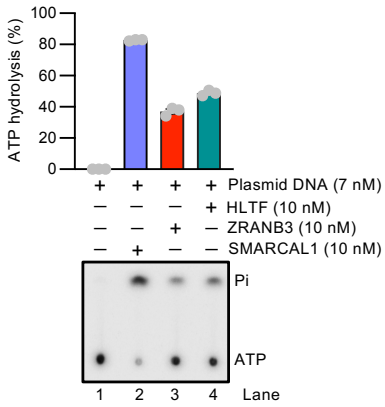
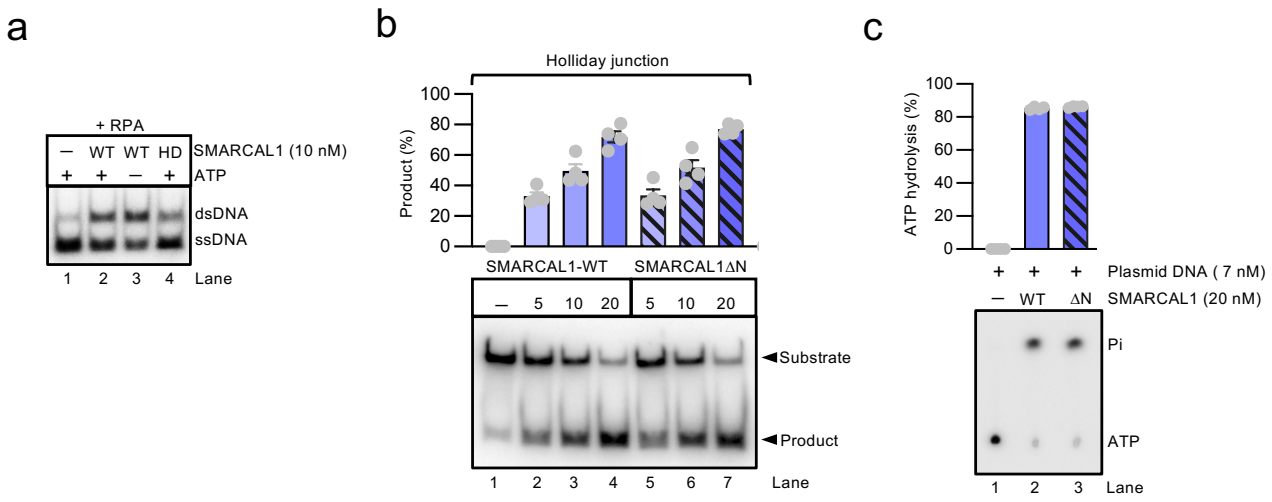


Supplementary Figure 1. A comparison of ATP hydrolysis by SMARCAL1, ZRANB3 and HLTF. Top, quantifications (error bars indicate SEM of three replicates); bottom, a representative experiment.

