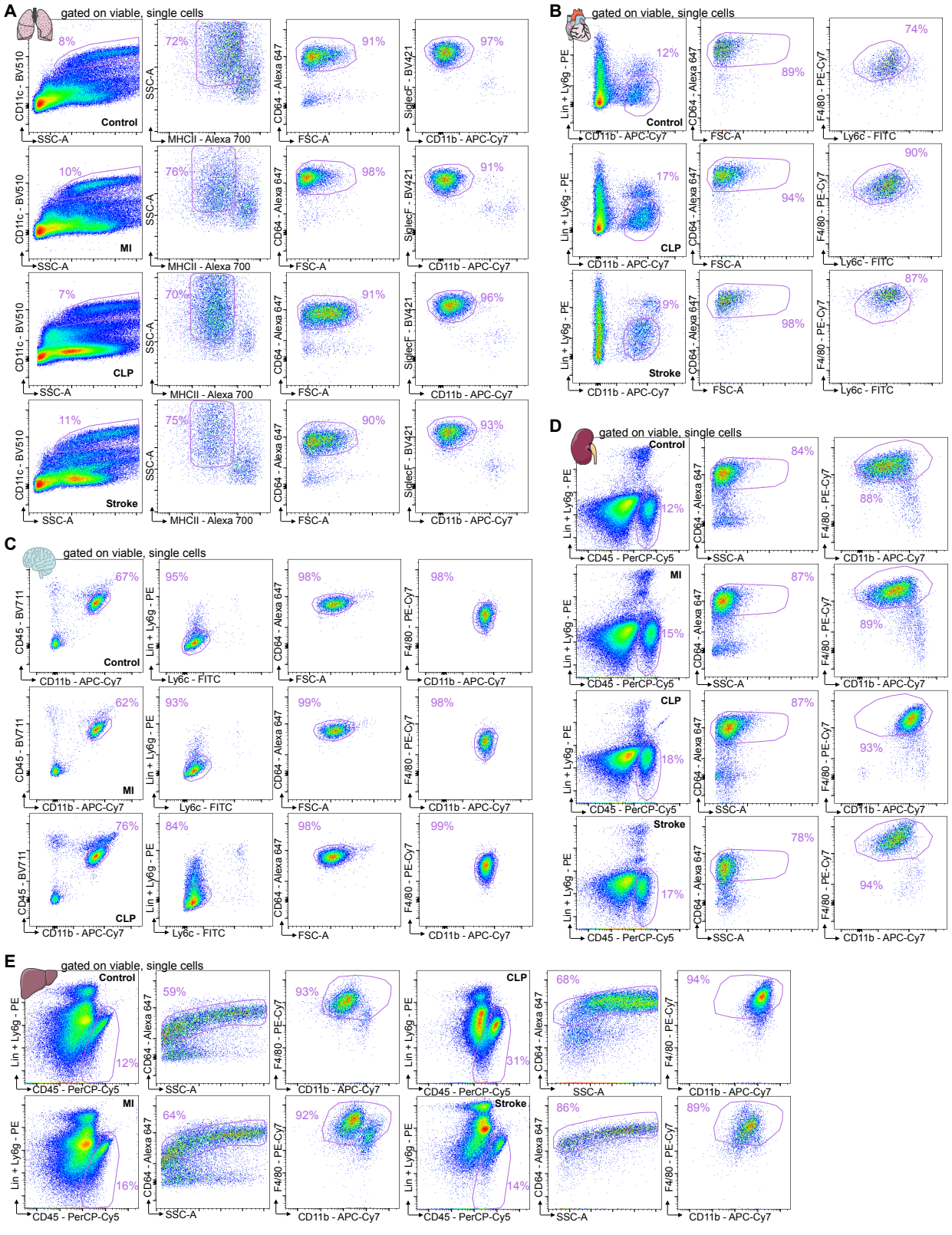
A
gated on viable, single cells
Control
MI
CLP
Stroke
CD11c - BV510
SSC-A
MHCII - Alexa 700
CD64 - Alexa 647
FSC-A
SiglecF - BV421
CD11b - APC-Cy7
B
gated on viable, single cells
Lin + Ly6g - PE
CD11b - APC-Cy7
CD64 - Alexa 647
FSC-A
F4/80 - PE-Cy7
Ly6c - FITC
C
gated on viable, single cells
CD45 - BV711
CD11b - APC-Cy7
Lin + Ly6g - PE
Ly6c - FITC
CD64 - Alexa 647
FSC-A
F4/80 - PE-Cy7
D
gated on viable, single cells
Lin + Ly6g - PE
CD45 - PerCP-Cy5
CD64 - Alexa 647
SSC-A
F4/80 - PE-Cy7
CD11b - APC-Cy7
E
gated on viable, single cells
Lin + Ly6g - PE
CD45 - PerCP-Cy5
CD64 - Alexa 647
SSC-A
F4/80 - PE-Cy7
CD11b - APC-Cy7

Figure S1. Flow cytometry gating strategy for all organs and conditions, Related to Figure 1-4.
Gating for macrophages in (A) lung, (B) heart, (C) brain, (D) kidney and (E) liver followed the Immgen guidelines whenever available. By design, the gates include both, resident and newly recruited macrophages.

