Extended data

Mechanism of BRCA1-BARD1 function in DNA end resection and DNA protection

Ilaria Ceppi, Maria Rosaria Dello Stritto, Stefan Braunshier, Martin Mütze, Giordano Reginato, Aurore Sanchez, Swagata Halder, Sean Michael Howard, Valentina Mengoli, Ralf Seidel and Petr Cejka

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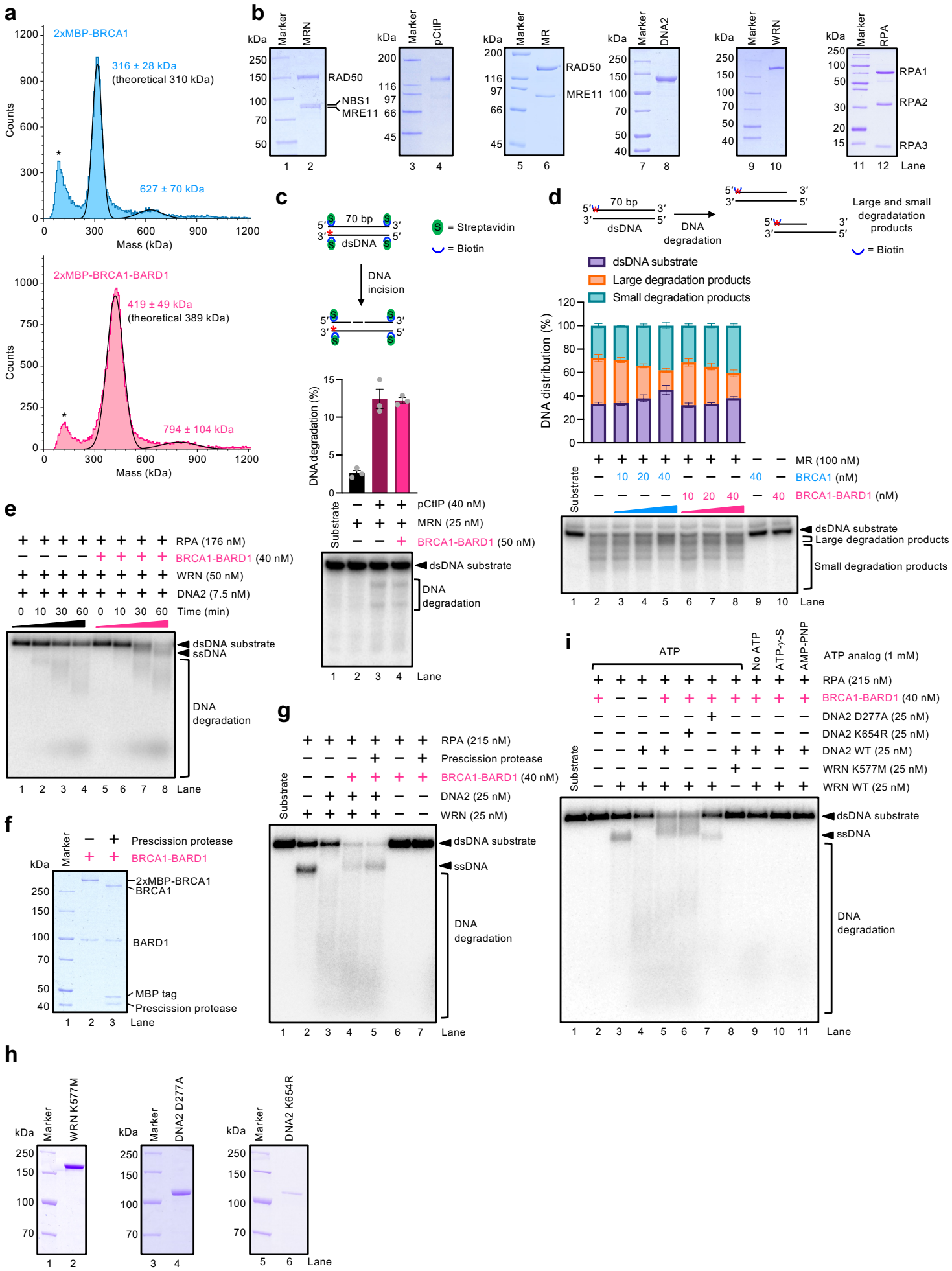

