Supplementary Methods

Bone marrow (BM) transplantation
Freshly dissected femora and tibiae were isolated from *Mx1*-Cre control, *Mx1*-Cre inv(3), *Mx1*-Cre *Sf3b1*<sup>K700E/WT</sup>, and *Mx1*-Cre inv(3) *Sf3b1*<sup>K700E/WT</sup> CD45.2<sup>+</sup> mice. The BM was spun at 0.5 g by centrifugation and red blood cells (RBCs) were lysed in ammonium chloride-potassium bicarbonate lysis buffer for 5 min. After centrifugation, cells were resuspended in PBS plus 3% fetal bovine serum (FBS), passed through a cell strainer, and counted. Finally, 1.0 x 10<sup>6</sup> total BM cells of *Mx1*-Cre control, *Mx1*-Cre inv(3), *Mx1*-Cre *Sf3b1*<sup>K700E/WT</sup>, and *Mx1*-Cre inv(3) *Sf3b1*<sup>K700E/WT</sup> CD45.2<sup>+</sup> mice were mixed with 1.0 x 10<sup>6</sup> WT CD45.1<sup>+</sup> support BM and transplanted via tail vein injection into lethally irradiated (4.5Gy, twice) CD45.1<sup>+</sup> recipient mice. Chimerism was measured by flow cytometry from the peripheral blood at 4 weeks after transplant (week 0, pre-pIpC [polyinosinic-polycytidylic acid] (p1530, Sigma-Aldrich)). After confirming the engraftment, the recipient mice were treated with pIpC (dissolved with PBS, intraperitoneal injection, 500 µg/body, every other day, three times). Chimerism was followed via flow cytometry from the peripheral blood every 4 weeks. Additionally, for each bleeding, whole blood cell counts were measured on a blood analyzer, and peripheral blood smears were scored. For the serial transplant, we collected whole BM samples eight months after the initial transplant and transplanted the viably frozen whole BM cells into sublethally irradiated (4.5Gy, once) wild-type recipients (1 x 10<sup>6</sup> cells per recipient). For noncompetitive transplantation experiments, 2.0 x 10<sup>6</sup> total BM cells of *Mx1*-Cre inv(3), *Mx1*-Cre *Sf3b1*<sup>K700E/WT</sup>, and *Mx1*-Cre inv(3) *Sf3b1*<sup>K700E/WT</sup> CD45.2<sup>+</sup> mice were injected into lethally irradiated (4.5Gy, twice) CD45.1<sup>+</sup> recipient mice. Five months after pIpC injection, whole BM cells (2.0 x 10<sup>6</sup> cells/recipient) were serially transplanted into lethally irradiated CD45.1<sup>+</sup> recipient mice.

Retroviral Infection
Retroviral vector pMYs-IRES-GFP encoding EVI1 (wild-type and +18) and SF3B1 (wild-type, K700E, K666N, and G740E) and, if necessary, pVSV-G vector (631530, TakaraBio) were transfected into Plat-E or GP2-293 (631530, Takara) cells using Xtremegene 9 (6365809001, Sigma-Aldrich). About 48 hours after transfection, the culture supernatant was filtered through a 0.22 µm filter unit (SLGVR33RS, Merck) and either used for infection or frozen for stock. For the infection into primary cells, the prestimulated cells were infected for 60 hours using 6-well dishes coated with RetroNectin (T100A, Takara Bio). For the infection into cell lines, cells were
incubated in complete media with 20% viral supernatant and 2.5 µg/ml polybrene (12996-81, nacalai tesque). The media was replaced with complete media 8-24 hours after infection.

**In vitro colony-forming assays**
Whole BM cells from *Mx1-Cre* control, *Mx1-Cre inv(3), Mx1-Cre *Sf3b1*<sup>K700E/WT</sup>, and *Mx1-Cre inv(3) Sf3b1*<sup>K700E/WT</sup> mice and seeded at a density of 20,000, 100,000, 200,000 cells/replicate into cytokine-supplemented methylcellulose medium (Methocult M3434, M3436, and M3630, respectively; STEMCELL Technologies). Colonies propagated in culture were scored at day 7-10 and M3434 colonies were replated into new M3434 semisolid media. For cDNA expression experiment, c-Kit<sup>+</sup> cells were selected by anti-mouse CD117 MicroBeads (Miltenyi Biotech) from 14-week-old primary CD45.1<sup>+</sup> mice, and were cultured overnight in Iscove’s Modified Dulbecco’s Medium (IMDM, I3390, Sigma-Aldrich) and 10% FBS medium supplemented with 50 ng/ml recombinant murine SCF (250-03; PeproTech), 20 ng/ml recombinant murine IL-3 (213-13; PeproTech), 20 ng/ml recombinant murine IL-6 (216-16; PeproTech), and 20 ng/ml recombinant murine TPO (315-14; Peprotech). The next day, those cells were infected with retroviral supernatent expressing pMYs-IRES-GFP empty vector, N-terminal 3X HA tagged full-length *EVI1* (NM_001105078.4), and *EVI1* +18 variant cDNA. Three days after infection, 2.0 x 10<sup>4</sup> of GFP<sup>+</sup> cells were FACS-sorted and plated in cytokine-supplemented methylcellulose medium (Methocult M3434; STEMCELL Technologies) in triplicate. For the *in vitro* competitive assay, 1.0 x 10<sup>4</sup> each of GFP<sup>+</sup> and tdTomato<sup>+</sup> cells were FACS-sorted and plated in M3434 semisolid media, followed by replating every 10 days.

