Figure S1

Related to Fig 1

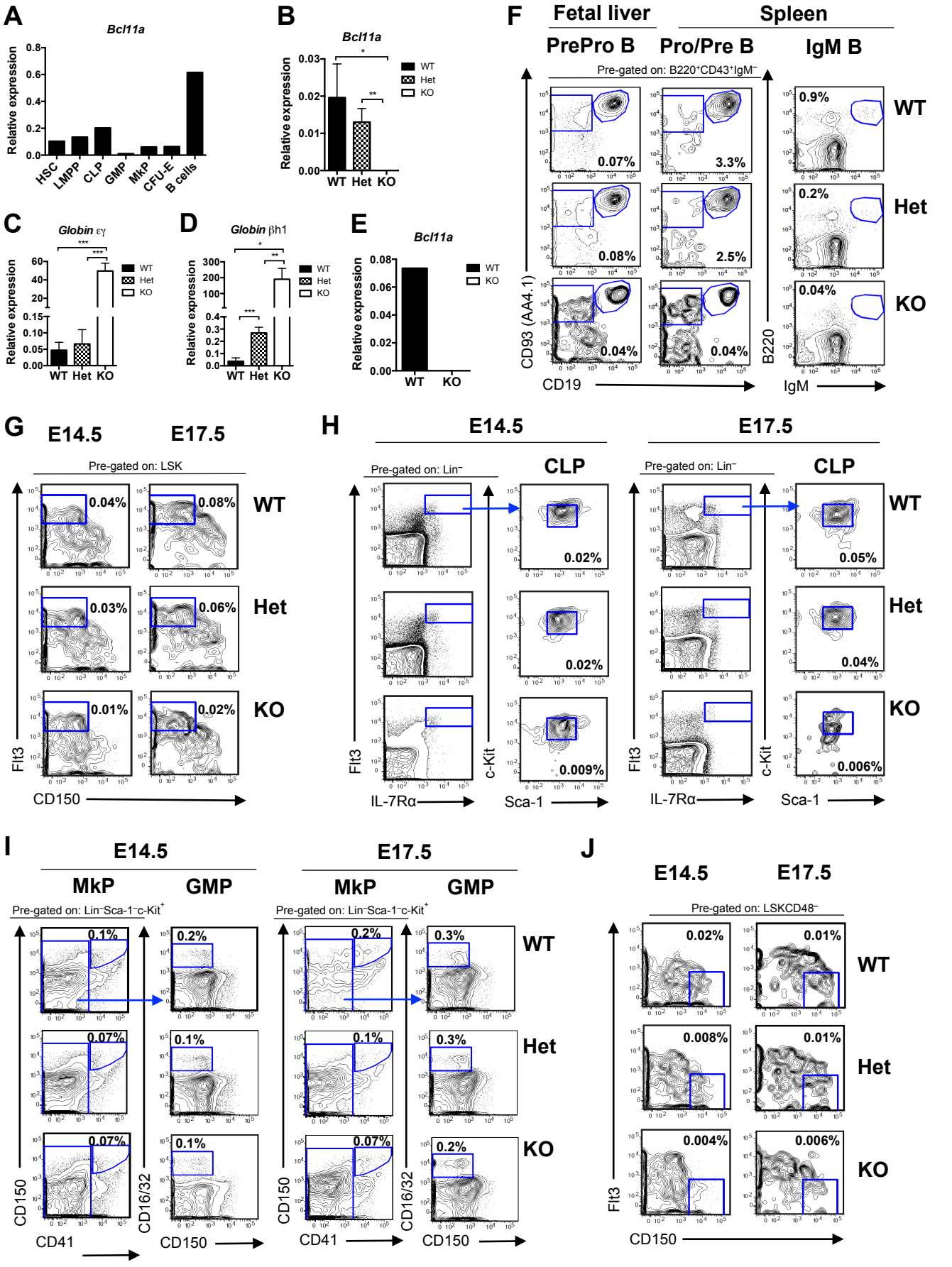

Figure S1. Analysis of *Bcl11a*$^{fl/\Delta}$ *x Gata1-Cre* embryos (Related to Figure 1)

(A) Relative mRNA expression of *Bcl11a* in sorted HSC, LMPP, CLP, GMP, MkP, CFU-E and B cells from 10-16 week old C57BL6 mice. Each replicate is a pool of cells from 2-3 mice.

(B) Relative mRNA expression of *Bcl11a* in WT, Het and KO fetal liver cells. n = 5 WT, 4 Het, 2 KO embryos.

(C) Relative mRNA expression of *Globin εγ* in WT, Het and KO E18.5 fetal liver cells. n = 5 WT, 4 Het, 2 KO embryos.

(D) Relative mRNA expression of *Globin βh1* in WT, Het and KO E18.5 fetal liver cells. n = 5 WT, 4 Het, 2 KO embryos.

(E) Relative mRNA expression of *Bcl11a* in sorted B220^{+}CD19^{+} fetal liver B cells from pooled E18.5 WT or KO embryos. Data is from one experiment.

(F) FACS profile of PreProB (B220^{+}CD43^{+}IgM^{-}CD19^{-}CD93^{+}), ProB/PreB (B220^{+}CD43^{+}IgM^{-}CD19^{+}CD93^{+}) and IgM B cells (B220^{+}IgM^{+}) from WT, Het and KO E18.5 fetal livers and fetal spleens.

(G) FACS profile of LMPP (Lin^{-}Sca-1^{+}c-Kit^{+}(LSK)Flt3^{+}CD150^{-}) from WT, Het and KO E14.5 and E17.5 fetal livers.

(H) FACS profile of CLP (Lin^{-}Flt3^{+}IL-7Ra^{+}c-Kit^{low}Sca-1^{low}) from WT, Het and KO E14.5 and E17.5 fetal livers.

(I) FACS profile of MkP (Lin^{-}Sca-1^{-}c-Kit^{+}CD150^{+}CD41^{+}) and GMP (Lin^{-}Sca-1^{-}c-Kit^{+}CD41^{-}CD150^{-}CD16/32^{+}) from WT, Het and KO E14.5 and E17.5 fetal livers.

(J) FACS profile of HSCs (LSKCD48^{-}Flt3^{-}CD150^{+}) from WT, Het and KO E14.5 and E17.5 fetal livers.

Data in (B-D) represent mean ± SD. Percentages in (F-J) represent total mean frequencies.

