# Supplementary Materials and Methods

# Inhibition of Neogenin Fosters Resolution of Inflammation and Tissue Regeneration

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**Supplementary Methods：**

*Generation of Chimeric Animals.*

Chimeric animals were generated to define the contribution of the myeloid and tissue-specific neogenin. In brief, WT and $Neo1^{-/-}$ donor mice (8–10 wk old; 20–25 g) were killed, and bone marrow from tibia and femur was extracted by flushing marrow cavity with sterile isotonic sodium chloride solution. Following irradiation of recipient mice with 10 Gy from a 137 Cs source, $10^7$ of washed bone marrow cells were injected in 0.2 mL 0.9% sodium chloride into the recipient mice (8–10 wk old; 20–25 g) via the tail vein. Afterward, chimeric mice were housed in microisolators for at least 8 weeks until hematologic engraftment was completed and fed with water containing tetracycline (100 μg/ml) in the first 2 weeks following bone marrow transplantation.

*Pediatric ICU patients with and without Abdominal Compartment Syndrom (ACS)*

EDTA plasma samples were taken from children (0-198 months of age) between January and August 2015, who had been admitted to the pediatric intensive care unit (PICU) at Hannover Medical School (MHH). The study was approved by the local IRB/ethics committee (MHH-no. 6677) and registered in the WHO ICTPR registry (no. DRKS00006556).

All infants, children and adolescents between 0 (≥37 week of gestational age [WOGA]) and 17 years of life, admitted to the PICU of MHH for at least 24 hours (after detailed enlightenment and written consent), were enrolled into the study when meeting enrollment criteria. Preterm infants (≤ 37 WOGA) and all children who had any pathological condition of the nasopharynx, the upper gastrointestinal tract or any neurogenic bladder dysfunction, were excluded.

Vital signs and functional organ paremeters were recorded through a digital patient record software (mlife, medisite, Hannover, Germany). Intraabdominal pressure (IAP) was measured in children at risk for the development of intraabdominal hypertension (IAH; intraabdominal hypertension, defined as IAP > 10 mmHg) (1). According to the 2013 WSACS

recommendations, IAP was quantified indirectly using tube-/catheter-based intra-vesical and/or intra-gastral pressure measurements (Spiegelberg® monitoring system, Hamburg, Germany). Severity of illness was assessed by the PRISM III score in each subject upon admission or discharge from PICU (2). Abdominal compartment syndrome (ACS) was defined according to 2013 WSACS definitions (3) (www.wsacs.org).

"ICU control subjects" were defined as any admitted patient during the study period with a PRISM III score ≤ 8 at admission, and the absence of ACS. The ill patients were further subdivided. "Critically ill patient without ACS" displayed a PRISM III score > 8 at admission and absence of ACS. Critically ill patient with ACS were defined according to the 2013 WSACS definitions(3). "Critically ill patient with ACS" presented with IAH and the emergence or aggravation of at least one organ dysfunction (3). Organ dysfunction was defined according to the 2005 definitions of the international pediatric sepsis consensus conference (IPSCC) (4).

Two ml EDTA blood were taken within the first 24h after admission to PICU through a central venous line. The blood was spinned down in a centrifuge at 1300 rpm for 10min at room temperature, and subsequently, the plasma aliquotted and immediately stored at -80 ° C.

*LC-MS/MS*

Peritoneal lavage samples were spiked with 4 µL of an internal standard solution (containing $PGE_4$-d4, $LTB_4$-d4 15-HETE-d8 and DHA-d6 at a concentration of 50 ng/ml in methanol). The samples were transferred to a 12-ml glass vial, and 1.75 ml of methanol was added. The samples were centrifuged at 4,000 rpm for 5 min at 6° C, and the supernatant was transferred to a fresh 12-ml glass vial. The pellet was re-extracted with 500 µl of methanol and centrifuged as described above, and the organic extracts were combined. The methanol was partially removed under a gentle stream of nitrogen at 40° C for 30 min. The remaining methanolic extract (approximately 1.5 ml) was diluted with 8 ml of water, and 20 µl of 6 M HCl was added. The

prepared samples were cleaned via solid-phase extraction (SPE) (SepPak C18 200 mg, Waters, MA, USA). The samples were loaded onto preconditioned SPE cartridges (2 ml methanol, followed by 2 ml water), the cartridges were washed with 3 ml of water followed by 3 ml of *n*-hexane, and then the samples were eluted with 3 ml of methylformate. The eluate was dried under a gentle stream of nitrogen, reconstituted in 200 µL of 40% methanol, and injected.

LC-MS/MS analysis was performed as described below. Briefly, a QTrap 6500 mass spectrometer operating in negative ESI mode (Sciex, Nieuwerkerk aan den Ijssel, The Netherlands) was coupled to an LC system employing two LC-30AD pumps, a SIL-30AC autosampler, and a CTO-20AC column oven (Shimadzu,'s-Hertogenbosch, The Netherlands). A 1.7 µm Kinetex $C_{18}$ 50 × 2.1 mm column protected with a C8 precolumn (Phenomenex, Utrecht, The Netherlands) was used, and the column was maintained at 50°C. A binary gradient of water (A) and MeOH (B) containing 0.01% acetic acid was generated as follows: 0 min 30% B, held for 1 min, then ramped to 45% B at 1.1 min, 53.5% B at 2 min, 55.5% B at 4 min, 90% B at 7 min, and 100% B at 7.1 min, and held for 1.9 min. The injection volume was 40 µl, and the flow rate was 400 µl/min. The MS was operated as previously described(5). For analyte identification, the mass transition used for each analyte was combined with its relative retention time (RRT). The calibration lines constructed with standard material for each analyte were used for quantification, and only peaks with a signal to noise (S/N) ratio > 10 were quantified.

**References:**


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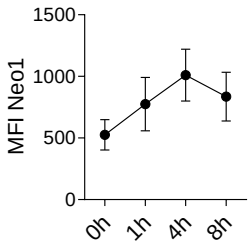
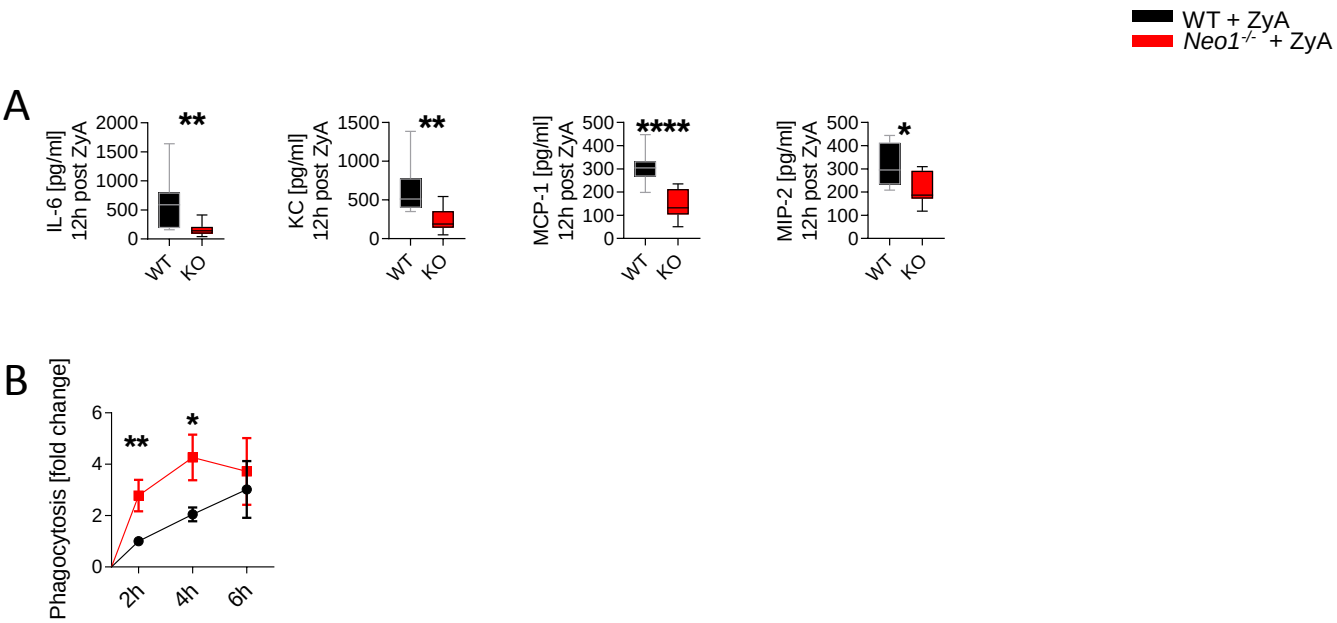
# Supplemental



