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Supplemental information

$Ym1^{+}$ macrophages orchestrate fibrosis, lesion growth, and progression during development of murine pancreatic cancer

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Figure S1, Related to Figure 2

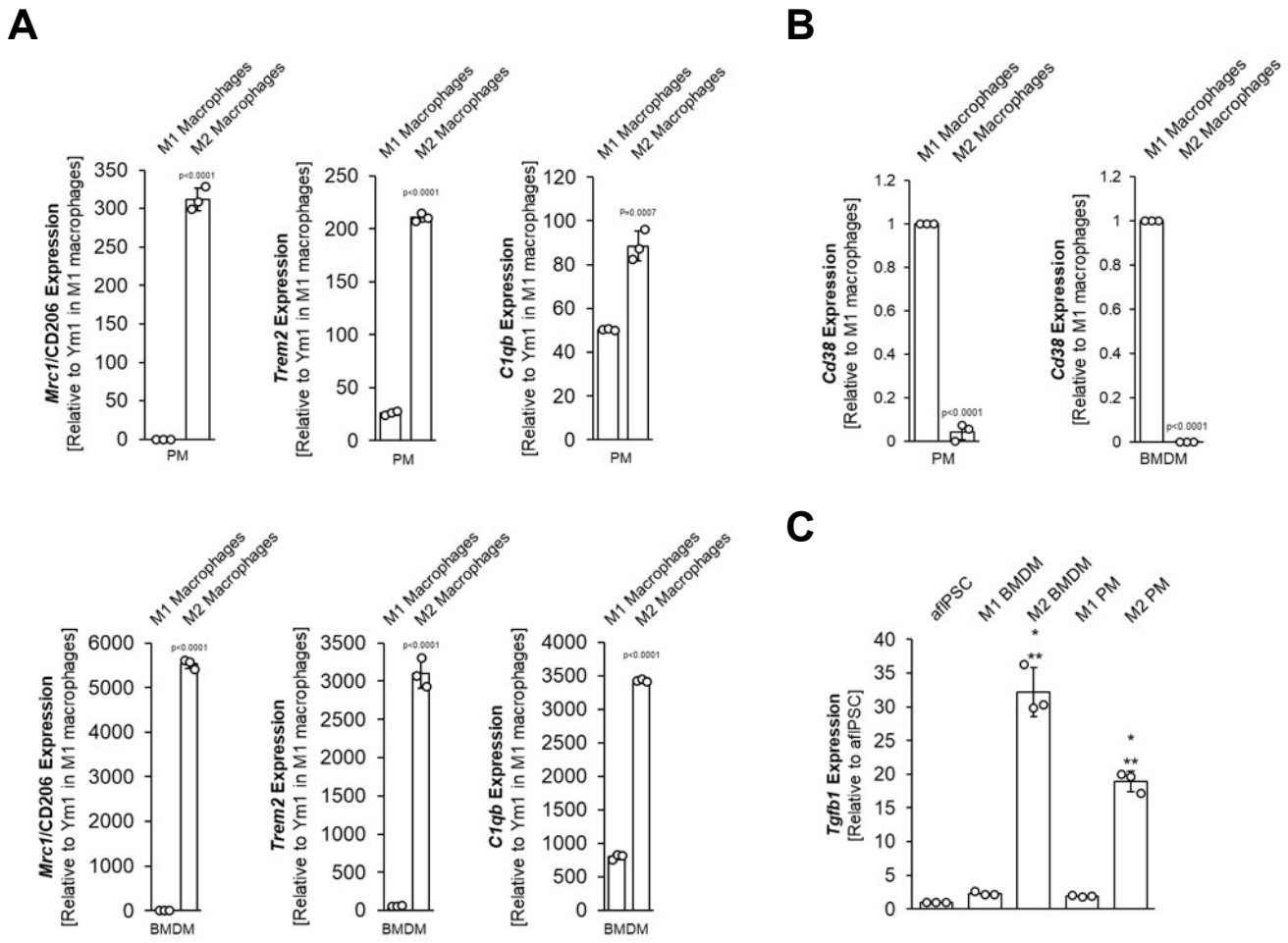

Figure S1, Related to Figure 2. **Ym1⁺ macrophages express *Tgfb1* and additional AAM markers. A-B:** qPCR analysis in peritoneal (PM) or bone-marrow-derived (BMDM) macrophages. Error bars represent the standard deviation. The t-test was used for statistical significance of M2 macrophage markers (A) and M1 macrophage markers (B). **C:** qPCR analysis for *Tgfb1* in aflPSC, BMDM, and PM. A one-way ANOVA was done for statistical analysis, followed by Tukey's multiple comparisons test. *indicates statistical significance compared to aflPSC and **indicates statistical significance compared to M1 macrophages (adjusted p-value < 0.0001 for each comparison). Error bars represent the standard deviation.

