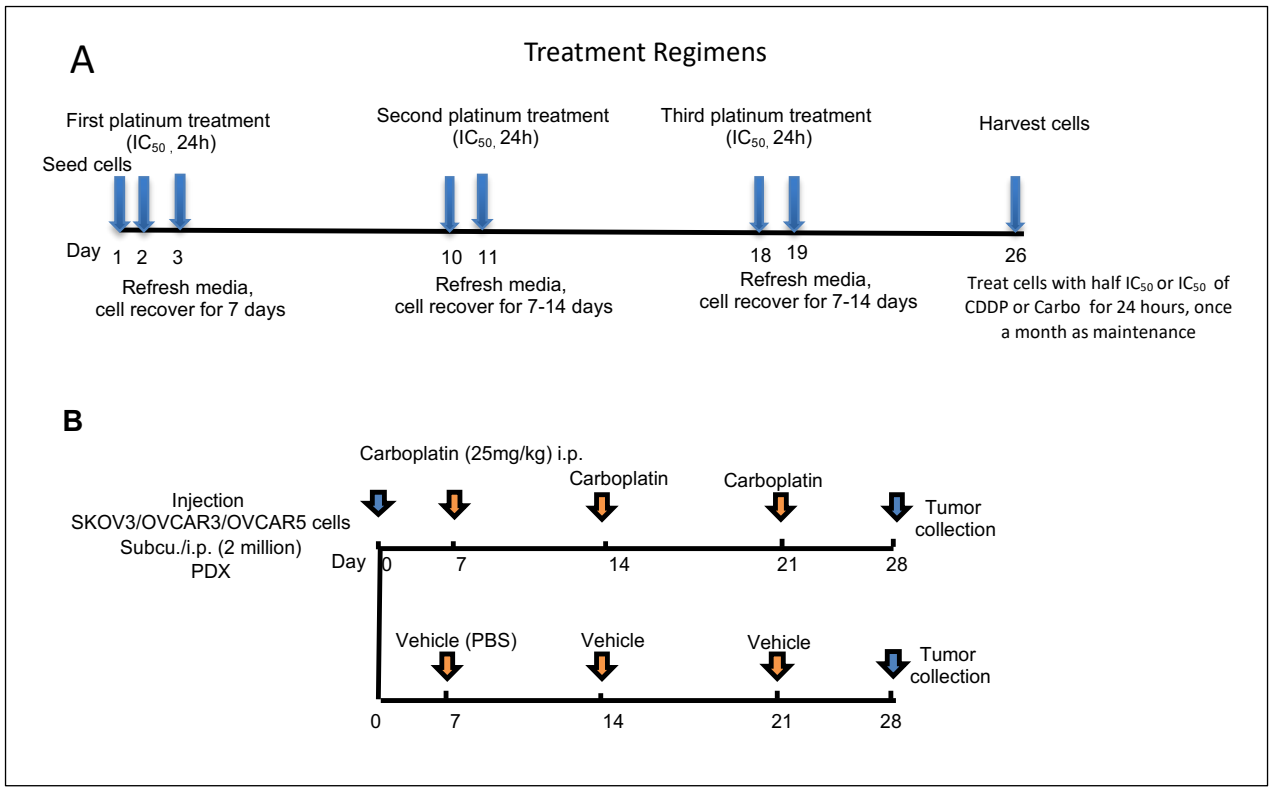
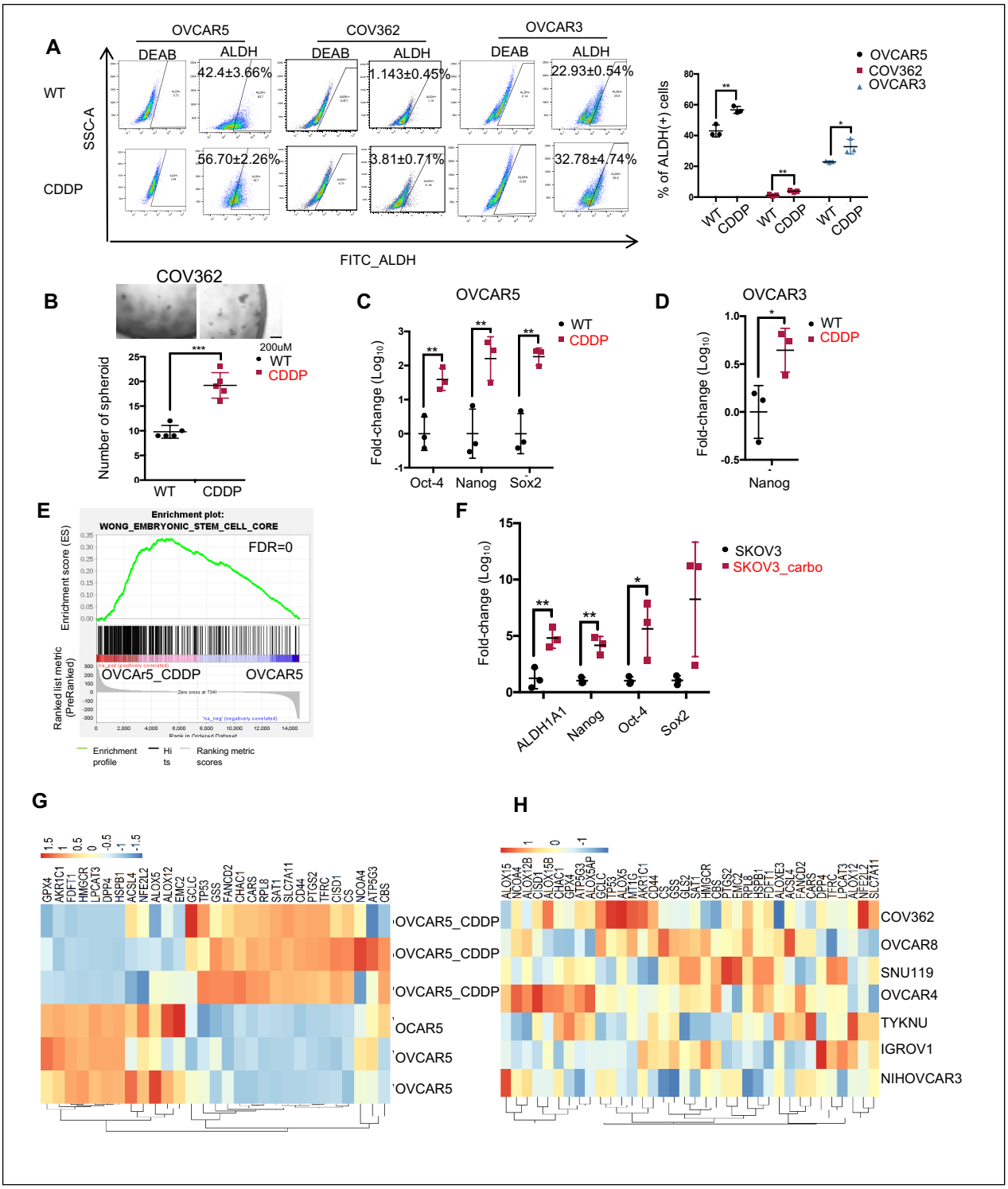
Supplemental Figures
Supplemental Figure S1



