SUPPLEMENTARY MATERIALS AND METHODS

Electron microscopy

NETs are composed of extracellular DNA, neutrophil-derived granule proteins, and histones. NETs formation in paraffin-embedded pancreatic tissue samples was examined by high resolution scanning electron microscopy. Specimens on coverslips were deparaffinized and fixed in 2.5 % glutaraldehyde in 0.15 M sodium cacodylate, pH 7.4, (cacodylate buffer) for 30 minutes at room temperature. Specimens were washed with cacodylate buffer and dehydrated with an ascending ethanol series from 50% (v/v) to absolute ethanol (10 minutes per step). The specimens were subjected to critical-point drying in CO_2, with absolute ethanol as intermediate solvent, mounted on aluminum holders, and finally sputtered with 30 nm palladium/gold. A Jeol/ FEI XL 30 FEG scanning electron microscope at the Core Facility for Integrated Microscopy at Panum Institute, University of Copenhagen, Denmark were used to examine specimens. Location of individual target molecules in tissue NETs were analyzed at high resolution by ultra-thin sectioning and transmission immunoelectron microscopy. Specimens on coverslips were embedded in Epon 812 and sectioned into 50-nm-thick ultrathin sections with a diamond knife in an ultramicrotome. Sections were incubated overnight (4°C) with primary antibodies against elastase (ab68672, abcam, Cambridge, UK), histone H4 (ab109463, abcam, Cambridge, UK), Mac-1 (clone M1/70, BD Pharmingen, San Jose, USA) and CD41 (clone MWReg30, BD Pharmingen, San Jose, USA). Controls without primary antibodies were included. Grids were then incubated with species-specific gold-conjugated secondary antibodies (Electron Microscopy Sciences, Fort Washington, MD). Finally, the sections were postfixed in 2% glutaraldehyde and post-stained with 2% uranyl acetate and lead citrate. A Jeol/FEI CM100 transmission electron microscope operated at 80 kV accelerating voltage was used to examine the specimens.

Amylase measurements

Blood amylase levels were determined in blood collected from the tail vein by use of a commercially available assay (Reflotron®, Roche Diagnostics GmbH, Mannheim, Germany).

Flow cytometry

For the analysis of PNA and the percentage of platelets, blood was collected into syringes pre-filled with 1:10 acid citrate dextrose 24 h after taurocholate challenge. Immediately after collection, blood samples were incubated with an anti-CD16/CD32 antibody blocking Fcγ III/II receptors to reduce non-specific labelling for 10 minutes at room temperature and then incubated with phycoerythrin- conjugated anti-Ly6G (clone 1A8, BD Pharmingen, San Jose, USA) and fluorescein isothiocyanate-conjugated anti-CD41 (clone MWReg30, BD Pharmingen, San Jose, USA) to detect the percentage of neutrophil-platelet aggregates by considering neutrophils as cells positive for Ly6G and platelets as $CD41^+$ cells. Cells were fixed with 2% formaldehyde solution, erythrocytes were lysed using ACK lysing buffer (Thermo Fisher Scientific,Somerset , NJ, USA) and neutrophils were recovered following centrifugation. Flow-cytometric determination of neutrophil-platelet aggregates was performed by first gating all viable cells and then the percentage of neutrophils ($Ly6G^+$) binding platelets ($CD41^+$) was analyzed in this population. For comparison of leukocytes subtypes and platelets, blood was collected from wild-type and IP6K-/- mice and incubated with an anti-CD16/CD32 antibody as described above. Samples were then incubated with PerCP-Cy5.5-conjugated anti-CD45 (clone 30-F11, Biolegend, San Diego, CA) antibody, PE-conjugated anti-Ly6G (clone 1A8, BD Pharmingen, San Jose, CA) antibody, APC-conjugated anti-CD4 (clone GK 1.5, eBioscience, San Diego, CA) antibody and a FITC-conjugated anti-CD41 (clone eBioMWReg30, eBioscience, San Diego, CA) antibody at 4°C for 20 minutes. Cells were fixed with 2% formaldehyde, erythrocytes were lysed and cells were recovered following centrifugation. Flow-cytometric analysis was performed according to standard setting on a Cytoflex flow cytometer (Beckman Coulter, Indianapolis, IN), and viable gate was used to exclude dead and fragmented cells. Data was analyzed using CytExpert version 2.0 (Beckman Coulter).

MPO assay

A piece of the pancreatic head and lung tissue were snap-frozen in liquid nitrogen for biochemical quantification of MPO. Before analysis, tissues were pre-weighed and homogenized for 1 minute in a 1 ml mixture (4:1) of PBS and aprotinin containing 10 000 Kallikrein inhibitor units per ml (Trasylol®,

Bayer HealthCare AG, Leverkusen, Germany). The homogenates were centrifuged (15300 g, 10 minutes) and the supernatant was stored at −20°C for subsequent ELISA. Pellets were resuspended in 0.02 M PB pH 7.4, centrifuged and frozen overnight in 0.05 M PB, pH 6.0 containing 0.5% hexadecyltrimethylammonium bromide before MPO assay. Briefly, samples were thawed, sonicated for 90 seconds, incubated in a water bath at 60°C for 2 hours and centrifuged for 5 minutes at 15300 g after which the MPO activity of the supernatant was measured. The enzyme activity was determined spectrophotometrically as the MPO-catalyzed change in absorbance in the redox reaction of hydrogen peroxide (450 nm, with a reference filter 540 nm, 25°C). Values are expressed as MPO units/g tissue.

Histology

Tissue pieces from the head of the pancreas were fixed in 4% formaldehyde overnight, dehydrated and embedded in paraffin. Sections (6 µm) were stained with hematoxylin and eosin and examined by light microscopy. The severity of pancreatitis was evaluated in a blinded manner by use of a pre-existing scoring system, quantifying edema, acinar cell necrosis, hemorrhage and neutrophil infiltration on a 0 (absent) to 4 (extensive) scales as previously described (1).

ELISA

Pancreatic levels of CXCL1, CXCL2, histone 3 and histone 4 were determined in stored supernatants from homogenized pancreatic tissue by use of double-antibody ELISA kits (R&D Systems Europe, Abingdon, Oxon, UK and USCN, Life Science Inc., Burlington, NC, USA). Blood collected from the inferior vena cava was diluted (1:10) in acid citrate dextrose, centrifuged (15300 g for 10 minutes at 4°C) and stored at -20°C until use. Commercially available ELISA kits were used to quantify plasma levels of CXCL1, CXCL2 (R&D Systems Europe), IL-6 (R&D Systems Europe) and MMP-9 (R&D Systems Europe). Plasma levels and in vitro samples were centrifuged at 15 300 g for 5 minutes at 4°C and a Cell Death Detection Elisa Plus kit (Roche Diagnostics, Mannheim, Germany) was used to quantify DNA-histone complexes according to the manufacturer's instructions.

