CRISPR-Cas9-induced t(11;19)/MLL-ENL translocations initiate leukemia in human hematopoietic progenitor cells *in vivo*

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Supplementary Methods

Plasmids and viral particle production

The previously described L-CRISPR vector[1] was modified by introducing CMV-immediate-early- and SV40-enhancers via PCR, enhanced gRNA[2], and an optimized H1 promoter[3]. The CMV immediate early enhancer sequence and SV40 enhancer sequence was synthesized (Integrated DNA Technologies, gBlock Gene Fragment) and inserted upstream of the RSV promoter. The mNeonGreen fluorescent protein[4] was codon optimized for human cells, synthesized (Integrated DNA Technologies, gBlock Gene Fragment) and inserted downstream of the S.pyogenes Cas9 open reading frame with a 2A sequence (P2A). The optimized sgRNA backbone with elongated stem-loop and A/T-switch has been described before[2], was synthesized (Integrated DNA Technologies, gBlock Gene Fragment) and inserted upstream of the EFS promoter. Unique EcoRI and XhoI sites were incorporated downstream of the RNA-Polymerase-III terminator sequence to allow insertion of a second RNA-Polymerase-III/sgRNA-cassette. In summary, these changes yielded the advanced lentiviral CRISPR-Cas9 vector L40C-CRISPR.

The Cas9-P2A-mNeonGreen cassette was replaced with the RFP657 fluorescent protein to generate the SGL40C.EFS.RFP657 vector for sgRNA-only delivery. To replace the human U6 promoter, the H1 promoter with enhanced sgRNA backbone was synthesized (Integrated DNA Technologies, gBlock Gene Fragment) and inserted (SGL40C-H1.EFS.RFP657). The H1 promoter cassette was flanked by a 5' EcoRI site and a 3' XhoI site to allow insertion into the L40C-CRISPR or SGL40C.EFS.RFP657 backbone.

CRISPR-Cas9 target sites were selected using the CCTop selection tool.[5] All vectors contain a short sequence harboring two BsmBI sites that allow cloning of spacer sequences. Spacer sequences were ordered as complementary oligonucleotides, were phosphorylated with T4-Polynucleotide-Kinase at 10µM concentration of each oligo, diluted 500-fold and ligated into the respective vector.

To obtain L-CRISPR-CTN vectors with dual sgRNA expression, tested H1-sgRNA-cassettes were inserted into the L40C-CRISPR vector via EcoRI and XhoI sites. Lentiviral vectors L40C and L-CRISPR-CTN are provided via Addgene (# 69146-69148, 69212, 69215, 89392-89395).

Lentiviral particles were produced by transient transfection of 293 T cells using PEI solution (PEI:DNA=3:1; w/w) (Polysciences). $5x10^6$ cells were plated per a 10cm dish. The constructs were co-transfected with 6µg vector, 2µg pMD2.G and 12µg pSPAX2 next day. After 6 hours medium was changed and the supernatant was collected after 24-48h. To improve viral titers, caffeine at 2mM concentration was added at the medium change 6h post transfection and for all subsequent medium changes, as described before[6].
The virus supernatant was concentrated 100x by ultracentrifugation.

Cell culture

The suspension cell lines HEL, TF1, K562 were cultured in RPMI 1640 supplemented with 10% FCS, 1% Penicillin/Streptomycin, 1% L-glutamine and 1% Sodium Pyruvate. TF1 received additionally 2ng/ml GM-CSF. All cells were maintained at 37°C in 5% humidified CO_2.

CD34 culture and transduction

For transduction, $CD34^+$ cells were prestimulated 48 hours before transduction with StemSpan supplemented with 1% Penicillin/Streptomycin, SCF (100ng/ml), FLT3 (100ng/ml), IL-6 (20ng/ml), THPO (50ng/ml), SR1 (750nM), and Ciprobay (10µg/ml). The HSPCs were transduced with virus corresponding to MOIs of 60/100 and hexadimethrin bromide (2µg/ml) (Polybrene, Life Technologies) on retronectin coated plates (Takara). 48 hours post transduction, the HSPCs were sorted by fluorescent activated cell sorting. After sorting the cells were cultured in IMDM supplemented with 10%FCS, 1% Penicillin/Streptomycin, 1% Natrium Pyruvate, SCF (25ng/ml), FLT3L (25ng/ml), IL-3 (10ng/ml), IL-6 (10ng/ml), THPO (20ng/ml), and Ciprobay (10µg/ml).
Colony-forming assays were performed using human methylcellulose base media (HSC003, R&D Systems or HSC002, R&D Systems supplemented with 50 ng/ml SCF, 10 ng/ml GM-SCF, 10 ng/ml IL-3, 3U/ml Epo, 1,5% Penicillin/Streptomycin and Ciprobay (10µg/ml)). 5,000 sorted GFP+ cells were plated. Colonies were counted and 30.000 cells if available were replated after 7-14 days.

References (Supplementary Methods)

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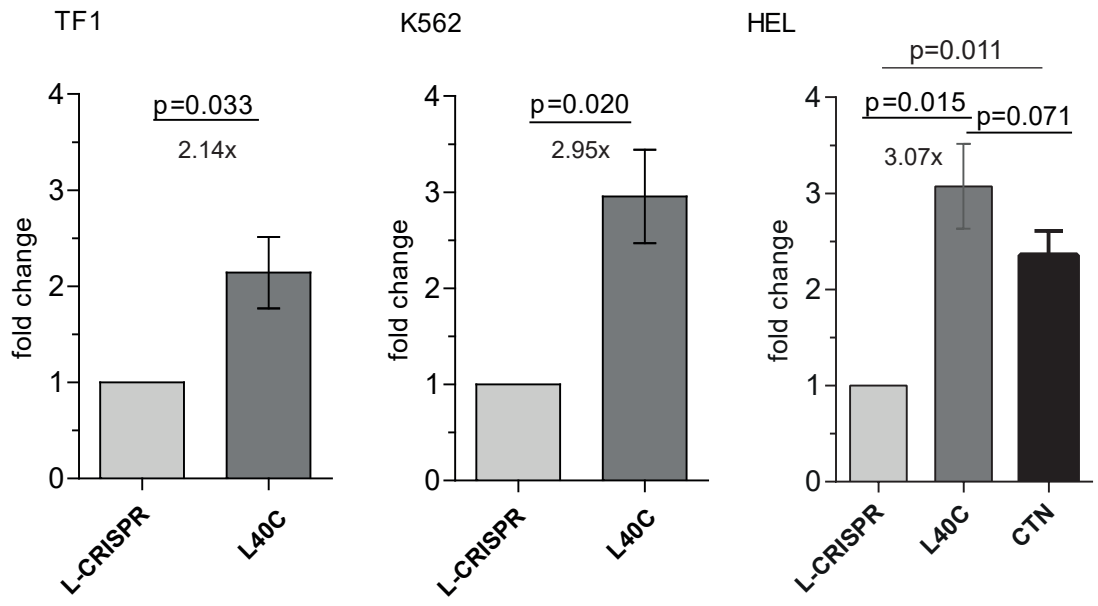
Supplementary Figure 1



Supplementary Figure 2

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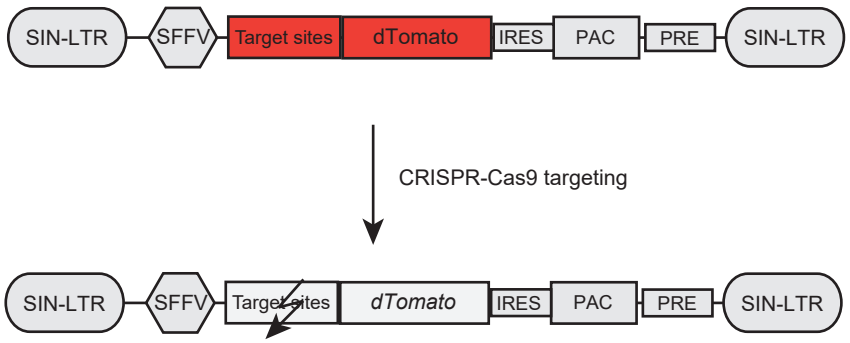

