RECQ1 possesses DNA branch migration activity

Dmitry V. Bugreev, Robert M. Brosh, Jr., Alexander V. Mazin

SUPPORTING ONLINE INFORMATION

This supplement contains:

Supplementary Figure Legends

Supplementary Figures 1-5

Supplementary Table S1

SUPPLEMENTARY FIGURE 1. **RECQ1 helicase promotes the 3-stranded DNA branch migration in the 3'→5' direction.** *A,* Scheme depicts the 3-stranded branch migration. Mutually heterologous terminal regions are marked by crosses. Unpaired ssDNA regions (4 bases) that arise during branch migration are shown as a bubble. These non-homologous regions were introduced to block spontaneous branch migration. The asterisk indicates ^{32}P label at the 5'-end DNA. *B*, The DNA products of the branch migration reactions were visuailized by electrophoresis in a 8% polyacrylamide gel. Branch migration was initiated by addition of indicated amount of RECQ1 to appropriate DNA substrates followed by incubation for 30 min at 37 °C. In controls, storage buffer was added instead of RECQ1. Lanes 13 and 14 show migration of DNA markers (379*+379*/117) and (201*+201*/71), respectively. *C*, The data from panel (*B*) presented as a graph.

SUPPLEMENTARY FIGURE 2. **RAD54 protein promotes 3-stranded branch migration in either 3'→5' or 5'→3'directions.** *A*, Scheme depicts 3-stranded branch migration reaction. Mutually heterologous terminal regions are marked by crosses. Non-homologous ssDNA parts arise during branch migration are shown as a bubble (4 bases). These non-homologous regions were introduced to block spontaneous branch migration. The asterisk indicates ^{32}P label at the 5'-end DNA. *B*, The DNA products of the branch migration promoted by RAD54 were visuailized by electrophoresis in a 8% polyacrylamide gel. To prepare substrates that migrate in either 3'→5' or 5'→3'direction, ^{32}P-labeled DNA intermediates #169*/71 or #193*/117 (^{32}P-labeled strands marked by asterisk) (32 nM, molecules) were annealed with #201 or #379 oligonucleotides

