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Supplemental information

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Figure S1

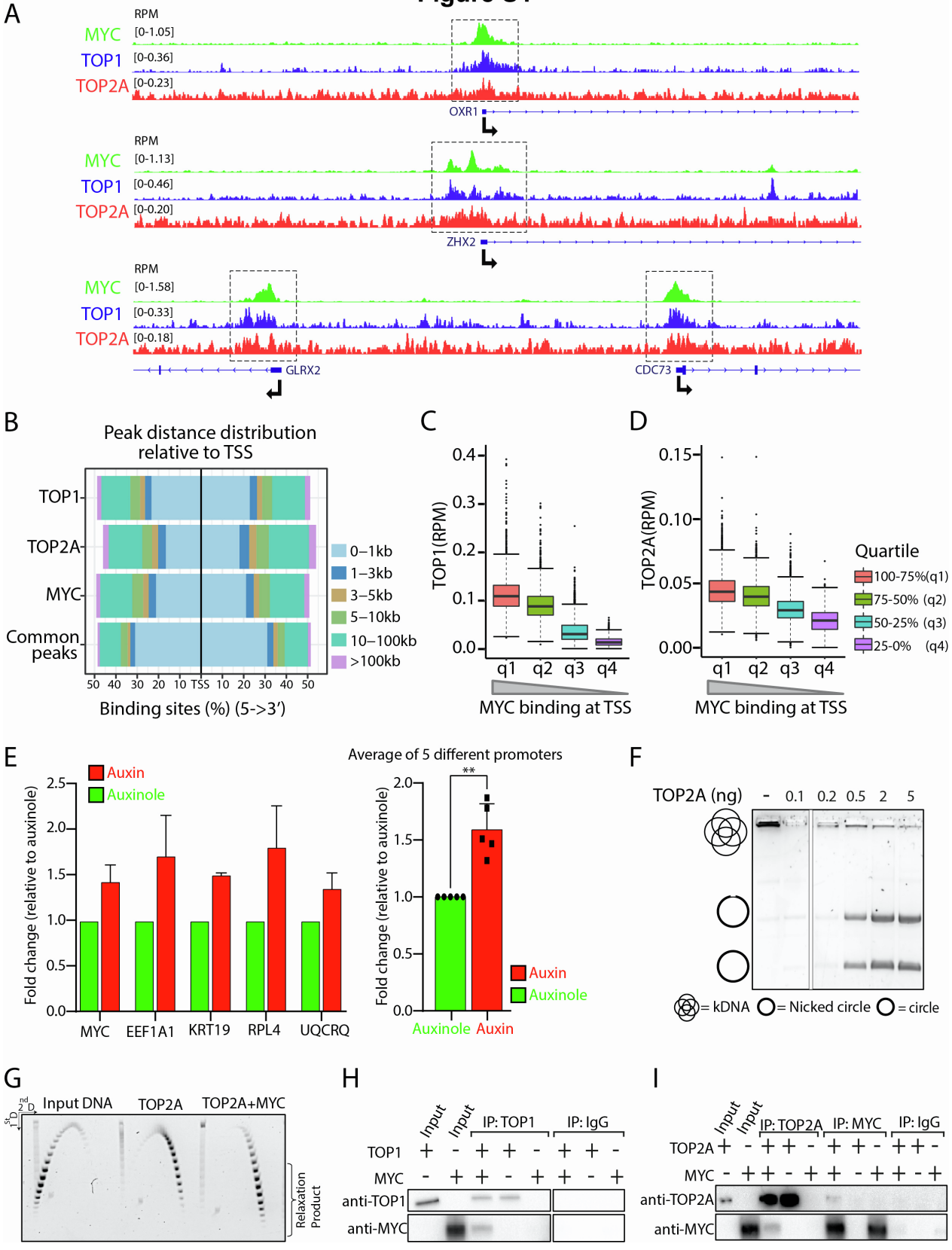
A
RPM
MYC
[0-1.05]
TOP1
[0-0.36]
TOP2A
[0-0.23]
OXR1
RPM
MYC
[0-1.13]
TOP1
[0-0.46]
TOP2A
[0-0.20]
ZHX2
RPM
MYC
[0-1.58]
TOP1
[0-0.33]
TOP2A
[0-0.18]
GLRX2
CDC73
B
Peak distance distribution relative to TSS
TOP1
TOP2A
MYC
Common peaks
0–1kb
1–3kb
3–5kb
5–10kb
10–100kb
>100kb
50 40 30 20 10 TSS 10 20 30 40 50
Binding sites (%) (5->3')
C
TOP1(RPM)
0.4
0.3
0.2
0.1
0.0
q1 q2 q3 q4
MYC binding at TSS
D
TOP2A(RPM)
0.15
0.10
0.05
0.00
q1 q2 q3 q4
MYC binding at TSS
Quartile
100-75%(q1)
75-50% (q2)
50-25% (q3)
25-0% (q4)
E
Auxin
Auxinole
Fold change (relative to auxinole)
2.5
2.0
1.5
1.0
0.5
0.0
MYC EEF1A1 KRT19 RPL4 UQCRQ
Average of 5 different promoters
**
Fold change (relative to auxinole)
2.0
1.5
1.0
0.5
0.0
Auxinole Auxin
Auxin
Auxinole
F
TOP2A (ng) - 0.1 0.2 0.5 2 5
= kDNA = Nicked circle = circle
G
Input DNA
TOP2A
TOP2A+MYC
1st D
2nd D
Relaxation Product
H
Input
Input
IP: TOP1
IP: IgG
TOP1 + - + + - + + -
MYC - + + - + + - +
anti-TOP1
anti-MYC
I
Input
Input
IP: TOP2A
IP: MYC
IP: IgG
TOP2A + - + + - + + - + + -
MYC - + + - + + - + + - +
anti-TOP2A
anti-MYC