**Antibodies, FACS, and Western blot analysis**
All FACS antibodies were purchased from BD Biosciences, eBioscience, or BioLegend. BM mononuclear cells were stained with a lineage cocktail comprised of antibodies targeting CD3, CD4, CD8, B220, CD19, NK1.1, Gr-1, CD11b, Ter119, and IL-7Rα. Cells were also stained with antibodies against c-Kit, Sca1, CD150, and CD48. Cell populations were analyzed using an LSR Fortessa (BD Biosciences) and a FACSLyric (BD Biosciences) and sorted with a FACSArray II instrument (BD Biosciences). We used the following antibodies: B220-APC-Cy7 (clone; RA3-6B2; BioLegend; catalog #: 103224; dilution: 1:200); B220-PerCP-Cy5.5 (RA3-6B2; eBioscience; 45-0452-82; 1:200); CD3-PE-Cy7 (17A2; BioLegend; 100220; 1:200); CD3-APC-Cy7 (17A2; BioLegend; 100222; 1:200); Gr1-APC (RB6-8C5; eBioscience; 25-5931-82; 1:500); CD11b-FITC (M1/70; Biolegend; 101206; 1:200); CD11b-APC-Cy7 (M1/70; BioLegend; 101226; 1:200); NK1.1-APC-Cy7 (PK136; BioLegend; 108724; 1:200); Ter119-APC-Cy7 (Ter119,
BioLegend; 116223; 1:200); c-Kit-APC (2B8; BioLegend; 105812; 1:100); c-Kit-PerCP-Cy5.5 (2B8; BioLegend; 105824; 1:100); c-Kit-BV605 (ACK2; BioLegend; 135120; 1:100); Sca1-PE-Cy7 (D7; BioLegend; 108102; 1:100); CD45.1-FITC (A20; BioLegend; 110706; 1:200); CD45.1-PerCP-Cy5.5 (A20; BioLegend; 110728; 1:200); CD45.1-BV711 (A20; BioLegend; 110739; 1:200); CD45.1-APC (A20; BioLegend; 110714; 1:200); CD45.2-PE (104; eBioscience; 12-0454-82; 1:200); CD45.2-Alexa700 (104; BioLegend; 109822; 1:200); CD45.2-BV605 (104; BioLegend; 109841; 1:200); CD48-PerCP-Cy5.5 (HM48-1; BioLegend; 103422; 1:100); CD150-PE (9D1; eBioscience; 12-1501-82; 1:100); CD135-APC (A2F10; BioLegend; 135310; 1:200); CD16/CD32 (FcγRII/III)-Alexa700 (93; eBioscience; 56-0161-82; 1:100); CD34-FITC (RAM34; BD Biosciences; 553731; 1:50); CD34-PerCP (8G12; BD Biosciences; 345803; 1:50); CD117-PE-Cy7 (104D2; eBioscience; 25-1178-42; 1:100); CD45-APC-H7 (2D1; BD Biosciences; 560178; 1:200); Ly-5.1-PE (BP-1; BD Bioscience; 553735; 1:200). For Western blot analysis, lysate of cultured cells with RIPA buffer (150 mM NaCl, 50 mM Tris, pH 8.0, 1% NP-40, 0.5% sodium deoxycholate, 0.1% SDS) with Halt Protease and Phosphatase Inhibitor Cocktail (78446, Thermo Fisher Scientific) was mixed with Pierce Lane Marker Reducing Sample Buffer (39000, Thermo Fisher Scientific) and denatured by boiling for 5 minutes. The mixture was loaded onto 4–12% Bis-Tris NuPAGE Gels (Thermo Fisher Scientific) followed by wet transfer. The following antibodies were used for Western blot analysis: HA (#3724, Cell Signaling Technology; 1:1,000), Actin (A-5441, Sigma-Aldrich, 1:5,000), rabbit IgG-HRP (#7074, Cell Signaling Technology; 1:10,000), and mouse IgG-HRP (#7076, Cell Signaling Technology; 1:10,000).

**Histological analysis**

Mice were sacrificed and autopsied, and the dissected tissue samples were fixed for 24 hours in 4% paraformaldehyde, dehydrated, and embedded in paraffin. Paraffin blocks were sectioned at 4 mm and stained with H&E. Images were acquired using an Axio Observer A1 microscope (Carl Zeiss).

**Peripheral blood analysis**

Blood was collected by retro-orbital bleeding using heparinized microhematocrit capillary tubes (02-668-25, Thermo Fisher Scientific). Automated peripheral blood counts were obtained using a HemaVet 950 (Drew Scientific) according to standard manufacturer’s instruction. Differential blood counts were realized on blood smears stained using Wright-Giemsa staining and visualized using an Axio Observer A1 microscope.
**Indisulam treatment and the IC50 measurements**

For *in vitro* experiments, indisulam (22759-5, Cayman Chemical) was dissolved in DMSO to make a 100 micromolar stock solution, and this was then added to complete culture media to the appropriate final concentration. Cell lines were plated in 96 well plates and incubated the media including indisulam at concentrations ranging from 0.1 to 100 micromolar with a minimum of three technical replicates per concentration per cell line. Cell viability was measured with the CellTiter Glo reagent (G7570, Promega) as per manufacturer’s instructions. Absolute viability values were converted to percentage viability versus DMSO control treatment, and then non-linear fit of log (inhibitor) versus response (three parameters) was performed in GraphPad Prism v7.0 to obtain an IC50 values.

**mRNA isolation and analysis**

For patient samples, cells were resuspended in TRIzol (Thermo Fisher) and chloroform. RNA was then extracted using RNeasy mini spin columns (Qiagen) per manufacturer’s instructions. The concentration of extracted RNA was determined via NanoDrop (Thermo Fisher) and the quality was assessed by TapeStation analysis (Agilent). Poly(A)-selected Illumina libraries were generated using the TruSeq RNA Library Kit v2 following the manufacturer’s protocol with 100 ng of input RNA. Libraries were amplified by PCR (13 cycles) and samples were submitted for a second round of TapeStation analysis to determine the purity and abundance of the expected ~300 bp PCR amplicon. Samples that met these criteria were subjected to paired-end sequencing on the Illumina HiSeq at a depth of ~50M reads per sample.

For sorted mouse cell populations (live, lineage-negative c-Kit⁺ cells), RNA was extracted using RNeasy columns (Qiagen) per manufacturer’s instructions. RNA was then Poly(A)-selected, and unstranded Illumina libraries were prepared with the standard TruSeq protocol. To select for fragments <400 bp, 0.5× AMPure XP beads were added to the library followed by 1× AMPure XP beads to select for fragments >100 bp. These fragments were then amplified by PCR (15 cycles) and separated by gel electrophoresis (2% agarose). 300 bp DNA fragments were isolated and sequenced on the Illumina HiSeq 2000 at a depth of ~100M 2×49 bp reads per sample. For differential expression analysis, sequenced reads were mapped to mm10 using nf-core/rnaseq v3.0 pipeline¹ with star_rsem aligner. RSEM gene counts were normalized and differentially expressed genes were identified with adjusted p-values < 0.1 by DESeq2 v1.26.0,² where independent hypothesis weighting was applied.³ Variance stabilizing transformed gene counts were used for hierarchical clustering and principal component
Hierarchical clustering was done using Euclidean distance and Complete linkage method. With differentially expressed gene sets, pathway analysis was performed by Enrichr. In parallel, RNA-seq reads were mapped to the transcriptome annotations assembled as described above with RSEM v1.2.4, modified to invoke Bowtie v1.0.0 with the ‘-v 2’ option. Remaining unaligned reads were then mapped to the genome as well as a database of possible splice junctions, consisting of all possible combinations of 5' and 3' splice sites annotated for each gene, using TopHat v2.0.8b. The resulting read alignments generated by TopHat were then merged with the output from RSEM. Human patient samples were accessed via the NCBI Sequence Read Archive. Reads were aligned to Gencode annotation v25 for human using STAR v2.4.1d and quantified by QoRTs v1.1.8, while alignment-free quantification was performed by Kallisto v0.43.0 accounting for hexamer bias and using 100 bootstrap iterations to estimate the uncertainty due to the finite depth of coverage. Differentially expressed genes were then identified from alignment-free data using Sleuth v0.28.1. Splicing defects at known loci (exons etc.) were identified using SUPPA v1.

