
Supplementary information

Helicase-mediated mechanism of SSU processome maturation and disassembly

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Supplementary Information

Helicase-mediated mechanism of SSU processome maturation and disassembly

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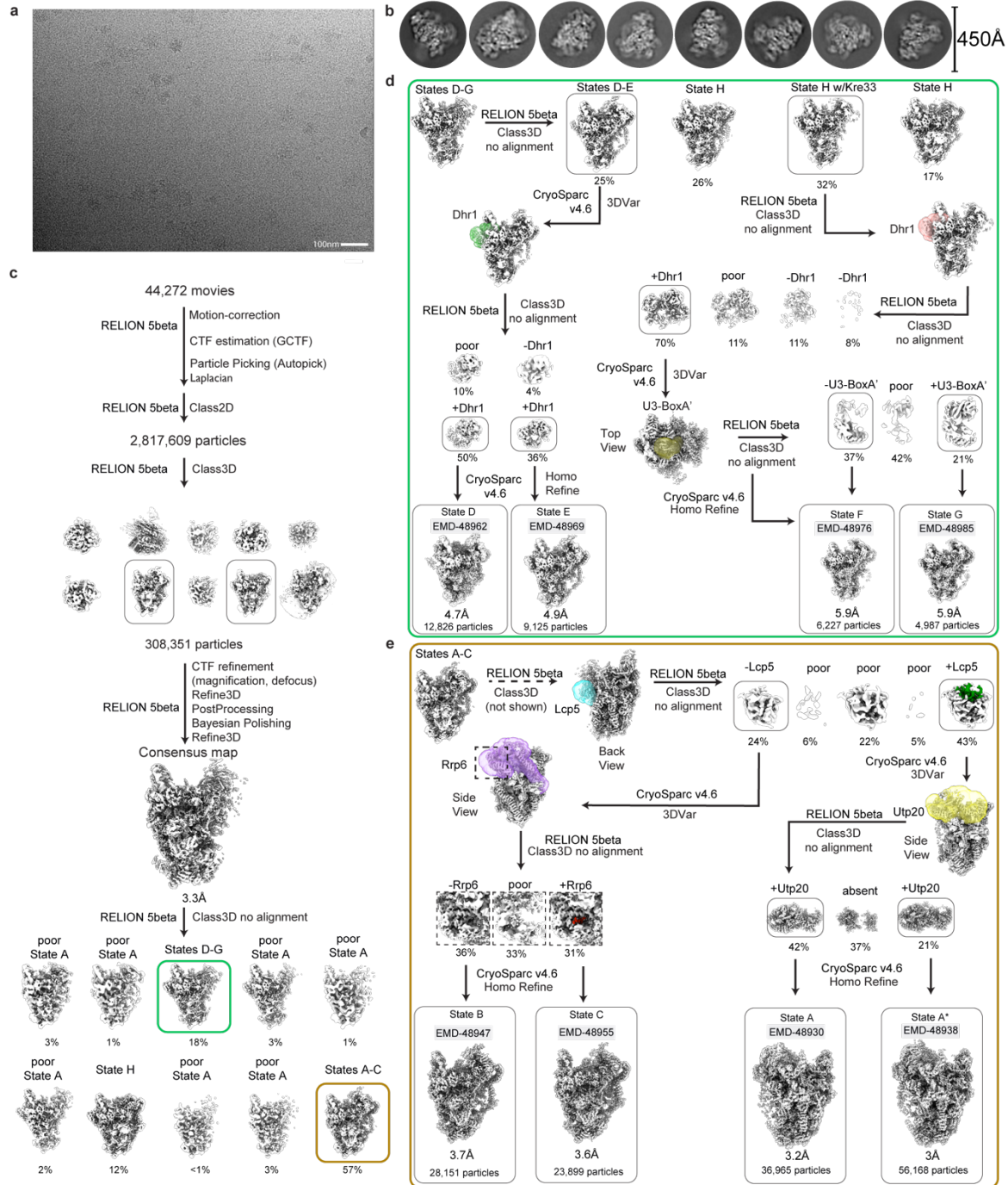
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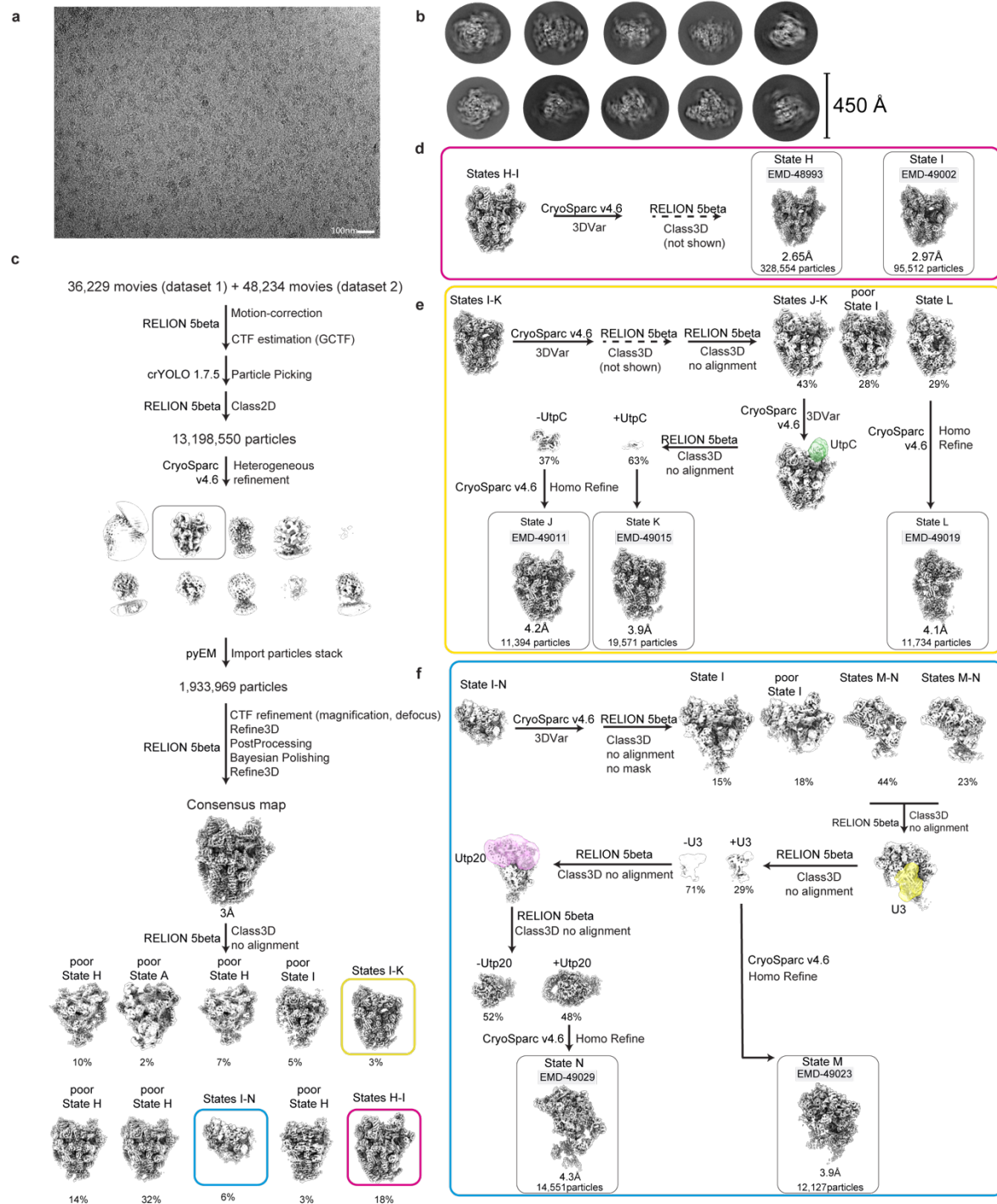
Dataset 1 Strain used for pulldown: Dhr1-linker-tev-ALFA-3c-mCherry + Kre33-linker-tev-GFP



Supplementary Fig. 1. Cryo-EM processing of Dhr1 and Kre33 dataset.

(a) Representative motion corrected cryo-EM micrograph from 44,272 total micrographs. (b) 8 representative 2D class averages (4x binned, 4.32Å/px). (c) Initial data processing workflow for determination of a consensus map and identification of 2 mixed state populations. (d) and (e) Workflow for the identification and reconstruction of 8 SSU processome intermediates (States A-G).

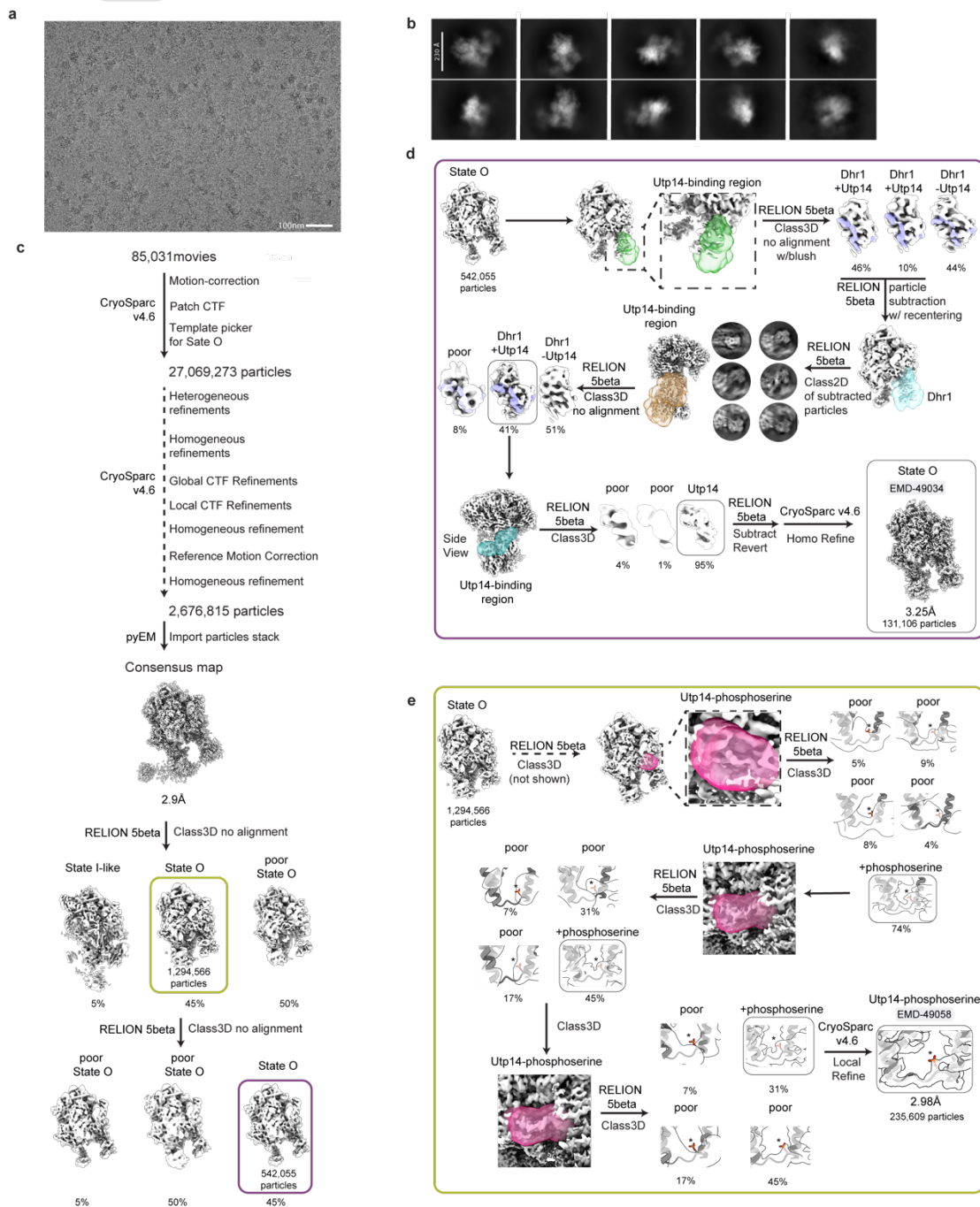
Dataset 2 Strain used for pulldown: Dhr1-linker-tev-ALFA-3c-mCherry



Supplementary Fig. 2. Cryo-EM processing of Dhr1 dataset.

(a) Representative motion corrected cryo-EM micrograph from 84,463 total micrographs. (b) 12 representative 2D class averages (4x binned, 4.32Å/px). (c) Initial data processing workflow for determination of a consensus map and identification of 3 mixed state populations. (d), (e) and (f) Workflow for the identification and reconstruction of 7 SSU processome intermediates (States H-N).

Dataset 3 Strain used for pulldown: Dhr1-linker-tev-ALFA-3c-mCherry and Utp7-linker-tev-GFP and Utp14-linker-sbp



(a) Representative motion corrected cryo-EM micrograph from 85,031 total

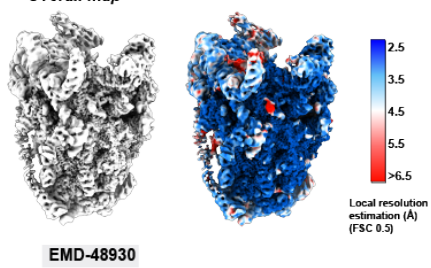
representative 2D class averages (4x binned, 4.32Å/px). **(c)** Initial data processing workflow for determination of a consensus map. **(d)** workflow for the identification and reconstruction of States O containing highest resolution Utp14 bound Dhr1. **(e)** Workflow for the identification of highest resolution focused map for Utp14-phosphoserine.

a

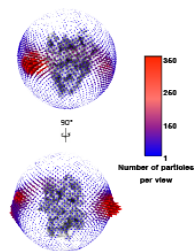
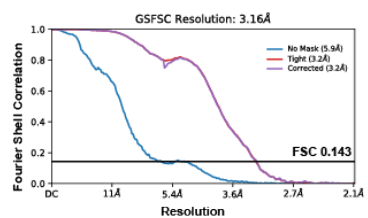
State A

State A

Overall map

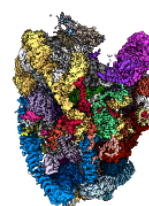


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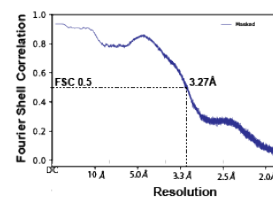


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Composite map



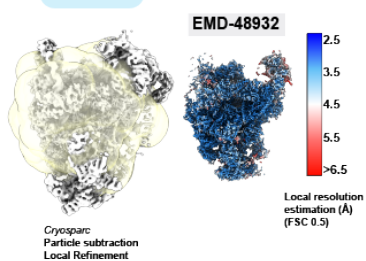
EMD-49075



C
Focused maps

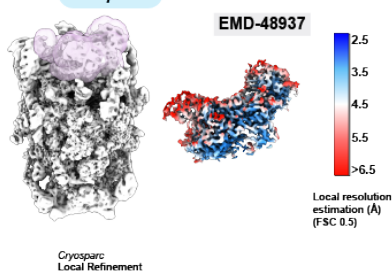
Focused maps

Core

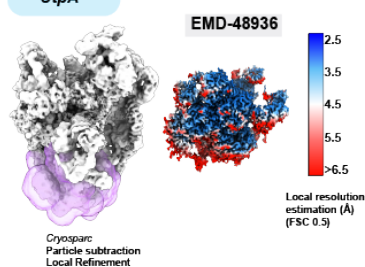


Cryosparc
Particle subtraction
Local Refinement

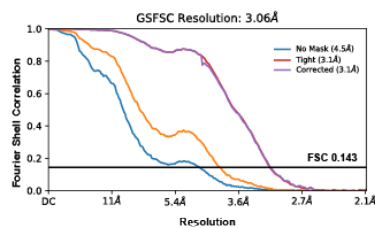
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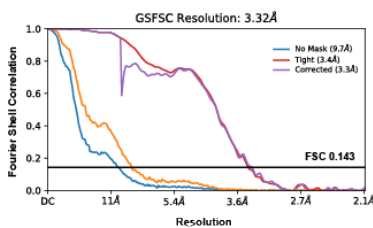
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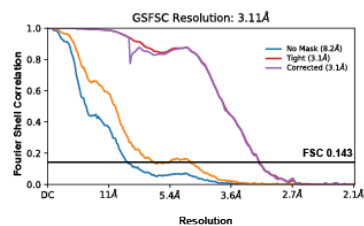
Cryosparc
Particle subtraction
Local Refinement



