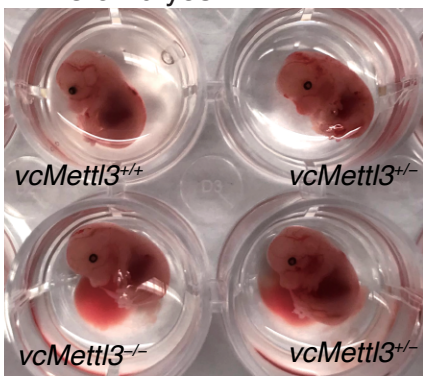
A

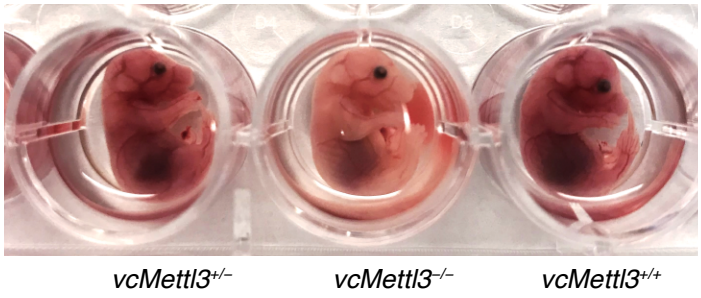
Vav-Cre^{+}-*Mettl3*$^{fl/wt}$ **x** *Vav*-Cre^{-}-*Mettl3*$^{fl/fl}$

Stage	Litters	*vcMettl3*$^{+/+}$	*vcMettl3*$^{+/-}$	*vcMettl3*$^{-/-}$	Expected *vcMettl3*$^{-/-}$
E12.5	3	13	4	7	6
E14.5	11	45	29	31	26.25
E16.5	4	21	6	8	8.75
Birth	3	12	7	4	5.75
P10	6	30	17	0	11.75

