Supplemental information.

Supplemental methods.

Cell lines, Primary T-ALL cells culturing and characterization.

DND41, HPB-ALL and Jurkat T-ALL cell lines were maintained in RPMI 1640 supplemented with 1% penicillin/streptomycin, 1% glutamine (all from Gibco) and 10% fetal calf serum (FCS) (Life Technologies).

Primary pediatric T-ALL samples were obtained from Hôpital Armand Trousseau and Hôpital Robert Debré, both in Paris and from Institut d'Hématologie et Oncologie Pédiatrique, in Lyon. Cells were co-cultured with MS5/DL1 in reconstituted alpha-MEM essential medium supplemented with 10% FCS (6450; StemCell Technologies, Vancouver, BC), 10% human AB serum (Jacques Boy, Reims, France), 50 ng/ml recombinant human stem cell factor (SCF), (Amgen, Thousand Oaks, CA), 20 ng/ml rhFlt3-ligand (Miltenyi Biotec), 20 nM Insulin (Sigma-Aldrich, St Louis, MO), and 10 ng/ml rhIL-7 (Miltenyi Biotec).

Identification of NOTCH1, FBXW7, N-RAS and PTEN mutations, gene overexpression and rearrangements in primary T-ALL samples.

Amplification and sequencing of *NOTCH1* exons 26, 27, 28 and 34; *FBXW7* exons 7, 8, 9, 10 and 11; *PTEN* exons 4, 5, 6, 7, 8 and *NRAS* was performed as previously described [1,2].

Expression of SIL-TAL1, TAL1, LMO1/2, TLX1, TLX3, ABL1, and ETS1 genes were assessed by TaqMan R-Q-PCR according to standard curves obtained with logarithmic dilutions of corresponding amplicons (from 10^2 to 10^5 copies). Absolute Copy Number (CN) of target transcript was normalized by the ABL1 housekeeping gene and expressed as % of ABL1. Deregulation of TAL1 was defined by increased

expression and/or rearrangements SIL-TAL1, t (1; 14) or t (1; 7). The absolute CN of TAL1 in SIL-TAL samples ranged from 28 to 229 (averaged value: 134, mean value: 140). Because the blasts percentage in the diagnostic samples varied between 90%-96% (mean: 92%) and no TAL1 expression is detected in normal thymocytes, samples with a TAL1 CN > 150, were considered as TAL1 positives. Primers and R-Q-PCR probes are detailed in).

For gene expression analysis, 400 ng of DNase-treated total RNA from T-ALL specimens were reverse transcribed with VILO Reverse Transcriptase (Life Technologies), starting RNA quantity from sorted T-cell fractions was 2 ng. Diluted cDNA was then used as input in the 7900 fast real-time PCR system (Life Technologies).

Primers used for sequence analysis.

Primer name	Primer sequence	target	Protein domain
NOTCH1 exon26 F	AGGAAGGCGGCCTGAGCGTGT	genomic	Heterodimerization domain (HD) N-terminal
NOTCH1 exon26 R	AGAGTTGCGGGGATTGACCGT	genomic	
NOTCH1 exon27 F	GGTGGGCGGGGGAGGAGGAAG	genomic	HD C-terminal
NOTCH1 exon27 R	GCAGGTGGGGGCGGAGTG	genomic	
NOTCH1 exon28 F	CGTAGCCGCTGCCTGATGTCC	genomic	Juxtemembrane (JM)
NOTCH1 exon28 R	GGGGGTGAGGGGTCGAGAAGTGA	genomic	
NOTCH1exon34a F	GCTGCACAGTAGCCTTGCT	genomic	Transactivation (TAD) domain and proline glutamate serine threonine (PEST) domain
NOTCH1 exon34a R	AAGGCTTGGGAAAGGAAGC	genomic	
NOTCH11exon34b F	CTTCCTCTGGTGATGGAACCT	genomic	
NOTCH1exon34b R	CATCCCAGGCAGGTGGTTGA	genomic	
NOTCH1 exon34c F	GCCCTCCCCGTTCCAGCAGTCT	genomic	

Primers and Probes used for quantitative reverse transcriptase polymerase chain reactions (R-Q-PCR).

SIL-TAL ENR-664	5’ CCG AGG AAG AGG ATG CAC A 3’
SIL-TAL ENF-601	5’ CGC TCC TAC CCT GCA AAC A 3’
SIL-TAL ENP-641	5’ FAM- ACC TCA GCT CCG CGG AAG TTG C-3’ TAMRA
ABL1 ENF-1003	5’TGG AGA TAA CAC TCT AAG CAT AAC TAA AGG 3’
ABL1 ENFR-1063	5’ GAT GTA GTT GCT TGG GAC CCA 3’
ABL1 ENP-1043	5’ FAM-CCA TTT TTG GTT TGG GCT TCA CAC CATT -3’TAMRA
TAL1F	5’ GAA GAG GAG ACC TTC CCC CT 3’
TAL1R	5’ GGT GAA GAT ACG CCG CAC A 3’
TAL1-P	5'-FAM-TGA GAT GGA GAT TAC TGA TGG TCC CCA-TAMRA-3'
LMO2F	5'-TAC AAA CTG GGC CGG AAG C-3'
LMO2R	5'-CTT GTC ACA GGA TGC GCA GA-3'
LMO2-P	5'-FAM-CGG AGA GAC TAT CTC AGG CTT TTT GGG C-TAMRA-3'
TLX1-F	5’ AAA TGA CCG ATG CGC AGG T 3’
TLX1R	5’ GTT CGC TTG CTG CCT CTC G3’
TLX1 P	5’ FAM -AAC CGG CGG ACA AAG TGG AGA CG-3’ TAMRA
TLX3-3F	5'-CAA GAC CTG GTT CCA AAA CCG –3'
TLX3-4R	5'-AGG CTG GAT GGA GTC GTT GA –3'
TLX3-P	5' FAM-CAG CTG CAA CAC GAC GCC TTC CAA -3'-TAMRA
ETS1	Hs00428287-m1, Life Technologies
CBFb	Hs00242386_m1 Life Technologies
Linc00478 “long” F	5’ TCA GGT GTC TGA TGA TTT GTG AGC C
Linc00478 “long” R	5’ TGA CTC ATG GCC TAG GGA AAG CTA A 3’
Linc00478 “short” F	5’ GCG GTG GCA AGC GTA TGG AA 3’
Linc00478 “short” R	5’ CTG AGA GGA GCG CAA CAA TGT 3’

Purification of human lymphocyte subsets.