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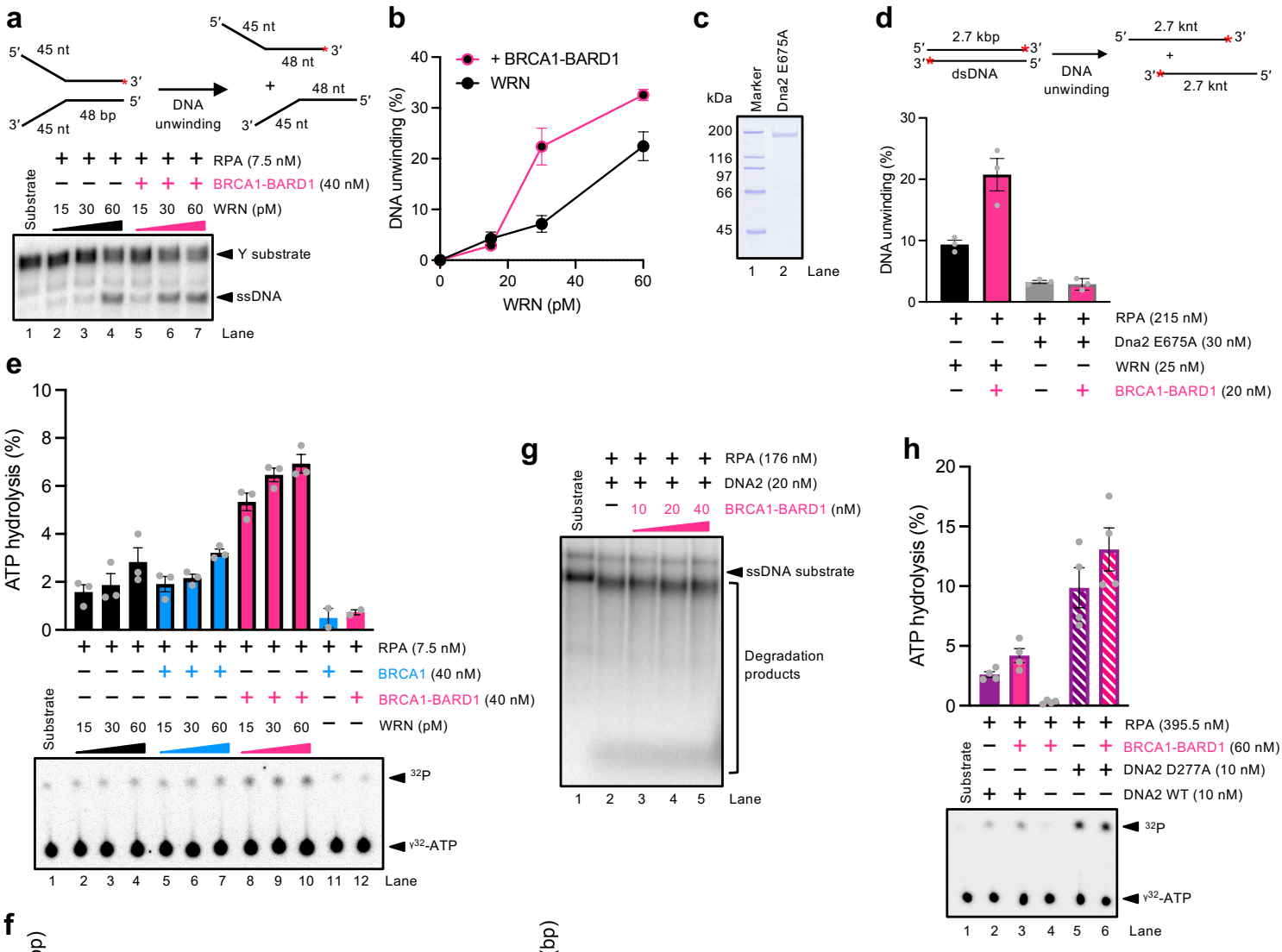
Extended Data Figure 1. BRCA1-BARD1 stimulates long-range resection by WRN-DNA2-RPA. **a**, Molecular weight distributions of 2xMBP-tagged BRCA1 alone or in complex with BARD1 measured by mass photometry. Black asterisks (*) indicate background noise. **b**, Polyacrylamide gels of the indicated recombinant proteins stained with Coomassie Brilliant Blue. **c**, DNA endonuclease assays on a 3'-labeled 70 bp-long dsDNA with all ends blocked with streptavidin with MRN-pCtIP, in the absence or presence of BRCA1-BARD1. Top, a schematic of the assay. Red asterisk (*) represents the position of the radioactive label. Middle, quantitation of the DNA degradation products. Averages shown; error bars, SEM; n=3. Bottom, representative resection assays after denaturing gel electrophoresis. **d**, DNA exonuclease assays on a 5'-labeled 70 bp-long dsDNA with MR, in the absence or presence of either BRCA1 or BRCA1-BARD1. Top, a schematic of the assay. Red asterisk (*) represents the position of the radioactive label. Middle, quantitation of the different reaction intermediates. Averages shown; error bars, SEM; n=3. Bottom, representative resection assays after denaturing gel electrophoresis. **e**, Representative kinetic resection assays on a randomly labeled 2.2 kbp-long dsDNA with WRN-DNA2-RPA, in the absence or presence of BRCA1-BARD1. **f**, 2xMBP-BRCA1-BARD1 either mock-treated or treated with Prescission protease (1:5 ratio, w:w) for 1 h at 4°C. The polyacrylamide gel was stained with Coomassie Brilliant Blue. **g**, Representative resection assays on a 3'-labeled 2.7 kbp-long dsDNA with WRN-DNA2-RPA, in the absence or presence of BRCA1-BARD1, either mock- or Prescission protease-treated. **h**, Polyacrylamide gels of the indicated recombinant WRN and DNA2 variants stained with Coomassie Brilliant Blue. **i**, Representative resection assays on a 3'-labeled 2.7 kbp-long dsDNA with wild type WRN or helicase-dead WRN K577M, wild type DNA2, helicase-dead DNA2 K654R or nuclease-dead DNA2 D277A, RPA, in the absence or presence of BRCA1-BARD1. No ATP or non-hydrolysable ATP analogs (ATP-γ-S or AMP-PNP) were used as indicated.



