MOHITO, a novel mouse cytokine-dependent T-cell line, enables studies of oncogenic signaling in T-cell context

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Online Supplementary Appendix

Files in this data supplement:

Online Supplementary Results

- **Genetic characterization of MOHITO cells**
- **Gene expression profile of MOHITO cells**
- **Aberrant gene expression of homeobox transcription factors Hoxb3 and Hoxb4**
- **Sensitivity to small molecule inhibitors**

Online Supplementary Design and Methods

Online Supplementary Tables

- **Supplementary Table S1.** Characteristics of MOHITO cells
- **Supplementary Table S2.** FISH probes
- **Supplementary Table S3.** Primer sequences
- **Supplementary Table S4.** Numbers of differentially expressed genes
- **Supplementary Table S5.** Most up-regulated probe sets in MOHITO cells
- **Supplementary Table S6.** Commonly down-regulated genes in leukemic cells compared to thymus

Supplementary Figures

- **Supplementary Figure S1.** Analysis of primary diseased Balb/c mouse
- **Supplementary Figure S2.** Growth characteristics of MOHITO cells *in vitro*
- **Supplementary Figure S3.** Additional genetic lesions identified in MOHITO cells
- **Supplementary Figure S4.** MOHITO expression pattern highly differs from thymus cells
- **Supplementary Figure S5.** High expression of homeobox transcription factors Hoxb3 and Hoxb4
- **Supplementary Figure S6.** Constitutive activation of the Notch1 pathway
- **Supplementary Figure S7.** A new transforming JAK1 mutation
- **Supplementary Figure S8.** Sensitivity to small molecule inhibitors
- **Supplementary Figure S9.** Downstream signaling of oncogenic kinases depends on the cell context

Supplementary References

Online Supplementary Results

Genetic characterization of MOHITO cells

We have performed a comprehensive genetic characterization of the novel T-ALL cell line MOHITO. Genetic alterations in MOHITO cells included a typical focal deletion on chromosome 6 involving the TCRβ chain locus **(data not shown)**, identifying a physiological TCR rearrangement, trisomy of chromosome 15 (also visible in the M-FISH in **Figure 1D**) and a mono-allelic deletion of a 12 Mb region of mouse chromosome 12 **(Online Supplementary Figure S3A)**. This region on mouse chromosome 12 embraced the genomic location of the *Dicer1* gene, which encodes an important RNA helicase required for microRNA (miRNAs) processing. The identification of spacious reduction of miRNAs in association with a typical expression profile in human cancer spotlighted *Dicer1* as tumor suppressor gene.[1] Indeed, different groups have recently reported a dosage effect of Dicer1 protein levels where partial reduction of protein expression levels promoted tumorigenesis.[2] Another tumor suppressor implicated in the development of T-cell diseases is the C2H2-type zinc finger protein B-cell lymphoma/leukemia 11B (Bcl11b). Bcl11b is encoded by the *Bcl11b* gene positioned within the terminal region of chr12. We found one copy of *Bcl11b* deleted **(Online Supplementary Figure S3A)**, whereas the other allele was affected by a reciprocal translocation between chr5 and 12 **(Figure 1D** and **Online Supplementary Figure S3B)**. Array CGH analysis depicted the breakpoint on chromosome 12q downstream of the 3'-prime end of *Bcl11b* **(Online Supplementary Figure S3B,** upper panel**)**. Several groups have reported head-to tail juxtaposition of *BCL11B* to transcription factors such as T-cell leukemia homeobox 3 (*TLX3*), resulting in ectopic expression of the partner gene.[3] Interestingly, it is proposed that overexpression is caused by enhancer elements located albeit downstream of 3'-*BCL11B*.[4] We located several potential partner genes clustered within the breakpoint region at chr5, namely GS homeobox 2 *(Gsx2)*, platelet derived growth factor receptor A *(Pdgfra)* and Kit receptor (*c-kit*) **(Online Supplementary Figure S3B,** lower panel**)**. All candidate genes lie in a 3' telomere-5' centromere orientation and therefore translocation of the distal region of chr5 would place the gene under the influence of the transcriptional enhancer elements normally positioned downstream of *Bcl11b*. Out of these three genes, only the *Kit* oncogene RNA levels were found to be significantly increased compared to different mouse T-ALL cell lines and thymus cells (highlighted in red in **Online Supplementary Table S5**).

Gene expression profile of MOHITO cells

We compared gene expression profiles of MOHITO cells with the profiles of 3 other T-cells (HOX11 or NUP214-ABL1 immortalized/transformed T-cells, normal mouse thymus cells). Strikingly, the overall expression pattern of MOHITO cells showed major differences in comparison to normal T-cells **(Online Supplementary Figure S4A** and **Online Supplementary Table S4)**. Notably, 104 out of 164 genes highly expressed in MOHITO cells when compared to normal mouse thymus ($\log 2$-ratio thymus/primary MOHITO cells <-3) were also significantly higher expressed in MOHITO cells than the two other analyzed murine leukemic T-cell lines **(Online Supplementary Figure S4B** and **Supplementary Table S5)**. 199 genes were found to be down-regulated in MOHITO cells with a difference in the log2 ratio greater than 5 **(Online Supplementary Table S6)**. However, down-regulated genes were generally lower expressed in all studied immortalized cell lines (129 out of 199) with only 14 genes uniquely detected in MOHITO cells, indicating that the subset of up-regulated genes is likely to be characteristic for MOHITO cells **(Online Supplementary Figure S4C)**.

Aberrant gene expression of homeobox transcription factors Hoxb3 and Hoxb4

In human T-ALL, a set of transcription factors, including *TLX1*, *TLX3*, *TAL1*, *LMO1/2, LYL1* and *HOXA* cluster genes, are frequently up-regulated and define the major subgroups of T-ALL.[5, 6] None of these transcription factors were found to be significantly up-regulated in MOHITO cells, but we detected 3 to 4-fold higher expression of the homeobox transcription factors Hoxb3 and Hoxb4, compared to the other mouse T-ALL cell lines **(Online Supplementary Figure S5A-B)**.

The *Hoxb4* and *Hoxb3* genes fulfill pivotal roles in developmental processes through regulation of self-renewal and differentiation of both embryonic and adult stem cells.[7, 8] Under normal conditions, expression of both factors is strictly regulated with highest expression in early hematopietic stem cells (HSCs) and declines as cells mature. Next to high expression levels of Hoxb3 and Hoxb4 **(Online Supplementary Figure S5A)**, we also found a profound up-regulation of Pbx3 specifically in MOHITO cells, whereas Pbx1 expression was reduced, which is in accordance with a previously reported functional interplay between Hoxb3 and Hoxb4 and members of the TALE family **(Online Supplementary Figure S5B)**.[9] We also investigated possible underlying mechanisms for the ectopic expression of these Hox genes. However neither the *Hoxb* cluster region itself nor *Mll1* (an upstream regulator of Hox expression) were genetically altered **(Online Supplementary Figure S5C-D)**. The *Hoxb4* promoter contains HxRE-1 and HxRE-2 sites and binding of transcriptional regulatory factors such as NF-Y and USF1/2 are known effectors of Hoxb4 expression.[10, 11] However, none was

abnormally expressed **(data not shown)**. These data suggest that ectopic expression of Hoxb3, Hoxb4 and Pbx3 may be important for MOHITO cells, but the mechanism by which these genes are affected remains unknown.

Sensitivity to small molecule inhibitors

In contrast to a high responsiveness of leukemic mouse T-cells, targeting of NOTCH1 receptor in human T-ALL cell lines showed only in a modest anti-leukemic effect questioning the effectiveness of single GSI therapy in the clinics. Indeed, first clinical trials showed only a limited clinical response, which was accompanied by severe gastrointestinal toxicity.[12] Overall, recent findings point to a benefit of dual targeted therapy combining GSI with glucocorticoids (GCs).[13] To test whether MOHITO cells are typically responsive to glucocorticoid therapy we exposed cells to increasing concentrations of dexamethasone (dexa). Analysis of proliferation and survival depicted high GC sensitivity of the MOHITO cell line. Presence of the GC derivate dexa completely blocked cell growth and induced profound cell death at a low 10 nM concentration **(Online Supplementary Figure S8A)**.

Lastly, we tested the anti-leukemic effect of a Janus kinase inhibitor (JAK inhibitor I, Calbiochem). All four members of the non-receptor Janus tyrosine kinase family (TYK2, JAK1, JAK2 and JAK3) mediate cytokine, interferon and growth-factor induced signaling through the JAK/STAT pathway. Notably, several decisive hematopoietic growth factors are dependent on distinct JAK members to transduce their signal to the nucleus. Anticipatory, aberrant activation of the JAK/STAT pathway is a phenomena shared among all forms of leukemia, but also found in other types of human cancer. As a result, several JAK inhibitors currently undergo pre-clinical and clinical trials.[14, 15] As pointed out above, MOHITO cells are not just dependent on exogenous cytokines for cell growth and survival, but also carry a missense mutation in the kinase domain of *Jak1*. As expected, addition of the JAK inhibitor blocked IL-2 and IL-7 induced activation of Jak1 and yielded a clear antiproliferative effect accompanied by cell death **(Online Supplementary Figure S8B)**.

Online Supplementary Design and Methods

Cell lines

The NUP214-ABL1 cell line was derived from a mouse T-ALL-like disease as described by De Keersmaecker and colleagues.[16] The HOX11 overexpressing cell line was derived from a transgenic mouse overexpressing HOX11 in T-cells at time the animals developed a T-

ALL/T-cell lymphoma like disease. The HOX11 cell line was kindly provided by Dr. A. Ferrando (Columbia University, New York, NY).

Histopathological analysis

Hematoxylin and eosin-stained histological sections of murine tissues were prepared by standard procedures. Histological images were obtained on a Leica DM2500 microscope equipped with a Leica DFC295 camera model (both Leica microsystems, Switzerland) and a 10x/25 ocular lens. Different objectives were applied for respective magnification (N-PLAN: 2.5/0.07; HCX-PL S-APO (5x/10.15, 10x/0.30, 20x/0.50, 40x/0.75, 63x/0.90).

Array CGH and gene array

Array-based comparative genomic hybridization (array CGH) was performed using the Agilent Mouse Genome CGH Microarray 244K platform (Agilent Technologies) according to manufacturer instructions. CGH analytics 3.4.40 software was used for analysis. Gene expression profiling of RNA isolated from murine leukemic T-cells, cultured cell lines or wildtype thymus cells was performed with the Mouse Genome 430A 2.0 Array (Affymetrix) at the VIB MicroArray Facility (Leuven, Belgium). Array CGH and RNA expression data will be deposited to GEO.

Microarray data analysis

Intensities below background signal were omitted and remaining RMA expression values were compared for all samples. Data were preprocessed using MAS 5.0 detection calls.[17] Assessment of differentially expressed genes was based on normalized intensity values of the different conditions with the limma package of Bioconductor.[18] In case of inhibitor studies, conditions were separately compared (JAK inhibitor versus DMSO) for both cell lines studied. Significant deviations from 0 for each contrast were tested with a moderated t-statistic and resulting p-values were corrected for multiple testing with Benjamini-Hochberg.[19] Genes were defined as differentially expressed based on the corrected p-values (<0.05) in combination with a cut-off on the fold-change of two (absolute $\log 2$-ratio >1).

