Supplemental Figure Legends

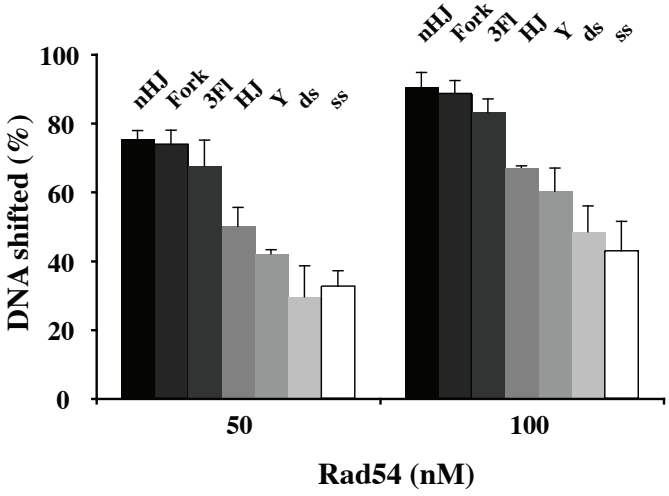
Supplemental Figure 1. Rad54 binds branched DNA substrates preferentially. Histogram showing the affinity of Rad54 (50 and 100 nM) towards the indicated substrates (12 nM).

Supplemental Figure 2. Protein concentration-dependent cleavage of 3' DNA flap by Mus81-Mms4 or Mus81-Eme1.
The 3' DNA Flap was incubated with Mus81-Mms4 (0.12, 0.25, 0.5, and 1.0 nM in **A**) or Mus81-Eme1 (0.12, 0.25, 0.38, and 0.5 nM in **B**) at 37°C for 20 min. (**C**) and (**D**), histograms showing the data from panel (A) and panel (B) and also those from the 10 min timepoint.

