Deletion of the protein tyrosine phosphatase gene *PTPN2* in T-cell acute lymphoblastic leukemia

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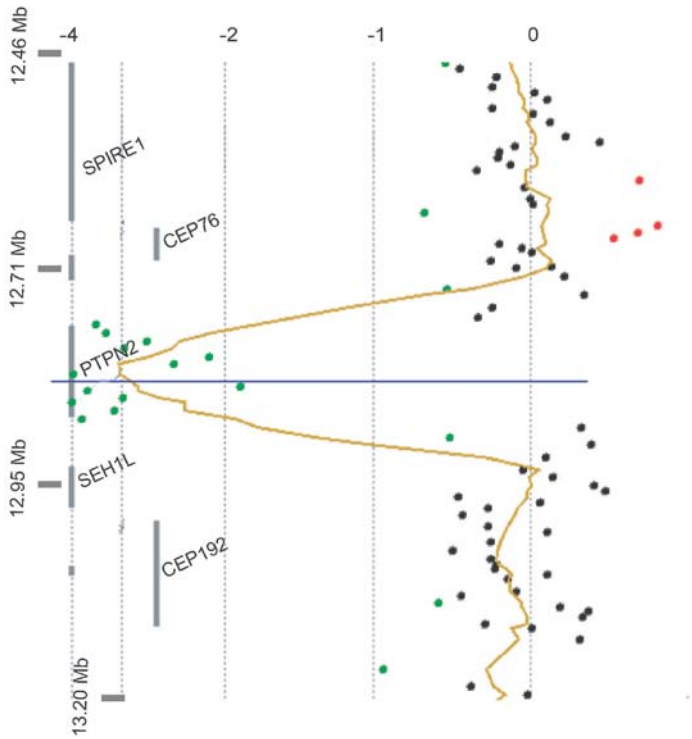
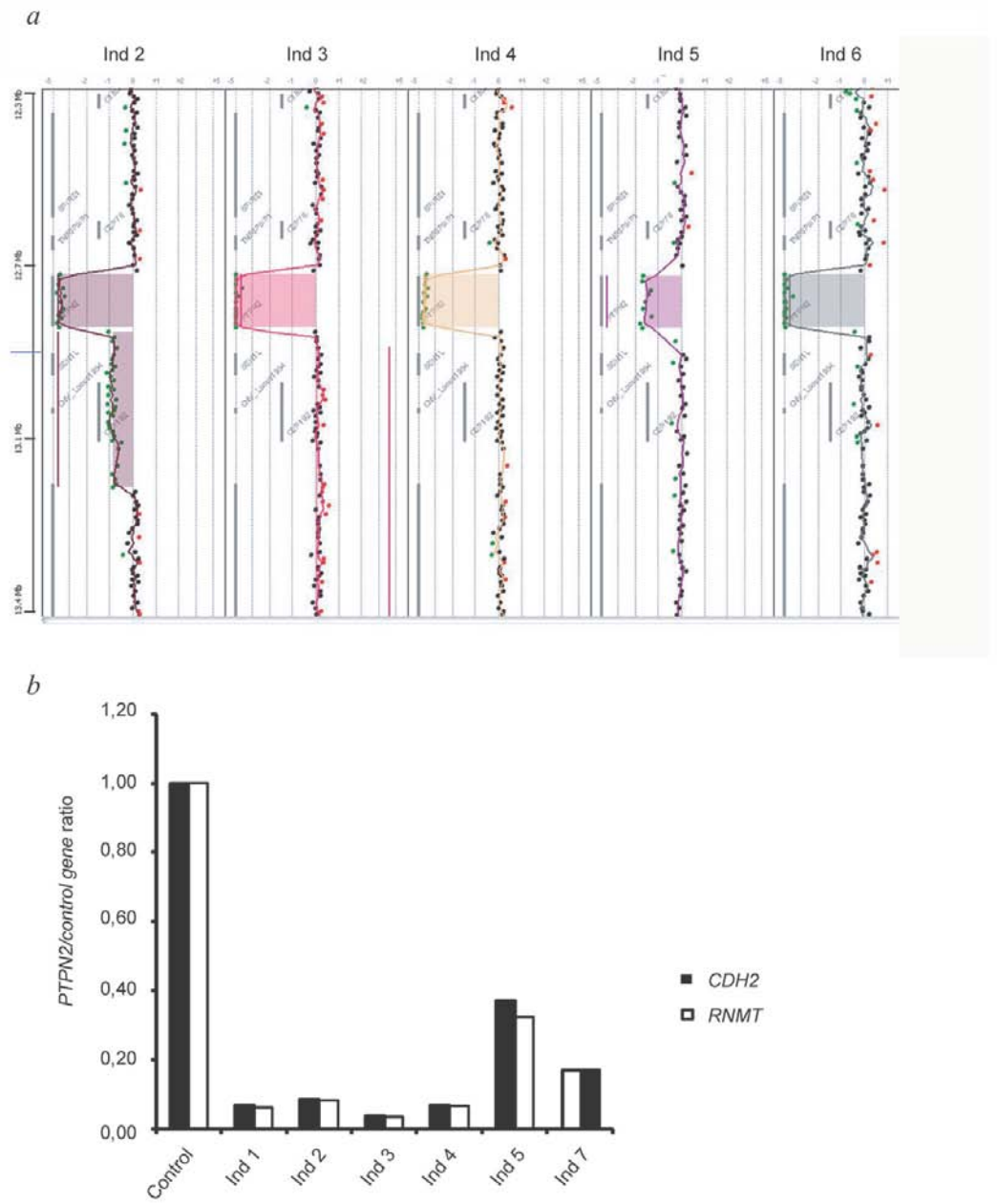


