Supplemental methods

MS5-derived adipocytes and MSC-derived adipocytes

Confluent MS5 cells were grown during 4 days in an induction medium containing α-MEM (Life technologies®, Carlsbad, CA, USA) and 10% FBS with 3-Isobutyl-1-methylxanthine (0.1mM, Sigma-Aldrich®), bovine insulin (1µg/mL, Sigma-Aldrich®) and indomethacin (0.1mM, Sigma-Aldrich®) followed by a PBS wash and a 5-day maintenance period in α-MEM (Life technologies®) + 10% FBS with bovine insulin (1µg/mL, Sigma-Aldrich®). MS5-derived adipocytes were stained using Oil Red O staining to visualize adipocyte differentiation and triglyceride content was evaluated using an enzymatic-based colorimetric assay as described above.Human MSC were obtained from BM of healthy donors after informed consent (department of Cell Therapy, Hôpital Saint Louis, Paris, France). Human adipocytes were obtained after a 3-week differentiation period of MSC with the StemPro® Adipogenesis Differentiation Kit (Life technologies®).

Flow cytometry

The leukemic phenotype in thoracic or tail vertebrae BM was determined among $hCD45^+$ cells using fluorescein (FITC-), phycoerythrin (PE-), PE-cyanin 7 (PC7-) and allophycocyanin (APC-) mouse anti-human monoclonal antibodies specific for CD7, CD4, CD8, sCD3, TCRαβ, CD49d, CD31 (eBioscience, San Diego, CA, USA), or CXCR4, CD34, CD44, CD127 (IL7-Rα) (Beckman Coulter International SA, Nyon, Switzerland). The leukemic ICN1 phenotype was evaluated in $mCD45.2^+CD45.1^-$ cells using using murine CD4, CD8, surface CD3, TCRβ, V-α2-TCR and CD127 (IL7-R) (eBioscience). Cell cycle analysis of leukemic cells from thoracic or tail vertebrae was performed in $hCD45^+$ / $hCD7^+$ cells or $mCD45.2^+$ ICN1 cells after fixation and permeabilization (BD Cytofix/cytoperm™) using Hoechst 33342 according to manufacturer's instructions (BD Bioscience) and Ki67 (BD Biosciences). After leukemic cells permeabilization, intracellular protein were stained with anti-human antibodies specific for Bax (eBioscience), Bcl2, Mcl1, P21 (Cell Signaling) and E2F1 (Abcam). During analysis, doublet exclusion was performed. To study leukemic apoptosis in thoracic or tail vertebrae, cells were washed in apoptosis buffer (BD Bioscience) and stained during 15 minutes at room temperature using hCD7-PC7, hCD45-APC and Annexin V-FITC (BD Bioscience). Hoechst 33342 (Life Technologies®) was added prior to flow cytometry analysis to detect dead cells. Flow cytometry was performed on FacsCalibur, LSRII or Canto cytometers (BD Biosciences) and plots were analysed using the FlowJo software (Tree Star Inc., Ashland, OR, USA).

Comparative Genomic Hybridization (CGH) arrays and NOTCH1 mutations

Genomic DNA of Thorax- and Tail-derived hT-ALL cells was isolated and deposited in CytoScan®HD (Affymetrix®, Santa Clara, CA, USA) after sample preparation according to manufacturer's instructions. CGH arrays were interpreted using the Chromosome analysis Suite (Affymetrix®) using a 50kb or a 10kb resolution. *NOTCH1* and *FBXW7* mutations were investigated using sequencing of PCR amplifications of exons 26-28, 34 of *NOTCH1* and exons 9-10 of *FBXW7* (list of primers provided in supplementary table 2).

Expression profiling on Affymetrix microarray

Total RNA from Thorax- or Tail-derived hT-ALL was purified using the RNAqueous Micro Kit (ambion, life technologies, USA). Labeling and hybridization to the Affymetrix Human Gene 1.0 ST gene expression microarrays were performed following standard Affymetrix procedures (**http://www.affymetrix.com**). The microarray raw data are deposited into GEO (accession number GSE100718).

Gene set enrichment analysis

Analysis was performed using gene sets of hallmark from GSEA MSigDB Collections C2analysis (Molecular Signatures Database v3.0; **http://www.broadinstitute.org/gsea/msigdb/index.jsp**) comparing micro-array data from Thorax- and Tail-derived hT-ALL.

Measurement of Cell respiration and glycolysis

To obtain a homogeneous monolayer of cells, 1.2×10^5 cells per well were plated in XFp cell culture microplates (XFp FluxPak, Ref: 103022-100, Seahorse Bioscience) pre-coated with 22.4 µg/mL of Cell-Tak (ref: 354240, Corning®). OCR was measured in cells incubated in Seahorse XF base medium (ref: 102353-100 Seahorse Bioscience) supplemented with 2 mM glutamine (ThermoFisher Scientific) at 37 °C in a CO_2-free incubator for 1h equilibration before the measurement was done. An overnight-equilibrated calibration cartridge (Seahorse Bioscience) was loaded with 5.55 mM Glucose (port A), 1 µM oligomycin (port B) and 50 mM 2-DG (port C) all obtained from Seahorse XFp Glycolysis Stress test kit. ECAR was measured in cells incubated in Seahorse XF media supplemented with 5.55 mM Glucose (Sigma-Aldrich®), 2 mM Glutamine and 1 mM Pyruvate (ThermoFisher Scientific) at 37 °C in a CO_2-free incubator for 1h equilibration before the measurement was done. An overnight-equilibrated calibration cartridge (Seahorse Bioscience) was loaded with 1 µM oligomycin (port A), 2 µM FCCP (port B) and 0.5 µM Rotenone/antimycin (port C) all obtained from Seahorse XFp Cell Mito Stress test kit. In all experiments, the protein concentration was determined in each well at the end of the measurements, using the Qubit® Protein assay kit (ref: Q33211, ThermoFisher Scientific) after cell lysis in RIPA buffer (Sigma–Aldrich®).

Measurement of Glucose 6 Phosphate Deshydrogenase activity

Thorax- and Tail-derived cells were purified then lysed in water (500 µL/ 3.10^6 cells). G6PD activity was analysed by using G6PD assay kit (ab176722, Abcam)

Isolation of Gonadal Adipose Tissue-Derived Cells

Gonadal Adipose Tissue (GAT) was subjected to mechanical dissociation. Visible blood vessels, and ganglia were carefully dissected and removed. GAT fragments were digested at 37°C in phosphate-buffered saline (PBS) containing 0.2% bovine serum albumin (BSA) and 2 mg/ml collagenase B (Roche Diagnostics, Meylan, France) for 30 minutes. After elimination of undigested fragments by filtration through 70-µm filters, cells were submitted to centrifugation (320 G, 10 minutes). Cells obtained were resuspended in PBS then, used for flow cytometry analysis.

Statistical analysis

Graphics were designed using GraphPad Prism (GraphPad Software, Inc. La Jolla, CA, USA). Continuous variables were compared using the t-test or the Mann-Whitney test. When comparing thoracic and tail-derived cells from the same mouse, the paired t-test or the paired Mann-Whitney tests were used. *, **, ***, **** correspond to a p-value <0.05, 0.01, 10^{-3} or 10^{-4}, respectively. Statistics were performed using R Package (R Development Core Team (2006). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org). The leukemic initiating capacity in limiting dilution assays was evaluated using L-Calc™ software (Stem Cell™ Technologies, Canada).