In (F) n = 5-6 WT, 4-8 Het, 2-4 KO from E18.5 embryos. In (G-J) n = 2 WT, 9 Het, 10 KO from E14.5 embryos and n = 2 WT, 2 Het, 4 KO from E17.5 embryos. *p<0.05; **p<0.01; ***p<0.001.

Figure S2

Related to Fig 2

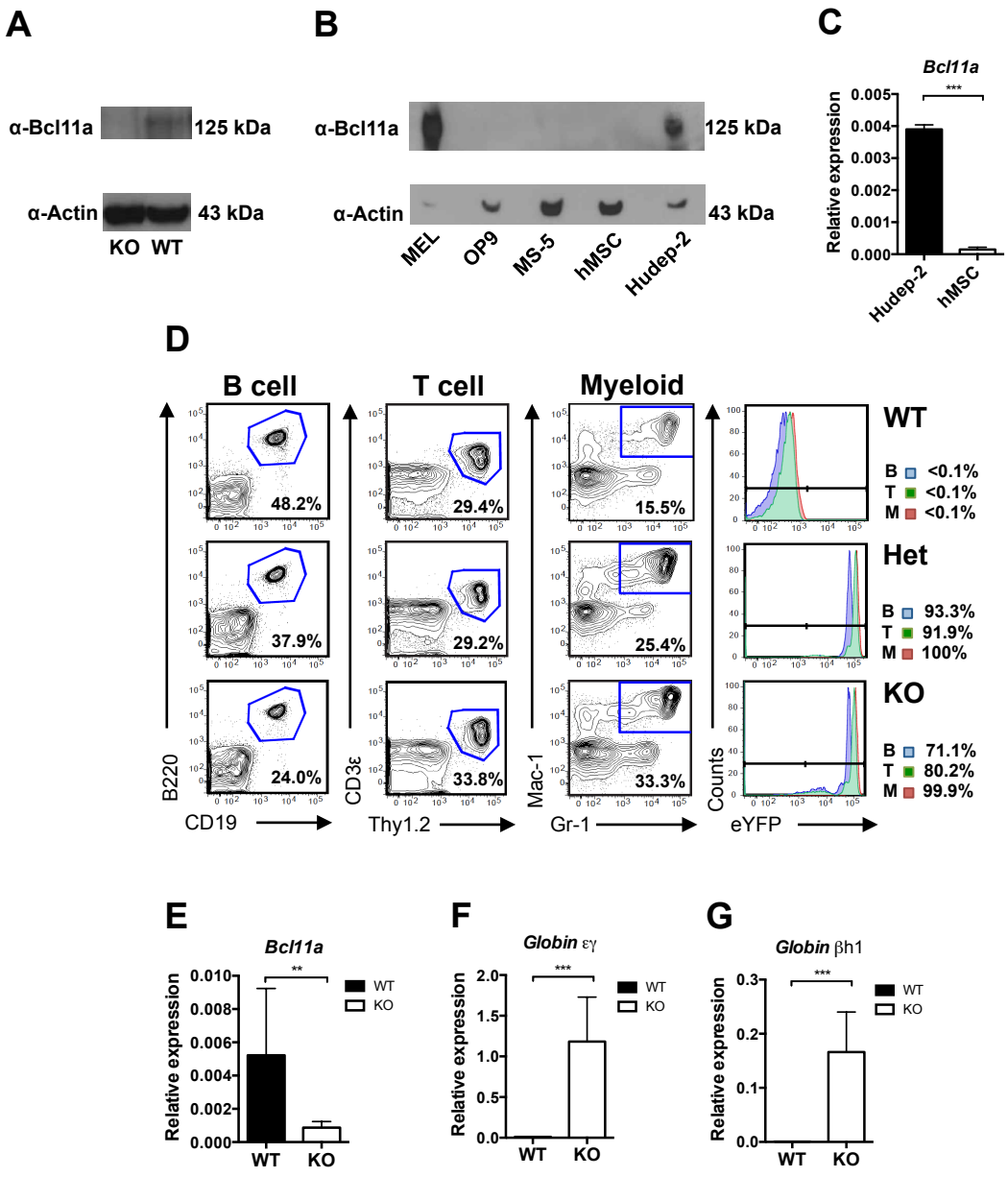

Figure S2. Bone marrow and peripheral blood analysis of *Bcl11a* deleted mice (Related to Figure 2)
(A-B) Western blot analysis of BCL11A in CD117-enriched BM from *Bcl11a* WT (WT) and *Bcl11a* KO (KO) mice 4 weeks post-p(I:C) treatment (A), and in the mouse and human BM stromal compartment (B). Mouse stromal cell lines OP9, MS-5 and primary human mesenchymal stem cells (hMSC) were analyzed in (B). Mouse erythroleukemia (MEL) and human umbilical cord blood-derived erythroid progenitor 2 (Hudep-2) cell lines were used as positive controls . Actin was used as a loading control.
(C) Relative mRNA expression of *Bcl11a* in hMSC and Hudep-2 cells.
(D) FACS profile of B cells ($B220^{+}CD19^{+}$), T cells ($Cd3e^{+}Thy1.2^{+}$) and myeloid cells ($Mac\text{-}1^{+}Gr\text{-}1^{+}$) in peripheral blood, and *R26*-eYFP deletion reporter expression in the lineages, 10 weeks post-p(I:C) treatment. Percentages represent total mean frequencies and mean frequencies from the indicated populations for eYFP expression.
(E) Relative mRNA expression of *Bcl11a* in peripheral blood from WT and KO mice.
(F) Relative mRNA expression of *Globin εγ* in peripheral blood from WT and KO mice.
(G) Relative mRNA expression of *Globin βh1* in peripheral blood from WT and KO mice.

In (C) n = 2 per cell type. In (D) n = 8 WT, 5 Het, 8 KO. In (E-G) n = 7 WT, 8 KO. Data in (C, E-G) represent mean ± SD. *p<0.05; **p<0.01; ***p<0.001.

