Supplemental Fig. S1. Quality Control (QC) Analysis at Key Steps in High Throughput Sequencing of TOP2A Cleavage Complexes.

(A) QC analysis of DNA fragmentation in representative input K562 cell lysate samples after sonication (Samples 1-A, 1-B, 1-C; Supplemental Table S1). K562 cells (10^7 per sample) in 1 mL RIPA buffer were sheared using a Diagenode Bioruptor (21 cycles of 30 sec on/ 2 min off at full power sonication). The gel image represents 0.2% of each sonicated lysate after DNA purification. The samples were size-separated on a 1.5% agarose gel in 1X TAE buffer. Note fragment size distributions in sheared samples ranging from 100 and 1500 bp. These results indicate the presence of substantial starting DNA fragments in the desired 200-350 bp range to generate the libraries.

(B) Proof of specificity of TOP2A immunocapture. The specificity of the anti-TOP2A antibody used for immunocapture (Kamiya PC-123, rabbit polyclonal) is confirmed by Western blot analysis of a 10 μL aliquot of the sonicated input (1% of total lysate), 10 μL aliquots representing ~1% of each of the three non-bound fractions, and 50% of the eluted TOP2A using an anti-TO2A antibody of a different species (Santa Cruz Biotechnology SC-56803, mouse monoclonal). The samples are also analyzed using an unrelated negative control antibody (anti-BECN1; Santa Cruz Biotechnology SC-48341, mouse monoclonal) to exclude non-specific protein binding on the beads. Sample 3-A (Supplemental Table S1) is shown as representative. The data show that TOP2A and BECN1 proteins are both present in the sonicated input sample and the non-bound fractions, and that only TOP2A but not BECN1 is present in the eluate, providing proof of specificity of the immunocapture and excluding non-specific protein binding on the beads.

(C) QC analysis of DNA fragment size distribution in representative libraries used for sequencing (Samples 1-A, 1-B, 1-C; Supplemental Table S1). A 1 μL aliquot of each 15 μL library was profiled on an Agilent Bioanalyzer DNA 1000 Chip. Left, analyzer traces; right, virtual gel representations of same libraries. The data show that the DNA sizes including adapters in each of the libraries is within the desired 225-375 bp range for sequencing.

(A-C) Abbreviations: VP16, etoposide; pBQ, p-benzoquinone; NB1, non-bound fraction 1; NB2, sequential non-bound fraction 2; NB3, sequential non-bound fraction 3.