Figure S1. The recruitment of MYC parallels TOP1 and TOP2A at highly expressed genes. Related to Figure 1. (A and **B)** Representative Genome Browser tracks of ChIP-seq showing co-localization (in dashed boxes) of MYC, TOP1 and TOP2A at promoters. **(B)** Bar graphs of peak distance distribution relative to TSSs of all MYC, TOP1, TOP2A peaks and the subset of peaks they have in common. **(C** and **D)** Box plots showing the recruitment of TOP1 **(C)** or TOP2A **(D),** with respect to MYC binding at the TSS ± 500 bps. TSSs are ranked by quartile with highest (100-75%), high (75-50%), medium (50-25%) or low (25-0%) MYC binding. Whiskers indicate lowest and highest values no further than 1.5 x interquartile. **(E)** Left. K562MYC_mAID cells were treated with auxin for 30 min followed by MG132 (10 μM) for 30 min and Eto (100 μM) in the last 5 min. TOP2A CAD-qPCR at TSSs of selected highly expressed genes, normalised on a non transcribed region (α-sat) and shown as fold change over auxinole treated-sample. Results presented from 3 independent replicas (n = 3; error bars represent the mean ± S.E.M.). Right. Average of the different promoters. Statistical significance was calculated by paired t-test (**: $p < 0.01$). **F)** Decatenation assay in presence of increasing amount of recombinant TOP2A. **(G)** Distribution of product DNA topoisomers after incubation with TOP2A with or without MYC (relaxation products, which run in the right/bottom corner, are indicated). Representative blot from 2 replicas. **(H)** Recombinant TOP1 and full length MYC were mixed and immunoprecipitated with anti-TOP1 and probed for MYC (N-term) or TOP1. **(I)** Recombinant TOP2A and full length MYC were mixed and immunoprecipitated with anti-TOP2A or anti-MYC and probed for TOP2A or MYC. Representative blots for 3 independent replicas are shown.

Figure S2

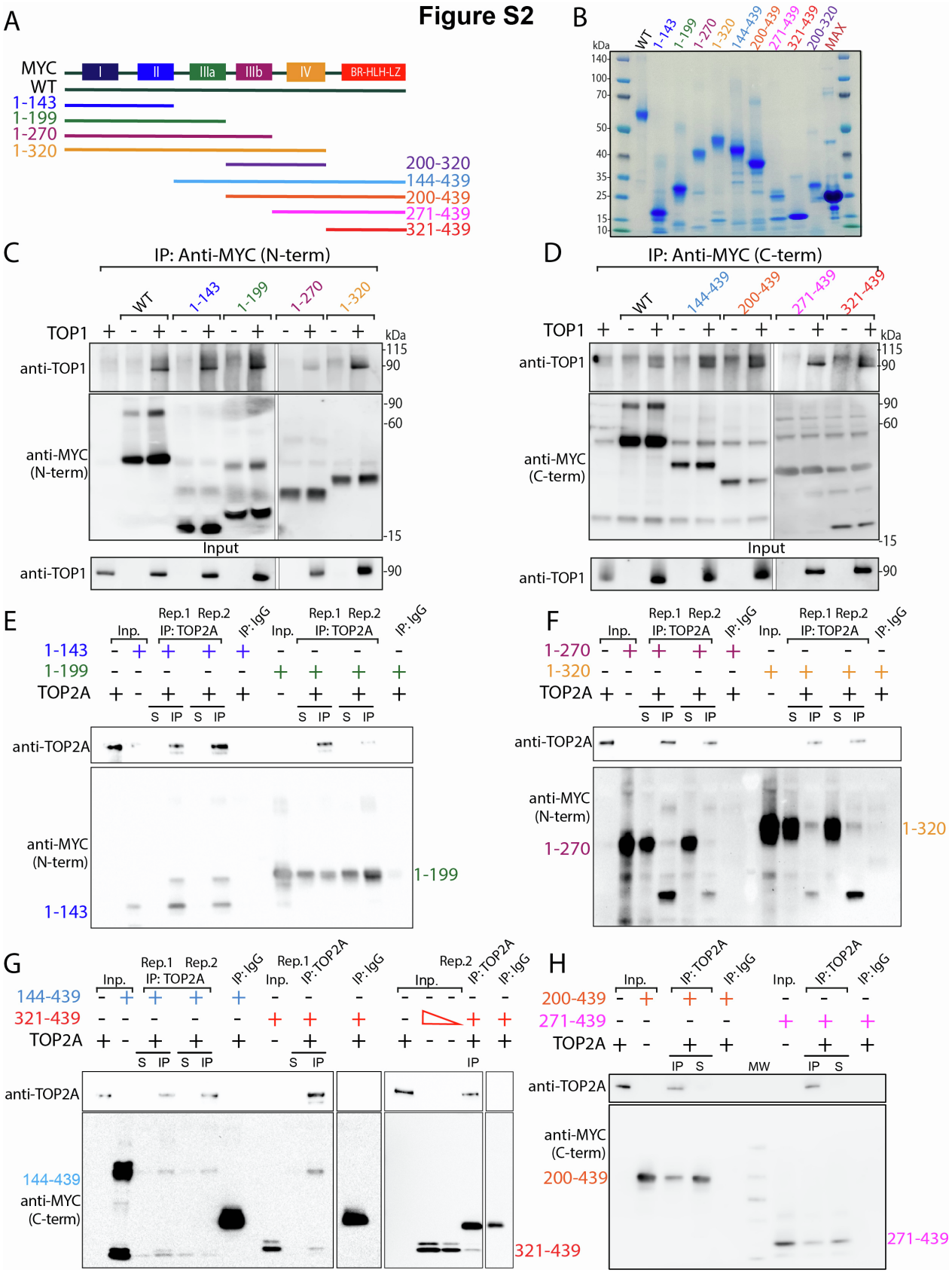
A
MYC
I
II
IIIa
IIIb
IV
BR-HLH-LZ
WT
1-143
1-199
1-270
1-320
200-320
144-439
200-439
271-439
321-439
B
kDa
WT
1-143
1-199
1-270
1-320
144-439
200-439
271-439
321-439
200-320
MAX
140
100
70
50
40
35
25
15
10
C
IP: Anti-MYC (N-term)
WT
1-143
1-199
1-270
1-320
TOP1
anti-TOP1
anti-MYC (N-term)
Input
anti-TOP1
D
IP: Anti-MYC (C-term)
WT
144-439
200-439
271-439
321-439
TOP1
anti-TOP1
anti-MYC (C-term)
Input
anti-TOP1
E
Inp.
Rep.1 Rep.2 IP: TOP2A
IP: IgG
1-143
1-199
TOP2A
S IP S IP
anti-TOP2A
anti-MYC (N-term)
1-199
1-143
F
1-270
1-320
TOP2A
anti-TOP2A
anti-MYC (N-term)
1-270
1-320
G
144-439
321-439
TOP2A
IP: TOP2A
IP: IgG
anti-TOP2A
144-439
anti-MYC (C-term)
321-439
H
200-439
271-439
TOP2A
IP S
MW
anti-TOP2A
anti-MYC (C-term)
200-439
271-439