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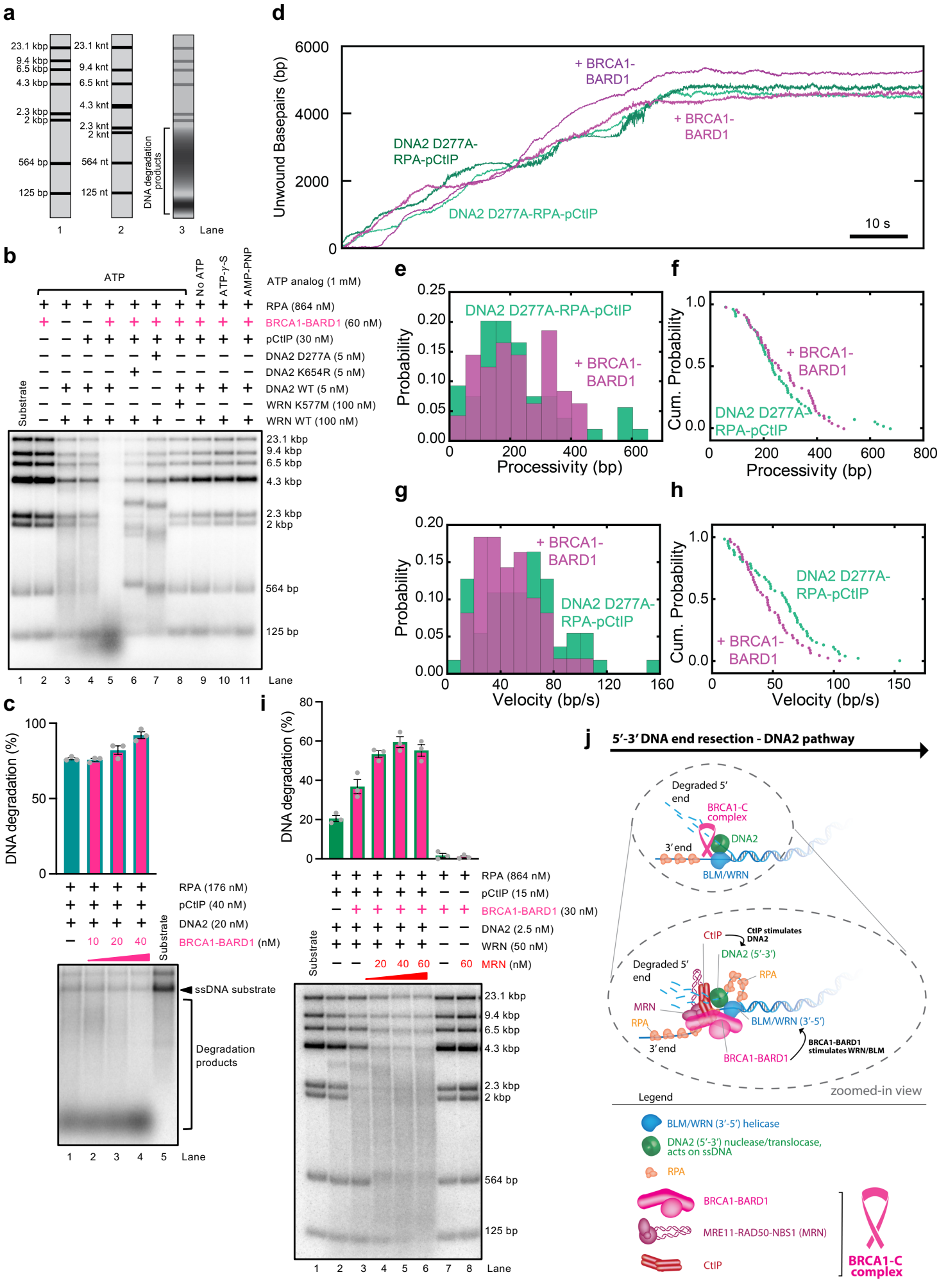
Extended Data Figure 2. BRCA1-BARD1 stimulates WRN helicase, but not DNA2 nuclease. a, Unwinding assays using a 3'-labeled Y-structured oligonucleotide-based DNA by WRN-RPA, in the absence or presence of BRCA1-BARD1. Top, a schematic of the assay. Red asterisk (*) represents the position of the radioactive label. Bottom, representative unwinding assays. **b**, Quantitation of DNA unwinding assays such as shown in a. Averages shown; error bars, SEM; n=3. **c**, Recombinant nuclease-dead yeast Dna2 E675A used in this study. The polyacrylamide gel was stained with Coomassie Brilliant Blue. **d**, Unwinding assays using a 3'-labeled 2.7 kbp-long dsDNA with WRN-RPA or nuclease-dead yeast Dna2 E675A and RPA, in the absence or presence of BRCA1-BARD1. Averages shown; error bars, SEM; n=3. **e**, ATPase assays with a 93 nt-long ssDNA, WRN and RPA, in the absence or presence of either BRCA1 or BRCA1-BARD1. Top, quantitation of ATP hydrolysis. Averages shown; bars, SEM; n=2 for BRCA1 and BRCA1-BARD1 alone controls and n=3 for all the other samples. Bottom, representative ATPase assays. **f**, Representative trajectories from magnetic tweezer experiments showing no apparent DNA unwinding with the reaction buffer (top) or BRCA1-BARD1 (40 nM) and RPA (25 nM) alone (bottom). **g**, Representative nuclease assays with a randomly labeled 2.2 knt-long ssDNA and DNA2-RPA, in the absence or presence of BRCA1-BARD1. **h**, ATPase assays in the presence of 10.3 knt-long ssDNA with wild type DNA2 or nuclease-dead DNA2 D277A and RPA, in the absence or presence of BRCA1-BARD1. Top, quantitation of ATP hydrolysis. Averages shown; error bars, SEM; n=4. Bottom, representative ATPase assays.



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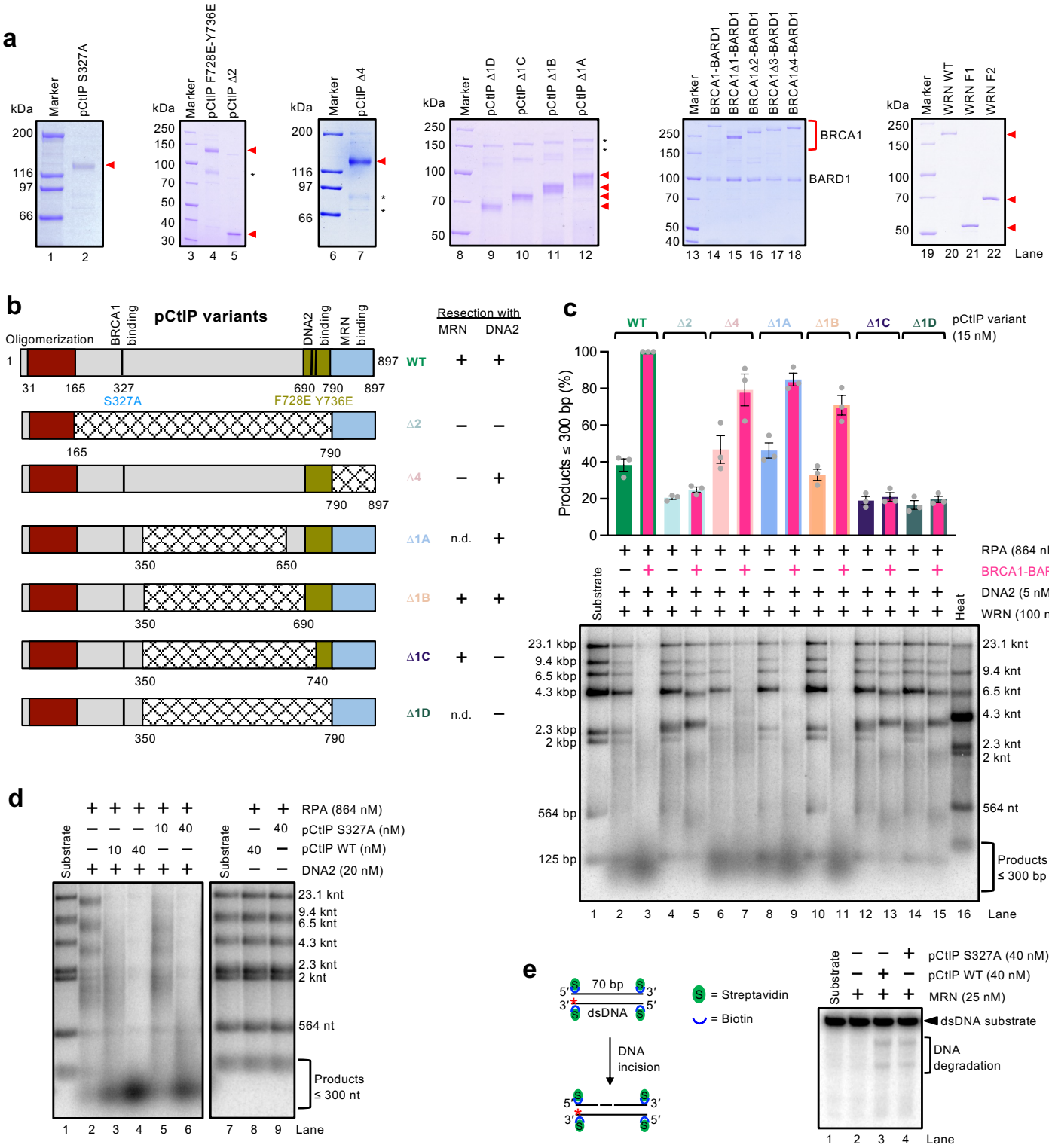
Extended Data Figure 3. BRCA1-BARD1 and MRN stimulate ATP hydrolysis-dependent resection by the WRN-DNA2-CtIP-RPA ensemble. a, A schematic of the λ DNA/HindIII-based substrate employed for the DNA end resection assays. Shown is the distribution of the DNA fragments in their double-stranded (left) or single-stranded (middle) forms. The right lane shows an example of a pattern upon partial DNA degradation. **b**, Representative resection assays with 3'-labeled λ DNA/HindIII-based dsDNA with wild type WRN or helicase-dead WRN K577M, wild type DNA2, or helicase-dead DNA2 K654R or nuclease-dead DNA2 D277A, pCtIP and RPA, in the absence or presence of BRCA1-BARD1. No ATP or non-hydrolysable ATP analogs (ATP-γ-S or AMP-PNP) were used where indicated to determine the ATP hydrolysis dependency of the reaction. **c**, Nuclease assays with randomly labeled 2.2 knt-long ssDNA and DNA2-pCtIP-RPA, in the absence or presence of BRCA1-BARD1. Top, quantitation of the DNA degradation products. Averages shown; error bars, SEM; n=3. Bottom, representative nuclease assays. **d**, Representative trajectories of DNA unwinding events by nuclease-dead DNA2 D277A (10 nM) with pCtIP (10 nM) and RPA (25 nM), in the absence (teal) or presence (pink) of BRCA1-BARD1 (25 nM). **e**, **f**, Processivity histogram and cumulative probability distribution (shown as survival probability) of the observed DNA unwinding events by nuclease-dead DNA2 D277A-pCtIP-RPA, in the absence (teal) or presence (pink) of BRCA1-BARD1, with mean values of 264 ± 38 bp (n=55) and 271 ± 32 bp (n=49), respectively. Error, 2SEM. **g**, **h**, Velocity histogram and cumulative probability distribution (shown as survival probability) of the observed DNA unwinding events by nuclease-dead DNA2 D277A-pCtIP-RPA, in the absence (teal) or presence (pink) of BRCA1-BARD1, with mean values of 57 ± 8 bp/s (n=55) and 46 ± 6 bp/s (n=49), respectively. Error, 2SEM. **i**, Resection assays with a 3'-labeled λ DNA/HindIII-based dsDNA, WRN-DNA2-pCtIP-RPA, BRCA1-BARD1, and the indicated concentration of MRN. Top, quantitation of DNA degradation, based on the disappearance of DNA fragments of ≥4.3 kbp in length. Averages shown; error bars, SEM; n=3. Bottom, representative resection assays. **j**, A model of DNA2-dependent DNA end resection pathway and the involvement of BRCA1-BARD1 within the BRCA1-C complex.



