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Supplemental Information

Mesothelial Cell HIF1α Expression Is Metabolically Downregulated by Metformin to Prevent Oncogenic Tumor-Stromal Crosstalk

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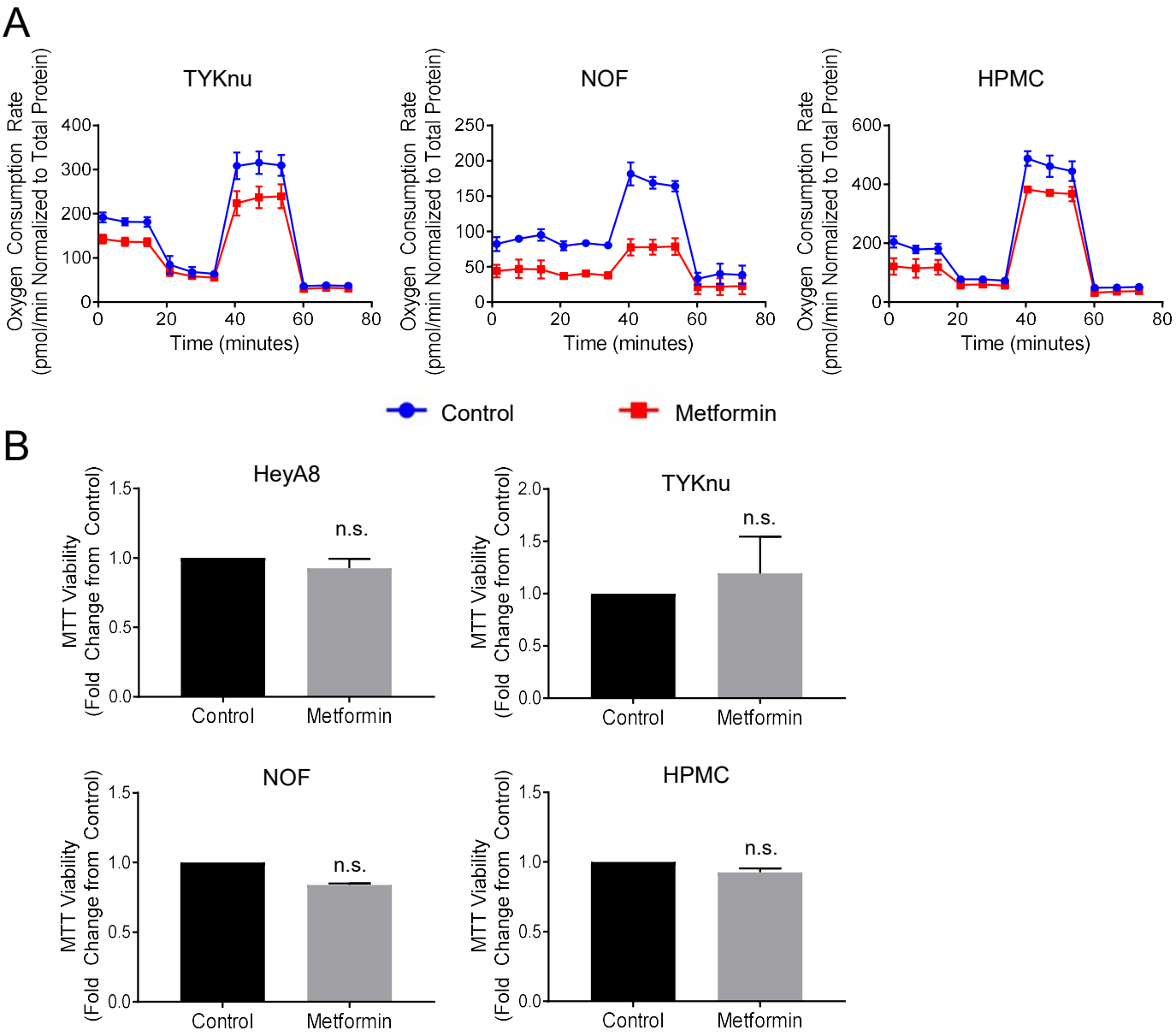
Figure S1

