JEM

SUPPLEMENTAL MATERIAL

Han et al., http://www.jem.org/cgi/content/full/jem.20090831/DC1

a

	hsa-let-7f	*hsa-miR-19b*	*hsa-miR-28*	*hsa-miR-30d*	*snoR-02*	*snoR-13*
hsa-let-7f	1					
hsa-miR-19b	0.91	1				
hsa-miR-28	0.93	0.91	1			
hsa-miR-30d	0.90	0.95	0.91	1		
snoR-02	0.92	0.92	0.91	0.93	1	
snoR-13	0.79	0.76	0.83	0.74	0.84	1

b

	hsa-let-7f	hsa-miR-19b	hsa-miR-28	hsa-miR-30d	snoR-02	snoR-13
Median Ct	24.0	16.2	24.7	21.0	19.0	11.3
stdev_all	6.4	3.0	5.5	3.7	3.0	2.2
stdev_AML	1.4	1.5	1.7	1.8	1.3	0.7
stdev_normal	5.7	2.7	5.1	2.6	2.3	2.2

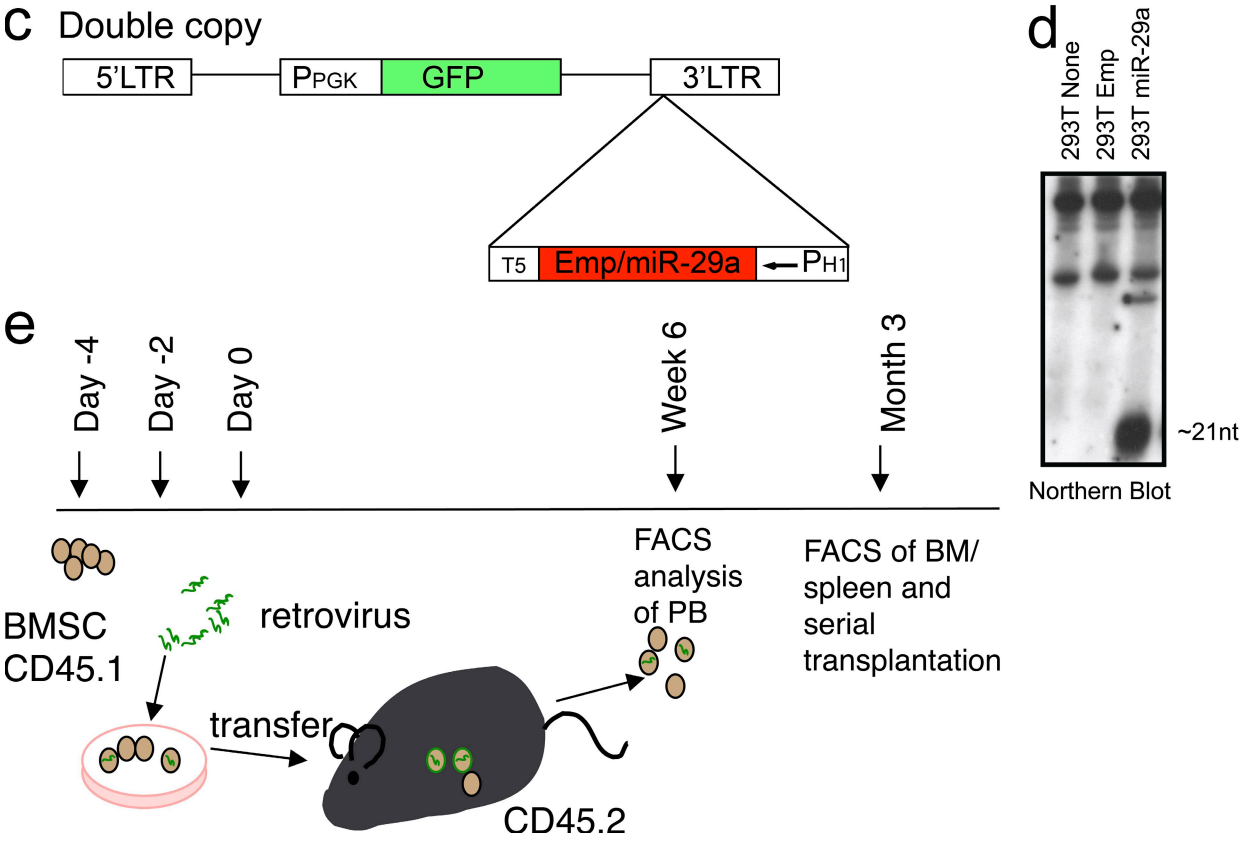


Figure S1. Normalization controls and experimental schematic. (a) Choice of snoR-02 as an endogenous control for normalization of miRNA expression levels. Summary of correlation coefficients from TaqMan-based RT-PCR analysis of six candidate small RNA endogenous controls in normal human HSC/progenitors and AML LSC and non-LSC blasts. (b) Summary of candidate small RNA expression variation among normal human HSC/progenitors and AML LSC and non-LSC blasts. (c) Schematic representation of the MSCV-based miR-29a expression construct used in expression studies and for generation of miR-29a