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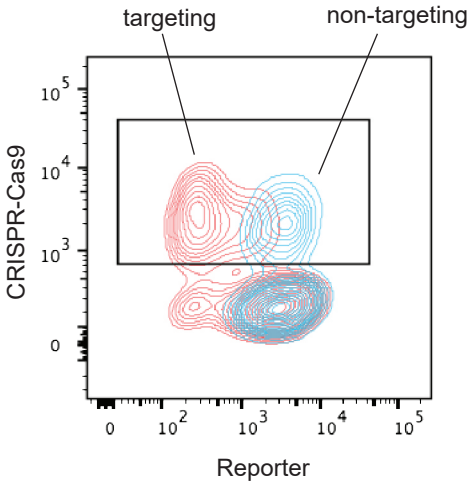

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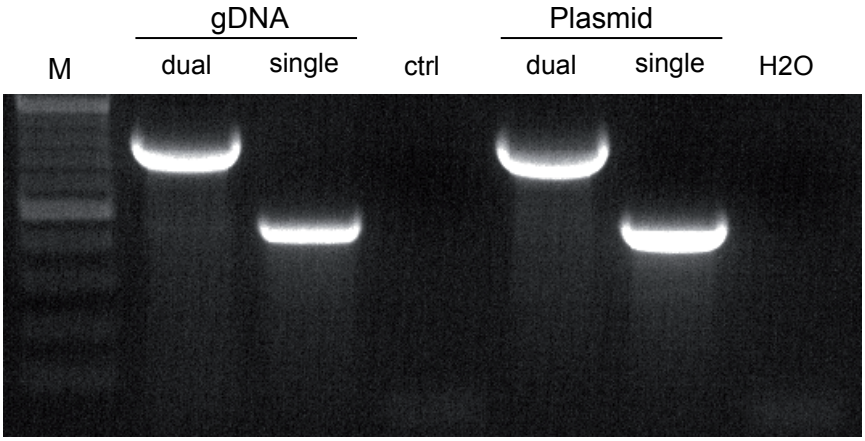

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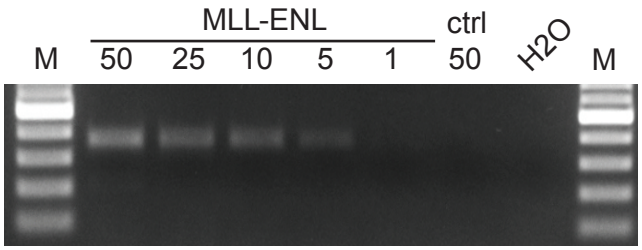