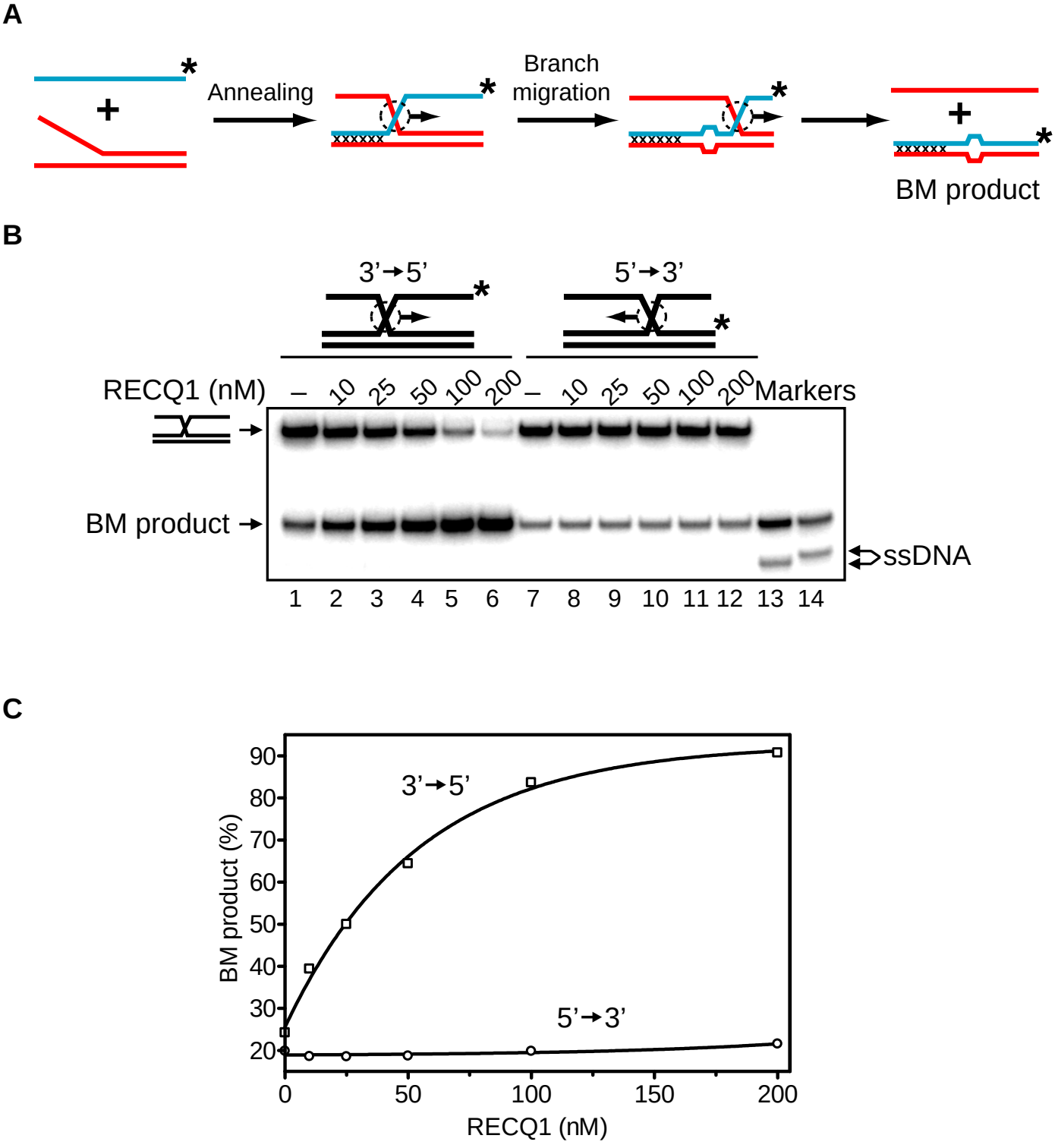
respectively (48 nM, molecules) by incubating in buffer containing 25 mM Tris acetate, pH 7.5, 2 mM ATP, 5 mM magnesium acetate, 2 mM DTT, BSA (100 μg/ml), 15 mM phosphocreatine and creatine phosphokinase (30 units/ml) for 15 min at 37 °C. Branch migration was initiated by addition of the indicated amounts of RAD54 to the reaction mixture containing branched structures followed by a 30-min incubation at 37 °C. *C*, The data from panel (*B*) presented as a graph. Error bars indicate s.e.m.

SUPPLEMENTARY FIGURE 3. **BLM helicase and RAD54 protein promote 4-stranded branch migration in either 3'→5' or 5'→3'directions.** *A*, Scheme represents 4-stranded branch migration. Mutually heterologous terminal regions are marked by crosses. Non-homologous ssDNA parts arise during branch migration are shown as a 1 base mismatch. These non-homologous regions were introduced to block spontaneous branch migration. The asterisk indicates ^{32}P label at the 5'-end DNA. *B*, The DNA products of the branch migration promoted by BLM were visualized by electrophoresis in a 8% polyacrylamide gel. To prepare substrates ^{32}P-labeled DNA intermediates #169*/71 or #193*/117 (^{32}P-labeled strands marked by asterisk) (32 nM, molecules) were mixed with #170/171 or #194/195 oligonucleotides, respectively (48 nM, molecules), and annealed by incubating in buffer containing 25 mM Tris acetate, pH 7.5, 2 mM ATP, 5 mM magnesium acetate, 2 mM DTT, BSA (100 μg/ml), 15 mM phosphocreatine and creatine phosphokinase (30 units/ml) for 15 min at 37 °C. Branch migration was initiated by addition of BLM (10 nM) to the reaction mixture containing branched DNA substrates followed by incubation for indicated periods of time at 37 °C. *C*, The data from panel (*B*) presented as a graph. *D*, The kinetics of 3-stranded branch

