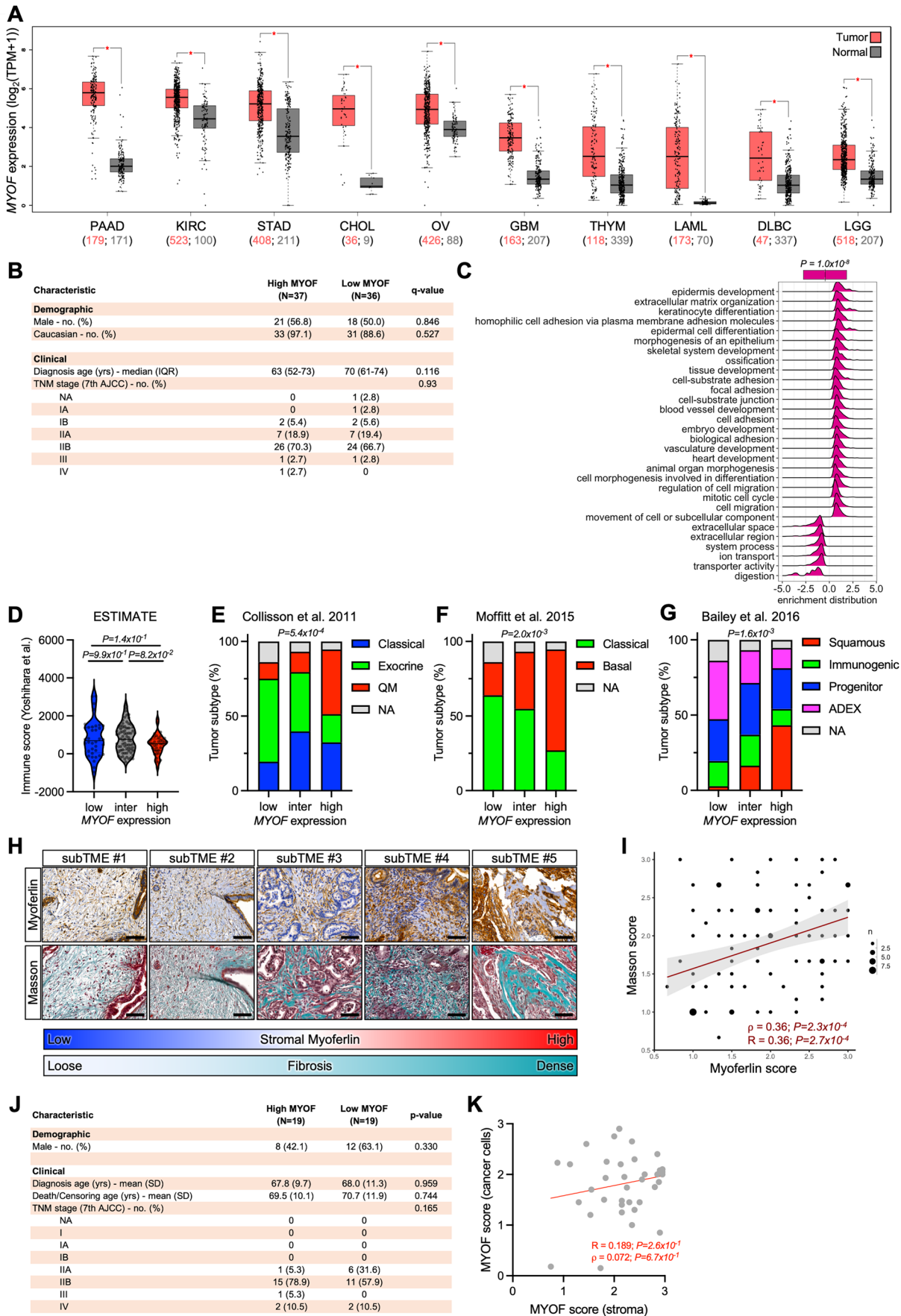


Expanded View Figures

Figure EV1. Myoferlin correlates with stromal features in pancreatic cancer.

