Expanded View Figures

Figure EV1. CMO BM exhibits increased cellularity and expansion of HSPCs in a time-dependent fashion.

A, B Percentage (A) and absolute number (B) of distinct BM subpopulations. Each symbol represents one biological replicate (mouse). Blue round symbols indicate values for WT mice and square orange symbols indicate values for CMO mice. *X*-axes indicate the age of the mice: 7 or 20 weeks old (w). Data represent mean ± s.d. At least six mice were used per group in two separate experiments. Statistical significance was assessed using two-tailed Student's *t*-tests (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$, ns, not significant).

C Tri-lineage reconstitution upon transplantation of WT and CMO HSCs. The *x*-axis indicates the dose of donor cells. The *y*-axis indicates the percentage of cells defined as $Gr1^+$ $CD11b^+$ myeloid cells (green), $B220^+$ B-cells (red), and $CD3^+$ T-cells (blue). Each column represents values for one mouse.

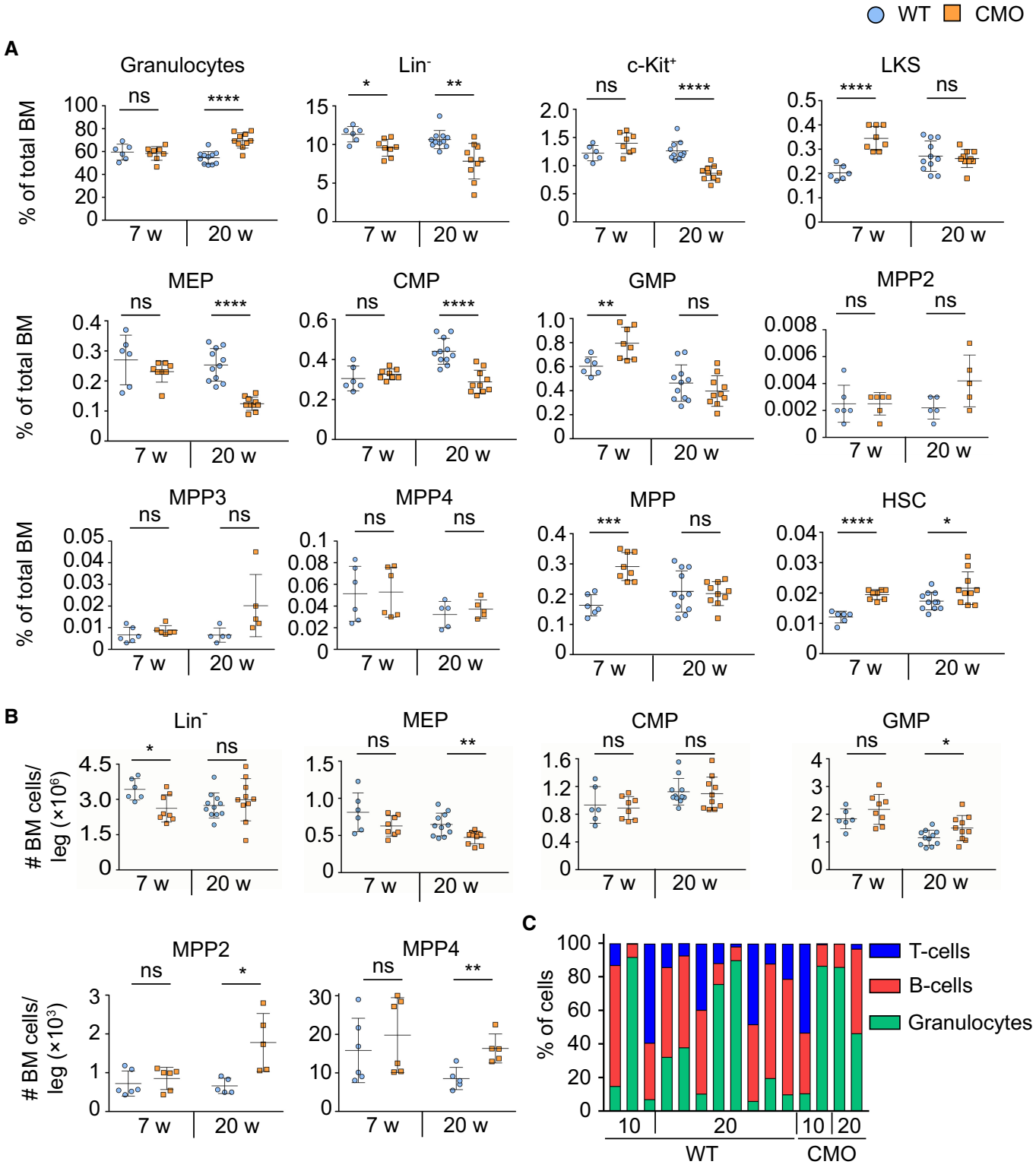

WT
CMO
A
Granulocytes
Lin-
c-Kit+
LKS
MEP
CMP
GMP
MPP2
MPP3
MPP4
MPP
HSC
% of total BM
7 w
20 w
ns
B
BM cells/ leg (×10^6)
BM cells/ leg (×10^3)
C
% of cells
T-cells
B-cells
Granulocytes
10
20
WT
CMO

Figure EV1.

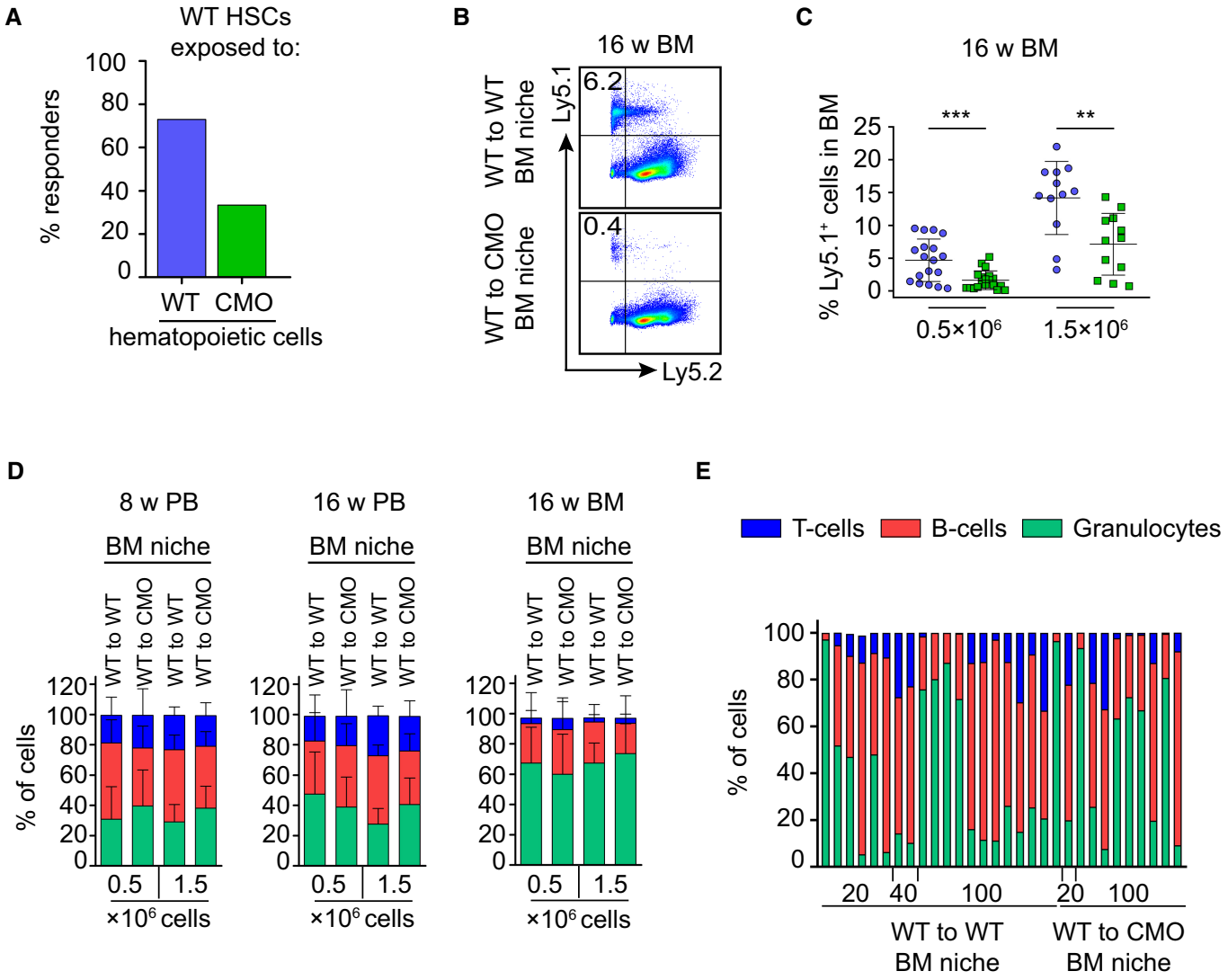


Figure EV2. CMO hematopoietic cells and the CMO BM niche exert detrimental effects on HSCs.