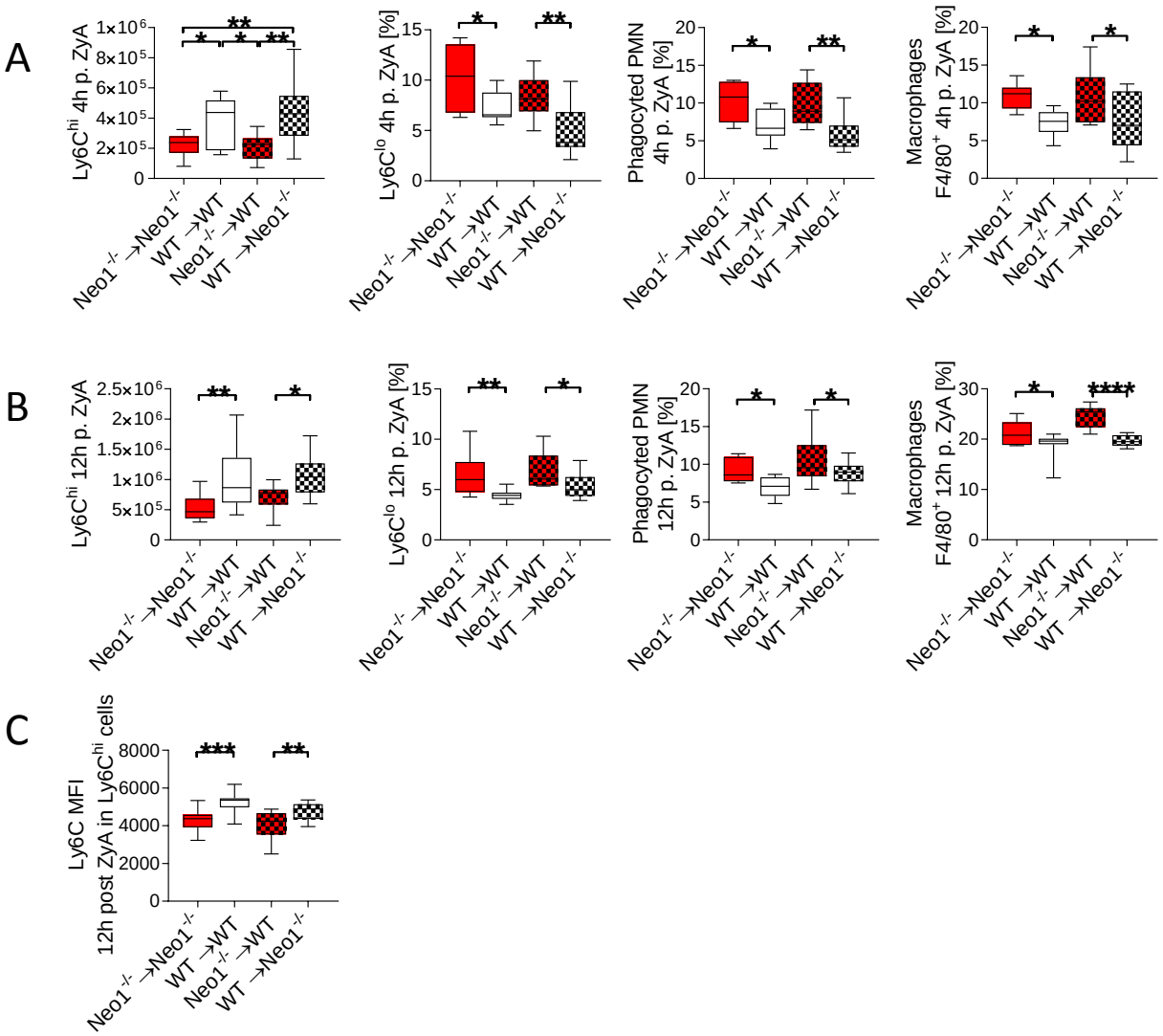

**Suppl. Figure 1. Neo1 Expression on human neutrophils.** Human whole blood was stimulated with 100 ng/ml TNF-α (100 ng/ml) and Neo1 expression was determined by flow cytometry at 0, 1, 4 and 8 h. Results represent at least two independent experiments and are expressed as the mean±SEM.

