Supplementary Materials

MMP1/PAR1/SP/NK1R paracrine loop modulates early perineural invasion of pancreatic cancer cells

Short title: MMP1 regulates early nerve invasion

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Methods

Cell lines

Human pancreas duct epithelia cell line hTERT-HPNE, human pancreatic cancer cell lines SW1990 and BXPC-3 were purchased from ATCC and maintained with DMEM containing 10% fetal bovine serum. All cells were incubated in 5% CO2 at 37℃.

Reagents

Primary antibodies: Anti-MMP1, anti-CK19 (rabbit monoclonal), anti-twist, anti-Neurokinin 1 receptor (rabbit polyclonal) were obtained from Abcam (Cambridge, USA). Anti-PAR1 and anti-Substance P (E-15) (all goat polyclonal) were purchased from Santa Cruz Biotechnology (Texas, USA). Anti-AKT, anti-pAKT, anti-ERK1/2, anti-pERK1/2, anti-vimentin (all rabbit monoclonal), anti-CD68 (mouse monoclonal) were purchased from Cell Signaling technology (Massachusetts, USA). Recombinant human MMP1 (MMP1) and Substance P (SP) were purchased from PeproTech (NJ, USA) and Sigma-Aldrich (Saint Louis, USA), respectively. ERK1/2 inhibitor U0126 and PI3K/Akt inhibitor LY294002 were purchased from Cell Signaling Technology. PAR 1 receptor antagonist SCH 79797 and NK1-R antagonist L-732,138 were obtained from TOCRIS Bioscience (Bristol, UK). Phorbolmyristate acetate (PMA) was purchased from Sigma-Aldrich. Recombinant human interleukin-4 (IL-4) was purchased from PeproTech. Iron oxide nanoparticles (IONPs) was purchased from Ocean NanoTech company (Springdale, AR, USA). Potassium hexacyanoferrte(II) trihydrate was purchased from Nanhua Company (Hunan, China).

Migration and invasion assays

BD were used to perform migration and invasion assays. For migration assay, PANC-1 and MIA PaCa-2 cells with/without co-cultured with TAMs were harvested and re-suspended in DMEM media. 2×10^4 cells were seeded in the upper chamber. Then complete media or DMEM containing 3% FBS and 100nM SP with/without NK1R antagonist L-732,138 (100 μM) or ERK inhibitor U0126 (10nM) was added to the bottom chamber as attractants. After incubation for 24 hours at 37°C, the migrated cells at the bottom of the chamber were fixed with 4% paraformaldehyde, stained with 0.1% crystal Violet and then counted 6 random fields under × 200 magnification using microscope. The average number of cells of per chamber was represented. Experiments were repeated three times. For invasion assay, the membrane of transwell chamber was pre-coated with Matrigel, and the incubation time was 48 hours.

Cell transfection

Plasmid for human MMP1 （NM_002421.3） expression was constructed by Vigene Bioscience (Shandong, China). PANC-1 and Mia PaCa-2 cells were transiently transfected with the DNAs or vectors by use of Lipofectamine 2000 (Invitrogen) following the manufacturer's protocol. The shRNA-MMP1 and shRNA-control plasmids were constructed and packaged into lentiviral virus vectors (GenePharma, Suzhou, China). For stable transfections, 2.5×10^5 PANC-1 cells per well were seeded in a six-well plate for 24 h. Cells were infected by LV3-shRNA-MMP1 and LV3-shRNA-control according to the manufacturer's protocol. Cells were selected with 500ng/ml puromycin (Invitrogen, Carlsbad, CA, USA) over 4 weeks. The MMP1 mRNA expression levels of the positive cell clones that stably expressed shRNA-MMP1 or shRNA-control were then identified using qRT-PCR. The clones stably expressing shRNA-

MMP1 or shRNA-control were maintained in DMEM medium containing 500ng/ml of puromycin for further experiments.

RT-PCR

Total RNA was extracted from cells using Trizol (Invitrogen), reverse-transcribed to cDNA using PrimeScript TM RT Master Mix Kit (TaKaRa, Dalian, China). And quantitative real-time PCR was performed on the LightCycler480 Real-time PCR System (F. Hoffman-La Roche Ltd, Basel, Switzerland) using SYBRTM PrimeScript RT-PCR kit (TaKaRa).

Western blot

Cells and DRGs were lysed in RIPA contained protease inhibitor cocktail (1:100, ComWin biotech, Beijing, China). Proteins were quantified referring to the instruction of BCA protein assay kit (Beyotime, Jiangsu, China). Proteins were separated by electrophoresis in 10% SDS-PAGE (Beyotime), transferred to polyvinylidene fluoride (PVDF) membranes (0.45μm, Merck Millipore, Darmstadt, Germany), incubated with primary antibody overnight at 4℃, and exposed to secondary antibody. Antibody binding was visualized by enhanced chemiluminescence (Merck Millipore).

Prepare for DRGs

Female BABL/c Mice (5±1weeks) were euthanized with CO2 and sterilized with 75% ethanol. The spinal cords were separated from the spinal column, and DRGs beside the spinal cords were harvested [1].

Immunofluorescent

Cells and DRGs were fixed and incubated with NK-1R or PAR-1 primary antibody at 4℃ overnight, respectively. Then the FITC-conjugated goat anti-rabbit or FITC-conjugated rabbit anti-goat secondary antibodies were added and incubated for 2 hours at room temperature. Nuclei were stained with DAPI. The Green fluorescent of FITC-labeled cells or DRGs was observed under fluorescence microscopy.

Cytotoxicity of IONPs on cell viability

To detect the cytotoxicity of IONPs on cell viability, PANC-1 cells were seeded in 96-well plates at the concentration of 5.0×10^3 per well in triplicate. After serum starvation for 12 hours, cells were growth in DMEM with different concentration of IONPs (0, 12, 24, 36, and 48 mg/L) in 5% CO_2 at 37℃ for 8 hours. Then the cell viability was detected as described as the cell proliferation assay.

MRI T2 values measurement of various concentration of IONP

The 100μl volume of MDEM containing IONPs in different concentration (0, 12, 24, 36, and 48 mg/L) were placed in 96-well plates ordinal. MRI was performed using a 3.0T imaging system MAGNETOM Verio (Siemenz Medical System, Erlangen, Germany) with a 40 mm Loop Coils. T2-mapping sequence (repetition time 1500 msec, echo time beginning at 13.4ms, echo spacing of 13.4ms, eight echoes, slice thickness 2.0 mm) was performed and T2 value was calculated by system automatically.

Prussian blue staining

PANC-1 cells were seeded in 24-wells plates for 24 hours, then incubated with DMEM containing IONPs (0, 12, 24, 36, and 48 mg/L) for 8 hours. Media were removed and washed twice with PBS. The cells were fixed with 4% paraformaldehyde for 30min, and then reacted with the mixture of equal 2%ferrous potassium choide Potassium hexacyanoferrte (II) trihydrate （Nanhua Company , China, Hunan) and 6% muriatic acid in 30min. The number of staining cells were counted under fluorescent inverted microscope.

