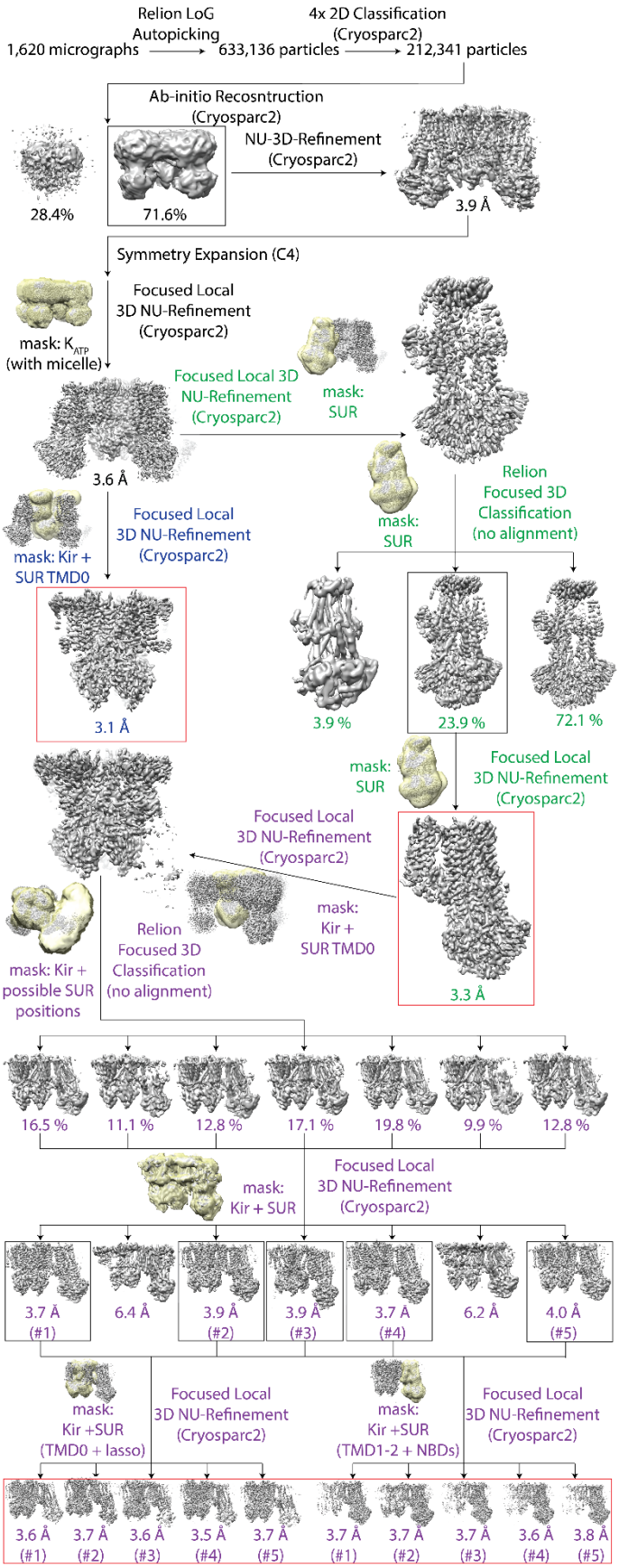
Relion LoG Autopicking
1,620 micrographs
633,136 particles
4x 2D Classification (Cryosparc2)
212,341 particles
Ab-initio Recosntruction (Cryosparc2)
28.4%
71.6%
NU-3D-Refinement (Cryosparc2)
3.9 Å
Symmetry Expansion (C4)
mask: K$_{ATP}$ (with micelle)
Focused Local 3D NU-Refinement (Cryosparc2)
mask: SUR
3.6 Å
Focused Local 3D NU-Refinement (Cryosparc2)
mask: Kir + SUR TMD0
3.1 Å
Relion Focused 3D Classification (no alignment)
mask: SUR
3.9 %
23.9 %
72.1 %
Focused Local 3D NU-Refinement (Cryosparc2)
mask: SUR
Focused Local 3D NU-Refinement (Cryosparc2)
mask: Kir + SUR TMD0
3.3 Å
Relion Focused 3D Classification (no alignment)
mask: Kir + possible SUR positions
16.5 %
11.1 %
12.8 %
17.1 %
19.8 %
9.9 %
12.8 %
mask: Kir + SUR
Focused Local 3D NU-Refinement (Cryosparc2)
3.7 Å (#1)
6.4 Å
3.9 Å (#2)
3.9 Å (#3)
3.7 Å (#4)
6.2 Å
4.0 Å (#5)
mask: Kir +SUR (TMD0 + lasso)
Focused Local 3D NU-Refinement (Cryosparc2)
mask: Kir +SUR (TMD1-2 + NBDs)
Focused Local 3D NU-Refinement (Cryosparc2)
3.6 Å (#1)
3.7 Å (#2)
3.6 Å (#3)
3.5 Å (#4)
3.7 Å (#5)
3.7 Å (#1)
3.7 Å (#2)
3.7 Å (#3)
3.6 Å (#4)
3.8 Å (#5)