B

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clone #1   ACTGGGGGAATGAATAAGAACTCCCATTAGCAGGTGGGTTTAGCGGAGGGAGCTGGGCTGCCCAGGCCACGGATCCACCTGGGACCGGTCACCAG
clone #2   ACTGGGGGAATGAATAAGAACTCCCATTAGCAGGTGGGTTTAG------GAGCTGGGCTGCCCAGGCCACGGATCCACCTGGGACCGGTCACCAG
clone #3   ACTGGGGGAATGAATAAGAACTCCCATTAGCAGGTGGGTTTAGG-----GAGCTGGGCTGCCCAGGCCACGGATCCACCTGGGACCGGTCACCAG
clone #4   ACTGGGGGAATGAATAAGAACTCCCATTAGCAGGTGGGTT----------TAGGCTGCCCAGGCCACGGATCCACCTGGGACCGGTCACCAG
clone #5   ACTGGGGGAATGAATAAGAACTCCCATTAGCAGGTGGG-----------AGCTGGGCTGCCCAGGCCACGGATCCACCTGGGACCGGTCACCAG
clone #6*  ACTGGGGGAATGAATAAGAACTCCCATTAGCAGGTGGG----------------------------------------ACCGGTCACCAG
clone #7   ACTGGGGGAATGAATAAGAACTCCCATTAGCAGGTGGG----------------CTGCCCAGGCCACGGATCCACCTGGGACCGGTCACCAG
clone #8   ACTGGGGGAATGAATAAGAACTCCCATTAGCAGG----------AGGGAGCTGGGCTGCCCAGGCCACGGATCCACCTGGGACCGGTCACCAG
clone #9   ACTGGGGGAATGAATAAGAACTCCCATTAGCAG----------AGGGAGCTGGGCTGCCCAGGCCACGGATCCACCTGGGACCGGTCACCAG
clone #10  ACTGGGGGAATGAATAAGAACTCCCATTAGCAGGTGGG--------AGGGAGCTGGGCTGCCCAGGCCACGGATCCACCTGGGACCGGTCACCAG
clone #11  ACTGGGGGAATGAATAAGAACTCCCATTAGCAGG----------TGGGCTGCCCAGGCCACGGATCCACCTGGGACCGGTCACCAG
clone #12  ACTGGGGGAATGAATAAGAACTCCCATTAGCGG----------AGGGAGCTGGGCTGCCCAGGCCACGGATCCACCTGGGACCGGTCACCAG
clone #13  ACTGGGGGAATGAATAAGAACTCCCATTAGCA----------GGCTGCCCAGGCCACGGATCCACCTGGGACCGGTCACCAG
clone #14  ACTGGGGGAATGAATAAGAACTCCCATTAGCA----------GGATCCACCTGGGACCGGTCACCAG
clone #15  ACTGGGGGAATGAATAAGAACTCCCATT----------ACGGATCCACCTGGGACCGGTCACCAG
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C

```
MLL-ENL    ACTGGGGGAATGAATAAGAACTCCCATTAGCAGGTGGGTT----TA-GCGGA-----GGGAGCTGGGCTGCCCAGGCCACGGGTCCACCTGGGACCGGTCACCAG
clone #16  ACTGGGGGAATGAATAAGAACTCCCATTAGCAGGTGGGTTACCATAAGCTCCCTCCTGGGAGCTGGGCTGCCCAGGCCACGGATCCACCTGGGACCGGTCACCAG
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Supplementary Figure 5

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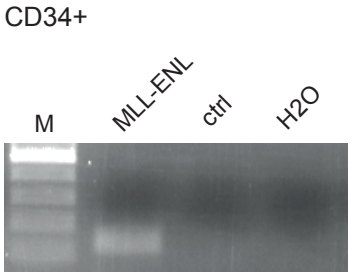

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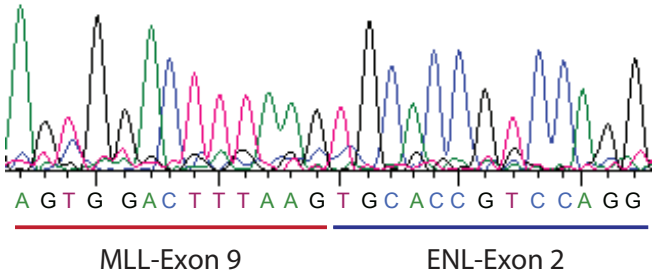

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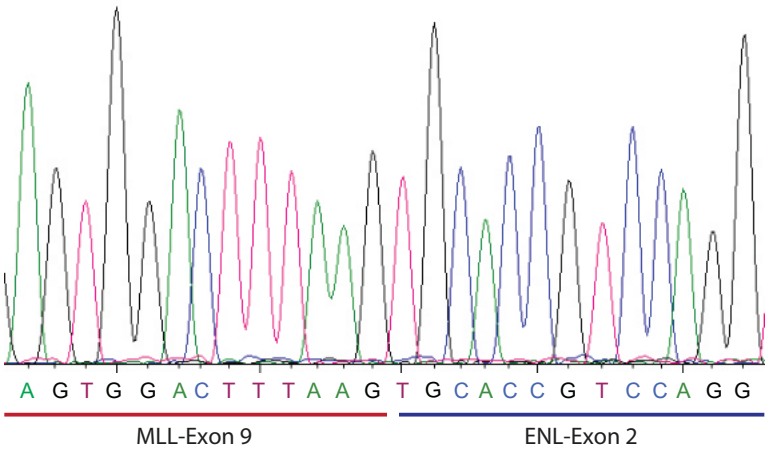

B

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MLL-Intron9   GAACTCCCATTAGCAGGTGGGTTTAG▶
ENL-Intron1                            CGGAGGGAGCTGGGCTGCCCAGGC
MLL-ENL       GAACTCCCATTAGCAGGTGGGTTTAGCGGAGGGAGCTGGGCTGCCCAGGC
Colony Assay  GAACTCCC-TT-GTATTTCAG---AGCAAAGGGAGCTGGGCTGCCCAGGC
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Supplementary Figure 7

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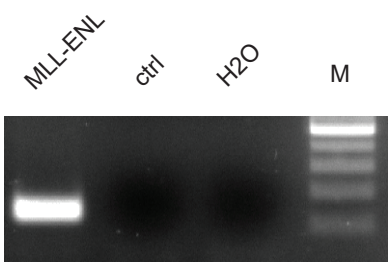

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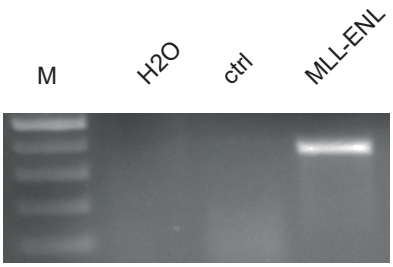