RT–PCR and quantitative RT–PCR
For cDNA synthesis, total RNA was reverse transcribed to cDNA with the Verso cDNA Synthesis kit (AB-1453/B; Thermo Scientific). The resulting cDNA was diluted 10–20-fold before use. EVI1 splice variants were detected via semiquantitative RT–PCR in the condition listed in Supplementary Table 1. Quantitative RT–PCR (RT–qPCR) was performed in 10-μl reactions with SYBR Green PCR Master Mix (Roche Life Science). All RT–qPCR analyses were performed on CFX Connect™ Real-Time PCR Detection System (BioRad). Relative gene expression levels were calculated using the comparative CT method, and the values were corrected with expression levels of the internal controls Gapdh. Primers used for RT–PCR are listed in Supplementary Table 1.

Chromatin immunoprecipitation (ChIP) and ChIP-seq
Chromatin fractions from HEK293T cells were prepared using the fanChIP method, as described previously. Cells were suspended in CSK buffer (100 mM NaCl, 10 mM PIPES [pH 6.8], 3 mM MgCl2, 1 mM EGTA, 0.3 M sucrose, 0.5% Triton X-100, 5 mM sodium butyrate, 0.5 mM DTT, and protease inhibitor cocktail) and centrifuged (400× g for 5 min, at 4°C) to remove the soluble fraction. The pellet was resuspended in MNase buffer (50 mM Tris-HCl [pH 7.5], 4 mM MgCl2, 1 mM CaCl2, 0.3 M sucrose, 5 mM sodium butyrate, 0.5 mM DTT, and protease inhibitor cocktail) and treated with MNase at 37°C for 3–6 min to obtain
oligonucleosomes. MNase reaction was then stopped by adding EDTA (pH 8.0) to a final concentration of 20 mM. An equal amount of lysis buffer (250 mM NaCl, 20 mM sodium phosphate [pH 7.0], 30 mM sodium pyrophosphate, 5 mM EDTA, 10 mM NaF, 0.1% NP-40, 10% glycerol, 1 mM DTT, and EDTA-free protease inhibitor cocktail) was added to increase solubility. The chromatin fraction was cleared by centrifugation (15,000 rpm for 5 min, 4°C) and subjected to immunoprecipitation with anti-HA antibody (3F10, Roche) and Protein-G magnetic microbeads (Invitrogen). Immunoprecipitates were then washed five times with washing buffer (1:1 mixture of lysis buffer and MNase buffer with 20 mM EDTA) and eluted in elution buffer (1% SDS and 50 mM NaHCO3). The eluted DNA material was fragmented by DNA shearing system (M220 Covaris) and analyzed by deep sequencing, which was performed using a TruSeq ChIP Sample Prep Kit (illumina) and HiSeq2500 (illumina) at the core facility of Hiroshima University. Approximately 29 to 33 million single end reads were obtained and trimmed using cutadapt v1.2.1. 27 to 31 million of reads were mapped to the hg19 reference genome with BWA v0.7.5 and were subjected to further. Peaks were called and differential peaks were detected using bdgdiff module in MACS2 software v2.0.10 with log likelihood ratio = 1. Enriched motifs in differential peaks were found using findMotifsGenome module with mask option and peaks were annotated using annotatePeaks module in HOMER software v4.11.16 Percentages of annotated peaks were shown as Pie chart. Bigwig files were made from bam files by bamCoverage in deepTools software v3.5.1,17 where counts in chrX were ignored and normalized in CPM. Bigwig files were converted to matrix files around the center of the peaks extended to 10 kb up-and-down stream regions by reference-point mode of computeMatrix software. ChIP peaks were then visualized as heatmap.

For ChIP-seq with anti-EVI1 antibody (2593; Cell signalling), we followed the protocol previously described.18 Cells were cross-linked with 1% formaldehyde. Chromatin was isolated using lysis buffer A (50mM Tris pH 8, 10mM EDTA, 1% SDS). At least 30 million cells were double crosslinked with 2mM disuccinimidyl glutarate followed by 1% formaldehyde. Chromatin of double crosslinked cells was isolated using lysis buffer B (10mM Tris pH 7.5, 74mM NaCl, 3mM MgCl2, 1mM CaCl2, 4% NP40, 0.32% SDS). The chromatin was sonicated with a Bioruptor device (Diagenode) using the following settings: 10 cycles of 30 s on, 30 s off. Immunoprecipitation of cross-linked chromatin was performed with antibodies against EVI1. Chromatin bound antibody was precipitated with prot G Dynabeads (Thermo Fisher Scientific) and washed with low salt buffer (20mM Tris pH 8, 2 mM EDTA, 1% Triton, 150mM NaCl), high salt buffer (20mM Tris pH 8, 2 mM EDTA, 1% Triton, 500mM NaCl), LiCl buffer (10mM Tris, 1mM EDTA, 0.25mM LiCl, 0.5% IGEPAL, 0.5% Sodium-Deoxycholate) and TE (10mM Tris pH
8, 1mM EDTA). The ChIP chromatin was eluted in elution buffer (0.1M Sodiumhydrogencarbonate, 1% SDS). Crosslinks were reversed overnight at 65 °C in the presence of proteinase K (New England Biolabs). De-crosslinked material was purified using a QIAGEN PCR Purification Kit. The purified DNA was processed according to the Nextflex ChIP Sample Preparation Protocol (Perkin Elmer) or the Microplex library preparation kit V2 (Diagnode C05010013) and sequenced on the Illumina NovaSeq6000 platform.

**EVI1 minigene construction**

The *EVI1* minigene construct was generated by inserting the DNA fragment containing the human *EVI1* genomic sequence from exon 12 to exon 13 between the BamHI and XhoI restriction sites of pcDNA3.1(+) vector. The sequences of the inserted fragments were verified by sanger sequencing. Mutagenesis of minigene constructs was performed with the Agilent QuikChange II site-directed mutagenesis kits (Agilent) or Q5 Site-Directed Mutagenesis Kit (New England Biolabs) according to the manufacturer’s directions. Primers used in mutagenesis are listed in **Supplementary Table 1**.

**Lariat sequencing**

Branchpoints of the alternative spliced intron of *MECOM/EVI1* were mapped by lariat RT-PCR amplifying branchpoint-spanning fragments from lariat RNAs arising from the splicing of this intron (intron 12 of **Figure 5**). Briefly, the RT reaction was performed with SuperScript IV first strain synthesis kit (Invitrogen) and a primer complementary to the intronic sequences downstream of the 5′ splice sites to generate cDNA from lariat RNAs. The branchpoint spanning fragments were amplified by nested PCR with pairs of outer primers (using RT primer as the reverse primer) and inner primers (listed in **Supplementary Table 1**), cloned into the pGEM-T vector (Promega) and sequenced by Sanger sequencing.

