Supplementary Material

DSS1 and ssDNA Regulate Oligomerization of BRCA2

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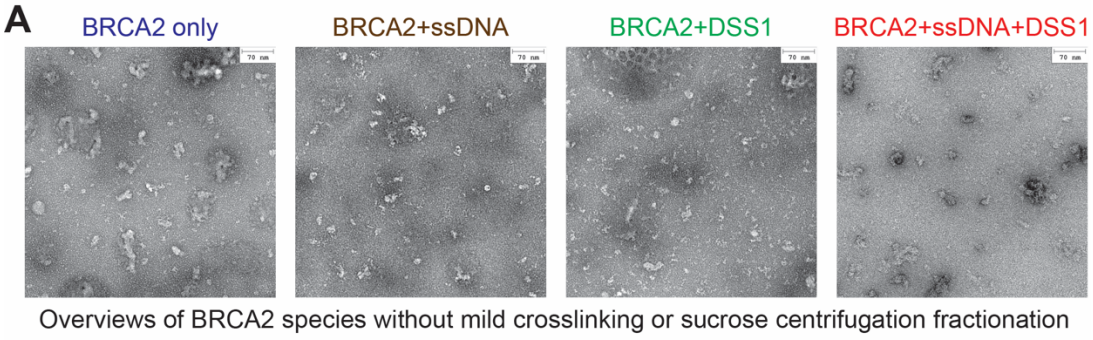
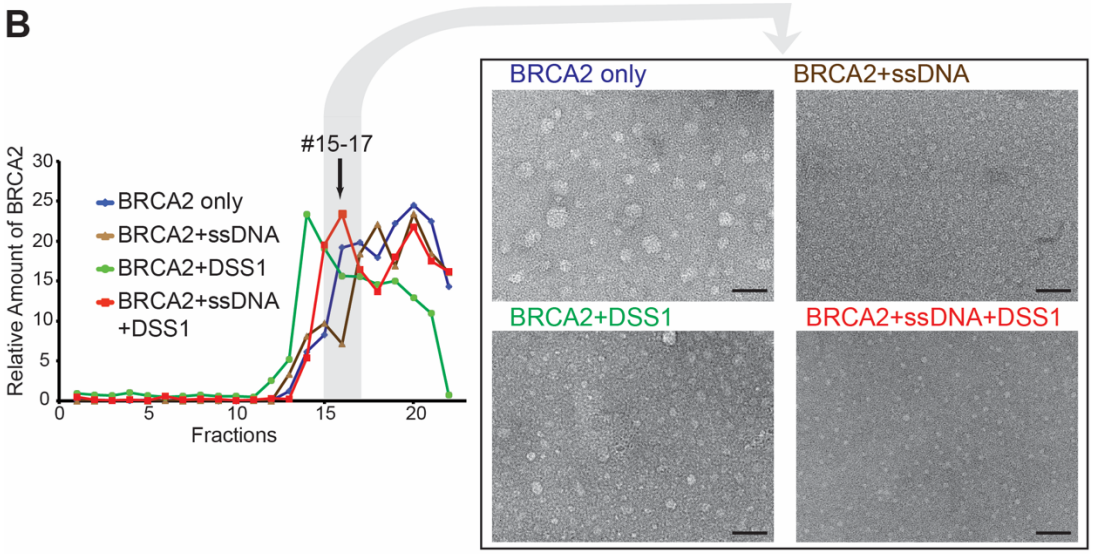
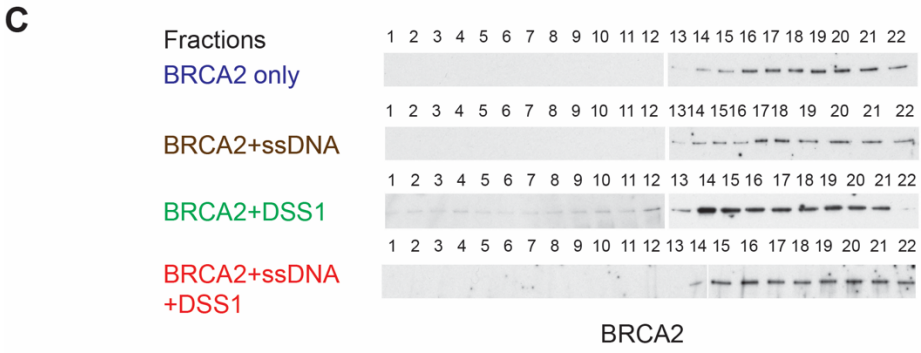
Table S1. Oligos and Peptides used in this study

