The Blk pathway functions as a tumor suppressor in chronic myeloid leukemia stem cells

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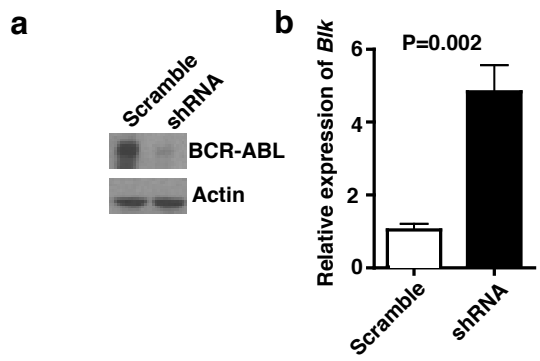
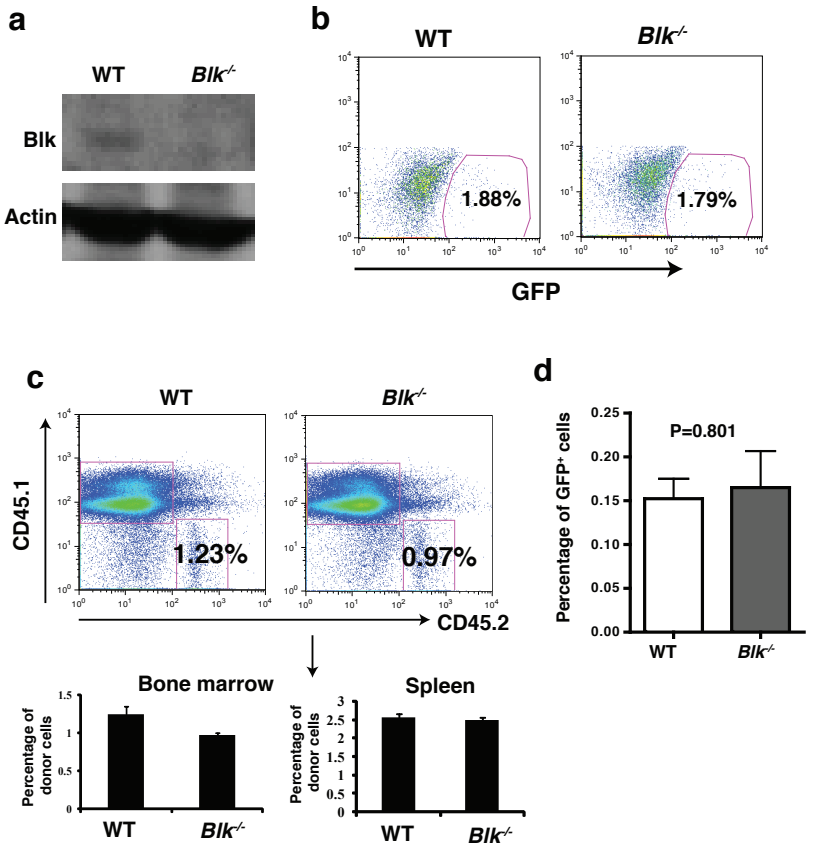
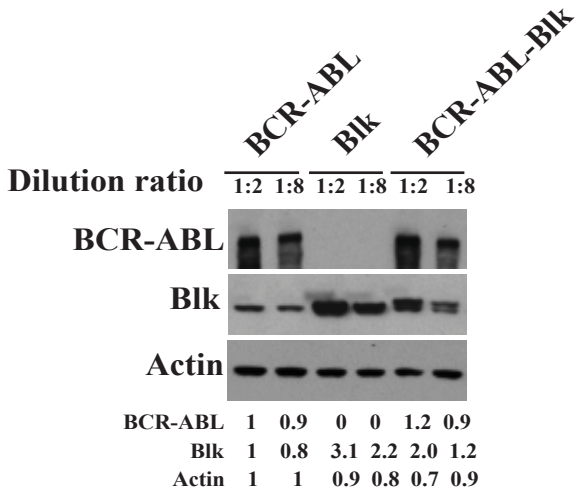


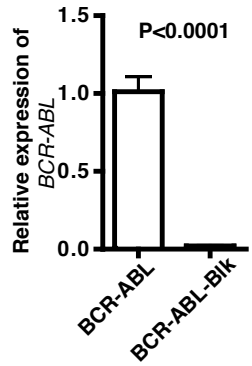
Supplementary Figure 1 Knockdown of BCR-ABL expression restores *Blk* expression. (a) Western blot analysis showed marked decrease of BCR-ABL expression after lentivirus shRNA infection. (**b**) Real time RT-PCR analysis showed increased *Blk* expression in leukemia cells after knockdown of BCR-ABL.



