Shyian_FigS2

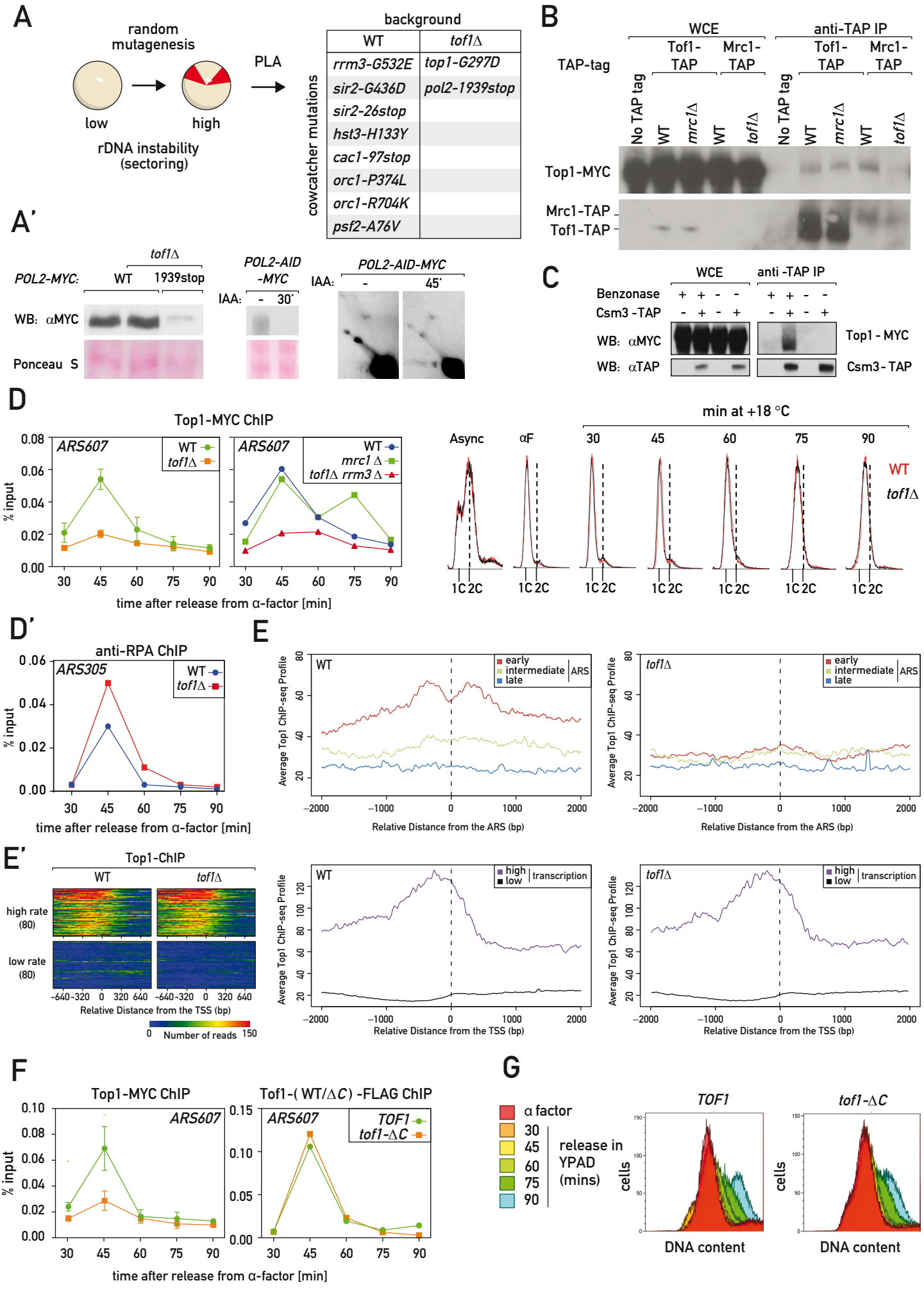

Figure S2. Related to Figure 2. Tof1-Csm3 recruits Top1 to the replisome

(A) An outline of the "cowcatcher" screen and mutants identified by Pooled Linkage Analysis (PLA) as leading to elevated rDNA instability in WT and *tof1*Δ backgrounds.
(A') *pol2-1939stop* allele led to low levels of expression of full-lenght Pol2 protein (left panel), perhaps due to stop codon read-through; degrading Pol2 protein with auxin-inducible degron (middle panel) did not affect fork pasuing at rRFB (right panel).
(B-C) Western blot detection of the Top1-MYC in Tof1-TAP and Mrc1-TAP (B) or Csm3-TAP (C) anti-TAP immunoprecipitates; DNA degradation by Benzonase was absolutely required to co-immunoprecipitate Top1 (C).
(D-E') Top1-MYC ChIP experiments followed by qPCR at *ARS607, ARS305* or high throughput DNA sequencing in cell cultures synchronously released in S phase from G1 (α-factor) arrest: Top1-MYC ChIP-qPCR in the strains of designated genotypes (D, left panel); representative flow cytometry prophiles of WT and *tof1*Δ strain cultures (D, right panel); anti-RPA ChIP to estimate origin activation timing (D'); average Top1-MYC ChIP-seq profiles (read distribution) in the vicinity of replication origins at 45' of release into S phase (E; see also Figure 2D) and in the vicinity of TSS: heat map (E', left panel) and average profiles (E', right panel).
(F-G) Top1-MYC, or Tof1-FLAG and Tof1-ΔC-FLAG ChIP-qPCR experiments in *TOF1* and *tof1*-Δ*C* cells: ChIP-qPCR on *ARS607* (F); representative flow cytometry profiles of *TOF1* WT and *tof1*-Δ*C* strain cultures (G).

PLA - pooled linkage analysis. IAA - Indole-3-acetic acid. TSS - transcription start site.