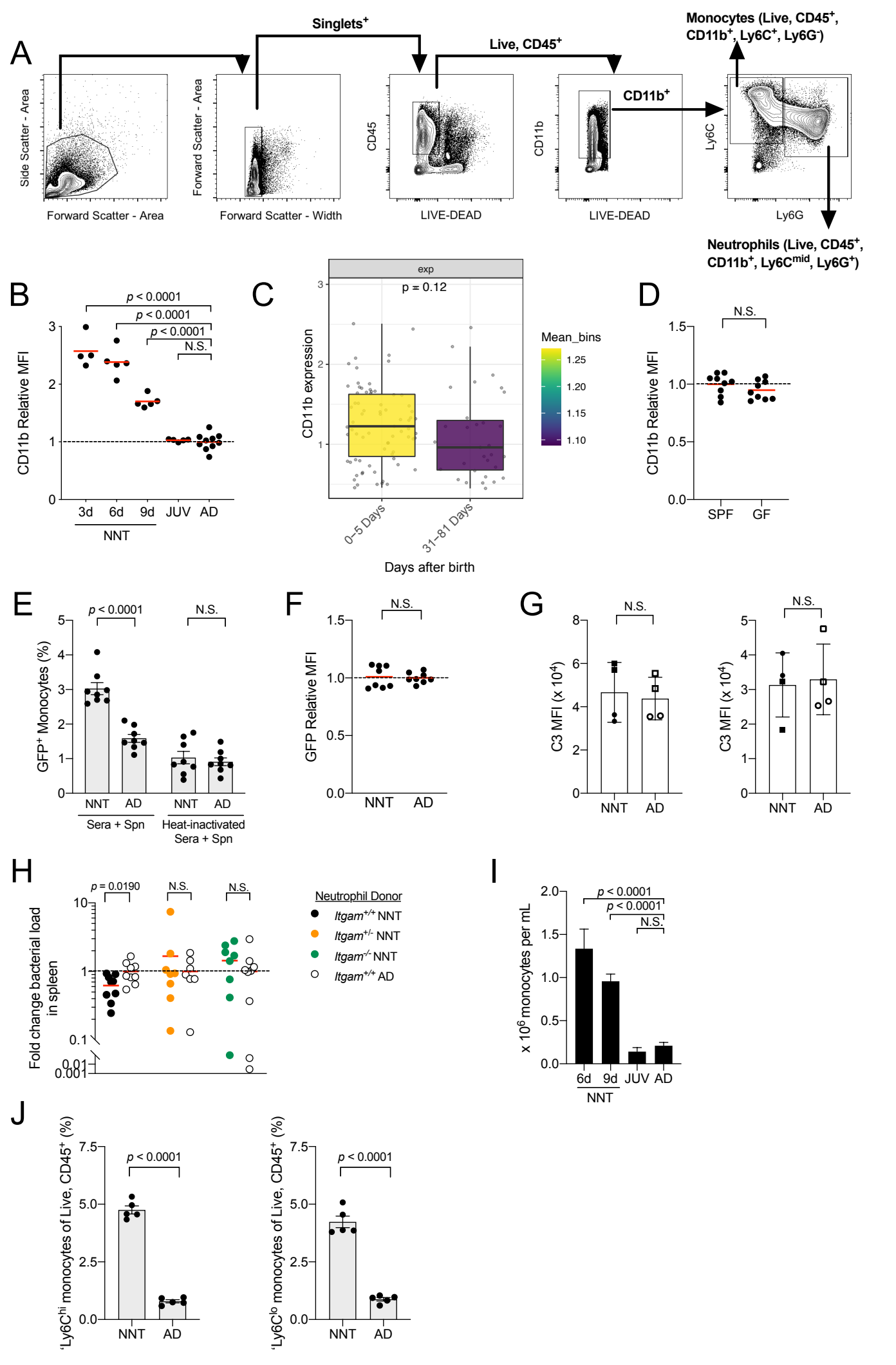

A
Singlets+
Live, CD45+
Monocytes (Live, CD45+, CD11b+, Ly6C+, Ly6G-)
CD11b+
Side Scatter - Area
Forward Scatter - Area
Forward Scatter - Area
Forward Scatter - Width
CD45
LIVE-DEAD
CD11b
LIVE-DEAD
Ly6C
Ly6G
Neutrophils (Live, CD45+, CD11b+, Ly6Cmid, Ly6G+)
B
p < 0.0001
p < 0.0001
p < 0.0001
N.S.
CD11b Relative MFI
3d
6d
9d
JUV
AD
NNT
C
exp
p = 0.12
CD11b expression
Mean_bins
1.25
1.20
1.15
1.10
0–5 Days
31–81 Days
Days after birth
D
N.S.
CD11b Relative MFI
SPF
GF
E
p < 0.0001
N.S.
GFP+ Monocytes (%)
NNT
AD
NNT
AD
Sera + Spn
Heat-inactivated Sera + Spn
F
N.S.
GFP Relative MFI
NNT
AD
G
N.S.
C3 MFI (x 10^4)
NNT
AD
N.S.
C3 MFI (x 10^4)
NNT
AD
H
p = 0.0190
N.S.
N.S.
Fold change bacterial load in spleen
Neutrophil Donor
Itgam+/+ NNT
Itgam+/- NNT
Itgam-/- NNT
Itgam+/+ AD
I
p < 0.0001
p < 0.0001
N.S.
x 10^6 monocytes per mL
6d
9d
JUV
AD
NNT
J
p < 0.0001
'Ly6Chi monocytes of Live, CD45+ (%)
NNT
AD
p < 0.0001
'Ly6Clo monocytes of Live, CD45+ (%)
NNT
AD

