**Supplementary Methods:**

**Pancreatitis Models and Experimental setup**:

The caerulein model of acute pancreatitis was induced by 12 hourly intra-peritoneal injections of caerulein (a decapeptide analogue of the potent pancreatic secretagogue cholecystokinin) at a dose of 50 µg/kg/h to 6-8 week old male wild type or MORKO mice, after which a subcutaneous pellet of either morphine or placebo (Supplementary Fig-1A) was placed as described earlier.[1] The plasma level of morphine using this method ranges between 0.6 to 1.2 µg/ml (blood levels seen in patients on opioids for moderate to severe pain).[2] This method has been extensively used to study opiates and their effects in animal models.[1] Mice were sacrificed at 48, 72 and 120 hours from the start of the experiment and blood and tissue samples collected for further analyses (Supplementary Fig-1A).

To study if naltrexone (16590-41-3; MedChem Express, Monmouth Junction, NJ, USA), an opioid receptor antagonist with highest affinity for mu-opioid-receptor, can reverse effects of morphine treatment on acute pancreatitis. After induction of caerulein AP mice were either given placebo, morphine alone or morphine and naltrexone (40mg/kg). Naltrexone was administered IP at 12, 24 and 36 hours after induction of caerulein pancreatitis. Mice were sacrificed at 48 hours and tissue samples were collected for analysis (Supplementary Fig-1E)

The L-Arginine model of acute pancreatitis was induced as previously described by our group.[3] Briefly 6-8 week old male wild type mice were injected intraperitoneally with L-arginine in two doses of 4 g/kg each, 1 hour apart. Subcutaneous pellet of either placebo or morphine was implanted at 72 hours. Animals were sacrificed at 108 hours from start of experiment. (Supplementary Fig-2A)

Ethanol-POA model of pancreatitis was induced as first described[4] and as adopted by Vigna et al[5]. Briefly 6-8 week old male wild type were injected intraperitoneally with 1.32 g/kg pure ethanol alone (1.673 µl/g body weight) followed 1 hour later by 2 mg/kg POA alone (1.1175 µl/g body weight of POA diluted 1:500 in DMSO). Subcutaneous pellet of either placebo or morphine was implanted at 12 hours. Animals were sacrificed at 48 hours, 36 hours after placing the pellet. (Supplementary Fig-3A)

Pancreatic necrosis and MPO activity for Caerulein, L-Arginine and Ethanol-POA models at the time of pellet insertion are shown in supplementary figure-1B-D, 2B-D and 3B-D respectively.

**Tissue culture and collection**

Liver, lungs, spleen and mesenteric lymph nodes were collected, homogenized in sterile PBS under aseptic conditions and aliquots cultured on blood agar plates, which were then incubated at 37 degree Celsius for 16 hours. The number of microbial colonies observed on the plates were counted and expressed as colony forming units (CFU).

Pancreatic tissue was fixed in 10% neutral phosphate-buffered formalin for histology. Lung and pancreatic tissue samples were snap frozen in liquid nitrogen and stored at -80C for later measurement of tissue myeloperoxidase (MPO) activity.

**Implantation of Morphine and Placebo Pellets.**

A subcutaneous pocket was created under anesthesia by a skin incision approximately 1 cm in length proximal and dorsal to the left posterior hind leg of the animal. Morphine sulfate (25 mg) or placebo pellets, generously provided by the National Institute on Drug Abuse [NIDA], Bethesda, MD, were placed in the pockets and the incisions were closed by surgical wound clips (9mm stainless steel).

**Intestinal permeability**

Intestinal permeability was determined by administering FITC-dextran (FD4000; Sigma-Aldrich, St. Louis, Missouri, USA) via gavage (500 mg/kg body weight, 125 mg/ml), sacrificing them 4 hrs later and measuring its levels in the blood as previously described.[6 7]

**BrdU incorporation in-vivo**

BrdU (Invitrogen catalog number 00-0103 and 93-3943) incorporation in the regenerating pancreatic tissue was evaluated by using the in-vivo protocol as described by the vendor. Briefly animals were injected via the intraperitoneal route with 1ml of reagent per 100 g body weight 2 hours before sacrificing the mice after which pancreatic tissue was harvested and paraffin embedded. Sections were then cut and stained using the protocol as described by the vendor.

