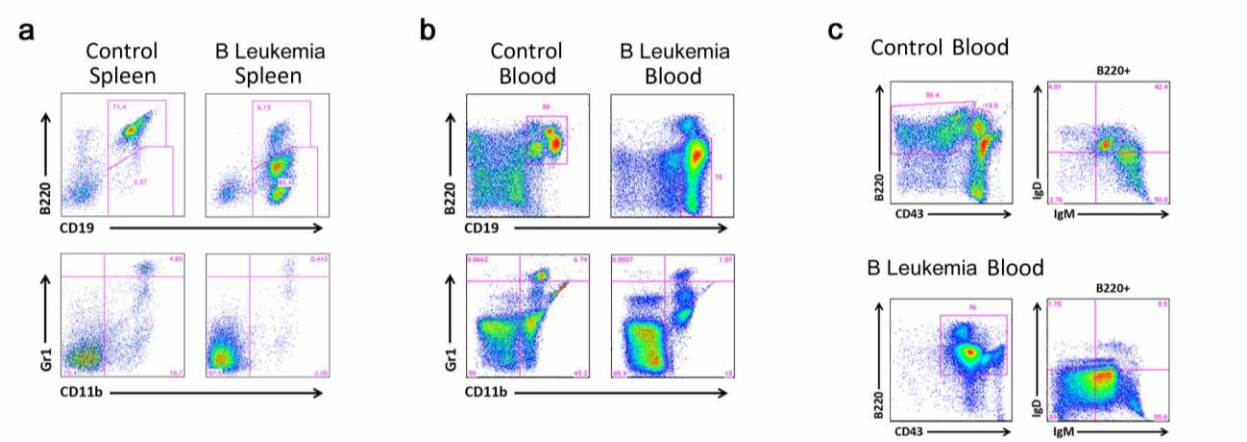
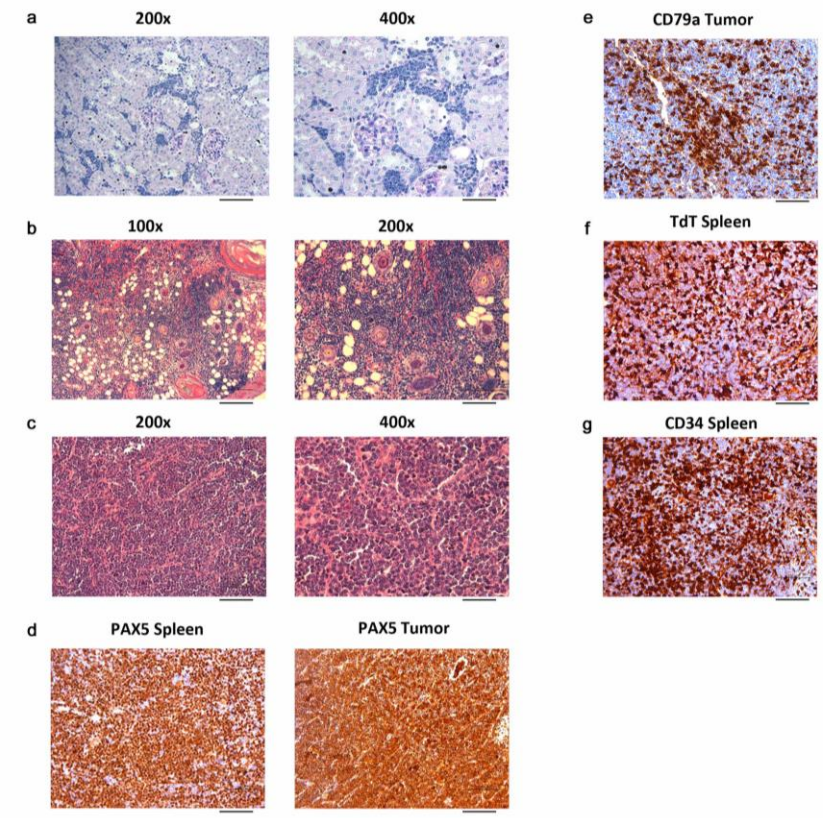
SUPPLEMENTARY INFORMATION



Supplementary Figure 1. Flow cytometric analysis of spleens and peripheral blood confirms expansion of aberrant B lymphoblasts in mutant mice.

