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Supplemental Information

Three-Dimensional Architecture of the Human BRCA1-A Histone Deubiquitinase Core Complex

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Supplementary Experimental Procedures

DUB activity assay

The DUB activity assay was carried out in 40 mM Tris pH=8.0, 100 mM NaCl, 1 mM TCEP. The active BRCA1 A complex core was combined with K63-linked penta-ubiquitin in a total volume of 15 µl and incubated at 37°C for 5, 10, 20, 30, 40, 50, 60, 90 and 120 min. The reaction was quenched by adding SDS sample buffer and by heating the sample to 100°C for 5 min. Samples were analysed by SDS-PAGE. The following enzyme concentrations were used in this DUB assay: [UB_5, K63-linked] =23.5 µM and [BRCA1-A] = 16.8 nM.

Tilt-pair analysis and determination of absolute hand

Tilt-pair images, at 20° and -20°, were obtained for the cross linked structure using the same grids and conditions as described above. Particle pairs were picked from tilted images using Xmipp tilt-pair picking function. Euler angles were assigned to both tilted and untilted particle stacks using 1 cycle of Frealign in mode 3 (global search) with the final map as a reference. Tilt-pair analysis (Rosenthal & Henderson, 2003) was performed using the tilt-service (Wasilewski and Rosenthal, 2014). The minimum in the tilt-pair parameter plot agreed with that expected for the known tilt-axis and tilt-angle used in the experiment confirmed by the presence of the expected defocus gradient in the image fields. However, the minimum was at a smaller angle than used in the experiment due to flattening of the particles in stain.

Figure S1

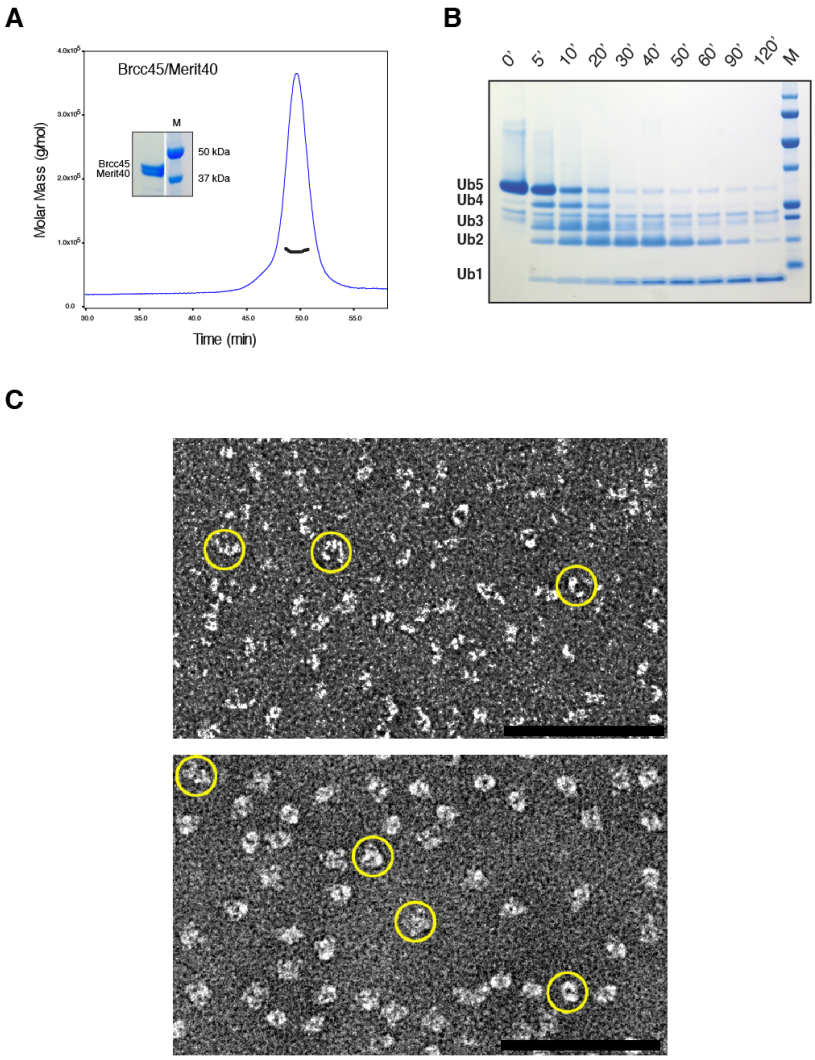

Figure S1 (Related to Figure 1) **BRCA1-A complex (A)** SEC-MALLS and SDS-PAGE analysis (inset) analysis of the purified BRCC45/MERIT40 complex. UV absorbance at 280 nm is shown in blue and the weight-averaged molecular mass across the peak in black. **(B)** Deubiquitinase assay of the BRCA1-A core complex with K63-linked penta-ubiquitin substrate analysed by SDS-PAGE. **(C)** Representative fields of raw negatively stained electron micrographs obtained from both un-crosslinked (Top) and crosslinked (Bottom) BRCA1-A complex, scale bars 100 nm. Representative views are circled.