Operation process of *in vivo* model of murine sciatic nerve invasion

Mice were anesthetized with 4% chloral hydrate (85μl/10g, intraperitoneal injection) and diethyl ether inhalation. The left sciatic nerves were exposed deep to the femorococcygeous and biceps femorismusces, and then 3μl of cells mixture in PBS were slowly microscopically injected under the perineurium using a microliter syringe (Hamilton, 10μl, 33G). The concentration of PANC-1 cells was 1×10^5/μl and TAMs concentration was 1×10^4/μl (PANC-1/TAMs = 10:1). 1×10^5/μl TAMs in a total volume of 3μl was injected under the perineurium to exclude the neoplastic proliferation of TAMs.

H&E and immunohistochemistry staining on tissue sections

The tissues were embedded in paraffin and sectioned into 3-4μm thick slices along the nerve for H&E and immunohistochemical staining. After sequential deparaffinization, antigen retrieval, background close, the slides were incubated with primary antibodies overnight at

4 ℃. Then, secondary antibodies were dropped and incubated at 37℃ for 15 minutes. At last, the sections were stained with the diaminobenzidine solution for 5 minutes and hematoxylin for 1 minute. We referred to the CAP (College of American Pathologists) Immunohistochemisty Survey MK system carrying out the semi quantitative analysis. The percentage of positive cells (PP) was divided as : 1 point (6-25% cells staining), 2 point (26-50% cells staining), 3 point (51-75% cells staining), and 4 point (over 75% cells staining). And the staining intensity (SI) was determined as: 0 (no staining); 1 point (weak staining); 2 point (moderate staining), and 3 point (strong staining). Added the PP and SI, we got the immunoreactive score (ISR). The IRS as defined into 4 groups: negative (0 point),1+ (2-3 point), 2+ (4-5 point), and 3+ (6-7 point).

References:

1. Ayala GE, Wheeler TM, Shine HD, et al. In vitro dorsal root ganglia and human prostate cell line interaction: redefining perineural invasion in prostate cancer. Prostate. 2001; 49: 213-23.

Figure Legends

Figure S1. (A) Morphology and immunotype of U937 monocytes changed to TAMs after induction. Before treatment, the U937 cells are circle morphology and suspended growth under microscope. After incubation with IL-4 and PMA, cells emerged pseudopodia and began to half-adherence. Immunophenotype analyzed with qPCR showed that CD68 and CD163 expression up-regulated. (B) After co-culture with TAMs, the migration and invasion abilities of PANC-1 and Mia PaCa-2 cells were enhanced. (C) After co-culture with TAMs, the perineural invasion abilities of PANC-1 and Mia PaCa-2 cells were enhanced. (D) The MMP1 mRNA upregulated significantly after MMP1-expression vector transfection and downregulated significantly after shMMP1 vector transfection both in PANC-1 and Mia PaCa-2 cells. The upregulation of MMP1 protein was detected by ELISA. Data showed as mean±SD. ** $P<0.01$, and *** $P<0.001$ compared with control.

Figure S2. Assessment of PAR1 expression in neuron and NK1R expression in pancreatic cancer cell lines. (A) The expression of PAR1 in neurons in PDAC tissues was observed using H&E staining and IHC. The expression of PAR1 in DRGs separated from mice were observed using immunofluorescence assay. (B) PAR1 and NK1R protein expression in neuron and pancreatic cell lines were evaluated by western blot assay. Both pancreatic cancer cells and DRG expressed PAR1 and NK1R. DRGs expressed high level of PAR1 protein, and pancreatic cancer cells PANC-1, Mia Paca2, SW1990, and Bxpc-3 expressed high levels of NK1R. Whereas immortalized human pancreas duct epithelia cell line hTERT-HPNE (H-H) didn't express PAR1 or NK1R.(C) In PANC-1 and Mia Paca-2 cells, immunofluorescence assay

exhibited that NK1R protein was expressed in the cytoplasm and on the cell membrane.

Figure S3. (A) SP induced phosphorylation of ERK both in PANC-1 and Mia PaCa-2 cells. SP upregulated Vimentin and TWIST1 expression both in PANC-1 (B) and Mia PaCa-2 cells (C), whereas the NK1R antagonist SP+L-732,138 and U0126 (ERK pathway inhibitor) attenuated the phosphorylation of ERK, indicating that SP enhanced pancreatic cancer cells EMT via SP/NK1R/ERK pathway. Data showed as mean±SD. * P<0.05, and ** P<0.01 compared with 0 minute in (A) and with control in (B and C). ΔP<0.05 compared to the SP+L-732,138 group.

Figure S4. (A) The histologic immunochemical staining of tumor sections from mice injected with PANC-1+TMAs exhibited $CK19^{+}$ and $CD68^{-}$. (B) Mice injected with TAMs alone didn't exhibit paralysis. In surgical images, there was not any neoplastic growth, or any changes in nerve caliber was observed. (C) H&E staining of nerve sections did not show any neoplasm invasion. All the above results excluded TAMs proliferation. (D) PANC-1 cells were pre-co-cultured with TAMs for 24 hours in vitro, then collected and injected into perineurium of left sciatic nerves. Even TAMs were not maintained in the inoculation microenvironment, transient co-culture with TAMs robustly enhanced the nerve invasion and migration ability of PANC-1 cells.

Figure S5. Labeling PANC-1 cells with IONPs. (A) Endocytosis of IONPs in PANC-1 cells. Eight hours after incubation with IONPs, Prussian-blue staining was performed. Blue

particles scattered in the cells plasma represented the up-taken IONPs. (B) MR signals changed with various IONPs concentrations. As a negative MRI contrast agent, the T2-signal value decreased with the increased concentration of IONPs. (C) MTS assay exhibited that IONPs did not affect cell viability apparently when used at the concentration ranging from 0 ~ 48 mg/L.

