

Supplementary Information

Gut-primed neutrophils activate Kupffer cells to promote hepatic injury in mouse sepsis

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Running title: Gut-primed neutrophils in septic liver

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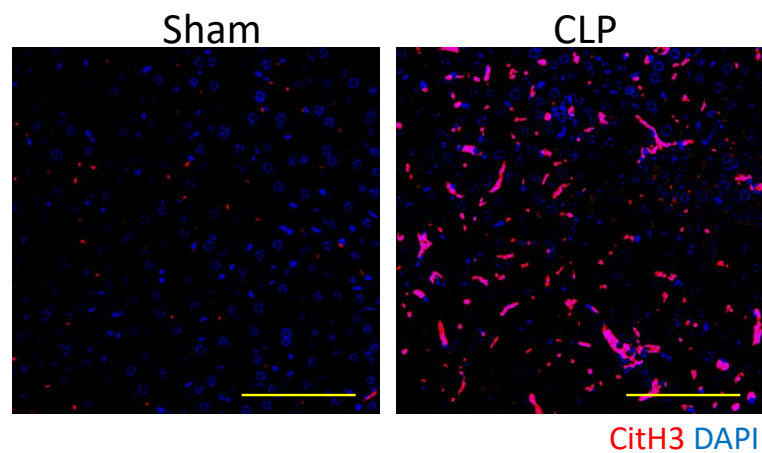
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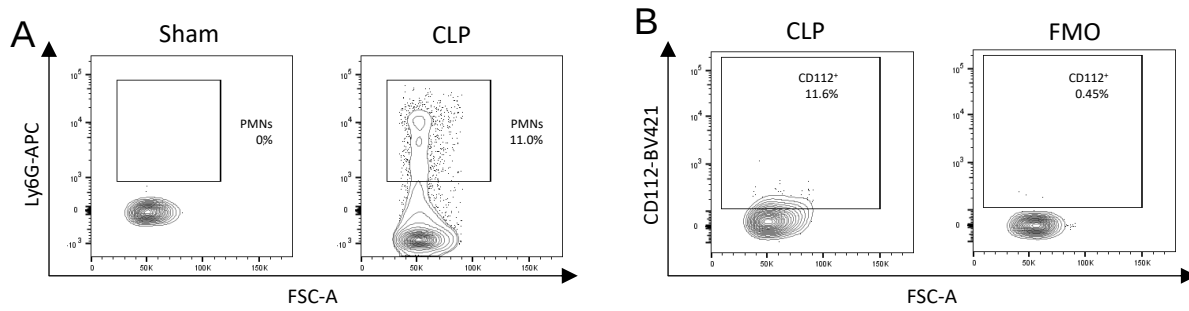
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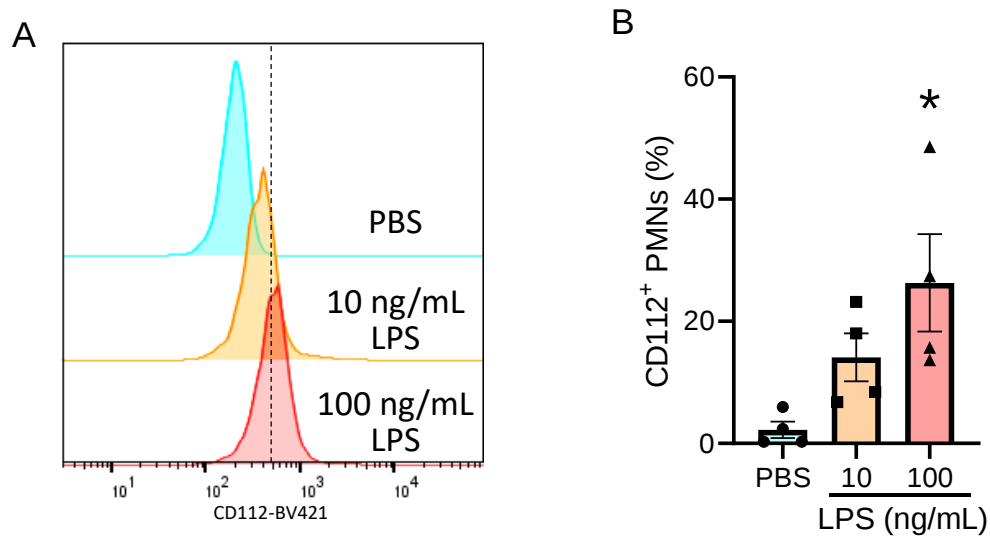
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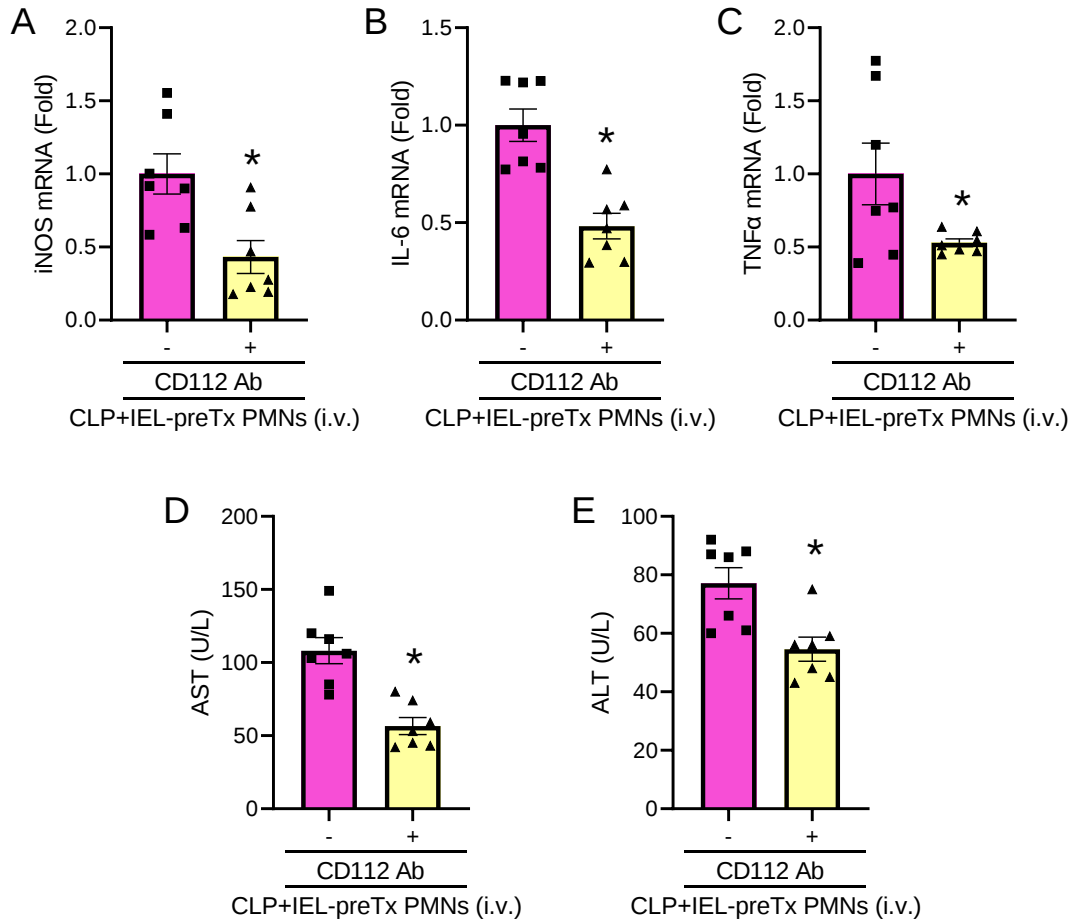
Supplementary Figure 1. Deposition of NETs in the septic liver. Microscopic images of liver NETs assessed by Cit-H3 staining (red) 20 h after sham or CLP surgery ($\times 200$; scale bar: 100 μm). Experiments were performed 3 times, and representative images are shown.



