Supplementary Information

Processive DNA Unwinding by RecBCD Helicase in the Absence of Canonical Motor Translocation

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and Timothy M. Lohman*

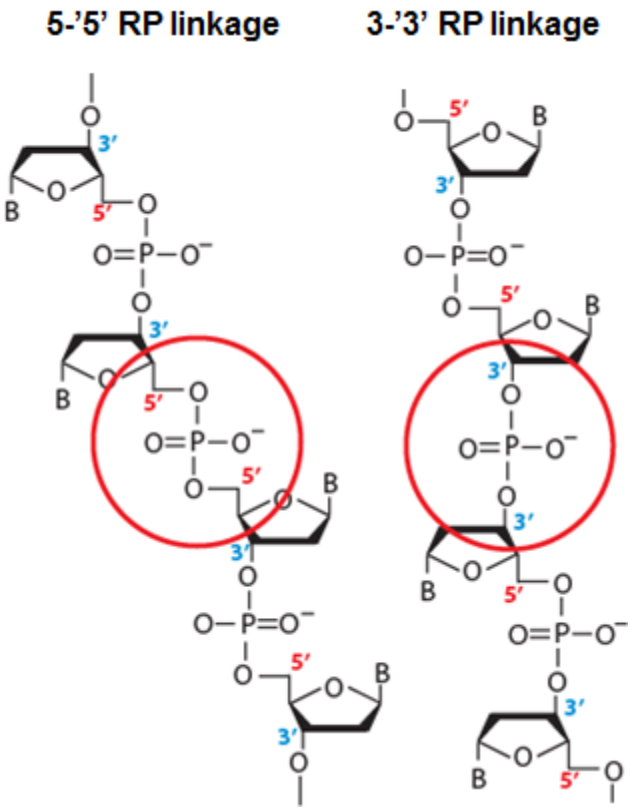

Figure S1. **Chemical structures of single stranded DNA with reversed polarity linkages in the phosphate backbone**. A 5'-5' linkage (left) and a 3'-3' linkage (right).

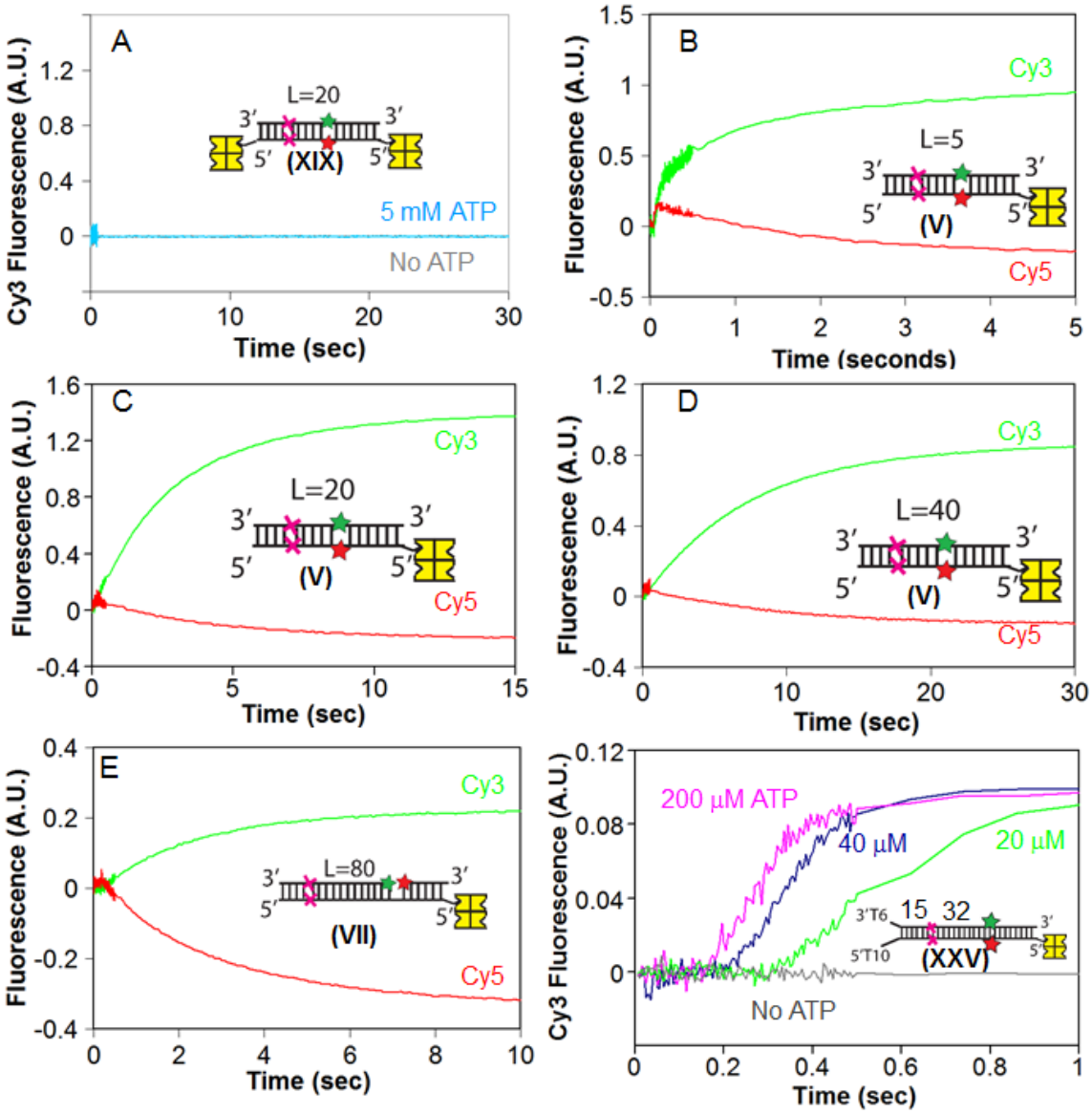
A
L=20
(XIX)
5 mM ATP
No ATP
Cy3 Fluorescence (A.U.)
Time (sec)
B
Cy3
L=5
(V)
Cy5
Fluorescence (A.U.)
Time (seconds)
C
Cy3
L=20
(V)
Cy5
Fluorescence (A.U.)
Time (sec)
D
Cy3
L=40
(V)
Cy5
Fluorescence (A.U.)
Time (sec)
E
Cy3
L=80
(VII)
Cy5
Fluorescence (A.U.)
Time (sec)
200 µM ATP
40 µM
20 µM
3'T6
15
32
5'T10
(XXV)
No ATP
Cy3 Fluorescence (A.U.)
Time (sec)

