Supplementary Information

for

Srs2 prevents Rad51 filament formation by repetitive motion on DNA

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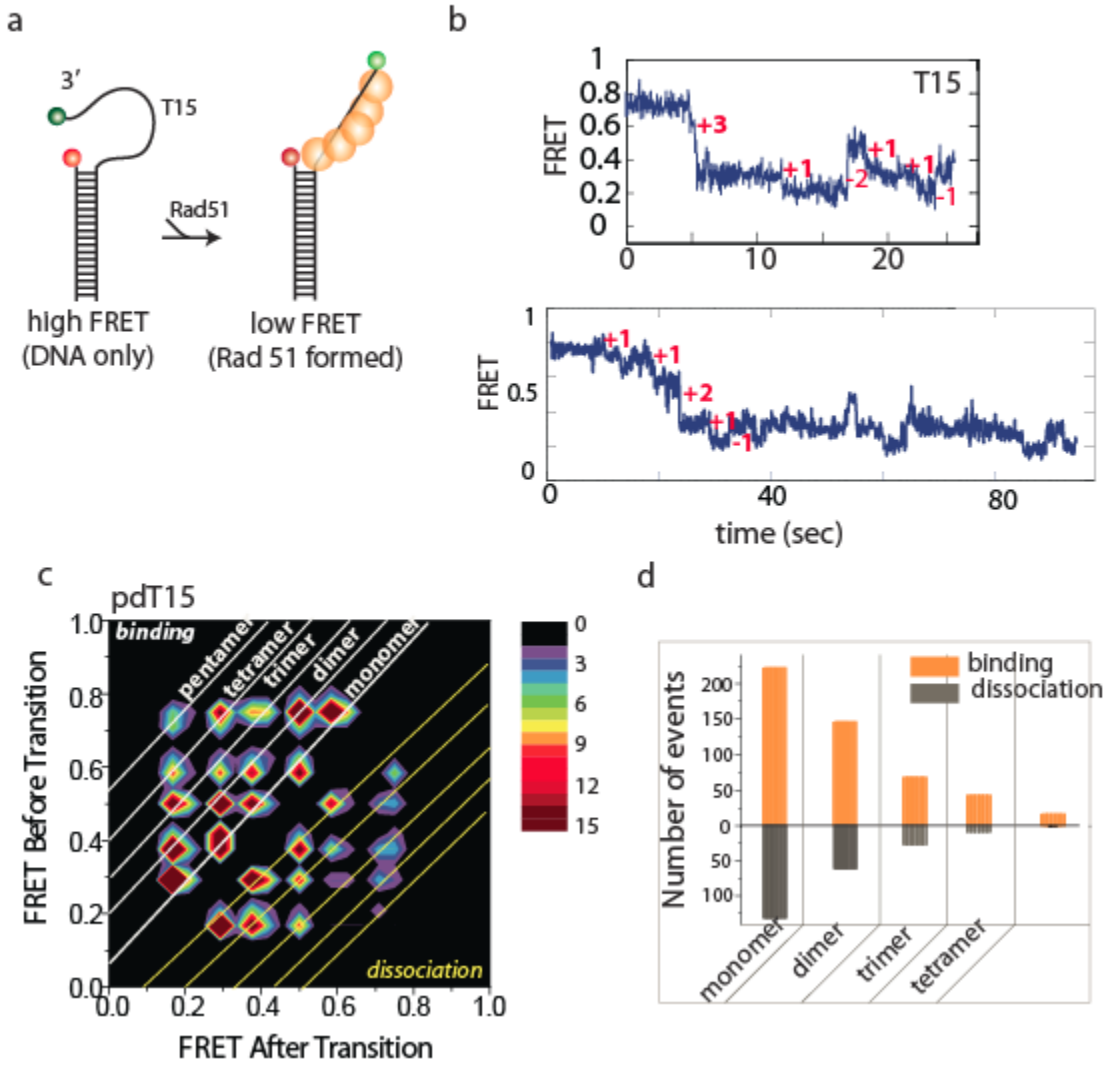
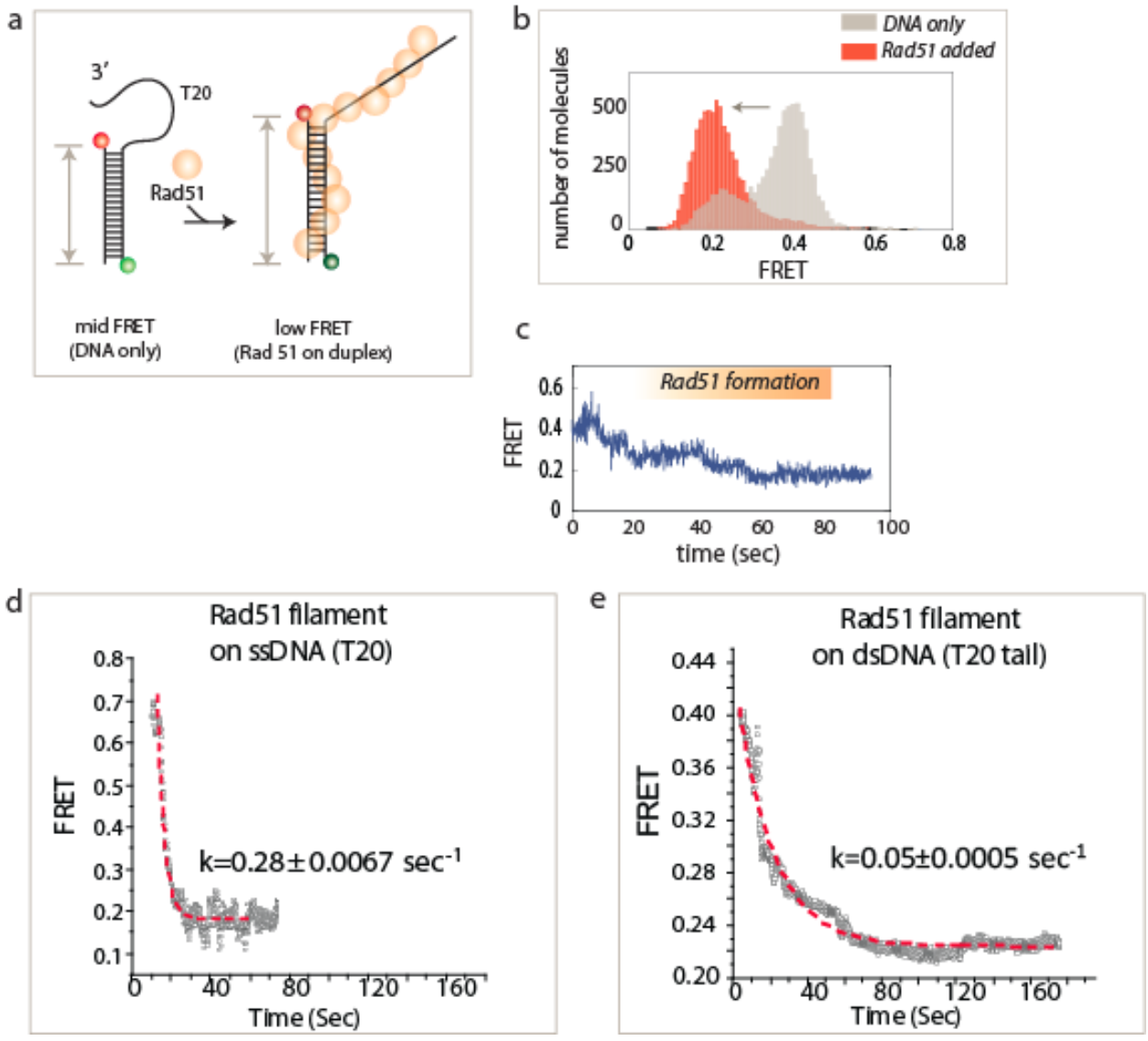
Supplementary Figure S1