Pancreatic acinar cells

Pancreatic acinar cells were prepared by collagenase digestion as previously described (2). HEPES-ringer buffer containing collagenase from *Clostridium histolyticum* type 1 (2.5 ml, 1%, Sigma-Aldrich) was gently infused into the pancreatic duct of male C57BL/6 mice. The animals were sacrificed through cervical dislocation and pancreatic tissue was collected. In order to achieve maximal exposure to collagenase, the pancreas was cut into pieces, gently shaken and incubated at 37°C for 15 minutes. The solute was then centrifuged and washed three times in cold HEPES-Ringer buffer, pH 7.4 to stop digestion and remove the collagenase. Next, the acinar cells were suspended in cold HEPES-Ringer buffer and the solute was passed through a 150 μm cell strainer (Partec, Canterbury, England). Trypan blue was used to check the viability of pancreatic acinar cells which was higher than 95%. The cell suspension was then divided in Eppendorf tubes and kept on ice until subsequent in vitro experiments. Isolated acinar cells were exposed to NETs, NETs depleted of MPs (calpain/caspase treatment), DNase I-treated NETs and neutrophil-derived MPs for 1 hour at 37°C. The cell suspension was centrifuged (1400 g, 5 minutes) and the supernatant was used for determination of amylase secretion assay. The cell pellets were homogenized and used for western blot of STAT-3 phosphorylation and quantitative RT-PCR, as described below.

Western blot

After incubation with NETs, NETs depleted of MPs (calpain/caspase treatment), DNase I-treated NETs and neutrophil-derived MPs, acinar cell pellets were collected and homogenized in ice-cold RIPA buffer (RIPA Lysis and Extraction Buffer; Thermo Fisher Scientific, Rockford, IL, USA) containing protease inhibitors (Halt Protease Inhibitor Cocktail; Pierce Biotechnology, Rockford, IL) for 20 minutes and then sonicated and centrifuged (16000 g for 5 minutes, 4°C). Supernatants were collected and stored at -20°C. Protein concentration of supernatants were determined by use of Pierce BCA Protein Assay Reagent (Pierce Biotechnology). Proteins (20 μg per lane) were separated by 8-16% Mini-PROTEAN® TGX

Stain-Free™ Gels (Bio-Rad) and transferred to polyvinylidene fluoride membranes (Novex, San Diego, CA, USA). Before blotting, total protein gel image was taken using Bio-Rad's stain-free gel chemistry. Membranes were blocked in TBS/Tween 20 buffer containing 5% non-fat dry milk powder. Protein immunoblots were performed using antibodies to phosphotyrosin (Tyr 705) stat3 and stat3 (Cat. #9131 and Cat. #4904, Cell Signaling Technology, Beverly, MA). Membranes were then incubated with peroxidase conjugated secondary antibodies. Protein bands were developed and analyzed using the BioRad ChemiDoc™ MP imaging system and Image Lab™ software version 5.2.1. In separated experiment, L-arginine was used to trigger AP and pancreatic samples were collected and homogenized as described above. Protein immunoblots were performed using antibodies to histone H3 (citrulline 2,8,17, ab5103, Abcam, Cambaridge, MA, USA). Membranes were then incubated with peroxidase conjugated secondary antibodies. The Image Lab™ software (version 5.2.1) was used to normalize the band signal against the total protein in the respective lane.

qRT-PCR

Acinar cells were incubated with NETs, NETs depleted of MPs (calpain/caspase treatment), DNase I-treated NETs and neutrophil-derived MPs, then cell pellets were collected and expression of IL-6 mRNA and TGFB1 mRNA was analyzed by RT-qPCR. Briefly, RNA samples were extracted using Direct-zol RNA extraction kit (Zymo Research, Irvine, CA, USA) kit according to manufacturer's recommendations. Total RNA concentration was determined using Nanodrop spectrophotometer at 260 nm absorbance and cDNA was synthesized using RevertAid First Strand cDNA synthesis kit (Thermo Fisher Scientific) in a final reaction volume of 10 µL according to the manufacturer's instructions. qRT-PCR was conducted in a final volume of 25 µL using SYBR Green dye (Takara Bio, USA) for relative expression of IL-6 mRNA and TGFB1 mRNA. The PCR primers used were as follows; mouse IL-6 mRNA sense; 5'-GAGGATACCACTCCCAACAGACC-3', antisense; 5'-AAGTGCATCATCGTTGTTCATACA-3' ; and mouse TGFB1 mRNA sense; 5′-TCCCGTGGCTTCTAGTGCTG-3′, antisense; 5′-ATTTTAATCTCTGCAAGCGCA-3′, and GAPDH

sense; 5′-GTCCCAGCTTAGGTTCATAG-3′, GAPDH antisense; 5′-GATGGCAACAATCTCCACTTTG-3′. Expression of IL-6 mRNA and TGFB1 mRNA relative to house-keeping gene GADPH were determined using 2- ΔΔ CT method.

Platelet-neutrophil interactions

Bone marrow neutrophils were freshly isolated from wild-type mice by density gradient centrifugation using a Ficoll-Paque gradient (GE Healthcare, Uppsala, Sweden). Blood platelets were also isolated from wild type and IP6K1-/- mice. Briefly, blood was collected from the inferior vena cava with 1:10 acid citrate dextrose anticoagulant and immediately diluted with equal volumes of modified Tyrode solution (1 μg/ml prostaglandin E1 and 0.1 U/ml apyrase), centrifuged at 200 *g* for 5 minutes. The resulting platelet-rich plasma (PRP) was collected and centrifuged at 800*g* for 15 minutes. Platelet pellets were washed twice with modified Tyrode solution and immediately used for the experiment. For immunofluorescence imaging, isolated neutrophils were co-incubated with isolated wild-type or IP6K1-/- platelets with or without thrombin (0.2 U/ml, Sigma, USA) and with or without polyP (10 and 100 μM, Kerafast, Boston, USA) over glass coverslips inside a 6-well plate for 3 hours at 37 °C. In separate experiments, wild-type and IP6K1-deficient neutrophils were stimulated with 50 nM PMA (Sigma-Aldrich, Stockholm, Sweden) for 3 h at 37°C in RPMI 1640. After stimulation, neutrophils were fixed with 2% formaldehyde and then permeabilized with 1% Triton X-100 for 10 minutes. After washing two times with PBS containing 2% fetal bovine serum, cells were incubated with primary antibodies: Fluorescein isothiocyanate (FITC) conjugated anti-MPO antibody (mouse: ab90812) and rabbit anti-H3cit (citrulline 2,8,17, ab5103; Abcam, Cambridge, MA) in PBS containing 5% donkey serum. After washing two times, cells were incubated with rat anti-rabbit allophycocyanin (APC) conjugated secondary antibody (A-21038, Thermo Scientific, Rockford, IL). After immunostaining, cells were counterstained with Hoechst 33342 and then coverslips were rinsed and mounted in fluoromount (Thermo Fisher Scientific). Confocal microscopy was performed using Zeiss LSM 800 (Carl Zeiss, Jena, Germany) by a × 63 oil immersion objective (numeric aperture = 1.25). The pinhole was ~ 1 airy unit and the scanning

frame was 1024×1024 pixels. Images were later processed using ZEN2012 software. In separate experiment, DNA-histone complex was measured by Cell Death Detection Elisa Plus kit (Roche Diagnostics, Mannheim, Germany). Briefly, platelets and neutrophils were co-incubated as described above in a volume of 200 µl, samples were centrifuged at 500 g for 5 min, and then supernatants were collected for determination of DNA-histone complex formation.

