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Supplementary Information for

Rad51 facilitates filament assembly of meiosis-specific Dmc1 recombinase

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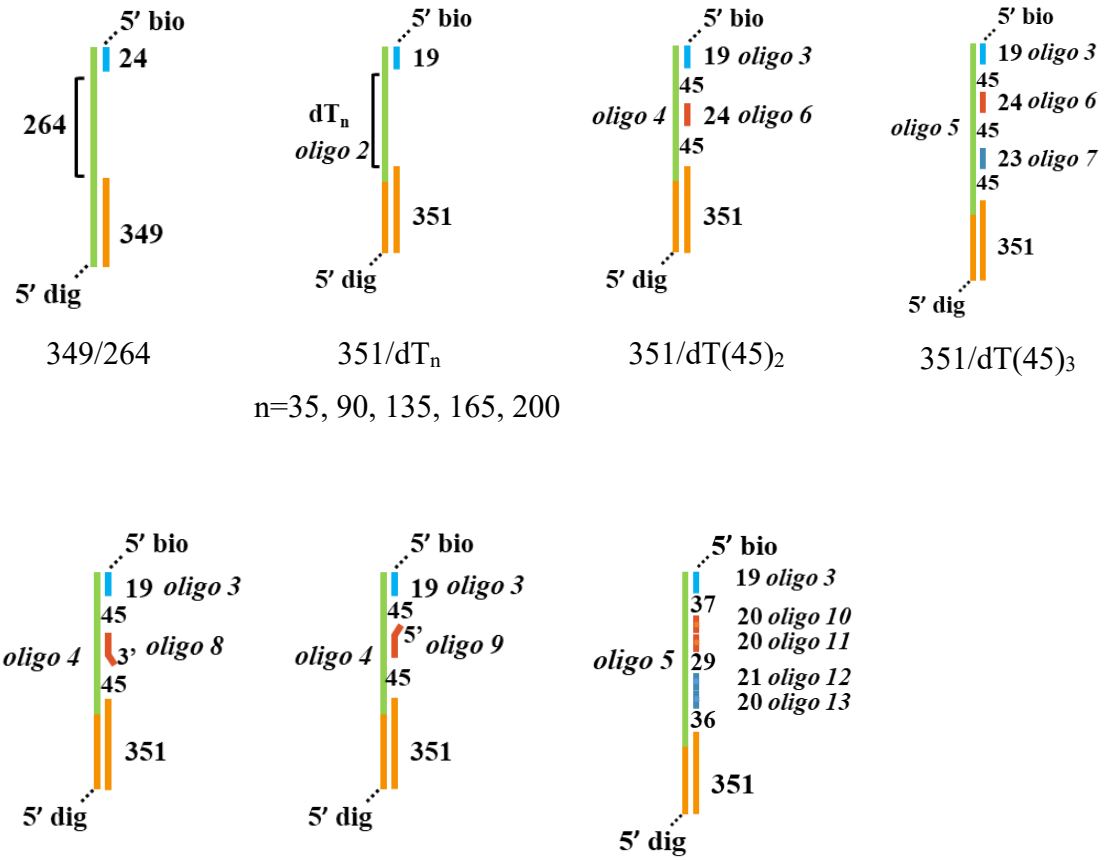

349/264 351/dT$_n$ 351/dT(45)$_2$ 351/dT(45)$_3$

n=35, 90, 135, 165, 200

351/dT(45)$_2$-3′flap 351/dT(45)$_2$-5′flap 351/dT(37+29+36)

Figure S1. DNA substrates used in single-molecule experiments. Oligo sequences are listed in Table S1. DNA substrate preparation is listed in the Methods and Materials section.

