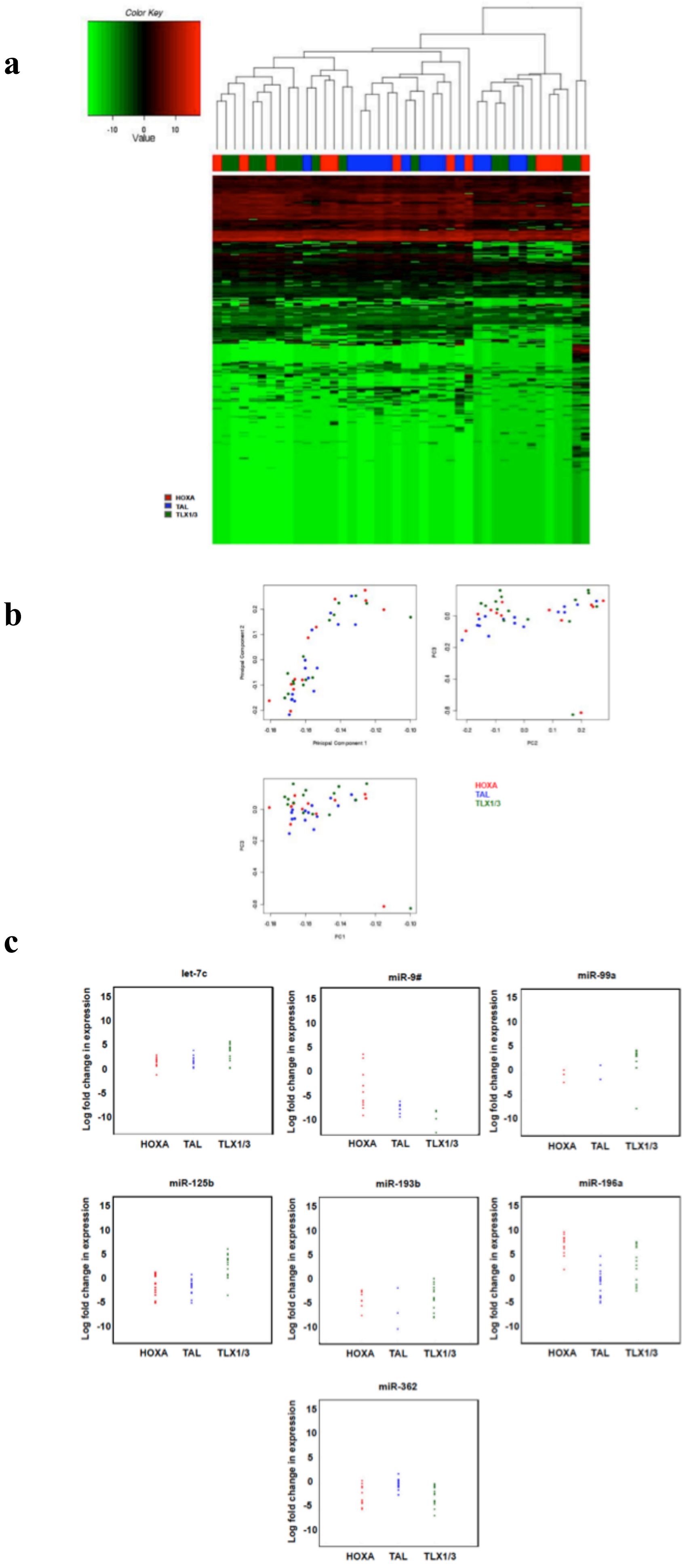
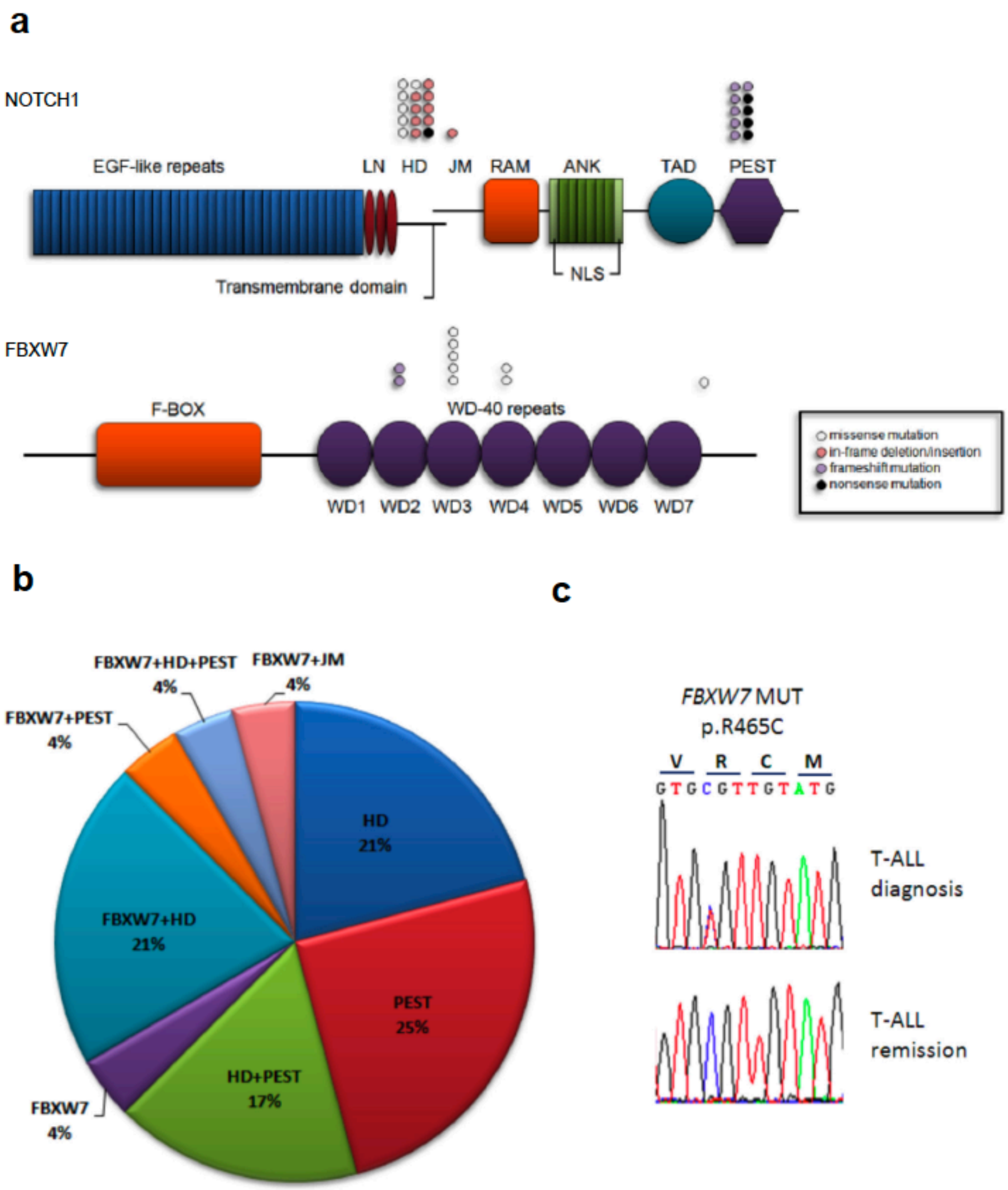
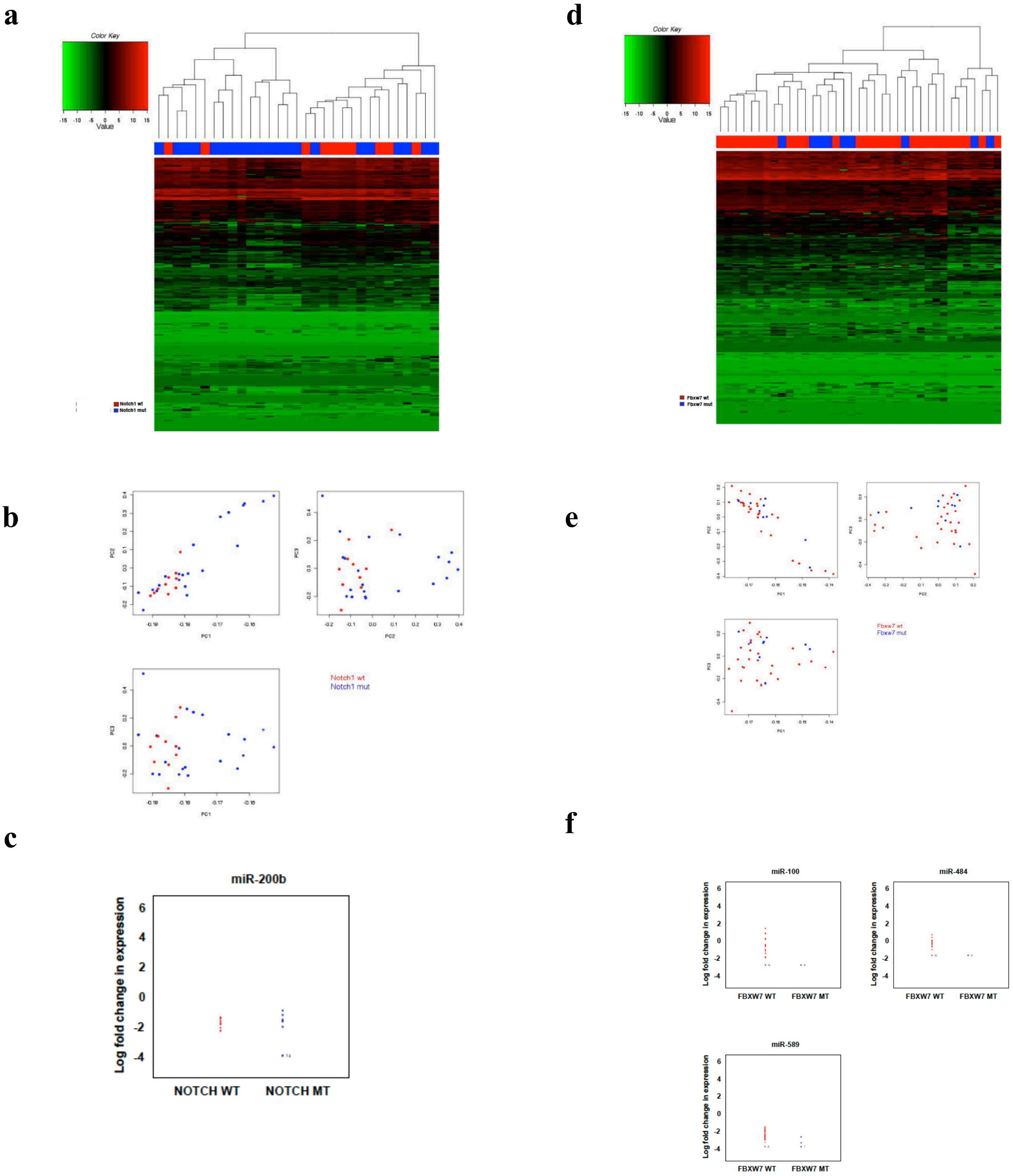


