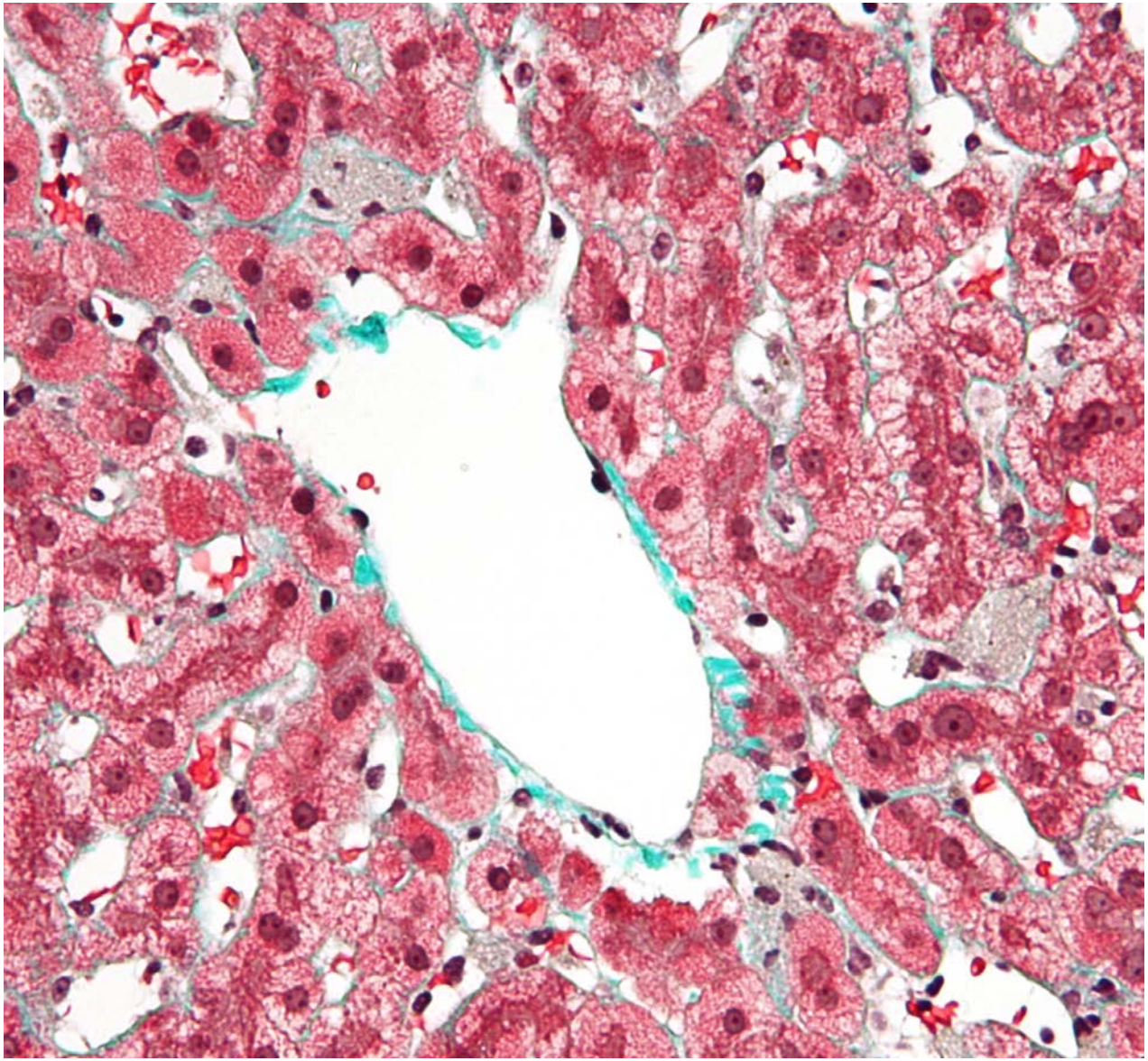
Liver-Resident Macrophage Necroptosis Orchestrates Type 1 Microbicidal Inflammation and Type-2-Mediated Tissue Repair during Bacterial Infection

Camille Blériot, Théo Dupuis, Grégory Jouvion, Gérard Eberl, Olivier Disson, and Marc Lecuit

Dominika Lukovic
PhD Student at the Department of Internal Medicine II



Introduction

Liver innate immune effectors against bacterial infection

Tissue-resident macrophages

- Known as Kupffer cells
- Local self-renewal activity
- Embryonic derived
- Type-2-like anti-inflammatory phenotype (M2-like)

Monocyte-derived macrophages

- Bone-marrow derived
- Type-1-like pro-inflammatory phenotype (M1-like)

M1-like vs. M2-like phenotype

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M1 macrophages (Monocyte-derived M.)

-activated by LPS and IFN gamma

-secret high levels of IL-12, low levels of IL-10

-inhibit cells proliferation and promote tissue damage

-pro-inflammatory response

-“Fight” program

-arginine-> nitric oxide

M2 macrophages (Kupffer cells)

-activated by IL-4

-secret high levels of IL-10, TGF-beta and low level of IL-12

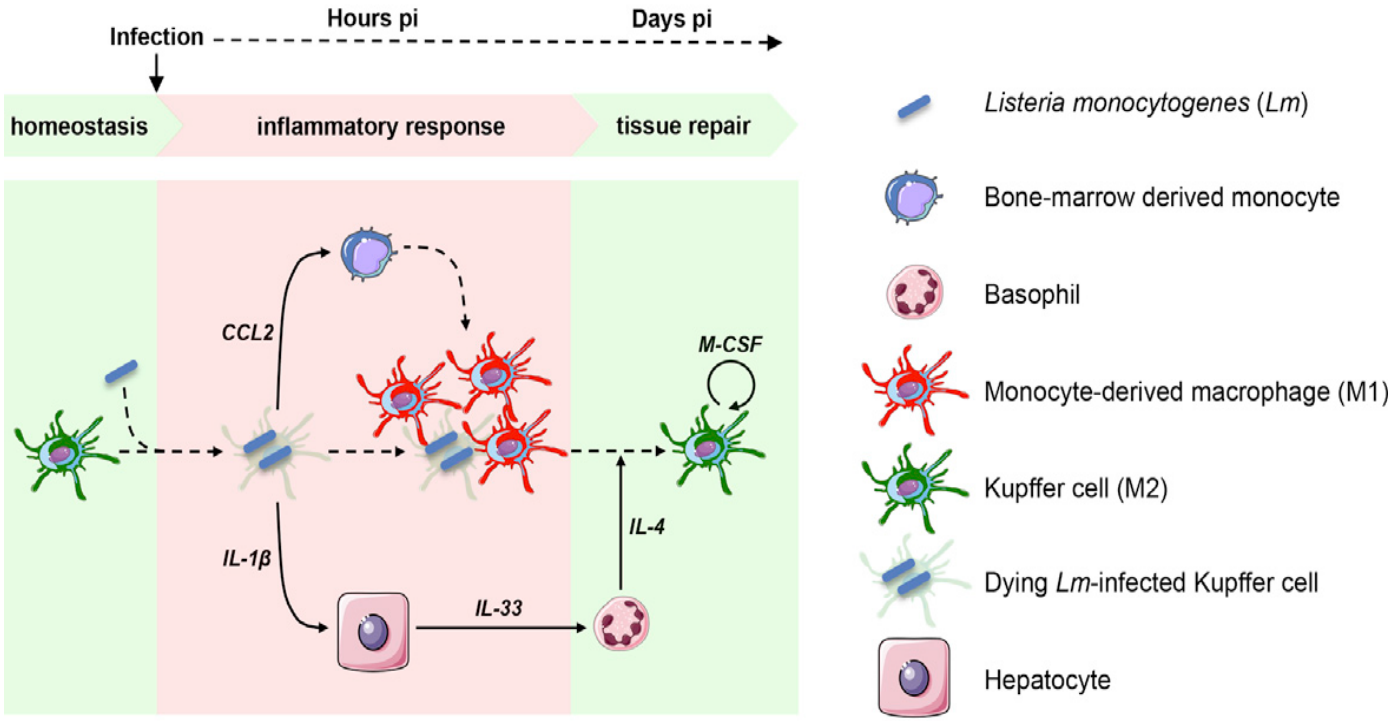
-Promote cell proliferation and tissue repair