Supplementary Figure 1 – Related to Figures 2 and 4: **Extended phenotypic and functional analyses of neonatal myeloid cells.**

[A] Gating strategy for phenotyping of myeloid cells in mouse bone marrow.

[B] MFI of CD11b on bone marrow monocytes from mice at different ages [3 days old (3d), n = 4; 6 days old (6d), n = 5; 9 days old (9d), n = 5; JUV, n = 5; AD, n = 10].

[C] CD11b marker expression of monocytes from mass cytometry experiment comparing newborns aged 0-5 days (n = 75) and 31-81 days after birth (n = 31), mean expression from binned days used for coloring.

[D] MFI of CD11b on bone marrow neutrophils from neonatal mice house in either germ-free or conventional, specific pathogen free facility (n = 9 for both groups).

[E] Opsonophagocytic uptake of mouse sera opsonized GFP-fluorescent Spn by monocytes from bone marrow suspensions of neonatal or adult mice at multiplicity of infection (MOI) of 25 (n = 8 for both groups).

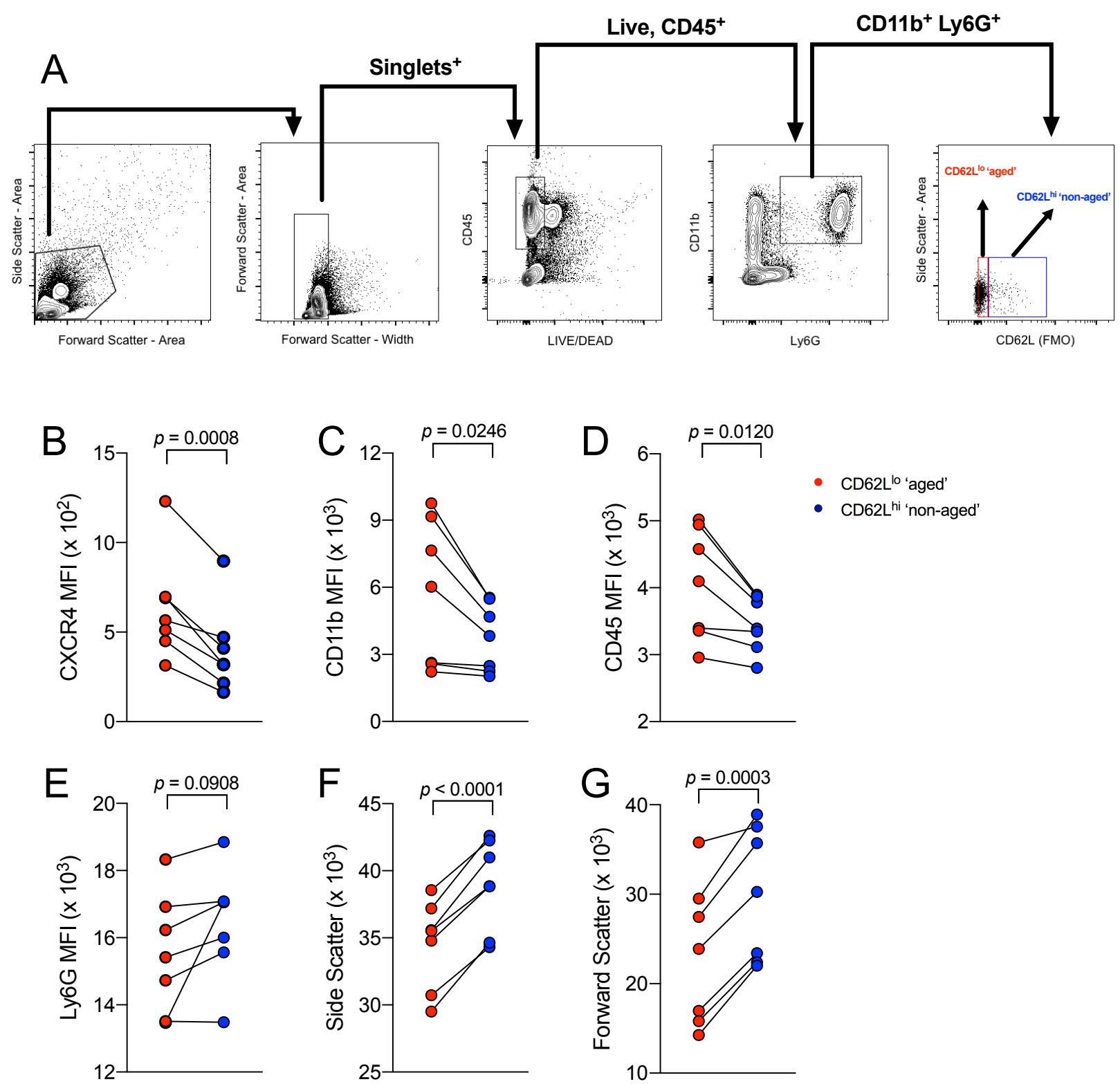
[F] MFI of GFP signal on GFP+ monocytes in [D].

