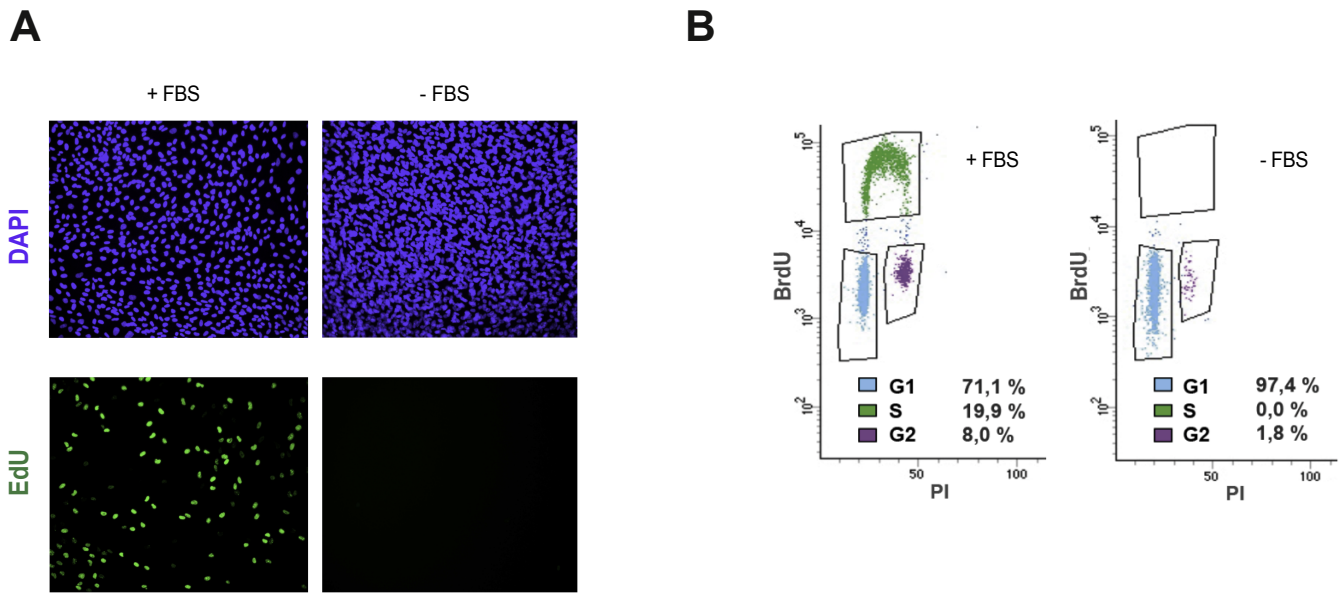


Figure S1. Serum-starved confluent RPE-1 cells synchronised in G0/G1. (**A**) Representative images of 5-Ethynyl-2'-deoxiuridine (EdU) pulse labelling in RPE-1 cells. Left, proliferating cells (+FBS). Right, serum-starved (-FBS) confluent cells. EdU (green) and DAPI counterstain (blue) are shown. (**B**) RPE-1 cells were stained for incorporated BrdU against total DNA content using Propidium Iodide (PI). Left, proliferating cells in presence of serum (+FBS). Right, serum-starved (-FBS) confluent cells. The percentage of each phase is indicated.

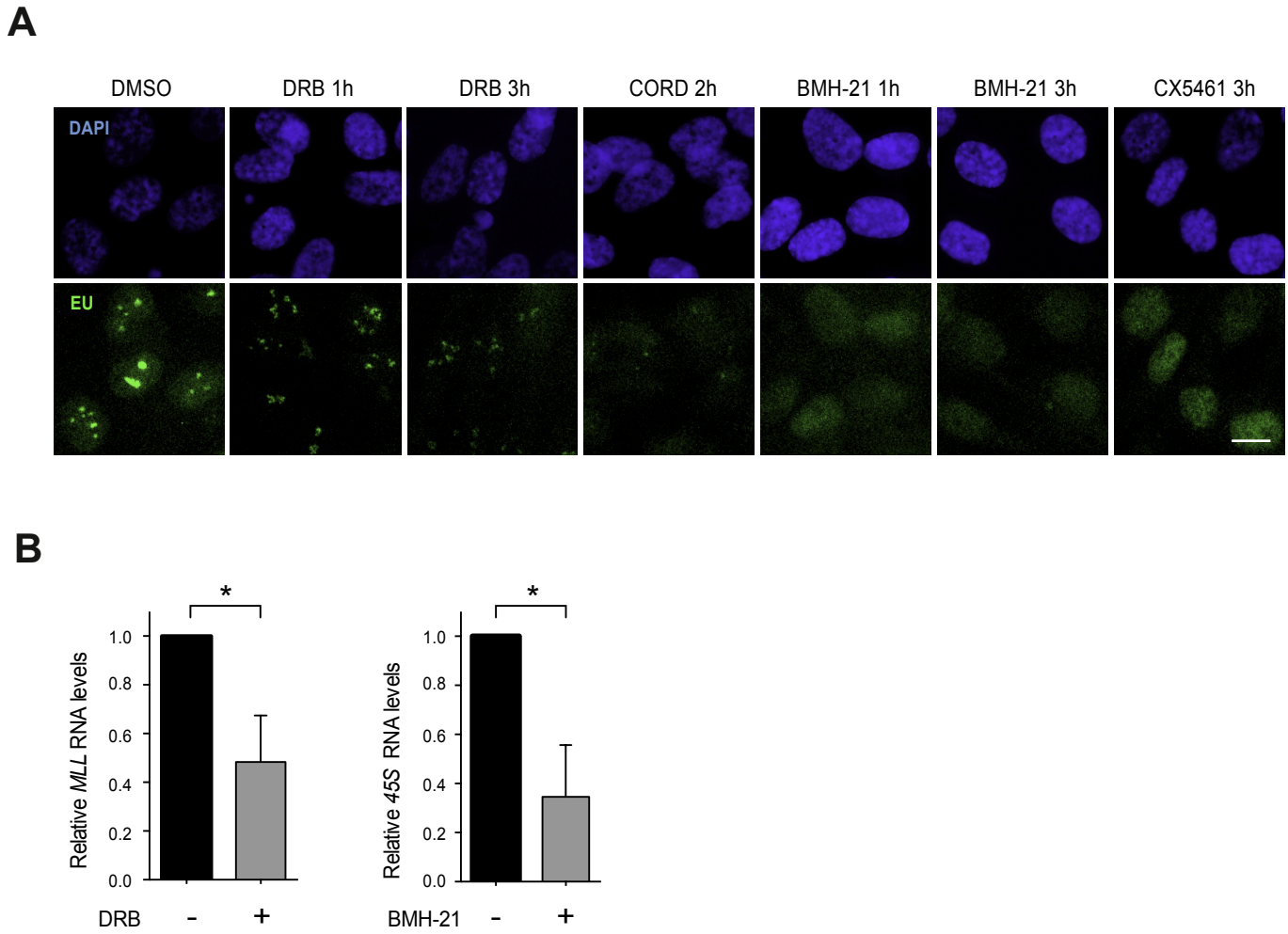


Figure S2. Transcription inhibition in RPE-1 cells. (**A**) Representative images of 5-Ethynyl Uridine (EU) pulse labelling in serum-starved RPE-1 cells after the indicated mock-treatment (DMSO) or treatment with 1 or 3 h with 100 μM DRB, 2 h with 100 μM cordycepin (CORD), 1 or 3 h with 100 μM BMH21, or 3 h with 20 μM CX5461. EU (green) and DAPI counterstain (blue) are shown. (**B**) *Left*, *MLL* (RNA polymerase II-transcribed gene) mRNA levels in serum-starved RPE-1 cells following mock-treatment (DMSO) or treatment with 100 μM DRB for 3 h. *Right*, 45S (RNA polymerase I-transcribed rDNA) RNA levels in serum-starved RPE-1 cells following mock-treatment or treatment with 2 μM BMH21 for 3 h. In all cases, RNA levels were quantified by qRT-PCR, normalized against 18S and then made relative to the mock-treated condition. Data are the mean (± s.e.m) of four independent experiments. Statistical significance was determined by *t*-tests (*P<0.05).

