Figure S1. Targeting epithelial ovarian cancer cell lines with carboplatin and birinapant *in vitro*. (A) Genetic alterations of cIAP1, cIAP2 and XIAP in ovarian cancer and other cancers treated with platinum-based chemotherapy. Data extracted from TCGA, PanCancer datasets through cBioPortal (http://www.cbioportal.org/). Gene amplification (shown in pink) was the most common alteration observed across all cancer types. (B) Three western blot analyses measuring the endogenous levels of cIAP1, cIAP2, and XIAP in 7 epithelial ovarian cancer cell lines. Recombinant (rec.) cIAP1, cIAP2, and XIAP protein were used as positive controls. GAPDH was used as loading control in each experiment. cIAP1 and XIAP bands were detected at approximately 62 and 53 kDa respectively. cIAP2 protein was not detected in these cell lines. (C) Viability plots for 6 epithelial ovarian cancer cell lines treated with carboplatin (CP) (0-50 μM) and birinapant (0-50 nM) in the 3D organoid bioassay. Data represents an average of 3 independent experiments plated by two investigators. Data for OVCAR8 cell line is shown in Fig. 2.

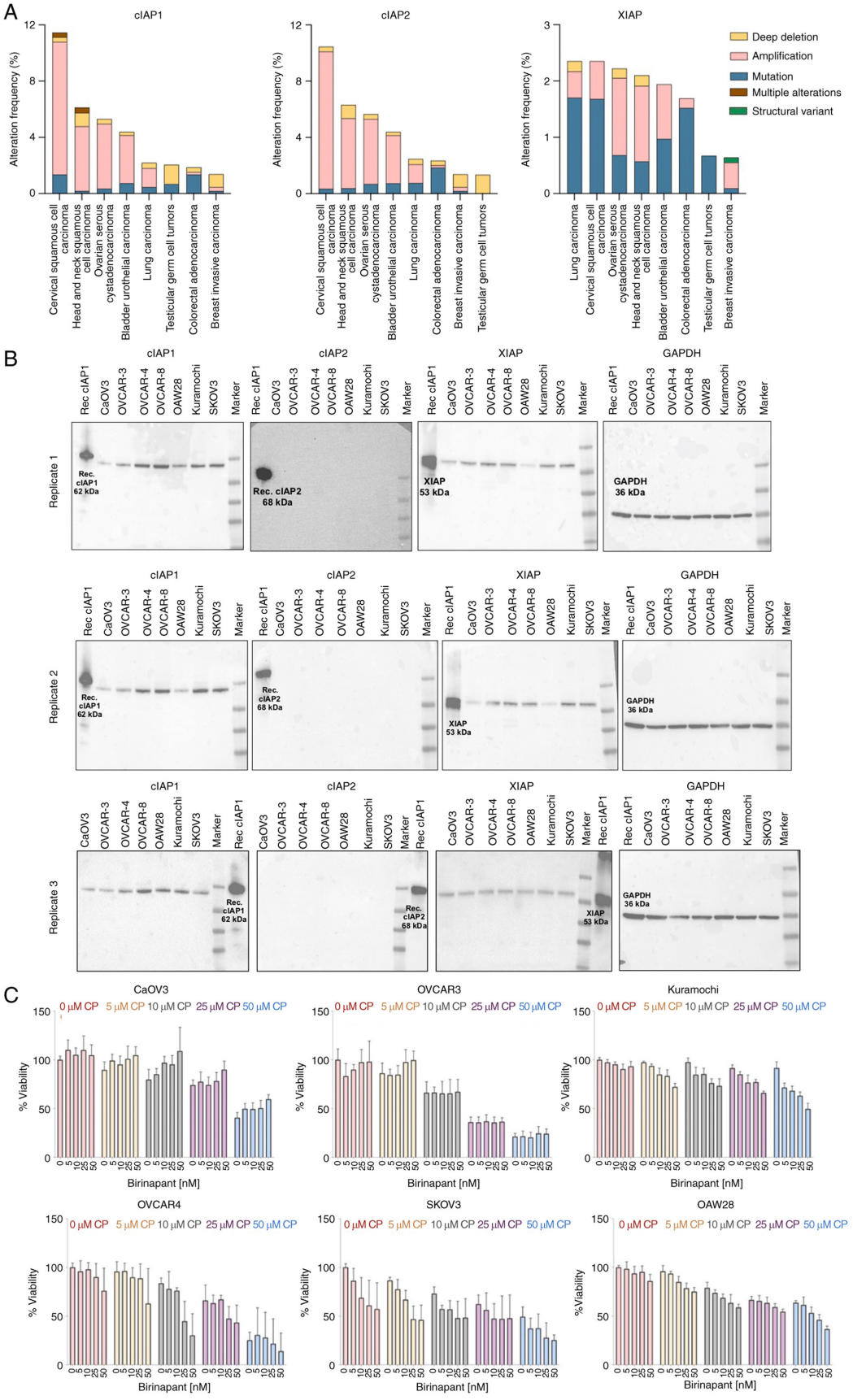


Figure S2. Birinapant degrades cIAP1 in OVCAR8 and SKOV3 cells. (A) Experimental schema. Cells were treated with carboplatin (CP), birinapant (BP), or the combination (CP+BP) for 24 h. After drug treatment, cells were harvested, lysed, and protein expression for birinapant targets were assessed by western blot analysis. (B and C) Three independent western blot analyses assessing the levels of cIAP1, cIAP2 and XIAP in OVCAR8 (B) and SKOV3 (C) cells after drug treatment. Birinapant-mediated degradation of cIAP1 was observed in both cell lines. No change in XIAP expression levels was observed. Both cell lines were treated with drugs at a concentration corresponding to the half maximal inhibitory concentration (IC_{50}) values of each drug [For SKOV3, (CP)=40 μm, (BP)=30 nM; OVCAR8: (CP)=100 μM, (BP)=100 nM]. Recombinant (rec.) cIAP1, cIAP2, and XIAP protein were used as positive controls. GAPDH was used as loading control in each experiment.