[G] Deposition of complement C3 on Spn (left, serotype 4; right, serotype 23F) in 5-10% sera from NNT versus AD mice (n = 2 with 2 technical replicates marked by the same symbol).

[H] Recoverable live bacteria in spleen of *Cd11b*$^{-/-}$ recipients receiving either *Cd11b*$^{+/+}$ neonatal neutrophils (solid circle, n = 9), *Cd11b*$^{+/-}$ neonatal neutrophils (solid orange circles, n = 8) or *Cd11b*$^{-/-}$ neonatal neutrophils (solid green circle, n = 8) relative to that of recipients receiving *Cd11b*$^{+/+}$ adult neutrophils (open circle; n = 9, n = 9, n = 7, respectively) at 20 hours post infection with Spn via IP.

[I] Monocyte counts in peripheral blood from NNT (6d old, n = 7; 9d old, n = 10), JUV (n = 6) and AD (n = 11) mice.

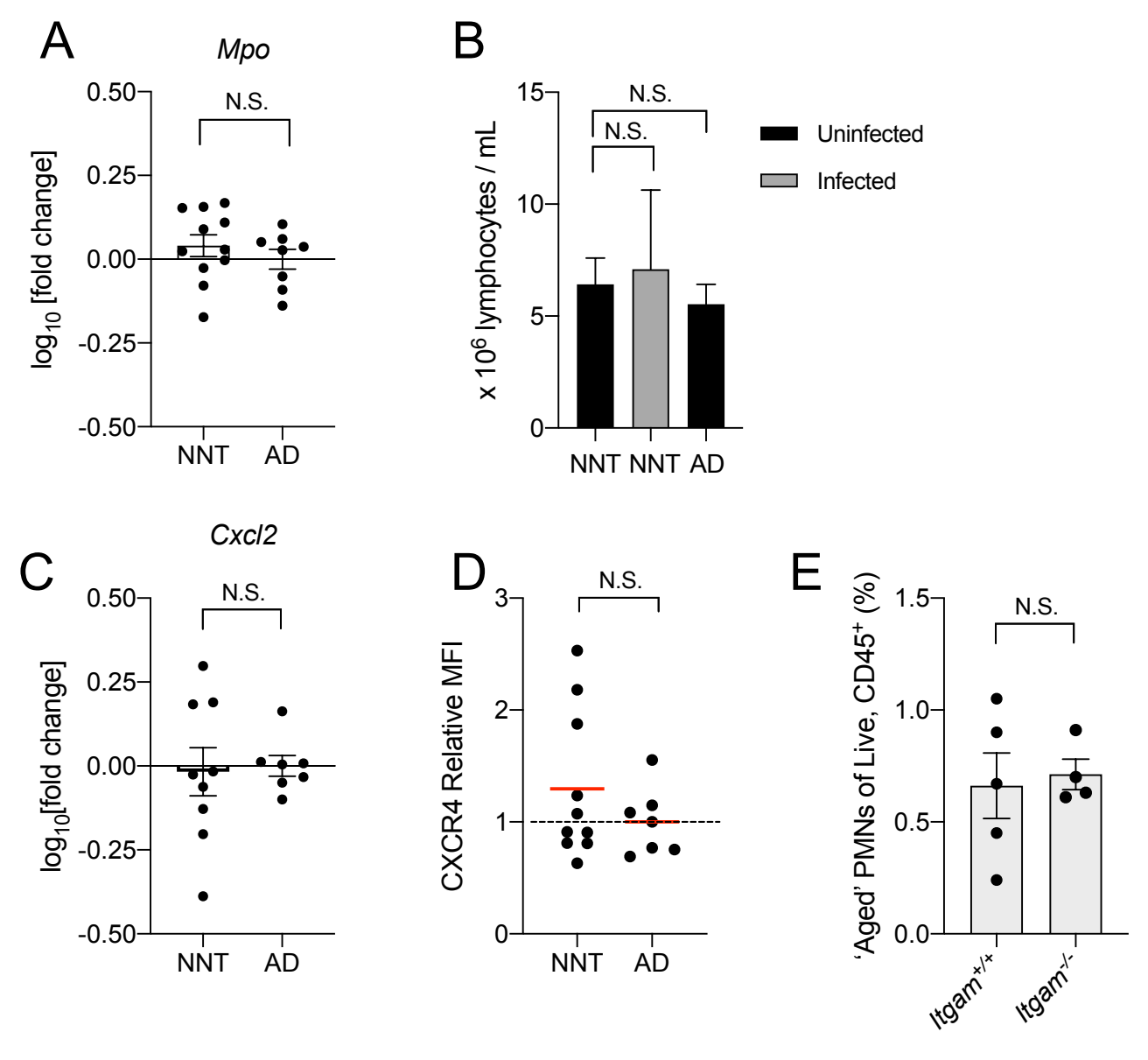
[J] Frequency of Ly6C^{hi} monocytes (left) and Ly6C^{lo} monocytes (right) from spleen of NNT (n = 5) versus AD (n = 5) mice. Data with error bars are presented as mean ± SEM. N.S., not significant.


A
Singlets+
Live, CD45+
CD11b+ Ly6G+
Side Scatter - Area
Forward Scatter - Area
Forward Scatter - Area
Forward Scatter - Width
CD45
LIVE/DEAD
CD11b
Ly6G
Side Scatter - Area
CD62L (FMO)
CD62Llo 'aged'