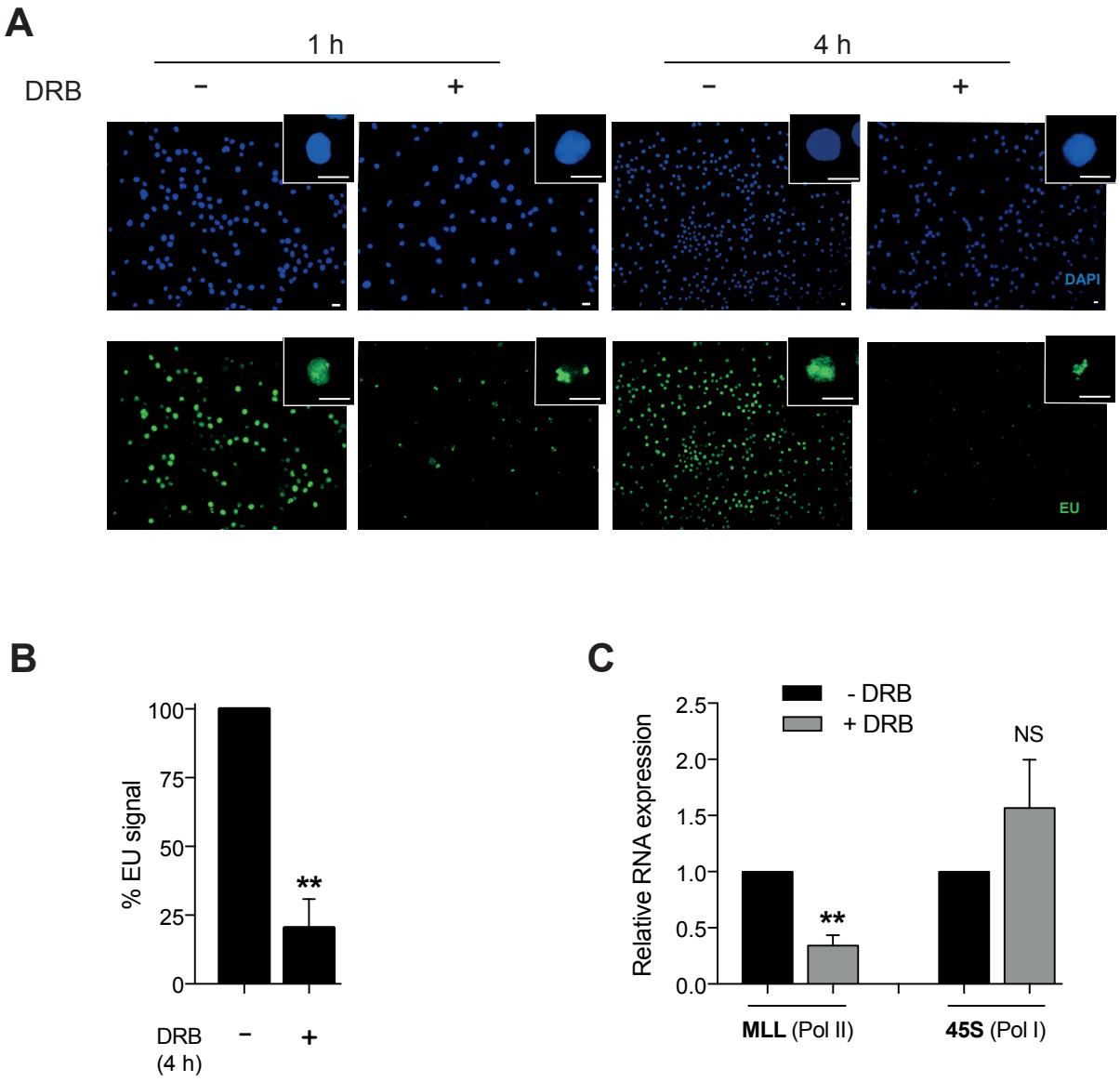


Figure S3. Transcription inhibition in KG1a cells. (**A**) Representative images of 5-Ethynyl Uridine (EU) pulse labelling in proliferating KG1a cells after treatment with 1 or 4 h with 100 μM DRB or vehicle. EU (green) and DAPI counterstain (blue) are shown. Scale bar, 10 μm. (**B**) Quantification of EU signal at 4 h DRB treatment (mean ± s.e.m.) of three independent experiments. Statistical significance was measured by *t*-test (**$P<0.01$). (**C**) Analysis of *MLL* (RNA polymerase II-transcribed gene) and 45S (RNA polymerase I-transcribed rDNA) mRNA levels in KG1a cells treated with 100 μM DRB for 4 h. In all cases, RNA levels were quantified by qRT-PCR, normalized against 18S expression and then made relative to the mock-treated condition. Data are the mean (± s.e.m) of three independent experiments. Statistical significance was measured by *t*-test (**$P<0.01$, NS, not significant).

