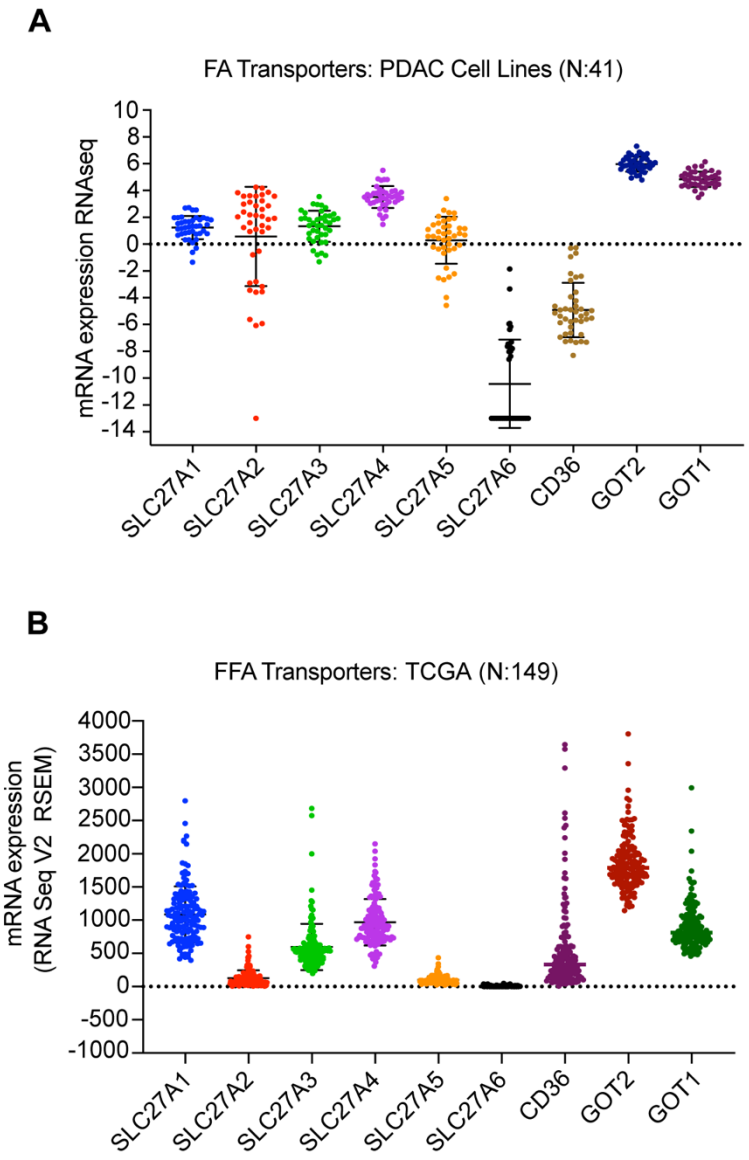
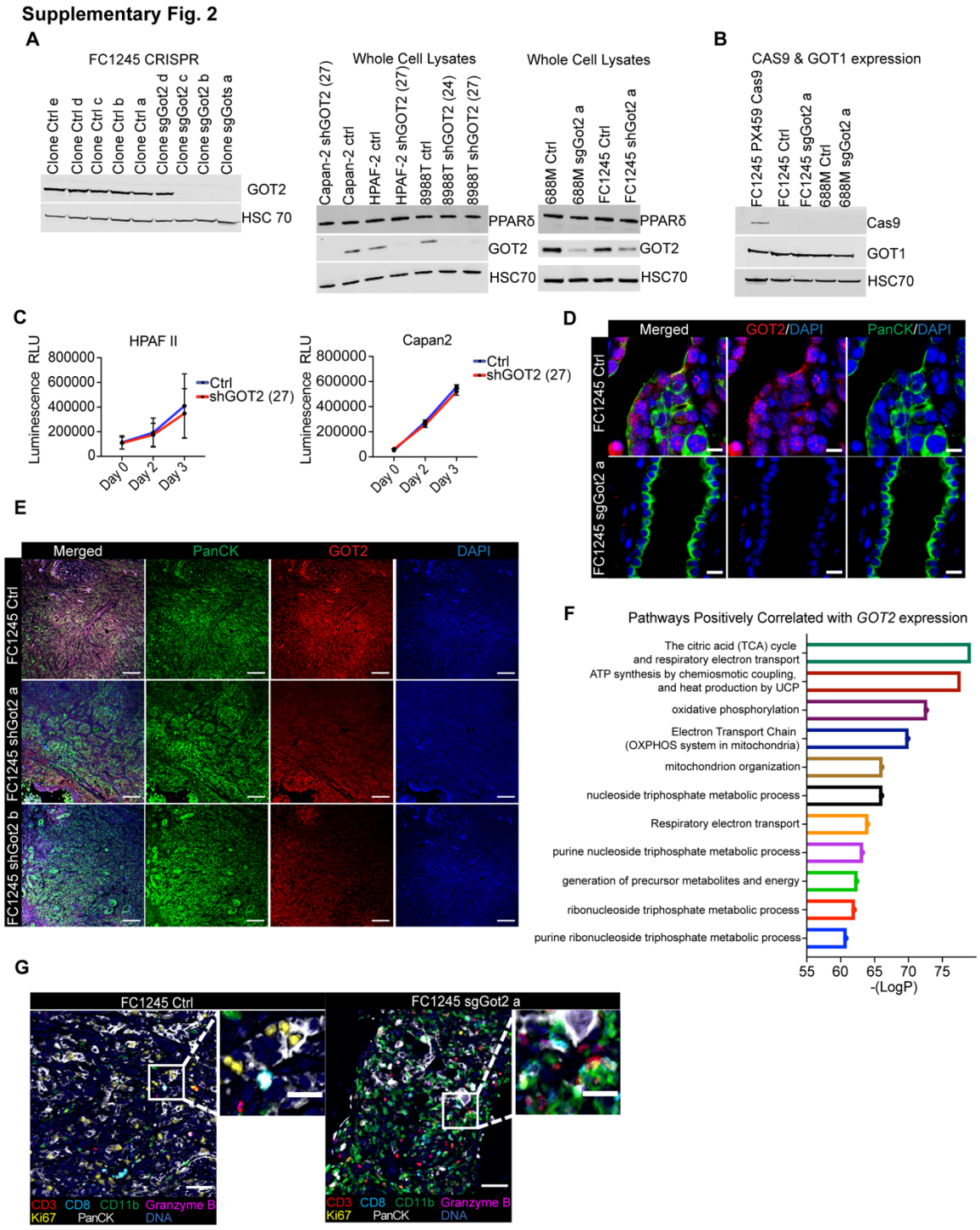
Supplementary Fig. 1