**Morphological examination**

5µm sections of formalin fixed pancreatic tissue samples were stained with haematoxylin/eosin and evaluated by a blinded morphologist. Ten microscopic fields (10x) were randomly selected per section. Necrosis and acinar cell damage quantified by morphometry as described.[8] Extent of injury was expressed as percentage of the total pancreatic tissue visualized. Edema was quantified using scoring system as describe by Schmidt et al.[9]

**Myeloperoxidase Activity**

Myeloperoxidase activity as a marker of neutrophil infiltration and inflammation was measured in both the pancreatic and lung tissues from tissue homogenates as previously described by us.[10]

**Immunohistochemistry for F4/80**

Immunohistochemistry for F4/80 (R&D Systems, Minneapolis, MN, USA) was performed on mouse pancreatic tissue after inducing caerulein pancreatitis in vivo. Briefly, 5-μm–thick cryosections of quick-frozen pancreatic tissue were fixed in acetone, quenched with 3% $H_2O_2$, and blocked with horse serum. After 3 washes with phosphate-buffered saline, the sections were treated with anti–F4/80 primary antibody (1:100) for 1 hour. Then, the sections were incubated with horseradish-peroxidase–conjugated secondary antibody for 30 minutes. Finally, color was developed using purple 3,3′-diaminobenzidine tetra hydrochloride as peroxidase substrate, and the slides were counterstained with methyl green for bright field microscopy.

**Immunofluorescence Staining**

For immunofluorescence, 4um thick sections were cut from paraffin–embedded tissue blocks. Antigen retrieval was done in reveal citrate buffer (Biocare, Concord, CA) using a steamer for 30 minutes. After blocking in protein block (Dako Carpenteria, CA) for 10 minutes, slides were incubated overnight in primary antibody solution in 1X Sniper (Biocare Medical CA). For negative control sections were incubated with antibody diluent only. This was followed by incubation with secondary fluorochrome-tagged antibodies for 1h. Sections were mounted using ProLong Gold antifade mountant with DAPI (Life Technologies).

All microscopic images were taken using Leica DM5500 B microscope (Leica Microsystems) at magnification of 20X. Quantification of immunofluorescent staining was performed using Image J software.

**Antibodies**:

Ki67 (1:100 dilution) Santa Cruz Cat # 15402

Shh (1:100 dilution) R & D systems Cat# AF445

Pdx1 (1:100 dilution) Millipore Cat # 07-696

**RNA Isolation, Measurement, and Analysis**

RNA was isolated from pancreatic tissue stored in RNA later (Qiagen) using TRIzol (Invitrogen) per manufacturer's protocol. Following quantitation, 2 µg of RNA was used to make cDNA using Retroscript first strand synthesis kit for RT-PCR (Applied Biosystems, Grand Island, NY) per the manufacturer's protocol. Real time PCR analysis for PDX-1, PTF-1, GLI-1, PTCH, TNF-α, IL-6, CXCR-2 was performed using cDNA in an ABI7300 instrument (Applied Biosystems). Primers for TNF-α, IL-6, CXCR-2 and PTCH were obtained from Qiagen. Primers for PDX-1 forward primer, 5′- GGA CAT CTC CCC ATA CGA AG -3′; reverse primer, 5′- TGG ATT TCA TCC ACG GGA AA -3′. PTF-1 forward primer, 5′- GCC TCT CCA AAG TAG ACA CG -3′; reverse primer, 5′- TAA CCT TCT GGG CCT GGT TA -3′ . GLI-1 forward primer, 5′- CTA CTC GGG GTT CAA TGA TG -3′; reverse primer, 5′- TAG ACA TGT CCC CTT CCA AA -3′ were from invitrogen. The data were normalized to 18 S rRNA expression levels and expressed as -fold change to control. Untreated wild type pancreata were used as control.

**Figure legend**

**Supplementary Figure 1: Experimental Design of studies involving caerulein induced model of acute pancreatitis**: (A) Schematic representation of experimental design for studying effect of morphine in caerulein induced acute pancreatitis. (B) Representative pictures showing pancreatic damage at the time of pellet insertion (12 hours). (C) Quantification of necrosis and edema at time of pellet insertion (12h), and at the end of experiment (48h). (D) Pancreatic neutrophil sequestration as shown by MPO activity at 12h. (E) Schematic representation of experimental design using naltrexone, an opioid receptor antagonist.