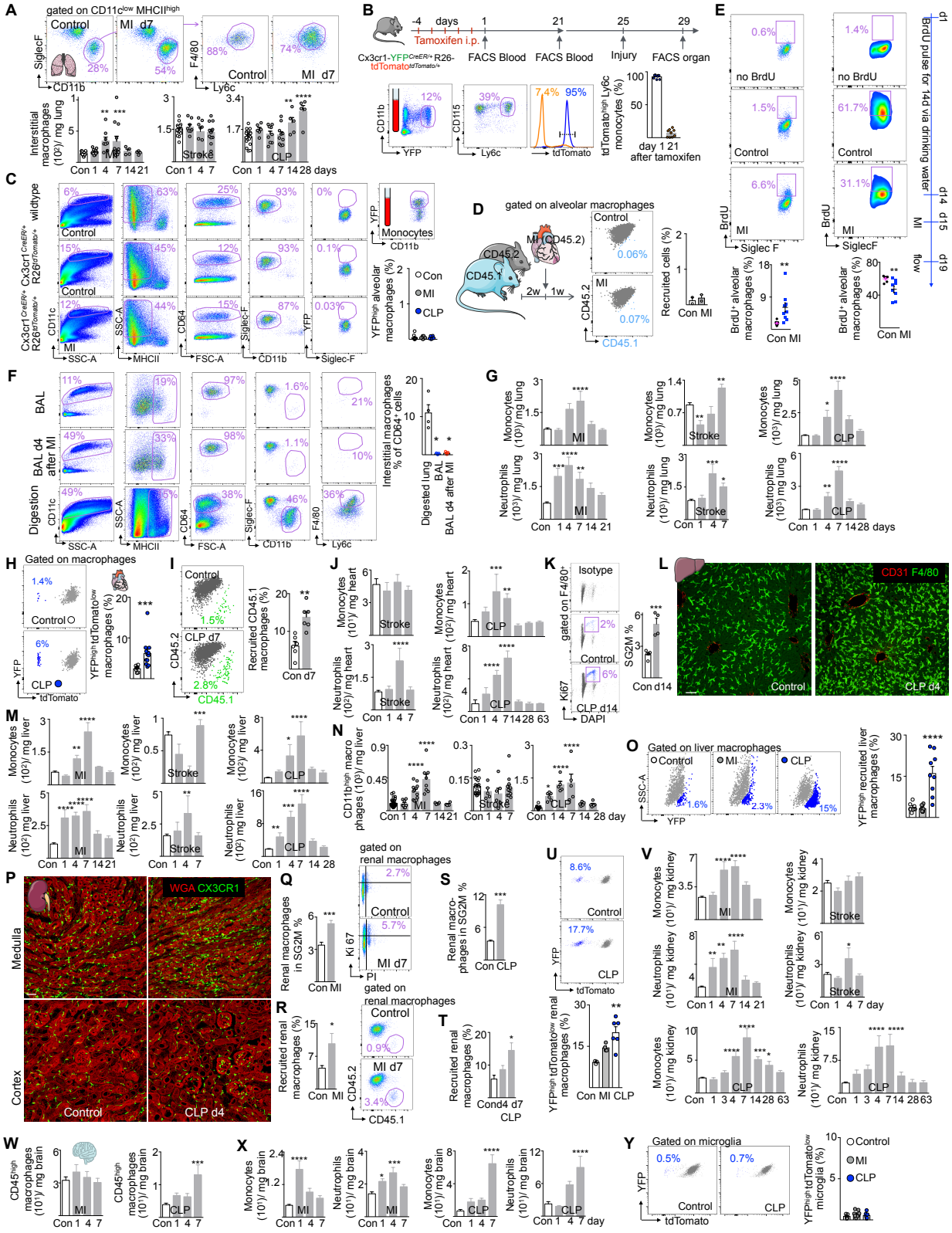
A
gated on CD11c^low MHCII^high
Control
MI d7
SiglecF
CD11b
28%
54%
F4/80
Ly6c
88%
74%
Interstitial macrophages (10^5)/ mg lung
Con 1 4 7 14 21
Stroke
CLP
Con 1 4 7 14 28 days
B
-4 days
Tamoxifen i.p.
1
21
25
29
Cx3cr1-YFP^CreER/+ R26-tdTomato^tdTomato/+
FACS Blood
FACS Blood
Injury
FACS organ
CD11b
YFP
12%
CD115
Ly6c
39%
7.4%
95%
tdTomato
tdTomato^high Ly6c monocytes (%)
day 1 21 after tamoxifen
C
wildtype
Cx3cr1^CreER/+ R26^tdTomato/+
6%
63%
25%
93%
0%
15%
45%
12%
93%
0.1%
12%
44%
15%
87%
0.03%
CD11c
SSC-A
MHCII
CD64
FSC-A
Siglec-F
CD11b
F4/80
Ly6c
YFP
Monocytes
YFP^high alveolar macrophages (%)
Con
MI
CLP
D
gated on alveolar macrophages
MI (CD45.2)
CD45.2
CD45.1
2w 1w
Control
0.06%
MI
0.07%
Recruited cells (%)
Con MI
E
no BrdU
0.6%
1.5%
Control
6.6%
MI
BrdU
Siglec F
1.4%
61.7%
31.1%
BrdU pulse for 14d via drinking water
d1
d14
d15
MI
flow
d19
BrdU^+ alveolar macrophages (%)
Con MI
F
BAL
BAL d4 after MI
Digestion
11%
19%
97%
1.6%
21%
49%
33%
98%
1.1%
10%
49%
38%
46%
36%
Interstitial macrophages % of CD64^+ cells
Digested lung
BAL
BAL d4 after MI
G
Monocytes (10^3)/ mg lung
Neutrophils (10^3)/ mg lung
MI
Stroke
CLP
Con 1 4 7 14 21
Con 1 4 7
Con 1 4 7 14 28 days
H
Gated on macrophages
1.4%
Control
6%
CLP
YFP
tdTomato
YFP^high tdTomato^low macrophages (%)
I
Control
1.5%
CLP d7
2.8%
CD45.2
CD45.1
Recruited CD45.1 macrophages (%)
Con d7
J
Monocytes (10^3)/ mg heart
Neutrophils (10^3)/ mg heart
Stroke
CLP
Con 1 4 7
Con 1 4 7 14 28 63
K
Isotype
gated on F4/80^+
2%
Control
6%
CLP d14
Ki67
DAPI
SG2M %
Con d14
L
CD31 F4/80
Control
CLP d4
M
Monocytes (10^3)/ mg liver
Neutrophils (10^3)/ mg liver
MI
Stroke
CLP
Con 1 4 7 14 21
Con 1 4 7
Con 1 4 7 14 28
N
CD11b^high macrophages (10^3)/ mg liver
MI
Stroke
CLP
Con 1 4 7 14 21
Con 1 4 7
Con 1 4 7 14 28 day
O
Gated on liver macrophages
Control
MI
CLP
SSC-A
YFP
1.6%
2.3%
15%
YFP^high recruited liver macrophages (%)
P
WGA CX3CR1
Medulla
Cortex
Control
CLP d4
Q
gated on renal macrophages
Renal macrophages in SG2M %
Con MI
Ki 67
PI
2.7%
Control
5.7%
MI d7
R
gated on renal macrophages
Recruited renal macrophages (%)
Con MI
Control
0.9%
MI d7
3.4%
CD45.2
CD45.1
S
Renal macrophages in SG2M %
Con CLP
T
Recruited renal macrophages (%)
Cond4 d7 CLP
U
8.6%
Control
17.7%
CLP
YFP
tdTomato
YFP^high tdTomato^low renal macrophages (%)
Con MI CLP
V
Monocytes (10^3)/ mg kidney
Neutrophils (10^3)/ mg kidney
MI
Stroke
CLP
Con 1 4 7 14 21
Con 1 4 7 day
Con 1 3 4 7 14 28 63
Con 1 3 4 7 14 28 63
W
CD45^high macrophages (10^3)/ mg brain
MI
CLP
Con 1 4 7
X
Monocytes (10^3)/ mg brain
Neutrophils (10^3)/ mg brain
MI
CLP
Con 1 4 7
Con 1 4 7 day
Y
Gated on microglia
0.5%
0.7%
Control
CLP
YFP
tdTomato
YFP^high tdTomato^low microglia (%)
Control
MI
CLP