CD62Lhi 'non-aged'
B
p = 0.0008
CXCR4 MFI (x 10²)
C
p = 0.0246
CD11b MFI (x 10³)
D
p = 0.0120
CD45 MFI (x 10³)
CD62Llo 'aged'
CD62Lhi 'non-aged'
E
p = 0.0908
Ly6G MFI (x 10³)
F
p < 0.0001
Side Scatter (x 10³)
G
p = 0.0003
Forward Scatter (x 10³)

Supplementary Figure 2 - Related to Figure 3: **Assessment of surface markers associated with CD62L^{lo} 'aged' neutrophils versus CD62L^{hi} 'non-aged' neutrophils in adult peripheral blood**.

[A] Gating strategy for phenotyping of neutrophils in mouse blood.

[B-G] MFI analysis of known markers between CD62L^{lo} 'aged' (red dots) and CD62L^{hi} 'non-aged' (blue dots) neutrophils in adult blood (n = 7).


A
Mpo
log10 [fold change]
0.50
0.25
0.00
-0.25
-0.50
N.S.
NNT
AD
B
x 10^6 lymphocytes / mL
15
10
5
0
N.S.
N.S.
NNT
NNT
AD
Uninfected
Infected
C
Cxcl2
log10[fold change]
0.50
0.25
0.00
-0.25
-0.50
N.S.
NNT
AD
D
CXCR4 Relative MFI
3
2
1
0
N.S.
NNT
AD
E
'Aged' PMNs of Live, CD45+ (%)
1.5
1.0
0.5
0.0
N.S.
Itgam+/+
Itgam-/-

Supplementary Figure 3 – Related to Figure 4: **Bias of neonatal neutrophils toward an 'aged' phenotype is unlikely driven by lifespan shortening, intrinsic signaling pathways or CD11b expression itself.**

