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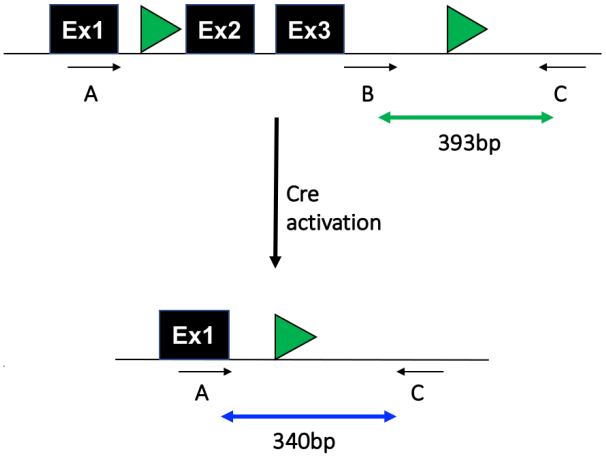

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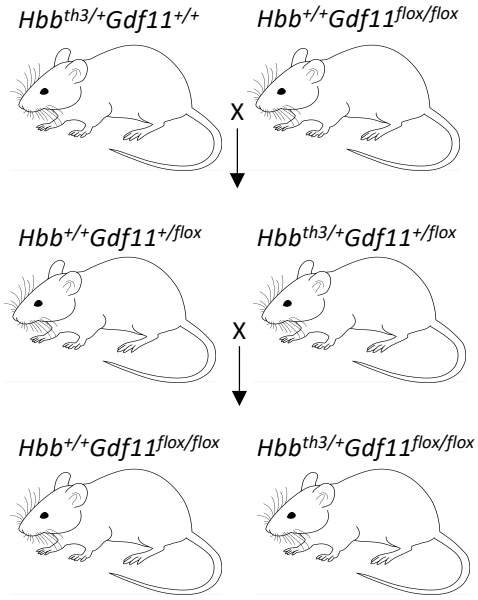
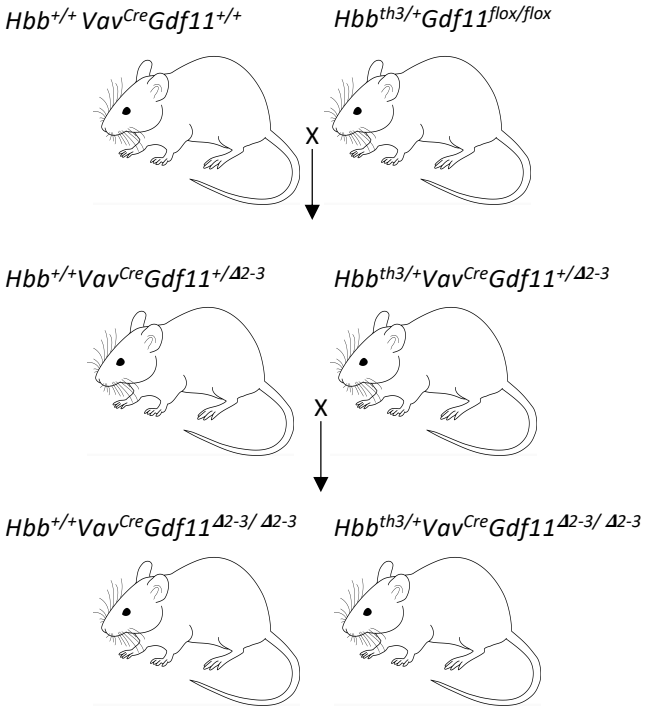
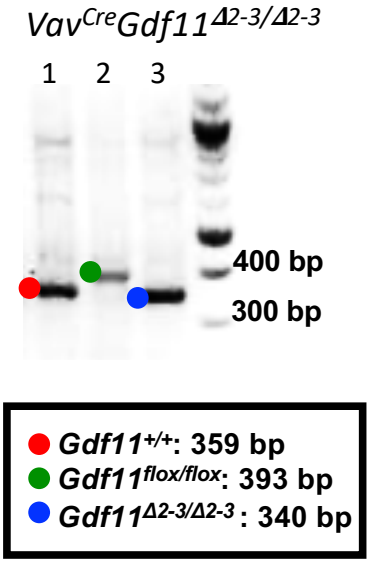


SF1c.



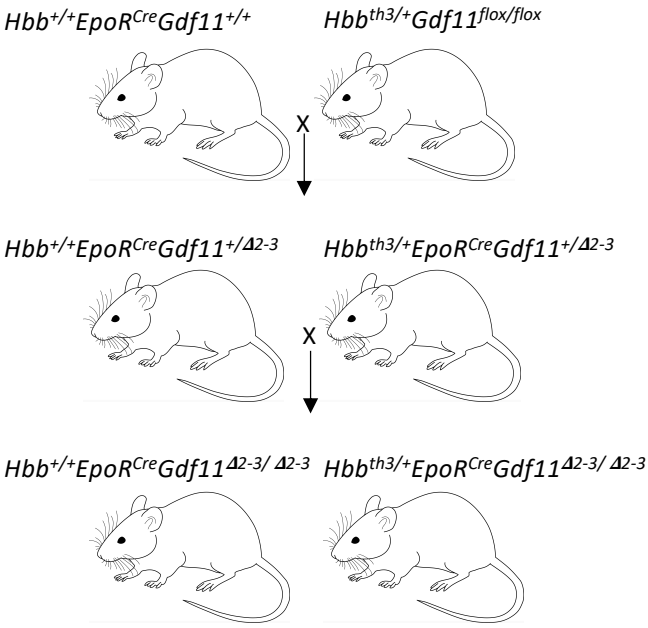
SF1d.



Supplementary Fig.1

Supplementary Figure 1 Strategy to generate conditional knockout mice by targeting loxP flanked *Gdf11* (*Gdf11*$^{flox/flox}$*)* (a). The exons are represented by black boxes and loxP flanking sites are indicated with green arrows. Recombination of the *Gdf11*$^{flox/flox}$ alleles removes the biologically-active carboxy-terminal domain located in exons 2 and 3. Oligonucleotide primers used to distinguish alleles are labeled A, B, and C. The *Hbb*$^{th3/+}$ trait was transferred onto the *Gdf11*$^{flox/flox}$ as shown in (b). *Hbb*$^{th3/+}$ homozygous for the *Gdf11* floxed allele were used to generate all subsequent lines presented in this manuscript. To generated mice lacking *Gdf11* in the hematopoietic compartments *Vav*^{Cre} mice were crossed to *Hbb*$^{th3/+}$*Gdf11*$^{flox/flox}$ mice (c). A representative example of the PCR analysis of gDNA collected from peripheral blood (PB) of *Vav*^{Cre}*Gdf11*$^{\Delta 2\text{-}3/\Delta 2\text{-}3}$, WT and *Gdf11*$^{flox/flox}$ controls (d) on an agarose gel shows WT *Gdf11* in red (lane 1), *Gdf11*$^{flox/flox}$ in green (lane 2) and a deletion of exon 2 and exon 3 (*Gdf11*$^{\Delta 2\text{-}3/\Delta 2\text{-}3}$) in blue (lane 3). Mice with *Gdf11* deleted in cells expressing Cre recombinase under the VAV promoter are denoted *Vav*^{Cre}*Gdf11*$^{\Delta 2\text{-}3/\Delta 2\text{-}3}$.