Supplementary Figure S1: GOT2 is upregulated in human PDAC.
(A) Relative expression of the indicated fatty acid transporters and trafficking factors among 41 human PDAC cell lines in the Broad Institute Cancer Cell Line Encyclopedia database. **(B)** Relative expression of the indicated fatty acid transporters and trafficking factors in human PDAC in The Cancer Genome Atlas database.

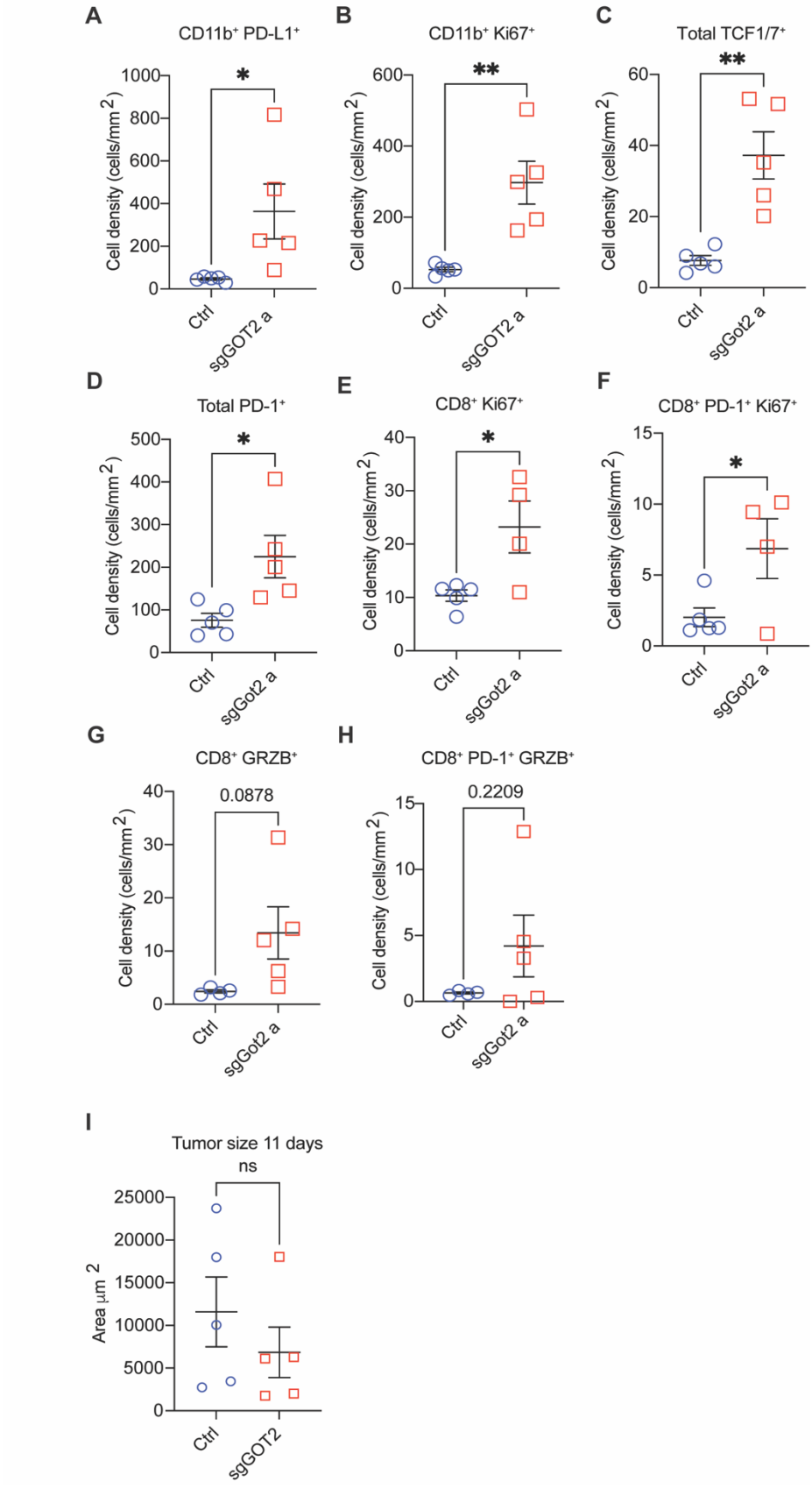


Supplementary Figure S2: PDAC cells maintain proliferative capacity with loss of GOT2 expression.

(A) Western blots indicating GOT2 levels in control and GOT2 loss-of-function PDAC lines. **(B)** Western blots for GOT1 and Cas9 in the indicated GOT2 loss-of-function PDAC lines. **(C)** Viable cell measurements in the indicated PDAC lines. Data are presented as mean ± s.e.m. from biological triplicates. **(D)** Immunohistochemical staining of FC1245 tumors at experimental endpoints, representative of n = 3 per cohort. Scale bar = 10 μm. **(E)** Immunohistochemical

staining of FC1245 tumors at experimental endpoint, representative of n = 3 per cohort. Scale bar = 50 μm. **(F)** Metascape pathway analysis depicting transcriptional programs positively correlated with GOT2 expression in human PDAC. **(G)** Multiplex immunohistochemical staining of control and sgGot2 FC1245 tumors for the indicated markers (large images, scale bar = 50 μm; insets, scale bar = 20 μm).

