Supplementary Figure Legends

Figure S1. Intrasplenic cDC1 migration in *Dock8*$^{-/-}$ mice is not impaired, related to Figure 1.

(A) Tracking of intrasplenic DC migration into the white pulp before (naïve) or 6 h after 1x10^8 live rLM-Ova infection (6 h *Listeria*) using in vivo labeling. Injection of anti-CD11c intravenously 3 m before sacrifice marks DCs in the red pulp and marginal zone (RP/MZ) but not the white pulp (WP). Percentage of $CD4^+$ cDC2 (upper panel) and $CD8\alpha^+$ cDC1 (lower panel) in the white pulp of the spleen before and after infection. Results are representative of four independent experiments with n= 3/group. (B) 1x10^6 CFSE labeled OT-1 T cells were adoptively transferred into control and DC-*Irf4*$^{-/-}$ (*Cd11c*^{cre}-*Irf4*$^{-/-}$) mice. 1 day later, the recipient mice were i.v. infected with 10^3 live rLM-OVA. 3 days later the spleens were analyzed for T cell proliferation by flow cytometry. Numbers indicate the percentage of proliferating cells. Results are representative of three independent experiments. (C) 1x10^6 CFSE labeled OT-2 T cells were adoptively transferred into WT, *Dock8*$^{-/-}$, and *Batf3*$^{-/-}$ mice. 1 day later, the recipient mice were i.v. injected with heat-killed 1x10^8 rLM-OVA. 3 days later mice were sacrificed and the spleens were collected for the analyses of OT-2 cell proliferation by flow cytometry. Results are representative of five independent experiments with n=2 or 3/group. *p<0.05; **p<0.01; ns, not significant. Data are means ± SD.

