Expanded View Figures

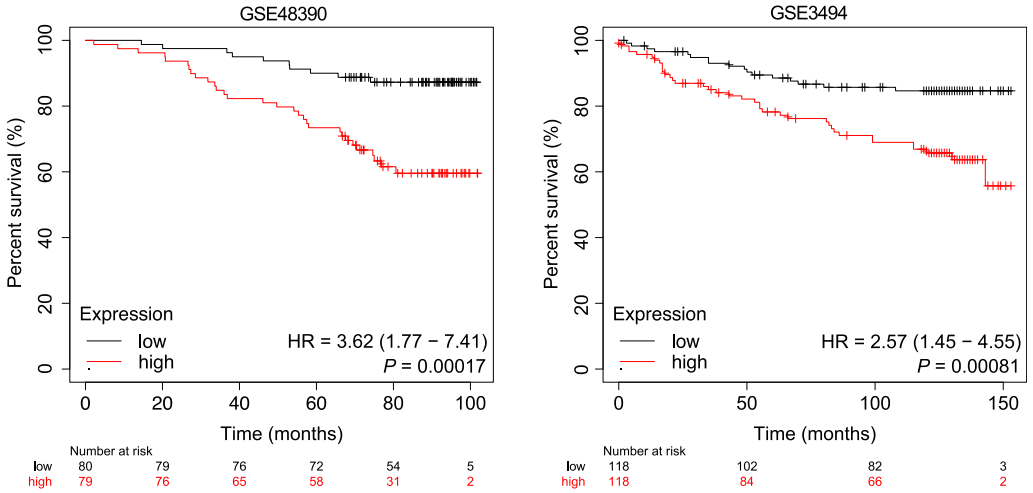

Figure EV1. mRNA levels of TOP2A positively correlate with overall survival in human breast cancer tissues.

Datasets used for the survival analysis were obtained from the indicated GEO datasets (GSE48390 and GSE3494). Patients with TOP2A mRNA expression greater than the median are indicated by the red line, and patients with TOP2A mRNA expression below the median are indicated by the black line. HR, hazard ratio. Statistical analysis was performed by the log-rank test. The P-values are indicated (n = 159 biological replicates for GSE48390, n = 236 biological replicates for GSE3494).

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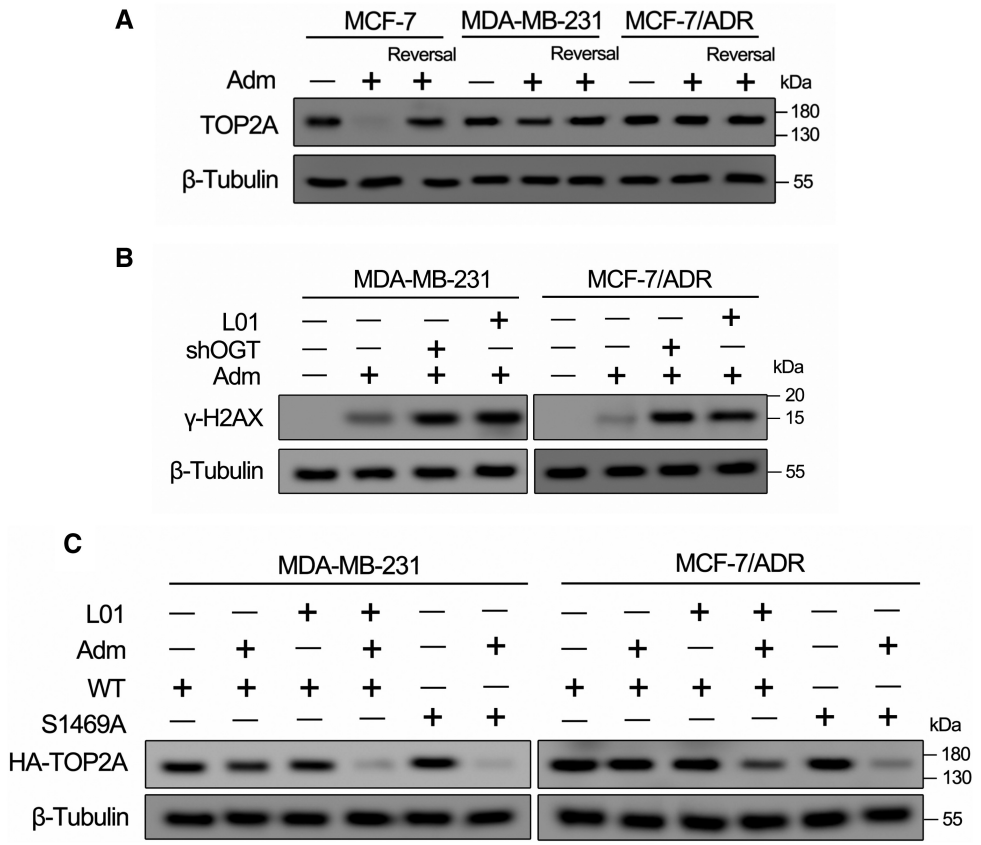

