Table S1. Runx1-bound genes deregulated in the peripheral blood of *Cbfb*$^{+/\Delta C95}$ embryos

Fold Change	Symbol	Entrez Gene Name	Deregulated in *Cbfb*$^{-/-}$ *embryos*[4]	Deregulated in Runx1 KD MEL cells[25]	Deregulated in *Runx1* KD K562 cells[26]
2.726	1600029D21Rik	RIKEN cDNA 1600029D21 gene			
-7.459	ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1			
-2.022	ACADM	acyl-CoA dehydrogenase, C-4 to C-12 straight chain			
-2.526	ACAP2	ArfGAP with coiled-coil, ankyrin repeat and PH domains 2			
-2.216	ACSL1	acyl-CoA synthetase long-chain family member 1			yes
-2.31	ACTR3	ARP3 actin-related protein 3 homolog (yeast)			
-2.613	AFF4	AF4/FMR2 family, member 4	yes		
-4.329	ALOX5AP	arachidonate 5-lipoxygenase-activating protein		yes	
-3.202	ANKRD13C	ankyrin repeat domain 13C			
-2.032	ARHGEF12	Rho guanine nucleotide exchange factor (GEF) 12			
-2.309	ASPM	asp (abnormal spindle) homolog, microcephaly associated (Drosophila)		yes	
-2.066	B3GALNT1	beta-1,3-N-acetylgalactosaminyltransferase 1 (globoside blood group)			
-2.24	BNIP3L	BCL2/adenovirus E1B 19kDa interacting protein 3-like			
-2.572	CCL2	chemokine (C-C motif) ligand 2			yes
-3.117	Ccl9	chemokine (C-C motif) ligand 9	yes		
-2.409	CCNG2	cyclin G2		yes	
-2.467	CCNL1	cyclin L1			
-2.256	CCR1	chemokine (C-C motif) receptor 1			
-2.22	CD47	CD47 molecule			
-2.497	CD48	CD48 molecule			
-5.261	CD53	CD53 molecule	yes	yes	yes
-2.076	CITED2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2		yes	
-3.721	CMTM7	CKLF-like MARVEL transmembrane domain containing 7		yes	
-2.505	COTL1	coactosin-like 1 (Dictyostelium)			
-2.447	CPA3	carboxypeptidase A3 (mast cell)	yes		
-2.20	CPEB4	cytoplasmic polyadenylation element binding protein 4		yes	
-2.195	CREG1	cellular repressor of E1A-stimulated genes 1			
-2.059	CSNK1G3	casein kinase 1, gamma 3			
-6.338	CTSS	cathepsin S	yes		
-2.361	DAPP1	dual adaptor of phosphotyrosine and 3-phosphoinositides	yes		
-2.076	DCUN1D1	DCN1, defective in cullin neddylation 1, domain containing 1 (S. cerevisiae)			