1



Resolution

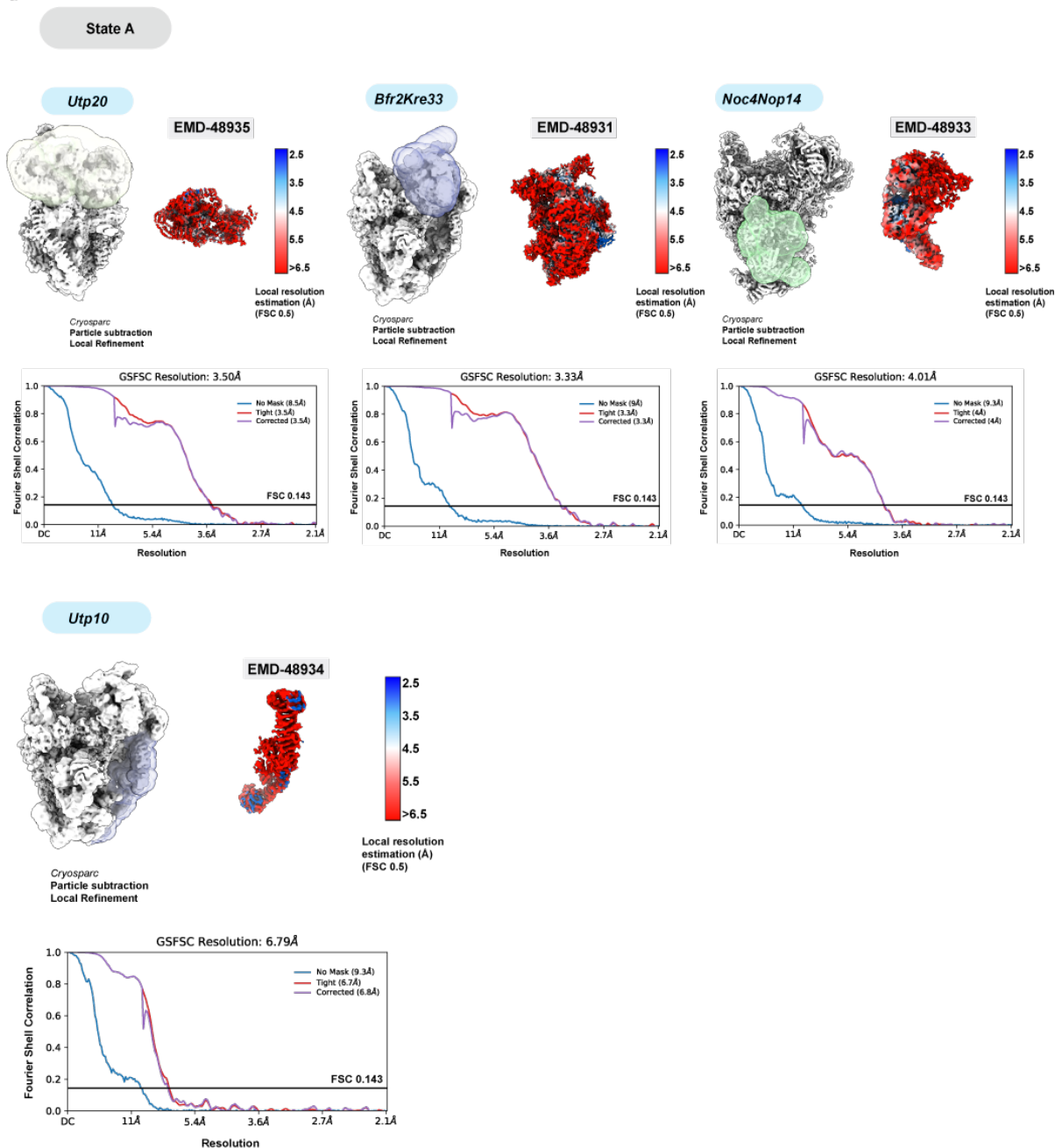


Resolution

Supplementary Fig. 4. Cryo-EM focused maps and composite reconstruction of State A.

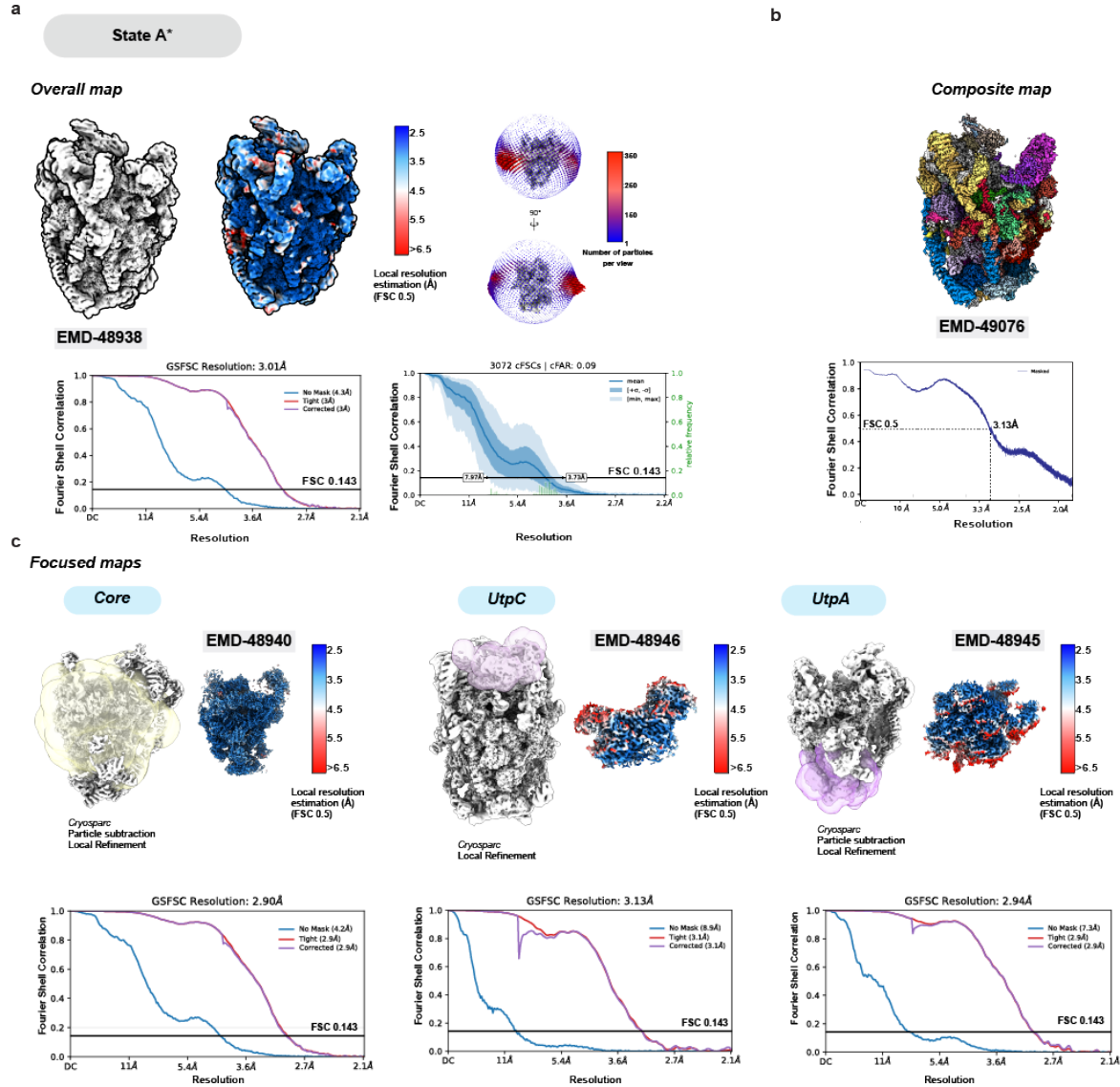
(a) Local-resolution filtered overall map of State A. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, UtpC and UtpA modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.

a



Supplementary Fig. 5. Cryo-EM focused maps of State A continued.

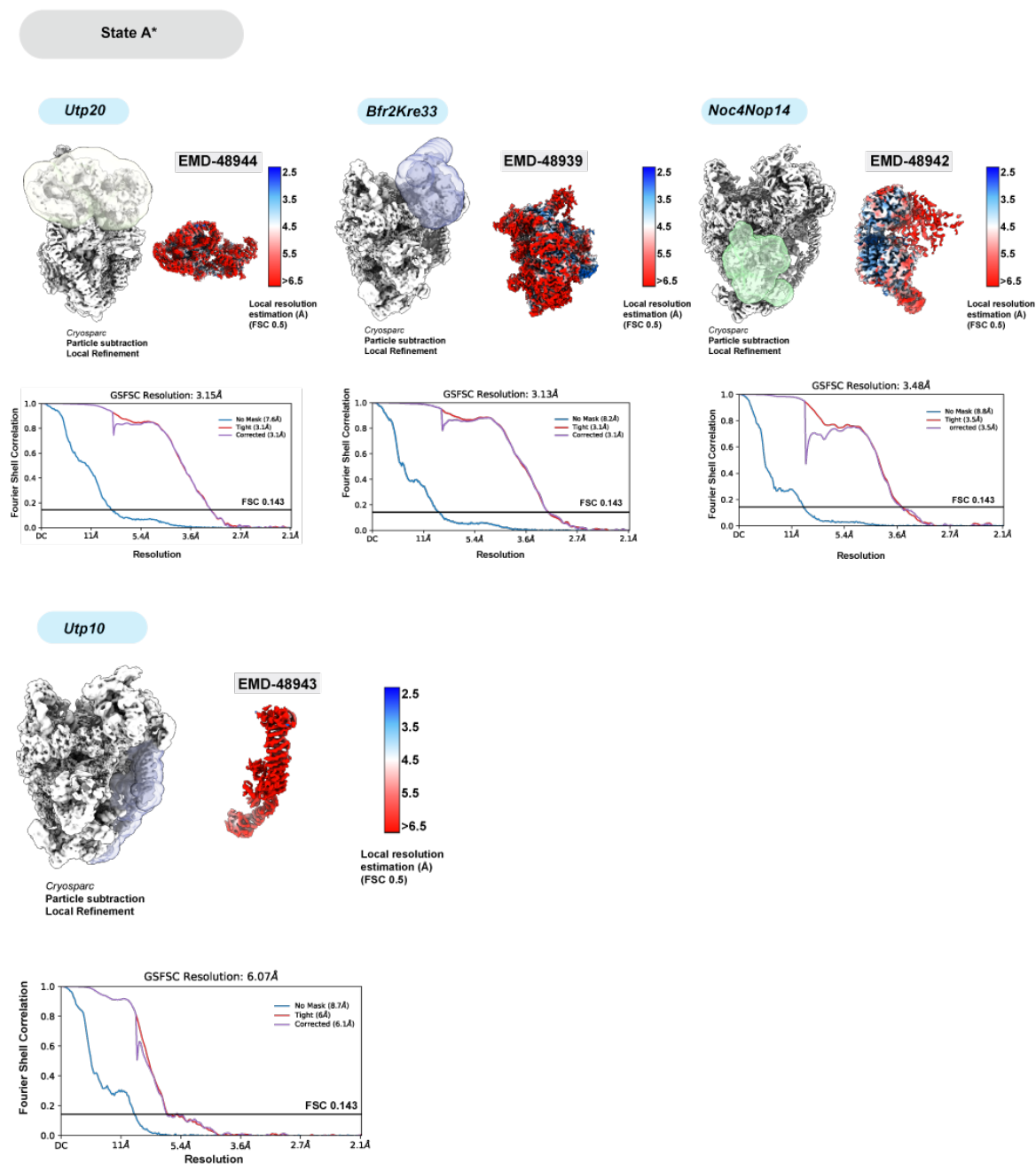
(a) Focused maps for the Utp20, Bfr2Kre33, Noc4Nop14, and Utp10 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.



Supplementary Fig. 6. Cryo-EM focused maps and composite reconstruction of State A*.

(a) Local-resolution filtered overall map of State A*. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, UtpC and UtpA modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.

a



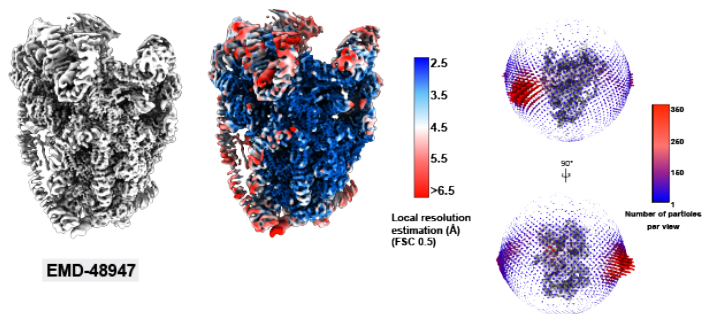
Supplementary Fig. 7. Cryo-EM focused maps of State A* continued.

