Cell Reports, Volume *26*

Supplemental Information

Myeloid Cells Restrict MCMV and Drive Stress-Induced Extramedullary Hematopoiesis through STAT1

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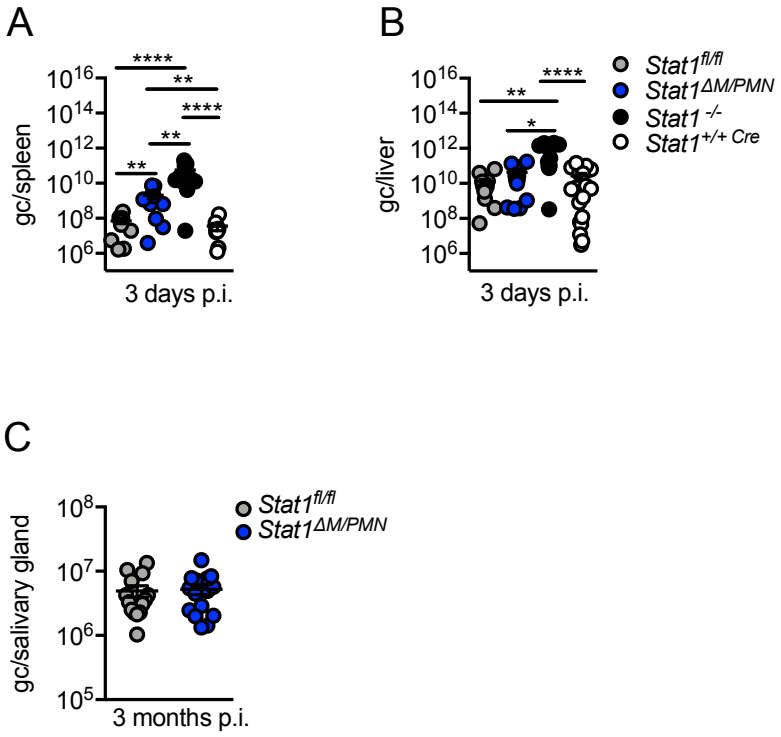

Figure S1. Related to Figure 1. *Myeloid cells suppress early MCMV replication in the spleen through STAT1.*

(A, B) MCMV genome copy number (gc) in **(A)** spleen (n=10-12, N=2) and **(B)** liver (n=12-18, N=2-3) of *Stat1*$^{fl/fl}$, *Stat1*$^{\Delta M/PMN}$, *Stat1*$^{-/-}$ and *Stat1*$^{+/+\ Cre}$ mice after i.p. infection with 5 x 10^4 PFU of MCMV. **(C)** MCMV genome copy number in salivary glands of *Stat1*$^{fl/fl}$ and *Stat1*$^{\Delta M/PMN}$ *mice* 3 months after i.p. infection with 2.5 x 10^4 PFU of MCMV (n=14-15, N=2). Mean values ± SEM are given. n, biological replicates; N, experimental repetitions; ** $p \leq 0.05$, ** $p \leq 0.01$, **** $p \leq 0.0001$.*

