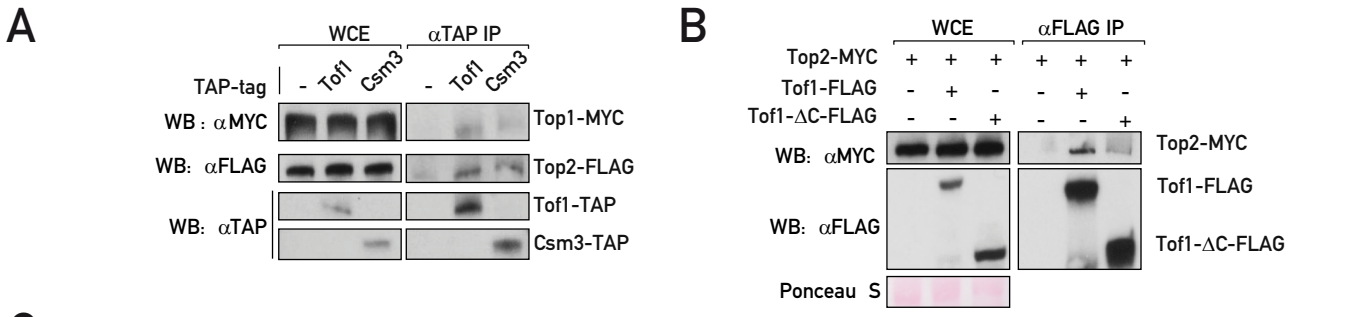
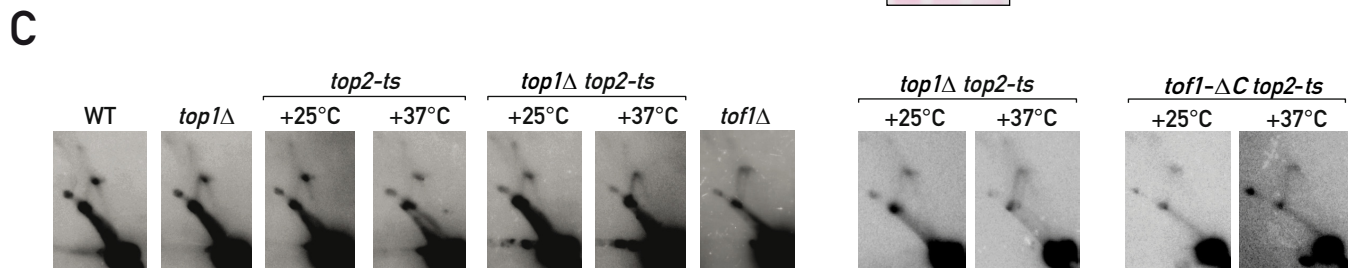
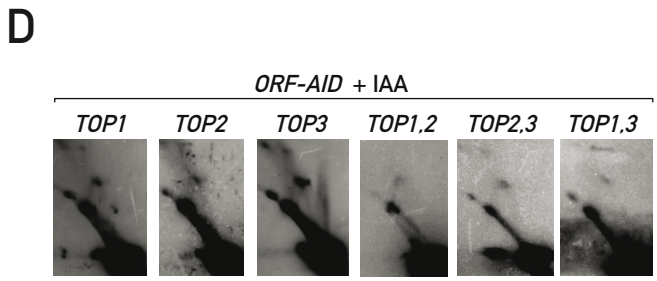
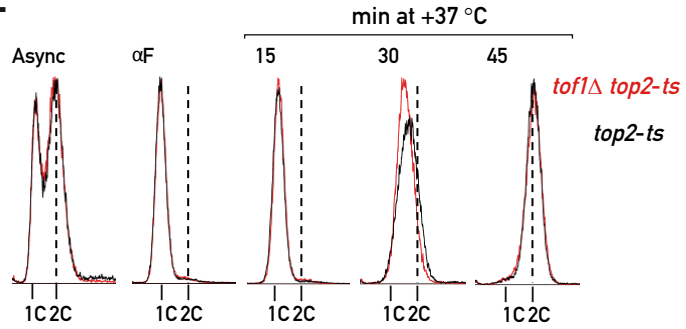
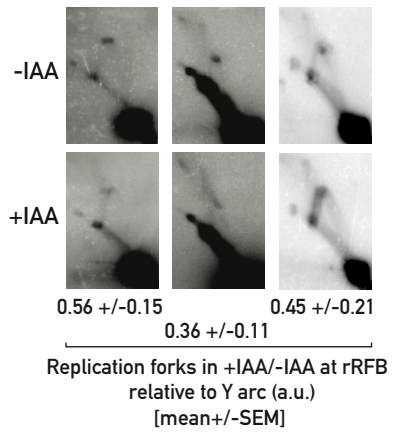
Shyian_FigS4