Supplementary table 1: patients' characteristics

	Age	Gender	White blood cell count (G/L)	CNS involvement	Karyotype	Genomic abnormalities	EGIL classification
hT-ALL #1	6	F	743	Yes	t(11;14)(11p13;q11)	*SIL-TAL1* deletion *NOTCH1 mutated*	mature
hT-ALL #2	8	M	48	No	Normal	*NOTCH1 mutated*	cortical
hT-ALL #3	16	M	362	No	t(11;14)(p13;q11)	*NOTCH1* mutated	cortical
hT-ALL #4	13	F	7	No	NA	*NOTCH1* mutated	cortical
hT-ALL #5	1	M	707	Yes	t(6;7) (q23;q34)	*MYB* *NOTCH1* mutated	cortical
hT-ALL #6	2	M	230	No	del(6)(q22q26)	*NA*	Pre-T
hT-ALL #10	12	F	239	No	del (6) (q14;q24)	*NOTCH1* mutated	pre-T
hT-ALL #11	12	M	27	No	der(6)t(1;6)(p3?5;q2?7)	*FBXW7* mutated *TLX3*	cortical
hT-ALL #12	10	F	440	No	45, X, -X[25]/46, XX [5]	*SIL-TAL1* deletion	Pre-T
hT-ALL #14	15	M	NA	NA	NA	NA	pre-T

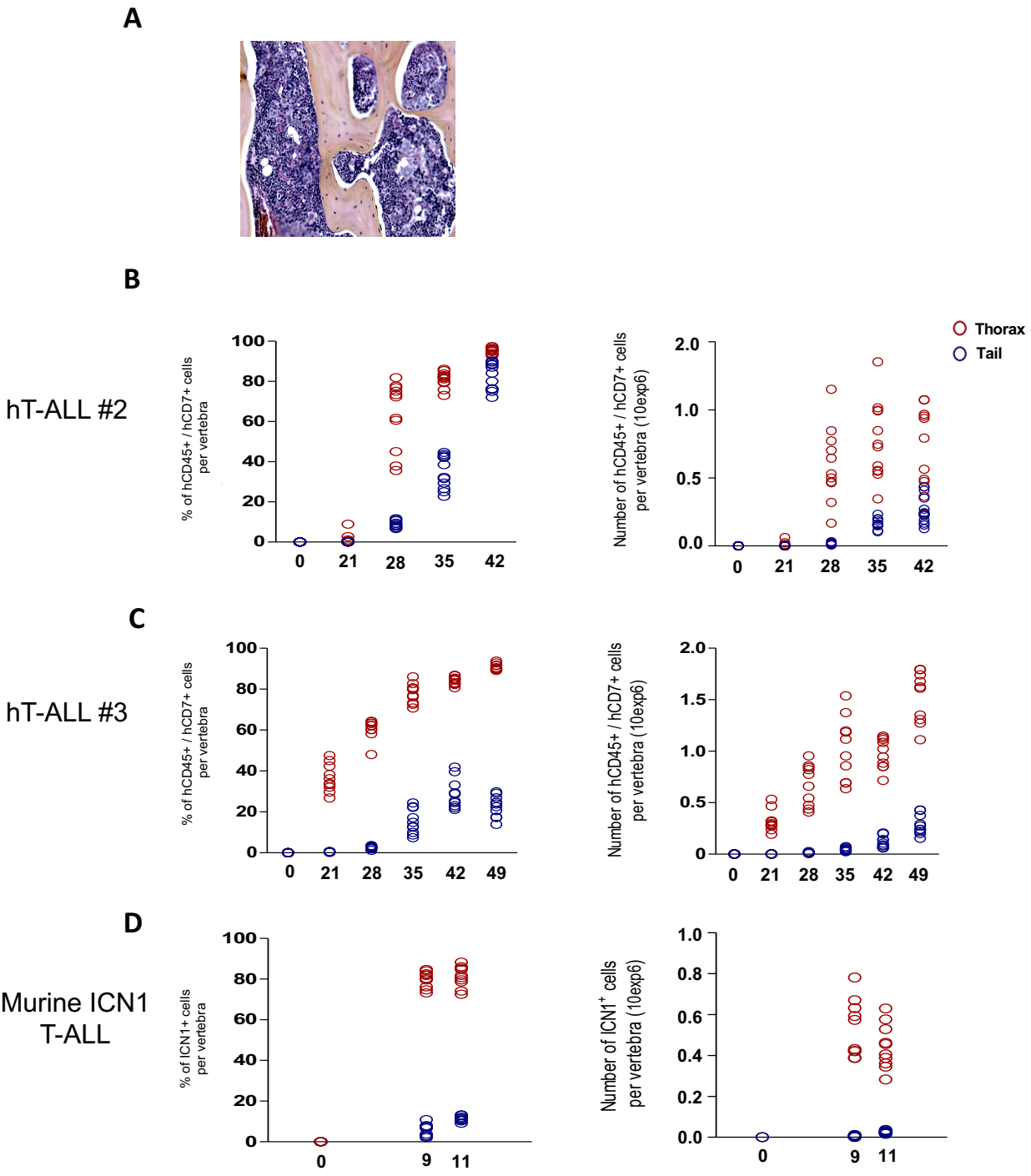
Supplementary table 2: *NOTCH1* and *FBXW7* primers

	Primers
NOTCH1	*Exon 26: NOTCH1 _X26_F1: AGTGGGCTGGAGGCACC* *NOTCH1 _X26_R1: CCCAGGAGAGTTGCGGG* *Exon 27: NOTCH1 _X27_F1: TGTCCTGCGGCAGCATC* *NOTCH1 _X27_R1: ACTGGCACAAACAGCCAGC* *Exon 28: NOTCH1 _X28_F1: CTGATGTCCGGGCACCTG* *NOTCH1 _X28_R1: GTGAGGATGCTCGGCCAG* *Exon 34: NOTCH1 _X34_F11: CATCGGGCACCTGAACGT* *NOTCH1 _X34_R11: GAACTGGGCTGCGGTCAC* *NOTCH1 _X34_F21: CAGTAGCCTTGCTGCCAGC* *NOTCH1 _X34_R21: CCTGGCATCCACAGAGCG*
FBXW7	*Exon 9: FBXW7_X09_F1: CCTTTCTACCCAAAAGTAATCATCTTAAG* *FBXW7_X09_R1: AGAGGAAGAAGTCCCAACCATG* *Exon 10: FBXW7_X10_F2: AACCTTGACTAAATCTACCATGTT* *FBXW7_X10_R2: CTGGATCAGCAATTTGACAGTG*

Supplementary table 3: CGH arrays of hT-ALL cells in Thoracic or Tail BM

	Thorax	Tail
hT-ALL #2*	Chromosome 9, p21.3 Homozygous deletion of CDKN2A and MTAP loci	Chromosome 9, p21.3 Homozygous deletion of CDKN2A and MTAP loci
hT-ALL #4*	Chromosome 9, p21.3 Homozygous deletion of the CDKN2A and CDKN2B locus	Chromosome 9, p21.3 Homozygous deletion of the CDKN2A and CDKN2B locus