Figure S2

Figure S2. Biotin Streptavidin at the duplex DNA ends blocks DNA unwinding by RecBCD.

A. Control experiment testing ability of RecBCD to unwind dsDNA with streptavidin blocks on both ends. Stopped-flow experiments were performed by pre-incubating RecBCD (15 nM) with DNA XVI (20 nM) and mixing with ATP (5 mM) and heparin (8 mg/mL) (blue) or no ATP (gray) and heparin (8 mg/mL) in Buffer M30 at 25° C. Cy3 was excited at 505 nm, and Cy3 and Cy5 fluorescence were monitored simultaneously.

B. Experiments were conducted as in panel **A**, with DNA Series V (L=5 bp).

C. Experiments were conducted as in panel **A**, with DNA Series V (L=20 bp).

D. Experiments were conducted as in panel **A**, with DNA Series V (L=40 bp).

E. Experiments were conducted as in panel **A**, with DNA Series VI (L=80 bp) Cy3 time courses (green) and Cy5 time courses (red) are shown.

F. Experiments were conducted as in panel A, with DNA XXV at 200 μM, 40 μM, 20 μM, and no ATP.

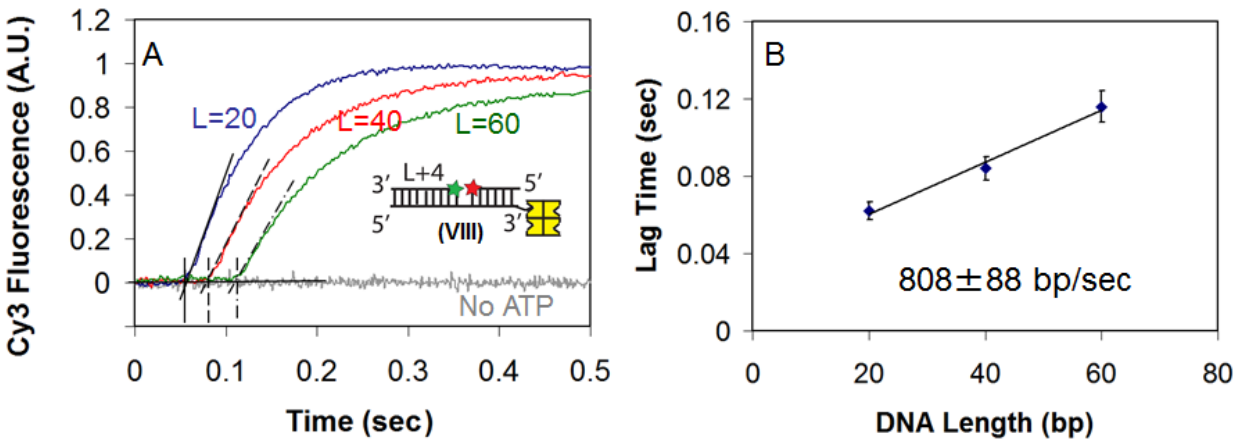

Figure S3. RecBCD unwinding of normal DNA.

A. Stopped-flow time experiments were performed by mixing pre-incubated RecBCD (15 nM) and DNA Series VIII (20 nM) with ATP (5 mM) and heparin (8 mg/mL) in Buffer M30 at 25° C. Cy3 was excited at 505 nm, and Cy3 and Cy5 fluorescence were monitored simultaneously. Cy3 fluorescence time course are shown for L=20 bp (blue), L=40 bp (red), and L=60 bp (green).