Figure S2. Myeloid cells and macrophage fate mapping after remote injury in the lung, heart, liver and kidney, Related to Figure 1.
(A) Interstitial macrophages after injury. Control *n*=19-28, MI *n*=4-12, stroke *n*=8-13, CLP *n*=5-10 biological replicates. (B) Fate mapping in Cx3cr1$^{CreER/+}$R26$^{tdTomato/+}$ mice and quantification of fluorescent proteins in blood monocytes, day 1 *n*=9, day 21 *n*=9. (C) Fate mapping for alveolar macrophages in *Cx3cr1*$^{CreER/+}$R26$^{tdTomato/+}$ mice after MI and CLP and naive *Cx3cr1*$^{CreER/+}$*R26*$^{tdTomato/+}$ controls. Control *n*=8, MI *n*=10, CLP *n*=6 biological replicates. (D) Fate mapping experiments for alveolar macrophages in CD45.1/2 parabionts. MI was induced in DC45.2 mouse, whose lung was then investigated. MI *n*=2 pairs, naive control *n*=2 pairs. (E) Proliferation of alveolar macrophages by BrdU flow cytometry. Left: BrdU was injected i.p. three hours before sacrifice, *n*=8-10. Right: BrdU pulse chase experiment, *n*=6-8. (F) FACS of bronchoalveolar lavage (BAL) in naive controls and day 4 after MI. Naive controls (digested) *n*=4, controls BAL *n*=5, MI *n*=5 biological replicates. (G) Lung monocytes and neutrophils after MI, *n*=4-30; after stroke, *n*=8-19; and after CLP, *n*=5-21 biological replicates. (H) Fate mapping for cardiac macrophages in *Cx3cr1*$^{CreER/+}$*R26*$^{tdTomato/+}$ mice after CLP and naive *Cx3cr1*$^{CreER/+}$*R26*$^{tdTomato/+}$ controls. Control *n*=8, CLP *n*=12 biological replicates. (I) Fate mapping for cardiac macrophages in CD45.1/2 parabionts after CLP. MI *n*=6 pairs, naive control *n*=6 pairs. (J) Cardiac monocytes and neutrophils after stroke, *n*=4-18 biological replicates per time point; and after CLP, *n*=5-20. (K) Cell cycle analysis of cardiac macrophages after CLP, naive control *n*=5, CLP *n*=4 biological replicates. (L) Fluorescence images of hepatic macrophages at baseline and 4 days after CLP. Scale bar, 50 µm. (M) Hepatic monocytes and neutrophils after MI, *n*=4-31; after stroke, *n*=5-19; and after CLP, *n*=5-16. (N) Hepatic CD11b^{high} macrophages, control *n*=16-32, MI *n*=4-12, stroke *n*=5-13, CLP *n*=5-6 biological replicates. (O) Fate mapping for hepatic macrophages in *Cx3cr1*$^{CreER/+}$*R26*$^{tdTomato/+}$ mice after CLP or MI and naive *Cx3cr1*$^{CreER/+}$*R26*$^{tdTomato/+}$ controls. Control *n*=7, MI *n*=9, CLP *n*=9 biological replicates. (P) Histology of renal macrophages after CLP, Scale bar, 50 µm. (Q) Proliferation of renal macrophages by Ki-67 and Propidium iodide (PI) flow cytometry on d7 after MI, *n*=7-12. (R) Recruitment of renal macrophages after MI assessed by parabiosis, *n*=5-8. (S) Cell cycle analysis in renal macrophage on d7 after CLP, *n*=4-6 biological replicates. (T) Recruitment of renal macrophages after CLP assessed by parabiosis, *n*=10-11; and (U) after MI and CLP in *Cx3cr1*$^{CreER/+}$*R26*$^{tdTomato/+}$ mice, *n*=3-6. (V) Renal monocytes and neutrophils after MI, *n*=4-34; after stroke, *n*=4-16; and after CLP, *n*=5-29. (W) Brain CD45^{high} macrophages after MI and CLP, *n*=8-14 biological replicates per time point. (X) Monocytes and neutrophils in the brain after MI, *n*=8 and after CLP, *n*=8-14. (Y) Fate mapping for microglia in *Cx3cr1*$^{CreER/+}$*R26*$^{tdTomato/+}$ mice after CLP, after MI and in and naive *Cx3cr1*$^{CreER/+}$*R26*$^{tdTomato/+}$ controls. Control *n*=5, MI *n*=8, CLP *n*=6 biological replicates. Data are mean ± s.e.m. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001. One-way ANOVA with Dunnett's multiple comparison test was applied for normally distributed data.