Figure S2. The N and C termini of MYC interact with TOP1 and TOP2A. Related to Figure 2. (A) Diagram of full length MYC (WT) depicting MYC-boxes (I - IV), basic region-helix-loop-helix-leucine zipper domain (bHLH-LZ) and different truncation mutants extending from either the carboxyl-terminus "(1–143, 1–199, 1–270, 1–320)" or the amino-terminus "(144–439, 200–439, 271–439, 321–439)" and from both ends "(200–320)". **(B)** SDS–PAGE stained with Coomassie brilliant blue of the recombinant MYC WT, MYC truncation mutants shown in **(A)**, and MAX used in this study. **(C** and **D)** Recombinant full length MYC (WT), or truncated from the N-terminus **(C)** or C-terminus **(D)** alone or together with recombinant TOP1 were immunoprecipitated with anti-MYC (N-term) or anti-MYC (C-term) as indicated, and probed for TOP1 and MYC (anti-N-term or anti-C-term). Representative blots from 3 independent replicas are shown. **(E-H)** Recombinant TOP2A was combined with the indicated truncated MYC, immunoprecipitated with anti-TOP2A and probed for MYC (anti-N-term or anti-C-term) and anti-TOP2A as described. Two immunoprecipitation experiments (Rep. 1 & Rep. 2) were performed for each IP, where both the unbound supernatant (S) and pulled down proteins (IP) were loaded. Expected sizes for each MYC truncation are indicated on the blots. IgG was used in the immunoprecipitation as a negative control.

Figure S3

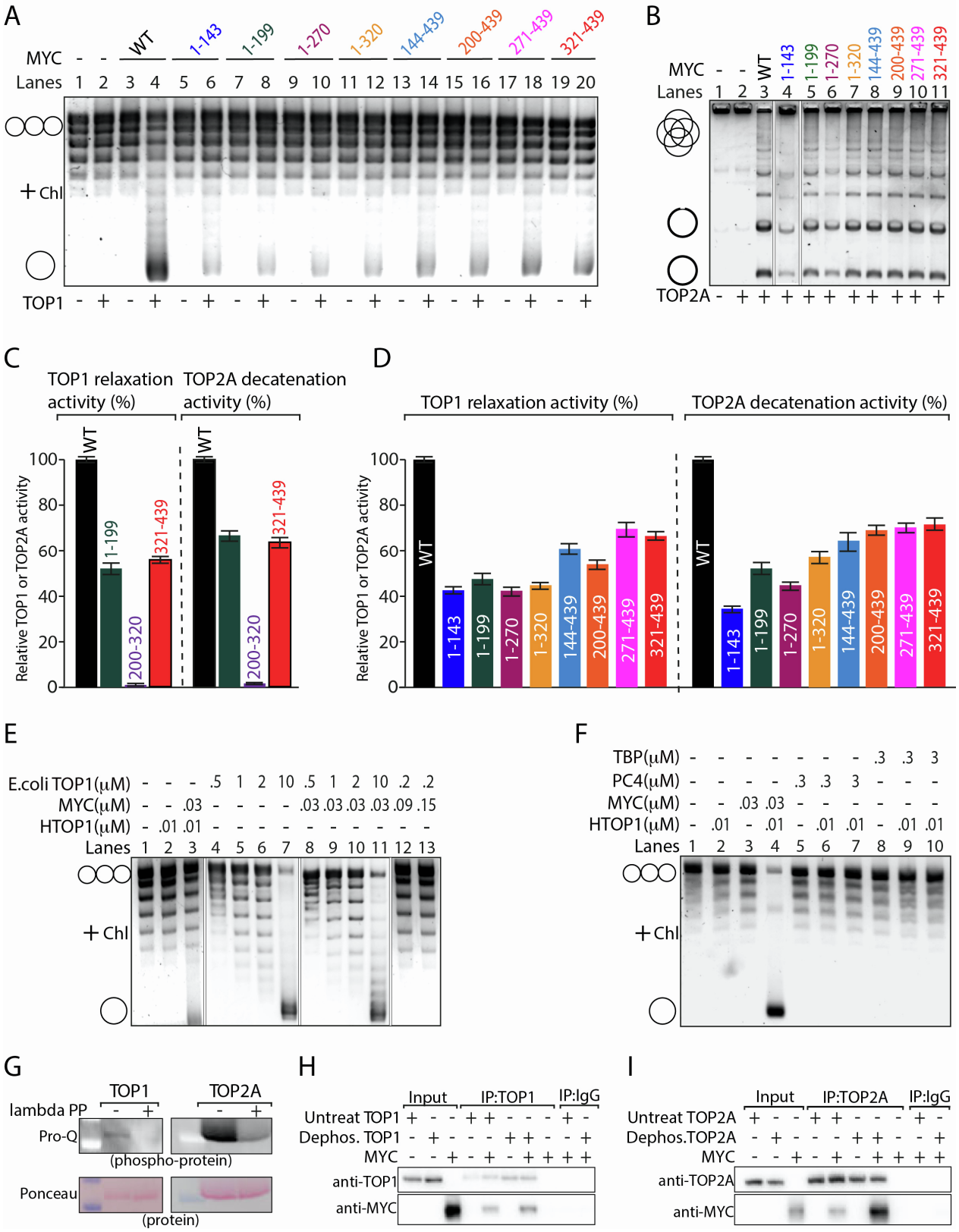
A
MYC - - WT 1-143 1-199 1-270 1-320 144-439 200-439 271-439 321-439
Lanes 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
+ Chl
TOP1 - + - + - + - + - + - + - + - + - + - +
B
MYC - - WT 1-143 1-199 1-270 1-320 144-439 200-439 271-439 321-439
Lanes 1 2 3 4 5 6 7 8 9 10 11
TOP2A - + + + + + + + + + +
C
TOP1 relaxation activity (%)
TOP2A decatenation activity (%)
Relative TOP1 or TOP2A activity
100
80
60
40
20
0
WT
1-199
200-320
321-439
WT
200-320
321-439
D
TOP1 relaxation activity (%)
TOP2A decatenation activity (%)
Relative TOP1 or TOP2A activity
100
80
60
40
20
0
WT
1-143
1-199
1-270
1-320
144-439
200-439
271-439
321-439
WT
1-143
1-199
1-270
1-320
144-439
200-439
271-439
321-439
E
E.coli TOP1(μM) - - - .5 1 2 10 .5 1 2 10 .2 .2
MYC(μM) - - .03 - - - - .03 .03 .03 .03 .09 .15
HTOP1(μM) - .01 .01 - - - - - - - - - -
Lanes 1 2 3 4 5 6 7 8 9 10 11 12 13
+ Chl
F
TBP(μM) - - - - - - - .3 .3 3
PC4(μM) - - - - .3 .3 3 - - -
MYC(μM) - - .03 .03 - - - - - -
HTOP1(μM) - .01 - .01 - .01 .01 - .01 .01
Lanes 1 2 3 4 5 6 7 8 9 10
+ Chl
G
TOP1
TOP2A
lambda PP - + - +
Pro-Q
(phospho-protein)
Ponceau
(protein)
H
Input
IP:TOP1
IP:IgG
Untreat TOP1 + - - + + - - - + -
Dephos. TOP1 - + - - - + + - - +
MYC - - + - + - + + + +
anti-TOP1
anti-MYC
I
Input
IP:TOP2A
IP:IgG
Untreat TOP2A + - - + + - - + -
Dephos.TOP2A - + - - - + + - - +
MYC - - + - + - + + + +
anti-TOP2A
anti-MYC