SF2a.



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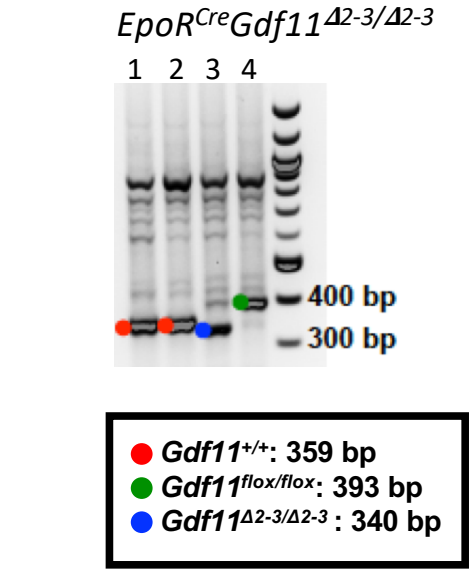

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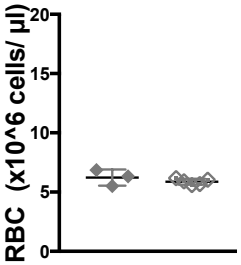

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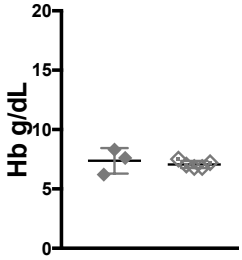

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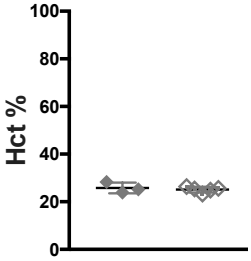

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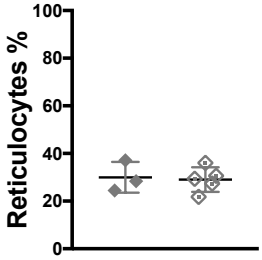

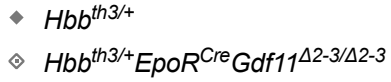

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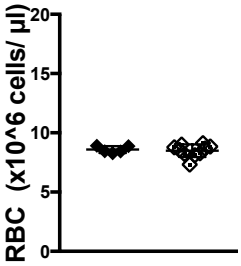

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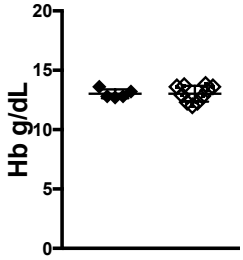

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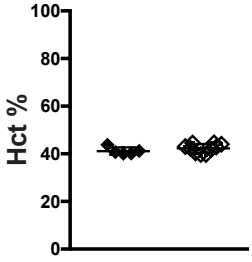

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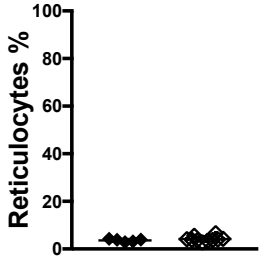
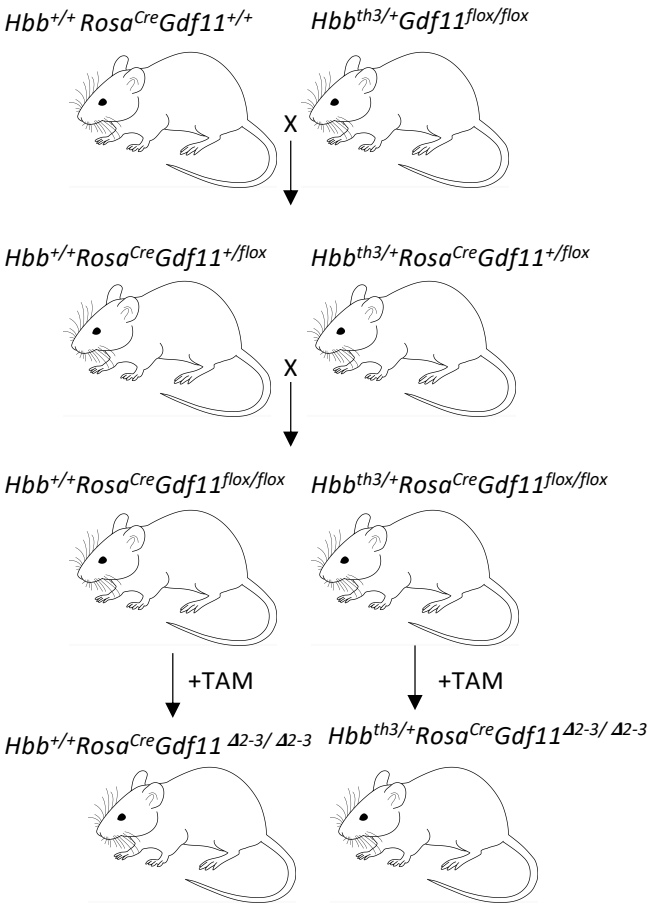


