Mmrn1 expression defines a novel subset of hematopoietic stem cells and leukemia stem cells with great self-renewal potential

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Supplementary Material

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Supplementary methods

HSC culture

For bulk HSC culture, 1×10^3 Mmrn1$^{-/low}$ and Mmrn1^{high} LT-HSCs were purified from the BM of Mmrn1$^{+/eGFP}$ mice and cultured in StemSpan SFEM medium (Stem Cell Technologies, Vancouver, Canada) containing 10 ng/ml SCF (PeproTech, Rocky Hill, NJ, USA), 20 ng/ml TPO (PeproTech), 10 μg/ml heparin (MedChemExpress, Monmouth Junction, NJ, USA) and 1% penicillin/streptomycin (Sigma, St. Louis, MO, USA). Ten days after culture, cells were collected for flow cytometric analysis. For single-cell colony formation assay, single Mmrn1$^{-/low}$ or Mmrn1^{high} LT-HSC was cultured with M3434 methylcellulose medium (Stem Cell Technologies) in U-bottom 96-well plates. Fourteen days after culture, colony size was analyzed under an inverted microscope.

Cell line culture

Human HS-5, HEL, HL-60, KG-1, NB-4, THP-1 and U-937 cells (Procell, Wuhan, China) were inoculated and cultured in RPMI-1640 medium (HyClone, South Logan, UT, USA) with 10% fetal bovine serum (HyClone).

qPCR analysis

This assay was carried out as we previously described.[1]

Lentiviral transfection

CD34$^+$ UCB cells were prestimulated for 12 hours in StemSpan SFEM medium (Stem Cell Technologies) with 100 ng/ml recombinant human SCF (PeproTech, Rocky Hill, NJ, USA), 100 ng/ml recombinant human Flt3L (PeproTech), 20 ng/ml recombinant human IL-6 (PeproTech), 20 ng/ml human recombinant TPO (PeproTech) and 1× penicillin/streptomycin (Beyotime Biotechnology, Shanghai, China). After that, the

transduction of lentivirus carrying shMMRN1 (labeled with mCherry) was performed in the same medium at a multiplicity of infection of 50-100 for 24 hours. Two to three days later, transduced cells were purified by flow cytometry based on mCherry expression and then used for further experiments. The core interference sequence of shRNA against MMRN1 is as follows: 5'-CTTGGACTATACCTGAGGAT-3'.

Stress treatment

For irradiation stress, Mmrn1$^{+/eGFP}$ mice were subjected to 5.0 Gy total body irradiation by a ^{60}Co irradiator (Third Military Medical University Irradiation Center,). For 5-FU treatment, Mmrn1$^{+/eGFP}$ mice were intraperitoneally injected with a single dose of 150 mg/kg 5-FU (Sigma). For LPS treatment, Mmrn1$^{+/eGFP}$ mice were intraperitoneally injected with a single dose of 5 mg/kg LPS (Beyotime Biotechnology, Shanghai, China). For *in vitro* stress, LT-HSCs from the BM of Mmrn1$^{+/eGFP}$ mice were cultured in SFEM medium with 1 μg/mL pI:pC (Sigma), 50 ng/mL IL-6 (PeproTech), 20 ng/mL IL-3 (PeproTech), 1000 units/mL IFNβ1 (R&D Systems, Minnesota, USA) or 5 μM SenexinB (MedChemExpress) for 48 h.

Transplantation assays

For HSCT assay, 5×10^2 freshly isolated Mmrn1$^{-/low}$ or Mmrn1^{high} LT-HSCs were mixed with 5×10^5 CD45.1 BM cells and then transplanted into lethally irradiated (10.0 Gy) CD45.1 mice. After 16 weeks, 1×10^6 BM cells from the first recipients were transplanted into the second recipients. At the indicated time points after the first and second HSCT, the chimerism levels in the recipients were detected by flow cytometry. For short-term transplantation assay, equal numbers of Mmrn1$^{-/low}$ or Mmrn1^{high} LT-HSCs (5×10^2) were transplanted into lethally irradiated CD45.1 mice, together with 5×10^5 CD45.1 BM cells. Two weeks post-transplantation, the PB and BM samples from recipients were analyzed by flow cytometry. For competitive

transplantation assay, 5×10^5 BM cells from WT or Mmrn1$^{-/-}$ mice, along with 5×10^5 CD45.1 BM cells, transplanted into lethally irradiated CD45.1 mice. After 16 weeks, 1×10^6 BM cells from the first recipients were transplanted into the second recipients. The third round of transplantation was carried out in the same manner. At the indicated time points after the first, second and third transplantation, the chimerism levels in the recipients were detected by flow cytometry.

Homing assay

This assay was performed as we previously described.[2,3] A total of 3×10^4 Mmrn1$^{-/low}$ and Mmrn1^{high} LT-HSCs freshly sorted from the BM of Mmrn1$^{+/eGFP}$ mice were labeled by 5,6-carboxyfluorescein diacetate succinimidyl ester (CFSE; Invitrogen, Carlsbad, CA, USA) and then transplanted into normal WT recipient mice irradiated with 10.0 Gy. After 16 h, CFSE$^+$ cell population in the BM of recipient mice was analyzed by flow cytometry. The homing efficiency was calculated as follows: homing efficiency = (CFSE$^+$ cell number in recipient BM/transplanted cell number)×100%.

Xenotransplantation assay

CD34$^+$ cells (2×10^5) isolated from the BM of AML patients with high or -/low expression of MMRN1 were transplanted into sublethally irradiated (2.5 Gy) M-NSG mice via intravenous injection. At 25 days after transplantation, the percentage of hCD45$^+$ cells in the PB of recipients were analyzed by flow cytometry. Meantime, the survival rates of mice were monitored.

Murine AML model establishment

For assessing the function of Mmrn1$^{-/low}$ and Mmrn1^{high} LSCs, retrovirus containing MSCV-MLL-AF9-IRES-mCherry vector was transduced into Lin$^-$ cells isolated from

the BM of Mmrn1$^{+/eGFP}$ mice. Next, pre-leukemic cells (mCherry^{+}) were sorted from the transduced cells and were transplanted into 7.5 Gy-irradiated WT mice. Twenty-five days after transplantation, 2×10^3 Mmrn1$^{-/low}$ and Mmrn1^{high} LSCs were purified from the first recipients and transplanted into the second WT recipients irradiated with 6.0 Gy. After another 25 days, 2×10^3 LSCs purified from the second recipients were transplanted into the third WT recipients irradiated with 6.0 Gy. For assessing the function of WT and Mmrn1$^{-/-}$ LSCs, MLL-AF9-induced leukemia model was established as mentioned above. After that, pre-leukemic cells (mCherry^{+}) were transplanted into 7.5 Gy-irradiated WT mice. Twenty-five days after transplantation, 2×10^3 LSCs purified from the first recipients and transplanted into the second WT recipients irradiated with 6.0 Gy.

Serial replating assay

Freshly sorted LSCs (5×10^2) were seeded in MethoCultTM M3434 medium (Stem Cell Technologies). After 7 days of culture, colonies were counted and then harvested for replating.

Limiting dilution assays

For assessing the functional HSC frequency, BM cells (5×10^3, 1×10^4, 2.5×10^4, 5×10^4 or 1×10^5) obtained from WT or Mmrn1$^{-/-}$ mice, along with CD45.1 BM cells (5×10^5), were transplanted into lethally irradiated CD45.1 recipient mice. In addition, CD34^{+} cells (1×10^4, 2.5×10^4, 5×10^4 or 1×10^5) transfected with shCtrl or shMMRN1 were transplanted into sublethally irradiated M-NSG mice. For assessing the functional LSC frequency, mCherry^{+} BM cells (5×10^2, 2.5×10^3, 5×10^3 or 1×10^4) were obtained from the first recipients reconstructed with Mmrn1$^{-/low}$ and Mmrn1^{high} LSCs. Then, these cells were transplanted into sublethally irradiated WT recipient mice. The frequency of functional HSCs or LSCs were analyzed as we previously reported.[4]

PB counts

PB samples were collected from the tail vein of leukemia mice and diluted in a 1% EDTA solution. White blood cell (WBC), red blood cell (RBC) and platelet (PLT) counts were measured by the Sysmex XT2000i hematology analyzer (Sysmex, Kobe, Japan).

Megakaryocytic differentiation induction

Freshly sorted Mmrn1^{high} and Mmrn1$^{-/low}$ LSCs were cultured in vitro in the presence of TPO stimulation, according to our previous protocol.[5] After 7 days of culture, CD41 expression was analyzed by flow cytometry.

Histology analysis

PB smears were fixed with methanol and stained with Wright-Giemsa (Beyotime Biotechnology) according to the manufacturer's protocol. Mouse spleen, lung and liver were fixed in zinc formalin (Solarbio, Shanghai, China) for 48 hours and then dehydrated in ethanol, embedded in paraffin, sectioned and stained with hematoxylin and eosin (Beyotime Biotechnology). These samples were scanned on a KF-PRO-020 Digital Pathology Slide Scanner (KFBIO, Ningbo, China).

