

Figure Supplement 1. Characterization of the K_{ATP} channel complex.

(A) Cartoon topology of SUR1-Kir6.2 fusion construct covalently linked by a flexible 39-residue linker (a dashed line). TMD, transmembrane domain; L0, loop 0; NBD, nucleotide-binding domain; OH, outer helix; PH, pore helix; IH, inner helix; β A, the first β strand of Kir6.2; IFH, interfacial helix; and CTD, cytoplasmic domain.

(B) Activation effect of Mg-ADP and NN414 (left panel), and inhibitory effect of GBM and ATP (right panel) on the macroscopic currents of SUR1-Kir6.2 fusion construct in an inside-out mode.

(C) Size exclusion chromatography (SEC) elution profiles of SUR1-Kir6.2 K_{ATP} fusion protein. Pooled fractions between dashed lines were used for cryo-EM sample preparation.

(D) SDS-PAGE of purified K_{ATP} fusion protein corresponding to the indicated SEC fractions. Fractions labeled with stars were pooled and concentrated for cryo-EM grids preparation.

Figure Supplement 2. Workflow for cryo-EM data processing of K_{ATP} in complex with ATPyS and GBM (ATP + GBM state).

(A) Representative raw micrograph.

(B) Representative two-dimensional class averages of the K_{ATP} channel.

(C) Flow-chart of EM data processing. Focused classification on Kir6.2 CTD and SUR1 NBD2, and focused refinement on SUR1 ABC transporter module were applied to improve the map quality of corresponding regions. Masked and corrected gold-standard Fourier shell correlation (FSC) curves of the final refinement are shown below the maps after post-processing. Resolution estimation was based on the criterion of FSC 0.143 cutoff.

Figure Supplement 3. Structure of K_{ATP} in complex with ATPyS (ATP state).

(A) Workflow for cryo-EM data processing of K_{ATP} in ATP state. Flow-chart of 3D classification, refinement, and focused 3D classification on Kir6.2 CTD and subsequent refinement for “T state” and “R state”. Masked and corrected gold-standard Fourier shell correlation (FSC) curves of the final refinement are shown below the maps after post-processing. Resolution estimation was based on the criterion of FSC 0.143 cutoff.

(B) Top view of structural comparison of “T state” (left) and “R state” (right) structures between the ATP (green) and ATP + GBM (gray) states.

Figure Supplement 4. Cryo-EM map of K_{ATP} in the ATP + GBM state and the GBM binding site.

(A) Local EM densities of ATPyS in “T state” (top) and “R state” (bottom) structures of the ATP + GBM state. Maps are colored the same as in Figure 1A.

(B) Cryo-EM map around the lasso motif in the ATP + GBM (top) and Mg-ADP states (bottom, described later). Extra density with unknown identity is colored in green.

(C) Different conformation of glibenclamide bound in K_{ATP} (top) and human AKR1C (bottom) (PDB: 4YVP).

(D) Cryo-EM density of GBM.

(E) Putative glibenclamide interactions with SUR1. Blue ovals represent residues on TMD1 and magenta ovals represent residues on TMD2.

(F) Superposition of SUR1 ABC transporter module structures in the ATP + GBM state solved using different constructs. Structure of SUR1-Kir6.2 fusion construct in this study is shown in grey. Structure of SUR1+Kir6.2 as separated peptides (PDB: 6BAA) is colored. Transmembrane helices from the first half of TMD of SUR1 ABC transporter module are aligned. Angles between helices M10 and M16 in the two structures are shown below.

Figure Supplement 5. Workflow for cryo-EM data processing of K_{ATP} in the Mg-ADP state.

Flow-chart of 3D classification and subsequent refinement. To improve the map quality certain regions, focused classification and refinement were employed similarly as in Extended Data Fig. 2c. Masked and corrected gold-standard Fourier shell correlation (FSC) curves of the final refinement are shown below the maps after post-processing. Resolution estimation was based on the criterion of FSC 0.143 cutoff.

Figure Supplement 6. Structural comparison of K_{ATP} in different states.