Figure S3. The N and C termini of MYC both stimulate topoisomerases. Related to Figure 2. **(A** and **B)** Recombinant full length MYC (WT) or the indicated truncations shown in Figure S2A were pre-incubated with or without TOP1 **(A)** or TOP2A **(B)** before addition of plasmid DNA or kDNA, respectively. TOP1 reaction products were checked by agarose gel electrophoresis in presence of chloroquine (Chl), and TOP2A products were run in presence of ethidium bromide. **(C)** Percentage of DNA relaxation by TOP1 (showed in **Figure 2D**) and decatenation by TOP2A (showed in **Figure 2E**) in presence of the indicated MYC variants. Each experiment was performed more than 3 times. (n=4; error bars represent the mean ± S.E.M.). **(D)** Percentage of plasmid DNA relaxation by TOP1 (showed in **Figure S3A**) and decatenation by TOP2A (showed in **Figure S3B**) in presence of the indicated MYC variants. Experiments were performed more than 3 times (n=4; error bars represent the mean ± S.E.M.). **(E)** Indicated concentrations of *E. coli* TOP1A or human TOP1 (HTOP1) were preincubated alone or with the indicated concentrations of purified MYC before addition of plasmid DNA. The products from relaxation assay were subjected to agarose gel electrophoresis in presence of Chl. Relaxed products migrate at the bottom of the gel. **(F)** Recombinant human TATA-binding protein (TBP) or positive cofactor 4 (PC4) or full length MYC were mixed with human TOP1 (HTOP1) as indicated, before addition of plasmid DNA. Reaction products were run on agarose gel electrophoresis with Chl. All experiments were performed at least 3 times. **(G)** Recombinant TOP1 and TOP2A were dephosphorylated by lambda protein phosphatase (lambda PP). Dephosphorylation was confirmed by western blotting and by probing the membrane for phosphoproteins (using the Pro-Q, upper panel) and counterstaining for total protein using Ponceau stain (lower panel). **(H** and **I)** Phosphorylated and dephosphorylated TOP1 and TOP2A were incubated with full length MYC, immunoprecipitated with anti-TOP1 **(H)** and anti-TOP2A **(I)** and blotted for detection of MYC, TOP1 **(H)** and TOP2A **(I)**. Experiments were performed in triplicates and representative blots are shown.

Figure S4

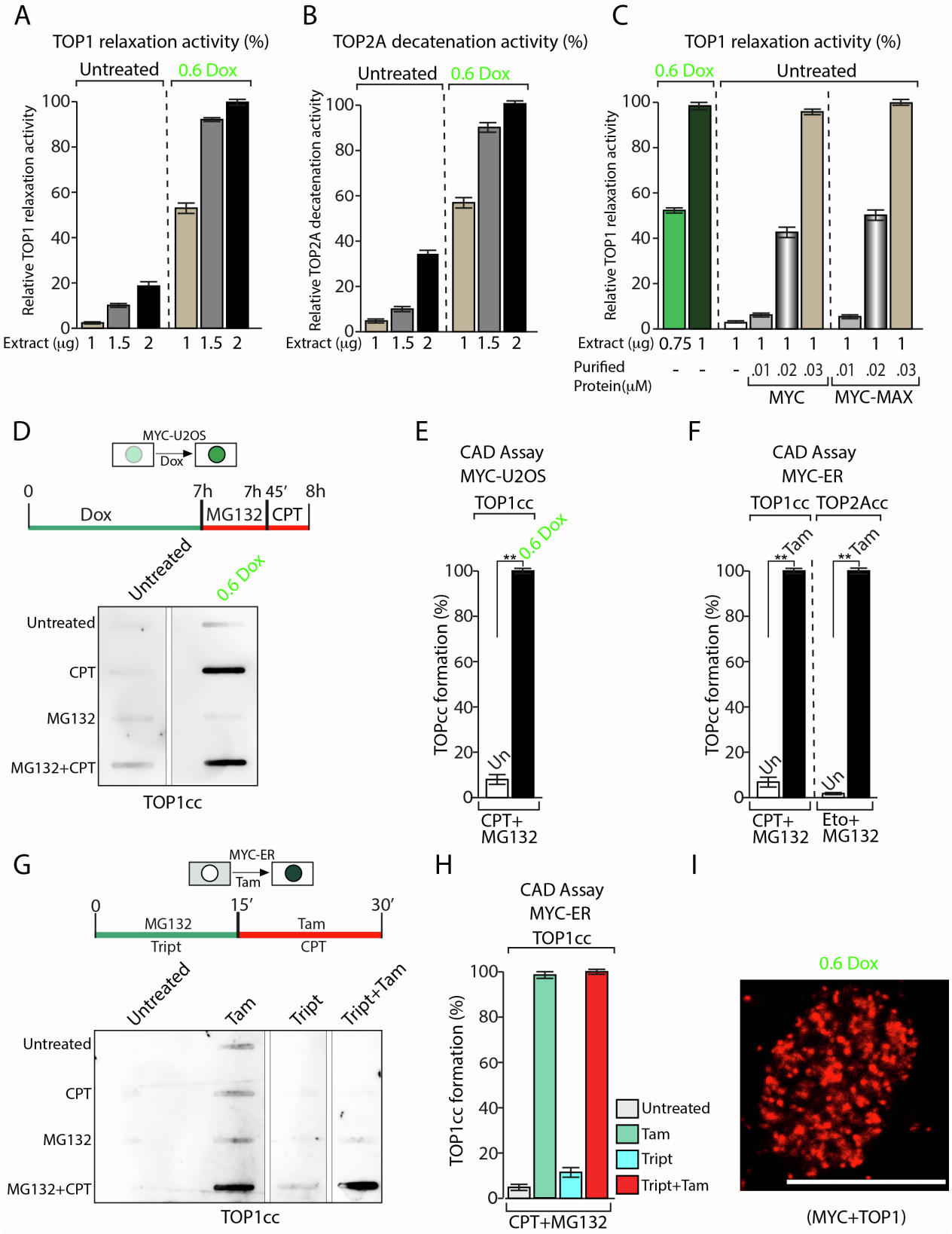
A
TOP1 relaxation activity (%)
Untreated
0.6 Dox
Relative TOP1 relaxation activity
Extract (µg) 1 1.5 2 1 1.5 2
B
TOP2A decatenation activity (%)
Untreated
0.6 Dox
Relative TOP2A decatenation activity
Extract (µg) 1 1.5 2 1 1.5 2
C
TOP1 relaxation activity (%)
0.6 Dox
Untreated
Relative TOP1 relaxation activity
Extract (µg) 0.75 1 1 1 1 1 1 1 1
Purified Protein(µM) - - - .01 .02 .03 .01 .02 .03
MYC
MYC-MAX
D
MYC-U2OS
Dox
0 7h 7h 45′ 8h
Dox
MG132
CPT
Untreated
0.6 Dox
Untreated
CPT
MG132
MG132+CPT
TOP1cc
E
CAD Assay
MYC-U2OS
TOP1cc
0.6 Dox
TOPcc formation (%)
Un
CPT+ MG132
F
CAD Assay
MYC-ER
TOP1cc TOP2Acc
Tam
TOPcc formation (%)
Un
CPT+ MG132
Eto+ MG132
G
MYC-ER
Tam
0 15′ 30′
MG132
Tript
Tam
CPT
Untreated
Tam
Tript
Tript+Tam
Untreated
CPT
MG132
MG132+CPT
TOP1cc
H
CAD Assay
MYC-ER
TOP1cc
TOP1cc formation (%)
Untreated
Tam
Tript
Tript+Tam
CPT+MG132
I
0.6 Dox
(MYC+TOP1)

