Supplemental Information

Downregulation of *Morrbid* in Tet2-deficient preleukemic cells overcomes resistance to inflammatory stress and mitigates clonal hematopoiesis

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Figure S1. Additional supporting data for Figure 1

Figure S1 is presented for hematologic changes in Monocyte, Lymphocyte, Platelet and Red Blood Cells (A), bone marrow and spleen histology (B), the impact on frequency (Freq.) of mature cells in the bone marrow and spleen (C), quantitative changes in MPPs and ST-HSCs in the bone marrow (D) and quantification of the Freq. and No. of CMP, LSK and HSCs on day 0 and day 2 post LPS treatment in juvenile wildtype and *Tet2*-KO mice (E) pre- and post-LPS treatment.

Data in (A-B) are pooled from multiple experiments (n=4-10 mice per group, mean ± s.e.m.). Data in (C) is a representative profile from a single experiment. Data in (D) and (E) is from a representative experiment (n=3-4 mice per group, mean ± s.e.m.).

Experiments were repeated twice. *P* value: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. n.s., not significant. Statistical analysis performed by unpaired, two-tailed Student's *t*-test.

Figure S2. Additional supporting data for Figure 2

Figure S2 is presented for representative flow cytometry plots of CD45.2/CD45.1 chimerism in peripheral blood (A) and bone marrow (B) as well as lineage chimerism (C). (D) Quantitative analysis of chimerism in various bone marrow subsets after 4 weeks, 8 weeks and 16 weeks of transplantation in primary recipients. (E) Comparable frequency of HSCs in the LSK pool in wildtype and *Tet2*-KO pre and post LPS challenge.

Data in (D) is from a representative experiment (n=5 recipients for cBMT analysis, mean ± s.e.m.). Data in (A-C) and (E) are representative profiles of each analysis. *P* value: * $P < 0.05$, ** $P < 0.01$. n.s., not significant. Statistical analysis performed by unpaired, two-tailed Student's *t*-test.

Figure S3. Additional supporting data for Figure 3

(A) Presence of Ki-67^+ proliferating cells within the Lin- and the LSK fraction of the bone marrow post LPS treatment.

(B) Schematic of the ~800-bp promoter sequence of pro-survival gene *Morrbid*. The "CCGG" island for 5-mC and 5-hmC analysis is highlighted in green. The four CpG sites (1-4) for bisulfite-sequencing are highlighted in red square.

(C) The genomic DNA derived from Lin- cells from wildtype and *Tet2*-KO mice was subjected to HpaII and MspI digestion, followed by PCR amplification using the primers for the ~800-bp promoter region of *Morrbid* gene. Note that HpaII but not MspI is 5-mC sensitive. A similar digestion efficiency in wildtype and *Tet2*-KO was observed for HpaII and MspI, indicating that the "CCGG" island is not associated with 5-mC modification in wildtype and *Tet2*-KO Lin- cells (comparison between blue stars).

(D) The genomic DNA derived from Lin- cells from wildtype and *Tet2*-KO were subjected to "5hmC to 5ghmC" conversion by hydroxymethylcytosine Glucosyltransferase (GT), then subject to digestion by MspI. Note that MspI is 5-ghmC sensitive. The "5hmC to 5ghmC" conversion by GT indeed prevents MspI mediated digestion. However, such conversion by GT in wildtype and *Tet2*-KO Lin- cells shows similar levels, indicating that the "CCGG" island has similar 5-hmC modification between wildtype and *Tet2*-KO cells (comparison between red stars).

(E) Subtle methylation modification at indicated four CpG sites in both wildtype and *Tet2*-KO HSPCs, revealed by Bisulfite Sequencing PCR (BSP). The genomic DNA of Lin- cells from wildtype and *Tet2*-KO cells was subjected to bisulfite treatment for "C to T" conversion, followed by PCR amplification, cloning and sequencing. See the Material and Method for more detailed experimental procedure.

Data in (A) are from a representative experiment (n=3-4 mice per group, mean ± s.e.m.). Data in (C-E) are from a representative profile (n = 2 mice per group). Experiments were repeated twice. *P* value: * $P < 0.05$. Statistical analysis performed by unpaired, two-tailed Student's *t*-test.

Figure S4. Additional supporting data for Figure 4

Figure S4 is presented for serum expression of the 31 cytokines/chemokines pre- and post-LPS treatment (A), mature cell population of $IL6^+$ bone marrow cells (B), representative ICFC histograms for TNFα, IL-1b and GM-CSF expression in Lin- cells and quantification of expression of TNFα, IL-1b and GM-CSF (C-E).

Serum level of cytokines were analyzed once and other experiments were repeated twice. Data in (A) is from a single experiment (n=4 mice per group, mean ± s.e.m.). Data in (B-E) are from a

representative profile (n=4 mice per group, mean ± s.e.m.). *P* value: * $P < 0.05$, ** $P < 0.01$. Statistical analysis performed by unpaired, two-tailed Student's *t*-test.

Figure S5. Additional supporting data for Figure 5

Figure S5 is presented for qRT-PCR analysis on Lin- cells derived from naïve wildtype and *Tet2*-KO mice showing the expression of *Tlr4, Ticam1, Nfkb1, Nfkbiz, Apex1, Stat1, Stat3 and Ly6a* (A), representative flow cytometry profile of NFkB (B), quantification of $TLR4^+$ cells and TLR4 expression in indicated bone marrow subsets (C), IL-6 stimulation of Lin- cells induces Stat3 phosphorylation (pStat3) (D), expression of Shp2E76K in Lin- cells induces pStat3 activation and enhances the binding of Stat3 to the *Morrbid* promoter relative to controls (E) and quantification of expression of IL-6 and pStat3 upon E3330 or SHP099 treatment in Lin- cells Analysis in (A-E) was performed twice. Experiment in (F) were performed once. Data in (A) and (D) are from 3 to 4 mice per group, mean ± s.e.m. Data in (B) are representative profiles. *P* value: * $P < 0.05$, ** $P < 0.01$. n.s., not significant. Statistical analysis performed by unpaired, two-tailed Student's *t*-test

Figure S6: Additional supporting data for Figure 6

(A) Expansion of LSK cells in *Tet2*-KO mice on day 2-post LPS treatment was repressed by E3330 or SHP099.

