

Table S1. Antibodies used in experiments. Marker column shows the cell surface antigen; 1 denotes BD Pharmingen as the source; 2 denotes eBiosciences. The clone numbers are shown.

Marker	Clone
CD45R/B220 ^{1,2}	RA3-6B2
CD19 ¹	1D3
CD4 ¹	GK1.5
CD8a ¹	53-6.7
Mac1 ^{1,2}	M1/70
Gr1 ^{1,2}	RB6-8C5
c-kit ^{1,2}	ACK2
Sca1 ¹	D7
Ter 119 ^{1,2}	TER-119
CD135 ²	A2F10
CD48 ¹	C7
CD150 ²	9D1
CD21/CD35 ¹	7G6
IgM ¹	R6-60.2
CD23 ¹	B3B4
CD127 ^{1,2}	SB/199
CD44 ¹	IM7
CD25 ¹	PC61
CD3e ¹	145-2C11
CD45.1 ¹	A20
CD45.2 ¹	104
CD34 ¹	ram34

Table S2. Cell surface markers defining specific B-cell subsets and stem cell populations.

Organ	B cell compartment	Surface marker
Spleen	T1 B cells	CD19+, IgM+, CD23-,CD21-
	T2 B cells	CD19+, IgMhi, CD23+, CD21int
	Follicular B cells	CD19+, IgMint, CD23+, CD21+
	Marginal zone b cells	CD19+, IgM+, CD23-,CD21+
	Marginal zone precursor B cells	CD19+, IgMint, CD23+, CD21hi
	CD93+ B cells	CD19+, IgM+, CD93+
	Anergic B cells	CD19+, IgMlo, CD93+, CD23lo
	Plasma cells	CD19+, CD13+
Bone marrow		
	Pro B cells	B220int, CD43+
	Pre B cells	B220int, CD25+
	Immature B cells	B220int, IgM+
	Recirculating B cells	B220hi,IgM+
	Plasma cells	B220+, CD138hi
Bone marrow		
	HSC	LSK CD34- CD48- CD150+ Flt3-
	MPP1	LSK CD34+ CD48- CD150+ Flt3-
	MPP2	LSK CD34+ CD48+ CD150+ Flt3-
	MPP3	LSK CD34+ CD48+ CD150- Flt3-
	MPP4	LSK CD34+ CD48+ CD150- Flt3+
	HSPC or LSK	Lineage-Sca1+c-kit+

Table S3. Tgfb1 target genes are enriched in Hhex cKO HSC compared to WT HSC. Tgfb1 was determined to be an upstream regulator enriched in Hhex cKO versus WT by whole transcriptome data. The differential expression of each target is shown by log₂ ratio. The predicted state of Tgfb1 is determined by the target's up- or downregulation in Hhex cKO HSCs.