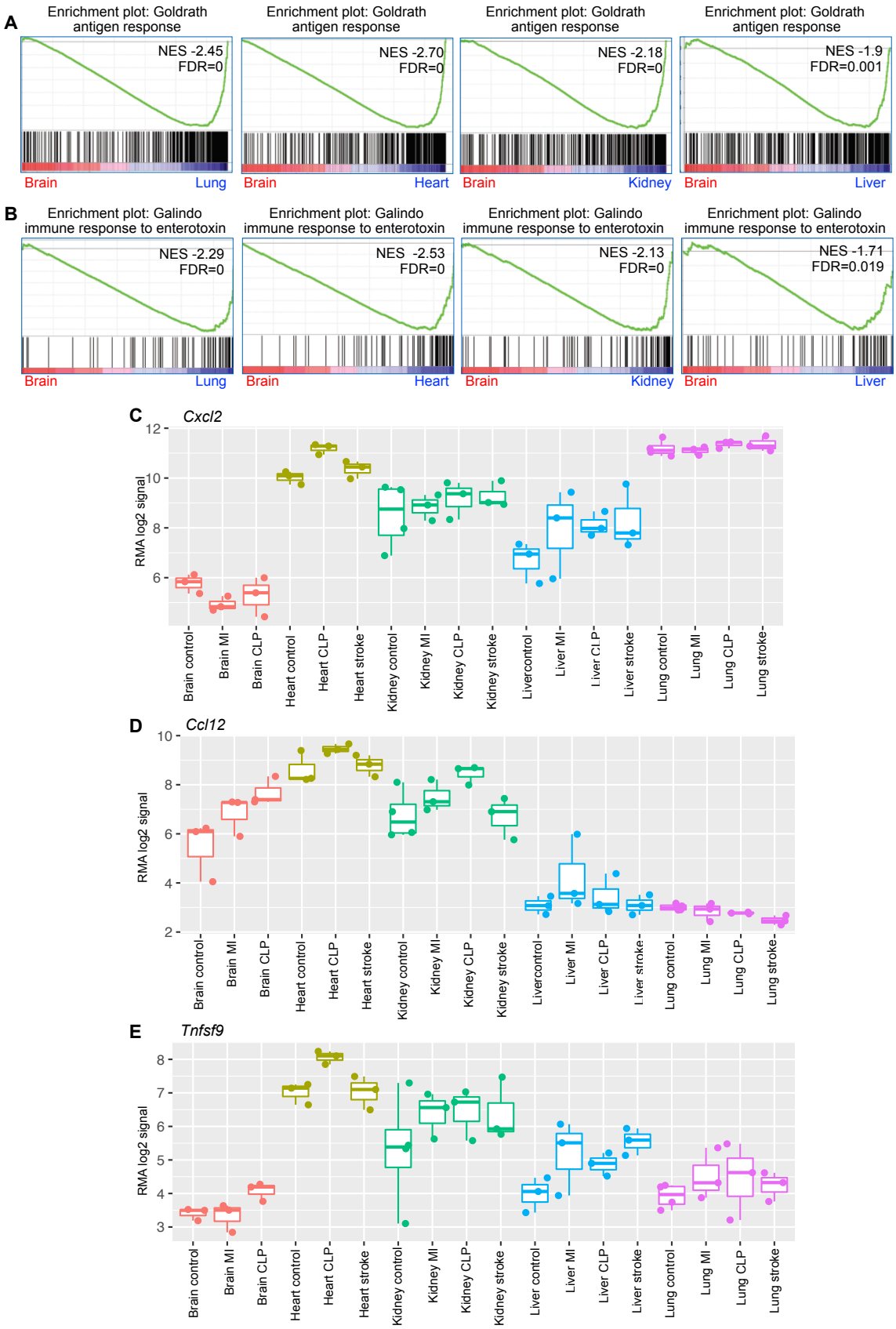
A
Enrichment plot: Goldrath antigen response
NES -2.45
FDR=0
Brain
Lung
Enrichment plot: Goldrath antigen response
NES -2.70
FDR=0
Brain
Heart
Enrichment plot: Goldrath antigen response
NES -2.18
FDR=0
Brain
Kidney
Enrichment plot: Goldrath antigen response
NES -1.9
FDR=0.001
Brain
Liver
B
Enrichment plot: Galindo immune response to enterotoxin
NES -2.29
FDR=0
Brain
Lung
Enrichment plot: Galindo immune response to enterotoxin
NES -2.53
FDR=0
Brain
Heart
Enrichment plot: Galindo immune response to enterotoxin
NES -2.13
FDR=0
Brain
Kidney
Enrichment plot: Galindo immune response to enterotoxin
NES -1.71
FDR=0.019
Brain
Liver
C
Cxcl2
RMA log2 signal
12
10
8
6
Brain control
Brain MI
Brain CLP
Heart control
Heart CLP
Heart stroke
Kidney control
Kidney MI
Kidney CLP
Kidney stroke
Livercontrol
Liver MI
Liver CLP
Liver stroke
Lung control
Lung MI
Lung CLP
Lung stroke
D
Ccl12
RMA log2 signal
10
8
6
4
2
Brain control
Brain MI
Brain CLP
Heart control
Heart CLP
Heart stroke
Kidney control
Kidney MI
Kidney CLP
Kidney stroke
Livercontrol
Liver MI
Liver CLP
Liver stroke
Lung control
Lung MI
Lung CLP
Lung stroke
E
Tnfsf9
RMA log2 signal
8
7
6
5
4
3
Brain control
Brain MI
Brain CLP
Heart control
Heart CLP
Heart stroke
Kidney control
Kidney MI
Kidney CLP
Kidney stroke
Liver control
Liver MI
Liver CLP
Liver stroke
Lung control
Lung MI
Lung CLP
Lung stroke

Figure S3. Organ specific macrophage expression changes after injury, Related to Figure 2 and 3.

