Supplementary information

Patients and sample collection

For the blood platelet count test, we performed a retrospective analysis on data collected from 656 PDAC patients from May 2014 to December 2017 from Fudan University Shanghai Cancer Center. Of these patients, 274 had stage I/II PDAC and underwent R0 resection, and 382 had stage III/IV PDAC with metastasis to local lymph nodes, large blood vessels or distant organs. Patients with hematological disorders and patients who received anticoagulant medical treatment were excluded from our study. A total of 3105 healthy people from the physical examination center were selected during the same period and matched to PDAC patients by age and sex, excluding the following: (1) patients taking anticoagulants; (2) patients with systemic diseases or taking medications for these diseases; (3) patients with autoimmune diseases or pregnancy; and (4) patients with infectious diseases or a history of alcohol abuse.

For flow cytometry analysis of platelets, we enrolled two independent cohorts containing 89 patients with stage I/II PDAC and 103 patients with stage III/IV PDAC from January 2018 to March 2018. A total of 95 healthy donors were matched to the PDAC patients by age and sex and selected as the control groups. Blood platelets were collected before treatments, such as surgery and anticancer therapy.

Overall survival (OS) was calculated as the interval between the date of surgery and the date of death. Relapse-free survival (RFS) was defined as the interval between the date of surgery and the date of tumor recurrence. All patients were followed up until June 2019. This study was approved by the Fudan University Shanghai Cancer Center Research Ethics Committee. Informed consent was obtained from all patients prior to the investigation.

Meta-analysis

Two independent reviewers searched PUBMED, EMBASE, and MEDLINE databases prior to 30 December 2018. The MeSH (Medical Subject Heading) search included headings that were all combinations of "platelet" and "pancreatic cancer." A pooled risk estimate was calculated with a random-effects model considering both intra-study and inter-study variances. All statistical analyses were conducted using STATA 11.0 for Windows (Stata, College Station, TX, USA).

Cell culture

The human pancreatic cancer cell line MIAPaCa-2 and mouse pancreatic cancer cell line Panc02 were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The MIAPaCa-2 and Panc02 cells were cultured in Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS; Gibco, Thermo Fisher Scientific, Waltham, MA) and 1% penicillin-streptomycin.

Animals

C57BL/6 mice were purchased from Charles River (Beijing, China). Panx1 KO mice were obtained from Meng GX (Institut Pasteur of Shanghai). Male C57BL/6 mice (specific pathogen-free (SPF)-grade) and Panx1-KO mice (aged 6–8 weeks) were bred at the animal facility of Institut Pasteur of Shanghai under pathogen-free conditions. Panx1 genotyping was performed by PCR. Panx1 genotyping F: 5'-CCTCTGGTCTGCTCTGTGTC-3', R: 5'-CACTGACAGACACCTGCCTG-3', Mut: 5'-ATCGCCTTCTATCGCCTTCT-3'. All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of

Laboratory Animals" issued by the National Institutes of Health (NIH publication 86-23 revised 1985).

Isolation of human and murine platelets

The healthy volunteers and PDAC patients were not receiving known platelet inhibitors, such as aspirin or nonsteroidal anti-inflammatory drugs (NSAIDs). Human whole blood was collected in vacutainer tubes containing EDTA anticoagulant for the analysis of patients' platelets and collected with sodium citrate solution for *in vitro* experiments. Platelet-rich plasma (PRP) was obtained from the supernatant of whole blood by centrifugation at 200 × g at room temperature for 20 min. The upper phase was transferred into a fresh tube and washed and resuspended in HEPES-modified Tyrode buffer.

Murine whole blood was drawn by cardiac puncture from C57BL and Panx1 KO mice terminally anesthetized with chloroform and taken with sodium citrate solution. The tube was centrifuged at 180 × g at room temperature for 10 min. PRP was collected and mixed with washing buffer containing 5 μg/mL prostaglandin E1 (PGE1; Cayman Chemical Co., Ann Arbor, MI, USA). The tube was centrifuged at 1250 × g for 10 min. The supernatant was removed and washed twice using wash buffer.

The above PRP stage, the contaminating leukocytes were always removed by CD45+ bead selection. The purity of isolated platelets was confirmed by flow cytometry and direct counting via a hemocytometer, where ,3 leukocytes were observed in each preparation containing 1x 10^7 platelets.

Activation of platelets

Thrombin (Sigma-Aldrich) or PDAC cells were used to activate platelets. Briefly, platelets were activated *in vitro* by exposure to 25 mM thrombin (Sigma-Aldrich) for 15 min and washed three times by PBS. After PDAC cells were seeded into 6-well plates at 5×10^{6}/mL for 12 h, platelets were added to tumor cell (100:1 ratio of platelets to PDAC cells) for 24 h at 37°C. The entire platelet was marked by an APC-conjugated anti-CD41 antibody (BD Biosciences). The activation status of platelets was determined by flow cytometry using an FITC-conjugated anti-CD62P (P-selectin) antibody (BD Biosciences) or indicated by changes in shape to a more amorphous form.

Platelet aggregation assay

Whole blood (500 μL) was prepared freshly from C57/BL6 mice and was dispensed into citrate anticoagulant tubes. Platelet aggregation was assessed at 37°C by PL-11 (SINNOWA Medical Science & Technology Co., Nanjing, China). Aggregation was initiated by the addition of 10 μL ADP solution (final concentration, 20 μM). The maximum aggregation ratio (MAR) was calculated according to the following formula: MAR = 100 − ([1st platelet count-2nd platelet count]/2-lowest platelet count) × 100%. The average aggregation ratio (AAR) was calculated as follows: AAR = (max MAR + min MAR)/2.

Co-culture of the platelets with tumor

Treatment of tumor cells with platelets for *in vitro* assay: pancreatic cancer cells were seeded in the corresponding medium with 10% FBS and incubated overnight. Platelets were added for

another 24 h (100:1 ratio of platelets to PDAC cells). Then, platelets were removed, and PDAC cells were washed with 1 × PBS.

In vivo: Panc-02 cells were seeded in a 10 cm plate. After growth to 50–60% confluence, platelets (100:1 ratio of platelets to PDAC cells) were added and cultured for another 24 h. Then, the platelets were removed, and PDAC cells were washed with 1 × PBS, harvested and resuspended in PBS at a concentration of 1×10^7 cells /mL. Cell suspension (0.1 mL) was injected into mice i.v. [1].

We used ^{10}Panx1, SB203580 or apyrase to block Panx1 channel, or p38 MAPK pathway or neutralize ATP on platelets, respectively. We treated platelets with 25 min with 100 μM 10Panx1 to block Panx1 channel (scrambled peptides for 25 min in 100% PBS as control) or 25 min with 10 μM SB203580 to block the platelet p38 MAPK pathway, or 10 min with 0.5 U/ml to neutralize the platelet ATP. The ATP rescue experiments with 1.0 mM was used. Platelets were treated with 4 μM NF449 (P2X1 receptor antagonist) for 2 h. Then, we performed centrifugation at 400 x g 20 min to remove supernatant and washed two times with Tyrode buffer.