T-cell receptor beta rearrangement

Analysis of the T-cell receptor (TCR) status in primary and cultured cells was performed using the procedure previously described by Baker *et al*.[20] 19 PCR reactions were performed combining a common C-beta primer (reverse) with 19 unique V-primer (forward) covering all TCR variable chain sequences. PCR reactions were conducted with a Taq DNA polymerase kit (Qiagen).

Quantitative PCR

Quantitative analysis of RNA expression levels of selected genes were performed using the Fast SYBR® Green Master Mix (Applied Biosystems) with the LightCycler® 480 Real-Time PCR System (*Roche* Diagnostics) applying standard protocols. HPRT were used as control gene for normalization. Data were analyzed with the LC480 software (Roche Diagnostics) before applying the comparative ddCT method (Bulletin 2; Applied Biosystems).[21] Primer sequences are listed in **Online Supplementary Table S3.**

Mutational analysis

Genomic DNA was isolated from cell lines, mouse tissue or primary cells. The complete coding region of murine *Jak1* and the HD and PEST domain of *Notch1* were amplified from genomic DNA using primers listed in **Online Supplementary Table S3**. PCR products were either directly sequenced (*Jak1*) or cloned into the pGEM-T Easy vector (Promega) and sequenced with appropriate vector primers at the Genetic Service Facility (VIB, Belgium). In case of the insertion mutation in the PEST domain of *Notch1*, a total of 10 clones of both primary and secondary recipient were analyzed.

Inhibitor experiments

Cells were seeded out in triplicate at an appropriate cell density (BaF3: $0.2x10^6$, MOHITO: $0.3x10^6$) and exposed to respective diluted inhibitors or DMSO (control). Relative cell proliferation compared to control cells (DMSO) was assessed at indicated timepoints using a Vi-cell™ XR cell viability analyzer (Beckman Coulter). Inhibitors used in this study: JAK Inhibitor I, Compound E (GSI), dexamethasone (dexa) (both Calbiochem), imatinib (ChemieTek) and CP-690550 (Axon).

Signaling pathway analysis

For pathway analysis, cells were treated with increasing concentrations of respective inhibitors for either 90 minutes (imatinib), 24 hours (GSI) or 6 hours (JAK inhibitor I and CP-690550). Cells were washed once in ice-cold PBS before lysed in complete cell lysis buffer and subsequently analyzed using standard protein blot procedures.

Knockdown experiments

Gene silencing experiments were performed as described previously (Kleppe, 2010) using the Gene Pulser Xcell Electroporation System (BioRad). Electroporation conditions: Ba/F3 cells 950µF/300V, MOHITO 500µF/350V. siRNA duplexes were resuspended at a stock concentration of 20mM and used at a final concentration of 300nM. siRNA duplexes: human ABL1 GGAAUGGUGUGAAGCCCAAACCAAA, human JAK1

GCCUUAAGGAAUAUCUUCCAAAGAA and mouse Jak1 CCAGUGGCGGCAGAAACCAAAUGUU. Respective Stealth RNAi™ siRNA Negative Controls (Invitrogen) was used as non-targeting control. In case of subsequent analysis of cell proliferation, cells were seeded out 24 hours after electroporation at appropriate cell concentration and cell numbers were determined 48 hours later.

Flow cytometry analysis

Cultured cells or single cell suspensions from thymus, spleen, lymph node, and bone marrow were prepared and stained as described previously.[16] The following antibodies were used: allophycocyanin (APC)-conjugated CD8, phycoerythrin (PE)-conjugated CD4, APC-conjugated B220, and phycoerythrin-Cy7 (PeCy7)-conjugated Mac1 (BD PharMingen), PE-conjugated CD3, PE-conjugated c-Kit (CD117), PE-conjugated TCR$\alpha\beta$, APC-conjugated TCR$\gamma\delta$ (eBioscience). All experiments were performed with the respective labeled isotype controls. Flow cytometry was performed on a FACSCalibur (BD Bioscience) and data were analyzed using CellQuest software (BD Bioscience). Annexin V positive cells were identified using the PE-Apoptosis Detection Kit (BD Bioscience) according to manufactures instructions.

Western blotting and immunoprecipitation

The following antibodies were used: anti-Lyn (44), anti-Fyn (3), anti-c-ABL (24-11), anti-ERK (C-16), anti-LCK (3A5), anti-LCK (2102), anti-phospho-JAK1 (Tyr1022/1023), anti-PTEN (A2B1), anti-NOTCH1 (C-20) (Santa Cruz Biotechnology), anti-phospho-STAT5 (C11C5), anti-phospho-SRC (Tyr416), anti-phospho-AKT (Ser473), anti-phospho-ERK1/2 (9101), anti-phospho-MEK (41G9), anti-c-ABL, cleaved NOTCH1 (2421) (Cell Signaling), anti-phosphotyrosine (4G10), anti-JAK1 (Upstate), beta-actin (AC15) (Sigma). Activated SFKs were immunoprecipitated using a pan-phospho-SRC (Tyr416) antibody (Cell Signaling) pre-coupled to Dynabeads protein G (Invitrogen).

Primer design

Primers were designed using Primer3 Input 0.4.0., PrimerExpress software (Applied Biosystems) or LightCycler Probe Design2.

Online Supplementary Tables

- **Table S1. Characteristics of MOHITO cells**

Immunophenotype		
Lineage	Cluster designation/antibody	Reactivity[¶]
Panleukocyte	CD45	+
Lymphoid	CD3	-
	CD4[ǂ]	+
	CD8	+
	TCRalpha	-
	TCRbeta	-
	CD127 (IL7R)	+
	CD25 (IL2Rα)	+
	CD19	-
Myeloid	CD11b (Mac-1)	-
	Gr-1	-
	CD45R (B220)	-
NK associated	CD16	-
Stem-cell	CD34	-
	CD48	+
	CD150	+
	Sca-1	-
	CD117 (c-kit)	+
Other characteristics		
Morphology	Round	
Diameter	~ 10 µm	
Doubling time	16-20 hours	
Karyotype	43,XX,t(5;12),+der(5)(5;12),+10,+14,+15	
Genes analyzed for mutations	*Notch1*	HD: 5123T>G, I1708S PEST: 7463-64insC, p.P2513fs*3
	Jak1	Kinase domain: 3125G>T, S1042I
	Pten	wt
Gene rearrangements	*IgH**	Nd
	*IgL**	Nd
	*IgK**	Nd

	β-chain of T-cell receptor	Vβ6
	MLL[§]	Negative
	Hoxb3[§]	Negative
	Hoxb4[§]	Negative
Transcription factors	Notch1	Constitutively active
	Hoxb3	Upregulated
	Hoxb4	Upregulated
	Pbx1	Downregulated
	Pbx2	Downregulated
	Pbx3	Upregulated
Copy number status*	*Dicer* (chr12)	Loss of one copy
	Bcl11b (chr12)	Loss of one copy
	Pten (chr19)	Normal copy number
	TCRα locus (chr14)	Interstitial deletion > indication for TCR rearrangement
	TCRβ locus (chr6)	Interstitial deletion > indication for TCR rearrangement
	IgK, IgH, IgK	No deletions detected

Nd: not detected; IgH: immunoglobulin heavy chain locus, IgL: immunoglobulin light chain locus, IgK: immunoglobulin kappa chain locus; TCR: T-cell receptor; Pten: phosphatase and tensin homolog; Hoxb4: homeobox B4, Hoxb3: homeobox B3; + : expressed by entire population; - : not expressed on the cell surface of cells; [¶] determined by flow cytometry; [+]decrease in CD4 positive cell population during ex vivo culture; * determined by array CGH; [§] determined by FISH; Notch1 mutations: numbering according to start codon of transcript ENSMUST00000028288; Jak1 mutations: numbering according start codon of transcript ENSMUST00000102781.

- **Table S2.** ***FISH probes (BAC clones)***

BAC clones covering regions upstream and downstream of respective gene

Gene	upstream	downstream
Hoxb3/Hoxb4 (chr11)	RP24-94L10	RP24-493P11
	RP24-106E16	RP24-67J12
Mll (chr9)	RP23-17H2	RP23-51L2
	RP23-432B12	RP24-419J13

All probes were designed based on the Ensembl Genome Browser (http://www.ensembl.org) and purchased from CHORI BACPAC Resources.

- **Table S3.** ***Primer sequences***

Sequence analysis of murine *Jak1*

Exon	forward primer	reverse primer
1	TCCTCAGTCTTGGGTCTTGG	GGGGCCATAAACAGGATGT
2	CTGCCTTCCCAGTGTCTCTC	CTGGGTGTCACGGTTTAAGG
3	CCTCCATAGCAAACAGCACA	ATGTGCTTCTCCCAGTGTCC
4	TGAGAGGATGCAGGTGACAG	TGAGAGGATGCAGGTGACAG
5	CTCTCCCAGATGAGCAGTCC	CAACTGCACAGGGACTCTGA
6	CATTTTGGCCAGCTCTTCTC	AGCCTGAGCTACACACAGCA
7	CGCTCTCTGTCAGGATTGTG	GAGAGATGAGTGGCCTCCAG
8	GCATGCTGGCTTTCTCTTCT	GCTTCAAAGGGACCATCAAA
9	AAGGTGCTTGTATGCCAGGT	CATGCAGAGCTAAGGGGACT
10	CACGCCAGAGAGGACCTTAG	CCTTTCTCCCACAGTTCCTG
11	TGGTTGGCTTGTTGATGAAG	ACAACTGGCAAAGGACAACC
12	GTGAGCTCCTGGAGGGTATG	TGGCCTTACCTCGTTGTTCT
13	CCCATCCCACTGTGTTCAG	AGACAGCACTGAAGGGAGGA
14	GGAGGTGACAGTGGTGAGGT	CACAAATGACCCCGATTTCT
15	AAGGTCCCTGGGAGAGGTAA	CAATACCCCTCACCTGCAAC
16	TGGAGTGGAGAAAGCTGACC	CTTCTTGTTGCTGGTGGTCA
17	GCTGTGTGTTGGACTCACCA	AAGACGACCAAGCTCACAGG
18/19	ACATTTGCCTTTTGGCAGAG	AAAGCCTCTGACGAAGGACA
20	AGGAGATCAGACCGAGGTGA	CCTACCAGCAAATGCCCTTA
21	GTCTACAGAGCCCCATGCTC	CTAACCATTTCCCCAGGACA
22	TGCTCTTAGGGGAAGGACTG	CCAGACTCTCCCATTCTCCA
23	CTGTGAGCCTCTGGACTGCT	CTGCACTTGTGAAGGCAAAC
24	CAATCCTTGAGAGCGCAAAT	CAGTGTAAGGCCTTCGGTTC
25	GTTACACTCGTGGGGCAAGT	CATAGGCATATCCACACACTGA

Sequence analysis of murine *Notch1*

Primary and secondary cells 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.