Figure EV2. Relationship between TOP2Acc and TOP2A *O*-GlcNAcylation-related Adm resistance.

A The amount of TOP2Acc in Adm-treated breast cells was measured by a band depletion assay. Indicated cells were treated with Adm (1 μM for MCF-7 cells, 5 μM for MDA-MB-231 and MCF-7/ADR cells) for 2 h. Cells were lysed either immediately or after reversal of the TOP2Acc (Reversal). Cell lysates were analyzed by Western blot.

B MDA-MB-231 or MCF-7/ADR cells were transfected with OGT shRNA (shOGT) or treated with 50 μM L01 for 48 h. The cells were further cultured in the presence or absence of Adm (5 μM) for 2 h. Cell lysates were analyzed by Western blotting using anti-γ-H2AX antibody.

C Band depletion assays were performed in MDA-MB-231 and MCF-7/ADR cells which stable transfected with HA-tagged TOP2A-WT or TOP2A-S1469A (in the presence or absence of 50 μM L01). Breast cancer cells were treated with Adm (5 μM) for 2 h. Cell lysates were analyzed by Western blot.

Source data are available online for this figure.

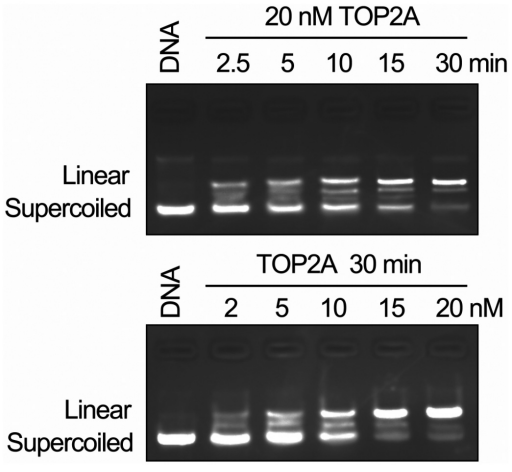

Figure EV3. DNA cleavage assays were performed using the endogenous TOP2A purified from MCF-7/ADR cells.

Supercoiled pBR322 plasmid DNA was used as the TOP2A substrate. TOP2A could retain DNA cleavage activity *in vitro* and catalyzed supercoiled plasmid DNA relaxation in a concentration- and time-dependent manner. The enzyme concentration and reaction time were indicated.

Source data are available online for this figure.