Figure S4. MYC sponsors the covalent engagement of topoisomerase-DNA complexes in cells. Related to Figure 3. (A and **B)** Percentage of plasmid DNA relaxation by TOP1 **(A)** and percentage of decatenation by TOP2A **(B)** using the indicated amount of lysate from untreated or Dox-induced (0.6 μg/ml) U2OS-MYC-EGFP cells showed in **Figure 3B** and **C**, respectively. Each experiment was performed at least 3 times (n=5; error bars represent the mean ± S.E.M.). **(C)** Percentage of plasmid DNA relaxation by TOP1 from experiment shown in **Figure 3D**. The amount of extract from untreated or Dox-induced (0.6 μg/ml) U2OS-MYC-EGFP cells is indicated as well as the amont of of purified MYC or MYC-MAX (n=3; error bars represent the mean ± S.E.M.). **(D)** Trapped TOP1cc detected by CAD assay. Untreated and Dox-induced (0.6 μg/ml) U2OS-MYC-EGFP cells were treated with CPT (10μM) in the presence/absence of MG132 (10μM) respectively for the indicated time (top) and were immunoblotted with anti-TOP1 antibody. Samples of the CAD assay are loaded every other lane for clarity. **(E)** Quantification of trapped TOP1 cleavage complexes (TOP1cc) detected in U2OS-MYC-EGFP cells treated with CPT (10μM) and MG132 (10μM) as showed in **Figure S4D**. Significance was assessed by two-way ANOVA using Dunnett's correction for multiple comparisons (n = 3; mean ± S.E.M., **P < 0.001; t test). **(F)** Quantification of trapped TOP1ccs or TOP2Accs detected in HO15.19-MYC-ER cells treated with Eto (25μM) and MG132 as indicated in **Figures 3E** and **3F**. Significance was assessed by two-way ANOVA using Dunnett's correction for multiple comparisons (n = 4; mean ± S.E.M., **P < 0.001; t test). **(G)** Immunoblot showing TOP1ccs detected by CAD assay in HO15.19-MYC-ER cells treated as indicated and probed with anti-TOP1. Experiments were performed in biological triplicates and representative blots are shown. **(H)** Quantification of trapped TOP1ccs detected in **Figure S4G** (n=3; error bars represent mean ± S.E.M.). Based on statistical test (two-way ANOVA using Dunnett's correction for multiple comparisons) the difference between Tam and Trip+Tam is not significant. **(I)** Representative image showing an enlarged picture of PLA using anti-MYC and anti-TOP1 in U2OS-MYC-EGFP cells induced with 0.6 μg/ml of Dox (relative to **Figure 3G**, left) (scale bars, 15 μm).

