Expanded View Figures

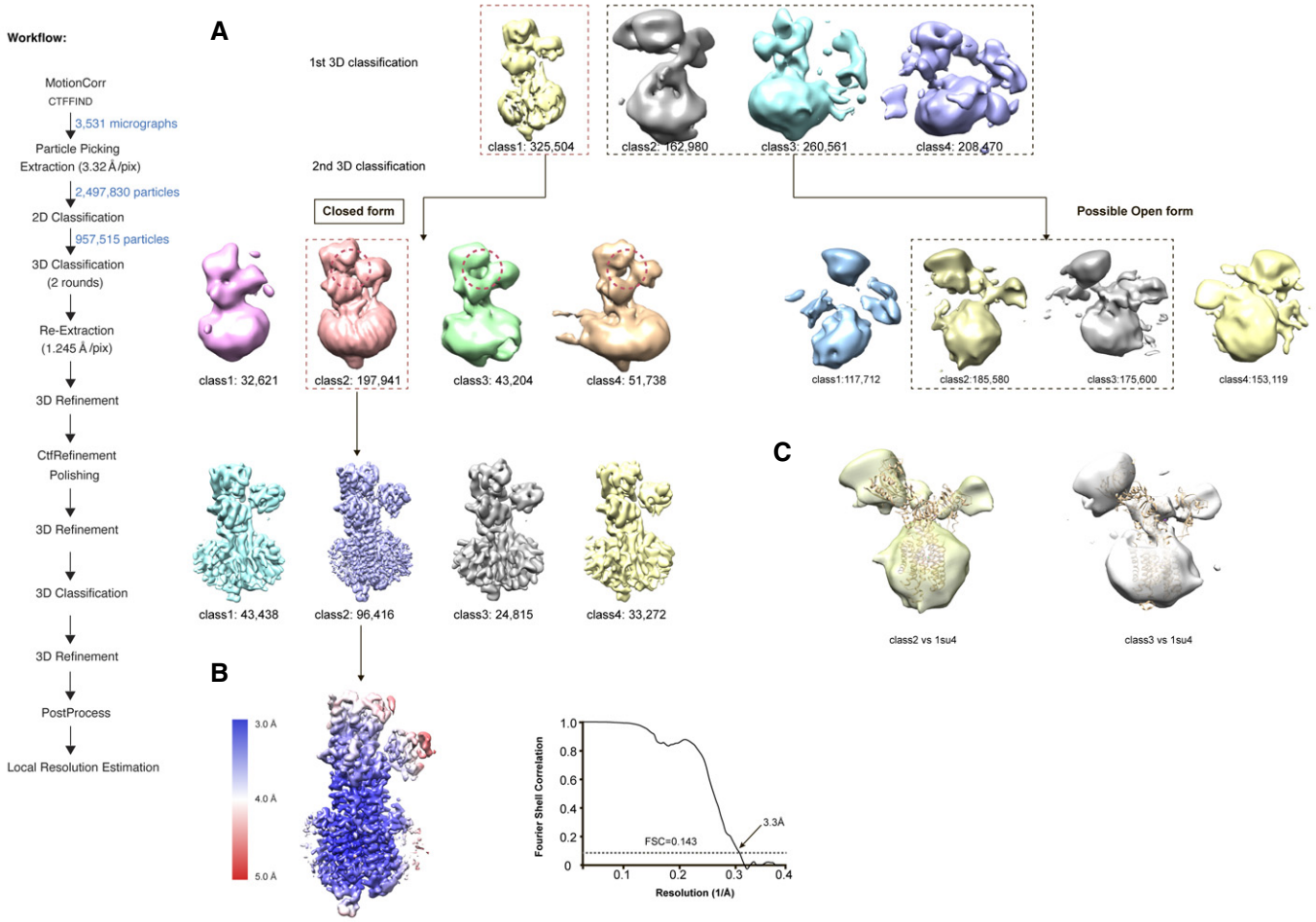

Figure EV1. Workflow of data processing for cryo-EM single-particle analysis and local resolution analysis of SERCA2b in the E1•2Ca^{2+} state.

A Multiple classes of density map were generated after the first round of 3D classification, which were divided into two classes during the second round of 3D classification. A workflow of data processing for the cryo-EM single-particle analysis is shown on the left.

