SUPPLEMENTAL MATERIAL

Chemical screen of primary human acute myeloid leukemia (AML) specimens.

Compound responses were determined for 20 primary AML specimens with diverse cytogenetic and molecular characteristics (Supplemental Table 1). AML cells were cultured in IMDM supplemented with 15% BIT (bovine serum albumin, insulin, transferrin; Stem Cell Technologies 09500), 100 ng/ml SCF (Shenandoah 100-04), 50 ng/ml FLT3L (Shenandoah 100-21), 20 ng/ml Il-3 (Shenandoah 100-80), 20 ng/ml G-CSF (Shenandoah 100-72), 10^{-4} M β-mercaptoethanol, Gentamicin (50 μg/ml), Ciprofloxacin (10 μg/ml), 500 nM StemRegenin (SR1, Chemie Tek 1227633-49-9), and 500 nM UM729 (Medicinal Chemistry Facility, IRIC). Cells were plated in 384-well plates (Greiner, 781091) at a density of 5000 cells in 50 μL/well. Compounds were tested at 2.5 μM. In addition to test compounds each plate comprised positive controls Daunorubicin (Tocris Bioscience 1467) and Cytarabine (Tocris Bioscience 4520), both at 50 nM and 1μM, and 0.1% DMSO (vehicle) as a negative control. After 6-day culture, cells were incubated with 10 nM Calcein AM (eBioscience 65-0853-39) for 45 min, and viable cells were enumerated using an automated microscopy system (Operetta®) High Content Imaging System, PerkinElmer). Growth inhibition was calculated as 1 - (cell count (compound) / cell count (DMSO)), where cell count (DMSO) corresponded to the average determined for 32 control wells.

Co-culture conditions

Briefly, pre-LSCs and leukemic cells were co-cultured on MS5-DL4 stromal cells in complete T-cell medium containing reconstituting α-MEM medium (12561, Gibco, Life Technologies, Burlington, Ontario, Canada) supplemented with 10% FBS (12318, Gibco), HEPES 10 mM

(15630-080, Gibco), sodium pyruvate 1 mM (11360-070, Gibco), β-mercaptoethanol 55 µM (21985-023, Gibco), glutamax 2 mM (15750-060, Gibco), penicillin/Streptomycin (15140-122, Gibco), 5 ng/mL FLT-3 Ligand (308-FK, R&D system) and 5 ng/mL IL-7 (407-ML, R&D system).

Apoptotic assay

Pre-LSCs from *SCL*^{tg}*LMO1*^{tg} mice or T-ALL cell lines were fixed and permeabilized (CytofixCytoperm Plus, BD Bioscience) during 30 minutes before the staining with Annexin V-FITC (BD Bioscience), in accordance with the manufacturer's instructions. Apoptosis were finally analysed by FACS.

Immunofluorescence and microscopy

JURKAT cells were washed twice in PBS and fixed with paraformaldehyde (PFA) 4% diluted in PBS 15 minutes at room temperature (RT). Cells were then washed twice in PBS and permeabilized 30 minutes with ice-cold Ethanol 66%. After 2 washes in PBS, cells were blocked for 1 h in PBS, 5% BSA, 0.5% triton and then labeled over night with primary antibodies. After 2 washes in PBS, cells were labeled for 1 h with solution containing the secondary antibodies conjugated with Cy3 or Alexa488. Following PBS washes and DAPI counterstaining, cells were mounted with Vectashield (Vector Laboratories, Burlingame, CA, USA) and analyzed by confocal microscopy. Images were analyzed using Zeiss LSM Image Examiner 2.50 (Carl Zeiss, Jena, Germany) and Adobe Phoposhop 5.0 (Adobe Systems Incorporated, San Jose, CA, USA) softwares. The corrected total cell fluorescence (CTCF) per nucleus of SCL (n=27-98) or histone

H3 (n=42-60) was calculated using Image J software according to the manufacturer instructions (http://sciencetechblog.com).

Western-blot

Cell nuclear extracts were prepared as previously described (1). The concentrations of protein extracts were determined by Bradford staining (Bio-Rad Laboratories, Hercules, CA, USA). Proteins were then separated on sodium dodecyl sulfate–PAGE (SDS-PAGE) and transferred on poly(vinylidene difluoride) (PVDF) membranes (Millipore Corporation, Billerica, MA, USA). The following immunodetection was revealed by means of ECL Plus (Amersham Biosciences, Piscataway, NJ, USA). The signal was quantified with Multi-Gauge (Fujifilm, Tokyo, Japan), Excel (Microsoft, Redmond, WA, USA) and Graph Pad Prism 4 (San Diego, CA, USA) softwares.

Staining and antibodies

For the FACS analysis, all antibodies were obtained from Pharmingen (BD Biosciences, Mississauga, Ontario, Canada). The PE-labelled anti-c-Kit (2B8), PE-labelled anti-Thy1.2 (30-H12), Pe-Cy5-labelled anti-CD4 (RM4-4), APC-labelled anti-CD8 (53-6.7), Pe-Cy7-labelled anti-CD25 (PC61.5), AF780-labelled anti-CD44 (IM7) were diluted at 1/500 and PerCPCy5.5-labelled anti-CD45.2 (104) and FITC-labelled anti-CD45.1 (A20) at 1/100 in staining buffer (PBS + 2% FBS) 15 minutes at 4°C. The antibodies anti-human FITC-labelled anti-hCD45 (HI30), PE-labelled anti-hCD7 (M-T701), PeCy7-labelled anti-hCD4 (RPA-T4), PeCy5-labelled anti-hCD8 (RPA-T8) and APC-labelled anti-hCD34 (581) were diluted at 1/200. For western-blot, the membranes were blocked and the antibodies were diluted in 5% dried milk in TBST (10 mM

Tris-HCl pH 8.0, 150 mM NaCl, 0.05% Tween-20). For the immunofluorescence assays, antibodies were diluted in blocking buffer (PBS, 5% BSA, 0.5% triton). The mouse monoclonal antibody SCL (BTL73) was generously provided by Dr D. Mathieu (Institut de Génétique Moléculaire, Montpellier, France). The rabbit polyclonal H3 antibody (abcam, ab1791) was diluted 1/200 for immunofluorescence. SCL (BTL73) was diluted 1/500 for western-blot, 1/100 for immunofluorescence. The c-MYC (9E10) sc-40 mouse monoclonal antibody, the RNA Pol II (N-20) sc-899 rabbit polyclonal antibody, the HoxA9 (HOX5I043) sc-81291 mouse monoclonal antibody and the E2A (YAE) sc-416 mouse monoclonal antibody were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA) and the HoxB4 antibody was obtained from the Developmental Studies Hybridoma Bank. The α-Tubulin (T5168) monoclonal mouse antibody, the α-Actin (A5044) monoclonal antibody and the Lamin A (L1293) polyclonal rabbit antibody were obtained from Sigma (Milwaukee, WI, USA). The S6 (5G10) and phosphor-S6 (S240/244) rabbit monoclonal antibodies were obtained form Cell Signaling Technology. For western-blot, α-Actin, α-Tubulin and Lamin A antibodies were diluted at 1/5000, HoxA9 antibody at 1/2000, E2A, RNA Pol II, S6 and phosphor-S6 antibodies at 1/1000, c-MYC antibody at 1/500 and HoxB4 antibody at 1/200. The secondary antibodies goat against mouse IgG(H+L)-HRP conjugate (170-6516) and goat against rabbit IgG(H+L)-HRP conjugate (170-6515) from Bio-Rad Laboratories (Hercules, CA, USA) were diluted at a concentration of 1/5000. As for the immunofluorescence assays, the secondary Cy3-conjugated goat anti-mouse antibody (Jackson ImmunoResearch Laboratories, West Grove, PA, USA) and the secondary Alexa488-conjugated goat anti-rabbit antibody (Invitrogen) were diluted 1/200 and 1/500 respectively. For the intracellular detection of SCL and MYC by flow cytometry, cells were fixed and permeabilized using TF Fix/Perm Buffer (BD Pharmingen, 51-9008100) for 30 minutes and washed twice in TF

Perm/Wash Buffer (BD Pharmingen, 51-9008102). Cells were then labeled with SCL (BTL73) and MYC (9E10) antibodies (dilution 1/500) during 45 minutes, washed extensively with TF Perm/Wash Buffer followed by the incubation of a secondary goat anti-mouse antibody coupled to FITC.

RT-qPCR

Total RNAs were prepared with RNeasy extraction kit (Qiagen, Mississauga, Ontario, Canada). First strand cDNA syntheses were performed by reverse transcription as described (2). The sequences of the primers used are listed in Supplemental Table 4. Real-time quantitative PCR was done with SYBR Green Master Mix (Applied Biosystems, Foster City, California, United States) on Stratagene Mx3000 apparatus (Stratagene, La Jolla, California, United States) or using PERFECTA QPCR FASTMIX II (Quanta) and detected with the Viia qPCR instrument (Life Technologies). DDCt values were calculated using Ct values from *β-actin* gene as reference.

