Expanded View Figures

Figure EV1. Sample preparation and cryo-EM of the doublet microtubules.

A The schematic of our isolation strategy for the intact doublet microtubules.

B, C Micrographs of doublet microtubules from *Tetrahymena* WT (B) and K40R mutant (C). The red arrowheads indicate the ODA complex falling off from the doublet. The red rectangle indicates the row of intact ODA in the K40R mutant. Scale bars represent 50 nm.

D, E 24 nm structure of doublet from WT and K40R showing the DC is intact in both cases while ODA is clearly present only in K40R. Red arrows indicate the docking complex. Scale bars, 50 nm.

F Fitting of our high-resolution structure into the tomographic map of *Tetrahymena* showing it is physiological (ΔRib72B mutant rescued with Rib72B-GFP) (EMD-7807, Stoddard *et al*, 2018).

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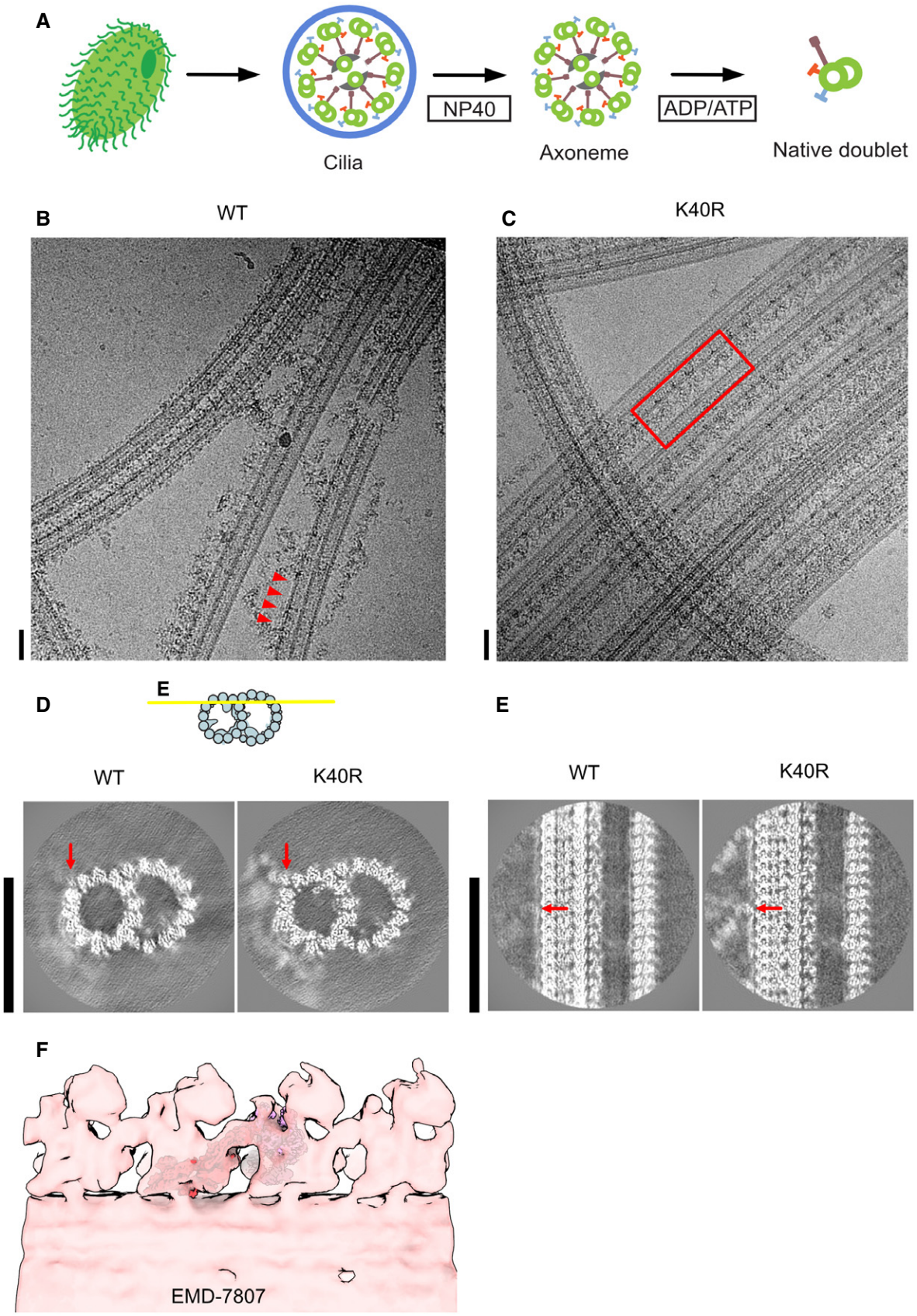

Figure EV1.