(A) *MYOF* expression in human matched healthy and neoplastic tissue, ranked according to tumor *MYOF* expression. PAAD = pancreatic adenocarcinoma, KIRC = kidney renal clear cell carcinoma, STAD = stomach adenocarcinoma, CHOL = cholangiocarcinoma, OV = ovarian serous cystadenocarcinoma, GBM = glioblastoma, THYM = thymoma, LAML = acute myeloid leukemia, DLBC = diffuse large B-cell lymphoma, LGG = lower grade glioma. Patient numbers are indicated for each group. Boxplot (P25-1.5*IQR; P25; Median; P75; P75 + 1.5*IQR). * $P < 0.01$. (B) TCGA PAAD cohort patient characteristics, low *MYOF* ($n = 36$) versus high *MYOF* ($n = 37$) patients. Chi-squared test for demographic characteristics and TNM stage; Wilcoxon test for diagnosis age. (C) Ridgeplot of most enriched gene sets ($P < 0.0001$) in *MYOF*^{high} patients. (D) ESTIMATE immune scores in TCGA PAAD cohort patients ($n = 146$) according to *MYOF* expression. Violin plot, one-way ANOVA (Tukey's test). (E) Percentages of tumor subtypes (Collisson et al, 2011) according to *MYOF* expression (QM = quasi-mesenchymal). Stacked bar plot, Chi-squared test. (F) Percentages of tumor subtypes (Moffitt et al, 2015) according to *MYOF* expression. Stacked bar plot, Chi-squared test. (G) Percentages of tumor subtypes (Bailey et al, 2016) according to *MYOF* expression. Stacked bar plot, Chi-squared test. (H) Myoferlin IHC and Masson trichrome staining (collagens stained in blue) in human PAAD sections. Representative images from 99 subTME regions across 33 patients. Scale bar = 100 μ m. (I) IHC analysis, correlation and linear regression between Masson trichrome scores and Myoferlin stromal scores ($n = 99$). Pearson (R) and Spearman (ρ) correlation. (J) Internal PAAD cohort patient characteristics, *MYOF*^{low} stroma ($n = 19$) versus *MYOF*^{high} stroma ($n = 19$) patients. Chi-squared test for demographic characteristics and TNM stage; Wilcoxon test for diagnosis age and death/censoring age. (K) Linear regression between stromal myoferlin IHC scores and tumor cell myoferlin IHC scores of PAAD patients ($n = 38$). Pearson (R) and Spearman (ρ) correlation. Source data are available online for this figure.