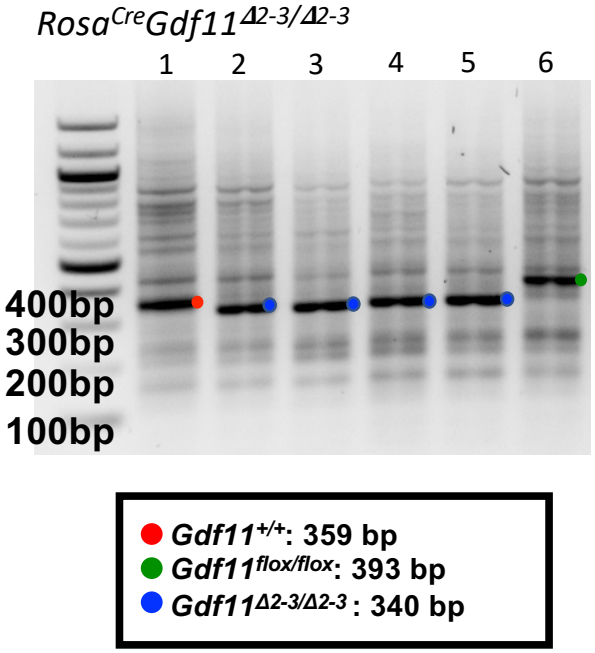
Supplementary Fig.2

Supplementary Figure 2 To generated mice lacking *Gdf11* in erythroid progenitors, *EpoR*^{Cre} mice were crossed to *Hbb*$^{th3/+}$*Gdf11*$^{flox/flox}$ mice (a). PCR analysis of gDNA collected from PB of *EpoR*^{Cre} mice (b) on an agarose gel shows wild-type (WT) *Gdf11* in red (lane 1 and 2) at 359 bp, *Gdf11*$^{flox/flox}$ in green (lane 4) at 393 bp and *Gdf11*$^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ in blue (lane 3) at 340 bp. Mice with *Gdf11* deleted in cells expressing Cre recombinase under the EPOR promoter are denoted *EpoR*^{Cre}*Gdf11*$^{\Delta 2\text{-}3/\Delta 2\text{-}3}$. Conditional deletion of *Gdf11* in erythroid progenitors showed no improvement in hematological parameters compared to controls. Red blood cell counts (c), Hb (d), Hct (e), and reticulocytes (f) remain unaffected in 2-month-old *Hbb*$^{th3/+}$ *EpoR*^{Cre}*Gdf1* $^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (n=5) males compared to sex and age matched *Hbb*$^{th3/+}$ controls (n=3). Similarly, no differences were observed in *Hbb*$^{+/+}$ *EpoR*^{Cre}*Gdf1* $^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (n=10) males compared to sex and age matched *Hbb*$^{+/+}$ controls (n=5).

SF3a.



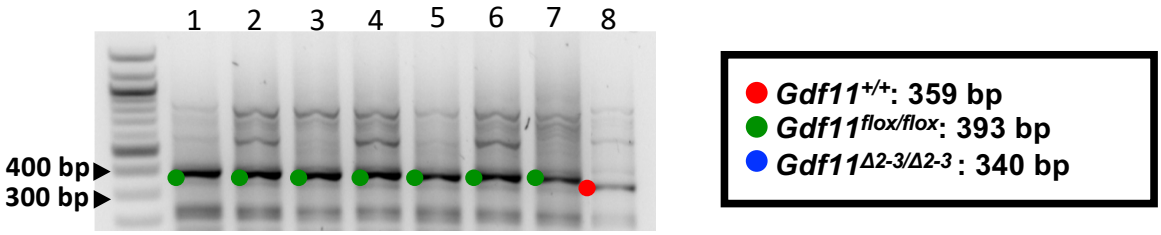
SF3b.



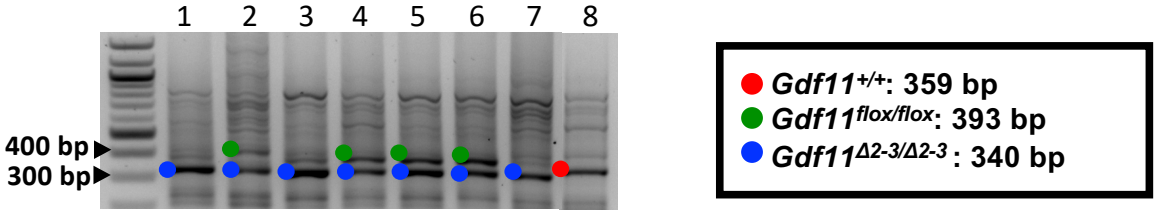
Supplementary Fig.3

Supplementary Figure 3 To generate pancellularly inactive *Gdf11* mice, $Rosa^{Cre}$ were crossed to $Hbb^{th3/+}Gdf11^{flox/flox}$ mice (a) and treated with TAM. After TAM treatment PCR analysis of gDNA extracted from tail and ear were assessed for Cre recombination (b). WT *Gdf11* (lane 1), $Gdf11^{flox/flox}$ in green (lane 6), and $Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (lanes 2-5) for $Rosa^{Cre}Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ are indicated. Mice with *Gdf11* deleted in cells expressing Cre recombinase under the ROSA26 promoter are denoted $Rosa^{Cre}Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$. Only animals that exhibited complete recombination were moved into treatment groups with PBS or RAP-536.

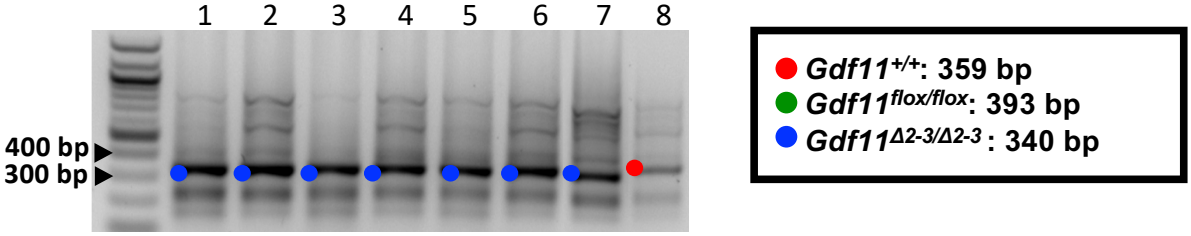
SF4a. *Gdf11*$^{flox/flox}$:



SF4b. *Rosa*^{Cre}*Gdf11*$^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (incomplete recombination)