**Supplementary Figure 2: Experimental Design L-Arginine model of pancreatitis**: (A) Schematic representation of experimental design. (B) Representative pictures showing pancreatic damage at the time of pellet insertion is shown (72 hours). (C) Quantification of necrosis at time of pellet insertion (72h), and at the end of experiment (108h) (D) Pancreatic neutrophil sequestration as shown by MPO activity at 72h.

**Supplementary Figure 3: Experimental Design Ethanol-POA model of pancreatitis**: (A) Schematic representation of experimental design. (B) Representative pictures showing pancreatic damage at the time of pellet insertion is shown (12 hours). (C) Quantification of necrosis and edema at time of pellet insertion (12h), and at the end of experiment (48h). (D) Pancreatic neutrophil sequestration as shown by MPO activity at 12h.

**Supplementary Figure 4:** Lung MPO activity for (A) L-Arginine and (B) Ethanol-POA model of pancreatitis at 108h and 48h respectively. (C) Control F4/80 at 0 hour.

**Supplementary Table 1:** p-values for Figure 1C

**Supplementary Table 2:** p-values for Figure 1D

**Supplementary Table 3:** p-values for Figure 2

**References**


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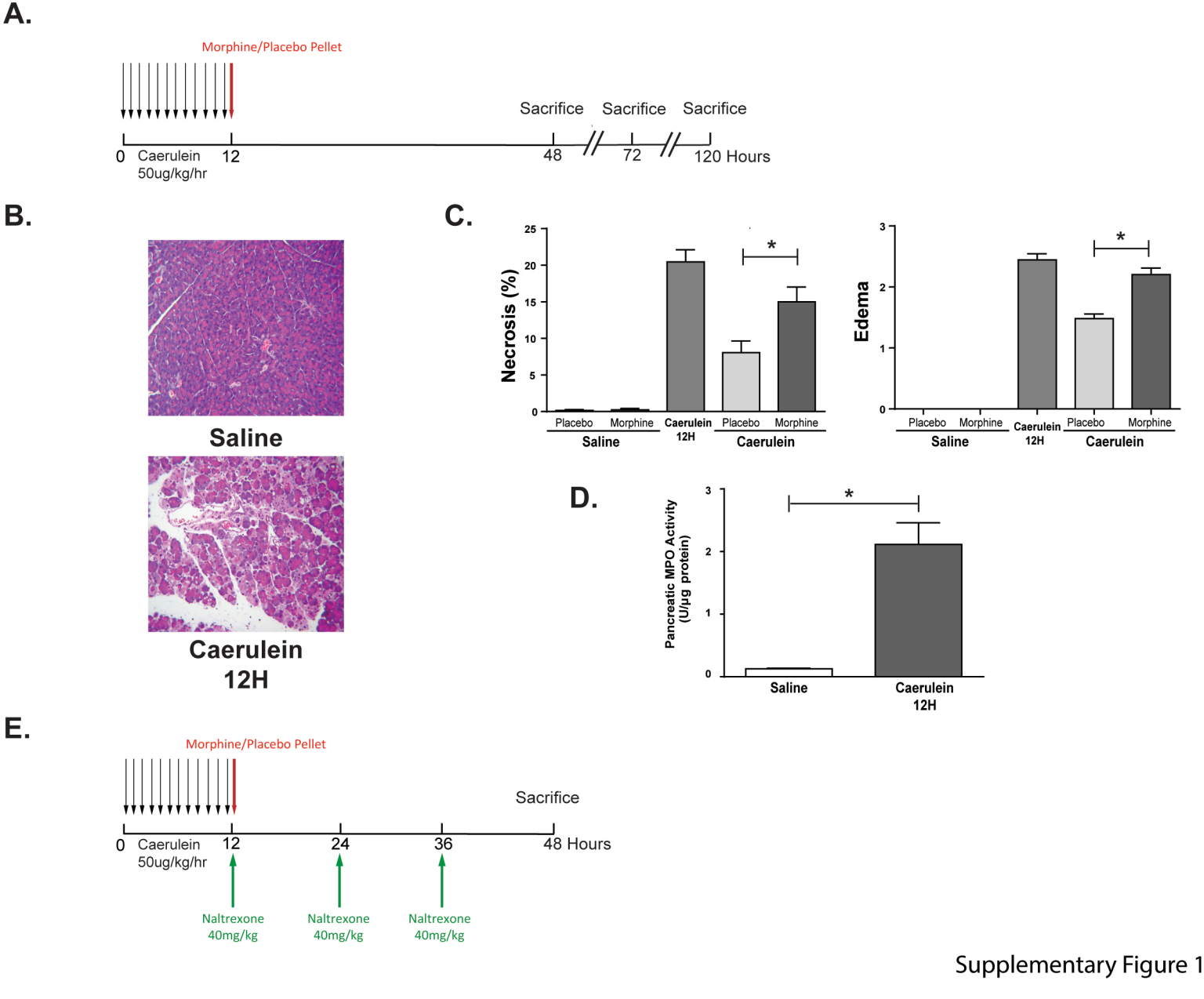
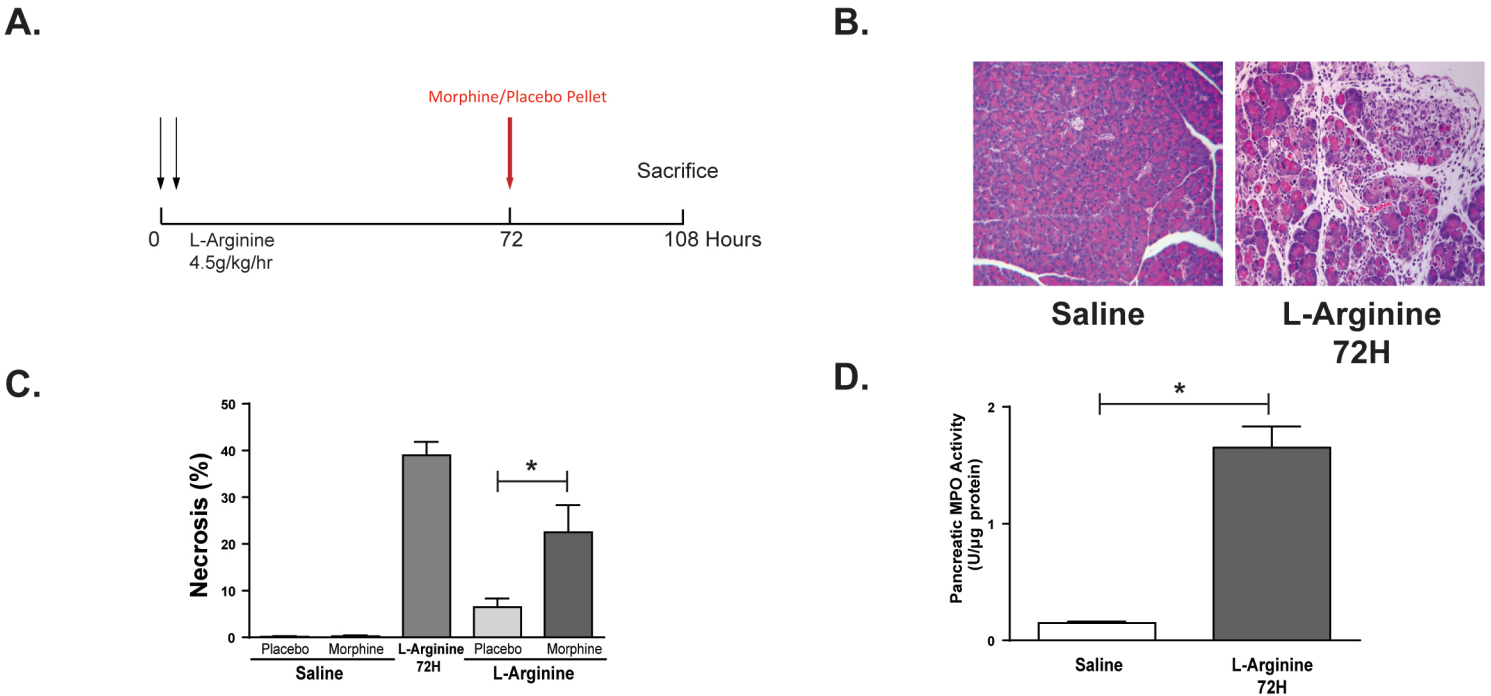
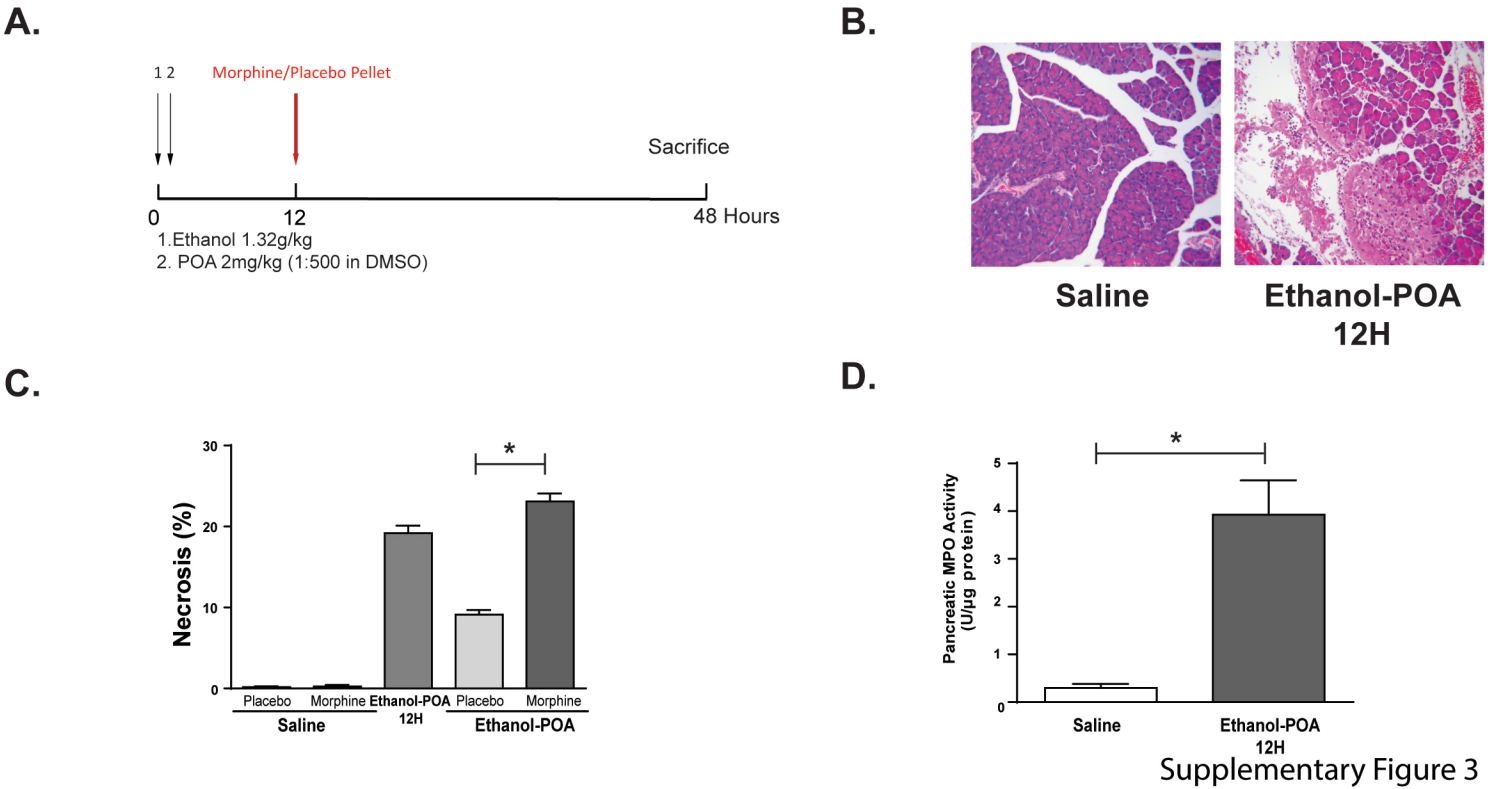



