Supplementary Materials for

Structural basis for the subtype-selectivity of K_{Ca}2.2 channel activators

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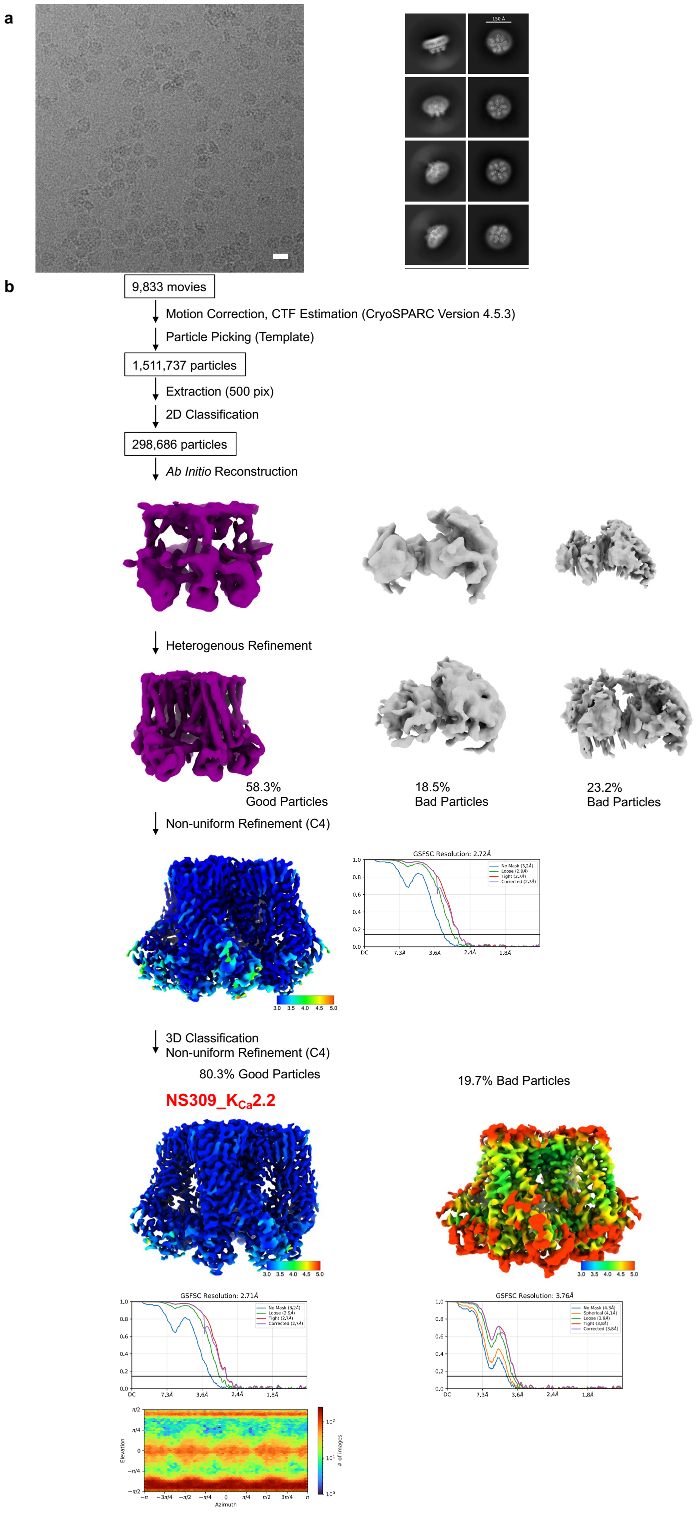
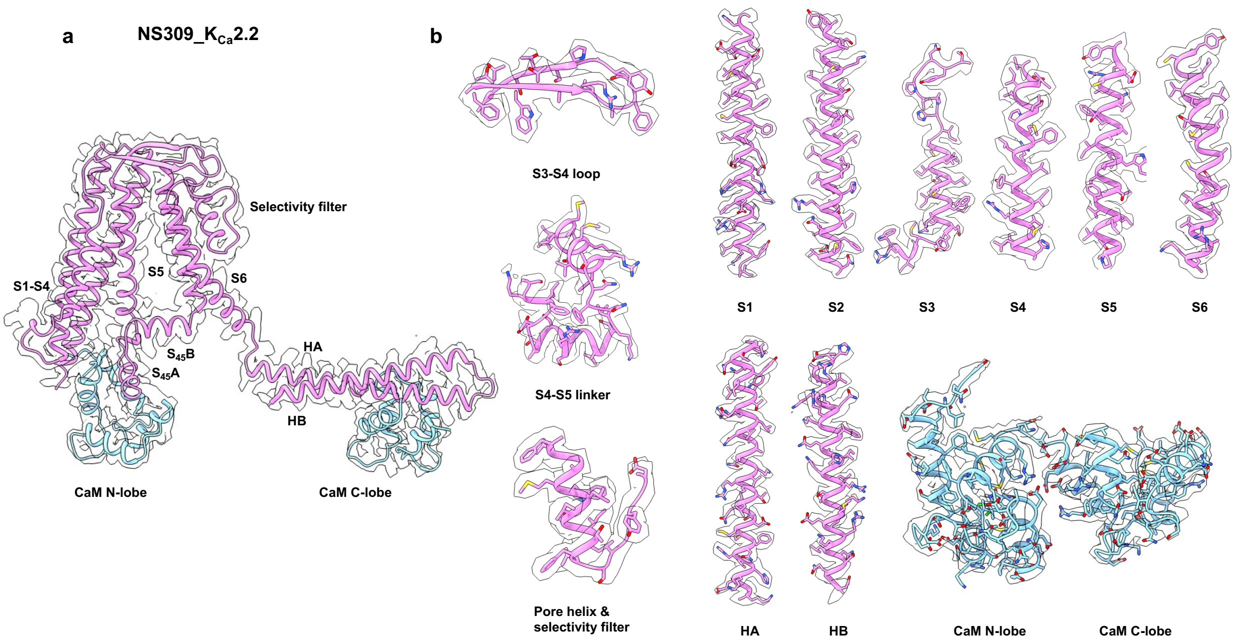
Supplementary Figs. 1 to 10
Supplementary Table 1

Supplementary Table 1 | Cryo-EM data collection, refinement and validation statistics structures

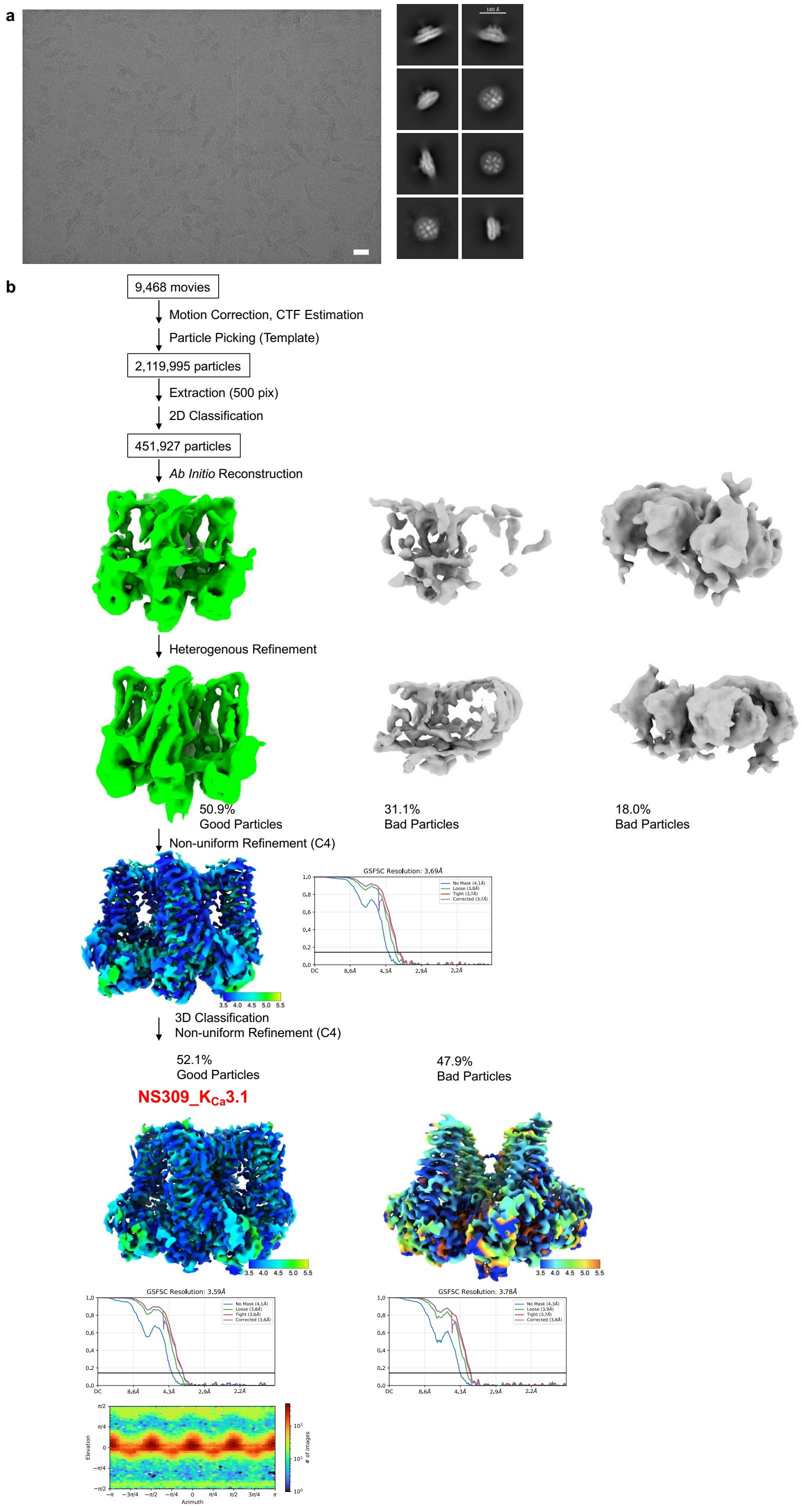
	NS309_K_{Ca}2.2 (EMDB-70207) (PDB-9O7S)	**NS309_K_{Ca}3.1** (EMDB-70275) (PDB-9OA8)	**Rimtuzalcap_K_{Ca}2.2_I** (EMDB-70217) (PDB-9O85)	**Rimtuzalcap_K_{Ca}2.2_II** (EMDB-70240) (PDB-9O93)
Data collection and processing				
Magnification	165,000	105,000	105,000	105,000
Voltage (kV)	300	300	300	300
Electron exposure (e–/Å^2)	50	50	50	50