Flow cytometry of MPO and citrullinated histone 3 in neutrophils

For detection of NETs by flow cytometry, neutrophils were fixed with 2% formaldehyde immediately after stimulation. After washing two times with PBS containing 2% fetal bovine serum, cells were incubated with primary antibodies: Phycoerythrin (PE) conjugated anti-Ly6G (clone 1A8, BD Pharmingen), Fluorescein isothiocyanate (FITC) conjugated anti-MPO antibody (mouse: ab90812) and rabbit anti-H3cit (citrulline 2,8,17, ab5103; Abcam, Cambridge, MA) in PBS containing 5% donkey serum. After washing two times, cells were incubated with rat anti-rabbit allophycocyanin (APC) conjugated secondary antibody (A-21038, Thermo Scientific, Rockford, IL). Flow cytometry analysis was performed according to standard setting on a CytoFLEX flow cytometer (Becton Dickinson, Mountain View, CA, USA), and viable gate was used to exclude dead and fragmented cells.

Confocal imaging of platelet-polyphosphate

Blood platelets were isolated from wild-type and IP6K1-/- mice as described above. Isolated platelets were fixed with 4% paraformaldehyde in PBS for 30 minutes. Fixed platelets were then washed with PBS. Platelet pellets were dissolved in water and 1 mg/ml DAPI was added. Samples were then mounted on the slides and confocal images were taken using Zeiss LSM 800 (Carl Zeiss, Jena, Germany) by a × 63 oil immersion objective (numeric aperture = 1.25). The fluorescence emission of DAPI shifts to higher wavelengths when binds with polyP (3, 4). Samples were excited at 405 nm and emission was observed at 530-570 nm. Images were processed using ZEN2012 software.

SUPPLEMENTARY REFERENCES

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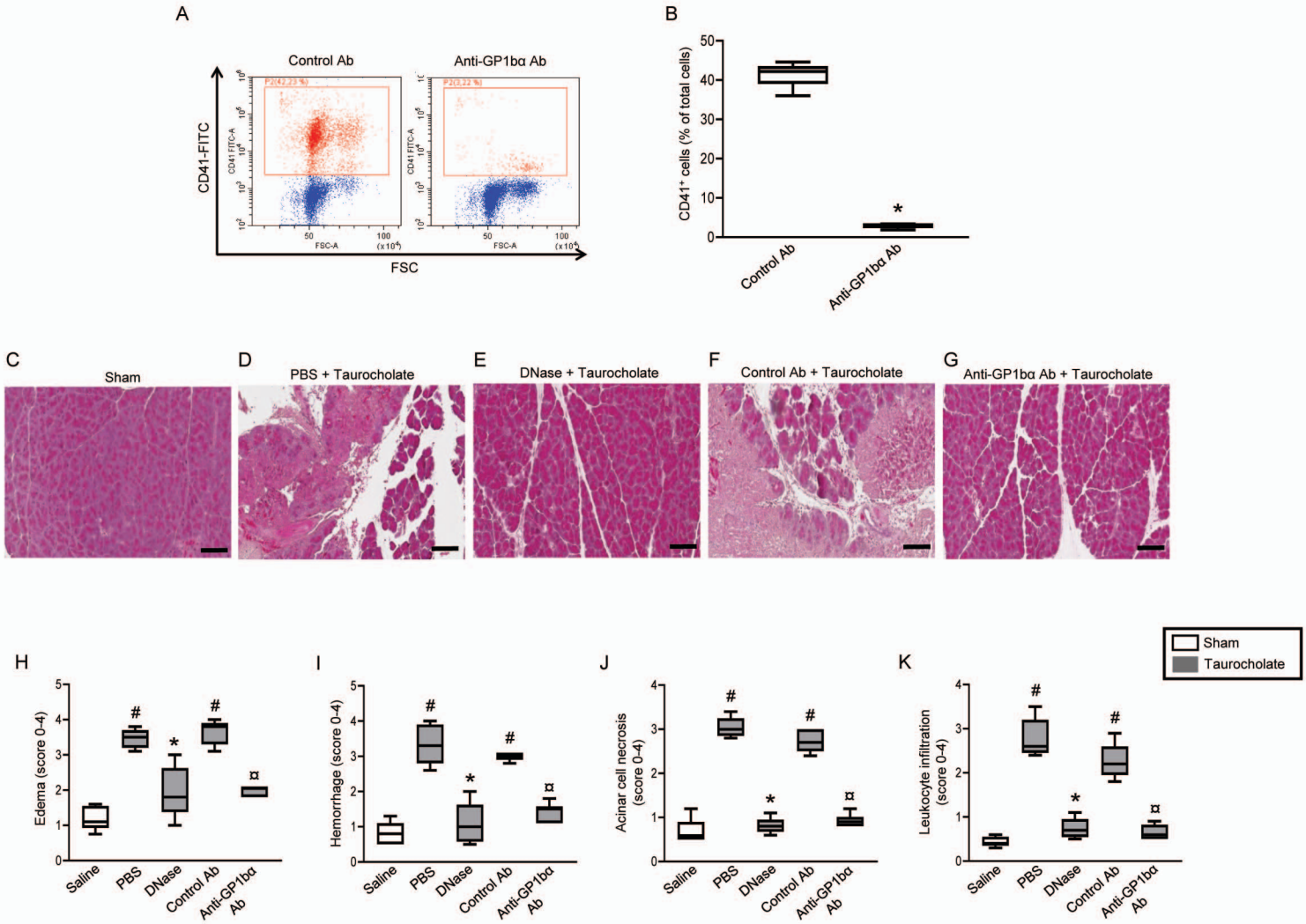
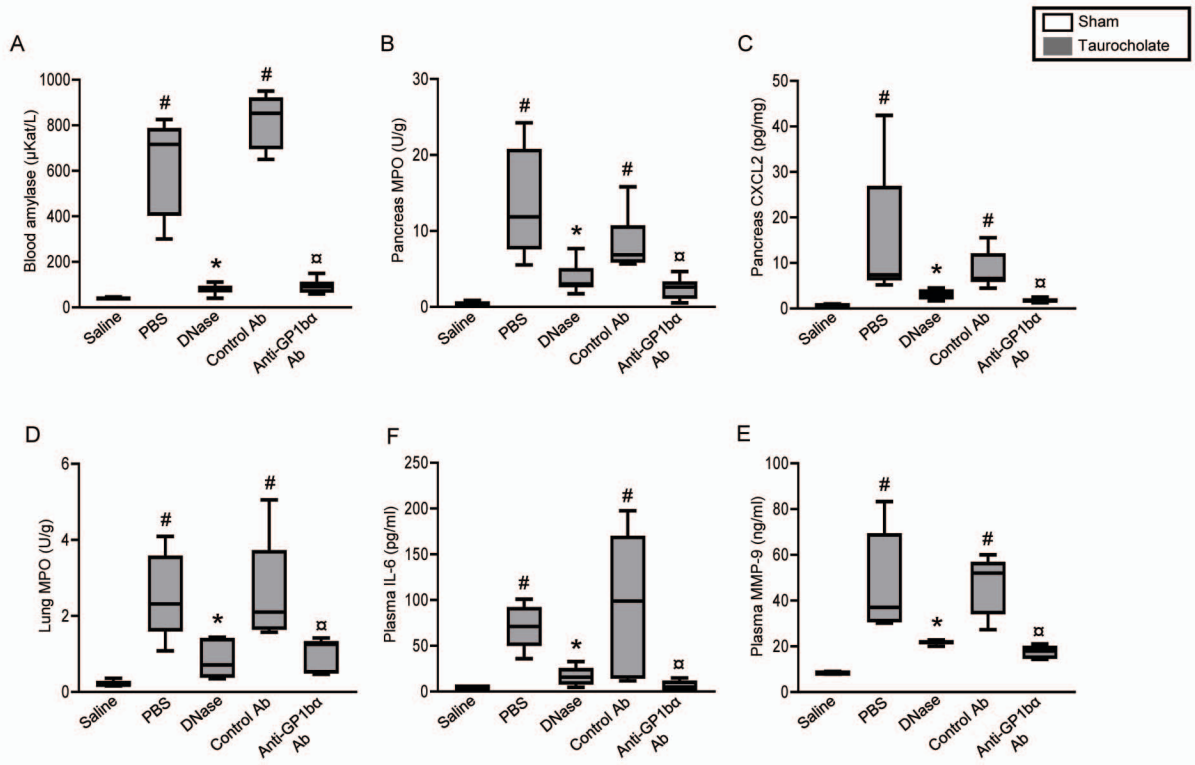
2. Perides G, Laukkarinen JM, Vassileva G, et al. Biliary acute pancreatitis in mice is mediated by the G-protein-coupled cell surface bile acid receptor Gpbar1. Gastroenterology 2010;138:715-25.
3. Jimenez-Nunez MD, Moreno-Sanchez D, Hernandez-Ruiz L, et al. Myeloma cells contain high levels of inorganic polyphosphate which is associated with nucleolar transcription. Haematologica 2012;97:1264-71.
4. Aschar-Sobbi R, Abramov AY, Diao C, et al. High sensitivity, quantitative measurements of polyphosphate using a new DAPI-based approach. J Fluoresc 2008;18:859-66.