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MLL-Intron9      AATAAGAACTCCCATTAGCAGGTGGGTTTAG
ENL-Intron1                                    CGGAGGGAGCTGGGCTGCCCAGGCCACGGGT
MLL-ENL          AATAAGAACTCCCATTAGCAGGTGGGTTTAGCGGAGGGAGCTGGGCTGCCCAGGCCACGGGT
MLL-ENL-liquid   AATAANAACTCCCATTAGCAGGT----------GGGAGCTGGGCTGCCCAGGCCACGGGT
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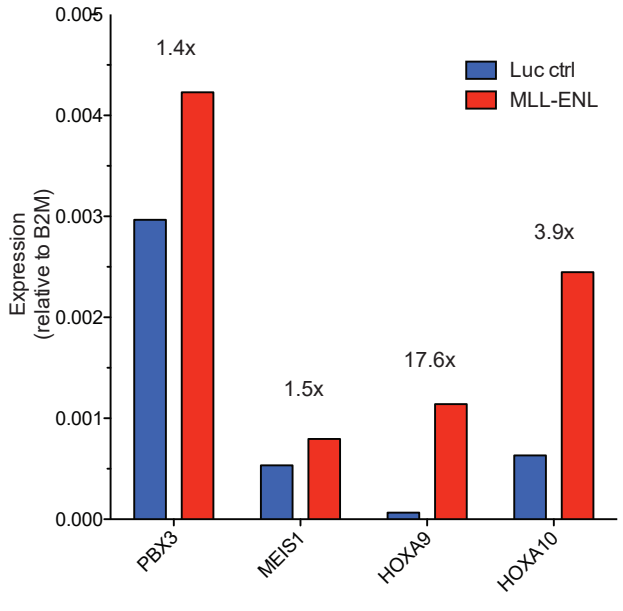
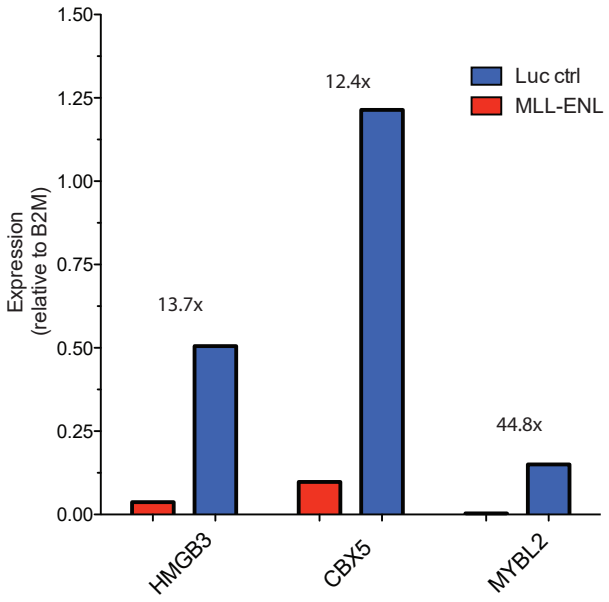
D





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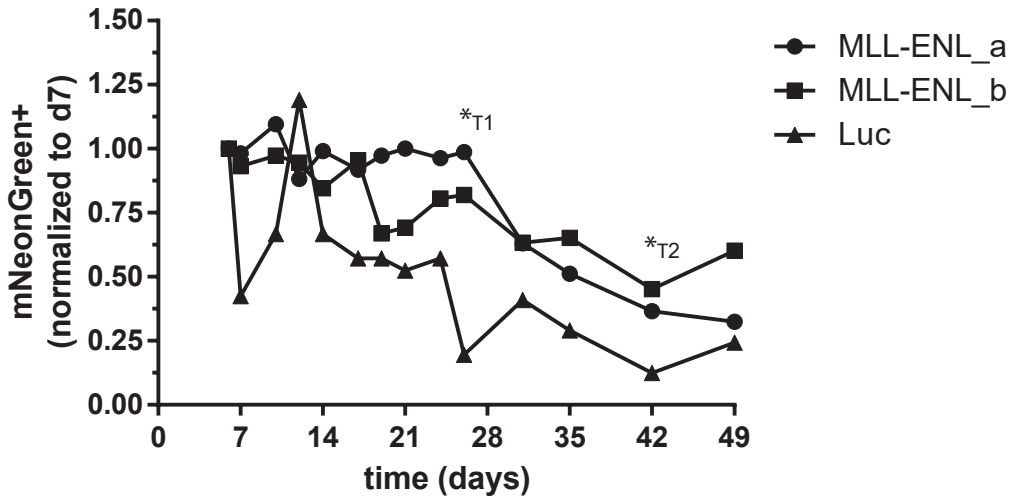

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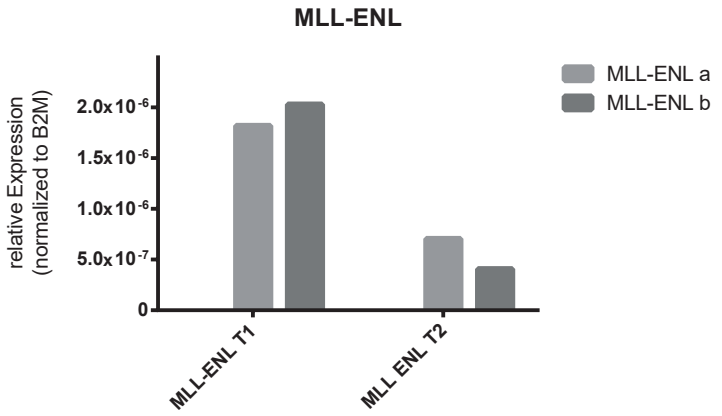

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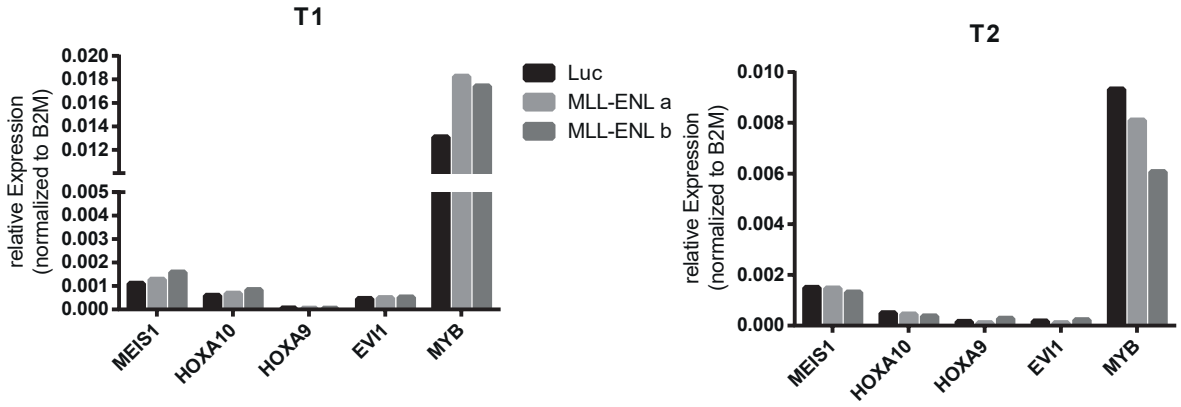

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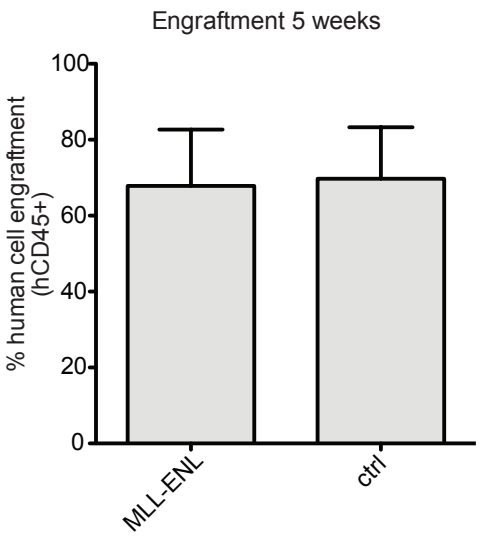

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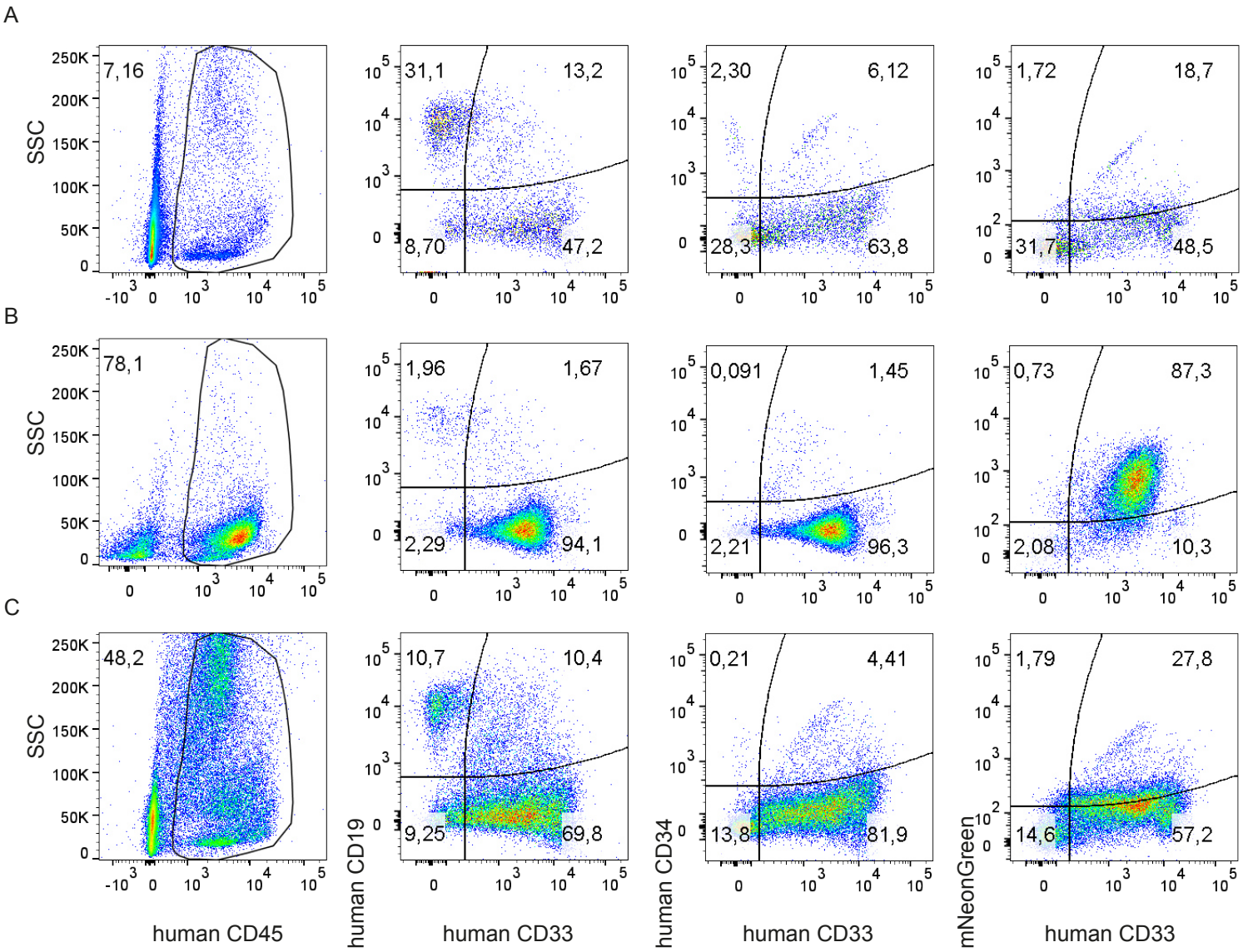

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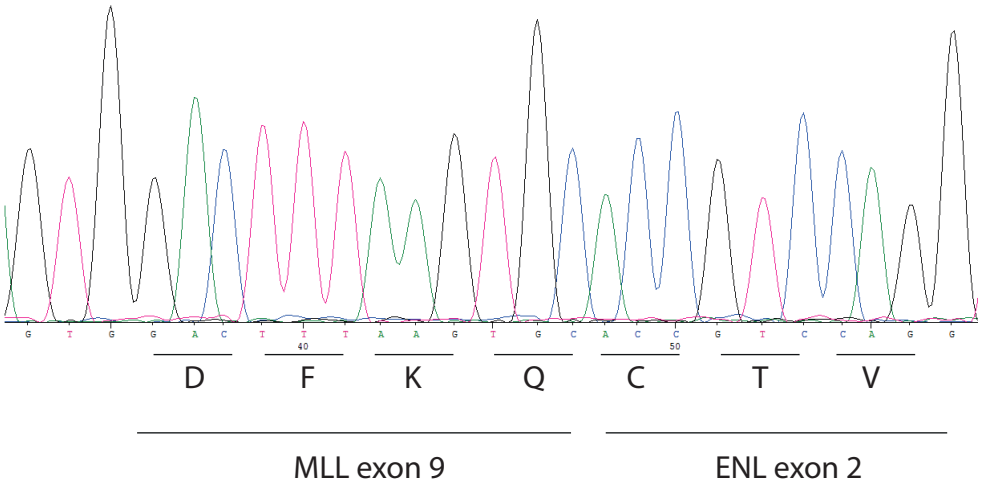

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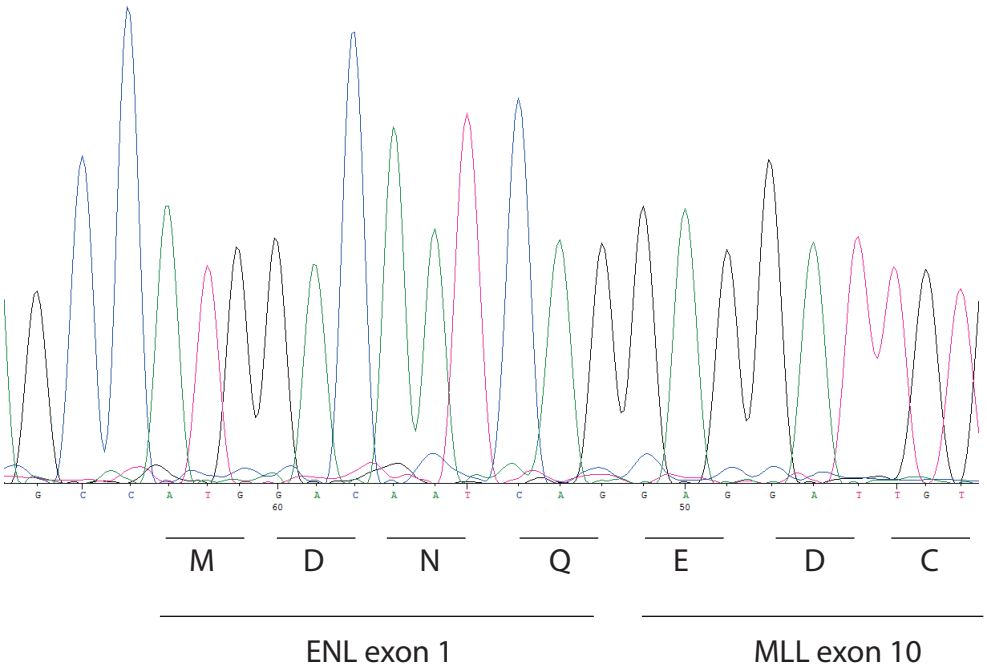

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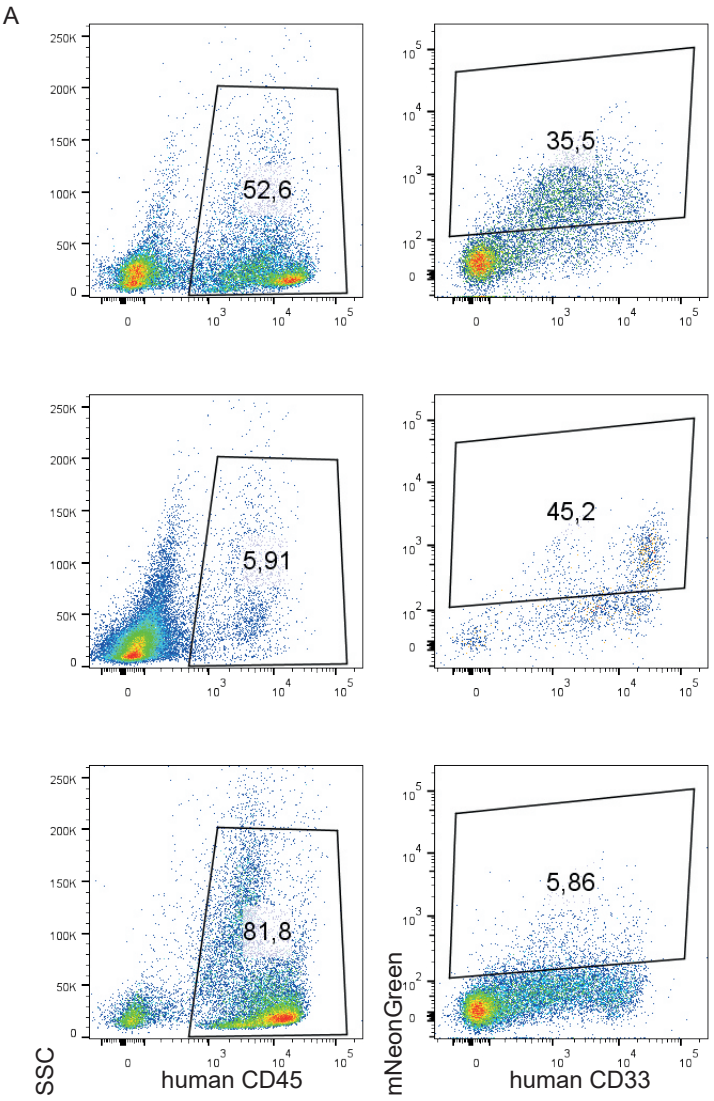

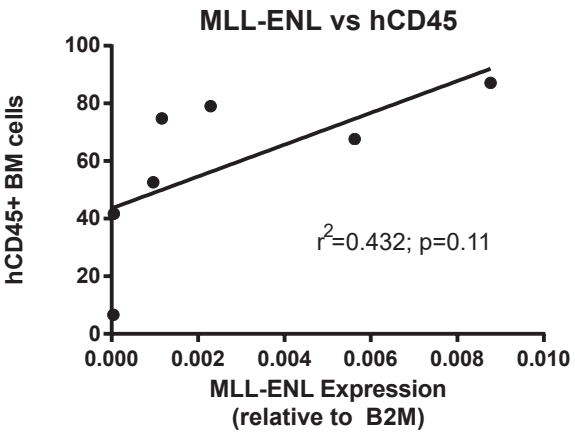

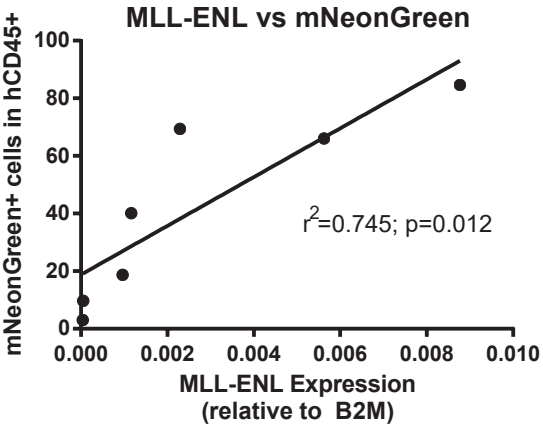

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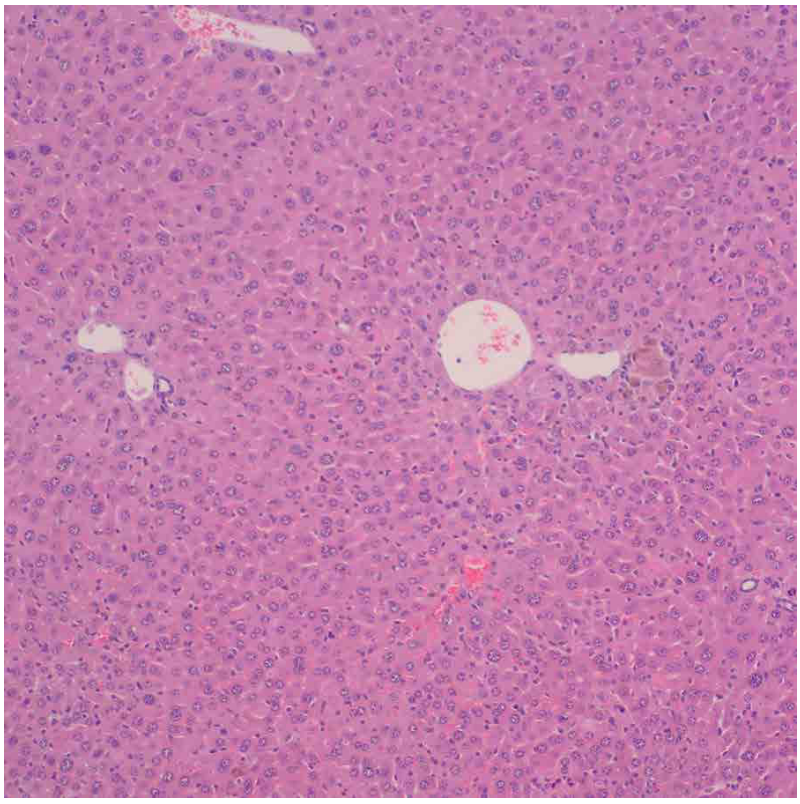

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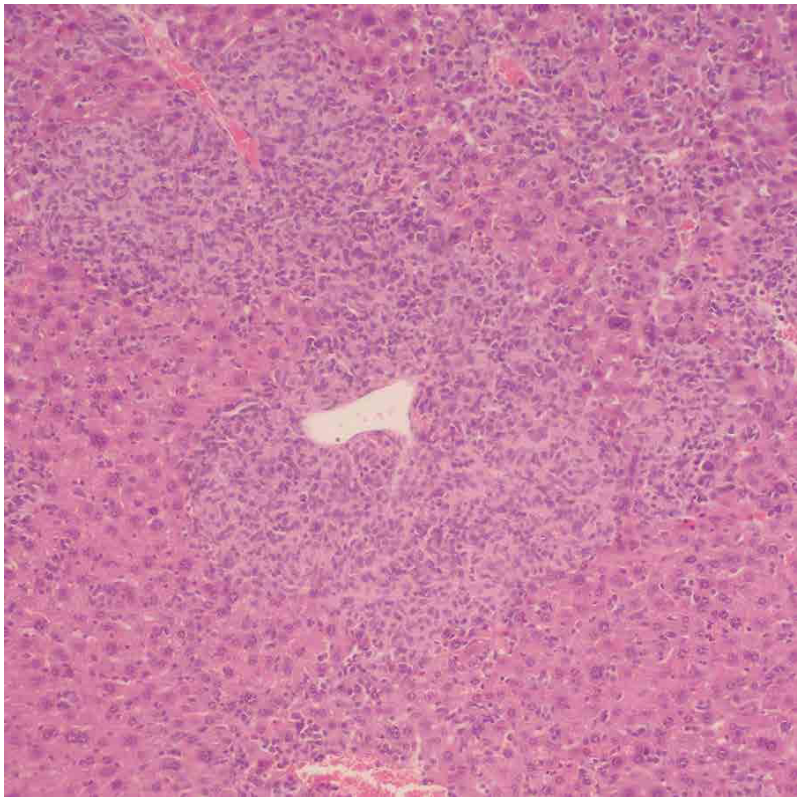

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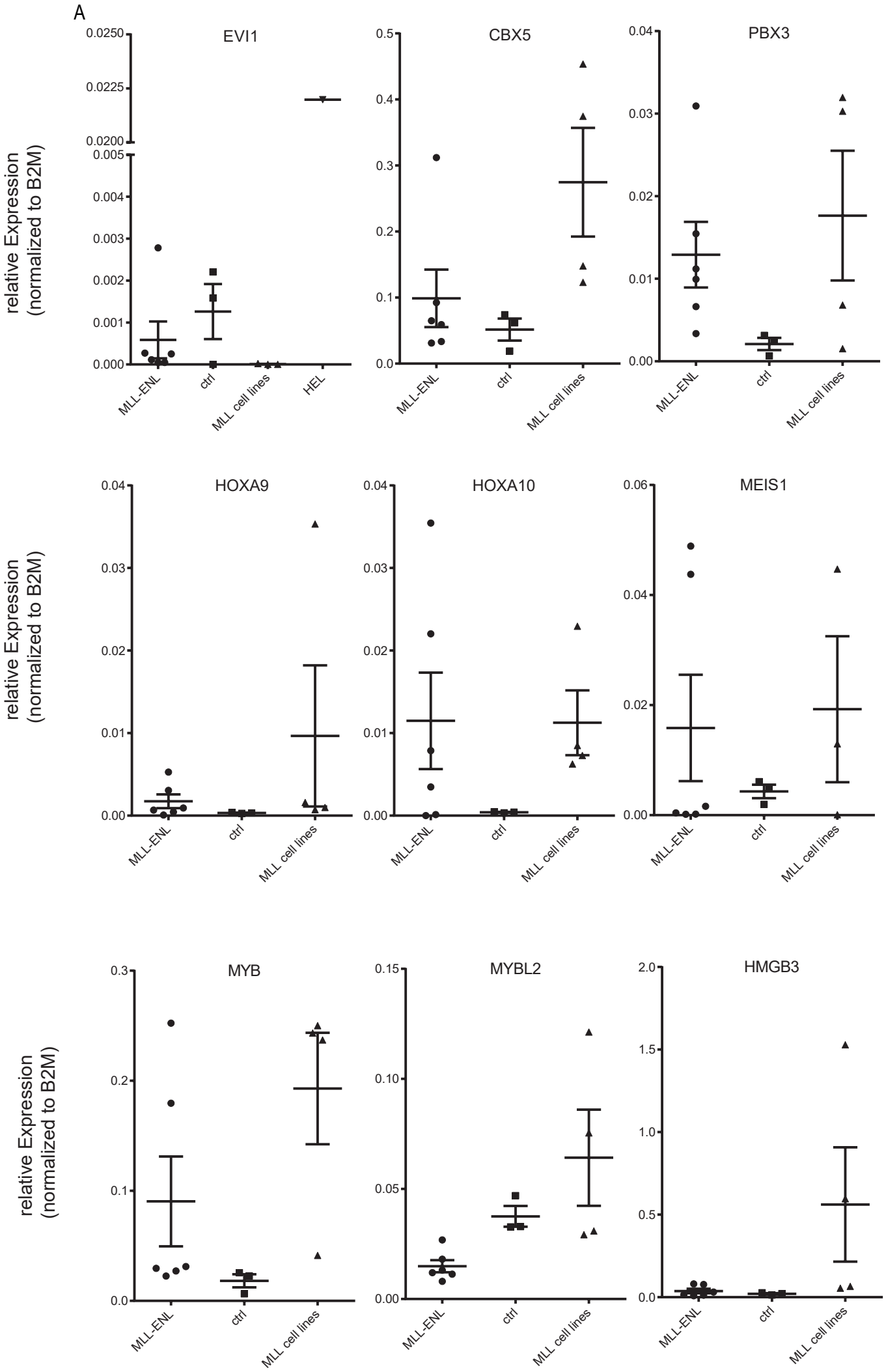

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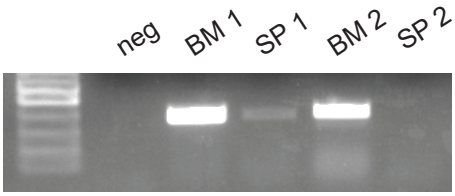

B

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MLL      GGAATGAATAAGAACTCCCATTAGCAGGTGGGTTTAG▶
ENL                                          ▮CGGAGGGAGCTGGGCTGCCCAGGCCACGGGTCCACCT
MLL-ENL  GGAATGAATAAGAACTCCCATTAGCAGGTGGGTTTAGCGGAGGGAGCTGGGCTGCCCAGGCCACGGGTCCACCT
BM 1     GGAATGAATAAGAACTCCCATTAGC----------------------AGCTGGGCTGCCCAGGCCACGGATCCACCT
BM 2     GGAATGAATAAGAACTCCCATTAG-----------------GAGGGAGCTGGGCTGCCCAGGCCACGGATCCACCT
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Supplementary Figure 1 – Comprehensive testing of optimized lentiviral CRISPR-Cas9 vectors for increased viral titer

Three different cell lines (TF1, K562 and HEL) were transduced with two different lentiviral CRISPR-Cas9 vectors. L40C vector includes an additive CMV, SV40 enhancer. The titer was measured by flow cytometry. Three independent sgRNAs were tested in three biological replicates on three cell lines (n=27). The titer of the improved L40C vector was normalized on L-CRISPR titer. L40C shows significant improvement of the titers in all tested cell lines, as indicated above (Student's t-test, two sided, unpaired). L-CRISPR-CTN titers were compared to L40C titers (HEL cells).