Figure S5

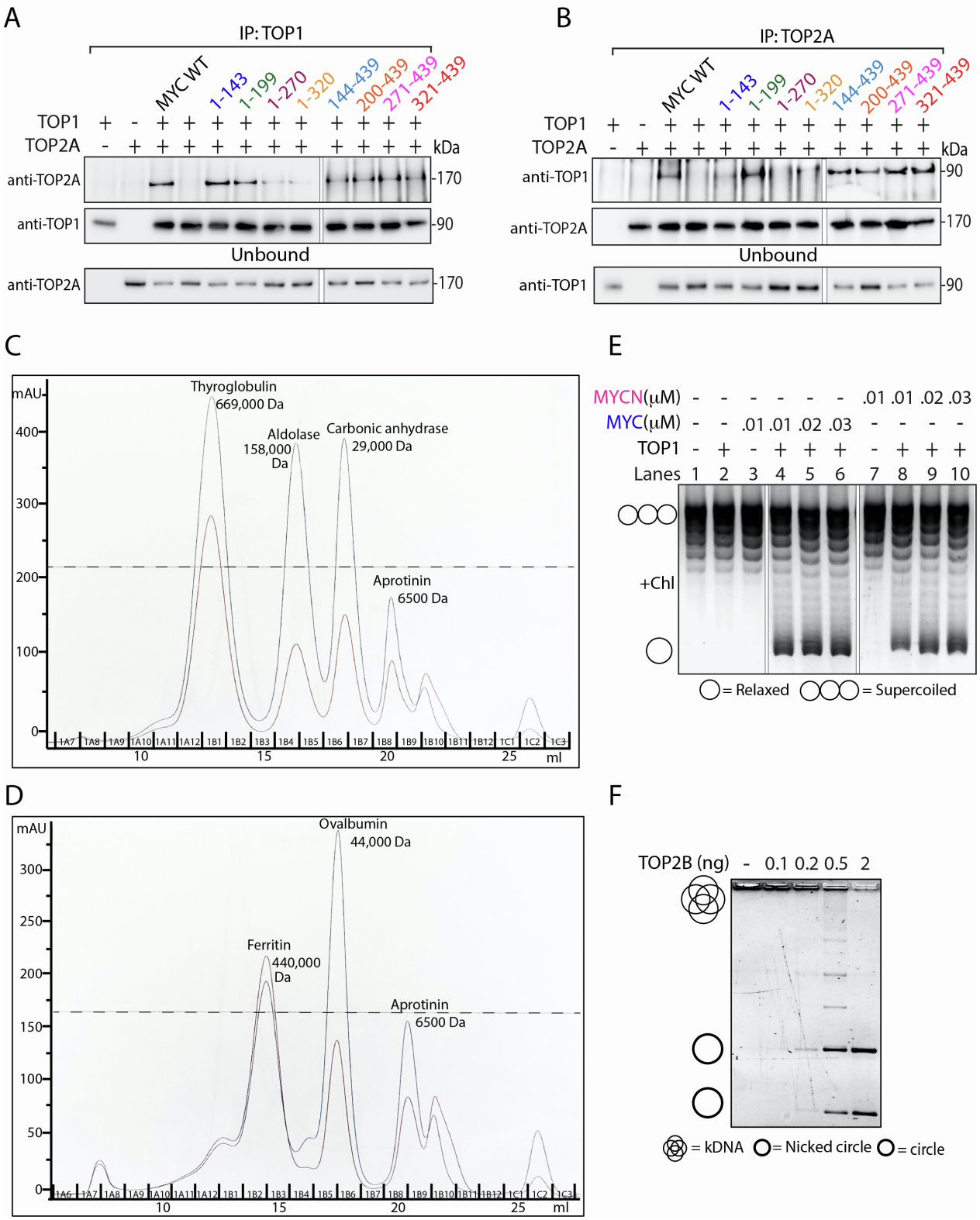
A
IP: TOP1
MYC WT
1-143
1-199
1-270
1-320
144-439
200-439
271-439
321-439
TOP1
TOP2A
kDa
anti-TOP2A
anti-TOP1
170
90
Unbound
anti-TOP2A
170
B
IP: TOP2A
MYC WT
1-143
1-199
1-270
1-320
144-439
200-439
271-439
321-439
TOP1
TOP2A
kDa
anti-TOP1
anti-TOP2A
90
170
Unbound
anti-TOP1
90
C
mAU
Thyroglobulin
669,000 Da
Aldolase
158,000
Da
Carbonic anhydrase
29,000 Da
Aprotinin
6500 Da
400
300
200
100
0
10
15
20
25
ml
D
mAU
Ovalbumin
44,000 Da
Ferritin
440,000
Da
Aprotinin
6500 Da
300
250
200
150
100
50
0
10
15
20
25
ml
E
MYCN(μM)
MYC(μM)
TOP1
Lanes 1 2 3 4 5 6 7 8 9 10
.01 .01 .02 .03
.01 .01 .02 .03
+Chl
= Relaxed
= Supercoiled
F
TOP2B (ng) - 0.1 0.2 0.5 2
= kDNA
= Nicked circle
= circle

Figure S5. Trunncated N- or C-terminal regions of MYC joins TOP1 and TOP2A to form a unique complex. Related to Figure 4 and Figure 5. (A and **B)** Recombinant TOP1or TOP2A were mixed with the indicated MYC full length or MYC truncations, immunoprecipitated with anti-TOP1 **(A)** or anti-TOP2A **(B)** and probed for TOP1 and TOP2A. Shown are representative blots from 3 independent replicas**. (C** and **D)** Elution profile of the marker proteins (high molecular weight marker kit, cytiva) with the indicated molecular mass. **(E)** Recombinant full length MYC or MYCN were pre-incubated with TOP1, as indicated, before addition of plasmid DNA and reaction products were checked by agarose gel electrophoresis with Chl. Experiment were performed more than 3 times. **(F)** Decatenation assay in presence of increasing amount of recombinant TOP2B. Represenative gel of 2 independent replicas.

