

Supplementary Information

Clonal hematopoiesis related TET2 loss-of-function impedes IL1 β -mediated epigenetic reprogramming in hematopoietic stem and progenitor cells

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INVENTORY FOR SUPPLEMENTAL INFORMATION

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SUPPLEMENTAL DATA

SUPPLEMENTAL DATA 1s (included separately as .xlsx file) <i>Cell number by sample.</i>
SUPPLEMENTAL DATA 2s (included separately as .xlsx file) <i>Differentially expressed genes in Tet2-KO relative to WT HSCs.</i>

SUPPLEMENTAL DATA 3s (included separately as .xlsx file)

Enrichr and STRING ontology analysis of Tet2-KO relative to WT HSCs.

SUPPLEMENTAL DATA 4s (included separately as .xlsx file)

Gene set enrichment analysis results for C2 molecular signature database in Tet2-KO relative to WT HSCs.

SUPPLEMENTAL DATA 5s (included separately as .xlsx file)

List of differentially methylated regions between Tet2-KO and WT LSK, CMP and GMP cells

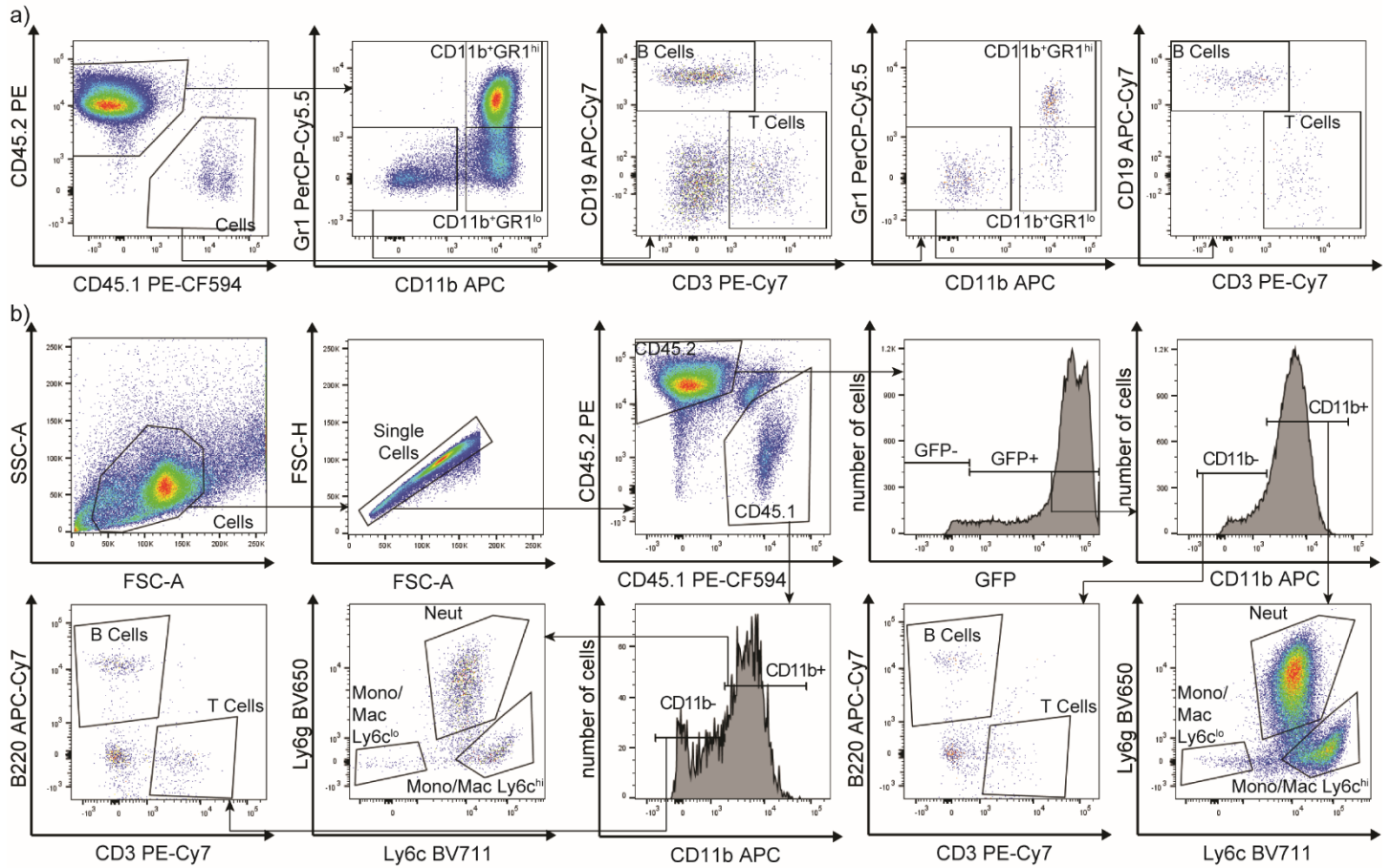
SUPPLEMENTAL DATA 6s (included separately as .xlsx file)

List of GEO accession numbers for ChIP-seq factors.

SUPPLEMENTAL DATA 7s (included separately as .xlsx file)

HOMER motif analysis of differentially methylated regions between Tet2-KO and WT LSK, CMP and GMP cells.

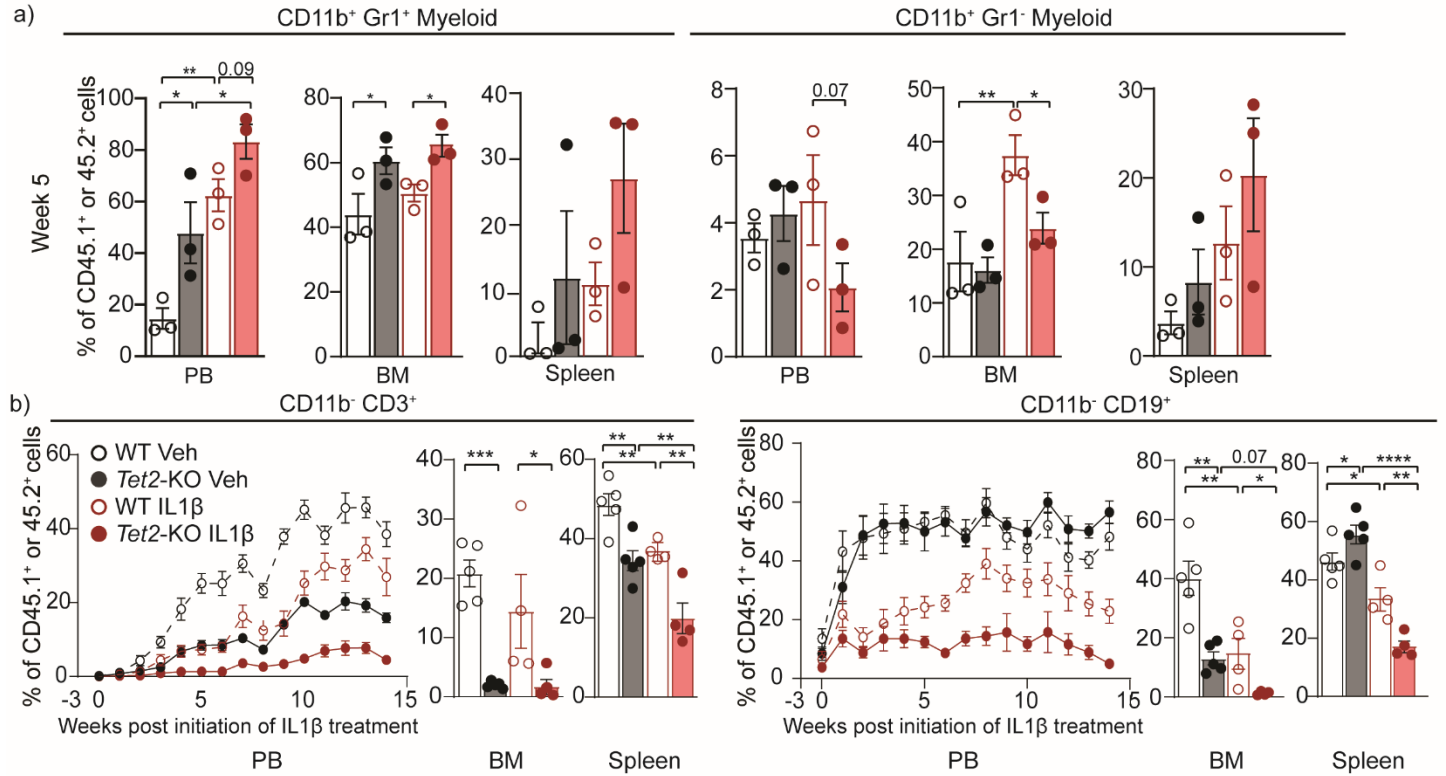
Supplementary Figure 1



Supplementary Figure 1: Schematics for flow cytometric analysis of differentiated hematopoietic populations.

