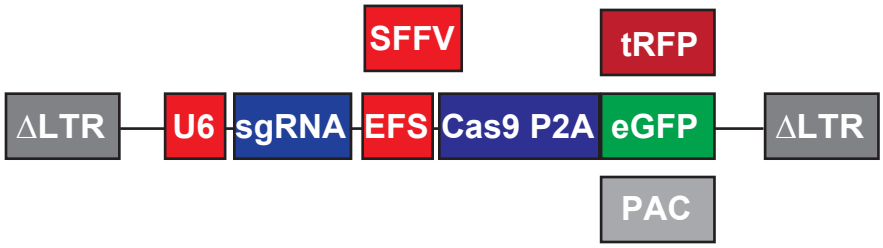
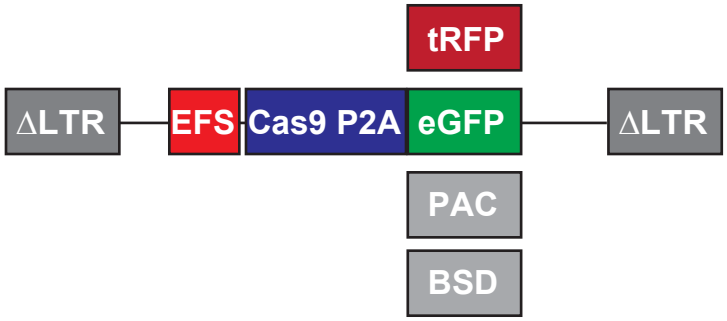
Supplementary Figures

A)



B)



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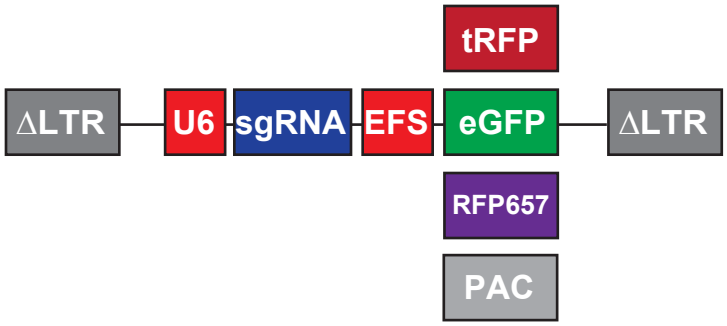
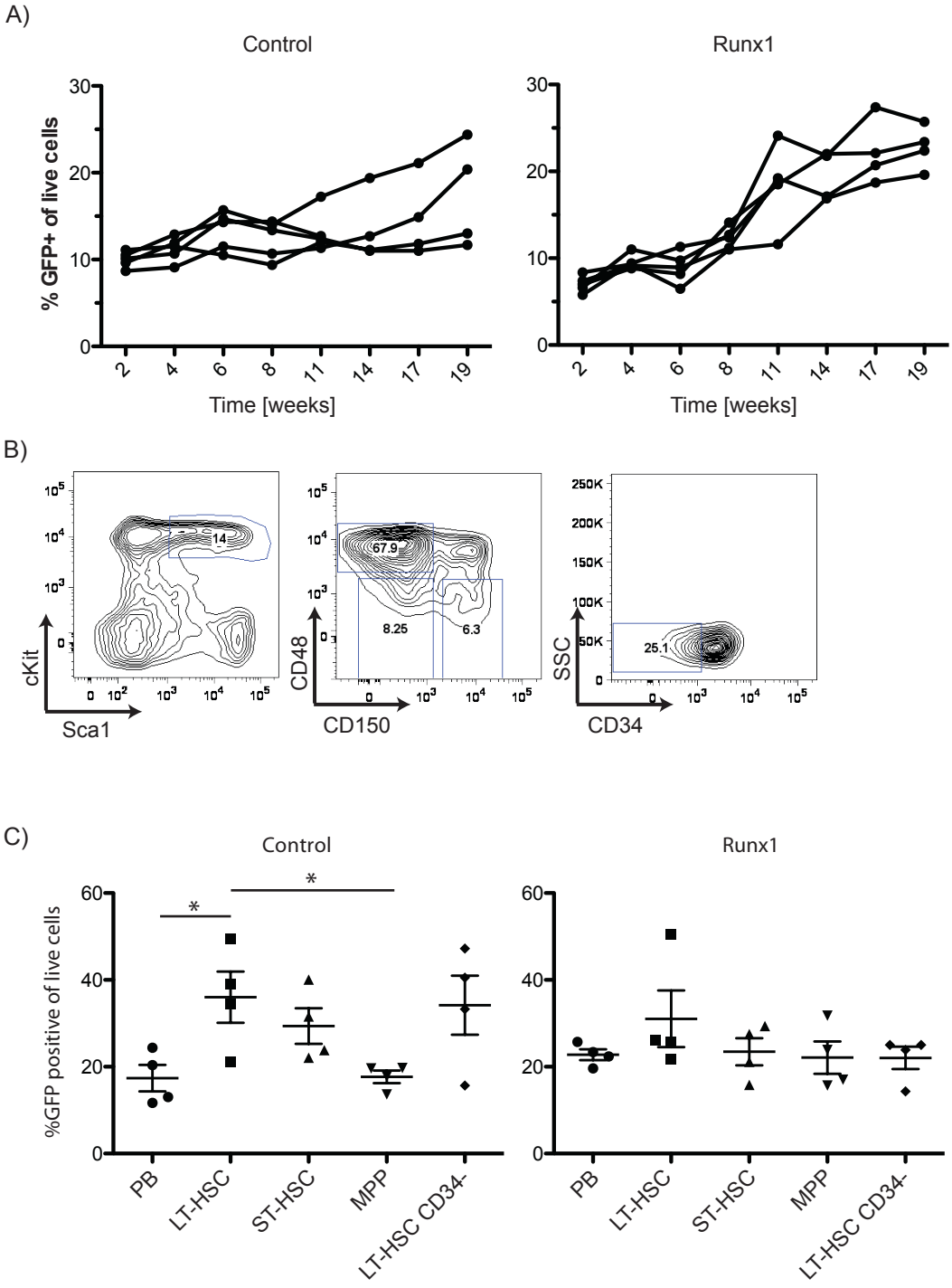


Supplementary Figure 1 - A modular CRISPR/Cas lentiviral vector system for genome engineering
A) Schematic depiction of a lentiviral vector for bi-cistronic expression of the sgRNA from a U6 promoter (U6) and expression of Cas9 from a short *EF1a* promoter (EFS) or Spleen Focus Forming Virus (SFFV) promoter with a fluorescent protein marker (eGFP or tagRFP) or drug selectable marker (Purmomycin acetyl transferase (PAC) or Blasticidin deaminase (BSD)) from a picorna virus derived 2A auto-cleavage site (P2A). B) Depiction of a lentiviral vector for expression of Cas9 without the sgRNA. C) Depiction of a lentiviral vector for expression of the sgRNA only with a fluorescent protein marker or selectable marker form an EFS promoter.



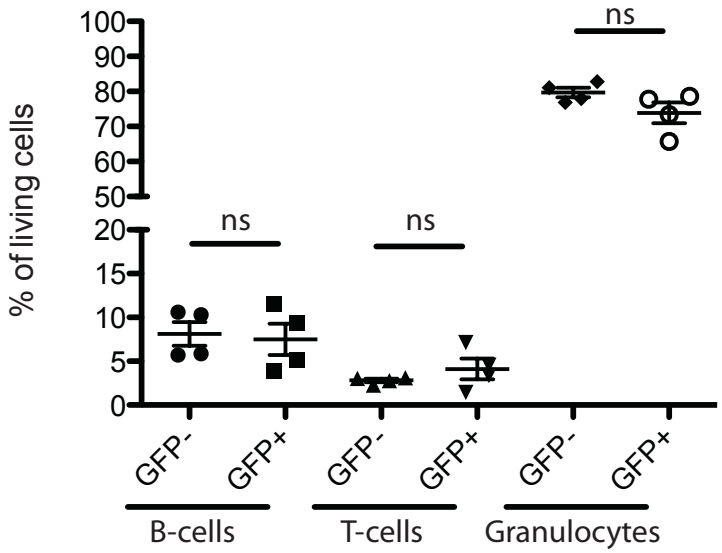
Supplementary Figure 2 - sgRNA:Cas9 mediated knock out of *Runx1* in Flt3-ITD heterozygous HSCs induces a competitive advantage

A) Flow cytometric assessment of sgRNA:Cas9 transduced cells in the peripheral blood of mice over 19 weeks (control sgRNA left; Runx1 sgRNA right).

B) Gating scheme of HSPC populations in mice transduced with sgRNA:Cas9 lentiviral vectors.