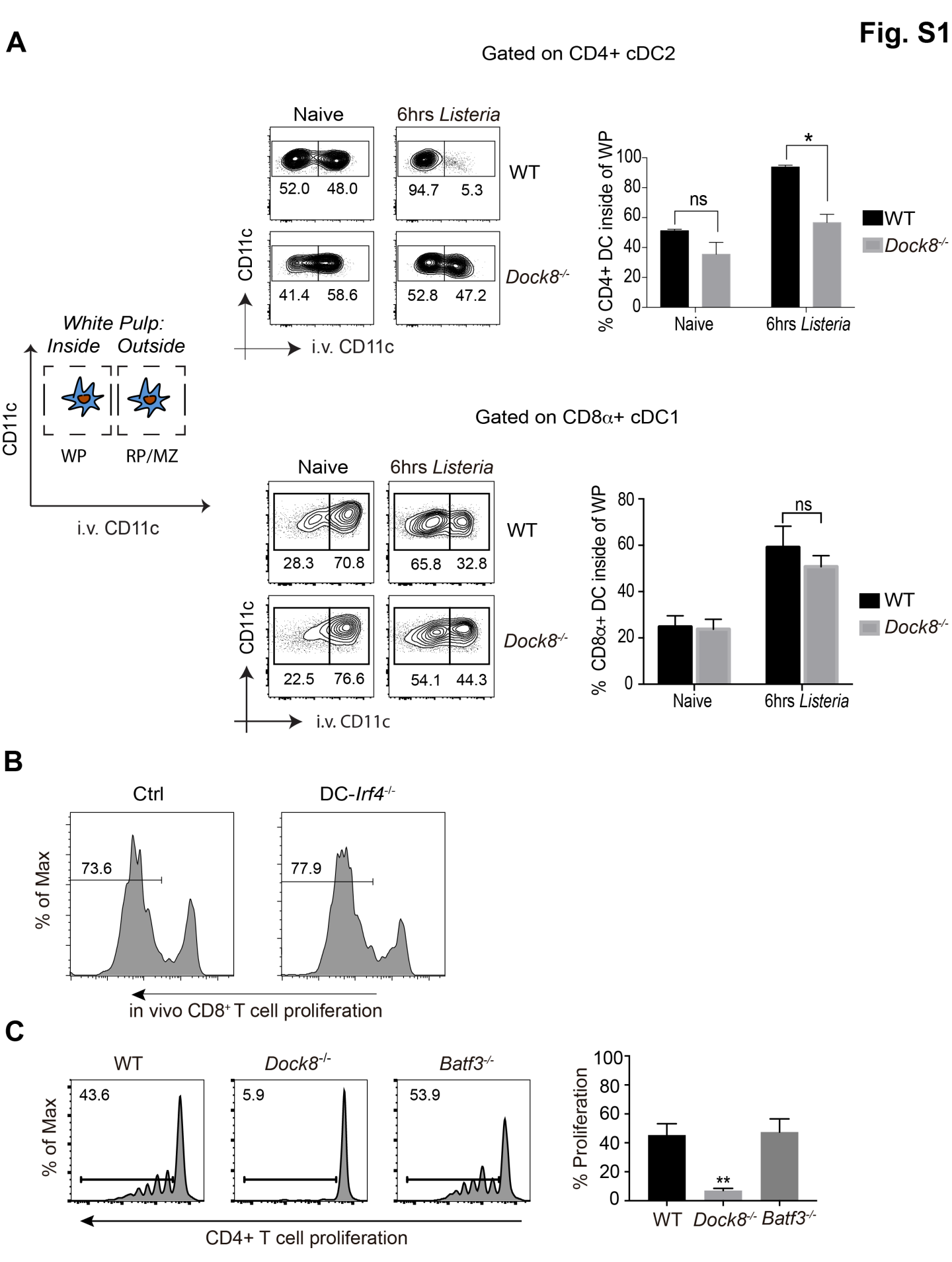

A
Gated on CD4+ cDC2
Naive
6hrs Listeria
WT
Dock8-/-
52.0
48.0
94.7
5.3
41.4
58.6
52.8
47.2
CD11c
i.v. CD11c
White Pulp:
Inside
Outside
WP
RP/MZ
% CD4+ DC inside of WP
ns
*
Naive
6hrs Listeria
Gated on CD8α+ cDC1
28.3
70.8
65.8
32.8
22.5
76.6
54.1
44.3
% CD8α+ DC inside of WP
B
Ctrl
DC-Irf4-/-
73.6
77.9
% of Max
in vivo CD8+ T cell proliferation
C
43.6
5.9
53.9
Batf3-/-
CD4+ T cell proliferation
% Proliferation
**

Figure S2. *Listeria* burden in the liver of *Dock8*$^{-/-}$ mice, related to Figure 2.

WT and *Dock8*$^{-/-}$ mice were i.v. infected with 10^5 live rLM-OVA. Burdens of rLM-OVA in liver were determined by colony forming units (CFUs) at indicated time points post infection. Dashed lines indicate detection limit. **p<0.01; ns, not significant; nd, not detectable. Results are representative of two independent experiments with n=4 or 5/group. Data are means ± SD.

Fig. S2

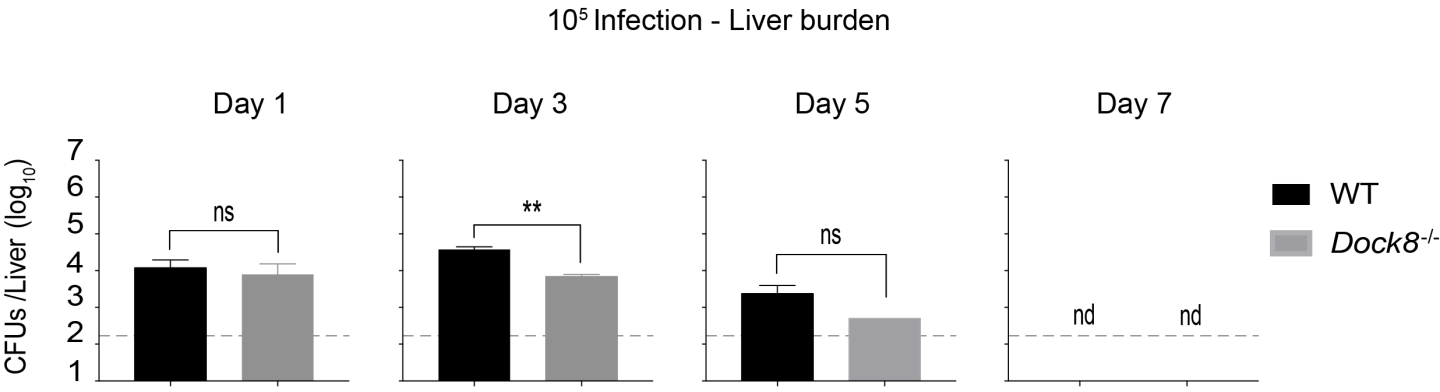

10⁵ Infection - Liver burden
Day 1
Day 3
Day 5
Day 7
CFUs /Liver (log10)
7
6
5
4
3
2
1
ns
**
ns
nd
nd
WT
Dock8-/-

Figure S3. Identification of intracellular *Listeria* in DCs, related to Figure 3.

(A) Naïve WT mice were infected with 5-10x10^8 rLM-OVA. 4 h later the mice were sacrificed to measure the *Listeria* infectivity on CD8α$^+$ cDC1s. (B) Naïve splenocytes were in vitro infected with live LM-GFP at M.O.I. 5 for 2.5 h, and CD4$^+$ cDC2 and CD8α$^+$ cDC1 intracellular bacteria were analyzed based on GFP expression by flow cytometry. Results are representative of three independent experiments. (C) WT and *Dock8*$^{-/-}$ mice were i.v. infected with 10^9 live LM-GFP. Burden of LM-GFP in the spleen was determined by colony forming units (CFUs) at 4 h post infection. *$p<0.05$. Results are representative of two independent experiments with n=3/group. (D) Control and DC-*Irf4*$^{-/-}$ (*Cd11c*^{cre}-*Irf4*$^{-/-}$) mice were i.v. infected with 10^5 live rLM-OVA. Burdens of rLM-OVA in spleen were determined by CFUs at day 3 post infection. ns, not significant. Results are representative of two or three independent experiments with n=4 or 5/group. Data are means ± SD.