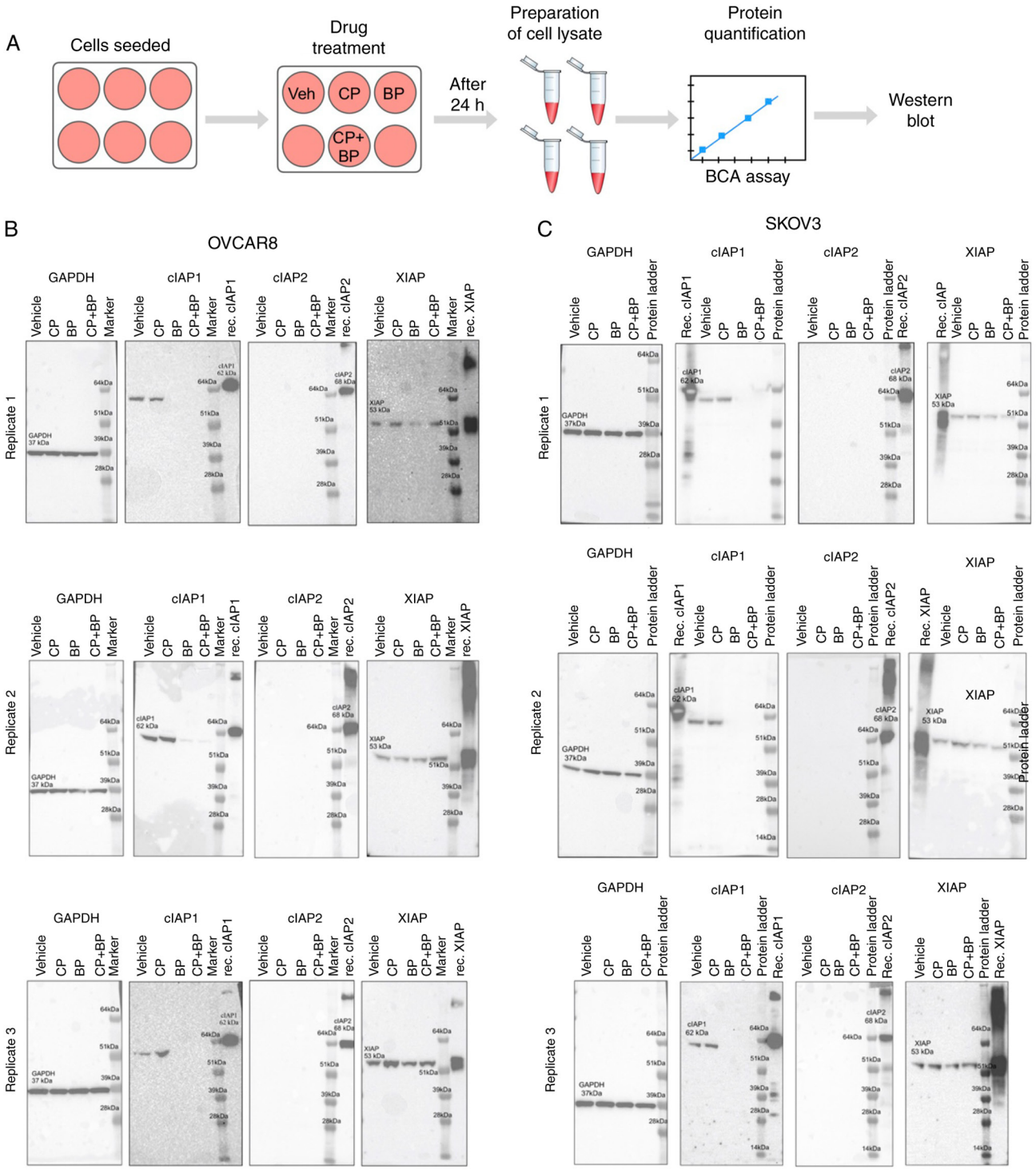