Supplementary Figure 2 – Fluorescent reporter-based sgRNA testing for selection of high knock-out rate CRISPR-Cas9 constructs

A) Schematic presentation of fluorescent reporter-based testing of sgRNAs. CRISPR-Cas9 target sites are integrated in-frame into the open reading frame of a fluorescent protein cDNA generating a fusion protein to label positive cells. Bi-cistronic co-expressed of a Puromycin acetyl transferase (PAC) gene by an internal ribosomal entry site allows generation of purified reporter cell lines with Puromycin. CRISPR-Cas9 targeting of the incorporated target sites followed by cellular NHEJ causes frame-shift mutations in the cDNA and loss of reporter fluorescence in the cell line, which can be quantified to assess knock-out efficacy and comparison of individual sgRNAs. B) Representative flow cytometry of a reporter based sgRNA testing. Delivery of the CRISPR-Cas9 components indicated on the Y-axis; reporter fluorescence marked on the X-Axis. Targeting sgRNA with reduced reporter fluorescence marked in red; non-target sgRNA with constant reporter fluorescence marked in red.

Supplementary Figure 3 – Stable transfer of Dual-sgRNA lentiviral CRISPR-Cas9 vectors for generation of chromosomal translocations

To test genetic stability of dual sgRNA delivery, HEL cells were transduced with L-CRISPR-CTN (dual) vectors or L40C (single). PCR was performed on genomic DNA of transduced HEL cells and on plasmids DNA of the corresponding vectors. The results show recombination free integration of the dual-sgRNA vector after reverse-transcription, compared to single-sgRNA vectors and plasmid DNA. Ctrl= non-transduced HEL genomic DNA.

Supplementary Figure 4 – Analysis of genomic MLL-ENL breakpoints in K562 cells

A) The genomic/intronic MLL-ENL translocation breakpoints generated by the CRISPR-Cas9 system in K562 cells were analyzed by PCR with limiting amounts of DNA (amount in ng indicated above each lane). PCR products were subcloned and sequenced. Alignment of the sequences obtained compared to the predicted MLL-ENL breakpoint are shown: B) the majority of clones presented with deletions at the breakpoint. Sequences detected twice are indicated (*); C) detected insertions.

At 100ng input DNA approximately equal to 17,000 diploid cells, 17 out of 18 analyzed bacterial clones presented with individual breakpoints, indicating polyclonal induction of MLL-ENL translocations with a minimum frequency of 0.1%.

Supplementary Figure 5 – Detection and verification of MLL-ENL transcript in HSPC colony forming unit assay

