Supplementary Information

*TET2* deficiency promotes MDS-associated leukemogenesis

Feiteng Huang¹,², Jie Sun¹, Wei Chen³, Lei Zhang¹, Xin He¹, Haojie Dong¹, Yuhui Wu¹, Hanying Wang¹, Zheng Li¹, Brian Ball⁴, Samer Khaled⁴, Guido Marcucci¹,⁴, Ling Li¹

¹Department of Hematological Malignancies Translational Science, Gehr Family Center for Leukemia Research, Hematologic Malignancies and Stem Cell Transplantation Institute, Beckman Research Institute, City of Hope Medical Center, Duarte, CA 91010, USA

²Department of Hematology, Sir Run Run Shaw Hospital, School of Medicine, Zhejiang University, Hangzhou 310016, China

³The Integrative Genomics Core, Beckman Research Institute, City of Hope Medical Center, Duarte, CA 91010, USA

⁴Department of Hematology and Hematopoietic Cell Transplantation (HCT), Beckman Research Institute, City of Hope Medical Center, Duarte, CA 91010, USA

**Correspondence:** Ling Li, Ph.D. Department of Hematological Malignancies Translational Science, Gehr Family Center for Leukemia Research, Hematologic Malignancies and Stem Cell Transplantation Institute, Beckman Research Institute of City of Hope, Monrovia Research Center 2005, 1500 E. Duarte Rd, Duarte, CA 91010. Tel: 626-218-2011, Fax: 1-626-301-8973, Email: lingli@coh.org
Materials and methods

Mice

The Nup98-HoxD13 (NHD13) transgenic mice were from Jackson Laboratory. The Tet2^{fl/fl} (Tet2^{fl}) and Mx1-Cre mice were mated on a C57BL/6J genetic background. The NHD13 mice were crossed with Tet2^{fl}/Mx1-Cre or Tet2^{fl} mice to generate NHD13/Tet2^{fl}/Mx1-Cre and control NHD13/Tet2^{fl} mice. Both genotypes were injected with poly(I:C) at 6-weeks-old. All mice were drug or test naive and not involved in previous procedures. Mice of the same gender and age were randomly divided into groups. Investigators were blinded to mouse genotype while performing treatment or monitoring engraftment or survival. Mice were bred at the City of Hope (COH) animal facility and received autoclaved water and clean food. All mice were subjected to 12-hour light/dark cycles and kept in controlled ambient room temperature and air humidity conditions. All animal procedures were conducted in accordance with established institutional guidance and approved protocols of the Institutional Animal Care and Use Committee at COH.

Cell line and primary cell culture

The human MDS cell line MDS-L was provided and developed by Dr. Kaoru Tohyama. According to the inventor, cells were cultured in RPMI 1640 medium with 10% FBS and 1% penicillin-streptomycin in the presence of 30 ng/mL human recombinant human IL-3. For MDS-L, no known reference STR profiling is available. To avoid misidentification, cross-contamination, or genetic drifting during the experiments, we replaced the cultured cells with original frozen stock (100 vials totals) which was originally directly provided from inventor Dr. Kaoru Tohyama. Human CB CD34+ and primary MDS CD34+ cells were maintained in StemSpan Serum-Free Expansion Media (StemCell Technologies, Inc.) supplemented with recombinant human SCF (50 ng/mL), Flt3 ligand (100 ng/mL), TPO (100 ng/mL), IL-3 (25 ng/mL), and IL-6 (10 ng/mL).

Flow cytometry
Single cell suspensions from BM, spleen and peripheral blood (PB) were stained with required antibodies for 20 minutes at 4 °C, washed and analyzed on a 5-laser, 15 detector LSRII. The DAPI (ThermoFisher Scientific)-negative cell population was selected as living cells. Mouse HSPCs were determined by staining with anti-mouse c-kit, Sca-1, CD16/32 and CD34 antibodies, biotin-linked lineage cocktails (including anti-mouse CD3, CD4, CD8, B220, IgM, CD19, CD11B, CD11c, NK1.1, Gr1, CD41 and Ter119 antibodies (eBioscience)) and FITC-labeled streptavidin. The lineage `c-kit+ Sca-1` population was defined as LK, and the lineage `c-kit+ Sca-1` population as LSK. CMP, GMP and MEP populations were further gated from the LK population based on CD16/32 and CD34 expression. Mouse BM differentiation was evaluated using anti-mouse CD11B, Gr1, B220, CD3 and Ter119 antibodies. Data were analyzed using BD FACS Diva or FlowJo software.

**BM c-kit+ cell selection**

BM c-kit+ cells were isolated using mouse CD117 microBeads (Miltenyi Biotec) according to the kit protocol. Briefly, total BM cells were counted and resuspended in MACS buffer. After staining with CD117 microbeads (20µL per 10^7 total cells, 15 min at 4°C), cells were washed and applied onto MS columns, which were rinsed and placed in a magnetic field. Columns were washed three times with 500 µL buffer and then removed from the separator. Magnetically-labeled cells were flushed out with 1mL buffer. c-kit+ cells were counted and used for further experiments.

