

Appendix

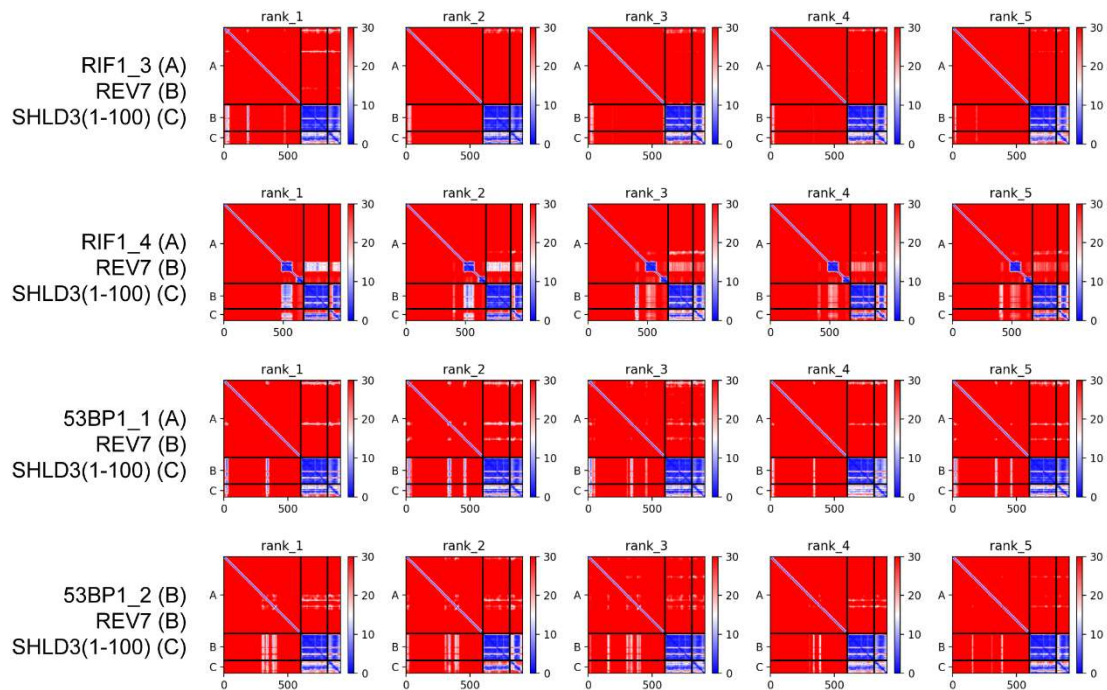
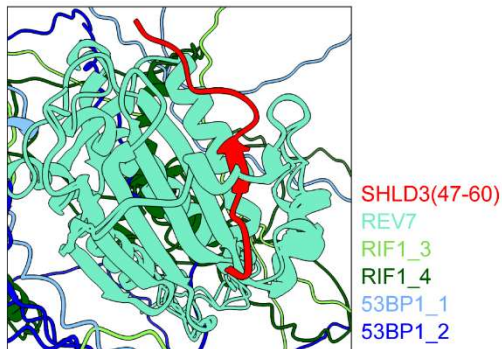
An AlphaFold 2 map of the 53BP1 pathway identifies a direct SHLD3-RIF1 interaction critical for shieldin activity

