

Supplementary Figure 1. FACS analysis can be used to reliably isolate CA125 positive versus negative cells from **high grade serous cancers. (a)** Quantitative-PCR measurement of CA125 transcripts confirmed reports that CA125 is highly expressed in Ovcar-3 but not Skov3 cells. Results are mean ± SD, n=3. To validate detection of CA125 antigen, mixed populations of Ovcar-3 and Skov-3 cells were stained with commercially available OC125 antibody, isolated using FACS and cytospun. Appropriate signal was detected by fluorescent microscopy in sorted cell populations. Probing these samples with a second anti-CA125 antibody directed against a different epitope of the antigen confirmed the accuracy of this isolation. **(b)** Human HGSCs were mechanically and enzymatically dissociated to single cells and analyzed by FACS. After elimination of hematopoietic, endothelial and red blood cells using the lineage markers CD31, CD45 and CD235a the proportions of CA125 positive and negative cells were determined. Post-sort purity analysis showed accurate separation of these cellular subpopulations (n=3) confirmed by western blot. Scale bars equal 5 mm for tumor and 100 µm for cells. **(c)** Immunostaining of human HGSCs confirmed presence of CA125 negative cells (arrows). Scale bars equal 5 mm for tumor and 100 µm for cells. **(d)** FACS analysis of CA125 subpopulations co-stained with the epithelial marker EpCAM and Müllerian stromal marker CD10 demonstrates the presence of cells expressing epithelial and/or stromal marker in each fraction. Results are median ± interquartile range [IQR], n=11. **(e)** Analysis of CD44 expression by FACS revealed the presence of CD44 positive cells in both the CA125 negative and CA125 positive HGSC subpopulations. Results are median ± IQR, n=16. **(f)** Analysis of 73 stage IIIC and IV HGSC from the TCGA database demonstrated a trend toward shorter overall survival in patients with lower CA125 mRNA levels in their tumors. Patients with mRNA Z score levels above or below 1 were included in this

Supplementary Figure 2. Live-bank cryopreservation did not alter the growth characteristics of primary human high-grade serous tumor cells. (a) Freshly dissociated, live-banked or live-banked and then sorted primary human HGSC cells gave rise to xenografts in vivo. **(b)** Tumors generated from equal numbers of fresh vs matched live-banked cryopreserved cells demonstrated similar size, serous histology and marker expression profile (n=3). **(c)** Equal numbers of cryopreserved or cryopreserved and lineage depleted FACS sorted serous tumor cells were injected in vivo (n=2). Results demonstrate that serous tumors could be generated from cryopreserved and FACS sorted cells. Scale bars equal 100 um unless noted.

Supplementary Figure 3. The CA125 negative tumor cells efficiently initiate the tumor and undergo multi-lineage differentiation in vivo. (a) Limiting dilution assays confirmed CA125 negative cells were the cells that initiate serous tumors. Gross tumors generated from 5 other independent chemo-naive human HGSCs used in this experiment are shown. Scale bars equal 5 mm. **(b)** In vivo lineage tracing experiments on a second independent specimen confirmed that the CA125 negative HGSC subpopulation could differentiate into both CA125 positive and CA125 negative cells, while progeny of CA125 positive cells were predominantly CA125 positive. Scale bars equal 5 mm for gross tumors and 50 µm for stained cells. **(c)** In the two ascites samples that exhibited growth from both CA125 subpopulations, tumors generated from the CA125 negative HGSC cells expressed CA125 on some but not all cells while tumors generated from CA125 positive cells uniformly expressed CA125. Scale bars equal 50 µm.

Supplementary Figure 4. Human high-grade serous cancers have a higher proportion of CA125 negative cells and increased tumor growth capacity after chemotherapy. (a) Equal numbers of matched enumerated bulk tumor cells (100,000 cells) obtained from the same patient before and after chemotherapy were injected in the subcutaneous space on opposing flanks of an immunocompromised mouse (n=3). **(b)** Matched human HGSC specimens obtained before and after platinum chemotherapy from 3 independent patients demonstrated an increased proportion of CA125 negative cells following chemotherapy. In each case, larger tumors were generated from the bulk tumor cells harvested after chemotherapy. Presence of tumor in all specimens was confirmed histologically and by staining for TP53. Scale bars equal 100 µm.