-anti-inflammatory response

-“Fix” program

-arginine-> ornithine

Elimination of microorganisms by liver



1.LM enters intestinal barrier and reaches the liver 2. LM is engulfed by KCs

3. Recruitment of monocytes 4. Micro-abscesses formation 5. pro-inflammatory response

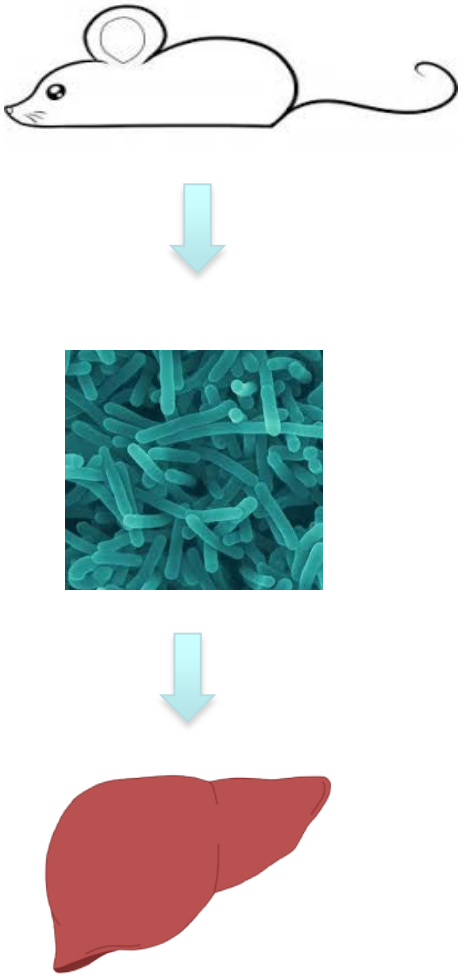
Lm Induces Local Proliferation of Liver Macrophages

- Macrophages (F4/80+)
- Liver cells (E-cadherin +)
- Neutrophils (Ly/6G+)

- After the infection increased total liver macrophages

- Macrophages proliferation was detectable in 24 hours, peaked at 3 dpi

- Bacteria were totally cleared before 10 dpi



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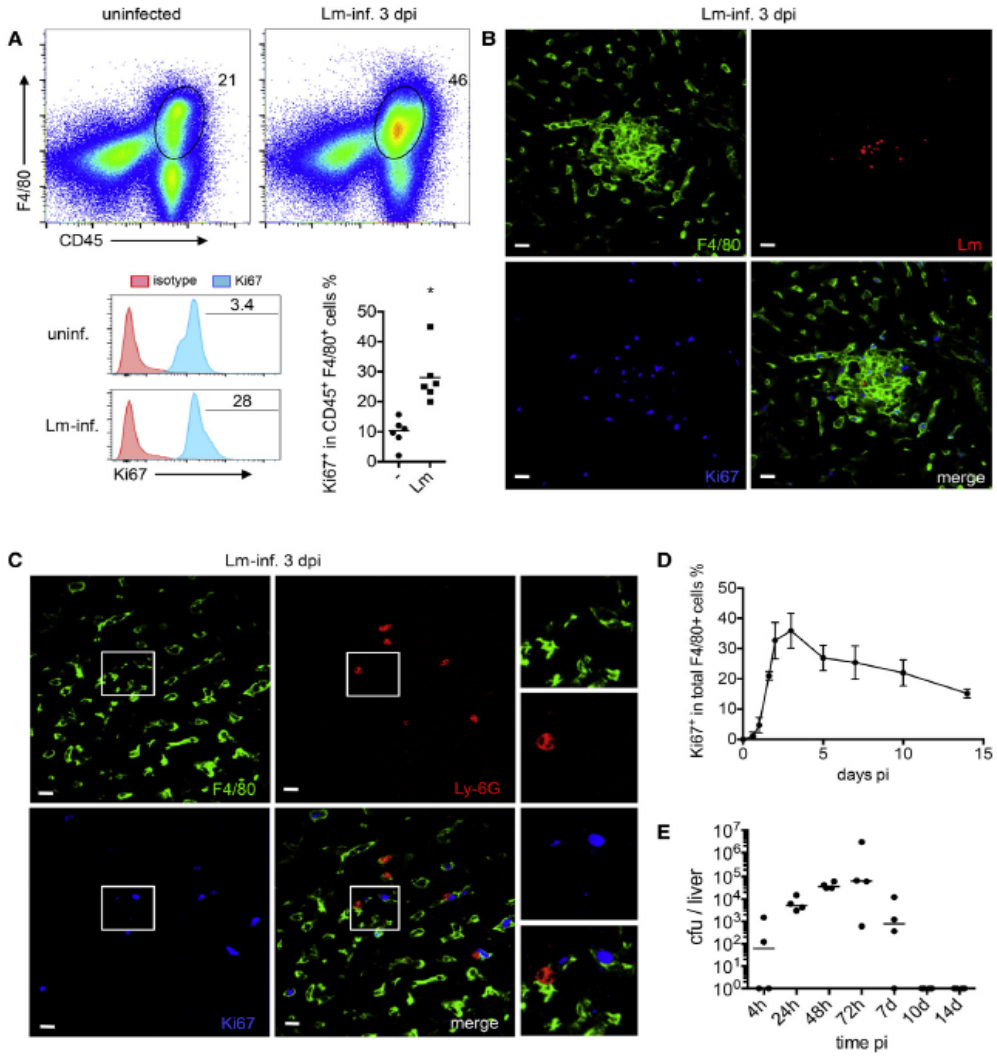


A- FACS analysis of liver cells (Percentage of Ki67+ cells out of CD45+F4/80+ cells)

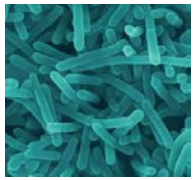
B+C- Confocal imaging of frozen liver sections (F4/80, Ki67, Listeria, Ly-6G)

D- Quantification over time of F4/80+Ki67+ cells in forzen liver sections

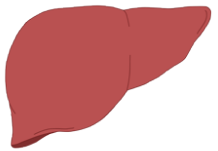
E- Kinetics of liver bacterial load from WT mice

