HUMANIZED MOUSE MODEL OF COOLEY'S ANEMIA

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Running head: Cooley's Anemia mouse model
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SUPPLEMENTAL INFORMATION

SUPPLEMENTAL FIGURE LEGENDS

Fig. S1. (A) Chimeric $\gamma\beta^0$ KI mouse. **(B)** Germline $\gamma\beta^0$ KI offspring produced from breeding the chimera in (A). **(C)** Sequence confirmation of the IVS1.1 G to A mutation in the human β globin gene in $\gamma\beta^0$ KI mice.

Fig. S2. Histology of erythroid hyperplasia in the bone marrow, increased iron deposition in the liver, and α globin chain inclusions in the peripheral blood of adult heterozygous $\gamma\beta^0$ KI mice. The femoral bone marrow cavity is expanded and filled with erythroid cells in adult $\gamma\beta^0$ globin KI mice. The liver of $\gamma\beta^0$ globin KI mice show increased levels of iron after Perl's iron staining. Vital staining of peripheral blood with methyl violet demonstrates the presence of α globin chain inclusions in the $\gamma\beta^0$ KI mice.

Fig. S3. Lack of erythroid hyperplasia in newborn heterozygous CA ($\gamma\beta^0$/+), homozygous CA ($\gamma\beta^0/\gamma\beta^0$), and mouse β globin knockout (thal3/+) mice. **(A)** Newborn livers from wild-type (Control) and humanized heterozygous ($\gamma\beta^0$/+) and homozygous ($\gamma\beta^0/\gamma\beta^0$) CA mice were disaggregated into single cells, stained with 7AAD for viability and with fluorescently labeled antibodies to CD71 and Ter119 to quantify the percentage of erythroblasts (Ter119+) in the hematopoietic newborn liver. Erythroblast cell numbers are similar in CA mice and control animals demonstrating the lack of erythroid hyperplasia at birth. **(B)** Histological sections of bone marrow and liver from newborn wild-type (+/+) and β globin knockout mice (thal3/+) demonstrate a lack of erythroid hyperplasia at birth. Similar to newborn humanized CA mouse bone marrow and liver (Fig. 5D), there are plenty of myeloid progenitors in the bone marrow at birth, but the majority of erythropoiesis is found in the liver of β globin knockout mice. Though β thalassemia initiates early in fetal life in the β KO mice, there is no obvious erythroid hyperplasia at birth.

SUPPLEMENTAL TABLES

Table S1:

PartA. QPCR of blood RNA in heterozygous $\gamma\beta^0$ KI mice through development

$\gamma\beta^0$ / +	E10.5	E14.5	E16.5	E18.5	NB	PB	BM
mβh1 / Tβ	30.0 ± 7.3	0.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0	ND
mεY / Tβ	49.5 ± 11	25.2 ± 9.8	0.1 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	0.0	ND
mβ / Tβ	2.0 ± 0.5	44.6 ± 6.1	70.1 ± 1.3	78.8 ± 1.2	95.0 ± 1.9	99.3 ± 0.1	98.1 ± 0.1
hγ / Tβ	18.5 ± 3.3	29.8 ± 3.8	29.5 ± 1.3	20.8 ± 1.2	4.6 ± 1.8	0.2 ± 0.0	0.1 ± 0.0
$h\beta^0$ / Tβ	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.6 ± 0.1	1.8 ± 0.1
hγ / Tα	53.0 ± 6.4	46.0 ± 20	17.1 ± 0.9	7.0 ± 0.6	1.9 ± 0.8	0.1 ± 0.0	0.0 ± 0.0
Tα / Tβ	41.0 ± 2.1	53.7 ± 16	102 ± 5.8	136 ± 15	135 ± 20	109 ± 2.5	115 ± 7.3

Data are presented as an average percent with standard error from at least three heterozygous KI mice per developmental time point. Each beta-like globin mRNA is normalized to gene copy number and total beta-like globin message (Tβ) per gene copy. Tα is the total mouse alpha-like globin gene expression. hγ/Tα and Tα/Tβ ratios are uncorrected for gene copy. NB indicates newborn; PB, adult peripheral blood; BM, adult bone marrow; and ND, not determined.

Part B. QPCR of fetal blood RNA in homozygous $\gamma\beta^0$ KI mice during fetal life

$\gamma\beta^0 / \gamma\beta^0$	E14.5	E15.5	E16.5	E17.5
mβh1 / Tβ	0.7 ± 0.0	0.2 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
mεY / Tβ	40.3 ± 9.3	19.6 ± 6.0	2.0 ± 0.5	5.7 ± 2.3
hγ / Tβ	59.3 ± 9.4	79.1 ± 6.1	96.1 ± 0.8	89.8 ± 2.7
$h\beta^0$ / Tβ	0.4 ± 0.0	1.1 ± 0.1	1.9 ± 0.5	4.5 ± 1.1
hγ / Tα	54.5 ± 8.4	51.2 ± 7.4	72.3 ± 17	35.6 ± 5.5
Tα / Tβ	110 ± 6.8	161 ± 18	149 ± 24	263 ± 38

Data are presented as in Part A. The hγ/Tα and Tα/Tβ ratios indicate β thalassemia in peripheral blood from homozygous KI fetuses.