Suppl. Figure 1

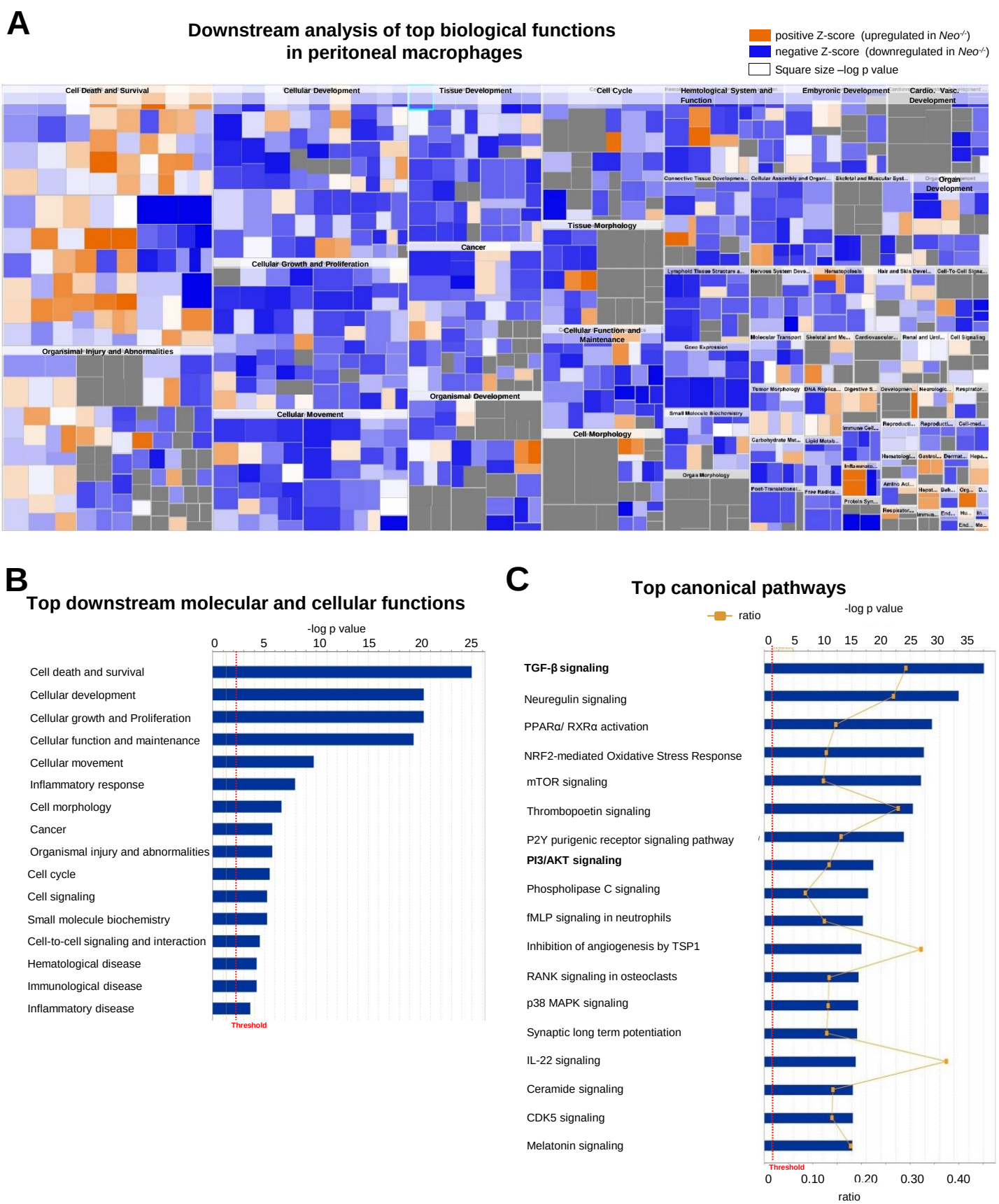



**Suppl. Figure 2. Neo1 is crucial for Resolution processes.** Peritoneal exudates were collected 12h post ZyA and levels of IL-6, KC, MCP-1 and MIP-2 measured by ELISA **(A)**. Peritoneal MΦ from Neo1$^{-/-}$ and WT-mice were collected and phagocytosis of fluorescent ZyA particles was determined at 2, 4 and 6 h **(B)**. Results represent at least two independent experiments and are expressed as the as the median±95% CI (A) and as the mean±SEM (B), n=8-10 per group, unpaired Student's t-test *P<0.05; **P<0.01; **** P<0.0001.

Suppl. Figure 2

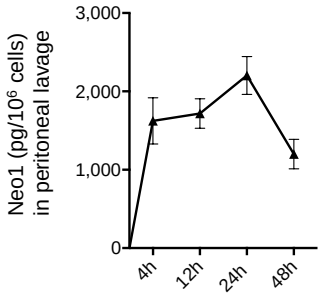



**Suppl. Figure 3. Loss of hematopoietic Neogenin (Neo1) is critical to dampen acute murine peritonitis**. Chimeric animals with C57BL/6 background expressing Neo1 only in bone marrow and blood (wild-type [WT]→Neo1$^{-/-}$), C57BL/6 mice with a targeted hematopoietic knockout of Neo1 (Neo1$^{-/-}$→ WT), and control mice (C57BL/6 background) (WT→WT; Neo1$^{-/-}$ →Neo1$^{-/-}$) were injected with 1mg ZyA i.p. and peritoneal exudates were collected at 4 **A)** and 12 h **B)**. Classical Ly6C$^{hi}$ monocytes and non-classical Ly6C$^{low}$ monocytes, F4/80+ peritoneal MΦ and monocyte-derived MΦ efferocytosis were determined by flow cytometry. Ly6C expression in peritoneal monocytes was determined by flow cytometry **C)**. The results represent two independent experiments and are expressed as the median±95% CI, n=10-13 per group, One Way- ANOVA followed by Bonferroni post-hoc test *P<0.05; **P<0.01; **P<0.01; ***P<0.001; ****P<0.001

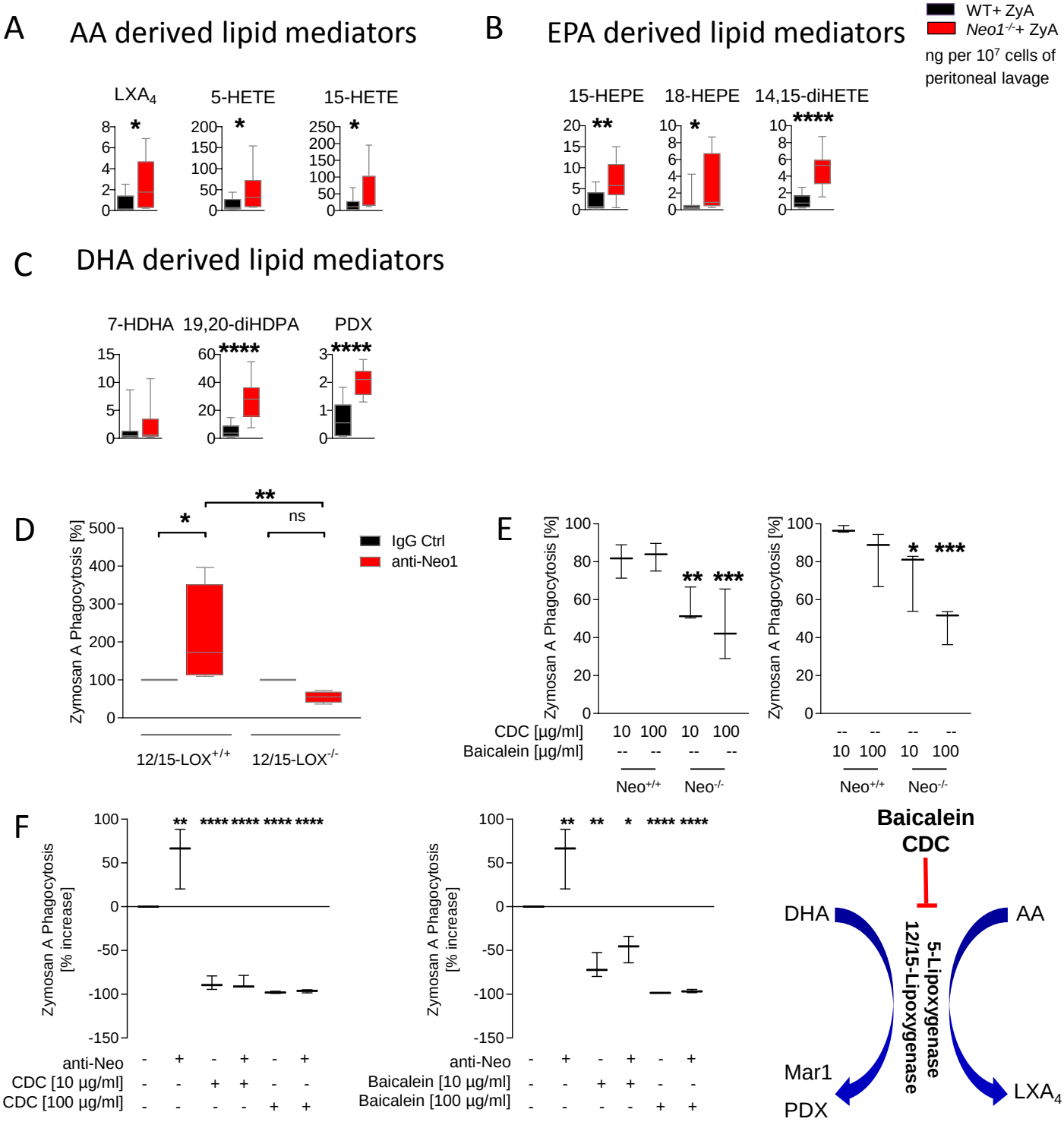



**Suppl. Figure 4. Top function, canonical pathways and networks derived from protein profiling of murine peritoneal monocytes of Neo1-deficient mice and WT.** Peritoneal lavages of Neo1$^{-/-}$ and WT mice were collected 12 h after ZyA treatment and protein expression and phosphorylation were measured in peritoneal monocytes by using a protein microarray. **A)** Downstream effect analysis of biological functions regulated in Neo1$^{-/-}$ mice and littermates. **B)** Top molecular and cellular functions regulated in peritoneal monocytes of Neo1-deficient and WT mice. **C)** Most relevant canonical pathways of differentially regulated proteins in Neo1$^{-/-}$ and WT peritoneal monocytes. Ratio calculated by the number of measured proteins compared with the total number of proteins involved in the pathway. Peritoneal monocytes from 4 mice/condition were pooled for analysis. The -log p value was calculated by the Fisher's exact test right-tailed.

