Figure S1, related to Figure 1

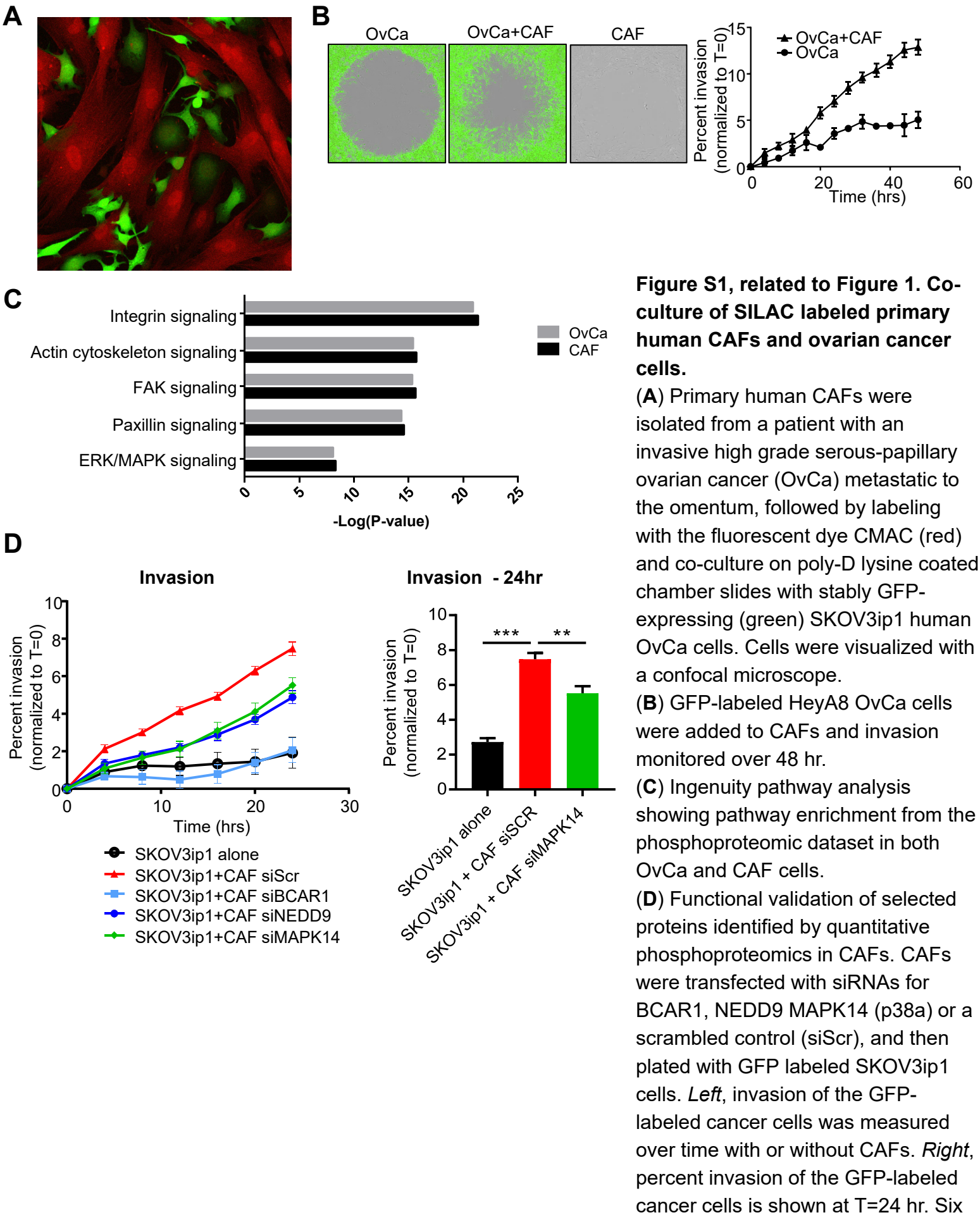


Figure S1, related to Figure 1. Co-culture of SILAC labeled primary human CAFs and ovarian cancer cells.

(**A**) Primary human CAFs were isolated from a patient with an invasive high grade serous-papillary ovarian cancer (OvCa) metastatic to the omentum, followed by labeling with the fluorescent dye CMAC (red) and co-culture on poly-D lysine coated chamber slides with stably GFP-expressing (green) SKOV3ip1 human OvCa cells. Cells were visualized with a confocal microscope.

(**B**) GFP-labeled HeyA8 OvCa cells were added to CAFs and invasion monitored over 48 hr.

(**C**) Ingenuity pathway analysis showing pathway enrichment from the phosphoproteomic dataset in both OvCa and CAF cells.

(**D**) Functional validation of selected proteins identified by quantitative phosphoproteomics in CAFs. CAFs were transfected with siRNAs for BCAR1, NEDD9 MAPK14 (p38a) or a scrambled control (siScr), and then plated with GFP labeled SKOV3ip1 cells. *Left*, invasion of the GFP-labeled cancer cells was measured over time with or without CAFs. *Right*, percent invasion of the GFP-labeled cancer cells is shown at T=24 hr. Six technical replicates were used per condition. **p< 0.01, ***p< 0.001.