Transwell migration assay

The invasive ability of cells was detected in 24-well invasion chambers (8 μm pore size). The transwell membrane of the upper chamber was coated with Matrigel (BD Biosciences Discovery Labware, Woburn, MA, USA). The chambers were rehydrated in an incubator with serum-free medium at 37°C for 2 h. The top chambers were subsequently hydrated with 200 μL of PDAC cell suspension (containing 1×10^5 cells). In the bottom chambers, 500 μL of medium containing platelets (containing 5×10^6 cells) as chemoattractant was added. After 24 h of culture at 37°C, the transmembrane cells on the lower surface of the membrane were fixed with formaldehyde at room

temperature for 5 min, stained with crystal violet for 20 min, washed three times with clean water, and counted under a microscope.

Wound healing assay

PDAC cells were seeded in a 6-well plate at 5×10^5 cells per well. After starvation for 24 h in 1% FBS medium, the cells were removed using 200 μL pipette tips. The PDAC cells were washed with PBS and cultured in growth medium (1% FBS) with platelets (2.5×10^6) for an additional 24 h. PDAC cells were then washed to remove platelets, and PDAC cell migration was observed using a Nikon TS100 microscope.

Cytokine assay

For the analysis of various cytokines, blood and culture supernatants were collected and centrifuged at 1000 × g for 30 min. The serum or culture supernatant levels of pro-inflammatory cytokines, chemokines and growth factors (G-CSF, GM-CSF, IFN-γ, IL-10, IL-12p70, IL-13, IL-1β, IL-2, IL-4, IL-6, and M-CSF/CSF1) were assessed with the customized Luminex-based multiplex Procarta Cytokine Assay Kit (Multimetrix, Heidelberg, Germany). Standard curves were prepared from the standards provided with the kit and serially (log 4) diluted from 20 ng/mL to 1.2 pg/mL.

Enzyme-linked immunosorbent assay (ELISA)

Peripheral blood was collected from patients, healthy people, and mice, and standard procedures were followed to centrifuge samples at 4°C and store aliquots at 80°C. Platelets were incubated as described above. Platelets were isolated by centrifugation after incubation. Supernatants were collected, and aliquots were stored at 80°C. Serum and supernatant were incubated for 1 h at room

temperature. IL-1β was assessed with an ELISA kit in accordance with the manufacturer's instructions.

Quantitative reverse-transcription PCR (qRT-PCR)

Total RNA from tissues and cells was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Approximately 1 μg of RNA was used for the synthesis of cDNA with the Superscript III Reverse-Transcription Kit (Invitrogen). Standard PCR amplification was performed, consisting of 95°C for 30 s followed by 40 cycles at 95°C for 5 s and 60°C for 30 s. β-Actin or GAPDH was used to normalize target gene expression. The primer pairs used in qRT-PCR are listed below.

Gene	**Sequence 5'-3'**
Human IL-1β Forward	AAATGTCGGGAAGGTACTCG
Human IL-1β Reverse	GCCAGGCAAGTGTTCCAC
Human Panx1 Forward	CGTGACCTTGACATGAGAGATG
Human Panx1 Reverse	CTGCTCCACAATTGGGTACTT
Human Panx2 Forward	TTCTGCGACATCAACATCCT
Human Panx2 Reverse	ACCACGTTGTCGTACATGAG
Human Panx3 Forward	AAGGCTCGGAAAGAACGATAC
Human Panx3 Reverse	GGAGGTGAAGATGAGCAAGAG
Human E-cadherin Forward	GAACAGCACGTACACAGCCCT
Human E-cadherin Reverse	GCAGAAGTGTCCCTGTTCCAG
Human Snail Forward	TGCAGGACTCTAATCCAAGTTTACC
Human Snail Reverse	GTGGGATGGCTGCCAGC

Human N-cadherin Forward	GACGGTTCGCCATCCAGAC
Human N-cadherin Reverse	TCGATTGGTTTGACCACGG
Human Vimentin Forward	TGTCCAAATCGATGTGGATGTTTC
Human Vimentin Reverse	TTGTACCATTCTTCTGCCTCCTG
Human NLRP3 Forward	CTTCCTTTCCAGTTTGCTGC
Human NLRP3 Reverse	TCTCGCAGTCCACTTCCTTT
Human GAPDH Forward	CCAACCGCGAGAAGATGA
Human GAPDH Reverse	CCAGAGGCGTACAGGGATAG
Mouse IL-1βForward	AACCTGCTGGTGTGTGACGTTC
Mouse IL-1βReverse	CAGCACGAGGCTTTTTTGTTGT
Mouse NLRP3 Forward	AGAGCCTACAG TTGGGTGAAATG
Mouse NLRP3 Reverse	CCACGCCTACCAGGAAATCTC
Mouse Panx1 Forward	AGCCAGAGAGTGGAGTTCAAAGA
Mouse Panx1 Reverse	CATTAGCAGGACGGATTCAGAA
Mouse E-cadherin Forward	AGGCTGGCTGAAAGTGACACA
Mouse E-cadherin Reverse	ACACGGCATGAGAATAGAGGATGT
Mouse Snail Forward	GGCGGAAGCCCAACTATAGC
Mouse Snail Reverse	AGGGCTGCTGGAAGGTGAA
Mouse N-cadherin Forward	AGCGCAGTCTTACCGAAGG
Mouse N-cadherin Reverse	TCGCTGCTTTCATACTGAACTTT
Mouse Vimentin Forward	CCGTTCAAGGTCAAGACGTGCCA
Mouse Vimentin Reverse	AGGAGGCCGAAAGCACCCTGC

Mouse GAPDH Forward	AACTTTGGCATTGTGGAAGG
Mouse GAPDH Reverse	ACACATTGGGGGTAGGAACA

Platelet/RNA isolation and RNA sequencing

The platelet lysates were kept at -80℃ for RNA isolation in preparation for sequencing. Total RNA from platelets was extracted using TRIzol (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. Sample preparation for Illumina sequencing was performed using the TruSeq DNA Sample Prep Kit (Illumina, cat No. FC-121-2001). Each sample was sequenced by Illumina HiSeq according to the manufacturer. In brief, the total RNA was extracted using TRIzol Reagent (Invitrogen)/RNeasy Mini Kit (Qiagen)/other kits followed by library construction. The poly(A) mRNA isolation was performed using Poly(A) mRNA Magnetic Isolation Module or an rRNA Removal Kit. Then, the libraries were sequenced with an Illumina HiSeq instrument (Illumina, San Diego, CA, USA) using a 2 x 150 bp paired-end (PE) configuration. The pass filter data in fastq format were processed by Cutadapt (V1.9.1) to yield high-quality clean data, then aligned to the reference genome via Hisat2 software (v2.0.1). Gene and isoform expression levels were estimated by HTSeq (v0.6.1). Differential expression analysis used the edgeR package from R with FDR < 0.05 and fold change > 2.

