SUPPLEMENTARY INFORMATION

MATERIALS AND METHODS

Human HSC, LSC and AML source data

To identify candidate regulators of self-renewal, we identified genes that were expressed in both human HSCs and LSCs as described by Eppert et al., defined as the HSC-LSC-core enriched gene set in that paper.[1] Ten candidate genes were identified for further expression analysis based on criteria that they encode potentially druggable proteins and that they had not been previously characterized in hematopoietic progenitors at the time that the study was initiated. *PRKCH* expression was analyzed in human hematopoietic progenitors and a separate cohort of LSCs using previously published microarray data from Gentles et al.[2] These data were obtained from Gene Expression Commons (https://gexc.riken.jp/).[3] *PRKCH*, *BAALC* and *MECOM* expression in human AML was characterized using data from The Cancer Genome Atlas.[4] Files with annotated RNA sequencing data (as Fragments per Kilobase of transcript per Million, FPKM) and patient characteristics (e.g. overall survival, mutation profile and cytogenetics) were obtained from David Spencer and Timothy Ley (Washington University School of Medicine). The source data used for these comparisons are included as Supplementary Table 2. Comparisons were performed as indicated in the text and figure legends.

Mouse strains

Prkch loss of function mice have been described previously and are available at the Jackson Laboratory (JAX 018988).[5] The *Flt3*^{ITD} (JAX 011112), *Runx1*^{f} (JAX 008772), *Vav1-Cre* (JAX 008610) and Ubc-CreER (JAX 008085) mouse strains have all been previously described and were obtained from The Jackson Laboratory.[6-9] These lines were all on a pure C57BL/6 background. All mice were housed in the Department for Comparative Medicine at Washington University. All animal procedures were approved by the Washington University Committees on the Use and Care of Animals.

Isolation of HSCs, HPCs and GMPs by flow cytometry

Bone marrow cells were obtained by flushing the long bones (tibias and femurs) or by crushing long bones, pelvic bones and vertebrae with a mortar and pestle in calcium and magnesium-free Hank's buffered salt solution (HBSS), supplemented with 2% heat inactivated bovine serum (Gibco). Single cell suspensions were filtered through a 40 μm cell strainer (Fisher). The cells were then stained for 20 minutes with fluorescently conjugated antibodies, washed with HBSS + 2% bovine serum and resuspended for analysis. Cell counts were measured by hemocytometer. The following antibodies were used for flow cytometry, all were from Biolegend except as indicated: CD150 (TC15-12F12.2), CD48 (HM48-1), Sca1 (D7), c-Kit (2B8), Ter119 (Ter-119), CD3 (17A2), CD11b (M1/70), Gr-1 (RB6-8C5), B220 (RA3-6B2), CD8a (53-6.7), CD34 (eBioscience, RAM34), CD2 (RM2-5), CD45.1 (A20), CD45.2 (104), CD127 (A7R340),

CD16/32 (93) and FLT3/CD135 (A2F10). Lineage stains for all experiments included CD2, CD3, CD8a, Ter119, B220 and Gr1. Except as indicated, the following surface marker phenotypes were used to define cell populations: HSCs ($CD150^+$, $CD48^-Lineage^-$, $Sca1^+$, $c\text{-}kit^+$), HPCs ($CD48^+Lineage^-$, $Sca1^+$, $c\text{-}kit^+$), and GMPs ($Lineage^-$, $Sca1^-$, $CD127^-$, $c\text{-}kit^+$, $CD34^+$, $CD16/32^+$). Non-viable cells were excluded from analyses by 4',6-diamidino-2-phenylindone (DAPI) staining (1 μg/ml). When progenitors were isolated for Western blotting or RNA collection, $c\text{-}kit^+$ cells were enriched prior to sorting by selection with paramagnetic beads (Miltenyi Biotec). Flow cytometry was performed on a BD FACSAria Fusion flow cytometer (BD Biosciences).

Western blots

Thirty thousand HSCs, HPCs, GMPs or AML cells were sorted into Trichoracetic acid (TCA), and the volume was adjusted to a final concentration of 10% TCA. Extracts were incubated for 15 minutes on ice and centrifuged at 16,100xg at 4°C for 10 minutes. Precipitates were washed in acetone twice and dried. The pellets were solubilized in 9M urea, 2% Triton X-100, 1% DTT. LDS loading buffer (Lifetech) was added and the pellet was heated at 70°C for 10 minutes. Samples were separated on Bis-Tris polyacrylamide gels (Lifetech) and transferred to PVDF membrane (Lifetech). All antibodies were from Cell Signaling Technologies except as indicated: P-STAT5 (4322), Total STAT5 (9363), P-STAT3 (9145), Total STAT3 (9139), P-ERK1/2 (4370), Total ERK1/2 (4696), P-AKT Ser473 (4060), P-AKT T308 (13038), Total AKT (4691), P-JNK

(4668), α-TUBULIN (3873), β-ACTIN (Santa Cruz Bioscience, clone AC-17), PKCη (Santa Cruz Bioscience, clone Sc-215), PKCα (2056), PKCδ (9616), PKCε (2683), PKCΘ (9616), PKCξ (9368), HRP-anti-Rabbit IgG (7074) and HRP-anti-mouse IgG (7076). Blots were developed with the SuperSignal West Femto or Pico chemiluminescence kits (Thermo Scientific). Blots were stripped (1% SDS, 25 mM glycine pH 2) prior to re-probing.

Quantitative RT-PCR

RNA was isolated from sorted progenitors with RNAeasy micro plus columns (Qiagen) and converted to cDNA with Superscript III reverse transcriptase (Lifetech). Quantitative RT-PCR assays were performed with SYBR green master mix (Roche) and primers listed in Supplementary Table S3. Samples were normalized based on β-actin expression.

