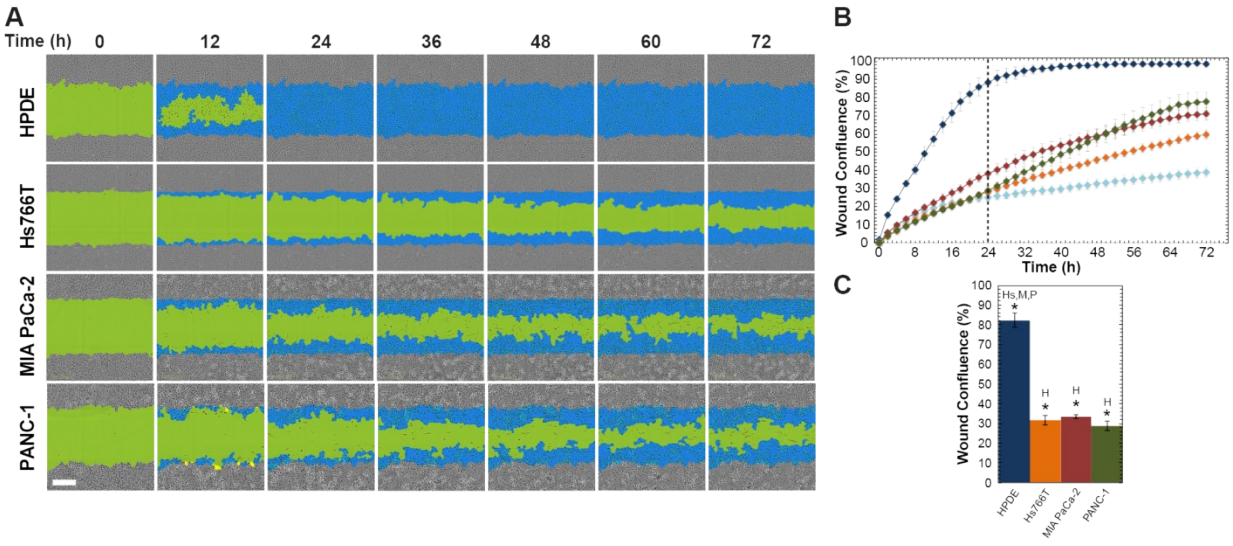
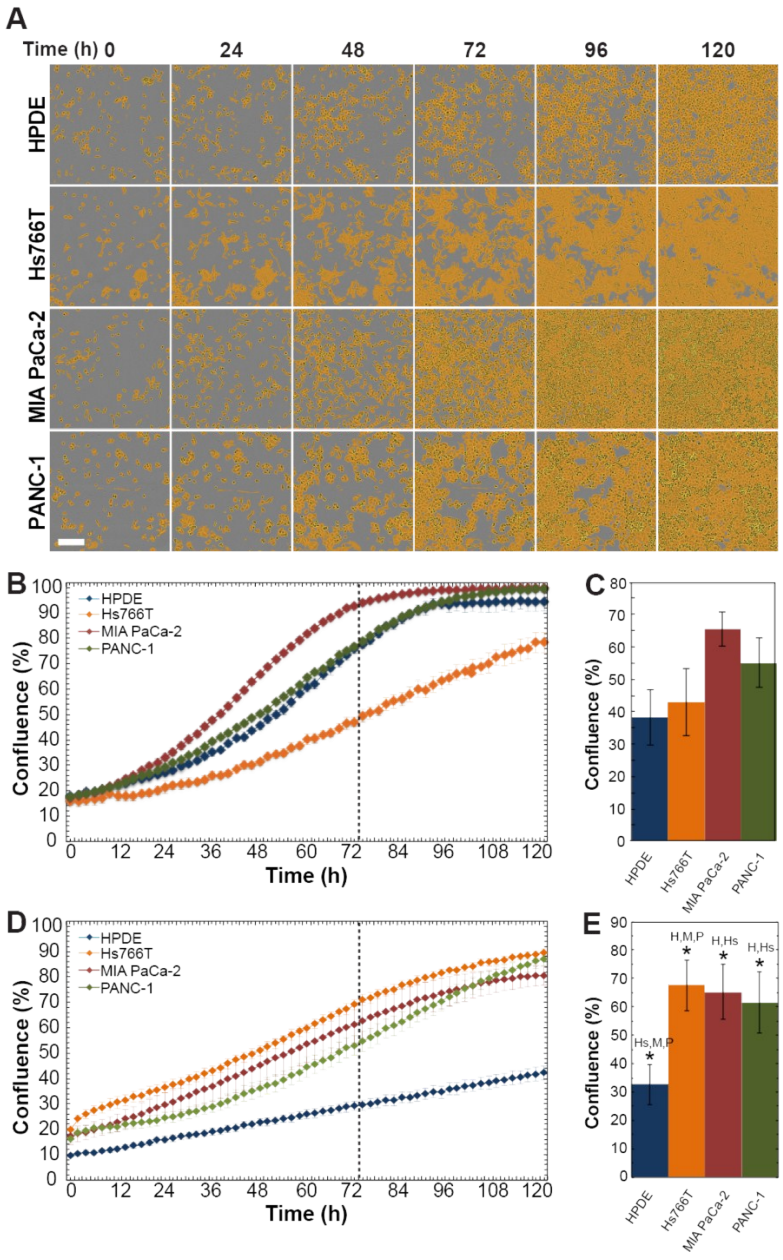
Electronic Supplementary Material (ESI) for Integrative Biology.

