Supplementary Methods and Tables

Table S1. *Sequences of oligonucleotide primers used*

Genomic PCR	
hGAPDH_Fw	5'-GGAAGGACTCATGAGCACAGTCC-3'
hGAPDH_Rv	5'-TCGCTGTTGAAGTCAGAGGAGACC-3'
mHPRT_Fw	5'-GGGGGCTATAAGTTCTTTGC-3'
mHPRT_Rv	5'-TCCAACACTTCGAGAGGTCC-3'
mIntegrin alpha4_Fw-1	5'-CCACCTGGTGTATGAAAGC-3'
mIntegrin alpha4_Fw-2	5'-CGGGATCAGAAAGAATCCA-3'
mIntegrin alpha4_Rv	5'-CTGGCATGGGGTTAAAATTG-3'

Quantitative RT-PCR	
murine BCR-ABL1_Fw[13]	5'-ATCGTGGGCGTCCGCAAGAC-3'
murine BCR-ABL1_Rv[13]	5'-GCTCAAAGTCAGATGCTACTG-3'
human MLL-AF4_Fw[14;15]	5'-AGAATCAGGTCCAGAGCAGAGC-3'
human MLL-AF4_Rv[14;15]	5'-ATGCTGAGAGTCCTTTGTAGGG-3'

Table S2. *Antibodies used for flow cytometry*

Surface antigen	**Clone ID**	**Purchased from**
Murine CD19 (FITC)	1D3	eBioscience
Human CD19 (FITC)	HIB19	BD Biosciences
Human CD10 (FITC)	HI10a	BD Biosciences
Murine CD45 (FITC)	30-F11	BD Biosciences
Human CD45 (APC)	HI30	BD Biosciences
Murine CD49d (PE)	R1-2	eBioscience
Murine B220 (PE)	RA3-6B2	eBioscience
Human CD49d (PE)	9F-10	BD Biosciences

Human CD49d (Purified)	9F-10	eBioscience
Human CD3 (FITC)	HIT3a	BD Biosciences
Human CD56 (PE)	CMSSB	eBioscience

Correlation of integrin alpha4 gene expression on leukemic blasts with clinical outcomes of pre-B ALL patients

Patient clinical and outcome data were obtained from the National Cancer Institute TARGET Data Matrix (http://target.nci.nih.gov/dataMatrix/TARGET_DataMatrix.html) of the Children's Oncology Group (COG) Clinical Trial P9906.[11] COG P9906 enrolled 272 eligible high-risk B-precursor ALL patients, which were treated uniformly with a modified augmented Berlin-Frankfurt-Münster Study Group (BFM) regimen. Patients with very high-risk features (*BCR-ABL1* or hypodiploidy) were excluded.[11] The gene expression microarray data were obtained from Gene Expression Omnibus (http://www.ncbi.nih.gov/geo) with accession number GSE11877. Kaplan-Meier survival analysis was used to estimate overall survival (OS) and relapse-free survival (RFS). Log-rank test was used to compare survival differences among patient groups. R package "survival" version 2.35-8 was used for the survival analysis (R Development Core Team, 2009). The majority of patients had MRD assessed by flow cytometry, as previously described; cases were defined as MRD positive or MRD negative at the end of induction therapy (day 29) using a threshold of 0.01%.[11]

Studies with Integrin alpha4 $^{fl/fl}$ bone marrow cells

Bone marrow cells from integrin alpha4$^{fl/fl}$ mice described previously[6] were cultured with IMDM and 20% FBS media supplemented with 10 ng/ml murine IL-7 (Peprotech, Rocky Hill,

NJ) and retrovirally transduced with *BCR-ABL1* p210 (Supplementary Figure S2A) and cultured under lymphoid conditions.[12;18;19] Oncogenically transformed $B220^{+}CD19^{+}$ murine leukemia cells were selected by withdrawal of IL-7 and subsequently transduced with $EmptyER^{T2}$ or $CreER^{T2}$ retrovirus (**Supplementary Figure S2B,C** and **S3A**). After 1 μg/ml puromycin selection, integrin alpha4 deletion was induced *in vitro* by addition of 1.0 μM Tamoxifen (Sigma-Aldrich) dissolved in 100% sterile ethanol. The adhesion assays for mouse cells were performed on immobilized recombinant mouse VCAM-1 (10 μg/ml, R&D Systems) as described for primary ALL cells. To test the effect of integrin alpha4 ablation on $p210^{+}$ leukemia cells in an *in vivo* model, non-deleted $CreER^{T2}$ cells and $EmptyER^{T2}$ cells were injected either via tail vein (**Figure 1F**) or via intrafemoral injection (**Supplementary Figure S4C, D**) into C57BL/6 $Ly5.1^{+}(CD45.1^{+})$ recipient mice ($5x10^{5}$ cells/mouse), which had been irradiated with 525 cGy prior to cell transfer. To induce deletion *in vivo*, mice were treated with 100-125 mg/kg/day Tamoxifen (Sigma-Aldrich) by oral plastic feeding tubes (Instech Solomon, Plymouth Meeting, PA) for 5 days at indicated times (**Supplementary Figure S4A, C**). Mice were treated daily for 4 weeks with 50 mg/kg/day Nilotinib (Novartis, Stein, Switzerland) by oral plastic feeding tubes. Lentiviral luciferase labeling of murine leukemia cells and bioimaging were performed as described below.

Quantitative Real-Time PCR

Quantitative real-time PCR was performed by mixing cDNA samples with the SYBR GreenER (Invitrogen) and primers listed in Table S1. The samples were loaded into an optical 96-well reaction plate (Applied Biosystems, Foster City, CA) and analyzed by ABI7900HT real-time PCR system (Applied Biosystems, Foster City, CA).

CBC Counts

Peripheral blood was withdrawn via the tail vein for CBC analysis from mice when they were sacrificed. Blood samples (~100 µl/sample) were collected in BD microtainer tubes with EDTA (BD Biosciences) and analyzed on a VetScan HM5 cell counter (Abaxis, Union City, CA).

CFU assays

BCR-ABL1^{+} (p210) CreER^{T2} or EmptyER^{T2} ALL cells were plated in triplicate in MethoCult GF^{+} M3434 (StemCell Tech, Vancouver, BC) and incubated at 37^{o}C in humidified 5% CO_2 for 10~14 days. Colonies were captured under confocal fluorescent microscopy (Carl Zeiss Microimaging, Thornwood, NY) equipped with an Orca C4742-80-12AG camera (Hamamatsu Photonics, Bridgewater, NJ) at room temperature under 400x magnification. The microscope and camera were controlled by Micro-Manager 1.4 software. Micrographs were acquired using QCapture software 2.98 (Quantitative Imaging Corp; Surrey, BC Canada) via a QImaging QiCam, mounted on an Olympus IX71 microscope. CFU assay for primary ALL cells (**Figure 2I,J**) were performed in triplicates by plating an equal number of viable white blood cells (WBC) isolated from femurs, spleen, liver, and lung, pooled from 2 animals (1x10^{5} cells/dish) as described below, and from peripheral blood by red blood cell lysis using ammonium chloride (1.2 ml/per dish). WBCs were cultured in MethoCult GF^{+} H4435 as described previously.[6] Images were taken with 2.5x magnification on Day 18 after tissues harvesting.

White blood cell isolation from mouse organs

For homing and mobilization assays, femurs, spleen, peripheral blood, lung, and liver were harvested in cold PBS. The white blood cell isolation from femurs, spleen, and peripheral blood

Xenograft model of primary leukemia

Under IACUC approved protocols, NOD.Cg-*Prkdc*^{scid} *IL2rg*^{tm1Wjl}/SzJ (NSG) or NOD/SCID mice of 5-7 weeks of age were conditioned with a single sub-lethal dose of 250 cGy of whole body irradiation (250cGy), followed by intravenous injection of about 1 – 5x10^6 pre-B ALL patient bone marrow cells per mouse.[12] Mice were monitored for weight change and presence of human CD45$^+$ cells in the peripheral blood by flow cytometry. Mice were sacrificed based on appearance, mobility, and over 15% body weight loss of the animal. White blood cells (WBC) from peripheral blood, spleen, and bone marrow were then isolated by gradient separation (Ficoll) and stained with mouse and human CD45 antibodies to confirm a full engraftment of primary ALL cells (>95% human CD45$^+$). Engrafted cells from mice directly receiving patient cells were considered as primary passage and serially xenotransplanted into new mice up to tertiary passage for cell expansion.

In vitro assays of primary ALL cells

Primary ALL cells (previously frozen) were co-cultured with irradiated murine OP9 and treated with anti-integrin alpha4 monoclonal antibodies as a single agent or in combination with chemotherapy, VDL (0.0005 μM Vincristine, 0.005 nM Dexamethasone, 0.0005 IU/ml L-

Asparaginase) for 4 days.[12] Experiments were performed in triplicates three times. Cell viability was determined by trypan blue exclusion. Statistical significance of differences between groups was determined by the unpaired t-test.