(a) Representative gating for differentiated subsets for flow cytometric analysis of the BM from chimeric mice, defining: CD11b⁺Gr1^{hi} (CD11b⁺Ly6g^{hi}Ly6c^{hi}), CD11b⁺Gr1^{lo} (CD11b⁺Ly6g^{lo}Ly6c^{lo}), B and T Cells (experimental design is shown in Fig. 1a). **(b)** Representative gating scheme for differentiated subsets for flow cytometric analysis of the BM from chimeric recipient mice, defining: neutrophils (Neut), ly6c^{lo} monocytes/macrophages (Ly6c^{lo} Mono/Mac), ly6c^{hi} monocytes/macrophages (Ly6c^{hi} Mono/Mac), B and T Cells (experimental design is shown in Fig. 1d).

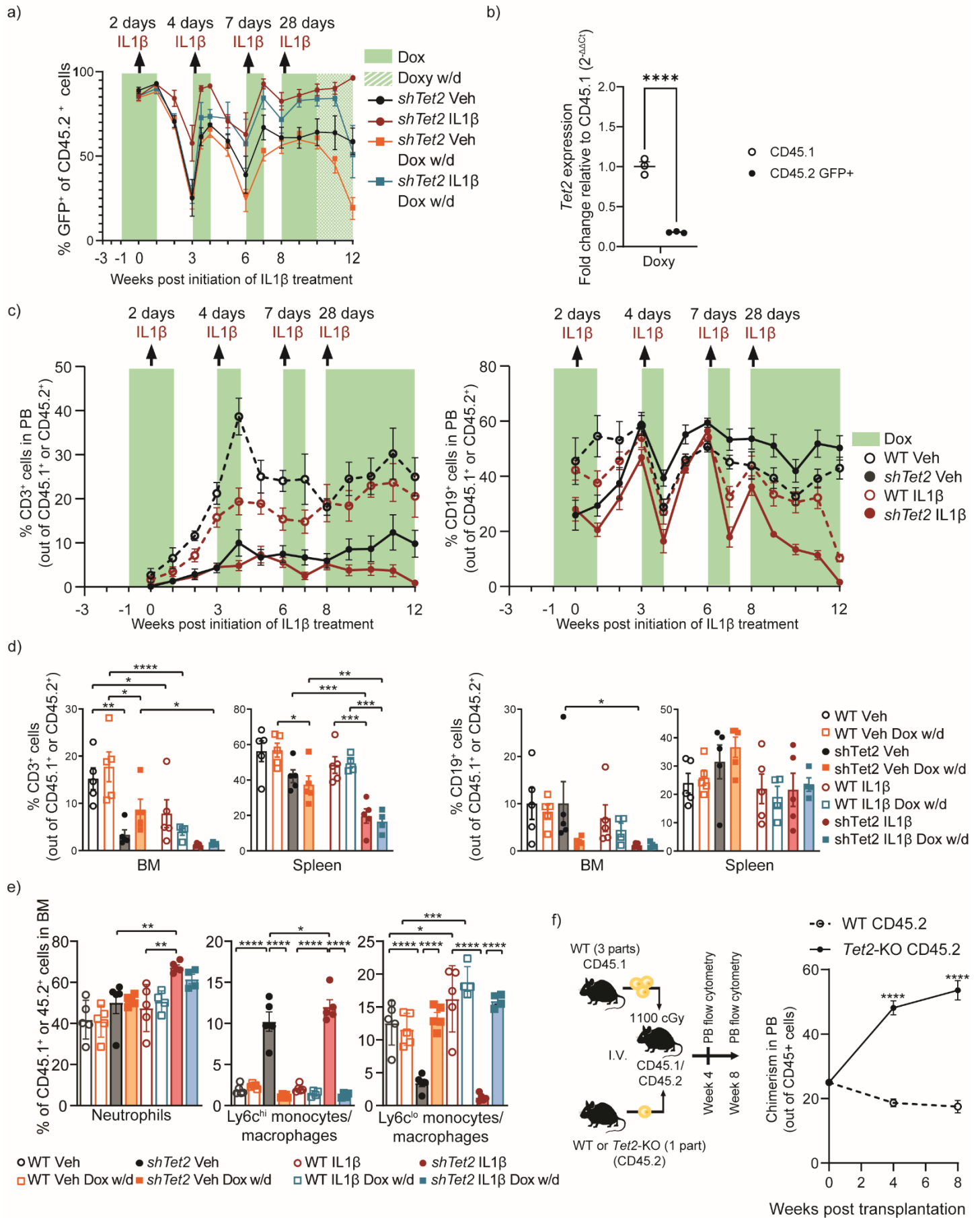
Supplementary Figure 2



Supplementary Figure 2: Chronic IL1 β exposure enhances myelopoiesis at the expense of B cell frequency.

(a-b) Lineage-depleted BM cells from WT CD45.1 and *Tet2*-KO CD45.2 mice were transplanted into lethally irradiated WT CD45.1/2 mice and after 3 weeks treated with IL1 β (500 ng/mouse/day) or vehicle daily. **(a)** The frequency of myeloid subsets (CD11b⁺Gr1^{hi}, CD11b⁺Gr1^{lo}) at week 5 ($n = 3$ mice/group, week 15 is shown in main Fig. 1) and **(b)** T cells (CD11b⁻CD3⁺) and B cells (CD11b⁻CD19⁺) at 15 weeks in PB, BM, and spleen ($n = 4-5$ mice/group). Error bars represent mean $\pm$ SEM. Two-factor ANOVA was used for the FWER adjusted p values: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