B. Lag times for RecBCD unwinding of normal DNA (VIII) increase linearly with duplex length, L, (5mM ATP).

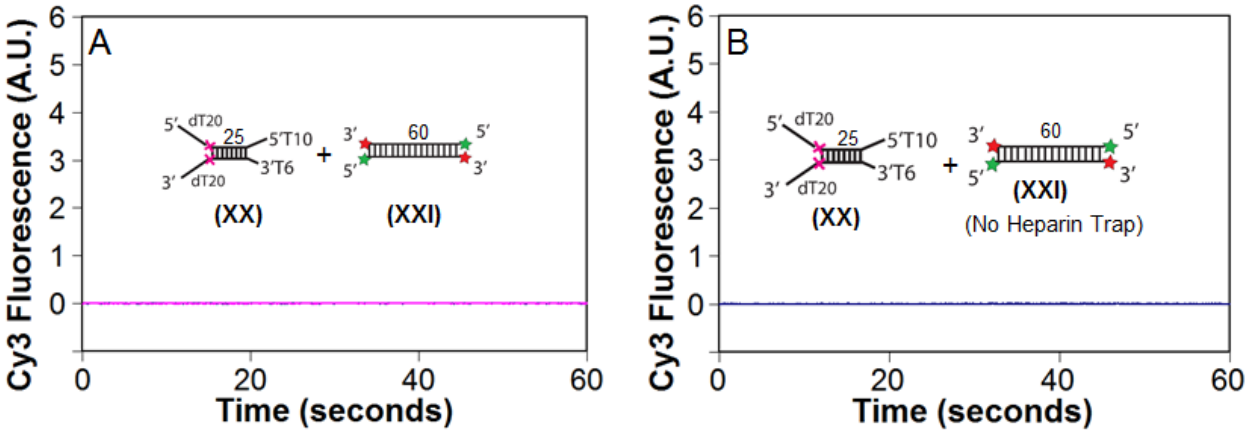

Figure S4. RecBCD remains at RP linkages in the absence of a heparin trap.

A. Experiments were conducted as in Fig. 1B, with RecBCD (18.75 nM) pre-incubated with DNA XX (25 nM) and XXI (25 nM).

B. Experiments were conducted as in panel **A**, except with no heparin trap.

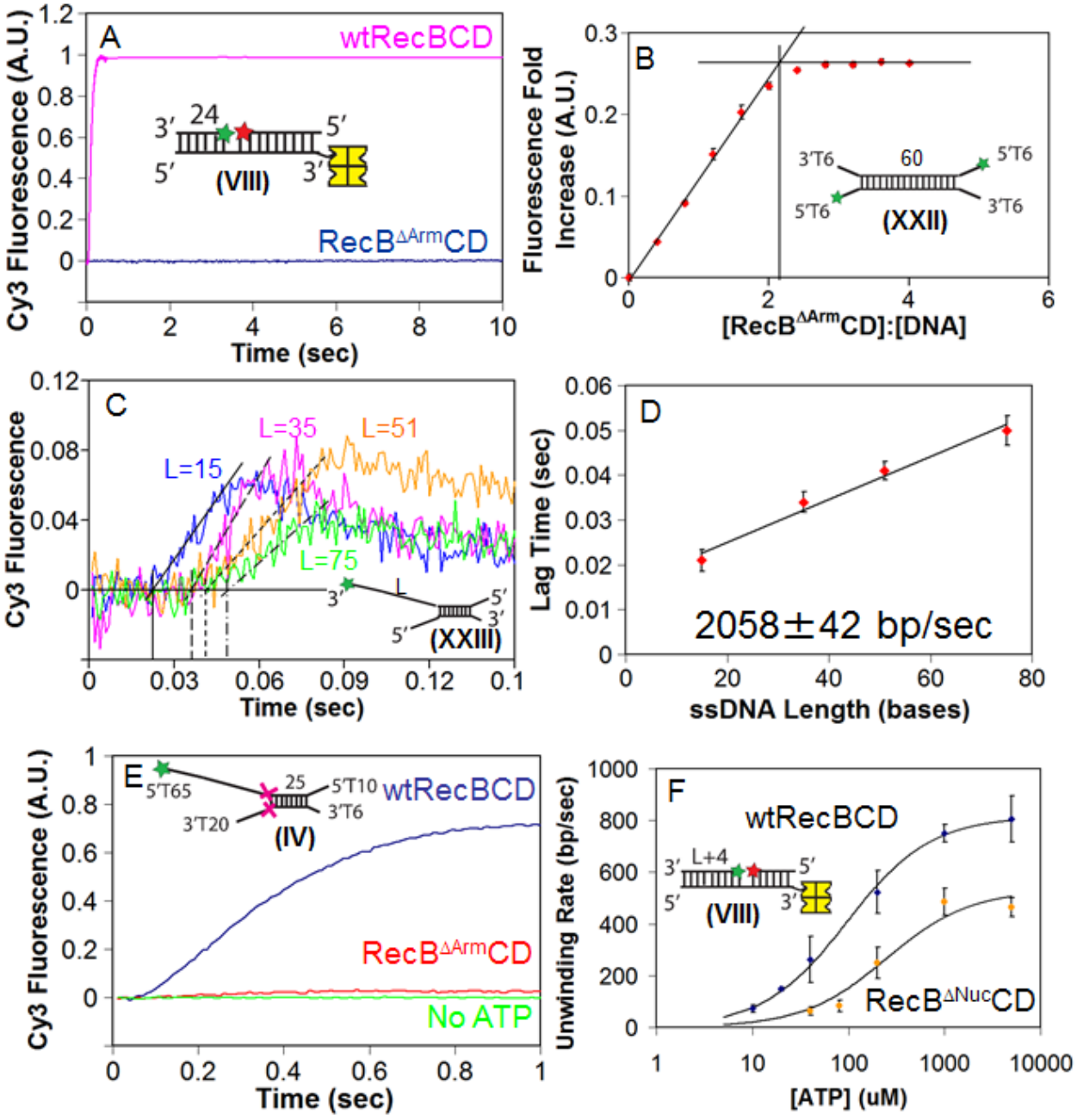
A
wtRecBCD
RecB^ΔArmCD
(VIII)
Cy3 Fluorescence (A.U.)
Time (sec)
B
Fluorescence Fold Increase (A.U.)
[RecB^ΔArmCD]:[DNA]
(XXII)
C
L=35
L=51
L=15
L=75
(XXIII)
Cy3 Fluorescence
Time (sec)
D
2058±42 bp/sec
Lag Time (sec)
ssDNA Length (bases)
E
(IV)
wtRecBCD
RecB^ΔArmCD
No ATP
Cy3 Fluorescence (A.U.)
Time (sec)
F
wtRecBCD
RecB^ΔNucCD
(VIII)
Unwinding Rate (bp/sec)
[ATP] (uM)

