Expanded View Figures

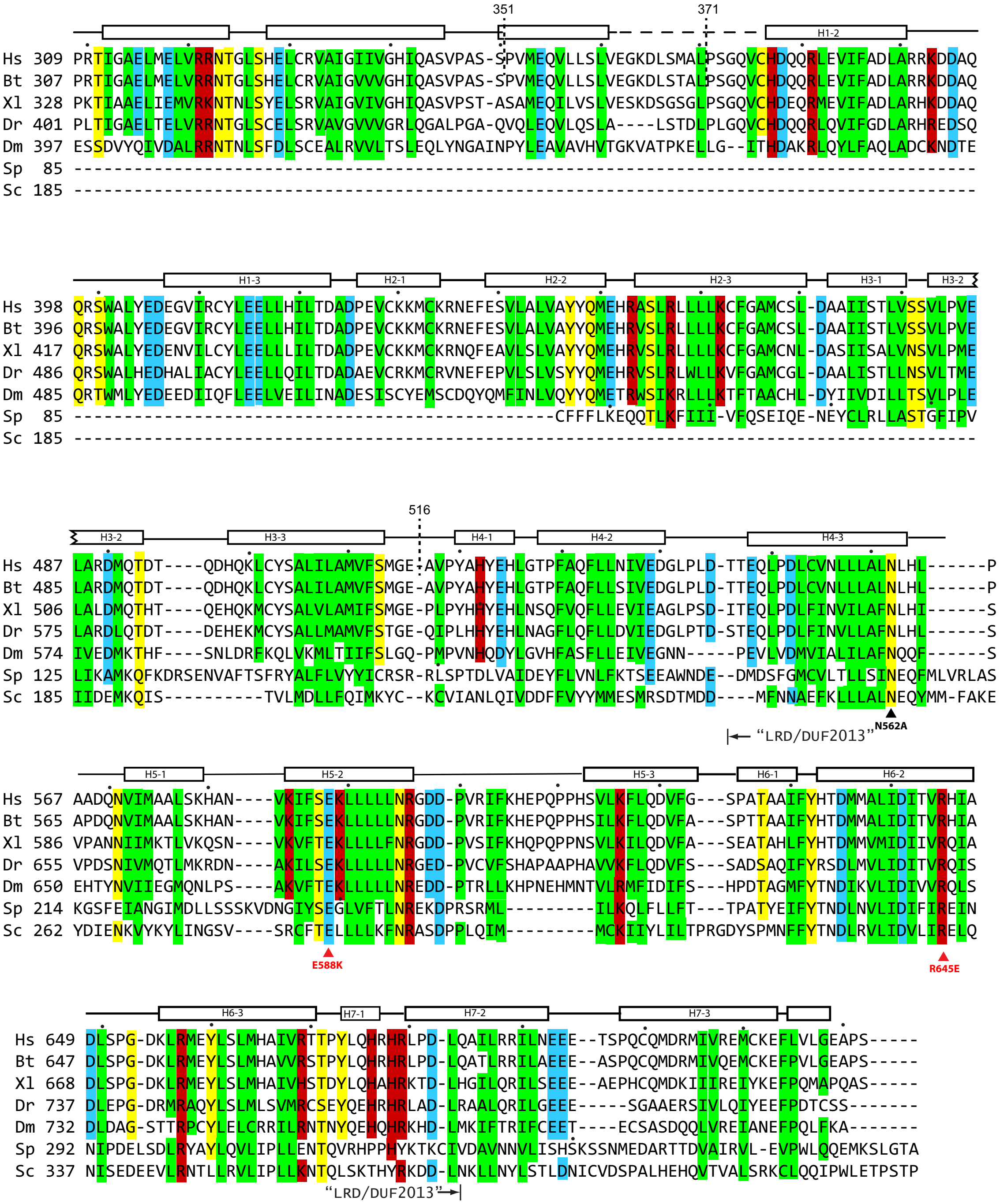

Figure EV1. Sequence alignment of SPIN90 from diverse species.

Conserved residues are indicated by colored boxes (green: hydrophobic; yellow: hydrophilic; red: basic; cyan: acidic). The highly conserved segment among WDS proteins, the “leucine-rich domain” (LRD)/DUF2013 segment, is indicated below the sequences, along with residues mutated in this study. Truncation sites are indicated with dashed lines. Alpha helices in the SPIN90 structure are shown above the sequence as rectangles. Hs, *H. sapiens*; Sc, *S. cerevisiae*; Sp, *S. pombe*; Bt, *B. taurus*; Dm, *D. melanogaster*; Xl, *X. laevis*; Dr, *D. rerio*.

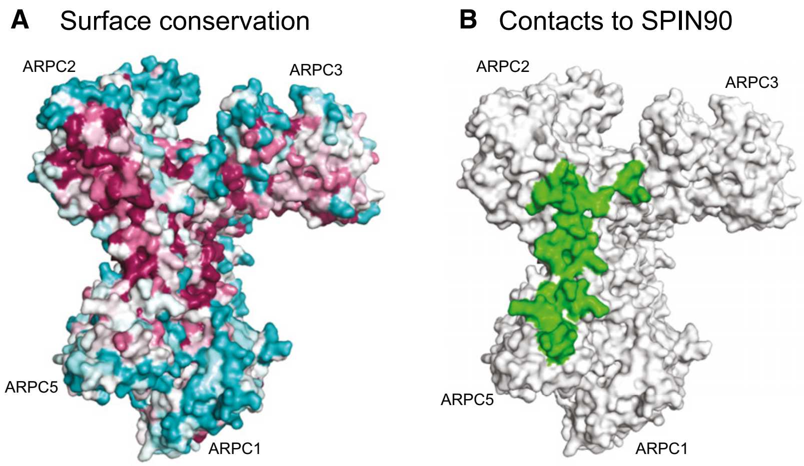

Figure EV2. The SPIN90 contact surface overlaps with a conserved patch of residues on Arp2/3 complex.