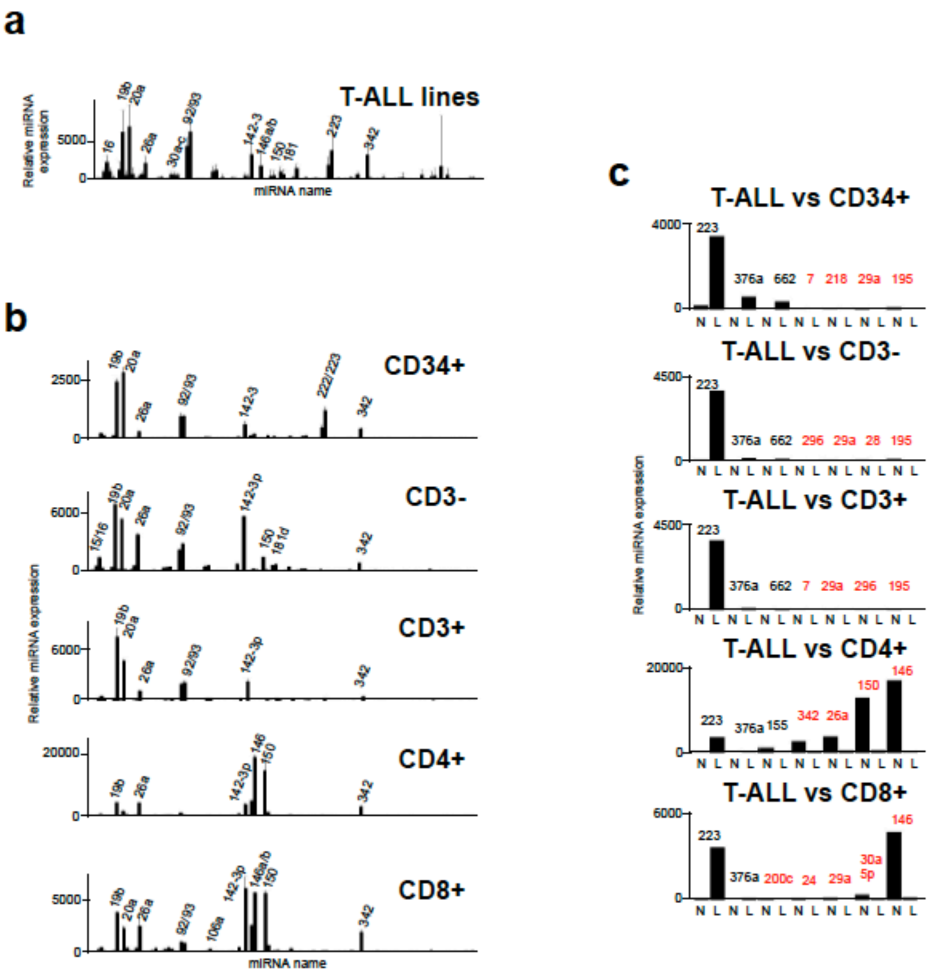
**Suppl. Figure 1 Analysis of miRNA expression and its association with cytogenetic subgroups.** **a,** Hierarchical clustering of miRNA expression analysis of HOXA, TAL and TNX1/3 subgroups. Unsupervised clustering does not recover the cytogenetic subgroups (Red-HOXA, Blue-TAL, Green-TLX1/3); **b,** Principle component analysis (PCA) of miRNA expression in cytogenetic subgroups. PCA by the three main components separates normal from leukemia samples (not shown), but does not separate the cytogenetic subgroups (Red-HOXA, Blue-TAL, Green-TLX1/3); **c,** Differentially expressed miRNA between cytogenetic subgroups. In a pair wise comparison miR-125b, miR-153, miR-196a and miR-196b show significantly different expression between subgroups ($p < 0.05$). (Red-HOXA, Blue-TAL, Green-TLX1/3).