**BM transplantation**

BM cells from CD45.2 mutant mice (test cells) and CD45.1 WT mice (support cells) were transplanted intravenously into 6- to 8-week-old CD45.1 recipients irradiated at a dose of 650 cGy. Chimerism of donor-derived hematopoietic cells was monitored by flow cytometry.

**RT-qPCR analysis**
For RT-qPCR, RNA was isolated using Trizol reagent (Invitrogen) or an RNeasy micro kit (QIAGEN), following standard manufacturers’ protocols. cDNA was amplified using a High Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Quantitative real time PCR was performed using TaqMan or SYBR Green master mix (Life Technologies) with 0.2 mM Taqman probe (Life Technologies) or gene-specific primers. Signals were detected with a QuantStudio 7 Flex Real-Time PCR system (Life Biotechnology). Relative expression levels were determined after normalization to GAPDH levels.

**Western blotting**

Cells were lysed in CytoBuster Protein Extraction Reagent (Millipore), and boiled lysates were then resolved on 7.5%, 10% or 12% SDS-PAGE gels and transferred to nitrocellulose membranes (Bio-Rad). The following primary antibodies were used: TET2 (ab124297) from Abcam, ARIH2 (15006-1-AP) from Proteintech, FGFR1 (9740T), P-STAT5 (9351L), P-AKT (4058S), AKT (9272S), P-ERK1/2 (9272S) and ERK1/2 (4695S) from Cell Signaling Technology, beta-actin (sc-69879), STAT5 (sc-74442) and Ub (sc-8017) from Santa Cruz Biotechnology.

**Lentivirus transduction and cell transfection**

First, 293T cells were transiently transfected with pCDH plasmids and pMD2G/pSAPX2 packaging plasmids. Then, 24-36 hours later, supernatants containing replication-incompetent lentiviruses were collected and concentrated using PEG-it (System Biosciences). After titration, target cells were exposed to virus (MOI=10), and 48 hours later, cells were sorted by flow cytometry based on GFP/RFP expression.

**In vitro colony forming assays**
Total BM or c-kit+ cells were obtained and suspended in methylcellulose medium (M3434, StemCell Technologies; colonyGEL 1201, ReachBio Research Labs) and then seeded into plates, according to the manufacturer’s protocol. Colonies were counted 7 days later. For replating assays, cells from each plate were harvested and replated at $5 \times 10^3$ per well.

**Cell viability analysis**

Cell viability was determined using the Cell Titer-Glo Luminescent Cell Viability Assay Kit (G7570, Promega). Briefly, cells were cultured under different treatment conditions and transferred to 96-well plates for 30 min. Kit substrate and buffer reagents were then added to each well and mixed to allow cell lysis. Plates were read on a Microplate reader (Beckman culture DTX880). Results were expressed as a percentage of untreated cells, for three replicates.

**Whole-exome sequencing (WES)**

Initial WES was carried out to identify mutations in gene exomes. Three $\textit{NHD13/}^{\text{fl/fl}}\text{Tet2}^{\text{fl/fl}}$ and five $\textit{NHD13/}^{\text{fl/fl}}\text{Tet2}^{\text{fl/fl}}/\text{Mx1-Cre}$ mice from the same pair of breeders ($\textit{NHD13/}^{\text{fl/fl}}\text{Tet2}^{\text{fl/fl}}$ and $\textit{Tet2}^{\text{fl/fl}}/\text{Mx1-Cre}$) were used to exclude the confounding results of germline mutations. Tet2 was deleted by injection of poly(I:C) at 6-weeks-old and c-kit+ cells were isolated at 20-weeks-old. Genomic DNA was captured with the Agilent SureSelect all mouse exon probes according to the manufacturer’s protocol. 100-bp paired-end sequencing was performed using an Illumina HiSeq 2500 system. Raw sequencing reads were mapped to the whole mouse genome (mm10) using PEMapper/PECaller (https://github.com/wingolab-org/pecaller) with default settings, and variant bases were annotated with SeqAnt (http://seqant.genetics.emory.edu/).

**HPRT mutation analysis**
The HPRT mutation assay was conducted to evaluate spontaneous forward mutation frequency. Briefly, K562 cells were selected in Hypoxanthine–aminopterin–thymidine (HAT) medium for 3 days to remove pre-existing HPRT mutants. Next, the cells were washed and seeded in normal culture medium for 13 days for mutant expression. After the phenotypic expression period, the cells were added to 96-well microplates (4 x 10^5 cells/well) in culture medium with selection using the toxic analogue 6-TG (from Sigma UK) at 0.6 μg/ml. Plating efficiency was determined by culturing 200 cells/well in the absence of 6-TG. After 14 days of culturing, colonies of 20+ cells in diameter were counted as viable expansion. The mutation frequency was determined by dividing the number of 6-TG-resistant colonies by the total number of cells plated, normalized by the plating efficiency.