(B) *Tet2*-KO mice develop age-dependent increased neutrophil counts, neutrophil frequency and serum IL-6 level.

(C) Treatment of Juvenile wildtype mice with E3330 or SHP099 on a daily basis does not impact hematologic parameters.

(D) Short-term treatment with E3330 or SHP099 of aged *Tet2*-KO mice partially reverses multiple early signs of CMML.

Experiments were repeated twice. Data in (A-D) are from a representative experiment (n=3 to 5 mice per group, mean ± s.e.m.). *P* value: * $P < 0.05$, ** $P < 0.01$. n.s., not significant. Statistical analysis performed by unpaired, two-tailed Student's *t*-test.

Figure S7: Additional supporting data for Figure 7 and a model for summarizing the finding of the present study

(A) A schematic showing the approach taken to target *Tet2*-KO Lin^- bone marrow cells using lentivirus shRNA against *Morrbid*. The growth advantage of *Morrbid shRNA*-targeted HSPCs was monitored in both CFU assay (*ex vivo*) and competitive transplantation assay (*in vivo*).

(B) Knocking-down *Morrbid* in *Tet2*-KO HSPCs results in reduced repopulating advantage in a serial CFU replating assay.

(C) Knocking-down *Morrbid* in *Tet2*-KO HSPCs results in reduced repopulating advantage in a cBMT assay.

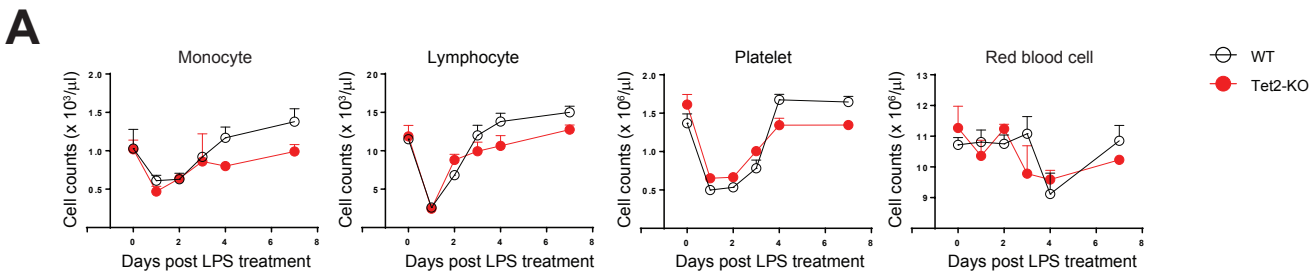
(D) $Tet2^{+/-}$; $Morrbid^{+/-}$ cells manifest increased expression of Bim and apoptosis as assessed by Annexin-V labeling of both mature myeloid cells and myeloid progenitors.

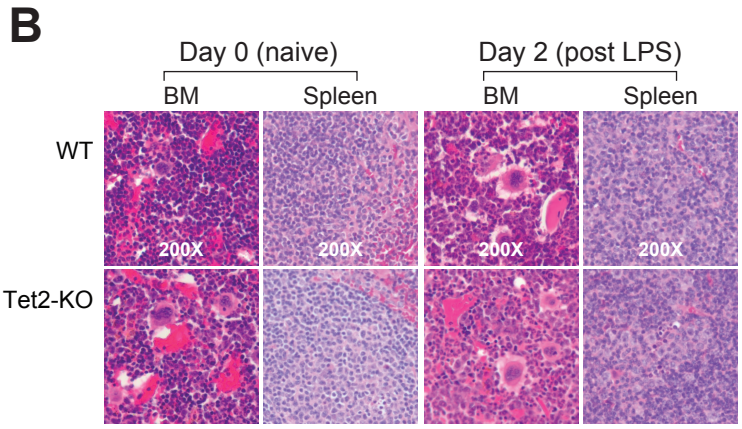
Knockdown of *Morrbid* by shRNA lentivirus, serial plating, transplantation analysis of $Tet2^{+/-}$;$Morrbid^{+/-}$ BM cells and LPS challenge of $Tet2^{+/-}$;$Morrbid^{+/-}$ mice along with controls was performed twice. *P* value: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. n.s., not significant. Statistical analysis performed by unpaired, two-tailed Student's *t*-test.