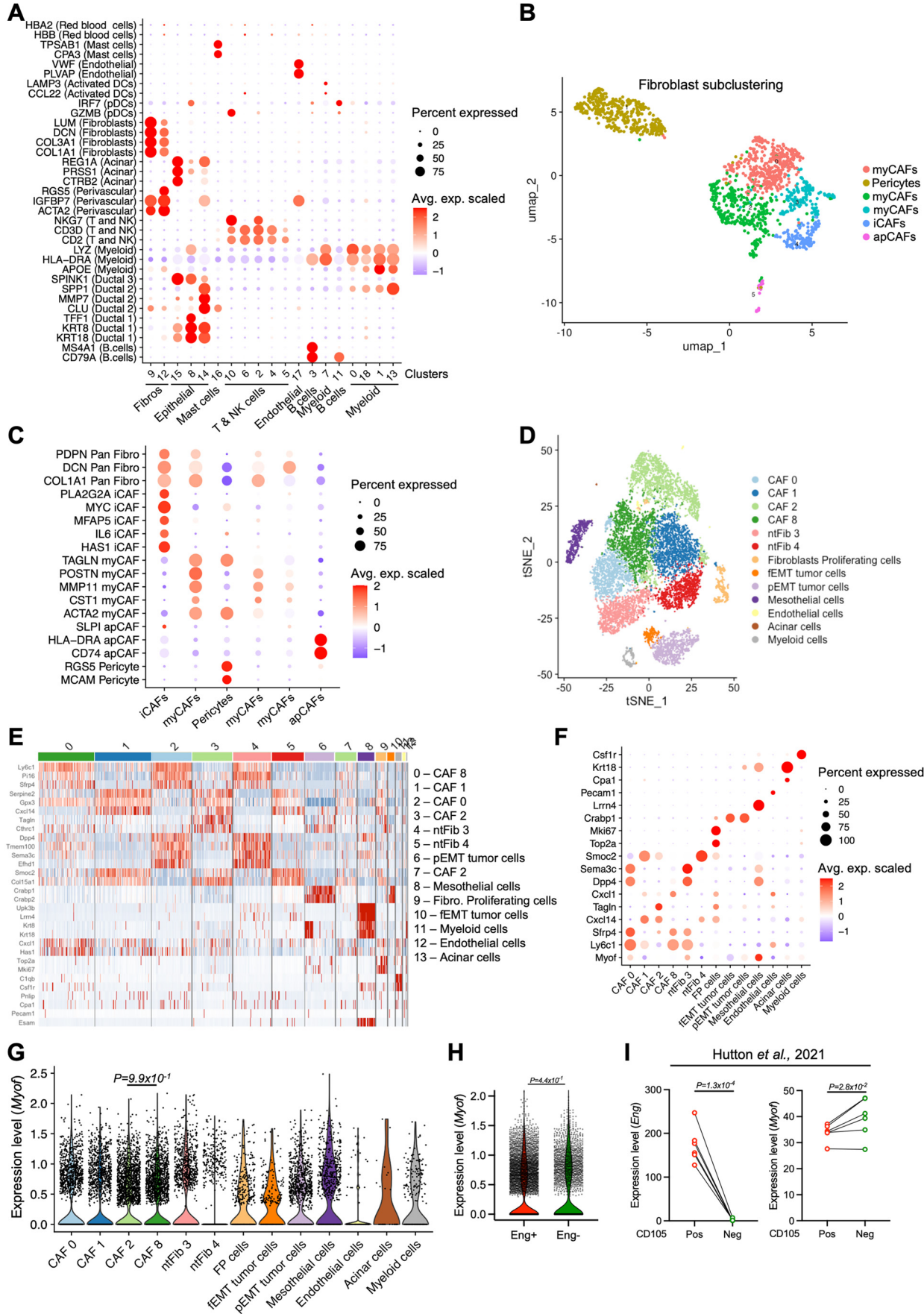
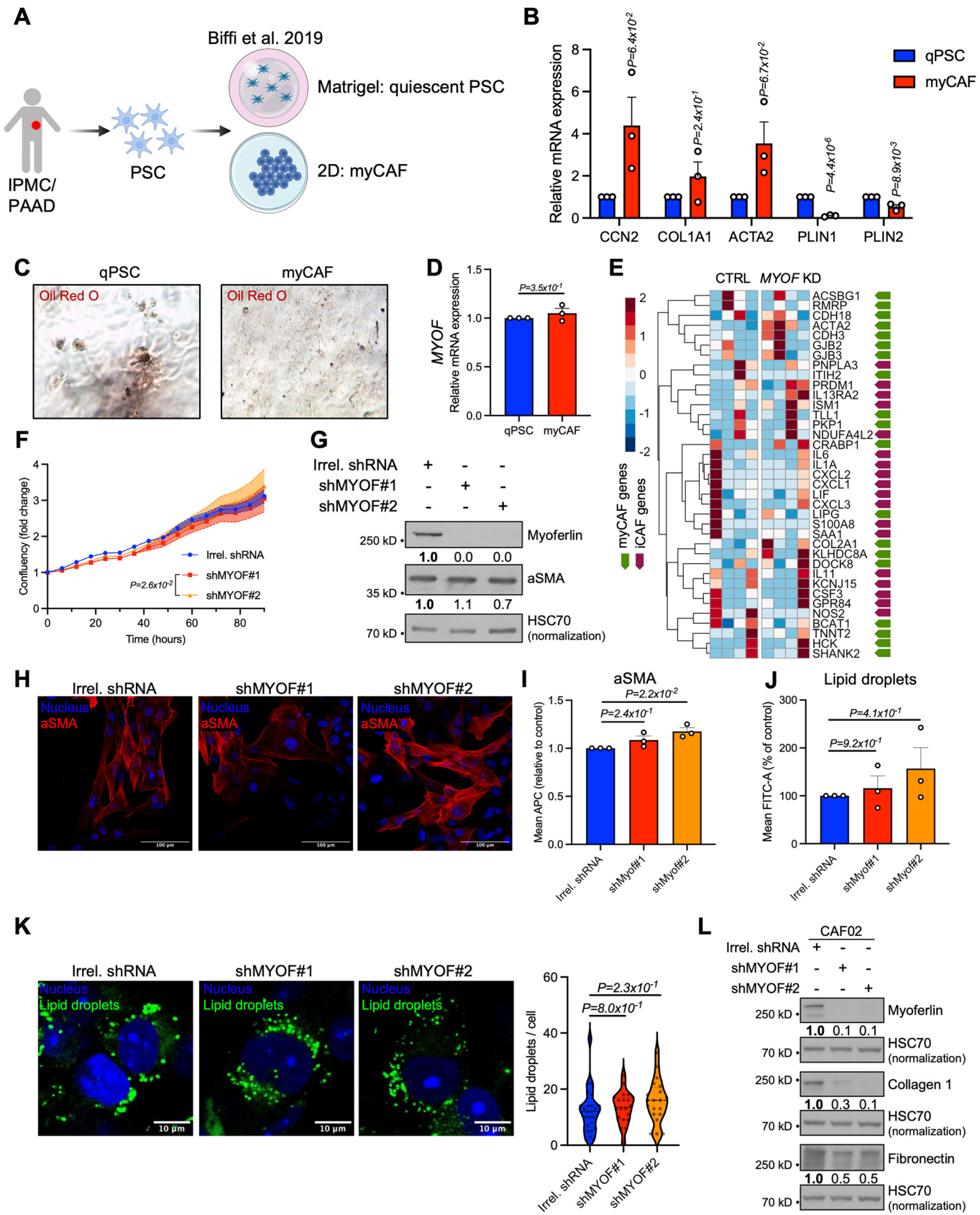


