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Supplemental Information

COMMD10-Guided Phagolysosomal Maturation Promotes Clearance of *Staphylococcus aureus* in Macrophages

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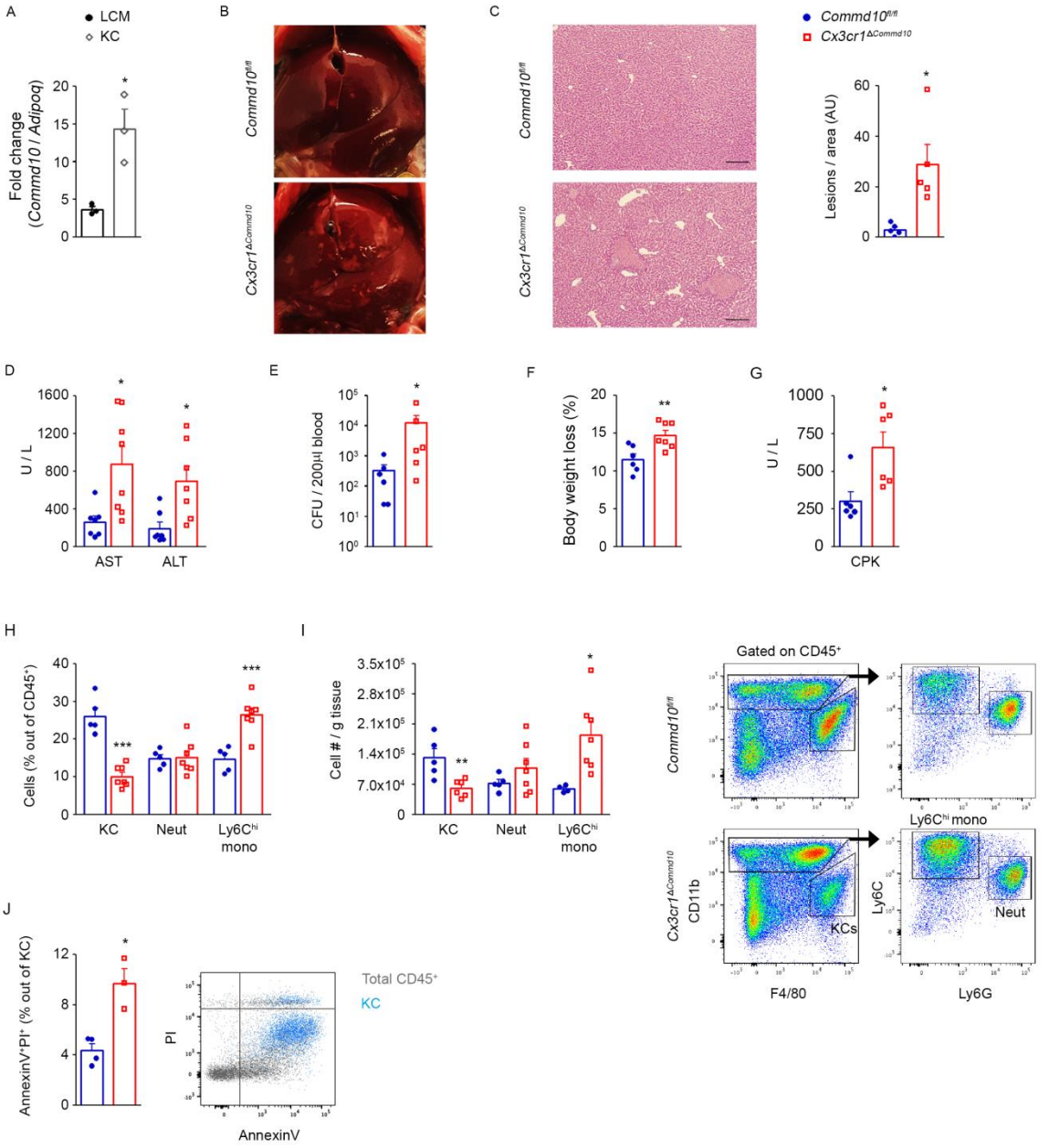
A
LCM
KC
Fold change (Commd10 / Adipoq)
B
Commd10fl/fl
Cx3cr1ΔCommd10
C
Commd10fl/fl
Cx3cr1ΔCommd10
Lesions / area (AU)
D
U / L
AST
ALT
E
CFU / 200µl blood
F
Body weight loss (%)
G
U / L
CPK
H
Cells (% out of CD45+)
KC
Neut
Ly6Chi mono
I
Cell # / g tissue
KC
Neut
Ly6Chi mono
Gated on CD45+
Commd10fl/fl
Ly6Chi mono
Cx3cr1ΔCommd10
CD11b
KCs
Ly6C
Neut
F4/80
Ly6G
J
AnnexinV+PI+ (% out of KC)
PI
AnnexinV
Total CD45+
KC