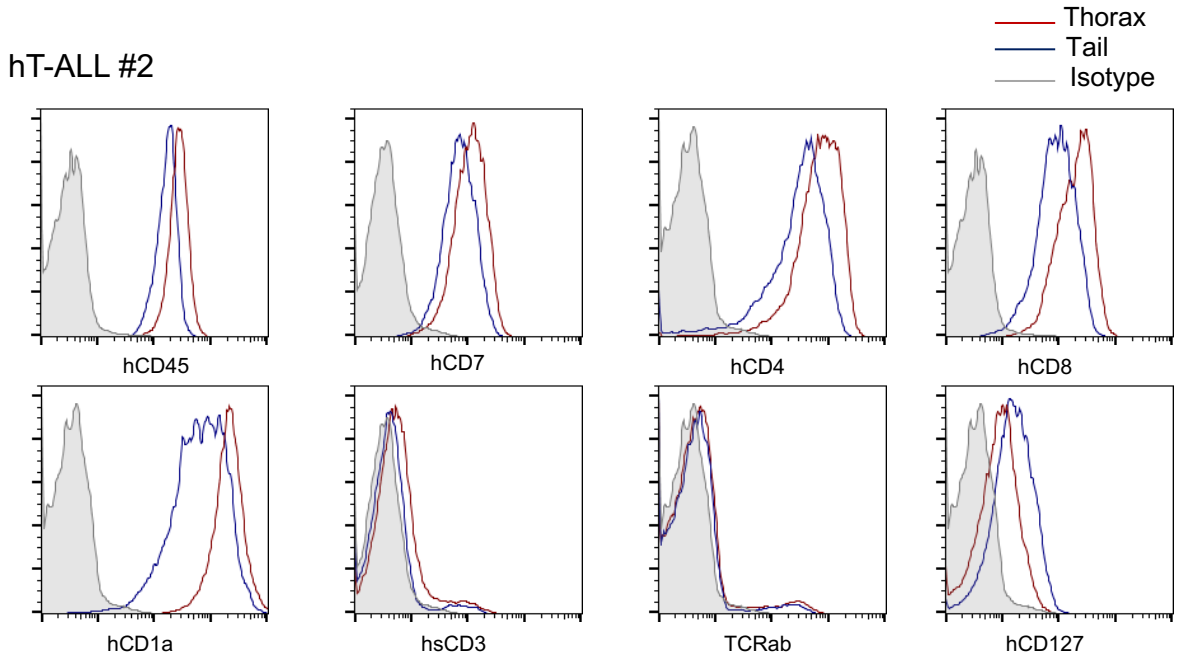
* 2 mice per hT-ALL sample.



Supplementary figure 1: Engraftment kinetics for hT-ALL #2, hT-ALL #3 in NSG mice and ICN1-murine T-ALL cells in C57BL/6 mice.

A. Shown is the femur section from an non manipulated NSG mouse colored with May-Grünwald Giemsa staining. NSG mice were thereafter injected with 10^6 diagnostic hT-ALL #2 (**B**), hT-ALL #3 (**C**) cells per mice. 10^5 murine CD45.2$^+$ ICN1 T-ALL cells were injected in CD45.1 C57BL/6 mice (**D**). At each time point, two mice were sacrificed until mice showed clinical signs of illness. 3 to 6 Thoracic or Tail vertebrae were isolated per mouse. The % of hCD45$^+$/hCD7$^+$ cells or CD45.2 ICN1 cells per vertebra was determined in Ter119$^-$ mononuclear cells (**B-D**, left graphs). Diagrams indicate mean numbers or % (+/- S.E.M.).

A



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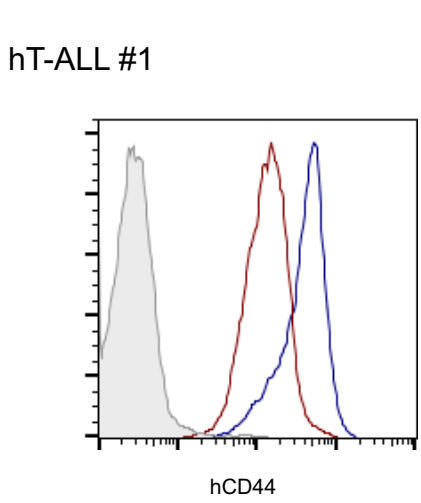
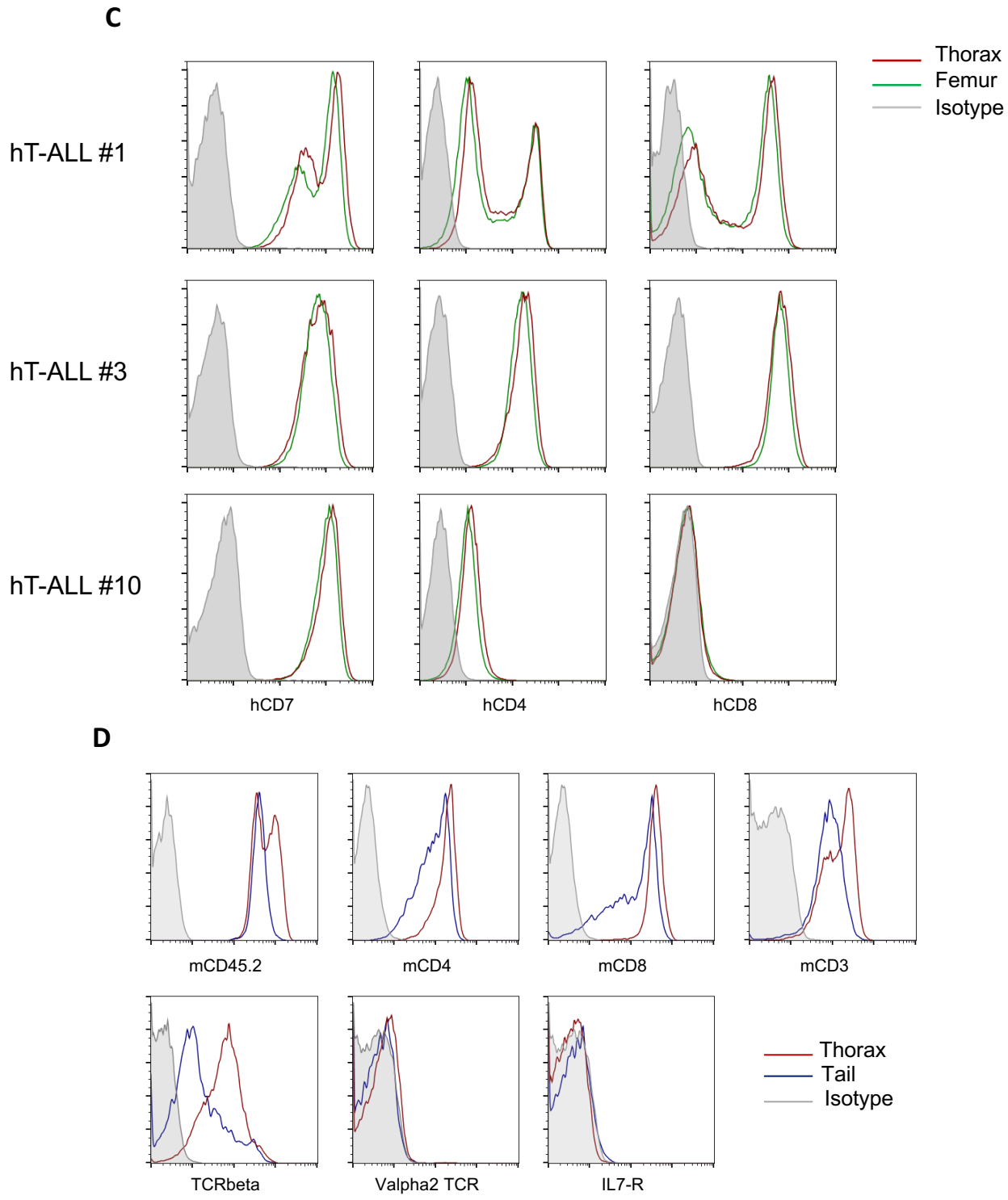
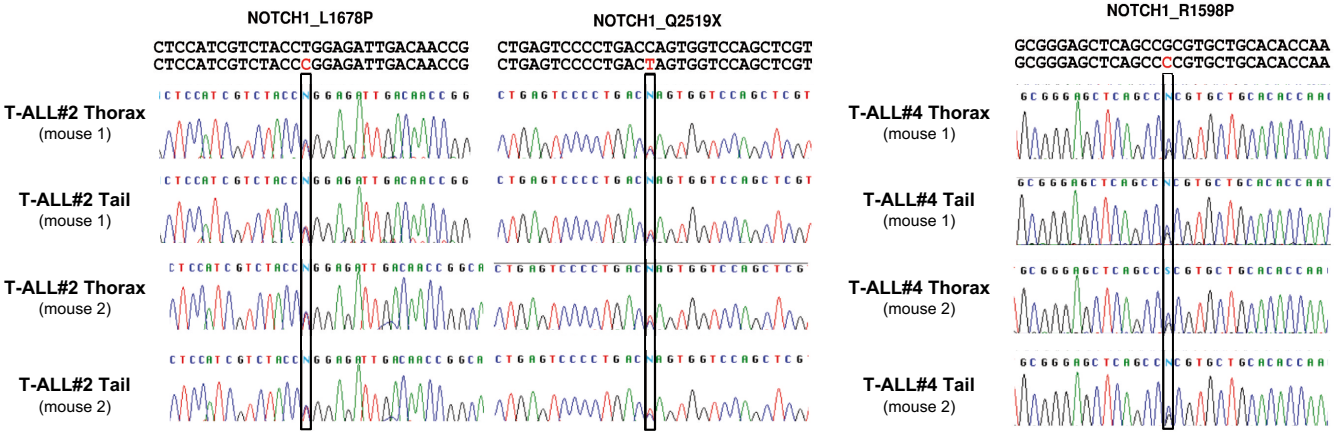


