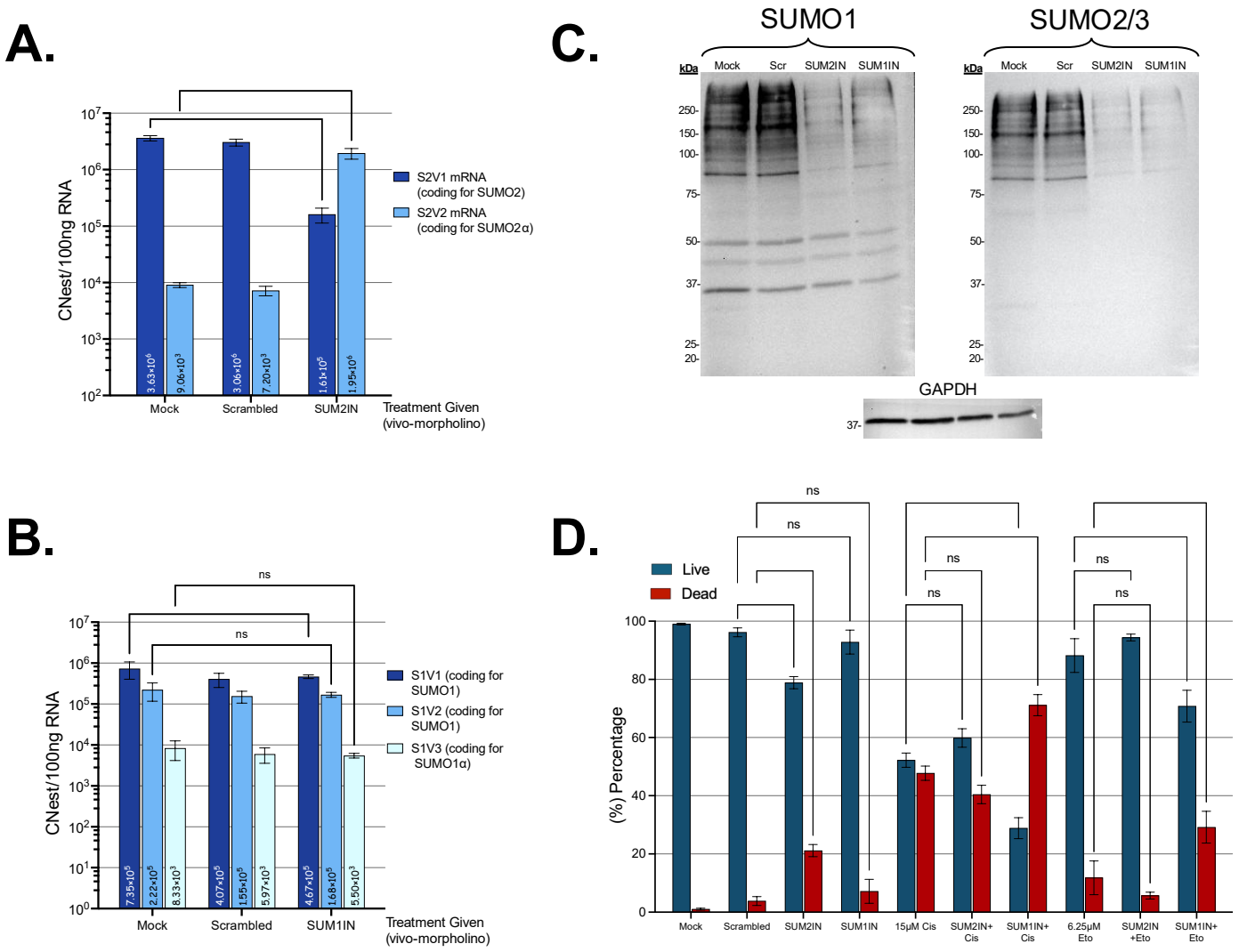
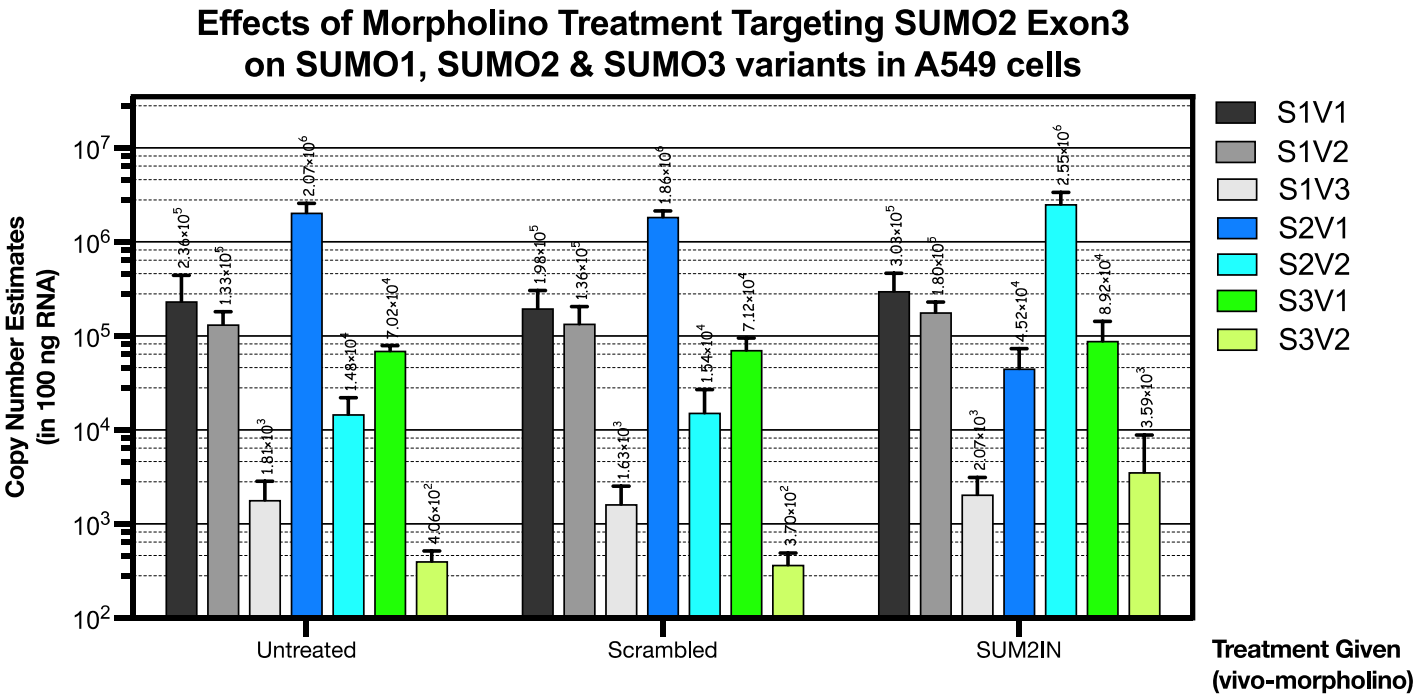
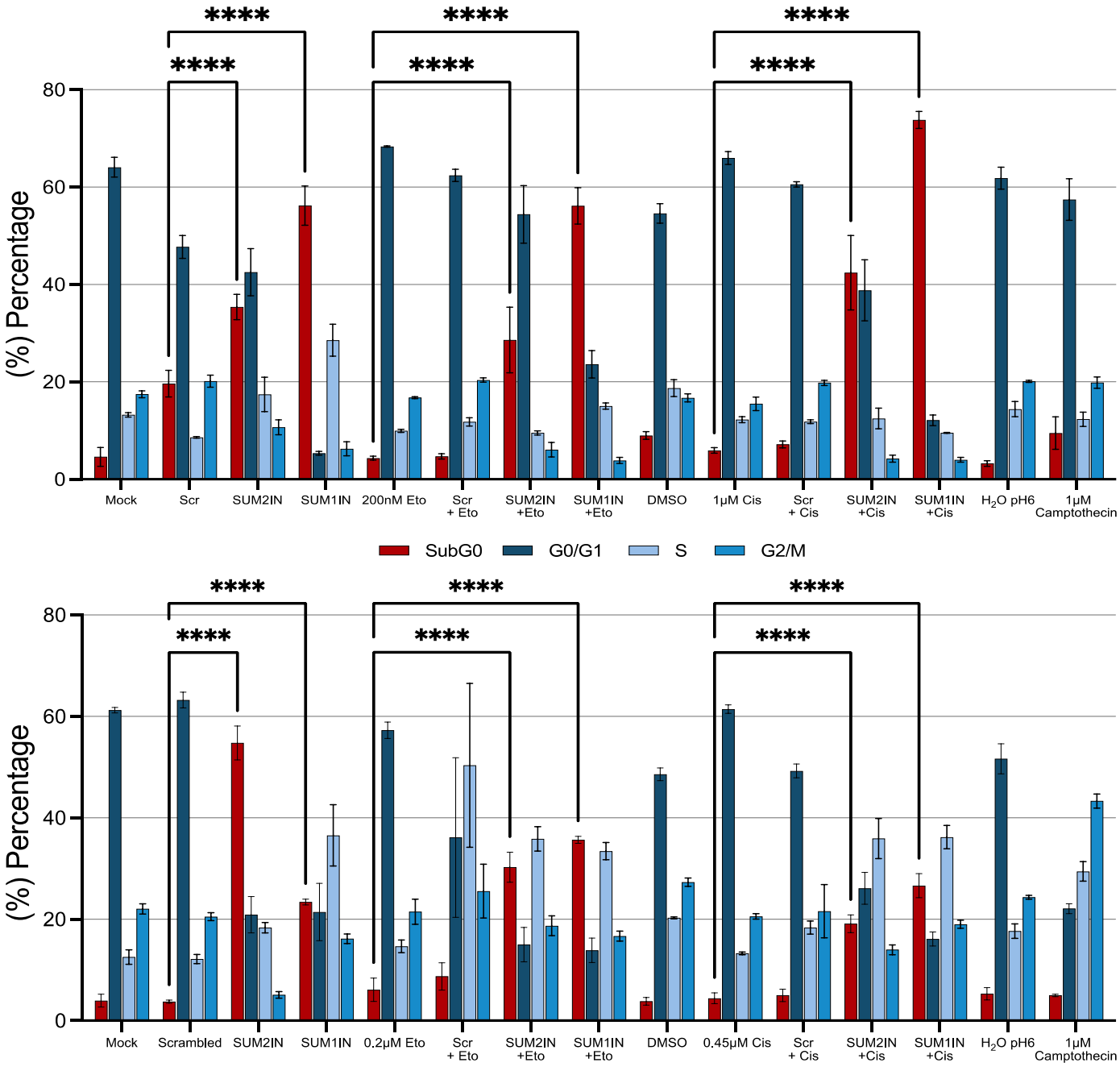


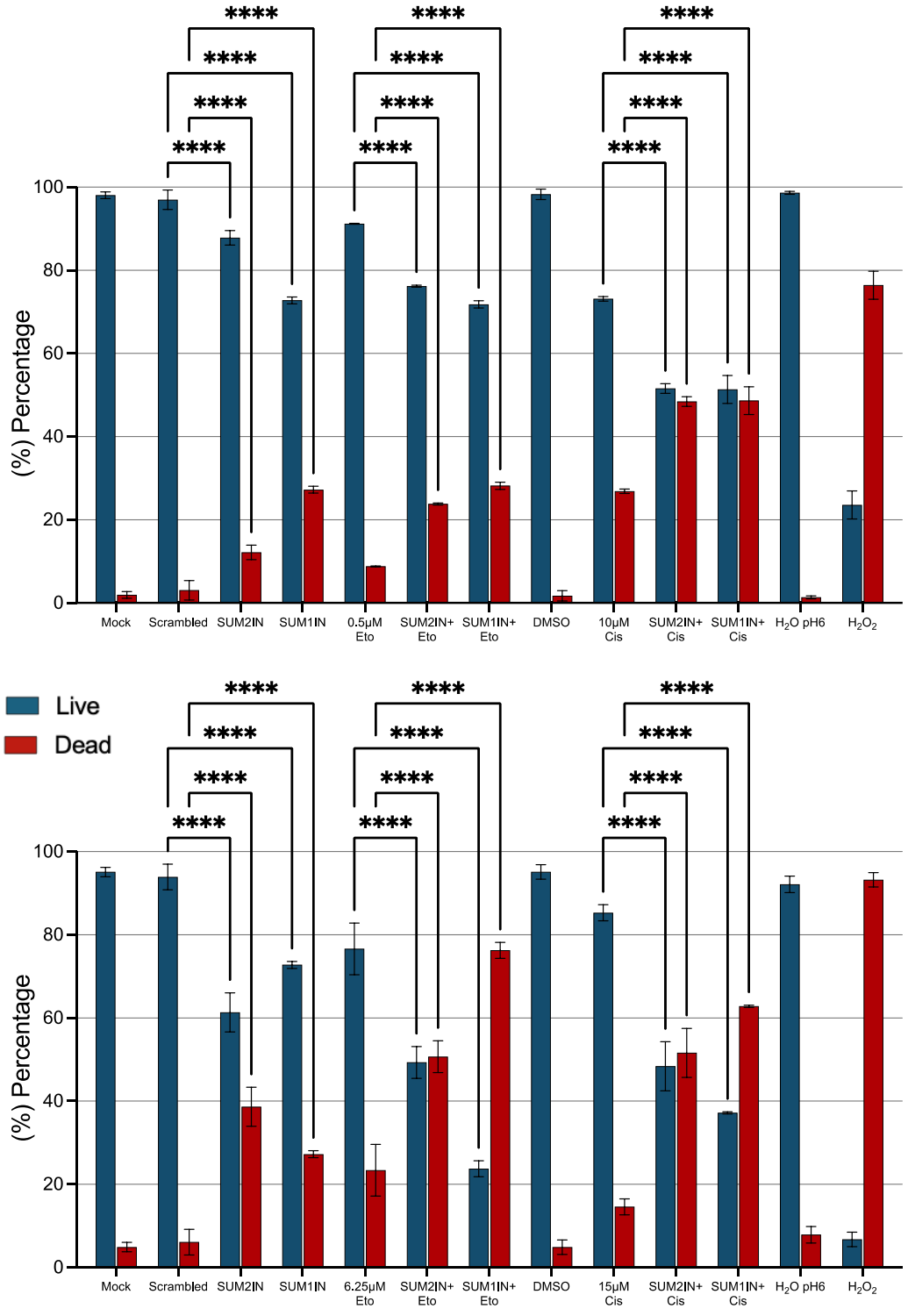
Supplemenatry Figure 1. Effects of SUM2IN and SUM1IN treatment in HEL299 cells measured by SUMO2 and SUMO1 transcript abundance, protein expression, and cytotoxicity. (A–B) At 24 hours post-treatment, cells were collected and RNA was purified. Transcript levels for