Adhesion of primary ALL cells

Primary pre-B ALL cells (previously frozen) were either pre-treated with function-blocking integrin alpha4 antibody (Ab) Natalizumab (NZM) or control IgG4 (G17-4) Ab (BD Biosciences) (**Supplementary Figure S7A**) for 30 minutes at 37 °C and washed once with PBS. Cells were then loaded in triplicates on 12-well non-tissue culture treated plates coated with or without human recombinant VCAM-1 (hVCAM-1; 10 μg/ml) (R&D Systems, Minneapolis, MN) and BD BioCoat human fibronectin (Fn) (BD Biosciences), plus 2% BSA (A2153, Sigma-Aldrich, St Louis, MO). Pre-treated ALL cells were also co-cultured with the mouse bone marrow stromal cell line OP9, or the human bone marrow stromal cell line HS-5 (ATCC No. CRL11882) in 12-well tissue culture treated plates (**Supplementary Figure S7B**). After 2 hours incubation, suspension cells in the supernatant were removed and the plate was washed once with PBS. Adherent cells were photographed with an Olympus IX71 microscope and then detached by pipetting 20 times. The cell count for adherent cells was assessed by trypan blue exclusion of dead cells (**Supplementary Figure S7B**).

In vivo integrin alpha4 blockade of primary ALL cells in combination with chemotherapy

Luciferase-labeled ALL cells were injected into sub-lethally irradiated NSG or NOD/SCID mice with $5x10^4$ cells per mouse as described above.[12] Mice were then treated intraperitoneally with Immunoglobulin (Ig) control or anti-Integrin alpha4 mAb, Natalizumab (NZM) (5 mg/kg) once

per week, or combined with chemotherapy consisting of VDL (V: Vincristine 0.8 mg/kg, once per week; D: Dexamethasone 10.5 mg/kg, 5 days/week; L: L-Asparaginase 800 IU/kg, 5 days/week) from Day 3 to Day 29 after cell transfer. Mice were weighed daily during the treatment and weekly after the treatment. Based on over 15% weight loss compared to the initial weight, mice were sacrificed. White blood cells in peripheral blood, spleen, and bone marrow were separated by gradient separation using Ficoll and analyzed by flow cytometry and immunohistochemistry. The survival time of mice is presented by a Kaplan-Meier survival curve and analyzed by Log-rank test.

Leniviral production, transduction, and bioluminescent imaging.

pCCL-MNDU3-LUC was a third generation HIV-1 based, lentiviral vector expressing the firefly luciferase gene (gift from Dr. Donald Kohn, UCLA). Lentiviral supernatant was produced using the transfection reagent polyetherimide (PEI; Sigma-Aldrich, St Louis, MO), for the triple transfection of confluent HEK293FT cells with the 8.9 packaging plasmid, pMDG-VSV-G, and the transfer plasmid. Plasmids were isolated using the Qiagen Endotoxin Free Maxiprep (Qiagen, Hilden, Germany) grown from stably transformed *E.coli*. Lentiviral supernatant was collected 72 hours post-transfection, and concentrated by ultracentrifugation. Primary ALL cells were transduced with pCCL-MNDU3-LUC lentivirus supernatant in plates coated with retronectin (Takara, Shiga, Japan) containing serum-free QBSF-60 medium (Quality Biologicals, Gaithersburg, MD) for 48 hours. Aliquots of all transduced cells were assayed to confirm bioluminescent signal prior to transplantation. The transduction efficiency of luciferase was analyzed by immunofluorescence.[12] Monitoring of leukemia progression by determining bioluminescence signal development in mice was performed as previously described. [12] A mouse

with no leukemia injection and treated only with luciferin at time of imaging was included as background control mouse (Ctrl).

Isolation of NK cells and Calcein-AM release assay

ADCC (Antibody-dependent cell-mediated cytotoxicity) of primary ALL cells was determined using a Calcein-AM release assay as previously described[24]. NK cells (>94% $CD56^{+}CD3^{-}$) were isolated from fresh filtered blood from healthy donors (CHLA Blood Bank) using Ficoll-plague plus (GE Healthcare, Pasadena, CA) density gradient centrifugation and further isolation with a MACS NK cell isolation kit (Miltenyi Biotec, Auburn, CA). Primary ALL cells were labeled with 5μM Calcein-AM (Invitrogen) for 30 minutes at 37 ̊C and then treated with NZM or IgG4 control Ab and washed with PBS once. Primary ALL cells as target cells (T) were mixed with isolated NK cells as effectors cells (E) at E/T ratio from 5:1 to 15:1. After 4 hours incubation, cells were transferred to a black ViewPlate-96 plate and read on Filter Max F3 Microplate reader (Molecular devices, Sunnyvale, CA) using 485 nm excitation/535 nm emission filter set. % specific lysis was calculated by the following equation[24]: % specific lysis = (mean experimental release–mean spontaneous release) / (mean maximal release–mean spontaneous release) x 100%.

Immunohistochemistry

Tissues, including spleen, bone marrow, lung, and intestine from experimental NOD/SCID or NSG mice were immersed in 10% formalin (VWR, Radnor, PA) for 24 hours and transferred to PBS. Paraffin embedding was done following standard procedures on a Tissue-TEK VIP processor (Miles Scientific). 4 μm sections were mounted on Apex superior adhesive slides (Leica Microsystems) and stained on a Ventana BenchMark automated IHC stainer (Tucson,

Arizona). The Bond™ ready-to-use hCD45 (X 16/99) primary antibody (Leica Biosystems, Newcastle, UK) was used and the antigen-antibody reaction were detected and visualized by Ventana iView DAB detection kit (Tucson, Arizona), which includes polymeric horseradish peroxidase (HRP) IgG secondary antibody, substrate chromogen 3,3'-Diaminobenzidine tetrahydrochloride (DAB), and other necessary reagents. For Ki-67 staining, slides were prepared as previously described[25] and first stained with the Bond™ ready-to-use human Ki-67 (K2) primary antibody (Leica Biosystems) followed by polymeric alkaline phosphatase (AP) IgG secondary antibody and substrate chromogen, Fast Red. Slides were also counterstained with routine hematoxylin for cell nuclei. Mounting medium (Pro-Long Gold Antifade Reagent; Invitrogen) was applied and coverslips sealed, prior to acquisition of fluorescent images at room temperature on a Zeiss Axiovert 200M inverted confocal microscope with a 40 Plan Neofluor objective using IP Lab 4.0 software (Scanalytics). Photomicrographs were acquired using a Hamamatsu ORCAER HAL100 digital camera (400x magnification) and brightness was enhanced using Adobe Photoshop 6.0.

Supplementary Figures and Tables

Table S1. *Tables of mean and standard deviation of three independent experiments for Figure 1*

Figure 1D

Cell viability of initial (%)

Control	EmptyER^{T2}	CreER^{T2}	EmptyER^{T2} +VDL	CreER^{T2} +VDL
Exp#1	97.2±6.3	85.4±9.1	52.7±2.5	27.9±6.6
Exp#2	93.6±3.1	84.8±4.1	47.0±4.9	31.9±4.8
Exp#3	93.6±3.1	92.9±2.2	51.4±6.8	45.1±4.0
Combined	94.7±3.9	87.7±6.5	50.3±5.0	34.8±9.0

Cell viability of initial (%)

mVCAM-1	EmptyER^{T2}	CreER^{T2}	EmptyER^{T2} +VDL	CreER^{T2} +VDL
Exp#1	92.8±3.3	80.3±4.4	46.0±2.3	28.3±5.0
Exp#2	93.1±2.8	80.5±4.2	43.6±2.8	26.0±2.3
Exp#3	92.1±3.2	88.3±3.3	56.5±3.5	30.2±2.8
Combined	92.8±2.6	83.1±5.2	48.8±6.4	28.0±3.6

Figure 1E

Numbers of Colonies

1st Plating	EmptyER^{T2}	CreER^{T2}
Exp#1	319±36	42±8

Numbers of Colonies

2nd Plating	EmptyER^{T2}	CreER^{T2}
Exp#1	223±56	1±1

Table S2. *Tables of mean and standard derivation of three independent experiments of Figure 2*

Figure 2A

Number of adhering cells (x10^4)

Control	IgG4	NZM	hVCAM-1	IgG4	NZM
Exp#1	0.3±0.3	0.3±0.3	Exp#1	9.7±1.2	0.5±0.5
Exp#2	0.8±0.3	0.7±0.3	Exp#2	10.2±0.3	0.4±0.1
Exp#3	0.8±0.3	0.7±0.3	Exp#3	10.0±1.3	0.7±0.3
Combined	0.7±0.4	0.6±0.3	Combined	9.9±0.9	0.5±0.3

Figure 2B

Cell viability of initial (%)

Control	IgG4	NZM	hVCAM-1	IgG4	NZM
Exp#1	78.2±5.5	80.4±7.5	Exp#1	83.4±9.2	80.3±5.9
Exp#2	73.8±5.0	78.1±4.5	Exp#2	80.0±3.4	79.7±2.6
Exp#3	76.2±2.7	82.2±6.5	Exp#3	77.5±1.9	83.2±4.9
Combined	76.1±4.4	80.3±5.7	Combined	80.3±5.6	81.1±4.4

