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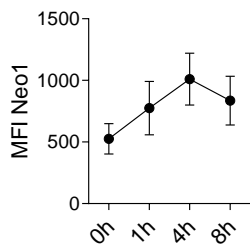
Suppl. Figure 1. Neo1 Expression on human neutrophils. Human whole blood was stimulated with 100 ng/ml TNF-α (100 ng/ml) and Neo1 expression was determined by flow cytometry at 0, 1, 4 and 8 h. Results represent at least two independent experiments and are expressed as the mean±SEM.

Suppl. Figure 1
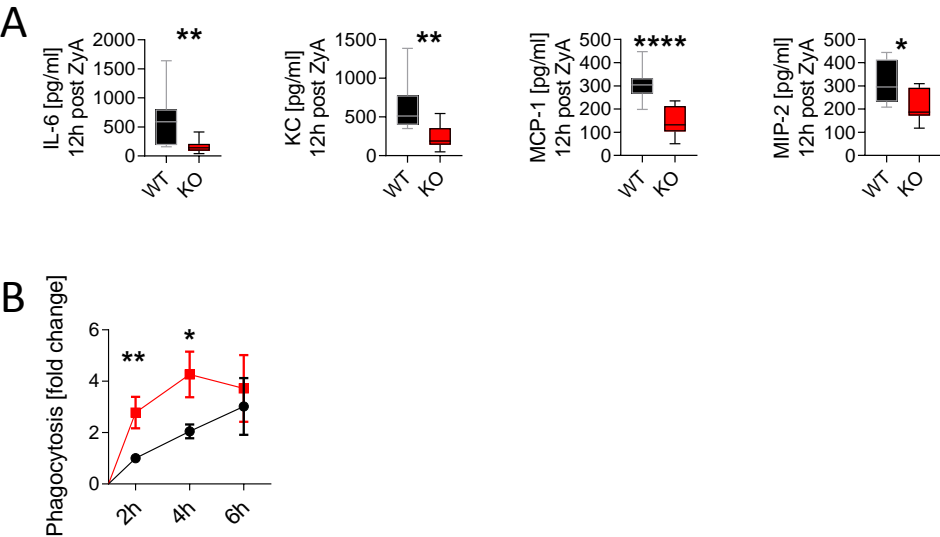
Suppl. Figure 2. Neo1 is crucial for resolution processes. Peritoneal exudates were collected 12h post ZyA and levels of