(a) Focused maps for the Utp20, Bfr2Kre33, Noc4Nop14, and Utp10 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.

a

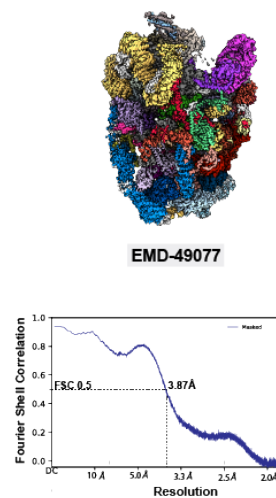
State B

Overall map



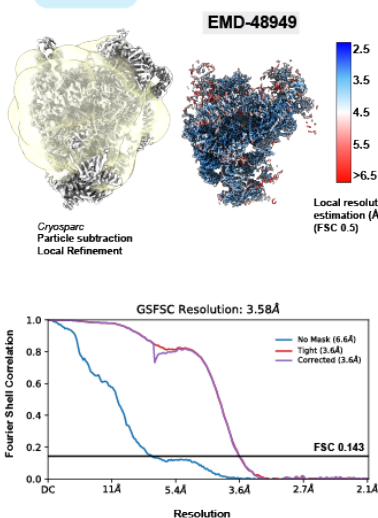
b

Composite map

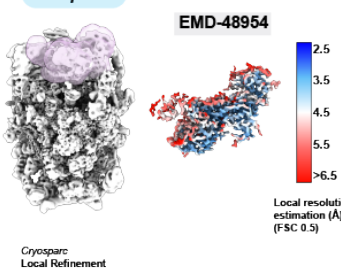


C Focused maps

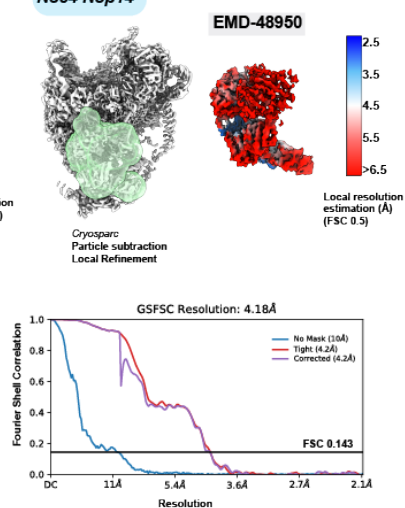
Core



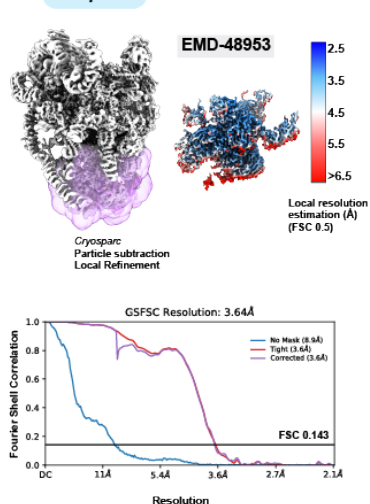
UtpC



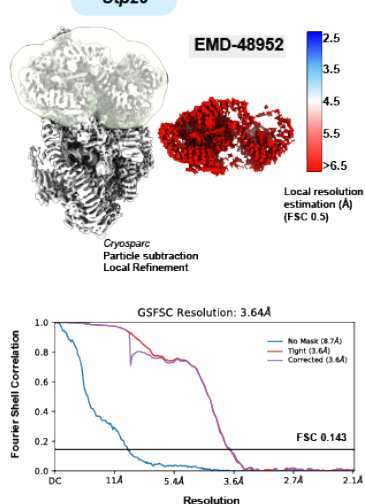
Noc4 Nop14



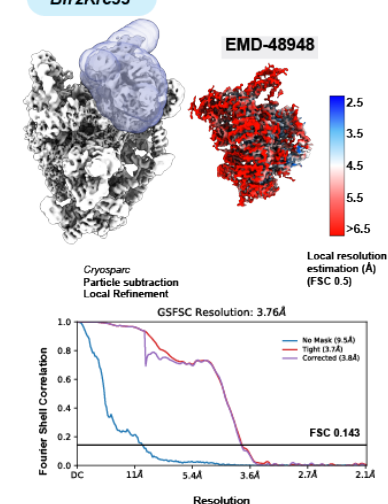
UtpA



Utp20



Bfr2Kre33



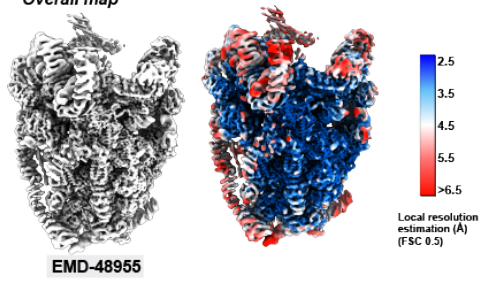
Supplementary Fig. 8. Cryo-EM focused maps and composite reconstruction of State B.

(a) Local-resolution filtered overall map of State B. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, UtpC, Noc4Nop14, UtpA, Utp20, and Bfr2Kre33 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.

a

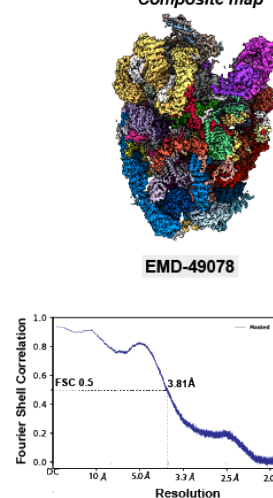
State C

Overall map



b

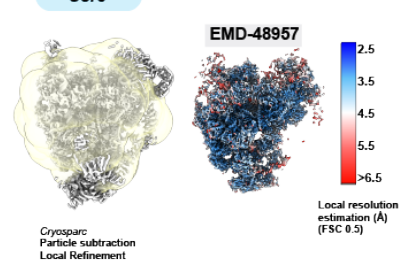
Composite map



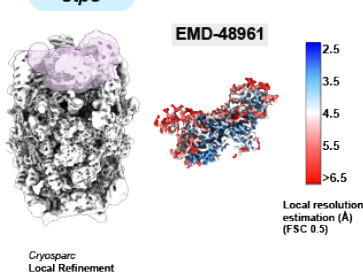
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Focused maps

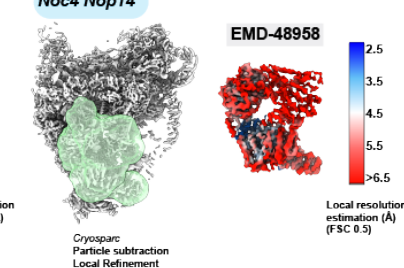
Core



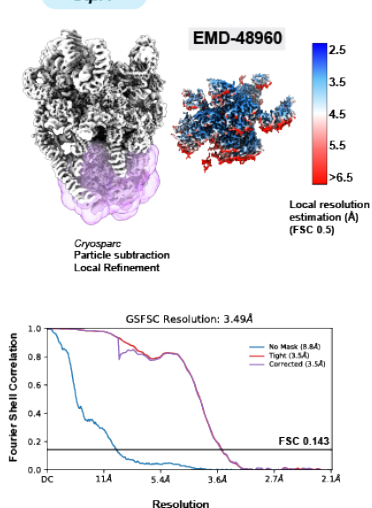
UtpC



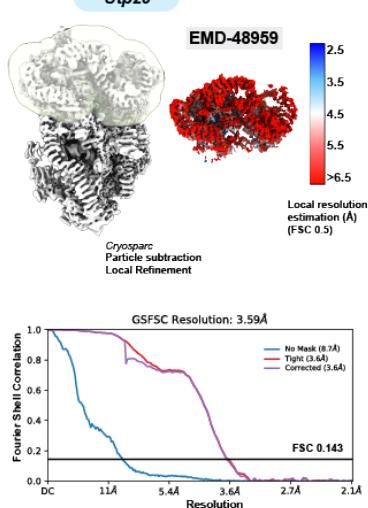
Noc4 Nop14



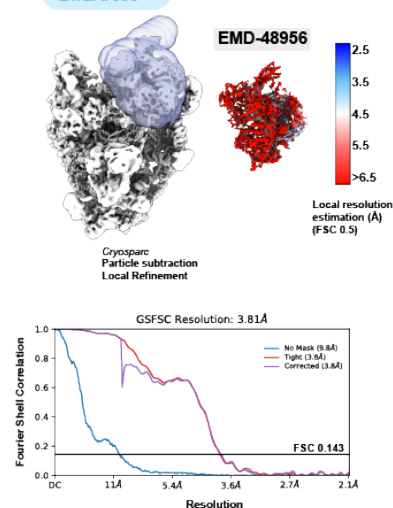
UtpA



Utp20



Bfr2Kre33



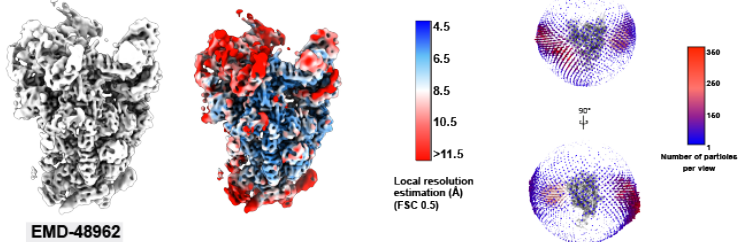
Supplementary Fig. 9. Cryo-EM focused maps and composite reconstruction of State C.

(a) Local-resolution filtered overall map of State C. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, UtpC, Noc4Nop14, UtpA, Utp20, and Bfr2Kre33 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.

a

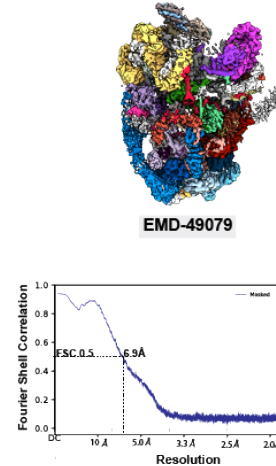
State D

Overall map



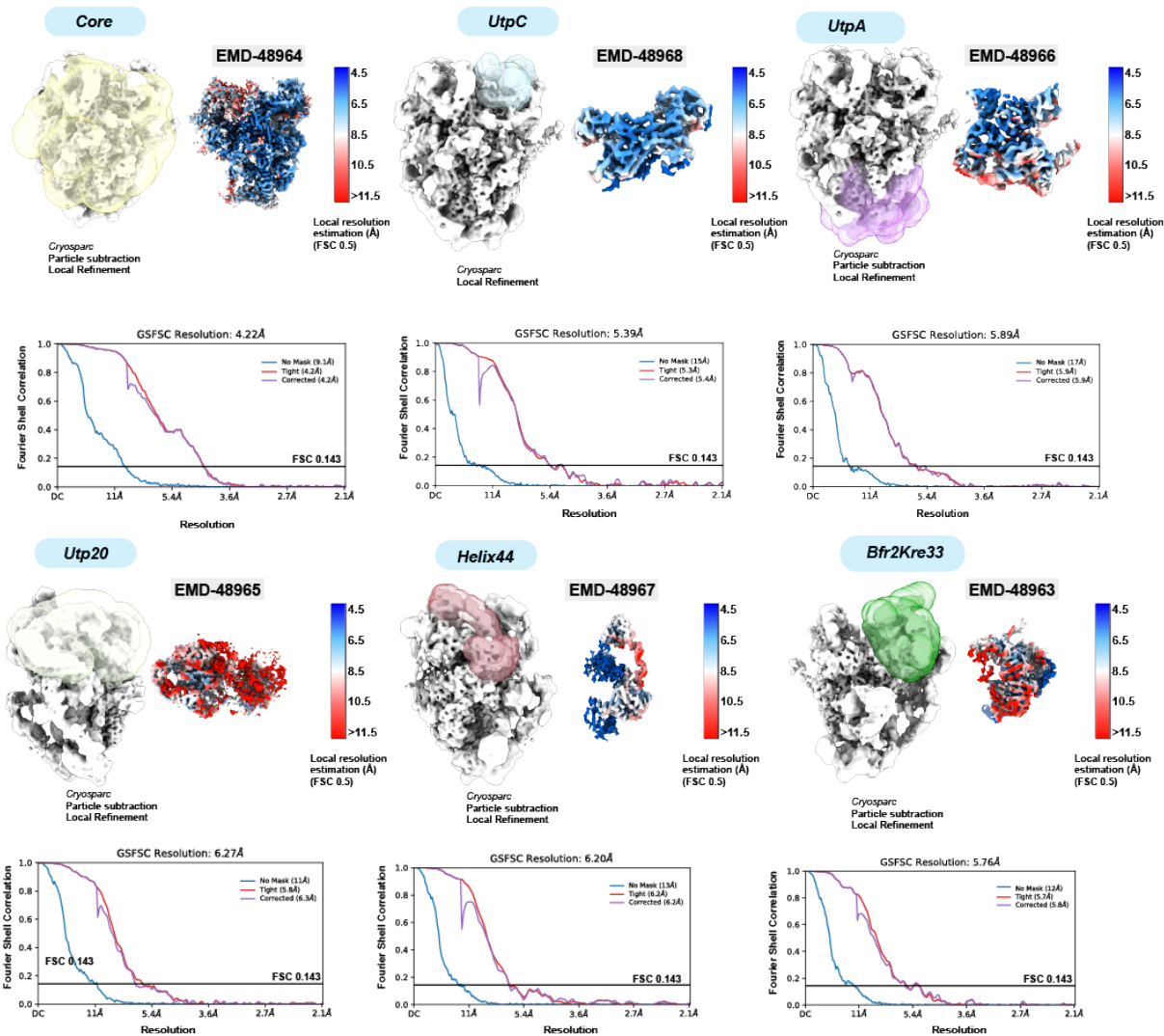
b

Composite map



c

Focused maps



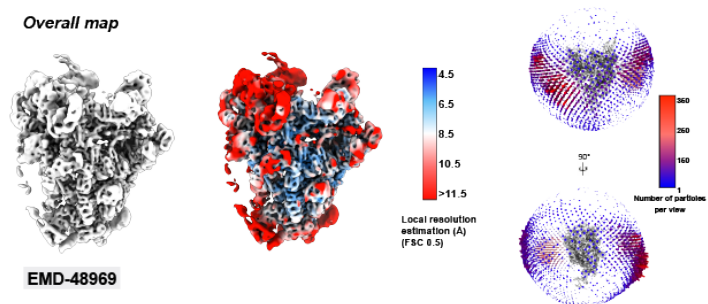
Supplementary Fig. 10. Cryo-EM focused maps and composite reconstruction of State D.

(a) Local-resolution filtered overall map of State D. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, UtpC, UtpA, Utp20, Helix44 and Bfr2Kre33 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.