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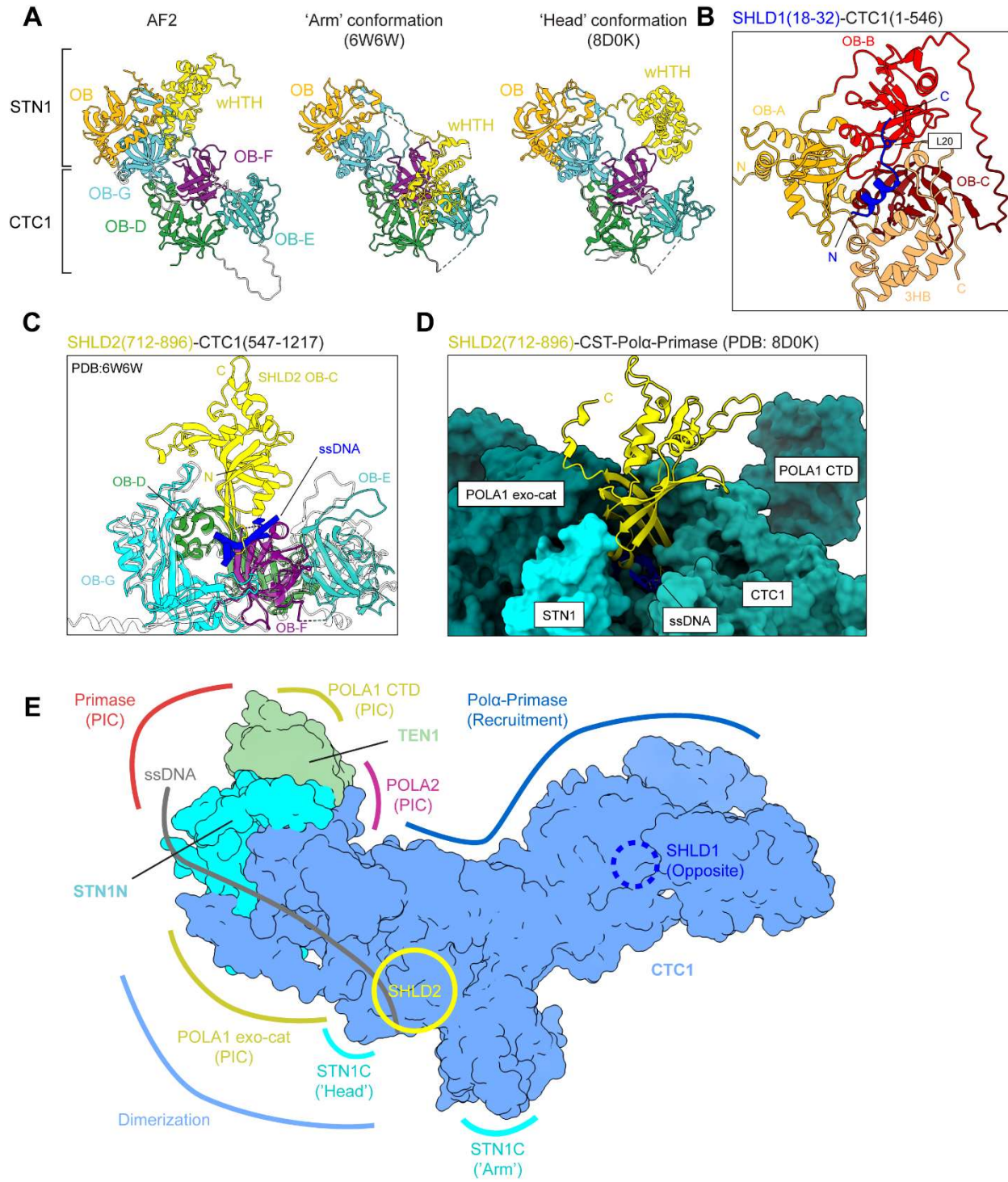
A**B**

Appendix Figure S1. AF2 prediction of 53BP1 and RIF1 association with the REV7 seatbelt in the presence of SHLD3.

A. Predicted aligned error plots of the indicated fragments ranked by predicted template model (pTM) scores. Each prediction corresponds to Fig EV1C except with the addition of SHLD3 residues 1-100.

B. Superimposition of predicted heterotrimeric structures between REV7, SHLD3(1-100), and RIF1 fragments 3 and 4 and 53BP1 fragments 1 and 2. Only rank_1 models are shown. See also [Fig EV1C](#).

Appendix Figure S2



Appendix Fig S2. Analysis of AF2-predicted shieldin-CST structures.

- A. Comparison of the AF2-predicted CTC1-STN1 structure with experimentally determined structures of CST in the ‘Arm’ and ‘Head’ conformations (PDB: 6W6W and 8D0K, respectively). Structures are aligned through the CTC1 subunit.
- B. Alternative view of the AF2-predicted SHLD1-CTC1 structure (Fig 1B panel vii) with the individual CTC1 domains (three-helix bundle 3HB, OB-A, OB-B, and OB-C) labeled.
- C. Superimposition of the AF2-predicted SHLD2-CTC1 structure with the experimentally determined structure of CST (PDB: 6W6W; translucent) showing steric incompatibility with CTC1-bound single-stranded DNA (ssDNA).
- D. Superimposition of the AF2-predicted SHLD2-CTC1 structure with the experimentally determined structure of CST-Pol α -Primase in the PIC conformation (PDB: 8D0K, surface representation) showing that the SHLD2 binding interface is not mutually exclusive with POLA1 exo-cat domain.
- E. Schematic of the AF2 predicted SHLD2 and SHLD1 binding sites (circles) on the experimentally determined structure of CTC1, STN1 N-terminal half (STN1N), and TEN1 (PDB: 8D0K; surface representation). The approximate binding interfaces of other CST-Pol α -Primase subunits are shown (PDB: 6W6W and 8D0K for PIC and recruitment conformations, respectively). SHLD1 interface is on the opposite face of the displayed model.