A Percentage of responder recipient mice that received WT HSCs, which co-habited with WT or CMO hematopoietic cells. A recipient was considered as a positive responder when engraftment was ≥ 0.01% Ly5.1$^+$ cells and contribution ≥ 0.5% in at least one out of three lineages (granulocytes, B-cells, and T-cells) at week 16 post-transplantation. Percentage of Ly5.1$^+$ donor-derived cells in blood of secondary recipients.

B Representative flow cytometry plots from secondary recipient mice transplanted with 0.5 × 10^6 WT BM exposed to WT (WT to WT) or CMO (WT to CMO) BM niche. *Y*-axis shows Ly5.1$^+$ cells and *x*-axis Ly5.2$^+$ cells in BM of recipient mice 16 weeks after transplantation. Numbers indicate the percentage of WT donor-derived Ly5.1$^+$ cells.

C Quantification of panel (B). *X*-axis indicates the dose of transplanted cells. At least 12 animals were used per group in three separate experiments. Values for WT BM exposed to WT BM niche are indicated with a blue symbol, and values for WT BM exposed to CMO BM niche are indicated in a green symbol. Each symbol represents one biological replicate (mouse). Data represent mean ± s.d. Statistical significance was assessed using two-tailed Student's *t*-tests (**$P < 0.01$, ***$P < 0.001$).

D Tri-lineage reconstitution from secondary transplantation of WT BM exposed to WT (WT to WT) or CMO (WT to CMO) BM niche. Graphics indicate analysis of peripheral blood (PB) 8 and 16 weeks after transplantation, and BM 16 weeks after transplantation. Each column indicates mean ± s.d. (at least 12 mice were used per group in three separate experiments). *Y*-axes indicate the percentage of donor-derived Ly5.1$^+$ Gr1$^+$ CD11b$^+$ granulocytes (green), B220$^+$ B-cells (red), and CD3$^+$ T-cells (blue). The *x*-axes indicate the number of donor cells.

E Tri-lineage reconstitution 16 weeks after transplantation in blood from secondary recipients transplanted with WT HSCs exposed to WT (WT to WT) or CMO (WT to CMO) BM niche. Each column represents values for one biological replicate (mouse). *Y*-axes indicate the percentage of donor-derived Ly5.1$^+$ Gr1$^+$ CD11b$^+$ granulocytes (green), B220$^+$ B-cells (red), and CD3$^+$ T-cells (blue). The *x*-axes indicate the number of donor cells.

Figure EV3. Phenotypic analysis of CMO mice and CMO mice lacking MyD88.

A *Y*-axis indicates IL-1β (pg/ml) in BM and paws isolated from WT (blue symbols) and symptomatic CMO (orange symbols) mice. Each symbol represents one biological replicate (mouse). At least five mice were used per group in two separate experiments. Data represent mean ± s.d. Statistical significance was assessed using two-tailed Student's *t*-tests (**P* < 0.05, ns, not significant).

B IkB-α levels in HSCs isolated from WT or CMO mice. *Y*-axis indicates IkB-α mean fluorescence intensity (MFI). Values are normalized to the average of WT levels. Each symbol represents one biological replicate (mouse). At least six mice were used per group in two separate experiments. Data represent mean ± s.d. Statistical significance was assessed using two-tailed Student's *t*-tests (ns, not significant).

C Percentage of distinct BM subpopulations in distinct murine lines. In this figure, each symbol represents one mouse (biological replicates). At least five mice were used per group in three separate experiments. Data represent mean ± s.d. Statistical significance was assessed using two-tailed Student's *t*-tests (**P* < 0.05, ***P* < 0.01, ****P* < 0.001, *****P* < 0.0001, ns, not significant).