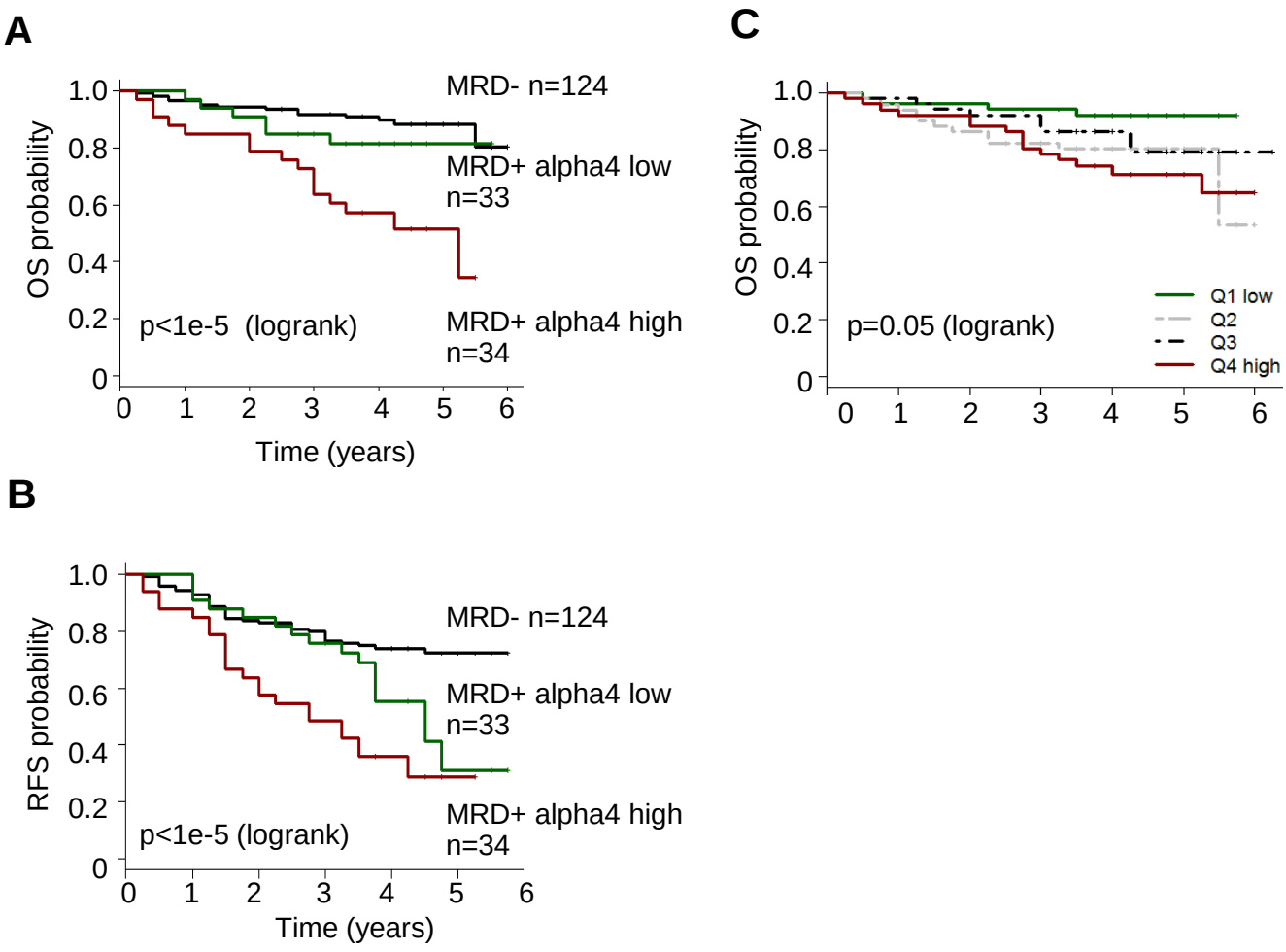
Figure 2C

Cell viability of initial (%)

Control	IgG4	NZM	IgG4 +VDL	NZM +VDL
Exp#1	69.8±3.3	65.7±1.0	36.6±9.5	30.4±2.4
Exp#2	74.5±1.8	70.8±1.7	40.4±6.3	35.7±7.6
Exp#3	78.3±2.0	74.8±3.6	49.5±11.9	48.8±8.2
Combined	74.2±4.3	70.0±4.6	42.2±10.0	38.3±10.0

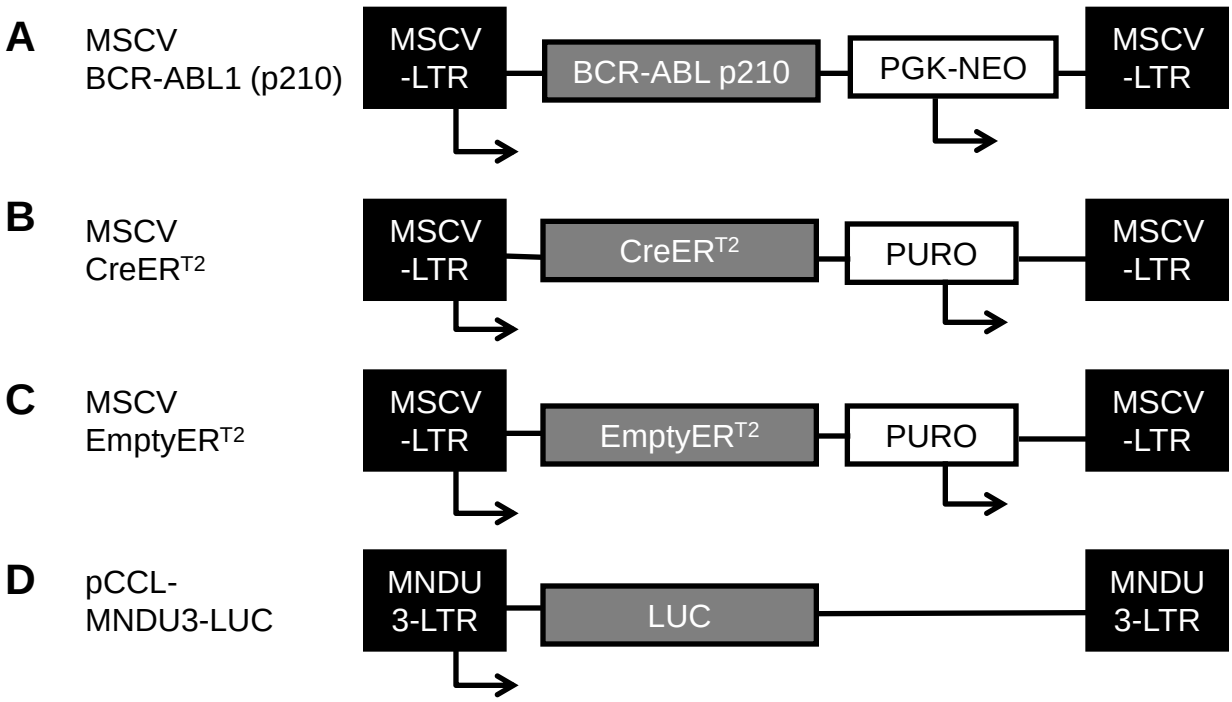
hVCAM-1	IgG4	NZM	IgG4 +VDL	NZM +VDL
Exp#1	74.5±6.5	62.5±6.6	50.5±1.9	38.2±5.3
Exp#2	72.6±2.0	70.8±1.7	52.8±6.3	32.5±1.5
Exp#3	79.9±3.0	74.0±5.6	43.4±8.4	23.9±2.2
Combined	75.7±4.9	69.1±6.8	48.9±6.8	31.5±6.9

Figure S1. *Kaplan-Meier estimates of OS and RFS based on end-induction (day 29) MRD and Integrin alpha4* (ITGA4, 205885_s_at) *expression.*



Legend. Kaplan-Meier estimates of OS and RFS based on MRD (day 29) and Integrin alpha 4 (alpha4) expression. The overlay of the Kaplan-Meier estimates of overall survival (OS) **(A)** and relapse-free survival (RFS) **(B)** for the MRD^{+} $alpha4^{low}$, MRD^{+} $alpha4^{high}$, and MRD^{-} cases showed significant p-values for the separation. The p-value is calculated from Log-rank test for differences among all groups. **(C)** The OS probability (COG P9996) was further divided into 4 groups based on the alpha4 expression (Q1: 1^{st} quartile with the lowest alpha4 expression; Q2: 2^{nd} quartile; Q3: 3^{rd} quartile; Q4: 4^{th} quartile with the highest alpha4 expression). p-value was calculated from Log-rank test for the difference in all groups.; p-value for Q1 versus Q4: p=0.007.