Supplementary figure 2: hT-ALL cell surface phenotype in thoracic or tail vertebrae BM.

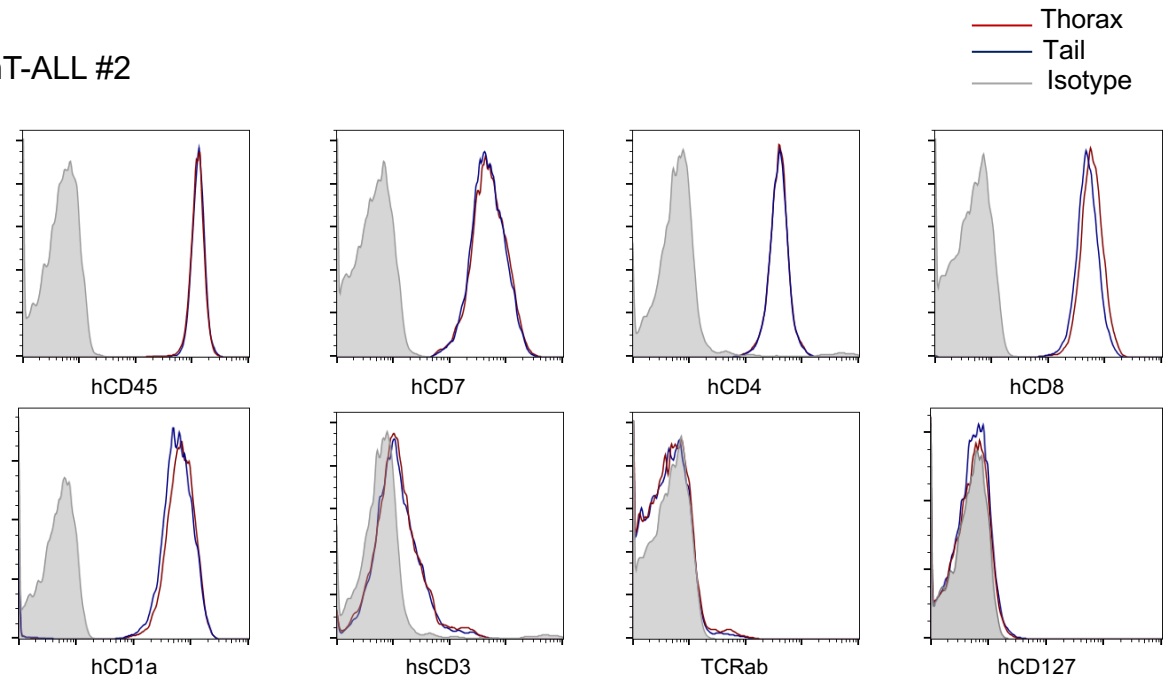


Supplementary figure 2: hT-ALL cell surface phenotype in thoracic or tail vertebrae BM. **A**. hT-ALL #2 cell surface phenotype (representative of #3, #4 and #5). Thoracic and tail histograms are in red and black, respectively (representative of 3 mice per hT-ALL). **B**. Cell surface expression of CD44 among thoracic or tail hT-ALL cells. Shown is hT-ALL #1. **C.** Cell surface phenotype of hT-ALL in thoracic vertebrae BM (red) and Femur (green). Isotype controls are colored in dashed yellow line. Representative of 3 mice per hT-ALL. **D.** Cell surface markers detected in ICN1 mouse T-ALL recovered from thoracic or tail vertebrae. Representative of 3 mice.

