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Supplemental Information

Crystal Structure of *Entamoeba histolytica* Cdc45 Suggests a Conformational Switch that May Regulate DNA Replication

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Supplemental Figures

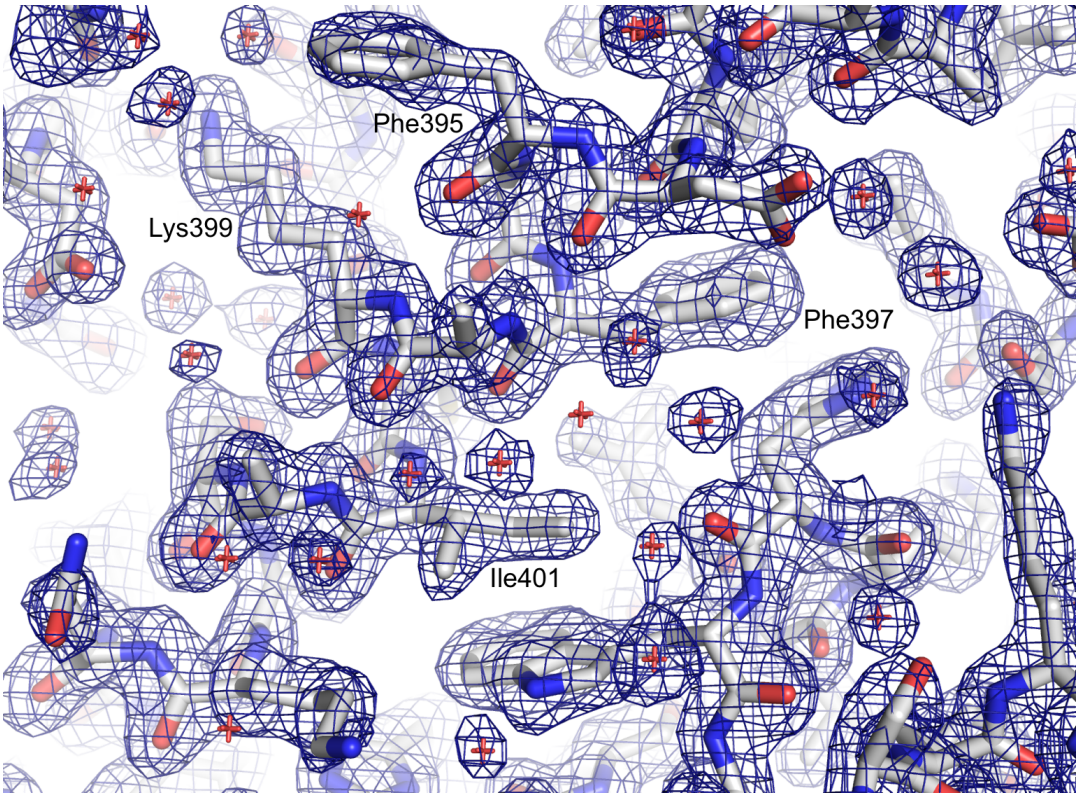

Figure S1. Representative electron density map, Related to Figure 2 and Transparent Methods. The 2mFo-DFc map contoured at 1.0 σ is shown for a portion of the connector helix (α17).