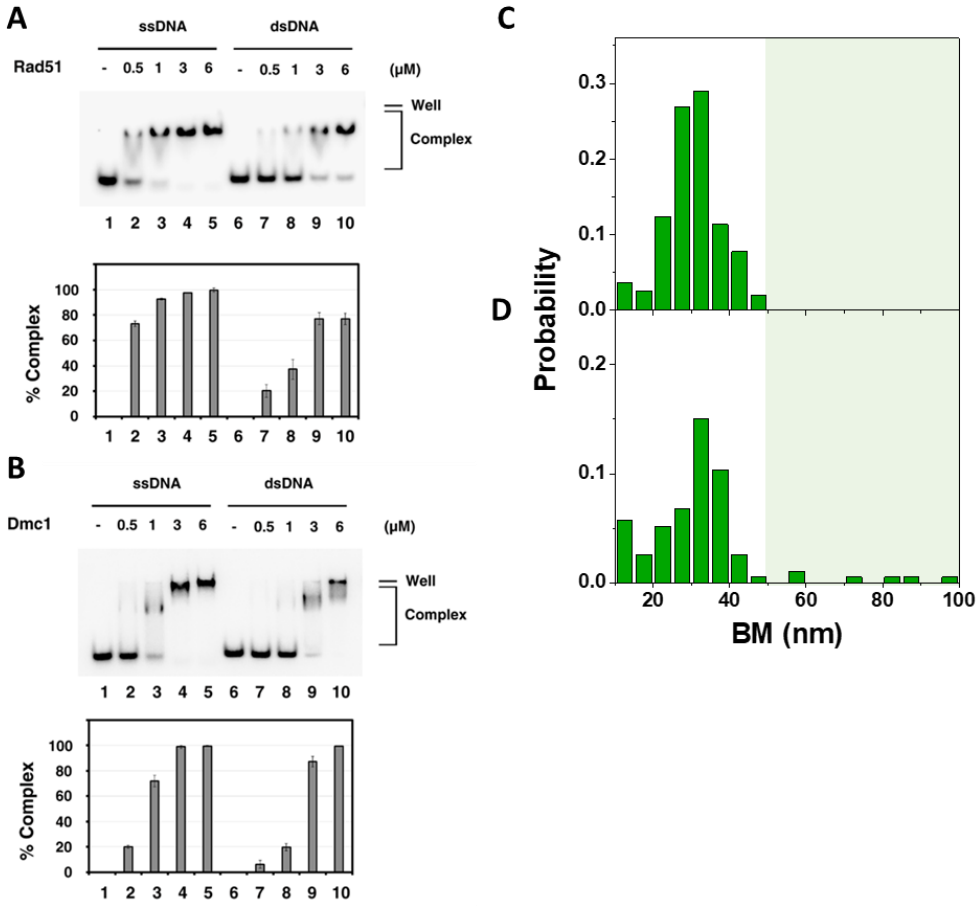

Figure S2. ScDmc1 preferentially binds to ssDNA segment in the presence of 50 mM KCl. (A-B) Electrophoresis mobility shift assay to determine DNA binding affinity for ScRad51 (A) and ScDmc1 (B) in the presence of 50 mM KCl. The 5'γ-32P-labeled 80-mer Oligo-1 ssDNA (3 μM nucleotides) or derived complementary 80-mer dsDNA (3 μM base pairs) were incubated with the indicated amounts of recombinases in 10 μl of reaction buffer A containing 1 mM ATP at 37 °C for 5 min. The reaction mixtures were run in 2% agarose gel in TAM buffer (30 mM Tris-HCl, 30 mM sodium acetate, and 5 mM magnesium acetate, pH 7.5) at 4 °C. The gels were dried on DE81 paper and the DNA species were quantified by phosphorimaging analysis in a Personal FX phosphorimager using the Quantity One software (Bio-Rad). The quantification was graphed as indicated. The error bars represent the standard deviation determined from at least three independent experiments. (C-D) BM Histograms of ScDmc1 assembling on fully duplex 349 bp DNA (ds349). (C) BM histogram of DNA substrate-only. (D) BM histogram at 10 minutes after ScDmc1 (1.1 μM) addition. Shaded region represents extended filament BM. No apparent ScDmc1 filament formation is observed within the 10-minute scale. Assembly reaction was done in the presence of 1 mM ATP, 1 mM phosphoenolpyruvate, 4 units/mL pyruvate kinase, 30 mM Tris (pH=7.5), 50 mM KCl, 2.5 mM $MgCl_2$ and 1 mM dithiothreitol.