Supplementary Figure 1. Array CGH analysis of one individual with T-ALL.

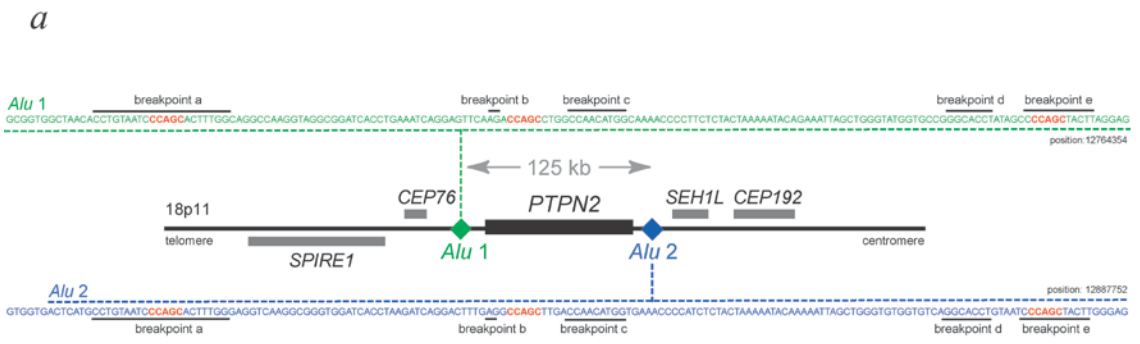
Array CGH analysis of individual 1 demonstrates a focal homozygous deletion of about 125kb restricted to the *PTPN2* locus.

