Supplemental Information

Immobilization rapidly selects for chemoresistant ovarian cancer cells with enhanced ability to enter dormancy

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Supplementary Materials:

Supplementary Tables S1-S3

Supplementary Figures S1-S7

Table S1. Detailed information for antibodies used in immunofluorescence (IF) and Western blotting (WB) experiments.

Target	Application	Vendor Information	Dilution
Ki67	Primary, IF	D3B5 Rabbit IgG, Cell Signaling Technology	1:500
--	Secondary, IF	AlexaFluor 594 Goat anti-Rabbit IgG, Thermo Fisher Scientific	1:1000
P-p38	Primary, WB	Phospho-p38 MAPK (Thr180/Tyr182), Cell Signaling Technology	1:1000
p38	Primary, WB	p38 MAPK, Cell Signaling Technology	1:1000
P-ERK1/2	Primary, WB	Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204), Cell Signaling Technology	1:1000
ERK1/2	Primary, WB	p44/42 MAPK (Erk1/2), Cell Signaling Technology	1:1000
β-actin	Primary, WB	Rabbut mAb (13E5) HRP-conjugated, Cell Signaling Technology	1:1000
--	Secondary, WB	Goat anti-Rabbit HRP-conjugated, Cell Signaling Technology	1:1000

Table S2. Canonical signaling pathways identified using Ingenuity Pathway Analysis for differentially expressed genes that were commonly up- or down-regulated between chemoresistant versus chemosensitive patients in Koti *et al.* (2013) and between our OVCAR-3 cells which survived immobilization and were extracted from silica gels ("Extracted") versus control OVCAR-3 cells.

Canonical Signaling Pathway	-log(P-Value)
EIF2 Signaling	8.25
Regulation of eIF4 and p70S6K Signaling	4.48
mTOR Signaling	2.73
Cell Cycle Control of Chromosomal Replication	2.71
Role of BRCA1 in DNA Damage Response	2.4
NER Pathway	2.19
BER pathway	1.85

Table S3. Canonical signaling pathways identified using Ingenuity Pathway Analysis for differentially expressed genes that were commonly up- or down-regulated between chemoresistant versus chemosensitive patients from the Koti *et al.* (2013) *in silico* analysis of The Cancer Genome Atlas ovarian cancer patient datasets and between our OVCAR-3 cells which survived immobilization and were extracted from silica gels ("Extracted") versus control OVCAR-3 cells.

Canonical Signaling Pathways	-log(p-value)
Ephrin B Signaling	4.59
Hepatic Fibrosis / Hepatic Stellate Cell Activation	3.36
Antiproliferative Role of Somatostatin Receptor 2	2.74
IL-15 Production	2.37
PTEN Signaling	2.34
STAT3 Pathway	2.28
Corticotropin Releasing Hormone Signaling	2.22
Relaxin Signaling	2.19
Axonal Guidance Signaling	2.17
Cardiac Hypertrophy Signaling (Enhanced)	2.17
Tec Kinase Signaling	2.11
Glioblastoma Multiforme Signaling	2.10
NF-κB Signaling	2.04
Synaptic Long Term Depression	2.00
IL-8 Signaling	1.95
Role of NFAT in Cardiac Hypertrophy	1.89
Sperm Motility	1.87
Huntington's Disease Signaling	1.80
Cardiac Hypertrophy Signaling	1.79
Colorectal Cancer Metastasis Signaling	1.75
Sonic Hedgehog Signaling	1.62
G Protein Signaling Mediated by Tubby	1.60
Inhibition of Angiogenesis by TSP1	1.56
Ephrin A Signaling	1.42
Protein Kinase A Signaling	1.39
Glutamate Receptor Signaling	1.34
PCP pathway	1.32
Wnt/Ca+ pathway	1.31