Western blot analysis

Standard western blot analysis was performed. Total proteins from the frozen tissues, PDAC cells and platelets were extracted using M-PER Mammalian Protein Extraction Reagent (Thermo Scientific, Rockford, IL, USA). The lysates were electrophoresed in 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels and then transferred onto

polyvinylidene difluoride membranes (EMD Millipore, Billerica, MA, USA). The membranes were incubated with primary antibodies for 1–2 h at room temperature or overnight at 4°C and then incubated with the appropriate secondary antibody at room temperature for 1 h. Protein detection was performed using an enhanced chemiluminescence kit (AbFrontier Co. Ltd., Seoul, South Korea), and the blots were exposed to X-ray film. The band intensities were quantified using ImageJ software (version 1.43; National Institutes of Health, Bethesda, MD, USA) with β-actin as the loading control.

Flow cytometry

The platelets were isolated and incubated with PE anti-CD41 antibody (Clone: VI-PL2, eBioscience), FITC anti-CD62P antibody (Clone: AK4, eBioscience), or isotype controls conjugated with each fluorescent dye. The flow cytometric analysis was performed using the BD LSRFortessa X-20 (BD Biosciences). The flow cytometry data were analyzed with FlowJo software Version 10 (Tree Star, Ashland, OR, USA).

Immunohistochemistry (IHC) and immunofluorescence (IF)

After formalin fixation, paraffin-embedded tumors were deparaffinized and rehydrated. The paraffin sections were incubated with primary antibody. Both the staining density and intensity were scored for each slide and were calculated using the semi-quantitative scoring method [2]. Specifically, staining scores were assigned according to the percentage of positive tumor cells: 1 (up to 25% positive cells), 2 (25%–50% positive cells), 3 (50%–75% positive cells), and 4 (more than 75% positive cells). The intensity scores ranged between 0 and 3 as follows: 0, no staining; 1, weak staining; 2, moderate staining; and 3, strong staining. A final score between 0 and 12 was calculated by multiplying the staining score with the intensity score. A score of 0–6 indicates low

expression, whereas a score of 7–12 indicates high expression.

For immunofluorescence, isolated platelets from mice were fixed with 4% formaldehyde for 20 min, attached to poly-lysine-coated coverslips and processed for immunofluorescence microscopy as previously described [3].

Lung tissues were embedded in optimal cutting temperature compound. Cryosections were air dried, fixed for 10 min at room temperature using a 1:1 mixture of acetone and methanol and subsequently blocked to eliminate non-specific binding using 0.5 % bovine serum albumin (BSA) in PBS for 1 h. The cryosections were incubated with primary antibody followed by secondary antibody, and the nuclei were stained with DAPI (Invitrogen, Carlsbad, CA). The tissues were prepared for immunofluorescence labeling using standard protocols [4]. The images were acquired using a Zeiss LSM 710 confocal microscope (Zeiss, Germany).

Live cell imaging

Panc02 pancreatic cancer cells were transduced with lentiviral vectors encoding GFP (green). A PKH26 red fluorescent cell linker kit was used for platelet membrane labeling. Panc02 cells and platelets were co-cultured (100:1 ratio of platelets to PDAC cells) in culture plates. Fluorescent images of each position were taken every 15 min with the ImageXpress® Pico Automated Cell Imaging System.

ATP measurements

The supernatants of wild-type platelets (WPs) and Panx1 KO platelets (KPs) were collected. ATP concentrations were measured using the ATP Determination Kit (Thermo Fisher Scientific, Waltham, MA, USA) and a luminometer according to the manufacturer's instructions.

Xenograft tumor growth

Panc02 cells (2×10^6 cells/mice) were subcutaneously injected into the right flanks of 6-week-old male nude mice to establish a tumor-bearing model (five mice per group). Every 7 days, WT platelets and $Panx1^{-/-}$ platelets were injected into nude mice by the tail vein. The tumor size was determined every 3 days by measuring the tumor diameter using Vernier calipers, and tumor weight was measured in grams after dissection. Tumor volume (cm^3) = $d^2 \times D/2$, where d and D represent the shortest and longest tumor diameters, respectively. All experimental mice were euthanized after 4 weeks. All tumors were excised, weighed, harvested, fixed, and embedded. All animals received humane care per the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" issued by the National Institutes of Health (NIH publication 86-23 revised 1985).

Bleeding time

The bleeding time in mice was usually measured by severing 2 mm from the tip of the tail of restrained animal, immersing the tail in normal saline (37°C). Bleeding time from the incision to cessation was recorded.

Adoptive platelet transfusion model

Platelet transfusions were performed as previously described [5]. The platelets in recipient mice were neutralized with a LEAF anti-mouse CD41 antibody (1 μg/g body weight, BioLegend, San Diego, CA) via tail vein injection every four days (0, 4, 8, 12 days, every four days). Twenty-four hours after the CD41 antibody injection, $5x10^8$ platelets isolated from one mouse from each donor group (WT and Panx1 KO mice) were resuspended in 200 μL of sterile phosphate-buffered saline

(PBS) and infused into one mouse from each recipient group via tail vein injection. Platelet transfusions were performed in recipient mice every 4 days (2, 6, 10 days, every four days) until the mice were sacrificed.

Orthotopic model of PDAC and lung metastasis model

Healthy 6–8-week-old male WT and Panx1 KO and nude mice were used (five mice in each group) for *in vivo* invasion and metastasis experiments. The mice were anesthetized intraperitoneally. After local shaving and disinfection, the abdominal cavity was opened by a 1.5-cm longer longitudinal incision into the left upper quadrant. The tail of the pancreas was identified after the spleen was lifted. Then, 100 mL of ice-cold Matrigel containing Panc02 cells (2×10^6 cells/mice) was slowly injected into the pancreatic parenchyma using an ice-cold 27-gauge needle and an ice-cold calibrated syringe. To further prevent leakage, the needle was kept on the injection site for 30 s prior to removal. Metastasis was analyzed using live imaging after 4 weeks.

For the lung metastasis model, WT mice and node mice were injected with 2 x 10^6 Panc02 cells in 200 μL of PBS via the tail vein. Metastasis was analyzed using live imaging every week. After 4 weeks, the mice were euthanized, and the lungs were excised, fixed, and embedded.

***In vivo* bioluminescence imaging (BLI) and histology of lung metastasis**

Bioluminescence images were analyzed using a small animal imaging system (IVIS Kinetics; Caliper Life Science, Waltham, MA, USA). BLI was performed 10-15 min after d-luciferin injection. Regions of interest were drawn, and luminescence intensity was quantified and processed using Living Image software (Version 4.2; Caliper Life Science). The mice were euthanized after 4 weeks. Paraffin sections of the lungs were prepared and analyzed after H&E staining.