Supplementary Figure 5. CA125 negative tumor cells isolated from primary human high-grade serous cancers differentiate and form self-renewing tumor organoids in vitro. (a) HGSC single cells plated in a 3-dimensional matrix gave rise to organoids which upon release and dissociation into single cells had sustained self-renewal capacity. **(b)** Organoids resembled the parent tumor based on histology and marker expression profile (n=3). Scale bars equal 100 µm. **(c)** FACS analysis of organoids generated from primary HGSC CA125 subpopulations demonstrated presence of CA125 positive and negative progeny in organoids arising from the CA125 negative fraction. Conversely, the CA125 positive tumor fraction gave rise to predominantly CA125 positive cells (n=3). **(d)** Equal numbers of CA125 negative, CA125 positive and unfractionated tumor cells from primary human chemo-naive HGSCs were plated in the 3D assay. CA125 negative serous tumor cells demonstrated significantly higher organoid forming capacity compared to CA125 positive counterparts (p<0.0001, paired two-sided *t-*test). Hatched bars indicate ascites samples (n=2) while intact bars represent samples from solid tumors (n=8). Matched ascites and solid tumors were available for 2 specimens (red and green bars). Organoid growth capacity was normalized to activity in bulk tumor cells for each sample. Results are mean ± SD, n=3 replicates per sample.

Supplementary Figure 6. Characterization of the low passage, patient derived HGSC cell lines S1-, S3- and S5-GODL. **(a)** Based on FACS analysis, subsets of low-passage patient derived HGSC cell lines (S1-, S3- and S5-GODL) are CA125 negative in comparable proportion to the parent tumor. **(b)** Only the CA125 negative subpopulation of these cell lines efficiently gave rise to tumors (n=3 replicates). Genomic copy number variation analysis revealed conserved amplifications and deletions between the cell lines and parent tumors, while clustering analysis confirms similarity between transcript expression patterns. Elevated expression of cIAP proteins was detected in the CA125 negative subpopulation of all lines by western blot. **(c)** Calculation of the correlation between cell lines and parent tumors indicated that each cell line closely resembled their parent HGSC (r^2 = 0.841 for S1-GODL, 0.996 for S3 GODL and 0.908 for S5-GODL). R^2 calculated using R function of hclust().

and apoptotic cleaved **Supplementary Figure 7. HGSC cell death induced by Birinapant and carboplatin cotherapy is directly mediated by degradation of cIAP. (a)** FACS analysis demonstrated that in S1-GODL, S3-GODL and S5-GODL cell lines, all cells including the CA125 negative population, could be eliminated with birinapant and carboplatin cotreatment in vitro. CA125 negative tumor cells were resistant to Carboplatin alone. Birinapant as a single agent caused only partial cell death. Averaging this data as a plot of cell survival for S1,3&5-GODL cell lines demonstrated that only combined birinapant and carboplatin cotreatment resulted in complete tumor cell kill (n=3 replicates per sample). Birinapant mediated degradation of cIAP induced PARP cleavage is confirmed by western blot in S1,3&5-GODL treated

cells. **(b)** To achieve selective knockdown of cIAP proteins, S1-GODL, S3-GODL, or S5-GODL cells were infected with lentivirus expressing short hairpin RNAs targeting cIAP1, cIAP2 , cIAP1&cIAP2 or a scrambled control. After elimination of uninfected cells by puromycin treatment, cells were treated in triplicates with either vehicle or carboplatin. Cell survival (determined by propidium iodide and annexin V negativity) and CA125 expression was quantified by FACS analysis. Graphs of cell survival for the bulk cells and the CA125 negative subpopulation is shown for each cell line. Results are mean ± SD, n=3. Knockdown of cIAP1 and cIAP2 together sensitized the CA125 negative cells to carboplatin treatment, resulting in complete cell death. Knockdown of neither cIAP alone could cause complete elimination of CA125 negative cells in response to carboplatin. Specific knockdown of cIAP proteins was verified by QPCR for cIAP1 and cIAP2 transcripts and western blot. Collectively, these results show sensitization of the CA125 negative HGSC cells is likely a direct result of cIAP degradation.

Supplementary Figure 8. Decreased tumor burden was observed in patient derived cell lines co-treated with birinapant and carboplatin. (a) Experimental schema for subcutaneous xenograft model. Mice in each cohort harbored 4 individual tumors to enable comparison of therapy effects between experimental and

control cells. Cell lines used in each cohort and their drug sensitivities are indicated. **(b)** Based on histology and expression of Pax8, combination therapy eliminated tumor cells in majority of subcutaneous xenografts (9 of 13).