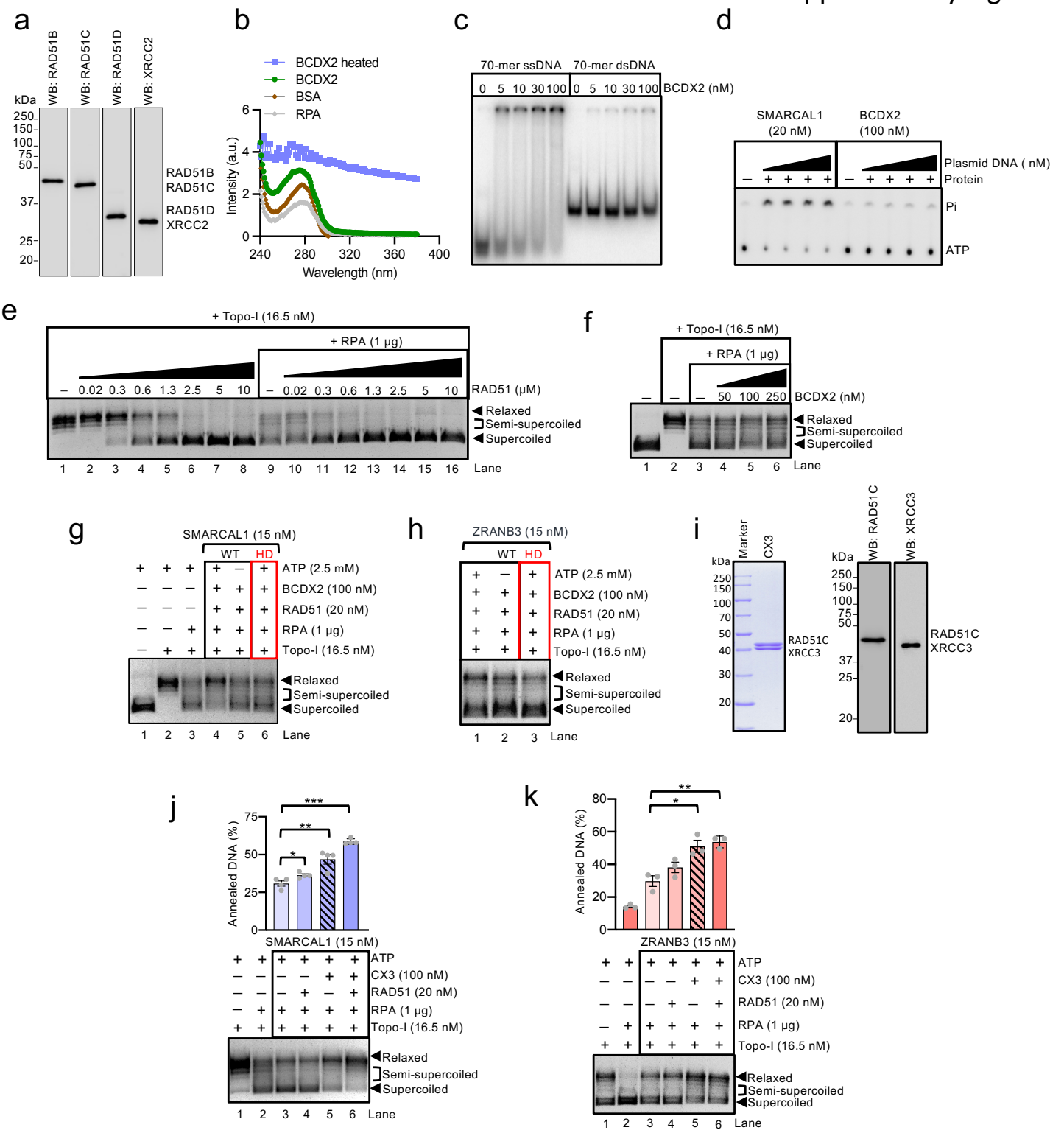


Supplementary Figure 2. Analysis of SMARCAL1 and SMARCAL1ΔN in DNA annealing and branch migration.

(a) Annealing of ssDNA by SMARCAL1 wild type or helicase-dead (D549A, E550A, SMARCAL1-HD) in the presence or absence of ATP.

(b) Holliday junction branch migration by SMARCAL1-WT (residues 1-954) and SMARCAL1ΔN (residues 31-954). Experiment was performed with 100 mM NaCl and 5 mM ATP.

(c) ATP hydrolytic capacity of SMARCAL1-WT and SMARCAL1ΔN. Top, quantifications (error bars indicate SEM of four replicates); bottom, representative experiment.



Supplementary Figure 3. Analyses of RAD51, BCDX2, CX3 and their interplay with SMARCAL1 and ZRANB3.

(a) Western blot analysis of the recombinant BCDX2 complex.

(b) Spectrophotometric analysis showing recombinant BCDX2 does not form aggregates. BSA and RPA were used as negative controls. However, when heated at 65 °C for 30 minutes, BCDX2 aggregated.

(c) Electrophoretic mobility shift assay showing BCDX2 has a much higher affinity towards 70-mer ssDNA (1 nM, in molecules) in contrast to 70-mer dsDNA (1 nM, in molecules).

(d) BCDX2 is a functional but weak ATPase. SMARCAL1 was used as a positive control. Plasmid DNA (2.961 kbp-long, 0.25, 0.5, 1 and 2 nM) was added as indicated in the figures a co-factor.