Exon	forward primer	reverse primer
26	ACGGGAGGACCTAACCAAAC	CAGCTTGGTCTCCAACACCT
27	GGGAGTCAGAGCTGGTGTGT	GGGATTTGAACCCTTGTCCT
34_1	GCTCCCTCATGTACCTCCTG	TAGTGGCCCCATCATGCTAT
34_2	GCCAGTACAACCCACTACGG	CTTCACCCTGACCAGGAAAA

Pten copy status analysis (quantitative PCR)

Fbxl11 (12qC1) and *Grk5* (12qD3) were used as control genes.

Gene	forward primer	reverse primer
Fbxl11	GGACAGAGAAGCCCGAC	TAATCCAGGTTCACTCCATTAGC
Grk5	CATTATGAATGGTGGTGACCTGA	AAGGCTCGCTCTTCCTC
Pten	TAGCATTTGCAGTATAGAGCGTG	TTGTTTGCTTTGTCAAGATCGT

Expression analysis (quantitative RT-PCR)

Gene	forward primer	reverse primer
Hprt1	CATTATGCCGAGGATTTGG.	GCAAGTCTTTCAGTCCTGT
Hoxb3	CCCTGGATGAAAGAGTCGAG	CACAACCTTCTGCTGTGC
Hoxb4	GCACGGTAAACCCCAATTA	GGCAACTTGTGGTCTTTTTT
Pbx1	CAGTGGAGCATTCCGAC	CTTCTCCAGCTCTGTGTGGTA
Pbx2	GAGCAACCCTTACCCTAGT	AACCTGAGAGACGGTGAT
Pbx3	CCAAGGGTCCCAAGTCG	GTAGCCTCCCGTCTGATTGATAA
IL7Ra	TTAAAGCCGAGGCTCCC	TGAGGTGCATTAAATGTCACCA
Socs1	CACCTTCTTGGTGCGCGACA	AAGCCATCTTCACGCTGAGC
Socs2	AGCTGGACCGACTAACC	GTCTGAATGCGAACTATCTCTAATCAA
Socs3	CAAACAGGATGGTACTGGG	TGGATGCGTAGGTTCTTGG
Dusp4	GAAGACAACCACAAGGCCGA	TCGACAGTCCTTTACTGCG
Dusp6	CGAGTTCAAATACAAGCAAATTCCTA	TTCATCTATGAAAGAAATGGCCTCAG
Pim2	CATTCCCTTCGAGAGAGACCA	AGCAGAATCTCCTCCAGTGA
Xaf1	CCTCTCTCCACTTCATGCTC	TCTTCACATTCTGGGCAAAGG
Gbp2	GTATAACCAGGCTCCTGGG	AACAACATCTGCCTTGGAATC
Spred2	CAGATGCACGAGCCTTTG	CTCAGCTTCGTTATGGAGAGT
Etv5	CAGGCTCTTGGTGCTAAGTA	AGGGAGGCTTCCTATCG
Id1	GACATGAACGGCTGCTACT	GATCTCCACCTTGCTCACT
Bcl2	TGTTTGATTTCTCCTGGCTGT	GGTATGCACCCAGAGTGA
Lck	ACAAGATCCGTAACCTAGACAAC	TGTAATGGCGGACTAGATCG
Lyn	TGCAGAAGGCATGGCGTA	AGAGACCAGGACGTTAGCA
Fyn	GTTACATTCCCAGCAATTACGTG	AGCTGTCTCTCAGCATCT
Hck	CCAAAGGGAGCTACTCGTT	CTTATAGTGCTTCACGGTGT
fgr	TGCTTTCCTCAACACTGGCAA	CCTGTCCTGGCCTCATAG
c-scr	TCCTCGTGAGGGAGAGT	TGCGGGAGGTGATGTAGA
c-yes	TAAAGCCAGGTACAATGATGCC	GCTCTTCAGAAACAACTGCATAG
blk	GTTATGTGCCCAGCAACTTT	GGCTGATGGTCCTGAAGAAC

Expression analysis of Dicer1 (semi-quantitative RT-PCR)

Gene	forward primer	reverse primer
Dicer1	GAATGGAAGATGCCCAAGAA	GAGGGTTTTCTCTGCGTCTG
18-S	CCTGAGAAACGGCTACCACATC	CATCTAAGGGCATCACAGACCTG

MLL partial tandem duplication

Presence of a partial tandem duplication of the Mll gene was analyzed using the following primer combinations:

Exon	forward primer	Exon	reverse primer
3	AGGCAGACAAGCTTCCAATG	8	TTTTTCTGCTTGCTTTGCTC
		9	TCTGCTGGGATTTTCTGCTT
		14	GCACTGCATCATCTTGCTCT

- **Table S4.** ***Numbers of differentially expressed probe sets***

Primary cells vs.	Log2 ratio **<** -1*	Log2 ratio **>** +1*
cultured MOHITO cells	162	356
cells isolated from secondary recipient	10	32
HOX11 positive cells	2401	2865
NUP214-ABL1 positive cells	1534	888
thymus wt	2429	3348

*Log2 ratios were calculated as described in **Online Supplementary Results**.

- **Table S5.** ***Most up-regulated probe sets in MOHITO cells***

	Gene Title[§]	cultured MOHITO cells	secondary cells	HOX11 positive cells	NUP214-ABL1 positive cells	thymus	Log2 ratio thymus/primary cells*
1	inactive X specific transcripts	0	0	-1	0	-1	-8,30
2	predicted gene 15529 /// HORMA domain containing 1 /// similar to HORMA domain containing 1	0	0	-1	-1	-1	-8,17
3	inactive X specific transcripts	0	0	-1	0	-1	-7,61
4	endothelial cell-specific molecule 1	0	0	-1	-1	-1	-7,20
5	CD163 molecule-like 1	0	0	-1	-1	-1	-6,21
6	dopa decarboxylase	0	0	-1	-1	-1	-6,20
7	inactive X specific transcripts	0	0	-1	0	-1	-6,14
8	fructose bisphosphatase 1	0	0	-1	-1	-1	-5,76
9	interleukin 2 receptor, alpha chain	0	0	-1	-1	-1	-5,47
10	RIKEN cDNA 2700008G24 gene	0	0	-1	-1	-1	-5,43
11	beta-1,4-N-acetyl-galactosaminyl transferase 2	0	0	-1	-1	-1	-5,40
12	**kit oncogene**	**0**	**0**	**0**	**-1**	**-1**	**-4,88**
13	cytotoxic T-lymphocyte-associated protein 4	0	0	-1	1	-1	-4,87
14	T-cell receptor gamma, variable 4	0	0	-1	0	-1	-4,80
15	aldo-keto reductase family 1, member C12 /// aldo-keto reductase family 1, member C13	0	0	-1	0	-1	-4,77
16	transforming growth factor, beta 3	0	0	-1	-1	-1	-4,77
17	CD160 antigen	0	0	-1	-1	-1	-4,77
18	tumor necrosis factor receptor superfamily, member 9	0	0	-1	-1	-1	-4,74
19	N-terminal EF-hand calcium binding protein 1	0	0	-1	-1	-1	-4,71
20	microfibrillar-associated protein 1A	0	0	0	-1	-1	-4,67
21	aldo-keto reductase family 1, member C13	0	0	-1	0	-1	-4,57
22	predicted gene 10451	0	0	0	-1	-1	-4,56
23	---	0	0	-1	-1	-1	-4,56
24	deltex 1 homolog (Drosophila)	0	0	-1	-1	-1	-4,54

25	SH3-domain GRB2-like (endophilin) interacting protein 1	1	0	-1	-1	-1	-4,52
26	Abelson helper integration site 1	0	0	-1	-1	-1	-4,48
27	leukemia inhibitory factor	0	0	-1	-1	-1	-4,44
28	DNA segment, Chr 1, ERATO Doi 564, expressed	0	0	-1	-1	-1	-4,43
29	CD160 antigen	0	0	-1	-1	-1	-4,41
30	CD163 molecule-like 1	1	0	-1	-1	-1	-4,39
31	solute carrier family 15 (oligopeptide transporter), member 1	1	0	-1	-1	-1	-4,37
32	family with sequence similarity 183, member B	0	0	-1	0	-1	-4,35
33	**kit oncogene**	**0**	**0**	**0**	**-1**	**-1**	**-4,29**
34	---	0	0	-1	-1	-1	-4,29
35	interferon induced transmembrane protein 1	0	0	0	-1	-1	-4,27
36	---	0	0	-1	-1	-1	-4,27
37	purinergic receptor P2X, ligand-gated ion channel, 7	0	0	-1	-1	-1	-4,27
38	ADP-ribosyltransferase 2a	0	0	-1	-1	-1	-4,25
39	RIKEN cDNA 3830403N18 gene /// X-linked lymphocyte-regulated complex	0	0	-1	-1	-1	-4,25
40	family with sequence similarity 109, member B	0	0	-1	-1	-1	-4,23
41	predicted gene 1060	0	0	-1	1	-1	-4,22
42	G protein-coupled receptor 83	0	0	-1	-1	-1	-4,21
43	transketolase-like 1	0	0	-1	0	-1	-4,12
44	hepatoma-derived growth factor, related protein 3	0	0	-1	-1	-1	-4,10
45	RIKEN cDNA 5830468F06 gene	0	0	-1	0	-1	-4,08
46	T-cell receptor gamma, variable 1	0	0	-1	-1	-1	-4,08
47	transmembrane protein 146	0	0	-1	-1	-1	-4,06
48	---	0	0	-1	0	-1	-4,02
49	interleukin 2 receptor, alpha chain	0	0	-1	-1	-1	-3,98
50	granzyme A	0	0	-1	0	-1	-3,97
51	retinitis pigmentosa GTPase regulator interacting protein 1	0	0	-1	-1	-1	-3,96
52	family with sequence similarity 71, member B	0	0	-1	-1	-1	-3,91

