SUPPLEMENTARY FIGURES

Supplementary Figure 1. *Tet2*^{HR} cooperates with additional myeloid malignancy mutations and accelerates AML.

A, Generation of a *Tet2*^{HR} Knock-in allele. A *Tet2* targeting vector directed against the exon 12 of *Tet2* and including the H1794R mutation was electroporated to ESCs. Upon homologus recombination, ESC clones carrying the FRT allele were used to generate mice by tetraploid complementation. By crossing the FRT mice with mice expressing Flp recombinase the NEO cassette was removed, leading to the expression of the *Tet2*^{HR} Konock-in allele under the *Tet2* endogenous locus.

B, Colony formation assay. Quantification of the number of colonies derived from serial plating of 2000 cKit+ bone marrow cells collected from *Tet2* $^{+/+}$ and *Tet2* $^{+/HR}$ (n=3).

C, Lethally irradiated CD45.1 mice were transplanted with wild-type 45.1 and *Tet2*^{HR} CD45.2 or wild-type 45.1 and *Tet2*^{WT} CD45.2 (50:50). After engraftment, mice were monitored over 20 weeks. Proportion of donor 45.2 cells in the peripheral blood was quantify by flow cytometry.

D, Kaplan-Meier curve showing cooperation between *Tet2*^{HR} driver *Flt3-ITD*. (n=5 per genotype)

E, Flow cytometry quantification of percentage of myeloid (CD11b+ Gr1+) cells in the peripheral blood of *Tet2*$^{+/+}$; *Flt3-ITD* and *Tet2*$^{+/HR\ Flt3\text{-}ITD}$ in 6 months old mice.

F, Kaplan-Meier curve showing cooperation between *Tet2*^{HR} and the AML-ETO translocation (AE). Kit+ cells bone marrow cells from *Tet2*^{WT} or *Tet2*^{HR} were transduced with the pMIGR1-AML-ETO-IRES-GFP plasmid and transplanted into lethally irradiated mice. Mice were monitored for signs of AML for a year (n=8, *Tet2*^{HR}-AE, n=9 AE).

G, Hemavet quantification of the White blood counts (WBC) in the peripheral blood (PB) of *Tet2*$^{+/+}$-AE and Tet2$^{+/HR}$-AE of 3-months-old mice.

H, Representative immunophenotyping of the bone marrow cells collected from a Tet2$^{+/HR}$-AE mice, showing expansion of a Lineage^{neg}, cKit^{+} and Sca-1^{-} blasts population (left panel), which carries the AML-ETO translocation (GFP^{+}, right panel).