A) Representative RT-PCR based detection of MLL-ENL expression in CD34+ HSPCs at the first replating. B) Verification of the MLL-ENL fusion mRNA with Sanger sequencing. MLL and ENL exonic sequences are indicated

Supplementary Figure 6 – Verification of the MLL-ENL translocation expression and breakpoint after clonal outgrowth of HSPC in colony forming unit assays

A) Sequence trace of the MLL-ENL transcript in CD34+ HSPCs at the fifth replating. B) Alignment of the genomic breakpoint detected in CD34+ HSPCs at the fifth replating.

Supplementary Figure 7 – Clonal outgrowth of MLL-ENL expressing cells in liquid culture

A) Detection of MLL-ENL expression in CD34+ HSPCs in liquid culture. ctrl = MLL-I9-#1 + anti-Luciferase sgRNAs; M=Marker. B) Detection of the genomic MLL-ENL breakpoint in CD34+ HSPCs in liquid culture. ctrl = MLL-I9-#1 + anti-Luciferase sgRNAs; M=Marker. C) Sequence alignment of the genomic breakpoint compared to the predicted breakpoint, the MLL-Intron9 and ENL-Intron1 sequence. Deletions indicate NHEJ induced scaring. ctrl = MLL-I9-#1 + anti-Luciferase sgRNAs; M=Marker. D) Quantitative PCR based testing of MLL target genes in MLL-ENL expressing cells derived from the liquid culture compared to control cells (ctrl= MLL-I9-#1 + anti-Luciferase sgRNAs).

Supplementary Figure 8 – Tracking of vector expression and MLL target gene expression in CD34+ cells *in vitro*

A) CB CD34+ cells were transduced with L-CRISPR-CTN(11;19) (MLL-ENL_a, MLL-ENL_b) or L-CRISPR-CTN(ctrl) (Luc) and analyzed via flow cytometry for mNeonGreen expression over time. Results were normalized to day7. Samples for qRT-PCR analysis were taken at indicated timepoints (*T1, *T2). B) Analysis of MLL-ENL (relative to B2M) expression at indicated timepoints. C) Analysis of MLL target gene expression at the indicated timepoints (relative to B2M).

Supplementary Figure 9 – Flow cytometry based detection of human cell engraftment 5 weeks post transplantation

Percentage of human cells in the peripheral blood of NSGS mice after transplantation with CD34+ HSPCs transduced with lentiviral CRISPR-Cas9 vectors for the induction of MLL-ENL or control.

Supplementary Figure 10 – Flow cytometry based analysis of immunophenotypes in diseased NSGS mice

A) Immunophenotype of bone marrow cells from a mouse transplanted with control vector transduced $CD34^+$-HSPCs at week 25 post transplantation. B)/C) Immunophenotype of bone marrow cells from two mice transplanted with t(11;19)/MLL-ENL vector transduced $CD34^+$-HSPCs. Percentages and markers are indicated.

Supplementary Figure 11 – Sequence and reading frame validation of MLL-ENL and ENL-MLL cDNA derived from diseased mice