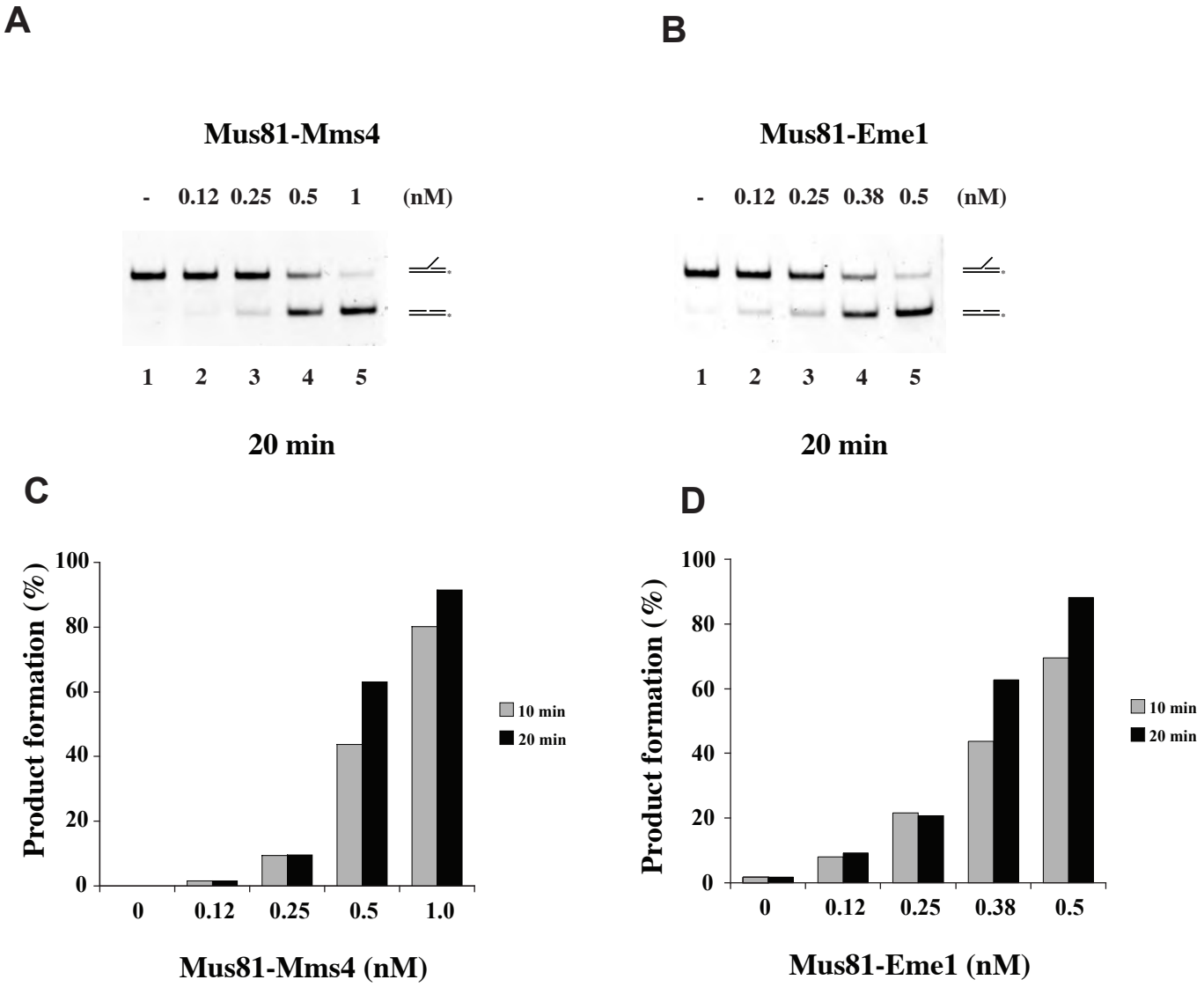
Supplemental Figure 3. The His_6-tag on Mms4 has no effect on the functionality of the Mus81-Mms4 complex.
(**A**) SDS-PAGE of Mus81-Mms4-$(His)_6$ treated without (lane 1) or with (lane 2) thrombin (0.1 unit, Novagen) in 10 µl of thrombin cleavage buffer (20 mM Tris-HCl, 150 mM NaCl, 2.5 mM $CaCl_2$, pH 8.4) for 2 hours at 16°C to remove the $(His)_6$ tag. (**B**) Mus81-Mms4-$(His)_6$ and Mus81-Mms4 (0.12, 0.25, 0.38, and 0.5 nM in panel I and panel II, as indicated) were assayed for nuclease activity using the 3' DNA flap (6 nM) as substrate. The reactions were incubated at 37°C for 30 min and then analyzed. (**C**) The 3' DNA flap (6 nM) was incubated with Mus81-Mms4-$(His)_6$ (0.25 nM in panel I) or Mus81-Mms4 (0.25 nM in panel II) and without or with Rad54 (1, 2, 4, or 8 nM) at 37°C for 30 min and then analyzed.

Supplemental Figure 4. Enhancement of Mus81-Mms4-mediated D-loop cleavage by Rad54.
(A) The D-loop substrate (6 nM) was incubated with Mus81-Mms4 (0.25 nM) and without or with Rad54 (0.5, 1, 2, 4, or 8 nM) at 37°C for 30 min. The reactions were stopped, deproteinized, resolved in a 20% denaturing PAGE gel containing 7 M urea in TBE buffer (90 mM Tris, 2 mM EDTA, 90 mM Boric acid) and analyzed. The incision point in the D-loop substrate is indicated by the arrow. Since the analysis was done under denaturing conditions, only the labeled ssDNA and cleavage product were detected. **(B)** Graphical representation of the data in (A).

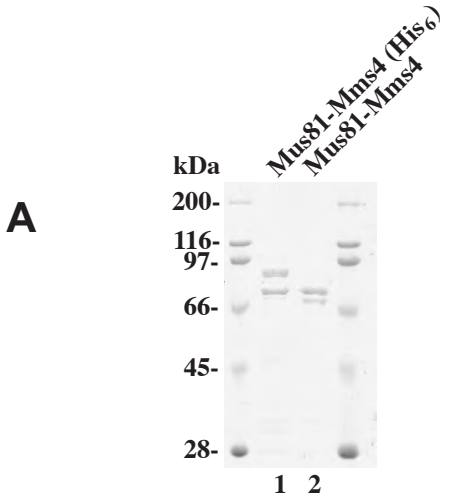
Supplemental Figure 1



Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4