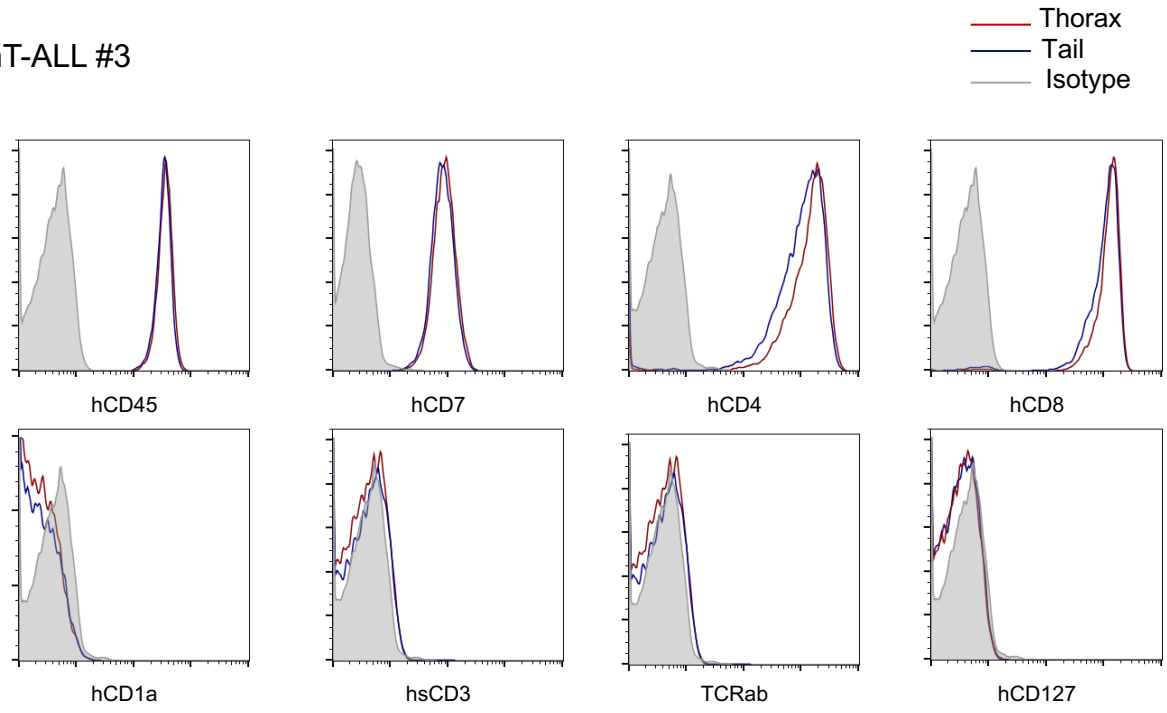


Supplementary figure 3: *NOTCH1* mutations in thoracic or tail BM hT-ALL #2 and #4. Genomic DNA from Thorax- or Tail-derived hT-ALL cells was isolated and a polymerase chain reaction was performed using specific primers (list provided in supplementary table 2) and Sanger sequencing was performed. The results show that sequences are identical between Thorax- and Tail-derived hT-ALL.

