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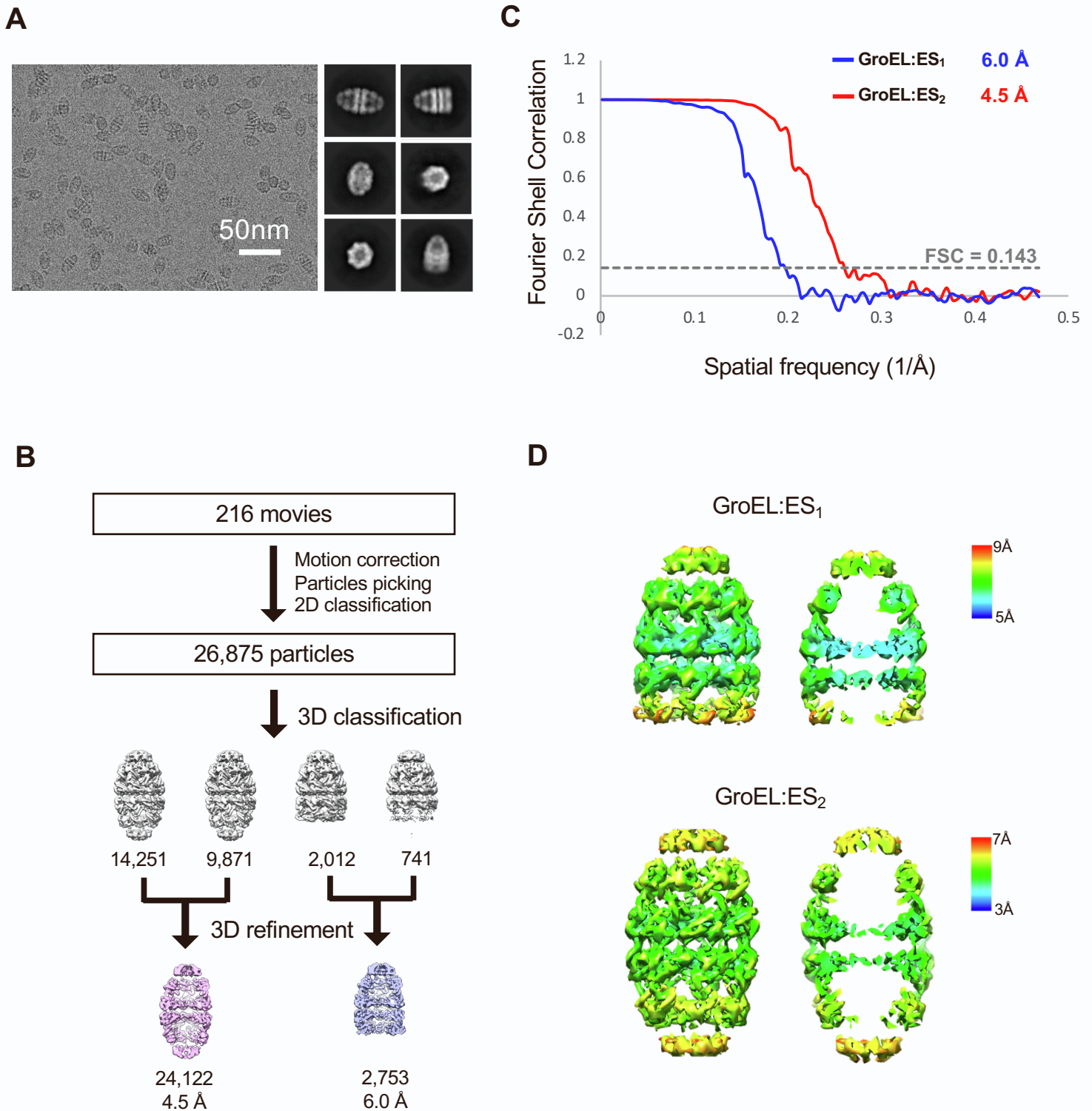
Supplemental information