Figure S5 (A-F)

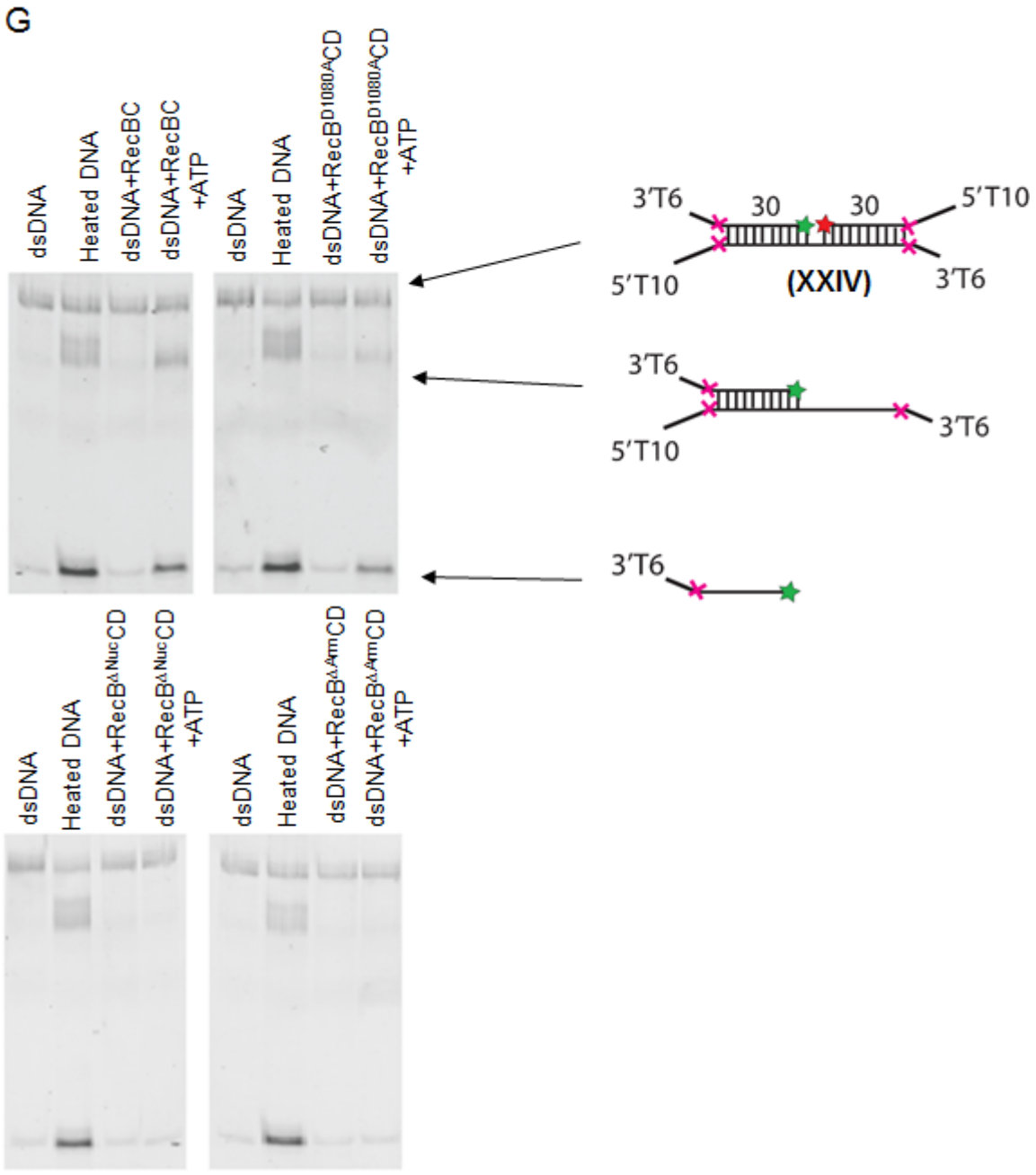
G
dsDNA
Heated DNA
dsDNA+RecBC
dsDNA+RecBC +ATP
dsDNA
Heated DNA
dsDNA+RecB D1080A CD
dsDNA+RecB D1080A CD +ATP
3'T6
30
30
5'T10
5'T10
(XXIV)
3'T6
3'T6
5'T10
3'T6
3'T6
dsDNA
Heated DNA
dsDNA+RecB ΔNuc CD
dsDNA+RecB ΔNuc CD +ATP
dsDNA
Heated DNA
dsDNA+RecB ΔArm CD
dsDNA+RecB ΔArm CD +ATP

