Expanded View Figures

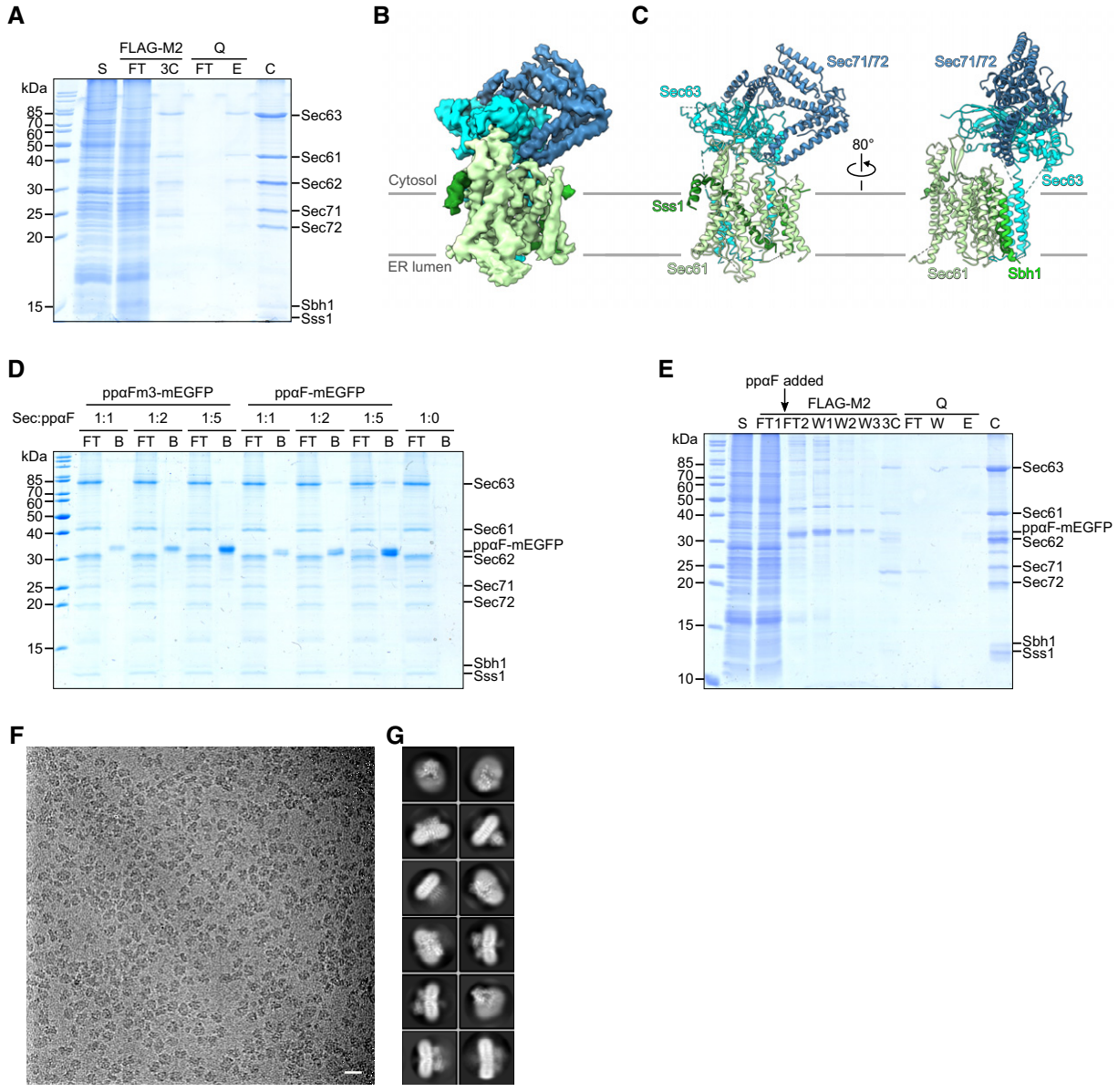

Figure EV1.

Figure EV1. Purification and cryo-EM of the Sec complex.

A SDS–PAGE analysis of the purification of the heptameric Sec complex containing FLAG-tagged Sec62. After solubilization, the tagged complex was applied to anti-FLAG-M2 resin and the eluate was subjected to ion-exchange chromatography using Q sepharose (S: solubilized microsomes; FT: flow-through from the anti-FLAG resin; 3C: eluate after 3C cleavage; E: eluate from Q sepharose; C: concentrated heptameric Sec complex sample).

