SUPPLEMENTARY DATA:

SUPPLEMENTARY METHODS:

Storage and processing of human samples: After collection, all samples were promptly transported to the laboratory or shipped overnight on ice to avoid repeated freeze thaws and consequent protein denaturation. Samples were spun at 300g for 5 minutes; the supernatant was sonicated, aliquoted and analyzed immediately, or frozen at -80˚C for subsequent analyses after a single thaw on ice. This reporting fulfills guidelines set by the Biospecimen Reporting for Improved Study Quality (BRISQ) criteria.[W1]

DNA quantification: The total amount of DNA was measured spectrophotometrically by ultraviolet absorbance at 260nm[W2] using an Eppendorf BioPhotometer (Hamburg, Germany), where one unit of optical density at 260nm equaled 50µg/ml. DNA concentration was reported in µg/ml units.

LDH release and Propidium Iodide uptake: These were done using a colorimetric assay as described previously[W3, 4] and expressed as a percentage of total.

Cytokine Assays: These were done as previously described[W3, 4] with the fluorescence-based capture sandwich immunoassay based on the Luminex FlowMetrix system (Luminex). The MILLIPLEX MAP Human Adipocyte Magnetic Bead Panel from Millipore was used for human fluids, MILLIPLEX MAP Rat Cytokine-Chemokine Magnetic beads Panel from Millipore was used for the rat serum samples. A Bio-Plex suspension array system including a fluorescent reader and Bio-Plex Manager analytical software (Bio-Rad Laboratories) was used to analyze samples at the University of Pittsburgh Cancer Institute.

Evaluation of Pancreatic Necrosis and special stains: Whole pancreas section slides stained by hematoxylin & eosin were examined by a trained pathologist blinded to the sample as described previously[W3, 5]. Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was done on paraffin sections of the lungs and kidneys as described previously[W3].

Broncho-alveolar lavage (BAL): FITC leakage, LDH leakage, Propidium Iodide positive cells, and protein content were analyzed in BAL fluid. Rodents were injected with 200 µl (5mg/ml) of fluorescein isothiocyanate-dextran (FD4; Sigma-Aldrich) by tail vein injection and sacrificed an hour later as

previously described[W6]. Briefly, trachea was exposed and intubated with a catheter, lavaged 3 times with HEPES buffer (pH 7.4). PI positive cells were measured and BAL fluid was centrifuged at 5000g for 5 minutes. The supernatant was used to measure FITC leakage, LDH leakage and protein amount.

Lipase activity in Ascitic fluid: Rodents were sacrificed 3 hours after the induction of Caerulein pancreatitis alone or with IL1β+KC-GRO, triolein or Triolein+Orlistat, (as described in the animal work sub-section in methods), and ascitic fluid was harvested from the peritoneal cavity. Lipase levels were measured in this following the manufacturer's (Pointe Scientific Inc, Canton, MI) instructions.

Immunohistochemical Studies: The horseradish-peroxidase immunohistochemical technique was used to detect Myeloperoxidase (MPO) in paraffin-embedded sections of pancreatic and lung tissue and Kidney Injury Molecule-1 (KIM-1) in paraffin-embedded kidney tissue. In brief, after deparaffinization and antigen epitope retrieval, tissues were incubated with a rabbit polyclonal antibody against MPO (1:50, Abcam, Cambridge, MA) or KIM-1 (15 µg/ml, R&D systems, Minneapolis, MN), followed by application of horseradish peroxidase conjugated (1:1000, Millipore Corp, Billercia, MA) secondary antibody. Staining was completed with chromogen incubation with AEC substrate kit for Peroxidase and Hematoxylin QS nuclear counterstain (Vector Laboratories, Burlingame, CA). Quantification of MPO positivity was done as MPO positive cells/HPF in the pancreas or percentage of AEC stained nuclear area to total nuclear area in the lung tissue, calculated by color thresh holding on Adobe Photoshop CS4.

Isolation of Peripheral blood mononuclear cells (PMBC) from rodent blood: This procedure was adapted from a method first described by Boyum et al [W7] using a customized density barrier medium made from commercially available density media Optiprep™. Whole rodent blood was collected by cardiac puncture and quickly mixed with an equal volume of isotonic buffer containing 4mM EDTA. Erythrocytes were removed by aggregation and sedimentation using 2% methyl cellulose. The resulting leukocyte rich plasma was layered over the customized density barrier medium and centrifuged at 700g for 20 minutes to separate PBMCs with some residual erythrocytes, which were lysed in isotonic ammonium chloride solution. PMBCs were washed in an iso-osmotic buffer and resuspended in appropriate media for downstream applications.

Flow Cytometry of Rodent PMBCs: Rodent PMBCs were incubated for 30 minutes at 37°C in serum free RPMI 1640 media containing 25mM HEPES either alone, with 10μM oleic acid or with 200ng each of the cytokines IL-1β and KC/GRO together. Cytotoxicity was evaluated by labelling cells with annexin V and propidium iodide following manufacturer's instructions for the FITC-Annexin V Apoptosis detection kit (BD Pharmigen™). Flow cytometry of labeled cells was carried out on the BD LSR Fortessa Analyzer running the BD FACSDiva Software. Acquisition of fluorescence signals was carried out after adjusting for forward and side scatter voltages, selecting appropriate fluorochrome channels and a minimum of 50,000 gated events per experimental condition were analyzed.