For Figure 1



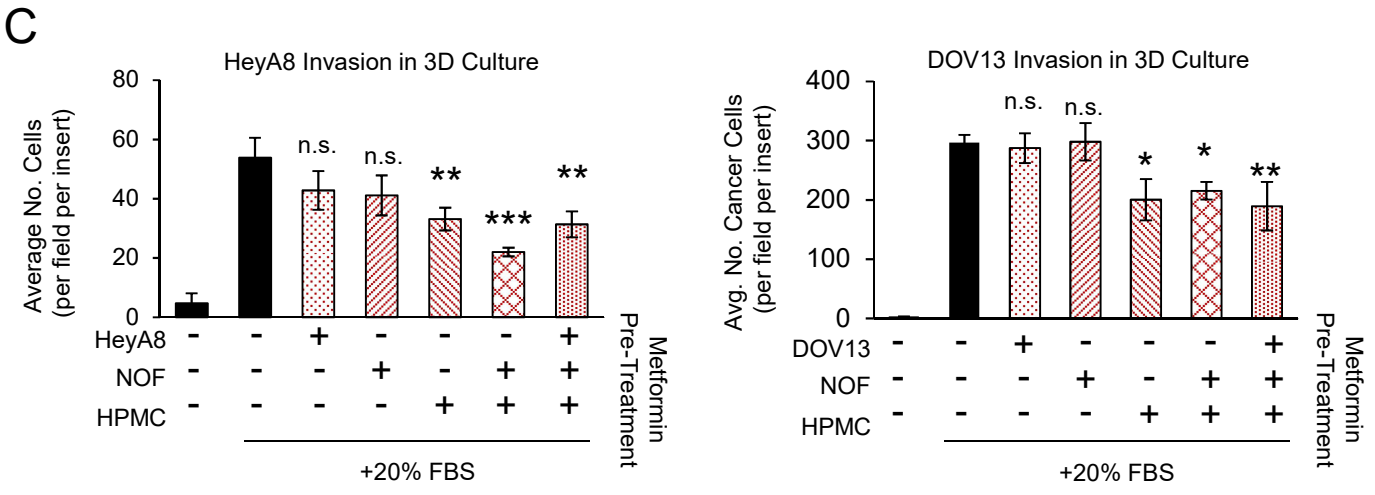
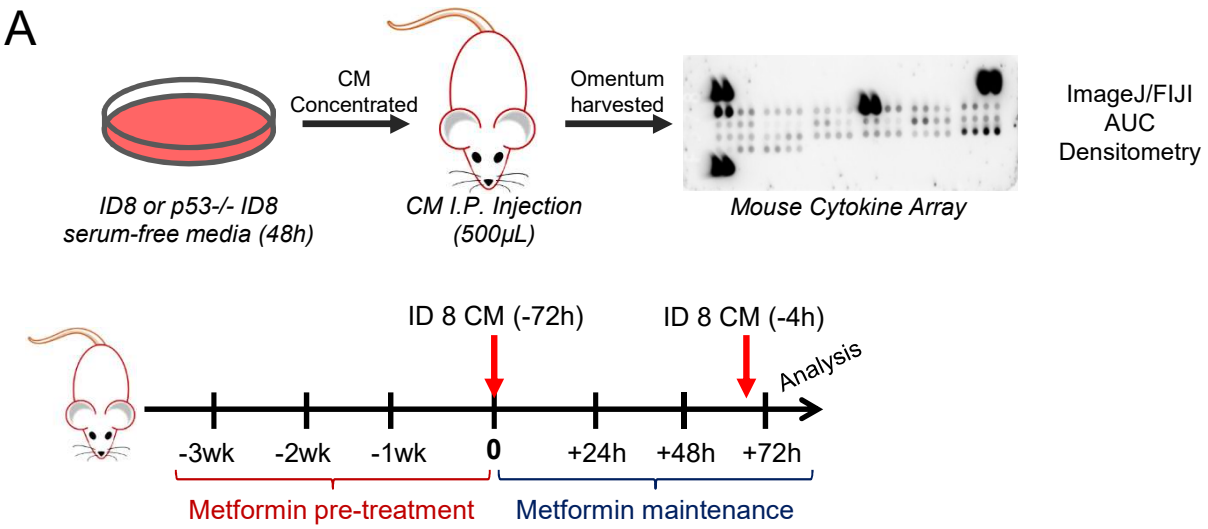


