SUPPLEMENTARY DATA

Roles of the C-terminal Domains of Topoisomerase IIα and Topoisomerase IIβ in Regulation of the Decatenation Checkpoint

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Supplementary Tables and Figures

Table S1. Expression of topo IIα and topo IIβ in the parental HTETOP cell line and the different topo IIα or topo IIβ (WT or mutant) transfected cell lines. In all of these cell lines expression of low concentrations of endogenous topo IIβ was observed, which was approximately 8-fold less than that observed in the WT-topo IIβ transfected cells.

HTETOP Cell Line	Expression of Topo II Isozymes		
	Parental topo IIα	Transfected topo IIα (WT or mutant)	Transfected topo IIβ (WT or mutant)
Parental -Tet	+	-	-
WT topo IIα + Tet	-	+	-
WT topo IIα – Tet	+	+	-
Y640F topo IIα + Tet	-	+	-
Y640F topo IIα – Tet	+	+	-
C-del topo IIα +Tet	-	+	-
C-del topo IIα –Tet	+	+	-
WT topo IIβ + Tet	-	-	+
WT topo IIβ – Tet	+	-	+
Y656F topo IIβ + Tet	-	-	+
Y656F topo IIβ – Tet	+	-	+
C-del topo IIβ +Tet	-	-	+
C-del topo IIβ -Tet	+	-	+

Table S2. Percent mitotic cells in the parental HTETOP cell line (-Tet) and topo II transfected HTETOP cell lines (+Tet) treated with colcemid for 30 minutes.

HTEOP cell line	Percent mitotic cells
Parental -Tet	3.25 ± 0.75
WT topo IIα + Tet	4.30 ± 0.43
Y640F topo IIα + Tet	3.52 ± 0.75
C-del topo IIα +Tet	3.00 ± 0.40
WT topo IIβ + Tet	4.88 ± 0.66
Y656F topo IIβ + Tet	4.17 ± 0.45
C-del topo IIβ +Tet	3.45 ± 0.44

Table S3. Percent mitotic cells with untangled normal chromosomes in ICRF-193 treated HTETOP cells

Transfectant	Normal mitotic cells in ICRF-193 treated cells (% of total cells)
WT topo IIα	0.030 ± 0.07
C-del topo IIα	0.0
Y640 F topo IIα	0.022 ± 0.05
WT topo IIβ	0.024 ± 0.06
C-del topo IIβ	0.015 ± 0.04
Y656 F topo IIβ	0.0

Table S4. Anchoring contacts in the folded "Greek-key"-like motif in topo IIα

Topo IIα		
R-1/R-2	contact with	Interaction type
L616	L468	hydrophobic
L616	D541	H-bond
Y686	K550	H-bond
Y590	I574	hydrophobic
T620	A624	H-bond
Y613	Y627	stacking
E594	R633	Salt bridge
Y686	R633	H-bond
G687	R633	H-bond
Q688	R633	H-bond
Q688	T690	H-bond
Y686	L693	hydrophobic
D683	K701	Salt bridge
K611	E741	Salt bridge
G617	Q789	H-bond
S619	Q789	H-bond
T618	Y805	H-bond
K599	N833	H-bond
Y612	N833	H-bond

Topo IIβ		
R-1/R-2	contact with	Interaction type
G633	S480	H-bond
K630	D557	Salt bridge
Y606	P592	stacking
S635	A637	H-bond
Y629	E639	H-bond
P697	R691	H-bond
Q699	K717	H-bond
Y628	D848	H-bond
K622	N849	H-bond