Defocus range (μm)	-0.6 to -2.2	-1.0 to -2.0	-1.3 to -2.3	-1.3 to -2.3
Pixel size (Å)	0.73	0.86	0.86	0.86
Symmetry imposed	C4	C4	C4	C4
Initial particle images (no.)	1,511,737	2,119,995	2,453,407	2,453,402
Final particle images (no.)	139,830	119,846	64,173	102,190
Map resolution (Å)	2.71	3.59	3.13	2.96
FSC threshold	0.143	0.143	0.143	0.143
Refinement				
Initial model used (PDB code)	8v2g	6cno	8v2g	8v2g
Model resolution (Å)	2.8	3.7	3.2	3.0
FSC threshold	0.5	0.5	0.5	0.5
Map sharpening *B* factor (Å^2)	-29.1	37.73	6.48	-16.18
Model composition				
Non-hydrogen atoms	15539	15255	16403	16057
Protein residues	2024	2024	2096	2096
Ligands	K: 3, CA: 8	K: 3, CA: 8	K: 3, CA: 8	K: 2
B factors (Å^2)				
Protein	76.31	25.41	42.57	88.95
Ligand	104.99	29.49	41.61	105.19
R.m.s. deviations				
Bond lengths (Å)	0.006	0.003	0.003	0.004
Bond angles (°)	0.561	0.465	0.505	0.519
Validation				
MolProbity score	1.51	1.21	1.32	1.33
Clashscore	4.26	2.09	2.35	2.84
Poor rotamers (%)	0.00	0.00	0.00	0.00
Ramachandran plot				
Favored (%)	95.67	96.50	5.67	96.15
Allowed (%)	4.33	3.50	4.33	3.85
Disallowed (%)	0.00	0.00	0.00	0.00



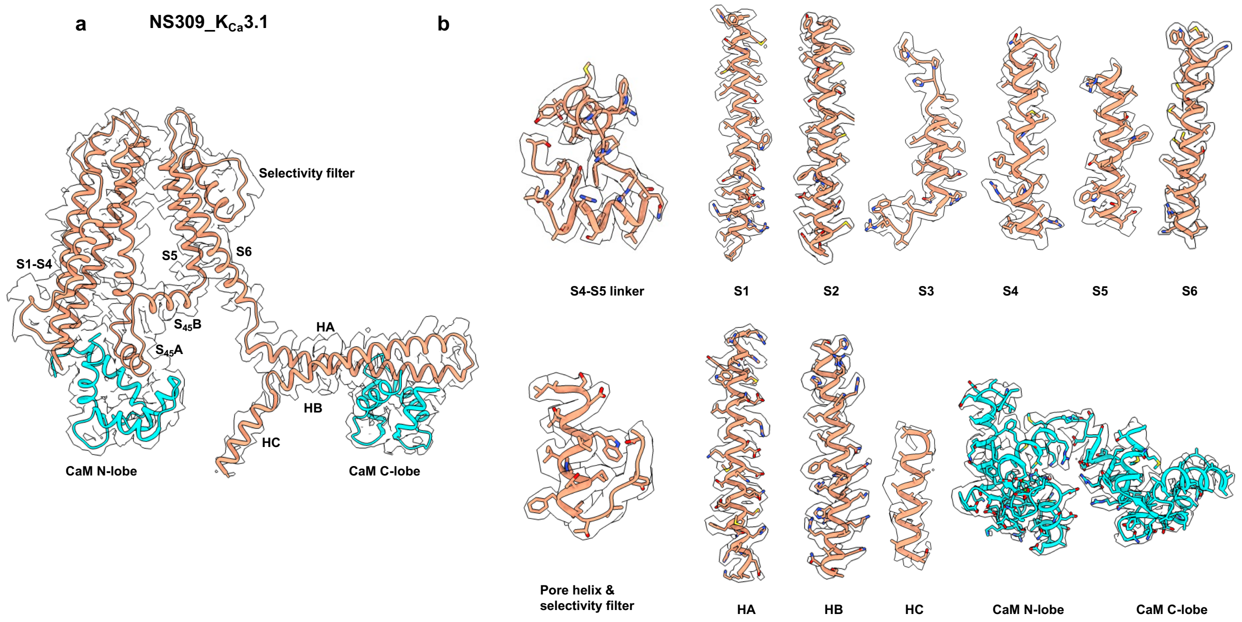
Supplementary Fig. 1 Single-particle cryo-EM analysis procedure for the NS309_K$_{Ca}$2.2 complex. a A representative cryo-EM micrograph and two-dimensional classification averages of the NS309_K$_{Ca}$2.2 complex. Scale bar (200 Å) is shown in white inside the micrograph. **b** Cryo-EM data processing workflow of the NS309_K$_{Ca}$2.2 complex. The cryo-EM map used for model building is labeled in red font.