Lm-induced Liver macrophage Proliferation Requires **M-CSF** and **Basophil-Derived IL-4**

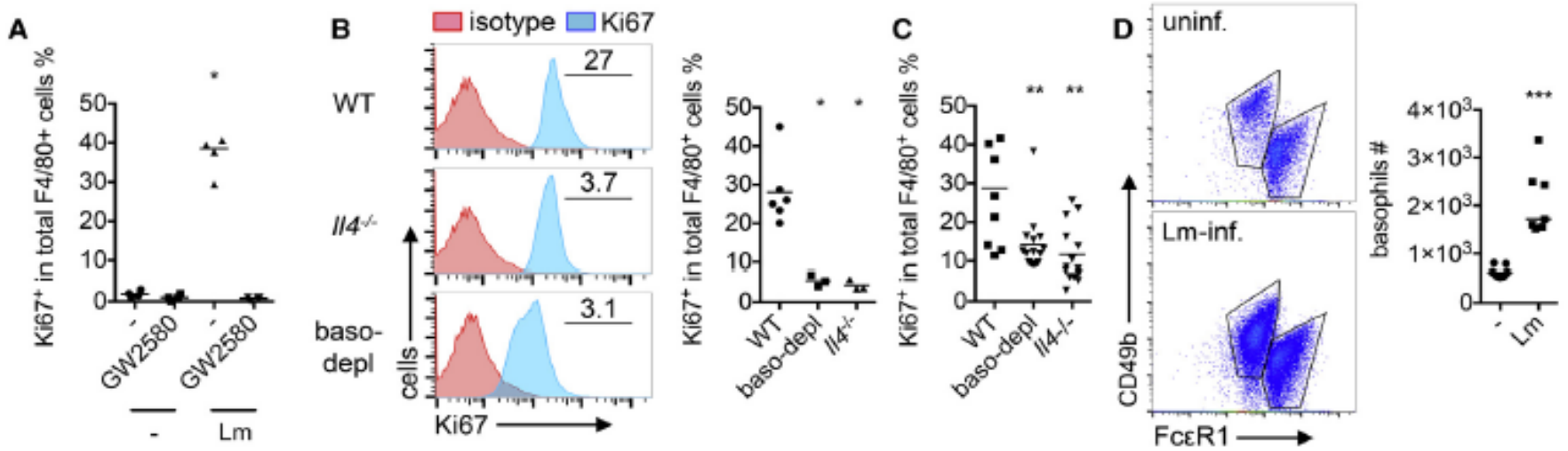
- GW2580 (inhibitor of M-CSFR)
- total liver macrophages decreased in infected and uninfected mouse
- In IL4$^{-/-}$ mice was decreased liver macrophage proliferation, whereas level of bone marrow monocyte was stable
- Basophils as a source of IL4 (CD49b^{int}FcεR1^{int}CD117^{-})



+GW2580



Lm-induced Liver macrophage Proliferation Requires **M-CSF** and **IL-4**



A- Quantification of F4/80+Ki67+ cells in liver sections (G2580 is M-CSFR inhibitor)

B-FACS analysis of gated CD45+F4/80+ liver cells, percentages of Ki67+ cells

C- Quantification of Ki67+ cells out of F4/80+ cells on frozen sections of the liver

D- FACS analysis of liver CD45+ cells (Basophils CD49b^{int}FcεR1^{int}CD117^{-})

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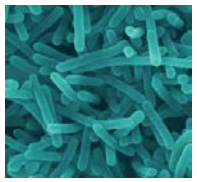
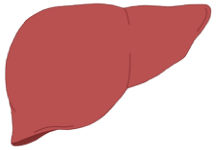
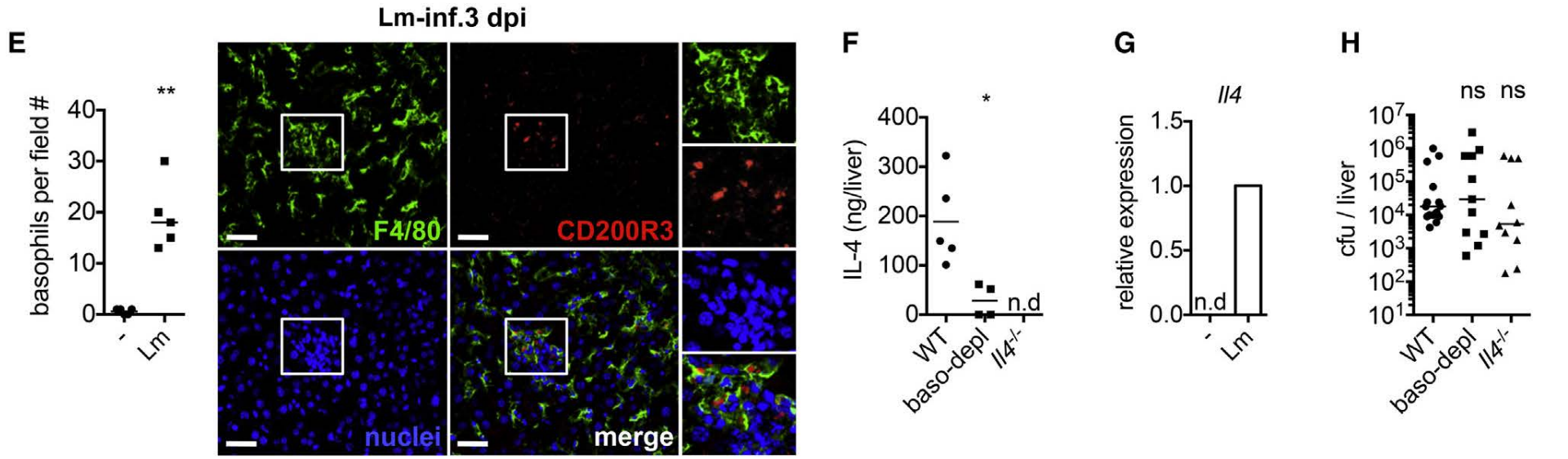




Lm-induced Liver macrophage Proliferation Requires **M-CSF** and **Basophil-Derived IL-4**