Figure S5 (G)

Figure S5. ssDNA translocation and DNA unwinding properties of RecB$^{\Delta Arm}$CD and RecB$^{\Delta Nuc}$CD.

A. Stopped-flow time experiments were performed by mixing pre-incubated RecBCD (15 nM) (pink) or RecB$^{\Delta Arm}$CD (blue) (15 nM) and DNA Series VIII (L=20 bp) (20 nM) with ATP (5 mM) and heparin (8 mg/mL) in Buffer M30 at 25° C. Cy3 was excited at 505 nm, and Cy3 and Cy5 fluorescence were monitored simultaneously.

B. RecB$^{\Delta Arm}$CD binds with high affinity (stoichiometrically) to DNA ends possessing 3'-dT6/5'-dT6 tails (DNA XXII). Increase in Cy3 fluorescence was monitored upon titration of Cy3-labeled DNA (20 nM, 40 nM DNA ends) with RecB$^{\Delta Arm}$CD in Buffer M (30 mM NaCl), 25˚C. Cy3 fluorescence was excited at 515 nm, emissions were collected at 570 nm.

C. Stopped-flow time experiments monitoring 5' to 3' ssDNA translocation of RecB$^{\Delta Arm}$CD. RecB$^{\Delta Arm}$CD (18.75 nM) pre-incubated with DNA series XXIII (25 nM) (with (dT_L) extensions) in Buffer M (250 mM NaCl) in a 1:10 volumetric ratio with ATP (various concentrations) and heparin (8 mg/mL) in buffer M (8 mM NaCl) yielding the final concentrations listed above and in the figure, and a final NaCl concentration of 30 mM at 25°C. Cy3 fluorescence time courses are shown for L=15 nt (blue), L=35 nt (pink), L=51 nt (orange), and L=75 nt (green).

D. Lag times from the experiments in panel **C** increase linearly with L yielding a rate of 2058±42 nt/sec for 5'-3' translocation of RecB$^{\Delta Arm}$CD along ssDNA at (5 mM ATP) RecB$^{\Delta Arm}$CD.

E. RecB$^{\Delta Arm}$CD shows little evidence for secondary translocase activity. Stopped-flow experiments monitoring Cy3 fluorescence performed by pre-incubating RecBCD or

RecB$^{\Delta Arm}$CD (18.75 nM) with DNA IV (25 nM) in Buffer M (250 mM NaCl) in a 1:10 volumetric ratio with ATP (5 mM) and heparin (8 mg/mL) in buffer M (8 mM NaCl) yielding the final concentrations listed above and a final NaCl concentration of 30 mM at 25°C. RecBCD (blue), RecB$^{\Delta Arm}$CD (red), or RecBCD in the absence of ATP (green).

F. ATP dependence of rates of normal DNA unwinding by RecBCD (blue) and RecB$^{\Delta Nuc}$CD (orange). Experiments were performed as described in Fig. 5C, at the indicated ATP concentrations.

G. RecB^{1080A}CD and RecBC, but not RecB$^{\Delta Arm}$CD or RecB$^{\Delta Nuc}$CD show DNA strand separation of RP DNA in a standard DNA helicase assay. DNA XXIV (25 nM), helicase (20 nM), and ATP (1 mM) were mixed in Buffer M (30 mM NaCl) at 22˚C, and the reaction was stopped with EDTA after 3 minutes. Bound protein was then removed by adding SDS (2.5%). Each reaction was loaded onto a non-denaturing 10% PAGE TBE gel. Control reactions lacked ATP (third lane) or ATP and enzyme (first and second lane). DNA in the second lanes were heated at 95 degrees for 3 minutes and then cooled before loading. The fourth lane contained DNA, ATP, and a variant of the RecBCD helicase as indicated.