**References**

1. Ewels PA, Peltzer A, Fillinger S, et al. The nf-core framework for community-curated bioinformatics pipelines. *Nat Biotechnol*. 2020;38(3):276-278.
2. Love MI, Huber W, Anders S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol*. 2014;15(12):550.
3. Ignatiadis N, Klaus B, Zaugg JB, Huber W. Data-driven hypothesis weighting increases detection power in genome-scale multiple testing. *Nat Methods*. 2016;13(7):577-580.
4. Chen EY, Tan CM, Kou Y, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics*. 2013;14:128.
5. Kuleshov MV, Jones MR, Rouillard AD, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic Acids Res.* 2016;44(W1):W90-97.

6. Xie Z, Bailey A, Kuleshov MV, et al. Gene Set Knowledge Discovery with Enrichr. *Curr Protoc.* 2021;1(3):e90.

7. Li B, Dewey CN. RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC bioinformatics.* 2011;12:323.

8. Langmead B, Trapnell C, Pop M, Salzberg SL. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome biology.* 2009;10(3):R25.

9. Trapnell C, Pachter L, Salzberg SL. TopHat: discovering splice junctions with RNA-Seq. *Bioinformatics.* 2009;25(9):1105-1111.

10. Hartley SW, Mullikin JC. QoRTs: a comprehensive toolset for quality control and data processing of RNA-Seq experiments. *BMC Bioinformatics.* 2015;16:224.

11. Bray NL, Pimentel H, Melsted P, Pachter L. Near-optimal probabilistic RNA-seq quantification. *Nat Biotechnol.* 2016;34(5):525-527.

12. Pimentel H, Bray NL, Puente S, Melsted P, Pachter L. Differential analysis of RNA-seq incorporating quantification uncertainty. *Nat Methods.* 2017;14(7):687-690.

13. Trincado JL, Entizne JC, Hysenaj G, et al. SUPPA2: fast, accurate, and uncertainty-aware differential splicing analysis across multiple conditions. *Genome Biol.* 2018;19(1):40.

14. Miyamoto R, Yokoyama A. Protocol for fractionation-assisted native ChIP (fanChIP) to capture protein-protein/DNA interactions on chromatin. *STAR Protoc.* 2021;2(2):100404.

15. Miyamoto R, Okuda H, Kanai A, et al. Activation of CpG-Rich Promoters Mediated by MLL Drives MOZ-Rearranged Leukemia. *Cell Rep.* 2020;32(13):108200.

16. Heinz S, Benner C, Spann N, et al. Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol Cell.* 2010;38(4):576-589.

17. Ramirez F, Ryan DP, Gruning B, et al. deepTools2: a next generation web server for deep-sequencing data analysis. *Nucleic Acids Res.* 2016;44(W1):W160-165.

18. Ottema S, Mulet-Lazaro R, Erpelinck-Verschueren C, et al. The leukemic oncogene EVI1 hijacks a MYC super-enhancer by CTCF-facilitated loops. *Nat Commun.* 2021;12(1):5679.
Supplementary Table 1. Primer sequences and PCR conditions for mice genotyping, RT-PCR, and minigene assays.

| Genotyping             | Primer sequences                          | PCR protocol                                      | initial | denature | annealing | extension | cycles | final extension |
|------------------------|-------------------------------------------|--------------------------------------------------|---------|----------|-----------|-----------|--------|-----------------|
| inv (3) transgene      | 5'-GCACCTGCTGCAACACAGCTTGC-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 94℃     | 2 min    | 94℃       | 30 sec    | 72℃    | 1 min 30       |
|                        | 5'-GCACCTGCTGCAACACAGCTTGC-3'             |                                                  |         |          |           |          |        |                 |
|                        | 5'-GCACCTGCTGCAACACAGCTTGC-3'             |                                                  | 95℃     | 3 min    | 95℃       | 30 sec    | 72℃    | 1 min 35       |
|                        | 5'-GCACCTGCTGCAACACAGCTTGC-3'             |                                                  |         |          |           |          |        |                 |
| F13b-1 flanked         | 5'-GCACCTGCTGCAACACAGCTTGC-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 94℃     | 2 min    | 94℃       | 30 sec    | 72℃    | 1 min 30       |
|                        | 5'-GCACCTGCTGCAACACAGCTTGC-3'             |                                                  |         |          |           |          |        |                 |
|                        | 5'-GCACCTGCTGCAACACAGCTTGC-3'             |                                                  | 95℃     | 3 min    | 95℃       | 30 sec    | 72℃    | 1 min 35       |
|                        | 5'-GCACCTGCTGCAACACAGCTTGC-3'             |                                                  |         |          |           |          |        |                 |
| Mx1-Cre                | 5'-GCACCTGCTGCAACACAGCTTGC-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 94℃     | 2 min    | 94℃       | 30 sec    | 72℃    | 1 min 30       |
|                        | 5'-GCACCTGCTGCAACACAGCTTGC-3'             |                                                  |         |          |           |          |        |                 |
|                        | 5'-GCACCTGCTGCAACACAGCTTGC-3'             |                                                  | 95℃     | 3 min    | 95℃       | 30 sec    | 72℃    | 1 min 35       |

RT-PCR for confirming F13b-1 K700E expression

| House F13b-1           | 5'-GCACCTGCTGCAACACAGCTTGC-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 94℃     | 2 min    | 94℃       | 30 sec    | 72℃    | 1 min 30       |
|                        | 5'-GCACCTGCTGCAACACAGCTTGC-3'             |                                                  |         |          |           |          |        |                 |
|                        | 5'-GCACCTGCTGCAACACAGCTTGC-3'             |                                                  | 95℃     | 3 min    | 95℃       | 30 sec    | 72℃    | 1 min 35       |
|                        | 5'-GCACCTGCTGCAACACAGCTTGC-3'             |                                                  |         |          |           |          |        |                 |

RT-PCR for detecting EVI1+8

| human EVI1 exon 8 to 13 from human inv(3) Tg | 5'-GCACCTGCTGCAACACAGCTTGC-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 94℃     | 2 min    | 94℃       | 30 sec    | 72℃    | 1 min 30       |
| human EVI1 exon 12 to 13 from human inv(3) Tg | 5'-GCACCTGCTGCAACACAGCTTGC-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 94℃     | 2 min    | 94℃       | 30 sec    | 72℃    | 1 min 30       |
| human EVI1 exon 12 inner | 5'-GCACCTGCTGCAACACAGCTTGC-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 94℃     | 2 min    | 94℃       | 30 sec    | 72℃    | 1 min 30       |
| human EVI1 exon 12 inner | 5'-GCACCTGCTGCAACACAGCTTGC-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 94℃     | 2 min    | 94℃       | 30 sec    | 72℃    | 1 min 30       |

RT-PCR for lentiviral sequencing

| human EVI1 intron 12 outer | 5'-GCACCTGCTGCAACACAGCTTGC-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 94℃     | 2 min    | 94℃       | 30 sec    | 72℃    | 1 min 30       |
| human EVI1 intron 12 inner | 5'-GCACCTGCTGCAACACAGCTTGC-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 94℃     | 2 min    | 94℃       | 30 sec    | 72℃    | 1 min 30       |