Figure S1. Increased hepatic and systemic damage and KC death in *Cx3Cr1*$^{\Delta Commd10}$ livers, Related to Figure 1.

(A) *Commd10* gene expression in Liver capsular macrophages (LCM) and KC as extracted from existing databases of sorted KCs (GSE55606) and LCMs (E-MTAB-5932). *Commd10* expression was normalized to the expression of the adipocyte marker *Adipoq* (n=3). (B-J) *Commd10*$^{fl/fl}$ (blue closed circle) or *Cx3Cr1*$^{\Delta Commd10}$ (red open square) mice were i.v. injected with *S. aureus* Rosenbach ($5x10^7$ CFU per animal) and sacrificed 24 h later. (B) Representative images of infected livers. Note the lesions appearing on the surface. (C) Left panel: representative images of Hematoxylin and eosin (H&E) staining of liver sections. Magnification x20, bar, 200 μM. Right panel: quantification of lesions per liver area (AU, arbitrary units) (n=5). (D) Serum AST and ALT levels (Units/Liter) (n=8). (E) CFU per 200 μl blood (n=8). (F) Body weight loss (n≥6). (G) Serum CPK levels (Units/Liter) (n=6). (H) Assessment of KC, neutrophil and Ly6C^{hi} monocyte fraction out of total CD45$^+$ immune cells (n≥5). (I) Assessment of liver-resident KC, neutrophil and Ly6C^{hi} monocyte numbers normalized to tissue mass (n≥5). Right panel, representative flow cytometry images showing the gating strategy of KCs, neutrophils and Ly6C^{hi} monocytes. (J) Assessment of AnnexinV$^+$ PI$^+$ KCs out of total population. Right panel, representative flow cytometry image showing AnnexinV$^+$PI$^+$ KCs (light blue) over total CD45$^+$ immune cells (grey) (n≥3). Data in A, E and J were analyzed by non-parametric Mann-Whitney test, and data in C, D, F-I were analyzed by unpaired two-tailed *t-test*, comparing each time between *Commd10*$^{fl/fl}$ and *Cx3cr1*$^{\Delta Commd10}$ groups. Results are presented as mean ± SEM with significance: *p<0.05 **p<0.01. ***p<0.001

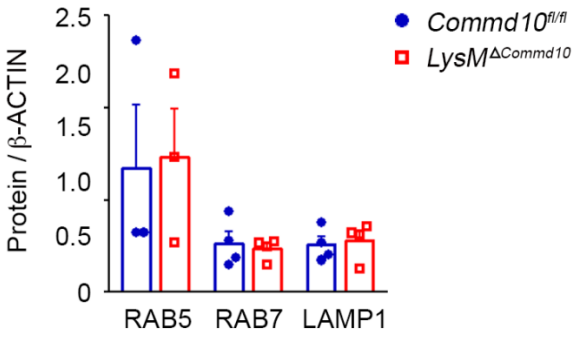
Protein / β-ACTIN
2.5
2.0
1.5
1.0
0.5
0
RAB5
RAB7
LAMP1
Commd10fl/fl
LysMΔCommd10

Figure S2. Baseline expression of phagolysosomal maturation markers, Related to Figure 5.