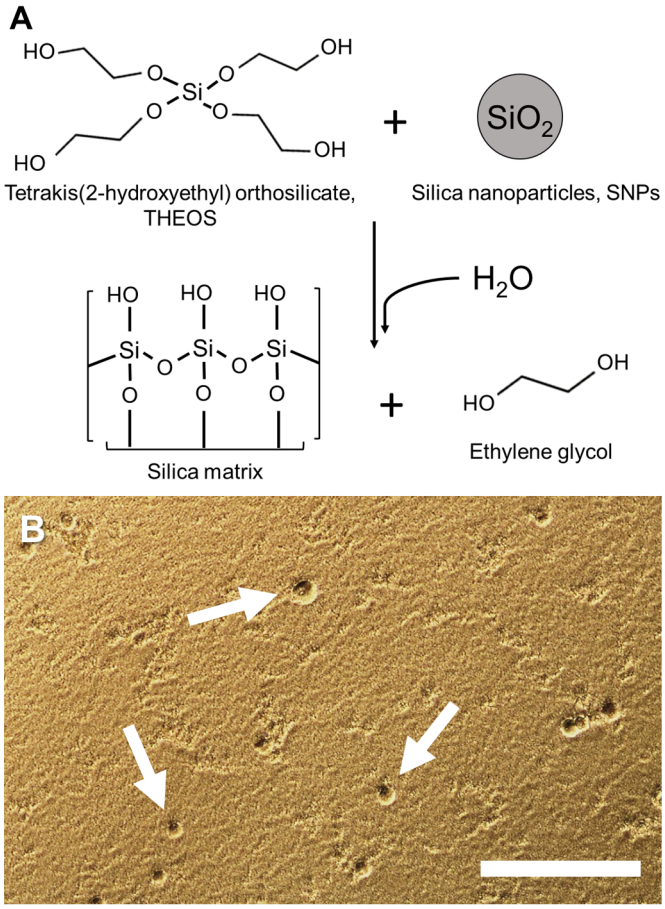

Figure S1. Silica gels formed from tetrakis(2-hydroxyethyl) orthosilicate (THEOS) and silica nanoparticles (SNPs) were used to immobilize OVCAR-3 cells. (**A**) Schematic of chemical formation of silica matrices by incorporating water with a silicon alkoxide, where addition of water causes hydrolysis of THEOS followed by condensation reactions producing ethylene glycol as a by-product. SNPs aid in gel network formation by acting as additional nucleation sites during gelation. (**B**) Phase-contrast image of cells (white arrows) after immobilization within a silica gel. Scale bar indicates 200 μm.

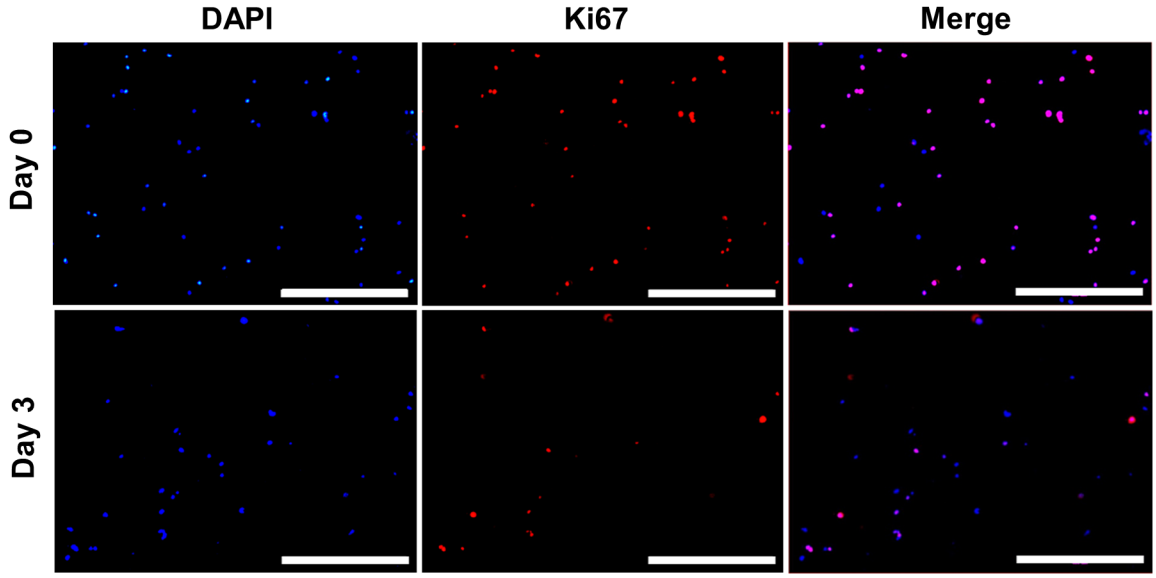

Figure S2. Percentage of Ki67-positive OVCAR-3 cells in silica gels decreases over time. Fluorescence images of immunostaining of nuclei (DAPI, blue) and Ki67 (red) at Day 0 and Day 3 of silica gel immobilization. Scale bar indicates 400 μm.