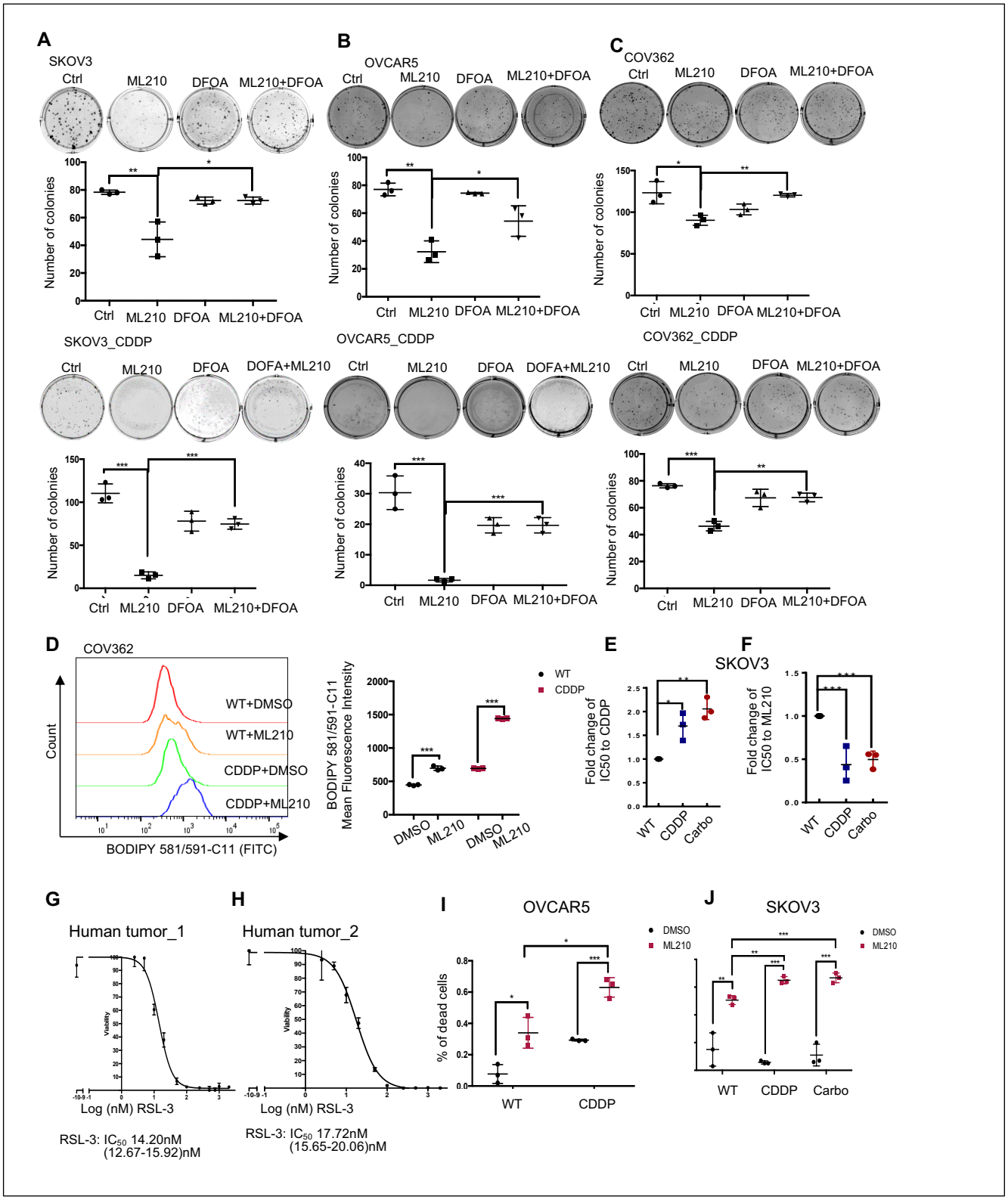
Supplemental Figure S1 (A) Schematic diagram describing the development of platinum (cisplatin or carboplatin) tolerant OC cells by using repeated treatment with platinum. **(B)** Schematic diagram describing the development of platinum tolerant ovarian xenografts.