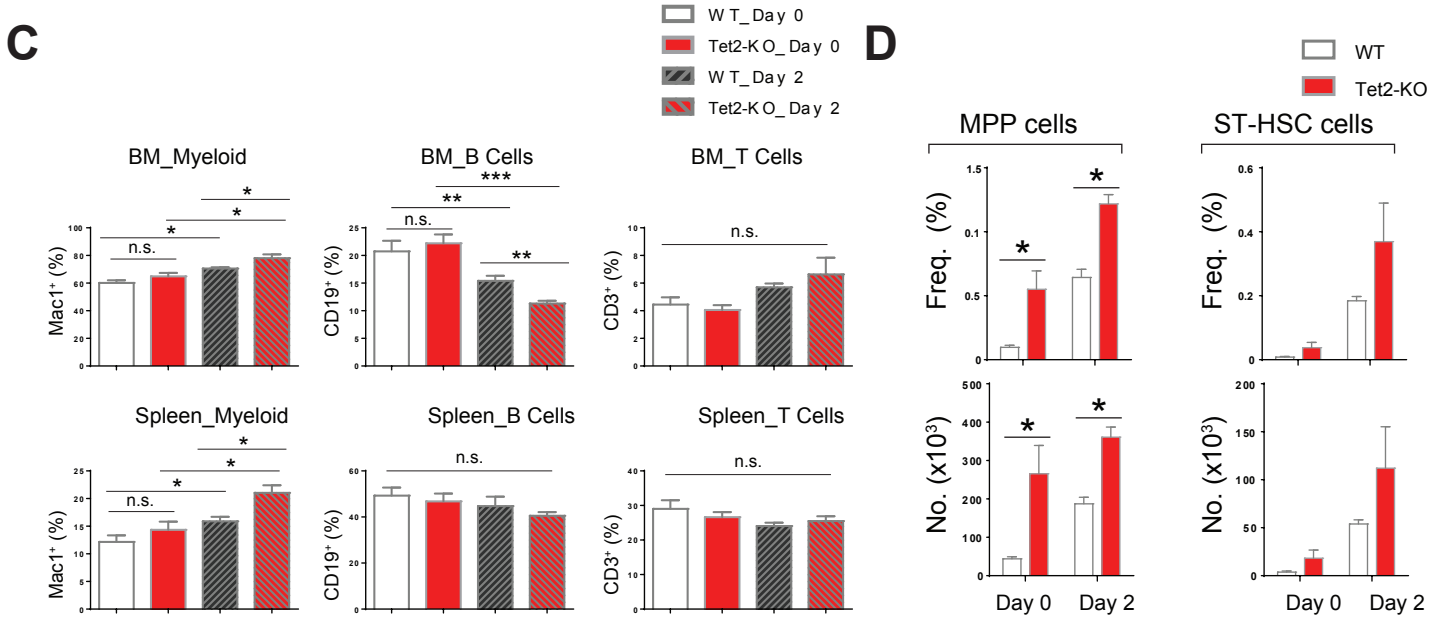
(E and **F)** Genetic loss of TLR4 or IL-6 prevents Tet2 deficiency-mediated aberrant "emergency granulopoiesis". $Tet2^{-/-}$; $Tlr4^{-/-}$ (E) or $Tet2^{-/-}$; $Il6^{-/-}$ (F) double knockout mice (3-4 month old)

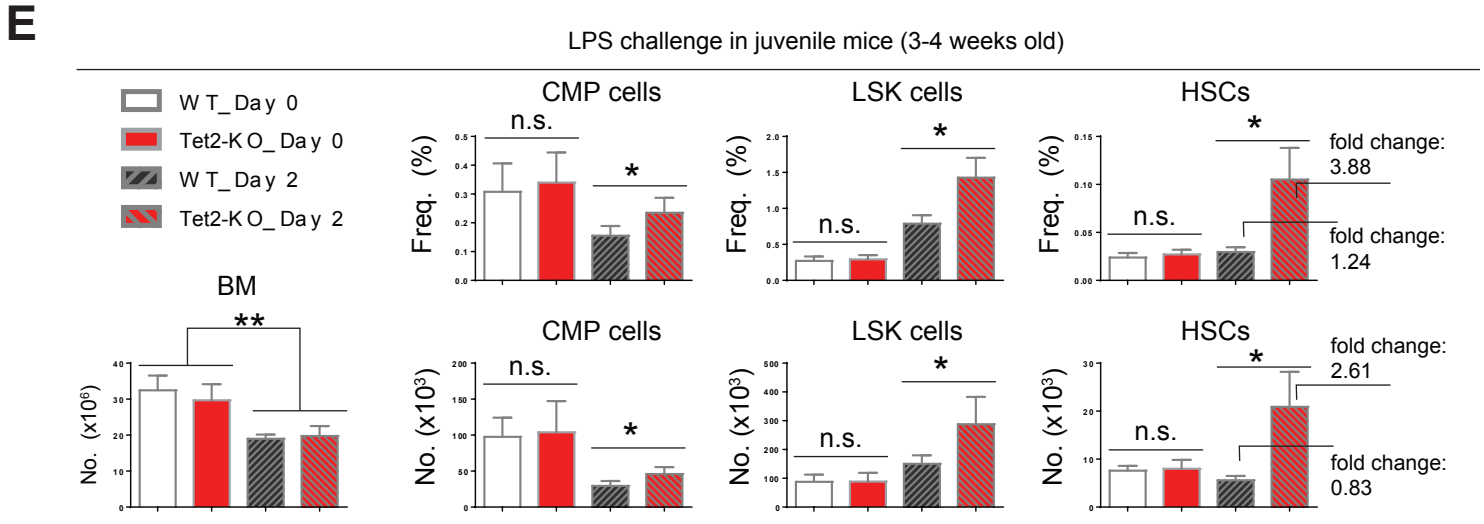
were subject to LPS challenge (0.8 mg/kg, one dose) and neutrophils in PB and hematopoiesis in bone marrow was analyzed. Data are mean ± s.e.m. One experimentation with n=3 biological repeats. *P* value: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$. n.s., not significant. Statistical analysis performed by unpaired, two-tailed Student's t-test.

(G) Model for a feed-forward loop and development of aberrant hematopoiesis induced by Tet2 loss, which can be modulated by the administration of E3330 or SHP099 or by repressing the expression of *Morrbid*. See discussion for detailed explanation.