chimeras. (d) Northern blot analysis of 293T cells transduced with the miR-29a retrovirus demonstrates miR-29a that is expressed and properly processed to its mature 21-nt length. (e) Time course of primary chimera generation and evaluation. After treatment of WT (CD45.1) mice with 5-FU, the BM was harvested and transduced with the miR-29a–expressing retrovirus. After transduction, bulk BM cells were transplanted into lethally irradiated congenic (CD45.2) recipients. Chimerism was evaluated beginning at 6–8 wk and monitored monthly.

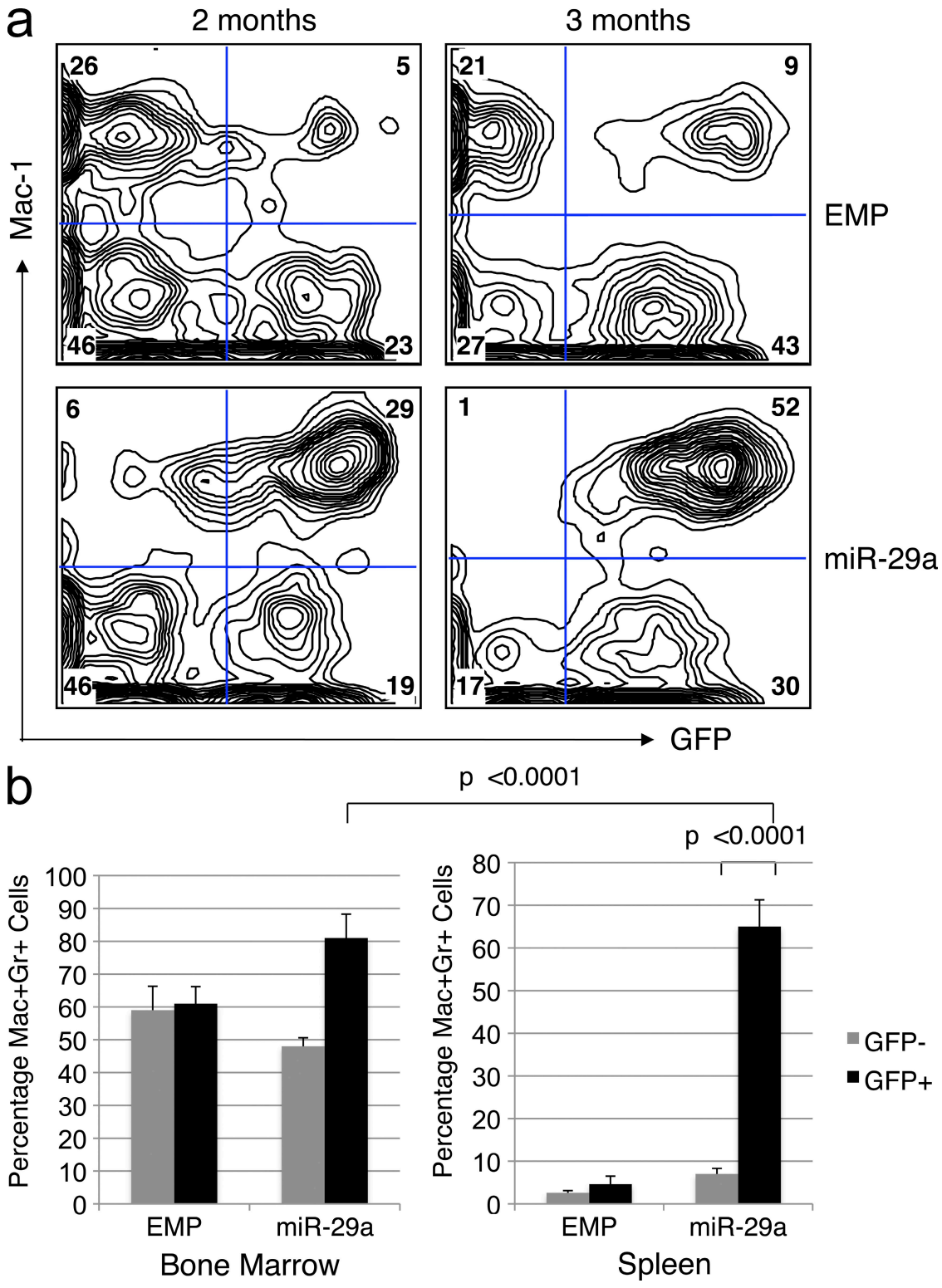


Figure S2. Progressive myeloid expansion in the peripheral blood of engrafted primary miR-29a chimeric mice. (a) Flow cytometric analysis was performed on peripheral blood leukocytes using Mac-1$^+$ at the indicated times. (b) Primary EMP and miR-29a chimeras exhibit statistically significant differences in myeloid chimerism in the BM and spleen. Percentages represent the percentage of granulocytes among donor-derived leukocytes in the indicated organ. Statistical analysis was performed using a two-tailed Student's *t* test. Error bars represent SD from at least three independent experiments.