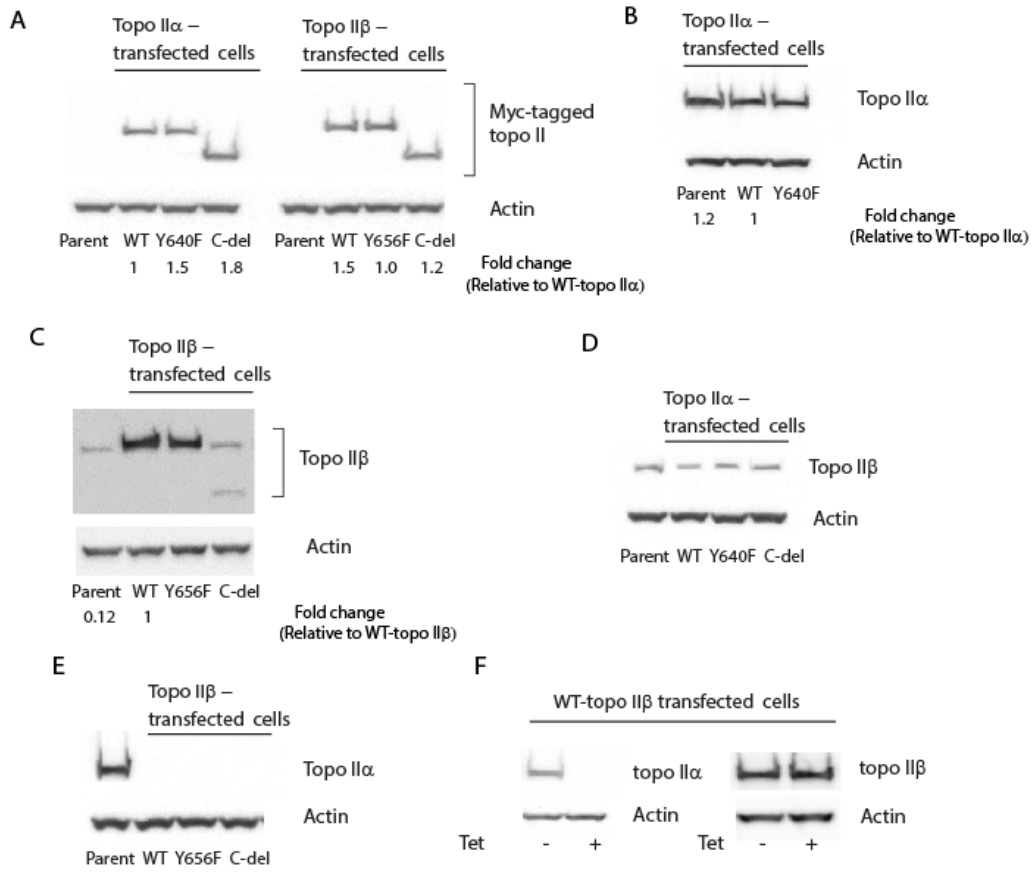


Figure S1: Ectopic expression of WT or mutant topo II proteins in HTETOP cells. (A) Expression of myc-tagged topo II protein in parent HTETOP cells (-Tet) or HTETOP cells (+Tet) stably expressing the indicated ectopic myc-tagged WT or mutant topo II protein. (B) Expression of topo IIα protein in parent HTETOP cells (-Tet) or HTETOP cells (+Tet) transfected with WT topo IIα or Y640F mutant topo IIα. (C) Expression of topo IIβ protein in parent HTETOP cells (-Tet) or HTETOP cells (+Tet) transfected with WT topo IIβ, Y656F mutant topo IIβ or C-del topo IIβ. (D) Expression of topo IIβ protein in parent HTETOP cells (-Tet) or HTETOP cells (+Tet) transfected with WT topo IIα, Y640F mutant topo IIα or C-del topo IIα. (E) Expression of topo IIα protein in parent HTETOP cells (-Tet) or HTETOP cells (+Tet) transfected with WT topo IIβ, Y656F mutant topo IIβ or C-del topo IIβ. (F) Expression of topo IIα and topo IIβ protein in WT topo IIβ-transfected HTETOP cells cultured in the absence or presence of Tet. The immunoblots were probed with topo IIα-, topo IIβ-, myc-tag- or actin-specific antibody, as indicated next to the bands. Since topo IIβ specific antibody was generated with antigens corresponding to the CTD, only a faint signal is observed for C-del topo IIβ protein (in which the NLS within the C-terminal domain was retained) when probed with topo IIβ specific antibody.

