SUPPLEMENTAL MATERIAL

The gut microbiota restricts NETosis in acute mesenteric ischemia-reperfusion injury

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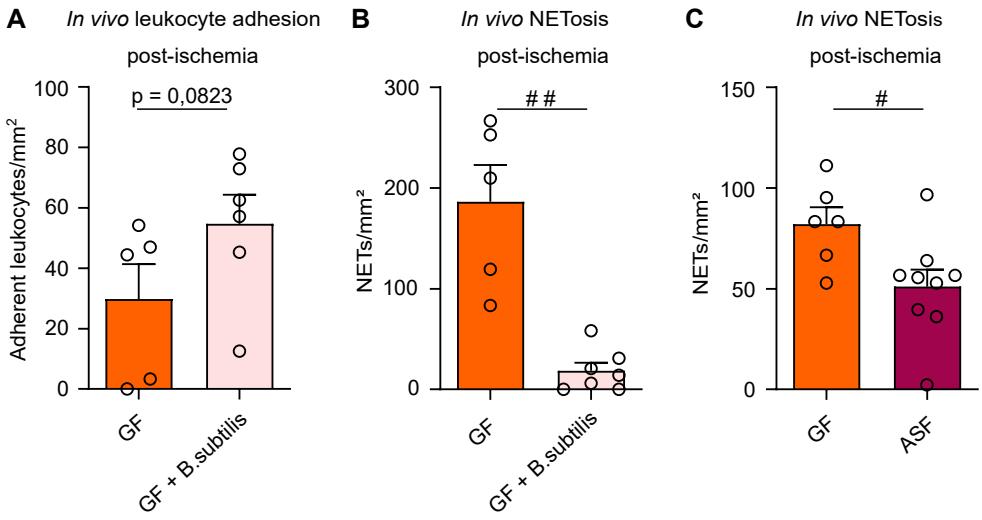
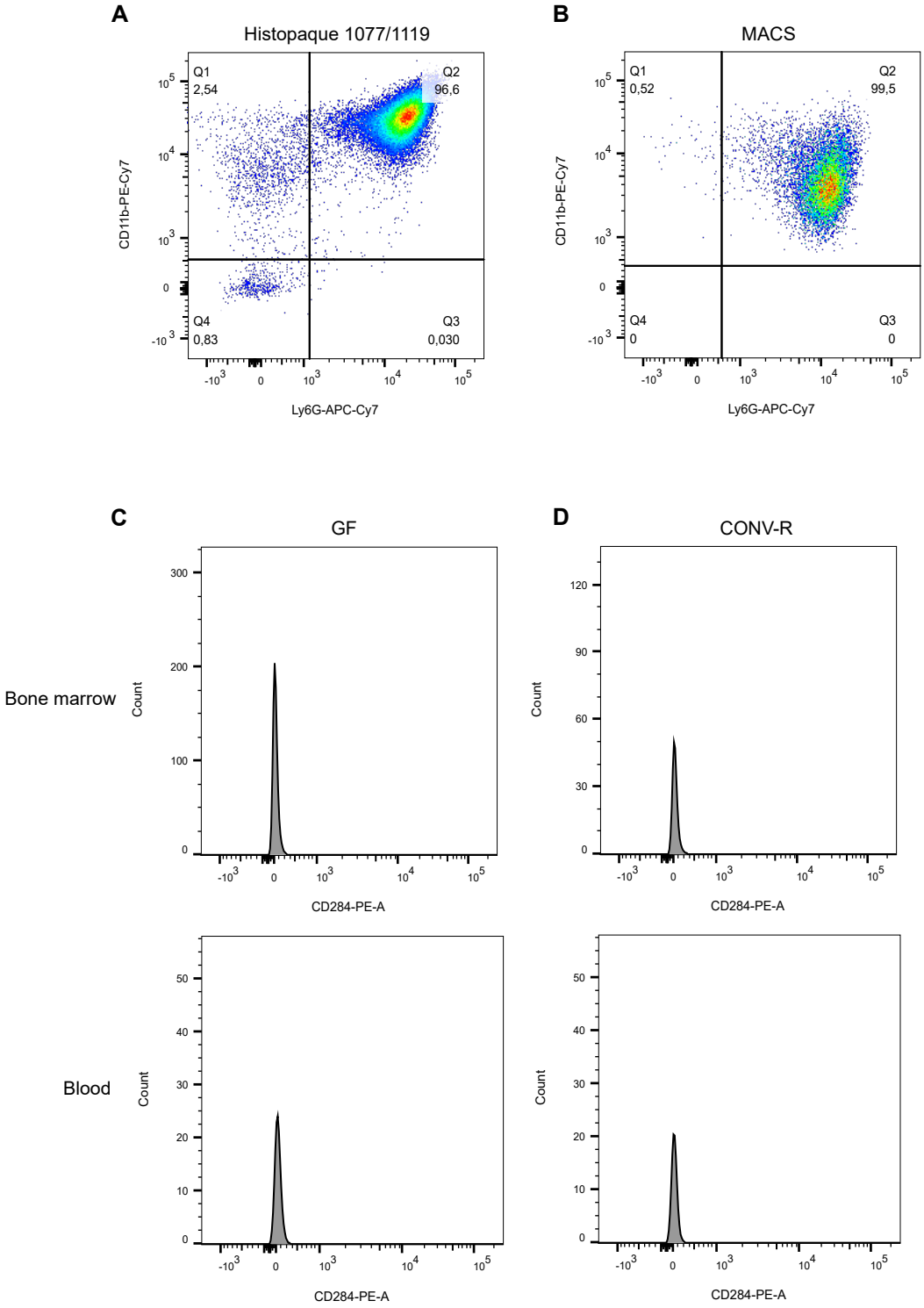
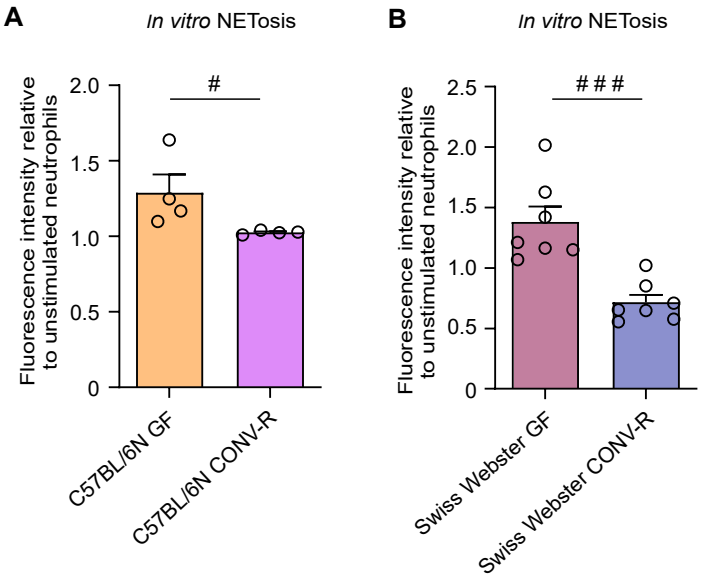
Supplemental Figures