Suppl. Figure 4

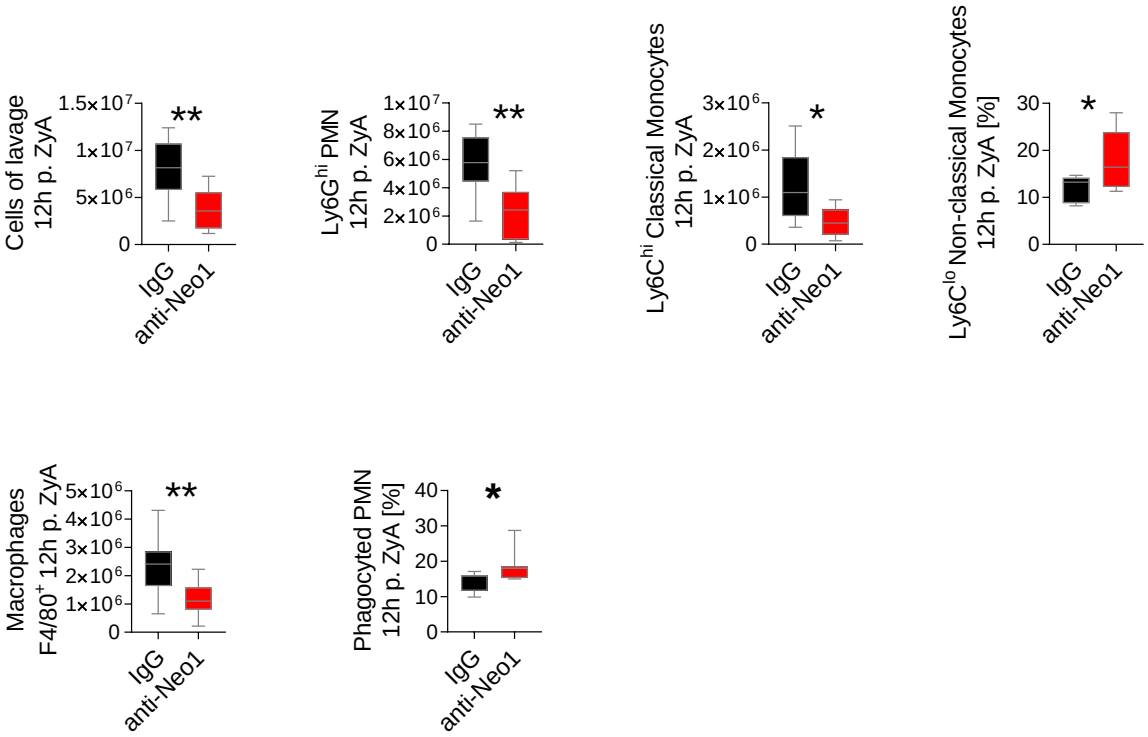



**Suppl. Figure 5. Temporal regulation of Neo1 in ZyA induced peritonitis.** WT mice were exposed to ZyA peritonitis and peritoneal lavages were collected. Neo1 expression in peritonitis cellular exudates measured by ELISA The results represent at least two independent experiments and are expressed as the mean±SEM, n=5-7 per group.

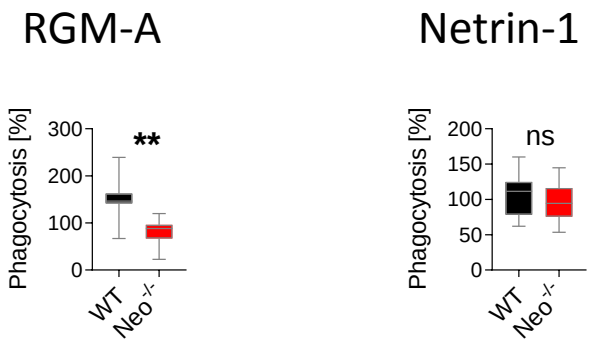



**Suppl. Figure 6. A-C) Endogenous biosynthesis of lipid mediators 12 h post ZyA in WT and Neo1$^{-/-}$ mice.** LC-MS/MS-based profile was performed in peritoneal lavages of WT and Neo1$^{-/-}$ mice. **A)** Bioactive lipid mediators and precursors derived from the arachidonic acid. **B)** Bioactive lipid mediators and precursors derived from eicosapentaenoic acid (EPA). **C)** Bioactive lipid mediators and precursors derived from the docosahexaenoic acid (DHA). All results are reported as ng/10$^7$ peritoneal cells. **D-F) The impact of Neo1 on MΦ Phagocytosis is 5-LOX and 12/15-LOX-dependent. D)** Peritoneal MΦ from *12/15-LOX$^{-/-}$* and WT-mice were collected and phagocytosis of fluorescent ZyA particles was determined after stimulation with anti-Neo1 or IgG control. **E)** Peritoneal MΦ from *Neo1$^{-/-}$* and WT-mice were collected and phagocytosis of fluorescent ZyA particles was determined after stimulation with 5-LOX and 12/15-LOX inhibitors CDC and Baicalein. Results are normalized to control. **F)** Human MΦ were incubated with Neo1 ab and baicalein or CDC and phagocytosis of fluorescent ZyA particles was determined. Results represent three independent experiments and are expressed as the as the median±95% CI, n= 6–8 per group, *P<0.05; **P<0.01; ***P<0.001; ****P<0.001, two-tailed t-test or One Way- ANOVA followed by Bonferroni post-hoc test.