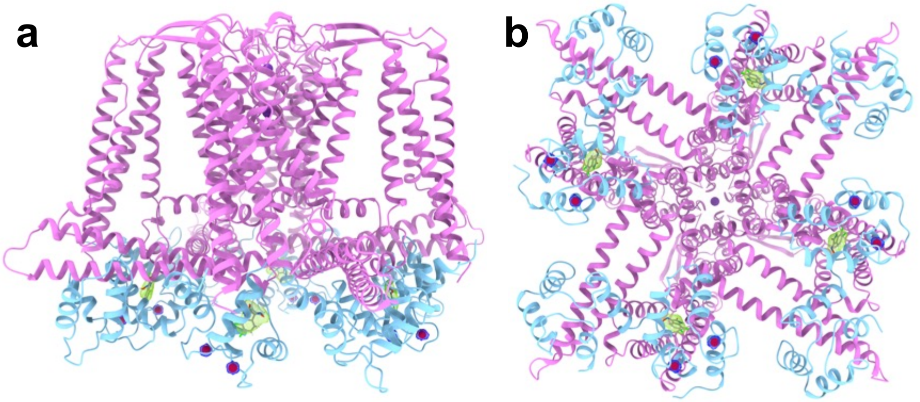
Supplementary Fig. 2 Cryo-EM density maps with fitted models of NS309_K$_{Ca}$2.2. a Single subunit of NS309_K$_{Ca}$2.2 including S1-S6 transmembrane domains and pore domain (purple). One CaM bound to the S4-S5 linker, and one CaM bound to the HA/HB helices in the C-terminus are shown in light blue. **b** Cryo-EM density map with the fitted model of NS309_K$_{Ca}$2.2 showing side chain densities in the S3-S4 loop, S4-S5 linker, S1 to S6 helices, pore helix and selectivity filter, HA/HB helices in the C-terminus, CaM N-lobe, and CaM C-lobe.



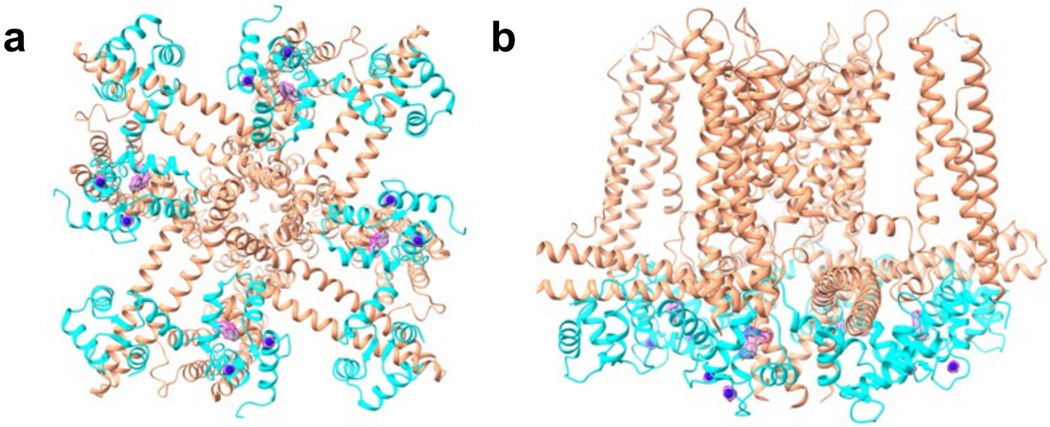
Supplementary Fig. 3 Single-particle cryo-EM analysis procedure for the NS309_K$_{Ca}$3.1 complex. a A representative cryo-EM micrograph and two-dimensional classification averages of the NS309_K$_{Ca}$3.1 complex. Scale bar (200 Å) is shown in white inside the micrograph. **b** Cryo-EM data processing workflow of the NS309_K$_{Ca}$3.1 complex. The cryo-EM map used for model building is labeled in red font.