Figure S1

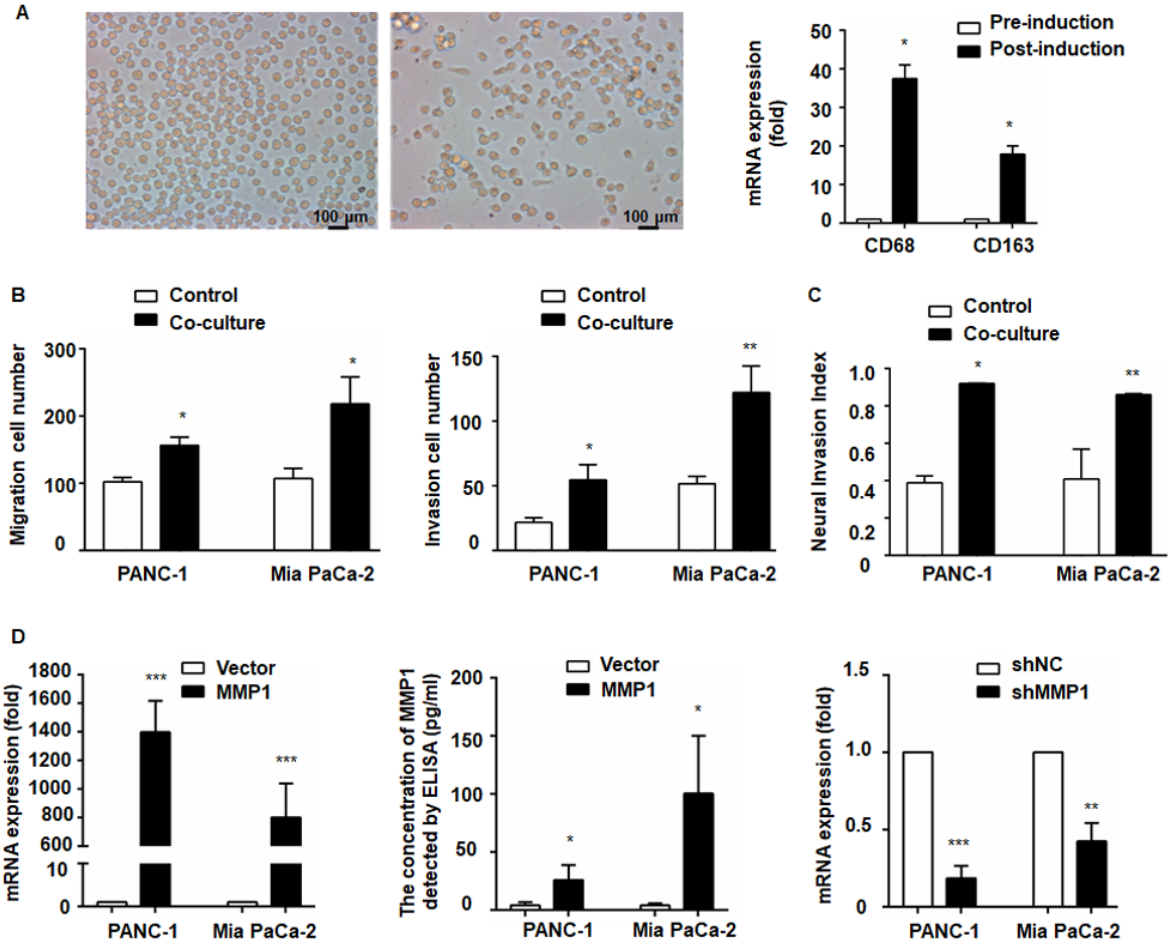
A
100 μm
100 μm
Pre-induction
Post-induction
mRNA expression (fold)
CD68
CD163
B
Control
Co-culture
Migration cell number
PANC-1
Mia PaCa-2
Invasion cell number
C
Neural Invasion Index
D
Vector
MMP1
mRNA expression (fold)
The concentration of MMP1 detected by ELISA (pg/ml)
shNC
shMMP1

Figure S2

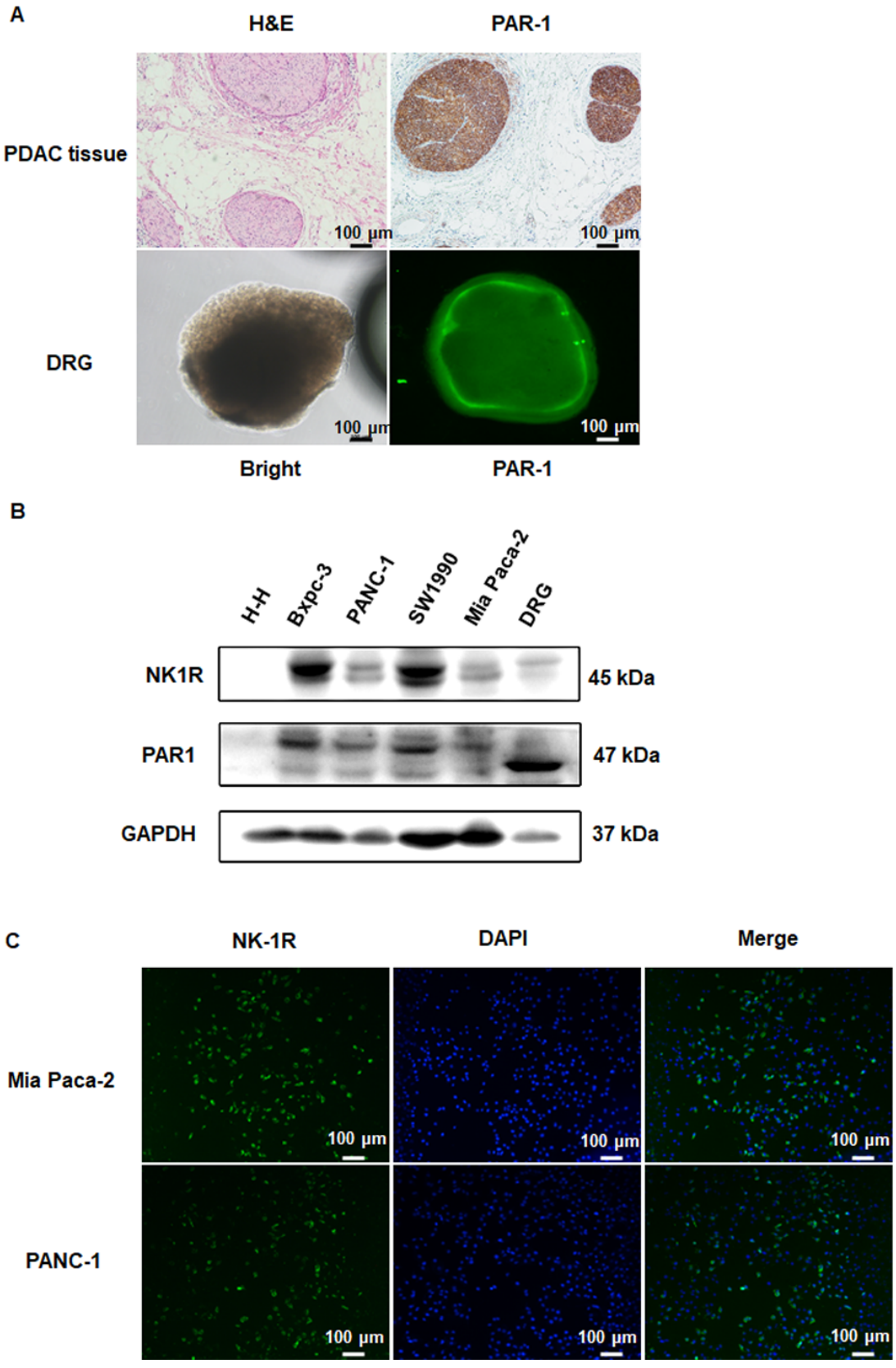
A
H&E
PAR-1
PDAC tissue
DRG
100 μm
Bright
PAR-1
B
H-H
Bxpc-3
PANC-1
SW1990
Mia Paca-2
DRG
NK1R
45 kDa
PAR1
47 kDa
GAPDH
37 kDa
C
NK-1R
DAPI
Merge
Mia Paca-2
PANC-1