Figure S2

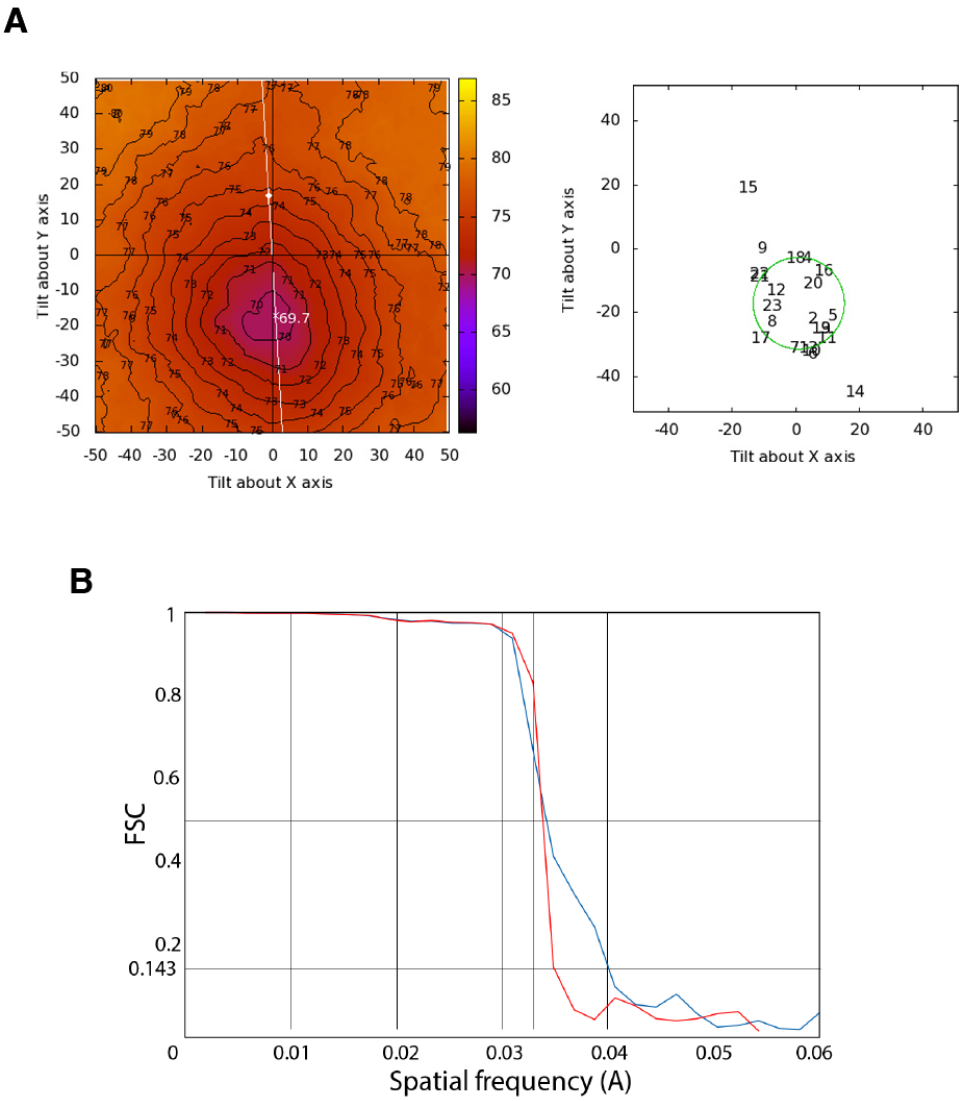

Figure S2 (Related to Figure 2) **EM analysis (A)** (Left) Tilt-pair phase residual plot and (Right) tilt-pair parameter plot shows a minimum and cluster respectively near the tilt-axis and tilt-angle (20 deg) used in the experiment. **(B)** Fourier–Shell Correlation for the crosslinked BRCA1-A complex between two maps calculated from different subsets of particle images (blue) and for refinement performed with phases randomized after 30A (red).