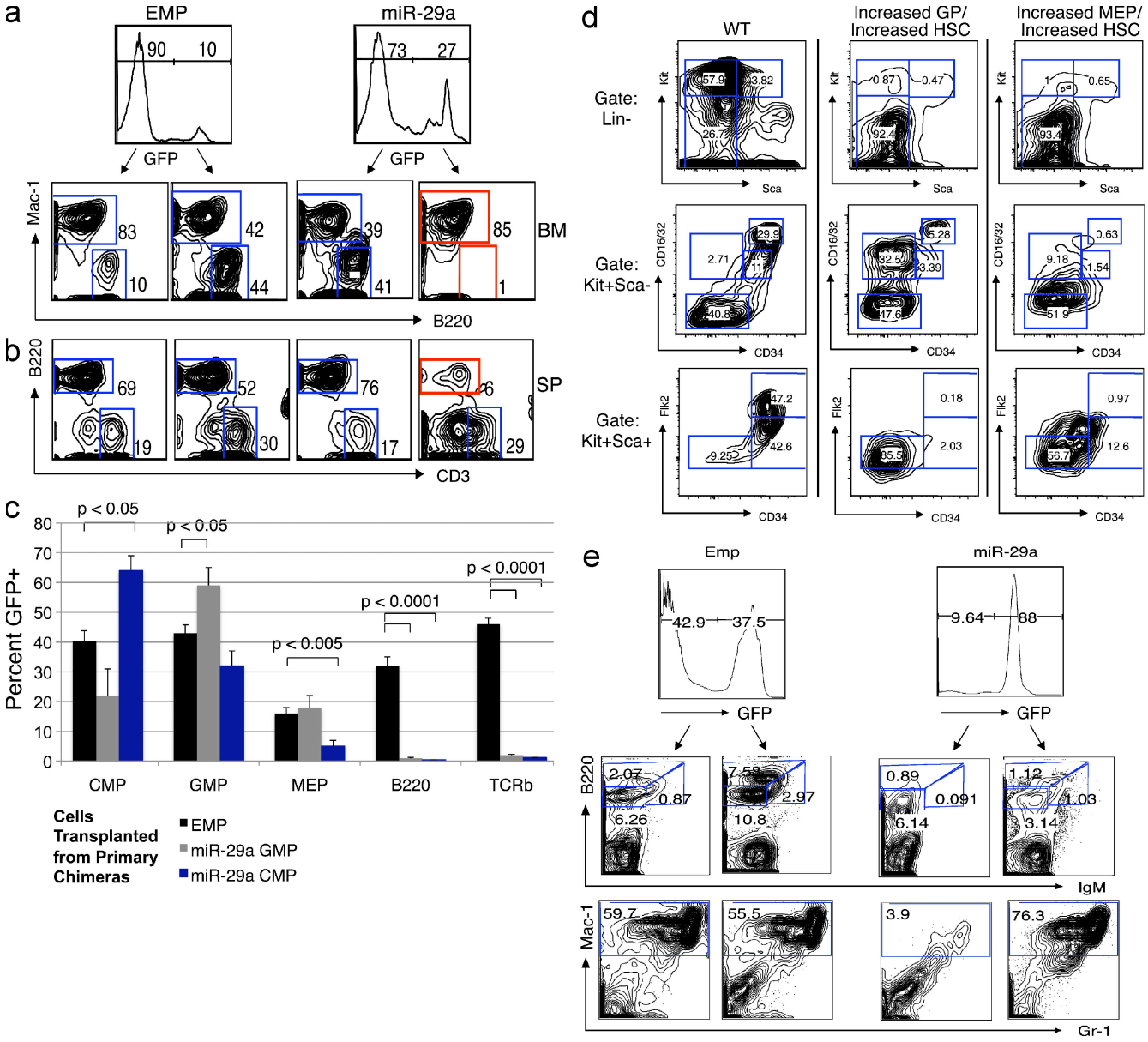


Figure S3. Expansion of myeloid cells and decrease in mature B cells in primary chimeric mice is seen 2–3 mo after transplant. (a) Representative BM of miR-29a recipient mice exhibits a marked increase in GFP$^+$ Mac-1$^+$ myeloid cells and near absence of GFP$^+$B220$^+$ B cells. (b) Representative spleen of secondary recipient mice shows similar features to the BM, with near-absence of B220$^+$ B cells. (c) Secondary transplant of committed progenitors purified from primary miR-29a chimeras reveals self-renewing myeloid progenitors. BM cell chimerism was determined >16 wk after transplant of FACS-purified CMP and GMP from control (EMP) and miR-29a primary chimeras. The percentage of cell composition was determined with flow cytometry by calculating the percentage of GFP$^+$ cells within the indicated cell population. Statistical analyses were performed using a two-tailed Student's *t* test. Error bars represent SD from at least three independent experiments. (d) Altered myeloid progenitor profiles in miR-29a induced myeloproliferative disease. Some primary chimeric mice exhibited expansion of a novel myeloid progenitor provisionally referred to as the granulocyte progenitor (GP; Lin$^-$Kit$^+$Sca$^-$CD16/32$^+$CD34$^-$), whereas others showed a relative expansion of immunophenotypic MEP. In both cases, notice the expansion of Lin$^-$Kit$^-$ cells, which is consistent with left-shifted myeloid maturation. (e) miR-29a expression promotes myeloid differentiation while inducing decreased commitment to the B cell lineage. Flow cytometric evaluation of the BM of primary chimeric mice demonstrates reduced numbers of progenitor B cells (total B220$^+$IgM$^-$ cells), although the ratios of maturing B cell progenitor subsets is relatively unaltered.

