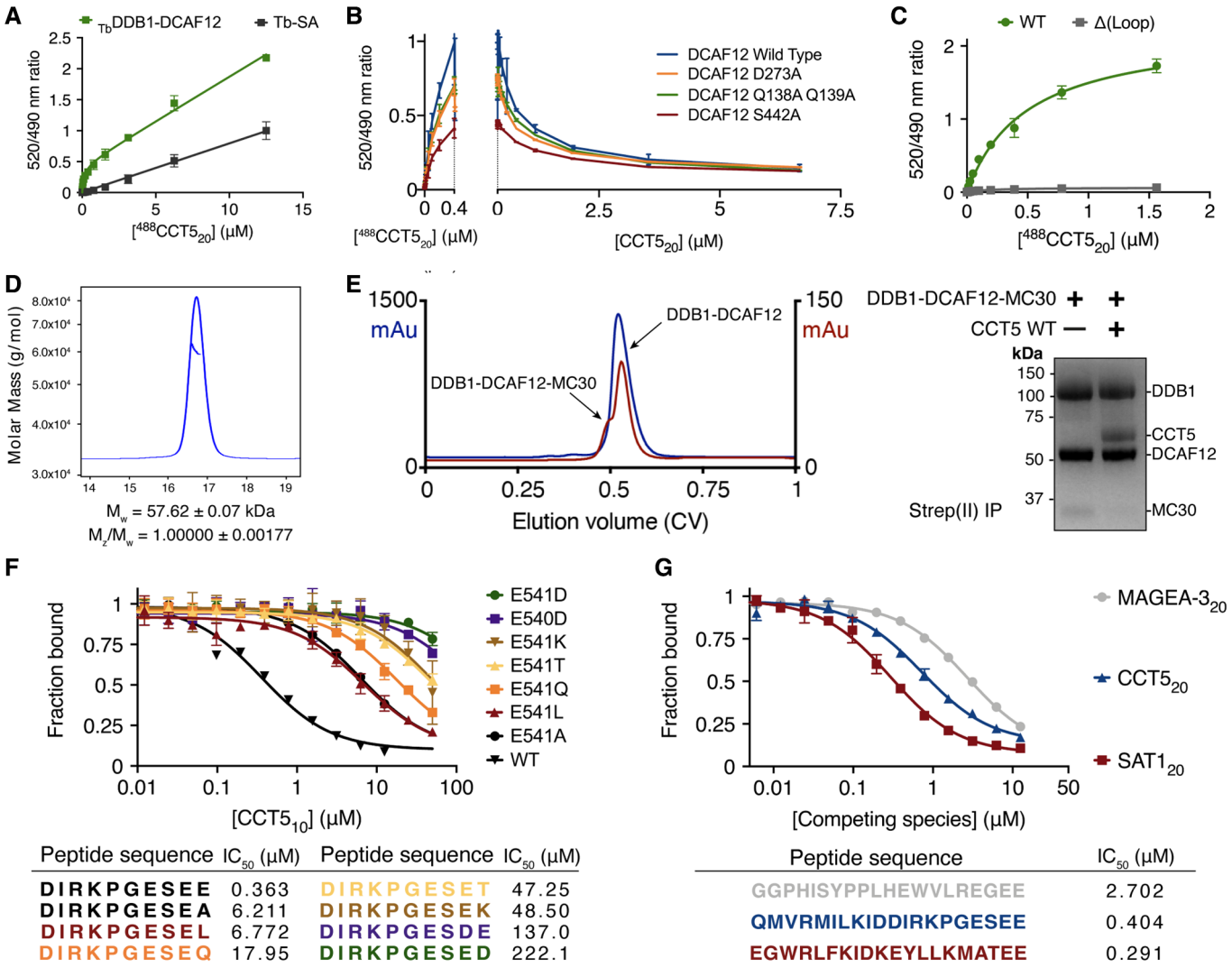
Expanded View Figures



Peptide sequence	IC_{50} (μM)	Peptide sequence	IC_{50} (μM)
DIRKPGESEE	0.363	DIRKPGESET	47.25
DIRKPGESEA	6.211	DIRKPGESEK	48.50
DIRKPGESEL	6.772	DIRKPGESDE	137.0
DIRKPGESEQ	17.95	DIRKPGESED	222.1

Peptide sequence	IC_{50} (μM)
GGPHISYPPLHEWVLREGEE	2.702
QMVRMILKIDDIRKPGESEE	0.404
EGWRLFKIDKEYLLKMATEE	0.291

Figure EV1. DCAF12 binds monomeric substrates.