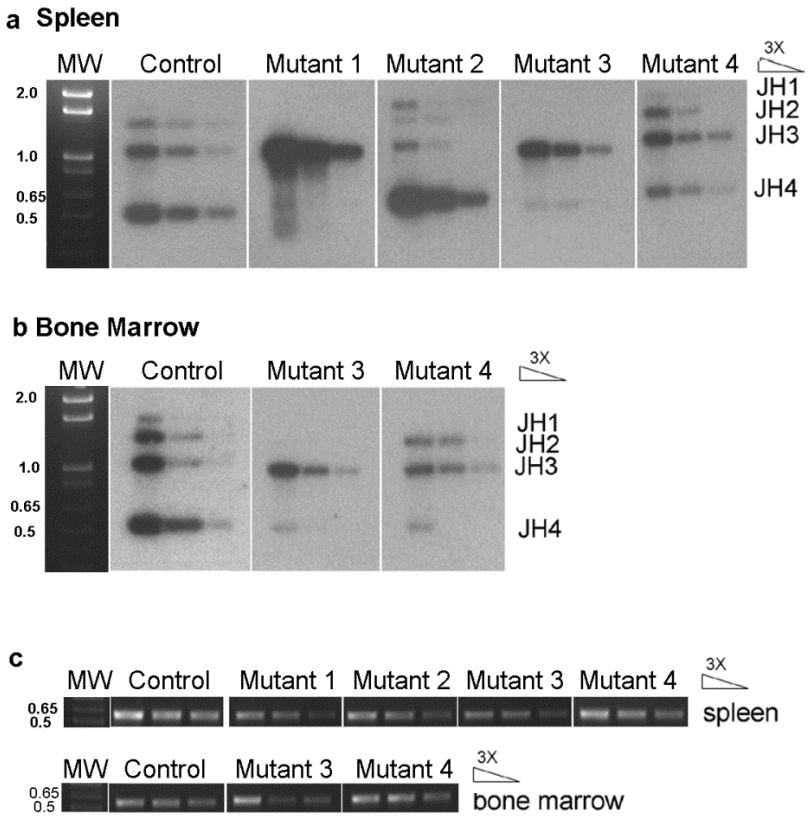
(a) Spleen cells from control animals (left column) consists of 71% phenotypically normal B-cells ($B220^{+}CD19^{+}$) (top), ~5% granulocytes ($Gr1^{+}CD11b^{+}$)(bottom), and ~20% monocytes ($Gr1^{-}CD11b^{+}$). In spleen cells from leukemic mice (right column), there is replacement of normal B-cells (0.13%), granulocytes (0.4%), and monocytes (2%) with an expanded population of lymphoblastss (83%) which have aberrant dim B220 expression ($B220^{Dim}CD19^{+}$). (b) In addition, the peripheral blood of mutant animals consists mostly (78%) of aberrant B-lymphoblasts which have $B220^{Dim}$ expression (right column) and less granulocytes (1%) and immature myeloid cells (13%) than control animals (left column).

(c) Blood from control animals show low CD43 expression (pro-B-cell marker) and almost all B-cells express IgM and/or IgD. However, blood cells from mutant animals retain CD43 expression and have less IgM/IgD expression, demonstrating an arrest in maturation and consistent with the results of bone marrow studies.



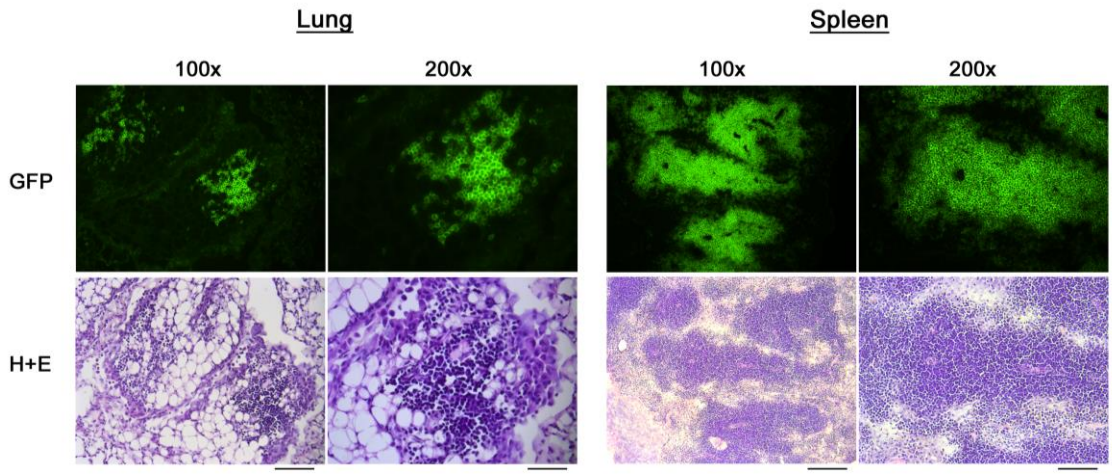
Supplementary Figure 2. Histological characterization of leukemic animals.