Fig. S2. Cryo-EM data processing procedure. Cryo-EM maps are shown in grey and masks are shown in yellow. Steps for obtaining hKir6.2 structure by focused refinement are colored in blue, steps for obtaining hSUR1 structure by focused refinement are colored in green, and steps for obtaining hKir6.2-hSUR1 structures with hSUR1 at different positions relative to hKir6.2 are colored in purple. Classes that were selected for further data processing are indicated by black boxes. Cryo-EM maps that were used for final model building are indicated by red boxes.

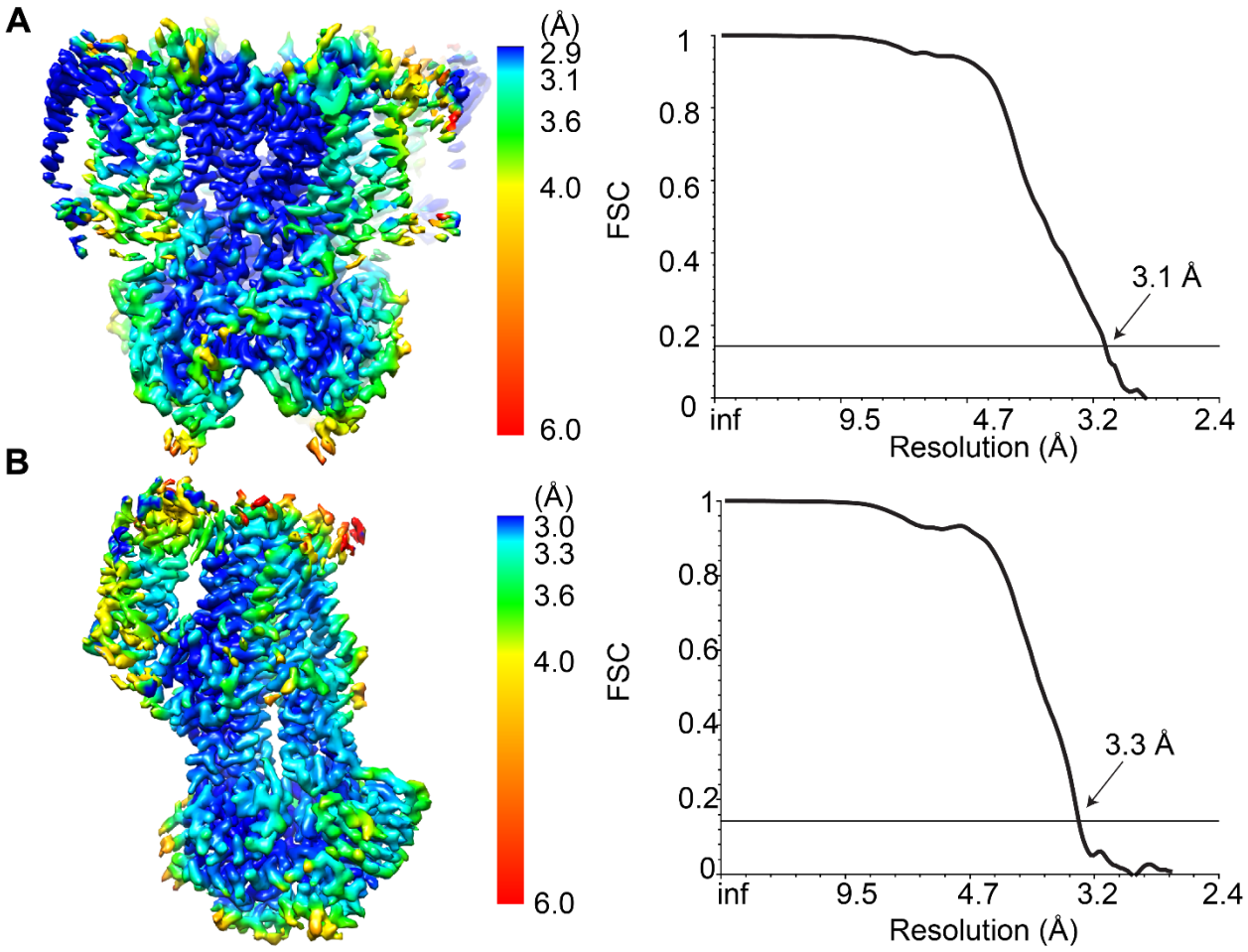
A
(Å)
2.9
3.1
3.6
4.0
6.0
FSC
1
0.8
0.6
0.4
0.2
0
3.1 Å
inf
9.5
4.7
3.2
2.4
Resolution (Å)
B
(Å)
3.0
3.3
3.6
4.0
6.0
FSC
1
0.8
0.6
0.4
0.2
0
3.3 Å
inf
9.5
4.7
3.2
2.4
Resolution (Å)