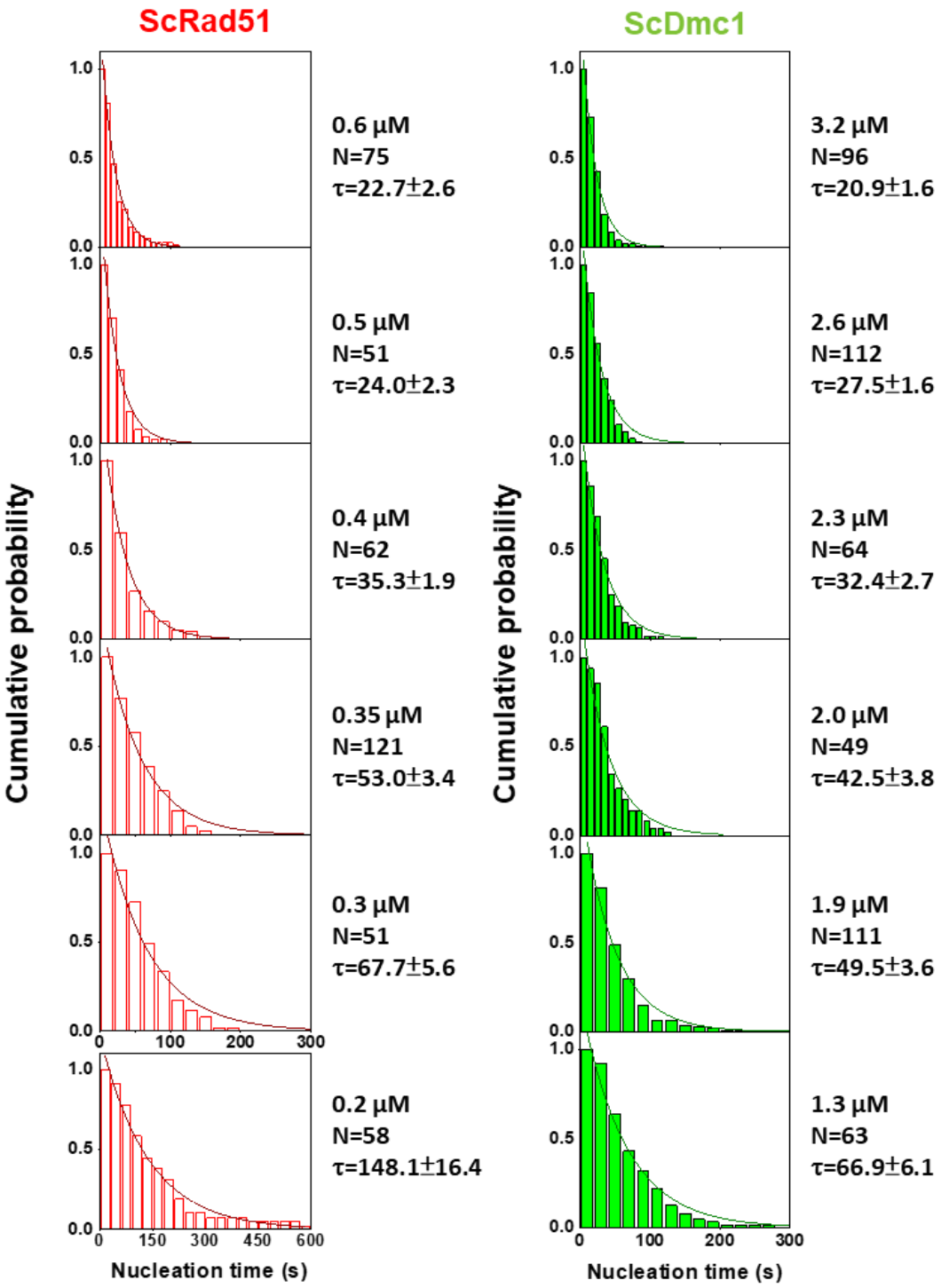

Figure S3. **Concentration-dependence of nucleation times of ScRad51 (left, in red) and ScDmc1 (right, in green) on the 349/264 DNA substrates.** The error bars of nucleation time are the standard deviation by bootstrapping 5,000 times.

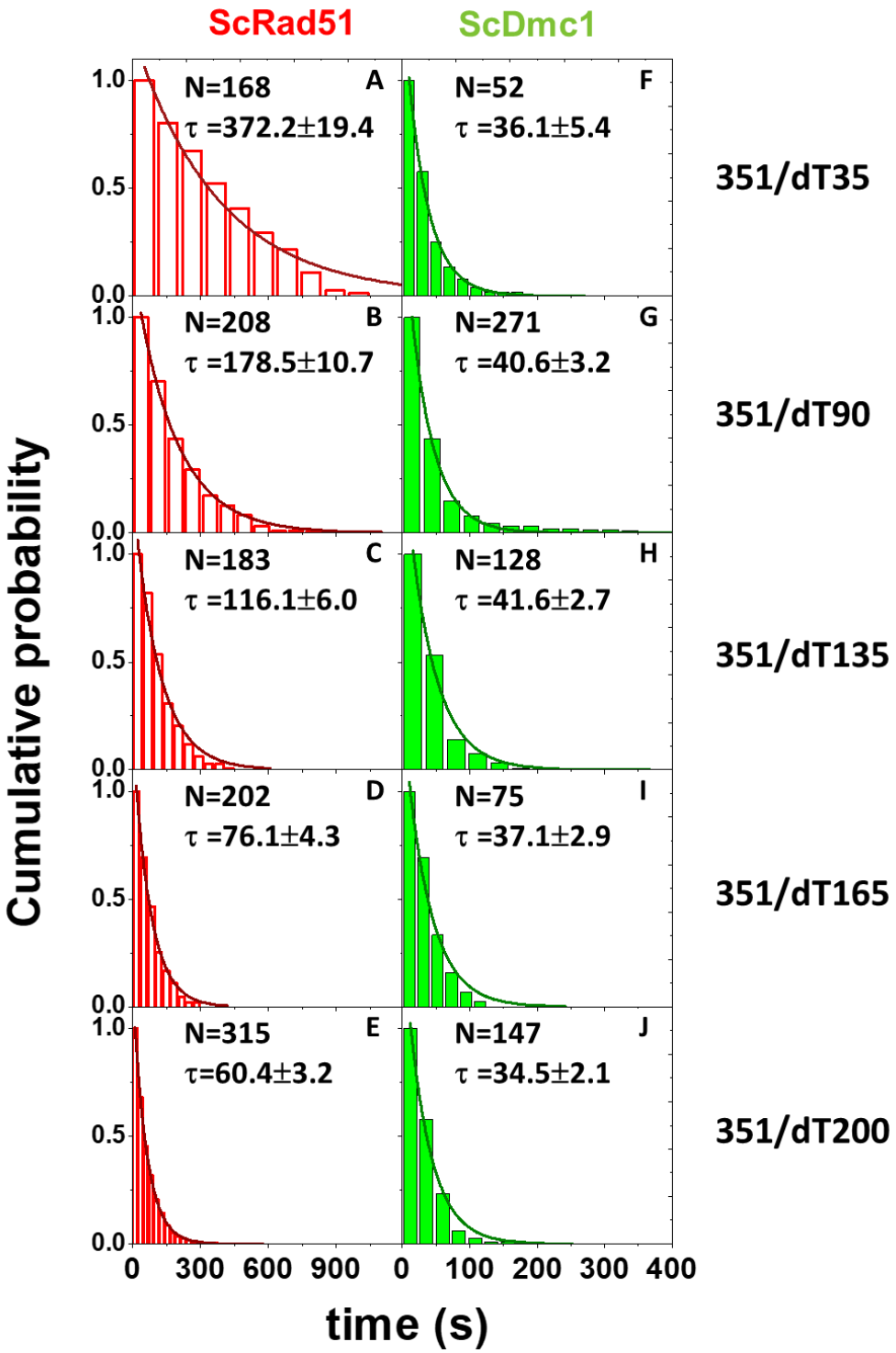

Figure S4. The nucleation times of ScRad51 (left, in red) and ScDmc1 (right, in green) at different ssDNA lengths (351/dT$_n$, n=35, 90, 135, 165 and 200). (A-E) Nucleation time determined in 0.4-μM ScRad51. (F-J) Nucleation time determined in 1.9-μM ScDmc1. The error bars of nucleation time are the standard deviation by bootstrapping 5,000 times.