B Cryo-EM map of the (heptameric) Sec complex (apo state) shown after focused refinement and post-processing. The map is shown at contour level 0.02 except for the Sec71/72 part, which is at 0.018. Individual subunits of the Sec complex are color-coded as in Fig 1C.

C Molecular model of the Sec complex in apo state.

D SDS–PAGE analysis of pull-down assays of the purified Sec complex with the signal sequence-containing mEGFP-tagged ppαF or its translocation defect mutant ppαFm3. Pull downs were performed at varying molar ratios (B: bead-bound fraction; FT, unbound flow-through). Semi-quantitative assessment of the Sec63 bands on the gel using ImageJ shows that the mutant signal sequence binding to the Sec complex appears weaker than the wildtype, as expected by weaker activity of the mutant in translocation (Allison & Young, 1989).

E SDS–PAGE analysis of the purification of the signal sequence-bound Sec complex. Lanes are labeled as in (A) except: FT1: flow-through after binding of the Sec complex on beads; FT2: flow-through after binding of ppαF-mEGFP to the Sec complex immobilized on beads; W1-3: washes after ppαF-mEGFP binding.

F A representative cryo-EM micrograph as obtained from TITAN KRIOS equipped with a K2 direct electron detector. The scale bar represents 20 nm.

G Selected 2D class averages. The box size was chosen to be 200 pixels resulting in a box width of 212 Å.