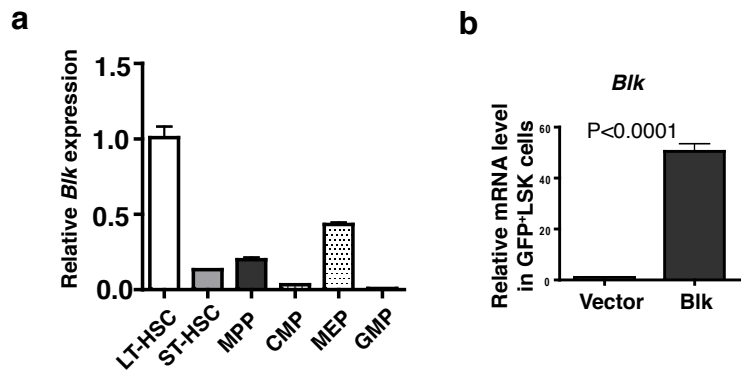
Supplementary Figure 2 Blk deletion does not affect the transfection efficiency and homing ability. (**a**) Detection of Blk expression in bone marrow cells of WT and *Blk*$^{-/-}$ mice by western blotting. (**b**) Blk does not affect the retroviral transduction efficiency of bone marrow cells. Bone marrow cells from 5-FU treated WT or *Blk*$^{-/-}$ mice were transduced with *BCR-ABL-GFP*, and the percentages of GFP^{+} cells before BMT were analyzed by FACS. (**c**) Homing assay of WT or *Blk*$^{-/-}$ bone marrow cells. 6□10^{6} WT or *Blk*$^{-/-}$ bone marrow cells (CD45.2) were transplanted into lethally irradiated recipients (CD45.1) and the donor-derived bone marrow cells (CD45.2) were detected by FACS in 3 hours after BMT. Mean values (± s.e.m.) are shown. (**d**) WT or *Blk*$^{-/-}$ bone marrow cells transduced with *BCR-ABL* retrovirus were cultured for 6 days. Equal number of GFP^{+} cells were transplanted into each lethally irradiated recipient mice, and the percentages of GFP^{+} cells in bone marrow of recipients were analyzed 3 hours after BMT (n=4). Mean values (± s.e.m.) are shown.



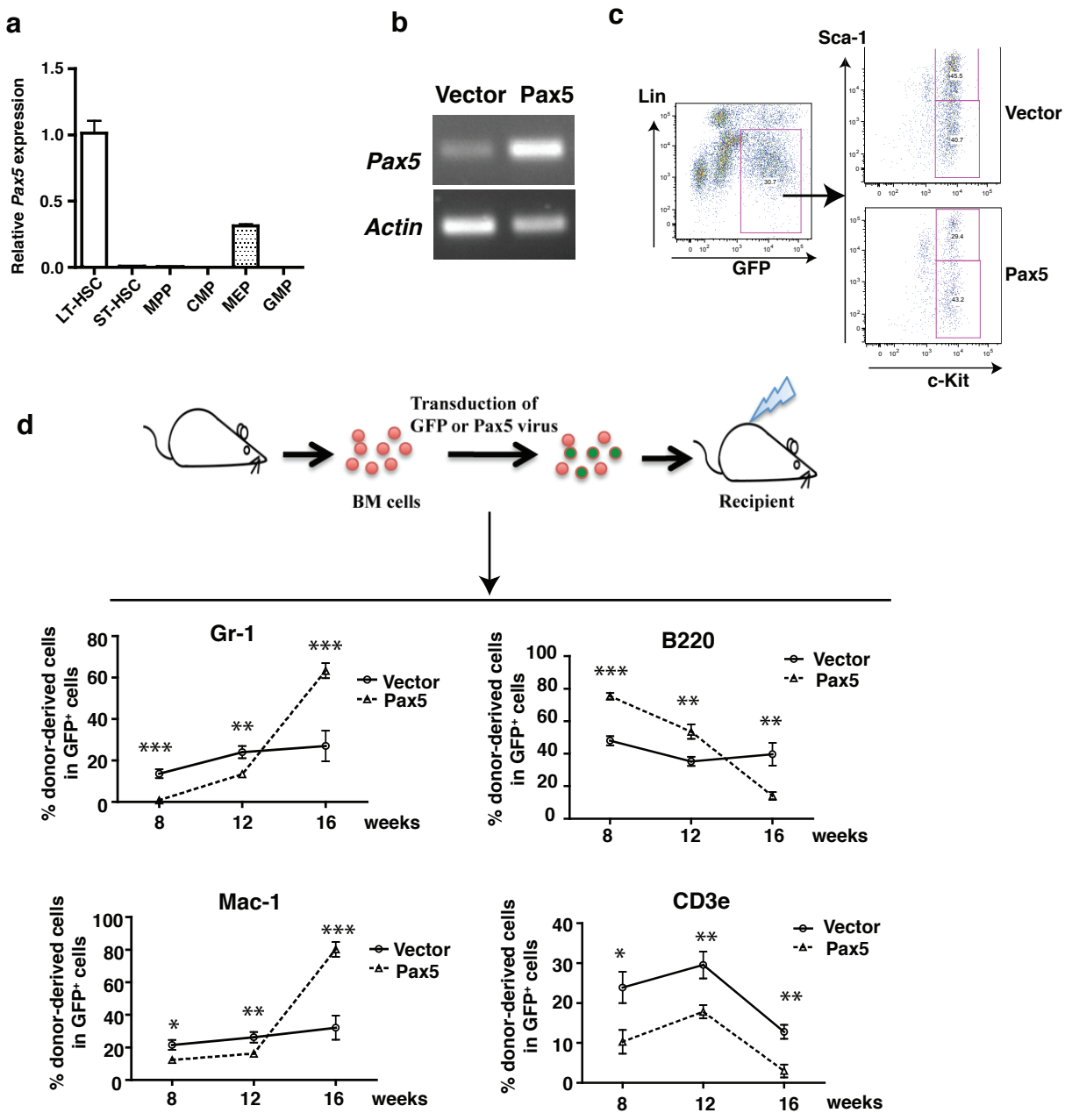
Supplementary Figure 3 Functional testing of *BCR-ABL* and *Blk* retroviruses in 3T3 cells. 3T3 cells were transduced with retrovirus (1:2 or 1:8 dilution) expressing *BCR-ABL-GFP, Blk-GFP, or BCR-ABL-Blk*. Expression of BCR-ABL and Blk proteins were detected by western blotting.