Cryo-EM structures of GroEL:ES₂ with RuBisCO

visualize molecular contacts of encapsulated

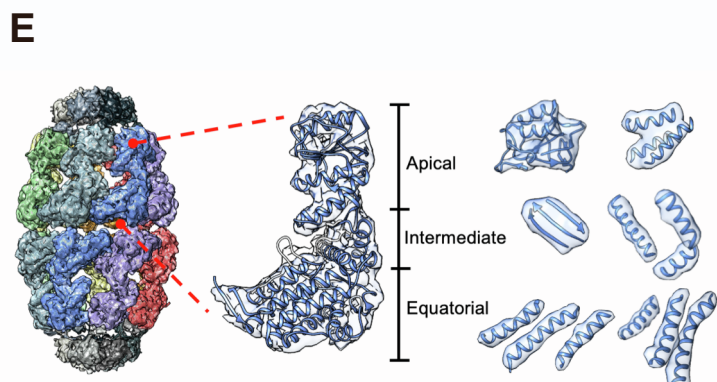
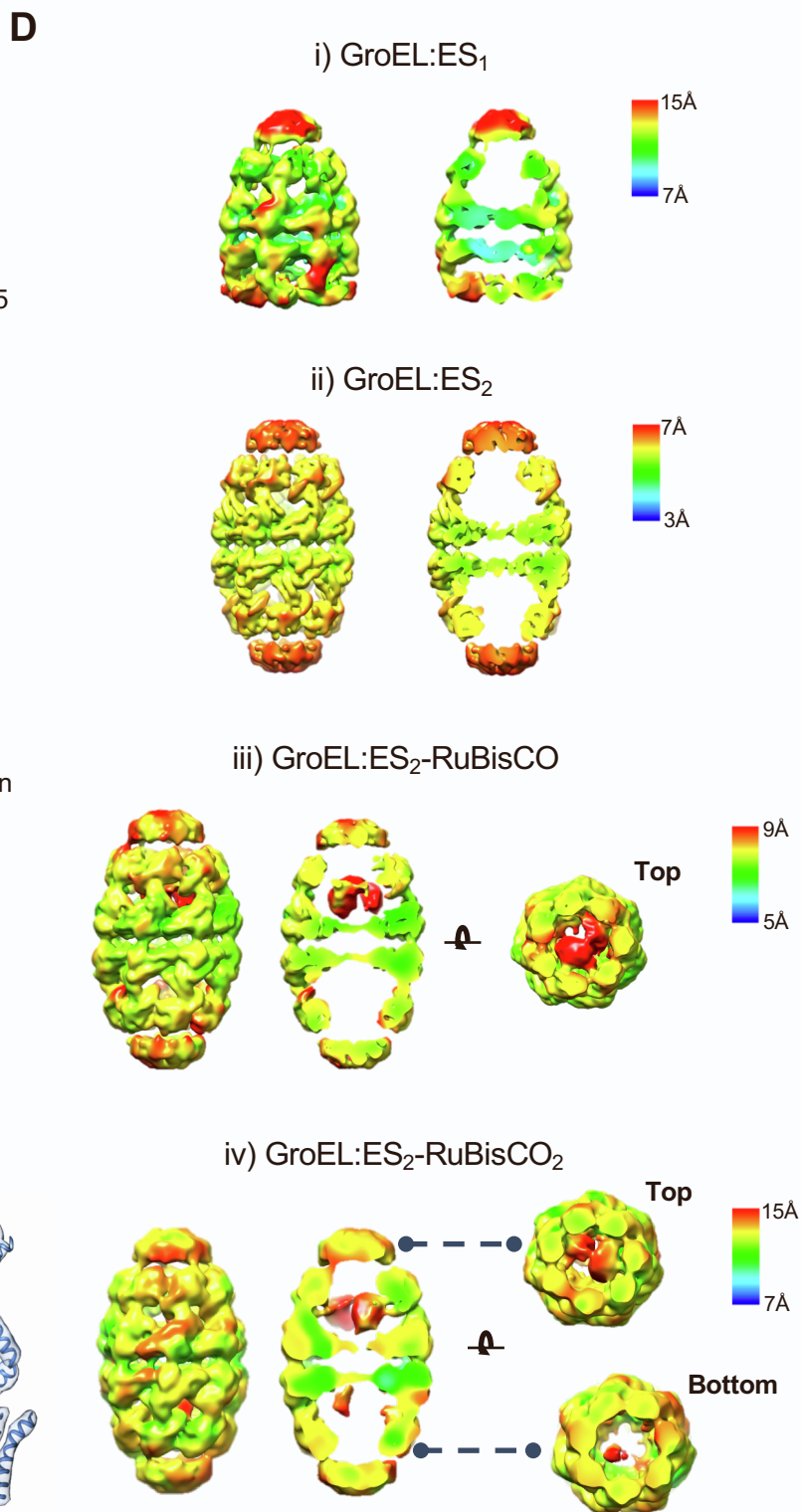
