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Supplemental information

Regeneration of PEG slide for multiple rounds of single-molecule measurements

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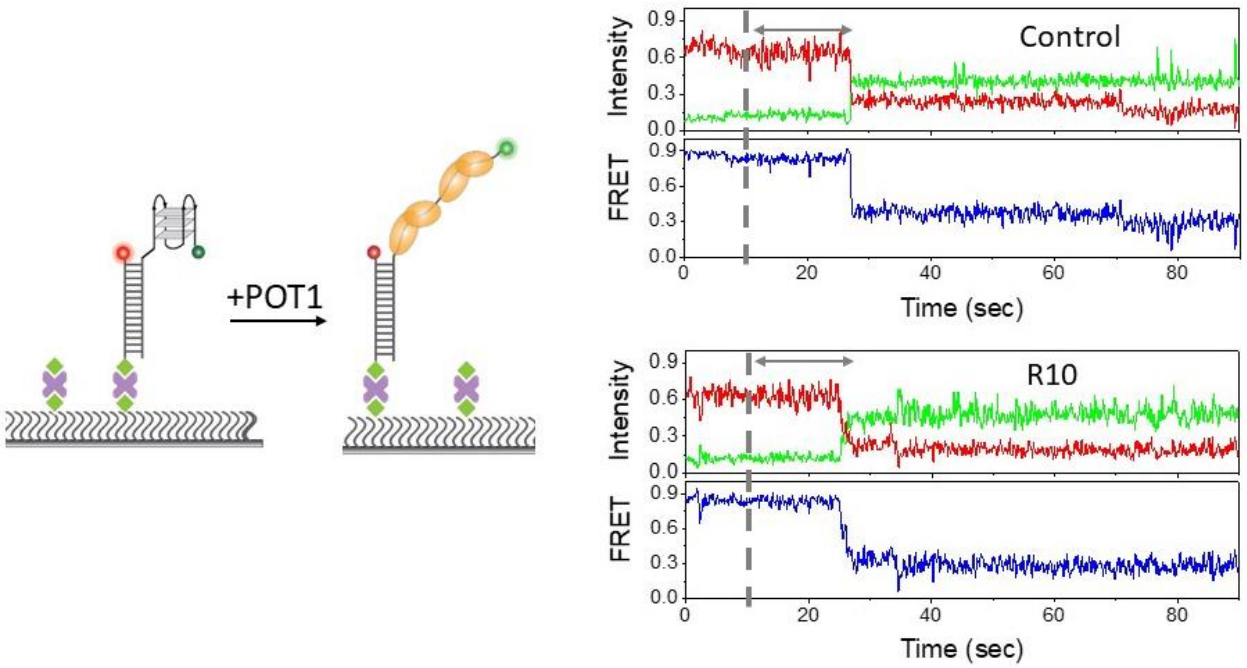

Figure S1 POT1 binding to telomeric G4. Schematic of smFRET experiment (left) and representative smFRET traces of real time POT1 binding to telomeric G4 of first and 10th (R10) repeat of experiment. Dwell time calculated from the moment of POT1 flow (dash line) and subsequent FRET decline from the smFRET traces.