-2.286	EDEM3	ER degradation enhancer, mannosidase alpha-like 3			
-2.885	ELF1	E74-like factor 1 (ets domain transcription factor)			
-2.199	ELL2	elongation factor, RNA polymerase II, 2			
-2.230	EMB	embigin			
-3.061	EMR1	egf-like module containing, mucin-like, hormone receptor-like 1			
-2.008	EPC2	enhancer of polycomb homolog 2 (Drosophila)			
-2.26	EVI5	ecotropic viral integration site 5			
-2.123	F2R	coagulation factor II (thrombin) receptor			
-2.081	FAHD1	fumarylacetoacetate hydrolase domain containing 1			
-2.319	FAM13B	family with sequence similarity 13, member B			
-3.433	FCER1G	Fc fragment of IgE, high affinity I, receptor for; gamma polypeptide	yes		
-4.935	Fcrls	Fc receptor-like S, scavenger receptor	yes		
-2.197	FNDC3B	fibronectin type III domain containing 3B			
-2.116	FRMD4A	FERM domain containing 4A			
-2.323	FRMD4B	FERM domain containing 4B			
-2.576	FYB	FYN binding protein	yes	yes	
-2.008	GLIPR1	GLI pathogenesis-related 1			
-4.709	Ifitm3	interferon induced transmembrane protein 3			
-2.357	ITGA6	integrin, alpha 6			
-2.057	ITGAL	integrin, alpha L (antigen CD11A (p180), lymphocyte function-associated antigen 1; alpha polypeptide)		yes	
-2.212	ITM2B	integral membrane protein 2B			yes
-3.67	KLF6	Kruppel-like factor 6	yes		yes
-2.498	KTN1	kinectin 1 (kinesin receptor)			
-4.307	LAPTM5	lysosomal protein transmembrane 5	yes		
-2.352	LCP1	lymphocyte cytosolic protein 1 (L-plastin)		yes	
-4.397	LIMD2	LIM domain containing 2			
-2.27	LIMS1	LIM and senescent cell antigen-like domains 1			
-2.304	LPIN2	lipin 2			
-2.758	LRRC33	leucine rich repeat containing 33	yes		
-3.330	LY86	lymphocyte antigen 86			
-3.70	MALAT1	metastasis associated lung adenocarcinoma transcript 1 (non-protein coding)	yes		
2.24	MCM5	minichromosome maintenance complex component 5	yes		yes
-2.041	MEF2C	myocyte enhancer factor 2C			
-5.727	MPO	myeloperoxidase	yes		
-3.562	MYB	v-myb myeloblastosis viral oncogene homolog (avian)	yes	yes	

-2.006	MYO1F	myosin IF			
-2.051	NRIP1	nuclear receptor interacting protein 1			
-2.36	OGFRL1	opioid growth factor receptor-like 1	yes		
-2.999	P2RY6	pyrimidinergic receptor P2Y, G-protein coupled, 6	yes		
-2.198	PDCD4	programmed cell death 4 (neoplastic transformation inhibitor)			
-2.621	PELI1	pellino E3 ubiquitin protein ligase 1			
-4.431	PF4	platelet factor 4	yes	yes	
-2.076	PHF20L1	PHD finger protein 20-like 1			
2.023	PHLDA1	pleckstrin homology-like domain, family A, member 1	yes		
-2.34	PIP4K2A	phosphatidylinositol-5-phosphate 4-kinase, type II, alpha		yes	
-4.31	PLEK	pleckstrin	yes		
-2.23	PPM1A	protein phosphatase, Mg2+/Mn2+ dependent, 1A	yes		
-2.10	PPP1R12A	protein phosphatase 1, regulatory subunit 12A			
-2.436	PPP3CA	protein phosphatase 3, catalytic subunit, alpha isozyme			
-2.317	PRKAR2B	protein kinase, cAMP-dependent, regulatory, type II, beta			
-3.138	PROKR1	prokineticin receptor 1	yes		
-2.06	PTPN12	protein tyrosine phosphatase, non-receptor type 12			
-2.447	RAB8B	RAB8B, member RAS oncogene family			
-2.428	RALA	v-ral simian leukemia viral oncogene homolog A (ras related)			
-2.124	RALBP1	ralA binding protein 1			
-2.129	RAPGEF6	Rap guanine nucleotide exchange factor (GEF) 6			
-2.114	RCBTB2	regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 2	yes		
-2.121	RHOB	ras homolog family member B			
-2.265	RICTOR	RPTOR independent companion of MTOR, complex 2			
-2.481	RNF130	ring finger protein 130			
-4.113	RTP4	receptor (chemosensory) transporter protein 4			
-2.547	SH3GLB1	SH3-domain GRB2-like endophilin B1			
-3.372	SLC18A2	solute carrier family 18 (vesicular monoamine), member 2	yes	yes	
-2.282	SLC2A1	solute carrier family 2 (facilitated glucose transporter), member 1			
-2.071	SLC2A1	solute carrier family 2 (facilitated glucose transporter), member 1			
-2.241	SLC38A2	solute carrier family 38, member 2			yes
-2.147	SLMAP	sarcolemma associated protein			
-5.188	SOX4	SRY (sex determining region Y)-box 4	yes		
-3.455	SRGN	serglycin	yes	yes	
2.037	SRM	spermidine synthase	yes	yes	