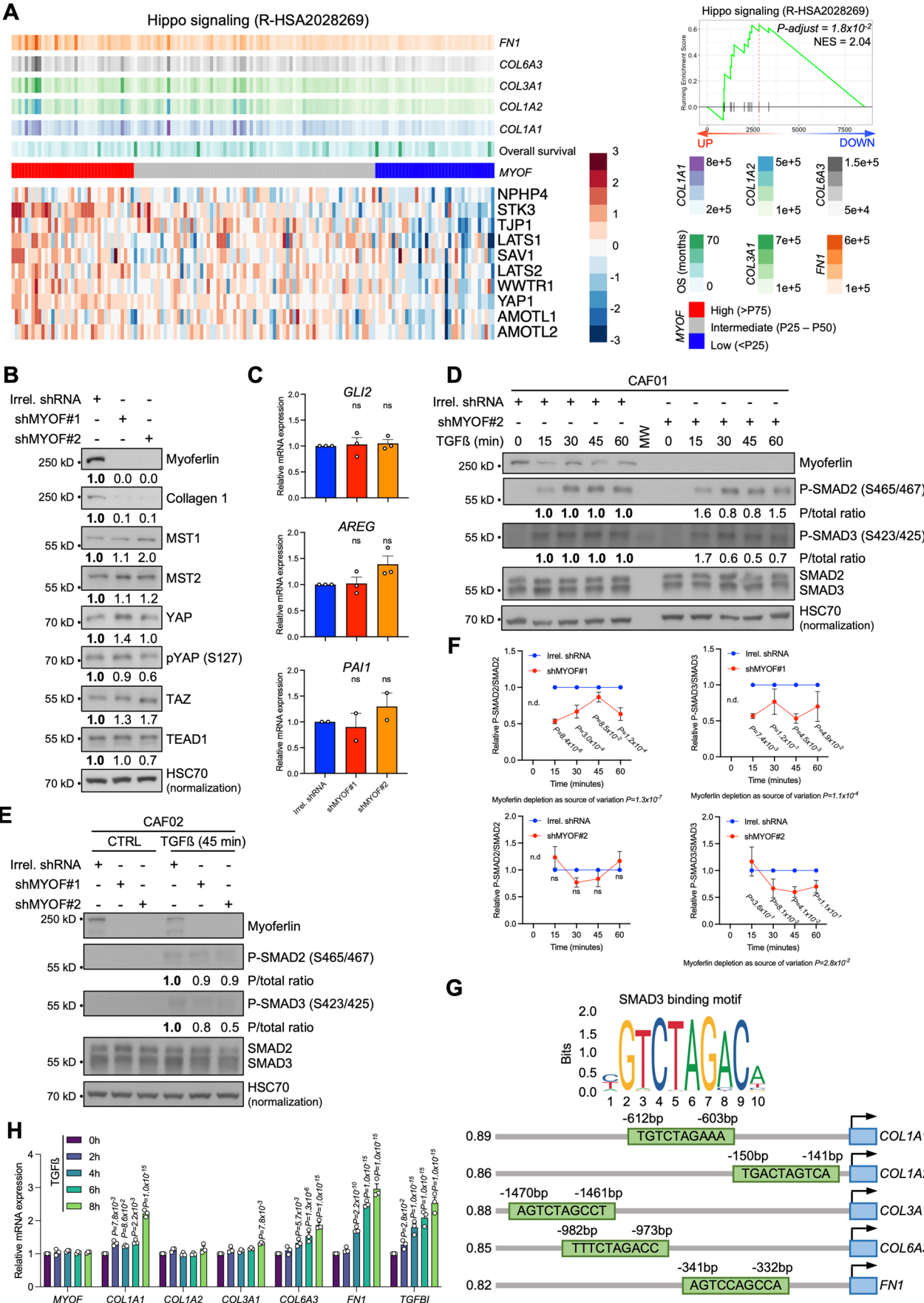

Figure EV2. Myoferlin expression in pancreatic cancer at single cell resolution.