each variant were quantified by RT-qPCR. SUM2IN specifically alters the splicing of the SUMO2 mRNA, leading to an increase in S2V2 and a decrease in S2V1. In contrast, SUM1IN does not affect SUMO1 mRNA splicing, as S1V3 levels remained unchanged. **(C)** At 48 hours post-treatment (Mock, scrambled control (Scr), SUM2IN, or SUM1IN), cells were collected and analyzed by immunoblotting. Membranes were sequentially probed with antibodies against SUMO1, SUMO2/3, and GAPDH. Both SUM2IN and SUM1IN reduced the abundance of SUMO1 and SUMO2/3 SUMOylation, indicating that these treatments may broadly suppress global SUMO conjugation. **(D)** HEL299 cells plated at 4,000 cells/well in 96-well plates were treated with SUM2IN, SUM1IN, cisplatin, and etoposide, alone or in combination, as indicated. At 96 hours post-treatment, cells were stained with Hoechst and Propidium Iodide and analyzed using a 1xPico Bioimager (MolDev). SUM2IN did not enhance the cytotoxic effects of cisplatin or etoposide. Conversely, cytotoxicity induced by cisplatin and etoposide increased when combined with SUM1IN. All data represent the average values obtained from six replicate wells per condition and summarize the means and standard deviations from three independent experiments. Each data point represents mean ± SD.



