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Supplementary Information for

Calcium Channel Blockers Potentiate Gemcitabine Chemotherapy in Pancreatic Cancer

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SUPPLEMENTAL MATERIALS AND METHODS

Chemicals and Reagents

For *in vitro* experiments, Gemcitabine, Amlodipine, Nifedipine, BAPTA-AM, KN-62, Paclitaxel, 5-Flurouracil, SN-38, and Oxaliplatin were purchased from Selleck Chem (Houston, TX), W-7 from Sigma Aldrich/Millipore (Burlington, MA), reconstituted in DMSO per the manufacturer instructions, and used as described. The NFAT-inhibitor peptide was purchased from Cayman Chemical (Ann Arbor, MI), reconstituted in PBS, and also used as described. For *in vivo* studies, Gemcitabine was purchased from the University of Illinois Hospital pharmacy, reconstituted in sterile saline, and used at 100 mg/kg. Amlodipine besylate was purchased from Selleck Chem, reconstituted in DMSO, then added to sterile saline with 20% polyethylene glycol to reach a final concentration of <1% DMSO. Amlodipine was used at 2 mg/kg.

Antibodies

All antibodies were purchased from established commercial vendors and were verified by the manufacturer for the specific species and applications for which they were used. A complete list of all antibodies used as well as the vendor, clone, and product numbers can be found in Table S1.

Drug Resistant Cell Lines

To generate the drug resistant PANC-1-GR cell line, cells were seeded at 50% confluence and incubated in 100nM Gemcitabine until 80% confluent. Cells were then passaged and seeded at 50%. After allowing a 24-hour recovery period without Gemcitabine, cells were incubated with 200nM of Gemcitabine and allowed to grow until 80% confluent. This process was repeated several times using

incremental increases in Gemcitabine concentrations including 300nM, 500nM, 1μM, 2μM, 3μM, 5μM, 7.5μM, and finally 10μM. Once cells were viable in 10μM, they were designated PANC-1-GR and maintained in high dose Gemcitabine.

Cell Culture and Viability Assays

Human pancreatic cancer cells (PANC-1, MiaPaCa-2, and MIA PaCa-2) were cultured in DMEM supplemented with 10% heat-inactivated FBS, penicillin (100U/mL), and streptomycin (100mg/ml). AsPC-1 cells were grown in RPMI 1640 supplemented with 10% heat-inactivated FBS, penicillin (100U/mL), and streptomycin (100mg/ml). Capan-1 cells were grown in IMDM1 supplemented with 10% heat-inactivated FBS, penicillin (100U/mL), and streptomycin (100mg/ml). Cell lines were purchased from the ATCC, used within six months, and kept under passage 10. All cell lines tested negative for mycoplasma via PCR within 6 months of use.

For cell viability assays, 2,000-4,000 cells were seeded into each well of a 96-well plate in serum free DMEM. After 16 hours, media/drug was added and cells cultured for 72 hours. At this time, 20ul of a 5mg/ml 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) solution was added to each well (1:10). After two hours, media was aspirated, crystals dissolved in DMSO, and 570 nm absorbance determined by plate reader.

Single Cell RNA Sequencing

Cells were trypsinized, collected in serum-free media and washed in PBS. Cells were re-suspended PBS and filtered through a 40-μm cell strainer. Cell counts were obtained using Trypan blue and hemocytometer. Cell suspension was

adjusted with PBS/BSA to 1.6×10^5 cells in a final volume of 1.5 mL for the flow. As previously, we followed the Macosko et al. Drop-seq protocol with minor modification, as described in our previous study (1). Pre- and post-tagmentation libraries were checked with Agilent TapeStation 4200, and sequencing performed using an Illumina NextSeq500, V2 High-output (400 M clusters) with custom Drop-seq read 1 primer.

For experiments using 10x genomics, cells were suspended in PBS/0.04% BSA targeting to generate 10,000 single cells, mixed with Master Mix and loaded onto a 10x Genomics Chromium Single Cell instrument. All the procedures were done using Single Cell 3' Reagent Kits v3 following the 10x Genomics user guide #CG000183 Rev B. Libraries were run on the Illumina NovaSeq 6000 also as described previously (1). For all experiments, extracted digital gene expression matrices were subjected to unsupervised clustering analysis using R package Seurat version 3.1.2, R version 3.5.322,79, with subsequent analyses performed as described (1). The number of sequenced cells and genes for each experiment is shown in Table S2.

scRNA-seq processing

Count matrices from either Drop-seq or 10x Genomics were processed using the Seurat R package (2, 3). The following parameters, which included basic statistics created by FastQC (version 0.11.5) report, were used for assessing cell quality: nFeature_RNA, nFeature_RNA to nCount_RNA distribution, and the percent of mitochondrial genes (percent.MT), Dispersion or Standardized Variance to Average Expression, the number of significant PCs, whether there were cells

predicted to be doublets, and the identity of top cluster markers (such as no irrelevant stress response genes).

For cells that have passed the quality criteria above, nFeature_RNA mitochondrial genes range Table S3 was used for filtering cells. We empirically determined the cutoff for the minimum number of genes per cell (nFeature_RNA), which was not high enough to disproportionately filter out the cells but also not low enough to affect the cell distribution by inclusion of low-quality cells, while avoiding rare cell doublets by setting the nFeature_RNA_max as indicated in Table S3. After filtering out low quality cells, only protein coding was used, and no mitochondrial genes were kept for further analysis.

In order to select the ideal number of clusters we took the following into consideration: (1) only principal components (PCs) with *P* value < 10^{-10}, calculated with the "JackStraw" function in Seurat; (2) highest average silhouette width (4) for the combined clusters; (3) preventing cells from treated and untreated mixed in the same cluster; (4) avoiding clusters for identical top genes (to prevent over-clustering); (5) overlapping or missed signatures from gene set enrichment analysis, that would indicate over-clustering or under-clustering, respectively. After clustering, the following clusters were excluded from further analysis:

- All treated and untreated PANC-1 cells: *Cluster 9* had only 18 cells from control sample and was removed from cluster presentations (e.g. UMAPS, GITools); *Cluster 8* was formed by only 20 cells and was removed from several UMAPs and lists of markers.