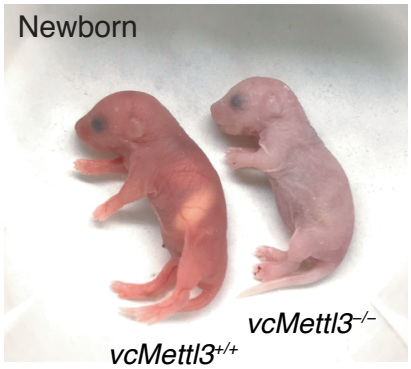
B



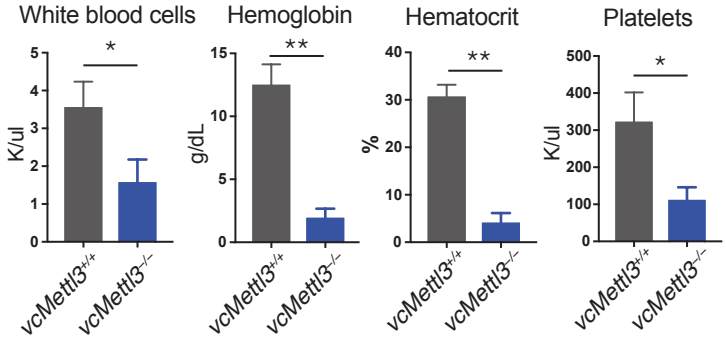
C



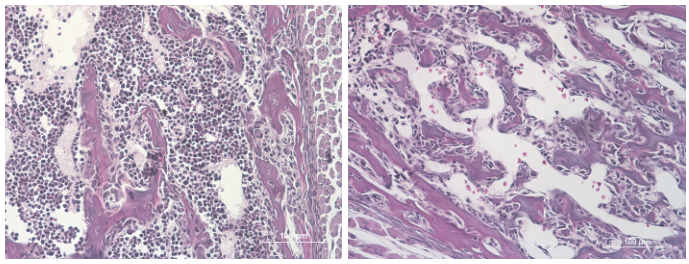
D



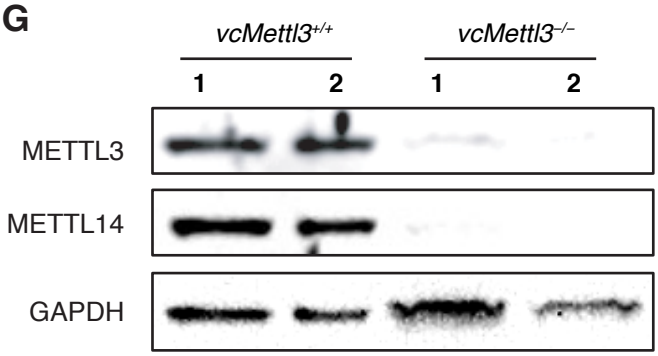
E



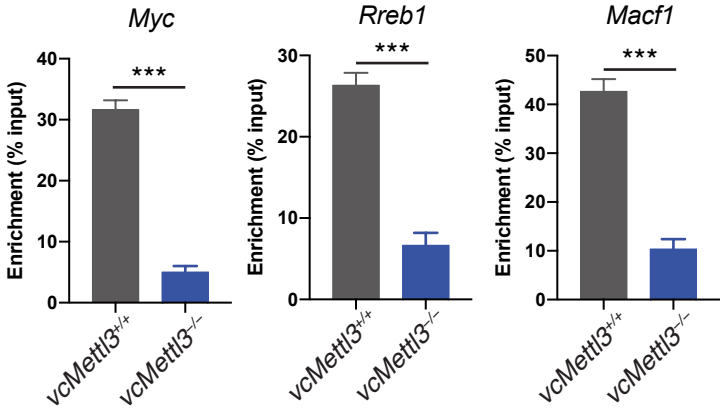
F



G



H



I

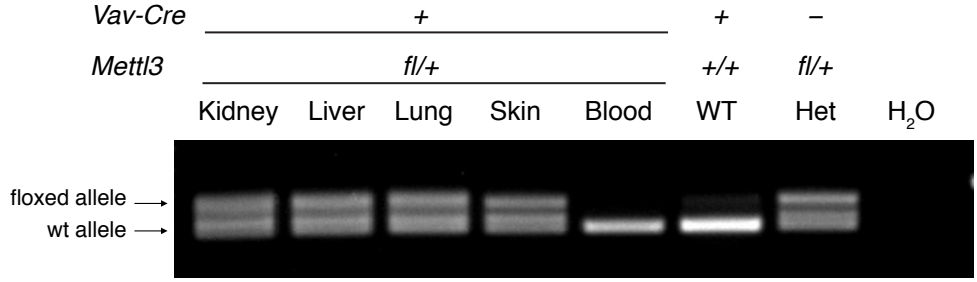


Figure S1. *Vav*-Cre mediated *Mettl3* deletion results in bone marrow failure and early demise after birth, related to Figure 1

(A) Mendelian ratio at the indicated stages. *vcMettl3*$^{-/-}$ were expected to be 1/4 of the total mice.

(B-D) Representative photograph of *vcMettl3*$^{+/+}$ and *vcMettl3*$^{-/-}$ embryos at E14.5 (B), E16.5 (C), and newborns (D).

(E) Peripheral blood counts of *vcMettl3*$^{+/+}$ and *vcMettl3*$^{-/-}$ newborn mice (n=3 biological replicates per group).

(F) Histology of *vcMettl3*$^{+/+}$ and *vcMettl3*$^{-/-}$ bone marrow in femur. H&E. Scale bar 100 μm.

(G) Western blot of METTL3 and METTL14 in *vcMettl3*$^{+/+}$ and *vcMettl3*$^{-/-}$ fetal livers.

(H) Determination of the m^{6}A depletion on m^{6}A enriched transcripts by m^{6}A-RIP-PCR (n=3 biological replicates per group).

(I) Determination of *Vav*-Cre specificity in hematopoietic system by genotyping of different tissues.

Data are represented as mean ± SEM, and representative of at least three independent experiments; The *P* values were calculated using two-tailed Student's *t* test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

