

SUPPLEMENTAL DATA

The MukB-Topoisomerase IV Interaction Is Required for Proper Chromosome Compaction*

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Running title: MukB-Topoisomerase IV Interaction

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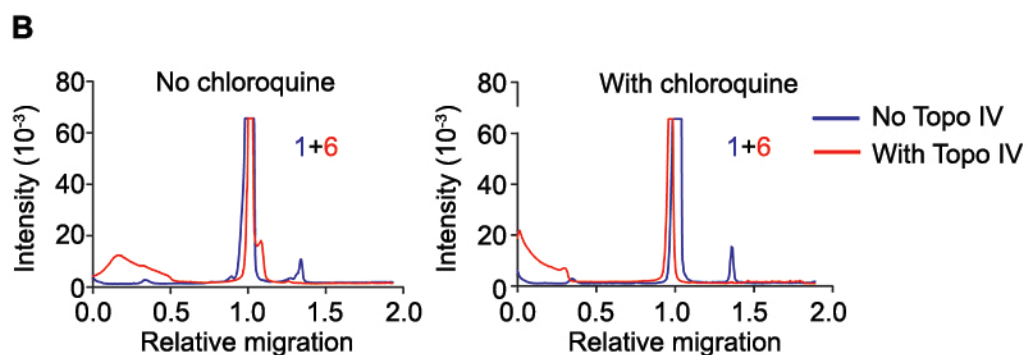
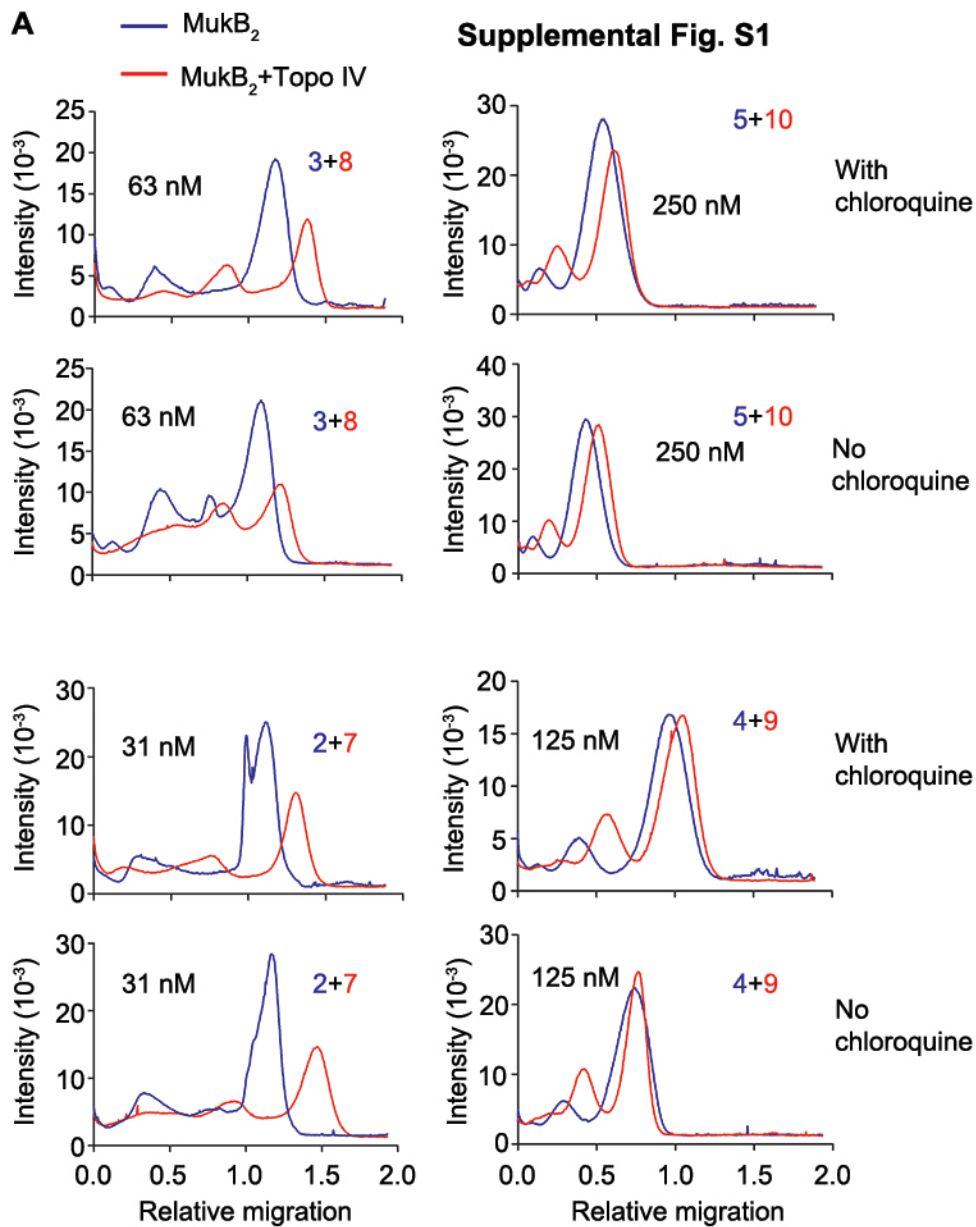
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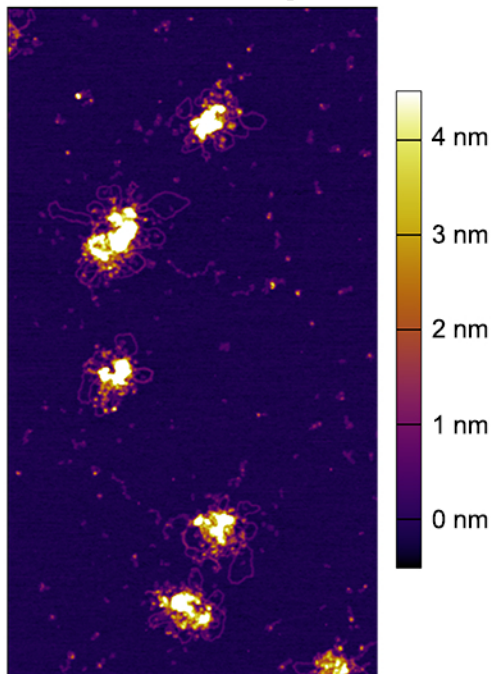
Legend for Supplemental Movie S1