- All treated and untreated PANC-1-GR cells: *Clusters 9 and 10 expressing IFIT1-3, B2M, ISG15, and Cluster 11 expressing PNISR, GADD45A, GOLGA8A,B, were removed*
- PANC-1-GR + Amlodipine, BAPTA-AM, or W-7: *Cluster 7 expressing IFIT1-3, B2M, ISG15 and Cluster 11, expressing high PNISR, GADD45A, GOLGA8A,B, were removed.* Clusters A and B are very similar to each other and somewhat different from W-7. In particular, W-7 does not have many cells in 1 2 3 but prominent 10 and 11, a likely reflection of the fact that A and B have other targets besides Calmodulin. The same is true for W-7.
- PANC-1-GR + Amlodipine: *Clusters expressing IFIT1-3, B2M, or high PNISR, GADD45A, GOLGA8A,B, were removed.* Clusters of both samples are very clearly separated, no overlap.
- PANC-1-GR + BAPTA-AM: *Clusters expressing IFIT1-3, B2M, or high PNISR, GADD45A, GOLGA8A,B, were removed.* SCD-positive clusters 12 and 7 contain cells from both samples. We attempted all possible combinations of dimensions and resolutions, and clusters do not change.
- PANC-1-GR + W-7: *Clusters expressing IFIT1-3, B2M, or high PNISR, GADD45A, GOLGA8A,B, were removed.* Cluster 4 (*UBE2S*, *PTTG1*/*securin*, *CCNB1* mitosis) and 9 (*BIRC3*, *PLAU*, *NFκB* factors) are similar between C and W. When the cell cycle genes were regressed, this removed cluster 4 but not cluster 9 (new cluster 6).

All clusters were kept in AverageExpression files.

Functional enrichment analysis

Functional annotation of genes was based on GO terms and transcription factor data sets available from MSigDB collections database v7.4 updated April

2021 (5). Enrichment analysis using z-score statistics was run on a data matrix representing avg_log2FC values. Thus, both positive and negative values were used to show upregulated genes and downregulated genes (in one condition such as treated cells versus another condition such as untreated cells), respectively. Sampling size was 10,000, and multiple test correction was performed using Benjamin and Hochberg's method. Heatmap of z-score values was generated using GiTools (6), with upregulated genes shown in red colors and downregulated genes shown in blue colors. The values < -1.96 and > 1.96 were considered significant.

10x Genomics

Cells were suspended in PBS/0.04% BSA targeting to generate 10,000 single cells, mixed with Master Mix, and loaded onto a 10x Genomics Chromium Single Cell instrument. All the procedures were done using Chromium Next GEM Single Cell 3' Reagent Kits v3.1 following the 10x Genomics user guide #CG000204 Rev D. Libraries were sequenced on two SP 28x90nt lanes in a NovaSeq 6000. Read1 is 28nt in length, Read2 is 90nt in length and I1 is 8nt in length. Fastq.gz files were generated and demultiplexed with CellRanger 6.0.1. Mapping was done using CellRanger 6.0.0 and human genome version GRCh37.p13 with Ensembl gene model (release 74) as the reference.

Western blot and immunoprecipitation

Cells were lysed in RIPA buffer followed and homogenized by sonication. Equal amounts of protein (15–50 µg) were mixed with loading dye, boiled for 8 minutes, separated on a denaturing SDS–PAGE gel and transferred to a PVDF

membrane. The membrane was blocked in 5% milk/TBS/0.1% Tween for 1 hour and incubated with antibodies against Calmodulin, pRB, pMEK, MEK, pERK, ERK, cleaved caspase 3 (Cell Signaling, Danvers, MA, USA), Pan-RAS (Thermo Fischer, Waltham, MA), KRAS (Genetex, Irvine, CA), the L-type Calcium Channel (abcam), or GAPDH (Santa Cruz Biotech, Santa Cruz, CA, USA). The membrane was washed with TBS-0.1% Tween and then incubated with HRP conjugated secondary antibody (Cell Signaling) at room temperature for 1 hour and rewashed. Protein bands were visualized by an enhanced chemiluminescence method (Thermo, Waltham, MA, USA) and resolved digitally per the manufacturer's specifications.

For immunoprecipitation, cell lysates were collected using IP buffer (25 mM Tris–HCl pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.1% NP-40 and 5% glycerol) with a protease and phosphatase inhibitory cocktail (Cell Signaling), and cell extracts were incubated overnight with the respective antibodies followed by incubation with protein A or G agarose beads for 4 h at 4°C. After washing 5–7 times with lysis buffer, immunocomplexes were resolved using SDS–PAGE and visualized by western blot. All antibodies were compared with isotype specific IgG controls to affirm specificity. All experiments were performed in triplicate unless otherwise specified.

Primary Cell Line-Derived Xenografts

The G-68 cell line was established from a non-Hispanic, white female with a T3N1, moderately differentiated, pancreatic ductal adenocarcinoma using methods described previously (7), cultured in DMEM/F12 media supplemented

with 10% heat-inactivated FBS, penicillin (100 U/mL), and streptomycin (100mg/ml). As described in our previous study, $5x10^6$cells were suspended in 100 µL of a 1:1 DMEM/F-12 with Glautamax media and matrigel (Corning, Corning, NY) and inoculated subcutaneously into the right flank of NSG mice (The Jackson Laboratory) (8). Tumor size was measured twice weekly with digital caliper. For treatment, mice were randomized into treatment groups when the tumors reached 100-200 mm^3, with an average tumor volume of 145 mm^3. Once randomized, animals were treated with either PBS vehicle or 40mg/kg Gemcitabine via IP injection. Mice were euthanized when moribund, when the maximum tumor size allowed per to institutional policy (2 cm), or when tumors became ulcerated. For euthanasia, animals were scarified by CO_2 suffocation followed by cervical dislocation, and tumors were subsequently harvested and processed as described above.

Orthotopic Xenografts

Luciferase expressing PANC-1-GR cells (PANC-1-GR^{Luc}) were generated and $0.8x10^6$ cells implanted into the pancreas of NSG mice (The Jackson Laboratory, Bar Harbor, ME). Disease progression was monitored by IVIS, and once animals developed 100mm^3 tumors, they were administered (IP) injections of a PBS vehicle, 100mg/kg Gemcitabine, 2mg/kg Amlodipine, or Gemcitabine and Amlodipine. Mice were sacrificed when showing clear signs of health decline such as weight loss or lethargy.

Flow Cytometry and Cell Death Assays

Cells were collected, washed with PBS, and stained using an Annexin-V and 7-AAD kit per manufacturer instruction (BioLegend, San Diego, CA). Cells were analyzed with a BD Fortessa Cytometer, gating exclusively to cells within acceptable FSC/SSC parameters. All subsequent flow plots correspond to single cells based SSC-W gating, and are representative of 50,000 events unless otherwise stated. All experiments were compared to both unstained controls.

