Supplementary Methods

Mice

Mettl3 floxed mice, *Vec-Cre* mice and *Vav-Cre* mice (gifts from B. Liu, Academy of Military Medical Sciences, Beijing, China) were generated as previously described [1, 2]. All mice were C57BL/6 genetic background and were bred under specific-pathogen free conditions. *Vec-Cre*; *Mettl3*$^{fl/+}$and *Vav-Cre*; *Mettl3*$^{fl/+}$ mice mated with *Mettl3*$^{fl/fl}$ mice, respectively, were utilized to obtain *Vec-Cre*; *Mettl3*$^{fl/fl}$ and *Vav-Cre*; *Mettl3*$^{fl/fl}$ embryos. The number of somite pairs (sp) of embryos was counted and the E10.5 embryos with 35-40 sp were chosen in this study. In the following transplantation experiments, the embryos we used were the late E10.5 embryos with 39-40 sp. This study was approved by the Ethical Review Committee of the Institute of Zoology, Chinese Academy of Sciences, China.

Immunofluorescence

The E10.5 embryo was fixed with 4% paraformaldehyde in PBS overnight at 4℃. After gradient dehydrating, the embryo was embedded in paraffin and sectioned to 5 μm thickness slides. The antigen-retrieved slides were blocked with 5% BSA containing 0.3% Triton X-100 in PBS for 2 h at room temperature. After washing in PBS three times, the slides were incubated with primary antibodies diluted in 1% BSA overnight at 4℃. After washing in PBST (containing 0.1% Tween-20) three times, the slides were then incubated with secondary antibodies diluted in 1% BSA for 2 h at room temperature. The cell nuclei of slides were stained with DAPI and the immunofluorescence images were obtained by a laser scanning confocal microscope (A1; Nikon). The antibodies used in the assays include: anti-Mettl3 antibody (Abnova, H00056339-B01P), anti-Runx1 antibody (abcam, ab92336), anti-CD31 antibody (abcam, ab56299), anti-Notch1 antibody (abcam, ab65297), anti-Dll4 (abcam, ab7280), anti-rabbit-Ig-fluorescein (Life Technologies, A11037), anti-rat-Ig-fluorescein (Life Technologies, A11006) and anti-mouse-Ig-fluorescein (Life Technologies, A11001).

Western blotting

The E10.5 AGM protein was extracted with RIPA lysis buffer. The procedure was performed

as previously reported [3]. The following antibodies were used to detect the protein levels: anti-Mettl3 antibody (abcam, ab98009) and anti-Dll4 antibody (abcam, ab7280).

qPCR

The total RNA of AGM region was extracted using TRIzol reagent (TIANGEN, DP405). The cDNA template of qPCR was obtained by M-MLV reverse transcription. The qPCR experiment was performed using Bio-Rad CFX96 detection systems. The housekeeping gene *Gapdh* was used as internal control. Three independent technique replicates were analyzed. The sequences of qPCR primers used in the study are listed in Table S1.

m^{6}A-RIP-qPCR

The m^{6}A immunoprecipitation procedure was performed according to the previously published protocol [4]. The level of m^{6}A enrichment was assessed by qPCR. The sequences of primers are listed in Table S1.

Colony-forming unit culture assay

The E10.5 AGM was dispersed into single-cell suspension by collagenase treatment and then cultivated in M3434 medium (Stem Cell Technologies, 03434). After cultured under 5% CO_2 at 37℃ for 7 d. The count of each type of colony was based on the colony morphology. More than three groups of independent experiments were analyzed.

Flow cytometry analysis

Single-cell suspension from the E10.5 AGM was dissociated with collagenase treatment and stained with antibodies for 30 mins at 4℃. Flow cytometry analysis was performed using MoFlo XDP (Beckman Coulter). Two parameters, FSC and SSC, were used to enrich the living and active cells, and the agminate cell populations were gated to analyze the proportion of target cells. The antibodies used in this study include: CD31-PE (BD, 561073), CD144-PE (eBioscience, 12-1441-80), c-Kit-APC (eBioscience, 17-1171-81), CD45-FITC (eBioscience, 11-0451-81), CD34-PE (BD, 551387), CD45.1-APC (eBioscience, 17-0453-82), and CD45.2-PE-Cy7 (eBioscience, 25-0454-80).