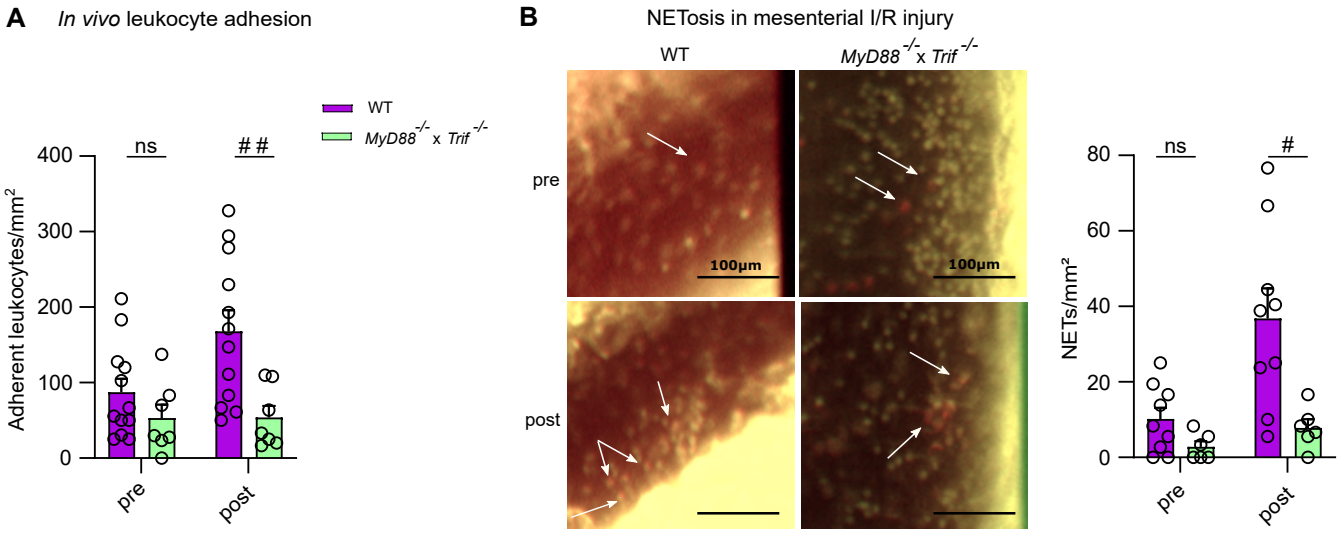
Supplemental Figure I. Colonization leads to supression of I/R-induced NETosis. (**A**) Number of adhering leukocytes one hour post-ischemia in GF and *B.subtilis* monocolonized GF mice (5 vs 6 mice/group). For GF male mice and *B.subtilis* monocolonized GF female mice were used. (**B**) NETosis in mesenteric venules one hour post-ischemia in GF and *B.subtilis* monocolonized GF mice (5 vs 6 mice/group). For GF male mice and *B.subtilis* monocolonized GF female mice were used. (**C**) NETosis in mesenteric venules one hour post-ischemia in GF and altered Schaedler flora (ASF) mice (6 vs 9 mice/group). For GF and ASF male and female mice were used Results are shown as means ± s.e.m. Statistical comparison were performed using the Mann-Whitney (#) test, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.



