

Supporting Information for

ZBP1 activation triggers hematopoietic stem and progenitor cell death resulting in bone marrow failure in mice

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Supplemental Table 1. Flow cytometry antibodies

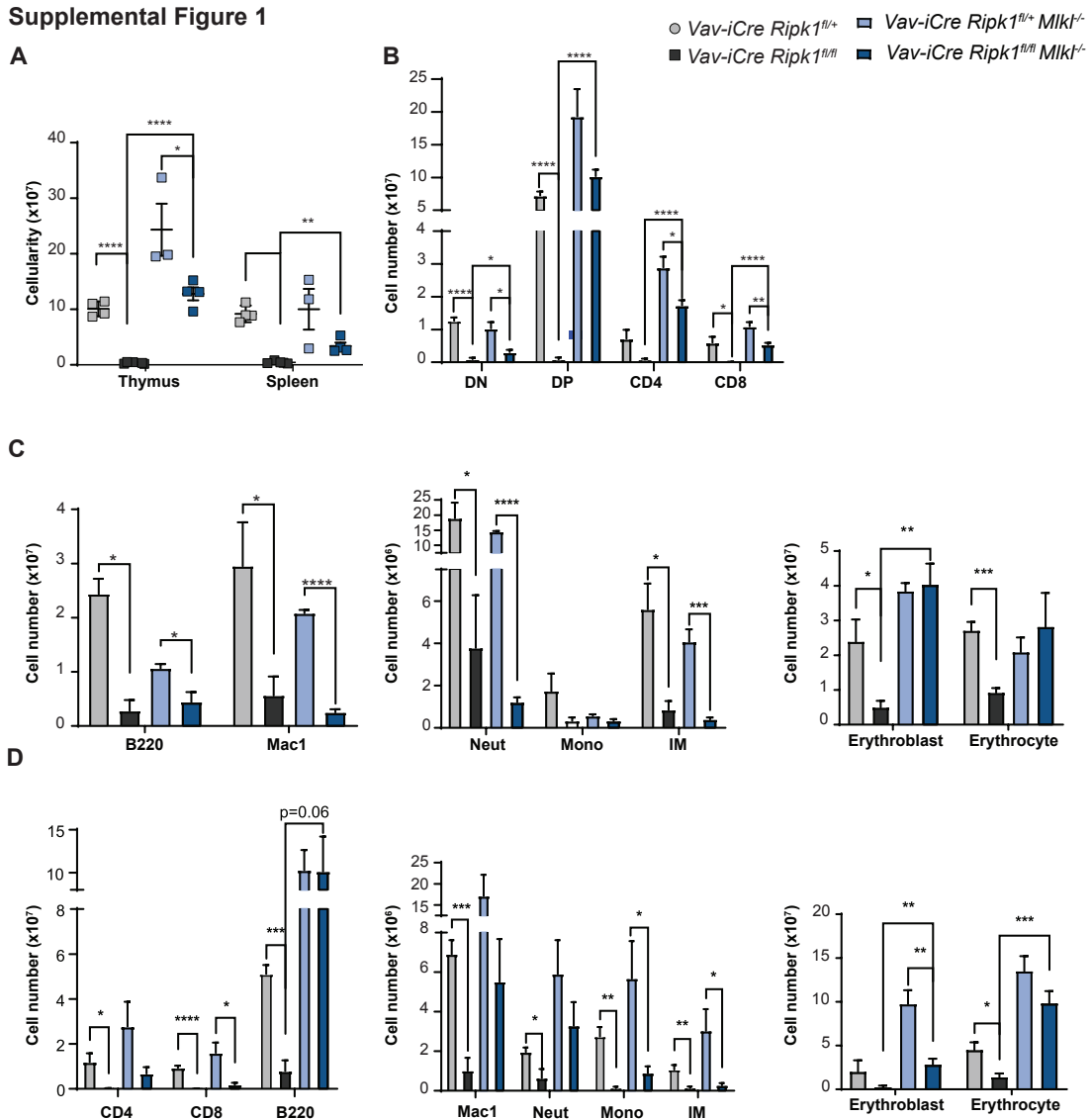
Antibody	Clone	Fluorophore	Source
CD11b	M1/70	FITC, biotin	BioLegend
Gr-1	RB6-8C5	PE, biotin	BD Biosciences
Ly-6C	HK1.4	APC	BioLegend
CD3	145-2C11	biotin	BioLegend
Ter119	TER-119	biotin	BioLegend
CD4	RM4-5	PerCP/Cy5.5, biotin	BioLegend
CD8	53-6.7	FITC	BD Biosciences
B220	RA3-6B2	Pe-Cy7, biotin	BD Biosciences, BioLegend
Sca-1	D7	APC/Cy7	BioLegend
CD34	RAM34	FITC	BD Biosciences
Flk2	A2F10	PE	BioLegend
CD150	TC15-12F12.2	Pe-Cy7	BioLegend
CD86	GL1	Pe-Cy7	Invitrogen

