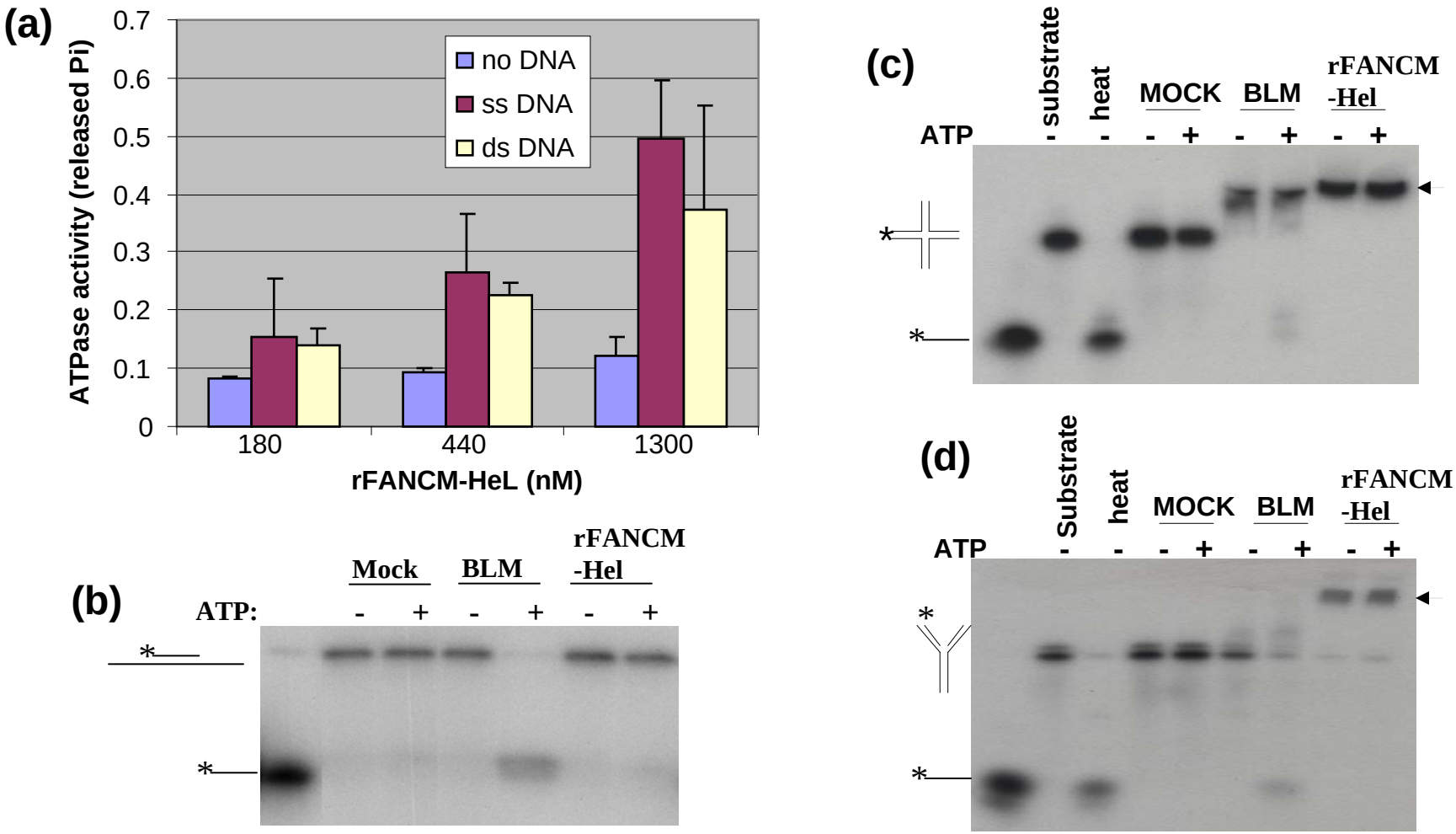
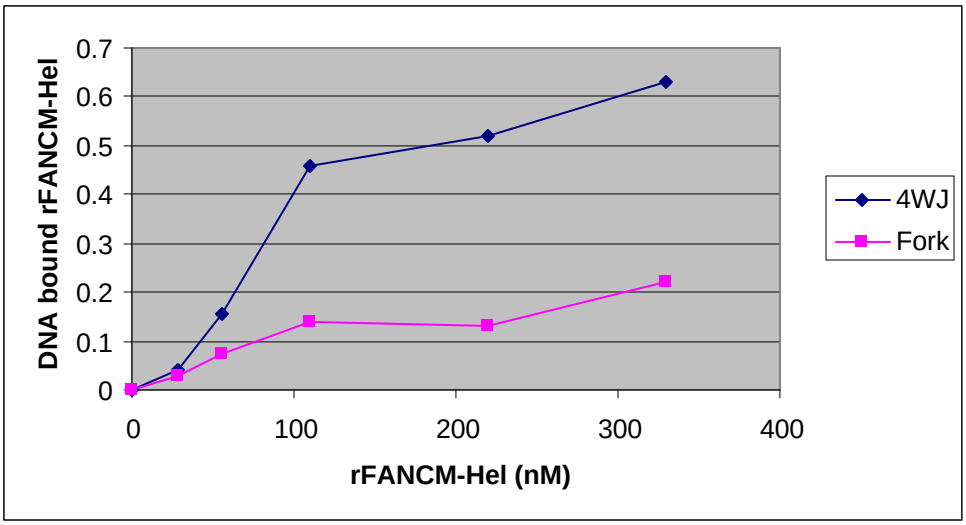
The FANCM Helicase domain has higher affinity to 4-way junction and fork DNA, but no helicase activity