53	---	0	0	-1	-1	-1	-3,91
54	RAB27b, member RAS oncogene family	0	0	-1	-1	-1	-3,90
55	sperm acrosome associated 1	-1	0	-1	-1	-1	-3,90
56	PDZ and LIM domain 4	0	0	-1	-1	-1	-3,90
57	SH3-domain GRB2-like (endophilin) interacting protein 1	0	0	-1	-1	-1	-3,85
58	distal-less homeobox 1	0	0	0	0	-1	-3,85
59	CD160 antigen	0	0	-1	-1	-1	-3,82
60	RAB27b, member RAS oncogene family	0	0	-1	-1	-1	-3,82
61	calcium channel, voltage-dependent, L type, alpha 1C subunit	0	0	-1	-1	-1	-3,80
62	G protein-coupled receptor 83	0	0	-1	-1	-1	-3,79
63	lipoma HMGIC fusion partner-like 3	0	0	-1	-1	-1	-3,78
64	T-cell receptor gamma, variable 4	0	0	-1	-1	-1	-3,78
65	ATPase, aminophospholipid transporter (APLT), class I, type 8A, member 1	0	0	0	-1	-1	-3,77
66	---	0	0	-1	-1	-1	-3,77
67	synaptotagmin-like 3	0	0	-1	-1	-1	-3,75
68	tribbles homolog 3 (Drosophila)	0	0	0	0	-1	-3,70
69	NLR family, pyrin domain containing 6	0	0	-1	-1	-1	-3,70
70	a disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 motif, 20	0	0	-1	-1	-1	-3,70
71	T-cell receptor gamma, variable 4	0	0	-1	-1	-1	-3,70
72	beta-site APP cleaving enzyme 1	0	0	1	-1	-1	-3,70
73	transketolase-like 1	0	0	-1	0	-1	-3,70
74	---	-1	0	0	-1	-1	-3,70
75	GTPase activating RANGAP domain-like 4	0	0	-1	-1	-1	-3,70
76	myosin, light polypeptide 4	0	0	-1	-1	-1	-3,68
77	ELAV (embryonic lethal, abnormal vision, Drosophila)-like 4 (Hu antigen D)	0	0	-1	-1	-1	-3,67
78	hemolytic complement	0	0	-1	-1	-1	-3,67
79	G protein-coupled receptor 114	0	0	-1	-1	-1	-3,66

80	sterol O-acyltransferase 2	0	0	0	-1	-1	-3,66
81	proteasome (prosome, macropain) subunit, alpha type, 8	0	0	-1	-1	-1	-3,65
82	B-cell leukemia/lymphoma 2	0	0	-1	0	-1	-3,65
83	transient receptor potential cation channel, subfamily M, member 1	0	0	-1	-1	-1	-3,64
84	zinc finger protein 105	0	0	-1	-1	-1	-3,63
85	expressed sequence W91776	0	0	-1	-1	-1	-3,62
86	---	0	0	0	-1	-1	-3,60
87	PRELI domain containing 2	0	0	-1	0	-1	-3,60
88	hepatoma-derived growth factor, related protein 3	0	0	-1	-1	-1	-3,60
89	cut-like homeobox 2	0	0	-1	-1	-1	-3,58
90	RIKEN cDNA 2900001G08 gene	0	0	0	-1	-1	-3,57
91	non-catalytic region of tyrosine kinase adaptor protein 2	0	0	-1	0	-1	-3,57
92	N-myc downstream regulated gene 1	0	0	-1	-1	-1	-3,56
93	myelin transcription factor 1-like	0	0	-1	0	-1	-3,55
94	RIKEN cDNA 6720477C19 gene	0	0	-1	-1	-1	-3,55
95	amyotrophic lateral sclerosis 2 (juvenile) chromosome region, candidate 12 (human)	0	0	-1	-1	-1	-3,55
96	tumor necrosis factor (ligand) superfamily, member 11	0	0	-1	-1	-1	-3,53
97	---	0	0	0	-1	-1	-3,53
98	Epithelial stromal interaction 1 (breast)	0	0	-1	-1	-1	-3,52
99	N-myc downstream regulated gene 1	0	0	-1	-1	-1	-3,51
100	transmembrane protein 45a	0	0	-1	-1	-1	-3,50
101	RAB27b, member RAS oncogene family	0	0	-1	-1	-1	-3,48
102	RIKEN cDNA 1700048O20 gene	0	0	-1	-1	-1	-3,47
103	N-myc downstream regulated gene 1	0	0	-1	-1	-1	-3,47
104	zinc finger, CCHC domain containing 18	0	0	-1	-1	-1	-3,41
105	insulin-like growth factor 2 mRNA binding protein 2	0	0	-1	0	-1	-3,40
106	N-myc downstream regulated gene 1	0	0	-1	-1	-1	-3,39
107	cysteinyl leukotriene receptor 1	0	0	-1	-1	-1	-3,39

108	---	0	0	-1	0	-1	-3,38
109	aldo-keto reductase family 1, member C12	0	0	-1	1	-1	-3,37
110	coiled-coil domain containing 102A	0	0	-1	-1	-1	-3,35
111	armadillo repeat containing, X-linked 4	0	0	0	-1	-1	-3,35
112	suppressor of cytokine signaling 2	0	0	-1	0	-1	-3,32
113	integrin beta 7	0	0	0	-1	-1	-3,31
114	eukaryotic translation initiation factor 3, subunit I pseudogene	0	0	-1	0	-1	-3,30
115	B-cell leukemia/lymphoma 2	0	0	-1	0	-1	-3,30
116	beta-1,4-N-acetyl-galactosaminyl transferase 4	0	0	-1	-1	-1	-3,29
117	oncostatin M	0	0	-1	-1	-1	-3,28
118	adenosine A3 receptor	0	0	-1	-1	-1	-3,28
119	epithelial stromal interaction 1 (breast)	0	0	-1	-1	-1	-3,27
120	purinergic receptor P2X, ligand-gated ion channel, 7	0	0	0	-1	-1	-3,27
121	amphiphysin	0	0	-1	-1	-1	-3,26
122	tumor necrosis factor receptor superfamily, member 9	0	0	-1	-1	-1	-3,26
123	B-cell leukemia/lymphoma 2	0	0	-1	0	-1	-3,23
124	Notch-regulated ankyrin repeat protein	0	0	-1	-1	-1	-3,22
125	protein tyrosine phosphatase, non-receptor type 13	0	0	-1	-1	-1	-3,22
126	ATPase, aminophospholipid transporter (APLT), class I, type 8A, member 1	0	0	-1	-1	-1	-3,22
127	stearoyl-Coenzyme A desaturase 2	0	0	-1	-1	-1	-3,22
128	cytochrome P450, family 39, subfamily a, polypeptide 1	0	0	0	-1	-1	-3,21
129	matrix-remodelling associated 7	-1	0	-1	-1	-1	-3,21
130	G protein-coupled receptor 34	0	0	-1	0	-1	-3,21
131	RIKEN cDNA 1700112E06 gene	0	0	-1	-1	-1	-3,21
132	lipoma HMGIC fusion partner-like 3	0	0	-1	-1	-1	-3,19
133	---	0	0	-1	-1	-1	-3,19
134	F-box protein 17	0	0	-1	0	-1	-3,19
135	RIKEN cDNA 5830474E16 gene	0	0	-1	0	-1	-3,18

136	---	0	0	-1	0	-1	-3,18
137	DEAD (Asp-Glu-Ala-Asp) box polypeptide 43	-1	0	-1	-1	-1	-3,18
138	ArfGAP with FG repeats 1	0	0	-1	-1	-1	-3,18
139	---	0	0	-1	0	-1	-3,17
140	ATP-binding cassette, sub-family B (MDR/TAP), member 1A	0	0	-1	-1	-1	-3,17
141	granzyme B	1	0	0	0	-1	-3,17
142	chymosin	-1	0	-1	-1	-1	-3,16
143	EGL nine homolog 3 (C. elegans)	0	0	0	-1	-1	-3,16
144	retinol dehydrogenase 10 (all-trans)	0	0	-1	-1	-1	-3,15
145	asparagine synthetase	0	0	0	0	-1	-3,15
146	isocitrate dehydrogenase 3 (NAD+) alpha	0	0	-1	-1	-1	-3,15
147	DENN/MADD domain containing 2D	0	0	-1	-1	-1	-3,13
148	Rap guanine nucleotide exchange factor (GEF) 2	0	0	-1	-1	-1	-3,13
149	---	0	0	-1	0	-1	-3,12
150	T-cell receptor gamma, variable 2 /// T-cell receptor gamma, variable 3	0	0	0	0	-1	-3,10
151	oligodendrocyte transcription factor 3	0	0	-1	1	-1	-3,09
152	DNA segment, Chr 10, Brigham & Women's Genetics 1379 expressed	0	0	-1	-1	-1	-3,09
153	N-terminal EF-hand calcium binding protein 1	-1	0	-1	-1	-1	-3,08
154	Predicted gene 2629	0	0	0	0	-1	-3,08
155	a disintegrin and metallopeptidase domain 19 (meltrin beta) /// similar to metalloprotease-disintegrin meltrin beta	0	0	-1	-1	-1	-3,08
156	RIKEN cDNA D930026N18 gene	0	0	-1	-1	-1	-3,08
157	EGL nine homolog 3 (C. elegans)	0	0	0	-1	-1	-3,07
158	integrin beta 3	0	0	-1	-1	-1	-3,07
159	suppressor of cytokine signaling 2	0	0	-1	0	-1	-3,06
160	ATPase, class I, type 8B, member 4	0	0	1	-1	-1	-3,05
161	WD repeat domain 25	0	0	-1	0	-1	-3,05
162	Transformation related protein 53	0	0	0	0	-1	-3,05

163	---	0	0	-1	0	-1	-3,03
164	B-cell leukemia/lymphoma 2	0	0	0	0	-1	-3,01
165	protein tyrosine phosphatase, receptor type, O	0	0	-1	-1	-1	-3,01

[§]Multiple probe sets map to a single gene.

*Log2 ratios were calculated as described in **Online Supplementary Results**.

- **Table S6.** ***Commonly down-regulated genes in leukemic cells compared to thymus***

	Gene Title[s]	cultured MOHITO cells	secondary cells	HOX11 positive cells	NUP214-ABL1 positive cells	thymus	Log2 ratio thymus/primary cells*
1	fatty acid binding protein 4, adipocyte	0	0	0	0	1	10,73
2	carbonic anhydrase 3	0	0	1	0	1	9,92
3	thyroid hormone responsive SPOT14 homolog (Rattus)	0	0	0	0	1	9,59
4	stearoyl-Coenzyme A desaturase 1	1	0	0	0	1	9,44
5	hemoglobin alpha, adult chain 1 /// hemoglobin alpha, adult chain 2	0	0	0	1	1	9,41
6	microsomal glutathione S-transferase 1	0	0	1	0	1	9,26
7	fatty acid binding protein 4, adipocyte	0	1	0	0	1	9,23
8	lysozyme 1	0	0	1	0	1	9,17
9	thymus, brain and testes associated	0	0	0	0	1	9,14
10	RIKEN cDNA 2010205A11 gene /// predicted gene 10883 /// predicted gene 1420 /// predicted gene 7202 /// immunoglobulin kappa chain complex /// immunoglobulin kappa chain, constant region /// immunoglobulin kappa chain variable 28 (V28) /// similar to Chain L, Structural Basis Of Antigen Mimicry In A Clinically Relevant Melanoma Antigen System	0	0	0	0	1	8,97
11	lysozyme 1	0	0	1	0	1	8,79
12	adiponectin, C1Q and collagen domain containing	0	0	0	0	1	8,76
13	carbonic anhydrase 3	0	0	0	0	1	8,40
14	thymus, brain and testes associated	0	0	0	0	1	8,40
15	predicted gene 10883 /// predicted gene 1420 /// predicted gene 7202 /// immunoglobulin kappa chain complex /// immunoglobulin kappa chain, constant region /// immunoglobulin kappa chain variable 28 (V28) /// similar to Chain L, Structural Basis Of Antigen Mimicry In A Clinically Relevant Melanoma Antigen System	0	0	0	0	1	8,34
16	predicted gene 10883 /// predicted gene 1420 /// predicted gene 7202 /// immunoglobulin kappa chain	0	0	0	0	1	8,33