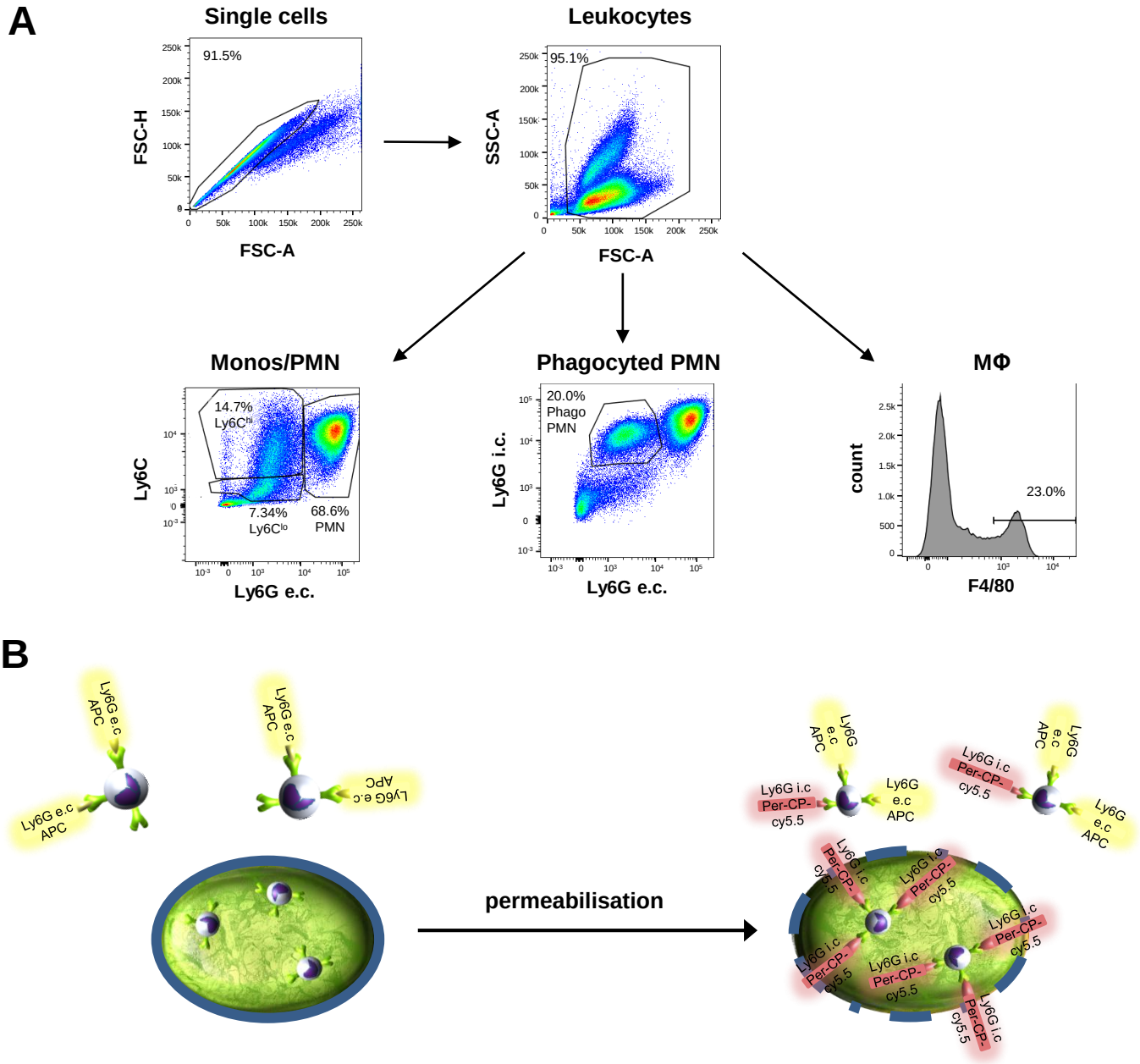
Suppl. Figure 6




**Suppl. Figure 7. Therapeutic inhibition of Neo1 fosters resolution programs.** The therapeutic potential of Neo1 blockade in resolution phase was evaluated by application of anti-Neo1 6 h after ZyA peritonitis induction and peritoneal exudates were collected at 12 h. The total leukocytes were enumerated by light microscopy. PMNs, classical Ly6C$^{hi}$ and non-classical Ly6C$^{low}$ monocytes, F4/80+ peritoneal MΦ and monocyte-derived MΦ efferocytosis were assessed by flow cytometry. Results represent at least two independent experiments and are expressed as the median±95% CI, n=8-10 per group, unpaired two-tailed Student's t-test *P<0.05; **P<0.01.




**Suppl. Figure 8. Neo1 actions in MΦ efferocytosis: ligand-receptor dependency.** Peritoneal MΦ from Neo1$^{-/-}$ and WT mice were collected and incubated with RGM-A or Netrin-1 and efferocytosis of fluorescent ZyA particles was determined. Results represent at least two independent experiments and are expressed as the median±95% CI, n=8-10 per group, unpaired two-tailed Student's t-test *P<0.05; **P<0.01.




Suppl. Figure 9

**Suppl. Figure 9. FACS gating strategy for Leukocyte Differentiation and Efferocytosis.** Peritoneal lavages were collected as described in the materials and methods. **A)** Doublets (FSC-H vs. FSC-A) and debris (FSC-A vs. SSC-A) were excluded. Leukocyte subtypes were differentiated into PMN (Ly6G$^{hi}$), classical inflammatory monocytes (Ly6C$^{hi}$) and non-classical monocytes (Ly6C$^{lo}$) and peritoneal MΦ (F4/80$^{+}$). Phagocytosis: For differentiation of intra- and extra-cellular PMN Ly6G-PerCP-Cy5.5 and Ly6G-APC antibodies were used. Phagocytosed PMN were positive for intracellular Ly6G-PerCP-Cy5.5 and negative for extracellular Ly6G-APC. MΦ were identified by F4/80 expression. **B)** Scheme of phagocytosis staining strategy.

