ERK2-topoisomerase II regulatory axis is important for gene activation in immediate early genes

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Supplementary Data 2: Mass spectrometry data- deTOP2B with ERK1

Supplementary Data 3: Mass spectrometry data- deTOP2B with ERK2

Supplementary Data 4: Mass spectrometry data- deTOP2B with ERK1m

Supplementary Data 5: Mass spectrometry data- deTOP2B with ERK2m

Supplementary Data 6: Mass spectrometry data- spectrum

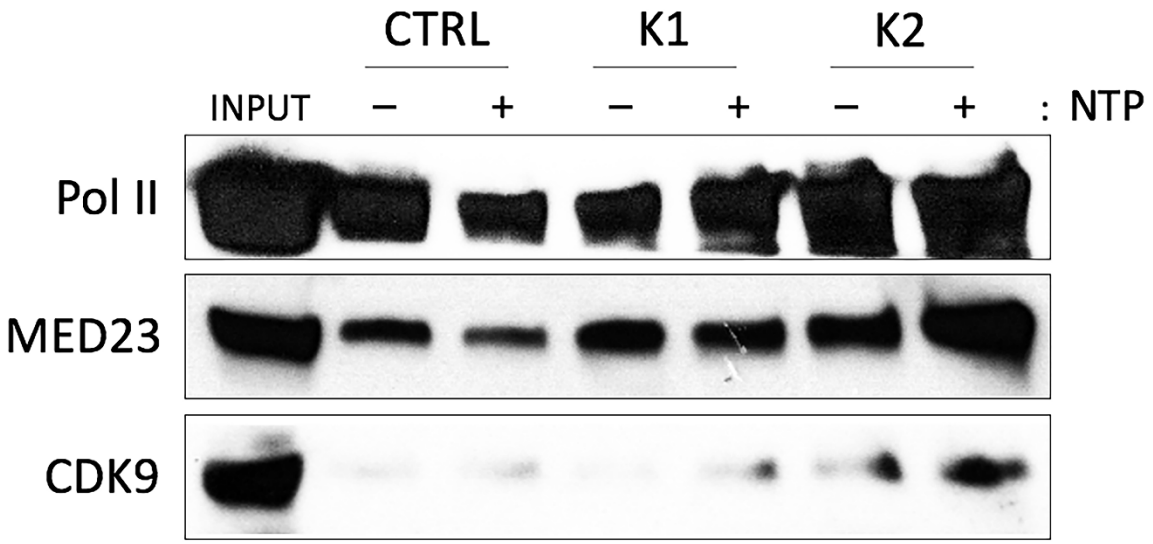
Supplementary Data 7: Mass spectrometry data-CTRL reaction against *E. coli* proteome

Supplementary Data 8: Mass spectrometry data-ERK1m reaction against *E. coli* proteome

Supplementary Data 9: Mass spectrometry data-ERK2m reaction against *E. coli* proteome

SUPPLEMENTARY FIGURES

A



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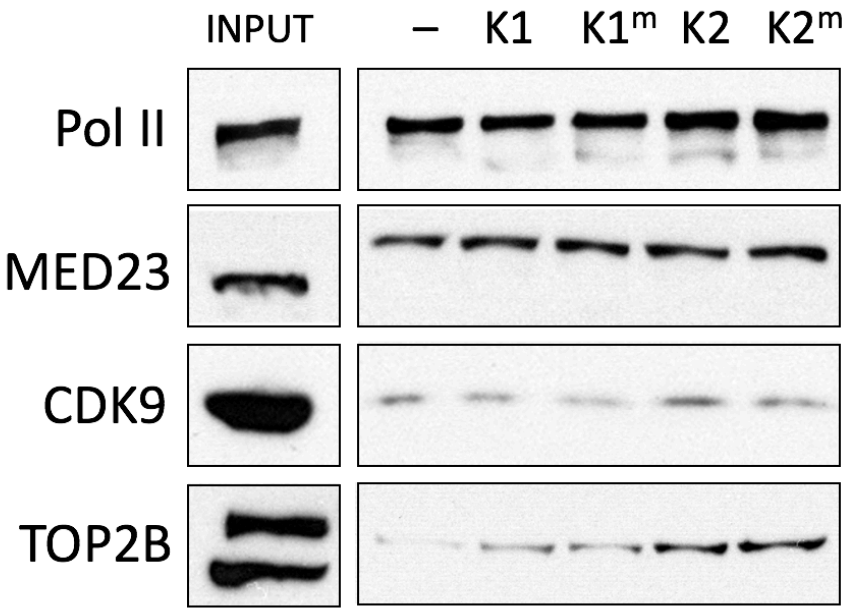
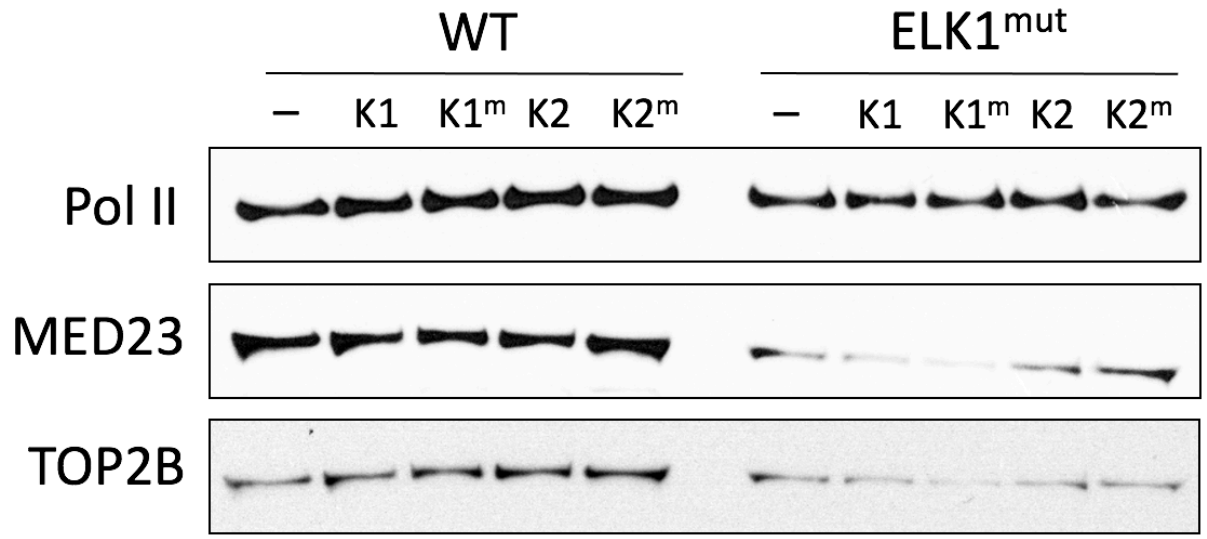