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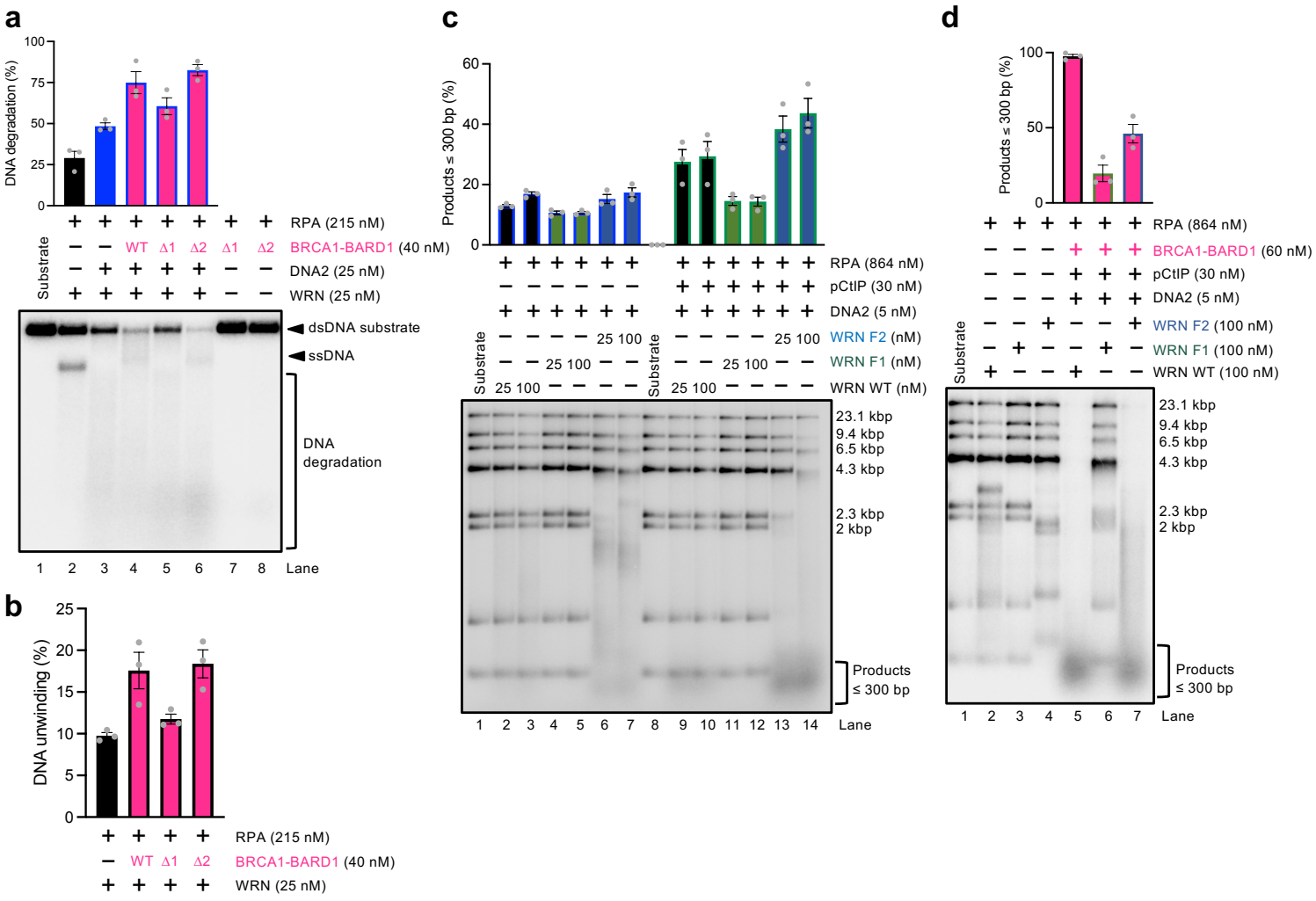
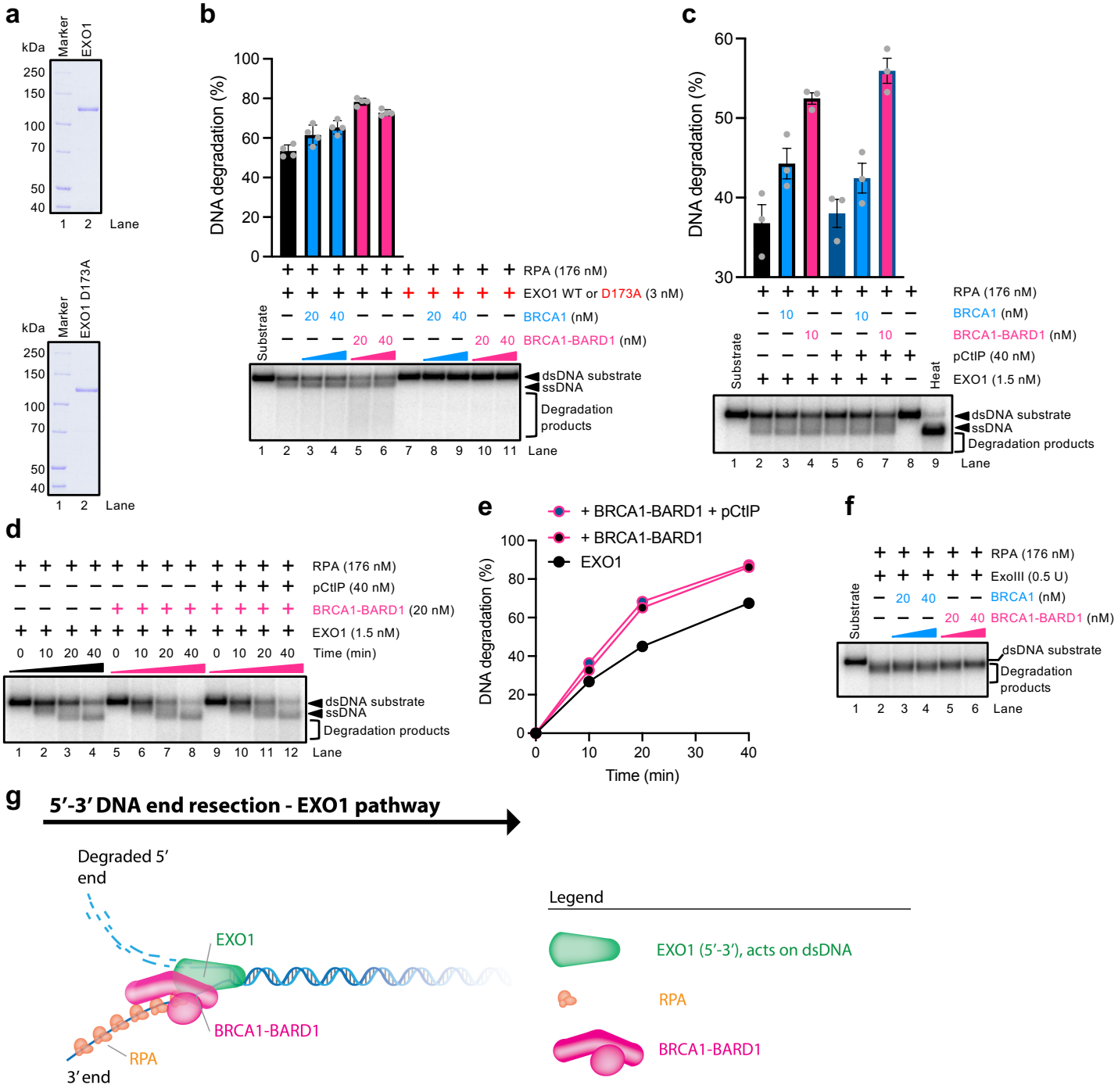
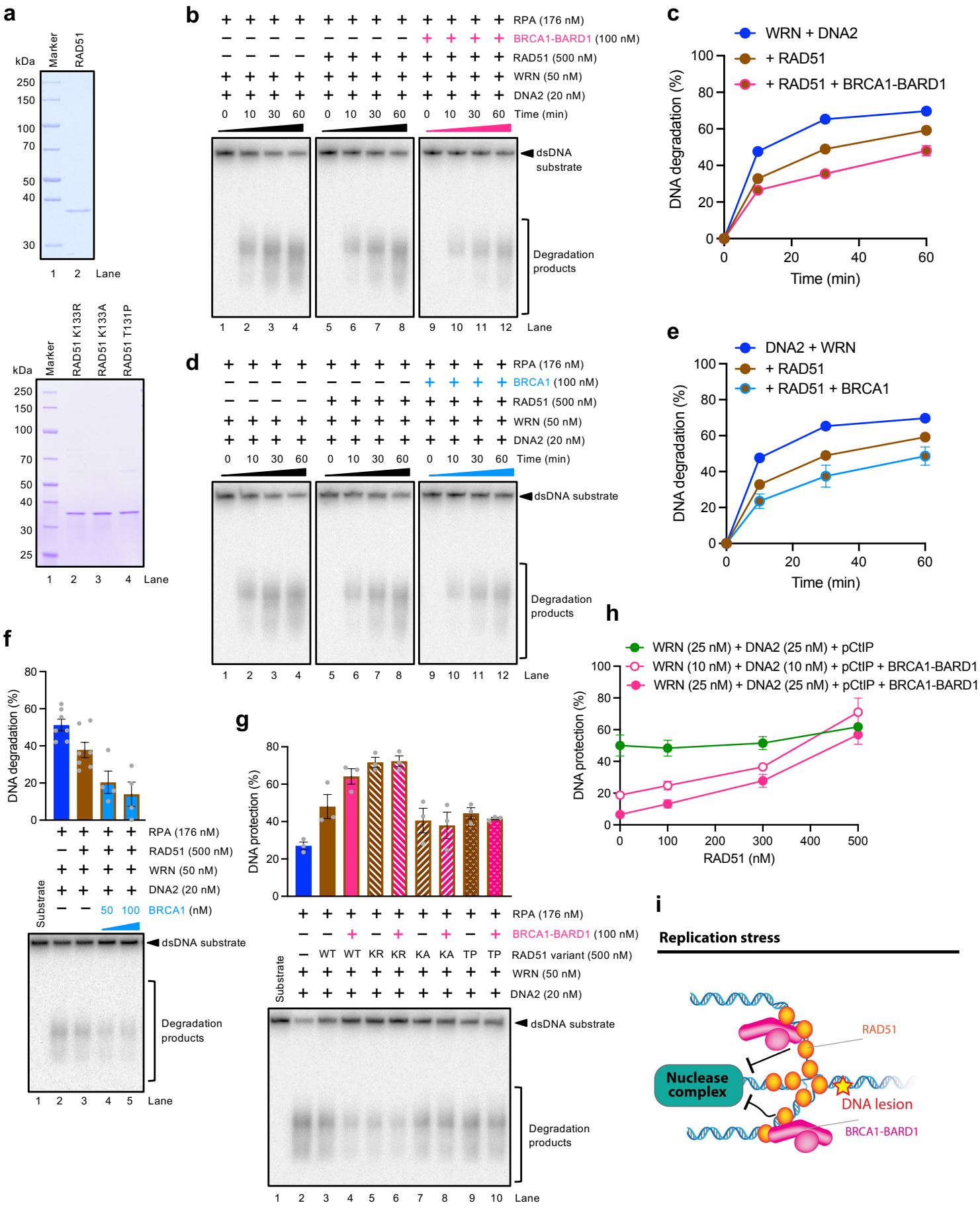
Extended Data Figure 4. CtIP interaction with BRCA1 and DNA2 is required for its function in the long-range DNA end resection ensemble. a, Polyacrylamide gels of the indicated recombinant proteins stained with Coomassie Brilliant Blue (relevant proteins indicated with red arrows). Black asterisks (*) represent truncations of the indicated proteins (lanes 5, 7), or proteins with uncleaved MBP-tag (lanes 9-12). **b**, Cartoon of the primary structure of the phosphorylated CtIP (pCtIP) variants used in this study. Deleted portions of the protein are indicated with diamond greed pattern. The capacity of each variant to stimulate MRN and/or DNA2-mediated resection, as reported from previous studies, is indicated on the right. n.d., not determined. **c**, Resection assays with a 3'-labeled λ DNA/HindIII-based dsDNA and WRN-DNA2-RPA, in the absence or presence of BRCA1-BARD1 and the indicated pCtIP variant (see panel b). Top, quantitation of DNA degradation products of ≤300 bp in length. Averages shown; error bars, SEM; n=3. Bottom, representative resection assays. **d**, Representative resection assays showing the degradation of a 3'-labeled λ DNA/HindIII-based ssDNA by DNA2-RPA, in the presence of either wild type pCtIP or pCtIP S327A variant. Quantitation of the data is shown in Fig. 4c. **e**, Representative endonuclease assays using a 5'-labeled 70 bp-long dsDNA with all ends blocked with streptavidin with MRN, in the presence of either wild type pCtIP or pCtIP S327A variants. Left, a schematic of the assay. Red asterisk (*) represents the position of the radioactive label. Right, representative endonuclease assays.