-2.458	ST6GAL1	ST6 beta-galactosamide alpha-2,6-sialyltranferase 1			yes
-2.609	TAX1BP1	Tax1 (human T-cell leukemia virus type I) binding protein 1			
-2.31	TBCEL	tubulin folding cofactor E-like			
-2.278	TGFBI	transforming growth factor, beta-induced, 68kDa			
-2.30	TMEM30A	transmembrane protein 30A			
-2.027	TNFAIP2	tumor necrosis factor, alpha-induced protein 2			
-2.426	TOB1	transducer of ERBB2, 1			
-2.280	TRAK2	trafficking protein, kinesin binding 2	yes		
-2.736	TREML2	triggering receptor expressed on myeloid cells-like 2	yes		
-9.284	TYROBP	TYRO protein tyrosine kinase binding protein	yes		yes
-2.066	Unkl	unkempt-like (Drosophila)	yes	yes	
-2.116	Zrsr1	zinc finger (CCCH type), RNA binding motif and serine/arginine rich 1			

The chromatin immunoprecipitation sequencing data from Wilson, et al. (Cell Stem Cell 7:532, 2010) was used to determine which differentially expressed genes in $Cbfb^{+/\Delta C95}$ mice are bound by Runx1. In addition, the indicated published gene expression datasets from other models of decreased Runx1 activity were queried to determine if individual genes showed deregulated expression in other studies. Genes that showed >2 fold change, and p< .05 in each study are indicated with a “yes”. KD= Knockdown.

Supplemental Figure Legends

Kamikubo et al., “The C-terminal of CBFβ-SMMHC is required for its dominant negative activity and leukemogenesis”

Figure S1. The C-terminal 95 amino acids of CBFβ-SMMHC are required for primitive blood differentiation defects. Bar graphs of (A) Ter119 and C-Kit and (B) Csf2rb staining of primitive blood cells from E10.5 embryos of the indicated genotypes and indicated lines. * indicates a statistically significant difference ($p<.05$) as compared to wildtype littermate embryos. N≥ 3 for all genotypes for both lines. (C) Colony assays with fetal liver (FL) cells from embryos of the indicated genotypes at E12.5. FL cells from *Cbfb*$^{+/\Delta C95}$ embryos gave rise to similar numbers of colonies (both total and different lineages) as those from wildtype embryos. On the other hand, FL cells from *Cbfb*$^{+/MYH11}$ embryos produced very few to no colonies. N≥3 for all genotypes.

Figure S2. Increased Mac1/Gr1 double positive cells in peripheral blood (PB) from aged *Cbfb*$^{+/\Delta C95}$ mice. (A) PB Mac1/Gr1 double positive cell percentage in mice from line #2 1 -1.5 years of age. (B) Representative PB FACS staining of Mac1 and Gr1.

Figure S3. Increased myelopoiesis in the bone marrow and spleen in adult *Cbfb*$^{+/\Delta C95}$ mice. H&E stained bone marrow and spleen sections from adult *Cbfb*$^{+/\Delta C95}$ mice.

Figure S4. Increased colony forming activity in bone marrow cells from adult *Cbfb*$^{+/\Delta C95}$ mice. Bar graph showing (A) total colony number and (B) percentage of the indicated colony types from in vitro differentiation of bone marrow cells from mice of the indicated genotype. p values are shown. NS= not significant. N=3 for each genotype.

Figure S5. Infiltration of myeloid cells in liver and kidney in adult *Cbfb*$^{+/\Delta C95}$ mice. H&E stained histological sections of the indicated organs from adult mice of the indicated genotypes. Black arrows indicate infiltrated myeloid cells.