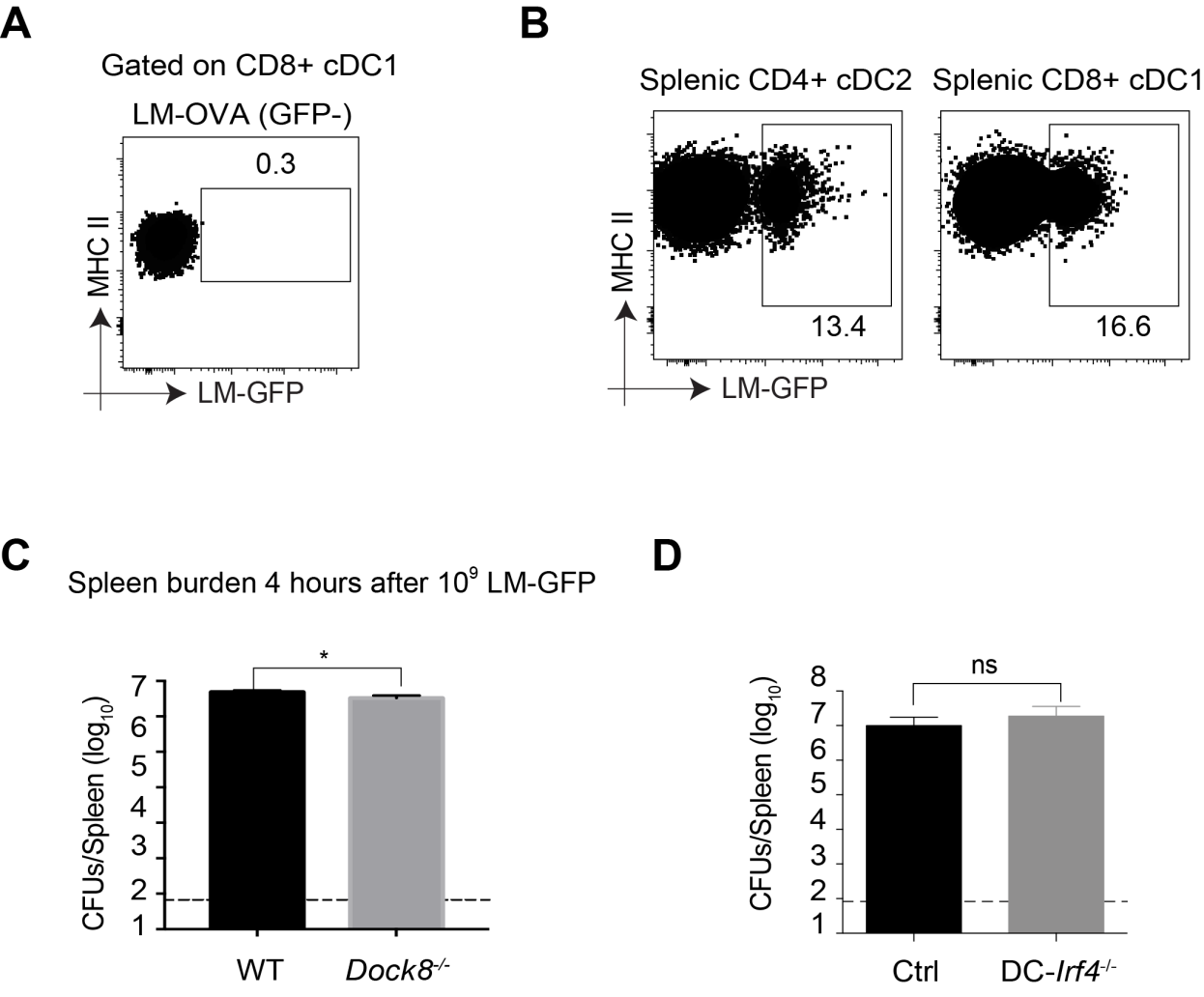
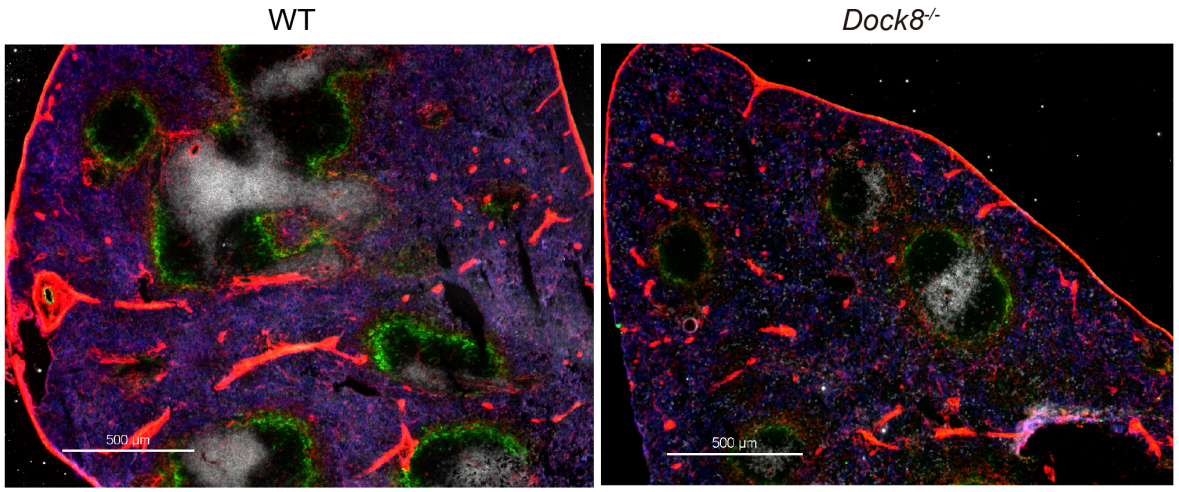