Supplemental Figure 1



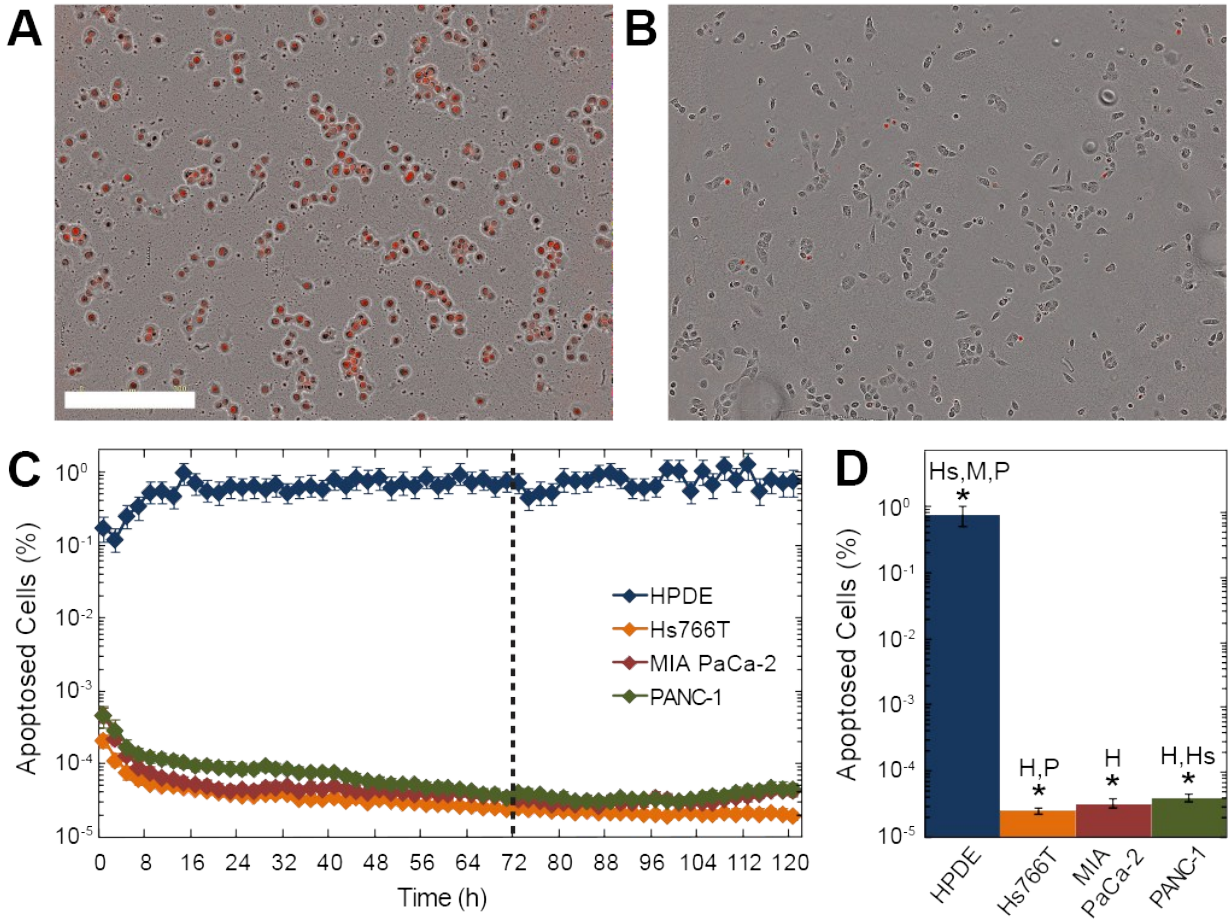
Migration behavior of pancreatic ductal cell lines. A. Time series of images showing scratch wound migration of pancreatic ductal cells without Matrigel. Wound confluence is the percentage of wound area covered by cells. Color legend: green is the wound area, grey represents the confluent cells outside of the wound area, and blue shows wound confluence in the wound area. Scale, 300 μm. **B.** Quantification of migration across three independent experiments. Dotted line indicates the 24-hour time point, which we use compare wound confluence values for statistical significance. **C.** Bar plot of migration data after 24 hours. Error bars represent standard error. P-values determined by student's T-test. *p < 0.05. The significance of pairwise comparisons between cell lines is shown in panel C by the initial(s) of the cell lines that are significantly different where H: HPDE, Hs: Hs766T, M: MIA PaCa-2, and P: PANC-1. For example, HPDE is significantly different (*p < 0.05) from Hs766T (Hs), MIA PaCa-2 (M), and PANC-1 (P).