A Titration curves between a fluorescent $^{488}CCT5_{20}$ degron peptide and 50 nM biotinylated DDB1-DCAF12 pre-mixed with 2 nM terbium-coupled streptavidin ($_{Tb}$DDB1-DCAF12) or 2 nM terbium-coupled streptavidin (Tb-SA) ($n = 3$). Signal originating in the absence of $_{Tb}$DDB1-DCAF12 is unspecific and becomes dominant at high $^{488}CCT5_{20}$ concentrations.

B Left: titrations between 0–0.4 μM $^{488}CCT5_{20}$ and mutant $_{Tb}$DDB1-DCAF12 complexes ($n = 3$). The maximum fluorescent signal originating from the titrations was out-competed with a label-free $CCT5_{20}$ peptide (right).

C Titration curves between $^{488}CCT5_{20}$ and wild-type (WT) $_{Tb}$DDB1-DCAF12 or a mutant with DCAF12 amino acids 370–416 replaced by a flexible glycine-serine linker (Δ(Loop)) ($n = 3$).

D SEC-MALS analysis of wild-type CCT5. The chromatogram displays Rayleigh ratio curves for CCT5 together with the molar mass in Da of the main peaks. The calculated molecular weight (M_w) corresponds to a CCT5 monomer. The polydispersity of the sample (M_z/M_w) indicates a uniform species in the peak.

E Left: a representative size exclusion chromatograph (blue) for DDB1-DCAF12. A fraction of DDB1-DCAF12 is bound to a contaminant of ~ 30 kDa in size, MC30, that ends in a Glu-Thr motif. The contribution of the DDB1-DCAF12-MC30 species to the chromatograph absorbance (measured in arbitrary absorbance units, mAu) increases in low-yield purifications (red). Right: untagged CCT5 displaces MC30 from his_6-DDB1-strep(II)-DCAF12 *in vitro*.

F TR-FRET counter-titrations of label-free $CCT5_{10}$ degron peptides with mutant C-terminal amino acids ($n = 3$).

G TR-FRET counter-titrations of label-free degron peptides of different DCAF12 substrates (Koren *et al*, 2018) into $_{Tb}$DDB1-DCAF12^{488} ($n = 3$).

Data information: In (A, B, C, F, G), data are presented as mean ± 95% CI. Where indicated, "*n*" represents biological replicates.