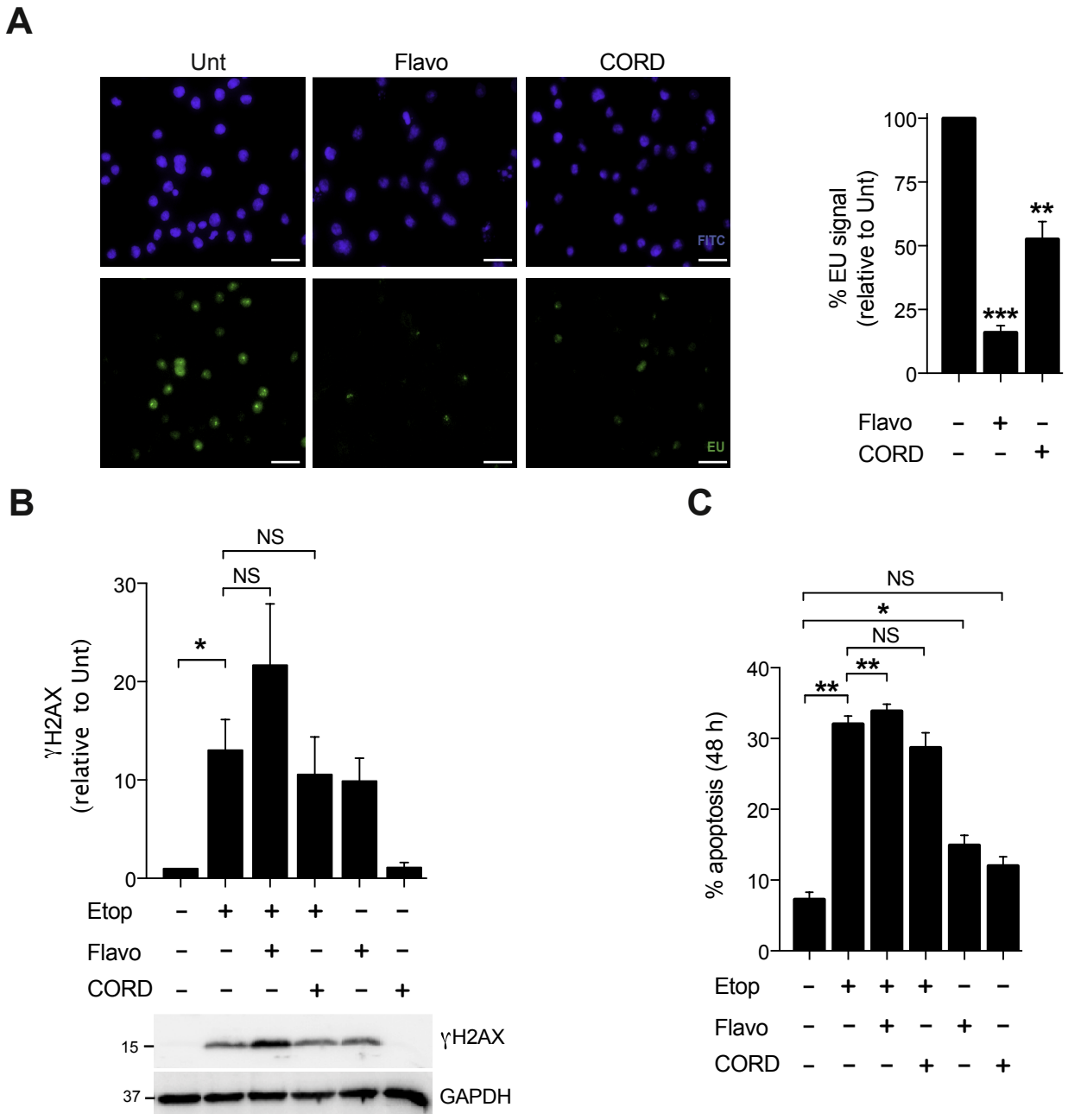


Figure S4. Effect of RNA Pol II inhibitors on etoposide survival in cycling cells (**A**) Representative images of 5-Ethynyl Uridine (EU) pulse labelling in proliferating KG1a cells after treatment with 500 μM flavopiridol or 100 μM cordycepin for 2 h. Left, representative images of EU (green) and DAPI counterstain (blue). Scale bar, 10 μM. Right, quantification of EU signal (mean ± s.e.m.) of three independent experiments. Statistical significance was measured by *t*-test (**P<0.01, ***P<0,001). (**B**) Protein blots showing γH2Ax and GAPDH in KG1a cells treated with 40 μM etoposide for 3 h. Where indicated, cells were pre-incubated with flavopiridol (500 μM) or cordycepin (100 μM) for 2 h, prior etoposide treatment. Representative images and quantification (mean ± s.e.m.) of three independent experiments. γH2Ax protein level was normalized to GAPDH. Statistical significance was measured by *t*-test (*P<0.05, NS, not significant). Molecular weight markers are in kDa. (**C**) FACS apoptosis analysis of KG1a cell line treated with 40 μM etoposide for 3 h. Where indicated, cells were pre-incubated with flavopiridol or cordycepin as in (B). Cells were incubated in drug-free medium for 48 h. Plot shows Annexin-V positive cells percentage (mean ± s.e.m.) of three independent experiments at 48 h. Statistical significance was determined by *t*-test (*P<0.05, **P<0.01, NS, not significant).

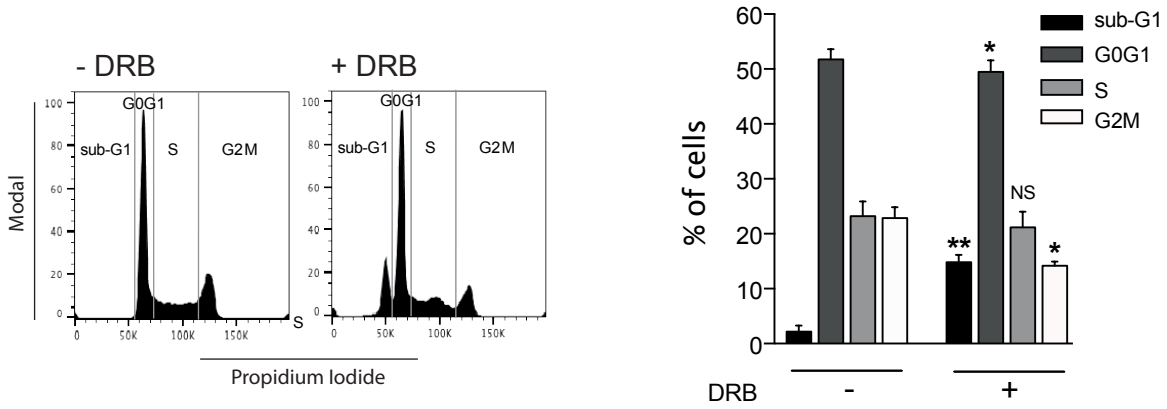


Figure S5. Cell cycle analysis of KG1a cells. Cell cycle analysis of proliferating KG1a cells after 4 h of 100 μM DRB by FACS. Left, histograms of cell cycle phases according to PI staining of untreated and DRB treated cells. Right, quantification (mean ± s.e.m.) of at least three independent experiments. Data shows percentage of cells in each cell cycle phase. Statistical significance was measured by *t*-test (**P*<0.05, ***P*<0.01, NS, not significant).

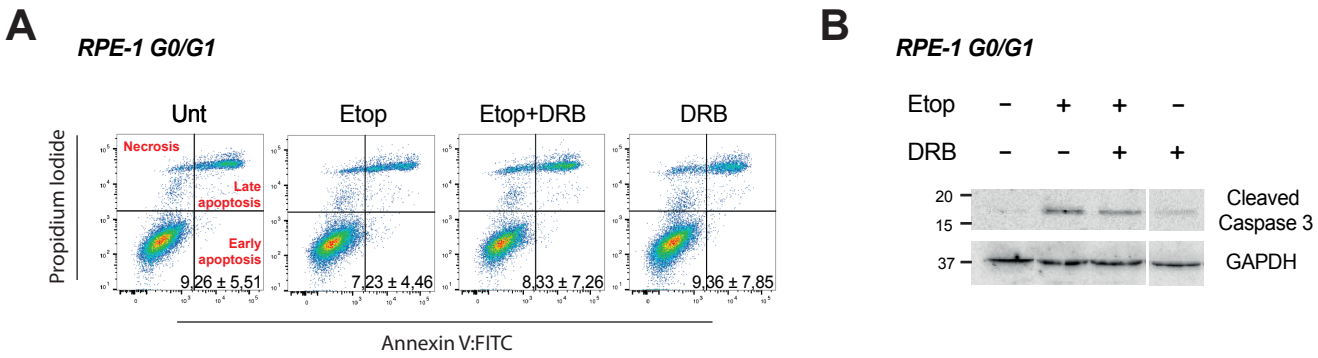


Figure S6. Etoposide-induced apoptosis is independent of transcription. (**A**) Apoptosis analysis by FACS of serum starved RPE-1 cells treated with 40 μM etoposide for 3 h. Cells were pre-incubated with 100 μM DRB for 1 h prior etoposide treatment. Following trypsinization, cells were cultured in serum containing drug-free medium for 48 h. Representative dot-plots (Annexin-V vs. Propidium Iodide) and Annexin-V positive cells percentage mean (± s.e.m.) of three independent experiments. Statistical significance was determined by T-test. Not significant differences were observed between conditions. (**B**) Protein blots of cleaved Caspase-3 in serum starved RPE-1 cells treated as in (A). Vinculin was used as a loading control. Molecular weight markers are in kDa.

