

SUPPLEMENTAL MATERIAL

Behrens et al., <https://doi.org/10.1084/jem.20160927>

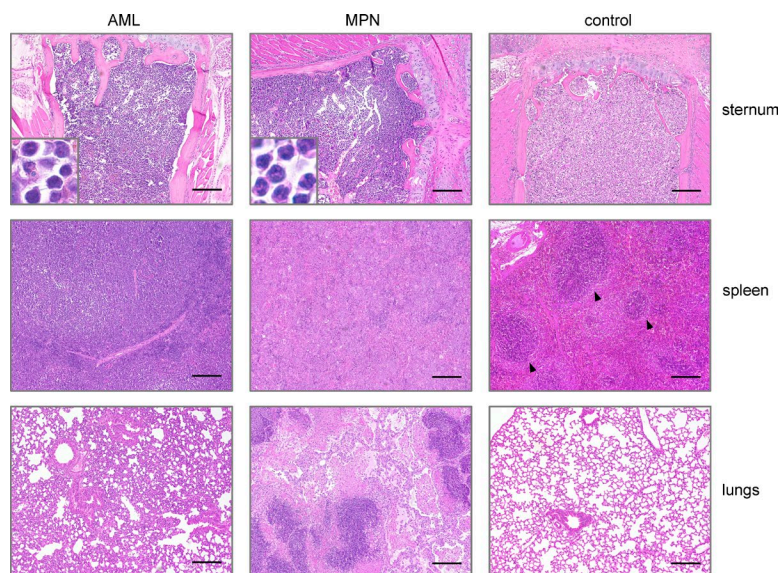


Figure S1. **Pathology and differential diagnosis of diseased animals.** Histological analysis of BM sections from sternum, spleen, and lungs from representative mice showing the shared and distinct pathology. (Left) AML cohort. $n = 3$. (Middle) MPN. $n = 3$. (Right) Nontransplanted control mouse. AML blasts lead to diffuse infiltration of all analyzed organs, whereas the more differentiated MPN progenitors focally infiltrate the spleen and the liver. Arrowheads indicate the white pulp of normal spleen. H&E staining was used. Bars, 600 μm.

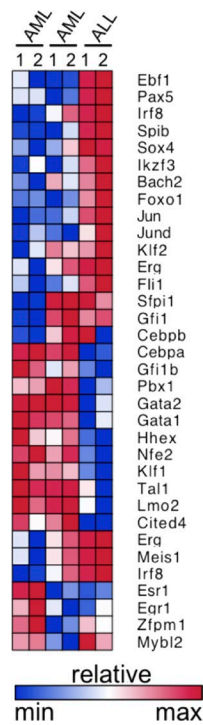


Figure S2. **Comparative gene expression analysis of leukemic cells.** Heat map showing the relative expression of genes encoding typical lymphoid and myeloid TFs in neoplastic blasts with the indicated phenotype as described in Fig. 2 K. Gene expression levels were determined by RNA sequencing. $n = 2$ per cohort.

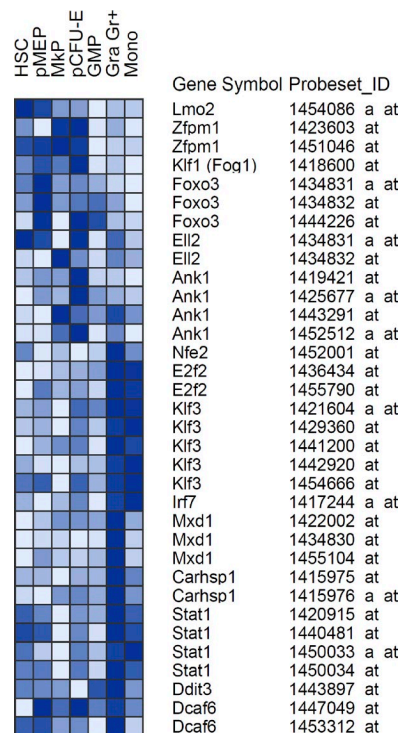


Figure S3. **Deregulated gene expression after RUNX1 inactivation.** Heat map showing the normal expression of genes encoding TFs significantly deregulated after inactivation of RUNX1 in several hematopoietic lineages. Dark blue represents high expression, and light blue represents low expression. Expression values are according to Gene Expression Commons. Gra Gr⁺, Gr1⁺ granulocyte; Mkp, Meg progenitor; mono, monocyte; pCFU-E, pre-CFU-Ery; pMEP, pre-Meg/Ery progenitor.