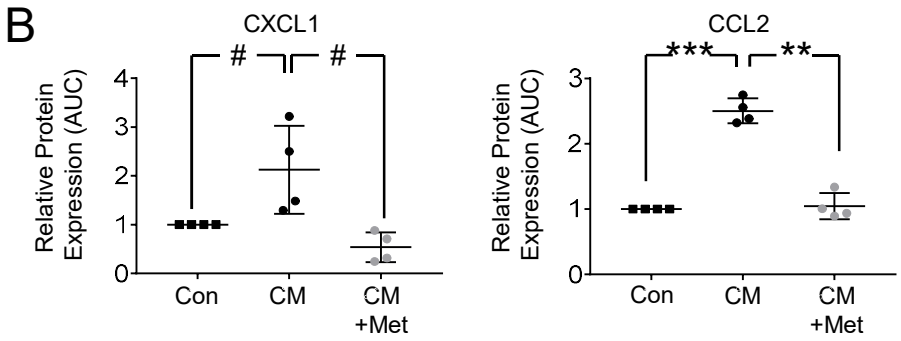
Figure S1 – (Related to Figure 1)

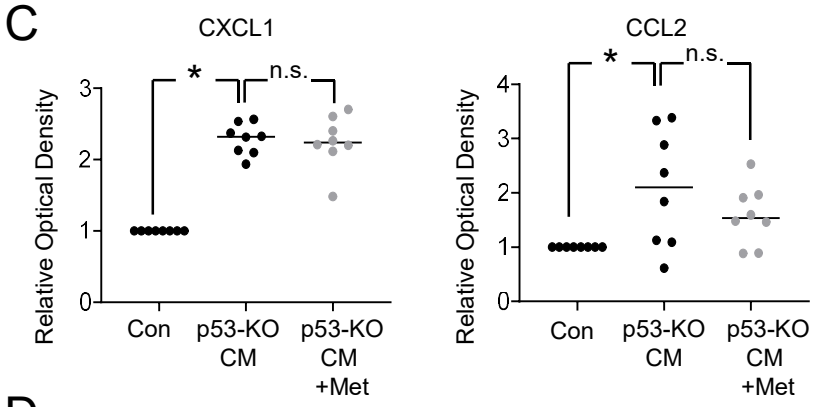
(A) Metformin targets mitochondrial respiration similarly across tumor cells, omental fibroblasts and mesothelial cells. Oxygen consumption rate (OCR)/oxidative phosphorylation measured by Seahorse electron flux assay in TYKnu OvCa cells, fibroblasts (NOFs) and mesothelial cells (HPMCs) assessing the canonical activity of metformin as a Complex I inhibitor (n = 2 patients in quadruplicate). **(B) Low dose of metformin does not affect cell viability in tumor cells, omental fibroblasts or mesothelial cells.** MTT viability assays in HeyA8, TYKnu, NOF or HPMC (n = 2 per group/patient in triplicate). **(C) Metformin inhibits HeyA8 and DOV13 OvCa cell invasion in the TME in a mesothelial cell dependent manner.** Invasion through 3D model after seeding HeyA8 cells (15h) or DOV13 (36h). Cells were individually treated with metformin (250μM, 72h) and then added to 3D model (n = 3 patients in triplicate). Data represent mean value ± S.D. * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.005$; n.s. = not significant.