a

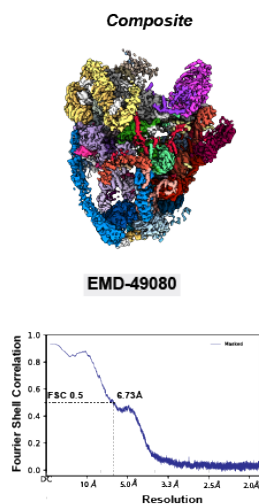
State E

Overall map



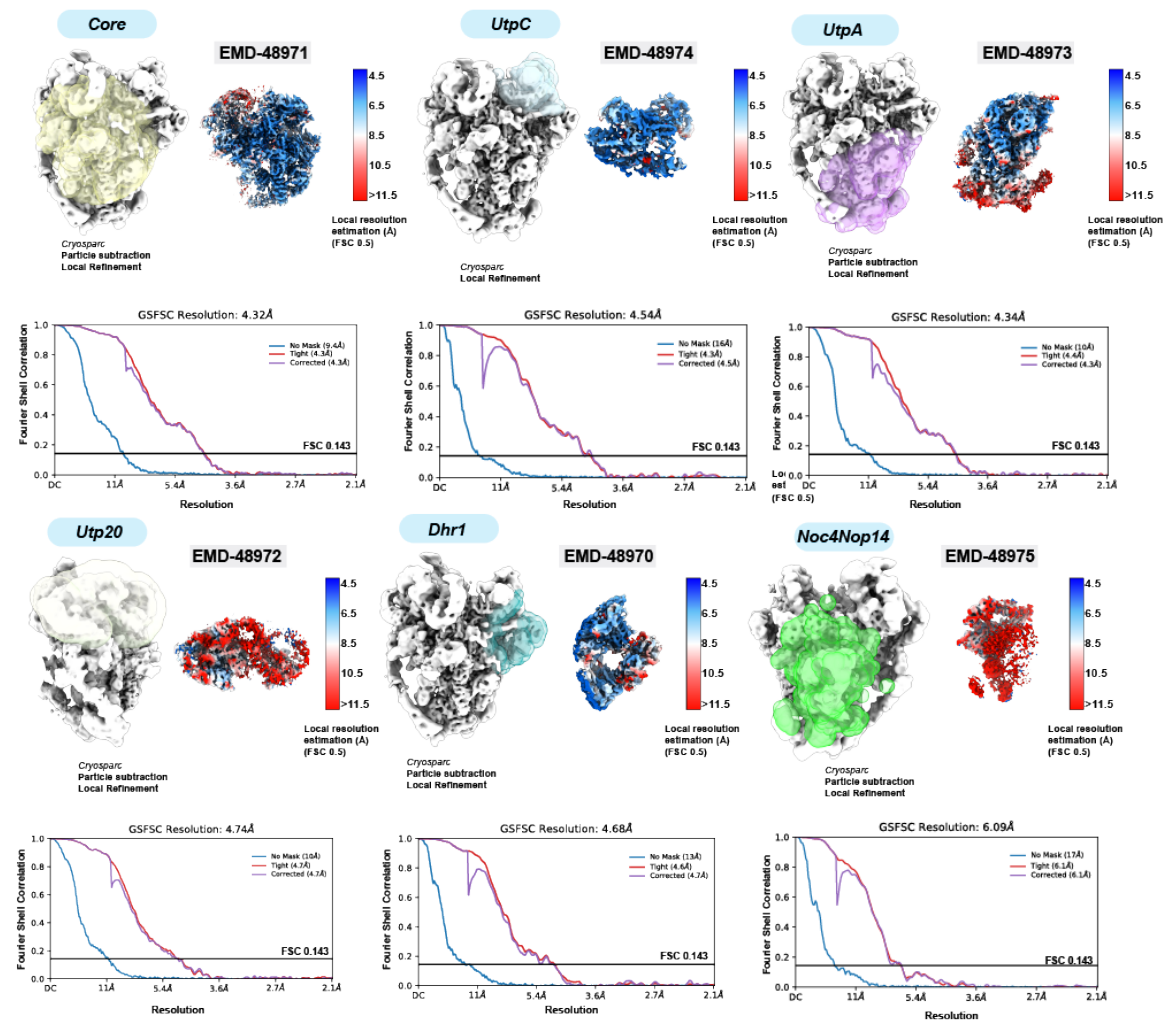
b

Composite



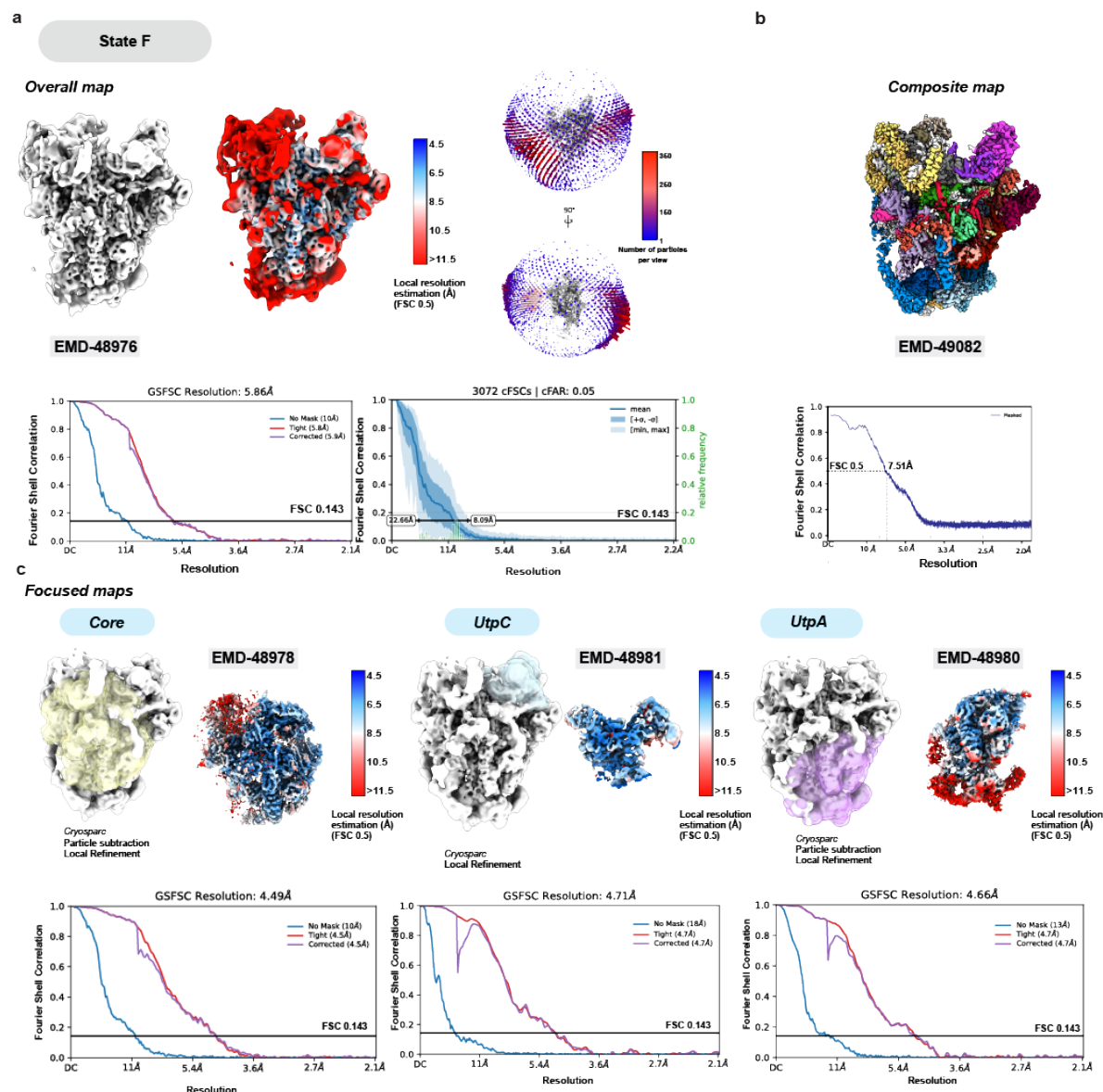
c

Focused maps



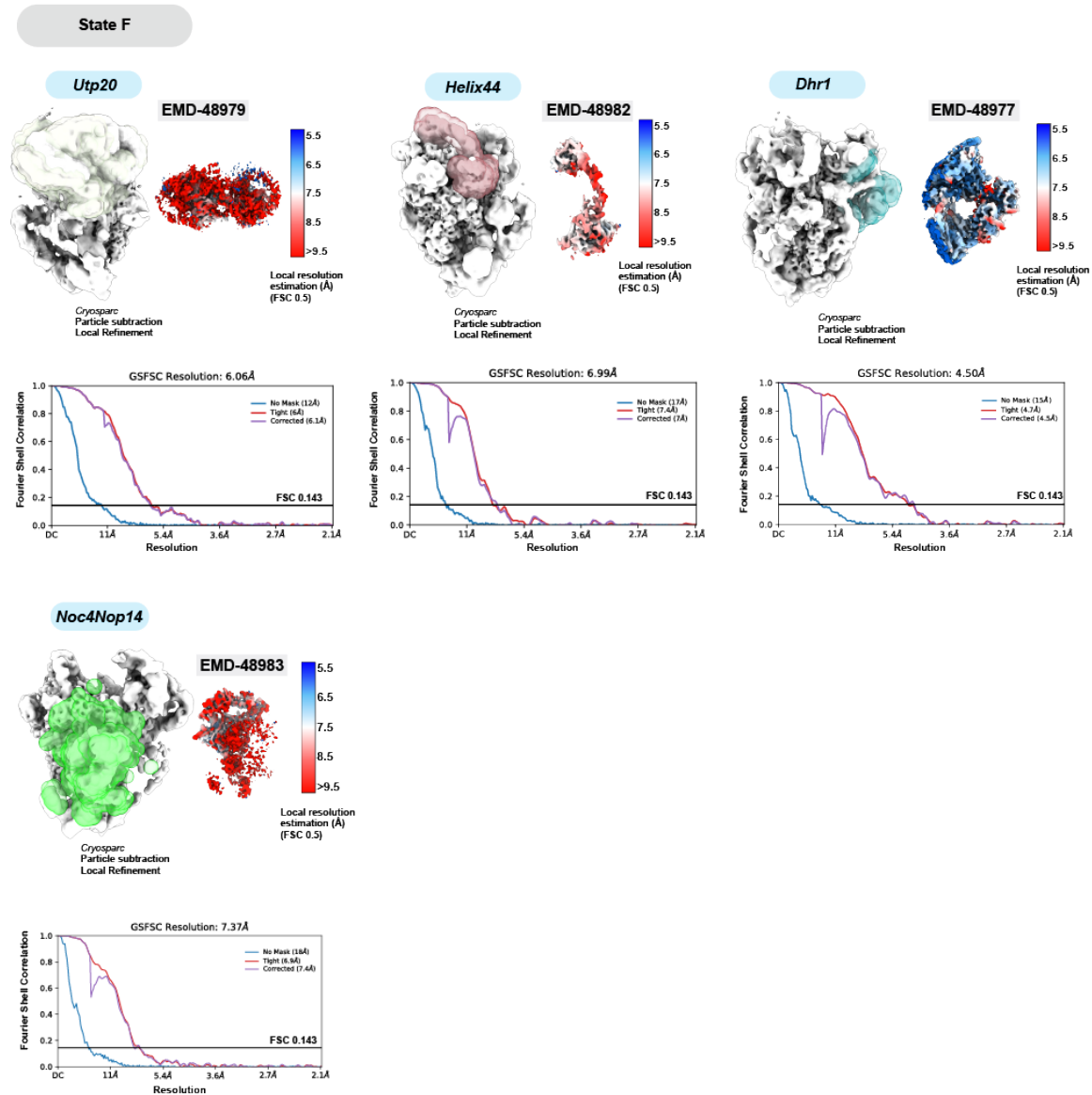
Supplementary Fig. 11. Cryo-EM focused maps and composite reconstruction of State E.

(a) Local-resolution filtered overall map of State E. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, UtpC, UtpA, Utp20, Dhr1 and Noc4Nop14 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.



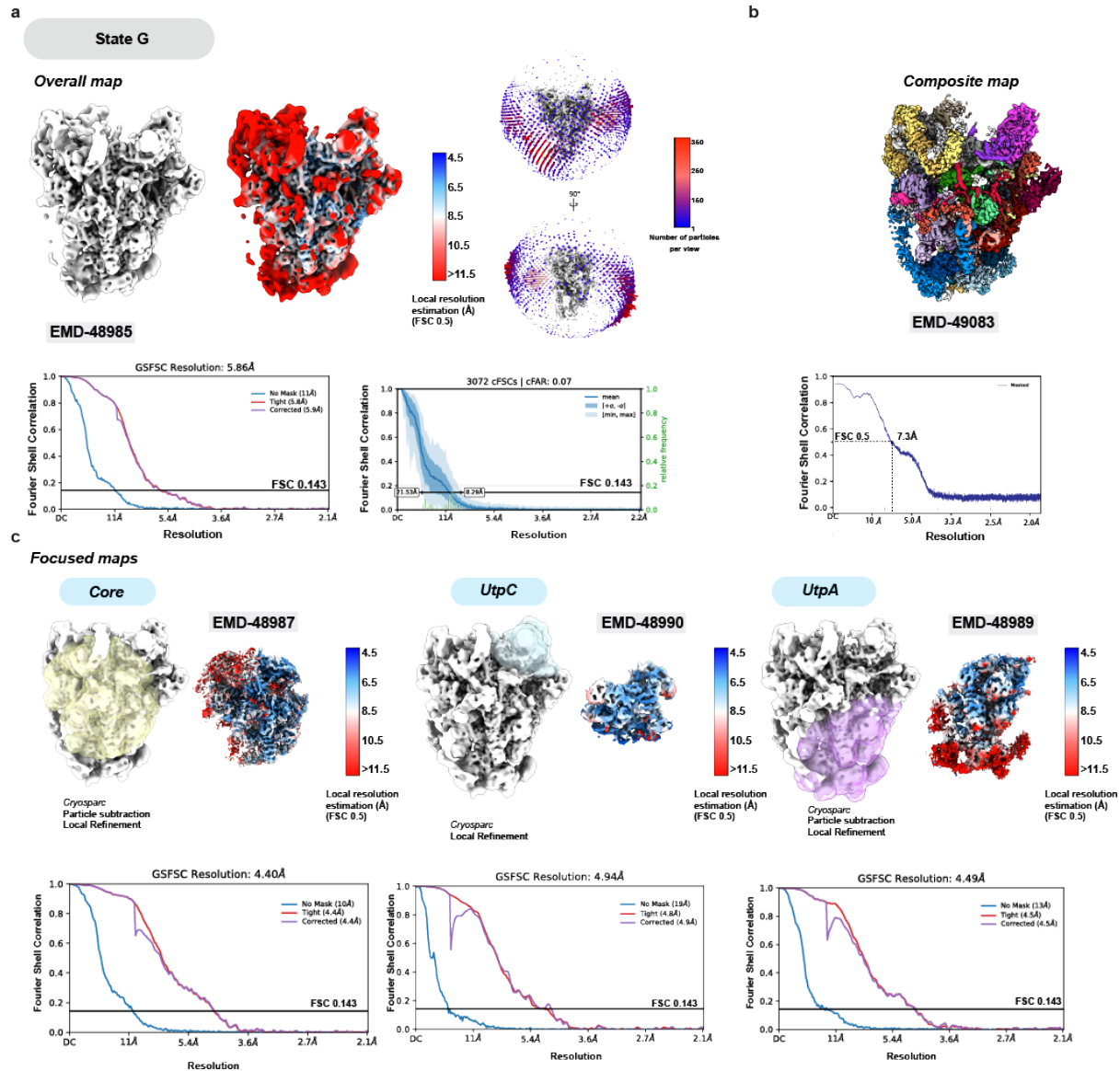
Supplementary Fig. 12. Cryo-EM focused maps and composite reconstruction of State F. (a) Local-resolution filtered overall map of State F. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, UtpC and UtpA modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.

a



Supplementary Fig. 13. Cryo-EM focused maps of State F continued.