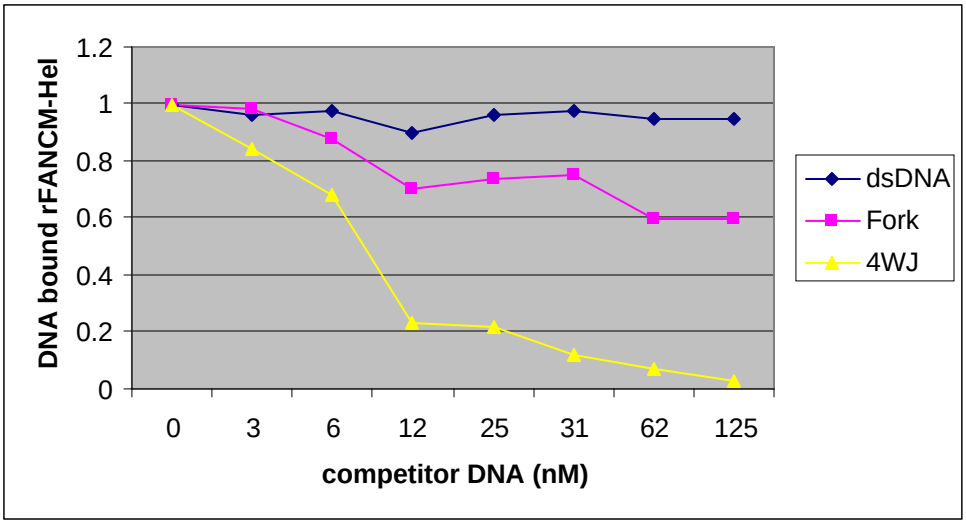
Legend: (a) A graph shows the ATPase activity of the recombinant FANCM helicase domain (rFANCM-Hel). The presence of double-strand (ds) or single-strand (ss) DNA is shown. The specific activity of this protein is at least an order of magnitude lower than the full-length FANCM from EBNA-293 cells. (b-d) DNA-displacement assays shows that rFANCM-Hel has no detectable helicase activity to a linear DNA with both 5' and 3' single-stranded DNA (b), or a 4-way Junction (4WJ) (c), or a replication fork (d). Notably, rFANCM-Hel shifted all the 4WJ and majority of fork (indicated with an arrow in 1c and 1d), but not the linear DNA containing both single and double strand regions, indicating that rFANCM-Hel has a higher binding affinity to 4WJ and fork DNA. The helicase complex containing BLM (BLM) was included as a positive control (Meetei et al., 2003b).

Quantification shows that the recombinant FANCM helicase domain has higher affinity to 4-Way Junction than fork DNA

(a)