BMDM from *Commd*$^{fl/fl}$ (blue closed cicrle) or *LysM*$^{\Delta Commd10}$ (red open square) mice were lysed and analyzed by immunoblotting for protein expression of indicated phagolysosome maturation markers (n≥3). β-actin served as control. Densitometry graph is shown based on the zero time-point in immunoblot from Figure 5A. Data were analyzed by non-parametric Mann-Whitney test.

Transparent Methods

Mice

Animal experiments were performed with male adult C57BL/6J mice (8–12-wk old). Animals were maintained in specific pathogen-free animal facility and experiments were performed according to protocols and regulatory standards required by the Animal Care Use Committee of the Sourasky Medical Center (24-8-18). $LysM^{\Delta Commd10}$ and $Cx3cr1^{\Delta Commd10}$ mice were generated by crossing $Lyz2^{cre}$ and $Cx3cr1^{cre}$ (Yona et al., 2013) mice with $Commd10^{fl/fl}$ mice that were purchased from the EUCOMM consortium (strain EM:05951) (C57BL/6J background). Experiments with $LysM^{\Delta Commd10}$ and $Cx3cr1^{\Delta Commd10}$ mice were performed on mice heterozygous for these genes.

BMDM and neutrophil preparation

BMDM were prepared by flushing BM from the femur and tibia and culturing in RPMI medium containing FBS (10%), penicillin (100 IU/ml), streptomycin (100 μg/ml) and macrophage-colony stimulating factor (M-CSF, 20 ng/ml), at 37°C in 5% CO_2. Media was supplemented every 2-3 days. On day 6, macrophages were harvested and plated overnight at an assay-dependent concentration. Neutrophils were isolated using the neutrophil isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany cat# 130-092-332). Neutrophils were enriched to high purity (above 99%) and identified using flow cytometry as $CD45^+CD11b^+$ $CD115^-Ly6G^+$ cells.

Isolation of liver KCs

Hepatic non-parenchymal cells were isolated by collagenase digestion buffer (0.5 mg/ml collagenase (C2139, Sigma), 5% Fetal bovine serum, PBS) followed by gradient centrifugation (Zigmond et al., 2014). KCs were enriched by selective adherence to plastic. Cells were seeded in DMEM containing 10% FBS, 1% L-Glutamine and 0.1% penicillin streptomycin, and incubated for 2 h in 5% CO2 at 37°C. Non-adherent cells were then

removed by gently washing with $PBS^{-/-}$. Cells were cultured for 7 days, (Gilboa et al., 2017). KC purity (88%) was determined by flow cytometry analysis. KCs were defined as $CD45^{+}CD11b^{int}F4/80^{+}CD64^{+}$ $Tim4^{+}MHCII^{+}$ cells.

Bacterial infection of KCs, BMDM and neutrophils

The following bacteria species were grown in LB broth at 37°C overnight: *S. xylosus*, *S. aureus Rosenbach* (clinical isolated ATCC-29213) and *SH1000-GFP S. aureus* ($rsbU^{+}$ NCTC8325-4 derivative (kindly provided by Dr. Ingo Schmitz, Helmholtz Center for Infection Research, Germany). Infection experiments were performed, unless indicated otherwise, at multiplicity of infection (MOI) = 5, with early log phase bacteria. At 30 min after infection, cells were washed and supplemented with gentamicin (100 ng/ml) to eliminate extracellular bacteria. At indicated times cells were washed twice with $PBS^{-/-}$ (without Ca^{++} and Mg^{++}), and collected for different analyses. The *SH1000-GFP S. aureus* were used in fluorescent assays; otherwise the *S. aureus Rosenbach* was used. With respect to TFEB, BMDM were infected with late log phase *S. aureus* at MOI=10.