Supplemental Figure II. FACS analysis of neutrophil isolaton methods and TLR4 surface expression. (**A**) Isolation protocol with Histopaque 1077/1119 density gradient centrifugation (representative FACS histogram). (**B**) Isolation with the magnetic sell sorting (MACS) isolation protocol (representative FACS histogram). (**C, D**) FACS quantification of neutrophil Toll-like receptor-4 (TLR4) surface expression. Bone marrow (upper panels) and blood (lower panels).



Supplemental Figure III. Enhanced NET formation in GF mice is not strain dependent. (**A**) LPS-induced *in vitro* NETosis of cultered bone marrow neutrophils from GF C57BL/6N and CONV-R C57BL/6N mice (4 vs 4 mice/group). For GF C57BL/6N and CONV-R C57BL/6N male and female mice were used. (**B**) LPS-induced *in vitro* NETosis of cultered bone marrow neutrophils from GF Swiss Webster and CONV-R Swiss Webster mice (7 vs 7 mice/group). For GF Swiss Webster and CONV-R Swiss Webster male and female mice were used. Results are shown as means ± s.e.m. Statistical comparison were performed using the Mann-Whitney (#) test, $^{\#}p < 0.05$, $^{\#\#\#}p < 0.001$.



Supplemental Figure IV. I/R-induced NETosis is diminished in *MyD88*$^{-/-}$ *x Trif*$^{-/-}$ double-deficient mice. (**A**) Number of adhering leukocytes pre-ischemia and one hour post-ischemia in CONV-R (WT) and *MyD88*$^{-/-}$ *x Trif*$^{-/-}$ double-deficient mice (12 vs 7 mice/group). For WT and *MyD88*$^{-/-}$ *x Trif*$^{-/-}$ male and female mice were used (**B**) NETosis in mesenteric venules pre- and post-ischemia in CONV-R (WT) and *MyD88*$^{-/-}$ *x Trif*$^{-/-}$ double-deficient mice (9 vs 6 mice/group). For WT and *MyD88*$^{-/-}$ *x Trif*$^{-/-}$ male and female mice were used. Adhering leukocytes were stained with acridine orange; NETs were visualized by SYTOX orange. Results are shown as means ± s.e.m. Scale bare: 100 µm. Statistical comparison were performed using the Mann-Whitney (#) test, $^{\#}p < 0.05$, $^{\#\#}p < 0.01$.

Major Resources Table

Animals (in vivo studies)

Species	Vendor or Source	Background Strain	Sex	Other Information
WT GF	Breeding in our lab	C57BL/6J	Male and Female	Sterile isolator
WT CONV-R	Breeding in our lab	C57BL/6J	Male and Female	SPF
CONV-D	-	C57BL/6J	Female	Conventionalisation of WT GF mice; SPF
WT CONV-R + Abx	-	C57BL/6J	Male and Female	Administration of broad-spectrum antibiotics; SPF
WT GF + *E. coli*	-	C57BL/6J	Male and Female	Monocolonisation with *Escherichia coli*; in a sterile isolator
WT GF + LPS	-	C57BL/6J	Male and Female	Administration of TLR4-agonist, in a sterile isolator
$TLR4^{-/-}$	Breeding in our lab	C57BL/6J	Male and Female	SPF
$MyD88^{-/-}$	Breeding in our lab	C57BL/6J	Male and Female	SPF
$Trif^{-/-}$	Breeding in our lab	C57BL/6J	Male and Female	SPF
$MyD88^{-/-}$ x $Trif^{-/-}$	Breeding in our lab	C57BL/6J	Male and Female	SPF
$TLR4^{wt/wt}$ x VE-Cdh-Cre^{+}	Breeding in our lab	C57BL/6J	Female	SPF
$TLR4^{fl/fl}$ x VE-Cdh-Cre^{+}	Breeding in our lab	C57BL/6J	Male and Female	SPF
ASF	Breeding in our lab	C57BL/6J	Male and Female	Sterile isolator
WT GF + *B. subtilis*	-	C57BL/6J	Male and Female	Monocolonisation with *Bacillus subtilis*; in a sterile isolator

