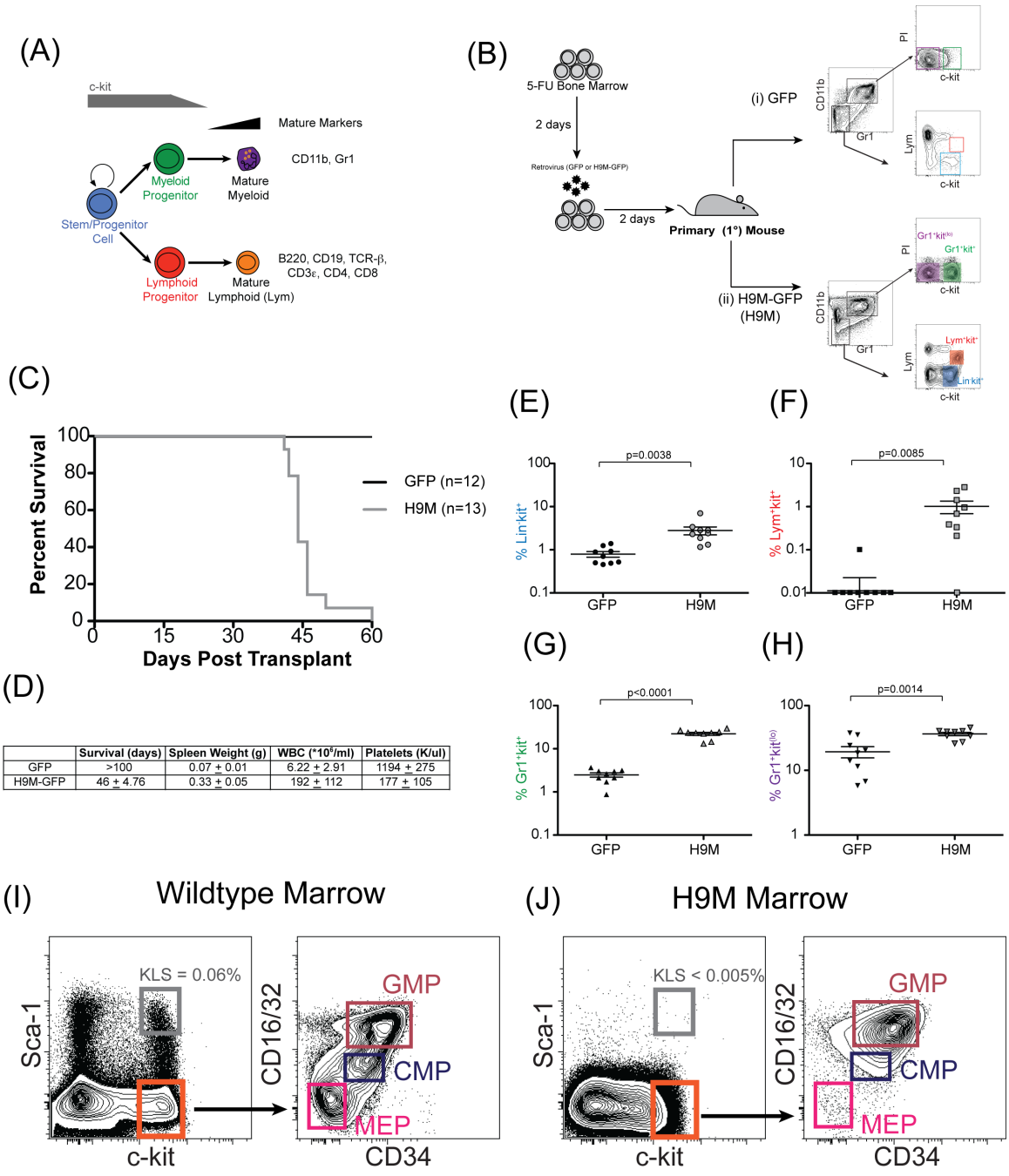
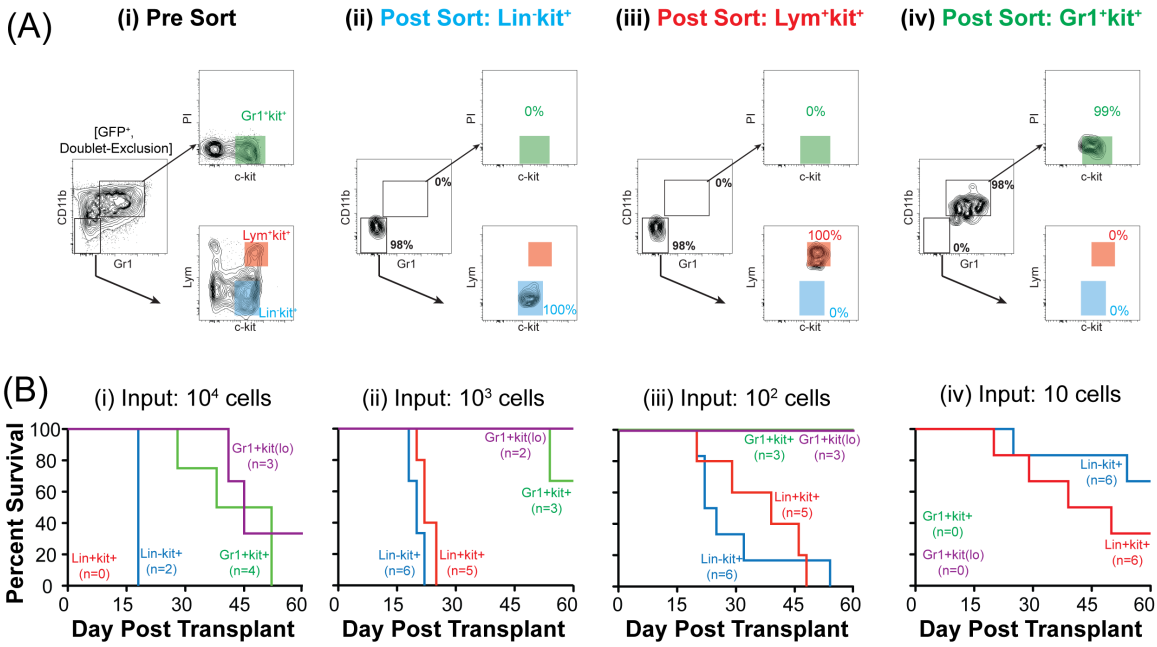