Thymic tissues were gently crushed over a 70µm filter (Falcon Cell Strainer) to free cells from connective tissue. Cell suspensions were filtered and washed with phosphate-buffered saline (PBS). Live thymus mononuclear cells (MNCs) were isolated by Pancoll centrifugation (Dutscher, Brumath, France) and $CD34^+$ cells were purified by immunomagnetic selection using a CD34 MicroBeads selection kit (MACS) (Myltenyi Biotec, Paris, France). $CD34^+$ cells were then labelled with monoclonal antibodies (mAbs) against CD34 (clone 4H11) conjugated to allophycocyanin (APC), CD1a-phycoerythrin (PE) (clone BL6) and CD4-PE-cyanin (PC7) (clone SK3) (Beckman Coulter, Villepinte, France). Pro-T ($CD34^+/CD1a^-/CD4^-$) and Pre-T ($CD34^+/CD1a^+/CD4^-$) cell subsets were isolated from these thymus-derived $CD34^+$ cells using INFLUX cell sorter (BD Biosciences, Le Pont de Claix, France). In parallel ISP ($CD34^-/CD4^+$ $/CD8^-/CD3^-$), DPI ($CD34^-/CD4^+/CD8^+/CD3^-$), DP ($CD34^-/CD4^+/CD8^+/CD3^+$), SP4 ($CD34^-/CD4^+/CD8^-/CD3^+$) and SP8 ($CD34^-/CD4^-/CD8^+/CD3^+$) cell subsets were sorted from $5x10^7$ thymus MNCs labelled with CD34-APC (4H11), CD8-PE (B9.11), CD4-PC7 (SK3) and CD3-fluorescein (FITC) (SK7) mAbs (all from Beckman Coulter). The purity of sorted cell fractions was always at least 98%, as estimated by analysis performed on a FacsCalibur cytometer (Becton Dickinson Biosciences).

Micro-RNA expression analysis.

In brief, 100 ng of total RNA was reverse transcribed using the megaplex stem-loop RT primer pool for miRNA cDNA synthesis of 738 miRNAs and 6 small RNA controls. Pre-amplification of cDNA was performed in a 14-cycle PCR reaction using TaqMan PreAmp Master Mix (2×) and PreAmp Primer Mix (5×) (Applied Biosystems, Life

Technologies). Finally, small RNAs were profiled for each sample using a 40-cycle PCR protocol. SDS software version 2.1 was used to calculate the raw Cq values, using automatic base line settings and a threshold of 0.05.

MiRNA Data processing and statistical analysis.

Minimal detection level was determined as fewer than 40 rounds of Q-PCR. We discarded all miRNA targets, which were unexpressed in more than 75% of the patients (i.e. more than 30 out of 41). This led to a final dataset of 532 miRNA targets out of the 762 initially available for analysis. MiRNA were expressed on the Ct scale and normalized, by patient, by subtracting the expression level of RNU48. RNU48 was chosen because of evenly expression across all samples in the range from Ct14 to Ct16 and good correlations with other internal controls (RNU44, RNU43, U6). We also considered alternative choice for normalization, i.e. by the average level of miRNAs expression computed patient-wise. In the analysis for differences in expression levels according to patient class, the results were little affected (data not shown).

For patients, differences in expression according to molecular group were tested by ANOVA. Correction for multiple testing was done according to the single step Westfall & Young max T procedure. We used LIMMA to test the differences in miRNA expression levels according to differentiation stage of normal T-cells. In this analysis, the false discovery rate was set at 5%.

MiR-125b, miR-99a, miR-100, let7c and let7a expression levels in specific human T-cell subsets and T-ALL samples were assessed by single miRCURY LNA TM microRNA PCR System was used (Exiqon). Ten nanograms of total RNA were

polyadenylated and reverse transcribed and diluted cDNA (1:20) was used in real-time PCR. MiRNA expression was normalized to RNU44.

Western blot analysis.

Total proteins were extracted with lysis buffer containing 50 mM Tris pH8.0, 300 mM NaCl, 2mM MgCl2, 1% NP-40, 0.5% DOC supplemented with a protease inhibitor mix. Proteins were separated by 10% SDS PAGE, transferred onto nitrocellulose membrane (Schleicher & Schuell) and immunoblotted in standard conditions. The antibodies used were against TLX3 (H-55, 1:500, Santa Cruz) and β–actin (clone AC-15; Sigma-Aldrich).

T cell differentiation.

$CD34^+/CD38^{-/low}/CD45RA^-/CD90^-$ HPCs were sorted from cord blood $CD34^+$ cells isolated by MACS immunomagnetic selection (Miltenyi Biotec) and labelled using the following mAbs: CD38-FITC (T16), CD45RA-PE (ALB11), CD34-PC7 (581) and CD90-APC (5E10) (Becton Coulter). Sorted cells were transduced with lentiviral vectors and co-cultured with MS5/DL1 cells in cytokines-containing medium. The efficiencies of transduction of $CD34^+/CD38^{-/low}/CD45RA^-/CD90^-$ HPCs were as follows: 75-95% for GFP and mCherry CTRL vectors, 80% for shCTRL vectors, 56-90% for TLX3/OE vector, 55-90% for miR-125b/OE depending on donors' cells. CD7+ T cell progenitors were sorted at day 21 of culture using CD7-PE-CY7 (8H8.1) mAb and vector-derived fluorescent markers (eGFP or mCherry). Equal quantities of progenitors were re-seeded with MS5/DL1 stromal cells at density $25x10^4$ cells with $5X10^4$ MS5-DL1 stromal cells per well in 24 well plate and then re-seeded every week in the same conditions. Cells were immuno-phenotyped every week starting

from day 21. All antibodies including isotype controls were from Beckman Coulter and eBiosciences.

Cloning of lentiviral constructs.

Two shRNAs directed to different exons of human TLX3 (Sh1: 5'-GCGGGATCTTACAGTGTGA-3' and Sh2: 5'-CTGCAACACGACGCCTTCCAA-3') driven by pol III H1 promoter were subcloned in lentiviral vector pTRIP/ΔU3-MND-eGFP, where expression of the enhanced green fluorescent protein (eGFP) is controlled by the modified myeloid proliferative sarcoma virus promoter (MND). The control irrelevant shRNA was directed to the human hepatitis B virus RNA [3]. TLX3-expressing IRES bi-cistronic vector pTRIPΔU3-Mnd-TLX3-IRES-eGFP was a kind gift of Dr. E. Clappier.

To generate miR-125b-1 and miR-125b-2 “double copy” 125b/OE expression vectors, genomic regions containing predicted pre-miRNAs and 150 nt flanking each side of the hairpin were amplified and cloned 3’ to H1 promoter in pSuper plasmid. The expression cassette was then subcloned in the U3 region of the viral 3’ long terminal repeat (LTR) of pTRIP/ΔU3-MND-mCherry vector, as in [4]. SpeI and SalI insertion sites were created using GeneTailor ™ Site-Directed Mutagenesis System (Invitrogen). Expression and maturation of ectopic miR-125b was confirmed by Northern analysis of transduced Jurkat cells.

Stable miR-125b knockdown was achieved using the sponge technology [5]. MiR-125b “sponge” cassette containing H1 promoter driving the expression of short RNA composed of four bulged miR-125b-target sequences: TCACAAGTTACCACTCAGGGA separated by short spacers and 5T termination signal was synthesized by GeneArt technology (Life Technology) and cloned into

SpeI/SalI restriction sites in U3 region of pTRIP/ΔU3-MND-mCherry vector to get a double-copy expression vector 125b/KO. Control "sponge" construct CTRL/KO contained four scrambled targets GGAGCTCCACCGCGGTGGCAT without sequence complementarity to any of the known miRNAs (adapted from [6]). The efficiency of 125/KD vector was tested using luciferase reporter. The cotransfection of 125/KD vector with a luciferase-reporter plasmid carrying miR-125b complementary site in the 3'UTR of luciferase gene relieved at least 60% of luciferase activity inhibited by endogenous miR-125b (Supplemental figure 2).