Figure S6

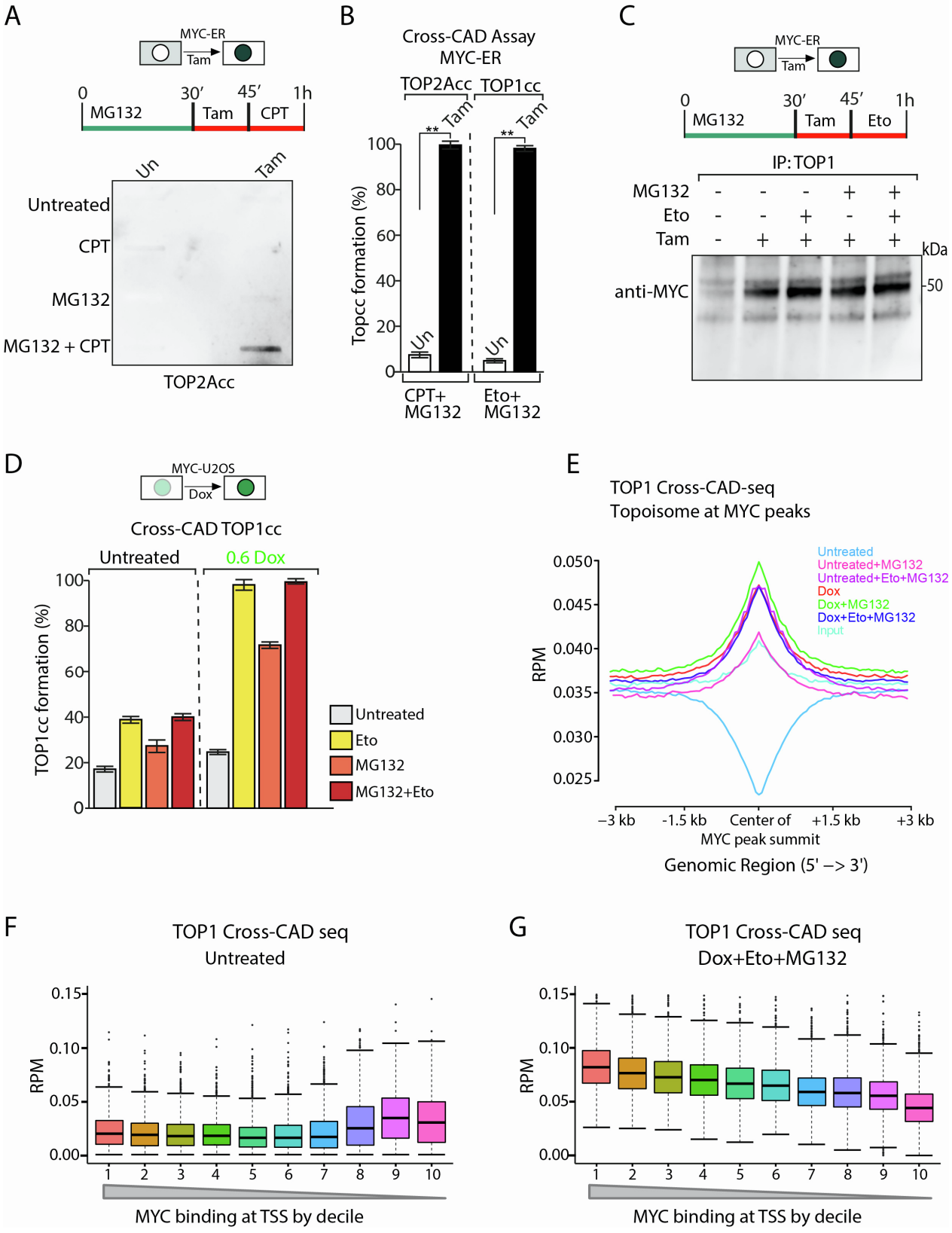
A
MYC-ER
Tam
0
30′
45′
1h
MG132
Tam
CPT
Un
Tam
Untreated
CPT
MG132
MG132 + CPT
TOP2Acc
B
Cross-CAD Assay
MYC-ER
TOP2Acc
TOP1cc
Tam
**
Topcc formation (%)
100
80
60
40
20
0
Un
CPT+
MG132
Eto+
MG132
C
MYC-ER
Tam
0
30′
45′
1h
MG132
Tam
Eto
IP:TOP1
MG132 - - - + +
Eto - - + - +
Tam - + + + +
kDa
anti-MYC
50
D
MYC-U2OS
Dox
Cross-CAD TOP1cc
Untreated
0.6 Dox
TOP1cc formation (%)
100
80
60
40
20
0
Untreated
Eto
MG132
MG132+Eto
E
TOP1 Cross-CAD-seq
Topoisome at MYC peaks
Untreated
Untreated+MG132
Untreated+Eto+MG132
Dox
Dox+MG132
Dox+Eto+MG132
Input
RPM
0.050
0.045
0.040
0.035
0.030
0.025
−3 kb
-1.5 kb
Center of
MYC peak summit
+1.5 kb
+3 kb
Genomic Region (5' –> 3')
F
TOP1 Cross-CAD seq
Untreated
RPM
0.15
0.10
0.05
0.00
1
2
3
4
5
6
7
8
9
10
MYC binding at TSS by decile
G
TOP1 Cross-CAD seq
Dox+Eto+MG132
RPM
0.15
0.10
0.05
0.00
1
2
3
4
5
6
7
8
9
10
MYC binding at TSS by decile

Figure S6. MYC adjusts the dynamics of topoisome engagement at genes to favor transcription. Related to Figure 6. (A) Detection of MYC-tethered TOP2Acc along with CPT (10μM)-trapped TOP1cc by cross-CAD assays in HO15.19-MYC-ER cells treated as indicated (top) and detected by immunoblotting with anti-TOP2A. Samples of the cross-CAD assay are loaded every other lane for clarity. **(B)** Quantification of MYC-tethered TOP2Acc along with CPT (10μM)-trapped TOP1cc or MYC-tethered TOP1cc along with Eto (25μM)-trapped TOP2Acc by cross-CAD assays in HO15.19-MYC-ER cells (representative blot is shown in **Figures 6B** and **S6A**). Each experiment was performed more than 3 times. Significance was assessed by two-way ANOVA using Dunnett's correction for multiple comparisons (n = 4; mean ± S.E.M., **P < 0.001; t test). **(C)** Nuclear extracts from HO15.19-MYC-ER cells treated with or without 200 nM Tam, Eto (25μM) or MG132 (50μM), were immunoprecipitated with anti-TOP1 and probed for MYC. **(D)** Quantification of MYC-tethered TOP1cc along with Eto (25μM)-trapped TOP2Acc by cross-CAD assays in in U2OS-MYC-EGFP cells in the presence or absence of 0.6 μg/ml Dox (representative blot is shown in **Figure 6C**). Each experiment was performed at least 3 times (n=3; error bars represent mean ± S.E.M.). **(E)** TOP1 cross-CAD-seq signal at MYC binding sites, centered around peak summits. MYC ChIP seq from (Lorenzin et al. 2016) was used to define MYC peak summit. **(F-G)** Box plots of TOP1 cross-CAD-seq (RPM) at TSSs of 70% most expressed genes (based on RNA-seq (Ibarra et al. 2016)). TSS ± 500 bps are sorted into deciles according to decreasing MYC binding (using the dataset +Dox from (Lorenzin et al. 2016)) from 1 to 10, and the cross-CAD-seq reads from untreated **(F)** and sample treated with Dox+MG132+Eto **(G)** are computed in each decile. Whiskers indicate lowest and highest values no further than 1.5 x interquartile.

