Splicing factor 3b subunit 1 (Sf3b1) haploinsufficient mice display features of low risk Myelodysplastic syndromes with ring sideroblasts

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Abstract

Background: The presence of somatic mutations in splicing factor 3b subunit 1 (SF3B1) in patients with Myelodysplastic syndromes with ring sideroblasts (MDS-RS) highlights the importance of the RNA-splicing machinery in MDS. We previously reported the presence of bone marrow (BM) RS in Sf3b1 heterozygous (Sf3b1+/−) mice which are rarely found in mouse models of MDS. Sf3b1+/− mice were originally engineered to study the interaction between polycomb genes and other proteins.

Methods: We used routine blood tests and histopathologic analysis of BM, spleen, and liver to evaluate the hematologic and morphologic characteristics of Sf3b1+/− mice in the context of MDS by comparing the long term follow-up (15 months) of Sf3b1+/− and Sf3b1+/+ mice. We then performed a comprehensive RNA-sequencing analysis to evaluate the transcriptome of BM cells from Sf3b1+/− and Sf3b1+/+ mice.

Results: Sf3b1+/− exhibited macrocytic anemia (MCV: 49.5 ± 1.6 vs 47.2 ± 1.4; Hgb: 5.5 ± 1.7 vs 7.2 ± 1.0) and thrombocytosis (PLTs: 911.4 ± 212.1 vs 878.4 ± 240.9) compared to Sf3b1+/+ mice. BM analysis showed dyserythropoiesis and occasional RS in Sf3b1+/− mice. The splenic architecture showed increased megakaryocytes with hyperchromatic nuclei, and evidence of extramedullary hematopoiesis. RNA-sequencing showed higher expression of a gene set containing Jak2 in Sf3b1+/− compared to Sf3b1+/+

Conclusions: Our study indicates that Sf3b1+/− mice manifest features of low risk MDS-RS and may be relevant for preclinical therapeutic studies.

Keywords: SF3B1 mice, Myelodysplasia, RNA-sequencing

Background

Myelodysplastic syndrome (MDS) is a heterogeneous group of hematopoietic stem cell disorders characterized by peripheral blood (PB) cytopenias, dysplastic bone marrow (BM), and increased risk of transformation to acute myeloid leukemia (AML). Within MDS, refractory anemia with ring sideroblasts (RARS) is a low-grade disease characterized by anemia, erythroid dysplasia, and the presence of 15% or more RS [1]. Some patients with RARS also present with marked thrombocytosis (RARS-T), a form of myelodysplastic/ myeloproliferative neoplasm (MDS/ MPN) associated with mutations in JAK2, TET2, and MPL genes [2-5]. The presence of RS is a key pathologic criterion for the diagnosis of both RARS and RARS-T. RS are erythroblasts with an abnormal localization of mitochondrial iron which appears in the shape of a blue ring by light microscopy. Studies investigating the mechanisms of RS formation in MDS implicated the mitochondrial genes ALAS2 and ABCB7 based on the gene expression differences detected in CD34-positive cells of RARS and RARS-T patients compared to healthy individuals [6,7]. The discovery of recurrent somatic mutations in splicing factor 3b, subunit 1

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(SF3B1), a component of the RNA splicing machinery in approximately 60% of RARS and 82% of RARS-T patients opened a new area of study in MDS [8-12].

**SF3B1** is a core component of the U2 small nuclear ribonucleoprotein (U2 snRNP). The function of **SF3B1** is to recognize the 3’ splice site at the intron-exon boundaries of pre-nascent RNAs. **SF3B1** protein interacts with the 3’-splice-site recognition of U2AF65 and other splicing factors such as SF3B14 to facilitate the successive steps of RNA splicing [13,14]. Although such as SF3B14 to facilitate the successive steps of RNA splicing, the mechanism for this decline is unclear at present and it is under active investigation.