Supplementary Figure 1



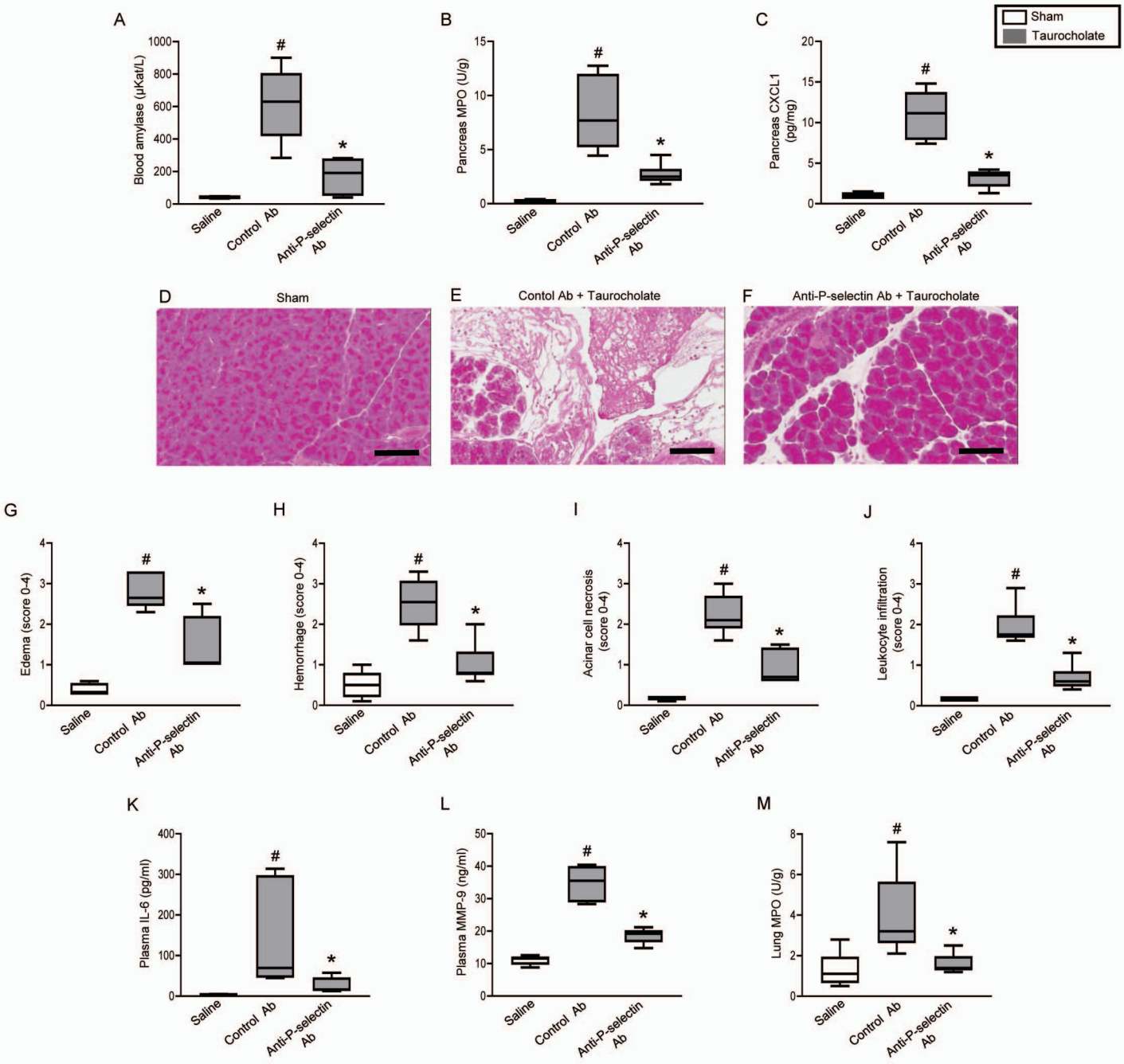
Supplementary Figure 1. Platelet regulates tissue damage in AP. Mice were pretreated with a control antibody (Ctrl Ab) and a platelet-depleting Ab against GP1BA (Anti-GP1b alpha). A) Dot plots showing $CD41^+$ platelets and B) aggregate data of CD41+ cells. C-G) Representative hematoxylin & eosin sections of the head of the pancreas from indicated groups. Scale bar = 100 µm. Histological quantification of H) edema, I) hemorrhage, J) acinar cell necrosis and K) leukocyte infiltration. Pancreatitis (grey boxes) was induced by infusion of sodium taurocholate (5%) into the pancreatic duct. Sham mice (white boxes) were infused with saline alone. Animals were treated with i.p. injections of the DNase I, a control antibody (Control Ab) or an antibody directed against GP1BA (anti-GP1b alpha Ab) or vehicle (PBS) as described in Materials and Methods. Samples were collected 24 hours after induction of pancreatitis. Data represent median (25-75 percentile); whiskers extend from the minimum to the maximum values and $n = 4$-6. $^{\#}P < 0.05$ versus sham mice, $^{*}P < 0.05$ versus PBS+taurocholate and $^{¤}P < 0.05$ versus Control Ab+taurocholate (Mann-Whitney test).