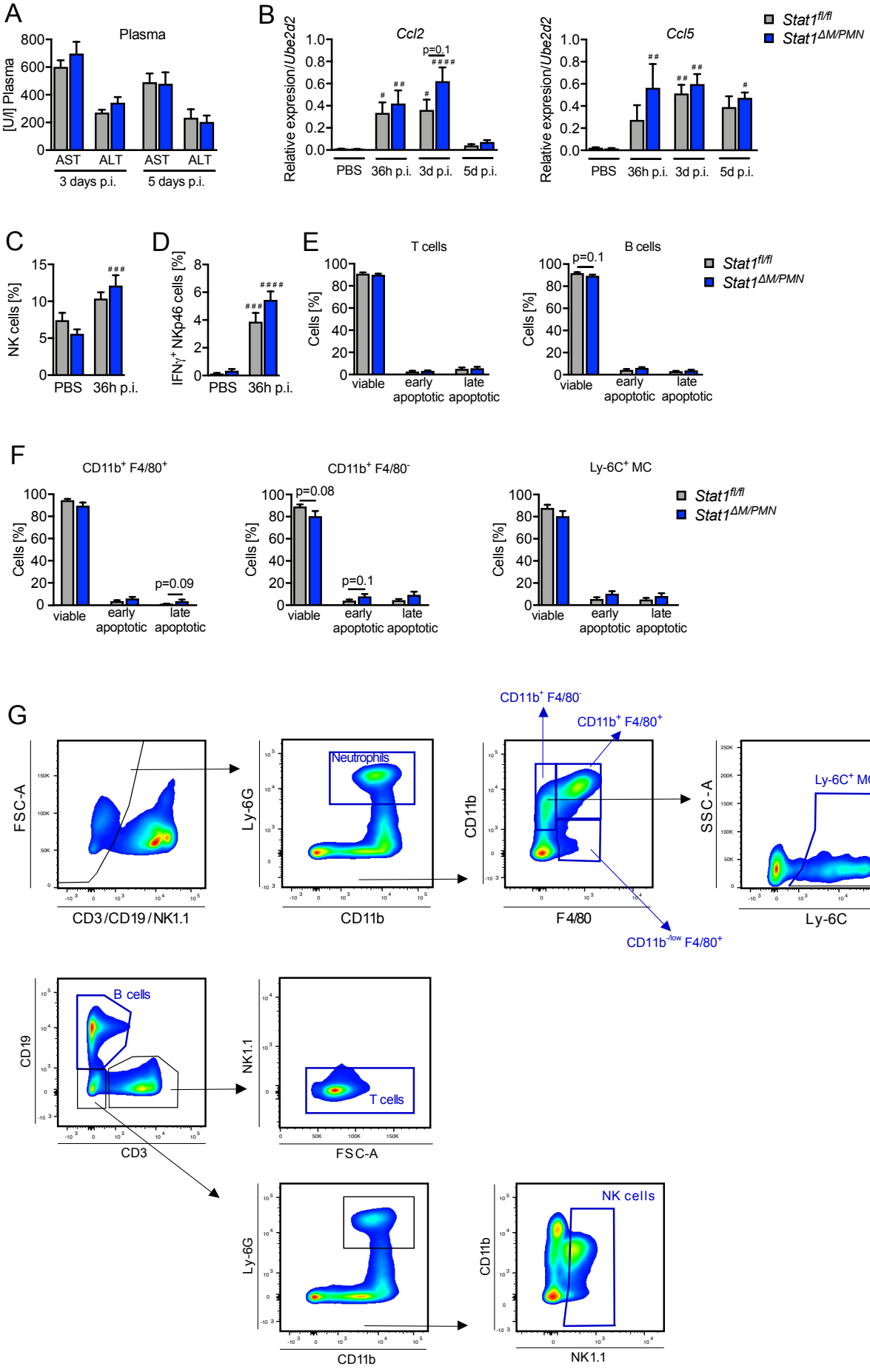
A
Plasma
[U/l] Plasma
AST
ALT
AST
ALT
3 days p.i.
5 days p.i.
B
Ccl2
Relative expresion/Ube2d2
p=0.1
PBS
36h p.i.
3d p.i.
5d p.i.
Ccl5
Stat1fl/fl
Stat1ΔM/PMN
C
NK cells [%]
PBS
36h p.i.
D
IFNγ+ NKp46 cells [%]
E
T cells
B cells
Cells [%]
viable
early apoptotic
late apoptotic
p=0.1
F
CD11b+ F4/80+
CD11b+ F4/80-
Ly-6C+ MC
p=0.09
p=0.08
G
FSC-A
CD3/CD19/NK1.1
Ly-6G
CD11b
Neutrophils
CD11b+ F4/80-
CD11b+ F4/80+
F4/80
CD11b-/low F4/80+
SSC-A
Ly-6C
Ly-6C+ MC
CD19
B cells
CD3
NK1.1
T cells
FSC-A
NK cells
NK1.1

Figure S2. Related to Figure 2. *Myeloid STAT1 protects from MCMV-induced spleen pathology.*