Figure EV2. Cryo-EM processing strategy.

A Alignment strategy for ODA particles. First, we obtained the 24-nm doublet structures. After that, we performed signal subtraction of the doublet microtubule. We centered and boxed out the ODA particles and performed refinement of the entire ODA particles.
B Focus refinement strategy for different regions of the ODA complex. The Dyh5 seems to be too flexible; therefore, we can only obtain Dyh4 head at 17 Å resolution.
C Fourier Shell Correlation of the doublet of WT and K40R & MEC17 combined data.
D Fourier Shell Correlation of the different regions of the ODA complex by focus refinement.

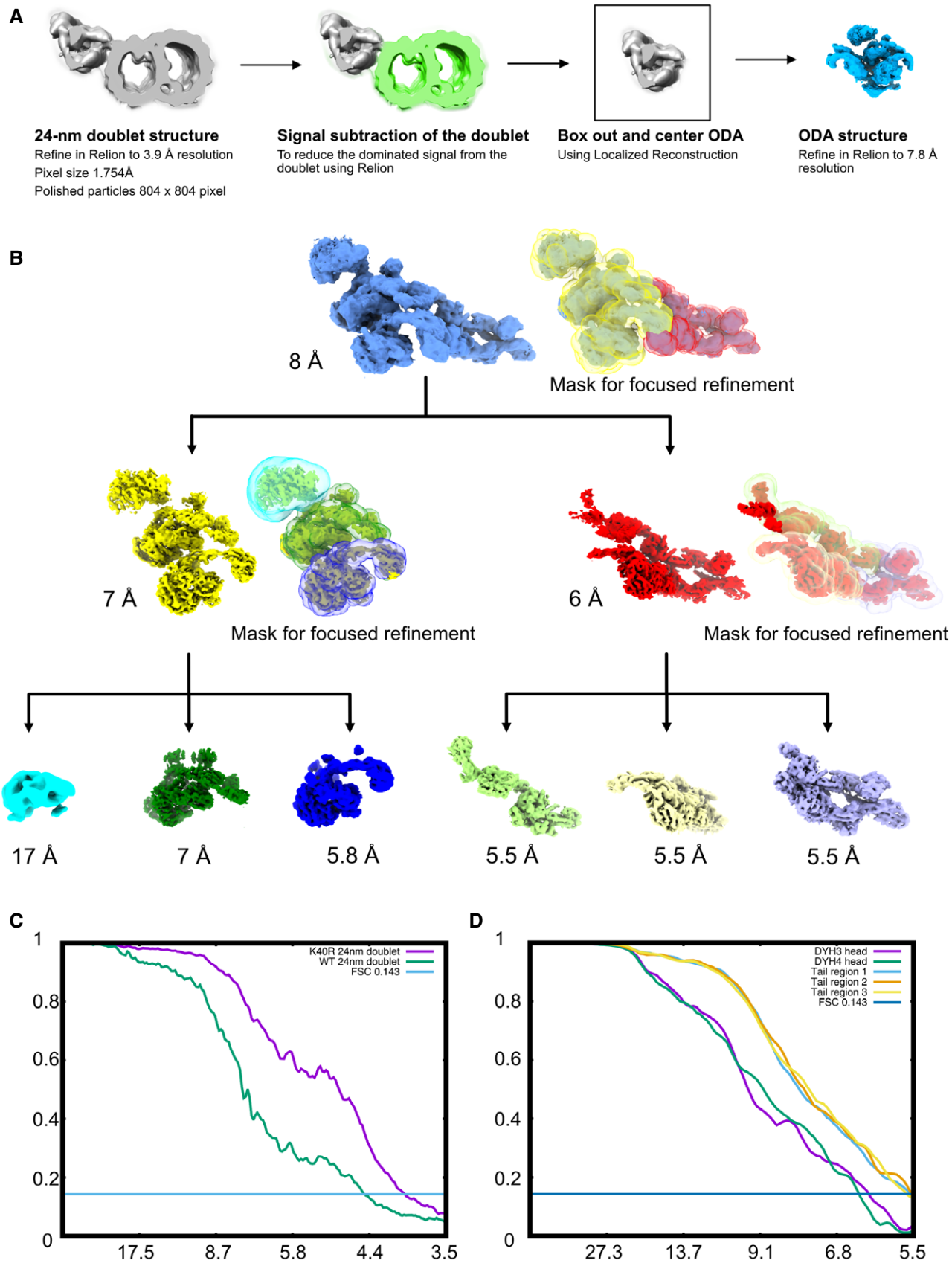

Figure EV2.