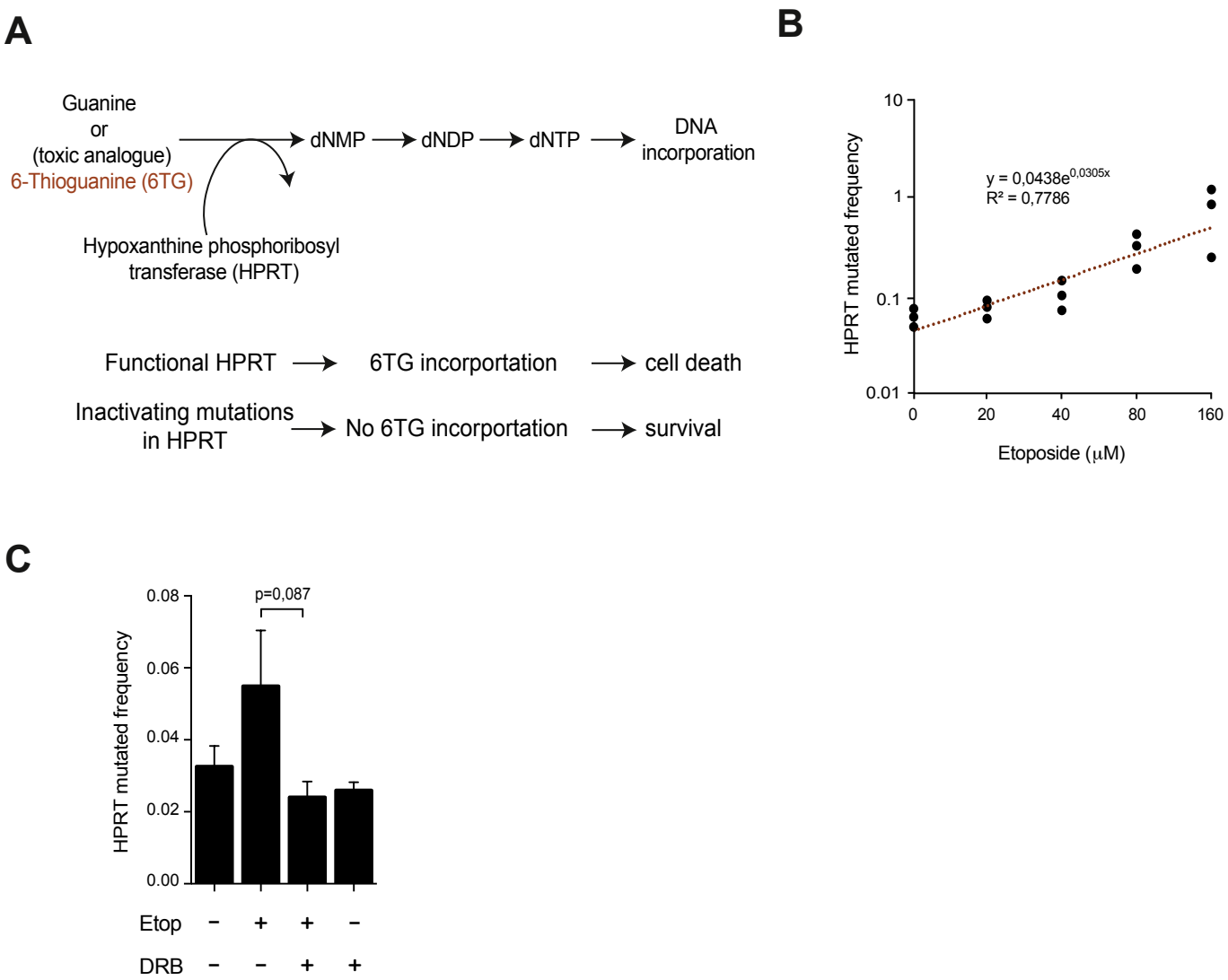


Figure S7. HPRT mutagenic assay. (**A**) Model for 6TG incorporation into DNA. (**B**) HPRT mutagenic assay in proliferating KG1a cells. Cells were treated with indicated concentrations of etoposide for 3h. Cells were cultured in 6TG containing drug-free medium for 10 days. Graph shows 6TG total cells with respect to the survival in a 6TG-free culture of three independent experiments. (**C**) HPRT mutagenic assay in serum-starved RPE-1 cell line. G0/G1 cells were treated with 40 μM etoposide 3h. Where indicated, cells were pre-incubated with 100 μM DRB for 1 h, prior etoposide. Cells were cultured in 6TG containing drug-free medium for 10 days. Other details as in B.

