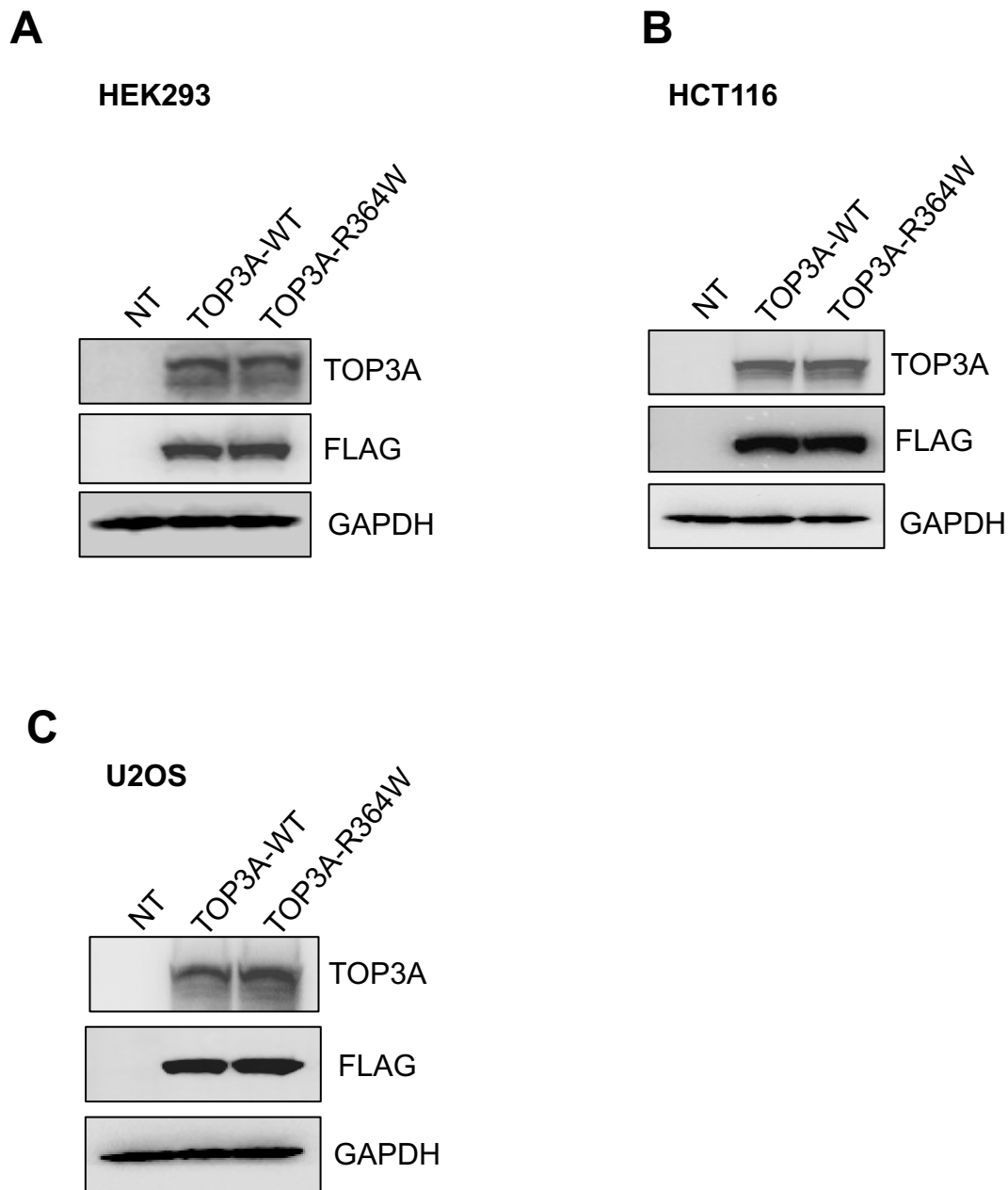
