Expanded View Figures

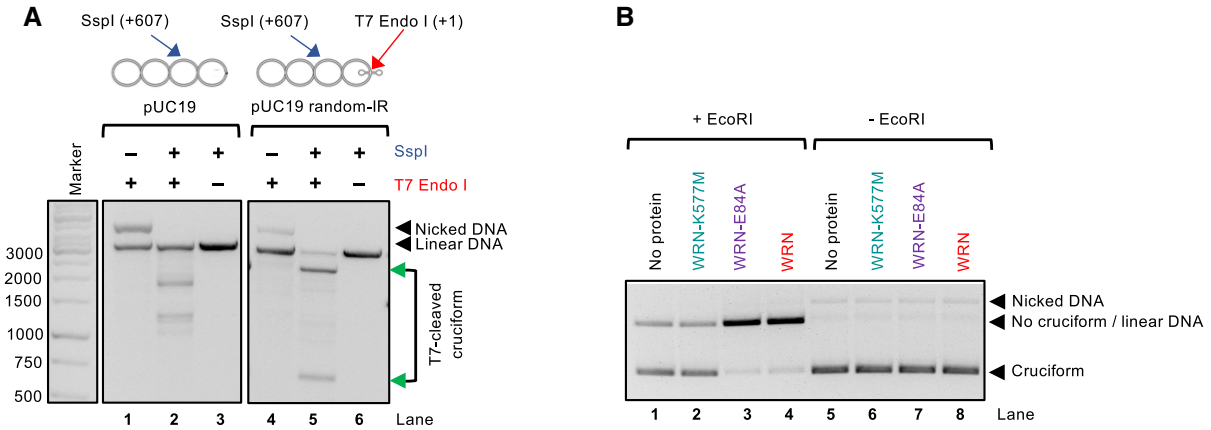

Figure EV1. WRN helicase unfolds cruciform DNA structures.

A Representative assay showing the activity of T7 Endonuclease I, which cleaves the 4-way junction formed at the cruciform DNA site. Incubation with T7 Endonuclease I and SspI results in the production of bands at the expected positions of 607 and 2,119 bp, indicated by the green arrows. The SspI restriction site is 607 bp away from the cruciform site. A plasmid without the inverted repeats is cleaved less efficiently and produces bands of different sizes (lane 2), resulting from T7 Endonuclease I activity at other sites that spontaneously extrude in pUC19 DNA. The DNA molecules were resolved by standard electrophoresis on a 1% native agarose gel, stained with GelRed.

B Representative cruciform unfolding assay with WRN, WRN-K577M, and WRN-E84A (all used at 10 nM). The assay was incubated either with or without EcoRI and the DNA species were separated on a 1% native agarose gel, stained with GelRed.

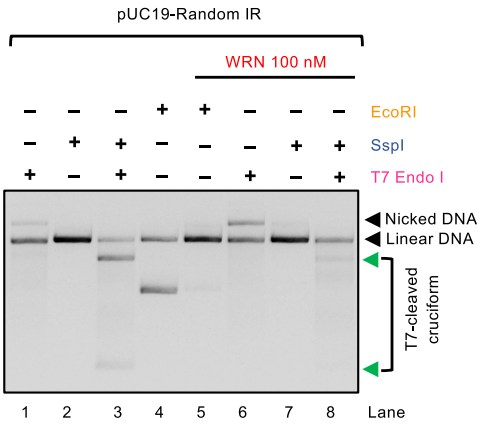

Figure EV2. WRN helicase prevents cruciform DNA cleavage by T7 Endonuclease I.

Representative assays detecting cruciform DNA (random-IR) by T7 Endonuclease I, pre-incubated or not with WRN. Preincubation with WRN reduces the intensity of bands resulting by cleavage with T7 Endonuclease I (607 and 2,119 bp, indicated by the green arrows), resulting in linearization by SspI. The DNA molecules were resolved by standard electrophoresis on a 1% native agarose gel, stained with GelRed.