Supplemental Table 2. Primer Sequences

Gene	Forward	Primer R	Reference
mouse β - <i>actin</i>	CGAGGCCAGAGCA AGAGAG	CGGTTGGCCTTAGGGTT CAG	(Simmons et al. 2012)
mouse <i>Zbp1</i>	AACCCTCAATCAAGT CCTTTACCGC	TCTTCCACGTCTGTCCGT CATAGCT	(Liu et al. 2009)
mouse <i>Stat1</i>	GCTGCCTATGATGT CTCGTTT	TGCTTTTCCGTATGTTGT GCT	Primerbank ID 328887937c2 (Spandidos et al. 2010)
mouse <i>Mkl1</i>	TCGATTCTCCCAACA TCTTGC	GGTGTAGCCTGTATAAG CCTCTG	Primerbank ID 141802525c2
human β - <i>ACTIN</i>	CGCGAGAAGATGAC CCAGAT	GATAGCACAGCCTGGAT AGCAAC	Kelliher lab stock
human <i>ZBP1</i>	AAAGCATGGACGAT TTACCG	ATGATGTTCCCGTGTCCA AT	<i>Previously not published</i>
human <i>MLKL</i>	AGAGCTCCAGTGGC CATAAA	TACGCAGGATGTTGGGA GAT	(Moriwaki et al. 2015)

Supplemental Figures

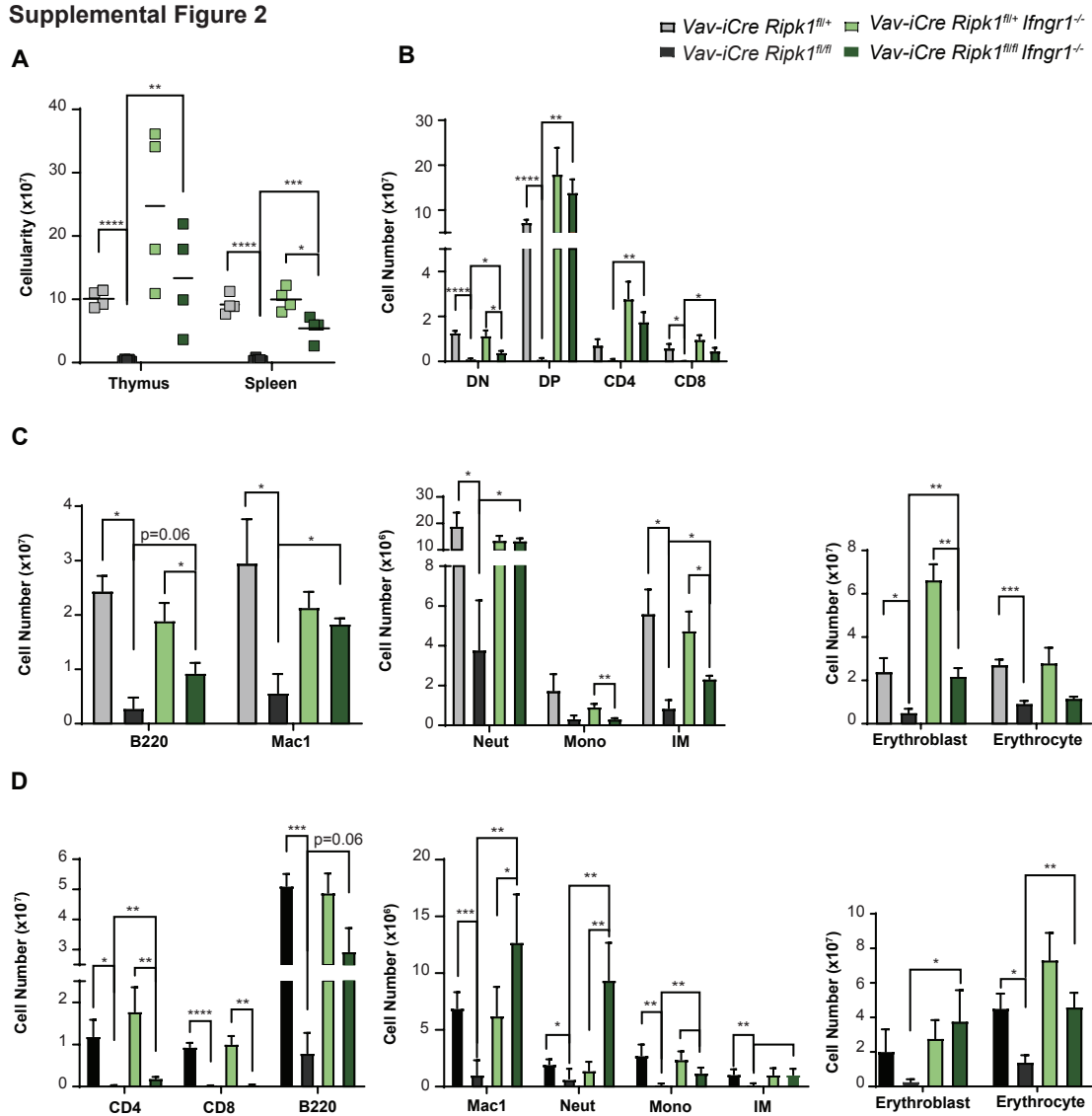
Supplemental Figure 1



Supplemental Figure 1. B lymphoid and myeloid lineages remain reduced in *Vav-iCre Ripk1^{fl/fl} Mlkl^{-/-}* bone marrow but splenic B cells, myeloid cells and erythroid cells increase.