Figure S3

Related to Fig 2

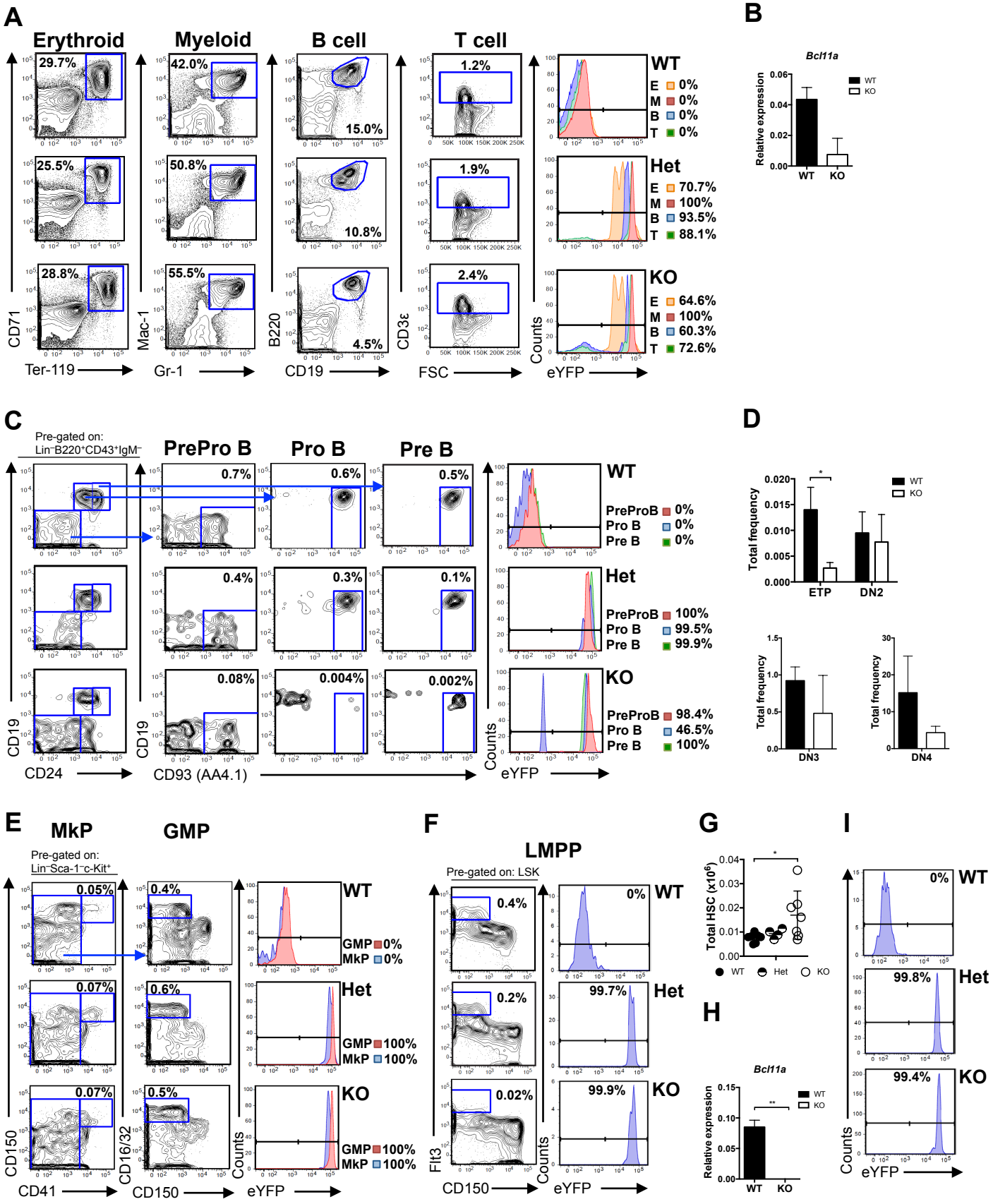

Figure S3. Analysis of the hematopoietic compartment of *Bcl11a* deleted mice (Related to Figure 2)
(A) FACS profile erythroid cells (Ter-119^{+}CD71^{+}), myeloid cells (Mac-1^{+}Gr-1^{+}), B cells (B220^{+}CD19^{+}) and T cells (Cd3e^{+}) in BM, and *R26*-eYFP deletion reporter expression in the lineages, 13 weeks post-p(I:C) treatment.
(B) Relative mRNA expression of *Bcl11a* in sorted B220^{+}CD19^{+} BM B cells from *Bcl11a* WT and KO mice 10 weeks post-p(I:C). n = 2 WT, 2 KO.
(C) FACS profile of PreProB (B220^{+}CD43^{+}IgM^{-}CD19^{-}CD24^{-}CD93^{+}), ProB (B220^{+}CD43^{+} IgM^{-}CD19^{+}CD24^{low}CD93^{+}) and PreB (B220^{+}CD43^{+}IgM^{-}CD19^{+}CD24^{hi}CD93^{+}) cells from *Bcl11a* WT and KO mice, and *R26*-eYFP deletion reporter expression in the lineages, 13 weeks post-p(I:C).
(D) Frequency of early thymic progenitor (ETP), double-negative (DN) 2, DN3 and DN4 progenitors in the thymus of *Bcl11a* WT and KO mice, 4 weeks post-p(I:C). n = 3 WT, 3 KO.
(E) FACS profile of MkP (Lin^{-}Sca-1^{-}c-Kit^{+}CD150^{+}CD41^{+}) and GMP (Lin^{-}Sca-1^{-}c-Kit^{+}CD41^{-}CD150^{-} CD16/32^{+}) from *Bcl11a* WT and KO mice, and *R26*-eYFP deletion reporter expression in the lineages, 13 weeks post-p(I:C).
(F) FACS profile of LMPP (LSKFlt3^{+}CD150^{-}) from *Bcl11a* WT and KO mice, and *R26*-eYFP deletion reporter expression, 13 weeks post-p(I:C).
(G) Total number of BM HSC 13 weeks following *Bcl11a* gene deletion.
(H) Relative mRNA expression of *Bcl11a* in sorted LSK BM cells from *Bcl11a* WT and KO mice. n = 2 WT, 2 KO.
(I) FACS profile of *R26*-eYFP expression in HSCs (LSKCD48^{-}Flt3^{-}CD150^{+}) as a measure of deletion efficiency, 13 weeks post-p(I:C).

Data in (B, D, H) represent mean ± SD. Percentages in (A, C, E-F, I) represent total mean frequencies, except for eYFP frequencies, which denote mean deletion efficiency from the indicated populations. In (A, C, E-I) n = 7 WT, 4 Het, 7 KO. *p<0.05; **p<0.01; ***p<0.001.