(e) and (f) Increasing concentration of RAD51 and BCDX2, respectively, does not promote annealing in the topoisomerase-coupled annealing assay.

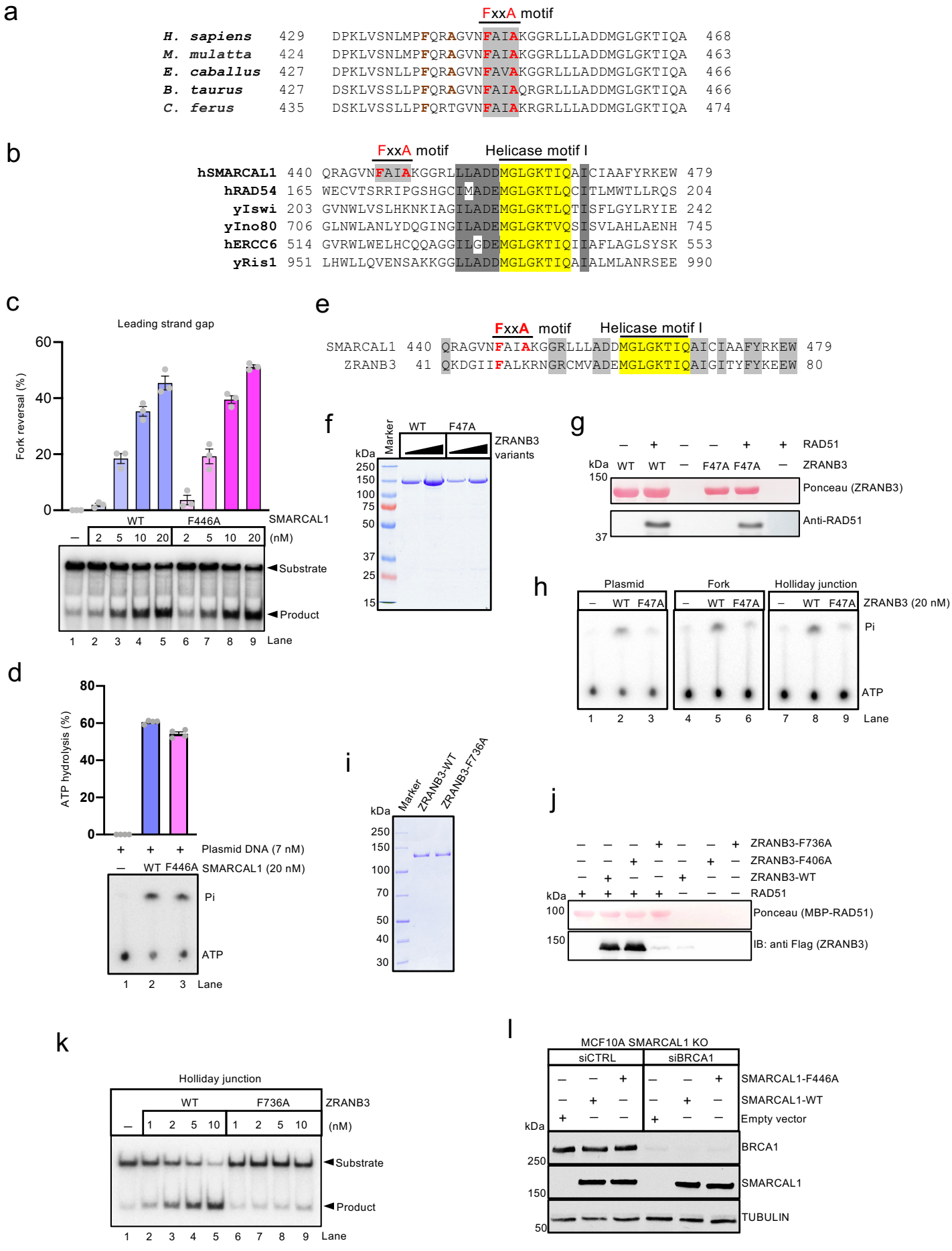
(g) Topoisomerase-coupled annealing assay with wild type and helicase-dead (D549A, E550A, SMARCAL1-HD) SMARCAL1 variants. Shown is a representative experiment.