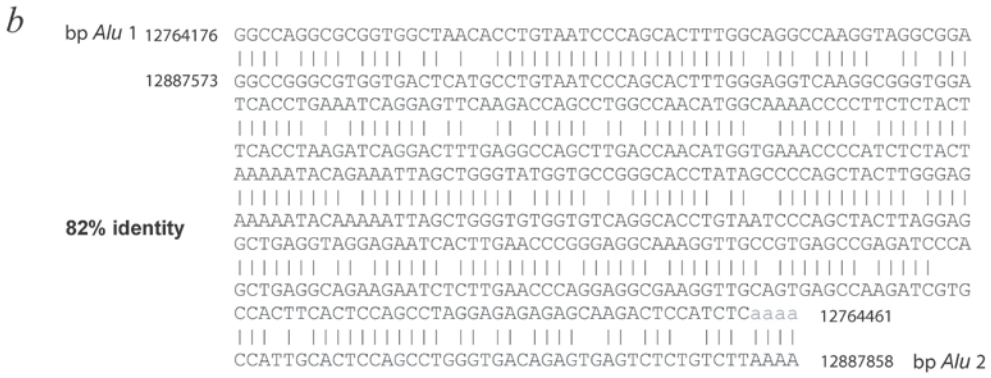


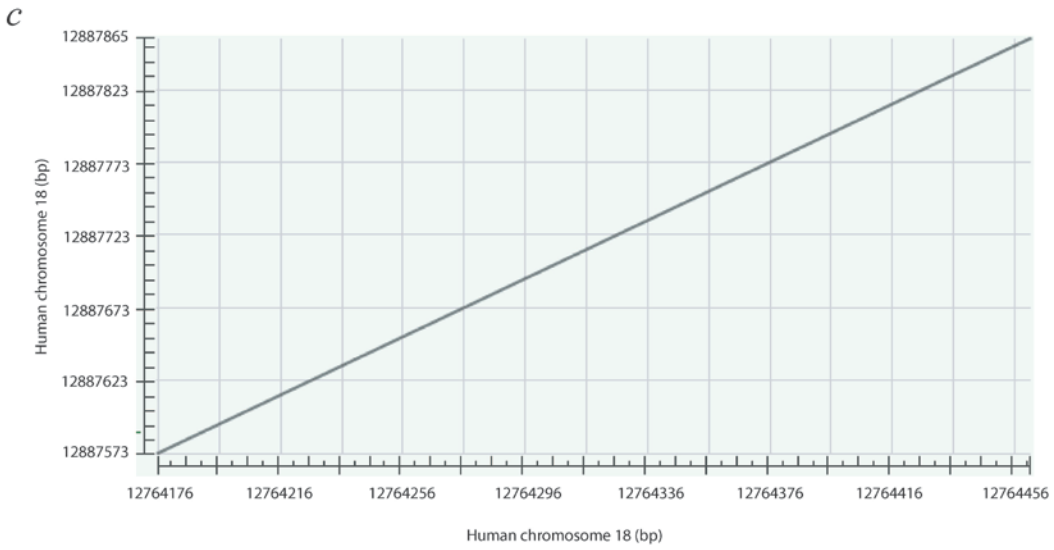
Supplementary Figure 2. Array CGH and quantitative PCR analysis confirms homozygous deletion of *PTPN2.*

(a) Array CGH analysis confirmed the presence of acquired homozygous deletions restricted to the *PTPN2* locus in all individuals with low expression levels. Array CGH profiles suggested highly similar telomeric as well as centromeric breakpoints in 4 of the 5 individuals. Individual 2 displayed a similar focal deletion on one allele, but a larger deletion on the other allele. Blast cell content of individual 5 was about 50% resulting in a higher log ratio value than expected for homozygous deletion of *PTPN2*.

(b) Quantitative PCR analysis of T-ALL individuals with homozygous loss of *PTPN2*. A primer set for *CDH2* (closed bars, 18q12) and RNMT (open bars, 18p11) was used for normalization.







Supplementary Figure 3. Alignment of the two *Alu* elements flanking the *PTPN2* gene.

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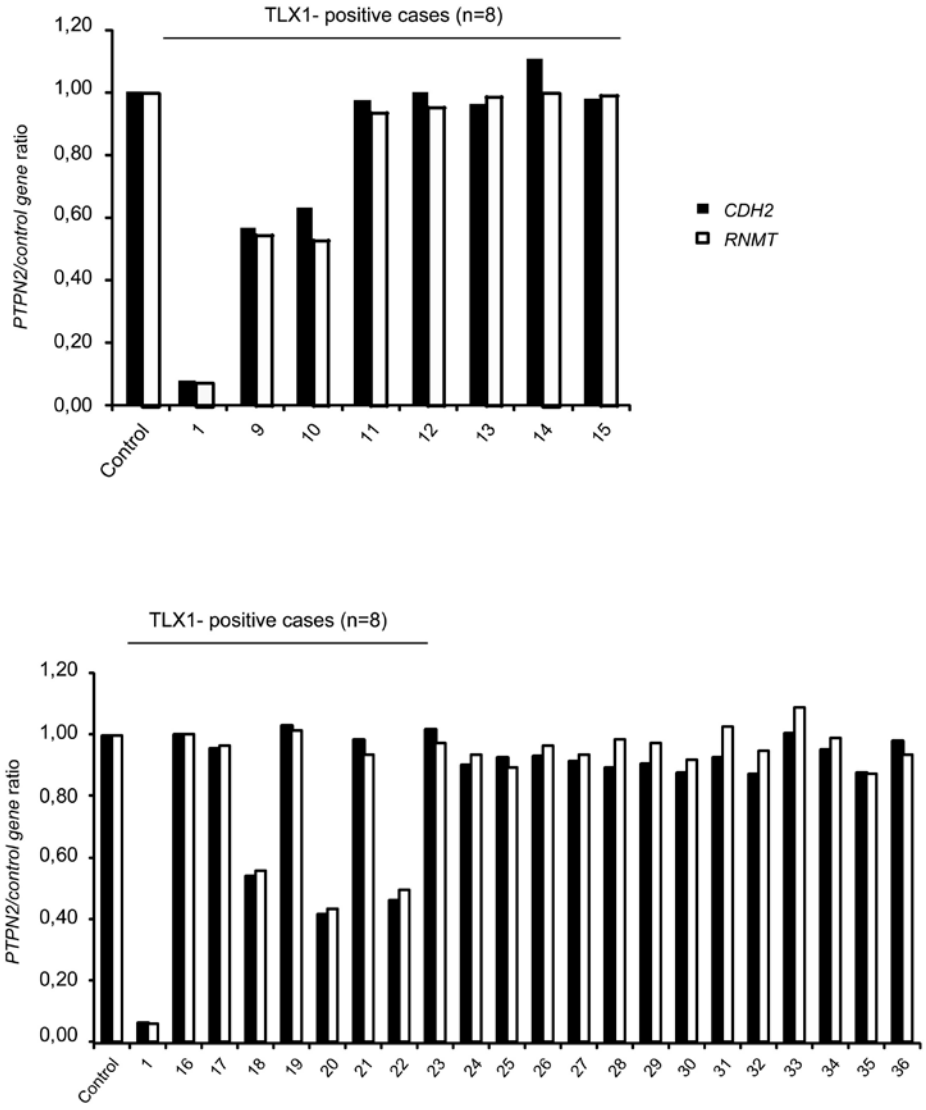
(a) Schematic presentation of the genetic region around the *PTPN2* locus and illustration of the breakpoint junctions. Sequence analysis of breakpoint junctions identified *Alu* elements at both sides with high sequence similarity. Figure depicts an alignment of the flanking *Alu* sequences. Recombinogenic hotspots are shown in red.[1] Identified breakpoint junctions are underlined.