D IL-1β levels in BM and paws. *Y*-axis indicated IL-1β (pg/ml) in BM and paws isolated from WT (blue symbols) and symptomatic CMO (orange symbols) mice. Each symbol represents one biological replicate (mouse). At least four mice were used per group in two separate experiments. Data represent mean ± s.d. Statistical significance was assessed using two-tailed Student's *t*-tests (**P* < 0.05, ***P* < 0.01, ns, not significant).

E Percentage (left panel) and absolute number (right panel) of LKS in BM. Each symbol represents one biological replicate (mouse). At least eight mice were used per group in three separate experiments. Data represent mean ± s.d. Statistical significance was assessed using two-tailed Student's *t*-tests (**P* < 0.05, ***P* < 0.01, ****P* < 0.001, ns, not significant).

F Tri-lineage reconstitution upon transplantation of CMO and CMO MyD88$^{-/-}$ HSCs. The *x*-axis indicates the dose of donor cells. The *y*-axis indicates the percentage of cells defined as Gr1^{+} CD11b^{+} myeloid cells (green), B220^{+} B-cells (red), and CD3^{+} T-cells (blue). Each column represents values for one mouse.

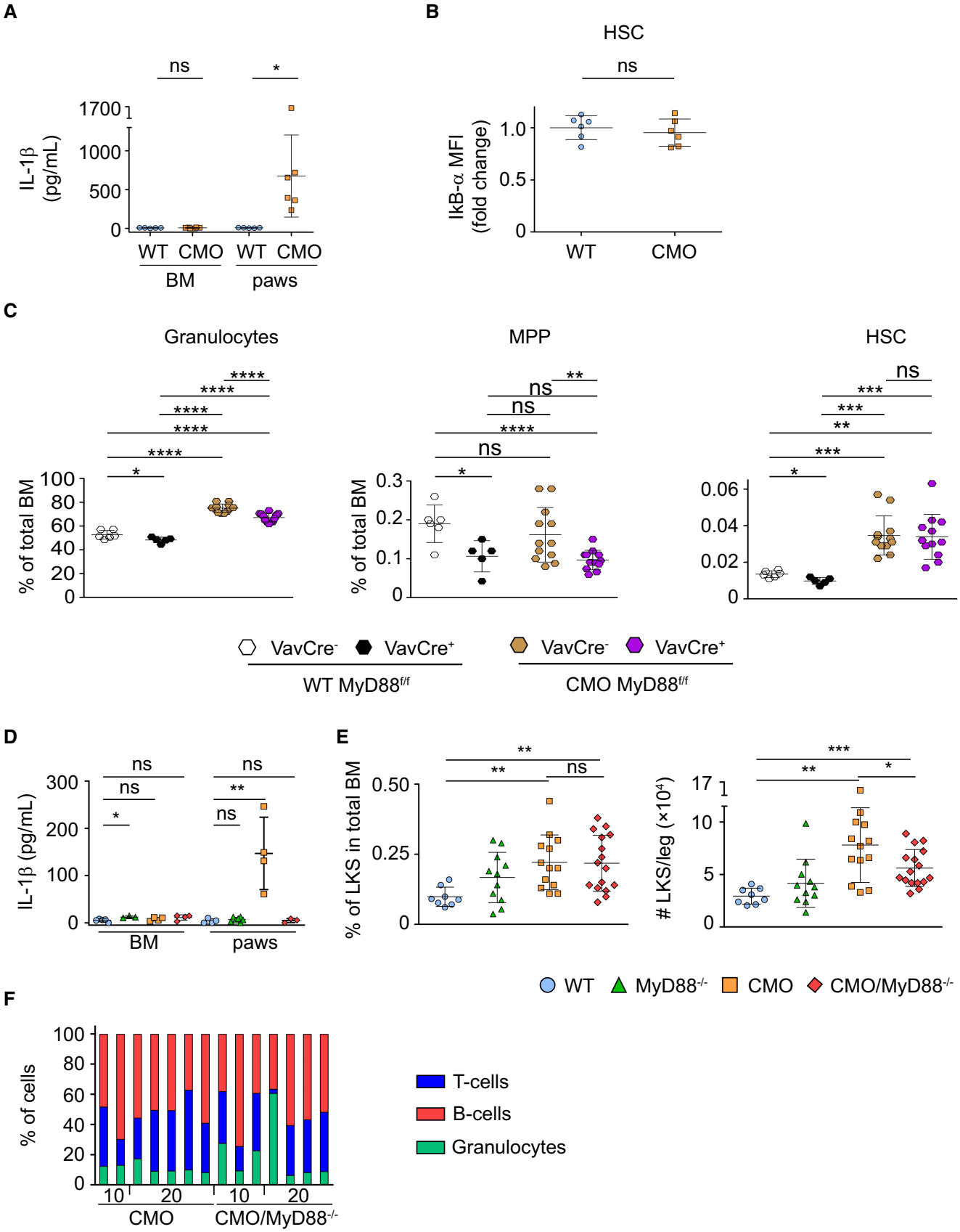


Figure EV3.