Supplemental Figure 2



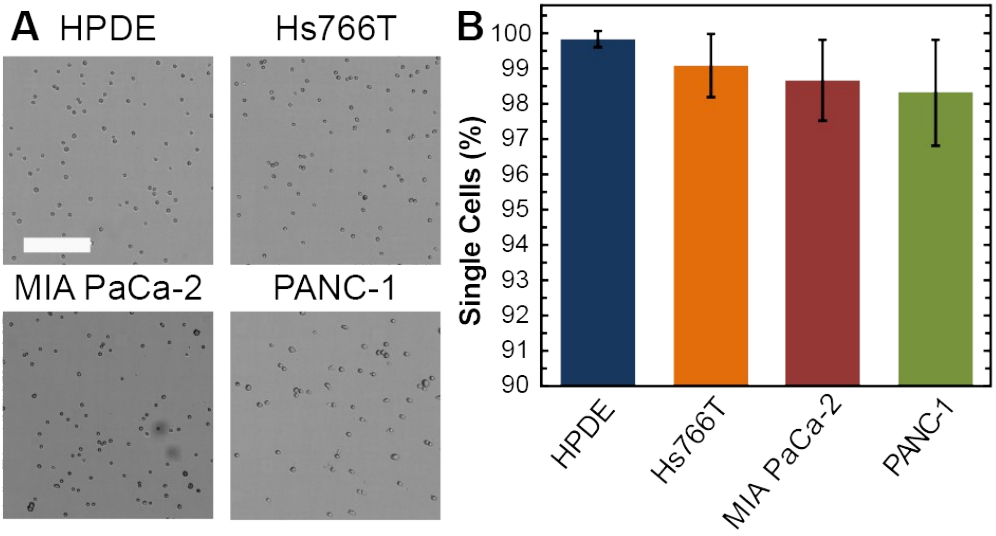
Proliferation of pancreatic ductal cell lines. A. Time series of images showing confluence of pancreatic ductal cells. Scale, 300 μm. **B.** Representative quantification from one experiment of percent confluence, which denotes the percentage area coverage of cells plated on a thin layer of Matrigel. Dotted line indicates the 72-hour time point, which we use compare confluence values for statistical significance. **C.** Bar plot of confluence data after 72 hours averaged across three individual experiments. Error bars represent standard error. P-values determined by student's T-test. None of the pairwise comparisons between pancreatic ductal cell lines are significant ($p > 0.05$). **D.** Representative quantification from one experiment of percent confluence of cells plated on a thin layer of Matrigel and overlaid with a thick layer of Matrigel. Dotted line indicates the 72-hour time point, which we use compare confluence values for statistical significance. **E.** Bar plot of confluence data after 72 hours averaged across three individual experiments. The significance of pairwise comparisons between cell lines is shown in panel E by the initial(s) of the cell lines that are significantly different where H: HPDE, Hs: Hs766T, M: MIA PaCa-2, and P: PANC-1. For example, HPDE is significantly different ($*p < 0.05$) from Hs766T (Hs), MIA PaCa-2 (M), and PANC-1 (P).