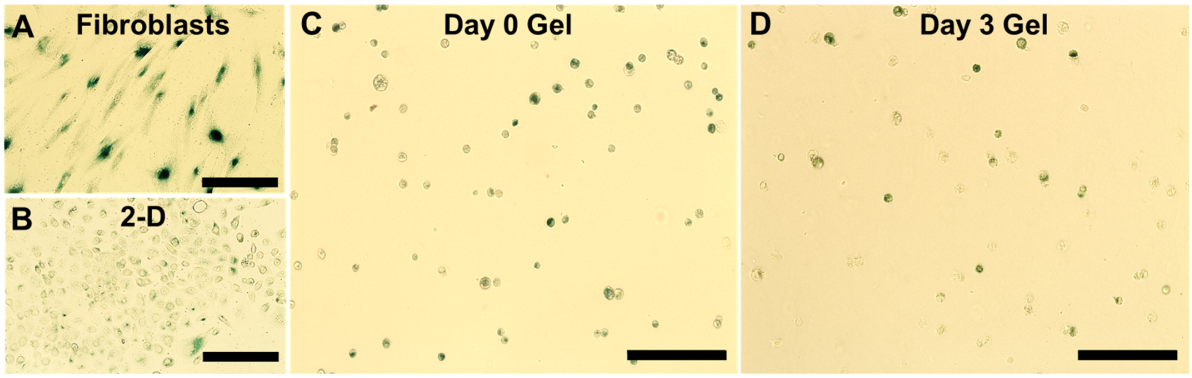

Figure S3. Surviving OVCAR-3 cells within silica gels do not exhibit senescence. Senescence-associated β-galactosidase staining was used to label senescent cells. (**A**) Human foreskin fibroblasts at high passage number were used as a positive control. (**B-D**) Images comparing staining of OVCAR-3 cells grown in 2-D (**B**) or immobilized within silica gels at Day 0 (**C**) and Day 3 (**D**) of immobilization. Scale bar indicates 200 µm.

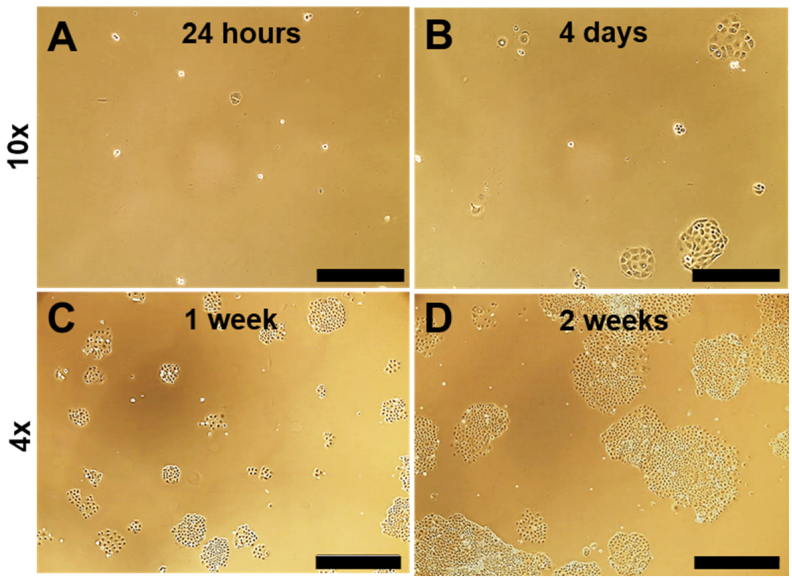

Figure S4. Immobilized cells can be extracted from silica gels and resume proliferation after being re-seeded in 2-D culture conditions. Primarily single cells adhered to flasks 24 hours after extraction and continued to grow in small colonies, which was clearly seen by four days post-extraction, and exhibited similar morphology as cells maintained in standard 2-D culture. (**A,B**) Phase-contrast images at 10x magnification of cells on 2-D surface 24 hours (**A**) or 4 days (**B**) after extraction. (**C,D**) Phase-contrast images at 4x magnification 1 week (**C**) or 2 weeks (**D**) after extraction. Scale bar indicates 400 µm and 1000 µm for 10x and 4x images, respectively.