RT-PCR for minigene assay

| minigene-derived human EVI1 | 5'-GCACCTGCTGCAACACAGCTTGC-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 95℃     | 2 min    | 94℃       | 30 sec    | 72℃    | 1 min 40       |
| endogenous human EVI1 | 5'-GCACCTGCTGCAACACAGCTTGC-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 95℃     | 2 min    | 94℃       | 30 sec    | 72℃    | 1 min 40       |

RT-PCR for minigene cloning

| EVI1 minigene, WT | 5'-GCACCTGCTGCAACACAGCTTGC-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 98℃     | 1 min    | 98℃       | 10 sec    | 72℃    | 1 min 30       |

PCR protocol

|                  | PfU Ultra DNA polymerase (20052, Agilent) |                                      | 95℃     | 30 sec   | 95℃       | 30 sec    | 72℃    | 1 min 9         |
|                  |                                          |                                      |         |          |           |          |        |                 |
|                  | PfU Ultra DNA polymerase (20052, Agilent) |                                      | 95℃     | 30 sec   | 95℃       | 30 sec    | 72℃    | 1 min 9         |
|                  |                                          |                                      |         |          |           |          |        |                 |
|                  | PfU Ultra DNA polymerase (20052, Agilent) |                                      | 95℃     | 30 sec   | 95℃       | 30 sec    | 72℃    | 1 min 9         |

PCR protocol

|                  | Q5 Hot Start High-Fidelity 2X Master Mix (E0552, New England Biolabs) |                                      | 98℃     | 30 sec   | 95℃       | 10 sec    | 72℃    | 1 min 5         |
|                  | Q5 Hot Start High-Fidelity 2X Master Mix (E0552, New England Biolabs) |                                      |         |          |           |          |        |                 |
|                  | Q5 Hot Start High-Fidelity 2X Master Mix (E0552, New England Biolabs) |                                      | 98℃     | 30 sec   | 95℃       | 10 sec    | 72℃    | 1 min 5         |
|                  | Q5 Hot Start High-Fidelity 2X Master Mix (E0552, New England Biolabs) |                                      |         |          |           |          |        |                 |
|                  | Q5 Hot Start High-Fidelity 2X Master Mix (E0552, New England Biolabs) |                                      | 98℃     | 30 sec   | 95℃       | 10 sec    | 72℃    | 1 min 5         |

Sanger sequence

| minigene of Pdx1, F wax | 5'-CCTTGTGAGAGCTGACACGATGACGT-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 94℃     | 2 min    | 94℃       | 30 sec    | 72℃    | 1 min 30       |
| polymeric of pdnA3, Rv | 5'-CCTTGTGAGAGCTGACACGATGACGT-3'             | 2xGoTaq GreenMaster Mix (MT123, Promega)          | 94℃     | 2 min    | 94℃       | 30 sec    | 72℃    | 1 min 30       |
**Supplementary Table 2.** Patient characteristics of *EVI1* rearranged myeloid neoplasms with or without *SF3B1* mutations.

|                          | Total cohort (n=46) | *SF3B1*-mut (n=18) | *SF3B1*-WT (n=28) | P-value* |
|--------------------------|--------------------|---------------------|-------------------|----------|
| Age at diagnosis (mean, SD) | 64.3 (13.4)        | 62.8 (12.6)         | 65.3 (14.0)       | 0.55     |
| Male sex (n, %)           | 29 (63.0%)         | 11 (61.1%)          | 18 (64.3%)        | 1.00     |
| Disease (n, %)            |                    |                     |                   |          |
| - AML                    | 25 (54.4%)         | 10 (55.6%)          | 15 (53.6%)        |          |
| - MDS                    | 16 (34.8%)         | 7 (38.9%)           | 9 (32.1%)         |          |
| - Other (blast-phase CML, CMML) | 5 (10.9%)         | 1 (5.6%)           | 4 (14.3%)         | 0.76     |
| Therapy-related MN (n, %) | 7 (15.2%)          | 1 (5.6%)           | 6 (21.4%)         | 0.22     |
| Number of lines of therapy (mean, SD) | 2.2 (1.8)   | 2.6 (1.6)          | 2.0 (1.9)         | 0.24     |
| Best response to first line therapy |                |                     |                   |          |
| - CR/mCR/CRI/PR/MLFS     | 9 (19.6%)          | 2 (11.1%)           | 7 (25.0%)         |          |
| - SD                     | 7 (15.2%)          | 3 (16.7%)           | 4 (14.3%)         |          |
| - Progressive disease/primary induction failure | 24 (52.3%) | 12 (66.7%)         | 12 (42.9%)        |          |
| - not evaluable          | 6 (13.0%)          | 1 (5.6%)           | 5 (17.9%)         | 0.35     |
| Allogeneic hematopoietic cell transplant (n, %) | 13 (28.9%) | 7 (38.9%)          | 6 (22.2%)         | 0.32     |
| Concurrent cytogenetic abnormalities (n, %) |       |                     |                   |          |
| - Del(5q)                | 6 (13.0%)          | 0                   | 6 (21.4%)         | 0.07     |
| - Monosomy 7             | 22 (47.8%)         | 9 (50.0%)           | 13 (46.4%)        | 1.00     |
| - 17p abnormality or monosomy 17 | 4 (8.7%) | 1 (5.6%)           | 3 (10.7%)         | 1.00     |
| - Complex karyotype      | 12 (26.1%)         | 4 (22.2%)           | 8 (28.6%)         | 0.74     |
| - Monosomal karyotype    | 20 (43.5%)         | 5 (27.8%)           | 15 (53.6%)        | 0.13     |
| - t(9;22)                | 3 (6.5%)           | 1 (5.6%)           | 2 (7.1%)          | 1.00     |

* Student’s t-test for continuous variables and Pearson chi-square or Fisher’s exact test for categorical variables
### Supplementary Table 3. *EVI1*-rearranged leukemia cell lines.

| cell line      | SF3B1     | EVI1 rearrangement | age | sex | diagnosis                          |
|----------------|-----------|--------------------|-----|-----|------------------------------------|
| HNT-34         | K700E     | t(3;3)(q21;q26)     | 45  | F   | CMMoL overt AML                    |
| MUTZ-3         | K666N     | inv(3)(q21q26)      | 29  | M   | AML                               |
| YCU-AML1       | K700E     | t(3;3)(q21;q26.2)   | 62  | M   | AML-MRC (MDS/AML)                 |
| OCI-AML-20     | wild-type | inv(3)(q21q26.2)    | 34  | M   | AML                               |
| MOLM-1         | wild-type | inv(3)(q21q26)      | 41  | M   | CML-BC                            |
| Kasumi-3       | wild-type | t(3:7) (q27q22)     | 57  | M   | AML (M0)                          |
| Kasumi-4       | wild-type | inv(3)(q21q26)      | 6   | F   | CML-BC                            |
| UCSD-AML1      | wild-type | t(3;3)(q21;q26)     | 73  | F   | AML                               |