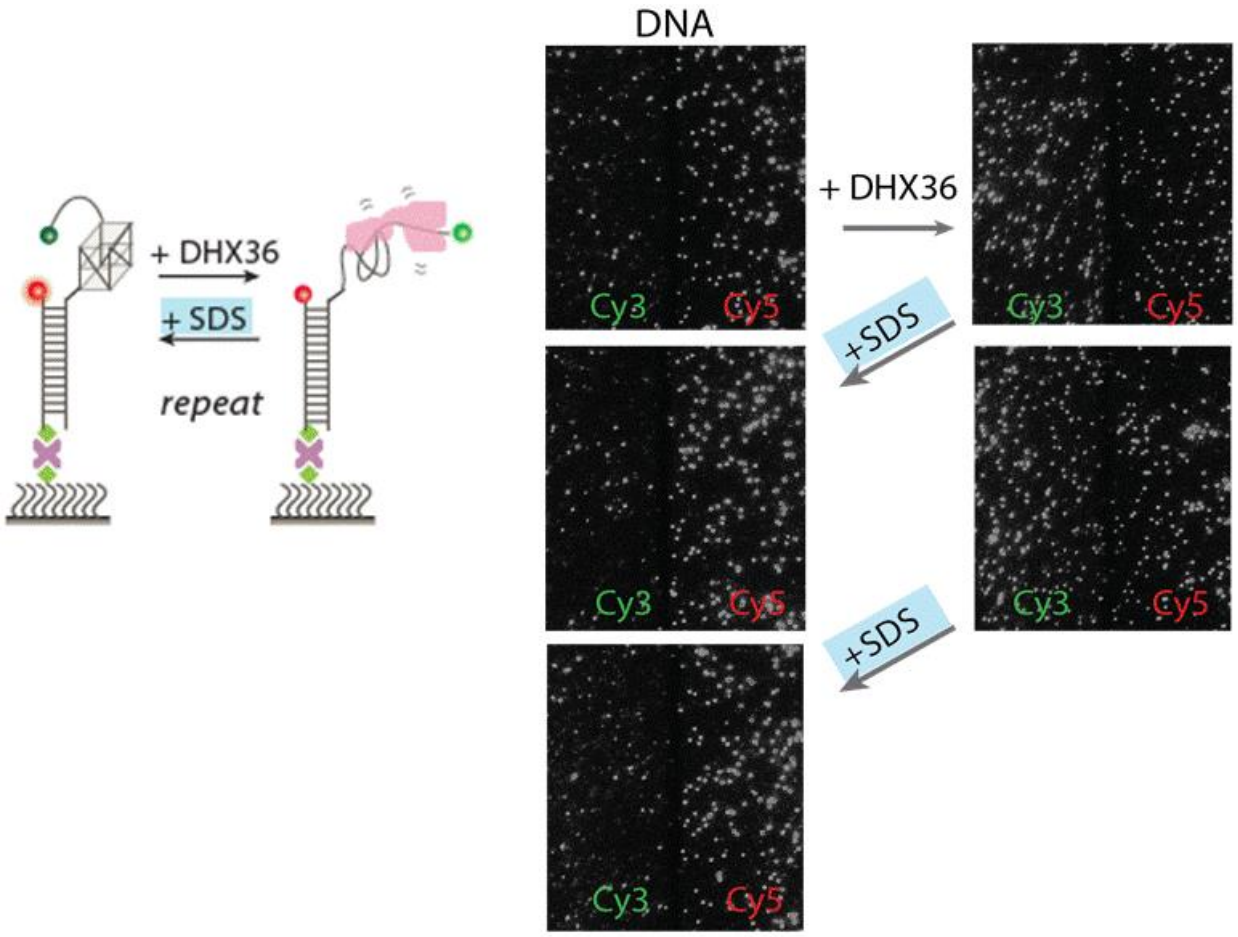

Figure S2 Representative field of view before and after DHX36 binding followed by 0.1% SDS wash.