Extended Data Figure 5. Mapping the BRCA1 and WRN domains required for their interplay during DNA end resection. **a**, Resection assays with a 3'-labeled 2.7 kbp-long dsDNA and WRN-DNA2-RPA, in the absence or presence of the indicated BRCA1-BARD1 variant (see Fig. 4e). Top, quantitation of the DNA degradation. Averages shown; error bars, SEM; n=3. Bottom, representative resection assays. **b**, Quantitation of unwinding of a 3'-labeled 2.7 kbp-long dsDNA by WRN-RPA, in the absence or presence of the indicated BRCA1-BARD1 variant (see Fig. 4e). Averages shown; error bars, SEM; n=3. **c**, Resection assays with a 3'-labeled λ DNA/HindIII-based dsDNA with the indicated WRN variant (see Fig. 4f), DNA2, RPA, in the absence or presence of pCtIP. Top, quantitation of DNA degradation products of ≤300 bp in length. Averages shown; error bars, SEM; n=3. The data points with 100 nM of each WRN variant are again shown as in Fig. 4f as a reference. Bottom, representative resection assays. Reactions were incubated for 2 h at 37°C. **d**, Resection assays with a 3'-labeled λ DNA/HindIII-based dsDNA and the indicated WRN variant (see Fig. 4f), DNA2-pCtIP-RPA, in the absence or presence of BRCA1-BARD1. Top, quantitation of DNA degradation products of ≤300 bp in length. Averages shown; error bars, SEM; n=3. The data points with 100 nM of each WRN variant are the same as shown in Fig. 4f as a reference. Bottom, representative resection assays.