Supplemental Figure 3



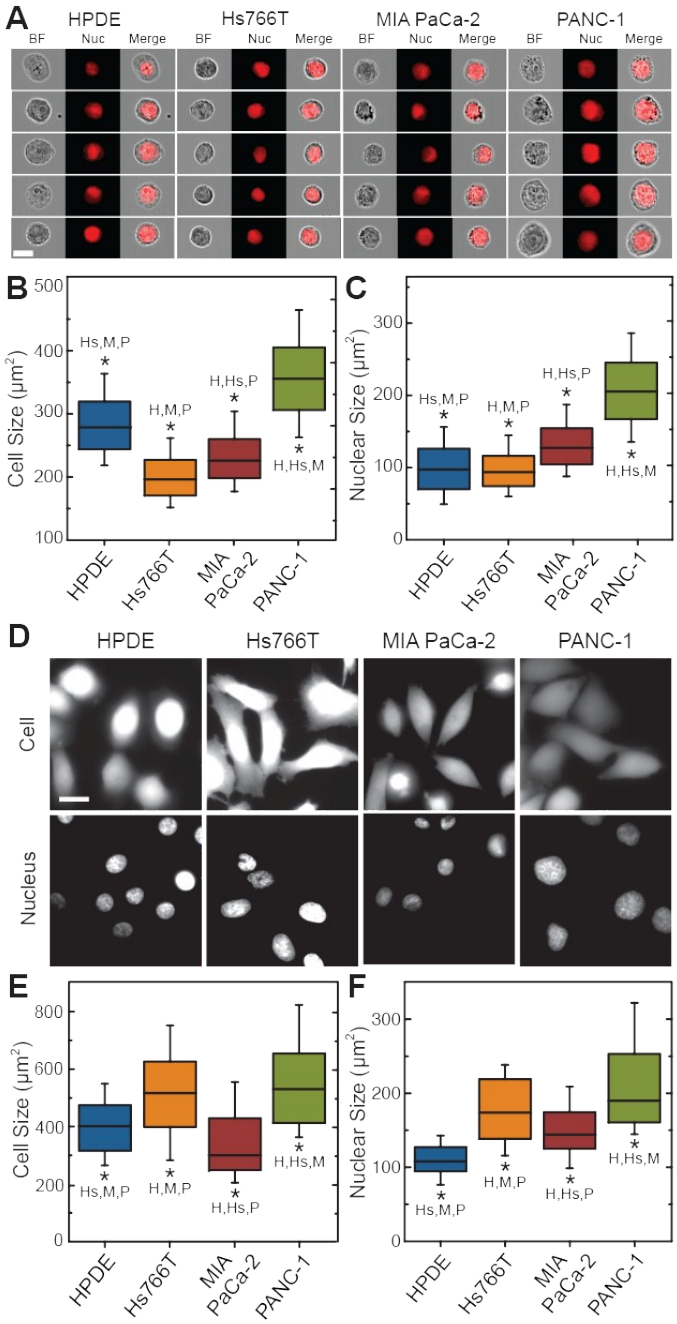
Apoptosis of pancreatic ductal cell lines with Matrigel overlay. Representative images of cells stained with DRAQ7 to quantify apoptosis: **A.** positive control (HPDE cells treated with DMEM, which is causes these cells to undergo apoptosis) and **B.** HPDE cells with a Matrigel overlay after 72 hours. Scale, 350 μm. **C.** Data of apoptosis across 4 pancreatic ductal cell lines over 3 independent experiments. Data points represent average % apoptosis over 3 independent experiments and error bars represent standard deviation. Dotted line indicates the 72-hour time point, which we use compare % apoptosis for statistical significance. **D.** Bar plot of apoptosis data after 72 hours averaged across three individual experiments. Error bars represent standard deviation. Statistical significance determined using a Student's t-test. *$p < 0.05$. The significance of pairwise comparisons between cell lines is shown in panel C by the initial(s) of the cell lines that are significantly different where H: HPDE, Hs: Hs766T, M: MIA PaCa-2, and P: PANC-1. For example, HPDE is significantly different (*$p < 0.05$) from Hs766T (Hs), MIA PaCa-2 (M), and PANC-1 (P).