Figure S3

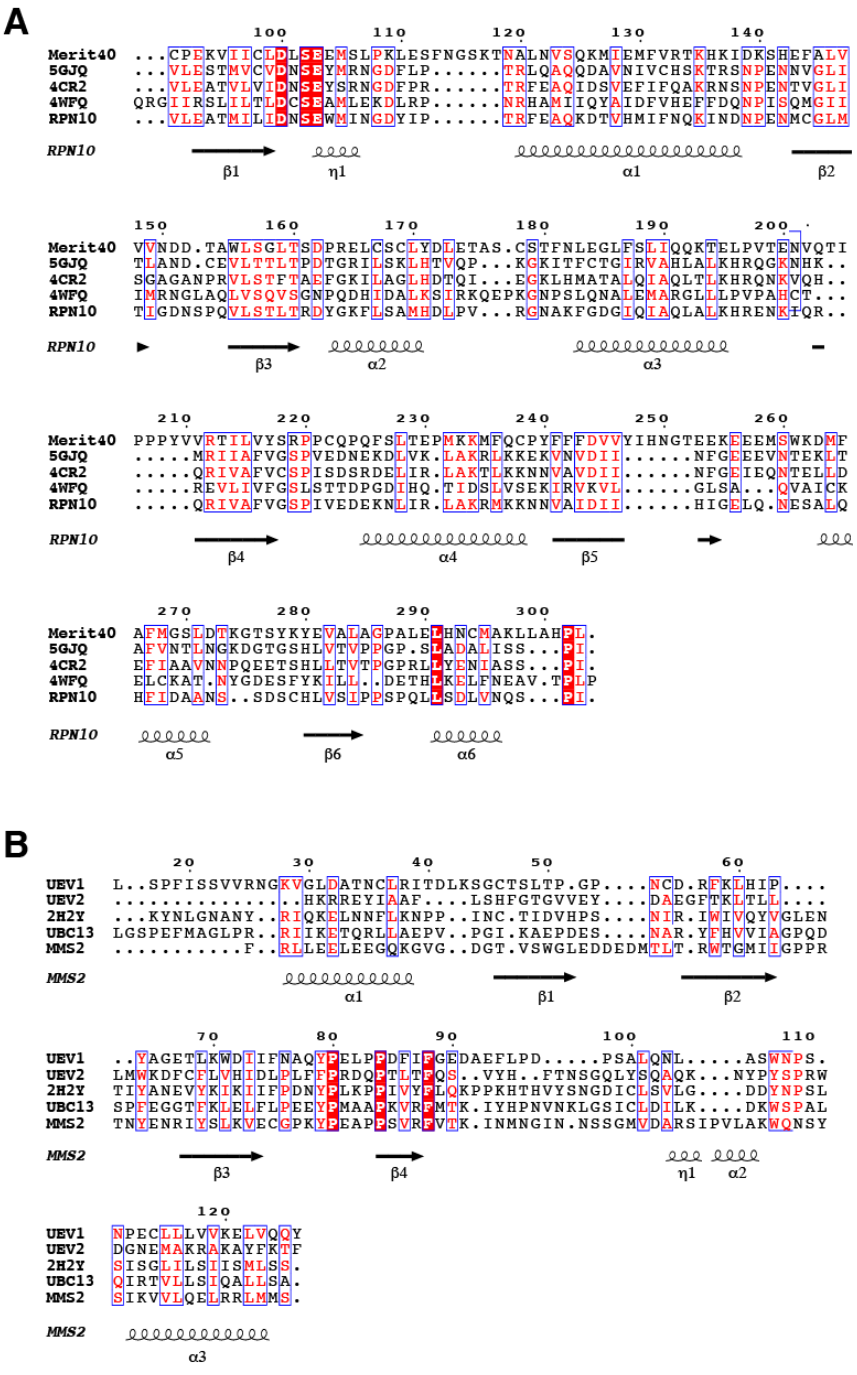

Figure S3 (Related to Figure 3) **Sequence homology** - Sequence alignments of the UEV domains of BRCC45 **(A)** and the VWA domain of MERIT40 **(B)** with homologous domains of known structure.

Supplementary References

Wasilewski S, Rosenthal PB Web server for tilt-pair validation of single particle maps from electron cryomicroscopy. *J Struct Biol* 2014, 186:122-131.

Rosenthal PB, Henderson R Optimal determination of particle orientation, absolute hand, and contrast loss in single-particle electron cryomicroscopy. *J Mol Biol* 2003, 333:721-745.