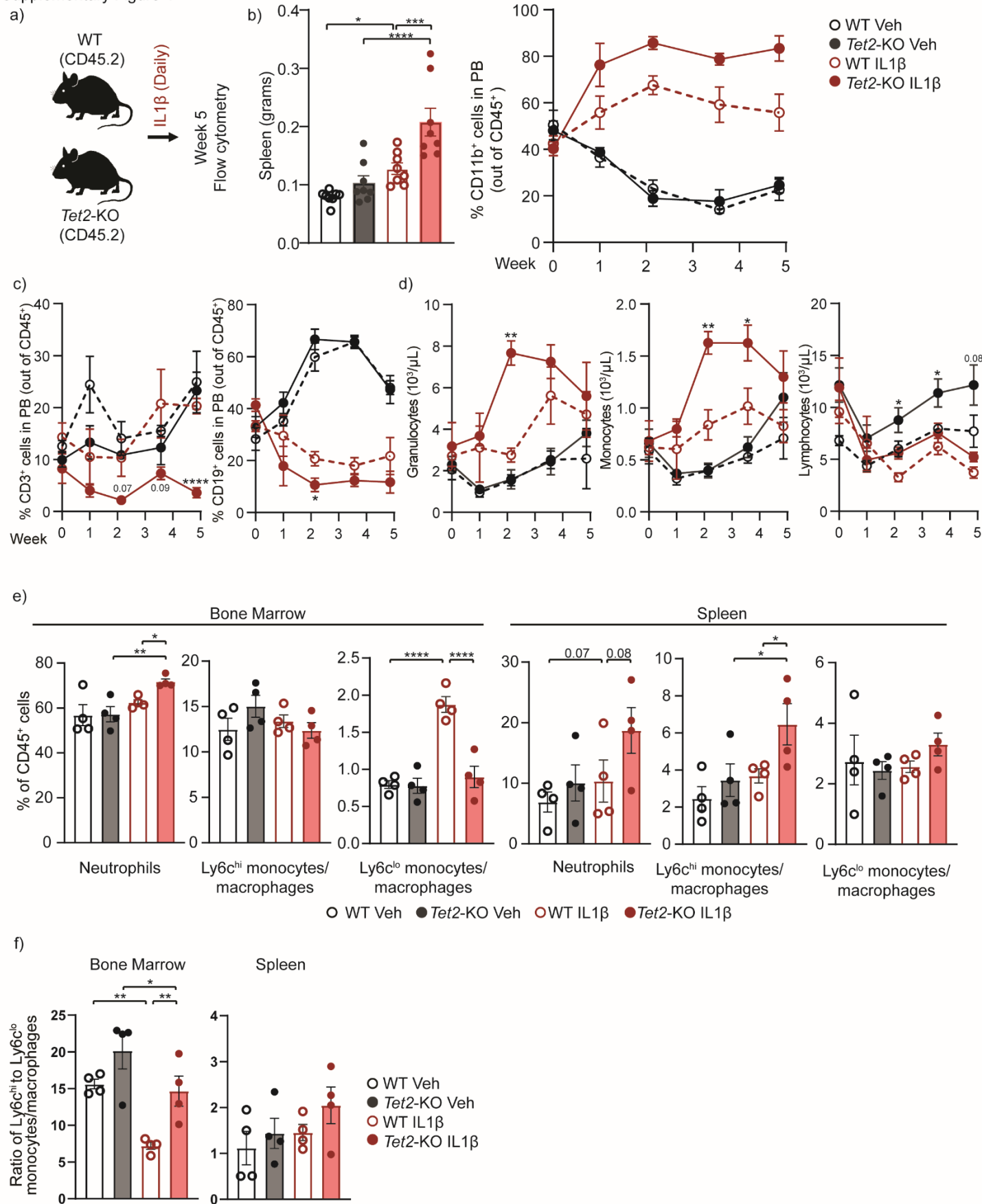
Supplementary Figure 3



Supplementary Figure 3: Within an inducible knockdown model chronic IL1 β exposure increases the ratio of ly6c^{hi} to Ly6c^{lo} monocytes/macrophages.

Lineage-depleted BM cells from wild-type (WT) CD45.1⁺ and Rosa-rtTA-driven inducible *Tet2* knockdown (*shTet2*) mice were transplanted into lethally irradiated WT CD45.1/2 mice, which were treated with doxycycline (dox) two weeks after transplantation and IL1 β or vehicle three weeks after transplantation for increasing intervals up to 10 weeks and then with and without doxycycline (withdrawal, w/d) for an additional two weeks, analyzed by flow cytometry (n = 4-5 mice/group). Arrows indicate the time relative to the initial treatment (week: 0, 3, 6, 8) and duration of periods (days: 2, 4, 7, and 28) during which daily treatment with IL1 β or vehicle occurred (n = 4-5 mice/group). The green color indicates the time and duration of doxycycline treatment, (described in Fig. 1d). **(a)** Percentage of CD45⁺ *shTet2* which are GFP⁺ in PB. **(b)** qPCR of *Tet2* expression from CD45.2⁺GFP⁺ and CD45.1⁺ cells sorted from the BM of IL1 β and doxycycline-treated mice (n = 3). **(c)** The percentage of WT CD45.1 or *shTet2* CD45.2 cells which are B (CD45⁺CD11b⁻B220⁺) or T (CD45⁺CD11b⁻CD3⁺) cells in the PB, **(d)** BM and Spleen **(e)** and the percentage of neutrophils (CD45⁺CD11b⁺Ly6c^{int}Ly6g⁺), ly6c^{hi} monocytes/macrophages (CD45⁺CD11b⁺Ly6c⁺Ly6g⁻) and ly6c^{lo} monocytes/macrophages (CD45⁺CD11b⁺Ly6c⁻Ly6g⁻) in the BM (n = 4-5 mice/group). **(f)** Lineage-depleted BM from CD45.2 (WT or *Tet2*-KO) cells and WT CD45.1 cells were transplanted into lethally irradiated WT CD45.1/2 recipients in a 1 to 3 ratio and analyzed 4 and 8 weeks post-transplantation by flow cytometry (n = 8 CD45.1 and n = 28 CD45.2 mice/group). Error bars represent mean $\pm$ SEM. For panel b, d, and e, two-factor ANOVA was used to determine the FWER-adjusted *p* values. For panel f a student's two-tailed t-Test was used to determine significance. For family-wise error rate (FWER) adjusted and regular *p* values: **p* < 0.05, ***p* < 0.01, ****p* < 0.001, *****p* < 0.0001.

Supplementary Figure 4

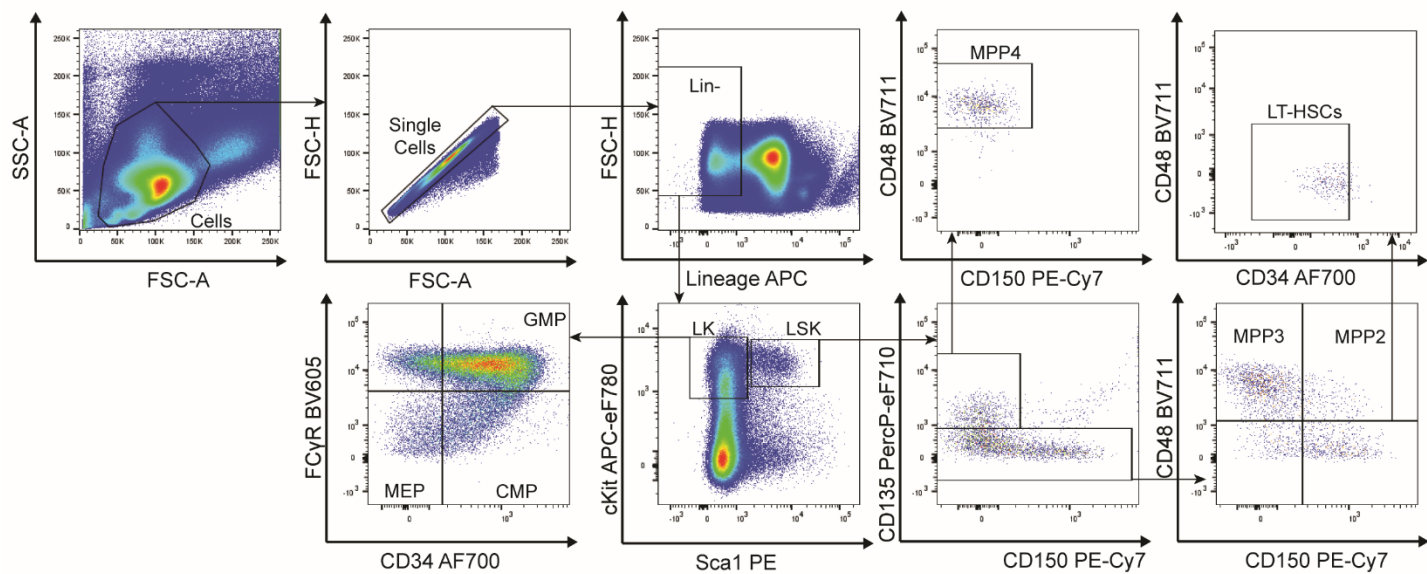


Supplementary Figure 4: Chronic IL1 β exposure enhances myeloid expansion of *Tet2*-KO cells in non-competitive murine models.

