Supplemental Fig. S4. Validation of TOP2A Cleavage Sites Detected by Sequencing in Areas of KMT2A Intron 10 – Exon 11 using TOP2A in vitro Cleavage Assays.

(Left) Cleavage sites found in sense strands within the KMT2A bcr by high throughput sequencing of libraries prepared from DNA released from immunocaptured TOP2A cleavage complexes. (Right) TOP2A cleavage sites found in sense strands of same areas by in vitro cleavage assays of naked DNA substrates. Locations of sequences resolved in the in vitro cleavage assays relative to the KMT2A bcr are shown in schematic in Fig. 1D (Top). Region is of interest because of intron 10 treatment-related and infant leukemia translocation breakpoint hotspots. Arrows at peaks in sense strand of the sequence detected in the cell-based assays (Left) indicate +1 positions of cleavage sites that also were detected in the corresponding in vitro cleavage assay autoradiographs (Right, dashes). Coordinates, NC_000011.10 (GRCh38/hg38). Bracket (Right) indicates inset of autoradiograph shown in Fig. 1D (Bottom Right). (Left) Colors, different treatments; symbols, different replicates. The data demonstrate overlap with cleavage sites from the independent in vitro assay, confirming that bona fide TOP2A cleavage sites were detected by the sequencing. See also Fig. 1D and Supplemental Fig. S3.