In terms of MCV, the **Sf3b1**+/− mice have a higher MCV compared to **Sf3b1**+/+ at 6 (46.72 ± 1.32 vs 44.98 ± 2.32) to 12 months (49.50 ± 1.58 vs 47.68 ± 1.40) of age. Levels of statistical significance were reached at 7 (P = 0.047) and 10 (P = 0.031) months of age (Figure 1A).

In terms of Hgb levels, **Sf3b1**+/− mice tend to have lower values compared to **Sf3b1**+/+ at 6, 8, 9, 11, and 12 months of age. Statistically significant difference was noted at month 11 (6.97 ± 1.60 vs 10.04 ± 0.73; P = 0.008) of age (Figure 1B). As expected, the trend of the RBC values paralleled the trend of the Hgb levels with statistical significance being reached at 11 (5.96 ± 0.64 vs. 8.28 M/uL ± 0.48; P = 0.008) and 12 (4.52 ± 0.83 vs 6.09 M/uL ± 0.82; P = 0.027) months of age (Figure 1C). PLT counts increased at month 6 until month 12 of age with a significant difference at month 10 (731 K/uL ± 579 K/uL ± 92.66; P = 0.008) (Figure 1D).

We also observed that after 12 months of age, some of the mice (n = 3) started to show a decline in overall activity characterized by reduced movements and difficulty walking which culminated in death a few weeks later. Two of the deaths were in the **Sf3b1**+/− group while 1 occurred in the **Sf3b1**+/+ cohort.

Since somatic heterozygous mutations in **SF3B1** were also identified in a specific cohort of chronic lymphocytic leukemia (CLL) patients [18] we also measured and analyzed the leukocyte counts of the mice. The leukocyte compartment was primarily enriched with lymphocytes. However, the values were variable over time in both mice groups (data not shown). Moreover, mast cells were also evaluated in BM cells derived from **Sf3b1**+/− (n = 2) and **Sf3b1**+/− (n = 2) by performing immunohistochemistry for CD117 (c-Kit) (Additional file 2: Figure S2). Mast cells noted as CD117 positive cells were rare and scattered and no difference was detected between both groups.

### Results

**Genomic analysis of **Sf3b1** mice**

Embryos of the **Sf3b1** mice were purchased from RIKEN. Mating of **Sf3b1** mice was conducted in-house at the Cleveland Clinic. All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Cleveland Clinic.

None of the **Sf3b1**+/− or **Sf3b1**+/+ mice died immediately after birth and no obvious skeletal abnormalities were noted. There were no reported early deaths in either cohort. A total of 78 mice were analyzed (**Sf3b1**+/−/**Sf3b1**+/+ = 33/45). There were no homozygous **Sf3b1**+/− mice. Tissues from tail and toes were taken in the first 10 days of life and used as a source of genomic DNA. PCR analysis showed that **Sf3b1**+/− mice carried 2 PCR products: a wild type (WT) band at 1.5 kb and knock-out (KO) band at 0.9 kb as shown for mice # 1, 2, 4, and 7 in Additional file 1: Figure S1.

**Hematologic findings of **Sf3b1**+/− mice**

Mouse models of MDS demonstrate specific features resembling human MDS disease albeit at variable time points [17]. This fact underlines the importance of long term follow-up of mouse models to accurately capture disease-related events. We examined the standard hematologic parameters of **Sf3b1**+/− (n = 5) and **Sf3b1**+/+ (n = 5) starting from 6 months of age every month. After 6 months of age, fertility of breeding pairs dropped dramatically. No progeny was produced by mice of this age or older. The mechanism for this decline was unclear at present and it is under active investigation.