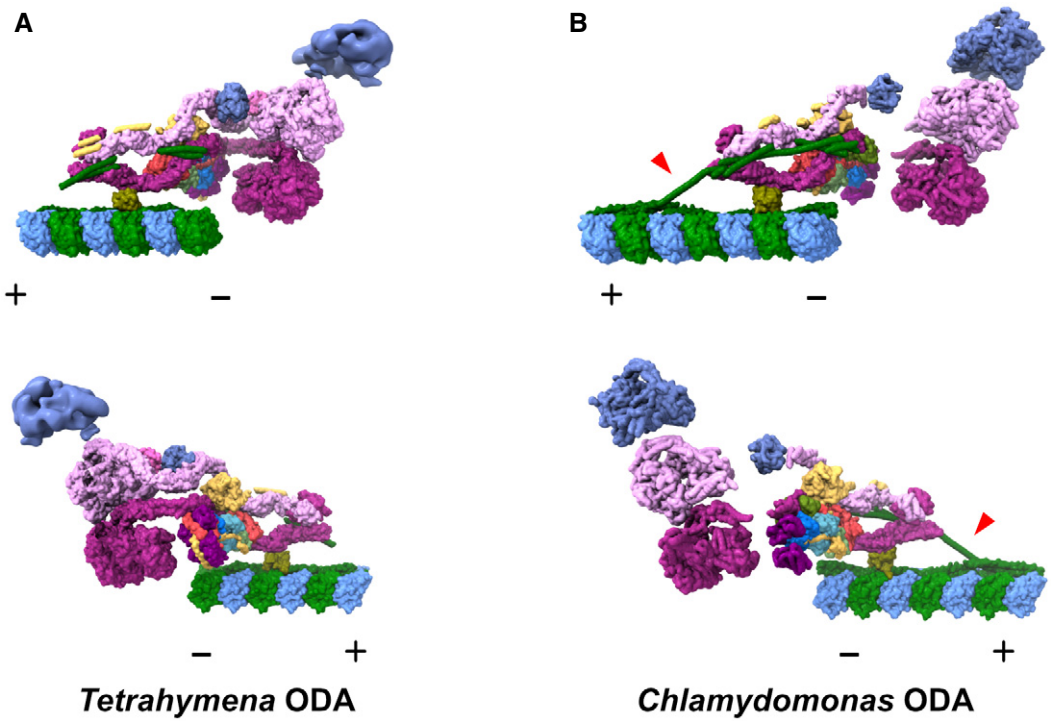

Figure EV3. Comparison of the ODA structures obtained by cryo-EM.

A, B The model of our *Tetrahymena* ODA complex (A) and Chlamydomonas ODA complex (PDB ID: 7KZM, Walton *et al*, 2021) (B) viewed from different sides. The Dyh5 head of *Tetrahymena* ODA is shown as 18-Å resolution surface rendering. α-Tubulin is shown in green and β-tubulin is shown in blue. The polarities of the doublet microtubules are indicated by + and −. In our *Tetrahymena* model, there is more modeled region around the linker region. In contrast, there is more modeled region in DC in the Chlamydomonas model from Walton *et al* (2021), especially the region connecting the DC1/2 on the doublet surface and the extended coiled-coil region associated with the ODA complex (red arrowheads). These differences could relate to different properties of the *Tetrahymena* and Chlamydomonas ODA or different ways of sample preparation.

Figure EV4. Modeling of the docking complex.

A The DC density on the doublet microtubule between PFs-A7 and A8.
B Prediction of coiled coil for DC1 and DC2 using COILS with a window size of 28 (Lupas *et al*, 1991).
C Sequence alignment of Chlamydomonas DC1 and *Tetrahymena* CCDC151 homolog (Q22T00).
D–G Structures of Chlamydomonas ODA reconstituted on microtubules with biotin carboxyl carrier protein (BCCP) tagged in different regions of DC2 (residue 76, 276, 412, and 507) from Oda *et al* (2016a) (EMD-6508, 6509, 6510, 6511). The enhanced signals of BCCP-tag are indicated in colors.
H Slice from a density map showing the docking complex (position indicated in the cartoon). The yellow line indicates one continuous DC1/2.
I The globular density at the end of the coiled coil of DC1/2 (black arrow and dotted line).
J The model of the DC based on our analysis.