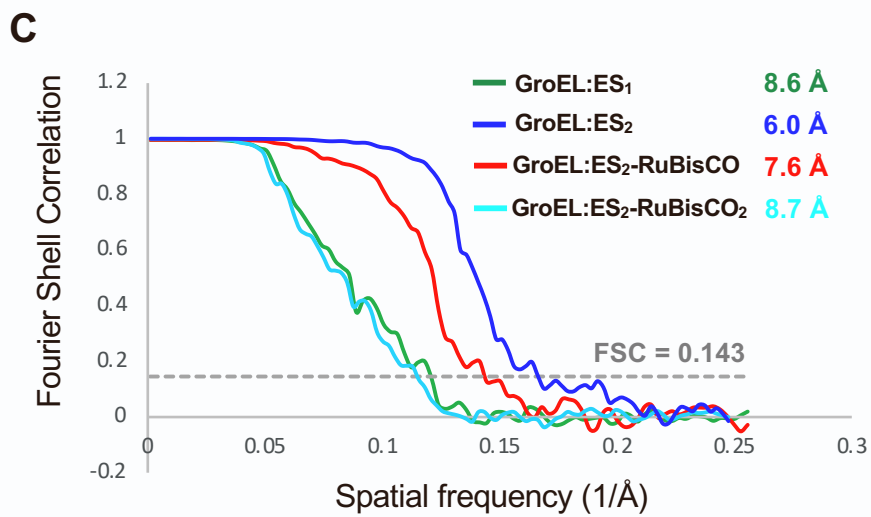
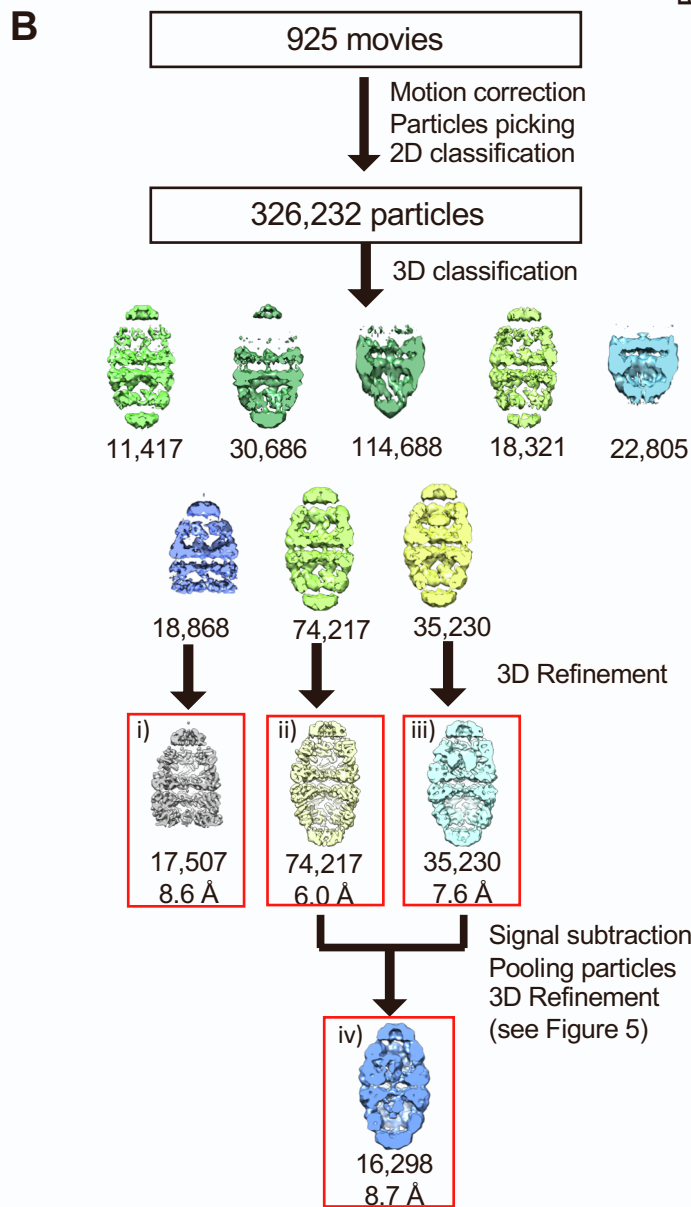
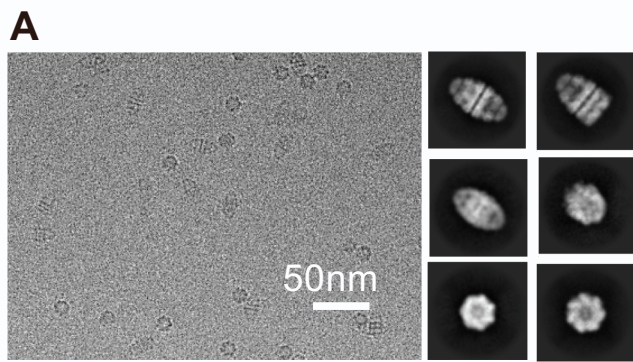
substrates in a double-cage chaperonin