(h) Topoisomerase-coupled annealing assay with wild type and helicase-dead (D157A, E158A, ZRANB3-HD) ZRANB3 variants. Shown is a representative experiment.

(i) Recombinant CX3-complex was analyzed by polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue or subjected to Western blot analysis with anti-RAD51C and anti-XRCC3 antibodies, respectively.

(j) RAD51 and CX3 heterodimer separately stimulate SMARCAL1-mediated strand annealing. Top, quantifications (error bars indicate SEM of four replicates); bottom, representative experiment. Statistical significance; *($P < 0.05$), **($P < 0.01$), ***($P < 0.001$), two-tailed t-test.

(k) RAD51 and CX3 heterodimer separately stimulate ZRANB3-mediated strand annealing. Top, quantifications (error bars indicate SEM of three replicates); bottom, representative experiment. Statistical significance; *($P < 0.05$), **($P < 0.01$), two-tailed t-test.



Supplementary Figure 4. Legend on next page.

Supplementary Figure 4. Mapping RAD51 interaction motifs in SMARCAL1 and ZRANB3.

(a) Multiple sequence alignment showing conservation of the FxxA motif in SMARCAL1 in vertebrates, highlighted in grey with bold red letters. A less conserved second FxxA motif, with no effect on interaction with RAD51, is shown in bold brown letters.

(b) Multiple sequence alignment showing conserved helicase motif I (highlighted in yellow) in Snf2 family members in multiple yeast (y) and human (h) proteins. Additional conserved residues are highlighted in dark grey. The RAD51 interaction motif FxxA, indicated with bold red letters and highlighted in light grey in SMARCAL1, is located just upstream, but is not a part of the conserved ATPase domain.

(c) SMARCAL1-WT and SMARCAL1-F446A have comparable biochemical activities on a leading strand gap substrate. Note that the experiment was performed without RPA and in presence of 120 mM NaCl. Top, quantifications (error bars indicate SEM of three replicates); bottom, representative experiment.

(d) ATP hydrolysis by SMARCAL1-WT or SMARCAL1-F446A with a plasmid dsDNA as a substrate in the presence of 120 mM NaCl. Top, quantifications (error bars indicate SEM of four replicates); bottom, representative experiment.

(e) Sequence alignment showing conservation between human SMARCAL1 and ZRANB3, highlighted in grey. FxxA motif is highlighted in bold red letters in SMARCAL1. The corresponding phenylalanine (F) is also indicated in ZRANB3 in bold red letter.

(f) Recombinant ZRANB3-WT and ZRANB3-F47A were analyzed by polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue.

(g) F47A substitution in ZRANB3 does not impair its interaction with RAD51. Soluble extract from *Sf*9 cells expressing FLAG-ZRANB3 variants (bait) was immobilized on M2 anti-FLAG affinity resin and tested for interaction with recombinant and purified RAD51 (prey). Samples were analyzed by polyacrylamide gel electrophoresis and either stained with Ponceau to show ZRANB3 or subjected to Western blot analysis with anti-RAD51 antibody.