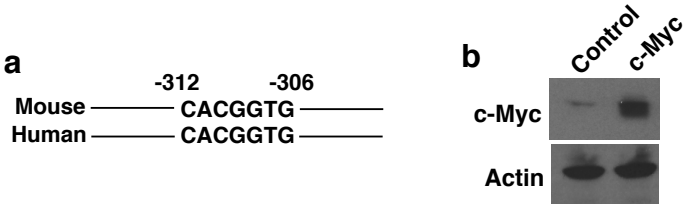
Supplementary Figure 4 Detection of *BCR-ABL* expression. RT-PCR analysis of *BCR-ABL* expression in peripheral blood of BCR-ABL or BCR-ABL-Blk induced CML mice treated with imatinib. Mean values (± s.e.m.) are shown



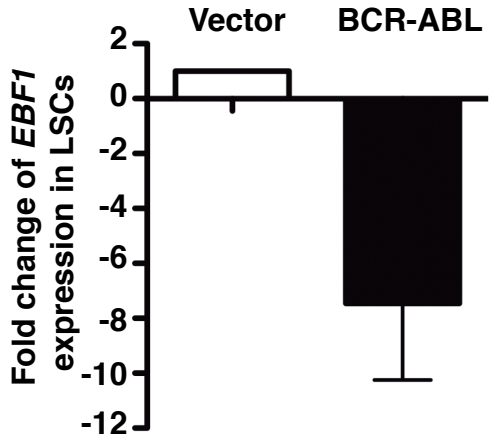
Supplementary Figure 5 Real time RT-PCR analysis of *Blk* expression (**a**) *Blk* expression in sorted LT-HSC, ST-HSC, MPP, CMP, MEP, and GMP population. **(b)** *Blk* expression in sorted GFP$^+$LSK cells from recipients of *GFP* or *Blk-GFP* transduced bone marrow cells. Mean values (± s.e.m.) are shown.



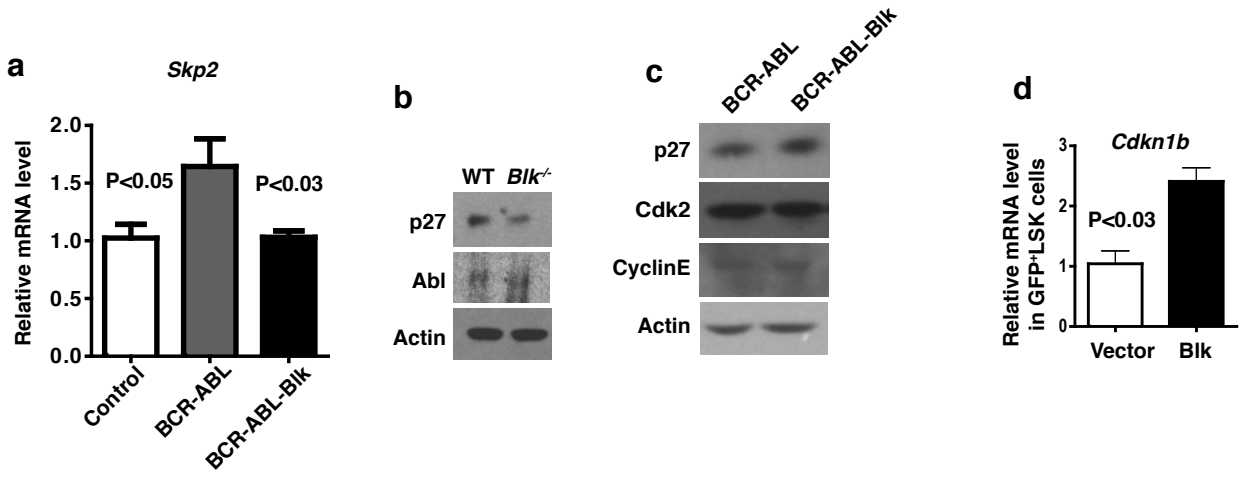
Supplementary Figure 6 The role of Pax5 in normal hematopoiesis. (a) Real time RT-PCR analysis of *Pax5* expression in different stem/progenitor cell populations. (**b**) Real time RT-PCR analysis showed *Pax5* expression in sorted LSK cells transduced with *Pax5-GFP* retrovirus. (**c**) Bone marrow cells were transduced with retrovirus expressing *Pax5-GFP* or *GFP* alone, and cultured for 4 days in vitro under the stem cell culture conditions. FACS analysis showed a lower percentage of LSK cells in Pax5-expressing bone marrow cells than in non-Pax5-expressing bone marrow cells. (**d**) Long-term reconstitution assay of Pax5-GFP or GFP-expressing HSC. Bone marrow cells were tranduced with *Pax5-GFP* or *GFP* virus, and injected into lethally irradiated mice. The distribution of different lingeages was analyzed by FACS at 8, 12, and 16 weeks after BMT. *p<0.05; **p<0.01; ***p<0.001.