Positron emission computed tomography combined with X-computed tomography (PET-CT)

Animal-PET-CT scans and image analyses were performed 1 h after an injection of radiolabeled tracer (via intraperitoneal injection with 5.55 MBq 18F-FDG in 0.1 mL saline) using an Inveon Animal-PET-CT (Siemens Preclinical Solution, Knoxville, TN). Animals were maintained under 2% isoflurane anesthesia during scanning period. The mice were placed in the prone position on the bed of the scanner (five-min CT scanning followed by ten-min PET scanning). The animal-PET and animal-CT images were generated separately and then fused using Inveon Research Workplace (Siemens Preclinical Solution, Knoxville, TN). The three-dimensional ordered-subset expectation-maximization (OSEM3D)/maximum algorithm was used for image reconstruction. The region of interest (ROI) was manually drawn covering the whole tumor on the fused images for further analysis. The highest uptake point of the entire tumor was included in the ROI, and no necrosis area was allowed. After the acquisition, SUVmax was assessed on the Siemens syngo MultiModality WorkPlace (MMWP) system by a single nuclear medicine physician. SUVmax was determined by manually placing a cylindrical ROI over the tumor of interest.

The ligation of PSGL-1 and carbenoxolone (PC63435)

Briefly, PSGL-1 (100 μg/mL) dissolved in morpholine ethanesulfonic acid (MES, pH = 5.6) was pre-activated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (pH 5.6, 5 mg/mL) for 2 h at 25°C. The activated PSGL-1 was added to the carbenoxolone in PBS (carbenoxolone, 1 mg/mL); then, N-hydroxyl succinimide (NHS, 5 mg/mL) was dissolved into the

mixture. The mixture was stirred at room temperature for 24 h to form an amide bond between the carboxyl group in the amino of Asn sequences in PSGL-1 and the carboxyl in carbenoxolone. The product was dialyzed in ultrapure water for 3 days and then lyophilized. The mouse was treated 1 mg/kg of PC63435 intraperitoneally every 7 days.

1H-NMR and electrospray mass spectrometry

The spatial distribution of molecules that combine PC63435 was measured by 1H-NMR and mass spectrometry. The NMR analyses were performed using the Avance II 400 MHz UltraShield Plus NMR Spectrometer (Bruker, Rheinstetten, Germany). The electrospray mass spectrometry (ESI-MS) analyses were performed in positive-ion mode using the Thermo LCQ Fleet Ion-trap Mass Spectrometer (Thermo Fisher Scientific Inc., San Jose, CA, USA). All the characterizations were repeated three times.

Scanning electron microscopy (SEM)

Collect platelet and then wash slide gently with PBS, followed by adding electron microscopy fixative into petri dish. Then transfer platelet blocks into 1% OsO4 in 0.1 M PB (pH 7.4) for 1-2 h at room temperature. After dehydrating and drying, specimens are attached to metallic stubs using carbon stickers and sputter-coated with gold for 30s. Observe and take images with scanning electron microscope.

Statistical analyses

Statistical analyses were performed using SPSS 24.0 (SPSS Inc., Chicago, IL). The continuous variables in different subgroups were compared using unpaired t-test and one-way analysis of variance. The categorical variables were compared using the chi-square test. OS and RFS were

displayed using Kaplan-Meier survival curves, and the differences between subgroups were compared using the log-rank test. Univariate and multivariate regression analyses were used to identify independent prognostic factors, and $P < 0.05$ was the criterion for variable deletion when performing backward stepwise selection. All tests were two sided, and $P < 0.05$ was considered statistically significant.

References

1. Yu LX, Yan L, Yang W, Wu FQ, Ling Y, Chen SZ, et al, Platelets promote tumour metastasis via interaction between TLR4 and tumour cell-released high-mobility group box1 protein. Nat Commun. 2014;5:5256.

2. Goethals L, Perneel C, Debucquoy A, De Schutter H, Borghys D, Ectors N, et al. A new approach to the validation of tissue microarrays. J Pathol. 2006;208:607-14.

3. Patel-Hett S, Richardson JL, Schulze H, Drabek K, Isaac NA, Hoffmeister K, et al. Visualization of microtubule growth in living platelets reveals a dynamic marginal band with multiple microtubules. Blood. 2008;111:4605-16.

4. Snegovskikh V, Mutlu L, Massasa E, Taylor HS. Identification of putative fallopian tube stem cells. Reprod Sci. 2014;21:1460-4.

5. Bergmeier W, Boulaftali Y. Adoptive transfer method to study platelet function in mouse models of disease. Thromb Res. 2014;133:S3-5.

Supplementary Figure Legends:

Supplementary Figure 1. (A) Kaplan-Meier survival analysis for the correlation between CD62P+ platelets and OS in stage III/IV patients (n = 103). (B) The mRNA levels of Panx1, Panx2, and Panx3 in non-activated platelets and activated platelets were detected by RT-PCR. (C) The protein levels of Panx1 in non-activated platelets and activated platelets were detected by western blotting. (D) The blood platelet count was detected in WT and Panx1 KO mice. (E) The bleeding time was detected in WT and Panx1 KO mice. (F-G) Platelet aggregation was analyzed in Panx1 KO and WT mice. (H) The platelet shape was detected in WT platelet and Panx1 KO platelet by IF. (I) Live cell imaging of the platelets in real time.

Supplementary Figure 2. (A-B) Platelets promote PDAC cell invasion and metastasis *in vitro* compared to no platelet co-culture by transwell assay and wound healing. (C-D) Platelets promote PDAC cell EMT marker expression in mRNA level and protein level according to qRT-PCR and western blotting. (E-F) ^{10}Panx1-treated platelets were washed and centrifuged to remove the remaining ^{10}Panx1 to exclude the direct effect of ^{10}Panx1 on PDAC cell invasion and metastasis *in vitro*.

Supplementary Figure 3. (A-C) Panc02 cells co-cultured with platelets or non-platelets for 24 h and then subcutaneously injected in nude mice (n = 4 for each group). Tumor volumes were monitored every week, and tumor weights were measured at the fourth week after mice were sacrificed. (D) Tumor tissues from subcutaneous xenograft tumors were immunohistochemically stained for EMT markers. (E) Panc02 cells co-cultured with non-platelets and platelets were injected into nude mice via the tail vein. The incidence of lung metastasis was observed. Hematoxylin and eosin-stained images of lung tissues harvested are shown. (F) The spleen

invasion in nude mice of the orthotopic model of PDAC with platelet and no platelet infusion (n = 5). (G-H) Tumor tissues from orthotopic tumors of nude mice were immunohistochemically stained for Ki-67 and EMT markers between non-platelet and platelet groups. (I) The survival rate of nude mice of the orthotopic model of PDAC with and without platelet infusion.

Supplementary Figure 4. (A) Adoptive platelet transfusion model was established. (B) The number of platelets was lowest in the two days after anti-CD41 injection and platelet started to rise on the fourth day. (C) The number of platelets was measured in continuous time points after anti-CD41 injection (0, 4, 8 days, every four days) and the transfused platelets (2, 6, 10 days, every four days) by routine blood examination, and transfused platelets accounted for most of the total platelet count. (D) Tumor tissues from subcutaneous xenograft tumors (adoptive platelet transfusion nude mice) were immunohistochemically stained for EMT markers. (E) Representative xenograft tumors derived from MIAPaCa-2 cells co-cultured with platelets isolated from PDAC patients and healthy people (n = 5). Tumor volume was monitored every week. (F) Immunofluorescence (IF) analysis of WT platelets Panx1$^{-/-}$ platelets and from the lung tissues, liver tissues, pancreatic tissues and blood of nude mice.