(A) Blood was collected at indicated time points and levels of AST and ALT were determined in the plasma of controls (PBS) and MCMV infected mice (n=9-14, N=2-4) **(B)** Relative mRNA expression levels of *Ccl2* and *Ccl5* in the liver at indicated time points p.i. (n=8, N=2). **(C)** Percentage of liver NK cells and **(D)** percentage of IFNγ-producing liver NK cells in infected and PBS treated *Stat1*$^{fl/fl}$ *and Stat1*$^{\Delta M/PMN}$ mice (n=5-6, N=2). **(E, F)** Flow cytometric analysis of apoptotic cells in spleens of MCMV-infected mice (day 3 p.i.). Percentage of viable, early apoptotic and late apoptotic **(E)** T cells and B cells and **(F)** CD11b$^+$ F4/80$^+$, CD11b$^+$ F4/80$^-$ and Ly-6C$^+$ monocytes (MC); n=7-8, N=2. **(A-F)** Mean values ± SEM are given. $^{\#}$ *p ≤ 0.05,* $^{\#\#}$ *p ≤ 0.01,* $^{\#\#\#}$ *p ≤ 0.001,* $^{\#\#\#\#}$ *p ≤ 0.0001,* statistical significance relative to the PBS control; n, biological replicates; N, experimental repetitions. (**G**) Gating strategy for neutrophils (CD3$^-$ CD19$^-$ NK1.1$^-$ Ly-6G$^+$ CD11b$^+$), CD11b$^+$F4/80$^-$ monocytes/DCs (CD3$^-$CD19$^-$ NK1.1$^-$ Ly-6G$^-$ CD11b$^+$ F4/80$^-$), CD11b$^+$ F4/80$^+$ macrophages/monocytes (CD3$^-$ CD19$^-$ NK1.1$^-$ Ly-6G$^-$ CD11b$^+$ F4/80$^+$), CD11b$^{-/low}$ F4/80$^+$ macrophages (CD3$^-$ CD19$^-$ NK1.1$^-$ Ly-6G$^-$ CD11b$^{-/low}$ F4/80$^+$), B cells (CD19$^+$ CD3$^-$), T cells (CD19$^-$ CD3$^+$ NK1.1$^-$) and NK cells (CD19$^-$ CD3$^-$ Ly-6G$^-$ CD11b$^{-/int}$ NK1.1$^+$).