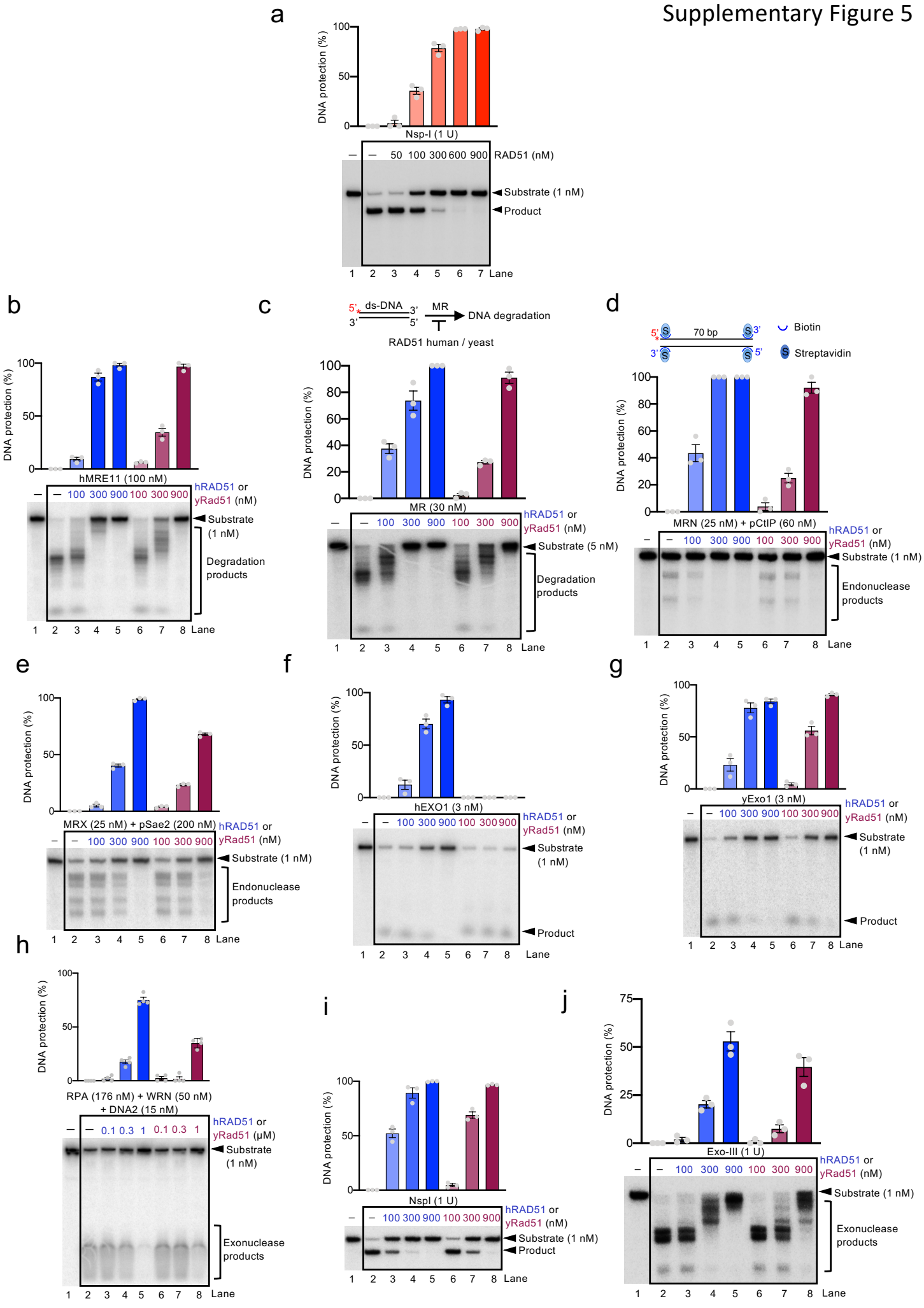
(h) F47A substitution in ZRANB3 impairs ATP hydrolytic capacity with co-factors such as plasmid dsDNA (7 nM, in molecules), replication fork (5 nM) and Holliday junction (5 nM).

(i) Recombinant ZRANB3-WT and ZRANB3-F736A were analyzed by polyacrylamide gel electrophoresis and stained with Coomassie Brilliant Blue.

(j) Soluble extract from *E. coli* containing MBP-RAD51 (bait) was immobilized on amylose resin and incubated with purified recombinant ZRANB3-WT and ZRANB3-F736A (prey) and ZRANB3-F406A (prey). Samples were analyzed by polyacrylamide gel electrophoresis and either stained with Ponceau to show RAD51 or subjected to Western blot analysis with anti-FLAG antibody to detect ZRANB3.

(k) F736A substitution in ZRANB3 severely affects its branch migration activity. Shown is a representative experiment.

(l) Western blot analysis showing the efficiency of siRNA-mediated BRCA1 depletion and the comparable expression levels of SMARCAL1-WT or -F446A in SMARCAL1 KO cells, as indicated.



Supplementary Figure 5. Legend on next page.

Supplementary Figure 5. DNA protection by human and yeast RAD51.

(a) Human RAD51 efficiently protects DNA against the endonuclease NspI on a 5′-end-labeled 50 bp-long dsDNA. Top, quantifications (error bars indicate SEM of three replicates); bottom, a representative experiment.