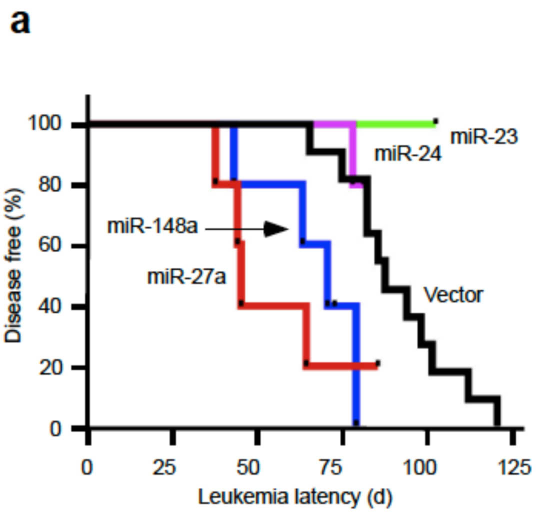
Suppl. Figure 2 **Sequence analysis of *NOTCH1* and *FBXW7* in 37 human T-ALL samples.** **a,** Diagram of *NOTCH1* and *FBXW7* indicating domain structure and sites of mutations; **b,** Relative frequency of *NOTCH1* and *FBXW7* mutations. Note that in our series *NOTCH1* PEST domain and *FBXW7* mutations are not mutually exclusive; **c,** Example of a new *FBXW7* mutation in diagnosis and not a remission sample.

a
Color Key
-15 -10 -5 0 5 10 15
Value
Notch1 wt
Notch1 mut
b
PC1
PC2
PC3
Notch1 wt
Notch1 mut
c
miR-200b
Log fold change in expression
6
4
2
0
-2
-4
NOTCH WT
NOTCH MT
d
Color Key
-15 -10 -5 0 5 10 15
Value
Fbxw7 wt
Fbxw7 mut
e
PC1
PC2
PC3
Fbxw7 wt
Fbxw7 mut
f
miR-100
miR-484
miR-589
Log fold change in expression
FBXW7 WT
FBXW7 MT

**Suppl. Figure 3 Association of miRNA expression and Notch or Fbxw7 mutations. a,** Hierarchical clustering of miRNA expression analysis of *NOTCH1* wild type and mutant cases. Unsupervised clustering produces a grouping of cases by *NOTCH1* status, although 7 wild type cases cluster with the mutant group. (Red-NOTCH MT, Blue-NOTCH WT). **b**, Principle component analysis (PCA) of miRNA expression by *NOTCH1* mutation status. The three plots show the pair wise comparisons of the first three principal components. (Red-NOTCH MT, Blue-NOTCH WT); c**,** Differential expression of miR-200b between *NOTCH1* wild type and mutant cases. Only miR-200b is significantly different ($p < 0.05$). (Red-NOTCH MT, Blue-NOTCH WT); **d,** Hierarchical clustering of miRNA expression analysis by *FBXW7* status. Unsupervised clustering does not recover the groups based on *FBXW7* status. (Red-FBXW7 MT, Blue-FBXW7 WT); **e,** Principle component analysis (PCA) of miRNA expression by *FBXW7* status. PCA does not separate the subgroups. (Red-FBXW7 MT, Blue-FBXW7 WT); **f,** Differentially expressed miRNA by *FBXW7* status. In a pair wise comparison miR-100, miR-484, and miR-589 show significantly different expression between wild type and mutant cases ($p < 0.05$). (Red-FBXW7 MT, Blue-FBXW7 WT).

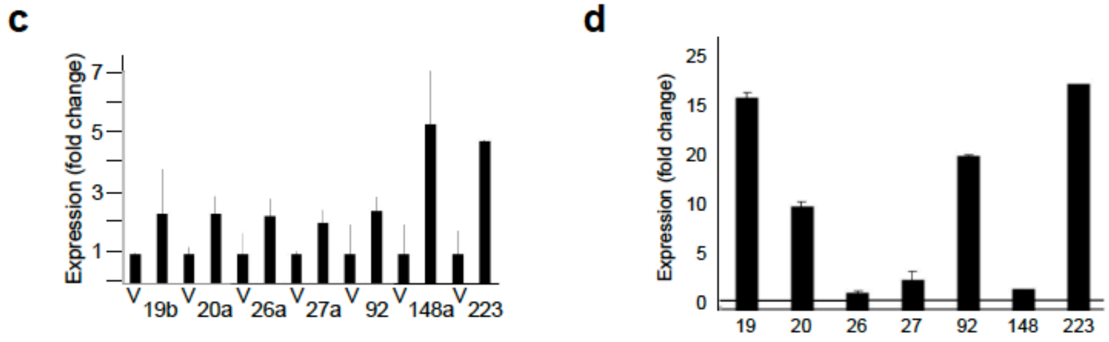


Suppl. Figure 4 **MiRNA expression analyses. a,** qRT-PCR measurement of miRNA expression in 18 human T-ALL cell lines (shown are mean and SD); **b,** qRT-PCR measurement of miRNA expression in FACS sorted normal cell populations (shown are mean and SD of two replicate profilings on one sample); **c,** Differential miRNA expression comparing the average value across all human T-ALL samples (L) to the indicated normal cell populations (N). Shown are only miRNAs with a fold change difference > 5 and detectable expression (relative value > 50) in either normal or leukemic sample; black numbers indicate higher expression in leukemic cells, red numbers indicate higher expression in normal cells.

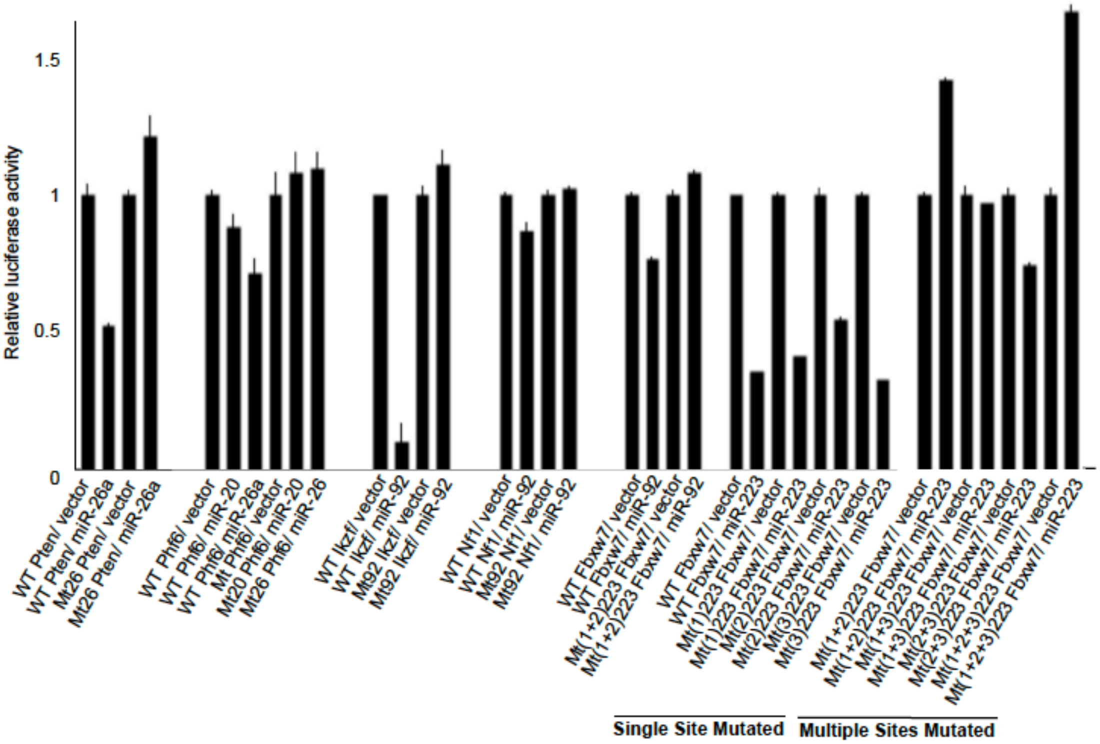


**b**

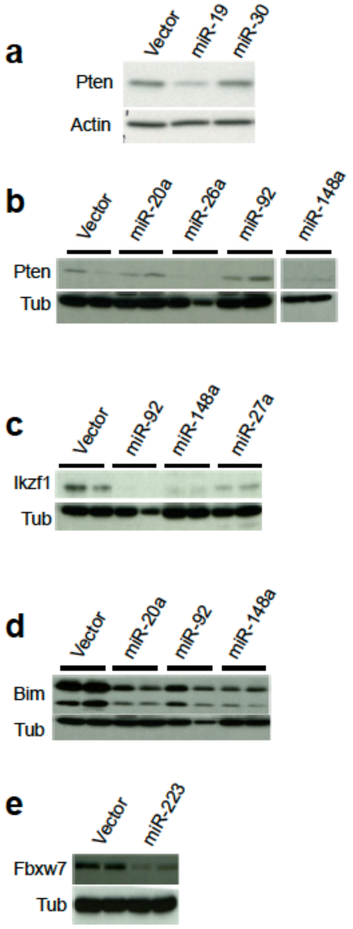
| | CD4 | CD8 | Thy1 | B220 | cKit |
|---|---|---|---|---|---|
| **miR-20a** | 64.5 | 68.5 | 70.5 | 32 | 34 |
| **miR-26a** | 86.5 | 91 | 84.5 | 34.5 | 41 |
| **miR-27a** | 65 | 83 | 85.3 | 78 | 38.5 |
| **miR-92a** | 52.5 | 63.5 | 62 | 48.5 | 37.5 |
| **miR-148** | 73.5 | 80.5 | 77.5 | 28 | 30 |
| **miR-223** | 39.5 | 78 | 86 | 1.8 | 1.4 |