Figure S2

For Figure 2







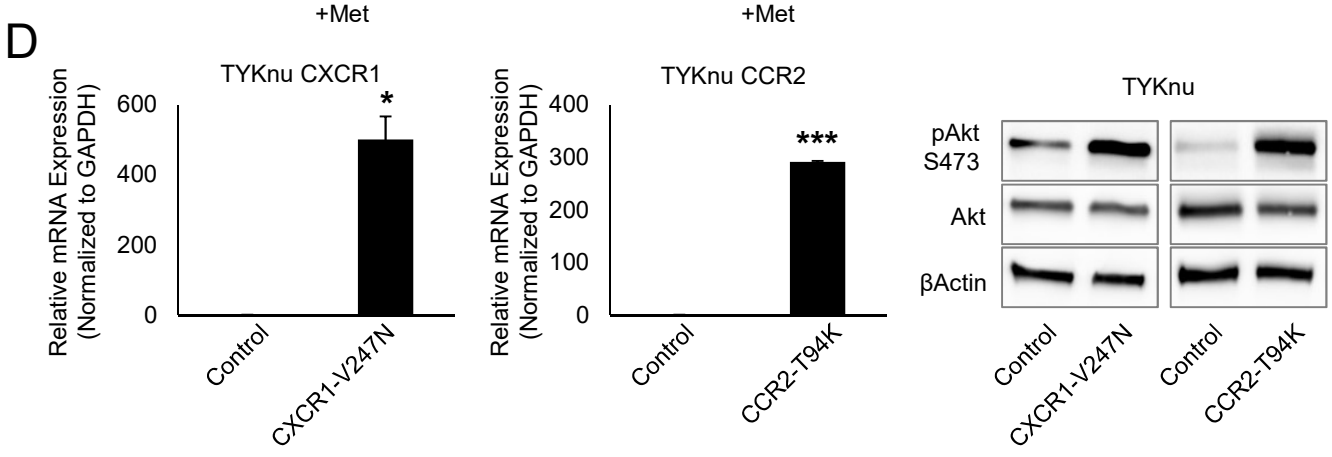


Figure S2 – (Related to Figure 2)

(A) Experimental design for *in vivo* omentum cytokine assessment. Mice were treated with metformin (300mg/kg, p.o.) for 3 weeks prior to i.p. injection of ID8 mouse tumor cell conditioned media (CM) at the indicated times. Mouse omentum was then harvested and cytokine production was measured using a cytokine array. **(B) Quantification of murine omental CXCL1 and CCL2 protein expression.** Quantification (densitometry AUC) of cytokine array (from Figure 2A) blot showing CXCL1 and CCL2 levels (n = 2 mice per group with duplicate AUC measurements). **(C) ELISA measurement of CXCL1 or CCL2 protein expression in murine omentum**. ID8 $p53^{-/-}$ derived CM was i.p. injected into mice pre-treated with metformin, and relative protein expression (normalized absorbance) of CXCL1 and CCL2 from mouse omentum was analyzed. (n = 8 mice per group). **(D) Validation of CXCR1 and CCR2 overexpression in ovarian cancer cells.** Left: qRT-PCR of either CXCR1 (IL8 receptor) or CCR2 (CCL2 receptor) in TYKnu cells transfected with constitutively active mutants (CXCR1-V247N or CCR2-T94K, respectively) (n = 3 per group). Right: Western immunoblot assessing active Akt at serine 473 (S473) phosphorylation in CXCR1 and CCR2 mutant cell lines (representative blot from n = 3 per group). Data represent mean value ± S.D. # = $p < 0.09$, * = $p < 0.05$, ** = $p < 0.01$ and *** = $p < 0.005$; n.s. = not significant.