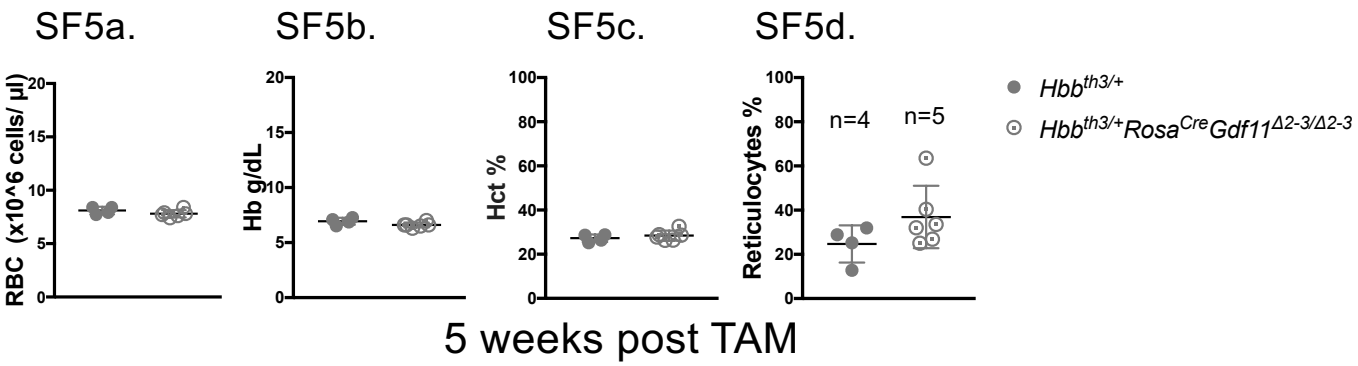


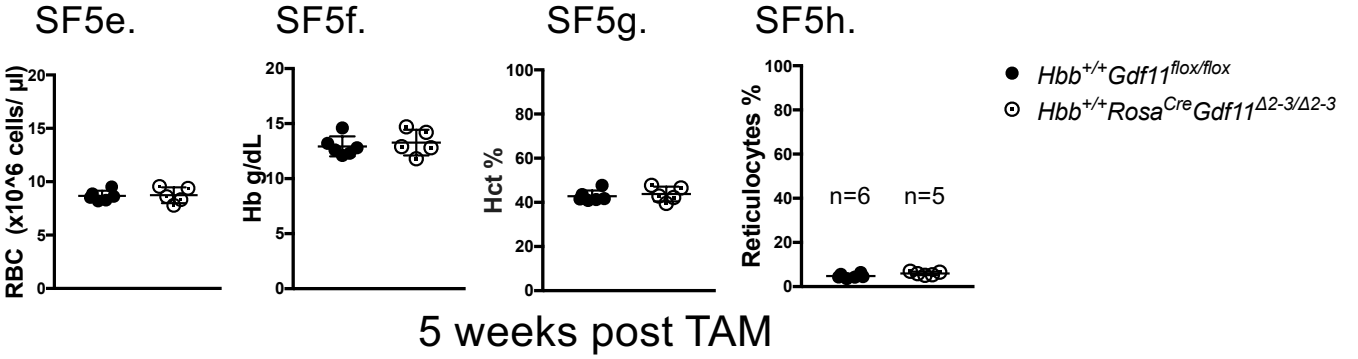
SF4c. *Rosa*^{Cre}*Gdf11*$^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (complete recombination):

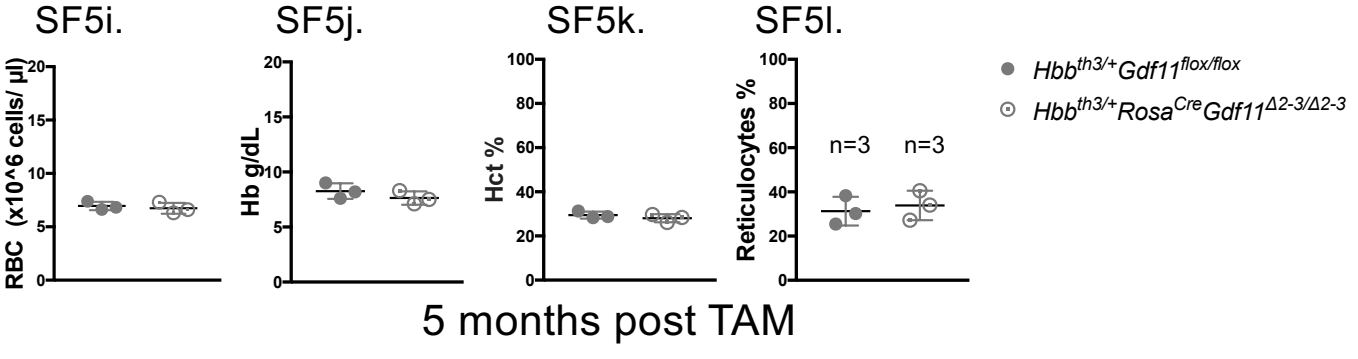


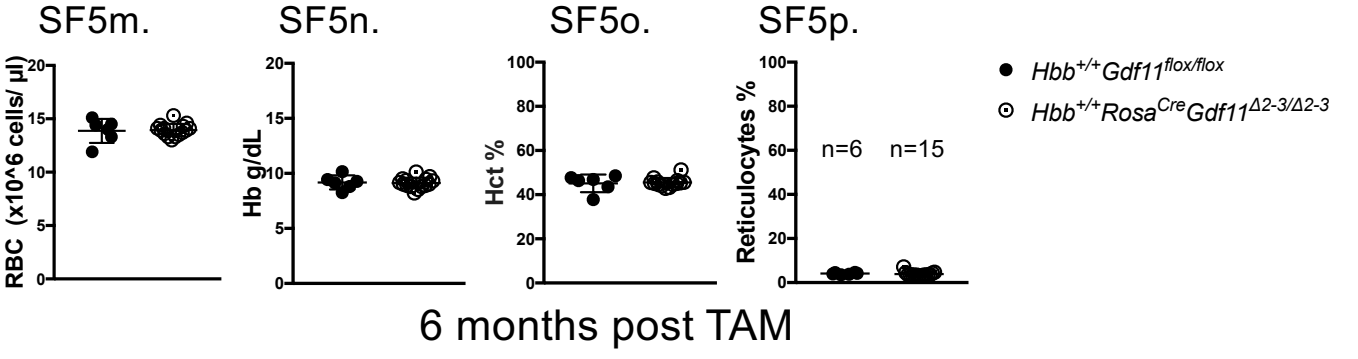
Supplementary Fig.4

Supplementary Figure 4 *Gdf11*$^{flox/flox}$ (a) and *Rosa*^{Cre}*Gdf11*$^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (b-c) animals were assessed for *Gdf11* recombination at end of PBS and RAP-536 treatment. Lanes indicate organs tested for recombination (1=liver 2=spleen 3=duodenum 4=heart 5=kidney 6=muscle 7=bone marrow 8= WT tail Control). Animals had high levels of recombination in all organs tested, some showing partial recombination in some tissues (b) and others maintaining complete recombination in all tissues tested (c).