Extended Data Figure 6. Stimulation of EXO1-dependent long-range DNA end resection by BRCA1-BARD1. **a**, Polyacrylamide gels of the indicated recombinant EXO1 variants stained with Coomassie Brilliant Blue. **b**, Resection assays using a randomly labeled 2.2 kbp-long dsDNA with the indicated EXO1 variant and RPA, in the absence or presence of either BRCA1 or BRCA1-BARD1. Top, quantitation of the DNA degradation products. Averages shown; error bars, SEM; n=4. Bottom, representative resection assays. **c**, Resection assays with a randomly labeled 2.2 kbp-long dsDNA, EXO1 and RPA, in the absence or presence of either BRCA1 or BRCA1-BARD1 and pCtIP. Top, quantitation of the DNA degradation products. Averages shown; error bars, SEM; n=3. Bottom, representative resection assays. **d**, Representative kinetic resection assays with a randomly labeled 2.2 kbp-long dsDNA, EXO1 and RPA, in the absence or presence of BRCA1-BARD1 and pCtIP. **e**, Quantitation of DNA resection assays such as shown in d. Averages shown; error bars, SEM; n=3. **f**, Representative resection assays with a randomly labeled 2.2 kbp-long dsDNA, *E. coli* ExoIII and RPA, in the absence or presence of either BRCA1 or BRCA1-BARD1. **g**, A model of EXO1-dependent DNA end resection pathway and the involvement of BRCA1-BARD1.