Public database analysis.

The bulk RNA-seq data for Mmrn1 expression analysis in human and mouse hematopoietic cells were obtained from the BloodSpot database (https://www.bloodspot.eu/). The scRNA-seq data for Mmrn1 expression analysis in human and mouse hematopoietic cells were obtained from the Atlas of blood cells database (http://abc.sklehabc.com/) under reference number GSE150774, GSE128743 and GSE139369. The data on Mmrn1 expression in mouse megakaryocytes were derived from the OMIX database (https://ngdc.cncb.ac.cn/omix/) under reference

number OMIX005321. The AML clinical data were obtained from the UCSC XENA database (https://xena.ucsc.edu/), UALCAN database (https://ualcan.path.uab.edu/) and Vizome database (http://www.vizome.org/). The data applied to survival curve analysis of AML patients were downloaded from the TCGA database (https://tcga-data.nci.nih.gov/tcga/), PrognoScan database (http://dna00.bio.kyutech.ac.jp/PrognoScan/) and Kaplan-Meier plotter database (https://kmplot.com/analysis/). The data used for KEGG enrichment analysis were obtained from the TCGA database, and the data used for transcription factor binding analysis were obtained from the BloodChIP Xtra database (https://bloodchipxtra.vafaeelab.com/). For analyzing the expression of MMRN1 in GMP-like LSCs and LMPP-like LSCs, the data derived from a previous study was employed.[6] The R packages include pROC (v1.18.0), ggplot2 (v3.3.6), survival (v3.3.1), survminer (v0.4.9), DESeq2 (v1.36.0), edgeR (v3.38.2) and clusterProfiler (v4.4.4).

References

1. Lu Y, Zhang Z, Wang S, et al. Srebf1c preserves hematopoietic stem cell function and survival as a switch of mitochondrial metabolism. Stem Cell Reports. 2022;17(3):599-615.

2. Chen N, Quan Y, Chen M, et al. Melanocortin/MC5R axis regulates the proliferation of hematopoietic stem cells in mice after ionizing radiation injury. Blood Adv. 2023;7(13):3199-3212.

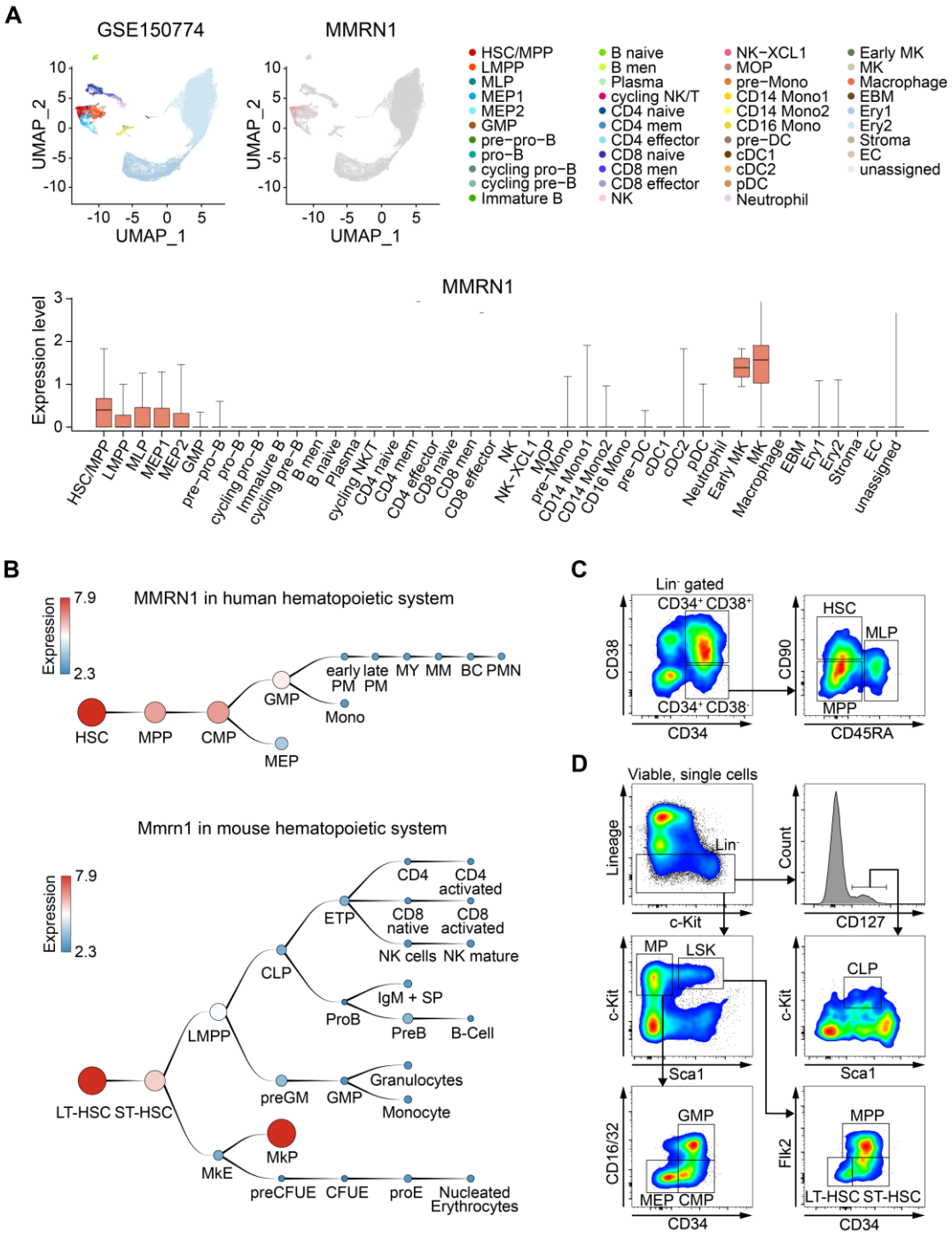
3. Hu M, Zeng H, Chen S, et al. SRC-3 is involved in maintaining hematopoietic stem cell quiescence by regulation of mitochondrial metabolism in mice. Blood. 2018;132(9):911-923.

4. Chen F, Lu Y, Xu Y, et al. Trim47 prevents hematopoietic stem cell exhaustion during stress by regulating MAVS-mediated innate immune pathway. Nat Commun. 2024;15(1):6787.

5. Chen S, Hu M, Shen M, et al. IGF-1 facilitates thrombopoiesis primarily through Akt activation. Blood. 2018;132(2):210-222.

6. Goardon N, Marchi E, Atzberger A, et al. Coexistence of LMPP-like and GMP-like leukemia stem cells in acute myeloid leukemia. Cancer Cell. 2011;19(1):138-52.

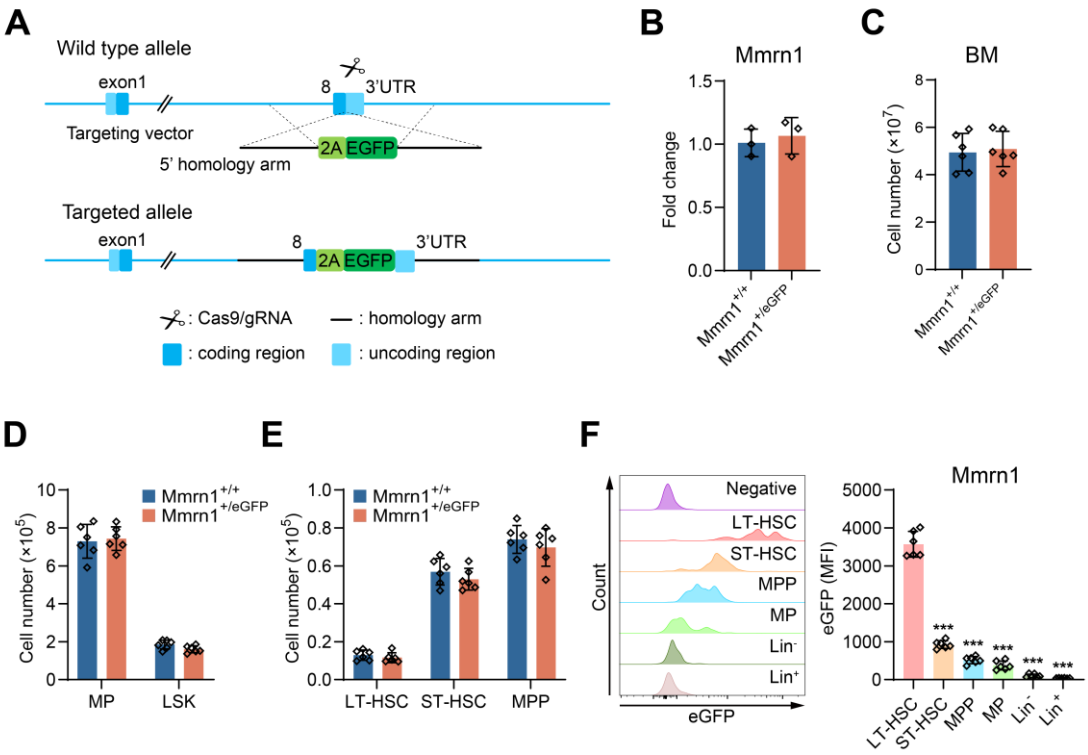
Supplementary Figure S1



Supplementary Figure S1. The expression profile of Mmrn1 in human and murine. (A) scRNA-seq analysis of the expression of MMRN1 in human BM and UCB cells (GSE150774) from the Atlas of Blood Cells database. (B) The expression of Mmrn1 in human (up) and mouse (down) hematopoietic system from the