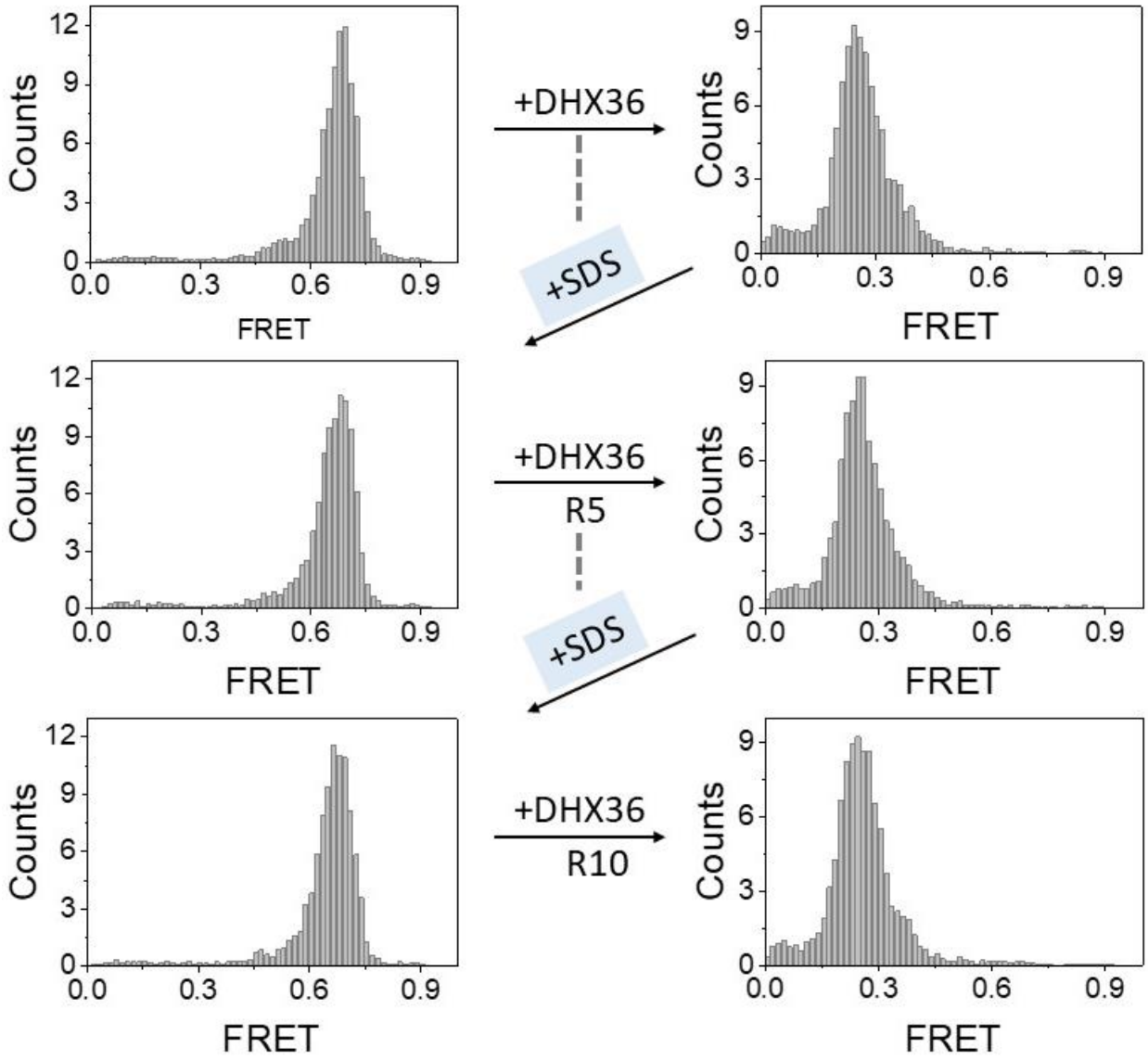

Figure S3 Multiple times of DHX36 protein binding and unbinding by applying 0.1% SDS to the RNA-G4 on the same flow channel. The FRET histogram of control, five and ten repeat of binding before and after addition of DHX36 protein.