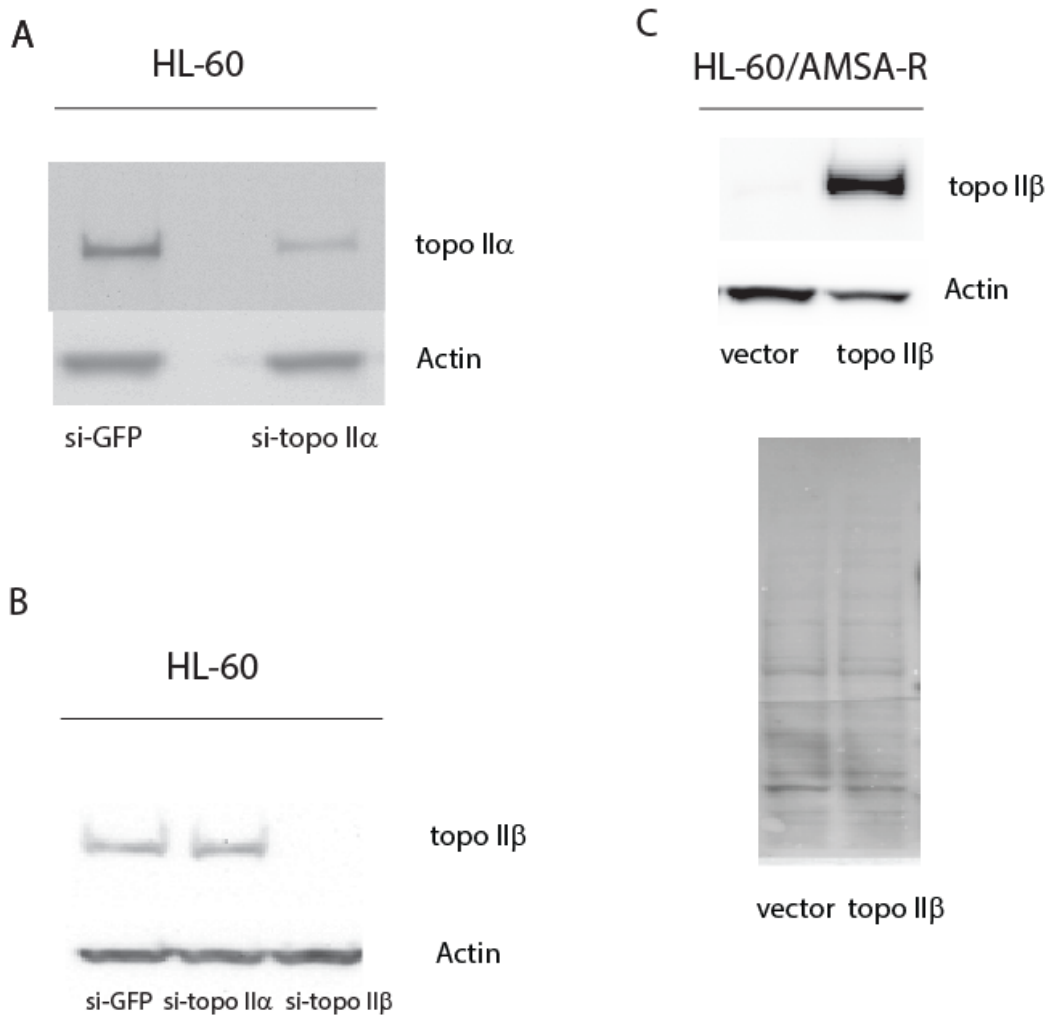


Figure S2: Altered expression of topo IIα or topo IIβ in HL-60 and HL-60/AMSA-R cells. Down regulation of (A) topo IIα or (B) topo IIβ in HL-60 cells following stable transfection of an shRNA to topo IIα (HL-60/si-topo IIα) or topo IIβ(HL-60/si-topo IIβ), respectively. Cells transfected with an shRNA to GFP (HL-60/si-GFP) was used as a control. The transfected cell lines were constructed according to the procedure of Gurova et al (Cancer Res, 64, 1951-1958, 2004) using the pBabe-puro retroviral vector for inserting the H1 promoter followed by a template of small interfering RNA (si-RNA) expression, designed according to the loop model (short hairpin RNA) described by Brummelcamp et al. (Science, 296,550-553, 2002). Since the template siRNA derived from the short hairpin RNA, is the functional entity that down regulates topo IIα and topo IIβ, the cell lines were named HL-60/si-topo IIα and HL-60/si-topo IIβ, respectively. The degree of stable down regulation was 72 ± 7.2% for topo IIα and 96 ± 4.12% for topo IIβ, even when these cells were maintained in the absence of the selection marker, puromycin. (C) Expression of topo IIβ in HL-60/ AMSA-R and HL-60/AMSA-R/topo IIβ cells, transfected with the PEIE vector or PEIE vector containing the topo IIβ cDNA, respectively. The upper panel shows the immunoblot probed with topo IIβ or actin-specific antibody as indicated next to the band. The lower panel shows the Ponceau S stained blot.