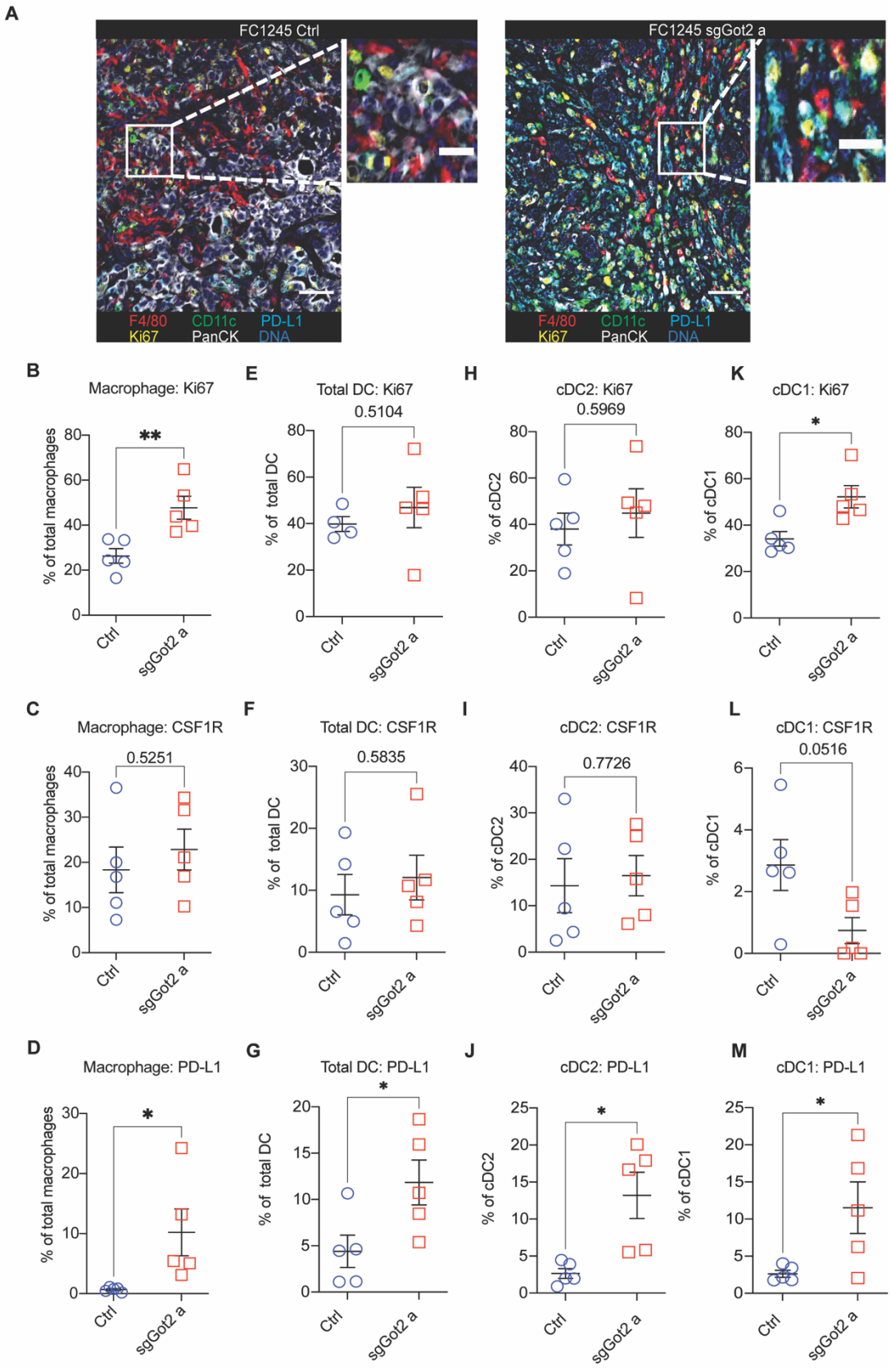
Supplementary Fig. 3



Supplementary Figure S3: GOT2 in cancer cells promotes an immune-suppressive PDAC immune microenvironment.

(A-H) Quantification of multiplex immunohistochemical staining for the indicated marker combinations or populations in control and sgGot2 FC1245 tumors (n = 4-5 per arm). Panels **C** and **D** reflect quantification of total T cells expressing the indicated markers. Data presented as mean ± s.e.m. *$p < 0.05$, **$p < 0.01$ by unpaired t-test. **(I)** Tumor size quantification at the day 11 time point depicted in Figure 2g (688M PDAC, Ctrl d11: n = 5, sgGot2 d11: n = 5).