A

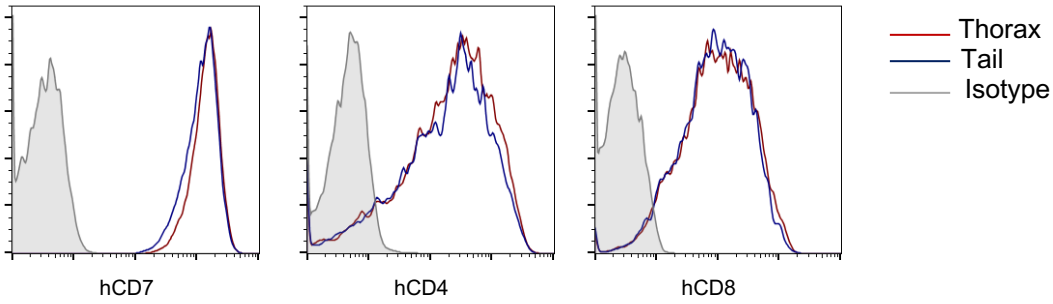

hT-ALL #2
Thorax
Tail
Isotype
hCD45
hCD7
hCD4
hCD8
hCD1a
hsCD3
TCRab
hCD127

B

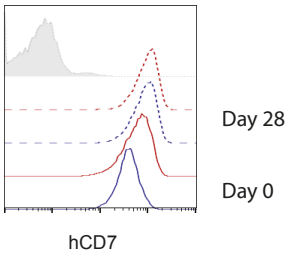

hT-ALL #3
Thorax
Tail
Isotype
hCD45
hCD7
hCD4
hCD8
hCD1a
hsCD3
TCRab
hCD127

C

hT-ALL #1

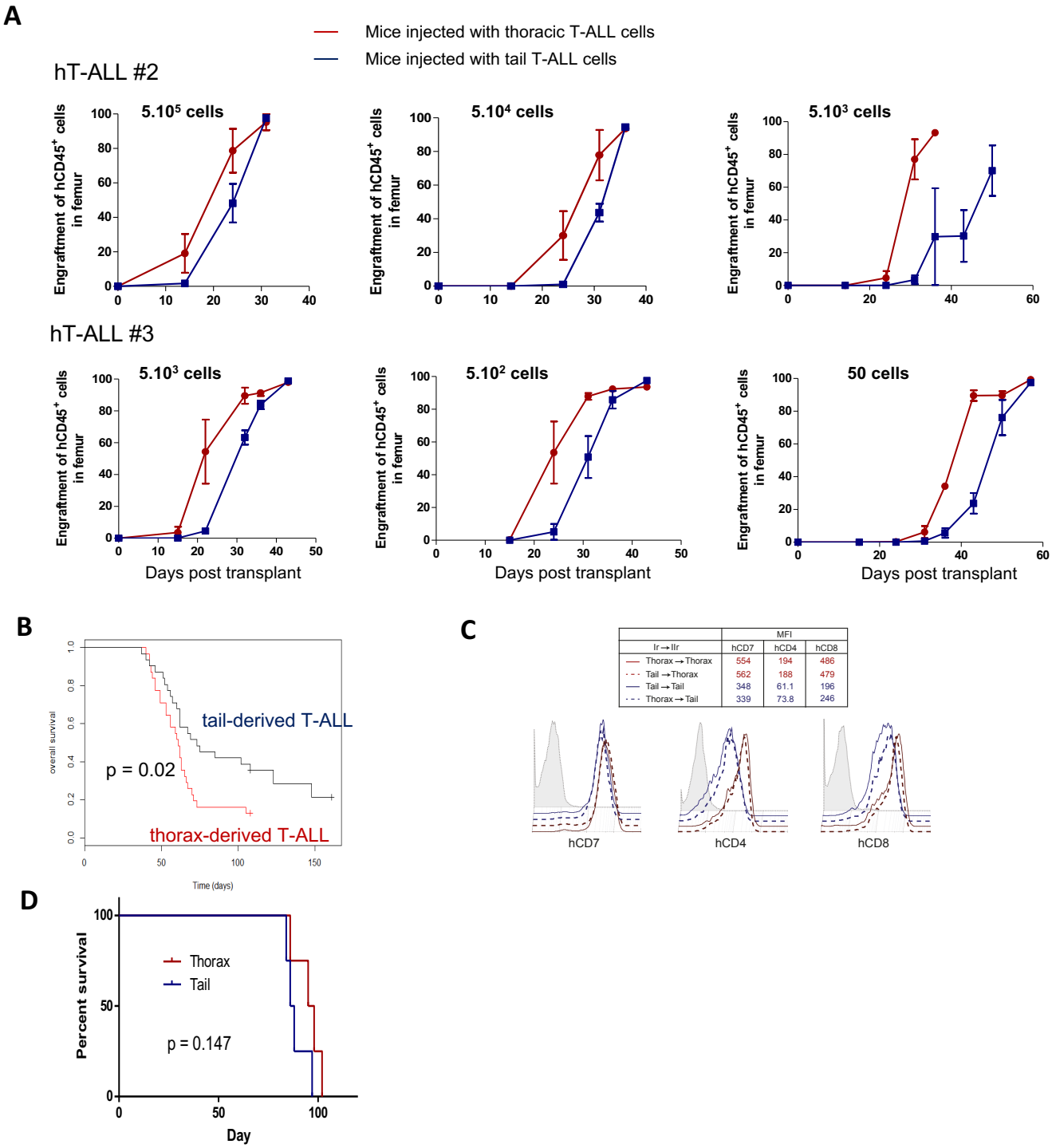


D



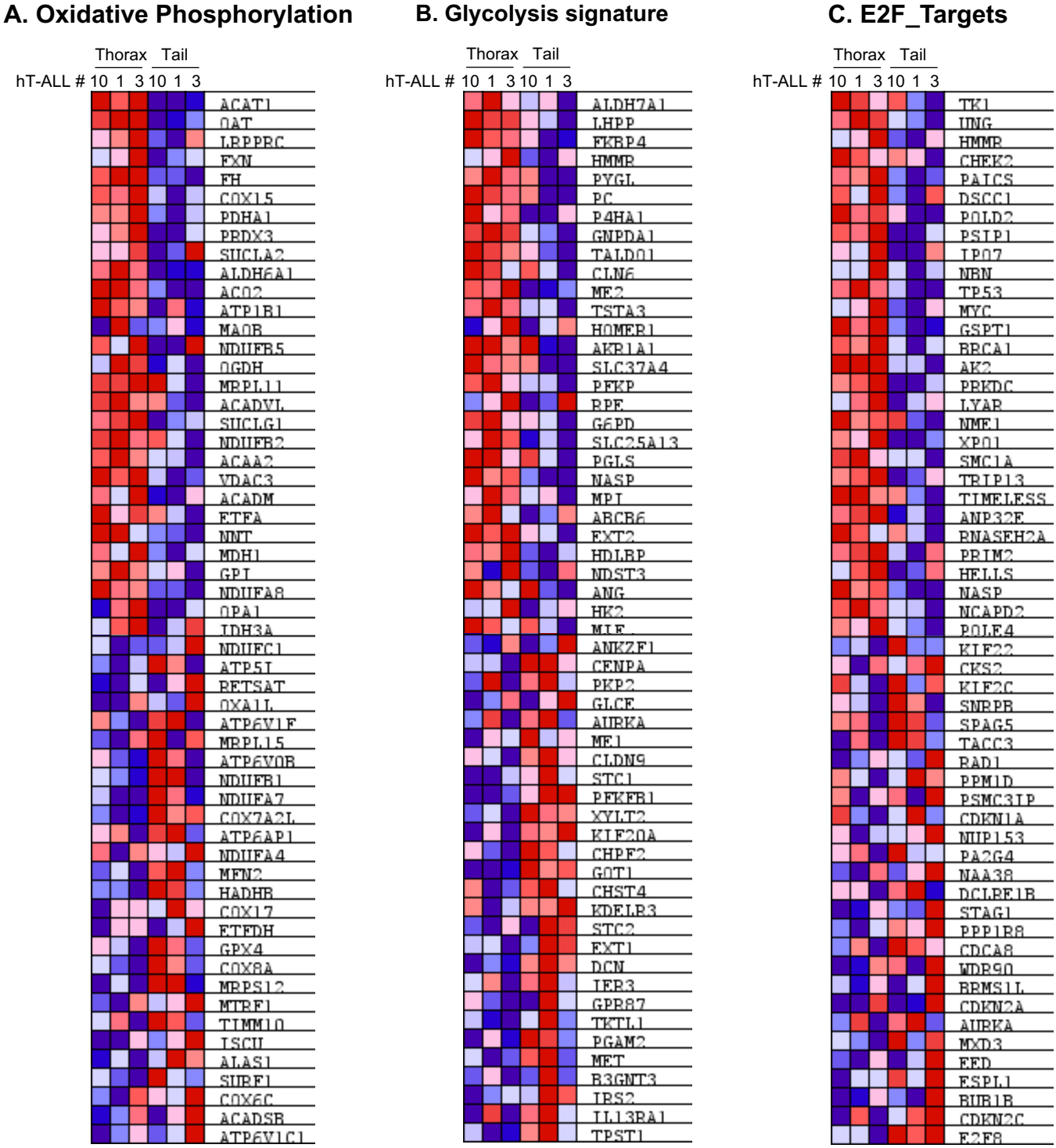
Supplementary figure 4: Cell surface phenotype of Thorax- and Tail-derived hT-ALL #2 and hT-ALL #3 after long-term coculture with MS5-DL1.

Cell surface phenotype of Thorax- or Tail-derived hT-ALL #2 (**A**) or hT-ALL #3 (**B**) after long-term coculture (28 days) of Thorax- or Tail-derived hT-ALL cells with MS5-DL1 stromal cells. Thoracic or tail histograms are in red and blue, respectively. Isotypes are colored in dashed yellow line. Each condition was performed in triplicate. **C-D**. Cell surface phenotype in Thorax- or Tail-derived BM after 12 (**C**) and 28 (**D**, dashed histograms) days of hT-ALL #1 in co-culture with MS5-DL1 stromal cells (performed in quadruplicate).

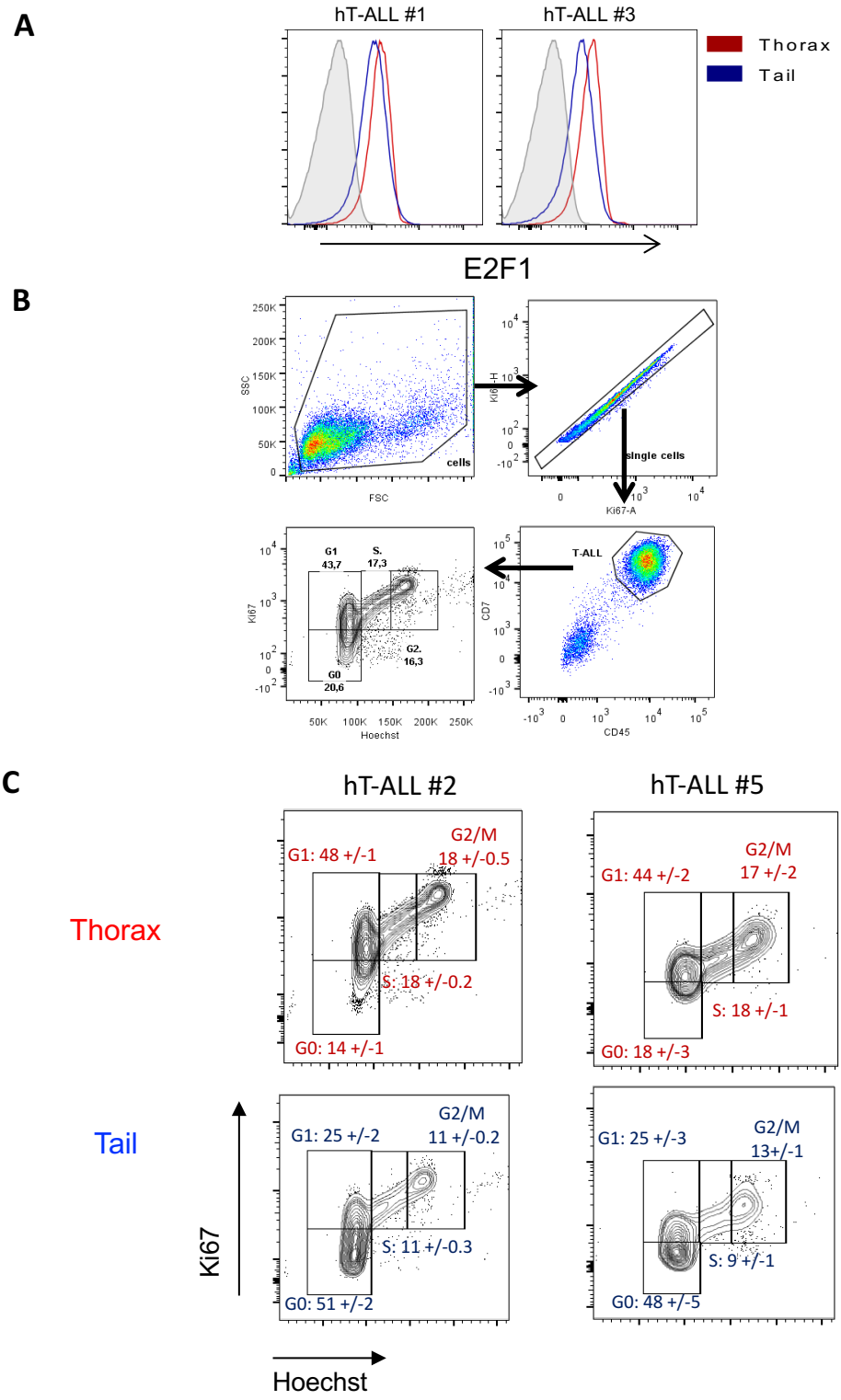


Supplementary figure 5: Secondary transplant of Thorax- or Tail-derived hT-ALL cells.