Figure S3

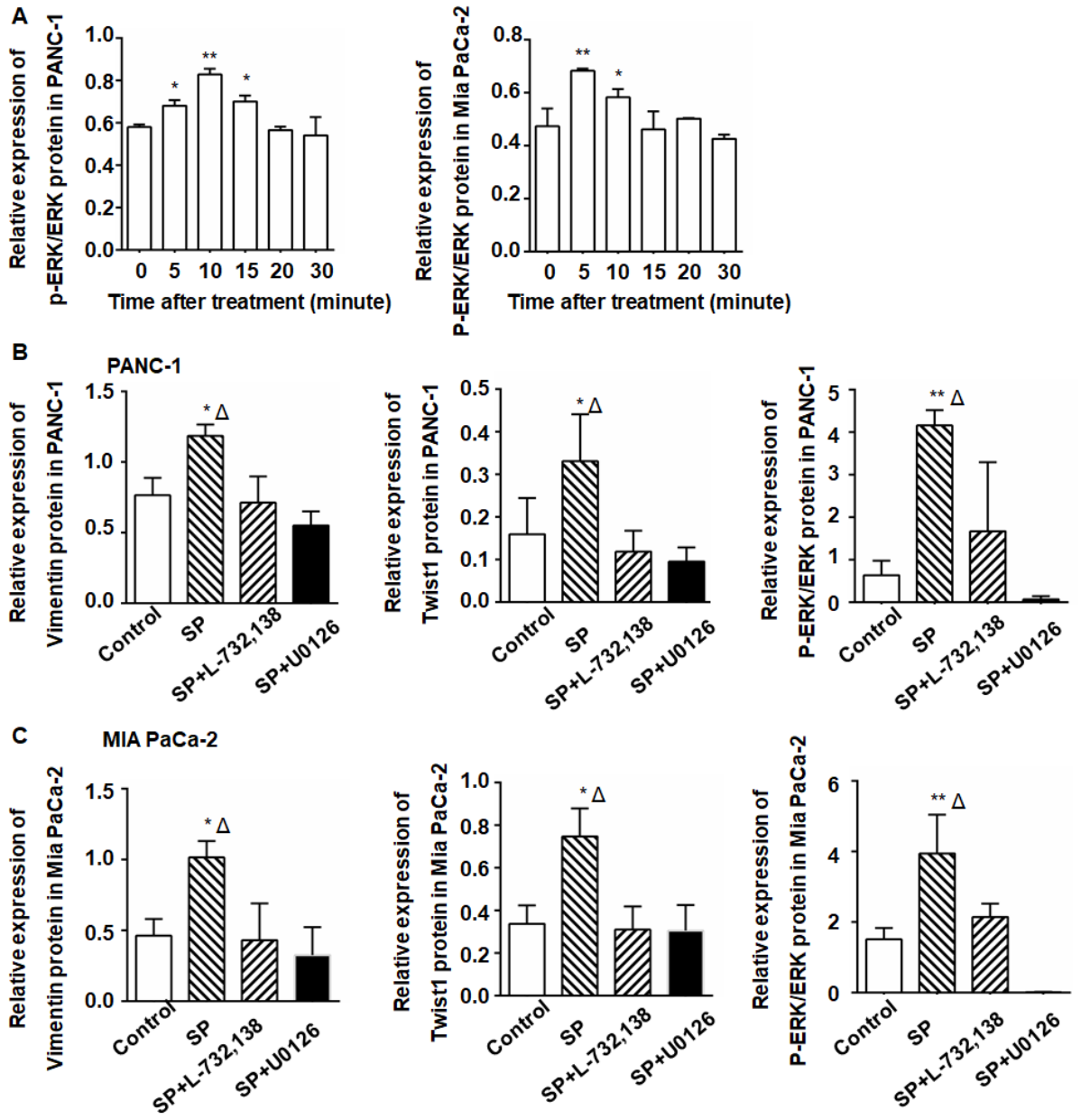
A
Relative expression of p-ERK/ERK protein in PANC-1
Time after treatment (minute)
Relative expression of P-ERK/ERK protein in Mia PaCa-2
Time after treatment (minute)
B
PANC-1
Relative expression of Vimentin protein in PANC-1
Relative expression of Twist1 protein in PANC-1
Relative expression of P-ERK/ERK protein in PANC-1
Control
SP
SP+L-732,138
SP+U0126
C
MIA PaCa-2
Relative expression of Vimentin protein in Mia PaCa-2
Relative expression of Twist1 protein in Mia PaCa-2
Relative expression of P-ERK/ERK protein in Mia PaCa-2

Figure S4

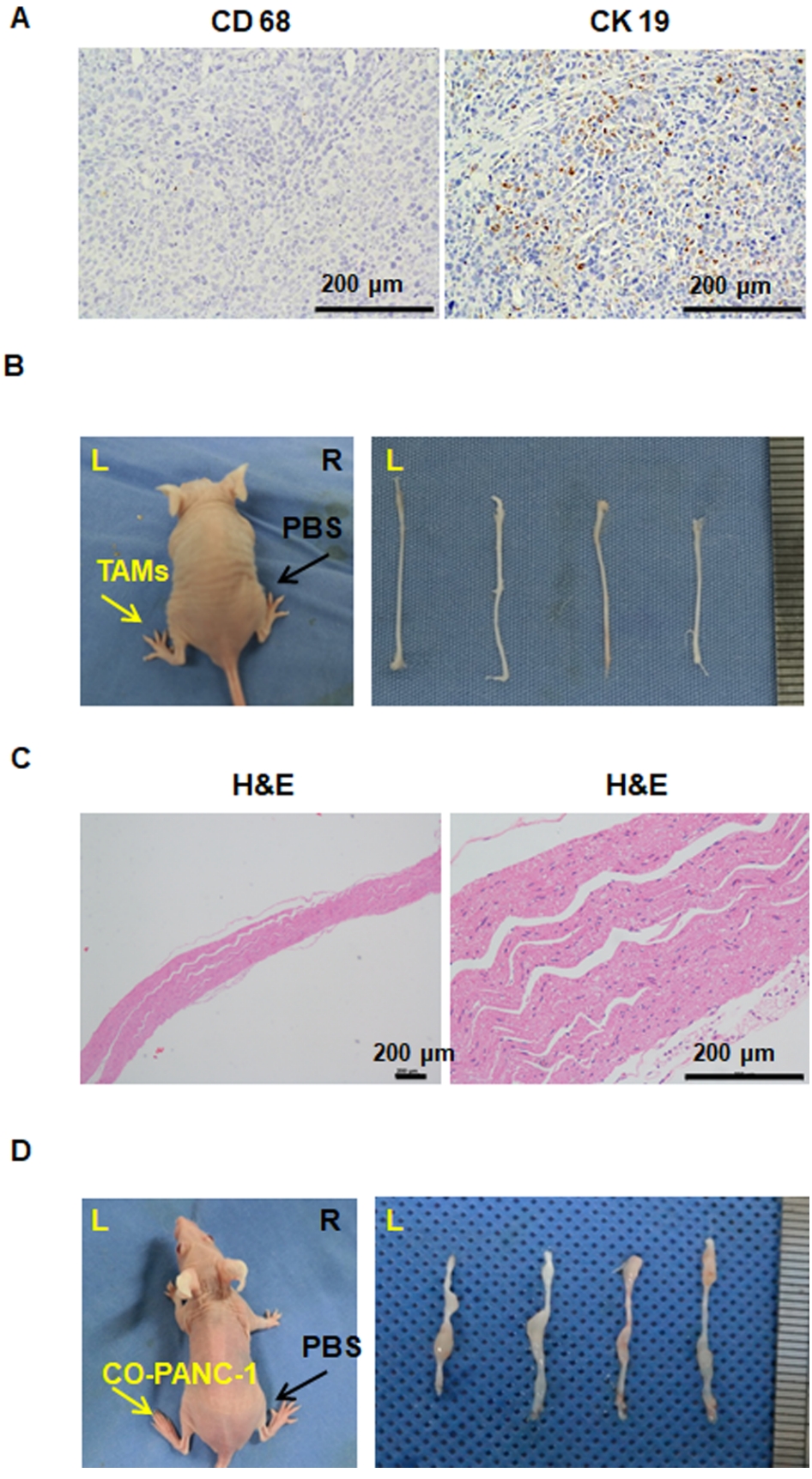
A
CD 68
CK 19
200 μm
200 μm
B
L
R
PBS
TAMs
L
C
H&E
H&E
200 μm
200 μm
D
L
R
PBS
CO-PANC-1
L