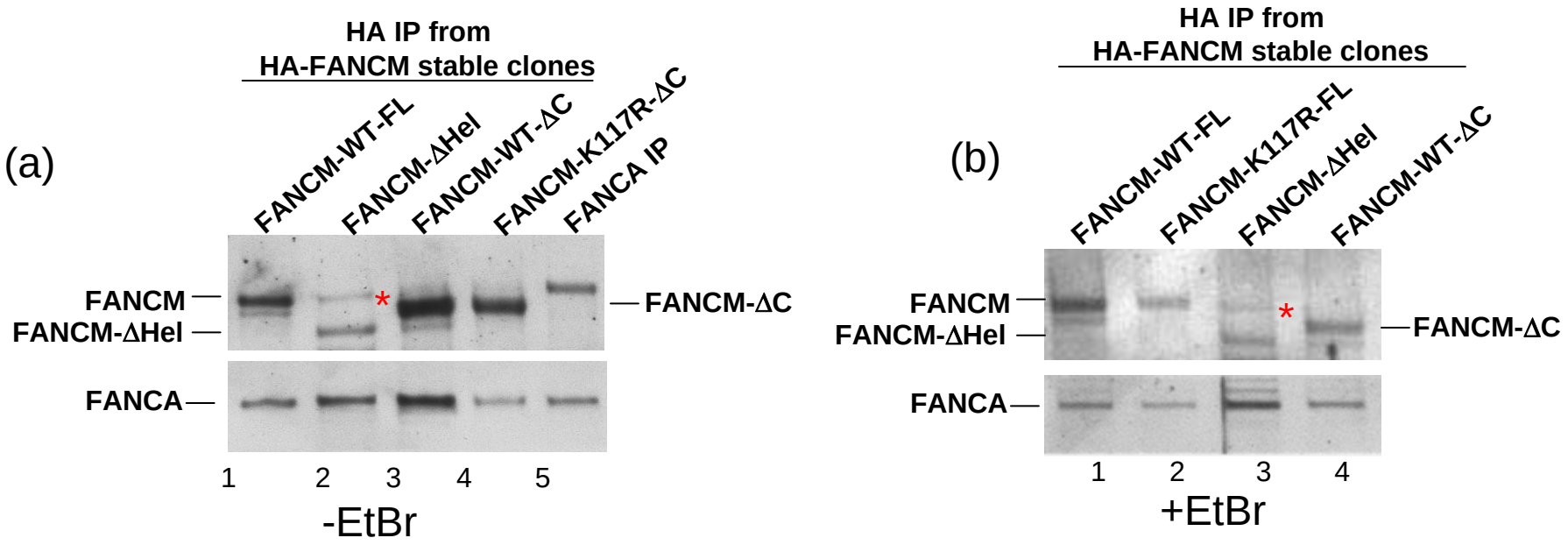


(b)



Legend: (a) A graph shows quantification of the DNA-binding data by recombinant FANCM-Hel in Figure 1c and 1d. (b) A graph shows the quantification of the competition data in Figure 1e. The Y-axis represents the ratio between the DNA-bound form of rFANCM-Hel and total protein (both bound and unbound). The use of 4-Way Junction (4WJ), fork, and double-stranded (ds) DNA are indicated.

The deletion mutant of FANCM helicase-domain, but not mutants of its C-terminal region, co-immunoprecipitate with exogenous FANCM: evidence for homodimerization of FANCM through its C-terminal region



Legend. (a)(b) Immunoprecipitation-coupled Western blotting shows that the deletion mutant of FANCM helicase domain (FANCM-ΔHel), but not mutants of the C-terminal region (FANCM-WT-ΔC or FANCM-K117R-ΔC), co-immunoprecipitated with endogenous FANCM (marked with an asterisk). The experiments were performed in the absence (a) or presence of ethidium bromide (EtBr)(b). The inclusion of EtBr in the nuclear extract should disrupt DNA-protein interactions, thus excluding the possibility that the interaction between FANCM molecules is through DNA. Immunoblotting of FANCA was included as a control.

Supplementary Figure 1. Oligo sequences used for DNA-binding and helicase assays.

Name	Length (nt)	Sequence (5' to 3')
Hel.20	20	CGCTAGCAATATTCTGCAGC
Hel.40	46	GCGCGGAAGCTTGGCTGCAGAATATTGCTAGCGGGAATTC GGCGCG
Hol.1	70	GTGACCGTCTCCGGGAGCTGCATGTGTCAGAGGTTTTCACC GTCATCACCGAAACGCGCGAGACGAAAGG
Hol.2	70	CCTTTCGTCTCGCGCGTTTCGGTGATGACGGTGAAAACCTC TGACACATGGCCAGCCCCGACACCCGCCA
Hol.3	70	TGGCGGGTGTCGGGGCTGGCCATGTGTCAGAGGTTTTCACC GTCATCACCCGCCGGGCAAGAGCAACTCG
Hol.4	70	CGAGTTGCTCTTGCCCGGCGGGTGATGACGGTGAAAACCTC TGACACATGCAGCTCCCGGAGACGGTCAC
Fork2	22	AATTCGTGCAGGCATGGTAGCT
Fork3	49	AGCTACCATGCCTGCACGAATTAAGCAATTCGTAATCATGG TCATAGCT
Fork5	47	AGCTATGACCATGATTACGAATTGCTTGGAATCCTGACGAA CTGTAG
Fork7	20	CTACAGTTCGTCAGGATTCC
H1	40	GTGACCGTCTCCGGGAGCTGGAAACGCGCGAGACGAAAGG
H5	40	CCTTTCGTCTCGCGCGTTTCCAGCTCCCGGAGACGGTCAC
H4	40	CGAGTTGCTCTTGCCCGGCGCAGCTCCCGGAGACGGTCAC
H3.5	20	CGCCGGGCAAGAGCAACTCG
H2.5	20	CCTTTCGTCTCGCGCGTTTC
H2	40	CCTTTCGTCTCGCGCGTTTCGCCAGCCCCGACACCCGCCA
H3	40	TGGCGGGTGTCGGGGCTGGCCGCCGGGCAAGAGCAACTCG

Methods:

These affinity-purified synthetic oligos are hybridized to each other to make the various substrates in Figure 1, as illustrated below:

1. The DNA with both single and double stranded regions in Figure 1b: Hel.20+Hel.40
2. The non-movable HJ substrate in Figure 1c: Hol. 1+2+3+4. Hol.1 was labeled
3. The fork substrate in Figure 1d: fork 2+3+5+7. Fork 2 was labeled.
4. The substrates in Figure 1e, 1f and 1g: double-stranded DNA: H1+H5; Y-shaped DNA: H1+H4; 3'-flap DNA: H1+H3.5+H4; replication fork: H1+H2.5+H3.5+H4; non-movable HJ: H1+H2+H3+H4.