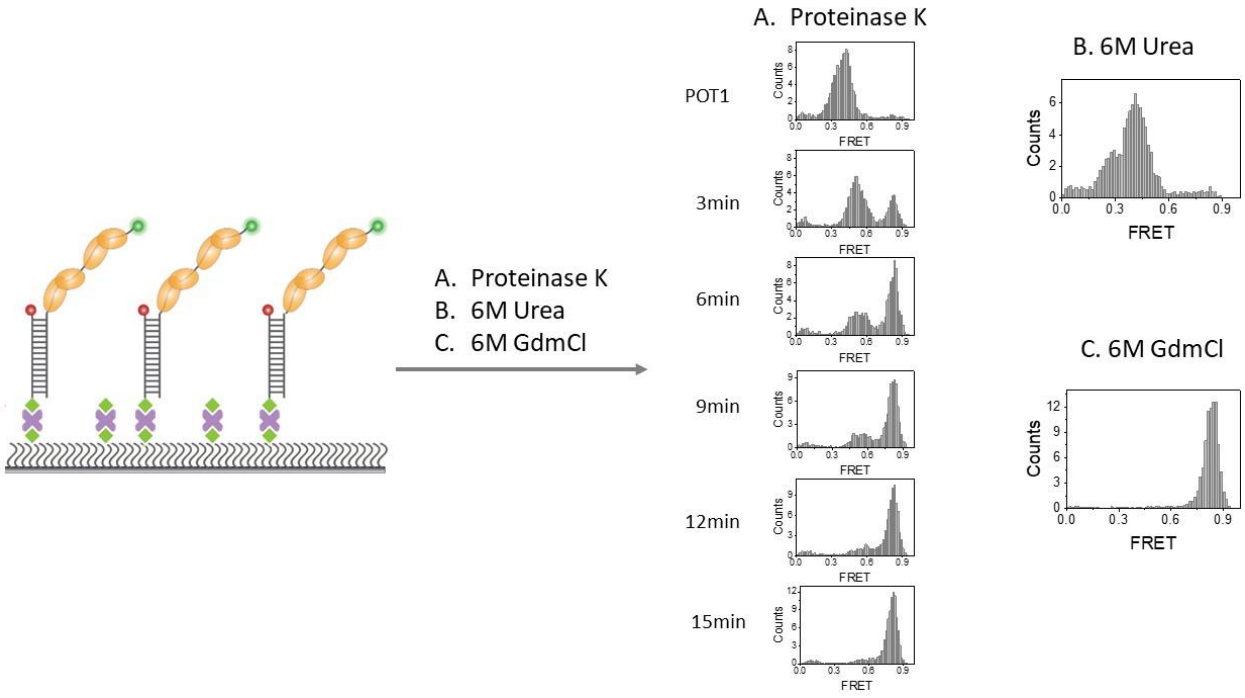

Figure S4 Proteinase K (100 μM), 6M urea and 6M GdmCl added to the POT1 bound telomeric G4 overhang. The percentage of recovery was calculated from shifted FRET peak from protein bound state to the free DNA/RNA only state (Fig. 3).