Luciferase assays

The *Gypa-84* promoter (1) (1500 ng) were transfected in NIH 3T3 cells with expression vectors for E47 (150 ng), LMO2 (750 ng), Ldb-1 (750 ng) and GATA-1 (150 ng), and the indicated amounts of SCL vectors (150 ng). Calcium phosphate was used to transfect NIH 3T3 cells 24 h after plating at 30,000 cells per milliliter. The amount of reporter was kept at 1.5 μg per well, and 100ng cytomegalovirus-βgal (CMV-βgal) was added as an internal control. Total DNA was kept constant at 4.5 μg per well with pGem4. The medium was changed 24h later and cells were treated or not (vehicle) with 2-ME2 (1μM) or DEXA (2nM). Luciferase and βgal activities were determined 24h after treatment. For all transfections, results are shown as the mean ± SD of

triplicate determinations of 2 independent experiments. Luciferase reporter activities were normalized to that of an internal control for transfection efficiency (CMV-βgal).

RNA-seq of murine leukemias

Poly-A mRNA libraries obtained from *Cd3*$^{-/-}$*SCL*^{tg}*LMO1*^{tg} leukemias (n=4) and *WT* thymocytes (n=2) were sequenced in the Illumina HiSeq2000. Paired-end 100 bp reads were aligned to the mouse genome mm10 using Tophat2/Bowtie2 v2.0.7 (3), and differentially expressed genes were determined using cuffdiff v.2.1.1.

Real-time imaging

2 x 10^5 DN3 thymocytes (pre-LSCs) from *Gfp*^{tg}*SCL*^{tg}*LMO1*^{tg} and 2 x 10^5 *SCL*^{tg}*LMO1*^{tg} leukemic cells were mixed and plated on 2.5 x 10^4 non-GFP OP9-DL1 stromal cells in 8-well microscopy slides (Ibidi) with CO_2-Independent Medium (Gibco) supplemented with 5ng/mL FLT-3 Ligand (308-FK, R&D system) and 5ng/mL IL-7 (407-ML, R&D system). Live-cell time-lapse images were acquired at 37°C, with 5% CO_2 on a DeltaVision Personal microscopy system with SoftWorx acquisition software (Applied Precision), equipped with a CoolSnap HQ2 camera (Photometrics) at 1 x 1 binning, and a 60X planApo objective (Olympus). DIC and fluorescent images were acquired on multiple adjacent fields of view at 5 minute intervals for 18 to 30 hours. Images were processed using imageJ software (NIH), and analysed manually.

HeLa cells transfected with SCL-GFP and E2A constructs were re-seeded onto an 8-well fluorescence microscopy microslide and were allowed to adhere overnight. Immediately prior to imaging (approximately 52 hours post-transfection), cells were treated with 2-ME2 or vehicle (DMSO). Time lapse images of treated HeLa cells were recorded every 30 minutes for over 14

hours. Acquired images were deconvoluted using the Softworx software and processed using MetaMorph. Average fluorescence intensity of SCL-GFP in the nucleus was measured from three representative regions for each time point before mitotic entry (if the cell entered mitosis during image acquisition). Due to individual variations in GFP expression, all measured intensities were normalized to the initial intensity of the corresponding cell which was set as 100%, and plotted against time. Data represent the average of 6 and 8 independent 2-ME2-treated and untreated cells, respectively in Figure 4D and normalized MFIs for each independent cell are shown in Supplemental Figure 6A.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1. Immunophenotypes and invasive behavior of leukemic cells. (**A**) Immunophenotypes of three T-ALL form *Cd3ε*$^{-/-}$*SCL*^{tg}*LMO1*^{tg} thymoma ranked according to the absolute number of blasts found in their thymoma (tumor load). (**B**) Purified pre-leukemic *Cd3ε*$^{-/-}$ DN3 (pre-LSCs) thymocytes and fully transformed leukemic blasts were transplanted as described in Figure 1E. The percentage of donor cells in Thy1.2^{+} populations was analysed in the spleen (*upper panel*) and in the bone marrow (BM, *lower panel*) of recipient mice 3 and 6 weeks later by FACS. Each dot represents one individual engrafted mouse and horizontal lines, the median of thymic engraftment of each experimental group. (**C**) Fully transformed leukemic cells (GFP^{-}) and SCL-LMO1 pre-LSCs (GFP^{+}) were mixed and cultured as described in Figure 1F and real-time cell imaging was performed by videomicroscopy as described in Figure 1G. Representative example of the time-lapse imaging of GFP^{+} and GFP^{-} cell division.

Supplemental Figure 2. Schematic of the screen configuration. Each plate contained sixteen wells with DN3 *SCL*^{tg}*LMO1*^{tg} thymocytes treated with 1μM of γ-secretase inhibitor (GSI) and sixteen wells treated with DMSO (vehicle) as internal positive and negative controls, respectively.

Supplemental Figure 3. Heatmap of primary AML blasts treated with hits from the screen on pre-LSCs. Effect of compounds of the three major groups of pre-LSC inhibitors on the ex vivo survival and proliferation of primary human acute myeloid leukemic cells. Numbers represent the fractions of non-viable cells compared to Vehicle (DMSO) control.

Supplemental Figure 4. Estrogen-like compounds with high-affinity for estrogen receptors do not inhibit efficiently pre-LSCs viability and 2-ME2 does not affect normal T cell differentiation in vitro. (**A**) Pre-LSCs from *SCL*^{tg}*LMO1*^{tg} thymocytes were co-cultured on MS5-DL4 stromal cells with a dose-response of Estradiol, Estrone and Nomegestrol for 5 days. The percentages of cell viability are shown and the IC50 was calculated for each condition (mean ± SE, n=6). (**B**) DN3 WT thymocytes were treated with a dose-response of 2-ME2 and the ratio of donor-derived DP to DN3 was calculated for each dose (n=6). Representative of two independent experiments.

Supplemental Figure 5. Limiting dilution analysis of pre-LSCs treated with 2-ME2 or DEXA. (**A-B**) Schematic of the functional assay (A). Pre-LSCs were exposed to 1μM 2-ME2 or 5nM DEXA (75% inhibition). Treated and untreated viable cells were transplanted by limiting dilution assay. Engraftment was monitor by the presence of donor-derived (CD45.2^{+}) thymocytes in the thymus of recipient mice and mice were scored positive when T-cell lineage reconstitution was more than 0.1% in the thymus and pre-LSCs frequency (± Confidence Interval) was calculated and shown in Figure 3G. Illustrated are the numbers of positive mice over the total numbers for each injected cell dose (B).

Supplemental Figure 6. 2-ME2 inhibits nuclear accumulation of SCL. (**A**) HeLa cells viability after 14h of treatment with 2-ME2 (1μM). (**B-C**) HeLa cells expressing SCL-GFP (B) or H2B-RFP (C) were filmed by video-microscopy in presence or not (Vehicle) of 2-ME2 (1μM) as described in Figure 4, D and E. Data represent the normalized MFIs of 5 to 8 independent 2-ME2-treated and untreated cells.

Supplemental Figure 7. The cochicine does not affect SCL protein levels. (**A**) JURKAT human T-ALL cell line was treated with a dose-response of Colchicine during 48h and the percentages of cell viability and the IC50 were then calculated (mean ± SE, n=6). (**B**) JURKAT cells were treated with an IC50 (2nM) of Colchicine for the indicated times. Nuclear extracts were subjected to immunoblotting. Representative of two independent experiments.

Supplemental Figure 8. 2-ME2 induces apoptosis of human cortico-resistant T-ALL cell lines without modifying their cortico-sensitivities. (**A**) 4 human T-ALL cell lines (JURKAT, KOPT1, P12-ICHIKAWA and HPB-ALL) were treated with a dose-response of DEXA (*left panel*) and 2-ME2 (*right panel*) for 48h. The proportion of apoptotic cells was then assessed by FACS (mean ± SE, n=3, *p≤0.05). (**B**) Representative histogram FACS profile of Annexin V staining in JURKAT cells after the treatment with a dose response (0-3 μM) of 2-ME2 during 48h. (**C**) The cortico-resistant (JURKAT) and cortico-sensitive (P12-ICHIKAWA) human T-ALL cell lines were treated with a dose-response of DEXA with or without (Vehicle) an IC20 dose of 2ME-2 during 48h. The percentages of cell viability were then calculated (mean ± SE, n=6). (**D**) Pre-LSCs from *SCL*^{tg}*LMO1*^{tg} thymocytes were co-cultured on MS5-DL4 stromal cells with a dose-response of DEXA with or without (Vehicle) an IC20 dose of 2ME-2 during 3 days. The percentages of cell viability were then calculated (n=3). (**E**) mRNA levels of *SCL* and its target gene *NKX3.1* in JURKAT (*left panel*) and of *MYC* in JURKAT and KOPTK1 (*right panel*) T-ALL cell lines after treatment with 3μM of 2-ME2 during 16h (n=3, **p≤0.01). Representative of two independent experiments.