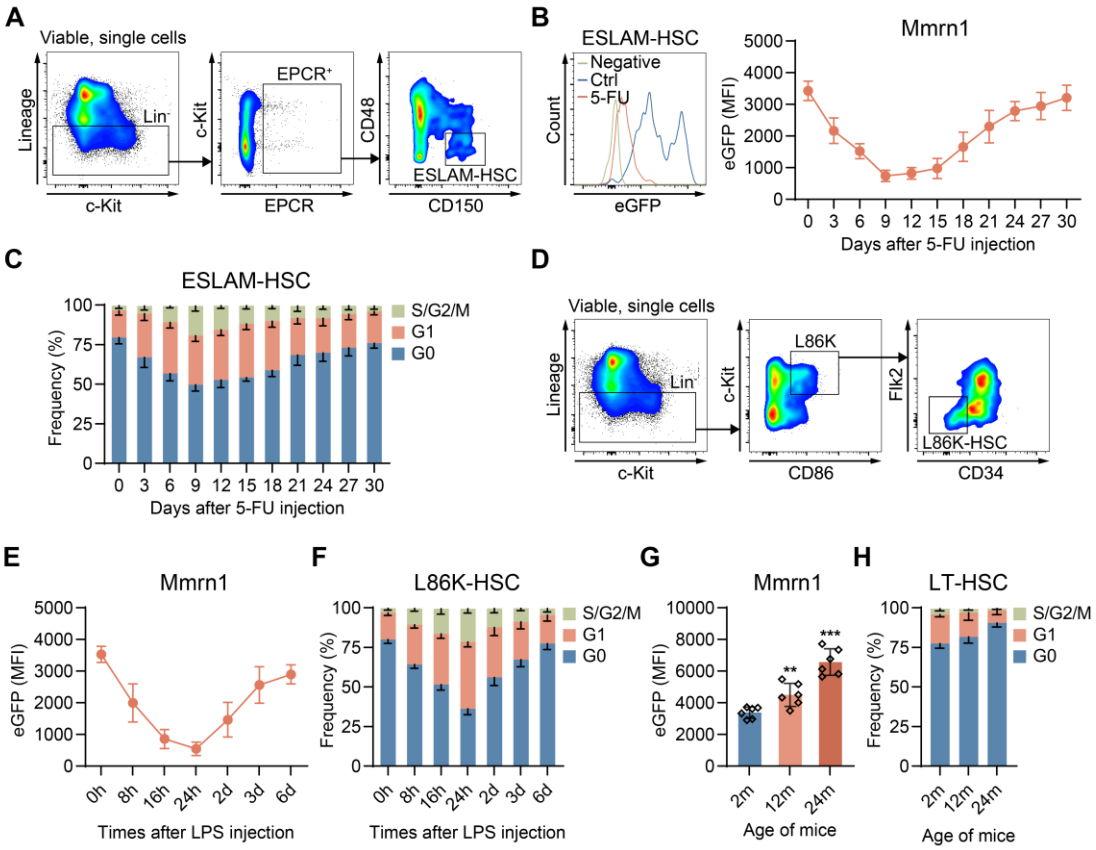
BloodSpot database. (C) The gating strategies for flow cytometric analysis or sorting of human HSPC populations from UCB: HSCs (Lineage$^-$ CD34$^+$ CD38$^-$ CD45RA$^-$ CD90$^+$), MPPs (Lineage$^-$ CD34$^+$ CD38$^-$ CD45RA$^-$ CD90$^-$) and MLPs (Lineage$^-$ CD34$^+$ CD38$^-$ CD45RA$^+$). (D) The gating strategies for flow cytometric analysis or sorting of mouse HSPC populations from BM: Lin$^-$ cells (Lineage$^-$), MPs (Lineage$^-$ Sca1$^-$ c-Kit$^+$), LSKs (Lineage$^-$ Sca1$^+$ c-Kit$^+$), LT-HSCs (Lineage$^-$ Sca1$^+$ c-Kit$^+$ CD34$^-$ Flk2$^-$), ST-HSCs (Lineage$^-$ Sca1$^+$ c-Kit$^+$ CD34$^+$ Flk2$^-$), MPPs (Lineage$^-$ Sca1$^+$ c-Kit$^+$ CD34$^+$ Flk2$^+$), CLPs (Lineage$^-$ CD127$^+$ Sca1^{low} c-Kit^{low}), CMPs (Lineage$^-$ Sca1$^-$ c-Kit$^+$ CD16/32$^-$ CD34$^+$), GMPs (Lineage$^-$ Sca1$^-$ c-Kit$^+$ CD16/32$^+$ CD34$^+$) and MEPs (Lineage$^-$ Sca1$^-$ c-Kit$^+$ CD16/32$^-$ CD34$^-$) .

Supplementary Figure S2



Supplementary Figure S2. The generation of Mmrn1$^{+/eGFP}$ mice. (A) The strategy for generating the Mmrn1$^{+/eGFP}$ mouse model. (B) qPCR analysis of Mmrn1 mRNA expression in LT-HSCs from the BM of Mmrn1$^{+/+}$ and Mmrn1$^{+/eGFP}$ mice (n = 3). (C-E) The numbers of (C) total BM cells and (D, E) indicated HSPC populations in two tibias and femurs from Mmrn1$^{+/+}$ and Mmrn1$^{+/eGFP}$ mice (n = 6). (F) Flow cytometric analysis of Mmrn1-eGFP expression in the indicated hematopoietic cell populations from the BM of Mmrn1$^{+/eGFP}$ mice (n = 6). WT mice served as negative controls. Representative flow cytometric plots are shown in the left. Statistical differences were analyzed by comparing each group with LT-HSCs. Data are shown as the mean ± SD. ***P < 0.001.