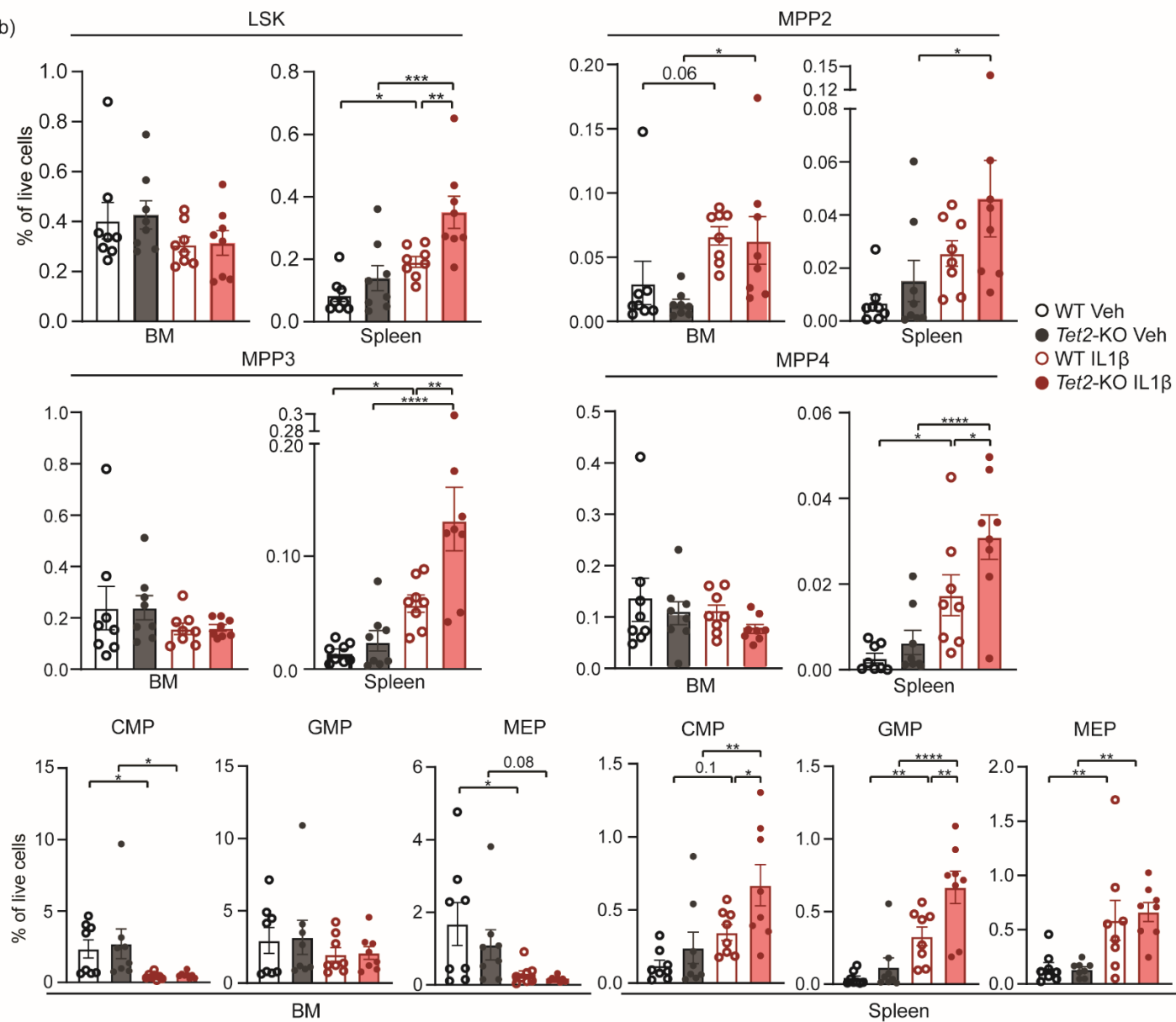
Tet2^{fl/fl} (WT) and *Vav-Cre Tet2*^{fl/fl} (*Tet2*-KO) mice were treated with IL1 β (500 ng/mouse/day) or vehicle (n = 4 mice/group) for five weeks and peripheral blood (PB), bone marrow (BM) and spleen samples were analyzed by flow cytometry. **(a)** Experimental design, **(b)** spleen weight in grams (left) and the percentage of the PB CD45⁺ cells which are myeloid (CD45⁺CD11b⁺, right), **(c)** T Cells or B Cells. **(d)** Differential blood cell counts representing granulocytes, monocytes, and lymphocytes in the PB. **(e)** Neutrophils, ly6c^{hi} monocytes/macrophages, and ly6c^{lo} monocytes/macrophages and **(f)** ratio of Ly6c^{hi} to ly6c^{lo} monocytes/macrophages in BM and spleen cells at week five by flow cytometry. Error bars represent mean $\pm$ SEM. For panel b (left), d and e two-factor ANOVA, was used to determine FWER adjusted p values. For panel b (right) and c a student's two-tailed t-Test was used to determine significance. For FWER adjusted and regular p values: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Supplementary Figure 5

a)



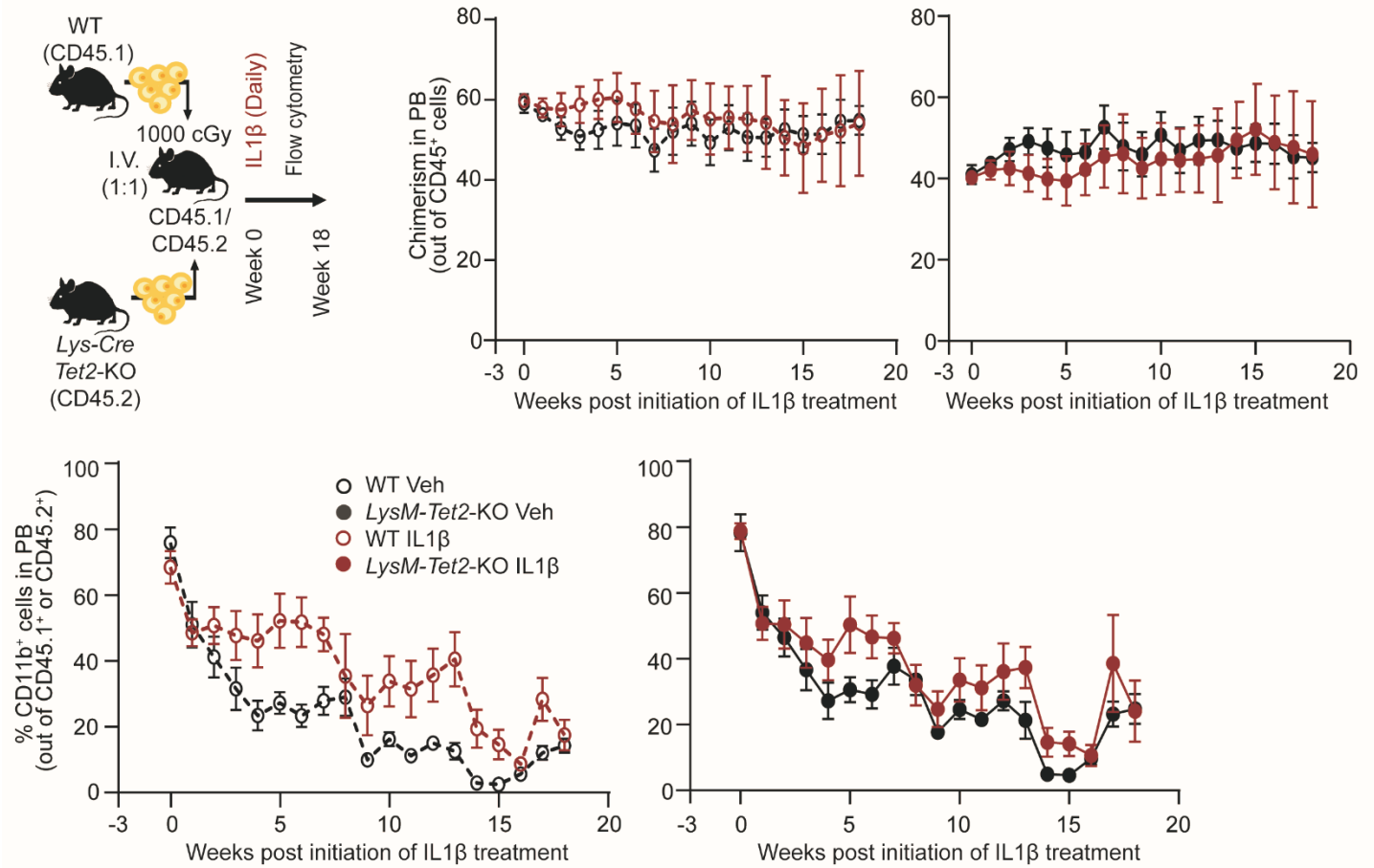
b)



Supplementary Figure 5: IL1 β stimulation promotes HSPC expansion in mice with *Tet2* knockdown relative to WT in a competition repopulation experiment.