Supplemental Figure 9. SCL and c-MYC half-life are not affected by 2-ME2. (**A**) NOTCH1 targets genes are not affected by the 2-ME2 in pre-LSCs. Pre-LSCs from *SCL*^{tg}*LMO1*^{tg} thymocytes were treated with 2-ME2 (1μM) for 16h and mRNA levels of *Notch1* and Notch1 target genes *Hes1*, *Dtx1*, *Ptrcα*, *Il7rα*, *Notch3* and *c-Myc* were determined by qRT-PCR and normalized to *β-actin* (Mean +/- SD, n=3, **p≤0.01). Representative of two independent experiments. (**B**) Pre-LSCs from *SCL*^{tg}*LMO1*^{tg} thymocytes were treated for 16h with the indicated doses of 2-ME2 and endogenous c-MYC protein expression levels were then determined by immunoblotting. α-Actin is used as a loading control. (**C**) Expression of the major oncogenes in the three T-ALL cell lines. The expression data are from (4). NOTCH1 mutation status of all T-ALL cell lines (5, 6) (http://cancer.sanger.ac.uk/cell_lines) are indicated. (**D**) 2-ME2 does not modify the stability of SCL and c-MYC. JURKAT (SCL^{+}c-MYC^{+}) and KOPTK1 (c-MYC^{+}) cells were treated with a time course (0-8h) of cyclohexymide (CHX, 20μg/mL) in presence or not (vehicle) of 2-ME2 (1μM). Total protein extracts were prepared and then subjected to immunobloting with the SCL and c-MYC antibodies. α-Actin is used as a loading control. Representative of two independent experiments. (**E**) α-Actin and α-Tubulin expression levels were assessed by immunobloting on labeled-JURKAT cells with [^{35}S]methionine treated or not (Vehicle) with 2-ME2 before SCL and MYC immunoprecipitation as loading control of the experiment in Figure 5H.

Supplemental Figure 10. 2-ME2 induces apoptosis in pre-LSCs and leukemic cells. (**A**) SCL complex is involved in the regulation of apoptosis in T-ALL. Genes bound by SCL (2kb within the transcription start site) were overlapped with those up-regulated in *SCL*^{tg}*LMO1*^{tg} leukemias (*left panel*), and over-represented GO terms were analyzed using MsigDB v5.0 enrichment tool

(*right panel*). (**B**) Pre-LSCs and leukemic blasts from *SCL*^{tg}*LMO1*^{tg} mice were treated with 2-ME2 (1μM) for 48h. The percentages of cell viability and apoptotic (Annexin V^{+}) cells were then calculated (n=5, ***p≤0.001, **p≤0.01). (**C**) T-ALL blasts (CD45.2^{+}) from *SCL*^{tg}*LMO1*^{tg} thymoma were transplanted into recipient CD45.1 mice as described in Figure 6A. Cells were collected by BM aspiration 7 and 12 days after transplantation and the proportion of CD45.2^{+} T-ALL cells was assessed by FACS.

Supplemental Figure 11. 2-ME2 does not affect normal T-cell differentiation in vivo. (**A**) Absolute numbers of normal host T-cell populations (CD45.2^{+}, n=5) from the thymus of mice treated or not with 2-ME2 with osmotic pumps in vivo as described in Figure 6A-C were calculated. (**B**) Normal DN3 thymocytes were purified and treated for 16h with the indicated doses of 2-ME2 and endogenous c-MYC protein expression levels were then determined by flow cytometry. (**C**) KSL cells from the BM of CD45.2^{+} mice were purified and treated or not with 2-ME2 (1μM) according to Figure 6G *left panel*. Absolute numbers of donor-derived CD45.2^{+} KSL, B, Myeloid (My) and T cells in the BM and of donor-derived CD45.2^{+} thymocytes (DN and DP) in the thymus of recipient mice were calculated after primary and secondary transplantation. There was no significant difference between controls and 2-ME2-treated conditions.

Supplemental Figure 12. 2-ME2 inhibits SCL and c-MYC expression in xenografted T-ALL patients. (**A**) Determination of the SIL-TAL1 deletion in patients #14H025 and #14H148 by Q-PCR. Genomic DNA from both patients was extracted and amplified for *SIL-TAL1* rearrangement in 1:10 dilution range. RPMI and P12-ISHIKAWA T-ALL cell lines were used as positive and

negative controls respectively. (**B**) NSG mice engrafted with T-ALL blasts from patients #14H025 and #14H148 were treated with 2-ME2 according to Figure 7G left panel, and percentage of human $CD45^+CD7^+$ blasts was monitored by FACS in the BM, the spleen and the blood (***$p \leq 0.001$, **$p \leq 0.01$, *$p \leq 0.05$). (C) SCL and c-MYC protein expression was assessed by immunobloting in the purified engrafted human T-ALL blasts of patient #14H025 after the in vivo treatment.

Supplemental Table 1. Primary human AML patient and sample characteristics.

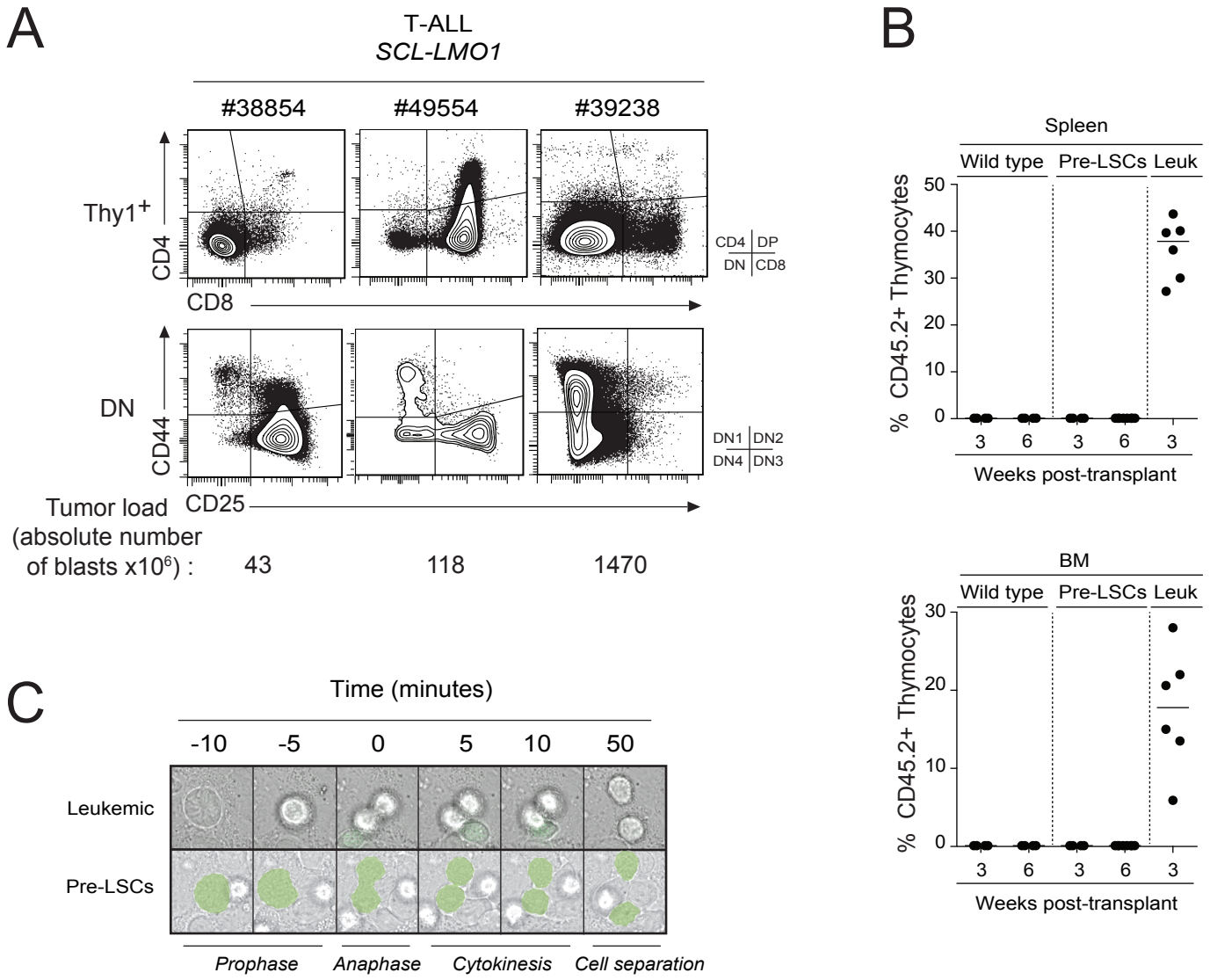
Supplemental Table 2. List of classified compounds related to Figure 3C.

Supplemental Table 3. Clinical and cytogenetic data of T-ALL patient samples. NOTCH1 mutations were determined by targeted sequencing of Exon 26, 27 and 34 as described (5).