A
Gated on CD8+ cDC1
LM-OVA (GFP-)
0.3
MHC II
LM-GFP
B
Splenic CD4+ cDC2
Splenic CD8+ cDC1
MHC II
13.4
16.6
LM-GFP
C
Spleen burden 4 hours after 10^9 LM-GFP
*
CFUs/Spleen (log10)
7
6
5
4
3
2
1
WT
Dock8-/-
D
ns
CFUs/Spleen (log10)
8
7
6
5
4
3
2
1
Ctrl
DC-Irf4-/-

Figure S4. Splenic structure and liver *Listeria* burden of MZB cell deficient mice, related to Figure 4.

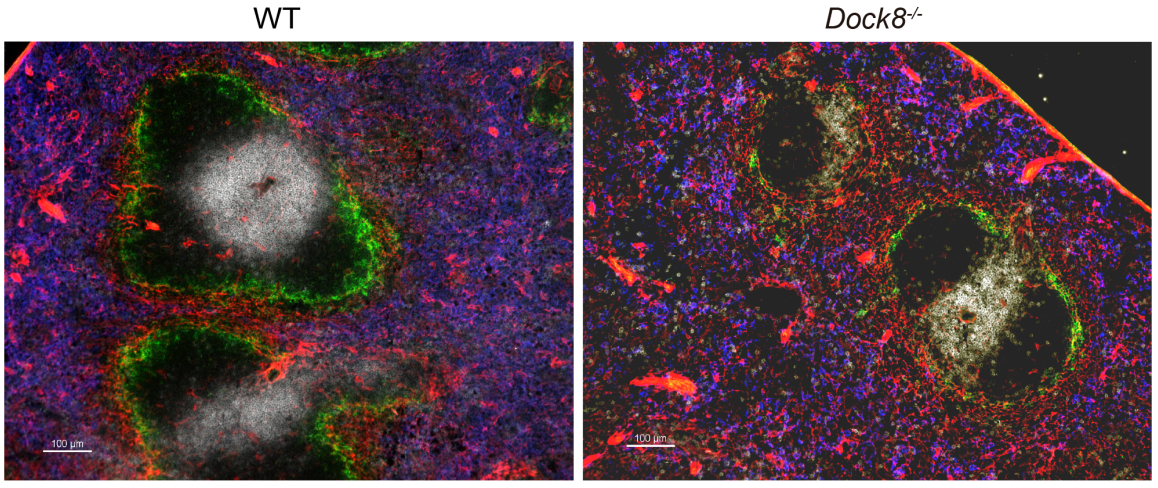
Fluorescence images of a spleen from a naïve WT mouse (left) and *Dock8*$^{-/-}$ mouse (right). Red pulp macrophages (F4/80 in blue), the reticular fibroblastic network (ER-TR7 in red), marginal zone metallophilic macrophages (MOMA in green) and T cell zone (TCRβ in white). (A) 4x images. Scale bar, 500 μm. (B) 10x images. Scale bar, 100 μm. A representative spleen from 3 different mice/group is shown. (C) Naïve WT (*Cd19*$^{+/+}$), *Cd19*$^{+/-}$, and *Cd19*$^{-/-}$ mice were analyzed for the percentages of the marginal zone B cell population among total B cells in the spleens by flow cytometry. (D) WT (*Cd19*$^{+/+}$) and *Cd19*$^{-/-}$ mice were i.v. infected with 10^5 live rLM-OVA. Burdens of rLM-OVA in liver were determined by colony forming units (CFUs) at day 3 post infection. *$p<0.05$. Results are representative of three (C) or two (D) independent experiments with n=4-5/group. Data are means ± SD.