Bacterial viability-colony forming unit assay

Tissues were weighed and homogenized (Polytron PT-MR 2100) in 1 ml $PBS^{-/-}$. One million BMDM or KCs were homogenized in 0.1% triton x-100. Appropriate dilutions were seeded on LB agar plates and incubated at 37°C for 24 h. Isolated neutrophils (500,000/24 multiwell plate) were seeded in RPMI containing 10% FBS, 1% L-Glutamine and 0.1% penicillin streptomycin, and incubated for 30min in a 5% CO2 at 37 °C. Non-adherent cells were removed by washing. Colonies were counted and presented as CFU per g tissue.

Protein immunoblotting

Total protein from one million BMDM was extracted in ice-cold RIPA buffer (C-9806S, Cell Signaling Tech. Beverly, Massachusetts) containing protease inhibitors (P8340, Sigma Aldrich St. Louis, Missouri). Proteins were detected by immunoblotting using standard

techniques. Antibodies used were: mTOR (2972), phosphorylated-mTOR ser2448 (2971) from Cell Signaling; GCN5 (sc-20698), RAB7 (sc-376362), RAB5 (sc-46692), β-ACTIN (sc-47778) from Santa Cruz; TFEB (A303-673A-T) from Bethyl Laboratories Inc; LAMP1 (ab24170), Cathepsin D [EPR3057Y] (ab75852) from Abcam; anti-COMMD10 antibody (GTX121488) from Genetex and RABAPTIN 5 (610676) from BD Biosciences, CCDC22 (16636-1-AP), CCDC93 (20861-1-AP) from ProteinTech Group, C16orf62 (PA5-28553) from Pierce. Blots were incubated with HRP-conjugated secondary antibodies, and subjected to chemiluminescent detection using the MicroChemi imaging system (DNR Bio-Imaging Systems, Israel). Densitometry was performed using ImageJ software. TFEB subcellular fractionation was performed using NE-PER nuclear/cytoplasmic extraction kit (78835, Thermo scientific, Paisley, UK) per manufacturer's instructions. Equivalent protein amounts were loaded for both nuclear and cytoplasmic fractions.

Confocal microscopy

BMDM and liver KCs were seeded (200,000/24 multiwell plate) overnight on cover glass. On the following day, cells were washed with medium and infected with *SH1000-GFP S. aureus* at MOI 20 for 1h. With respect to KCs, cells were infected at MOI 5 for 1h. Cells were then washed 3 times with $PBS^{-/-}$ and supplemented with 100 ng/ml gentamycin until indicated time points. Cells were fixed with 4% PFA for 10 minutes at 37°C. Slides were blocked and permeabilized with 0.1% saponin in blocking reagent (B10710, Thermofisher Scientific) for 45 min at room temperature, followed by overnight incubation at 4°C with specific antibodies for LAMP1 (ab24170) and COMMD10 (bs-8181R, Bioss, Woburn, Massachusetts). Slides were washed and then incubated for 1 h with the secondary antibody anti-rabbit Alexa Flour 647 (A31573, Life Technologies). Subsequently, slides were washed and mounted with fluorescent mounting medium without DAPI (E18-18, GBI labs, Bothell, Washington). For LysoTracker Red DND-99 (Invitrogen) staining, infected cells were

incubated with 50 nM dye for 30 min, washed with PBS, fixed with 2% PFA for 15 minutes and observed under the fluorescence microscope. Images were acquired using Zeiss LSM 700 confocal microscope with x100 (1.4 Oil DIC) and x40 (1.3 Oil DIC) oil objectives. Quantification of bacteria was performed utilizing ImageJ software on ≥10 separate fields (1 cell per field) for each group.