	Survival (days)	Spleen Weight (g)	WBC (*10⁶/ml)	Platelets (K/ul)
GFP	>100	0.07 ± 0.01	6.22 ± 2.91	1194 ± 275
H9M-GFP	46 ± 4.76	0.33 ± 0.05	192 ± 112	177 ± 105

Figure S1, related to Figure 1. Experimental System. (A) Simplified model of normal hematopoiesis. (B) Experimental scheme for retroviral transduction. The bone marrow from mice injected 4 days prior with 5-fluorouracil was harvested, cultured for 2 days, transduced, and cultured for two additional days (to allow for GFP expression). GFP$^+$ cells were sorted, and 10^5 cells were transplanted into lethally irradiated primary recipients along with 10^5 helper marrow cells animals.

Phenotypic analysis of GFP^+ compartment of bone marrow from animals transplanted with (i) GFP transduced or (ii) H9M-GFP transduced cells. (C) Survival curve of animals transplanted with GFP (black) or H9M (grey) cells. (D) Characteristics of mice transplanted with GFP or H9M-GFP cells. Values represent mean $\pm$ SD. (E-G) Percentage of (E) Lin^-kit^+, (F) Lym^+kit^+, (G) $Gr1^+kit^+$, and (H) $Gr1^+kit^{(lo)}$ cells in the bone marrow of animals transplanted with GFP or H9M cells. (I,J) Flow cytometric hematopoietic progenitor analysis of bone marrow from (I) wildtype or (J) H9M mice.