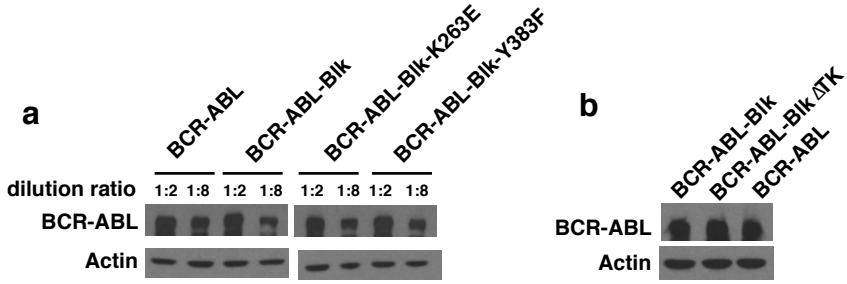
Supplementary Figure 7 The c-Myc binding site in the *Pax5* promoter. (a) The sequence of c-Myc binding site in the *Pax5* promoter is completely conserved in mouse and human. **(b)** Western blot analysis showed c-Myc expression.



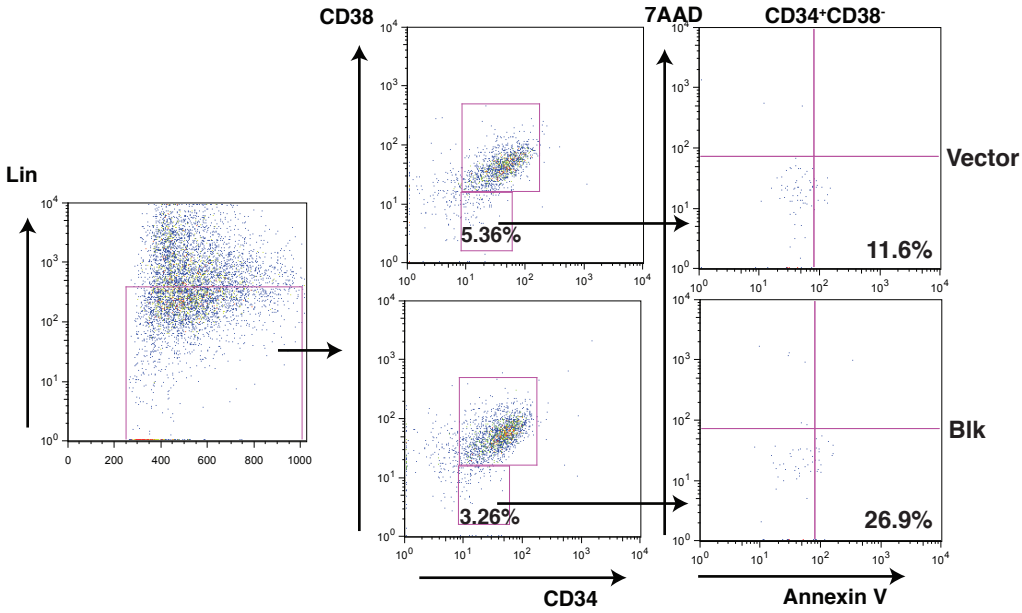
Supplementary Figure 8 *EBF1* expression was down-regulated in BCR-ABL expressing LSCs. Microarray analysis showing *EBF1* expression in vector- and *BCR-ABL*-transduced LSCs.
Mean values (± s.e.m) are shown. $p<0.05$.



Supplementary Figure 9 Blk regulates the expression of Skp2 and p27. (a) Real time RT-PCR analysis showed Blk inhibited the induction of *Skp2* expression by BCR-ABL. (**b**) p27 was downregulated in myeloid leukemia cells. Myeloid leukemia cells were sorted from the spleen of CML mice receiving BCR-ABL transduced WT or *Blk*$^{-/-}$ bone marrow cells two weeks after CML induction, and p27 levels in the cells were detected by western blot. (**c**) Western blot analysis showed that Blk induced expression of p27 expression but not CDK2 and cyclin E in bone marrow cells from CML mice which were cultured for 4 days in vitro under the stem cell culture conditions. (**d**) Real-time RT-PCR data showed a significant upregulation of *Cdkn1b* in sorted Blk-expressing LSK cells. Mean values (± s.e.m.) were shown (P<0.01).



Supplementary Figure 10 Normalization of the retrovirus titers of *BCR-ABL, BCR-ABL-Blk, BCR-ABL-Blk-K263E, BCR-ABL-Blk-Y383F, and BCR-ABL-BlkΔTK*. **(a** and **b)** NIH3T3 cells were infected with retroviruses, and 48h later, the levels of BCR-ABL proteins were analyzed by western blotting.