5-hydroxymethylcytosine DNA-IP (hMeDIP) sequencing and analysis

hMeDIP was performed according to a protocol provided in the SimpleDIP Hydroxymethylated DNA IP kit (Cell Signaling Technology Inc.). First, illumina barcode adapters were ligated to sonicated genomic DNA prior to hMeDIP, and DNA was then denatured and incubated with anti-5hmC antibody at 4 °C overnight. The DNA/antibody complex was then captured on protein G magnetic beads. Enriched DNA was purified and sequenced followed by standard Illumina protocols on the Illumina HiSeq 2500 platform (Illumina). Sequencing reads were aligned to the mouse genome build mm10 using the Burrows-Wheeler Aligner (BWA, v0.7.5a).

hMeDIP-qPCR analysis

hMeDIP assays were performed based on the manufacturer's instructions using the SimpleDIP kit. DNA was denatured and IP’d with anti-5hmC or IgG control antibodies and protein G magnetic beads. After three washes, 5hmC-modified DNA was eluted from beads, purified and used as template for qPCR.
**Statistical analysis**

Data obtained from independent experiments were expressed as means ± SEM, and \( n \) represented the number of samples. Unless otherwise specified, two-tailed Student’s \( t \)-tests were used to compare differences between groups. GraphPad Prism 8 software (La Jolla, CA) was used for statistical analyses. \( P<0.05 \) represented a statistically significant difference.

**Sequences of shRNAs**

**shArih2 #1:**

Sense: 5'-CCGGCCCAGGAAGACTGGTTTGAACGTAAACAGCTTTCTTCCTGAGGTTTTG-3'. Antisense: 5'-AATTCAAAAACCCAGGAAGACTGGTTTGAACGTAAACAGCTTTCTTCCTGAGG-3'.

**shArih2 #2:**

Sense: 5'-CCGGCGCTACCTCTCTCTTTAGGGACTATCTCGAGATAGTCCCTAAAGAGGCCTTTTG-3'. Antisense: 5'-AATTCAAAAATTTGACTCTCTCTCTCGAGATAGTCCCTAAAGAGGCCTTTTG-3'.

**shSetd2 #1:**

Sense: 5'-CCGGTTTGACTCTGGATATAACCTCAGAGATGTCCCTAAAGAGGCCTTTTG-3'. Antisense: 5'-AATTCAAAAATTTGACTCTCTCTCGAGATGTCCCTAAAGAGGCCTTTTG-3'.

**shSetd2 #2:**

Sense: 5'-CCGGATAGTGTGACCTCGCCTTATTCTCGAGATAGTCCCTTTTG-3'. Antisense: 5'-AATTCAAAATAGTGTGACCTCGCCTTATTCTCGAGATAGTCCCTTTTG-3'.

**shTet3 #1:**

Sense: 5'-CCGGTACCTAGACACACTAAGCTCGAGTTTAGTGCTCTAGGTTATTTTG-3'. Antisense: 5'-AATTCAAAATACCTAGACACACTAAGCTCGAGTTTAGTGCTCTAGGTTATTTTG-3'.

**shTet3 #2:**

Sense: 5'-CCGGCCCAACCAAGAACACACTAAGCTCGAGTTTAGTGCTCTAGGTTATTTTG-3'. Antisense: 5'-AATTCAAAATACCTAGACACACTAAGCTCGAGTTTAGTGCTCTAGGTTATTTTG-3'.

**shTET2 #1:** Sigma Cat#TRCN0000192770; **shTET2 #2:** Sigma Cat#TRCN0000418976.
Figure S1

A: Survival of HR-MDS
B: Survival of LR-MDS
C: Survival of AML

E: % Each AML subset

F: Relative TET2 protein level

H: NHD13/Tet2^fl/fl Mx1-Cre

I: Relative TET2 mRNA level

J: Cell number (10^6)

M: HGB

L: Weight (g)