Figure EV4. RNA-seq analysis of WT HSCs exposed to WT recipient mice (WT exposed to WT BM niche) or WT HSC exposed to CMO recipient mice (WT exposed to CMO BM niche).

A Principal component analysis of four samples of WT HSC exposed to WT recipient mice (WT exposed to WT BM niche, blue symbols) and four samples of WT HSC exposed to CMO recipient mice (WT exposed to CMO BM niche, green symbols).

B Heatmap of unsupervised hierarchical cluster analysis of genes differentially expressed in WT HSC exposed to WT or CMO recipient mice ($P < 0.05$, log2 fold change > 0.5). Data is normalized to *z*-scores for each gene. Red color indicates increased and blue color decreased gene expression in comparison to the universal mean for each gene.

C Representative enrichment plots showing upregulated pathways in WT HSC exposed to CMO recipient mice from MSigDB Hallmark gene set v.7 and MSigDB GO Biological Process gene set v.7.

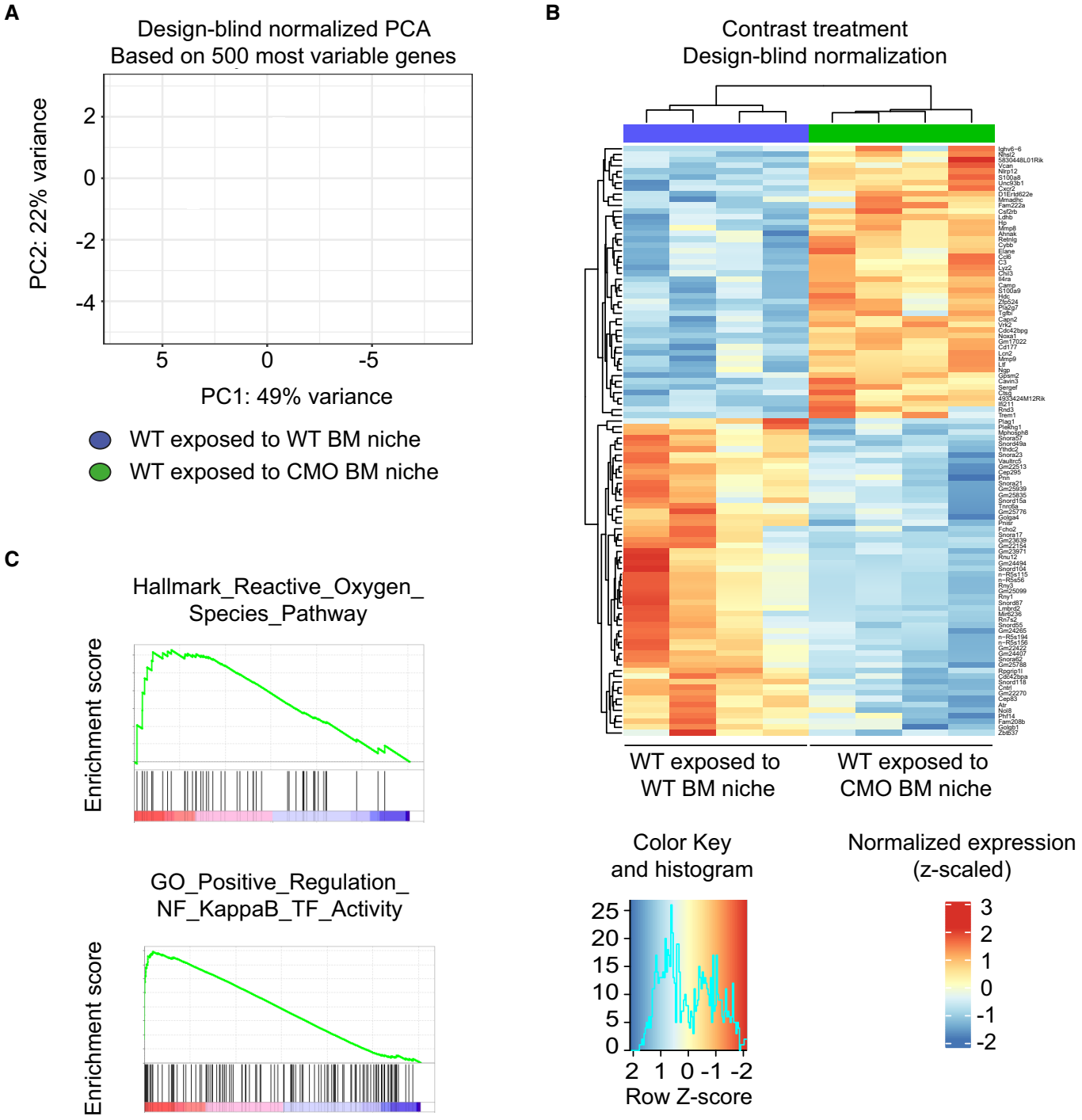


Figure EV4.

Figure EV5. Analysis and inhibition of IL-6 and Stat3 *in vivo*.

A IL-6 levels in BM of WT ($n = 4$) and CMO ($n = 4$) recipient mice upon transplantation of WT BM cells. *Y*-axis indicates the amount of IL-6 (pg/ml).

B Quantitative RT–PCR in WT LKS cells exposed to WT (blue symbols) or CMO (green symbols) BM niche. The *y*-axes represent *Mmp9*, *Bcl2l11*, *Bcl2*, and *Casp1* expression relative to *Gapdh*.

C Representative flow cytometry histograms of pStat3 signal from WT (blue) and CMO (orange) c-Kit$^+$ cells.