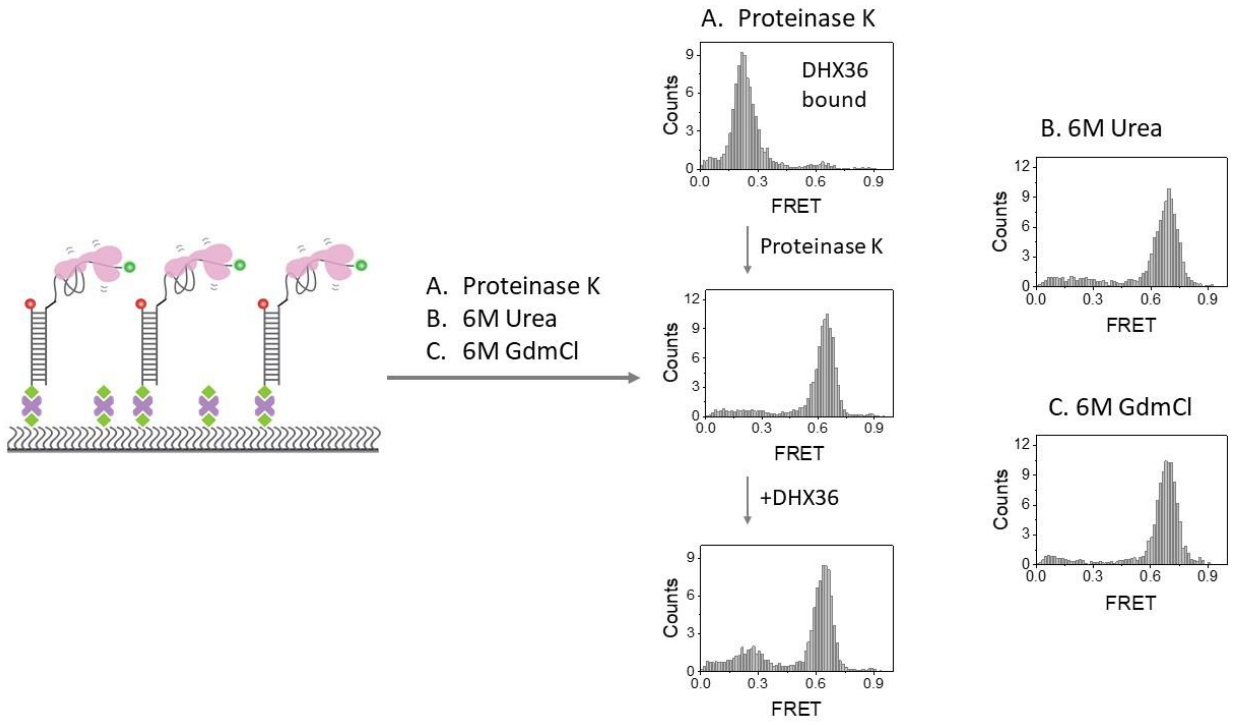

Figure S5 Proteinase K (100 µM), 6M urea and 6M GdmCl added to the DHX36 bound G4with overhang. In case of proteinase K, further addition of DHX36 cannot bind G4, likely due to the remnant of previously bound DHX36 after the proteinase K treatment. The percentage of recovery was calculated from shifted FRET peak from protein bound state to the free DNA/RNA only (Fig. 3).

