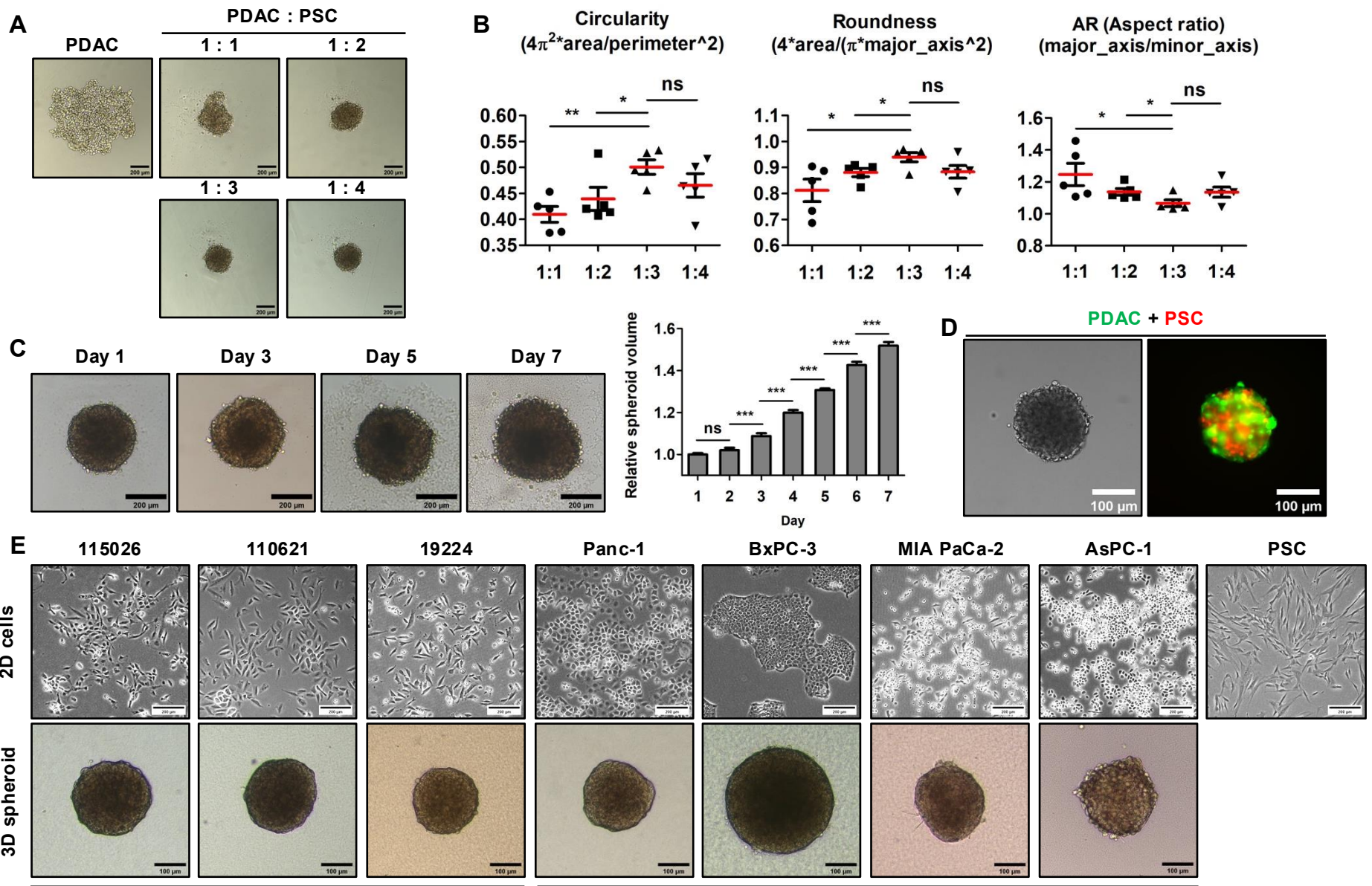
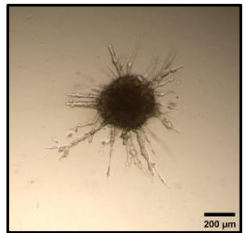
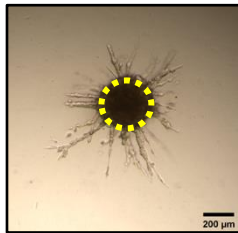
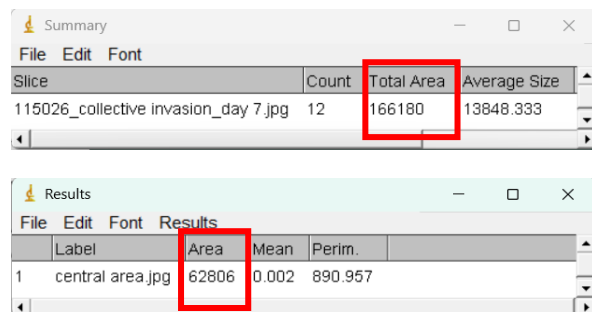
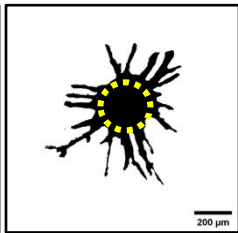


Supplementary Figures (1-13)



Supplementary Figure S1. Establishment of co-culture spheroid models using PDAC cells and PSCs

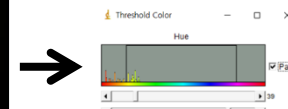
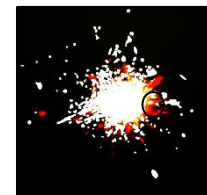
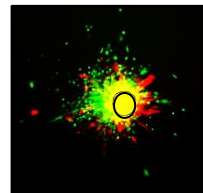
(A and B) Generation of co-culture spheroids with various ratios of Panc-1 cells and PSCs using the ULA method. Morphological analysis of co-culture spheroids, including circularity, roundness, and aspect ratio (AR). **(C)** Representative images of 115026 co-culture spheroids captured on days 1, 3, 5, and 7; quantification was conducted by measuring the relative spheroid volume. **(D)** Representative images of co-culture spheroids using fluorescence-labeled PDAC cells and PSCs. **(E)** Representative images of PDAC 2D-cultured PDAC cells and 3D co-culture spheroids. Patient-derived PDAC cells: 115026, 110621, and 19224. Pancreatic cancer cell lines: Panc-1, BxPC-3, and AsPC-1. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