SSC-A
Figure S1. Tet2 deficiency accelerates leukemia transformation in murine model of MDS.
(A) Histogram of TET2 mRNA levels in BM CD34+ cells from 183 MDS patients (GSE19429). (B and C) Overall survival of patients with high-risk MDS (RAEB I, II cases, termed as HR-MDS, GSE19429) and low-risk MDS (RA and RARS, termed as LR-MDS) stratified by TET2 expression into TET2-high and TET2-low groups. Statistical significance was evaluated by log-rank test. (D) Overall survival of patients with AML (BEAT AML) stratified by TET2 mutation status into TET2-WT (n = 427; median survival, 15.76 months) and TET2-Mut (n = 48; median survival, 10.39 months) groups. (E) Proportion of patients either de novo AML or secondary AML in both TET2-WT and -Mutant populations. (F and G) Western blot and q-PCR showing TET2 levels in BM c-kit+ cells from NHD13 mice, as indicated. (H) The scheme shown to generate NHD13/Tet2-KO mice and further analysis. (I) Western blot and q-PCR showing respective TET2 protein and mRNA levels in total BM cells from indicated mice. (J) CBC analysis of indicated mice at 30 weeks old. (K) Total BM cell number of indicated mice at the leukemia stage. (L) Different size and weight of spleens from WT (n = 11), Tet2-KO (n = 11), NHD13 (n = 13) and NHD13/Tet2-KO (n = 12) mice at 30 weeks old. (M) Representative c-kit expression profile of PB, BM and spleen (SP) cells isolated from indicated mice at 30 weeks old. (N) CBC of recipient mice receiving BM cells from NHD13 (n = 5) or NHD13/Tet2-KO (AML) (n = 5) mice at 8-weeks post-transplantation. (O) Survival of mice receiving BM cells from NHD13 (n = 5; median survival, 191 days) or NHD13/Tet2-KO (AML) (n = 5; median survival, 77 days) mice. (P to R) Overall survival of MDS patients (GSE19429) stratified by HOXA9 and TET2 expression levels. (S and T) Overall survival of AML patients with HOXA9high or HOXA9low (BEAT AML) stratified by TET2 mutation status. (U) Survival of HoxA9/Tet2-WT (n = 8; median survival, undefined) and HoxA9/Tet2-KO (n = 8; median survival, 134 days) transplants. (V) Representative Wright Giemsa-stained BM cytospin from HoxA9/Tet2-WT or HoxA9/Tet2-KO transplants. Leukemia blast cells are highlighted by red arrows. *P<0.05; **P<0.01; ***P<0.001, ****P<0.0001.
Figure S2

A

B

C

D

E

F

G

H

I

J

K

L

M

N
Figure S2. Tet2 deletion expands the stem/progenitor pool of murine MDS.
(A) CBCs of indicated mice at a pre-leukemic stage (20 weeks old). (B and C) Gating strategy and representative morphology of monocytes (B) and neutrophils (C) in BM. (D) Frequencies of monocytes and neutrophils in BM of indicated primary mice at a pre-leukemic stage. (E) Total cell number, frequency and apoptosis percentage of LSK subsets in BM of indicated primary mice at a pre-leukemic stage (20-weeks-old). (F and G) Numbers (F) and frequencies (G) of CMP, GMP and MEP subsets in BM of indicated primary mice at a pre-leukemic stage. (H) Representative FACS profiles and cumulative results for cell cycle analysis of BM c-kit+ cells from NHD13/Tet2-WT or NHD13/Tet2-KO mice. (I) 5 X 10^4 total BM cells isolated from NHD13/Tet2-WT or NHD13/Tet2-KO mice at a pre-leukemic stage were plated in duplicate for CFC analysis. Shown are representative morphologies of myeloid colonies grown in the absence of cytokines. (J and K) BM cells isolated from indicated mice were plated in duplicate for CFC analysis, the colony number ratio in each cytokine concentration relative to physiological concentration of cytokines (10ng/mL mIL-3, 10ng/mL mIL-6 and 30ng/mL mSCF) were indicated. (L) Serial replating assays performed using c-kit+ BM cells from indicated primary mice at a pre-leukemic stage. (M to Q) LK cells (2 X 10^5, CD45.2+) from pre-leukemic NHD13/Tet2-WT or NHD13/Tet2-KO mice were mixed together with support cells (2 X 10^5 unfractionated BM cells, CD45.1+) from normal control mice and injected into lethally-irradiated recipient mice. (M) WBC in PB of recipient mice at different time points. (N and O) Quantification of neutrophils and monocytes (N) and frequency of c-kit+ cells (O) in PB of recipient mice at 16-weeks post-transplantation. (P) Wright-Giemsa-stained PB smear shows leukemia blast cells (red arrows) in recipient mice that received LK cells from NHD13/Tet2-KO mice. Scale bars, 20 μm. (Q) Kaplan-Meier comparative survival analysis of NHD13/Tet2-WT and NHD13/Tet2-KO BM reconstituted mice. (R and S) c-kit+ cells (2 X 10^5, CD45.2+) from WT or Tet2-KO mice were mixed together with support cells (2 X 10^5 unfractionated BM cells, CD45.1+) from normal control mice and injected into lethally-irradiated recipient mice. Shown are chimerism of donor-derived cells (CD45.2+) (R) and WBC (S) in PB of WT and Tet2-KO recipient mice at different time points.
Figure S3. Whole-exome sequencing analysis reveals more genetic alterations in HSPCs from pre-leukemic NHD13/Tet2-KO mice.