(a) Hematoxylin and eosin (H+E) stainings at low (left) and high (right) magnification demonstrating infiltration of lymphoblasts in kidney. Scale bars equal 100 µm for 200x magnification and 50 µm for 400x magnification. (b) H+E staining of skin at low and high power. Scale bars equal 200 µm for 100x magnification and 100 µm for 200x magnification. (c) H+E staining of tumor sections. Scale bars equal 100 µm for 200x magnification and 50 µm for 400x magnification. (d) Pax5 staining of a leukemic spleen and tumor demonstrating intense staining consistent with B-cell lineage. Scale bars equal 100 µm. (e) CD79a staining of tumor sample demonstrating heterogeneous staining, again consistent with a B-cell lineage. Scale bar equals 100 µm. (f) TdT staining of leukemic spleen showing infiltration of cells positive for TdT, indicating a precursor population. Scale bar equals 100 µm. (g) CD34 staining of leukemic spleen showing infiltration of cells positive for CD34, again suggesting a precursor population. Scale bar equals 100 µm.



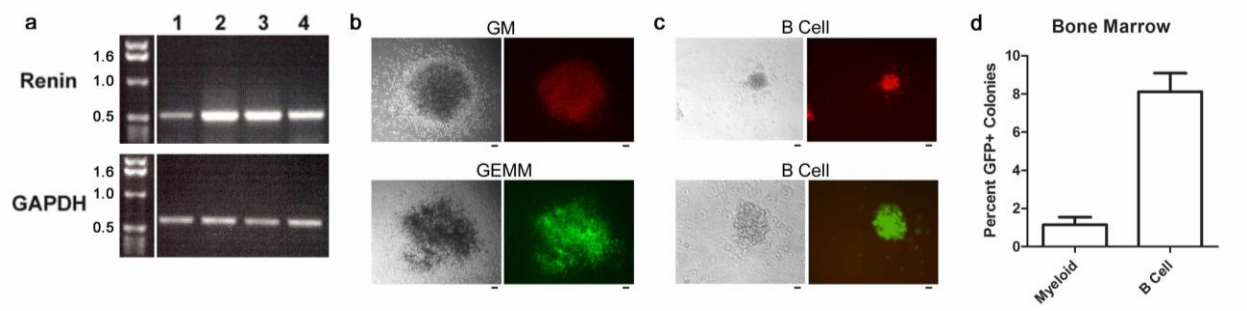
Supplementary Figure 3. V(D)J recombination in control and leukemic mice.

(a-c) Genomic DNA from control and mutant leukemic spleens and bone marrow was isolated and 3-fold dilutions were subjected to PCR analysis. IgH V(D)J recombination was assessed using a reverse primer downstream of JH4 and a forward primer recognizing the VH7183 family. PCR products were blotted and hybridized with the JH4 probe. Rearrangements can occur to JH1, JH2, JH3, or JH4. (a) Spleen samples from all of the mutants exhibit mono or oligo clonal rearrangements. Mutant 1 shows one rearrangement event while Mutant 3 shows two, with JH3 being the predominant recombinant. Mutant 2 has predominantly JH4 and Mutant 4 has reduced JH4. (b) In bone marrow samples, Mutants 3 and 4 have recombination patterns similar to that found in the spleens. (c) DNA input was normalized to DLG5 PCR product. MW: The molecular weight ladder on the ethidium bromide stained gel before transfer with band sizes indicated in kb.



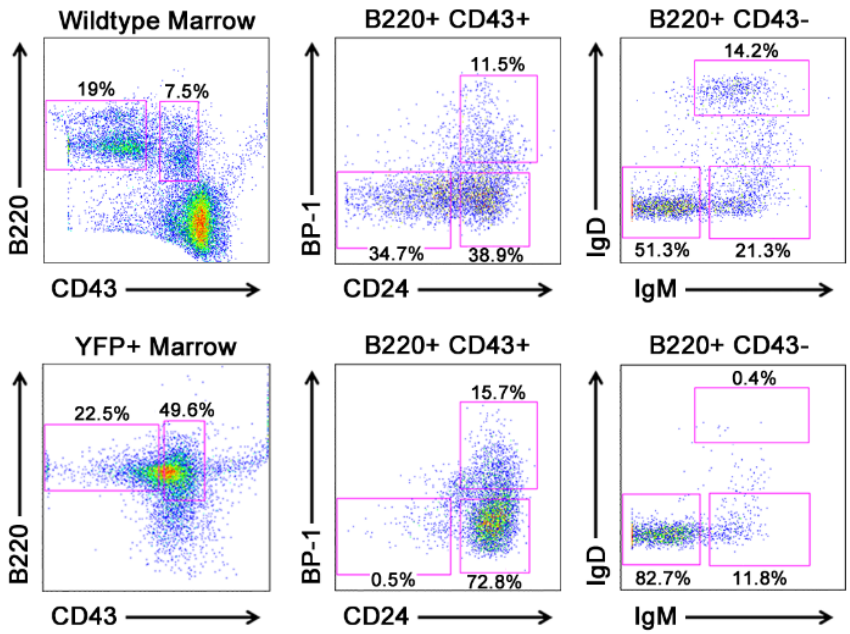
Supplementary Figure 4. Transplanted leukemic cells infiltrate recipient organs.