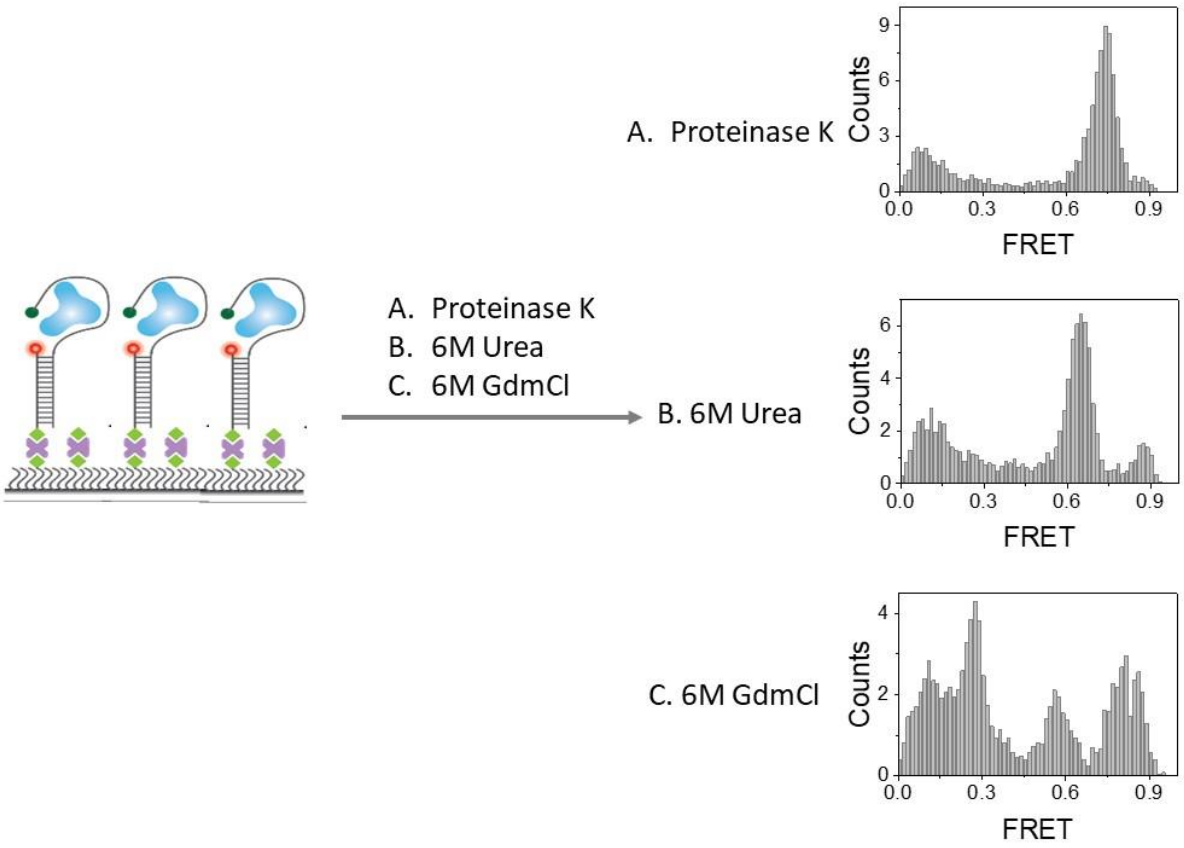

Figure S6 Proteinase K (100 μM), 6M urea and 6M GdmCl added to the FUS bound poly U-50 ssRNA tail and all the histograms are taken after 20 minutes of incubation. The percentage of recovery was calculated from shifted FRET peak from protein bound state to the free DNA/RNA only state (Fig. 3).