Supplementary Figure 11 Blk expression induces apoptosis of CML stem cells. Lin^{-}CD34^{+} cells were sorted and infected with *Blk* lentivirus. After puromycin selection, FACS analysis showed increased apoptosis in the presence of Blk.

Supplementary Table 1. Long-term repopulation potential of control and Blk-expressing LSK cells

Number of LSK cells	Control	Blk
	Positive for donor engraftment/Total mice	
10	0/6	0/6
50	3/7	3/7
100	4/6	3/6
500	6/6	6/6

Supplementary Table 2. Lists of genes regulated by Blk in leukemia stem cells
List of upregulated genes (P ≤ 0.05)

Symbol	Gene name	Relative fold change
Igkv19-93	immunoglobulin kappa chain variable 19-93	2.89
Myo1h	myosin 1H	2.1
Ufsp1	UFM1-specific peptidase 1	2.07
Them4	thioesterase superfamily member 4	1.92
Igl-V2	immunoglobulin lambda chain, variable 2	1.89
Clec9a	C-type lectin domain family 9, member a	1.88
Cldn22	claudin 22	1.78
Rab1b	RAB1B, member RAS oncogene family	1.78
Gnrh1	gonadotropin releasing hormone 1	1.77
Tsga14	testis specific gene A14	1.75
Plekho2	pleckstrin homology domain containing, family O member 2	1.73
Hist1h2ak	histone cluster 1, H2ak	1.73
Cyb5d1	cytochrome b5 domain containing 1	1.72
Prl7c1	prolactin family 7, subfamily c, member 1	1.7
Zfp459	zinc finger protein 459	1.66
Kcnrg	potassium channel regulator	1.65
Cmtm3	CKLF-like MARVEL transmembrane domain containing 3	1.65
Gcet2	germinal center expressed transcript 2	1.62
Olfr43	olfactory receptor 43	1.62
Rfc5	replication factor C (activator 1) 5	1.6
Tek	endothelial-specific receptor tyrosine kinase	1.59
Lrrc28	leucine rich repeat containing 28	1.59
Slc41a1	solute carrier family 41, member 1	1.59
Mxd4	Max dimerization protein 4	1.59
Bclp2	chitinase like protein 2	1.59
Serpinb9	serine (or cysteine) peptidase inhibitor, clade B, member 9	1.59
Hist2h2ab	histone cluster 2, H2ab	1.58
Ppt2	palmitoyl-protein thioesterase 2	1.58
Mylc2pl	myosin light chain 2, precursor lymphocyte-specific	1.58
Nlrp1a	NLR family, pyrin domain containing 1A	1.57
Dppa3	developmental pluripotency-associated 3	1.57
Cypt6	cysteine-rich perinuclear theca 6	1.57
Zfp313	zinc finger protein 313	1.55
Zfp7	zinc finger protein 7	1.54
Tut1	terminal uridylyl transferase 1, U6 snRNA-specific	1.54
Eif4h	eukaryotic translation initiation factor 4H	1.54

Defb5	defensin beta 5	1.54
Stk40	serine/threonine kinase 40	1.53
Mpzl1	myelin protein zero-like 1	1.52
Nfya	nuclear transcription factor-Y alpha	1.5
Tmem110	transmembrane protein 110	1.5
Olfr517	olfactory receptor 517	1.5
Zfat	zinc finger and AT hook domain containing	1.49
Cops8	COP9 (constitutive photomorphogenic) homolog, subunit 8 (Arabidopsis thaliana)	1.49
Selm	selenoprotein M	1.49
Lman2l	lectin, mannose-binding 2-like	1.48
Zfyve16	zinc finger, FYVE domain containing 16	1.48
Srl	Sarcalumenin	1.47
Ube2m	ubiquitin-conjugating enzyme E2M (UBC12 homolog, yeast)	1.47
Cldn12	claudin 12	1.46
Lrig2	leucine-rich repeats and immunoglobulin-like domains 2	1.46
Olfr1128	olfactory receptor 1128	1.45
Enpep	glutamyl aminopeptidase	1.45
Sbf1	SET binding factor 1	1.45
Wdr22	WD repeat domain 22	1.45
Il15	interleukin 15	1.45
Map4k2	mitogen-activated protein kinase kinase kinase kinase 2	1.44
Il17ra	interleukin 17 receptor A	1.44
Efcab4b	EF-hand calcium binding domain 4B	1.43
Csnk1g1	casein kinase 1, gamma 1	1.43
Sh2d3c	SH2 domain containing 3C	1.43
Nfic	nuclear factor I/C	1.42
Kctd9	potassium channel tetramerisation domain containing 9	1.42
Nfkbiz	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta	1.42
Mknk2	MAP kinase-interacting serine/threonine kinase 2	1.42
Rdh13	retinol dehydrogenase 13 (all-trans and 9-cis)	1.41
Olfr1045	olfactory receptor 1045	1.41
Blk	B lymphoid kinase	1.41
Zfp354c	zinc finger protein 354C	1.4
Grlf1	glucocorticoid receptor DNA binding factor 1	1.4
Mettl1	methyltransferase-like 1	1.4
Asf1b	ASF1 anti-silencing function 1 homolog B (S. cerevisiae)	1.4
Tnf	tumor necrosis factor	1.4
Zbtb24	zinc finger and BTB domain containing 24	1.4