- A Surface representation of the "front side" of Arp2/3 from the apo Arp2/3 complex structure (1k8k; Robinson *et al*, 2001) showing residue conservation based on analysis with the ConSurf server (Ashkenazy *et al*, 2016). Residues are colored according to conservation, with the following order, going from most to least conserved: dark magenta > pink > light pink > gray > light cyan > cyan.
- B Surface representation of Arp2/3 complex (1k8k) showing residues (green) that are within 6 Å of SPIN90 in the co-complex structure.

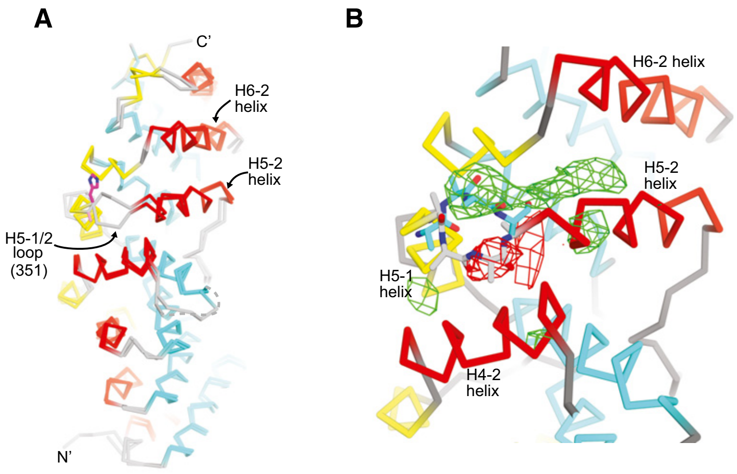

Figure EV3. The H5-1/2 loop is flipped toward the C-terminus of the ARM domain in Arp2/3-bound SPIN90.

- A $C\alpha$ trace of SPIN90 (351–722) superposed onto the structure of SPIN90 (269–722) from the co-complex structure. His580 is shown in stick representation. H5-1/2 is flipped away from the C-terminus in the inactive 351–722 structure and toward it in the co-complex structure.
- B Zoomed in view from panel (A) showing the difference in conformation of the H5-1/2 loop in the inactive (351–722, sticks with gray carbon atoms) versus active (269–722, sticks with cyan carbon atoms) SPIN90 structures. Positive (green) or negative (red) $F_o - F_c$ electron density maps are contoured at $\pm 2.0\ \sigma$ and were generated from the co-complex reflection file plus a PDB file in which H5-1/2 loop (residues 577–585) of the co-complex structure was replaced with the same residues from chain A in the 351–722 structure.

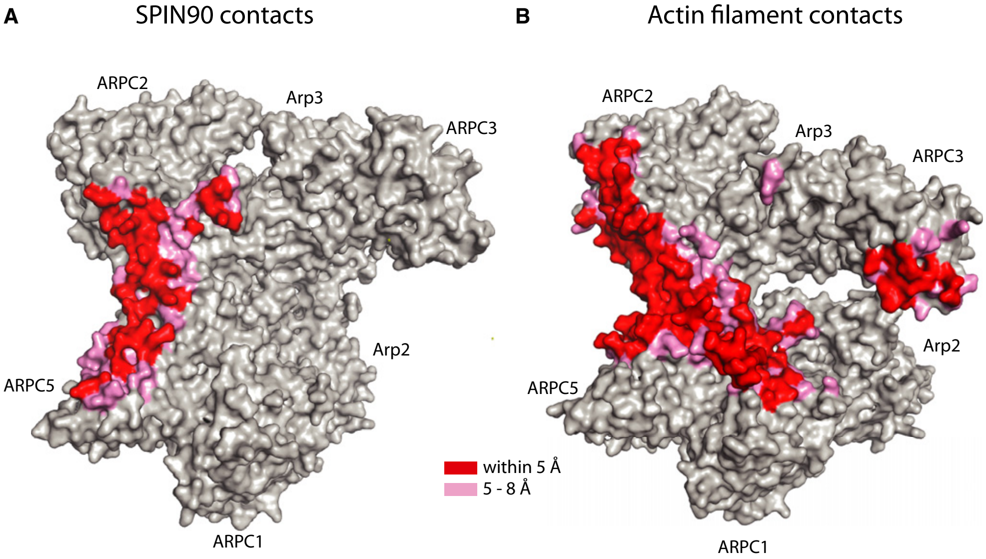

Figure EV4. SPIN90 binds to the same surface of Arp2/3 complex as actin filaments.

A Surface representation of the "front side" of Arp2/3 from SPIN90 co-complex structure showing residues that are within 5 Å of SPIN90 (red) or between 5 and 8 Å (pink). Side chains were modeled onto the co-complex structure before generating the surface.

B Surface representation of the "front side" of Arp2/3 from the branch junction model colored as described in (A) (Rouiller *et al*, 2008), except close contacts between the complex and actin filaments are colored.