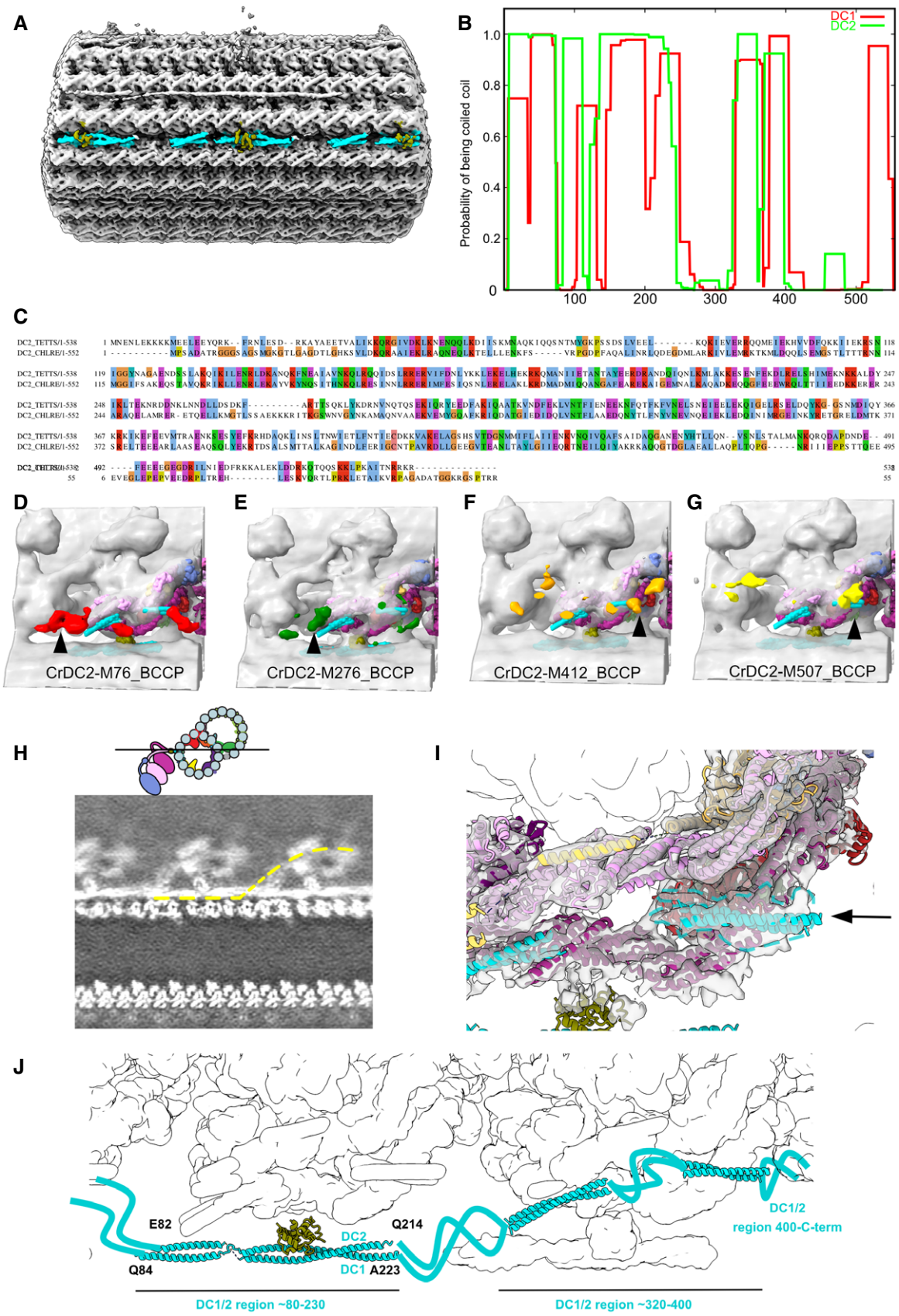

Figure EV4.

Figure EV5. Data related to the remodeling of the ODA complex.