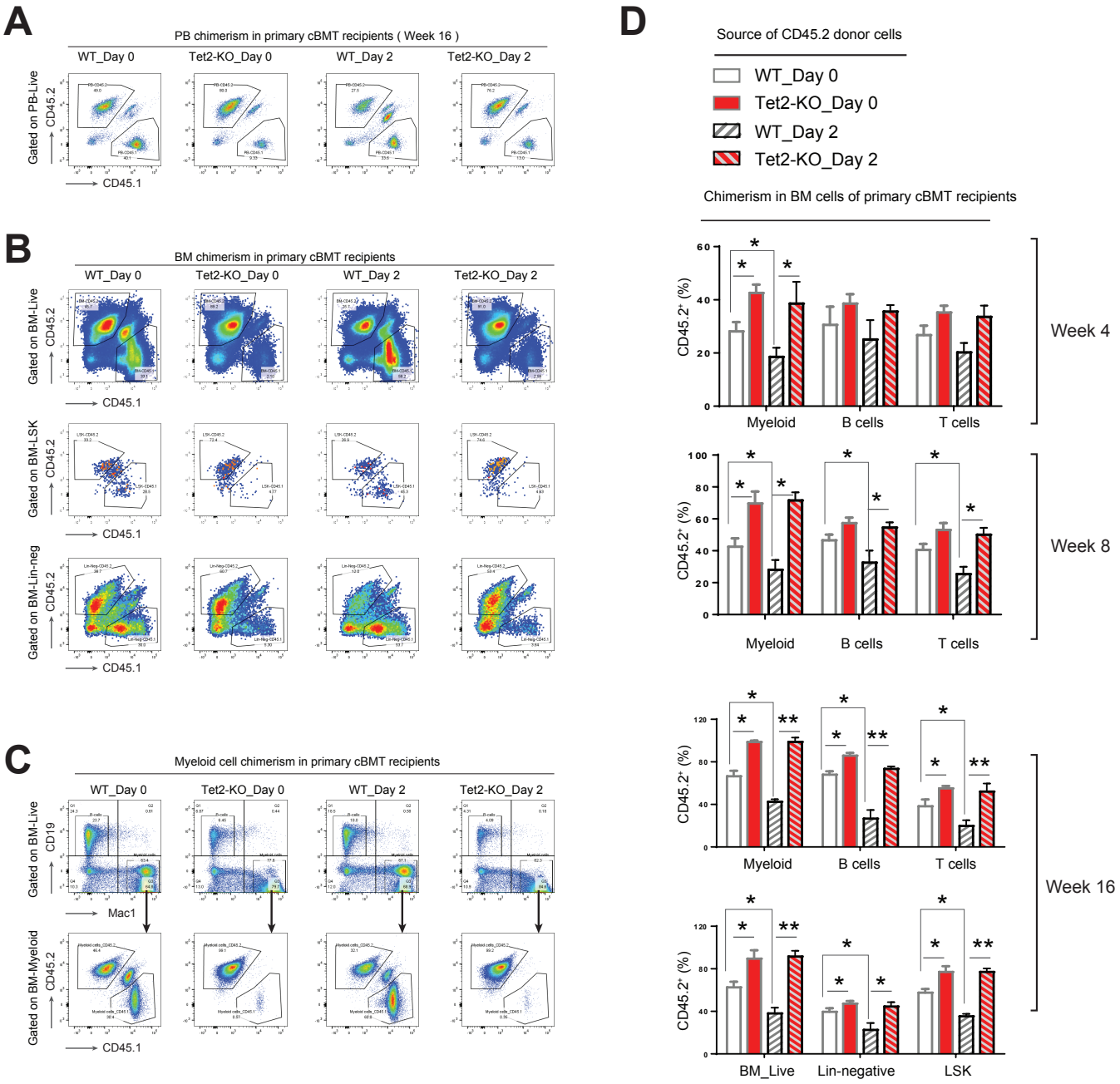




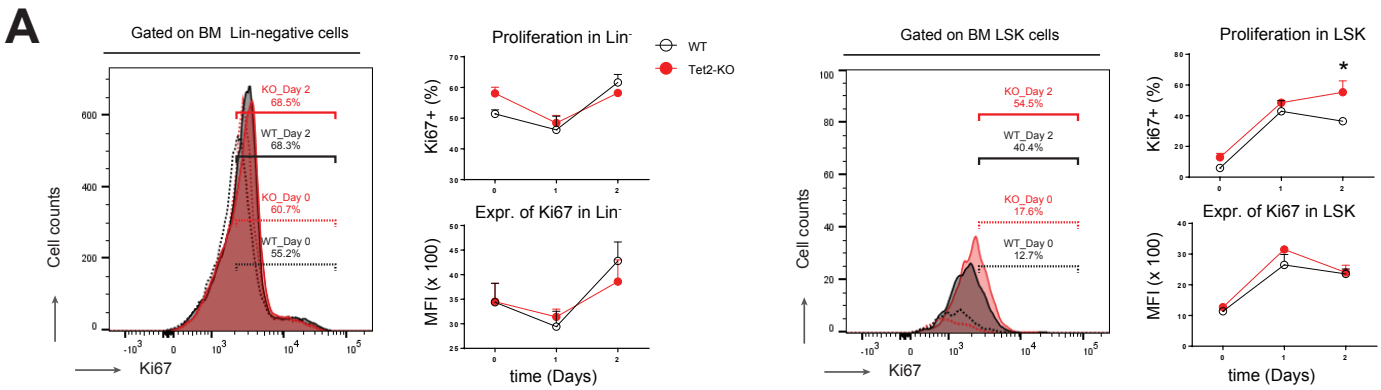




Cai *et al.* Supplemental Figure 1., related to Figure 1.



Cai *et al.* Supplemental Figure 2., related to Figure 2.