Fig. S4

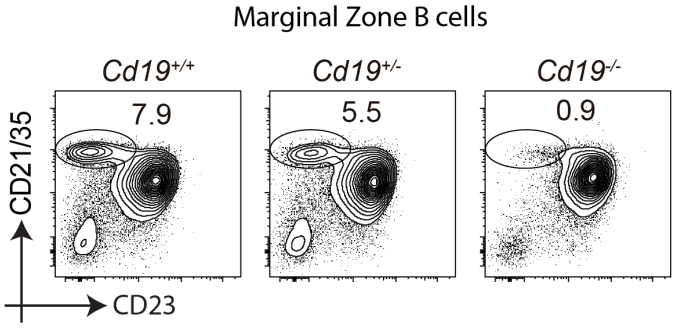
A


WT
Dock8-/-
500 µm
500 µm
4X
F4/80
ER-TR7
CD169
TCRβ (white)

B


WT
Dock8-/-
100 µm
100 µm
10X
F4/80
ER-TR7
CD169
TCRβ (white)

C


Marginal Zone B cells
Cd19+/+
Cd19+/-
Cd19-/-
7.9
5.5
0.9
CD21/35
CD23

D

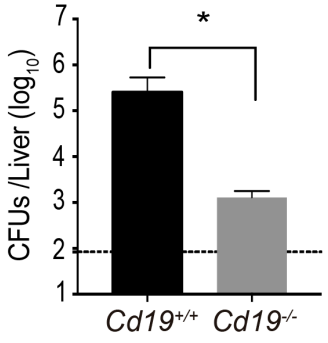

*
CFUs /Liver (log10)
7
6
5
4
3
2
1
Cd19+/+
Cd19-/-

Figure S5. Characterization of *Dock8* B cell conditional deficient mice, related to Figure 5.

(A) Naïve WT, B-*Dock8*$^{-/-}$ (*Mb1*^{cre}-*Dock8*$^{fl/fl}$) and *Dock8*$^{fl/fl}$ (Ctrl) mice were analyzed for the percentages of T cell subsets and dendritic cell subsets in the spleens. Results are representative of two independent experiments with n=4/group. (B) B-*Dock8*$^{-/-}$ and control mice were analyzed for the percentages of the marginal zone B cell (MZB) population among total B cells in the spleens. Results are representative of three independent experiments with n=3/group. (C) WT and *Dock8*$^{-/-}$ mice were sub-lethally irradiated before reconstitution with 2x10^{7} enriched B cells from *Cd19*$^{-/-}$ or WT mice. 8-10 weeks post transfer, the recipient mice were analyzed for the percentages of the MZB population among total B cells in the spleens.