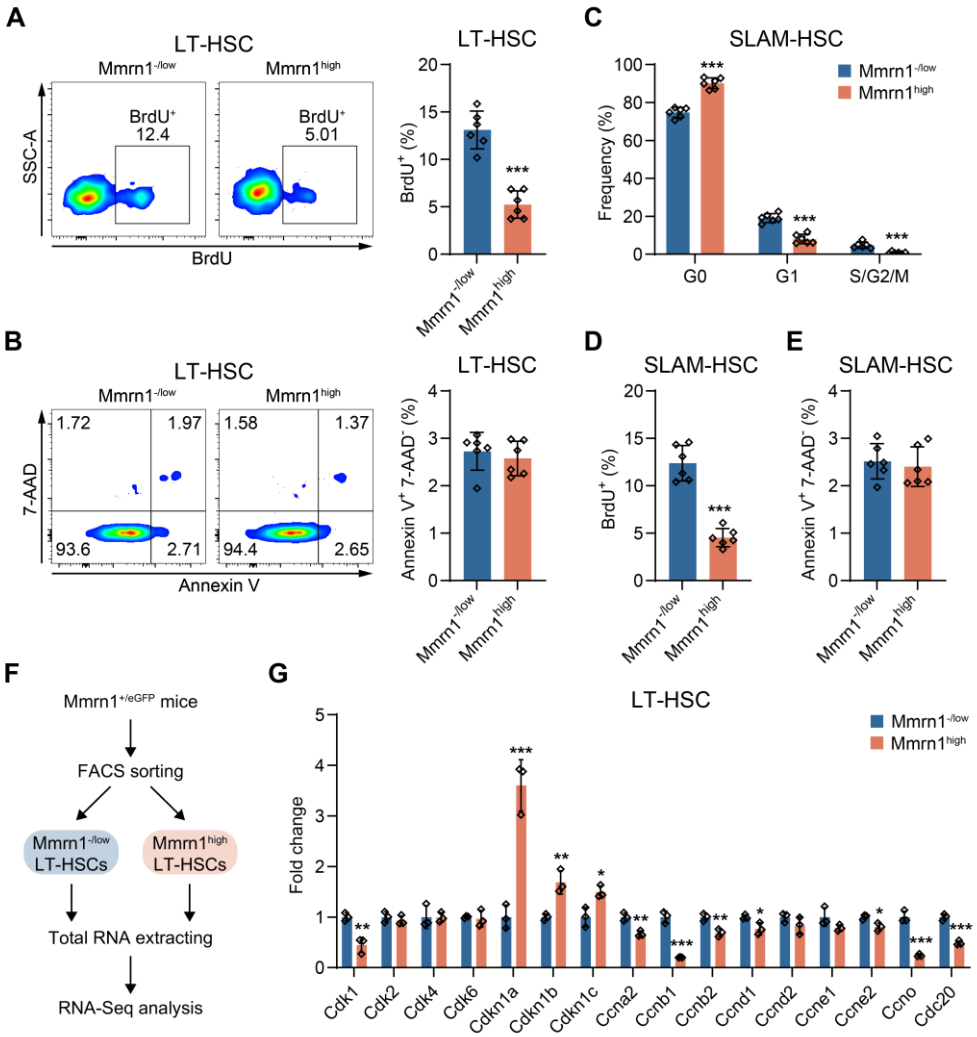
Supplementary Figure S3



Supplementary Figure S3. Mmrn1 expression is changed in HSCs after various stresses. (A) The gating strategies for flow cytometric analysis of ESLAM-HSCs (Lineage$^-$ EPCR$^+$ CD150$^+$ CD48$^-$) after IR or 5-FU treatment. (B) Flow cytometric analysis of Mmrn1-eGFP expression in ESLAM-HSCs from the BM of Mmrn1$^{+/eGFP}$ mice at the indicated time points after 5-FU injection (n = 6 per time point). WT mice were served as negative controls. Representative flow cytometric plots at 9 days after 5-FU injection are shown in the left. (C) Cell cycle analysis of ESLAM-HSCs from the BM of Mmrn1$^{+/eGFP}$ mice at the indicated time points after 5-FU injection (n = 6 per time point). (D) The gating strategies for flow cytometric analysis of L86K-HSCs (Lineage$^-$ CD86$^+$ c-Kit$^+$ Flk2$^-$ CD34$^-$) after LPS treatment. (E, F) Flow cytometric analysis of (E) Mmrn1-eGFP expression and (F) cell cycle state in L86K-HSCs from

the BM of Mmrn1$^{+/eGFP}$ mice at the indicated time points after LPS injection (n = 6 per time point). h, hour. (G, H) Flow cytometric analysis of (G) Mmrn1-eGFP expression and (H) cell cycle state in LT-HSCs from the BM of Mmrn1$^{+/eGFP}$ mice at the indicated age (n = 6 per time point). m, month. Data are shown as the mean ± SD. **P < 0.01, ***P < 0.001.

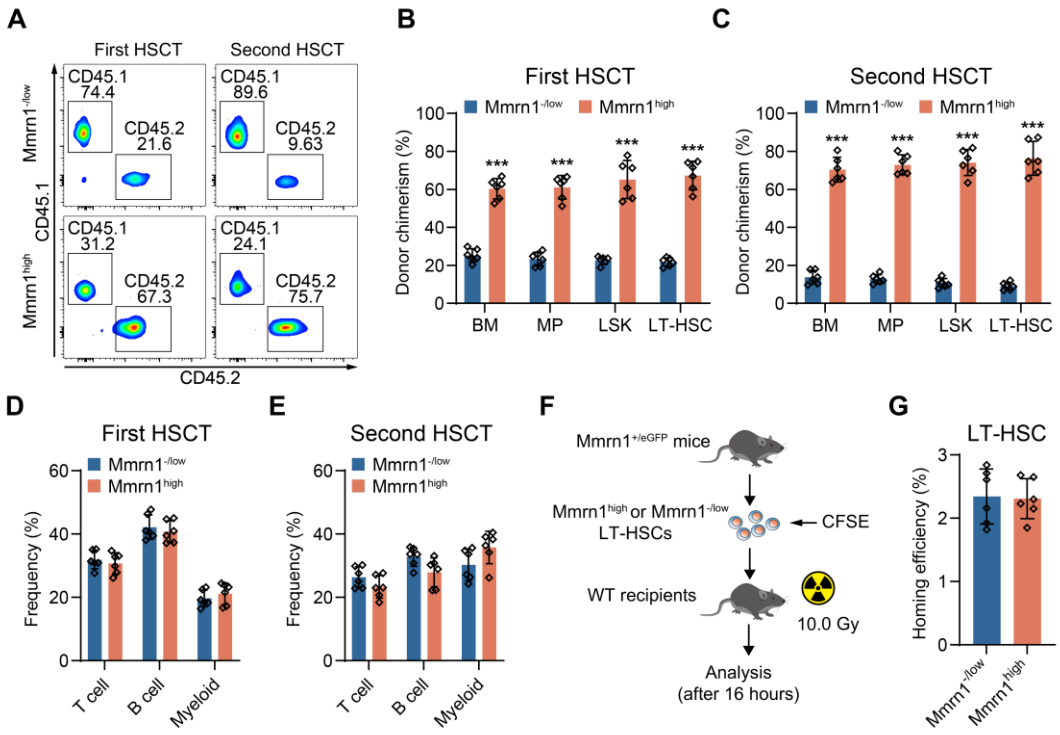
Supplementary Figure S4



Supplementary Figure S4. Mmrn1 expression distinguishes different subgroups of HSCs. (A) The percentage of BrdU^{+} cells in Mmrn1$^{-/low}$ and Mmrn1^{high} LT-HSCs from the BM of Mmrn1$^{+/eGFP}$ mice (n = 6). (B) Apoptosis analysis of Mmrn1$^{-/low}$ and Mmrn1^{high} LT-HSCs from the BM of Mmrn1$^{+/eGFP}$ mice (n = 6). (C) Cell cycle analysis of Mmrn1$^{-/low}$ and Mmrn1^{high} SLAM-HSCs from the BM of Mmrn1$^{+/eGFP}$ mice (n = 6). (D) The percentage of BrdU^{+} cells in Mmrn1$^{-/low}$ and Mmrn1^{high} SLAM-HSCs from the BM of Mmrn1$^{+/eGFP}$ mice (n = 6). (E) Apoptosis analysis of Mmrn1$^{-/low}$ and Mmrn1^{high} SLAM-HSCs from the BM of Mmrn1$^{+/eGFP}$ mice (n = 6). (F) Schematic diagram of the RNA-seq analysis (n = 3). (G) qPCR analysis of mRNA

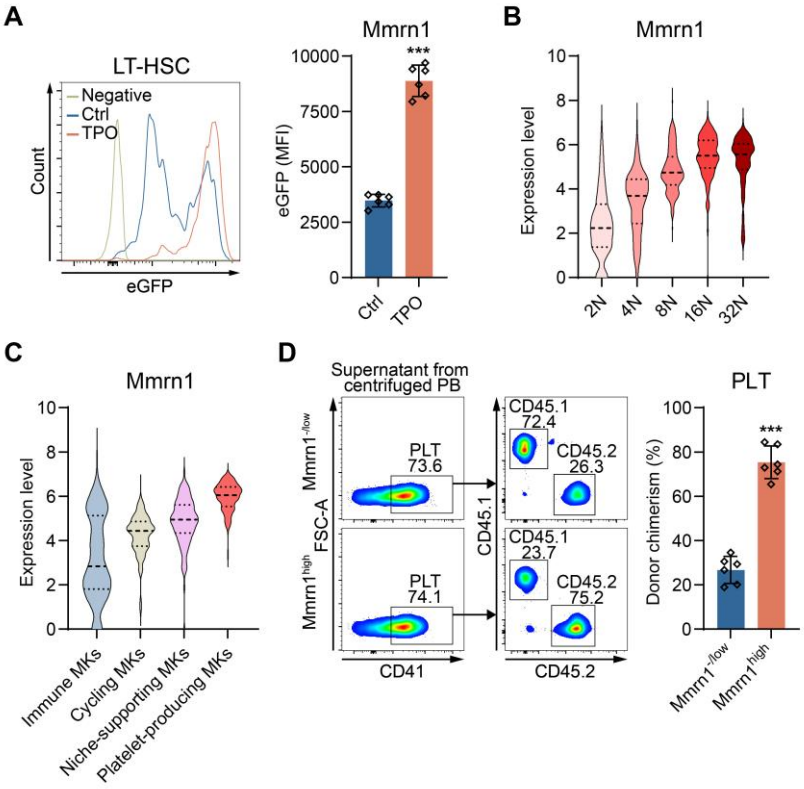
expression of the indicated genes in Mmrn1$^{-/low}$ and Mmrn1^{high} LT-HSCs sorted from the BM of Mmrn1$^{+/eGFP}$ mice (n = 3). Data are shown as the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