Luciferase reporter analyses.

Fragments amplified from genomic DNA were cloned into KpnI/XhoI sites of pGL3 promoter vector. 293T cells were transduced with TLX3/OE vector or empty GFP control vector at equal efficiency (95%) and then co-transfected with 100 ng pGL3 reporters and 10 ng of pRenilla/TK plasmid for transfection control. Relative Luciferase activity was measured 36h after transfection using Dual-Luciferase Reporter Assay system, Promega, according to the manufacture protocol.

ChIP-seq and ChIP-Q-PCR experiments.

Sequencing of TLX3 ChIP and input DNA samples was performed on an AB SOLiD v4.0 (Life Technologies) according to the manufacturer's protocol and mapped onto the HG19 reference genome using the SOLiD pre-processing pipeline. To reduce the risk of potential bias, we discarded non-uniquely mapped tags and tags with exactly the same coordinates. Reads were then elongated to the average size of the sequenced material and the values were summed up in non-overlapping 100 bp bins along each chromosome. Histone modification profiles in the DND41 cell line were obtained from the ENCODE consortium ChIP-seq profiles were visualized using the UCSC genome Browser (http://genome.ucsc.edu)

Enrichment of TLX3-binding regions was analyzed by Q-PCR using following oligonucleotide primers: Linc00478 +1,3 kb Forward:

5'-TGTGCTTTCACCCTGTTGATCTGA -3'; Linc00478 +1,3 kb Reverse:

5'-ACGCACGAAGTAACCACACAGACC -3'; Linc00478 +29 kb Forward:

5'-TCCTGTCATCCTGAGGCAGCCAA -3', Linc00478 +29 kb Reverse:

5'-ACGTGCATCTGTGCTTTCTGAGCTT -3'; and ChIP-qPCR Human hIGX1A Negative Control (Qiagen) primers were used for data normalization.

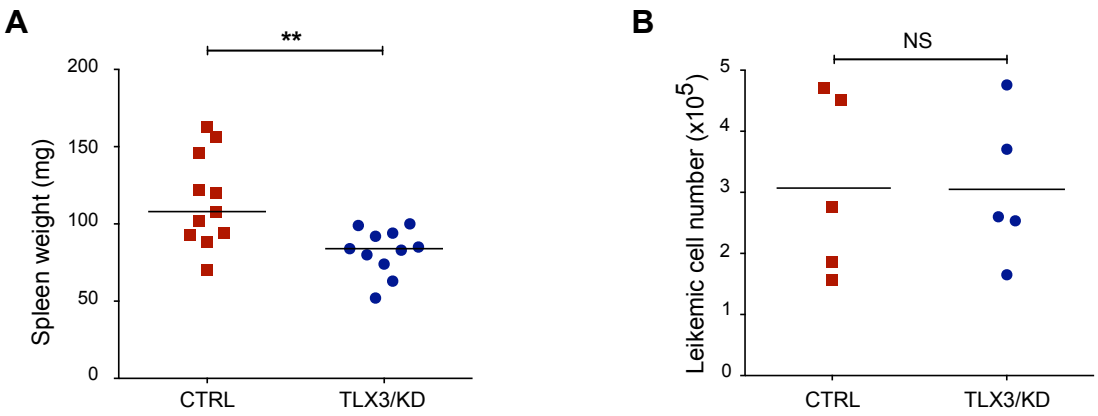
Mice.

NOD.Cg-Prkdc^{scid} Il2rg^{tm1Wjl}/SzJ (abbreviated NSG) mice (The Jackson Laboratory, Bar Harbor, USA) were housed in pathogen-free animal facilities at CEA, Fontenay-aux-Roses, France. Mice were anesthetized with isoflurane prior intravenous injection of transduced DND41 human leukemic cells ($3x10^5$ per animal). Experimental procedures were performed in compliance with the French Agriculture Ministry and local ethics committee guidelines (Authorization number 12-015). Mice were euthanized at 6 weeks after leukemic cell injection or when mice showed signs of sickness. Leukemia progression was monitored using anti–human CD45-PE (2D1) and CD7-PC7 mAbs (Beckman Coulter and eBioscience).

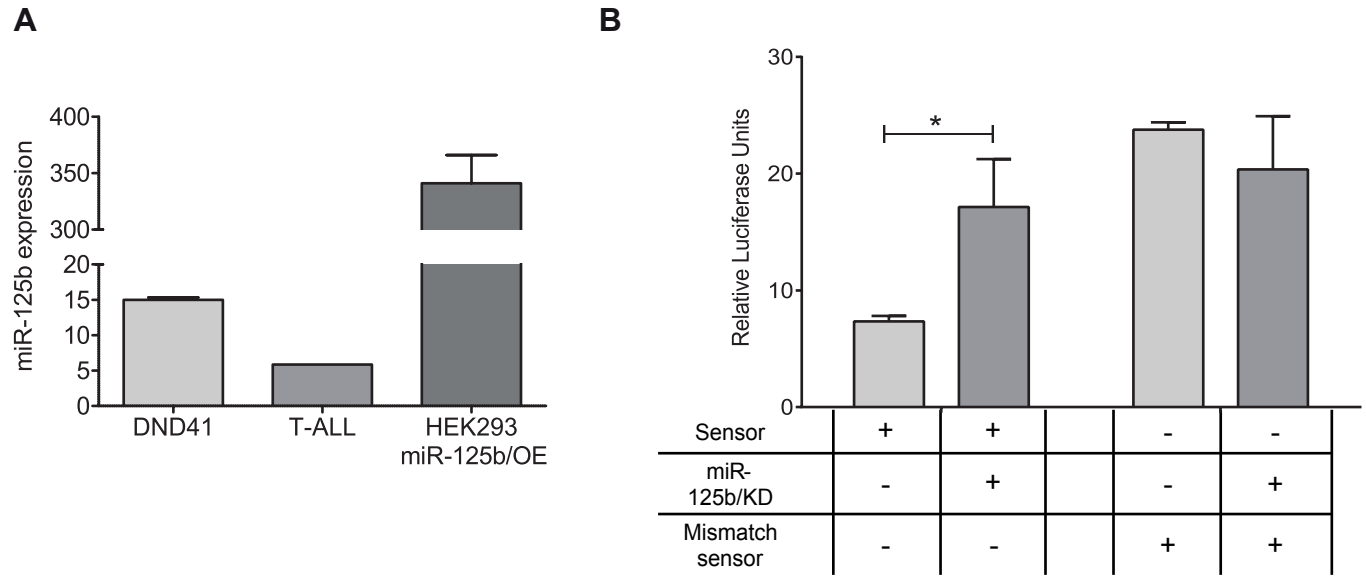
Statistical Analysis

Data were analyzed by the Mann-Whitney test, if appropriate. Differences were considered statistically significant at $p < 0.05$. Unless otherwise indicated, data are expressed as mean ± SEM.