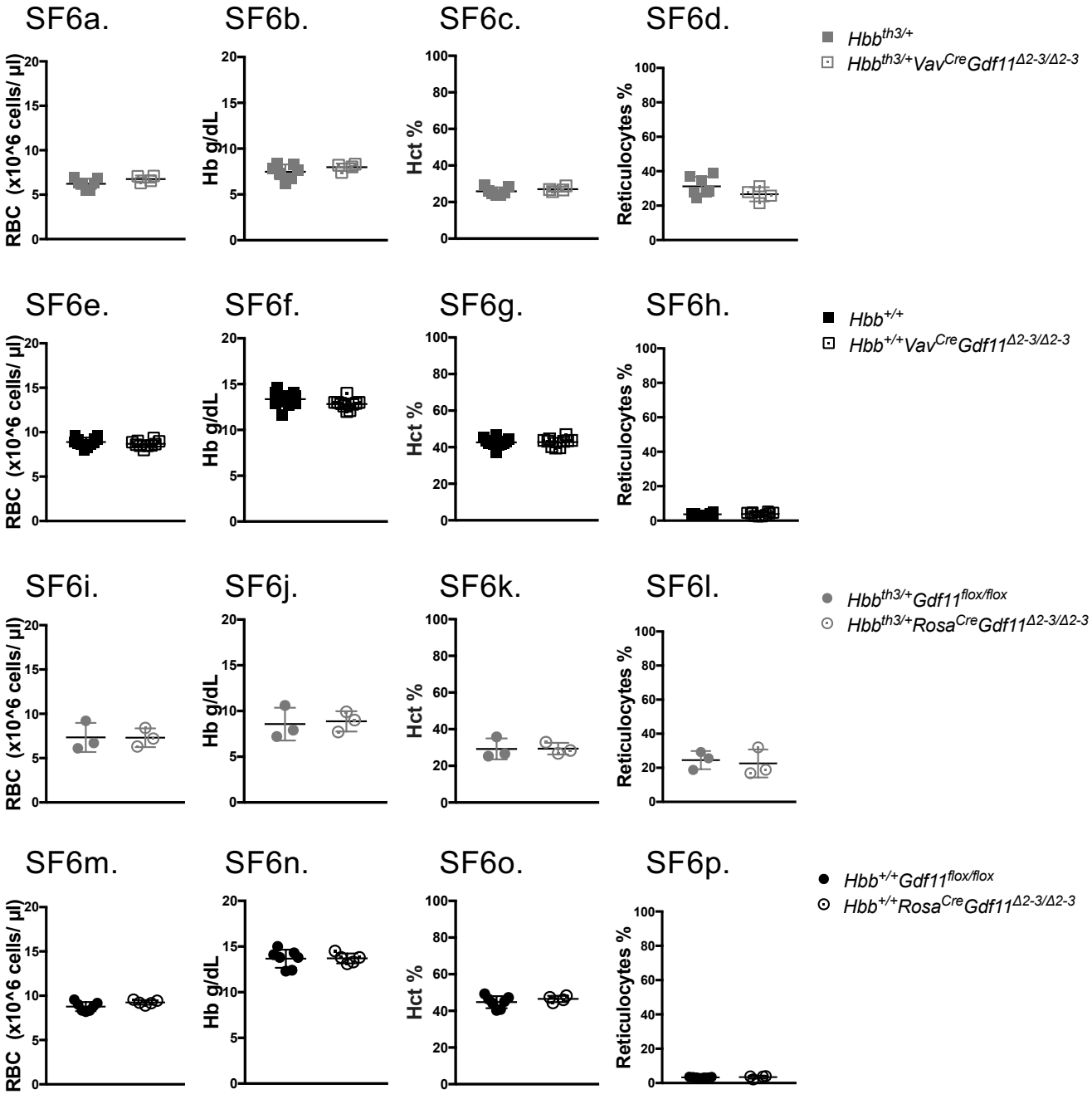




Supplementary Fig. 5

Supplementary Figure 5 Analysis of TAM treated animals 5-weeks post treatment showed no changes in hematological parameters of 4-5-month-old males in *Hbb*$^{th3/+}$*Rosa*^{Cre}*Gdf11*$^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (a-d) (n=6) *or Hbb*$^{+/+}$*Rosa*^{Cre}*Gdf11* $^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (e-h) (n=5) compared to *Hbb*$^{th3/+}$ (n=3) and *Hbb*$^{th3/+}$ (n=6). Analysis of 11-month-old males, at 5-6 months post TAM treatment, showed no changes in hematological parameters for *Hbb*$^{th3/+}$*Rosa*^{Cre}*Gdf11*$^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (i-l) or *Hbb*$^{+/+}$*Rosa*^{Cre}*Gdf11*$^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (m-p) (n=15) compared to *Hbb*$^{th3/+}$*Gdf11*$^{flox/flox}$ (n=3) or *Hbb*$^{+/+}$*Gdf11*$^{flox/flox}$ (n=6) animals.