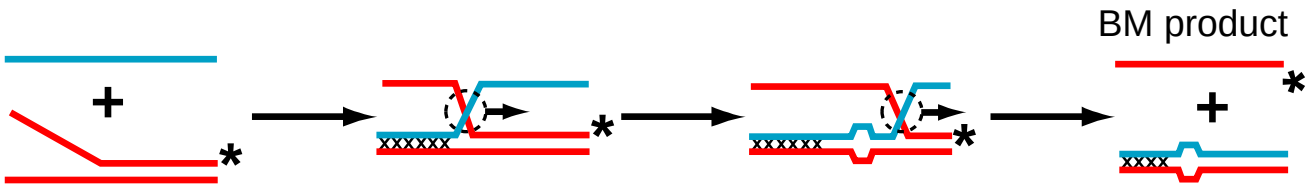
migration promoted by human RAD54 protein (50 nM), shown as a graph. Error bars in (*C*) and (*D*) indicate s.e.m.

SUPPLEMENTARY FIGURE 4. **ssDNA binding proteins, SSB and RPA, do not affect D-loop dissociation by RECQ1.** *A,* Experimental scheme. The asterisk indicates ^{32}P label at the 5'-end DNA. *B* and *D*, Dissociation of deproteinized D-loops (150 pM) by RECQ1 helicase (in indicated concentrations) in the presence of SSB (400 nM) or RPA (100 nM) was analyzed in a 1% agarose gel. RECQ1 was added to the D-loop with prebound SSB or RPA protein. The reactions with SSB and RPA were carried out for 30 min at 37 °C in buffers contained 1 mM and 5 mM magnesium acetate, respectively. In reactions with RPA, higher Mg^{2+} concentration was used because RPA has its own D-loop disruption activity at lower Mg^{2+} concentrations. *C* and *E*, The data from panel (*B* and *D*) are presented as a graph.

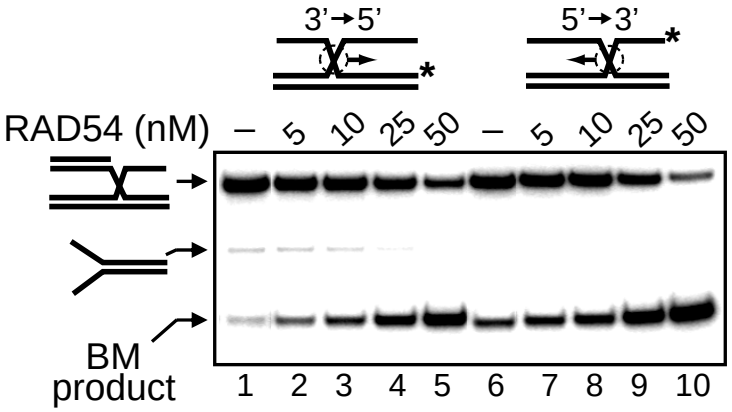
SUPPLEMENTARY FIGURE 5. **BLM helicase catalyzes dissociation of the 3'- and 5'-invaded joint molecules (D-loops) with similar efficiency.** *A,* Experimental scheme depicting reaction of dissociation of D-loops formed by the 3'- and 5'-invading ssDNA. The asterisk indicates ^{32}P label at the 5'-end DNA. *B*, Dissociation of deproteinized D-loops (150 pM) by BLM helicase (in indicated concentrations) was analyzed in a 1% agarose gel. *C*, The data from panel (*B*) are presented as a graph.


A
Annealing
Branch
migration
BM product
B
3'→5'
5'→3'
RECQ1 (nM)
–
10
25
50
100
200
Markers
BM product
ssDNA
1 2 3 4 5 6 7 8 9 10 11 12 13 14
C
BM product (%)
90
80
70
60
50
40
30
20
3'→5'
5'→3'
0
50
100
150
200
RECQ1 (nM)