Supplementary Figure 2



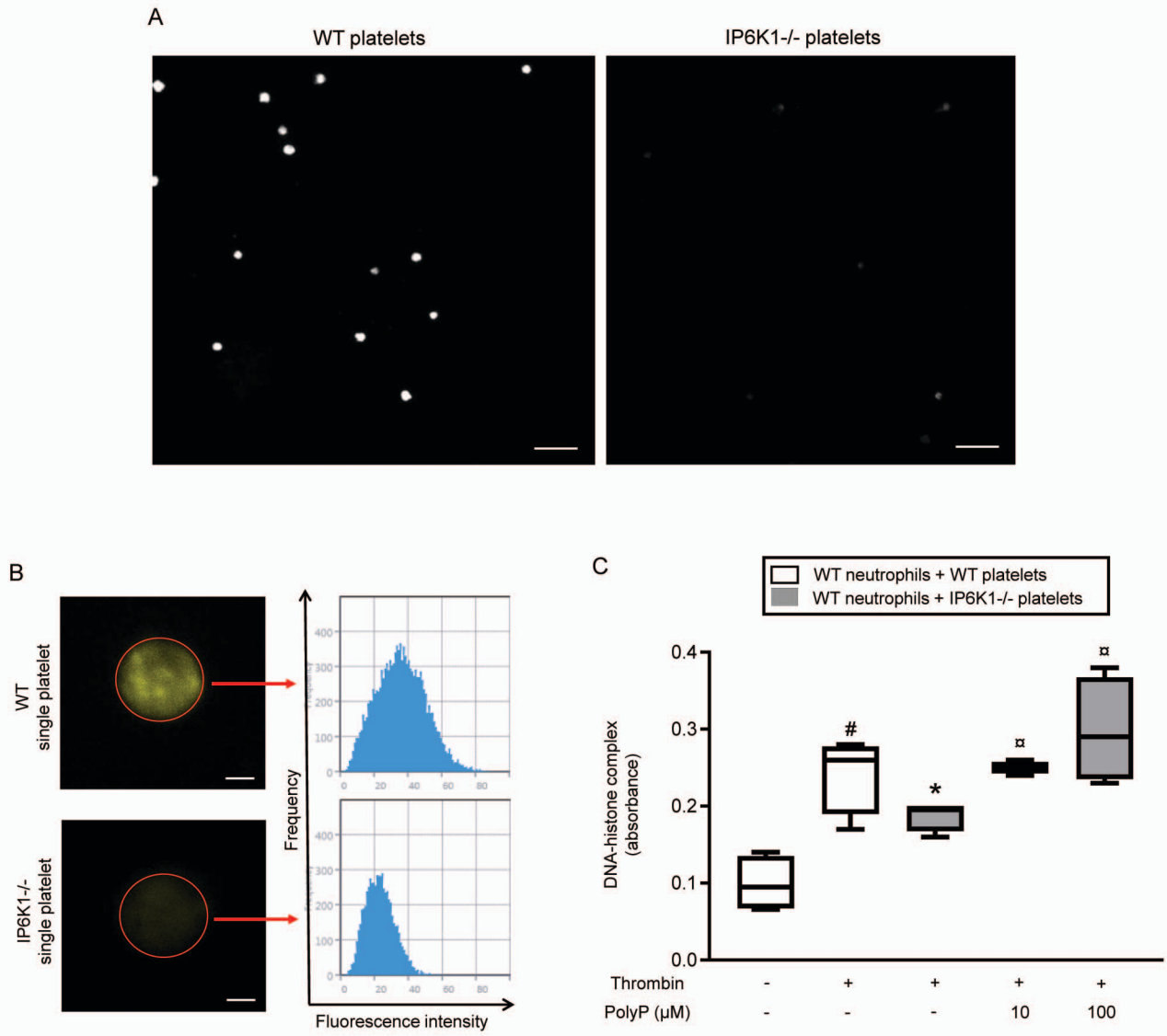
Supplementary Figure 2. Platelet-dependent inflammation and tissue damage in AP. Quantitative measurements of A) blood amylase levels, pancreatic B) MPO and C) CXCL2 levels. D) Lung levels of MPO, plasma levels of E) IL-6 and F) MMP-9. Pancreatitis (grey boxes) was induced by infusion of sodium taurocholate (5%) into the pancreatic duct. Sham mice (white boxes) were infused with saline alone. Animals were treated with i.p. injections of the DNase I, a control antibody (Control Ab) an antibody directed against GP1BA (anti-GP1b alpha Ab) or vehicle (PBS) as described in Materials and Methods. Samples were collected 24 hours after induction of pancreatitis. Data represent median (25-75 percentile); whiskers extend from the minimum to the maximum values and $n = 5$-6. $^{\#}P < 0.05$ versus sham mice, $^{*}P < 0.05$ versus PBS+taurocholate and $^{¤}P < 0.05$ versus Control Ab+taurocholate (Mann-Whitney test).