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We next examined the morphology of BM cells derived from $Sf3b1^{+/−}$ and $Sf3b1^{+/+}$ mice. BM (2–3 × 10^5) cells were spotted on cytospin slides and stained with Wright-Giemsa. BM cells from $Sf3b1^{+/−}$ showed dyserythropoietic features including nuclear budding or nuclear irregularity (Figure 2, red arrows) similar to what is observed in human MDS. Similar features were also noted in slides stained with Prussian blue (Additional file 4: Figure S4, black arrows). We originally reported the presence of rare RS in BM slides from $Sf3b1^{+/−}$ mice [15]. We confirmed this observation by performing Prussian blue staining on fresh BM cytospin slides and finding occasional RS (Figure 3, black arrows) in the BM of $Sf3b1^{+/−}$ mice while BM cells from $Sf3b1^{+/+}$ only showed iron accumulation in histiocytes. RS were noted in several BM slides as shown in Additional file 5: Figure S5, black arrows.

The spleen and liver from both groups of mice were also dissected, measured, and histopathologically examined at the end of the study. Spleen and liver weights were compared between $Sf3b1^{+/−}$ (n = 4) and $Sf3b1^{+/+}$ (n = 3) (0.10 ± 0.02 vs. 0.08 ± 0.01; $P = 0.08$; 1.13 ± 0.15 vs. 1.36 ± 0.21; $P = 0.14$). Microscopic examination of the spleen tissues showed significant expansion in the red pulp of $Sf3b1^{+/−}$ mice with finding of extramedullary hematopoiesis (EMH) with hematopoietic elements, increased megakaryocytes with hyperchromatic nuclei, increased hemosiderin deposits and signs of fibrosis (Figure 4) but no hepatomegaly or microscopic abnormalities in the liver were noted (Additional file 6: Figure S6).

**RNA-sequencing analysis showed overexpression of Jak2 and other hematopoietic-related gene sets**

We performed RNA-sequencing to characterize and compare the transcriptome profile of BM cells derived from 2 female $Sf3b1^{+/−}$ and 2 female $Sf3b1^{+/+}$ mice. Total 100-bp reads (mapped to mm10 genome reads) in millions for the four mice were 35.54 (22.29), 31.18 (18.50), 35.07 (21.09) and 48.48 (26.28), of which 17.69, 15.54, 16.70 and 22.52 million reads, respectively, mapped to 20,207 mouse genes. After filtering by gene intensity, 17.67, 12.46, 16.67 and 22.49 million reads, respectively, mapped to 10,330 genes.

Global gene level differential expression analysis of these 10,330 genes did not find any significant differential expression in $Sf3b1^{+/−}$ compared to $Sf3b1^{+/+}$ mice (Additional file 7: Table S1). The target gene $Sf3b1$ showed evidence of...
Figure 2 Bone marrow morphology in Sf3b1+/− compared to Sf3b1+/+ mice. Bone marrow (BM) cells were extracted by flushing femurs of Sf3b1+/− (n = 5) and Sf3b1+/+ (n = 5) in media supplemented with 10% fetal bovine serum. Cells (2–3 x 10⁵) were washed and spotted on cytospin slides prior immersion in buffered Wright-Giemsa staining solution. Budding and irregular nuclei are indicated in red arrows and are also magnified in the right quadrants. This feature was also observed in slides of BM cells from Sf3b1+/− mice subjected to iron staining (Additional file 4: Figure S4). The image is presented for 1 Sf3b1+/− mouse.