B Local resolution estimation of the "closed-form" cryo-EM map of SERCA2b in the E1•2Ca^{2+} state. The panel on the right shows the FSC curve for post-processing of SERCA2b WT in the E1•2Ca^{2+} state.

C Superposition of the crystal structure of SERCA1a in the E1•2Ca^{2+} state (PDB ID: 1SU4) onto the present "possible-open-form" class 2 and class 3 cryo-EM maps.

Figure EV2. Close-up views of the C-terminal part, Ca^{2+}-binding sites, and ATP-binding pocket of SERCA2b.

A TM11 and the LE of SERCA2b are highlighted in red, while TM1-TM10 are colored wheat. L7/8 is colored blue. The backbone model of TM11 in SERCA2b in the E1•2Ca^{2+} state was placed based on the cryo-EM map (left inset). Density is shown at a contour level of 3.5 RMSD. The backbone model of the LE in SERCA2b in the E1•2Ca^{2+} state was placed based on the cryo-EM map (bottom inset). Density is shown at a contour level of 2.7 RMSD.

B Close-up view of the Ca^{2+}-binding sites of SERCA2b (yellow) and SERCA1a (green) in the E1•2Ca^{2+} state, in which bound Ca^{2+} ions are depicted as spheres. Ca^{2+} binding residues are shown as sticks. Note that the mode of Ca^{2+} binding is almost identical between these two states.

C Close-up view of the Ca^{2+}-binding sites of SERCA2b in E1•2Ca^{2+} (yellow) and E1•2Ca^{2+}-AMPPCP (cyan) states, in which bound Ca^{2+} ions are depicted as spheres. Ca^{2+} binding residues are shown as sticks. Note that the mode of Ca^{2+} binding is almost identical between these two states.

D Close-up view of the Ca^{2+}-binding sites in the E1•2Ca^{2+} state, in which bound Ca^{2+} ions and their density are represented by purple spheres and violet meshes, respectively. Neighboring residues are shown as sticks. Density is shown at a contour level of 5.0 α.

E Density maps of the residues that constitute the ATP-binding pocket of SERCA2b in the E1•2Ca^{2+} state, shown at a contour level of 5.0 α. The residues are shown as sticks.

