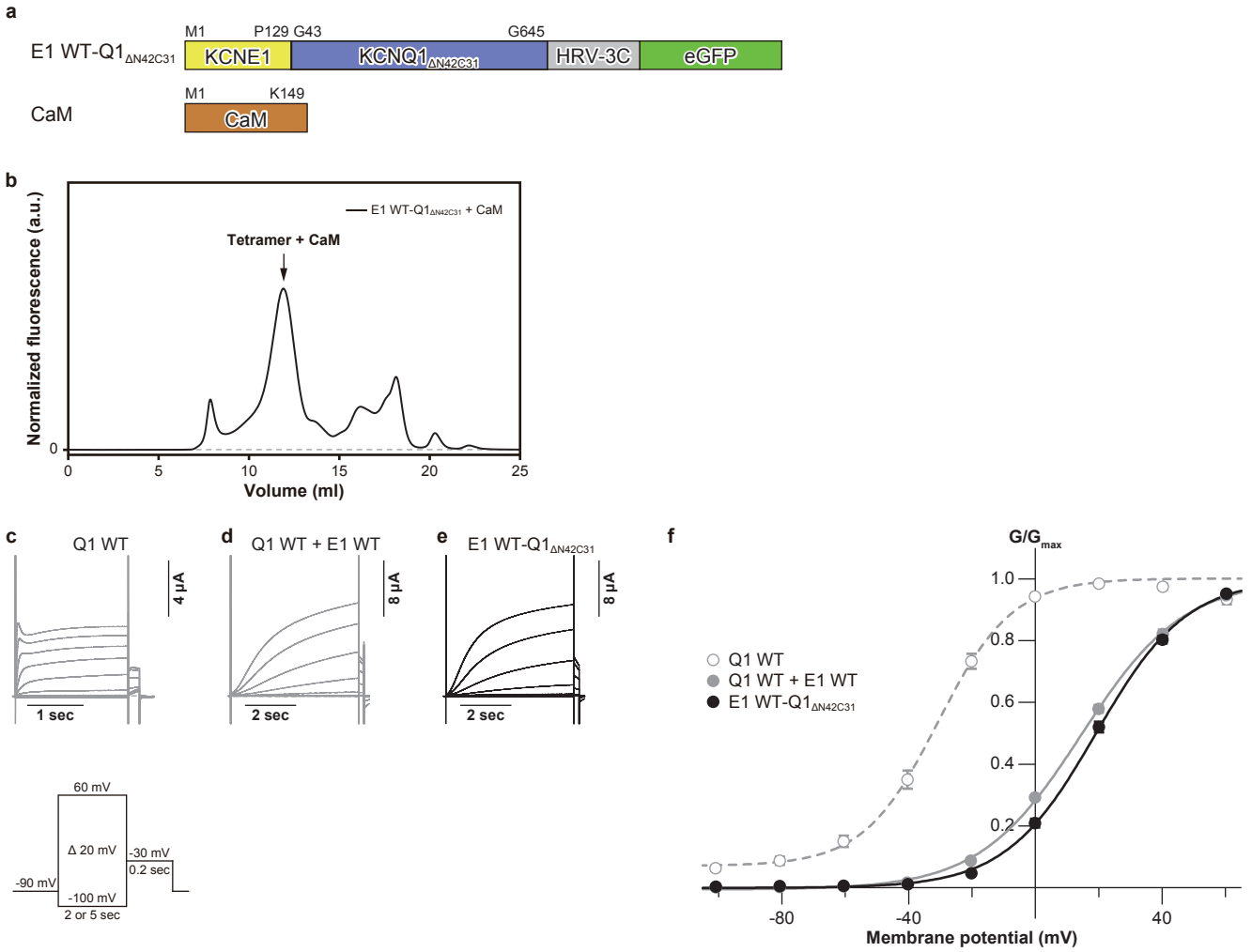
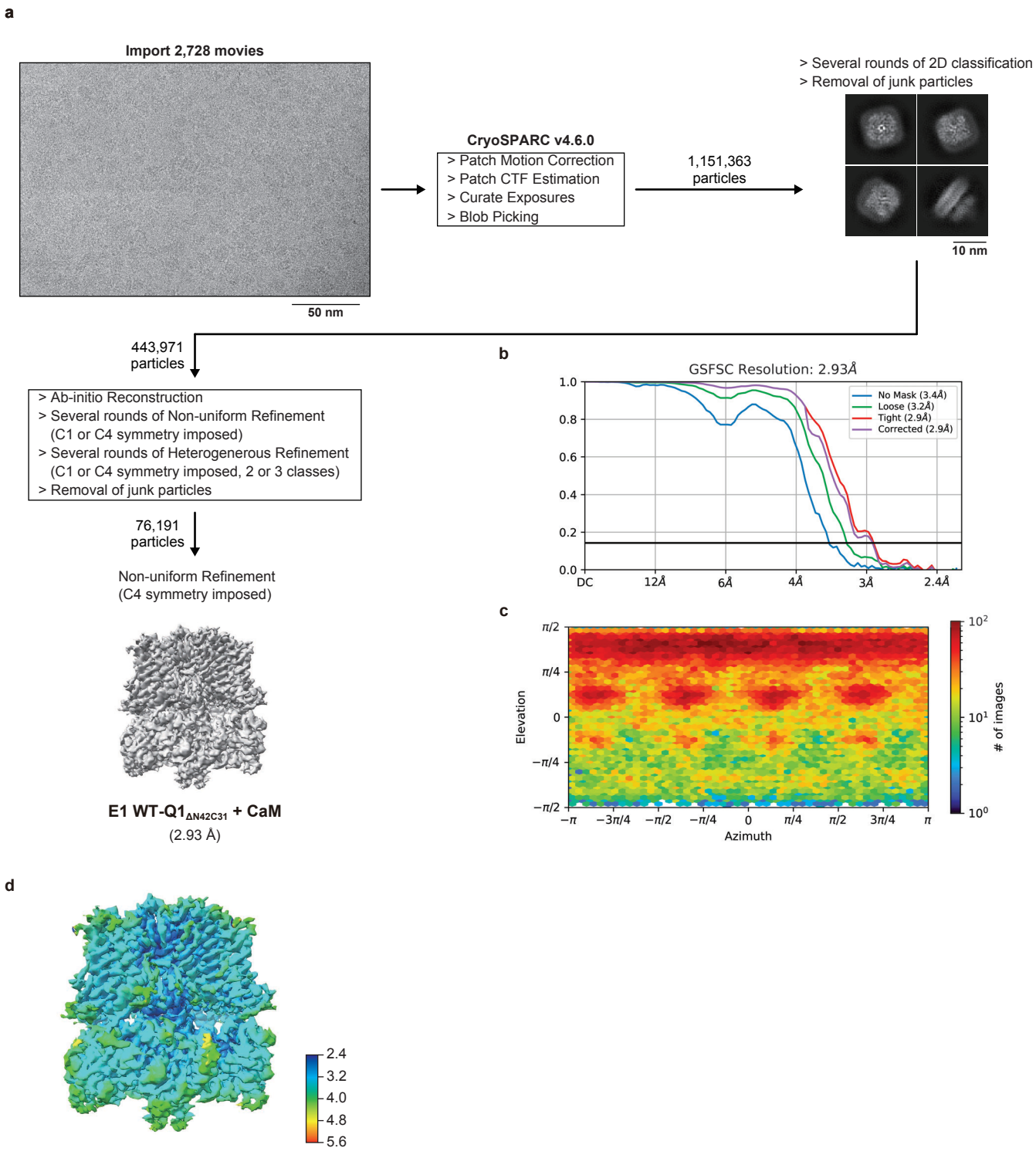
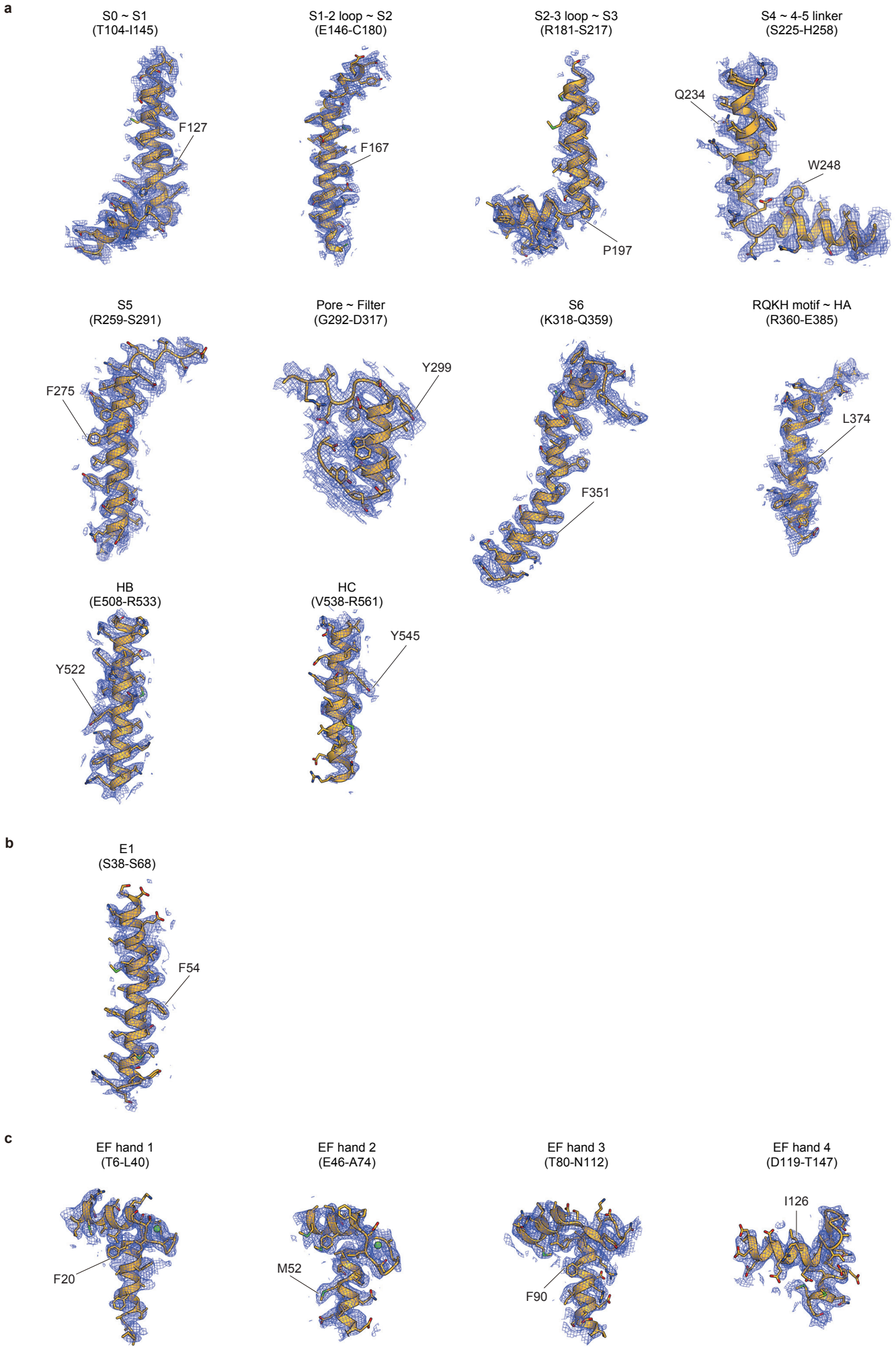