Long-term repopulation assays

Eight to ten week old C57BL/6Ka-Thy-1.2 (CD45.1) recipient mice were given two doses of 550 rad delivered at least 3 hours apart. Donor *Prkch*$^{+/+}$ or *Prkch*$^{-/-}$ marrow cells were mixed with competitor bone marrow cells (300,000 donor and competitor cells) and injected via the retroorbital sinus. To assess donor chimerism, peripheral blood was obtained from the submandibular veins of recipient mice at the indicated times after transplantation. Blood was subjected to ammonium-chloride lysis of the red blood cells and leukocytes were stained with antibodies to CD45.2, CD45.1, B220, CD3, CD11b and Gr-1 to assess

multilineage engraftment. For secondary transplants, mice were injected with 3 million cells from the bone marrow of primary recipient mice.

Mouse survival analyses and limiting dilution studies

To generate leukemias, 1 million bone marrow cells from *Ubc-CreER; Nras^{G12D}; Runx1$^{f/f}$* , *Ubc-CreER; Flt3^{ITD}; Runx1$^{f/f}$*; *Prkch$^{+/+}$* control or *Ubc-CreER; Flt3^{ITD}; Runx1$^{f/f}$*; *Prkch$^{-/-}$* mice were transplanted into lethally irradiated recipient C57BL/6Ka-Thy-1.2 (CD45.1) mice. *Runx1* was deleted and, where applicable *Nras^{G12D}* expression was induced, 4 weeks after the transplants by administering tamoxifen. Tamoxifen (T5648; Sigma, St. Louis, MO) was dissolved in 90% corn oil/10% ethanol at 20 mg/ml, and injected at 80 mg/kg/day i.p. for 5 consecutive days. Mice were then monitored daily until they became moribund. Sick mice were euthanized. Spleens were fixed in 10% formalin and bone marrow cells were analyzed by flow cytometry and cytospin. In all cases tested, leukemias expressed myeloid surface markers CD11b and Gr1. Survival curves were compared with the log rank test. For limiting dilution assays, AML cells were transplanted at the indicated doses in sublethally irradiated (600 cGy) CD45.1 mice. Recipients were followed for 16 weeks for evidence of leukemia (CD45.2 AML cells), and mice were euthanized when they became moribund. LSC frequencies were calculated by Extreme Limiting Dilution Analysis.[10]

Peripheral blood counts

Blood was collected into BD Microtainer EDTA tubes (Becton Dickinson) and analyzed on a Hemavet (Drew Scientific).

***Prkch* silencing in 32D cells**

The 32D cell line was maintained in RPMI 1640 media (Thermo Fisher Scientific) supplemented with 10% heat inactivated fetal bovine serum (Thermo Fisher Scientific), penicillin and streptomycin (Thermo Fisher Scientific), and 1 ng/mL recombinant mouse Interleukin 3 (Peprotech). To silence *Prkch*, an shRNA was obtained from the Washington University RNAi core to target the sequence CGACAAGGACTTCAGTGTAAA. Control viruses targeted either the LacZ sequence GCGGATCGTAATCACCCGAGT or the Luciferase sequence CTTCGAAATGTCCGTTCGGTT. In each case, the Puromycin cassette of the pLKO.1-puro lentiviral plasmid was replaced with an EGFP cDNA. Cells were infected with packaged lentiviral particles, and *Prkch* silencing was confirmed by Western blotting (Supplementary Figure S1A). The percentage of EGFP+ cells was measured over time by flow cytometry (Supplementary Figure S1B).

SUPPLEMENTARY TABLES AND FIGURES

Table S1. Expression of HSC/LSC-related genes is conserved in mouse HSCs.

Fourteen genes from the Eppert et al. HSC-LSC-core enriched gene set were evaluated by quantitative RT-PCR in mouse HSCs, GMPs and unfractionated bone marrow cells. Numbers reflect average fold changes for HSCs relative to GMPs and for HSCs relative to unfractionated bone marrow.

Table S2. Expression data and clinical data for TCGA patients analyzed in Figure 2.

Expression values (FPKM) for *PRKCH*, *BAALC* and *MECOM* are shown for 179 TCGA samples that had RNAseq data, survival and mutation panels available. FAB subtypes, cytogenetic risk categories, survival times, and *TP53* and *RUNX1* mutation profiles are also included.

Table S3. Quantitative RT-PCR primer sequences for genes analyzed in Table S1.

Table S4. Limiting dilution analysis for AML specimens from Figure 2H.

Cell doses are shown in the top row. The fraction of mice with AML is shown in each column for 4 independent *Prkch*$^{+/+}$ and 5 independent *Prkch*$^{-/-}$ AML. LSC frequency was calculated by ELDA.[10] For specimen WT-4, an LSC frequency

could not be calculated because all recipient mice developed AML at the dose tested.

Figure S1. *In vitro* analysis of *Prkch* regulation of 32D cell growth.

As a preliminary assessment of *Prkch* function, mouse *Prkch* was silenced in 32D cells. (A) *Prkch* silencing led to reduced expression of PKCη by Western blot. (B) After infection, the percentage of EGFP-positive shRNA-expressing cells was measured at 4, 5, 8, 12 and 18 days after infection. The fraction of EGFP positive cells is shown as a ratio relative to the day 4 value. Error bars reflect standard deviations for n=3 technical replicates, and the panel is representative of 3 independent experiments. P values were calculated by the two-tailed Student's t-test comparing shPrkch cells to shLacZ controls, ***p<0.001.

Figure S2. Peripheral blood counts in *Prkch* deficient mice.

Peripheral white blood cell (A), hemoglobin (B) and platelet counts (C) in peripheral blood collected at 8-10 weeks after birth from mice of the indicated genotypes (n=5-7). Error bars reflect standard deviations.