(A) Thymus and spleen cellularity and (B-D) lineage analysis of thymus (B), bone marrow (C), and spleen (D) of *Vav-iCre Ripk1^{fl/+} Mlkl^{-/-}* (n=3) and *Vav-iCre Ripk1^{fl/fl} Mlkl^{-/-}* (n=4) mice at Day 35. Values compared to *Vav-iCre Ripk1^{fl/+}* (n=3) and *Vav-iCre Ripk1^{fl/fl}* (n=3) mice at day 35 are from Roderick J et al¹⁶. *Abbreviations:* double negative (DN), CD4 and CD8 double positive (DP), Mac1⁺ myeloid cells (Mac1), neutrophils (Neut), monocytes (Mono), and inflammatory monocytes (IM). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

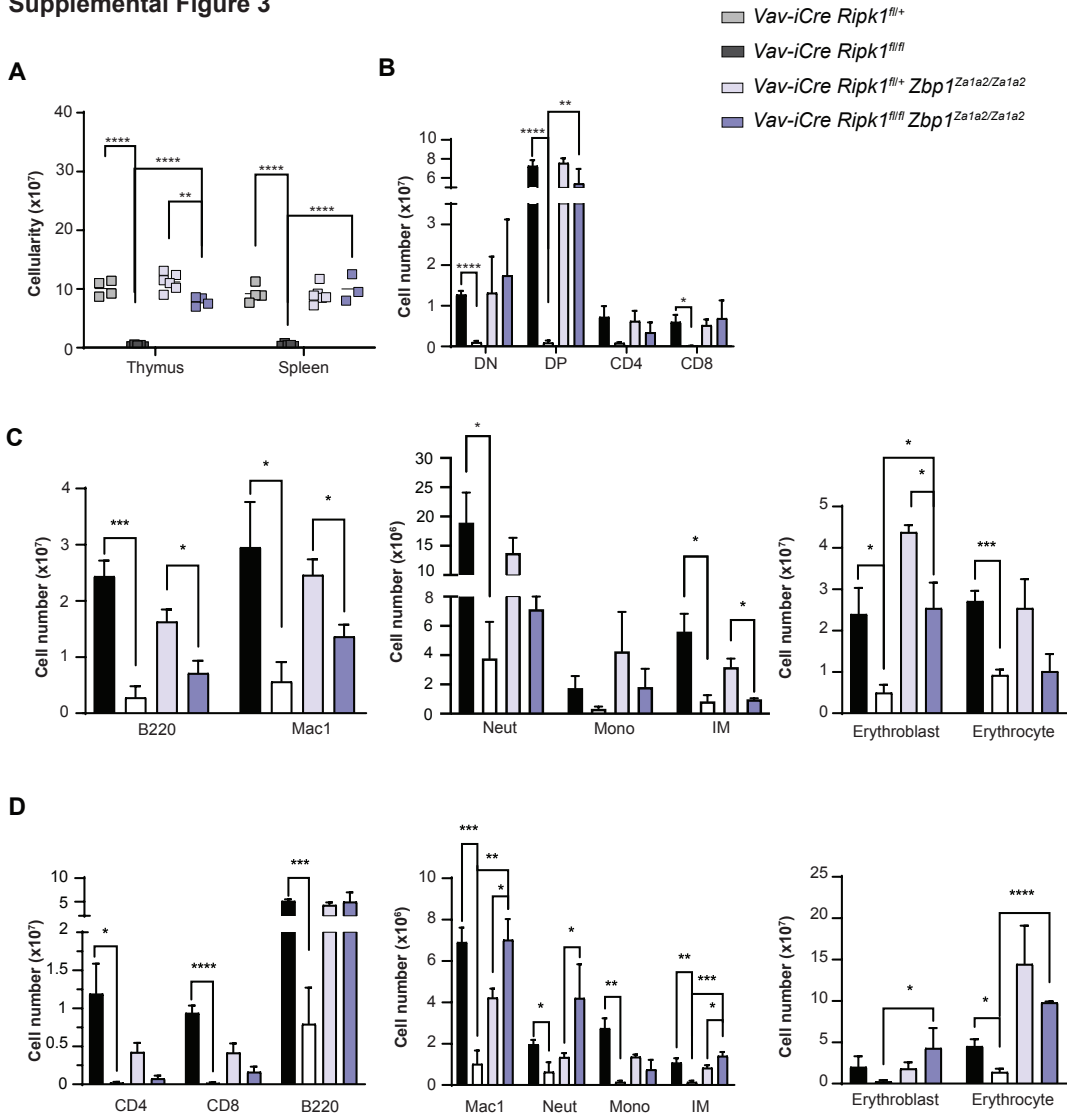
Supplemental Figure 2



Supplemental Figure 2. Thymopoiesis and hematopoiesis in bone marrow and spleen is rescued in *Vav-iCre Ripk1^{fl/fl} Ifngr1^{-/-}* mice.