Histology, IHC/immunofluorescence, and Slide Scoring

Age-matched mice were euthanized and the pancreas, colon, small bowel, liver, and spleen were subjected to pathologic examination. Tissues were fixed in 10% formalin, paraffin-embedded, and sectioned at 4 mm interval. Tissues were then stained with hematoxylin and eosin (H&E), or via immunohistochemistry (IHC) or immunofluorescence (IF). For IHC, slides were deparaffinized by xylenes and rehydrated by ethanol gradient, then heated in a pressure cooker using DAKO retrieval buffer. Endogenous peroxidases were quenched in 3% hydrogen peroxide in methanol for 30 minutes. Tissues were blocked with 0.5% BSA in PBS for 30 minutes and incubated with primary antibodies against Calmodulin (abcam, Cambridge, MA), pERK, Cleavaed Caspase 3 (Cell Signaling Technology), or Ck19 (University of Iowa Hybridoma Bank, Iowa City, IA) at 1:50–1:200 overnight at 4°C. Slides were developed using either Streptavidin or secondary antibodies, followed by DAB substrate/buffer (DAKO).

For IF, cultured cells were fixed with methanol and paraffin-embedded slides were heated via pressure cooker in DAKO retrieval buffer and blocked with

0.5% BSA in PBS for 1 hour at room temperature. Cells/sections were exposed to primary antibodies against Calmodulin (abcam), E-Cadherin, (Cell Signaling Technology), PCNA (Santa Cruz Biotechnology), or CK19 (University of Iowa Hybridoma Bank) at 1:50–1:200 overnight at 4°C. Slides were developed using AlexaFluor 488- or 594–conjugated secondary antibodies (1:200–1:1,000, Abcam), mounted in DAPI-containing media (Santa Cruz Biotechnology), exposed to DAPI, FITC, and Texas Red filters, and images superimposed. All counts and scores were performed by two blinded investigators. For any contradicting scores, a third investigator was consulted.

Tissue Slide Counts, Scores, and Measurements

All counts were performed by a minimum of three blinded investigators, and each value displayed includes the average of minimum of three high power fields per specimen. All counts from each investigator were averaged and value distributions were visualized via Minitab express software, showing the median value as a solid line, as well as each individual value excluding any statistical outliers.

Antibody	Company	Clone	Product Number
Calmodulin 1,2,3	abcam	EP799Y	ab45689
L-Type Calcium Channel	-	EPR23267-8	ab253190
Anti-Pancreatic Amylase	-	Not Provided	ab21156
Anti-Rat 488	-	Not Provided	ab150153
Anti-Mouse 488	-	Not Provided	ab150117
Anti-Rat 488	-	Not Provided	ab96971
Anti-Rabbit 594	-	Not Provided	ab150084
Anti-Rat HRP	-	Not Provided	ab97057
Anti-pRB	CST	D20B12	8516S
Anti-pERK1/2	-	Not Provided	9101S
Anti-ERK1/2	-	137F5	4695S
Anti-pMEK1	-	Not Provided	9127S
Anti-MEK1	-	61B12	2352S
Anti-E-Cadherin	-	4A2	14472S
Anti-Cleaved Caspase 3	-	Asp175	9661S
Calmodulin 1,2,3	-	D1F7J	35944S
Anti-PCNA	SCBT	PC10	sc-56
Anti-GAPDH	-	0411	sc-47724
Anti-CK19	University of Iowa	TROMA-III	TROMA-III-c
Anti-Mouse HRP	DAKO	Not Provided	K4001
Anti-Rabbit HRP	-	Not Provided	K4003
Calmodulin 1,2,3	Thermo	2D1	MA3-917
Pan-RAS	-	Ras10	MA1-012
KRAS	Genetex	AT2F8	GTX31957

Table S1. Antibodies arranged by vendor

10xGenomics	# Genes	# Cells with > 200 genes	# Cells with > 800 genes
PANC-1-GR	23766	12849	8687
Amlodipine	24362	11451	9177
BAPTA-AM	22754	8233	6051
W-7	23066	9085	7075

DropSeq	# Genes	# Cells with > 200 genes	# Cells with > 600 genes
PANC-1	17983	2734	853
Gemcitabine	18824	4545	751

Table S2. Number of sequenced cells and genes in single cell RNAseq experiments

					Primary Analysis		Subset Analysis		
Dataset	nFeature_RNA_min	nFeature_RNA_max	% Mitochondrial genes	FindVariableFeatures	Dim used	Resolution	Primary clusters removed	Dim used	Resolution
PANC1-GR vs Amlodipine-Treated	>800	<6000	<20%	selection.method = "mvp", nfeatures = 2000	1:13	0.9	9,10	1:15	1
PANC1-GR vs BAPTA-AM-Treated	>800	<6000	<20%	selection.method = "mvp", nfeatures = 2000	1:13	0.9	8,13	1:20	1.3
PANC1-GR vs W-7-Treated	>800	<6000	<20%	selection.method = "mvp", nfeatures = 2000	1:13	0.9	9,12	1:15	1
Amlodipine vs BAPTA-AM vs W-7-Treated	>800	<6000	<20%	selection.method = "mvp", nfeatures = 2000	1:13	0.9	7,11	1:20	1.3
ANC1-GR vs Amlodipine vs BAPTA-AM vs W-7-Treated	>800	<6000	<20%	selection.method = "mvp", nfeatures = 2000	1:13	0.9	9,19,11	1:20	1.3
PANC-1 vs Gemcitabine-Treated	>600	<10000	<12.5%	selection.method = "mvp", nfeatures = 2000	1:10	0.67	9	1:10	0.6