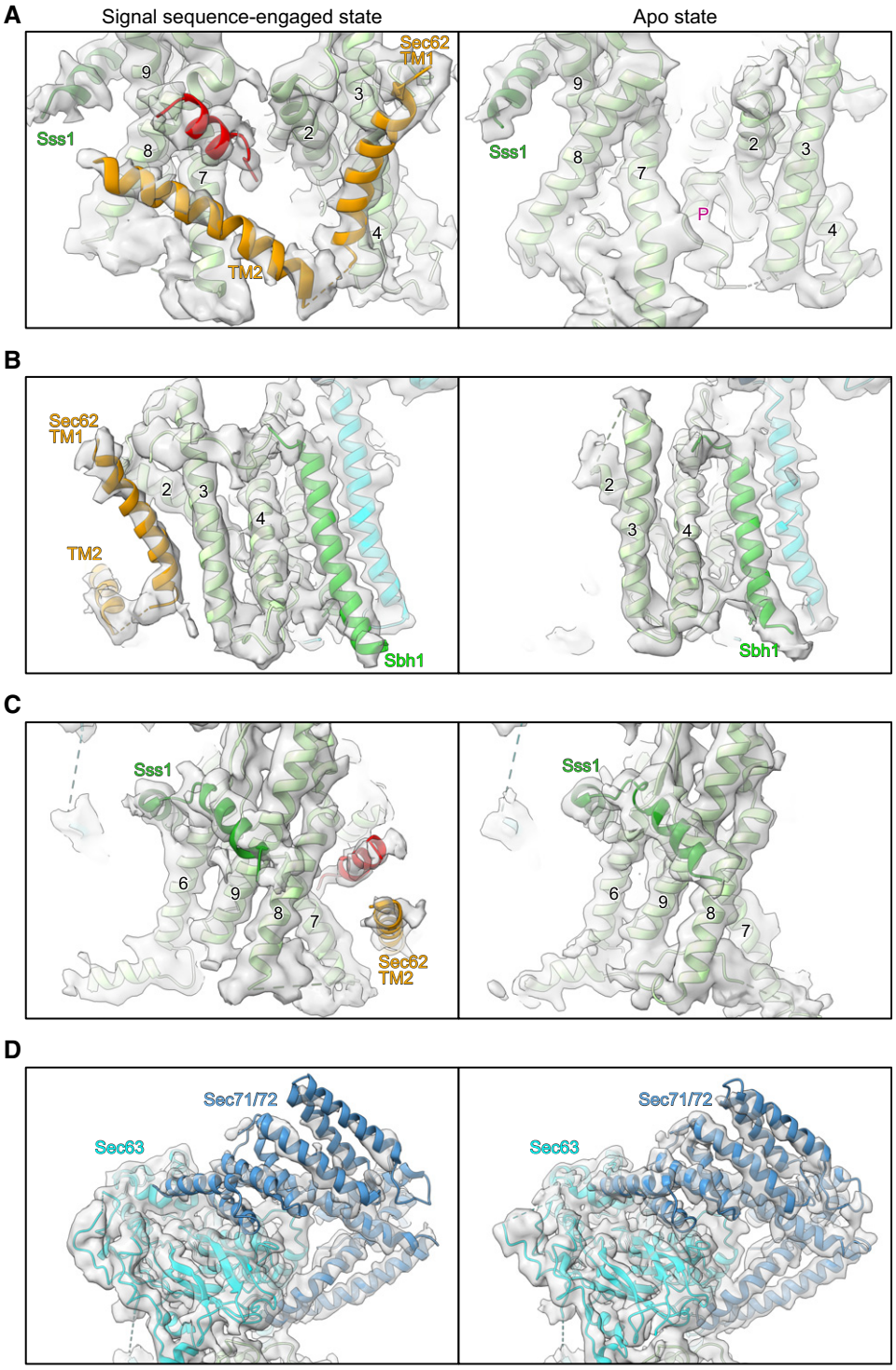

Figure EV2. Fitting of molecular models into the cryo-EM maps of ss-bound and apo Sec complex.

A View focusing on the lateral gate of Sec61α. In the ss-bound state (left), the signal sequence of ppαF (red) is inserted into the lateral gate (TM2 and TM7) and the two Sec62 TM helices are stabilized. In the apo state (right), density for the plug (P) is present below the central pore.

B View focusing on the N-terminal half of Sec61α (TMs 2, 3 and 4). In the ss-bound state (left), Sec62 TM1 is bound near Sec61α TM2.

C View focusing on the C-terminal half of Sec61α (TMs 6-9).

D View focusing on the cytosolic domains of the Sec complex.

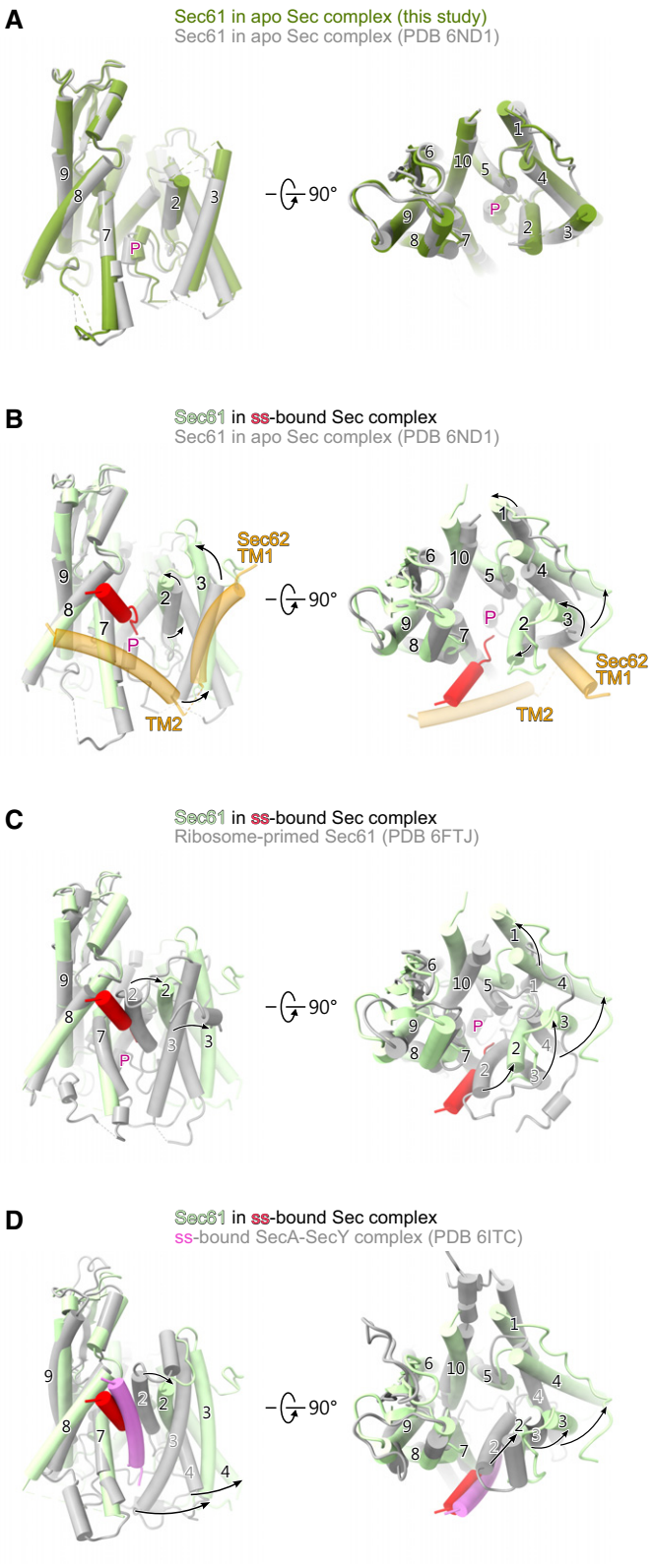

Figure EV3. Comparison of lateral gate conformation.

