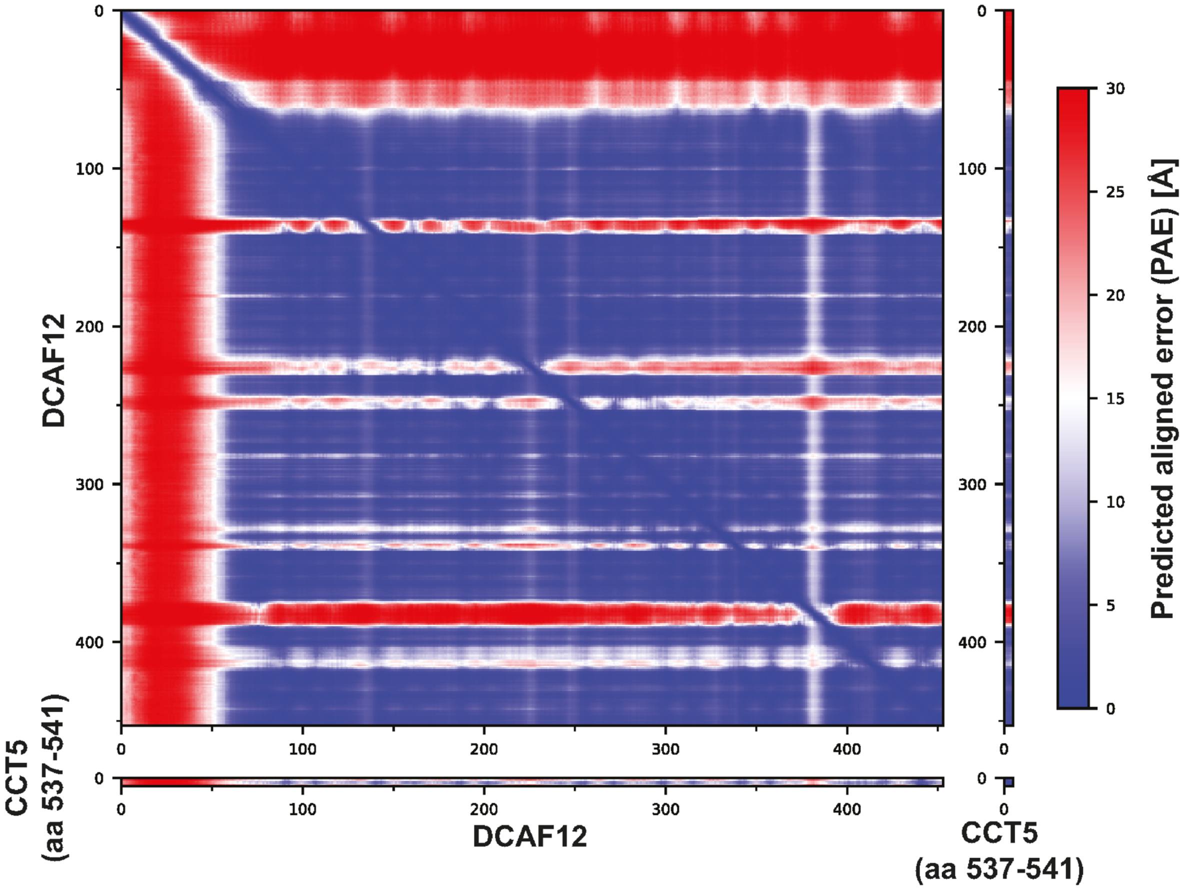
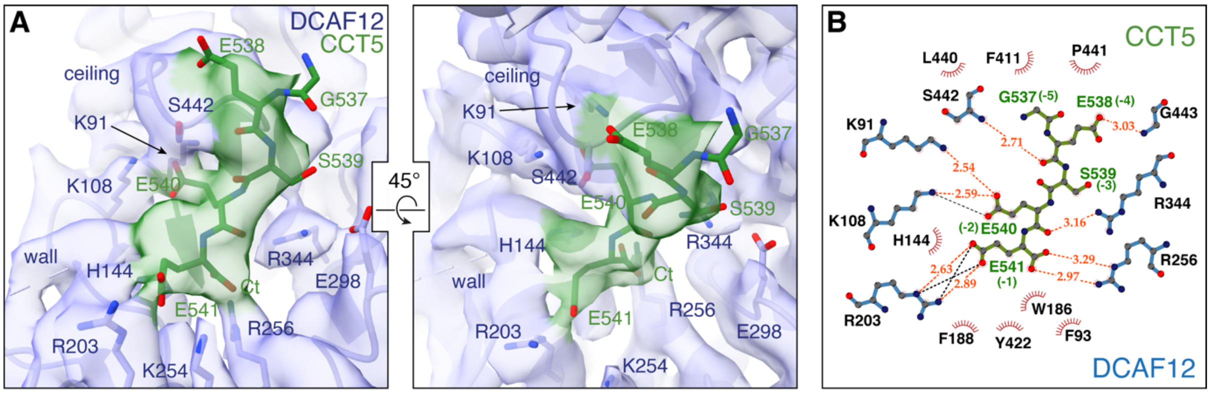
APPENDIX