(a) Representative gating scheme for HSPCs for flow cytometric analysis of the BM from a WT mouse. **(b)** WT and *Tet2*-KO mice were treated with IL1 β or vehicle for five weeks (Supplementary Fig. 4a) and analyzed by flow cytometry for the percentage of live CD45⁺ cells which are LSKs, MPP2, MPP3, MPP4, CMP, GMP and MEP in the spleen or BM (4 mice/group from two separate experiments, total n = 8 mice/group). Error bars represent mean $\pm$ SEM. Two factor ANOVA was used to determine the FWER adjusted p values: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

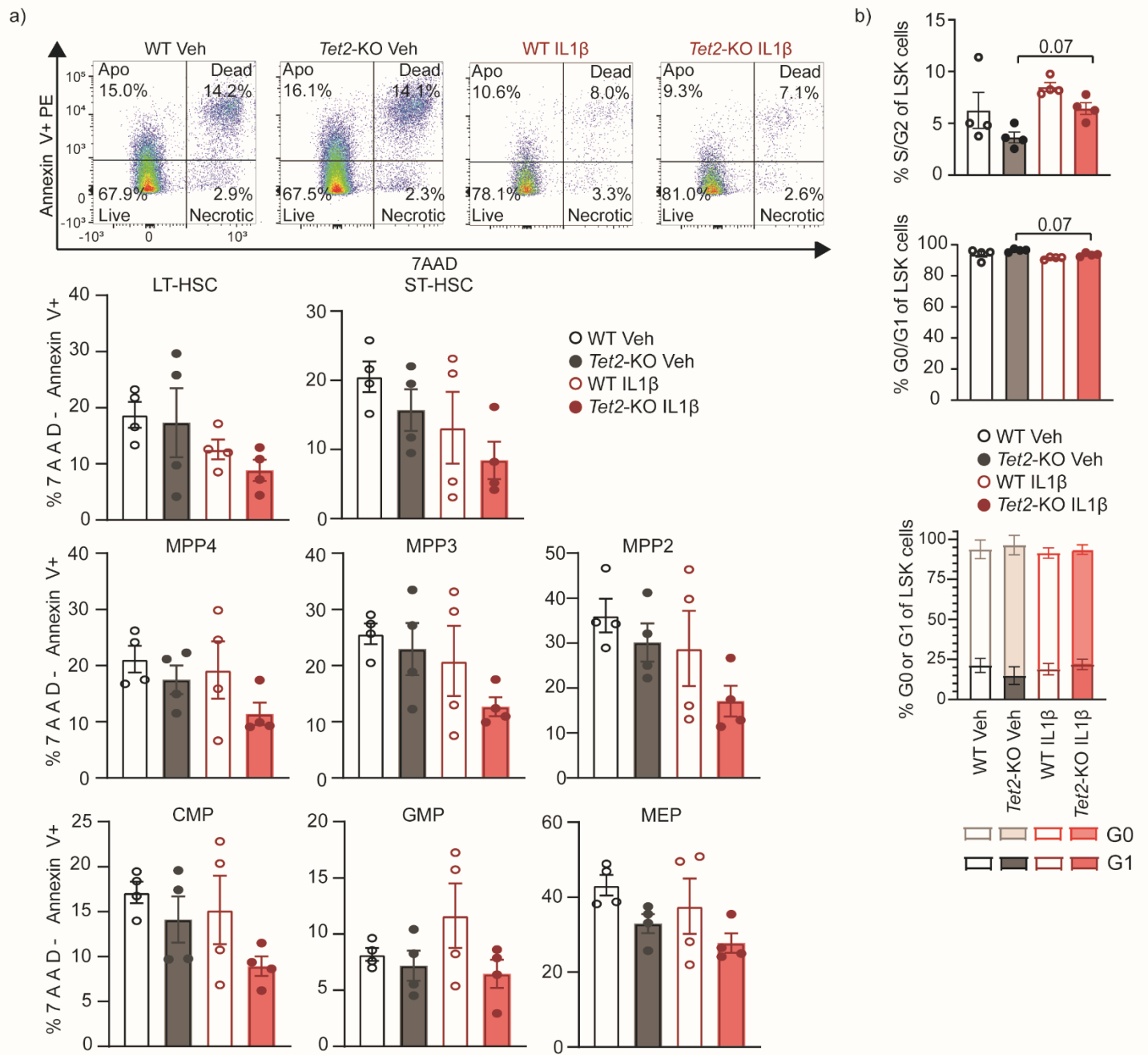
Supplementary Figure 6



Supplementary Figure 6: IL1 β stimulation promotes myeloid bias of *Tet2*-KO CMPs and deletion of *Tet2* within HSPCs is necessary for their expansion and myeloid bias.

Lineage-depleted BM cells were harvested from WT CD45.1 and *LysM-Cre Tet2*^{-/-} CD45.2 (*LysM-Cre Tet2*-KO) mice into lethally irradiated WT CD45.1⁺CD45.2⁺ mice which after 3 weeks were treated with IL1 β or vehicle. The percentage of WT CD45.1 and *LysM-Cre Tet2*-KO CD45.2 cells out of all CD45⁺ cells (chimerism) and the percentage WT CD45.1 and *Tet2*-KO CD45.2 cells which are myeloid in the PB. Error bars represent mean $\pm$ SEM. Student's two-tailed t-Test was used to determine significance.