Fig. S5

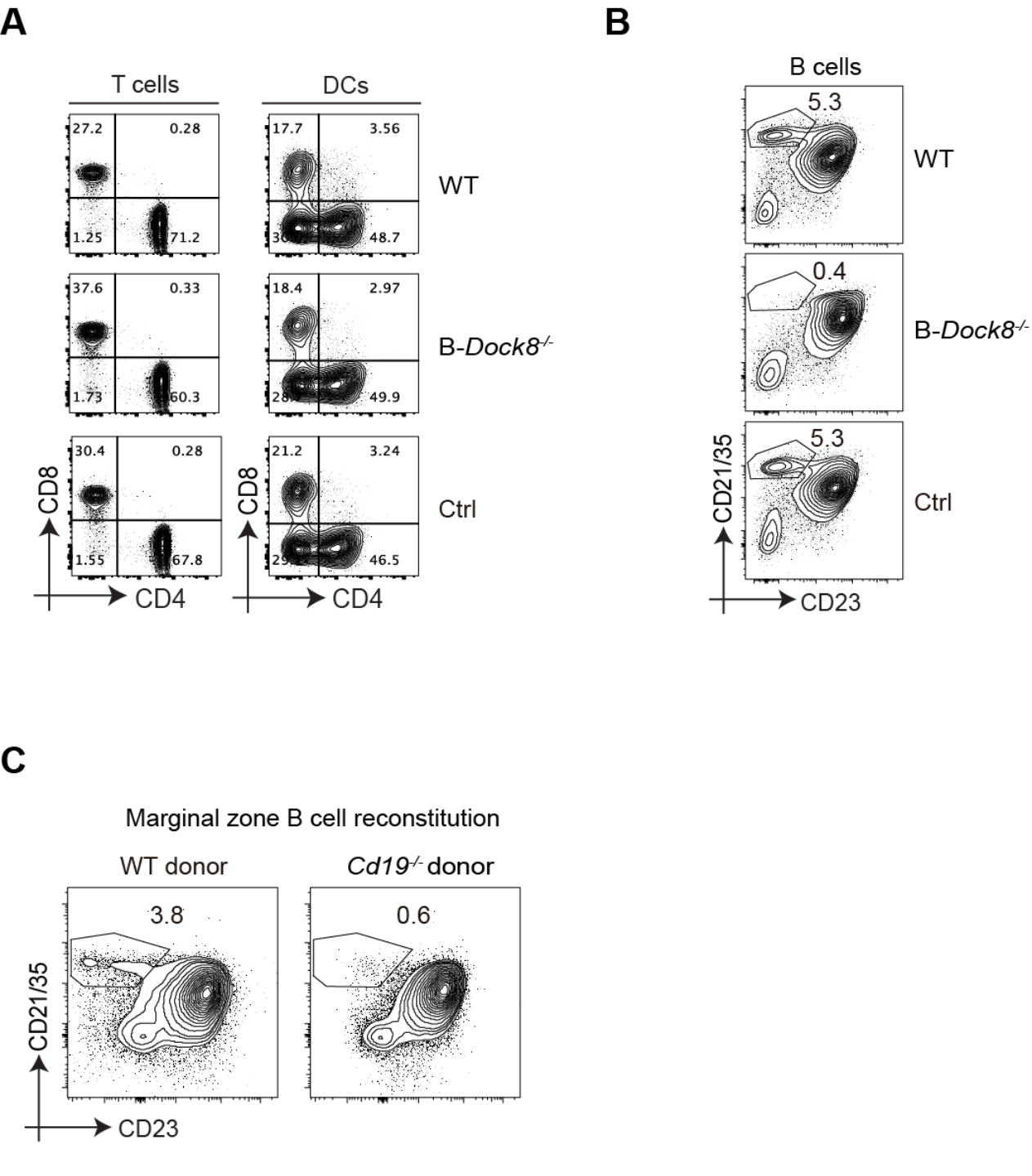

A
T cells
DCs
27.2
0.28
1.25
71.2
17.7
3.56
48.7
WT
37.6
0.33
1.73
60.3
18.4
2.97
49.9
B-Dock8-/-
30.4
0.28
1.55
67.8
21.2
3.24
46.5
Ctrl
CD8
CD4
B
B cells
5.3
WT
0.4
B-Dock8-/-
5.3
Ctrl
CD21/35
CD23
C
Marginal zone B cell reconstitution
WT donor
Cd19-/- donor
3.8
0.6
CD21/35
CD23

Figure S6. IL-10 receptor expression after *Listeria* infection and MZB cells reconstitution of mixed BM recipients, related to Figure 6.

(A) IL-10 receptor expression before and after *Listeria* infection. WT mice were infected with 5-10x10^8 rLM-OVA. 4 h later the mice were sacrificed to measure surface expression of IL-10Rα on different cell subsets in the spleen. Results are representative of two independent experiments with n=3 or 5/group. (B) 2x10^5 bone marrow cells from either WT or *Il10*$^{-/-}$ mice and 8x10^5 bone marrow cells from B-*Dock8*$^{-/-}$ (*MB1*^{cre}-*Dock8*$^{fl/fl}$) mice were adoptively transferred into lethally irradiated CD45.1 B6 recipient mice. 8 weeks later, the recipient mice were analyzed for the percentages of the MZB cell population among total B cells in the spleens. (C) 1x 10^6 $CFSE^+$ OT-1 T cells were adoptively transferred into WT+B-*Dock8*$^{-/-}$ (WT MZB cells) and *Il10*$^{-/-}$+B-*Dock8*$^{-/-}$ (*Il10*$^{-/-}$ MZB cells) chimeric mice. 1 day later, the recipients were i.v. infected with 10^3 live rLM-OVA. 3 days later, mice were sacrificed and the spleens were collected for analyses of T cell proliferation by flow cytometry. Results are representative of two independent experiments with n=4-5/group. (D) WT and *C3*$^{-/-}$ mice were infected with 5-10x10^8 LM-GFP. 4 h later, MZB cells were enriched and used for *Il10* mRNA analysis by qPCR. Results are representative of two independent experiments with n=3-5/group. (E) B-*MyD88*$^{-/-}$ (*Mb1*^{cre}-*MyD88*$^{fl/fl}$) and Cre- control mice were infected with 5-10x10^8 LM-GFP. 4 h later, *Listeria* load in MZB cells were measured by flow cytometry. Results are representative of two independent experiments with n=4-5/group. **p<0.01; ns, no significance. (C-D) Data are mean ± SD or (E) mean ± SEM.