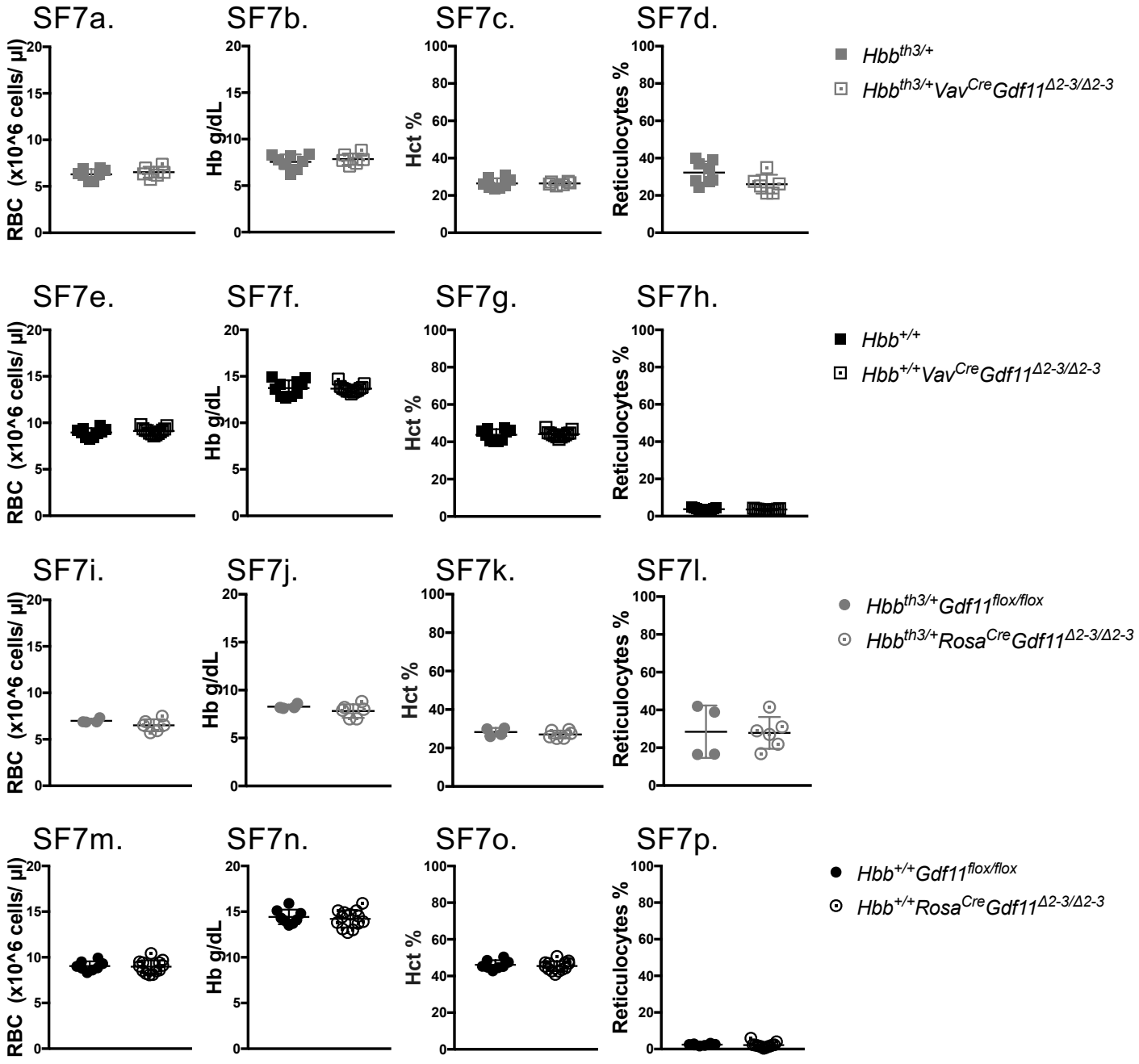
Females



Supplementary Fig.6

Supplementary Figure 6 CBC analyses in females. Conditional deletion of *Gdf11* in the entire hematopoietic compartment in $Hbb^{th3/+}$ $Vav^{Cre}Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (n=3) shows no differences in RBC count (a), Hb (b), Hct (c) or reticulocytes compared to $Hbb^{th3/+}$ controls (n=7). Conditional deletion of *Gdf11* in erythroid cells did not result in altered hematopoietic parameters in $Hbb^{+/+}$ $Vav^{Cre}Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (n=10) compared to $Hbb^{+/+}$ controls (n=12) (e-h). CBCs were analyzed at 2 months of age. Ubiquitous deletion of *Gdf11* in $Hbb^{th3/+}Rosa^{Cre}Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ mice (n=3) after treatment TAM did not improve hematological parameters (i-l) compared to $Gdf11^{flox/flox}$ controls (n=3). No hematological differences were detected in $Hbb^{+/+}Rosa^{Cre}$ $Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (n=5) compared to $Hbb^{+/+}$ $Gdf11^{flox/flox}$ controls (n=7) (m-p). $Rosa^{Cre}$ $Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ and $Gdf11^{flox/flox}$ controls were analyzed between 3-6 months of age. CBCs were analyzed 1-week post TAM treatment.

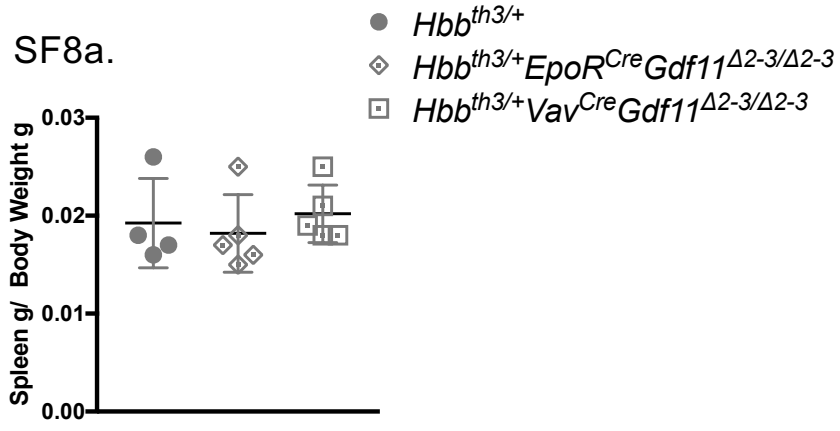
Males



Supplementary Fig.7

Supplementary Figure 7 CBC analyses in males. Conditional deletion of *Gdf11* in the entire hematopoietic compartment in $Hbb^{th3/+}$ $Vav^{Cre}Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (n=3) showed no differences in RBC count (a), Hb (b), Hct (c) or reticulocytes, (d) compared to $Hbb^{th3/+}$ controls (n=7). Conditional deletion of *Gdf11* in erythroid cells did not result in altered hematopoietic parameters in $Hbb^{+/+}$ $Vav^{Cre}Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (n=10), compared to $Hbb^{+/+}$ controls (n=12) (e-h). CBCs were analyzed at 2 months of age. Ubiquitous deletion of *Gdf11* in male $Hbb^{th3/+}Rosa^{Cre}Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ mice (n=6) after treatment TAM did not improve hematological parameters (i-l) compared to $Gdf11^{flox/flox}$ controls (n=4). No hematological differences were detected in $Hbb^{+/+}Rosa^{Cre}$ $Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (n=13) compared to $Hbb^{+/+}$ $Gdf11^{flox/flox}$ controls (n=10) (m-p). $Rosa^{Cre}$ $Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ and $Gdf11^{flox/flox}$ controls were analyzed between 3-6 months of age CBCs were analyzed 1-week post TAM administration.

SF8a.



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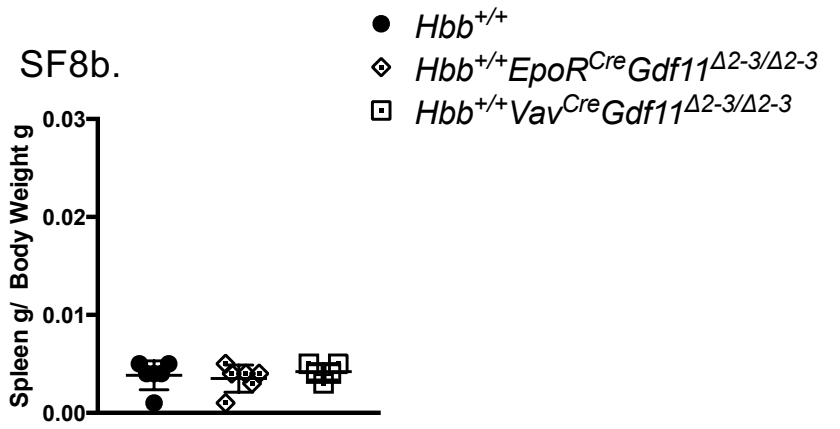