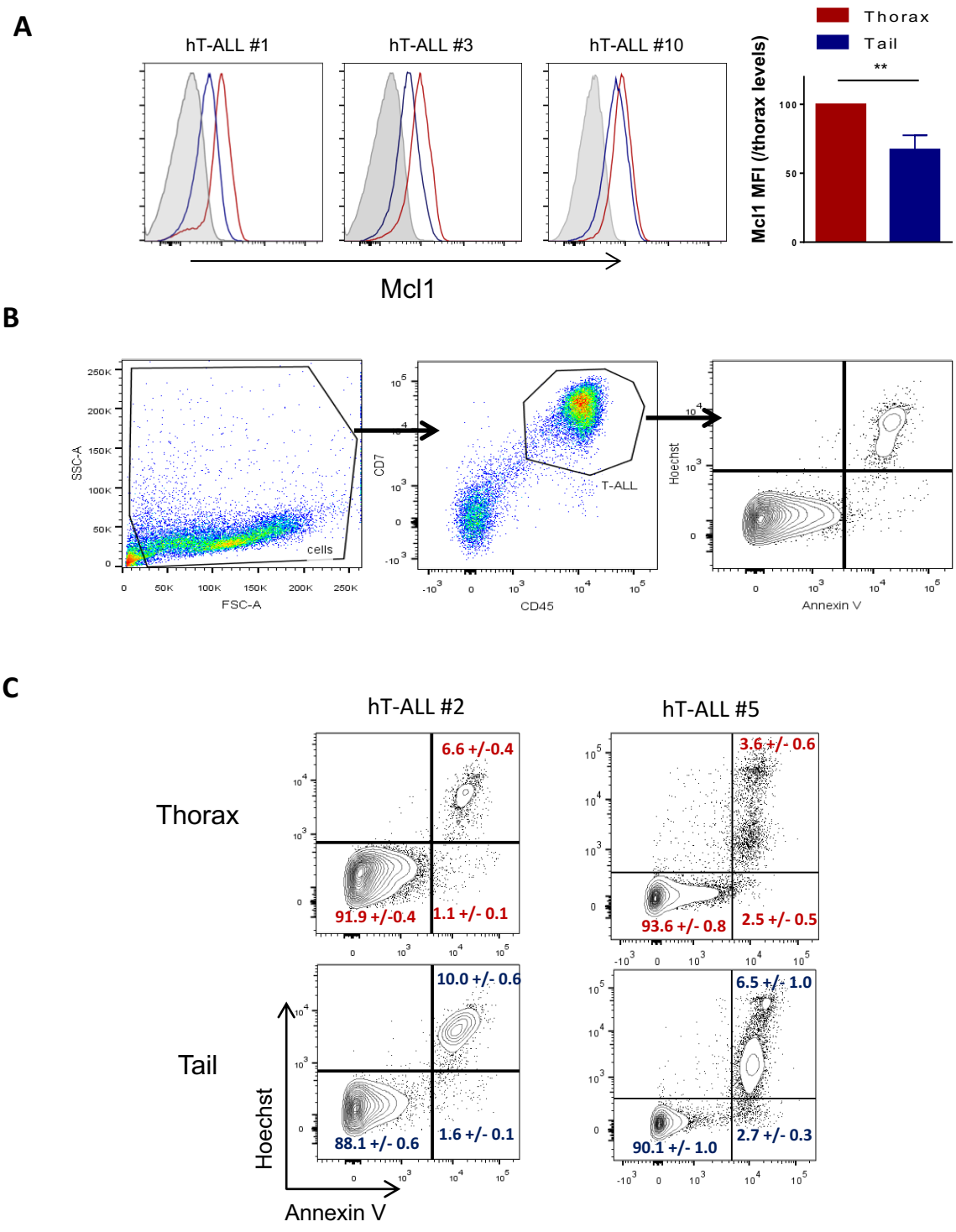
A-C. hT-ALL were isolated and purified from thoracic or tail vertebrae BM and injected intravenously into secondary NSG mice. **A**. Engraftment was monitored after sampling BM cells from Femurs. 3 mice per niche (thoracic or tail) were injected. Mean data (+/- S.E.M.) are indicated. **B.** Overall survival in secondary NSG mice (31 mice in each group; 62 mice in total). The p-value corresponds to the Log-rank test. **C.** Human CD7, CD4 and CD8 surface expression in a representative mouse after secondary transplant of Thorax- or Tail-derived hT-ALL. **D.** Thorax- and Tail-derived hT-ALL #1 cells were grown in co-culture with MS5-DL1 cells during 12 days before transplantation into secondary mice (500 cells/mouse, 3 mice/niche). Survival of mice is indicated. Representative of two experiments. Mean data (+/- S.E.M.) are indicated.



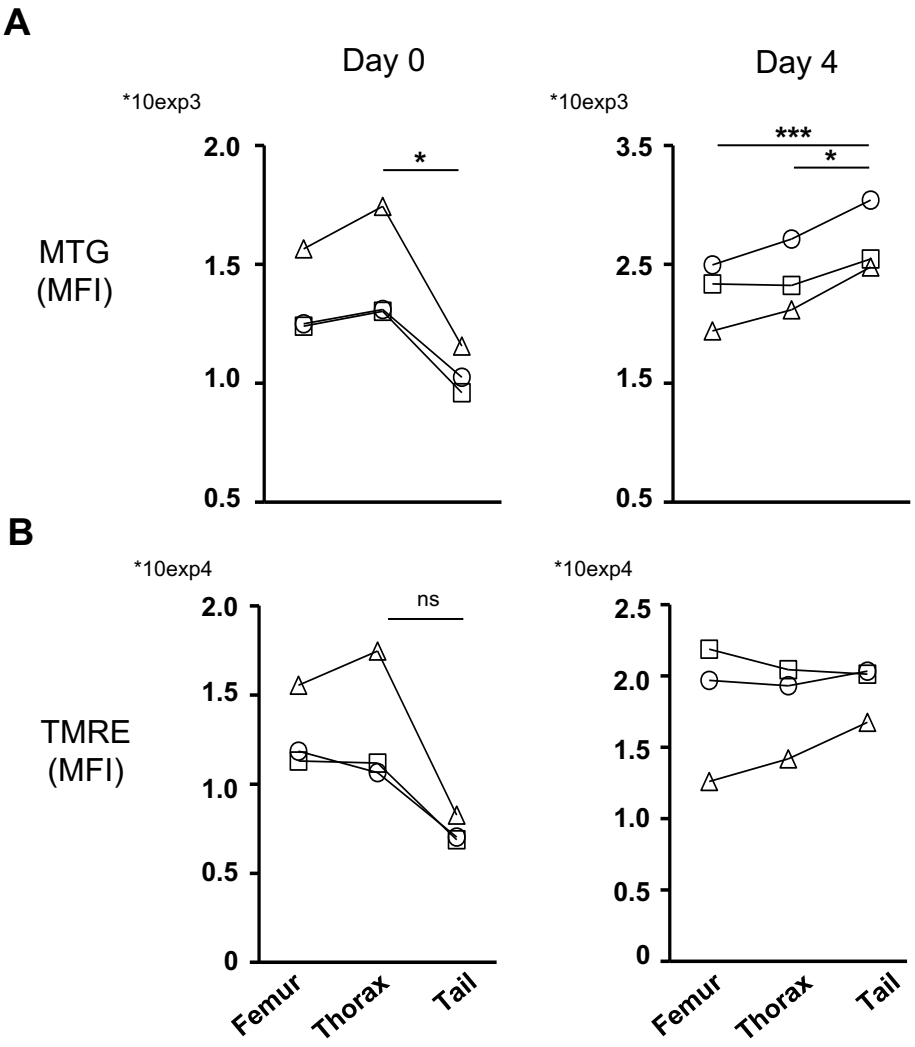
Supplementary figure 6: List of genes inversely expressed in Thorax- and Tail-derived hT-ALL. Data from 3 hT-ALL (#1, #3, #10) recovered from NSG mouse BM sites. Shown are comparative levels of 56 (29(+) and 27(-)) genes.