Figure S5

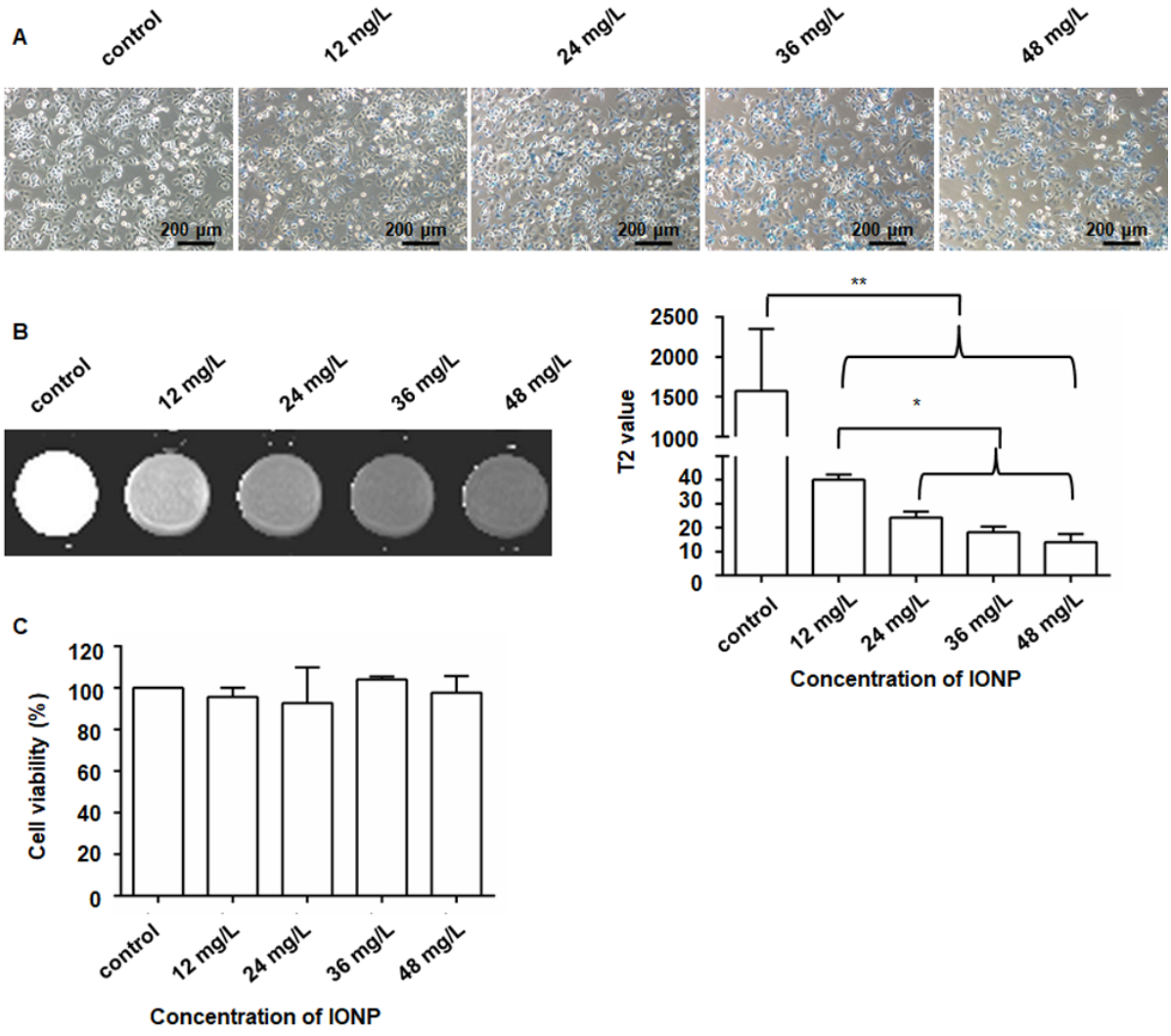
A
control
12 mg/L
24 mg/L
36 mg/L
48 mg/L
200 μm
B
control
12 mg/L
24 mg/L
36 mg/L
48 mg/L
T2 value
2500
2000
1500
1000
40
30
20
10
0
**
*
control
12 mg/L
24 mg/L
36 mg/L
48 mg/L
Concentration of IONP
C
Cell viability (%)
120
100
80
60
40
20
0
control
12 mg/L
24 mg/L
36 mg/L
48 mg/L
Concentration of IONP

Table S1. The sequences of primers and shRNA

Gene	Sequence
CD68 primers	forward 5’- GACCCACGACTGCCACTC-3’ reverse 5’-GTGCTGTTGCTTGTTGGATG-3’
CD163 primers	forward 5’-CGAGTT AACGCCAGTAAGG-3’ reverse 5’-GAACATGTCACGCCAGC-3’
MMP1 primers	forward 5’-ACACGCCAGATTTGCCAAGAGC-3’ reverse 5’-GGAGAGTTGT CCCGATGATCTCCCC-3’
MMP2 primers	forward 5’-GATGATGCCTTTGCTCGTGC-3’ reverse 5’-CAAAGGGGTATCCACGCCA-3’
MMP9 primers	forward 5’-CAAA GGGGTATCCACGCCA-3’ reverse 5’-TCGTAGTTGGCGGTCGTG-3’
GAPDH primers	forward 5’-AAGGTGAAGGTCGGAGTC AAC-3’ reverse 5’-CATGAGTCCTTCCACGATACC-3’
MMP1 shRNA	5’-CTGACCTACAGGATTGAAA-3’
LV3-NC	5’-TTCTCCGAACGTGTCACGTTTC-3’