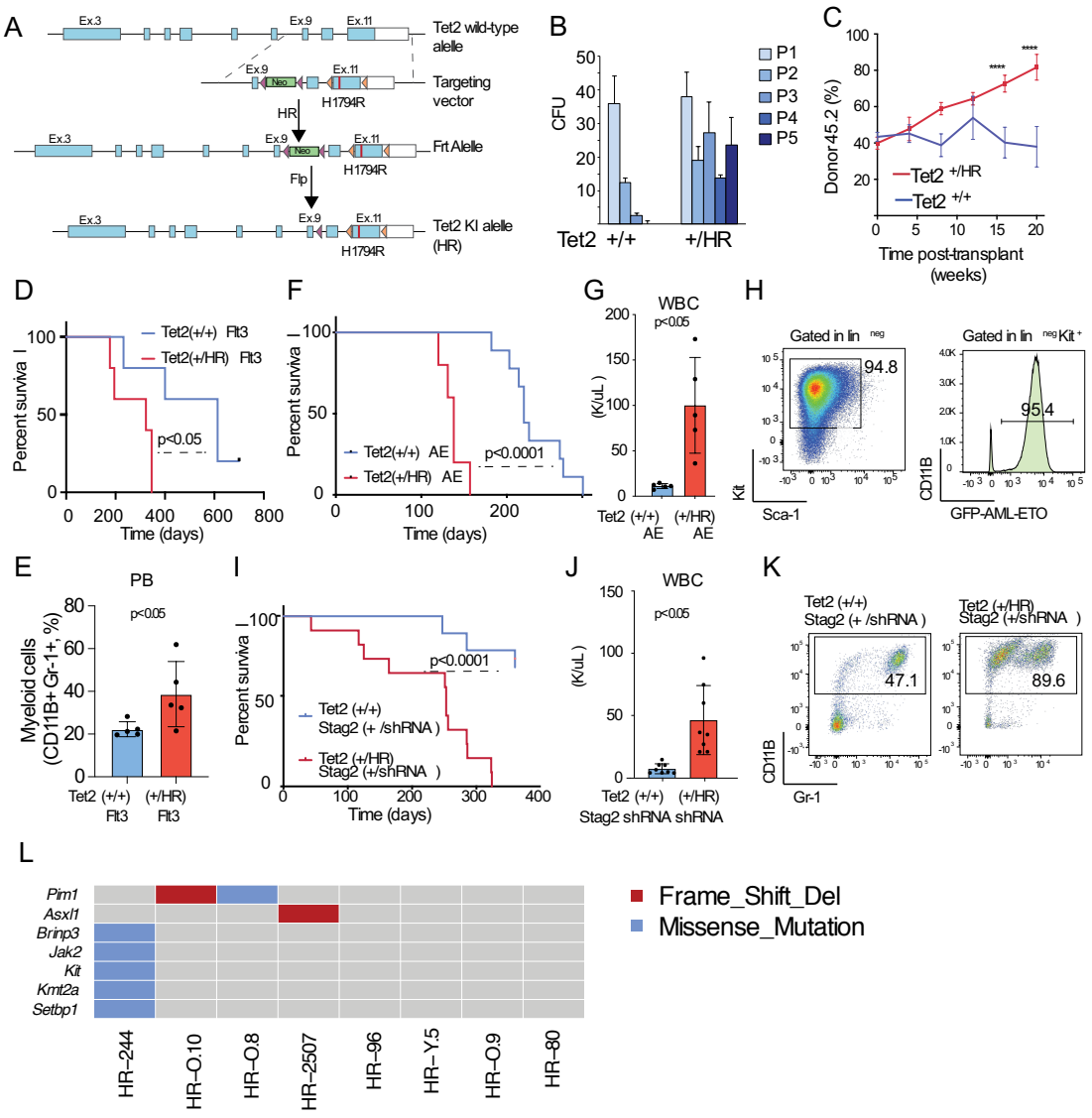
I, Kaplan-Meier survival curve of *Tet2*$^{+/+}$;*Stag2 (shRNA)* vs *Tet2*$^{+/HR}$;*Stag2* (shRNA) (n=12 per genotype)

J, Hemavet analysis of WBC in the peripheral blood of *Tet2* $^{+/+}$;*Stag2* (shRNA) and *Tet2*$^{+/HR}$;*Stag2* (shRNA) mice.

K, Representative flow cytometry plot of CD11b^{+} and Gr-1^{+} cells in the peripheral blood of *Tet2* $^{+/+}$;*Stag2* (shRNA) and *Tet2*$^{+/HR}$;*Stag2* (shRNA) mice.

L, Hematologic malignancy related mutations found in *Tet2*^{HR} animals from paired whole exome sequencing of Tet2^{HR} mice (n=8). For each mouse, tumor and germline control (ear) was sequenced.

Supplementary Figure 1.



Supplementary Figure 2. Aged *Tet2*^{HR} mice show myeloid expansion and spleen infiltration.

A, Quantification of the percentage of lineage^{neg}, cKit^{+} cells (LK) (on left) and lineage^{neg}, Sca-1^{+}, cKit^{+} (on right) calculated by flow cytometry analysis (see panel D).

B, Quantification of the percentage of hematopoietic stem cells (HSC, CD150^{+}, CD135^{-}, CD150^{-}, CD48^{-}), erythroid biased- Multipotent progenitor (MPP2, CD135^{-}, CD150^{+}, CD48^{+}), myeloid biased- multipotent progenitor (MPP3, CD135^{-}, CD150^{-}, CD48^{+}), and Lymphoid biased-multipotent progenitor (MPP4, CD135^{+}, CD48^{+}, CD150^{-}) in the LSK compartment (see panel D)

C, Quantification of the percentage of common myeloid progenitor (CMP, CD34^{+}, CD16/32^{-}), megakaryocyte-erythrocyte progenitor (MEP, CD34^{-}, CD16/32^{-}), and granulocyte-monocyte progenitors (GMP, CD16/32^{+}, CD34^{+}) in the LK compartment.