D Western blot analysis for pStat3 in WT and CMO c-Kit$^+$ cells. Gapdh expression was used as a loading control. Positions of m.w. standards are indicated (KDa). (E and F right panels) HSCs are measured by limiting dilution competitive repopulation unit assays and calculated using ELDA online software based on Poisson distribution statistics. Graphs show the curve fit of the log fraction of nonresponding mice (solid lines) and confidence intervals (dashed lines) versus the number of mice tested. Logarithmic plot; *X*-axis indicates the dose of transplanted cells and *Y*-axis percentages of negative responders. Reconstitution was evaluated in blood of recipient mice 16 weeks after transplantation. A responder mouse was defined as engraftment $\geq$ 0.5% Ly5.2$^+$ cells and contribution $\geq$ 0.5% in at least two out of three lineages (granulocytes, B-cells, and T-cells).

E Number of phenotypically enumerated HSCs per leg (left panel) and frequency of functional HSCs (right panel) in CMO mice treated with PBS control (orange) or IL-6-blocking antibody (green).

F Number of phenotypically enumerated HSCs per leg (left panel) and frequency of functional HSCs (right panel) in CMO mice treated with PBS control (orange) or IL-6 receptor-blocking compound (purple).

G pStat3 levels in LKS cells isolated from CMO mice treated as indicated. *Y*-axis indicates pStat3 mean fluorescence intensity (MFI). Values are normalized to the average of PBS treatment.