B

Sequence of *Morrbid* promoter: ~800 bp (amplified by the oligos in red)

```
GCCAGCGTGT TACAGCACCC GTGTGCATGT GAGATCTCTG CCTTTTATTT TCAGTTACCC
CCAAGTCTCC TAACCAAAGC GTCTCCTCCC TGATGAGGGT GATCACTGGC TACAAGCCTG
GGGCTTCCAC ACCCAAGGTC TGGGAAGCTG CTCTTTCCGG AAGGGTGGTA GAGACCCTGG
GAACCAGACC AGGCGGGCTC ACAAAGTATG AAGAGAATGT CGGGAGAGGA AGTGGTGGCT
CTGAGTCAGC ACGAGTCATC TGGTTCCAGT GACAATGAAG ACTGAGGTGT CTGCTTTGCA
GTTTTTTTTT TTCTTTTCTT GTCTTTTTTT TTTTTTTTCC TAATCATGTG GACATCTCTG
AGAATGAGGG GACTGGGTGA CTTTGCAGTG TCTGCTTTCT AAGTTTCAAA GTGACTTTCC
AGGCAAGCTG CGCATATCTT GGGATCTTCA CAGCACAGTG GCTGGGAAGG CAGGCGGACC
CGTCTATCAG CTTCTCCCTG GTGAGTACTC TCAGTCAAAG ATGTTTTCCG TGCACCTCTG
AGCTGTGACA GCTGCTGTGT TCTGGGGTAG ACACTGCTTC TCCCAGACTT TGAAGGTTTT
GTGAGGTCAG AAAAGTGGCT CCCTCTGCCT CCAGCCCACC TGTTGTGTCT GTTGCGGGAC
TCTTGGAGGA AGTTTCTGAG CTGTGCCTTG TGGATGGTGG CTGTGTGTTA TGCTGTCTCA
GAGGAGAGCG TGAGGCTTGC CTGGGAAGGA AGCCACCACG GATTCCTAGC TAGAAGCTAA
AGTGAAGGTC AGAGACGGAG GTGGGTGAGT TTGTGTTAAC TGGGCACTGA GGTTGAACTA