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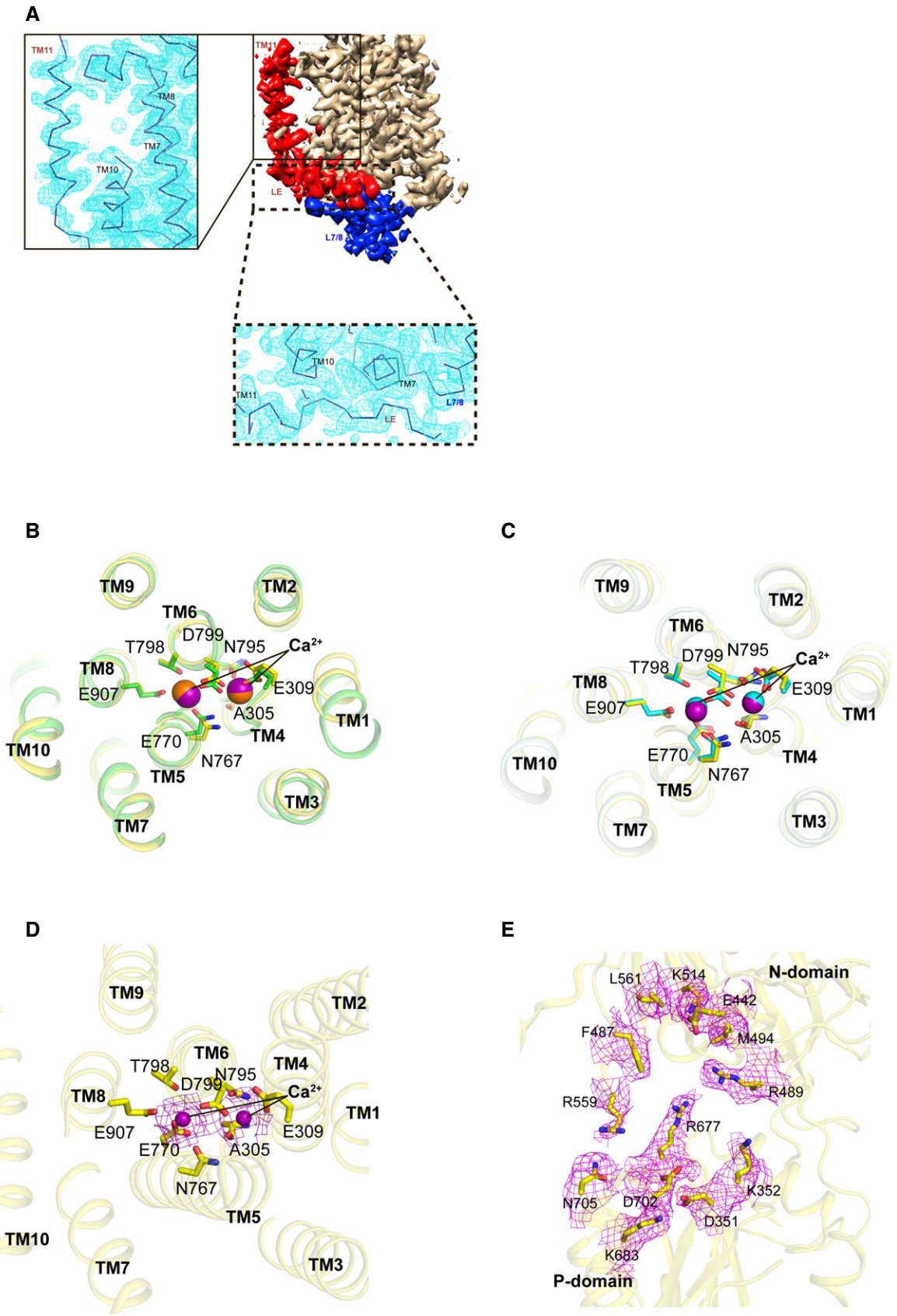
A
TM11
TM8
TM7
TM10
LE
L7/8
B
TM9
TM2
TM6
D799
T798
N795
Ca2+
TM8
E907
E309
A305
TM1
E770
TM4
TM10
N767
TM5
TM3
TM7
C
TM9
TM2
TM6
D799
N795
T798
Ca2+
TM8
E309
E907
TM1
A305
E770
TM4
TM10
N767
TM5
TM7
TM3
D
TM9
TM2
TM6
T798
N795
TM4
D799
Ca2+
TM8
TM1
E907
E309
E770
A305
N767
TM5
TM10
TM7
TM3
E
N-domain
L561
K514
E442
F487
M494
R489
R559
R677
K352
N705
D702
D351
K683
P-domain

Figure EV2.

Figure EV3. Cytosolic domain interface in the "closed-form" of SERCA2b and critical residues for the cytosolic domain interactions in the E1·2Ca^{2+} state.

A Closed-up view of the interface between the A and N domains in the "closed-form" cryo-EM structure of SERCA2b. The amino acid pairs in which Cα atoms are located within a distance of 8.5 Å are represented by sticks, and their cysteine mutants were prepared in this study.

B Six kinds of SERCA2b mutants including Q138C/D426C, V137C/D426C, A154C/G438C, V155C/G438C, T171C/E486C, or T171C/L577C were treated with no reagent (upper), DTT (middle) or $K_3Fe(CN)_6$ (lower). Among them, T171C/L577C showed a significant upward band shift relative to WT after oxidative treatment with $K_3Fe(CN)_6$.

C Close-up views of the interface between the A and P domains in the "open-form" crystal structure of SERCA1a in the E1•2Ca^{2+} state (upper) (PDB ID: 1SU4), and the interfaces between the cytosolic A, P, and N domains in the "closed-form" cryo-EM structure of SERCA2b in the E1•2Ca^{2+} state (lower). Critical residues for the cytosolic domain interactions are represented by sticks. Dotted lines indicate hydrogen bonds or salt bridges formed between those residues. Magenta spheres represent two bound Ca^{2+} ions.