Supplementary Figure 1

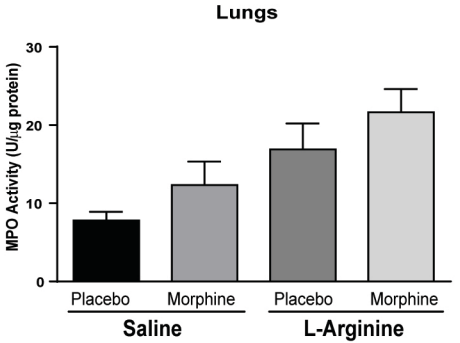



Supplementary Figure 2




Supplementary Figure 3

A.

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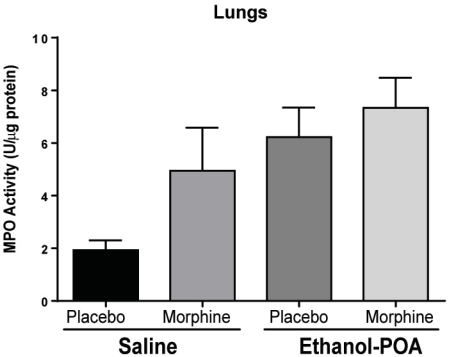
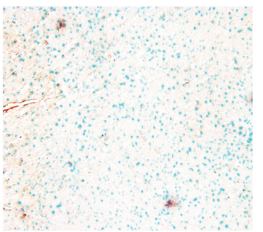



C.



Control F4/80

Supplementary Figure 4

## Figure 1C: p-values

| **48 Hour** | **p-values** |
|---|---|
| Saline+ Placebo vs Caerulein + Placebo | 0.0007 |
| Saline + Placebo vs Caerulein + Morphine | 0.0005 |
| Caerulein + Placebo vs Caerulein + Morphine | 0.0027 |
| | |
| **72 Hour** | |
| Saline+ Placebo vs Caerulein + Placebo | 0.3730 |
| Saline + Placebo vs Caerulein + Morphine | 0.0112 |
| Caerulein + Placebo vs Caerulein + Morphine | 0.0070 |
| | |
| **120 Hour** | |
| Saline+ Placebo vs Caerulein + Placebo | 0.0040 |
| Saline + Placebo vs Caerulein + Morphine | 0.0263 |
| Caerulein + Placebo vs Caerulein + Morphine | 0.6294 |

Supplementary Table 1

**Figure 1D: p-values**

| **48 Hour** | **p-values** |
|---|---|
| Saline+ Placebo vs Caerulein + Placebo | 0.0105 |
| Saline + Placebo vs Caerulein + Morphine | < 0.0001 |
| Caerulein + Placebo vs Caerulein + Morphine | 0.0051 |
| | |
| **72 Hour** | |
| Saline+ Placebo vs Caerulein + Placebo | 0.0076 |
| Saline + Placebo vs Caerulein + Morphine | 0.0074 |
| Caerulein + Placebo vs Caerulein + Morphine | 0.8400 |
| | |
| **120 Hour** | |
| Saline+ Placebo vs Caerulein + Placebo | 0.7278 |
| Saline + Placebo vs Caerulein + Morphine | 0.0743 |
| Caerulein + Placebo vs Caerulein + Morphine | 0.3231 |

Supplementary Table 2

| **Figure 2A** | **p-value** |
|---|---|
| Saline + Placebo vs Saline + Morphine | 0.0005 |
| Saline+ Placebo vs Caerulein + Placebo | 0.0006 |
| Saline + Placebo vs Caerulein + Morphine | < 0.0001 |
| Saline + Morphine vs Caerulein + Morphine | 0.0343 |
| Caerulein + Placebo vs Caerulein + Morphine | 0.0015 |
| | |
| **Figure 2B** | |
| Saline + Placebo vs Saline + Morphine | 0.0006 |
| Saline+ Placebo vs Caerulein + Placebo | 0.0017 |
| Saline + Placebo vs Caerulein + Morphine | 0.0001 |
| Saline + Morphine vs Caerulein + Morphine | 0.0004 |
| Caerulein + Placebo vs Caerulein + Morphine | 0.0254 |
| | |
| **Figure 2C** | |
| Saline + Placebo vs Saline + Morphine | < 0.0001 |
| Saline+ Placebo vs Caerulein + Placebo | 0.1300 |
| Saline + Placebo vs Caerulein + Morphine | < 0.0001 |
| Saline + Morphine vs Caerulein + Morphine | < 0.0001 |
| Caerulein + Placebo vs Caerulein + Morphine | < 0.0001 |
| | |
| **Figure 2D** | |
| Saline + Placebo vs Saline + Morphine | > 0.9999 |
| Saline+ Placebo vs Caerulein + Placebo | 0.8607 |
| Saline + Placebo vs Caerulein + Morphine | 0.0127 |
| Saline + Morphine vs Caerulein + Morphine | 0.0094 |
| Caerulein + Placebo vs Caerulein + Morphine | 0.0549 |

Supplementary Table 3