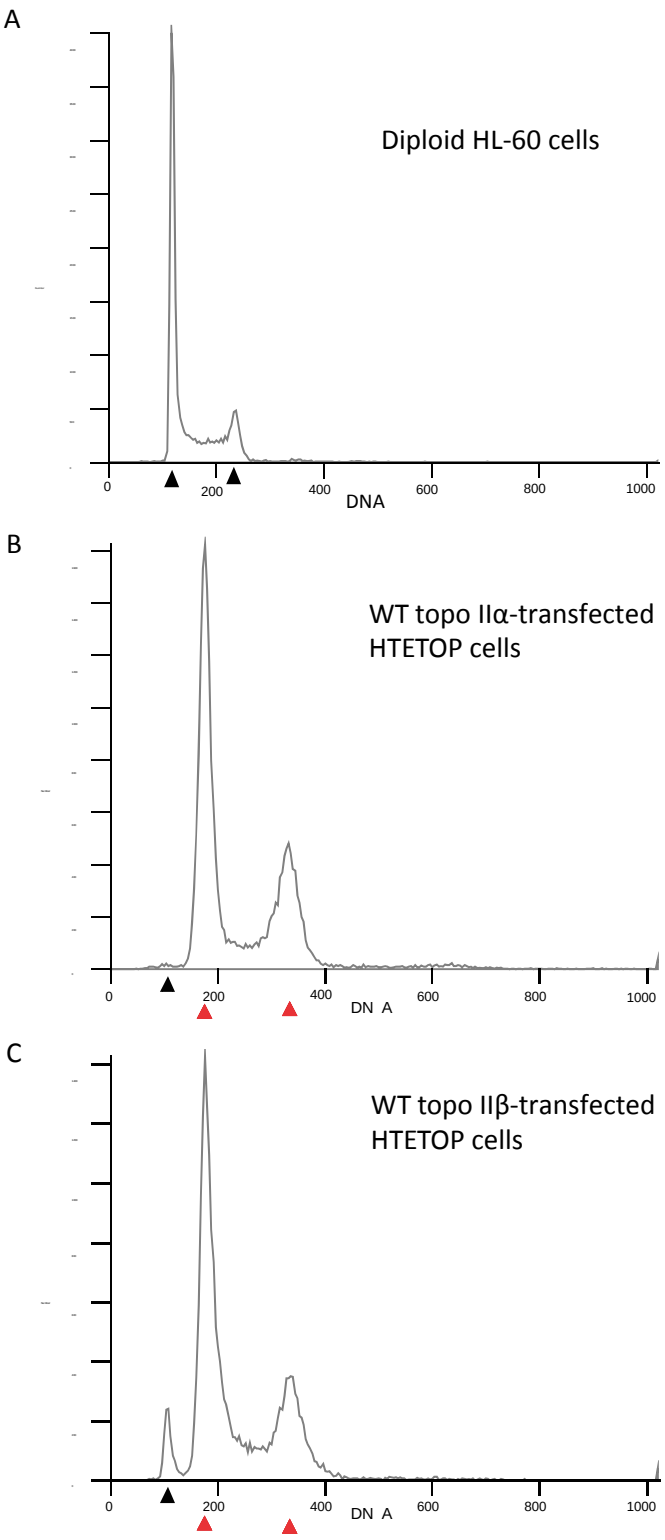


Figure S3: Majority of WT topo IIα- and topo IIβ-transfected HTETOP cells are aneuploid, while a small, but distinct population, of WT topo IIβ transfected cells are diploid. (A) DNA distribution profile of diploid HL-60 cells. The black arrowheads mark the position of the 2n and 4n DNA corresponding to the G1 and G2/M peaks, respectively of the diploid HL-60 cells. (B) DNA distribution profile of aneuploid topo IIα-transfected HTETOP cells. The red arrowheads at positions >2n and >4n DNA, correspond to the G1 and G2/M peaks of the aneuploid WT-topo IIα transfected HTETOP cells. No diploid G1 peak at 2n DNA (black arrowhead) is observed. (C) DNA distribution profile of mostly aneuploid topo IIβ-transfected HTETOP cells. The red arrowheads at positions >2n and > 4n DNA, correspond to the major G1 and G2/M peaks of the aneuploid WT-topo IIβ transfected HTETOP cells. The minor peak at the position of 2n DNA (black arrow) corresponds to the G1 peak of the minor diploid population.