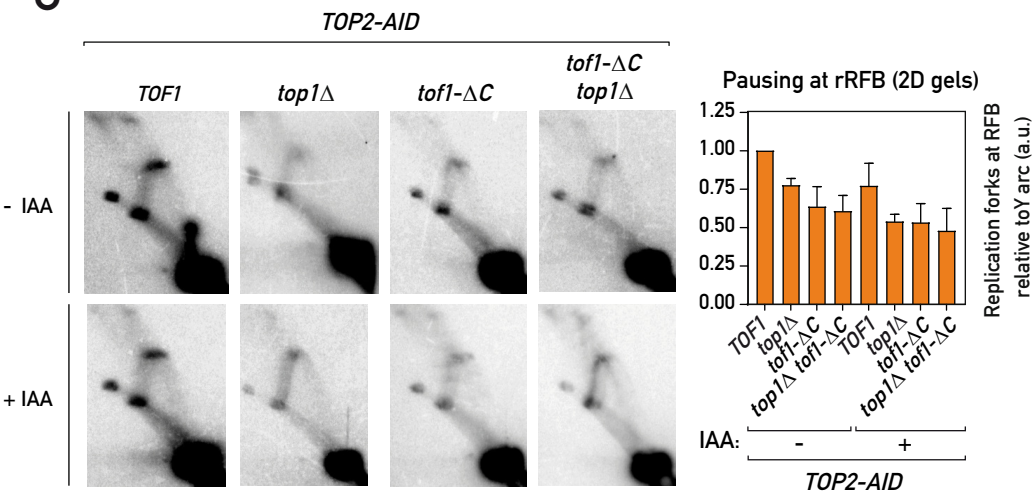












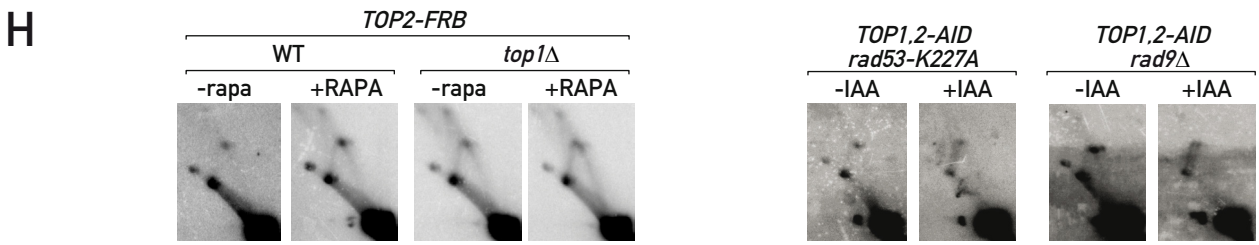


Figure S4. Related to Figure 4. Tof1-Csm3 engages Top1 and Top2 to pause the replisome (A-B) Co-immunoprecipitation experiments: Immunoprecipitates of Tof1 and Csm3 contained Top1 and Top2 (A), Top2 in the immunoprecipitates of Tof1-FLAG and Tof1-Δ C-FLAG (B). (C-D) 2D gels of the *Bgl* II digested DNA isolated from asynchronous cell cultures and probed with rDNA rRFB probe: DNA from asynchronous cultures of control strains (grown at +30 °C) or *top2-ts* strains (grown at +25 °C and shifted or not to +37 °C for 1 hour) (C); DNA from strains harboring AID-tagged topoisomerase genes *TOP1*, *TOP2* and *TOP3* from asynchronous cultures treated for 1 hour with 1 mM IAA (Indole-3-acetic acid) to degrade respective proteins (D). (E) Flow cytometry DNA content profile of the *tof1*Δ *top2-ts* (red) and *top2-ts* (black) strains upon release in S phase at +37 °C from G1 (αF) arrest. (F-H) 2D gels as in Figure S4D): upon Tof1, Top1 or Top1 and Top2 degradation (F); upon Top2 degradation (left panel – representative images; right panel – quantifications) (G); upon Top2 anchoring away from the nucleus (H, four left panels); fork pausing in strains additionally harboring mutations of the DNA damage checkpoint genes (*rad53-K227A* and *rad9*Δ) (H, right four panels). RAPA - rapamycin. Values plotted and statistics as in Figure 1.