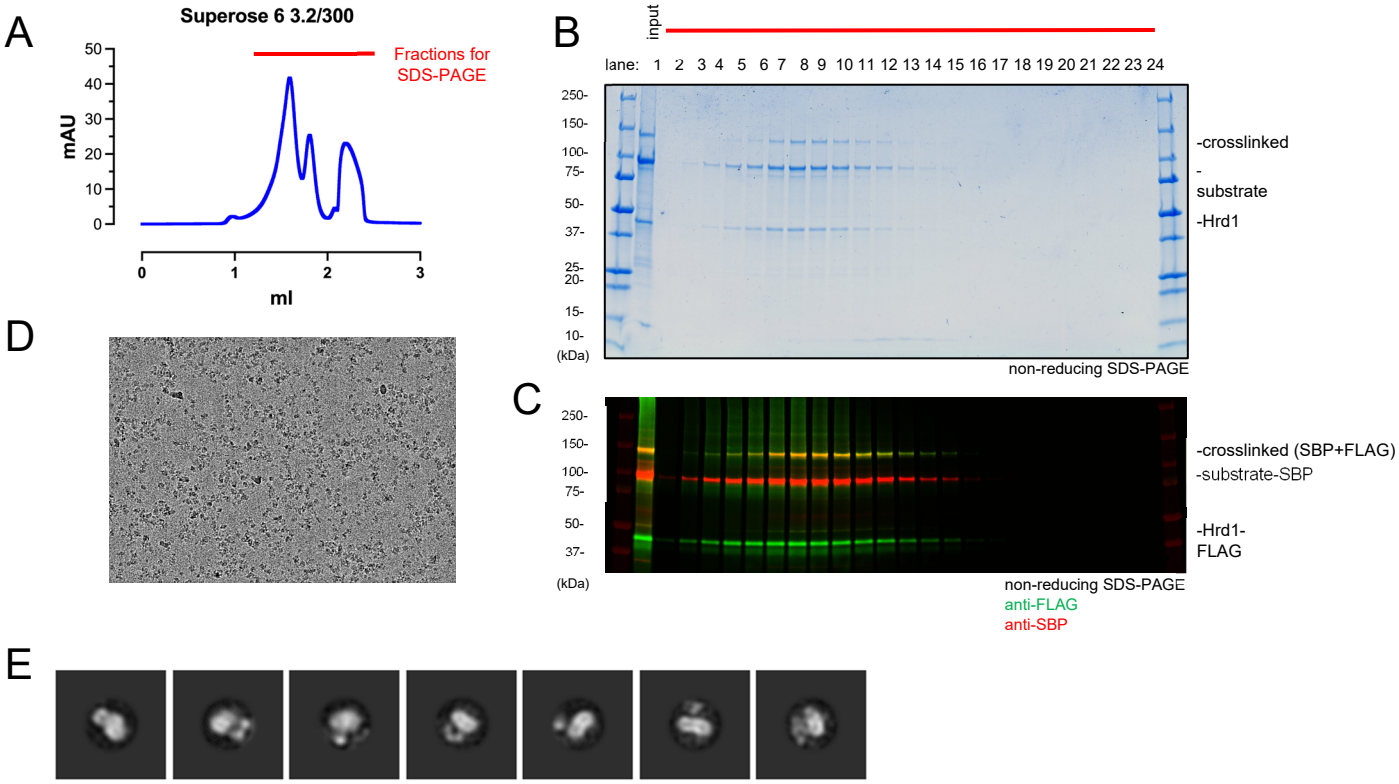

Figure 1. A) Size exclusion chromatography profile of the Hrd1-substrate complex. Cells over-expressing Hrd1-FLAG and substrate-SBP were lysed, and the Hrd1-substrate complex was isolated by binding to anti-SBP beads. Biotin-eluted fractions containing the Hrd1-substrate complex were pooled, concentrated, and further purified by size exclusion chromatography using a Superose 6 column. B) Non-reducing SDS-PAGE of fractions from size exclusion chromatography in A) stained with Coomassie blue. C) Immunoblotting of fractions from A) with anti-FLAG and anti-SBP antibodies. D) Representative cryo-EM image of the Hrd1-substrate sample acquired on Talos 200kV microscope. E) Representative 2D class averages of particles picked from D).