Figure S4

Related to Fig 3

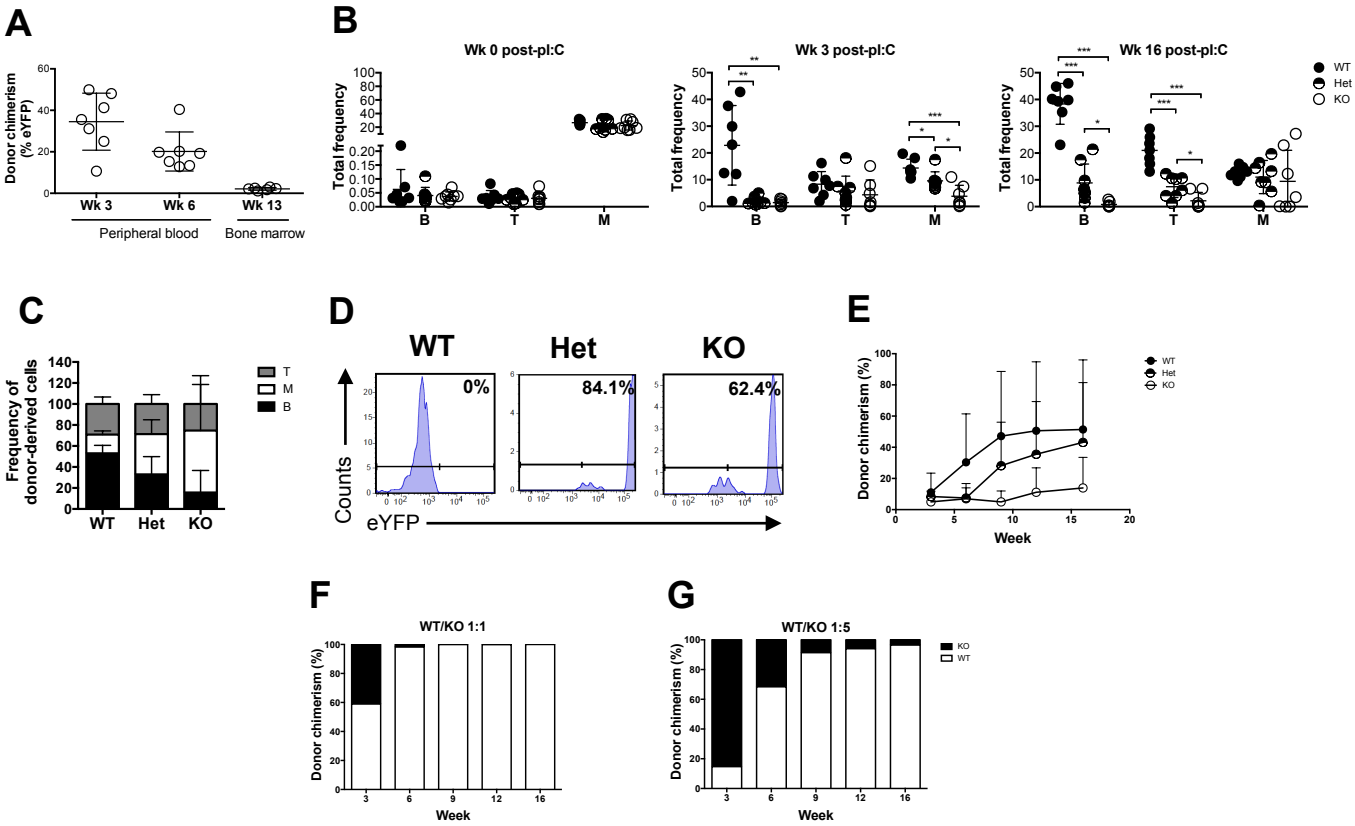

Figure S4. Transplantation of *Bcl11a*-deficient HSCs (Related to Figure 3)
(A) Donor chimerism, measured as frequency of *R26*-eYFP in the peripheral blood at 3 and 6 weeks, and in the BM at 13 weeks, following non-competitive transplantation of $1x10^6$ *Bcl11a*-deficient BM cells. n = 6-7 mice.
(B) Total frequency of peripheral blood B cells, T cells and myeloid cells before p(I:C) treatment (0 weeks) and 3 and 16 weeks after p(I:C) treatment, following transplantation of *Bcl11a* WT, Het and KO HSCs. n = 7 WT, 8 Het, 7-8 KO.
(C) Distribution of B cells, T cells and myeloid cells within donor-derived cells in the peripheral blood, 16 weeks after p(I:C) treatment, following transplantation of *Bcl11a* WT, Het and KO HSCs. n = 7 WT, 8 Het, 7-8 KO.
(D) FACS profiles of *R26*-eYFP expression in HSCs, as a measure of deletion efficiency, from *Bcl11a* WT, Het and KO HSC transplanted mice, 18 weeks post-p(I:C). n = 6 WT, 7 Het, 6 KO. Percentages represent mean frequencies.
(E) Donor-chimerism in peripheral blood following transplantation of *Bcl11a* WT, Het and KO HSCs into sublethally irradiated C57BL/6J-Ly5.1-$Kit^{W\text{-}41/W\text{-}41}$-$Gpi^{a/a}$ mice. n = 3 WT, 3 Het, 2 KO.
(F) Donor-chimerism in peripheral blood following competitive transplantation of *Bcl11a* WT and KO HSCs at 1:1. Data is from one transplanted mouse.
(G) Donor-chimerism in peripheral blood following competitive transplantation of *Bcl11a* WT and KO HSCs at 1:5. Data is from one transplanted mouse.

Data in (A-B, D) represent mean ± SD. *$p<0.05$; **$p<0.01$; ***$p<0.001$.