Tumor foci in carboplatin treated xenografts were predominantly CA125 negative. Scale bars equal 50 µm. **(c)** Histologic examination of xenografts demonstrated the presence of tumor foci in all xenografts treated with birinapant

Birinapant + Carboplatin

One month off therapy

S1-GODL

c At completion of therapy

Solid tumor

S3-GODL

500 uM

Tumor foci

Solid tumor

Tumor foci

海

Solid

Solid tumor

Tumor foci

S5-GODL

Tumor foci

V

Tumor foci

 \mathbb{Z}^{mo}

Tumor foci

Tumor Tumor foci

Birinapant + Carboplatin

Birinapant + Carboplatin

Ovcar-3

bars equal 50 µm. **(d)** While obvious tumor was detected in all carboplatin treated xenografts, disease was detected in only 5 of 11 subcutaneous xenografts in the co-therapy arm one month after the cessation of therpy, and these foci were small compared to carboplatin treated tumors. Resurgence of CA125 positive cells was observed in carboplatin treated grafts after therapy was stopped. Scale bars equal 50 µm **(e)** Serial measurement of tumor volume demonstrates that in contrast to other treatment groups, xenografts treated with birinapant and carboplatin co-therapy do not increase in size following the cessation of treatment. Results are mean ± SEM, n=4 per group. **(f)** Full xenograft images are shown to demonstrate that when disease foci were detected in birinapant and carboplatin treated grafts released from therapy; these foci were very small compared to tumors in the carboplatin treated arm. Scale bars equal 500 μ m monotherapy. Scale

Subcutaneous xenografts 1 month off therapy

Supplementary Figure 9. Response of control cell lines to in vivo therapy correlated with their known drug **sensitivities (a)** Serial measurement of tumor volumes demonstrated that Skov3 cells responded only to birinapant therapy, Ovcar-3 cells responded to carboplatin, and MCF7 cells exhibited minimal response to either drug. Results are mean ± SEM, n=4 **(b)** Based on histology and immunohistochemistry, tumor was detected in all subcutaneous xenografts at the completion of therapy**.** Scale bars equal 50 µm. **(c)** Tumor was also detected in all control grafts one month after cessation of therapy. Scale bars equal 50 μ m.

Supplementary Figure 10. Serum CA125 correlated with tumor burden only when majority of tumor cells expressed CA125 on their cell surface. (a) Gross tumors from mice bearing only S3-GODL or S5-GODL xenografts and treated with vehicle, carboplatin, birinapant or the combination therapy are shown. Residual xenografts in the cotherapy arm were significantly smaller compared to monotherapy treated tumors immediately after treatment and 6 weeks following cessation of treatment (p<0.01 by one-way ANOVA, n=3 per group). Scale bars equal 1 cm **(b)** In both cohorts, mice treated with birinapant and carboplatin co-therapy showed no progression of disease after cessation of therapy vastly different from results seen in mono-therapy arms (n=3 per group). **(c)** Human serum CA125 levels detected in vehicle treated mice (n=3 per group) correlated with tumor burden. In carboplatin treated mice (n=3 per group), human CA125 was detected only after mice had been released from therapy and their tumors expressed CA125. This biomarker was undetectable immediately after therapy despite detection of CA125 negative tumor cells in the carboplatin treated xenografts. Scale bars equal 50 μ m. R² calculated by linear regression.

Blood chemistry and CBC values for mice treated with in vivo therapies a

Supplementary Figure 11. Birinapant and carboplatin co-therapy is safe and well-tolerated. (a) Blood chemistry and complete blood count analysis demonstrates that addition of birinapant to carboplatin therapy did not increase carboplatin induced neutropenia or thrombocytopenia. No additional effects on liver or renal function were observed when birinapant was added to carboplatin therapy Results are mean ± SEM (n=9, p value calculated by unpaired, twosided *t-*test). **(b)** Weight loss with combined carboplatin and birinapant co-therapy was less than 10% of original body weight, was statistically similar to weight loss with carboplatin treatment alone, and was reversed with cessation of treatment. Results are mean ± SEM, n= 6 per group).

Supplementary Figure 12. (a) Full length images of western blots for Figure 3

14

αERK

Supplementary Figure 12. Full length images of western blots for Figure 5c

Supplementary Table 1. Specimen characteristics

Supplementary Table 2. Results of limiting dilution analysis

† Indicates the median value for the group (i.e. Bulk, CA125 - or CA125 +)

Supplementary Table 3. TP53 mutations detected by RNA sequencing

Supplementary Table 4. Fold change in drug efflux transcripts

Supplementary Table 5. Fold change in autophagy transcripts

Supplementary Table 6. Frequency or tumor foci in xenografts

Supplementary Table 8. Cell surface transcripts highly expressed in CA125- cells

Supplementary Table 9. Antibodies used for FACS

Supplementary Table 10. Antibodies used for immunohistochemistry

Supplementary Table 11. Antibodies used for western blot

Supplementary Table 12. Quantitative-PCR primer sets