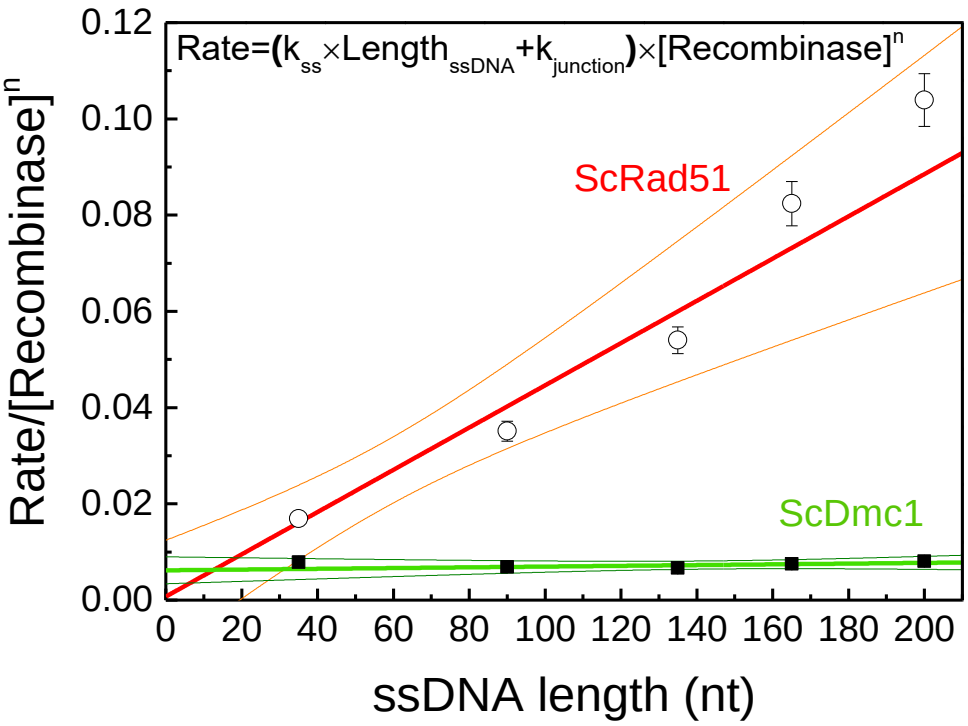

Figure S5. **Nucleation rates of ScRad51 and ScDmc1 as a function of ssDNA lengths.** Replotting data from Figure S3 but using nucleation size of two (n=2) for both ScRad51 and ScDmc1 returns with the same trend as in Figure 3B. Fitting parameters are listed in Table S2. The error bars of nucleation rate are the standard deviation by bootstrapping 5,000 times.