A**Microscopy image****Central grid****Mask**

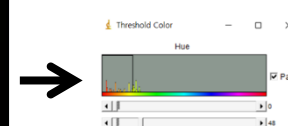
Measuring
the area

* Relative invasion area

= (total invasion area - central area) / central area

B**Fluorescence image**

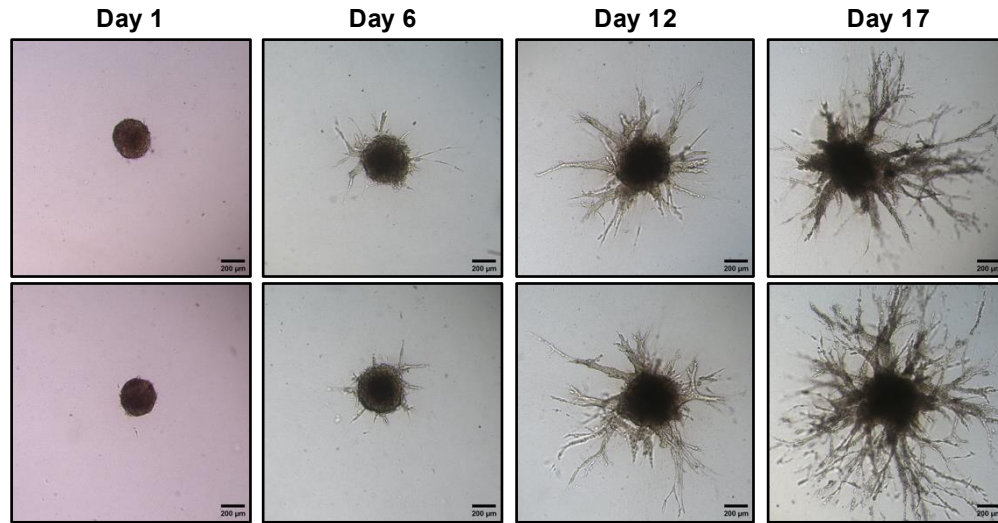
Measuring area of RFP signal



Measuring area of GFP signal

Supplementary Figure S2. Quantification methods of collective invasion and spheroid-based migration assays

(A) Quantification method for collective invasion. **(B)** Quantification method for the spheroid-based migration assay.

A

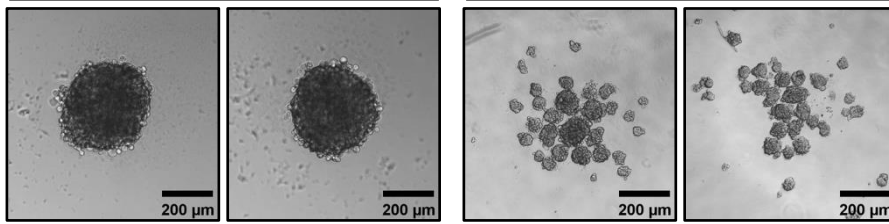
Supplementary Figure S3. Long-term collective invasion model

(A) Representative images of the collective invasion model of 115026 co-culture spheroids at days 1, 6, 12, and 17.