Figure S1

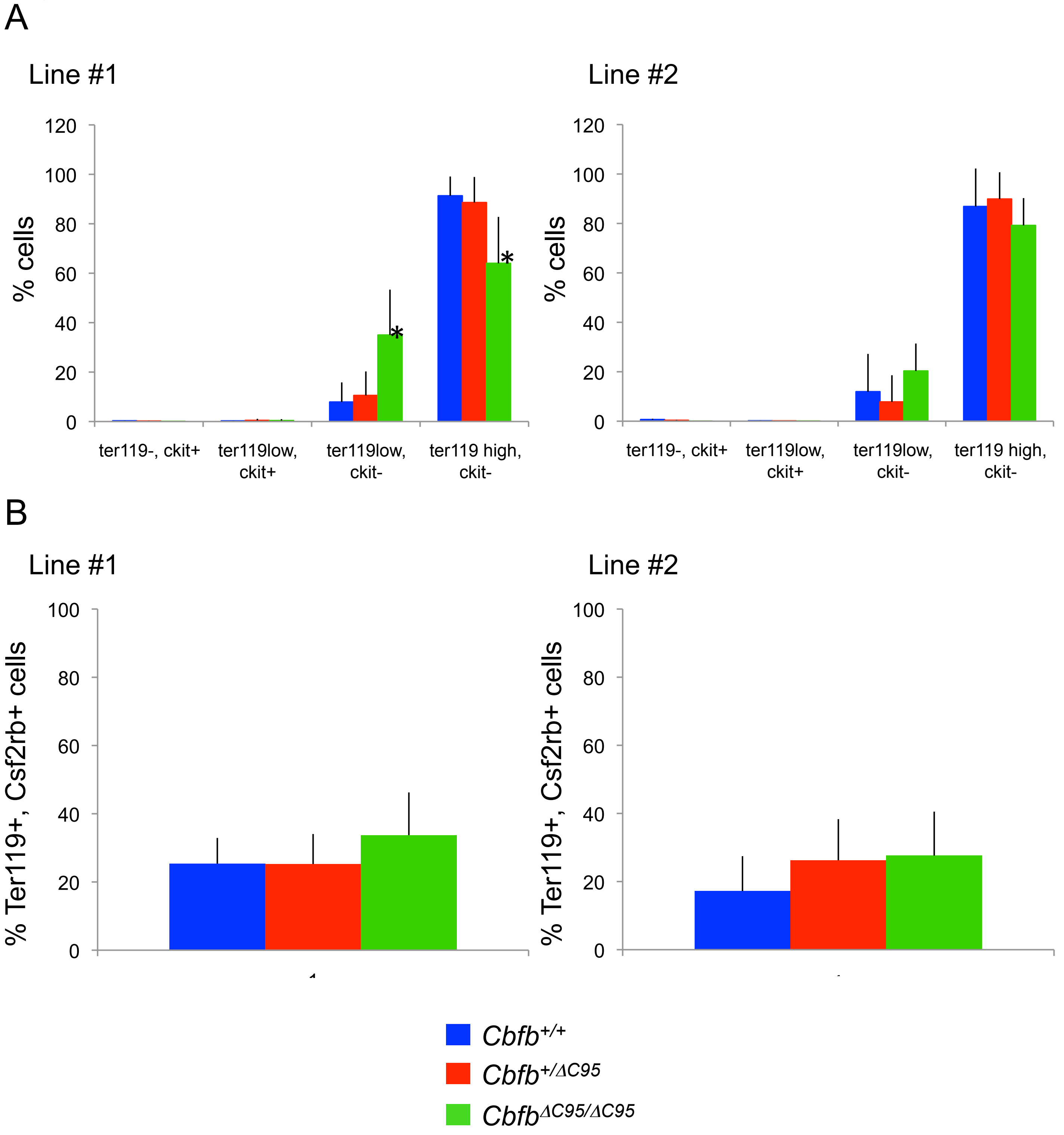