Transplanted leukemic cells, which express GFP, infiltrate numerous organs of irradiated mice including the lung (left panel) and spleen (right panel). Scale bars equal 200 µm for 100x magnification and 100 µm for 200x magnification.



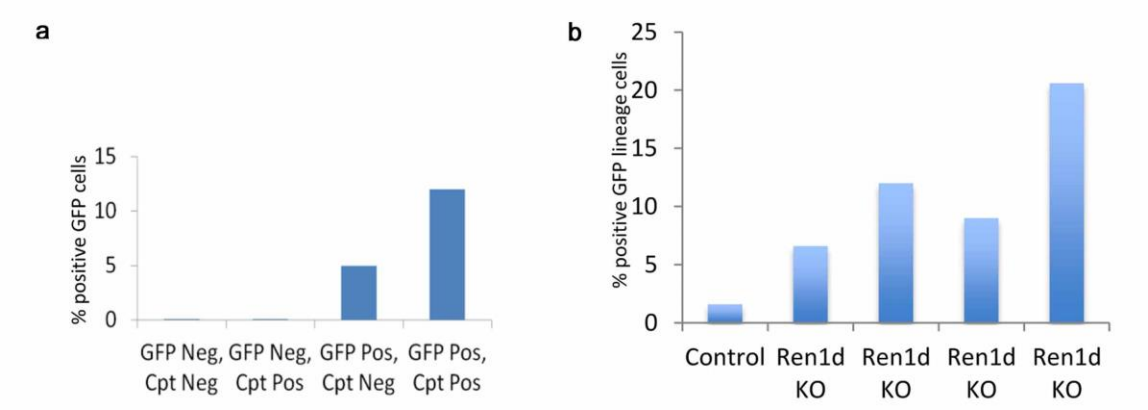
Supplementary Figure 5. Renin is actively expressed in bone marrow precursors.

(a) RT-PCR for renin and GAPDH from bone marrow samples of 4 wildtype mice (1-4) demonstrating the presence of renin mRNA in cells from the bone marrow. (b-d) Methylcellulose colony assays of bone marrow cells from *Ren1*$^{dcre/+}$*;mT/mG* mice. (b) Example of a RFP^{+} (renin-lineage negative) GM colony (top) and a GFP^{+} (renin-lineage positive) GEMM colony grown in conditions permissive to myeloid/erythroid precursors. Scale bars equal 100 µm. (c) Examples of RFP^{+} (top) and GFP^{+} (bottom) colonies grown in conditions permissive to pre-B precursors. Scale bars equal 25 µm (top) and 12.5 µm (bottom). (d) Quantification of the number of colonies derived from renin precursors (GFP^{+}) showed a higher percentage of GFP^{+} colonies in media permissive to B cell growth. Error bars are SEM (n=6 for each group).



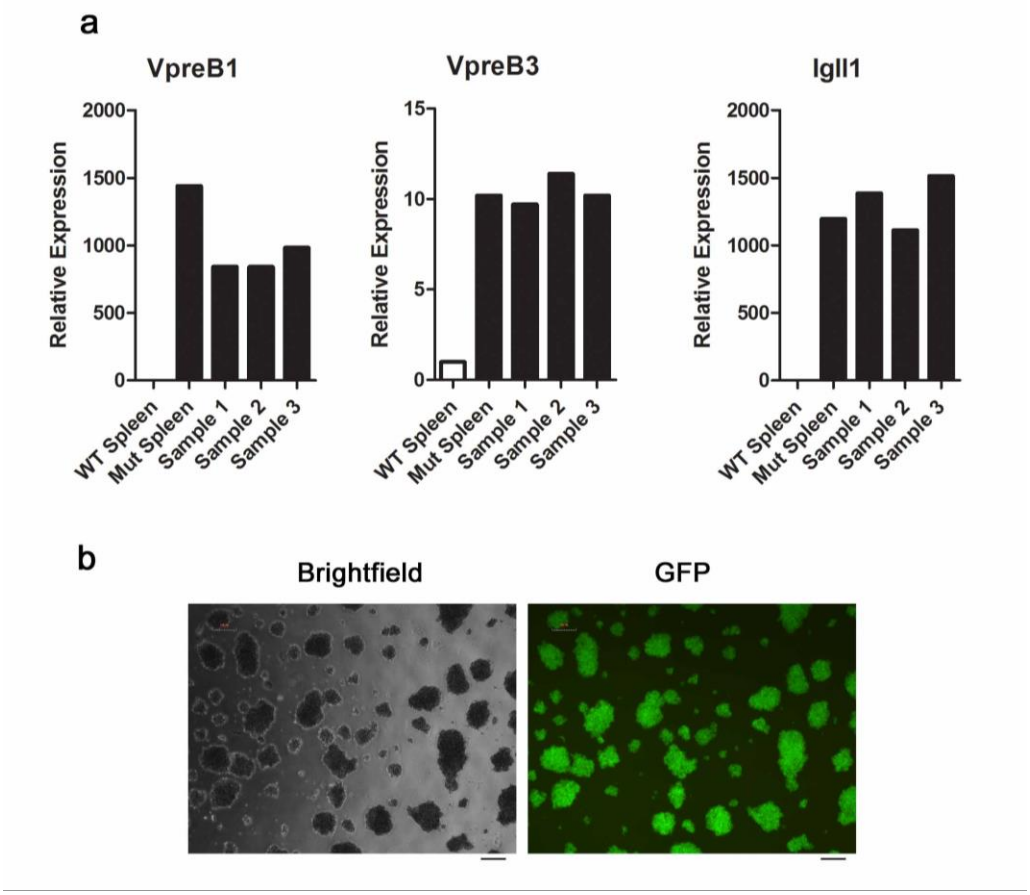
Supplementary Figure 6. Renin expression during B cell development.