Supplementary Figure S1. Rad51 binds primarily as monomer and dimer (a) Schematic of FRET-DNA construct, same as Figure 1a. (b) Single molecule FRET traces that exhibit Rad51 monomer mixed with higher oligomer binding to T15. (c) TDP built out of all the binding events found in Rad51 binding to T15 DNA. Each cluster in TDP can be classified into monomer, dimer, trimer, tetramer and pentamer binding as shown. (d) The majority of (>75%) of Rad51 bind as monomer or dimer whereas less than 25% bind as a higher oligomer.

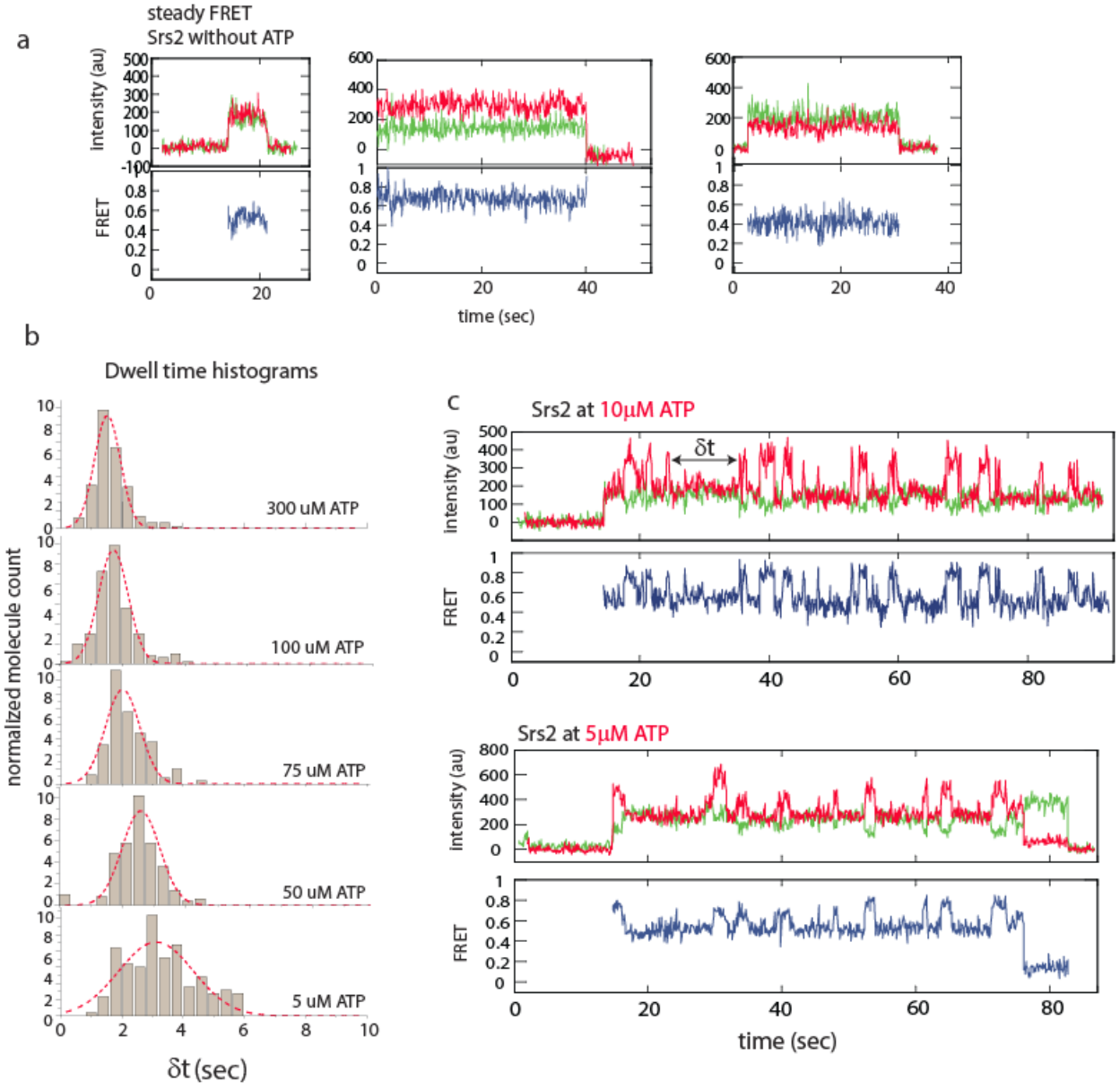
Supplementary Figure S2



Supplementary Figure S2. 5' to 3' directionality is not due to Rad51 formation on DNA duplex (a) Schematic diagram of DNA used for Rad51 formation on duplex DNA. Cy3 and Cy5 are located across the duplex DNA such that Rad51 formation results in FRET decrease. (b) FRET histogram of DNA only (gray) vs. Rad51 formed (orange) shows a transition from mid to low FRET induced by Rad51 formation on duplex DNA. (c) Single molecule trace showing a slow FRET decrease due to Rad51 formation on duplex DNA. (d, e) Rad51 filament formation rate deduced from fitting FRET traces collected from over fifty traces. Errors in fit results are in SEM. (The rate in Fig. S2d is higher than that shown in Fig. 2e (3'Cy3) likely due to the different