A

ULA method

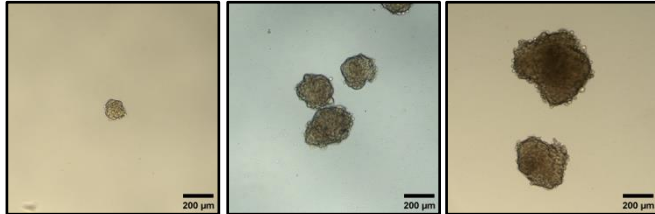
Hanging-drop method

**B**

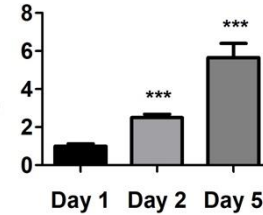
Day 1

Day 2

Day 5



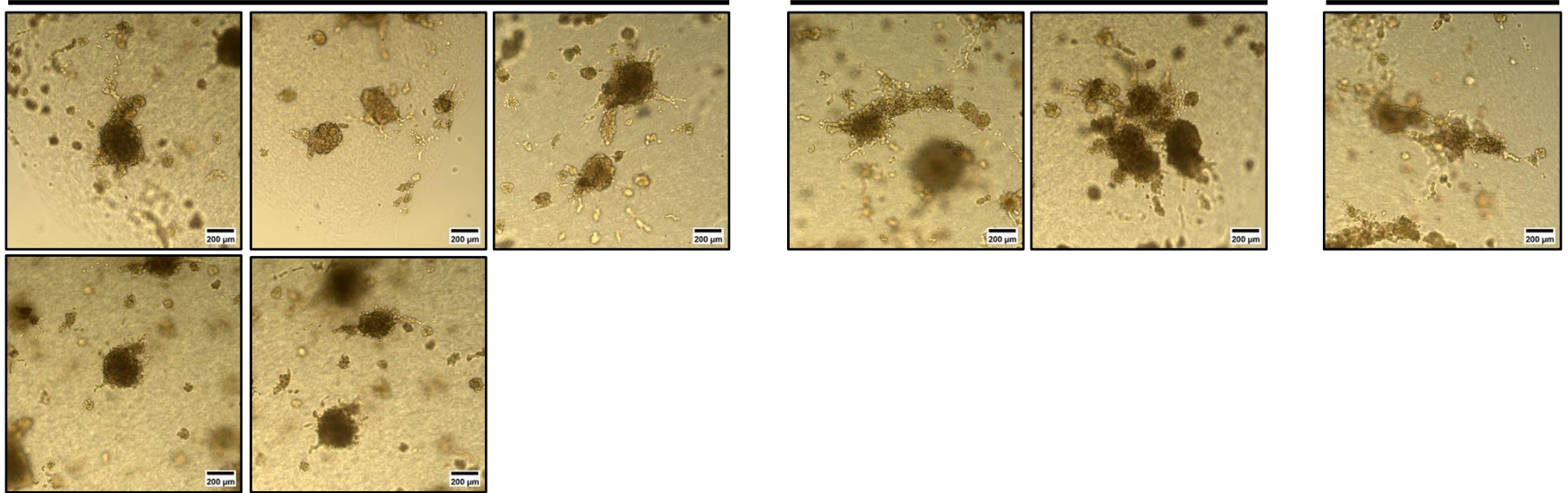
Relative spheroid volume

**C**

Heterogenous phenotype

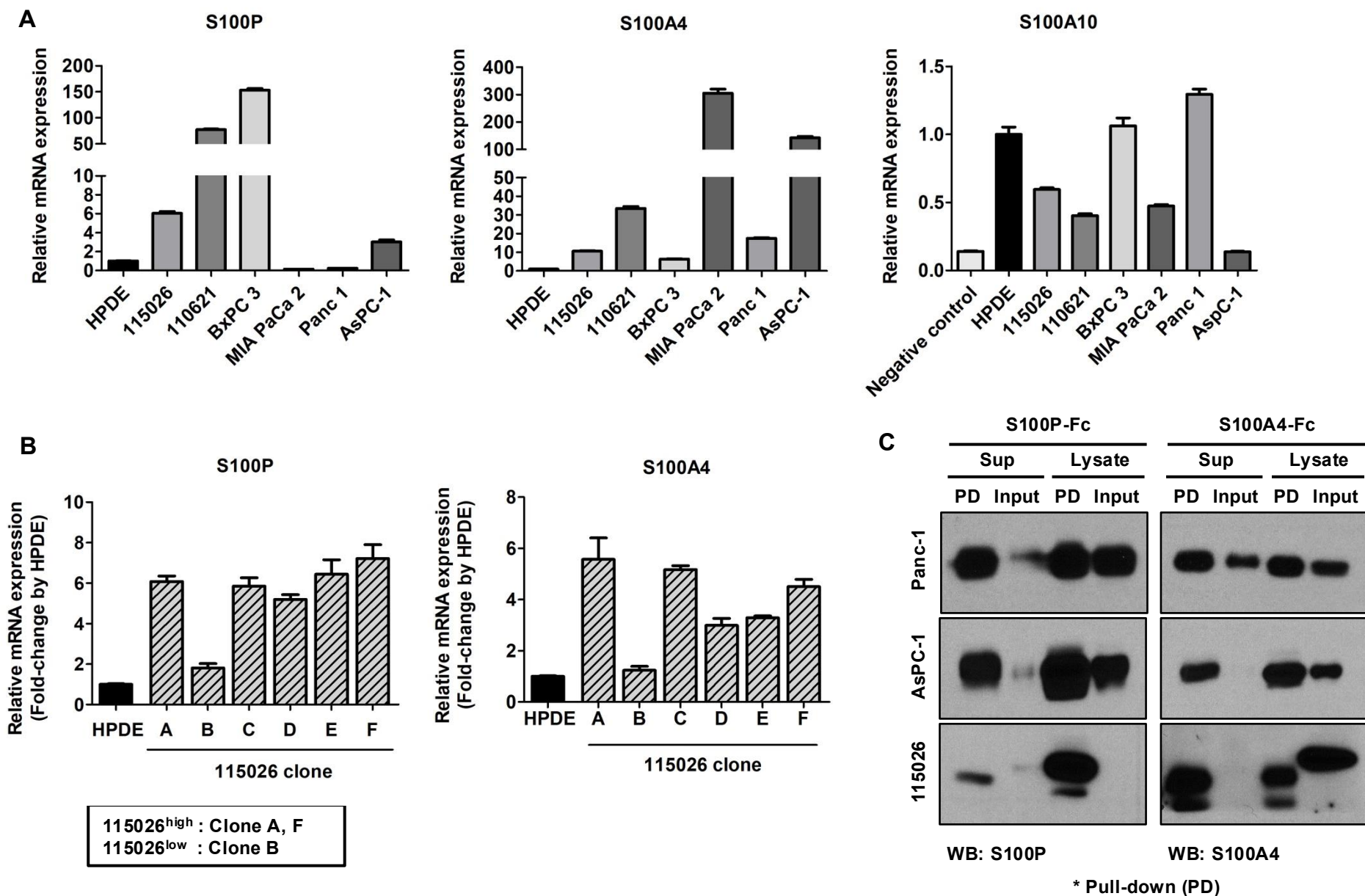
Aggregation & Bridging

Dirty background



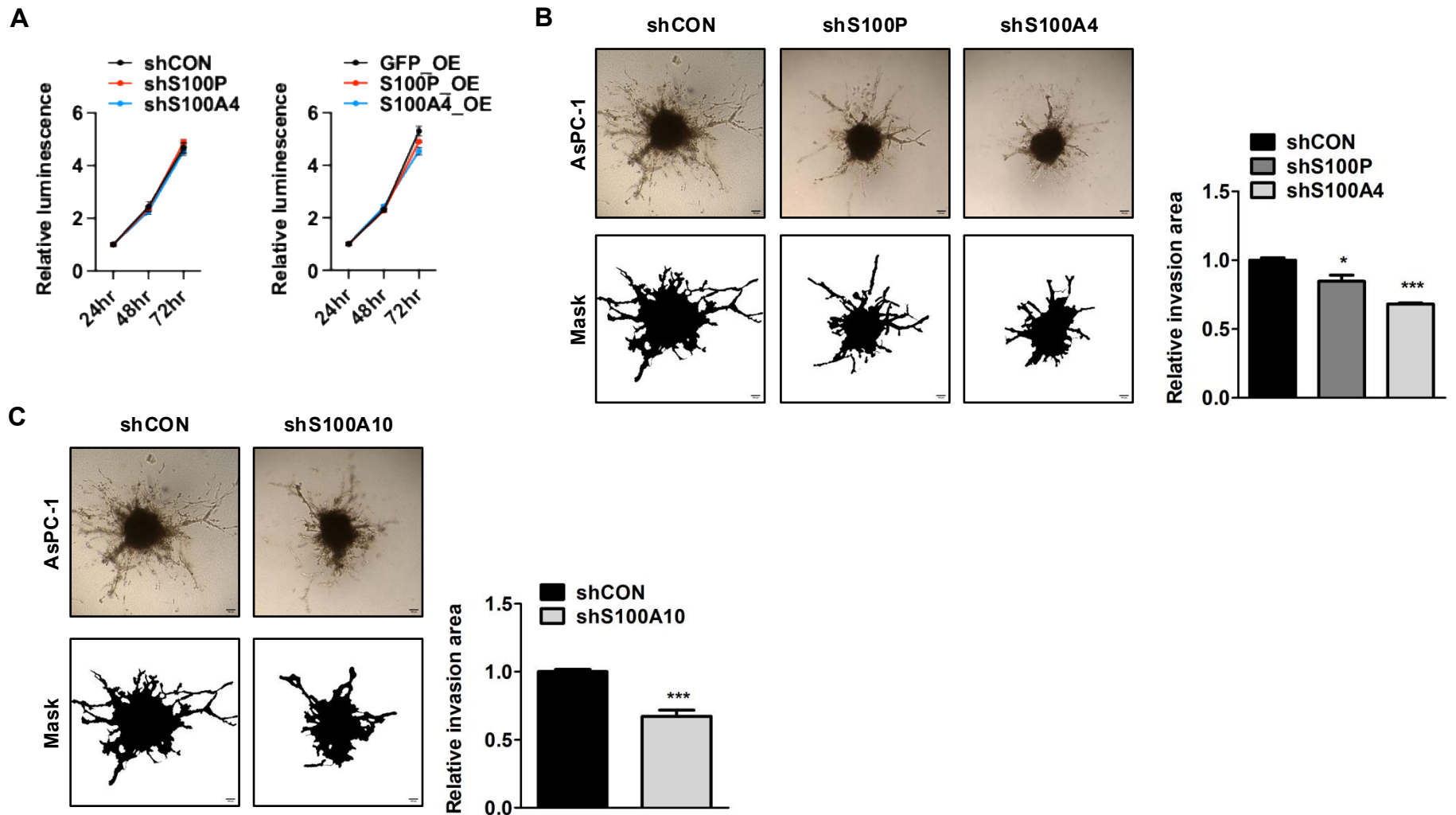
Supplementary Figure S4. Co-culture spheroids and collective invasion assay using the HD method

(A) Representative images of 115026 co-culture spheroids generated by the ULA method (left) and hanging-drop (HD) method (right). **(B)** Representative images of 115026 co-culture spheroids generated by the HD method on days 1, 2, and 5, with quantification of volume growth. **(C)** Issues encountered in the collective invasion assay using 115026 co-culture spheroids generated by the HD method. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.