Figure S5

Related to Fig 4

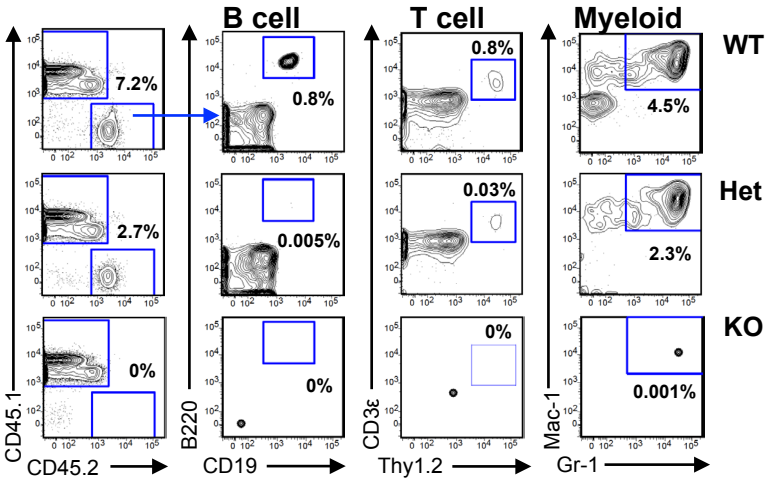

Figure S5. Peripheral blood analysis of secondary transplanted mice
(Related to Figure 4)

FACS profile of donor chimerism ($CD45.2^+$), B cells ($B220^+CD19^+$), T cells ($CD3e^+Thy1.2^+$) and myeloid cells ($Mac\text{-}1^+Gr\text{-}1^+$) in peripheral blood, 16 weeks post-transplantation. Percentages represent total mean frequencies.

Figure S6

Related to Fig 5

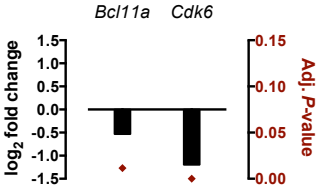

Figure S6. Expression of *Bcl11a* and *Cdk6* in microarray analysis
(Related to Figure 5)
Fold-change expression of *Bcl11a* and *Cdk6* in *Bcl11a*-deficient HSCs from microarray analysis. n = 3/genotype.

Figure S7

Related to Fig 7

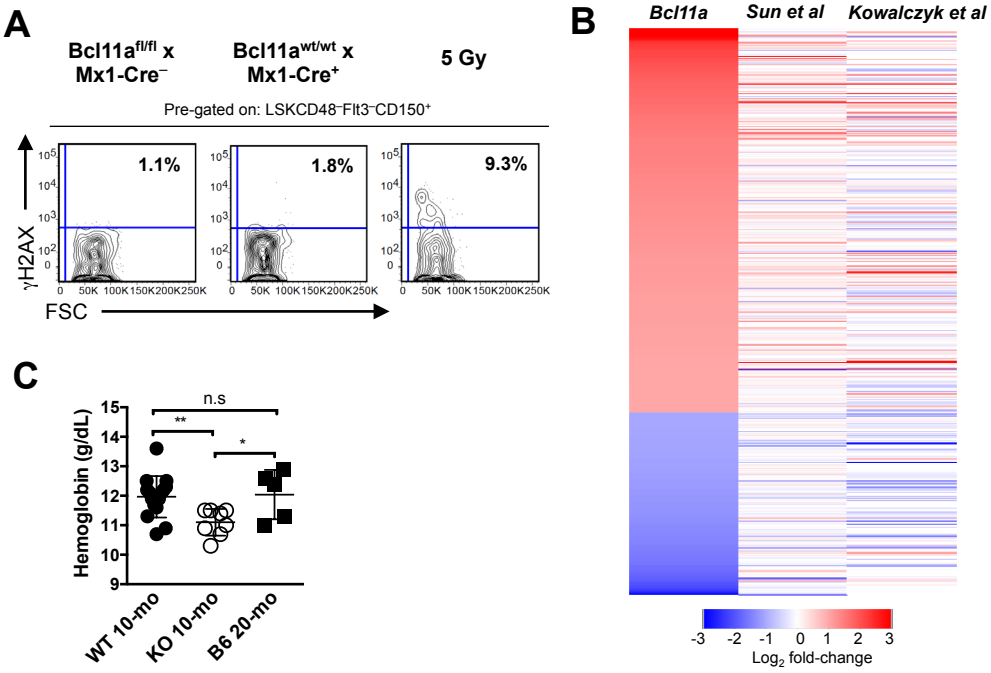

Figure S7. HSCs from *Bcl11a*-deficient mice resemble aged HSCs
(Related to Figure 7)

(A) FACS profiles of gH2AX staining in HSCs, showing mean frequencies from *Bcl11a*$^{fl/fl}$ *x Mx1-Cre*$^{-}$ and *Bcl11a*$^{wt/wt}$ *x Mx1-Cre*$^{+}$ mice and an irradiated control mouse (5 Gy). n = 4 Mx1-Cre^{-}, 4 Mx1-Cre^{+}, 1 irradiated control.

(B) Gene expression change of the *Bcl11a* KO associated differentially expressed genes (≥2 fold; $p<0.01$) in the *Bcl11a* KO (versus WT) or in the aged HSCs (versus young) (Kowalczyk et al., 2015 and Sun et al., 2014).

(C) Hemoglobin levels (g/dL) in 10-month (10-mo) old *Bcl11a* WT and KO mice, and 20-month (20-mo) old C57BL6 (B6) mice. n = 15 WT, 8 KO, 5 B6.

Table S1. Upregulated genes in *Bcl11a* KO HSC

#	Pathway maps (Figure reference)	Pathway maps (Full name)	p-value	FDR	Total # genes	# genes in pathway	Genes in pathway
1	Development	Development: Regulation of endothelial progenitor cell differentiation from adult stem cells	2.503E-05	1.279E-02	59	7	von Willebrand factor, VEGFR-2, AMPK alpha 1 subunit, CD34, CSF1, TIE2, P-selectin
2	Neutrophil migration	Inhibition of neutrophil migration by proresolving lipid mediators in COPD	7.675E-05	1.446E-02	70	7	p38 MAPK, IL-1RI, TNF-R2, ICAM2, PKC, CD34, PECAM1
3	Development	Development: Inhibition of angiogenesis by PEDF	8.491E-05	1.446E-02	31	5	p38 MAPK, VEGFR-2, Presenilin 1, Caspase-3, Presenilin
4	Immune response	Immune response: Role of integrins in NK cells cytotoxicity	2.307E-04	2.947E-02	38	5	p38 MAPK, Pyk2(FAK2), ICAM2, PKC-epsilon, c-Fos
5	Immune response	Immune response: ETV3 affect on CSF1-promoted macrophage differentiation	1.38E-03	1.410E-01	33	4	p38 MAPK, DDX20, CSF1, c-Fos
6	Platelet	Role of platelets in the initiation of in-stent restenosis	3.709E-03	2.369E-01	43	4	von Willebrand factor, PPBP, ICAM2, P-selectin
7	Tissue factor signaling	Regulation of tissue factor signaling in cancer	3.709E-03	2.369E-01	43	4	VEGFR-2, EPAS1, TNF-R2, P-selectin
8	Cell adhesion	Role of cell adhesion in vaso-occlusion in sickle cell disease	3.709E-03	2.369E-01	43	4	ITGAM, PKC-epsilon, PECAM1, P-selectin
9	Cell adhesion	Cell adhesion: ECM remodeling	7.341E-03	3.280E-01	52	4	Kallikrein 1, Syndecan-2, Kallikrein 2, Kallikrein 3 (PSA)
10	Development	Development: Thrombospondin-1 signaling	8.068E-03	3.280E-01	28	3	p38 MAPK, VEGFR-2, Caspase-3