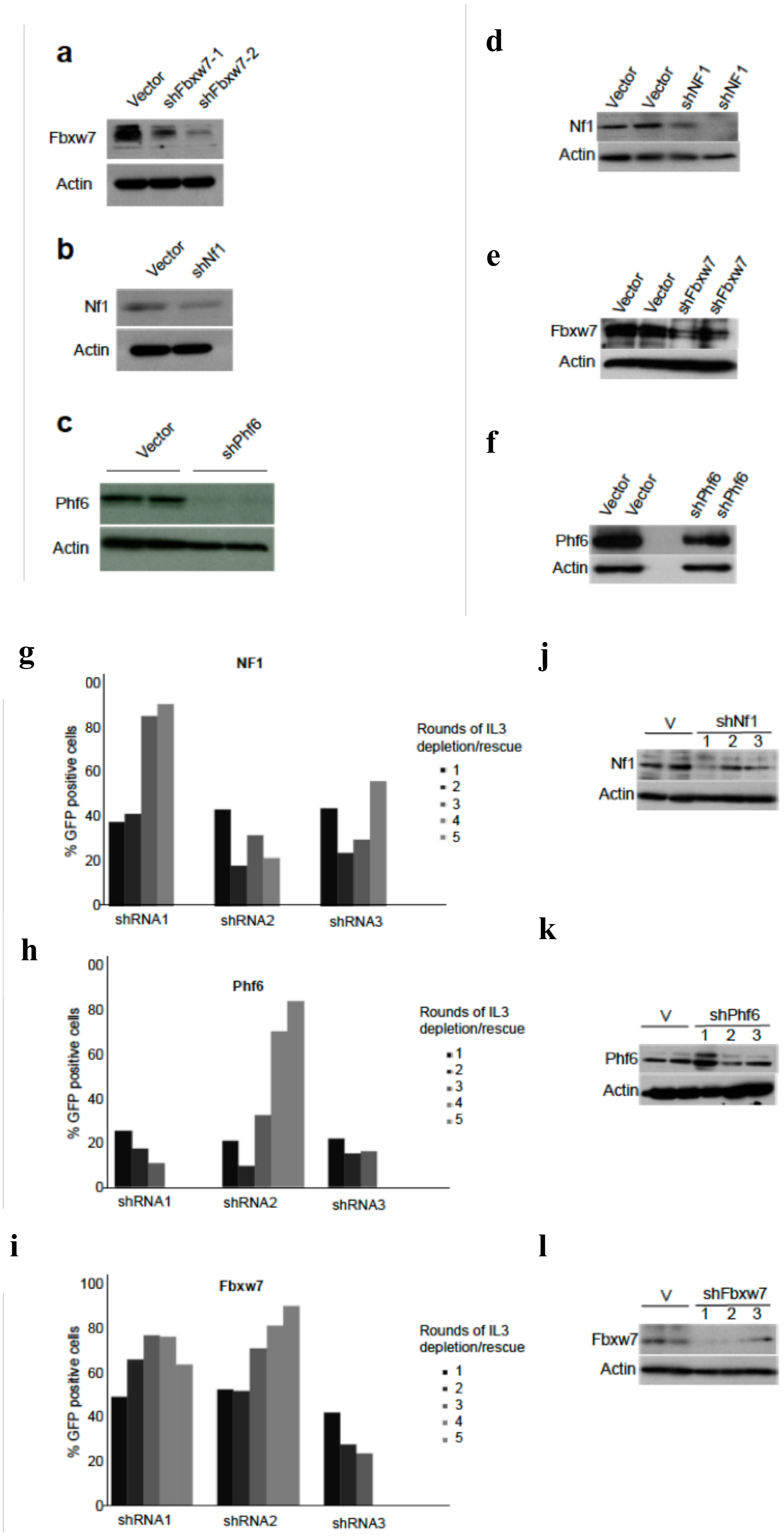
Suppl. Figure 5 **Candidate miRNAs act as oncogenes in a murine T-ALL model. a,** Kaplan-Meier analysis of leukemia free survival after transplantation of HPCs expressing *NOTCH1-ICN* and vector (black, n=13), or miR-27a (red, n=5; $p < 0.05$ compared to vector), miR-24 (magenta, n=5; $p = 0.5$), miR-23a (green, n=5; $p < 0.05$), miR-148a (blue, n=7; $p < 0.05$); **b,** Analysis of surface marker expression on murine T-ALLs, indicated is the percentage of cells staining positive for the indicated marker; **c**, qRT-PCR measurement of miRNA expression in murine leukemic cells expressing the indicated miRNA compared to leukemias arising in vector controls; **d,** qRT-PCR measurement of miRNA expression in murine leukemic cells expressing the indicated miRNA compared to murine thymocytes.



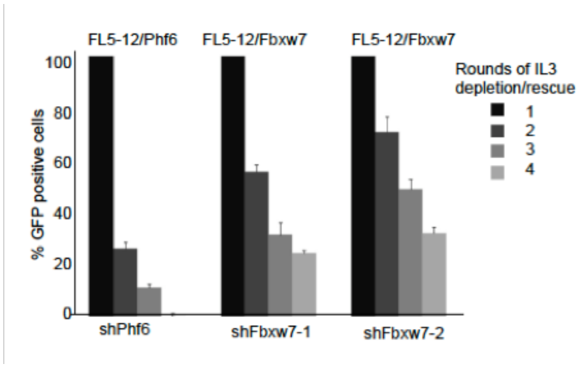
Suppl. Figure 6 **Reporter assay for 3'UTR repression**. Inhibition of the 3'UTR by the indicated miRNA compared to vector as mean +/- SD ratio of Renilla to Firefly luciferase activity (RLuc/Luc). Shown are effects of the indicated miRNAs on wild type (WT) and mutated (Mt) miRNA 3'UTR binding sites of *Pten, Phf6, Ikzf, Nf1, Fbxw7*. The *Ikzf* Mt 3'UTR is truncated and missing the miR-92 site, and in the case of *Fbxw7* we mutated single and combinations of all three binding sites for miR-223 (labelled Mt1-3).