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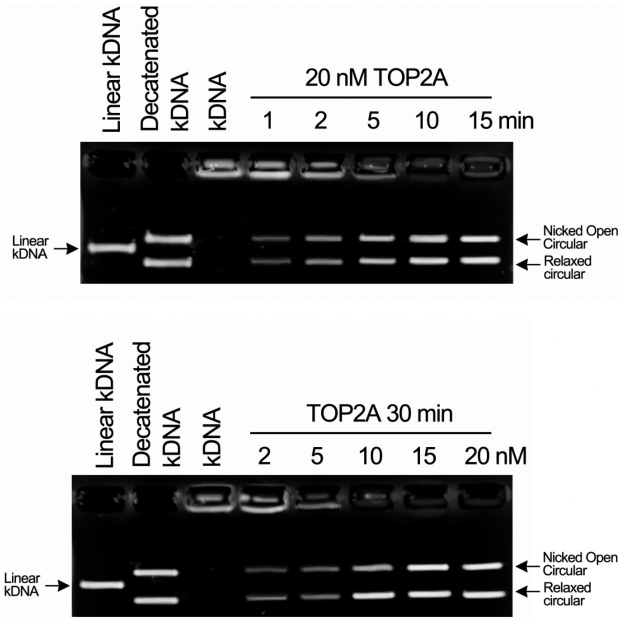

Figure EV4. kDNA decatenation assays were measured using the endogenous TOP2A purified from MCF-7/ADR cells.

kDNA was used as the TOP2A substrate. TOP2A could retain catalytic activity *in vitro* and catalyzed kDNA decatenation in a concentration- and time-dependent manner. Linear kDNA was used as the negative control, and decatenated kDNA was used as the positive control. The enzyme concentration and reaction time were indicated.

Source data are available online for this figure.

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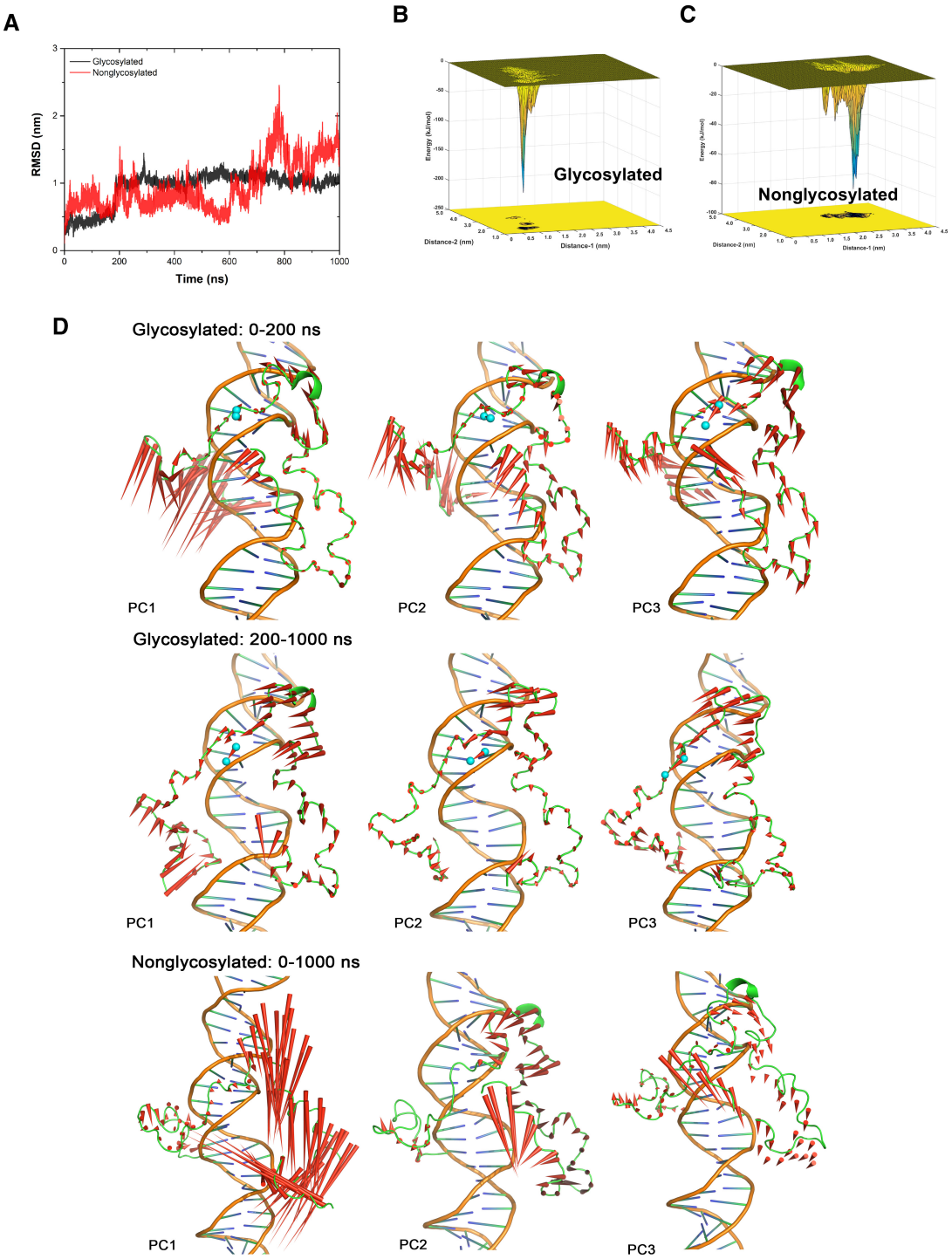