Figure S3

For Figure 3

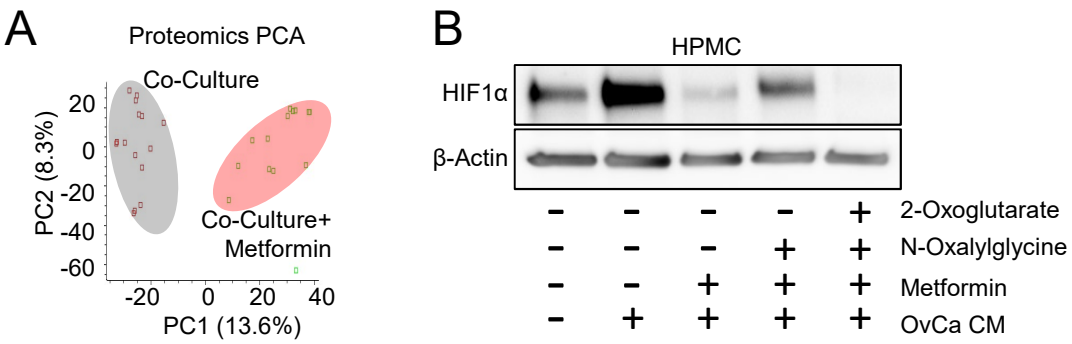
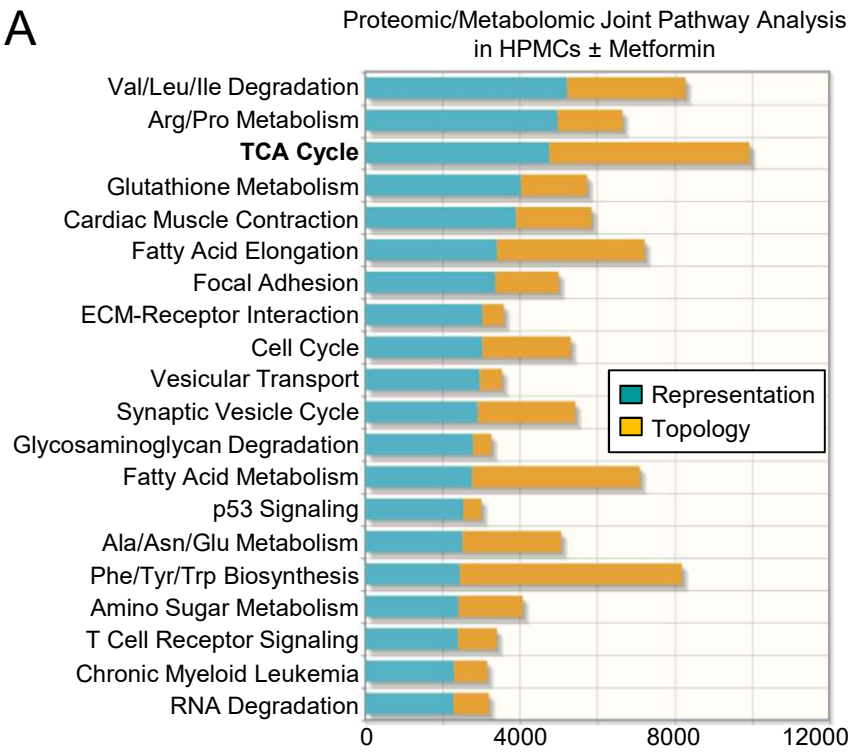


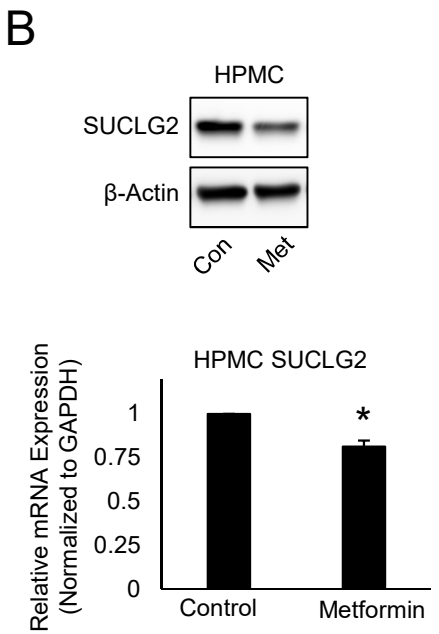
Figure S3 – (Related to Figure 3)