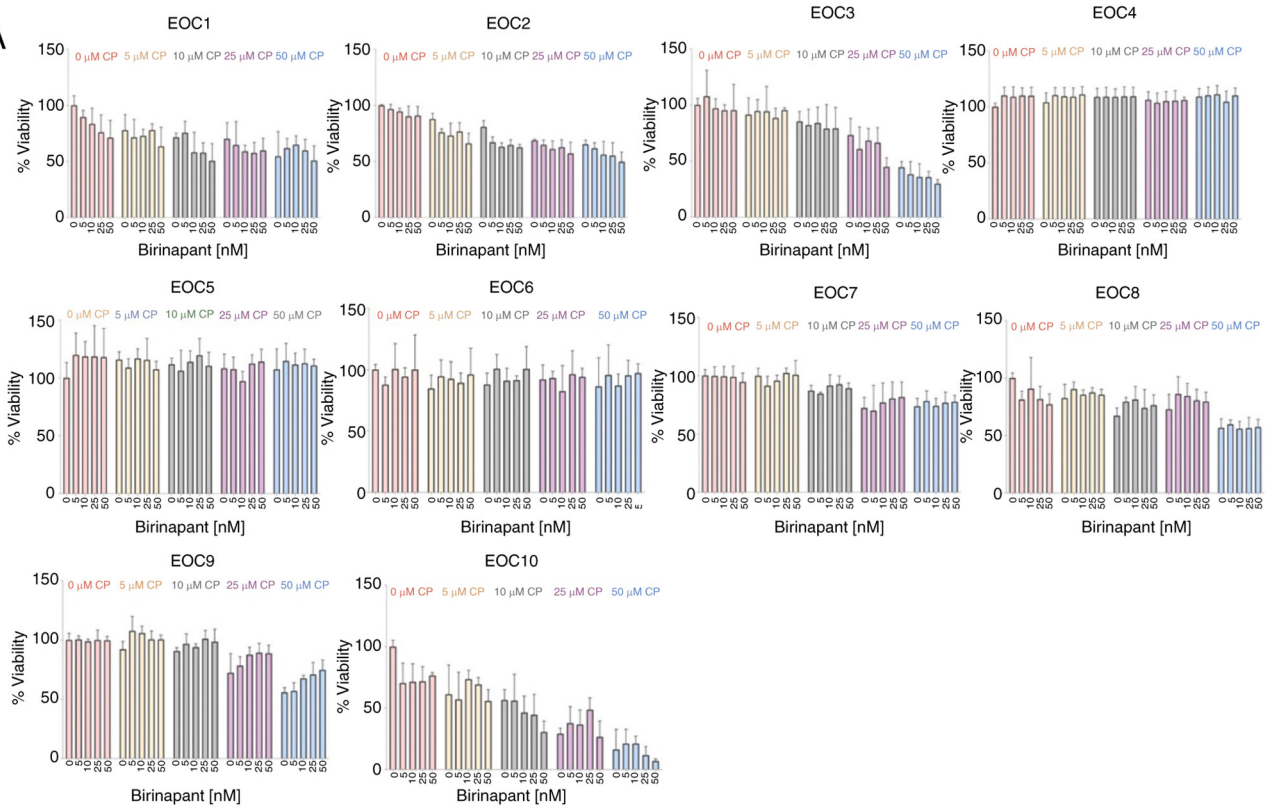