Transplantation

The late E10.5 (39-40 sp) embryos were transferred into medium at 37℃ to maintain their robust activity and the tail tissues were obtained from the embryos for genotyping. Then the CD45.1 recipients received a split lethal dose (9 Gy) of X-Ray irradiation, and bone marrow (BM) cells were obtained from CD45.1 mice. When these preparations had been done, the AGM tissues were separated from donor embryos and dispersed into single-cell suspensions. Next, the single-cell suspensions were mixed with 2×10^6 BM cells and intravenously injected into the tail of recipients. The chimerism of survived recipient was analyzed using peripheral blood (PB).

mRNA purification and RNA sequencing

Total RNAs from control and *Vec-Cre*; *Mettl3*$^{fl/fl}$ AGM region of embryos at E10.5 were extracted with TRIzol (TIANGEN, DP405). cDNA libraries were constructed by using KAPA Stranded mRNA-Seq Kit (KAPA) as manufacturer's instructions. Sequencing was performed on Illumina HiSeq2500 with paired end 150-bp read length.

Sequencing data analysis

For RNA-seq, adaptor sequences were trimmed off for all raw reads using the Cutadapt software (version 1.2.1) [5]. Reads that were less than 35 nt in length or contained an ambiguous nucleotide were discarded by Trimmomatic (version 0.32) [6]. The remaining reads were aligned to the mouse genome (version mm10) using HISAT2 (version 2.0.1) [7]. Only uniquely-mapped reads with mapping quality score ≥ 20 were kept for the subsequent analysis for each sample. The number of reads mapped to each Ensembl gene (release 69) was counted using the HTSeq python package [8], with the 'union' overlap resolution mode and unstranded count feature by setting '--mode=union' and '--stranded=rev', respectively. The expression of transcripts was quantified as reads per kilobase of exon model per million mapped reads (RPKM).

Statistical analysis of differentially expressed genes

Differentially-expressed genes between control and *Vec-Cre*; *Mettl3*$^{fl/fl}$ embryos were determined using the R-package DEGseq with the method MARS (MA-plot-based method with random sampling model), fold change cutoff = 1.2, p value cutoff = 1×10^{-3} and RPKM $\geq$ 1 for each sample [9].

KEGG pathway and GSEA analysis

KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway analysis of genes with significantly differential expression changes was performed using DAVID (http://david.abcc.ncifcrf.gov/). Pathways with $p < 0.05$ were determined to be statistically significant. And the expression levels of total genes involved in Notch signaling pathway (annotated as mmu04330 in KEGG database) were analyzed using Gene-Set Enrichment Analysis (GSEA) [10, 11].

m^{6}A peak and Ythdf2 cluster

m^{6}A peak data was obtained from Gene Expression Omnibus (GEO) with accession number GSE61995 [12]. Ythdf2 cluster identified by PAR-CLIP-seq in human was derived from GSE49339 [13] and converted to mouse genome by coordinates and annotation files between assemblies using online toolkit LiftOver. (https://www.genome.ucsc.edu/cgi-bin/hgLiftOver).

Data availability statement

The RNA-seq data was deposited in the Genome Sequence Archive [14] in BIG Data Center [15], Beijing Institute of Genomics (BIG), Chinese Academy of Sciences, under accession number PRJCA000601. All other data generated or analyzed during this study were included in the article and Supplementary information.

Statistical analyses

All experiments were performed at least three times. The data were shown as the mean $\pm$ standard error of the mean (SEM). Student's t-test was used for statistical comparisons, p

values were used to examine significance.

Supplementary Table 1. qPCR primers list used in this work.

Primer name	Primer sequence (5' to 3')	Length (bp)
Gapdh F	TCCCACTCTTCCACCTTCGATGC	247
Gapdh R	GGGTCTGGGATGGAAATTGTGAGG	
Runx1 F	GCCTCTCTGCAGAACTTTCC	88
Runx1 R	GACGGCAGAGTAGGGAACTG	
Gfi1 F	TCCGAGTTCGAGGACTTTTG	122
Gfi1 R	CATGCATAGGGCTTGAAAGG	
Notch1 F	CCGTGTAAGAATGCTGGAACG	254
Notch1 R	AGCGACAGATGTATGAAGACTCA	
Hey1 F	GCGCGGACGAGAATGGAAA	190
Hey1 R	TCAGGTGATCCACAGTCATCTG	
Hey2 F	AAGCGCCCTTGTGAGGAAAC	104
Hey2 R	GGTAGTTGTCGGTGAATTGGAC	
Hes1 F	CCAGCCAGTGTCAACACGA	126
Hes1 R	AATGCCGGGAGCTATCTTTCT	
Hes5 F	AGTCCCAAGGAGAAAAACCGA	141
Hes5 R	GCTGTGTTTCAGGTAGCTGAC	
Dll4 F	GCACCTTTGGCAATGTCT	105
Dll4 R	GCTTGGATGTTGAGTGAGA	
Mettl3 F	TGATTGAGGTAAAGCGAGGTC	310
Mettl3 R	TCCTGACTGACCTTCTTGCTC	
Notch1 m^{6}A-RIP F	GCATCCCTCCAAACAGCATC	185
Notch1 m^{6}A-RIP R	TCCACCCACATTCCAGCAC	