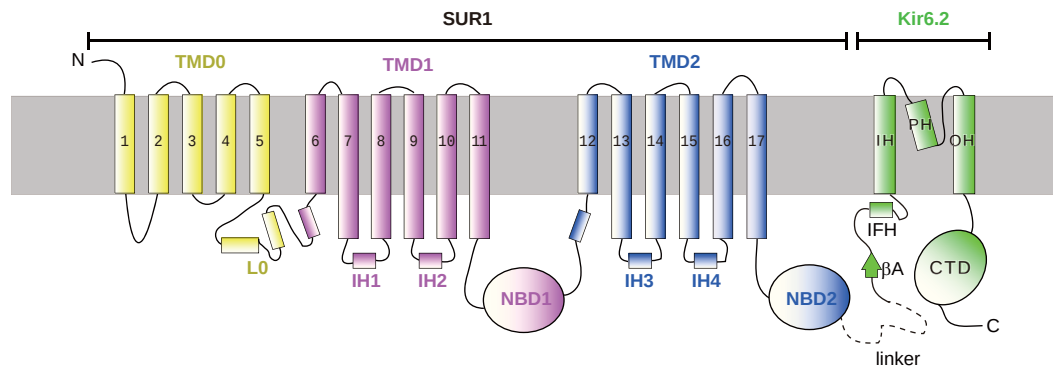
(A) Top view of structural comparison between the Mg-ADP ("T state") and ("propeller form", PDB: 6C3P) states by aligning Kir6.2.
(B) Superposition of the ABC module between the Mg-ADP (colored) and Mg-ATP&ADP (grey) states.
(C) Local EM density maps of inhibitory site bound with ADP (top), ATPγS (middle), and ATP (EMD-7073 and PDB: 6BAA) (bottom) in different states. Maps of Kir6.2 are colored in green. Nucleotide densities are shown as blue meshes with nucleotide model shown as sticks.
(D) Similar asymmetric NBD dimer induced by the Mg-ADP (colored spheres) or Mg-ATP/Mg-ADP (grey spheres) molecules.

Figure Supplement 7. Extra densities on the map of SUR1 in the Mg-ADP state.

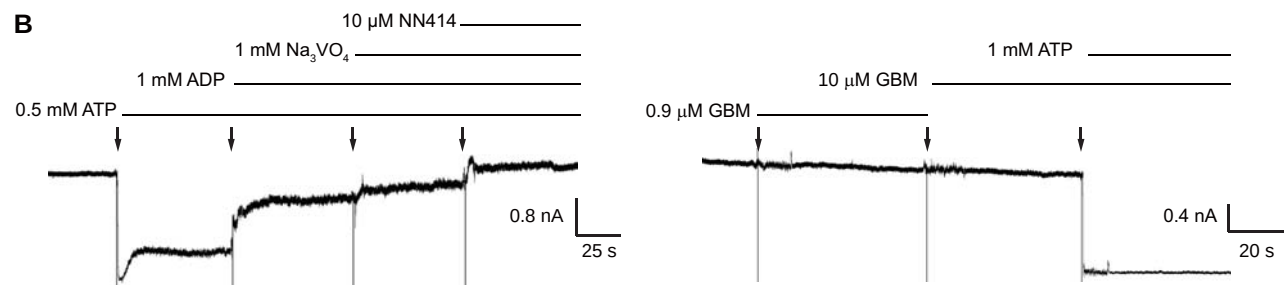
(A) Chemical structure of NN414 (6-chloro-3-[[1-methylcyclopropyl]amino]-4H-thieno[3,2-e]-1,2,4-thiadiazine 1,1-dioxide tifenazoxide).
(B) Local EM density maps surrounded by M10-12 and M17 in the Mg-ADP state (top) with one extra density (density 1: yellow). The same site in the Mg-ATP&ADP state (bottom, EMD-7338, PDB: 6C3O) is apo.
(C) Local EM density maps surrounded by M8 and M15–17 in the Mg-ADP state (top) the other extra density (density 2: red). The same site in the Mg-ATP&ADP state EMD-7338, PDB: 6C3O) is apo. Figures are created from 4.2 Å, focused refinement map of SUR1 ABC transporter module. The cryo-EM density maps are contoured to the same level as in panel (B). Densities of SUR1 molecule are colored the same as in Figure 1.
(D) Superposition of the structures with (Mg-ADP state) and without NN414 (Mg-ATP&ADP state) by aligning the TMD domain of SUR1. Local conformational changes of transmembrane helices (M9-11) at density 1 are indicated by red arrows. A surface representation of density 1 is shown in yellow.

Figure S1

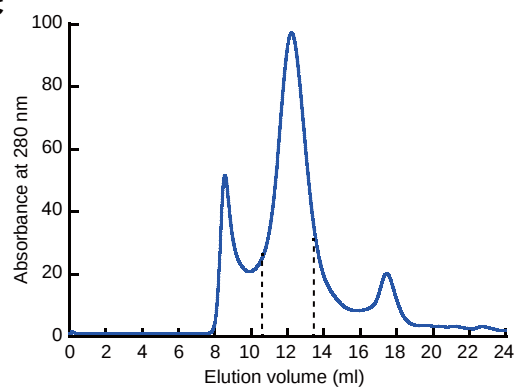
A



B



C



D

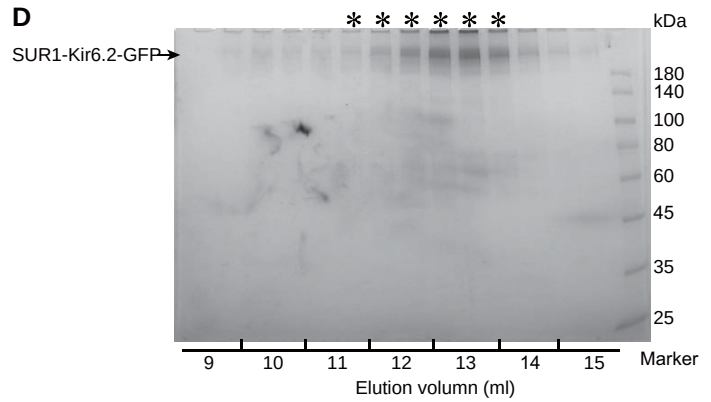
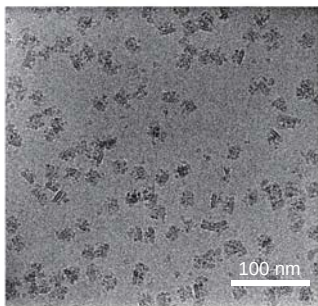