(C)

H9M Population	Derived from Primary/Secondary AML	Cells	Leukemic	Survival (d)	Spleen Weight (g)	WBC (*10⁶/ml)
Total GFP+	Primary	10^4	3/3	24 ± 0	0.21 ± 0.08	33.4 ± 17.8
Lin-kit+	Primary	10^4	2/2	18 ± 0	0.27 ± 0.01	95.5 ± 48.9
	Primary	10^3	6/6	20 ± 1.8	0.25 ± 0.05	66.8 ± 45.1
	Primary	10^2	6/6	29.1 ± 12.9	0.28 ± 0.03	105.2 ± 127
	Secondary	10^2	7/7	22.86 ± 1.7	.34 ± .08	106.4 ± 86.6
	Primary	10	2/6	39.5 ± 20.5	0.21 ± 0.07	8.2 ± 6.7
Lym+kit+	Primary	10^3	5/5	22.8 ± 2.2	0.27 ± 0.05	124.8 ± 66.0
	Primary	10^2	6/6	33.7 ± 12.5	0.24 ± 0.02	71.6 ± 68.2
	Secondary	10^2	7/7	22.71 ± 1.6	.39 ± .05	178.7 ± 108.9
	Primary	10	4/6	34.5 ± 12.9	0.21 ± 0.05	7.21 ± 5.47
Gr1+kit+	Primary	10^4	4/4	42.5 ± 11.7	0.24 ± 0.09	56 ± 40.5
	Primary	10^3	1/3	54	0.23	7.12
	Primary	10^2	0/3	>120 days	ND	ND
	Secondary	10^2	6/6	38 ± 13.2	.31 ± .07	109.2 ± 132.4
Gr1+kitlo	Primary	10^4	2/3	43 ± 2.8	0.18 ± 0.05	30 ± 27.0
	Primary	10^3	0/2	>120 days	ND	ND
	Primary	10^2	0/3	>120 days	ND	ND
	Secondary	10^2	0/4	>120 days	ND	ND

Figure S2, related to Figure 1. Potency of TICs by Cell Number in Primary & Secondary H9M AML. (A) High efficiency FACS purification of TICs. Representative flow cytometric analysis of the GFP+ fraction H9M bone marrow (i) before sorting, (ii) post sort for Lin-kit+, (iii) post sort for Lym+kit+, and (iv) post sort for Gr1+kit+. (B) Survival curves for mice transplanted with (i) 10^4, (ii) 10^3, (iii) 10^2, or (iv) 10 cells from the indicted populations from primary H9M AML. (C) Characteristics of leukemic mice transplanted with indicated compartments derived from primary or secondary H9M AML. Values represent mean ± SD.