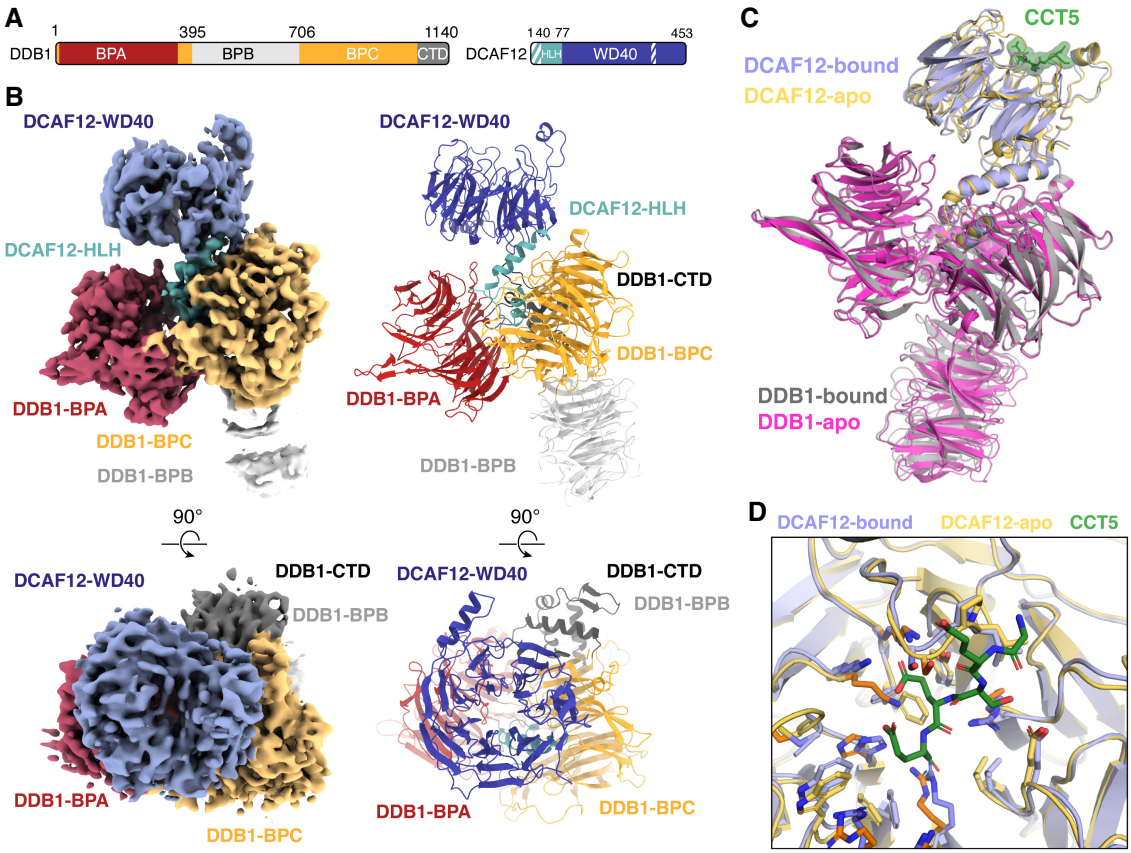


Figure EV2. Cryo-EM structure of DDB1-DCAF12 in the absence of CCT5.

A Domain organization of the proteins present in the sample. Unmodeled regions are shown as stripes.

B Different views of the DDB1-DCAF12 cryo-EM map (left) with fit structures (right). The map and models are colored as in (A).

C Superposition of the DDB1-DCAF12 (apo) and DDB1-DCAF12-CCT5 (bound) structures. Density corresponding to the CCT5 peptide (shown as green sticks with surface representation) was only observed in the DDB1-DCAF12-CCT5 structure (Fig 2). The root-mean-square deviation (RMSD) between the two structures is 1.2 Å between all atoms and 1.0 Å when excluding the flexible BPB domain of DDB1.

D Superposition of the residues forming the DCAF12 pocket between the DDB1-DCAF12 (apo) and DDB1-DCAF12-CCT5 (bound) cryo-EM structures.