Figure S3. Above-median *PRKCH* expression predicts poor overall survival more effectively than *BAALC* or *MECOM* expression in the TCGA cohort.

(A) Survival curves are shown for patients with above- and below-median expression of *BAALC* and *MECOM*. Unlike *PRKCH* (Fig. 2B), patient segregation based on the median expression of *BAALC* or *MECOM* does not resolve an

adverse risk population. (B) A higher threshold of 20th or 10th percentile expression begins to resolve patients with poor prognosis based on *BAALC* or *MECOM* expression. High *PRKCH* expression predicts poor prognosis irrespective of the threshold chosen. In all panels, survival curves were compared by the log rank test, and p-values are shown in each panel. Source data are in Supplementary Table S2.

Figure S4. *Prkch* deletion does not lead to changes in the morphology or surface marker phenotypes of *Flt3^{ITD}/Runx1* mutant AML. (A) Wright-Giemsa stained cytospins of representative *Prkch$^{+/+}$* and *Prkch$^{-/-}$* AML cells. (B) Surface marker phenotypes of *Prkch$^{+/+}$* and *Prkch$^{-/-}$* AML cells (N=9 per genotype). Several different populations were assayed, including CD11b^{+}, c-Kit^{+}, GMP-like (Lineage^{-}c-Kit^{+}Sca1^{-}CD34^{+}CD16/32^{+}) and LSK-like (Lineage^{-}c-Kit^{+}Sca1^{+}) populations. (C) Representative flow plots and gating strategies for the data summarized in Figure S4B.

Figure S5. *Prkch* deletion does not result in reproducible changes in signal transduction in *Flt3^{ITD}/Runx1* mutant AML. (A) Western blot showing phosphorylation of several signal transduction proteins in *Flt3^{ITD}/Runx1* mutant AML with the indicated *Prkch* genotypes, as well as HPC and GMP controls. This blot is representative of 3 independent experiments that analyzed a total of 10 independent *Prkch$^{+/+}$* and 10 *Prkch$^{-/-}$* AMLs. While there were differences in signal transduction (e.g. ERK1/2 phosphorylation) among the lines, consistent

differences between *Prkch*$^{+/+}$ and *Prkch*$^{-/-}$ leukemias were not observed. (B, C) Compensatory changes in PKCε, PKCδ (B) or PKCα (C) were not observed in *Prkch*$^{-/-}$ leukemias. Other PKC family members, including theta and zeta, were not detectable by Western blot.

SUPPLEMENTARY REFERENCES

1. Eppert K, Takenaka K, Lechman ER, Waldron L, Nilsson B, van Galen P, et al. Stem cell gene expression programs influence clinical outcome in human leukemia. *Nat Med*. 2011;17(9):1086-93.

2. Gentles AJ, Plevritis SK, Majeti R, Alizadeh AA. Association of a leukemic stem cell gene expression signature with clinical outcomes in acute myeloid leukemia. *JAMA*. 2010;304(24):2706-15.

3. Seita J, Sahoo D, Rossi DJ, Bhattacharya D, Serwold T, Inlay MA, et al. Gene Expression Commons: an open platform for absolute gene expression profiling. *PLoS One*. 2012;7(7):e40321.

4. Cancer Genome Atlas Research Network. Genomic and epigenomic landscapes of adult de novo acute myeloid leukemia. *N Engl J Med*. 2013;368(22):2059-74.

5. Fu G, Hu J, Niederberger-Magnenat N, Rybakin V, Casas J, Yachi PP, et al. Protein kinase C eta is required for T cell activation and homeostatic proliferation. *Sci Signal*. 2011;4(202):ra84.

6. Lee BH, Tothova Z, Levine RL, Anderson K, Buza-Vidas N, Cullen DE, et al. FLT3 mutations confer enhanced proliferation and survival properties to multipotent progenitors in a murine model of chronic myelomonocytic leukemia. *Cancer Cell*. 2007;12(4):367-80.

7. Taniuchi I, Osato M, Egawa T, Sunshine MJ, Bae SC, Komori T, et al. Differential requirements for Runx proteins in CD4 repression and epigenetic silencing during T lymphocyte development. *Cell*. 2002;111(5):621-33.

8. Siegemund S, Shepherd J, Xiao C, Sauer K. hCD2-iCre and Vav-iCre mediated gene recombination patterns in murine hematopoietic cells. *PLoS One*. 2015;10(4):e0124661.

9. Ruzankina Y, Pinzon-Guzman C, Asare A, Ong T, Pontano L, Cotsarelis G, et al. Deletion of the developmentally essential gene ATR in adult mice leads to age-related phenotypes and stem cell loss. *Cell Stem Cell*. 2007;1(1):113-26.

10. Hu Y, Smyth GK. ELDA: extreme limiting dilution analysis for comparing depleted and enriched populations in stem cell and other assays. *J Immunol Methods*. 2009;347(1-2):70-8.

Supplementary Table S1

Gene Symbol	Gene Name	HSC vs. GMP	HSC vs. BM
Prkch	Protein kinase C eta	10	21
Yes1	yamaguchi sarcoma oncogene homolog	176	24
Smarca1	SWI/SNF related, matrix associated chromatin regulator	109	164
Plscr4	phospholipid scramblase 4	10086	31
Rbpms	RNA binding protein with multiple splicing	2.8	7.6
Hlf	Hepatic leukemia factor	0.8	5.9
Socs2	suppressor of cytokine signaling 2	180	18
Dapk1	death associated protein kinase	2.8	8.8
Dram	DNA damage related autophagy modulator 1	0.1	0.2
Elk3	ETS domain protein, SRF accessory	2.3	11
Ppp1r16b	protein phosphatase 1R16B	58.0	4.6
Inpp4b	Inositol polyphosphate 4 phosphatase	29	10
Meis1	Meis homeobox 1	59	116
Mecom	MECOM MDS1 and EVI1 complex locus	1953	3650