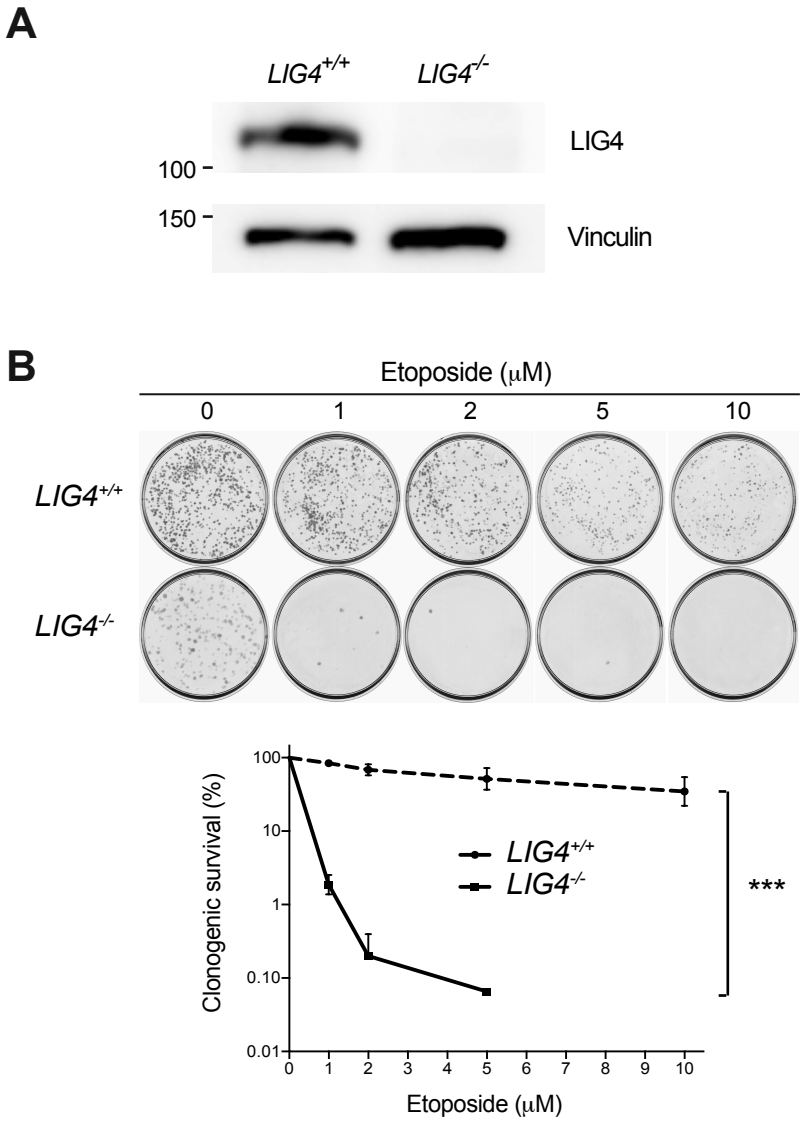


Figure S8. *LIG4*$^{-/-}$ RPE-1 cells. (**A**) LIG4 western blot in wild-type (*LIG4*$^{+/+}$)and *LIG4*$^{-/-}$ RPE-1 cells. Vinculin was used as loading control. (**B**) Clonogenic survival of wild-type and *LIG4*$^{-/-}$ RPE-1 cells following treatment (3 h) with the indicated concentrations of etoposide. Data are the mean (±s.e.m.) of three independent experiments. Statistical significance was determined by two-way ANOVA (***$P < 0.005$).

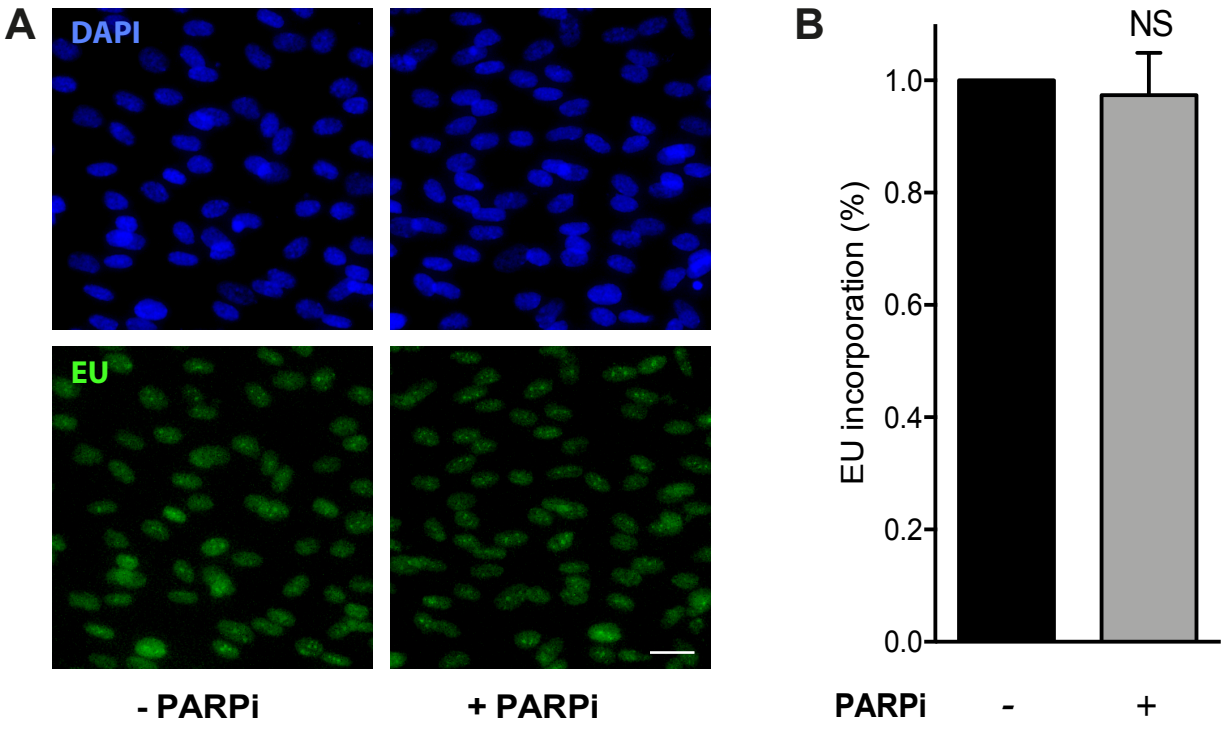
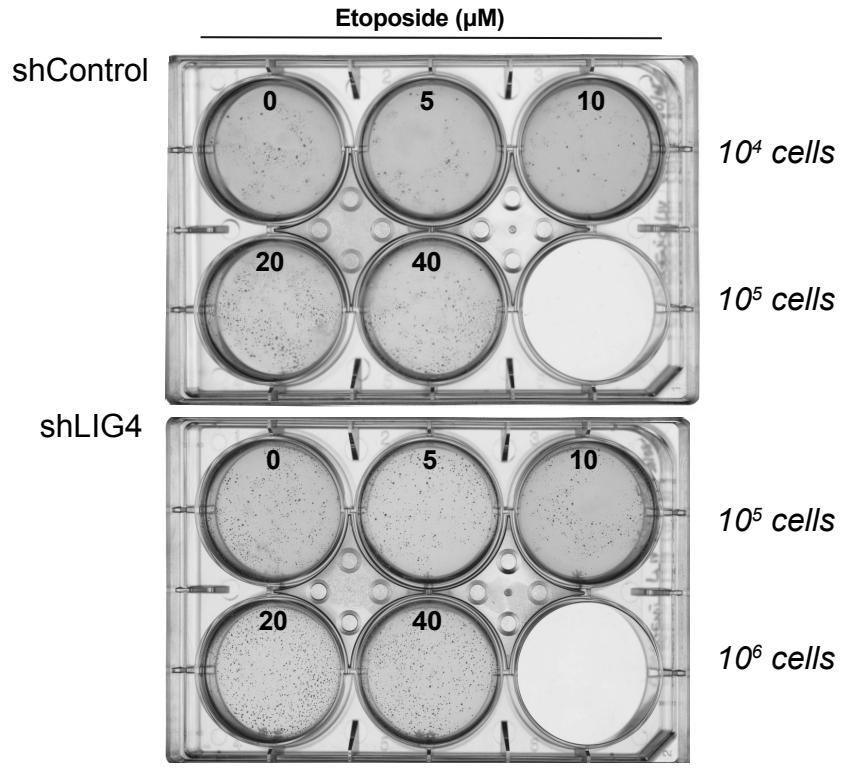


Figure S9. Transcription inhibition in RPE-1 cells. (**A**) Representative images of 5-Ethynyl Uridine (EU) pulse labelling in RPE-1 cells after treatment with PARP inhibitor KU58948 (1 μM) for 1h. EU (green) and DAPI counterstain (blue) are shown. Scale bar, 10μm. (**B**) Quantification of EU signal (mean ± s.e.m.) of three independent experiments. Statistical significance was measured by *t*-test (NS, not significant).