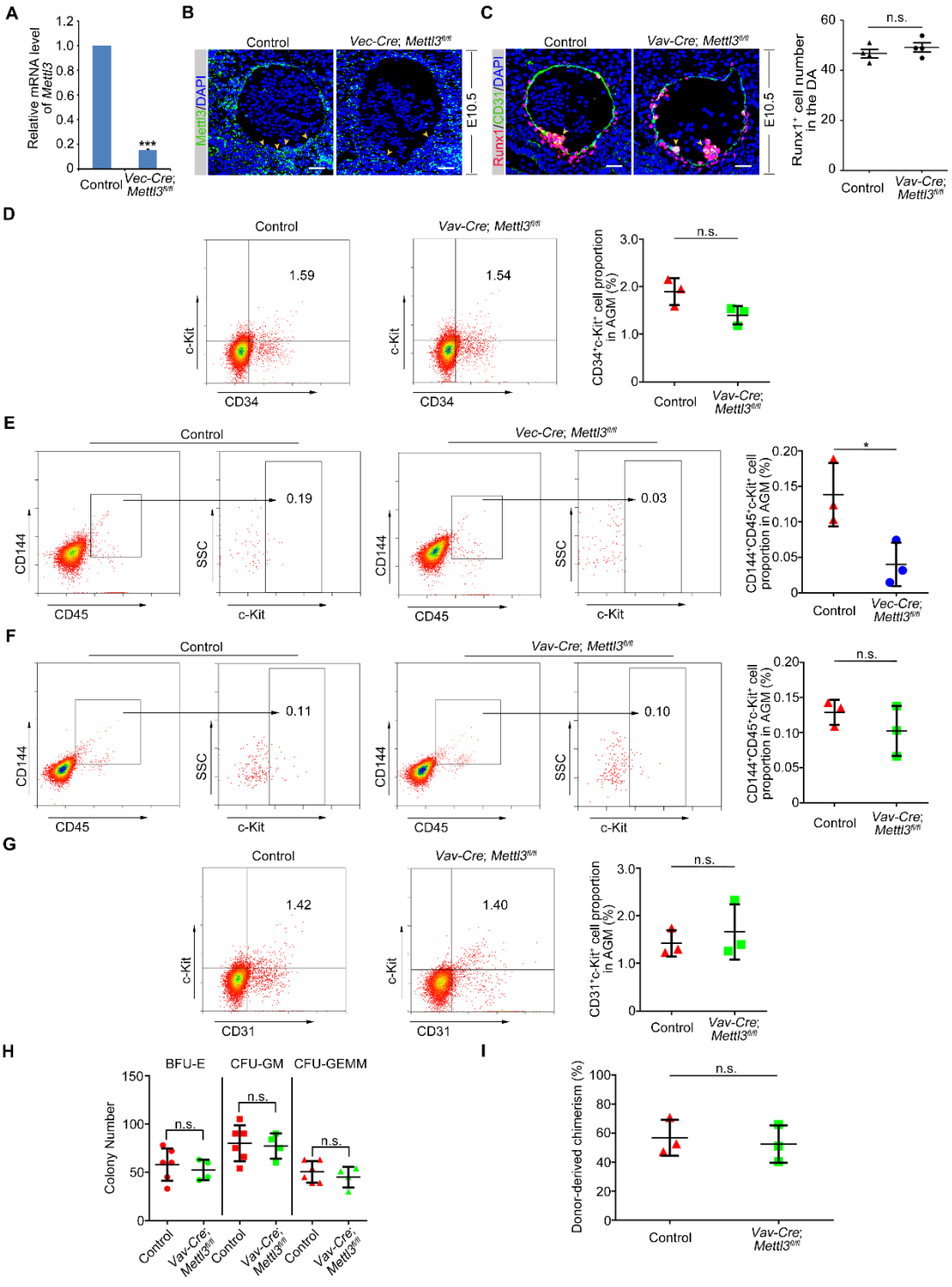
A
Relative mRNA level of Mettl3
1.2
1.0
0.8
0.6
0.4
0.2
0
***
Control
Vec-Cre; Mettl3fl/fl
B
Control
Vec-Cre; Mettl3fl/fl
Mettl3/DAPI
E10.5
C
Control
Vav-Cre; Mettl3fl/fl
Runx1/CD31/DAPI
E10.5
Runx1+ cell number in the DA
60
40
20
0
n.s.
Control
Vav-Cre; Mettl3fl/fl
D
Control
Vav-Cre; Mettl3fl/fl
1.59
1.54
c-Kit
CD34
CD34+c-Kit+ cell proportion in AGM (%)
3.0
2.0
1.0
0
n.s.
Control
Vav-Cre; Mettl3fl/fl
E
Control
Vec-Cre; Mettl3fl/fl
0.19
0.03
CD144
CD45
SSC
c-Kit
CD144+CD45-c-Kit+ cell proportion in AGM (%)
0.20
0.15
0.10
0.05
0
*
Control
Vec-Cre; Mettl3fl/fl
F
Control
Vav-Cre; Mettl3fl/fl
0.11
0.10
CD144
CD45
SSC
c-Kit
CD144+CD45-c-Kit+ cell proportion in AGM (%)
0.20
0.15
0.10
0.05
0
n.s.
Control
Vav-Cre; Mettl3fl/fl
G
Control
Vav-Cre; Mettl3fl/fl
1.42
1.40
c-Kit
CD31
CD31+c-Kit+ cell proportion in AGM (%)
3.0
2.0
1.0
0
n.s.
Control
Vav-Cre; Mettl3fl/fl
H
BFU-E
CFU-GM
CFU-GEMM
Colony Number
150
100
50
0
n.s.
n.s.
n.s.
Control
Vav-Cre; Mettl3fl/fl
Control
Vav-Cre; Mettl3fl/fl
Control
Vav-Cre; Mettl3fl/fl
I
Donor-derived chimerism (%)
100
80
60
40
20
0
n.s.
Control
Vav-Cre; Mettl3fl/fl