Table S2. Downregulated genes in *Bcl11a* KO HSC

#	Pathway maps (Figure reference)	Pathway maps (Full name)	p-value	FDR	Total # genes	# genes in pathway	Genes in pathway
1	Apoptosis and survival	Apoptosis and survival: Role of IAP-proteins in apoptosis	9.820E-03	3.090E-01	31	2	Cytochrome c, NAIP
2	Cell cycle	Cell cycle: Start of DNA replication in early S phase	1.044E-02	3.090E-01	32	2	RPA3, Histone H1
3	Cell cycle	Cell cycle: ESR1 regulation of G1/S transition	1.108E-02	3.090E-01	33	2	Skp2/TrCP/FBXW, CDK6
4	Apoptosis and survival	Apoptosis and survival: Cytoplasmic/mitochondrial transport of proapoptotic proteins Bid, Bmf, Bim	1.174E-02	3.090E-01	34	2	Cytochrome c, FLASH
5	DNA damage repair	DNA damage: Nucleotide excision repair	1.310E-02	3.090E-01	36	2	POLD reg (p12), RPA3
6	Cholesterol and sphingolipid transport	Cholesterol and Sphingolipid transport Distribution to the intracellular membrane compartments (normal and CF)	1.453E-02	3.090E-01	38	2	NPC2, MENTHO
7	Cell cycle	Cell cycle: Regulation of G1/S transition (part 1)	1.453E-02	3.090E-01	38	2	Skp2/TrCP/FBXW, CDK6
8	Apoptosis and survival	Apoptosis and survival: TNFR1 signaling pathway	1.838E-02	3.090E-01	43	2	Cytochrome c, FLASH
9	Apoptosis and survival	Apoptosis and survival: FAS signaling cascades	1.920E-02	3.090E-01	44	2	Cytochrome c, FLASH
10	Neurophysiological process	Neurophysiological process: Receptor-mediated axon growth repulsion	2.003E-02	3.090E-01	45	2	Pleiotrophin (OSF1), Cofilin

Table S3. Antibodies for flow cytometry

Antigen	Fluorochrome	Company	Clone
Lineage antibodies			
B220 (CD45R)	eFluor450	eBioscience	RA3-6B2
CD3e	eFluor450	eBioscience	145-2C11
CD5 (Ly-1)	eFluor450	eBioscience	53-7.3
CD11b (Mac-1)	eFluor450	eBioscience	M1//70
Gr-1 (Ly-6G)	eFluor450	eBioscience	RB6-8C5
TER-119	eFluor450	eBioscience	TER-119
NK1.1	eFluor450	eBioscience	PK136
Ly-6C	eFluor450	eBioscience	HK1.4
CD11c	eFluor450	eBioscience	N418
CD19	PE-Cy5	BioLegend	6D5
CD3e	PE-Cy5	BioLegend	145-2C11
CD11b (Mac-1)	PE-Cy5	BioLegend	M1//70
TER-119	PE-Cy5	BioLegend	TER-119
Bone marrow , fetal liver, fetal spleen and peripheral blood staining			
Sca-1	AlexaFluor700	eBioscience	D7
Sca-1	BV605	BioLegend	D7
Sca-1	PE	BD Biosciences	D7
CD117 (c-Kit)	APC-eFluor780	eBioscience	2B8
Flt3	PE	BioLegend	A2F10
CD48	AlexaFluor700	BioLegend	HM48-1
CD48	PerCP-Cy5.5	BioLegend	HM48-1
CD150	PE-Cy7	BioLegend	TC15-12F12.2
CD150	APC	BioLegend	TC15-12F12.2
IL-7R	APC	eBioscience	A7R34
CD41	PE	eBioscience	MWReg30
CD105	PE-Cy7	BioLegend	MJ7/18
CD16/32	AlexaFluor700	eBioscience	93
B220	FITC	BD Biosciences	RA3-6B2
B220	APC	BioLegend	RA3-6B2
B220	BV605	eBioscience	RA3-6B2
CD19	APC	BD Biosciences	1D3
CD19	PerCP-Cy5.5	eBioscience	1D3
CD43	PE	eBioscience	eBioR2/60
CD93 (AA4.1)	PE-Cy7	BioLegend	AA4.1
CD24	APC-eFluor780	eBioscience	M1/69
IgM	APC	eBioscience	ll/41
IgM	FITC	eBioscience	Il-41
IgM	PerCP-Cy5.5	BD Biosciences	R6-60.2
CD71	PE	BD Biosciences	C2
NK1.1	PE-Cy7	BioLegend	PK136
Ter119	APC	BioLegend	TER-119
CD11b (Mac-1)	APC-Cy7	BioLegend	M1/70
Gr-1	BV570	BioLegend	RB6-8C5
Gr-1	APC	BioLegend	RB6-8C5
CD11b (Mac-1)	BV510	BioLegend	M1/70
CD90.2 (Thy1.2)	APC-eFluor780	eBioscience	53-2.1

Transplantation staining			
CD45.2	AlexaFluor700	BioLegend	104
CD45.2	BV510	BioLegend	104
CD45.2	Biotin	BD Biosciences	104
CD45.2	PE	BioLegend	104
CD45.1	PE	eBioscience	A20
Thymus staining			
CD8a	PE-Cy7	BioLegend	53-6.7
CD4	AlexaFluor700	BioLegend	RM4-5
CD25	APC	BioLegend	PC61
Intracellular staining			
Annexin V	APC	BioLegend	
BrdU	APC	BD Biosciences	
Ki-67	eFluor660	eBioscience	
phospho-H2AX (S139)	eFluor660	eBioscience	
Viability dyes			
7-AAD		BD Biosciences	
DAPI		ThermoFisher Scientific	