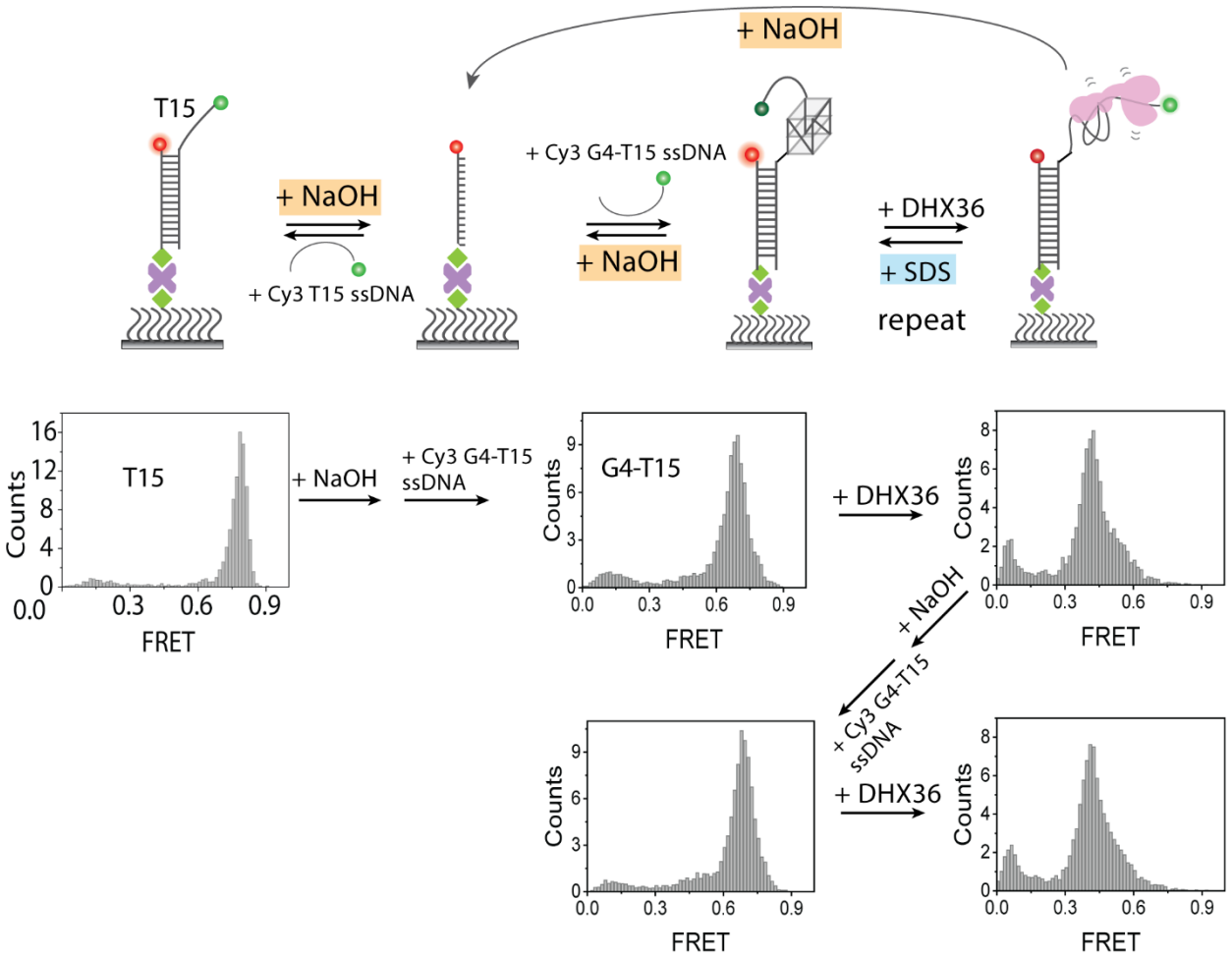

Figure S7 NaOH induced unzipping of immobilized partial duplex followed by reannealing with ssDNA. The T15 tail containing partial duplex was immobilized on surface, washed with 50 mM NaOH to unzip the partial duplex, leaving behind the biotinylated surface tethered Cy5 strand. Then another complementary ssDNA containing G4-T15 was applied to be annealed to the tethered ssDNA, followed by DHX36 binding. Further cycles of unzipping followed by reannealing with any complementary sequence can be continued.

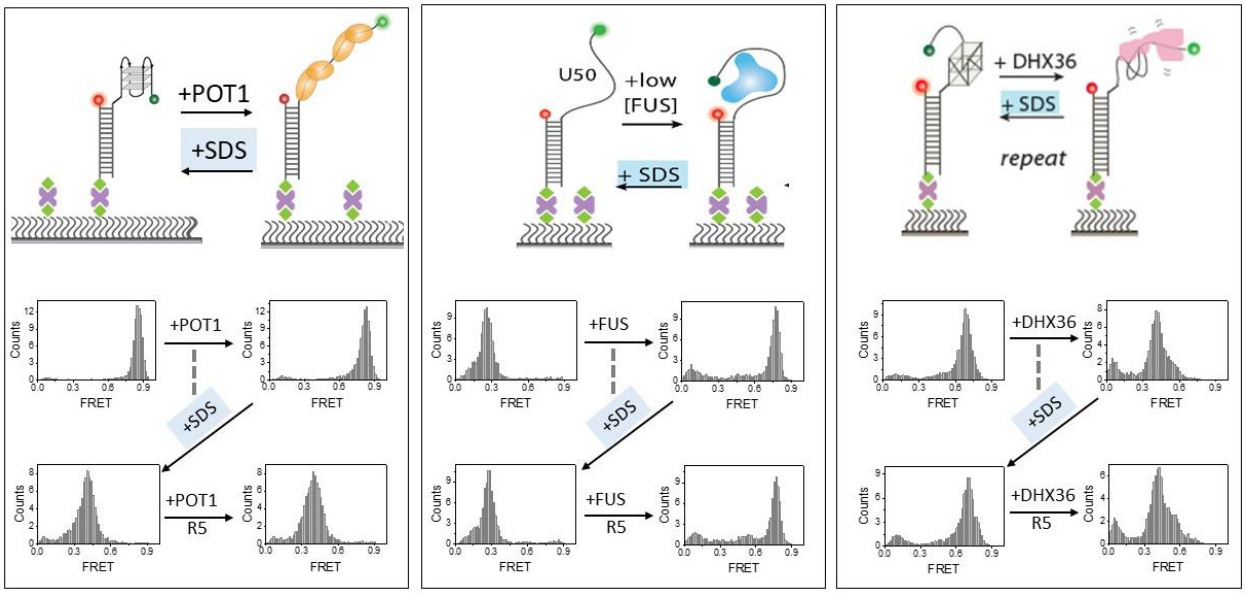

Figure S8 NaOH (7M) induced breaking of the biotin-NeutrAvidin linkage and recycling of the surface multiple times. Telomeric G4 construct in a form of a partial duplex was immobilized on the surface (left) to which POT1 can be added and removed by SDS. The surface can be treated with NaOH to have poly-U50 partial duplex (middle) to which FUS can be bound for measurement and removed by SDS. In the same way, the same surface can be prepared with immobilized G4-T15 tail (right) to which DHX36 can be applied.