Supplemental Figure 4



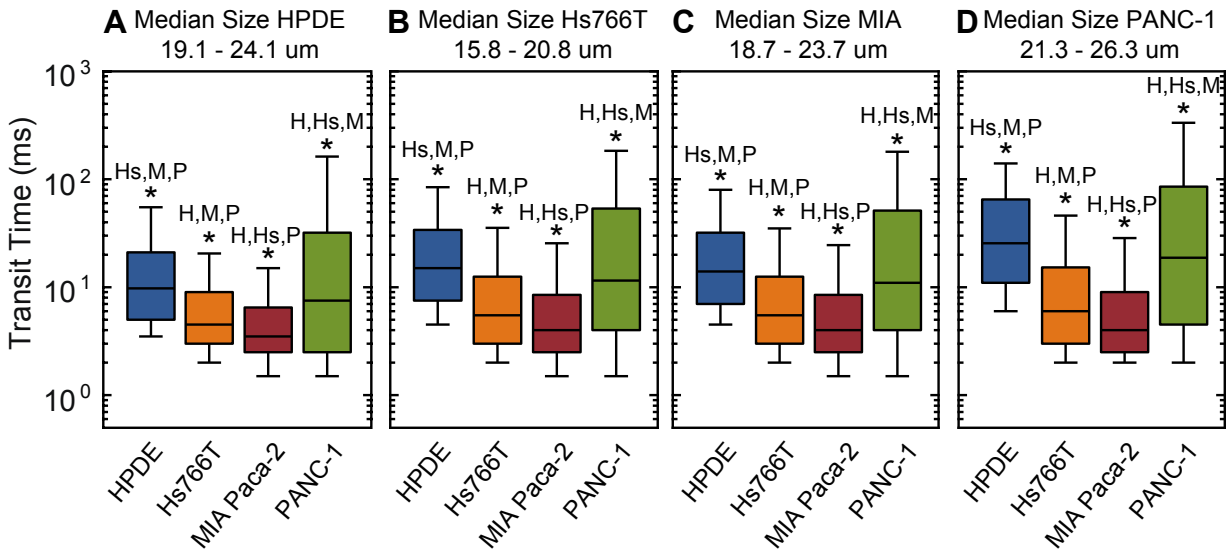
Cell suspensions for PMF assay contain over 98% single cells. A. Representative images of cell suspensions prior to PMF. Scale, 300 μm. B. Quantification of single cells determined by image analysis. Bar plot represents the average from 3 independent experiments. Error bars represent standard deviation. Statistical significance determined using a Student's t-test. No significance is observed ($p > 0.05$) for all pairwise comparisons between cell lines.