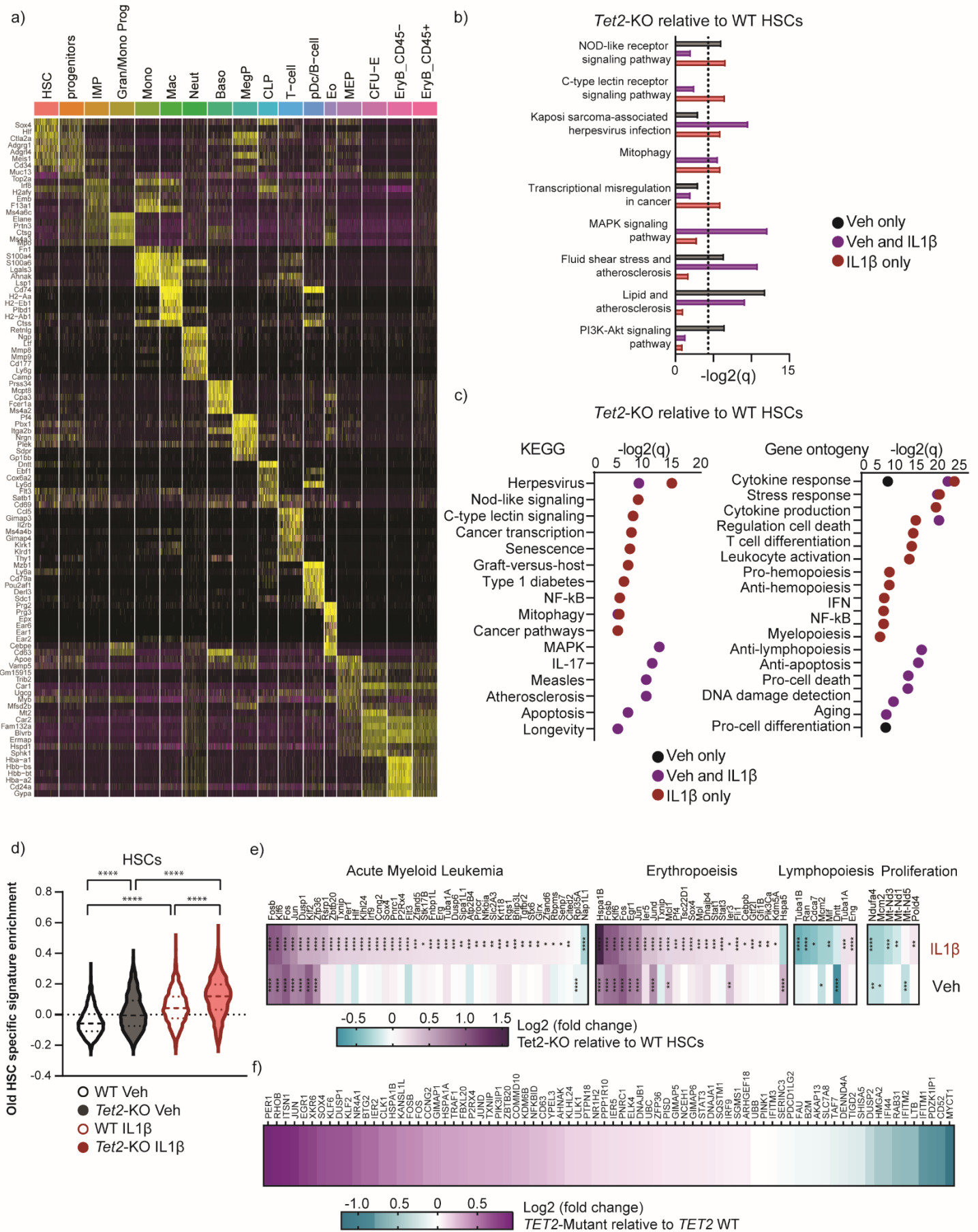
Supplementary Figure 7



Supplementary Figure 7: IL1 β -mediated expansion of *Tet2*-KO HSPCs is associated with reduced S/G₂ frequency without significant differences in the frequency of apoptosis.

(a-b) WT and *Tet2*-KO mice were treated with IL1 β for 5 weeks (as described in Supplementary Fig. 4a; n = 4 mice/group). The percentage of apoptotic (7AAD⁺Ann V⁺) cells for HSPC subsets was quantified by flow cytometry. **(b)** Flow cytometry analysis of LSK cells isolated from the BM of *Tet2*-KO and WT mice treated with and without IL1 β for 5 weeks, with cell cycle proportions using KI-67 and DAPI (right, n = 4 mice/group). Error bars represent mean $\pm$ SEM. Two-factor ANOVA was used to determine the FWER adjusted *p* values.

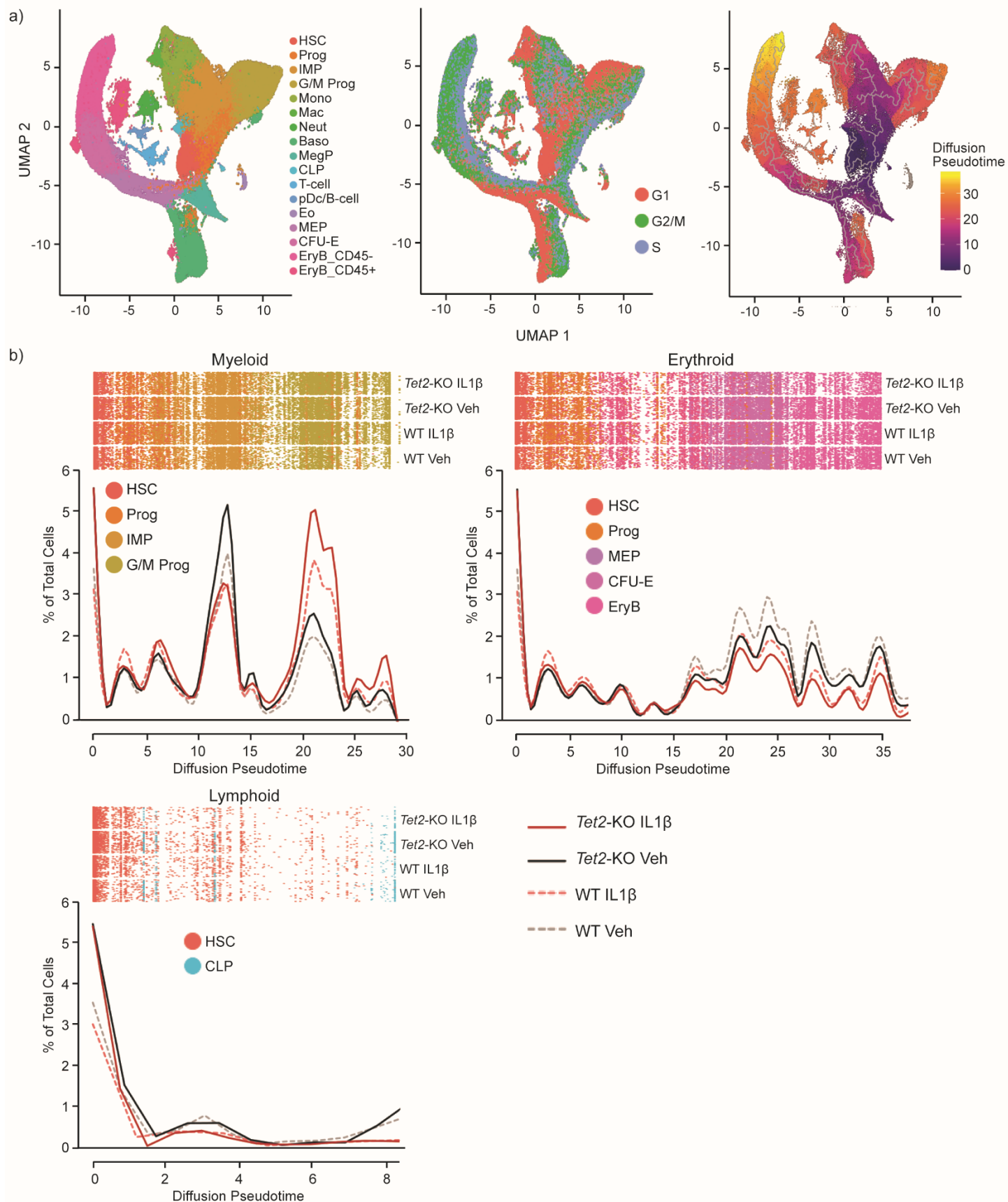
Supplementary Figure 8



Supplementary Figure 8: 10x single cell RNA sequencing to identify cellular clusters from *Tet2*-KO and WT mice with and without IL1 β stimulation *in vivo*.

(a) Heatmap showing gene expression of cluster-defining genes for individual cells from each cluster. **(b)** Enrichr ontology (KEGG 2021 human) of DEGs upregulated in *Tet2*-KO relative to WT HSCs in vehicle alone (black), IL1 β alone (red) or in both (purple) with *q* values calculated by Enrichr. **(c)** STRING KEGG response to cytokine from biological process gene ontology (GO:0034097) and MAPK signaling pathway from KEGG Pathways (mmu04010) of DEGs upregulated in *Tet2*-KO relative to WT only under vehicle alone (black), IL1 β alone (red), or both conditions (purple) with *q* values calculated by STRING. **(d)** Enrichment of transcriptional signature specific to old HSCs (Kirschner et al., Cell Rep, 2017) within HSCs. **(e)** Heatmap of upregulated DEGs in *Tet2*-KO relative to WT HSCs with and without IL1 β stimulation. The genes shown are the lead genes for enriched pathways by GSEA analysis, with genes identified in multiple pathways shown multiple times. *q* values were determined by DESeq2. **(f)** Differential gene expression in *TET2*-mutant relative to *TET2*-WT de novo primary AML for the top 100 human orthologous genes that are upregulated in *Tet2*-KO relative to WT murine HSCs stimulated with IL1 β . For *q* values: **q* < 0.05, ***q* < 0.01, ****q* < 0.001, *****q* < 0.0001.

