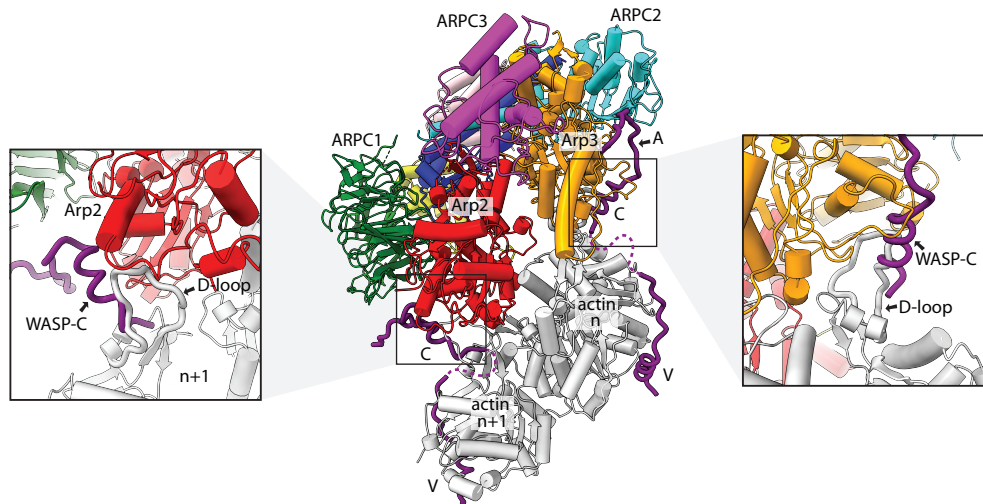
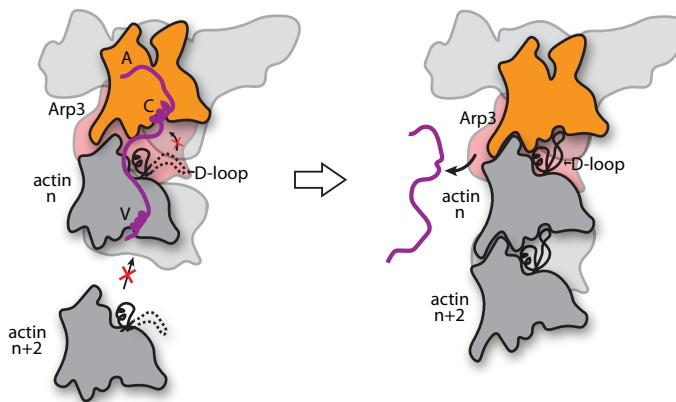


Supplementary Fig. 1 | Density maps of the barbed end grooves of Arp2 and Arp3 showing distance measurements x_2 and x_3 . **a**, Stereo view of Arp2 barbed end groove with density map shown around Arp2. Atoms used as endpoints for measurements are shown as spheres with dashed lines indicating distance x_2 (I136 C α to V169 C α) and x_3 (S148 C α to D383 C α). A portion of the D-loop from actin n+1 is shown in stick representation with cyan carbon atoms. **b**, Stereo view of Arp3 barbed end groove with density map shown around Arp3. Atoms used as endpoints for measurements are shown as spheres with dashed lines indicating distance x_2 (V159 C α to Y200 C α) and x_3 (L179 C α to R418 C α). A portion of the D-loop from actin n is shown in stick representation with cyan carbon atoms.

a



b



Supplementary Fig. 2 | The WASP-C segment likely blocks engagement of the D-loop of actins with the barbed end grooves of Arp2 or Arp3. **a**, Hypothetical model of WASP bound to the activated Arp2/3 complex. The model was made by superposing the cross-linking and mass spectrometry-based WASP-CA binding model²⁸ onto the activated structure presented here. The WASP-V region was modeled into position on actins n and n+1 based on the structure PDB 2A40⁵⁶. **b**, Diagrammatic representation showing WASP blocking long pitch interactions in the protofilament containing Arp3. In addition to WASP-C blocking the D-loop of actin subunit n, the WASP-V region may block association of the D-loop actin n+2 with actin n.