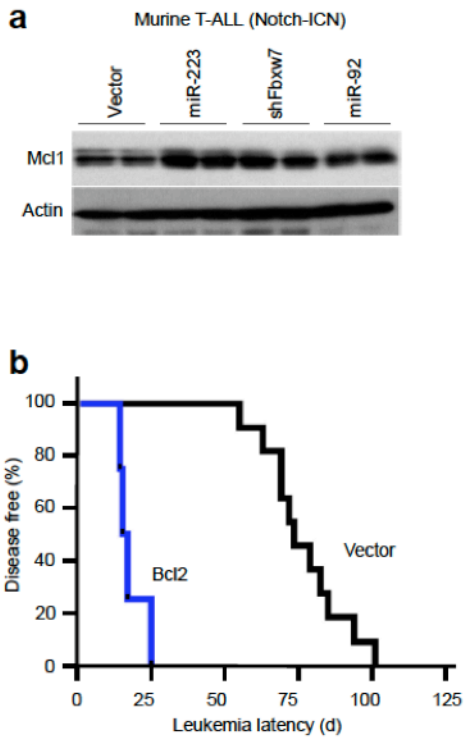
Suppl. Fig. 7 **MiRNAs affect the expression of several tumor suppressor genes in murine FL5-12 cells. a-e,** Lysates of GFP-sorted FL5-12 cells expressing either empty vector or the indicated miRNA were probed for the indicated proteins.


a
Vector
shFbxw7-1
shFbxw7-2
Fbxw7
Actin
b
Vector
shNf1
Nf1
Actin
c
Vector
shPhf6
Phf6
Actin
d
Vector
Vector
shNF1
shNF1
Nf1
Actin
e
Vector
Vector
shFbxw7
shFbxw7
Fbxw7
Actin
f
Vector
Vector
shPhf6
shPhf6
Phf6
Actin
g
NF1
% GFP positive cells
00
80
60
40
20
0
shRNA1
shRNA2
shRNA3
Rounds of IL3 depletion/rescue
1
2
3
4
5
h
Phf6
% GFP positive cells
00
80
60
40
20
0
shRNA1
shRNA2
shRNA3
Rounds of IL3 depletion/rescue
1
2
3
4
5
i
Fbxw7
% GFP positive cells
100
80
60
40
20
0
shRNA1
shRNA2
shRNA3
Rounds of IL3 depletion/rescue
1
2
3
4
5
j
V
shNf1
1 2 3
Nf1
Actin
k
V
shPhf6
1 2 3
Phf6
Actin
l
V
shFbxw7
1 2 3
Fbxw7
Actin

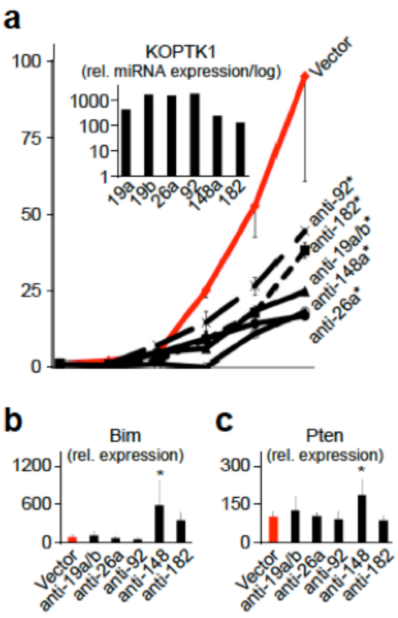
**m**



Suppl. Figure 8 **shRNA validation and control experiments; a-c,** Protein knockdown by shRNAs in FL5-12 cells. Lysates of GFP-sorted FL5-12 cells expressing either empty vector or the indicated shRNAs were probed for the indicated proteins; **d-f**, Protein knockdown by shRNAs in murine leukemias expressing the shRNAs or vector. Lysates of murine leukemic cells expressing either empty vector or the indicated shRNAs were probed for the indicated proteins; **g, I, k,** Evaluation of mutiple shRNAs against Nf1, Phf6 and Fbxw7. Enrichment of shRNA and GFP expressing FL5-12 cells in subsequent rounds of IL3 depletion and rescue by addition of IL3; **h, j l,** corresponding immunoblots on lysates of shRNA expressing FL5-12 cells probed as indicated; **m,** Reversion of shRNA effect by cDNA expression. Expression of indicated cDNAs reverses the protective effect of shRNAs and causes a negative selection (loss of cDNA expressing cells) upon IL-3 depletion.



Suppl. Fig. 9 **Deregulation of Mcl1 in miR-223 and miR-92 expressing murine leukemias. a)** Immunoblot on lysates from *Notch1*-driven leukemias that express the indicated miRNAs or shRNA; **b)** *Bcl2* (blue; n = 4) cooperates with *Notch1-ICN* in murine leukemogenesis compared to *Notch1-ICN/* vector (black, n = 13; p < 0.001).



Suppl. Figure 10 **Antagomir studies in the human KOPTK1 T-ALL cell line. a,** Cell number during in vitro culture of KOPTK1 cells expressing the indicated antagomirs (shown are mean and SD for each time point, *indicates significant ($p < 0.05$) growth delay; **b**, and **c**, qRT-PCR of *Bim* (b) and *Pten* (c) mRNA levels in KOPTK1 cells expressing the indicated antagomirs (mean and SD, * is $p < 0.05$ compared to vector).

**Suppl. Table 2) Comparison of miRNA expression between cytogentic subgroups** (only significant differences are shown)

| | p-value | HOXA mean | Other mean | TAL mean |
|---|---|---|---|---|
| **hsa-mir-125b** | 0.0124 | -2.2938 | 2.1695 | -1.819 |
| **hsa-mir-153** | 0.0076 | -5.4164 | -5.5594 | -9.1937 |
| **hsa-mir-196a** | 0.0045 | 7.1794 | 3.3939 | -0.7036 |
| **hsa-mir-196b** | 0.0045 | 7.2014 | 3.3183 | -0.7178 |

## Suppl. Table 3) Mutation analysis for NOTCH1 and FBXW7 in human T-ALL samples

| ID | Age | Gender | Genetic subtype | ***NOTCH1* mutation** | | | ***FBXW7* mutation** | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | *Nucleotide change* | *Predicted protein lesion* | *Domain* | *Nucleotide change* | *Predicted protein lesion* | *Domain* |
| 1 | 16 | M | Unknown | c.4737G_4738CinsGTG | p.V1579_L1580insV | HD | c.1393C>T<br>c.2065C>T | p.R465C<br>p.R689W | WD3 |
| 2 | 29 | M | HOXA (inv(7)) | c.4802T>C | p.L1601P | HD | c.1393C>T | p.R465C | WD3 |
| 3 | 11 | M | TLX3 | c.7510C>T | p.Q2504* | PEST | c.[1318delGATCGGACAC; 1317_1318insCACCT] | p.D440fs*30 | WD2 |
| 4 | 8 | M | Unknown | c.5229C_5230GinsAGATTCCCTTATGGGACC | p.A1743_A1744insRFPYGT | JM | c.1513C>T | p.R505C | WD4 |
| 5 | 16 | M | TAL/LMO (LMO2) | c.4858_4864TACTACG>CCCGTCC | p.1620_1622YYG>PDR | HD | c.1513C>T | p.R505C | WD4 |
| 6 | 13 | F | Unknown | c.7504C>T<br>c.5036T>C | p.Q2502*<br>p.L1679P | PEST<br>HD | c.1270_1271insGGAG<br>- | p.V424fs*14 | WD2 |
| 7 | 3 | F | Unknown | c.4748_4749insCCCCCCTTATAC | p.P1583_E1584insPPYT | HD | c.1436G>A | p.R479Q | WD3 |
| 8 | 2 | F | Unknown | c.[4749_4752delGGAG; 4748_4749insC] | p.E1584del | HD | c.1436G>A | p.R479Q | WD3 |
| 9 | 11 | M | HOXA (inv(7)) | - | | | c.1436G>A | p.R479Q | WD3 |
| 10 | 14 | F | TAL/LMO (LMO2) | c.4732_4737delGTGGTG<br>c.7462C>T | p.V1578_V1579del<br>p.Q2488* | HD<br>PEST | - | | |
| 11 | 5 | F | HOXA (CALM-AF10) | c. [7545_7548delTGAG; 7545_7546insCTTT;7552_7553insGAGC] | p.[E2516F;P2518fs*3] | PEST | - | | |
| 12 | 4 | M | TAL/LMO (SIL-TAL) | c.7331_7332insCG | p.V2444fs*35 | PEST | - | | |
| 13 | 16 | M | TLX3 | c.7318delG | p.P2439fs*39 | PEST | - | | |
| 14 | 7 | M | TLX3 | c.4728_4729insTTTCTCTTG<br>c.7563G>A | p.V1576_V1577insFLL<br>p.W2521* | HD<br>PEST | - | | |
| 15 | 18 | F | TAL/LMO (LMO2) | c.[7372delC; 7372_7374insGGT] | p.L2458fs*21 | PEST | - | | |
| 16 | 3 | M | TAL/LMO (SIL-TAL) | c.4802T>C<br>c.7393delC | p.L1601P<br>p.L2465fs*13 | HD<br>PEST | - | | |
| 17 | 17 | M | TAL/LMO (LMO2) | c.7462C>T | p.Q2488* | HD | - | | |
| 18 | 12 | M | TAL/LMO (LMO2) | c.4821_4822insGGATTC | p.F1607>LDS | HD | - | | |
| 19 | 8 | M | TLX3 | c.4802T>C | p.L1601P | HD | - | | |
| 20 | 4 | M | TLX1 | c.4781T>C | p.L1594P | HD | - | | |
| 21 | 7 | M | TAL/LMO (SIL-TAL) | c.4782_4783insCCC | p.L1594_R1595insP | HD | - | | |
| 22 | 6 | M | TLX1 | c.4802T>A | p.L1601Q | HD | - | | |
| 23 | 1 | F | HOXA (MLL) | - | | | - | | |
| 24 | 16 | M | HOXA (MLL) | - | | | - | | |
| 25 | 3 | M | TAL/LMO (LMO2) | - | | | - | | |
| 26 | 5 | M | TAL/LMO (LMO2) | - | | | - | | |
| 27 | 16 | M | TAL/LMO (SIL-TAL) | - | | | - | | |
| 28 | 14 | M | TAL/LMO (SIL-TAL) | - | | | - | | |
| 29 | 9 | F | TAL/LMO (SIL-TAL) | - | | | - | | |
| 30 | 13 | M | TAL/LMO (SIL-TAL) | - | | | - | | |
| 31 | 4 | M | TLX3 | - | | | - | | |
| 32 | 6 | M | TLX3 | - | | | - | | |
| 33 | 9 | M | TLX1 | - | | | - | | |
| 34 | 22 | F | TLX1 | - | | | - | | |
| 35 | 13 | M | Unknown | - | | | - | | |
| 36 | 14 | F | Unknown | - | | | - | | |
| 37 | 6 | M | Unknown | - | | | - | | |