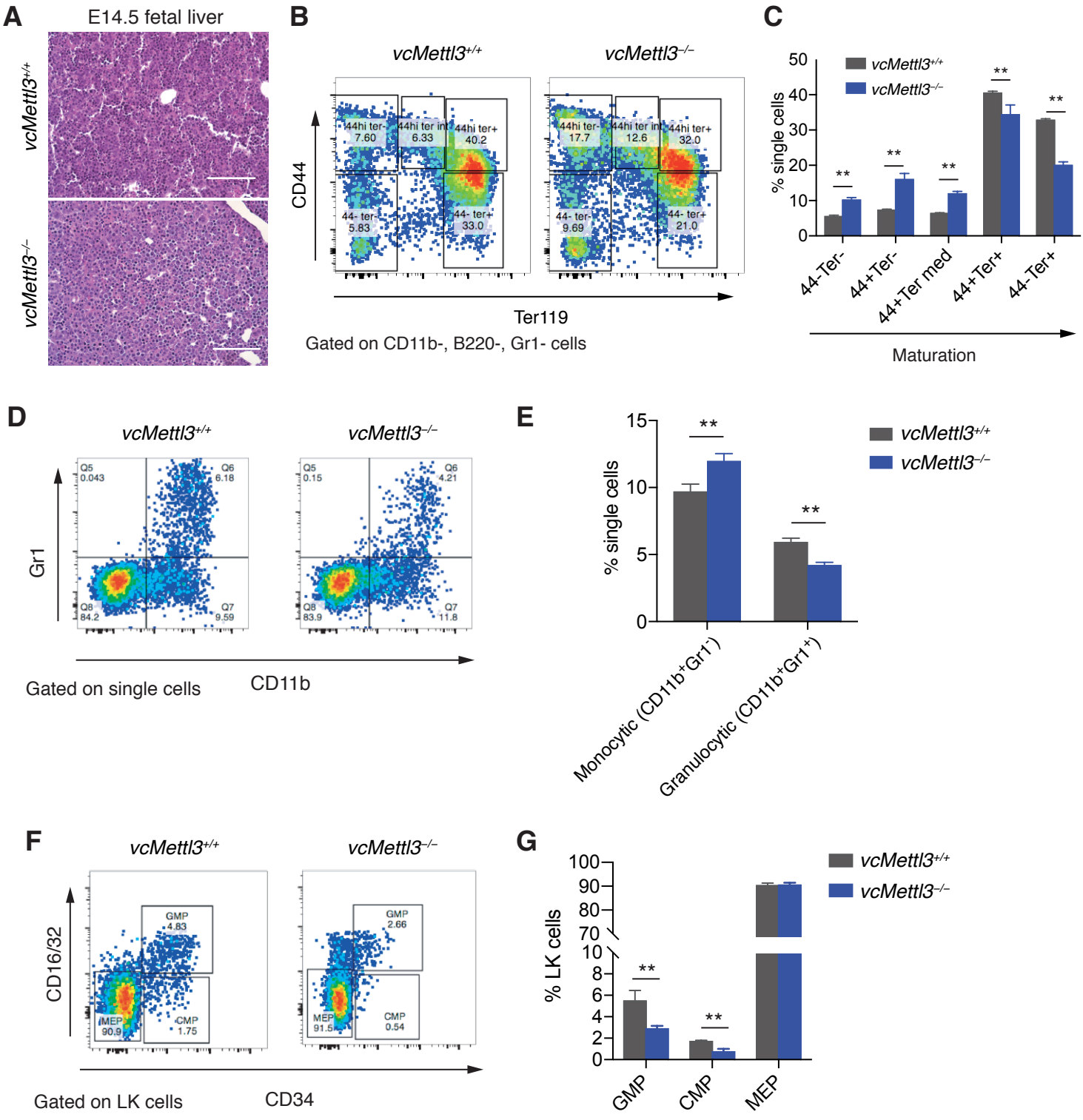

A
E14.5 fetal liver
vcMettl3+/+
vcMettl3−/−
B
vcMettl3+/+
vcMettl3−/−
CD44
Ter119
44hi ter- 7.60
44hi ter int 6.33
44hi ter+ 40.2
44- ter- 5.83
44- ter+ 33.0
44hi ter- 17.7
44hi ter int 12.6
44hi ter+ 32.0
44- ter- 9.69
44- ter+ 21.0
Gated on CD11b-, B220-, Gr1- cells
C
vcMettl3+/+
vcMettl3−/−
% single cells
44-Ter-
44+Ter-
44+Ter med
44+Ter+
44-Ter+
Maturation
D
vcMettl3+/+
vcMettl3−/−
Gr1
CD11b
Q5 0.043
Q6 6.18
Q8 84.2
Q7 9.59
Q5 0.15
Q6 4.21
Q8 83.9
Q7 11.8
Gated on single cells
E
vcMettl3+/+
vcMettl3−/−
% single cells
Monocytic (CD11b+Gr1-)
Granulocytic (CD11b+Gr1+)
F
vcMettl3+/+
vcMettl3−/−
CD16/32
CD34
GMP 4.83
iMEP 90.9
CMP 1.75
GMP 2.66
MEP 91.5
CMP 0.54
Gated on LK cells
G
vcMettl3+/+
vcMettl3−/−
% LK cells
GMP
CMP
MEP

Figure S2. *vcMettl3*$^{-/-}$ fetal liver cells have defective progenitor differentiation and maturation potentials, related to Figure 2

(A) Histology of *vcMettl3*$^{+/+}$ and *vcMettl3*$^{-/-}$ E14.5 fetal livers. Scale bar, 100 μm.

(B, C) Flow-cytometric evaluation of erythroid differentiation in E14.5 fetal livers, quantified in (C) (n=3 biological replicates per group).

(D, E) Flow-cytometric evaluation of myeloid differentiation in E14.5 fetal livers, quantified in (E) (n=3 biological replicates per group).

(F, G) Determination of myeloid progenitor distribution, GMP (CD16/32^{+}CD34^{+} LK), CMP (CD16/32^{-}CD34^{+} LK), and MEP (CD16/32^{-}CD34^{-} LK) in E14.5 fetal livers (n=3 biological replicates per group).

Data are represented as mean ± SEM, representative of at least three independent experiments; The *P* values were calculated using two-way ANOVA. ** $p<0.01$.

