Supplemental material

Abbreviations:

CPT, camptothecin; CTD, C-terminal domain; DRB, 5,6-dicholoro-1-β-D-ribobenzimidazole; DSBs, double-strand breaks; etoposide, 4'-demethylepipodophyllotoxin ethylidene-β-D-glucoside; pMEFs, primary mouse embryonic fibroblasts; MG132: carbobenzoxy-L-leucyl-L-leucyl-L-leucinal; RNAPII, RNA polymerase II; RNAPII LS, the large subunit of RNA polymerase II; Ser2-P, serine-2 phosphorylated; Ser5-P, serine-5 phosphorylated; SSB, single-strand breaks; t-AML, therapy-related acute myeloid leukemia; Top1, topoisomerase I; Top2, topoisomerase II; Ub, ubiquitin.

Supplemental Figure Legend

Fig. S1 (A) Over-expression of UBA domain of Rad23 (ΔUBL) did not abolish VP-16-induced Top2β degradation. HeLa cells were transfected with the plasmid (pcDNA3) expressing truncated Rad23 ΔUBA or ΔUBL. Empty vector served as a vehicle control. Cells were then treated with 250 μM VP-16 or 25μM CPT for time periods as indicated. Cell lysates were prepared and immunoblotted with anti-Top1, Top2β or α-tubulin antibody. CPT-induced Top1 degradation served as positive control for Ub-dependent degradation (24). **(B)** siRNA-mediated silencing of PA28γ regulatory particle does not impact VP-16-induced degradation of Top2β. HeLa cells were transfected with control or PA28γ siRNA. 72 hrs post-transfection, cells were treated with 250 μM VP16 or 25 μM CPT in the presence or absence of proteasome inhibitor MG132 for time periods as indicated. Cell lysates were immunoblotted with anti-Top1, Top2β or α-tubulin antibody. Silencing of PA28γ was evaluated by immunoblotting against PA28γ protein.

Fig. S2 (A) The knockdown efficiencies of 19S siRNAs were evaluated by the decrease of its corresponding protein. HeLa cells were transfected with control, Rpt5, Rpt6, Rpn2, Rpn11or S5a siRNA. 72 hrs post-transfection, cell lysates were immunoblotted with anti-Rpt5, Rpt6, Rpn2, Rpn11or S5a antibody. **(B)** The knockdown efficiencies of 19S siRNAs were further confirmed by the accumulation of Ub-conjugates in whole cell lysates. HeLa cells were transfected with control, Rpt5, Rpt6, Rpn2, Rpn11or S5a siRNA. 72 hrs post-transfection, cell lysates with same protein concentration were immunoblotted with anti-Ub antibody.

Fig. S3 A model that schematically depicts the two different polarities of the Top1 roadblock and the subsequent responses of RNAPII.