Figure S2

A



B

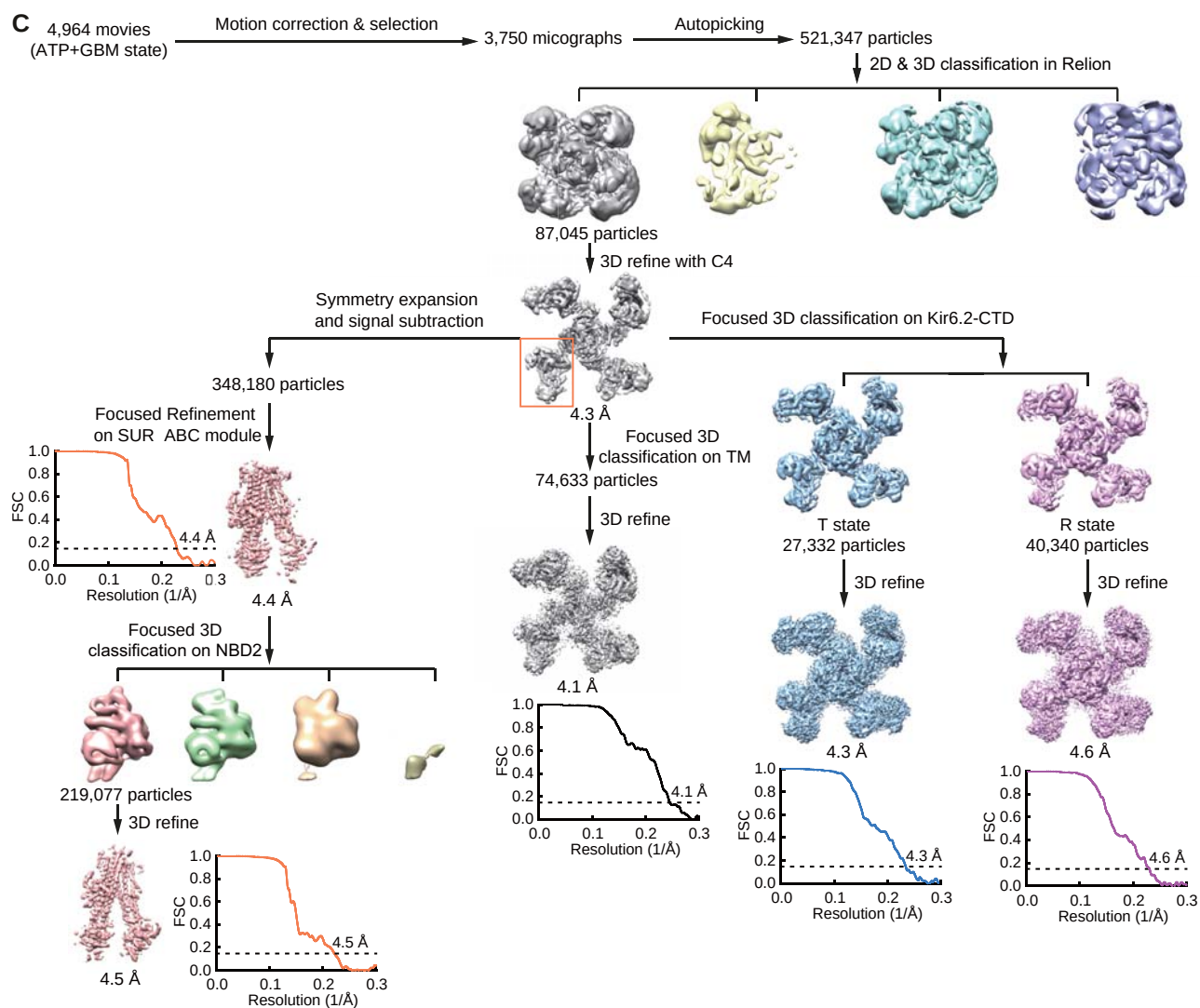


Figure S3