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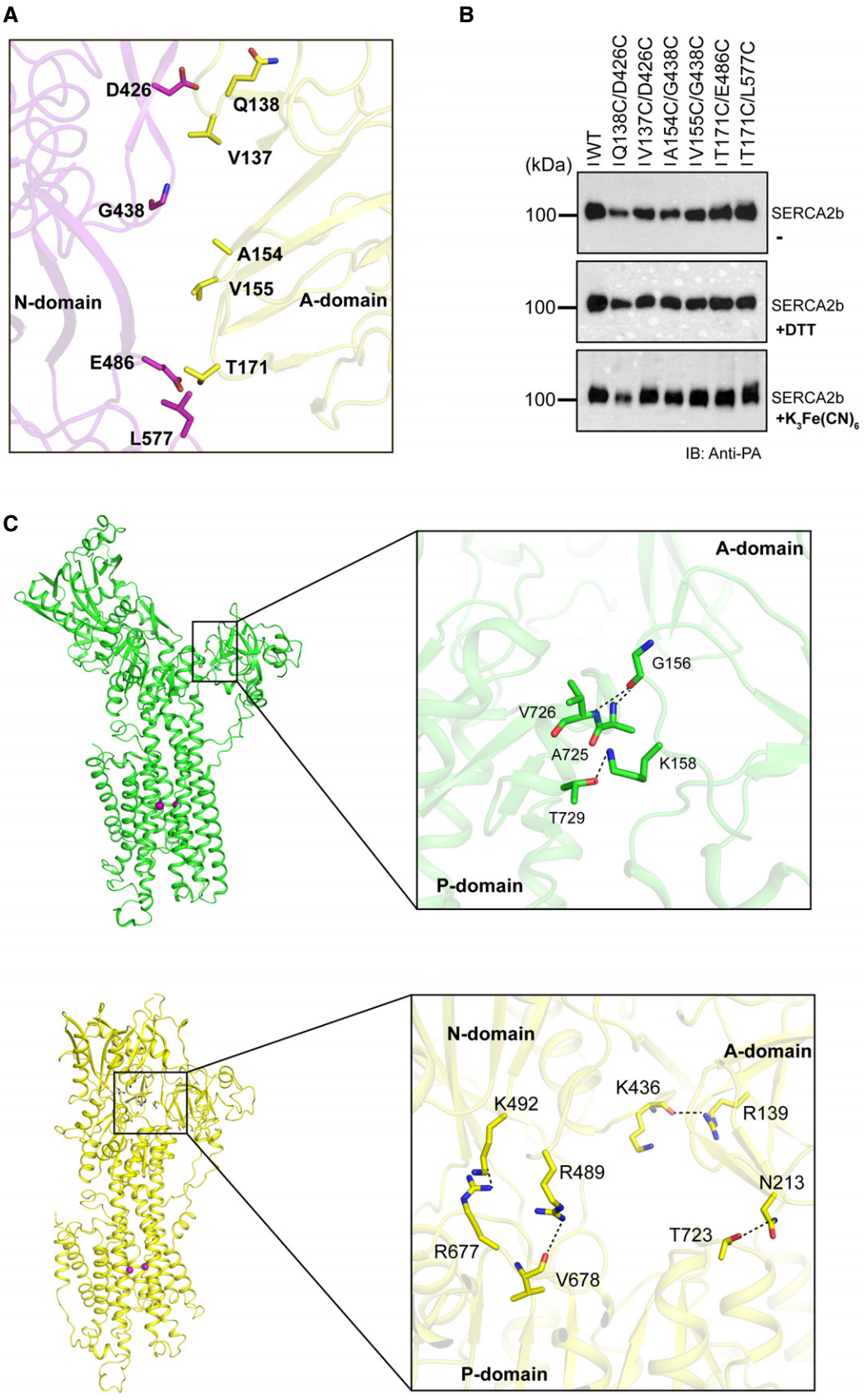
A
D426
Q138
V137
G438
A154
V155
N-domain
A-domain
E486
T171
L577
B
IWT
IQ138C/D426C
IV137C/D426C
IA154C/G438C
IV155C/G438C
IT171C/E486C
IT171C/L577C
(kDa)
100
SERCA2b
-
SERCA2b
+DTT
SERCA2b
+K3Fe(CN)6
IB: Anti-PA
C
A-domain
G156
V726
A725
K158
T729
P-domain
N-domain
A-domain
K436
K492
R139
R489
N213
T723
R677
V678
P-domain

Figure EV3.

Figure EV4. Effects of Ca^{2+} and ATP on formation of the closed headpiece cluster of the cytosolic domains, and autophosphorylation assay with BSA.

A Closed-up view of the interface between the A and N domains in SERCA. The Cβ-Cβ distance between Thr171 (A domain) and Leu577 (N domain) in the E2 state of SERCA1a (left upper), the E2-AMPPCP state of SERCA2a (right upper), the E1•2Ca^{2+} state of SERCA2b (left lower), or E1•2Ca^{2+}-AMPPCP state of SERCA2b (right lower) is shown in each panel.

