SUPPLEMENTAL MATERIAL

Role of double-stranded DNA translocase activity of human HLTF in replication of damaged DNA

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SUPPLEMENTAL METHODS

DNA substrates

To generate oligonucleotide-based DNA substrates we annealed oligonucleotides listed in Supplementary Table 1 in various combinations followed by purification from polyacrylamide gel as described (1, 2). The heterologous fork (HetF) term refers to replication fork-like structures with non-complementary leading and lagging arms, and homologous fork (HomF) term to forks with complementary leading and lagging arms. Substrate compositions that are not specified in the text are described below, where we underlined the 5' ^{32}P-labelled oligonucleotide(s).

HetF 30/30: O1054/ O1118/ O1175/ O1176 (Fig. 1B)

HomF 30/30: O1054/ O1056/ O1058/ O1118 (Fig. 1B, Fig. 1C, and Fig. 2B)

The four-way junctions, X0 and X12, used in Fig. 2B were made by annealing O1111, O1112, O1113, O1114, and O1114, O1115, O1116, O1117 oligonucleotides, respectively, in which O1114 was 5' ^{32}P-labeled.

Substrates that were used for Supplemental Figure 1 are:

O1058/ O1118 (Fig. S1B lanes 1-6)

O1056/ O1058 (Fig. S1A panel I, Fig. S1B lanes 7-12)

O1054/ O1118 (Fig. S1A panel II, Fig. S1B lanes 13-18)

O1175/ O1118 (Fig. S1A panel III, Fig. S1B lanes 19-24)

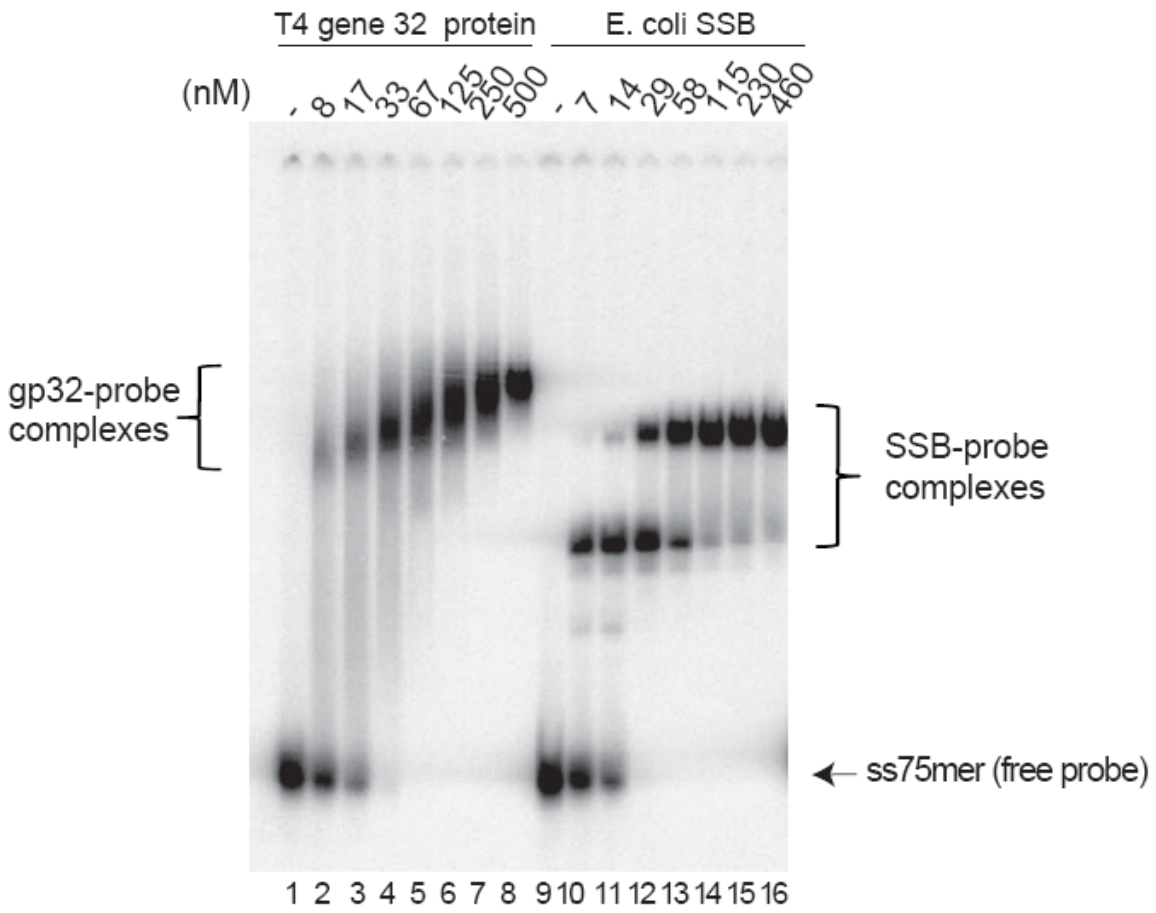
O1176/ O1175/O1118 (Fig. S1A panel IV, Fig. S1B lanes 25-30)