ID	Genes in dataset	Prediction (based on expression direction)	Log Ratio	Findings from literature
ENSMUSG0000005339	FCER1A	Inhibited	6.094	Downregulates (1)
ENSMUSG00000091971	HSPA1A/HSPA1B	Inhibited	4.020	Downregulates (0)
ENSMUSG00000032323	CYP11A1	Activated	3.187	Upregulates (2)
ENSMUSG00000024680	MS4A2	Inhibited	3.017	Downregulates (2)
ENSMUSG00000037759	PTGER2	Activated	2.766	Upregulates (3)
ENSMUSG00000020592	SDC1	Activated	2.578	Upregulates (2)
ENSMUSG00000007613	TGFBR1	Activated	2.399	Upregulates (14)
ENSMUSG00000017950	HNF4A	Inhibited	2.308	Downregulates (8)
ENSMUSG00000025804	CCR1	Activated	2.117	Upregulates (4)
ENSMUSG00000015437	GZMB	Inhibited	1.971	Downregulates (0)
ENSMUSG00000034957	CEBPA	Activated	1.897	Upregulates (2)
ENSMUSG00000024190	DUSP1	Activated	1.835	Upregulates (2)
ENSMUSG00000041112	ELMO1	Activated	1.758	Upregulates (1)
ENSMUSG00000055148	KLF2	Inhibited	1.719	Downregulates (1)
ENSMUSG00000054932	AFP	Inhibited	1.645	Downregulates (2)
ENSMUSG00000056758	HMGA2	Activated	1.637	Upregulates (0)
ENSMUSG00000003032	KLF4	Inhibited	1.635	Downregulates (5)
ENSMUSG00000027776	IL12A	Activated	1.599	Upregulates (2)
ENSMUSG00000058355	ABCE1	Affected	1.571	Regulates (2)
ENSMUSG00000044734	SERPINB1	Activated	1.514	Upregulates (1)
ENSMUSG00000021701	PLK2	Affected	1.508	Regulates (2)
ENSMUSG00000031207	MSN	Activated	1.487	Upregulates (1)
ENSMUSG00000021065	FUT8	Activated	1.475	Upregulates (1)
ENSMUSG00000024381	BIN1	Affected	1.433	Regulates (2)
ENSMUSG00000053931	CNN3	Activated	1.417	Upregulates (1)
ENSMUSG00000058587	TMOD3	Activated	1.394	Upregulates (1)
ENSMUSG00000049103	CCR2	Activated	1.389	Upregulates (0)
ENSMUSG00000024697	GNA14	Inhibited	1.352	Downregulates (1)
ENSMUSG00000018930	CCL4	Activated	1.337	Upregulates (1)
ENSMUSG00000030830	ITGAL	Inhibited	1.335	Downregulates (2)
ENSMUSG00000016494	CD34	Inhibited	1.277	Downregulates (7)
ENSMUSG00000056515	RAB31	Activated	1.262	Upregulates (1)
ENSMUSG00000035473	GALM	Inhibited	1.233	Downregulates (1)
ENSMUSG00000037235	MXD4	Activated	1.202	Upregulates (4)
ENSMUSG00000076431	SOX4	Activated	1.174	Upregulates (6)
ENSMUSG00000075602	Ly6a (includes others)	Inhibited	1.121	Downregulates (1)
ENSMUSG00000029777	GARS	Activated	1.079	Upregulates (1)
ENSMUSG00000049382	KRT8	Activated	-2.354	Downregulates (3)
ENSMUSG00000037362	NOV	Activated	-2.394	Downregulates (21)
ENSMUSG00000036905	C1QB	Inhibited	-2.405	Upregulates (1)
ENSMUSG00000023034	NR4A1	Inhibited	-2.430	Upregulates (5)
ENSMUSG00000022037	Clu	Inhibited	-2.797	Upregulates (0)

ENSMUSG00000037411
 ENSMUSG00000040152
 ENSMUSG00000035936
 ENSMUSG00000053475
 ENSMUSG00000029380
 ENSMUSG00000021091
 ENSMUSG00000019997
 ENSMUSG00000022887
 ENSMUSG00000026077
 ENSMUSG00000048126
 ENSMUSG00000005237
 ENSMUSG00000032561
 ENSMUSG00000024803
 ENSMUSG00000003665

SERPINE1	Inhibited	-3.012	Upregulates (211)
THBS1	Inhibited	-3.142	Upregulates (13)
ALDH5A1	Affected	-3.431	Regulates (2)
TNFAIP6	Inhibited	-3.474	Upregulates (2)
CXCL2	Inhibited	-3.749	Upregulates (1)
SERPINA3	Inhibited	-3.807	Upregulates (3)
CTGF	Inhibited	-3.846	Upregulates (139)
Masp1	Inhibited	-4.977	Upregulates (1)
NPAS2	Affected	-4.990	Regulates (2)
COL6A3	Inhibited	-5.083	Upregulates (4)
DNAH2	Affected	-5.240	Regulates (2)
ACPP	Inhibited	-5.468	Upregulates (1)
ANKRD1	Inhibited	-6.051	Upregulates (2)
HAS1	Inhibited	-	Upregulates (8)

Table S4. Network analysis of pathways enriched in *Hhex* cKO HSC by IPA. Upstream transcriptional regulators implicated in *Hhex* cKO versus WT were found by comparing whole transcriptome data. The differential expression of each regulator is shown by log₂ ratio. The predicted state of the regulator is determined by the consistency of its targets being up- or downregulated in *Hhex* cKO HSCs. Z score and the P values were generated by the IPA software. The last column shows the regulators' target gene lists.