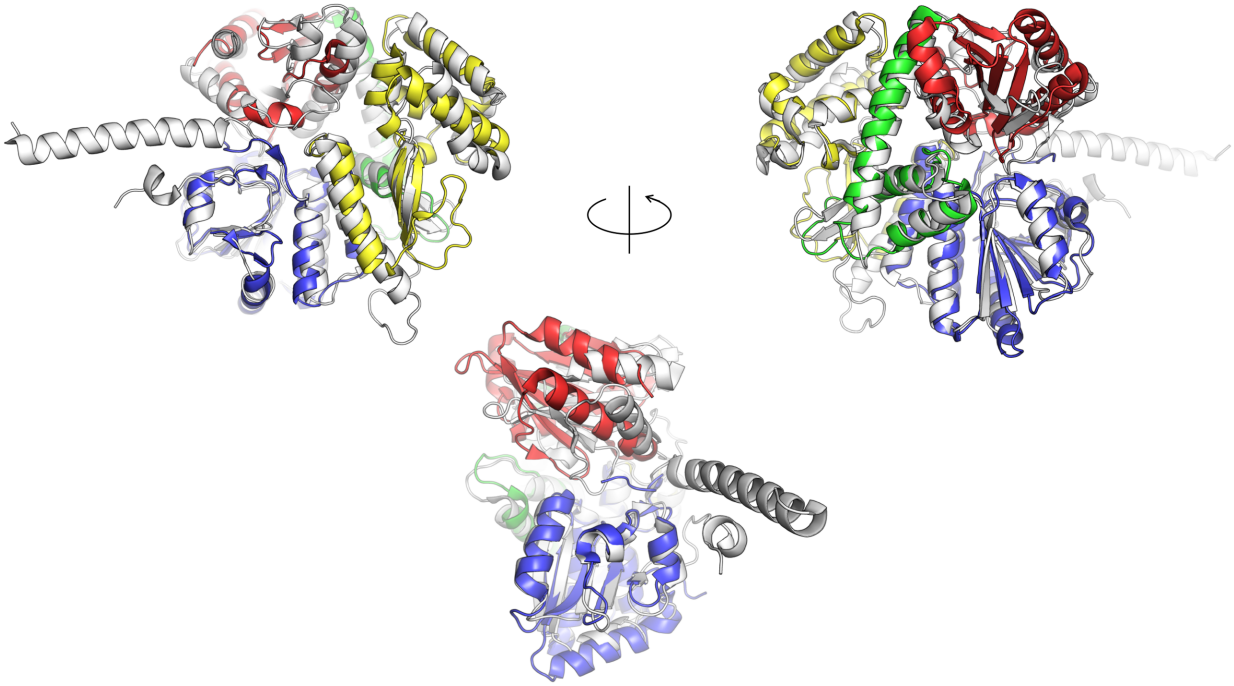

Figure S2. Superposition between *E. histolytica* (colored) and human (light gray) Cdc45 structures, Related to Figure 3. The structures were superimposed globally to minimize the overall RMSD (3.2 Å over 373 Cα atoms). 3 different views related by rotation about a vertical axis are shown. The human Cdc45 structure was reported previously (PDB ID: 5dgo) (Simon et al., 2016).

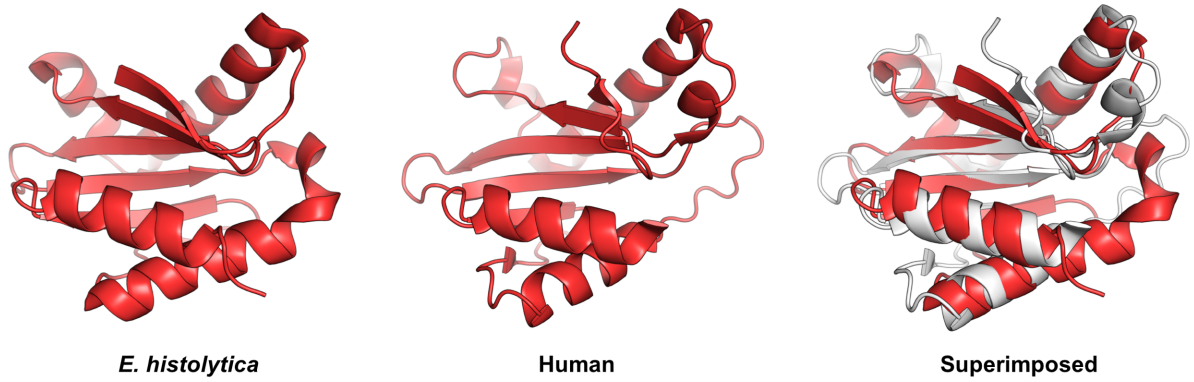

Figure S3. *E. histolytica* and human Cdc45 C-terminal DHHA1 domains share the same fold, Related to Figure 3. The two structures are shown in the same orientation to highlight their similarity. In the superposition (RMSD of 1.75 Å over 77 C α atoms), human Cdc45 DHHA1 domain (Simon et al., 2016) is shown in light gray.