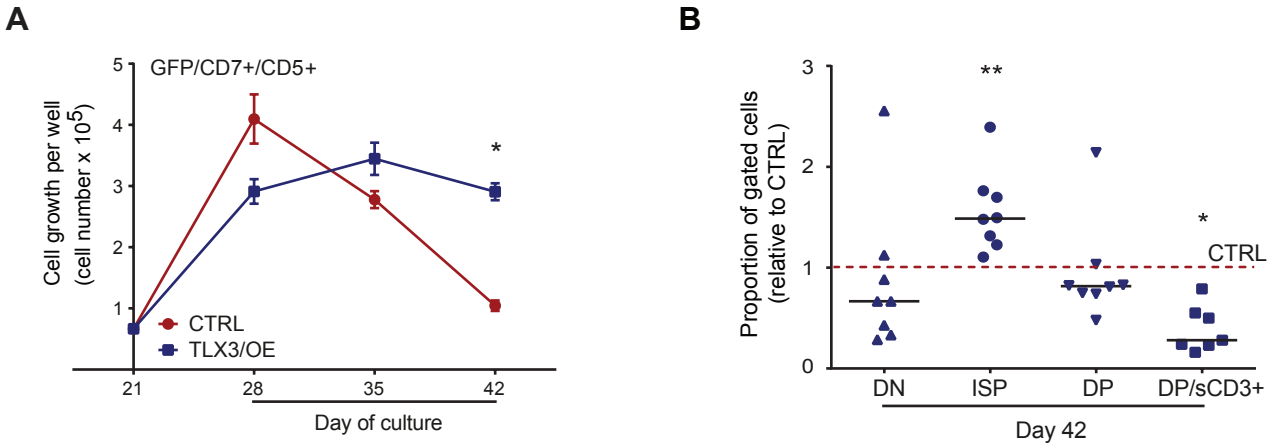
1. Asnafi V, Buzyn A, Le Noir S, et al. NOTCH1/FBXW7 mutation identifies a large subgroup with favorable outcome in adult T-cell acute lymphoblastic leukemia (T-ALL): a Group for Research on Adult Acute Lymphoblastic Leukemia (GRAALL) study. *Blood*. 2009;113(17):3918-3924.
2. Zuurbier L, Homminga I, Calvert V, et al. NOTCH1 and/or FBXW7 mutations predict for initial good prednisone response but not for improved outcome in pediatric T-cell acute lymphoblastic leukemia patients treated on DCOG or COALL protocols. *Leukemia*. 2010;24(12):2014-2022.
3. Brunet de la Grange P, Armstrong F, Duval V, et al. Low SCL/TAL1 expression reveals its major role in adult hematopoietic myeloid progenitors and stem cells. *Blood*. 2006;108(9):2998-3004.
4. Chen CZ, Li L, Lodish HF, Bartel DP. MicroRNAs modulate hematopoietic lineage differentiation. *Science*. 2004;303(5654):83-86.
5. Ebert MS, Neilson JR, Sharp PA. MicroRNA sponges: competitive inhibitors of small RNAs in mammalian cells. *Nat Methods*. 2007;4(9):721-726.
6. Gentner B, Schira G, Giustacchini A, et al. Stable knockdown of microRNA in vivo by lentiviral vectors. *Nat Methods*. 2009;6(1):63-66.



Supplemental Figure 1. TLX3/KD reduces engraftment of DND41 cells in spleen of NSG mice. DND41 cell were transduced with shTLX3 or shCTRL GFP vectors with equal efficiency (95%) and 500 000 cells were IV injected in mice. CD45+/GFP+ cells were counted 4 weeks later, at sacrifice.
A) Spleen weight. Bars represent median values.
(n=2) ** P<0.01, Mann-Whitney test.
B) Leukemic infiltration of bone marrow (BM).



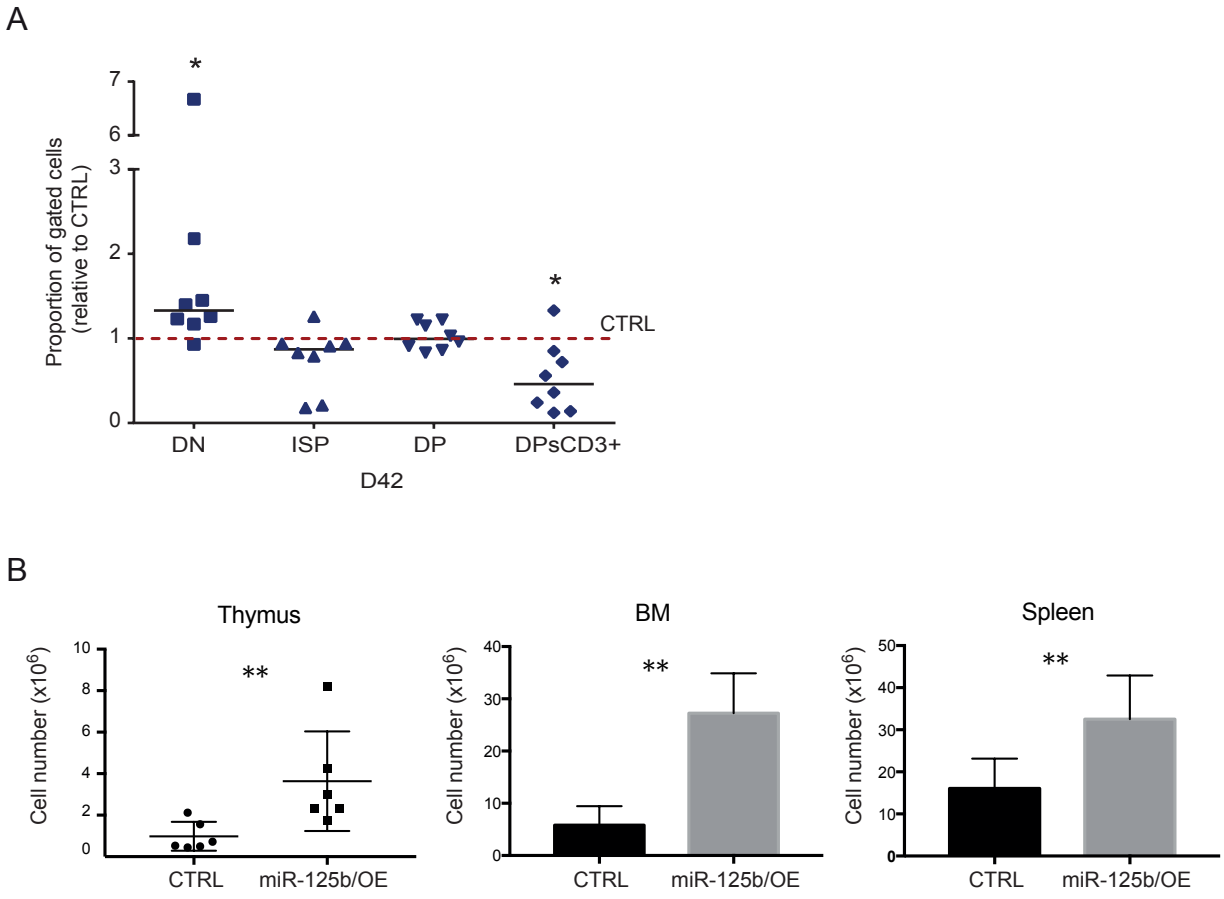
Supplemental Figure 2. Efficiency of miR-125b knockdown (125b/KD)
HEK293 cell line constitutively expressing miR-125b (125b/OE) was prepared using lentiviral transduction and used in reporter experiments.
(A) Comparison of miR-125b levels in DND41 cell line, primary T-ALL and HEK293 (125b/OE) cells. miR-125b expression is normalized to RNU44.
(B) Dual luciferase assay of HEK293 (125b/OE) cells cotransfected with sensor or control mismatch plasmid together with 125b/KD vector. Sensor pmirGLO plasmid contained two miR-125b complementary sites in 3'UTR of Firefly luciferase gene. Mismatch plasmid contained four mutations in miR-125b binding sites of sensor. *Firefly* activity was normalised to activity of *Renilla* luciferase gene (internal pmirGLO control).
Data were avereged from (n=3) experiments. * P<0.05, Mann-Whitney test.