Figure S3. Targeting platinum-resistant primary patient ovarian cancer tumor samples with carboplatin and birinapant co-therapy using the 3D organoid bioassay. (A) Viability plots of 10 patient tumor specimens after treatment with combinations of carboplatin (CP) (0-50 μM) and birinapant (0-50 nM). Data shown represent an average of 2 independent experiments plated by two investigators. (B) Half maximal inhibitory concentration (IC_{50}) values for carboplatin and birinapant for each primary patient tumor sample tested. For determination of IC_{50} values, patient-derived organoids were treated with increasing concentrations of carboplatin (0-250 μM) or birinapant (0-500 nM) in 2 separate 3D organoid bioassays. After 72 h of drug treatment, IC_{50} values were obtained from individual drug-response curves. In cases where the IC_{50} values were not within the concentration range tested, it was determined by extrapolation of the dose-response curves using GraphPad Prism software. OR*, outside the range calculable by GraphPad Prism software.

A



B

Sample ID	Sample site	Carboplatin IC50	Birinapant IC50
EOC1	Ovary	249.1 μM	4.043 μM
EOC2	Pleural effusion	83.25 μM	763.5 nM
EOC3	Ovary	41.4 μM	714.9 nM
EOC4	Ovary	83.16 μM	OR*
EOC5	Pleural effusion	291.8 μM	1.468 μM
EOC6	Omentum	153.10 μM	OR*
EOC7	Pleural effusion	111.4 μM	OR*
EOC8	Ovary	85.59 μM	265.2 nM
EOC9	Ascites	73.18 μM	OR*
EOC10	Ovary	162.50 μM	OR*

Figure S4. Generation of a platinum-resistant PDX model to test the efficacy of carboplatin and birinapant co-therapy. (A) Workflow for establishment of a platinum-resistant PDX model. Pleural effusion tumor cells from a patient diagnosed with recurrent, platinum-resistant high grade serous ovarian cancer were injected into immunocompromised mice and serially passaged *in vivo*. (B) *In vitro* testing of PDX cells with carboplatin (CP) (0-50 μM) and birinapant (0-50 nM) using the 3D organoid bioassay demonstrated drug additivity based on Loewe score. PDX cells also demonstrated high half maximal inhibitory concentration (IC_{50}) values for carboplatin and birinapant. (C) Two technical replicates of western blot analyses measuring endogenous levels of cIAP1, cIAP2, and XIAP in the PDX subcuticular tumors, PDX cells, and primary pleural effusion sample (EOC2). Recombinant cIAP1, cIAP2, and XIAP protein were used as positive controls. Total protein isolated from the OVCAR8 cell line was also used as a positive control. GAPDH was used as loading control in each experiment. (D) Workflow to test the *in vivo* efficacy of birinapant and carboplatin co-therapy in platinum-resistant PDX subcuticular tumor model. $1x10^6$ PDX cells each were injected into the subcuticular space of n=17 female NSG mice. Before treatment, one mouse was euthanized to confirm the established tumor. Once the average tumor volume of all remaining mice reached approximately 125 mm^3, mice were randomized into treatment groups to receive either vehicle, carboplatin, birinapant or the co-therapy for four weeks. During the treatment phase, tumor growth and mouse weight were recorded twice each week. (E) Establishment of PDX subcuticular tumors confirmed by euthanizing one mouse pre-therapy. Formalin-fixed, paraffin-embedded tumor tissue was histologically sectioned and stained with hematoxylin and eosin (H&E) and anti-Pax8 antibody. Pax8 expression confirms malignancy of Mullerian origin. (F) Comparison of tumors resected at the end of therapy showed reduced weight of the birinapant-treated (BP) and co-therapy-treated (CP+BP) tumors (mean ± SEM, n=4 per treatment group, one-way ANOVA: *P=0.0390, **P=0.0042). (G) Representative images of PDX subcuticular tumors harvested from each cohort after drug treatment. Resected tumor fragments were analyzed for expression of Ki-67 (proliferation marker) and Pax8 by IHC staining. (H) Percentage of Ki-67-positive cells were quantified by visually counting the total number of Ki-67 positive-stained tumor cells in each tumor (mean ± SEM, n=4 per treatment group, one-way ANOVA). (I) Number of mitoses were visually counted in 10 random high-power fields (HPF) in H&E stained histologic sections of tumor fragments (mean ± SEM, n=4 per treatment group, one-way ANOVA). A decreasing trend was observed in the birinapant-treated (BP) and co-therapy-treated (CP+BP) tumors. (J) Body weights of the mice in each cohort during drug treatment. No change in body weight was observed.