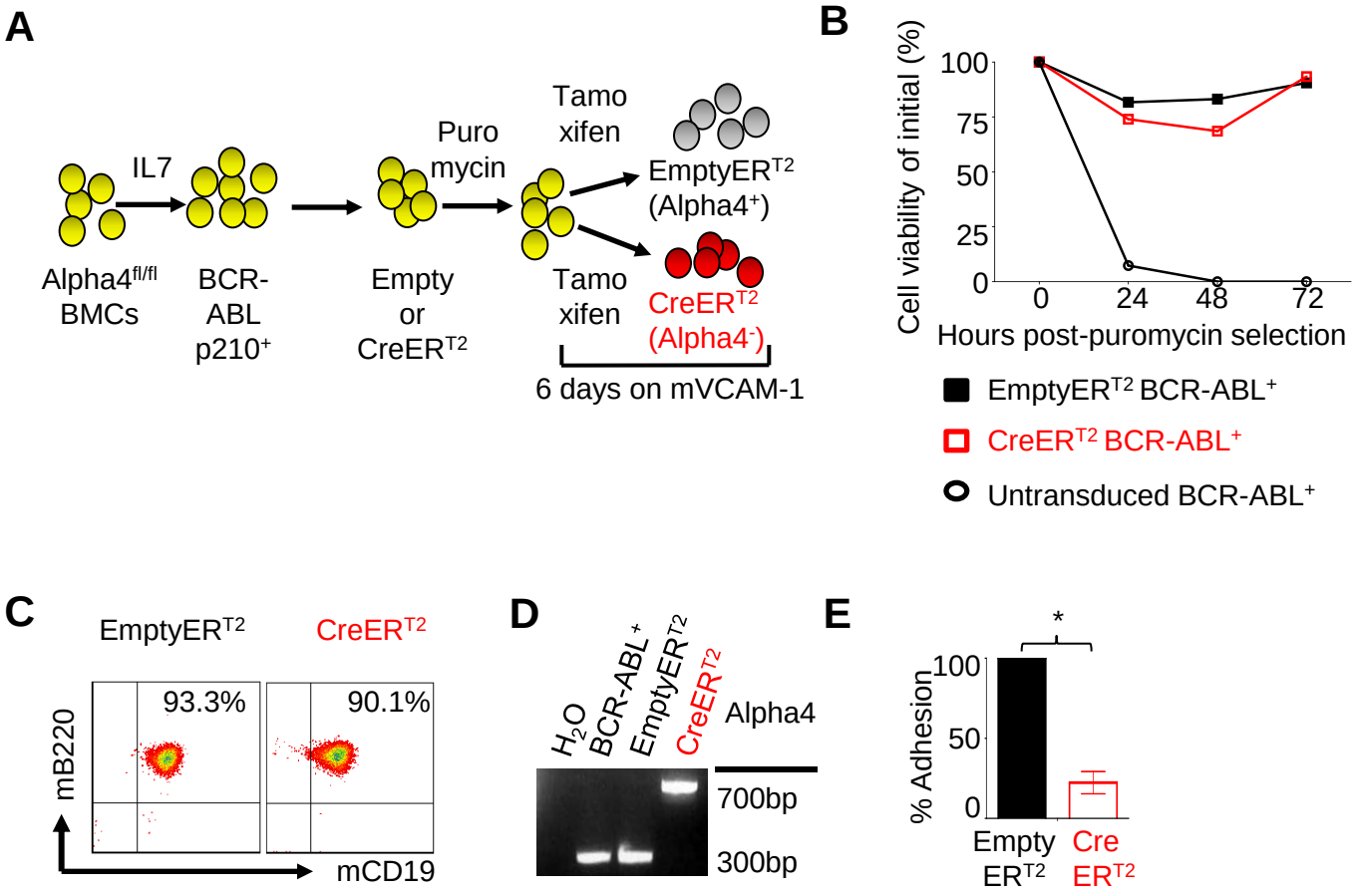
Figure S2. *Retroviral and lentiviral vectors and transduction methods*



Legend: Schematic of the MSCV-based retroviral vectors used for oncogenetic tranduction of **(A)** BCR-ABL1 p210^{+}; for conditional deletion of alpha4 **(B)** CreER^{T2} and **(C)** EmptyER^{T2} (Control) transduction. **(D)** Lentiviral pCCL-MNDU3 backbone vector was used for luciferase transduction.

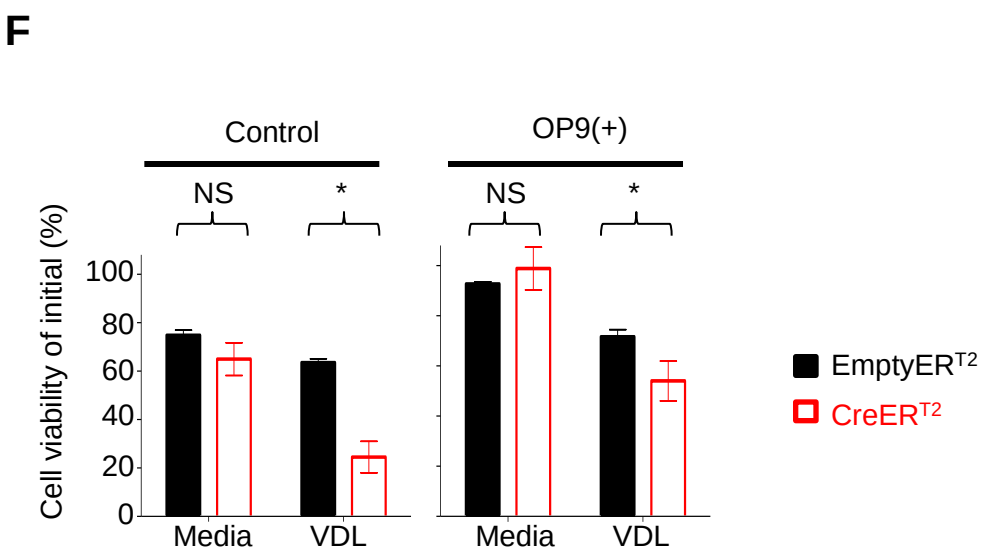
Transfections of the above MSCV-based retroviral constructs encoding BCR-ABL1, EmptyER^{T2} and Cre-ER^{T2} were performed using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) with Opti-MEM media (Invitrogen). Retroviral supernatant was produced as described previously[12]. Lentiviral supernatant was produced as described in Supplementary Methods.

Figure S3. *Conditional knockout of Integrin alpha4 in murine (BCR-ABL1⁺) leukemia*



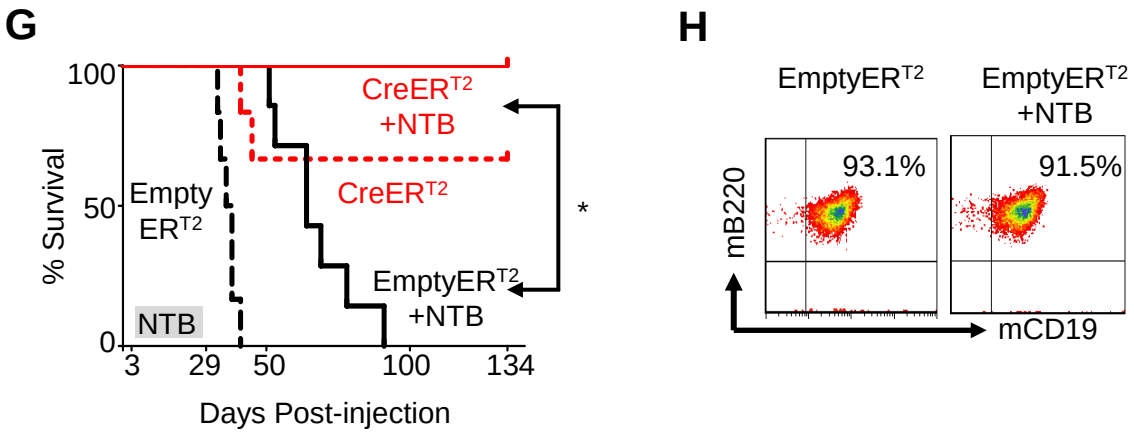
Legend. (A) Schematic of oncogenic transformation of alpha4$^{fl/fl}$ cells with BCR/ABL1 p210^{+}. **(B)** After viral transduction, integrin alpha4 EmptyER^{T2} BCR-ABL^{+} (Black) and CreER^{T2} BCR-ABL^{+} (red) cells were selected by puromycin (1µg/ml) for 72hours (Vector map). Viability of selected cells was assessed by trypan blue exclusion of dead cells and compared to control untransduced BCR-ABL1^{+} cells (Black circle). **(C)** Alpha4 EmptyER^{T2} BCR-ABL^{+} and CreER^{T2} BCR-ABL^{+} cells were stained with mouse B220 and CD19 for FACS analysis. **(D)** Deletion of alpha4 induced by Tamoxifen was confirmed 6 days post Tamoxifen incubation by PCR of genomic DNA. **(E)** Adhesion of alpha4 deleted CreER^{T2} and non-deleted EmptyER^{T2} cells on mVCAM-1 coated plates.

Figure S3. *Conditional knockout of Integrin alpha4 in murine (BCR-ABL1^{+}) leukemia*