Supplemental Figure S2



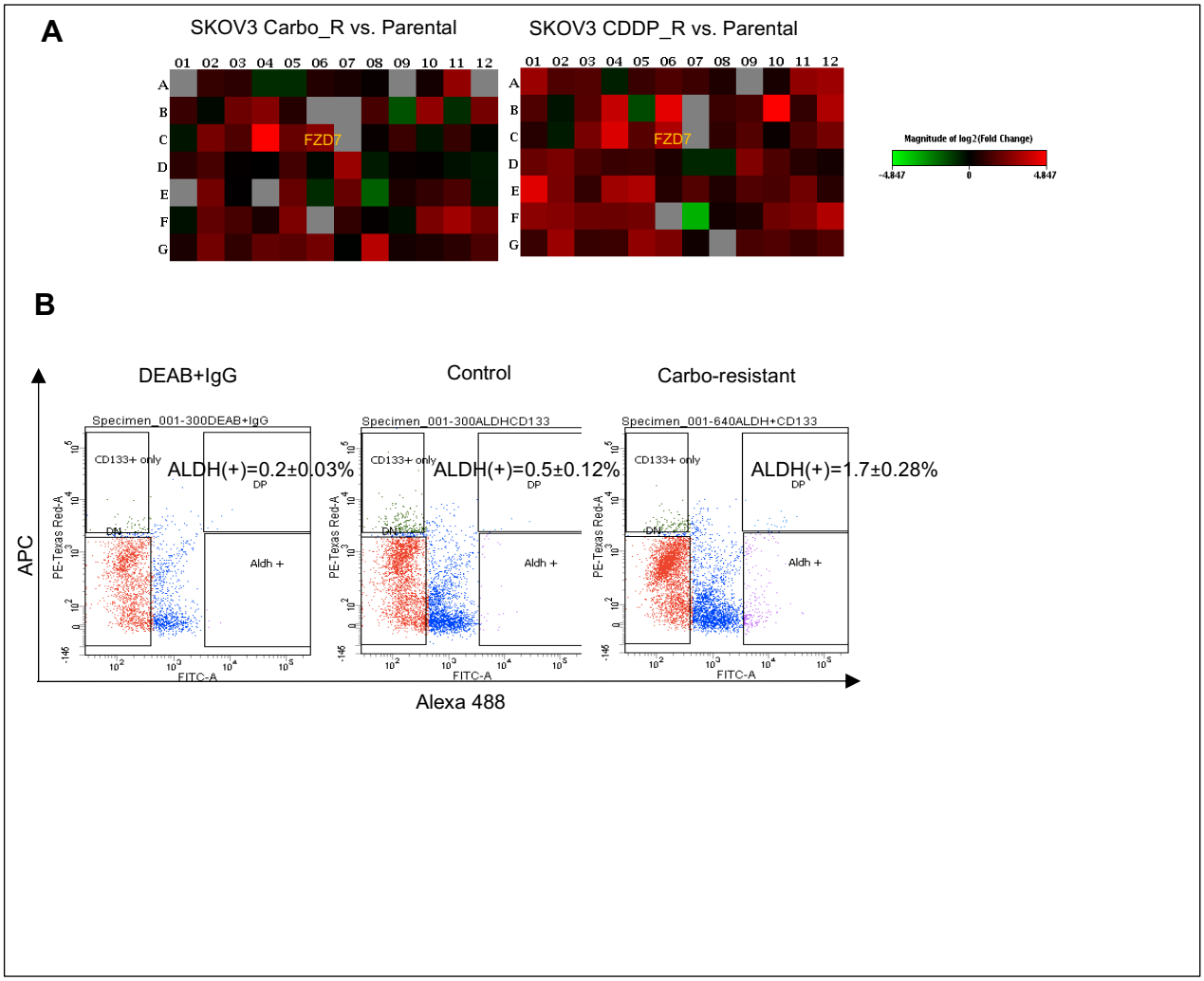
Supplemental Figure S2. OVCAR5, COV362 and OVCAR3 cells were repeatedly treated with cisplatin (n = 3 - 4 times). **(A)** Side scatter of FACS shows percentage of ALDH(+) cells in wild type (WT) and cisplatin (CDDP) tolerant cells and mean percentages of ALDH(+) cells (± SD). **(B)** Mean (± SD, n=5) numbers of spheroids generated from 10,000 COV362 CDDP-tolerant cells versus wild type cells (*P< 0.05, **P<0.01, and ***P<0.001). **(C)** Average (± SD, n=3) fold change in mRNA expression levels of stemness associated TFs (*Oct4, Nanog* and *Sox2*) in OVCAR5 CDDP-tolerant vs. parental cells (*P< 0.05, **P<0.01, and ***P<0.001). **(D)** Average (± SD, n=3) fold change of *Nanog* mRNA expression in OVCAR3 CDDP-tolerant vs. wild type cells **(E)** GSEA analysis of Wong Embryonic Stem Cell Core in OVCAR5 CDDP-tolerant vs. wild type cells. Gene list was ranked using signed (from log2FC) likelihood ratio from OVCAR5 CDDP-tolerant vs.parental cells (FDR=0). **(F)** Average fold changes (± SD, n=3) in the expression of *ALDH1A1, Nanog, Oct4* and *Sox2* in carboplatin treated SKOV3 xenografts compared to control tumors (*P< 0.05, **P<0.01, and ***P<0.001). **(G)** Heatmap shows differential expression (FDR<0.05) of ferroptosis related genes between OVCAR5 CDDP-tolerant and parental cells. **(H)** Heatmap highlights differential expression (FDR<0.05) of ferroptosis related genes in platinum resistant HGSOC cells (COV362, OVCAR8, SNU119, and OVCAR4, IC_{50} >5μM) and platinum sensitive HGSOC cells (TYKNU, IGROV1, and OVCAR3, IC_{50} <5μM).