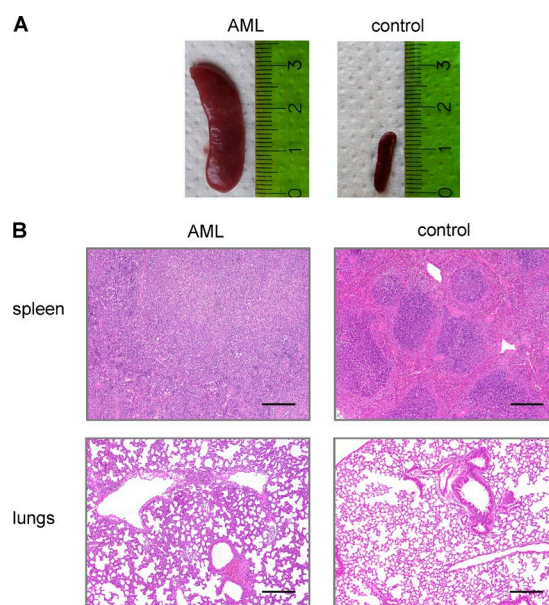


Figure S4. **Pathology of AML induced by HHEX/FLT3-ITD in transplanted mice.** (A) Images showing the spleen of a representative mouse developing an HHEX/FLT3-ITD⁺ AML (left) and a nontransplanted control mouse. (B) Histological analysis of spleens and lungs. Abundant and diffuse infiltration of leukemic blasts was observed in both organs. H&E staining was used. Bars, 600 μ m.

Table S1. List of antibodies used for FACS

Antigen	Conjugate	Clone
CD4	APC	RM4-5
CD8a	PE/Cy7	53-6.7
CD11b	PE/Cy7	M1/70
CD19	APC	6D5
CD19	PE/Cy7	6D5
CD45R/B220	APC	RA3-6B2
CD117/kit	APC	2B6
Gr1	APC	RB6-8C5
Sca	PE/Cy7	D7
Ter119	PE/Cy7	TER-119

Table S2. Western blot antibodies

Antigen	Clone	Species
Erk1	K-23	rabbit
Flt3	8F2	rabbit
Gapdh	6C5	mouse
GFP	FL	rabbit
pErk	Thr202/Tyr204	goat
pFlt3	Tyr591	rabbit
Runx1	D4A6	rabbit

Table S3. Oligonucleotides used for RT-PCR

Primer	Direction	Sequence (5'-3')
Hprt	fwd	GCTGGTGAAAAGGACCTCT
Hprt	rev	CACAGGACTAGAACACCTG
Hhex	fwd	GAGGTTCTCCAACGACCAGA
Hhex	rev	GTCCAACGCATCCTTTTGT
hCXCR4	fwd	TGACGGACAAGTACAGGCTGC
hCXCR4	rev	CCAGAAGGGAAGCGTGATG
hGAPDH	fwd	GTTTCGACAGTCAGCCGCATCTTCTTT
hGAPDH	rev	GTGACCAGGCGCCAATACGACCAA

Abbreviations used: fwd, forward; rev, reverse.

Table S4. shRNA sequences

shRNA	Sequence (5'-3')
RUNX1_sh_I	ACGATCAGTCCTACCAATCTCAAGAGAGATTGGTAGGACTGATCGT
RUNX1_sh_II	CGAAGACATCGGCAGAACTTCAAGAGAGTTCTGCCGATGCTTCG
scr_sh	GACGAAGGAATAACGCCATTCAAGAGATGGCGTTATTCCTTCGTC