(A) Venn diagram showing overlap of mutations in NHD13/Tet2-WT and NHD13/Tet2-KO mice at a pre-leukemic stage, as detected by whole-exome sequencing. (B) KEGG pathway analysis of newly acquired mutations and higher VAF mutations (VAF fold change >1.5) in NHD13/Tet2-KO mice. Pathways are ranked based on number of enriched genes. (C) Circos plots depicting the relative frequencies and pairwise co-occurrences of selected genetic alterations in NHD13/Tet2-KO mice. The length of the arc corresponds to the frequency of the first gene mutation, and the width of the ribbon corresponds to the proportion of co-occurrence with the second gene mutation. (D) c-kit+ BM cells from NHD13 mice were transduced with shRNA against indicated genes, q-PCR showing relative mRNA levels. (E) c-kit+ BM cells from NHD13 mice were transduced with shRNA targeting indicated genes and then plated for CFC. Shown are colony number after second plating.
**Figure S4. ARIH2 deficiency promotes MDS cell proliferation.**

(A) Overall survival of AML patients (GSE12417) stratified by ARIH2 expression levels (ARIH2\textsuperscript{high}, n = 40, median survival, 1118 days; ARIH2\textsuperscript{low}, n = 39, median survival, 388 days) groups. (B and C) Overall survival of MDS patients (GSE19429) stratified by SETD2 (B) and TET3 (C) expression. Statistical significance was evaluated by a log-rank test.

(D) Western-blot analysis of indicated proteins in K562 cells transduced with either shRNA targeting endogenous ARIH2 (shARIH2) or control shRNA (shCtrl). (E) and cell growth (F) were monitored as indicated. Each point is the mean of three determinations with error bars indicating standard deviation. (G) Western blot showing respective ARIH2 protein level in MDS-L/TET2-KD cells overexpressed with full-length ARIH2 or MOCK controls. (H and I) Full-length ARIH2 was overexpressed in MDS-L/TET2-KD cells, then cells were assessed for CFC replating (H) and cell number during the culture as indicated (I). (J) MDS-L/TET2-KD cells transduced with full-length ARIH2 (Flag-ARIH2\textsuperscript{WT}) or MOCK control (Flag-Ctrl) were transplanted into NSGS mice, in-vivo engraftment of human CD45\textsuperscript{+} cells in mouse BM was assessed at 4 weeks post-transplantation.
Figure S5

A

Number of somatic mutations per case

MDS  

AML

B

Number of somatic mutations per case

MDS  

AML

C

Number of somatic mutations per case

MDS  

AML

D

Number of somatic mutations per case

MDS  

AML

E

TMB (nonsynonymous)

AML

F

TMB (nonsynonymous)

AML

G

TMB (nonsynonymous)

AML

| K562 cells | Mutation Frequency (10^{-6}) | Fold Change | p-value |
|------------|-----------------------------|-------------|---------|
| shCtrl     | 3.116±0.9251                | 1           | 0.0129  |
| shTET2 #1  | 59.90±13.24                 | 19.22       | 0.0129  |
| shTET2 #2  | 71.93±18.57                 | 23.08       | 0.0208  |

H

Relative read density

I

Arih2  

Setd2  

Tet3
**Figure S5. Tet2 deficiency induces a state of hypermutagenicity.**

(A and B) Numbers of nonsynonymous somatic mutations in MDS/AML cases with or without TET2 (A) or ASXL1 (B) mutations (Wilcoxon’s rank-sum test). (C) Numbers of nonsynonymous somatic mutations in MDS patients with or without DNMT3A mutations. (D) Numbers of nonsynonymous somatic mutations in AML patients with or without DNMT3A, NRAS or FLT3 mutations. (E and F) Tumor mutational burdens (TMB) in TCGA AML cases with or without indicated mutations. (G) HPRT mutation frequencies in shCtrl or shTET2 K562 cells. (H) Distribution of average 5hmC enrichment at all genes in BM c-kit$^+$ cells from Tet2-WT or Tet2-KO NHD13 mice. (I) hMeDIP-qPCR analysis of 5hmC enrichment at mutant loci of indicated genes in BM c-kit$^+$ cells from Tet2-WT or Tet2-KO NHD13 mice. Bars represent mean enrichment over input.

**Figure S6**

![Figure S6](image)