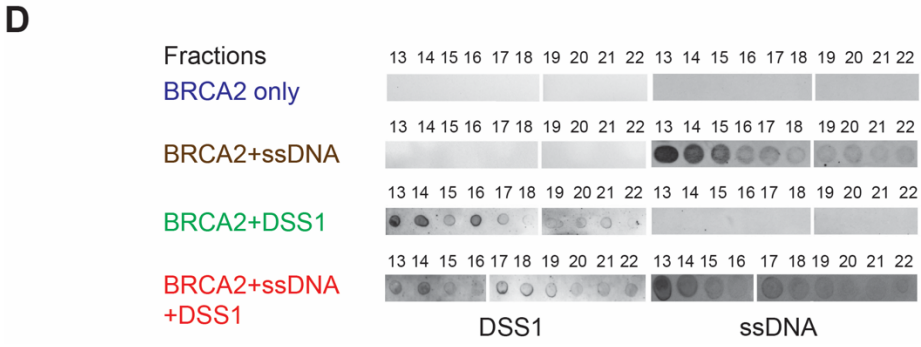
Name	Size	Sequence	Reference
Bio-100	100 mer	5'-biotinylated AACGACGTTTGGTCAGTTCCATCAACATCA TAGCCAGATGCCCAGAGATTAGAGCGCATG ACAAGTAAAGGACGGTTGTCAGCGTCATAA GAGGTTTTAC	This study
olWDH1728	20 mer	5'-AAGTAAATGAATAAAAAGCG-3'	This study
RJ-Oligo2	40 mer	5'-CTTTATTTTCTCTGTTTATTCATTTACTTA TTTTGTATTA-3'	(1)
olWDH1724	80 mer	5'-CTGCTTTATCAAGATAATTTTTCGACT CATCAGAAATATCCGTTTCCTATATTTATT CCTATTATGTTTTATTCATTTAC-3'	This study
olWDH1726	100 mer	5'-CTGCTTTATCAAGATAATTTTTCGACT CATCAGAAATATCCGTTTCCTATATTTATT CCTATTATGTTTTATTCATTTACTTATTCT TTATGTTCATTTT-3'	This study
RJ-167-mer	167 mer	5'-CTGCTTTATCAAGATAATTTTTCGACT CATCAGAAATATCCGTTTCCTATATTTATT CCTATTATGTTTTATTCATTTACTTATTCT TTATGTTCATTTTTTATATCCTTTACTTTA TTTTCTCTGTTTATTCATTTACTTATTTTG TATTATCCTTATCTTATTTA-3'	(1)
DSS1-N		MSEKKQPVDLGLLEEDDEFEEFPAEDWAG	This study
DSS1-C		LDEDEDAHVWEDNWDDDNVEDDFSNQLRA ELEKHGYKMETS	This study