Figure S7

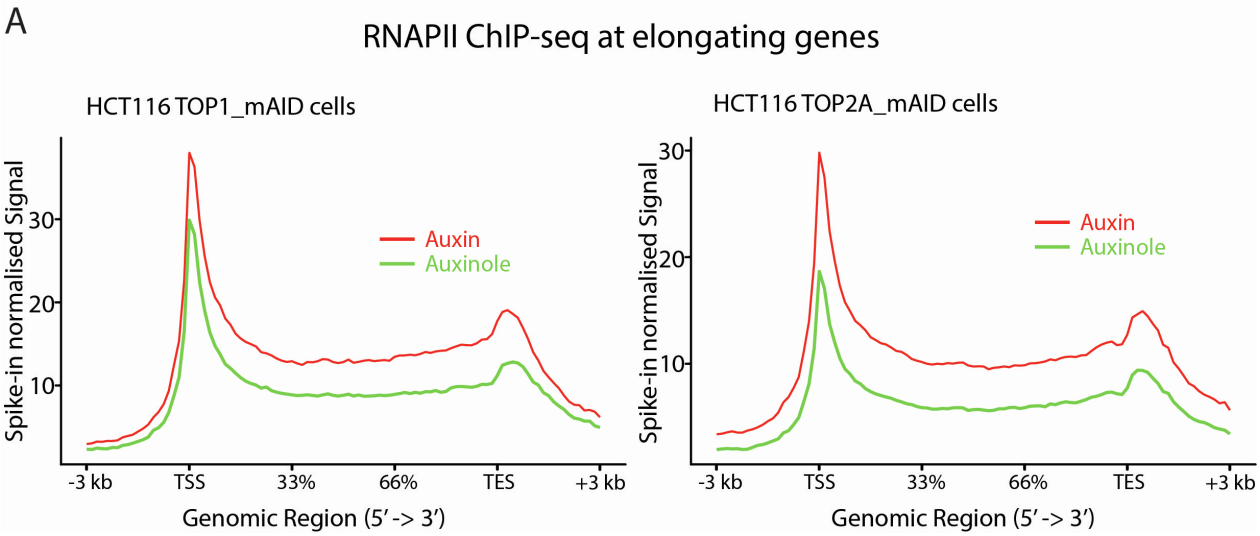

Figure S7. Degradation of TOP1 or TOP2A triggered marked accumulation of RNAPII. Related to Figure 7. (A) RNAPII signal (spike-in normalized) from HCT116TOP1_mAID cells (left) or HCT116TOP2A_mAID cells (right) treated with auxin or auxinol was plotted at elongating genes identified by pausing index (calculated in the auxinole sample). Pausing index is the ratio between RNAPII bound at the TSS and RNAPII bound in the gene body (see **STAR Methods**).

Supplementary Tables

Table S1.

ChIP-seq	Peak caller	Total number of peaks	Number of peaks after filtering for blacklisted hg38 regions
TOP1	MACS3	20694	19834
TOP2A	EPIC2	14623	13383
MYC	MACS3	36983	34917

Table S1. Number of TOP1, TOP2A and MYC ChIP-seq peaks. Related to Figure 1.

For detailed peak files please access the GEO database, accession number GSE181447.
File name: GSE181447_Supplementary_table_ChIP-seq_peaks.xlsx

Table S2.

ChIP-seq

Sample name	Total sequenced reads	Unique deduplicated dm6 spike-in reads
TOP2A_ChIP-seq_1	24,904,071	
TOP2A_ChIP-seq_2	29,071,653	
MYC_ChIP-seq_1	25,897,232	
MYC_ChIP-seq_2	35,267,961	
MYC_ChIP-seq_TOP1-mAID_auxin_1	37,690,949	34,706
MYC_ChIP-seq_TOP1-mAID_auxin_2	33,892,390	40,396
MYC_ChIP-seq_TOP1-mAID_auxinole_1	37,143,594	14,396
MYC_ChIP-seq_TOP1-mAID_auxinole_2	53,559,918	51,015
MYC_ChIP-seq_TOP2A-mAID_auxin_1	29,090,462	81,244
MYC_ChIP-seq_TOP2A-mAID_auxin_2	31,105,771	69,421
MYC_ChIP-seq_TOP2A-mAID_auxinole_1	28,727,255	94,588
MYC_ChIP-seq_TOP2A-mAID_auxinole_2	27,832,569	75,608
RNAPII_ChIP-seq_TOP1-mAID_auxin_1	33,375,643	22,900
RNAPII_ChIP-seq_TOP1-mAID_auxin_2	38,017,226	33,504
RNAPII_ChIP-seq_TOP1-mAID_auxinole_1	24,159,772	21,853
RNAPII_ChIP-seq_TOP1-mAID_auxinole_2	37,606,468	27,539
RNAPII_ChIP-seq_TOP2A-mAID_auxin_1	22,495,459	24,324
RNAPII_ChIP-seq_TOP2A-mAID_auxin_2	35,800,690	37,847
RNAPII_ChIP-seq_TOP2A-mAID_auxinole_1	20,276,516	38,269
RNAPII_ChIP-seq_TOP2A-mAID_auxinole_2	22,259,976	35,195

CAD-seq

Sample name	Total sequenced reads
TOP1cc_CAD-seq_MYC-mAID_auxin_1	26,415,581
TOP1cc_CAD-seq_MYC-mAID_auxin_2	25,498,562
TOP1cc_CAD-seq_MYC-mAID_auxinole_1	31,002,490
TOP1cc_CAD-seq_MYC-mAID_auxinole_2	26,804,708

Cross-CAD-seq

Sample name	Total sequenced reads
TOP1cc_cross-CAD-seq_input	101,109,808
TOP1cc_cross-CAD-seq_untr_1	96,607,282
TOP1cc_cross-CAD-seq_untr_2	127,543,246
TOP1cc_cross-CAD-seq_untr_MG132_1	121,400,934
TOP1cc_cross-CAD-seq_untr_MG132_2	111,485,132
TOP1cc_cross-CAD-seq_untr_ETO_MG132_1	124,507,963
TOP1cc_cross-CAD-seq_untr_ETO_MG132_2	117,292,679
TOP1cc_cross-CAD-seq_DOX_1	94,460,687
TOP1cc_cross-CAD-seq_DOX_2	106,474,424
TOP1cc_cross-CAD-seq_DOX_MG132_1	105,026,363
TOP1cc_cross-CAD-seq_DOX_MG132_2	102,845,262
TOP1cc_cross-CAD-seq_DOX_ETO_MG132_1	123,477,453
TOP1cc_cross-CAD-seq_DOX_ETO_MG132_2	166,490,961

Table S2. Number of reads for all the sequencing datasets and number of *Drosophila Melanogaster* genome assembly Release 6 (dm6) spike in reads. Related to Figures 1,6 and 7.

Table S3.

	Protein	Fraction	IgG	Untreated (UN)	Fraction of Total	0.6 Dox	Fraction of Total
TOP1 IP	TOP1	Input	107.8	102.5	1.00	101.34	
		Sup	107.1	11.6	0.03	7.53	0.02
		pellet	0.0	89.2	0.22	90.29	0.22
	TOP2A	Input	56.0	54.4	1.00	47.58	
		Sup	50.5	39.5	0.18	14.73	0.08
		pellet	0.0	6.0	0.03	25.97	0.13

	Protein	Fraction	IgG	Untreated	Fraction of Total	0.6 Dox	Fraction of Total
TOP2A IP	TOP1	Input	91.3	87.4		90.32	
		Sup	83.3	63.5	0.18	44.32	0.12
		pellet	0.0	17.3	0.05	50.34	0.14
	TOP2A	Input	109.2	111.7		104.71	
		Sup	102.7	2.1	0.00	3.06	0.01
		pellet	0.0	104.7	0.23	99.79	0.24

Table S3. Percentage of cellular TOP1 and TOP2A present in the topoisome. Related to Figure 4.

N.B: All values for each fraction are mean of three independent experiments
Fraction of total = Fraction/(Input*4) as IP samples were 4x volume compared to Input