Supplemental Figure 5



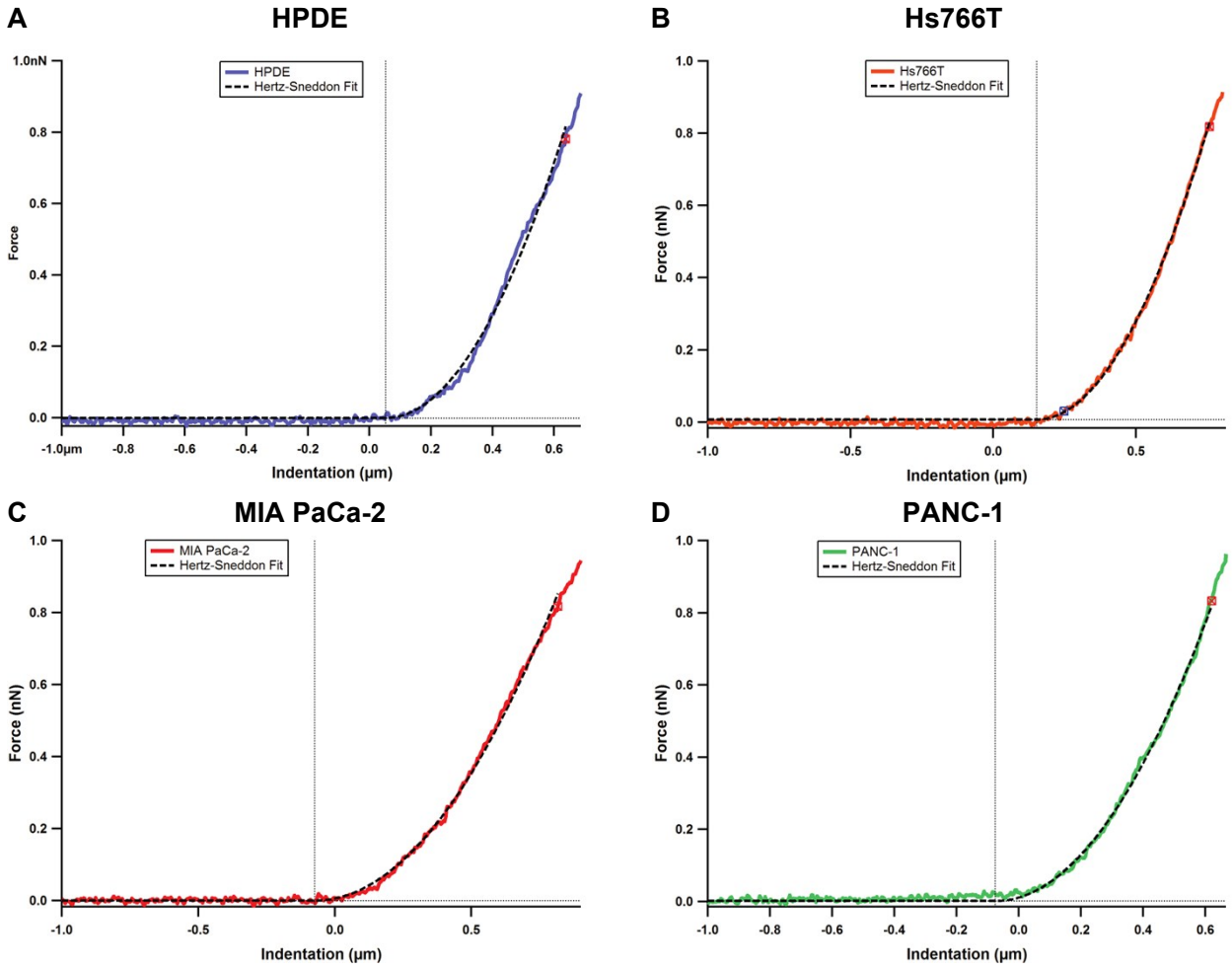
Cell and nuclear size of PDAC cells. A. Imaging flow cytometry of HPDE and PDAC cells that are fixed in a suspended state. Nuclei are labeled with DRAQ5. Scale, 15 μm. We use this data to quantify **B.** Cell size and **C.** Nuclear size. **D.** Images of adhered pancreatic ductal cells obtained by confocal microscopy. From this data, we measure **E.** Cell size and **F.** Nuclear size. Boxes represent the 25-75 percentile, whiskers represent the 10-90 percentile, and the horizontal line represents the median. P-values are calculated with a Mann-Whitney U test. *p < 0.05. All error bars represent standard errors. The significance of pairwise comparisons between cell lines is shown in panels B, C, E, and F by the initial(s) of the cell lines that are significantly different where H: HPDE, Hs: Hs766T, M: MIA PaCa-2, and P: PANC-1. For example, in panel B, HPDE is significantly different (*p < 0.05) from Hs766T (Hs), MIA PaCa-2 (M) and PANC-1 (P).