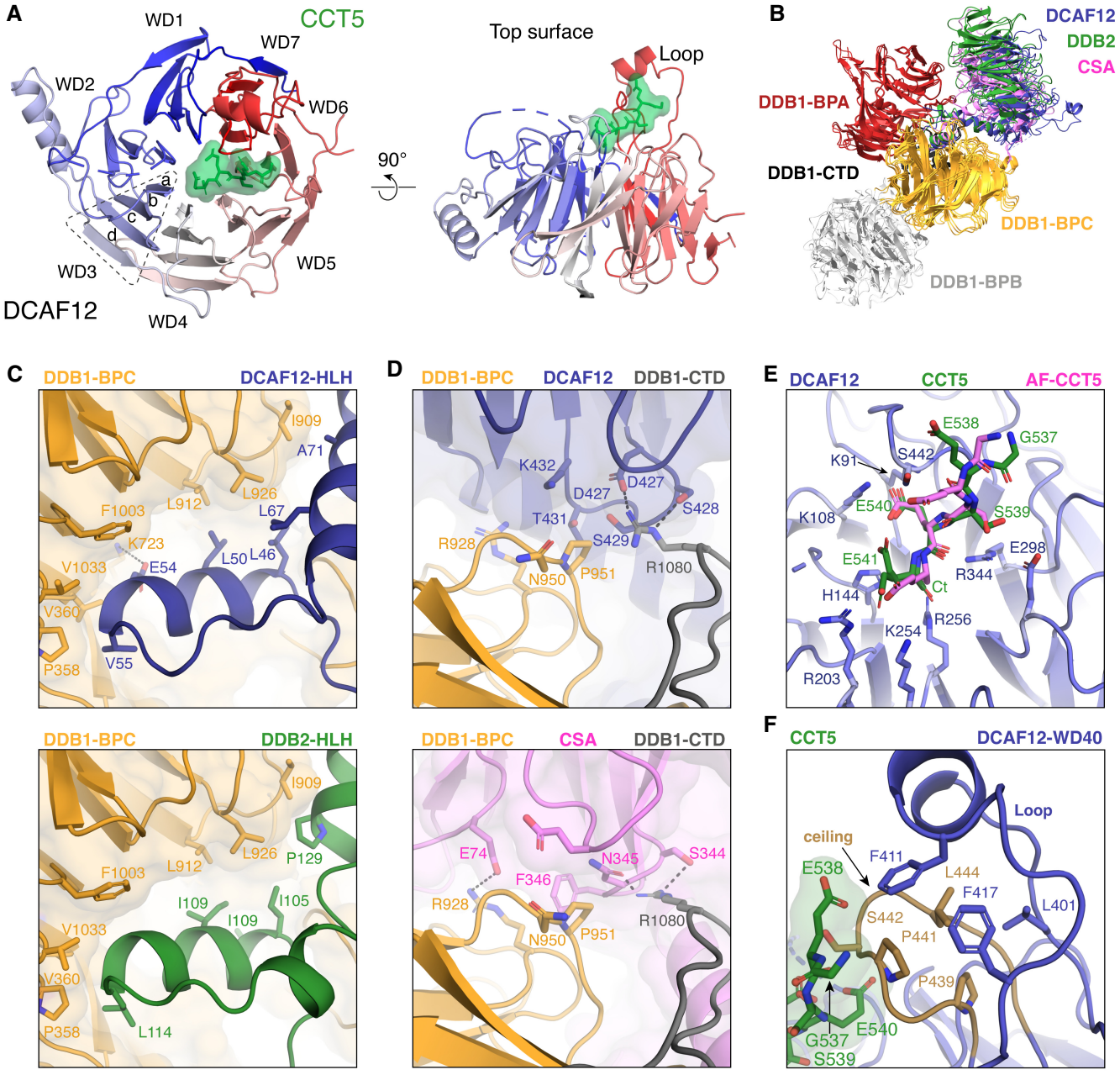


Figure EV3. DCAF12 assembles into a CRL4 ligase.

A Structural organization of the DCAF12 WD40 domain. The blades of the DCAF12 ß-propeller are labeled WD1-WD7 and colored according to their proximity to the N- or C-terminus. Each blade is composed of four ß strands labeled a-d in the outward direction. The CCT5 degron peptide is shown as green sticks and surface representation.

B Superposition between the coordinates of DDB1-DCAF12 (this study), DDB1-DDB2 (PDB ID 4A0K; Fischer *et al*, 2011) and DDB1-CSA (PDB ID 4A11; Fischer *et al*, 2011).

C, D Close-up of DDB1 residues contacted by the HLH motif (C) or ß-propeller (D) of DCAF12 (top) that are involved in binding other substrate receptors (bottom).

E Superposition of the AlphaFold2 prediction for the CCT5 peptide (AF-CCT5, violet) onto the cryo-EM coordinates of DDB1-DCAF12-CCT5 (CCT5 shown in green). The two modeled conformations of the CCT5 Glu541 side chain are depicted.

F Close-up of the hydrophobic residues mediating the interaction between the DCAF12 Loop (amino acids 370–416) and ceiling (amino acids 438–447).