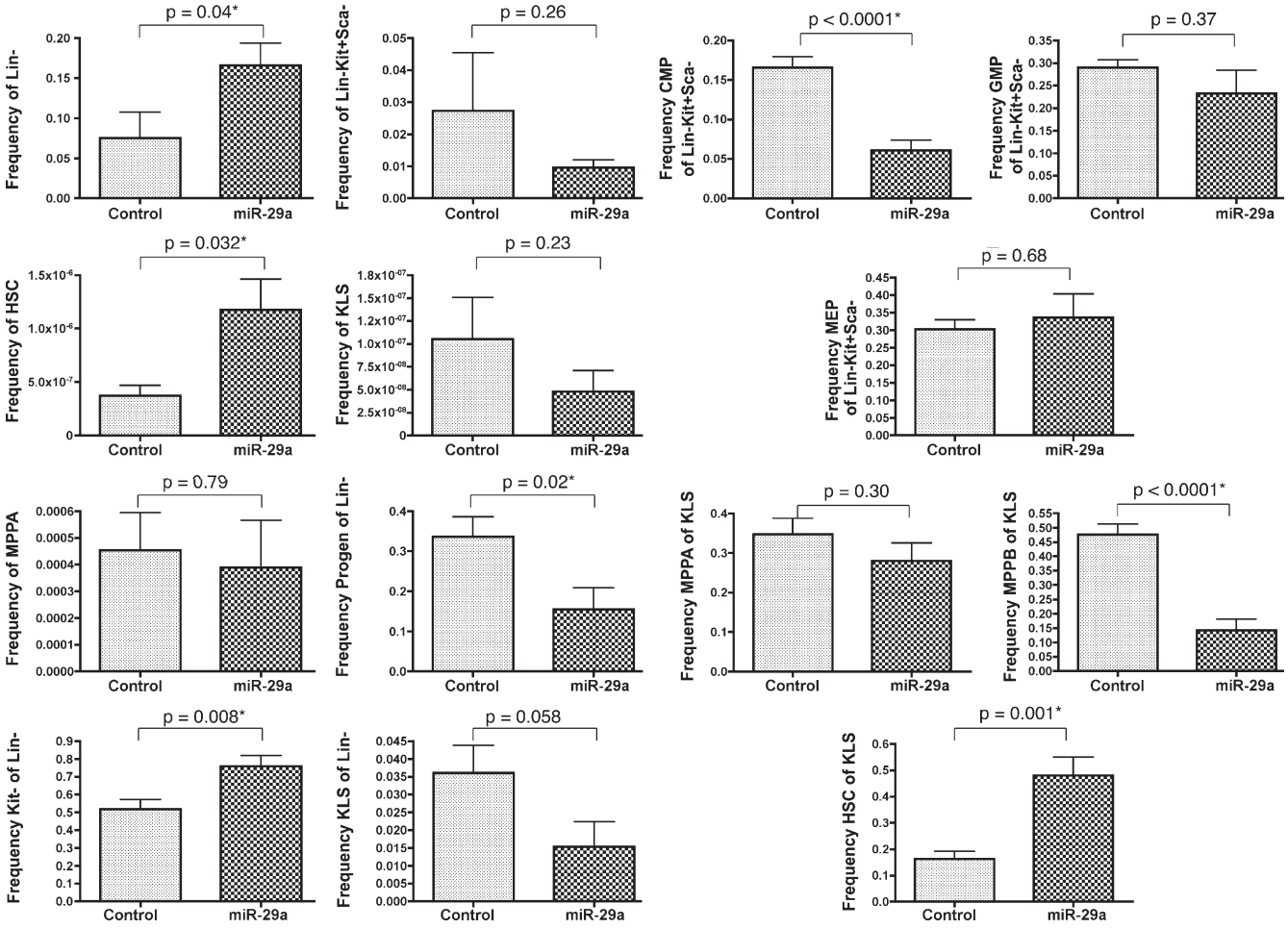


Figure S4. Summary of HSC/progenitor cell composition in miR-29a chimeric mice exhibiting myeloproliferative disease. The control group included WT (untransduced) and EMP retrovirus-transduced mice. miR-29a mice included those exhibiting signs of myeloproliferative disease, which is defined as splenomegaly with expanded immature myeloid compartment, and no increase in blasts, as assessed by morphology. Data were analyzed by a two-tailed Student's *t* test, and statistically significant differences are denoted with an asterisk. Error bars represent SD from at least five independent experiments.

a

Primary Chimera	A	B1	B2