Supplemental Figure 6



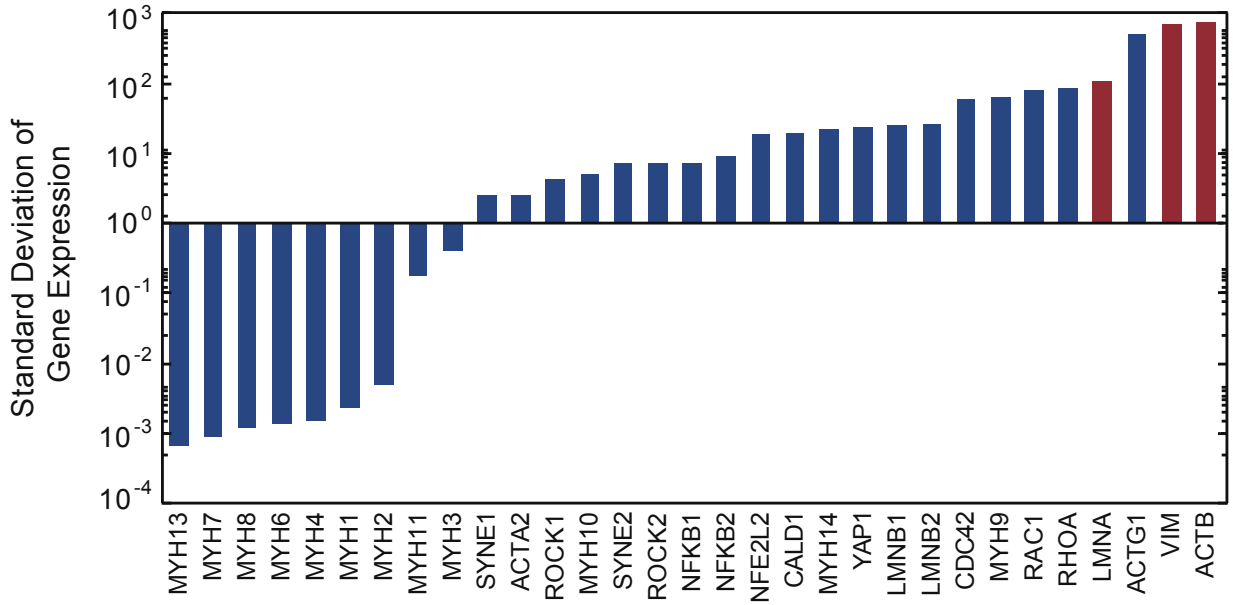
Microfluidics data gated for cell size. Each plot shows transit time data obtained by microfluidic deformability cytometry for pancreatic ductal cell lines within a 5 μm range of the median size of each cell line: **A.** HPDE, **B.** Hs766T, **C.** MIA PaCa-2, and **D.** PANC-1. Boxes represent the 25th and 75th percentiles, whiskers represent the 10th and 90th percentiles, and the horizontal line represents the median. n > 350 cells for each box. The significance of pairwise comparisons between cell lines is shown in panels B, C, E, and F by the initial(s) of the cell lines that are significantly different where H: HPDE, Hs: Hs766T, M: MIA PaCa-2, and P: PANC-1. For example, in panel A, HPDE is significantly different (*$p < 0.05$) from Hs766T (Hs), MIA PaCa-2 (M), and PANC-1 (P).

Supplemental Figure 7



Hertz-Sneddon fits for AFM force curves. Each plot shows an example force curve obtained by atomic force microscopy (AFM) for pancreatic ductal cell lines: **A.** HPDE, **B.** Hs766T, **C.** MIA PaCa-2, and **D.** PANC-1. Hertz-Sneddon fits are indicated by black dashed lines.

Supplemental Figure 8



RNAseq analysis of mechanome proteins across cohort of 41 pancreatic cancer cell lines. Bar plot represents the standard deviation in gene expression levels of proteins that contribute to cell mechanotype.