Table S2. All gene and the relative expression assessed in the array

GenbankAccession	GeneSymbol	foldchange	type
NM_001039500	VWA5B1	340.5143	mRNA
NM_152423	MUM1L1	285.5048	mRNA
S75894		242.7293	mRNA
NM_002043	GABRR2	236.6134	mRNA
NM_002421	MMP1	155.7959	mRNA
NM_152337	C16orf46	151.7916	mRNA
BX097862	XLOC_l2_013506	149.8562	mRNA
AK098312	IL20RA	136.3074	mRNA
NM_001199219	INMT	89.87091	mRNA
NM_175878	XKR3	78.90831	mRNA
AK098398	HMP19	73.29284	mRNA
DB550107	XLOC_l2_008396	69.28253	mRNA
NM_016383	LUZP4	64.80661	mRNA
D13078		64.78482	mRNA
NM_001195272	LOC100129520	63.58293	mRNA
NM_181610	KRTAP19-4	60.12266	mRNA
AK092754	SPATA13	59.95894	mRNA
NM_000735	CGA	53.80423	mRNA
NR_028045	KLRAP1	52.48699	mRNA
NM_005118	TNFSF15	52.25233	mRNA
NM_000840	GRM3	52.1503	mRNA
XM_001714987	ARMCX3-AS1	46.9051	mRNA
AK131244	AKD1	44.94223	mRNA
		42.60285	mRNA
AK093200	XLOC_001259	42.13821	mRNA
NM_002585	PBX1	41.31057	mRNA
NM_001144058	NTM	40.67006	mRNA
NM_001004440	FAM19A3	39.99677	mRNA
NM_007038	ADAMTS5	39.68226	mRNA
NM_144992	VWA3B	39.50031	mRNA
NM_001004472	OR10R2	38.72748	mRNA
JN120858		36.37209	mRNA
NR_033945	LOC647107	36.07351	mRNA
NM_198687	KRTAP10-4	34.67572	mRNA
NM_001085420	PLSCR5	34.1537	mRNA
BX100197		33.92456	mRNA
BC031871	CSMD2	33.24908	mRNA
EU154352		31.40358	mRNA
NM_003154	STATH	29.86743	mRNA

NM_001005286	OR6F1	29.55381	mRNA
NM_001193502	TCF24	29.4981	mRNA
		29.12926	mRNA
BC008001	XLOC_003427	28.98743	mRNA
NM_003264	TLR2	27.91328	mRNA
AK130276		26.58965	mRNA
NM_001114734	PABPC4L	26.12827	mRNA
		25.71948	mRNA
NM_001136002	TMEM229A	24.66118	mRNA
NM_176817	TAS2R38	24.60632	mRNA
XM_291007	HEATR7B1	24.26947	mRNA
NM_173857	VN1R4	23.84094	mRNA
NM_198795	TDRD1	23.14781	mRNA
		22.59221	mRNA
		20.1139	mRNA
NM_000040	APOC3	19.6467	mRNA
AK127184	LOC100131129	19.58904	mRNA
NM_152402	TRAM1L1	19.53713	mRNA
NM_145259	ACVR1C	19.53134	mRNA
NR_038239	LOC100507629	19.35226	mRNA
NR_033460	PAGE3	19.15738	mRNA
NM_001035256	POMC	19.05149	mRNA
NM_001204118	CLEC17A	18.96375	mRNA
NM_020663	RHOJ	18.65978	mRNA
NM_001013355	OR2G6	18.53143	mRNA
NM_002196	INSM1	18.29421	mRNA
NM_014058	TMPRSS11E	18.23555	mRNA
BC019904	XLOC_009358	18.03082	mRNA
NM_001906	CTRB1	17.49797	mRNA
NM_016540	GPR83	16.89886	mRNA
NR_003581	ATP8B5P	16.6394	mRNA
NM_000379	XDH	16.44097	mRNA
AK125988	XLOC_004040	16.24305	mRNA
NM_016734	PAX5	16.01752	mRNA
		15.5648	mRNA
XR_109007		15.49039	mRNA
AK124269	BAIAP2L1	15.31691	mRNA
	ZFP64	14.86324	mRNA
NM_014395	DAPP1	14.33479	mRNA
NR_001564	XIST	14.01264	mRNA
NM_002121	HLA-DPB1	13.97449	mRNA
NM_001004463	OR10G7	13.70065	mRNA
NM_001080523	ARRDC5	13.57659	mRNA

		13.56628	mRNA
NM_006668	CYP46A1	13.41727	mRNA
NM_033181	CNR1	13.35937	mRNA
NR_033923	C10orf136	13.23658	mRNA
NR_027455	LOC100131434	13.12596	mRNA
NM_175737	KLB	13.1152	mRNA
NM_001113523	PARP15	13.08623	mRNA
NM_000250	MPO	13.06912	mRNA
AK127982		12.96185	mRNA
NR_038919	LOC100128993	12.83782	mRNA
NM_001192	TNFRSF17	12.42775	mRNA
BC036297	FLJ45983	12.33999	mRNA
		12.0339	mRNA
NM_001136219	FCGR2A	11.75227	mRNA
NM_000584	IL8	11.71158	mRNA
NM_006871	RIPK3	11.6045	mRNA
NM_000128	F11	11.44076	mRNA
NM_004666	VNN1	11.32854	mRNA
		11.15781	mRNA
NM_032126	C1orf49	11.02634	mRNA
BF229395	XLOC_l2_007096	11.02167	mRNA
AY181245	EMR4P	10.97635	mRNA
NR_036519	C17orf62	10.84139	mRNA
NM_031440	RTP3	10.8277	mRNA
		10.75933	mRNA
NR_036676	IFNA22P	10.71745	mRNA
AY766452		10.69504	mRNA
NM_178434	LCE3C	10.67245	mRNA
BC035381	XLOC_l2_003414	10.66568	mRNA
NR_038839	LOC645355	10.26352	mRNA
CR749827	MEIS1	10.20236	mRNA
BX648982	DNAJC16	10.18331	mRNA
AK125887	XLOC_007813	9.850361	mRNA
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NR_015392	FLJ40852	9.62231	mRNA
NM_173550	C9orf93	9.617543	mRNA
NR_038434	LOC100506474	9.560641	mRNA
NM_024726	IQCA1	9.390121	mRNA
NR_036445	OR5AK4P	9.37276	mRNA
		9.273648	mRNA
NM_206965	FTCD	9.26044	mRNA
NR_034007	LOC339894	9.250949	mRNA

BC143323	SLC17A2	9.21982	mRNA
BC042433	XLOC_l2_010586	9.119917	mRNA
NR_036444	LOC400550	9.082806	mRNA
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NM_003155	STC1	8.734215	mRNA
NM_001086	AADAC	8.671047	mRNA
NM_032972	PCDH11Y	8.590037	mRNA
NR_040105	FLJ46066	8.367218	mRNA
NM_001141919	XG	8.356159	mRNA
NM_021226	ARHGAP22	8.351188	mRNA
		8.286636	mRNA
BU570946	XLOC_l2_002271	8.281887	mRNA
NR_026860	LINC00473	8.177215	mRNA
NR_034115	LOC729178	8.154344	mRNA
NM_014334	C9orf4	8.1444	mRNA
AA454051	XLOC_013796	8.122709	mRNA
NM_001195685	LYPD6	8.10247	mRNA
AW630386	XLOC_004430	8.013116	mRNA
AF547222	LOC280665	7.975397	mRNA
NM_024785	FAM124B	7.962754	mRNA
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NM_001142483	C5orf13	0.19287	mRNA
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AL713682	SYNE1	0.1921	mRNA
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NM_006174	NPY5R	0.190676	mRNA
BI833485		0.190433	mRNA