Supplementary figure 7: hT-ALL cell cycle analysis in thoracic and tail bone marrow vertebrae. NSG mice injected with hT-ALL #2 and #5 were sacrificed at late time points - determined by iterative bone marrow samplings showing leukemic infiltration > 90% - to ensure that hT-ALL cells had engrafted into Tail vertebrae. Cells were fixed, permeabilized and further stained with anti-E2F1 antibody, or anti-Ki67 antibody and Hoechst. **A**. Levels of E2F1 in hT-ALL from thoracic (red) and from tail (blue) vertebrae. Of note P21 was equally expressed in hT-ALL from both sites. Unstained controls are shown as shadowed histograms. **B.** Gating strategy for the cell cycle measurement. **C.** Cell cycle plots for hT-ALL #2 and #5. These results are representative of 6 T-ALL samples (136 vertebrae from 20 mice).

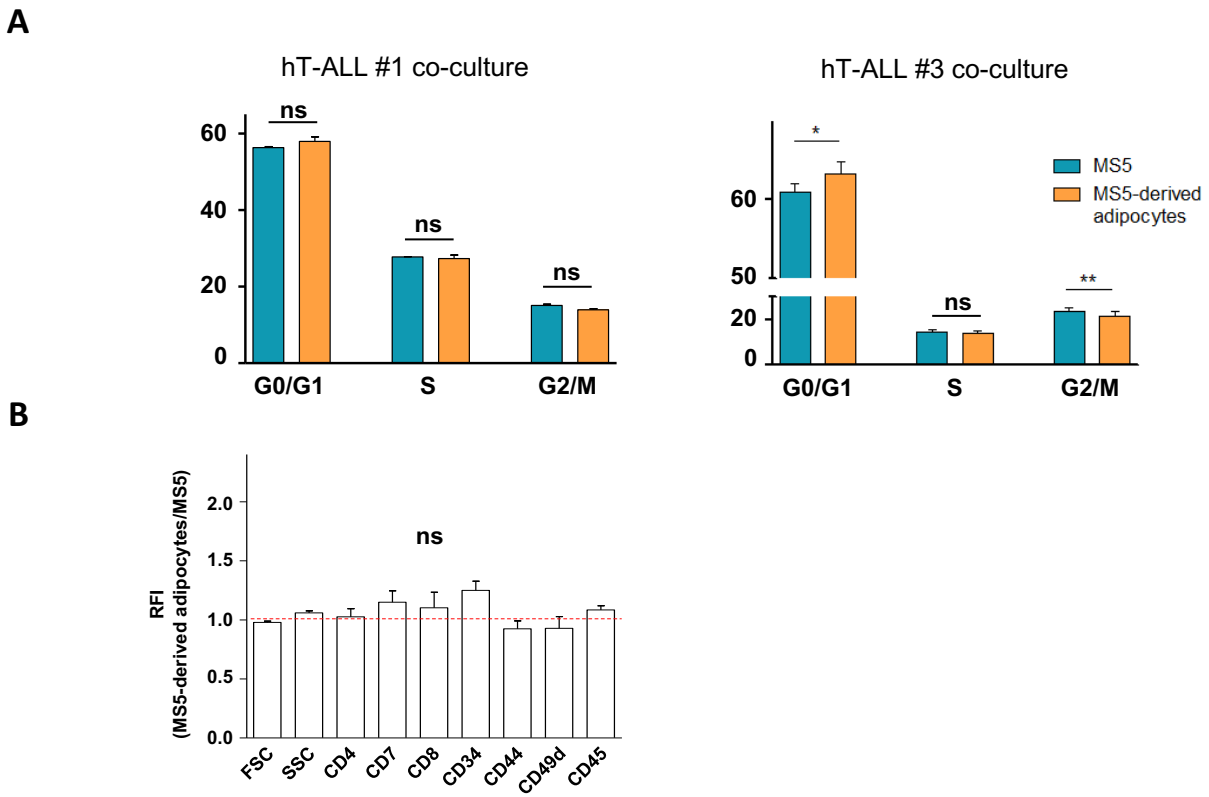


Supplementary figure 8: hT-ALL apoptosis in thoracic and tail vertebrae BM.
A. MCL1 expression in hT-ALL from thoracic (red) and from tail (blue) vertebrae. Of note BCL2 and Bax expression were similar in hT-ALL from both sites. Unstained controls are shown as shadowed histograms. **B.** Gating strategy. **C.** Apoptosis plots for hT-ALL #2 and #5. Representative of a total of 5 studied hT-ALL samples (>100 vertebrae from a total of 16 analysed mice).



Supplementary figure 9: Rescue of the mitochondrial mass and membrane potential during *ex vivo* permissive cultures.

NSG mice (n=3) injected with hT-ALL #1 were sacrificed at late time points - determined by iterative bone marrow samplings showing leukemic infiltration > 90% - to ensure that hT-ALL cells had engrafted into tail vertebrae. Cells were fixed, permeabilized and further stained with **A**. MTG and **B.** TMRE. Flow cytometry analyses show that Tail-derived hT-ALL exhibit a decreased MTG and TMRE labelling, indicative of mitochondrial loss compared to Femur or Thorax-derived hT-ALL, after being isolated from their BM niche (day 0). These features were lost after 4 days in co-cultures with MS5-DL1 stromal cells. Statistics are calculated according to non-parametric Friedman test (*, $p<0.05$; ***, $p<0.001$).



Supplementary figure 10: Adipocytes influence hT-ALL characteristics

A. Cell cycle of hT-ALL 6 days (#1; 3) co-cultures with MS5 and MS5-derived adipocytes. **B.** Cell surface expression of a panel of antigens. The results are Ratio of Fluorescent Intensities (RFI) calculated by dividing the MFI of every marker on cells recovered from co-cultures wit MS5-derived adipocytes over the co-cultures with MS5 cells. The results are Mean +/- S.E.M. from 3 hT-ALL tested in triplicate during 7 days co-cultures.