DOI [to be added]

Cultured Cells (in vitro studies)

Name	Species	Vendour or Source	Background Strain	Sex	Other Information
Bone marrow-derived neutrophils	WT GF	Breeding in our lab	C57BL/6J	Male and Female	Sterile isolator
	WT CONV-R	Breeding in our lab	C57BL/6J	Male and Female	SPF
	WT GF + LPS	-	C57BL/6J	Male and Female	Administration of TLR4-agonist, in a sterile isolator
	WT CONV-R + Abx	-	C57BL/6J	Male and Female	Administration of broad-spectrum antibiotics; SPF
	$TLR4^{-/-}$	Breeding in our lab	C57BL/6J	Male and Female	SPF
	C57BL6-N GF	Breeding in our lab	C57BL/6NRj	Male and Female	Sterile isolator
	C57BL6-N CONV-R	Janvier	C57BL/6NRj	Male and Female	SPF
	Swiss Webster GF	Breeding in our lab	Swiss Webster	Male and Female	Sterile isolator
	Swiss Webster CONV-R	Breeding in our lab	Swiss Webster	Male and Female	SPF

Antibodies

Target antigen	Vendor or Source	Working concentration	Catalog #
Anti-Histone H3 (citrulline R2+R8+R17)	Abcam	4 µg/ml	ab5103
Alexa Fluor 555 donkey anti-rabbit IgG	Life Technologies	4 µg/ml	A-31572
PE Rat Anti-Mouse CD162 Clone 2PH1	BD Bioscience	6 µg/ml	555306
PerCP-CyTM5.5 Rat Anti-Mouse CD62L Clone MEL-14	BD Bioscience	6 µg/ml	560513
V450 Rat anti-Mouse LY-6G and LY-6C Clone RB6-8C5	BD Bioscience	6 µg/ml	560453
APC Mouse Anti-Human CD11b Clone ICRF44	BD Bioscience	6 µg/ml	550019
APC anti-mouse Ly-6A/E (Sca-1) Clone D7	Biolegend	2,5 µg/ml	108112
APC anti-mouse CD117 (c-Kit) Clone 2B8	Biolegend	1 µg/ml	105812
APC anti-mouse NK-1.1 Clone PK136	Biolegend	2 µg/ml	108710
APC anti-mouse TER-119/ Erythroid Cells Clone TER-119	Biolegend	1 µg/ml	116212
APC anti-mouse CD3ε Clone 145-2C11	Biolegend	1 µg/ml	100312
APC anti-mouse CD19 Clone 6D5	Biolegend	2 µg/ml	115512

DOI [to be added]

APC/Cy7 anti-mouse Ly-6G Clone 1A8	Biolegend	1 µg/ml	127624
PE/Cy7 anti-mouse/human CD 11b Clone M1/70	Biolegend	0,5 µg/ml	101216
Brilliant Violet 510™ anti-mouse Ly-6C Clone HK1.4	Biolegend	2 µg/ml	128033
PE Rat IgG2a, κ Clone RTK2758	Biolegend	2,5µg/ml	400508
APC/Cy7 Rat IgG2a, κ Clone RTK2758	Biolegend	1,2 µg/ml	400524
PE/Cy7 Rat IgG2b, κ Clone RTK4530	Biolegend	0,5 µg/ml	400618
PE anti-mouse CD284 (TLR4) Clone SA15-21	Biolegend	2,5 µg/ml	145404
IRF-3 (D83B9) Rabbit mAb	Cell Signaling	1:750	4302S
Phospho-IRF3 (Ser386) (E7J8G) XP Rabbit mAb	Cell Signaling	1:750	37829S
α-Actinin (D6F6) XP Rabbit mAb	Cell Signaling	1:1000	6487S

DOI [to be added]