INPUT
–
K1
K1m
K2
K2m
Pol II
MED23
CDK9
TOP2B

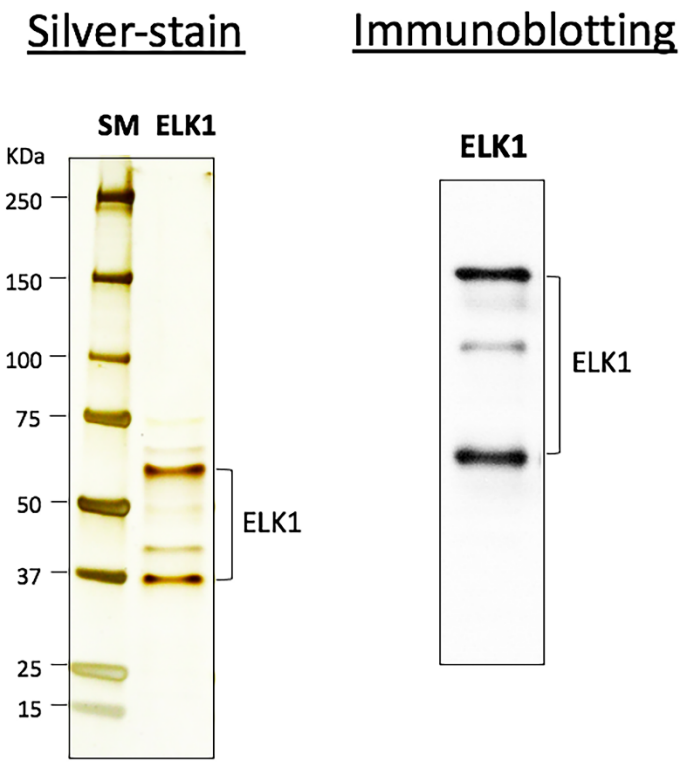
C


WT
ELK1mut
–
K1
K1m
K2
K2m
–
K1
K1m
K2
K2m
Pol II
MED23
TOP2B

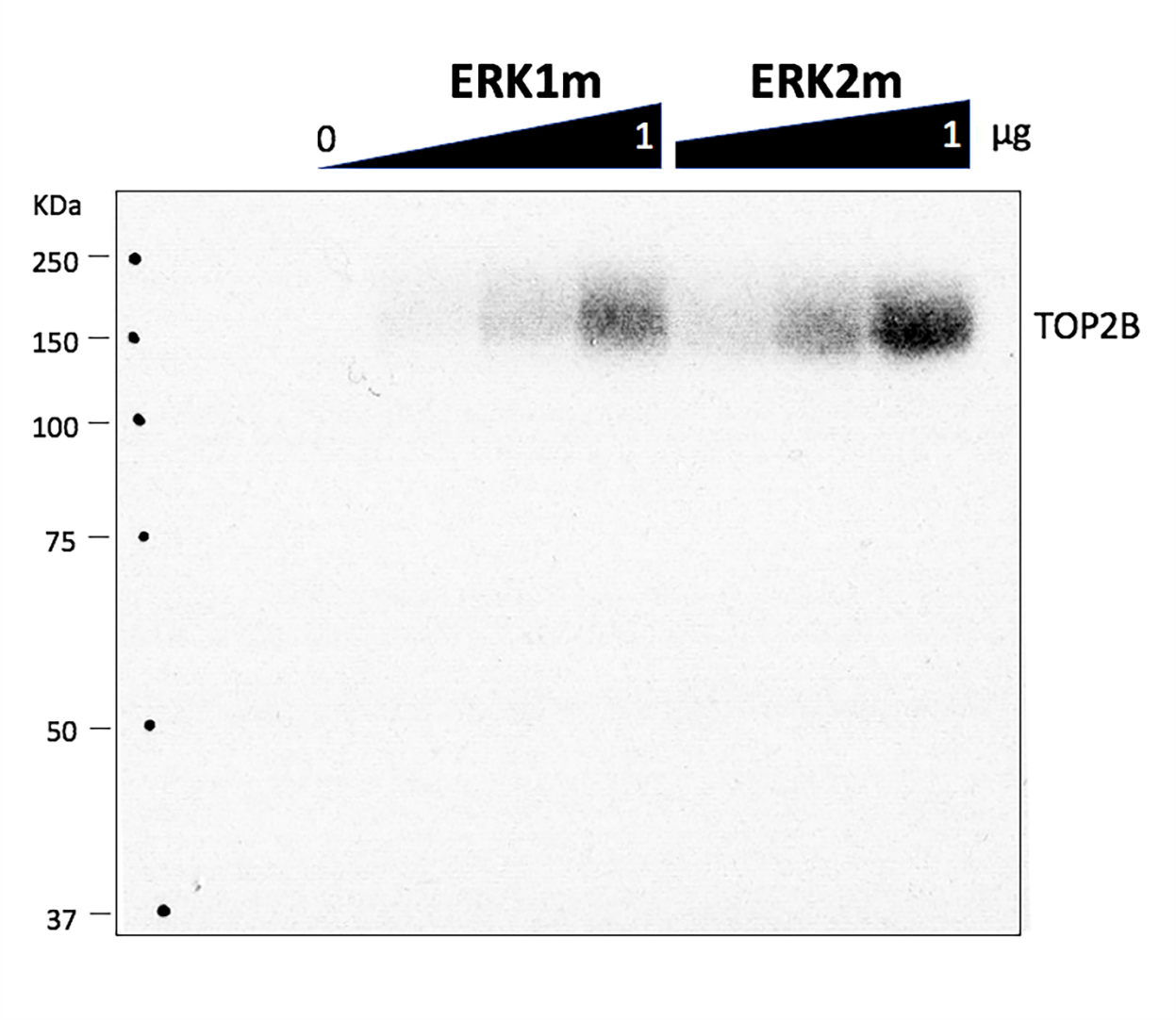
Supplementary Figure 1. ERK2 activates EGR1 transcription.

(**A**) Immobilized template assay results showing more prominent effects of ERK2 on recruiting Pol II, MED23, and CDK9 to the *EGR1* TSS. ERKs were supplemented with HeLa NE and before NTP addition. CTRL, ERK storage buffer only without ERKs.
(**B**) Immobilized template assay results indicating that ERK2 and ERK2m are more effective to recruit transcription factors, such as Pol II, MED23, TOP2B, and CDK9, on the *EGR1* template than ERK1 and ERK1m (upper panel). Input (HeLa nuclear extracts, NE), 20%. –, ERK storage buffer only control.
(**C**) The stimulating effects by ERK2 and ERK2m is dependent on ELK1 binding on the template. –, ERK storage buffer only control. For the representative immunoblotting image data, the experiments were repeated at least 3 times with similar results. Original blot images are provided as additional Supplementary Figures.

A


Silver-stain
Immunoblotting
SM
ELK1
KDa
250
150
100
75
50
37
25
15
ELK1
ELK1
ELK1