Quantitative RT-PCR

Total RNA was extracted from *S. aureus* infected BMDM using the RNeasy Mini Kit (QIAGEN, Hilden, Germany). RNA was reverse transcribed with a high-capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, California). All PCR reactions were performed with SYBR Green PCR Master Mix kit (Applied Biosystems) and Applied Biosystems 7300 Real-Time PCR machine. Quantification of PCR signals of each sample was performed by the ΔCt method normalized to *Gapdh*. Gene primers are listed in table**:**

	Forward	Reverse
Acp5	ATCATGTCTCTGGGGGACAA	AGAGACGTTGCCAAGGTGAT
Commd1	TGGCGAAGATGAGAGGACTT	GTGATGCCACCTTGCTTTTT
Ccdc22	CCTTGGAGCTTGGCTATCAG	AATGGCCCGAAGGAAAATAG
Ccdc93	GCAGCTGGCTATTTCAGAGC	AAGAGCAAATCCACGTCCAC
Commd10	TGAGAAGTTCCGCCAGAG	TTGCTCACTCCCAGTTGC
Commd2	CTC AAA GCT CAT GAT TTC	TGT CCA GAT AAA GCT GAA G
Commd3	TGA CAG AGA GCG AAT AGA AC	TGC CTA TAC TTC CCA GTA G
Commd4	CCA AGA TGT CCT CTG TGA AG	ACA TCG CCT GAC TCA AAC
Commd5	CTT TCA GAT GGG TCA GCA TAC	TTC AGA TAT CGC TTG AAG AGC
Commd7	GGC GCG CAG CAG TTC TCA	CGA TGC TTC TGA GGG AGC CAA G
Commd8	GTT TGG GAA TCA GAA GAA TG	GCT GAA ATA TCT CTT CAT CAG
Commd9	CCTCCTCTGACAACATCAGC	GGAGGGTTTCTCTCCACAC
Ctsb	AAATCAGGAGTATACAAGCATGA	GCCCAGGGATGCGGATGG
Ctsd	CTGAGTGGCTTCATGGGAAT	CCTGACAGTGGAGAAGGAGC
Ctsk	ATGTGAACCATGCAGTGTTGGTGG	ATGCCGCAGGCGTTGTTCTTATTC
Gapdh	TGCAGTGGCAAAGTGGAGAT	TGCCGTGAGTGGAGTCATACT
Il1b	GCTGAAAGCTCTCCACCTCA	AGGCCACAGGTATTTTGTCG
Il6	GTTCTCTGGGAAATCGTGGA	TTTCTGCAAGTGCATCATCG
Lamp1	AGGCCACTGTGGGAAACTCATACA	TTCCACAGACCCAAACCTGTCACT
Lamp2	ATTGGGGTATTCACCTGCAA	TTGGAGTTGGAGTGGGTGTT
Lipa	CACCTGGTCTCTGAAGCACA	GCCTTGAGAATGACCCACAT
Scarb2	GGTGTGTTCTTTGGCTTGGT	TAGGTTCGTATGAGGGGTGC
Nox2	CCCTTTGGTACAGCCAGTGAAGAT	CAATCCCAGCTCCCACTAACTCA

ELISA

Supernatants from BMDM were collected. The level of IL-1β was assessed with the DuoSet ELISA kit (R&D Systems).

Flow cytometry analysis

The following anti-mouse antibodies were used (dilutions are indicated): CD45 (clone 30-F11, 1:100), CD11b (clone M1/70, 1:300), CD64 (clone X54-5/7.1, 1:50), TIM4 (clone RMT4-54, 1:50), all purchased from BioLegend, San Diego, USA. Anti-mouse F4/80 (clone A3-1, 1:50) was purchased from BIORAD. The staining for ROS was performed with 0.1 mM of 2,7-dichlorodihydrofluoresceindiacetate (Molecular Probes Invitrogen). Staining for apoptosis and necrosis markers with Annexin V and propidium iodide (PI) was performed with MEBCYTO-Apoptosis Kit (MBL International Corporation). Cells were analyzed with BD FACSCanto™ II (BD Bioscience). Flow cytometry analysis was performed using FlowJo software (TreeStar, Ashland, OR, USA).