Supplementary Figure S5



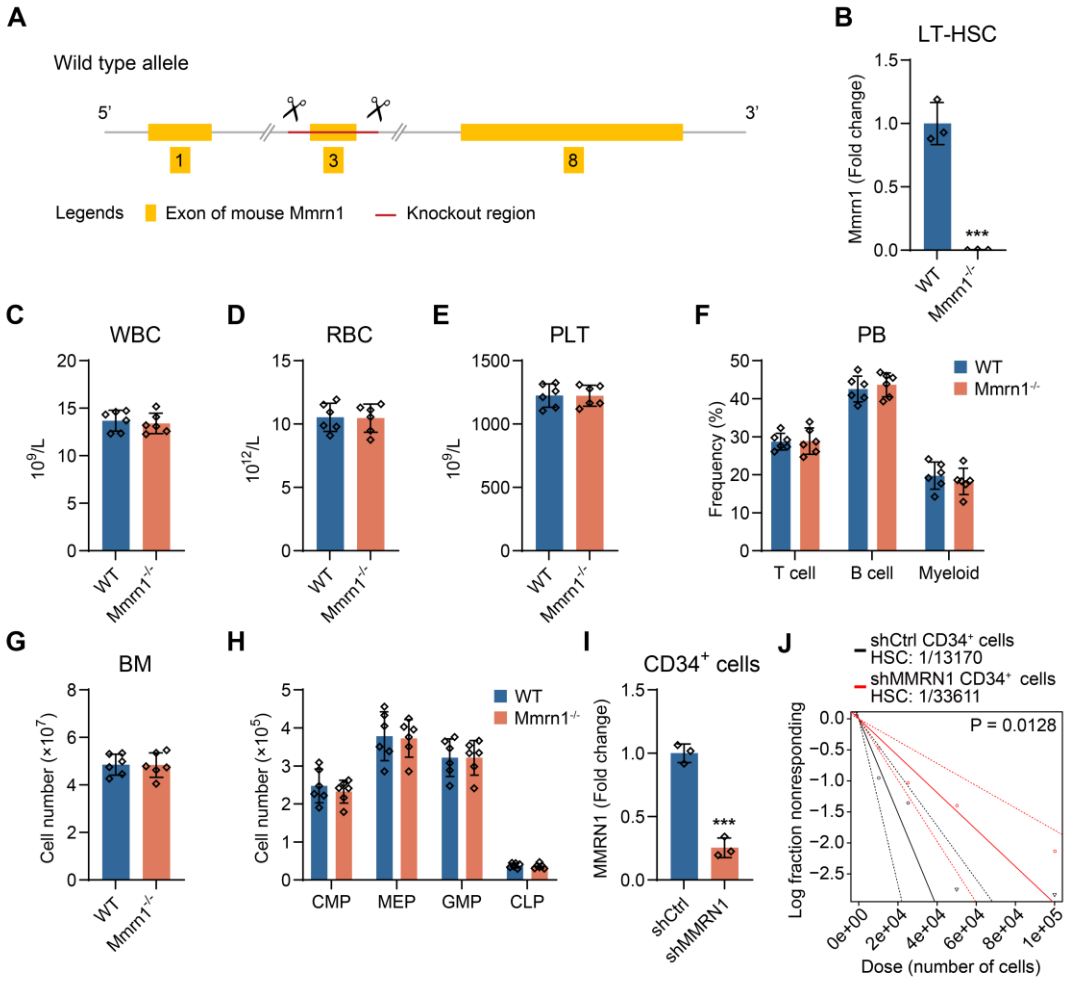
Supplementary Figure S5. Mmrn1$^{-/low}$ and Mmrn1^{high} HSCs show different abilities in long-term reconstitution but not in homing and differentation. (A) Representative flow cytometric plots showing the chimerism levels of LT-HSCs in the BM of recipients at 16 weeks after the first and second HSCT. (B, C) The chimerism levels of donor-derived BM cells, MPs, LSKs and LT-HSCs in the recipients at 16 weeks after the (B) first and (C) second HSCT (n = 6). (D, E) The lineage distribution of donor-derived cells in the PB of recipients at 16 weeks after the (D) first and (E) second HSCT (n = 6). (F) The schematic diagram of homing assay. (G) The homing efficiency of Mmrn1$^{-/low}$ and Mmrn1^{high} LT-HSCs from the BM of Mmrn1$^{+/eGFP}$ mice (n = 6). Data are shown as the mean ± SD. ***P < 0.001.

Supplementary Figure S6



Supplementary Figure S6. Mmrn1 marks megakaryocytic lineage commitment. (A) Flow cytometric analysis of Mmrn1-eGFP expression in LT-HSCs from the BM of Mmrn1$^{+/eGFP}$ mice at 48 hours after 50 μg/kg TPO injection (n = 6). WT mice were served as negative controls. Representative flow cytometric plots are shown in the left. (B, C) Mmrn1 expression in (B) the ploidy clusters and (C) the subpopulations of mouse megakaryocytes from the OMIX database under reference number OMIX005321. Mk, megakaryocyte. (D) The chimerism levels of PLT in the PB of recipient mice were determined at 2 weeks after transplanted with Mmrn1$^{-/low}$ and Mmrn1^{high} LT-HSCs (n = 6). Representative flow cytometric plots are shown in the left. Data are shown as the mean ± SD. ***P < 0.001.

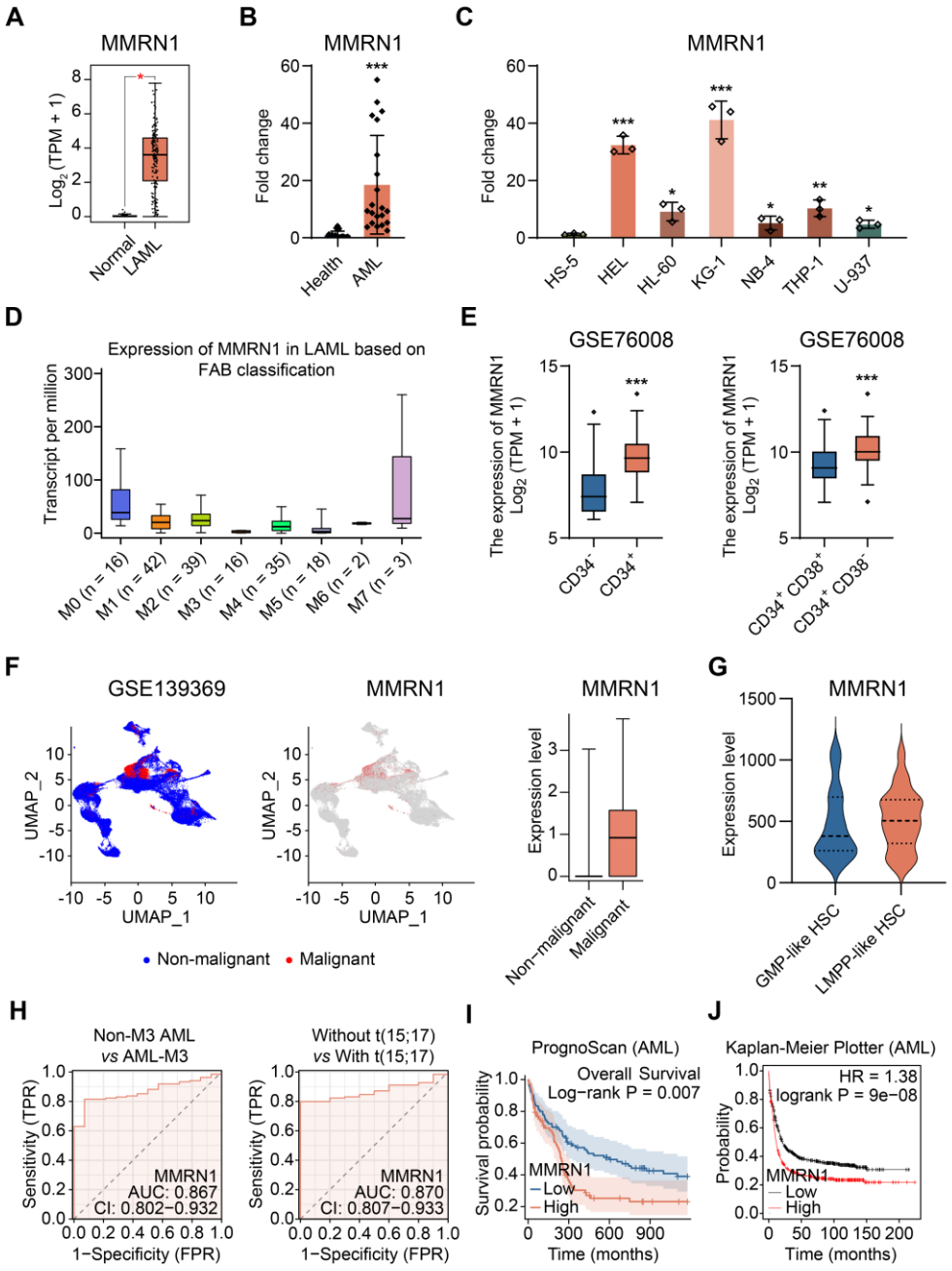
Supplementary Figure S7



Supplementary Figure S7. Mmrn1 deficiency does not significantly affect normal hematopoietic parameters. (A) The strategy of generation of the Mmrn1$^{-/-}$ mouse model. (B) qPCR analysis of Mmrn1 mRNA expression in LT-HSCs from the BM of WT and Mmrn1$^{-/-}$ mice (n = 3). (C-E) The counts of (C) WBC, (D) RBC and (E) PLT in the PB of WT and Mmrn1$^{-/-}$ mice (n = 6). (F) The percentages of T cells, B cells and myeloid cells in PB of WT and Mmrn1$^{-/-}$ mice (n = 6). (G) The number of total BM cells in two tibias and femurs from WT and Mmrn1$^{-/-}$ mice (n = 6). (H) The numbers of CMPs, MEPs, GMPs and CLPs in two tibias and femurs from WT and Mmrn1$^{-/-}$ mice (n = 6). (I) qPCR analysis of MMRN1 mRNA expression in human