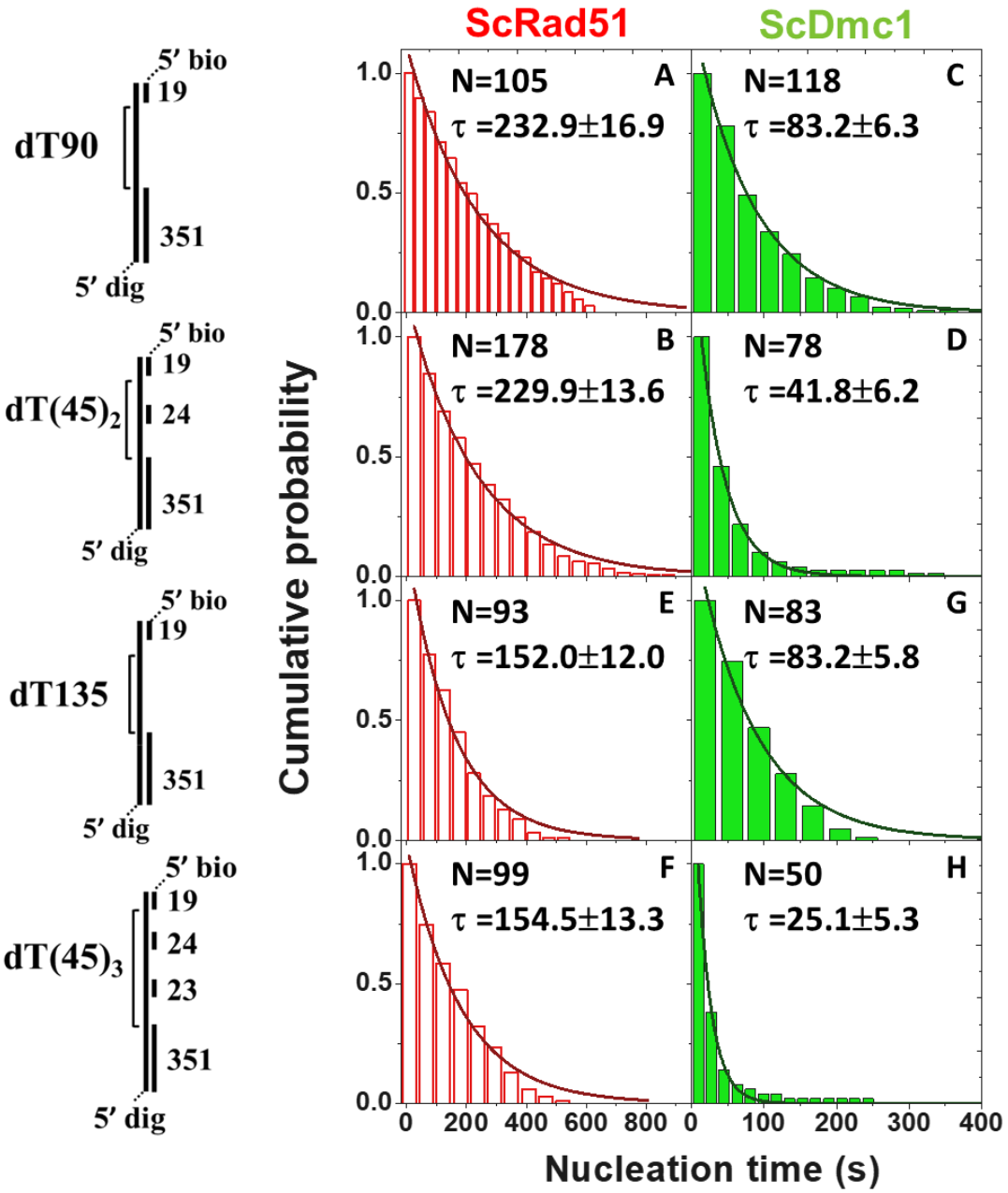

Figure S6. The nucleation times of ScRad51 (left, in red) and ScDmc1 (right, in green) at different DNA substrates containing one ssDNA gap (dT90, dT135), two gaps (dT(45)$_2$) or three gaps (dT(45)$_3$). (A-B, E-F) Nucleation time determined in 0.4-μM ScRad51. (C-D, G-H) Nucleation time determined in 1.1-μM ScDmc1. The error bars of nucleation time are the standard deviation by bootstrapping 5,000 times.

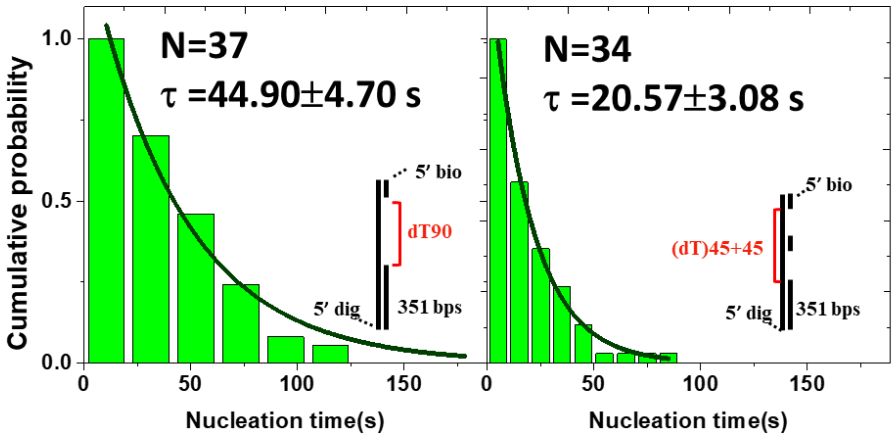

Figure S7. The nucleation times of ScDmc1 351/dT90 DNA and 351/dT(45)$_2$ DNA at high [K$^+$] (150 mM). The reduced nucleation time is also seen at the double gap substrate at this high salt condition. Therefore, the ss/dsDNA junction nucleation preference of ScDmc1 is insensitive of ionic strength. Nucleation time determined in 1.7-μM ScDmc1. The error bars of nucleation time are the standard deviation by bootstrapping 5,000 times.