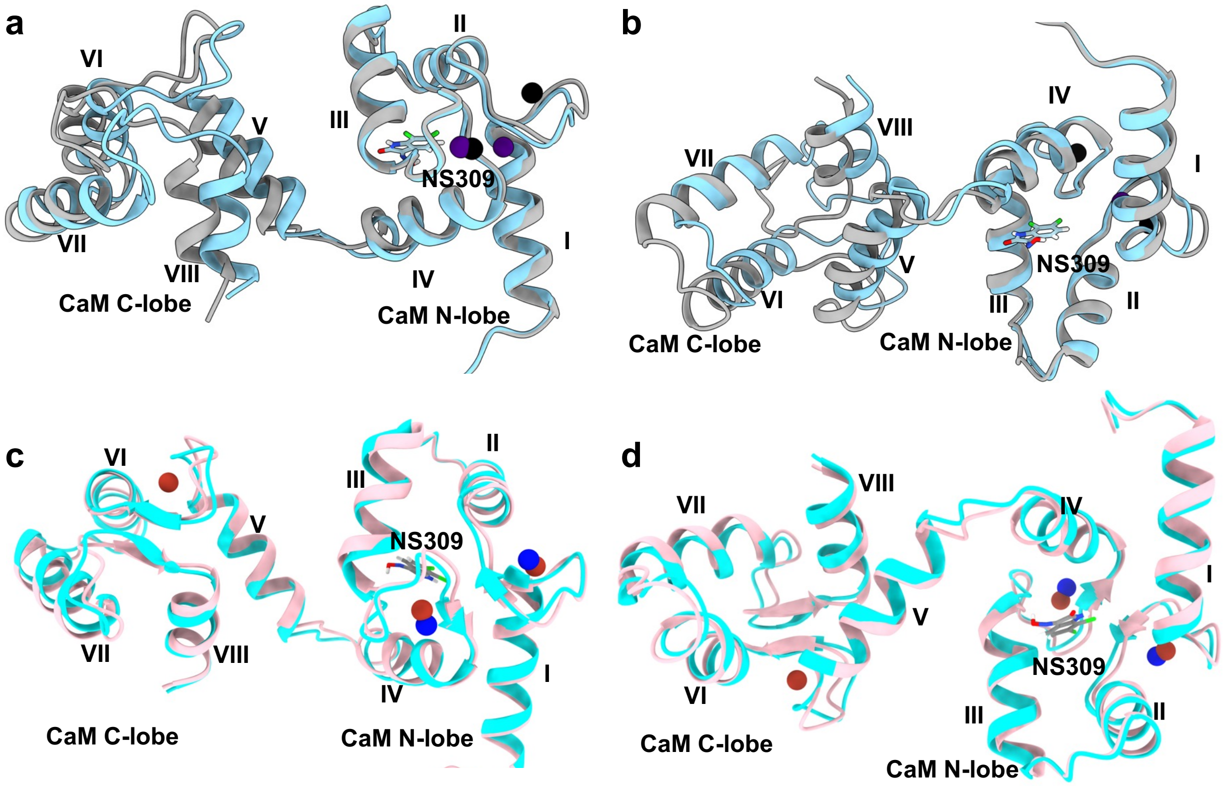
Supplementary Fig. 4 Cryo-EM density map with fitted models of NS309_K_{Ca}3.1. a Single subunit of NS309_K_{Ca}3.1 including S1-S6 transmembrane domains and pore domain (salmon). One CaM bound to the S4-S5 linker and one CaM bound to the HA/HB helices in the C-terminus are shown in cyan. **b** Cryo-EM density map with the fitted model of NS309_K_{Ca}3.1 showing side chain densities in the S4-S5 linker, S1 to S6 helices, pore helix and selectivity filter, HA, HB and HC helices in the C-terminus, CaM N-lobe, and CaM C-lobe.