Strand I-1	3'- TTT TTT GGT ACC GAG GAC TCG ATC GAC GTC TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TT **Cy3**-5′
Strand I-2	5'- T TTT TTT TTT CCA TGG CTC CTG AGC TAG CTG CAG TTT TTT TTT TTT TTT TTT TT-3′
Strand II-1	3'- TTT TTT GGT ACC GAG GAC TCG ATC GAC GTC T -5'-5'- TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TCC ATG GAT ACG TCG AGT CGC ATC C **Cy3** T-3'
Strand II-2	5'- T TTT TTT TTT CCA TGG CTC CTG AGC TAG CTG CAG T -3'-3'- TTT TTT TTT TTT TTT TTT T-5′
Strand III-1	5'-T TTT TTT TTT CCA TGG CTC CTG AGC TAG CTG CAG TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT T TCC ATG GAT ACG TCG AGT CGC ATC C **Cy3** T-3'
Strand III-2	3'-TTT TTT GGT ACC GAG GAC TCG ATC GAC GTC TTT TTT TTT TTT TTT TTT TT-5'
Strand IV-1	5'-T TTT TTT TTT CCA TGG CTC CTG AGC TAG CTG CAG T-3'-3'-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TCC ATG GAT ACG TCG AGT CGC ATC C **Cy3**-5'
Strand IV-2	3'-TTT TTT GGT ACC GAG GAC TCG ATC GAC GTC T-5'-5'-TTT TTT TTT TTT TTT TTT T-3'
DNA Series V	
L=1 Strand 1	3'-GGT C-5'-5'-G **Cy3** C TCC TGA GCT AGC TGC AGT AGC CTA TGC TCC AG-3'
L=1 Strand 2	5'-CCA G-3'-3'-C **Cy5** G AGG ACT CGA TCG ACG TCA TCG GAT ACG AGG TC-biotin-5'
L=3 Strand 1	3'-GGT C-5'-5'-GCT **Cy3** CCT GAG CTA GCT GCA GTA GCC TAT GCT CCA G-3'
L=3 Strand 2	5'-CCA G-3'-3'-CGA **Cy5** GGA CTC GAT CGA CGT CAT CGG ATA CGA GGT C-biotin-5'
L=5 Strand 1	3'-GGT C-5'-5'-GCT CC **Cy3** TGA GCT AGC TGC AGT AGC CTA TGC TCC AG-3'
L=5 Strand 2	5'-CCA G-3'-3'-CGA GG **Cy5** ACT CGA TCG ACG TCA TCG GAT ACG AGG TC-biotin-5'
L=20 Strand 1	3′-GGT C-5'-5'-GC TCC TGA GCT AGC TCC AGT **Cy3** AG CCT ATG GTC CAG CTC ACC TCC ATC GCG TTG GCA CTG CAC TGA CGT GAG AGA CGG-3′
L=20 Strand 2	5'-CCA G-3'-3'-CG AGG ACT CGA TCG AGG TCA **Cy5** TC GGA TAC CAG GTC GAG TGG AGG TAG CGC AAC CGT GAC GTG ACT GCA CTC TCT GCC-biotin-5'
L=40 Strand 1	3′-GGT C-5'-5'-GC TCC TGA GCT AGC TCC AGT AGC CTA TGG TCC AGC TCA CC **Cy3** TCC ATC GCG TTG GCA CTG CAC TGA CGT GAG AGA CGG-3′
L=40 Strand 2	5'-CCA G-3'-3'-CG AGG ACT CGA TCG AGG TCA TCG GAT ACC AGG TCG AGT GG **Cy5** AGG TAG CGC AAC CGT GAC GTG ACT GCA CTC TCT GCC-biotin-5'
DNA Series VI	
Series VI Template	5'-CCA G-3'-3'-C GAG GAC TCG ATC GAG GTC ATC GGA TAC CAG GTC GAG TGG AAG TAG CGC AAC CGT GAC GAG ACT GCA CTC TCT GCC-biotin-5'
L=20n Strand 1	3′-GGT C-5'-5'-G CTC CTG AGC TAG CTC CAG **Cy3** T-3'
L=20n Strand 2	5'-**Cy5** AG CCT ATG GTC CAG CTC ACC TTC ATC GCG TTG GCA CTG CTC TGA CGT GAG AGA CGG-biotin-3′
L=40n Strand 1	3′-GGT C-5'-5'-G CTC CTG AGC TAG CTC CAG TAG CCT ATG GTC CAG CTC ACC T **Cy3** T-3'
L=40n Strand 2	5'-**Cy5** CC ATC GCG TTG GCA CTG CTC TGA CGT GAG AGA CGG-biotin-3′
L=60n Strand 1	3′-GGT C-5'-5'-G CTC CTG AGC TAG CTC CAG TAG CCT ATG GTC CAG CTC ACC TTC ATC GCG TTG GCA CTG C **Cy3** T-3'
L=60n Strand 2	5'-**Cy5** C TGA CGT GAG AGA CGG-biotin-3′
DNA VII	
Substrate VII Template	5'-CCA G-3'-3'-C GAG GAC TCG ATC GAG CTG ATC GGA TAC CAG GTC GAG TGG AAG TAG CGC AAC CGT CAG GTG ACT GCA CTC TCT GCA AGA ACC TCG TGA GCG AGG CC-biotin-5'
L=80n Strand 1	3′-GGT C-5'-5'-G CTC CTG AGC TAG CTC CAG TAG CCT ATG GTC CAG CTC ACC TTC ATC GCG TTG GCA CTG CAC TGA CGT GAG AGA CGT TCT **Cy3** T-3'
L=80n Strand 2	5'-**Cy5** GG AGC ACT CGC TCC GG-biotin-3'
DNA Series VIII	
Series VII Template	5'-CCA GCG AGG ACT CGA TCG AGA TCA ACG GAT ACC AGG TCG AAT GGA GGT AGC GCA ACC GTG ACG TAA CTG CAC TCT CTG CC-biotin-3'
L=20n Strand 1	3'-GGT CGC TCC TGA GCT AGC TCT AGT-**Cy3**-5'
L=20n Strand 2	3'-T **Cy5** GC CTA TGG TCC AGC TTA CCT CCA TCG CGT TGG CAC TGC ATT GAC GTG AGA GAC GG-5'
L=40n Strand 1	3'-GGT CGC TCC TGA GCT AGC TCT AGT TGC CTA TGG TCC AGC TTA CC-**Cy3**-5'
L=40n Strand 2	3'-T **Cy5** CCA TCG CGT TGG CAC TGC ATT GAC GTG AGA GAG GG-5'
L=60n Strand 1	3'-GGT CGC TCC TGA GCT AGC TCT AGT TGC CTA TGG TCC AGC TTA CCT CCA TCG CGT TGG CAC TGC A **Cy3**-5'
L=60n Strand 2	3'-T **Cy5** T GAC GTG AGA GAC GG-5'