C) Summarized sgRNA:Cas9 vector expression in different HSPC populations at 19 weeks post transplantation for control sgRNA (left) or Runx1 sgRNA (right). Significant differences are indicated where applicable (t-test, two sided, unpaired; Welch corrected for comparisons with significantly different variances)

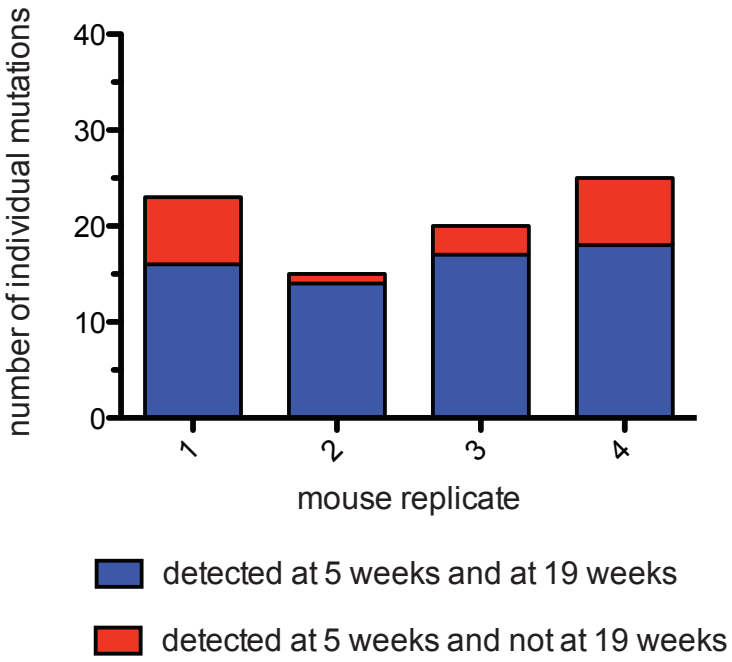
MPP = Multipotent progenitors (LSK CD150- CD48+); ST-HSC = short-term HSCs (LSK CD150- CD48-); LT-HSC = long-term HSCs (LSK CD150+ CD48-) ; LT-HSC CD34- = most quiescent long-term-HSCs (LSK CD150+ CD48- CD34-)



Supplementary Figure 3 - sgRNA:Cas9 expression does not alter lineage maturation in the BM

Flow cytometry based quantification of mature hematopoietic lineages within the GFP negative and GFP positive population in the BM of mice expressing Cas9 (GFP+) and a control sgRNA shows no significant differences (t-test, two sided, unpaired) in the maturation potential of Cas9 expressing cells.

A)



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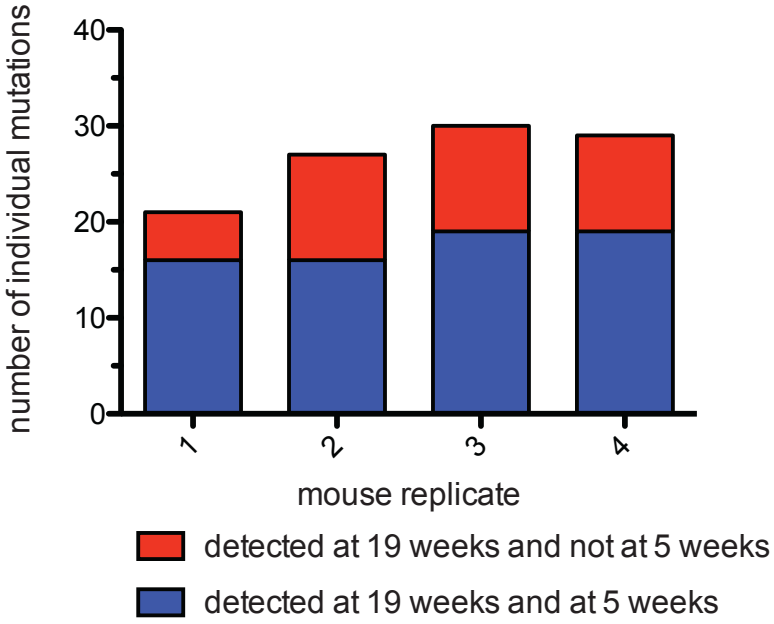
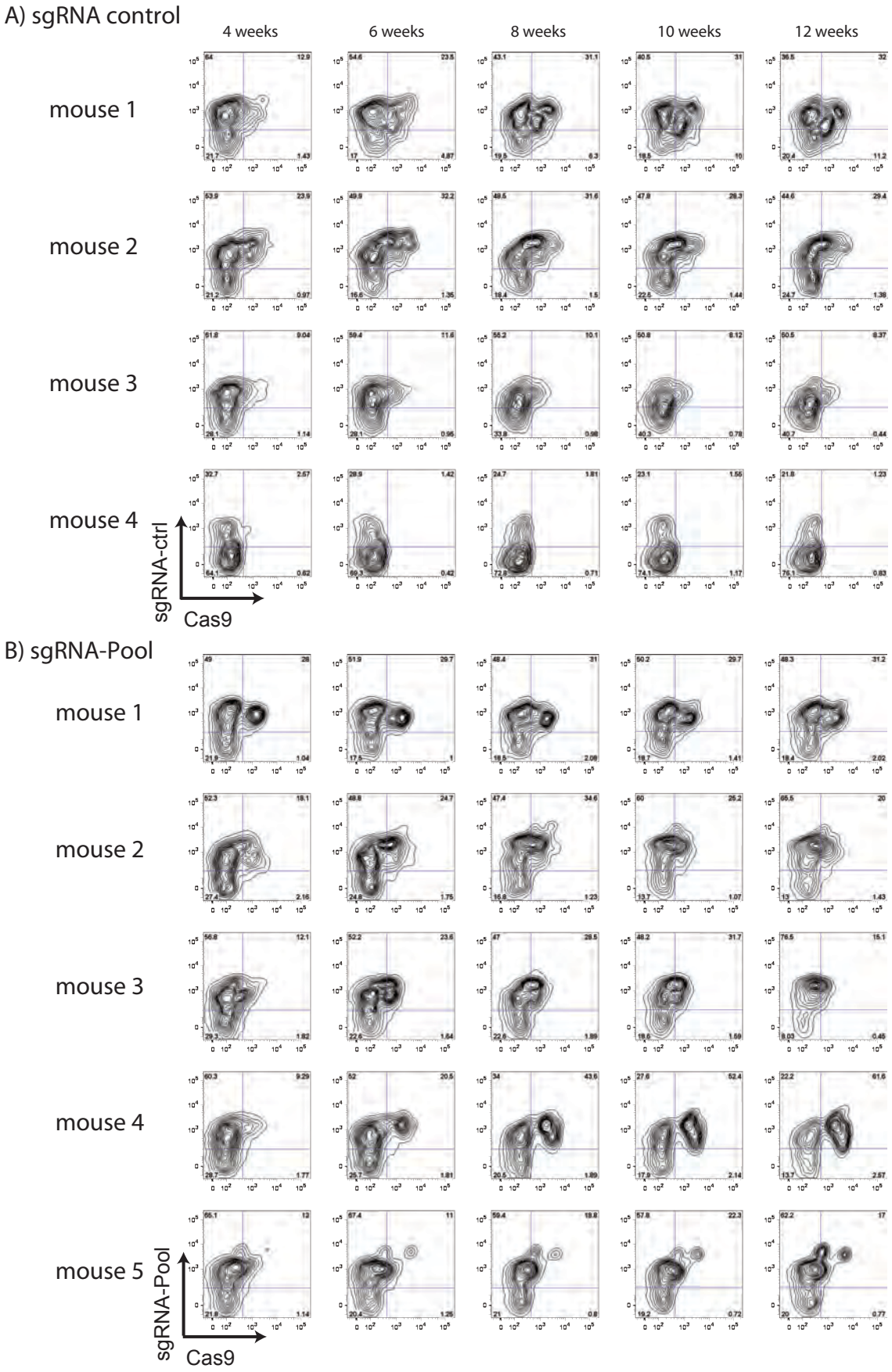
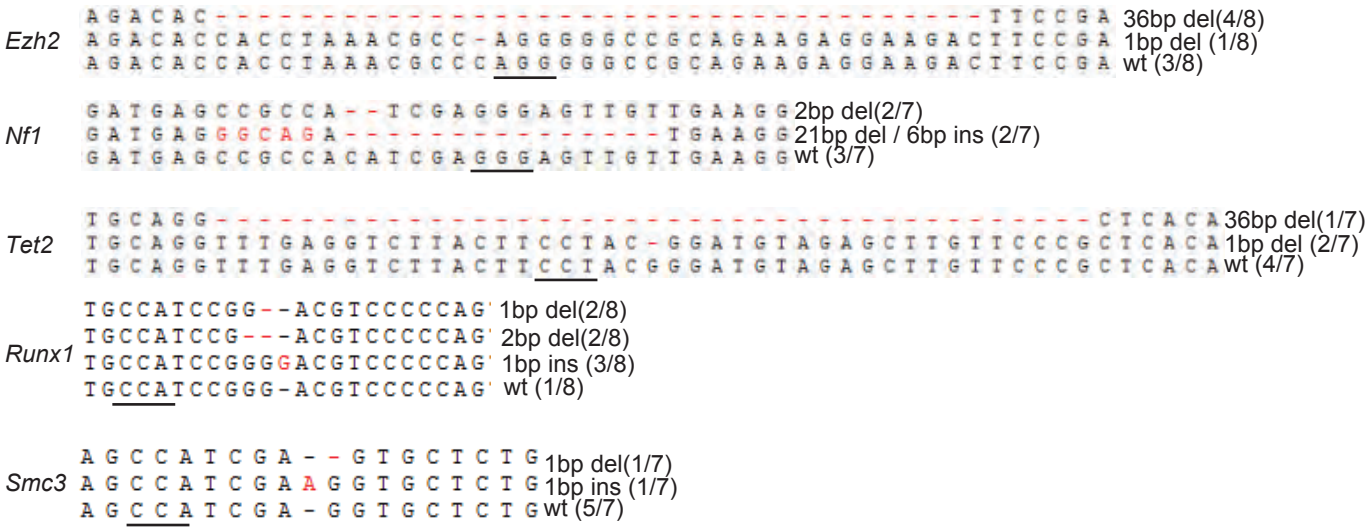


Supplementary Figure 4 - Next Generation Sequencing shows stable genome modification in long-term repopulating HSCs *in-vivo*

Mutational analysis of peripheral blood samples at 5 weeks post-transplant and 19 weeks post-transplant. A) Samples were analyzed to detect all unique mutations from each mouse at 5 weeks and at 19 weeks for mutations at the *Runx1* target site. Mutations detected at both time points and only at 5 weeks are shown. B) Mutations found at 19 weeks but not at 5 weeks are shown.