Table S3. Seurat parameters used in single cell RNAseq experiments

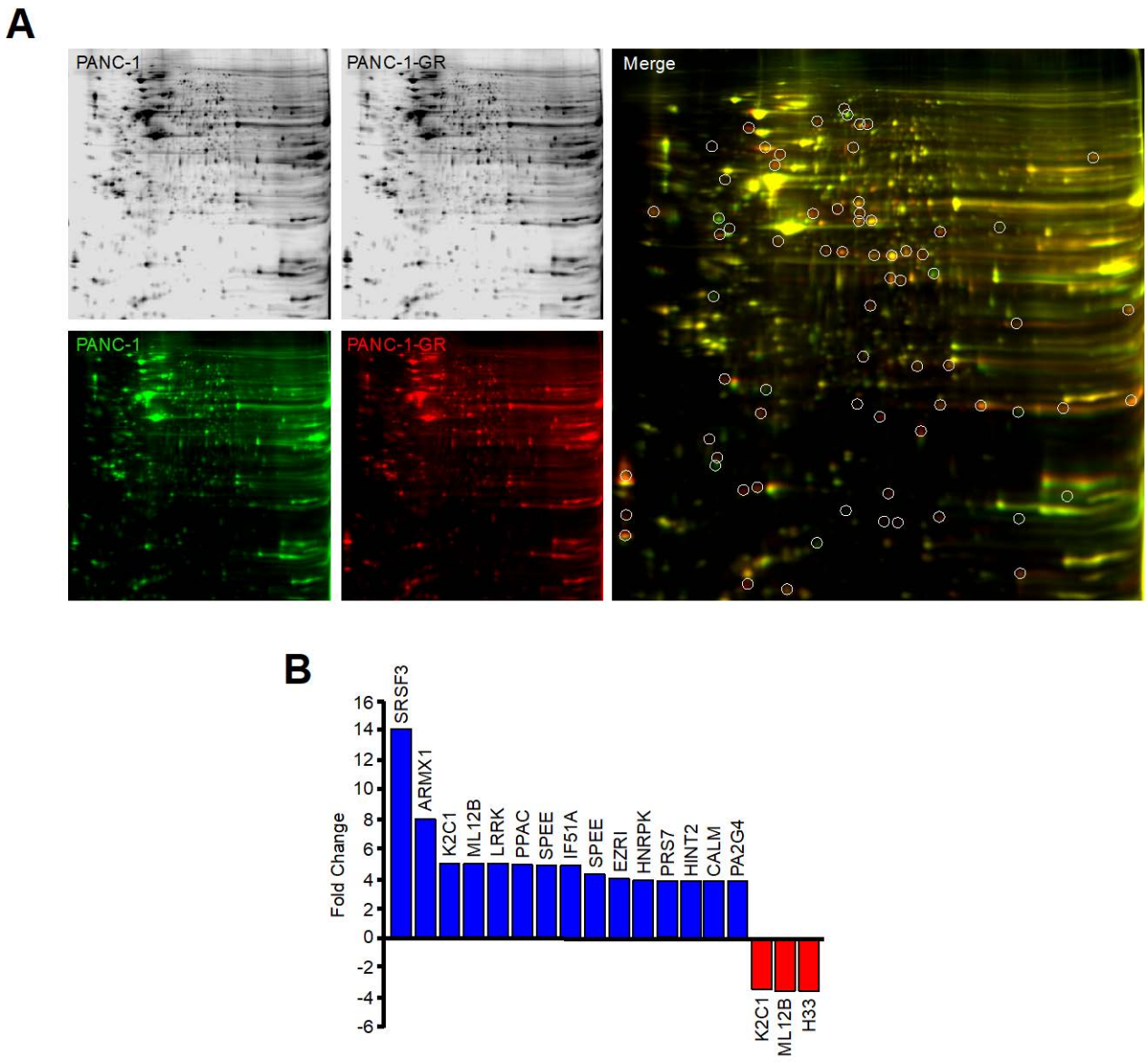

Figure S1. ***Whole proteome analysis comparing PANC-1 and PANC-1-GR cells***

(A) PANC-1 and PANC-1-GR cells were subjected to whole proteome analysis by 2D gel electrophoresis, and representative images shown for each. **(B)** The top 15 differentially expressed proteins were identified by mass spectrometry and shown by fold change, with blue representing proteins overrepresented in PANC-1-GR cells and red representing proteins overrepresented in PANC-1 cells.

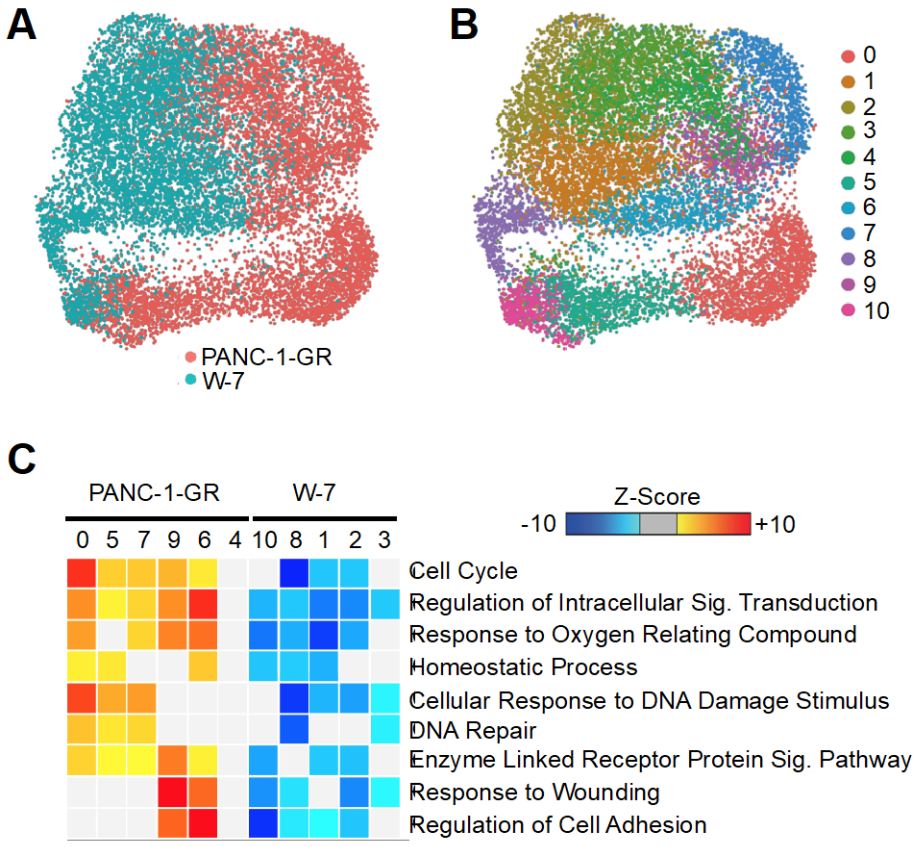

Figure S2. ***W-7-treated PANC-1-GR cells display similar cell clustering following regression of cell cycle genes***

(A,B) PANC-1-GR cells were incubated with a DMSO vehicle or 10μM W-7 for 24 hours, at which time cells were collected and evaluated by single cell RNA sequencing. To determine if the clustering shown in Figure 2 was dominated by cell positioning in the cell cycle, analyses were also performed after regression of cell cycle genes. **(C)** These populations were visualized via UMAP scatterplot, transcriptionally distinct clusters were identified, and each subjected to enrichment analysis for cell processes identified in Figure 1.