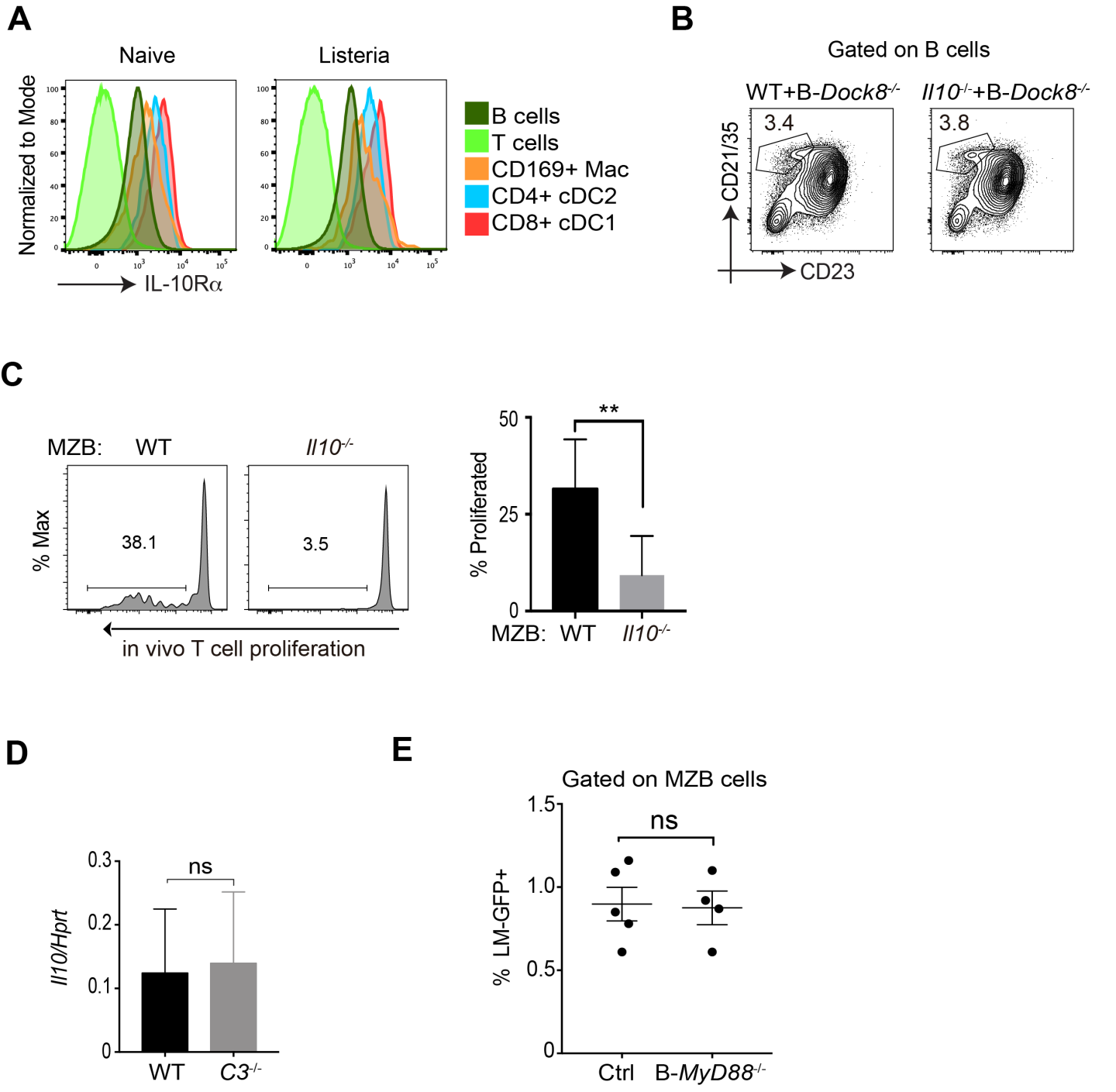

A
Naive
Listeria
Normalized to Mode
IL-10Rα
B cells
T cells
CD169+ Mac
CD4+ cDC2
CD8+ cDC1
B
Gated on B cells
WT+B-Dock8-/-
Il10-/-+B-Dock8-/-
3.4
3.8
CD21/35
CD23
C
MZB: WT
Il10-/-
% Max
38.1
3.5
in vivo T cell proliferation
% Proliferated
**
MZB: WT Il10-/-
D
Il10/Hprt
ns
WT
C3-/-
E
Gated on MZB cells
% LM-GFP+
ns
Ctrl
B-MyD88-/-

Figure S7. IL-10 and MZB cells enhance intracellular *Listeria* in macrophages, related to Figure 7.

(A) WT bone marrow derived macrophages or (B) splenic cDC1s were (+) or were not (-) treated with IL-10 (200 ng/ml) for 2 h in vitro. Half of the groups were infected with LM-GFP at M.O.I. 5 and *Listeria* load was analyzed based on GFP expression. Results are representative of 3 independent experiments. (C) WT and *Cd19*$^{-/-}$ female mice were i.v. infected with or without 20x10^{8} LM-GFP. 4 h later the mice were sacrificed to measure intracellular *Listeria* in XCR1^{+} cDC1s and CD169^{+} MMMs. **p<0.01. Results are representative of two independent experiments with n=3 /group. (D) Comparison of IL-10 receptor staining by flow cytometry on splenic MMM in *Il10r* floxed mice crossed to *Itgax* Cre^{+} or Cre^{-} mice. **p<0.01. (E) WT mice receiving bone marrow from DC-*Il10r*$^{-/-}$ (*Cd11c*^{cre}-*Il10r*$^{fl/fl}$) or Cre- control female mice were i.v. infected with 5-10x10^{8} LM-GFP. 4h later, *Nos2* mRNA analysis was performed by qPCR on total splenocytes. Results are representative of two independent experiments with n=4/group. ** p<0.01. Data are means ± SD.