Supplemental Figure S3



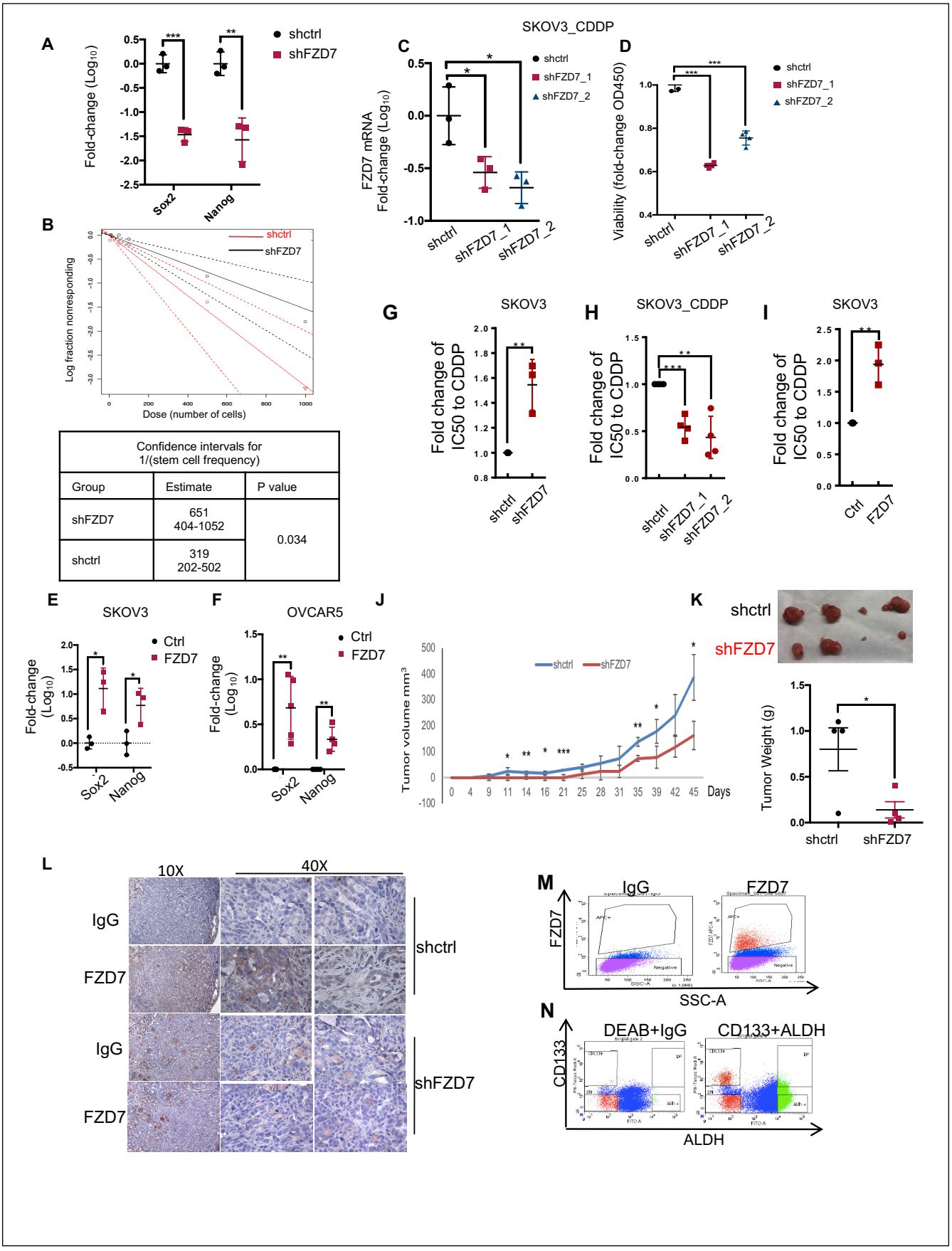
Supplemental Figure S3. (A) Colony formation derived from SKOV3, **(B)** OVCAR5, **(C)** COV362 wild type (upper panels) and CDDP-tolerant cells (lower panels) seeded at a density of 1000 cells per well and treated with DMSO, ML210 (SKOV3 and OVCAR5, 1μM; COV362, 2μM), DFOA (800nM) or ML210 and DFOA for 24 hours. Colonies were fixed, crystal violet stained and imaged on Day 14. Average numbers of colonies (± SD) were quantified for each condition (n = 3; *P< 0.05, **P<0.01, and ***P<0.001); **(D)** Lipid peroxidation was assessed in COV362 cells (CDDP-tolerant vs. parental) treated with DMSO and ML210 (2μM for 20 hours) by flow cytometry using BODIPY staining. Histograms are show on the left and mean (± SD, n=3) fluorescence intensities (MFI) of BODIPY 581/591-C11 are shown on the right (***P<0.001). **(E-F)** Average fold change (± SD) in IC_{50} for SKOV3 CDDP resistant and carboplatin resistant cells to CDDP **(E)** and ML210 **(F)** compared to SKOV3 parental cells (n = 3; *P< 0.05, **P<0.01, and ***P<0.001). **(G-H)** Cell survival curve of primary platinum-resistant HGSOC tumor cells after treatment with RSL-3 (from 0 to 2000nM) (n=3-4). IC_{50} to RSL-3 are shown. **(I-J)** Average percentage of dead cells (± SD) in **(I)** OVCAR5 and **(J)** SKOV3 parental (WT) cells, cisplatin resistant and carboplatin resistant cells treated with DMSO or ML210 (1 uM) for 24 hours. Dead cells were stained by trypan blue.