Supplementary Figure 5. (A) Cytokines and chemokines were detected by the Luminex-based multiplex Procarta Cytokine Assay Kit (Multimetrix, Heidelberg, Germany) in KO (Panx1$^{-/-}$) and WT (wild-type) mice. (B) rmIL-1β promotes Panc 02 cell invasion and metastasis *in vitro* compared to IL-1βAb and IgG without platelets. (C) rmIL-1β promotes Panc 02 cell EMT marker expression compared to IL-1βAb and IgG without platelets.

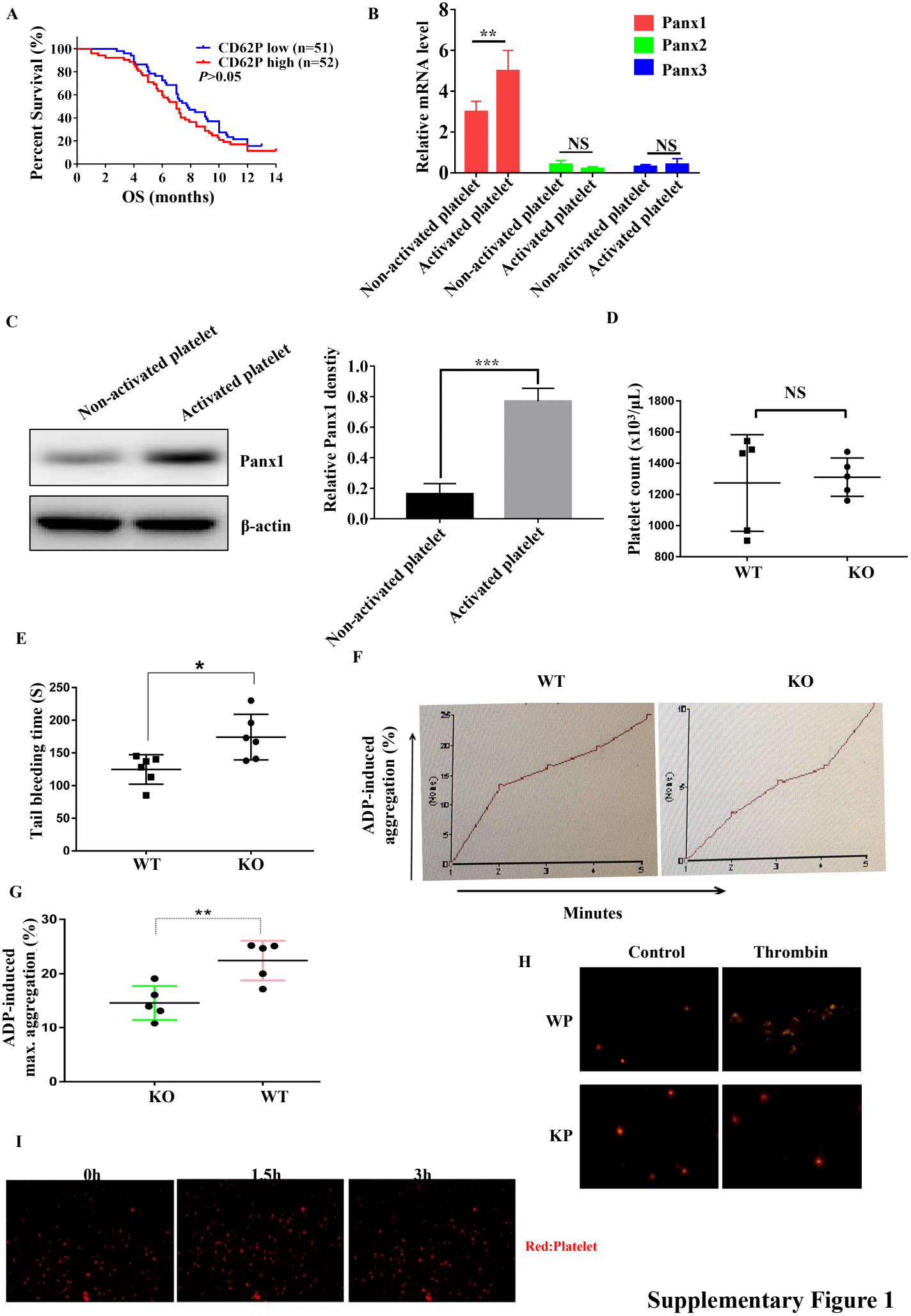
Supplementary Figure 6. (A) Interaction networks of Panx1 with other genes based on the RNA-Seq data of platelets. (B) Western blot analysis of P-p38 MAPK, P38 MAPK expression on

human platelets stimulated with 10Panx1. (C) Western blot analysis of the p-p38 MAPK expression in PDAC patient platelets and healthy patient platelets after thrombin stimulation. (D-E) Western blot and qRT-PCR analysis of p38 MAPK, NLRP3, and IL-1β expression in human platelets treated with 10Panx1. (F) Western blot analysis of NLRP3 and IL-1β expression after blocking the p38 MAPK pathway in human platelets. (G-H) SB203580-treated platelets were washed and centrifuged to remove the remaining SB203580 to exclude the direct effect of SB203580 on PDAC cell invasion and metastasis *in vitro*. (I) ATP levels were detected in the supernatant of WPs and KPs after thrombin stimulation. (J) The bleeding time was detected in PC63435-injected WT mice. (K) Platelet aggregation was analyzed in WT+Control mice and WT+PC63435 mice. (L) Platelet counts were analyzed in WT+Control mice and WT+PC63435 mice. (M) *In vivo* lung metastasis in WT mice injected with PC63645 or carbenoxolone (CBX) as a control (n = 5 per group).