# Secondary Recipients	SP → 3 mo 3 mice	BM → 2 mo 5 mice	BM → 2 mo 5 mice
Outcomes	24 wks: 2 exp after sick 26 wks: 1 died	20 wks: 3 exp after sick 24 wks: 2 died	25 wks: 2 exp after sick 28 wks: 3 died

Primary Chimera	C1	C2	D1	D2	D3
# Secondary Recipients	BM → 3 mo. 5 mice	BM → 3 mo. 5 mice	BM → 2 mo 5 mice	BM → 2 mo. 5 mice	BM → 2 mo. 5 mice
Outcomes	18 wks: 2 exp prior sick 20 wks: exp after sick 28 wks: 1 died	8 wks: 2 exp prior sick 24 wks: 3 exp after sick	12 wks: 2 exp prior sick 24 wks: 3 exp after sick	12 wks: 2 exp prior sick 20 wks: 2 exp after sick 28 week: 1 died	22 wks: 2 exp after sick 15 wks: 1 died 19 wks: 1 died 30 wks: 1 died

b

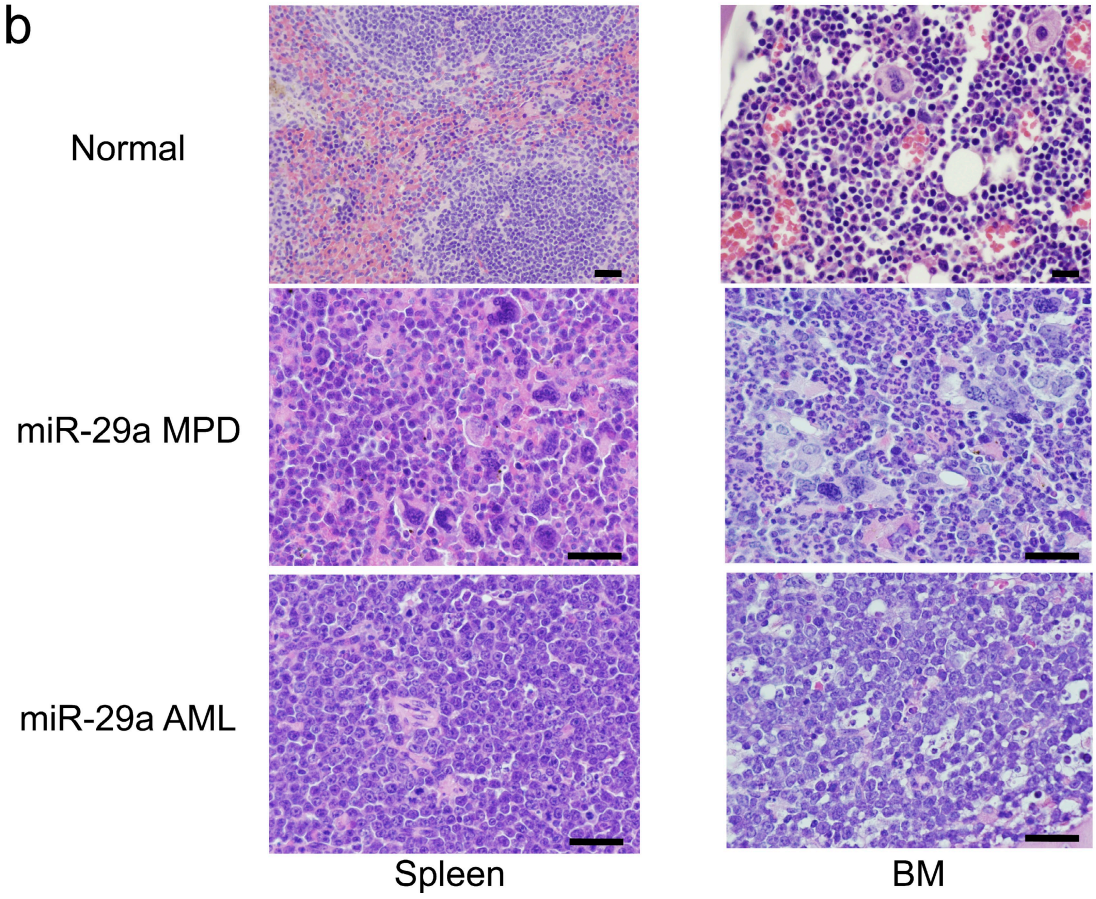
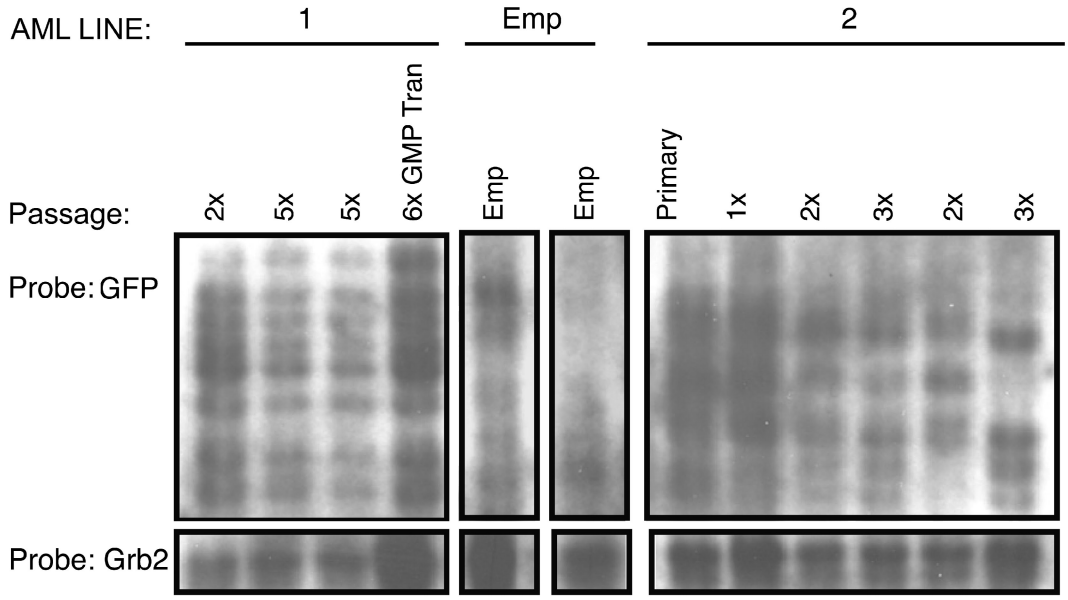