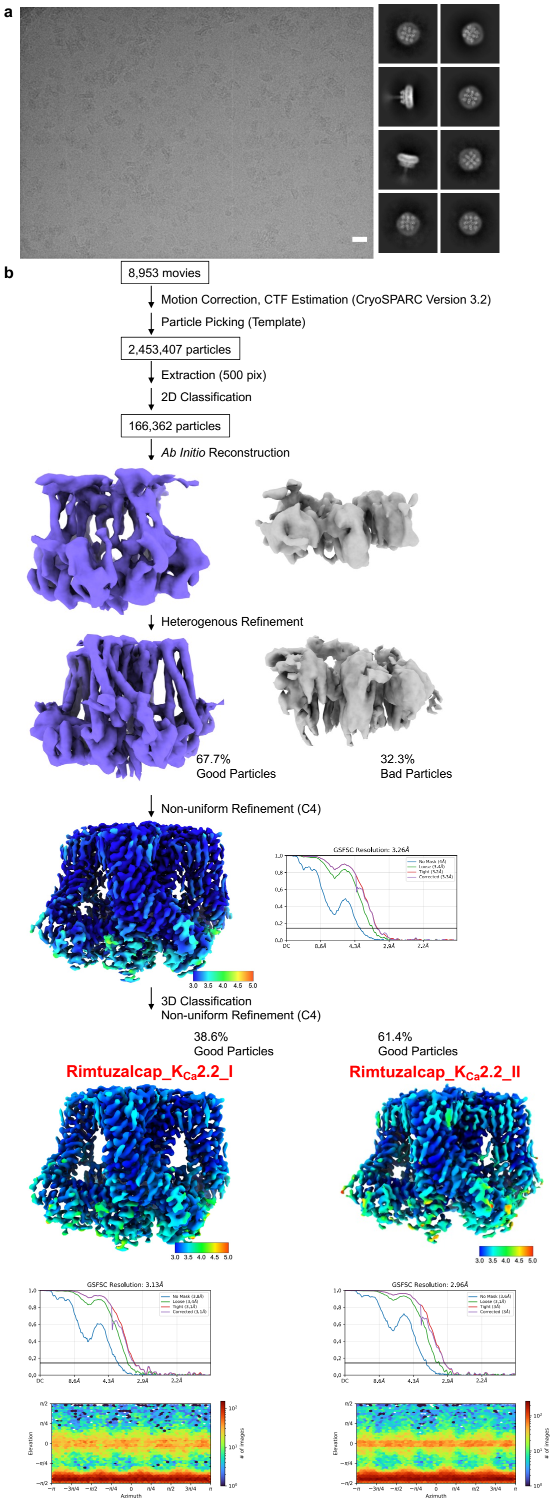
Supplementary Fig. 5 Densities of Ca^{2+} and NS309 in NS309_K$_{Ca}$2.2. Side view (**a**) and intracellular view (**b**) of NS309_K$_{Ca}$2.2 (K$_{Ca}$2.2: purple/CaM: light blue). Cryo-EM densities for Ca^{2+} and NS309 are shown as blue and green mesh, respectively. The densities are contoured at $\sigma = 6$.



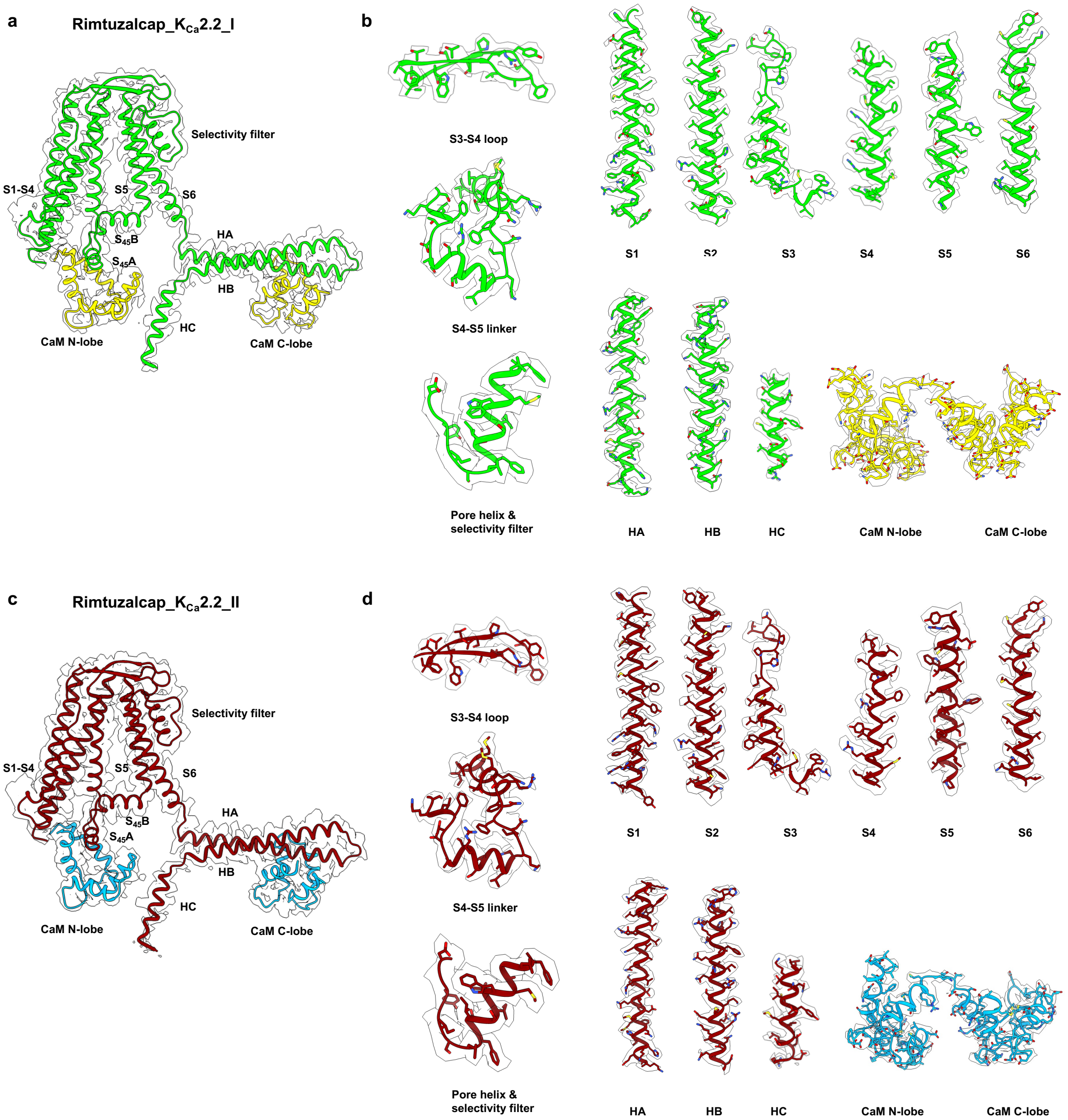
Supplementary Fig. 6 Densities of Ca^{2+} and NS309 in NS309_K$_{\mathrm{Ca}}$3.1. Side view (**a**) and intracellular view (**b**) of NS309_K$_{\mathrm{Ca}}$3.1 (K$_{\mathrm{Ca}}$3.1: salmon/CaM: cyan). Cryo-EM densities for Ca^{2+} and NS309 are shown as magenta mesh. The densities are contoured at $\sigma = 6$.



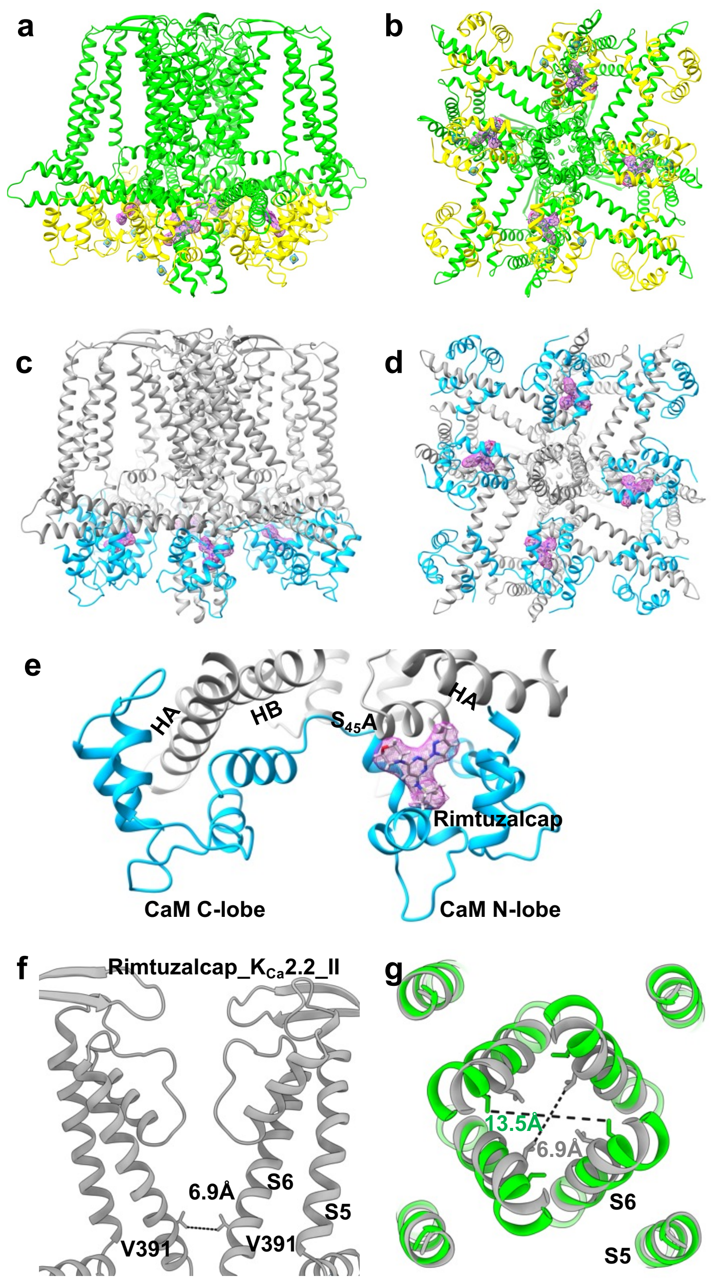
Supplementary Fig. 7 Conformations of CaM in NS309_K_{Ca}2.2 and NS309_K_{Ca}3.1. a A Ca^{2+}-bound CaM molecule of NS309_K_{Ca}2.2 (CaM: blue/Ca^{2+}: indigo blue) superimposed on a Ca^{2+}-bound CaM of apo_K_{Ca}2.2 (CaM: gray/Ca^{2+}: black) with the hydrophobic surfaces at the N- and C-lobes facing away. **b** One Ca^{2+}-bound CaM molecule of NS309_K_{Ca}2.2 superimposed on a Ca^{2+}-bound CaM of apo_K_{Ca}2.2 with the hydrophobic surfaces at the N- and C-lobes facing the viewer. **c** A Ca^{2+}-bound CaM molecule of NS309_K_{Ca}3.1 (CaM: cyan/Ca^{2+}: blue) superimposed on a Ca^{2+}-bound CaM of