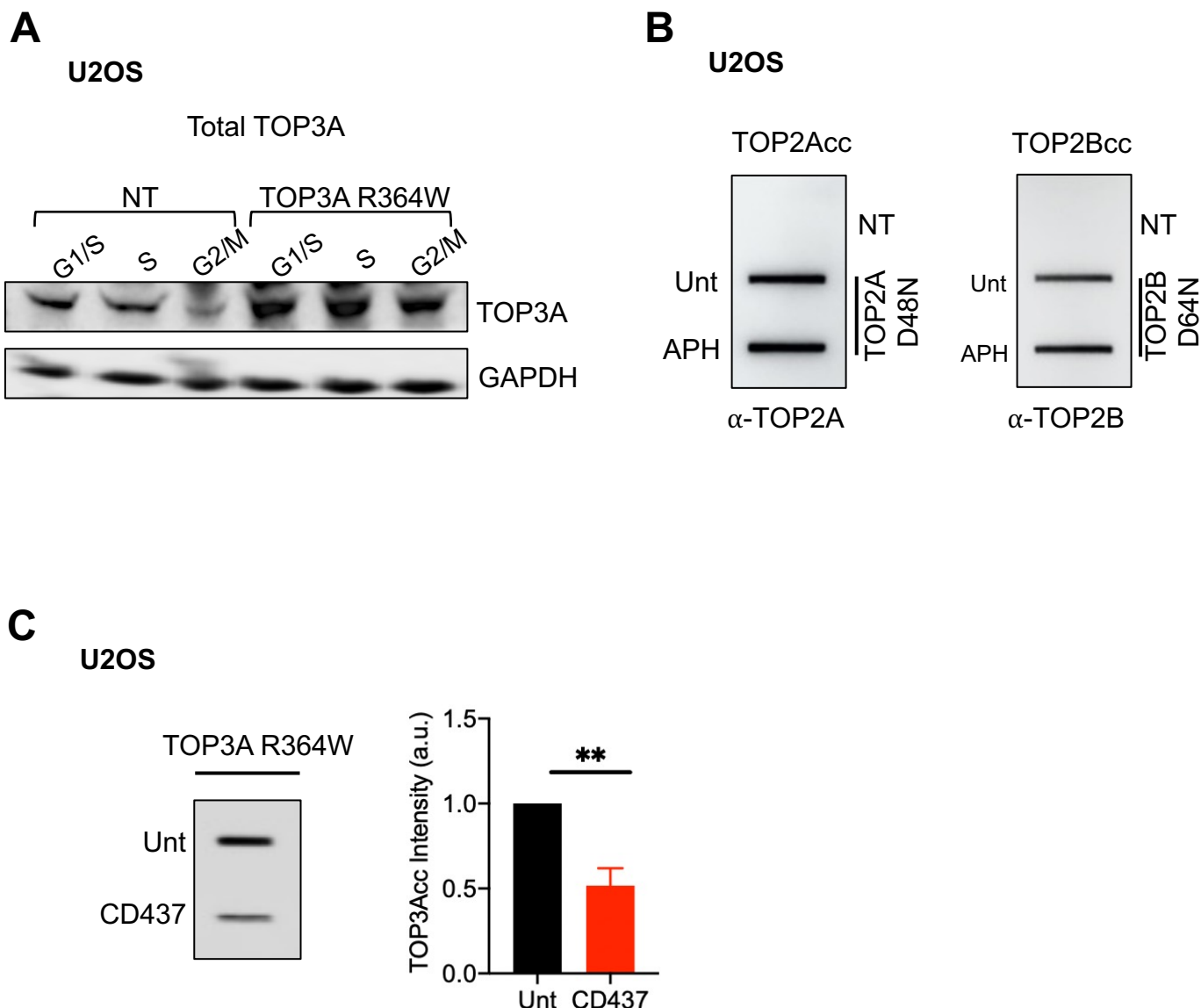