Supplementary Figure 1



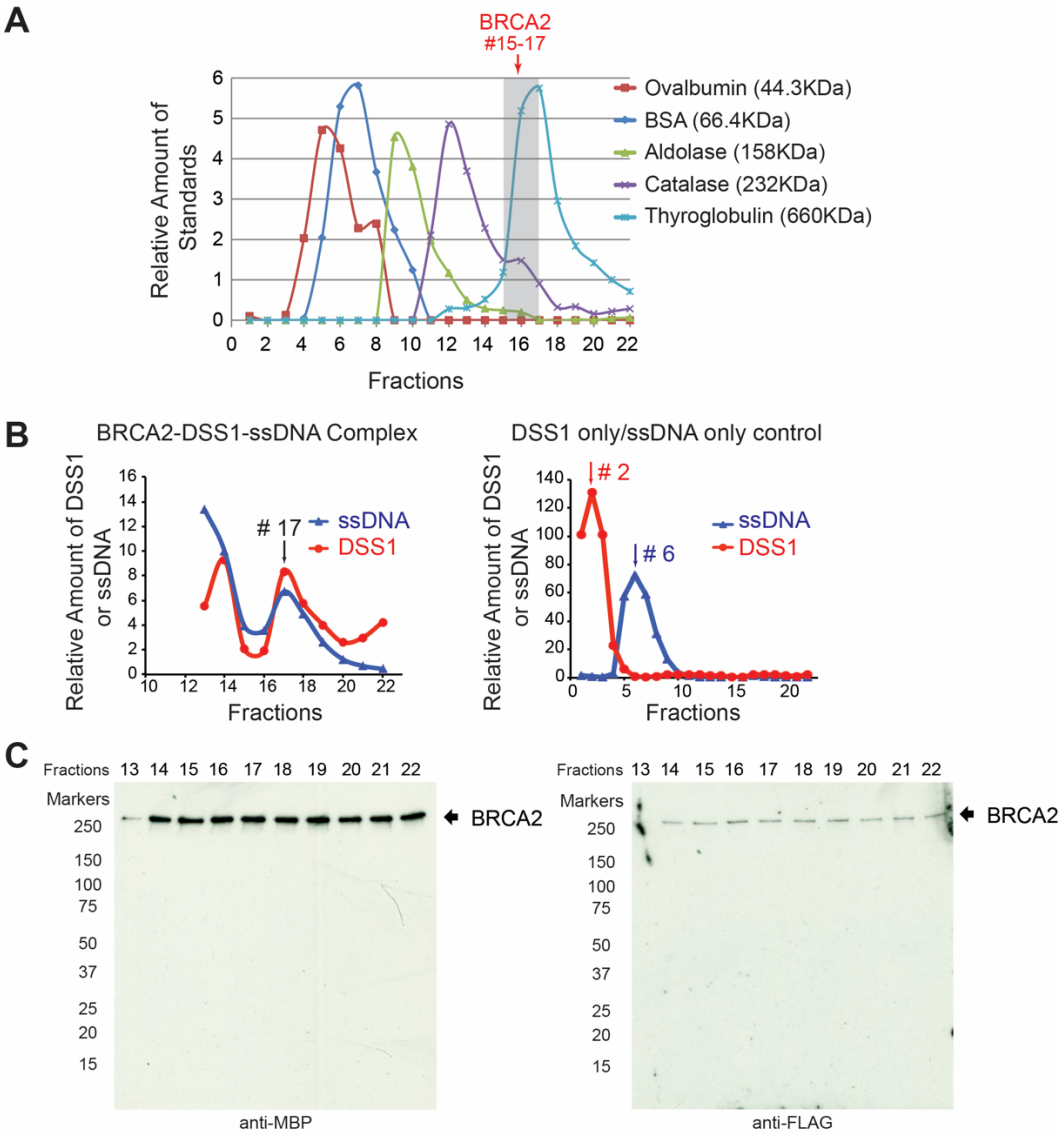






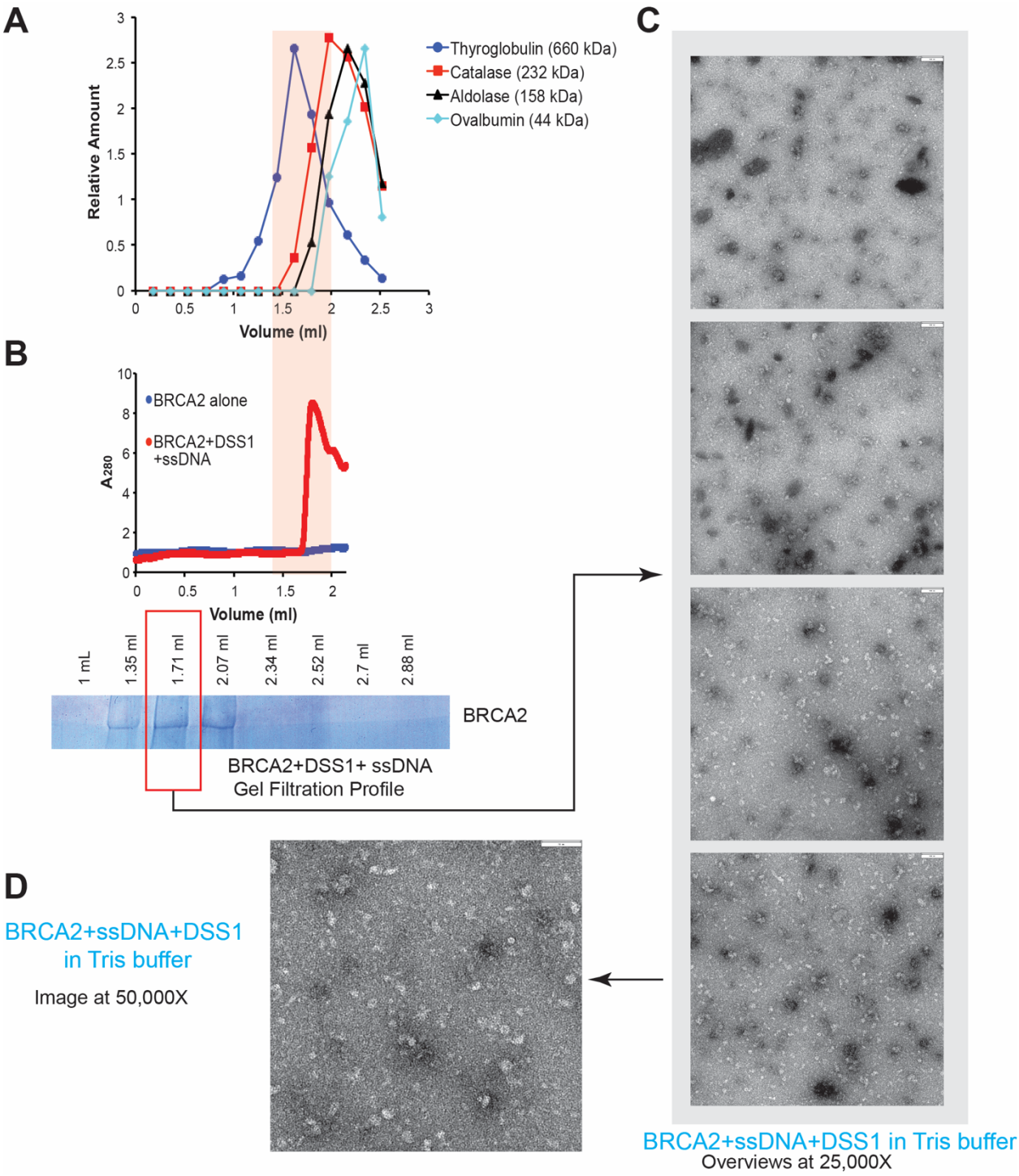
Supplementary Figure 1. DSS1 and ssDNA block BRCA2 multimerization. (**A**) BRCA2 aggregation is alleviated by DSS1 and ssDNA. BRCA2 or BRCA2 complexes were assembled at 37 ˚C degree and then deposited on carbon-coated grids for staining with 2 % uranyl acetate. Representative micrograph overviews of negatively stained BRCA2, BRCA2 + ssDNA, BRCA2 + DSS1, and BRCA2 + ssDNA + DSS1 are shown. Scale bar, 70 nm. (**B**) Sucrose gradient centrifugation profiles of BRCA2 in four conditions: BRCA2 (blue diamonds), BRCA2 + ssDNA (brown triangles), BRCA2 + DSS1 (green circles), and BRCA2 + ssDNA + DSS1 (red squares). Two overlapping peaks of BRCA2 signal were identified in each centrifugation curve, and quantitation was based on semi-quantitative immunoblots shown in **C**. After manual screening of every fraction, Fr. #15-17 of all four conditions were pooled and analyzed for simplicity of comparison. The material from #15-17 was negatively stained and examined with representative micrograph overviews shown on the right. Scale bar, 100 nm. (**C**) Immunoblots of BRCA2 in each fraction of the four different conditions from **B** with anti-BRCA2 (H-300) antibody (see **Fig. 1A**). Quantitation is shown in **B** (left). (**D**) Immunodotblots of DSS1 and biotinylated ssDNA for fractions 13-22 of the four different conditions in **B**. Left: DSS1 blots with anti-DSS1 antibody. Right: ssDNA blots recognized by HRP-conjugated streptavidin protein.