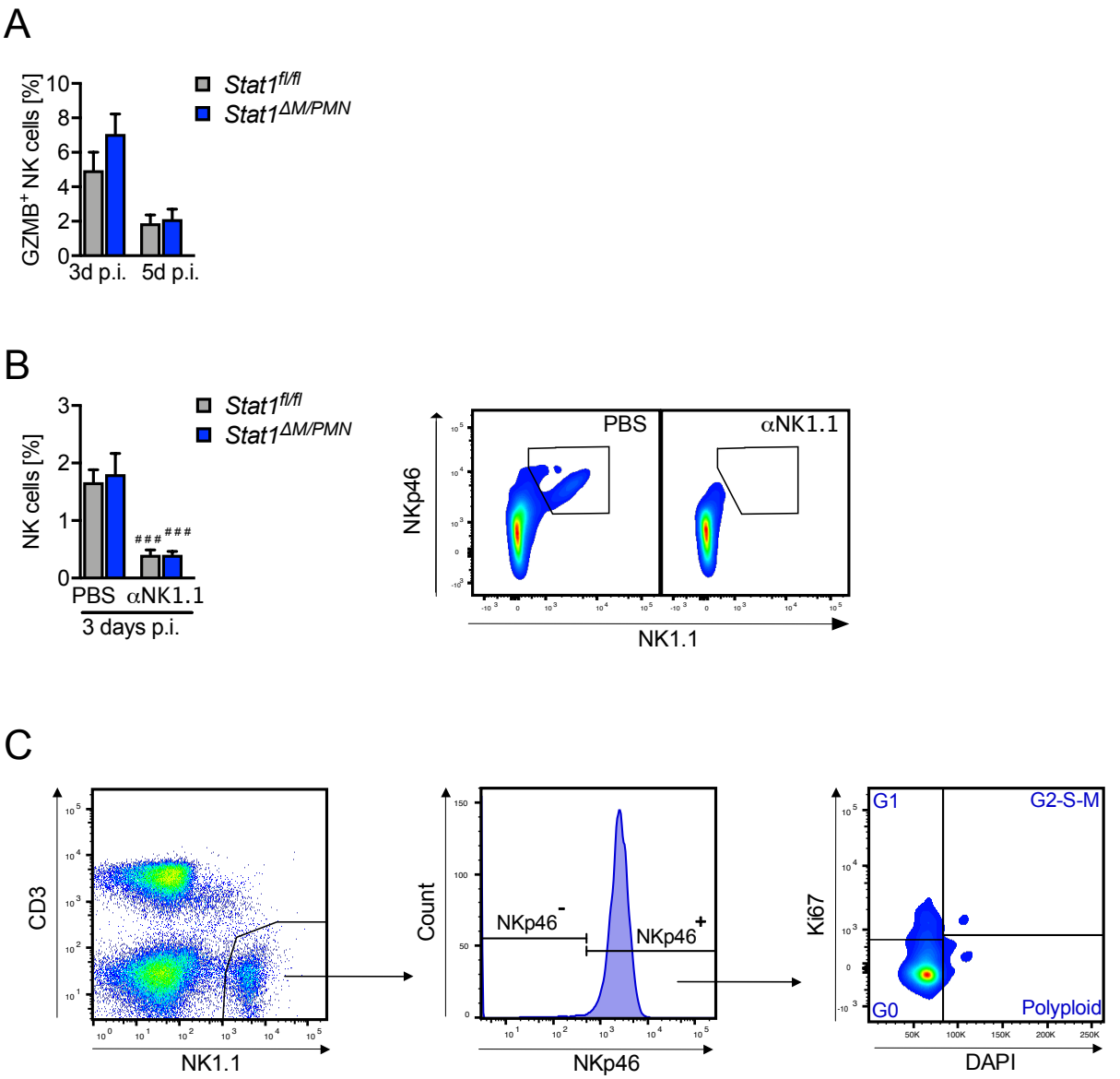

Figure S3. Related to Figure 3. *Impaired early control of MCMV infection in Stat1$^{\Delta M/PMN}$ mice is partially NK cell independent.*

(A) Percentage of Granzyme B (GZMB) positive NK cells in the spleen 3 and 5 days after i.p. infection with 5 x 10^4 PFU MCMV (n=8, N=2). **(B)** Quantification and representative FACS plots of NK cell frequencies before and after depletion (n=10, N=2). **(C)** Representative FACS plots showing gating strategy for DAPI/Ki67 staining of NKp46$^+$ NK cells (CD3$^-$ NK1.1$^+$). **(A, B)** Mean values ± SEM are given. $^{###}p \leq 0.001$, statistical significance relative to the PBS control; n, biological replicates; N, experimental repetitions.