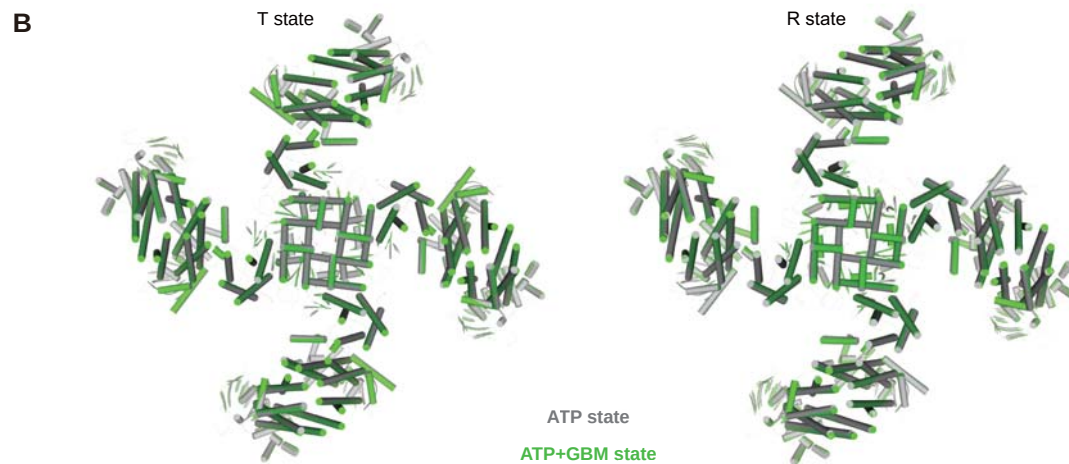
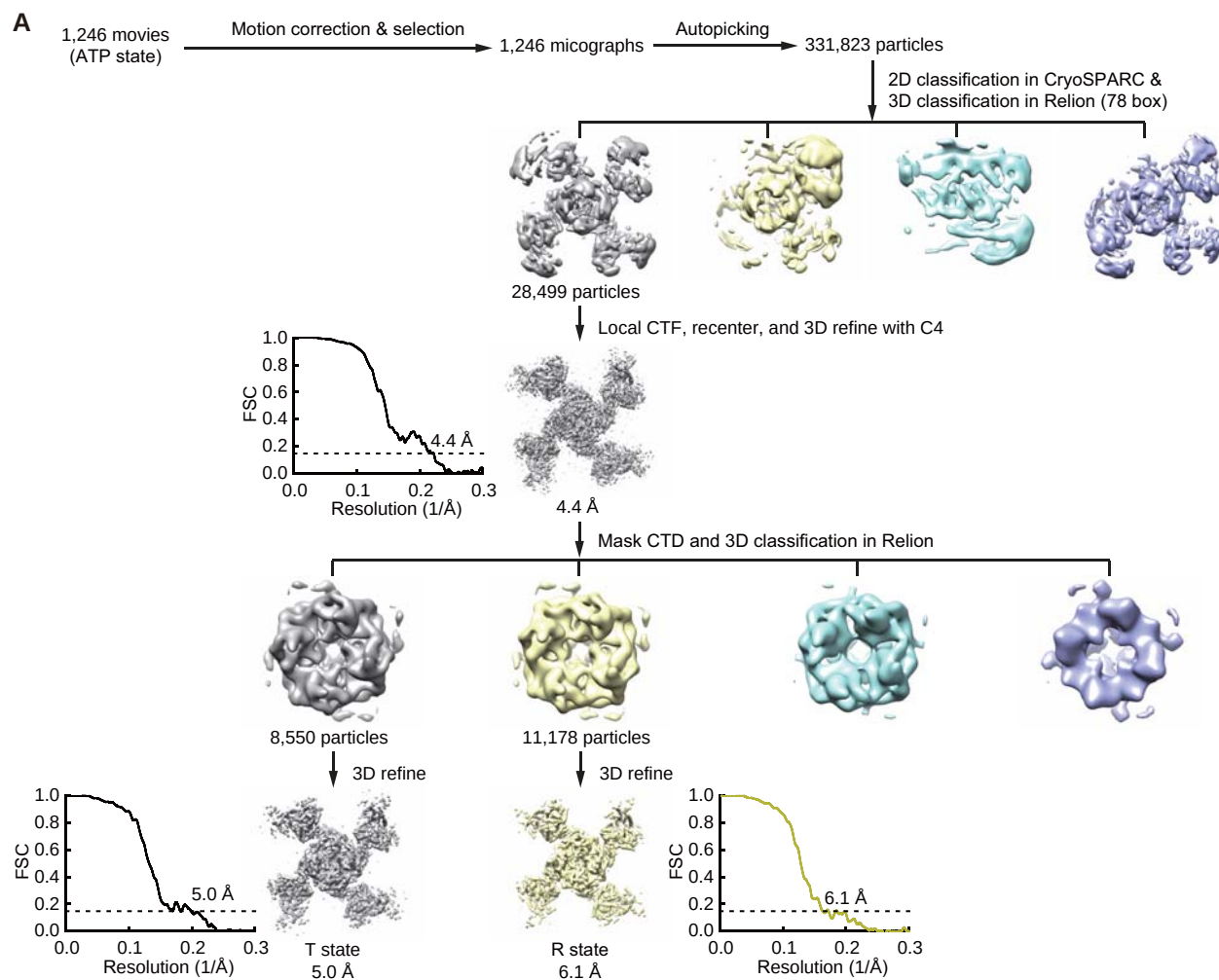