Figure S3

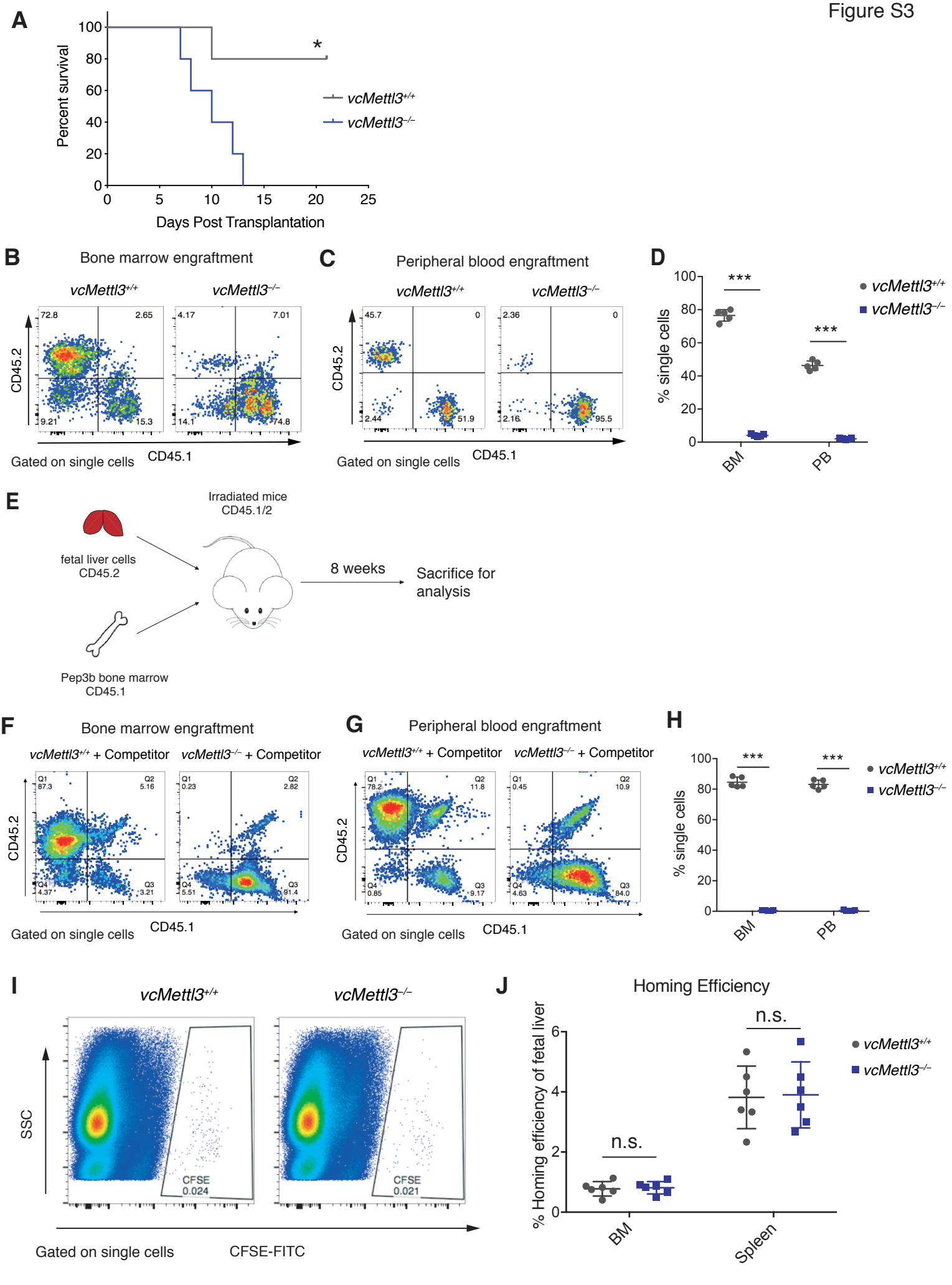

A
Percent survival
Days Post Transplantation
vcMettl3+/+
vcMettl3−/−
B
Bone marrow engraftment
CD45.2
CD45.1
Gated on single cells
C
Peripheral blood engraftment
D
% single cells
BM
PB
E
Irradiated mice
CD45.1/2
fetal liver cells
CD45.2
Pep3b bone marrow
CD45.1
8 weeks
Sacrifice for analysis
F
Bone marrow engraftment
+ Competitor
G
Peripheral blood engraftment
H
I
SSC
CFSE-FITC
CFSE 0.024
CFSE 0.021
J
Homing Efficiency
% Homing efficiency of fetal liver
n.s.
Spleen

Figure S3. *vcMettl3*$^{-/-}$ fetal liver HSPCs fail to repopulate lethally irradiated recipients, related to Figure 2

(A) Kaplan-Meier survival curves of recipient mice transplanted with *vcMettl3*$^{+/+}$ and *vcMettl3*$^{-/-}$ fetal liver cells (n=5 mice per group).

(B-D) Contribution of CD45.2^{+} *vcMettl3*$^{+/+}$ and *vcMettl3*$^{-/-}$ cells in lethally irradiated congenic CD45.1^{+} Pep3b recipient mouse bone marrow (B) and peripheral blood (C) 8 days after transplantation, quantified in (D) (n=5 mice per group).

(E) Scheme of competitive transplantation of *vcMettl3*$^{+/+}$ and *vcMettl3*$^{-/-}$ fetal liver cells against Pep3b bone marrow into lethally irradiated recipient mice.

(F-H) Contribution of CD45.2^{+} *vcMettl3*$^{+/+}$ and *vcMettl3*$^{-/-}$ fetal liver cells in competition with CD45.1^{+} bone marrow cells in lethally irradiated congenic CD45.1/2 recipient mouse bone marrow (F) and peripheral blood (G), quantified in (H) (n=5 mice per group).

(I, J) Flow-cytometric detection of homing of CFSE labeled fetal liver hematopoietic cells in recipient bone marrow (I, J) and spleen (J) (n=6 mice per group).

Data are represented as mean ± SEM, and representative of at least three independent experiments; The *P* value in (A) was calculated using log-rank test. *$p < 0.05$. The *P* values in (D), (H) and (J) were calculated using two-tailed Student's t test; n.s., not statistically significant; *** $p<0.001$.