Upstream Regulator	Log ₂ Ratio	Predicted State	z-score	P	Target molecules in dataset
<i>Hoxa7</i>	0.958	Activated	2.596	2.04E-08	<i>CD34, CD93, DDX4, FLT3, LAT2, SOX4, Tmsb4x</i> (includes others)
<i>E. coli</i> B4 LPS	n/a	Activated	2.197	5.35E-08	<i>C11orf82, C1QB, CCL4, Cd52, CLEC14A, CXCL2, F5, FLT3, GNA14, HLA-B, Ifi204</i> (includes others), <i>IL12A, LCP1, Ly6a</i> (includes others), <i>RTP4, USP18</i>
<i>Runx1</i>	0.432	Activated	2.136	1.01E-06	<i>CD34, CD53, CEBPA, CPA3, CTSG, CYP11A1, GZMB, ITGAL, PF4</i>
<i>Hoxa9</i>	0.808	Activated	2.760	3.08E-06	<i>CD34, CD93, CTSG, DDX4, FCGRT, FLT3, LAT2, RAB31, SOX4, TACC1, Tmsb4x</i> (includes others)
<i>Ifnb1</i>	0.000	Activated	2.197	4.56E-06	<i>CCL4, CLEC4E, CXCL2, Gbp6</i> (includes others), <i>HMGA2, Ifi204</i> (includes others), <i>IFI27L2, IL12A, THBS1, TLR7, UBA7, UCHL1, USP18</i>

Table S5. Competitive bone marrow transplantation. Whole bone marrow was flushed from donor mouse strain (CD45.2) and mixed with B6.Ly5.1 congenic marrow (CD45.1) at various input ratios. The mixed whole bone marrow was intravenously injected into lethally irradiated B6.Ly5.1 congenic host mice. The peripheral blood was analyzed for CD45.1/CD45.2 chimerism at the time points shown post-transplant. Figure 4 in the paper shows the 16 week post-transplant analyses at an input ratio of 6:1. The blank cells in this table show time points that were not analyzed for a given group. The CD45.1/CD45.2 congenic system is established in the field¹³⁻¹⁵.

donor bone marrow	Input ratio (45.1:45.2)	4 weeks		6 weeks		8 weeks		12 weeks	
Hhex cKO (Hhex ^{lox/lox} ;vav-Cre)		%45.1	%45.2	%45.1	%45.2	%45.1	%45.2	%45.1	%45.2
Hhex cKO	1:1	99.01	0.99	98.69	1.3	99.87	0.13	100	0
Hhex cKO	1:1	100	0	94	6	100	0	99.9	0.1
Hhex cKO	1:4	99.64	0.36	96	4	99.85	0.14	100	0
Hhex cKO	1:4	99.77	0.23	98.45	1.55	100	0	100	0
Hhex cKO	1:4	100	0	99.87	0.12	99.98	0.02	100	0
Hhex cKO	1:1	99.66	0.34	98.9	1.05	99.66	0.33	99.8	0.2
Hhex cKO	1:1	99.87	0.13	96.2	3.78	99.83	0.17	100	0
Hhex cKO	1:4	99.72	0.28	99.95	0.05	100	0	100	0
Hhex cKO	1:4	100	0	99.91	0.09	100	0	100	0
B6									
B6	1:1	26	74					32.4	67.6
B6	1:4	16	84					8	92
Hhex^{lox/lox}									
Hhex ^{lox/lox}	1:5	29.4	70.6	30.6	69.4	22	88		
Hhex ^{lox/lox}	1:5	36.9	63	31.2	68.8	25.5	74.5		
Hhex ^{lox/lox}	1:5	55.1	44.9	71.2	28.8	20.7	79.3		
Hhex ^{lox/lox}	1:8	41.9	58.1	35	65	31.5	68.5		