Supplemental Figure S4.



Supplemental Figure S4. (A) Heatmap of differentially expressed genes from the cancer stem cell RT-PCR based platform indicates stemness associated genes in the carboplatin (left) or cisplatin (right) tolerant SKOV3 vs parental cells. Red corresponds to upregulated and green to downregulated genes in platinum-tolerant vs. parental cells. **(B)** NSG mice bearing subcutanous PDX ovarian tumors were treated with carboplatin (Sigma) at 15mg/kg, or PBS (n = 5 mice per group). FACS histograms indicate ALDH (+) cells in single cell suspensions derived from PBS or carboplatin-treated PDX tumors; percentages ± SD of ALDH(+)cells were quantified (n=3).

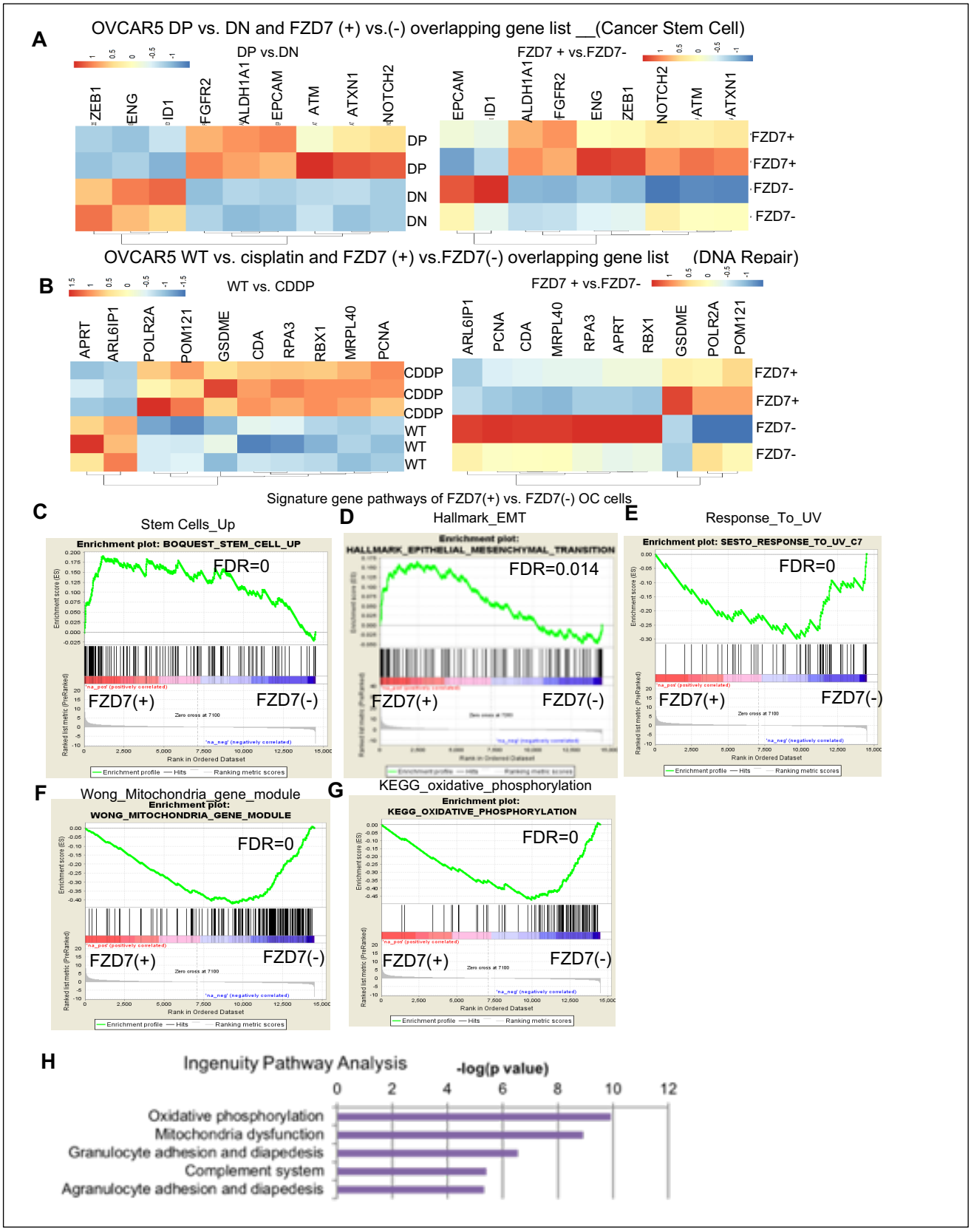
Supplemental Figure S5.