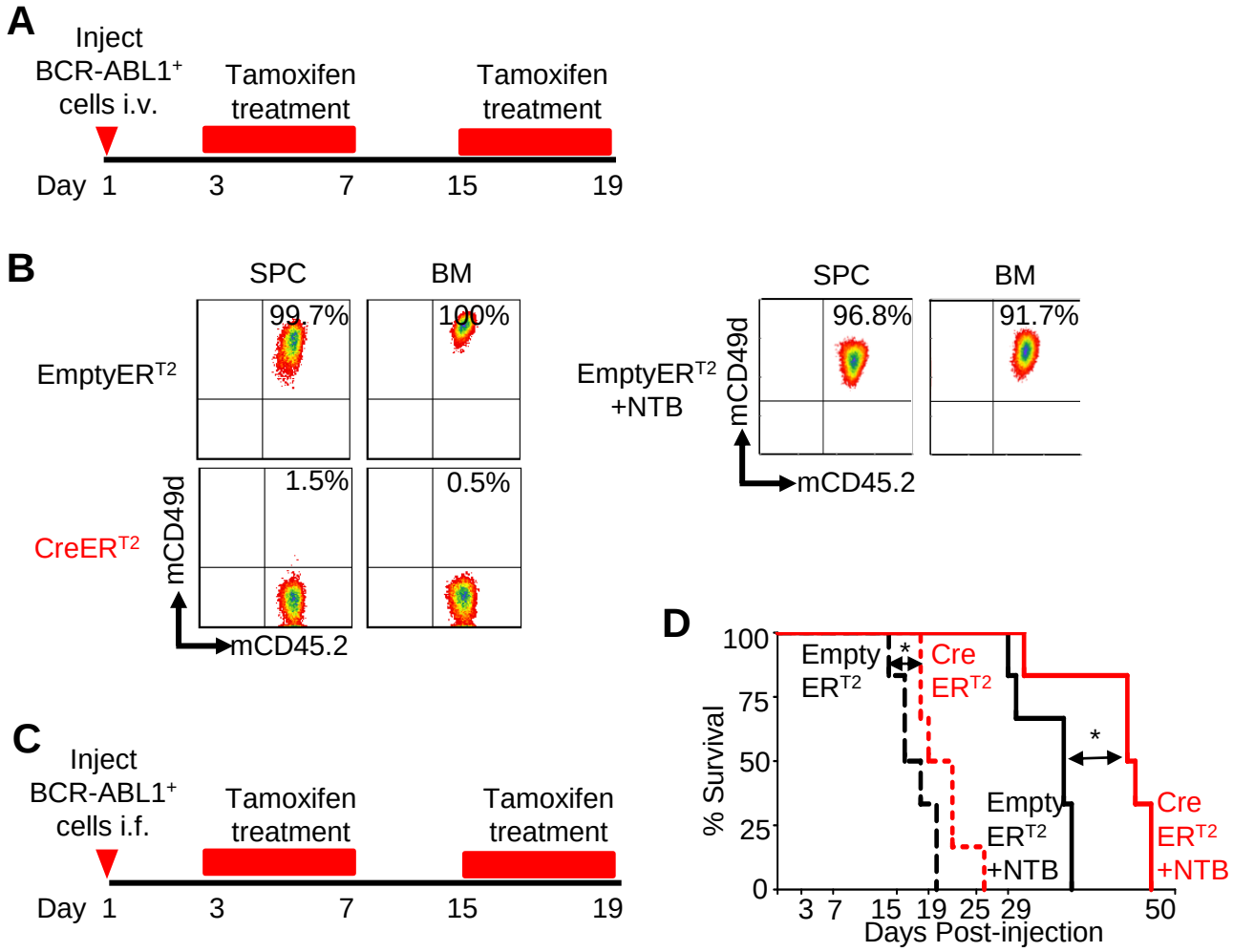
Legend. (F) Alpha4 deleted cells (CreER^{T2}:Red) and non-deleted control cells (EmptyER^{T2}: Black) were cultured with OP9(+) or without OP9 (Control). Cells were then treated with standard chemotherapy VDL (0.005 µM Vincristine, 0.05 nM Dexamethasone, 0.005 IU/ml L-Asparaginase) for 4 days. Cell viability of initial viability on Day 0 was assessed by trypan blue exclusion of dead cells. *p<0.05, performed in triplicates, mean± s.d., unpaired t-test. NS= non-significant (p>0.05).

Figure S3. *Conditional knockout of integrin alpha4 in murine (BCR-ABL1^{+}) leukemia*



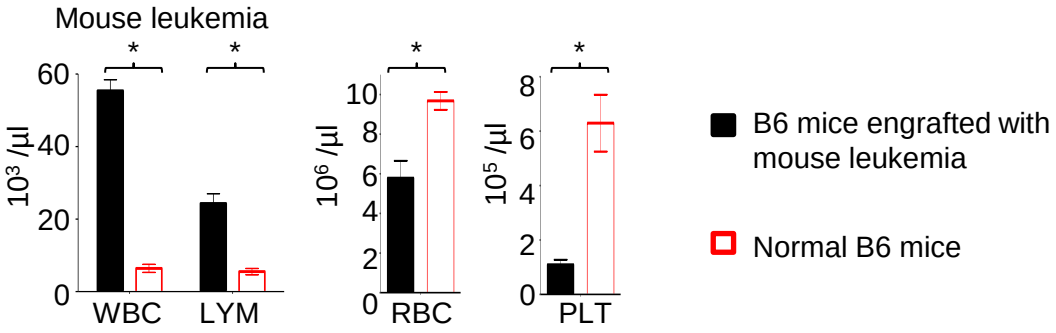
Legend. (G) Kaplan-Meier survival curve of alpha4-deleted CreER^{T2} and non-deleted EmptyER^{T2}. Cells (5x10^{5} cells/mouse) were injected into NSG mice intravenously and mice were treated with or without 50mg/kg Nilotinib (NTB). MST was calculated for each group by Log-rank Test (MST for EmptyER^{T2} = 37 days; MST for CreER^{T2} = undefined; MST for EmptyER^{T2} +NTB = 64 days MST for CreER^{T2} +NTB = undefined). * p<0.0001, n=6 /group. **(H)** Bone marrow cells from EmptyER^{T2} and EmptyER^{T2}+NTB mice at time of death were stained with mouse B220^{+}/CD19^{+} antibodies and analyzed by flow cytometry.

Figure S4. *In vivo deletion of alpha4 in murine (BCR-ABL1+) leukemia*



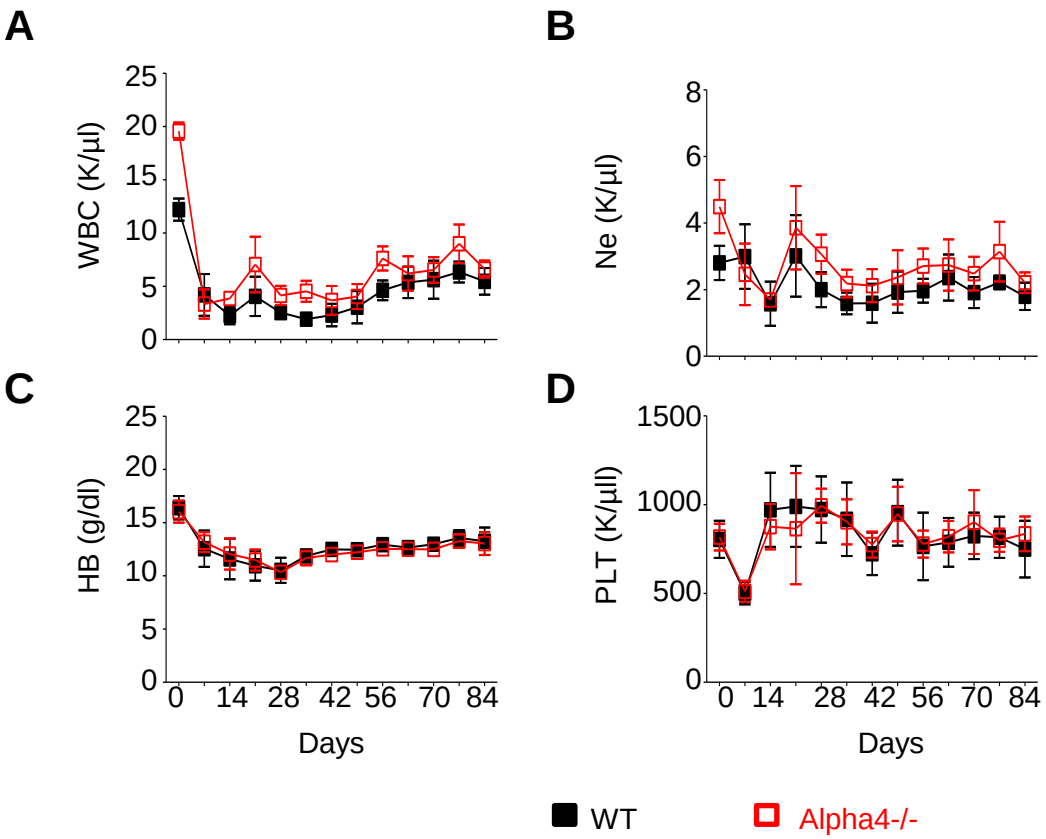
Legend. (A) Schematic for alpha4 *in vivo* deletion induced by Tamoxifen treatment (100-125 mg/kg/day). BCR-ABL1$^+$ mouse cells were intravenously (i.v.) injected into C57/BL6 Ly5.1$^+$ mice. **(B)** EmptyER^{T2} and CreER^{T2} mice of the *in vivo* experiment in Figure 1 were sacrificed on Day 15 and Day 32 post-injection, based on the sacrificing criteria. The injected murine leukemia cells were distinguished from endogenous mouse cells (CD45.1$^+$) by mouse CD45.2 expression by FACS analysis. The FACS dot plot shows complete *in vivo* deletion of mouse CD49d in spleen cells (SPC) and bone marrow (BM) cells. **(C)** Schematic for alpha4 *in vivo* deletion induced by Tamoxifen treatment (100-125 mg/kg/day). BCR-ABL1$^+$ mouse cells (1x10^5 cells/mouse) were intrafemorally (i.f.) injected into C57/BL6 Ly5.1$^+$ mice. **(D)** Kaplan-Meier survival curve. Mice were treated with or without 50mg/kg Nilotinib (NTB). MST was calculated for each group by Log-rank Test (MST for EmptyER^{T2} = 17 days; MST for CreER^{T2} = 20.5 days; MST for EmptyER^{T2} +NTB = 36 days MST for CreER^{T2} +NTB = 44.5 days). * p=0.001, n=6 /group.