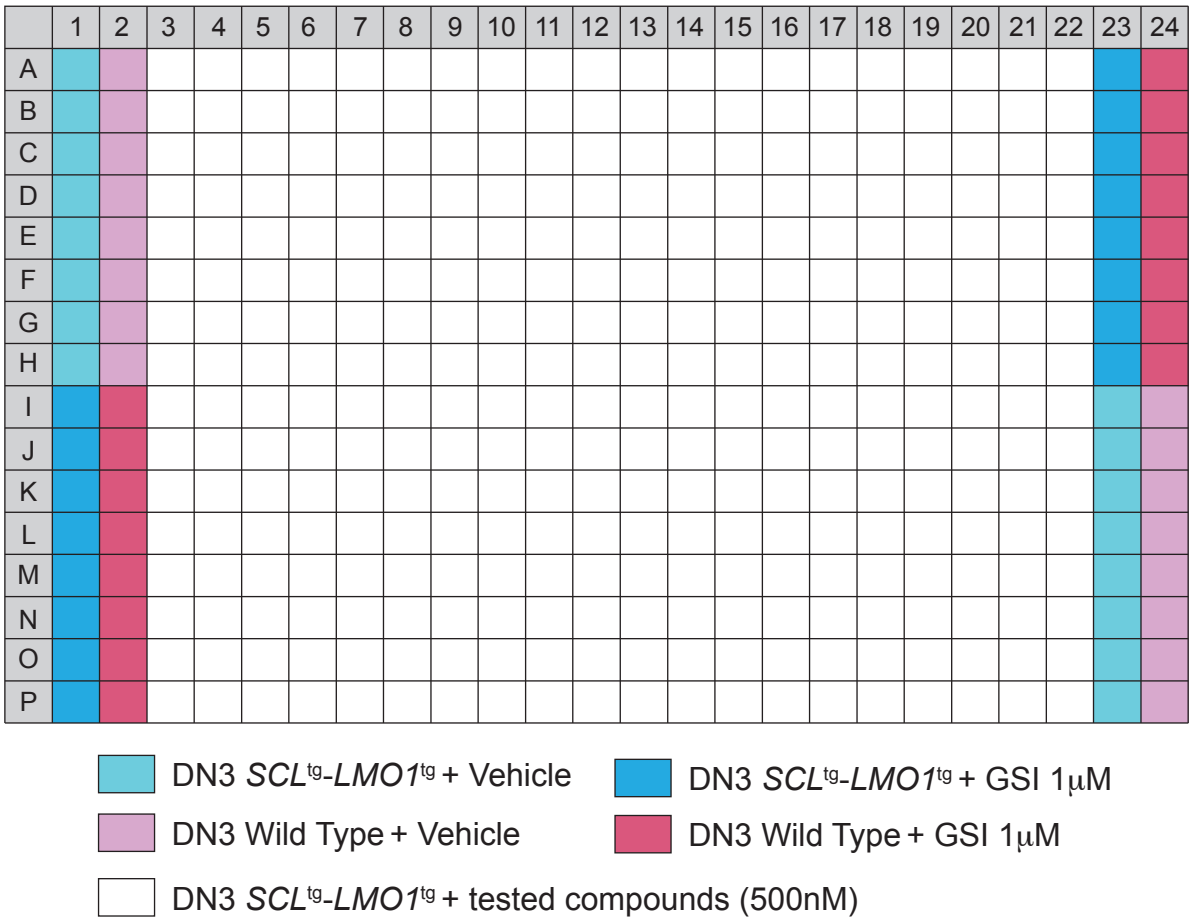
Supplemental Table 4. **Sequences of oligonucleotide primers used for Real-time quantitative PCR.**

SUPPLEMENTAL REFERENCES

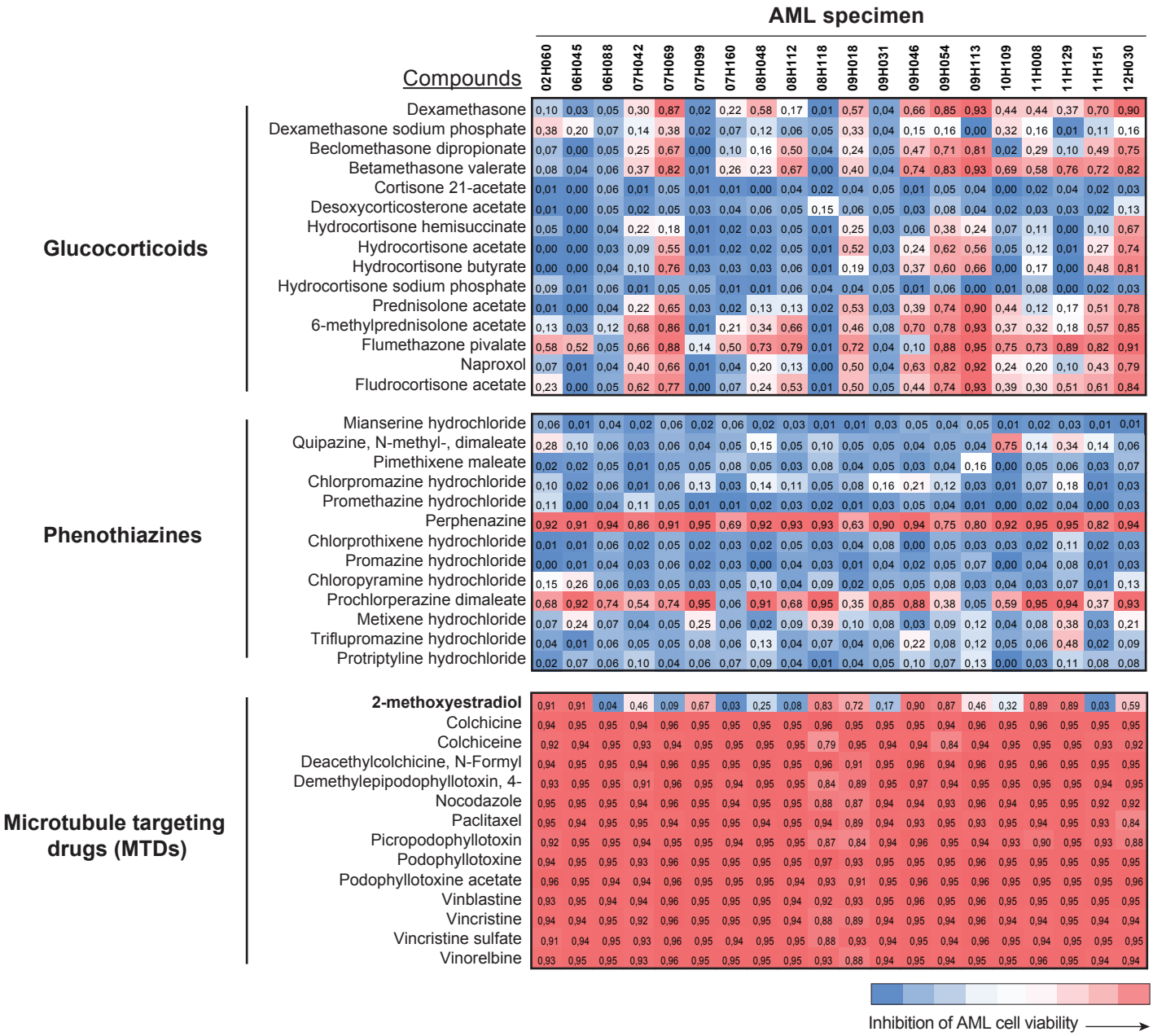
1. Lahlil R, Lecuyer E, Herblot S, and Hoang T. SCL assembles a multifactorial complex that determines glycophorin A expression. *Mol Cell Biol.* 2004;24(4):1439-52.
2. Herblot S, Steff AM, Hugo P, Aplan PD, and Hoang T. SCL and LMO1 alter thymocyte differentiation: inhibition of E2A-HEB function and pre-T alpha chain expression. *Nat Immunol.* 2000;1(2):138-44.
3. Kim D, Pertea G, Trapnell C, Pimentel H, Kelley R, and Salzberg SL. TopHat2: accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions. *Genome Biol.* 2013;14(4):R36.
4. Palomero T, Sulis ML, Cortina M, Real PJ, Barnes K, Ciofani M, Caparros E, Buteau J, Brown K, Perkins SL, et al. Mutational loss of PTEN induces resistance to NOTCH1 inhibition in T-cell leukemia. *Nat Med.* 2007;13(10):1203-10.
5. Weng AP, Ferrando AA, Lee W, Morris JPt, Silverman LB, Sanchez-Irizarry C, Blacklow SC, Look AT, and Aster JC. Activating mutations of NOTCH1 in human T cell acute lymphoblastic leukemia. *Science.* 2004;306(5694):269-71.
6. Sulis ML, Williams O, Palomero T, Tosello V, Pallikuppam S, Real PJ, Barnes K, Zuurbier L, Meijerink JP, and Ferrando AA. NOTCH1 extracellular juxtamembrane expansion mutations in T-ALL. *Blood.* 2008;112(3):733-40.