O1054/ O1175/O1118 (Fig. S1A panel V, Fig. S1B lanes 31-36)

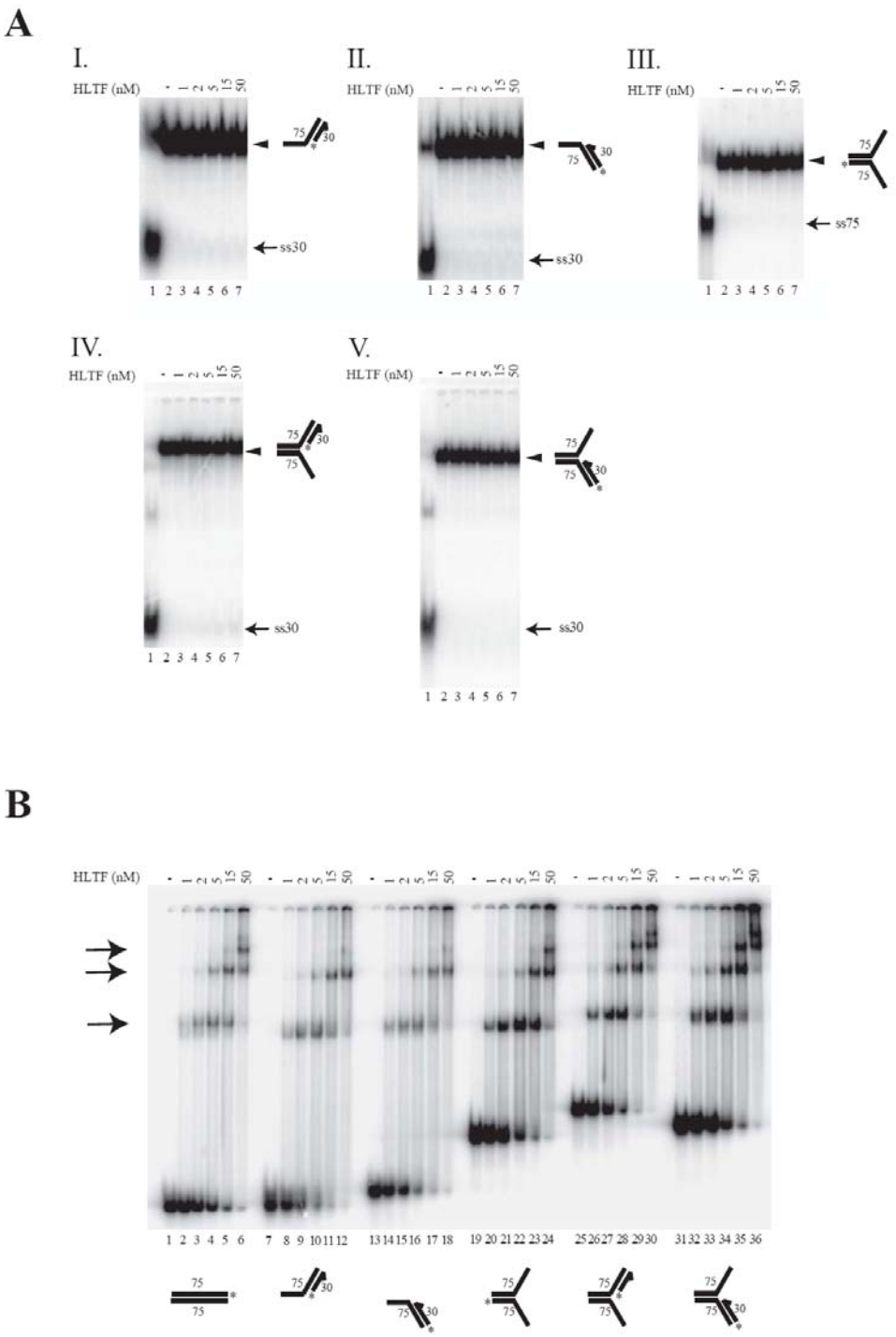
Supplemental Table S1. List of oligonucleotides used for the construction of various DNA substrates.