Supplementary Table S2

TCGA	PRKCH	BAALC	MECOM	FAB	RISK CYTO	OS 3.31.12	Expired 3.31.12	TP53	RUNX1
2842	0.9	0.5	22.0	M4	Intermediate	0.3	Y		
2877	2.6	0.2	0.2	M1	Intermediate	41.4			
2811	5.2	0.2	0.2	M4	Intermediate	8.2	Y		
2893	2.1	1.0	3.5	M5	Poor	6.6	Y		
2879	3.2	1.1	0.4	M1	Intermediate	31.9			
2931	13.6	5.2	0.3	M2	Intermediate	0.1	Y		
2956	4.7	0.0	0.3	M5	Intermediate	5.7	Y		
2896	5.9	2.3	0.3	M5	Intermediate	7.5	Y		
2818	4.2	0.4	0.5	M2	Intermediate	10.2	Y		
2990	3.1	0.2	0.3	M1	Intermediate	42.1			
2895	9.1	0.7	0.2	M1	unknown	5.7	Y		
2883	0.9	0.2	7.1	M5	Poor	47.2			
2832	1.4	25.3	0.5	M5	Intermediate	11.8	Y		
2885	22.7	44.5	0.4	M0	Poor	7	Y	p.H193Y	
2869	10.0	1.6	0.3	M2	Intermediate	27.1	Y		
2835	1.0	0.3	1.0	M5	Intermediate	77.3			
2855	1.8	8.1	0.6	M1	Poor	7.2	Y		
2900	3.7	1.9	0.4	M1	Intermediate	6.3	Y		
2920	12.2	10.2	0.7	M2	Poor	11.8	Y		
2988	10.0	3.9	0.4	M1	Intermediate	0.8	Y		
2932	3.7	0.5	0.4	M5	Intermediate	42.1			
2925	3.2	0.6	0.3	M5	Intermediate	7.9	Y		
2871	25.3	0.1	0.3	M1	Intermediate	22.6	Y		
2913	5.3	1.6	0.4	M0	Intermediate	52.6	Y		
2812	4.6	0.3	0.5	M2	Intermediate	11.5	Y		
2884	5.3	0.3	0.4	M1	Intermediate	24.8	Y		
2929	23.1	59.6	51.3	M0	Poor	4.6	Y		
2919	1.9	0.0	0.5	M1	Intermediate	26.8			
2853	8.8	0.2	0.2	M1	Intermediate	3.1	Y		
2928	35.8	92.8	0.3	M1	Poor	4	Y		
2861	2.7	0.0	1.8	M5	Poor	0.6	Y		
2963	4.3	0.6	0.7	M1	Intermediate	53.9	Y		
2848	2.0	0.2	0.4	M4	Intermediate	14.5	Y		
2972	7.3	0.2	0.6	M2	Intermediate	1.5	Y		
2859	19.5	1.9	0.3	M2	Intermediate	10.2	Y		
2976	5.5	4.2	1.6	M1	Intermediate	30	Y		
2949	28.9	171.9	0.4	M0	Poor	32.6	Y		p.S314fs

2824	4.9	0.1	0.2	M5	Intermediate	0.3	Y		
2944	43.8	126.3	63.4	M0	Poor	2.4	Y		
2903	0.3	0.1	0.6	M1	Intermediate	0.4	Y		
2995	25.3	14.1	8.9	M2	Intermediate	73.6			
2810	1.5	0.4	0.6	M2	unknown	1.3	Y		
2839	8.0	3.1	0.5	M2	Intermediate	16.3	Y		
2910	1.9	0.1	0.3	M5	Poor	0	Y		
2882	19.6	22.9	0.6	M2	Poor	11	Y		
2820	19.9	79.4	0.7	M0	Poor	0.3	Y	p.P223fs	
2965	4.4	0.2	0.3	M4	Intermediate	11.3	Y		
2993	4.7	2.3	0.5	M1	Intermediate	24.6	Y		
2941	16.3	0.4	2.7	unknown	Poor	0.2	Y	p.I195S	
3006	6.9	1.1	0.4	M1	Intermediate	15.5	Y		
2826	2.7	0.1	0.5	M4	Intermediate	24.1	Y		
2909	20.7	215.9	1.0	unknown	Intermediate	26.1			
2881	23.0	30.8	0.3	M1	Good	29.7	Y		
2986	6.7	0.8	1.5	M2	Intermediate	6.6	Y		
2924	6.3	0.7	0.4	M4	Intermediate	26			
2870	15.5	69.0	0.2	M1	Good	4.5	Y		
2917	7.7	7.7	11.7	M0	Poor	57.3	Y		
3011	2.7	0.4	0.4	M1	Intermediate	81.9			
2890	29.6	148.4	0.8	M0	Intermediate	0.5	Y		p.R201*
2851	6.0	2.2	0.6	M5	Intermediate	7.7	Y		
2969	3.1	0.9	0.3	M5	Intermediate	62.3			
2971	5.4	0.0	0.4	M4	Intermediate	25.8	Y		
2967	1.9	0.8	0.3	M4	Intermediate	56.5			
2865	11.1	60.9	0.4	M1	Intermediate	2.3	Y		p.D198N,p.S141L
2894	5.6	0.9	4.9	M5	Poor	5.6	Y		
2938	11.1	0.3	2.0	M7	Intermediate	9.9	Y	p.R342fs,p.H179R	
2836	6.2	0.3	0.2	M4	Intermediate	17	Y		
2838	14.0	18.6	0.3	M2	Poor	12.4	Y	p.T125	
2816	4.9	1.7	3.4	M4	Intermediate	7.5	Y		
2825	3.2	0.1	0.1	M5	Intermediate	7.5	Y		
2984	0.4	2.5	0.3	M1	Intermediate	71.3			
2887	25.1	200.0	0.2	M1	Poor	17.4	Y		
2822	12.7	57.1	0.4	M0	Intermediate	32.3	Y		