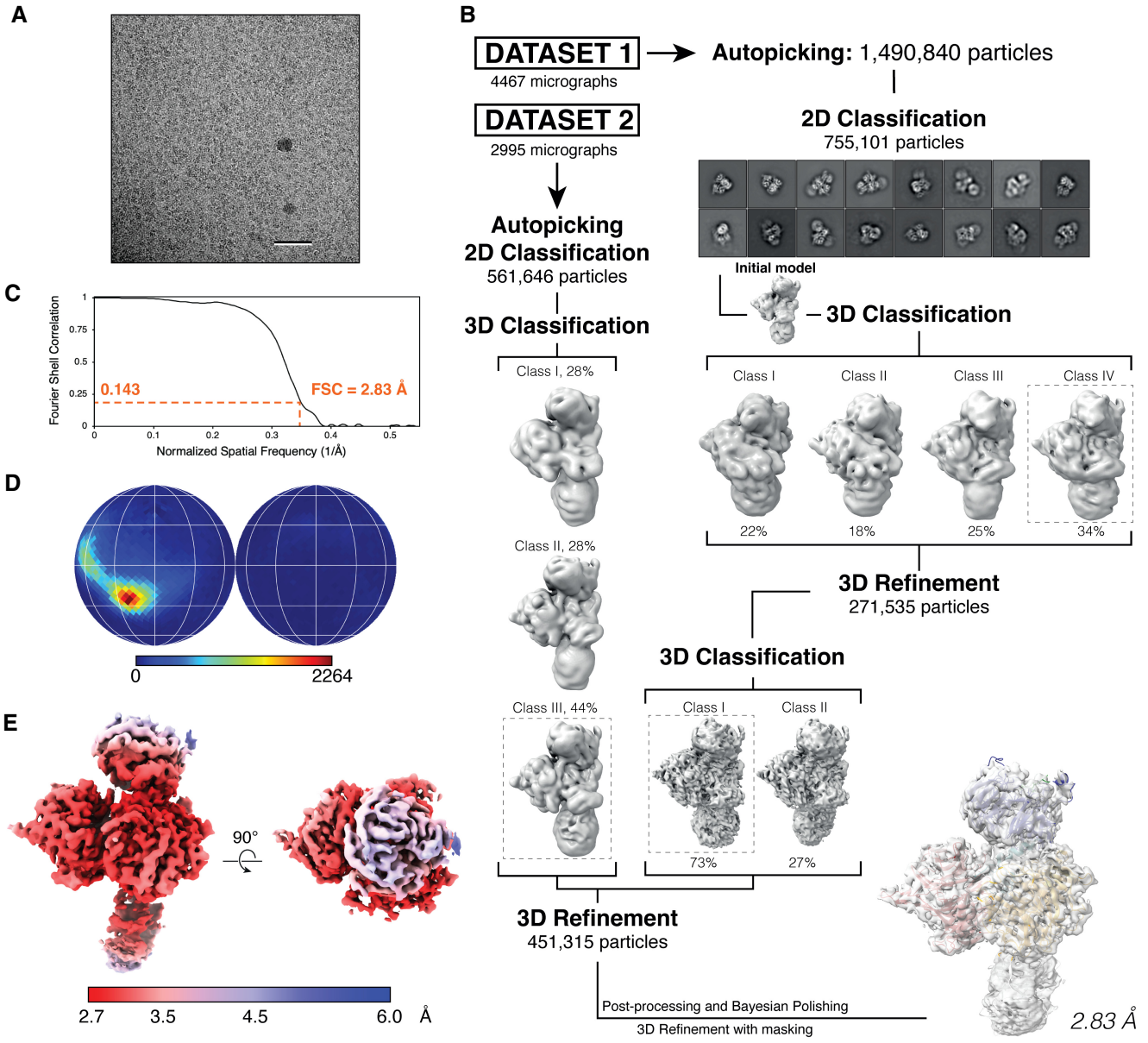


Figure EV4. DDB1-DCAF12-CCT5 cryo-EM structure determination.

A Representative micrograph from the DDB1-DCAF12-CCT5 collection. Scale bar: 50 nm.
B Workflow of cryo-EM data analysis for the DDB1-DCAF12-CCT5 cryo-EM map.
C Gold standard Fourier shell correlation (FSC) curve for the DDB1-DCAF12-CCT5 reconstruction.
D Angular distribution of DDB1-DCAF12-CCT5.
E Final cryo-EM map colored according to its local resolution, in angstroms.