Supplementary Figure 2. Assessment of neutrophils in the gut epithelium by flow cytometry. Representative dot plots showing **(A)** neutrophils (PMNs) in the gut epithelium and **(B)** CD112 expression within the neutrophil population evaluated by flow cytometry. FMO, Fluorescence Minus One.



Supplementary Figure 3. LPS upregulates CD112 expression on neutrophils. Neutrophils were treated with different doses of LPS for 12 h and stained with BV421 anti-CD112 Ab to be evaluated by flow cytometry. **(A)** Representative histograms and **(B)** frequency of CD112⁺ neutrophils are shown. Experiments were performed 2 times, and all data were used for analysis. Data are expressed as mean ± SEM (n=4 samples/group) and compared by one-way ANOVA and SNK test. *p<0.05 vs. PBS. P value: 2.37E-2.



Supplementary Figure 4. CD112 mediates proinflammatory neutrophil-IEL interaction in sepsis. Neutrophils (PMNs) were pretreated with LPS and IELs (IEL preTx) in the presence and absence of anti-CD112 Ab for 12 h and sorted by fluorescence-activated cell sorting (FACS). Sorted neutrophils were adoptively transferred into CLP mice via i.v. to collect the liver and blood after 20 h. mRNA expressions of **(A)** iNOS, **(B)** IL-6, and **(C)** TNF α in Kupffer cells and **(D)** AST **(E)** ALT levels in the serum are shown. Experiments were performed 3 times, and all data were used for analysis. Data are expressed as mean $\pm$ SEM (n=7 samples/group) and compared by paired two-tailed Student's t-test. P values: 7.91E-3 (A); 3.68E-4 (B); 4.75E-2 (C); 3.98E-4 (D); 5.74E-3 (E). *p<0.05 vs. CD112 Ab(-).

Supplementary Table 1. Antibodies used.

Antibody	Dilution/ Amount	Supplier	Catalog number	Clone	Lot number
APC anti-Ly6G	1:100	BioLegend	127614	1A8	B448088
PerCP-Cy5.5 anti-CD11b	1:100	BioLegend	101228	M1/70	B423267
FITC anti-MPO	1:50	Abcam	ab90812	2D4	110654-1
anti-Histone H3	1:200	Abcam	ab5103	N/A	1116477-2
PE anti-rabbit IgG	1:200	BioLegend	406421	Poly4064	B440363
APC anti-F4/80	1:100	BioLegend	123116	BM8	B422785
PE anti-iNOS	1:100	Santa Cruz	sc-7271	C-11	C2125
BV421 anti-CD112	1:100	BD Biosciences	748046	828038	2151227
anti-CD112	10 µg/mL	R&D Systems	MAB3869	N/A	CHTE0123041

Supplementary Table 2. Primer sequences.

β -Actin (NM_007393)	F: 5'CGTGAAAAGATGACCCAGATCA3'
	R: 5'TGGTACGACCAGAGGCATACAG3'
iNOS (NM_010927)	F: 5'GGCAAACCCAAGGTCTACGTT3'
	R: 5'GAGCACGCTGAGTACCTCATTG3'
IL-6 (NM_031168)	F: 5'CCGGAGAGGAGACTTCACAG3'
	R: 5'CAGAATTGCCATTGCACAAC3'
TNF (NM_031168)	F: 5'AGACCCTCACACTCAGATCATCTTC3'
	R: 5'TTGCTACGACGTGGGCTACA3'