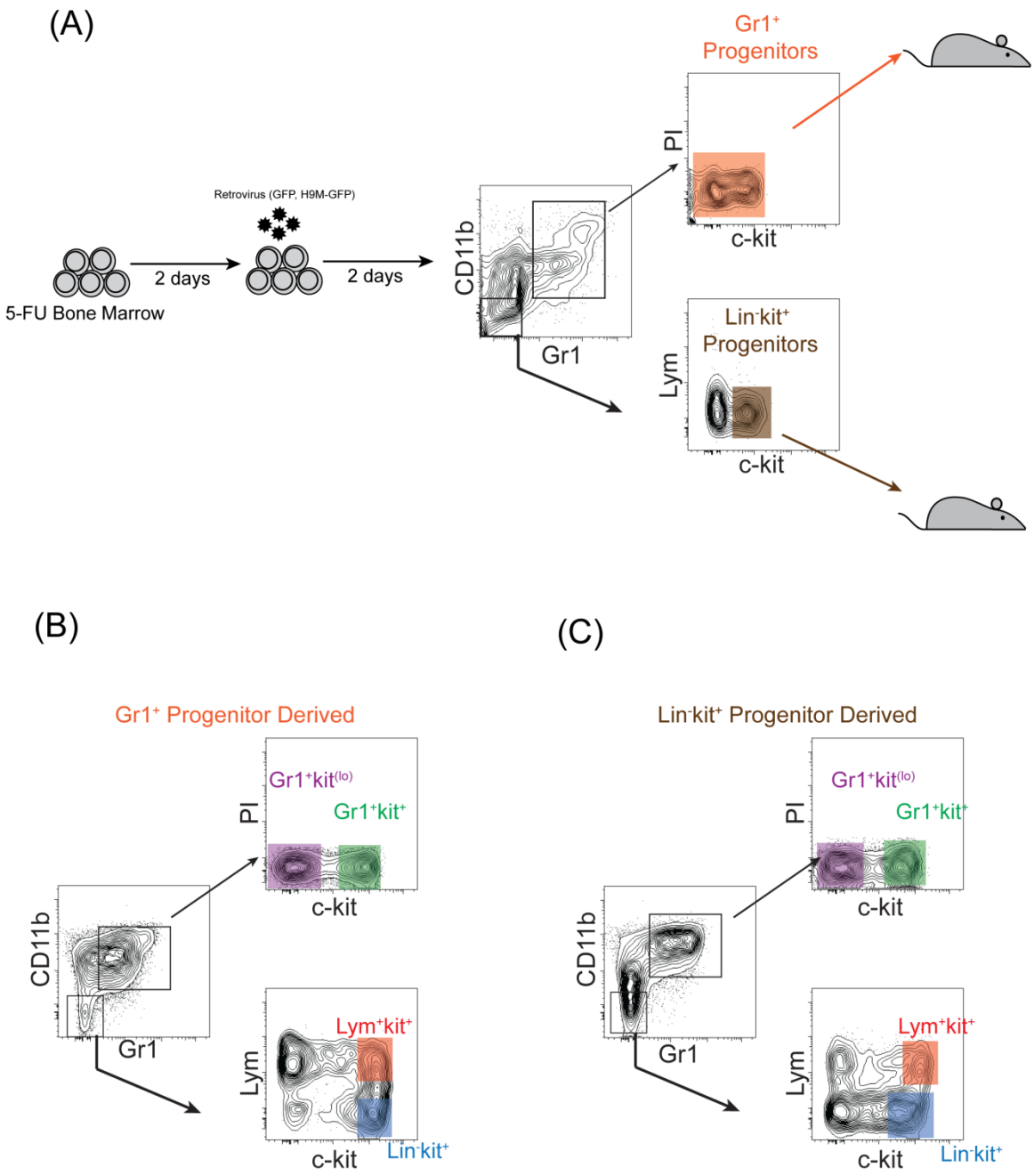
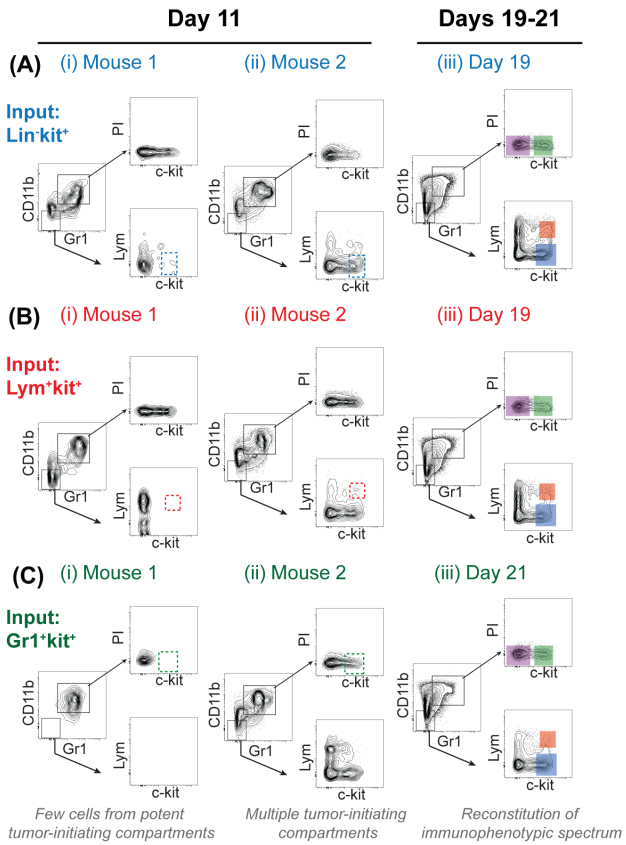


Figure S3, related to Figure 2. Enforced H9M Expression Permits Surface Marker Plasticity. (A) Experimental scheme. The bone marrow from mice injected 4 days prior with 5-fluorouracil was harvested, cultured for 2 days, transduced, and cultured for two additional days to allow for GFP expression. GFP^+ cells from these transduced progenitors were FACS purified into $Gr1^+$ progenitors (orange) or Lin^-kit^+ progenitors (brown), and ~$3*10^3$ cells from these purified populations were transplanted into lethally irradiated recipients along with 10^5 helper marrow cells. Flow cytometric of analysis of the bone marrow of mice that received (B) $Gr1^+$ progenitors or (C) Lin^-kit^+ progenitors.