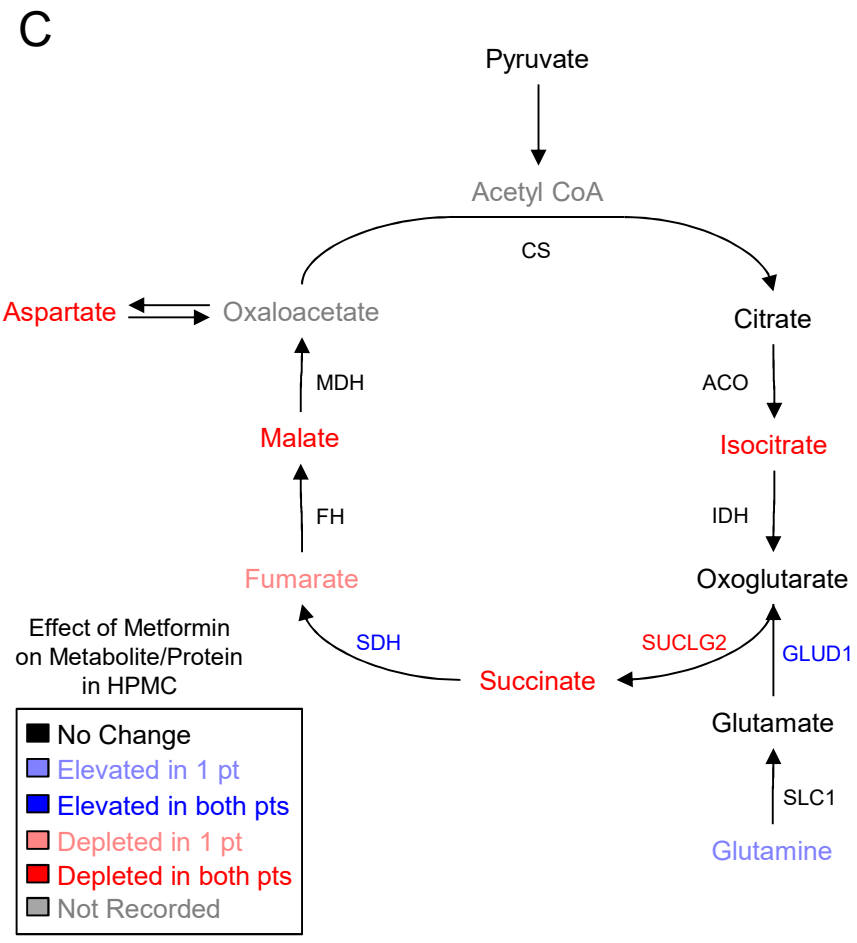
(A) Principal component analysis (PCA) of data presented in Figure 3A. Characterization of proteomic profiles obtained by mass spectrometry of mesothelial cells (HPMCs) co-cultured with TYKnu OvCa cells and treated with metformin (1mM, 48h) (n = 4 patients in triplicate; each box represents an individual sample/replicate). **(B) Modulation of HIF1α levels in mesothelial cells using pharmacologic manipulation of PHDs.** Western blot of HIF1α expression in HPMCs treated with TYKnu OvCa CM in the presence of the indicated compounds: metformin (1mM) ± the PHD antagonist N-oxalylglycine (1mM) ± the PHD substrate 2-oxoglutarate (5mM) for 48h (representative blot from n = 2 patients in duplicate).

Figure S4

For Figure 4







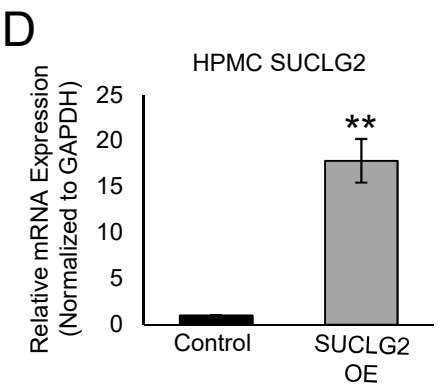


Figure S4 – (Related to Figure 4)

Metabolomic and proteomic profiling of mesothelial cells treated with metformin. (A) Pathway representation determined by Joint Pathway Analysis, which integrates proteomic and metabolomics data from Figures 4A and 4B. Shown are significantly altered metabolites and proteins in HPMCs treated with metformin (1mM, 48h). Representation is the number of overlapping proteins and metabolites per pathway, while topology refers to the strength of interactions between represented proteins/metabolites in a given pathway. **(B)** Top: Effect of metformin on SUCLG2 protein expression in HPMCs was verified by Western blot (representative blot from n = 2 patients in duplicate). Bottom: qRT-PCR of SUCLG2 mRNA expression in HPMCs treated with metformin (1mM) for 72h (n = 2 patients in duplicate). **(C)** Schematic representation showing alterations of primary TCA metabolites and their key regulatory enzymes by metformin (see **Supplementary Tables 3 and 4**). Metabolites/proteins in blue are significantly increased in HPMCs from both patients, decreased in red, unchanged in black and undetected/unrecorded in gray. Pale red or blue was used for fumarate and glutamine, respectively, as only one of the two patients had significant alteration in these metabolites. **(D)** qRT-PCR of SUCLG2 mRNA expression in control and SUCLG2 transfected HPMCs (n = 2 patients in duplicate). Data represent mean value ± S.D. * = $p < 0.05$ and ** = $p < 0.01$.