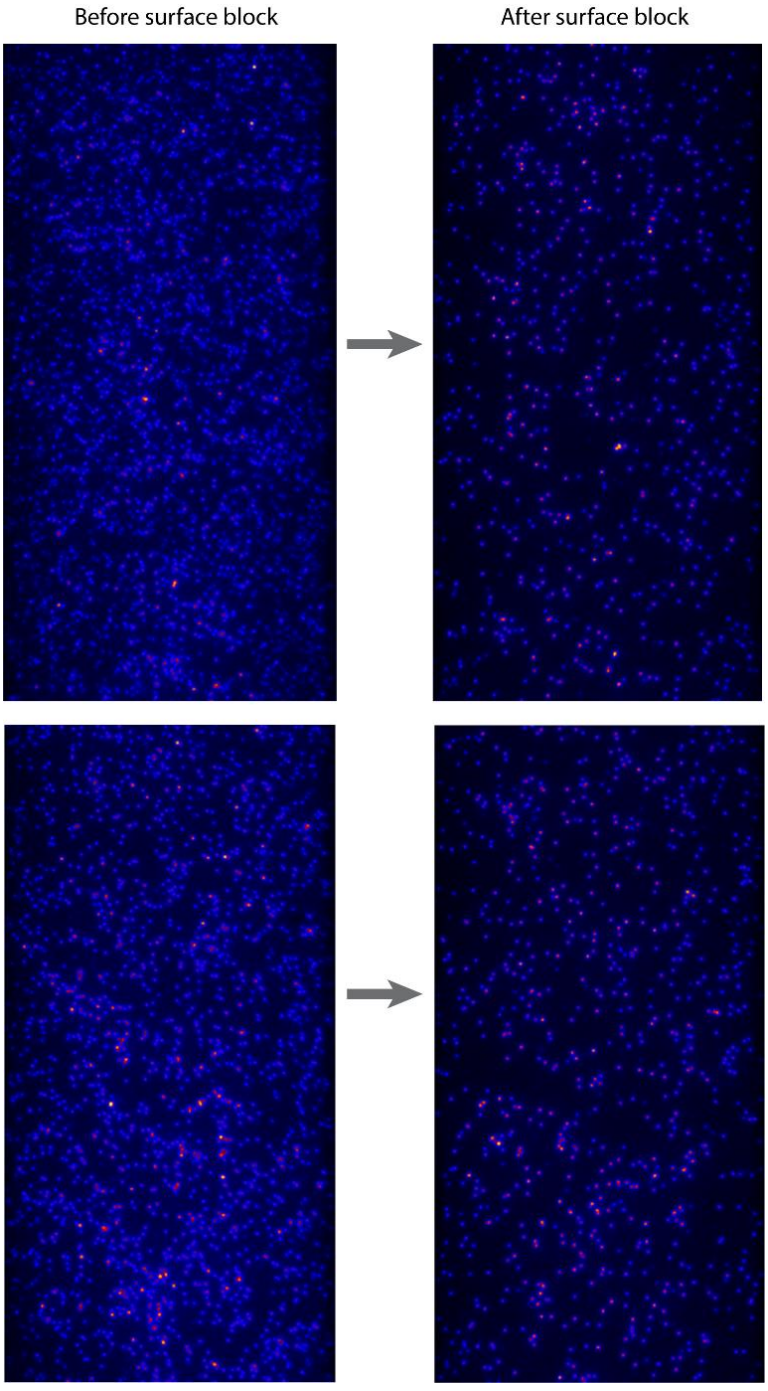

Figure S9 The images of field of view during 7M NaOH treatment before (left side images) and after (right side images) surface block with BSA and yeast t-RNA.