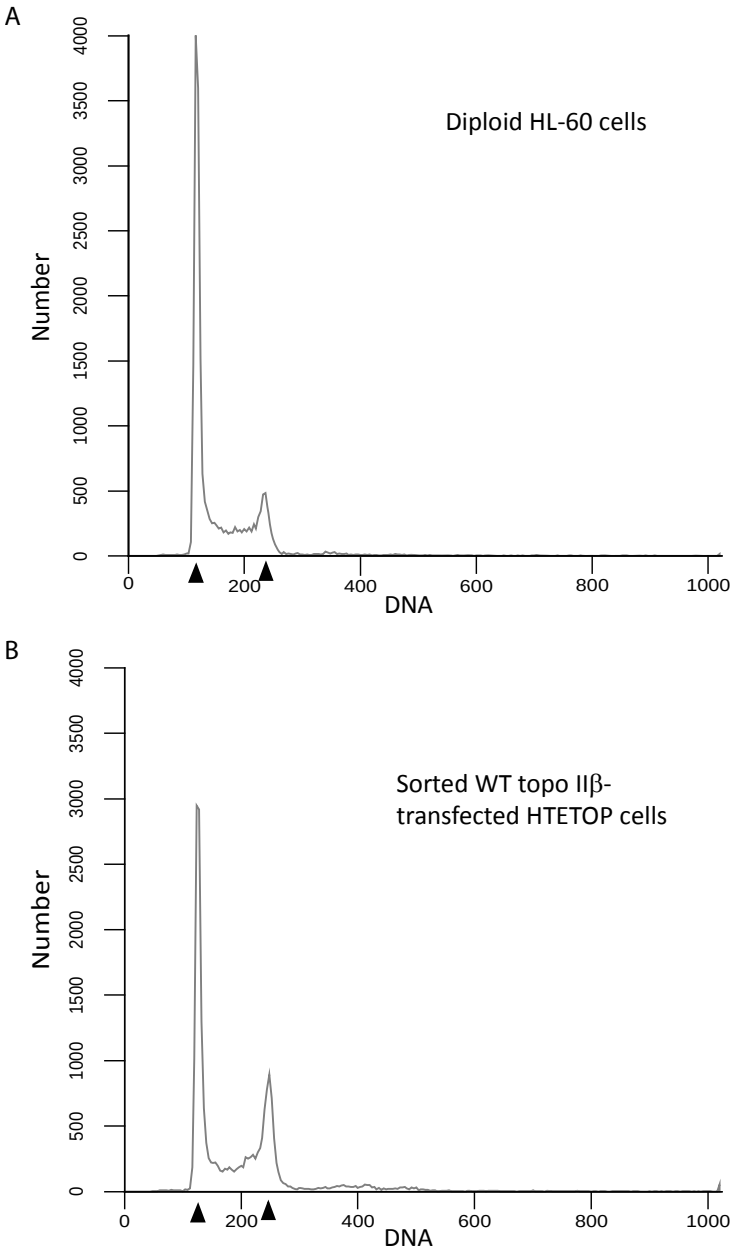


Figure S4: Sorted WT topo IIβ-transfected HTETOP cells are diploid. (A) DNA distribution profile of diploid HL-60 cells. The arrowheads mark the position of the G1 and G2/M peaks corresponding to 2n and 4n DNA, respectively. (B) DNA distribution profile of sorted topo IIβ – transfected HTETOP cells. The G1 and G2/M peaks (marked by arrowheads) are in the same position as that of the diploid HL-60 cells and correspond to 2n and 4n DNA respectively.