(A) Thymus and spleen cellularity and (B-D) lineage analysis of thymus (B), bone marrow (C), and spleen (D) of control *Vav-iCre Ripk1^{fl/+} Ifngr1^{-/-}* (n=4) and *Vav-iCre Ripk1^{fl/fl} Ifngr1^{-/-}* (n=3) mice at day 35. Values compared to *Vav-iCre Ripk1^{fl/+}* (n=3) and *Vav-iCre Ripk1^{fl/fl}* (n=3) mice at day 35 as published in¹⁶. *Abbreviations:* double negative (DN), CD4 and CD8 double positive (DP), Mac1⁺ myeloid cells (Mac1), neutrophils (Neut), monocytes (Mono), and inflammatory monocytes (IM). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

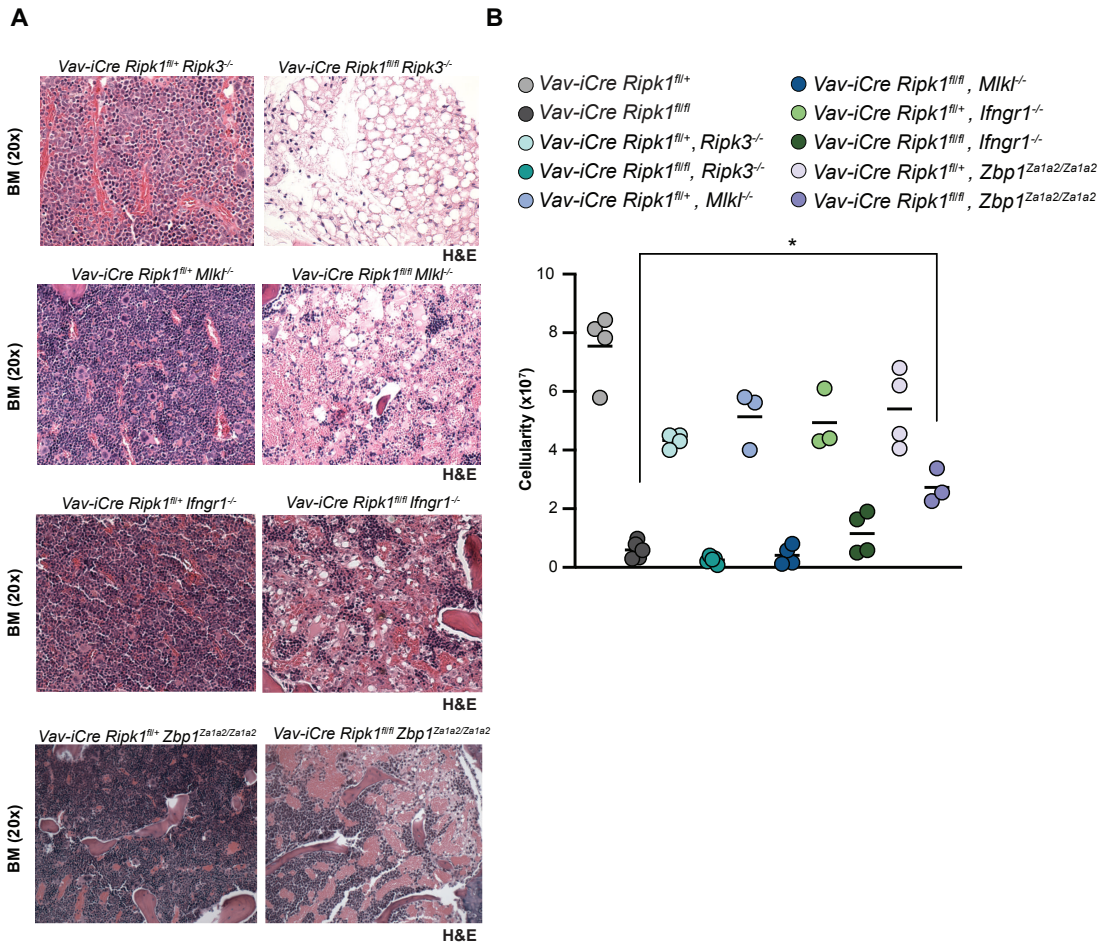
Supplemental Figure 3



Supplemental Figure 3. Expression of mutant *Zbp1* that cannot sense Z-nucleic acids rescues thymopoiesis and hematopoiesis in *Vav-iCre Ripk1^{fl/fl}* mice.