IL-6, KC, MCP-1 and MIP-2 measured by ELISA (**A**). Peritoneal MΦ from Neo1^{-/-} and WT-mice were collected and phagocytosis of fluorescent ZyA particles was determined at 2, 4 and 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
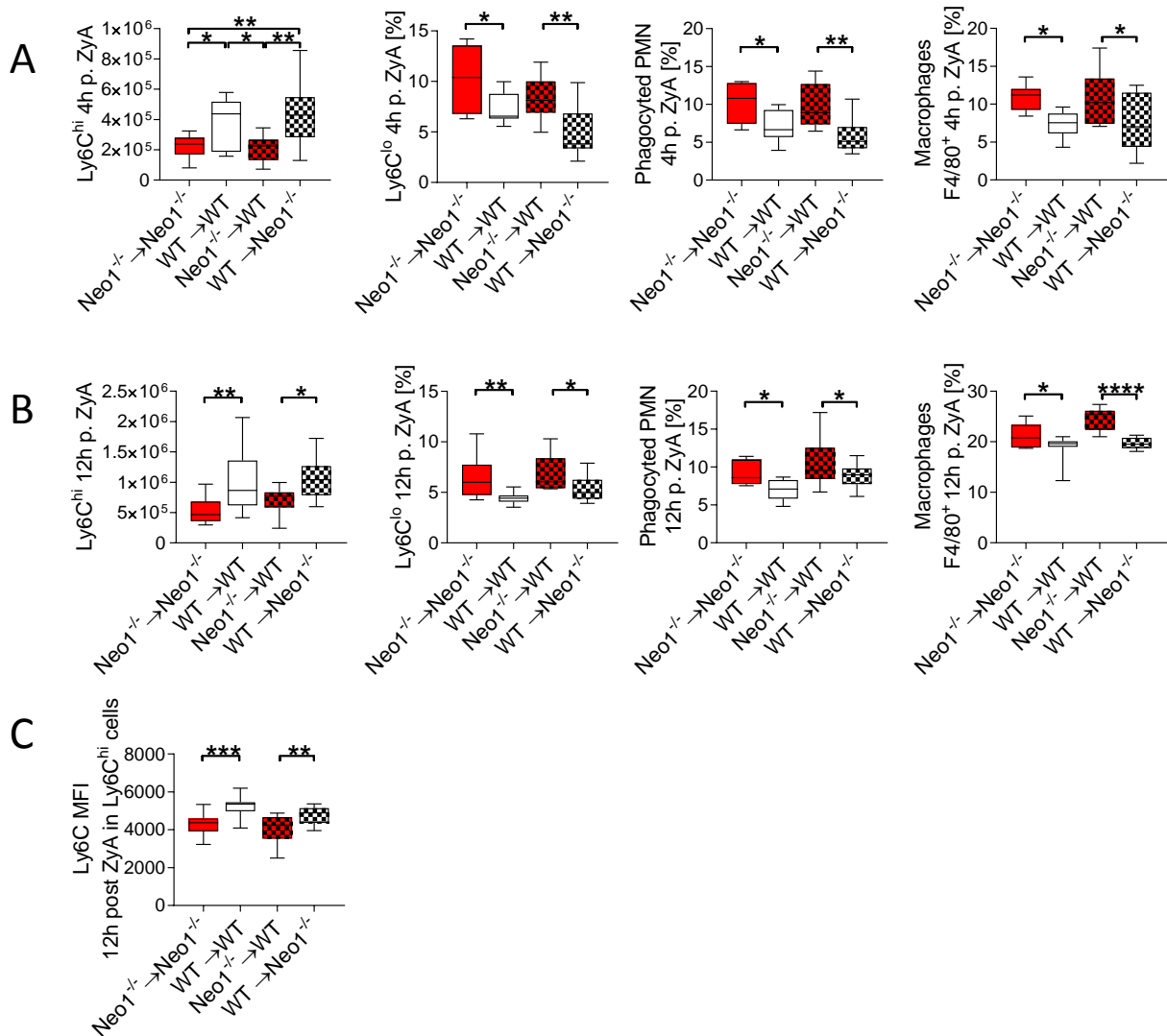
Suppl. Figure 3. Loss of hematopoietic Neogenin (Neo1) is critical to dampen acute murine peritonitis. Chimeric animals with C57BL/6 background expressing Neo1 only in bone marrow and blood (wild-type [WT]→Neo1$^{-/-}$), C57BL/6 mice with a targeted hematopoietic knockout of Neo1 (Neo1$^{-/-}$→ WT), 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