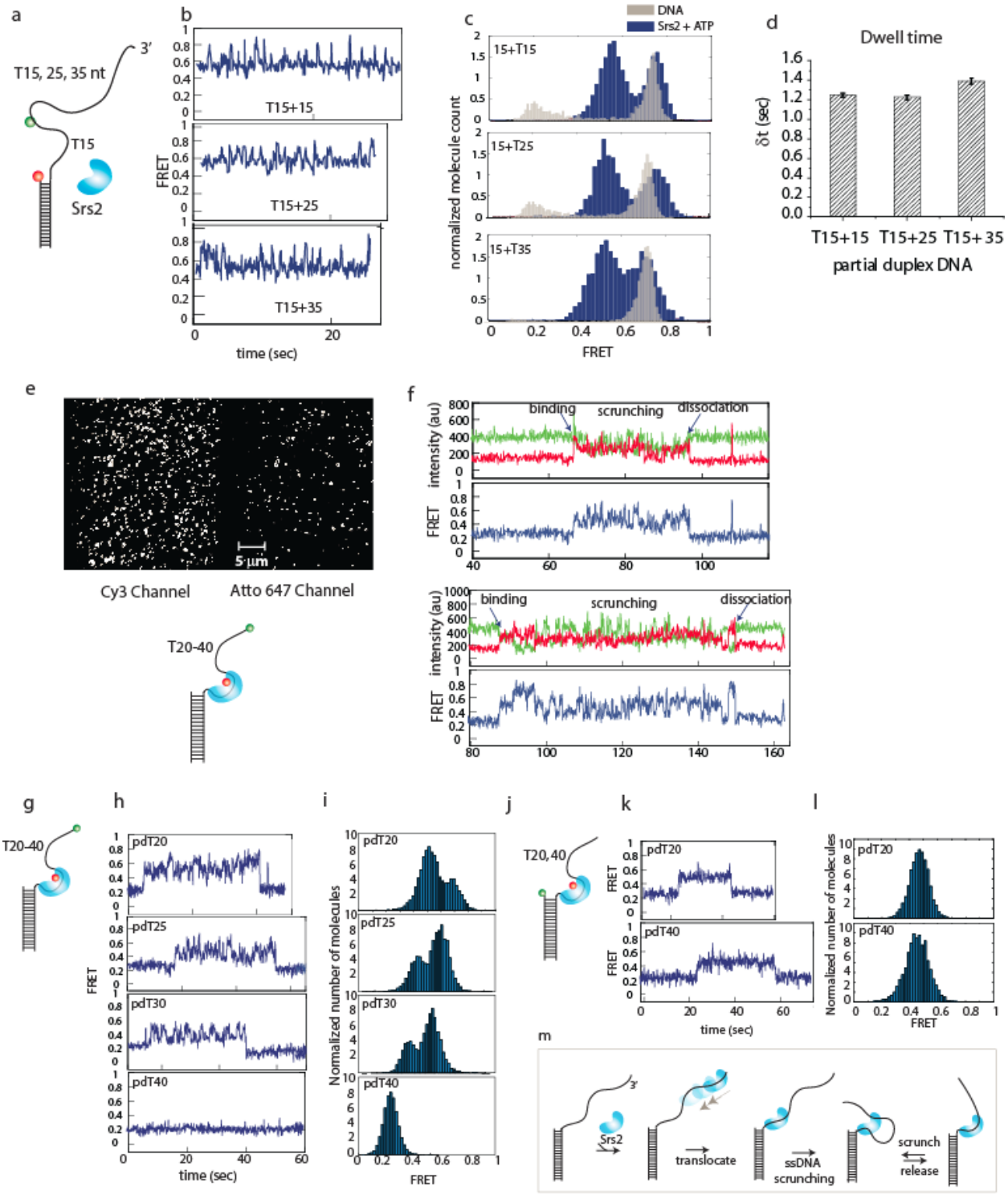
distance sensitivity exhibited by FRET (3-8 nm) and PIFE (0-4nm) i.e. FRET is not sensitive to last monomers of Rad51 binding.)

Supplementary Figure S3



Supplementary Figure S3. Srs2 repetitive movement is ATP dependent (a) In the absence of ATP, Srs2 shows only a static binding to DNA, but no fluctuation. (b) FRET peak to peak dwell times collected in varying ATP concentrations were built as histograms and fit with a Gaussian distribution fit. (c) Single molecule traces collected at low ATP concentrations show markedly slower FRET fluctuations.