Fig. S3. Local resolution and FSC curves for (A) hKir6.2 and (B) hSUR1. Local resolution was calculated by Cryosparc V2.

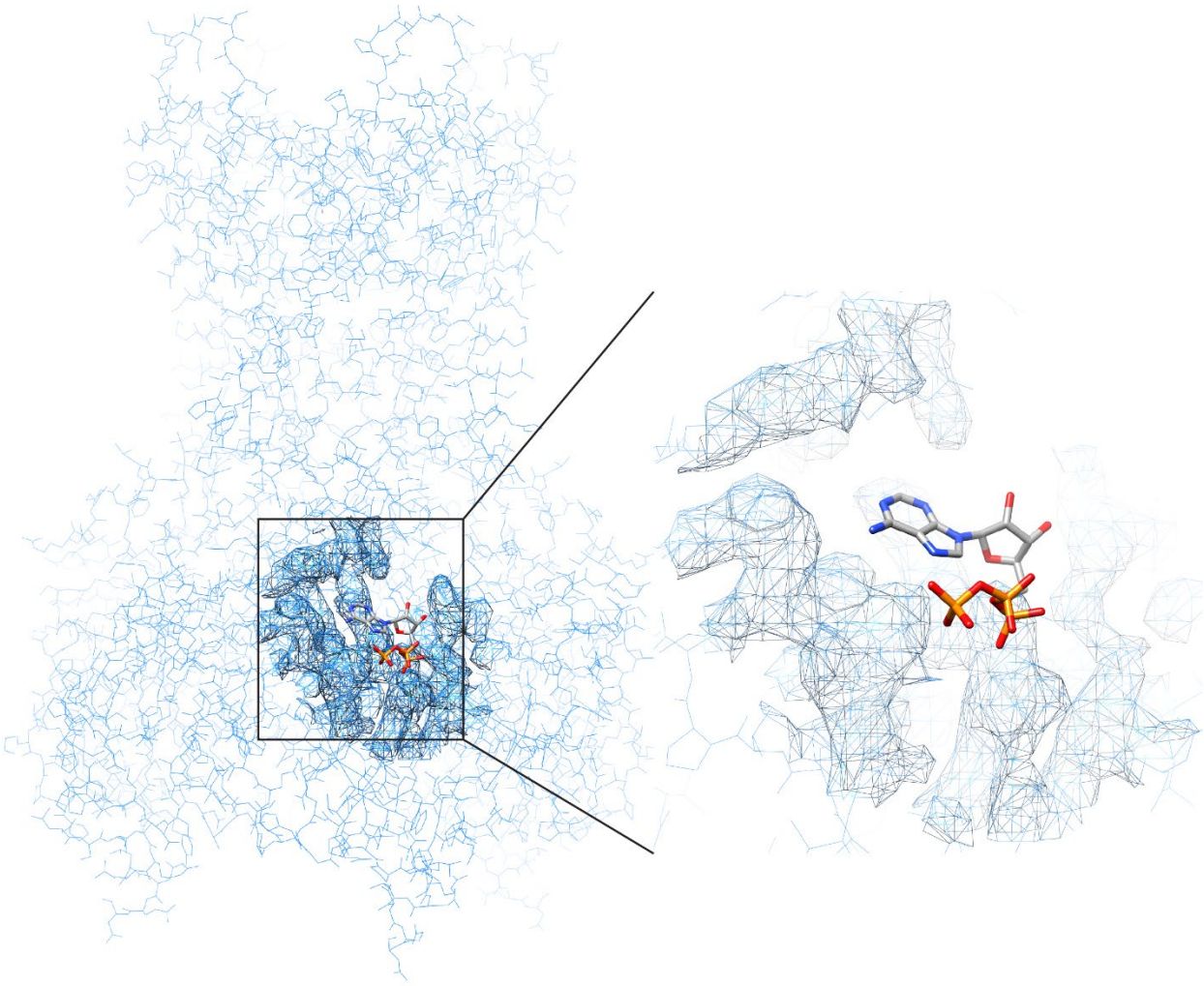

Fig. S4. ATP is absent in its inhibitory binding site in hKir (C166S, G334D). ATP molecule in hKir6.2 (WT) is shown as sticks. Cryo-EM density of hKir6.2 (C166S, G334D) within 10 Å radius centered at the location of ATP in hKir6.2 (WT) is shown as meshes.