Figure 3 Detection of ring sideroblasts by Prussian blue staining in Sf3b1+/− compared to Sf3b1+/+ mice. Bone marrow cells were extracted from femurs of Sf3b1+/− (n = 5) and Sf3b1+/+ (n = 5) and cells (2–3 x 10⁵) spotted on cytospin slides prior staining with Prussian blue. Ring sideroblasts (RS) were detected in Sf3b1+/− compared to Sf3b1+/+ mice. Images were taken from 2 mice per group. RS were also detected in additional mice as shown in Additional file 5: Figure S5.
down-regulation [fold change (FC) = 0.75; \( P = 0.075 \), rank = 309] in Sf3b1\(^{-/-}\) vs Sf3b1\(^{+/+}\) mice. Since this Sf3b1\(^{-/-}\) mouse model was originally developed to study the interaction of Sf3b1 protein and proteins of the polycomb (PcG) complex, we also evaluated the status of known PcG genes, finding a trend towards lower mRNA levels of Ezh2 (FC = 0.02; \( P = 0.185 \), rank = 1387). We also found higher mRNA levels of Bmi1 (FC = 1.69; \( P = 0.138 \), rank = 471), a component of the PcG repressive complex, which is involved in axial skeletal development. This is likely a consequence of repression of the Hox genes. Bmi1 has been associated with progressive loss of proliferative capacity of hematopoietic stem cells and anemia. In addition, gene expression analysis of genes important in MDS pathogenesis showed weak evidence for lower mRNA expression levels of Npm1 (FC = 0.01; \( P = 0.184 \), rank = 1363) and no evidence of changes for Asxl1 and Runx1 (FC = 1.25; \( P = 0.296 \) and FC = 1.21; \( P = 0.471 \)) in Sf3b1\(^{-/-}\) vs Sf3b1\(^{+/+}\).

Because in human MDS, SF3B1 clones are found in early hematopoietic stem cells, [19] we interrogated gene sets and genes related to hematopoietic stem cell function and signaling. In total 39 gene sets were selected from the MSigDB c2 collection (gene set results in Additional file 8: Table S2; gene results for members of the gene sets in Additional file 9: Table S3) and showed that hematopoietic receptors mainly expressed in myeloid cells like Trem1 and transcriptional factors involved in hematopoietic development like Ptsg2 (Cox2) were over-expressed in Sf3b1\(^{-/-}\) (FC = 2.80, \( P = 0.011 \) and 2.43, \( P = 0.028 \)). Thrombospondin-1 (Thbs1), a glycoprotein involved in the in-vitro proliferation of megakaryocytes was also one of the highest ranked genes and was found to be over-expressed (FC = 2.67, \( P = 0.008 \)). Haploinsufficiency of Nr4a1 and Nr4a3, two nuclear receptors expressed in hematopoietic stem and myeloid cells, has been shown to cause MDS/MPN and leukemic evolution in mice [20]. In patients with MDS carrying SF3B1 mutations, the risk of AML transformation is less compared to those with WT SF3B1. In this study, Nr4a1 was found to be over-expressed in Sf3b1\(^{-/-}\) mice compared to Sf3b1\(^{+/+}\) mice (FC = 2.29, \( P = 0.038 \)) but Nr4a3 was not detected. We also observed some evidence of down-regulation in Sh2b3 (Lnk) and Calr in Sf3b1\(^{-/-}\) (FC = 0.22, \( P = 0.261 \) and FC = 0.33, \( P = 0.193 \)) compared to Sf3b1\(^{+/+}\) mice. Mutations in both genes have been found in human MPNs. In addition, a Stat5 target gene set showed some evidence of increased expression in Sf3b1\(^{-/-}\) mice (gene set \( P = 0.064 \)).