**Suppl. Table 1. Protein and phosphorylation profile of murine peritoneal monocytes.**

WT monocytes — *Neo1*$^{-/-}$ monocytes

| Antibodies | Uniprot ID | Phosphory. Site | Protein Express. | Phosphoryl. |
|---|---|---|---|---|
| Abl1 (Ab-204) | P00520 | Y204 | 1.08 | -1.00 |
| Abl1 (Ab-754/735) | P00520 | T735 | -1.17 | -1.05 |
| Abl1 (Ab-754/735) | P00520 | T754 | | -1.05 |
| Abl1 (Ab-754/735) | P00520 | Y412 | | 1.23 |
| AKT (Ab-308) | P31750/Q60823 | T308 | 1.19 | 1.08 |
| AKT (Ab-326) | P31750/ Q60823 | Y326 | 1.09 | 1.22 |
| AKT (Ab-473) | P31750/Q60823 | S473 | 1.19 | 1.29 |
| AKT1 (Ab-124) | P31750 | S124 | 1.04 | 1.09 |
| AKT1 (Ab-246) | P31750 | S246 | -1.13 | 1.25 |
| AKT1 (Ab-450) | P31750 | T450 | 1.16 | 1.38 |
| AKT1 (Ab-474) | P31750 | T474 | -1.56 | 1.05 |
| AKT1 (Ab-72) | P31750 | T72 | 1.12 | 1.06 |
| AKT2 (Ab-474) | Q60823 | S474 | 1.04 | 1.42 |
| Abl2 (Ab-412) | Q4JIM5 | Y412 | -1.09 | 1.31 |
| Abl2 (Ab-412) | Q4JIM5 | Y245 | | 1.07 |
| CREBBP (Inter) | P45481 | | -1.05 | |
| cofilin (Ab-3) | P18760 | S3 | -1.05 | 1.32 |
| ERK1/2 (N-term) | Q63844 | | 1.14 | |
| ERK2 | P63085 | T202 | 1.14 | 1.12 |
| ERK1-p44/42 MAP Kinase (Ab-202) | Q63844/P63085 | Y204 | -1.18 | 1.00 |
| ERK1-p44/42 MAP Kinase (Ab-204) | Q63844/P63085 | | 1.06 | |
| ERK3 (Ab-189) | Q61532 | S189 | -1.35 | 1.24 |
| ERK8 (Phospho-Thr175/Tyr177) | Q80Y86 | T175 | | 1.22 |
| ERK8 (Phospho-Thr175/Tyr177) | Q80Y86 | Y177 | | 1.22 |
| Gab2 (Ab-623) | Q9Z1S8 | Y643 | -1.59 | 1.02 |
| JNK1/2/3 (Ab-183/185) | Q61831/ Q91Y86/ Q9WTU6 | T183/ Y185 | 1.01 | 1.12 |
| JNKK (MKK4) (Inter) | P47809 | | 1.06 | |
| LIMK1 (Ab-508) | P53668 | T508 | 1.03 | -1.22 |
| MAP3K1/MEKK1 (Phospho-Thr1381) | P53349 | T1381 | | -1.18 |
| MKK3 (Ab-189) | O09110 | S189 | -1.14 | -1.60 |
| MKK3/MAP2K3 (Ab-222) | O09110 | T222 | -1.29 | -1.03 |
| MKK6 (Ab-207) | P70236 | S207 | 1.09 | 1.25 |
| mTOR (Ab-2446) | Q9JLN9 | T2446 | 1.11 | 1.07 |
| mTOR (Ab-2448) | Q9JLN9 | S2448 | -1.36 | 1.09 |
| mTOR (Ab-2481) | Q9JLN9 | S2481 | -1.07 | 1.11 |
| Myc (Ab-358) | P01108 | T358 | -1.17 | 1.19 |
| Myc (Ab-373) | P01108 | S373 | 1.07 | -1.06 |
| Myc (Ab-58) | P01108 | T58 | -1.08 | 1.02 |
| Myc (Ab-62) | P01108 | S62 | 1.12 | 1.06 |
| p300/CBP (C-term) | B2RWS6 | | -1.55 | |
| p38 MAPK (Ab-180) | P47811 | T180 | -1.45 | -1.12 |
| p38 MAPK (Ab-182) | P47811 | Y182 | 1.13 | 1.14 |
| p38 MAPK (Ab-322) | P47811 | Y322 | 1.42 | 1.02 |
| PAK1 (Ab-204) | O88643 | S204 | 1.20 | 1.19 |
| PAK1 (Ab-212) | O88643 | T212 | 1.01 | 1.27 |
| PAK1/2 (Ab-199) | O88643 / Q8CIN4 | S199 | -1.04 | 1.40 |
| PAK1/2/3 (Ab-141) | O88643 / Q8CIN4/ Q61036 | S141 | 1.05 | 1.06 |
| PAK1/2/3 (Ab-423/402/421) | O88643 / Q8CIN4/ Q61036 | T421/ T423/T402 | -1.15 | -1.13 |
| PAK2 (Ab-192) | Q8CIN4 | S192 | 1.03 | 1.08 |
| PAK2 (Ab-197) | Q8CIN4 | | -1.28 | |
| PAK2 (Phospho-Ser20) | Q8CIN4 | S20 | | 1.07 |
| PAK3 (Ab-154) | Q61036 | S154 | -1.33 | 1.09 |
| PAK4/PAK5/PAK6 (Ab-474) | Q8BTW9/ Q8C015/ Q3ULB5 | | 1.19 | |

| Antibodies | Uniprot ID | Phosphory. Site | Protein Express. | Phosphory. |
|---|---|---|---|---|
| PI3-kinase p85-alpha (Phospho-Tyr607) | P26450 | Y607 | | 1.18 |
| PI3-kinase p85-subunit alpha/gamma (Ab-467/199) | P26450/ Q64143 | T199/ Y467 | 1.05 | 1.21 |
| PKC alpha (Ab-657) | P20444 / P68404 | Y657 | -1.18 | -1.08 |
| PKC alpha/beta II (Ab-638) | P20444 / P68404 | T638 | -1.08 | 1.14 |
| PKC delta (Ab-505) | P28867 | T505 | -1.03 | 1.10 |
| PKC delta (Ab-645) | P28867 | S645 | 1.42 | -1.11 |
| PKC epsilon (Ab-729) | P16054 | S729 | -1.36 | 1.11 |
| PKC theta (Ab-538) | Q02111 | T538 | -1.19 | -1.05 |
| PKC theta (Ab-676) | Q02111 | S676 | 1.16 | 1.00 |
| PKC zeta (Ab-410) | Q02956 | T410 | 1.16 | -1.03 |
| PKC zeta (Ab-560) | Q02956 | T560 | -1.19 | 1.13 |
| PP2A-alpha (Ab-307) | P63330 | Y307 | 1.17 | 1.09 |
| Rac1/cdc42 (Ab-71) | P63001 | S71 | -1.27 | -1.04 |
| RAS (p21 H and K) (Inter) | Q61411/ P32883 | | -1.09 | |
| RAS (p21 H and K) (Inter) | P08556 | | -1.10 | |
| RASE (Inter)(GPTase Eras) | Q7TN89 | | -1.01 | |
| RASF4 (Inter) | Q8CB96 | | -1.10 | |
| Ras-GRF1 (Ab-916) | P27671 | S916 | -1.04 | -0.99 |
| Rho/Rac guanine nucleotide exchange factor 2 (Ab-885) | Q60875 | S885 | -1.58 | 1.13 |
| RhoA (Ab-188) | Q9QUI0 | | -2.51 | |
| S6 Ribosomal Protein (Ab-235) | P62754 | S235 | -1.12 | 1.14 |
| S6K beta(Inter) | Q8BSK8 | | -1.45 | |
| S6K-α6 (Inter) | Q7TPS0 | | -1.26 | |
| SAPK/JNK (Ab-183) (MAPK8) | Q91Y86/ Q9WTU6/ Q61831 | T183 | 1.06 | 1.07 |
| SAPK/JNK (Ab-185) | Q91Y86/ Q9WTU6/ Q61831 | Y185 | 1.12 | -1.34 |
| SEK1/MKK4 (Ab-261) | P47809 | T261 | -1.11 | -1.70 |
| SEK1/MKK4 (Ab-80) | P47809 | S80 | -1.08 | -1.65 |
| Shc (Ab-349) | P98083 | Y349 | 1.30 | -1.34 |
| Shc (Ab-427) | P98083 | Y427 | 1.19 | 1.11 |
| Smad1 (Ab-187) | P70340 | S187 | -1.30 | -1.03 |
| Smad1 (Ab-465) | P70340 | S465 | -1.12 | -1.42 |
| Smad1/5/9 (Inter) | P70340/ P97454/ Q9JIW5 | | 1.02 | |
| Smad2 (Ab-220) | Q62432 | T220 | -1.25 | -1.03 |
| Smad2 (Ab-245) | Q62432 | S250 | -1.32 | -1.01 |
| Smad2 (Ab-250) | Q62432 | S467 | -1.14 | 1.25 |
| Smad2 (Ab-255) | Q62432 | | -1.02 | |
| Smad2 (Ab-467) | Q62432 | | 1.01 | |
| Smad2/3 (Ab-8) | Q62432/ Q8BUN5 | T8 | -1.18 | -1.01 |
| Smad3 (Ab-179) | Q8BUN5 | T179 | -1.09 | -1.05 |
| Smad3 (Ab-204) | Q8BUN5 | | 1.05 | -1.58 |
| Smad3 (Phospho-Ser208) | Q8BUN5 | S208 | | -1.46 |
| Smad3 (Ab-213) | Q8BUN5 | S213 | -1.34 | 1.04 |
| Smad3 (Ab-425) | Q8BUN5 | S425 | -1.30 | 1.24 |
| Smad4 (Inter) | P97471 | | -1.46 | |
| SP1 (Ab-739) | O89090 | T739 | 1.12 | -1.27 |
| TAK1 (Ab-184) | Q62073 | T184 | 1.01 | 1.06 |
| TGF alpha (C-term) | P48030 | | -1.06 | |
| TGF beta receptor II (internal) | Q62312 | | -1.26 | |
| TGF beta1 (C-term) | P04202 | | -1.36 | |
| TGF beta2 (C-term) | P27090 | | -1.39 | |
| TGF beta3 (internal) | P17125 | | -1.25 | |
| TGFBR1 (Ab-165) | Q64729 | | 1.06 | |
| TGFBR2 (Ab-250) | Q62312 | | -1.13 | |