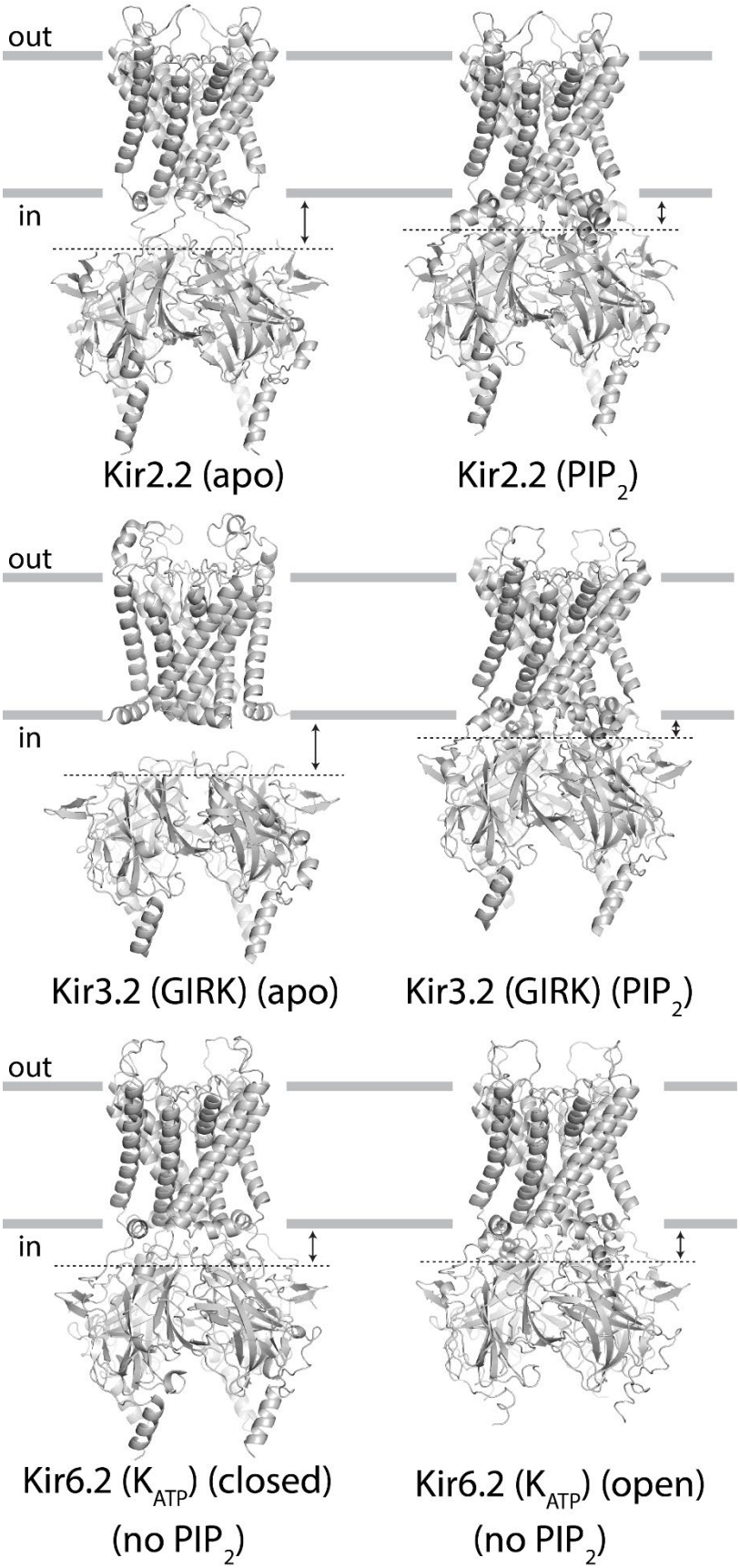
out
in
Kir2.2 (apo)
Kir2.2 (PIP2)
out
in
Kir3.2 (GIRK) (apo)
Kir3.2 (GIRK) (PIP2)
out
in
Kir6.2 (KATP) (closed)
(no PIP2)
Kir6.2 (KATP) (open)
(no PIP2)

Fig. S5. PIP_2-induced conformational changes in Kir channels. PDB IDs: Kir2.2 (apo) 3JYC, Kir2.2 (PIP_2) 3SPI, Kir3.2 (apo) 6XIS, Kir3.2 (PIP_2) 6XIT, Kir6.2 (closed, no PIP_2) 6C3P.