Figure S4

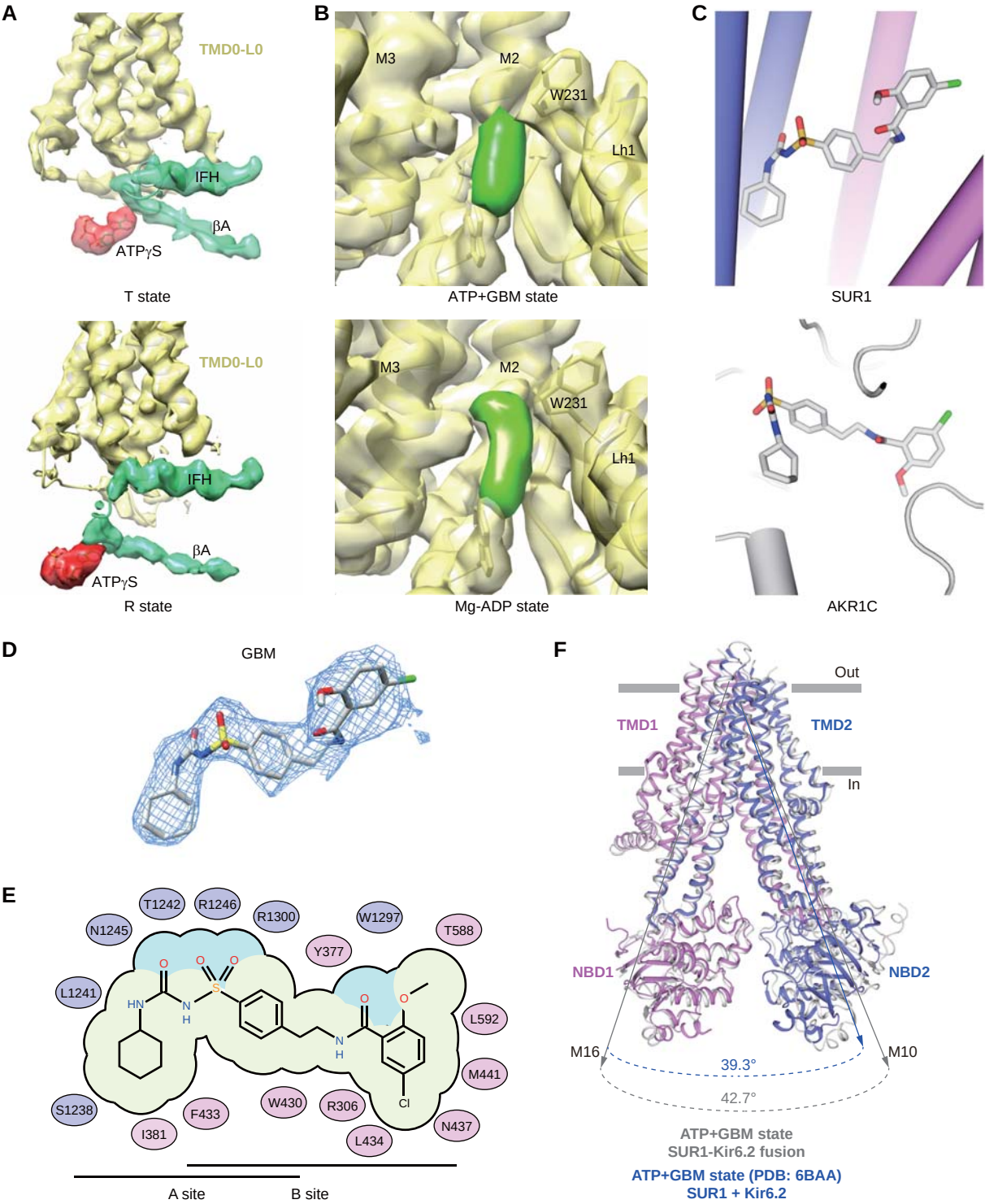


Figure S5

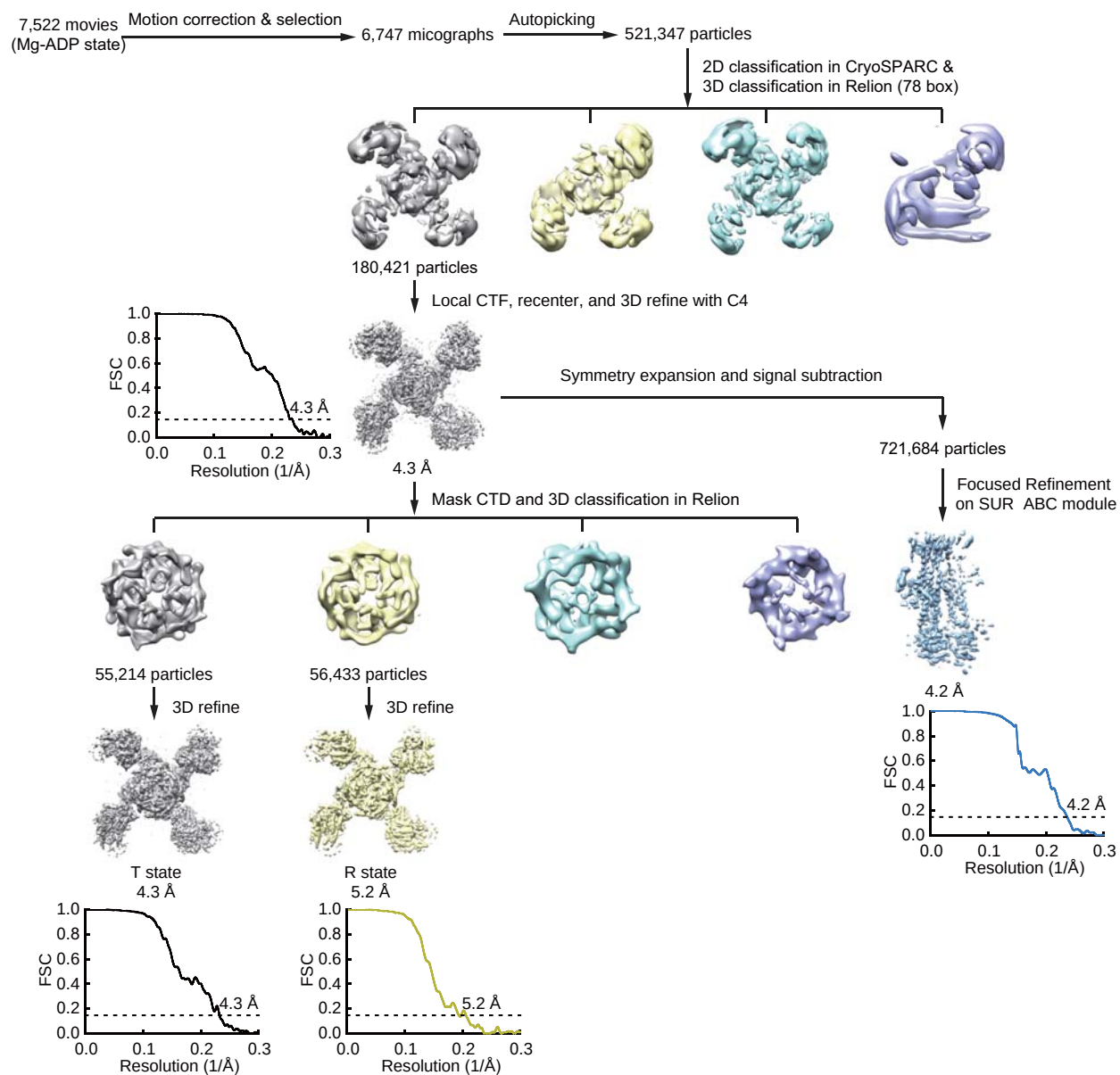