2942	16.0	48.9	0.9	M4	Good	43.5			
2981	3.4	1.1	0.3	M5	Intermediate	44.4			
2952	23.6	4.7	0.6	M1	Poor	1.9	Y	p.E286G	
2878	10.7	1.8	0.7	M2	Poor	12.2	Y	p.S215G ,p.V173fs	
2936	36.3	70.0	0.3	M0	Intermediate	13.8	Y		p.R320*
2915	17.0	5.7	25.2	M2	Poor	0.5	Y		
2985	7.2	4.9	7.9	M5	Intermediate	9.2	Y		
2973	5.7	0.8	0.5	M4	Intermediate	20.5	Y		
2975	5.6	4.4	2.1	M2	Intermediate	34	Y		
2943	28.7	12.5	0.2	M1	Poor	4.2	Y	p.R273C	
2837	4.5	0.0	0.6	M5	Intermediate	0.7	Y		
2959	25.2	101.8	0.8	M0	Intermediate	15.4	Y		p.R201Q
2889	15.0	27.4	0.3	M4	Good	34.3	Y		
2978	12.6	5.6	1.2	M2	Intermediate	44.8			p.R201*
3005	4.1	7.3	0.6	M5	Intermediate	100.5			
2814	26.6	27.1	0.3	M0	Poor	26.3	Y		
2933	11.0	13.4	1.0	M4	Intermediate	4.6	Y		p.R162G
2873	11.7	0.1	0.9	M5	Intermediate	32.7			
2918	4.8	31.3	0.4	M1	Intermediate	7.4	Y		
2935	14.9	26.7	0.3	M4	Poor	1.6	Y	p.R248Q	
2914	17.2	29.1	0.6	M2	Good	48.3			
2904	17.2	9.9	0.7	M0	Poor	17.1	Y	p.R337C	
2930	6.8	22.3	0.7	M2	Intermediate	7.1	Y		
2847	15.2	28.7	0.5	M1	Poor	52.7	Y		
2844	1.7	0.1	9.8	M2	Poor	3.9	Y		
2846	11.7	45.7	0.5	M4	Good	45.8	Y		
2868	15.8	2.7	0.3	M7	Poor	4.5	Y	e4-1	
2815	8.2	14.3	0.6	M4	Good	26.3	Y		
2817	23.7	98.4	9.1	M2	Poor	9.3	Y		
2996	9.0	20.0	0.4	M2	Intermediate	73			
2921	7.1	11.0	0.1	M4	Intermediate	8.1	Y		
2833	8.3	17.3	0.9	M4	Intermediate	15.8	Y		
2830	12.6	31.8	0.6	M1	Intermediate	8.1	Y		
2856	7.4	9.7	0.5	M4	Intermediate	5.3	Y		
2948	7.1	0.1	0.6	M4	Intermediate	18.1	Y		
2807	16.1	31.0	1.1	M1	Intermediate	4.9	Y		p.SG167in_frame_del
2911	2.4	0.6	9.2	M4	Poor	61.2			
2834	3.6	0.4	18.1	M4	Poor	7.7	Y		
2892	8.1	12.8	0.5	M4	Good	59.3			

2860	16.1	11.9	0.4	M7	Poor	13.6	Y	p.M39fs	
2843	13.0	55.3	0.5	M6	Intermediate	6.3	Y		
2992	5.6	1.9	0.4	M1	Intermediate	56.3	Y		
2964	7.1	0.4	0.5	M1	Intermediate	59.5			
2970	8.9	12.2	0.1	M4	Intermediate	10.7	Y		p.P113L
2954	10.6	43.8	0.5	M4	Good	68.3			
2934	12.6	45.6	0.7	M1	Intermediate	6.6	Y		
2927	22.0	176.4	2.8	M1	Intermediate	2.4	Y		p.R201*,p.R162S
2908	18.8	84.3	0.1	M2	Poor	1.4	Y	p.Q317*,p.C141W	
2857	18.4	1.1	1.3	M6	Poor	10	Y	e0+1	
2912	5.1	13.5	1.0	M4	Intermediate	8.4	Y		p.R162K
3002	17.3	18.7	1.4	M2	Intermediate	46.8	Y		
2898	7.3	20.6	0.3	M1	Intermediate	36.1			
2916	13.6	8.0	1.7	M4	Intermediate	45.3			
2821	16.0	13.3	0.2	M1	Intermediate	27.4	Y		p.D123fs
2805	11.6	55.3	0.4	M0	Intermediate	18.5	Y		p.R201*,p.A142fs
2866	14.7	8.5	0.3	M1	Intermediate	5.5	Y		
2987	15.2	0.2	0.8	M5	Intermediate	6.3	Y		
2854	7.7	7.2	0.6	M4	Intermediate	11.1	Y		
2899	13.8	67.0	0.1	M4	Intermediate	22.3	Y		p.R162G
2863	7.3	62.2	0.4	M1	Poor	1.2	Y		
2828	5.9	3.2	0.4	M4	Good	99.9			
2888	14.2	6.0	0.2	M4	Good	33.5			
2983	10.4	9.2	0.7	M0	Intermediate	11.2	Y		p.A149P
2813	7.0	0.9	0.9	M4	Poor	1.3	Y	p.C176Y	
3001	7.9	5.9	0.3	M3	Good	76.2			
2841	5.2	9.4	0.6	M3	Good	46.5	Y		
2980	5.2	0.7	0.3	M3	Good	47.5			
2849	24.6	11.7	1.6	M0	Poor	95.5			
2939	8.1	36.6	0.5	M1	Poor	36.9			
2950	4.0	35.7	0.4	M2	Good	40.3			
3012	2.6	8.0	0.6	M3	Good	86.6			
2867	11.6	4.8	0.2	M2	Intermediate	5.2	Y		
2808	3.4	13.0	0.2	M2	Intermediate	118.1			
2999	4.4	2.5	1.0	M3	Good	83.3			
2872	1.4	0.2	0.5	M3	Good	43.4			
2946	4.1	7.1	1.7	M2	unknown	24.4			
2977	11.7	13.7	3.1	M2	Intermediate	0.8	Y		
2940	6.1	0.9	0.2	M2	Intermediate	55.4	Y		