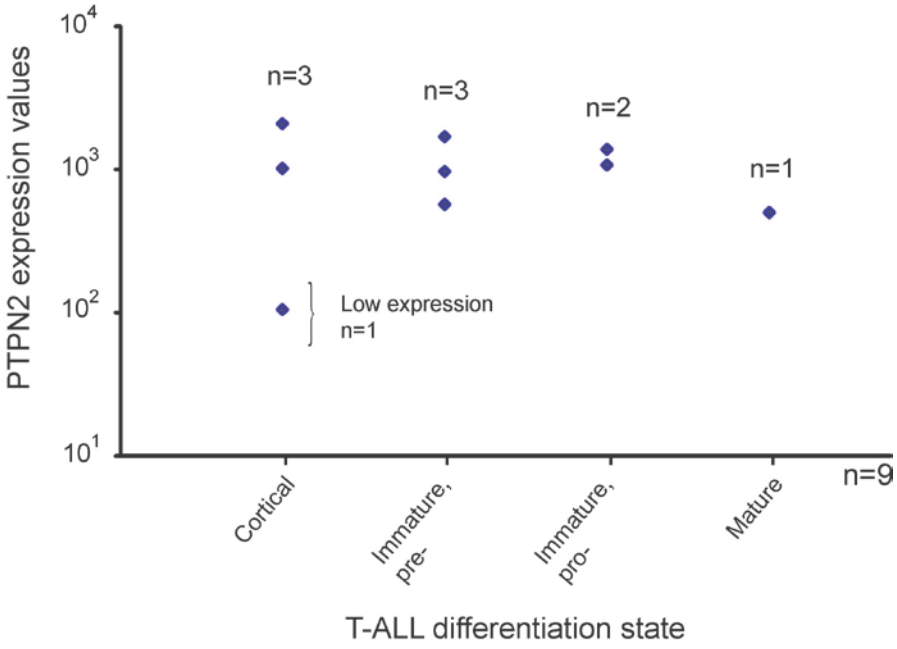
(b) BLAST2 analysis of the *Alu* sequences flanking the *PTPN2* locus (telomeric: *Alu* 1, centromeric: *Alu* 2) detected 82% identity between both elements. *Alu* elements were oriented in the same direction (strand=plus/plus) and both about 300 bp long.

(c) Dot matrix view showing region of similarity based upon Blast2 results. X-axis and y-axis represent *Alu* sequence 1 and 2, respectively. Numbers indicate position on chromosome 18p11 (bp). Blast analysis identified one alignment through the entire length as shown in a single line.



Supplementary Figure 4. Heterozygous deletion of *PTPN2* in *TLX1* positive T-ALL.

Quantitative PCR analysis of additional T-ALL samples identified 5 individuals with loss of one copy of *PTPN2*. A primer set for *CDH2* (closed bars, 18q12.1) and RNMT (open bars, 18p11.21) was used for normalization.



Supplementary Figure 5. Gene expression analysis of T-ALL individual 7.