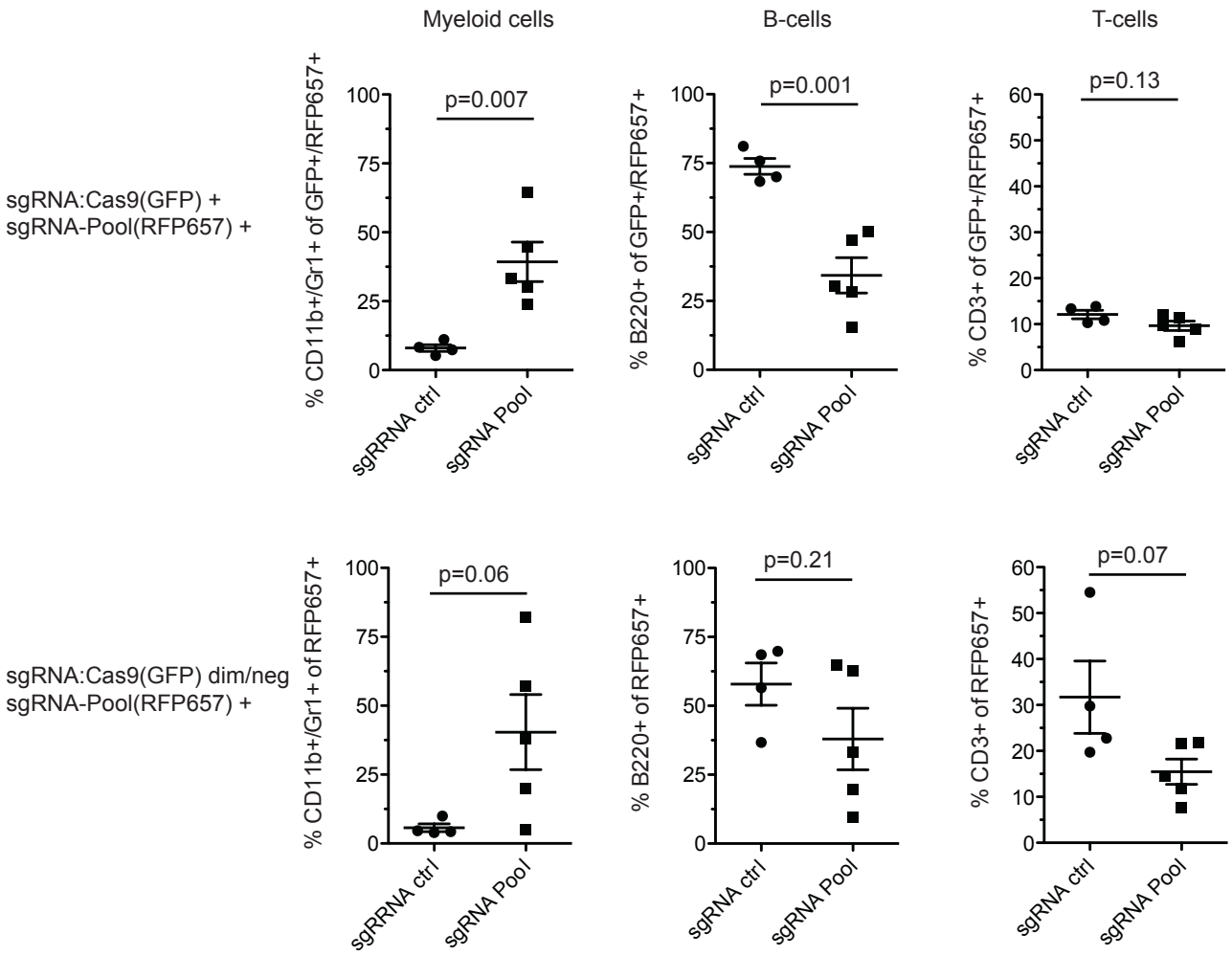


Supplementary Figure 5 - Flow cytometry based clonality analysis of C57Bl/6 mice shows expansion of sgRNA:Cas9 engineered populations over time
Flow cytometric assessment of sgRNA:Cas9 vector expression (eGFP) and sgRNA-only vector expression (RFP657) for control sgRNA (A) and pooled sgRNAs (B) in C57Bl/6 wild-type mice. Each row shows vector expression at the given time point from an individual mouse.



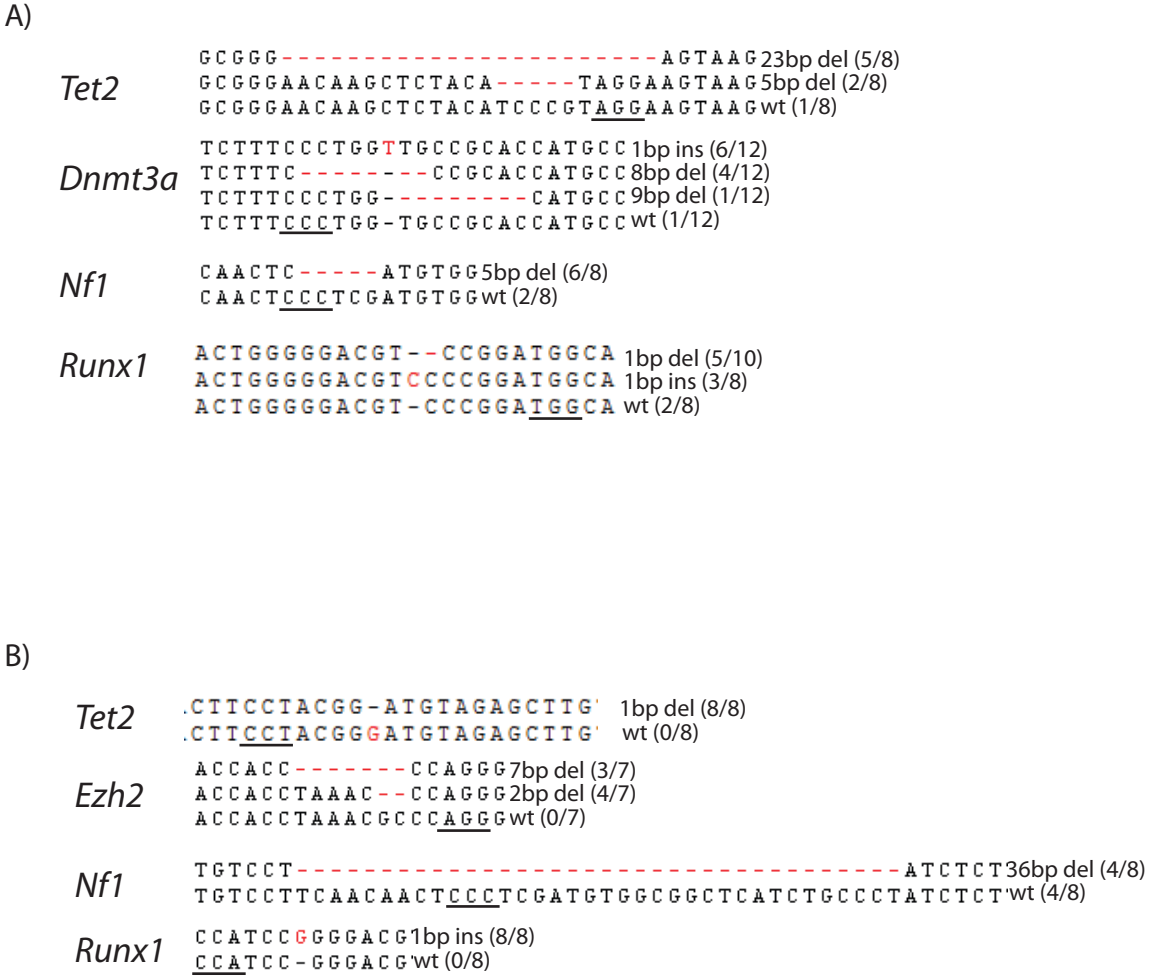
Supplementary Figure 6 - Sequence alignment of targeted gene loci in a putatively clonal sgRNA:Cas9 modified population with dim Cas9-eGFP expression shows gene editing

Alignment of sequences obtained for targeted genes from sorted cell populations shown in Figure 2. Ratio of bacterial clones with presented adjacent sequence out of the total clones sequenced for the respective gene shown behind each sequence A) Genes found mutated in the clone shown in Figure 2C. B) Genes found mutated for the clone shown in Figure 2D. PAM underlined



Supplementary Figure 7 - Myeloid lineage skewing induced by multiplex CRISPR/Cas in eGFP-high and eGFP-dim sgRNA:Cas9 expressing populations

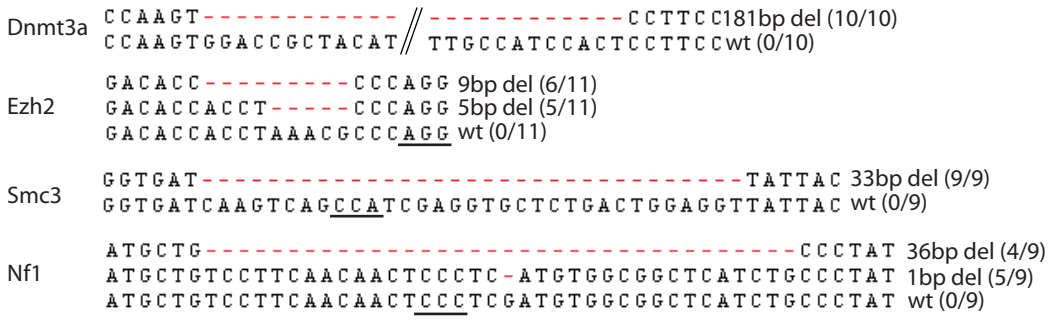
Flow cytometric assessment of mature hematopoietic lineage distribution in mice transduced with sgRNA-control or sgRNA-pool 10 weeks post transplant (myeloid = CD11b+/Gr1+; B-cells=B220+; T-cells=CD3+. Mice are shown in Supplementary Figure 5.
Upper panel shows lineage distribution in the GFP+ / RFP657+ population of mice with the targeting sgRNA-Pool (sgRNA-Pool) compared to the non-targeting control (sgRNA-ctrl). Lower Panel shows lineage distribution in the GFP-dim/neg / RFP657+ population of mice with the targeting sgRNA-Pool (sgRNA-Pool) compared to the non-targeting control (sgRNA-ctrl). Statistical differences are indicated (t-test, two sided, unpaired; Welch corrected for comparisons with significantly different variances).