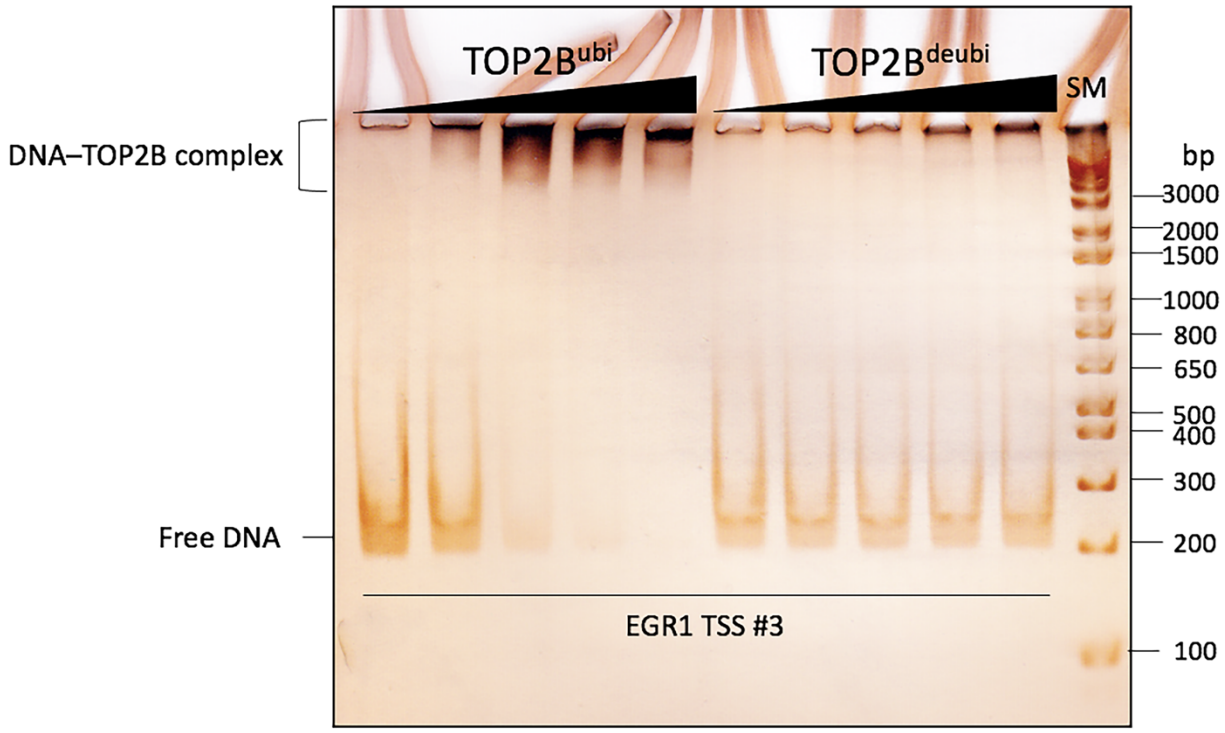
B



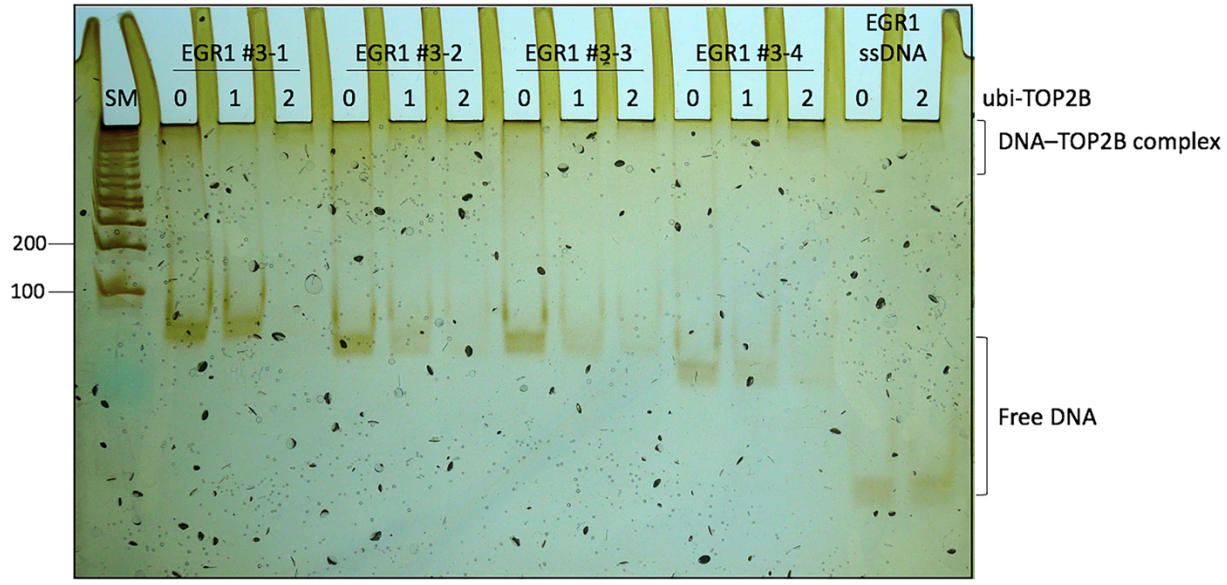
Supplementary Figure 2. *In vitro* kinase assay and autoradiography of a SDS PAGE gel.

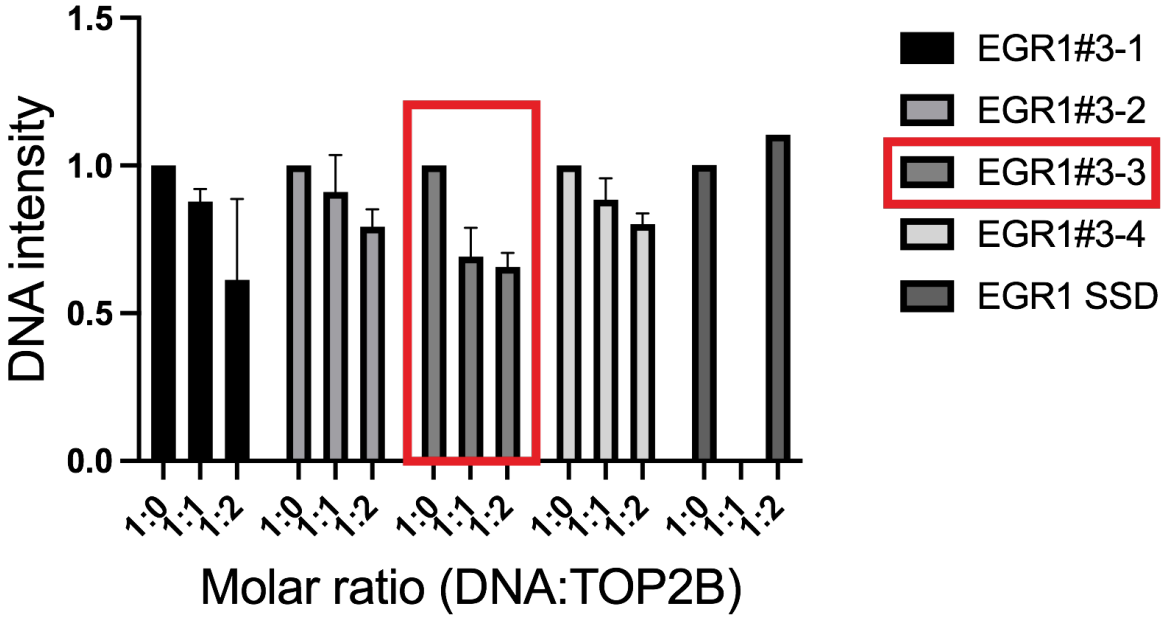
(**A**) Recombinant ELK1 that was purified from *E. coli* and used for the *in vitro* kinase assays in this study is shown on a SDS-PAGE gel stained with silver (left) and on an immunoblot (right).
(**B**) Titration of ERK1m and ERK2m in the *in vitro* kinase assay showing a dose-dependent increase of phosphorylation of TOP2B. Nine µg of TOP2B and 0, 0.25, 0.5 and 1 µg of ERK1m or ERK2m were incubated for 1 h at 30 °C. A ^{32}P autoradiography of SDS-PAGE gel. For the representative image data, the experiments were repeated at least 3 times with similar results. Original images are provided as additional Supplementary Figures.

A


TOP2B^ubi
TOP2B^deubi
SM
DNA–TOP2B complex
Free DNA
EGR1 TSS #3
bp
3000
2000
1500
1000
800
650
500
400
300
200
100