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Supplementary Figure 1. cryoEM data processing workflow for GroEL+GroES, related to Figure 1

A Electron micrograph of ice-embedded GroEL+GroES. Scale bar, 50nm. Averaged 2D projections showing top, tilted and side views of bullet- and football-shaped complexes. **B** The data-processing workflow. **C** Gold-standard Fourier shell correlation (GS-FSC) curves depicting the resolution. **D** Local resolution estimation of bullet and football forms.



Supplementary Figure 2. cryoEM data processing workflow for GroEL+GroES with RuBisCO, related to Figure 1

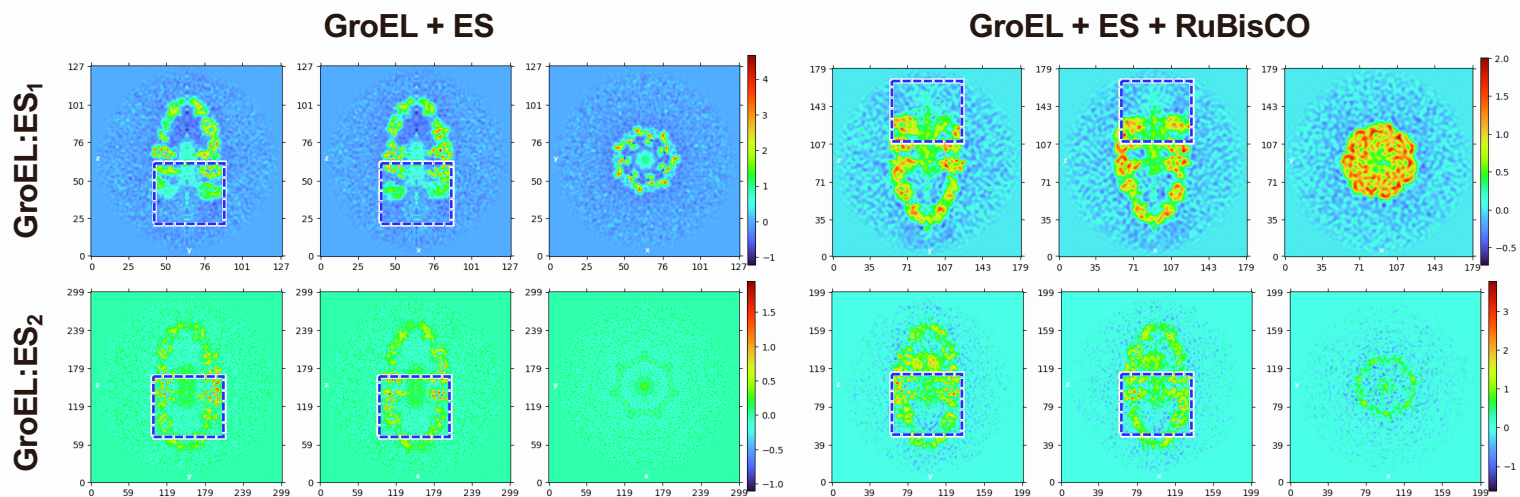
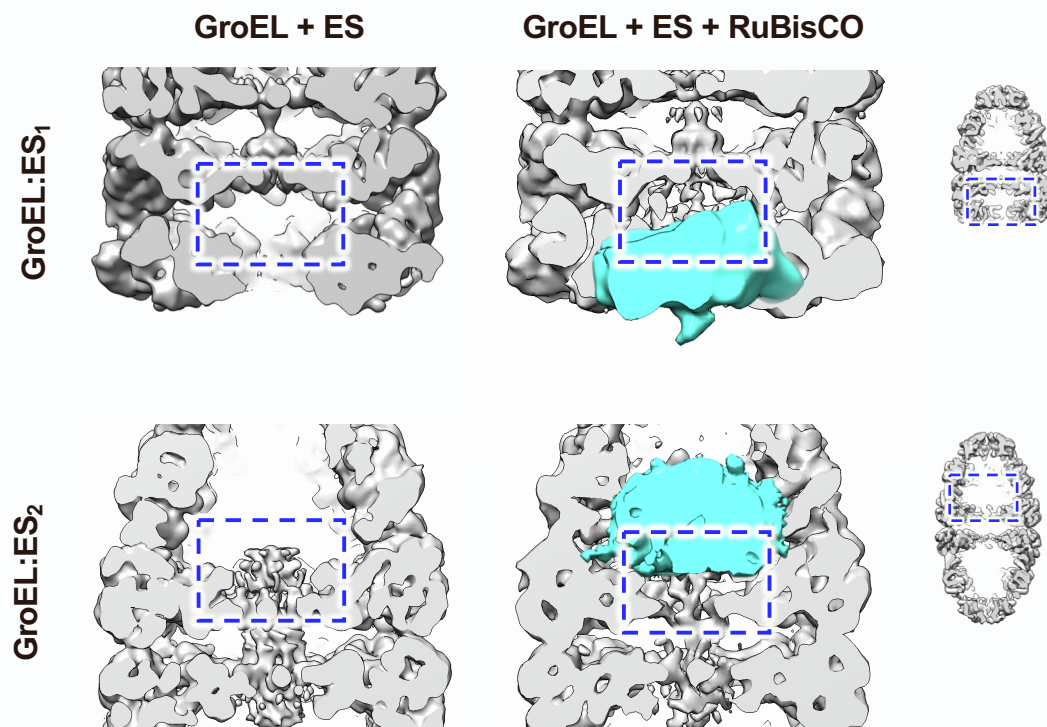
A Electron micrograph of ice-embedded GroEL+GroES with RuBisCO. Scale bar, 50nm. Averaged 2D projections showing side, tilted and top views of football and bullet forms.

B The data-processing workflow. 326,232 particles were subjected to 3D classification after motion correction and 2D classification. 3 out of 8 classes, i) GroEL:ES1, ii) GroEL:ES2, and iii) GroEL:ES2-RuBisCO were determined from the particles. iv) GroEL:ES2-RuBisCO2 was further refined from football-shaped complex after signal subtraction, pooling particles, and 3D refinement.