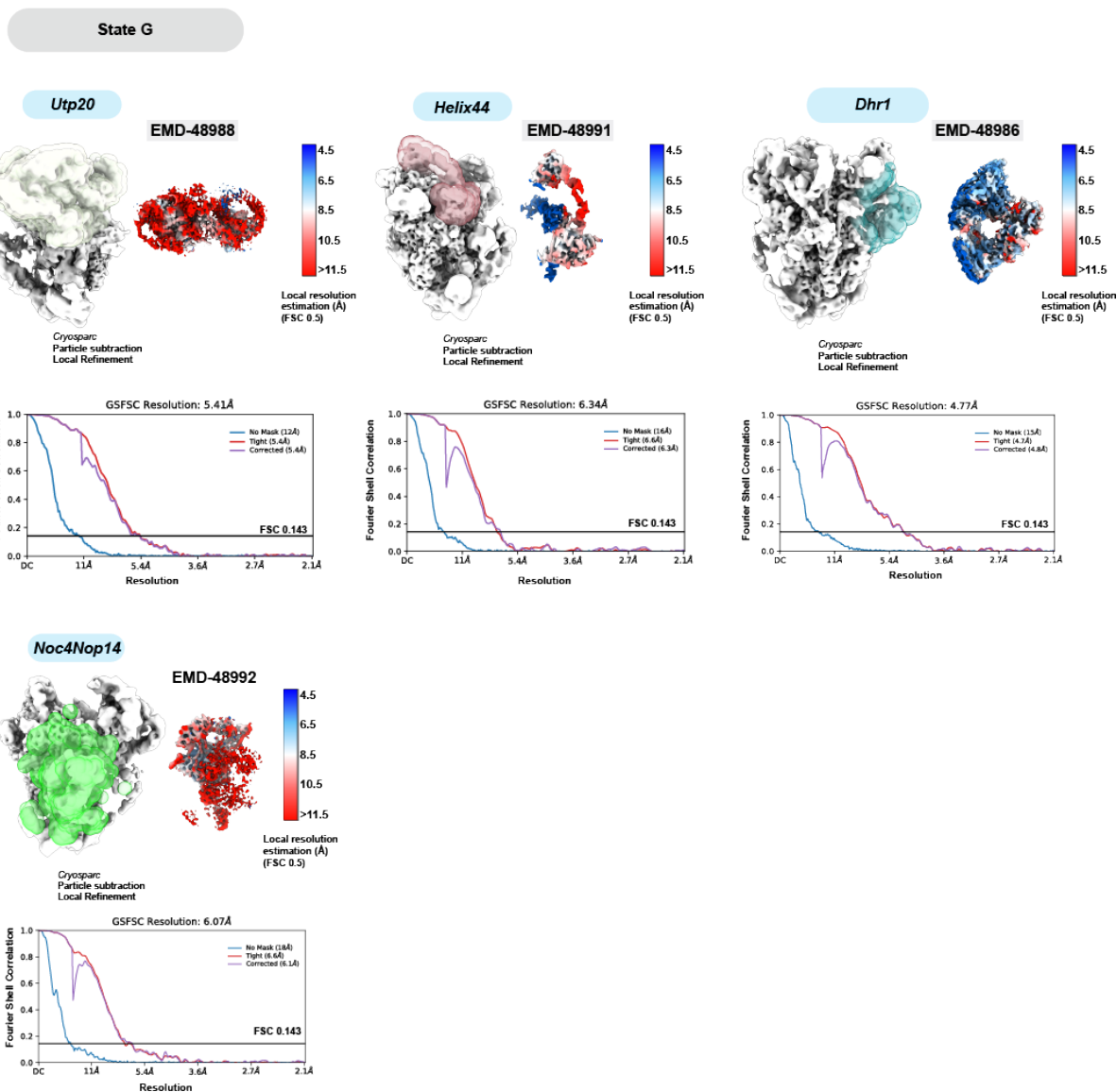
(a) Focused maps for the Utp20, Helix44, Dhr1 and Noc4Nop14 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.



Supplementary Fig. 14. Cryo-EM focused maps and composite reconstruction of State G.

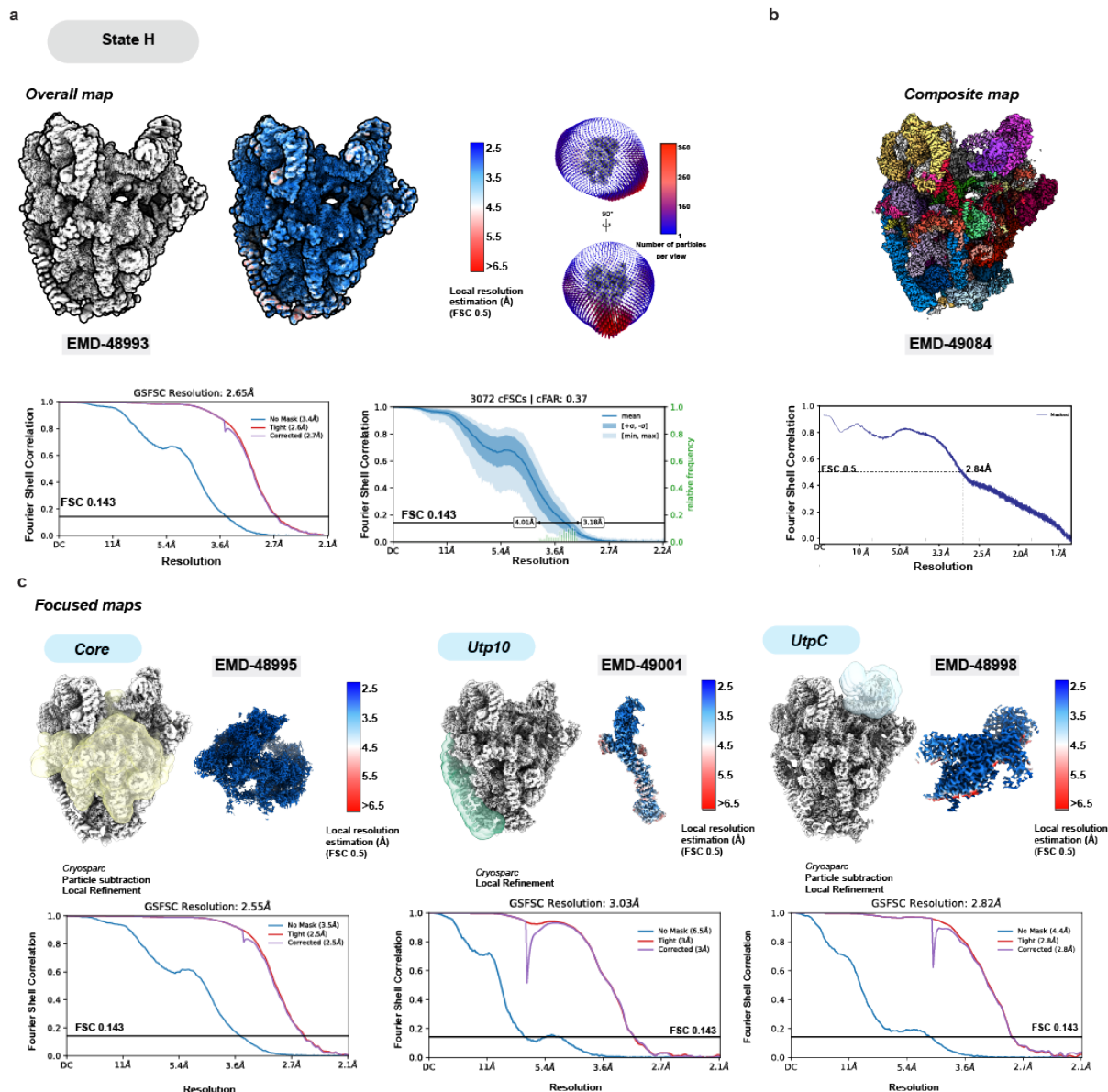
(a) Local-resolution filtered overall map of State G. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, UtpC and UtpA modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.

a



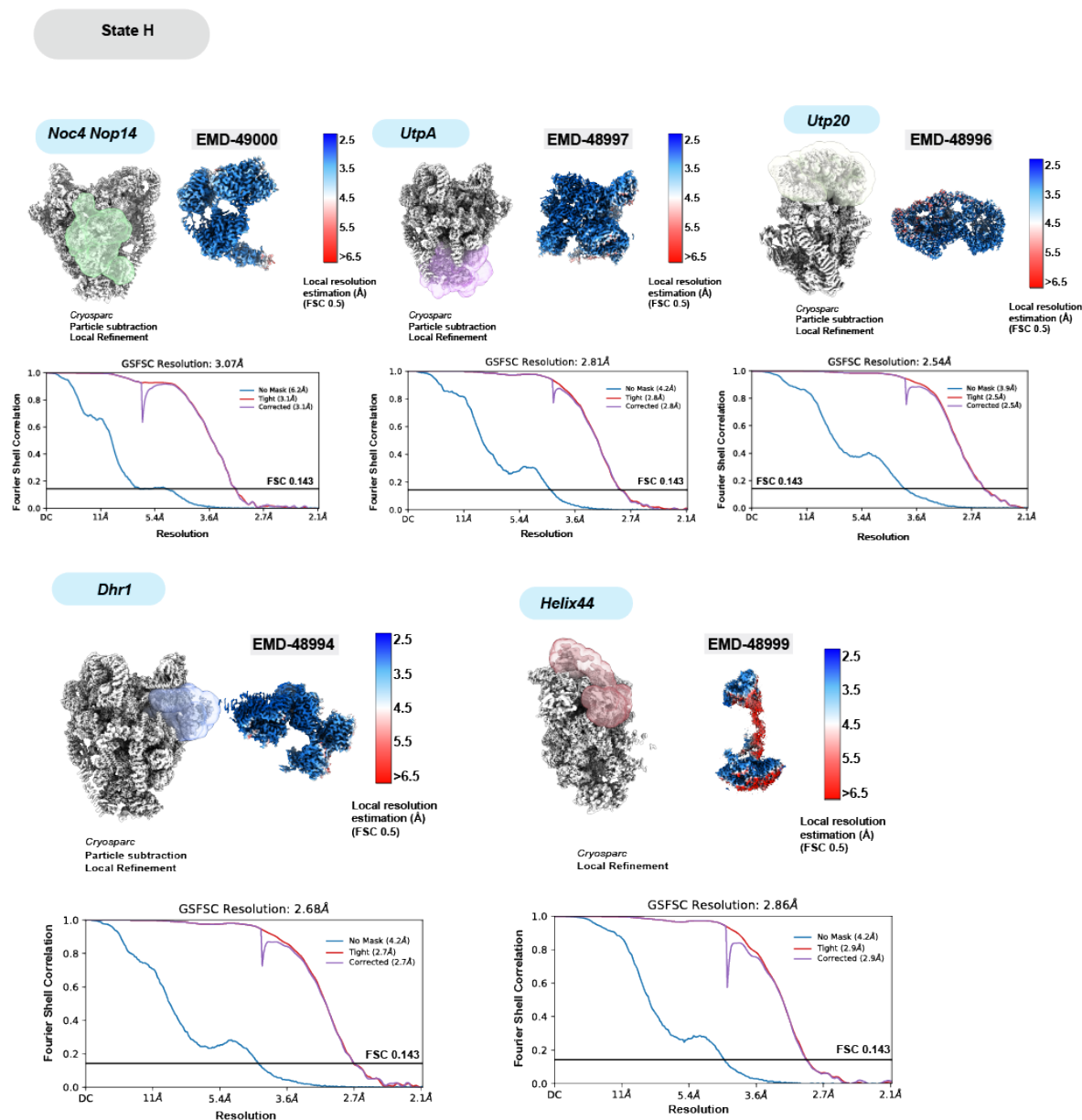
Supplementary Fig. 15. Cryo-EM focused maps of State G continued.

(a) Focused maps for the Utp20, Helix44, Dhr1 and Noc4Nop14 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.



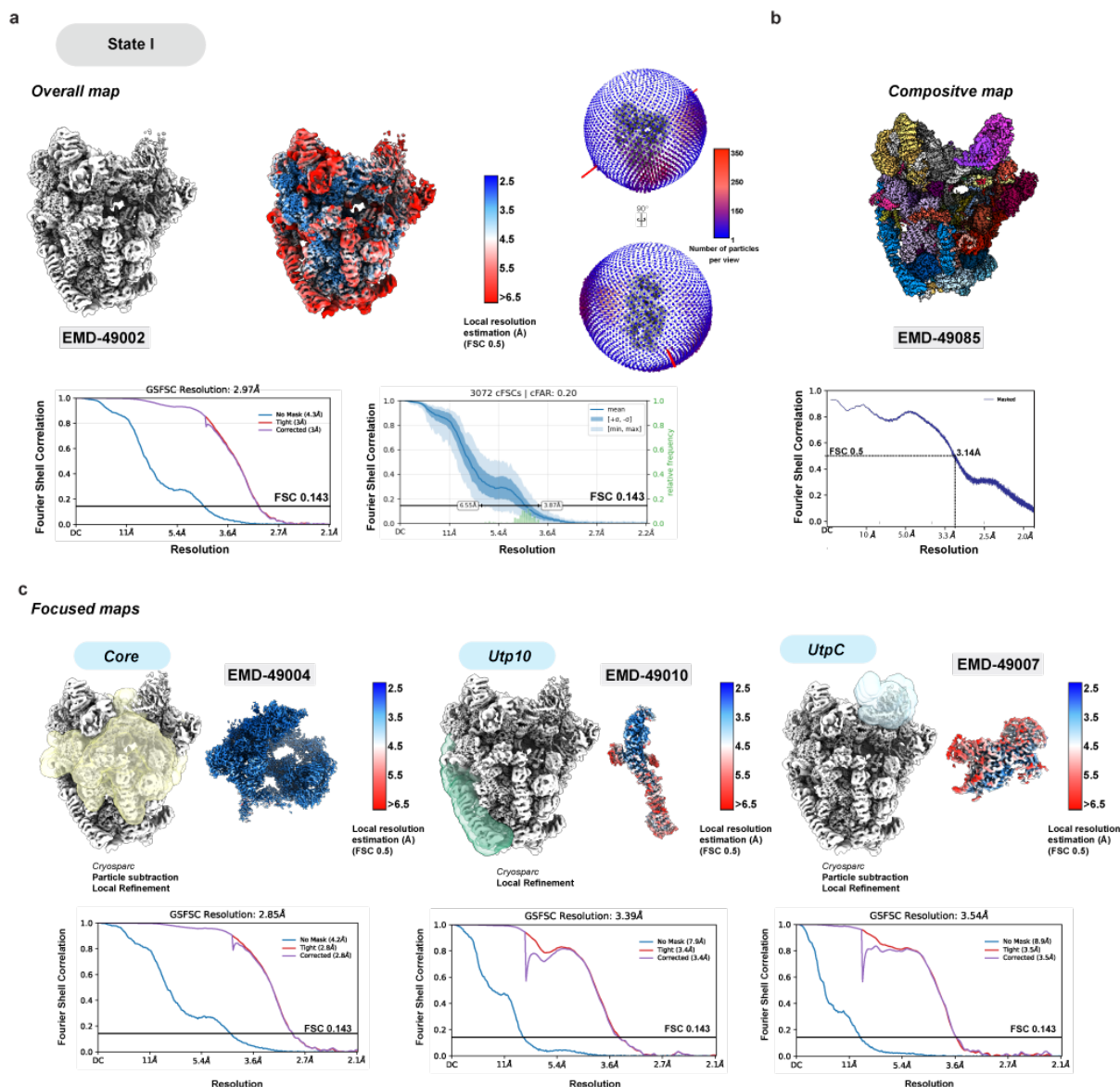
Supplementary Fig. 16. Cryo-EM focused maps and composite reconstruction of State H. (a) Local-resolution filtered overall map of State H. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, Utp10 and UtpC modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.

a



Supplementary Fig. 17. Cryo-EM focused maps of State H continue.