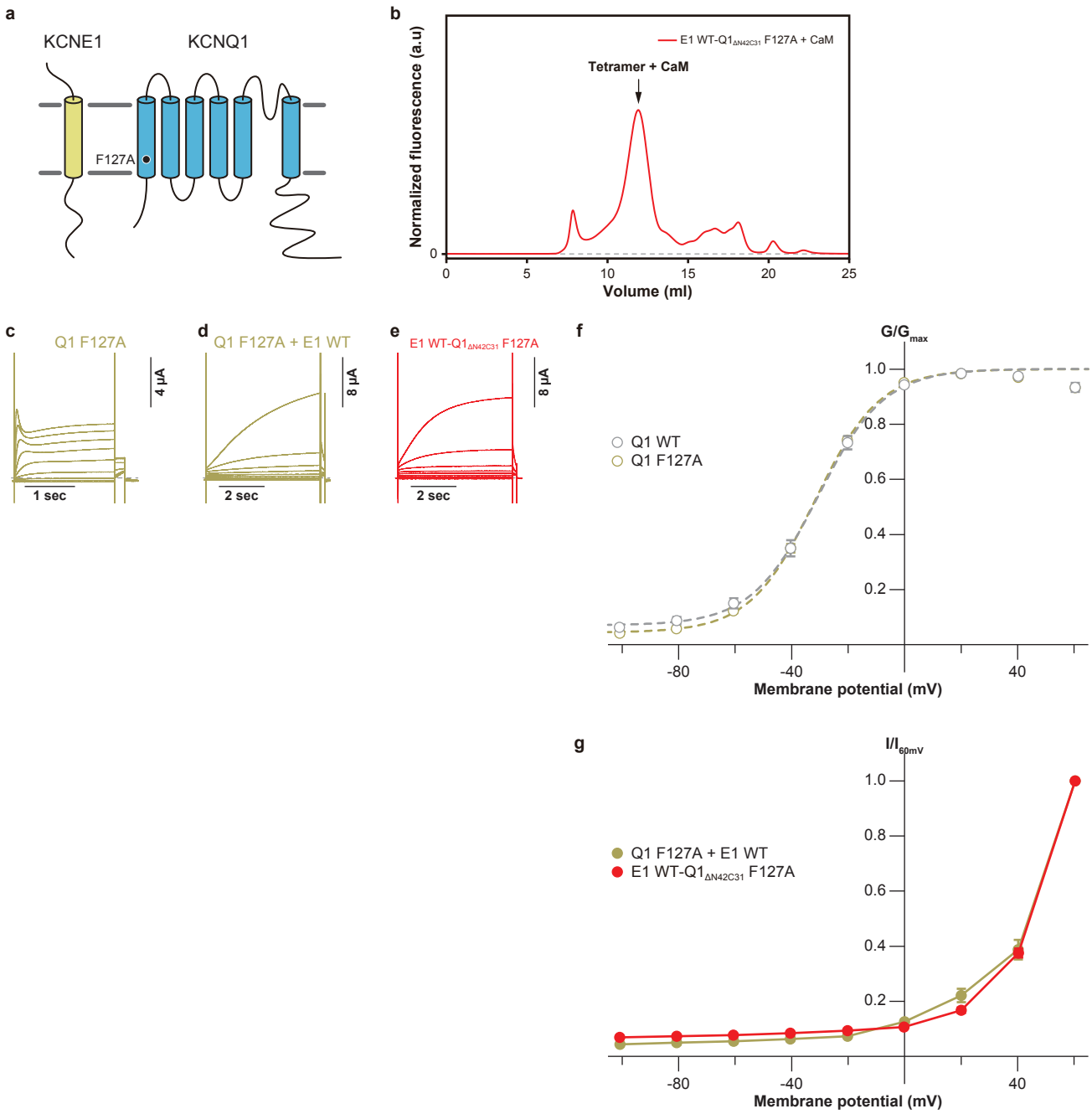
Supplementary Figure 1



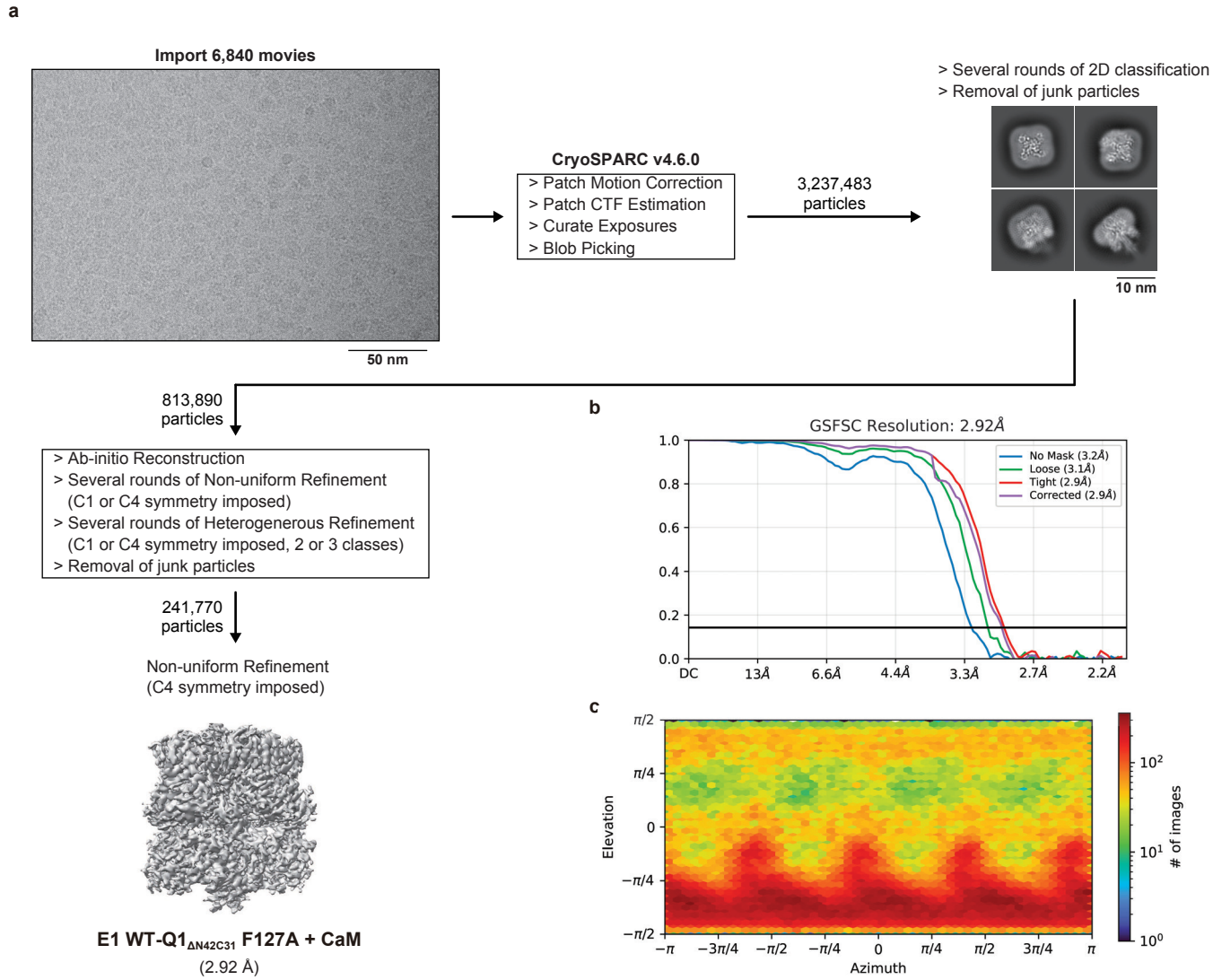
Supplementary Figure 2

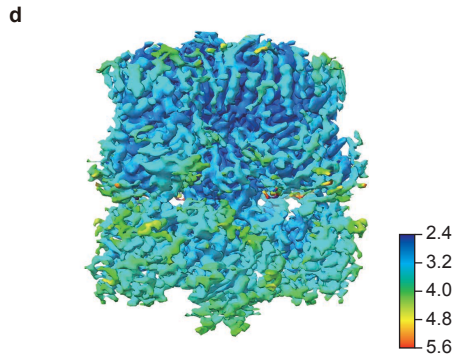


Supplementary Figure 3

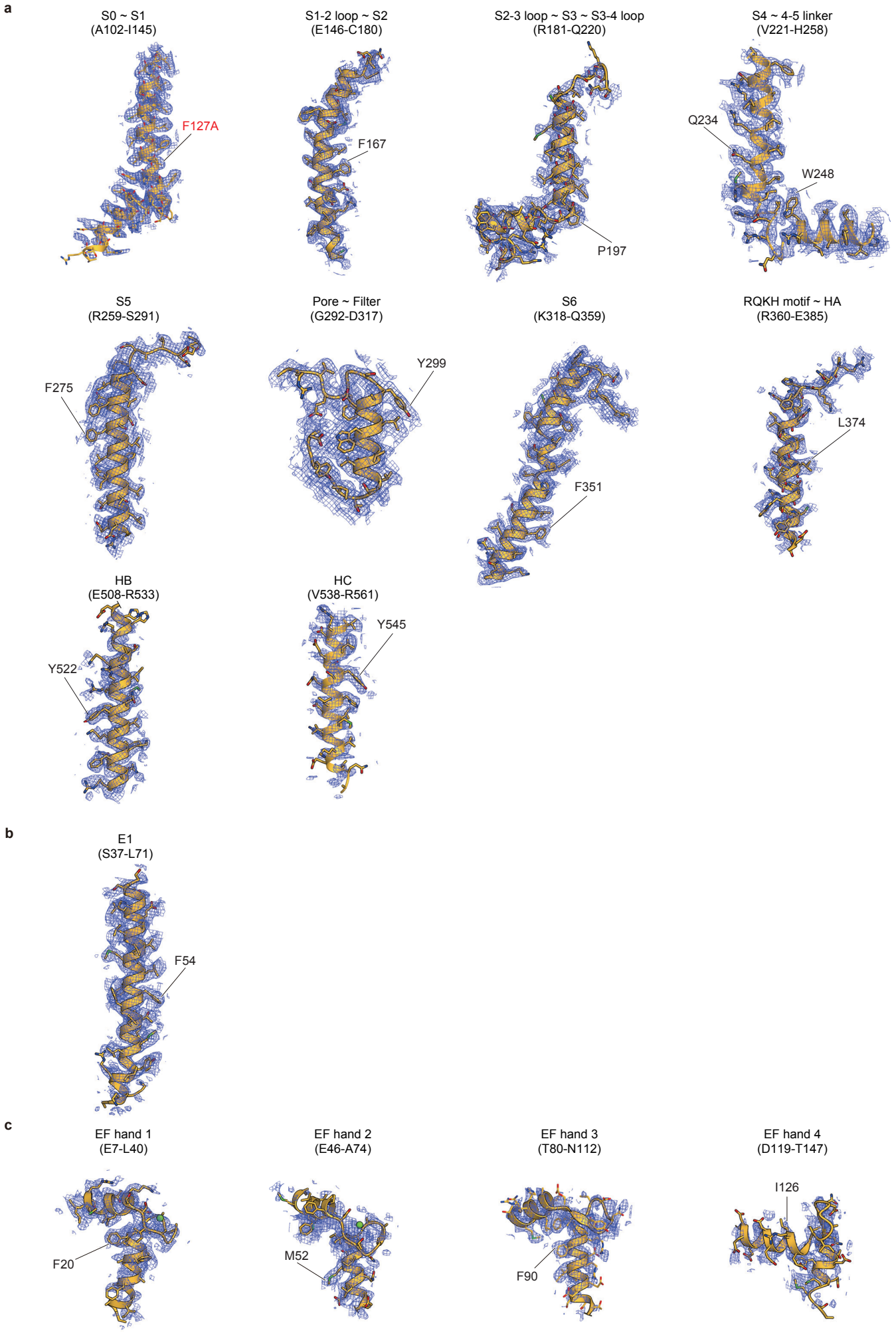


Supplementary Figure 4

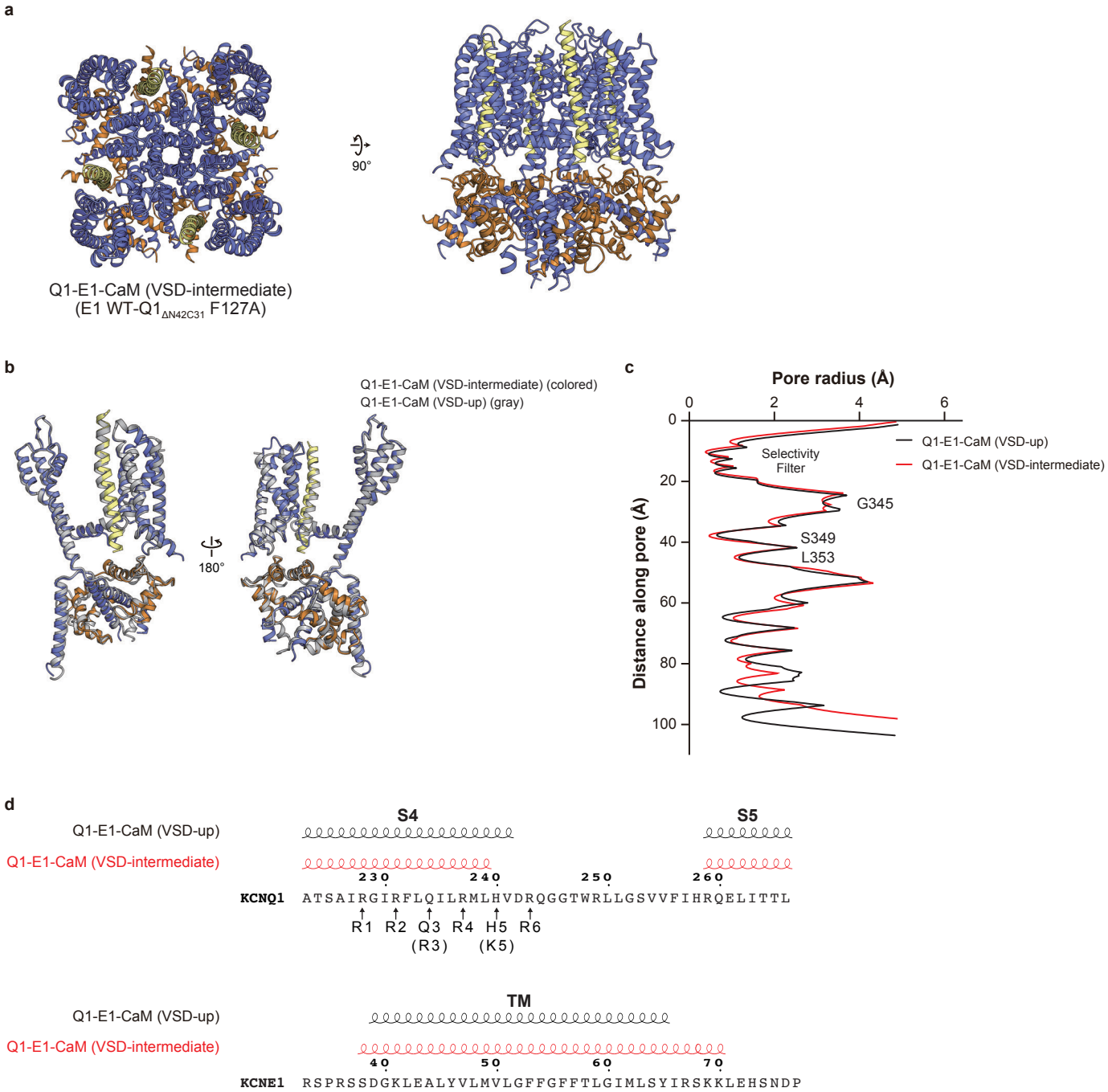




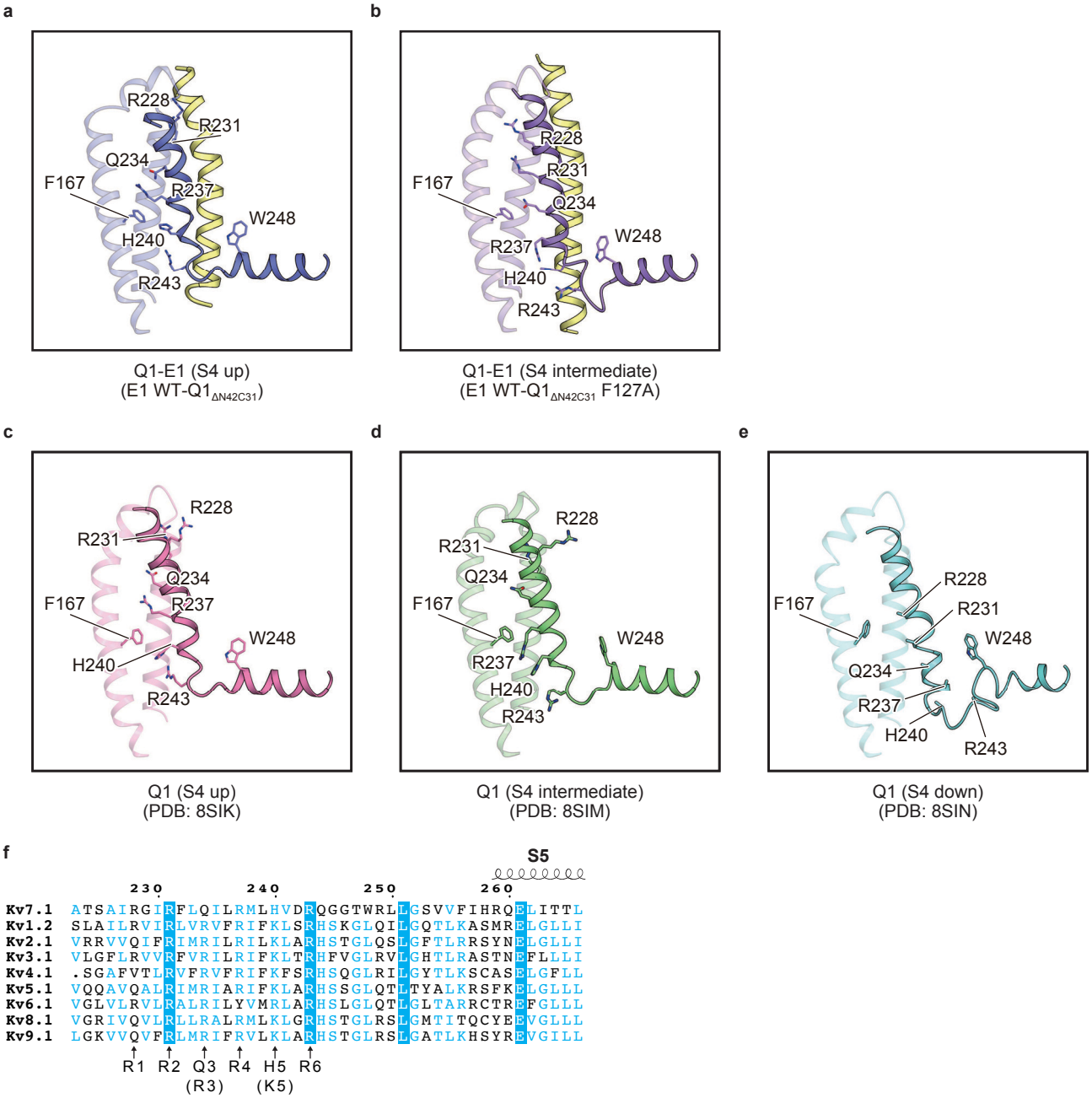
Supplementary Figure 5



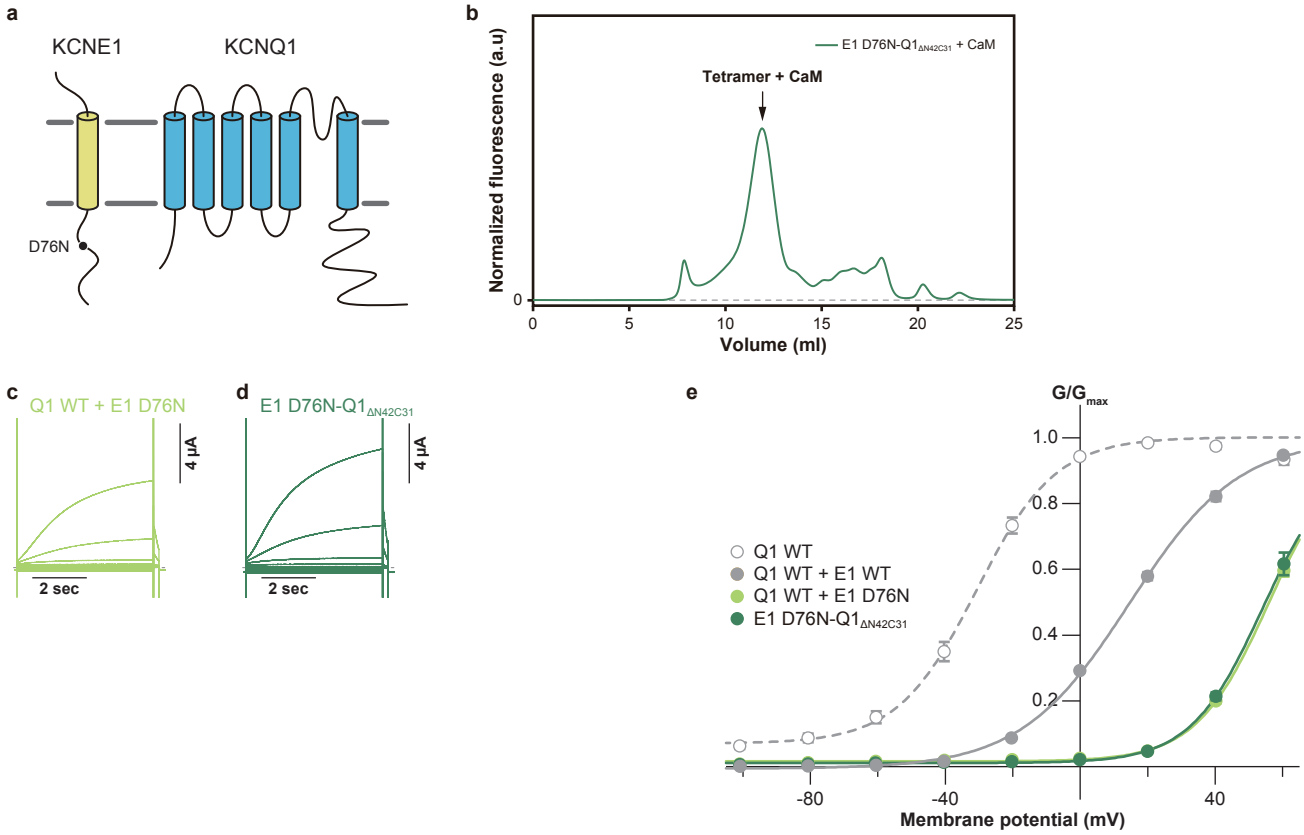
Supplementary Figure 6



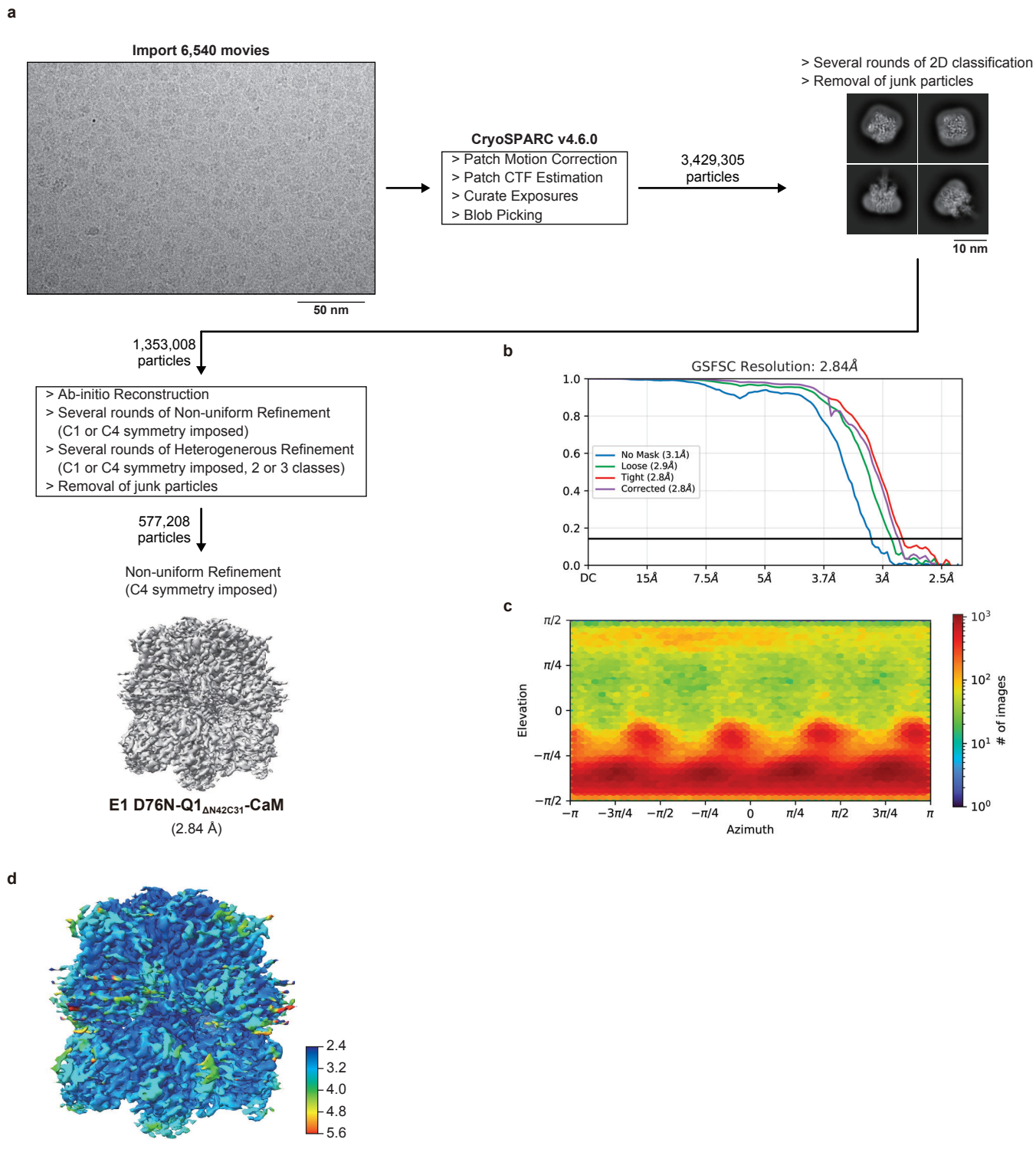
Supplementary Figure 7



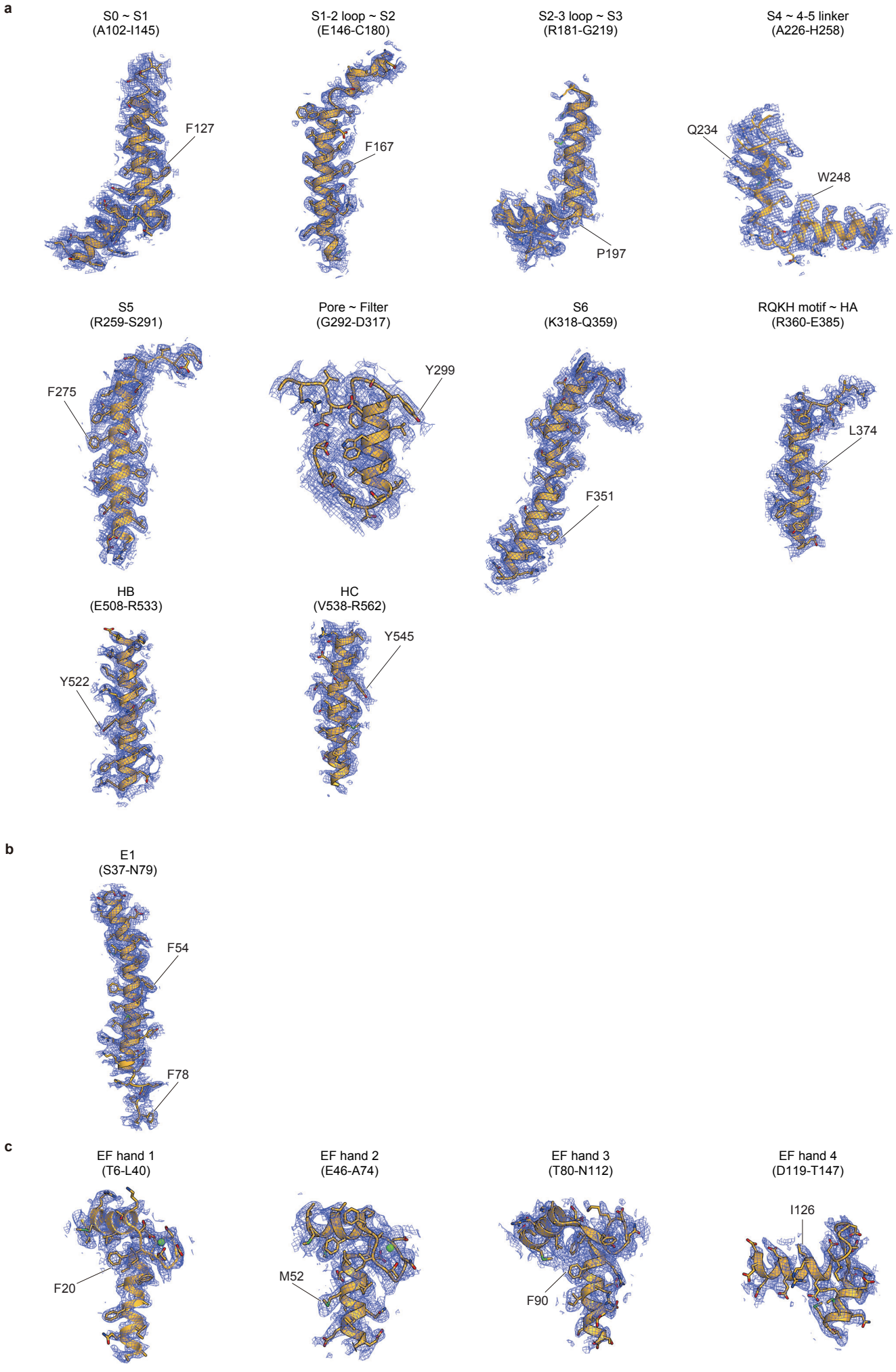
Supplementary Figure 8



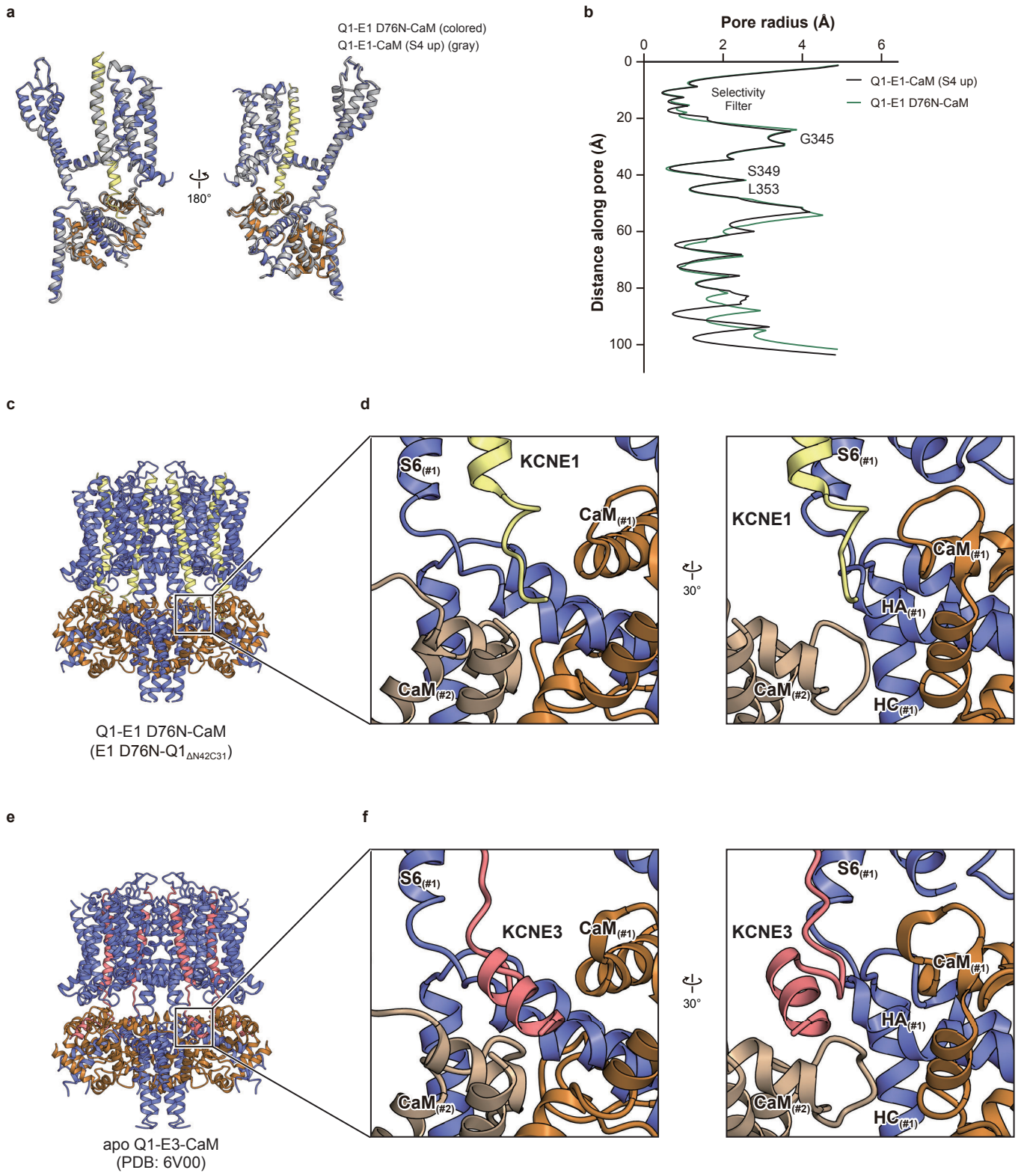
Supplementary Figure 9