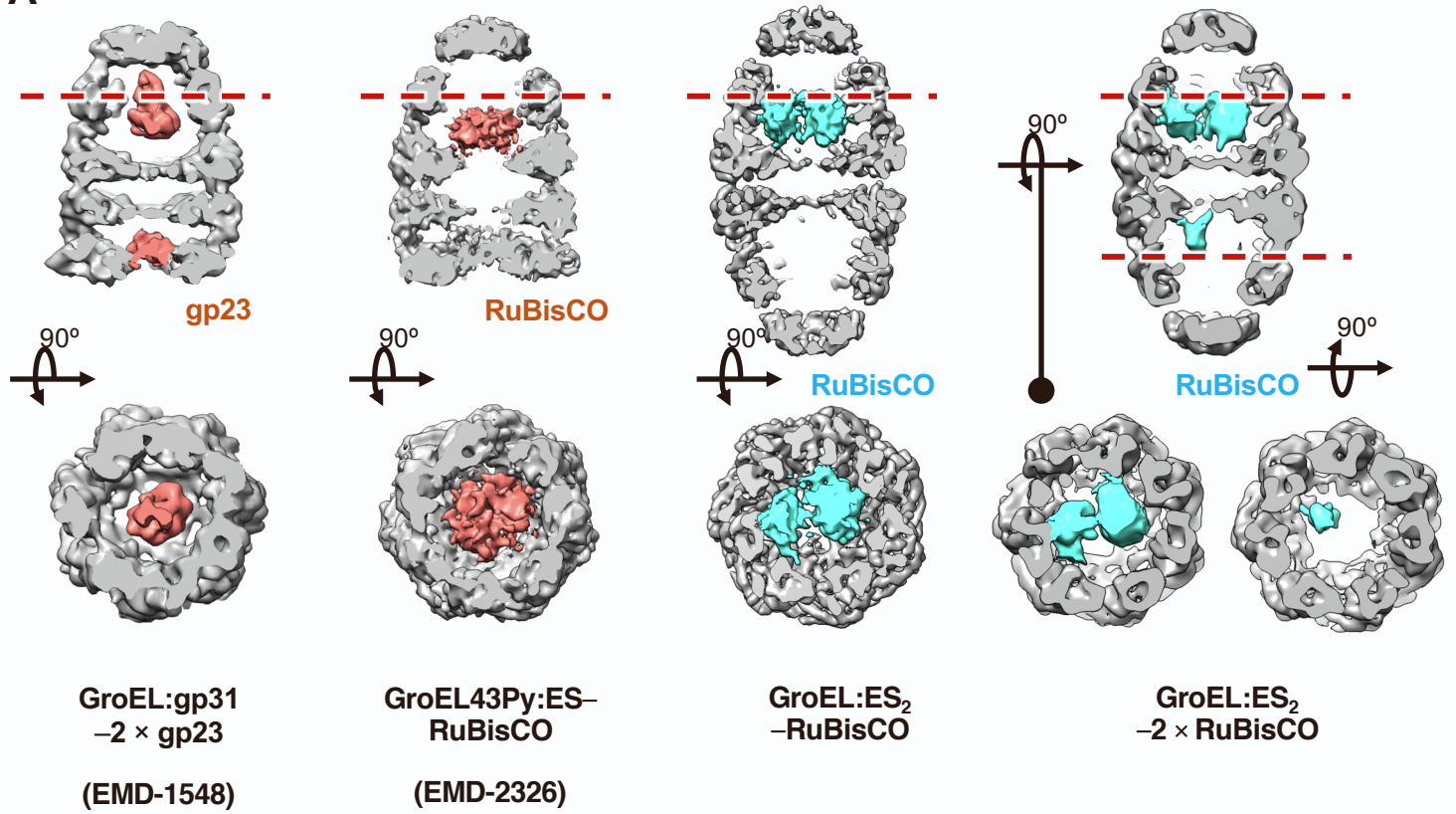
C Gold-standard Fourier shell correlation (GS-FSC) curves depicting the resolution.

D Local resolution estimation of each maps.