(b) DNA protection by human RAD51 and yeast Rad51 against degradation by human MRE11 exonuclease using 5′-end-labeled 50 bp-long dsDNA. Top, quantifications (error bars indicate SEM of three replicates); bottom, a representative experiment.

(c) Comparison of DNA protection by human RAD51 and yeast Rad51 against degradation by human MRE11-RAD50 (MR). 5′-end-labeled 50 bp-long dsDNA was used as a substrate. Asterisk indicates the position of the ^{32}P label. Top, quantifications (error bars indicate SEM of three replicates); bottom, representative experiment. The level of DNA protection by RAD51 is presented as a relative value with respect to DNA degradation without RAD51 (lane 2).

(d) Comparison of DNA protection by human RAD51 and yeast Rad51 against degradation by human MRE11-RAD50-NBS1 (MRN) and phosphorylated CtIP (pCtIP). Top, quantifications (error bars indicate SEM of three replicates); bottom, a representative experiment. The level of DNA protection by RAD51 is presented as a relative value with respect to DNA degradation without RAD51 (lane 2).

(e) DNA protection by human RAD51 and yeast Rad51 against degradation by the endonuclease activity of yeast MRX-pSae2 complex on a quadruple blocked 5′-end-labeled 70 bp-long dsDNA. Top, quantifications (error bars indicate SEM of three replicates); bottom, representative experiment. Note that experiment was performed at 30 °C.

(f) The exonuclease activity of human EXO1 and its inhibition by human RAD51 and yeast Rad51 on a 5′-end-labeled 50 bp-long dsDNA. Top, quantifications (error bars indicate SEM of three replicates); bottom, representative experiment.

(g) Similar experiments as in panel (f), but using yeast Exo1. Note that experiment was performed at 30 °C.

(h) The exonuclease activity of WRN-DNA2-RPA ensemble and its inhibition by human RAD51 and yeast Rad51, using a 2.2-kilobase pair (kbp)-long randomly labeled dsDNA. Top, quantifications (error bars indicate SEM of four replicates); bottom, representative experiment.

(i) DNA protection by human RAD51 and yeast Rad51 against endonuclease NspI on a 5′-end-labeled 50 bp-long dsDNA. Top, quantifications (error bars indicate SEM of three replicates); bottom, representative experiment.

(j) Comparison of DNA protection by human RAD51 and yeast Rad51 against *E. coli* Exo-III. Top, quantifications (error bars indicate SEM of three replicates); bottom, representative experiment. The level of DNA protection by RAD51 is presented as a relative value with respect to DNA degradation without RAD51 (lane 2).