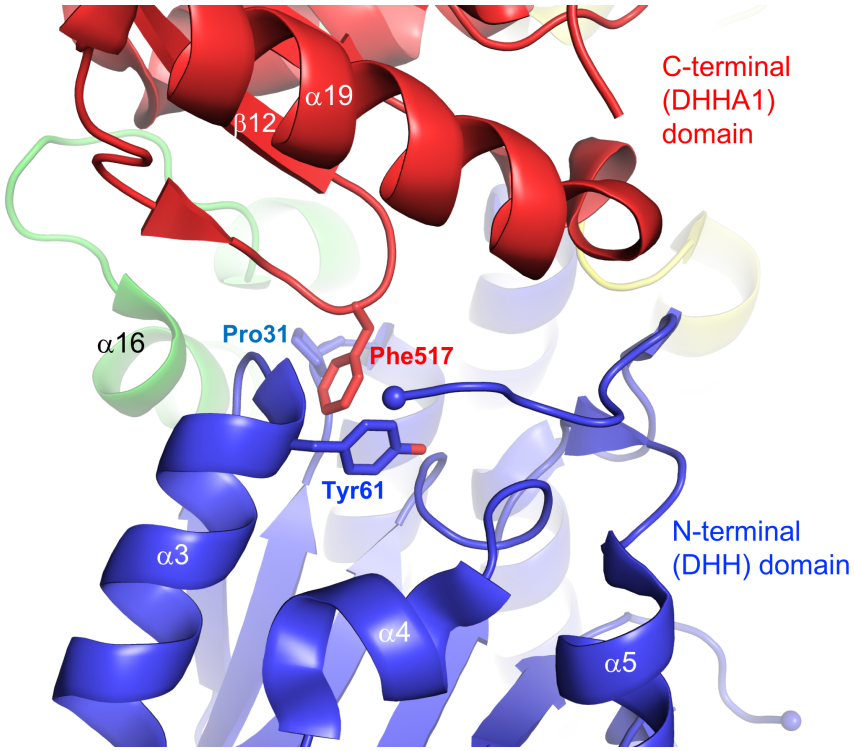

Figure S4. A close-up view, Related to Figure 2. Phe517 from the C-terminal DHHA1 domain of *E. histolytica* Cdc45 is inserted in a hydrophobic pocket in the N-terminal DHH domain.