| Common name | Lipidmaps ID | WT | | Neo1$^{-/-}$ | | |
|---|---|---|---|---|---|---|
| | | Mean | SEM | Mean | SEM | P value |
| AdA | LMFA01030178 | 296.5 | 69.7 | 821.2 | 154.3 | 0.0030 ** |
| ALA | LMFA01030152 | 6048 | 1440 | 11740 | 2336 | 0.0374 * |
| **Arachidonic Acid Bioactive Metabolome** | | | | | | |
| AA | LMFA01030001 | 3117 | 369 | 7424 | 1202 | 0.0008 *** |
| TXB2 | LMFA03030002 | 9.21 | 1.39 | 10.5 | 1.20 | 0.5778 ns |
| $PGD_2$ | LMFA03010004 | 3.63 | 0.64 | 7.40 | 1.81 | 0.0223 * |
| $PGF_2$ | LMFA03010002 | 2.38 | 0.37 | 7.81 | 1.34 | < 0.0001 *** |
| 8-iso-$PGF_{2\alpha}$ | LMFA03110015 | 1.51 | 0.52 | 1.92 | 0.32 | 0.5882 ns |
| $PGE_2$ | LMFA03010003 | 7.39 | 1.46 | 20.18 | 5.36 | 0.0074 ** |
| 5-HETE | LMFA03060002 | 21.50 | 4.71 | 67.05 | 13.45 | 0.0005 *** |
| $LTB_4$ | LMFA03020001 | 12.37 | 2.69 | 35.75 | 10.10 | 0.0103 * |
| 6-trans-$LTB_4$ | LMFA03020013 | 3.92 | 0.62 | 10.7 | 2.10 | 0.0005 *** |
| 6t.12epi-$LTB_4$ | LMFA03020014 | 5.09 | 0.99 | 18.8 | 4.22 | 0.0004 *** |
| 8-HETE | LMFA03060006 | 2.33 | 0.97 | 3.64 | 1.11 | 0.3982 ns |
| 11-HETE | LMFA03060003 | 5.29 | 1.00 | 19.5 | 4.67 | 0.0007 *** |
| 12-HETE | LMFA03060007 | 35.0 | 12.3 | 57.2 | 11.5 | 0.1212 ns |
| 15-HETE | LMFA03060001 | 10.5 | 2.66 | 43.1 | 11.4 | 0.0014 ** |
| $LXA_4$ | LMFA03040001 | 0.46 | 0.12 | 2.53 | 0.58 | < 0.0001 *** |
| 14,15-diHETE | LMFA03060077 | 2.59 | 0.42 | 14.1 | 3.42 | 0.0002 *** |
| **Eicosapentaenoic Acid Bioactive Metabolome** | | | | | | |
| EPA | LMFA01030759 | 417.3 | 164.8 | 826.6 | 165 | 0.0995 ns |
| 15-HEPE | LMFA03070009 | 5.34 | 1.56 | 25.7 | 4.83 | < 0.0001 *** |
| 18-HEPE | LMFA03070038 | 0.70 | 0.18 | 2.88 | 0.93 | 0.0061 ** |
| **Docosahexaenoic Acid Bioactive Metabolome** | | | | | | |
| DHA | LMFA01030185 | 3031 | 528 | 6179 | 1171 | 0.0091 ** |
| PDX | LMFA04000047 | 2.07 | 0.52 | 6.35 | 0.17 | 0.0090 ** |
| MaR1 | LMFA04050001 | 0.164 | 0.031 | 0.776 | 0.188 | 0.0047 ** |
| 19,20-diHDPA | LMFA04000043 | 14.2 | 2.54 | 99.2 | 24.0 | 0.0001 *** |
| 10-HDHA | LMFA04000027 | 0.971 | 0.418 | 1.87 | 0.57 | 0.2045 ns |
| 7-HDHA | LMFA04000025 | 0.44 | 0.07 | 3.71 | 0.99 | 0.0017 ** |
| 17-HDHA | LMFA04000072 | 28.7 | 6.01 | 139 | 23.1 | < 0.0001 *** |

Suppl. Table 2

**Table S2. Lipid mediator levels in murine peritoneal fluids following administration of ZyA in *Neo1*$^{-/-}$ and WT mice.** WT and Neo1 deficient animals were challenged with ZyA. Lavages were collected at 4 h and LC-MS/MS-based profiling was performed. Levels of bioactive lipid mediators and precursors derived from the AA, DHA and EPA pathways. All results are reported as ng/$10^7$ peritoneal cells. Results represent three independent experiments with n=8-14 mice/group and are expressed as the mean±SEM, unpaired student's t-test, *P<0.05; **P<0.01; ***P<0.001.

**Table S3. Clinical characteristics and demographic profile of study patients.**

| | PICU control patients | Critically ill PICU patients | Critically ill PICU patients without ACS | Critically ill PICU patients with ACS |
|---|---|---|---|---|
| N | 25 | 34 | 20 | 14 |
| Age (months) | 15.0 (0-198.4) | 31.5 (0-197.5) | 31.5 (0-198.9) | 28 (0-190) |
| Male sex (%) | 9 (36) | 17 (50) | 10 (50) | 7 (50) |
| Height (m) | 0.72 (0.48-1.71) | 0.85 (0.49-1.79) | 0.92 (0.48-1.91) | 0.76 (0.54-1.69) |
| Weight (kg) | 9.0 (2.7-78.1) | 11.5 (14.0-29.7) | 12.4 (12.1-35.8) | 11.0 (8.2-29.9) |
| BMI (kg/m$^2$) | 14.7 (9.9-30.5) | 16.7 (10.7-24.3) | 15.5 (12.2-24.4) | 18.2 (9.4-23.9) |
| Days of PICU stay | 1.0 (0.3-19.8) | 7.5 (1.0-199.2) | 4.0 (1.0-17.85) | 19.5 (1.0-332.0) |
| PRISM III score at admission | 5.0 (0.0-7.0) | 18.0 (6.5-32.0) | 16.0 (8.1-30.7) | 18.0 (2.0-35.0) |
| PRISM III score at discharge | 0.0 (0.0-8.0) | 3.0 (0.0-17.4) | 3.0 (0.0-12.0) | 5.0 (0.0-21.0) |
| ICU-mortality (%) | 0 (0) | 6 (17.6) | 2 (10) | 4 (28.6) |
| IAH incidence (%) | 7 (28) | 22 (65) | 8 (40) | 14 (100) |
| IAH grade (%) | | | | |
| 0 | 18 (72) | 12 (35.3) | 12 (60) | 0 (0) |
| I | 5 (20) | 5 (14.7) | 4 (20) | 1 (7.1) |
| II | 2 (8) | 6 (17.6) | 1 (5) | 5 (35) |
| III | 0 (0) | 4 (11.7) | 0 (0) | 4 (28.6) |
| IV | 0 (0) | 7 (20,.6) | 3 (15) | 4 (28.6) |
| Primary reason for admission on PICU (%) | | | | |
| post surgery | 7 (28) | 14 (41.1) | 6 (30) | 8 (57.2) |
| hemi-oncology | 3 (12) | 1 (2.8) | 0 (0) | 1 (7.1) |
| cardiology | 6 (24) | 17 (50) | 13 (65) | 4 (28.6) |
| sepsis | 0 (0) | 1 (2.8) | 0 (0) | 1 (7.1) |
| post neurosurgery | 9 (36) | 1 (2.8) | 1 (5) | 0 (0) |

Values represent the number of patients or median with 95% CI, as appropriate.