From our global gene set analysis of collections c1 through c7, we found 1 significant gene set in the human
higher expression in Sf3b1 consistently identified although in small numbers in the evidence of RS in erythroid precursors were once again enlarged spleen and an evidence of EMH. Definitive evidence of RS in human RARS and RARS-T. The RNA-sequencing of Sf3b1+/- mice describing that besides a decrease in the number of hematopoietic cells and a reduced capability of hematopoietic reconstitution, no features of MDS were observed. Based on their results there was no change in the number of WBC and PLTs and in the content of Hgb up to 44 weeks (Additional file 1: Figure S1, panel A) [22]. Morphologically, Matsunawa et al. did not detect any RS and any change in spleen size by weight estimation. Although, the same mouse model was used, there are key differences in the methodology that significantly affected the outcomes of both studies. Our current study aimed to study specifically the morphologic features of this mouse model using conventional routine techniques used in the assessment of clinico-pathologic features of human MDS and MPN and during long term follow-up. This is an important difference since some mouse models exemplified by Sall4 (14.5 months), Evi1/Evi1t (12 months), NPM-1 (6–18 months) and Arid4a (12–22 months) did not show their respective phenotypes until the mouse models were much older and had longer follow-up [17]. This is in keeping with human MDS, where the vast majority of patients are diagnosed at an elderly age with a median age of diagnosis of 71 years old [23]. Next Matsunawa et al. did not analyze specifically the dysplastic morphologies and no images of cellular morphology of the BM aspirates have been shown. The tabulated hematologic results presented in their study showed a lower percentage of erythroid cells in Sf3b1+/- mice compared to Sf3b1+/- which are important clinical features of RARS-T patients. In humans, somatic mutations in JAK2 have been associated with disorders characterized by increased number of PLTs like RARS-T and related MPNs [5]. Patients with JAK2 mutations are also frequently found to have an enlarged spleen and an evidence of EMH. Definitive evidence of RS in erythroid precursors were once again consistently identified although in small numbers in the Sf3b1+/- and not in the Sf3b1+/- mice supporting our initial report that demonstrated rare RS in this mouse model. Somatic mutations in SF3B1 have also been found in 7-15% of CLL patients and associated with aggressive phases of the disease, relapsed and chemotherapy resistant CLL [18]. The link between SF3B1 mutations and CLL pathogenesis remain unclear. In MDS, mutations have been associated with a better survival outcome and a lower rate of AML transformation. Interestingly, we noticed an enrichment of the lymphocyte compartment in our mouse model although the increase was variable over time. Studies of Sf3b1 haploinsufficiency identified a reduction of hematopoietic stem cell pool confined in the myeloid compartment. Our data differ from a recent paper where Sf3b1+/- haploinsufficiency appears to only lead to an impairment in the stem cell function but does not lead to MDS features in the same mouse model [22]. Matsunawa et al. investigated the functional role of Sf3b1 in normal hematopoiesis in this mouse model describing that besides a decrease in the number of hematopoietic cells and a reduced capability of hematopoietic reconstitution, no features of MDS were observed. Based on their results there was no change in the number of WBC and PLTs and in the content of Hgb up to 44 weeks (Additional file 1: Figure S1, panel A) [22]. Morphologically, Matsunawa et al. did not detect any RS and any change in spleen size by weight estimation. Although, the same mouse model was used, there are key differences in the methodology that significantly affected the outcomes of both studies. Our current study aimed to study specifically the morphologic features of this mouse model using conventional routine techniques used in the assessment of clinico-pathologic features of human MDS and MPN and during long term follow-up. This is an important difference since some mouse models exemplified by Sall4 (14.5 months), Evi1/Evi1t (12 months), NPM-1 (6–18 months) and Arid4a (12–22 months) did not show their respective phenotypes until the mouse models were much older and had longer follow-up [17]. This is in keeping with human MDS, where the vast majority of patients are diagnosed at an elderly age with a median age of diagnosis of 71 years old [23]. Next Matsunawa et al. did not analyze specifically the dysplastic morphologies and no images of cellular morphology of the BM aspirates have been shown. The tabulated hematologic results presented in their study showed a lower percentage of erythroid cells in Sf3b1+/- mice compared to Sf3b1+/- further supporting our findings (P = 0.07). The histomorphologic features of the spleen, a frequently affected organ in human RARS-T were also not studied in the prior study. Our study showed that the spleen of the Sf3b1+/- was not just enlarged but displayed architectural changes consistent with EMH akin to patients with human RARS-T. The RNA-sequencing results also support the fact that Sf3b1+/- mice have a pattern more close to low rather than to high-risk MDS. In human MDS, ASXL1 mutations have been found enriched in patients with high-risk rather than in low-risk MDS and are correlated with unfavorable outcomes and AML transformation. In addition patients with ASXL1 mutations carry concomitant RUNX1 mutations and lower incidence of SF3B1 mutations [24]. Studies in mice showed that Asxl1 haploinsufficiency leads to a reduced hematopoietic stem cell pool, decreased hematopoietic repopulating capacity, and mild features of MDS [25]. On the same line, mice expressing the RUNX1 frameshift mutation (S291fs) develop signs of MDS including excess of blasts and dysplasia of the erythroid compartment [26]. In our mouse model, we observed minimal changes in the expression levels of both Asxl1 and Runxl, factors traditionally associated with more inferior outcomes in
patients with MDS further supporting the natural history of human RARS-T probably due to the fact that $Sf3b1^{+/−}$ mice do not manifest a late stage higher risk MDS disease. Indeed we did not observe any increased in blasts percentage and any sign of AML development despite the long term follow-up.