Part C. QPCR of newborn blood RNA in fully humanized CA mice

$\gamma\beta^0/\gamma\beta^0$	NB
$h\gamma$ / $h\alpha$	8.7
$h\beta^0$ / $h\alpha$	1.7
$h\alpha$ / $T\beta$	960

Human γ and β^0 globin RNAs are shown as a percentage of human α globin gene expression. $h\alpha/T\beta$ is the percentage of human α globin relative to total beta-like globin expression. Human γ globin mRNA levels are low in newborn peripheral blood of humanized CA mice compared to human α globin.

Table S2:
Genotyping of fetal and adult offspring of heterozygous $\gamma\beta^0$ globin KI mice.

Age	Litters analyzed	+/+	$\gamma\beta^0$/+	$\gamma\beta^0/\gamma\beta^0$
E14.5	2	4	12	4
E15.5	2	5	11	5
E16.5	2	4	10	5
E17.5	5	12	20	6
E18.5	3	5	14	7
Total Fetus	14	30	67	27
Adult	15	24	45	0

Figure S1

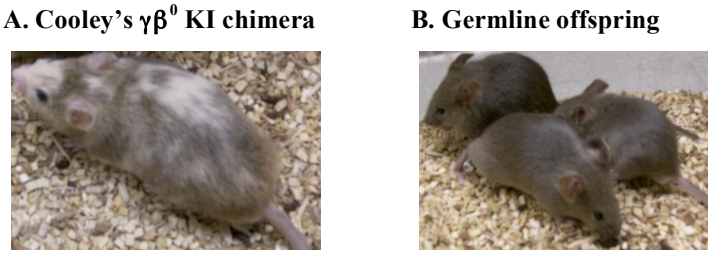


Figure S2

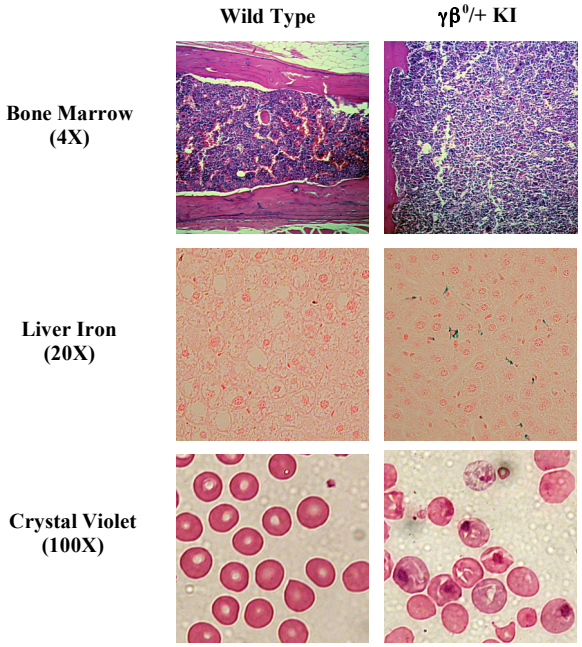
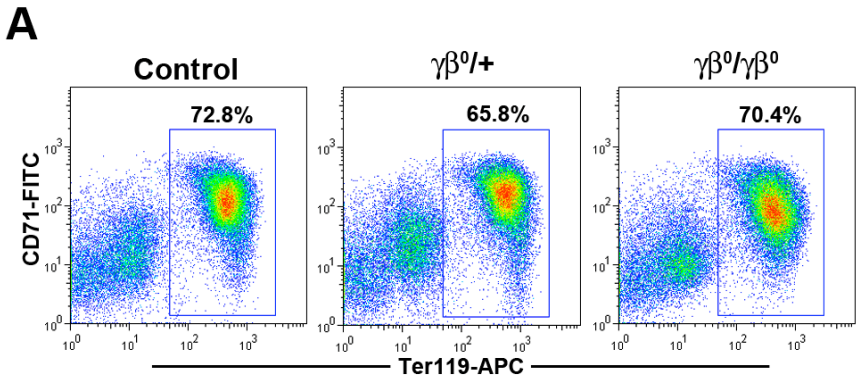
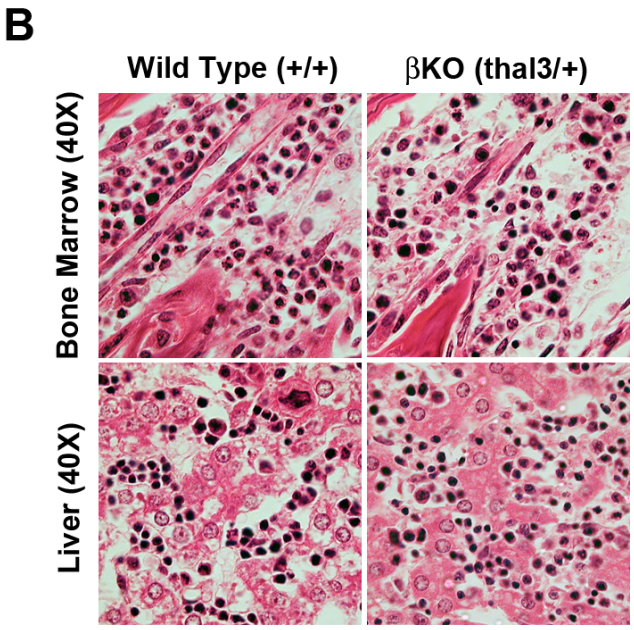


Figure S3


A
Control
γβ⁰/+
γβ⁰/γβ⁰
72.8%
65.8%
70.4%
CD71-FITC
Ter119-APC


B
Wild Type (+/+)
βKO (thal3/+)
Bone Marrow (40X)
Liver (40X)