Supplementary Figure 8 - Sequence alignments of genes mutated in putatively clonal hematopoietic sgRNA:Cas9 modified populations identified after multiplex sgRNA:Cas9 genome engineering

A) Alignment of sequences obtained for targeted genes from sorted cell populations shown in Figure 2. The ratio of the number of bacterial clones with each sequence over the total clones sequenced for the respective gene is shown following each sequence. A) Genes found to be mutated in the clone shown in Figure 2C. B) Genes found to be mutated for the clone shown in Figure 2D.

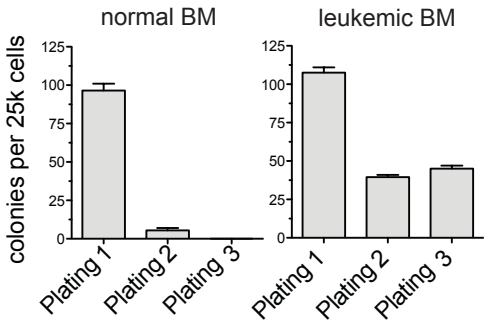
PAM underlined



Supplementary Figure 9 - Sequence alignments of *Dnmt3a, Ezh2, Smc3*, and *Nf1* mutations in AML cells

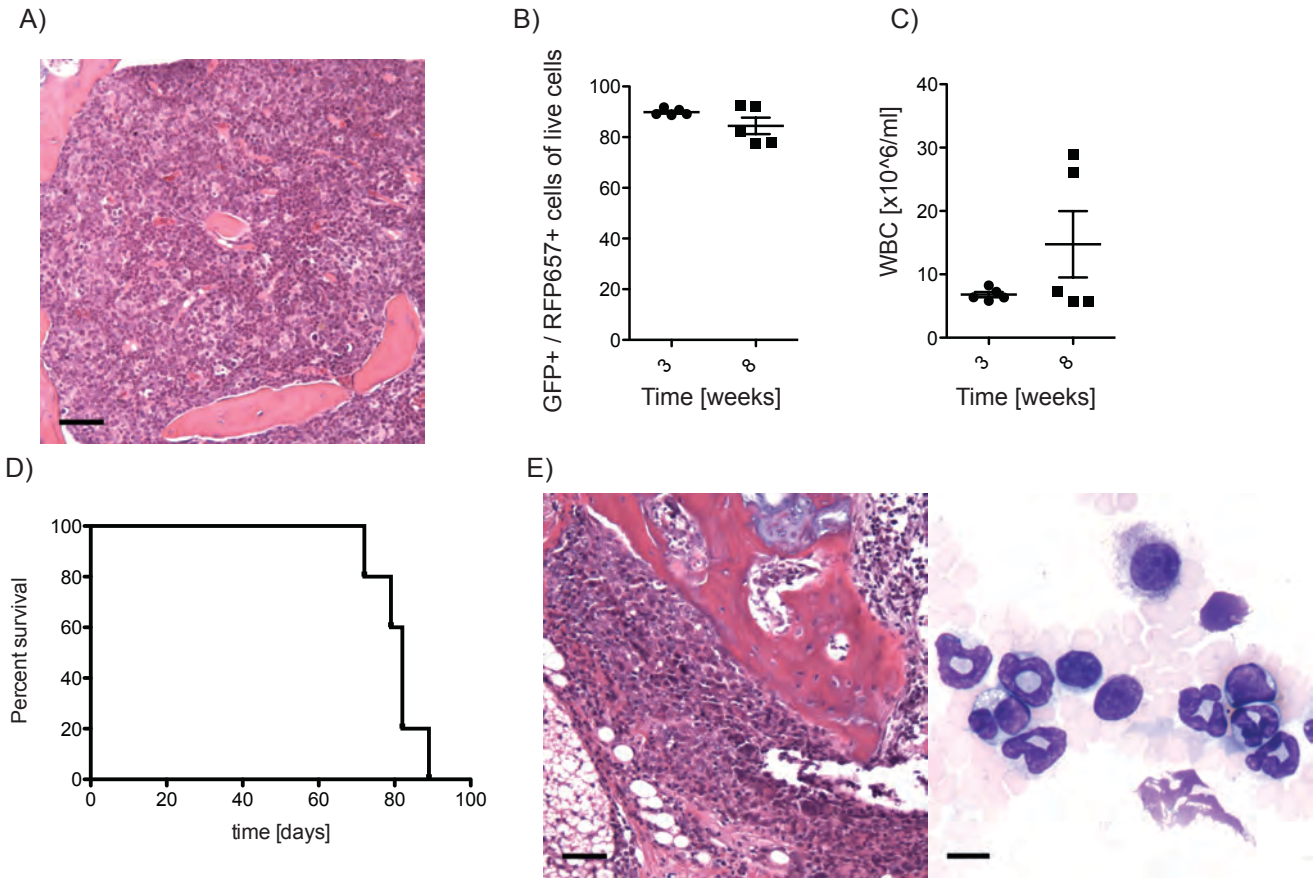
Alignment of sequences obtained for targeted genes from sorted leukemic cells shown in Figure 3A. The ratio of the number of bacterial clones with each sequence over the total clones sequenced for the respective gene is shown following each sequence.

PAM underlined (due to deletion size not possible for *Dnmt3a*)



Supplementary Figure 10 - Leukemia induced by sgRNA:Cas9 genome editing shows enhanced replating capacity

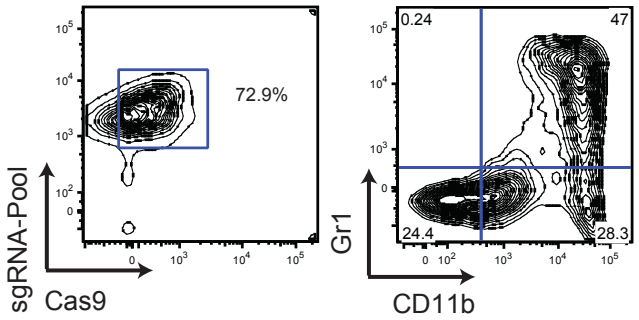
Serial replating capacity of normal BM compared to the leukemic BM cells from the leukemia presented in Figure 3.



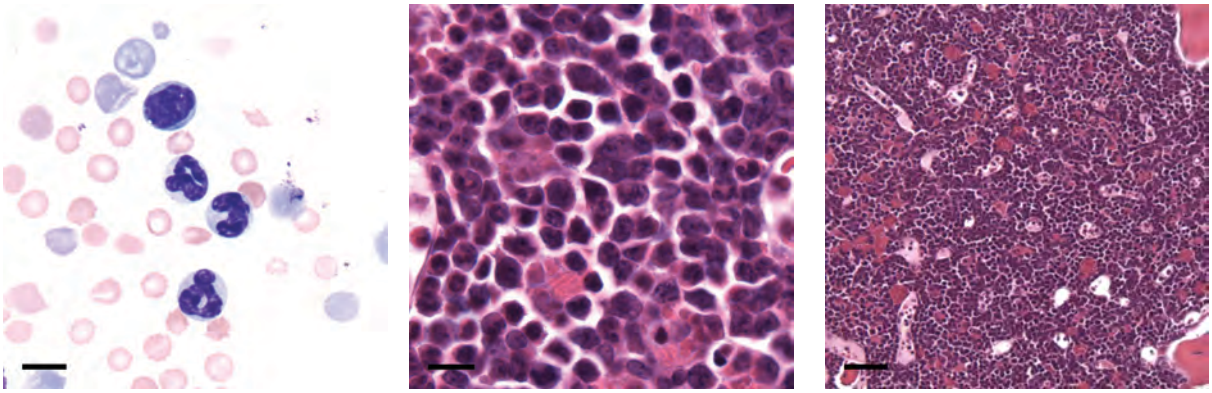
Supplementary Figure 11 - Primary sgRNA:Cas9 induced leukemia engrafts in secondary mice and re-initiates disease

A) Low magnification (200x, HE) BM histology of the leukemic mouse presented in Figure3. Scale bar (25µm) given in the lower right. B) Engraftment of primary leukemia cells in sublethally irradiated secondary recipients as assessed by flow cytometry for eGFP and RFP657 at 3 and 8 weeks post transplant. C) WBC counts of the same secondary mice at 3 and 8 weeks post transplant. D) Survival of the secondary mice. E) Low magnification (200x, HE) BM histology of a leukemic secondary mouse (left) and high magnification peripheral blood smear (MGG, 1000x) of a leukemic secondary mouse (right).Scale bar (25µm for 200x; 5µm for 1000x) given in the lower left.