A Comparison of Sec61α in the apo state (this study) with Sec61α in the apo Sec complex as shown in the Wu study (PDB 6ND1).

B Comparison of Sec61α in the ss-bound Sec complex with Sec61α in the apo Sec complex as shown in the Wu study (PDB 6ND1). The ppαF-ss and Sec62 TM helices are shown in addition.

C Comparison of Sec61α in the ss-bound Sec complex with ribosome-primed apo Sec61 complex as shown in the Braunger study (PDB 6FTJ).

D Comparison of Sec61α in the ss-bound heptameric Sec complex with SecY as found in the study of Ma *et al* (2019) showing the ss-bound SecA-SecY complex (PDB 6ITC).

Data information: All structures are shown from a view focusing on the lateral gate (left) and from the top (cytosolic side; right). Structure alignments are based on TMs 7–9 of Sec61α. Black arrows indicate the movement of the helices of the Sec61 complex. The plug is indicated by "P".

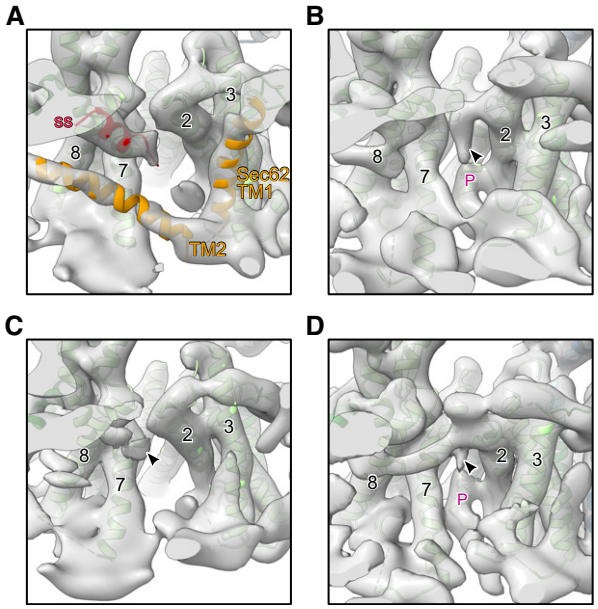

Figure EV4. Detergents or lipids bound to the lateral gate of Sec complex.

A Cryo-EM map and fitted model of the ss-bound Sec complex from this study.

B Cryo-EM map and fitted model of the apo Sec complex from this study.

C, D Cryo-EM maps and fitted models of the apo Sec complexes from the Itskanov study ((C); PDB 6N3Q and EMD-0336) and Wu study (D)); PDB 6ND1 and EMD-0440).

Data information: All maps are low-pass filtered to 8 Å. The black triangle marks extra density likely attributing for a bound detergent or lipid molecule and "P" marks the plug.

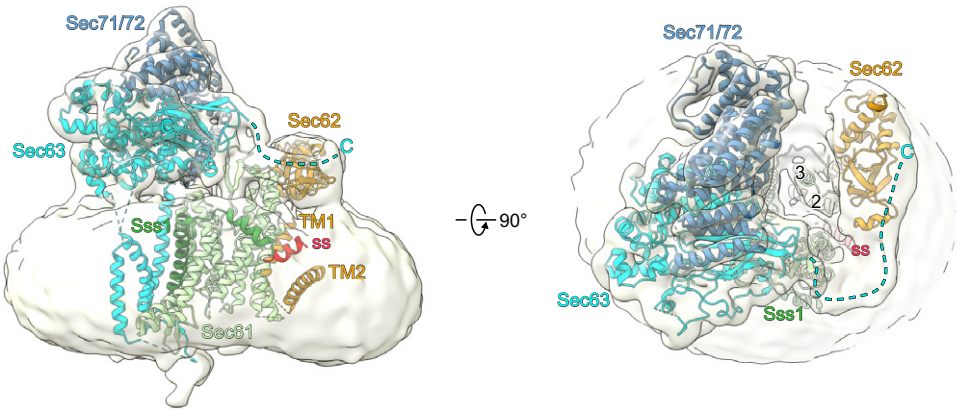

Figure EV5. Localization of Sec62 in the Sec complex.

Cryo-EM reconstruction of the ss-bound Sec complex low-pass filtered to 8 Å to visualize the rather flexible C-terminus of Sec63 (highlighted by a cyan dashed line).