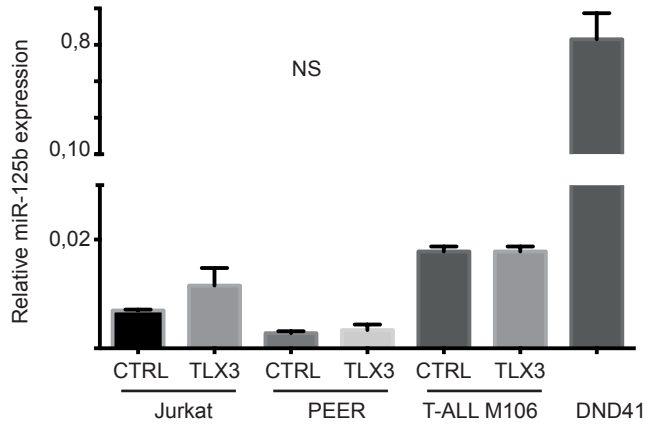
Supplemental Figure 3. Overexpression of TLX3 correlates with expansion and differentiation arrest of T-cell progenitors.

A) Dynamics of T-cell growth in a representative experiment. All data points show mean +- SEM of quadruplicates. Mann-Whitney test, * P<0.05.

B) Ectopic TLX3 induces accumulation of ISP progenitors and blocks sCD3 acquisition by DP cells. Proportion of different T-cell fractions in TLX3/OE condition relatively to CTRL (n=8). Bars represent median value. * P<0.05, ** P<0.01, Wilcoxon test.

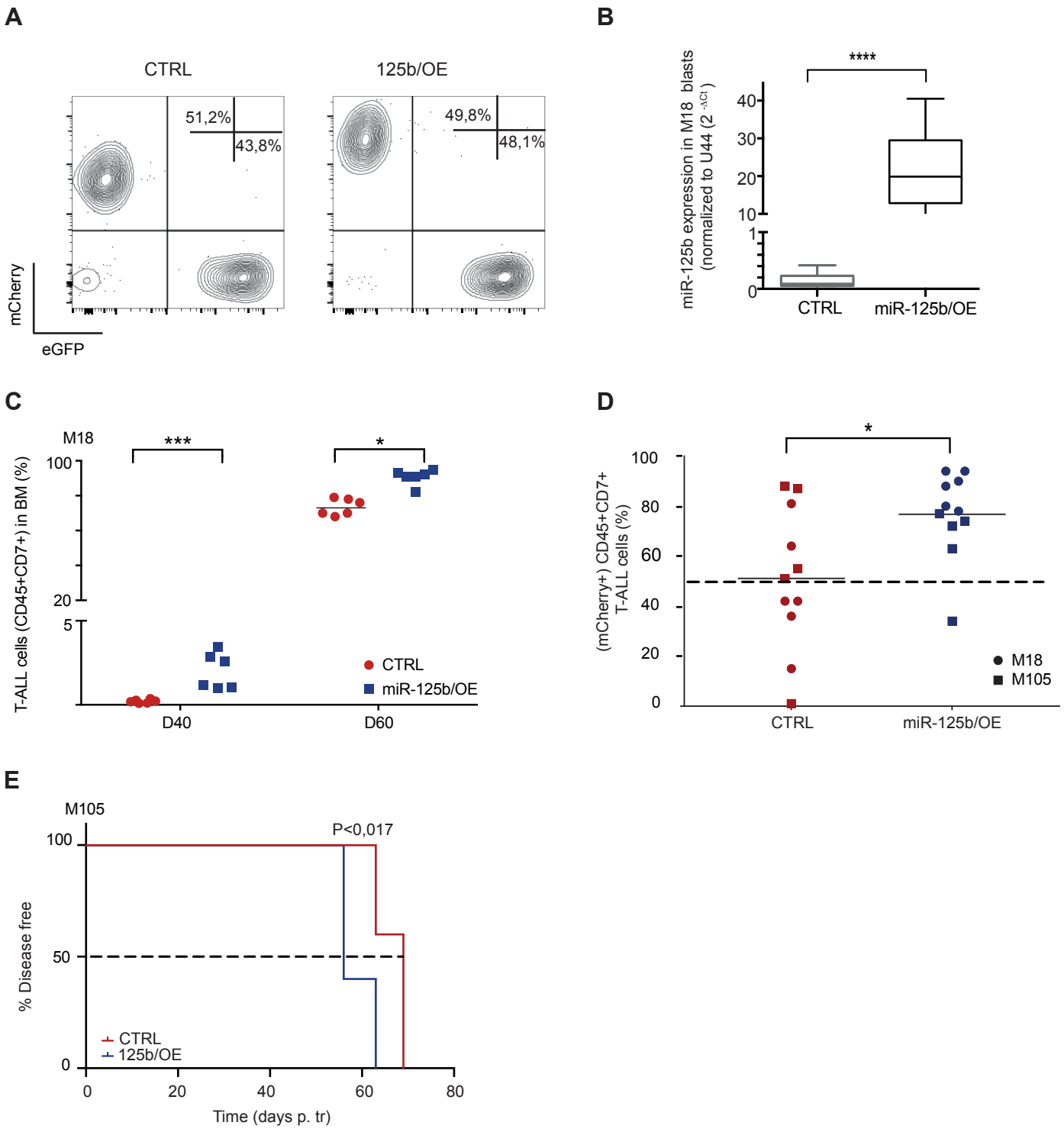


Supplemental Figure 4. Effects of ectopic expression of miR-125b in HPC *in vitro* and *in vivo*.
(**A**) MiR-125b/OE induces accumulation of immature DN cells and blocks sCD3 acquisition by DP cells. Proportions of T-cell fractions in 125b/OE vs CTRL conditions. Bars represent median values, (n=8), * P<0.05, Wilcoxon test.
(**B**) Ectopic expression of miR-125b in HPC induces cell proliferation in thymus, bone marrow (BM) and spleen. CD45+ cells were counted in the respective organs 90 days after injection into NSG recipients. MiR-125b/OE: cells transduced with lentivirus vector encoding miR-125b. N=6 per group. Mann-Whitney test, ** P<0,01.