A The labeling of helix bundles in the inactive and active Dyh3 and Dyh4. Inactive and active structures are aligned on helix bundle 4 of Dyh4 (residues 414–513).
B Alignment of the inactive and active Dyh3 at helix bundle 3 (residue 448–536) showing ~ 90-degree rotation of the head domain (top).
C Alignment of inactive and active Dyh4 at helix bundle 3 (residue 414–513) showing compressing conformational changes (bottom).
D Bending conformational change in the LC tower. LC tower from the active ODA complex is in colors, and the LC tower from the Shulin–ODA is shown in transparent. LC towers are aligned based on either Lc8B/10 (left) or Lc8e/f (right) as indicated by green dashed circles.
E Regions of the ODA that interact with Shulin in the inactive conformation (green regions with green arrowhead) are spread out in the active conformation. The dash arrow indicates the region of Dyh3 head interacting with C3 domain of Shulin, now at the back of the view.

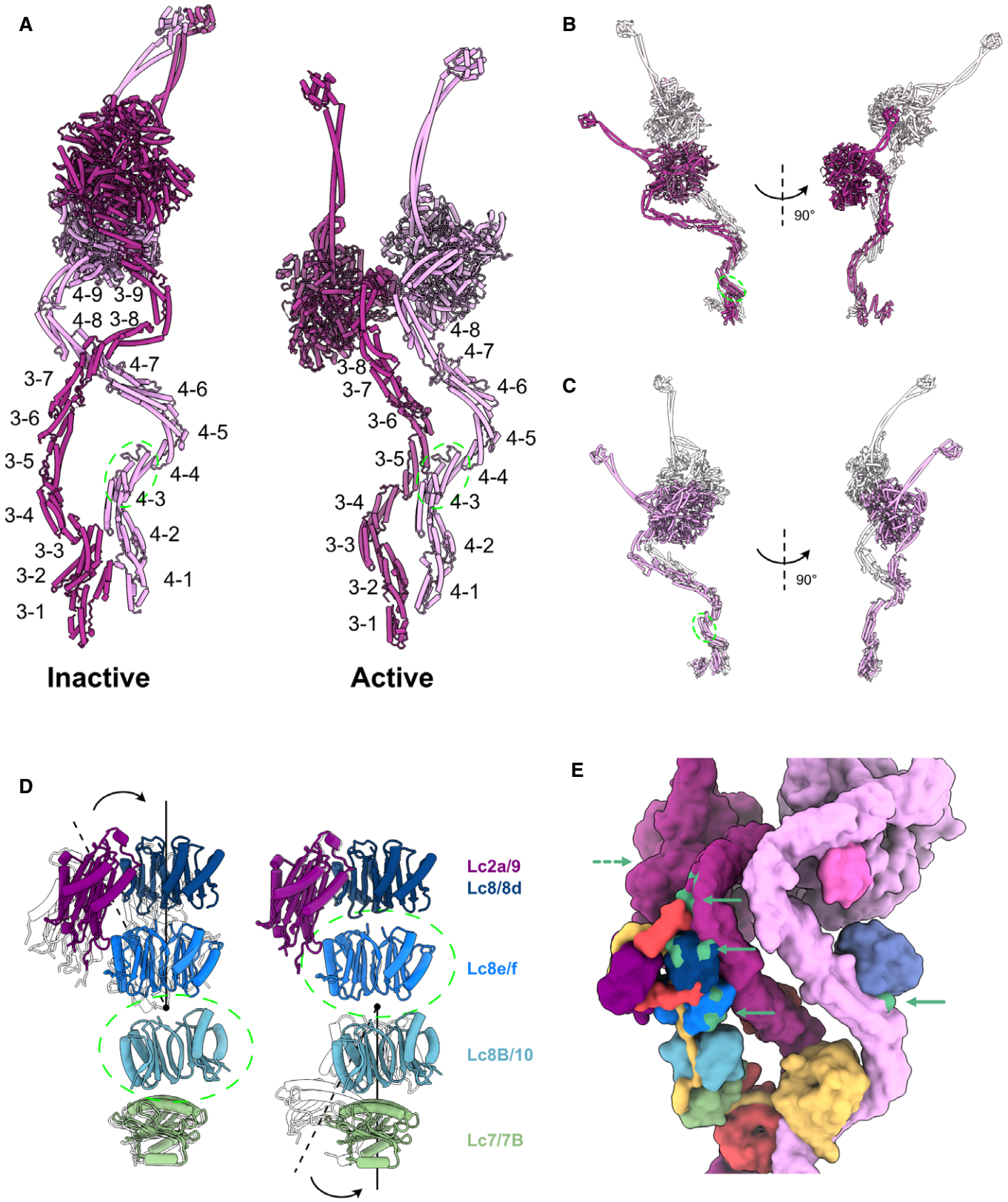

Figure EV5.