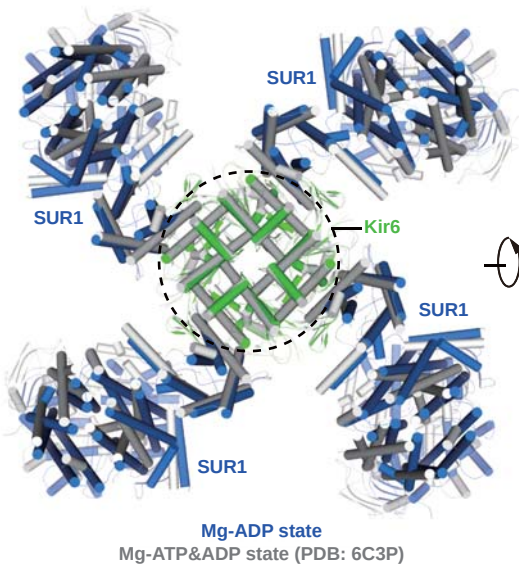
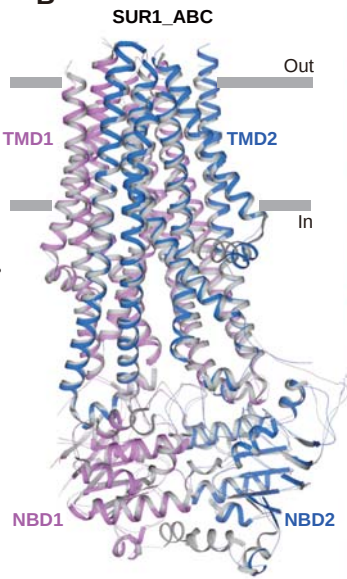