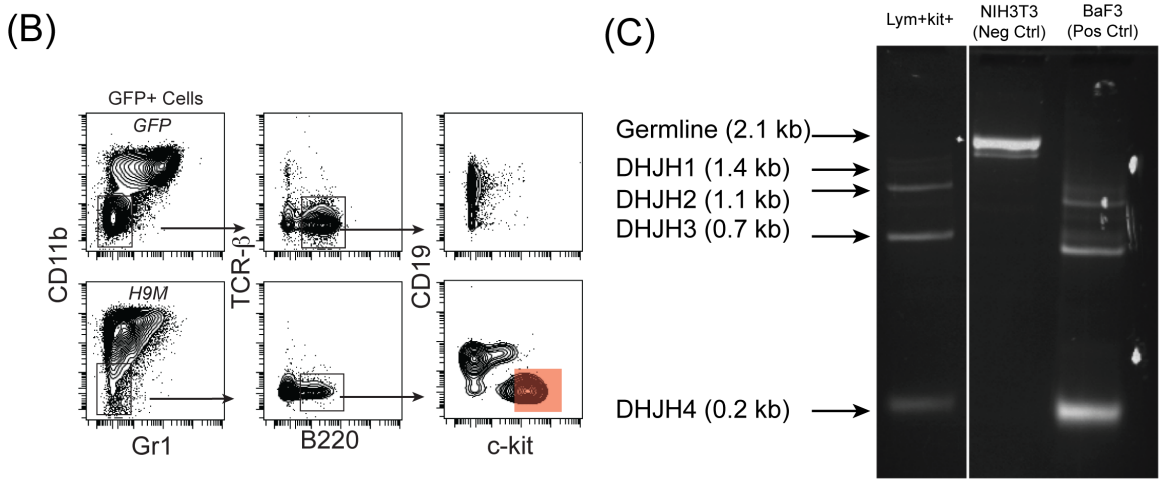
(D)

Population Transplanted	Day Post Transplant	% GFP⁺	%Lin⁻kit⁺	%Lym⁺kit⁺	%Gr1⁺kit⁺	% Gr1⁺kit^lo
Lin⁻kit⁺	7	0.488	0	0	0	85.358
	7	0.558	0.219912	0	3.29868	65.5452
	7	0.106	0	0	2.39784	72.7888
	8	0.336	21.84	1.82	32.7374	5.4626
	11	0.498	18.4992	0.53874	12.1878	22.0698
	11	0.735	0.66424	0.33304	10.434	25.345
	11	18.1	2.08314	0.12714	14.4184	34.3782
	11	19.1	2.05486	0.214385	7.7976	56.1564
	11	25.8	2.25362	0.107468	13.9722	44.5962
	14	65.7	7.8528	0.138432	13.064	20.976
	14	81.3	7.8288	0.125121	10.0794	18.84
	19	94	5.8905	1.3464	5.863	21.3928
	19	94.7	5.5776	0.83552	6.82	25.7114
Lym⁺kit⁺	7	0.233	0	0	0	96.4243
	7	0.326	0	0	2.55474	69.8445
	7	0.309	0	0	5.78088	63.612
	8	8.63	14.69	0.118876	7.0119	10.9998
	8	2.95	8.8392	0.51852	7.6241	23.4146
	11	7.05	2.02038	0.39471	2.2977	34.188
	11	0.913	0.105485	0.31501	19.24	30.992
	11	34.4	0.179267	0	3.07968	70.8096
	11	8.24	0.287371	0	2.63424	62.9748
	11	38.2	5.0875	0.27935	14.7026	26.4737
	14	79.7	7.2828	0.224532	11.5648	13.9834
	14	70.6	9.7776	0.196425	11.2217	15.2397
	19	95.1	5.6576	0.41327	4.4232	23.571
	19	93.4	3.3456	0.612	3.813	26.04
Gr1+kit+	7	0.0974	0	0	0	83.6734
	7	0.343	0	0	0.95648	74.088
	7	0.118	0	0	0.83665	54.3534
	11	2.7	0	0	0.235276	88.2734
	11	14.6	2.6969	0.63176	14.3472	39.102
	14	61	5.6	0.81312	10.0624	18.0576
	14	29.2	6.208	0	11.7261	23.7231
	21	93.4	5.244	0.3312	9.3026	27.9078
	21	92.7	6.2409	0.42174	6.417	23.498