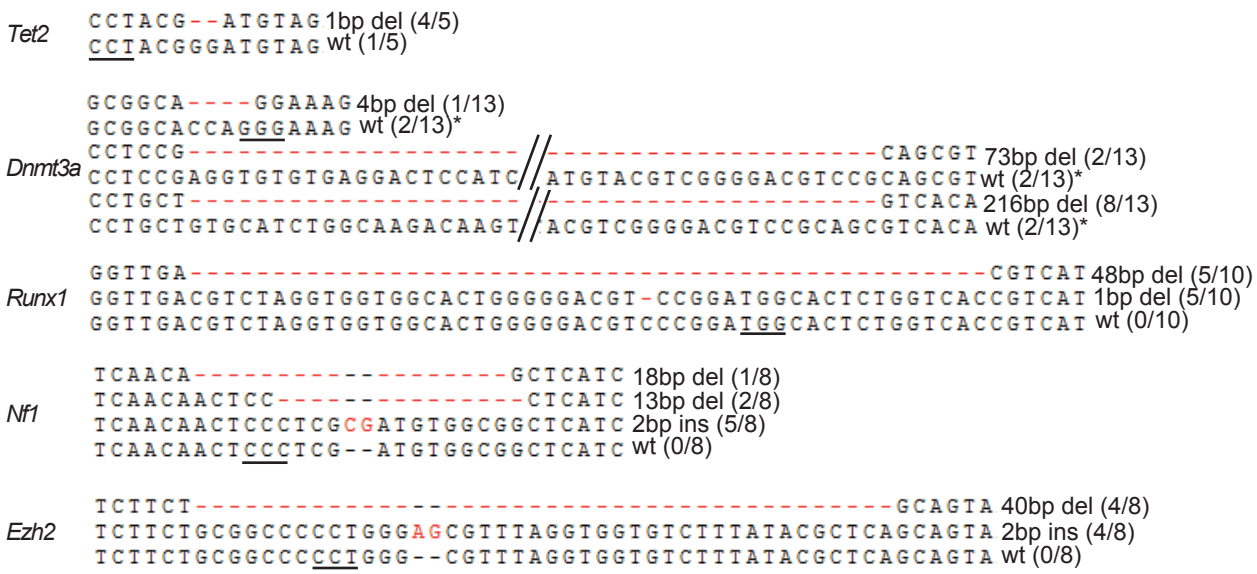
A)



B)

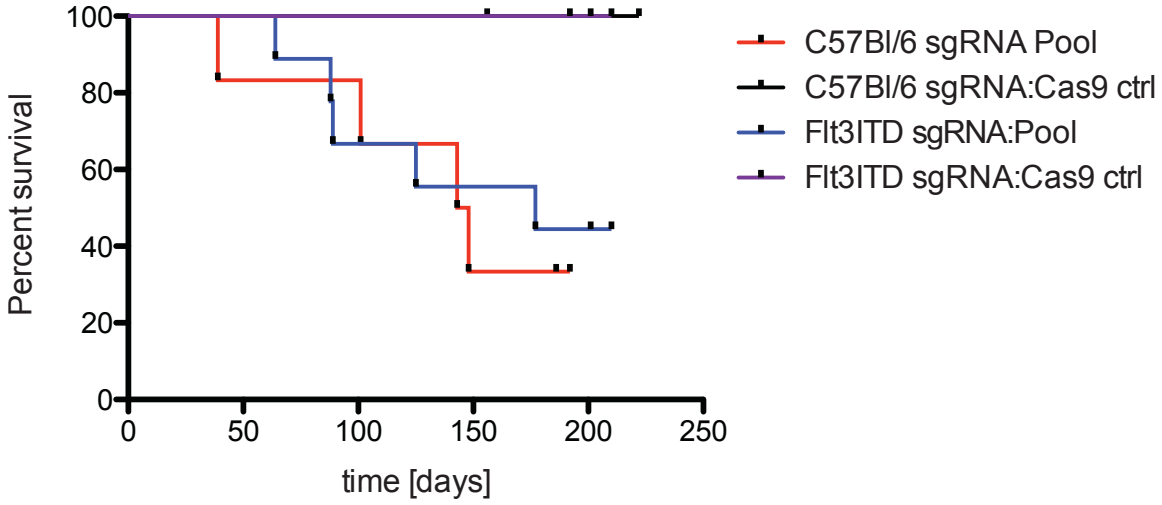


C)



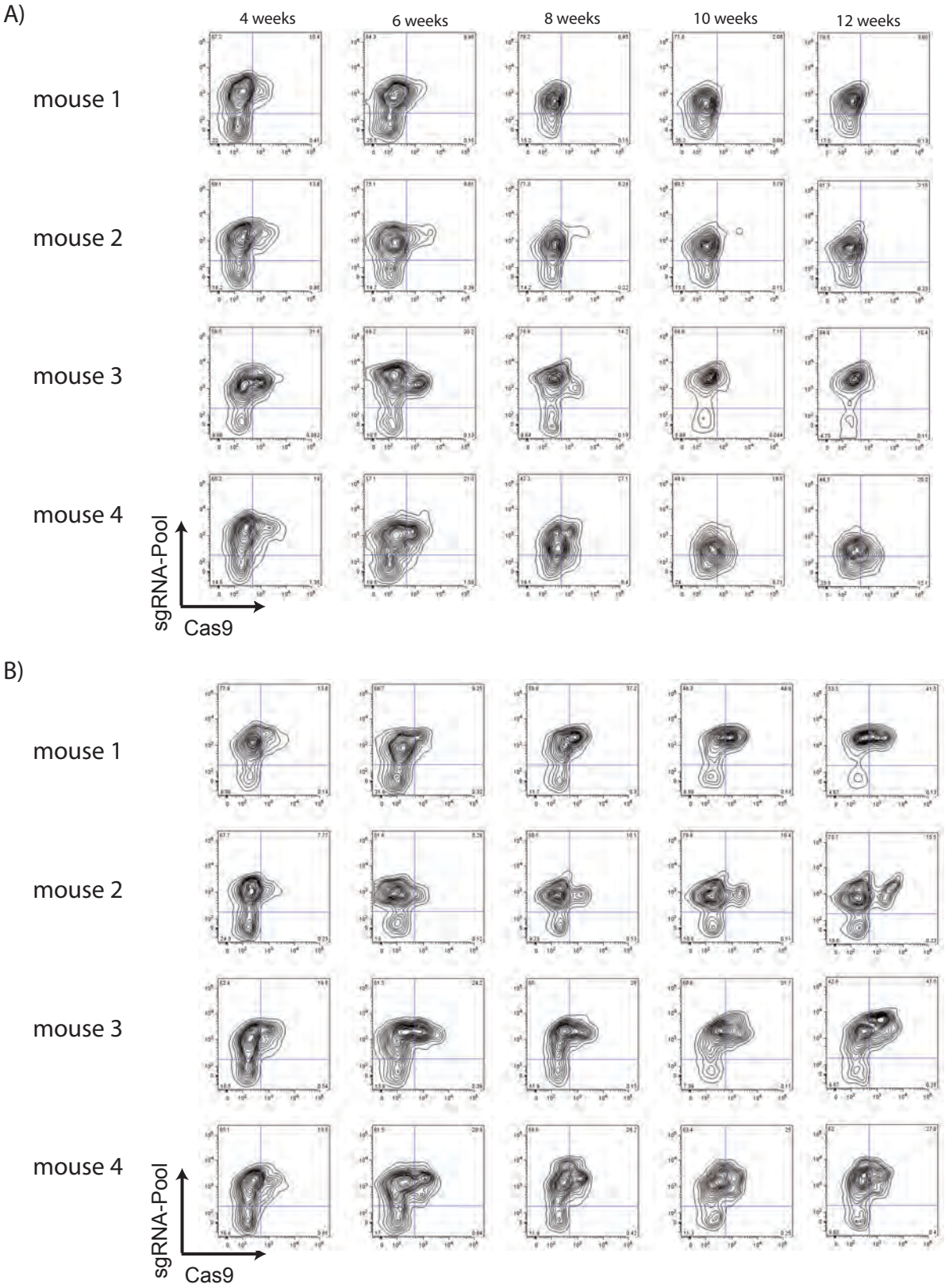
Supplementary Figure 12 - Characterization of a CRISPR/Cas induced myeloid leukemia in a C57Bl/6 with *Tet2, Dnmt3a, Runx1, Nf1,* and *Ezh2* mutation

A) Flow cytometric assessment of sgRNA:Cas9 vector expression (eGFP),sgRNA-only vector expression (RFP657) for pooled sgRNAs (left), and myeloid lineage marker expression (right) in a leukemic mouse transplanted with C57Bl/6 donor cells that presented with leukocytosis (33.6x10^6/ml) and splenomegaly (412mg). B) High magnification peripheral blood smear (MGG, 1000x) of the leukemic mouse (left). Low magnification (200x, HE) BM histology (middle) and high magnification BM histology (1000x, HE) (right) of the leukemic mouse. Scale bars (25µm for 200x; 5µm for 1000x) are given in the lower left C) Mutation analysis on targeted genomic regions showed mutations in *Tet2, Dnmt3a*, *Runx1*, *Nf1*, and *Ezh2.*
PAM underlined (due to deletion size not possible for some *Dnmt3a sequences*)



Supplementary Figure 13 - Survival of mice transplanted with pooled sgRNAs targeting tumor supressor genes

Survival of C57Bl/6 mice with the targeting sgRNA-pool and Cas9 or non-targeting sgRNA and Cas9, and Flt3-ITD heterozygous mice transplanted targeting sgRNA-Pool.



Supplementary Figure 14 - Flow cytometry based clonality analysis of Flt3-ITD heterozygous mice shows expansion of sgRNA: Cas9 engineered populations over time

Flow cytometric assessment of sgRNA:Cas9 vector expression (eGFP) and sgRNA-only vector expression (RFP657) for pooled sgRNAs in Flt3-ITD heterozygous mice. A) The *Runx1* targeting sgRNA was provided with the Cas9. B) The *Tet2* sgRNA was provided with the Cas9. Each row shows vector expression at the given time point from an individual mouse.