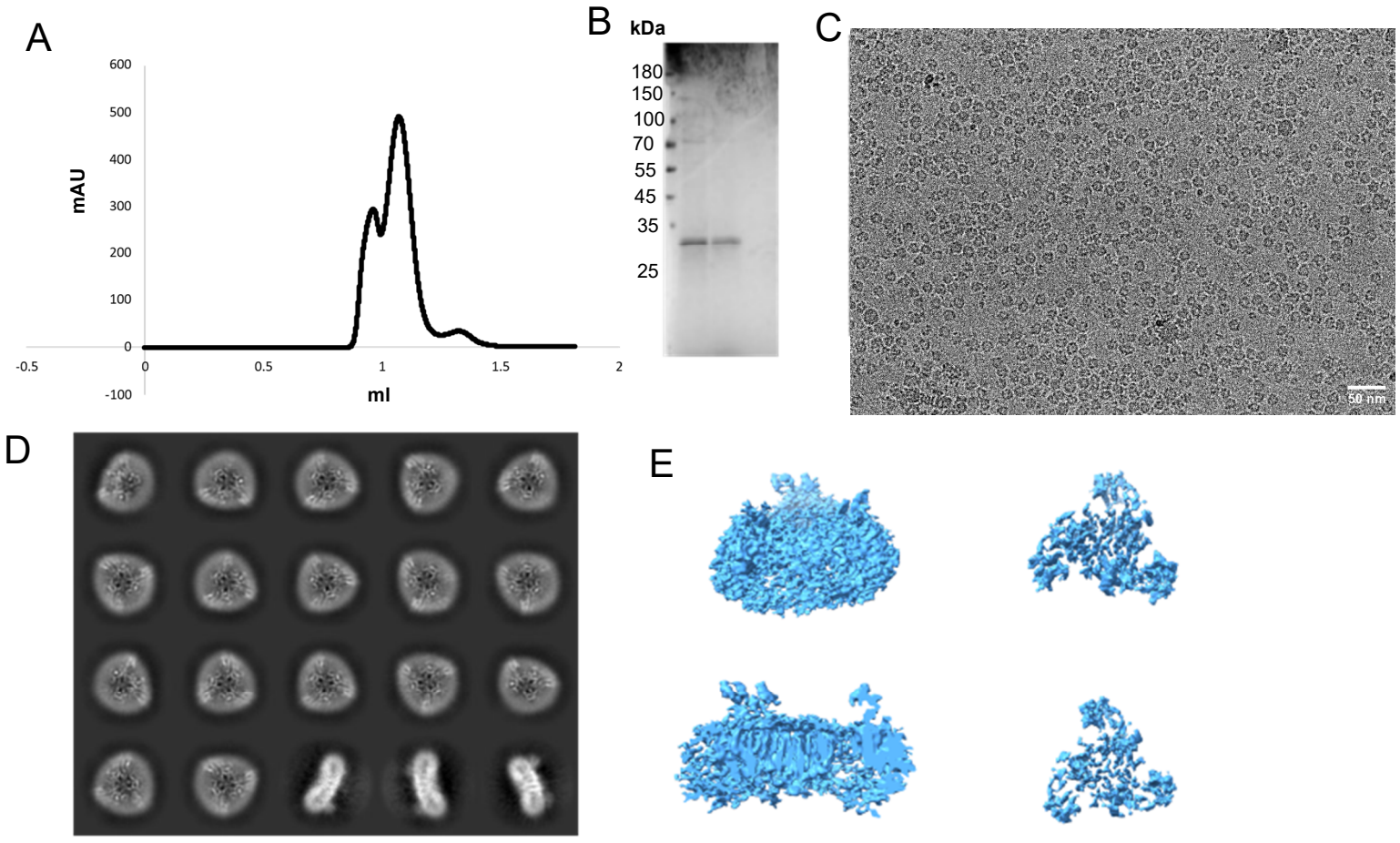

Figure 2. A) SEC profile of TMEM129-Derlin1 complex Superdex200 3.2 B) SDS-PAGE of peak fractions from SEC in A stained with Coomassie blue. C) Cryo-EM image of peak fraction acquired on Talos 200kV microscope. D) Representative 2D-class averages of particles picked from C. E) Different views (full and cut micelle right panel, top and bottom view left panel, and on a preliminary density map of 2D-class averages from D. homologous refinement of ab-initio model.