(A) Gene set enrichment analysis of the gene set “GOLDRATH_ANTIGEN_RESPONSE“ in brain microglia vs. all other organs. FDRs are calculated by gene set permutation. (B) Gene set enrichment analysis of the gene set “GALINDO_IMMUNE_RESPONSE_TO_ENTEROTOXIN“ in brain microglia vs. all other organs. (C) Expression (RMA log2 signal) of *Cxcl2,* (D) *Ccl12*, (E) *Tnfsf9* across the entire data set.

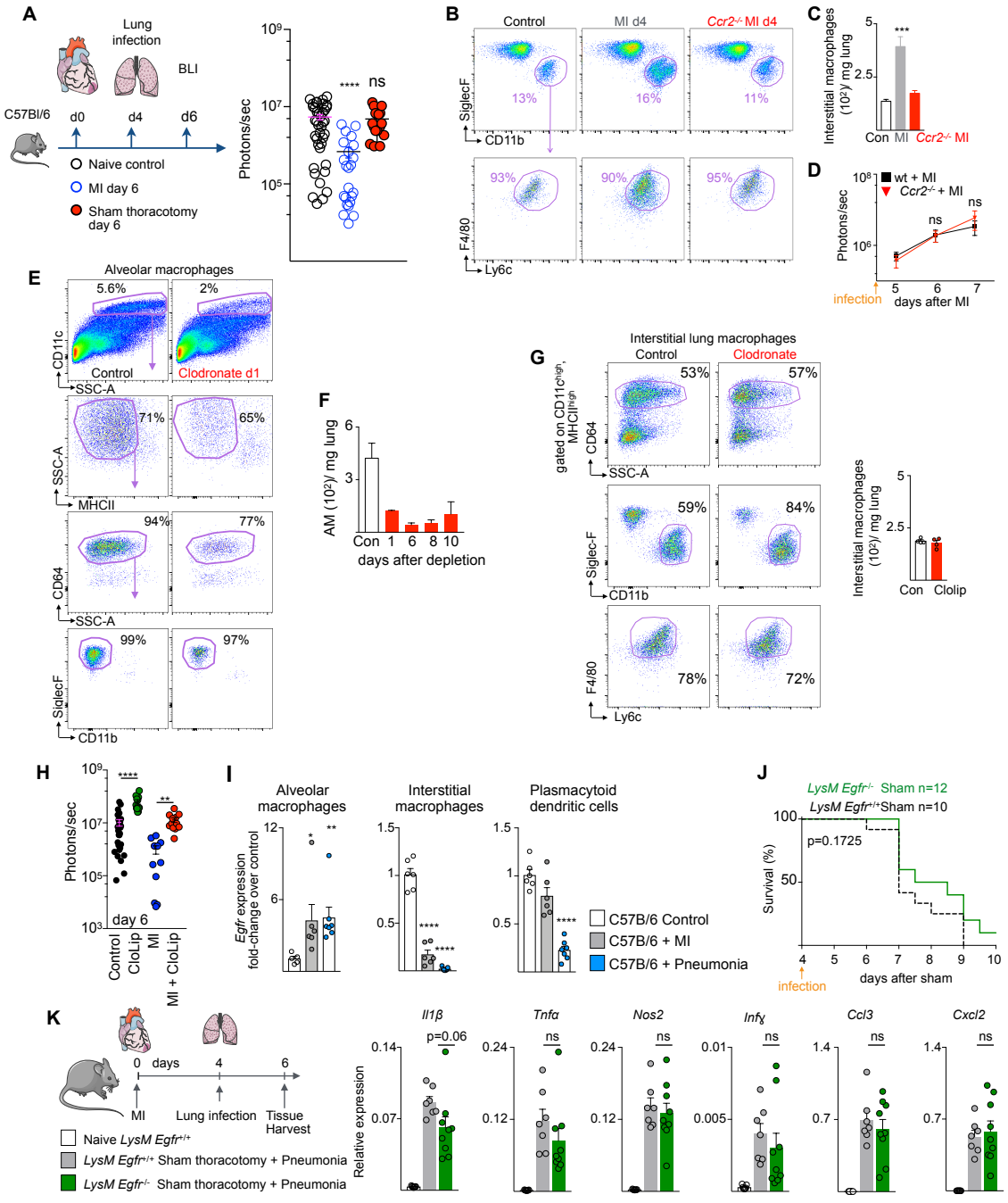
A
Lung infection
BLI
C57Bl/6
d0
d4
d6
Naive control
MI day 6
Sham thoracotomy day 6
Photons/sec
****
ns
B
Control
MI d4
Ccr2-/- MI d4
SiglecF
CD11b
13%
16%
11%
F4/80
Ly6c
93%
90%
95%
C
Interstitial macrophages (10²)/ mg lung
***
Con MI Ccr2-/- MI
D
wt + MI
Ccr2-/- + MI
ns
Photons/sec
infection
days after MI
E
Alveolar macrophages
5.6%
2%
Control
Clodronate d1
CD11c
SSC-A
71%
65%
MHCII
94%
77%
CD64
99%
97%
CD11b
F
AM (10²)/ mg lung
Con 1 6 8 10
days after depletion
G
Interstitial lung macrophages
Control
Clodronate
gated on CD11c^high, MHCII^high
53%
57%
59%
84%
Siglec-F
78%
72%
Interstitial macrophages (10²)/ mg lung
Con Clolip
H
Photons/sec
day 6
Control CloLip MI MI + CloLip
I
Alveolar macrophages
Interstitial macrophages
Plasmacytoid dendritic cells
Egfr expression fold-change over control
C57B/6 Control
C57B/6 + MI
C57B/6 + Pneumonia
J
LysM Egfr-/- Sham n=12
LysM Egfr+/+ Sham n=10
p=0.1725
Survival (%)
infection
days after sham
K
0 days 4 6
MI
Lung infection
Tissue Harvest
Naive LysM Egfr+/+
LysM Egfr+/+ Sham thoracotomy + Pneumonia
LysM Egfr-/- Sham thoracotomy + Pneumonia
Il1β
p=0.06
Tnfα
Nos2
Infγ
Ccl3
Cxcl2
ns
Relative expression