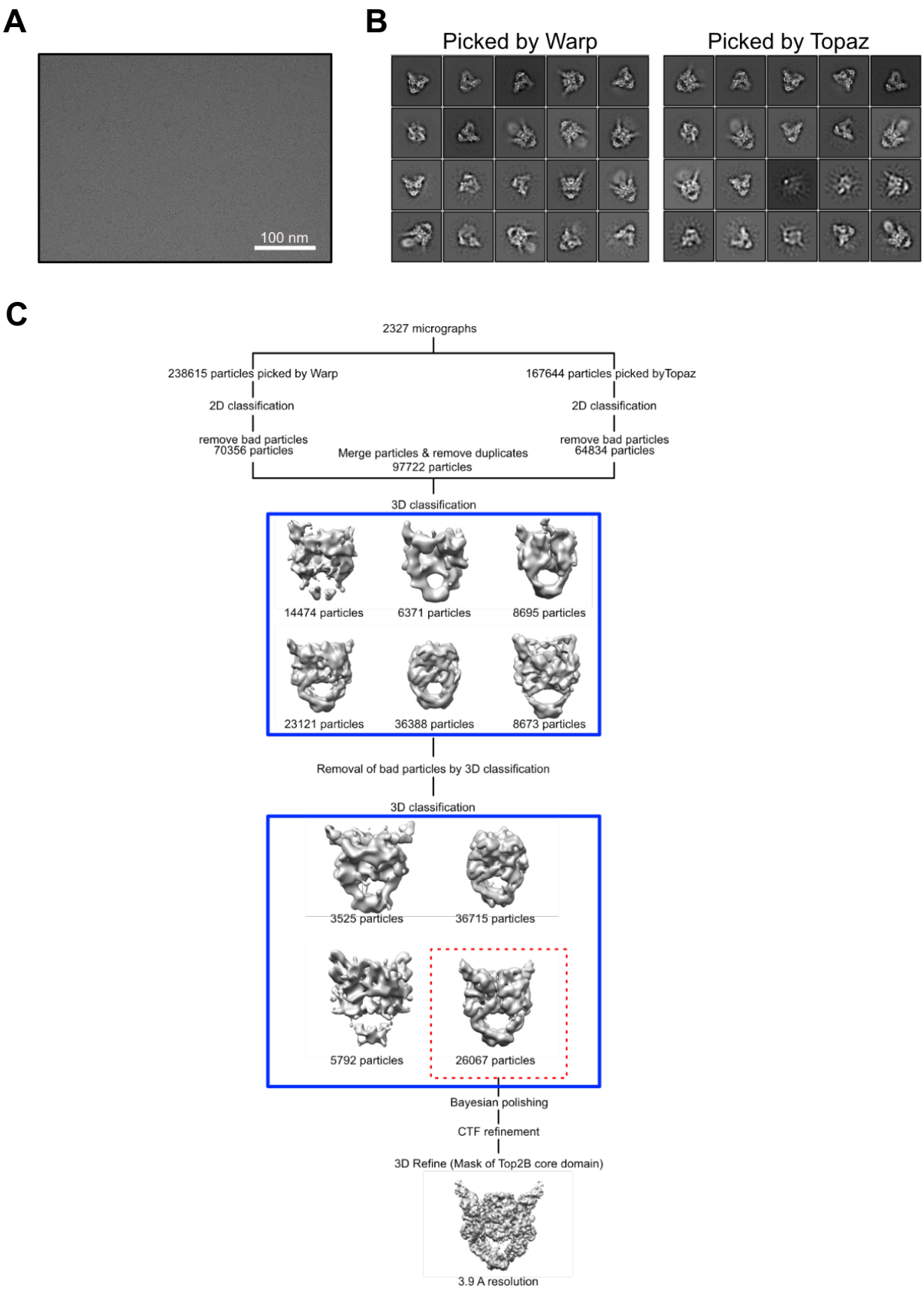
B

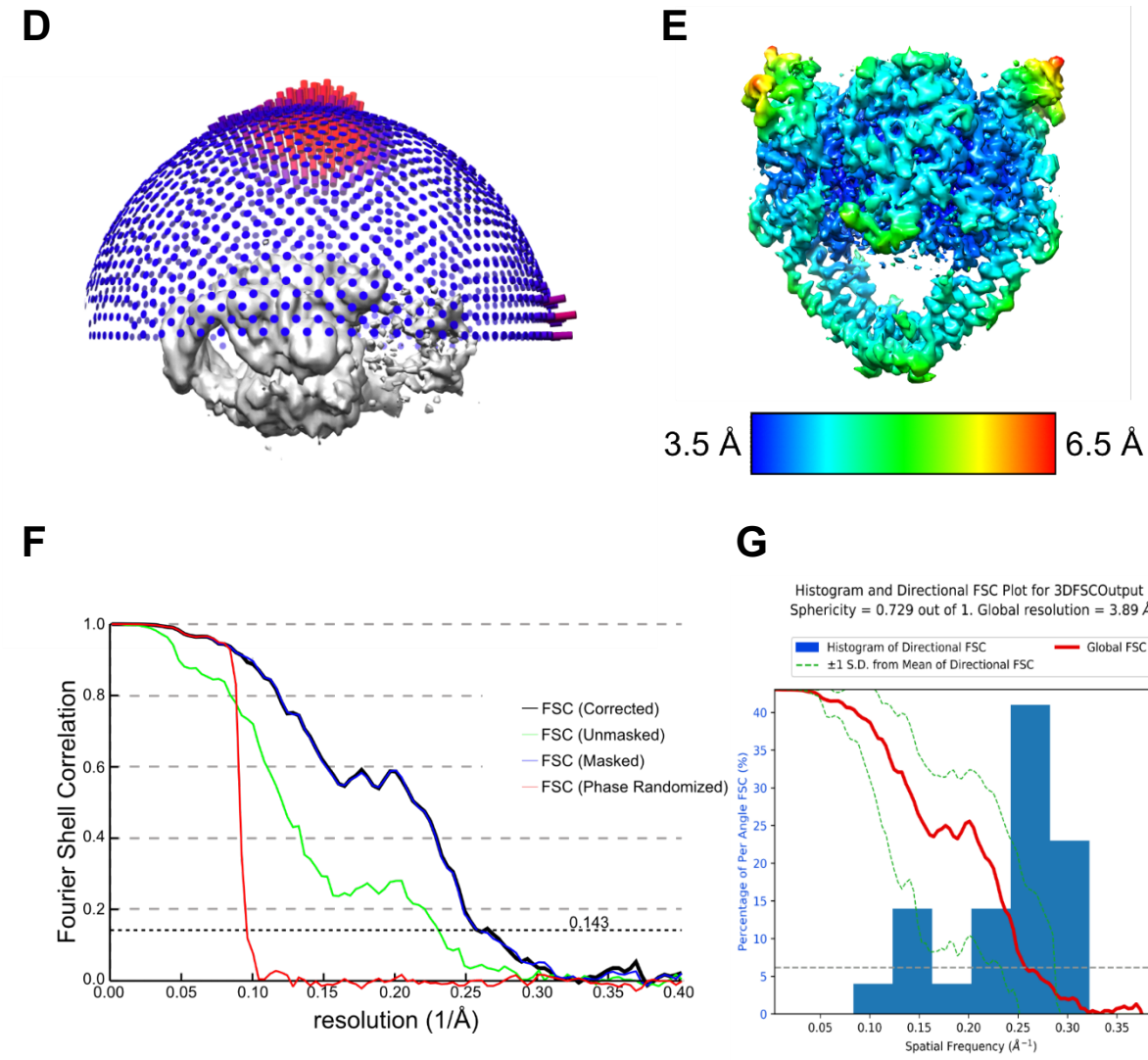

EGR1 #3-1
EGR1 #3-2
EGR1 #3-3
EGR1 #3-4
EGR1 ssDNA
SM
0 1 2 0 1 2 0 1 2 0 1 2 0 2
ubi-TOP2B
DNA–TOP2B complex
200
100
Free DNA


DNA intensity
1.5
1.0
0.5
0.0
1:0 1:1 1:2 1:0 1:1 1:2 1:0 1:1 1:2 1:0 1:1 1:2 1:0 1:1 1:2
Molar ratio (DNA:TOP2B)
EGR1#3-1
EGR1#3-2
EGR1#3-3
EGR1#3-4
EGR1 SSD

Supplementary Figure 3. Electrophoretic mobility shift assay (EMSA) using TOP2B and *EGR1* TSS fragments.