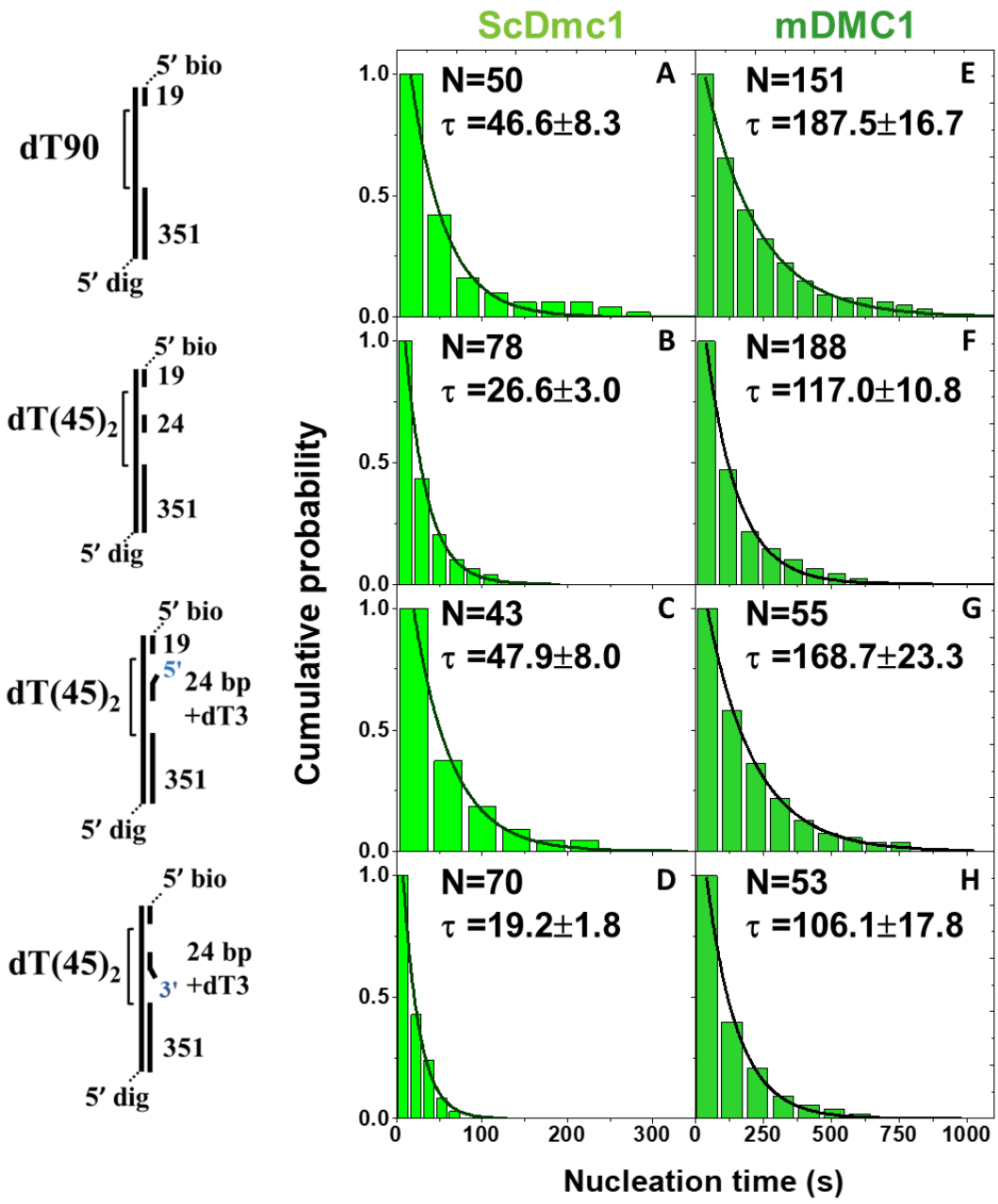

Figure S8. The nucleation times of ScDmc1 and mDMC1 at different DNA substrates containing one ssDNA gap (dT90) and two gaps (dT(45)$_2$) with or without additional dT3 flap. (A-D) Nucleation time determined in 1.7-μM ScDmc1. (E-H) Nucleation time determined in 5-μM mDMC1. The error bars of nucleation time are the standard deviation by bootstrapping 5,000 times.

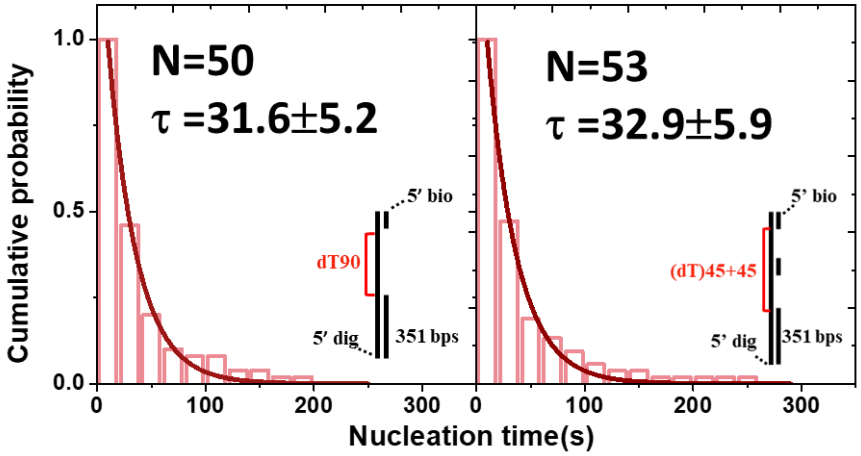

Figure S9. **The nucleation times of mRAD51 351/dT90 DNA and 351/dT(45)$_2$ DNA.** The similar nucleation times suggest that mRAD51 doesn't prefer assembling on the ss/dsDNA junction. Nucleation time is determined using 0.5-μM mRAD51. The error bars of nucleation time are the standard deviation by bootstrapping 5,000 times.