(A) Marker genes for broad cell type labeling from Fig. 2A. Dot plot. (B) UMAP plot for fibroblasts subclustering from human PAAD scRNAseq data. (C) Marker genes for fibroblasts subclustering from (B). Dot plot. (D) t-SNE plot and cluster labeling for murine PAAD scRNAseq data. iCAF lineage: ntFib3 = normal Fibroblasts; CAF0 = early CAFs; CAF8 = IL1 CAFs. myCAF lineage: ntFib4 = normal Fibroblasts; CAF1 = early CAFs; CAF2 = TGF β CAFs. (E) Marker genes for clusters from (D). Heatmap. (F) *Myof* expression and marker genes for clusters from (D). Dot plot. (G) *Myof* expression in clusters from (D). Violin plot, pairwise comparisons using Wilcoxon Rank test (Bonferroni correction). (H) Normalized *Myof* expression levels in fibroblasts clustered according to CD105 (Eng) expression (0.05 threshold for Eng +). Violin plot, pairwise comparisons using Wilcoxon Rank test (Bonferroni correction). (I) Normalized *Eng* and *Myof* expression levels in CyTOF-isolated murine CD105^{pos} and CD105^{neg} CAFs. Paired *T* test. Source data are available online for this figure.



◀ **Figure EV3. Myoferlin knockdown does not induce CAF quiescence.**

(A) Culture model for PSC-derived myCAFs. (B) RT-qPCR analysis of *CCN2*, *COL1A1*, *ACTA2*, *PLIN1* and *PLIN2* mRNA levels in qPSCs ($n = 3$) and myCAFs ($n = 3$). Mean $\pm$ SEM, unpaired T test (P values relative to qPSC). (C) Oil Red O staining of lipid droplets in qPSCs and myCAFs. (D) RT-qPCR analysis of *MYOF* mRNA levels in qPSCs ($n = 3$) and myCAFs ($n = 3$). Mean $\pm$ SEM, unpaired T test. (E) Heatmap and unsupervised clustering of myCAF and iCAF signature genes in CTRL myCAFs ($n = 4$) and *MYOF*^{KO} myCAFs (shMYOF#1; $n = 4$). Gene expression values are z-score normalized. (F) Confluency-based cell proliferation analysis of CTRL myCAFs (Irrel. shRNA; $n = 3$) and *MYOF*^{KO} myCAFs (shMYOF#1 and shMYOF#2; $n = 3$ each). Mean $\pm$ SEM (dashed lines), two-way ANOVA (Tukey's test). (G) Western blot analysis of CTRL myCAFs (Irrel. shRNA) and *MYOF*^{KO} myCAFs (shMYOF#1 and shMYOF#2). HSC70 was used as loading control. (H) Immunofluorescence microscopy of CTRL myCAFs (Irrel. shRNA) and *MYOF*^{KO} myCAFs (shMYOF#1 and shMYOF#2). Representative pictures are shown. Nuclei = blue, α SMA = red, scale bar = 100 μ m. (I) Flow cytometry analysis of α -SMA (APC-conjugated antibody) in CTRL myCAFs (Irrel. shRNA; $n = 3$) and *MYOF*^{KO} myCAFs (shMYOF#1 and shMYOF#2; $n = 3$ each). Mean APC signal was expressed as % of control (Irrel. shRNA). Mean $\pm$ SEM, one-way ANOVA (Dunnett's test). (J) Flow cytometry analysis of lipid droplets (FITC-Bodipy staining) in CTRL myCAFs (Irrel. shRNA; $n = 3$) and *MYOF*^{KO} myCAFs (shMYOF#1 and shMYOF#2; $n = 3$ each). Mean FITC-A signal was expressed as % of control (Irrel. shRNA). Mean $\pm$ SEM, one-way ANOVA (Dunnett's test). (K) Confocal microscopy analysis of lipid droplets (FITC-Bodipy staining) in CTRL myCAFs (Irrel. shRNA; $n = 24$ cells) and *MYOF*^{KO} myCAFs (shMYOF#1 and shMYOF#2; $n = 20$ each). Scale bar = 10 μ m. Violin plot, one-way ANOVA (Sidak's test). (L) Western blot analysis (CAF02) of total-cell lysates from CTRL myCAFs (Irrel. shRNA; $n = 3$) and *MYOF*^{KO} myCAFs (shMYOF#1 and shMYOF#2; $n = 3$ each). One representative western blot of three independent experiments is shown, HSC70 was used as loading control. Source data are available online for this figure.