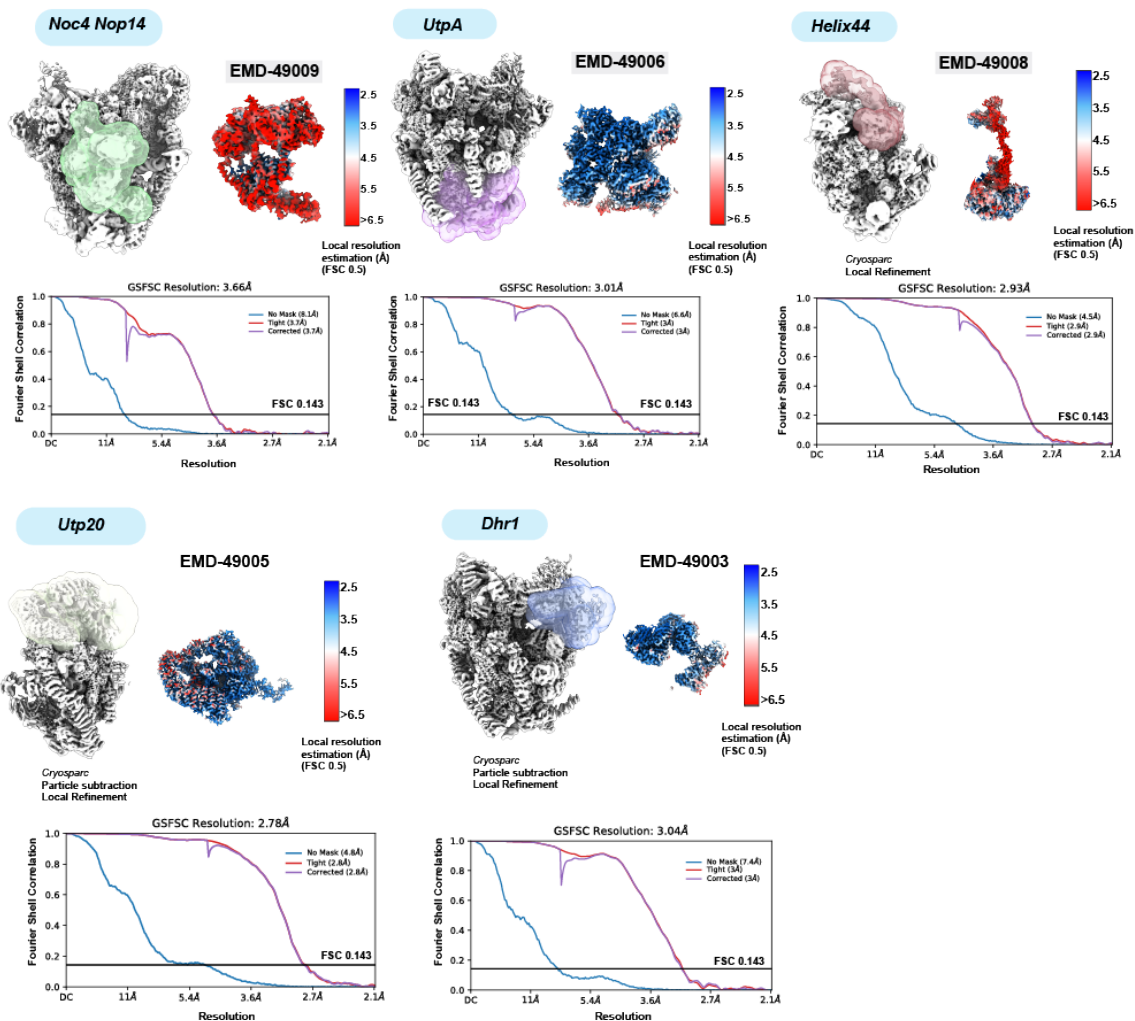
(a) Focused maps for the Noc4Nop14, UtpA, Utp20, Dhr1, and Helix44 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.



Supplementary Fig. 18. Cryo-EM focused maps and composite reconstruction of State I. (a) Local-resolution filtered overall map of State I. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, Utp10 and UtpC modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.

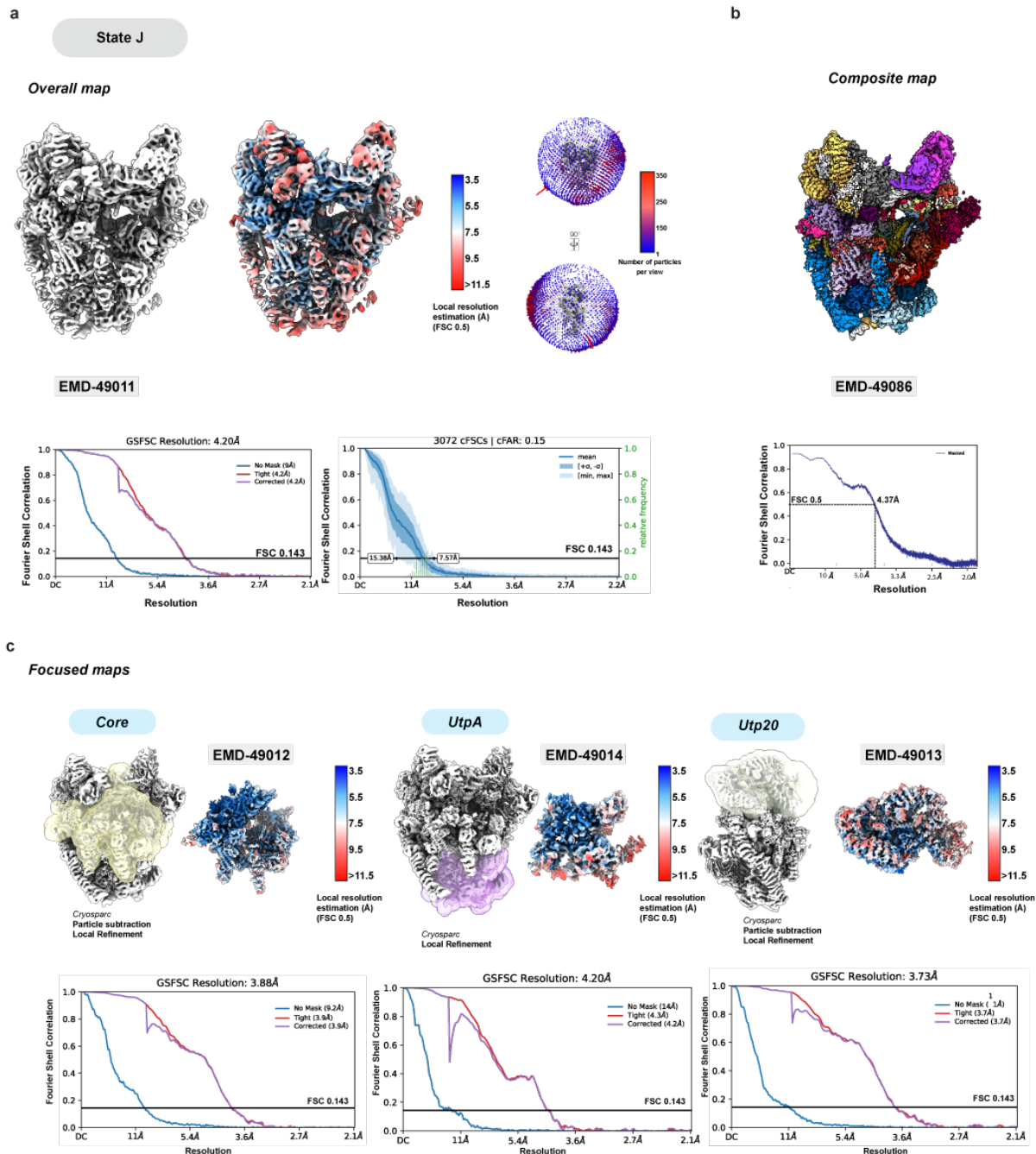
c

State I

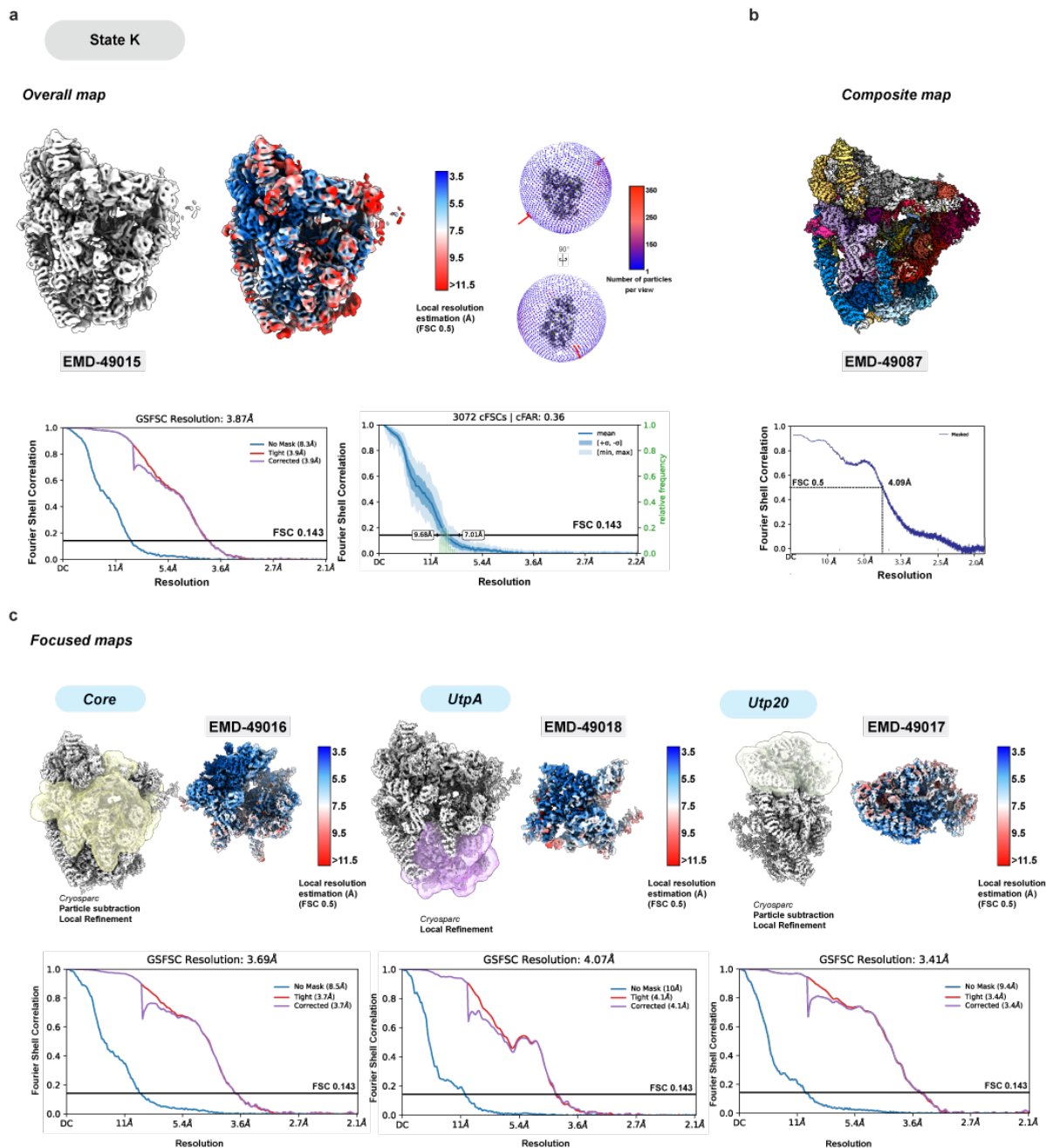


Supplementary Fig. 19. Cryo-EM focused maps of State I continued.

(a) Focused maps for the *Noc4Nop14*, *UtpA*, *Helix44*, *Utp20*, and *Dhr1* modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.

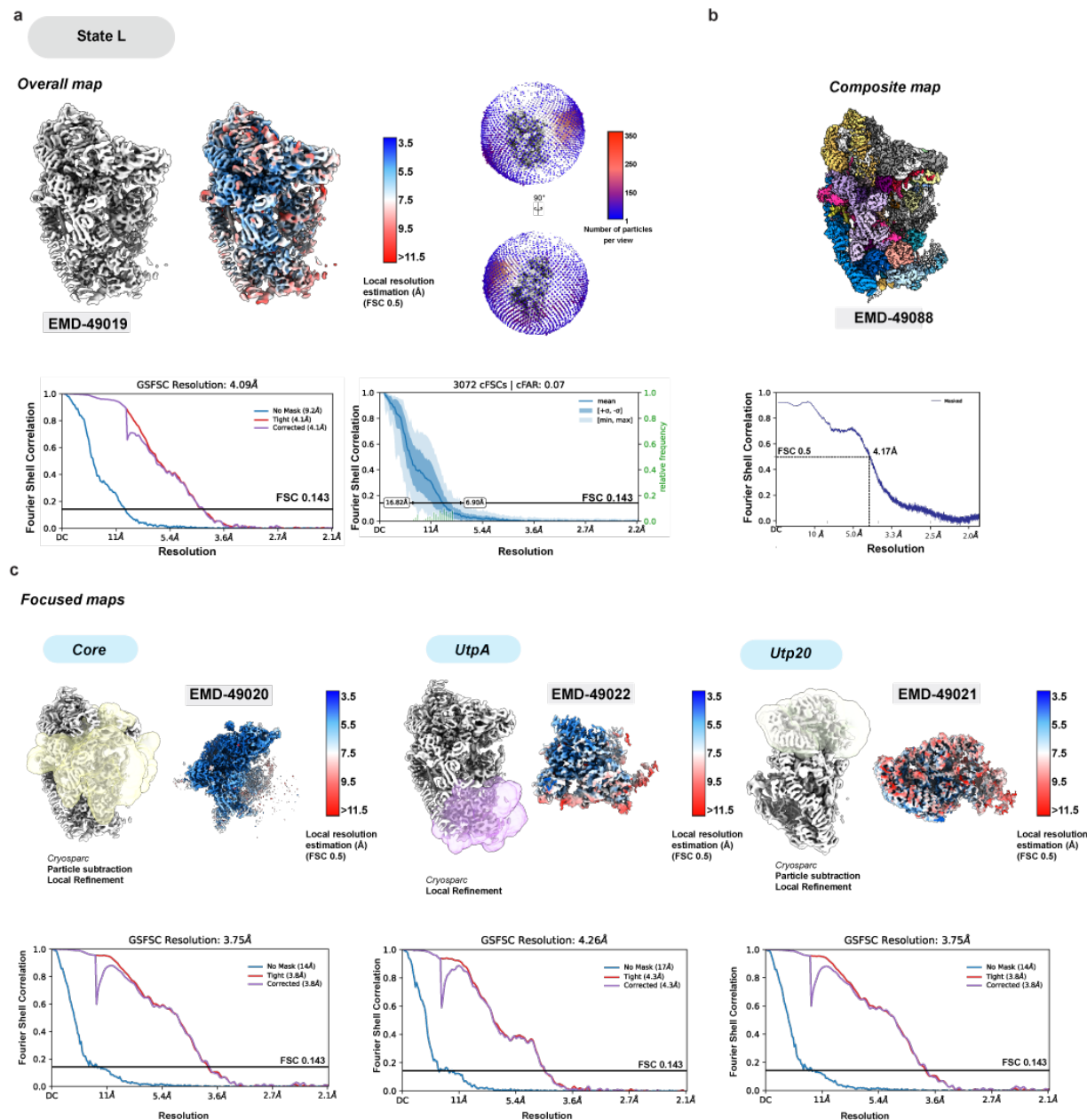


Supplementary Fig. 20. Cryo-EM focused maps and composite reconstruction of State J. (a) Local-resolution filtered overall map of State J. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, UtpA and Utp20 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.



Supplementary Fig. 21. Cryo-EM focused maps and composite reconstruction of State K.

(a) Local-resolution filtered overall map of State K. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, UtpA, and Utp20 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.

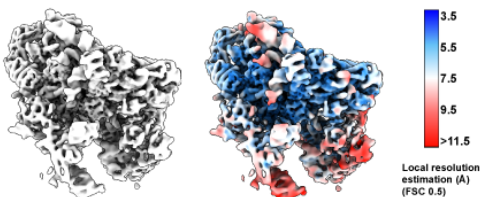


Supplementary Fig. 22. Cryo-EM focused maps and composite reconstruction of State L. (a) Local-resolution filtered overall map of State L. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, UtpA, and Utp20 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.