PTPN2 expression level was analyzed in 9 individuals with T-ALL using Affymetrix gene expression arrays (probeset 213136_at) and categorized according to the stage of differentiation (x-axis). Y-axis displays PTPN2 expression levels as a logarithmic scale. N indicates number of individuals analyzed. The case with low expression corresponds to individual 7 in the manuscript and was confirmed to harbor a bi-allelic deletion of PTPN2.

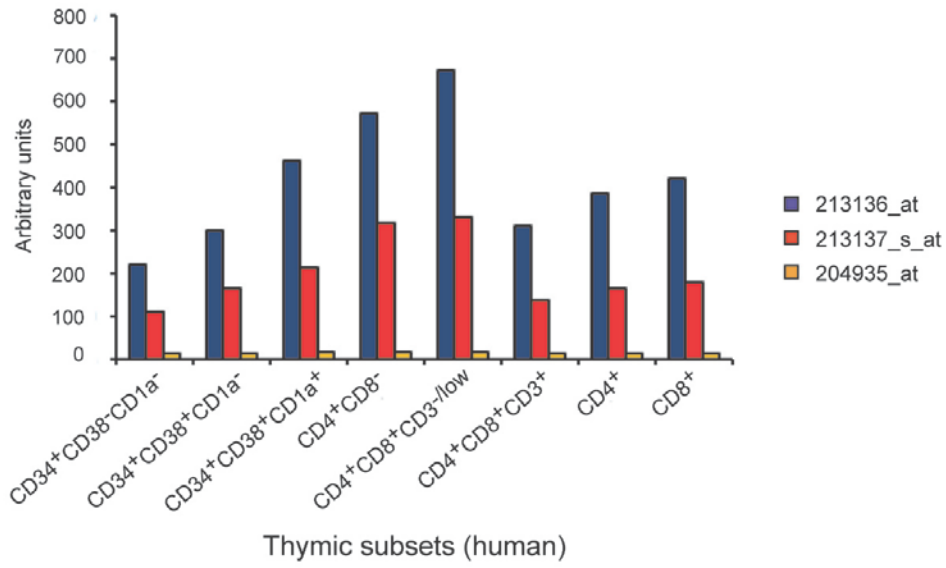
Supplementary Figure 6. Schematic representation of CpG-rich regions near the PTPN2 transcription start analyzed for DNA methylation.

All individuals with heterozygous deletion of *PTPN2* (9, 10, 18, 20 and 22) were analyzed for the methylation status of CpG-rich regions located between sites -940 to +255 bp (red arrows). Translation start site of *PTPN2* was designated as 0 (**ATG**).

PCR amplicon -940 to -640: for all individuals both methylated and unmethylated CpGs were detected and therefore considered as partially methylated. Neither the methylation pattern nor the degree of methylation was found to be different between the groups and not specifically associated with low expression levels of PTPN2.