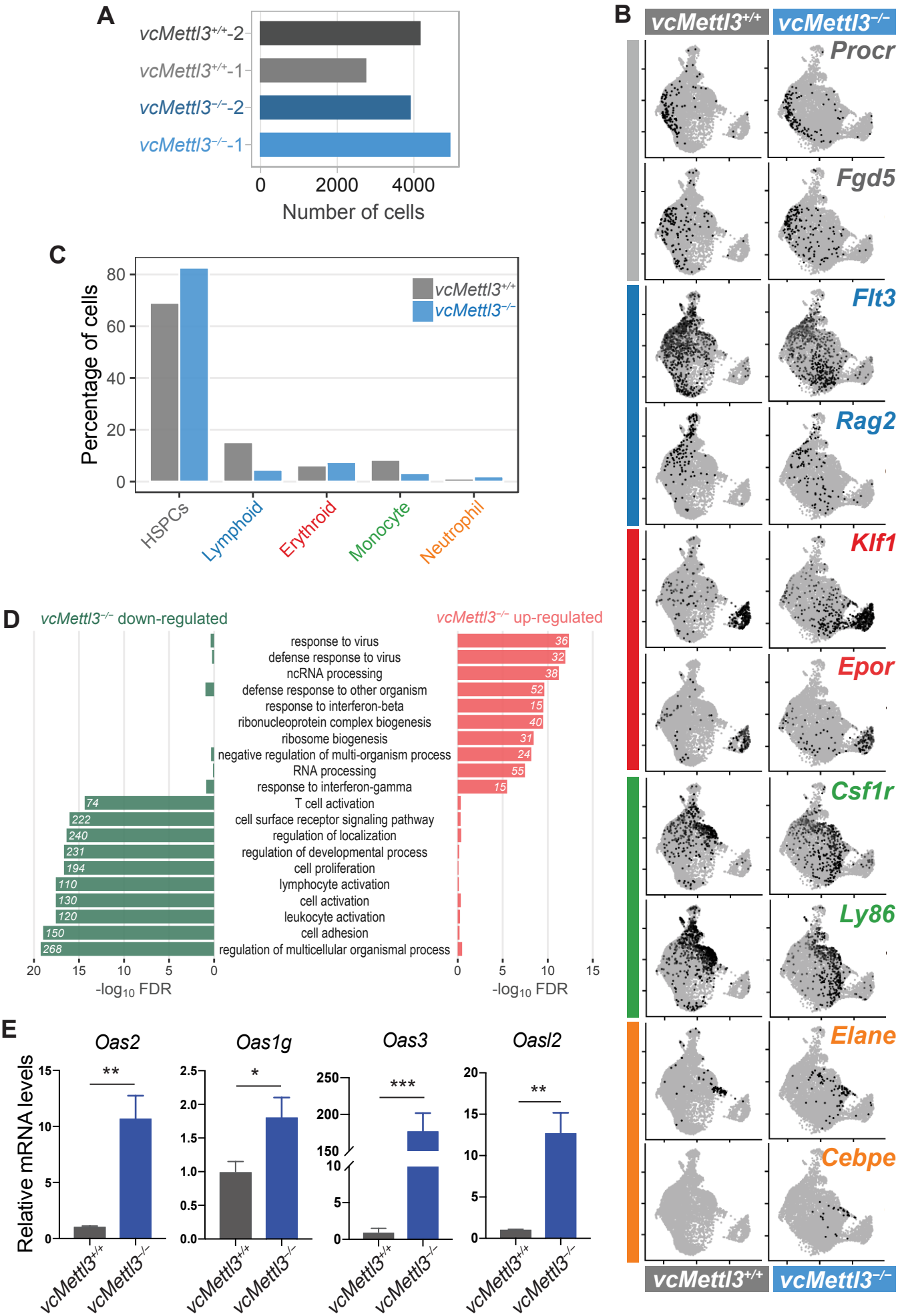

A
vcMettl3+/+-2
vcMettl3+/+-1
vcMettl3−/−-2
vcMettl3−/−-1
0
2000
4000
Number of cells
B
vcMettl3+/+
vcMettl3−/−
Procr
Fgd5
Flt3
Rag2
Klf1
Epor
Csf1r
Ly86
Elane
Cebpe
C
Percentage of cells
HSPCs
Lymphoid
Erythroid
Monocyte
Neutrophil
D
vcMettl3−/− down-regulated
vcMettl3−/− up-regulated
response to virus
defense response to virus
ncRNA processing
defense response to other organism
response to interferon-beta
ribonucleoprotein complex biogenesis
ribosome biogenesis
negative regulation of multi-organism process
RNA processing
response to interferon-gamma
T cell activation
cell surface receptor signaling pathway
regulation of localization
regulation of developmental process
cell proliferation
lymphocyte activation
cell activation
leukocyte activation
cell adhesion
regulation of multicellular organismal process
-log10 FDR
E
Oas2
Oas1g
Oas3
Oasl2
Relative mRNA levels

Figure S4. Gene expression changes upon *Mettl3* deletion in fetal liver LSK cells, related to Figure 3

(A) Number of unique LSK cells with more than 500 quantified genes, used for further single cell RNA-Seq (scRNA-seq) analysis. scRNA-Seq was performed in duplicate.

(B) Expression of marker genes used for the assignation of cell clusters to hematopoietic populations and differentiation entry points: HSPCs (grey), lymphoid (blue), erythroid (red), monocyte (green), neutrophil (orange). Marker expression is plotted on the UMAP representation of *vcMettl3*$^{+/+}$ and *vcMettl3*$^{-/-}$ LSK cells (light grey: low expression, black: maximum expression). Two markers for each clusters are shown.

(C) Percentage of *vcMettl3*$^{+/+}$ and *vcMettl3*$^{-/-}$ LSK cells composing each cluster identified by scRNA-Seq.

(D) Gene ontology (GO) enrichment analysis of genes significantly upregulated or significantly down-regulated in bulk RNA-seq of *vcMettl3*$^{-/-}$ LSK compared to *vcMettl3*$^{+/+}$ LSK cells. The number of differentially expressed genes within each category is displayed.

(E) Q-RT-PCR determination of expression of members of the OAS family of genes in *vcMettl3*$^{-/-}$ versus *vcMettl3*$^{+/+}$ E14.5 fetal liver cells (n=3 biological replicates per group). Data are representative of three independent experiments.

Data are represented as mean ± SEM; The *P* value was calculated using two-tailed Student's *t* test. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Figure S5