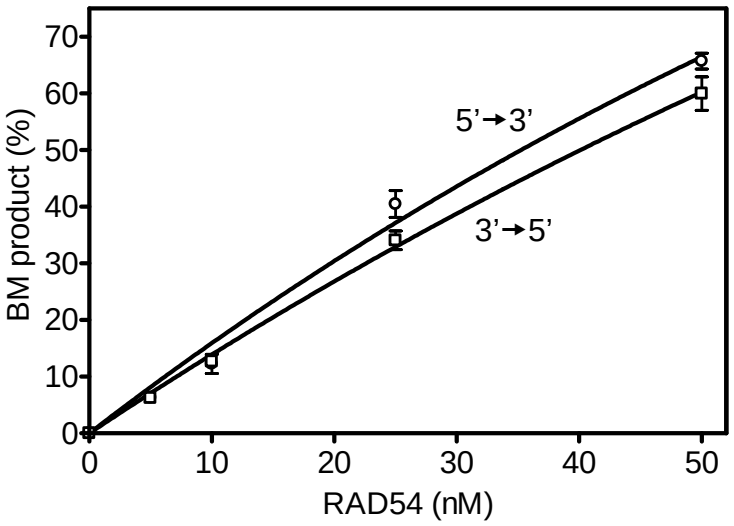
A

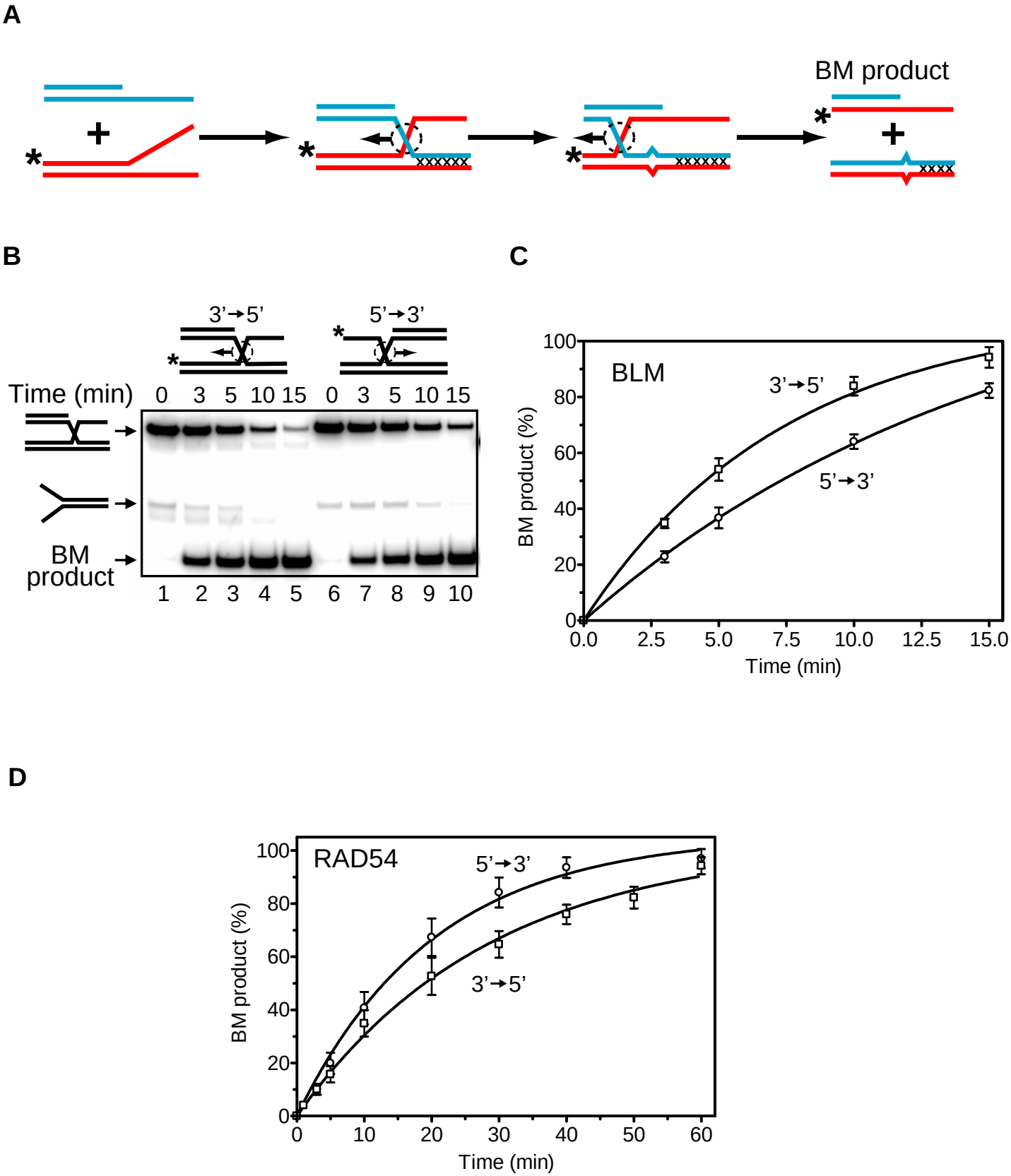

BM product

B

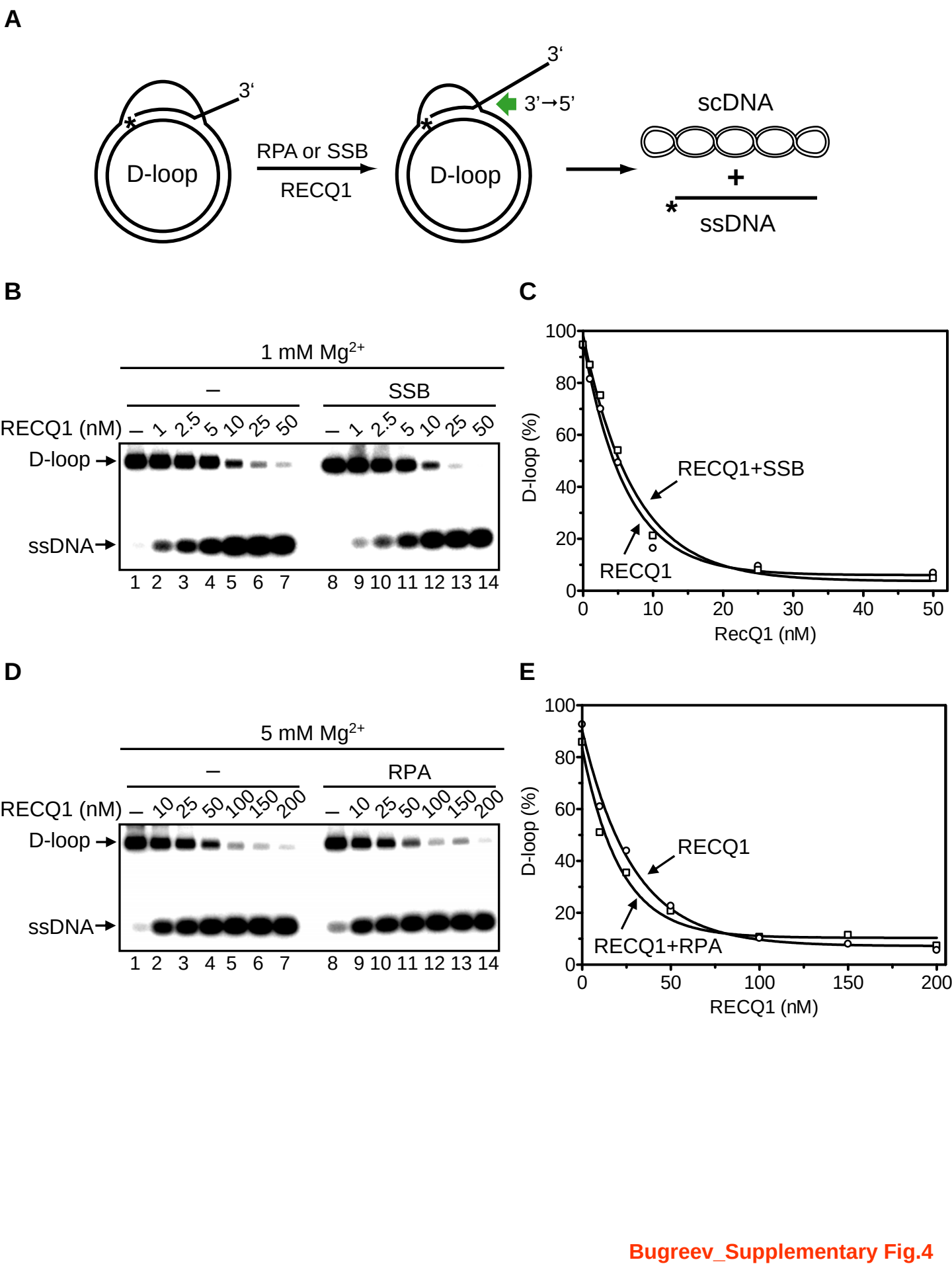

3'→5'
5'→3'
RAD54 (nM)
–
5
10
25
50
–
5
10
25
50
BM product
1
2
3
4
5
6
7
8
9
10

C

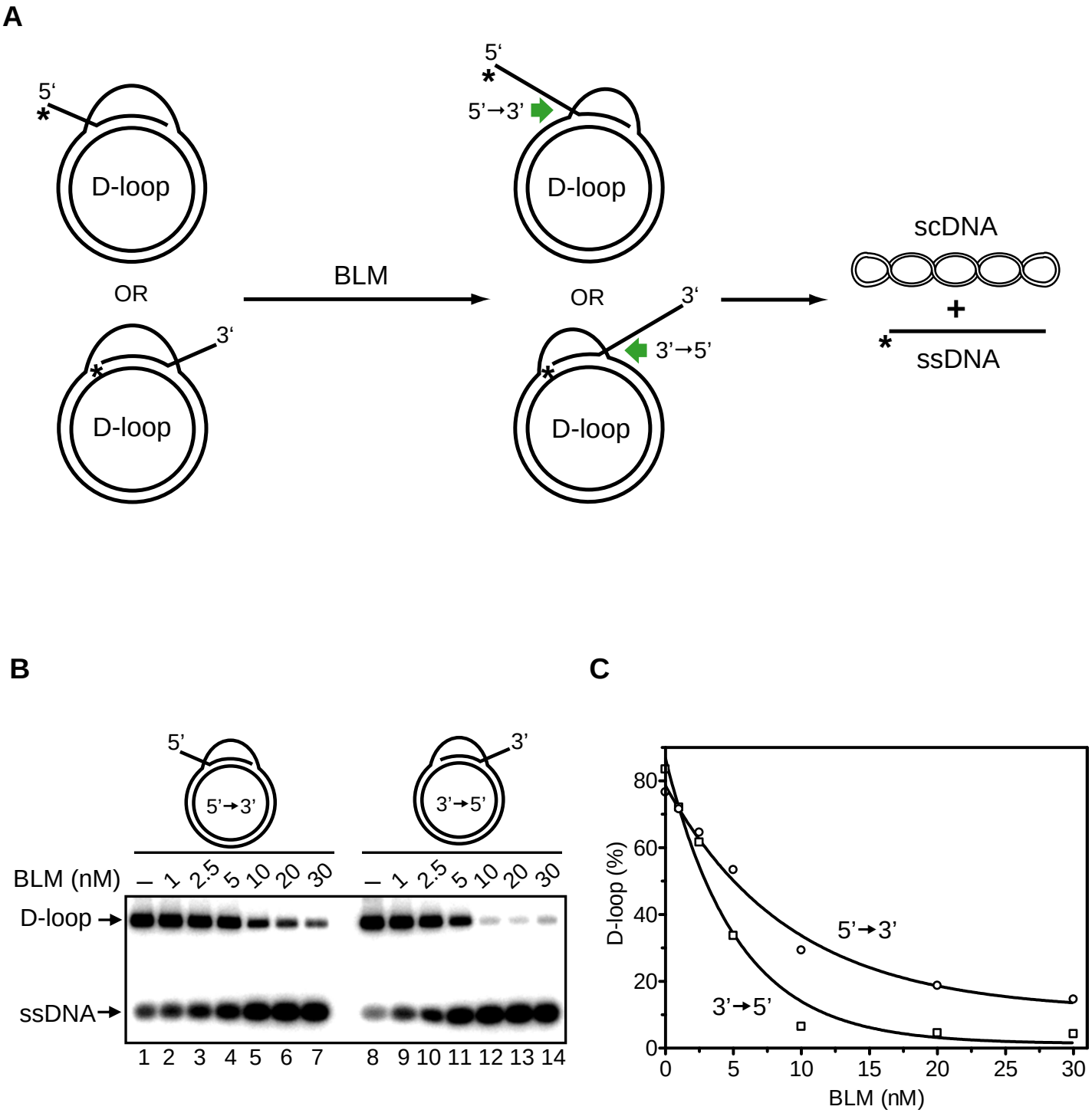

BM product (%)
70
60
50
40
30
20
10
0
5'→3'
3'→5'
0
10
20
30
40
50
RAD54 (nM)



Bugreev_Supplementary Fig.3



Bugreev_Supplementary Fig.4



Bugreev_Supplementary Fig.5

SUPPLEMENTARY TABLE S1. **Sequences of the oligonucleotides used in this study**

N	Length, nt	Sequence (5'→3')
2	63	TCCTTTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAATTGCTGAATCTGGTGCTGT