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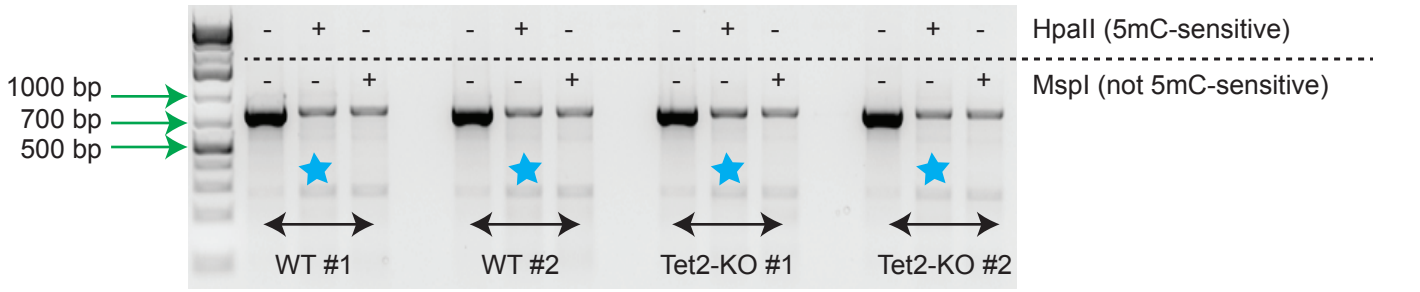
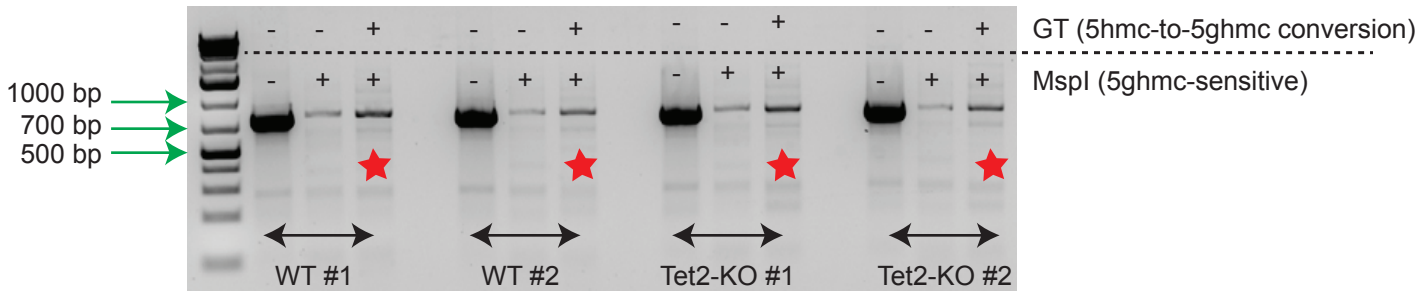
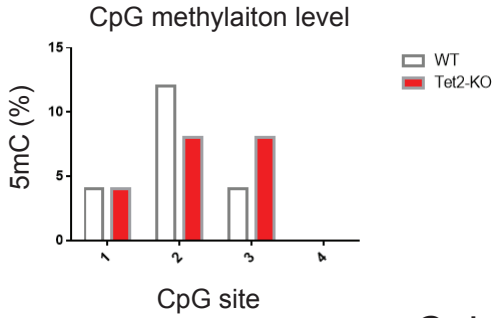
1 2 3 4

This "CCGG" island was used for 5-mC and 5-hmC analysis by restriction enzyme digestion (Figure **C** and **D**)

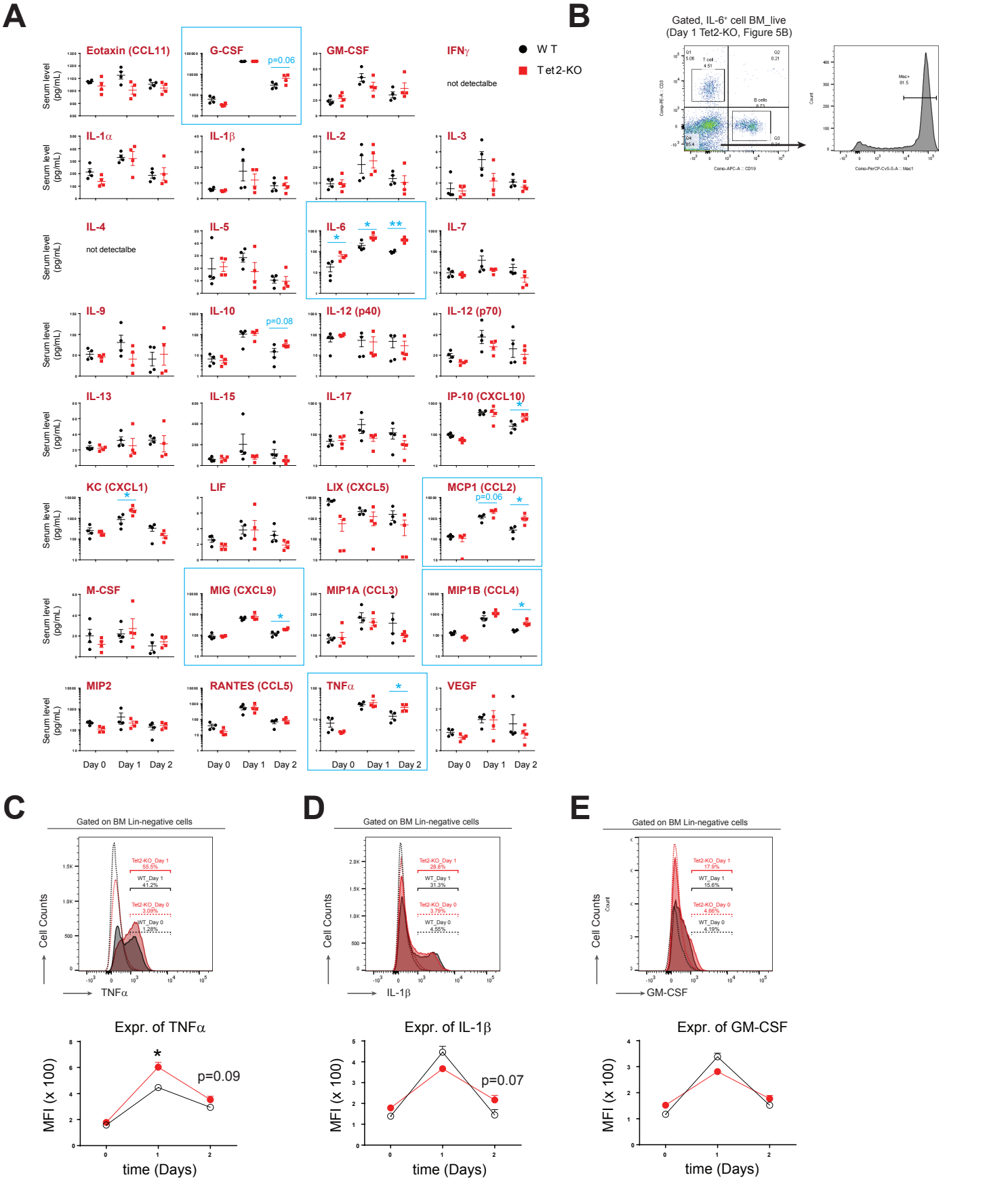
The sequence between the oligos in blue was used for 5-mC analysis by Bisulfite Sequencing PCR (BSP). The four CpG sites in this region are highlighted in red square. (Figure **E**)



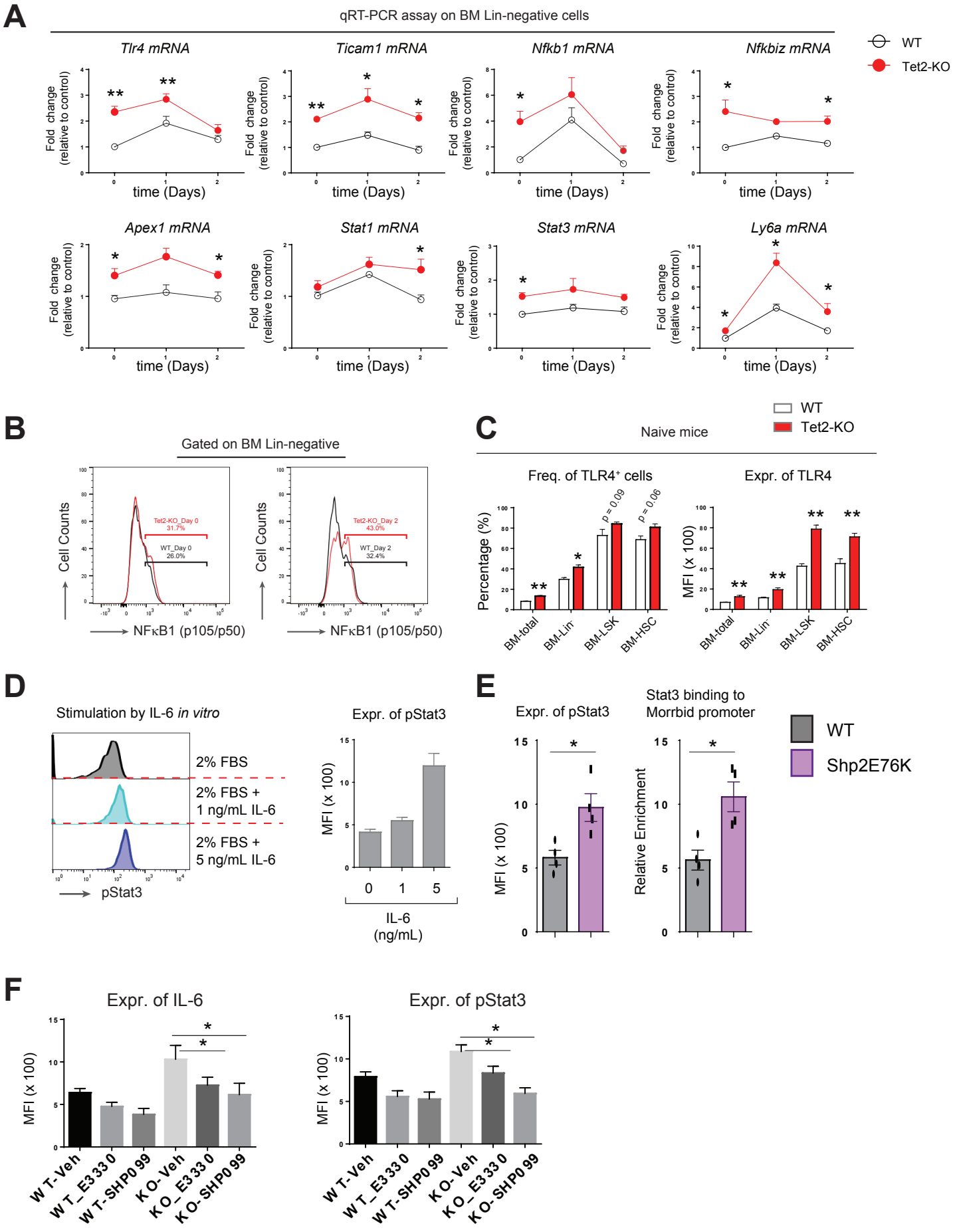




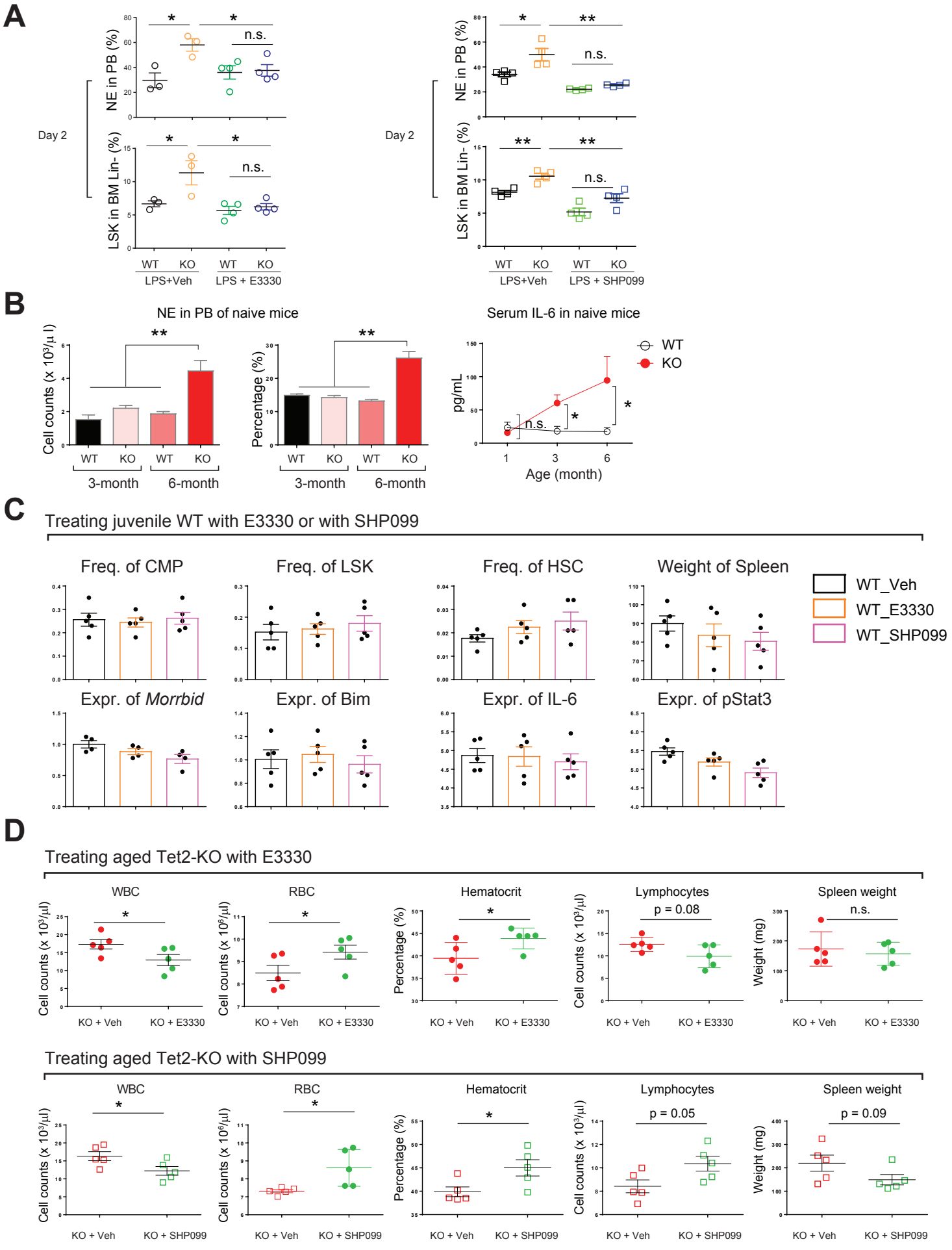
Cai *et al. Supplemental* Figure 3., related to Figure 3.



Cai et al. Supplemental Figure 4, related to Figure 4.



Cai et al. Supplemental Figure 5., related to Figure 5.



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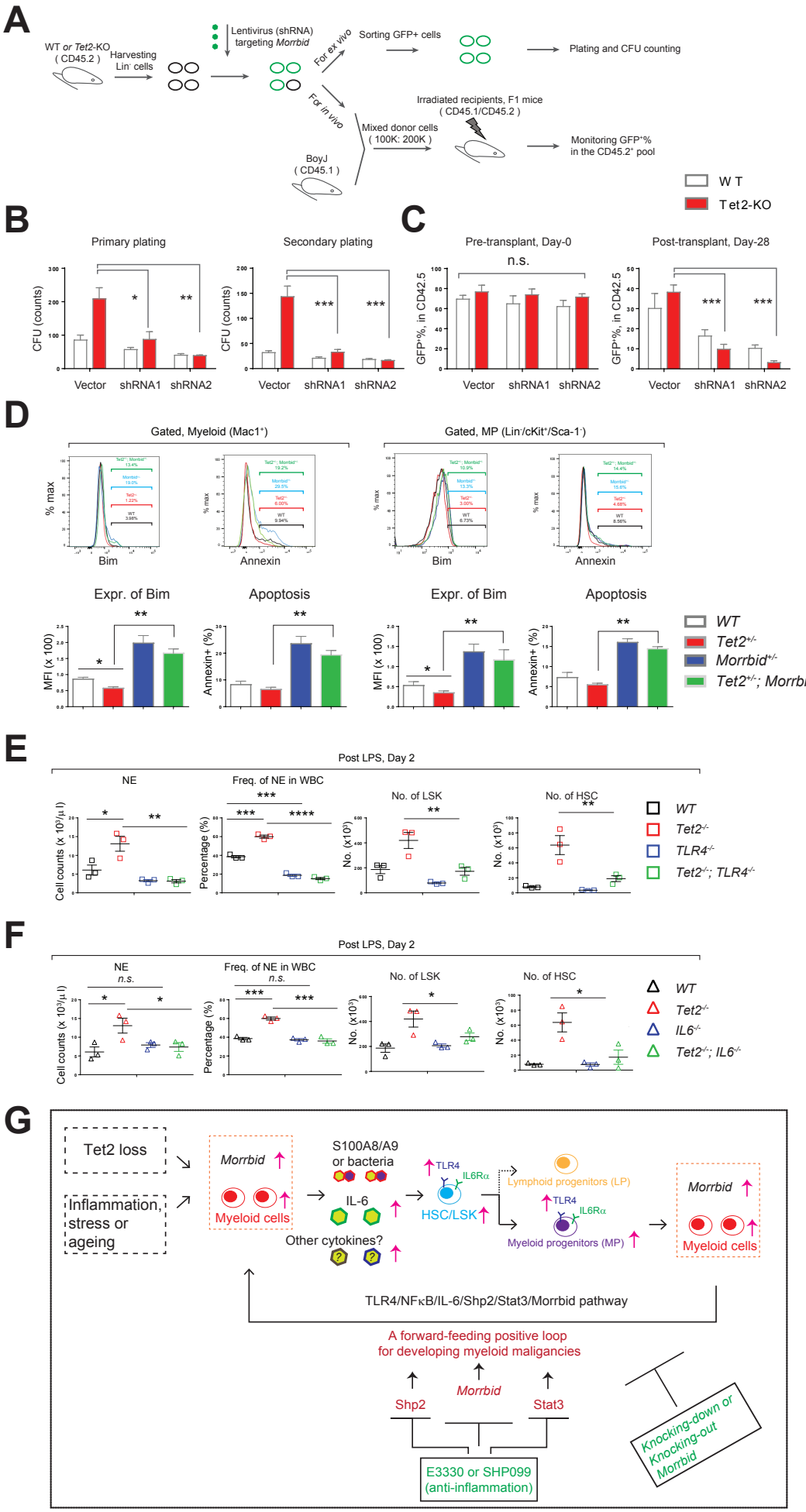

Cai et al. Supplemental Figure 7, related to Figure 7.

Supplementary Table 1. Related to STAR Methods