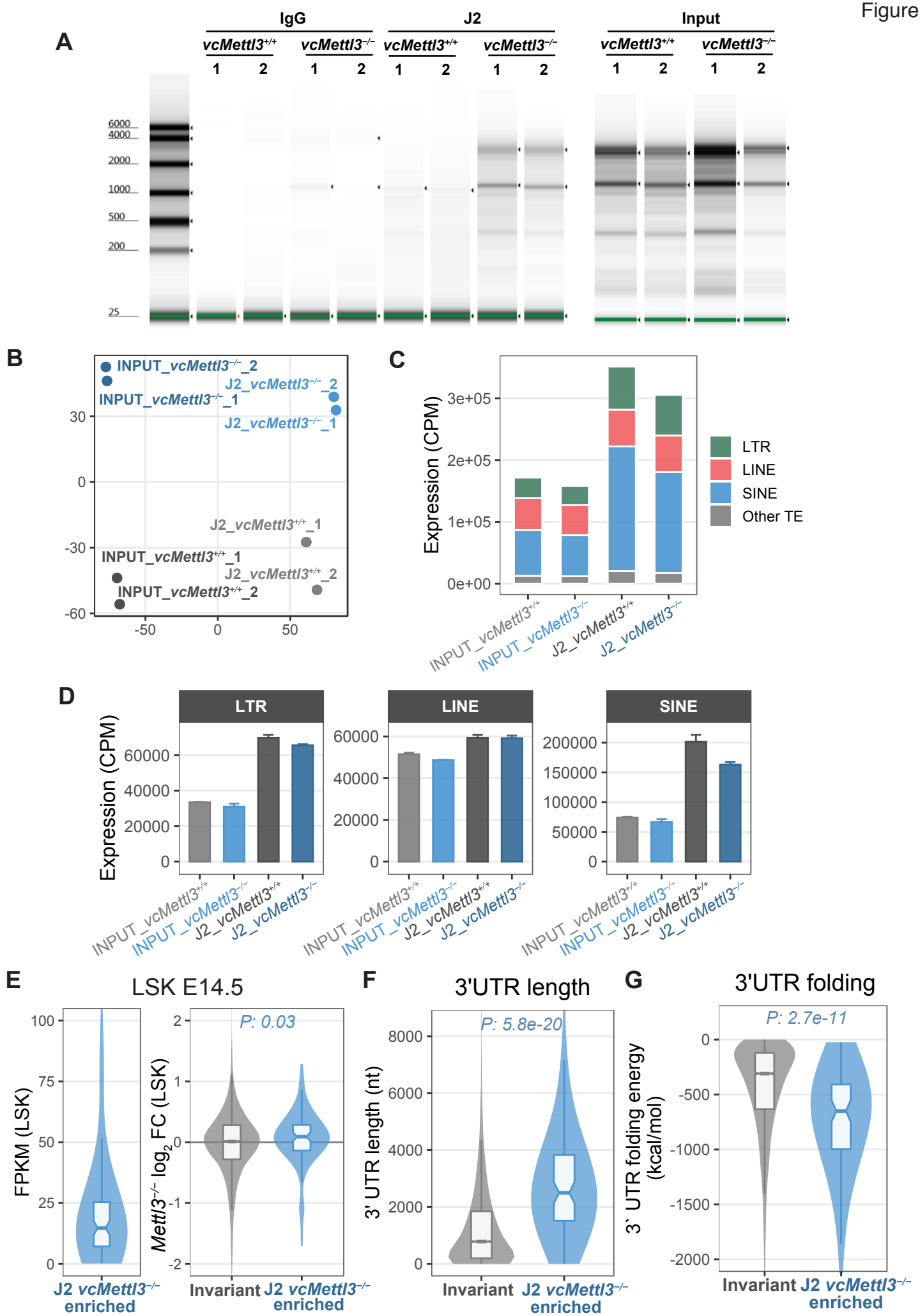

A
IgG
J2
Input
vcMettl3+/+
vcMettl3−/−
1
2
6000
4000
2000
1000
500
200
25
B
INPUT_vcMettl3−/−_2
J2_vcMettl3−/−_2
INPUT_vcMettl3−/−_1
J2_vcMettl3−/−_1
J2_vcMettl3+/+_1
INPUT_vcMettl3+/+_1
J2_vcMettl3+/+_2
INPUT_vcMettl3+/+_2
30
0
-30
-60
-50
0
50
C
Expression (CPM)
3e+05
2e+05
1e+05
0e+00
LTR
LINE
SINE
Other TE
INPUT_vcMettl3+/+
INPUT_vcMettl3−/−
J2_vcMettl3+/+
J2_vcMettl3−/−
D
LTR
LINE
SINE
Expression (CPM)
60000
40000
20000
0
200000
150000
100000
50000
E
LSK E14.5
FPKM (LSK)
100
75
50
25
0
Mettl3−/− log2FC (LSK)
2
1
0
-1
-2
P: 0.03
J2 vcMettl3−/− enriched
Invariant
J2 vcMettl3−/− enriched
F
3'UTR length
3' UTR length (nt)
8000
6000
4000
2000
0
P: 5.8e-20
Invariant
J2 vcMettl3−/− enriched
G
3'UTR folding
3` UTR folding energy (kcal/mol)
0
-500
-1000
-1500
-2000
P: 2.7e-11
Invariant
J2 vcMettl3−/− enriched

Figure S5. J2-RIP specifically isolates dsRNAs in fetal livers, related to Figure 5

(A) RNA chip analysis of J2-RIP, IgG-RIP and input RNA of *vcMettl3*$^{+/+}$ and *vcMettl3*$^{-/-}$ fetal livers.

(B) Multidimensional Scaling plot of samples from the J2-RIP-Seq experiment (*vcMettl3*$^{+/+}$ and *vcMettl3*$^{-/-}$, J2 and INPUT), based on gene-specific signals.

(C) Average expression levels of transposable element classes in *vcMettl3*$^{+/+}$ and *vcMettl3*$^{-/-}$ fetal livers in J2-RIP and INPUT samples. CPM: Counts Per Million. LTR: Long Terminal Repeat. LINE: Long INterspersed Elements. SINE: Short INterspersed Elements.

(D) Expression levels of specific retrotransposon classes (LTR, LINE, SINE) in J2-RIP samples (n=2 biological replicates per group). CPM: Counts Per Million.

(E) Distribution of the expression levels (left) and *vcMettl3*$^{-/-}$ fold changes (right) of *vcMettl3*$^{-/-}$ J2-RIP enriched genes in LSK cells. FPKM: Fragments Per Kilobase of transcript per Million.

(F) Distribution of the 3'UTR length of *vcMettl3*$^{-/-}$ J2-RIP enriched genes compared with invariant genes.

(G) Distribution of the predicted 3'UTR folding energy of *vcMettl3*$^{-/-}$ J2-RIP enriched genes compared with invariant genes.

The *P* values in (E, F, G) were calculated using two-tailed Wilcoxon rank-sum test.