apo_K_{Ca}3.1 (CaM: pink/Ca^{2+}: maroon) with the hydrophobic surfaces at the N- and C-lobes facing away. **d** One Ca^{2+}-bound CaM molecule of NS309_K_{Ca}3.1 superimposed on a Ca^{2+}-bound CaM of apo_K_{Ca}3.1 with the hydrophobic surfaces at the N- and C-lobes facing the viewer.



Supplementary Fig. 8 Single-particle cryo-EM analysis procedure for the rimtuzalcap_$\mathrm{K_{Ca}}$2.2 complex. a A representative cryo-EM micrograph and two-dimensional classification averages of the rimtuzalcap_$\mathrm{K_{Ca}}$2.2 complex. Scale bar (200 Å) is shown in white inside the micrograph. **b** Cryo-EM data processing workflow of the rimtuzalcap_$\mathrm{K_{Ca}}$2.2 complex. The cryo-EM maps used for model building are labeled in red font.



Supplementary Fig. 9 Cryo-EM density map with fitted models of rimtuzalcap_K_{Ca}2.2_I and rimtuzalcap_K_{Ca}2.2_II. a Single subunit of rimtuzalcap_K_{Ca}2.2_I including S1-S6 transmembrane domains and pore domain (green). One CaM bound to the S4-S5 linker, and one CaM bound to the HA/HB helices in the C-terminus are shown in yellow. **b** Cryo-EM density map with the fitted model of rimtuzalcap_K_{Ca}2.2_I showing side chain densities in the S3-S4 loop, S4-S5 linker, S1 to S6 helices, pore helix and selectivity filter, HA, HB, and HC helices in the C-terminus, CaM N-lobe, and CaM C-lobe. **c** Single subunit