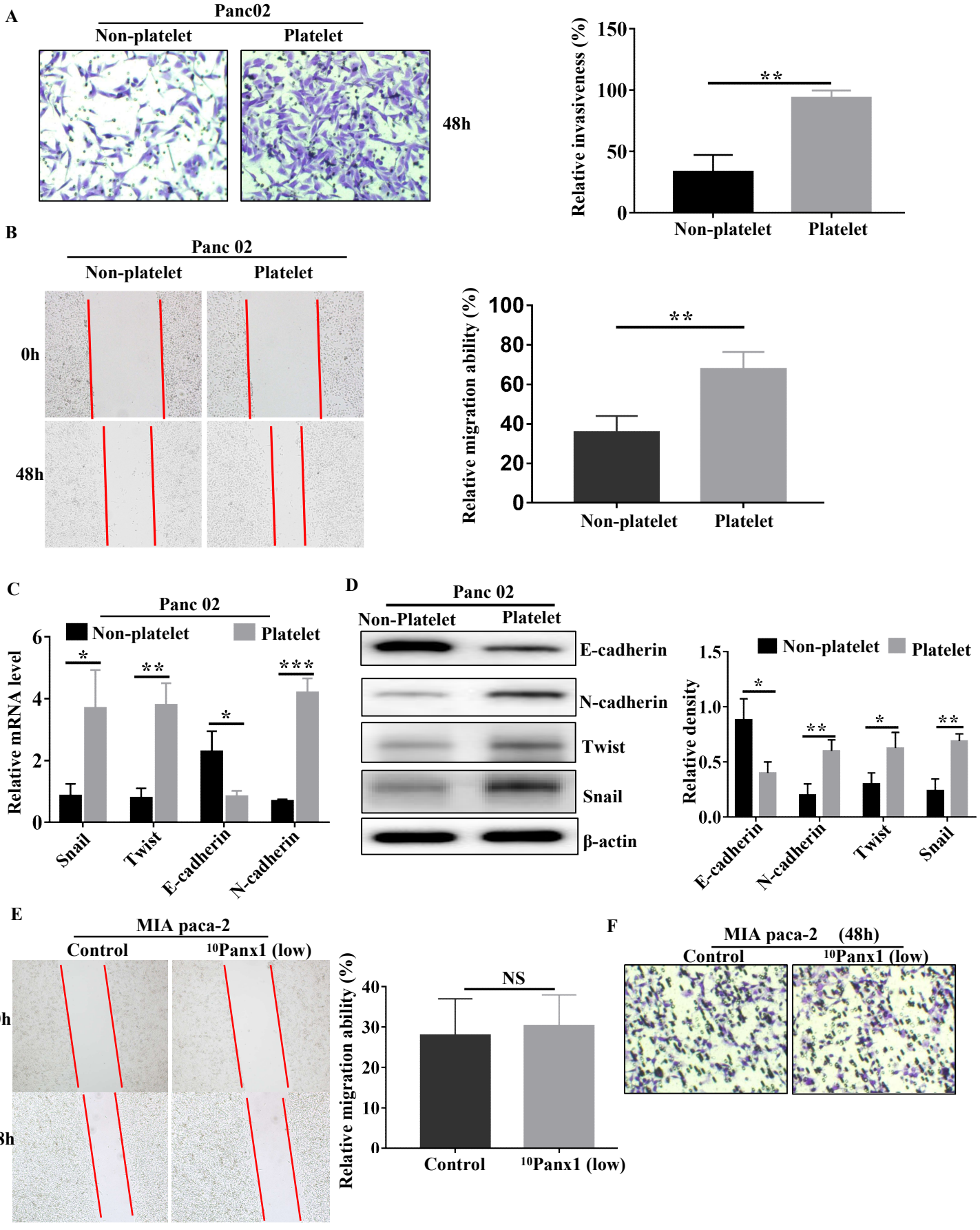
Supplementary Figure Tables:

Supplementary Table 1. Relationship between CD41+/CD62P+ platelet count and clinicopathological features of patients with PDAC (I/II and III/IV)

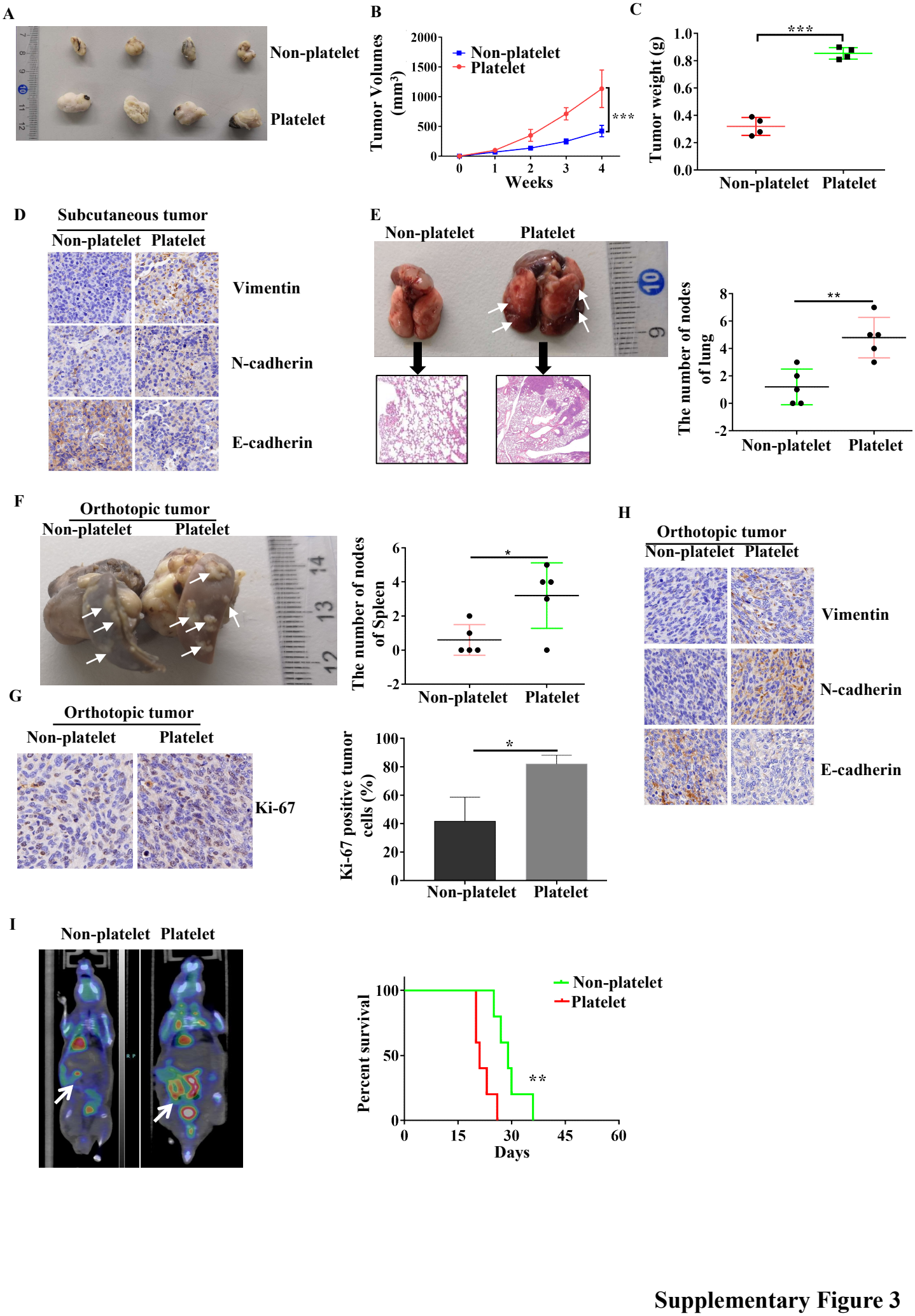
Supplementary Table 2. Multivariate Cox regression analyses of OS and RFS in PDAC patients