(A) Thymus and spleen cellularity, (B-D) lineage analysis of thymus (B), bone marrow (C), and spleen (D) of control *Vav-iCre Ripk1^{fl/+} Zbp1^{Za1a2/Za1a2}* (n = 4) and *Vav-iCre Ripk1^{fl/fl} Zbp1^{Za1a2/Za1a2}* (n = 3) mice at day 35. Values compared to *Vav-iCre Ripk1^{fl/+}* (n=4) and *Vav-iCre Ripk1^{fl/fl}* (n=3) mice at day 35 as published in¹⁶. *Abbreviations:* double negative (DN), CD4 and CD8 double positive (DP), *Mac1*⁺ myeloid cells (*Mac1*), neutrophils (Neut), monocytes (Mono), and inflammatory monocytes (IM). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

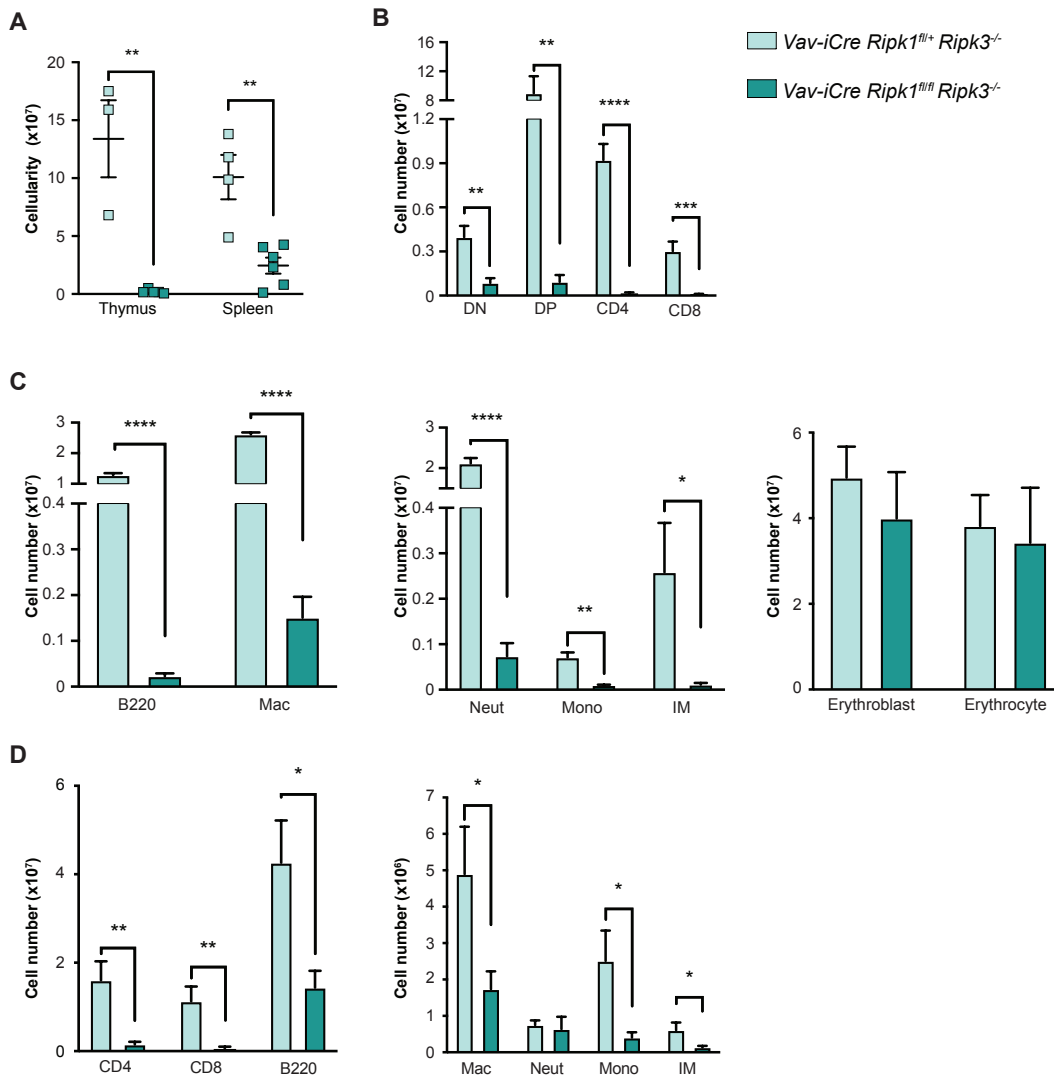
Supplemental Figure 4



Supplemental Figure 4. *Vav-iCre Ripk1^{fl/fl} Ripk3^{-/-}, Mlkl^{-/-}, Ifngr1^{-/-} or Zbp1^{Za1a2/Za1a2}* mice eventually succumb to BMF.

(A) Representative images of H&E-stained BM from littermate controls (left) of *Vav-iCre Ripk1^{fl/fl} Ripk3^{-/-}, Mlkl^{-/-}, Ifngr1^{-/-} or Zbp1^{Za1a2/Za1a2}* mice at time of disease. (B) BM cellularity of littermate controls (n=3–4) compared to *Vav-iCre Ripk1^{fl/fl} Ripk3^{-/-}, Mlkl^{-/-}, Ifngr1^{-/-} or Zbp1^{Za1a2/Za1a2}* mice (n=3–4 mice/genotype). *p<0.05.