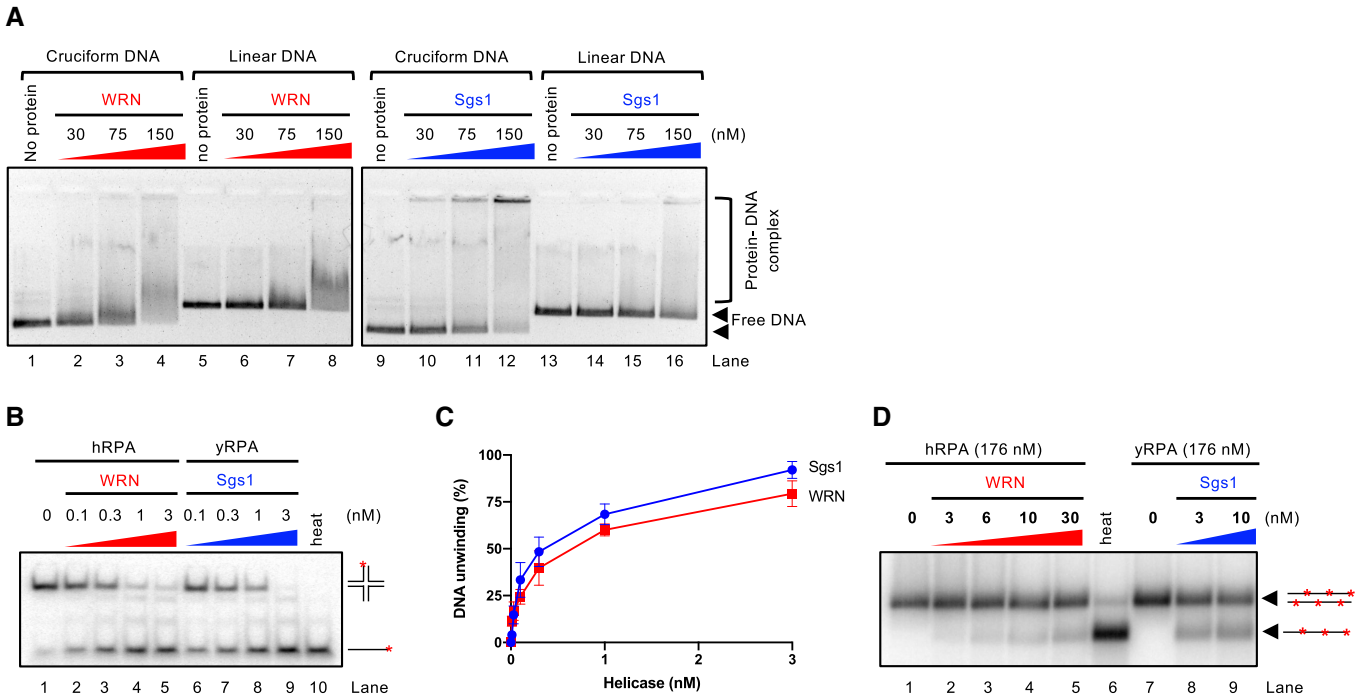

Figure EV3. Comparison of DNA binding and unwinding by WRN and Sgs1 DNA helicases.

A Representative electrophoretic mobility shift assay with WRN and Sgs1, using either pUC19 harboring the random inverted repeat-based cruciform structure (circular), or linearized pUC19. The samples were run on a 0.8% unstained native agarose gel. The gel was stained after the run with GelRed.

B Representative helicase assay with increasing concentrations of WRN and Sgs1, using an oligonucleotide-based Holliday junction as a substrate. Reactions were supplemented with either human or yeast RPA (15 nM) and analyzed by 10% native acrylamide gel electrophoresis. Red asterisk indicates the position of the radioactive label.

C Quantitation of DNA unwinding from assays such as in (B). Averages shown; $n \geq 3$; error bars, SEM.

D Representative helicase assay with increasing concentrations of WRN and Sgs1, using a 2.2 kbp-long dsDNA substrate. Reactions were supplemented with either human or yeast RPA and analyzed by electrophoresis on a 1% agarose gel.

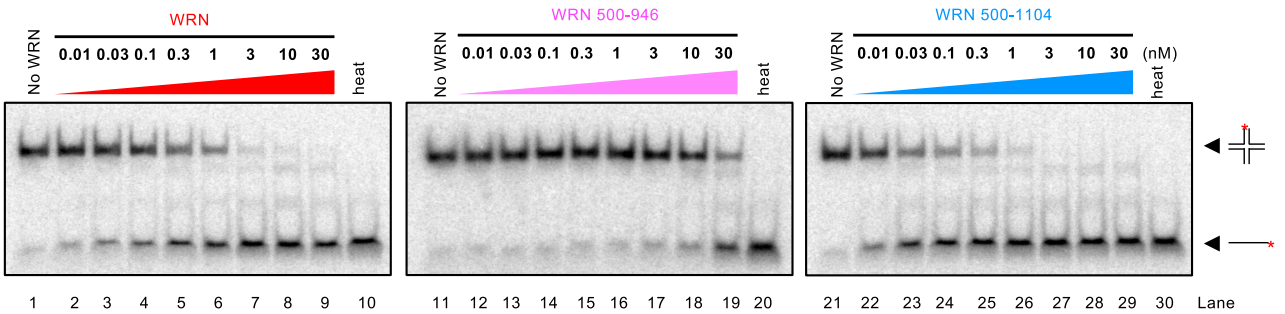

Figure EV4. DNA unwinding by WRN helicase variants.

Representative helicase assay with increasing concentrations of WRN variants, using an oligonucleotide-based Holliday junction as a substrate. Reactions were supplemented with human RPA (15 nM) and analyzed by 10% native acrylamide gel electrophoresis. Red asterisks indicate the position of the radioactive label.