Staining of bacteria with pHrodo

S. aureus Rosenbach were incubated with 10μM pHrodo (P36600, Life Technology) in the dark at 37°C, with shaking (250 rpm) for 30 min. Bacteria were centrifuged at 9300g for 1 min and the pellet was washed and resuspended in $PBS^{-/-}$ (Jubrail et al., 2016). BMDM were infected with the pHrodo-tagged bacteria as mentioned above and fixed in 0.2% PFA for 30 min at RT. About 80 macrophages were counted and the number of pHrodo bright/dim bacteria per macrophage was counted. Images were acquired using the confocal microscope (1.4 oil DIC lens). Additionally, pHrodo fluorescence intensity in infected macrophages was measured by a fluorescent plate reader.

Measurement of intracellular ROS

BMDM were infected with *S. aureus* (MOI=5). At indicated times, cells were washed with $PBS^{-/-}$ and incubated with 20μM 2′,7′-dichlorofluorescin diacetate (DCFH-DA) (D6883, Sigma Aldrich) for 30min at 37°C. Cells were analyzed by flow cytometry.

Cathepsin D activity measurement

BMDM were infected with *S. aureus* (MOI=5) as described above for 2 h. Cathepsin D activity (fluorescence released by cleavage, HiLyte Fluor^{TM} 488) was measured by fluorescence microplate reader using SensoLyte 520 Cathepsin D assay kit (AS-72170, Anaspec EGT Group, Fremont, CA).

Internalization of *S. aureus* bioparticles

S. aureus internalization into BMDM was assessed by using opsonized *S. aureus* bioparticles. Briefly, *S. aureus*, Wood strain without protein A (S-2851, Molecular probes), were opsonized with bioparticles opsonizing reagent (S-2860, Molecular probes) at a 1:1 ratio for 1 h at 37ºC, followed by 3 washes with $PBS^{-/-}$. Bioparticles were added to BMDM at MOI=50 for 3 h. Cells were washed, detached, and fluorescence intensity was analyzed by flow cytometry. Additionally, 500,000 BMDM were seeded in bottom glass cell culture dish and challenged as described above. Internalization was imaged by confocal microscopy (1.3 Oil DIC lens). Cells with 10 or more bacteria bioparticles were imaged.

In vivo S. aureus infection model

Mice were injected with early log phase *S. aureus*, $5x10^7$ CFU, in 200μl saline via the tail vein. Mice were sacrificed at indicated time points and liver and blood were collected for different analyses.

Gene-expression data mining

Gene expression of *Commd10* and *Adipoq* was extracted out of existing databases of sorted steady state KCs (Zigmond et al., 2014) and LCMs (Sierro et al., 2017), deposited at the

National Center for Biotechnology Information Gene Expression Omnibus public database2 (GSE55606) and ArrayExpress database (E-MTAB-5932), respectively.

Quantification of hepatic damage

Liver samples were obtained at 24 h after *S. aureus* infection, fixed (4% para-formaldehyde), paraffin-embedded, sectioned, and stained with H&E. Pathologic evaluation was performed by an expert pathologist. The number of necrotic lesions was calculated and normalized to sample area as measured by ImageJ software in scanned slides. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were measured using a Hitachi 747 Automatic Analyzer.

Statistical analysis

Statistical differences between two groups were determined accordingly: in cases where samples distributed normally according to Kolmogorov-Smirnov Test we used unpaired two-tailed t-test with GraphPad. When the sample did not distribute normally, we used Mann Whitney Test with GraphPad. Statistical differences between three groups were determined using one way ANOVA with Tukey post-tests using Graphpad. Significance was defined if p-value was less than 0.05 as following: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.