Figure S4, related to Figure 3. *In Vivo* Tumorigenic Differentiation Does Not Proceed along a Strictly Defined Pathway. Defined numbers of each tumor-initiating compartment in primary AML were transplanted into secondary recipients. Animals were sacrificed every 3-4 four days thereafter to assess for surface phenotype of engrafted cells. Flow cytometric analysis of representative bone marrow of mice transplanted with (A) 500 Lin^-kit^+ cells, (B) 500 Lym^+kit^+ cells, or (C) 2500 $Gr1^+kit^+$ cells. Dashed boxes in on day 11 represent the phenotypic compartment transplanted. Shaded regions for mice transplanted on days 19-21 correspond to phenotypic compartments present in primary leukemia. All plots were previously gated on GFP^+ cells. (D) Percentages of each phenotypic compartment in all transplanted.

(A)

Rank		Gene Name	Description
1		Rag1	recombination activating gene 1 Gene [Source:MGI Symbol;Acc:MGI:97848]
2		Pou2af1	POU domain, class 2, associating factor 1 Gene [Source:MGI Symbol;Acc:MGI:105086]
3		Igk-C	immunoglobulin kappa chain, constant region Complex/Cluster/Region [Source:MGI Symbol;Acc:MGI:96495]
4		Gfra2	glial cell line derived neurotrophic factor family receptor alpha 2 Gene [Source:MGI Symbol;Acc:MGI:1195462]
5		Ebf1	early B-cell factor 1 Gene [Source:MGI Symbol;Acc:MGI:95275]
6		Vpreb1	pre-B lymphocyte gene 1 Gene [Source:MGI Symbol;Acc:MGI:98936]
7		Vpreb2	pre-B lymphocyte gene 2 Gene [Source:MGI Symbol;Acc:MGI:98937]
8		Sdc4	syndecan 4 Gene [Source:MGI Symbol;Acc:MGI:1349164]
9		Igll1	immunoglobulin lambda-like polypeptide 1 Gene [Source:MGI Symbol;Acc:MGI:96529]
10		St3gal6	ST3 beta-galactoside alpha-2,3-sialyltransferase 6 Gene [Source:MGI Symbol;Acc:MGI:1888707]
11		Btbd11	BTB (POZ) domain containing 11 Gene [Source:MGI Symbol;Acc:MGI:1921257]
12		Dntt	deoxynucleotidyltransferase, terminal Gene [Source:MGI Symbol;Acc:MGI:98659]
13		Blnk	B-cell linker Gene [Source:MGI Symbol;Acc:MGI:96878]
14		AC079817.3	
15		Igl-C2	immunoglobulin lambda chain, variable 1 Gene [Source:MGI Symbol;Acc:MGI:96530]
16		AC079817.4	Ig lambda-3 chain C region [Source:UniProtKB/Swiss-Prot;Acc:P01845]
17		Tnnt2	troponin T2, cardiac Gene [Source:MGI Symbol;Acc:MGI:104597]
18		Gfra1	glial cell line derived neurotrophic factor family receptor alpha 1 Gene [Source:MGI Symbol;Acc:MGI:1100842]
19		Ncam1	neural cell adhesion molecule 1 Gene [Source:MGI Symbol;Acc:MGI:97281]
20		Cd19	CD19 antigen Gene [Source:MGI Symbol;Acc:MGI:88319]
		Defined role in lymphocyte development	



Supplemental Figure S5, related to Figure 4. Lym$^+$kit$^+$ Fraction represents a B-specified progenitor that initiates myeloid leukemia. (A) Table of top 20 genes over-expressed in primary Lym$^+$kit$^+$ in comparison to primary Lin$^-$kit$^+$ cells. Grey boxes indicate genes with a defined role in lymphoid development. (B) Representative mass cytometric analysis of GFP$^+$ bone marrow of mice transplanted with progenitors transduced with (top) control GFP vector or (bottom) H9M-GFP vector. All surface markers used in analysis found in Table S4. (C) PCR product of genomic immunoglobulin DH-JH rearrangements in Lym$^+$kit$^+$, NIH3T3 (negative control), or BaF3 (pro B-cell line).