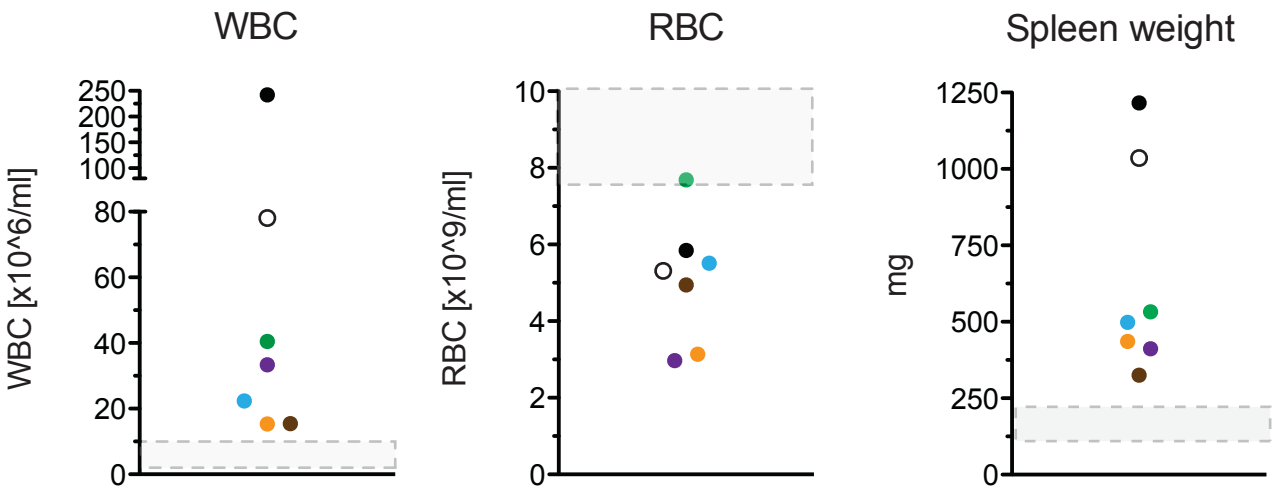
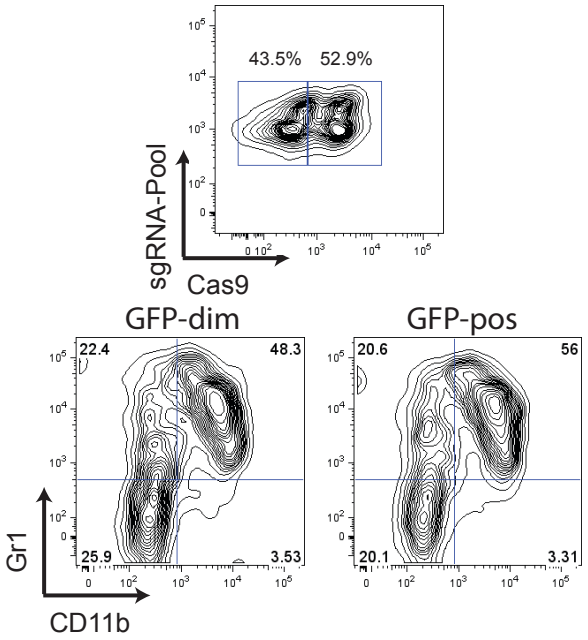


Figure	Donor	Colour
Figure 3	C57Bl/6	
Supplementary Figure 12	C57Bl/6	
Supplementary Figure 16	Flt3-ITD het	
Supplementary Figure 17	Flt3-ITD het	
Supplementary Figure 18	Flt3-ITD het	
not further analyzed	Flt3-ITD het	
not further analyzed	Flt3-ITD-het	

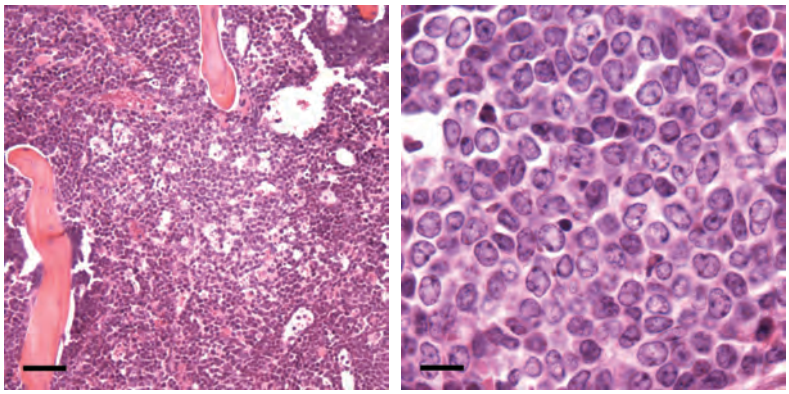
Supplementary Figure 15 - Summarized blood counts and spleen weights from leukemic mice

Summarized WBC (left), RBC (middle), and spleen weight of leukemic mice presented in the study. Each mouse is presented in all three plots by one colour. A legend where data has been presented is given below.
Range of control mice for each value is given as grey boxes. Control spleen weights are from Flt3-ITD control mice presented in Figure 1E/F and Supplementary Figure 3.

A)



B)



C)

Tet2 GFP dim
```
AGGTCT----------CACAAG 33bp del (3/7)
AGGTCTTACTTCCTTA----------CACAAG 25bp del/2bp ins (4/7)
AGGTCTTACTTCCTACGGGATGTAGAGCTTGTTCCCGCTCACAAG wt (0/7)
```

Tet2 GFP pos
```
TGCAGG----------CTCACAAG 41bp del (4/7)
TGCAGGTTTGAGGTCTTACTTCCTTA----------CACAAG 25bp del/2bp ins (3/7)
TGCAGGTTTGAGGTCTTACTTCCTACGGGATGTAGAGCTTGTTCCCGCTCACAAG wt (0/7)
```

Nf1 GFP dim
```
CCGCTACATGCTGATGCTGTCCTTCAACAACTCCCTCGAAACCATGTGGCGGCTCATCTGCCCTATCTCTT 5bp ins (1/7)
CCGCTACATGCTGATGCTGTCCT----------ATCTCTT 37bp del (2/7)
CCGCTA----------TCTCTT 54bp del(3/7)
CCGCTACATGCTGATGCTGTCCTTCAACAACTCCCTCGA-----TGTGGCGGCTCATCTGCCCTATCTCTT wt (1/7)
```

Nf1 GFP pos
```
CCGCTA----------TCTCTT 54bp del (4/8)
CCGCTACATGCTGATGCTGTCCT----------ATCTCTT 37bp del/(4/8)
CCGCTACATGCTGATGCTGTCCTTCAACAACTCCCTCGATGTGGCGGCTCATCTGCCCTATCTCTT wt (0/8)
```

Dnmt3a GFP dim
```
TGGGCA----------CGTCGGGG 30bp del (1/7)
TGGGCATGGTGCGGCAACCAGGGAAAGATCATGTACGTCGGGG 1bp ins (3/7)
TGGGCATGGTGCG----------TCGGGG 23bp del(3/7)
TGGGCATGGTGCGGCA-CCAGGGAAAGATCATGTACGTCGGGG wt (0/8)
```

Dnmt3a GFP pos
```
GGTGCG----------TCGGGG 30bp del (1/7)
GGTGCGG-A-CCAGGGAAAGATCATGTACGTCGGGG 1bp del (2/7)
GGTGCGGCAACCAGGGAAAGATCATGTACGTCGGGG 1bp ins (3/7)
GGTGCGGCA-CCAGGGAAAGATCATGTACGTCGGGG wt (1/7)
```

Runx1 GFP dim
```
GACGTCCCCGGATGG 1bp ins (2/8)
GACGTC---GGATGG 2bp del (4/8)
GACGTCC--GGATGG 1bp del (2/8)
GACGTCCC-GGATGG wt (0/7)
```

Runx1 GFP pos
```
AGGTGG----------ATGGCA 20bp del (2/8)
AGGTGGTGGCACTGGGGGACGTC--GGATGGCA 2bp del (1/8)
AGGTGGTGGCACTGGGGGACGTCC-GGATGGCA 1bp del (5/8)
AGGTGGTGGCACTGGGGGACGTCCCGGATGGCA wt (0/7)
```

Ezh2 GFP dim
```
CCTAAACG--CAGGGG 2bp del(2/7)
CCTAAA--CCCAGGGG 2bp dell (5/7)
CCTAAACGCCCAGGGG wt (0/7)
```

Ezh2 GFP pos
```
CACCTA----CCCAGGG 4bp del(3/5)
CACCTAAA--CCCAGGG 2bp del (2/5)
CACCTAAACGCCCAGGG wt (0/5)
```

Supplementary Figure 16 - Characterization of a Cas9:sgRNA induced myeloid leukemia in a Flt3-ITD heterozygous mouse

A) Flow cytometric assessment of sgRNA:Cas9 vector expression (eGFP), sgRNA-only vector expression (RFP657) (upper panel) in a leukemic mouse that presented with mild leukocytosis (15.4x10^6/ml) and splenomegaly (326mg). Myeloid marker expression (lower panel) was assessed both in GFP-dim cells (left) and GFP-pos cells (right).

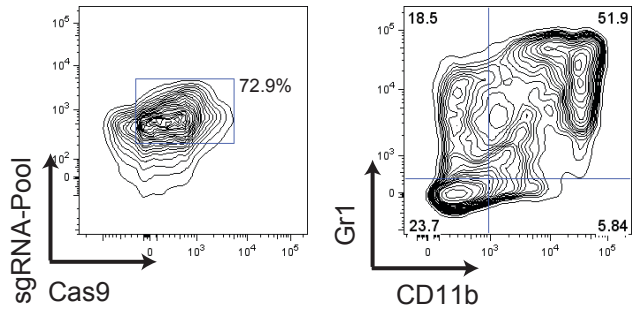
B) Low magnification (200x, HE) BM histology (left) and high magnification BM histology (1000x, HE) (right) of the leukemic mouse.