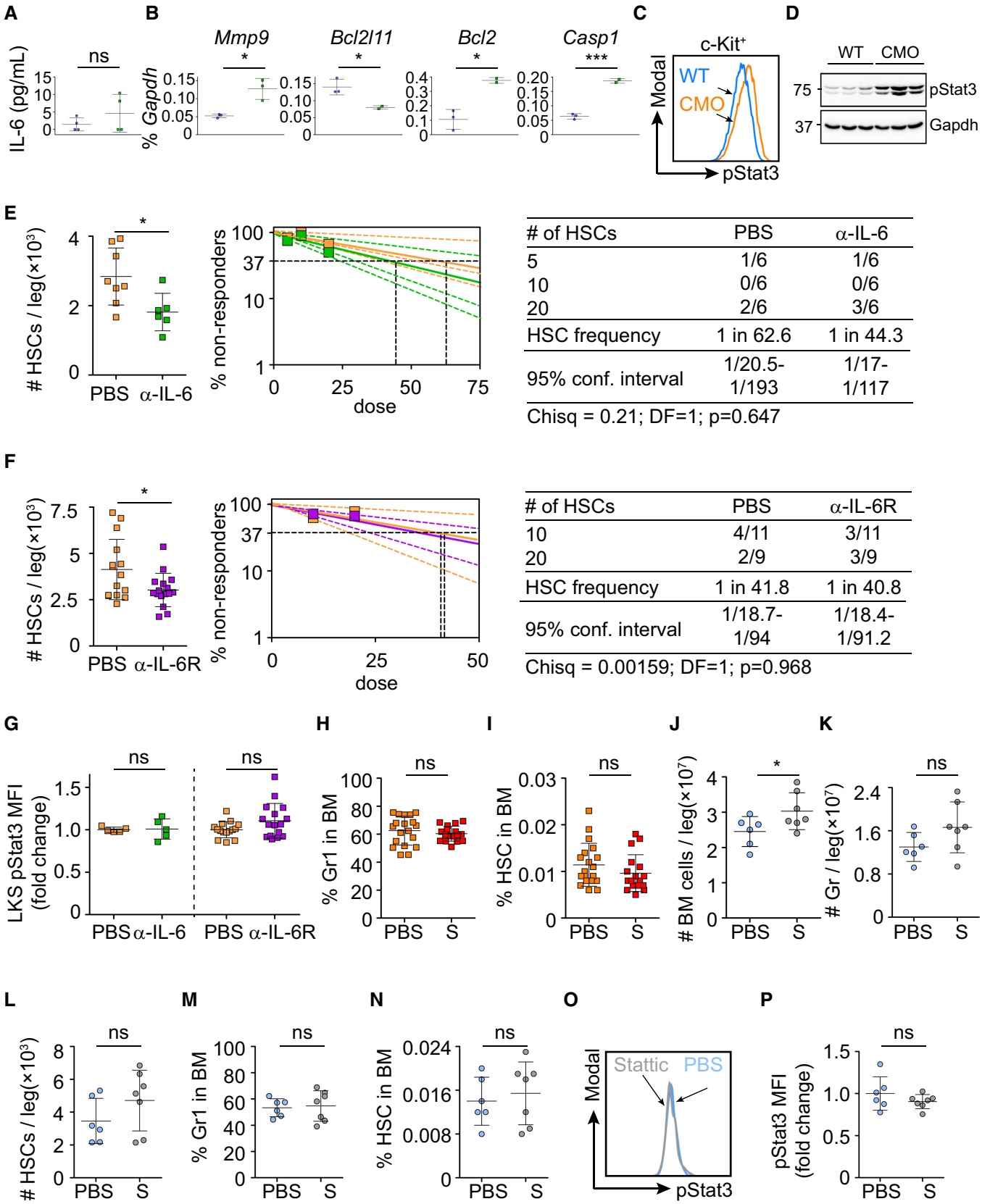
H, I Percentage of granulocytes (H) and HSCs (I) in BM of CMO mice treated with PBS control or Stattic.

J–L Absolute number of cells (J), granulocytes (K), and HSCs (L) per leg isolated from WT mice treated with PBS control (blue) or Stattic (S; gray).

M, N Percentage of granulocytes (M) and HSCs (N) in BM isolated from WT mice treated with PBS control (blue) or Stattic (S; gray).

O Representative flow cytometry histograms of pStat3 signal in LKS cells from WT mice treated with PBS control (blue) or Stattic (gray).

P Quantification of panel (K). *Y*-axis indicates pStat3 mean fluorescence intensity (MFI). Values are normalized to the average of the WT. In this figure, each symbol represents value for one biological replicate. Data represent mean $\pm$ s.d. Unless otherwise indicated, statistical significance was assessed using two-tailed Student's *t*-tests (*$P < 0.05$, ns, not significant).



# of HSCs	PBS	α-IL-6
5	1/6	1/6
10	0/6	0/6
20	2/6	3/6
HSC frequency	1 in 62.6	1 in 44.3
95% conf. interval	1/20.5-1/193	1/17-1/117

Chisq = 0.21; DF=1; p=0.647

# of HSCs	PBS	α-IL-6R
10	4/11	3/11
20	2/9	3/9
HSC frequency	1 in 41.8	1 in 40.8
95% conf. interval	1/18.7-1/94	1/18.4-1/91.2

Chisq = 0.00159; DF=1; p=0.968

Figure EV5.