◀ **Figure EV4. TGF β signaling but not Hippo signaling is altered upon myoferlin knockdown.**

(A) GSEA analysis and heatmap of TCGA PAAD cohort patients ($n = 146$) and Hippo-related genes ($P < 0.05$). Patients are segregated based on *MYOF* expression. P value assessed via Benjamini-Hochberg procedure. (B) Western blot analysis of total-cell lysates (CAF01) from CTRL myCAFs (Irrel. shRNA; $n = 1$) and *MYOF*^{KD} myCAFs (shMYOF#1 and shMYOF#2; $n = 1$ each). HSC70 was used as loading control. (C) RT-qPCR analysis (CAF01) of *GLI2*, *AREG*, and *PAI1* mRNA levels in CTRL myCAFs (Irrel. shRNA; $n \geq 2$) and *MYOF*^{KD} myCAFs (shMYOF#1 and shMYOF#2; $n \geq 2$ each). Mean $\pm$ SEM, one-way ANOVA (Tukey's test). (D) Western blot analysis of total-cell lysates (CAF01) from CTRL myCAFs (Irrel. shRNA; $n = 3$) and *MYOF*^{KD} myCAFs (shMYOF#2, $n = 3$) stimulated with human recombinant TGF β 1 (5 ng/mL) for indicated timepoints. One representative western blot of three independent experiments is shown, HSC70 was used as loading control. (E) Western blot analysis of total-cell lysates (CAF02) from CTRL myCAFs (Irrel. shRNA; $n = 3$) and *MYOF*^{KD} myCAFs (shMYOF#1 and shMYOF#2, $n = 3$ each) stimulated with human recombinant TGF β 1 (5 ng/mL) for 45 min. One representative western blot of three independent experiments is shown, HSC70 was used as loading control. (F) Quantification of Fig. 4D and (D). Mean $\pm$ SEM, two-way ANOVA (full model including time effect, shRNA effect and interaction effect). Individual pair comparisons were performed using the Fisher's LSD test, P value relative to control group (Irrel. shRNA). (G) SMAD3 consensus binding motif and predicted binding sites in promoter regions of *COL1A1*, *COL1A2*, *COL3A1*, *COL6A3* and *FN1* genes. Relative binding site scores were computed with Jaspar. (H) RT-qPCR analysis (CAF01) of *MYOF*, *COL1A1*, *COL1A2*, *COL3A1*, *COL6A3*, *FN1* and *TGFBI* mRNA levels in TGF β 1 (5 ng/mL) stimulated CTRL myCAFs (Irrel. shRNA). Mean $\pm$ SEM (3 technical replicates), two-way ANOVA (Tukey's test; P values relative to 0 h condition for each gene). Source data are available online for this figure.



◀ **Figure EV5. Myoferlin knockdown impairs TGFBR1 trafficking and activity.**

(A) Cycloheximide (CHX) chase upon TGFBR1 stimulation and western blot of total-cell lysates (CAF01) from CTRL myCAFs (Irrel. shRNA) and MYOF^{KD} myCAFs (shMYOF#1 and shMYOF#2). HSC70 was used as loading control. (B) COPII vesicle trafficking gene expression (*SAR1A*, *SEC13*, *SEC31A*, *SEC23A*, *SEC24A*, *SEC24B*, *SEC24C*) in human healthy pancreas (grey) and PAAD tissue (red). Boxplot (P25-1.5*IQR; P25; Median; P75; P75 + 1.5*IQR). **P* < 0.05. (C) Western blot analysis of total-cell lysates (CAF01) from CTRL myCAFs (Irrel. shRNA, *n* = 1) and MYOF^{KD} myCAFs (shMYOF#1 and shMYOF#2, *n* = 1 each). HSC70 was used as loading control. (D) Immunofluorescence microscopy of myCAFs (CAF02). Representative pictures are shown. Nuclei = blue, COPII vesicles (Sec24C) = green, myoferlin (MYOF) = red, scale bar = 50 μm. (E) Immunofluorescence microscopy of myCAFs (CAF02). Representative pictures are shown. Nuclei = blue, TGFBR1 = green, myoferlin (MYOF) = red, scale bar = 50 μm. (F) Proximity ligation assay (PLA) (CAF01) between myoferlin (MYOF) and COPII vesicles (Sec31) in CTRL myCAFs and MYOF^{KD} myCAFs (shMYOF#1 and shMYOF#2). PLA control (CTRL^{neg}) without primary antibodies is included. Representative pictures are shown. Nuclei = blue, PLA-dots = green, scale bar = 10 μm. (G) Quantification of PLA-dots shown in Fig. 5E. Violin plot, one-way ANOVA (Tukey's test). (H) Visualization of TGFBR1 and myoferlin amino acid sequence with Sec24C cargo motifs highlighted in the cytoplasmic region. (I, J) Differentially phosphorylated proteins (CAF01) upon TGFBR1-stimulation (8 h) between CTRL myCAFs (Irrel.shRNA) and MYOF^{KD} myCAFs (shMYOF#1). Proteins in bold mark TGFBR1 kinase targets. Source data are available online for this figure.

