

Day 0 CD11c+ CD11bhi Ly6Chi 1 66.8 CD11c+ CD11bhi Ly6Clo 2 10.2 41.5 10.5 CD11c⁺ CD11b^{lo} Ly6C^{hi} 3 17.5 27.7 19.7 34.1 65.9 33.8 18.0 CD11c+ CD11blo Ly6Clo 4 Day 1 CD11c⁻ CD11b^{hi} Ly6C^{hi} 5 18.2 27.0 24.7 29.4 6 CD11c⁻ CD11b^{hi} Ly6C^{lo} CD11c⁻ CD11b^{lo} Ly6C^{hi} 7.9 29.8 43.4 44.8 55.2 7 15.8 21.5 Day 7 CD11c⁻ CD11b^{lo} Ly6C^{lo} 8 9.6 51.9 14.2 25.2 С Population 1 **Population 2 Population 3 Population 4 Population 5 Population 6 Population 7 Population 8** Day 0 2.1 3.7 6.1 3.1 1.6 2.1 0.0 4.0 ø 41-96.0 98.4 97.9 96.3 93.9 96.9 100.0 97.9 Day 1 2.2 2.6 2.5 5.9 0.5 0.3 3.9 0.8 8 4 À) H 96.1 99.7 97.8 97.5 94.1 97.4 99.2 99.5 Day 7 12.4 3.4 12.4 3.4 2.7 2.7 3.7 3.7 97.3 97.3 96.6 96.6 96.3 87.6 87.6 96.3 **Podoplanin Isotype** Day 0 0.0 7.0 0.0 5.7 0.0 0.0 2.6 0.0 97.4 100.0 100.0 94.3 93.0 100.0 100.0 100.0 Day 1 1.0 2.1 0.0 0.5 0.0 0.0 0.0 2.8 4 97.2 100.0 1 99.0 97.9 100.0 100.0 99.5 100.0 Day 7 1.0 1.5 0.8 0.3 3.6 0.9 0.5 0.4 Podoplanin 99.5 99.7 98.5 99.6 96.4 99.2 99.1 99. CD11b

Hitchcock Supplementary Figure 2

F4/80

30.2

CD11c

69.8

-y6C

ÇD11b

-y6C

 F480+ CD11c+
 F480+ CD11c

 17.0
 4.4

 37.8
 4.4

CD11b

F4/80

17.6

Ly6G

Α

В

Population

Phenotype

1		Population 1	Population 2	Population 3	Population 4	Population 5	Population 6	Population 7	Population 8
	Day 0	0.0	0.0	9.1	0.0	0.0	0.0	0.0	0.0
lls									
י⁺ כפ	Day 1	28.6	11.8	62.5	0.0	100.0	100.0	75.0	0.0
odplanir									
P	Day 7	15.5	11.9	50.0	18.7	11.0	6.7	68.0	43.3



CD11b

В



A CD11b^{hi} Lv6C^{hi} Pdpn⁺ IFN γ^+ CD11b^{hi} Lv6C^{lo} Pdpn⁺ IFN γ^+ CD11b^{lo} Lv6C^{hi} Pdpn⁺ IFN γ^+ CD11b^{lo} Lv6C^{lo} Pdpn⁺ I

Hitchcock Supplementary Figure 5





1 Supplemental Figure Legends

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3 Supplementary Figure 1. Podoplanin is expressed by macrophages throughout infection

WT mice were infected i.p. with 5 x 10^5 CFU STm and podoplanin expression was examined during infection in parenchymal (top panel) and vascular (bottom panel) regions by IHC on frozen liver sections using podoplanin (brown), F4/80 (blue). Images are representative of 3 experiments where $n \ge 4$ mice in each group. Scale bars represent 100 µm; arrows = inflammatory foci; V = blood vessel, T = thrombus.

9 Supplementary Figure 2. Podoplanin is expressed by multiple macrophage populations 10 during infection

11 WT mice were infected i.p. as above. Leukocytes were isolated from livers and podoplanin 12 expression by macrophage populations was measured by flow cytometry. A) Representative 13 FACS plots: $F4/80^+$ Ly6G^{lo} cells were subdivided by expression of CD11c, then further 14 characterised into 8 populations according to expression of CD11b and Ly6C (listed in B). C) 15 Representative FACS plots of podoplanin expression in each population (top panel) and 16 isotype control (bottom panel). All FACS plots are from a representative experiment, (one of 17 4 repeat experiments), with each group containing ≥ 4 mice.

18 Supplementary Figure 3. IFNγ is produced by macrophage populations in the liver 19 during infection

EYFP IFN γ reporter mice were infected as above for 7 days and IFN γ production was examined by flow cytometry in leukocytes isolated from the liver. Macrophage populations were classified according to the gating strategy outlined in Figure 7;IFN γ -producing cells were identified by EYFP expression. Representative FACS plots are shown from experiments 24 performed twice with ≥ 4 mice per group at each time-point. Absolute numbers of cells are 25 shown in Supplementary Figure 4.

Supplementary Figure 4. IFNγ is produced by macrophage populations in the liver during infection

EYFP IFNy reporter mice were infected as above for 7 days and IFNy production was 28 examined by flow cytometry in leukocytes isolated from the liver, according to the gating 29 strategy defined in Figure 8. Representative FACS plots are shown in Supplementary Figure 30 3. Absolute numbers of EYFP⁺ cells from A) podoplanin⁺ and B) podoplanin⁻ subsets of 31 populations 1-8 (top panel: $CD11c^+$ populations; bottom panel: $CD11c^-$ populations). Data 32 are representative of experiments performed twice with ≥ 4 mice per group per time-point. 33 Statistical significance (One-way ANOVA with Dunnett's test) was determined relative to NI 34 mice $** = p \le 0.01$, $*** = p \le 0.001$. 35

Supplementary Figure 5. Absence of CLEC-2 on platelets does not affect podoplanin expression

WT and PF4.Cre.CLEC-2^{f1/f1} mice were infected as above for 7 days. Podoplanin expression was measured by flow cytometry on leukocytes isolated from the liver. A) The gating strategy used to broadly define podoplanin-expressing populations. B) Representative FACS plots of podoplanin expression by CD11c⁺ F4/80⁻ (red), CD11c⁻ F4/80⁺ (blue) and CD11c⁺ F4/80⁺ (green) populations. Data are representative of 4 repeat experiments, with each group containing \geq 4 mice.

44 Supplementary Figure 6. Von Willebrand Factor co-localises with CD41⁺ platelet 45 thrombi

46 WT mice were infected as above for 7 days and frozen liver sections were examined by 47 confocal fluorescence microscopy; CD31 (green), CD41 (red), von Willebrand factor (VWF) 48 (blue), Hoechst (grey); right panel = higher magnification of boxed region. Images are 49 representative of a minimum of 3 experiments, where $n \ge 4$ mice per group. Scale bars 50 represent 100µm unless otherwise stated.

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