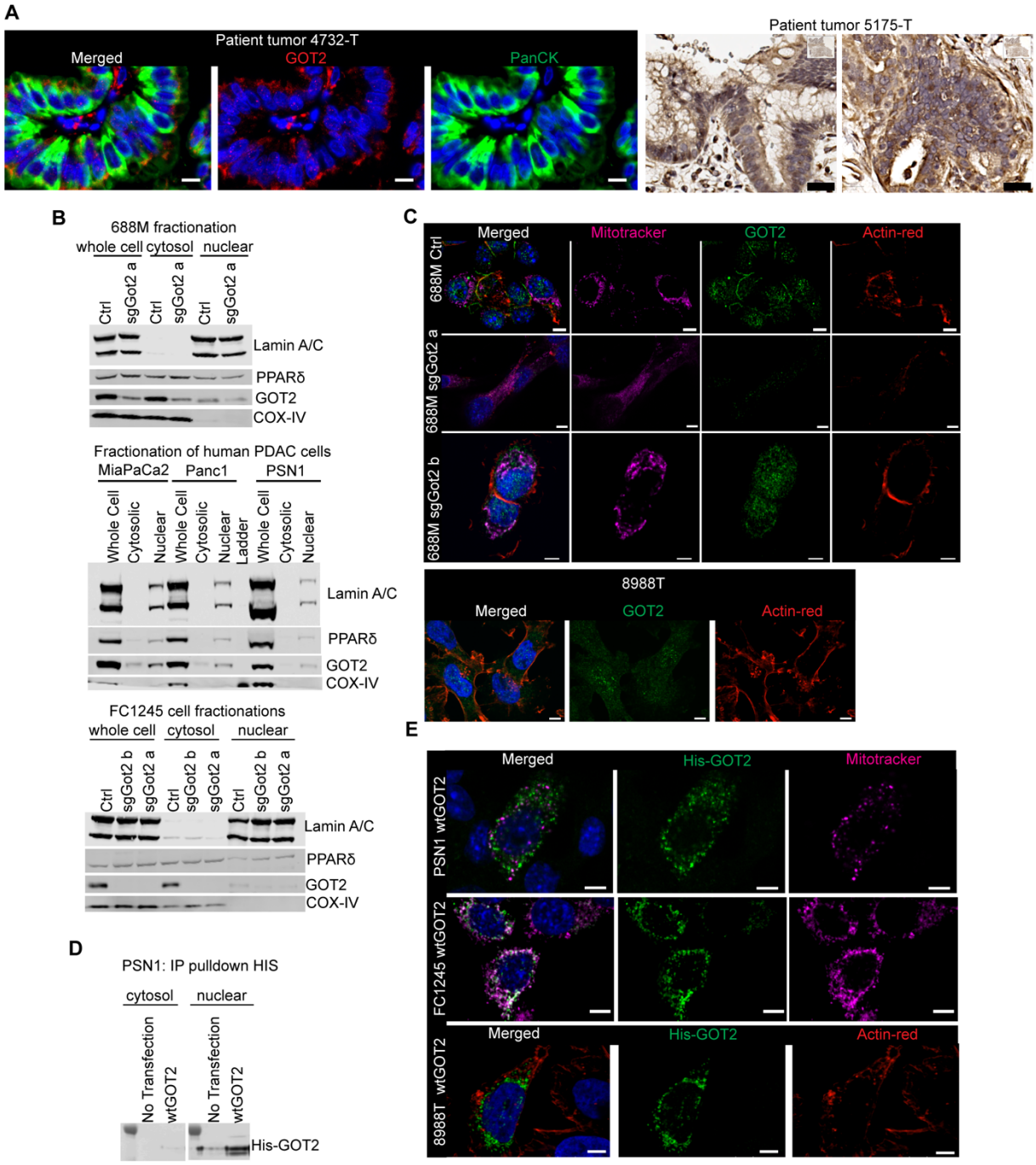
Supplementary Fig 4



Supplementary Figure S4: GOT2 in cancer cells promotes immune-suppressive phenotypes in macrophages and dendritic cells.

(A) Multiplex immunohistochemical staining of control and sgGot2 FC1245 tumors for the indicated markers (large images, scale bar = 50 μm; insets, scale bar = 20 μm). **(B-M)** Quantification of multiplex immunohistochemical staining for the indicated marker combinations or populations in control and sgGot2 FC1245 tumors (n = 4-5 per arm). Data are presented as mean ± s.e.m. *$p < 0.05$, **$p < 0.01$ by unpaired t-test. Macrophages were defined as $CD45^{+}$ $CD11b^{+}$ $Ly6G^{-}$ $F4/80^{+}$ $CSF1R^{+/-}$ $MHCII^{+/-}$. Dendritic cells were defined as $CD45^{+}$ $CD11b^{+/-}$ $Ly6G^{-}$ $F4/80^{-}$ $CD11c^{+}$ $MHCII^{+}$, with cDC2s defined as $CD11b^{+}$ and putative cDC1s defined as $CD11b^{-}$.