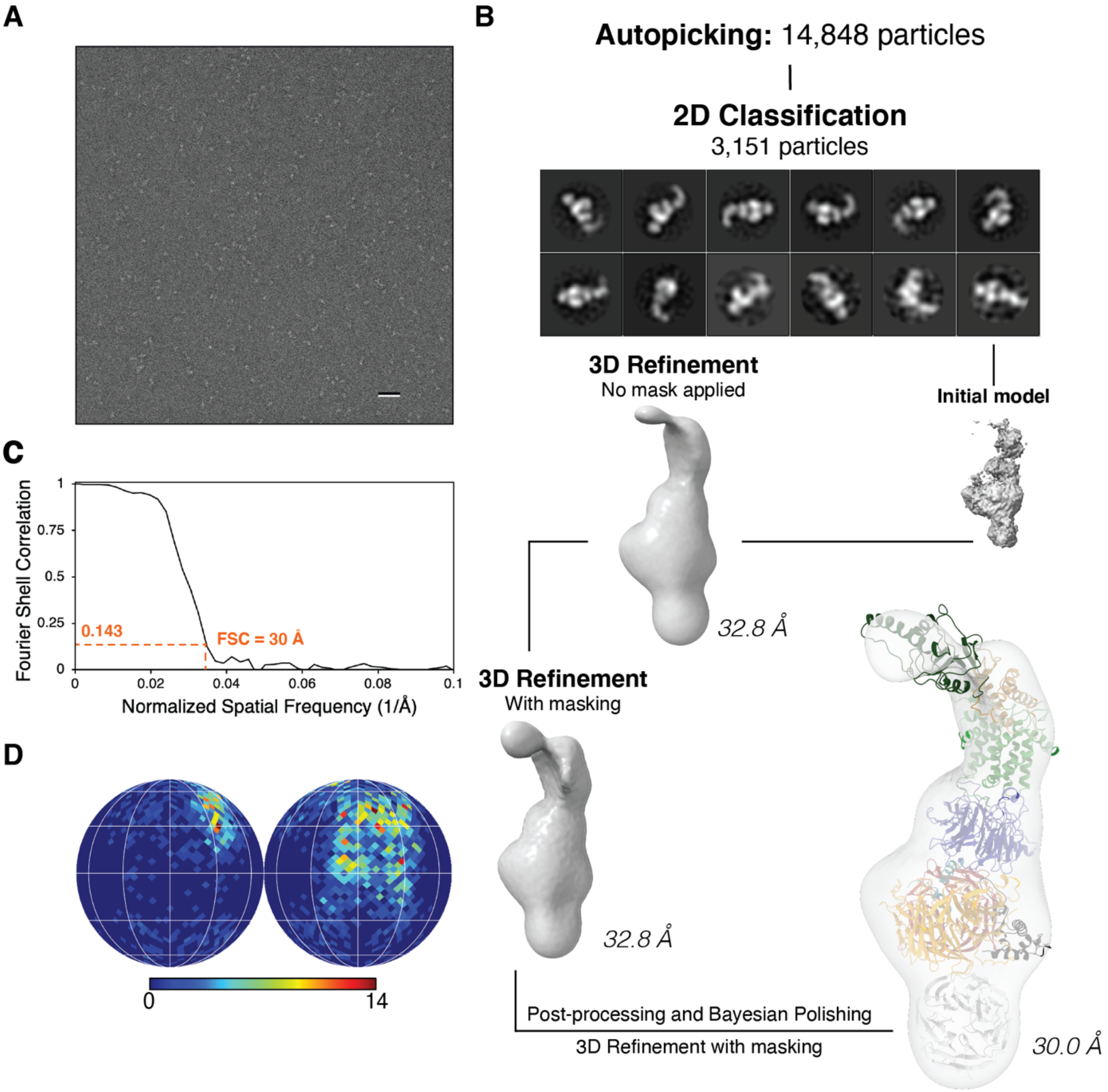


Appendix Figure S1. AlphaFold-Multimer prediction for the DDB1-DCAF12-CCT5 complex. Predicted aligned error (PAE) from AlphaFold-Multimer for complex prediction of DCAF12 (full-length) and CCT5 degron (amino acids 537-541). For each subunit pair, the PAE values are shown in different sub-plots.



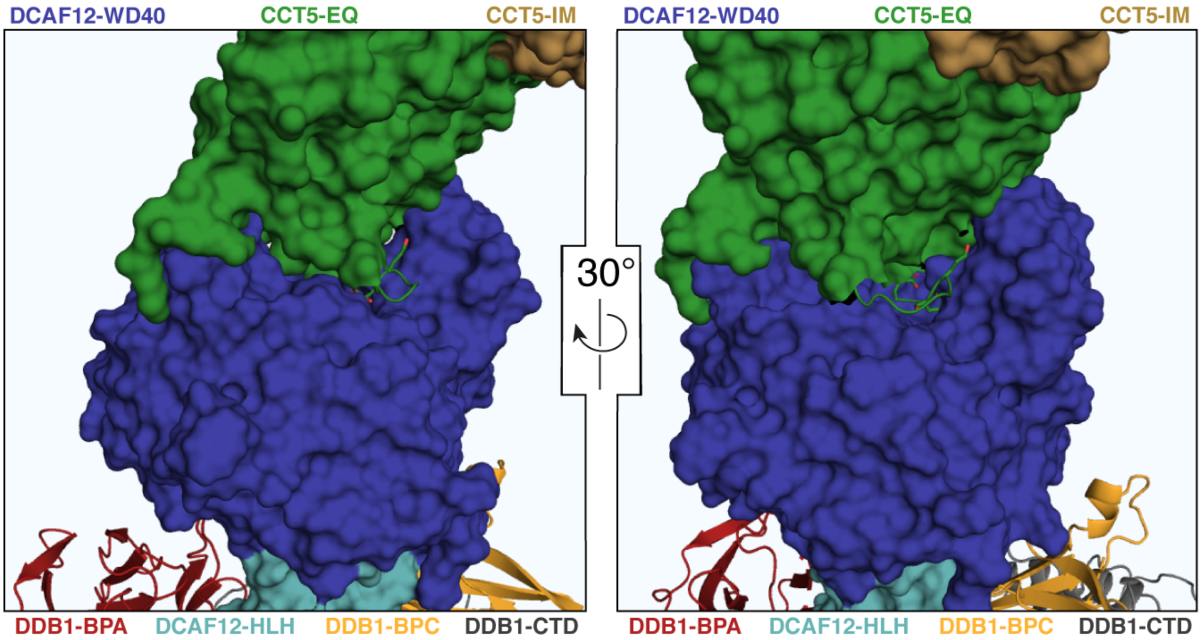
Appendix Figure S2. Alternative conformation of the CCT5 Glu541 side chain.

(A) A close-up view of the electron density around the DCAF12 pocket with an alternative conformation for the CCT5 Glu541 side chain modelled. DCAF12 is shown in light blue as cartoons, with key pocket residues shown as sticks. CCT5 residues are shown as green sticks. The electron density map is shown at a higher contour level than Fig 3A **(B)** LigPlot+ diagram of the interactions between DCAF12 and the alternative conformation of the CCT5 di-Glu degron (Laskowski & Swindells, 2011). The DCAF12 residues forming hydrogen bonds with CCT5 are shown in blue. DCAF12 residues involved in van der Waals packing are shown with eyelashes in red. CCT5 residues are shown in green with degron positions in parentheses. Hydrogen bonds and salt bridges are shown as orange and black dashed lines, respectively.