CMMoL, Chronic myelomonocytic leukemia; AML-MRC, acute myeloid leukemia with myelodysplasia-related changes (AML-MRC); CML-BC, chronic myeloid leukemia-blast crisis.
| Gene     | Cancer type | Tumor | Panel Change | Allele Change | Chromosome | Transcript Ref | Seq | Seq | Allele Freq | Annotations |
|----------|-------------|-------|--------------|---------------|------------|----------------|-----|-----|-------------|-------------|
| RECQL4   |             |       | A164Gfs*42   | C             | 5          | N/A            | 0   | 23  | 0.077568134 |             |
|          |             |       |              | A             | 12         | 1295228       |     |     |             |             |
|          |             |       |              | C             | 12         | 47045185      |     |     |             |             |
|          |             |       |              | G             | 12         | 37687220      |     |     |             |             |
|          |             |       |              | A             | 12         | 71247548      |     |     |             |             |
|          |             |       |              | C             | 12         | 123878886     |     |     |             |             |
|          |             |       |              | A             | 12         | 159171695     |     |     |             |             |
|          |             |       |              | C             | 12         | 139267179     |     |     |             |             |
|          |             |       |              | A             | 12         | 168927312     |     |     |             |             |
|          |             |       |              | C             | 12         | 203030899     |     |     |             |             |
|          |             |       |              | A             | 12         | 198746698     |     |     |             |             |
|          |             |       |              | C             | 12         | 198422062     |     |     |             |             |
|          |             |       |              | A             | 12         | 239506021     |     |     |             |             |
|          |             |       |              | C             | 12         | 239506021     |     |     |             |             |
|          |             |       |              | A             | 12         | 239506021     |     |     |             |             |
|          |             |       |              | C             | 12         | 239506021     |     |     |             |             |
|          |             |       |              | A             | 12         | 239506021     |     |     |             |             |
|          |             |       |              | C             | 12         | 239506021     |     |     |             |             |

**Table 4. Mutational analysis of EVI1-rearranged leukemia cell lines.**
Supplementary Table 5. Fraction of EVI1+18 variant in SF3B1-mutated and wildtype cases.

| SF3B1 | SF3B1 -mutation VAF | EVI1 Canonical | EVI1 Novel | Fraction of EVI1 novel (%) |
|-------|----------------------|----------------|------------|--------------------------|
| p.G740E | 35.90% | 80 | 54 | 40.30% |
| p.G740E | 44.00% | 785 | 483 | 38.09% |
| p.G740E | 49.12% | 33 | 20 | 37.74% |
| p.R625C | 47.00% | 381 | 186 | 32.80% |
| p.H662Q | 51.27% | 217 | 73 | 25.17% |
| p.K700E | 54.31% | 66 | 19 | 22.35% |
| p.K700E | 49.00% | 282 | 68 | 19.43% |
| p.K700E | 45.82% | 180 | 37 | 17.05% |
| p.K700E | 22.92% | 258 | 50 | 16.23% |
| p.K700E | 49.12% | 158 | 29 | 15.51% |
| p.K700E | 43.75% | 172 | 29 | 14.43% |
| p.K700E | 55.67% | 65 | 6 | 8.45% |
| p.K666N | 42.01% | 41 | 3 | 6.82% |
| p.K666T | 43.60% | 197 | 11 | 5.29% |

SF3B1-mutated:

| SF3B1 | SF3B1 -mutation VAF | EVI1 Canonical | EVI1 Novel | Fraction of EVI1 novel (%) |
|-------|----------------------|----------------|------------|--------------------------|
| wild-type | (-) | 72 | 1 | 1.37% |
| wild-type | (-) | 82 | 1 | 1.20% |
| wild-type | (-) | 178 | 2 | 1.11% |
| wild-type | (-) | 107 | 1 | 0.93% |
| wild-type | (-) | 253 | 2 | 0.78% |
| wild-type | (-) | 140 | 1 | 0.71% |
| wild-type | (-) | 185 | 1 | 0.54% |
| wild-type | (-) | 263 | 1 | 0.38% |
| wild-type | (-) | 264 | 1 | 0.38% |
| wild-type | (-) | 542 | 2 | 0.37% |
| wild-type | (-) | 567 | 2 | 0.35% |
| wild-type | (-) | 1282 | 2 | 0.16% |
| wild-type | (-) | 18 | 0 | 0.00% |
| wild-type | (-) | 132 | 0 | 0.00% |
| wild-type | (-) | 781 | 0 | 0.00% |
| wild-type | (-) | 147 | 0 | 0.00% |
| wild-type | (-) | 298 | 0 | 0.00% |
| wild-type | (-) | 27 | 0 | 0.00% |
| wild-type | (-) | 384 | 0 | 0.00% |
| wild-type | (-) | 37 | 0 | 0.00% |
| wild-type | (-) | 55 | 0 | 0.00% |
| wild-type | (-) | 162 | 0 | 0.00% |
| wild-type | (-) | 132 | 0 | 0.00% |
| wild-type | (-) | 73 | 0 | 0.00% |
| wild-type | (-) | 146 | 0 | 0.00% |
| wild-type | (-) | 85 | 0 | 0.00% |
| wild-type | (-) | 139 | 0 | 0.00% |
| wild-type | (-) | 114 | 0 | 0.00% |
| wild-type | (-) | 15 | 0 | 0.00% |
| wild-type | (-) | 117 | 0 | 0.00% |
| wild-type | (-) | 130 | 0 | 0.00% |
| wild-type | (-) | 168 | 0 | 0.00% |
| wild-type | (-) | 2 | 0 | 0.00% |
| wild-type | (-) | 467 | 0 | 0.00% |
| wild-type | (-) | 277 | 0 | 0.00% |
| wild-type | (-) | 127 | 0 | 0.00% |

SF3B1-wild-type:
Supplementary Figure 1. The genetic characteristics of EVI1-rearranged (EVI1-r) myeloid neoplasms. (A) Schematic image of inv(3) resulting in EVI1 expression by GATA2 distal hematopoietic enhancer (G2DHE). The bacterial artificial chromosome (BAC) we utilized in the murine model is also shown. (B) Diagram of location of 18 SF3B1 mutations identified in EVI1-r myeloid neoplasms of MSKCC cohort (n=46). HD, HEAT-repeat domain. (C) Correlations between driver mutations in entire MDS/AML cohorts (left) and EVI1-r myeloid neoplasms (right). Significantly co-occurring and mutually exclusive mutations are shown in red and blue circles, respectively. Odds ratio and associated -log10(Q-value) are indicated by the color gradient and size of circles, respectively. Q-values were calculated by Benjamini-Hochberg (BH) adjustment from p-values obtained from Fisher's exact test. (D) Overall survival (OS) from the time of diagnosis and from the time of inv(3) detection in inv(3) AML with (red) or without (blue) SF3B1 mutations.