**Table S4. Laboratory profile of study patients.**

| | PICU control patients | Critically ill PICU patients | Critically ill PICU patients without ACS | Critically ill PICU patients with ACS |
|---|---|---|---|---|
| N | 25 | 34 | 20 | 14 |
| pH | 7.38 (7.27-7.46) | 7.25 (7.04-7.38) | 7.26 (7.11-7.37) | 7.24 (7.02-7.38) |
| $HCO3^-$ (mmol/l) | 23.0 (19.3-25.0) | 20.5 (19.3-28.0) | 21.0 (18.0-28.0) | 19.5 (9.0-24.0) |
| Base deficit (mmol/l) | -1.4 (-6.2- 1.8) | -5.6 (-17.2- -0.4) | -5.5 (-9.3-4.0) | -6.1 (-20.9- -1.9) |
| Lactate (mmol/l) | 1.1 (0.4-3.9) | 3.7 (1.3-24.0) | 4.7 (1.6-22.6) | 2.6 (0.5-27.0) |
| $p_aO2$ (mmHg) | 118 (48-258) | 108 (37-266) | 109 (42-194) | 92 (32-482) |
| $p_aCO2$ (mmHg) | 40 (34-50) | 52 (33-80) | 49 (33-80) | 56 (31-80) |
| FiO2 | 0.21 (0.21-0.43) | 0.40 (0.21-1.0) | 0.35 (0.21-1.0) | 0.65 (0.21-1.0) |
| PO/FiO2 ratio | 467 (187-812) | 237 (45-671) | 286 (71-693) | 154 (32-662) |
| SaO2 (%) | 99.0 (90.0-100) | 98.0 (83.0-100) | 98.5 (86.4-100) | 98.0 (74.0-100) |
| mvSO2 (%) | 73.0 (67.0-85.0) | 73.0 (33.8-85.8) | 76.0 (54.0-87.0) | 62.5 (32-85) |
| avDO2 (%) | 25.0 (14.0-32.0) | 24.0 (13.0-64.6) | 22.0 (10.0-44.0) | 31.0 (15.0-67.0) |
| $K^+$ (mmol/l) | 3.9 (3.2-5.5) | 4.6 (3.6-6.4) | 4.4 (3.4-6.4) | 4.9 (3.7-7.4) |
| $Ca^{2+}$ (mmol/l) | 1.3 (1.1-1.4) | 1.2 (0.9-1.6) | 1.2 (0.7-2.1) | 1.3 (1.0-1.5) |
| Glucose (mmol/l) | 5.8 (4.3-9.2) | 10.0 (5.1-18.6) | 10.6 (5.6-19.1) | 8.0 (4.5-18.4) |
| Bilirubine (µmol/l) | 13 (6-158) | 28 (8-175) | 23 (6-139) | 33 (8-230) |
| ASAT (U/l) | 44 (15-751) | 128 (39-3020) | 106 (32-3538) | 162 (41-2801) |
| ALAT (U/l) | 22 (4-210) | 32 (15-1160) | 27 (13-1098) | 69 (18-1295) |
| LDH (U/l) | 335 (138.3-777) | 497 (170.3-3138) | 497 (200.9-4281) | 458.5 (93-2709) |
| C reactive protein (mg/l) | 2.0 (0.9-99.1) | 9.0 (0.9-294) | 2 (0.9-64.75) | 62 (0.9-354) |
| Interleukin 6 (ng/l) | 61 (5.1-24085) | 135 (18-19759) | 114 (18-4887) | 179 (18-24716) |
| Creatinine (µmol/l) | 30 (15-84) | 34 (21-200) | 37 (21-143) | 65 (27-311) |
| WBC ($x10^9$/l) | 11.7 (6.0-24.9) | 11.1 (3.8-30.7) | 11.3 (2.78-30.4) | 10.0 (4.2-30.8) |
| Platelets ($x10^9$/l) | 270 (158-691) | 122 (23-395) | 156 (43-408) | 90 (4-216) |
| INR | 1.19 (1.05-1.83) | 1.37 (1.14-3.71) | 1.39 (1.11-3.71) | 1.36 (1.15-3.15) |
| PTT (sec) | 36 (24-61) | 44 (29-141) | 40 (28-114) | 58 (33-160) |

From 59 PICU children with and without ACS blood samples were drawn within the first 24 h after admission to PICU and laboratory test were acquired. Values represent the number of patients or median with 95% CI, as appropriate.

| | Neo1 (n=58) | | CRP (n=58) | | IL-6 (n=44) | | PCT (n=6) | | Leuco (WBC) (n=58) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | rho | p-Value | rho | p-Value | rho | p-Value | rho | p-Value | rho | p-Value |
| Crea | 0.33 | 0.016 | 0.334 | 0.01 | 0.177 | 0.251 | 0.714 | 0.111 | 0.274 | 0.037 |
| Bili | 0.25 | 0.072 | 0.193 | 0.147 | 0.136 | 0.379 | 0.406 | 0.425 | -0.061 | 0.65 |
| LDH | 0.204 | 0.124 | 0.316 | 0.016 | 0.299 | 0.048 | 0.314 | 0.544 | 0.148 | 0.268 |
| Lactate | 0.34 | 0.01 | 0.232 | 0.086 | 0.268 | 0.086 | -0.029 | 0.957 | 0.221 | 0.101 |
| LOS-PICU | 0.18 | 0.17 | 0.38 | 0.004 | -0.005 | 0.974 | 0.657 | 0.156 | 0.155 | 0.25 |
| IAH-Grade | 0.21 | 0.113 | 0.511 | 0 | 0.06 | 0.701 | 0.309 | 0.552 | -0.034 | 0.8 |
| PRISM-III | 0.2 | 0.125 | 0.353 | 0.007 | 0.209 | 0.174 | 0.551 | 0.257 | -0.103 | 0.441 |

**Table S5. Correlations of Neo1 and conventional inflammatory parameters with descriptive, organ and outcome parameters of study patients.** From 59 PICU children with and without ACS blood samples were drawn within the first 24 h after admission to the interdisciplinary PICU. Spearman's rank correlation coefficient Rho was calculated and is shown.

Suppl. Table 5

| PICU contol patients (n=24) | | Critically ill PICU patients without ACS (n=20) | | Critically ill PICU patients with ACS (n=14) | | P values | | |
|---|---|---|---|---|---|---|---|---|
| median | 95%-CI | median | 95%-CI | median | 95%-CI | NCI vs CI-ACS | NCI vs CI+ACS | CI-ACS vs CI+ACS |
| 353.9 | 138.3-777 | 497 | 220.9-4281 | 458.5 | 93-2709 | p = 0.008 | p = 0.22 | p = 0.99 |

**Table S6. Lactate dehydrogenase [LDH] levels (U/L) of study patients. From 59 PICU children with and without ACS blood samples were drawn within the first 24 h after admission to PICU**. Values represent the median with 95%-confidence interval (CI). P-Values were calculated using Dunn's multiple comparisons test. PICU-controls (=NCI): Pediatric patients with intensive care needs (PICU), but not critically ill (NCI) in the strictest sense. CI-ACS: Critically ill (CI) children in intensive care (PICU) who do not meet the criteria for an abdominal compartment syndrome (IAP > 10mmHg *PLUS* newly-emergent or aggravated organ dysfunction). CI+ACS: Critically ill children in intensive care with the full picture of abdominal compartment syndrome (ACS).

Suppl. Table 6