B SDS–PAGE analysis of purified SERCA2b T171C/L577C (0.5 μg) in the presence or absence of 1 mM Ca^{2+} and 1.5 mM AMPPCP. Protein bands were visualized by staining with Coomassie Brilliant Blue. "SS" and "no SS" indicate disulfide-bonded and non-disulfide-bonded SERCA2b species, respectively. Note that after oxidative treatment with 5 mM $K_3Fe(CN)_6$ for 1hr at pH6.8, the "SS" spices significantly increased in the presence of Ca^{2+}, whereas AMPPCP gave marginal effect on the "SS" spices formation regardless of the presence or absence of Ca^{2+}.

C BSA (40 μg) was used as a negative control in the autophosphorylation assay, and no phosphorylation was observed with this protein. Experimental conditions were the same as those conducted with SERCA2b WT and T171C/L577C.

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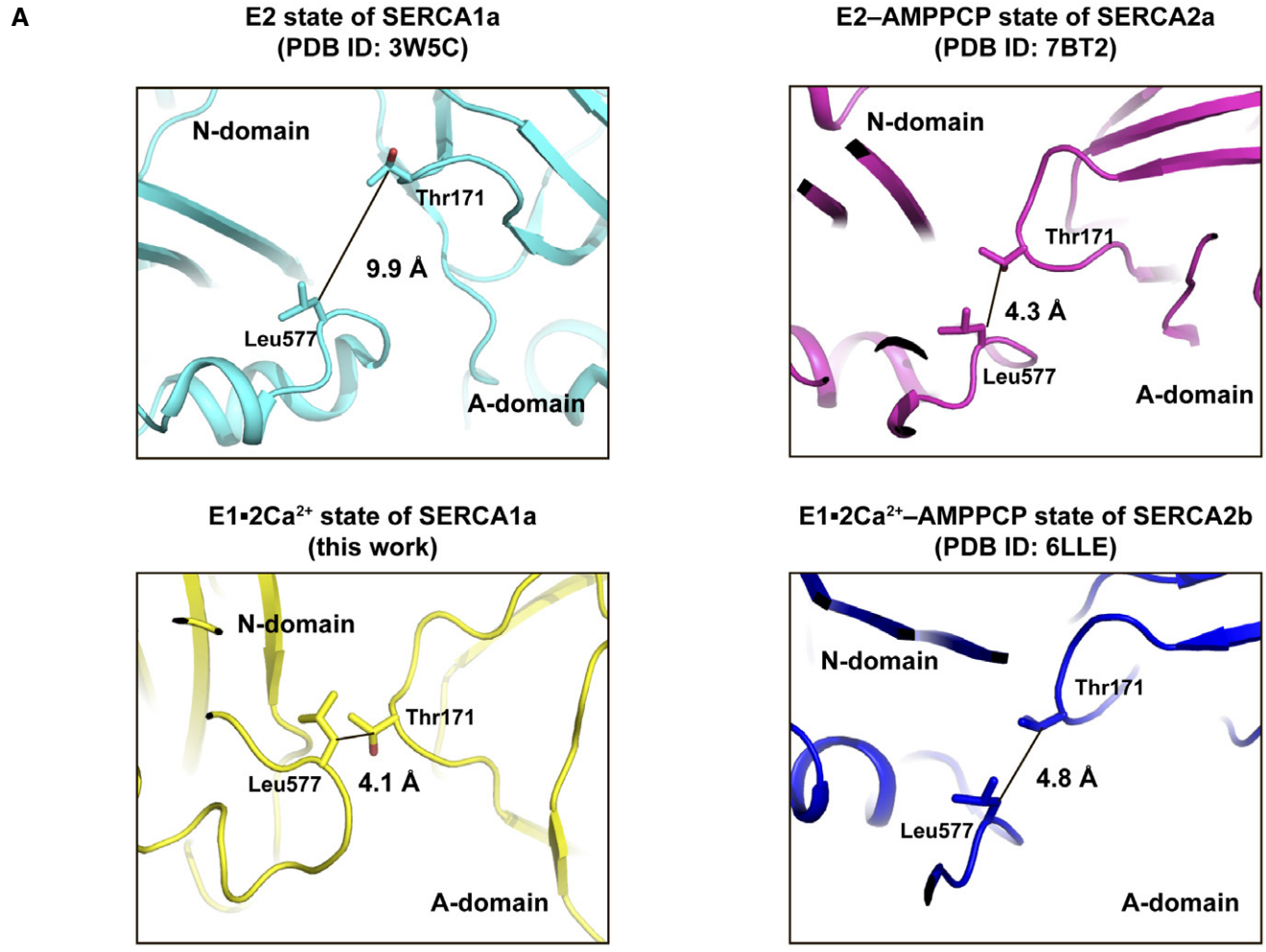
A
E2 state of SERCA1a
(PDB ID: 3W5C)
N-domain
Thr171
9.9 Å
Leu577
A-domain
E2–AMPPCP state of SERCA2a
(PDB ID: 7BT2)
N-domain
Thr171
4.3 Å
Leu577
A-domain
E1▪2Ca2+ state of SERCA1a
(this work)
N-domain
Thr171
Leu577
4.1 Å
A-domain
E1▪2Ca2+–AMPPCP state of SERCA2b
(PDB ID: 6LLE)
N-domain
Thr171
4.8 Å
Leu577
A-domain