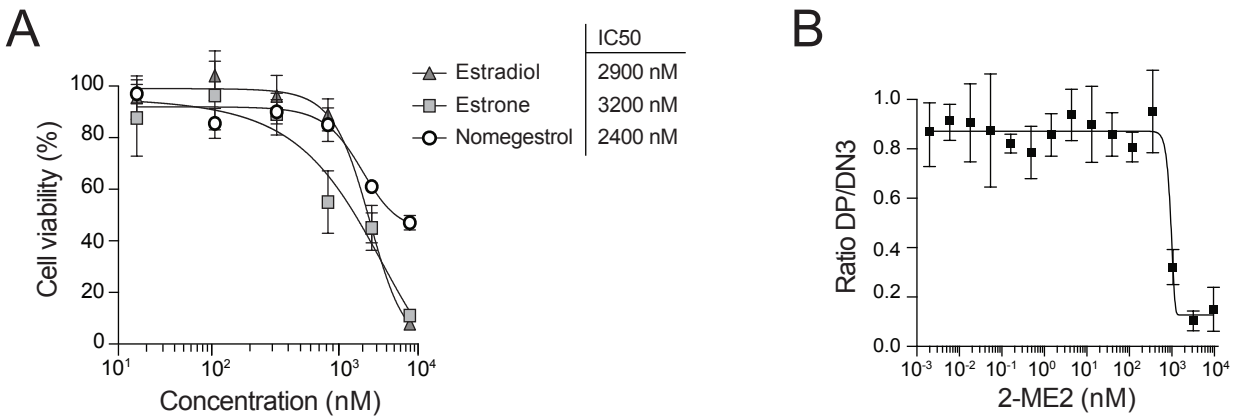
Supplemental Figure 1. Immunophenotypes and invasive behavior of leukemic cells. (**A**) Immunophenotypes of three T-ALL form *Cd3ε*$^{-/-}$*SCL*^{tg}*LMO1*^{tg} thymoma ranked according to the absolute number of blasts found in their thymoma (tumor load). (**B**) Purified pre-leukemic *Cd3ε*$^{-/-}$ DN3 (pre-LSCs) thymocytes and fully transformed leukemic blasts were transplanted as described in Figure 1E. The percentage of donor cells in Thy1.2^{+} populations was analysed in the spleen (*upper panel*) and in the bone marrow (BM, *lower panel*) of recipient mice 3 and 6 weeks later by FACS. Each dot represents one individual engrafted mouse and horizontal lines, the median of thymic engraftment of each experimental group. (**C**) Fully transformed leukemic cells (GFP^{-}) and SCL-LMO1 pre-LSCs (GFP^{+}) were mixed and cultured as described in Figure 1F and real-time cell imaging was performed by videomicroscopy as described in Figure 1G. Representative example of the time-lapse imaging of GFP^{+} and GFP^{-} cell division.



Supplemental Figure 2. Schematic of the screen configuration. Each plate contained sixteen wells with DN3 *SCL*^{tg}*LMO1*^{tg} thymocytes treated with 1μM of γ-secretase inhibitor (GSI) and sixteen wells treated with DMSO (vehicle) as internal positive and negative controls, respectively.

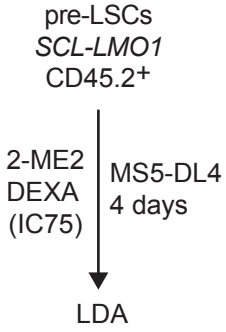


Supplemental Figure 3. Heatmap of primary AML blasts treated with hits from the screen on pre-LSCs. Effect of compounds of the three major groups of pre-LSC inhibitors on the *ex vivo* survival and proliferation of primary human acute myeloid leukemic cells. Numbers represent the fractions of non-viable cells compared to Vehicle (DMSO) control.



Supplemental Figure 4. Estrogen-like compounds with high-affinity for estrogen receptors do not inhibit efficiently pre-LSCs viability and 2-ME2 does not affect normal T cell differentiation in vitro. (**A**) Pre-LSCs from *SCL*^{tg}*LMO1*^{tg} thymocytes were co-cultured on MS5-DL4 stromal cells with a dose-response of Estradiol, Estrone and Nomegestrol for 5 days. The percentages of cell viability are shown and the IC50 was calculated for each condition (mean ± SE, n=6). (**B**) DN3 WT thymocytes were treated with a dose-response of 2-ME2 and the ratio of donor-derived DP to DN3 was calculated for each dose (n=6). Representative of two independent experiments.

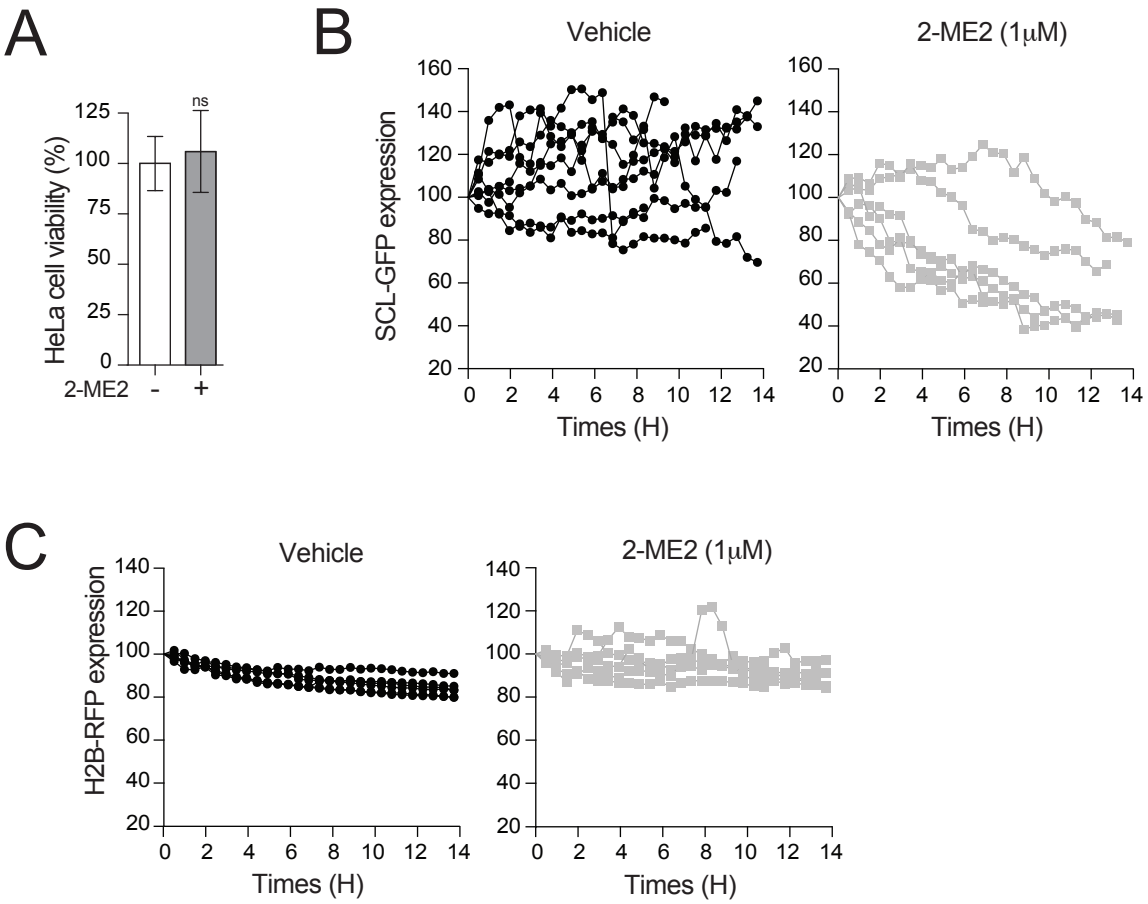
A



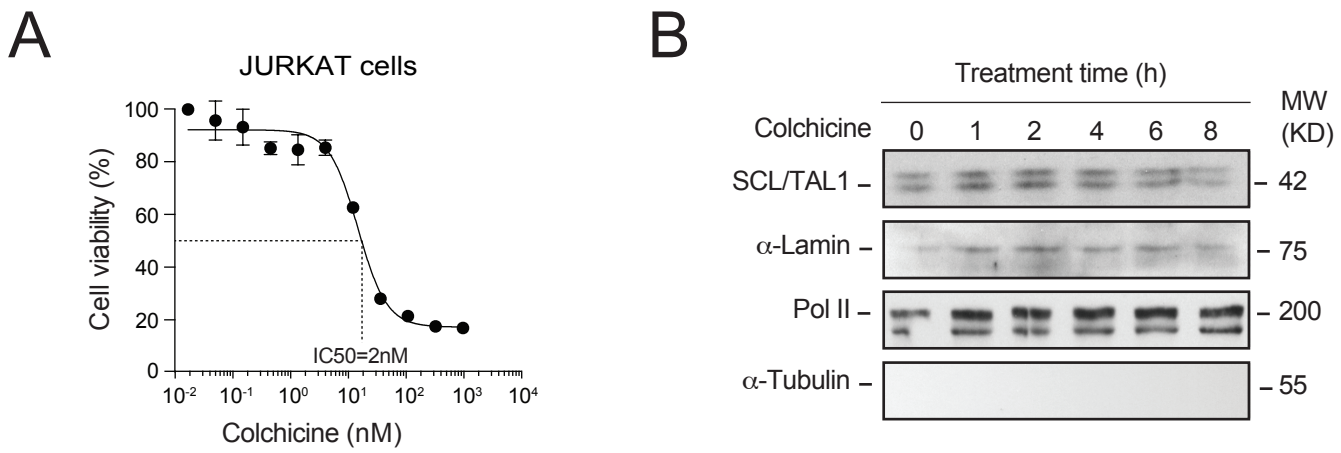
B

	Transplanted pre-LSCs				
	10^6	5.10^5	10^5	5.10^4	10^3
Vehicle	2/2	11/12	2/5	0/6	0/3
2-ME2	2/2	1/10	0/3	0/3	
DEXA		2/2	2/3	0/3	0/3