Renin-expressing cells from the bone marrow of *Ren1^{c}-YFP* transgenic mice were sorted and stained with fluorescently-labelled antibodies to B220, CD43, BP-1, CD24, IgD, and IgM. Wildtype bone marrow was used as a control. YFP^{+} bone marrow cells demonstrated increased expression of B220^{+}CD43^{+} cells compared to wildtype (left column). B220^{+}CD43^{+} cells were assessed for expression of BP-1 and CD24 (middle column), representing Hardy Fraction A (BP-1^{-}CD24^{-}), B (BP-1^{-}CD24^{+}), and C (BP-1^{+}CD24^{+}). Similarly, B220^{+}CD43^{-} cells were assessed for expression of IgM and IgD (right column), corresponding to Hardy Fraction D (IgM^{-}IgD^{-}), E (IgM^{+}IgD^{-}), and F (IgM^{+}IgD^{+}). Compared to wildtype bone marrow, YFP^{+} bone marrow cells had a higher frequency of Hardy Fraction B subset, suggesting that renin-expressing cells are pro-B cells.



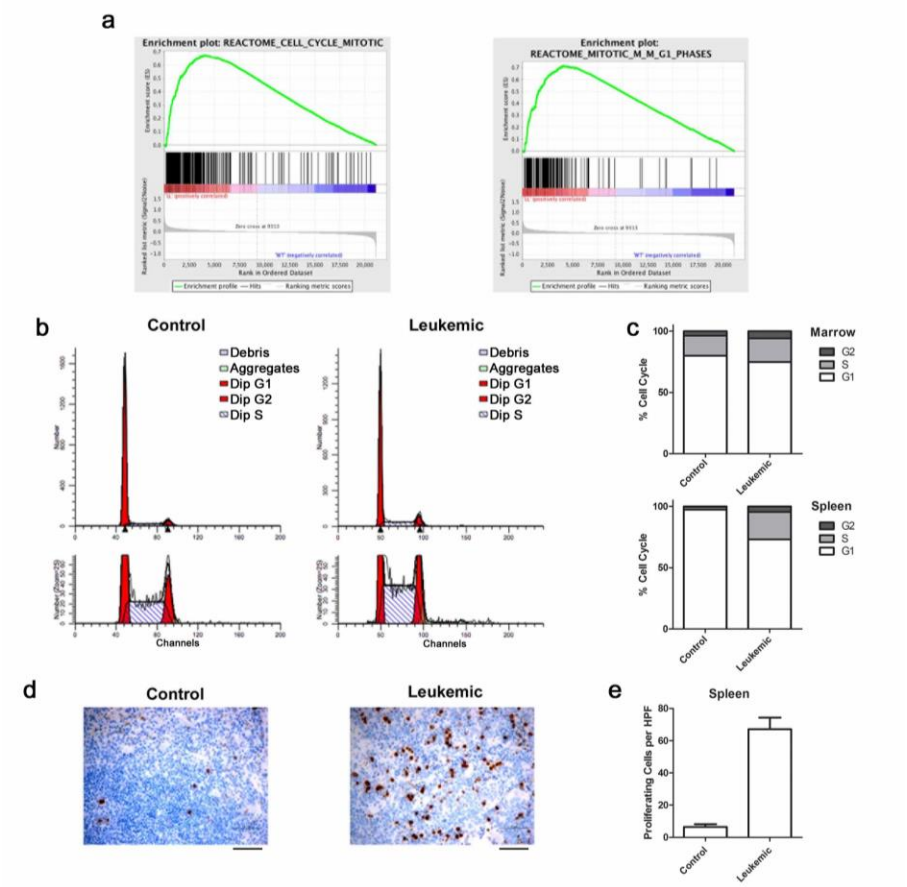
Supplementary Figure 7. Renin progenitors increase in number in response to homeostatic stress.

