

Supplemental Material

Topoisomerase III Acts at the Replication Fork to Remove Precatenanes

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Running Head: Topo III Removes Precatenanes

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SUPPLEMENTAL FIGURE LEGENDS

FIG S1 Expression level of Topo III and Topo III Y328F in CL109 and CL113, respectively.

Overnight cultures of CL109 (*topB-gfp mCherry-dnaN*) and CL113 (*topBY328F-gfp mCherry-dnaN*) grown at 30 °C in M9 minimal medium supplemented with 20 µg/ml thiamine, thymine, and uracil and 0.2% glycerol were diluted to O.D.₆₀₀ = 0.006 with fresh medium and grown at the same temperature to O.D.₆₀₀ = 0.9. Lysates and Western blotting using anti-GFP antibody (Invitrogen) were performed as described in reference 28 of the main text. Two left-hand lanes, CL109 and CL116, respectively. Right-hand lane, recombinant GFP (Clontech).

FIG S2 Flow cytometric analysis of DNA replication progression in synchronized cells of W3110 (wild type) and CL083 (W3110 Δ *topB*). Details of the methodology can be found in (1). Briefly, cells growing in M9 minimal medium were synchronized by the addition of DL-serine hydroxamate for 90 min. Cells were recovered and resuspended in fresh medium containing cephalixin. Cell growth was continued and at the indicated times aliquots were treated with rifampicin for 3 h. Cells were harvested, fixed, stained with Pico Green, and sorted with a BD Biosciences FAC-Scalibur flow cytometer. The fraction of cells containing 1N and 2N amounts of DNA were then plotted versus time from when the cells were released from the serine hydroxamate.

FIG S3 The number of individual movie frames that were averaged for each time point to make the average kymographs shown in Figs. 4B (wild type) and 4D (Δ *topB*).

FIG S4 Individual intensity plots for the 18-26 min frames of the averaged kymographs shown in Figs. 4B and 4D. Red, wild type. Blue, Δ *topB*. AU, arbitrary units.

SUPPLEMENTAL MOVIE LEGENDS

Movie S1. An example of time lapse imaging of MG1655*hupA::mcherry:frtKan* (CL269) growing in MOPS EZ Medium with 2% glucose used to derive the kymographs shown in Figs. 5A and 5B of the main text. Frames are every two min. Shown is the merge of the phase and Texas Red channels.

Movie S2. An example of time lapse imaging of MG1655 Δ *topB hupA::mcherry:frtKan* (CL272) growing in MOPS EZ Medium with 2% glucose used to derive the kymographs shown in Figs. 5C and 5D of the main text. Frames are every two min. Shown is the merge of the phase and Texas Red channels.

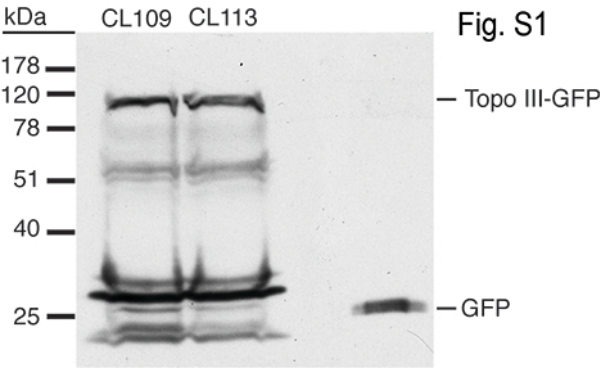
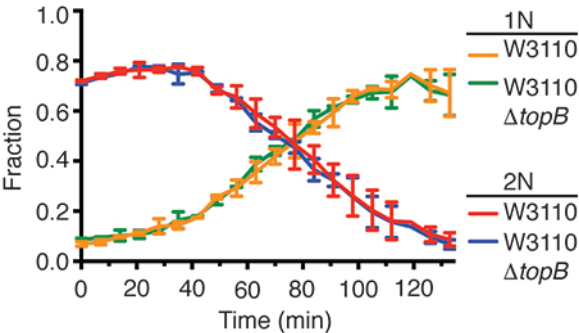
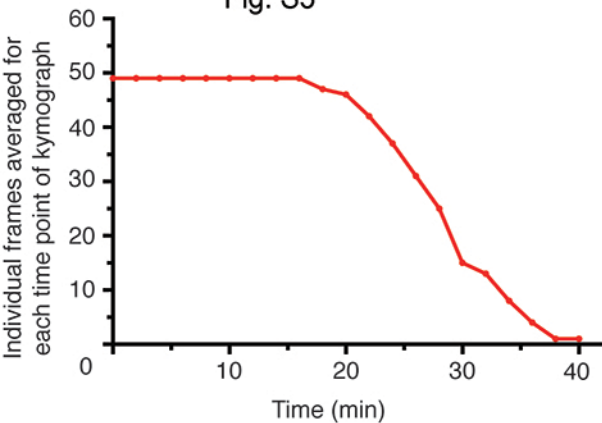
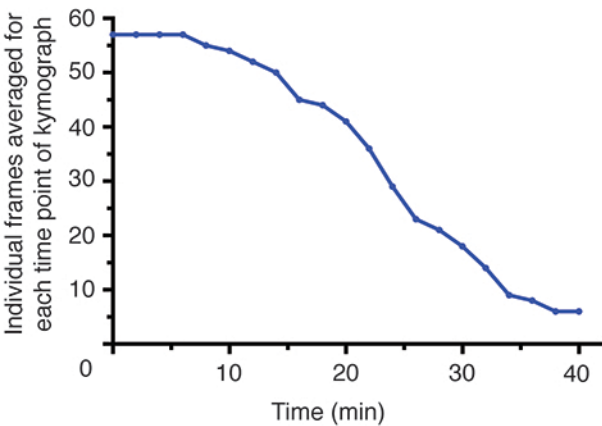


Fig. S2



A

Fig. S3

**B**

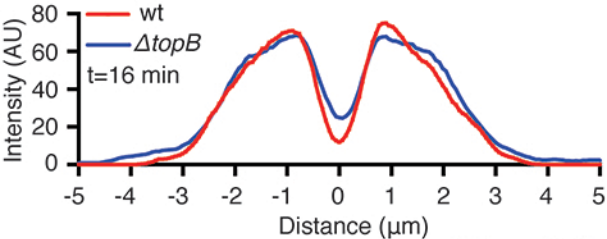


Fig. S4