**Figure S6. Vitamin C treatment mimics Tet2 restoration and blocks leukemogenesis.**

(A) Vitamin C treatment of mice reconstituted with NHD13/Tet2$^{fl/fl}$ and NHD13/Tet2$^{fl/fl}$/Mx1-Cre BM. Tet2 was deleted by injection of poly(I:C) at 6 weeks post-transplant. Mice were intraperitoneally injected with normal saline (vehicle) or ascorbate (ASC, 4 g/kg), 5 days per week for 16 weeks. Expression profile of c-kit$^+$ cells in recipient BM were monitored at 24 weeks post-transplant. (B) Leukemia occurrence in vehicle or ASC treated NHD13/Tet2-KO recipients by 24 weeks post-transplant. (C) K562/TET2-KD cells were treated with vehicle or ASC (0.25 mM) for 13 days (during the mutant expression period), HPRT mutation frequencies were evaluated. (D) CFC assay in methylcellulose-containing medium of MDS (MDS-18 and MDS-23, 1 X 10$^5$ CD34$^+$) and normal (cord blood [CB], 1 X 10$^4$ CD34$^+$) cells treated with ASC at indicated dose for 7 days.
| ID  | Diagnosis  | Genotype     | Gender | Age (weeks) | Spleen weight (g) | Total BM (10⁶) | WBC (10⁹) | HGB (g/dL) | MCV (fL) | PLT (10⁹) | c-kit% | c-kit% PB | c-kit% SP | c-kit% BM | Giemsa Staining |
|-----|------------|--------------|--------|-------------|-------------------|----------------|-----------|------------|----------|-----------|--------|------------|-----------|----------|-----------------|
| 359 | Leukemia   | NHD13/Tet2-KO| M      | 24          | 0.27              | 187.32         | 40.71     | 10.5       | 51.5     | 249       | 12.7   | 38.9       | 22.9      |          |                 |
| 165 | Leukemia   | NHD13/Tet2-KO| F      | 30          | 1.275             | 191.35         | 434.6     | 9.9        | 61.1     | 489       | 5.2    | NA         | 6.86      |          |                 |
| 175 | Leukemia   | NHD13/Tet2-KO| F      | 30          | 0.57              | 294.35         | 31.85     | 10.2       | 60       | 1143      | 3.59   | 22.1       | 17.6      |          |                 |
| 132 | Leukemia   | NHD13/Tet2-KO| M      | 27          | 0.247             | 202.32         | 109.4     | 8.7        | 54.6     | 531       | 1.07   | 2.48       | 0.28      |          |                 |
| 149 | Leukemia   | NHD13/Tet2-KO| M      | 30          | 0.2               | 91.35          | 14.44     | 7.2        | 62.1     | 538       | NA    | NA         | 13.3      |          |                 |
| 365 | MDS        | NHD13/Tet2-KO| M      | 30          | 0.15              | 154.92         | 6.61      | 10.9       | 50.1     | 619       | NA    | NA         | 10.74     |          |                 |
| 376 | MDS        | NHD13/Tet2-KO| M      | 30          | 0.15              | 159.65         | 3.82      | 11.4       | 50.3     | 618       | NA    | NA         | 2.169     |          |                 |
| 381 | MDS        | NHD13/Tet2-KO| M      | 30          | 0.13              | 180.45         | 2.33      | 13.3       | 49.2     | 1204      | NA    | NA         | 4.066     |          |                 |
| 384 | MDS        | NHD13/Tet2-KO| F      | 30          | 0.08              | 121.65         | 3.3       | 13         | 51.9     | 668       | NA    | NA         | 2.863     |          |                 |
| 388 | MDS        | NHD13/Tet2-KO| M      | 28          | 0.35              | 118.25         | 4.13      | 9.3        | 49.6     | 2019      | NA    | NA         | 4.912     |          |                 |
| ID | Diagnosis | Genotype | Gender | Age (weeks) | Spleen weight (g) | Total BM (10^6) | WBC (10^9) | HGB (g/dL) | MCV (fL) | PLT (10^9) | c-kit% PB | c-kit% SP | c-kit% BM | Giemsa Staining |
|----|-----------|----------|--------|-------------|------------------|----------------|------------|------------|----------|------------|-----------|-----------|-----------|-----------------|
| 49 | MDS       | NHD13    | M      | 30          | 0.05             | 102.4          | 1.88       | 11.8       | 52.1     | 604        | NA        | NA        | 1.399    | ![Image]         |
| 50 | MDS       | NHD13    | M      | 30          | 0.05             | 101.85         | 2.29       | 11.8       | 52       | 563        | NA        | NA        | 1.497    | ![Image]         |
| 372| MDS       | NHD13    | F      | 30          | 0.05             | NA             | 2.18       | 12.4       | 55.4     | 603        | NA        | NA        | 2.591    | ![Image]         |
| 383| MDS       | NHD13    | F      | 30          | 0.07             | 117.8          | 2.39       | 14.2       | 51.4     | 437        | NA        | NA        | 2.834    | ![Image]         |
| 392| MDS       | NHD13    | M      | 30          | 0.1              | 117.1          | 2.85       | 9.5        | 55.2     | 524        | NA        | NA        | 1.018    | ![Image]         |
| 73 | MDS       | NHD13    | M      | 30          | 0.06             | 104.4          | 2.57       | 10.7       | 52       | 842        | NA        | NA        | 1.547    | ![Image]         |
| 80 | MDS       | NHD13    | F      | 30          | 0.09             | 77.8           | 1.42       | 11.4       | 49.7     | 623        | NA        | NA        | 2.324    | ![Image]         |
| 81 | MDS       | NHD13    | F      | 30          | 0.12             | 94.25          | 1.51       | 11.3       | 54.1     | 541        | NA        | NA        | 2.271    | ![Image]         |
| 137| MDS       | NHD13    | F      | 30          | 0.1              | 79.75          | 1.94       | 11.7       | 56.2     | 712        | NA        | NA        | 2.389    | ![Image]         |
| 145| MDS       | NHD13    | F      | 30          | 0.06             | 92.2           | 0.99       | 7.5        | 51.2     | 406        | NA        | NA        | 2.551    | ![Image]         |