A) MLL-ENL transcript (electropherogram) with indicated reading frame (single letter amino acid code) and respective MLL and ENL Exons B) ENL-MLL transcript (electropherogram) with indicated reading frame (single letter amino acid code) and respective ENL and MLL Exons

Supplementary Figure 12 – Flow cytometry based analysis of vector expression in xenotransplated mice

A) Expression of mNeonGreen in non-diseased mice transplanted with L-CRISPR-CTN(11;19) transduced cells. B) Correlation of MLL-ENL expression (qRT-PCR relative to B2M) and human (CD45+) cell content (BM) in xenotransplanted mice. Linear correlation was assumed and r^2 calculated. C) Correlation of MLL-ENL expression (qRT-PCR relative to B2M) and vector expressing (mNeonGreen+) human (CD45+) cell content (BM) in xenotransplanted mice. Linear correlation was assumed and r^2 calculated.

Supplementary Figure 13 – Histopathological analysis of secondary recipients transplanted with primary monocytic leukemia-like cells or control vector transduced samples

Histopathological analysis of liver tissue from a secondary recipient transplanted with control vector transduced cells (ctrl = MLL-I9-#1 + Luc sgRNAs) (top) and from a mouse with monocytic leukemia-like disease transplanted with L-CRISPR-CTN(11;19) containing $CD34^+$-HSPCs (bottom) (HE, 100x).

Supplementary Figure 14 – qRT-PCR analysis of MLL fusion oncogene target genes in xenotransplanted mice with detectable MLL-ENL expression

Quantitative real time PCR of indicated genes relative to the housekeeping gene B2M. Control (MLL cell lines) cell lines ML2, NOMO1, THP1 (not available for MEIS1) and MOLM13 as reference for MLL rearranged acute leukemia. HEL cells as reference for high EVI1 expression. Xenotransplanted mice with L-CRISPR-CTN(11;19) (MLL-ENL) and L-CRISPR-CTN(ctrl) (ctrl) are indicated below each graph.

Supplementary Figure 15 – t(11;19)/MLL-ENL genomic breakpoint analysis in pre-leukemic primary recipients

A) Primary recipients sacrificed 8-10 weeks post transplantation (pre-leukemic) were analyzed for presence of the t(11;19)/MLL-ENL translocation in the bone marrow and spleen. Mice with detectable translocation are shown. B) Clonality analysis in the bone marrow of the pre-leukemic was performed using Sanger sequencing. Alignment to the predicted t(11;19)/MLL-ENL is shown.

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Supplementary Table 1. CRISPR-Cas9 target sites

Targeted gene	Targeted sequence	Knockout efficacy, %
ENL-I1-#1	CACAACGATGTTCTTCAGCG *GGG*	86.06
ENL-I1-#2	GACGCTCAAGCCACGTCAAC *GGG*	63.12
ENL-I1-#3	AGATGCGAAGTTCGGGTGCC *CGG*	23.34
ENL-I1-#4	ACACGACATGTGCTCAGCGG *AGG*	86.30
MLL-I7-#1	TTAAACTCTAGGCTTCGAAC *AGG*	25.93
MLL-I8-#1	TTAGGCTTGGCAAGGCGCAG *CGG*	51.61
MLL-I8-#2	GGTAATCCCAACACTTAGTG *AGG*	77.83