## Suppl. Table 4) Differential microRNA expression by FBXW7 and NOTCH mutation status in T-ALL patient samples

1. Fbxw7 wild type versus Fbxw7 mutant.

| **microRNA** | **p-value** | **mutant mean exp** | **wt mean exp** |
|---|---:|---:|---:|
| miR-100 | 0.0143 | -5.718 | -2.6865 |
| miR-484 | 7.00E-04 | -3.369 | -1.307 |
| miR-589 | 0.0363 | -7.3454 | -5.467 |

2. Notch1 wild type versus Notch1 mutant

| **microRNA** | **p-value** | **mutant mean exp** | **wt mean exp** |
|---|---:|---:|---:|
| miR-200b | 0.0143 | -6.4442 | -3.3669 |

## Suppl. Table 6) Differential microRNA expression by mutation status in T-ALL cell lines

1. Fbxw7 wild type versus Fbxw7 mutant: No significant differences.
2. Notch1 wild type versus Notch1 mutant H: No significant differences.
3. Notch1 wild type versus Notch1 mutant (any).

| **microRNA** | **p-value** | **mutant mean exp** | **wt mean exp** |
|---|---|---|---|
| miR-449b | 0.0339 | -8.1629 | -4.7352 |

4. NCID Status: No significant differences
5. Pten wild type versus Pten mutant: No significant differences.
6. GSI S/R: No significant differences.
7. Phf6 wild type versus Phf6 mutant.

| **microRNA** | **p-value** | **mutant mean exp** | **wt mean exp** |
|---|---|---|---|
| miR-125b | 0.0339 | 3.6653 | -5.5518 |

**Suppl. Table 10) Results of primary screen: Myc-induced apoptosis in MEFs**

| MicroRNA | # sequences | % of sequences |
|---|---|---|
| miR-30 | 10 | 9.803921569 |
| let7 | 9 | 8.823529412 |
| miR-34 | 9 | 8.823529412 |
| miR-25 | 6 | 5.882352941 |
| miR-148 | 5 | 4.901960784 |
| miR-19b | 4 | 3.921568627 |
| miR-223 | 4 | 3.921568627 |
| miR-125/351 | 4 | 3.921568627 |
| miR-23 | 4 | 3.921568627 |
| miR-21 | 2 | 1.960784314 |
| miR-22 | 2 | 1.960784314 |
| miR-106 | 2 | 1.960784314 |
| miR-138 | 2 | 1.960784314 |
| miR-146 | 2 | 1.960784314 |
| miR-154 | 2 | 1.960784314 |
| miR-217 | 2 | 1.960784314 |
| miR-101 | 2 | 1.960784314 |
| miR-221 | 2 | 1.960784314 |
| miR-7 | 1 | 0.980392157 |
| miR-9 | 1 | 0.980392157 |
| miR-16 | 1 | 0.980392157 |
| miR-99 | 1 | 0.980392157 |
| miR-100 | 1 | 0.980392157 |
| miR-103 | 1 | 0.980392157 |
| miR-122 | 1 | 0.980392157 |
| miR-124 | 1 | 0.980392157 |
| miR-128 | 1 | 0.980392157 |
| miR-135 | 1 | 0.980392157 |
| miR-136 | 1 | 0.980392157 |
| miR-140 | 1 | 0.980392157 |
| miR-150 | 1 | 0.980392157 |
| miR-181 | 1 | 0.980392157 |
| miR-189 | 1 | 0.980392157 |
| miR-196 | 1 | 0.980392157 |
| miR-200 | 1 | 0.980392157 |
| miR-201 | 1 | 0.980392157 |
| miR-206 | 1 | 0.980392157 |
| miR-211 | 1 | 0.980392157 |
| miR-295 | 1 | 0.980392157 |
| miR-302 | 1 | 0.980392157 |
| miR-322 | 1 | 0.980392157 |
| miR-325 | 1 | 0.980392157 |
| miR-328 | 1 | 0.980392157 |
| miR-339 | 1 | 0.980392157 |
| miR-337 | 1 | 0.980392157 |
| miR371 | 1 | 0.980392157 |
| miR-27 | 1 | 0.980392157 |

## Suppl. Table 11) Result of secondary screen

| | **Sequences** |
|---|---|
| miR-148/152 | 32 |
| miR-34a | 20 |
| miR-19b | 8 |
| miR-223 | 8 |
| mi-30 | 4 |
| miR-320 | 4 |
| miR-9 | 4 |
| miR-96 | 4 |
| miR-25/32/92 | 3 |
| miR-20/106 | 2 |
| miR-26 | 1 |
| miR-23 | 1 |
| miR-24 | 1 |
| miR-27 | 1 |
| miR-101 | 1 |
| miR-128 | 1 |
| miR-15 | 1 |
| miR-16 | 1 |
| miR-181c | 1 |
| miR-200 | 1 |
| miR-221 | 1 |
| miR-137 | 1 |
| miR-217 | 1 |
| miR-22 | 1 |
| miR-182 | 1 |