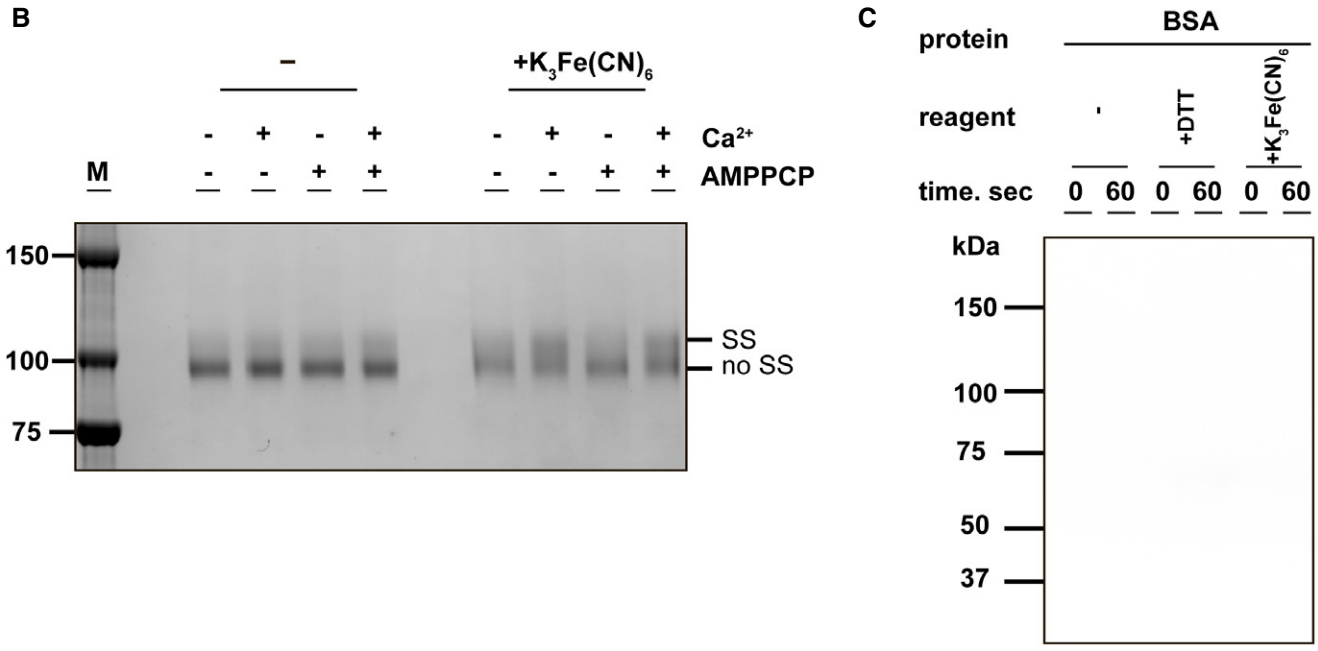
B
–
+K3Fe(CN)6
- + - + - + - + Ca2+
M - - + + - - + + AMPPCP
150
100
75
SS
no SS
C
protein
BSA
reagent
-
+DTT
+K3Fe(CN)6
time. sec 0 60 0 60 0 60
kDa
150
100
75
50
37

Figure EV4.