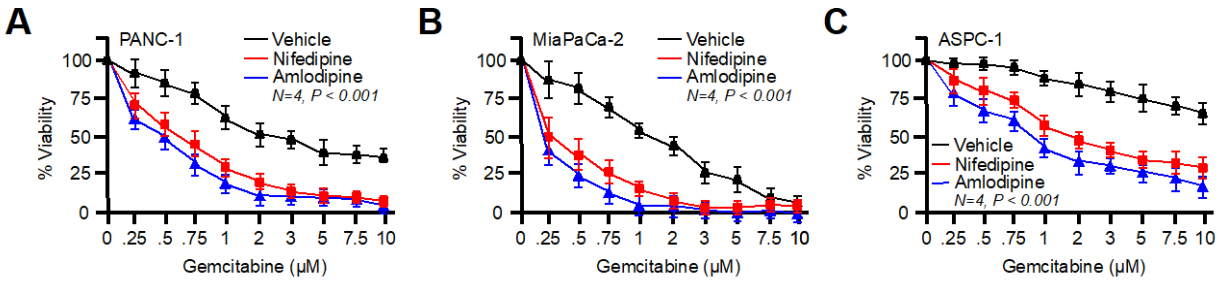

Figure S3. ***Calcium channel blockers enhance the effects of Gemcitabine in additional PDAC cell lines***

(A-C) PANC-1, MiaPaCa-2, and ASPC-1 cells were incubated with a fixed concentration of either a DMSO vehicle (1:1000), or calcium channel blockers Nifedipine (5μM) or Amlodipine (5μM). After two hours, cells were challenged with increasing concentrations of Gemcitabine, and cell viability evaluated after 48 hours by MTT assay.

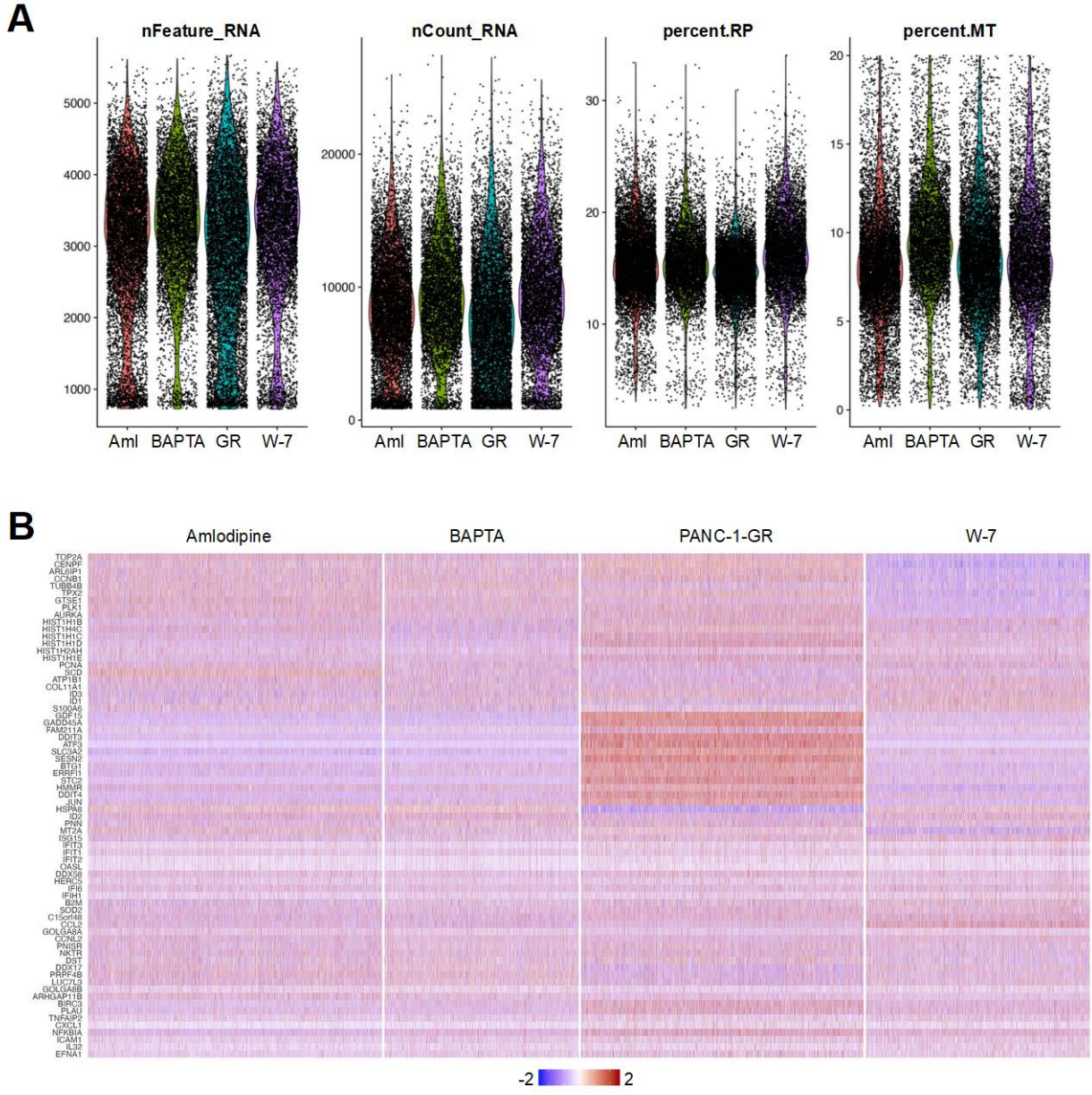

Figure S4. ***Marker expression and cell clustering in scRNA-seq samples across different treatments***

(A) Distribution of the number of sequenced genes (nFeature), RNA molecules as well as percent of ribosomal and mitochondrial genes per cell, per sample. Shown are the cells with at least 800 detected genes. **(B)** Expression heat map of top cluster markers.