(A)

	p-value (One-way ANOVA)	
Phospho-node	G-CSF	S36GM
Erk (pT202/Y204)	0.3565	0.0685
Stat3 (pY705)	0.4563	0.3836
Akt (pS473)	0.0918	0.9935
S6 (pS235/S236)	*0.0376*	0.1383
CamKII (pT286)	0.2206	0.4603
Stat5 (pY694)	0.3186	0.7748
Src (pY418)	0.8436	0.673
PLCg2 (pY759)	0.0649	0.4614
LCK (pY505)	0.9512	0.8278
Stat1 (pY701)	0.3318	0.2873
Crkl (pY207)	0.6831	0.2048
CREB (pS133)	0.0748	0.3446
Rb (pS807/S811)	0.9503	0.9587
p38 (pT180/Y182)	0.3845	*0.0289*

(B)

Stimulation	G-CSF	SCF	IL-3	IL-6	GM-CSF
p-value across phospho-nodes	**0.047***	0.15	**0.016***	0.054	**0.014***

Figure S6, related to Figure 4. Statistical Analysis of Variance in Fold-Change Values between Distinct Tumor-Initiating Compartments and WT Compartments. (A) One primary and two secondary H9M AML bone marrow samples were stimulated with G-CSF or a cocktail of SCF, IL-3, IL-6, and GM-CSF (S36GM) for 15 minutes. Cells were then fixed to halt signaling, stained for surface markers, permeabilized, stained for intracellular markers and subjected to mass cytometry analysis. Signal-induction values for each tumor-initiating compartment (Lin^-kit^+, Lym^+kit^+, or $Gr1^+kit^+$) were calculated, and ANOVA was employed to test for differences between fold-change values between compartments within tumors. Statistically significant differences are shown in red. (B) Wildtype bone marrow cells and splenocytes were stimulated with either G-CSF, SCF, IL-3, IL-6 or GM-CSF for 15 minutes. Cells were then fixed to halt signaling, permeabilized, and stained for surface markers and intracellular markers. Fold-change values for Lym^+ and $Gr1^+$ cells were calculated, and p-values were determined by pairwise comparison across all intracellular markers (Erk (pT202/Y204), S6 (pS235/S236), Stat1 (pY701), Stat3 (pY705), and Stat5 (pY694)). Values in red are significantly different.

Abca1	Fads2	L3mbtl3	Ssbp2
Abhd1	Fads3	Limd1	Stat5a
Aff3	Fam102b	Lmna	Tcf4
AI451557	Fam49a	Luzp1	Tgtp1
Akap12	Fam65a	Lyrm5	Tgtp2
Angpt1	Fam76b	Marcks	Tmem176a
Ap1s3	Fam84b	Mef2c	Tmem176b
Arap2	Flnb	Mex3a	Tns1
Arhgap23	Flt3	Mex3b	Tpm2
Armcx2	Foxp1	Mgat4a	Trim2
Auts2	Frmd4b	Mmd	Trps1
Bcat1	Fyn	Mndal	Tsc22d1
Bcl11a	Galnt6	Msi2	Tspan13
Bcl2	Gata2	Mtap7	Ttc3
Bend4	Gm11663	Mycbp	Tubb2b
Bpil2	Gm336	Noc3l	Zbed3
Brd1	Gpr171	Nol9	Zfp260
C230081A13Rik	Gpr174	Nsmaf	Zmym5
C2cd3	Gpr56	Oosp1	2010001M09Rik
Calcrl	Gramd4	Pde4b	2610307P16Rik
Capn5	H2-Ob	Pdgfrb	4930453N24Rik
Ccnd2	Haghl	Pdzd4	5730469M10Rik
Ccr2	Hba-a1	Plek	
Cd163	Hba-a2	Psd3	
Cd180	Hbb-b1	Ptar1	
Cd27	Hbb-b2	Ptger4	
Cd34	Hlf	Ptms	
Cd72	Hmgn1	Rgs12	
Clec10a	Hmgn3	Rhoq	
Cnn3	Hpvc-ps	Rnf220	
Cpa3	Ict1	Runx1	
Ctla2a	Ifi203	Satb1	
Ctr9	Il17rb	Sdc1	
Cux1	Il2rg	Sdcbp	
Cxxc5	Il6st	Sepw1	
Dab2ip	Il7r	Serpini1	
Ddx4	Ints5	Slc15a2	
Dnmt3a	Itgax	Slc44a1	
Dpysl2	Jak3	Slfn5	
Ednra	Jakmip1	Smad1	
Elk3	Kank2	Socs2	
Emid1	Kcnn4	Sox4	
Epb4.1l4b	Kif16b	Spred1	