A



B

	0	10	20	40	Replicate
shControl	315	276	131	72	1
	222	107	55,3	35	2
	227	70	51,8	30	3
	117	50	35,3	19,7	4
shLIG4	155	86	40	6	1
	78	19	14,5	8,4	2
	32	15	3,7	2,3	3
	341	164	57,4	43,4	4

Figure S10. LIG4 promotes survival following TOP2-induced DSBs. Clonogenic survival of KG1a shRNA LIG4 and control cells treated with the indicated concentration of etoposide for 3 h. (**A**) Representative images of cultures. The starting number of cells is indicated. (**B**) Colony counts of four independent experiments (corrected by the starting number of colonies).

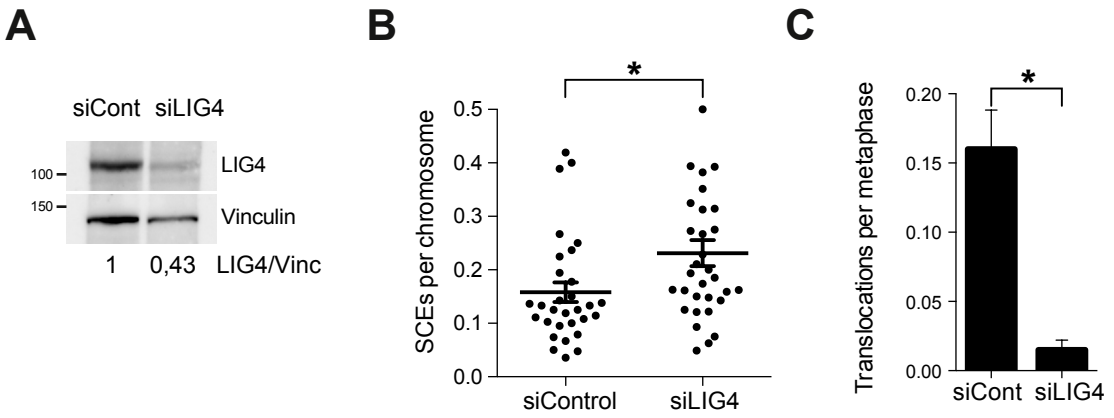


Figure S11. LIG4 promotes TOP2-induced chromosomal translocations. (**A**) Analysis of LIG4 protein level 48 h after Control or LIG4 siRNA transfection. Molecular weight markers are in kDa. bottom, LIG4 signal normalized with respect to Vinculin. LIG4 protein relative to the control cells (siControl). (**B**) Sister chromatid exchanges of RPE-1 siControl or siLIG4 cells treated with 5 μM etoposide for 30 min. After that, cells were washed and cultured in drug-free medium for 8 h, previous to metaphase spread preparation. Data are mean (± s.e.m.) of SCE events per chromosome per metaphase from two independent experiments. Statistical significance was determined by T-test (*P<0.05). (**C**) Translocation frequencies (translocations per metaphase) in chromosome 8 and 11 were quantified in asynchronous RPE-1 siControl or siLIG4 cells 24 h after etoposide treatment (1 h, 25 μM). Mean (± s.e.m.) from three independent experiments. Statistical significance was determined by t-test (*P<0.5).

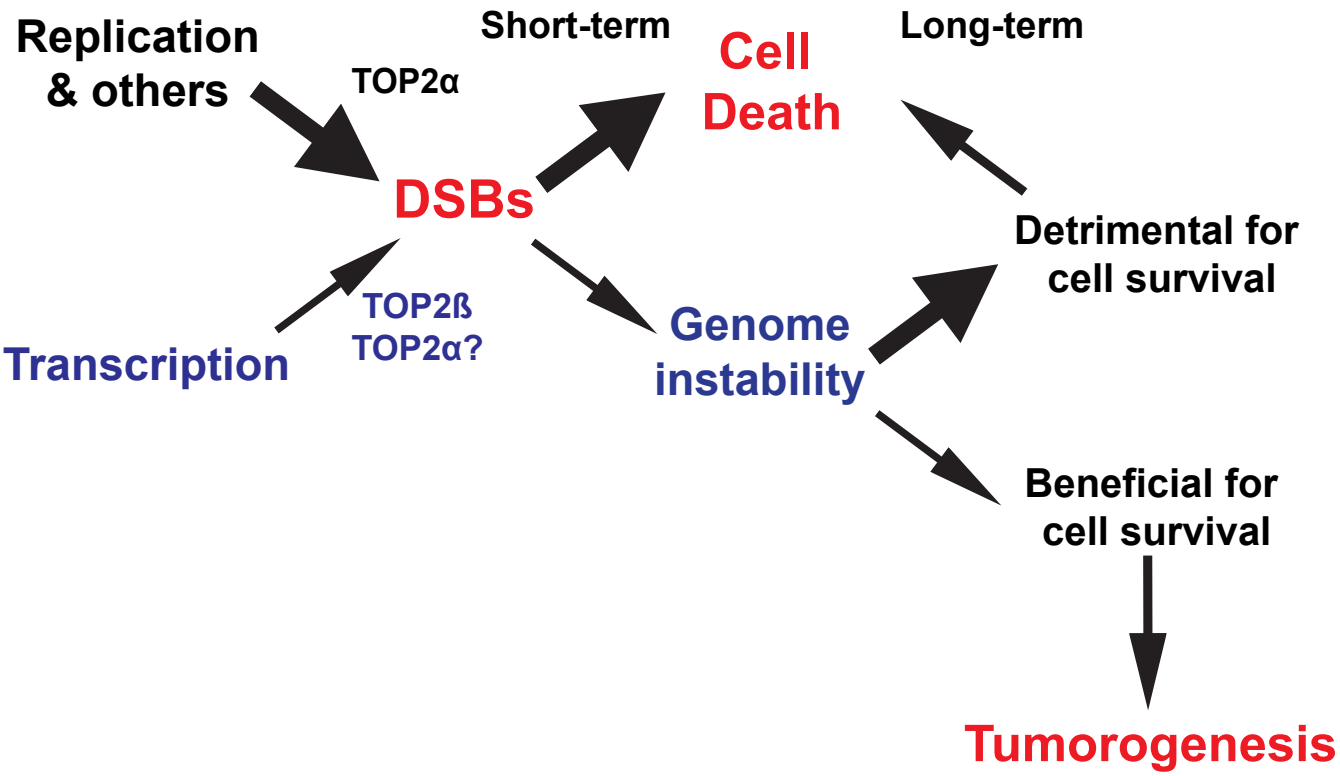
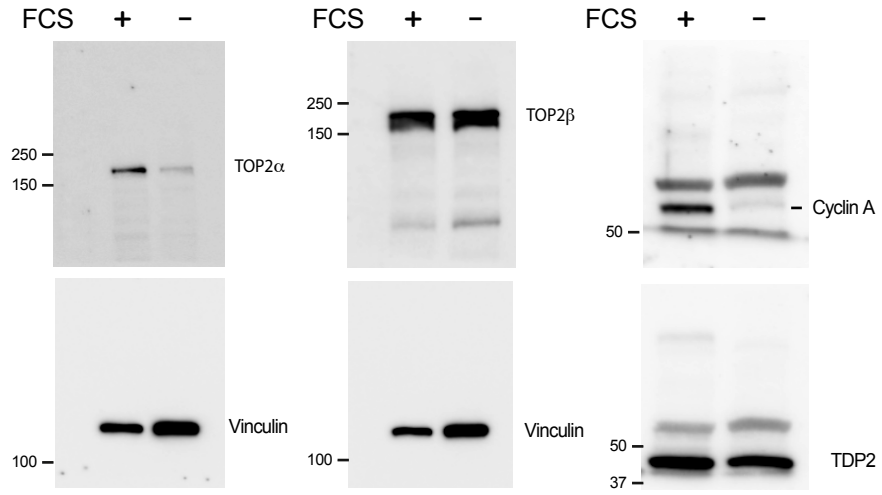
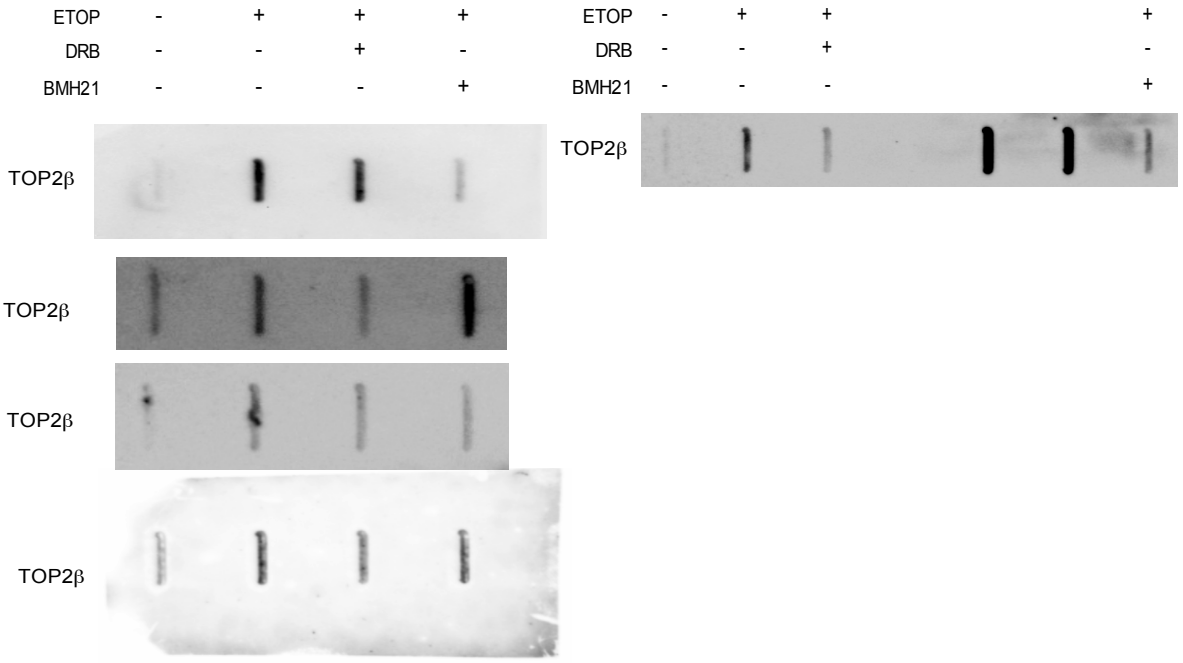