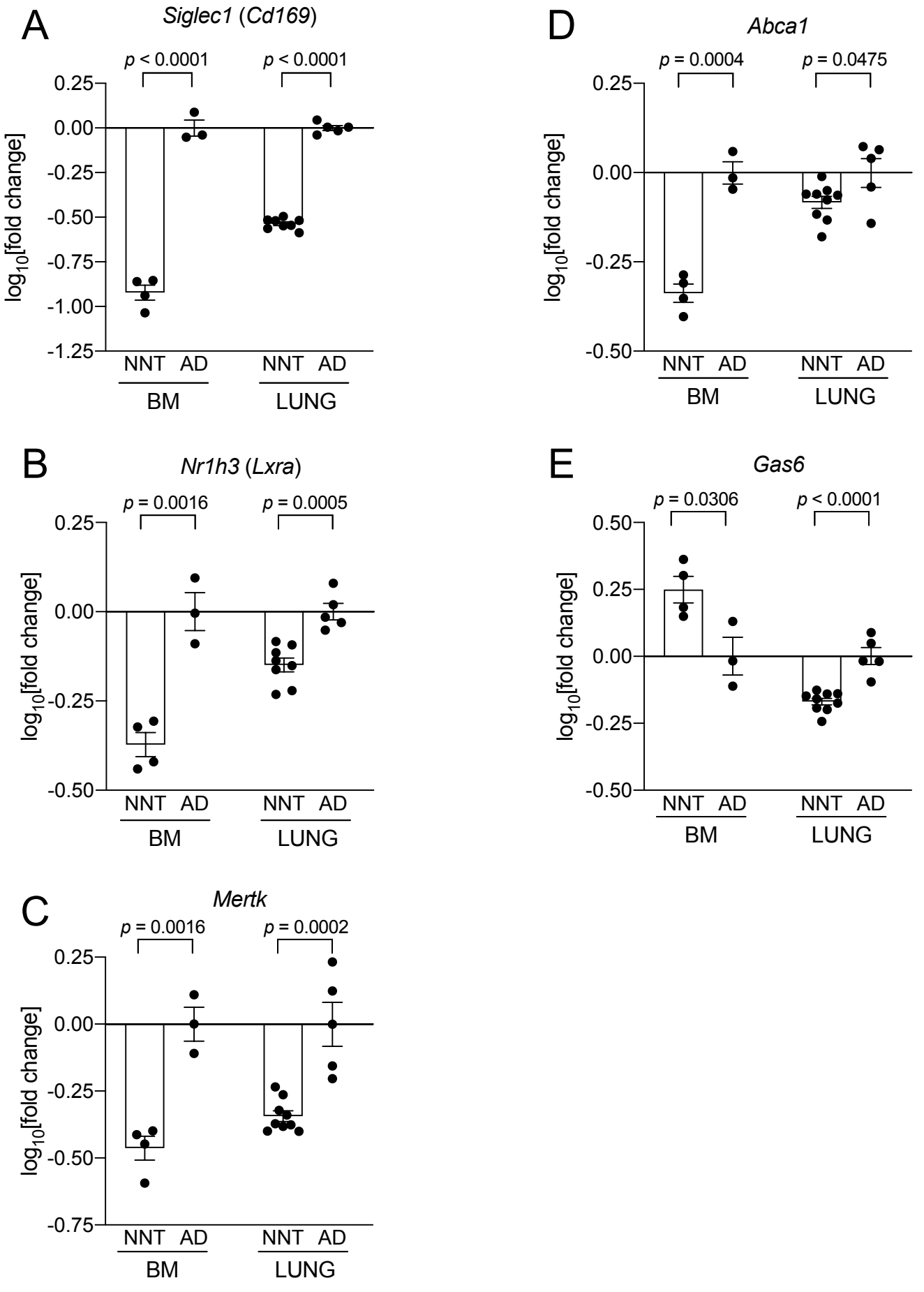
[A] RT-PCR analysis of myeloperoxidase (MPO) mRNA transcript in NNT (n = 11) versus AD (n = 8) neutrophils.

[B] Lymphocyte counts in peripheral blood from uninfected NNT mice (n = 5), NNT mice infected with Spn at 24 hpi (n = 4) and uninfected AD mice (n = 6).

[C] RT-PCR analysis of Cxcl2 mRNA transcript in NNT (n = 9) versus AD (n = 7) neutrophils.