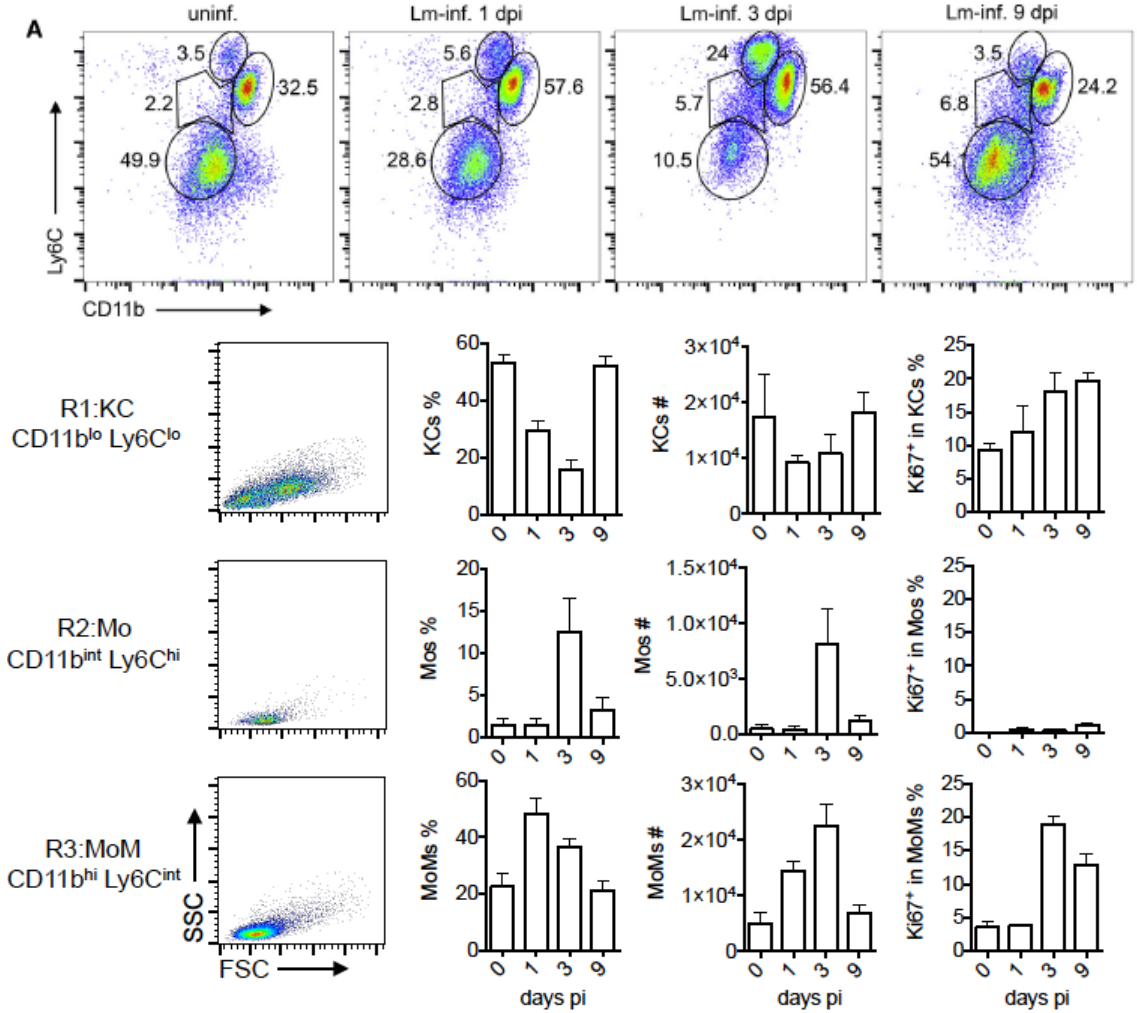
E- Confocal imaging on frozen liver sections (CD200R3 basophils)

F-ELISA of Il-4 in supernatants of the homogenized liver of of the LM-infected mice

G- relative expression of IL-4 in sorted liver basophils obtained in uninfected and infected mice

H- *LM* bacterial burden in the liver of *LM*-infected mice

Proliferating Liver Macrophages Derive from Recruited Monocytes



A-FACS analysis of gated CD45+/F4/80+ liver cells

KCs ($F4/80^{hi}CD11b^{lo}Ly6C^{lo}$);

inflammatory monocytes ($F4/80^{lo}CD11b^{int}Ly6C^{hi}$);

monocyte –derived macrophages ($F4/80^{int}CD11b^{hi}Ly6C^{int}$)

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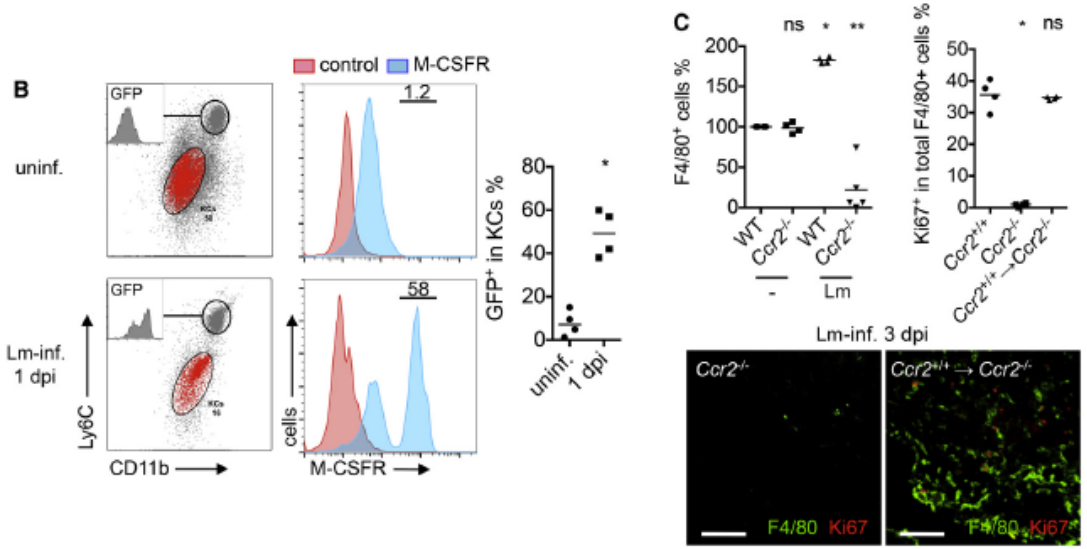
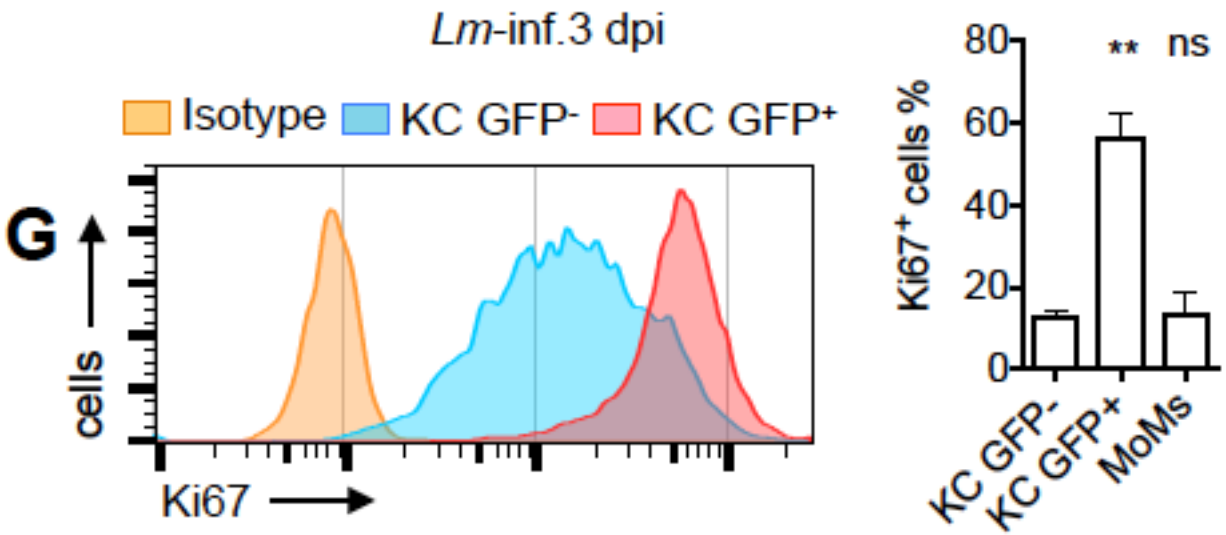