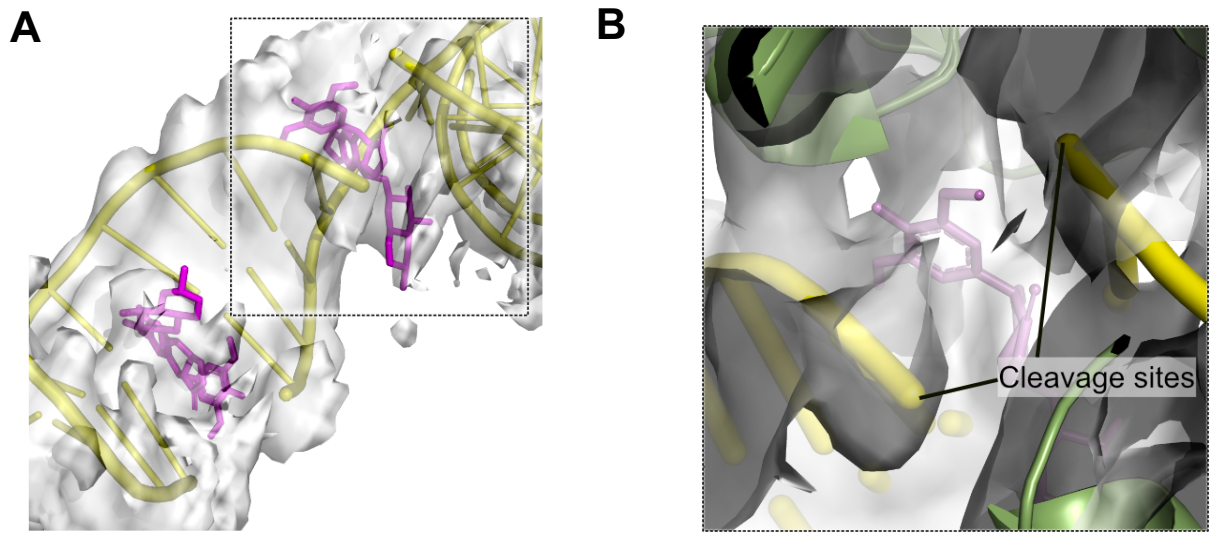
(**A**) EMSA results showing the *EGR1* TSS fragment (–132 to +62, EGR1 TSS #3) that was identified to have the strongest affinity to TOP2B from our previous study (Bunch et al. Open Biology 2021). DNA, 100 ng; ubiquitinated TOP2B ($TOP2B^{ubi}$) or deubiquitinated TOP2B ($TOP2B^{deubi}$) titration of 0, 150, 300, 450, and 600 ng. The gel was silver-stained to visualize both DNA and protein. SM, DNA size marker in bp. (**B**) *EGR1* TSS #3 was further dissected into 4 fragments, generating EGR1 #3-1, #3-2, #3-3, and #3-4. The four fragments were compared for binding to TOP2B, using EMSA (Bunch et al. Open Biology 2021). The reactions were separated on the DNA polyacrylamide gel and the resultant gel was stained with silver nitrate. The top panel shows a representative gel and the bottom graph shows the means and standard deviations (n = 2 independent experiments). Control, *EGR1* TSS ssDNA (or SSD). Per a reaction, 100 ng of DNA was used. 0, no protein; 1 and 2, DNA : TOP2B = 1:1 or 1:2 molar ratio.


A
100 nm
B
Picked by Warp
Picked by Topaz
C
2327 micrographs
238615 particles picked by Warp
167644 particles picked byTopaz
2D classification
2D classification
remove bad particles
70356 particles
remove bad particles
64834 particles
Merge particles & remove duplicates
97722 particles
3D classification
14474 particles
6371 particles
8695 particles
23121 particles
36388 particles
8673 particles
Removal of bad particles by 3D classification
3D classification
3525 particles
36715 particles
5792 particles
26067 particles
Bayesian polishing
CTF refinement
3D Refine (Mask of Top2B core domain)
3.9 A resolution



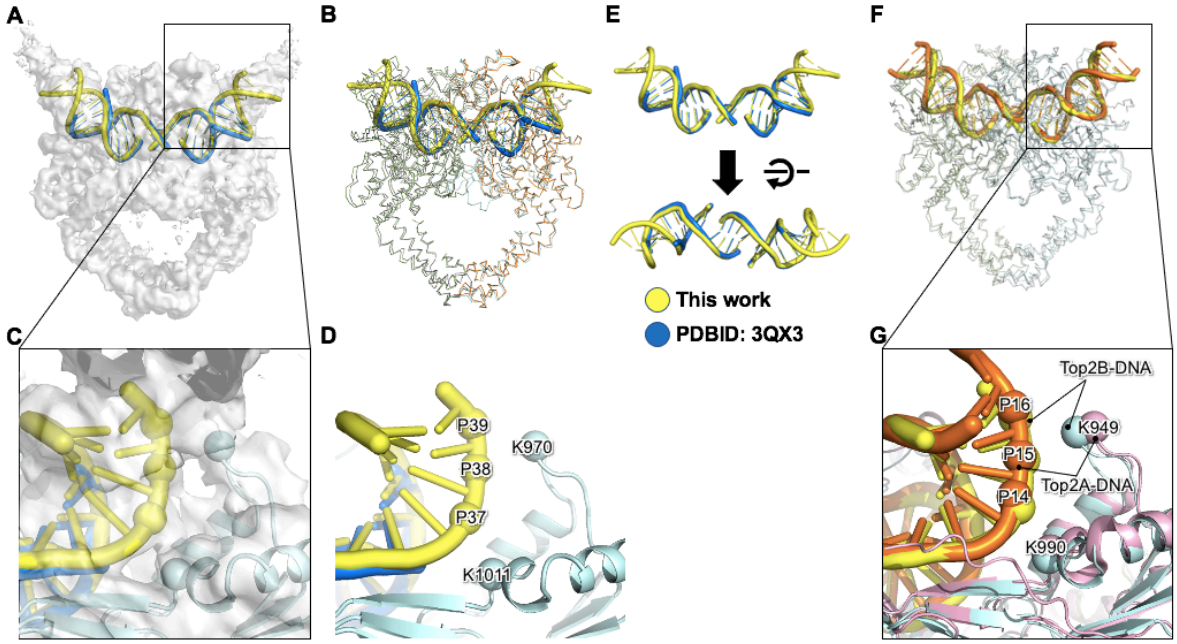
Supplementary Figure 4. Cryo-EM data collection and image processing.

(**A**) Representative cryo-EM micrograph.
(**B**) Representative 2D class averages from the reference-free 2D classification calculated after removing bad particles.
(**C**) Flowchart of the image processing.
(**D**) Euler distribution plot of the overall reconstruction.
(**E**) Local resolution estimation of the cryo-EM reconstruction. The map is colored according to the local resolution values. Local resolutions are estimated using the RELION postprocess.
(**F**) Fourier shell correlation curves for the TOP2B-ternary complex. The overall resolution is estimated to be 3.9 Å at an FSC = 0.143.
(**G**) 3DFSC plots of Top2B-DNA-Etoposide complex. The overall resolution is estimated to be 3.9 Å at an FSC = 0.143 with a sphericity of 0.729.


A
B
Cleavage sites
C

Supplementary Figure 5. Density maps and models of TOP2B-DNA-etoposide complex.