Legend for Supplemental Movie S2



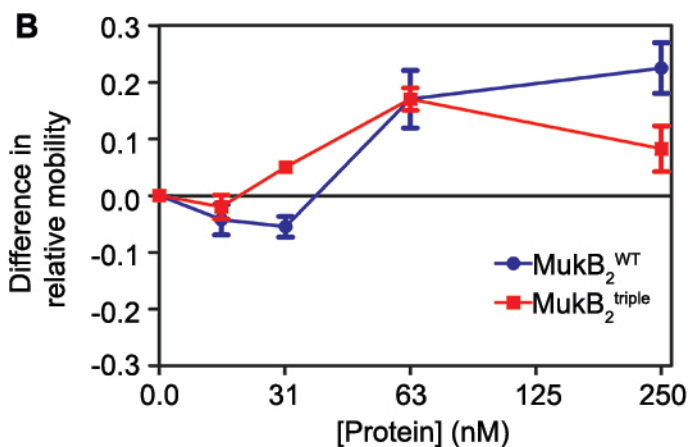
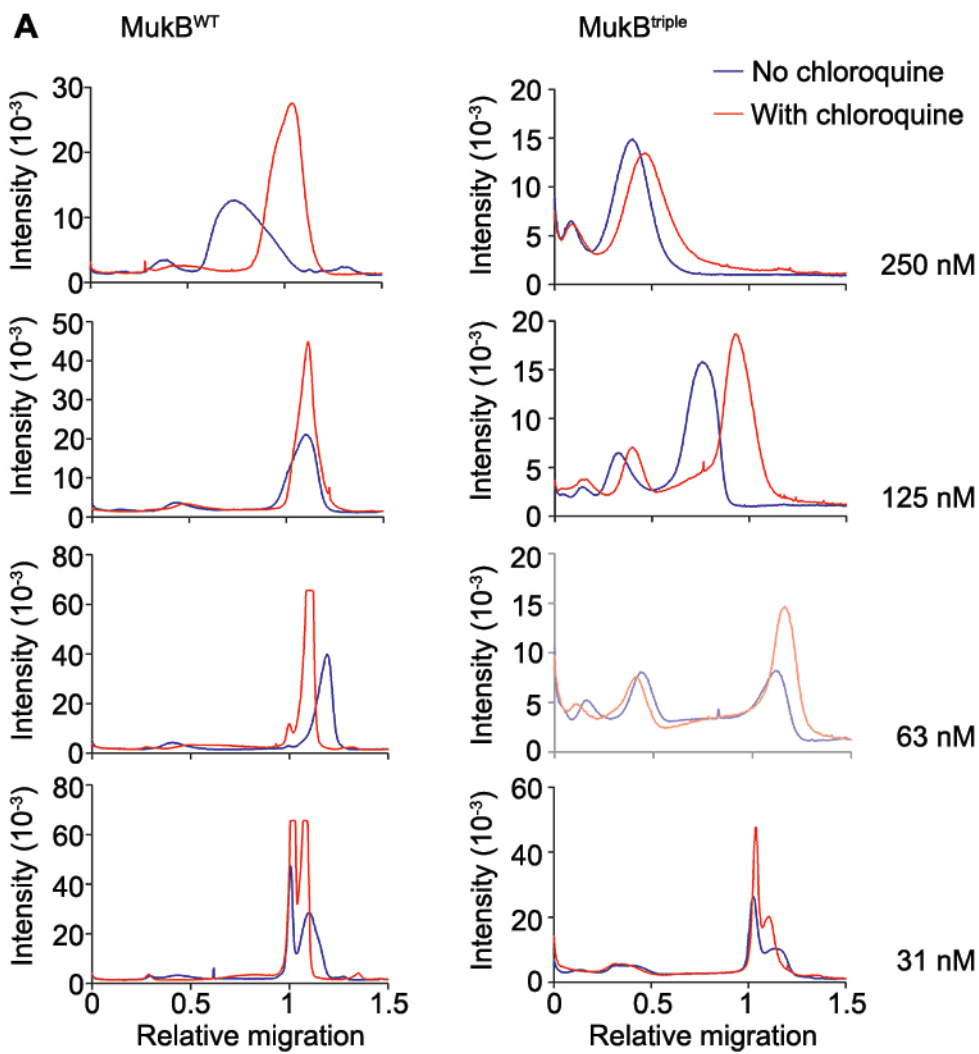
SUPPLEMENTAL FIGURE S1. MukB and Topo IV cooperate to maximally condense DNA.
A, Lane profiles from the gel shown in Fig. 1A. Clockwise from bottom left panel: lanes 2 and 7, Fig. 1A; lanes 2 and 7, Fig. 1B; lanes 3 and 8, Fig. 1A; lanes 3 and 8, Fig. 1B; lanes 5 and 10, Fig. 1B; lanes 5 and 10, Fig. 1A; lanes 4 and 9, Fig. 1B; lanes 4 and 9, Fig. 1A; respectively.
B, Comparison of the mobility of the nicked DNA in the presence and absence of chloroquine. Left panel, lanes 1 and 6, Fig. 1A; right panel, lanes 1 and 6, Fig. 1B, respectively.