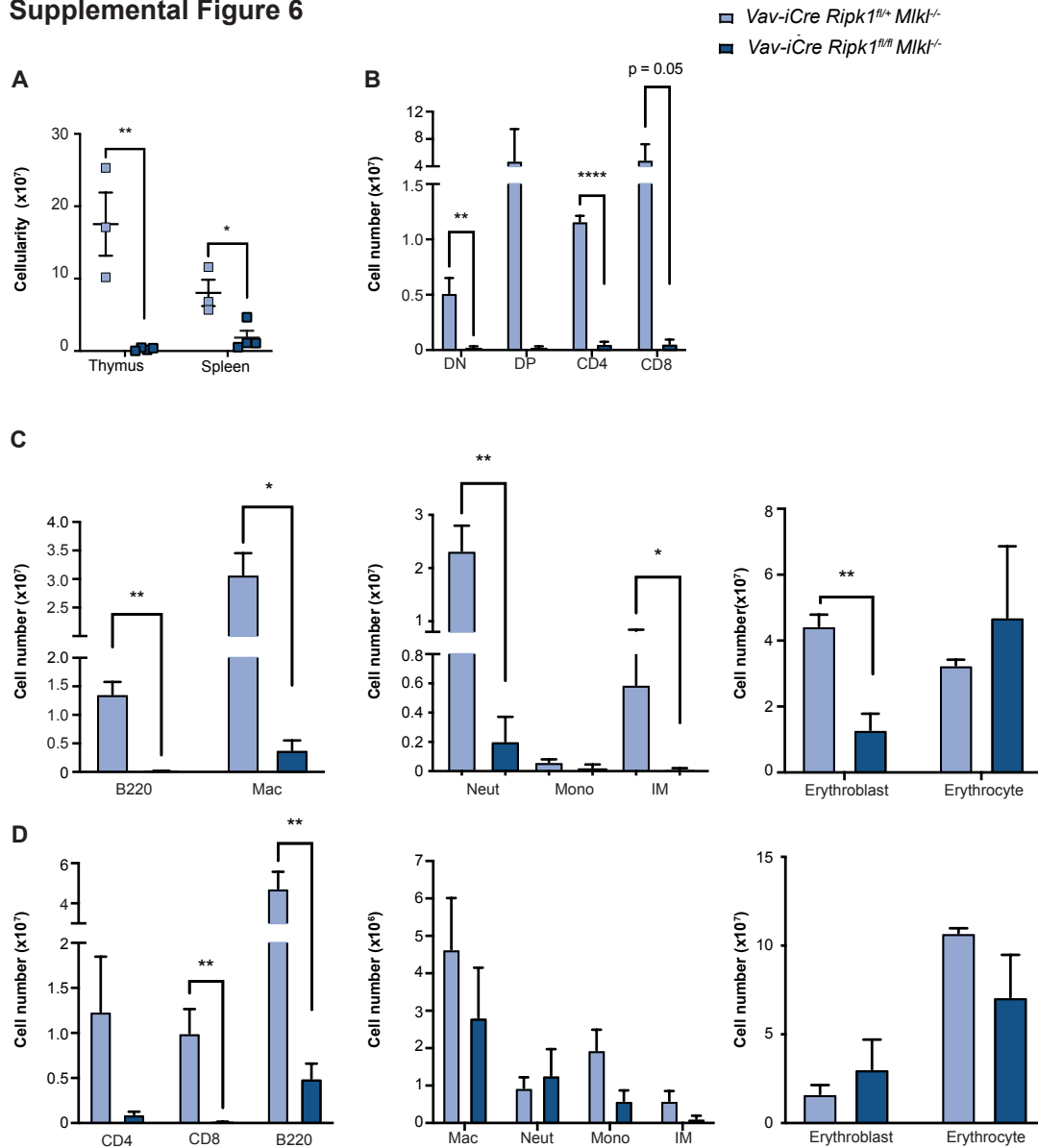
Supplemental Figure 5



Supplemental Figure 5. *Vavi-Cre Ripk1^{fl/fl} Ripk3^{-/-}* mice exhibit lymphoid and myeloid lineage loss in all hematopoietic compartments at time of disease.

(A) Thymus and spleen cellularity, (B-D) lineage analysis of thymus (B) bone marrow (C) and spleen (D) of control *Vavi-Cre Ripk1^{fl/fl} Ripk3^{-/-}* (n=3–4) and *Vavi-Cre Ripk1^{fl/fl} Ripk3^{-/-}* (n = 4–5) mice at time of disease. *Abbreviations:* double negative (DN), CD4 and CD8 double positive (DP), Mac1⁺ myeloid cells (Mac1), neutrophils (Neut), monocytes (Mono), and inflammatory monocytes (IM). *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001.