Gfi1b	growth factor independent 1B	1.39
Saps1	SAPS domain family, member 1	1.38
Rftn1	raftlin lipid raft linker 1	1.38
Cacnb4	calcium channel, voltage-dependent, beta 4 subunit	1.37
Abca1	ATP-binding cassette, sub-family A (ABC1), member 1	1.37
Gtsf1	gametocyte specific factor 1	1.37
Krt85	keratin 85	1.37
Uqcc	ubiquinol-cytochrome c reductase complex chaperone, CBP3 homolog (yeast)	1.36
Gm266	gene model 266, (NCBI)	1.36
Slc27a1	solute carrier family 27 (fatty acid transporter), member 1	1.36
Ccnt2	cyclin T2	1.36
Ccdc21	coiled-coil domain containing 21	1.36
Gabarapl1	gamma-aminobutyric acid (GABA(A)) receptor-associated protein-like 1	1.36
Slc12a6	solute carrier family 12, member 6	1.36
Ccdc52	coiled-coil domain containing 52	1.36
Nlgn2	neuroligin 2	1.36
Fahd2a	fumarylacetoacetate hydrolase domain containing 2A	1.36
Hmbs	hydroxymethylbilane synthase	1.35
Rnf122	ring finger protein 122	1.35
Gmeb1	glucocorticoid modulatory element binding protein 1	1.35
Usp49	ubiquitin specific peptidase 49	1.35
Arhgef5	Rho guanine nucleotide exchange factor (GEF) 5	1.35
Xkr5	X Kell blood group precursor-related family, member 5	1.35
Shkbp1	Sh3kbp1 binding protein 1	1.34
Nbr1	neighbor of Brca1 gene 1	1.34
Cdkn2c	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	1.34
Parp14	poly (ADP-ribose) polymerase family, member 14	1.34
Hist1h1b	histone cluster 1, H1b	1.34
Svil	supervillin	1.34
Rogdi	rogdi homolog (Drosophila)	1.34
Syap1	synapse associated protein 1	1.34
Zfp276	zinc finger protein (C2H2 type) 276	1.34
Nfatc1	nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1	1.33
Cdkn1b	cyclin-dependent kinase inhibitor 1B	1.33
Gdf6	growth differentiation factor 6	1.33
Prkacb	protein kinase, cAMP dependent, catalytic, beta	1.33
Nek7	NIMA (never in mitosis gene a)-related expressed kinase 7	1.33

Tmem35	transmembrane protein 35	1.32
Gk5	glycerol kinase 5 (putative)	1.32
Gtf2e1	general transcription factor II E, polypeptide 1 (alpha subunit)	1.32
Hrnr	hornerin	1.32
Apol10a	apolipoprotein L 10a	1.32
Tnrc6c	trinucleotide repeat containing 6C	1.31
Prkcsh	protein kinase C substrate 80K-H	1.31
Ppard	peroxisome proliferator activator receptor delta	1.3
Zbtb3	zinc finger and BTB domain containing 3	1.3
Inpp4a	inositol polyphosphate-4-phosphatase, type I	1.3

List of downregulated genes (P ≤ 0.05)

Symbol	Gene name	Relative fold change
Khdc1a	KH homology domain containing 1A	4.40
Il1f9	interleukin 1 family, member 9	2.78
Ublcp1	ubiquitin-like domain containing CTD phosphatase 1	2.48
Lcn2	lipocalin 2	2.39
Scrn3	secernin 3	2.26
Fabp4	fatty acid binding protein 4, adipocyte	2.21
Trem3	triggering receptor expressed on myeloid cells 3	2.17
Chi3l3	chitinase 3-like 3	2.17
Dhrs7	dehydrogenase/reductase (SDR family) member 7	2.16
Gldc	glycine decarboxylase	2.10
S100a5	S100 calcium binding protein A5	2.08
Ifitm6	interferon induced transmembrane protein 6	2.06
Clec4d	C-type lectin domain family 4, member d	2.03
Serpina3f	serine (or cysteine) peptidase inhibitor, clade A, member 3F	1.97
Nrg4	neuregulin 4	1.95
Hdc	histidine decarboxylase	1.94
Bank1	B-cell scaffold protein with ankyrin repeats 1	1.89
Mt1	metallothionein 1	1.84
Il6	interleukin 6	1.83
Gem	GTP binding protein (gene overexpressed in skeletal muscle)	1.81
Olfr56	olfactory receptor 56	1.80
Ceacam1	CEA-related cell adhesion molecule 1	1.80
Fpr2	formyl peptide receptor 2	1.79
Rad51l1	RAD51-like 1 (S. cerevisiae)	1.79
Ly96	lymphocyte antigen 96	1.78