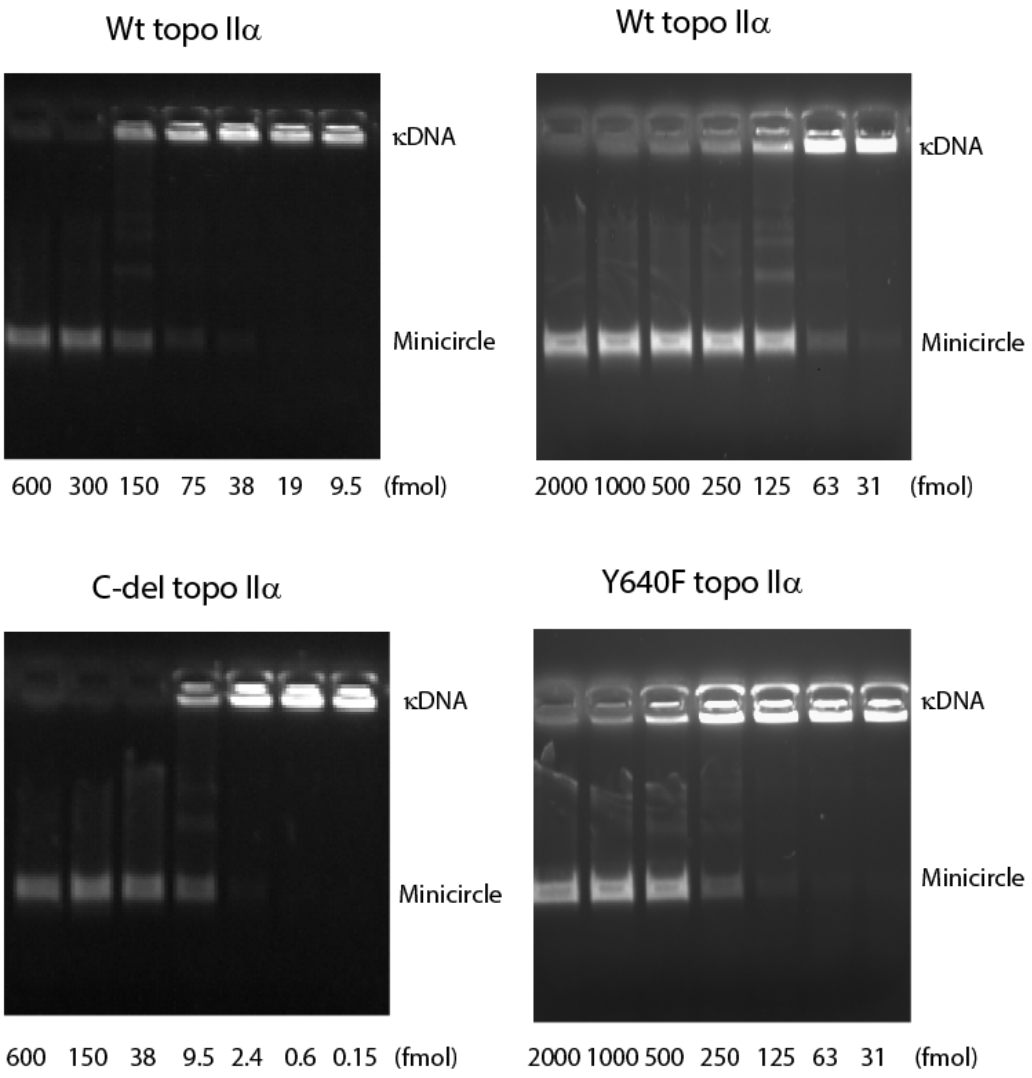


Figure S5: Decatenation of κDNA by human WT topo IIα, C-del topo IIα or Y640F topo IIα. Ethidium bromide stained gels showing decatenation of 150 ng of kDNA in 30 minutes by different concentrations of WT or mutant topo IIα.

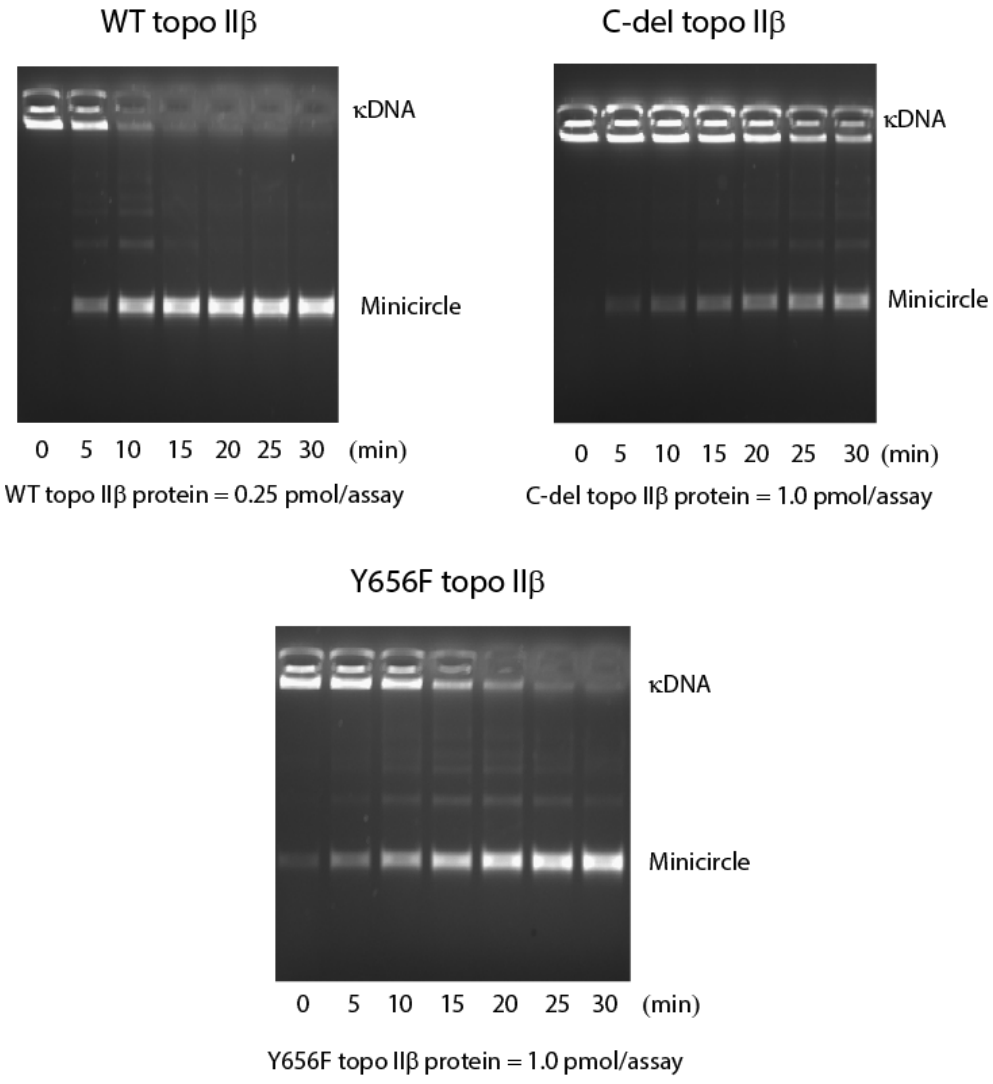


Figure S6: Decatenation of κDNA by human WT topo IIβ, C-del topo IIβ or Y656F topo IIβ. Ethidium bromide stained gels showing time course of decatenation of 150 ng of κDNA by the indicated concentration of WT or mutant topo IIβ