Figure S6

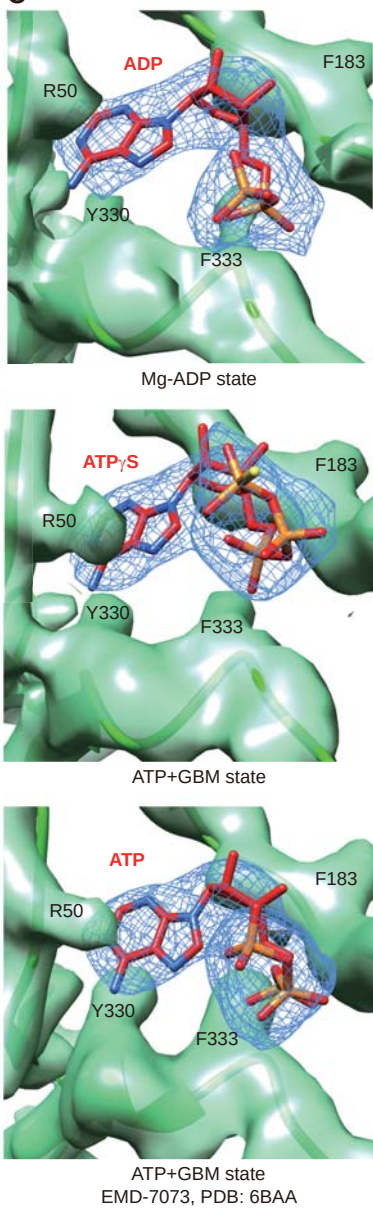
A



B



C



D

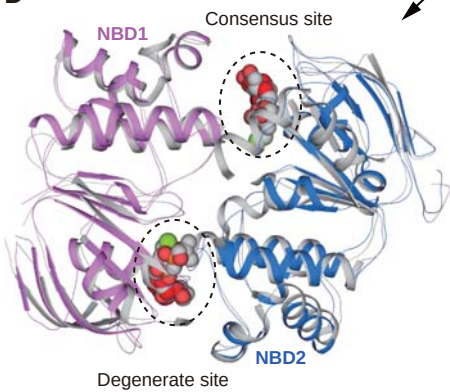
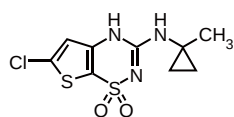


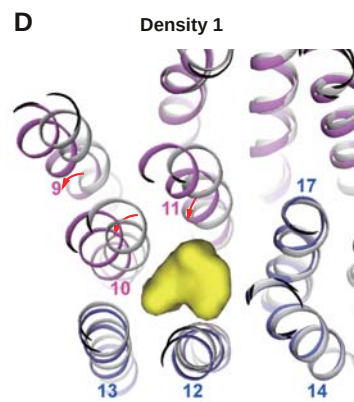
Figure S7

A



NN414

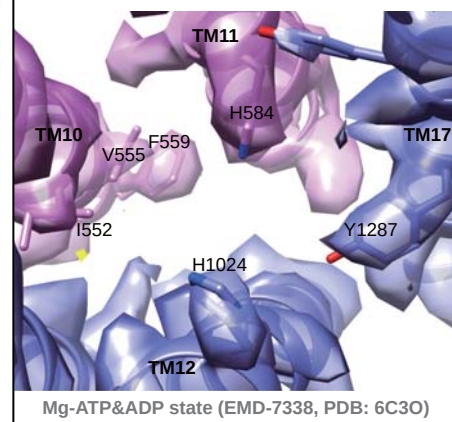
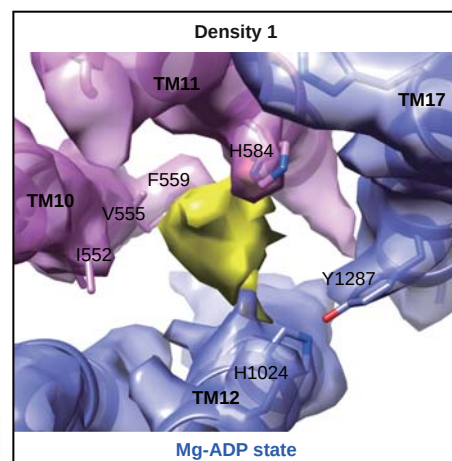
D



Mg-ADP state

Mg-ATP&ADP state
(EMD-7338, PDB: 6C3O)

B



C

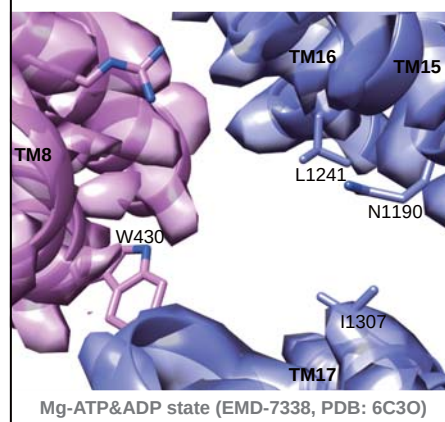
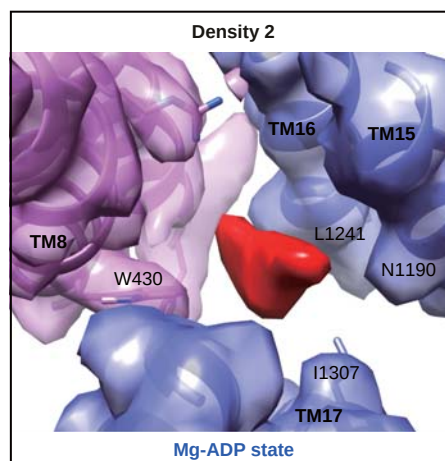


Table S1.
Cryo-EM data collection, refinement and validation statistics of the ATP+GBM state of K_{ATP}.