UCB $CD34^+$ cells after transfected with shCtrl or shMMRN1 (n = 3). (J) Limiting dilution analysis of the functional HSC frequency in human UCB $CD34^+$ cells after transfected with shCtrl or shMMRN1 (n = 8). Data are shown as the mean ± SD. ***P < 0.001.

Supplementary Figure S8



Supplementary Figure S8. High expression of MMRN1 portends a poor prognosis in patients with AML. (A) The expression of MMRN1 in 173 AML patients and 70 healthy donors from the GEPIA2 database. (B) qPCR analysis of MMRN1 expression in the BM samples obtained from 21 AML patients and 17 healthy donors. (C) qPCR analysis of MMRN1 mRNA expression in HS-5, HEL, HL-

60, KG-1, NB-4, THP-1 and U-937 cells (n = 3). Statistical differences were analyzed by comparing each group with HS-5 cells. (D) The expression of MMRN1 in LAML samples based on FAB classification from the UALCAN database. (E) The expression of MMRN1 in (left) $CD34^-/CD34^+$ and (right) $CD34^+$ $CD38^+/CD34^+$ $CD38^-$ AML samples (GSE76008). (F) scRNA-seq analysis of the expression of MMRN1 in AML samples (GSE139369) from the Atlas of Blood Cells database. (G) The expression of MMRN1 in GMP-like LSCs and LMPP-like LSCs (E-TABM-978). (H) ROC analysis of the diagnostic performance of MMRN1 for the indicated binary classification from the UCSC XENA database. (I, J) Survival curves of AML patients with low or high expression of MMRN1. Data were obtained from the (I) PrognoScan database and (J) Kaplan-Meier plotter database. Data are shown as the mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

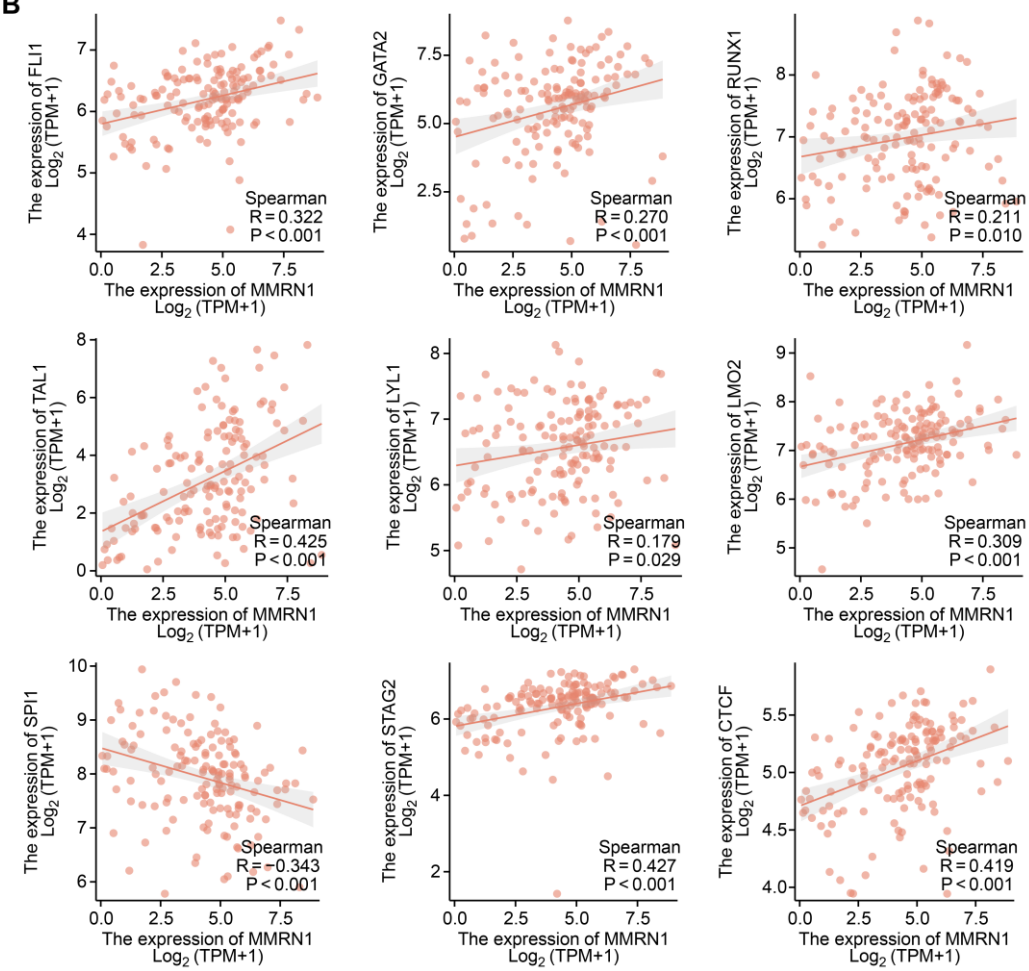
Supplementary Figure S9

A

Transcription Factor Binding at the MMRN1 locus

Cell Type	HSC	CMP	GMP	MEP	KG1	ME1	KAS1	TSU
ERG	×	✓	×	×	×	✓	✓	✓
FLI1	✓	✓	✓	✓	✓	×	✓	✓
GATA2	✓	✓	✓	✓	✓	×	×	✓
RUNX1	✓	✓	✓	✓	✓	✓	✓	✓
TAL1	✓	✓	×	✓	×	×	✓	✓
LYL1	✓	✓	✓	✓	✓	×	✓	✓
LMO2	✓	✓	✓	✓	×	×	×	✓
PU.1	×	✓	✓	✓	✓	×	✓	✓
STAG2	✓	✓	×	✓	▢	▢	▢	▢
CTCF	✓	✓	✓	✓	▢	▢	▢	▢