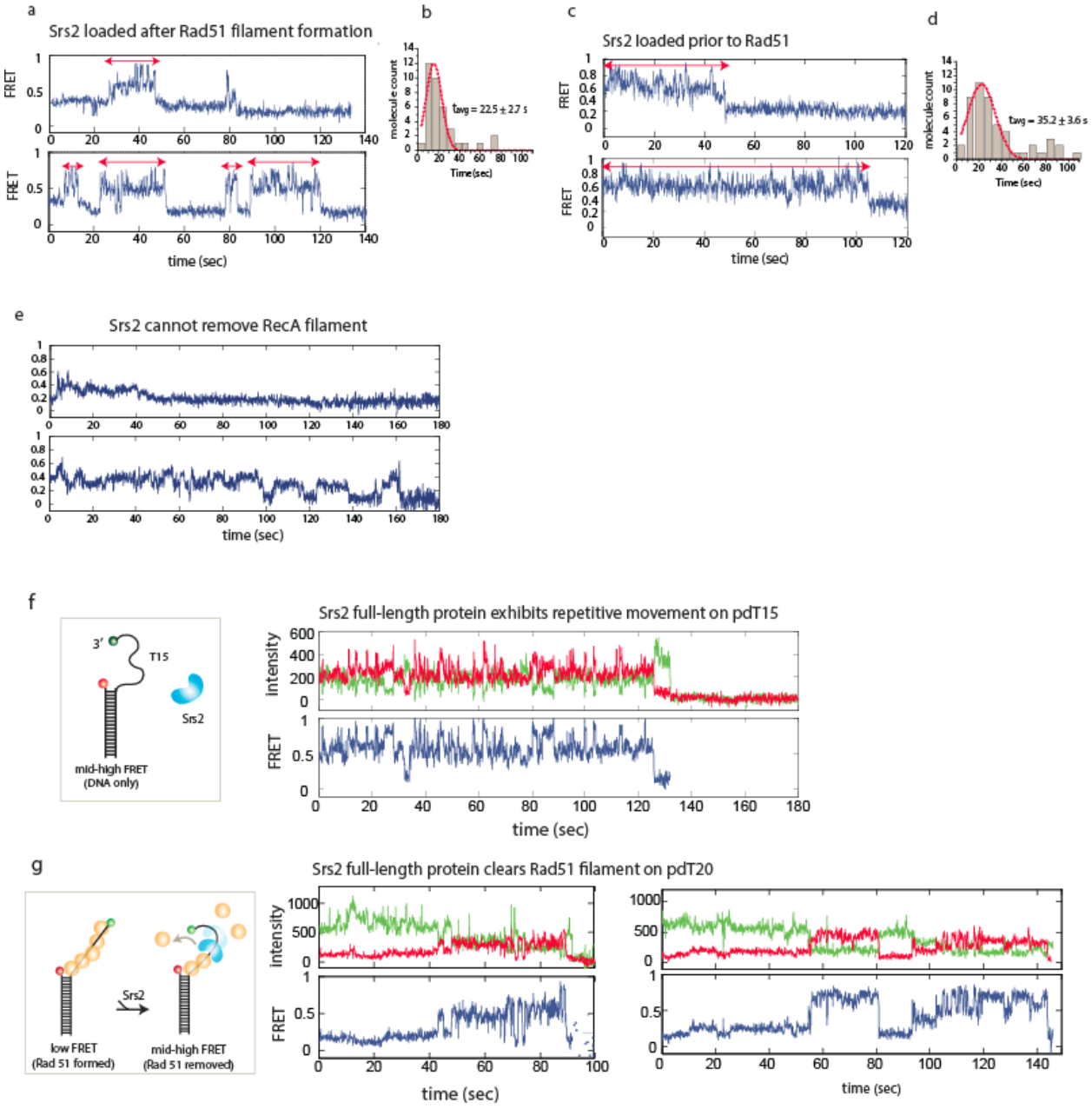
Supplementary Figure S4



Supplementary Figure S4. Srs2 anchors near duplex junction while pulling in a finite length of ssDNA (a) Schematic diagram of FRET-DNA construct. Three DNA substrates had