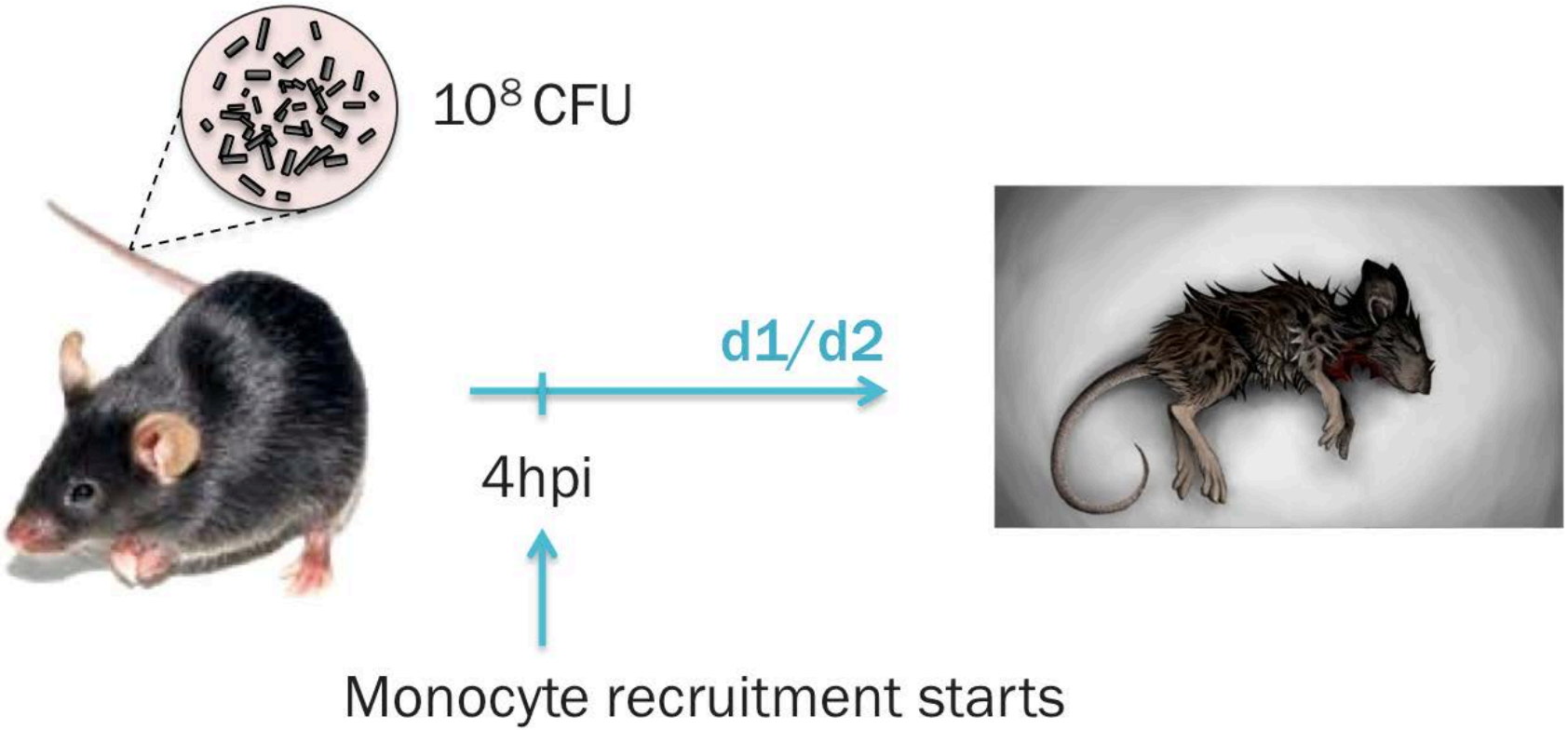


B- FACS analysis of liver KCs from WT mice.