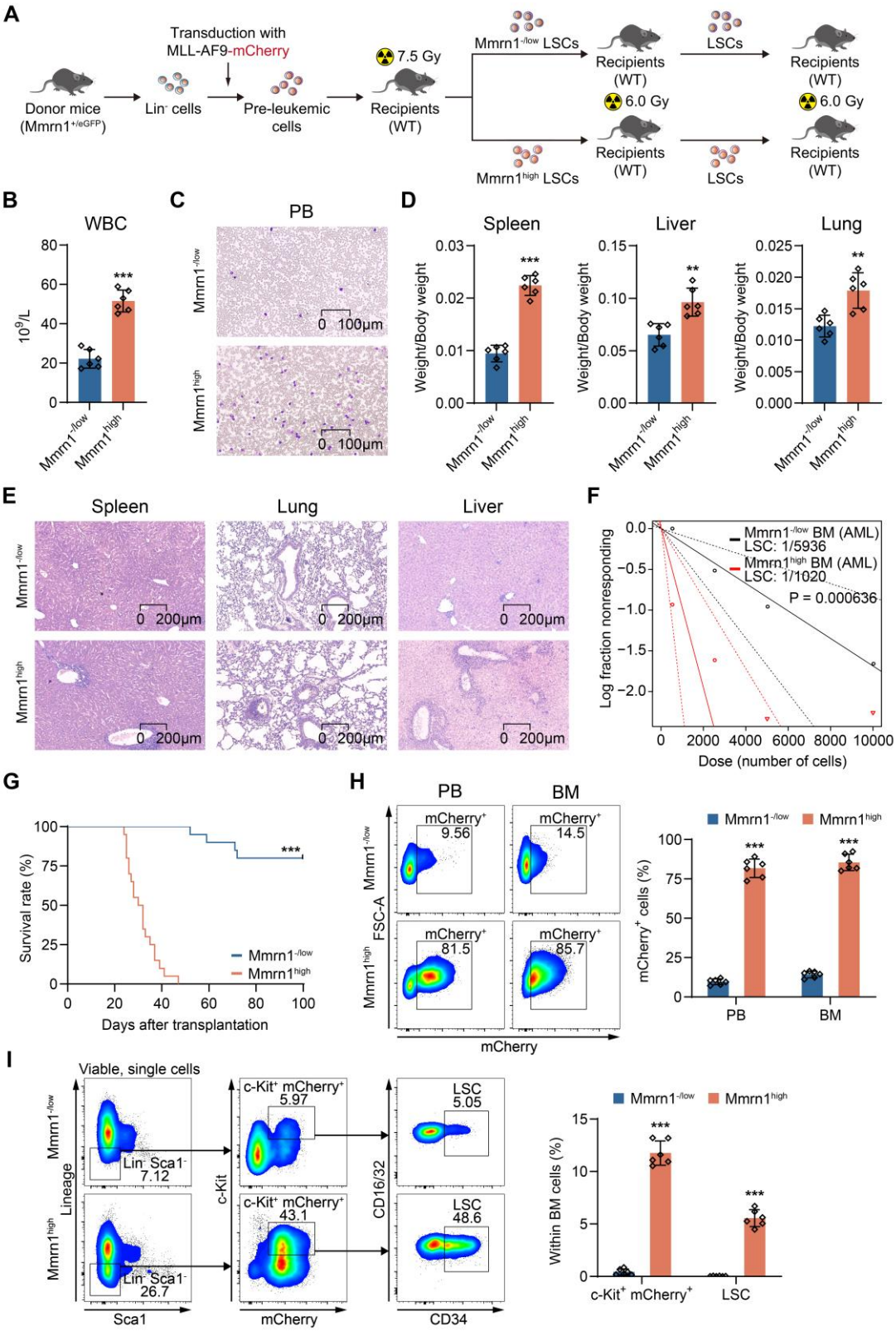
B



Supplementary Figure S9. The correlation of MMRN1 with several hematopoiesis-related transcription factors. (A) Analysis of transcription factor

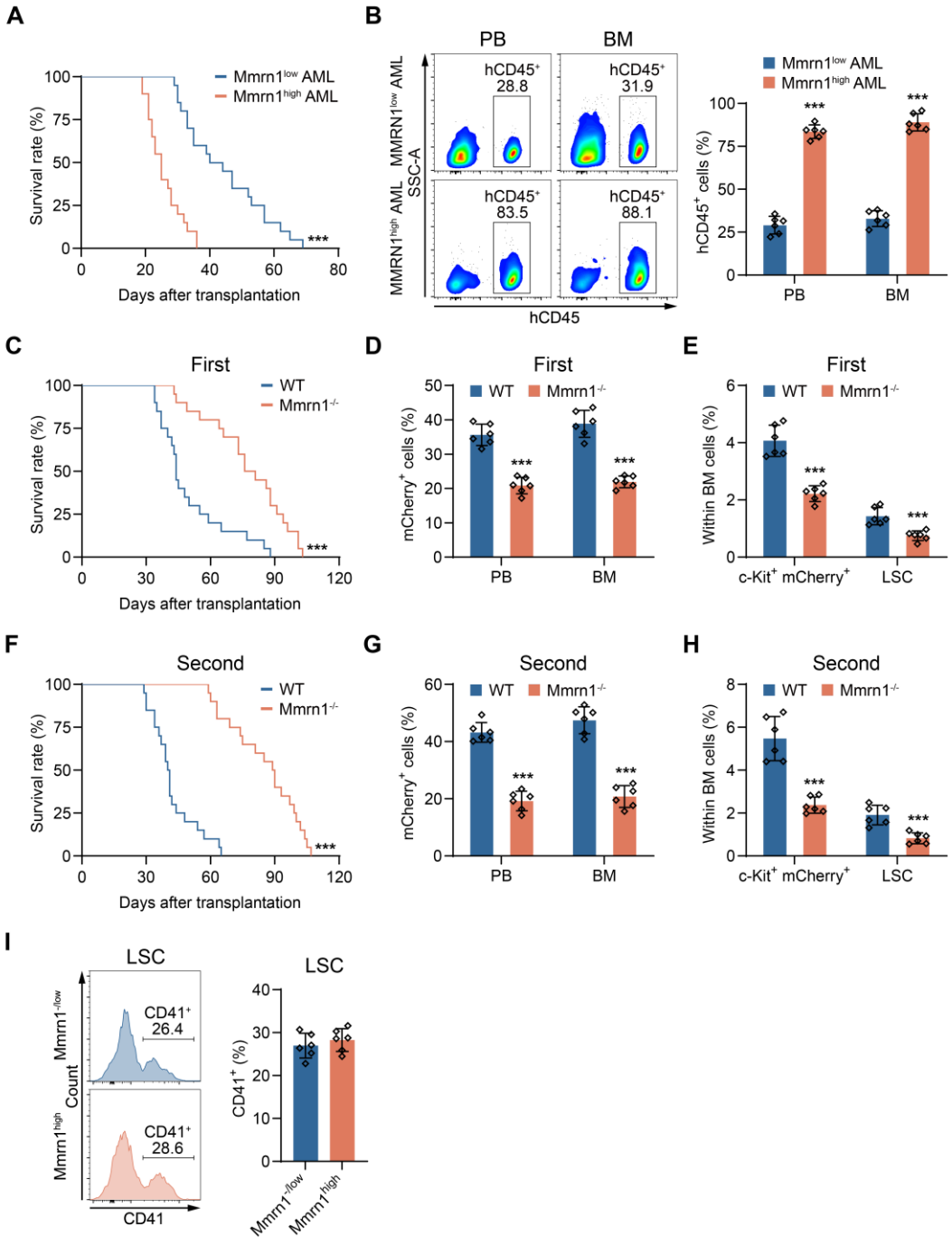
binding at the MMRN1 locus in the indicated types of cells from the BloodChIP Xtra database. (B) The correlation analysis of the expression of MMRN1 and the indicated transcription factors in AML samples from the TCGA database. The transcription factor PU.1 is encoded by SPI1.

Supplementary Figure S10



Supplementary Figure S10. Mmrn1^{high} LSCs are responsible for AML development. (A) Schematic diagram of generating MLL-AF9-induced leukemia mouse model. (B) The count of WBC in the PB of recipients at 25 days after transplanted with Mmrn1$^{-/low}$ and Mmrn1^{high} LSCs (n = 6). (C) Wright-Giemsa staining of PB smears from the second recipients at 25 days after transplanted with Mmrn1$^{-/low}$ and Mmrn1^{high} LSCs. (D) The spleen, liver and lung index (weights/body weights) of recipient mice at 25 days after transplanted with Mmrn1$^{-/low}$ and Mmrn1^{high} LSCs (n = 6). (E) Hematoxylin & eosin (H&E) staining of spleen, lung and liver of recipients at 25 days after transplanted with Mmrn1$^{-/low}$ and Mmrn1^{high} LSCs. (F) Limiting dilution analysis of the functional LSC frequency in the second recipients at 25 days after transplanted with Mmrn1$^{-/low}$ and Mmrn1^{high} LSCs (n = 5). (G) Kaplan-Meier curves of the third recipients after transplanted with Mmrn1$^{-/low}$ and Mmrn1^{high} LSCs (n = 20). (H) The percentages of mCherry^{+} cells in the PB and BM from the third recipients at 25 days after transplanted with Mmrn1$^{-/low}$ and Mmrn1^{high} LSCs (n = 6). Representative flow cytometric plots are shown in the left. (I) The percentages of c-Kit^{+} mCherry^{+} cells and LSCs in the BM of third recipients at 25 days after transplanted with Mmrn1$^{-/low}$ and Mmrn1^{high} LSCs (n = 6). Representative flow cytometric plots are shown in the left. **P < 0.01, ***P < 0.001

Supplementary Figure S11



Supplementary Figure S11. Mmrn1 deficiency delays AML development. (A, B) $CD34^+$ cells isolated from human AML cases with high or low expression of MMRN1 were transplanted into sublethally irradiated (2.5 Gy) M-NSG mice. (A) Kaplan-Meier curves of the recipients after transplanted with $MMRN1^{low}$ and

MMRN1^{high} CD34^{+} AML cells (n = 20). (B) The percentage of hCD45^{+} cells in the PB and BM of recipients at 25 days after transplantation. (C-H) Lin^{-} cells isolated from WT and Mmrn1$^{-/-}$ mice were transduced with MLL-AF9 retrovirus to generate pre-leukemic cells, which were then transplanted into 7.5 Gy irradiated WT mice. Twenty-five days after transplantation, LSCs were purified from the first recipients and were transplanted into 6.0 Gy irradiated second recipients. (C, F) Kaplan-Meier curves of the (C) first and (F) second recipients after transplanted with WT and Mmrn1$^{-/-}$ LSCs (n = 20). (D, G) The percentage of mCherry^{+} cells in the PB and BM of the (D) first and (G) second recipients at 25 days after transplantation. (E, H) The percentages of c-Kit^{+} mCherry^{+} cells and LSCs in the BM of the (E) first and (H) second recipients at 25 days after transplanted with WT and Mmrn1$^{-/-}$ LSCs (n = 6). (I) The expression of CD41 in Mmrn1$^{-/low}$ and Mmrn1^{high} LSCs after TPO stimulation *in vitro* (n = 6). Data are shown as the mean ± SD. ***P < 0.001.