2979	9.3	25.4	0.2	M1	Intermediate	47			
2982	7.5	2.2	0.4	M3	Good	37.1			
2819	4.9	3.0	1.2	M2	Good	95.6	Y		
2876	2.3	12.2	1.7	M2	Poor	59			
2994	4.6	0.8	0.3	M3	Good	83.5			
2891	14.8	6.8	0.7	M1	Poor	21.5	Y		
2901	8.6	18.1	0.4	M1	Poor	10.5	Y		
2991	4.1	3.1	0.3	M3	Good	88.3			
2858	4.4	28.5	1.2	M2	Good	19.2	Y		
2875	9.6	80.7	0.7	M2	Good	27.7			
3009	11.0	6.7	0.3	M4	Intermediate	19	Y		p.G164fs
2803	1.0	0.5	1.0	M3	Good	25.7	Y		
2823	1.4	0.8	0.2	M3	Good	0.2	Y		
2845	4.4	19.7	0.3	M2	Intermediate	9.3	Y		
2874	5.3	8.9	0.6	M2	Poor	35.2			
2862	3.8	2.3	0.6	M3	Intermediate	69			
3008	6.4	11.6	0.5	M1	Intermediate	27	Y		
3007	5.4	0.1	0.2	M3	Good	75.8			
2897	3.3	0.1	4.0	M3	Good	30.5			
2880	3.9	9.0	0.3	M1	Intermediate	20.5	Y		
2886	6.1	152.4	0.5	M2	Good	29.4			
2955	1.5	1.5	0.2	M2	Intermediate	16.4	Y		
2806	5.7	71.0	0.2	M1	Good	30.6	Y		
2998	1.2	0.5	0.4	M3	Good	1	Y		
2966	11.3	11.5	0.3	M2	Intermediate	28.4	Y		
2937	3.7	34.4	1.8	M1	Good	8	Y		
3000	6.5	19.5	0.4	M2	Intermediate	67.7			
2840	2.2	0.2	0.5	M3	Good	0.7	Y		

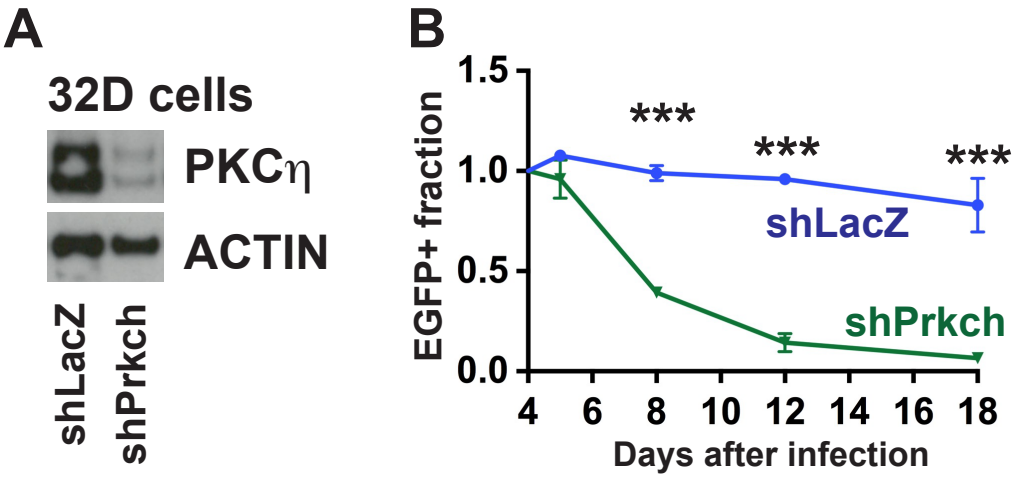
Supplementary Table S3

Gene	Primer sequence
Prkch	ccacagggatcctcaagtct
	aaagggtgtctcaggatctcat
Yes1	ttaagaagggtgaacgatttcaa
	attgatcttgcttcccacca
Smarca1	ccgtgttatttgtttcgttgg
	ctggcatcatttcatcacga
Plscr4	gacaacatacacgttcttcagca
	ttattagtttcaaaacgtgtcatcagt
Rbpms	agccgagaaggagaacacc
	cagaccgctgacaaataggg
Hlf	ttcattcccgatgatttgaag
	cggattgcgatctggttc
Socs2	cgcgagctcagtcaaacag
	agttccttctggagcctctttt
Dapk1	aacagcacgagcatgtgg
	gagtcctcggtgtgtgtcct
Dram	cttggattggtgggatgc
	acaggcacggctaactcct
Elk3	gagcagccttagtcctgtcg
	gttgagcagtgtggggaact
Ppp1r16b	gatctcgactggatcgatgg
	gctgctcgcagatatccatt
Inpp4b	gacagtcacaacgaagaaggtg
	cagctgttcataccagtcctca
Meis1	ttgtaatggacggtcagcag
	gctacatactcccctggcata
Mecom	cacagaaagtccaaatcacagg
	gccacacgttggaggaac