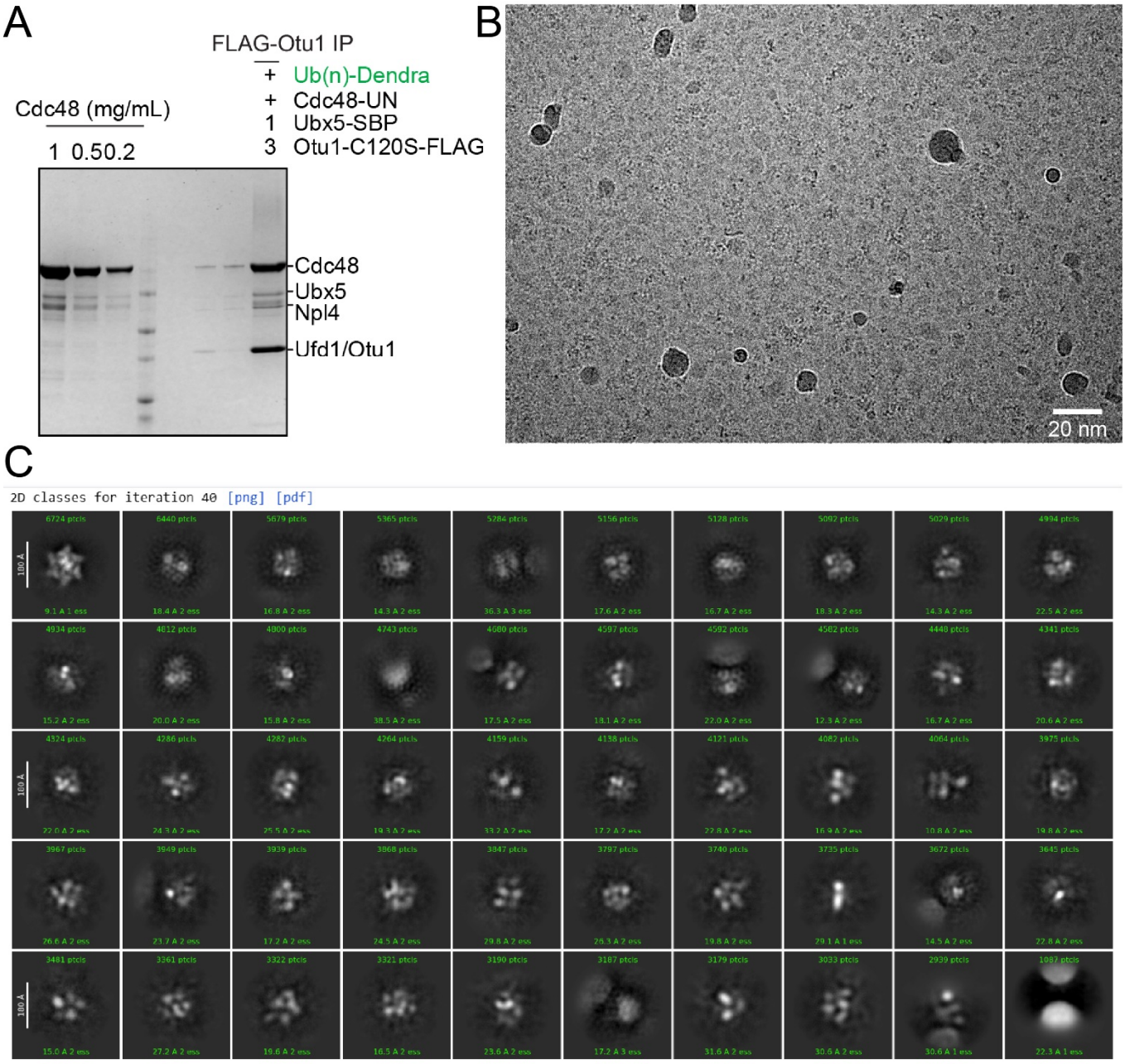

Figure 3. A) FLAG-tagged catalytic inactive mutant of Otu1 (Otu1-C120S-FLAG) was incubated with Cdc48, Ufd1-Npl4 (UN), SBP-tagged Ubx5, and polyubiquitinated Dendra protein (Ub(n)-Dendra) in ATP. After immunoprecipitation with FLAG antibodies, the samples were concentrated and analyzed by SDS-PAGE. The concentrated sample was used to prepare Cryo-EM grids. B) A representative cryo-EM image collected using a Talos electron microscope. C) A gallery of 50 representative 2D-class averages, which are ordered according to the decreasing number of particles assigned to each class.