Figure S4. Alveolar macrophages after MI, Related to Figure 5.
(A) Bioluminescence signal 2 days after instillation of bioluminescent bacteria in naive controls, on day 6 after MI and day 6 after sham thoracotomy surgery. Control *n*=39, MI *n*=23, sham thoracotomy *n*=18. (B) Flow plots of lung interstitial macrophages in wildtype and *Ccr2*$^{-/-}$ mice after MI. (C) Quantification of lung interstitial macrophages in wildtype and *Ccr2*$^{-/-}$ mice after MI, *n*=4-5. (D) *Ccr2*$^{-/-}$ mice were subjected to MI and received bioluminescent streptococcus 4 days later, *n*=8-15. (E) Alveolar macrophages were depleted via intra-tracheal instillation of clodronate liposome. Flow plots shows macrophages in naive control and after liposome treatment. (F) Quantification of alveolar macrophages over time after clodronate depletion, *n*=2-4 biological replicates per time point. (G) Flow cytometry for interstitial lung macrophages after intratracheal clodronate liposome instillation. Con *n*=4, Clolip *n*=4 biological replicates. (H) Bioluminescence signal 2 days after instillation of bioluminescent bacteria in naive controls, controls that received intratracheal clodronate liposomes, and on day 6 after MI with and without intratracheal clodronate. Naive control *n*=27, control with clodronate *n*=12, MI *n*=11, MI with clodronate *n*=12. (I) Expression of Egfr in flow sorted alveolar macrophages, interstitial lung macrophages and plasmacytoid dendritic cells. Naive control *n*=5-6 biological replicates, MI *n*=6, Pneumonia *n*=6. (J) Survival analysis in mice with sham thoracotomy. Sham *LysM Egfr*$^{-/-}$ *n*=12, *LysM Egfr*$^{+/+}$ *n*=12. (K) Gene expression in lung tissue harvested from mice that underwent sham thoracotomy control procedures and induction of pneumonia. Naive *LysM Egfr*$^{+/+}$ *n*=x, Sham *LysM Egfr*$^{-/-}$ *n*=8, *LysM Egfr*$^{+/+}$ *n*=7. Data are mean ± s.e.m. **P*<0.05, ***P*<0.01, ****P*<0.001, *****P*<0.0001. Unpaired t-test or ordinary one-way ANOVA with Dunnett's multiple comparison test was used for analysis.