Figure S5. miR-29a–transduced BM cells give rise to an MPD that progresses to AML, resulting in significant mouse morbidity. (a) Summary of transplant outcomes for eight miR-29a–transduced primary chimeras generated from four independent transductions, each designated by a different letter. Secondary transplants were performed at the indicated times, and secondary recipient mice are referred to as the following: exp prior sick, mice euthanized for analysis before falling sick; exp after sick, mice that were sick and euthanized for analysis; died, mice that were found dead before performing analysis. (b) High-power photomicrographs of histological sections of spleen and BM from normal, miR-29a MPD, and miR-29a AML mice. Bars, 50 µm.

a



b

# GMP-L cells transplanted	Number of mice engrafted
2	0/3
20	5/8
200	8/13
2,000	5/5
20,000	5/5

c

Sample	FAB category	WHO classification	% CD34 blasts	Karyotype	FLT3-ITD status
AML1	M0/M1	AML, NOS	95	46,XY[20]	na
AML2	M5b	AML, NOS	94	46,XX[20]	Neg.
AML3	M5	AML,NOS	86	46,XY[20]	Neg.
AML4	M1	AML/MLD	93	46,XY[20]	Neg.
AML8	M2	AML, NOS	71	46,XY[20]	Neg.
AML10	M2	AML, NOS	73	46,XY[20]	Neg.
AML11	M4	AML/MLD	35	46, XY[20]	Neg.
AML12	M4/M5	AML from MDS	24	46,XX[20]	Neg.
AML13	M2/M4	Therapy-related AML	32	46,XX[21]	na
AML14	M5	AML from CMML	24	46,XY[20]	Neg.
AML15	M1	AML from MPD	99	46,XX[20]	na
AML16	M2	AML with inv16	28	inv16	na