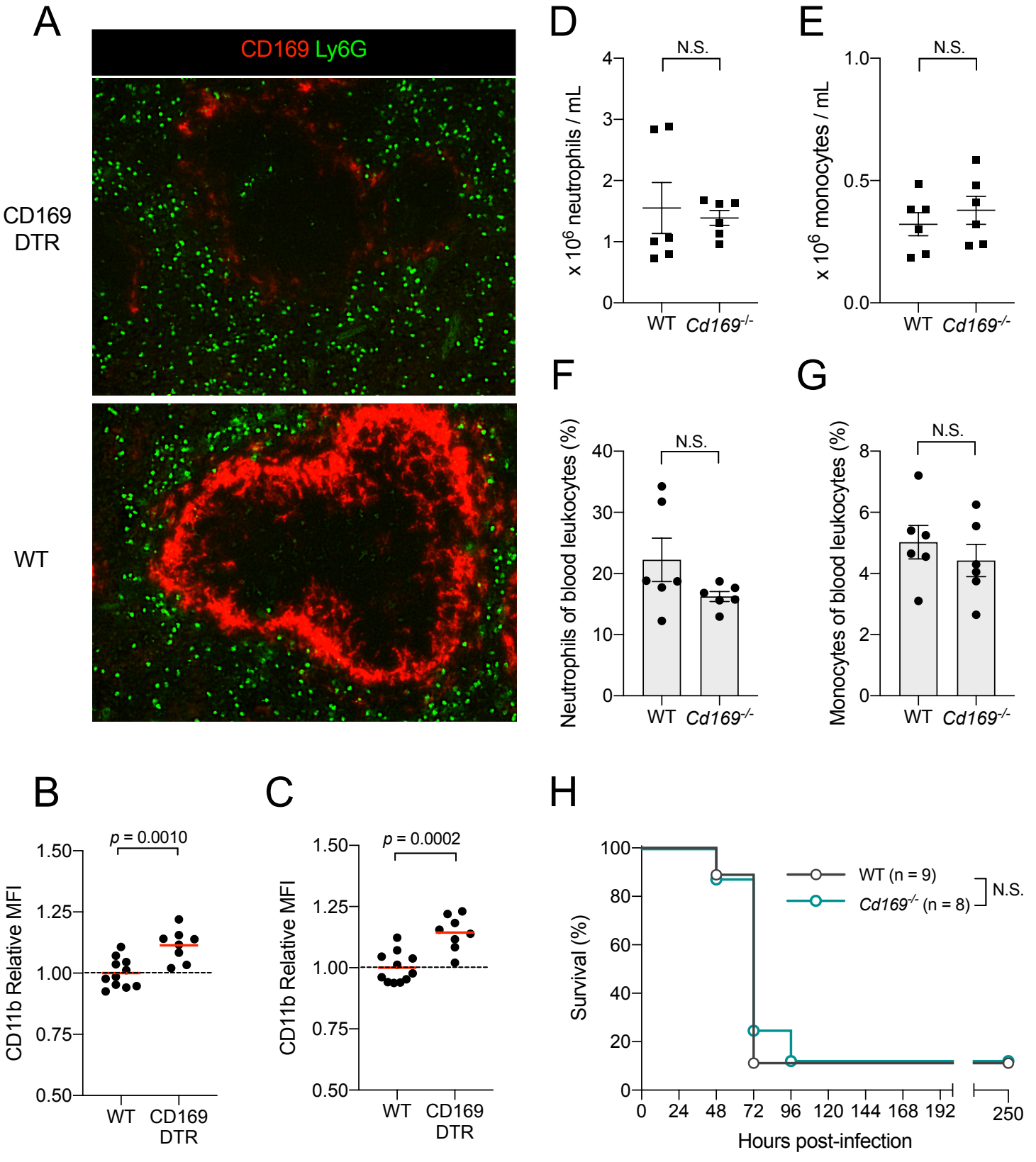
[D] MFI of CXCR4 on peripheral blood neutrophils from NNT (n = 10) versus AD mice (n = 7).

[E] Frequency of $CD62L^{lo}$ 'aged' neutrophils from peripheral blood of $Cd11b^{+/+}$ AD mice (n = 5) versus $Cd11b^{-/-}$ AD mice (n = 4). Data with error bars are presented as mean ± SEM. N.S., not significant.