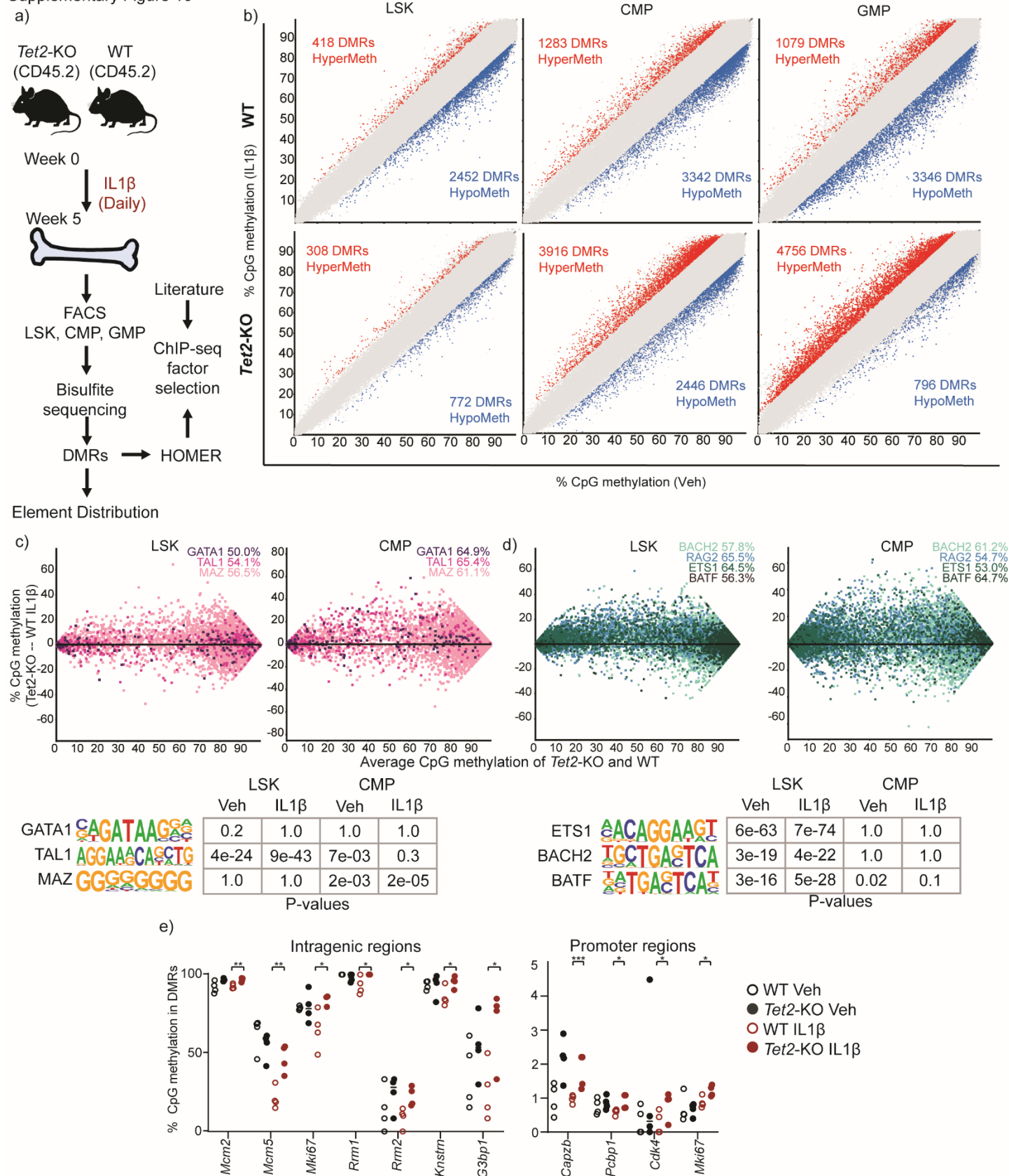
Supplementary Figure 9



Supplementary Figure 9: *Tet2*-KO exaggerates frequency of GMPs over pseudotime.

Cells analyzed by 10X single cell RNA (scRNA) sequencing (described in Figure 3a) were assigned pseudotime values using Monocle3. **(a)** UMAP from cell cycle G0/1, S, G2 regressed populations, showing clusters labelled as in Figure 3b (left), Cell Cycle (middle), and Monocle3 derived pseudotime values (right). **(b)** Pseudotime as HSCs differentiate over myeloid (HSC, Prog, IMP, GMP), erythroid (HSC, Prog, MEP, CFU-E, EryB) and lymphoid (HSC, CLP) lineage trajectories.

