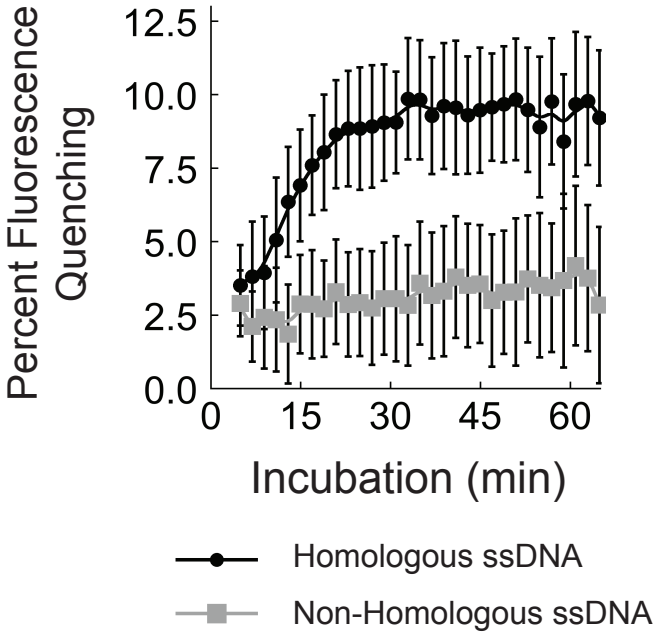
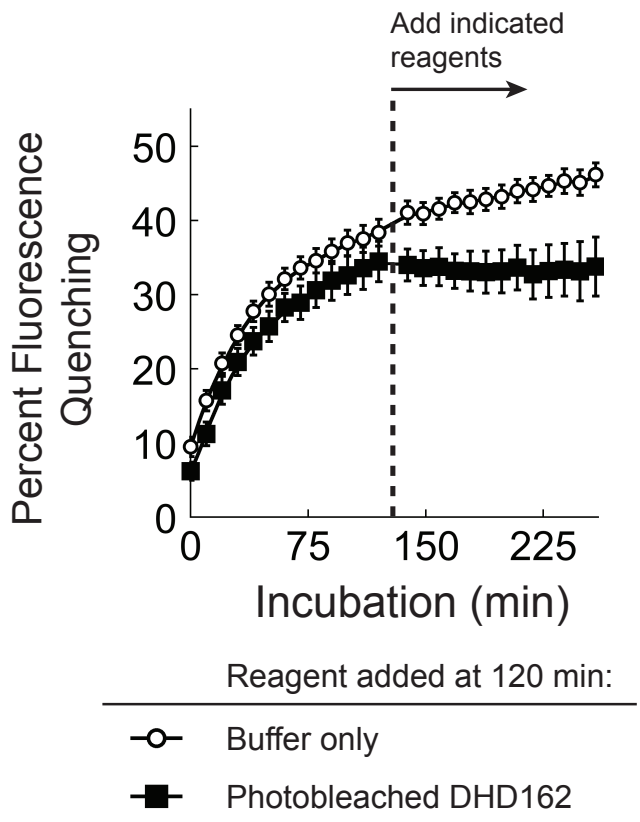
Oligo name	Length (nt)	Modification	Sequence (5' to 3')
DHD162-HP-L	85		TTT TCT AGA TCA TTT CTT TTG ATA AGA GGT CAT TTT TGC GGA TGG GAG ACC ATC CGC AAA AAT GAC CTC TTA TCA AAA GAA ATG A
DHD162-HP-R	73		AGT CTC GAG CTT TTG ATA AGA GGT CAT TTT TGA GGA TGG GAG ACC ATC CTC AAA AAT GAC CTC TTA TCA AAA G
DHD162-CD-O	87		TCT AGA AAA TAA GTA TAA AAG CTT AAA TGA ATA AAC ATA GAA AAT AAA GTA AAG GAT ATA AAT GGC ATT GAG GGT TAC GGA TAA ATA
DHD162-CD-CF	87	Internal Fluorescein between position 21 and 22	CTC GAG ACT TAT TTA TCC GTA**[Fl]** ACC CTC AAT GCC ATT TAT ATC CTT TAC TTT ATT TTC TAT GTT TAT TCA TTT AAG CTT TTA TAC TTA
DHD-HB	51	3' Biotin	AAA TGA ATA AAC ATA GAA AAT AAA GTA AAG GAT ATA AAT GGC ATT GAG GGT**[Bio]**
DHD-NB	51	3' Biotin	GTT ATG TAC ATT GAC GTA TAT TAT ATA TAC TCT AGT AGT TAT CGA AGA CAT**[Bio]**
DHD-HQ	51	3' Black Hole Quencher 1	AAA TGA ATA AAC ATA GAA AAT AAA GTA AAG GAT ATA AAT GGC ATT GAG GGT**[BHQ]**
DHD-NQ	51	3' Black Hole Quencher 1	GTT ATG TAC ATT GAC GTA TAT TAT ATA TAC TCT AGT AGT TAT CGA AGA CAT**[BHQ]**
RG1(+)A	84	5' Alexa Fluor 488	**[AF]**GGT AGC GGT TGG GTG AGT GGT GGG GAG GGT CGG GAG GTG GCG TAG AAA CAT GAT AGG AAT GTG AAT GAA TGA AGT ACA AGT AAA
RG1(-)	84		TTT ACT TGT ACT TCA TTC ATT CAC ATT CCT ATC ATG TTT CTA CGC CAC CTC CCG ACC CTC CCC ACC ACT CAC CCA ACC GCT ACC
RG1(-)B	84	3' Biotin	TTT ACT TGT ACT TCA TTC ATT CAC ATT CCT ATC ATG TTT CTA CGC CAC CTC CCG ACC CTC CCC ACC ACT CAC CCA ACC GCT ACC**[Bio]**
RG1(-)Q	84	3' Black Hole Quencher 1	TTT ACT TGT ACT TCA TTC ATT CAC ATT CCT ATC ATG TTT CTA CGC CAC CTC CCG ACC CTC CCC ACC ACT CAC CCA ACC GCT ACC**[BHQ]**
RG(rand)	84		CTC CCA GTG TTA TTA TCA AAT TGG ATT ACA CAT TAT AGG ATA ACT TGG TTA CAA GAC TAT TAG AGA GCA AGG TAA ATG AGT TAA
RG(rand)B	84	3' Biotin	CTC CCA GTG TTA TTA TCA AAT TGG ATT ACA CAT TAT AGG ATA ACT TGG TTA CAA GAC TAT TAG AGA GCA AGG TAA ATG AGT TAA**[Bio]**
RG(rand)Q	84	3' Black Hole Quencher 1	TCC AGT GAT TGT GGT AAG GGA CTA CGA ACA TGC GGA CAT TAG AGA CGT ACA GCT ACC TAT CCT GAG CAC GGT TGG ATT TCA GAA**[BHQ]**
306.90	90		TAC GAA TGC ACA CGG TGT GGT GGG CCC AGG TAT TGT TAG CGG TTT GAA GCA GGC GGC AGA AGA AGT AAC AAA GGA ACC TAG AGG CCT TTT