71	94	CTTTAGCTGCATATTTACAACATGTTGACCTACAGCACCAGATTCAGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATCAAAAGGA
117	94	TCCTTTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAATTGCTGAATCTGGTGCTGTAGGTCAACATGTTGTAAATATGCAGCTAAAG
169	93	TCCTTTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAATTGCTGAATCTGGTGCTGTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
170	94	TCCTTTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAATTGCTAAATCTGGTGCTGTAGGTCAACATGTTGTAAATATGCAGCTAAAG
171	63	ACAGCACCAGATTTAGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATCAAAAGGA
193	94	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCACAGCACCAGATTCAGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATCAAAAGGA
194	94	CTTTAGCTGCATATTTACAACATGTTGACCTACAGCACCAGATTCAGAAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTTATCAAAAGGA
195	63	TCCTTTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTTAATTTCTGAATCTGGTGCTGT

196	94	TCCTTTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTT AATTGCTGAATCTGGTGCTGTTGAGAGTGCACCATATGCGG TGTGAAATACC
201	94	TCCTTTTGATAAGAGGTCATTTTTGCGGATGGCTTAGAGCTT AATTATAAAATCTGGTGCTGTAGGTCAACATGTTGTAAATATG CAGCTAAAG
209	100	AATTCTCATTTTACTTACCGGACGCTATTAGCAGTGGGTGAG CAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGG GCGACACGGAAATGTTG
248	94	CTGAACGCCATGACTCGACCCACGTACTTCATAGACTTCAAC TTGCATAAAATCTGGTGCTGTAGGTCAACATGTTGTAAATAT GCAGCTAAAG
254	124	TAATATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGT GTCGCCCTTATTCCCTTTTTTGCGGCATTTTGCCTTCCTGTTT TTGCTCACCCAGAAACGCTGGTGAAAGTAAAAGATGCTG
255	124	CAGCATCTTTTACTTTCACCAGCGTTTCTGAAACGCTGGTCG TCATCCTAGTGGCGAATAAGTGCGCATTCTAGCAAATCCACA TATGGCTGaCAATACTCATACTCTTCCTTTTTCAATATTA
293	124	CAGCATCTTTTACTTTCACCAGCGTTTCTGACATAGCAAAAA CAGGAAGGCAAAATGCCGCAAAAAAGGGAATAAGGGCGACA CGGAAATTCGTAATACTCATACTCTTCCTTTTTCAATATTA
373	100	GGTGAGCAAAAACAGGAAGGCAAAATGCCGCAAAAAAGGGA ATAAGGGCGACACGGAAATGTTGAATTCTCATTTTACTTACC GGACGCTATTAGCAGTG
379	94	CTTTAGCTGCATATTTACAACATGTTGACCTACAGCAAAGAA TTCAGCAATTAAGCTCTAAGCCATCCGCAAAAATGACCTCTT ATCAAAAGGA