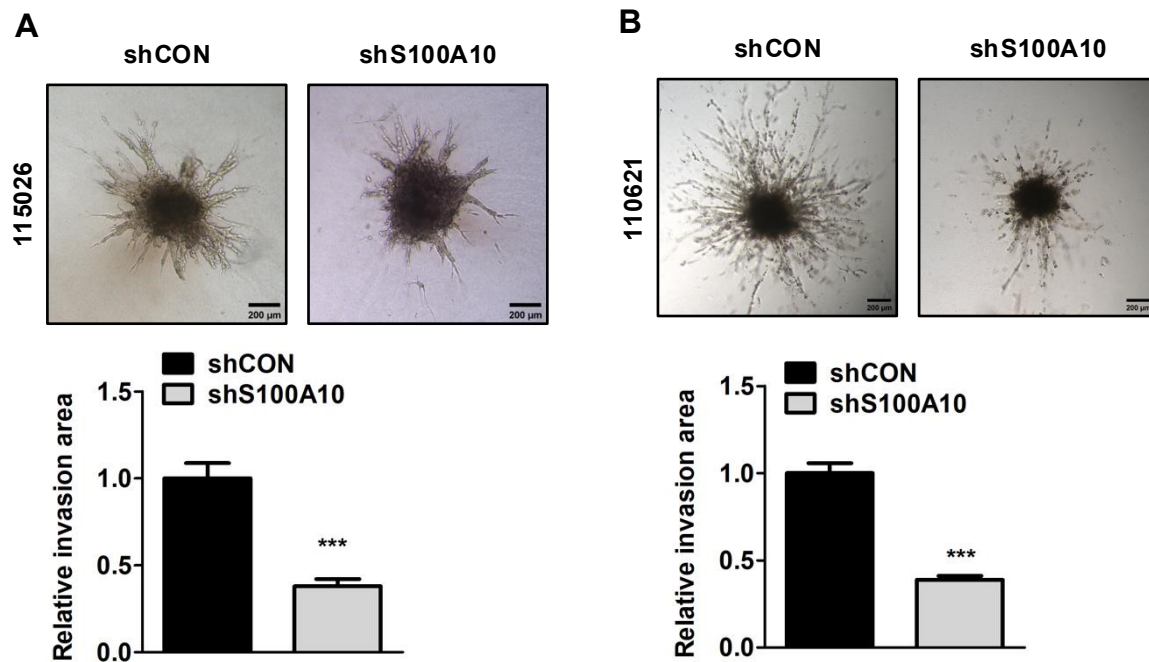
Supplementary Figure S5. Upregulation and secretion of S100P, S100A4, and S100A10 in PDAC cell lines

(A) Gene expression of S100P, S100A4, and S100A10 in PDAC primary cells (115026 and 110621), PDAC cell lines (BxPC-3, MIA PaCa-2, Panc-1, and AsPC-1), normal human pancreatic ductal epithelial cells (HPDE), and HEK 293T as a negative control. **(B)** Gene expression of S100P and S100A4 in six clones (clones A-F) of 115026 cells and HPDE cells. **(C)** Pull-down assay and western blot analysis for S100P and S100A4 using cell supernatants and lysates of Panc-1, AsPC-1, and 115026 expressing S100P or S100A4 tagged with the human IgG Fc region. These fusion proteins were pulled down by protein A/G beads.



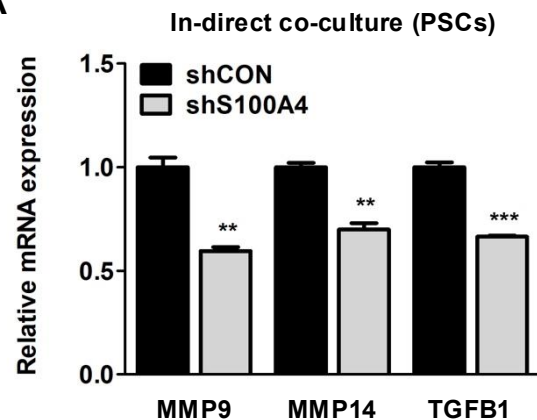
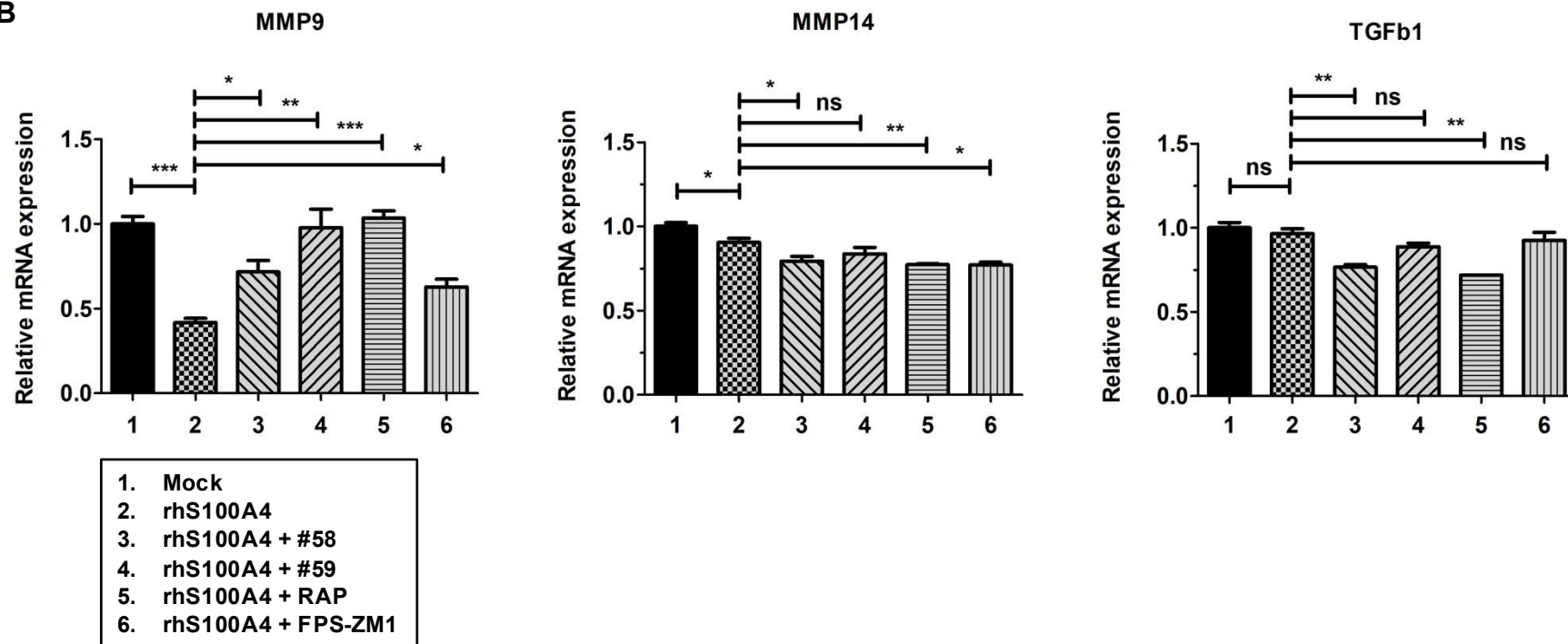
Supplementary Figure S6. Collective invasion assay using AsPC-1 co-culture spheroids with knockdown of S100P, S100A4, and S100A10

(A) Cell viability assay using 115026 cell line with knockdown or overexpression of S100P or S100A4. **(B and C)** Representative images and quantification of collective invasion assay using AsPC-1 co-culture spheroids with knockdown of S100P or S100A4 and S100A10. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.