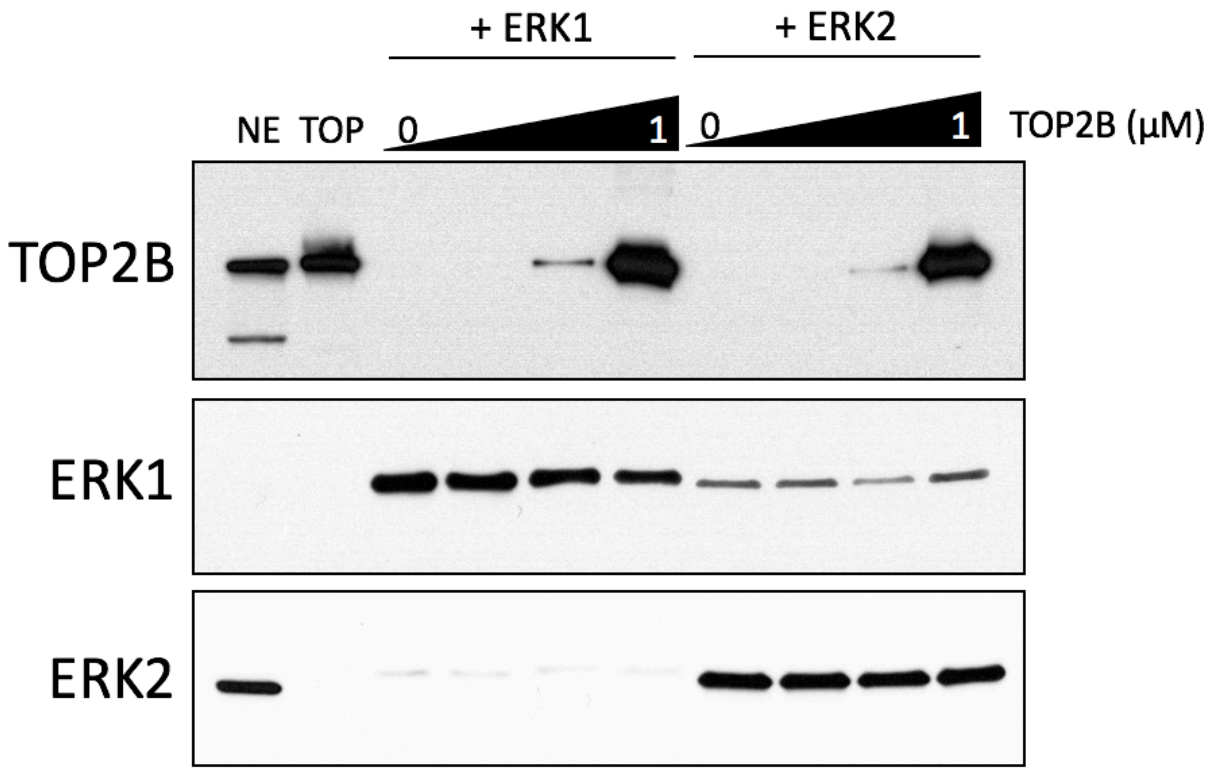
(**A**) Density map around the etoposide molecules and the cleavage sites of DNA. Etoposide molecules are shown as magenta sticks. DNA molecules are shown as yellow tubes.
(**B)** A close-up view of the square region in the panel A.
(**C**) Density of TOP2B-DNA-etoposide complex without imposing C2 symmetry. The ribbon model of TOP2B-DNA-Etoposide complex refined using the density with imposing C2 symmetry is superposed. There are no notable differences between the densities with and without imposing C2 symmetry.



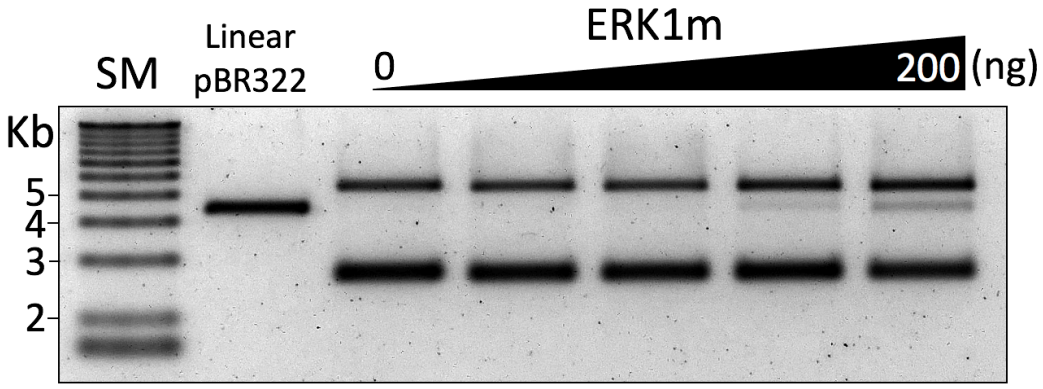
Supplementary Figure 6. Comparison of other TOP2 structures.

(**A**) The cryo-EM density map of the TOP2B-DNA-etoposide complex is shown with a cartoon model of DNA colored yellow. The DNA model in the crystal structure of Top2B-DNA-etoposide complex (PDB 3QX3 [https://www.rcsb.org/structure/3qx3]) is overlayed and colored blue.
(**B**) The backbone-trace model of the current TOP2B-DNA-etoposide complex was superimposed with that of the crystal structure (PDB 3QX3) by one of the TOP2B subunits (the right half of the molecules).
(**C**) A close-up view of the region enclosed by a rectangle in panel A. The viewing angle was changed for the visibility. The Top2B protein is represented as a light blue cartoon model. The Cα atoms of Top2B (K970 and K1011) and the DNA phosphate atoms (P37, P38, and P39) are shown as spheres.
(**D**) The cryo-EM density was removed from panel C. The atoms shown as spheres are labeled.
(**E**) Comparison of DNAs in panels A and B.
(**F**) The TOP2B-DNA-etoposide complex structure was superimposed with the TOP2A-DNA-etoposide complex structure by one of the protein subunits. The TOP2 proteins are shown as backbone-trace models. The DNA molecules in the TOP2B and the TOP2A complexes are represented as yellow and orange tubes, respectively.
(**G**) A close-up view of the region enclosed by a rectangle in panel F. The TOP2B and TOP2A proteins are represented as a light blue and pink cartoon models, respectively.

A



B



Supplementary Figure 7. Representative immunoblotting and ERK1m titration results for DNA relaxation assays.

(**A**) TOP2B was used at concentrations of 0, 0.005, 0.05, and 1 μM and ERK1 and ERK2 was added at 0.2 μM per each reaction for DNA relaxation assays. One tenth of the reaction was assays through immunoblotting. NE, HeLa nuclear extracts; TOP, TOP2B used for the assay. ERK1 antibody showed some cross-reactivity to ERK2. (**B**) ERK1m was titrated in increasing amounts of 0, 0.2, 2, 20, 200 ng in the DNA relaxation assay. Negatively supercoiled pBR322 (500 ng per each reaction) was used. The reaction was allowed for 30 min at 30 °C before termination and was subjected to agarose gel (0.8%) electrophoresis. pBR322 was linearized by a restriction enzyme Hind III (3 h incubation at 37 °C). DNA on the gel was visualized by ethidium bromide staining. For the representative image data, the experiments were repeated at least twice with similar results. Original images are provided as additional Supplementary Figures.

SUPPLEMENTARY TABLES

Supplementary Table 1.