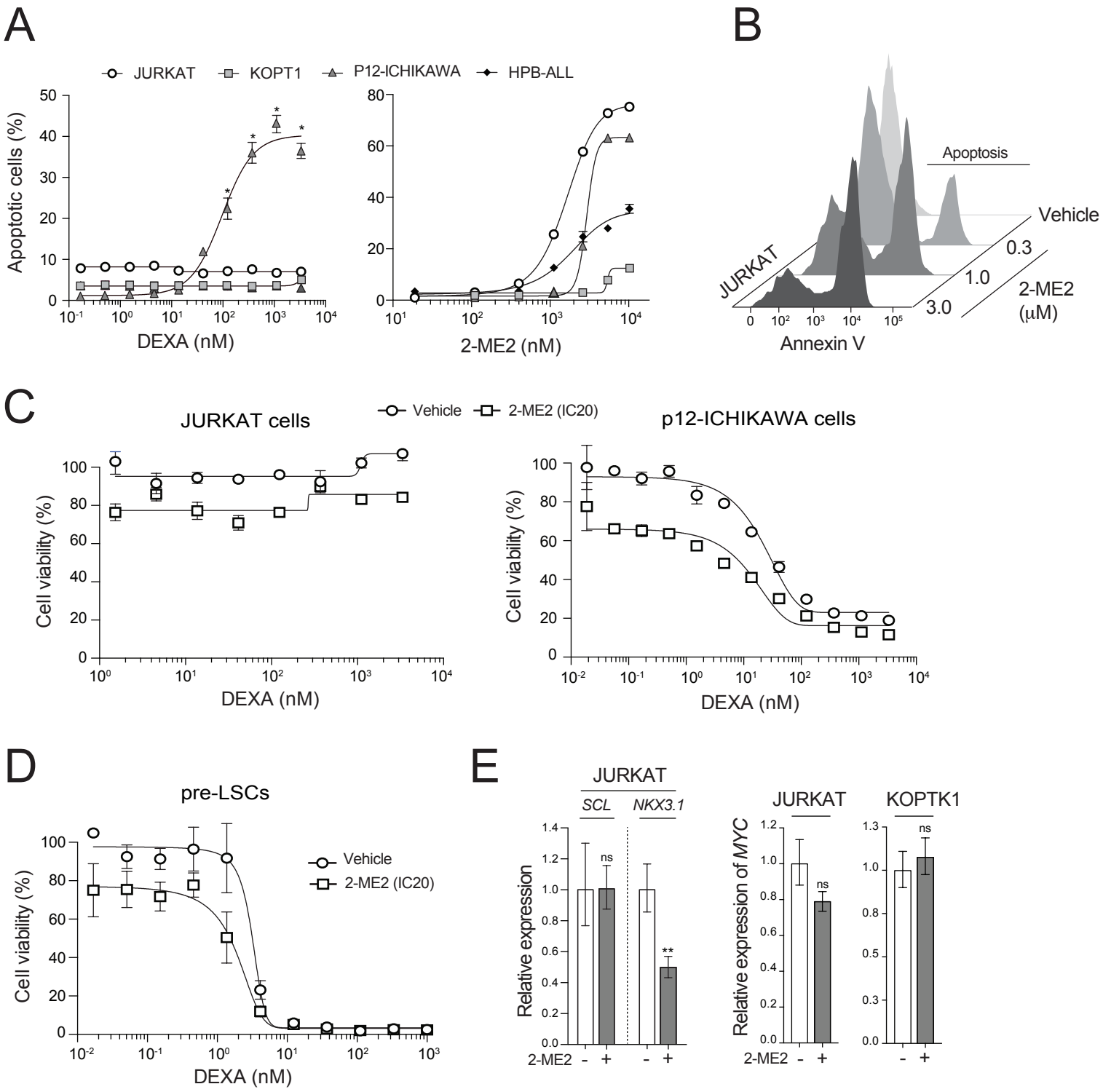
Supplemental Figure 5. Limiting dilution analysis of pre-LSCs treated with 2-ME2 or DEXA. (**A-B**) Schematic of the functional assay (A). Pre-LSCs were exposed to 1μM 2-ME2 or 5nM DEXA (75% inhibition). Treated and untreated viable cells were transplanted by limiting dilution assay. Engraftment was monitor by the presence of donor-derived (CD45.2+) thymocytes in the thymus of recipient mice and mice were scored positive when T-cell lineage reconstitution was more than 0.1% in the thymus. Illustrated are the numbers of positive mice over the total numbers for each injected cell dose (B).



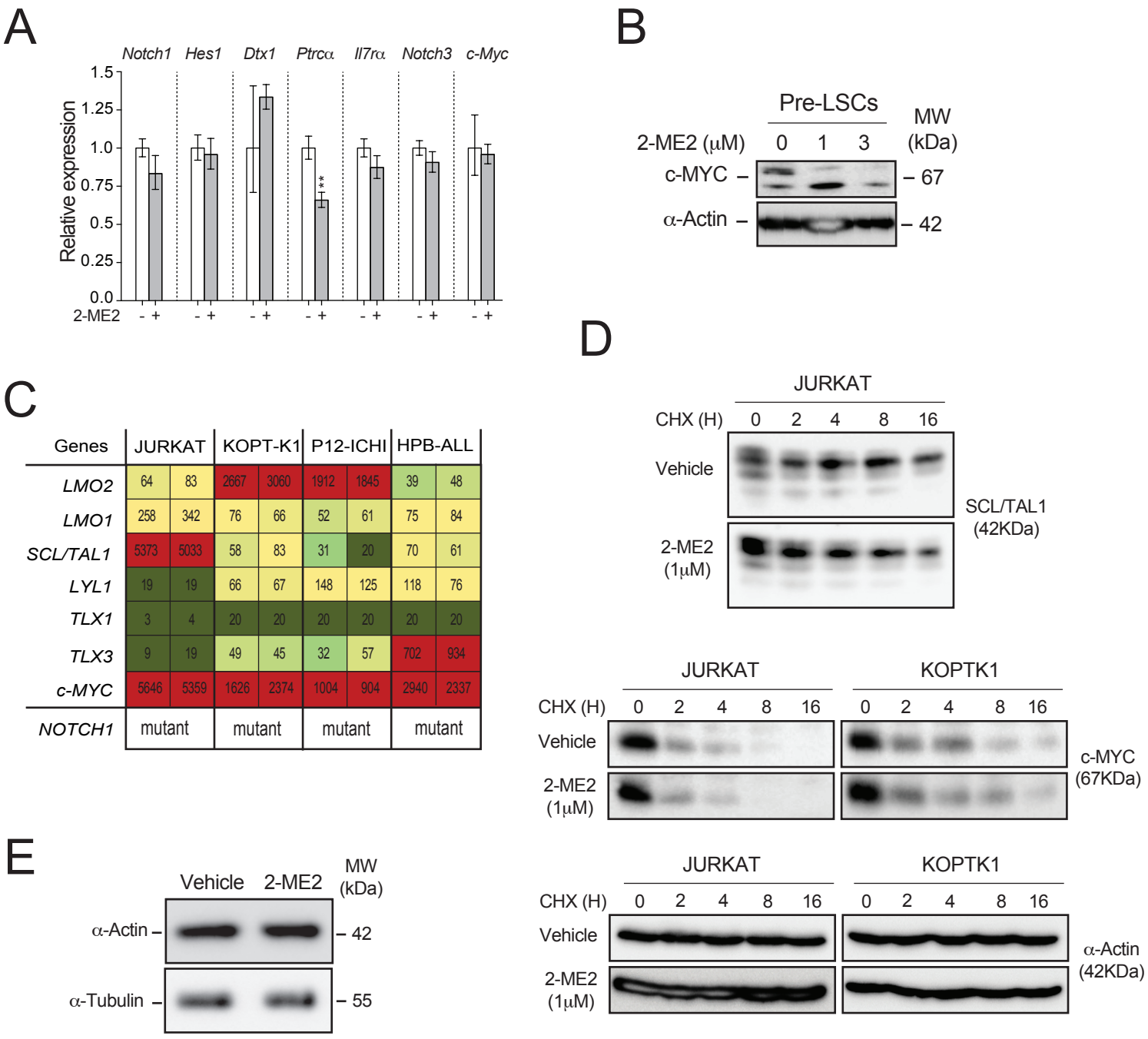
Supplemental Figure 6. 2-ME2 inhibits nuclear accumulation of SCL. (**A**) HeLa cells viabililty after 14h of treatment with 2-ME2 (1μM). (**B-C**) HeLa cells expressing SCL-GFP (B) or H2B-RFP (C) were filmed by video-microscopy in presence or not (Vehicle) of 2-ME2 (1μM) as described in Figure 4, D and E. Data represent the normalized MFIs of 5 to 8 independent 2-ME2-treated and untreated cells.