E



Legend. (E) *Blood counts of recipient mice of murine pre-B ALL.* To determine the cause of death in mice, blood samples from C57BL/6 mice engrafted with BCR-ABL1+ murine leukemia were collected via tail vein bleeding for blood count analysis when mice were sacrificed. Blood samples (~100µl /mouse, 3 mice/group) were collected in BD microtainer tubes with EDTA (BD Biosciences) and analyzed by VetScan HM5 cell counter (Abaxis, Union City, CA). WBC = White blood cell, LYM = Lymphocyte, RBC = Red blood cell, PLT = Platelet. *$p<0.05$, mean±s.d. (unpaired two-tailed t-test). Experiments were performed in triplicates.

Figure S5. *Chemo-toxicity of Integrin alpha4 ablation*



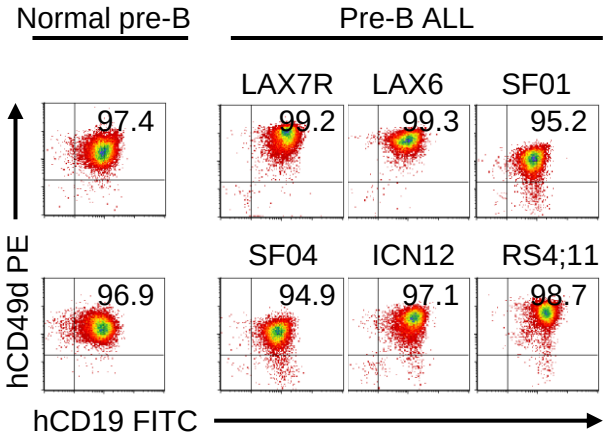
Legend. Mice with hematopoietic-specific ablation (Tie2cre-driven) of alpha4 have been previously described[7]. WT or alpha4-/- mice received sublethal irradiation followed by a 28-day course of VDL (0.55 mg/kg Vincristine, 11.5 mg/kg Dexamethasone, 880 IU/kg L-Asparaginase). Blood counts (CBC) were regularly monitored using a Hemavet hemacytometer (Drew Scientific, Barrow in Furness, UK). Indicated are total white blood cells (WBC) **(A)**, neutrophils **(B)**, hemoglobin **(C)** and platelets **(D)** after VDL treatment. n=5/group, mean±s.d. The chemotherapy was only modestly hematotoxic; the kinetics of leukocyte and erythrocyte recovery were indistinguishable.

Figure S6. *Karyotype and Integrin alpha4 expression of pre-B ALL.*

A

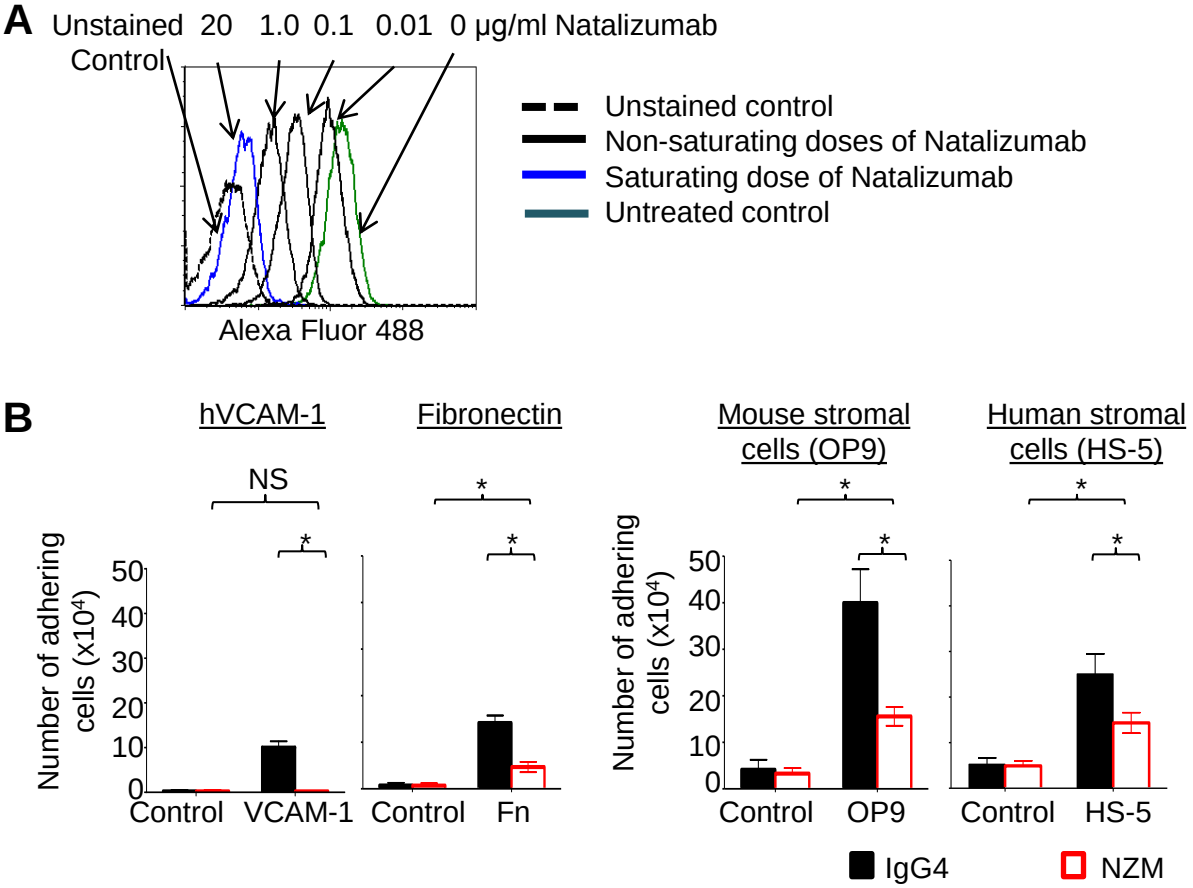
ALL #	Sample	Clinical Course	Karotypes	Alpha4 Expression
1	LAX7R	Relapse	Normal	99.2%
2	LAX6	Unknown	Unknown	99.3%
3	SF01	Relapse	Unknown	95.2%
4	SF04	Relapse	Unknown	94.9%
5	ICN12	Diagnosis	E2A-PBX	97.1%
6	RS4;11	Diagnosis	MLL-AF4	98.7%

B



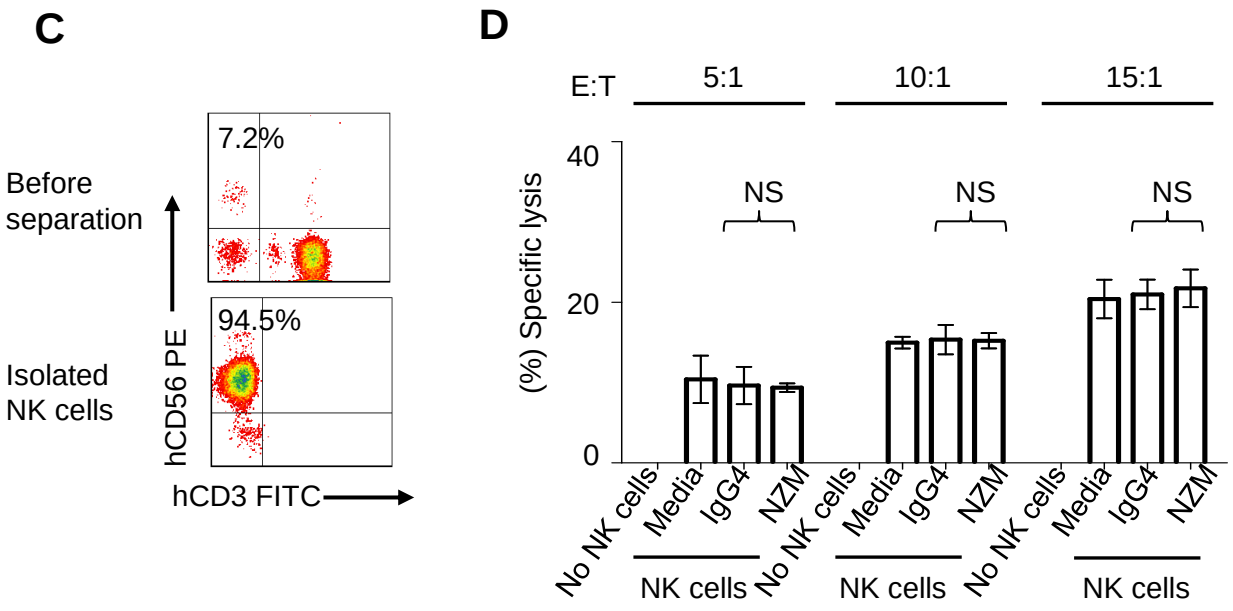
Legend: (A) Information on 5 primary pre-B ALL samples, and one ALL cell line, RS4;11. **(B)** FACS analysis of 2 normal donors and 6 pre-B ALL samples stained with anti-human CD19 FITC and anti-human CD49d PE. Percentages of CD19 and CD49d double positive populations are given.