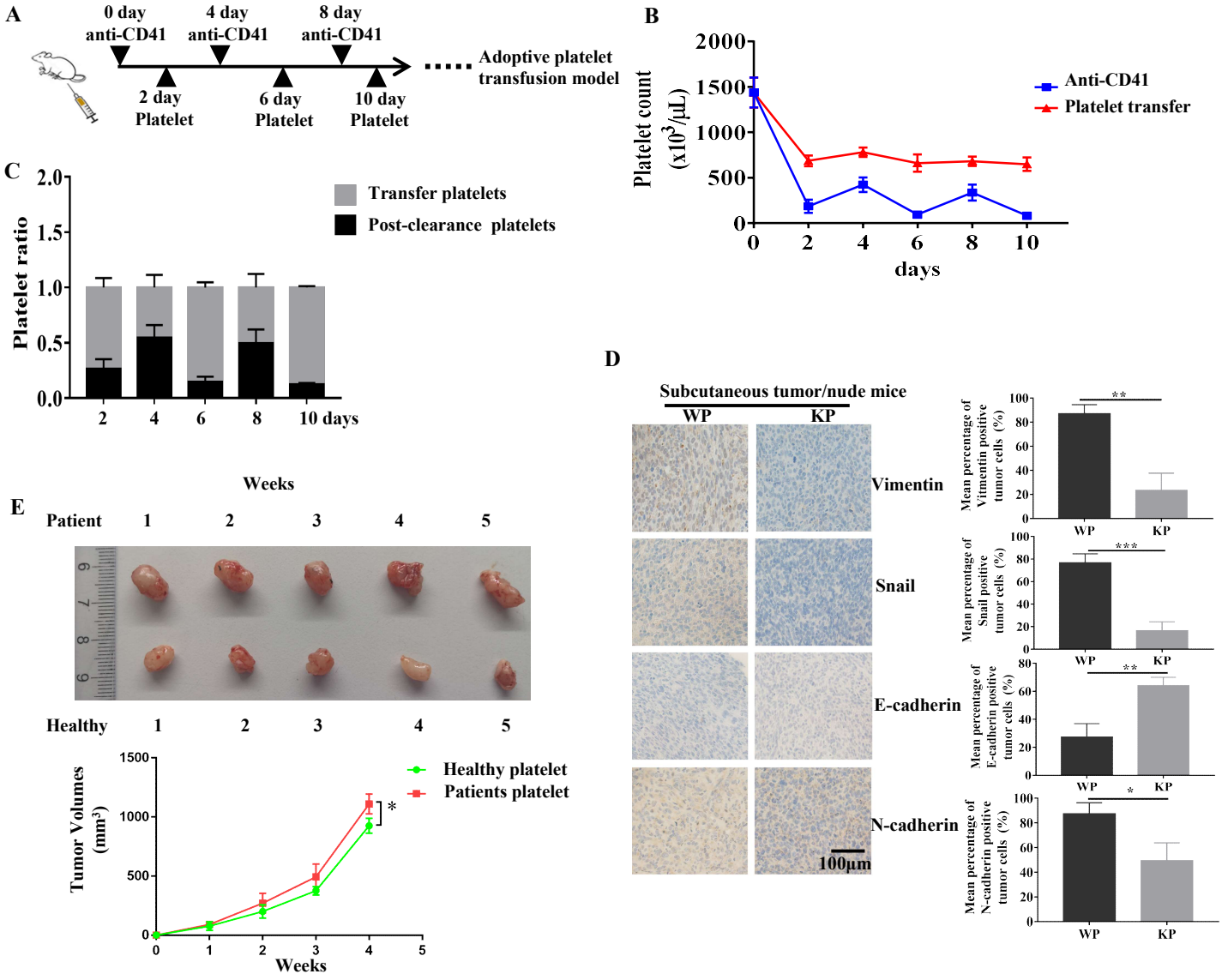
Supplementary Figure 1



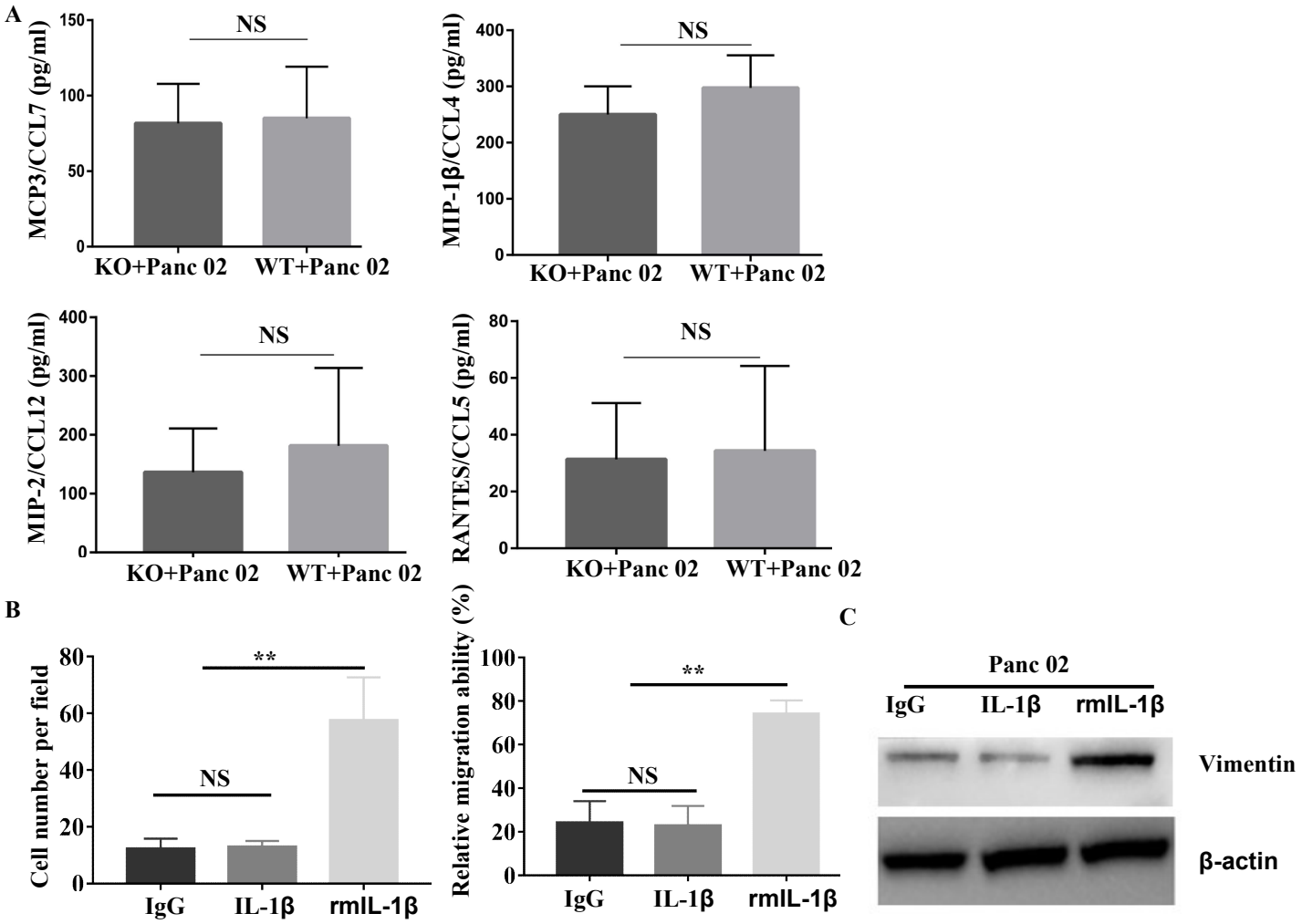
Supplementary Figure 2



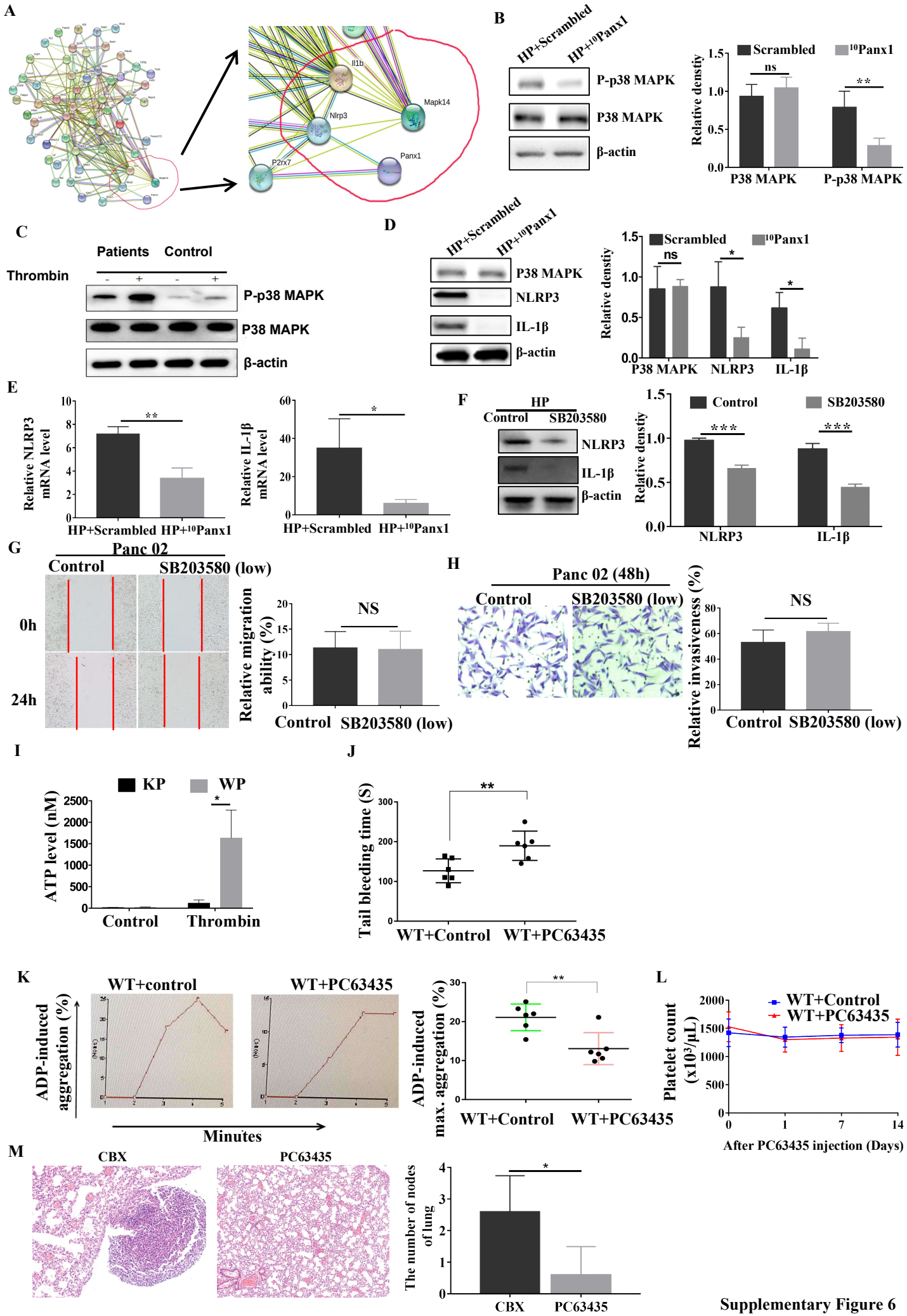
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6

Supplementary Table 1. Relationship between CD41+/CD62P+ platelet count and clinicopathological features of patients with PDAC (I/II and III/IV)

Features	CD41+/CD62P+ platelet (I/II)		P value	CD41+/CD62P+ platelet (III/IV)		P value
	High (n = 44)	Low (n = 45)		High (n = 52)	Low (n = 51)	
Age			0.741			0.323
≤62 years	19	21				
>62 years	25	24				
≤61 years				19	14	
>61 years				33	37	
Gender			0.603			0.481
Male	20	18		27	30	
Female	24	27		25	21	
Tumor location			0.232			0.280
Head	28	23		30	24	
Body/Tail	16	22		22	27	
Neural invasion			0.545			
Yes	31	29				
No	13	16				
Microvascular invasion			0.002			

Yes	31	17				
No	13	28				
Metastasis						**<0.001**
Yes				45	14	
No				7	37	
8th AJCC stage			**<0.001**			**<0.001**
I	11	29				
II	33	16				
III				12	32	
IV				40	19	
CA-199 (U/mL)			**0.027**			**0.380**
≤37	9	19		6	9	
>37	35	26		46	42	