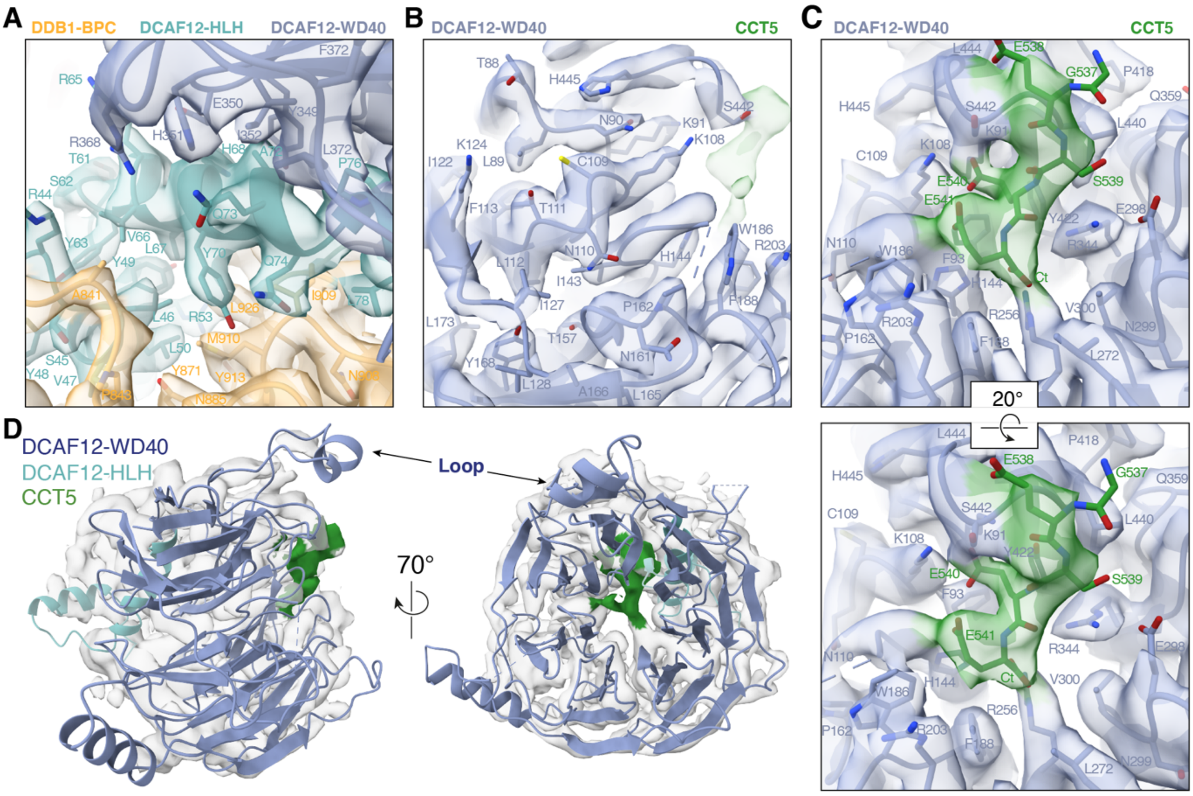
Appendix Figure S3. DDB1-DCAF12-CCT5 negative-stain EM structure determination.

(A) Representative micrograph from the DDB1-DCAF12-CCT5 negative-stain collection. Scale bar: 50 nm. **(B)** Workflow of cryo-EM data analysis for the DDB1-DCAF12-CCT5 negative-stain map. **(C)** Gold standard Fourier shell correlation (FSC) curve for the DDB1-DCAF12-CCT5 negative-stain reconstruction. **(D)** Angular distribution of DDB1-DCAF12-CCT5.



Appendix Figure S4. Binding of CCT5 to DCAF12 covers the pocket.

Different views of the interface between DCAF12 and CCT5 from the negative-stain EM map, shown in surface representation. DDB1 and the CCT5 C-terminal tail are shown as cartoons. Side chains are shown for CCT5 residues seen interacting with DCAF12 in the cryo-EM structure (Figure 3.6A).



Appendix Figure S5. Model-map fitting of the CCT5-bound DDB1-DCAF12 complex.