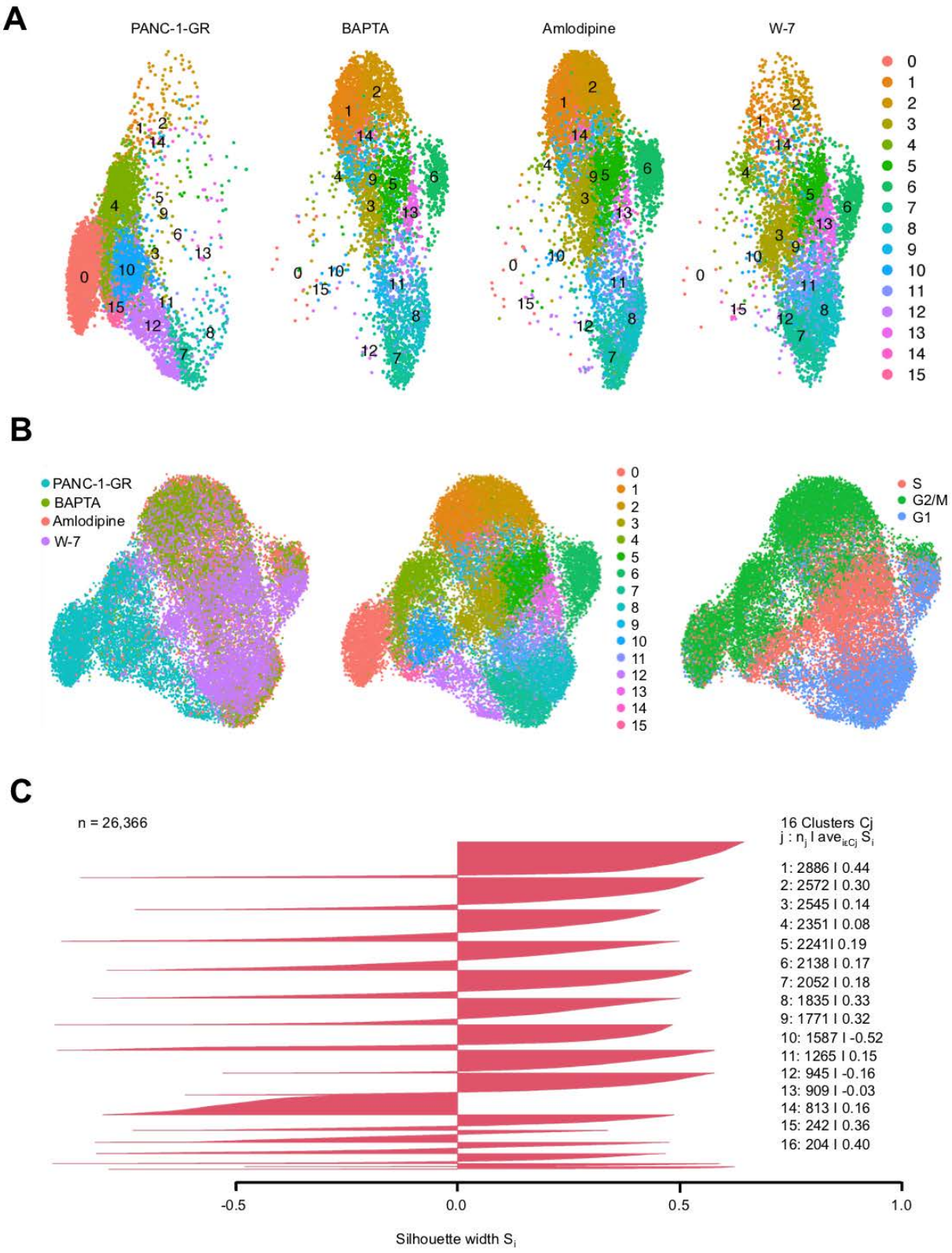
A
PANC-1-GR
BAPTA
Amlodipine
W-7
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
B
PANC-1-GR
BAPTA
Amlodipine
W-7
S
G2/M
G1
C
n = 26,366
16 Clusters Cj
j : n_j l ave_{i∈Cj} S_i
1: 2886 l 0.44
2: 2572 l 0.30
3: 2545 l 0.14
4: 2351 l 0.08
5: 2241l 0.19
6: 2138 l 0.17
7: 2052 l 0.18
8: 1835 l 0.33
9: 1771 l 0.32
10: 1587 l -0.52
11: 1265 l 0.15
12: 945 l -0.16
13: 909 l -0.03
14: 813 l 0.16
15: 242 l 0.36
16: 204 l 0.40
-0.5
0.0
0.5
1.0
Silhouette width S_i

Figure S5. ***Differences between treated and untreated cells at the sub-population level***

(A) PANC-1-GR cells were treated as described, and UMAP representation of each individual sample shown. **(B)** UMAP representation of four scRNA-seq samples altogether, with cells colored by treatment (left panel), clusters (middle panel) or cell cycle distribution (right panel).
(C) Silhouette widths calculated for each cluster in (A) and the resulting average silhouette width. The applied resolution was 0.9.

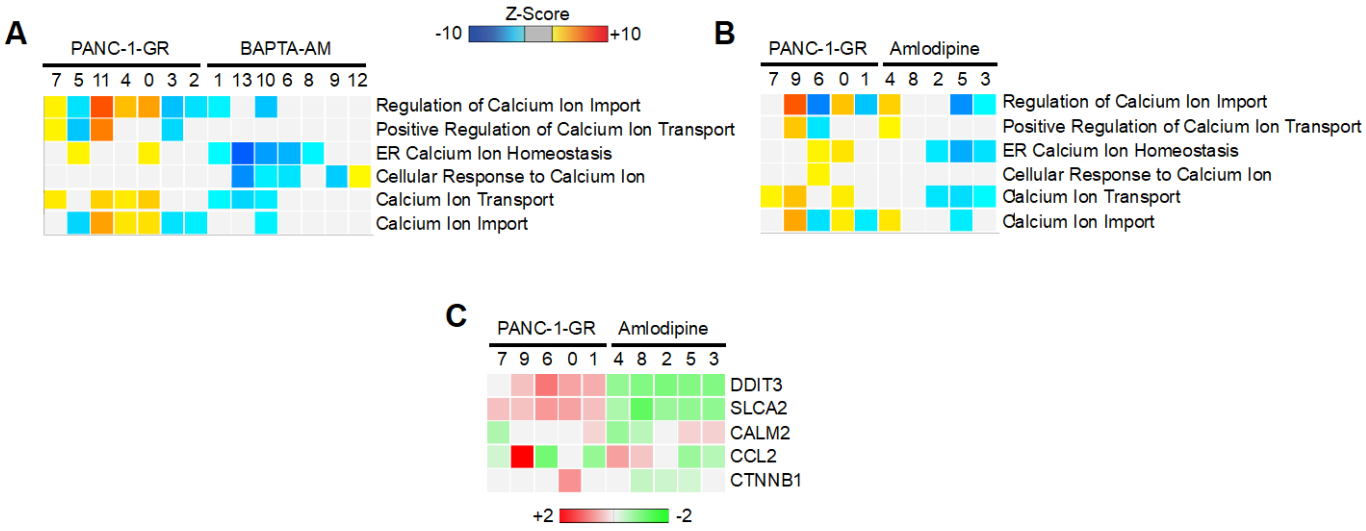

Figure S6. ***Amlodipine reduces calcium ion transport and ERK signaling pathways***