	complex /// immunoglobulin kappa chain, constant region /// immunoglobulin kappa chain variable 28 (V28) /// similar to Chain L, Structural Basis Of Antigen Mimicry In A Clinically Relevant Melanoma Antigen System						
17	choline phosphotransferase 1	0	0	1	1	1	8,15
18	immunoglobulin joining chain	0	0	0	1	1	8,03
19	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	0	0	0	1	1	7,92
20	vascular cell adhesion molecule 1	0	0	0	0	1	7,89
21	histocompatibility 2, class II antigen E alpha	0	0	0	0	1	7,83
22	chemokine (C-C motif) ligand 25	0	0	1	1	1	7,66
23	profilin 2	0	0	0	0	1	7,63
24	chemokine (C-X-C motif) ligand 12	0	0	0	0	1	7,63
25	T-cell receptor beta, joining region	0	0	0	0	1	7,62
26	keratin 18	0	0	0	0	1	7,62
27	complement factor D (adipsin)	0	0	0	0	1	7,59
28	thyroid hormone responsive SPOT14 homolog (Rattus)	0	0	0	0	1	7,56
29	cold shock domain protein A	0	0	0	1	1	7,55
30	cytochrome P450, family 2, subfamily e, polypeptide 1	0	0	0	0	1	7,53
31	lysozyme 2	0	0	1	0	1	7,53
32	T-cell receptor beta, joining region	0	0	1	1	1	7,48
33	histocompatibility 2, class II antigen A, alpha	0	0	0	0	1	7,45
34	choline phosphotransferase 1	0	0	1	1	1	7,45
35	complement component 1, q subcomponent, beta polypeptide	0	0	0	0	1	7,45
36	leukemia inhibitory factor receptor	1	0	0	0	1	7,34
37	carboxylesterase 3	0	0	1	0	1	7,22
38	S100 calcium binding protein A9 (calgranulin B)	0	0	1	0	1	7,21
39	keratin 8	0	0	0	0	1	7,20
40	keratin 8	0	0	0	0	1	7,15
41	S100 calcium binding protein A8 (calgranulin A)	0	0	1	0	1	7,12

42	hemoglobin alpha, adult chain 1 /// hemoglobin alpha, adult chain 2	0	0	0	0	1	7,11
43	CD36 antigen	0	0	0	0	1	7,02
44	lipoprotein lipase	0	0	1	0	1	6,99
45	complement component 1, q subcomponent, beta polypeptide	0	0	0	0	1	6,97
46	expressed sequence BB144871	0	0	0	0	1	6,96
47	protease, serine, 16 (thymus)	0	0	1	0	1	6,94
48	RIKEN cDNA 2010309G21 gene /// immunoglobulin lambda chain, constant region 2 /// immunoglobulin lambda chain, constant region 3	0	0	0	0	1	6,93
49	MAD homolog 1 (Drosophila)	0	0	0	0	1	6,91
50	RIKEN cDNA A630038E17 gene	0	0	0	0	1	6,91
51	signal peptidase complex subunit 3 homolog pseudogene /// signal peptidase complex subunit 3 homolog pseudogene	0	0	0	1	1	6,90
52	lysosomal-associated protein transmembrane 4B	0	0	1	0	1	6,88
53	CD36 antigen	0	0	0	0	1	6,87
54	ubiquitin D	0	0	0	0	1	6,82
55	insulin-like growth factor binding protein 5	0	0	0	0	1	6,81
56	hemoglobin alpha, adult chain 1 /// hemoglobin alpha, adult chain 2	0	0	0	0	1	6,75
57	collagen, type III, alpha 1	0	0	0	0	1	6,64
58	decorin	0	0	0	0	1	6,58
59	immunoglobulin heavy chain complex /// immunoglobulin heavy chain 2 (serum IgA) /// immunoglobulin heavy chain (J558 family) /// similar to immunoglobulin mu-chain	0	0	0	0	1	6,56
60	FK506 binding protein 9	0	0	0	0	1	6,53
61	heat shock protein 1B	0	0	1	0	1	6,52
62	histocompatibility 2, class II antigen A, alpha	0	0	0	0	1	6,51
63	RIKEN cDNA 5730469M10 gene	0	0	0	0	1	6,50
64	eukaryotic translation initiation factor 2, subunit 3, structural gene Y-linked	0	0	0	1	1	6,50

65	regulator of G-protein signaling 5	0	0	0	0	1	6,48
66	gap junction protein, alpha 1	0	0	1	0	1	6,46
67	cell death-inducing DNA fragmentation factor, alpha subunit-like effector A	0	0	0	0	1	6,46
68	complement component 1, q subcomponent, alpha polypeptide	0	0	0	0	1	6,45
69	T-cell receptor beta, joining region	0	0	0	0	1	6,43
70	choline phosphotransferase 1	0	0	1	0	1	6,43
71	PERP, TP53 apoptosis effector	0	0	1	0	1	6,41
72	interferon induced transmembrane protein 3	0	0	1	0	1	6,40
73	hemoglobin, beta adult major chain /// hemoglobin, beta adult minor chain	0	0	0	0	1	6,30
74	heat shock protein 1B	0	0	1	0	1	6,30
75	RIKEN cDNA 2010309G21 gene /// immunoglobulin lambda chain, constant region 2	0	0	0	0	1	6,29
76	cytochrome c oxidase, subunit VIIIb	0	0	0	0	1	6,28
77	ATP-binding cassette, sub-family A (ABC1), member 1	0	0	1	0	1	6,28
78	carboxylesterase 3	0	0	1	0	1	6,27
79	annexin A1	0	0	1	0	1	6,27
80	gap junction protein, alpha 1	0	0	1	0	1	6,25
81	immunoglobulin heavy chain complex /// immunoglobulin heavy chain 2 (serum IgA) /// immunoglobulin heavy chain (J558 family) /// similar to immunoglobulin mu-chain	0	0	0	0	1	6,24
82	RIKEN cDNA 5730469M10 gene	0	0	0	0	1	6,24
83	retinol binding protein 4, plasma	0	0	0	0	1	6,24
84	thymus, brain and testes associated	0	0	0	0	1	6,23
85	serum deprivation response	0	0	0	0	1	6,22
86	palate, lung, and nasal epithelium associated	0	0	0	0	1	6,20
87	caveolin 1, caveolae protein	0	0	0	0	1	6,18
88	lipoprotein lipase	0	0	0	0	1	6,18
89	coiled-coil-helix-coiled-coil-helix domain containing 6	0	0	1	1	1	6,15

90	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	0	0	0	1	1	6,15
91	caldesmon 1	0	0	0	0	1	6,14
92	carbonyl reductase 2	0	0	0	0	1	6,14
93	amyloid beta (A4) precursor protein	0	0	1	0	1	6,13
94	complement component 1, q subcomponent, beta polypeptide	0	0	0	0	1	6,13
95	MAD homolog 1 (Drosophila)	0	0	0	0	1	6,13
96	uncoupling protein 1 (mitochondrial, proton carrier)	0	0	0	0	1	6,11
97	gap junction protein, alpha 1	0	0	1	0	1	6,10
98	four and a half LIM domains 1	0	0	0	0	1	6,10
99	growth hormone receptor	0	0	0	0	1	6,10
100	matrix Gla protein	0	0	0	0	1	6,09
101	FXYD domain-containing ion transport regulator 2	0	0	0	0	1	6,09
102	glutamyl aminopeptidase	0	0	0	0	1	6,09
103	orosomucoid 1	0	0	1	0	1	6,06
104	casein beta	0	0	0	0	1	6,05
105	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	0	0	0	1	1	6,05
106	lysine (K)-specific demethylase 5D	0	0	0	1	1	6,03
107	stearoyl-Coenzyme A desaturase 1	1	0	0	0	1	6,01
108	melanoregulin	0	0	0	0	1	6,00
109	gap junction protein, alpha 1	0	0	1	0	1	5,99
110	Kruppel-like factor 9	0	0	1	1	1	5,97
111	transmembrane protein 45b	0	0	0	0	1	5,95
112	cytochrome c oxidase, subunit VIIa 1	0	0	0	0	1	5,95
113	TSC22 domain family, member 1	1	0	1	1	1	5,94
114	T-cell receptor beta, joining region	0	0	0	0	1	5,90
115	immunoglobulin lambda chain, variable 1	0	0	0	0	1	5,90
116	plastin 3 (T-isoform)	0	0	0	0	1	5,88
117	retinol binding protein 1, cellular	0	0	0	0	1	5,87

118	heat shock protein 1B	0	0	1	0	1	5,84
119	elongation factor RNA polymerase II 2	0	0	0	0	1	5,83
120	G protein-coupled receptor 177	0	0	1	0	1	5,82
121	synaptonemal complex protein 3	0	0	0	0	1	5,81
122	keratin 8	0	0	0	0	1	5,76
123	RIKEN cDNA 2810405K02 gene	0	0	0	0	1	5,76
124	TSC22 domain family, member 1	1	0	1	1	1	5,76
125	pyruvate dehydrogenase kinase, isoenzyme 4	0	0	0	0	1	5,75
126	homeodomain interacting protein kinase 2	0	0	1	0	1	5,73
127	T-cell receptor beta, joining region	0	0	1	0	1	5,72
128	eosinophil-associated, ribonuclease A family, member 1 /// eosinophil-associated, ribonuclease A family, member 12 /// eosinophil-associated, ribonuclease A family, member 2 /// eosinophil-associated, ribonuclease A family, member 3	0	0	0	0	1	5,70
129	ATPase, Na+/K+ transporting, alpha 2 polypeptide	0	0	1	0	1	5,66
130	fatty acid binding protein 3, muscle and heart	0	0	0	0	1	5,66
131	epidermal growth factor-containing fibulin-like extracellular matrix protein 1	0	0	0	0	1	5,65
132	fibroblast growth factor 13	0	0	0	0	1	5,64
133	STEAP family member 4	0	0	0	0	1	5,62
134	TSC22 domain family, member 1	1	0	1	1	1	5,62
135	Kruppel-like factor 9	0	0	1	1	1	5,61
136	metallothionein 1	1	1	1	1	1	5,59
137	fermitin family homolog 2 (Drosophila)	0	0	0	0	1	5,58
138	potassium channel tetramerisation domain containing 12	0	0	1	0	1	5,58
139	transforming growth factor, beta induced	0	0	1	0	1	5,57
140	RIKEN cDNA 1110067D22 gene	0	0	0	0	1	5,57
141	osteoglycin	0	0	0	0	1	5,55
142	immunoglobulin heavy chain 6 (heavy chain of IgM)	0	0	0	1	1	5,54