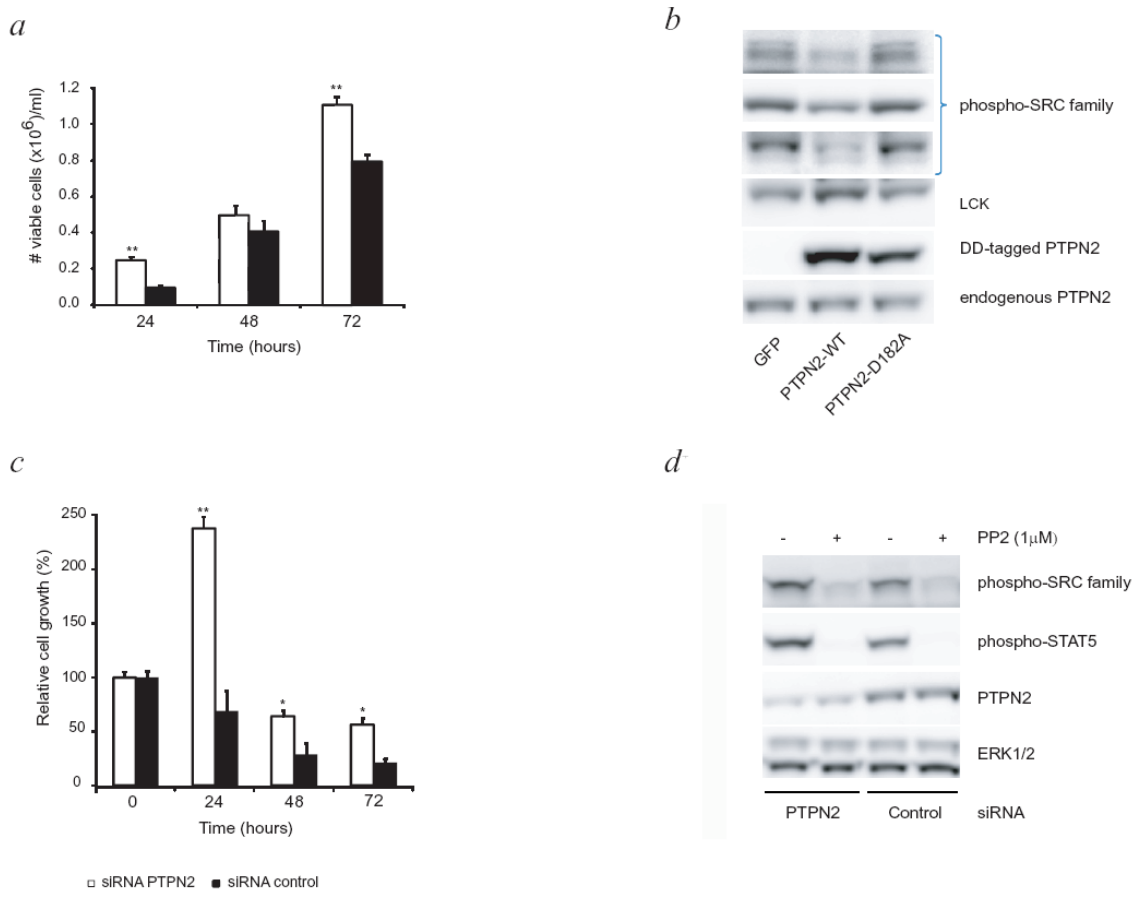
PCR amplicon -572 to -318: only thymidine peaks were observed independent of PTPN2 expression levels and the entire CpG islands was counted as unmethylated.

PCR amplicon -240 to +255: only thymidine peaks were observed independent of PTPN2 expression levels and the entire CpG islands was counted as unmethylated.



Supplementary Figure 7. *PTPN2* expression peaks at the $CD4^+CD8^+CD3^{-/low}$ stage of T-cell development.

Expression of PTPN2 was detected at all stages of T-cell development with highest levels measured in the $CD4^+CD8^+CD3^{-/low}$ subset. Expression levels were determined using 3 different probesets 213136_at, 213137_s_at and 204935_at. Probeset 213136_at showed the highest sensitivity resulting in the highest values, whereas no results were obtained with probeset 213137_s_at.



Supplementary Figure 8. Proliferative advantage and decreased sensitivity to LCK inhibitor PP2 due to knockdown of PTPN2.

HSB-2 cells were electroporated with siRNA targeting PTPN2 (open bars) or control siRNA (closed bars). Treatment with PP2 was initiated 24 hours after electroporation and viability as well as proliferation was followed for 72 hours. For inhibitor experiments cell number of untreated control cells (siRNA control) was set as 100% and y-axis displays relative cell growth. All values represent the average ± s.e.m. of three determinations.

(a) Viable cell numbers of untreated cells were determined 24, 48 and 72 hours after electroporation with respective siRNAs. Decrease in PTPN2 protein levels resulted in a proliferative advantage of the cells compared to control cells.

(b) HSB-2 cells were exposed to Shield1 compound for 24 hours to induce overexpression of PTPN2 wild type (PTPN2-WT) or mutant (PTPN2-D182A). Overexpression of PTPN2 wild type, but not the inactive trapping mutant caused reduced phosphorylation of LCK kinase and other members of the SRC-family kinase. Blots were stripped and reprobed with LCK antibody as loading control.

(c) Relative cell growth of HSB-2 cells treated with LCK inhibitor PP2 (1 μM).

(d) WB analysis of HSB-2 cells detected increased activation of STAT5 and a member of the SRC kinase family due to reduction in expression of PTPN2. ERK1/2 was used to ensure equal loading

Supplementary Tables

Supplementary Table 1. Cytogenetic and molecular findings in individuals with *PTPN2* deletion