Table S4. Primer sequences for real-time PCR

		5' – 3'
mGapdh	Fwd	TGGTGAAGGTCGGTGTGAAC
	Rev	CCATGTAGTTGAGGTCAATGAAGG
mBcl11a	Fwd	AACCCCAGCACTTAAGCAAA
	Rev	ACAGGTGAGAAGGTCGTGGT
hGapdh	Fwd	ACCCAGAAGACTGTGGATGG
	Rev	TTCAGCTCAGGGATGACCTT
hBcl11a	Fwd	AACCCCAGCACTTAAGCAAA
	Rev	GGAGGTCATGATCCCCTTCT
Globin εγ	Fwd	TGGCCTGTGGAGTAAGGTCAA
	Rev	GAAGCAGAGGACAAGTTCCCA
Globin βh1	Fwd	TGGACAACCTCAAGGAGACC
	Rev	ACCTCTGGGGTGAATTCCTT
Cdk6	Fwd	GCTTCGTGGCTCTGAAGCGCG
	Rev	TGGTTTCTGTGGGTACGCCGG
Ccnd1	Fwd	TGTTACTTGTAGCGGCCTGTTG
	Rev	CCGGAGACTCAGAGCAAATCC
Ccnd2	Fwd	CACGACTTCATTGAGCACATCCT
	Rev	GCGGATCAGGGACAGCTTCT
Ccna2	Fwd	CAGCATGAGGGCGATCCTT
	Rev	GCAGGGTCTCATTCTGTAGTTTATATTCT
Ccne1	Fwd	GCAGCGAGCAGGAGACAGA
	Rev	GCTGCTTCCACACCACTGTCTT
Ccnb1	Fwd	TGTGAACCAGAGGTGGAACTTGC
	Rev	ATCGGGCTTGGAGAGGGATTATC
Ccnb2	Fwd	GCATCATGGACCGGTTCCT
	Rev	TCCCGACCACCTGCAGTTT
p16	Fwd	GGTTCTTGGTCACTGTGAGGA
	Rev	GCAGAAGAGCTGCTACGTGAA
p21	Fwd	TGACCCACAGCAGAAGAG
	Rev	ACCAGCCTGACAGATTTCTA
p27	Fwd	TGGACCAAATGCCTGACTC
	Rev	GGGAACCGTCTGAAACATTTTC
p57 (Cdkn1c)	Fwd	CAGCGGACGATGGAAGAACT
	Rev	CTCCGGTTCCTGCTACATGAA
Mcm2	Fwd	AGAAGTTCAGCGTCATGCGGAGTA
	Rev	CCCAAAGCGGTTGCGTTGATATGT
Mcm3	Fwd	AGGAAGACTCATGCCAAGGATGGA
	Rev	TGGGCTCACTGAGTTCCACTTTCT
Mcm4	Fwd	ACAGGAATGAGTGCCACTTCTCGT
	Rev	AAAGCTCGCAGGGCTTCTTCAAAC
Mcm5	Fwd	CTGGATGCTGCTTTGTCTGGCAAT
	Rev	TGTGTTCAGACACCTGAGAGCCAA
Mcm6	Fwd	GGACCAAGTTGCTATTCA
	Rev	ATTCAGAGTTGCCTTCAC
Mcm7	Fwd	CCCTGCCCAATTTGAACCTTTGGA
	Rev	TCTCCACATATGCTGCGGTGATGT

Table S5. Growth factors

Growth factor	Company	Final concentration
Recombinant mouse Stem Cell Factor (rmSCF)	R&D Systems	50 ng/ml
Recombinant mouse Fms-like tyrosine kinase 3 ligand (rmFlt3L)	Peprotech	50 ng/ml
Recombinant mouse Thrombopoietin (rmTpo)	R&D Systems	50 ng/ml
Recombinant mouse Interleukin 3 (rmIL-3)	R&D Systems	20 ng/ml

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Experimental animals

The Bcl11a floxed (*Bcl11a*$^{fl/fl}$) mouse strain, containing loxP sites flanking exon 1 has previously been described (Ippolito et al., 2014; Sankaran et al., 2009). *Bcl11a fl/fl* mice were crossed with the *Mx1-Cre* strain (Kühn et al., 1995) and *Rosa26*-stop-EYFP (*R26*-eYFP; The Jackson Laboratory). Hematopoietic specific deletion of *Bcl11a* was achieved by intraperitoneal administration of poly(I:C) (InvivoGen). Five doses of poly(I:C) (12.5mg/g body weight) were given every other day. Deletion efficiency was assessed by RT-PCR and flow cytometric evaluation of eYFP expression. Animals were typically treated at 4-6 weeks of age and analyzed at least 3-4 weeks following poly(I:C) treatment. B6.SJL-*Ptprc*^{a}/BoyAiTac (Taconic) or C57BL/6J-Ly5.1-Kit$^{W\text{-}41/W\text{-}41}$-Gpi$^{a/a}$ (Benveniste et al., 2010) mice were used as recipients in transplantation experiments. Germline deletion of *Bcl11a* was achieved by mating *Bcl11a*$^{fl/\Delta}$ mice with *Gata1-Cre* mice (Jasinski et al., 2001). Fetal livers and fetal spleens were isolated from timed pregnant mice where the day of the vaginal plug was set to embryonic day (E) 0.5. All experiments using adult mice were performed with mouse strains backcrossed on a C57BL6 background (>9 generations) unless stated otherwise. The Institutional Animal Care and Use Committee at Boston Children's Hospital approved all experiments.

Flow cytometry experiments

Bone marrow cells were prepared from isolated femurs, tibiae and iliac crest bones and Fc-blocked with purified CD16/32 (E-Bioscience) followed by staining with antibodies against cell surface antigens. See **Table S3** for a complete list of antibodies. For HSC analysis, BM cells were enriched for CD117 using MACS beads (Miltenyi Biotec) prior to staining.

Peripheral blood was collected from the tail vein. RBC was separated from leukocytes by Dextran (Sigma-Aldrich) sedimentation followed by RBC-depletion of the leukocyte fraction with ammonium chloride (Stem Cell Technologies). Isolated leukocytes were Fc-blocked prior to staining with antibodies against cell surface markers. See **Table S3** for a complete list of antibodies.