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Extended Data Figure 7. DNA protection by RAD51-BRCA1-BARD1 ensemble. a, Polyacrylamide gels of the indicated recombinant RAD51 variants stained with Coomassie Brilliant Blue. **b**, Representative kinetic protection assays using a randomly labeled 2.2 kbp-long dsDNA and WRN-DNA2-RPA, in the absence or presence of BRCA1-BARD1 and RAD51. **c**, Quantitation of assays such as shown in b. Averages shown; error bars, SEM; n=7 (in the absence of BRCA1-BARD1) and n=4 (in the presence of BRCA1-BARD1). **d**, Experiments as in b, but with BRCA1 instead of BRCA1-BARD1. **e**, Quantitation of assays such as shown in d. Averages shown; error bars, SEM; n=7 (in the absence of BRCA1) and n=4 (in the presence of BRCA1). The data points in the absence of BRCA1 are the same as shown in c. **f**, Protection assays using a randomly labeled 2.2 kbp-long dsDNA and WRN-DNA2-RPA, in the absence or presence of BRCA1 and RAD51. Top, quantitation of the DNA degradation products. Averages shown; error bars, SEM; n=7 (in the absence of BRCA1) and n=4 (in the presence of BRCA1). Bottom, representative protection assays. **g**, Protection assays using a randomly labeled 2.2 kbp-long dsDNA and WRN-DNA2-RPA, in the absence or presence of BRCA1-BARD1 and the indicated RAD51 variant. KR, K133R, defective in ATP hydrolysis; KA, K133A, defective in ATP binding; TP, T131P, impaired in DNA binding. Top, quantitation of DNA protection. Averages shown; error bars, SEM; n=3. Bottom, representative protection assays. **h**, Quantitation of assays such as shown in Fig. 6c. Averages shown; error bars, SEM; n=3. **i**, A model for DNA protection function of BRCA1-BARD1 in the presence of RAD51 upon replication stress.

Extended Data Tables

Extended Data Table 1. Full-length sequence of *BRCA1* gene (in black) codon optimized for the expression in *Sf*9 cells. NheI and XmaI sites at the N- and C-terminus of the *BRCA1* gene, respectively, are highlighted in bold. The 2x MBP-tag and the 10x his-tag at the N- and C-terminus, respectively, are underlined.

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TGGAGATCTGCTGCTACGGTCCTTTCACCAACATGCCTACCGACCAGCTGGAGTGGATGGTGCAGCTGTGCGGTGCTTC
CGTGGTGAAGGAGCTGTCCTCCTTCACCCTGGGTACCGGTGTGCACCCTATCGTGGTGGTGCAGCCTGACGCTTGGAC
CGAGGACAACGGTTTCCACGCTATCGGTCAGATGTGCGAGGCTCCTGTGGTGACCCGCGAGTGGGTGCTGGACTCCGT
GGCTCTGTACCAGTGCCAGGAGCTGGACACCTACCTGATCCCTCAGATCCCTCACTCCCACTAC**CCCGGG**CTCGAG<u>CAC
CATCACCATCACCATCACCATCACCATT</u>GA

Extended Data Table 2. Full-length sequence of the *BARD1* gene codon optimized for the expression in *Sf*9 cells. BamHI and XmaI sites at the N- and C-terminus of the *BARD1* gene, respectively, are highlighted in bold. The FLAG-tag at the C-terminus is underlined.

GGATCCACCATGCCTGACAACCGCCAGCCTCGCAACCGCCAGCCTCGCATCCGCTCCGGTAACGAGCCTCGCTCCGCTC
CTGCTATGGAGCCTGACGGTCGCGGTGCTTGGGCTCACTCCCGCGCTGCTCTGGACCGCCTGGAGAAGCTGCTGCGCT
GCTCCCGCTGCACCAACATCCTGCGCGAGCCTGTGTGCCTGGGTGGTTGCGAGCACATCTTCTGCTCCAACTGCGTGTC
CGACTGCATCGGTACCGGTTGCCCTGTGTGCTACACCCCTGCTTGGATTCAAGACCTGAAGATCAACCGCCAGCTGGAC
TCCATGATCCAGCTGTGCTCCAAGCTGCGCAACCTGCTGCACGACAACGAGCTGTCCGACCTGAAGGAGGACAAGCCT
CGCAAGTCCCTGTTCAACGACGCTGGTAACAAGAAGAACTCCATCAAGATGTGGTTCTCCCCTCGCTCCAAGAAGGTGC
GCTACGTGGTGTCCAAGGCTTCCGTGCAGACCCAGCCTGCTATCAAGAAGGACGCTTCCGCTCAGCAGGACTCCTACG
AGTTCGTGTCCCCTTCCCCTCCTGCTGACGTGTCCGAGCGCGCTAAGAAGGCTTCCGCTCGCTCCGGTAAGAAGCAGAA
GAAGAAGACCCTGGCTGAGATCAACCAGAAGTGGAACCTGGAGGCTGAGAAGGAGGACGGTGAGTTCGACTCCAAG
GAGGAGTCCAAGCAGAAGCTGGTGTCCTTCTGCTCCCAGCCTTCCGTGATCTCCTCCCCTCAGATCAACGGTGAGATCG
ACCTGCTGGCTTCCGGTTCCCTGACCGAGTCCGAGTGCTTCGGTTCCCTGACCGAGGTGTCCCTGCCTCTGGCTGAGCA
GATCGAGTCCCCTGACACCAAGTCCCGCAACGAGGTGGTGACCCCTGAGAAGGTGTGCAAGAACTACCTGACCTCCAA
GAAGTCCCTGCCTCTGGAGAACAACGGTAAGCGCGGTCACCACAACCGCCTGTCCTCCCCTATCTCCAAGCGCTGCCGC
ACCTCCATCCTGTCCACCTCCGGTGACTTCGTGAAGCAGACCGTGCCTTCCGAGAACATCCCTCTGCCTGAGTGCTCCTC
CCCTCCTTCCTGCAAGCGCAAGGTGGGTGGTACCTCCGGTCGCAAGAACTCCAACATGTCCGACGAGTTCATCTCCCTG
TCCCCTGGTACCCCTCCTTCCACCCTGTCCTCCTCCTCCTACCGCCGCGTGATGTCCTCCCCTTCCGCTATGAAGCTGCTG
CCTAACATGGCTGTGAAGCGCAACCACCGCGGTGAGACCCTGCTGCACATCGCTTCCATCAAGGGTGACATCCCTTCCG
TGGAGTACCTGCTGCAGAACGGTTCCGACCCTAACGTGAAGGACCACGCTGGTTGGACCCCTCTGCACGAGGCTTGCA
ACCACGGTCACCTGAAGGTGGTGGAGCTGCTGCTGCAGCACAAGGCTCTGGTGAACACCACCGGTTACCAGAACGACT
CCCCTCTGCACGACGCTGCTAAGAACGGTCACGTGGACATCGTGAAGCTGCTGCTGTCCTACGGTGCTTCCCGCAACGC
TGTGAACATCTTCGGTCTGCGCCCTGTGGACTACACCGACGACGAGTCCATGAAGTCCCTGCTGCTGCTGCCTGAGAAG
AACGAGTCCTCCTCCGCTTCCCACTGCTCCGTGATGAACACCGGTCAGCGCCGCGACGGTCCTCTGGTGCTGATCGGTT
CCGGTCTGTCCTCCGAGCAGCAGAAGATGCTGTCCGAGCTGGCTGTGATCCTGAAGGCTAAGAAGTACACCGAGTTCG
ACTCCACCGTGACCCACGTGGTGGTGCCTGGTGACGCTGTGCAGTCCACCCTGAAGTGCATGCTGGGTATCCTGAACG
GTTGCTGGATCTTGAAGTTCGAGTGGGTGAAGGCTTGCCTGCGCCGCAAGGTGTGCGAGCAGGAGGAGAAGTACGAG
ATCCCTGAGGGTCCTCGCCGCTCCCGCCTGAACCGTGAGCAGCTGCTGCCTAAGCTGTTCGACGGTTGCTACTTCTACCT
GTGGGGTACCTTCAAGCACCACCCTAAGGACAACCTGATCAAGCTGGTGACCGCTGGTGGTGGTCAGATCCTGTCCCG
CAAGCCTAAGCCTGACTCCGACGTGACCCAGACCATCAACACCGTGGCTTACCACGCTCGCCCTGACTCCGACCAGCGC
TTCTGCACCCAGTACATCATCTACGAGGACCTGTGCAACTACCACCCTGAGCGCGTGCGCCAGGGTAAGGTGTGGAAG
GCTCCTTCCTCCTGGTTCATCGACTGCGTGATGTCCTTCGAGCTGCTGCCTCTGGACTCCGATTACAAGGATGACGACGA
TAAGTGA**CCCGGG**