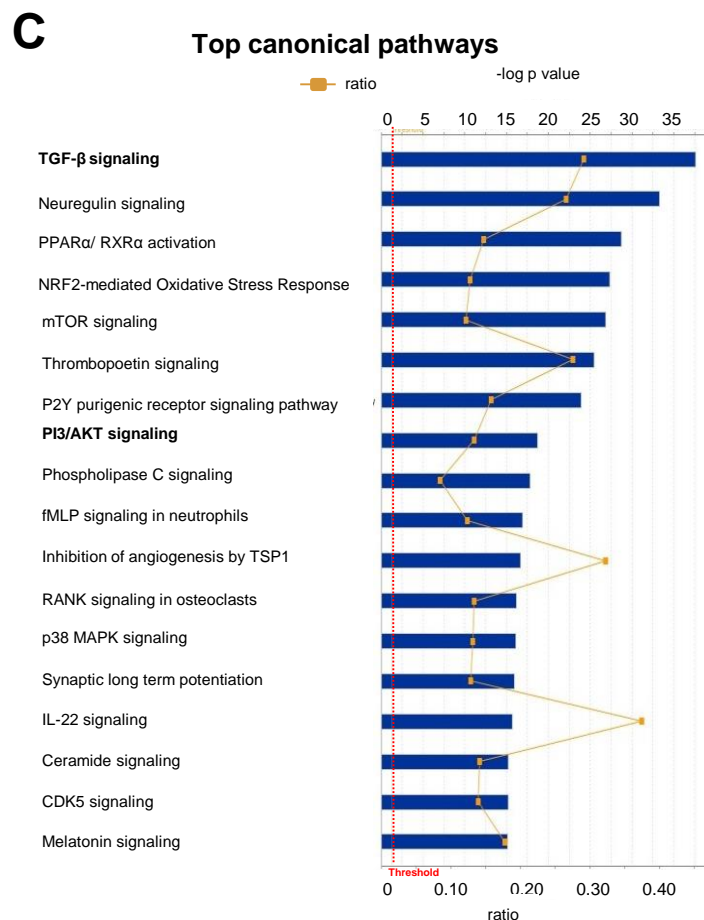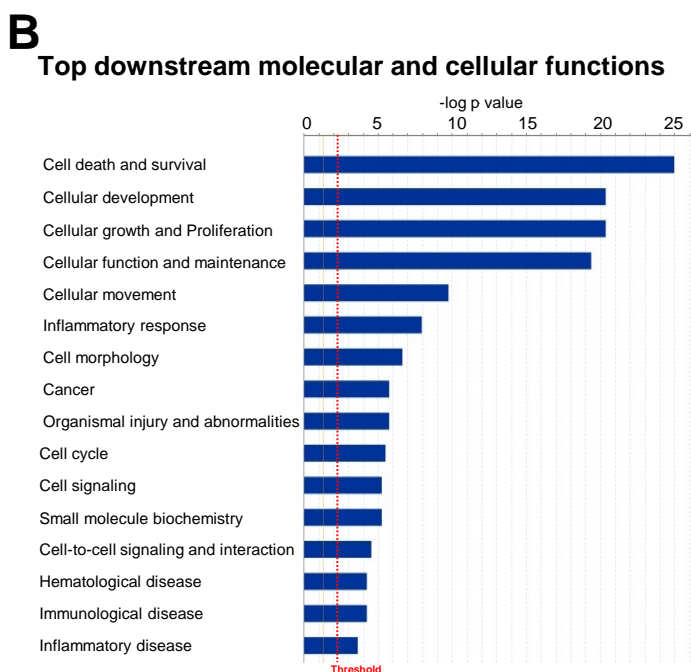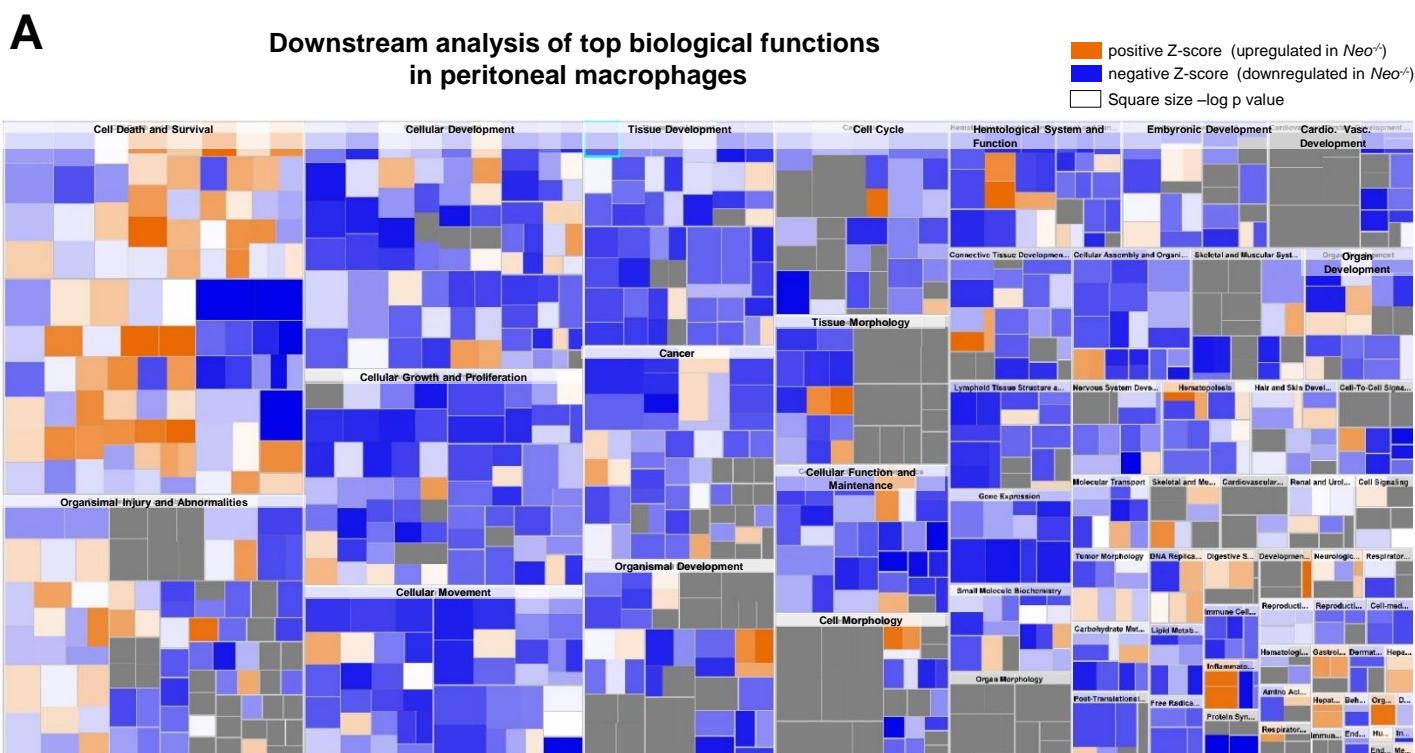
Suppl. Figure 4. Top function, canonical pathways and networks derived from protein profiling of murine peritoneal monocytes of Neo1-deficient mice and WT. Peritoneal lavages of Neo1^{-/-} and WT mice were collected 12 h after ZyA treatment and protein expression and phosphorylation were measured in 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 5