Supplementary Figure 1. Ectopic expression of TOP3A-WT and TOP3A-R364W

(A-C) Western blots analysis of cells over-expressing TOP3A-WT and TOP3A-R364W in HEK293 (A), HCT116 (B) and U2OS cells (C). Cells were transfected with the indicated FLAG-tagged TOP3A constructs for 48 h and subjected to Western blotting with indicated antibodies. GAPDH is loading control.

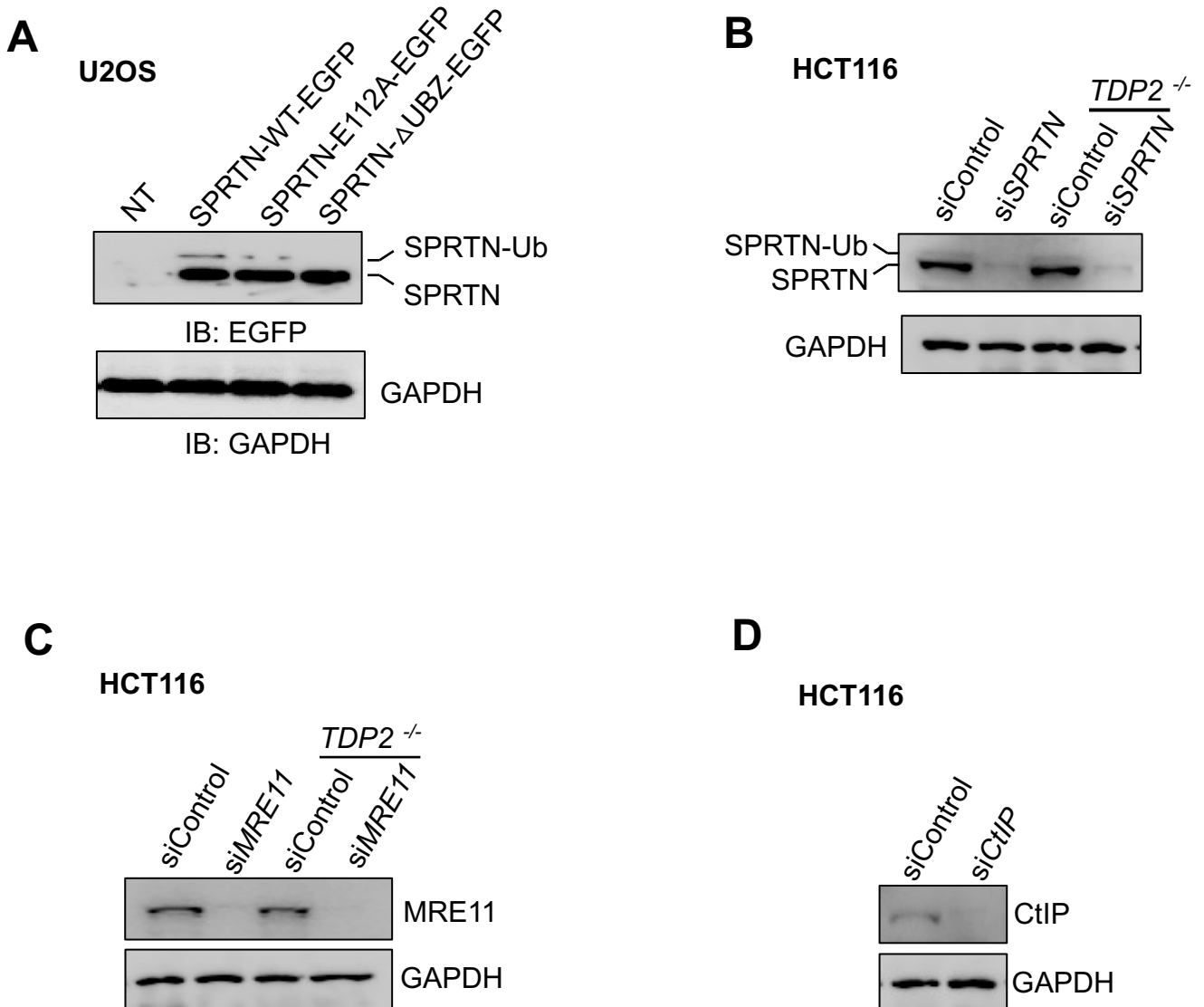


Supplementary Figure 2. Cell cycle phase dependent expression of TOP3A-R364W and impact of polymerase α inhibition on TOP3Accs.

(A) Western blots analysis of level of TOP3A in U2OS cells over-expressing TOP3A-R364W and mock-transfected (NT) in different phases of the cell cycle.

(B) Aphidicolin treatment causes no reduction in TOP2Accs and TOP2Bccs level. Representative slot blot for the detection of TOP2Accs and TOP2Bccs by RADAR assay in U2OS cells transfected with the indicated self-trapping TOP2A and TOP2B plasmid constructs respectively for 48 h. TOP2Accs and TOP2Bccs were detected with anti-TOP2A and anti-TOP2B antibody respectively.

(C) TOP3A-R364W-transfected U2OS cells were pretreated with 1.5 μ M DNA polymerase α inhibitor, CD437 for 1 h before cell harvesting and RADAR assays. CD437 treatment reduces TOP3Accs levels. Left panel: representative slot blot probed with anti-TOP3A antibody. Right panel: quantitation for the 3 independent experiments as shown on the left panel. P-values were obtained by two-tailed unpaired *t*-test. ** $p < 0.01$.



Supplementary Figure 3. Ectopic expression of SPRTN-EGFP plasmids and verification of depletion of SPRTN, TDP2 and MRE11.

(A) Western blot analysis of U2OS cells expressing EGFP-tagged SPRTN-WT, SPRTN-E112A (catalytic dead) and SPRTN-ΔUBZ plasmids. Cells were transfected with the indicated FLAG-tagged SPRTN constructs for 48 h and subjected to Western blotting with α-EGFP antibody. GAPDH is loading control.

(B-D) Western blot analysis of the expression of SPRTN (B), MRE11 (C), and CtIP (D) in indicated genotypes. GAPDH is loading control.