(a) Quantification of GFP cells from the bone marrow of *Bac-Ren1^{c}-GFP* transgenic mice. Captopril treatment results in increased GFP expression demonstrating that physiological stress results in recruitment of renin-expressing cells in the bone marrow. Bars denote results from individual animals. (b) Similarly, homozygous replacement of the *Ren1* locus with *Cre recombinase* (*Ren1^{d}* KO) results in recruitment of renin expression and thus increased GFP expression in the peripheral blood. Bars indicate the average of measurements done in triplicate from individual animals.



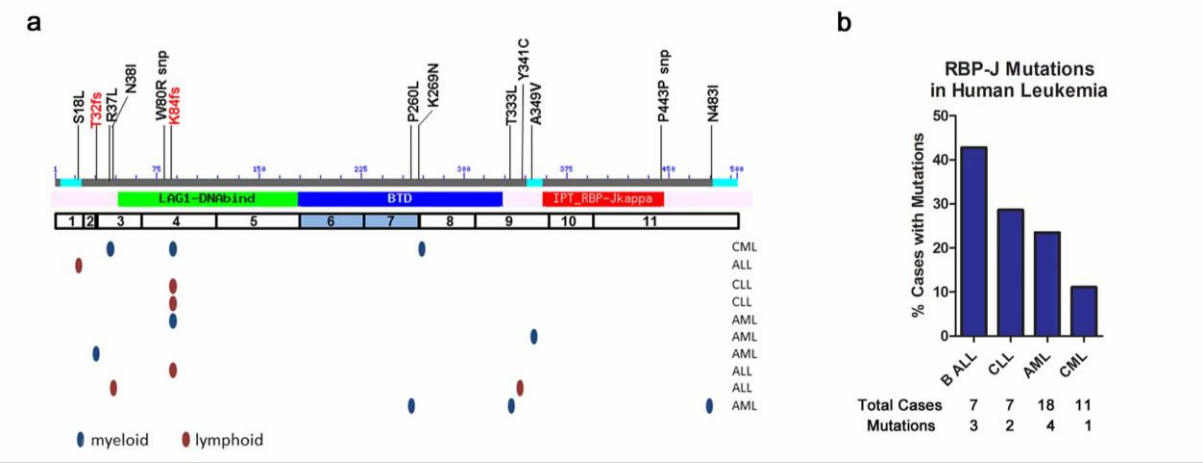
Supplementary Figure 8. Gene expression of leukemic cell lines.

(a) qRT-PCR of differentially expressed genes in RNA from control and mutant spleens compared to 3 leukemic cell samples derived from the bone marrow of the same mutant mouse and grown independently in culture. RNA was isolated from cells at passage 4. Genes important for early B cell development are upregulated in the cell samples, similar to the whole spleen, n=3 for leukemic cells. (b) All cell colonies were GFP^+, confirming that these cells represented a homogeneous population. Scale bars equal 100 µm.



Supplementary Figure 9. Deletion of *RBP-J* in renin-lineage cells leads to enhanced cell cycle progression and increased cell proliferation.

(a) Gene set enrichment analysis was performed on microarray data comparing control and leukemic spleens. The top two differentially expressed gene signatures include “Cell cycle mitotic” and “Mitotic M-M/G1 phases”. (b) Formal cell cycle analysis was performed on bone marrow and spleen from control (left) versus leukemic (right) mice. (c) Leukemic mice had increased percentage of cells in S and G2 phase compared to control mice. (d) Phosphohistone H3 immunohistochemistry was performed to evaluate cell proliferation on spleen samples from control and leukemic mice. Scale bars equal 50 µm. (e) Phosphohistione H3 immunohistochemistry demonstrated increased cellular proliferation in mice with B-cell leukemia versus mice control mice. Error bars represent SEM (n=4 for control animals and n=8 for leukemic animals).



Supplementary Figure 10. *RBP-J* variants occur in human hematopoietic malignancies.

(a) Schematic representation of the RBP-J gene and protein structure with domains and exons indicated. Exons 6 and 7 represent the region that is deleted in our study mice. The location of discovered *RBP-J* variants and resulting amino acid changes are denoted by a small oval (red for B-lymphoid malignancies and blue for myeloid malignancies) at their position on the *RBP-J* gene, and the diagnosis for that patient is listed to the right. Two known single nucleotide polymorphisms are included for reference. The most common variant was a frameshift deletion which occurred at position 188 of the coding region within the LAG1-DNA binding domain (exon 4). (b) The frequency of *RBP-J* variants within distinct hematologic malignancies. 44 leukemia samples were analyzed including 7 B-ALL, 7 CLL, 18 AML, 11 CML, and 1 mixed leukemia (which did not have an *RBP-J* variant and is not included in this graph).