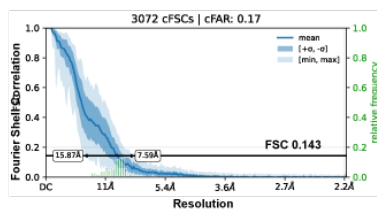
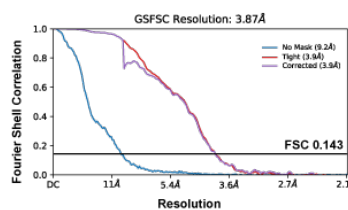
a

State M

Overall map

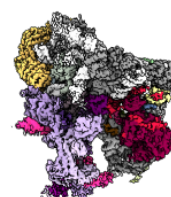


EMD-49023

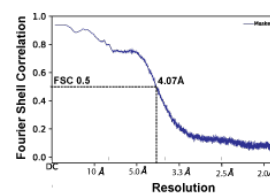


b

Composite map



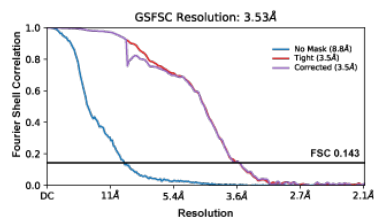
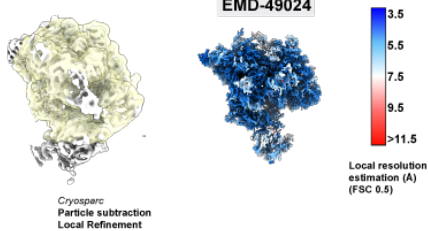
EMD-49089



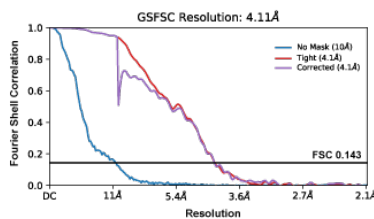
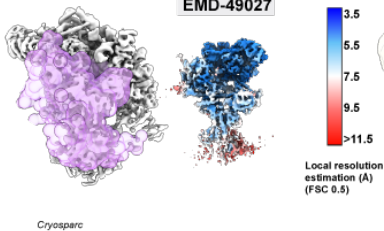
c

Focused maps

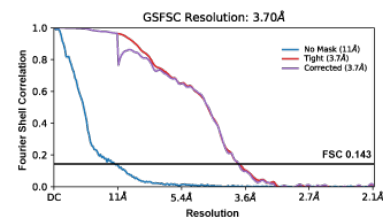
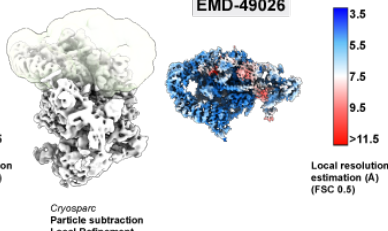
Core



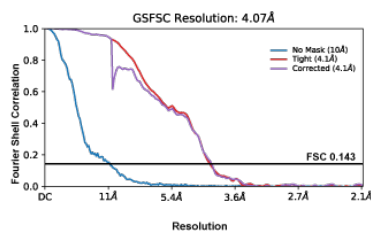
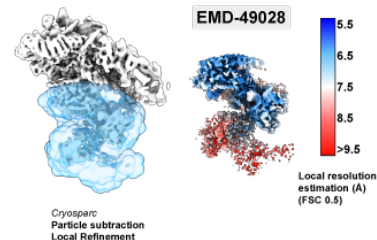
U3



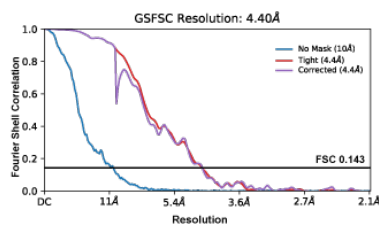
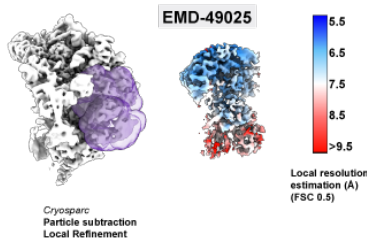
Utp20



Noc4Nop14



Dhr1



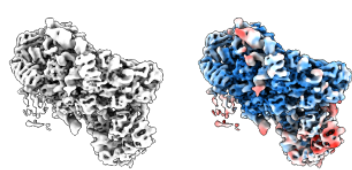
Supplementary Fig. 23. Cryo-EM focused maps and composite reconstruction of State M.

(a) Local-resolution filtered overall map of State M. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, U3, Utp20, Noc4nop14, and Dhr1 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.

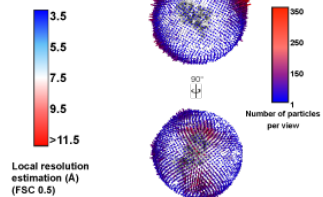
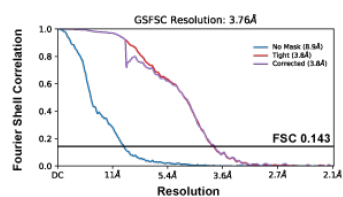
a

State N

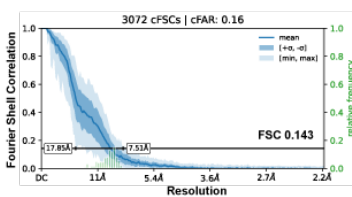
Overall map



EMD-49029



Local resolution estimation (Å) (FSC 0.5)

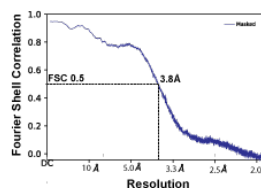


b

Composite



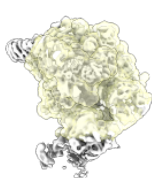
EMD-49090



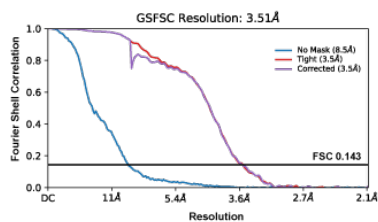
c

Focused maps

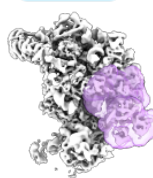
Core



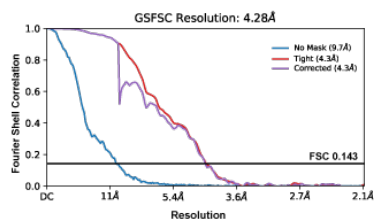
EMD-49030

Cryosparc
Particle subtraction
Local Refinement

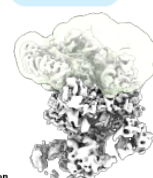
Dhr1



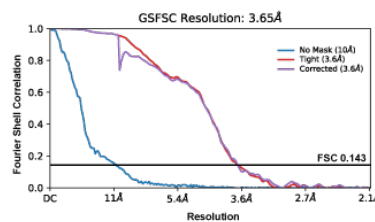
EMD-49031

Cryosparc
Particle subtraction
Local Refinement

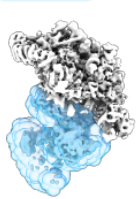
Utp20



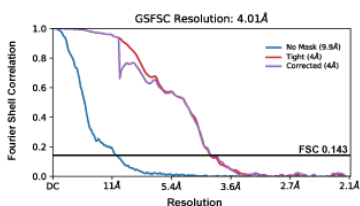
EMD-49032

Cryosparc
Particle subtraction
Local Refinement

Noc4Nop14

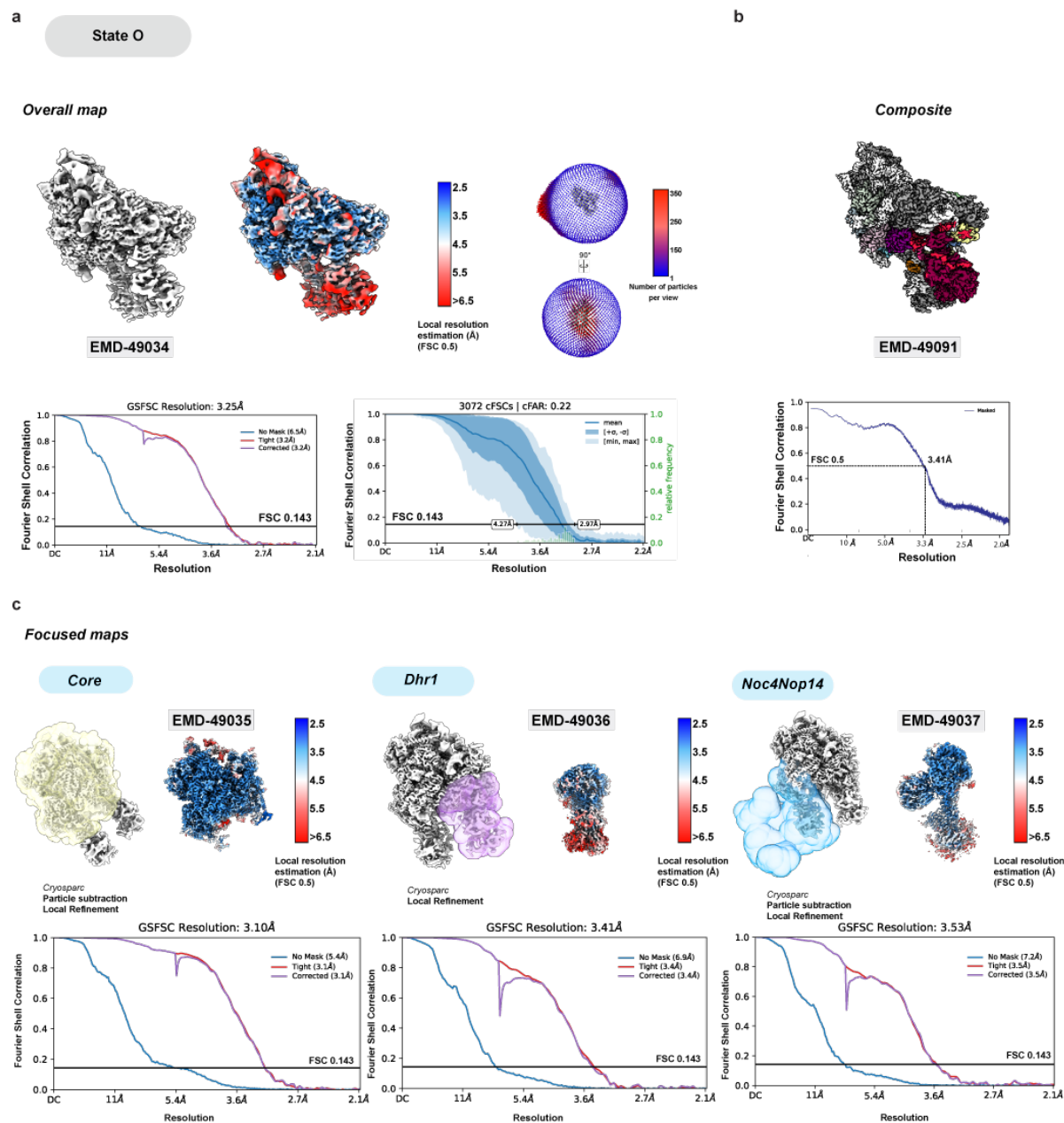


EMD-49033

Cryosparc
Particle subtraction
Local Refinement

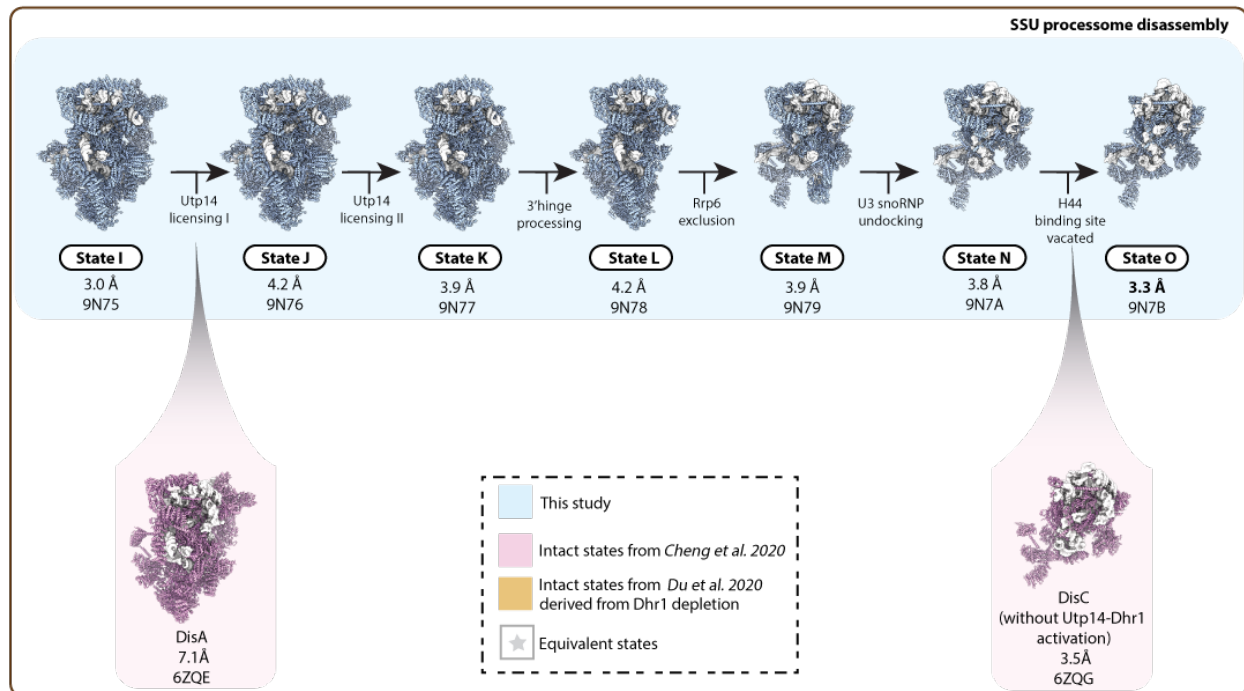
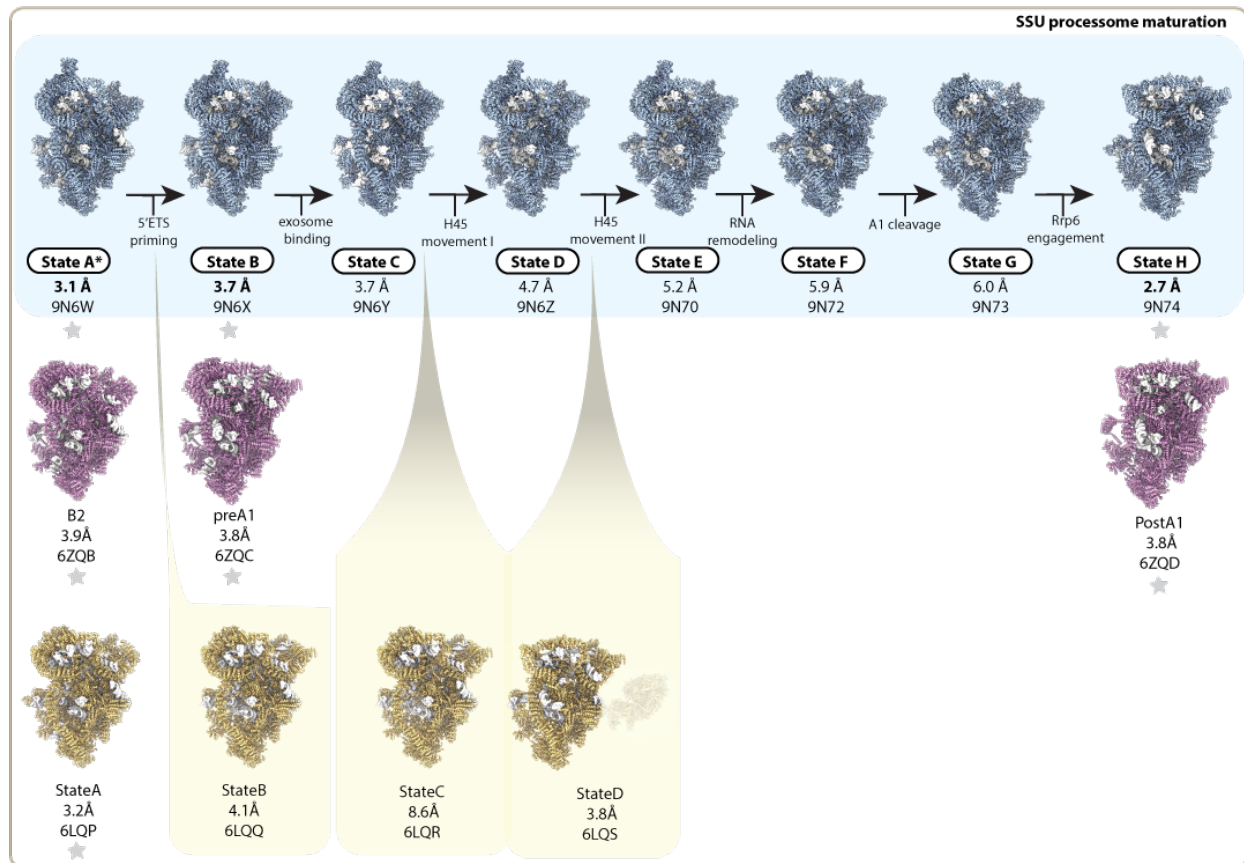
Supplementary Fig. 24. Cryo-EM focused maps and composite reconstruction of State N.