Figure EV5.

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Figure EV5. Molecular simulation of *O*-GlcNAcylated TOP2A CTD with DNA.

A The curves of root-mean-square deviation of CTD Cα atoms along with the time traces from the trajectories of glycosylated and nonglycosylated CTD-DNA systems. The fluctuation of glycosylated CTD-DNA system's RMSD was smaller than nonglycosylated system and became steady around 1 nm after 200 ns MD simulation.

B, C The conformational energy surface of glycosylated (B) and nonglycosylated CTD-DNA systems (C). The energy surface was computed as a function of Distance-1 (center of mass distance between S1469 and 1487-TSK-1489, to indicate the distance between GlcNAc group and adjacent residues) in nm and Distance-2 (center of mass distance between 1441-TKR-1443 and 1510-AKS-1512, to indicate the distance between two terminals of CTD) in nm against the overall 1 μs trajectory of each system. Isosurfaces were shown every 1 kJ/mol. There are several small ensemble states and one dominate ensemble state in the energy surface of glycosylated CTD-DNA system that the deepest energy minimum corresponds to Distance-1 about 0.8 nm, Distance-2 about 1.5 nm and energy about −225 kJ/mol. Three consecutive energy minimums in the energy surface of nonglycosylated CTD-DNA system, as shown in Fig 4G, correspond to Distance-1 from 1.5 to 2.5 nm, and Distance-2 around 3.3 nm with energy worse than −90 kJ/mol. This result indicated that glycosylated S1469 enables a tighter conformation ensemble and significantly stabilizes the association between CTD and DNA. Distance-1: center of mass distance between S1469 and 1487-TSK-1489. Distance-2: center of mass distance between 1441-TKR-1443 and 1510-AKS-1512.

D The difference in protein motion between the first 200 ns and the rest 800 ns simulation of glycosylated CTD-DNA system was obvious from the PCA analysis. The protein is quite dynamic, especially the N-terminal of CTD in the first 200 ns simulation. The interaction network around the glycosylation S1469 (the GlcNAc group shown as cyan sphere) gave the N-terminal loop (residue 1,435–1,450) enough time to close and enter the major groove of DNA that polar and charged sidechain residues (K1439, 1441-TKRD-1444) in this loop could make strong interactions with DNA. The protein dynamics were more stable in the rest 800 ns trajectory. In contrast, the movement in nonglycosylated system was enormous and inhomogeneous in different regions. The residues around S1469 performed strong tendency to move away from DNA, which was unbeneficial for the stable interaction.

Source data are available online for this figure.

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