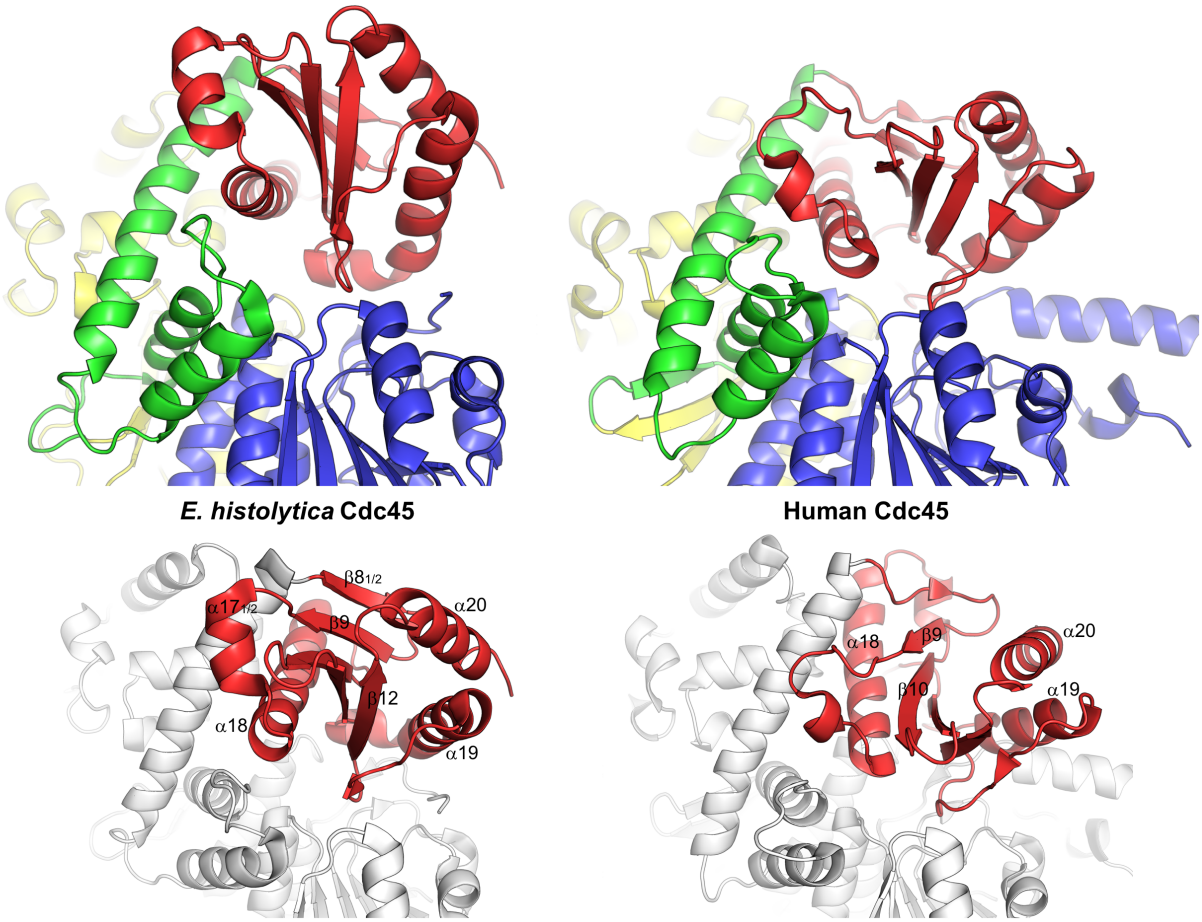

Figure S5. Additional views of structural comparison between *E. histolytica* and human Cdc45, Related to Figure 3.