Table S1. *Tet2* loss accelerates leukemogenesis in the *NHD13* mouse model. Included shows all mice from the survival analyses (Figure 1D). CBC, flow cytometry and morphologic analyses were included in the determination of cause of death. In stained panels, leukemia blasts are indicated by green arrows and dysplastic cells by red.
Table S2

| Newly acquired mutations |
|--------------------------|
| Mks1 | Wiz | Tsc22d1 | Dnend3 |
| Rps19bp1 | Abr | Prpf18 | Atpl3a2 |
| Sx6 | Cdk14 | Elf3 | Cypr113 |
| Ascn1 | Gm10375 | Dnah1/b | H2-Q2 |
| Mmrn3 | Pume5 | Rabb5 | Itf2 |
| Mcr3 | Atad2 | Lbr14a | Eip5 |
| Hdg1 | 92300020A06Rik | Myo1h | Sula2 |
| Shkbp1 | Cngb1 | Apo | A430089119Rik |
| Mbd5 | Tars2 | Tnsf15 | Smo3a |
| Fam38h | Babam1 | Ngr2 | Qk |
| Cox41 | Bbtd35/27 | Ype4 | Nova2 |
| Ato29892 | Longp1 | Tnfrsf11b | Tnfrsf11b |
| Tmm40 | A830010M20Rik | Vmmr2116 | Plfn5 |
| Cln6a | Atp10 | Cpgph2a | NONE |
| Fancd2 | Cola5 | Lhpl3 | Glb1 |
| Del2f5 | Creg2 | Mapk8p2 | Asb16 |
| Wast3 | Rappel4 | Zgpat | Rd3 |
| Zfhx1 | Pmm1 | Wip11 | Selenow |
| Lrnf4 | Cyp4a12a | Scl8a1 | Oclnka |
| 2610301B20Rik | Padi2 | Alp4a | Sbr2 |
| Acet1 | Aridc1 | Pchd3 | Aco8 |
| Parp3 | Dcpp2 | 943006910Rik | Gm10510 |
| Dpp10 | Ep400 | Col2a1 | Emer3 |
| Pkk4n | Gm1965 | Ablin3a | Nkx16 |
| Ninm2 | Zp462 | IIs5 | Adarb2 |
| Sh2d5 | Aldh7a1 | Osp1 | S6x3 |
| Setd2 | Hdc9 | Abca13 | Gm16573 |
| Mmnel | Ube4b | Pea15a | A730008H23Rik |
| Pel1 | Cdh16 | Olfr648 | Shn1 |
| Phip4a | Pkh3 | Cdc8 | Smbl1 |
| Pni2n | Tmem2 | Xpot1 | Myb |
| 4930452B06Rik | Itlf1b1 | LOC100862268 | Zp464 |
| Grm3 | Sper2/j | Celsr1 | Mthch2 |
| Edil3 | Acadvl | 4930550C14Rik | Spdy4c |
| Nijn2 | Rlcb | Thsd4 | Onn1 |
| Spala3 | Cola4a | Cntnap1 | Rnt216 |
| Ugt2b36 | Cnpe | Zlp41 | Olfr1010 |
| Ubxod2a | H1965 | Pex19 | Chln1a2 |
| Dzip1 | Rtn4 | Cyp2c88 | Tnsf11b1 |
| Zfand1 | Sacm11 | Fgfl1 | Stry5 |
| Tmem121 | Obbl1 | Dyrk1a | Phf1 |
| Spag5 | Clasp1 | Ngrf | Tspyl4 |
| Tin1 | Triob | Sic2a1 | Adams18 |
| Dun3a5 | Bad | Gmrp2 | Fkbpl1 |
| Dmdw | Svm1n | Cat7 | Cld9 |
| Bdp1 | Plx1n2 | Wd2c | Gbg1 |
| Heq1 | Cxcl2 | Yf6n3 | Lin28a |
| Wnk2 | Araf | Pparc1a | Bcl7b |
| Adam5 | Ot2 | Cntn2 | Mrtfa |
| Oflr20 | Plaur | Aham1 | Adams18 |
| Mpegc | Tmem225 | Eising | Upp9 |
| Gm37956 | Hox15 | Rabb9a | Itf2 |
| Fat3 | Zp467 | Gm875 | Samd5 |
| Dlap2 | Gm4884 | Lce1b | Qcpl |
| Srbk1 | Pdzn4 | Nkap | Mfg8e |
| Rad51c | Vara2 | Nell1 | Rab11fip4 |
| Rps17 | Slic35g3 | Pramel5 | Nav1 |
| Clik2 | Ifq4a | Carmsap3 | Ati2 |
| Slic6a6 | P81 | Hmct2b | Bmp10 |
| Tet3 | Mr5098 | Ubxn2b |