C- Quantification of F4/80+ and F4/80+Ki67+ cells

G- FACS analysis of liver KCs obtained from GFP+ monocyte-transferred WT

Lm induces Kupffer Cell Necroptosis



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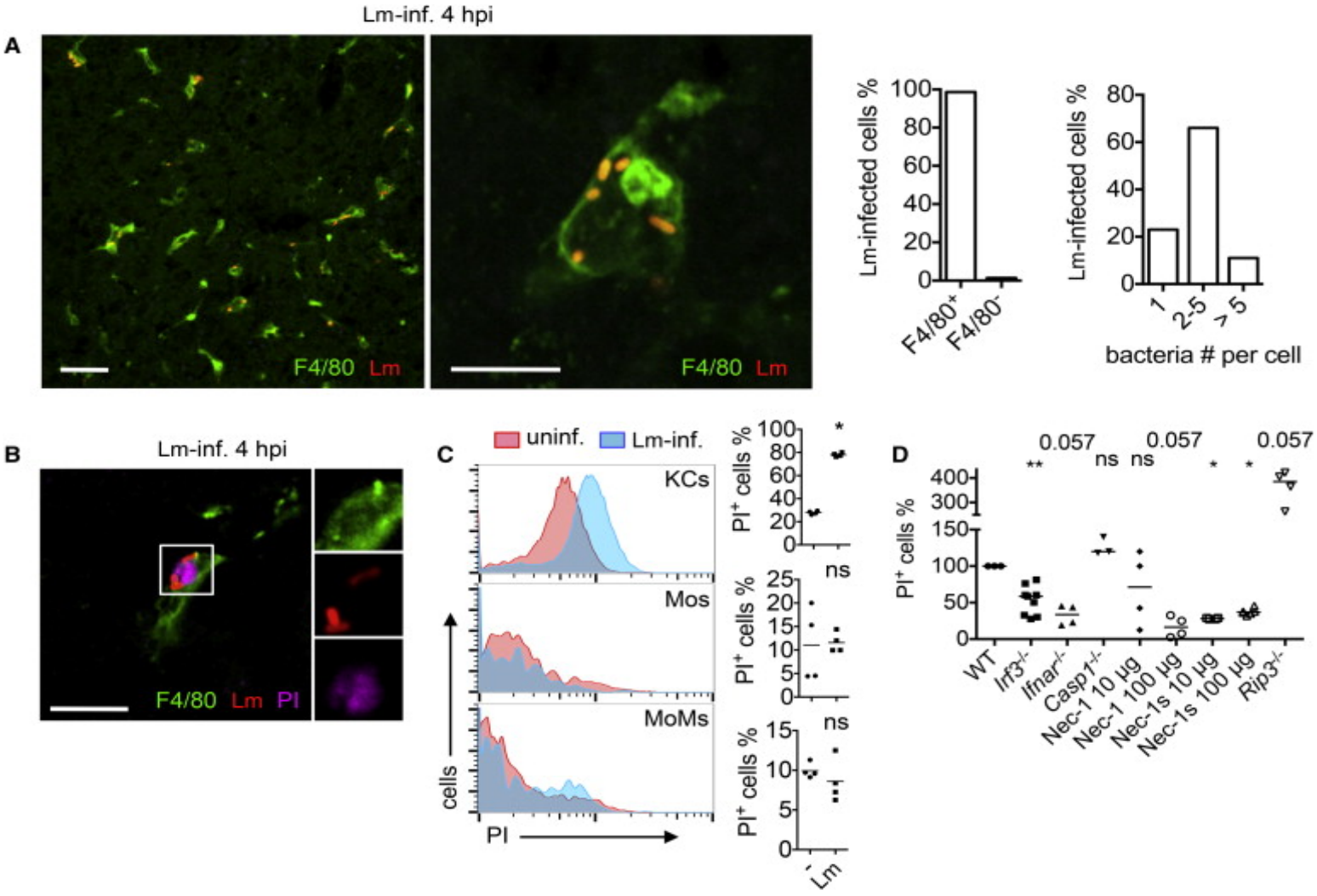
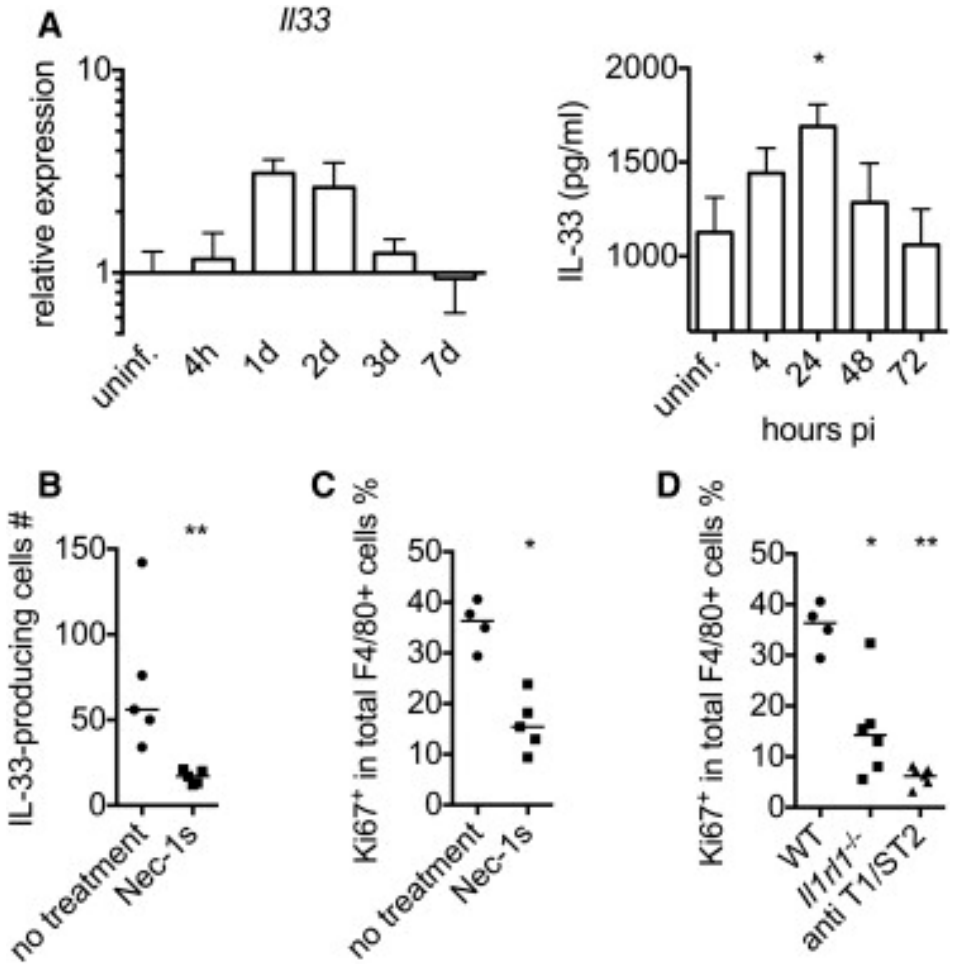


Hepatocytes-derived IL-33 induces monocyte-derived macrophage proliferation



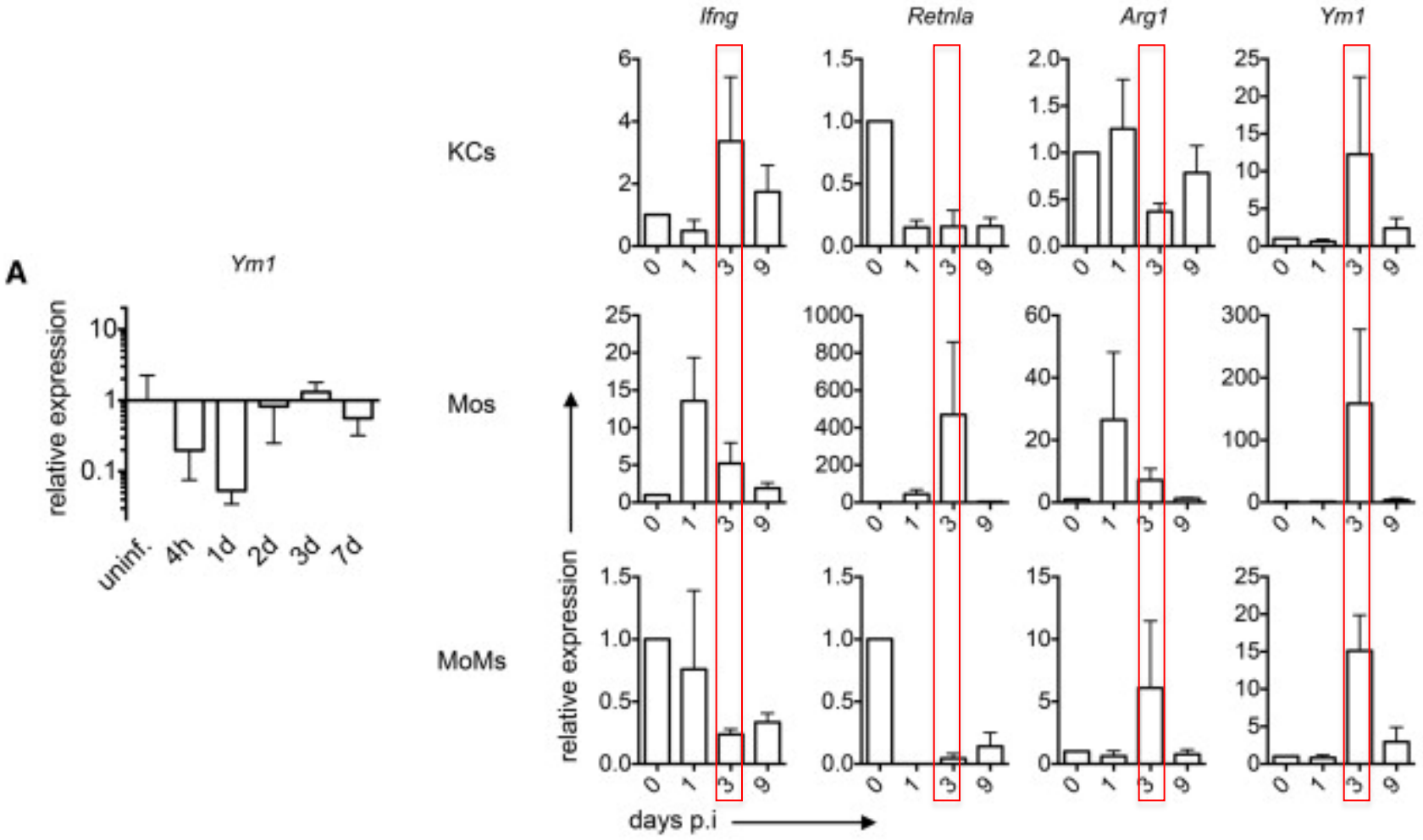
A: IL-33 production peaks at 24h (expression & ELISA)