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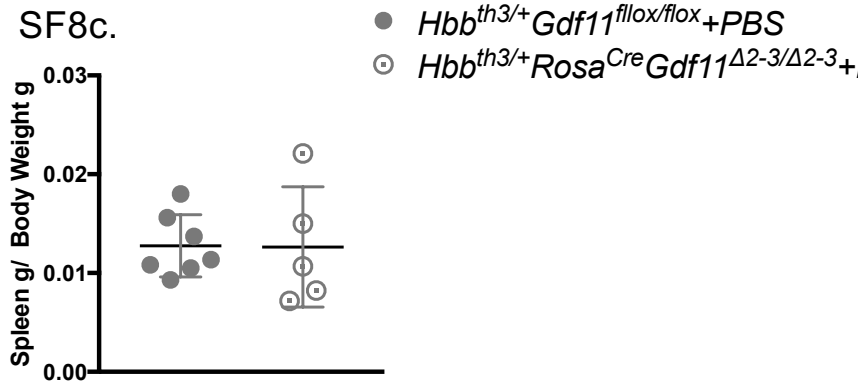

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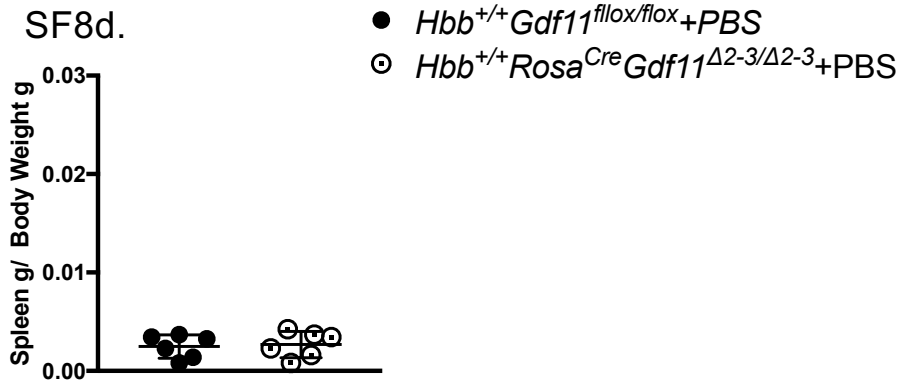
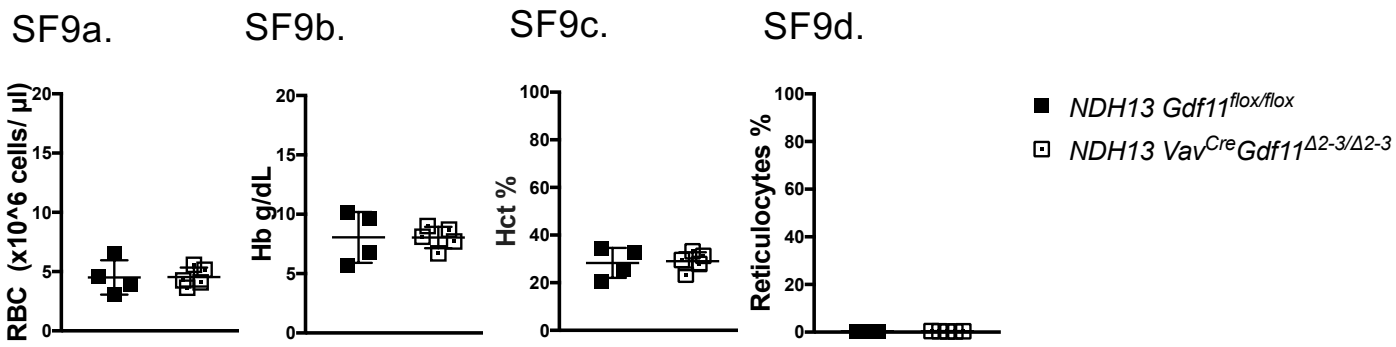


Supplementary Fig. 8

Supplementary Figure 8 Reduction of *Gdf11* did not result in decreased splenomegaly in $Hbb^{th3/+}$ animals. $Hbb^{th3/+}EpoR^{Cre}Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (n=5) and $Hbb^{th3/+}\ Vav^{Cre}Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (n=5) males did not show improvements in spleen size compared to $Hbb^{th3/+}$ controls (n=4) (a). No statistical differences were observed in the spleen sizes of $Hbb^{+/+}EpoR^{Cre}Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (n=6) and $Hbb^{+/+}\ Vav^{Cre}Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (n=6) compared to controls (n=6). Animals were 4-month old. $Hbb^{th3/+}Rosa^{Cre}Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ (n=5) males did not show improvement in spleen size compared to $Hbb^{th3/+}$ controls (n=7) (c). No statistical differences were observed in the spleen sizes of $Hbb^{+/+}Rosa^{Cre}Gdf11^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ males (n=6) compared to controls (n=6). Animals were 6-7 months old.



Supplementary Fig.9

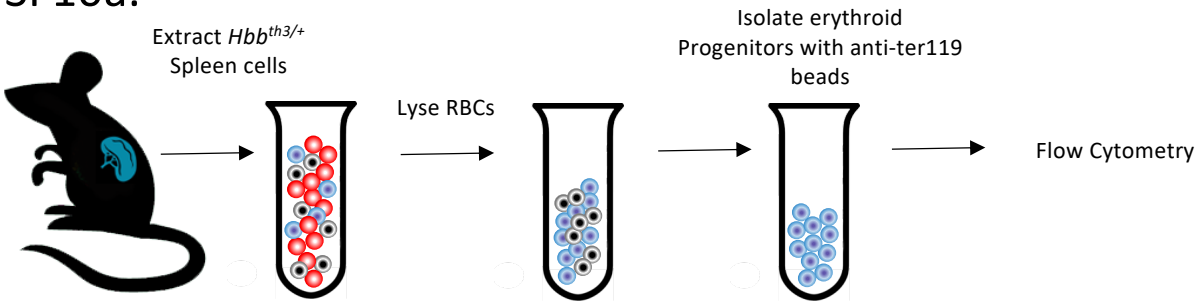
Supplementary Figure 9 Animals from an *NUP98-HOXD13* (*NDH13*) (a mouse model of MDS) line were crossed with *Vav*^{Cre}*Gdf11*$^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ animals to produce an MDS model with a *Gdf11* deletion in the entire hematopoietic compartment. *NHD13 Vav*^{Cre}*Gdf1* $^{\Delta 2\text{-}3/\Delta 2\text{-}3}$ did not show improvements in RBC counts (a), Hb (b), Hct (c) or reticulocytes (d) (n=6) compared to *Gdf11*$^{flox/flox}$ controls (n=4).