(A) PANC-1-GR cells were treated with either a DMSO vehicle or BAPTA-AM (10μM) for 24 hours. Cells were then collected and evaluated by single cell RNA sequencing as described. Cell populations were visualized via UMAP scatterplot, transcriptionally distinct clusters identified, and each subjected to gene set enrichment analysis (GSEA) showing significant BAPTA-AM-associated downregulation of several gene sets related to Calcium ion transport and homeostasis. **(B)** PANC-1-GR cells were treated with either a DMSO vehicle or Amlodipine (5μM) and RNA sequencing/GSEA conducted similarly, also showing Amlodipine-associated downregulation of several gene sets related to Calcium ion transport and homeostasis. **(C)** Individual genes in the Calcium ion transport gene set.

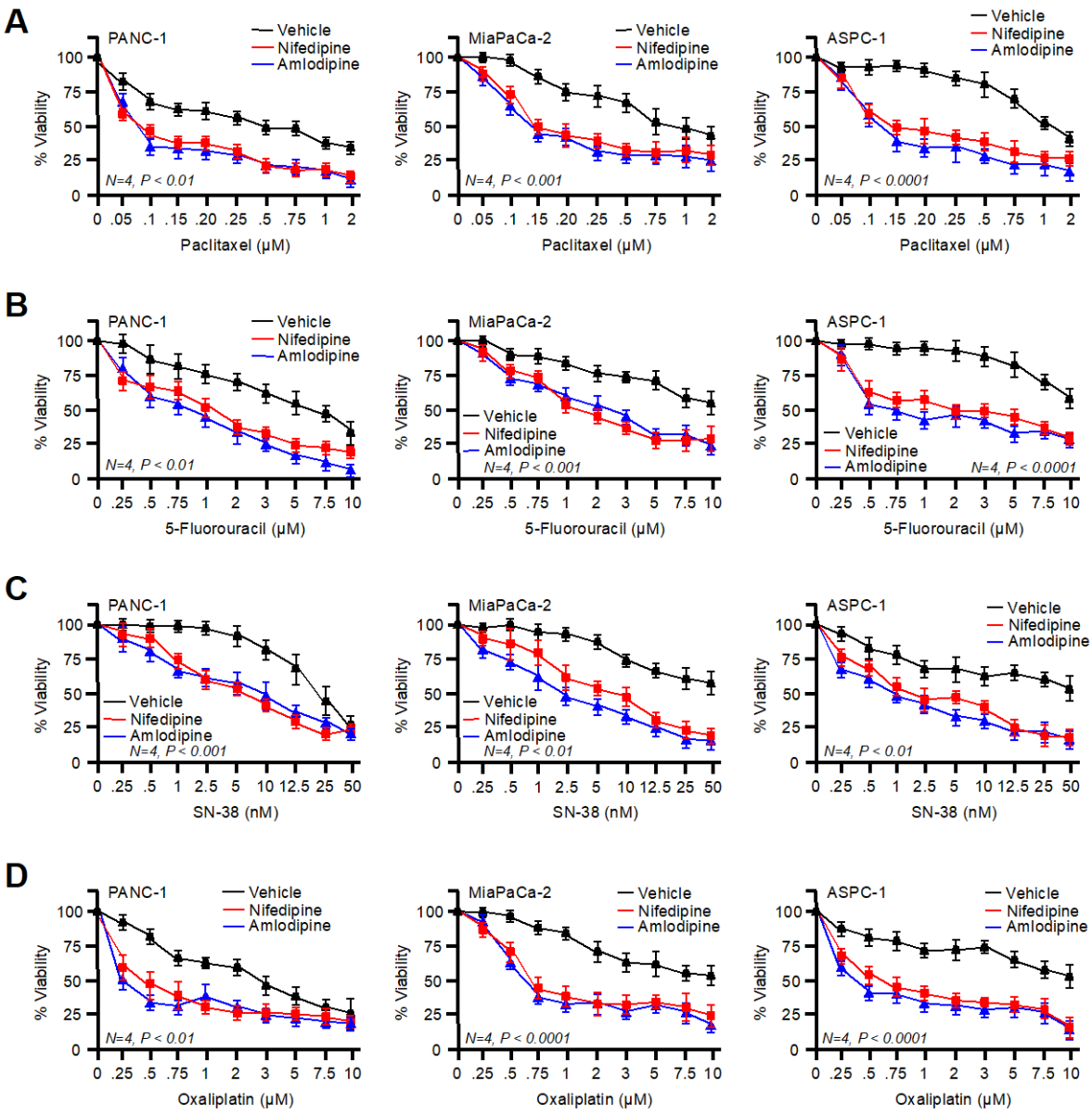

Figure S7. ***Calcium channel blockers potentiate the effects of additional chemotherapy medications in PDAC cells***

PANC-1-GR, MiaPaCa-2, and ASPC-1 cells were incubated with a fixed concentration of either a DMSO vehicle (1:1000), or calcium channel blockers Nifedipine (5μM) or Amlodipine (5μM). After two hours, cells were challenged with increasing concentrations of either **(A)** Paclitaxel, **(B)** 5-Fluorouracil, **(C)** the Irinotecan metabolite SN-38, or **(D)** Oxaliplatin. Cell viability was evaluated after 48 hours by MTT assay.