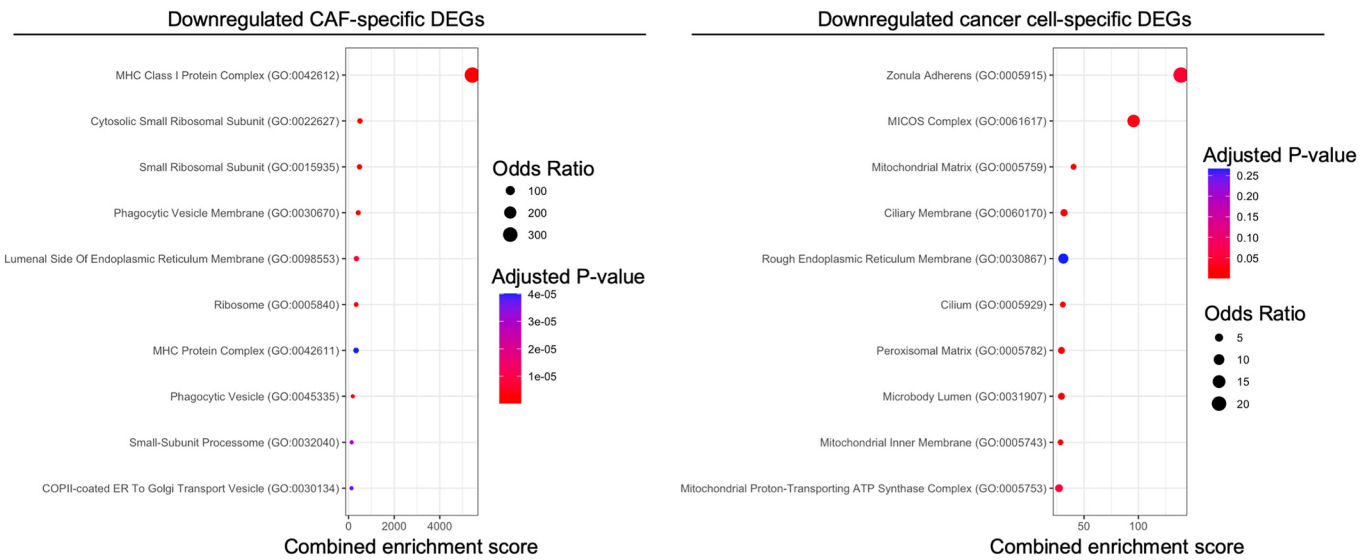


Figure EV6. Downregulated genesets upon myoferlin knockdown in CAFs and cancer cells.

ORA results of CAF-specific and cancer cell-specific downregulated DEGs. Genesets extracted gene ontology (GO - cellular compartments). Source data are available online for this figure.