Supplementary Figure 2. The impact of combined inv(3) and Sf3b1K700E mutations on hematopoiesis and leukemogenesis. (A) Mean number of colonies derived from bone marrow mononuclear cells from Mx1-Cre inv(3) Sf3b1K700E/WT mice and controls. Pre-B colonies are shown on left and BFU-E are on right. Mean ± SD. (B) Peripheral blood counts overtime following transplantation. (C) As in Figure 2E, but for LT-HSC, ST-HSC, GMP, and MEP. (D) BM cytospms of MDS-derived AML mice indicating immature blasts and dysplastic cells (black and red arrows, respectively) within the Mx1-Cre inv(3) Sf3b1K700E/WT group. Scale Bars, 20 μm; x1,000 magnification. (E) The cause of death within the Mx1-Cre inv(3) Sf3b1K700E/WT group. WBC, white blood cell count; Hb, hemoglobin; MCV, mean corpuscular volume; PLT, platelet count; LT-HSC, long-term hematopoietic stem cells, CD150^CD48^LSK; ST-HSC, short-term hematopoietic stem cells, CD150^CD48^LSK; MEP; megakaryocyte-erythrocyte progenitor, CD34^FcyR^-Lin^Kit^-Scal^-; GMP, granulocyte-monocyte progenitor, CD34^FcyR^-Lin^Kit^-Scal^+. P values were calculated by two-sided t-test, *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001.

Supplementary Figure 3. Bone marrow cytomorphology of inv(3) Sf3b1 double mutant recipient mice and controls. (A) Hematoxylin and eosin stain of bone marrow sections of 24-week-old recipient CD45.1 mice transplanted with bone marrow cells of mice with each of the indicated genotypes. The samples were collected 12 weeks after pIpC injection. The BM of the Mx1-Cre inv(3) Sf3b1K700E/WT animals exhibited hypercellularity and a monomorphic cell population. Scale Bars, 100 μm; x100 magnification. (B) BM cytospms of mice from (A) indicating blasts (red arrows) within the Mx1-Cre inv(3) Sf3b1K700E/WT group. Scale Bars, 20 μm; x600 magnification.

Supplementary Figure 4. The inv(3) allele rescues the self-renewal defect of mutant SF3B1. (A) Schema of competitive transplantation of CD45.2 Mx1-Cre inv(3) Sf3b1K700E/WT mice and single mutant controls. (B) % of CD45.2^+ peripheral blood cells in primary (1^st) and secondary competitive transplantation. Mean ± standard deviation shown. (C) % of donor-derived (CD45.2^+) B220^+, CD11b^-Gr1^+, and LSK cells in bone marrow (BM) and/or spleen following 5 months of transplantation. (D) As in (C), but for stem and progenitor fractions. MPP, multipotent progenitors, CD150^-CD48^-LSK; CMP, common myeloid progenitor, CD34^-FCyR^-Lin^-Kit^-Scal^-; P values were calculated by two-sided t-test, *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001.

Supplementary Figure 5. Combined impact of mutations in SF3B1 and EVI1 rearrangement on gene expression and splicing. (A) Significantly dysregulated Gene Ontology (GO) pathways in the transcripts of Mx1-Cre inv(3), Mx1-Cre Sf3b1K700E/WT, and Mx1-Cre inv(3) Sf3b1K700E/WT, compared to those of Mx1-Cre control. Three samples were
independently collected in each group. $\log_{10}(P\text{-values})$ are color-coded. (B) As in Figure 3F, but for the murine model. Each circle shows the number of aberrant splicing events of the indicated model.

**Supplementary Figure 6. The roles of novel EVI1+18 variant.** (A) As in Figure 4C, but for K562 cells expressing SF3B1 wild-type (WT), K666N, K700E, and G740E. (B) As in Figure 4D, sanger sequencing of cDNA showing that the same +18 nucleotides were inserted between exon 12 and 13 in the transcript of Mx1-Cre SF3B1<sup>K700E/WT</sup>; inv(3) mice. (C) The predicted structure of the 2<sup>nd</sup> ZF domain in the EVI1+18 variant. A superposition of AlphaFold2 models of three tandem ZF domains in the C-terminus (residues 909 - 1229) of human MECOM (UniProt accession Q03112) wild-type (grey) and mutant (orange). The 6 amino acid residues (FLLHTG) inserted in the mutant were highlighted by magenta. (D) The relative mRNA expression evaluated by qRT-PCR. (E) Representative FACS plot at day0 and day14 in the competition assay between HSPCs transduced with EVI1 (GFP<sup>+</sup>) and EVI1+18 (tdTomato<sup>+</sup>). (F) The chimerism evaluated by flow cytometry (GFP<sup>+</sup> vs tdTomato<sup>+</sup>) in the replating assay in M3434. (G) GSEA plots for the comparison of the transcripts derived from K562 cells expressing EVI1+18 (left) and EVI1 wild-type (right). P values were calculated by two-sided t-test, **P<0.01, ***P<0.001, and ****P<0.0001.

**Supplementary Figure 7. Spliceosomal disruption may be therapeutically effective against EVI1-rearranged AML with SF3B1 mutations.** (A) The relative growth of each cell line under the condition with the indicated dose (µM) of indisulam for 72 hours. Each growth was calculated in comparison with that of DMSO. (B) IC50 (µM) of indisulam in each cell line.

**Supplementary Figure 8. Anti-EVI1 ChIP-seq of human EVI1-rearranged leukemia cells.** (A) Venn diagram of EVI1 peaks from MUTZ-3, MOLM-1, and HNT-34 cell lines. (B) Transcription factor enrichment analysis in the genes associated with 5,698 peaks (orange section in (A)). (C) Coverage tracks showing EVI1 ChIP-seq occupancy at the indicated genomic loci.

**Supplementary Figure 9. Anti-HA ChIP-seq of 293T cells expressing HA-tagged EVI1 wild-type and +18 mutant.** (A) Immunoprecipitation (IP) with HA followed by immunoblotting with HA in K562 cells that express 3×HA tagged EVI1 wild-type and EVI1+18 cDNA. (B) Pie charts representing the distribution of HA (EVI1 and EVI1+18) binding sites on the genome of 293T cells. (C) Metaplots and heatmaps illustrating log2 ratios of HA peak counts per million (CPM) values of wildtype EVI1 and EVI1+18 against mock ChIP-seq data. (D) Metaplots and heatmaps illustrating CPM values of EVI1 and EVI1+18 ChIP-seq data. (E) As in (D), but for where the peaks were merged and used for illustrating both panels.