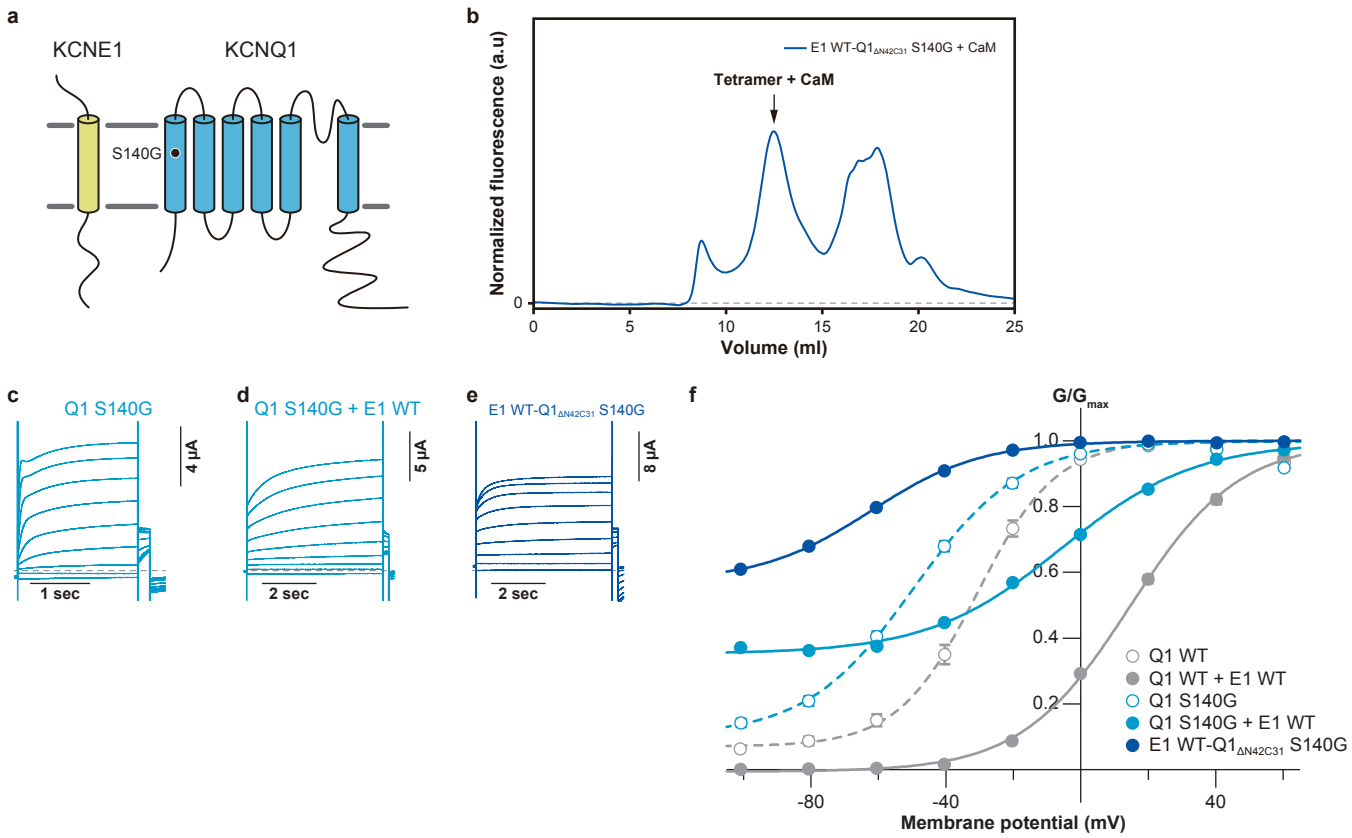
Supplementary Figure 10



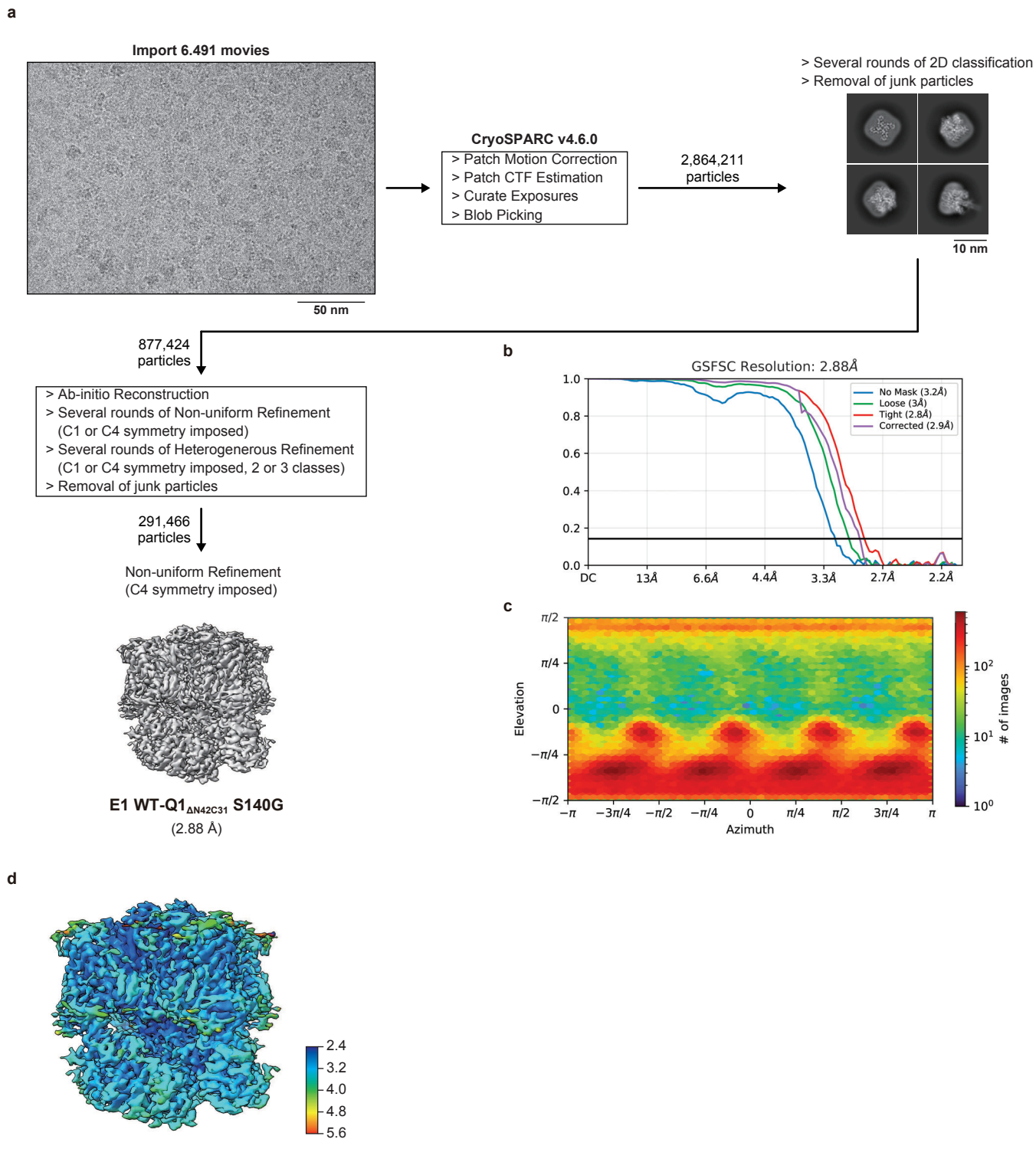
Supplementary Figure 11



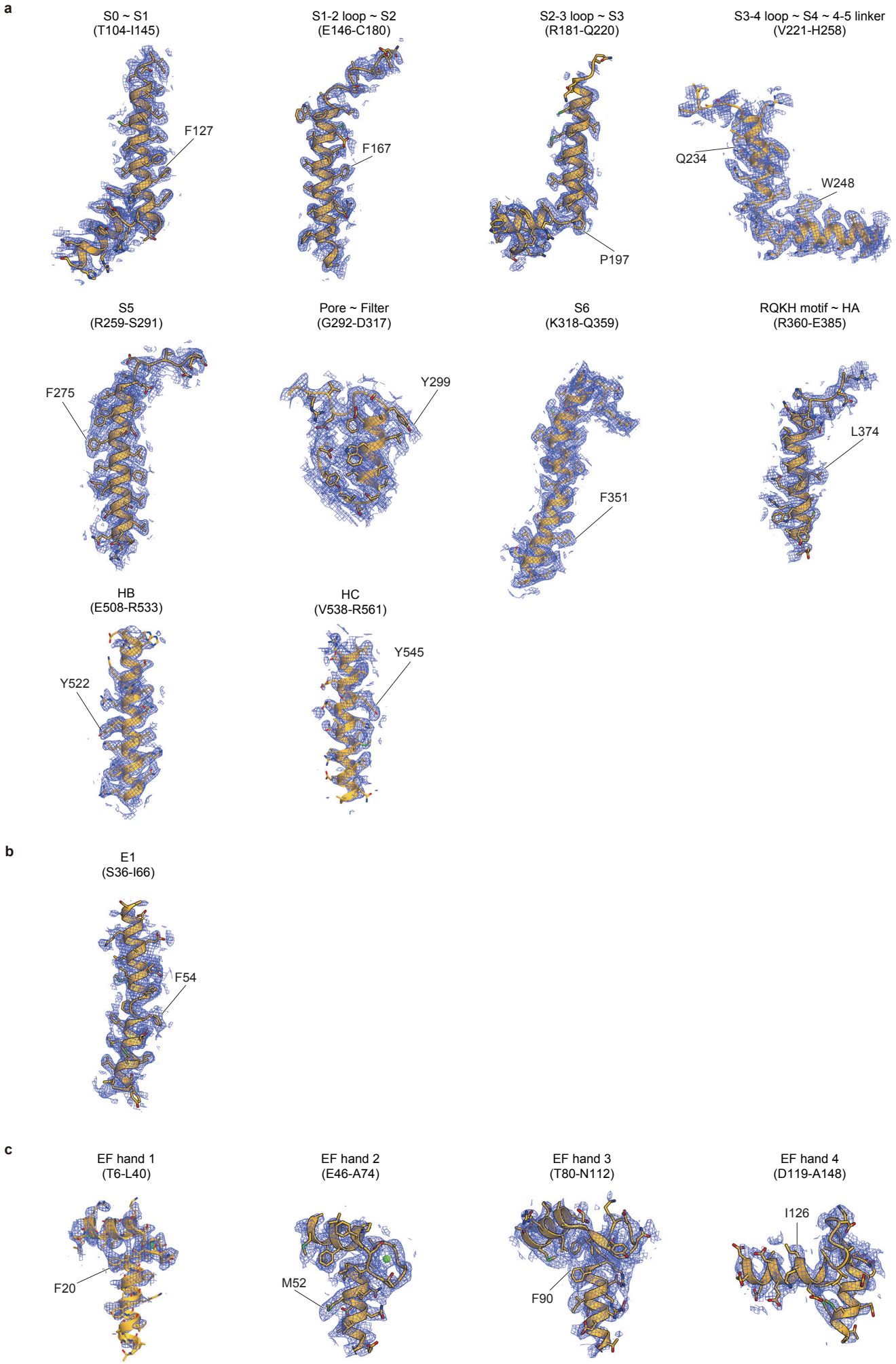
Supplementary Figure 12



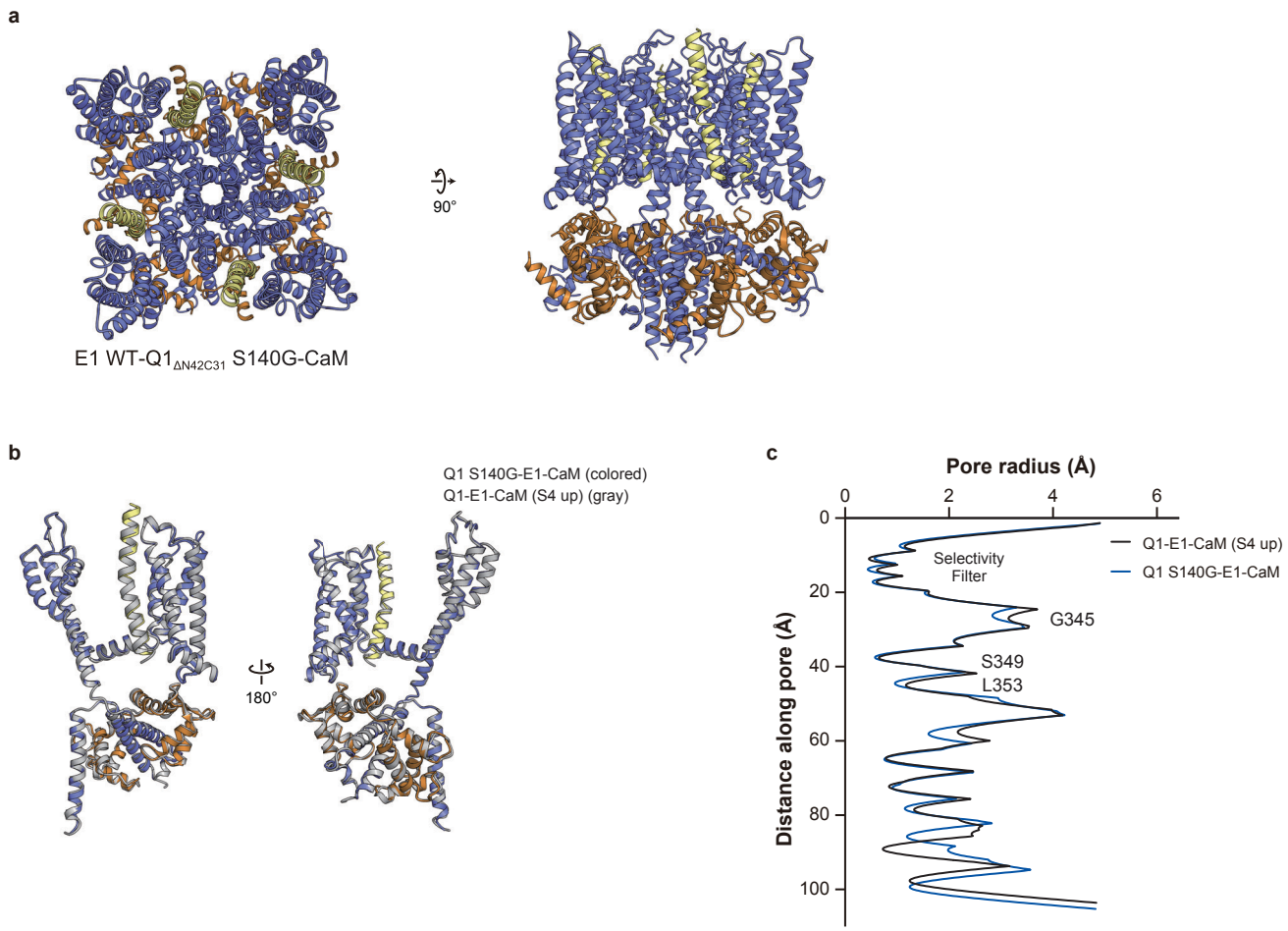
Supplementary Figure 13



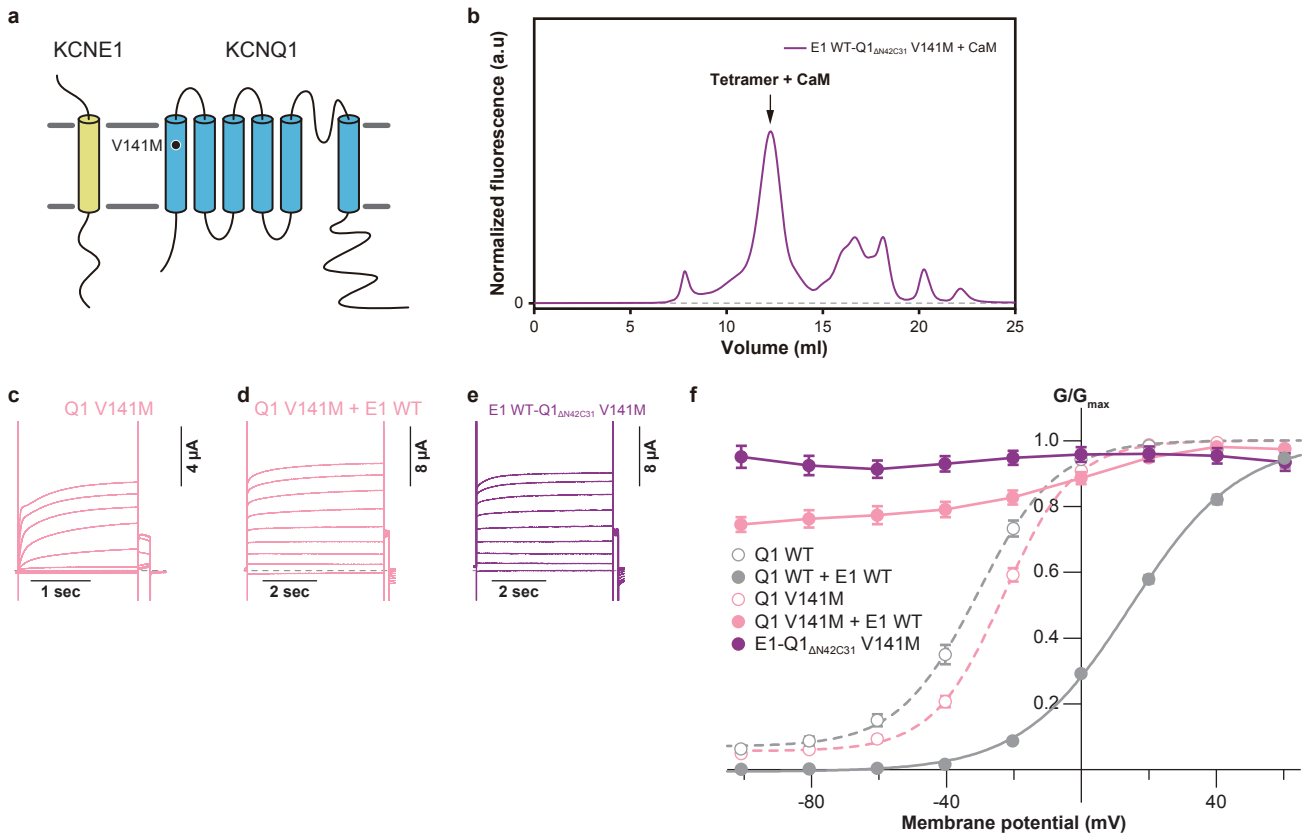
Supplementary Figure 14



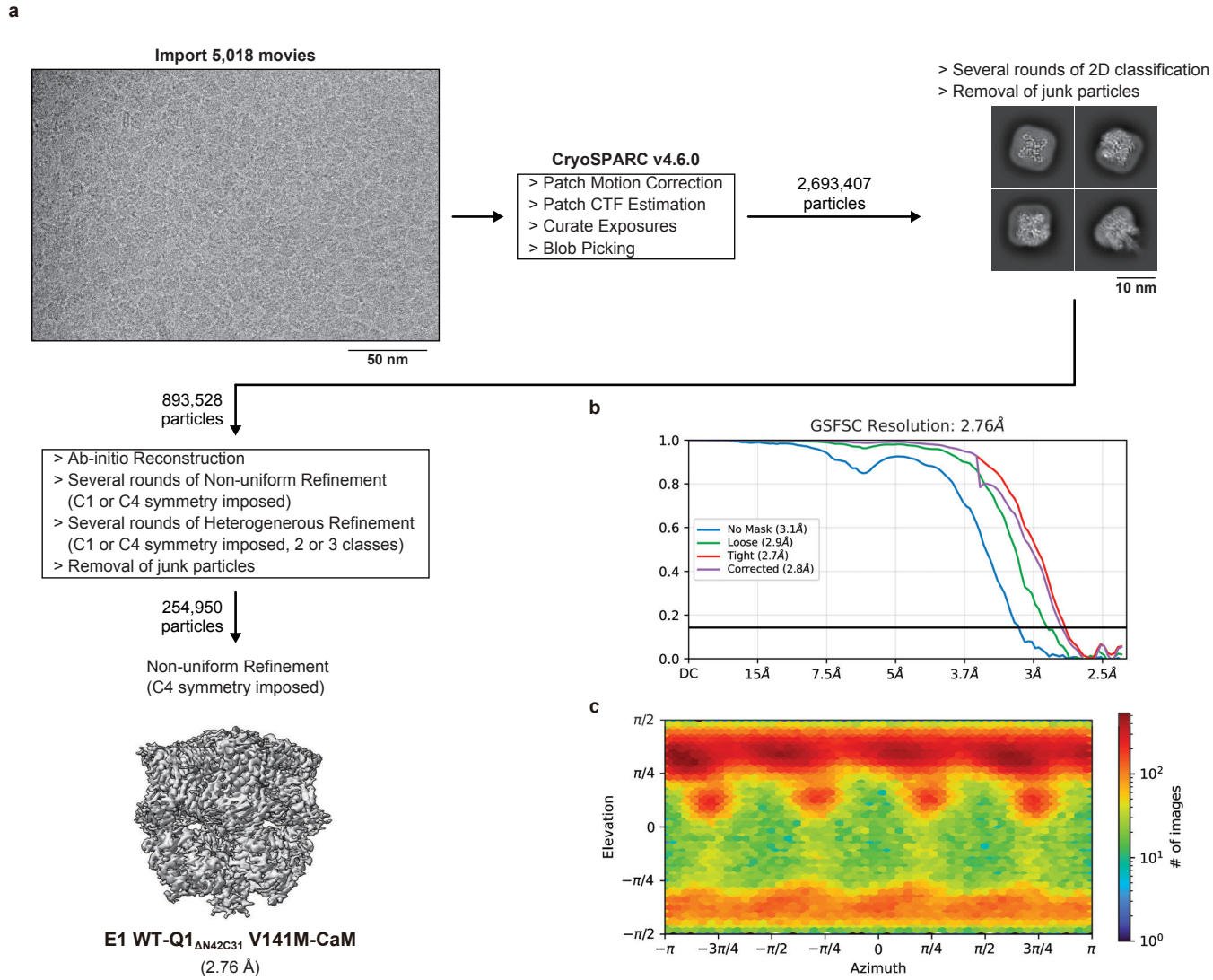
Supplementary Figure 15

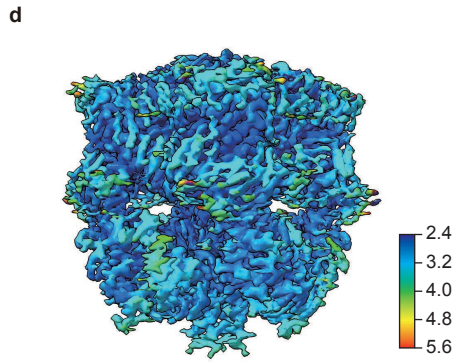


Supplementary Figure 16

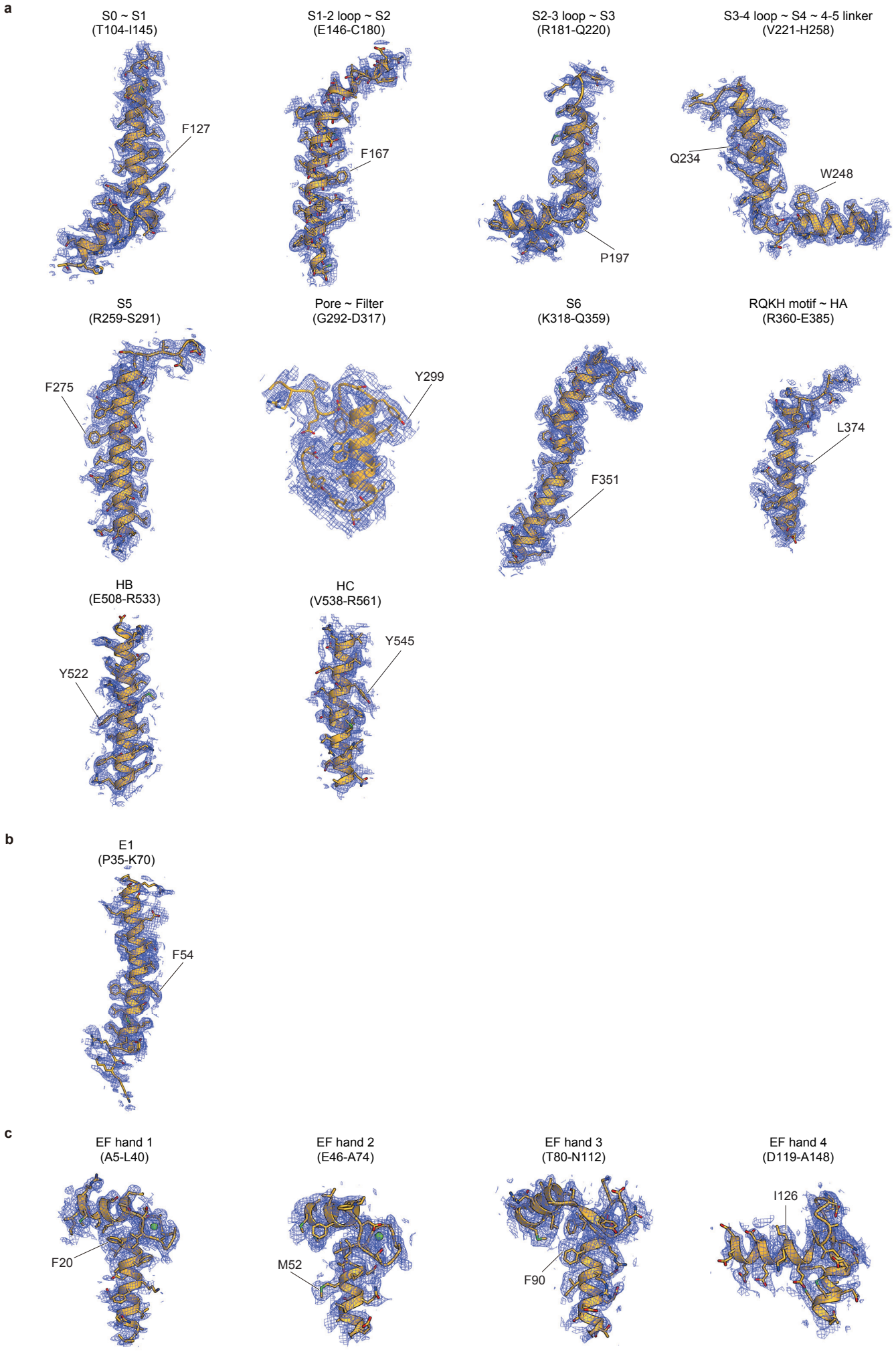


Supplementary Figure 17

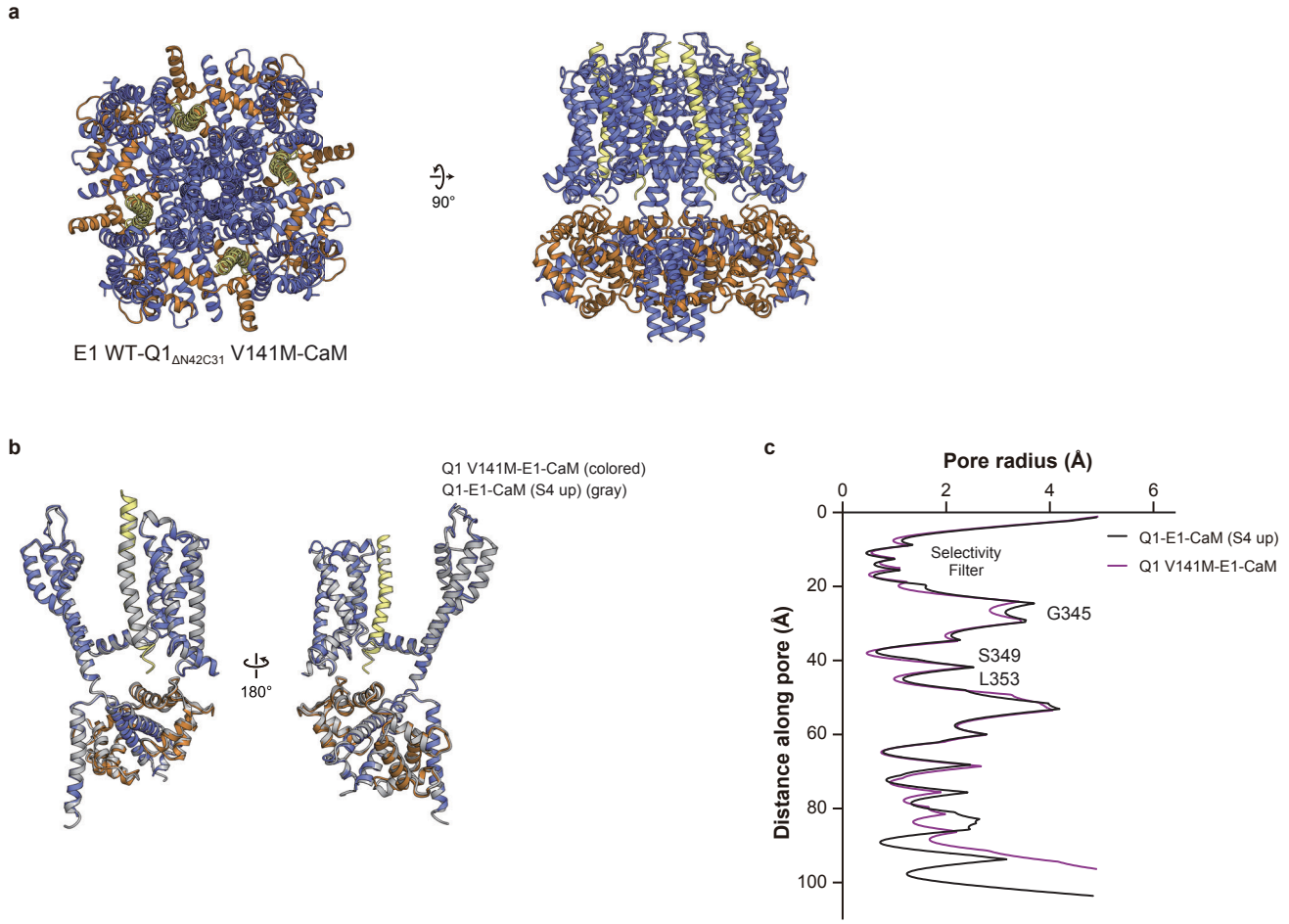




Supplementary Figure 18



Supplementary Figure 19



Supplementary Figure 20