Supplementary Table S1

Antibodies used for flow cytometry (FC)

Antibodies for mouse	**Cat. No**	**Origin**
Alexa Fluor 700 anti-mouse CD34	56-0341-82	eBioscience
APC anti-mouse CD201 (EPCR)	141505	BioLegend
APC anti-mouse CD86	105113	BioLegend
APC anti-mouse Sca1	108112	BioLegend
APC-eFluor 780 anti-mouse CD3e	47-0031-82	eBioscience
APC-eFluor 780 anti-mouse c-Kit	47-1171-82	eBioscience
Biotin anti-mouse Lineage Panel	133307	BioLegend
Brilliant Violet 510 Annexin V	640937	BioLegend
Brilliant Violet 510 anti-mouse TER-119	116237	BioLegend
Brilliant Violet 510 Streptavidin	405234	BioLegend
eFluor 450 anti-mouse BrdU Staining Kit	8848-6600-42	Thermo Fisher
eFluor 450 anti-mouse CD16/32	48-0161-82	eBioscience
eFluor 450 anti-mouse CD45.1	48-0453-82	eBioscience
eFluor 450 anti-mouse Lineage Cocktail	88-7770-72	eBioscience
eFluor 660 anti-mouse CD34	50-0341-82	eBioscience
PE anti-mouse B220	12-0452-82	eBioscience
PE anti-mouse CD127	12-1271-82	eBioscience
PE anti-mouse CD135	12-1351-82	eBioscience
PE anti-mouse CD150	115904	BioLegend
PE anti-mouse Ki67	151210	BioLegend
PE/Cyanine5 anti-mouse CD16/32	156618	BioLegend
PE/Cyanine7 anti-mouse CD48	103424	BioLegend
PE-Cyanine7 anti-mouse Ki67	25-5698-82	eBioscience
PE-Cyanine7 anti-mouse CD45.2	25-0454-82	eBioscience
PE-Cyanine7 anti-mouse c-Kit	25-1171-82	eBioscience
PerCP-Cyanine5.5 anti-mouse CD11b	45-0112-82	eBioscience
PerCP-Cyanine5.5 anti-mouse Sca1	45-5981-82	eBioscience
Antibodies for human	**Cat. No**	**Origin**
APC anti-human CD38	303510	BioLegend
Brilliant Violet 510 anti-human Lineage Cocktail	348807	BioLegend
PE anti-human CD34	343506	BioLegend
PE-Cyanine5 anti-human CD90	328112	BioLegend
PE-Cyanine7 anti-human CD45RA	304126	BioLegend
APC anti-human CD45	304012	BioLegend

Supplementary Table S2

Primers for mRNA expression analysis

Gene in mouse	**Forward primer (5'-3')**	**Reverse primer (5'-3')**
Cdkn1a	CCTGGTGATGTCCGACCTG	CCATGAGCGCATCGCAATC
Cdkn1b	TCAAACGTGAGAGTGTCTAACG	CCGGGCCGAAGAGATTTCTG
Cdkn1c	CGAGGAGCAGGACGAGAATC	GAAGAAGTCGTTCGCATTGGC
Ccnb1	AAGGTGCCTGTGTGTGAACC	GTCAGCCCCATCATCTGCG
Ccnb2	GCCAAGAGCCATGTGACTATC	CAGAGCTGGTACTTTGGTGTTC
Ccnd1	GCACAACGCACTTTCTTTCCA	GATGGAGGGGGTCCTTGTTTAG
Ccnd2	CATTCAGACACAGGACTT	GTATAGATGCCAAGAAGGAA
Ccne1	CCGTCTTGAATTGGGGCAATA	GAGCTTATAGACTTCGCACACC
Ccne2	TCAGCCCTTGCATTATCATTGAA	CCAGCTTAAATCTGGCAGAGG
Cdk1	AGAAGGTACTTACGGTGTGGT	GAGAGATTTCCCGAATTGCAGT
Cdk2	ACTGAGACTGAAGGTGTA	TTGACGATATTAGGGTGATTA
Ckd4	TGATGGATGTCTGTGCTA	TCCTGGTCTATATGCTCAA
Ckd6	TGGACATCATTGGACTCCCAG	TCGATGGGTTGAGCAGATTTG
Ccno	GTCTGTGACCTTTTCGAGTCC	CTCTGGCCGTATTCTCGGA
Cdc20	TTCGTGTTCGAGAGCGATTTG	ACCTTGGAACTAGATTTGCCAG
Gata1	TGGGGACCTCAGAACCCTTG	GGCTGCATTTGGGGAAGTG
Gata2	CGACGAGGTGGATGTCTTCT	ACAAGTGTGGTCGGCACAT
Fli1	ATGGACGGGACTATTAAGGAGG	GAAGCAGTCATATCTGCCTTGG
Runx1	GATGGCACTCTGGTCACCG	GCCGCTCGGAAAAGGACAA
Mpl	AACCCGGTATGTGTGCCAG	AGTTCATGCCTCAGGAAGTCA
Gp9	TGCGACCACAGATACTCAGG	ACTGAACGCAGGCTATTGTTG
Zfpm1	CCTTGCTACCGCAGTCATCA	ACCAGATCCCGCAGTCTTTG
Tal1	CGCTGCTCTATAGCCTTAGCC	CTCTTCACCCGGTTGTTGTT
Mmrn1	ACCACCATTCTGATAGGCCG	GATCTCCCGCTGATGCATGT
Gapdh	CCTCGTCCCGTAGACAAAATG	TCTCCACTTTGCCACTGCAA
Gene in human	**Forward primer (5'-3')**	**Reverse primer (5'-3')**
MMRN1	TACAGAGAGGCCAAGAGGTT	TCGGTTTCCTGTTTCTGTAGGG
GAPDH	GGAGTCCACTGGCGTCTTCA	GTCATGAGTCCTTCCACGATACC

Supplementary Table S3

Baseline patient characters with respect to MMRN1 expression

Clinical characters	$MMRN1^{low}$ (n=75)	$MMRN1^{high}$ (n=75)	*p* value
Gender, n (%)			0.622
Female	35 (23.3%)	32 (21.3%)	
Male	40 (26.7%)	43 (28.7%)	
Age, n (%)			0.620
<= 60	45 (30%)	42 (28%)	
> 60	30 (20%)	33 (22%)	
WBC count (×10^9/L), n (%)			0.933
<= 20	38 (25.5%)	38 (25.5%)	
> 20	36 (24.2%)	37 (24.8%)	
BM blasts (%), n (%)			0.012
<= 20	37 (24.7%)	22 (14.7%)	
> 20	38 (25.3%)	53 (35.3%)	
PB blasts (%), n (%)			0.252
<= 70	32 (21.3%)	39 (26%)	
> 70	43 (28.7%)	36 (24%)	
Cytogenetic risk, n (%)			< 0.001
Favorable	23 (15.5%)	7 (4.7%)	
Intermediate/normal	44 (29.7%)	38 (25.7%)	
Poor	7 (4.7%)	29 (19.6%)	
FAB classifications, n (%)			< 0.001
M0	3 (2.9%)	12 (11.8%)	
M1	14 (13.7%)	21 (20.6%)	
M2	13 (12.7%)	25 (24.5%)	
M3	14 (9.4%)	0 (0%)	
M4	19 (12.8%)	10 (6.7%)	
M5	11 (7.4%)	4 (2.7%)	
M6	1 (0.7%)	1 (0.7%)	
M7	0 (0%)	1 (0.7%)	
Cytogenetics, n (%)			< 0.001
Normal	38 (32.2%)	31 (26.3%)	
inv(16)	2 (1.7%)	6 (5.1%)	
t(15;17)	10 (8.5%)	0 (0%)	
t(8;21)	6 (5.1%)	1 (0.8%)	
Complex	6 (5.1%)	18 (15.3%)	
FLT3 mutation, n (%)			0.751
Negative	51 (34.9%)	50 (34.2%)	
Positive	24 (16.4%)	21 (14.4%)	
NPM1 mutation, n (%)			0.012
Negative	52 (34.9%)	64 (43%)	
Positive	23 (15.4%)	10 (6.7%)	
RAS mutation, n (%)			0.702
Negative	72 (48.3%)	69 (46.3%)	
Positive	3 (2%)	5 (3.4%)	

n, number of patients; FAB, French-American-British