Details of the structure of the CCT5-bound DDB1-DCAF12 complex fit into the 2.8 Å cryo-EM map are shown around the DCAF12 helix-loop-helix (HLH) motif **(A)**, and DCAF12 β-propeller blades one and two, which create the pocket wall **(B)**. DDB1 and DCAF12 are shown as cartoons, with individual residues shown as sticks. **(C)** Two close-up views of the 2.8 Å cryo-EM map around the DCAF12 pocket. DCAF12 is shown as cartoons, with individual residues shown as sticks. The CCT5 peptide is shown as sticks. **(D)** Side (left) and top (right) views of the 2.8 Å cryo-EM map around DCAF12, shown as cartoons. Density corresponding to the CCT5 peptide is colored green.

Appendix Table S1. IC$_{50}$ values of degron peptides.

Peptide name	Sequence	Kd (µM)	95% CI (symmetric)	95% CI (asymmetric)
^{ATTO488}CCT5$_{20}$	ATTO488-QMVRMILKIDDIRKPGESEE	0.215	0.080 – 0.350	0.108 – 0.473

Peptide name	Sequence	IC50 (µM)	95% CI (symmetrical)	95% CI (asymmetrical)
CCT5$_{2}$	EE	383.8	13.74 – 753.90	193.30 – 6448.00
CCT5$_{5}$	GESEE	24.64	20.51 – 28.76	20.98 – 29.36
CCT5$_{6}$	PGESEE	15.59	11.95 – 19.23	12.50 – 19.86
CCT5$_{7}$	KPGESEE	4.955	4.137 – 5.965	4.060 – 5.850
CCT5$_{8}$	RKPGESEE	0.771	0.621 – 0.920	0.631 – 0.942
CCT5$_{10}$	DIRKPGESEE	0.363	0.285 – 0.441	0.291 – 0.453
CCT5$_{15}$	ILKIDDIRKPGESEE	0.614	0.564 – 0.664	0.567 – 0.666
CCT5$_{20}$	QMVRMILKIDDIRKPGESEE	0.404	0.361 – 0.457	0.363 - 0.461
CCT5$_{10}$ D532A	AIRKPGESEE	0.222	0.198 – 0.247	0.198 – 0.250
CCT5$_{10}$ I533A	DARKPGESEE	0.209	0.184 – 0.234	0.185 – 0.236
CCT5$_{10}$ R534A	DIAKPGESEE	0.395	0.327 – 0.464	0.330 – 0.474
CCT5$_{10}$ K535A	DIRAPGESEE	0.208	0.182 – 0.234	0.182 – 0.236
CCT5$_{10}$ P536A	DIRKAGESEE	0.417	0.364 – 0.470	0.366 – 0.475
CCT5$_{10}$ G537A	DIRKPAESEE	0.100	0.089 – 0.111	0.089 – 0.112
CCT5$_{10}$ E538A	DIRKPGASEE	0.571	0.468 – 0.674	0.473 – 0.688
CCT5$_{10}$ S539A	DIRKPGEAEE	0.125	0.110 – 0.139	0.110 – 0.141
CCT5$_{10}$ E540A	DIRKPGESAE	53.810	35.83 – 72.60	40.08 – 79.85
CCT5$_{10}$ E541A	DIRKPGESEA	6.211	3.182 – 9.228	3.923 – 10.490
CCT5$_{10}$ E541L	DIRKPGESEL	6.772	5.305 – 8.684	5.112 – 8.432
CCT5$_{10}$ E541Q	DIRKPGESEQ	17.95	15.06 – 21.50	14.76 – 21.15
CCT5$_{10}$ E541T	DIRKPGESET	47.25	35.44 – 64.86	33.25 – 61.25
CCT5$_{10}$ E541K	DIRKPGESEK	48.50	40.16 – 59.28	38.61 – 58.39
CCT5$_{10}$ E540D	DIRKPGESDE	137.0	88.36 – 250.3	67.49 – 206.4
CCT5$_{10}$ E541D	DIRKPGESED	222.1	102.1 – 1981	0.000 – 448.5
MAGEA3$_{20}$	GGPHISYPPLHEWVLREGEE	2.702	2.109 – 3.295	2.170 – 3.385
SAT1$_{20}$	EGWRLFKIDKEYLLKMATEE	0.291	0.253 – 0.330	0.257 – 0.332