Figure S2, Related to Figure 3

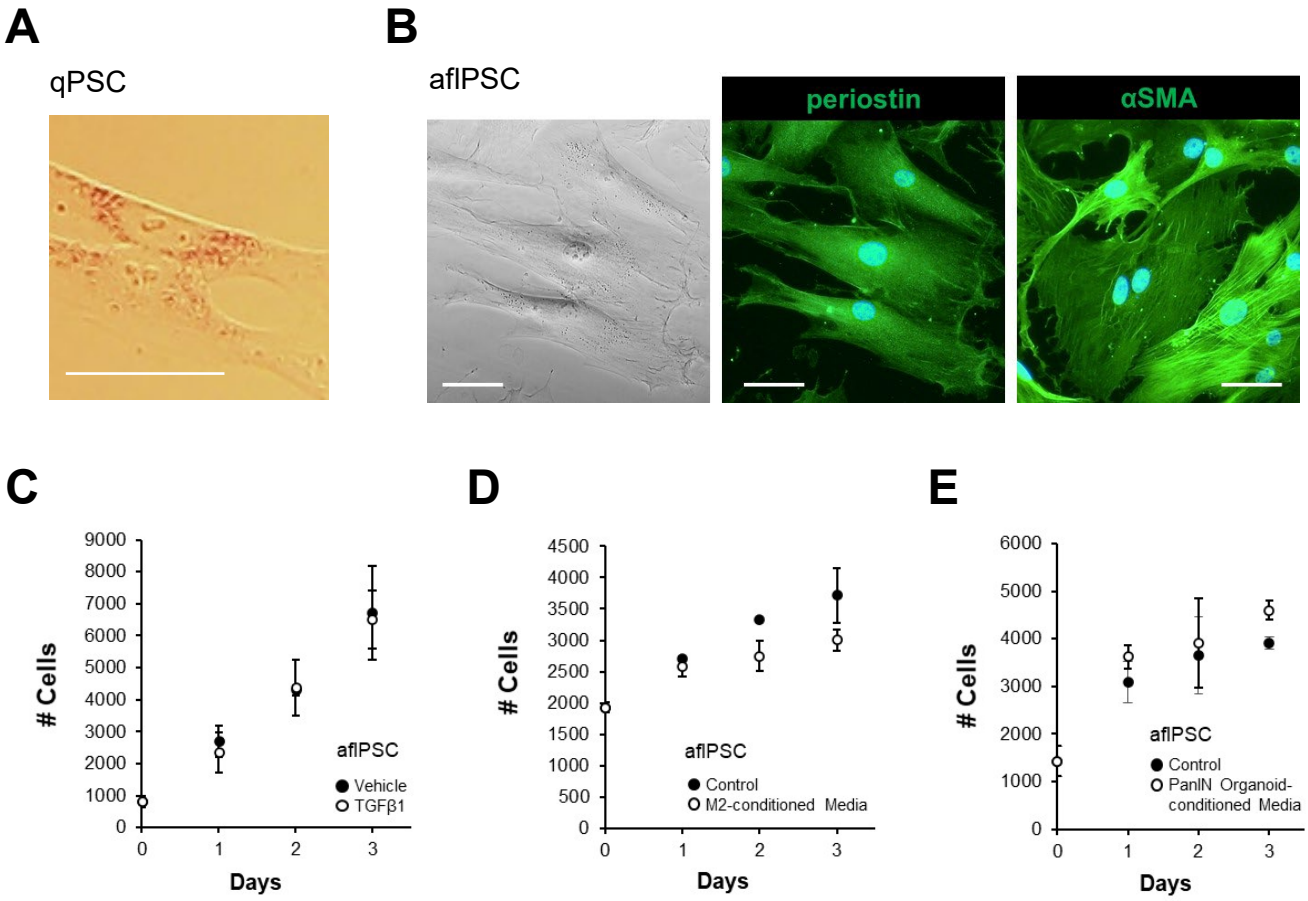

Figure S2, Related to Figure 3. **Proliferation of aflPSCs is not driven by AAMs or PanIN organoid cells. A:** Oil Red O staining in freshly isolated qPSCs. Scale bar indicates 50 μm. **B:** Brightfield and IF images of aflPSC stained with periostin or αSMA. Scale bar indicates 50 μm. **C-E:** Proliferation of aflPSCs treated with TGFβ1 (C), Peritoneal-derived M2-conditioned media (D), or PanIN organoid-conditioned media (E). Error bars indicate standard deviation.

Figure S3, Related to Figures 4 and 5