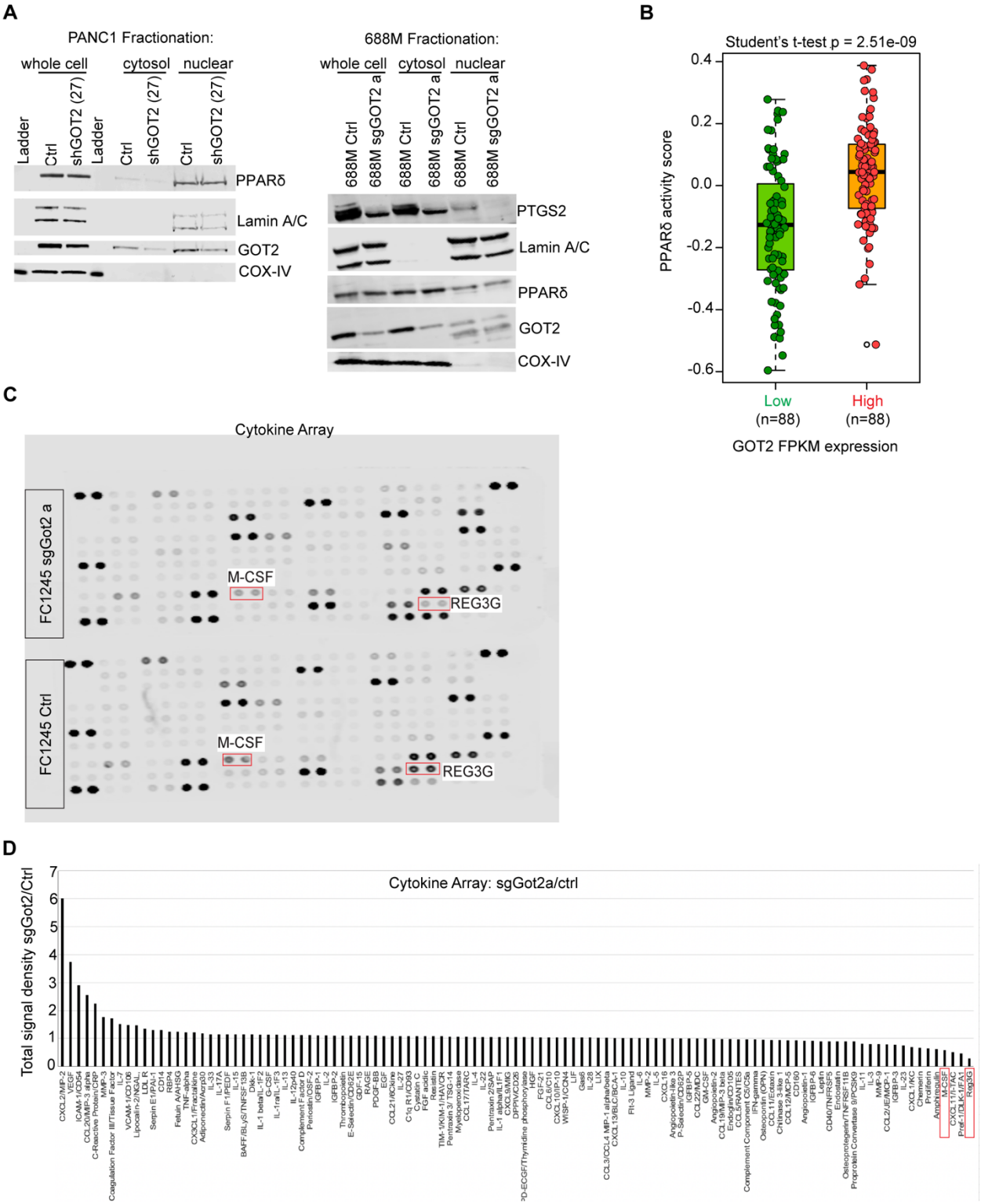
Supplementary Fig. 5



Supplementary Figure S5: A pool of GOT2 protein localizes to the nucleus in PDAC cells. **(A)** Immunohistochemical staining for GOT2 or GOT2 and panCK in human PDAC (representative of n = 5), showing additional samples to complement Figure 3B. Fluorescent images: scale bar = 2 µm, brightfield image: scale bar = 20 µm. **(B)** Western blots in PDAC cell lines indicating GOT2 protein levels in the indicated cellular fractions. Lamin A/C is a loading control for nuclei, COX-IV is a loading control for cytoplasm and indicates an absence of

mitochondrial protein in the nuclear fraction. **(C)** Immunofluorescent staining of the indicated PDAC cell lines for endogenous GOT2. Mitotracker indicates mitochondria, Actin-Red indicates F-actin, and nuclei are counterstained with DAPI. Scale bar = 5 μm. **(D)** Immunoprecipitation of transiently transfected, His-tagged GOT2 from the indicated cellular fractions in PSN-1 human PDAC cells. **(E)** Immunofluorescent staining of the indicated PDAC cell lines for transiently transfected, His-tagged GOT2. Scale bar = 5 μm.

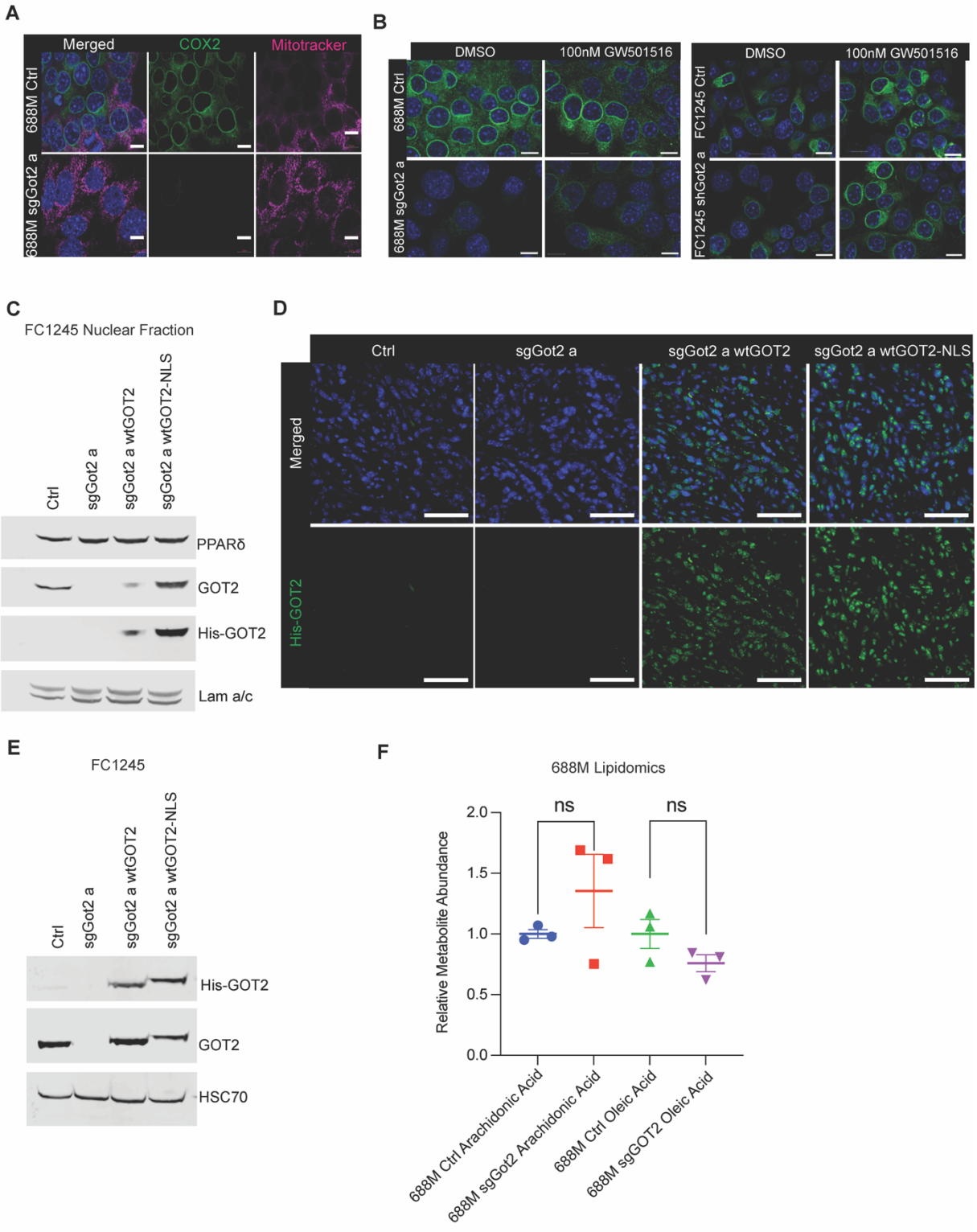
Supplementary Fig. 6



Supplementary Figure S6: GOT2 positively regulates PPARδ activity, target gene expression, and immune-modulatory cytokine expression.