Our clinicopathologic results are further supported by RNA sequencing analysis where we found an over-expression of Jak2 and a down-regulation of Sh2b3 and Calr mRNA levels consistent with what is observed in human RARS-T. In regards to RS we consistently identified RS in the BM of these mice by using two blinded independent hematopathologists and this is unlikely to be simply a matter of chance. Lastly, using the guidelines established by the hematopathology subcommittee of the *Mouse Models of Human Cancers Consortium*, [17,21], it clearly shows that this mouse model fulfills the criteria for an MDS mouse model (Additional file 10: Figure S7).

**Conclusions**

In conclusion, our current data show that $Sf3b1$ haploinsufficiency in mice causes biological and morphological features resembling low risk MDS patients with RS specifically RARS and RARS-T opening the possibility that this mouse model can be helpful in testing therapeutic approaches in low risk MDS.

**Methods**

**Mice**

All procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of the Cleveland Clinic. $Sf3b1^{+/−}$ mice were originally developed by Isono *et al.* [16] Cryopreserved embryos of $Sf3b1^{+/−}$ mice were purchased from Dr. H. Koseki and Dr. K. Isono from the Center for Integrative Medical Sciences (IMS) RIKEN (Japan) in early 2012. Embryos were successfully implanted in foster mothers and rederived mice were genotyped.

**Genotyping**

DNA derived by tail and toe clippings was extracted using a Puregene Core kit A (Qiagen, Valencia, CA) following the manufacturer's instruction. DNA (100 ng) was used for PCR amplification using 3 sets of primers: primer #1 [specific for the *neo* gene (5′ GCGTGCAATCCATCTTG)], primer #2 [specific for $Sf3b1$ (5′ AAGAATTGCTATTGACACTTCTTCA)], and primer #3 [specific for $Sf3b1$ (5′ GACTGAGCTGATAACATG)]. PCR conditions were: initial denaturation at 98°C for 1 min, 35 cycles (94°C for 1 min, 60°C for 1 min, 72°C for 2 min) and a final extension at 72°C for 7 min. PCR products were resolved on 1.2% agarose gels. Gel micrographs were acquired using a Quantity One 1D-analysis software (Bio-Rad Laboratories, Hercules, CA).

**Long-term evaluation of $Sf3b1^{+/−}$ mice**

A total of 5 $Sf3b1^{+/−}$ (3 females/ 2 males) and 5 $Sf3b1^{+/+}$ (3 females/ 2 males) were maintained on a regular diet. Blood was collected by retro-orbital puncture in heparinized tubes every month. Blood was diluted 1:1 with PBS containing 2.7 mM EDTA and standard blood parameters [leucocyte counts, mean corpuscular volume (MCV), red blood cells (RBC), hemoglobin (Hgb), and platelets (PLTs)] were measured using a Hemavet 950 FS analyzer (Drew Scientific Incorporation, Dallas, TX). Mice were sacrificed at the endpoint of the study and tissues were collected as following: femurs from 2 mice per genotype were submitted for hematoxylin and eosin (H&E) stain, BM from all mice was flushed with Iscove’s Modified Dulbecco’s media plus 10% fetal bovine serum using a 25-gauge needle syringe from femurs and evaluated for cell count using a Vi-Cell™ XR cell viability analyzer (Beckman Coulter, Brea, CA). The spleen and liver were also fixed in 4% formaldehyde/PBS and stained with H&E.