Non Esterified Fatty Acid Analysis: Long chain fatty acid analysis was done using gas chromatography as described previously[W3, 4]. UFA amounts were calculated by adding individual C16:1, C18:1.C18:2 and C20:4 fatty acids. Saturated fatty acids (SFA) amounts were calculated by adding individual C12:0, C14:0, C16:0 and C18:0 fatty acids.

Biochemical Assays: Amylases, Lipase, Blood Urea Nitrogen (BUN), Calcium, and Glycerol were measured following the manufacturer's (Pointe Scientific Inc, Canton, MI) instructions. Tests were carried out and analyzed using the Chemwell-T chemistry analyser (GMI Inc., Ramsey, MN).

Statistical Analysis: All values unless otherwise specified are reported as means ± standard error of mean (SEM). Data were entered manually and statistically assessed using the IBM SPSS version 21.0 (SPSS Inc., Chicago, IL) and JMP version 10.0 (SAS Institute, Cary, NC). Normality of data sets was determined using SPSS Explore and Descriptive functions. Histograms and box plots were used to evaluate variable distributions and assess for outliers. Frequency distributions were evaluated for all categorical variables. Analysis of covariance tests were performed to evaluate group differences in demographic variables such as age and BMI between groups followed by Tukey's HSD post-hoc tests. Tests for proportionality between groups were made using the Chi–square test. Most continuous outcome variables were positively skewed due to a few high values. We, therefore, compared between the groups using nonparametric Kruskal-Wallis one-way analysis of variance (ANOVA) based on ranks and multiple comparisons were carried out using the Dunn's method. Subsequently pairwise comparisons between

two groups were made using Mann Whitney-U tests. In secondary analyses, skewed measures were transformed (base -10 log + 1) and analysis of covariance (ANCOVA) was completed controlling for the influence of age, gender, and BMI. Significant omnibus F-tests from the ANCOVAs were further evaluated using Tukey's HSD (Tukey-Kramer method) post hoc tests. Significantly different groups have little or no overlap between the comparison-circles derived from Tukey Kramer post-hoc analysis. For the animal experiments, All groups/ subgroups were compared using the multivariate analysis by ANOVA.

SUPPLEMENTARY FIGURE LEGENDS:

Figure 1: Levels of cytokines IL-6, MCP1, NGF and HGF are similar among NCs, PCs and PCNs. Box plots showing levels of (A) IL-6, (B) MCP-1, (C) NGF and (D) HGF on a log scale to be similar in necrotic collections (NC), pseudocysts (PC) and pancreatic cystic neoplasms (PCN).

Figure 2: Inflammatory collections including NCs and PCs are associated with increased cell death and are enriched in unsaturated fatty acids and inflammatory mediators compared to pancreatic cystic neoplasms. Box plots showing (A) DNA, (B) UFAs, (C) SFAs, (D) IL-1β, and (E) Resistin being elevated in inflammatory collection, while (F) IL-8 remained similar compared to non-inflammatory PCNs. Proportionately, as shown in panel (G), inflammatory collections have more UFAs than SFAs.

Figure 3: Dose response of mortality, lung and renal injury with increasing triolein doses in caerulein pancreatitis (Preliminary dosing studies). While mortality (A) trended to be higher with 2% triolein, this reached statistical significance at 3% triolein. BAL fluid LDH (B) and FITC leakage (C), serum BUN (D) are significantly increased with 3%triolein. A double asterisk (**) indicates $P<0.05$ on ANOVA versus controls (Con). A single asterisk (*) indicates statistical significance on Students t-test versus controls.

Figure 4: Ascitic fluid - Lipase activity is inhibited by Orlistat. Bar graph comparing lipase activity in the ascitic fluid of different groups. Note that Orlistat significantly reduces lipase activity in the peritoneal cavity of rats with caerulein pancreatitis.

Figure 5: Comparison of Control and Triolein groups show no difference in parameters. Table comparing parameters of AP initiation, mortality, serum oleic acid, BUN, Pancreatic MPO and Lung MPO, TUNELs between control and triolein groups (A). Values are depicted as means ± SEM. There is no

significant difference with triolein compared to controls. Gross appearance of peritoneal cavity (B1,2), pancreas (B3,4), pancreatic histology (B5,6) and pancreatic MPO (B7,8) show no difference between the two groups. Lung histology (C1,2), MPO (C3,4) and TUNELs in lungs (C5,6) and kidneys (D1,2) are similar between controls and triolein groups.

Figure 6: Table comparing Koch's postulates (left side) to which of these are fulfilled by oleate or IL-1β and KC/GRO on the right side. Note that while oleate fulfilled all 4 criteria, IL-1β and KC/GRO did not fulfil the last 2 criteria.

References in Supplementary Section:

W1 Moore HM, Kelly AB, Jewell SD, McShane LM, Clark DP, Greenspan R*, et al.* Biospecimen reporting for improved study quality (BRISQ). Cancer cytopathology;**119**:92-101.

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W3 Navina S, Acharya C, DeLany JP, Orlichenko LS, Baty CJ, Shiva SS*, et al.* Lipotoxicity causes multisystem organ failure and exacerbates acute pancreatitis in obesity. Sci Transl Med 2011;**3**:107ra10.

W4 Acharya C, Cline RA, Jaligama D, Noel P, Delany JP, Bae K*, et al.* Fibrosis Reduces Severity of Acute-on-Chronic Pancreatitis in Humans. Gastroenterology 2013;**145**:466-75.

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W7 Boyum A LD, Tresland L, Nordlie EM. Separation of leucocytes: improved cell purity by fine adjustments of gradient medium density and osmolality. Scandinavian journal of immunology 1991;**34**:697-712.