Supplementary Figure 3



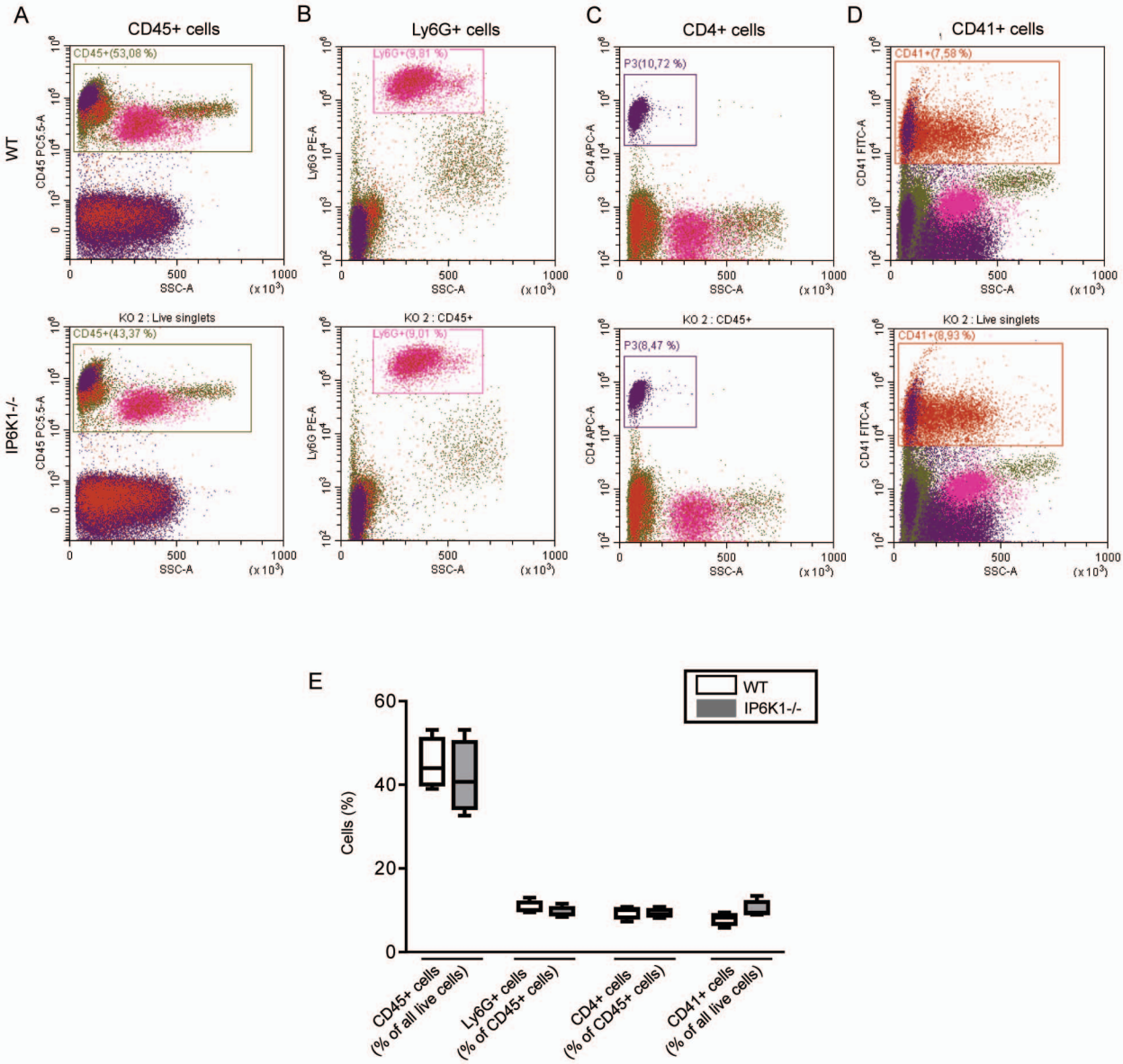
Supplementary Figure 3. Inhibition of NPA formation reduces inflammation and tissue damage in AP. Quantitative measurements of A) blood amylase levels, pancreatic B) MPO and C) CXCL1 levels. D-F) Representative hematoxylin & eosin sections of the head of the pancreas from indicated groups. Scale bar = 100 µm. Histological quantification of G) edema, H) hemorrhage, I) acinar cell necrosis and J) leukocyte infiltration. Plasma levels of K) IL-6 and L) MMP-9 as well as M) lung levels of MPO. Pancreatitis (grey boxes) was induced by infusion of sodium taurocholate (5%) into the pancreatic duct. Sham mice (white boxes) were infused with saline alone. Animals were treated with i.v. injections of a control or an anti-P-selectin antibody (Ab) as described in Materials and Methods. Samples were collected 24 hours after induction of pancreatitis. Data represent median (25-75 percentile); whiskers extend from the minimum to the maximum values and n = 5-6. $^{\#}P < 0.05$ versus sham mice and $^{*}P < 0.05$ versus Control Ab+taurocholate (Mann-Whitney test).

Supplementary Figure 4



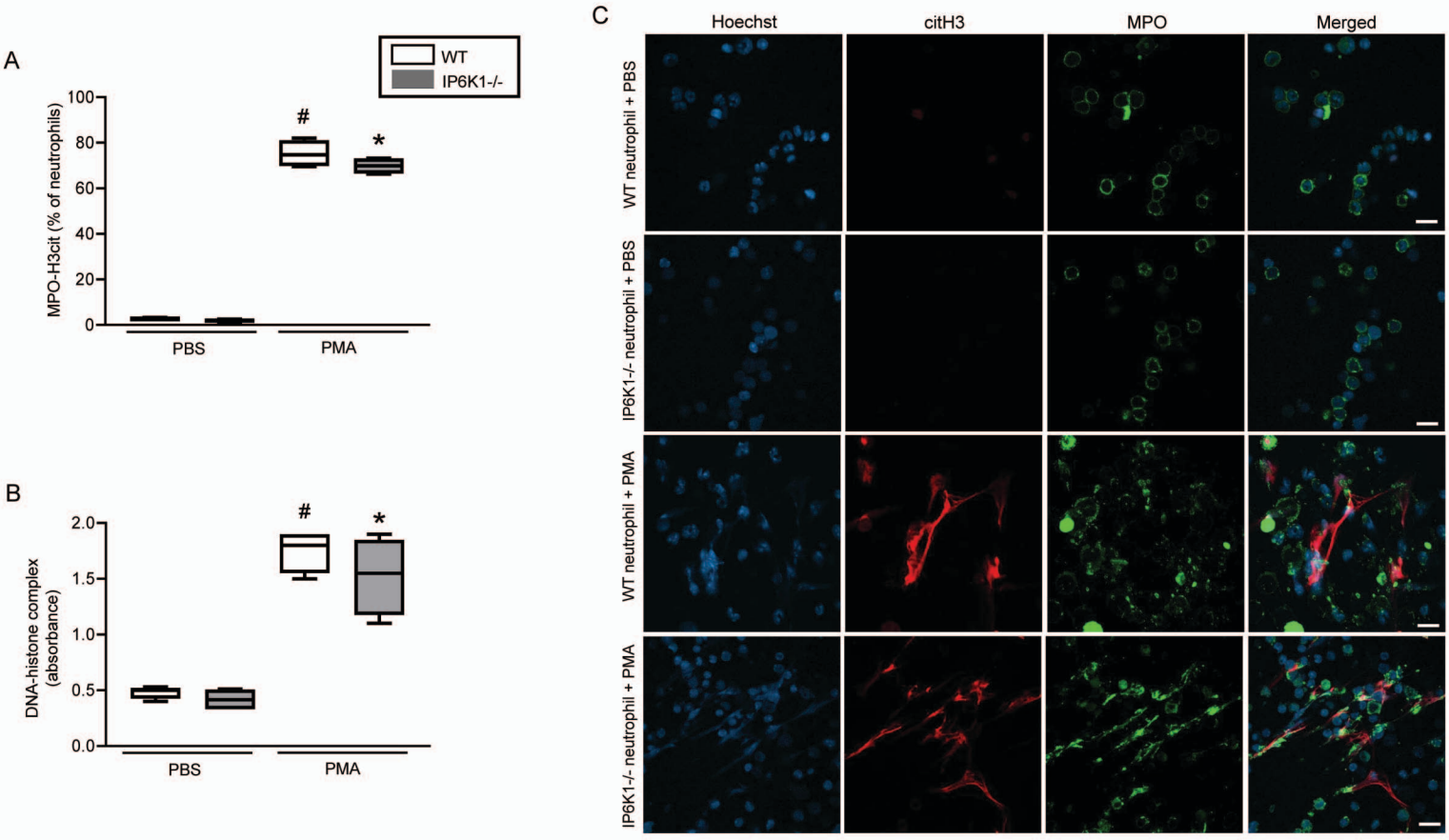
Supplementary Figure 4. Platelet IP6K1 regulates DNA-histone complex formation in vitro. A) Confocal fluorescence microscopy of platelets showing cellular PolyP after staining with 4′,6-diamidino-2-phenylindole (DAPI). Scale bars = 10 μm. DAPI emits higher wavelengths light after binding with PolyP. B) Higher magnification imaging of single platelets and corresponding histograms showing DAPI fluorescence intensity. Scale bars = 0.2 μm. C) Isolated neutrophils were co-incubated with isolated wild-type or IP6K1-/- platelets with or without thrombin (0.2 U/ml) and with or without PolyP (10 or 100 μM) for 3 hours at 37°C. DNA-histone complexes in the supernatant were measured by ELISA. Neutrophils with non-stimulated wild-type platelets served as a control. Data represent median (25-75 percentile); whiskers extend from the minimum to the maximum values and $n = 4$. $^{\#}P < 0.05$ versus WT neutrophils + WT platelets, $^{*}P < 0.05$ versus WT neutrophils + WT platelets + thrombin and $^{¤}P < 0.05$ WT neutrophils + IP6K1-/- platelets + thrombin (ANOVA on ranks followed by Dunnett's multiple comparisons test).