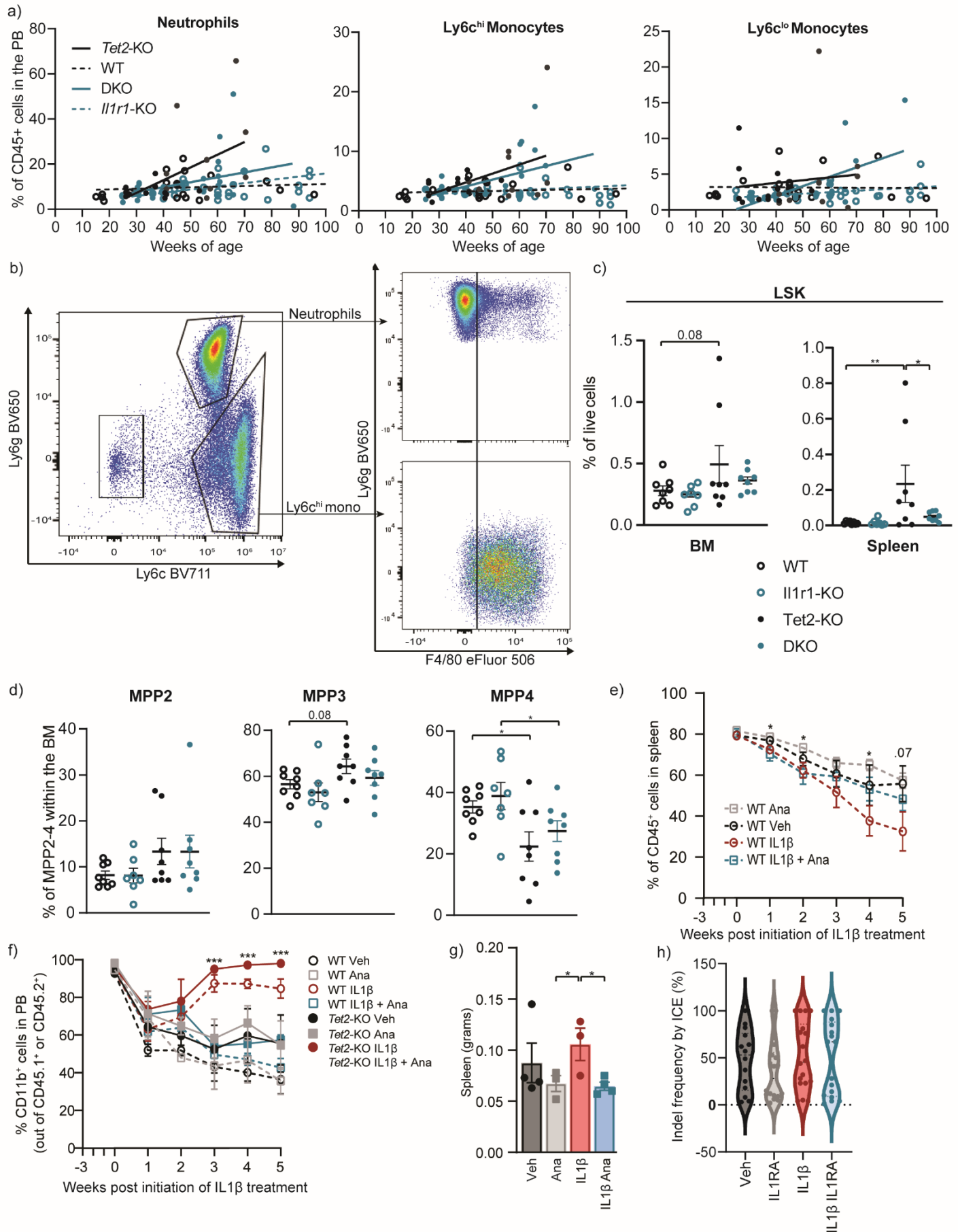
Supplementary Figure 10



Supplementary Figure 10: Resistance to IL1 β driven demethylation in *Tet2*-KO progenitors promotes differential hypermethylation associated with cellular fate.

WT and *Tet2*-KO mice were treated for 5 weeks with or without IL1 β , purified BM-derived LSK, CMP, and GMP cells were analyzed by whole genome bisulfite sequencing (n = 4 mice/group). **(a)** Experimental design, **(b)** differentially methylated regions (DMRs) from 50 CpGs tiling of the whole genome (>10% methylation difference, $q < 0.05$, as determined by SeqMonk) between vehicle or IL1 β treatment in WT or *Tet2*-KO cells. **(c)** Top, MA plots showing methylation difference for LSK and CMP cells derived from *Tet2*-KO and WT mice treated with IL1 β using literature-derived ChIP-seq binding sites of erythroid/megakaryocytic lineage specifying transcription factors (TFs). The percentage of sites with higher methylation in *Tet2*-KO than WT are shown in the top right corner of each graph. Bottom, the significance of motif enrichment using HOMER analysis tool within DMRs for TFs. With q values calculated by Homer. **(d)** As in Supplementary Fig. 10c, but showing lymphoid lineage specifying TFs. **(e)** Selected examples of genes showing methylation differences within promotor (top) or intragenic (bottom) regions. Student's two-tailed t-test was used to determine significance except where otherwise specified in the figure legend: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Supplementary Figure 11



Supplementary Figure 11: Disruption of IL1R1 signaling suppresses myeloid bias in *Tet2*-KO mice.

(a-d) *Vav-cre Tet2^{fl/fl}* (*Tet2*-KO), *Il1r1^{-/-}* (*Il1r1*-KO), and *Vav-cre Tet2^{fl/fl}Il1r1^{-/-}* (double knockout; DKO) mice over 60 weeks of age. **(a)** Frequency of neutrophils, Ly6c^{hi} monocytes, and Ly6c^{lo} monocytes in the PB. **(b)** Representative gating within BM of the percentage of Ly6c^{hi} monocytes/macrophages which are F4/80⁺. **(c)** The frequency out of live cells of LSK cells within the BM and spleen. **(d)** Frequency out of live cells of MPP2, MPP3, and MPP4 out of MPP2-4 within the BM. **(e-g)** Lineage-depleted BM cells derived from WT CD45.1 and *Tet2*-KO CD45.2 mice were transplanted into lethally irradiated WT CD45.1/2 mice. 3 weeks later, mice were treated with IL1 β (500 ng/mouse/day) or vehicle as well as with or without an IL1R1 antagonist (anakinra, 100mg/kg) (n = 3-4 mice/group). **(e)** Percentage of WT CD45.1 donor cells out of all CD45⁺ cells (chimerism) in PB. **(f)** Percentage of WT CD45.1 and *Tet2*-KO CD45.2 donor cells which are myeloid in the PB. **(g)** Spleen weight in grams. **(h)** Indel frequency of individually picked colonies for vehicle, IL1RA, IL1 β , and IL1 β with IL1RA is determined by Sanger sequencing for infer CRISPR edits (ICE) analysis. Error bars represent mean $\pm$ SEM. For panels c and d, two-factor ANOVA was used to determine FWER-adjusted p values. For panels g and h, one factor was used to determine the FWER-adjusted p values. For panels e and f, a student's two-tailed t-Test was used to determine significance. For FWER adjusted and regular p values: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Supplementary Data 1: Cell number by sample. WT and *Tet2*-KO mice were treated for 5 weeks with and without IL1 β (n = 4 mice/group). BM cells were harvested and lineage-negative cells were enriched by magnetic selection. Cells were analyzed by 10X single cell RNA (scRNA) sequencing as described in figure 3a. The data shows cell number metadata such as cell number per mouse per cell type.

Supplementary Data 2: Differentially expressed genes in *Tet2*-KO relative to WT HSCs. WT and *Tet2*-KO mice were treated for 5 weeks with and without IL1 β (n = 4 mice/group). BM cells were harvested and lineage-negative cells were enriched by magnetic selection. Cells were analyzed by 10X single cell RNA (scRNA) sequencing as described in figure 3a. The data shows differential gene expression with a q value of 0.05 cut off between *Tet2*-KO and WT HSCs as determined using DESeq2.

Supplementary Data 3: Enrichr and STRING ontology analysis in *Tet2*-KO relative to WT HSCs. WT and *Tet2*-KO mice were treated for 5 weeks with and without IL1 β (n = 4 mice/group). BM cells were harvested and lineage cells were depleted by magnetic selection. Cells were analyzed by 10X single cell RNA (scRNA) sequencing as described in figure 3a. Enrichr (KEGG 2021 Human), STRING KEGG Pathways (mmu04010), or STRING biological process gene ontology (GO:0034097) identified pathways from genes upregulated in *Tet2*-KO relative to WT HSCs treated with or without IL1 β , or in both conditions. q values were determined through Enrichr and STRING.

Supplementary Data 4: Gene set enrichment analysis results for C2 molecular signature database in *Tet2*-KO relative to WT HSCs. WT and *Tet2*-KO mice were treated for 5 weeks with and without IL1 β (n = 4 mice/group). BM cells were harvested and lineage cells were depleted by magnetic selection. Cells were analyzed by 10X single cell RNA (scRNA) sequencing as described in figure 3a. Gene set enrichment analysis (C2) of differential expressed genes between HSCs in *Tet2*-KO and WT with and without IL1 β administration. q values were determined by GSEA v4.2.1 using an empirical phenotype-based permutation test procedure.

Supplementary Data 5: List of differentially methylated regions between *Tet2*-KO and WT LSK, CMP and GMP cells. WT and *Tet2*-KO mice were treated for 5 weeks with and without IL1 β (n = 4 mice/group). BM cells were harvested and LSK, CMP and GMP cells were flow sorted and bisulfite sequenced as described in Supplementary Figure 7a. Data shows differentially methylated regions in *Tet2*-KO relative to WT LSK, CMP, and GMP cells in *Tet2*-KO relative to WT with and without IL1 β administration and in the vehicle, relative IL1 β treated mice. >10% methylation difference, q < 0.05, statistical significance determined by SeqMonk.

Supplementary Data 6: List of GEO accession numbers for ChIP-seq factors. GEO accession numbers for ChIP-seq databases whose called peaks were acquired from cistrome.

Supplementary Data 7: HOMER motif analysis of differentially methylated regions between *Tet2*-KO and WT LSK cells. Differentially methylated regions for fluorescently activated cell sorted LSK, CMP, and GMP cells described in Supplementary Data 4 underwent HOMER motif analysis for *Tet2*-KO relative to WT mice treated with vehicle or IL1 β . Statistical significance of motif enrichment was determined by hypergeometric distribution using the HOMER analysis tool.