Supplementary Figure S7. Collective invasion assay using S100A10-knockdown patient-derived PDAC cells and S100A10 expression in normal pancreatic cells [55]

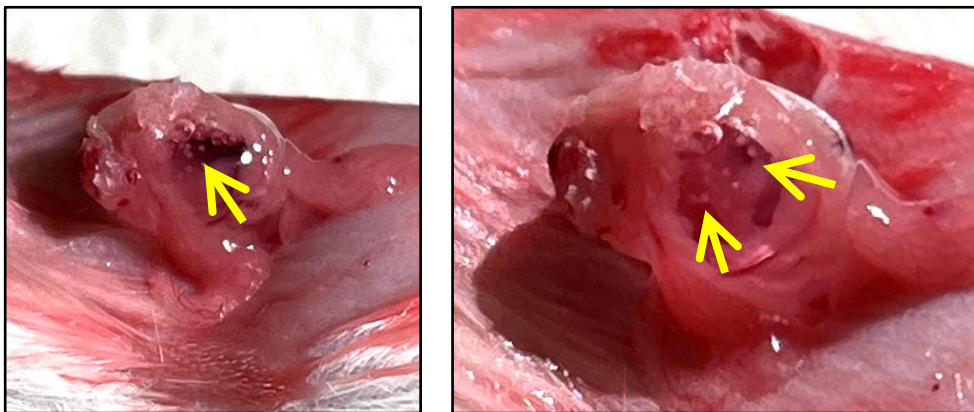
(A and B) Representative images and quantification of collective invasion assay using 115026 (A) and 110621 (B) co-culture spheroids with S100A10 knockdown.

A**B**

Supplementary Figure S8. Gene expression of MMP9, MMP14, and TGFB1 under indirect co-culture and co-treatment with rhS100A4 and S100A4 antagonists

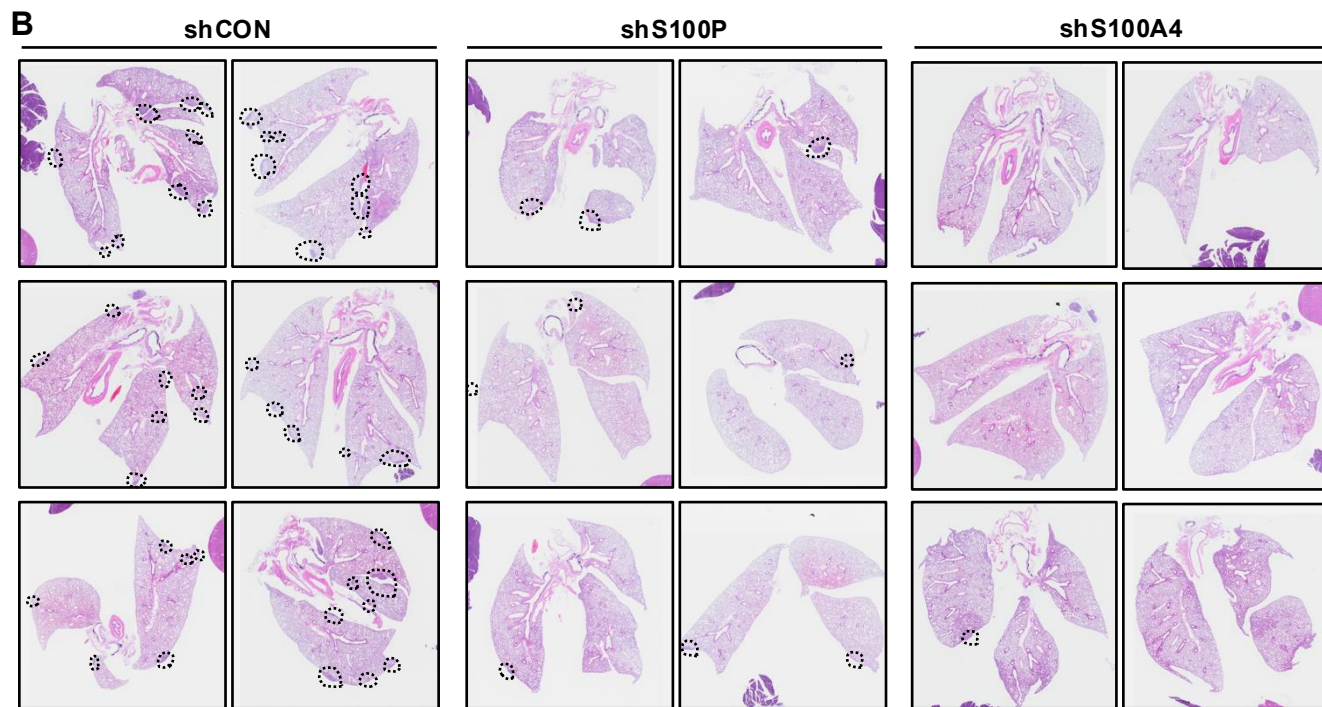
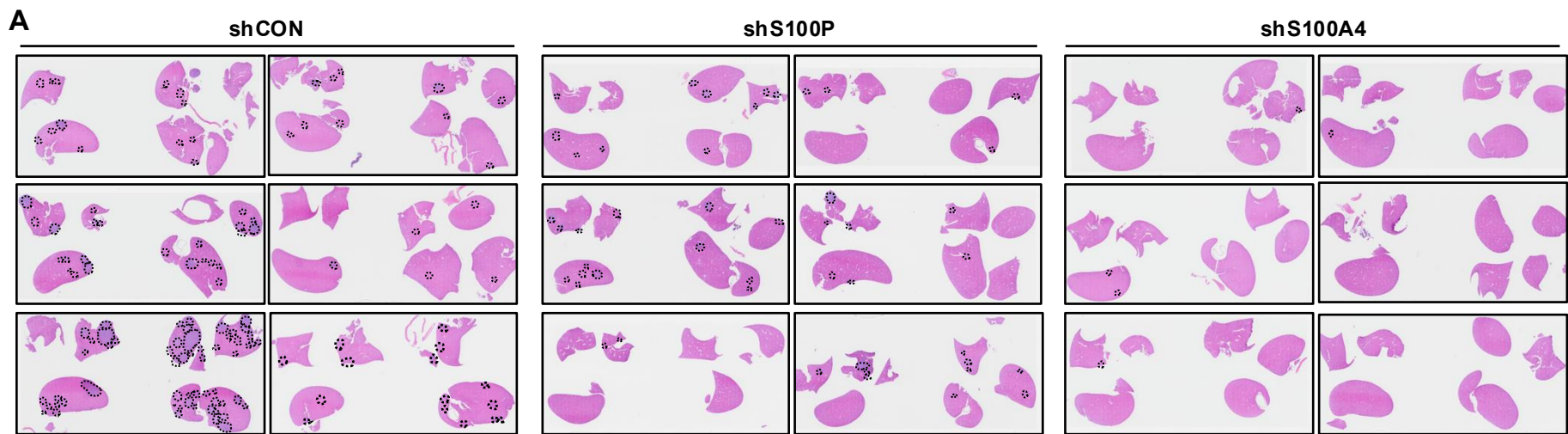
(A) Expression of MMP9, MMP14, and TGFB1 in PSCs under indirect co-culture conditions. **(B)** Expression of MMP9, MMP14, and TGFB1 in PSCs following treatment with 1 µg/mL rhS100A4 alone or co-treatment with 1 µg/mL of rhS100A4 and 2 µM S100A4 antagonists, including S100A4-targeting peptides #58, #59, RAP, and FPS-ZM1. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