Figure S12. Model depicting the effect of TOP2 induced DSBs in cell death and tumorogenesis.

WB Figure 1A



Slot blots Figure 1B



WB Figure 1F

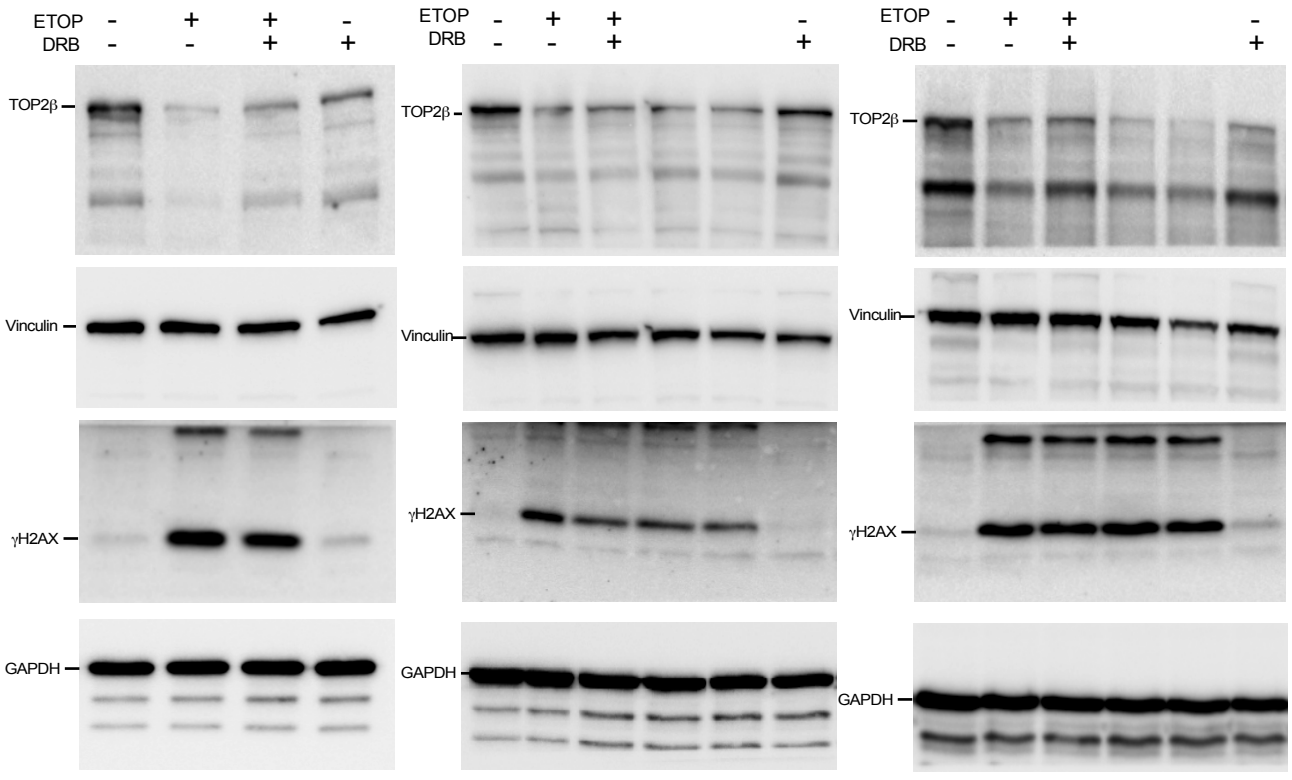
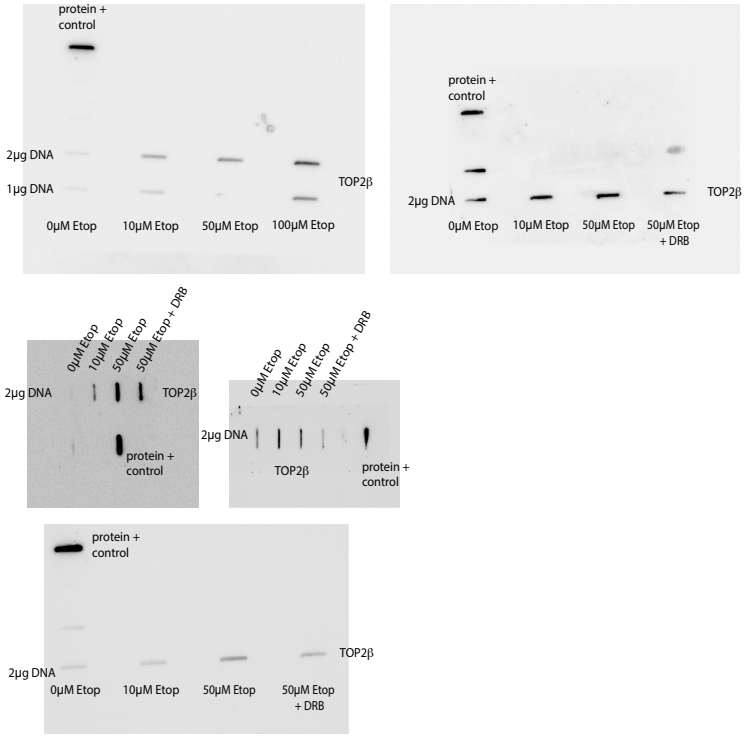
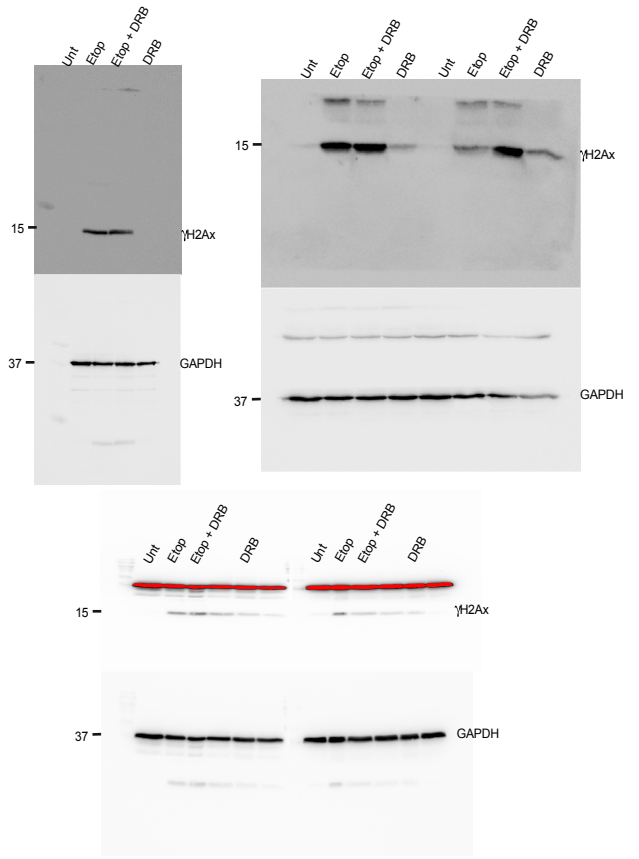