B: blocking cell death by necrostatin-1s >> decreased IL-33 production

C: necrostatin-1s >> decreased proliferation of macrophages

D: IL-33-receptor deficient mice shows decreased MoMs proliferation.
Same with Ab inhibition of IL33 receptor.

The Type 1 inflammatory liver response to Lm is counterbalanced by type 2 response



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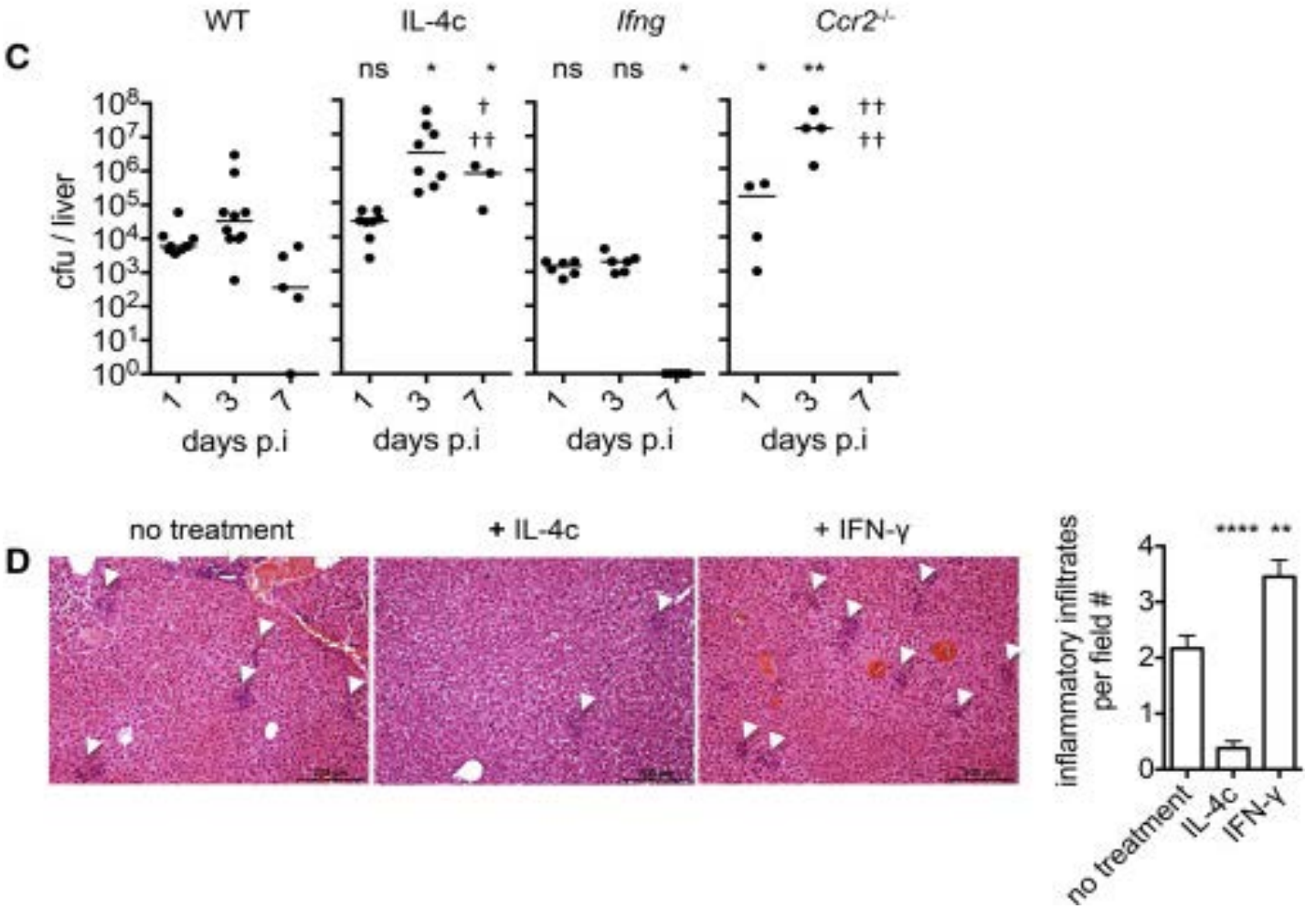
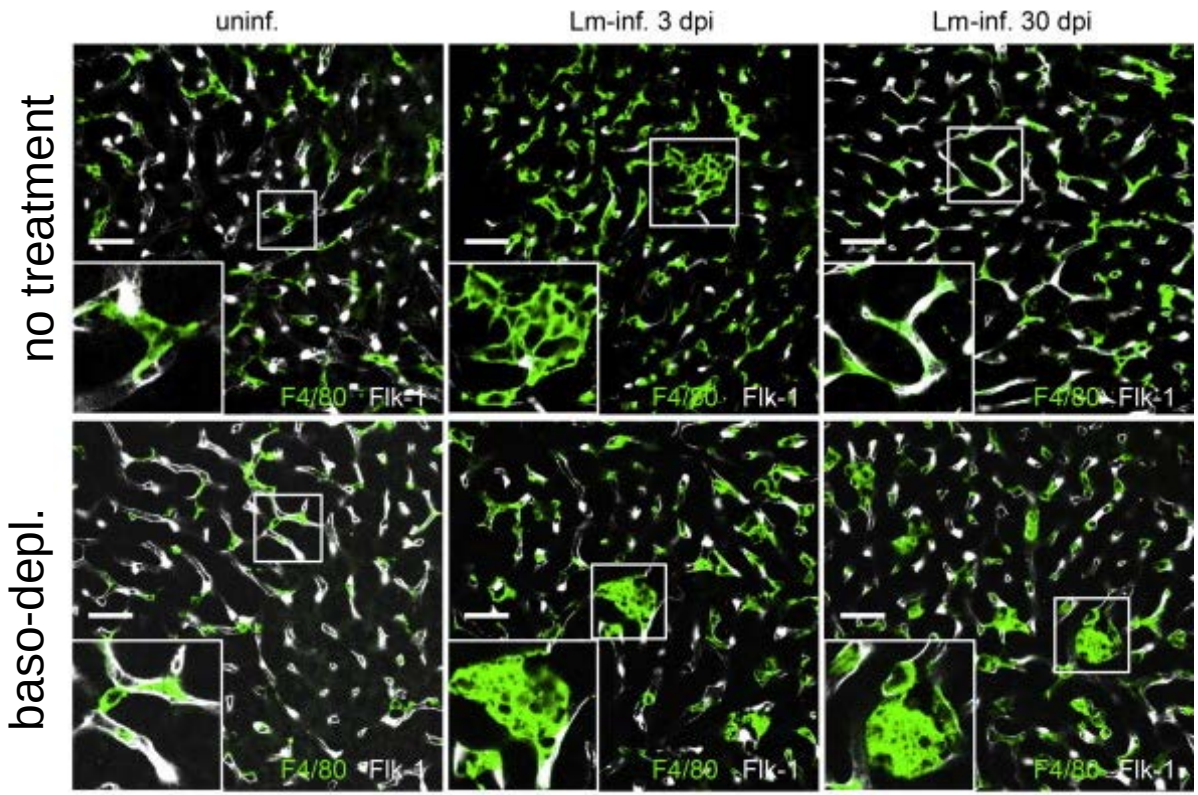
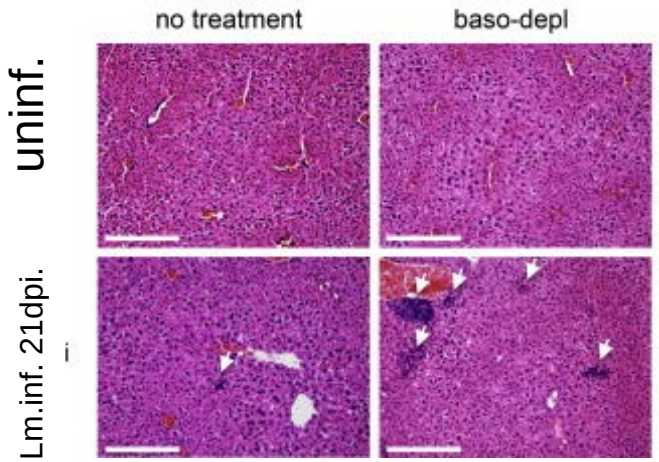
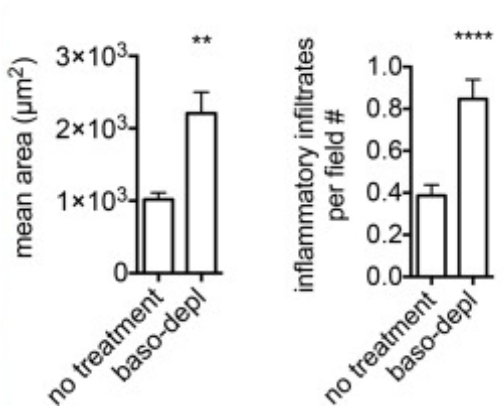