Non-Erythroid (Ter119-)

Erythroid progenitor (Ter119+)

RBC (Ter119+)

SF10a.



SF10b.

Taqman Gene Expression Assays (Applied Biosystems)	
Human	
Gene Name	**Sequence Access No.**
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ACVR2B	HS00609605_m1
TFRC	HS00951083_m1
GDF11	Hs00195156_m1
GYPA	HS00266777_m1
HPRT	Hs02800695_m1
RHO	Hs00892431_m1

Taqman Gene Expression Assays (Applied Biosystems)	
Mouse	
Gene Name	**Sequence Access No.**
Acvr2a	Mm01331094_m1
Acvr2b	Mm00431665_m1
Tfrc	Mm00441941_m1
Gdf11 (Exon 1)	Mm01159973_m1
Gdf11 (Exon 2)	Mm01159974_m1
Gypa	Mm00494848_m1
Hprt	Mm03024075_m1
Rho	Mm01184405_m1

Supplementary Fig.10

Supplementary Figure 10 Schematic of erythroid progenitors Ter119^{+} isolation from Hbb$^{th3/+}$ spleen cells (a). Table of Taqman probes used for mRNA analysis (b).

Materials and Methods

Ethics statement regarding mouse studies

All experiments involving mouse models were reviewed by the IACUC at The Children's Hospital of Philadelphia (protocol no. IACUC IAC 18-001173_AM03). Animals were handled and treated according to the approved protocols and procedures.

Transgenic mouse models and genotyping

Gdf11$^{flox/flox}$ mice were obtained from the laboratory of Dr. Se-Jin Lee and were genotyped using the primers as mentioned here.[1] Vav^{Cre} (Strain # 008610) and Rosa^{Cre}(Strain # 008463) were purchased from Jackson Laboratories and genotyped using the primer specified. We utilized *Hbb*$^{th3/+}$ animals as a model of β–thalassemia intermedia.[2] This model for thalassemia intermedia were received from Jackson Laboratories (Strain # 2683). EpoR^{Cre} mice were generated by Dr. U. Klingsmuller.[3] All animals began treatment between 1 to 3 months of age.

Tamoxifen Treatment

Hbb$^{th3/+}$ *Rosa*^{Cre} were mated with *Gdf11*$^{flox/flox}$ and treated with tamoxifen at a dose of 75mg/kg for 3-5 days (as recommended by Jackson labs) starting at 1 month of age followed by tamoxifen diet from Envigo at a dose of 40mg/kg for a week.[4,5] They were then allowed to rest for 2 weeks to 5 months before testing. The controls for this group (*Gdf11*$^{flox/flox}$, *Rosa*^{Cre} *Gdf11*$^{flox/flox}$ and *Rosa*^{Cre}) were treated with tamoxifen in the same manner. Various tissues (liver, duodenum, kidney, heart, muscle, bone marrow and spleen) for the *Rosa*^{Cre} were genotyped at the end of the experiments to ensure *Gdf11* recombination.

RAP-536 treatment

Hbb$^{+/+}$*Vav*^{Cre}*Gdf11*$^{flox/flox}$, *Hbb*$^{th3/+}$*Vav*^{Cre}*Gdf11*$^{flox/flox}$, *Hbb*$^{+/+}$ *Rosa*^{Cre} *Gdf11*$^{flox/flox}$, *Hbb*$^{th3/+}$ *Rosa*^{Cre} *Gdf11*$^{flox/flox}$ mice were subcutaneously treated with PBS or RAP -536 (provided by Acceleron Pharma) at a dose of 10mg/kg (as published previously)[6] for 6 weeks (2 doses per week) for a total of 12 doses.

Blood Collection

Blood samples were collected by retro-orbital bleeding. Briefly, mice were anesthetized (2.5% Isoflurane) and blood was collected by capillary action (about 50µl per mouse) using a non-heparinized capillary tube. Blood samples were analyzed at the Children's Hospital of Philadelphia by the Institutional Clinical and Translational Science Award Research Center (Grant UL1TR000003 and Grant UL1TR001878).

Ter119+ isolation from *Hbb*$^{th3/+}$ mice and FACs analysis

Spleens from *Hbb*$^{th3/+}$ mice were collected as previously described.[7] Single cell suspensions were incubated RBC lysis buffer as indicated by manufacture instructions (Cat. No. 420301, BioLegend San Diego, CA). After RBC lysis Ter119^{+}cells were isolated using anti-Ter119 MicroBeads as instructed in manufacturers protocol (Cat. No. 130-049-901, Miltenyi Biotec Inc., Auburn, CA). Ter119^{+} and Ter119^{-} fractions were then incubated in FITC-conjugated anti-mouse Cd71 (Cat. No. 553266, BD Pharmigen, San Diego, CA) and APC-conjugated anti-mouse Ter119 antibodies (Cat. No. 557909, BD Pharmigen, San Diego, CA) to ensure isolation was successful. Samples were washed and incubated with 2% FBS in Iscoves DMEM. Multicolor

data acquisition for progenitor subsets was performed on a FACSCanto II flow cytometer (BD Franklin Lakes, NJ). Data were analyzed with FlowJo 10.2 (Tree Star, Inc., Ashland, OR).

Three-phase liquid culture and treatment with RAP

$CD34^+$ cells selection from blood samples was performed by immunomagnetic separation, using the CD34 microbeads kit (Miltenyi Biotec Inc., Auburn, CA). Cells were then expanded and differentiated following a 3-phase cell culture system that minimizes HbF induction, as previously described.[8] While phase I can span from 10 to 14 days, and allow us to maintain cells undifferentiated and with high proliferative potential, phase II and III last 8 days altogether. In phaseII-III cells are induced to terminally differentiate into mature red cells. Treatment with RAP (5µg/mL or 150µg/mL) was done on the day cells were moved from expansion to differentiation condition, which coincide with the transition from phase I to phase II. The level of differentiation was assessed by benzidine staining.[9]

Messenger RNA analysis of $CD34^+$ cells and *Hbb*$^{th3/+}$ $Ter119^+$ isolated cells

mRNA analyses were performed by quantitative-PCR as elsewhere described.[8] A table with the access number for mouse and human *GAPDH, ACVR2A, ACVR2B, TFRC, GDF11, GYPA, HPRT,* and *RHO* is provided in **Supplemental Figure 10b**.

Statistics

For two-group comparisons we used either paired or unpaired t-tests (if normal distributions and equal variances were met) or Mann Whitney and Wilcoxon tests. All tests were done using GraphPad Prism software, version 6.0

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