Figure S7. *Blockade of integrin alpha4 in primary ALL by Natalizumab*



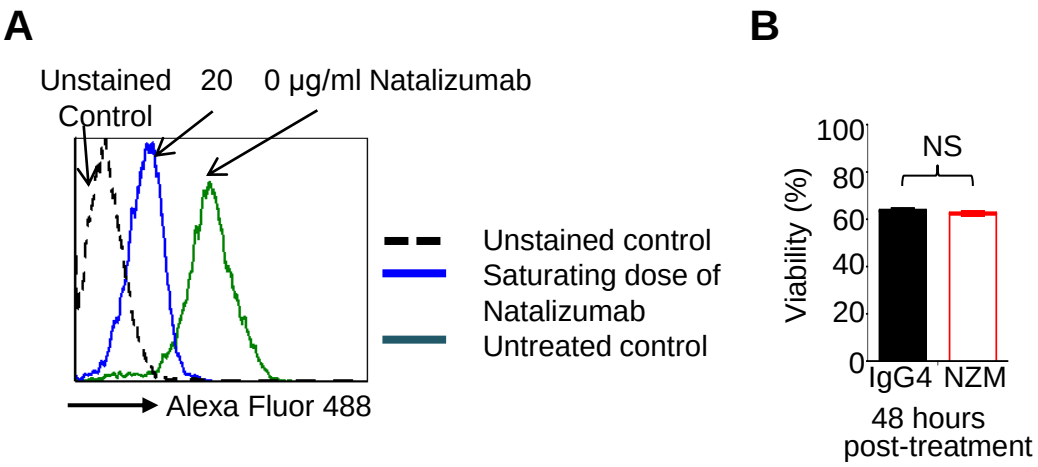
Legend. (A) To determine the saturating dose of anti-functional anti-alpha4 Ab for ALL cells, LAX7R ($1x10^6$) were treated with different doses Natalizumab (NZM) (0-20 µg/ml) for 30 minutes and washed with PBS once, subsequently stained with Alexa Fluor 488 labeled NZM for 30 minutes and again washed once with PBS. Histograms show unstained control (Black dashed line), non-saturating doses of NZM (black), the saturating dose of NZM (red line) and untreated control (green). NZM: p=0.0001 (0, 0.01 and 0.1 µg/ml), p=0.008 (1.0 µg/ml) compared with saturating dose (20 µg/ml). **(B)** LAX7R cells were pre-incubated with NZM or control IgG4 Ab and subsequently seeded on plates coated with or without human (h)VCAM-1, fibronectin (Fn), mouse stromal cells OP9, or human stromal cells HS-5 for 2 hours. Control groups for VCAM-1 and Fn were cultured in 2% BSA-coated non-tissue culture plates. For mouse or human stromal cells groups, tissue culture plates were used as the control. Number of adhering cells were counted by trypan blue exclusion of dead cells. NZM treatment significantly decreased the binding of primary ALL cells to hVCAM-1, Fn, and mouse and human stromal cell lines. *p<0.05. NS= non-significant (p>0.05), performed in triplicates, mean± s.d., unpaired t-test.

Figure S7. *Blockade of integrin alpha4 in primary ALL by Natalizumab*



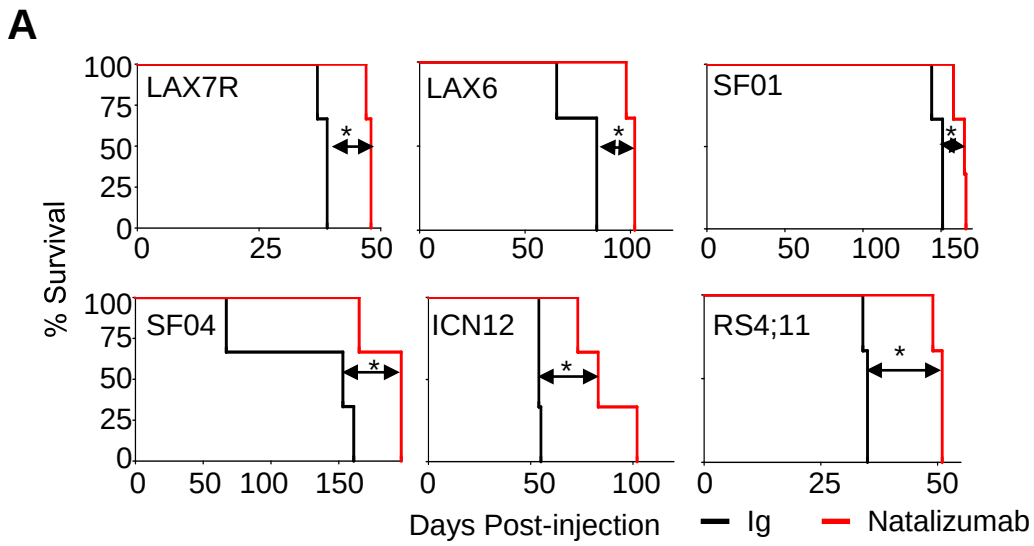
Legend: *Natalizumab (NZM) does not induce antibody-dependent cell-mediated cytoxicity (ADCC) on human leukemia cells.* **(C)** NK cells were >94% $CD56^{+}CD3^{-}$ as determined by flow cytometry**. (D)** Calcein-AM release assay was performed in triplicates by using Calcein-AM labeled human leukemia cells (target cells, T) with isolated NK cells (effector cells, E). Cells were incubated for 4 hours and % of specific lysis was calculated as described in Supplementary Method. NS = non-significant ($p>0.05$), mean± s.d., unpaired t-test.

Figure S8. *Viability of normal pre-B cells is unaffected by Natalizumab*



Legend. Normal pre-B cells were sorted by magnetic separation using human CD19 microbeads (Miltenyi Biotec, Auburn, CA). **(A)** The saturating dose of NZM for normal pre-B cells was determined as 20 µg/ml by staining cells with Alexa Fluor 488 labeled NZM, similarly to primary ALL cells in Figure S7. **(B)** 48 hours *in vitro* incubation with NZM showed no difference in viability as compared to the control IgG4-treated cells. NS= non-significant ($p>0.05$). This experiment was performed in triplicates.

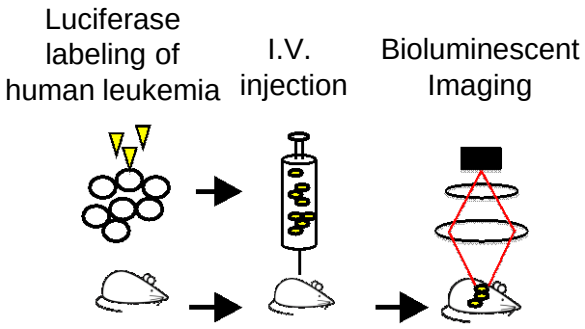
Figure S9. *Integrin alpha4 blockade prolongs survival of NSG mice engrafted with primary pre-B ALL and demonstrates de-adhesion effect of this treatment in vivo.*



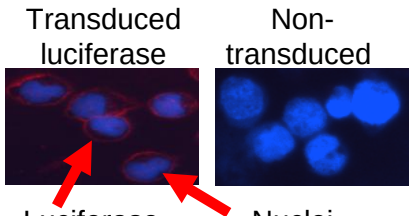
Legend: 6 pre-B ALL samples labeled with lentiviral luciferase were pre-treated with either Natalizumab or control AB for 30 minutes and injected intravenously into NSG mice. **(A)** Kaplan-Meier survival curves of 6 pre-B ALL samples pre-treated with Natalizumab (Red) or control AB (black) (n=3/per leukemia case; n=18 /group) were analyzed for significance using the Log-rank Test. *p<0.05. (**B)** Pooled Kaplan-Meier survival curve with median survival time: Natalizumab (102 days) vs. Ig (67 days). *p= 0.03, Log-rank Test.

Figure S10. *Lentiviral transduction efficiency and leukemogenesis of primary ALL cells*

A



B

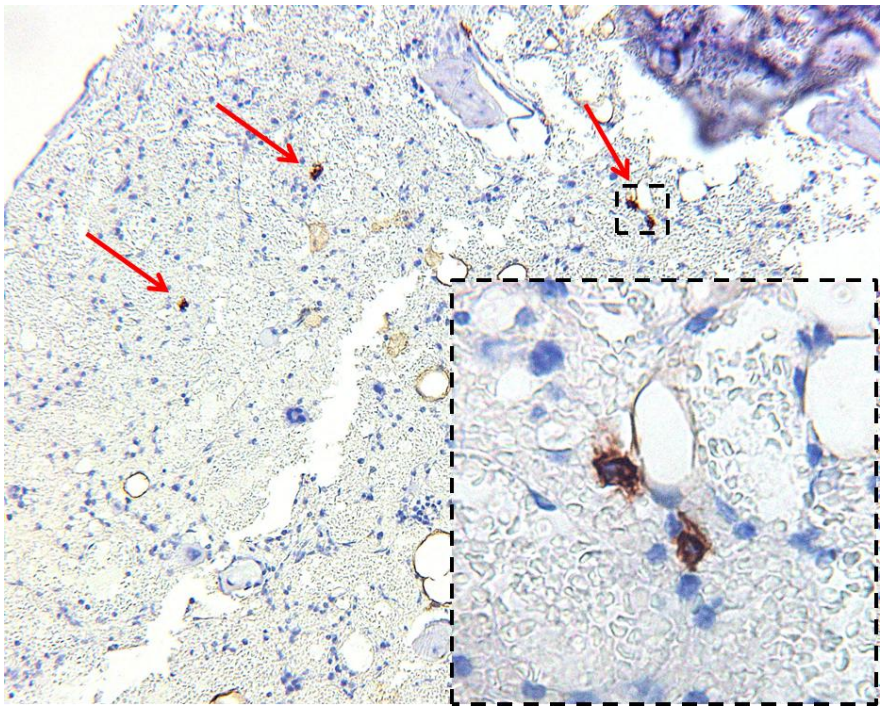


Total Number of cells	Transduced cells	Transduction efficiency (%)
47±11	37±11	79±9