Figure EV5. Representative densities and Cross-validation FSC curves for map-to-model.

A Representative densities of the A, N, and P cytosolic domains and TM helices in the present cryo-EM map of SERCA2b in the E1•2Ca^{2+} state. Segments are indicated by the residue number in parentheses. All densities are shown at a contour level of 5.0 σ.

B Cross-validation FSC curves for map-to-model fitting of SERCA2b WT in the E1•2Ca^{2+} state.

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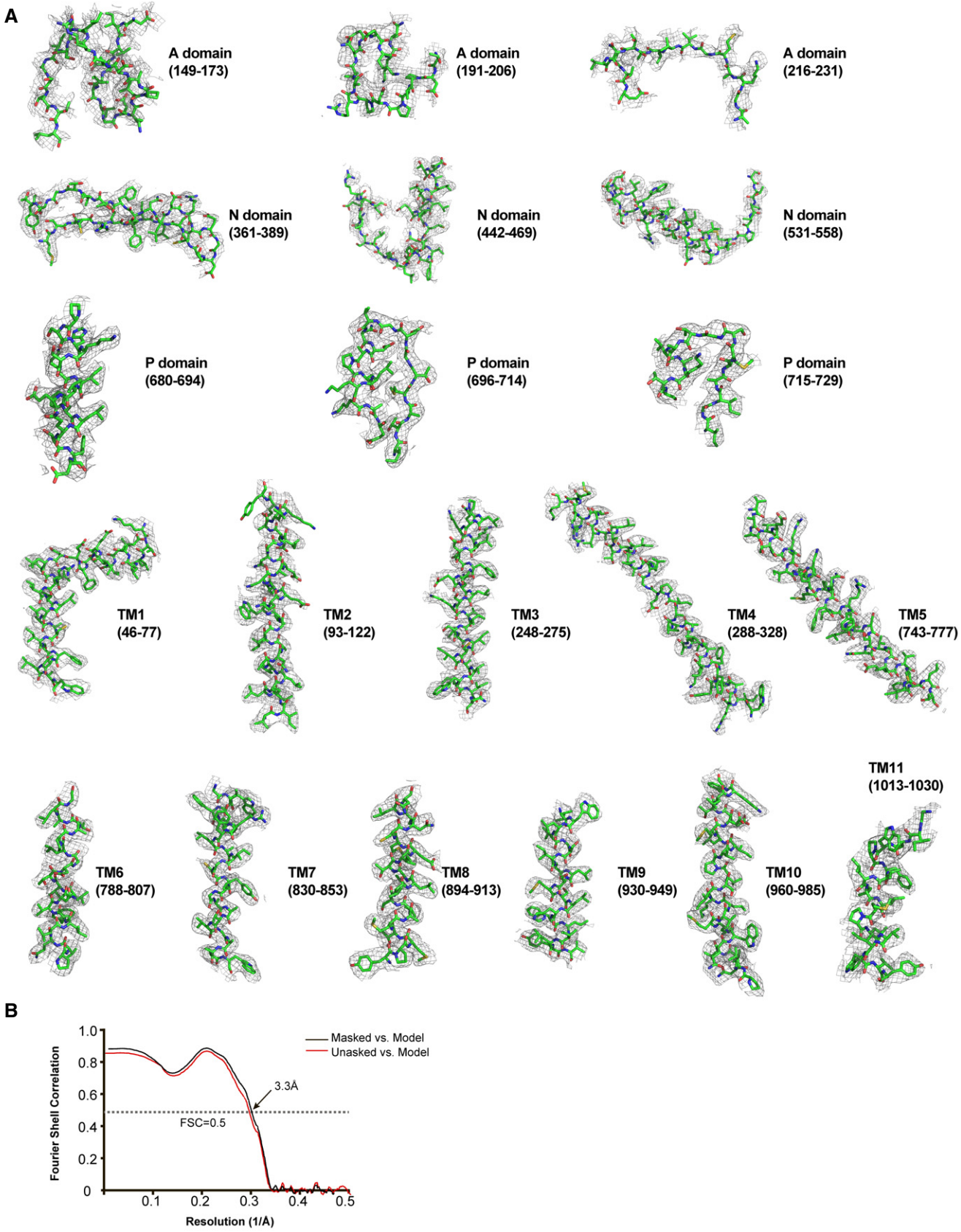

Figure EV5.

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