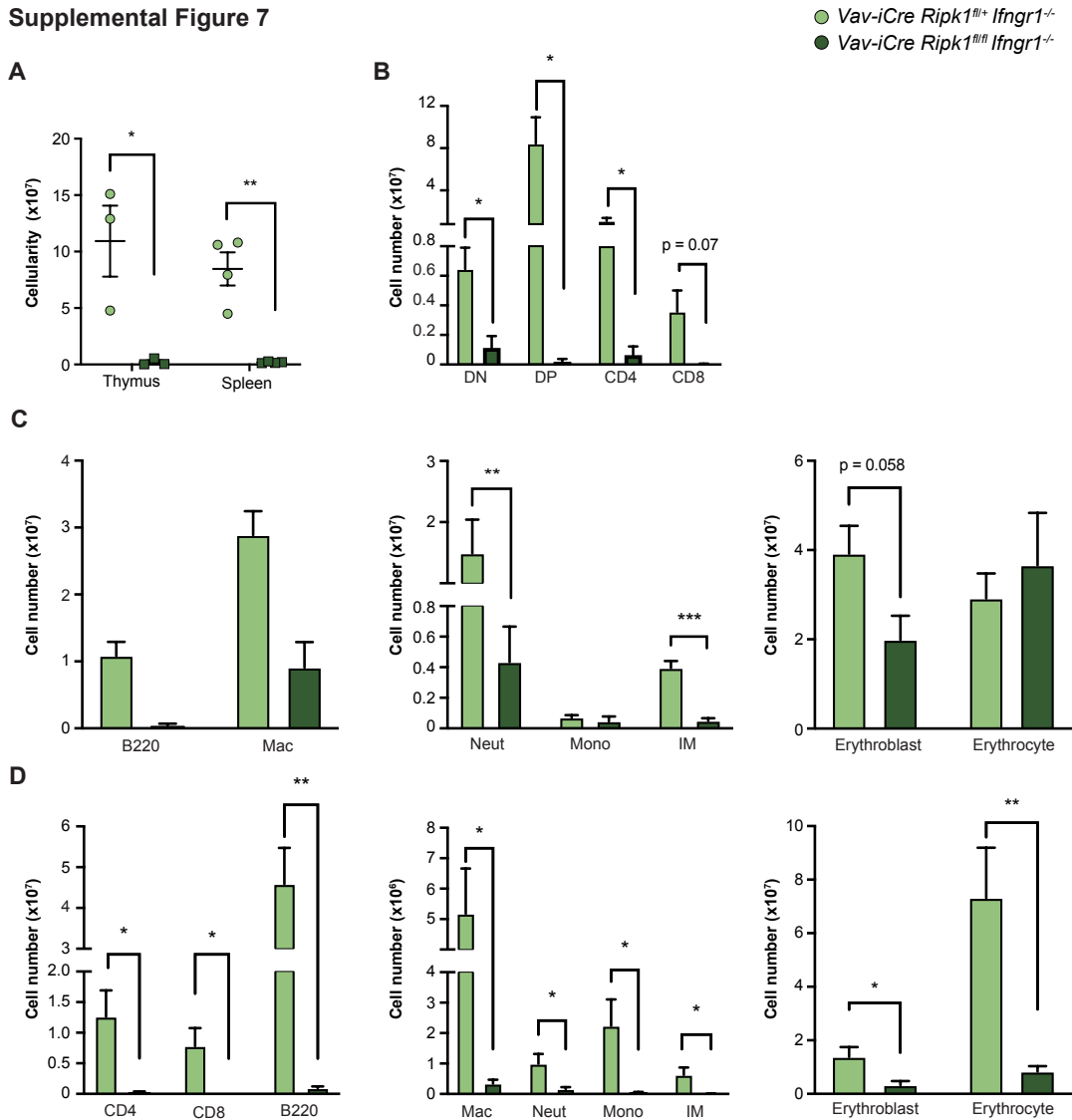
Supplemental Figure 6



Supplemental Figure 6. *Vav-iCre Ripk1^{fl/fl} Mik1^{-/-}* mice show reductions in all hematopoietic compartments at time of disease.

(A) Thymus and spleen cellularity, (B-D) lineage analysis of thymus (B), bone marrow (C), and spleen (D) of *Vav-iCre Ripk1^{fl/fl} Mik1^{-/-}* (n=3) and *Vav-iCre Ripk1^{fl/fl} Mik1^{-/-}* (n=4) mice at time of disease. *Abbreviations:* double negative (DN), CD4 and CD8 double positive (DP), Mac1⁺ myeloid cells (Mac1), neutrophils (Neut), monocytes (Mono), and inflammatory monocytes (IM). *p<0.05, **p<0.01, ****p<0.0001.