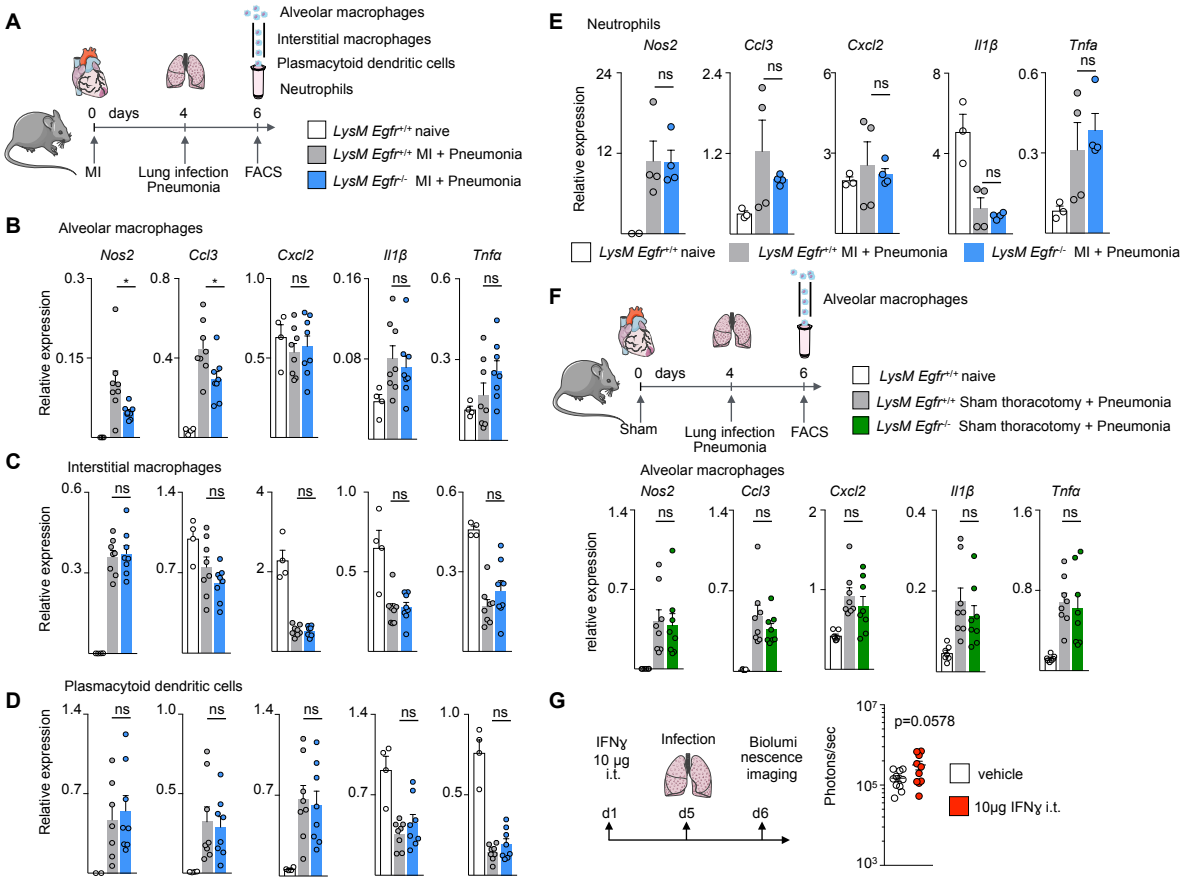

Figure S5. Gene expression changes in lung and heart macrophages, Related to Figure 5. (A) Experimental outline. (B) Gene expression in alveolar macrophages, (C) interstitial lung macrophages, (D) plasmacytoid dendritic cells, (E) neutrophils in naive *LysM Egfr*$^{+/+}$ *n*=2-4, *LysM Egfr*$^{+/+}$ with MI and pneumonia n=4-8, *LysM Egfr*$^{-/-}$ with MI and pneumonia *n*=4-8 biological replicates. (F) Gene expression in alveolar macrophages after sham thoracotomy control procedure and pneumonia. *LysM Egfr*$^{+/+}$ naive *n*=4, *LysM Egfr*$^{+/+}$ Sham thoracotomy + Pneumonia *n*=4-8, *LysM Egfr*$^{-/-}$ Sham thoracotomy + Pneumonia *n*=4-8 biological replicates. (G) Bioluminescence imaging after 10µg of intratracheal IFNγ and intratracheal streptococcus pneumoniae instillation. Data are mean ± s.e.m. **P*<0.05. Unpaired t-test or ordinary one-way ANOVA with Dunnett's multiple comparison test was used for analysis.

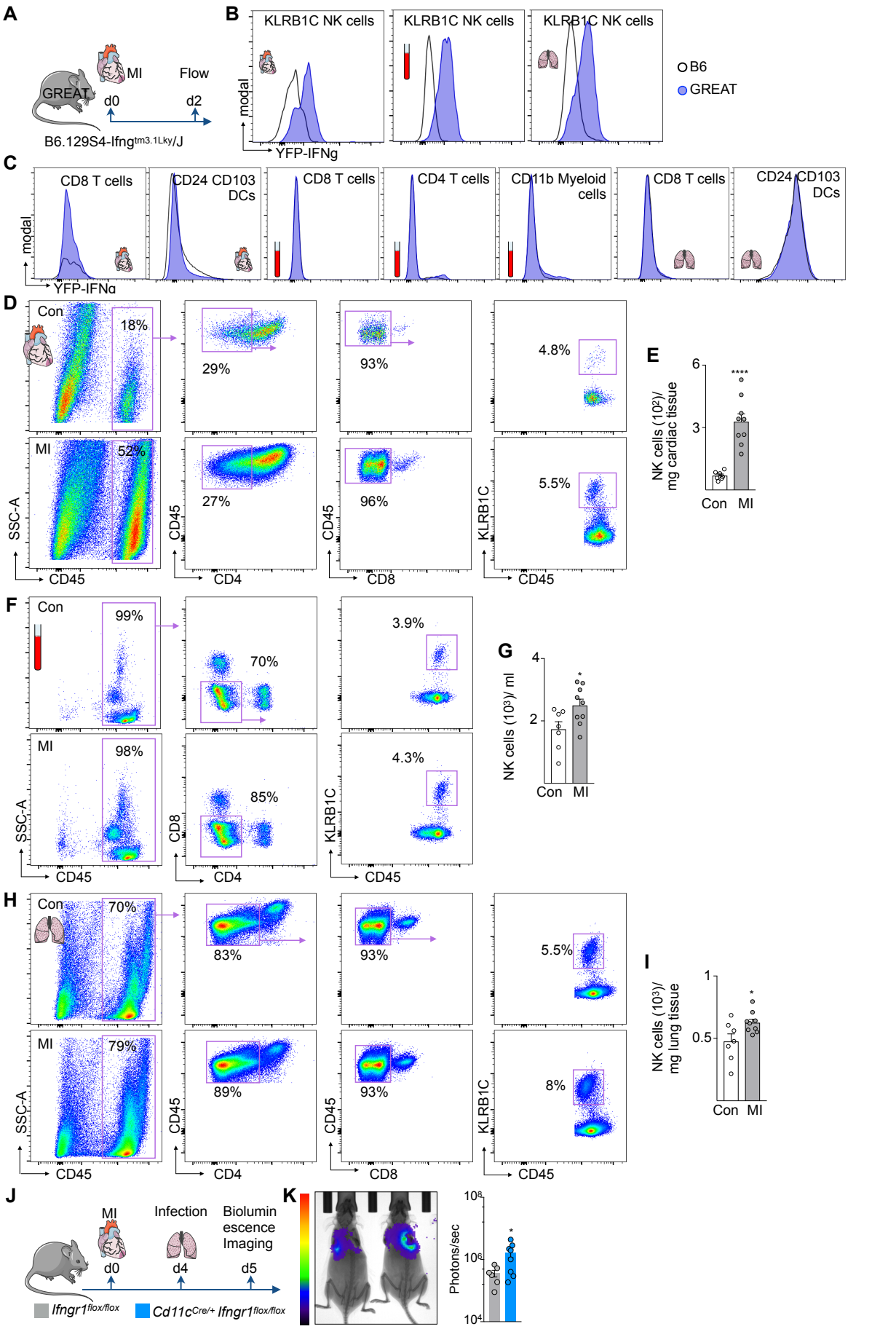
A
MI
Flow
GREAT
d0
d2
B6.129S4-Ifng tm3.1Lky/J
B
KLRB1C NK cells
KLRB1C NK cells
KLRB1C NK cells
modal
YFP-IFNg
B6
GREAT
C
CD8 T cells
CD24 CD103 DCs
CD8 T cells
CD4 T cells
CD11b Myeloid cells
CD8 T cells
CD24 CD103 DCs
modal
YFP-IFNg
D
Con
18%
29%
93%
4.8%
MI
52%
27%
96%
5.5%
SSC-A
CD45
CD4
CD8
KLRB1C
E
NK cells (10²)/ mg cardiac tissue
****
Con
MI
F
Con
99%
70%
3.9%
MI
98%
85%
4.3%
SSC-A
CD45
CD4
CD8
KLRB1C
G
NK cells (10³)/ ml
*
Con
MI
H
Con
70%
83%
93%
5.5%
MI
79%
89%
93%
8%
SSC-A
CD45
CD4
CD8
KLRB1C
I
NK cells (10³)/ mg lung tissue
*
Con
MI
J
MI
Infection
Biolumin escence Imaging
d0
d4
d5
Ifngr1flox/flox
Cd11cCre/+ Ifngr1flox/flox
K
Photons/sec
*