A



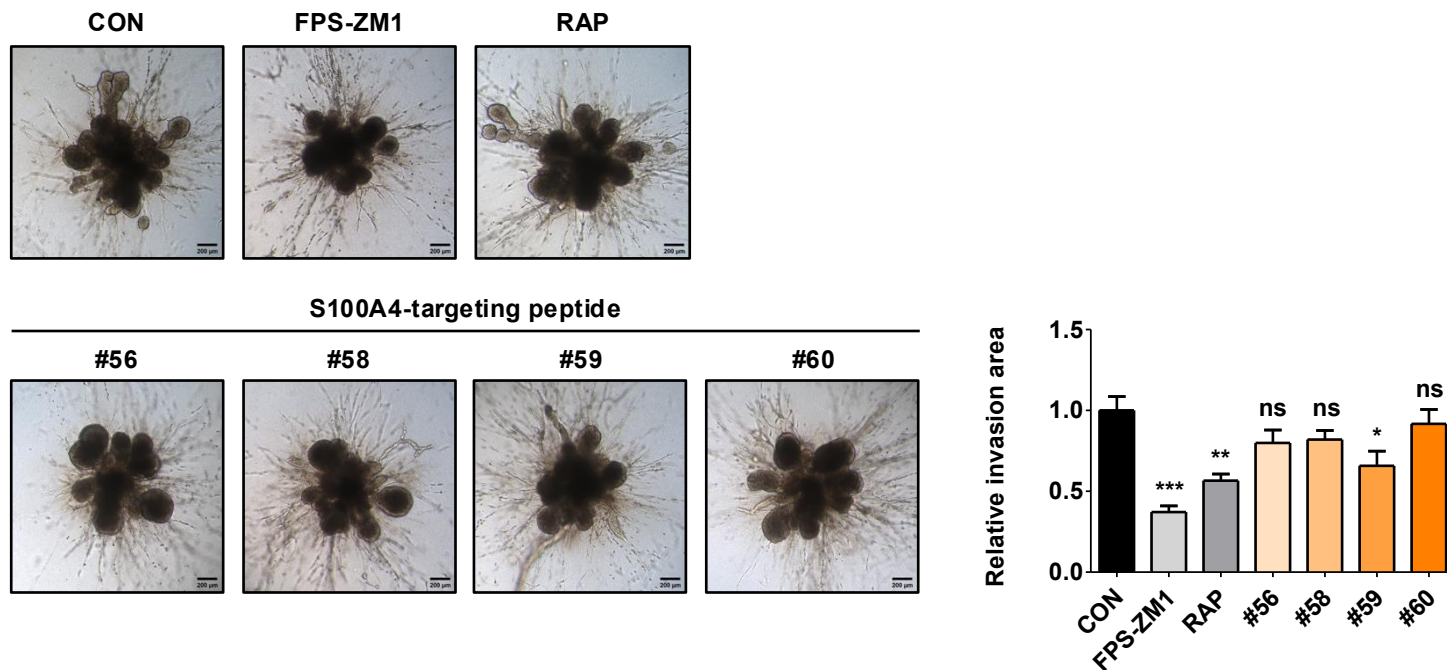
Supplementary Figure S9. Verification of injected spheroids in the pancreatic tumor orthotopic model

Injected spheroids (yellow arrow) were detected in the pancreas following the pancreatic orthotopic injection.



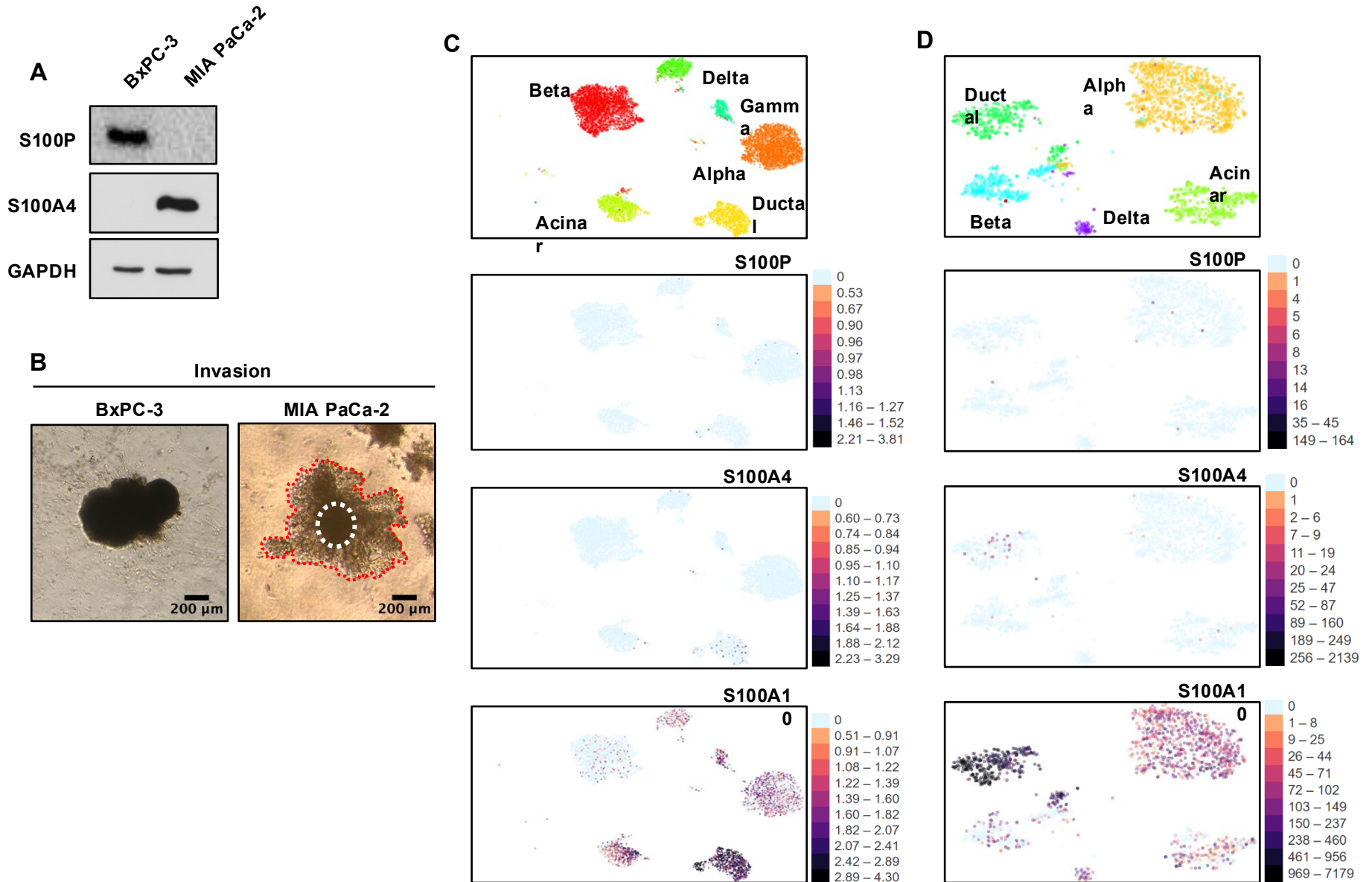
Supplementary Figure S10. Raw data of H&E staining for liver and lung of AsPC-1 orthotopic metastasis model