(A) Western blots indicating levels of GOT2, PPARδ, and PPARδ target PTGS2/COX2 in the indicated PDAC lines. **(B)** Box plot depicting expression levels of PPARδ target genes in human PDAC with high (n = 89) versus low (n = 88) expression of *GOT2* per TCGA RNA-seq data. **(C,D)** Cytokine array measuring abundance of the indicated secreted factors in conditioned media from control or sgGot2 688M PDAC cells, with dot blot results in **C** and quantification in **D**.

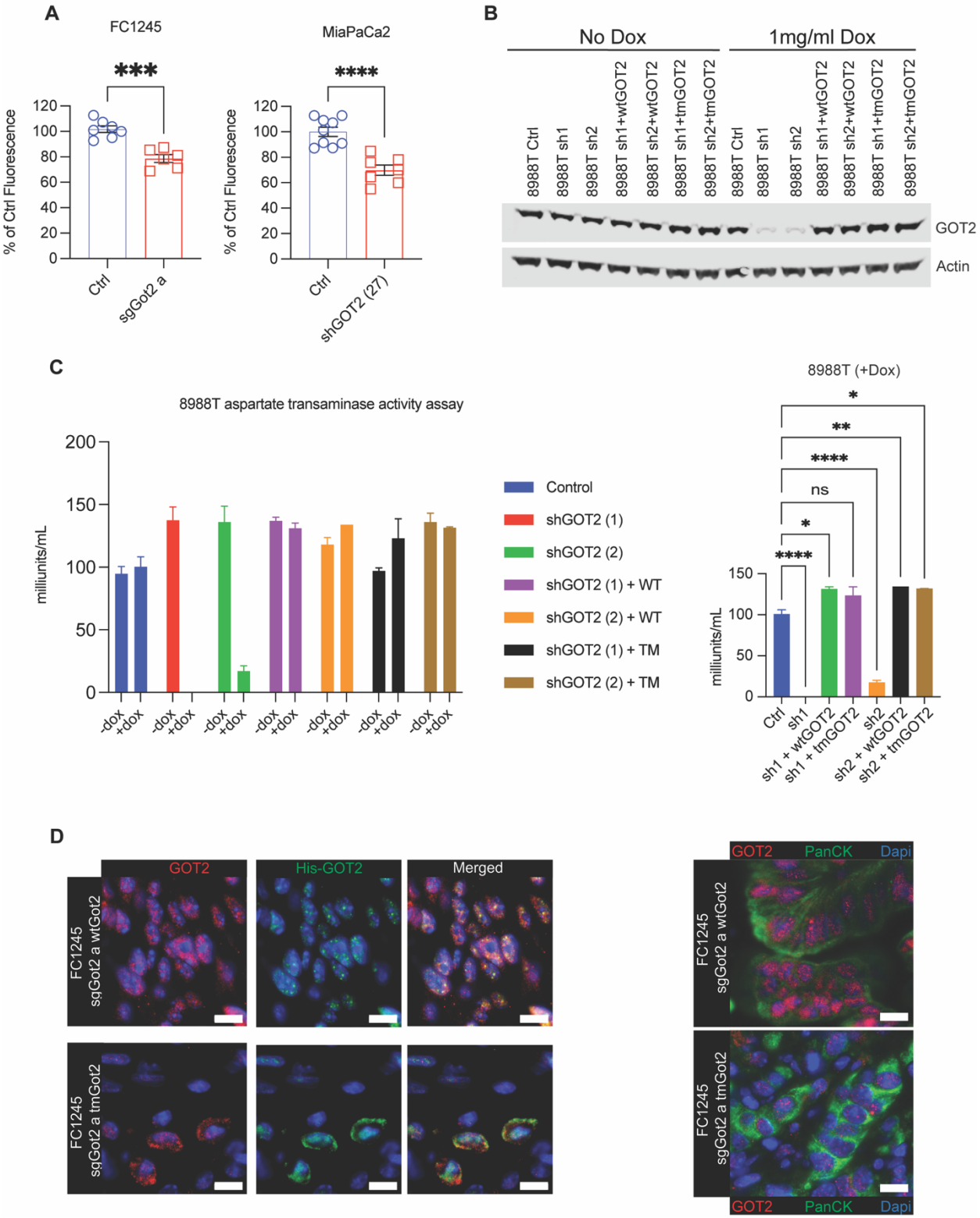
Supplementary Fig. 7



Supplementary Figure S7: GOT2 fatty acid binding and PPARδ activation support tumor growth.