**Histomorphological analysis and Prussian blue staining**

BM cells from femurs of $Sf3b1^{+/−}$ and $Sf3b1^{+/+}$ mice were flushed with Iscove’s Modified Dulbecco’s medium plus 10% fetal bovine serum (FBS). Cells (2x10⁶) were washed once with PBS supplemented with 2% FBS and spotted on cytopsin slides before Wright-Giemsa and Prussian blue stains were performed using standard histopathology staining procedures. Spleen and liver from $Sf3b1^{+/−}$ and $Sf3b1^{+/+}$ mice were fixed in 4% formaldehyde/PBS and embedded in paraffin before H&E staining.

**RNA Sequencing (RNA-Seq) analysis**

**Mapping**

Total RNA was extracted from whole BM of 6-month-old female $Sf3b1^{+/−}$ (n = 2) and $Sf3b1^{+/+}$ (n = 2) mice using NucleoSpin RNA II (Clontech Laboratories). PolyA cDNA was prepared from 3 μg of RNA and mouse RNA-sequencing was run on Illumina HiSeq2000 by Otogenetics (Norcross, GA). 100 basepair paired-end RNA-sequencing reads were mapped to the mm10 RefSeq mouse transcriptome and spliceome by DNAnexus (http://dnanexus.com) using a Bayesian method where a read was mapped when its posterior probability of mapping exceeded 0.9. These filtered posterior probabilities were summed to generate fractional read counts per gene and per exon, with probabilities from splice-junction spanning reads counted for each relevant exon. We used rounded gene and exon read counts as inputs for our differential expression analyses.

**Differential gene expression analysis**

We used TMM [27] normalization and the voom-limma approach [28] from the R package limma version 3.17 with R version 3.0.1 in order to perform differential gene
expression analysis for $S_{β}β_{1}^{+/−}$ versus $S_{β}β_{1}^{+/+}$ samples. Before testing, we dropped all genes with read counts per million reads less than or equal to 1 in at least 2 samples to improve testing power while maintaining type I error rates. We used the limFit function with empirical Bayes shrinkage to estimate fold changes, p-values and adjusted p-values obtained using the Benjamini-Hochberg method [29] for each filtered gene under the null hypothesis of common expression intensity across groups. Genes with adjusted p-values less than 0.10 were declared significant.

**Differential exon usage analysis**
We used the R package DEXSeq, version 1.6 (http://www.bioconductor.org/packages/release/bioc/html/DEXSeq.html), to perform differential exon usage analysis of $S_{β}β_{1}^{+/−}$ versus $S_{β}β_{1}^{+/+}$ samples. DEXSeq uses a negative binomial (NB) distribution to model the exon read counts and shrinkage estimators to estimate the per-exon NB dispersion parameters. We defined a testable exon as one that had a total sum of at least 8 mapped reads across samples and was in a gene with no more than 70 exons. Before exon usage testing, we dropped any exons that were not testable or were in genes with less than 2 testable exons to improve testing power while maintaining type I error rates. We used the testForDEU function, which compares deviations from generalized linear model fits (assuming NB likelihood) to a chi-squared reference distribution, to estimate p-values and adjusted p-values obtained using the Benjamini-Hochberg method for each exon under the null hypothesis of common usage across groups. Exons with adjusted p-values less than 0.10 were considered significant. Logarithm base 2 fold changes ($S_{β}β_{1}^{+/−}$/$S_{β}β_{1}^{+/+}$) for each exon were estimated using the function estmateLog2FoldChanges.

**Gene set differential expression analysis**
We used CAMERA [30], Competitive Gene Set Test Accounting for Inter-Gene Correlation approach, as implemented in the camera function from the R package limma version 3.17.17, on TMM normalized and voom weighted expression analysis for all tested genes not in the gene set. Any gene sets with adjusted p-values less than 0.10 were declared significant.