(A and B). H&E images of whole Liver(A) or Lung (B) from mouse injected with control (shCON), S100P or S100A4 depleted spheroids (shS100P or shS100A4) orthotopically. Metastasized lesions are marked with black dotted circles (N=6 for each group)

A

Supplementary Figure S11. Collective invasion of co-culture spheroids treated with S100A4-targeting inhibitory peptides

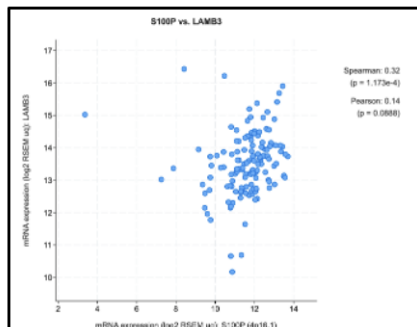
(A) Representative images and quantification of collective invasion assays using 110621 co-culture spheroids treated with 10 μ M S100A4-targeting inhibitory peptides (#56, #58, #59, and #60), RAP, or FPS-ZM1. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.



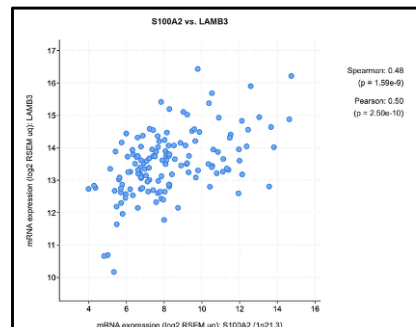
Supplementary Figure S12. Expression pattern of endogenous S100P and S100A4 in PDAC cell lines or normal pancreatic cells

(A) Western blot analysis of S100P and S100A4 in BxPC-3 cells and MIA PaCa-2 cells. **(B)** Representative images of collective invasion of BxPC-3 and MIA PaCa-2. **(C and D)** Single-cell RNA sequencing data analysis was performed using the UCSC Cell Browser (GSE81547 and GSE84133). Gene expression of S100P, S100A4, and S100A10 in normal pancreatic cells, including ductal cells, alpha cells, beta cells, gamma cells, delta cells, and acinar cells. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