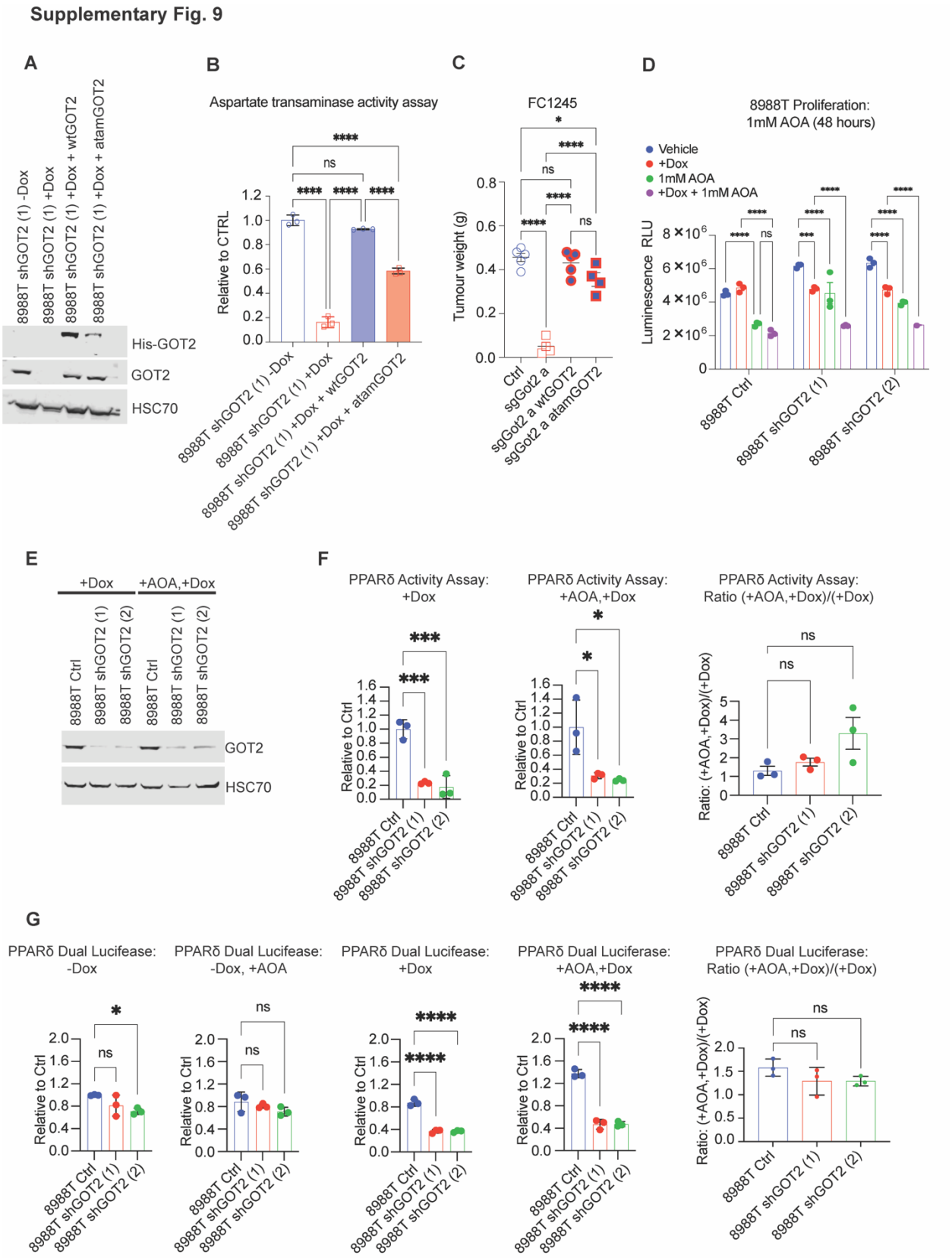
(A,B) Immunofluorescent staining of ctrl and sgGot2 688M cells, as well as ctrl and shGot2 FC1245 cells, for PTGS2/COX2 with or without 100 nM GW501516 treatment. Scale bar = 10 μm. **(C)** Western blots on nuclear lysates from sgGot2 FC1245 PDAC cells reconstituted with wtGOT2 or NLS-wtGOT2. **(D)** Immunohistochemical staining for His in WT-GOT2 and NLS-GOT2 tumors (n = 3 per arm, scale bar = 50 μm). **(E)** Western blots on whole cell extracts of FC1245 cells used for orthotopic implantations in **D. (F)** Quantification of fatty acid levels in ctrl and sgGot2 688M cells, measured in whole cells by LCMS.

Supplementary Fig. 8



Supplementary Figure S8: GOT2 has separable fatty acid binding and transaminase activities.

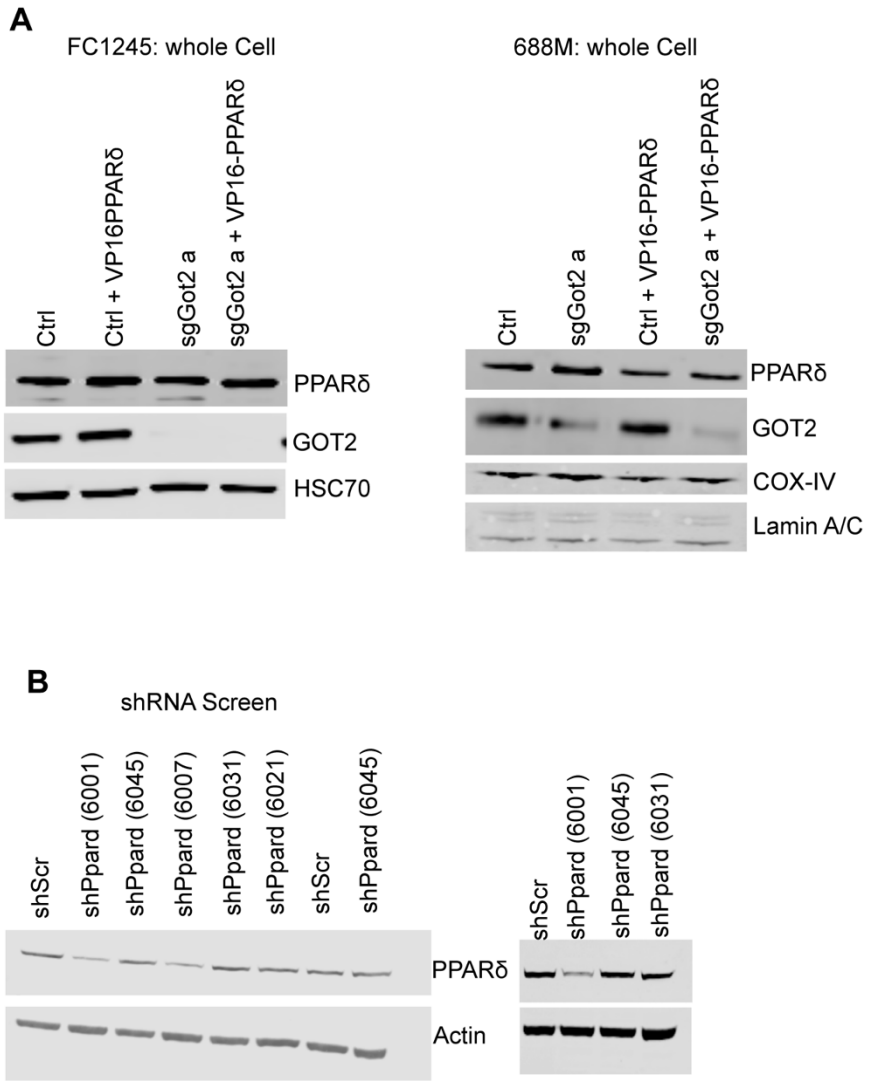
(A) Nuclear accumulation of NBD-arachidonic acid after the indicated cell lines were incubated with 2.5 μM NBD-aa for 2 hr (MiaPaCa2) or 2 μM NBD-aa for 15 min (FC1245). Data are presented as mean ± s.e.m. *$p < 0.05$, ****$p < 0.0001$ by unpaired t-test. **(B)** Western blots indicating GOT2 levels in doxycycline-inducible GOT2 knockdown 8988T cells reconstituted with wtGOT2 or tmGOT2. **(C)** Aspartate aminotransferase activity assay (also known as glutamate-oxaloacetate transaminase activity assay) on the cells indicated in **B**. Data are plotted as mean ± s.e.m. from biological triplicates. *$p < 0.05$, **$p < 0.01$, ****$p < 0.0001$ by one-way ANOVA. **(D)** Immunohistochemical staining for GOT2 in PDAC arising from FC1245 sgGot2 cells reconstituted with wtGot2 or tmGot2 (scale bar = 10 μm; representative of n = 4 mice per arm).