Extended Data Table 3. List of oligonucleotides for cloning and site-directed mutagenesis used in this study.

Name	**Sequence (5' to 3')**
fw_WRN_ATPase domain_NheI	TAGGAAGCTAGCAATCTGGGTCTTCCTACTAAAGAAG
rev_WRN_ATPase-Helicase_core_XhoI	TAGGAACTCGAGGCAATGATCCAATCTGGACCTGCAA
rev_WRN_RQC domain_XhoI	TAGGAACTCGAGAGACTTCTTCTCTGTACTTAATTCA
PforWRN_K577M	GCAACTGGATATGGAATGAGTTTGTGCTTCCAGTATCC
PrevWRN_K577M	GGATACTGGAAGCACAAACTCATTCCATATCCAGTTGC
hDNA2c_D277A_for	GGCCTGAAGGGAAAGATCGCTGTCACAGTTGGAGTGAAG
hDNA2c_D277A_rev	CTTCACTCCAACTGTGACAGCGATCTTTCCCTTCAGGCC
DNA2_K654R_F	GGCATGCCGGGAACTGGCAGGACAACCACTATCTGCACA
DNA2_K654R_R	TGTGCAGATAGTGGTTGTCCTGCCAGTTCCCGGCATGCC
CtIP_S327A_F	AATTACCTACTCGAGTGTCAGCTCCTGTATTTGGAGCTACCTC
CtIP_S327A_R	GAGGTAGCTCCAAATACAGGAGCTGACACTCGAGTAGGTAATT
CtIP_F728E_For	AATGATAGCTTGGAAGATATGGAAGATCGGACAACACATGAAGAG
CtIP_F728E_Rev	CTCTTCATGTGTTGTCCGATCTTCCATATCTTCCAAGCTATCATT
CtIP_Y736E_For	GATCGGACAACACATGAAGAGGAAGAATCCTGTTTGGCAGACAG
CtIP_Y736E_Rev	CTGTCTGCCAAACAGGATTCTTCCTCTTCATGTGTTGTCCGATC
AS86_EXO1_DA_For	CCATAATTACAGAGGACTCGGATCTCCTAGCTTTTGGCTGTAAAAAGG
AS87_EXO1_DA_Rev	CCTTTTTACAGCCAAAAGCTAGGAGATCCGAGTCCTCTGTAATTATGG
Human_RPA1_FP	CCTGCCGGATCCATGGTCGGCCAGCTGAGCGAGG
Human_RPA1_RP	CCTGCCGCTAGCTCACATCAATGCACTTCTCCTG
Human_RPA2_FP	CCTGCCGGATCCATGTGGAACAGTGGATTCG
Human_RPA2_RP	CCTGCCGCTAGCTTATTCTGCATCTGTGGATTTAAAATG
Human_RPA3_FP	CCTGCCGTCGACGTGGACATGATGGACTTGCCCAGG
Human_RPA3_RP	CCTGCCTCTAGATCAATCATGTTGCACAATCCC

Extended Data Table 4. List of oligonucleotides for substrate preparation used in this study. The bold **T** represents the site of the biotin modification.

Name	**Sequence (5′ to 3′)**
X12-3HJ3	GAGATCTATCTGGTGCCTTCTGACAGTGAATGGGTAACGAATCGTAATAGTCTCTAGAC AGCATGTCCTAGCAATGTAATCGTCTATGACGTC
X12-3TOPLbis	GACGTCATAGACGATTACATTGCTAGGACATGCTGTCTAGAGACTATCGCGACTTACGT TCCATCGCTAGGTTATTTTTTTTTTTTTTTTTTT
PC210	G**T**AAGTGCCGCGGTGCGGGTGCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCC ACCTCATGCA**T**C
PC211	GA**T**GCATGAGGTGGAGTACGCGCCCGGGGAGCCCAAGGGCACGCCCTGGCACCCGCAC CGCGGCACT**T**AC
PC206	G**T**AAGTGCCGCGGTGCGGGTGCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCC ACCTCATGCATC
PC217	GATGCATGAGGTGGAGTACGCGCCCGGGGAGCCCAAGGGCACGCCCTGGCACCCGCA CCGCGGCACTTAC
NBS1_F	GGCTAGATGGATCCTCTAGAGCTAGCATGTGGAAACTGCTGCCCGC
NBS1_R	GATTTGCGCTCGAGTTACCCGGGTCTTCTCCTTTTTAAATAAG
Main fragment Forward	CATTATGGGGATCCTCAACTGTGAGGAG
Main fragment Reverse	GTCATTCGCCCTCCTAAGAGACGGGATTTA
Dig handle Forward	GACCGAGATAGGGTTGAGTG
Dig handle Reverse	CAGGGTCGGAACAGGAGAGC
Bio handle Forward	ATGACGGACCACGACAGGCCCGCAGTTATC
Bio handle Reverse	GCCGGGCGCGGTTGCGGTATGAGCCGGGTC
Overhang	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCAGCTAGCCTCAGCCTACAATCACC