Figure S6. Southern blot analysis of serially passaged leukemias, limiting dilution studies to determine LSC frequency, and the AML samples included in this study. (a) Southern blot analysis of sequential transplants of miR-29a–transduced BM. Genomic DNA was prepared from total BM cells from lines 1 and 2. Both lines represent miR-29a–transduced cells in which primary chimeras showed MPD features and in which the MPD evolve to AML during passage 2. Line 2 shows pattern differences in a subline between the second and third passages, suggesting the possibility of the clonal selection. The probe: GFP. (b) Summary of GMP-L transplants. FACS-purified GMP-L were transplanted into nonirradiated or irradiated mouse recipients and monitored for leukemia development for up to 8 mo. Engraftment was assessed in the peripheral blood or in the spleen and BM of engrafted (>16 wk) or moribund mice. Positive engraftment for leukemia was determined by flow cytometry as well as morphological evaluation of total BM and/or spleen cells. (c) Selected patient characteristics for AML samples used in this study. FAB, French-American British; NOS, not otherwise specified; WHO, World Health Organization; ITD, internal tandem duplication; MLD, multilineage dysplasia; MDS, myelodysplastic syndrome; CMML, chronic myelomonocytic leukemia; na, not available.

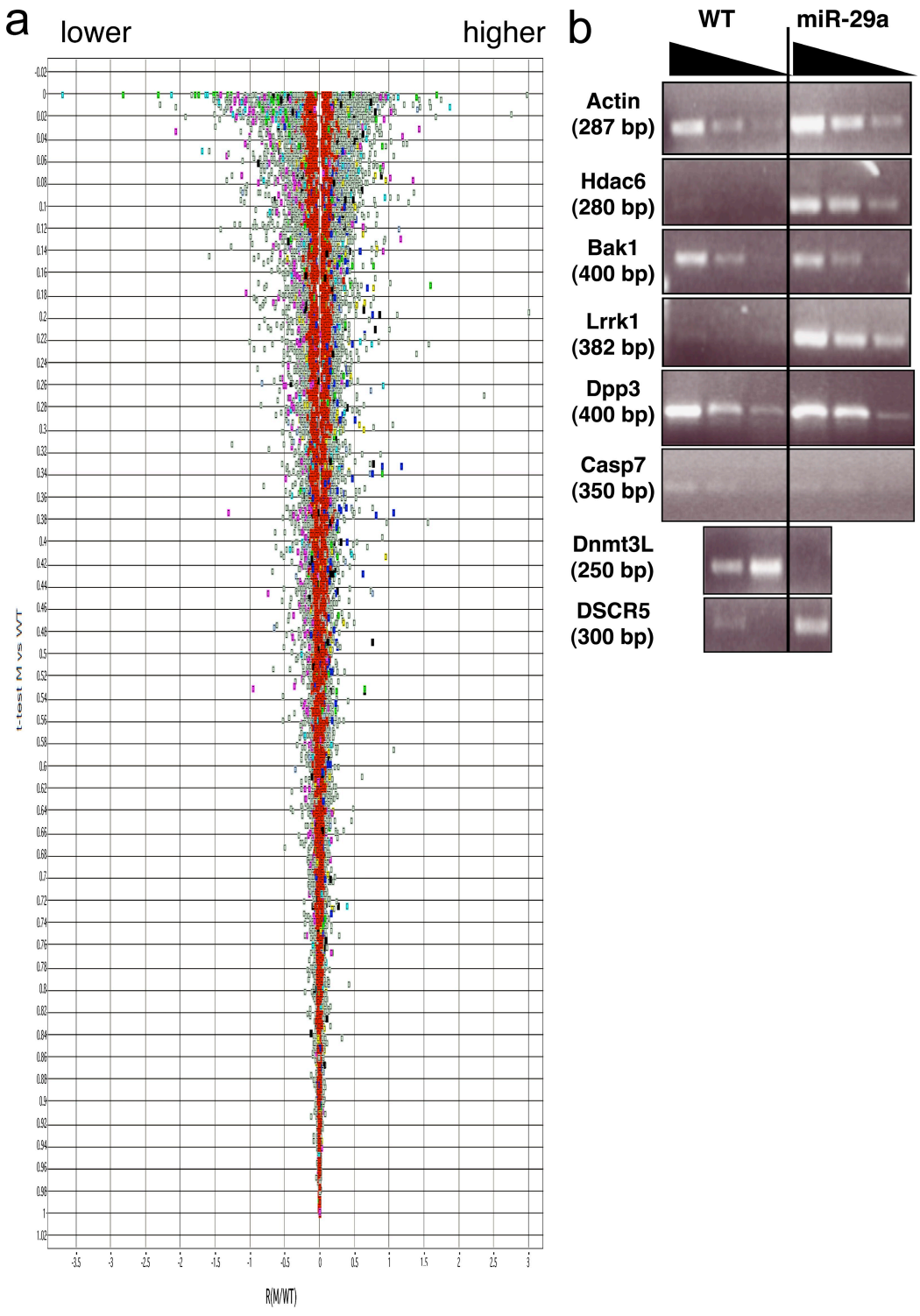


Figure S7. miR-29a expression alters mRNA expression. (a) mRNA microarray analysis was conducted using Mac-1$^+$Gr-1$^+$ cells purified from the spleens of miR-29a primary chimeras and empty vector control mice. (b) Semiquantitative PCR performed on total RNA purified from sorted miR-29a–expressing Mac-1$^+$Gr-1$^+$ cells confirms decreased mRNA expression for Dnmt3l and increased expression of Hdac6 and Lrrk1. Bak1, Dpp3, and Casp7 are not significantly decreased.