Supplementary table 1: List of ssDNA oligonucleotides used in this study.

SUPPLEMENTAL FIGURES:



Supplemental Figure 1: D-loop formation by RecA using ATP as a cofactor. D-loops are detected as the fluorescence quenching that occurs upon pairing of the BHQ-labeled ssDNA with the complementary strand of the dsDNA, which contains an internal fluorescein label. The reaction was performed by combining 31 mM Tris-HCl (pH 7.5), 1.3 mM ATP, 13 mM $MgCl_2$, 1.8 mM DTT, 1.32 µM BSA, 7.84 nM ssDNA, 11.14 nM DHD162, and 0.4 µM RecA in 25 µl and taking fluorescence measurements as described in the Methods section. DHD-HQ is used as the homologous ssDNA and DHD-NQ is used as the non-homologous ssDNA control. Kinetics of D-loop formation by *E. coli* RecA are displayed as the percent decrease in fluorescence, relative to the protein-free control. Error bars represent the standard error of four replicates.



Supplemental Figure 2: Stability of D-loops formed by human RAD51. D-loops are detected as the fluorescence quenching that occurs upon pairing of the BHQ-labeled ssDNA with the complementary strand of the dsDNA, which contains an internal fluorescein label. The reaction was performed as described in the Methods section for fluorescence-based D-loop assay of RAD51. At 120 minutes, a 5 µl aliquot of annealing buffer containing 1 µM DHD162 (final concentration equimolar to DHD162 already in the reaction), which was photobleached to 14% of its original fluorescence, or 5 µl of annealing buffer alone was added to the ongoing reactions and incubation was continued for another 120 minutes at 37 ºC. Kinetics of D-loop formation are displayed as the percent decrease in fluorescence, relative to the protein-free control. Error bars represent the standard error of four replicates.