Cryo-EM data collection, refinement and validation statistics

	E1 WT-Q1$_{\Delta N42C31}$	E1 WT-Q1$_{\Delta N42C31}$ F127A	E1 N76N-Q1$_{\Delta N42C31}$	E1 WT-Q1$_{\Delta N42C31}$ S140G	E1 WT-Q1$_{\Delta N42C31}$ V141M
EMDB code	XXX	XXX	XXX	XXX	XXX
PDB code	XXX	XXX	XXX	XXX	XXX
Data collection and processing					
Magnification	105,000	105,000	105,000	105,000	105,000
Voltage (kV)	300	300	300	300	300
Electron exposure (e–/Å^2)	47.8	49.8	49.8	48.9	49.8
Defocus range (μm)	-0.8 to -1.6	-0.8 to -1.6	-0.8 to -1.6	-0.8 to -1.6	-0.8 to -1.6
Pixel size (Å)	0.83	0.83	0.83	0.83	0.83
Symmetry imposed	C4	C4	C4	C4	C4
Movies (no.)	2,728	6,840	6,540	6,491	5,018
Initial particle images (no.)	1,151,363	3,237,483	3,429,305	2,864,211	2,693,407
Final particle images (no.)	76,191	241,770	577,208	291,466	254,950
Map resolution (Å)	2.93	2.92	2.84	2.88	2.76
FSC threshold	0.143	0.143	0.143	0.143	0.143
Map resolution range (Å)	2.44-47.17	2.42-36.24	2.61-32.35	2.29-35.01	2.61-27.64
Map sharpening *B* factor (Å^2)	-87.2	-135.2	-127.5	-125.2	-114.2
Refinement					
Initial model used (PDB code)	ModelAngelo	E1 WT-Q1$_{\Delta N42C31}$	E1 WT-Q1$_{\Delta N42C31}$	E1 WT-Q1$_{\Delta N42C31}$	E1 WT-Q1$_{\Delta N42C31}$
Model resolution (Å)	2.9	2.9	2.8	2.8	2.7
FSC threshold	0.5	0.5	0.5	0.5	0.5
Model composition					