DB045402	XLOC_002571	0.19023	mRNA
NM_173651	FSIP2	0.19021	mRNA
NR_036678	LOC283299	0.190102	mRNA
BX111391	XLOC_l2_010061	0.189691	mRNA
	XLOC_l2_014217	0.189471	mRNA
NM_001017930	DCAF8L1	0.188908	mRNA
	SNTG1	0.188607	mRNA
NM_001038705	GPR149	0.18845	mRNA
NM_005959	MTNR1B	0.188033	mRNA
NM_003547	HIST1H4G	0.187409	mRNA
NM_203451	SERTM1	0.187123	mRNA
		0.185241	mRNA
NM_014213	HOXD9	0.184699	mRNA
AL705284	XLOC_l2_012983	0.184636	mRNA
NR_027713	KRT8P41	0.182903	mRNA
NM_003638	ITGA8	0.182126	mRNA
NM_001080467	MYO5B	0.182031	mRNA
AL705861	XLOC_011173	0.179394	mRNA
NM_001144871	VSTM5	0.178739	mRNA
BC035370	XLOC_l2_000941	0.178591	mRNA
	XLOC_002151	0.178356	mRNA
NM_002838	PTPRC	0.177788	mRNA
NM_001013732	C6orf138	0.177043	mRNA
NM_001100111	LOC286238	0.176556	mRNA
NM_001042575	TMPRSS7	0.176386	mRNA
AK126029		0.172921	mRNA
NM_025213	SPTBN4	0.172741	mRNA
NM_001207026	POU2F2	0.172347	mRNA
AK055023	LOC219690	0.171691	mRNA
NM_198074	OR2C3	0.171139	mRNA
NM_016562	TLR7	0.170401	mRNA
	XLOC_007458	0.169261	mRNA
	XLOC_000902	0.168601	mRNA
NR_024464	LINC00426	0.167477	mRNA
		0.16733	mRNA
AK126787	ERV18-1	0.166663	mRNA
NM_015336	ZDHHC17	0.16662	mRNA
NM_207345	CLEC9A	0.166353	mRNA
NR_027151	C10orf108	0.166288	mRNA
AK128720	PKP4	0.166071	mRNA
NM_001243467	UBASH3A	0.165937	mRNA
AK125770	XLOC_009948	0.164773	mRNA
BC040863	P39189	0.164565	mRNA

NR_038863	LOC285758	0.163668	mRNA
NR_033878	LOC387895	0.163552	mRNA
	XLOC_005179	0.163375	mRNA
CD675678		0.162638	mRNA
NM_005142	GIF	0.162347	mRNA
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NM_080868	ASB17	0.162052	mRNA
NM_001005519	OR6C68	0.161763	mRNA
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NR_027312	LOC153910	0.160352	mRNA
NM_005235	ERBB4	0.158975	mRNA
NM_020822	KCNT1	0.158647	mRNA
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NM_001004063	OR4K1	0.155146	mRNA
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NM_024034	GDAP1L1	0.154673	mRNA
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AK026386	XLOC_l2_013302	0.15208	mRNA
NM_003813	ADAM21	0.15032	mRNA
NM_020344	SLC24A2	0.149151	mRNA
BG184208	XLOC_003765	0.147847	mRNA
NM_176875	CCKBR	0.146336	mRNA
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CR609644	XLOC_011918	0.144222	mRNA
AB305952		0.143075	mRNA
NR_027390	POLR2M	0.14296	mRNA
DA429566	XLOC_011064	0.142642	mRNA
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NM_000730	CCKAR	0.142434	mRNA
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NM_000458	HNF1B	0.1311	mRNA
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NM_001001916	OR52J3	0.130332	mRNA

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NM_052917	GALNT13	0.124958	mRNA
NM_000359	TGM1	0.123504	mRNA
BC048118	XLOC_l2_014219	0.121208	mRNA
NM_001166254	TBXAS1	0.120946	mRNA
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NM_001004745	OR5T1	0.118177	mRNA
NM_001198834	PDE4DIP	0.117873	mRNA
BX096383	XLOC_005105	0.117863	mRNA
NM_032598	SPATA22	0.117187	mRNA
DB088362	XLOC_014175	0.117165	mRNA
NR_034007	LOC339894	0.115319	mRNA
NM_033064	ATCAY	0.114173	mRNA
		0.112583	mRNA
		0.11256	mRNA
NR_026863	LINC00313	0.112397	mRNA
NR_040094	LOC348120	0.11198	mRNA
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NM_018990	SASH3	0.109885	mRNA
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NM_000759	CSF3	0.108037	mRNA
NM_001204424	RGS6	0.10726	mRNA
NM_001136234	FAM48B1	0.10711	mRNA
AY122474		0.105874	mRNA
NM_005020	PDE1C	0.105066	mRNA
	XLOC_l2_013730	0.10431	mRNA
NM_004291	CARTPT	0.103912	mRNA
BC026225	LOC100287221	0.103577	mRNA
NM_005211	CSF1R	0.10351	mRNA
BE875542	LINC00165	0.101911	mRNA
NR_027038	LOC401093	0.101763	mRNA
		0.100106	mRNA
BC032033	LOC414300	0.099714	mRNA
A25969		0.098007	mRNA
NR_027104	LOC285401	0.097863	mRNA
X95463	AFF2	0.09747	mRNA
AL598157	XLOC_l2_010011	0.096695	mRNA

NR_026838	DSCR8	0.094061	mRNA
		0.093461	mRNA
		0.093436	mRNA
		0.09161	mRNA
NM_001164442	FAM159B	0.091206	mRNA
NR_027481	ZNF876P	0.091179	mRNA
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NM_001113228	NTNG1	0.086386	mRNA
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NM_003106	SOX2	0.08554	mRNA
NR_027276	LOC100128239	0.085132	mRNA
NM_198451	FOXR2	0.083262	mRNA
NM_018280	C22orf26	0.081564	mRNA
NR_027249	GNN	0.080676	mRNA
NM_001001919	OR13C4	0.080045	mRNA
XM_003403488	LOC100652763	0.077533	mRNA
NR_033976	LOC401134	0.075904	mRNA
NM_001143939	ZNF534	0.075891	mRNA
DA729442	XLOC_006040	0.075802	mRNA
NM_178540	C1QTNF9	0.075776	mRNA
CF454975	XLOC_001070	0.075405	mRNA
NM_014692	SEC14L5	0.074674	mRNA
NR_026794	LOC731789	0.074365	mRNA
NM_018995	MOV10L1	0.07368	mRNA
NM_001004473	OR10K1	0.070883	mRNA
NM_001462	FPR2	0.069783	mRNA
DB515342	XLOC_004093	0.069752	mRNA
NM_174914	UGT3A2	0.069655	mRNA
NM_000844	GRM7	0.069174	mRNA
NR_028137	LOC286002	0.069126	mRNA
NM_015009	PDZRN3	0.068303	mRNA
AK024188		0.068181	mRNA
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NM_052898	XKR4	0.066065	mRNA
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NM_001013646	C20orf107	0.064437	mRNA
XM_003403443	LOC100509263	0.063714	mRNA
NM_000609	CXCL12	0.063144	mRNA
NM_001033019	DEFB134	0.063084	mRNA
BX486480	XLOC_013823	0.062503	mRNA
XM_003119104	LOC100287188	0.061905	mRNA
NM_001166242	C22orf39	0.061735	mRNA
AY928977	XLOC_001360	0.061417	mRNA