**Statistical analysis**
Comparison of hematologic parameters between $S_{β}β_{1}^{+/−}$ and $S_{β}β_{1}^{+/+}$ mice were analyzed using two-sample Wilcoxon signed rank test and presented as mean ± standard deviations. Statistical analyses were performed using R (www.r-project.org). Data were considered statistically significant if the P value was ≤ 0.05.

### Additional files

**Additional file 1: Figure S1.** Genotyping of wild type (Sββ1+/+) and Sββ1 haploinsufficient (Sββ1−/+ ) mice. Genomic DNA was extracted from tails and toes of Sββ1+/+ pups after rederivation and subjected to PCR amplification by using specific primers as described in Methods. Examples of Sββ1+/+ mice (1, 2, 4, and 7) identified by the presence of amplicons corresponding to wild type (WT: 1.5 Kb) and knock-out (KO: 0.9 Kb) alleles on a 1.2% agarose gel. Lane marked with MW indicates 1Kb Plus DNA ladder.

**Additional file 2: Figure S2.** Sββ1−/+ have no difference in the mast cell compartment compared to Sββ1+/+ mice. Immunohistochemistry (IHC) was used to evaluate the presence of mast cells in Sββ1−/+ (n = 2) and Sββ1+/+ (n = 2). Bone marrow cells (5 x 10⁷) were spotted on cytospin slides and IHC for CD117 (c-Kit) was performed.

**Additional file 3: Figure S3.** Sββ1−/+ have no difference in bone marrow cellularity compared to Sββ1+/+ mice. (a) Hematoxylin/eosin (H&E) was performed on bone marrow (BM) cells (3–5 x 10⁷) from Sββ1+/+ (n = 3) and Sββ1−/+ (n = 3). An H&E representative image showed normal trilineage hematopoiesis and no changes in BM cellularity in both groups of mice. (b) A bar graph shows mean ± standard deviations of the number of BM cells at the end of the follow-up between Sββ1+/+ (n = 4) and Sββ1−/+ (n = 3) mice.

**Additional file 4: Figure S4.** Sββ1−/+ mice have dyserythropoietic features in the bone marrow. Bone marrow (BM) cells (3–5 x 10⁷) were spotted on cytospin slides and iron stain (Prussian blue) was performed according to common pathology stain’s protocols. A representative image taken by light microscopy shows that BM cells from Sββ1−/+ showed specific dys erythropoietic features such as nuclear budding or nuclear irregularity (black arrows) that were not seen in Sββ1+/+ mice.

**Additional file 5: Figure S5.** Sββ1−/+ mice have ring sideroblasts in the bone marrow. Iron stain (Prussian blue) was performed on bone marrow (BM) cells (3–5 x 10⁷) derived from Sββ1−/+ and Sββ1+/+ mice. Images taken by light microscopy show presence of ring sideroblasts (black arrows) in the BM of Sββ1−/+ (n = 3) and absence in Sββ1+/+ (n = 2) mice.

**Additional file 6: Figure S6.** Sββ1−/+ mice do not have any liver abnormalities. Representative images from Hematoxylin & Eosin stain of liver sections from 2 Sββ1−/+ and 2 Sββ1+/+ mice show absence of hepatomegaly or abnormalities in the liver.

**Additional file 7: Table S1.** Differential gene level between Sββ1−/+ and Sββ1+/+ mice. The expression level of all genes is presented as mean fold change between Sββ1−/+ and Sββ1+/+ mice. In total 10330 genes were found in the comparison Sββ1+/+ versus Sββ1−/+ mice.

**Additional file 8: Table S2.** Comparison of hematopoietic-related gene sets between Sββ1−/+ and Sββ1+/+ mice. Gene set analysis shows hematopoietic-related genes between both groups of mice.

**Additional file 9: Table S3.** Gene cluster in hematopoietic-related gene sets between Sββ1−/+ and Sββ1+/+ mice. The genes related to the specific gene set are summarized.
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