D, Representative flow cytometry analysis plots of the hematopoietic stem and progenitor populations in young (2-months old) and old (>12 months-old) mice with the indicated genotypes.

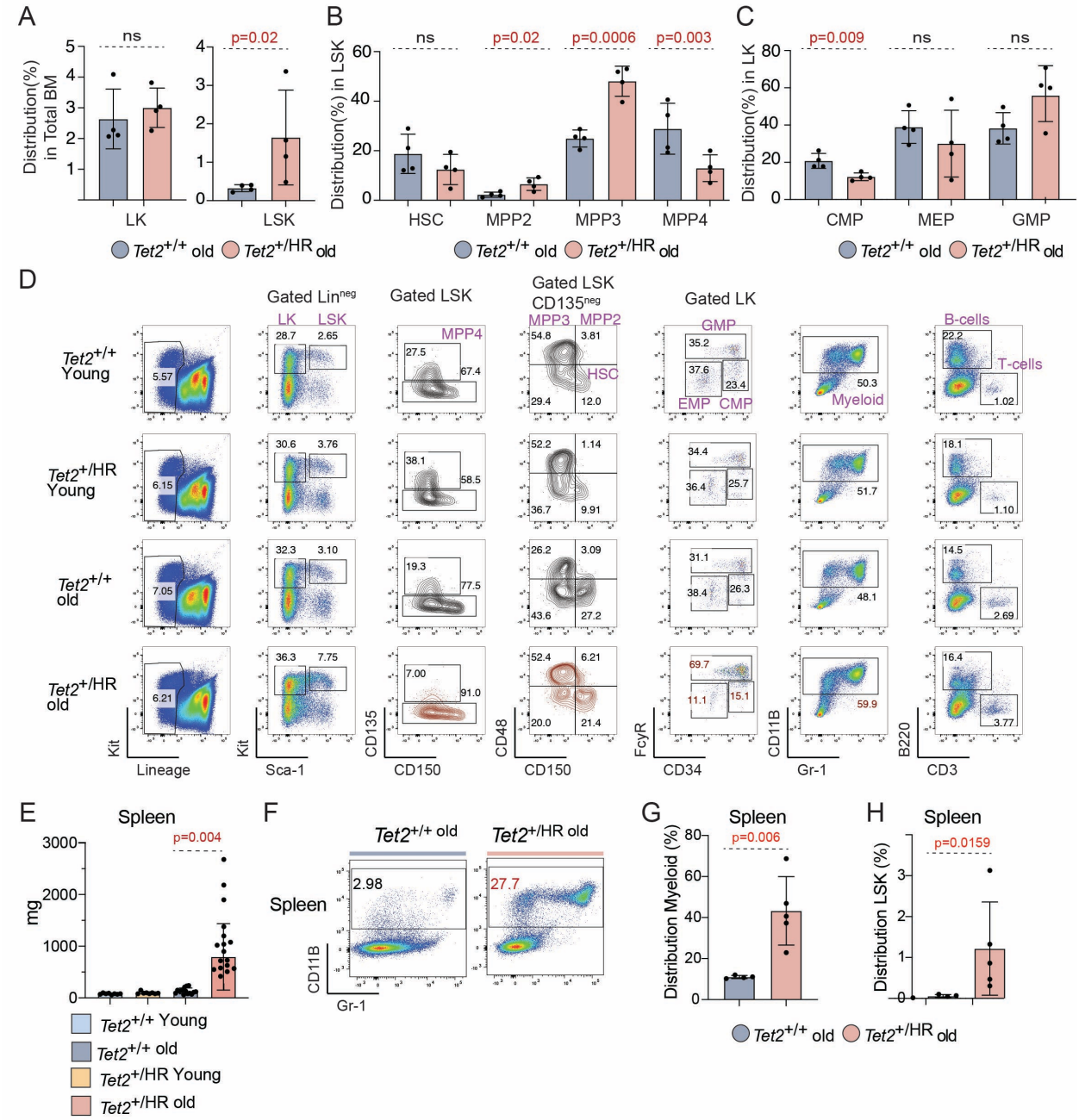
E, Spleen weights quantification of *Tet2*^{WT} or *Tet2*^{HR} young (2-4 months old) and old (>12 months-old) mice.