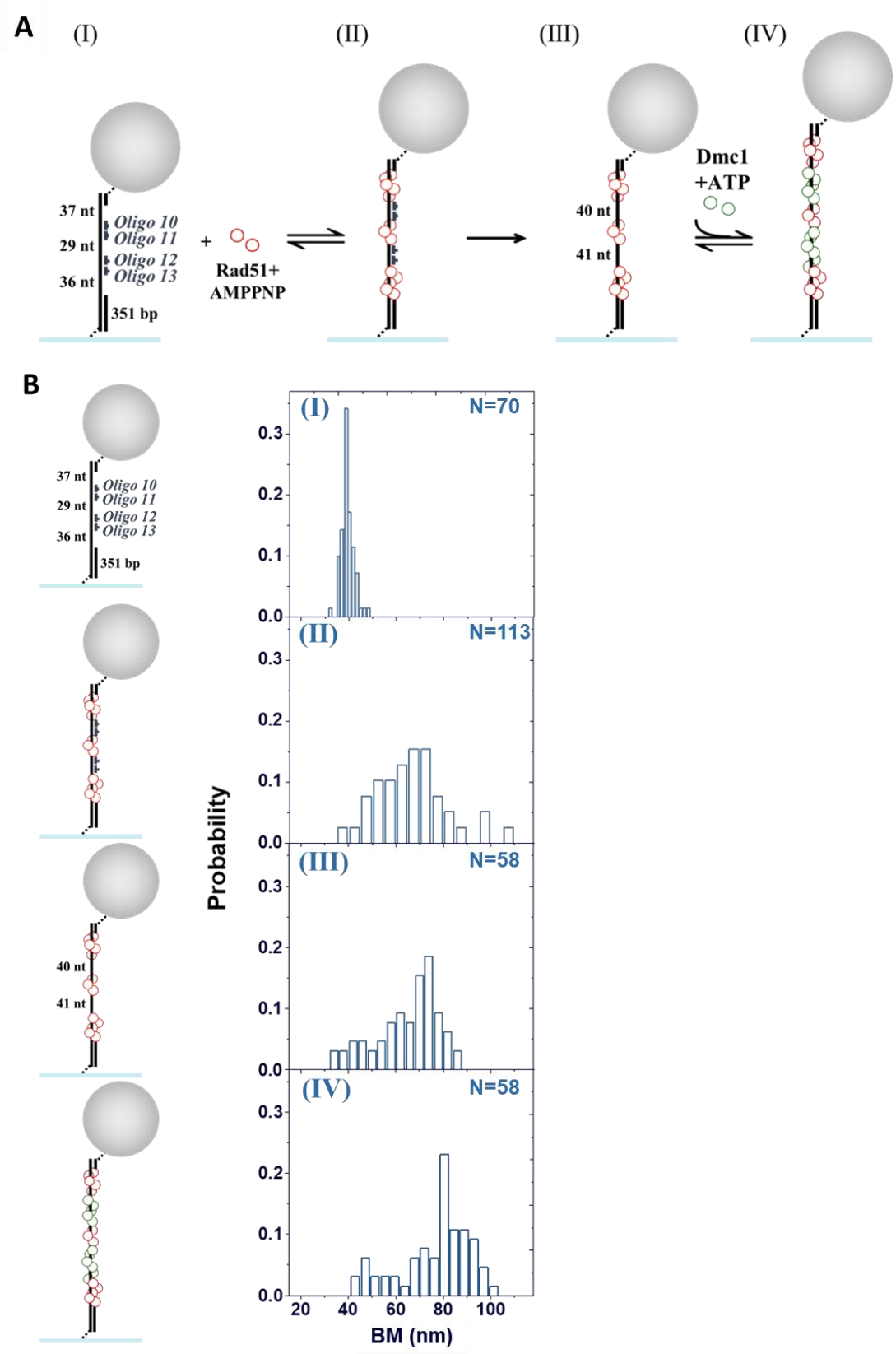

Figure S10. Preparation and BM histograms for Dmc1 assembly on 351/ss(40+41)/Rad51 DNA substrates (A) Schematic of DNA construct used in ScDmc1 assembly on ssDNA containing short Rad51 filament patches experiment. (B)

BM histograms of (I)~(IV) DNA substrates. **(I)** BM of bare DNA is 35~45 nm. **(II)** After ScRad51 formed nucleoprotein filament on ssDNA, the distribution shifts to higher BM (~ 70 nm). **(III)** BM distribution after Oligo 10-13 were removed. **(IV)** The assembly of Dmc1 on 351/ss(40+41)/Rad51 DNA substrate leads to the higher BM value (~ 90 nm).

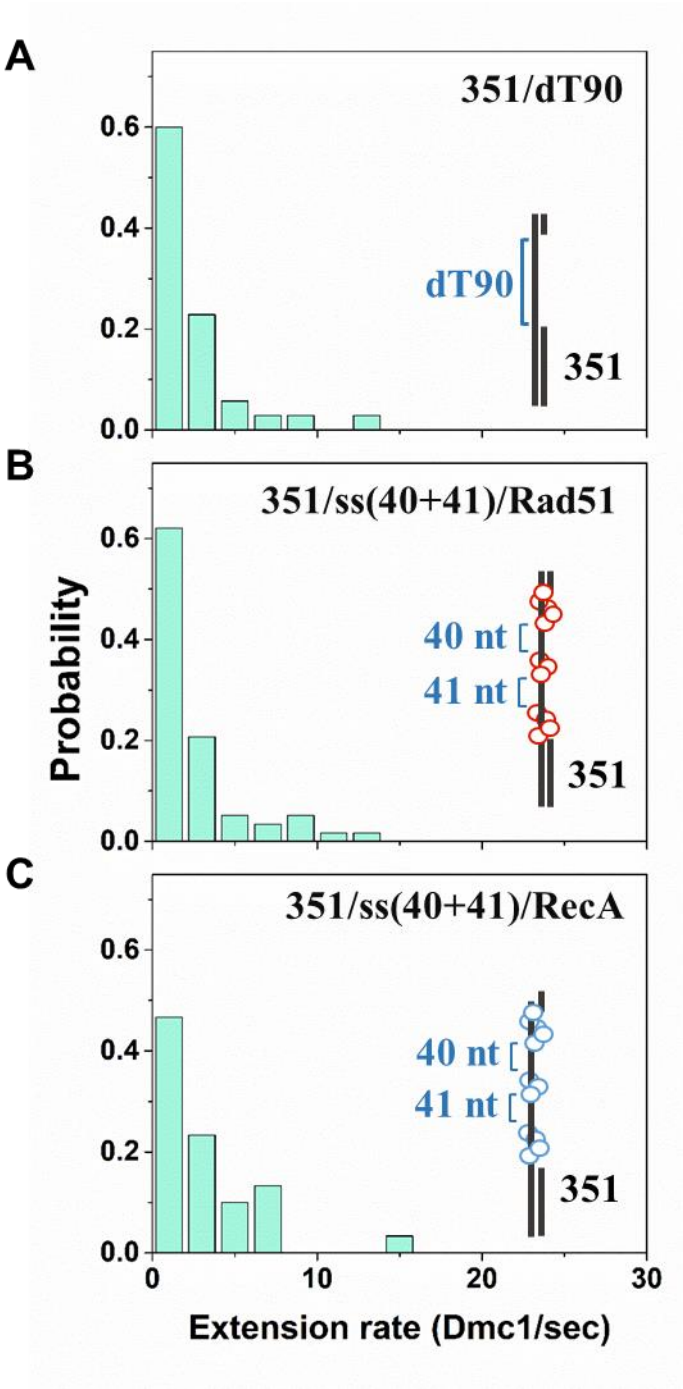

Figure S11. Extension rate histograms determined from Figure 6A-C. (A-C) Extension rate of ScDmc1 on 351/dT90 (A), 351/ss(40+41)/Rad51 (B) and 351/ss(40+41)/RecA (C), respectively. There is no statistically significant difference in extension rates among these three different DNA substrates.