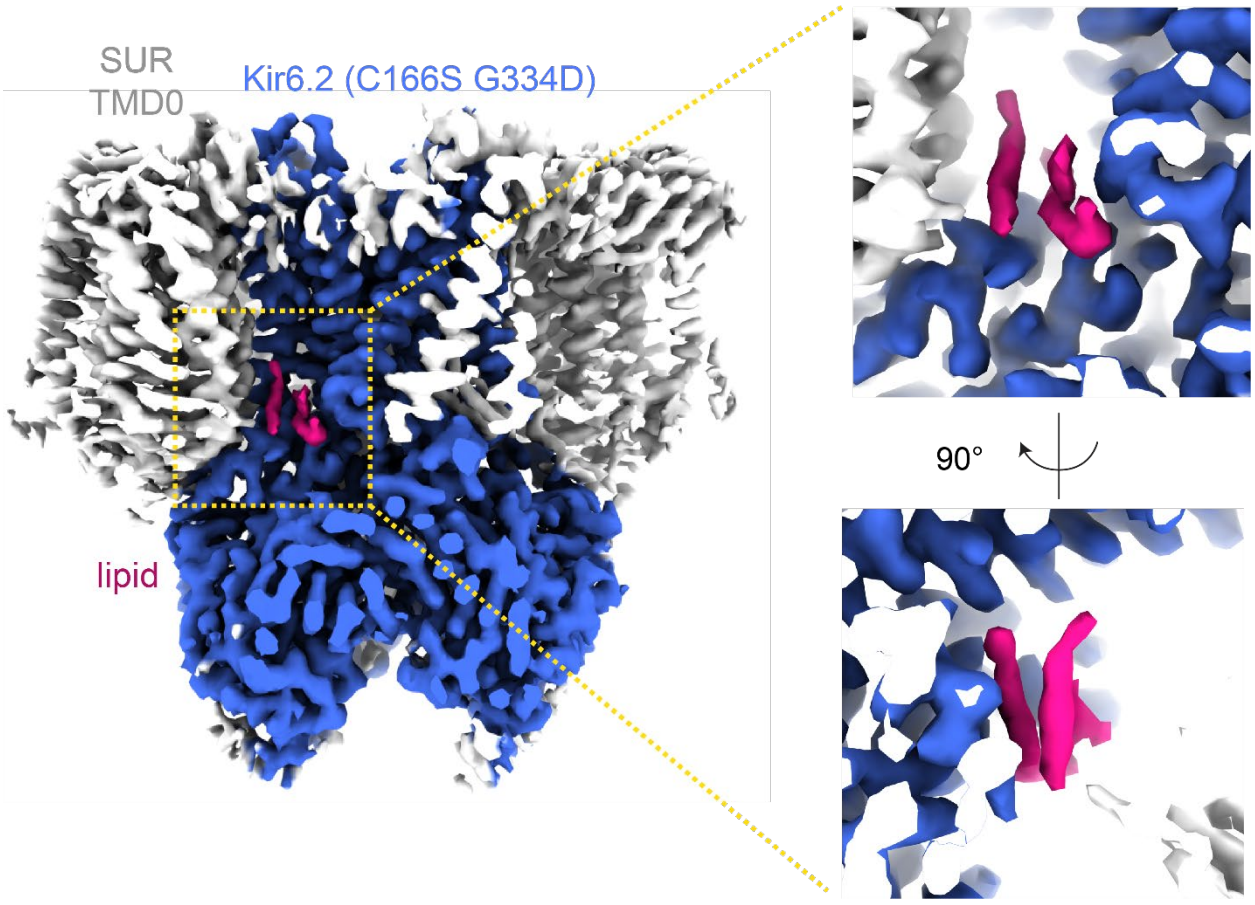
SUR
TMD0
Kir6.2 (C166S G334D)
lipid
90°

Fig. S6. Lipid densities in the potential PIP_2 binding pocket in hKir6.2 (C166S G334D). Lipid densities are colored in magenta, hKir6.2 (C166S G334D) is colored in blue, and TMD0 from SUR is colored in grey.