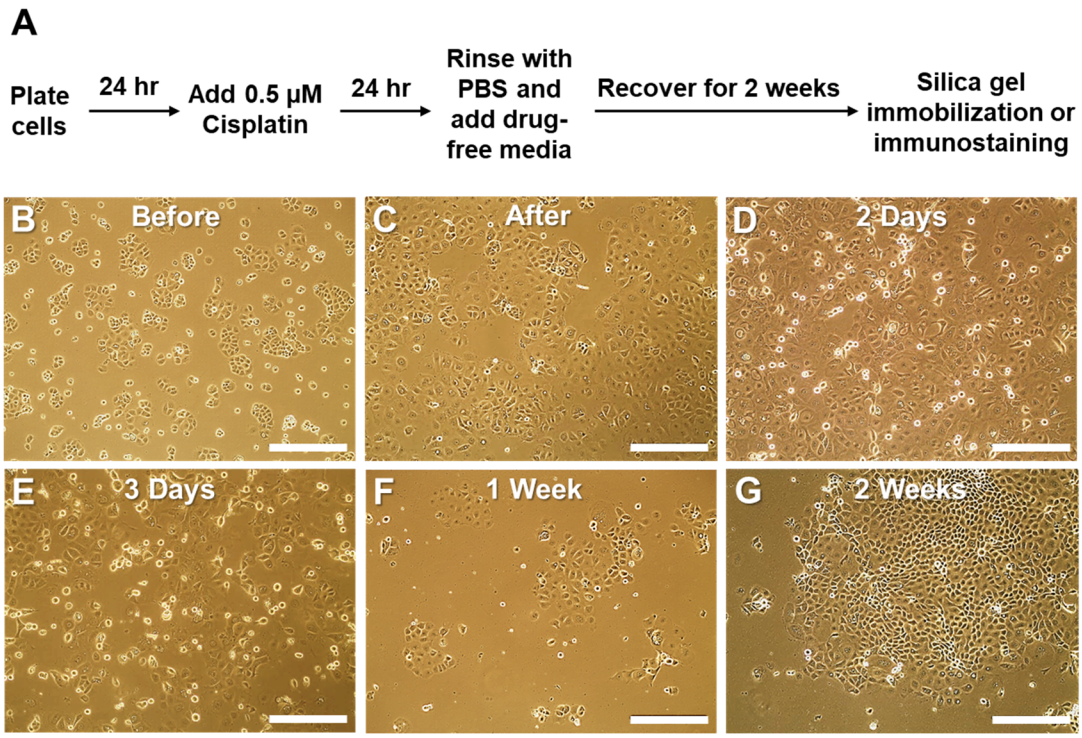

Figure S5. OVCAR-3 cells were treated with 0.5 µM cisplatin for 24 hours for significant cell death and recovery of a subset of cells. A 2 week-recovery period was required after drug removal because cells continued to detach and die for up to one week, and it was desired for cells to be back to proliferative state prior to encapsulation. (**A**) Timeline for selection of drug-resistant phenotype prior to silica gel immobilization or immunostaining. (**B-G**) Phase-contrast images of cells before treatment (**B**), immediately after drug was removed (**C**), and 2 days (**D**), 3 days (**E**), 1 week (**F**), and 2 weeks (**G**) after treatment. Scale bars indicate 400 µm.