Figure S1. The function of HSPC is relatively normal in E10.5 *Vav-Cre*; *Mettl3*$^{fl/fl}$ embryos.

(A, B) mRNA and protein levels of Mettl3 in the E10.5 control and *Vec-Cre*; *Mettl3*$^{fl/fl}$ AGM. (C) Immunofluorescence on the sections of control and *Vav-Cre*; *Mettl3*$^{fl/fl}$ AGM at E10.5 with anti-Runx1 and anti-CD31 antibodies. Yellow arrowheads mark hematopoietic clusters. The right panel is the quantification of Runx1 positive cells in the DA. (D) Flow cytometry analysis of the percentage of HSPCs (CD34^{+}c-Kit^{+}) in the E10.5 *Vav-Cre*; *Mettl3*$^{fl/fl}$ AGM compared with the control. (E, F) Flow cytometry analysis of the percentage of pre-HSCs (CD144^{+}CD45^{+}c-Kit^{+}) in the E10.5 *Vec-Cre*; *Mettl3*$^{fl/fl}$ and *Vav-Cre*; *Mettl3*$^{fl/fl}$ AGM compared with the control. (G) Flow cytometry analysis of the percentage of HE (CD31^{+}c-Kit^{+}) cells in the E10.5 *Vav-Cre*; *Mettl3*$^{fl/fl}$ AGM compared with the control. (H) *In vitro* colony-forming unit culture assay to detect the colony-forming ability of HSPCs in the E10.5 control and *Vav-Cre*; *Mettl3*$^{fl/fl}$ AGM. (I) Donor-derived chimerism in PB of recipients 4 weeks post transplantation (n=3 for each group). Student's t-test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. n.s., not significant. Scale bar, 10 μm. The statistical results are presented as mean ± SEM.