A AA derived lipid mediators

B EPA derived lipid mediators

WT+ ZyA
$Neo1^{-/-}$+ ZyA
ng per 10^7 cells of
peritoneal lavage

LXA$_4$ | 5-HETE | 15-HETE

15-HEPE | 18-HEPE | 14,15-diHETE

C DHA derived lipid mediators

7-HDHA | 19,20-diHDPA | PDX

D Zymosan A Phagocytosis [%]

12/15-LOX$^{+/+}$ | 12/15-LOX$^{-/-}$

IgG Ctrl
anti-Neo1

E Zymosan A Phagocytosis [%]

CDC [µg/ml] 10 100 10 100
Baicalein [µg/ml] -- -- -- --
Neo$^{+/+}$ Neo$^{-/-}$

CDC [µg/ml] -- -- -- --
Baicalein [µg/ml] 10 100 10 100
Neo$^{+/+}$ Neo$^{-/-}$

F Zymosan A Phagocytosis [% increase]

anti-Neo - + - + - +
CDC [10 µg/ml] - - + + - -
CDC [100 µg/ml] - - - - + +

anti-Neo - + - + - +
Baicalein [10 µg/ml] - - + + - -
Baicalein [100 µg/ml] - - - - + +

Baicalein
CDC

DHA ⟶ 5-Lipoxygenase / 12/15-Lipoxygenase ⟵ AA

Mar1
PDX ⟵ ⟶ LXA$_4$

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.