Supplemental Figure 7

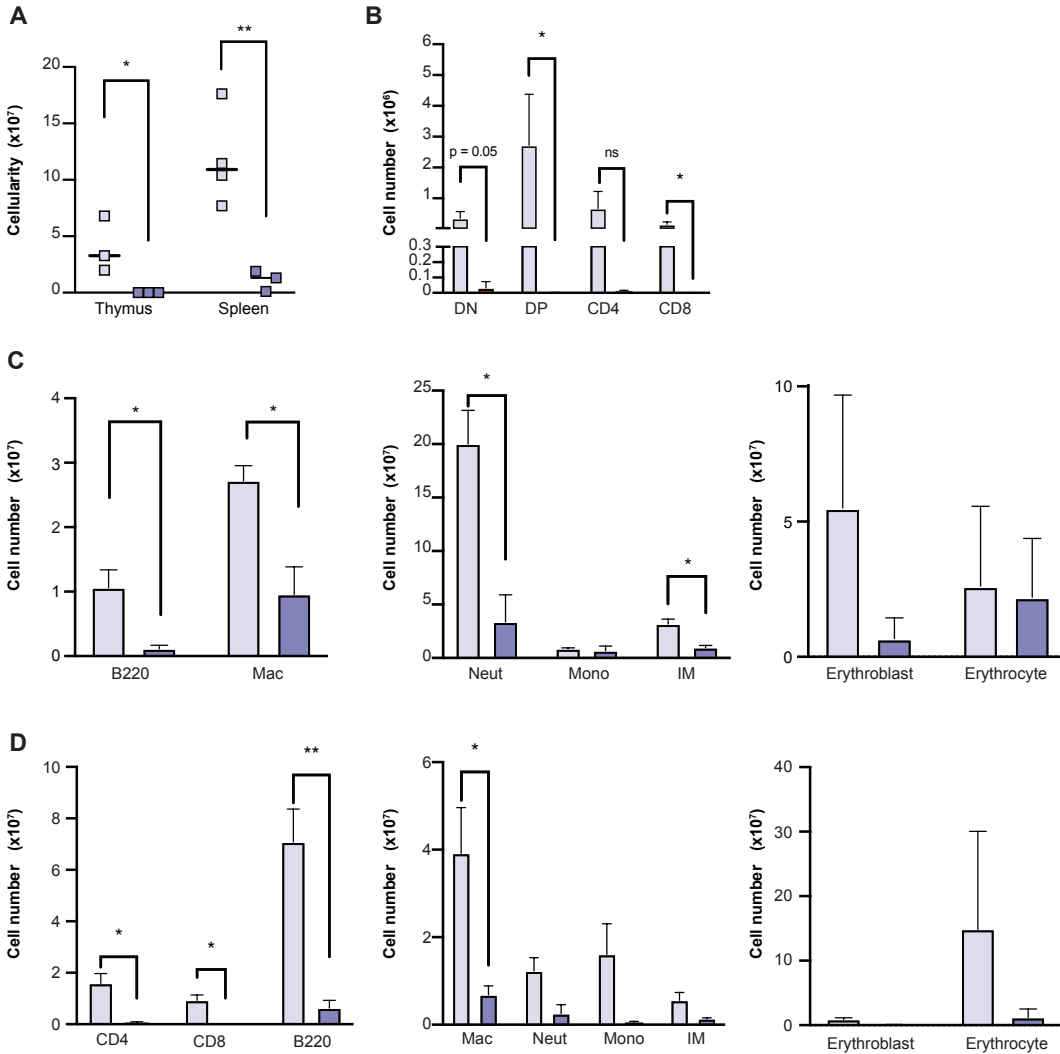


Supplemental Figure 7. *Vavi-Cre Ripk1^{fl/fl} Ifngr1^{-/-}* mice exhibit lymphoid and myeloid lineage loss in all hematopoietic compartments at time of disease

(A) Thymus and spleen cellularity and (B-D) lineage analysis of thymus (B), bone marrow (C), and spleen (D) of *Vavi-Cre Ripk1^{fl/fl} Ifngr1^{-/-}* (n=3) and *Vavi-Cre Ripk1^{fl/fl} Ifngr1^{-/-}* (n=4) mice at time of disease. *Abbreviations:* double negative (DN), CD4 and CD8 double positive (DP), Mac1⁺ myeloid cells (Mac), neutrophils (Neut), monocytes (Mono), and inflammatory monocytes (IM). *p < 0.05, **p < 0.01, ***p < 0.001.

Supplemental Figure 8

○ *Vav-iCre Ripk1^{fl/+} Zbp1^{Za1a2/Za1a2}*
 ● *Vav-iCre Ripk1^{fl/fl} Zbp1^{Za1a2/Za1a2}*



Supplemental Figure 8. *Vav-iCre Ripk1^{fl/fl} Zbp1^{Za1a2/Za1a2}* mice exhibit lymphoid and myeloid lineage loss in all hematopoietic compartments at time of disease.

(A) Thymus and spleen cellularity and (B-D) lineage analysis of thymus (B), bone marrow (C), and spleen (D) of *Vav-iCre Ripk1^{fl/+} Zbp1^{Za1a2/Za1a2}* (n=4) and *Vav-iCre Ripk1^{fl/fl} Zbp1^{Za1a2/Za1a2}* (n=3) mice at time of disease. *Abbreviations:* double negative (DN), CD4 and CD8 double positive (DP), Mac1⁺ myeloid cells (Mac1), neutrophils (Neut), monocytes (Mono), and inflammatory monocytes (IM). *p<0.05, **p<0.01.