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Additional Oligonucleotides for qRT-PCR		
Casp1, Forward AGTCCTGGAAATGTGCCATC	https://mouseprimerdepot.nci.nih.gov/	N/A
Casp1, Reverse TCAGCTCCATCAGCTGAAAC	https://mouseprimerdepot.nci.nih.gov/	N/A
Bcl2l11, Forward CGCAGATCTTCAGGTTCCTC	Kotzin, J. J. et al. , Nature, 2016	N/A
Bcl2l11, Reverse ACAAACCCCAAGTCCTCCTT	Kotzin, J. J. et al. , Nature, 2016	N/A
Bcl2, Forward GGTCTTCAGAGACAGCCAGG	https://mouseprimerdepot.nci.nih.gov/	N/A
Bcl2, Reverse GATCCAGGATAACGGAGGCT	https://mouseprimerdepot.nci.nih.gov/	N/A
Ccl2, Forward ATTGGGATCATCTTGCTGGT	https://mouseprimerdepot.nci.nih.gov/	N/A
Ccl2, Reverse CCTGCTGTTCACAGTTGCC	https://mouseprimerdepot.nci.nih.gov/	N/A
Tlr4, Forward TGTCATCAGGGACTTTGCTG	https://mouseprimerdepot.nci.nih.gov/	N/A
Tlr4, Reverse TGTTCTTCTCCTGCCTGACA	https://mouseprimerdepot.nci.nih.gov/	N/A
Ticam1, Forward TGTCCAGCGGTGTGTTACAT	https://mouseprimerdepot.nci.nih.gov/	N/A