Strand IX-1	3'-TTT TTT-5'-5'-GCT CCT GAG CTA GCT GCA GT **Cy3** AGC CTA TGC TCC AGC TCG GTC-3'-3′-TTT TTT-5′
Strand IX-2	5'-TTT TTT-3'-3'-CGA GGA CTC GAT CGA CGT CA **Cy5** TCG GAT ACG AGG TCG AGC CAG-5'-5'-TTT TTT-3'
Strand X-1	3 '-T-**Cy3** TT TTT GCT CCT GAG CTA GCT CCA GTA GCT ATG GTC CAG CTC ACC TCC ATC GG TTG GCA CTG CAC TGA CGT GAG AGA CGG-5'
Strand X-2	5'-T TTT TTT TTT CGA GGA CTC GAT CGA GGT CAT CGA TAC CAG GTC GAG TGG AGG TAG CG AAC CGT GAC GTG ACT GCA CTC TCT GCC -biotin-3'
Strand XI-1	3′- T **Cy3** TT TTT-5'-5'-G CTC CTG AGC TAG CTC CAG TAG CCT ATG GTC CAG CTC ACC TCC ATC GCG TTG GCA CTG CAC TGA CGT GAG AGA CGG-3′
Strand XI-2	5'-T TTT TTT TTT TTT-3'-3'-C GAG GAC TCG ATC GAG GTC ATC GGA TAC CAG GTC GAG TGG AGG TAG CGC AAC CGT GAC GTG ACT GCA CTC TCT GCC -biotin-5'
Strand XII-1	3'-TTT TTT G **Cy5** CTC CTG AGC TAG CTC CAG TAG CCT ATG GTC CAG CTC ACC TCC ATC GCG TTG GCA CTG CAC T **Cy3** GA CGT GAG AGA CGG-5'
Strand XII-2	5'-T TTT TTT TTT C GAG GAC TCG ATC GAG GTC ATC GGA TAC CAG GTC GAG TGG AGG TAG CGC AAC CGT GAC GTG ACT GCA CTC TCT GCC-biotin-3'
Strand XIII-1	3′- TTT TTT-5'5'-G **Cy5** CTC CTG AGC TAG CTC CAG TAG CCT ATG GTC CAG CTC ACC TCC ATC GCG TTG GCA CTG CAC T **Cy3** GA CGT GAG AGA CGG-3′
Strand XIII-2	5'-T TTT TTT TTT C GAG GAC TCG ATC GAG GTC ATC GGA TAC CAG GTC GAG TGG AGG TAG CGC AAC CGT GAC GTG ACT GCA CTC TCT GCC-biotin-3'
Strand XIV-1	3'-TTT TTT TAT ACA GAT UCA AGT TCC AGT AGC CTA TGG TCC AGC TCA C **Cy3** CT CCA TCG CGT TGG CAC TGC ACT GAC GTG AGA GAC GG -5'
Strand XIV-2	5'-T TTT TTT TTT ATA TGT CTA AGT TCA AGG TCA TCG GAT ACC AGG TCG AGT G **Cy5** GA GGT AGC GCA ACC GTG ACG TGA CTG CAC TCT CTG CC-biotin-3'
Strand XV-1	3'-GGAG-5'-5'-GCT CCT GAG CTA GCT GCA GT **Cy3** AGC CTA TGC TCC AGC TCG GTC-3'-3′-GACC-5′
Strand XV-2	5'-CCTC-3'-3'-CGA GGA CTC GAT CGA CGT CA **Cy5** TCG GAT ACG AGG TCG AGC CAG-5'-5'-CTGG-3'
Strand XVI-1	5'-T TTT TTT TTT CCA TGG CTC CTG AGC TAG CTG CAG TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTC CAT GGA TAC GTC GAG TCG CAT CC **Cy3** T-3'
Strand XVI-2	3'-TTT TTT GGT ACC GAG GAC TCG ATC GAC GTC TTT TTT TTT TTT TTT TTT TT-5'
Strand XVI-3	3'-G GTA CCT ATG CAG CTC AGC GTA GG **Cy5** -5'
Strand XVII-1	5'-T TTT TTT TTT CCA TGG CTC CTG AGC TAG CTG CAG T-3'-3'-TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TCC ATG GAT ACG TCG AGT CGC ATC C **Cy3**-5'
Strand XVII-2	3'-TTT TTT GGT ACC GAG GAC TCG ATC GAC GTC T-5'-5'-TTT TTT TTT TTT TTT TTT T-3'
Strand XVII-3	5'-GG TAC CTA TGC AGC TCA GCG TAG G **Cy5** T-3'
Strand XVIII-1	5'- T TTT TTT TTT CCA TGG CTC CTG AGC TAG CTG CAG T -3'-3'- TTT TTT TTT TTT TTT TTT T-5′
Strand XVIII-2	3'- TTT TTT GGT ACC GAG GAC TCG ATC GAC GTC T -5'-5'- TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TCC ATG GAT ACG TCG AGT CGC ATC C **Cy3** T-3'
Strand XVIII-3	3'-GG TAC CTA TGC AGC TCA GCG TAG G **Cy5** -5'
Strand XIX-1	3'-GGT C-5'-5'-GCT CCT GAG CTA GCT GCA GT **Cy3** AGC CTA TGC TCC AG-3'
Strand XIX-2	5'-biotin-CCA G-3'-3'-CGA GGA CTC GAT CGA CGT CA **Cy5** TCG GAT ACG AGG TC-biotin-5'
Strand XX-1	3'-TTT TTT GGT ACC GAG GAC TCG ATC GAC GTC T-5'-5'-TTT TTT TTT TTT TTT TTT T-3'
Strand XX-2	5'- T TTT TTT TTT CCA TGG CTC CTG AGC TAG CTG CAG T-3'-3'-TTT TTT TTT TTT TTT TTT T-5′
Strand XXI-1	5'-**Cy3** CAG GAG CTC CTG AGC TAG CTG CAG TAG CCT AAA GGA TGA AAC TAG GAT CTT ATG CTC CTG **Cy5** T-3'
Strand XXI-2	3'-T **Cy5** GTC CTC GAG GAC TCG ATC GAC GTC ATC GGA TTT CCT ACT TTG ATC ATC GAA TAC GAG GAC **Cy3**-5'
Strand XXII-1	5'-**Cy3** TTT TTT CAG GAG CTC CTG AGC TAG CTG CAG TAG CCT AAA GGA TGA AAC TAG GAT CTT ATG CTC CTG TTT TTT-3'
Strand XXII-2	3'-TTT TTT GTC CTC GAG GAC TCG ATC GAC GTC ATC GGA TTT CCT ACT TTG ATC ATC GAA TAC GAG GAC TTT TTT **Cy3**-5'
DNA Series XXIII	
L=15 Strand 1	5'-T TTT TTT TTT CCA TGG CTC CTG AGC TAG CTG CAG TTT TTT TTT TTT TTT **Cy3** T-3'
L=35 Strand 1	5'-T TTT TTT TTT CCA TGG CTC CTG AGC TAG CTG CAG TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT **Cy3** T-3'
L=51 Strand 1	5'-T TTT TTT TTT CCA TGG CTC CTG AGC TAG CTG CAG TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT **Cy3** T-3'
L=75 Strand 1	5'-T TTT TTT TTT CCA TGG CTC CTG AGC TAG CTG CAG TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT TTT **Cy3** T-3'
Series XXIII Strand 2	3'-TTT TTT GGT ACC GAG GAC TCG ATC GAC GTC TTT TTT TTT TTT TTT TTT TT-5'