Supplementary Figure 5



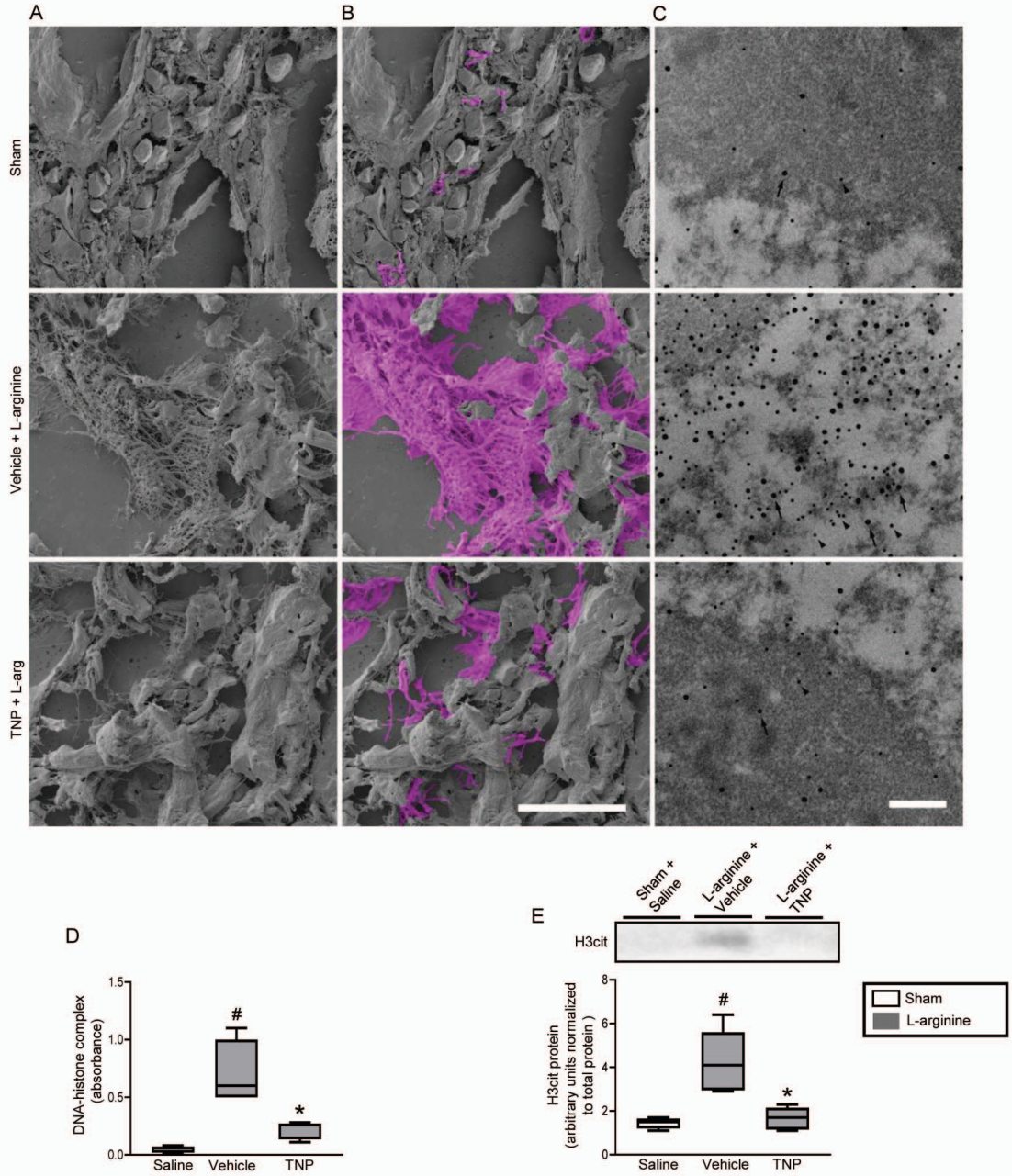
Supplementary Figure 5. Comparison of wild-type and IP6K1-/- leukocyte subtypes and platelets using flow cytometry. Representative dot plots of A) CD45+ cells, B) Ly6G+ cells, C) CD4+ cells and D) CD41+ cells. E) Aggregate data showing wild type and IP6K-/- leukocyte subtypes and platelets relative percentage. Data represent median (25-75 percentile); whiskers extend from the minimum to the maximum values and $n = 4$. No significant difference observed.