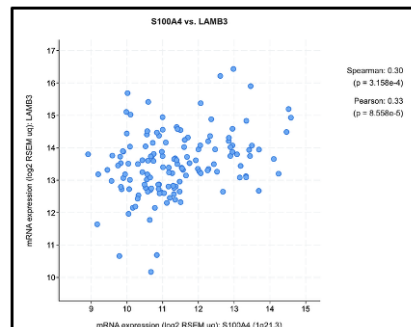
S100P-LAMB3



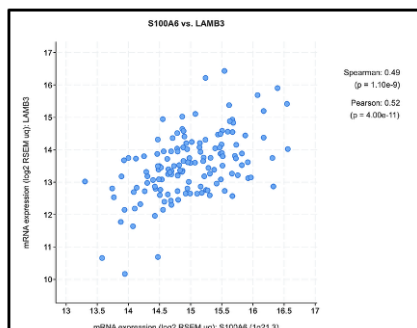
S100A2-LAMB3



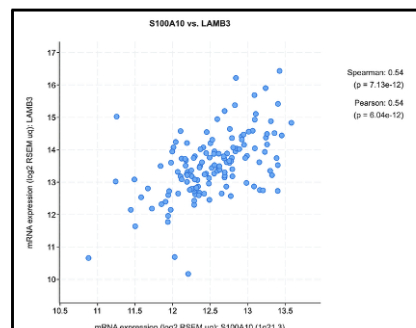
S100A4-LAMB3



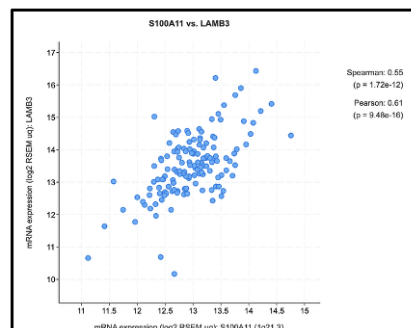
S100A6-LAMB3



S100A10-LAMB3

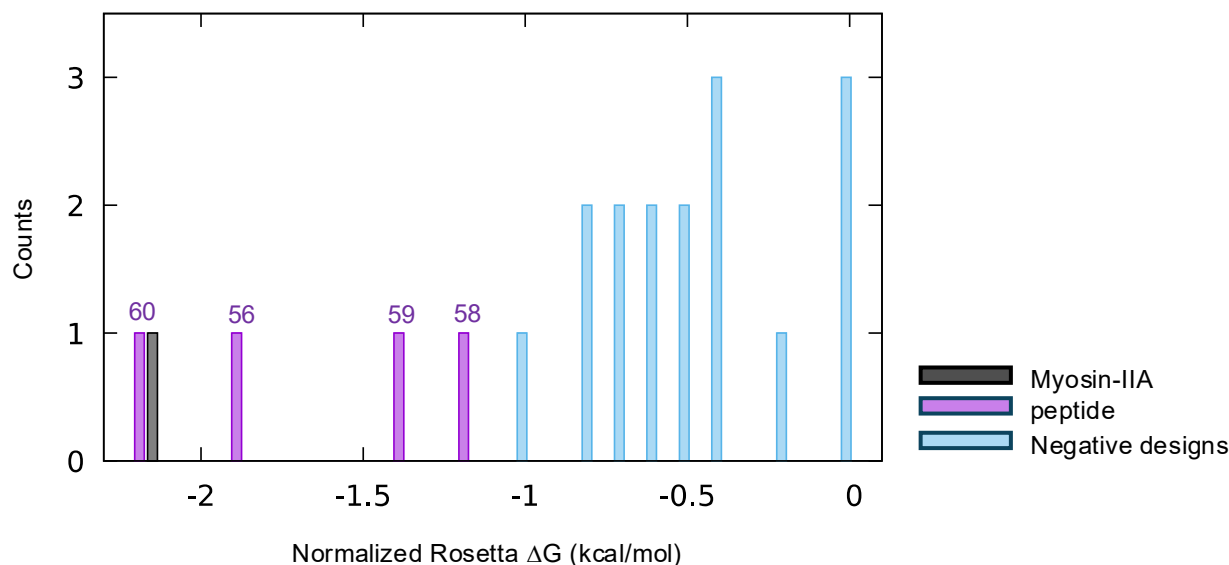


S100A11-LAMB3



Supplementary Figure S13. Correlation between S100 family members and LAMB3

Expression correlation analysis between S100 family members and LAMB3 in PDAC tissues, using cBioPortal data (CPTAC, Cell 2021)

A

Supplementary Figure S14. Predicted binding energetics of positive peptide designs and negative design backgrounds

(A) Distribution of calculated Rosetta binding energies for a known binder, Myosin-IIA peptide (black), four positive designed binders (purple), and 15 negative designs representing the background energy distribution (blue). The negative designs were generated using the same backbone structures as the positive binders, but their sequences were designed in the unbound state using ProteinMPNN [24]. Binding energies were computed using the Rosetta energy function [56] and defined as the energy difference between the bound and unbound states. To enable comparison across peptides of different lengths, the resulting values were normalized by peptide residue count. More negative normalized ΔG values indicate more favorable predicted binding energetics.

Supplementary Tables 1-4

Supplementary Table S1. Primer list

Primers for qPCR	
S100P_F	5'-AGGTGCTGATGGAGAAGGAG-3'
S100P_R	5'-ACTCACTGAAGTCCACCTGG-3'
S100A4_F	5'- ACCTTCCACAAGTACTCGGG-3'
S100A4_R	5'-GCTGTCCAAGTTGCTCATCA-3'
S100A10_F	5'-CGCTGGGGATAAAGGCTACT-3'
S100A10_R	5'-AGCCCACTTTGCCATCTCTA-3'
MMP9_F	5'-GCCACTACTGTGCCTTTGAGTC-3'
MMP9_R	5'-CCCTCAGAGAATCGCCAGTACT-3'
MMP14_F	5'-CCTTGGACTGTCAGGAATGAGG-3'
MMP14_R	5'-TTCTCCGTGTCCATCCACTGGT-3'
ITGB4_F	5'-CTCCTACCGCACACAGGATG-3'
ITGB4_R	5'-GCTTCACCTGCAGCTCTTTC-3'
LAMB3_F	5'-GTCACAGAGCAGGAGGTGGCT-3'
LAMB3_R	5'-GCTTCTGTCAAGACTCTCCAGG-3'
LAMA3_F	5'-TAGAGGAAGCCTCTGACACAGG-3'
LAMA3_R	5'-CCGATAGTATCCAGGGCTACAAC-3'
TGFB1_F	5'-CCCCTACATTGGAGCCTGG-3'
TGFB1_R	5'-GCACGATCATGTTGGACAGC-3'
GAPDH_F	5'- CCCATGTTTCGTCATGGGTGT-3'
GAPDH_R	5'-TGGTCATGAGTCCTTCCACGATA-3'
Primers for shRNA cloning	
shS100P_F	5'-CCGGGTGTACCTCTGATTAACTCGAGTTATTAATCAGAGGTACACTTTTT-3'
shS100P_R	5'- AATTAAAAAGTGACCTCTGATTAACTCGAGTTATTAATCAGAGGTACAC-3'
shS100A4_F	5'-CCGGGCTGAGCAAGTTC AATAAACTCGAGTTATTGAACTTGCTCAGCTTTTT-3'
shS100A4_R	5'- AATTAAAAAGCTGAGCAAGTTC AATAAACTCGAGTTTATTGAACTTGCTCAGC-3'
shS100A10_F	5'-CCGGGCCCTTGCCCTCAATAAATACTCGAGTATTATTGAGGGCAAGGCTTTTT-3'
shS100A10_R	5'- AATTAAAAAGCCTTGCCCTCAATAAATACTCGAGTATTATTGAGGGCAAGGC-3'
Primers for gateway cloning	
S100P_F	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGATGTGCAGTTCTCTG-3'
S100P_R	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCGGGTCGGTCTGGGCAGCC-3'
S100A4_F	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCGTGCCCTCTGGAG-3'
S100A4_R	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCCTTCTTCCTGGGCTGCTT-3'
GFP_F	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGTGAGCAAGGGCGAG-3'
GFP_R	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCCTTGTACAGCTCGTCCAT-3'
RFP_F	5'-GGGGACAAGTTTGTACAAAAAAGCAGGCTCTATGGTGTCTAAGGGCGAA-3'
RFP_R	5'-GGGGACCACTTTGTACAAGAAAGCTGGGTCATTAAGTTTGTGCCCCAG-3'

Supplementary Table S2. List of antibodies used in this study

Name	Cat.No.	Dilution
S100P	sc-374547 (Santa Cruz Biotechnology)	1:1000 (WB)
S100A4	ab197896 (Abcam)	1:1000 (WB)
S100A10	11250-1-AP (Proteintech)	1:1000 (WB)
PanCK	Ab86734 (Abcam)	1:200 (IHC-P)
GAPDH	Sc-47724 (Santa Cruz Biotechnology)	1:2000 (WB)
Anti-mouse IgG, HRP-linked	7076 (Cell Signaling technology)	1:5000 (WB)
Anti-rabbit IgG, HRP-linked	7074 (Cell Signaling technology)	1:5000 (WB)

Supplementary Table S3. Amino Acid Sequences of the S100A4-targeting inhibitory peptides selected by AI

#56	SFNELQIKTLKTFADLCEERGLKEKCLKAL EELKKLAEVLNDE ELIKEVEERIKKLN
#58	SFKEFSFNQLLQCAKIAKALGKEELMEKLLKEAEKIVKKLNDEELKKRLEEFKKKIN
#59	SPNELLIQQLKTYRLSKDLGREELAKKALEKCLKELAKKLNDEELLKEVEEEEEKEN
#60	SVDELLIKQLETAAKLAEDFGSKEKAEEKYKKLIEKLLKGDEEAEKRAEELEEELK

Supplementary Table S4. Summary of the in-silico selectivity test.

	iPAE* from AplhaFold2			
Peptide	S100a4	S100a1	S100a6	S100p
#60	6.3	20.3	19.0	19.9
#59	9.9	20.8	20.3	19.4
#58	7.7	18.9	16.6	7.5 (RMSD=32.5Å)
#56	8.7	18.5	15.0	16.5

* interface Predicted Aligned Error : score for the inface prediction in Alphafold-multimer. A lower iPAE value indicates a higher confidence