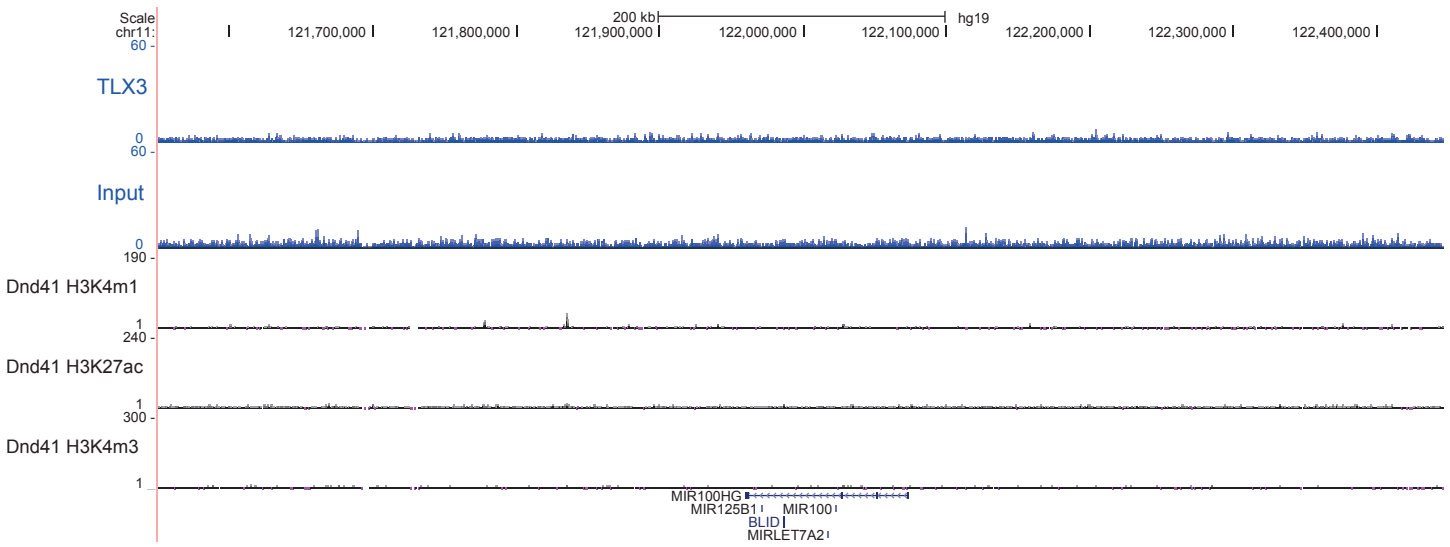


Supplemental Figure 5. TLX3/OE in negative cell lines and in mature cortical SIL-TAL1 rearranged primary T-ALL does not induce miR-125b.

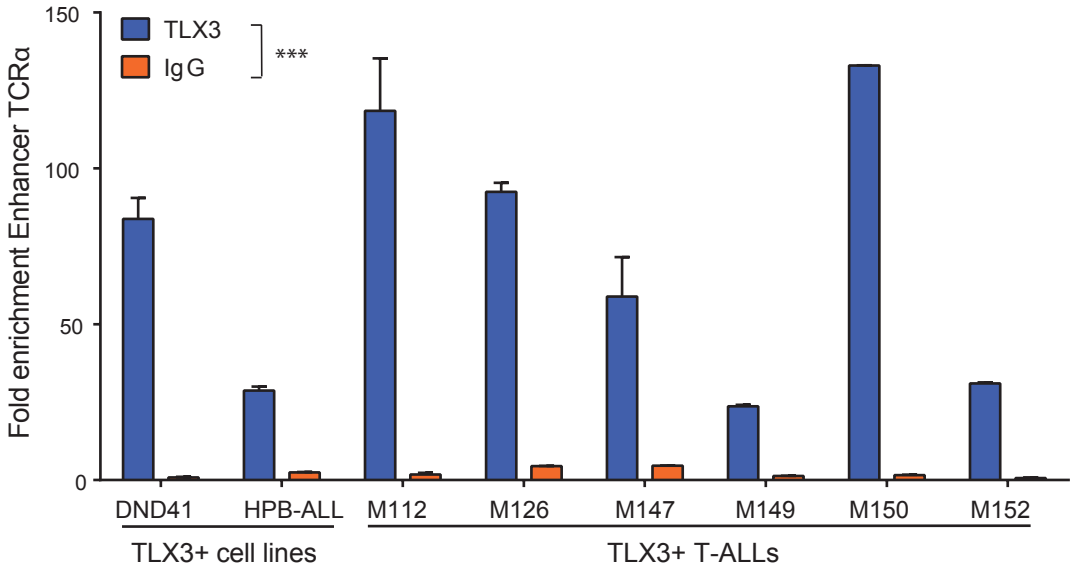
Expression (RT-Q-PCR) of miR-125b is shown in Jurkat and PEER cell lines and primary T-ALL at day 5 after transduction of TLX3 or CTRL expressing vectors and sorting of eGFP+ cells. MiR-125b levels were normalized to RNU44 expression ($2^{-\Delta Ct}$). MiR-125b expression in DND41 cell line is given for comparison.



Supplemental Figure 6. MiR-125b accelerates T-ALL development.
Sorted T-ALL (M18 or M105) infected cells (mCherry+) expressing or not miR-125b were mixed at ratio 1:1 with sorted control (eGFP+) cells and transplanted into NSG recipients. Engrafment was monitored as described in Figure 5F for M106 T-ALL. (CTRL (n=6); 125b/OE (n=6) for M18; CTRL (n=5), 125b/OE (n=5) for M105).
(A) Proportions of mCherry+ and eGFP+ cells at day of injection (shown for M18). (B) miR-125b expression in blasts recovered from BM of recipient mice at sacrifice (shown for M18). (C) T-ALL cell expansion in periphery assessed by CD45+ CD7+ cells in BM at two time points. (shown for M18).
(D) Percentage of (mCherry+) CD45+ CD7+ T-ALL cells in BM at sacrifice. Shown are combined results for M18 (circles) and M105 (squares).The dashed line indicates the percentage of mCherry+ cells (50%) at the day of injection.****P<0,0001; ***P<0.001; *P<0.05: (B-D): Mann-Whitney test.
(E) Kaplan-Meier survival plot depicting M105 leukemia onset in the mouse recipients. CTRL: (n=5); 125b/OE: (n=5) Mantel-Cox test. Survival plot was not done for M18.