Figure S2, related to Figure 2

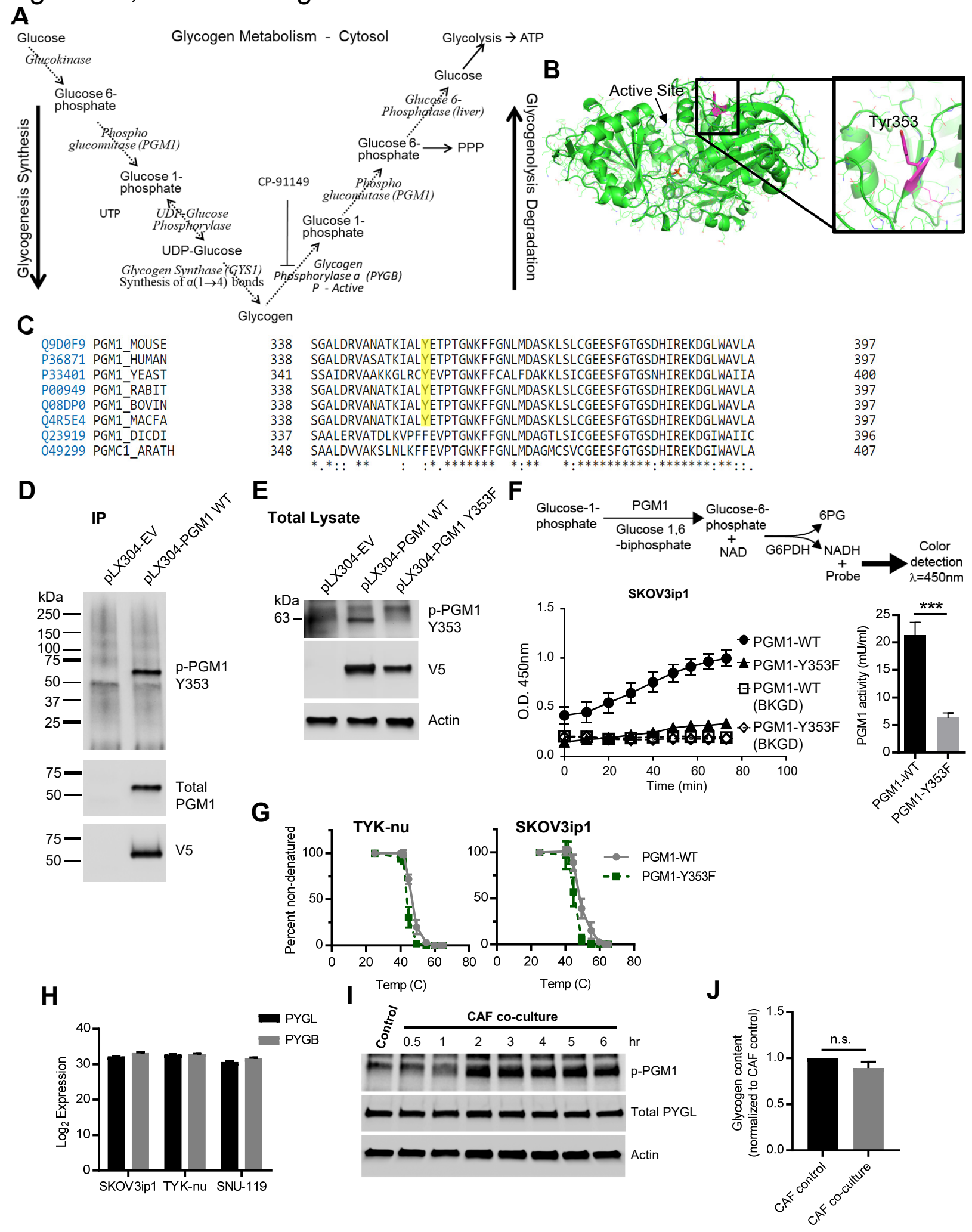


Figure S2, related to Figure 2. PGM1 activity is modulated by phosphorylation of Y353.
(**A**) Schematic of glycogen synthesis and degradation.
(**B**) Crystal structure of rabbit muscle PGM1 created using PyMOL. PDB ID: 3PMG. Y353 is shown on the outside of the structure in magenta. The active site is indicated.
(**C**) Alignment of the PGM1 sequence from several species indicates that the PGM1 Y353 phospho-site is evolutionary conserved in eukaryotes suggesting its importance. PGM1 protein sequences from different species were aligned using the Clustal Omega program. Y353 is highlighted in yellow. Uniprot ID is shown for each sequence. MOUSE, HUMAN, YEAST, RABIT (rabbit), BOVIN (cow), MACFA (cynomolgus monkey), DICDI (slime mold: Dictyostelium discoideum), ARATH (plant: Arabidopsis thaliana - Mouse-ear cress). The numbers in the column on the right are amino acid numbers of the protein sequence.
(**D**) A lentiviral construct containing the PGM1 cDNA was transfected in HEK293T cells and the supernatant used to infect SKOV3ip1 to express V5-tagged PGM1. PGM1 protein was immunoprecipitated using a V5 antibody and detected using the newly raised PGM1 Y353 phospho-antibody.
