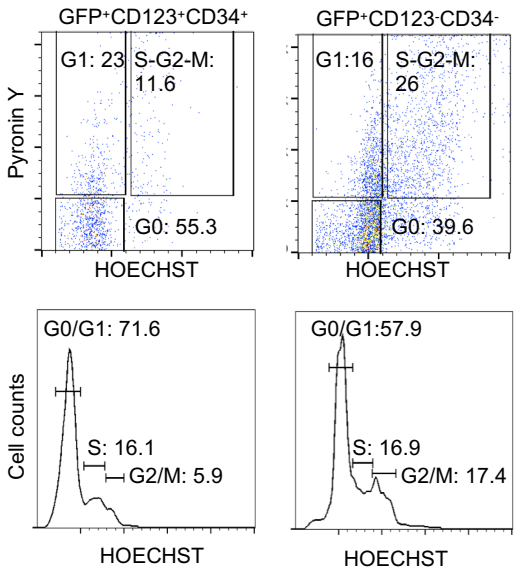
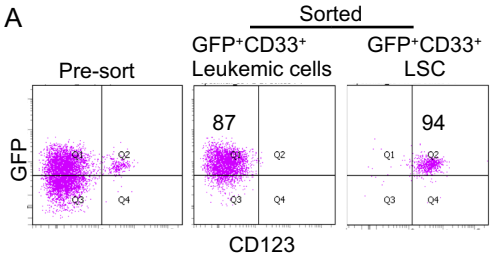


Supplementary Fig. S1. Cell cycle analysis.
GFP+CD123+CD34+ and GFP+CD123+CD34- cells were purified from the bone marrow of four NPM1c+ mice by cell sorting and stained with Pyronin Y and HOECHST and processed for flow cytometry. Shown are Pyronin Y vs HOECHST staining profiles (top) and HOECHST histograms (bottom) of the sorted cell populations. Gated areas are labeled with cell cycle stage and their percentages. Shown are representative results from one of two experiments.

A



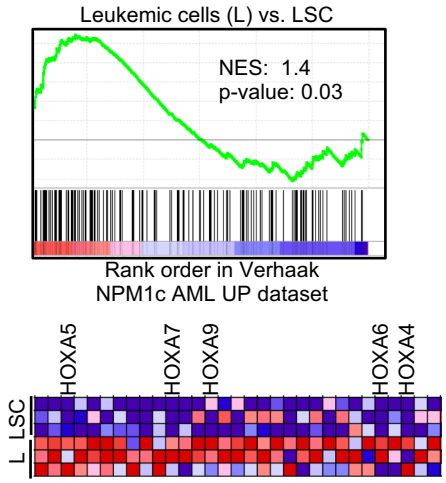
B

Sample ID	Total reads	% Mapped	% rRNA
NPM1c-bulk-m1	36,251,182	84.92%	0.55%
NPM1c-LSC-m1	65,194,666	83.73%	1.02%
NPM1c-bulk-m2	39,283,718	85.34%	1.14%
NPM1c-LSC-m2	41,450,150	91.54%	1.37%
NPM1c-bulk-m3	45,120,642	92.18%	0.27%
NPM1c-LSC-m3	41,466,764	91.28%	0.72%

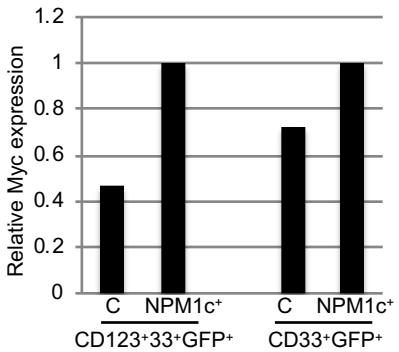
C

Gene ID	Verhaak et al	Alcalay et al	Fold upregulation in NPM1c bulk leukemic cells
EMR1	Y	Y	2.2
HOXA9	Y	Y	2.8
HIST1H2BC	Y	Y	2.99
LTBP1	Y	Y	3.5
PLS1	Y	Y	1.69
PTX3	Y	Y	3.95
TLR4	Y	Y	1.55
NGFRAP1	Y	Y	3.35
CDC42BPB	N	Y	1.75
DSC2	N	Y	2.96
GRB10	N	Y	2.34
ICA1	N	Y	3.38
ITGA5	N	Y	2.57
LRIG1	N	Y	2.56
WDFY3	N	Y	2.02
WT1	N	Y	2.04
MARCKS	Y	N	1.61
PILRA	Y	N	2.1
PRKAR2B	Y	N	2.06
RHAG	Y	N	3.62
RNASE2	Y	N	2.79
SERPINA1	Y	N	2.8
SERPINB2	Y	N	1.59
THBS1	Y	N	1.7
CYP1B1	Y	N	2.8
ACSL1	Y	N	1.94
AIM2	Y	N	3.4
CD163	Y	N	3.2
FCGR2A	Y	N	1.86
FCN1	Y	N	1.5
HIST1H1C	Y	N	1.88
HIST1H3D	Y	N	2.7
KCNJ2	Y	N	2.4
LGALS3BP	Y	N	2.89
% Match to Verhaak:		31%	65%
% Match to Alcalay:	50%		81%

D



E



Supplementary Fig. S2. Transcriptional analysis.

A) Staining profiles of CD123 vs. GFP gating on live CD33+ cells before and after sorting. Numbers indicate percentages of cells in the gated areas. B) Summary of RNA sequencing data. C) A list of genes that are up-regulated in two published datasets (Alcalay et al. and Verhaak et al.) and in the bulk leukemic cells from NPM1c+ mice. "Y" indicates transcript that is upregulated 2-fold or more between LSCs and bulk leukemic cells in the two published datasets. "N" indicates transcript that is not upregulated 2-fold or more between LSCs and bulk leukemic cells in the two published datasets. The last column list fold upregulation of listed genes in NPM1c+ bulk leukemic cells. The percentages of matched genes between two datasets are indicated. Genes with up-regulation of 2-fold or more in *de novo* NPM1c+ leukemic cells are used in calculation of percentages of match. D) GSEA analysis of genes expressed in leukemic cells (L) vs. LSCs compared to genes upregulated in patient NPM1c+ AML from Verhaak et al. Heat-map shows a subset of genes that are up-regulated in the dataset and in leukemic cells from NPM1c+ mice. Red, up-regulation; blue, downregulation; NES, normalized enrichment score. E) Relative levels of Myc in the indicated cell populations in the control and NPM1c+ mice as assayed by qRT-PCR.