Supplemental Figure S2

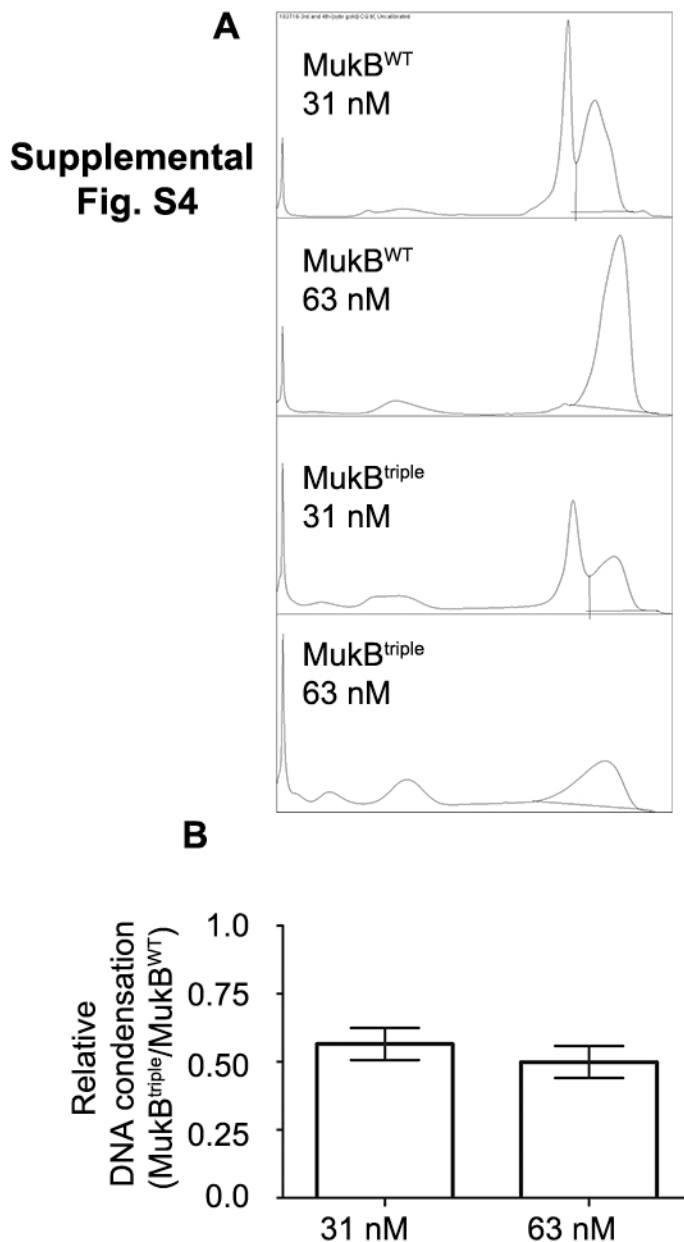


Supplemental Figure S2. **Scanning Force Microscopic Image of MukB-Topoisomerase IV Complex.** SFM was performed as described in the accompanying article (Kumar et al., submitted) using 30 nM MukB₂ and 60 nM Topo IV.