Supplementary Table 1. Stage of B cell development of Renin-lineage and Renin-expressing cells

	Common Lymphocyte Progenitor	Pro B Cell	Premature B Cell	Immature B Cell	Mature B Cell
Renin Expressing	3.01% ± 1.76	72.9% ± 8.4	16.9% ± 6	8.65% ± 1.85	0.92% ± 0.03
Renin Lineage	.014% ± 0.01	4.51% ± 0.44	37.15% ± 4.35	25.5% ± 1.2	28.75% ± 5.15

Data is presented as mean ± SEM, n=2 for all conditions.

Supplementary Table 2. Gene signatures enriched or repressed in Leukemic versus Control mice

Control versus Leukemic	Gene Signatures Enriched in Leukemic	Gene Signature Repressed in Leukemic
1	Cell cycle mitotic	Immunoregulatory interactions between a lymphoid and a non-lymphoid cell
2	Mitotic M – M/G Phases	Chemokine receptors and chemokines
3	Processing of capped intron containing pre-mRNA	Cytokine-cytokine receptor interaction
4	Spliceosome	Hematopoietic cell lineage
5	Cell cycle checkpoints	Signaling in immune system
6	DNA replication pre initiation	Natural killer cell mediated cytotoxicity
7	G1 – S transition	Cell adhesion molecules
8	Formation and maturation of mRNA transcript	Co-stimulation by the CD28 family
9	Mitotic prometaphase	Class A1 rhodopsin like receptors
10	S phase	T cell receptor signaling pathway

Supplementary Table 3. Patient Characteristics

Patient Number	Diagnosis	Age	Sex	Mutation Type	Other Cytogenetics
4	CML	47	M	Missense Frameshift Missense	t(9;22)
5	CMML	77	M		Trisomy 8
8	CLL	44	M		
16	CML	50	F		t(9;22)
20	CML	37	F		Abnormal
22	AML	86	M		Trisomy 8
23	Mixed Leukemia	65	M		t(9;22)
27	ALL	27	F	Missense	t(9;22)
30	CML	61	M		t(2;13)
33	APML	65	M		Trisomy 8 t(15;17)
36	ALL	44	F		Abnormal
37	AML	81	F		Abnormal
39	AML	46	F		inv(16)
40	AML	37	F		Trisomy 8
41	CML	81	M		t(9;22)
45	CML	73	M		Abnormal
48	CLL	59	M		del p53
50	CLL	62	M		Abnormal
55	CML	75	F		t(9;22)
56	AML	47	M		Normal
59	CML	64	F		t(9;22;14)
63	AML	63	M		Trisomy 13 Trisomy 15
69	CLL	60	M	Frameshift	del MYB(6q23)
73	CMML	62	M		Normal
74	CLL	51	M	Frameshift	del 13q14
75	AML	75	M		Normal
82	AML	55	M	Frameshift	t(9;22), +8, +10
83	AML	58	M		del(5) -20
86	AML	60	F	Missense	+8
87	CML	62	F		t(9;22)
90	MDS	21	M		del(20)
91	CLL	53	M		-13
96	ALL	55	F		t(9;22)
99	AML	75	F		Loss of RUNX1
101	AML	53	M		Normal
102	ALL	<21	F		Abnormal
105	AML	<21	M	Frameshift	Abnormal
106	ALL	26	M	Frameshift	Abnormal
113	AML	59	M		Normal
115	AML	42	M		Abnormal
118	ALL	51	M	Missense Missense	Normal
124	AML	50	M	Missense Missense Missense	Normal
130	ALL	<21	M		Abnormal
132	CLL	64	F		Abnormal
133	AML	<21	F		Abnormal

Supplementary Table 4. Variants occurring in patients with hematopoietic malignancies

Patient Number	Diagnosis	Age	Sex	Mutation Type	Mutation Position	Frequency of Detection	Damage Prediction	Other Cytogenetics
4	CML	47	M	Missense Frameshift Missense	26407808 26417145 26430362	14.4 13.5 9.2	5/5 Truncated 5/5	t(9;22)
27	ALL	27	F	Missense	26322431	9.8	0/5	t(9;22)
69	CLL	60	M	Frameshift	26417145	12.9	Truncated	del MYB(6q23)
74	CLL	51	M	Frameshift	26417145	12.3	Truncated	del 13q14
82	AML	55	M	Frameshift	26417145	14.6	Truncated	t(9;22) +8,+10
86	AML	60	F	Missense	26431638	51.4	2/5	+8
105	AML	8	M	Frameshift	2638008	9.9	Truncated	Abnormal
106	Pre B ALL	26	M	Frameshift	26417145	12	Truncated	Abnormal
118	B Cell Leukemia / Lymphoma	51	M	Missense Missense	26407811 26431614	11.6 13.6	3/5 2/5	Normal
124	AML	50	M	Missense Missense Missense	26426358 26431589 26432574	20 28.6 33.3	5/5 4/5 3/5	Normal