143	nudix (nucleoside diphosphate linked moiety X)-type motif 7	0	0	1	0	1	5,53
144	cell death-inducing DFFA-like effector c	0	0	0	0	1	5,53
145	cortactin	0	0	0	0	1	5,53
146	myosin, light polypeptide 1	0	0	0	0	1	5,52
147	growth arrest specific 6	0	0	0	0	1	5,52
148	T-cell receptor beta, joining region	0	0	0	0	1	5,51
149	immunoglobulin heavy chain complex /// Immunoglobulin heavy chain (gamma polypeptide)	0	0	0	0	1	5,50
150	carboxylesterase 3	0	0	1	0	1	5,49
151	T-cell receptor alpha chain	1	0	1	1	1	5,48
152	CD83 antigen	0	0	0	0	1	5,46
153	histocompatibility 2, class II antigen A, alpha	0	0	0	0	1	5,45
154	chemokine (C-X-C motif) ligand 13	0	0	0	0	1	5,44
155	predicted gene 10883	0	0	0	0	1	5,44
156	pantothenate kinase 1	0	0	0	0	1	5,43
157	apolipoprotein E	0	0	0	0	1	5,43
158	ubiquitously transcribed tetratricopeptide repeat gene, Y chromosome	0	0	0	1	1	5,40
159	cold shock domain protein A	0	0	0	1	1	5,37
160	UDP galactosyltransferase 8A	0	0	0	0	1	5,33
161	FXYD domain-containing ion transport regulator 2	0	0	0	0	1	5,31
162	transmembrane protein 176B	0	0	1	0	1	5,31
163	vesicular, overexpressed in cancer, prosurvival protein 1	0	0	0	0	1	5,31
164	formin binding protein 1-like	1	0	1	0	1	5,30
165	---	0	0	0	0	1	5,30
166	amylase 1, salivary	0	0	0	0	1	5,29
167	guanine nucleotide binding protein (G protein), alpha inhibiting 1	0	0	0	0	1	5,29
168	hydroxyprostaglandin dehydrogenase 15 (NAD)	0	0	0	0	1	5,28
169	complement component factor h /// similar to complement	0	0	0	0	1	5,27

	component factor H						
170	dodecenoyl-Coenzyme A delta isomerase (3,2 trans-enoyl-Coenyme A isomerase)	0	0	0	0	1	5,27
171	B-cell leukemia/lymphoma 2 related protein A1a /// B-cell leukemia/lymphoma 2 related protein A1b /// B-cell leukemia/lymphoma 2 related protein A1d	0	0	0	0	1	5,26
172	immunoglobulin kappa chain variable 1 (V1)	0	0	0	0	1	5,24
173	cytoplasmic FMR1 interacting protein 1	0	0	1	0	1	5,22
174	crystallin, alpha B	0	0	0	0	1	5,21
175	profilin 2	0	0	0	0	1	5,20
176	CD302 antigen	0	0	0	0	1	5,19
177	carbonic anhydrase 2	0	0	0	0	1	5,18
178	myosin, heavy polypeptide 1, skeletal muscle, adult	0	0	0	0	0	5,17
179	melanocortin 2 receptor accessory protein	0	0	0	0	1	5,16
180	serum deprivation response	0	0	0	0	1	5,16
181	monoglyceride lipase	0	0	1	0	1	5,15
182	chemokine (C-C motif) ligand 21A /// chemokine (C-C motif) ligand 21B /// chemokine (C-C motif) ligand 21C (leucine)	0	0	0	0	1	5,15
183	protein phosphatase 1, regulatory (inhibitor) subunit 3C	0	0	0	0	1	5,15
184	ERBB receptor feedback inhibitor 1	0	0	0	0	1	5,14
185	amine oxidase, copper containing 3	0	0	0	0	1	5,13
186	monoglyceride lipase	0	0	1	0	1	5,13
187	G protein-coupled receptor 177	0	0	1	0	1	5,11
188	secreted acidic cysteine rich glycoprotein	0	0	0	0	1	5,11
189	serine (or cysteine) peptidase inhibitor, clade G, member 1	0	0	0	0	1	5,10
190	immunoglobulin lambda chain complex /// immunoglobulin lambda chain, variable 1 /// similar to Ig lambda-1 chain C region	0	0	0	0	1	5,09
191	cysteine dioxygenase 1, cytosolic	0	0	0	0	1	5,09
192	formin-like 2	0	0	0	0	1	5,08

193	colony stimulating factor 1 receptor	0	0	0	0	1	5,08
194	caveolin 2	0	0	0	0	1	5,06
195	prostaglandin E receptor 3 (subtype EP3)	0	0	1	1	1	5,06
196	myosin, heavy polypeptide 1, skeletal muscle, adult	0	0	0	0	0	5,04
197	protein kinase inhibitor, alpha	0	0	0	0	1	5,03
198	aldehyde dehydrogenase family 1, subfamily A1	0	0	0	0	1	5,03
199	gamma-aminobutyric acid (GABA) A receptor-associated protein-like 1	1	0	1	1	1	5,02

[§]Multiple probe sets map to a single gene.

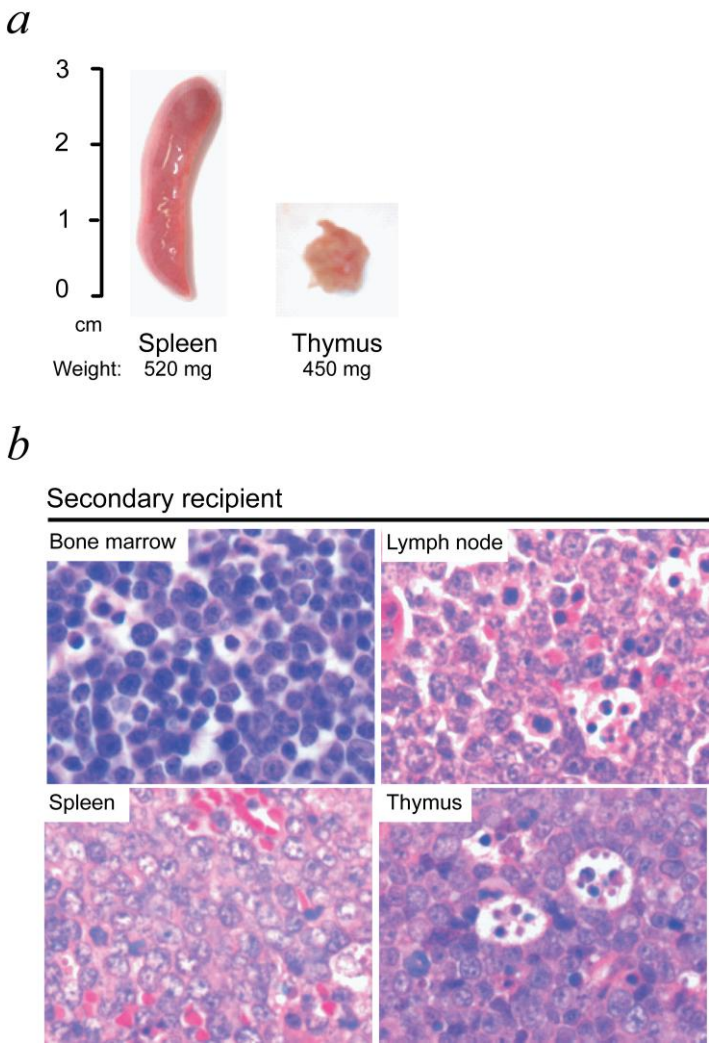
*Log2 ratios were calculated as described in **Online Supplementary Results**.

Online Supplementary Figures

- **Supplementary Figure S1.** ***Analysis of diseased primary Balb/c mouse***

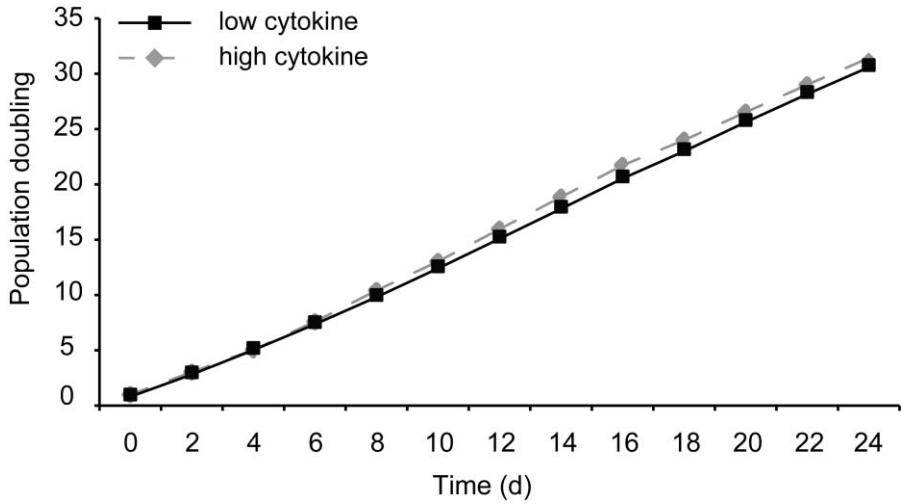
(A) Photograph showing a marked splenomegaly (left, 520 mg) and enlarged thymus (right, 450 mg) of primary mouse.

(B) Transplantation of isolated leukemic cells into secondary recipients resulted in a T-ALL like disease with massive lymphoblastic infiltration in the bone marrow, spleen, thymus and lymph nodes. Mitotic figures and presence of numerous tingible body macrophages (with starry sky pattern in lymph node and thymus) indicate high proliferative activity. H&E stainings of one representative secondary transplant are shown. Magnification: 400X.



- **Supplementary Figure S2.** ***Growth characteristics of MOHITO cells***

  Isolated cells were cultured *ex vivo* in presence of cytokines IL-7 and IL-2. Doubling curves for two different concentrations are shown. High cytokines (grey): 30 ng/ml IL-7 + 25 ng/ml IL-2; low cytokines (black): 10 ng/ml IL-7 + 5 ng/ml IL-2. Cell numbers were monitored every other day for a period of 24 days.



- **Supplementary Figure S3.** ***Additional genetic lesions identified in MOHITO cells***

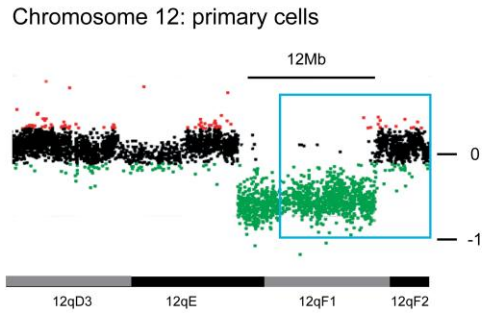
(A) Array CGH analysis detected a heterozygous deletion of about 12 Mb on chromosome 12 in primary cells. Probes covering chromosome region 12qD3 till 12qF2 are shown. The boxed chromosomal region was found to be involved in a balanced translocation in cultured MOHITO cells and as part of the generated der(5)t(5;12) duplicated **(Figure S3B)**.