Supplemental Fig. S3

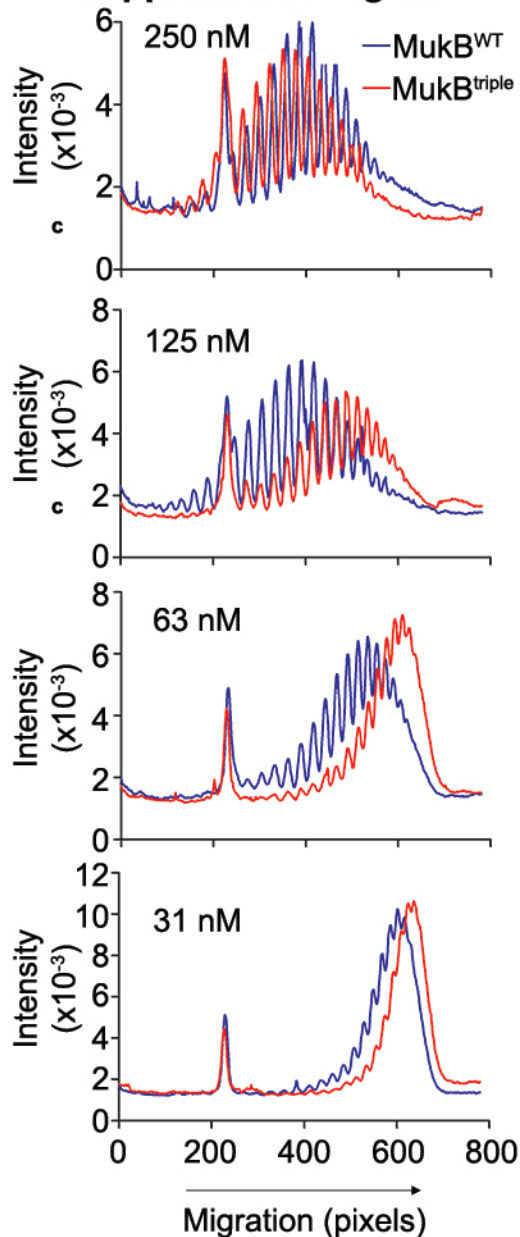


SUPPLEMENTAL FIGURE 3. THE MUKB D697K D745K E753K variant condenses DNA but does not form maximally condensed DNA with Topo IV. **A**, Effect of chloroquine on the mobility of protein-DNA complexes. Shown are the lane profiles for the gel presented in Fig. 5A. Left panels, top to bottom: lanes 5, lanes 4, lanes 3, and lanes 2, respectively. Right panels, top to bottom: lanes 9, lanes 8, lanes 7, and lanes 6, respectively. **B**, Relative change in mobility for the protein-DNA complexes in the presence and absence of chloroquine. The mean and standard deviation is shown for three experiments. The difference in relative mobility is given as the mobility of the protein-DNA complex in chloroquine divided by the mobility of the protein-DNA complex in the absence of chloroquine.



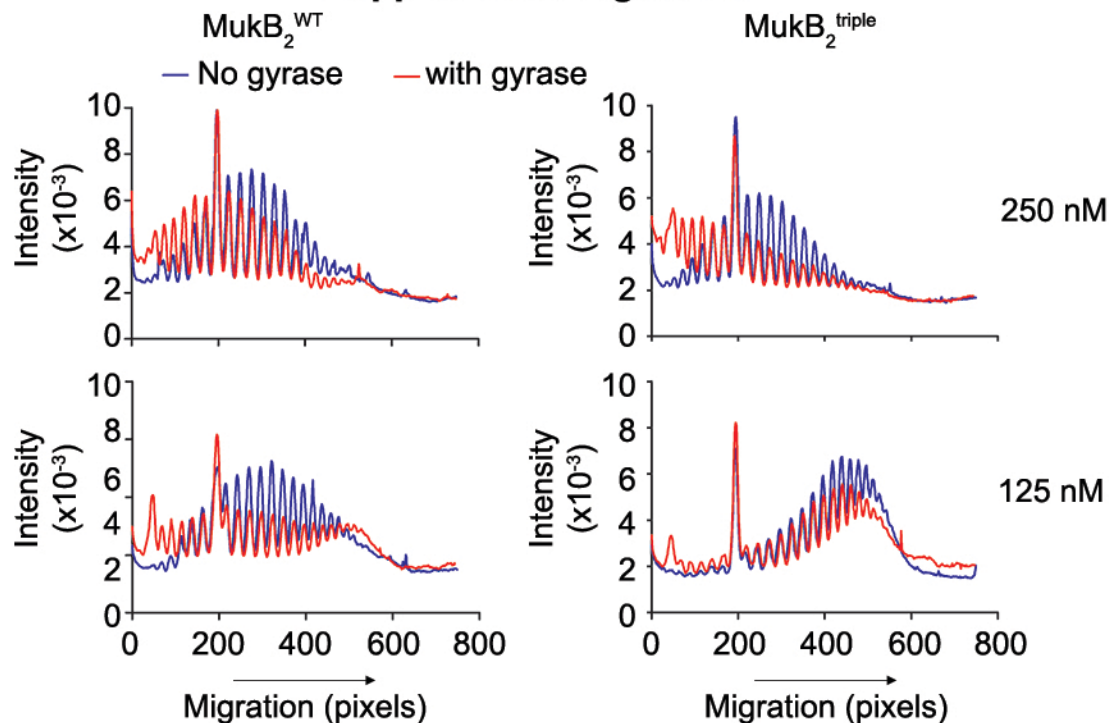
SUPPLEMENTAL FIGURE S4. The MukB D697K D745K E753K variant condenses DNA but does not form maximally condensed DNA with Topo IV. MukB^{triple} is roughly 50% as efficient as MukB^{wt} in condensing DNA. The fraction of DNA in protein-DNA complexes formed at 31 and 63 nM MukB that had a mobility greater than that of nicked DNA was determined and used as a measure of the extent of DNA condensation. *A*, Lane profiles from the gel shown in Fig. 5A. Top to bottom: lanes 2, 3, 6, and 7, respectively. *B*, Quantification of the extent of DNA condensation. Shown is the mean and standard deviation from three different experiments.