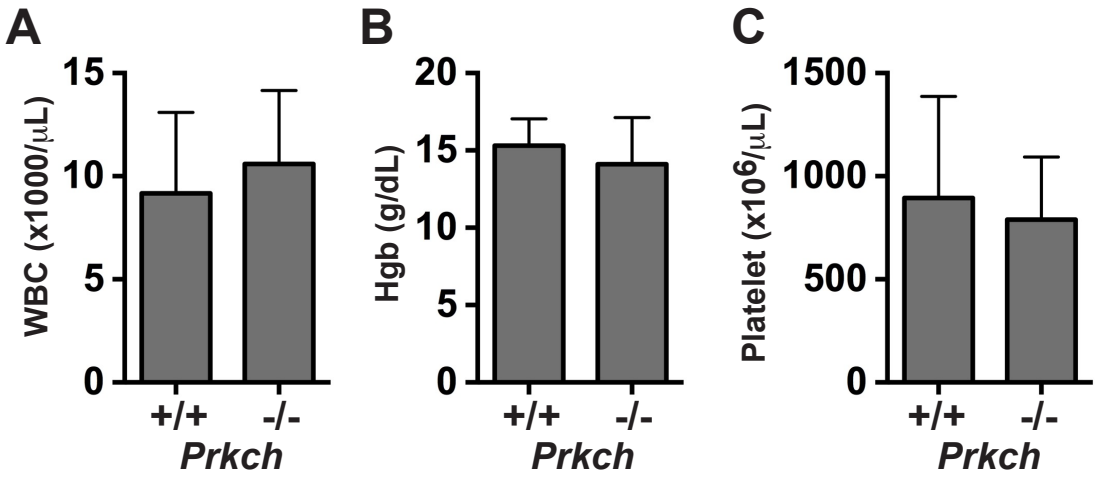
Supplementary Table S4

Specimen	1 million	100,000	10,000	LSC frequency
WT-1	3/5	0/5	0/5	1.3 million
WT-2	5/5	0/5	0/5	430,000
WT-3	2/6			2.5 million
WT-4	4/4			N/D
KO-1	5/5	2/5	1/5	140,000
KO-2	5/5	0/5	0/5	430,000
KO-3	4/5			620,000
KO-4	3/5			1.1 million
KO-5	1/3			2.5 million