Individual	Previously published (ID)	*CDKN2A*	*NOTCH1 mutation*	*NUP214-ABL1*	*TLX1, TLX3*	Others
1	No	*CDKN2A* -/-	4799 T>C, L1600P	-	*TLX1*	
2	Yes (TL52)[2]	*CDKN2A* -/-	na	+	*TLX1*	
3	Yes (TL54)[2]	*CDKN2A* -/-	na	-	*TLX1*	
4	Yes (TL57)[2]	*CDKN2A* -/-	4851insGAG, F1617LS	-	*TLX1*	
5	Yes (TL59)[2]	*CDKN2A* -/-	no mutation	+	*TLX1*	*FBXW7:* C1542T, R465C; *MYB* duplication
6	Yes (TL71)[2]	*CDKN2A* -/-	C7177T, Q2393*	-	*TLX3*	
7	No	*CDKN2A-/-*	5039 T>A, I1680N 6555 C>T, D2185D	-	*TLX1*	
8	No	*CDKN2A-/-*	4727 T>A, V1576D 5094 C>T, D1698D 6555 C>T, D2185D	-	*TLX1*	

Individual	**Previously published (ID)**	***CDKN2A***	***NOTCH1 mutation***	***NUP214-ABL1***		**Others**
9	Yes (1944)[3]	*CDKN2A-/-*	5025 C>G, I1675M; 5026 G>T, V1676F; 7516 G>T, Q2506*	+	*TLX1*	
10	Yes (2737)[3]	*CDKN2A-/-*	4733T>A, V1578E	-	*TLX1*	
18	No	*CDKN2A+/-*	4793 G>C, R1598P; 6555 C>T, D2185D	-	*TLX1*	*MYB* duplication; *RB1* deletion
20	No	*CDNK2A-/-*	4793 C>G, R1598P	+	*TLX1*	
22	No	*CDKN2A-/-*	5153 T>C, I1718T; 7383_7384insGC, P2461Afs*15	-	*TLX1*	

Molecular information on CDKN2A (FISH or array data), NOTCH1, NUP214-ABL1 (RT-PCR, array and/or FISH data), common transcription factor overexpression (RT-PCR or gene expression profiling data for TAL1, LMO1, LMO2, LYL1, TLX1, TLX3, HOXA, MLL fusions, or CALM-AF10), MYB duplication, RB1 deletion, PTEN deletion or mutation, FBXW7 mutation.[2,4,5] Individuals identified with homozygous deletion of *PTPN2* are referred to as individuals 2-6 in this manuscript and have been referred to TL52, TL54, TL57, TL59, and TL71 respectively by Soulier *et al*., 2005. NOTCH1 mutations: numbering according to start codon of transcript ENST00000277541. FBXW7 mutations: numbering according to transcript ENST00000281708.

Supplementary Table 2. Primer sequences

Sequence analysis of *PTPN2* in T-ALL individuals and cell lines

Coding region of *PTPN2* was amplified and subsequently analyzed applying a nested sequence strategy unless otherwise indicated*. Exon 1 was sequenced with the indicated reverse primer only. Exon 9: refers to exon 9 of PTPN2 variant 1 (NM_002828), exon 9s refers to exon 9 of PTPN2 variant 2 (NM_080422), exon 9v refers to exon 10 of PTPN2 variant 2 (NM_080422) and exon 9 of PTPN2 variant 3 (NM_080423).

Exon	Forward amplification primer (5'-3')	Reverse amplification primer (5'-3')
1	CTCTTGTCGGAAGACGCAAG	AGGAGCAAGAGAGCGGTCAG
2	TTACGCTGGCTGGGAAGATA	CCAAGCCCTCCTTTTCACTA
3	CAAGATTGCATTTTATACTCCTTACAA	AAGCTTCAAAACCTTATAATGTCCA
4	TCAGGTAGATTGGAAGTCTTGG	TCCGGACAGAAACACTTTGA
5	GTGACTTCTTAGTTTTGGGTACTTGA	CCTCAGAAAGCTACTAGATGCAGA
6	TGGGATTGTCAGAAAACAAATG	GCAACGGTGGAAATGAGATT
7	GATGTGGGGAGGGATGAGTA	GACAGCCAGCTGGATTTACC
8	TGTTACAAGCTACAGTATGGCAAA	CAGGCGTGATTGTATTTTCCT
9	CCCTCATTATTCCAGTGTTCAA	ACAGTTGCAAAATTTACCAGTTTTT
9s	TTCCAGTGTTCAAATAGGGTGA	ACTGCACCGTTTTTGGGATA
9v/10	CCAGCCGTTTTACCCTAAGA	CTCGGCAGTATGTCAATTGCT