Scale bar (25µm for 200x; 5µm for 1000x) are given in the lower left.

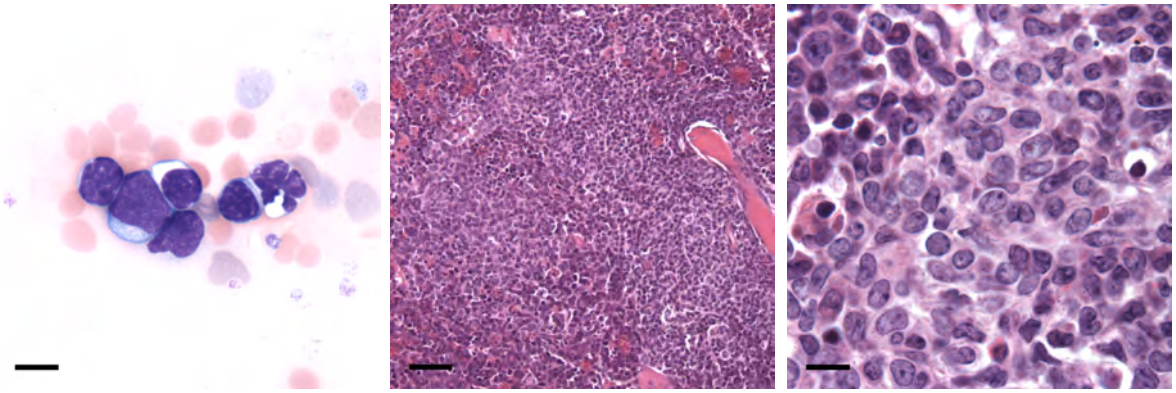
C) Mutation analysis on targeted genomic regions. Mutations were assessed for both flow sorted GFP-dim and GFP-pos cells. Redundant mutation on one allele in *Tet2*, *Nf1*, *Dnmt3a*, *Runx1*, and *Ezh2* suggests subclonal architecture.

PAM underlined

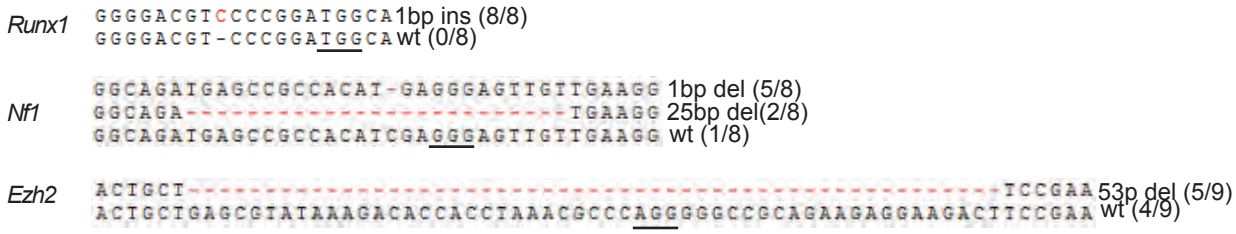
A)



B)



C)



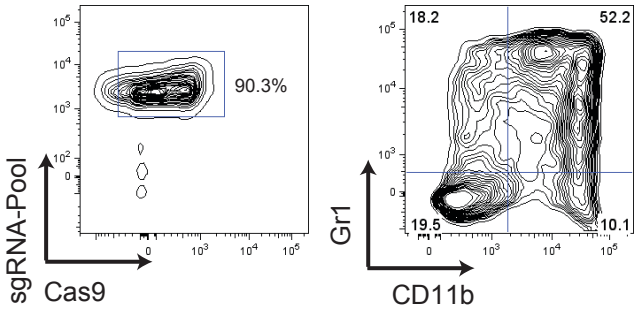
Supplementary Figure 17 - Characterization of a sgRNA:Cas9 induced myeloid leukemia in a Flt3-ITD heterozygous mouse

A) Flow cytometric assessment of sgRNA:Cas9 vector expression (eGFP), sgRNA-only vector expression (RFP657) (left) and myeloid lineage marker expression (right) in a leukemic mouse in a leukemic mouse with leukocytosis (40.4x10^6/ml) and splenomegaly (533mg). Expression of myeloid markers (right).

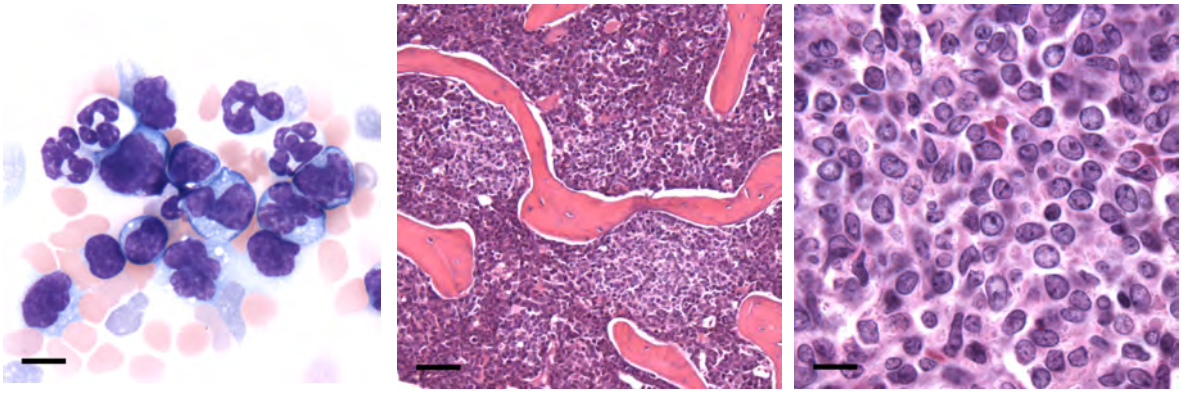
B) High magnification peripheral blood smear (MGG, 1000x) of the leukemic mouse (left). Low magnification (200x, HE) BM histology (middle) and high magnification BM histology 1000x, HE) (right) of the leukemic mouse. Scale bar (25µm for 200x; 5µm for 1000x) are given in the lower left.

C) Mutation analysis on targeted genomic regions showed mutations in *Runx1*, *Nf1*, and *Ezh2.*
PAM underlined

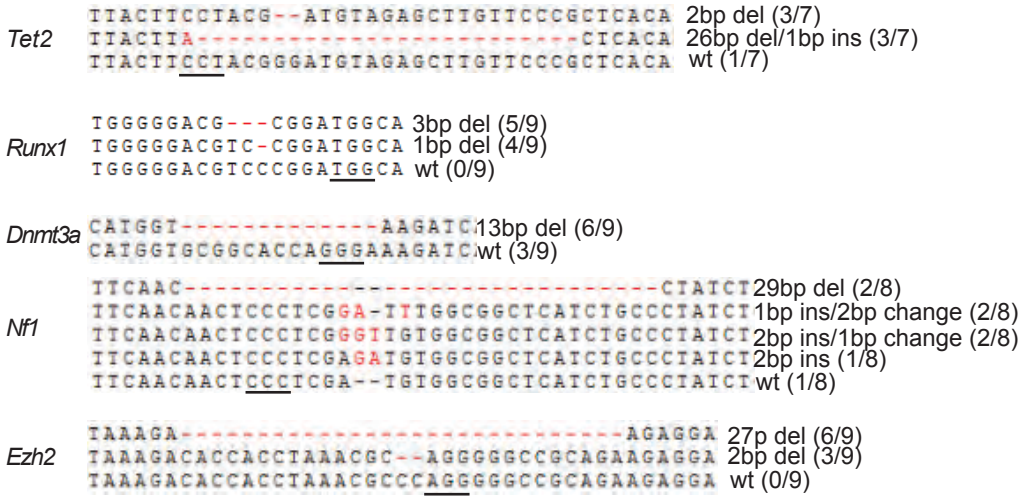
A)



B)



C)



Supplementary Figure 18 - Characterization of a sgRNA:Cas9 induced myeloid leukemia in a Flt3-ITD heterozygous mouse with

A) Flow cytometric assessment of sgRNA:Cas9 vector expression (eGFP), sgRNA-only vector expression (RFP657) (left) and myeloid lineage marker expression (right) in a leukemic mouse in a leukemic mouse with leukocytosis (78.1^6/ml) and splenomegaly (1036mg) and myeloid marker expression. Expression of myeloid markers (right).

B) High magnification peripheral blood smear (MGG, 1000x) of the leukemic mouse (left). Low magnification (200x, HE) BM histology (middle) and high magnification BM histology 1000x, HE) (right) of the leukemic mouse. Scale bar (25µm for 200x; 5µm for 1000x) are given in the lower left.

C) Mutation analysis on targeted genomic regions showed mutations in *Tet2*, *Runx1*, *Dnmt3a*, *Nf1*, and *Ezh2*.