A
Siglec1 (Cd169)
p < 0.0001
p < 0.0001
log10[fold change]
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
NNT AD
NNT AD
BM
LUNG
B
Nr1h3 (Lxra)
p = 0.0016
p = 0.0005
log10[fold change]
0.25
0.00
-0.25
-0.50
NNT AD
NNT AD
BM
LUNG
C
Mertk
p = 0.0016
p = 0.0002
log10[fold change]
0.25
0.00
-0.25
-0.50
-0.75
NNT AD
NNT AD
BM
LUNG
D
Abca1
p = 0.0004
p = 0.0475
log10[fold change]
0.25
0.00
-0.25
-0.50
NNT AD
NNT AD
BM
LUNG
E
Gas6
p = 0.0306
p < 0.0001
log10[fold change]
0.50
0.25
0.00
-0.25
-0.50
NNT AD
NNT AD
BM
LUNG

Supplementary Figure 4 – Related to Figure 4: **Transcriptional analysis of efferocytosis-associated markers in bone marrow and lung tissues.**

Bone marrow and lung expression of **[A]** *Cd169*, **[B]** *Lxra*, **[C]** *Mertk*, **[D]** *Abca1* and **[E]** *Gas6* in NNT (bone marrow, n = 4; lung, n = 8-9) versus AD (bone marrow, n = 3; lung, n = 5). Expression was calculated as fold change in NNT relative to AD. Data with error bars are presented as mean ± SEM.


A
CD169 Ly6G
CD169 DTR
WT
D
N.S.
x 10^6 neutrophils / mL
WT
Cd169-/-
E
N.S.
x 10^6 monocytes / mL
WT
Cd169-/-
F
N.S.
Neutrophils of blood leukocytes (%)
WT
Cd169-/-
G
N.S.
Monocytes of blood leukocytes (%)
WT
Cd169-/-
B
p = 0.0010
CD11b Relative MFI
WT
CD169 DTR
C
p = 0.0002
CD11b Relative MFI
WT
CD169 DTR
H
WT (n = 9)
Cd169-/- (n = 8)
N.S.
Survival (%)
Hours post-infection

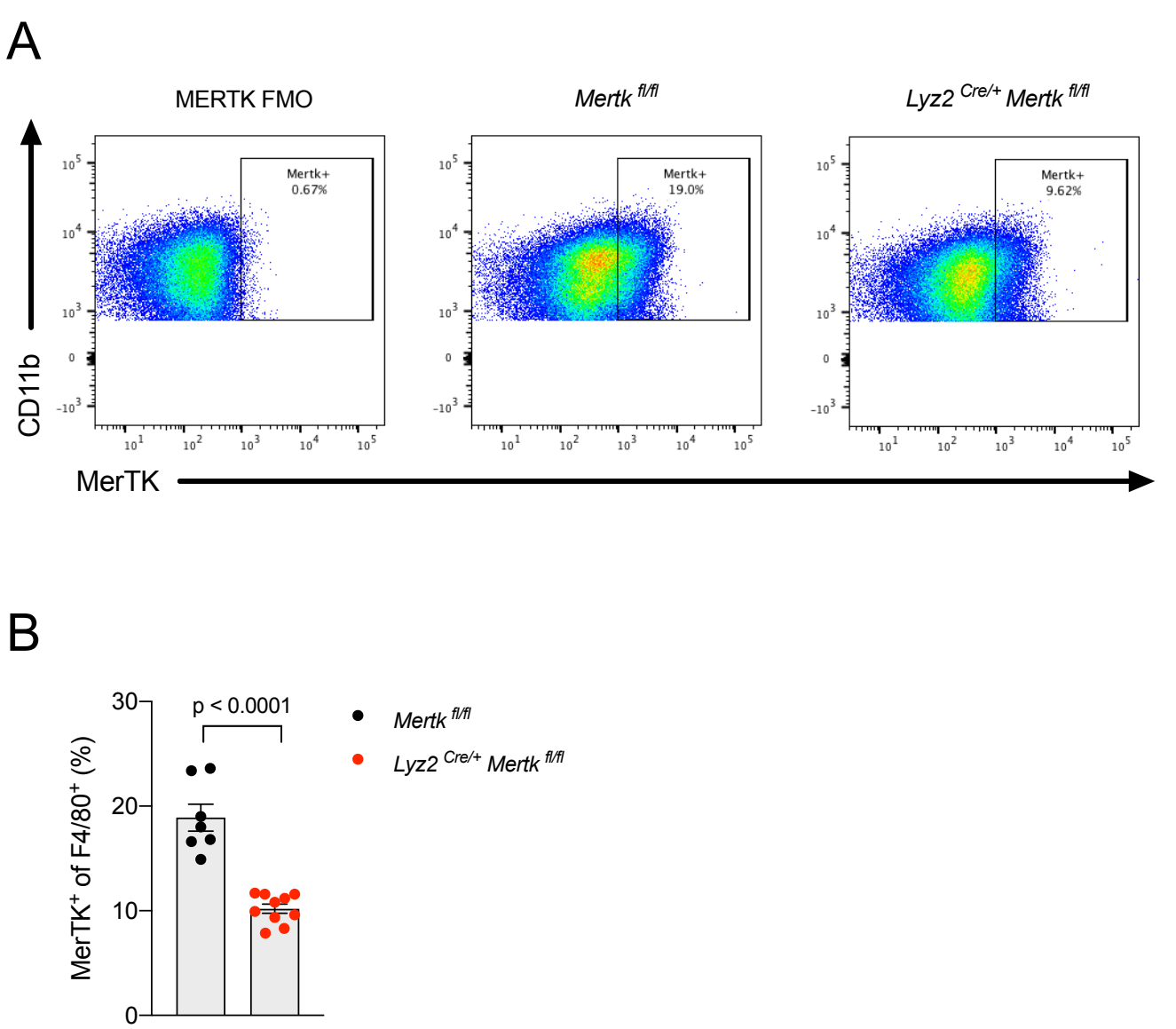
Supplementary Figure 5 – Related to Figure 5: **Extended phenotypic analyses of CD169-DTR and *Cd169*$^{-/-}$ adult mice.**