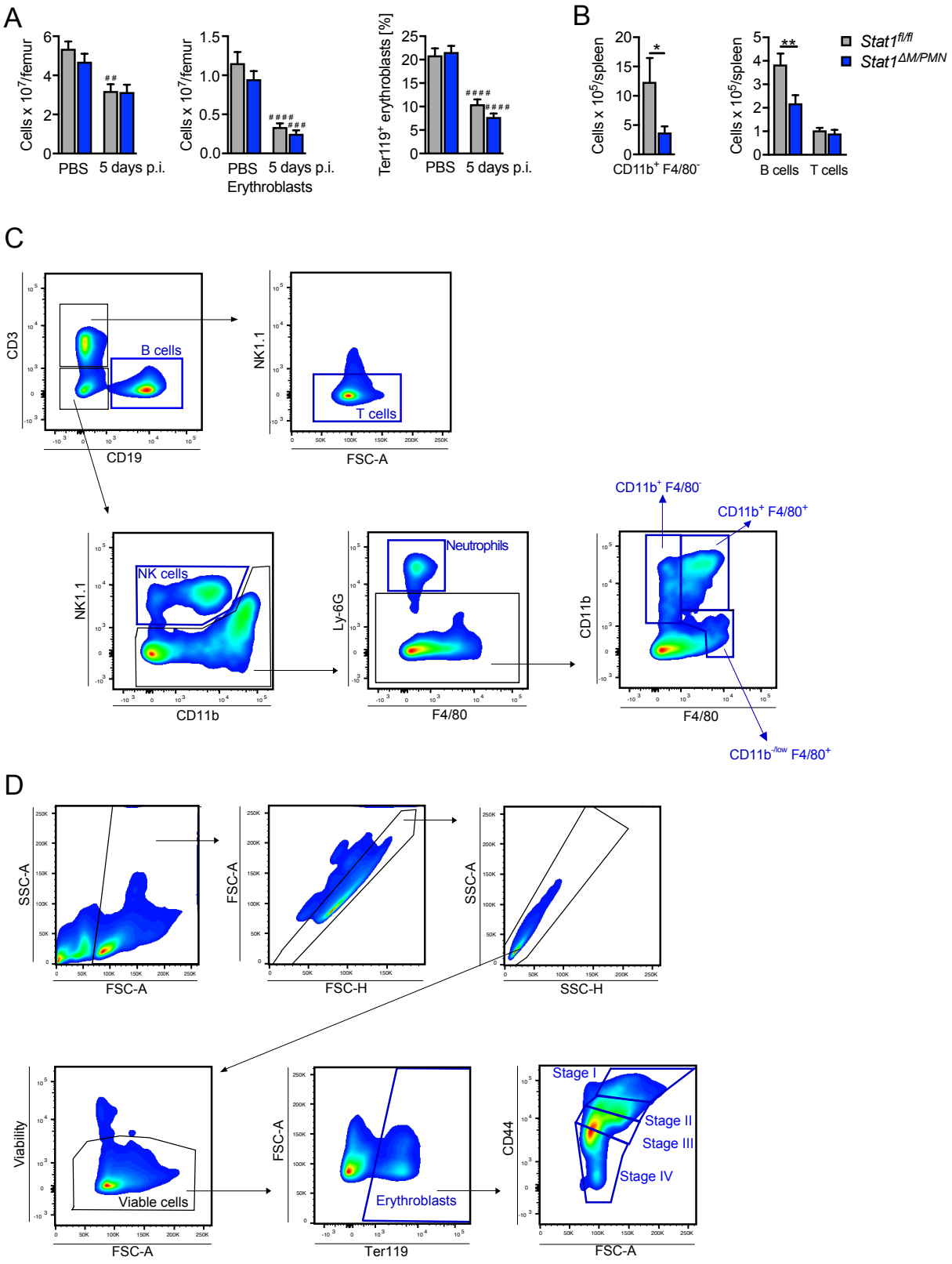

Figure S4. Related to Figure 4. *Stat1$^{\Delta M/PMN}$ mice have impaired extramedullary hematopoiesis during MCMV infection.*

(A) Total number of cells, erythroblasts and percentage of erythroblasts per femur in *Stat1$^{fl/fl}$* and *Stat1$^{\Delta M/PMN}$* mice 5 days p.i. with MCMV. (n=3-4, N=1). **(B)** Total numbers

of CD11b^{+} F4/80^{-} cells, B cells and T cells in *Stat1$^{fl/fl}$* and *Stat1$^{\Delta M/PMN}$* mice 5 days p.i. with MCMV. **(A, B)** Mean values ± SEM are given (n=8, N=2). n, biological replicates; N, experimental repetitions; * $p \leq 0.05$, ** $p \leq 0.01$, statistical significance between the genotypes; $^{\#\#}$ $p \leq 0.01$, $^{\#\#\#}$ $p \leq 0.001$, $^{\#\#\#\#}$ $p \leq 0.0001$, statistical significance relative to the PBS control. **(C)** Gating strategy for B cells (CD19^{+} CD3^{-}), T cells (CD19^{-} CD3^{+} NK1.1^{-}), NK cells (CD19^{-} CD3^{-} CD11b$^{-/int}$ NK1.1^{+}), neutrophils (CD3^{-} CD19^{-} NK1.1^{-} F4/80^{-} Ly-6G^{+}), CD11b^{+} F4/80^{-} monocytes/DCs (CD3^{-} CD19^{-} NK1.1^{-} Ly-6G^{-} CD11b^{+} F4/80^{-}), CD11b^{+} F4/80^{+} macrophages/monocytes (CD3^{-} CD19^{-} NK1.1^{-} Ly-6G^{-} CD11b^{+} F4/80^{+}) and CD11b$^{-/low}$F4/80^{+} macrophages (CD3^{-} CD19^{-} NK1.1^{-} Ly-6G^{-} CD11b$^{-/low}$ F4/80^{+}). **(D)** Gating strategy for erythroblasts (Ter119^{+}) and erythroblast precursors, namely stage I (FSC^{hi} CD44^{++}), stage II (FSC^{int} CD44$^{+/int}$), stage III (FSC^{int} CD44$^{int/-}$) and stage IV (FSC^{low} CD44^{-}). Size exclusion was done for mature erythrocytes and reticulocytes.

A

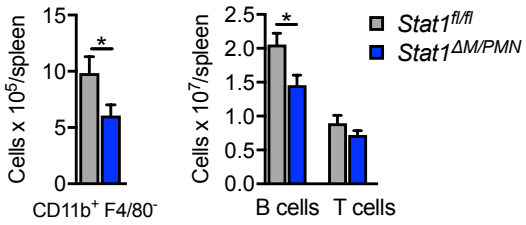

Figure S5. Related to Figure 6. *Stat1$^{\Delta M/PMN}$ mice have impaired extramedullary hematopoiesis during sterile inflammation.*

(A) Total numbers of CD11b^{+} F4/80^{-} monocytes/DCs, B and T cells in *Stat1$^{fl/fl}$* and *Stat1$^{\Delta M/PMN}$* mice 6 days post CpG-ODN injection. Mean values ± SEM are given (n=6-8; N=2). n, biological replicates; N, experimental repetitions; * $p \leq 0.05$.