Table S1, related to Figure 4. List of Genes shared, enriched in primary H9M Tumor-Initiating Compartments (Lin^-kit^+ and Lym^+kit^+) relative to $Gr1^+kit^+$.

Antigen	Supplier	Clone	Element	Isotope	Final conc. (µg/mL)
B220	Invitrogen	RA3-6B2	Cd	110,111, 112, 114	1:500 dilution of 1 mM stock
Gr-1	BD Biosciences	RB6-8C5	La	139	2
PLCgamma2 (pY759)	BD Biosciences	K86-689.37	Pr	141	1
GFP	Biolegend	FM264G	Nd	142	2
p38 (pT180/Y182)	BD Biosciences	clone 36/p38	Nd	144	2
CD4	Biolegend	RM-4.5	Nd	145	2
CD8a	Biolegend	53-6.7	Nd	146	2
CD45	Biolegend	30-F11	Sm	147	2
CD11b	BD Biosciences	M1/70	Nd	148	2
Stat5 (pY694)	BD Biosciences	47	Nd	150	2
Erk 1/2 (pT202/Y204)	BD Biosciences	20A	Eu	151	1
Akt (pS473)	Cell Signaling Techonology	193H12	Sm	152	4
Stat1 (pY701)	BD Biosciences	4a	Eu	153	2
Stat3 (pY705)	BD Biosciences	4	Gd	158	2
Sca-1	Biolegend	E13-161.7	Dy	162	2
CD19	Biolegend	1D3	Dy	164	2
Rb (pS807/S811)	BD Biosciences	J112-906	Ho	165	0.4
cPARP	BD Biosciences	F21-852	Er	167	1
pCAMKII	Cell Signaling Techonology	polyclonal	Er	168	2
TCR-β	BD Biosciences	H57-597	Tm	169	2
c-kit	Biolegend	2B8	Er	170	2
LCK (pY505)	BD Biosciences	4	Yb	171	2
S6 (pS235/S236)	BD Biosciences	N7-548	Yb	172	2
Src (pY418)	BD Biosciences	K98-37	Yb	174	2
CrkL (pY207)	Cell Signaling Techonology	polyclonal	Lu	175	2
Creb (pS133)	Cell Signaling Techonology	87G3	Yb	176	1.5

Table S2, related to Figure 4. A Summary of Antibodies used in Mass Cytometry Analysis.

Supplemental Experimental Procedures

Flow Cytometric Analysis

The following antibodies and clones were used to analyze and FACS sort leukemic marrow and splenocytes: CD11b (M1/70), Gr1 (RB6-8C5), c-kit (2B8), B220 (RA3-6B2), TCR-β (H57-597), CD3ε (145-2C11), CD4 (RM-4.5), CD8a (53-6.7), and CD19 (1D3). Lymphoid (Lym) cocktail included B220, CD19, CD3ε, CD4, CD8a, and TCR-β.

PCR

Detection of genomic immunoglobulin DH-JH rearrangement was conducted as previously described (Deshpande et al., 2006). Briefly, two upstream degenerate primers binding 50 of the DFL/DSP element or the DQ52 element and a reverse primer complementary to a binding site downstream of the JH4 segment were used in a single PCR reaction (denaturation at 94°C for 1 minute followed by 35 cycles of 1 minute at 94°C, 1 minute at 60°C, and 1 minute 45 seconds at 72°C with a final extension at 72°C for 10 minutes).

In vivo pharmacologic inhibition

Inhibitors and doses used for inhibition were chosen based on previously described work (Milella et al., 2001; Ozawa et al., 2008; Schmelz et al., 2005; Solit et al., 2006; Stirewalt and Meshinchi, 2010; Wang et al., 2011; Xu et al., 2005; Yilmaz et al., 2006; Zorn et al., 2007).

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