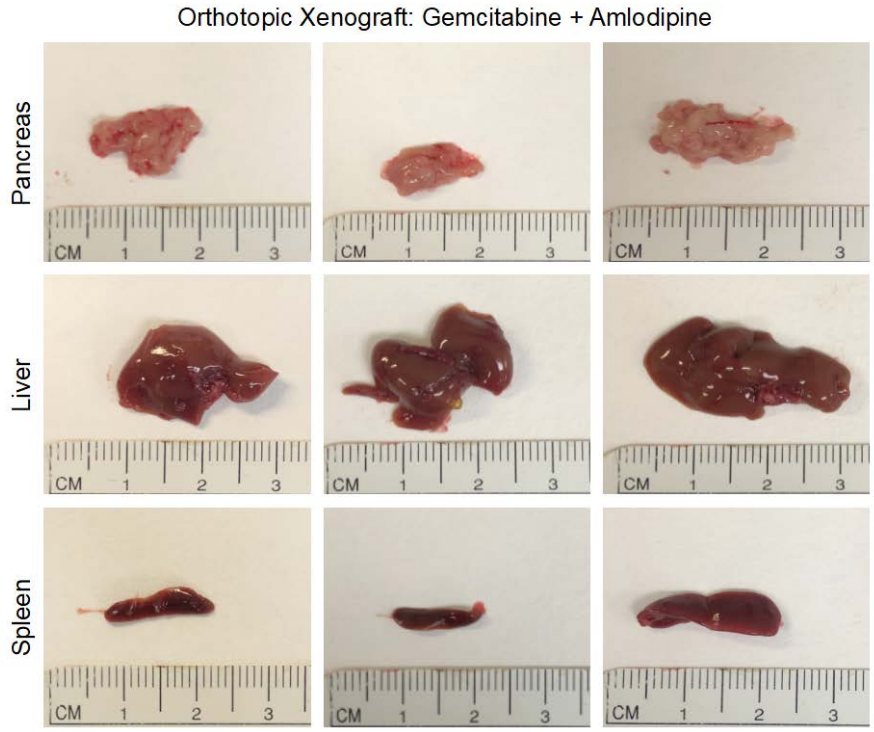

Figure S8. ***Additional gross pathology for PANC-1-GR orthotopic xenografts treated with Gemcitabine and Amlodipine***

PANC-1-GR cells were transduced with stable expression of firefly luciferase (PANC-1-GR^{Luc}), and 0.8x10^{6} cells implanted into the pancreas of NSG mice. Disease progression was monitored by IVIS, and once animals developed 100mm^{3} tumors, they were enrolled into one of four treatment groups. Mice were given intraperitoneal (IP) injections of a PBS vehicle (N = 5), 100mg/kg Gemcitabine twice per week (N = 7), 2mg/kg Amlodipine daily (N = 5), or twice weekly Gemcitabine with daily injections of Amlodipine (N = 9). Mice were sacrificed when showing clear signs of health decline such weight loss or lethargy, and the pancreata from three additional dual-treated mice shown above.

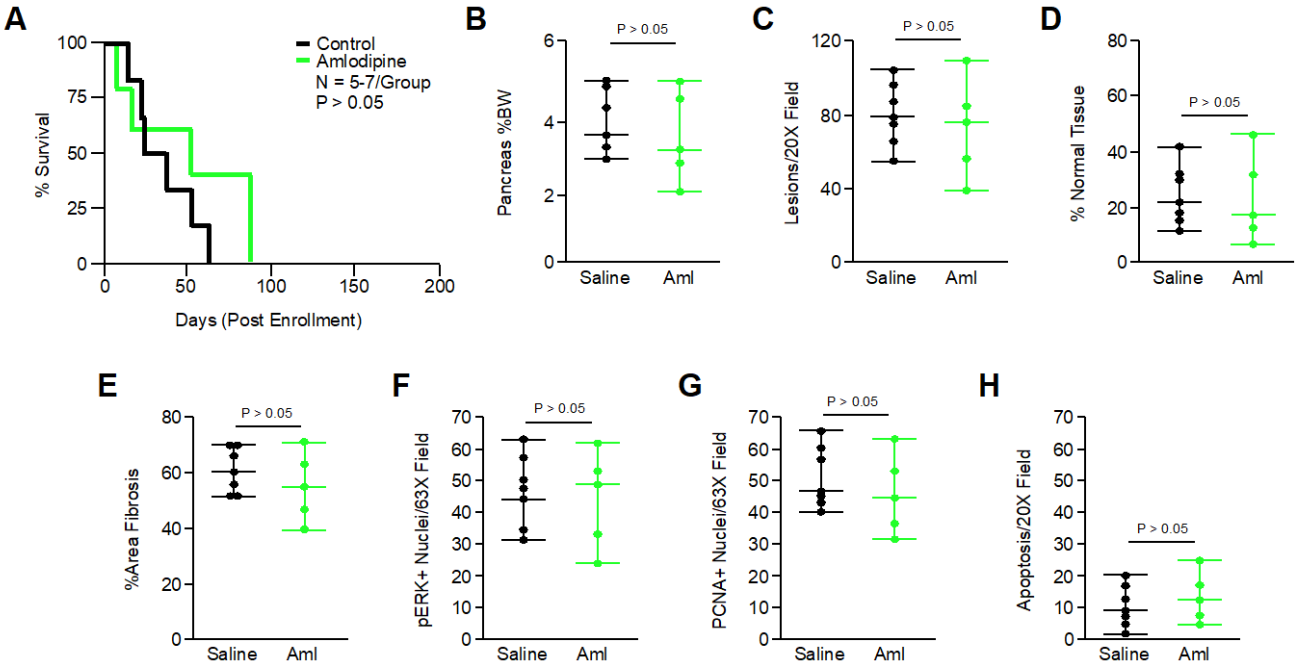

Figure S9. ***Amlodipine monotherapy fails to alter the disease course of KPC mice***

(A) Pdx1-Cre x LSL-*Kras*^{G12D} x LSL-*TP53*$^{R172H+/-}$ (KPC) mice were generated as a model of advanced PDAC. Starting at 15 weeks of age, KPC mice were enrolled into one of four treatment groups. Mice were either treated with intraperitoneal (IP) injections of a saline vehicle or daily injections of 2mg/kg Amlodipine. Mice were sacrificed when showing clear signs of health decline such weight loss or lethargy, and survival for the Amlodipine monotherapy group shown via the Kaplan-Meier method. **(B)** At the study endpoint, the pancreas gland was weighed, normalized to each animal's body weight, and results displayed as individual value plots. **(C-H)** Pancreas tissues were stained with H&E or via immunohistochemistry for CK19, pERK, E-Cadherin and PCNA, or cleaved caspase 3. Tissues were quantified as described and results displayed as individual value plots.

Supplementary References

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