tail length of 30, 40, 50 nucleotides and the same fixed Cy3-Cy5 distance of 15 nucleotide as shown. (b) Single molecule traces taken from Srs2 on three DNAs are highly analogous to one another. (c) FRET histograms obtained from the three DNA experiments are identical. (d) Dwell time collected form the three experiments are also the same. Error bars denote SEM. (e) Single molecule surface of Cy3 and Cy5 channel show single molecules of Cy3 labeled DNA and Atto 647N labeled Srs2 side by side. The presence of the single dye is confirmed by single step photobleaching as well as intensity corresponding to one fluorophore. (f) Single molecule intensity and corresponding FRET traces. As indicated by arrows, binding of Srs2 shows up as an abrupt appearance of FRET, followed by FRET fluctuations resulting from the repetitive activity of Srs2. Red signal disappears when the protein dissociates from DNA. (g) DNA constructs (3'-Cy3 labeled partial duplex with T20-40 nucleotide ssDNA) were used to probe the repetitive activity of fluorescently labeled Srs2 (NTA-Atto 647N). (h) Single molecule FRET traces from pdT20, 25, 30 and 40. (i) FRET histograms collected from 30-70 molecules that exhibit FRET fluctuations. (j) DNA construct (same partial duplex DNA with Cy3 at duplex junction) were used to visualize movement of labeled Srs2. (k) Single molecule FRET traces from pdT20 and pdT40. (l) FRET histograms collected from 50 traces that show FRET arising from Srs2 binding and repetitive motion. (m) Proposed model for Srs2 translocation and repetitive motion on a short segment of ssDNA while anchoring near the duplex junction.

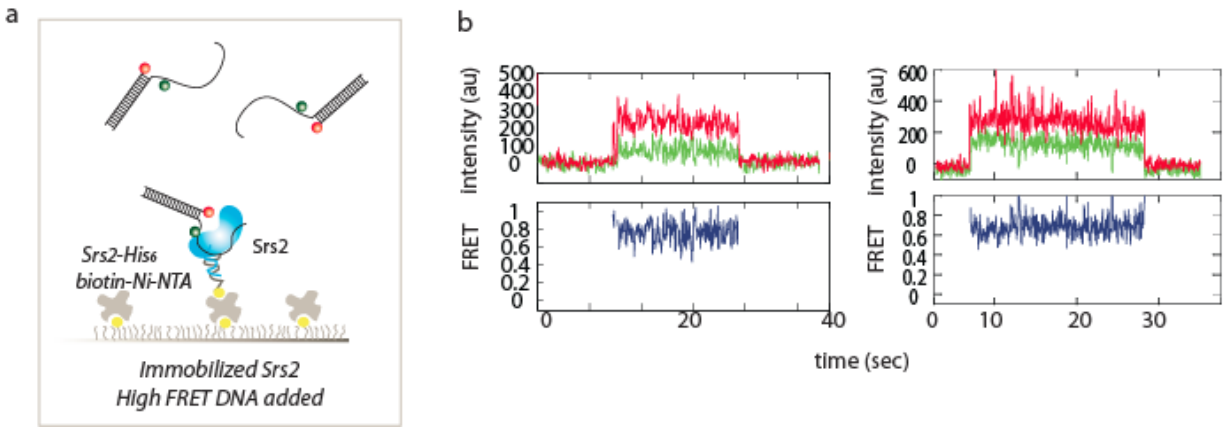
Supplementary Figure S5



Supplementary Figure S5. Srs2 removal strength and specificity (a) Single molecule FRET traces obtained from an experiment where $Srs2^{C\Delta276}$ was added to preformed Rad51. (b) Dwell time of $Srs2^{C\Delta276}$ movement duration (marked in red arrow in (a)) which represents the period $Srs2^{C\Delta276}$ prevents Rad51 reformation. (c) Single molecule FRET traces from an experiment where $Srs2^{C\Delta276}$ was preloaded to DNA then Rad51 was added. (d) Histogram and a Gaussian distribution fit of dwell time of $Srs2^{C\Delta276}$ motion duration which represents the period $Srs2^{C\Delta276}$

prevents Rad51 initiation. Error in fit result is in SEM. (e) Single molecule trace of $Srs2^{C\Delta276}$ subject to RecA removal shows that $Srs2^{C\Delta276}$ cannot remove RecA filament. (f) Srs2 full length (wildtype) protein exhibits the same repetitive movement as the C-terminal truncation mutant. (g) Srs2 full length protein shows the same Rad51 removal activity as the C-terminal truncation mutant.

Supplementary Figure S6



Supplementary Figure S6. Srs2 monomer cannot unwind DNA (a) FRET-DNA applied to surface tethered Srs2. (b) Single molecule traces show steady high FRET, indicating no unwinding by Srs2 monomer.