(B) Array CGH results for MOHITO cells depicting translocation breakpoints on chromosome 12 (upper panel) and chromosome 5 (lower panel). Balanced translocation typically remain undetected by sole array CGH analysis, however MOHITO cells featured a duplication of der(5)t(5;12) thereby allowing us to precisely map the breakpoints upstream of the *Bcl11b* gene on chromosome 12 and downstream of *Pdgfra* on chromosome 5. Even though the terminal part of chromosomal band 12qF1 is duplicated as part of der(5)t(5,12) array CGH profile displayed a normal copy number status for this region due to concomitant deletion of this region on the other allele **(Figure S3A)**. Schematic representations of respective breakpoint regions for each chromosome (chr12: blue, chr5: red) are shown.

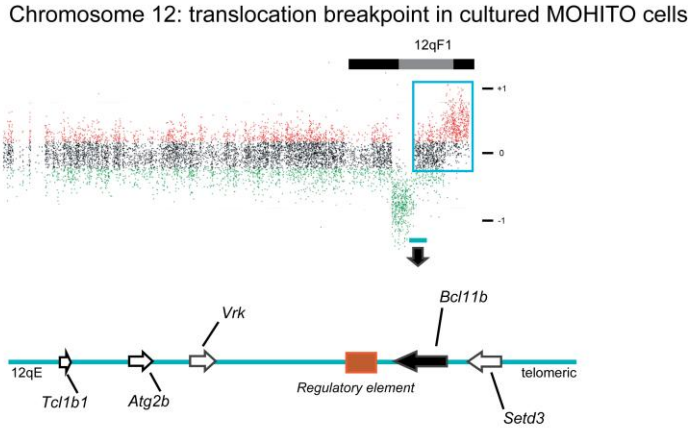
(C) Illustration of generated derivates resulting from a balanced translocation involving chromosome 5 and 12. Derivate chromosome 5 with translocation of chromosome 12 (der(5)t(5;12), upper panel) and derivate 12 (der(12)t(5;12), lower panel) are shown.

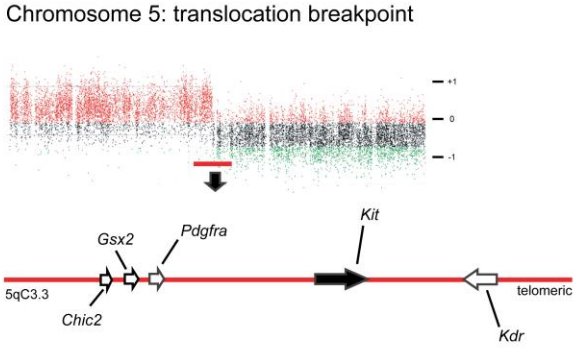
Green and red dots represent signal with negative or positive fluorescent ratios, respectively. Direction of arrows indicates gene orientation.

a


Chromosome 12: primary cells
12Mb
0
-1
12qD3
12qE
12qF1
12qF2

b


Chromosome 12: translocation breakpoint in cultured MOHITO cells
12qF1
Vrk
Bcl11b
12qE
telomeric
Tcl1b1
Atg2b
Regulatory element
Setd3


Chromosome 5: translocation breakpoint
Kit
Gsx2
Pdgfra
5qC3.3
telomeric
Chic2
Kdr

c

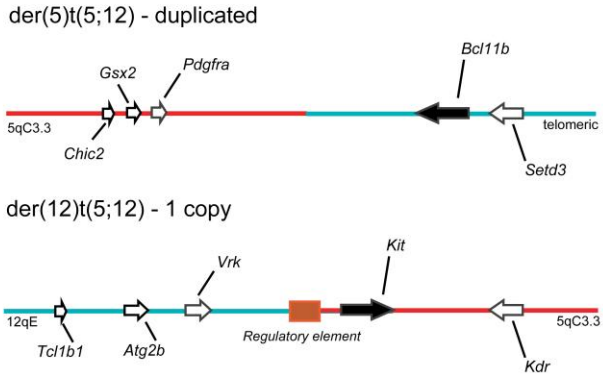

der(5)t(5;12) - duplicated
Bcl11b
Gsx2
Pdgfra
5qC3.3
telomeric
Chic2
Setd3
der(12)t(5;12) - 1 copy
Kit
Vrk
12qE
5qC3.3
Tcl1b1
Atg2b
Regulatory element
Kdr

Figure S4. ***MOHITO expression pattern highly differs from thymus cells***

(A) Volcano blot visualizing differentially expressed probes when comparing primary MOHITO cells to wildtype thymus. Estimated base 2 log ratios are blotted against minus the $\log_{10}$ p-value. Dots are colored in red or green if they are classified as down- or up-regulated, respectively, based on the criteria described in **Online supplementary Design and Methods**.

(B) Heat map highlighting severe differences in overall expression between primary cells and other mouse cells analyzed in this study. Gene expression values for probes differing more than 3 in their $\log 2$ ratio from thymus cells are shown. Samples are shown in duplicate. Red: high RMA expression values; blue: low RMA expression values.

(C) Down-regulated genes are highly conserved among mouse T-ALL cells. Heat map displaying gene probes with $\log 2$ expression ratios greater than five (thymus wt/ primary cells) are shown. Samples are shown in duplicate. Red: high RMA expression values; blue: low RMA expression values.

Detailed gene lists and values for each probe shown are listed in **Online Supplementary Table 5** and **Supplementary Table 6**, respectively.

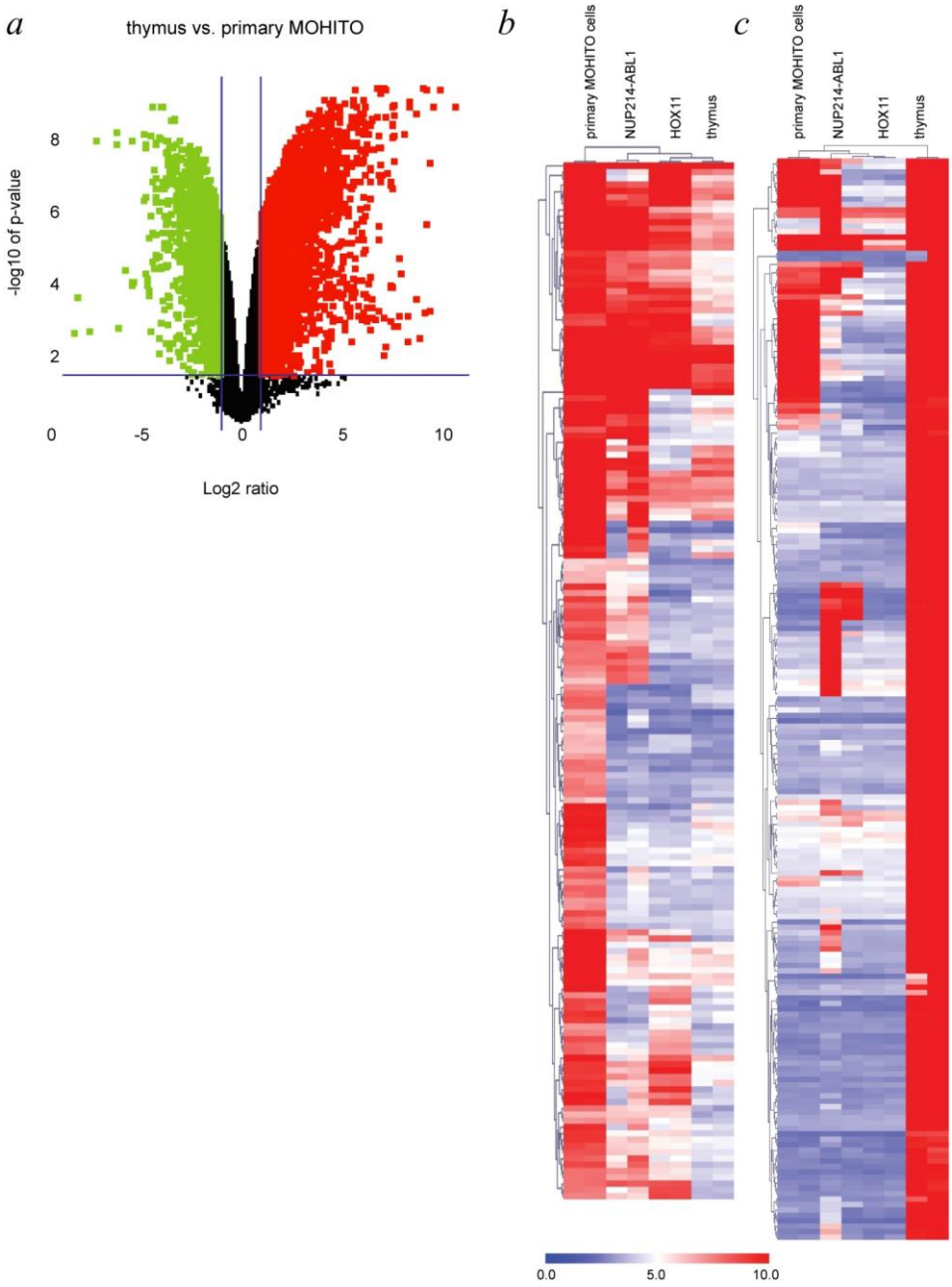

a
thymus vs. primary MOHITO
-log10 of p-value
8
6
4
2
0
-5
0
5
10
Log2 ratio
b
primary MOHITO cells
NUP214-ABL1
HOX11
thymus
c
primary MOHITO cells
NUP214-ABL1
HOX11
thymus
0.0
5.0
10.0