Non-hydrogen atoms	16,432	16,592	16,908	16,480	16,624
Protein residues	2,080	2,112	2,128	2,092	2,112
Ligands	8	8	8	8	8
B factors (Å^2)					
Protein	63.63	26.02	25.82	29.14	26.45
Ligand	82.23	33.41	40.37	43.83	35.77
R.m.s. deviations					
Bond lengths (Å)	0.002	0.002	0.002	0.003	0.002
Bond angles (°)	0.446	0.476	0.458	0.438	0.435
Validation					
MolProbity score	1.22	1.27	1.33	1.36	1.30
Clashscore	4.40	5.06	6.00	6.60	5.53
Poor rotamers (%)	0.00	0.00	0.00	0.00	0.00
Ramachandran plot					
Favored (%)	99.02	99.62	98.66	98.30	99.42
Allowed (%)	0.98	0.38	1.34	1.70	0.58
Disallowed (%)	0.00	0.00	0.00	0.00	0.00

Supplementary Table 1

Summary of the electrophysiological properties of constructs used in this study

Conditions	I_{max} (μA)	$V_{1/2}$ (mV)	z	n
KCNQ1 WT	2.8 ± 0.3	-30.9 ± 1.2	2.2 ± 0.1	5
KCNQ1 WT + KCNE1 WT	5.3 ± 0.8	14.7 ± 0.6	1.6 ± 0.1	5
E1 WT-Q1$_{\Delta N42C31}$	6.2 ± 0.6	19.1 ± 1.0	1.8 ± 0.0	5
KCNQ1 F127A	1.8 ± 0.2	-31.8 ± 0.8	2.2 ± 0.1	5
KCNQ1 F127A + KCNE1 WT	*3.3 ± 0.9	n.d.	n.d.	5
E1 WT-Q1$_{\Delta N42C31}$ F127A	*6.6 ± 0.4	n.d.	n.d.	5
	* I@60mV			
KCNQ1 WT + KCNE1 D76N	5.2 ± 0.8	56.4 ± 0.8	2.3 ± 0.1	5
E1 D76N-Q1$_{\Delta N42C31}$	3.8 ± 0.4	55.4 ± 1.4	2.3 ± 0.1	5
KCNQ1 S140G	2.5 ± 0.0	-49.5 ± 1.2	1.6 ± 0.1	5
KCNQ1 S140G + KCNE1 WT	4.1 ± 0.1	-6.2 ± 1.6	1.2 ± 0.1	5