Supplementary Figure 2



Supplementary Figure 2. Presence of full-length BRCA2, DSS1, and ssDNA in the BRCA2-DSS1-ssDNA complex after sucrose gradient separation. (**A**) Sucrose gradient centrifugation profiles of protein standards. Five globular protein standards were centrifuged at identical conditions of BRCA2 to show the distribution of their sizes. Positions of fractions 15-17 of BRCA2 species were labeled for comparison purpose. The predicted molecular weight of dually tagged human BRCA2 is ~470 KDa. (**B**) Sucrose gradient centrifugation profiles of DSS1 (red curve) and ssDNA (blue curve) in BRCA2-DSS1-ssDNA complex (left) or alone (right). When in complex with BRCA2, both DSS1 and ssDNA show a second peak in fraction #17 (black arrow). For control, DSS1 alone peaks in fraction #2 (red arrow), and ssDNA alone peaks in #6 (blue arrow). (**C**) Full-length BRCA2 is verified through immunoblots. The nitrocellulose membrane was blotted first with α-MBP antibody for N-terminal tag recognition (Left), and then stripped before blotting with α-FLAG antibody for the C-terminal tag recognition (Right).

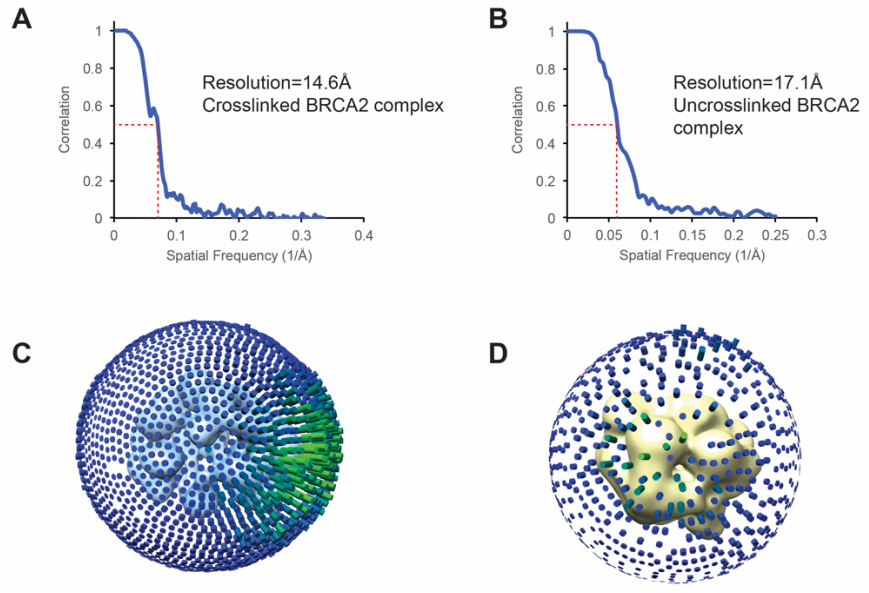
Supplementary Figure 3



Supplementary Figure 3. Large BRCA2 multimers are disrupted by DSS1 and ssDNA. (**A**) Gel filtration profiles of protein standards. Four globular protein standards were loaded on top of a superose 6 increase column under identical conditions of BRCA2 to show the distribution of their sizes. (**B**) Gel filtration profiles of BRCA2 alone (blue) or BRCA2-DSS1-ssDNA complex