[A] Representative images of spleens from CD169-DTR and WT mice following DT administration as described in STAR Methods.

[B-C] MFI expression of CD11b on bone marrow Ly6C^{hi} [B] and Ly6C^{lo} [C] monocytes in WT (n = 11) versus CD169-DTR mice (n = 8).

[D-G] Absolute counts (neutrophils in [D], monocytes in [E]) or percentage population of all leukocytes (neutrophils in [F] monocytes in [G]) in blood from WT versus *Cd169*$^{-/-}$ adults (n = 6 for both groups).

[H] Survival of WT (open circle grey line, n = 9) versus *Cd169*$^{-/-}$ adults (open circle teal line, n = 8) following IP challenge with Spn. Data with error bars are presented as mean ± SEM. N.S., not significant.


A
MERTK FMO
Mertk fl/fl
Lyz2 Cre/+ Mertk fl/fl
Mertk+
0.67%
Mertk+
19.0%
Mertk+
9.62%
CD11b
MerTK
B
p < 0.0001
Mertk fl/fl
Lyz2 Cre/+ Mertk fl/fl
MerTK+ of F4/80+ (%)
30
20
10
0

Supplementary Figure 6 – Related to Figure 5: **Assessment of MerTK expression on macrophages in the *Lyz2*$^{Cre/+}$ *Mertk*$^{fl/fl}$ conditional knockout mice.**

[**A**] Representative flow plots assessing frequency of MerTK-expressing cells as proportion of macrophages (gated on Live, Singlets, CD11b^{+} F4/80^{+}) from bone marrow of *Mertk*$^{fl/fl}$ versus *Lyz2*$^{Cre/+}$ *Mertk*$^{fl/fl}$ mice; FMO, fluorescence-minus-one control.

[**B**] Quantification of data described in [A] of *Mertk*$^{fl/fl}$ adult mice (n = 6) and *Lyz2*$^{Cre/+}$ *Mertk*$^{fl/fl}$ adult mice (n = 9). Data with error bars are presented as mean ± SEM.

Table S1 – Related to Figures 2 and 4: **List of mouse primer sequences used in this study**

Gene	**Primer Sequence (5' – 3')**
Gapdh	Forward: AGG TCG GTG TGA ACG GAT TTG
	Reverse: TGT AGA CCA TGT AGT TGA GGT CA
Itgam (Cd11b)	Forward: ATG GAC GCT GAT GGC AAT ACC
	Reverse: TCC CCA TTC ACG TCT CCC A
Cd169	Forward: CTT GGG TCA GCC AAC AGT TC
	Reverse: GGT GAT GGT GAA ACC TGG AC
Lxra	Forward: CAA CAG TGT AAC AGG CGC T
	Reverse: TGC AAT GGG CCA AGG C
Mertk	Forward: GAG GAC TGC TTG GAT GAA CTG TA
	Reverse: AGG TGG GTC GAT CCA AGG
Abca1	Forward: GGT TTG GAG ATG GTT ATA CAA TAG TT
	Reverse: CCC GGA AAC GCA AGT CC
Gas6	Forward: GGA TTT GCT ACC TAC AGG CTC A
	Reverse: TTA ACT TCC CAG GTG GTT TCC
Mpo	Forward: GGA AGG AGA CCT AGA GGT TGG
	Reverse: TAG CAC AGG AAG GCC AAT G
Cxcl2	Forward: CCA CCA ACC ACC AGG CTA C
	Reverse: GCT TCA GGG TCA AGG GCA AA