(**E**) Western blot showing the specificity of the phospho-Y353 PGM1 antibody. SKOV3ip1 cells were infected with lentivirus expressing either the WT (Y353) or the phenylalanine mutant (Y353F) and lysates were blotted with phospho-Y353 PGM1 antibody.
(**F**) PGM1 activity assay. PGM1 enzyme was immunoprecipitated using a V5 antibody from SKOV3ip1 cells stably expressing the V5-tagged WT or Y353F mutant constructs and analyzed for PGM1 activity using glucose-1-phosphate as the substrate (***$p < 0.001$). Background (BKGD). 6-phosphogluconate (6PG).
(**G**) CETSA assay on TYK-nu (*left*) and SKOV3ip1 (*right*) cells expressing either wild-type (WT) or the Y353F mutant PGM1. Data shown is an average of 4 biological replicates.
(**H**) Protein expression of glycogen phosphorylase isoforms PYGL and PYGB in three ovarian cancer cell lines. Data was extracted from a previously published proteomic dataset (Coscia et al., 2016).
(**I**) Western blot of p-PGM1 and total glycogen phosphorylase (PYGL) in TYK-nu cells following co-culture with CAFs over a time-course. Images are representative of 3 biological replicates.
(**J**) Glycogen content in primary human CAFs with or without co-culture with TYK-nu cells in a transwell insert. Values are mean + SEM from 4 biological replicates.