E1 WT-Q1$_{\Delta N42C31}$ S140G	5.4 ± 0.1	-62.2 ± 0.8	1.6 ± 0.1	5
KCNQ1 V141M	3.1 ± 0.3	-23.2 ± 0.8	2.4 ± 0.1	5
KCNQ1 V141M + HsKCNE1 WT	**5.1 ± 0.5	n.d.	n.d.	5
E1 WT-Q1$_{\Delta N42C31}$ V141M	**3.4 ± 0.7	n.d.	n.d.	5
	** Normalized to I_{max} with the recorded voltage range (-100 mV to + 60 mV)			
KCNQ1 Q244S	0.6 ± 0.1	-33.3 ± 1.0	2.2 ± 0.1	5
KCNQ1 Q244S + HsKCNE1 WT	3.5 ± 0.9	-3.7 ± 2.1	1.7 ± 0.4	5

Conditions	I_{max} (μA)	$V_{1/2}$ (mV)	z	$V_{1/2\ (F1)}$ (mV)	$V_{1/2\ (F2)}$ (mV)	n
KCNQ1$_{vcf}$ F127A	2.7 ± 0.6	-51.0 ± 0.6	1.9 ± 0.1	-46.3 ± 0.8	n.d	5
KCNQ1$_{vcf}$ F127A + KCNE1 WT	17.9 ± 1.2	74.7 ± 0.9	1.5 ± 0.0	-126.2 ± 2.6	>80	5

Supplementary Table 2