Supplemental Figure S5



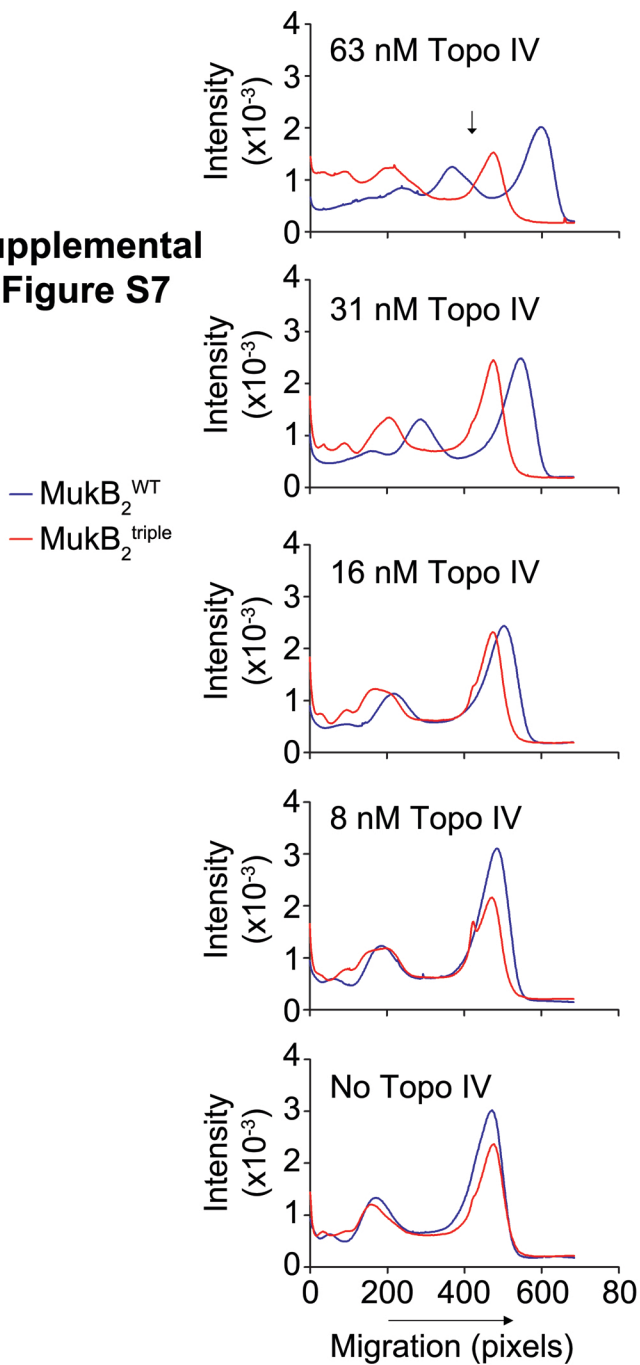
SUPPLEMENTAL FIGURE S5. The MukB D697K D745K E753K variant condenses DNA but does not form maximally condensed DNA with Topo IV. At low concentrations, MukB^{triple} does not protect negative supercoils as efficiently as MukB^{wt}. Lane profiles from the gel shown in Fig. 5B. Top to bottom panels: lanes 6 and 10, lanes 5 and 9, lanes 4 and 8, and lanes 3 and 7, respectively.