Confidence intervals for 1/(stem cell frequency)		
Group	Estimate	P value
shFZD7	651 404-1052	0.034
shctrl	319 202-502	

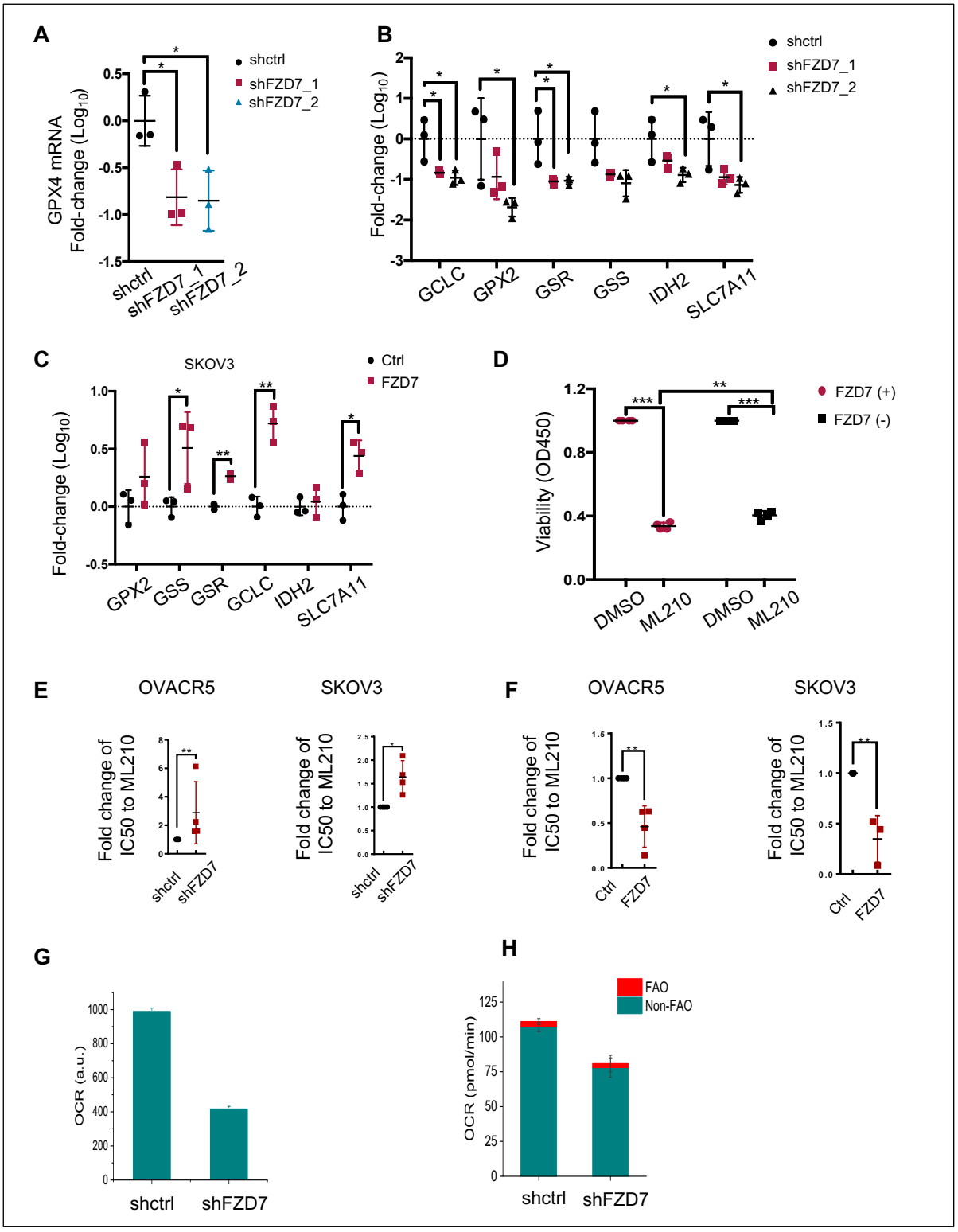
Supplemental Figure S5. (A) Mean fold changes (± SD, n=3) of *Sox2* and *Nanog mRNA* expression levels in SKOV3 transduced with shRNA targeting FZD7 (shFZD7) vs. control (shctrl). **(B)** Limited dilution assay used shctrl and shFZD7 transduced OVCAR5 cells. Serially diluted numbers (5, 10, 50, 100, 500 1000) of shctrl or shFZD7 OVCAR5 cells were cultured under spheroid conditions for 7 days (n=10 replicates per condition). Stem cell frequencies were calculated by using the Extreme Limiting Dilution Analysis (http://bioinf.wehi.edu.au/software/elda/) and shown in the table (P= 0.034). **(C)** Average fold changes ($Log_{10}FC$) (± SD, n=3) of *FZD7* mRNA levels in CDDP-tolerant SKOV3 cells transduced with shRNA targeting FZD7 (2 sequences) or control. **(D)** Spheroid formation in CDDP-tolerant SKOV3 cells transduced with shRNA targeting FZD7 (2 sequences) or control. Cell viability was measured and average fold changes (± SD, n=3) are shown (*P< 0.05, **P<0.01, and ***P<0.001). **(E)** Average fold changes (± SD, n=3) of *Sox2* and *Nanog mRNA* levels in SKOV3 and **(F)** OVCAR5 cells transfected with FZD7-pcDNA3.1 vs. empty vector. **(G-I)** Average fold change (± SD) in IC50 of SKOV3 **(G)** and SKOV3 cisplatin (CDDP) resistant cells **(H)** with *FZD7* KD to CDDP compared to control cells. **(I)** Average fold change (± SD) in IC50 of SKOV3 with overexpression of *FZD7* to CDDP compared to control cells (n = 3-4; *P< 0.05, **P<0.01, and ***P<0.001). **(J)** Growth curve of sc xenografts in nude mice generated from 2 X 10^6 OVCAR5 stably transduced with shRNA targeting FZD7 (shFZD7) vs. control (shctrl). Average tumor volumes (± SD) are shown (n=3/group). **(K)** Pictures and weights (mean ± SD, n=4) of tumor xenografts induced by OVCAR5_shctrl and OVCAR5_FZD7 cells. **(L)** Representative images of IgG and FZD7 IHC staining in OVCAR5_shctrl cells and OVCAR5_shFZD7 cells derived subcutaneous xenograft tumors. Magnification 10X (Left) and 40X (Right). **(M)** Gating strategy for FACS sorting of FZD7 (+) and FZD7 (-) OC cells or **(N)** CD133(+) ALDH(+) OCSCs vs. CD133(-) ALDH(-) non-OCSCs from OVCAR5 cells used for RNA sequencing.