Supplementary Table 1
List of oligonucleotides used in this study

Primer Name	DNA sequence (5'-3')
RAD51 K133A F	TTGGAGAATTCCGAACTGGG GCGACCCAGATCTGTCATACGCT
RAD51 K133A R	AGCGTATGACAGATCTGGGTCGCCCCAGTTCGGAATTCTCCAA
RAD51 K133R F	TTGGAGAATTCCGAACTGGGCGGACCCAGATCTGTCATACGCT
RAD51 K133R R	AGCGTATGACAGATCTGGGTCCGCCCAGTTCGGAATTCTCCAA
RAD51 Y232A F	CCGCCCTTTACAGAACAGACGCCTCGGGTCGAGGTGAGCTTTC
RAD51 K232A R	GAAAGCTCACCTCGACCCGAGGCGTCTGTTCTGTAAAGGGCGG
RAD51 T131P F	AAATGTTTGGAGAATTCCGACCTGGGAAGACCCAGATCTGTCA
RAD51 T131P R	TGACAGATCTGGGTCTTCCCAGGTCGGAATTCTCCAAACATTT
RAD51 Y232W F	CCGCCCTTTACAGAACAGACTGGTCGGGTCGAGGTGAGCTTTC
RAD51 Y232W R	GAAAGCTCACCTCGACCCGA CCAGTCTGTTCTGTAAAGGGCGG
SMARCAL1 F439A F	ATTGACTCCAGCTCTCTGAGCGGGCATCAGATTAGACACG
SMARCAL1 F439A R	CGTGTCTAATCTGATGCCCGCTCAGAGAGCTGGAGTCAAT
SMARCAL1 F446A F	GCCCTTTCAGAGAGCTGGAGTCAATGCTGCCATAGCCAAA
SMARCAL1 F446A R	TTTGGCTATGGCAGCATTGACTCCAGCTCTCTGAAAGGGC
ZRANB3 F47A F	CTACTTCCATTCCAGAAAGATGGCATCATTGCTGCCCTCAAAAGAAAT
ZRANB3 F47A R	ATTTCTTTTGAGGGCAGCAATGATGCCATCTTTCTGGAATGGAAGTAGC
ZRANB3 F736A F	TCAGTATTCCTACTTGCACAGGCCATTAAGGTGTCATACACTGG
ZRANB3 F736A R	CCAGTGTATGACACCTTAATGGCCTGTGCAAGTAGGAATACTGA
SMARCAL1ΔN F	ATCGTACGACTAGTGGCGGAGAACAGCATCAGAGGACTAGC
SMARCAL1ΔN R	AGCAGGCTCGAGTTACAGGGGAGACGTAAAGCTGTC
NBS1 F	GGCTAGATGGATCCTCTAGAGCTAGCATGTGGAAACTGCTGCCCGC
NBS1 R	GATTTGCG CTCGAG TTA CCCGGG TCTTCTCCTTTTTAAATAAG
XO2	TGGGTAAACCTGCAGGTGGGCAAAGATGTCCATCTGTTGTAATCGTCAAGCTTTATGCCGTT
XO1	GACGCTGCCGAATTCTACCAGTGCCTTGCTAGGACATcTTTGCCCACCTGCAGGTTCACCC
XO1C.MM2	GGGTGAACCTGCAGGTGGGCAAAAATGTCCTAGCAAGGCACTGGTAGAATTCGGCAGCGTC
XO2C.MM	GAACGGCATAAAGCTTGACGATTACAACAGATGGACATTTTTGCCCACCTGCAGGTTCACCC
#DC1	CGTGACTTGATGTTAACCCTAACCCTAAGATATCGCGTTATCAGAGTGTGAGGATACATGT AGGCAATTGCCACGTGTCTATCAGCTGAAGTTGTTCGCGACGTGCGATCGTCGCTGCGACG
#DC2	CGTCGCAGCGACGATCGCACGTCGCGAACAACTTCAGCTGATAGACACGTGGCAATTGCCT ACATGTATCCTCACACTCTGAATACGCGATATCTTAGGGTTAGGGTTAACATCAAGTCACG
#DC3	CGTCGCAGCGACGATCGCACGTCGCGAACAACTTCAGCTGATAGACACGTGGCAATTGCCT ACATGTATCCTCACACTCTGA
#DC4	CGTCGCAGCGACGATCGCACGTCGCGAACAACTTCAGCTGATAGACACGTGG
#DC5	CCACGTGTCTATCAGCTGAAGTTGTTCGCGACGTGCGATCGTCGCTGCGACG
#DC6	TCAGAGTGTGAGGATACATGTAGGCAATTGCCACGTGTCTATCAGCTGAAGTTGTTCGCGAC GTGCGATCGTCGCTGCGACG
X12-3	GACGTCATAGACGATTACATTGCTAGGACATGCTGTCTAGAGACTATCGC
X12-4C	GCGATAGTCTCTAGACAGCATGTCCTAGCAATGTAATCGTCTATGACGTC
X12-4SC	GCGATAGTCTCTAGACAGCATGTCCTAGCAA
X12-4NC	GCGATAGTCTCTAGACAGCATGTCCTAGCAAGCCAGAATTCGGCAGGCTA
X12-3SC	TTGCTAGGACATGCTGTCTAGAGACTATCGC
PC-210	GTAAGTGCCGCGGTGCGGGTGCCAGGGCGTGCCCTTGGGCTCCCCGGGCGCGTACTCCAC CTCATGCATC
PC-211	GATGCATGAGGTGGAGTACGCGCCCGGGGAGCCCAAGGGCACGCCCTGGCACCCGCACCG CGGCACTTAC