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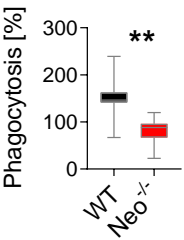
Netrin-1
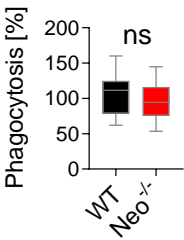
Suppl. Figure 8. Neo1 actions in MΦ efferocytosis: ligand-receptor dependency. Peritoneal MΦ from Neo1^{-/-} and WT mice were collected and incubated with RGM-A or Netrin-1 and efferocytosis of fluorescent ZyA particles was determined. Results represent at least two independent experiments and are expressed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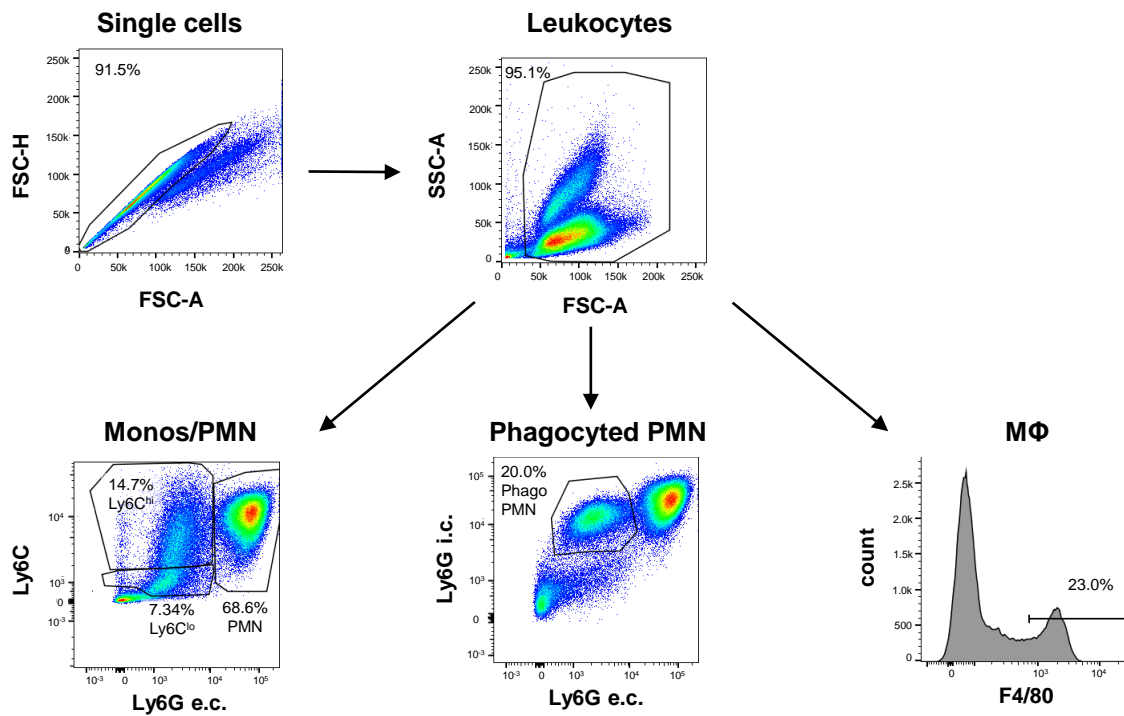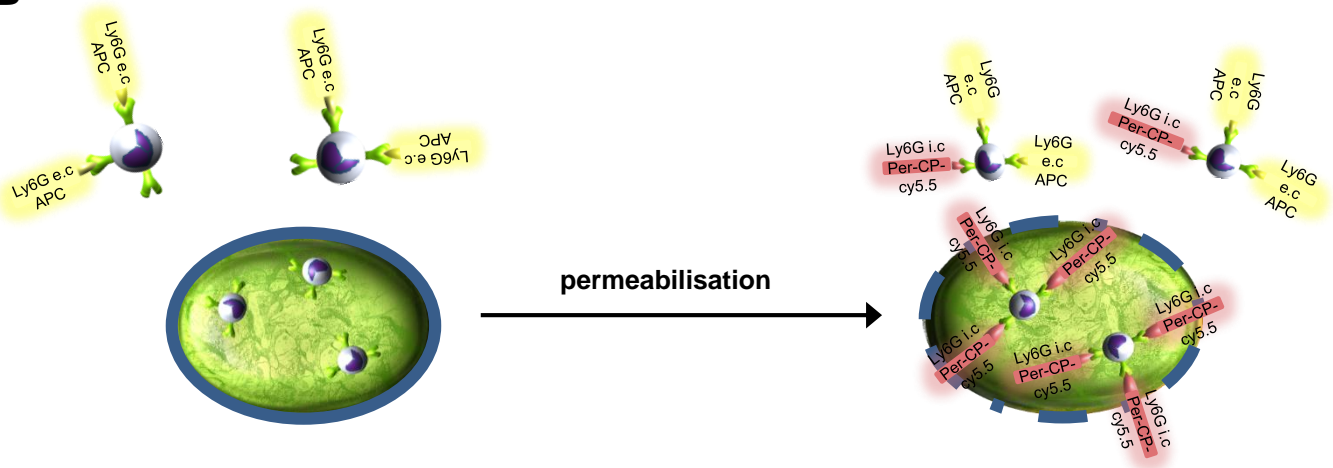
Suppl. Figure 8

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	

Suppl. Table 1

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/m²)	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.

Suppl. Table 3

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$_a$O2 (mmHg)	118 (48-258)	108 (37-266)	109 (42-194)	92 (32-482)
p$_a$CO2 (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)
Ca²⁺ (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 (x10⁹/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 (x10⁹/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.

Suppl. Table 4

	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