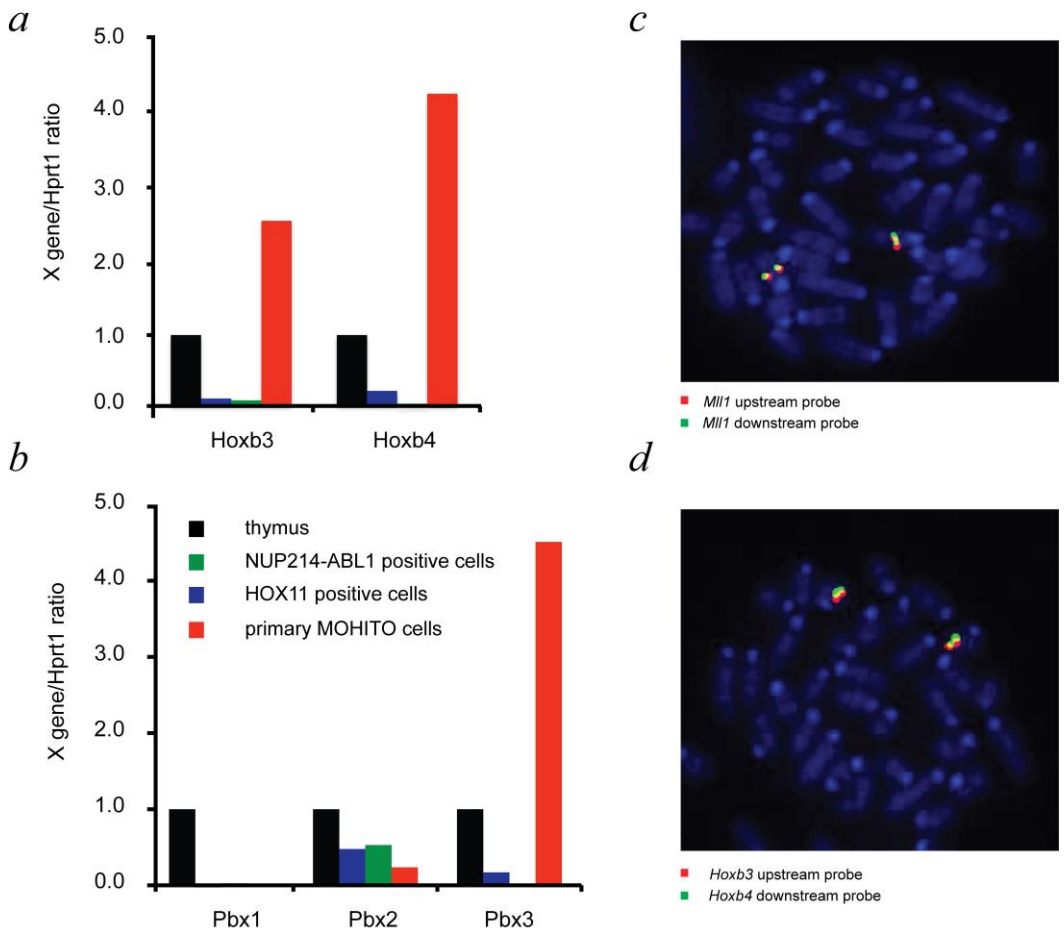
Figure S5. ***High expression of homeobox transcription factors Hoxb3 and Hoxb4***

(A) qRT-PCR results illustrating strikingly high expression of homeobox transcription factors Hoxb3 and Hoxb4 in MOHITO cells whereas nearly undetectable in other mouse T-cells.

(B) qRT-PCR analysis showing expression profile of the TALE family members Pbx1, Pbx2 and Pbx3.

Expression values were normalized for Hprt1 and are shown as fold expression compared to thymus cells. Red bars: MOHITO cells; black bars: thymus cells; green: NUP214-ABL1 positive cells; blue bars: HOX11 positive cells.

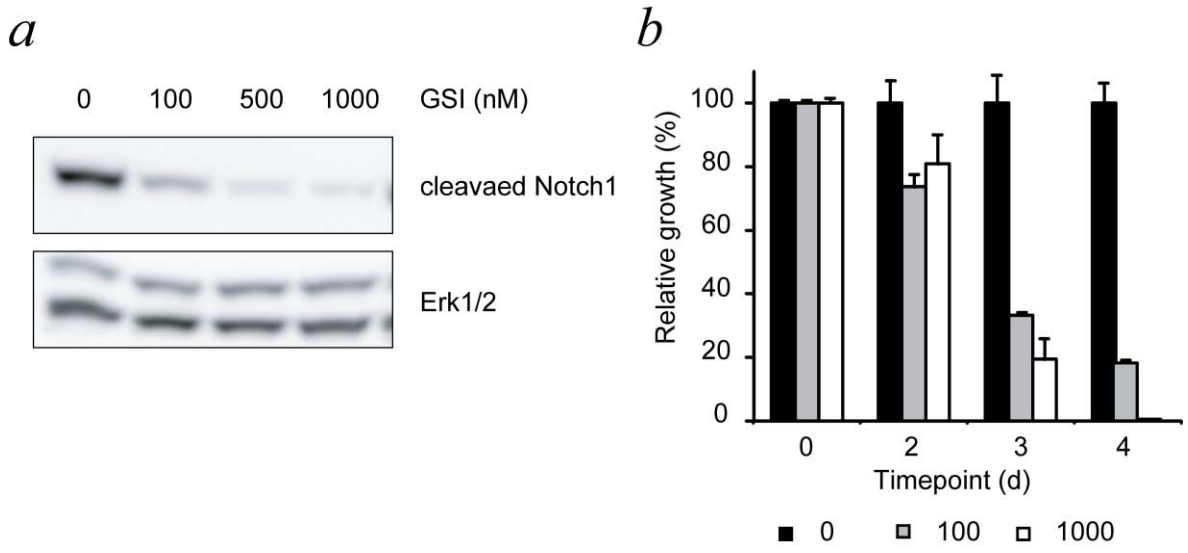
(C) FISH analysis for Mll or (D) Hoxb3/Hoxb4 gene rearrangements. Upstream probes: Spectrum Orange- dUTP; downstream probes: Spectrum-green-dUTP (both Abbott Molecular).



- **Figure S6.** ***Constitutive activation of the Notch1 pathway in MOHITO cells***

(A) Western blot analysis confirmed constitutive activation of Notch1 and exposure to a gamma-secretase inhibitor (GSI, 24 hours) dose dependently reduced activated Notch1 protein levels. Activated Notch1 was detected with an antibody detecting endogenous levels of Notch1 only when cleaved between Gly1743 and Val1744. Erk1/2 is shown as loading control.

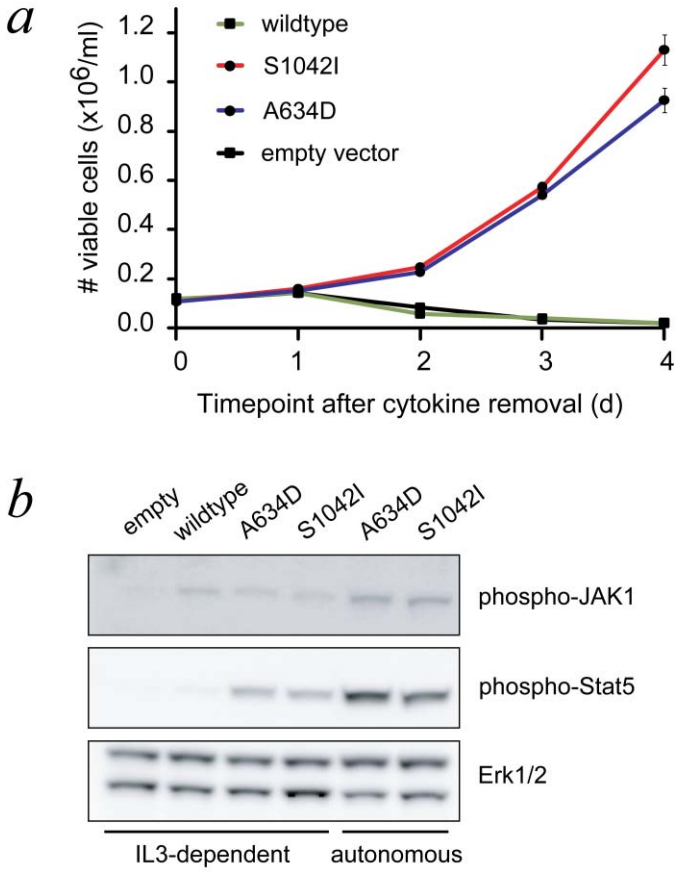
(B) Inhibition of Notch1 in MOHITO cells affected cell proliferation in a dose dependent fashion. Cells were exposed to compound E for four days (x-axis) and cell numbers were monitored daily (n=3). Y-axis displays relative cell growth (%) compared to DMSO treated control cells. Averages ± s.e.m. are shown.



- **Figure S7.** ***Transformation capacity of novel JAK1 mutant S1042I***

(A) Ectopic expression of Jak1 mutant S1042I confers growth factor independent growth to Ba/F3 cells. JAK1(A634D) is known to easily transform Ba/F3 cells and shown as positive control.[22] Respective cell lines were depleted from IL-3 and absolute cell numbers (x-axis) were monitored every 24 hours. Mean values of three determinations ± s.e.m. are shown.

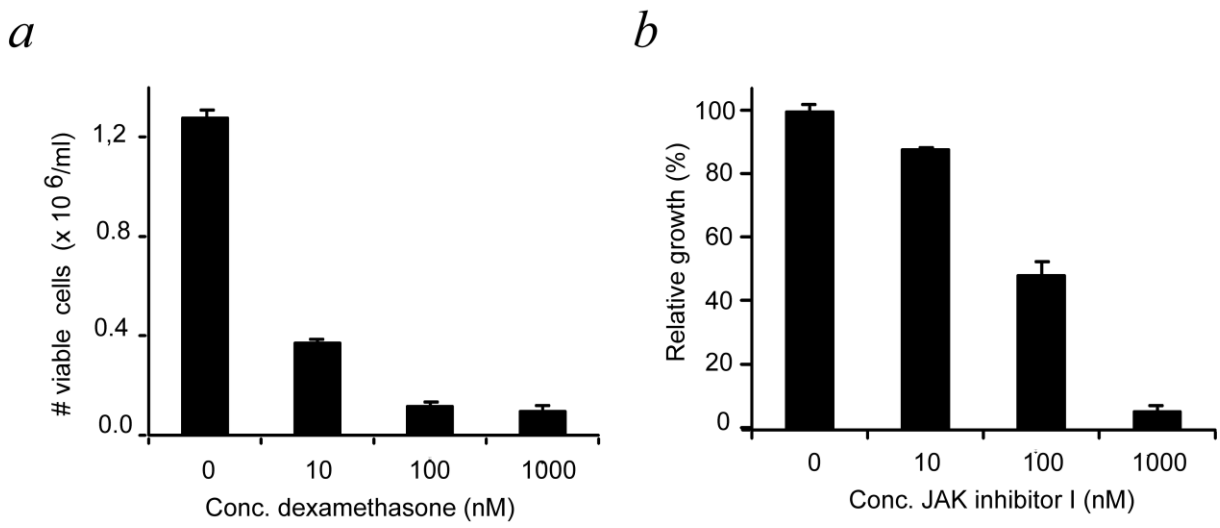
(B) Protein analysis of Ba/F3 cells stably expressing JAK1 variants. In case of cytokine dependency, IL-3 was removed for 4 hours prior to cell lysis (IL-3 dependent). Erk1/2 was used to ensure equal loading.



- **Figure S8.** ***Drug sensitivity of MOHITO cells***

(A) Growth inhibition of MOHITO cells by dexamethasone (dexa). Cells were treated with increasing concentrations dexamethasone (x-axis, nM) or DMSO (control cells). Viable cell numbers were assessed after 48 hours using an automatic cell analyzer (Vi-cell^{TM} XR cell viability analyzer, Beckman Coulter). Results shown are representative for two independent experiments. All values represent the average ± s.e.m. of three determinations.

(B) Blockage of Jak1 signaling transduction results in dose dependent reduction of proliferation. Cell number of untreated control cells (DMSO) was set as 100% and y-axis displays relative cell growth (%). All measurements were made in triplicate. All values represent the average ± s.e.m. of three determinations.



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