Supplemental Figure S6



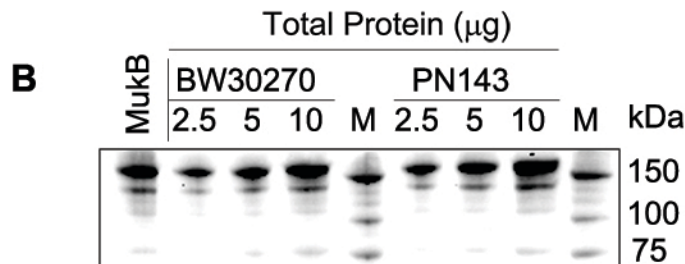
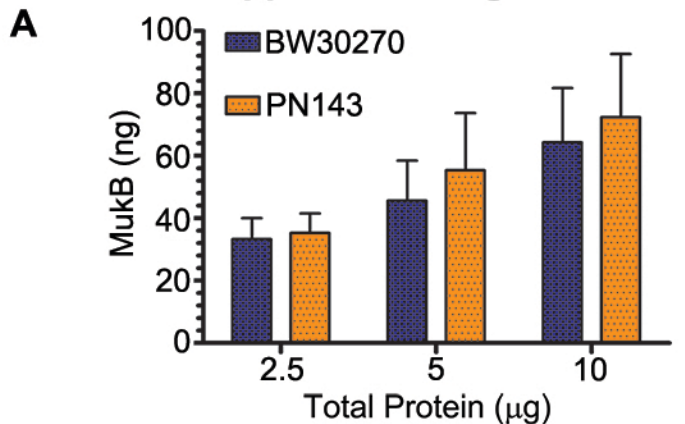
SUPPLEMENTAL FIGURE S6. The MukB D697K D745K E753K variant condenses DNA but does not form maximally condensed DNA with Topo IV. Topological loop stabilization by MukB^{triple} is less efficient than MukB^{wt} at low concentrations, but is equivalent at high concentrations. Lane profiles from the gel shown in Fig. 5C. Clockwise from bottom left panel: lanes 3 and 4, lanes 7 and 8, lanes 9 and 10, and lanes 5 and 6, respectively.

**Supplemental
Figure S7**



SUPPLEMENTAL FIGURE S7. The MukB D697K D745K E753K variant condenses DNA but does not form maximally condensed DNA with Topo IV. Lane profiles from the gel shown in Fig. 5D. Top to bottom panels: lanes 6 and 11, lanes 5 and 10, lanes 4 and 9, lanes 3 and 8, and lanes 2 and 7, respectively.

Supplemental Figure S8



SUPPLEMENTAL FIGURE S8. Determination of the relative levels of MukB^{triple} and MukB^{wt} in vivo. MukB levels were determined by Western blotting as described in Experimental Procedures. *A*, Quantification of MukB levels in cell extracts. Shown is the mean and standard deviation from three independent experiments. *B*, An example of one of the Western blots.

SUPPLEMENTAL MOVIE S1. Time-lapse imaging of the nucleoid replication/division cycle for PN124. Cells were grown in MOPS medium to $O.D._{600} = 0.2$. One to two microliters were spread on an agarose pad formed on a slide that had grooves formed in it by pressing down on the agarose with a glass optical diffraction grating (37). The agarose pad was formed in MOPS medium. The agarose pad was covered with a cover slip and immediately placed on the microscope stage. The entire microscope stage and objective turret was surrounded by an environmental control chamber held at 37 °C. After allowing temperature equilibration for 15 min, movies were commenced capturing the phase and mCherry red channels every two min.

SUPPLEMENTAL MOVIE S2. Time-lapse imaging of the nucleoid replication/division cycle for PN143. As in the legend for Movie S2.