Supplementary Figure S9: Point mutations within the transaminase domain impair GOT2 catalytic activity.

(A) Western blots indicating GOT2 levels in doxycycline-inducible GOT2 knockdown 8988T cells reconstituted with wtGOT2 or atamGOT2 (aspartate transaminase mutant). **(B)** Aspartate aminotransferase activity assay on the cells indicated in **A**. **(C)** PDAC tumor weight at experimental endpoint, 18 days after orthotopic transplantation of the indicated FC1245 cells. **(D)** Viable cell measurements from doxycycline-inducible control or shGOT2 8988T PDAC cells treated with 5 mg/mL doxycycline and/or 1 mM AOA for 48 h. Data are presented as mean ± s.e.m. ****$p < 0.0001$ by one-way ANOVA. **(E)** Western blots indicating GOT2 protein levels in the indicated cell lines and conditions. **(F)** PPARδ transcription factor activity assay from cells treated as in **D**. Data are presented mean ± s.e.m. *$p < 0.05$, ***$p < 0.001$ by one-way ANOVA. **(G)** PPRE luciferase assay from cells treated as in **D, F**. Data are presented mean ± s.e.m. *$p < 0.05$, ****$p < 0.0001$ by one-way ANOVA.

Supplementary Fig. 10



Supplementary Figure S10: Validation of PPARδ loss- and gain-of-function cell lines.
(A,B) Western blots indicating GOT2 and PPARδ expression in the indicated stable cell lines.

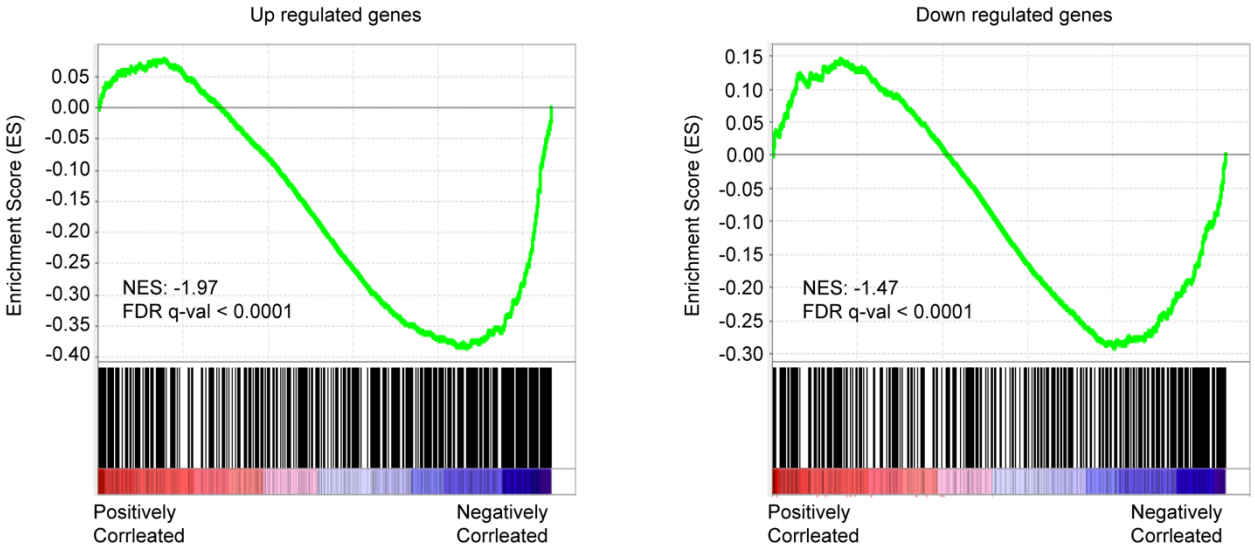
Supplementary Fig. 11

A

Top My Lists

Name	p-value	Overlap
Inflammation	8.41E-25	30.4% 673/2213
synapse	6.73E-22	31.3% 527/1685
Lipid metabolism	1.46E-18	28.5% 726/2545
Innate immune response	1.21E-16	29.8% 516/1732
Adaptive immune response	5.28E-11	29.8% 338/1134

B



C

Name	P-value
Interferon Alpha Response	0.0003044
Estrogen Response Early	0.0004117
Epithelial Mesenchymal Transition	0.001011
Angiogenesis	0.008045
Estrogen Response Late	0.01105
Inflammatory Response	0.02204
TNF-alpha Signaling via NF-kB	0.0416
Myogenesis	0.0416
UV Response Dn	0.0553
Hedgehog Signaling	0.1018

Supplementary Figure S11: RNA-seq reveals broad regulation of inflammatory gene expression by GOT2 and PPARδ in PDAC cells.
(A) Pathway analysis of differentially expressed genes in control versus sgGot2 PDAC cells per RNA-seq. **(B)** Gene set enrichment analysis comparing differentially expressed genes in control versus sgGot2 PDAC cells to a previously defined list of PPARδ-regulated genes. **(C)** MSigDB pathway analysis of genes downregulated by sgGot2 (padj < 0.01, logFC < -1) and not upregulated by GW501516 (padj < 0.01, logFC > 1).