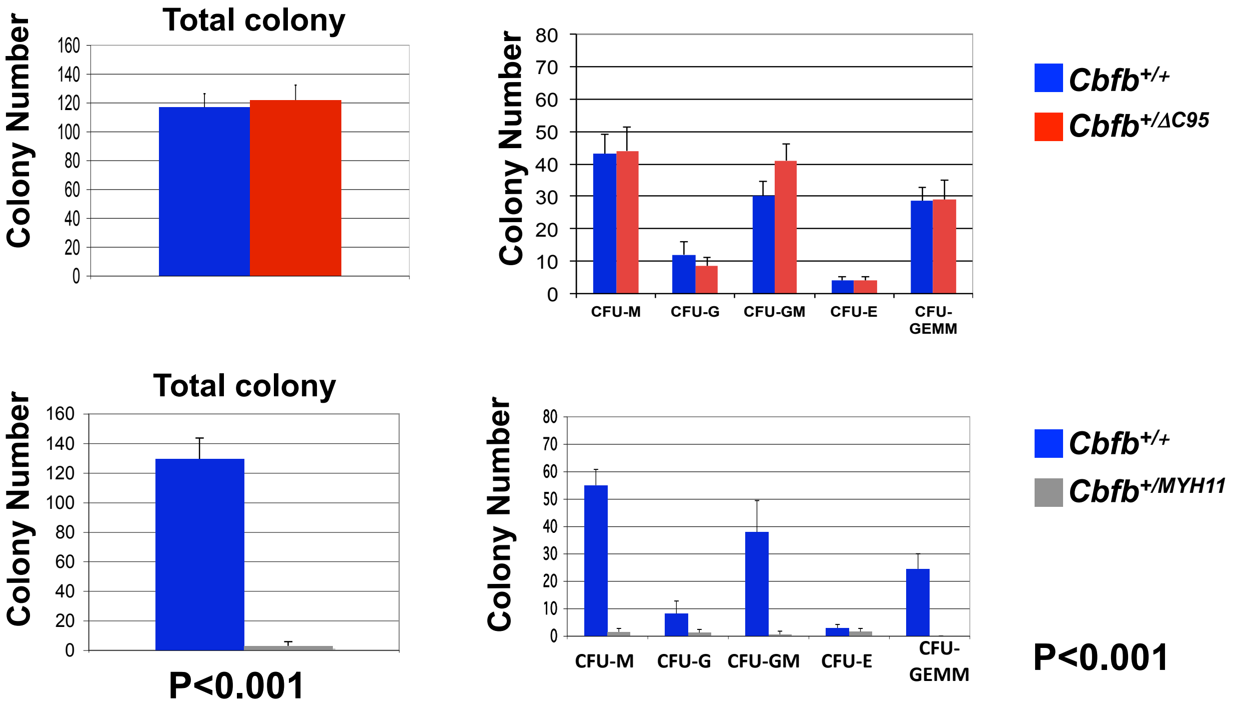