Figure S5

For Figure 5

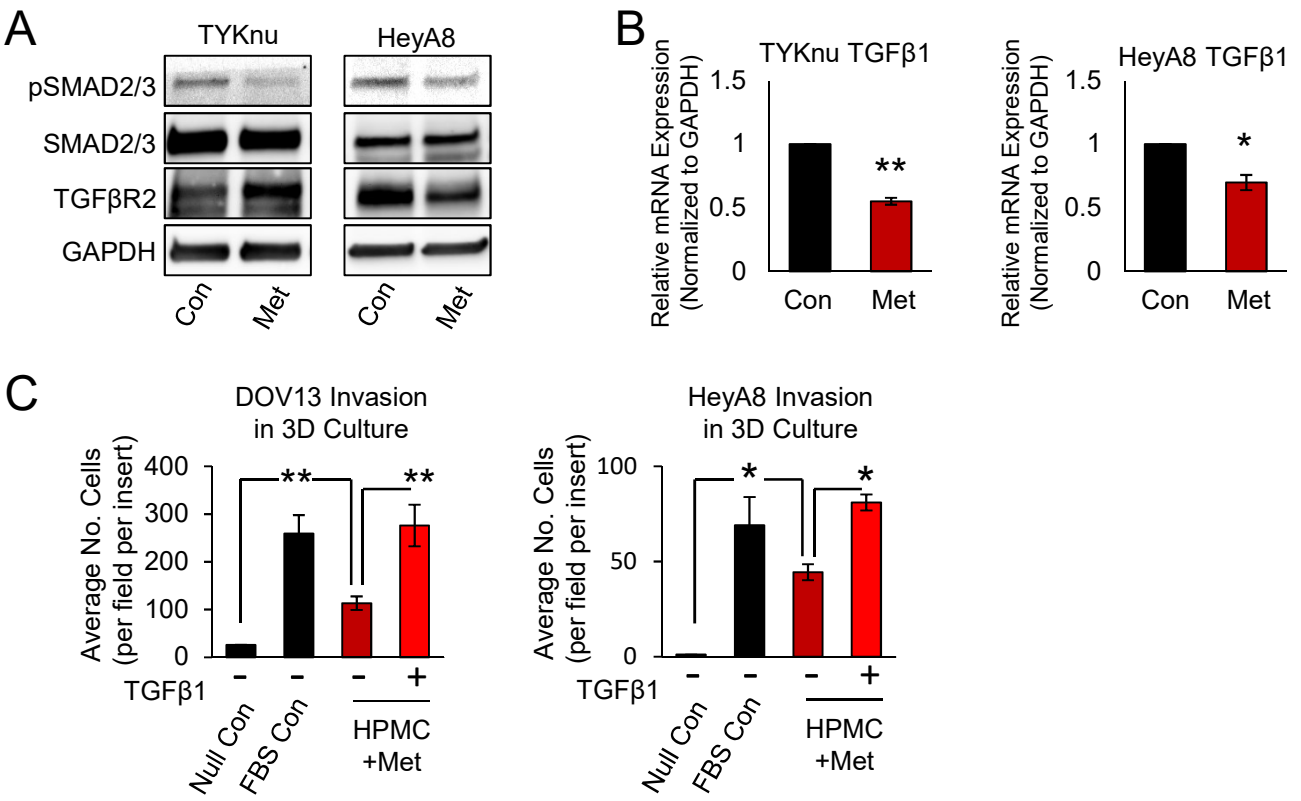


Figure S5 – (Related to Figure 5)

Metformin inhibits TGFβ1 signaling in ovarian cancer cell lines. (A) Western blot of TGFβ-related proteins in TYKnu and HeyA8 OvCa cells ± metformin (1mM, 72h) (representative blot from n = 6 per group). **(B)** qRT-PCR of TGFβ1 mRNA expression in TYKnu and HeyA8 OvCa cells treated with metformin (1mM, 72h) (n = 3 per group in duplicate). **(C)** Invasion of DOV13 (36h) or HeyA8 (15h) through the 3D model ± metformin pretreatment of HPMCs (250μM for 72h) ± recombinant human TGFβ1 protein (n = 2 patients in triplicate). Data represent mean value ± S.D. * = $p < 0.05$ and ** = $p < 0.01$.