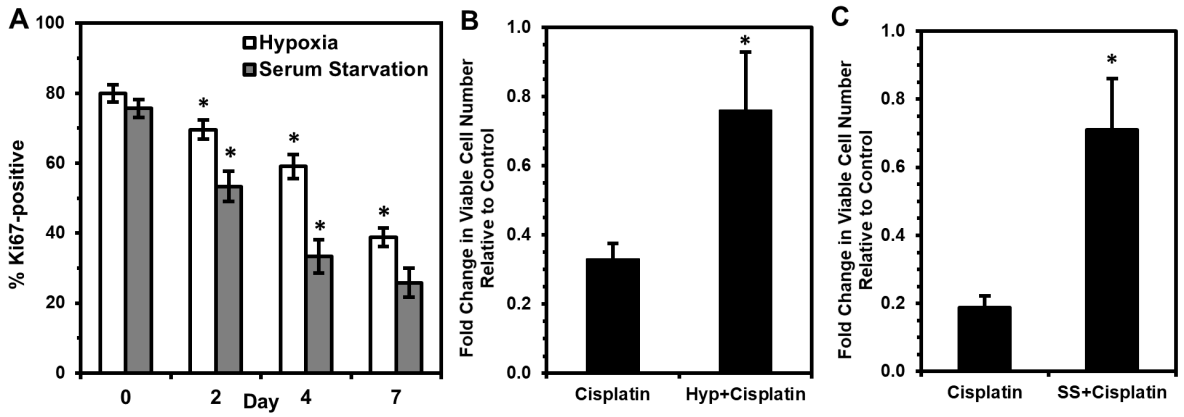

Figure S6. OVCAR-3 cells induced into a quiescent state are less susceptible to cisplatin treatment. (**A**) Percentage of Ki67-positive cells (number of Ki67-expressing cells normalized to number of DAPI-labeled cells) in cells cultured in 1% oxygen ("Hypoxia") or serum-free media ("Serum Starvation") conditions over a one-week period (*P < 0.05 compared to previous timepoint). Quiescent cells were obtained by culturing cells in 1% oxygen conditions or serum-free media for 3 days prior to drug addition. (**B,C**) Comparison of fold change in viable cell number for (**B**) hypoxia-treated cells ("Hyp+Cisplatin") or (**C**) serum-starved cells ("SS+Cisplatin") against cells grown in standard 10% FBS-supplemented media conditions ("Cisplatin") following treatment with 0.5 μM cisplatin for 24 hours. Fold change in viable cell number indicates cell number at Day 3 post-cisplatin treatment relative to untreated controls for each respective condition (*P < 0.05 compared to "Cisplatin").

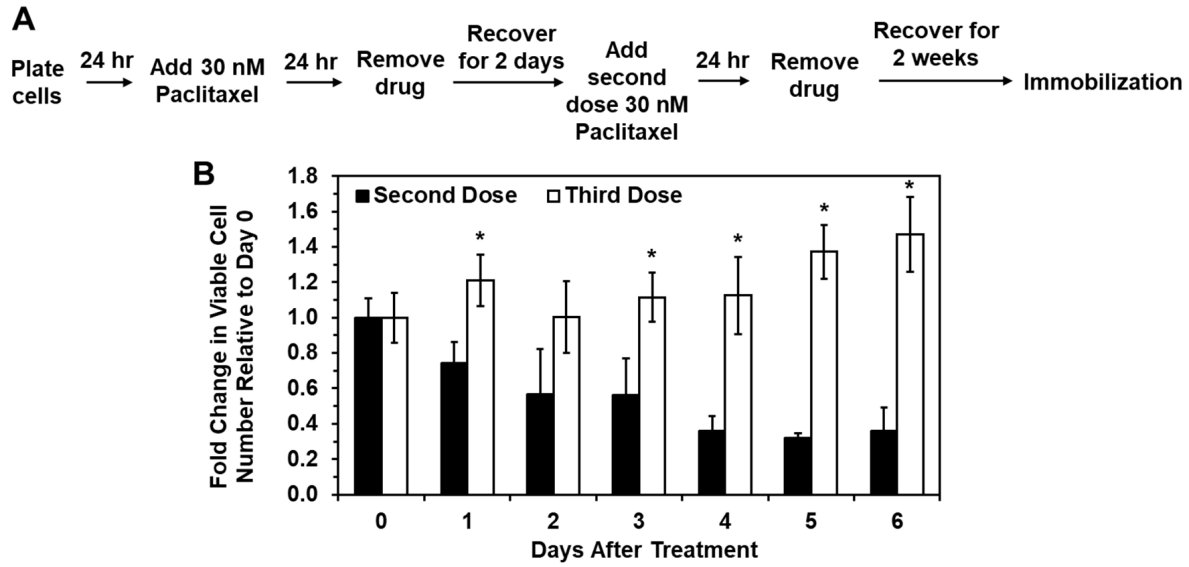

Figure S7. OVCAR-3 treatment with multiple doses of paclitaxel results in survival of a subset of cells that can recover and are less susceptible to an additional round of treatment. (**A**) Timeline for selection of more paclitaxel-resistant phenotype prior to silica gel immobilization. (**B**) Cells treated with a third dose of paclitaxel are less susceptible to treatment than cells undergoing secondary treatment. Each treatment dose is 30 nM paclitaxel for 24 hours, and cells receiving the third dose were allowed a 2 week-recovery period before treatment with the third dose. Viable cell number at Day 0 is the number of live cells counted immediately after drug removal. (*$P < 0.05$ compared to "Second Dose").