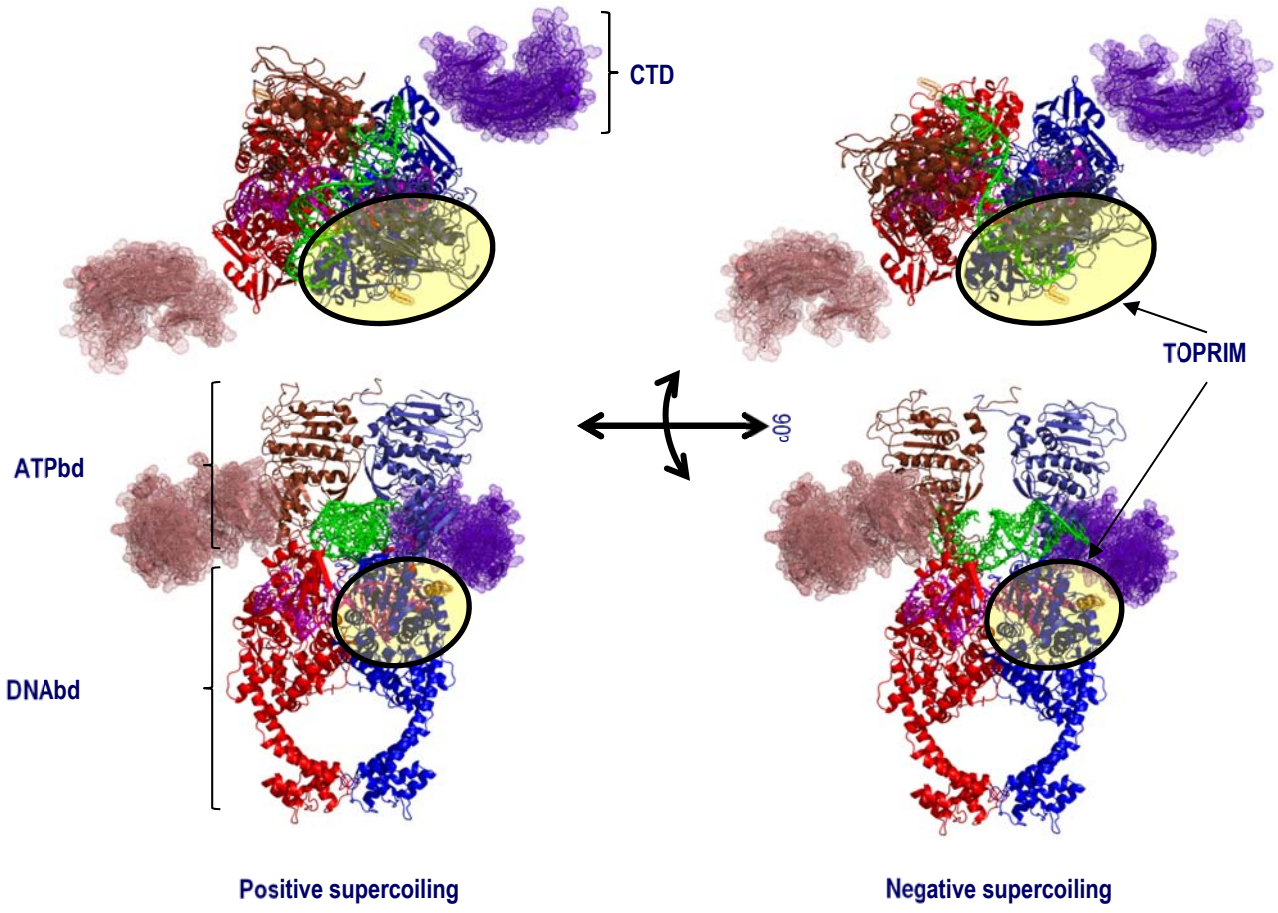
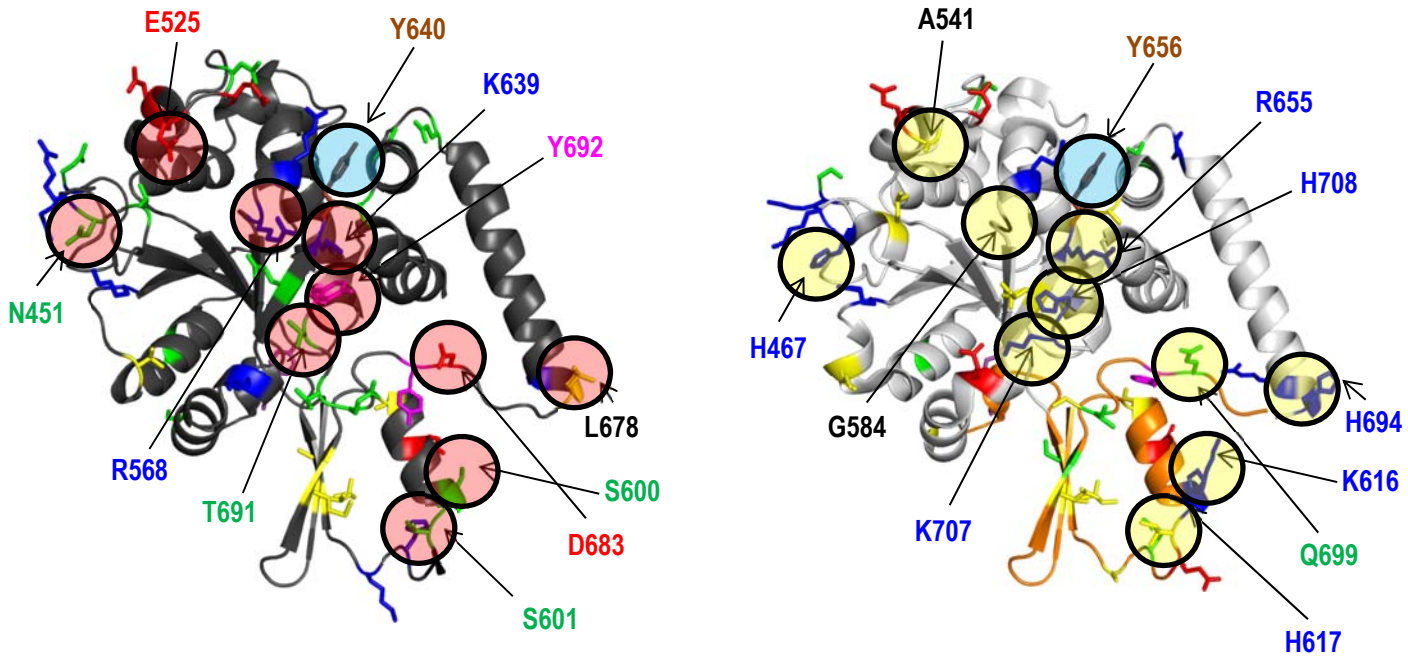