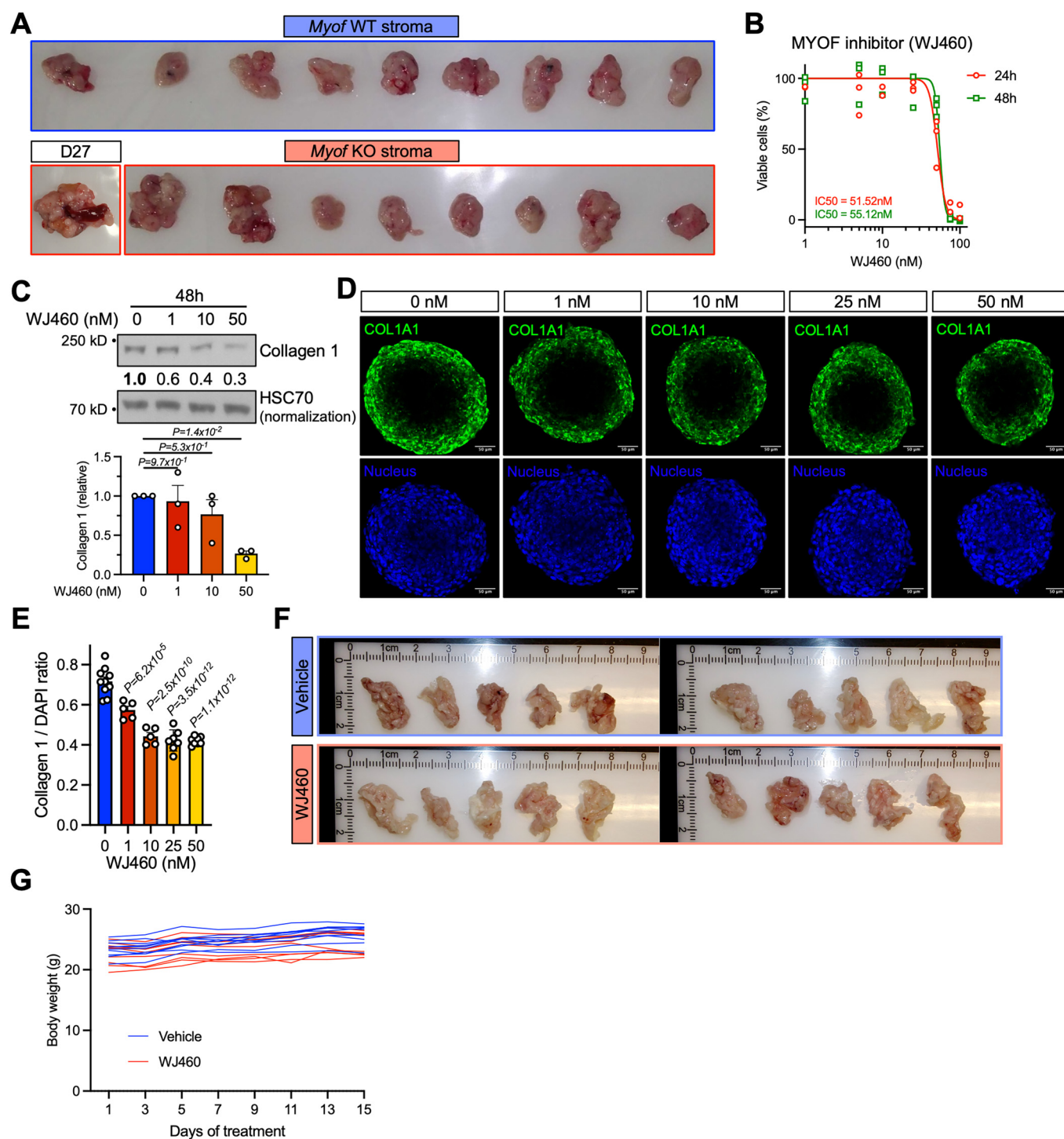


Figure EV7. Pharmacological targeting of myoferlin impairs ECM production.

(A) Resected tumors (D28) of orthotopic KPC allografts into *Myof*^{WT} ($n = 9$) or *Myof*^{KO} ($n = 9$) mice. (B) IC₅₀ analysis of myCAFs treated for 24 h or 48 h with increasing concentrations of WJ460 ($n = 3$). (C) Western blot analysis and quantification of total-cell lysates (CAF01) from 48 h WJ460-treated myCAFs ($n = 3$). One representative western blot of three independent experiments is shown, HSC70 was used as loading control. Mean $\pm$ SEM, one-way ANOVA (Tukey's test), P value relative to control group (0 nM). (D) Immunofluorescence microscopy of homotopic spheroids ($n \geq 5$) generated from myCAFs and treated with increasing concentrations of WJ460. Representative pictures are shown. Nuclei (DAPI) = blue, collagen 1 = green, scale bar = 50 μ m. (E) Mean fluorescence quantification of spheroids ($n \geq 5$) shown in (D), collagen 1 intensity was normalized to DAPI. Mean $\pm$ SEM, one-way ANOVA (Tukey's test, P values relative to 0 nM). (F) Resected pancreata of vehicle (DMSO, $n = 10$) and WJ460-treated ($n = 10$) mice orthotopically injected with KPC cells (D21). (G) Body weight (g) evolution of vehicle ($n = 10$) and WJ460-treated ($n = 10$) mice orthotopically injected with KPC cells. Source data are available online for this figure.