Supplemental Table 1. Founder mutations in PDAC cell lines.
Adapted from Deer, E. L., González-Hernández, J., Coursen, J. D., Shea, J. E., Ngatia, J., Scaife, C. L., … Mulvihill, S. J. (2010). Phenotype and genotype of pancreatic cancer cell lines. *Pancreas*, *39*(4), 425–35.

Gene	HPDE	HPAF	MIA PaCa-2	PANC-I	Hs766T
KRAS	WT	Mut	Mut	Mut	Mut
TP53	WT	Mut	Mut	Mut	Mut
CDKN2A/p16	WT	Mut	Mut	Mut	Mut
SMAD4/DPC4	WT	WT	WT	WT	Mut

Supplemental Table 2. Pearson's values (top) and significance values (bottom) for correlation heat map as shown in Figure 5.

		Invasive Potential		Deformability			Structural Proteins				
	PEARSON'S CORRELATIONS	Invasion	Transwell Migration	Young's Modulus	Retention	Transit Time	Vimentin	F-actin	Beta-actin	Lamin A	Lamin C
Invasive Potential	Invasion	1	0.98711	0.97116	0.2098	-0.23968	0.62	-0.94657	0.99489	0.95849	0.55184
	Transwell Migration	0.98711	1	0.92047	0.0506	-0.39198	0.73759	-0.88274	0.99823	0.90049	0.41124
Deformability	Young's Modulus	0.97116	0.92047	1	0.43688	-0.00128	0.41505	-0.99616	0.94211	0.99883	0.73476
	Retention	0.2098	0.0506	0.43688	1	0.89896	-0.63706	-0.51392	0.10998	0.47987	0.93117
	Transit Time	-0.23968	-0.39198	-0.00128	0.89896	1	-0.91033	-0.08623	-0.33651	0.04709	0.67738
Structural Proteins	Vimentin	0.62	0.73759	0.41505	-0.63706	-0.91033	1	-0.33384	0.69608	0.37056	-0.31218
	F-actin	-0.94657	-0.88274	-0.99616	-0.51392	-0.08623	-0.33384	1	-0.90915	-0.99923	-0.7913
	Beta-actin	0.99489	0.99823	0.94211	0.10998	-0.33651	0.69608	-0.90915	1	0.92479	0.46479
	Lamin A	0.95849	0.90049	0.99883	0.47987	0.04709	0.37056	-0.99923	0.92479	1	0.76671
	Lamin C	0.55184	0.41124	0.73476	0.93117	0.67738	-0.31218	-0.7913	0.46479	0.76671	1

		Invasive Potential		Deformability			Structural Proteins				
	SIGNIFICANCE	Invasion	Transwell Migration	Young's Modulus	Retention	Transit Time	Vimentin	F-actin	Beta-actin	Lamin A	Lamin C
Invasive Potential	Invasion	--	0.10234	0.15327	0.86544	0.84591	0.57426	0.20905	0.06441	0.18407	0.62786
	Transwell Migration	0.10234	--	0.2556	0.96777	0.74358	0.47192	0.31139	0.03793	0.28641	0.73019
Deformability	Young's Modulus	0.15327	0.2556	--	0.71217	0.99918	0.72753	0.05578	0.21767	0.0308	0.47459
	Retention	0.86544	0.96777	0.71217	--	0.28865	0.5603	0.65639	0.92984	0.68137	0.23758
	Transit Time	0.84591	0.74358	0.99918	0.28865	--	0.27165	0.94503	0.78151	0.97001	0.52623
Structural Proteins	Vimentin	0.57426	0.47192	0.72753	0.5603	0.27165	--	0.78331	0.50986	0.75833	0.79788
	F-actin	0.20905	0.31139	0.05578	0.65639	0.94503	0.78331	--	0.27346	0.02498	0.4188
	Beta-actin	0.06441	0.03793	0.21767	0.92984	0.78151	0.50986	0.27346	--	0.24848	0.69226
	Lamin A	0.18407	0.28641	0.0308	0.68137	0.97001	0.75833	0.02498	0.24848	--	0.44378
	Lamin C	0.62786	0.73019	0.47459	0.23758	0.52623	0.79788	0.4188	0.69226	0.44378	--