Ticam1, Reverse CAGCCACCTAGAGATCAGCC	https://mouseprimerdepot.nci.nih.gov/	N/A
Nfkb1, Forward GAACGATAACCTTTGCAGGC	https://mouseprimerdepot.nci.nih.gov/	N/A
Nfkb1, Reverse CATCACACGGAGGGCTTC	https://mouseprimerdepot.nci.nih.gov/	N/A
Nfkbiz, Forward TATCGGGTGACACAGTTGGA	https://mouseprimerdepot.nci.nih.gov/	N/A
Nfkbiz, Reverse TGAATGGACTTCCCCTTCAG	https://mouseprimerdepot.nci.nih.gov/	N/A
Apex1, Forward AGCACTTGGTCTCTTGGAGG	https://mouseprimerdepot.nci.nih.gov/	N/A
Apex1, Reverse GCAAATCTGCCACACTCAAG	https://mouseprimerdepot.nci.nih.gov/	N/A
Stat1, Forward CTGAATATTTCCCTCCTGGG	https://mouseprimerdepot.nci.nih.gov/	N/A
Stat1, Reverse TCCCGTACAGATGTCCATGAT	https://mouseprimerdepot.nci.nih.gov/	N/A
Stat3, Forward CTGCTCCAGGTAGCGTGTGT	https://mouseprimerdepot.nci.nih.gov/	N/A
Stat3, Reverse CTCAGCCCCGGAGACAGT	https://mouseprimerdepot.nci.nih.gov/	N/A
Ly6a, Forward GGCAGATGGGTAAGCAAAGA	https://mouseprimerdepot.nci.nih.gov/	N/A
Ly6a, Reverse CAATTACCTGCCCCTACCCT	https://mouseprimerdepot.nci.nih.gov/	N/A