Lm-induced macrophage proliferation dampens inflammation allowing the liver to return to homeostasis







Lm hepatic infection

Lm infection

KCs death

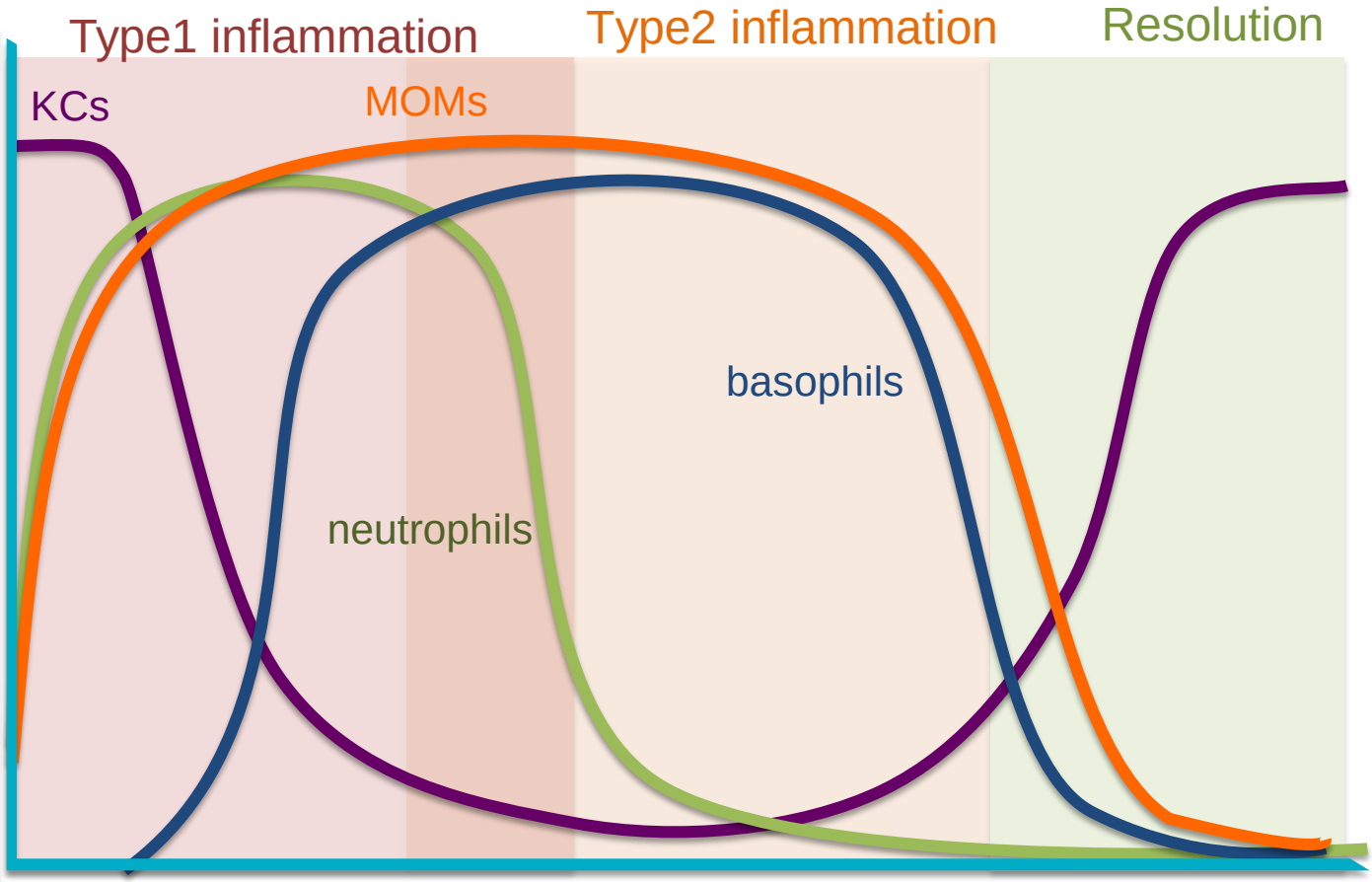
Recruitment & differentiation

(M1) **MoMs**

Phagocyt.
Protective responses

M2

Replace KCs



Summary

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- Phagocytized bacteria induce necroptosis of liver- resident macrophages
- Macrophages necroptosis triggers both type 1 and type2 responses
- Monocyte-derived macrophages replace dead tissue resident macrophages
- Sequential type 1 and type2 responses orchestrate liver return to homeostasis

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Thank you for attention