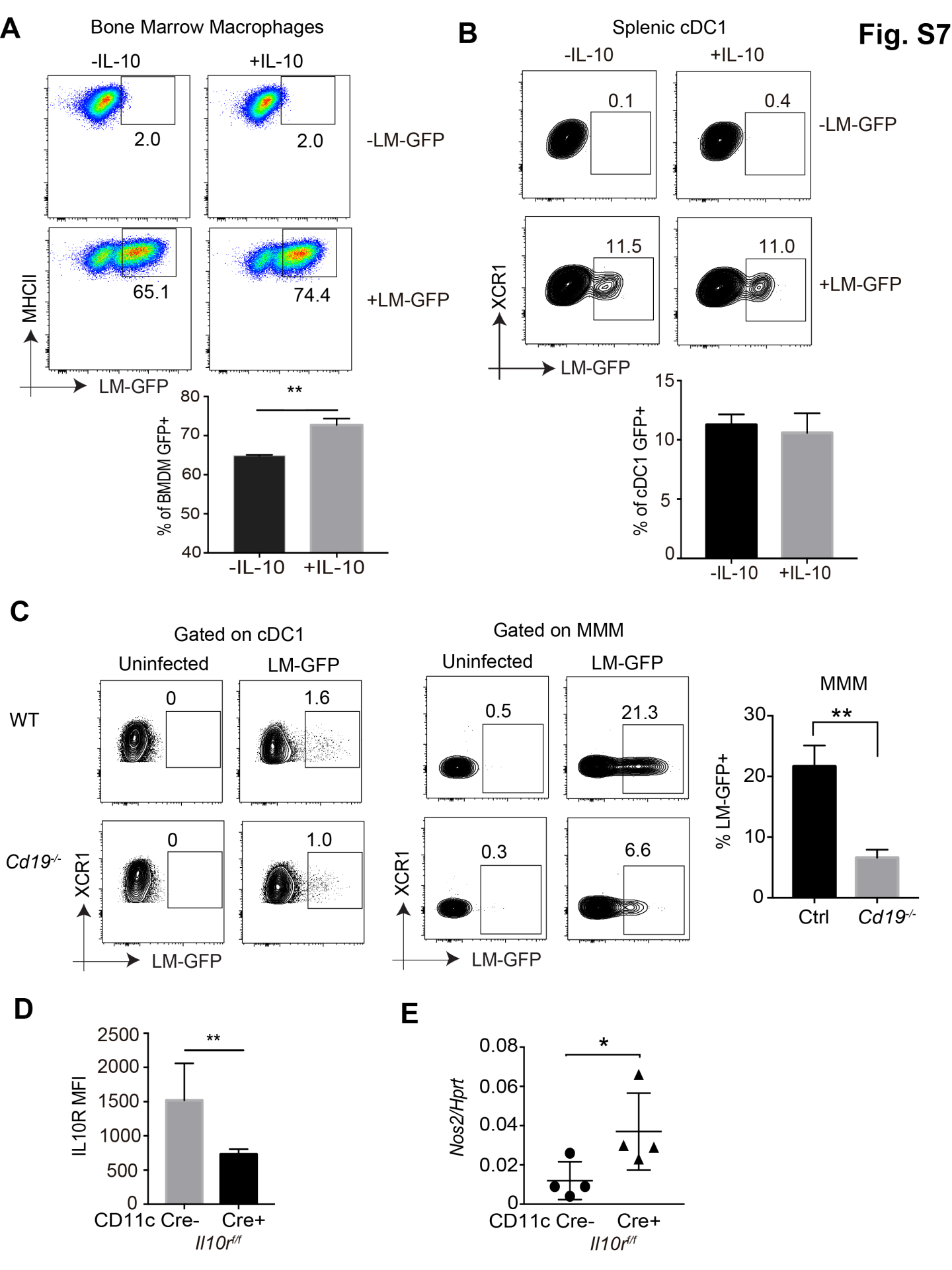


Fig. S7
A
Bone Marrow Macrophages
-IL-10
+IL-10
2.0
2.0
-LM-GFP
65.1
74.4
+LM-GFP
MHCII
LM-GFP
**
% of BMDM GFP+
80
70
60
50
40
-IL-10
+IL-10
B
Splenic cDC1
-IL-10
+IL-10
0.1
0.4
-LM-GFP
11.5
11.0
+LM-GFP
XCR1
LM-GFP
% of cDC1 GFP+
15
10
5
0
-IL-10
+IL-10
C
Gated on cDC1
Gated on MMM
Uninfected
LM-GFP
Uninfected
LM-GFP
WT
0
1.6
0.5
21.3
Cd19-/-
0
1.0
0.3
6.6
XCR1
LM-GFP
XCR1
LM-GFP
MMM
**
% LM-GFP+
30
20
10
0
Ctrl
Cd19-/-
D
**
IL10R MFI
2500
2000
1500
1000
500
0
CD11c Cre-
Cre+
Il10rf/f
E
*
Nos2/Hprt
0.08
0.06
0.04
0.02
0
CD11c Cre-
Cre+
Il10rf/f