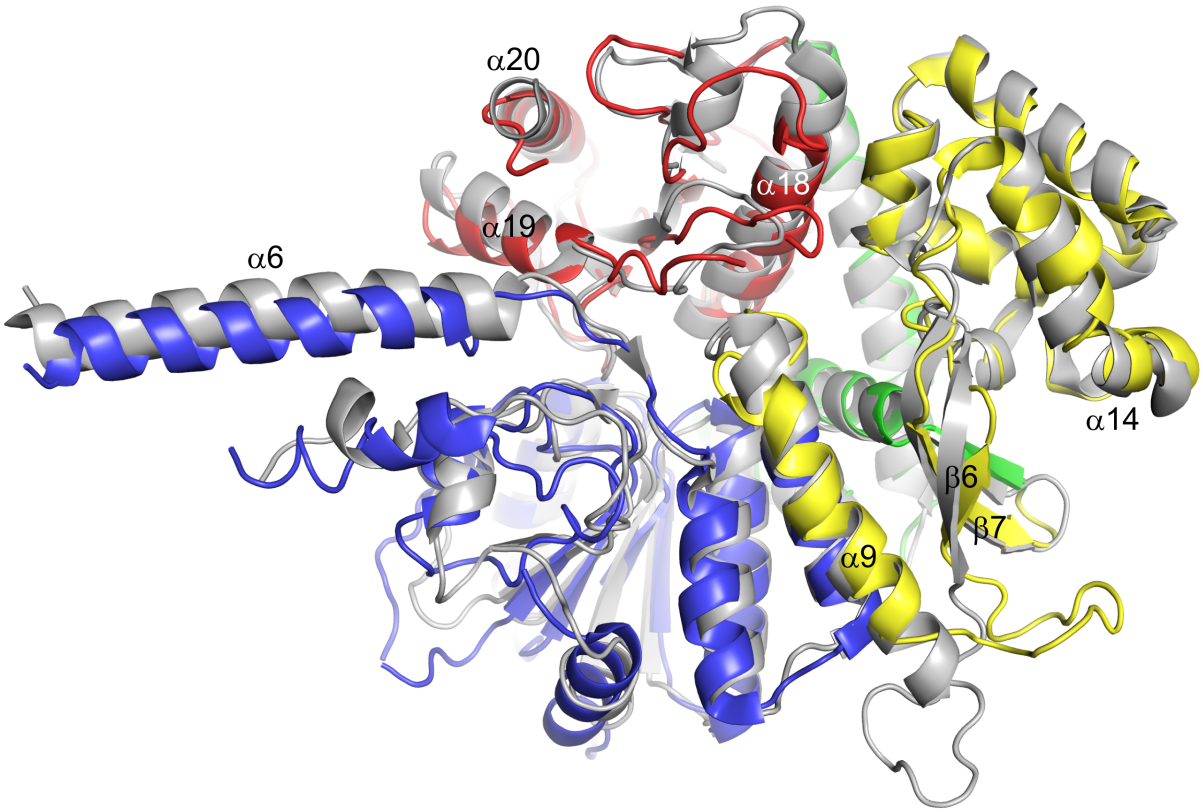

Figure S6. Additional structural comparison, Related to Figure 3. Superposition between yeast Cdc45 in the CMG complex (PDB ID: 3jc6, colored)(Yuan et al., 2016) and human Cdc45 (5dgo, gray)(Simon et al., 2016) showing their high similarity including the protruding α6. The structures were superimposed globally to minimize the overall RMSD (1.4 Å over 359 Cα atoms).

Transparent Methods

Full-length *E. histolytica* Cdc45 protein was expressed in *E. coli* strain BL21(DE3) as a 6xHis-SUMO-fusion protein and purified using nickel-NTA and Superdex 200 size-exclusion chromatography (SEC). The 6xHis-SUMO tag was removed by a SUMO protease (Ulp1) treatment between the two column chromatography steps. *E. histolytica* Cdc45 eluted as an apparent monomer in SEC. The peak fractions were pooled and concentrated by ultrafiltration to ~20 mg ml^{-1} in a buffer condition: 20 mM Tris-HCl (pH 7.4), 0.15 M NaCl , and 5 mM β-mercaptoethanol for use in crystallization experiments. Selenomethionine-labeled protein was expressed in the M9 minimal medium using the metabolic inhibition method (Doublie, 1997) and purified as described above. We obtained *E. histolytica* Cdc45 crystals by the sitting drop vapor diffusion method using a well solution consisting of 0.26 M sodium thiocyanate (pH 6.9) and 25 % polyethyleneglycol 3,350. To form the sitting drop, 0.1 μL each of the protein and well solutions were mixed. Crystals were harvested into the well solution supplemented with 20% ethylene glycol and flash cooled by plunging into liquid nitrogen. Diffraction data were collected at the Advanced Photon Source (APS) Northeastern Collaborative Access Team (NE-CAT) beamline 24-ID-C, using the selenium K-absorption edge x-ray wavelength. The data were processed with XDS (Kabsch, 2010) and the structure solved by the SAD phasing method using PHENIX AutoSol (Terwilliger et al., 2009). 13 Se atoms were located and the initial FOM was 0.39. AutoBuild (Terwilliger et al., 2008) built 384 amino acids into the SAD-phased map. Continuing manual model building and refinement were done using COOT (Emsley et al., 2010) and PHENIX (Adams et al., 2010). The refined electron density map suggested a disulfide bond formation between Cys420 and Cys430 within the C-terminal DHHA1 domain, which was refined with partial occupancy of both oxidized and reduced species, and modification of Cys539 by β-mercaptoethanol. The final R_{work} and R_{free} are 15.8% and 19.3% respectively. A summary of data collection and model refinement statistics is shown in **Table 1**. Molecular graphics images were produced using PYMOL (https://pymol.org/2/).

Data Availability

Atomic coordinates and structure factors have been deposited in the Protein Data Bank with the accession code 6CC2.

Supplemental References

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