F, Representative flow cytometry analysis plots of the myeloid (CD11b+, Gr-1) cells collected from the spleen of *Tet2*^{WT} or *Tet2*^{HR} mice

G, Percentage quantification of myeloid (CD11b+, Gr-1) in the spleen of *Tet2*^{WT} or *Tet2*^{HR} mice.

H, Percentage quantification of the LSK population in the spleen of *Tet2*^{WT} or *Tet2*^{HR} mice.

Supplementary Figure 2.



Supplementary Figure 3. Transcriptional landscape of *Tet2*^{HR} bone marrow cells.
A, Uniform manifold approximation and projection (UMAP) representation of 37,355 myeloid cells collected from total bone marrow of 18 mice, including *Tet2*^{WT} young (n=3), *Tet2*^{HR} young (n=3), *Tet2*^{WT} old (n = 4), and *Tet2*^{HR} old (transformed n = 6, chronic n = 2). Colored by cell cycle state.

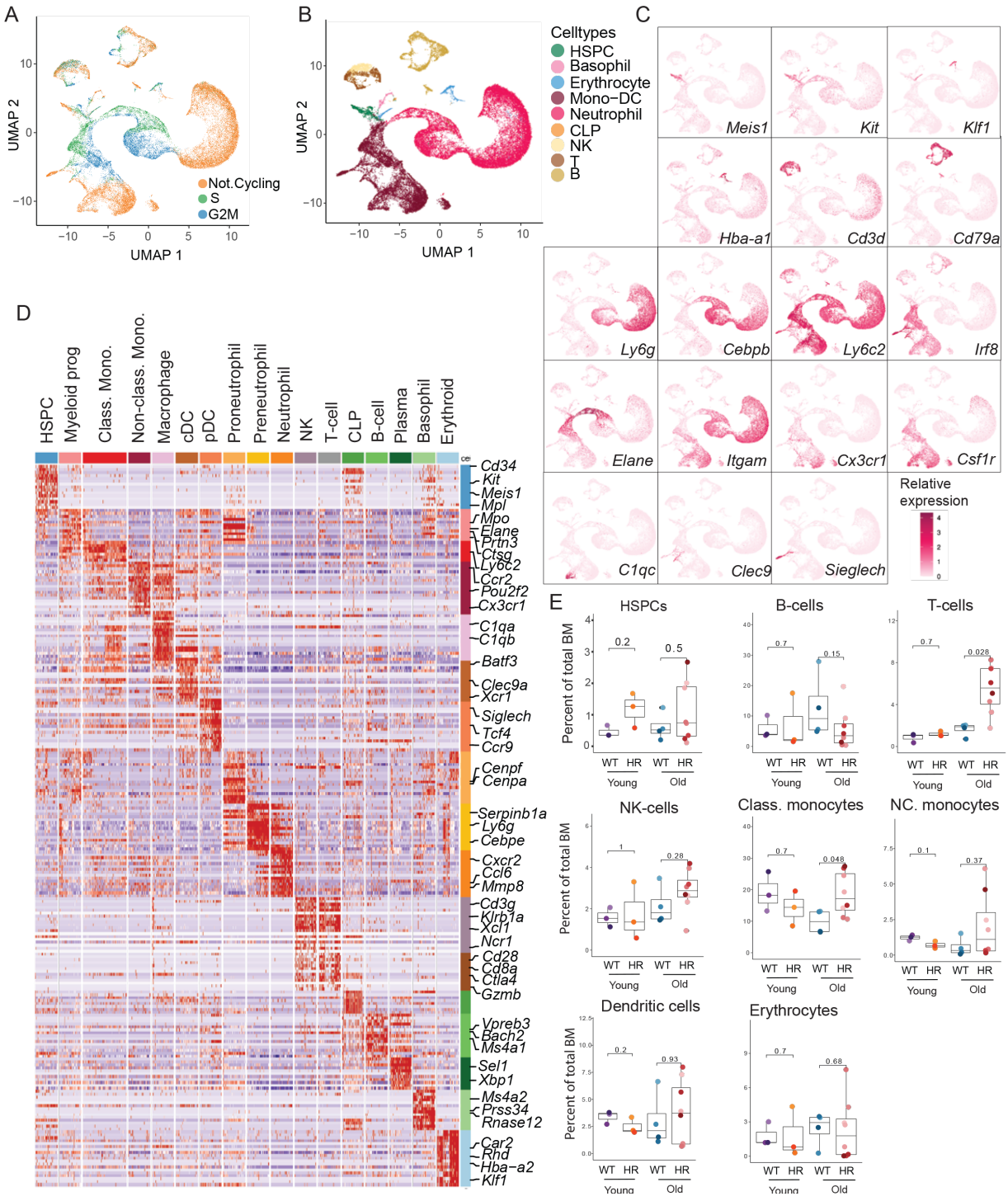
B, UMAP representation of 37,355 cells colored by the indicated broad cell types.