Gp1ba	glycoprotein 1b, alpha polypeptide	1.77
Clcn5	chloride channel 5	1.77
Icam4	intercellular adhesion molecule 4, Landsteiner-Wiener blood group	1.76
Car1	carbonic anhydrase 1	1.76
Camp	cathelicidin antimicrobial peptide	1.75
Fkbp11	FK506 binding protein 11	1.74
Met	met proto-oncogene	1.74
Rnd3	Rho family GTPase 3	1.74
Klhl4	kelch-like 4 (Drosophila)	1.73
Mt2	metallothionein 2	1.72
Rabl3	RAB, member of RAS oncogene family-like 3	1.70
Ddah1	dimethylarginine dimethylaminohydrolase 1	1.70
Il1r2	interleukin 1 receptor, type II	1.70
Fgl2	fibrinogen-like protein 2	1.70
Nqo2	NAD(P)H dehydrogenase, quinone 2	1.69
Tirap	toll-interleukin 1 receptor (TIR) domain-containing adaptor protein	1.68
Gzmb	granzyme B	1.67
Tnfsf9	tumor necrosis factor (ligand) superfamily, member 9	1.65
Osgepl1	O-sialoglycoprotein endopeptidase-like 1	1.65
Olfr1396	olfactory receptor 1396	1.64
Il4	interleukin 4	1.63
Stfa3	stefin A3	1.62
Zfp456	zinc finger protein 456	1.62
Ccr1	chemokine (C-C motif) receptor 1	1.62
Vldlr	very low density lipoprotein receptor	1.62
Hopx	HOP homeobox	1.62
Slc40a1	solute carrier family 40 (iron-regulated transporter), member 1	1.61
Retnla	resistin like alpha	1.60
Irak1bp1	interleukin-1 receptor-associated kinase 1 binding protein 1	1.59
Ero1l	ERO1-like (S. cerevisiae)	1.59
Stx18	syntaxin 18	1.59
Elp4	elongation protein 4 homolog (S. cerevisiae)	1.59
Slc7a11	solute carrier family 7 (cationic amino acid transporter, y+ system), member 11	1.58
Mtmr9	myotubularin related protein 9	1.57
Mrps18b	mitochondrial ribosomal protein S18B	1.56
Tmem34	transmembrane protein 34	1.56

Clybl	citrate lyase beta like	1.54
Kcne3	potassium voltage-gated channel, Isk-related subfamily, gene 3	1.54
Wdr32	WD repeat domain 32	1.54
Akap7	A kinase (PRKA) anchor protein 7	1.51
Tnfrsf9	tumor necrosis factor receptor superfamily, member 9	1.51
Slc35f2	solute carrier family 35, member F2	1.51
Maff	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein F (avian)	1.50
Gstm1	glutathione S-transferase, mu 1	1.49
Trdmt1	tRNA aspartic acid methyltransferase 1	1.48
Hsp90aa1	heat shock protein 90, alpha (cytosolic), class A member 1	1.48
Rabl4	RAB, member of RAS oncogene family-like 4	1.47
S100a8	S100 calcium binding protein A8 (calgranulin A)	1.47
Ston2	stonin 2	1.47
Tspyl3	TSPY-like 3	1.47
Lrrc42	leucine rich repeat containing 42	1.46
Dmc1	DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination (yeast)disrupted meiotic cDNA 1	1.46
Abhd5	abhydrolase domain containing 5	1.46
Clec4a2	C-type lectin domain family 4, member a2	1.46
Tlr4	toll-like receptor 4	1.46
Als2cr2	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 2 (human)	1.46
Abtb1	ankyrin repeat and BTB (POZ) domain containing 1	1.46
Sqrdl	sulfide quinone reductase-like (yeast)	1.45
Snx16	sorting nexin 16	1.45
Nme6	non-metastatic cells 6, protein expressed in (nucleoside-diphosphate kinase)	1.45
Stxbp6	syntaxin binding protein 6 (amisyn)	1.44
C4a	complement component 4A (Rodgers blood group)	1.44
Slc39a8	solute carrier family 39 (metal ion transporter), member 8	1.44
Ccr2	chemokine (C-C motif) receptor 2	1.44
Arrdc4	arrestin domain containing 4	1.44
Fcgr2b	Fc receptor, IgG, low affinity IIb	1.43
Mtrf1	mitochondrial translational release factor 1	1.43
Tacstd1	tumor-associated calcium signal transducer 1	1.42
Ppfibp1	PTPRF interacting protein, binding protein 1 (liprin beta 1)	1.42
Zfp280b	zinc finger protein 280b	1.42
Alox5	arachidonate 5-lipoxygenase	1.42