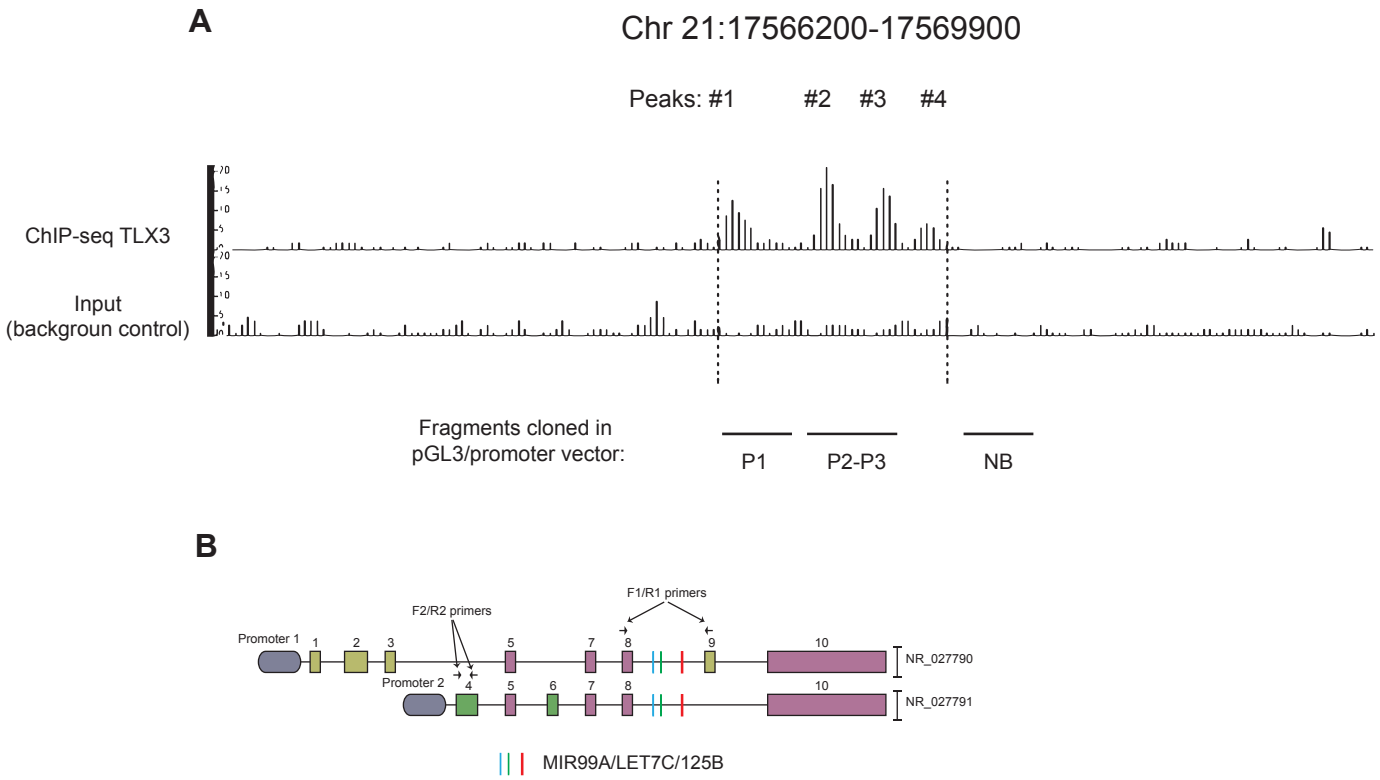


Supplemental Figure 7. Absence of TLX3 binding sites (upper panel) and marks of an active chromatin (lower panel) in the vicinity of MIR125B1 locus on the chromosome 11. ChIP-sec analysis of a TLX3 T-ALL.

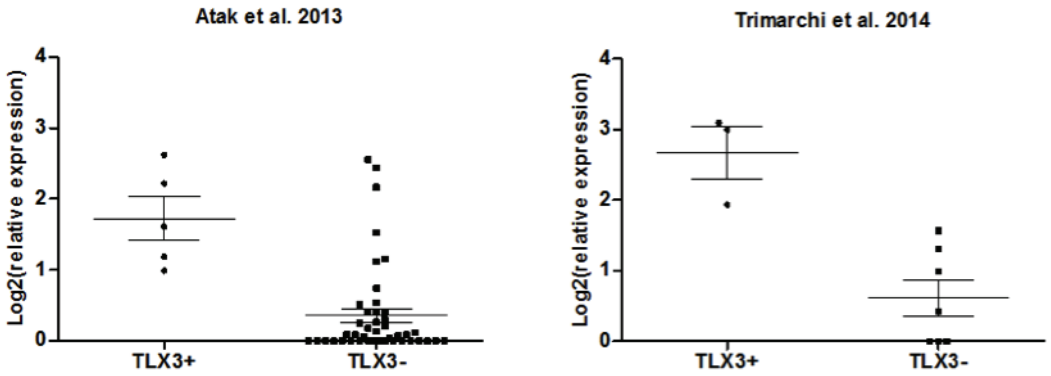


Supplemental Figure 8. ChIP-Q-PCR analysis of TCRa enhancer sequence in TLX3 T-ALLs. These locus were used as positive control in all ChIP experiments. Show are fold enrichments relative to IGX1A intergenic region (n=4), *** P<0.001, Mann-Whitney test.

Ref.
Dadi S, Le Noir S, Payet-Bornet D, Lhermitte L, Zacarias-Cabeza J, Bergeron J, Villarese P, Vachez E, Dik WA, Millien C, et al. TLX homeodomain oncogenes mediate T cell maturation arrest in T-ALL via interaction with ETS1 and suppression of TCRalpha gene expression. Cancer cell. 2012;21(4):563-76.



Supplemental Figure 9. Luciferase reporter assay and LINC00478 expression.
(A) Two fragments of TLX3 binding region corresponding to peak 1 (948 bp), peak 2-3 (1460 bp) and downstream non-binding region (980 bp) were cloned into pGL3 promoter vector. Enhancer activity of loci were assaied by luciferase reporter analysis.
(B) Positions of PCR primers designed to discriminate the “Long” (F1/R1) and “Short” (F2/R2) Linc00478 transcripts are indicated. Blue, green and red bars show positions of respectively MIR99a, LET7C and MIR125B2.



Supplemental figure 10. Assessment of *LINC00478* expression in T-ALL samples using RNA-seq data. RNA-seq data from T-ALL samples were obtained from previously published studies (Atak et al., 2013) (Trimarchi et al., 2014) and were downloaded from the European Genome-Phenome Archive under the accession number EGAS0000100536 (left panel) and from the NCBI Gene Expression Omnibus under the accession number GSE57982 (right panel). RNA-seq data were aligned to hg19 using Tophat v2.0.5 (Trapnell et al., 2009) et al. 2009) with default parameters. Relative expression was quanti Cuffdiff v2.1.1 (Trapnell et al., 2010), followed by read count normalization using DEseq2 - R package (Anders and Huber, 2010). Expression of *LINC00478* was compared between TLX3+ and TLX3- T-ALL samples. The statistical analysis was performed using unpaired t-test. The data are presented with the Mean +/- SD.

References:

Anders, S., *et al.* (2010). Differential expression analysis for sequence count data. Genome biology *11*, R106.
Atak, Z.K., *et al.* (2013). Comprehensive analysis of transcriptome variation uncovers known and novel driver events in T-cell acute lymphoblastic leukemia. PLoS genetics *9*, e1003997.
Trapnell, C., *et al.* (2009). TopHat: discovering splice junctions with RNA-Seq. Bioinformatics *25*, 1105-1111.
Trapnell, C., *et al.* (2010). Transcript assembly and quantification by RNA-Seq reveals unannotated transcripts and isoform switching during cell differentiation. Nat Biotechnol *28*, 511-515.
Trimarchi, T., *et al.* (2014). Genome-wide mapping and characterization of Notch-regulated long noncoding RNAs in acute leukemia. Cell *158*, 593-606.

Supplemental table 1. Characteristics of T-ALL patients included in the miRnome analysis.