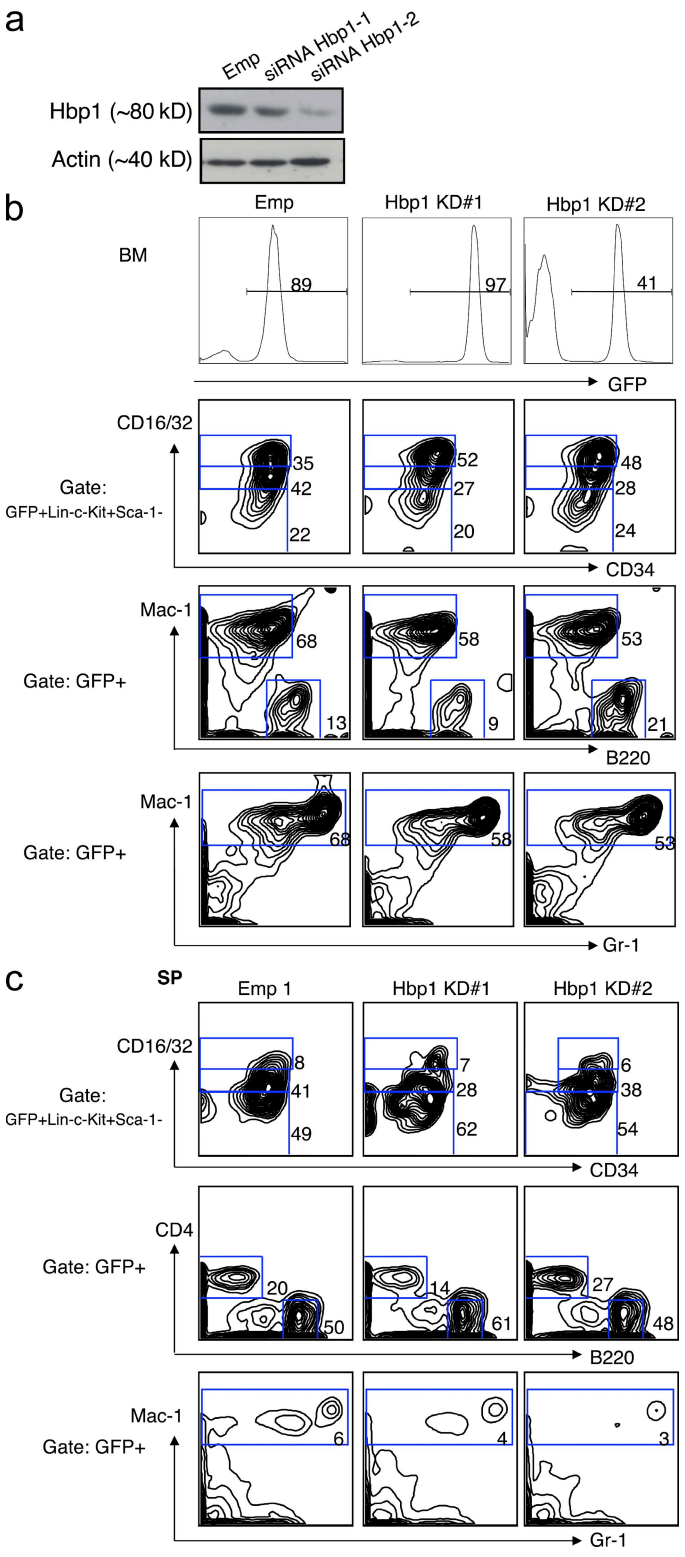


Figure S8. Hbp1 knockdown in hematopoietic progenitors does not alter ratios of myeloid progenitors or mature hematopoietic cells in engrafted mice. (a) Western blot analysis of 3DO cells reveals that Hbp1 shRNA construct (Hbp1-2) efficiently decreases Hbp1 expression. Construct Hbp1-2 was used for all subsequent in vivo experiments. (b) Myeloid progenitor and mature myeloid cell composition in BM were evaluated by flow cytometry in primary chimeric mice stably engrafted with Hbp1shRNA GFP$^+$ cells. (c) Hbp1 knockdown does not alter mature myeloid or lymphoid output in primary chimeric engrafted mice. The percentage of Hbp1 shRNA expressing GFP$^+$ granulocytes and lymphoid cells in SP were evaluated in the spleen using the indicated markers.