## Suppl. Table 12) Surface marker analysis on murine leukemias

| | | | Marker | | |
|---|---|---|---|---|---|
| Tumor | CD4 | CD8 | Thy-1 | C-Kit | B220 |
| 27a-1 | 64 | 83 | 83.6 | 77 | 34 |
| 27a-2 | 66 | 83 | 87 | 79 | 43 |
| mean | 65 | 83 | 85.3 | 78 | 38.5 |
| sdev | 1.414213562 | 0 | 2.404163056 | 1.414213562 | 6.363961031 |
| | | | | | |
| 92a-1 | 69 | 83.3 | 86 | 78 | 52 |
| 92a-2 | 36 | 44 | 38 | 19.2 | 23 |
| mean | 52.5 | 63.65 | 62 | 48.6 | 37.5 |
| sdev | 23.33452378 | 27.7892965 | 33.9411255 | 41.57787873 | 20.50609665 |
| | | | | | |
| 20a | 57 | 53 | 66 | 10 | 15 |
| 20a-2 | 72 | 84 | 75 | 54 | 53 |
| | 64.5 | 68.5 | 70.5 | 32 | 34 |
| sdev | 10.60660172 | 21.92031022 | 6.363961031 | 31.11269837 | 26.87005769 |
| | | | | | |
| 26a | 81 | 86 | 87 | 7 | 30 |
| 26a-2 | 92 | 96 | 82 | 62.1 | 52 |
| mean | 86.5 | 91 | 84.5 | 34.55 | 41 |
| sdev | 7.778174593 | 7.071067812 | 3.535533906 | 38.96158364 | 15.55634919 |
| | | | | | |
| 148a | 68 | 76 | 72 | 21 | 40 |
| 148a-2 | 79 | 85 | 83 | 35 | 20 |
| mean | 73.5 | 80.5 | 77.5 | 28 | 30 |
| sdev | 7.778174593 | 6.363961031 | 7.778174593 | 9.899494937 | 14.14213562 |
| | | | | | |
| 223-1 | 50.24 | 70.48 | 78.46 | 1.71 | 2.02 |
| 223-2 | 28.85 | 85.77 | 93.43 | 1.97 | 0.72 |
| mean | 39.545 | 78.125 | 85.945 | 1.84 | 1.37 |
| sdev | 15.12501405 | 10.81166268 | 10.58538851 | 0.183847763 | 0.919238816 |
| | | | | | |
| 19b-1 | 81.98 | 65 | 99.97 | 1.1 | 11.38 |
| 19b-2 | 97.46 | 80.7 | 99.32 | 1.83 | 13.16 |
| mean | 89.72 | 72.85 | 99.645 | 1.465 | 12.27 |
| sdev | 10.94601297 | 11.10157646 | 0.459619408 | 0.51618795 | 1.258650071 |
| | | | | | |
| Vector-1 | 49.98 | 82.62 | 85.08 | 1.98 | 76.66 |
| Vector-2 | 86.83 | 94.72 | 76.92 | 1.2 | 12.44 |
| mean | 68.405 | 88.67 | 81 | 1.59 | 44.55 |
| sdev | 26.05688489 | 8.555992052 | 5.769991334 | 0.551543289 | 45.41039749 |

### Suppl. Table 13) Results of an unbiased machine learning approach to identify target genes that discriminate oncogenic and non-oncogenic miRNAs.

Lasso regression was run 50 times comparing experimentally confirmed oncomirs to randomly selected groups of 30 miRNAs that did not pass the screen.
The number of non-zero coefficients indicates how often (out of 50 runs) the gene was identified in this analysis.
The Mean coefficient is the actual coefficient for each gene; a coeeficient >0 implies positive association with the oncomir group.

| Gene | No. of non zero coefficients | Mean coefficient |
|---|---:|---:|
| FBXW7 | 46 | 0.2919 |
| CHIC1 | 36 | 0.1114 |
| MAP3K2 | 31 | 0.0566 |
| ABL2 | 30 | 0.0725 |
| SNN | 29 | 0.0815 |
| BIM | 21 | 0.0707 |
| MED12L | 20 | 0.0728 |
| BBX | 19 | 0.0733 |
| ANKIB1 | 18 | 0.0422 |
| PPARA | 12 | 0.0274 |
| PTEN | 11 | 0.0587 |
| SLC8A1 | 11 | 0.031 |
| DMXL1 | 11 | 0.0271 |
| PTPRD | 10 | 0.0306 |
| DCP2 | 8 | 0.0067 |
| LPP | 7 | 0.0152 |
| CREB1 | 6 | 0.006 |
| MKL2 | 5 | 0.0054 |
| MFHAS1 | 5 | 0.0122 |
| EIF2C1 | 5 | 0.0138 |
| TNRC6B | 5 | 0.006 |
| FBXL17 | 4 | -0.006 |
| NFIC | 3 | -0.0022 |
| STRBP | 3 | -0.004 |
| TULP3 | 3 | -4.00E-04 |
| NUFIP2 | 3 | 0.0103 |
| RAP2C | 2 | 0.0041 |
| ADCY1 | 2 | 3.00E-04 |
| PPP1R9A | 2 | 9.00E-04 |
| CELF2 | 2 | -0.0164 |
| SLC24A2 | 2 | 2.00E-04 |
| FAM53C | 2 | -9.00E-04 |
| SCAI | 2 | -0.0012 |
| PRX | 2 | -0.0014 |
| FUT9 | 2 | 1.00E-04 |
| STEAP2 | 2 | -2.00E-04 |
| TARDBP | 2 | -3.00E-04 |
| MYLK4 | 2 | -5.00E-04 |
| PLXNA4 | 2 | -0.0037 |
| WASL | 2 | 8.00E-04 |
| DOCK5 | 1 | 0 |
| HIF3A | 1 | -0.0012 |
| ANKRD45 | 1 | 0 |
| AAK1 | 1 | 9.00E-04 |
| WNK3 | 1 | 0 |
| TMEM170B | 1 | -9.00E-04 |
| RFNG | 1 | -0.0032 |
| TBL1XR1 | 1 | 0 |
| ATXN1L | 1 | 0.0021 |
| MDGA2 | 1 | -4.00E-04 |
| YWHAZ | 1 | -4.00E-04 |
| SP1 | 1 | -0.0011 |
| CBX5 | 1 | -3.00E-04 |
| CLMN | 1 | -0.0021 |
| SPAG9 | 1 | -0.0018 |
| DENND5B | 1 | 1.00E-04 |
| GNAO1 | 1 | -4.00E-04 |
| CLOCK | 1 | 0.0053 |
| SYNJ2BP | 1 | -3.00E-04 |
| ONECUT2 | 1 | -4.00E-04 |
| KRAS | 1 | -0.0038 |
| TET3 | 1 | -0.0012 |
| MAN2A1 | 1 | 5.00E-04 |
| SH3PXD2B | 1 | -2.00E-04 |
| UNC5C | 1 | 0 |
| CYYR1 | 1 | -2.00E-04 |
| LAPTM5 | 1 | -1.00E-04 |
| OTUD7B | 1 | -0.0015 |
| CDC14B | 1 | 0 |
| TBX22 | 1 | -1.00E-04 |
| QSER1 | 1 | 1.00E-04 |
| FRS2 | 1 | 5.00E-04 |

| | | |
|---|---|---|
| FZD5 | 1 | -0.0017 |
| PRTG | 1 | -0.0012 |
| NEURL1B | 1 | 0.006 |
| TLN2 | 1 | -1.00E-04 |
| HOOK3 | 1 | -4.00E-04 |
| GPM6B | 1 | -6.00E-04 |
| CACNB4 | 1 | 0 |
| AKAP13 | 1 | -2.00E-04 |
| SYNPO | 1 | -3.00E-04 |
| RUFY2 | 1 | 0 |
| STXBP5L | 1 | -0.0011 |
| XPO7 | 1 | 0 |
| RASAL2 | 1 | -9.00E-04 |
| RGS17 | 1 | 0 |
| SRF | 1 | -7.00E-04 |
| PTPN2 | 1 | 0 |
| TMF1 | 1 | 0 |
| SHISA7 | 1 | -6.00E-04 |
| PGR | 1 | 0 |
| PHIP | 1 | 6.00E-04 |
| DENND1B | 1 | 0.0074 |
| ZYG11B | 1 | -2.00E-04 |
| BTBD3 | 1 | -6.00E-04 |
| ZBTB41 | 1 | 0 |
| SLC1A2 | 1 | 0.0012 |
| ARIH1 | 1 | -1.00E-04 |
| INO80D | 1 | 0.0036 |
| SHISA6 | 1 | 0 |
| PAPPA | 1 | -7.00E-04 |
| RAP1GAP2 | 1 | -5.00E-04 |
| CDS1 | 1 | 1.00E-04 |
| PACS1 | 1 | 0 |
| DNM3 | 1 | -6.00E-04 |
| ARRB1 | 1 | -4.00E-04 |
| NUCKS1 | 1 | -3.00E-04 |
| NAV1 | 1 | -0.0022 |
| AMOTL1 | 1 | -6.00E-04 |
| PRKCA | 1 | -0.002 |