7-aminoactinomycin D (7-AAD; BD Biosciences) or DAPI (4',6-Diamidino-2-Phenylindole, Dilactate; ThermoFisher Scientific) was used to discriminate non-viable cells.

In all flow cytometry analyses and purifications, *Bcl11a* Het and KO mice were always gated positively for eYFP in addition to the indicated immunophenotypes to analyze or isolate *Bcl11a*-deleted cells.

Cell sorting experiments were performed on a BD FACSAria II SORP and all analyses were performed on a BD LSRFortessa (BD Biosciences). Post-acquisition data analyses were done with the FlowJo data analysis software (FlowJo, LLC). Cell counting experiments assessing cellular expansion was performed on a BD Accuri C6 (BD Biosciences).

Western blot analysis

Whole-cell lysates from primary CD117-enriched BM cells and primary human mesenchymal cells (ATCC, PCS-500-012), murine erythroleukemia (MEL), OP9, MS-5 and Hudep-2 cells were separated by SDS-PAGE. Proteins were detected by a BCL11A mouse monoclonal antibody (Ctip1, 14B5, Abcam) and HRP-conjugated donkey anti-mouse IgG1 (Santa Cruz Biotechnology) or HRP-conjugated goat polyclonal IgG against Actin (C-11, Santa Cruz Biotechnology).

Transplantation experiments

B6.SJL-*Ptprc*^{a}/BoyAiTac (CD45.1; 10-12 weeks old) mice were lethally irradiated (two split doses of 500cGy). Competitive transplantation was performed by the intravenous injection of 200 HSCs (LSKCD48^{-}Flt3^{-}CD150^{+}) isolated by FACS from *Bcl11a*$^{fl/fl}$ *x Mx1-Cre* mice (CD45.2) and 200,000 whole BM cells (CD45.1). C57BL/6J-Ly5.1-Kit$^{W\text{-}41/W\text{-}41}$-Gpi$^{a/a}$ mice were used in transplantation experiments with sublethally irradiated (400cGy) hosts. Non-competitive transplantation was performed by the intravenous injection of $1x10^{6}$ whole BM cells from *Bcl11a*$^{fl/fl}$ *x Mx1-Cre* mice on a mixed background, into irradiated (700cGy) *Rag2*-deficient recipients. Peripheral blood was withdrawn from the tail vein of transplanted animals at regular intervals to assess reconstitution levels.

Gene expression experiments

Cells were sorted into TRIzol (ThermoFisher Scientific) and RNA was isolated using the RNeasy Plus Mini Kit or RNeasy Plus Micro Kit (Qiagen) according to the manufacturer's protocol. cDNA was synthesized with the iScript cDNA synthesis kit (Bio-Rad) from purified RNA. Real-time (RT) PCR was performed using the iQ SYBR Green Supermix (Bio-Rad). Relative expression was quantified using the ΔΔCt method and normalized to *Gapdh*. See **Table S4** for primer sequences.

For global gene expression analysis RNA was isolated as described. Purified RNA was processed as follows: Labeling Protocol, targets were prepared with the NuGEN Ovation Pico WTA System V2 with Ribo-SPIA® amplification for cDNA. The NuGEN Encore® Biotin Module was used to label the samples prior to loading on to Affymetrix Mouse Gene 2.0 ST chips. Hybridization Protocol: Chips were hybridized for 16-18 hours in Hybridization Oven 645 at 45°C. Chips were stained with the Affymetrix GeneChip(r) Hybridization, Wash, and Stain Kit on Fluidics Station 450 with Fluidics Script 002. Scanning Protocol: All chips were scanned with the Affymetrix GeneChip(r) Scanner 3000 7G System using the standard protocol.

Bioinformatics analyses

Affymetrix CEL files were normalized using RMA (Irizarry et al., 2003). Differentially expressed genes were detected by the lmFit function in the limma package (Smyth, 2004) with a threshold of fold change ≥2 and adjusted p-value ≤0.01. Pathway enrichment analyses were performed using GeneGo Metacore from Thomson Reuters (Version 6.24 build 67895, https://portal.genego.com/) on differentially expressed genes. GSEA was performed using GSEA software (http://www.broadinstitute.org/gsea) (Subramanian et al., 2005) with default parameters.

Comparative analyses with other published gene expression data derived on different platforms, were performed by comparison of differentially expressed genes (log_2 fold-change).

Cell cycle and cell proliferation experiments

Cell cycle analyses were performed by CD117 enrichment of BM cells followed by staining of HSC cell surface markers, and fixation and permeabilization by the BD Cytofix/Cytoperm Kit (BD Biosciences) according to manufacturer's protocol. Following fixation and permeabilization, cells were incubated with Ki-67 antibody and DAPI before flow cytometry analysis.

Cell proliferation assays were performed by the administration of a single intraperitoneal injection of 5-Bromo-2'-deoxyuridine (BrdU) to *Bcl11a*$^{fl/fl}$ *x Mx1-Cre* mice. Bone marrow cells were isolated from treated mice at different time-points. CD117 enrichment of BM cells was performed, followed by staining of HSC cell surface markers. Fixation and permeabilization and BrdU visualization was performed using the BD BrdU Flow Kit (BD Biosciences) according to manufacturer's protocol.

Cell division analyses

For flow cytometric evaluation of cell division, HSCs (LSKCD48$^-$Flt3$^-$CD150$^+$) were isolated by FACS and incubated with CellTrace Far Red (ThermoFisher Scientific) according to manufacturer's protocol. Following Cell Trace incubation, cells were cultured in X-VIVO15 media (Lonza) supplemented with 10% fetal calf serum (FCS, HyClone), BIT 9500 (Stemcell Technologies), β-mercaptoethanol, L-glutamine and growth factors (see **Table S5**). The number of cell divisions was assessed by flow cytometry after 6-7 days. Evaluation of cell division kinetics was performed by plating single HSCs (LSKCD48$^-$Flt3$^-$CD150$^+$) in 60-well plates (Nunc), in X-VIVO15 media (Lonza) supplemented with 10% fetal calf serum (FCS, HyClone), BIT 9500 (Stemcell Technologies), β-mercaptoethanol, L-glutamine and growth factors (see **Table S5**). The presence of single cells in the wells was confirmed 12-16 hours after cell plating. The number of cells in the wells was scored 24 and 48 hours after cell plating with an inverted microscope. Cell counting experiments assessing cellular expansion was performed using CountBright absolute counting beads (ThermoFisher Scientific).

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