(a) Local-resolution filtered overall map of State N. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, Dhr1, Utp20, and Noc4nop14 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.



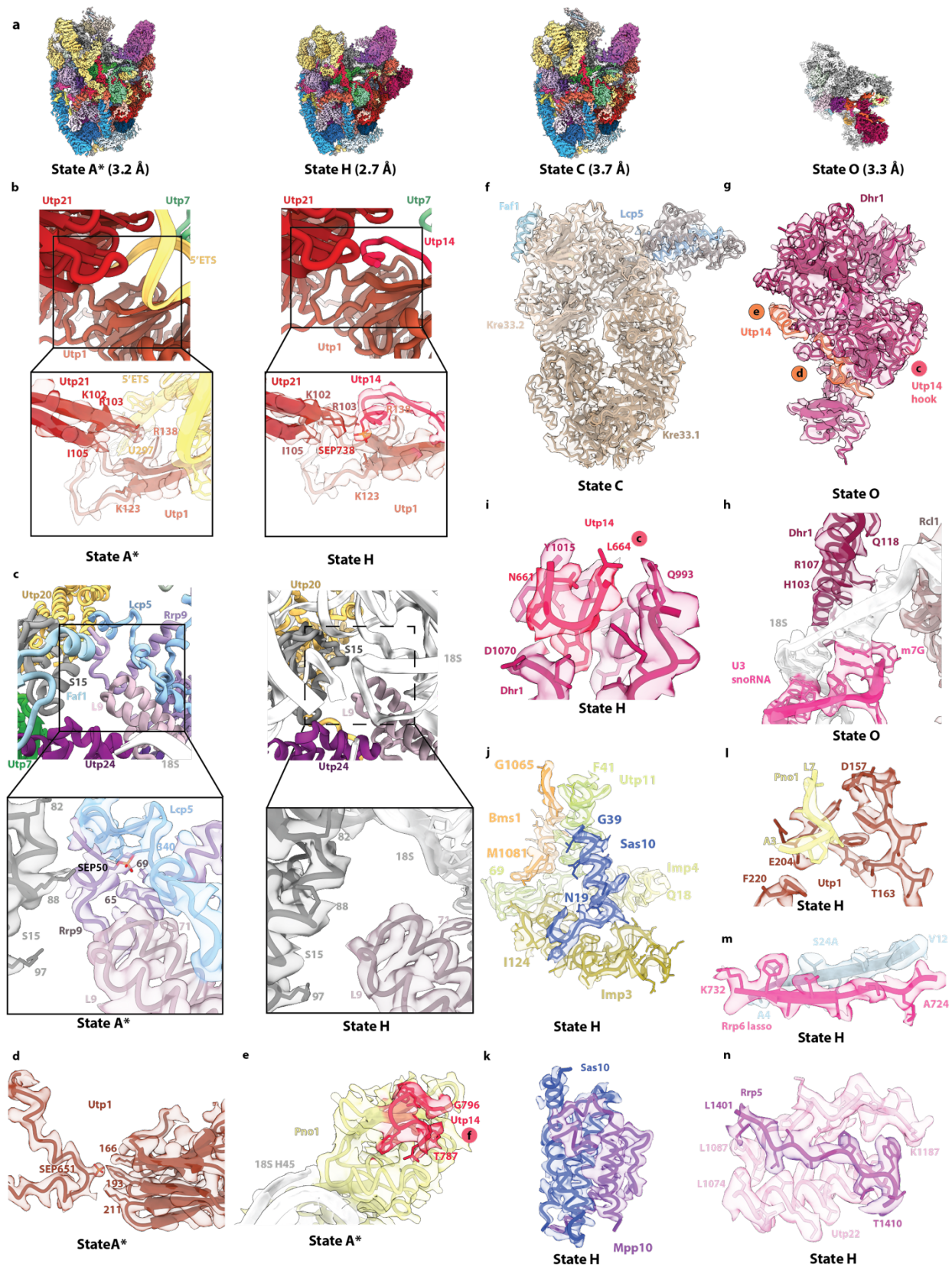
Supplementary Fig. 25. Cryo-EM focused maps and composite reconstruction of State O.

(a) Local-resolution filtered overall map of State O. FSC curves (no mask, tight mask and solvent corrected and 3D) are displayed on the bottom and Euler angle distribution is displayed on the right. (b) Composite map and FSC map-to-model curve displayed on the bottom. (c) Focused maps for the core, Dhr1, and Noc4nop14 modules used to generate the composite map are displayed along with the respective FSC curves (no mask, tight mask and solvent corrected) on the bottom.



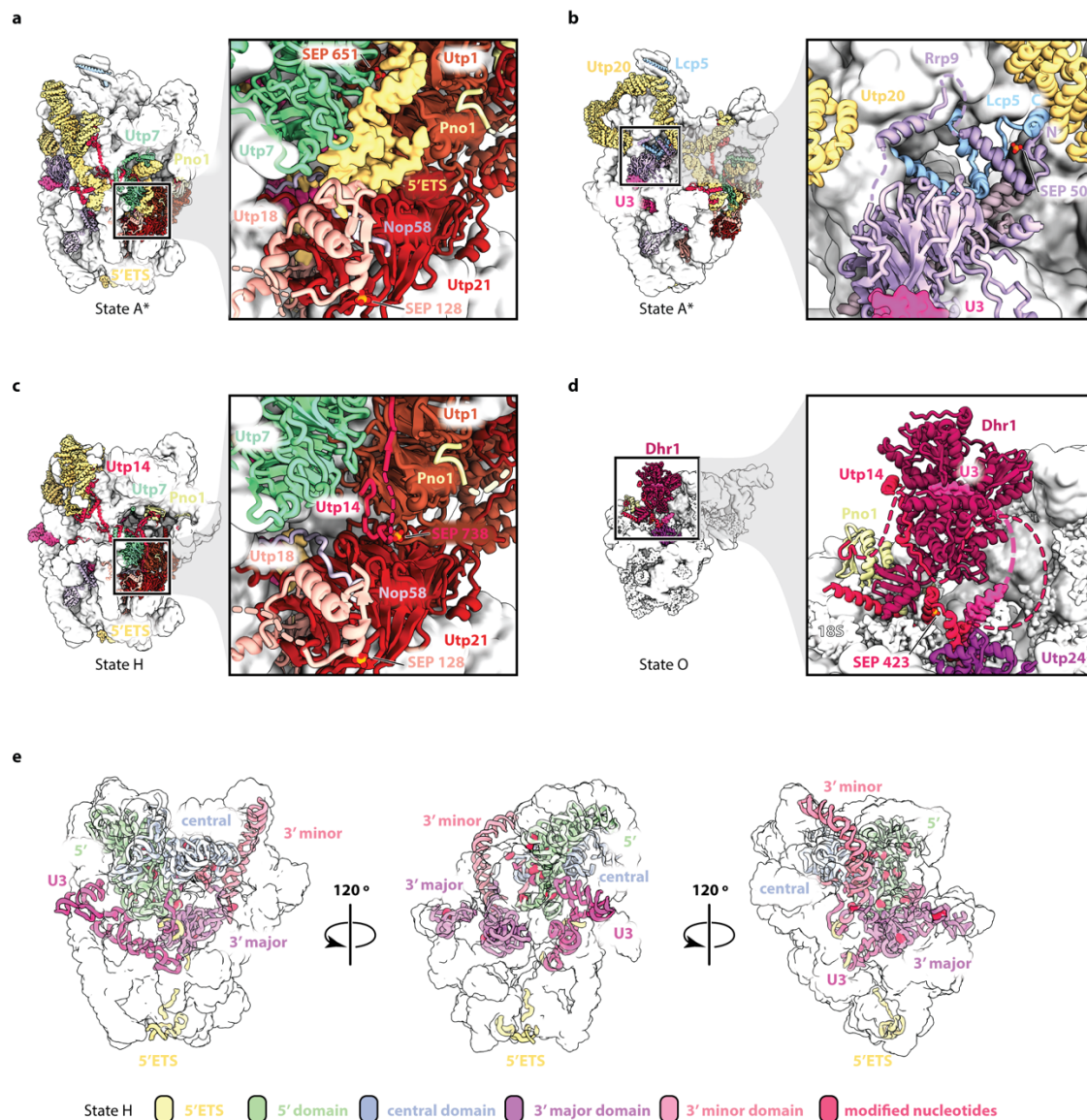
Supplementary Fig. 26. Comparative view of yeast SSU maturation and disassembly pathways.

Schematic representation of the *Saccharomyces cerevisiae* SSU processome maturation (top) and disassembly (bottom). Only intact states are depicted, states from this study are highlighted with blue background, intact states from other studies are highlighted with yellow and pink backgrounds and similar states are indicated with a grey star. States from other studies that follow between those observed in this study are indicated with a zoom out. Poor density for the RNA exosome in State D (6LQS) relative to the rest of the map is depicted with shading. Resolutions and PDB codes are indicated.



Supplementary Fig. 27. Representative Cryo-EM densities and models.

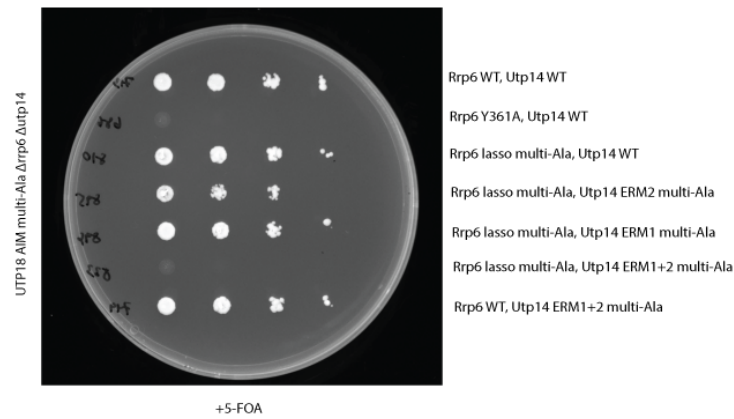
(a) Representative composite cryo-EM maps for States A*, State H, State C and State O with resolutions indicated in parenthesis below each state. **(b-n)** Selection of representative densities for assembly factors and RNAs from States A*, State H, State C and State O. The states from which density and models are derived are shown below each panel. Densities illustrated as continuous transparent volumes and models are shown as ribbons and sticks.



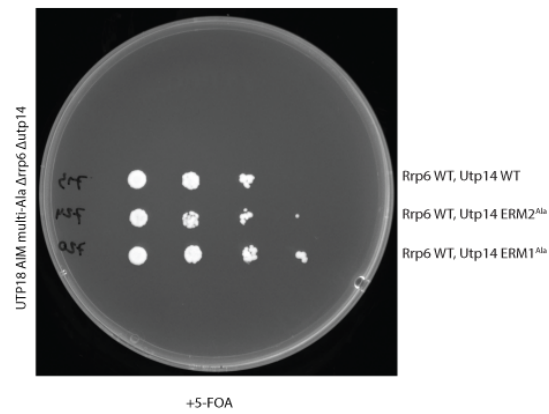
Supplementary Fig. 28. Modified residues in the SSU processome.

(a,b) Phosphoserines (SEP) present in State A*. (c) Phosphoserines present in State H. (d) Phosphoserines present in State O. (e) Locations of modified nucleotides in State H. RNA domains are color-coded and modified nucleotides are shown as red spheres.

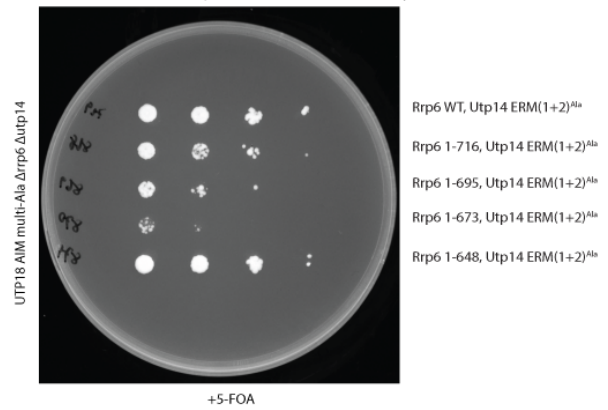
a Figure 2e Yeast Growth Assay



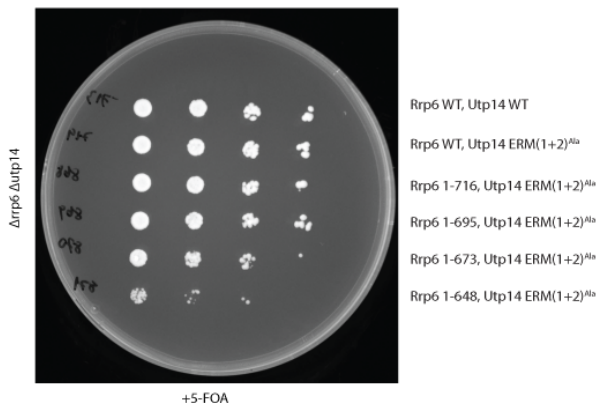
b Extended Data Fig. 5b Yeast Growth Assay



c Extended Data Fig. 5b Yeast Growth Assay



d Extended Data Fig. 5b Yeast Growth Assay



Supplementary Fig. 29. Uncropped yeast growth assays.

Uncropped yeast growth assay plates relating to: (a) Fig. 2e, (b-d) Extended Data Fig.5b