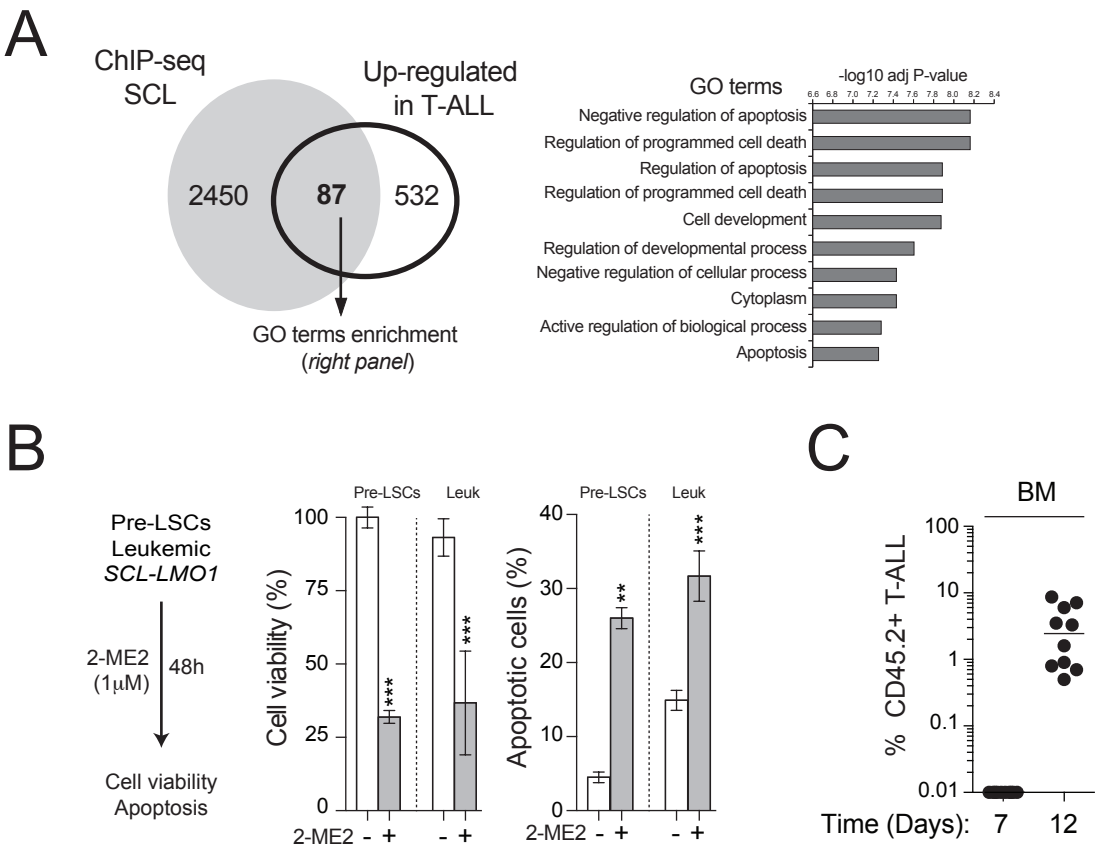
Supplemental Figure 7. The cochicine does not affect SCL protein levels. (**A**) JURKAT human T-ALL cell line was treated with a dose-response of Colchicine during 48h and the percentages of cell viability and the IC50 were then calculated (mean ± SE, n=6). (**B**) JURKAT cells were treated with an IC50 (2nM) of Colchicine for the indicated times. Nuclear extracts were subjected to immunoblotting. Representative of two independent experiments.



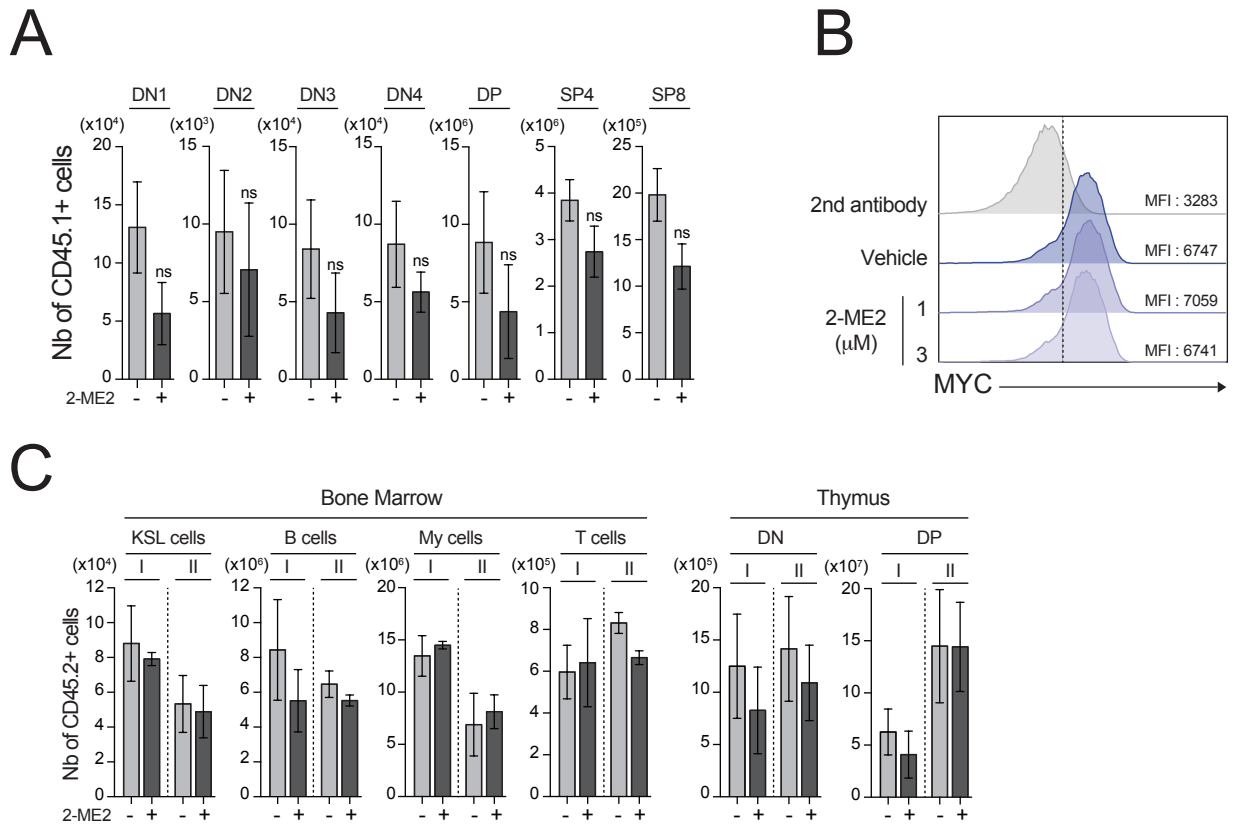
Supplemental Figure 8. 2-ME2 induces apoptosis of human cortico-resistant T-ALL cell lines without modifying their cortico-sensitivities. (**A**) 4 human T-ALL cell lines (JURKAT, KOPT1, P12-ICHIKAWA and HPB-ALL) were treated with a dose-response of DEXA (*left panel*) and 2-ME2 (*right panel*) for 48h. The proportion of apoptotic cells was then assessed by FACS (mean ± SE, n=3, *p≤0.05). (**B**) Representative histogram FACS profile of Annexin V staining in JURKAT cells after the treatment with a dose response (0-3 μM) of 2-ME2 during 48h. (**C**) The cortico-resistant (JURKAT) and cortico-sensitive (P12-ICHIKAWA) human T-ALL cell lines were treated with a dose-response of DEXA with or without (Vehicle) an IC20 dose of 2ME-2 during 48h. The percentages of cell viability were then calculated (mean ± SE, n=6). (**D**) Pre-LSCs from *SCL*^{tg}*LMO1*^{tg} thymocytes were co-cultured on MS5-DL4 stromal cells with a dose-response of DEXA with or without (Vehicle) an IC20 dose of 2ME-2 during 3 days. The percentages of cell viability were then calculated (n=3). (**E**) mRNA levels of *SCL* and its target gene *NKX3.1* in JURKAT (*left panel*) and of *MYC* in JURKAT and KOPTK1 (*right panel*) T-ALL cell lines after treatment with 3μM of 2-ME2 during 16h (n=3, **p≤0.01). Representative of two independent experiments.