The sequences of oligos and primers used in this study

EGR1 template and cloning	
EGR1 –423 Biotin-Forward	5Biotin-GG AGC AGG AAG GAT CCC CCG CCG GAA CAA CCC
EGR1 +332-Reverse	CCG AAC GGG TCA GAG ATC TGC AGC GGG GAC ATC AGC
EGR1-ELK1^{mut}-Forward	GGA GTG GCC CGA TAT GGC CCG GCC TAA ATT AAC TCT GGG AGG AGG GAA GAA GGC GG
EGR1-ELK1^{mut}-Reverse	CCG CCT TCT TCC CTC CTC CCA GAG TTA ATT TAG GCC GGG CCA TAT CGG GCC ACT CC
ELK1 Cloning-Forward	CGC CAT ATG ATG GAC CCA TCT GTG ACG CTG TGG CAG TTT CTG CTG C
ELK1 Cloning-Reverse	CGA TCT CGA GTT AAT GAT GAT GAT GAT GGT GTG GCT TCT GGG GCC CTG GGG AGA GC
ERK2 GA Insertion-Forward	GCG GCG GCG GCG GGT GCA GGC CCG GAG ATG GTC
ERK2 GA Insertion-Reverse	GAC CAT CTC CGG GCC TGC ACC CGC CGC CGC CGC
ERK2 L46V QC-Forward	GGT TTG TTC TGC TTA TGA TAA TGT CAA CAA AGT TCG AGT TGC
ERK2 L46V QC-Reverse	GCA ACT CGA ACT TTG TTG ACA TTA TCA TAA GCA GAA CAA ACC
qRT-PCR	
EGR1-Forward	CTT CAA CCC TCA GGC GGA CA
EGR1-Reverse	GGA AAA GCG GCC AGT ATA GGT
FOS-Forward	CAC AGA CCC AGG CCT GGC TCA ACA TGC TAC
FOS-Reverse	CAC CAG GCT GTG GGC CTC AAG GAC TTG AAA GC
HSP70-Forward	ATG TCG GTG GTG GGC ATA GA
HSP70-Reverse	CAC AGC GAC GTA GCA GCT CT
ACTIN-Forward	GCC GAC AGG ATG CAG AAG GAG ATC A
ACTIN-Reverse	AAG CAT TTG CGG TGG ACG ATG GA
EMSA & CryoEM	
EGR1 –133 to –71 (EGR1 #3-1) duplex 1	GTC GTG ACG TAC ATG GCC ATA TAT GGG AAG CAG GAA GCC CTA ATA TGG CAG GAC CGG CCG GGA
EGR1 –133 to –71 (EGR1 #3-1) duplex 2	TCC CGG CCG GTC CTG CCA TAT TAG GGC TTC CTG CTT CCC ATA TAT GGC CAT GTA CGT CAC GAC
EGR1 –70 to –21 (EGR1 #3-2) duplex 1	CCC GGA TCC GCC TCT ATT TGA AGG GTC TGG AAC GGC ACG GGT CCG CCT CC
EGR1 –70 to –21 (EGR1 #3-2) duplex 2	GGA GGC GGA CCC GTG CCG TTC CAG ACC CTT CAA ATA GAG GCG GAT CCG GG
EGR1 –20 to +30 (EGR1 #3-3) duplex 1	ATG GCG GCG GCG GCT CCC CAA GTT CTG CGC GCT GGG ATC TCT CGC GAC TC
EGR1 –20 to +30 (EGR1 #3-3) duplex 2	GAG TCG CGA GAG ATC CCA GCG CGC AGA ACT TGG GGA GCC GCC GCC GCC AT

EGR1 +31 to +70 (EGR1 #3-4) duplex 1	GGG CCG GTC CTG CGG CGG CGG AAG CTG GCT GCG GCG GCG G
EGR1 +31 to +70 (EGR1 #3-4) duplex 2	CCG CCG CCG CAG CCA GCT TCC GCC GCC GCA GGA CCG GCC C
EGR1 TSS ssDNA	CCT GCT TCC CAT ATA TGG CCA TGT ACG
ChIP-qPCR	
EGR1 TSS-Forward	CCT GCT TCC CAT ATA TGG CCA TGT ACG
EGR1 TSS-Reverse	CCT GCG GCG GCG GAA GCT GGC TGC
FOS TSS-Forward	GAC CGT GCT CCT ACC CAG CTC TGC TCC ACA GCG CCC
FOS TSS-Reverse	GAG TGG TAG TAA GAG AGG CTA TCC CCG GCC
***In vitro* transcription**	
EGR1 +1 Forward	GCG CAG AAC TTG GGG AGC CGC CGC CGC C
EGR1 +332-Reverse	CCG AAC GGG TCA GAG ATC TGC AGC GGG GAC ATC AGC

Supplementary Table 2.

Cryo-EM information

Data collection and processing	
Magnification	105000
Voltage (kV)	300
Electron exposure (e−/A2)	49
Defocus range (um)	1.2-2.5
Pixel size (Å)	0.83
Symmetry imposed	C2
Initial particle images (no.)	238615
Final particle images (no.)	26067
Map resolution (Å)	3.9 (FSC = 0.143), 4.7 (FSC = 0.5)
Refinement	
Initial model used	alphafold2 (UniProt Q02880)
Map sharpening B factor ($Å^2$)	-102.69
Model composition	
Non-hydrogen atom	13036
Protein residues	1460
DNA residues	56
Ligands	2 EVP, 2 MG
B factors ($Å^2$)	
Protein residues	164.16
DNA residues	181.07
Ligands	169.93
R.m.s. deviations	
Bond length (Å)	0.003
Bond angles (°)	0.576
Validation	
MolProbity score	1.87
Clashscore	13.05
Poor rotames (%)	0
Ramachandran plot	
Favored (%)	96.35
Allowed (%)	3.65
Disallowed (%)	0

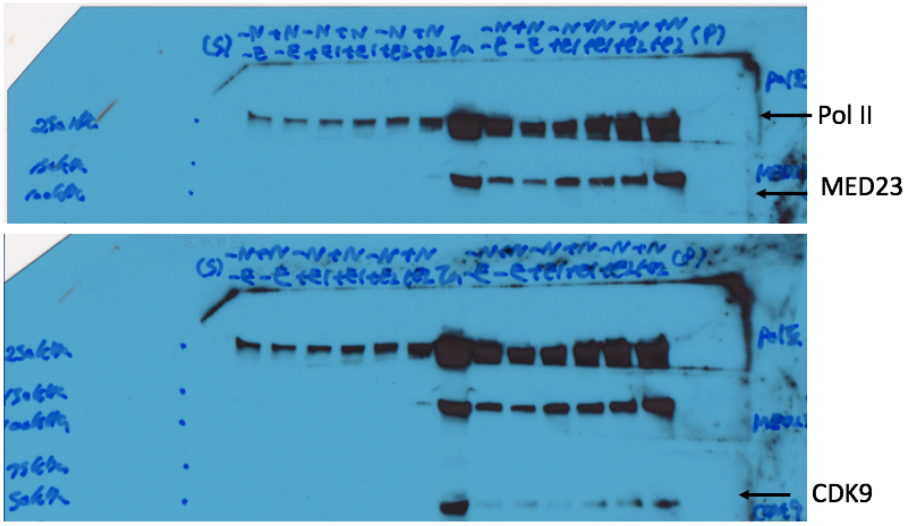
Supplementary Table 3.

Cryo-EM data collection, refinement and validation statistics

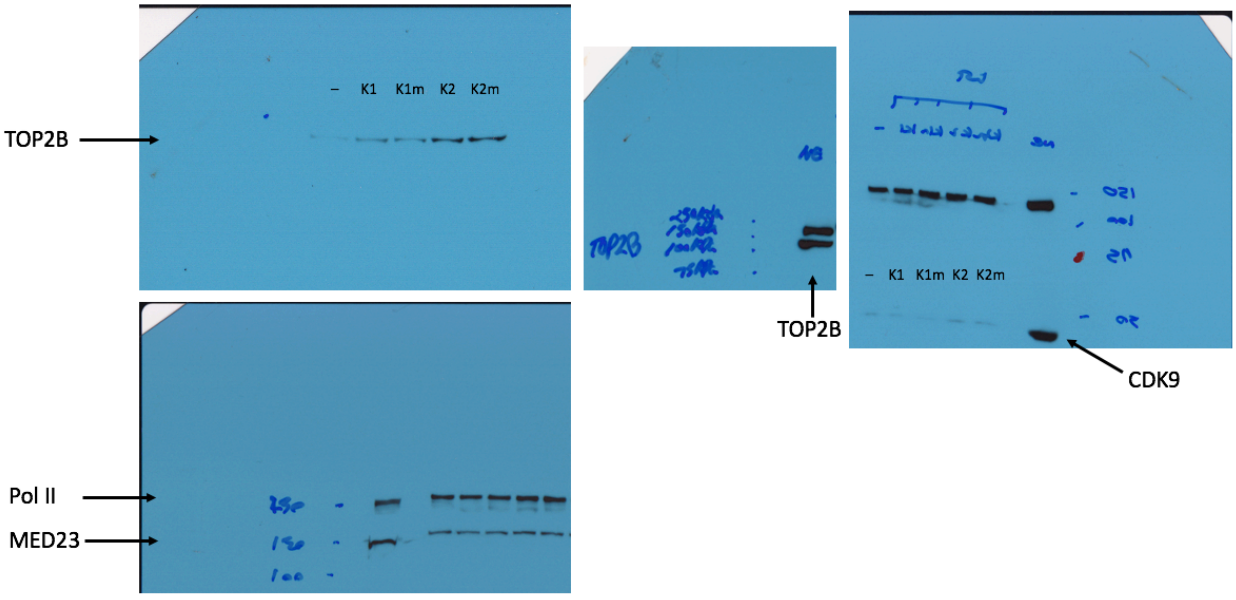
	Top2B-etoposide (EMDB-34022) (PDB 7YQ8)
Data collection and processing	
Magnification	105,000
Voltage (kV)	300
Electron exposure (e–/Å^2)	49
Defocus range (μm)	−1.2 to −2.5
Pixel size (Å)	0.83
Symmetry imposed	C2
Initial particle images (no.)	238,615
Final particle images (no.)	26,067
Map resolution (Å)	3.9 (FSC = 0.143), 4.7 (FSC = 0.5)
FSC threshold	
Map resolution range (Å)	3.4 to 6.8
Refinement	
Initial model used (PDB code)	AlphaFold2 (UniProt Q02880)
Model resolution (Å)	4.0 (FSC=0.5)
FSC threshold	
Map sharpening *B* factor (Å^2)	−102.69
Model composition	
Non-hydrogen atoms	13,036
Protein residues	1,460
Ligands	56
B factors (Å^2)	
Protein	164.16
DNA	181.07
Ligand	169.93
R.m.s. deviations	
Bond lengths (Å)	0.003
Bond angles (°)	0.576
Validation	
MolProbity score	1.87
Clashscore	13.05
Poor rotamers (%)	0
Ramachandran plot	
Favored (%)	96.35
Allowed (%)	3.65
Disallowed (%)	0