Figure S13_1. Full blots

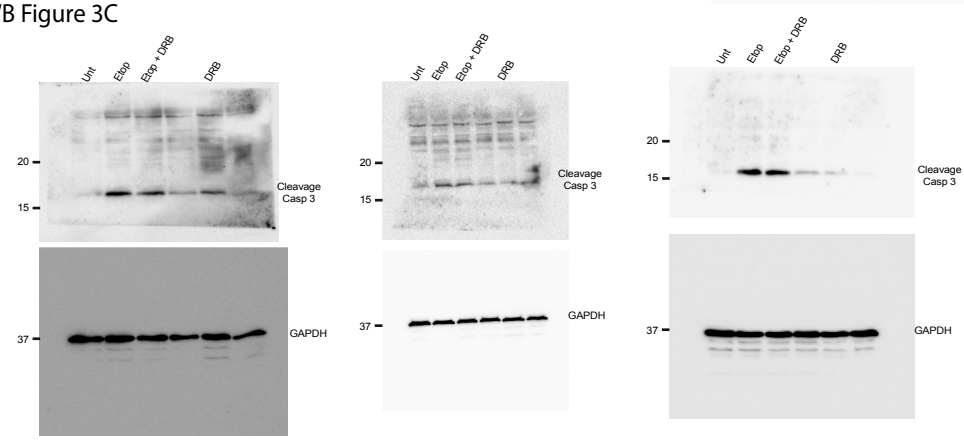
Slot blots Figure 2A



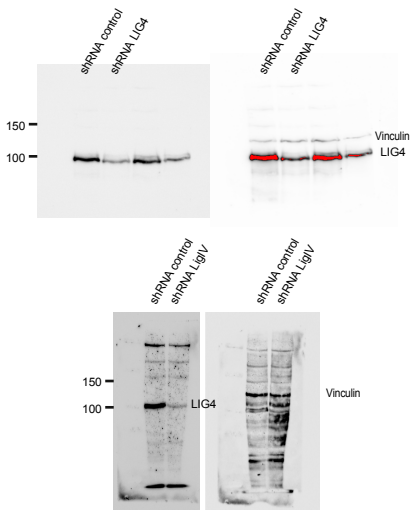
WB Figure 2B



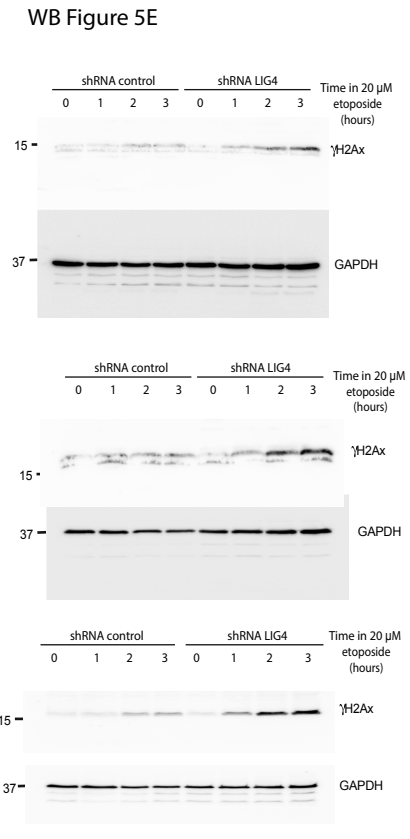
WB Figure 3C



WB Figure 5D



WB Figure 5E



WB Figure S4B

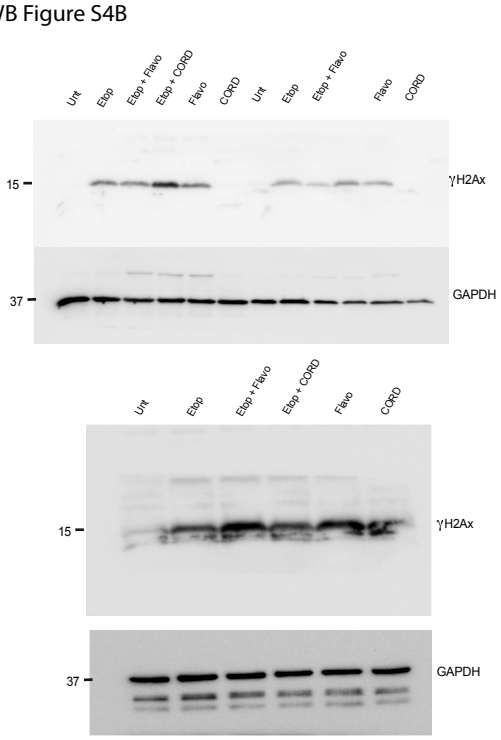


Figure S13_2. Full blots