Schip1	schwannomin interacting protein 1	1.42
Klf1	Kruppel-like factor 1 (erythroid)	1.41
Rnf17	ring finger protein 17	1.41
Car9	carbonic anhydrase 9	1.41
Esm1	endothelial cell-specific molecule 1	1.41
Napsa	napsin A aspartic peptidase	1.40
Vmn2r51	vomeronasal 2, receptor 51	1.40
Spry2	sprouty homolog 2 (Drosophila)	1.40
Tmem119	transmembrane protein 119	1.40
Igfbp7	insulin-like growth factor binding protein 7	1.39
Mospd1	motile sperm domain containing 1	1.39
Ctsl	cathepsin L	1.39
Hacl1	2-hydroxyacyl-CoA lyase 1	1.39
Lat2	linker for activation of T cells family, member 2	1.39
Hbegf	heparin-binding EGF-like growth factor	1.39
Angptl3	angiopoietin-like 3	1.39
Rhbdl2	rhomboid, veinlet-like 2 (Drosophila)	1.39
Pih1d2	PIH1 domain containing 2	1.39
Cd300a	CD300A antigen	1.38
Tmem41a	transmembrane protein 41a	1.38
Ceacam10	CEA-related cell adhesion molecule 10	1.38
Zfp296	zinc finger protein 296	1.37
Tmem67	transmembrane protein 67	1.37
Ralb	v-ral simian leukemia viral oncogene homolog B (ras related)	1.37
Slc16a1	solute carrier family 16 (monocarboxylic acid transporters), member 1	1.36
Iscu	IscU iron-sulfur cluster scaffold homolog (E. coli)	1.36
Perp	PERP, TP53 apoptosis effector	1.36
Pla2g12a	phospholipase A2, group XIIA	1.36
Prmt6	protein arginine N-methyltransferase 6	1.36
Acot1	acyl-CoA thioesterase 1	1.35
Dhx35	DEAH (Asp-Glu-Ala-His) box polypeptide 35	1.35
Ears2	glutamyl-tRNA synthetase 2 (mitochondrial)(putative)	1.35
Slc35b1	solute carrier family 35, member B1	1.35
Mgl1	macrophage galactose N-acetyl-galactosamine specific lectin 1	1.34
Slc24a5	solute carrier family 24, member 5	1.34
Vrk2	vaccinia related kinase 2	1.33
St7	Suppression of tumorigenicity 7	1.33
Fbxo18	F-box protein 18	1.33

Ofd1	oral-facial-digital syndrome 1 gene homolog (human)	1.33
Metrnl	meteorin, glial cell differentiation regulator-like	1.33
Rps6kc1	ribosomal protein S6 kinase polypeptide 1	1.33
Hif1a	hypoxia inducible factor 1, alpha subunit	1.33
Tnfsf4	tumor necrosis factor (ligand) superfamily, member 4	1.32
Vnn1	vanin 1	1.32
Hs6st2	heparan sulfate 6-O-sulfotransferase 2	1.32
Cebpb	CCAAT/enhancer binding protein (C/EBP), beta	1.32
Cftr	cystic fibrosis transmembrane conductance regulator homolog	1.32
Nmt1	N-myristoyltransferase 1	1.31
Smok3c	sperm motility kinase 3C	1.31
Pus3	pseudouridine synthase 3	1.31
Grina	glutamate receptor, ionotropic, N-methyl D-aspartate-associated protein 1 (glutamate binding)	1.31
Fads1	fatty acid desaturase 1	1.31
Slco4c1	solute carrier organic anion transporter family, member 4C1	1.31
Taf1b	TATA box binding protein (Tbp)-associated factor, RNA polymerase I, B	1.31
Fibp	fibroblast growth factor (acidic) intracellular binding protein	1.31
Evi2a	ecotropic viral integration site 2a	1.31
Scn1b	sodium channel, voltage-gated, type I, beta	1.30
Glb1	galactosidase, beta 1	1.30

Supplementary Table 3. Sequences of real time PCR primers.

1. *Pax5* sense: GTACAGCAGCCCCCCAATC
   antisense: ACCTTCATCCCTCTTGCGTTT
2. *Blk* sense: TGTGGTCACCAGAGAGCCCATTTA
   antisense: TGTCAATCAGCCTTGGAAGGGACA
3. *EBF1* sense: TGCAGATCTGGTTGAAGCCCTGTA
   antisense: ATCCCTGCATGGACCGAAGTGTTA
4. *Skp2* sense: AAACCTTTGTGGGTGCTCTG
   antisense: CTTAAGTCGAGGCGGATGAG
5. *BCR* sense: CGTGTGTGAAACTCCAGACTGTCA
   ABL antisense: CTTCAGCGGCCAGTAGCATCT
6. *Actin* sense: ATGGAATCCTGTGGCATCCA
   antisense: CGCTCAGGAGGAGCAATGAT
7. Myc-chip sense: GACGTGTCTGGGGACATCTTG
   antisense: GTCCGAAGGCACCGTGAAATG
8. EBF1-chip sense: ACTACGGAGACTACTTGGTGG
   antisense: CACCGGACATCGCAAATTCAC

Supplementary Note

In discussion, although a ten-fold overexpression of *Pax5* inhibited normal HSCs in some degree, we believe that restoration of *Pax5* expression to a normal endogenous level in HSCs would not significantly inhibit proliferation. In contrast to the effect of Pax5 on HSCs, *Pax5* overexpression strongly inhibited proliferation of LSCs and CML development. On the other hand, our observation that a higher percentage of B cells were found following *Pax5* overexpression at 8 and 12 weeks after BMT suggests that *Pax5* promotes progenitor differentiation into B cells, which is consistent with the known role of *Pax5* in initiating B-cell commitment. Moreover, the increase of myeloid cells at 16 weeks implies that *Pax5* overexpression could regulate cell fate determination of HSCs. Thus, further investigation is required to elucidate the complete role of Pax5 in HSCs.