MLL-I9-#1	TTAGCAGGTGGGTTTAGCGC *TGG*	76.64
MLL-I9-#2	GAATAAGAACTCCCATTAGC *AGG*	33.56
MLL-I10-#1	CATGCTAAGTGACCTAAGAG *TGG*	32.01
MLL-I10-#2	ACGGAGTCTCGCTGTCGCCC *AGG*	4.34

italic: PAM sequence
bold: target sites used for experiments

Supplementary Table 2. Off-target sites of sgRNA used in the study

Target	On-target sequences	Off-target name	Off-target sequence
MLL-I9-#1	TTAGCAGGTGGGTTTAGCGC *TGG*	MLL-I9-#1-OT-1	**GCG**GCA**T**GTGGGTTTAGCGC *AGG*
		MLL-I9-#1-OT-2	T**AG**GC**T**G**C**TGGGTTTAGCGC *TAG*
		MLL-I9-#1-OT-3	**CG**AGCA**C**GTGGGT**C**TAGCGC *AAG*
		MLL-I9-#1-OT-4	TTAG**ACA**GTGGGT**G**TAGCGC *AGG*
		MLL-I9-#1-OT-5	TT**G**GC**CA**GTGGGTT**C**AGCGC *TGG*
ENL-I1-#4	ACACGACATGTGCTCAGCGG *AGG*	ENL-I1-#4-OT-1	**GG**A**A**GACA**C**GTGCTCAGCGG *TGG*
		ENL-I1-#4-OT-2	**C**CA**T**G**G**CA**G**GTGCTCAGCGG *GGG*
		ENL-I1-#4-OT-3	ACA**G**GA**TGG**GTGCTCAGCGG *AGG*
		ENL-I1-#4-OT-4	**G**CAC**AG**CATGT**A**CTCAGCGG *TGG*
		ENL-I1-#4-OT-5	ACACG**G**CA**G**GTGC**C**CAGCGG *TGG*

italic: PAM sequence
bold: mismatches compared to on-target sites

Supplementary Table 3. Primers

	Primer name	Primer sequence
rt-PCR	MLL_exon9_fw	AGGAGAATGCAGGCACTTTGA
	ENL_exon2_rev	CCAGCTCTAACCTCACCTGGA
	ENL_exon1_fw	CCAGCCATGGACAATCAG
	MLL_exon10_rev	CTGGCACAGAGAAAGCAAAC
genomic DNA PCR	MLL_intron9_fw	GCAGATGGAGTCCACAGGAT
	ENL_intron1_rev	GGTGTCTCTGCCACCTAAGC
	ENL_intron1_fw	ATTTGATAGCGGGTGTCAGG
	MLL_intron9_rev	TGCTCAGGATGGATAGGAATG
T7-Endonuclease-I Assay	MLL_on_fw	CACCTCCGGTCAATAAGCAG
	MLL_on_rev	GCTTCACAATCCTCCTGTGGA
	MLL_OT-1_fw	TCAAGGAGCAGGCACATTGT
	MLL_OT-1_rev	CTTAGTGGCCGTGAGAGTCC
	MLL_OT-2_fw	TGGAGGGAAGAGAGCAGGAA
	MLL_OT-2_rev	ATAGCAAGTGCAAAGGCCCA
	MLL_OT-3_fw	GTAGGGGTGAGGGGAGTAGG
	MLL_OT-3_rev	TAAATGTCCTGCCGGCTCTG
	MLL_OT-4_fw	TCCTGCCATATCCACCTCCA
	MLL_OT-4_rev	GGAATCTCCTGGGTTGTGGG
	MLL_OT-5_fw	ACAATCGGGTTTCTTGGCCA
	MLL_OT-5_rev	ACGCGGGGAATTAACTGAGG
	ENL_on_fw	CCCTGACCACCGATCCAAAA
	ENL_on_rev	CGCTTGGCTGCTGCTTTATT
	ENL_OT-1_fw	ACAAACCAGTGTCTGTGGCA
	ENL_OT-1_rev	CACTCCCCCACATCCAGAAC
	ENL_OT-2_fw	TCATAACGGTGGCCTCCCTA
	ENL_OT-2_rev	TGTGCCATTTCCTCGACCTC
	ENL_OT-3_fw	TGGCGCCTCAACAGAATCTT
	ENL_OT-3_rev	AAGCTTGCTCCTTGGCTAGG
	ENL_OT-4_fw	GGTAGGAAGATCTGGGCTGC
	ENL_OT-4_rev	CCCCACTTCTGCTACCAAGG
	ENL_OT-5_fw	GGTACAACCTCGTGTCCAGG
	ENL_OT-5_rev	CATGGGGCGATGTGAGTGAT
qRT-PCR	PBX3_fw	TTCTTACCAAGGGTCCCAAG
	PBX3_rev	GAATTTCCTCCAAGGCCATC
	MEIS1_fw	GCATCTTTCCCAAAGTAGCC
	MEIS1_rev	TGTGCCAACTGCTTTTTCTG
	HOXA9_fw	TAC GTG GAC TCG TTC CTG CT
	HOXA9_rev	CGT CGC CTT GGA CTG GAA
	HOXA10_fw	TTCCGAGAGCAGCAAAGC
	HOXA10_rev	CAGTTGGCTGCGTTTTCAC

	HMGB3_fw	GGCAAAGGCAGATAAAGTGC
	HMGB3_rev	GCGGAATTCTGAACAGAACAG
	CBX5_fw	TCTCAAACAGTGCCGATGAC
	CBX5_rev	TCACCACAGGAATCTGTTGC
	MYBL2_fw	CAGAAACGAGCCTGCCTTAC
	MYBL2_rev	TACTCAGGTCACACCAAGCATC