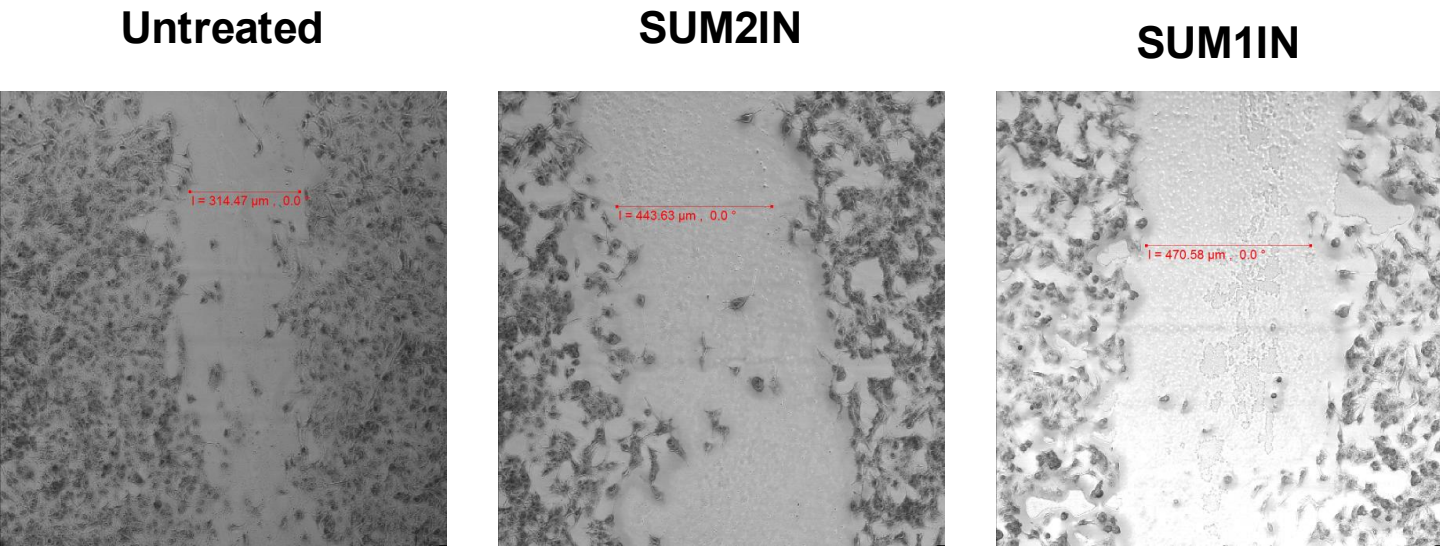
Supplementary Figure 2. SUM2IN trigger a 172x increase in S2V2, a 45x decrease in S2V1, and a 10x increase in S3V3. A549 cells were treated with the indicated morpholinos. At 24h post-treatment, cells were collected, RNA was purified, and the copy number estimates for the variant transcripts listed were calculated by RT-qPCR as previously described

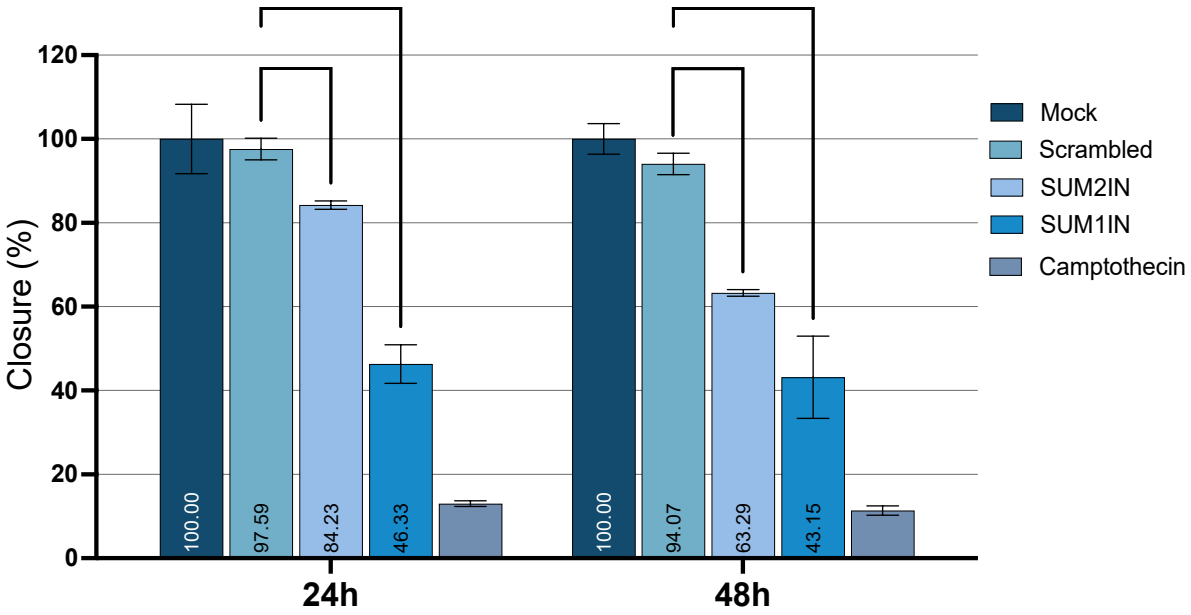


Supplementary Figure 3. Increasing the expression of SUMO2 alpha and SUMO1 alpha results in cell cycle arrest on S phase and cell death. A549 and HCC827 were treated as indicated, stained with NIM-DAPI, and analyzed using a flow cytometer at 48 hours post-treatment. Camptothecin, DMSO, H_2O pH6, and scrambled morpholinos alone or in combination with either cisplatin or etoposide, were used as controls. SUM2IN, SUM1IN, etoposide, and cisplatin were used at 200nM, 1μM, and 4μM, respectively. Each data point represents the mean ± SD of three independent experiments.



Supplementary Figure 4. **Treatment with SUM2IN and SUM1IN increases the killing effect mediated by Cisplatin and Etoposide as measured by a Hoechst and Propidium Iodide staining method.** A549 and HCC827 cells plated at 4,000 cells/well in 96-well plate were treated with SUM2IN, SUM1IN, cisplatin, and etoposide, alone or in combination as indicated; including all the controls used for this specific test. 96h post-treatment, the cells were stained with Hoechst and Propidium Iodide, and read using a 1xPico Bioimager MolDev. All data represent the average values obtained from six different wells per concentration pear each, and summarizes the averages and standard deviations from 3 independent experiments. Each data point represents the mean ± SD of three independent experiments.





Supplementary Figure 5. SUMO2α overexpression via SUM2IN and SUM1IN reduces migration and invasion of A549 cells in vitro. (A) A549 cells were treated with 4 µM SUM1IN, 4 µM SUM2IN, Camptothecin (10 µM), or a Scrambled Morpholino control, and migration was assessed at 24 and 48 hours post-treatment. **(B)** Bar graph quantifying the percentage of wound closure in A549 cells following treatment with SUM2IN, SUM1IN, Camptothecin (10 µM), or Scrambled Morpholino. Data represent means ± SD from three independent experiments. It is noted that SUM2IN inhibits cell migration by 20% and SUM1IN by 60%.