Additional Supplementary Figures

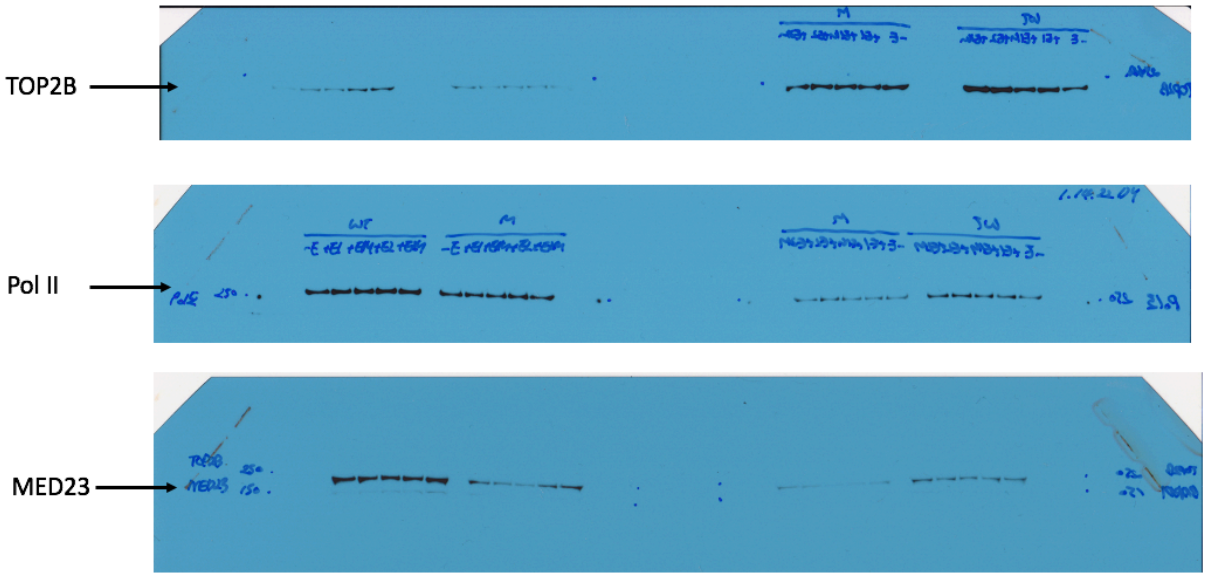
Supplementary Figure 1A: Raw data.



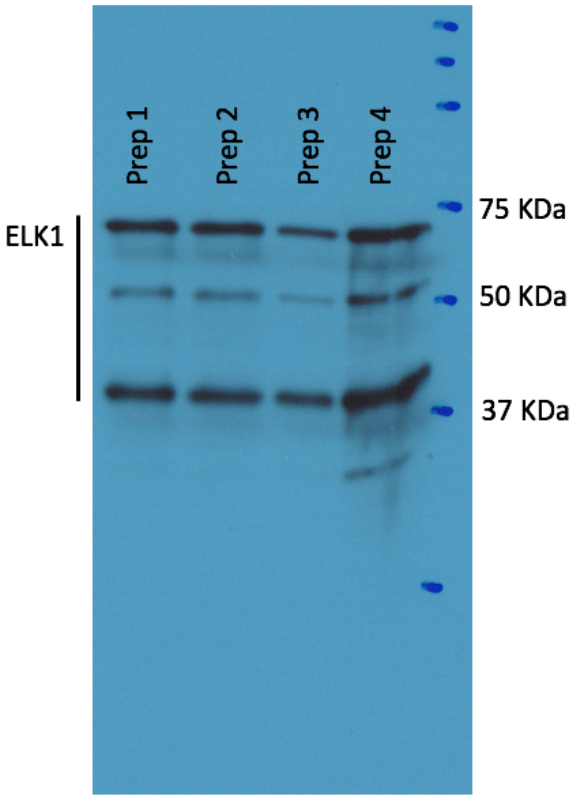
Supplementary Figure 1B: Raw data.



Supplementary Figure 1C: Raw data.



Supplementary Figure 2A: Raw data.

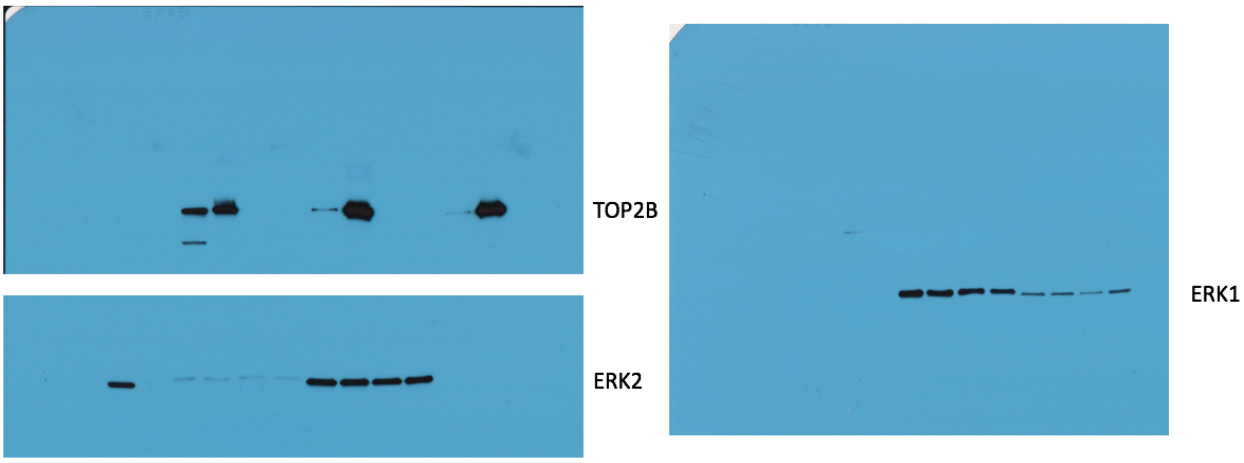


Prep 3 was shown in Fig. S2A and used in this study

Supplementary Figure 2B: Raw data.

TOP2B phosphorylated by ERK1 and ERK2

Supplementary Figure 7A: Raw data.



Supplementary Figure 7B: Raw data.