Figure S3, related to Figure 2

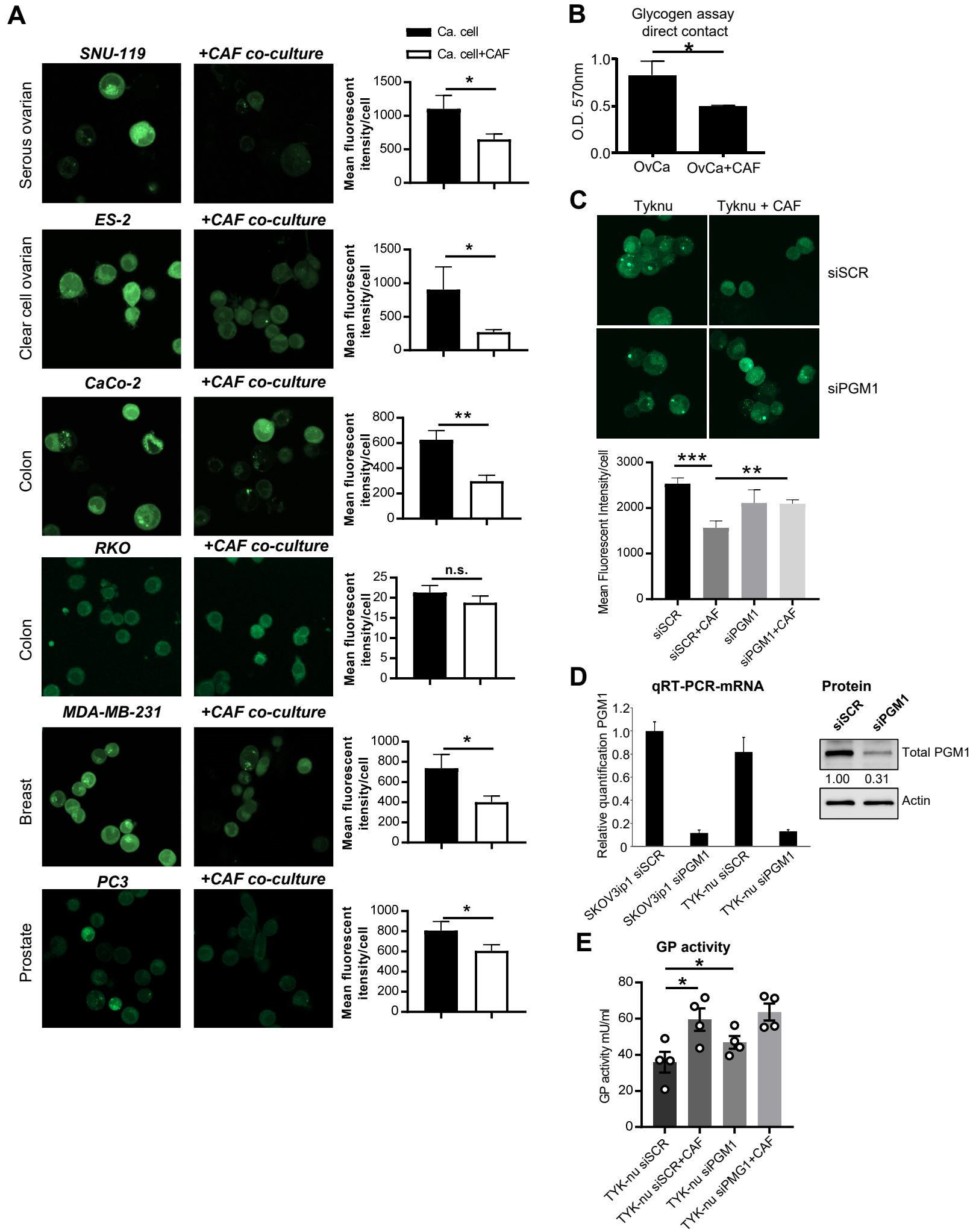


Figure S3, related to Figure 2. CAFs induce glycogen hydrolysis in multiple cancer cell types
(**A**) Several cancer cell lines store glycogen and use glycogen upon co-culture with CAFs. Glycogen content was visualized by 2-NBDG fluorescence and detected by confocal microscopy. Positive glycogen stain is seen as granular, green stain in the cytoplasm. Quantification is shown on the right.
(**B**) Glycogen content was quantified by measuring the hydrolysis of glycogen to glucose in FACS sorted SKOV3ip1-GFP cells with or without CAF co-culture for 4 hr. *p-value < 0.05.
(**C**) Glycogen levels were detected using 2-NBDG fluorescence after siRNA-mediated knocked down of PGM1 (siPGM1) in TYK-nu OvCa. Positive glycogen stain is seen as granular, green stain in the cytoplasm. Quantification is shown below.
(**D**) *Left*, qRT-PCR for PGM1 transcript levels following transfection with PGM1 siRNA (siPGM1) or scrambled control (siSCR) in both, TYK-nu and SKOV3ip1 OvCa cells. *Right*, western blot for total PGM1 in TYK-nu cells following siRNA transfection with indicated siRNAs. The image is representative of three biological replicates.
(**E**) Glycogen phosphorylase activity in TYK-nu cells transfected with PGM1 siRNA or scrambled siRNA and co-cultured with primary human CAFs separated by a transwell cell culture insert. Values are mean + SEM from 4 independent experiments (n=3/group/experiment) *p<0.05.