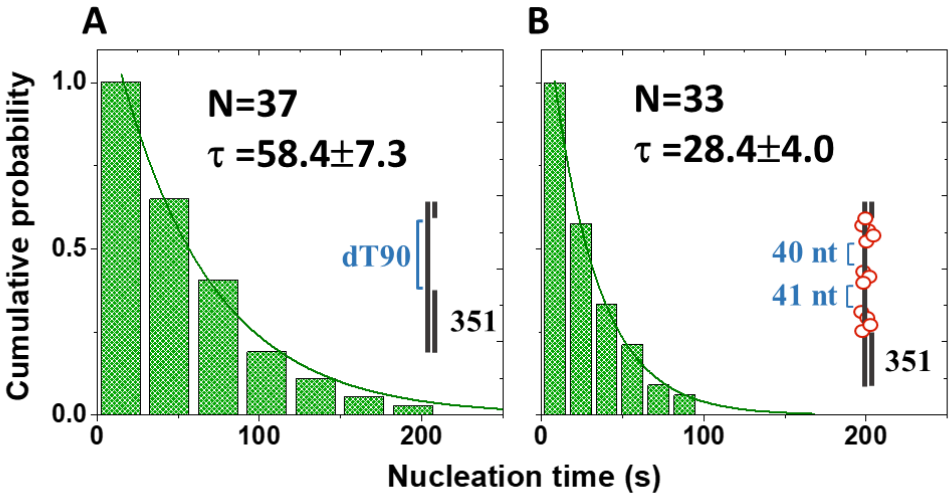

Figure S12. ScRad51 patches stimulate ScDmc1 nucleoprotein filament assembly in the presence of Ca^{2+} ions. Experiments are carried out similarly as shown in Figure 6A and 6B, but in the presence of 1 mM $CaCl_2$ and 4 mM ATP. DNA substrates used are 351/dT90 (A) and 351/ss(40+41)/ScRad51 (B) 1.1-μM ScDmc1 was used to measure the nucleation times. The error bars of nucleation time are the standard deviation by bootstrapping 5,000 times.

Table S1. Sequences of oligos used in protection assay and single-molecule experiments.

Name	Sequence (5′ to 3′)	Length
Oligo 1	TTATGTTCATTTTTTATATCCTTTACTTTATTTTCTCTGTTTATTCATTTACTTATTTTGTATTATCCTTATCTTATTTA	80 nt
Oligo 2	AAGGGAGAGCGTCGACCGAT-Tn-CTTACTGTCATGCCAT CCG, n=35, 90, 135, 165, 200	(39+n) nt
Oligo 3	biotin-CGGATGGCATGACAGTAAG	19 nt
Oligo 4	AAGGGAGAGCGTCGACCGAT-T45-CACAGAGACGACCATAAGATTCCA-T45-CTTACTGTCATGCCATCCG	152 nt
Oligo 5	AAGGGAGAGCGTCGACCGAT-T45-CCATTATGTTCCGGATCTGCAGT-T45-CACAGAGACGACCATAAGATTCCA-T45- CTTACTGTCATGCCATCCG	221 nt
Oligo 6	TGGAATCTTATGGTCGTCTCTGTG	24 nt
Oligo 7	ACTGCAGATCCGGAACATAATGG	23 nt
Oligo 8	TGGAATCTTATGGTCGTCTCTGTGTTT	27 nt
Oligo 9	TTTTGGAATCTTATGGTCGTCTCTGTG	27 nt
Oligo 10	AAAAAAAATGGTATCTTATG	20 nt
Oligo 11	GTCGTCTCAGTGTAAAAAAA	20 nt
Oligo 12	AAAAAAAAAACTCCTGATCCG	21 nt
Oligo 13	GAACATAAAGGAAAAAAAAA	20 nt
Oligo 14	CATAAGATACCATTTTTTT	20 nt
Oligo 15	TTTTTTTACACTGAGACGAC	20 nt
Oligo 16	CGGATCAGGAGTTTTTTTTT	21 nt
Oligo 17	TTTTTTTTCCTTTATGTTC	20 nt

Table S2. Fitting parameters in Figure 3B and Figure S3. The observed apparent nucleation rate can be expressed as: rate=[**recombinase**]^{n}*($k_{junction}$+k_{ss}*L_{ssDNA}). k_{ss} and $k_{junction}$ represents the nucleation rate constant on ssDNA and ss/dsDNA junction, respectively.

	ScRad51 (n=1.83)	**ScDmc1 (n=1.41)**
k_{ss} ($s^{-1}\cdot nt^{-1}\cdot \mu M^{-n}$)	$(3.78 \pm 0.44) \times 10^{-4}$	$(1.14 \pm 0.84) \times 10^{-5}$
$k_{junction}$ ($s^{-1}\cdot \mu M^{-n}$)	$(0.47 \pm 3.14) \times 10^{-3}$	$(9.01 \pm 1.26) \times 10^{-3}$
	ScRad51 (n=2)	**ScDmc1 (n=2)**
k_{ss} ($s^{-1}\cdot nt^{-1}\cdot \mu M^{-n}$)	$(4.39 \pm 0.52) \times 10^{-4}$	$(7.74 \pm 5.86) \times 10^{-6}$
$k_{junction}$ ($s^{-1}\cdot \mu M^{-n}$)	$(0.73 \pm 3.68) \times 10^{-3}$	$(6.19 \pm 0.88) \times 10^{-3}$