A
Line #1
Line #2
% cells
120
100
80
60
40
20
0
ter119-, ckit+
ter119low, ckit+
ter119low, ckit-
ter119 high, ckit-
*
*
B
Line #1
Line #2
% Ter119+, Csf2rb+ cells
100
80
60
40
20
0
$Cbfb^{+/+}$
$Cbfb^{+/\Delta C95}$
$Cbfb^{\Delta C95/\Delta C95}$

Figure S1

C



Total colony
Colony Number
160
140
120
100
80
60
40
20
0
Colony Number
80
70
60
50
40
30
20
10
0
CFU-M
CFU-G
CFU-GM
CFU-E
CFU-GEMM
🟦 Cbfb+/+
🟥 Cbfb+/ΔC95
Total colony
Colony Number
160
140
120
100
80
60
40
20
0
P<0.001
Colony Number
80
70
60
50
40
30
20
10
0
CFU-M
CFU-G
CFU-GM
CFU-E
CFU-GEMM
🟦 Cbfb+/+
⬜ Cbfb+/MYH11
P<0.001

Figure S2

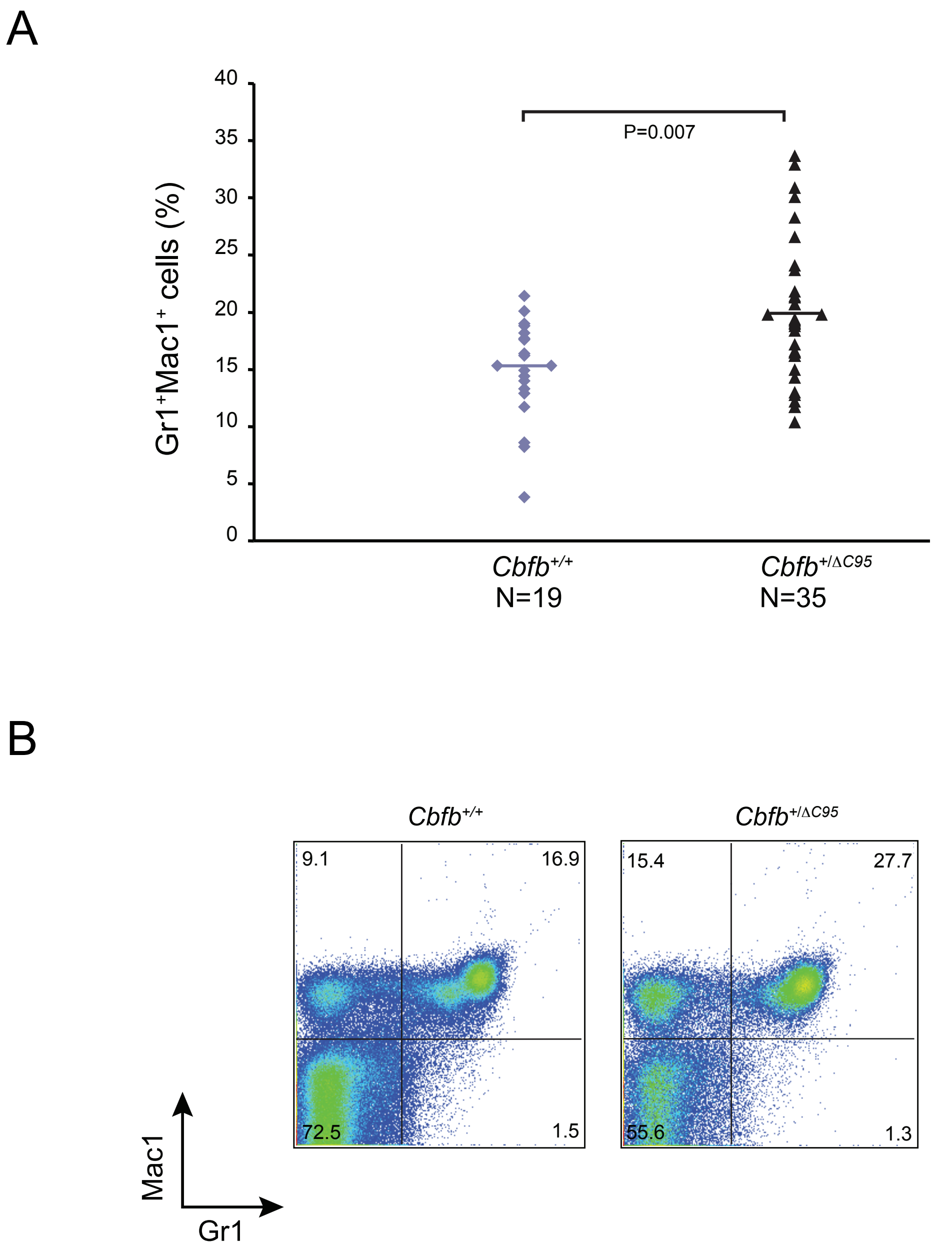

A
40
35
30
25
20
15
10
5
0
$Gr1^{+}Mac1^{+}$ cells (%)
P=0.007
$Cbfb^{+/+}$
N=19
$Cbfb^{+/\Delta C95}$
N=35
B
$Cbfb^{+/+}$
9.1
16.9
72.5
1.5
$Cbfb^{+/\Delta C95}$
15.4
27.7
55.6
1.3
Mac1
Gr1