	Focus on TM	Class 1 of Kir6.2 CTD	Class 2 of Kir6.2 CTD	Focus on SUR1 ABC module	Class 1 of SUR1 NBD2
	EMD-6831 5YKE	EMD-6832 5YKF	EMD-6833 5YKG	EMD-6847 5YW7	
Data collection and processing					
Magnification			47,400×		
Voltage (kV)			300		
Electron exposure (e ⁻ /Å ²)			52		
Defocus range (μm)			-1.5 to -3.5		
Pixel size (Å)			1.055		
Symmetry imposed	<i>C4</i>	<i>C4</i>	<i>C4</i>	<i>C1</i>	<i>C1</i>
Final particle images (no.)	74,633	27,332	40,340	348,180	219,077
Map resolution (Å)	4.1	4.3	4.6	4.4	4.5
FSC threshold	0.143	0.143	0.143	0.143	0.143
Map sharpening B factor (Å ²)	-204	-187	-210	-300	-307
Refinement					
Model composition					
Non-hydrogen atoms	30,636	50,988	50,868	8,944	
B factors (Å ²)					
Non-hydrogen atoms	130.49	151.43	152.64	168.47	
R.m.s. deviations					
Bond lengths (Å)	0.005	0.005	0.003	0.003	
Bond angles (°)	0.780	0.765	0.654	0.658	
Validation					
MolProbity score	2.52	2.71	2.61	2.57	
Clashscore	11.31	15.14	13.19	13.99	
Poor rotamers (%)	7.60	8.16	7.90	6.49	
Ramachandran plot					
Favored (%)	95.93	95.15	95.65	95.72	
Allowed (%)	3.65	4.42	4.23	4.28	
Disallowed (%)	0.42	0.44	0.12	0.00	

Table S2.
Cryo-EM data collection, refinement and validation statistics of the ATP state of K_{ATP}.

	All particles EMD-6848 5YW8	Class 1 of Kir6.2 CTD EMD-6849 5YW9	Class 2 of Kir6.2 CTD EMD-6850 5YWA
Data collection and processing			
Magnification		37,300×	
Voltage (kV)		300	
Electron exposure (e ⁻ /Å ²)		52	
Defocus range (μm)		-1.5 to -3.5	
Pixel size (Å)		1.34	
Symmetry imposed		C4	
Final particle images (no.)	28,499	8,550	11,178
Map resolution (Å)	4.4	5.0	6.1
FSC threshold	0.143	0.143	0.143
Map sharpening B factor (Å ²)	-198	-161	-195
Refinement			
Model composition			
Non-hydrogen atoms	50,524	50,524	50,496
B factors (Å ²)			
Non-hydrogen atoms	219.24	219.24	505.25
R.m.s. deviations			
Bond lengths (Å)	0.005	0.005	0.004
Bond angles (°)	0.796	0.796	0.693
Validation			
MolProbity score	2.81	2.81	2.72
Clashscore	19.01	19.00	17.20
Poor rotamers (%)	8.11	8.11	7.91
Ramachandran plot			
Favored (%)	95.13	95.13	95.65
Allowed (%)	4.43	4.43	4.17
Disallowed (%)	0.44	0.44	0.19

Table S3.
Cryo-EM data collection, refinement and validation statistics of the Mg-ADP state of K_{ATP}.

	All particles	Class 1 of Kir6.2 CTD EMD-6852 5YWC	Class 2 of Kir6.2 CTD EMD-6851 5YWB	Focus on SUR1 ABC module EMD-6853 5YWD
Data collection and processing				
Magnification		47,400×		
Voltage (kV)		300		
Electron exposure (e ⁻ /Å ²)		52		
Defocus range (μm)		-1.5 to -3.5		
Pixel size (Å)		1.055		
Symmetry imposed	<i>C4</i>	<i>C4</i>	<i>C4</i>	<i>C1</i>
Final particle images (no.)	180,421	55,214	56,433	721,684
Map resolution (Å)	4.3	4.3	5.2	4.2
FSC threshold	0.143	0.143	0.143	0.143
Map sharpening <i>B</i> factor (Å ²)	-227	-188	-265	-269
Refinement				
Model composition				
Non-hydrogen atoms		51,108	50,928	8,997
<i>B</i> factors (Å ²)				
Non-hydrogen atoms		283.99	300.02	203.33
R.m.s. deviations				
Bond lengths (Å)		0.004	0.004	0.003
Bond angles (°)		0.796	0.776	0.718
Validation				
MolProbity score		2.69	2.62	2.49
Clashscore		14.70	13.27	12.19
Poor rotamers (%)		7.97	7.66	6.20
Ramachandran plot				
Favored (%)		95.26	95.50	95.87
Allowed (%)		4.47	4.07	4.13
Disallowed (%)		0.37	0.43	0.00