Supplementary Figure 6



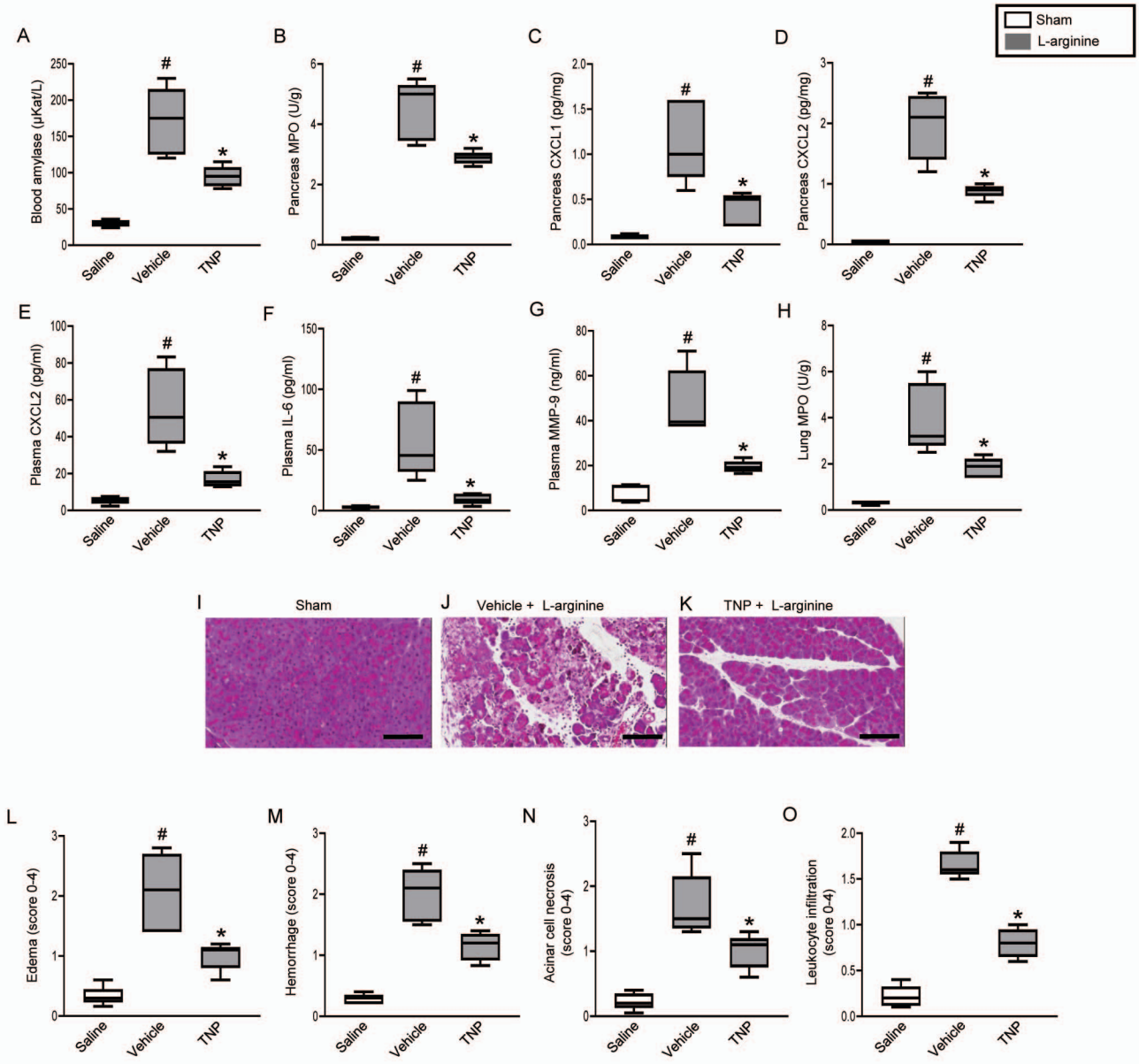
Supplementary Figure 6. PMA-induced NET formation. NETs were generated from isolated wild-type and IP6K1-/- neutrophils by stimulation with PMA (50 nM, 3h). Non-stimulated neutrophils served as a control. A) Levels of citrullinated histone 3 and MPO in isolated neutrophils was detected by FACS and B) DNA-histone complexes in the supernatant was determined by ELISA as described in Materials and Methods. Data represent median (25-75 percentile); whiskers extend from the minimum to the maximum values and n = 4. C) Neutrophils were immune-stained with antibodies to citrullinated histone 3 (H3Cit), myeloperoxidase (MPO), and Hoechst for nuclear staining. Images were taken using LSM 800 confocal microscope. One representative experiment of five independent experiments. Scale bars = 10 µm. $^{\#}P < 0.05$ versus PBS+WT and $^{*}P < 0.05$ versus PBS+ IP6K-/- (Mann-Whitney test).

Supplementary Figure 7



Supplementary Figure 7. TNP inhibits NET formation in L-arginine-induced AP. A) Scanning electron microscopy exhibiting extracellular web-like structures in the inflamed pancreas. Scale bar = 25 μm. B) NETs denoted in pink color. C) Transmission electron microscopy of area of interest from Figure 1A incubated with gold-labeled anti-histone 4 (large gold particles, arrows) and anti-elastase (small gold particles, arrowheads) antibodies. Scale bar = 0.25 μm. D) Plasma levels of DNA-histone complexes. E) Western blot of pancreatic citrullinated histone 3 and aggregate data showing H3Cit protein normalized with stain-free total protein load. AP was induced by administration of 4 g/kg/dose of L-arginine i.p. twice at an interval of one h. Mice were treated with i.p. injections of vehicle or TNP as described in Materials and Methods before the first dose of L-arginine (grey boxes). Sham mice (white boxes) were infused with saline alone. Samples were collected 72 hours after induction of pancreatitis. Data represent median (25-75 percentile); whiskers extend from the minimum to the maximum values and $n = 5$. $^{\#}P < 0.05$ versus Saline+Sham and $^{*}P < 0.05$ versus Vehicle+L-arginine (Mann-Whitney test).

Supplementary Figure 8



Supplementary Figure 8. TNP inhibits inflammation and tissue damage in L-arginine-induced AP. Quantitative measurements of A) blood amylase levels, pancreatic B) MPO, C) CXCL1 and D) CXCL2 levels. Plasma levels of E) CXCL2, F) IL-6 and G) MMP-9 as well as H) lung levels of MPO. I-K) Representative haematoxylin & eosin sections of the head of the pancreas from indicated groups. Scale bar = 100 μm. Histological quantification of L) edema, M) haemorrhage, N) acinar cell necrosis and O) leukocyte infiltration. AP was induced by administration of 4 g/kg/dose of L-arginine i.p. twice at an interval of one h. Mice were treated with i.p. injections of vehicle or TNP as described in Materials and Methods before the first dose of L-arginine (grey boxes). Sham mice (white boxes) were infused with saline alone. Samples were collected 72 hours after induction of pancreatitis. Data represent median (25-75 percentile); whiskers extend from the minimum to the maximum values and $n = 5$. $^{\#}P < 0.05$ versus Saline+Sham and $^{*}P < 0.05$ versus Vehicle+L-arginine (Mann-Whitney test).