Note： AJCC: American Joint Committee on Cancer; TNM: tumor node metastasis; CA19-9: carbohydrate antigen 19-9.

Supplementary Table 2. Multivariate Cox regression analyses of OS and RFS in PDAC patients

	OS		RFS	
Factors	**HR (95% CI)**	**P value**	**HR (95% CI)**	**P value**
Age	0.901	0.907	1.008	0.945
(>62 years/≤62 years)	(0.521-1.435)		(0.667-1.712)	
Gender (male/female)	1. 120	0.936	1.009	0.902
	(0.573-1.802)		(0.744-1.438)	
Tumor location	1.113	0.498	1.388	0.682
(head/body, tail)	(0.883-1.903)		(0.715-2.011)	
Neural invasion (yes/no)	1.311	0.084	1.003	0.649
	(0.535-2.163)		(0.331-1.775)	
Microvascular invasion (yes/no)	1.459	0.098	1.092	0.449
	(1.003-2.454)		(0.560-2.309)	
Preoperative CA19-9	2.956	0.003	1.503	**0.019**
(U/mL, >37/≤37)	(0.472-3.836)		(0.843-2.174)	
8th edition TNM stage (I/II)	2.250	<0.001	2.994	**<0.001**
	(1.693-3.942)		(1.029-3.544)	
CD41+CD62P+	2.180	<0.001	3.361	**<0.001**
(High/Low)	(1.733-4.705)		(1.963-5.975)	

Note: CI: confidence interval; HR: hazard ratio; OS: overall survival; RFS: recurrence-free survival; TNM: tumor node metastasis; CA19-9: carbohydrate antigen 19-9.