Figure S4, related to Figure 3

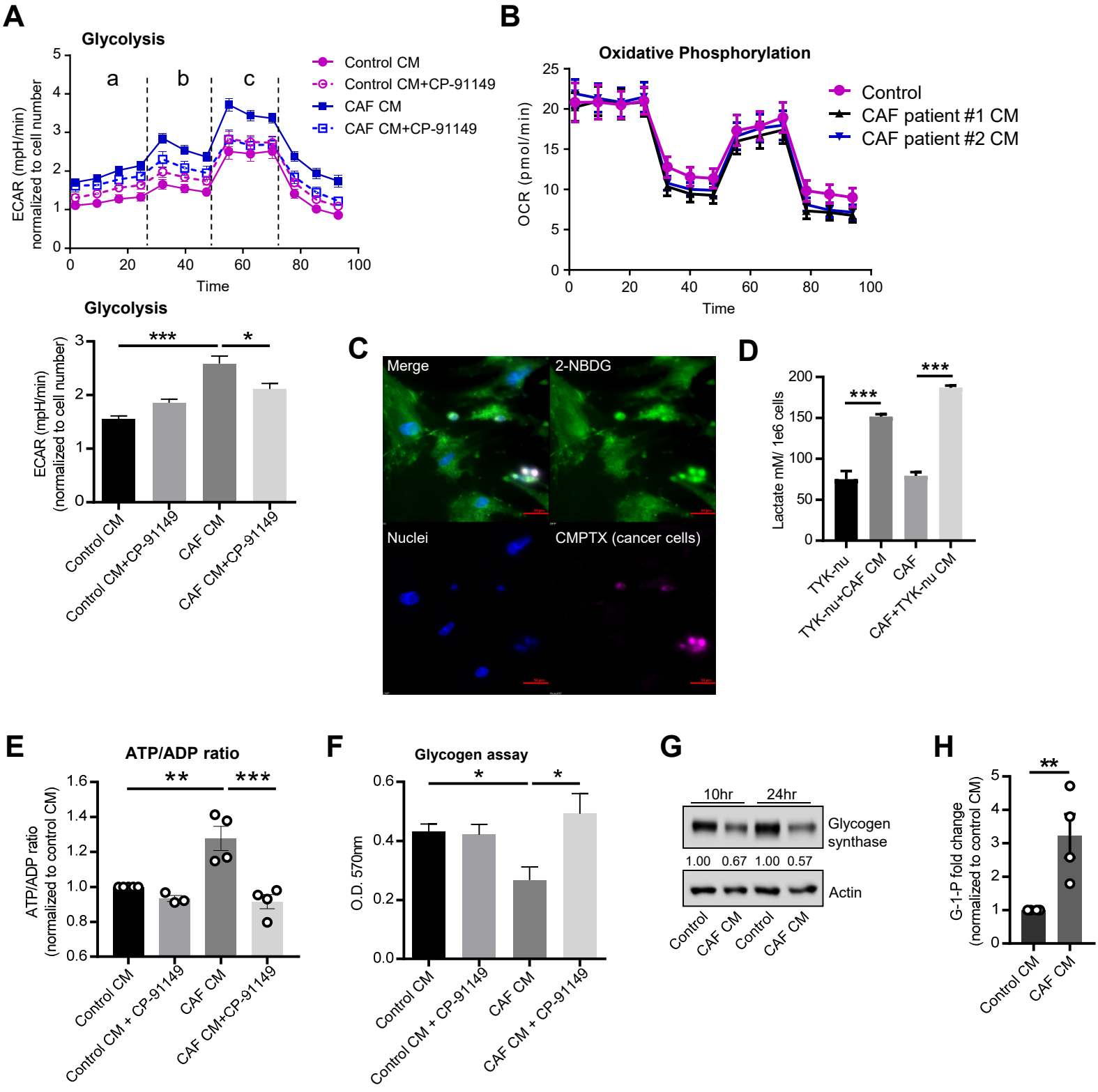


Figure S4, related to Figure 3. CAF-induced glycolysis is dependent on glycogen phosphorylase activity.

(**A**) Glycolysis. SKOV3ip1 cells were pretreated with the glycogen phosphorylase inhibitor CP-91149 for 4 hr followed by treatment with conditioned media (CM) from primary human CAFs or control CM for 16 hr. Glycolysis was measured using the Seahorse SF96 Extracellular Flux Analyzer. Extracellular acidification rates (ECAR) are shown normalized to cell number. 'a', basal glycolysis; 'b', glycolysis; 'c', glycolytic capacity. Lower panel shows the average of the 3 glycolysis time points for each group, *p<0.05, ***p<0.001.

(**B**) Oxygen consumption rate (OCR)/oxidative phosphorylation in cancer cells. SKOV3ip1 cells were treated with CM from 2 different primary CAFs or control conditioned media (CM) for 24 hr then OCR was measured (Seahorse XF96 Analyzer).

(**C**) Glucose uptake was assessed by uptake of the fluorescent glucose analog 2-NBDG (green). TYK-nu cells labeled with CMPTX were co-cultured with primary human CAFs overnight and then incubated with 2-NBDG for 30min prior to imaging. Scale bar = 50μM. Images are representative of two independent experiments.

(**D**) Lactate concentration in media from TYK-nu cells cultured alone or treated with CAF CM or CAF cultured alone or CAF cultured with TYK-nu CM for 24 hours. Concentrations of lactate in the CM were subtracted from these values. The concentration was normalized to cell number. Data is representative of two biological replicates

(**E**) ATP/ADP ratios in SKOV3ip1 cells treated with CAF or control CM pretreated with or without CP-91149, an inhibitor of glycogen phosphorylase. Values are mean + SEM from 4 independent experiments (n=4/group) and are normalized to the control. Comparisons were made to CAF CM using a Paired two tailed *t* test, ** p<0.01, ***p<0.001.

(**F**) Glycogen assay on SKOV3ip1 cancer cells pretreated with the glycogen phosphorylase inhibitor CP-91149, followed by stimulation with control or CAF CM.

(**G**) Western blot analysis of total glycogen synthase in TYK-nu cells treated with control or CAF CM. Relative band intensity is shown normalized to actin.

(**H**) Glucose-1-phosphate quantification in TYK-nu cells 4 hours after stimulation with control or CAF conditioned media (CM). Data is from 4 biological replicates with CM from human primary CAFs from 4 patients normalized to control CM. **, p-value <0.01.