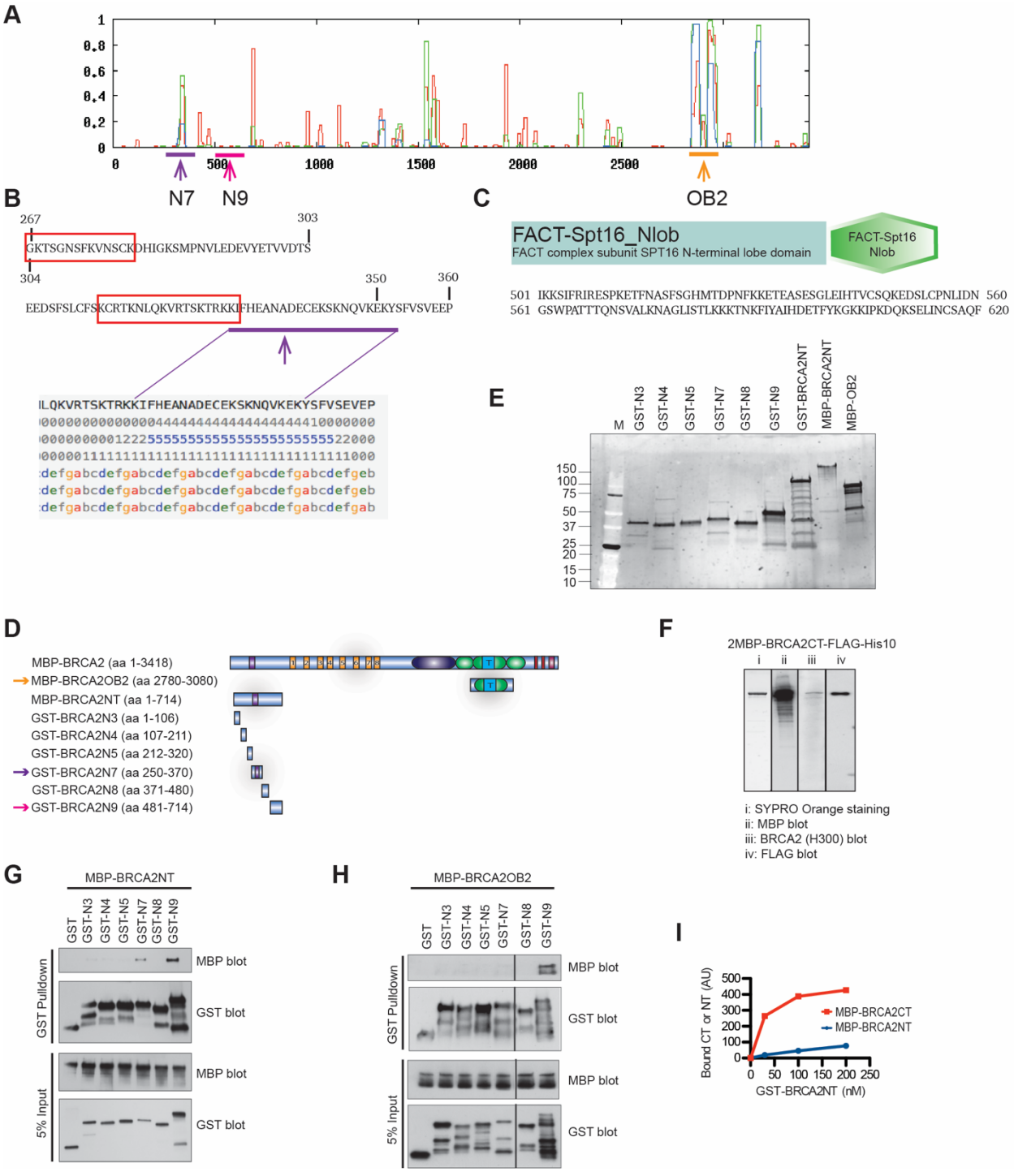
(red). Fractions were concentrated and analyzed by SDS-PAGE and Denville Blue staining, shown below for BRCA2-DSS1-ssDNA complex. The absorbance signal is very low for BRCA2 alone (**B**), and no BRCA2 band could be detected by SDS-PAGE and Denville Blue staining (data not shown), suggesting loss into large aggregates on the column. (**C**) Representative micrograph overviews of negatively stained BRCA2-DSS1-ssDNA from indicated peak fraction. A low magnification of 25,000X was used to assess particle distribution for large areas. Scale bar, 100 nm. (**D**) Representative micrograph of negatively stained BRCA2-DSS1-ssDNA from indicated peak fraction at a high magnification of 50,000X. Scale bar, 70 nm.