C, UMAP representation of common cell type marker genes (HSPC: *Meis1*, *Kit*, Erythrocytes: *Klf1*, *Hba-a1*, T cells: *Cd3d*, B cells: *Cd79a*, Myeloid progenitors: *Elane*, Neutrophils: *Ly6g*, *Cebpb*, Monocytes and Dendritic cells: *Irf8*, Monocytes: *Cx3cr1*, *Csf1r*, pDC: *Siglech*, cDC: *Clec9*, Macrophages: *C1qc*)

D, Heatmap of top 50 genes specific to the indicated cell types.

E, Barplot quantification of percentages of the indicated populations in young and old *Tet2*$^{WT/}$ or *Tet2*^{HR} mice.

Supplementary Figure 3.



Supplementary Figure 4. Emergence of a MHC II^{high} monocytic population correlates with transformed disease.

A, UMAP representation of cells in the monocyte lineage colored by genes specific to monocyte differentiation (Meis1, Mpo, Elane, Ly6c2, Cx3cr1, Csf1r, C1q) and to cell cycle (Mki67) (n=5597 cells).

B, Percentage of the indicated myeloid population per analyzed mouse.

C, Dot plot of marker genes for each monocyte subpopulation.

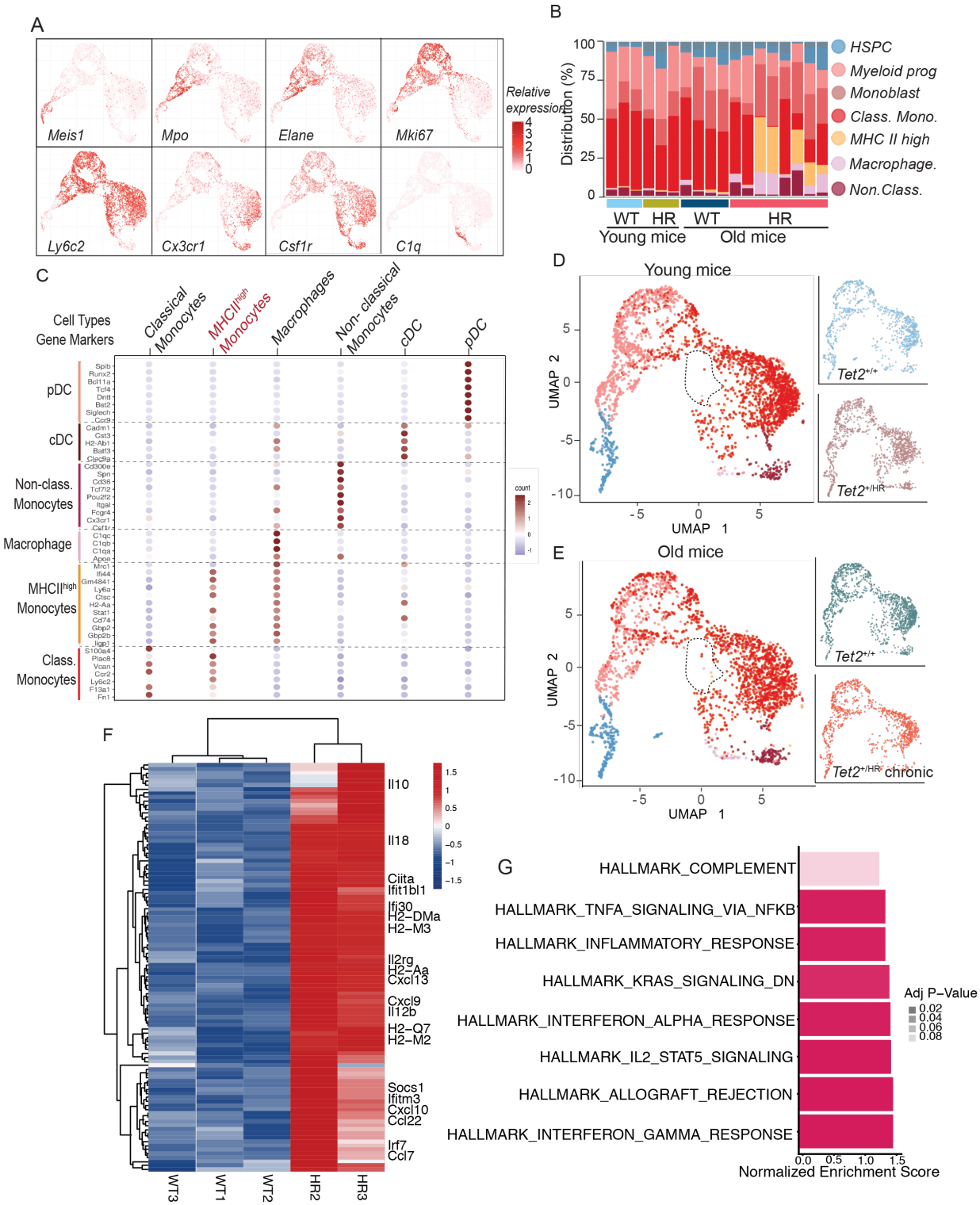
D, UMAP representation showing only transcriptomes from young mice. Left: all cells. Right : Tet2WT young mice (blue) and Tet2HR young (brown).