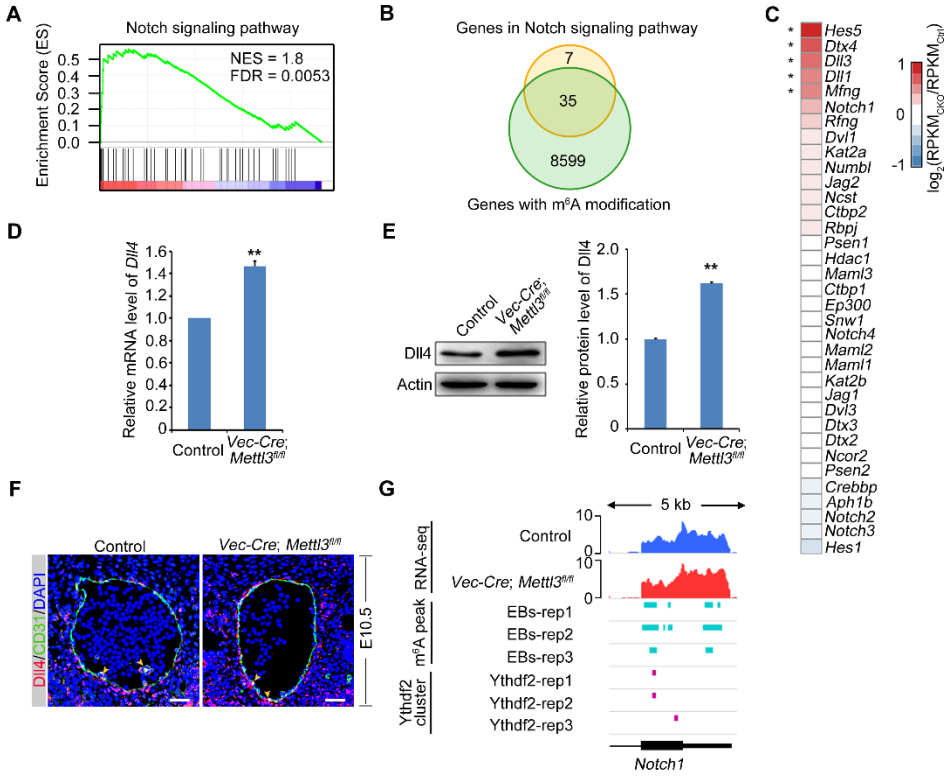
A
Notch signaling pathway
Enrichment Score (ES)
NES = 1.8
FDR = 0.0053
B
Genes in Notch signaling pathway
7
35
8599
Genes with m6A modification
C
Hes5
Dtx4
Dll3
Dll1
Mfng
Notch1
Rfng
Dvl1
Kat2a
Numbl
Jag2
Ncst
Ctbp2
Rbpj
Psen1
Hdac1
Maml3
Ctbp1
Ep300
Snw1
Notch4
Maml2
Maml1
Kat2b
Jag1
Dvl3
Dtx3
Dtx2
Ncor2
Psen2
Crebbp
Aph1b
Notch2
Notch3
Hes1
log2(RPKMCKO/RPKMCtrl)
D
Relative mRNA level of Dll4
Control
Vec-Cre; Mettl3fl/fl
E
Control
Vec-Cre; Mettl3fl/fl
Dll4
Actin
Relative protein level of Dll4
F
Control
Vec-Cre; Mettl3fl/fl
Dll4/CD31/DAPI
E10.5
G
5 kb
Control
Vec-Cre; Mettl3fl/fl
RNA-seq
m6A peak
Ythdf2 cluster
EBs-rep1
EBs-rep2
EBs-rep3
Ythdf2-rep1
Ythdf2-rep2
Ythdf2-rep3
Notch1

Figure S2. Notch signaling pathway is over-activated in E10.5 *Vec-Cre*; *Mettl3*$^{fl/fl}$ AGM.

(A) Gene-Set Enrichment Analysis (GSEA) plots to evaluate the changes of specific genes in Notch signaling pathway in the E10.5 *Vec-Cre*; *Mettl3*$^{fl/fl}$ compared with the control. (B) Venn plots showing about 83.3% (35/42) expressed genes in Notch signaling pathway with m^{6}A modifications. m^{6}A peak data were downloaded from GSE61995 [12]. (C) The expression levels of genes with m^{6}A modifications in the control and *Vec-Cre*; *Mettl3*$^{fl/fl}$ AGM. The asterisk represents genes within significantly differential expression levels upon *Mettl3* deficiency. (D) qPCR showing the mRNA level of *Dll4* in the AGM of control and *Vec-Cre*; *Mettl3*$^{fl/fl}$ embryos at E10.5. (E) Western blotting analysis of protein level of Dll4 in the E10.5 control and *Vec-Cre*; *Mettl3*$^{fl/fl}$ AGM. The right panel is the quantification of the western blotting results. (F) Immunofluorescence analysis of protein level of Dll4 in the E10.5 control and *Vec-Cre*; *Mettl3*$^{fl/fl}$ AGM. (G) Integrative Genomics Viewer (IGV) tracks displaying RNA-seq reads distribution in the control (blue) and *Vec-Cre*; *Mettl3*$^{fl/fl}$ (red) AGM as well as m^{6}A peaks (green) (m^{6}A peak data were downloaded from GSE61995 [12]) and Ythdf2 cluster (purple) (Ythdf2 cluster data were downloaded from GSE49339 [13]) in the *Notch1* last exon. The details of public data were described in Methods. Student's *t*-test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. Scale bar, 10 μm. The statistical results are presented as mean ± SEM.

Supplementary References

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