Supplementary Figure 1

***TOP3* and *TOP3*^{Y356F} are overexpressed in the presence of 2% galactose**

Wild-type strains transformed with pYES2-*TOP3* or pYES2-*TOP3*^{Y356F} plasmids were grown in medium containing 2% glucose. Galactose was added to the cultures at a final concentration of 2%, and protein extracts were prepared at the indicated times. Equivalent amounts of cell extract were separated by SDS-PAGE, and the Top3 and Top3^{Y356F} proteins were detected by Western blotting. Rad53 was also detected in the same samples to verify that equivalent protein levels were loaded and that overexpression of *TOP3* or *TOP3*^{Y356F} do not elicit a DNA damage response.

Supplementary Figure 2

Overexpression of *TOP3*^{Y356F} causes a *top3*-like phenotype that is suppressed by mutation of *SGS1*

Wild-type and *sgs1* strains transformed with pYES2, pYES2-*TOP3* or pYES2-*TOP3*^{Y356F} plasmids were grown in medium containing 2% glucose. Cultures were spotted onto 2% glucose (control) or 2% glucose + 2% galactose plates (to induce overexpression from the pYES2 *GAL1* promoter). Spots from left to right represent serial 1 in 10 dilutions of yeast cultures. Plates were grown at 30°C for up to 4 days.

Supplementary Figure 3

Rad53 activation occurs normally in cells overexpressing *TOP3*^{Y356F} following DNA damage

Wild-type strains transformed with pYES2 or overexpressing *TOP3*^{Y356F} were released from G_1–arrest into fresh medium containing 0.033% MMS. Protein extracts were prepared at the indicated times and Rad53 phosphorylation status was monitored by Western blotting. The positions of the unphosphorylated Rad53 and slower-migrating phosphorylated forms are shown on the right.

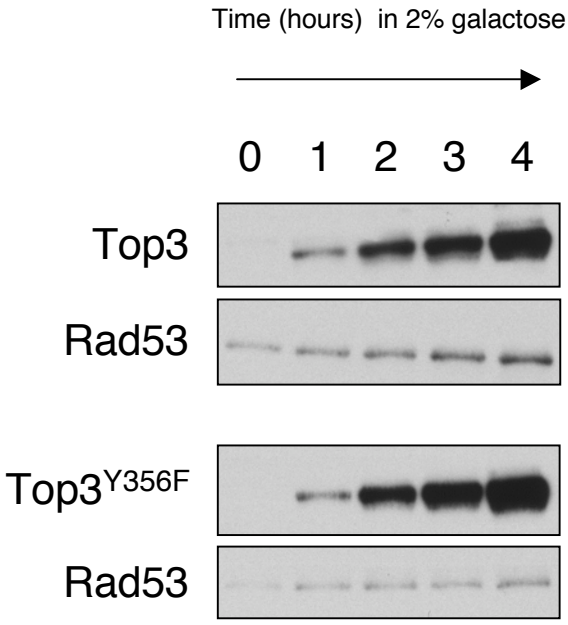
Supplementary Figure 4

Mutation of *RAD51* suppresses the poor growth caused by overexpression of *TOP3*^{Y356F}

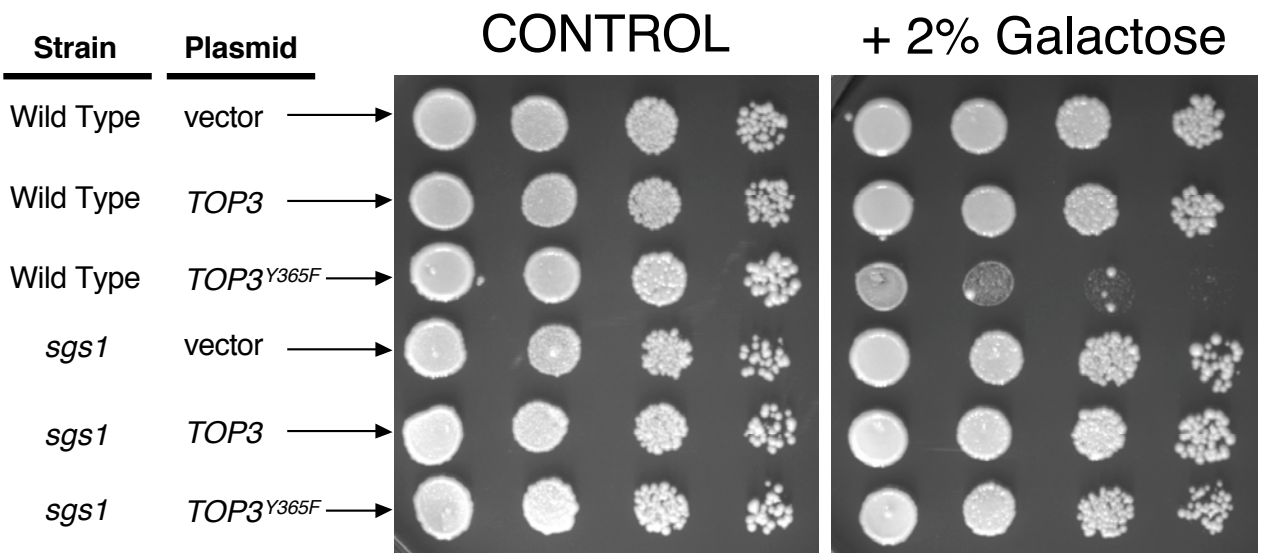
Wild-type and *rad51* strains transformed with empty pYES2 vector, pYES2-*TOP3* or pYES2-*TOP3*^{Y356F} were grown in medium containing 2% glucose. Cultures were spotted onto 2% glucose (control) or 2%

glucose + 2% galactose plates (to induce overexpression from the pYES2 *GAL1* promoter). Spots from left to right represent serial 1 in 10 dilutions of yeast cultures. Plates were grown at 30°C for up to 4 days.

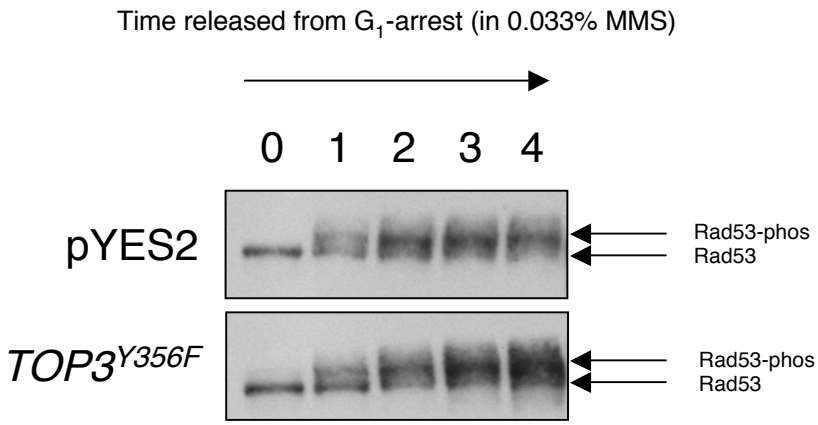
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4