Figure S5, related to Figures 3 and 6

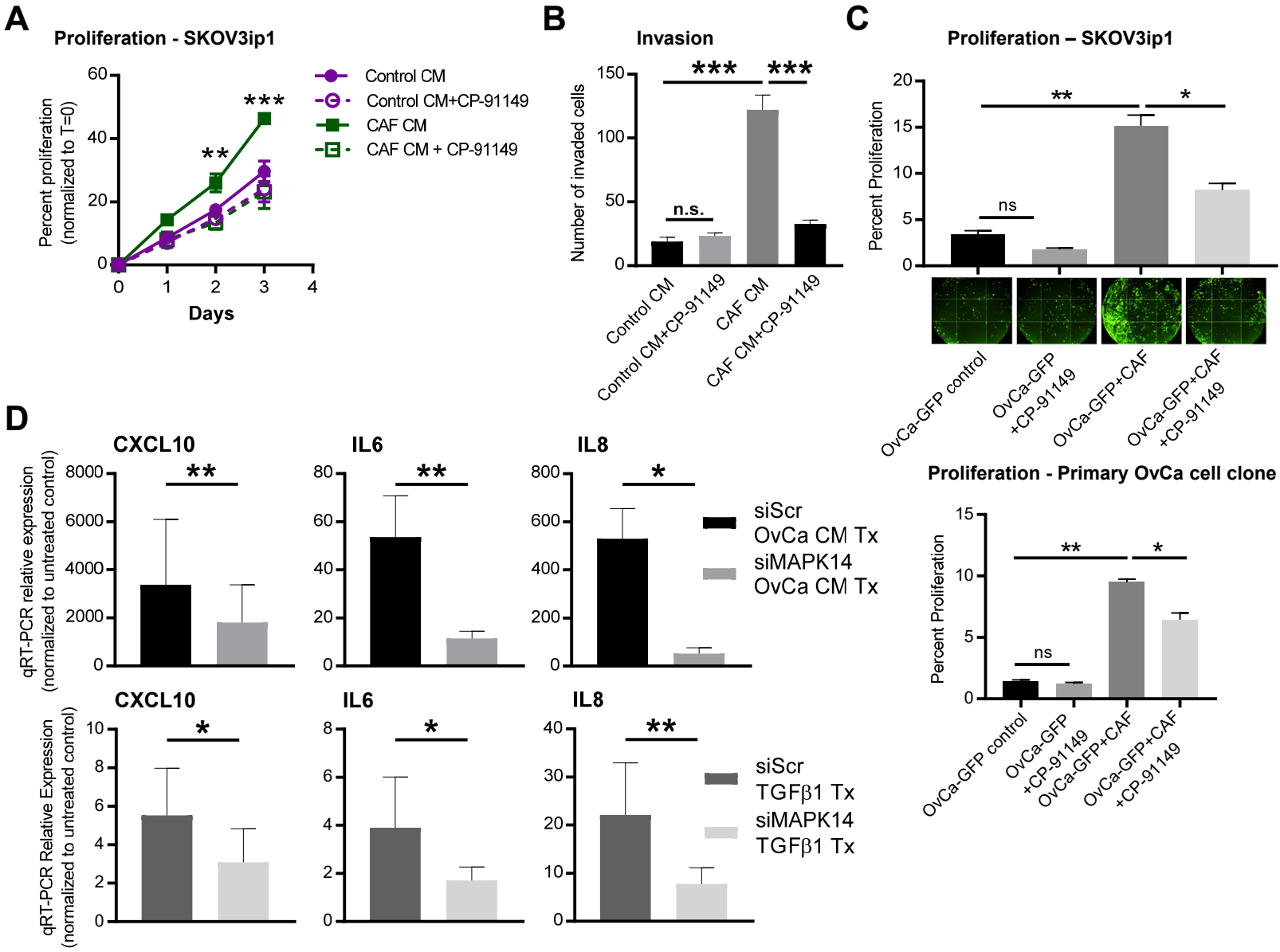


Figure S5, related to Figures 3 and 6. CAF-induced glycogen mobilization induces cancer cell proliferation and invasion.

(**A**) Proliferation of SKOV3ip1 cells exposed to CAF CM with or without the glycogen phosphorylase inhibitor CP-91149. Values are mean + SEM from 4 independent experiments (n=6/group). Comparisons were made to CAF CM using a Two-way ANOVA (** p<0.01, ***p<0.001).

(**B**) Boyden chamber invasion of SKOV3ip1 cells exposed to CAF CM or control with or without CP-91149. Data is representative of 3 independent experiments. Values are mean + SEM (n=3/group), ***p<0.001.

(**C**) Proliferation of SKOV3ip1-GFP cells (*upper*) and primary OvCa cell clone (*lower*) in direct contact with CAFs with or without CP-91149 after 48 hr. Data is representative of 3 independent experiments. Values are mean + SEM (n=6/group), ***p<0.001.

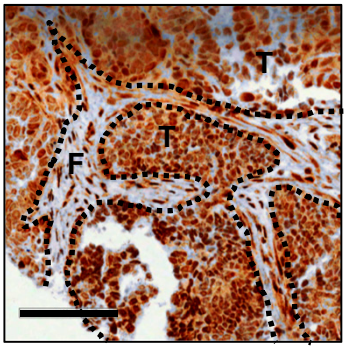
(**D**) Validation of cytokine expression by qRT-PCR in CAFs. The primary CAFs were transfected with a siRNA targeting p38α (siMAPK14) or a scrambled siRNA (siScr) and either stimulated with TYK-nu (OvCa) CM or TGF-β1. RNA was extracted and cytokine expression detected using the indicated TaqMan probes. Values are mean + SEM from 4 independent experiments with primary CAFs quantified relative to untreated control cells. Comparisons were made using a Paired one-tailed *t* test (*p<0.05, ** p<0.01, ***p<0.001).