Supplemental Figure S6



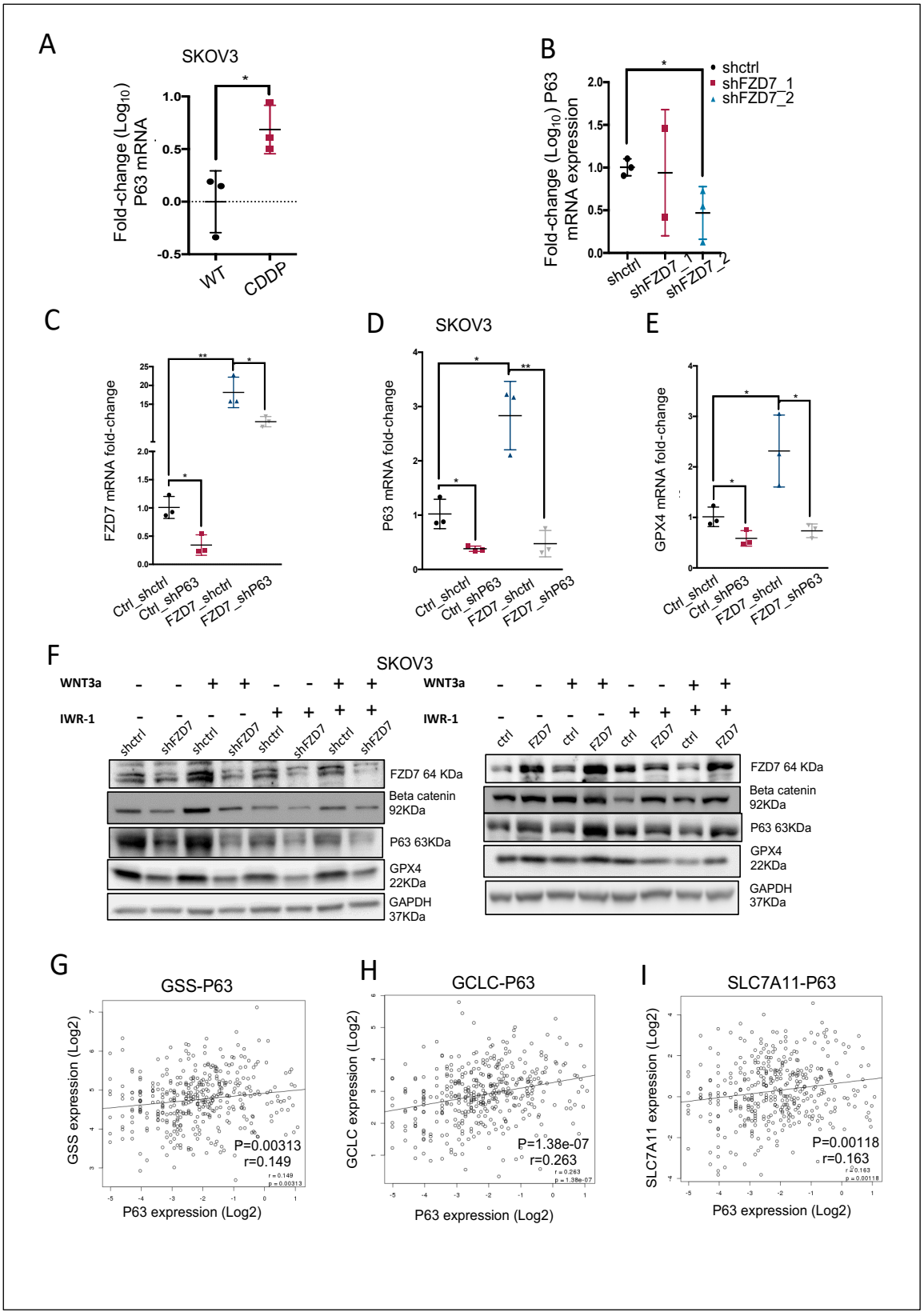
Supplemental Figure S6. (A) Heatmap of overlapping differentially expressed genes (FDR<0.05) related to "*Cancer Stem Cells"* pathway between OVCAR5_DP (ALDH+CD133+) OCSCs vs. OVCAR5_DN (ALDH-CD133-) non-OCSCs and OVCAR5_FZD7+ vs. OVCAR5_FZD7- OC cells. **(B)** Heatmap of overlapping differentially expressed genes (FDR<0.05) related to "*DNA Repair*" pathway between OVCAR5 CDDP-tolerant vs. parental and OVCAR5_FZD7(+) vs. OVCAR5_FZD7(-) cells. **(C)** GSEA plots of OVCAR5 derived FZD7(+) vs. FZD7(-) cells related to *Stem Cell UP* (FDR=0), **(D)** *Hallmark Epithelial Meshenchymal Transition* (FDR=0.014), **(E)** *Response to UV gene sets* (FDR=0), **(F)** *Mitochondria Gene_Module* (FDR=0), and **(G)** *KEGG Oxidative Phosphorylation* (FDR=0). **(H)** Ingenuity Pathway Analysis of differentially expressed genes between FZD7(+) vs. FZD7(-) OC cells indicates top canonical pathways enriched in FZD7(+) cells.

