Supplementary Materials and Methods

Methods:

Quantitative PCR

RT-PCR was performed for 40 cycles followed by gel electrophoresis 2% agarose gels. Primers are indicated in Supplementary Table 1. Primers for detection of Ebf1 and Actin-beta have been obtained from Qiagen (Qiagen GmbH, Hilden, Germany).

Supplementary Table 2: Primer

Primer sequence (5'>3')	Abreviation	Employed for
GCA GGA CTC GGC TTG CTG AAG	HIV SD fw	Detection of splicing from the HIV SD to Ebf1 Exon 9
GAC GCT CTG TGC CTT CGG AGG	GPIbaP SD fw	Detection of splicing from GPIbaP Intron 1 SD to Ebf1-exon9
CCC ATC AAA gAA ATT gTC CCC	Ebf1 Exon 9 rev	Detection of Splicing to Exon9
CGG ATG GCA TGA GGA GTT ATC	Ebf1 Exon10 rev	Detection of Splicing to Exon9
GCA CTG ATA ATT CCG TGG TGT TG	Pre# fw	Detection of Splicing from PRE SD
TGC AGG AAA CCC ACG TGA C	Ebf1 Exon7 fw	Detection of Splicing from Ebf1 Exon8 to GPIba SA qRT-PCR: Ebf1-readthrough in Intron 8
TGA ACA GCT CCT CGC CCT TG	GFP_rev	Detection of Splicing from Ebf1 Exon8 to GPIba SA
TCA CAA GTA GCA ATG CCA ACC	Ebf1 Intron 8 rev	qRT-PCR: Ebf1-readthrough in Intron 8
TCG GAA GGT ACG CCC TCT TA	Ebf1-GFP Fusion qPCR fw	qRT-PCR: Ebf1-exon8 fusion to GPIbaP SA
GCT GAA CTT GTG GCC GTT TA	Ebf1-GFP Fusion qPCR rev	Detection of Splicing from Ebf1 Exon8 to GPIba SA qRT-PCR: Ebf1-exon8 fusion to GPIbaP SA
AAA AGC TGT GGC AAC CGA AA	Ebf1fw	qRT-PCR: Ebf1
TGC ATT TAA GGA AAA ACT TCA GGA A	Ebf1rev	qRT-PCR: Ebf1
GCC ACC CTG TGT AGA AGA GC	Nhs_fw	qRT-PCR: Nhs
GGT AGG CTG GTA GTG GTG GA	Nhs_rev	qRT-PCR: Nhs

LM-PCR

Lentiviral vector integrations in leukemic cells were analyzed by ligation-mediated (LM) PCR, as previously described [1, 2]. Here, genomic DNA was isolated from total bone marrow cells with the QIAamp DNA Blood Mini Kit (QIAGEN). 500ng DNA were used for restriction digest with Tsp509I (New England Biolabs). For primer extension (95°C 5min; 64°C 30min; 72°C 15min) Native Pfu DNA polymerase (Stratagene) and a 5'-biotinylated primer (lvLTR1 5'-GAACCCACTGCTTAAGCCTCA-3') binding in the LTR were used. Amplified fragments were captured with streptavidin-coated magnetic beads (Invitrogen) and ligated with a linker before running a nested PCR (95°C 2min; 94°C 15sec, 60°C 30sec, 68°C 1min, for 30 cycles; 68°C 10min; lvLTR2 5'-AGCTTGCCTTGAGTGCTTCA-3' and #357 5'-GACCCGGGAGATCTGAATTCG -3'; lvLTR3 5'-AGTAGTGTGTGCCCGTCTGT-3' and #358 5'-AGTGGCACAGCAGTTAGGACG-3') using the Extensor High-Fidelity PCR Master Mix (AB gene). Amplified fragments were analyzed by agarose gel electrophoresis; bands were cut out with the QIAquick Gel Extraction Kit (QIAGEN) and sequenced.

Chromosome preparation and spectral karyotyping (SKY)

Leukemic cells were short-term cultivated for 2 day followed by chromosome preparation using standard protocols [3]. Spectral karyotyping (SKY) was performed as described previously [3] and according to the manufacturer´s instructions (ASI; Applied Spectral Imaging, Ltd., Migdal HaEmek, Israel). Spectral images were acquired using an epifluorescence microscope equipped with an interferometer (SpectraCube™ ASI), a custom-designed optical filter and the SkyView™ software (ASI).

Comparative genome hybridisation (Array-CGH)

Array-CGH was performed using the Agilent Mouse Genome Microarray Kit 4x180k (Agilent Technologies, Santa Clara, CA, USA), a high resolution 60-mer oligonucleotide based microarray with median overall probe spacing of about 10 kb. Labelling and hybridization of genomic DNA was performed according to the protocol provided by Agilent. Briefly, 0.75 µg of test DNA were labelled by random priming using the Agilent Genomic DNA Labelling Kit Plus, test DNA with Cy3-dUTP and reference DNA with Cy5-dUTP. Labelled products were purified by Amicon Ultra 30 k filters (Millipore, Billerica, MA, USA), combined and then mixed with mouse Cot-1 DNA (50 µg), Agilent 10X Blocking Agent, and Agilent 2X Hybridization Buffer. This solution was hybridized to Agilent's 4x180k Mouse Genome CGH microarray at 65°C with 20 rpm rotation for 24 h. Washing steps were performed according to the Agilent protocol. Microarray slides were scanned immediately using an Agilent microarray scanner at a resolution of 2 µm. For image analysis, default CGH settings of Feature Extraction Software (Agilent Technologies, Waldbronn, Germany) were applied. Output files from Feature Extraction were subsequently imported into Agilent's CGH data analysis software, DNA-Workbench. The Aberration Algorithm ADM2 was applied and Aberration Filters were set to: threshold 4.0, at least 5 probes with mean $\log_2$ ratio of -0.6

References

1. Schmidt, M., Hoffmann, G., Wissler, M., Lemke, N., Mussig, A., Glimm, H., et al. (2001). Detection and direct genomic sequencing of multiple rare unknown flanking DNA in highly complex samples. *Hum Gene Ther* **12**: 743-749.
2. Kustikova, O. S., Modlich, U., and Fehse, B. (2009). Retroviral insertion site analysis in dominant haematopoietic clones. *Methods Mol Biol* **506**: 373-390.
3. Rudolph, C., and Schlegelberger, B. (2009). Spectral karyotyping and fluorescence in situ hybridization of murine cells. *Methods Mol Biol* **506**: 453-466.