Figure S3

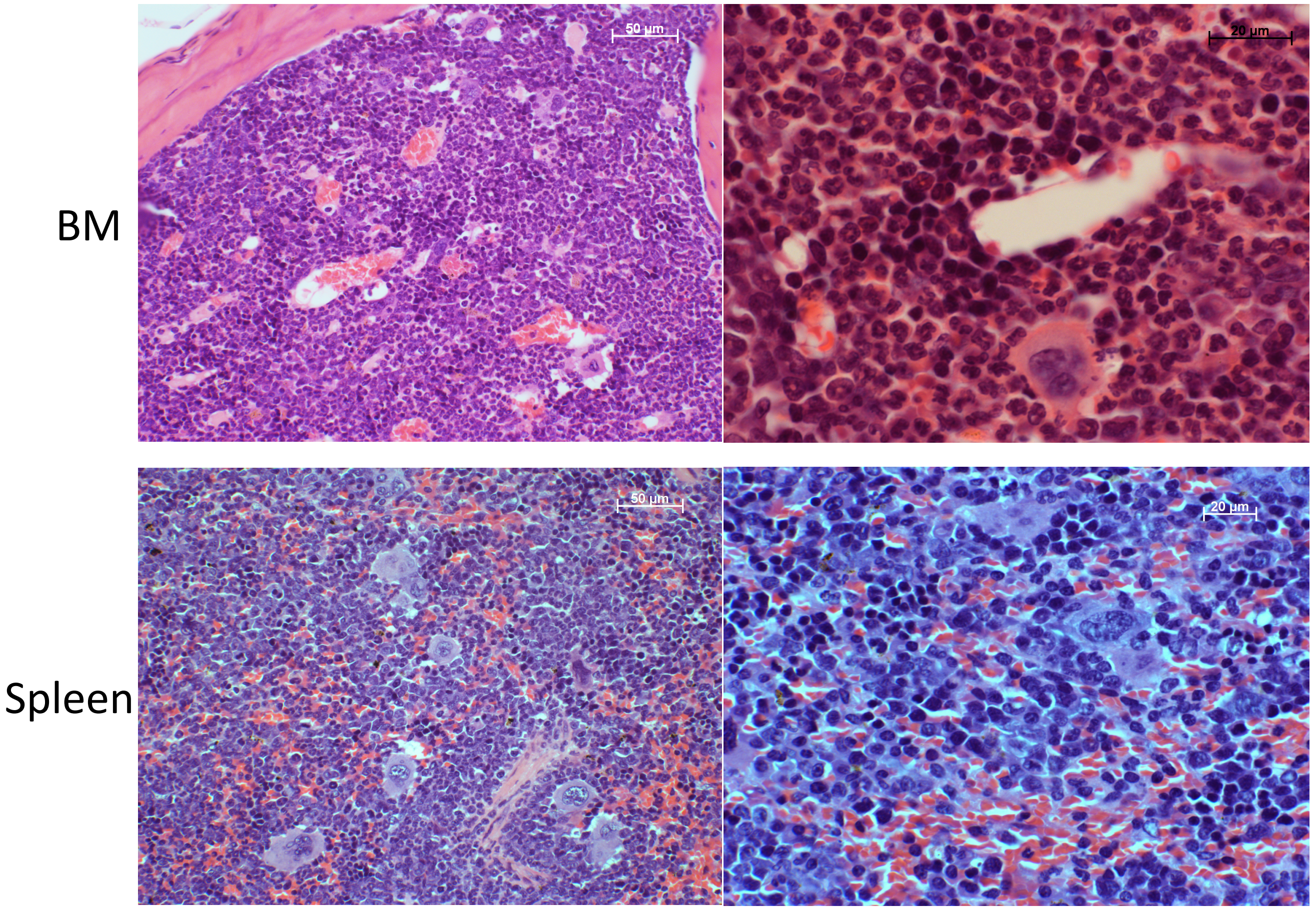


Figure S4

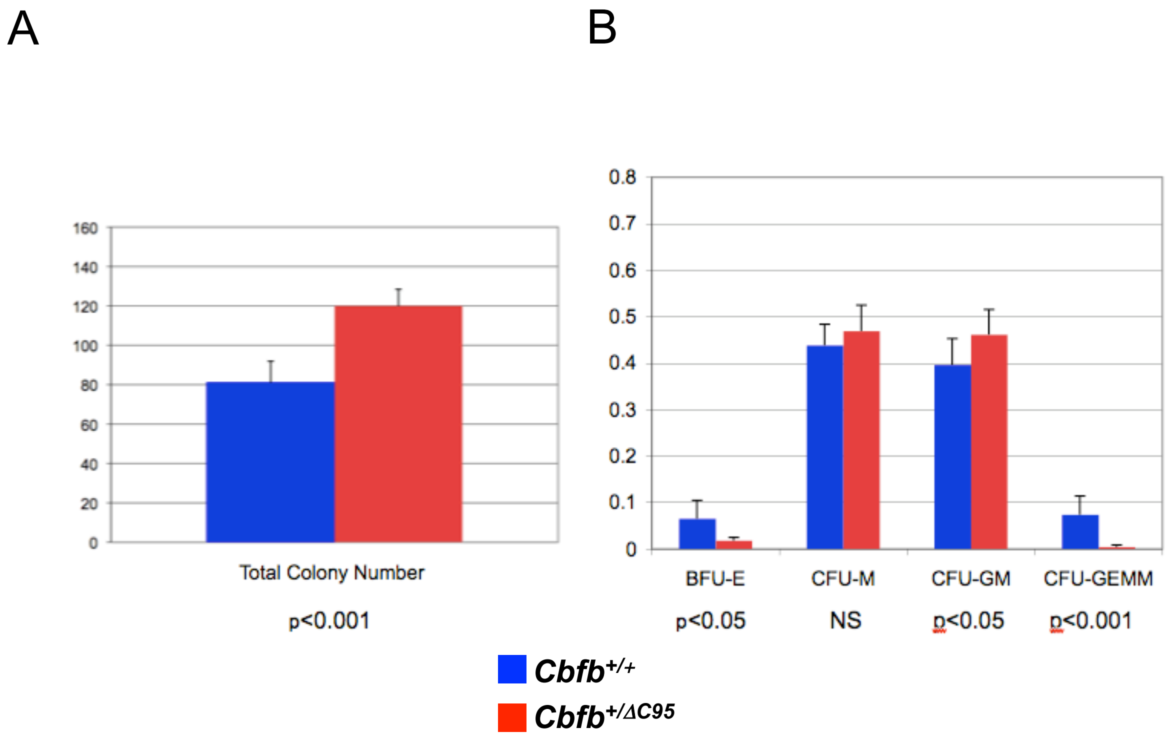

A
B
160
140
120
100
80
60
40
20
0
Total Colony Number
p<0.001
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
BFU-E
CFU-M
CFU-GM
CFU-GEMM
p<0.05
NS
p<0.05
p<0.001
🟦 $Cbfb^{+/+}$
🟥 $Cbfb^{+/\Delta C95}$

Figure S5