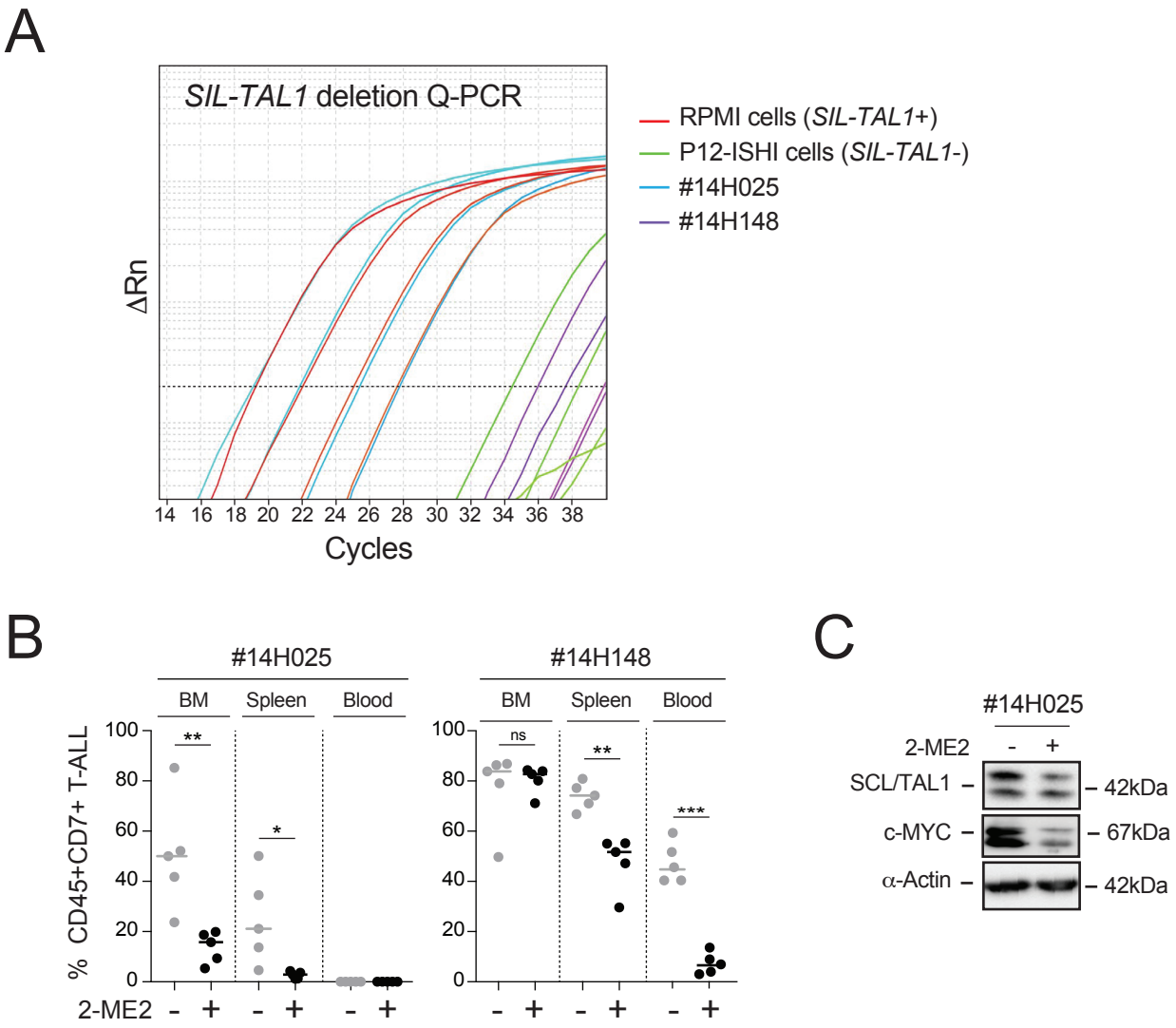
Supplemental Figure 9. SCL and c-MYC half-life are not affected by 2-ME2. (**A**) NOTCH1 targets genes are not affected by the 2-ME2 in pre-LSCs. Pre-LSCs from *SCL*^{tg}*LMO1*^{tg} thymocytes were treated with 2-ME2 (1μM) for 16h and mRNA levels of *Notch1* and Notch1 target genes *Hes1, Dtx1, Ptrcα, Il7rα, Notch3* and *c-Myc* were determined by qRT-PCR and normalized to *β-actin* (Mean +/- SD, n=3, **p≤0.01). Representative of two independent experiments. (**B**) Pre-LSCs from *SCL*^{tg}*LMO1*^{tg} thymocytes were treated for 16h with the indicated doses of 2-ME2 and endogenous c-MYC protein expression levels were then determined by immunoblotting. α-Actin is used as a loading control. (**C**) Expression of the major oncogenes in the three T-ALL cell lines. The expression data are from (4). NOTCH1 mutation status of all T-ALL cell lines (5) (http://cancer.sanger.ac.uk/cell_lines) are indicated. (**D**) 2-ME2 does not modify the stability of SCL and c-MYC. JURKAT (SCL+c-MYC+) and KOPTK1 (c-MYC+) cells were treated with a time course (0-8h) of cyclohexymide (CHX, 20μg/mL) in presence or not (vehicle) of 2-ME2 (1μM). Total protein extracts were prepared and then subjected to immunobloting with the SCL and c-MYC antibodies. α-Actin is used as a loading control. Representative of two independent experiments. (**E**) α-Actin and α-Tubulin expression levels were assessed by immunobloting on labeled-JURKAT cells with [^{35}S]methionine treated or not (Vehicle) with 2-ME2 before SCL and MYC immunoprecipitation as loading control of the experiment in Figure 5H.



Supplemental Figure 10. 2-ME2 induces apoptosis in pre-LSCs and leukemic cells. (**A**) SCL complex is involved in the regulation of apoptosis in T-ALL. Genes bound by SCL (2kb within the transcription start site) were overlapped with those up-regulated in *SCL*^{tg}*LMO1*^{tg} leukemias (*left panel*), and over-represented GO terms were analyzed using MsigDB v5.0 enrichment tool (*right panel*). (**B**) Pre-LSCs and leukemic blasts from *SCL*^{tg}*LMO1*^{tg} mice were treated with 2-ME2 (1μM) for 48h. The percentages of cell viability and apoptotic (Annexin V+) cells were then calculated (n=5, ***p≤0.001, **p≤0.01). (**C**) T-ALL blasts (CD45.2^{+}) from *SCL*^{tg}*LMO1*^{tg} thymoma were transplanted into recipient CD45.1 mice as described in Figure 6A. Cells were collected by BM aspiration 7 and 12 days after transplantation and the proportion of CD45.2^{+} T-ALL cells was assessed by FACS.



Supplemental Figure 11. 2-ME2 does not affect normal T-cell differentiation in vivo. (**A**) Absolute numbers of normal host T-cell populations (CD45.2$^+$, n=5) from the thymus of mice treated or not with 2-ME2 with osmotic pumps in vivo as described in Figure 6A-C were calculated. (**B**) Normal DN3 thymocytes were purified and treated for 16h with the indicated doses of 2-ME2 and endogenous c-MYC protein expression levels were then determined by flow cytometry. (**C**) KSL cells from the BM of CD45.2$^+$ mice were purified and treated or not with 2-ME2 (1μM) according to Figure 6G *left panel*. Absolute numbers of donor-derived CD45.2$^+$ KSL, B, Myeloid (My) and T cells in the BM and of donor-derived CD45.2$^+$ thymocytes (DN and DP) in the thymus of recipient mice were calculated after primary and secondary transplantation. There was no significant difference between controls and 2-ME2-treated conditions.



Supplemental Figure 12. 2-ME2 inhibits SCL and c-MYC expression in xenografted T-ALL patients. (**A**) Determination of the *SIL-TAL1* deletion in patients #14H025 and #14H148 by Q-PCR. Genomic DNA from both patients was extracted and amplified for *SIL-TAL1* rearrangement in 1:10 dilution range. RPMI and P12-ISHIKAWA T-ALL cell lines were used as positive and negative controls respectively. (**B**) NSG mice engrafted with T-ALL blasts from patients #14H025 and #14H148 were treated with 2-ME2 according to Figure 7G *left panel*, and percentage of human $CD45^{+}CD7^{+}$ blasts was monitored by FACS in the BM, the spleen and the blood (***p≤0.001, **p≤0.01, *p≤0.05). (**C**) SCL and c-MYC protein expression was assessed by immunobloting in the purified engrafted human T-ALL blasts of patient #14H025 after the in vivo treatment.