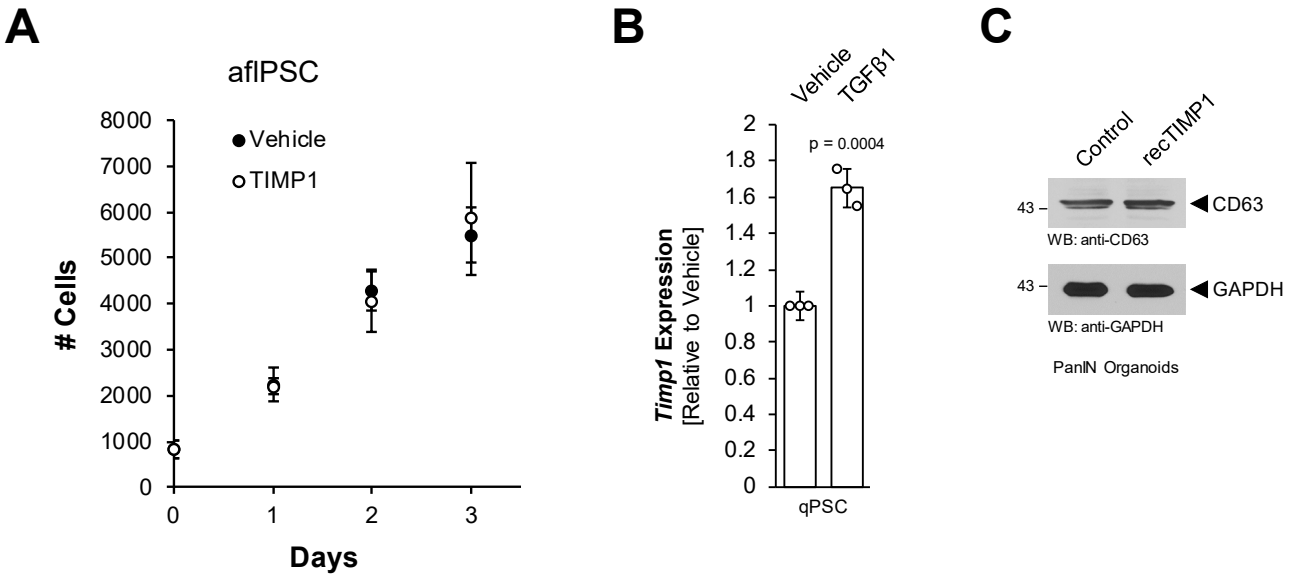

Figure S3, Related to Figures 4 and 5. **TIMP1 does not alter proliferation or αSMA in aflPSCs. A:** Proliferation of aflPSCs treated with TIMP1 or vehicle control. Error bars indicate standard deviation. **B:** qPCR analysis of *Timp1* in qPSCs treated with TGFβ1. Error bars indicate standard deviation. Statistical significance determined using the t-test. **C:** Western blot analysis of CD63 expression in PanIN organoids treated with vehicle control or recombinant TIMP1. GAPDH serves as a loading control.