of rimtuzalcap_K_{Ca}2.2_II including S1-S6 domains and pore domain (green). One CaM bound to the S4-S5 linker, and one CaM bound to the HA/HB helices in the C-terminus are shown in yellow. **d** Cryo-EM density map with the fitted model of rimtuzalcap_K_{Ca}2.2_II showing side chain densities in the S3-S4 loop, S4-S5 linker, S1 to S6 helices, pore helix and selectivity filter, HA, HB, and HC helices in the C-terminus, CaM N-lobe, and CaM C-lobe.



Supplementary Fig. 10 Comparison of rimtuzalcap_K_{Ca}2.2_I and rimtuzalcap_K_{Ca}2.2_II. Side view (**a**) and intracellular view (**b**) of rimtuzalcap_K_{Ca}2.2_I (K_{Ca}2.2: green/CaM: yellow). Side view (**c**) and intracellular view (**d**) of rimtuzalcap_K_{Ca}2.2_II (K_{Ca}2.2: gray/CaM: cyan). Cryo-EM densities for Ca^{2+} and rimtuzalcap are shown as magenta mesh. **e** Cryo-EM densities for rimtuzalcap bound to one CaM molecule is shown as magenta mesh. In rimtuzalcap_K_{Ca}2.2_II, density for Ca^{2+} is not present. The densities are contoured at $\sigma = 6$. **f** In the side view, the dimension of the inner gate are measured as distances between Val391 in the transmembrane S6 helices of the opposite K_{Ca}2.2 subunits in rimtuzalcap_K_{Ca}2.2_II. **g** The intercellular views of the inner gate in the superimposed rimtuzalcap_K_{Ca}2.2_I (green) and rimtuzalcap_K_{Ca}2.2_II (gray) structures.