**Supplementary Figure 10. Cis elements within EVI1 required for generation of the EVI1+18 splice variant by mutant SF3B1.** (A) Schematic image of lariat RT-PCR using RNA derived from SF3B1-K700E knocked-in K562 cells and mapped branch sites by lariat RNA. For detailed methods, see Supplemental Methods. (B) Mapped branch sites by lariat-sequencing RNA derived from K562 cells with endogenous knock-in of the SF3B1-K700E mutation. (C) RT-PCR analysis of the +18 nucleotides inclusion in a minigene (top) or endogenous (bottom) context following transfection of minigenes with the illustrated mutation (MT3-1) into SF3B1-K666N knocked-in K562 cells. (D) RT-PCR analysis of the +18 nucleotides inclusion in a minigene (top) or endogenous (bottom) context following transfection of minigenes with the illustrated mutations into SF3B1-K666N knocked-in K562 cells and SF3B1 wild-type K562 cells. Each individual minigene construct is shown in Figure 5A. (E) BRD9 transcripts when the poison exon
(14a) is excluded (top) or included (bottom). Green and Red A indicate the branchpoint for canonical and aberrant transcripts, respectively. The exonic splicing enhancer (ESE) in the poison exon is indicated by the blue square. The mutant minigene which alters the normal AG dinucleotide (to AA, top green box) and results in addition of +18 nucleotides (blue rectangle; bottom) and use of an aberrant, intron proximal AG (orange box, bottom). Green and Red A indicate the branchpoint for canonical and aberrant transcripts, respectively. (F) As in (C), but for K562 parental (wild-type) cells with the minigene mutagenized at 3’ splice site (AG to AA). (G) Sanger sequencing of cDNA arising from the top band in (F). The nucleotide sequences are indicated. (H) As in (C), but for MT14 and MT15 described in Figure 5A.
Supplementary Figure 2

A

pre-B colony #

BFU-E #

Colony number

Mxt-Cre

Mxt-Cre Inv(3)

Mxt-Cre Inv(3) S536P/C003

Mxt-Cre Inv(3) S536P/C003 Y296E

B

WBC (x10^9/L)

Hb (g/dL)

Months following transplantation

Mxt-Cre

Mxt-Cre Inv(3)

Mxt-Cre Inv(3) S536P/C003

Mxt-Cre Inv(3) S536P/C003 Y296E

MCV (fL)

PLT (x10^9/L)

Months following transplantation

C

LT-HSC (%)

ST-HSC (%)

GMP (%)

MEP (%)

% of live cells

Mxt-Cre

Mxt-Cre Inv(3)

Mxt-Cre Inv(3) S536P/C003

Mxt-Cre Inv(3) S536P/C003 Y296E

D

E

Mxt-Cre Inv(3) S536P/C003 Y296E

AML

MDS

(n=9)
Supplementary Figure 3

A

Mx1-Cre  
Mx1-Cre inv(3)

Mx1-Cre Sf3b1^{K700E}  
Mx1-Cre inv(3) Sf3b1^{K700E}

B

Mx1-Cre  
Mx1-Cre inv(3)

Mx1-Cre Sf3b1^{K700E}  
Mx1-Cre inv(3) Sf3b1^{K700E}
Supplementary Figure 4

A

B

1\textsuperscript{st} transplant

2\textsuperscript{nd} transplant

\begin{align*}
\text{Months} & \quad 0 & 1 & 2 & 3 & 4 & 5 & 6 & 7 & 8 & 9 & 10 & 11 & 12 & 13 & 14 & 15 \\
\% \text{CD45.2} & \quad \bullet & \quad \circ & \quad \diamond & \quad \triangle & \quad \square & \quad \star & \quad \% & \quad \% & \quad \% & \quad \% & \quad \% & \quad \% & \quad \% & \quad \% & \quad \% & \quad \% \\
\end{align*}

Mx1-Cre inv(3)

Mx1-Cre inv(3) Sf3b1\textsuperscript{K700E/WT}

Mx1-Cre Sf3b1\textsuperscript{K700E/WT}

C

D

LTHSC (%) \quad STHSC (%) \quad MPP (%) \quad CMP (%) \quad GMP (%) \quad MEP (%)

\begin{align*}
\text{Donor-derived chimerism (CD45.2 - %)} & \quad \% & \quad \% & \quad \% & \quad \% & \quad \% & \quad \% \\
\end{align*}
Supplementary Figure 5

A

B

Mx1-Cre St1b1^{loxo6/wt} vs Control

Mx1-Cre inv(3)

St1b1^{loxo6/wt} vs Control

St1b1^{loxo6/wt} vs Control

Mx1-Cre inv(3)

regulation of I-kappaB kinase/NF-kappaB signaling (GO:0034112)
lysosome (GO:0005764)
protein kinase binding (GO:0019021)
eutrophil-mediated immunity (GO:0022446)
eutrophil degranulation (GO:0043312)
eutrophil activation involved in immune response (GO:0022833)
type I interferon signaling pathway (GO:0006337)
GTPase activator activity (GO:0026096)
GTPase binding (GO:0010120)
protein transport (GO:0015021)
regulation of interferon-beta production (GO:0032546)
regulation of innate immune response (GO:0045036)
positive regulation of toll-like receptor 4 signaling pathway (GO:0034145)
cytokine-mediated signaling pathway (GO:0018221)
positive regulation of receptor catalytic process (GO:0000445)
positive regulation of myeloid leukocyte mediated immunity (GO:0022886)
granulocyte activation (GO:0026230)

log_{10}(P-value)

organelle inner membrane (GO:0015800)
mitochondrial inner membrane (GO:0005743)
DNA-dependent DNA replication (GO:0006251)
mitochondrial matrix (GO:0005759)
mitochondrial translation (GO:0032543)
mitochondrial translational elongation (GO:0070126)
DNA replication (GO:0006286)
mitochondrial translational termination (GO:0070126)
RNA binding (GO:0003723)
DNA metabolic process (GO:0006269)
mitotic spindle organization (GO:0070126)
miRNA transport (GO:0011021)
RNA export from nucleus (GO:0006405)
mRNA transport (GO:0011026)
NADH dehydrogenase (quinone) activity (GO:00350136)
Fc receptor signaling pathway (GO:0021980)
mitochondrial respiratory chain complex I (GO:0006747)
herm biogenic process (GO:006783)
chromatin remodeling at centromere (GO:0031055)
Supplementary Figure 6

A

B

Mouse PB tissue (Mef1-Cre SCl17a67 KO, Env(3) mice)

Exon 12  +18bp  Exon 13

D

E

F

G

EVI1  EVI1+18

Bcl11a  Cd34  Hes1  Meis1

Relative expression

% of GFP- or tdTomato+ cells

Ev11 (GFP)  EVI1+18 (tdTomato)

day0  day14

47.9  52.1

9.94  90.1

0  10  20  30

days

0  20  40  60

% of GFP- or tdTomato+ cells
Supplementary Figure 10

A

B

C

D

E

F

G

H

Supplementary Figure 10:

A. Diagram of RT-PCR results from Supplementary Figure 10A.

B. Diagram showing changes in RT-PCR data.

C. Western Blot data comparing SF3B1-K666N with Endogenous.

D. Western Blot data comparing SF3B1-wild type with SF3B1-K666N.

E. Diagram showing changes in RT-PCR data.

F. Western Blot data comparing SF3B1-K666N with Endogenous.

G. Diagram showing changes in RT-PCR data.

H. Western Blot data comparing SF3B1-K666N with Endogenous.