Supplementary Figure 4



Supplementary Figure 4. **3D reconstruction of crosslinked and uncrosslinked BRCA2-DSS1-ssDNA complexes.** (**A-B**) The Fourier shell correlation (FSC) plots of crosslinked (**A**) and uncrosslinked (**B**) complexes. The 0.5 FSC (red dashed lines) falls at a resolution of 14.6 Å (crosslinked) or 17.1 Å (uncrosslinked) for the 3D maps (blue solid line). (**C-D**) Angular distribution of the crosslinked (**C**) and uncrosslinked (**D**) BRCA2-DSS1-ssDNA complex with reconstructed volume shown inside. Almost all angles were covered with a slight preference for one orientation. The height of the bar is proportional to the number of images at that particular angle.

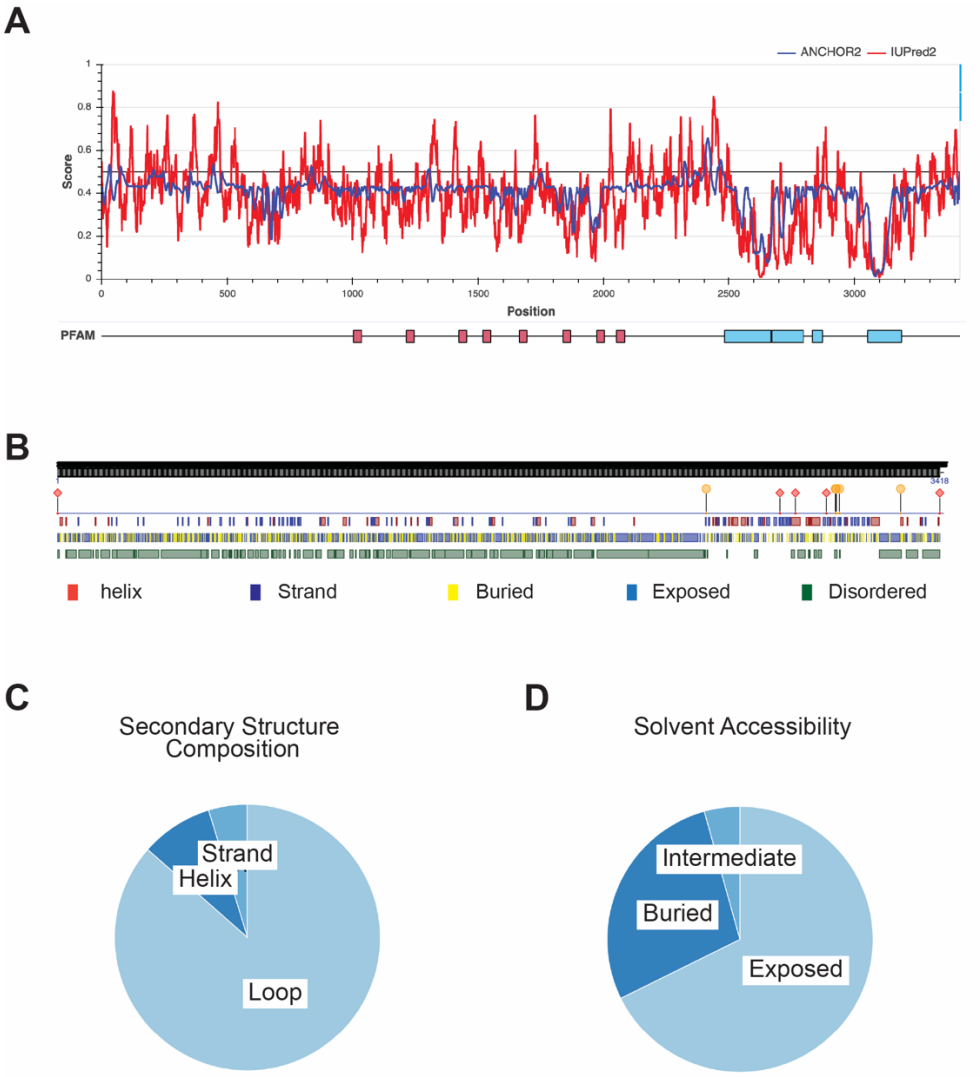
Supplementary Figure 5



Supplementary Figure 5. Three putative self-interaction regions (SIRs) in BRCA2. (A) Coils output of BRCA2 as predicted by the Lupas algorithm (https://npsa-prabi.ibcp.fr/cgi-bin/primanal_lupas.pl). The score between 0 and 1 for every residue corresponds to the probability of the given residue being part of a coiled-coil region in windows of 14 (green), 21

(blue) and 28 (red) residues. Putative SIRs were highlighted as N7 (purple), N9 (magneta) and OB2 fragment (orange). **(B)** Overlap between the N-terminal DNA-binding site (red boxes) (2) and a putative coiled-coil region (N7 fragment) (purple underline) in BRCA2 N-terminus. **(C)** Structural prediction by SMART showed a region from aa 501 to aa 620 (N9 fragment) shared a structural similarity with human histone chaperone FACT-Spt16 N-terminal lobe (Nlob) domain, which is responsible for protein-protein interaction (3) **(D)** Scheme of BRCA2 truncation mutants for protein interaction pull-down assay. Three putative SIRs are highlighted with corresponding