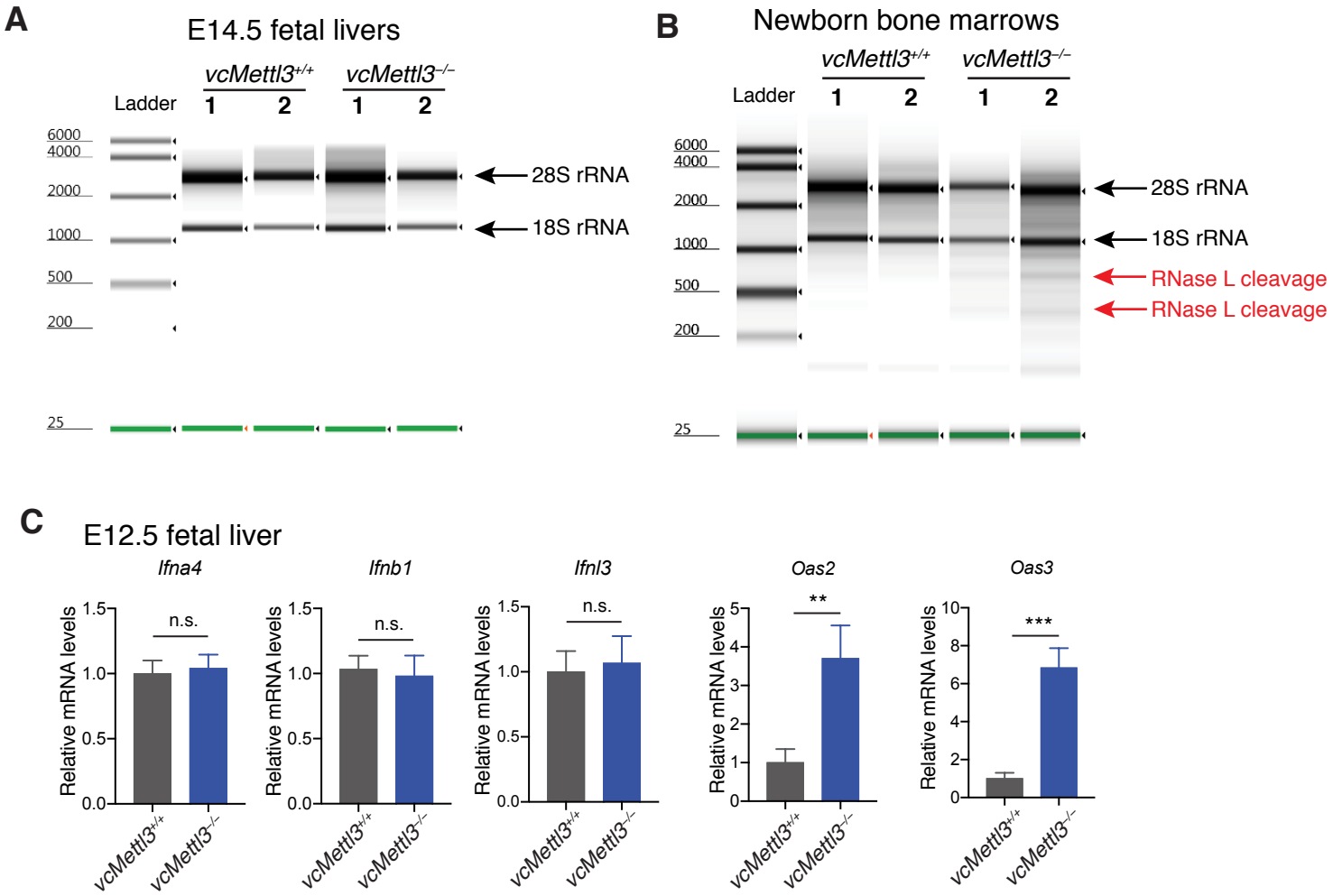

A
E14.5 fetal livers
vcMettl3+/+
vcMettl3−/−
Ladder
1
2
1
2
6000
4000
2000
1000
500
200
25
28S rRNA
18S rRNA
B
Newborn bone marrows
vcMettl3+/+
vcMettl3−/−
Ladder
1
2
1
2
6000
4000
2000
1000
500
200
25
28S rRNA
18S rRNA
RNase L cleavage
RNase L cleavage
C
E12.5 fetal liver
Ifna4
Ifnb1
Ifnl3
Oas2
Oas3
Relative mRNA levels
n.s.
n.s.
n.s.
**
***
vcMettl3+/+
vcMettl3−/−

Figure S6. Innate immune response is activated in *vcMettl3*$^{-/-}$ fetal livers, related to Figure 6

(A) RNA chip analysis for cleavage of ribosomal RNA (rRNA) by RNase L in E14.5 fetal livers.

(B) RNA chip analysis for cleavage of rRNA by RNase L in bone marrows of newborn mice.

(C) Determination of IFN and OAS family expression levels at E12.5 fetal livers (n=3 biological replicates per group).

Data are represented as mean ± SEM, and representative of three independent experiments; The *P* values were calculated using two-tailed Student's *t* test. n.s. not statistically significant, ** $p<0.01$, *** $p<0.001$.