NM_025239	PDCD1LG2	0.061076	mRNA
AW968277	XLOC_004534	0.060627	mRNA
NR_040013	LOC644554	0.060552	mRNA
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AK092662	LOC100132005	0.057332	mRNA
DA674107	XLOC_013531	0.056783	mRNA
AF007131	LOC100129973	0.056335	mRNA
NM_006258	PRKG1	0.055972	mRNA
NM_175721	TPO	0.055177	mRNA
NM_001145004	GOLGA6L6	0.052473	mRNA
CR625825	XLOC_005384	0.052343	mRNA
NR_033983	LOC400654	0.051705	mRNA
	C1QTNF9	0.051266	mRNA
		0.049986	mRNA
DQ983818	C1orf81	0.049395	mRNA
		0.048434	mRNA
NM_006902	PRRX1	0.047676	mRNA
NR_015430	C14orf64	0.045765	mRNA
NM_031956	TTC29	0.042656	mRNA
DA760426		0.041951	mRNA
AF461896		0.041617	mRNA
NR_033740	CES1P2	0.041542	mRNA
AK123052	LOC100131821	0.04001	mRNA
NR_033368	GRIK1-AS2	0.038689	mRNA
NM_033267	IRX2	0.038674	mRNA
NM_001184714	SLAMF6	0.038288	mRNA
NR_027104	LOC285401	0.037551	mRNA
NR_033985	FLJ26245	0.037303	mRNA
NR_038372	LOC402779	0.037247	mRNA
XM_001723012		0.037086	mRNA
NM_001001952	OR5D18	0.034613	mRNA
NM_005302	GPR37	0.032962	mRNA
NM_001004064	OR8J3	0.032288	mRNA
NM_002761	PRM1	0.032073	mRNA
NM_080475	SERPINB11	0.031705	mRNA
AJ245419		0.031422	mRNA
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AK307375		0.030028	mRNA
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NM_001002006	NT5C1B	0.029161	mRNA
AK098126		0.028933	mRNA
NR_001281	PCDHB18	0.027993	mRNA

BX096530	XLOC_005551	0.027437	mRNA
NM_001025076	CELF2	0.027146	mRNA
NM_197954	CLEC7A	0.026969	mRNA
		0.025913	mRNA
NM_001080484	KIAA1751	0.025411	mRNA
BC008585		0.025265	mRNA
NM_001005487	OR13G1	0.024988	mRNA
NM_006422	AKAP3	0.023898	mRNA
NM_016366	CABP2	0.023378	mRNA
NM_016945	TAS2R16	0.023367	mRNA
DB307521		0.022601	mRNA
		0.022193	mRNA
NM_001721	BMX	0.020277	mRNA
NM_001005160	OR52A5	0.019971	mRNA
NM_033401	CNTNAP4	0.019842	mRNA
NM_138379	TIMD4	0.01853	mRNA
NM_080615	GCNT7	0.014586	mRNA
AK097358	FLJ40039	0.013565	mRNA
NR_036490	LOC284648	0.013389	mRNA
NR_001591	psiTPTE22	0.012178	mRNA
NM_002365	MAGEB3	0.011704	mRNA
AK091000	XLOC_002322	0.011671	mRNA
BC043541	LOC339539	0.011127	mRNA
NM_001198986	SPINLW1-WFDC6	0.010847	mRNA
NM_014996	PLCH1	0.010108	mRNA
NM_001005489	OR5B17	0.010021	mRNA
NM_183058	LYZL2	0.009954	mRNA
DA230376	XLOC_002943	0.009056	mRNA
NM_001206626	LOC283116	0.008698	mRNA
NM_014495	ANGPTL3	0.008129	mRNA
NM_145027	KIF6	0.008125	mRNA
NM_001102470	ADH6	0.008061	mRNA
	NUBPL	0.007711	mRNA
NM_138715	MSR1	0.007126	mRNA
		0.00535	mRNA
NM_152404	UGT3A1	0.005332	mRNA
NM_001142800	EYS	0.004908	mRNA
NM_015393	PARM1	0.003905	mRNA
NR_029389	LOC100134317	0.00334	mRNA
NR_024072	MRS2P2	0.001821	mRNA

Table S3. Clinicopathological features of PDAC patients.

Patient	Gender	Age	Site*	MMP1	PNI number	PNI degree (ne)**	Grade	Stage
1	M	28	1	0	1	1	3	IIA
2	F	66	1	0	0	0	1	IV
3	F	58	1	0	0	0	2	IIA
4	M	44	1	0	1	1	1	IV
5	M	65	2	0	3	1	2	IIIA
6	M	73	1	0	2	1	1	IV
7	F	55	1	1	5	2	2	IIA
8	M	68	2	1	2	1	2	IIB
9	M	83	1	1	2	1	2	IIA
10	M	65	1	1	1	1	3	IIA
11	F	60	1	1	0	0	2	IIA
12	M	44	2	1	0	0	1	IIB
13	M	54	1	1	20	3	2	IIIA
14	M	48	1	1	1	1	1	IIB
15	M	54	1	1	3	1	2	IIA
16	F	55	2	1	0	0	2	IV
17	M	38	1	1	1	1	3	IIB
18	F	44	1	1	4	2	1	IIB
19	M	70	1	1	8	2	2	IIB
20	M	58	1	1	6	2	1	IIB
21	F	62	2	1	8	2	2	IIA
22	M	68	2	2	12	3	3	III
23	F	49	1	2	12	3	3	IIB
24	F	56	1	2	4	2	3	IIA
25	M	51	1	2	10	3	3	IIB
26	M	54	1	2	4	2	3	IIB
27	M	68	2	2	8	2	1	IIB
28	M	63	1	3	3	1	3	IIB
29	F	56	2	3	10	2	1	IIA
30	M	63	1	4	6	2	2	IIB

* 1 = head; 2 = body/tail

** The degree of PNI was defined microscopically as follows: ne0, no perineural invasion; ne1, perineural invasion is difficult to find, occurrences of lesions ≤ 3; ne2, perineural invasion that was easy to find and between ne1 and ne3; and ne3, perineural invasion that was even easier to find with more massive occurrences of lesions and extension beyond the border of the main tumor mass.