Figure S6, related to Figure 7

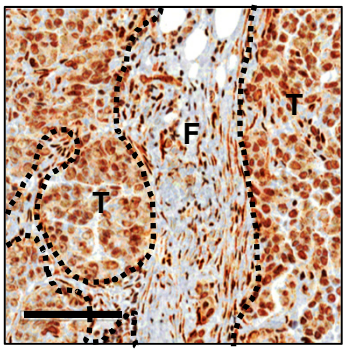
A

HGSOC omental metastasis

Patient# 1

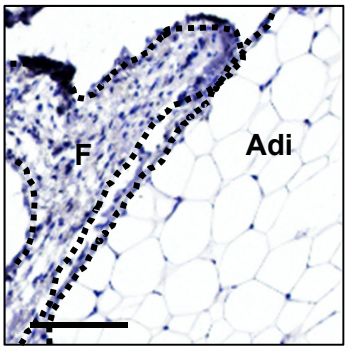


Patient# 2

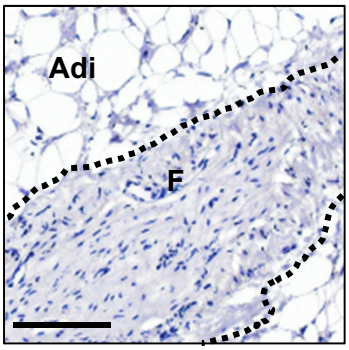


Normal omentum

Patient# 1

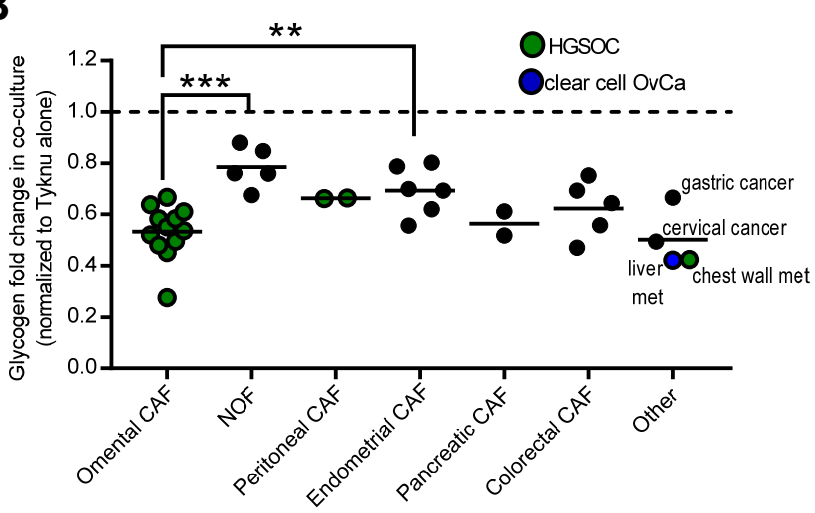


Patient# 2



p-p38 IHC scoring	HGSOC omental metastasis	Normal omentum
Positive cases	50/50	0/10
Average cells percent positive/case (SD)	86.5 (16.5)	
Average score 0-3 (SD)	1.8 (0.56)	

B



C

H&E staining

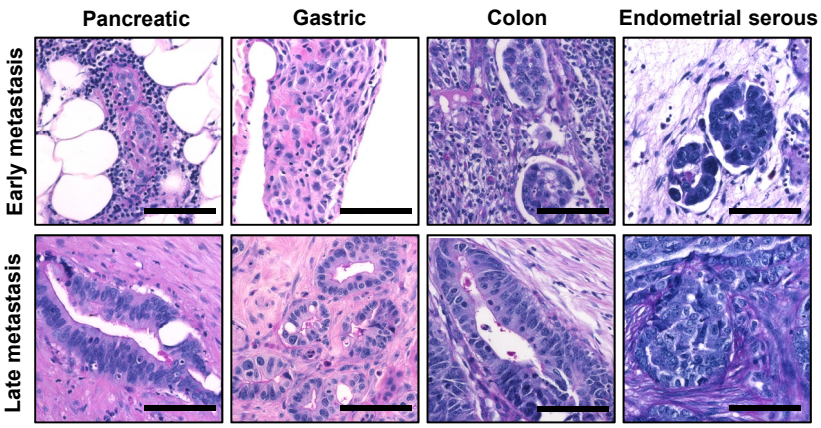


Figure S6, related to Figure 7. p-p38α in human metastasis and glycogen content in human cancer.

(**A**) Representative images of p-p38α MAPK immunostaining in the stroma of patients with invasive OvCa and omentum from normal patients 'F' indicates areas of fibroblasts, 'T' indicates areas of tumor cells, 'Adi' indicated adipocytes. Scale bar is equal to 100μm l*eft)*. Analysis of immunohistochemical phospho-p38α staining in 50 HGSOC omental tumors *(right*). Digital scoring was done on fibroblasts in the stroma using Aperio software and results were confirmed by a gynecologic pathologist. Ten normal omentums from human patients were also reviewed by a pathologist for p-38α staining in fibroblasts. SD – standard deviation.

(**B**) Glycogen assay on TYK-nu cells co-cultured with primary CAFs isolated from the indicated tumor types originating from their primary sites within the abdominal cavity. Subtypes of ovarian tumors are indicated by color. Data is normalized to TYK-nu alone set at 1 and is indicated by the dashed line. Each dot represents primary CAFs isolated from an individual patient. Normal omental fibroblasts (NOF) extracted from patients without cancer were used as control. Comparisons with omental CAFs were made using an unpaired, two-way, Mann Whitney test, **p< 0.01, ***p< 0.001. HGSOC, high grade serous ovarian cancer.