PAM underlined

Supplementary Table 1 – Target sites and efficacies

Targeted Gene	Targeted Sequence	Efficacy (Reporter)
Runx1	GCACTGGGGGACGTCCCGGATGG	0.17
Runx1	CCAGCGACACCCATTTCACCCGG	0.20
Runx1	CCGTGCCAGCGGCATGACCAGCC	0.81
Tet2	CCGTGCAGAGTACCGCATCTCAT	0.66
Tet2	AATACTATCCTAGTTCCGACCGG	0.10
Tet2	GAACAAGCTCTACATCCCGTAGG	0.11
Dnmt3a	GTGGGCATGGTGCGGCACCAGGG	0.44
Dnmt3a	CACC GCATGATGCGCGGCCCAAGG	0.50
Dnmt3a	CACC GCTTACCAGTATGACGACGA	0.86
Nf1	CCAGTTACCGGGACCGCTCCTTC	1.03
Nf1	CCCTCGATGTGGCGGCTCATCTG	0.55
Nf1	CCTCGATGTGGCGGCTCATCTGC	0.66
Ezh2	CTGAGAAGGGACCGGTTTGTTGG	0.77
Ezh2	CCGGTTTGTTGGCGGAAGCGTGT	1.10
Ezh2	AAGACACCACCTAAACGCCCAGG	0.61
Smc3	AAAGCTGATAAAGCGGCAAGAGG	0.34
Smc3	CCATCGAGGTGCTCTGACTGGAG	0.39
Asxl1	CAACAGTGCCATTCGAGGCCAGG	0.88
Asxl1	GATACTAAAACCGACTTAGCAGG	1.01
Asxl1	AGCCCAAAGTCCCGCCCATCCGG	1.07
p53	AGAAGAAAATTTCCGCAAAAAGG	1.01
p53	CCCTGAACTGCCCCCAGGGAGCG	1.10
p53	CCACTACAAGTACATGTGTAATA	1.11

PAM sequence underlined

bold: target sites used for in-vivo experiments

Efficacy describes the relative fluorescence compared to control target site

Supplementary Table 2 – Genotyping of methylcellulose colonies

	Primary genotype								
	Tet2	Runx1	Dnmt3a	Nf1	Ezh2	Smc3	Colonies with primary genotype	Colonies wt	Colonies with divergent genotype
Figure3	wt	wt	181bp del	36bp del / 1bp del	5bp del / 9 bp del	33bp del	5/5	0/5	0/5
Suppl. Figure 15	wt	1bp ins	wt	1bp del / 25 bp del	53 bp del / wt	wt	4/5	1/5	0/5

Supplementary Table 3 – Off-target sites of sgRNA target sites used for in-vivo studies

target	guide sequence	on-target locus	off target name	off target sequence	mismatches	UCSC gene	locus	genic location
Runx1.1	GCACTGGGGGACGTCCCGGA**TGG**	chr16:+92689348	Runx1_OT1	GCCCTGGGGGATGTGCCGGATGG	3MMs [3:12:15]	NM_019732	chr4:+134711201	Runx3 exon
			Runx1_OT2	GCCCTGGGGACCGTCCGGGAGAG	4MMs [3:10:11:17]	NM_011801	chr8:+114377958	Cfdp1 intron,CpG island
			Runx1_OT3	GGACTGGTGGAGGTTCCGGATGG	4MMs [2:8:12:15]	NM_001136058	chr5:-37680166	Crmp1 exon
			Runx1_OT4	GGACGGCGGGACGGCCCGGAGAG	4MMs [2:5:7:14]	NM_145837	chr14:+58143823	Il17d intron, CpG island
			Runx1_OT5	GCTCTGGGGGCCCTCCCGGGGAG	4MMs [3:11:13:20]	NM_027711	chr13:+96661671	Iqgap2 exon, CpG island
Nf1.2	CAGATGAGCCGCCACATCGA**GGG**	chr11:-79360625	Nf1_OT1	CCGAGGAGCCGCAACATCGTAAG	4MMs [2:5:13:20]	NM_177213	chr7:-127505021	Abca15 exon
			Nf1_OT2	GAGATGAGCAGCGACATCAATGG	4MMs [1:10:13:19]	NM_178017	chr8:-77547985	Hmgxb4 exon
			Nf1_OT3	CAGGTGAGCTGCAACATCTACGG	4MMs [4:10:13:19]	NM_001201378	chr6:+29355866	Ccdc136 exon
			Nf1_OT4	CAGATGATCGGGCAGATCGATGG	4MMs [8:10:12:15]	NM_019694	chr5:-34087729	Letm1 exon
			Nf1_OT5	CAGCTGGGCCCCCACATTGACGG	4MMs [4:7:11:18]	NM_023633	chr12:-85293126	R2410016O06Rik exon
Tet2.4	GAACAAGCTCTACATCCCGT**AGG**	chr3:+133149224	Tet2_OT1	GGACAAGTTCCACATCCCGCAAG	4MMs [2:8:11:20]	NM_146086	chr18:+61416955	Pde6a exon
			Tet2_OT2	GAAGCAGCTCTCCATCCCTTCGG	4MMs [4:5:12:19]	NM_145741	chr14:-34745345	Gdf10 exon
			Tet2_OT3	GAAGAAGCTCAGCATCTCGTCAG	4MMs [4:11:12:17]	NM_028816	chr7:+133252384	Xpo6 exon
			Tet2_OT4	GAGGAAGCTCTACATCCTGGAGG	4MMs [3:4:18:20]	NM_031257	chr8:-26182382	Plekha2 intron
			Tet2_OT5	TACCAAGCTCTACATCCTCTTGG	4MMs [1:3:18:19]	NM_199252	chr17:-13302577	Unc93a exon
Smc3.3	CTCCAGTCAGAGCACCTCGA**TGG**	chr19:-53708484	Smc3_OT1	CTCAAGTCAGAGAACCTCGCGGG	3MMs [4:13:20]	NM_198642	chr14:-75415982	5031414D18Rik gene, CpG island
			Smc3_OT2	CCCCACACACAGCACCTCGACAG	4MMs [2:6:7:10]	NM_144803	chr14:+66771517	Chrna2 exon
			Smc3_OT3	CTTCAGGCTGAGCACCTCGCAGG	4MMs [3:7:9:20]	NM_011904	chr19:-41163120	Tll2 intron, CpG island
			Smc3_OT4	CTCCAGAAAGAACACCTCGCTAG	4MMs [7:8:12:20]	NM_023878	chr14:-119260844	Cldn10 intron
			Smc3_OT5	ACCCAGTCTGAGCACCTGGAAGG	4MMs [1:2:9:18]	NM_001080815	chr7:+19744847	Gipr intron-exon
Dnmt3a.1	GTGGGCATGGTGCGGCACCA**GGG**	chr12:+3901625	Dnmt3_OT1	GTGAGCCAGGTGGGGCACCACAG	4MMs [4:7:8:13]	NM_013908	chr2:+25359855	Fbxw5 intron
			Dnmt3_OT2	GTGGGCAAGGGGCGGCAACACAG	3MMs [8:11:18]	NM_001220499	chr4:-155506697	Rnf223 gene
			Dnmt3_OT3	ATGGGCATGAAGTGGCACCATGG	4MMs [1:10:11:13]	NM_178627	chr15:+82967944	Poldip3 exon, unbalanced
			Dnmt3_OT4	ATGGACATGGGGCGGCACAAAGG	4MMs [1:5:11:19]	NM_201407	chr3:+90072469	Dennd4b, unbalanced
			Dnmt3_OT5	GTGGCCTTGTTGCGTCACCATGG	4MMs [5:7:10:15]	NM_001081302	chr15:-27781515	Trio intron
Ezh2.3	AAGACACCACCTAAACGCCC**AGG**	chr6:+47495847	Ezh2_OT1	CAGCTACCACCCAAACGCCCCAG	4MMs [1:4:5:12]	NM_172434	chr3:+94282696	Celf3 intron
			Ezh2_OT2	GTGACACCCCCTAAACTCCCTAG	4MMs [1:2:9:17]	NM_027627	chr16:+90831218	C21orf63 exon
			Ezh2_OT3	AACTCAGCACCTAAAAGCCCAAG	4MMs [3:4:7:16]	NM_133762	chr12:-117663077	Ncapg2 intro-exon
			Ezh2_OT4	GAGAGACCACATAAAAGCCCAAG	4MMs [1:5:11:16]	NM_001081467	chr5:+134868172	Gtf2ird1 intron
			Ezh2_OT5	AAGACACAGGCTAAAAGCCCGGG	4MMs [8:9:10:16]	NM_027667	chr19:+41847484	Arhgap19 intron

Supplementary Table 4 – Percentages of mutated sequencereads at off-target sites

	WT	Figure 3	Supplementary Figure 12	Supplementary Figure 15	Supplementary Figure 16
Runx1_OT1	0%	0%	0%	0%	0%
Runx1_OT2	0%	0%	0%	0%	0%
Runx1_OT3	0%	0%	0%	0%	0%
Runx1_OT4	0%	0%	0%	0%	0%
Runx1_OT5	0%	0%	0%	0%	0%
Nf1_OT1	0%	0%	0%	0%	0%
Nf1_OT2	0%	0%	0%	0%	0%
Nf1_OT3	0%	0%	0%	0%	0%
Nf1_OT4	0%	0%	0%	0%	0%
Nf1_OT5	0%	0%	0%	0%	0%
Tet2_OT1	0%	0%	0%	0%	0%
Tet2_OT2	0%	0%	0%	0%	0%
Tet2_OT3	0%	0%	0%	0%	0%
Tet2_OT4	0%	0%	0%	0%	0%
Tet2_OT5	0%	0%	0%	0%	0%
Smc3_OT1	0%	0%	0%	0%	0%
Smc3_OT2	0%	0%	0%	0%	0%
Smc3_OT3	0%	0%	0%	0%	0%
Smc3_OT4	0%	0%	0%	0%	0%
Smc3_OT5	0%	0%	0%	0%	0%
Dnmt3_OT1	0%	0%	0%	0%	0%
Dnmt3_OT2	0%	0%	0%	0%	0%
Dnmt3_OT3	0%	0%	0%	0%	0%
Dnmt3_OT4	0%	0%	0%	0%	0%
Dnmt3_OT5	0%	0%	0%	0%	0%
Ezh2_OT1	0%	0%	0%	0%	0%
Ezh2_OT2	0%	0%	0%	0%	0%
Ezh2_OT3	0%	0%	0%	0%	0%
Ezh2_OT4	0%	0%	0%	0%	0%
Ezh2_OT5	0%	0%	0%	0%	0%

Off-target sites (column 1) are listed in supplementary Table 3. Top row indicates where respective leukemias are presented. A C57Bl/6 (WT) control was included.