Supplemental Figure S7



Supplemental Figure S7. (A) Mean fold changes (± SD, n=3) of *GPX4 mRNA* expression levels in SKOV3 cells transduced with shRNA targeting FZD7 (shFZD7) vs. control (shctrl) (*P< 0.05, **P<0.01, and ***P<0.001). **(B)** Mean fold change (± SD, n=3) of mRNA expression levels for *GCLC, GPX2, GSR, GSS, IDH2* and *SLC7A11* in SKOV3 transfected with shFZD7 versus SKOV3 transfected with shctrl (*P< 0.05, **P<0.01, and ***P<0.001). **(C)** Average fold change of *GPX2, GSS, GSR, GCLC, IDH2,* and *SLC7A11* mRNA expression (± SD, n=3) in SKOV3 cells transfected with FZD7-pcDNA3.1 vs. empty vector (*P< 0.05, **P<0.01, and ***P<0.001). **(D)** Viability of FZD7(+) and FZD7(-) cells sorted by FACS from SKOV3 cells and treated daily with DMSO or the GPX4 inhibitor ML210 (0.25μM) for 72 hours. Cell viability was determined with a CCK8 assay. Data are presented as average fold-change (± SD, n = 4) of absorbance values relative to control. **(E)** Average fold change (± SD) in IC50 of OVCAR5 (left) and SKOV3 (right) cells transfected with shRNA targeting FZD7 to ML210 compared with cells transfected with shRNA control. **(F)** Average fold change (± SD) in IC50 of OVCAR5 (left) and SKOV3 (right) cells transfected with FZD7 vector to ML210 compared with cells transfected with control vector (n = 3; *P< 0.05, **P<0.01, and ***P<0.001)**.(G)** Oxygen consumption rate (OCR) in OVCAR5 cells transduced with shRNA targeting FZD7 (shFZD7) vs. control (shctrl), measured by using the oxygen consumption rate assay kit and **(H)** Seahorse assay (*P< 0.05, **P<0.01, and ***P<0.001)

Supplemental Figure S8.



Supplemental Figure S8. (A) Mean fold changes (± SD, n=3) of *P63 mRNA* expression levels in SKOV3 CDDP-tolerant vs. parental cells and **(B)** in SKOV3 stably transduced with shFZD7 vs. shctrl (*P< 0.05, **P<0.01, and ***P<0.001). **(C-E)** *FZD7***(C)**, *P63* **(D)**, and *GPX4* **(E)** mRNA expression levels (fold-change ± SD, n=3) in SKOV3 cells transduced with control shRNAs (Ctrl_shctrl, Ctrl_shP63, FZD7_shctrl) or transfected with FZD7 expression vector and subsequently transduced with shRNA targeting *P63* (FZD7_shP63). *mRNA levels* were determined by real-time RT-PCR. For all comparisons: *P<0.05, **P<0.01, ***P<0.001. **(F)** Western blot for FZD7, β-catenin, P63, GPX4 and GAPDH in SKOV3 cells transduced with shRNA control, shRNA targeting FZD7, FZD7 vector and control vector. Cells were stimulated with WNT3a (150ng/ul) and were treated with the small molecule inhibitor IWR-1-endo (1 μM 24 hours; n=2). **(G)** Correlations between expression levels of *P63* and gluthatione-related metabolism genes, *GSS* (P=0.00313), **(H)** *GCLC* (P=1.38e-07), and **(I)** *SLC7A11* (P=0.00118), as measured in the TCGA ovarian cancer dataset (n=419).