Exon	Forward sequence primer (5'-3')	Reverse sequence primer (5'-3')
1	-	GAGCGAGAGGCTAGAGGCGAGA
2	TACGCTGGCTGGGAAGATAA	GCCCTCCTTTTCACTACATCC
3	GATTGCATTTTATACTCCTTACAAAAA	AGCTTCAAAACCTTATAATGTCCA
4	CAGGTAGATTGGAAGTCTTGGTTT	CACTTTGACCGCTAGCCTTT
5	TGACTTCTTAGTTTTGGGTACTTGAA	TCCTCAGAAAGCTACTAGATGCAG
6	TCAGAAAACAAATGGAAAAATCATAGT	ACTGCCAGTGGAAGCAATTT
7	GAGGGTTAAATTATCGTTGTTCTTT	ACCACAAAAATTCAAATACACTCTTT
8	GGCAAAATGCCTTTTTCTTTT	CCTGAAATCCTATGGCACCT
9	TTCCAGTGTTCAAATAGGGTGA	CAGTTGCAAAATTTACCAGTTTTT
9s	-*	-*
9v/10	-*	CGGCAGTATGTCAATTGCTTG

Sequence analysis of *NOTCH1* in T-ALL individuals

All individuals with homozygous or heterozygous deletion of *PTPN2* were analyzed for mutations in HD-N (exon 26), HD-C (exon 27) and PEST domain (exon 34) of *NOTCH1* using the following primer pairs. Nested primers were used for sequence analysis of amplicons unless indicated otherwise*. Sequence analysis of Individuals 4, 5 and 6 were performed as described previously.[6]
Ampl: amplification primer; seq: sequence primer.

Exon	Forward primer (5'-3')	Reverse primer (5'-3')
26_{ampl}	TGAGGGAGGACCTGAACTTG	TGGAATGCTGCCTCTACTCC
26_{seq}	-*	CACGCTTGAAGACCACGTT
27_{ampl}	GTTGGTGGGTATCTGGGATG	CGGAGTGCCATTCAGAAAAT
27_{seq}	CGGGGGAGGAGGAAG	CTGCAGGCAGAGCCTGTT
34_{ampl}	CCATGGCTACCTGTCAGACG	TGGCTCTCAGAACTTGCTTGT
34_{seq}	-* / GCTGCACAGTAGCCTTGCTG	-*

Quantitative PCR

CDH2 (18q12.1) and *RNMT* (18p11.21) were used as control genes to determine the copy number status of *PTPN2*.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
CDH2	TGAGAGAATCTGGCTCCTTGAAC	GCCCAATGCCTGCACTACA
RNMT	TGCAGTTGTCAGTTTGTCTGTCA	CACGCATTTCTCAGCATCATG
PTPN2	TGAGAGAATCTGGCTCCTTGAAC	GCCCAATGCCTGCACTACA

Genomic analysis of breakpoints

PTPN2 breakpoints were analyzed using nested PCR with the following primer combinations in six individuals: ampl =amplification primer; seq: sequencing primer

Pair	Forward primer (5'-3')	Reverse primer (5'-3')
1_{ampl}	ACCAACTGCATCCACCAATC	CCTTCCTGTTGGTCCACATT
2_{seq}	ACATGACACCTGGGGCTAAG	AAAGCAGACCCTGAGACCAA

Expression analysis (quantitative RT-PCR)

Gene	Forward primer (5'-3')	Reverse primer (5'-3')
HPRT	TGACACTGGCAAAACAATGCA	GGTCCTTTTCACCAGCAAGCT
PTPN2	CCAGTTTAGTTGACATAGAAGAGG	GCAGCATGTGTTAGGAAGT

Primer sequences used for analysis of the methylation status of *PTPN2*

PCR fragment	Forward primer (5'-3')	Reverse primer (5'-3')
-940 to -640_{ampl}	GGTTGGTGTTATTGAGGAATTA	AAAAAAAAAACAACCAACCTCT
-940 to -640_{seq}	TTGGTGTTATTGAGGAATTAAGTTAA	AAAAAAAAACAACCAACCTCT
-572 to -318_{ampl}	GAGGAATTTGTTTTTAAGTTGGTT	ATAAACCRCCCCTTAAACTTA
-572 to -318_{seq}	AGGAATTTGTTTTTAAGTTGGTT	-*
-420 to $+255_{ampl}$	TTTTAGGGGATTTTTTTAGGGAGT	TTTTAAACCAAAAAAAACAAAAAAAC
-420 to $+74_{seq}$	TTTAGGGGATTTTTTTAGGGAGTT	CTCACCAAATACAARGACTACCAAC

References

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2. Soulier, J. et al. HOXA genes are included in genetic and biologic networks defining human acute T-cell leukemia (T-ALL). *Blood* **106**, 274-86 (2005).
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6. Asnafi, V. 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* **113**, 3918-24 (2009).