(**C**) Representative histology (H&E stain) of early and late omental metastases of pancreatic, gastric, colon, and endometrial serous cancers demonstrating the distribution of the fibroblast rich stroma in the cases used in Figure 7B. Scale bar = 100μm.

Table S3, related to Figure 1

a

OvCa pY Results

Gene name	phosphosite	log2 fold increase	Q-value
PXN	S85Y88*	4.03	0.007
LDHA	Y10	3.69	0.0114
MAPK1	Y187*	3.66	0.0057
PXN	Y88*	3.32	0.009
ANXA2	Y318*	3.06	0.0193
LPP	Y317*	2.21	0.0067
BCAR1	Y234*	2.19	0.0796
MYH9	Y754*	2.11	0.085
PTK2	Y598Y599*	2.00	0.0193
PPP1R12A	Y911*	1.99	0.0612
DYRK1A	Y273*	1.88	0.0121
PGM1	Y353	1.87	0.0543
ANXA2	Y317*	1.82	0.0445
EEF1A1	Y29*	1.81	0.0405
PEAK1	Y387	1.78	0.0207
PXN	Y118*	1.71	0.0021
PTK2	T597Y599*	1.52	0.0067
ARHGAP12	T231S240Y243*	1.51	0.0976
ARHGAP12	T230S240Y243*	1.51	0.0976
ARHGAP42	Y376	1.50	0.0651
TLN1	Y26*	1.44	0.0268
TLN2	Y1665*	1.36	0.007
PARD3	Y388*	1.23	0.0067
ERBB2IP	Y1104*	1.12	0.088
GSK3B	Y216S219*	1.10	0.0068
BCAR1	Y128*	1.04	0.0687
CDK1	T14Y15*	1.04	0.009
KIAA1598	Y24	-3.18	0.0182
PAG1	Y359*	-2.47	0.0023
TUBB2C	Y51	-2.32	0.0021
DCBLD2	Y732	-2.06	0.0021
DSP	Y56*	-1.89	0.0194
CFL1	Y89*	-1.69	0.0021
ATP1A1	Y260*	-1.69	0.005
APP	Y762*	-1.64	0.0044
PAG1	Y417*	-1.43	0.0021
VIM	Y276	-1.43	0.0093
SEPT7	Y319*	-1.40	0.0145
APP	Y757Y762*	-1.40	0.0232
RAB34	Y247	-1.36	0.0126
KRT8	Y267*	-1.36	0.0329
CLDN3	Y198	-1.36	0.0568
SLC7A11	Y15	-1.29	0.0048
EIF3L	Y36	-1.29	0.0145
TGM2	Y369*	-1.25	0.0232
APP	Y757*	-1.18	0.0505
KIRREL	Y461*	-1.15	0.0023
KIRREL	Y458*	-1.15	0.0023
EZR	Y354*	-1.15	0.0177
CAV1	Y25	-1.12	0.0021
CTNND1	Y803	-1.09	0.0067
PPP1R12A	Y766	-1.09	0.0121
LDHA	Y239*	-1.09	0.0755
PKP4	Y306	-1.06	0.0048
HNRNPA0	Y180*	-1.06	0.0117
HIPK2	Y352*	-1.06	0.0121
KIRREL	Y560*	-1.03	0.0021
S100A11	Y30*	-1.03	0.0145
SLC25A4	Y191	-1.03	0.0932

b

CAF pY Results

Gene name	phosphosite	log2 fold increase	Q-value
EPHA2	Y588Y594*	4.15	0.021
EPHA2	Y960	3.05	0.021
BCAR1	Y128*	2.69	0.0466
EPHA2	Y594*	2.57	0.0444
DYRK1A	Y273*	2.34	0.0444
ARHGAP42	Y376	2.28	0.0263
ARHGAP42	Y792	2.27	0.021
PXN	S85Y88*	1.66	0.0475
NEDD9	Y345*	1.51	0.021
AHNAK	Y160	1.51	0.0878
PPP1R12A	Y911*	1.49	0.0643
MAPK14	Y182*	1.45	0.0444
KIRREL	Y557*	1.33	0.0704
TLN1	Y70*	1.24	0.0444
PLD3	Y7	-2.32	0.0067
ITGB4	Y1207*	-1.69	0.0153
TSG101	Y390	-1.40	0.0153
PSMA2	Y57*	-1.36	0.0678
ALB	Y164*	-1.25	0.0446
PKP4	Y420*	-1.00	0.0217

Table S3. Significant changes in phosphopeptides detected by quantitative phosphoproteomics

a, Significantly altered phosphopeptides in OvCa cells after co-culture with CAFs.

b, Significantly altered phosphopeptides in CAFs after co-culture with OvCa cells. The Q-value is an estimate of the false discovery rate (FDR) based on the calculated p-values corrected for multiple hypothesis testing. Red indicates increased phophosite while, green indicates decreased phosphosite. Asterisks indicate a known Human Protein Reference Database (HPRD) phosphorylation site.