Figure S7: Coarse grain model of the position of the C-terminal domain in relation to the rest of the topo II for positive and negative supercoiled DNA**.** The C-terminal domains are represented starting from a similar domain in bacteria (pdb: 1ZVU) which shows approximately 12% identity to the human topo IIα C-terminal domain and approximately 8% identity to the human topo IIβ C-terminal domain.



Topo IIα

Topo IIβ

Figure S8: Model showing main differences between topo IIα and topo IIβ on the surface of TOPRIM domain in the proximity of Y640 topo IIα/Y656 topo IIβ**.** Equivalent amino acids between the two isoforms are shown with arrows and labels and colored based on their chemical properties. Basic amino acids are colored in blue, acidic amino acids are colored in red, hydrophobic amino acids are colored in yellow, aromatic amino acids are colored in magenta and polar amino acids are colored in green. R-1 and R-2 in topo IIβ are colored in orange whereas Y640 topo IIα /Y656 topo IIβ are colored in brown.

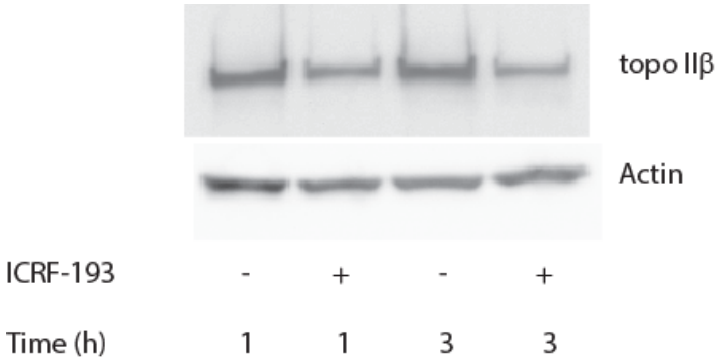


Figure S9: Degradation of topo IIβ following treatment of WT topo IIβ-transfected HTETOP cells with 2 μM ICRF-193 for the indicated times.