Supplementary Table 5. Antibodies used in Flow Cytometry Analysis

Antibody		Fluorochrome	Concentration
B220	Pan B cell marker expressed from pro-B cells through mature B cells	Alexa Fluor 488	2 ug per 10^6 cells
B220	Pan B cell marker expressed from pro-B cells through mature B cells	APC/Cy7	1 µg per 10^6 cells
BP-1	Expressed on B cell progenitors	Alexa Fluor 647	2 ug per 10^6 cells
CD11b	Expressed on granulocytes, monocytes, and macrophages	PerCP/Cy5.5	0.25 µg per 10^6 cells
CD19	Pan B cell marker expressed from pro-B cells through mature B cells	Alexa Fluor 647	0.25 µg per 10^6 cells
CD21	Expressed on B cells, forming part of the B-cell antigen receptor complex	APC	0.25 µg per 10^6 cells
CD23	Expressed on mature B cells including follicular B cells	PE/Cy7	0.05 µg per 10^6 cells
CD24	Expressed on B cells	Brilliant Violet 421	0.5 µl per 10^6 cells
CD3	Expressed on T cells and NK-T cells	PE	0.25 µg per 10^6 cells
CD34	Expressed on hematopoietic progenitors	PE	0.25 µg per 10^6 cells
CD43	Expressed on Pro-B cells	APC	0.25 µg per 10^6 cells
CD43	Expressed on Pro-B cells	PerCP/Cy5.5	0.2 µg per 10^6 cells
CD45	Leukocyte common antigen, expressed on all hematopoietic cells except platelets and red cells	APC/Cy7	0.25 µg per 10^6 cells
CD5	Expressed on a subset of B cells, called B-1 cells and T cells	Alexa Fluor 488	0.25 µg per 10^6 cells
Gr1	Expressed on mature granulocytes	PE/Cy7	0.05 µg per 10^6 cells
IgM	Expressed on immature and mature B cells	Brilliant Violet 421	5 µl per 10^6 cells
IgD	Expressed on mature B cells	PerCP/Cy5.5	0.25 µg per 10^6 cells
IgD	Expressed on mature B cells	APC	
IL-7Rα	Expressed on common lymphocyte progenitors	Alexa Fluor 647	1 µg per 10^6 cells
Lineage Cocktail	Includes antibodies reacting to cells from the major hematopoietic cell lineages including B cells, T cells, granulocytes, macrophages, NK cells, and erythrocytes	Brilliant Violet 421	20 µl per 10^6 cells

Supplementary Table 6. Primers and conditions for qRT-PCR

Gene	Primer sequences
Igll1	forward 5’ CCAAGGGAAGAAGATGCTGA
	reverse 5’ ACGTCTGTCCTGCTCATGC
Enpep	forward 5’ TTCATGGTCAGTGGCAGCTA
	reverse 5’ GTTTAAAGGCTGGCTGAACG
Rag1	forward 5’ CTGAAGCTCAGGGTAGACGG
	reverse 5’ CAACCAAGCTGCAGACATTC
VpreB1	forward 5’ TGCTCAGGGTACAGGAGAGG
	reverse 5’ GCTGCTGGCCTATCTCACAG
VpreB3	forward 5’ AGCTGAGATGAGCGTCTTGG
	reverse 5’ CCTGCCTCTGCTCCTGATAG
Myb	forward 5’ GTCCTCTGTCTTCCCACAGG
	reverse 5’ TGTCCTCAAAGCCTTTACCG
Ebf1	forward 5’ AGGCAATTTTGGTTGCATTT
	reverse 5’ TGACAAGAAAAGCTGTGGCA
E2A	forward 5’ AAGGAGGAGCTGCTCTGGTC
	reverse 5’ CTTCAGCATGATGTTCCCG
CD7	forward 5’ GTTGACAGAATCCCCCTCAG
	reverse 5’ TGGCTTTGCTGCTTACACTG
Ccr9	forward 5’ CATGCCAGGAATAAGGCTTG
	reverse 5’ ACTCACCTCCGCCGTACAT
GAPDH	forward 5’ TTGATGGCAACAATCTCCAC
	reverse 5’ CGTCCCGTAGACAAAATGGT

PCR conditions were: 95°C, 58°C, 72°C, 40 cycles, except for Igll1 and Ccr9 which required annealing at 59°C and 60°C respectively.