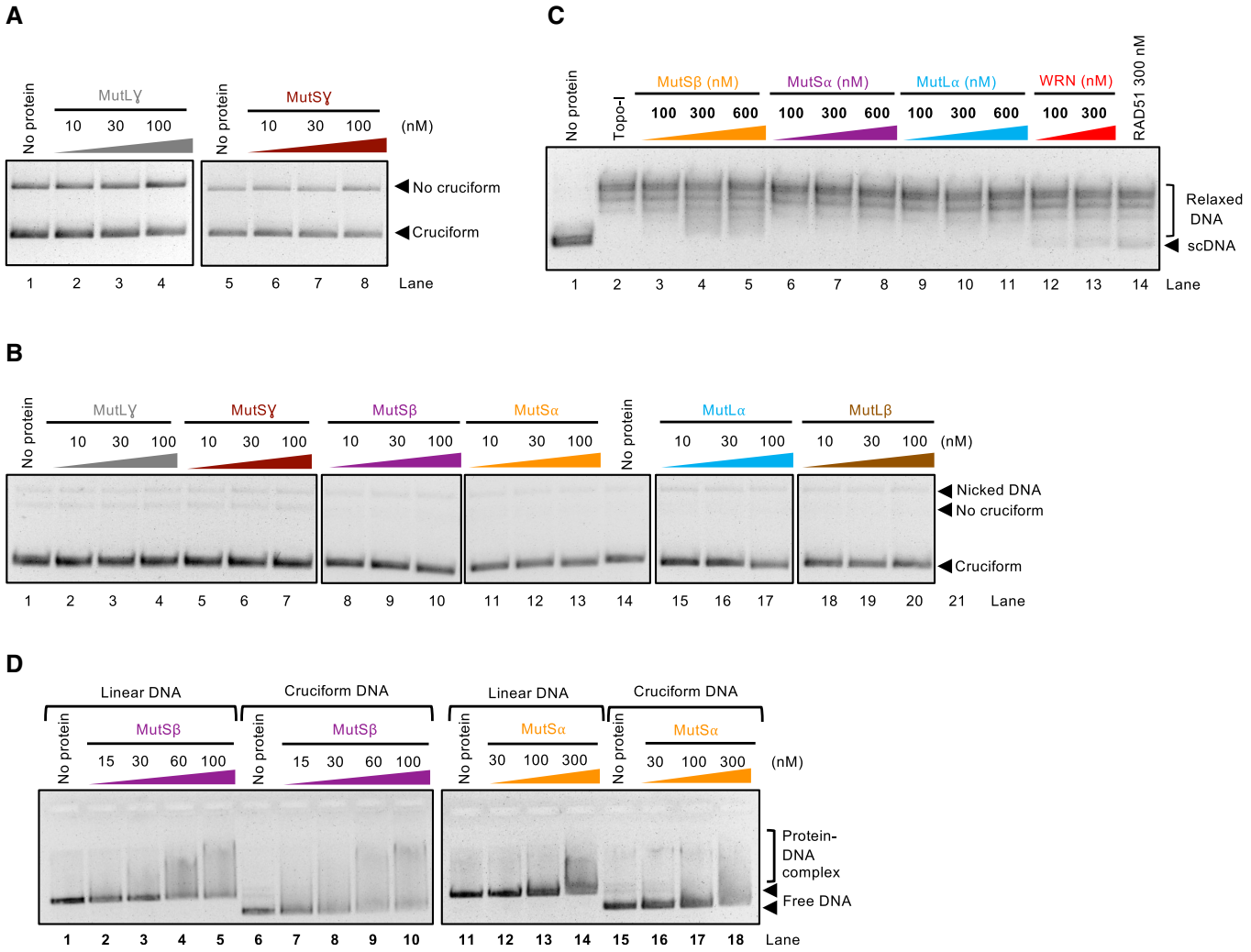

Figure EV5. Cruciform DNA binding by mismatch repair complexes.

A Representative cruciform unfolding assay with MutLγ and MutSγ, using the random-IR cruciform as a substrate.

B Representative cruciform unfolding assay with the MutL and MutS homolog heterodimers, carried out in the absence of EcoRI. The reactions indicate that the proteins do not cut DNA under the assay conditions.

C Representative Topoisomerase-I-coupled supercoiling assay with MutSβ, MutSα, MutLα, WRN, and RAD51. The reactions were analyzed by standard electrophoresis on a 1% native agarose gel. The gel was stained with GelRed after electrophoresis.

D Representative electrophoretic mobility shift assay with MutSβ and MutSα, using either circular pUC19 with the random inverted repeat cruciform structure, or linear pUC19 as a substrate. 0.8% unstained native agarose gel was used to separate the protein and DNA species.