Figure S4, Related to Figure 6

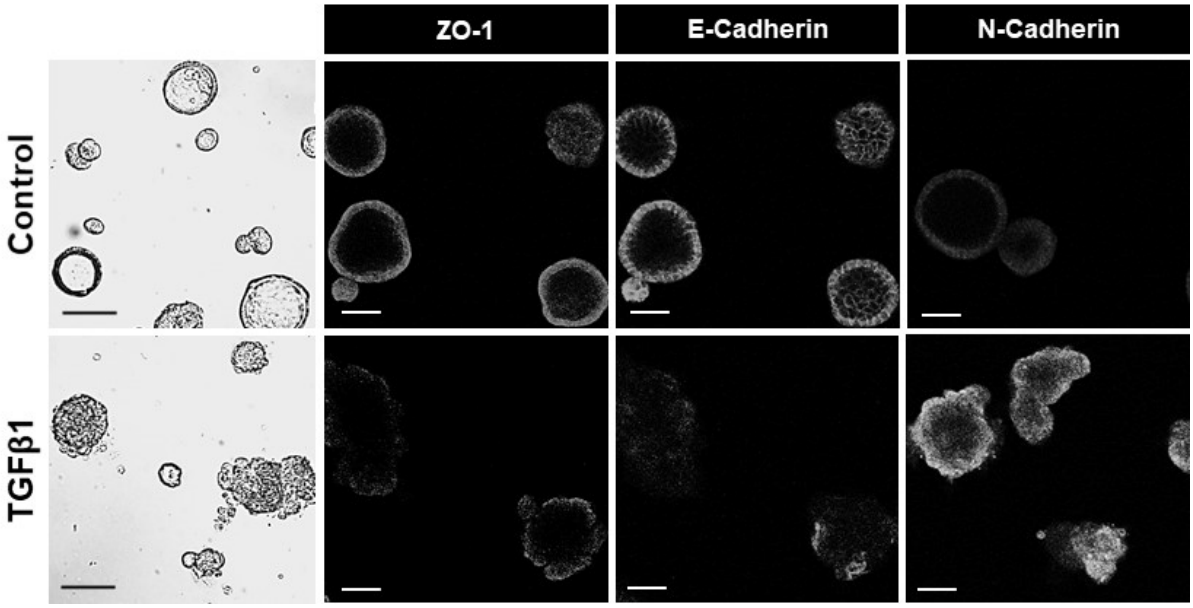

Figure S4, Related to Figure 6. **TGFβ1 drives EMT-like structural changes in PanIN Cells.** PanIN organoids were treated with vehicle control or TGFβ1 (50 ng/ml) for two days. Cells were fixated and analyzed for expression of ZO-1 & E-Cadherin or N-Cadherin via immunofluorescence. Scale bars indicate 100 µm (bright field) or 50 µm (IF).

Table S1

Antibody	Company/Source	Catalog Number	Species	IHC	IF-IHC	IF	WB	3D Assay
CD63	Sigma-Aldrich	SAB5700799	rabbit				1:1000	
CD63	Abcam	ab134045	rabbit	1:100				
CD63 (H5C6)	Novus	NBP2-42225	mouse					1µg/mL
E-Cadherin	BD Biosciences	610181	mouse			1:200		
GAPDH	Cell Signaling Technology	5174	rabbit				1:2000	
IgG1 Mouse	R&D Systems	MAB002	mouse					1µg/mL
N-Cadherin	Abcam	ab76011	rabbit			1:200		
N-Cadherin	R&D Systems	AF6426	sheep		1:200			
Periostin	Abcam	ab14041	rabbit			1:600		
SMAD4	Cell Signaling Technology	9515	rabbit	1:30	1:30			
TIMP1	R&D Systems	AF980	goat	1:750	1:1500			
Y369-TGFβ	Boster Bio	A00019Y369	rabbit	1:200	1:200			
Ym1	R&D Systems	AF2446	goat		1:500			
Ym1	STEMCELL Technologies	60130	rabbit	1:200				
ZO-1	Invitrogen	33-9100	mouse			1:200		
αSMA	Abcam	ab5694	rabbit	1:200	1:200	1:200		

Table S1, Related to Figures 1-6 and Figures S1-S4. **Antibodies and dilutions used.** Antibodies used were from the following sources: Abcam (Cambridge, MA), BD Biosciences (Franklin Lakes, NJ), Boster Bio (Pleasanton, CA), Cell Signaling Technology (Danvers, MA), Invitrogen (Carlsbad, CA), Novus Biologicals (Centennial, CO), R&D Systems (Minneapolis, MN), Sigma-Aldrich (St. Louis, MO), and STEMCELL Technologies (Vancouver, Canada).