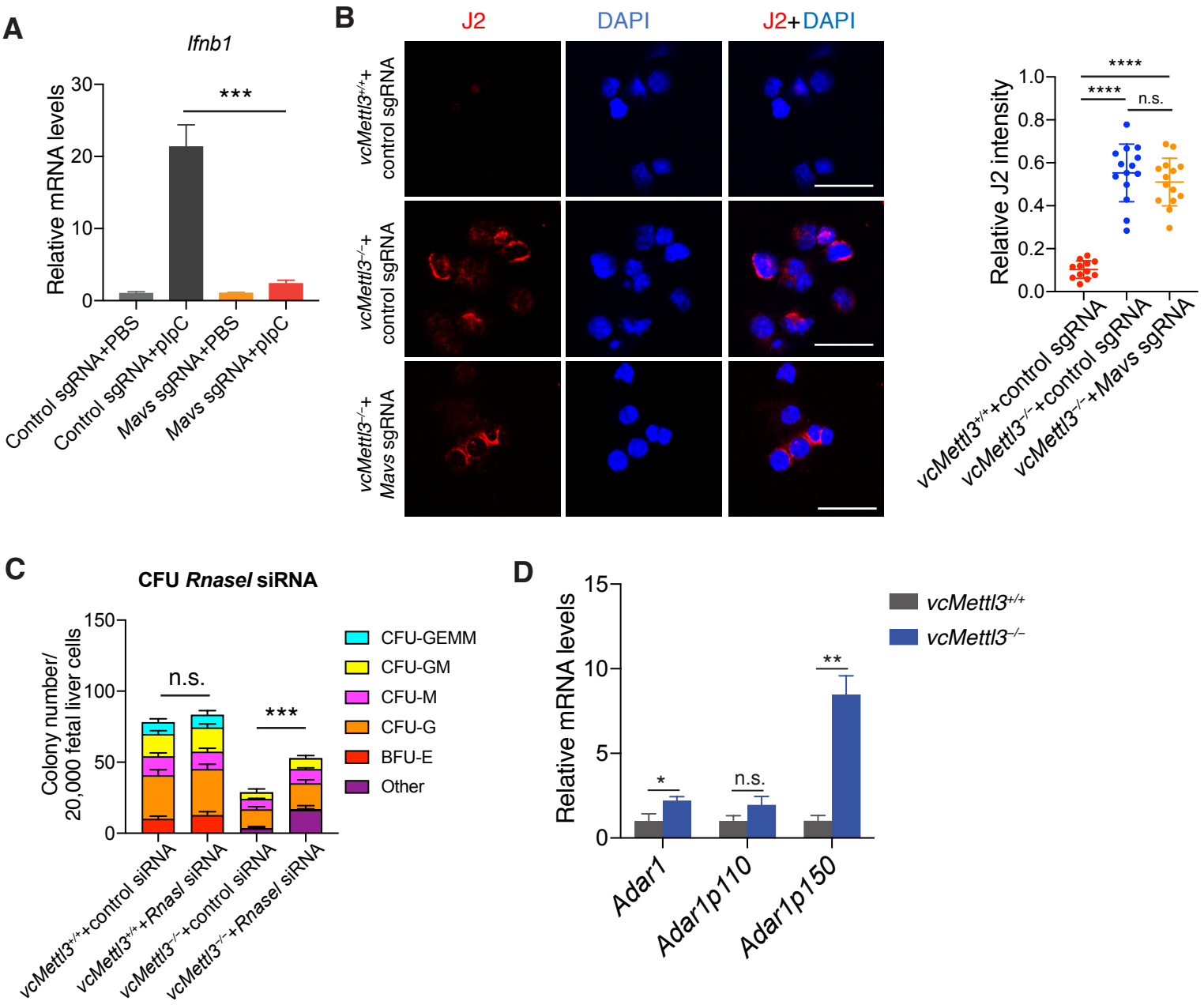

A
Ifnb1
Relative mRNA levels
30
20
10
0
***
Control sgRNA+PBS
Control sgRNA+plpC
Mavs sgRNA+PBS
Mavs sgRNA+plpC
B
J2
DAPI
J2+DAPI
vcMettl3+/+ + control sgRNA
vcMettl3−/− + control sgRNA
vcMettl3−/− + Mavs sgRNA
Relative J2 intensity
1.0
0.8
0.6
0.4
0.2
0.0
****
****
n.s.
vcMettl3+/+ +control sgRNA
vcMettl3−/− +control sgRNA
vcMettl3−/− +Mavs sgRNA
C
CFU Rnasel siRNA
Colony number/ 20,000 fetal liver cells
150
100
50
0
n.s.
***
CFU-GEMM
CFU-GM
CFU-M
CFU-G
BFU-E
Other
vcMettl3+/+ +control siRNA
vcMettl3+/+ +Rnasl siRNA
vcMettl3−/− +control siRNA
vcMettl3−/− +Rnasel siRNA
D
Relative mRNA levels
15
10
5
0
vcMettl3+/+
vcMettl3−/−
*
n.s.
**
Adar1
Adar1p110
Adar1p150
E
E12.5 Myc
E14.5 Myc
Newborn Myc
Relative mRNA levels
n.s.
*
*
vcMettl3+/+
vcMettl3−/−

Figure S7. Inhibition of innate immune response partially rescues the hematopoietic failure caused by *Mettl3* deletion, related to Figure 7

(A) Determination of *Ifnb1* expression in *vcMettl3*$^{-/-}$ cells after deletion of *Mavs* under the treatment of pIpC (n=3 biological replicates per group).

(B) Measurement of dsRNA in lineage depleted fetal liver cells with deletion of *Mavs* by J2 immunofluorescent staining (*vcMettl3*$^{+/+}$+control sgRNA n=12, *vcMettl3*$^{-/-}$+control sgRNA n=14, *vcMettl3*$^{-/-}$+*Mavs* sgRNA n=14).

(C) Colony formation unit (CFU) of E14.5 fetal liver cells transfected with control siRNA or *Rnasel* siRNA (n=3 biological replicates per group).

(D) Time course of *c-Myc* expression levels in fetal livers at E12.5, E14.5 and bone marrows of newborn mice (n=3 biological replicates per group).

(E) Determination of the expression levels of *Adar1* and its isoforms in fetal livers by Q-RT-PCR (n=3 biological replicates per group).

Data are represented as mean ± SEM, and representative of three independent experiments; The *P* values were calculated using two-tailed Student's *t* test. n.s. not statistically significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.