E, UMAP representation showing only transcriptomes from Tet2WT old and Tet2HR old with chronic disease. Left: all cells. Right: Tet2WT old (green) and Tet2HR old with chronic disease (orange).

F, Heatmap of differentially expressed, inflammation related genes, high in Tet2HR monocytes.

G, Gene set enrichment of upregulated genes in Tet2^{HR} monocytes.

Supplementary Figure 4.



Supplementary Figure 5. Inflammatory monocytes in mouse AML models and Tet2^{HR} tissues.

A, Representative flow cytometry gating strategy in peripheral blood of Tet2^{WT}, Tet2^{HR}, and Tet2^{KO} collected at 11 months.

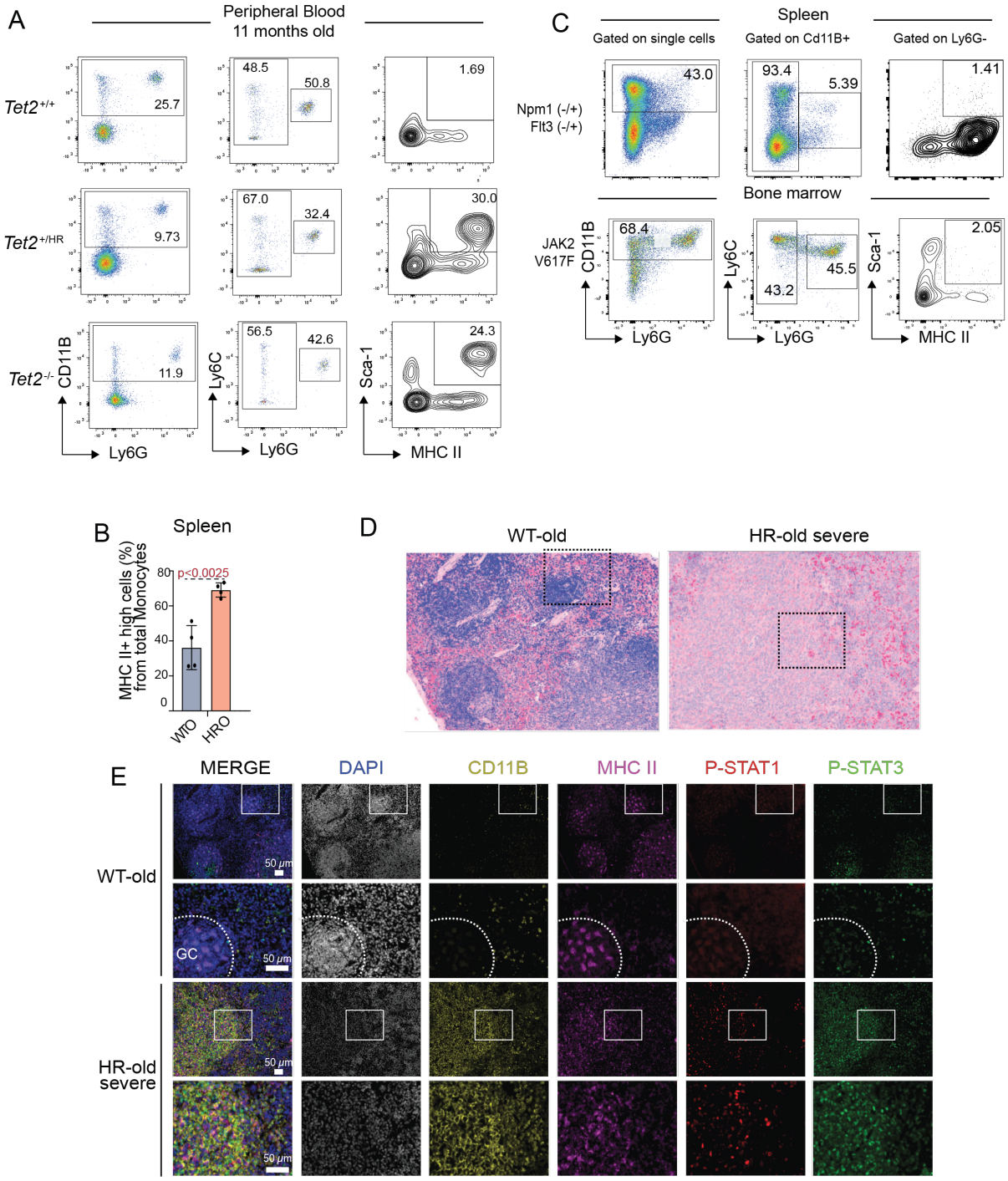
B, Representative flow cytometry gating strategy in spleen of Npm1$^{+/-}$ Flt3$^{+/-}$, and bone marrow of Stag2 $^{+/-}$Npm1$^{+/-}$, and Jak2V617F mice

C, Quantification of MHC II^{high} monocytes in spleen of Tet2^{WT} and Tet2^{HR}

D, Pseudo-Hematoxylin and Eosin (H&E) staining of spleen of old *Tet2* $^{+/+}$ or old *Tet2*$^{+/HR}$ with transformed disease phenotype used for C.

E, Multiplex Immunohistochemistry/Immunofluorescence for the simultaneous detection of the indicated antibodies in *Tet2*^{WT} and *Tet2*^{HR} spleen samples. Bottom panels are magnification from the indicated white square (GC, center of spleen)

Supplementary Figure 5.



Supplementary Figure 6. Extramedullary hematopoiesis and increased Saa3 expression observed in LPS treated Tet2^{HR} mice.

A, White blood count of Tet2^{WT} and Tet2^{HR} treated with PBS or LPS after 8 months following treatment.

B, Quantification of LSK analysis of spleen samples from Tet2^{WT} and Tet2^{HR} treated with PBS or LPS after 8 months following treatment.

C, Saa3 quantification of serum of Tet2^{WT} and Tet2^{HR} treated with PBS or LPS after 8 months following treatment.

D, Experimental design of antibiotic experiment. Young unfractionated bone marrow cells from CD45.2 *Tet2*^{HR} mice were transplanted into young CD45.1 *Tet2*^{WT} mice and allowed to recover for 4 weeks. Mice were bled retro-orbitally to monitor progression of MHC II^{high} monocyte percentage since beginning of IRAK 1/ 4 treatment or antibiotic treatment.

E, Quantification of MHC II^{high} monocyte percentage at week 12.

F, Progression of the MHC II^{high} monocyte percentage over 12 weeks since beginning of treatment.

Supplementary Figure 6.