## Suppl. Table 14) Primers used to generate 3'UTR fragments

| UTR | Primer Sequence Forward | Primer Sequence Reverse |
|---|---|---|
| Bim | 5'-GATCTCGAGTAAAGTGACTTCACCTC-3' | 5'-ATGCGGCCGCTTTTAGTCGCAAGTTT-3' |
| fbxw7 | 5'-GATCTCGAGGTGATGAGGGGCAGCTT-3' | 5'-ATGCGGCCGCCACCTTAAGTTATAAG-3' |
| PHF6 | 5'-CCGCTCGAGCCTTTTTCTGCCACTAGCAAC-3' | 5'-CGACTGCGGCCGCATGAGAGGAATGTTTGATTTTTGAC-3' |
| NF1 | 5'-CCGCTCGAGGGTTATATATGCTAGAATTGCATTTAA-3' | 5'-CGACTGCGGCCGCAAGTTTAGTAAATTGCACCTGTCTTC-3' |

Primers Used for Site-Directed Mutagenesis of 3'UTR miRNA binding sites

| | |
|---|---|
| PHF6-miR-20a site | 5'-CTGCCACTAGCAACCAGAATAGCACTTTACCTTTTGGTTGGCTAGATAAG-3' |
| PHF6-miR-26a site | 5'-CTATTGAATCAGAATCTGTGGATGGGAACAAATGTGTGAATCTC-3' |
| NF1-miR-92a site | 5'-GTTGGAAATACTGAAGACAGTTTAGATTTACTAAACTTTTG-3' |
| FBXW7-miR-92a site 1 | 5'-TGAGACTTTCTAAATCAAACCAGTTTAGATTATTTCTTTATTTTCTTCTCCAGTG-3' |
| FBXW7-miR-92a site 2 | 5'-ATTTTCTTATAACTTAATTTTGATAAAATGTGTTTTTTC-3' |

| Hairpin | stem-loop Sequence |
|---|---|
| Nf1 | 5'-CACCTTTGTTTGGAATATA-3' |
| Fbxw7 | 5'-CACGTTAGAATCTGTGACATA-3' |
| PHF6 | 5'-TTTCTTACTAACATTTCTGGT-3' |

**Suppl. Table 15) Mutagenesis of 3'UTR miRNA binding sites**

PHF6 mir-20a binding site

Position 2330-2336 of PHF6 3' UTR

```
                      T GTG
                      X XXX
5'  ...CUAGCAACCAGAAUAGCACUUUA...-Phf6 3'UTR
                      |||||||
3'     GAUGGACGUGAUAUUCGUGAAAU   -miR-20a
```

PHF6 mir-26a binding site

Position 3152-3158 of PHF6 3' UTR

```
                      G TGG
                      X XXX
5'  ...GAAUCAGAAUCUGUGUACUUGAA...-Phf6 3'UTR
                      |||||||
3'      UCGGAUAGGACCUAAUGAACUU   -miR-26a
```

NF1 miR-92a binding site

Position 1810-1816 of NF1 3' TTR

```
                     T TAG
                     X XXX
5' ...AAATACTGAAGACAGGTGCAATT...  -NF1 3'UTR
                     |||||||
3'     TGTCCGGCCCTGTTCACGTTAT     -miR-92a
```

FBXW7 miR-92a binding site

Position 285-291 of FBXW7 3' UTR

```
                     T TTG
                     X XXX
5' ...TCTAAATCAAACCAGGTGCAATT...  -FBXW7 3'UTR
                     |||||||
3'     TGTCCGGCCCTGTTCACGTTAT     -miR-92a
```

FBXW7 miR-92a binding site
Position 1595-1601 of FBXW7 3' UTR

```
                    T TTG
                    X XXX
5' ...TTTCTTATAACTTAAGTGCAATA...   -FBXW7 3’UTR
                    ||||||||
3'    TGTCCGGCCCTGTTCACGTTAT       -miR-92a
```

FBXW7 miR-223 binding site 1
Position 189-195 of FBXW7 3' UTR

```
                     C GT
                     X XX
5' ...GAGAUGACAAACCAUAACUGACA... -FBXW7 3’UTR
                     |||||||
3'    ACCCCAUAAACUGUUUGACUGU     -miR-223
```

FBXW7 miR-223 binding site 2
Position 1144-1150 of FBXW7 3' UTR

```
                     C GT
                     X XX
5' ...GAACGUACAUCGUAAAACUGACA... -FBXW7 3’UTR
                     |||||||
3'    ACCCCAUAAACUGUUUGACUGU     -miR-223
```

FBXW7 miR-223 binding site 3
Position 1260-1266 of FBXW7 3' UTR

```
                      C GT
                      X XX
5'    ...UUUAUUGCUCUGAGUAACUGACA...-FBXW7 3’UTR
                      |||||||
3'       ACCCCAUAAACUGUUUGACUGU     -miR-223
```

**Suppl. Table 16**

| Primer name | Primer sequence | Protein domain |
|---|---|---|
| NOTCH1 exon26 F | AGGAAGGCGGCCTGAGCGTGT | Heterodimerization domain (HD) N-terminal |
| NOTCH1 exon26 R | AGAGTTGCGGGGATTGACCGT | |
| NOTCH1 exon27 F | GTGGCGTCATGGGCCTCA | HD C-terminal |
| NOTCH1 exon27 R | GCACAAACAGCCAGCGTGT | |
| NOTCH1 exon28 F | GATCGGTGTCATGTGAAGT | Juxtamembrane (JM) domain |
| NOTCH1 exon28 R | TCCCGGTGAGGATGCTCGG | |
| NOTCH1 exon34a F | CTTCCTCTGGTGATGGAACCT | Transactivation (TAD) domain and proline glutamate serine threonine (PEST) domain |
| NOTCH1 exon34a R | CATCCCAGGCAGGTGGTTGA | |
| NOTCH1 exon34b F | GCCCTCCCCGTTCCAGCAGTCT | |
| NOTCH1 exon34b R | GCCTGGCTCGGCTCTCCACTCA | |
| NOTCH1 exon34c F | AGCCGCACCTTGGCGTGAGC | |
| NOTCH1 exon34c R | TGGTCGGCCCTGGCATCCAC | |
| FBXW7 exon7 F | TTTATGCCTTCATTTTTCTCTT | WD40-repeats |
| FBXW7 exon7 R | GGGGAAAAAAGCTAAGTTATG | |
| FBXW7 exon8 F | TTTTCCAGTGTCTGAGAACAT | |
| FBXW7 exon8 R | CCCAAATTCACCAATAATAGA | |
| FBXW7 exon9 F | TAAACGTGGGTTTTTTTGTT | |
| FBXW7 exon9 R | TCAGCAATTTGACAGTGATT | |
| FBXW7 exon10 F | CCTGGCATTACCTGTTTC | |
| FBXW7 exon10 R | AGGCTCCATATTTCTCTTGA | |
| FBXW7 exon11 F | GGACATGGGTTTCTAAATATGTA | |
| FBXW7 exon11 R | CTGCACCACTGAGAACAAG | |