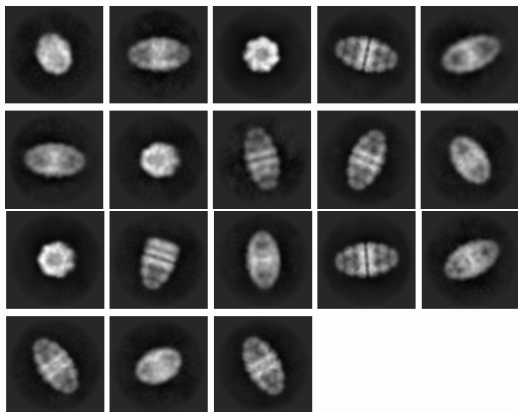
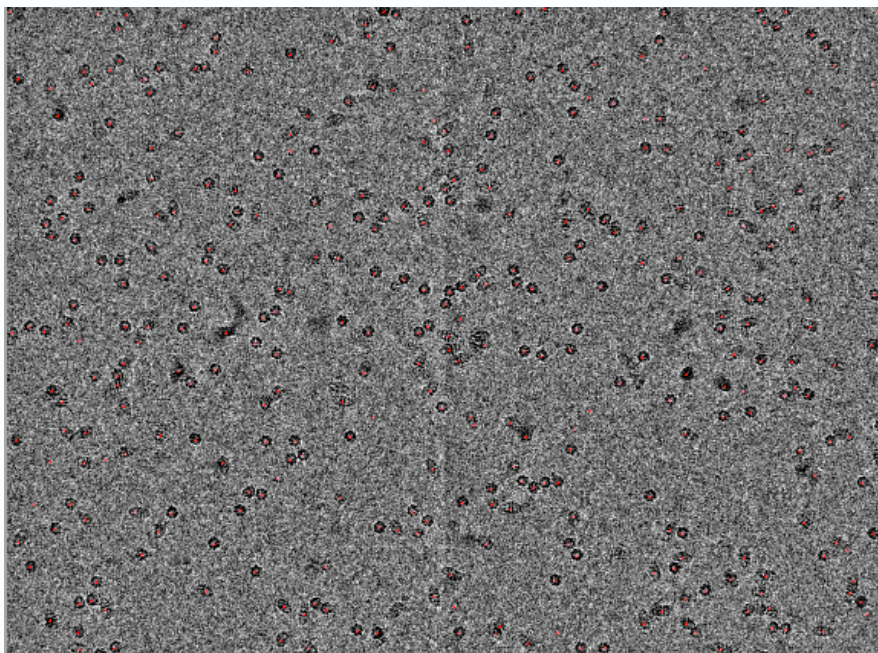
E Segmented GroEL subunit is shown as a representative for the quality of the map and model.

A**B**

Supplementary Figure 3. C-terminal tail inside the GroEL chamber, related to Figure 2. A Real space slices view of various GroEL:ES complexes. **B** Focused view on C-terminal tail of each GroEL:ES complex. Extended C-terminal tails are visualized except for bullet-shaped GroEL:ES complex without RuBisCO.

A

Supplementary Figure 4. Comparison of structures of GroEL/ES chaperone encapsulating substrate inside its chamber, related to Figure 2. A Structures of GroEL, whose chamber is bound to substrates and is sealed with its lid-like domain GroES, are shown.

A**B**

Supplementary Figure 5. Automatic particle picking from templates generated, related to Figure 1. A Templates generated from picked particles. **B** particles automatically picked on electron micrograph using generated templates.

Table S1.

EMDB code (resolution)	EMD-1548	EMD-2326	GroEL:ES2- RuBisCO	GroEL:ES2- 2 * RuBisCO
Resolution	10.1 Å	9.2 Å	7.6 Å	8.7 Å
Substrate	gp23	RuBisCO		RuBisCO
GroEL:GroES (input)	1:2.5*	1:1		1:2
GroEL:GroES (in structure)	1:1*	1:1		1:2
GroEL:Substrate (input)	1:2	1:1		1:10
GroEL:Substrate (in structure)	1:2	1:1	1:1	1:2
GroEL Construct	Wild-type	EL43Py398A		Wild-type
Used ATP analog	ADP-AIFx	ATP		ADP-BeFx
Released year	2009	2013		2021

*This structure used viral protein gp31 instead of GroES

Supplementary Table 1, related to Figure 2. List of structures of GroEL/ES chaperone with substrates encapsulated.