Oligo name	Length	Sequence	Reference
O1054	30	AgCTACCATgCCTgCCTCAAgAATTCgTAA	(Blastyak *et al.* 2007)
O1056	30	TTACgAATTCTTgAggCAggCATggTAgCT	(Blastyak *et al*. 2007)
O1058	75	AgCTACCATgCCTgCCTCAAgAATTCgTAATATgCCTACACTggAgTACCggAgCATCgTCgTgACTgggAAAAC	(Blastyak *et al.* 2007)
O1107	17	gTTTTCCCAgTCACgAC	This study
O1108	17	CAggAAACAgCTATgAC	This study
O1118	75	gTTTTCCCAgTCACgACgATgCTCCggTACTCCAgTgTAggCATATTACgAATTCTTgAggCAggCATggTAgCT	(Blastyak *et al.* 2007)
O1175	75	gATCgTTgCATTCATTCTggAggCCTACggTATgCCTACACTggAgTACCggAgCATCgTCgTgACTggAAAAC	(Blastyak *et al.* 2007)
O1176	30	CCgTAggCCTCCAgAATgAATgCAACgATC	(Blastyak *et al.* 2007)
O1301	29	gCTCACCTggTAgTCgACTTACgTgATCg	(Blastyak *et al.* 2007)
O1302	29	AgCCgATCACgTAAgTCgACTACCAggTg	(Blastyak *et al*. 2007)
O1377	34	TCTCTTTTTCTTTCTTTTCTTCTTTTTTCTTTCT	This study

O1111	50	gTCggATCCTCTAgACAgCTCCATgATCACTgg CACTggTAgAATTCggC	(McGlynn *et al.* 2000)
O1112	50	CAACgTCATAgACgATTACATTgCTACATggAg CTgTCTAgAggATCCgA	(McGlynn *et al.* 2000)
O1113	50	TgCCgAATTCTACCAgTgCCAgTgATggACATC TTTgCCCACgTTgACCC	(McGlynn *et al.* 2000)
O1114	50	TgggTCAACgTgggCAAAgATgTCCTAgCAATgT AATCgTCTATgACgTT	(McGlynn *et al.* 2000)
O1115	49	gACgCTgCCgAATTCTggCTTgCTAggACATCTT TgCCCACgTTgACCC	(McGlynn *et al.* 2000)
O1116	51	CAACgTCATAgACgATTACATTgCTAggACATg CTgTCTAgAgACTATCgA	(McGlynn *et al.* 2000)
O1117	50	ATCgATAgTCTCTAgACAgCATgTCCTAgCAAg CCAgAATTCggCAgCgT	(McGlynn *et al.* 2000)