Table S2. KEGG pathway analysis of mutations with increased VAF in NHD13/Tet2-KO mice. 470 genetic alterations with significantly increased VAF (fold change > 1.5) or newly acquired mutations in NHD13/Tet2-KO mice were analyzed for enrichment in KEGG gene sets using DAVID Functional Annotation Tools. Pathways were ranked based on the number of enriched genes.
Table S3

| #CHROM | POS   | REF | ALT  | Gene Name  | Z-score | Survival relevant | Average VAF | Mutation Ratio |
|--------|-------|-----|------|------------|---------|--------------------|-------------|----------------|
| chr1   | 85630543 | C   | G    | SP140      | -4.5    | 0.1191             | 0.6         |                |
| chr6   | 83403661 | CCT | C    | TET3       | -3.16   | 0.5879             | 0.6         |                |
| chr7   | 19081324 | G   | A    | DMWD       | -2.64   | 0.7500             | 0.4         |                |
| chr18  | 37747302 | A   | AAAGG| PCDHGA10   | -1.96   | 0.4421             | 0.4         |                |
| chr9   | 60394218 | G   | C    | THSD4      | -1.92   | 0.2414             | 0.2         |                |
| chr15  | 73523439 | GGT | G    | DENND3     | -1.82   | 0.0675             | 0.4         |                |
| chr8   | 72180049 | A   | ACTT | RAB8A      | -1.62   | 0.2917             | 0.2         |                |
| chr10  | 21154678 | A   | TTTT | MYB        | -1.48   | 0.3056             | 0.2         |                |
| chr7   | 19081324 | G   | A    | DMWD       | -2.64   | 0.7500             | 0.4         |                |
| chr11  | 69798282 | GGA | G    | FGF11      | -1.24   | 0.0624             | 0.4         |                |
| chr13  | 21468303 | C   | G    | NKAPL      | -1.2    | 0.4681             | 0.2         |                |
| chr9   | 110548233| CAG | C    | SETD2      | -1.1    | 0.7523             | 0.6         |                |
| chr1   | 136952050| GA  | G    | NR5A2      | -1.2    | 0.6126             | 0.6         |                |
| chr2   | 84849932 | A   | C    | SLC43A1    | -0.88   | 0.0777             | 0.4         |                |
| chr5   | 110685333| GA  | GA   | EP400      | -0.77   | 0.3077             | 0.2         |                |
| chr4   | 100333765| G   | A    | ROR1       | -0.48   | 0.4118             | 0.2         |                |
| chr15  | 101461017| T   | C    | KRT81      | -0.31   | 0.0511             | 0.2         |                |
| chr2   | 136952050| GA  | GA   | EP400      | -0.77   | 0.3077             | 0.2         |                |
| chr15  | 101461017| T   | C    | KRT81      | -0.31   | 0.0511             | 0.2         |                |
| chr4   | 143949820| G   | C    | PRAMEF25   | 0       | 0.3200             | 0.2         |                |
| chr17  | 23710640 | C   | T    | SSTR5      | 0.06    | 0.2143             | 0.2         |                |
| chr12  | 23710640 | C   | T    | SSTR5      | 0.06    | 0.2143             | 0.2         |                |
| chr5   | 35669434 | G   | C    | PCDHGA10   | 1.73    | 0.4167             | 0.6         |                |
| chr17  | 35669434 | G   | C    | PCDHGA10   | 1.73    | 0.4167             | 0.6         |                |
| chr4   | 23710640 | C   | T    | SSTR5      | 0.06    | 0.2143             | 0.2         |                |
| chr7   | 136952050| G   | A    | ARAP2      | 0.4     | 0.4154             | 0.4         |                |
| chr1   | 136952050| G   | A    | ARAP2      | 0.4     | 0.4154             | 0.4         |                |

Table S3. Top altered genes with AML survival data in PRECOG.

470 genes (newly acquired mutations or higher VAF mutations) in NHD13/Tet2-KO mice were selected and the top 70 altered genes (VAF FC>2, P<0.05) were imported into PRECOG. Shown are 37 genes with meta-z-scores indicating their AML prognostic significance.
| Sample  | MDS18 | MDS23 |
|---------|-------|-------|
| Age (yrs) | 74 | 78 |
| Sex | M | F |
| Subtype | RAEB-I | RAEB-I |
| Cytogenetics | Trisomy 8 | Normal Cytogenetic |
| TET2 mutation | c. 5284A>G (p.I1762V) | c. 2599T>C (p.Y867H) |
| WBC | 7.5 | 14.4 |
| %Blasts (PB) | 1 | 3 |
| % Blasts (BM) | 6 | 11.2 |