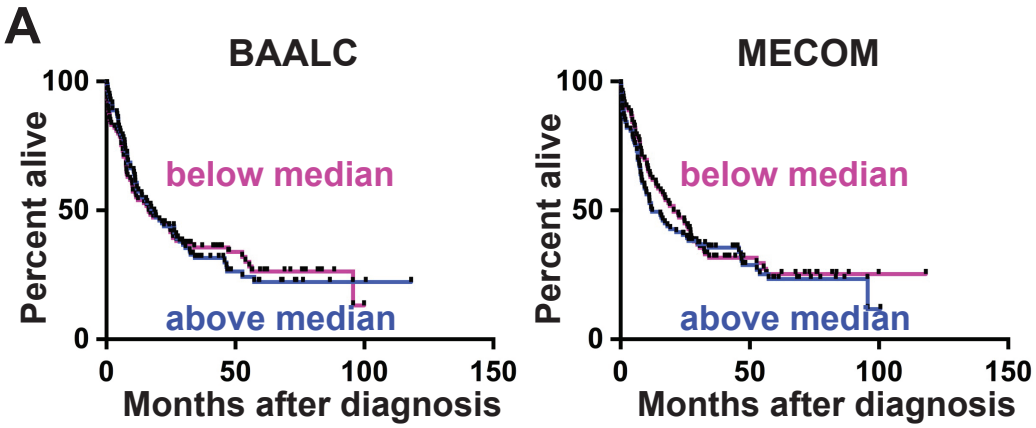
Supplementary Figure S1

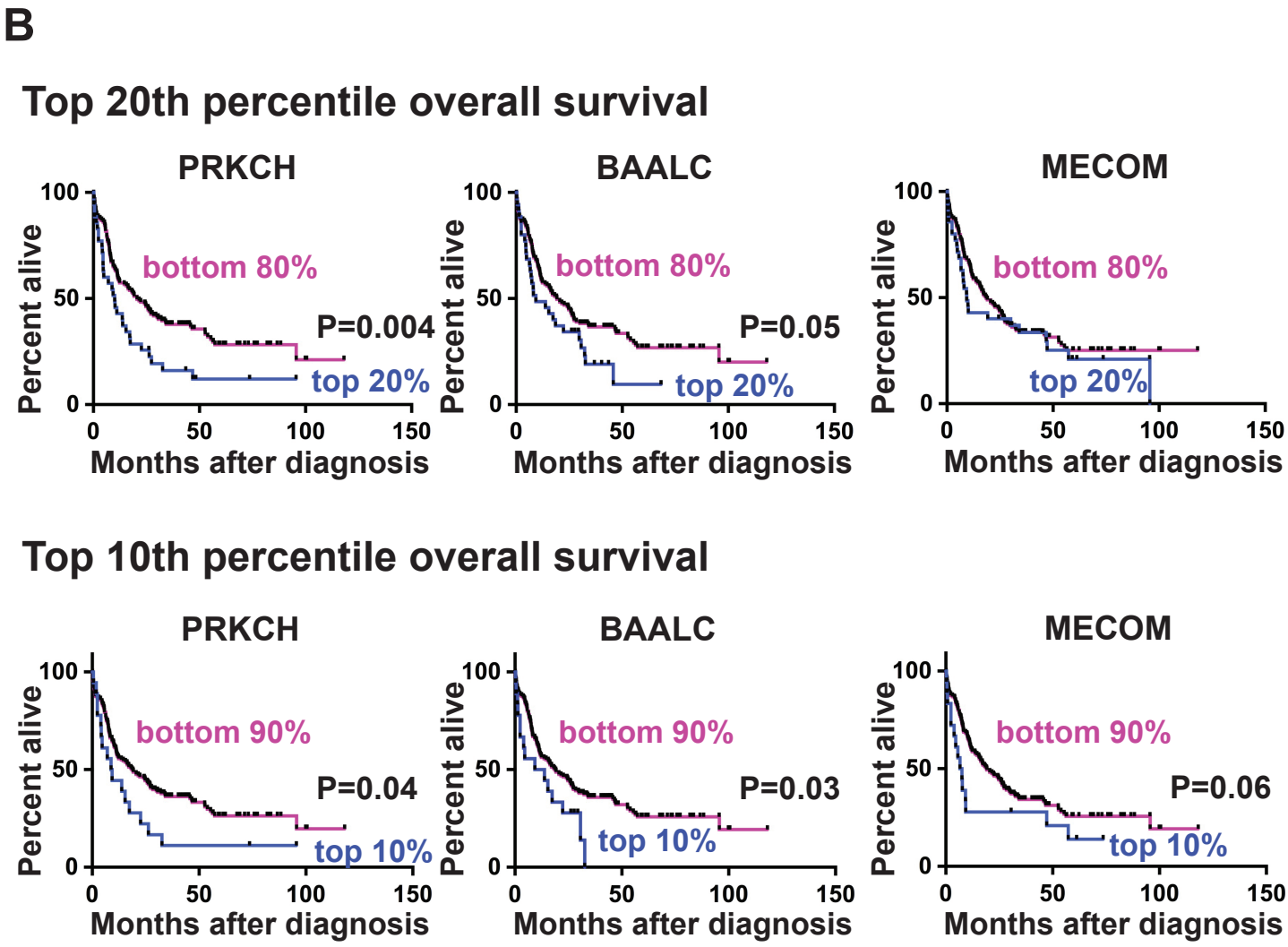


Supplementary Figure S2

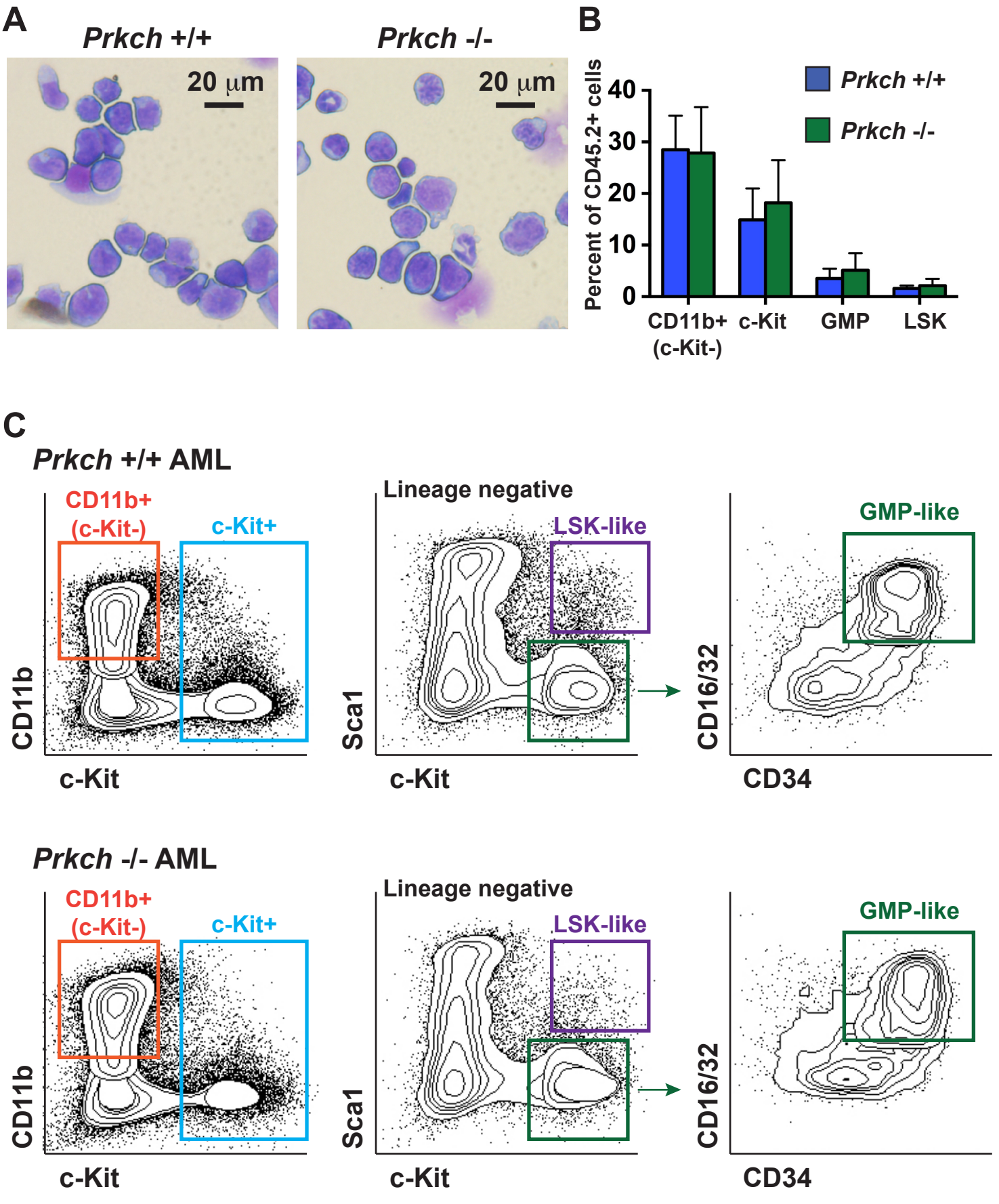


Supplementary Figure S3





Supplementary Figure S4



Supplementary Figure S5