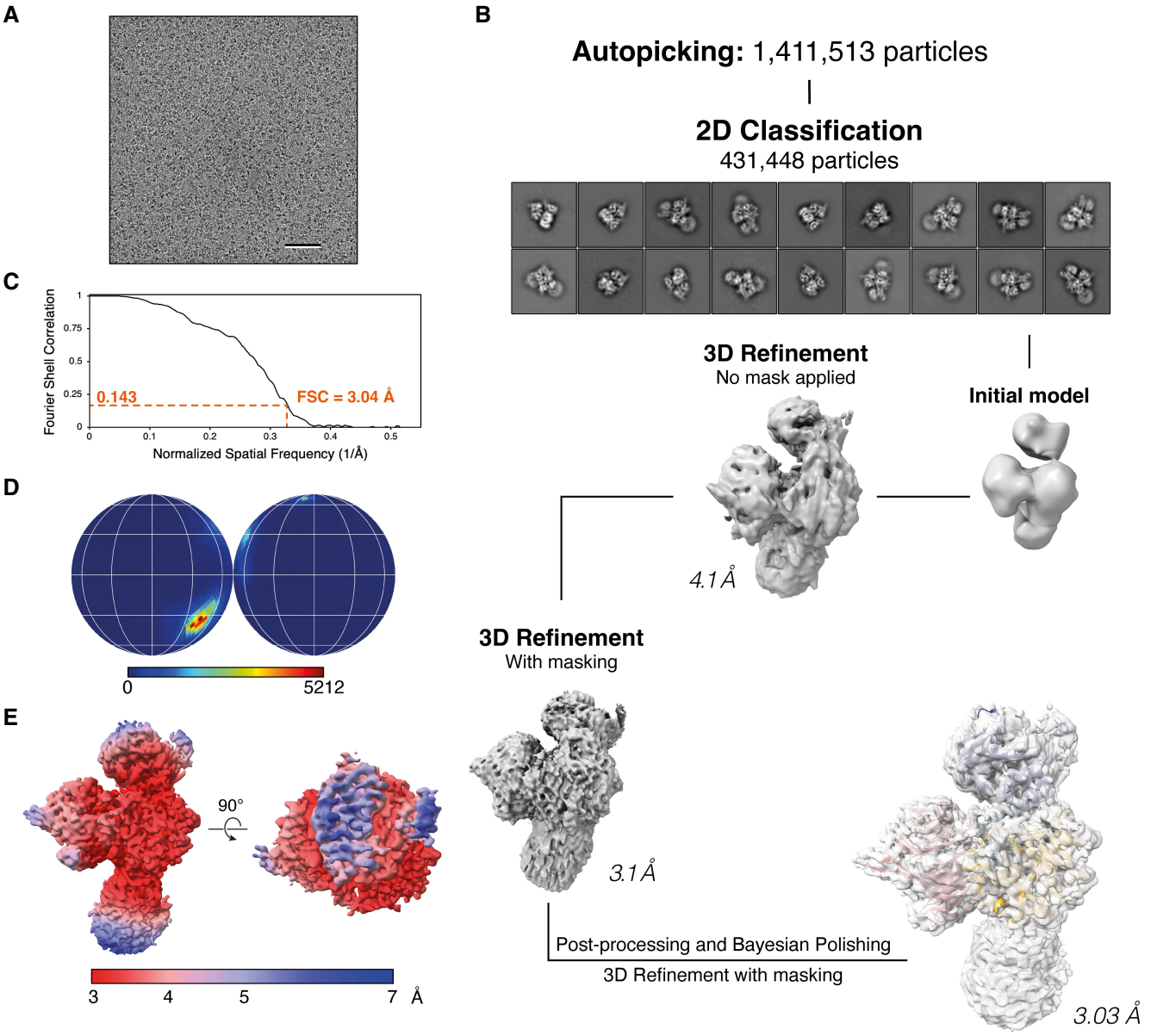


Figure EV5. DDB1-DCAF12 cryo-EM structure determination.

A Representative micrograph from the DDB1-DCAF12 collection. Scale bar: 50 nm.
B Workflow of cryo-EM data analysis for the DDB1-DCAF12 cryo-EM map. 4,568 micrographs were collected.
C Gold standard Fourier shell correlation (FSC) curve for the DDB1-DCAF12 reconstruction.
D Angular distribution of DDB1-DCAF12.
E Final cryo-EM map colored according to its local resolution, in angstroms.