arrows. **(E)** Purified BRCA2 fragments visualized on a 4-20% gradient SDS-PAGE gel by SYPRO Orange staining. **(F)** Purified C-terminal fragment of BRCA2, MBP-BRCA2CT, visualized on a 4-20% gradient SDS-PAGE gel by SYPRO Orange staining (i), immunoblotting with α-MBP (ii), α-BRCA2 (iii), or α-FLAG (iv) antibodies. **(G-H)** Immunoblots of the pulldown assay between 500 nM short BRCA2 N-terminal truncations GST-BRCA2Nx (x=3, 4, 5, 7, 8, 9) and 800 nM full-length BRCA2 N-terminal fragment (MBP-BRCA2NT) (**G**) or 800 nM BRCA2 OB2 region (MBP-BRCA2OB2) (**H**). **(I)** Quantification of **Fig. 4D** and **4E**. The signals were quantified from the same blot with the same exposure time.

Supplementary Figure 6



Supplementary Figure 6. **BRCA2 is composed of a significant amount of intrinsically disordered regions.** (**A**) Prediction of intrinsically unstructured regions of BRCA2 by IUPred2A. The score between 0 and 1 for every residue corresponds to the probability of the given residue being part of a disordered region. (**B**) Prediction of secondary structures, solvent accessibility, and disordered regions by PredictProtein. (**C-D**) Prediction of compositions of secondary structures (**C**) and solvent accessibility (**D**) by PredictProtein.

References:

1. Jensen, R.B., Carreira, A. and Kowalczykowski, S.C. (2010) Purified human BRCA2 stimulates RAD51-mediated recombination. *Nature*, **467**, 678-683.
2. von Nicolai, C., Ehlen, A., Martin, C., Zhang, X. and Carreira, A. (2016) A second DNA binding site in human BRCA2 promotes homologous recombination. *Nat Commun*, **7**, 12813.
3. Winkler, D.D. and Luger, K. (2011) The histone chaperone FACT: structural insights and mechanisms for nucleosome reorganization. *J Biol Chem*, **286**, 18369-18374.