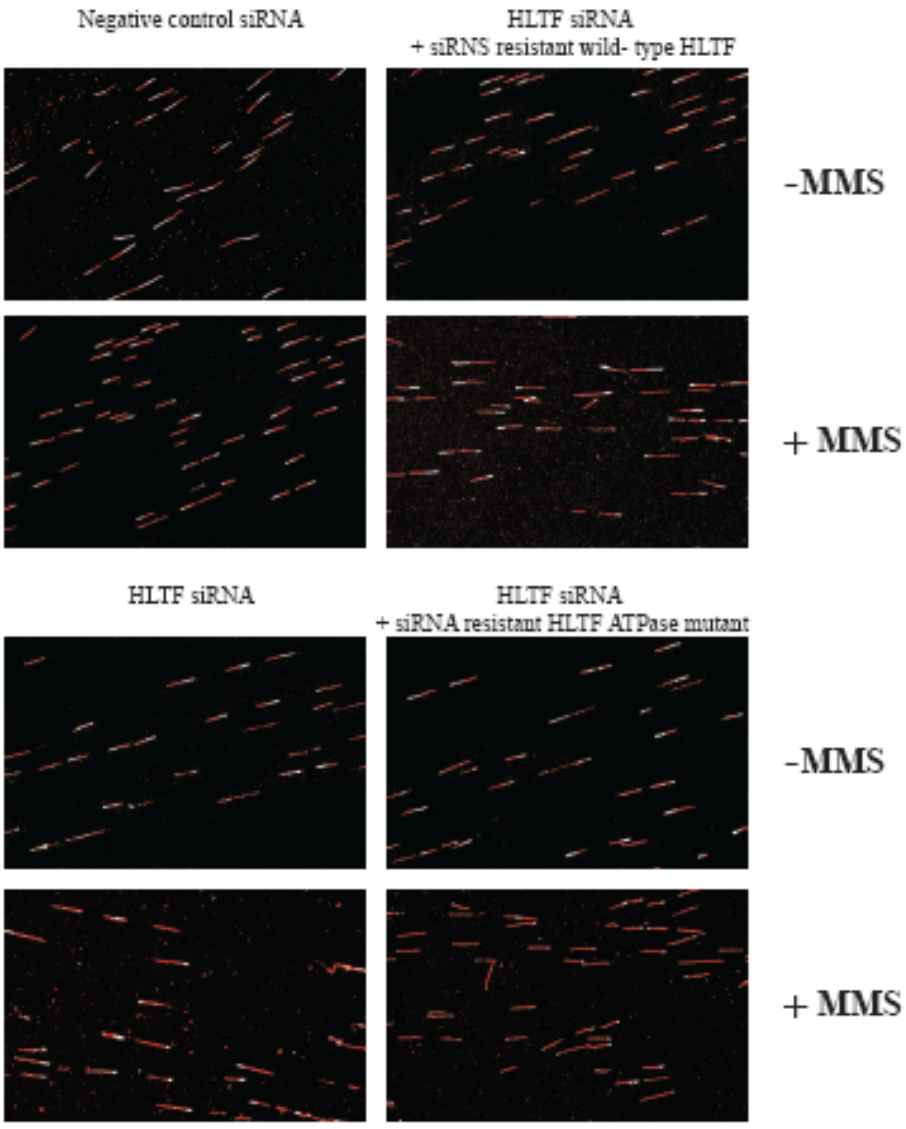


Supplemental FIG. 1. Activity test of single stranded DNA binding proteins. The ssDNA binding activity of T4 gene 32 protein and E. coli SSB was tested in gel mobility shift assay under the same conditions described for HLTF assays in the manuscript. Concentrations (nM) of ssDNA binding proteins are indicated at the top of each lane. The position of unbound ssDNA probe is indicated by an arrow and protein-DNA complexes are marked by brackets. Note, that the actual concentration of both ssDNA binding proteins in HLTF assays was fairly over the minimal concentration required for saturation of the ssDNA probe.



Supplemental FIG. 2. (A) Activity of HLTF was tested on the indicated set of canonical helicase substrates shown by an arrowhead on each panel. Asterisks and numbers refer to the position of radioactive label and length of oligonucleotides, respectively. Helicase assay was carried out in the presence of increasing HLTF concentration under the same condition described for the fork

reversal assays in the manuscript. Lane 1 contains a boiled sample that marks the mobility of the labeled ssDNA. Note, that HLTF shows no sign of canonical helicase activity. (B) We considered the possibility, that lack of helicase activity in (A) might be due to lack of substrate binding. In order to rule out this possibility we performed gel shift analysis with HLTF and the substrates used above at identical experimental conditions but in the absence of cofactor. The substrate structure is shown at the bottom of each set. Note, that all substrates formed DNA-protein complexes with HLTF (indicated by arrows), therefore lack of helicase activity is not due to reduced affinity of HLTF to these substrates. Each substrate is bound by HLTF with similar affinity that indicates the lack of structure specific DNA binding. The common denominator of this substrate set is dsDNA that is the possible recognition site for HLTF. This conclusion is in full agreement with the dsDNA translocase activity of HLTF demonstrated in the manuscript.



Supplemental FIG. 3. Images of labeled chromosome fibers from HLTF knock-down cells. HeLa cells were transfected with HLTF-specific siRNAs (HLTF siRNA) or for complementation, with HLTF-specific siRNAs and subsequently with siRNA resistant wild-type or ATPase mutant HLTF expressing plasmids as indicated. Between the two labeling, cells were not treated or treated with 0.01% MMS for 20 min as indicated on the right. Fibers were prepared after 40 min of the second labeling and visualized as described in the methods.

REFERENCES

1. **Blastyak, A., L. Pinter, I. Unk, L. Prakash, S. Prakash, and L. Haracska.** 2007. Yeast Rad5 protein required for postreplication repair has a DNA helicase activity specific for replication fork regression. Mol.Cell **28:**167-175.

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