Strand XXIV Template	3'-TTT TTT-5′-5'-CG TCA GTG CAG TGC CAA CGC GAT GGA GGT GAG CTG GAC CAT AGG CTA CTG GAG CTA GCT C-3'-3'-TTT TTT TTT T-5'
Strand XXIV-1	3'-T **Cy3** C GAC CTG GTA TCC GAT GAC CTC GAT CGA G-5'-5'-TTT TTT-3'
Strand XXIV-2	5'-T TTT TTT TTT-3'-3'-GC AGT CAC GTC ACG GTT GCG CTA CCT CCA C **Cy5**-5'

Strand XXV-1	3′-TTT TTT GCT CCT GAG CTA GCT-5'-5'-C CAG TAG CCT ATG GTC CAG CTC ACC TCC ATC G **Cy3** CG TTG GCA CTG CAC TGA CGT GAG AGA CGG-3'
Strand XXV-2	5'-T TTT TTT TTT CGA GGA CTC GAT CGA-3'-3'-G GTC ATC GGA TAC CAG GTC GAG TGG AGG TAG C **Cy5** GC AAC CGT GAC GTG ACT GCA CTC TCT GCC-biotin-5'

Supplementary Table S1:

Supplementary Table S1:

Table of oligonucleotide sequences annealed to construct substrates for experiments in this paper. Dyes are indicated by "Cy3" or "Cy5 in the sequence. Reverse polarity linkages are indicated by "-3'-3'-" or "-5'-5'-" in the sequence.