Figure S6. Disrupted IFNɣ signaling abrogates lung protection after MI, Related to Figure 5.

(A,B) IFNɣ expression of NK cells in heart, blood, and lung in control and YFP-IFNɣ reporter mice. (C) No change in IFNɣ expression in indicated cells in heart, blood and lung. (D) Gating and (E) quantification of cardiac NK cells in control mice and on day 4 after MI. Control *n*=7, MI *n*=9. (F) Gating and (G) quantification of blood NK cells of control mice on day 4 after MI. Control *n*=7, MI *n*=9 biological replicates. (H) Gating and (I) quantification of lung NK cells in control mice and on day 4 after MI. Control *n*=7, MI *n*=9 biological replicates. (J) Bioluminescence/x-ray images and (K) quantification of control and $CD11c^{Cre}$ $Ifngr1^{/flox}$ mice. Control *n*=6, $CD11c^{Cre}$ $Ifngr1^{/flox}$ *n*=8. Data are mean ± s.e.m. **P*<0.05, ***P*<0.01, ****P*<0.001. Unpaired t-test and Welch's t-test were used for statistical analysis.

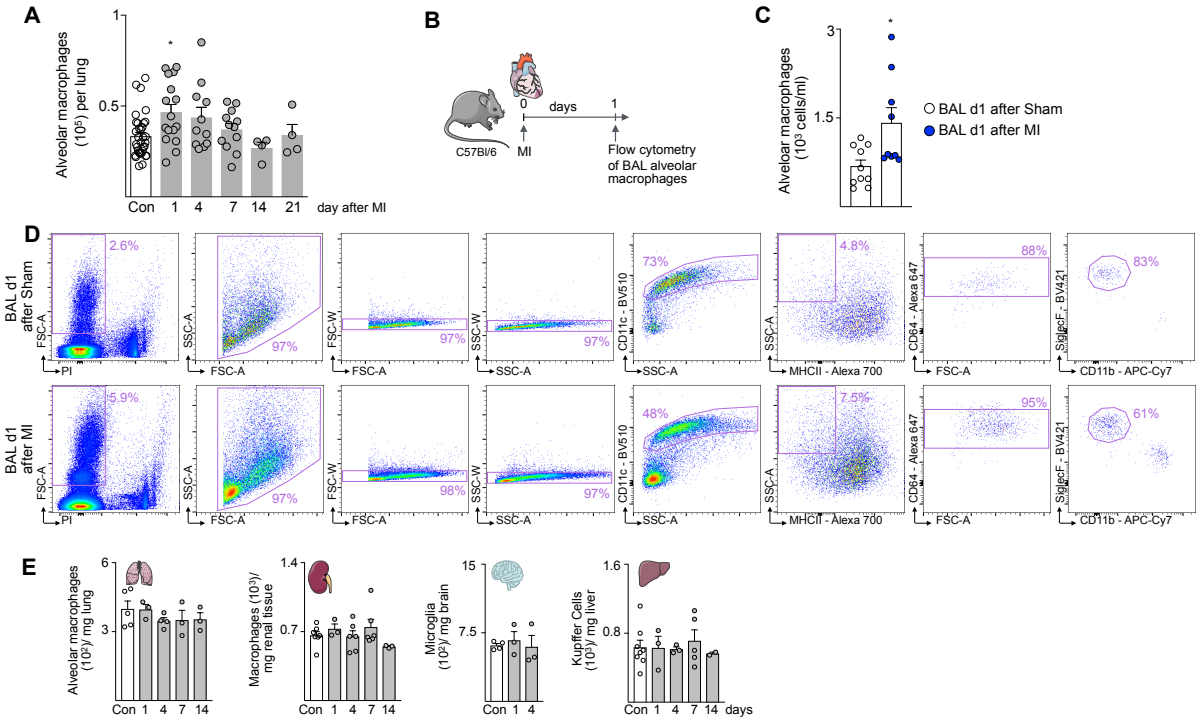

Figure S7. Macrophage enumeration controls, Related to STAR methods.
(A) Enumeration of alveolar macrophage numbers expressed per lung after MI. (B-C) Enumeration of alveolar macrophages in the broncho-alveolar lavage on day 1 after MI compared to Sham operated controls. Data are mean±SEM, P=0.014, two-tailed Mann-Whitney test, n=9-10 mice per group. (E) Macrophage numbers measured by flow cytometry in naive mice and after sham thoracotomy. *n*=4-8 naive controls, *n*=3-6 sham thoracotomy.