Id	Gender	age	WBC	Genetic Subtype	TAL overexpression (Absolute Copy Number)	NOTCH1 status	FBXW7 status	PTEN status	N-RAS status	Cytogenetic Analysis	FISH ABL
TRS 1	M	12	171 000	unknown	21	WT	WT	WT	WT	46, XY [25]	Neg.
TRS 2	M	5,0	80 000	sil-tal	161	WT	WT	$E7^{mut}$	WT	46, XY (26]	Neg.
TRS 3	M	9,4	302 000	TLX3	0	WT	WT	WT	WT	47, XY, +8 [26]/ 46, XY [1]; FISH TLX3+	POS (a)
TRS 4	M	9	46 600	TLX3	10	WT	WT	WT	c.35G>A	46, XY, inc(9)c [20]/ 46, XY [5]; FISH TLX3+	Neg.
TRS 5	M	9	146 500	unknown	3	$PEST^{mut}$	WT	WT	WT	46, XY [28]	Neg.
TRS 6	F	12	121 000	unknown	46	HD^{mut}	WT	WT	WT	46, XX [27]	Neg.
TRS 7	M	6	115 000	sil-tal	113	$PEST^{mut}$	WT	WT	WT	46, XY del(9)(p21) [10]/ 46, XY[10]	Neg.
TRS 8	F	8,4	117 000	sil-tal	229	WT	WT	WT	WT	46, XX + mar [24]	Neg.
TRS 9	M	14,0	371 000	unknown	188	HD^{mut}	WT	WT	WT	del(2)inv(2)	Neg.
TRS 10	M	13,9	512 000	siltal	86	JMmut	WT	WT	WT	46, XY [21]	Neg.
TRS 11	M	12,8	223 000	TAL-R*	6150	HD^{mut}	WT	WT	WT	47, XY, t(1;7)(p22;7q32), -6, +8, del(9)(q13-21), add(17)(p13), r(6) [8]/ 48,idem, +r(6) [1]/ 46, XY [14]	Neg.
TRS 12	M	8,7	35 000	unknown	286	WT	WT	WT	WT	46, XY [24]	Neg.
TRS 13	M	7	375 000	unknown	11	WT	WT	$E7^{mut}$	WT	failed	Neg.
TRS 14	M	3,6	97 000	sil-tal	173	$PEST^{mut}$	WT	WT	WT	failed	Neg.
TRS 15	F	5	81 000	sil-tal	89	$PEST^{mut}$	WT	$E7^{mut}$- $E8^{mut}$- $E9^{mut}$	WT	46, XX, t(11;14)(p15;q11) [6] / 46, XX [16]	Neg.
TRS 16	F	5,1	552 000	TLX3	2	Hd^{mut} and $PEST^{mut}$	WT	WT	WT	failed; RQ-PCR TLX3+	Neg.
TRS 17	M	10,7	74 000	TLX3	9	HD^{mut}	WD40-3 mut	WT	WT	46, XY [24]; FISH TLX3+	Neg.
TRS 18	M	11,0	359 000	sil-tal	152	Hd^{mut} and $PEST^{mut}$	WT	$E7^{mut}$	WT	failed	Neg.
TRS 19	M	3,5	50 000	unknown	246	HD^{mut}	WT	WT	WT	46, XY [22]	Neg.
TRS 20	M	16	50 100	unknown	56	HD^{mut}	WD40-3 mut	WT	WT	46,XY,t(1;7)(p32;p13)[11]46,idem,del(6)(q12q16)[4]/ 46,XY[7]	Neg.
TRS 21	M	13	217 000	sil-tal	170	WT	WT	WT	WT	failed	Neg.
TRS 22	M	9,9	230 000	unknown	247	$PEST^{mut}$	WT	WT	WT	46, XY [24]	Neg.
TRS 23	M	4,6	30 000	unknown	133	HD^{mut}	WD40-3 mut	WT	WT	46, XY [22]	Neg.
TRS 24	M	2,9	91 900	unknown	44	WT	WD40-3 mut	WT	WT	46, XY [24]	Neg.
TRS 25	M	6,8	104 000	TLX3	1	intronic mutation	WT	WT	WT	NA; RQ-PCR TLX3+	Neg.
TRS 26	M	9,0	53 000	unknown	50	WT	WT	WT	WT	46, XY, add(2)(q31), -5, -7, -11, -12, +mar1, +mar2, +mar3 (5]/46, XY (16]	Neg.
TRS 27	M	6,6	600 000	unknown	53	intronic mutation	NA	WT	WT	failed	Neg.
TRS 28	F	1,0	999 000	unknown	5	WT	NA	$E7^{mut}$	c.35G>A	46, XX [20]	Neg.
TRS 29	M	10,0	133 000	unknown	6	WT	NA	$E7^{mut}$	WT	46, XY [28]	Neg.
TRS 30	M	8,0	720 000	TLX3	1	$PEST^{mut}$	WT	WT	c.35G>A	failed; RQ-PCR TLX3+	Neg.
TRS 31	M	8,5	105 000	sil-tal	141	HD^{mut}	WD40-3 mut	WT	WT	46, XY [22]	Neg.
TRS 32	M	15,0	21 000	TAL-R*	666	NA	WT	$E7^{mut}$	WT	46, XY, t(1;14)(p32;q11) [16]/46, XY [6]	Neg.
TRS 33	M	14,3	50 000	TAL-R*	200	NA	WT	WT	WT	46,XY,t(1;14) [12] /46, XY [8]	Neg.
TRS 34	M	12	171 000	unknown	21	WT	WT	WT	WT	46, XY [18]	Neg.
TRS 35	F	15	75 400	TLX3	18	NA	WD40-3 mut	WT	WT	46,XX[15], FISH TLX3+	Neg.
TRS 36	M	8,5	117 000	unknown	189	HD^{mut}	WT	NA	WT	46,XY[22]	Neg.
TRS 37	M	14	103 000	unknown	18	WT	WD40-3 mut	E7mut- $E8^{mut}$ - $E9^{mut}$	WT	46, XY [22]	Neg.
TRS 38	M	3	N.A.	unknown	39	HD^{mut}	NA	WT	WT	NA	Neg.
TRS 39	M	10,8	31 700	sil-tal	28	HD^{mut}	NA	NA	WT	46,XY,del(6)(q13q22)[1]/46,XY[4]	Neg.
TRS 40	M	12	4 000	unknown	287	$PEST^{mut}$	inversion	$E6^{mut}$-$E7^{mut}$-$E9^{mut}$	WT	46, XY [24]	Neg.
TRS 41	M	3,7	195 000	TLX3	56	Hd^{mut} and $PEST^{mut}$	WT	$E6^{mut}$-$E7^{mut}$-$E9^{mut}$	WT	46, XY [18]	POS (b)

a) NUP214-ABL1 rearrangement
b/ ABL rearranged with unknow partner

Supplemental table 2. Distribution of frequent mutations among three oncogenic groups of T-ALL.

	TAL1 (n=19)	TLX3 (n=8)	Other (n=14)
Notch1 mutated	n=13 (13/17)	n=5 (5/7)	n=7 (7/14)
%	76.40%	71.40%	50%
FBXW7 mutated	n=2 (2/18)	n=2 (2/8)	n=4 (4/10)
%	11%	25%	40%
Notch&FBXW7	n=2	n=1	n=2
N-RAS mutated	0	n=2 (2/7)	n=1 (1/14)
%		28.50%	7%
PTEN mutated	n=4 (4/15)	n=1 (1/7)	n=5 (5/14)
%	26%	14.30%	36%