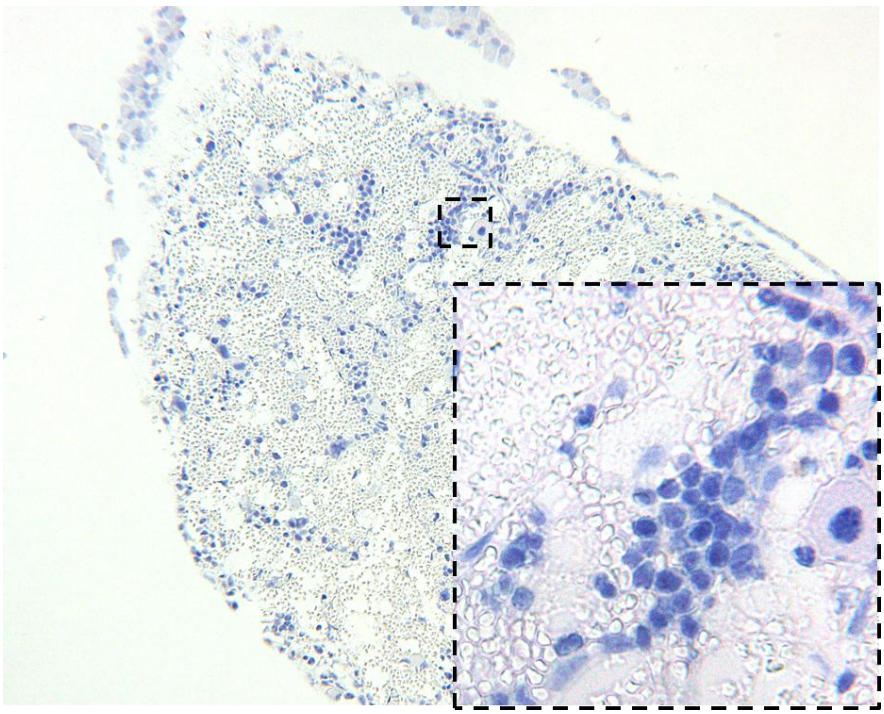
Legend. (A) Schematic of bioluminescent imaging **(B)** Transduced luciferase LAX7R cells showed red cytoplasm and blue nuclei by immunofluorescence compared with non-transduced cells as the control. 200X magnification. Transduction efficiency of LAX7R was calculated by dividing the number of transduced cells by the number of total cells.

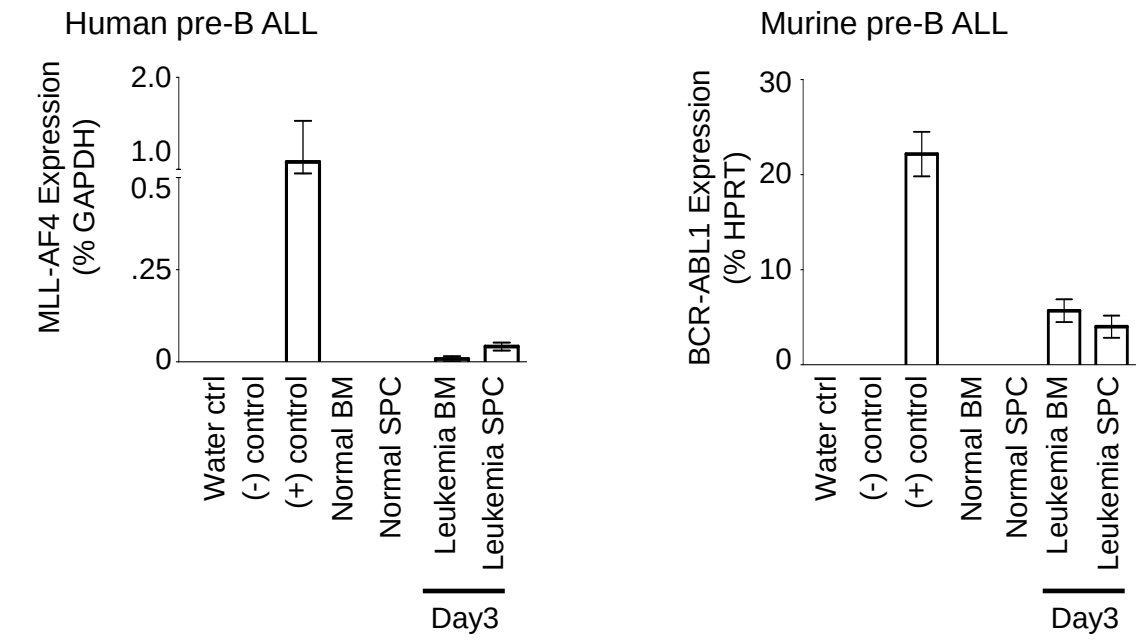
Figure S11. *Engraftment of leukemia cells in vivo 3 days after injection*

A 3 days after injection of primary ALL (LAX7R) ($CD45^{+}$) into NOD/SCID $IL2R\gamma^{-/-}$ mouse



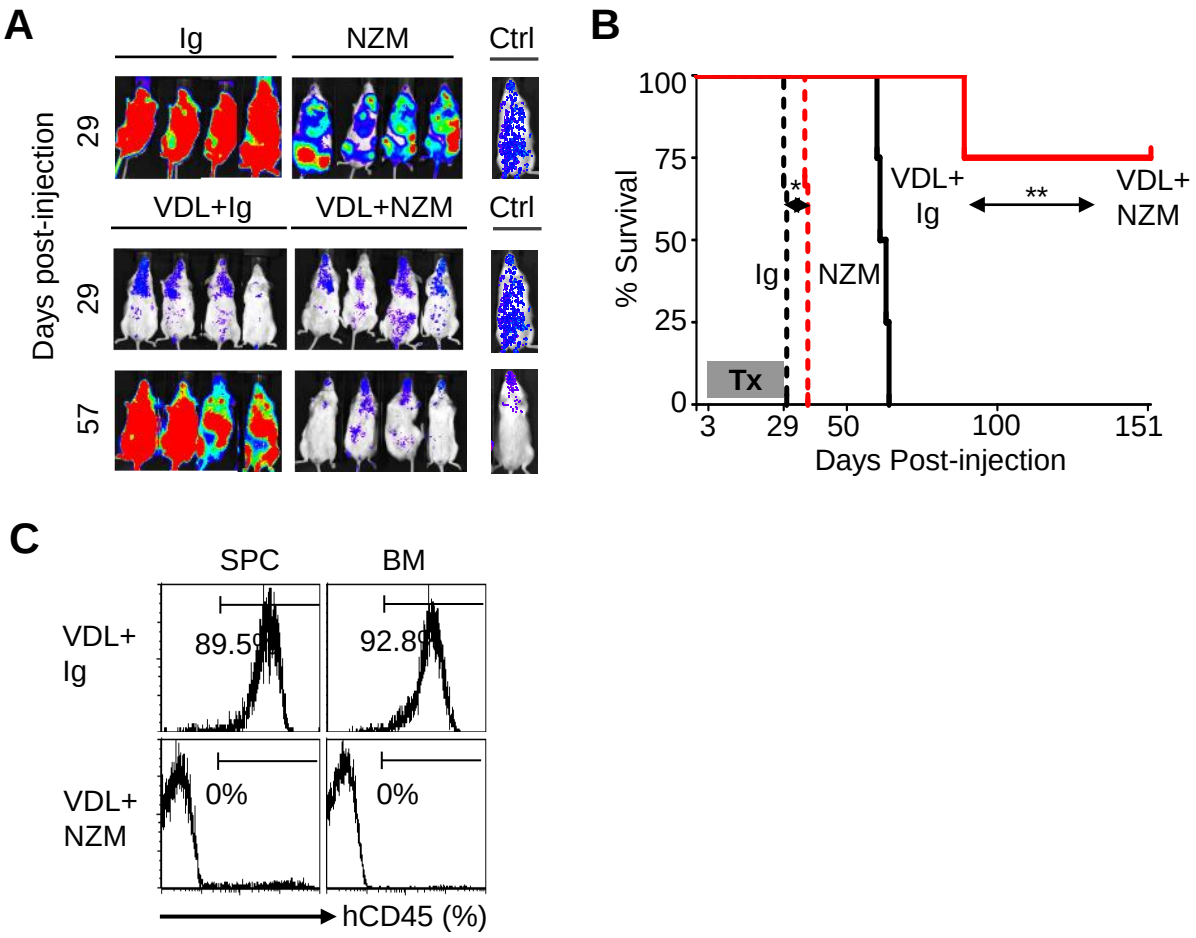
B Control NOD/SCID $IL2R\gamma^{-/-}$ mouse: No leukemia injected.





Legend. (A) Primary leukemia cells (LAX7R) were injected into NSG mice ($5x10^4$ cells/mouse) (n=3) and sacrificed on Day 3 post-cell injection. Femoral bones were immunostained with human CD45 antibody (Brown) to detect the injected primary leukemia cells (Red arrows) with counterstain by hematoxylin. **(B)** Control mouse did not receive primary leukemia cells (n=3). 400x magnification. To determine the engraftment of injected leukemia cells in animals, **(C)** human pre-B ALL RS4;11 (MLL-AF4) cells were injected into three NSG mice. Mice were sacrificed on Day 3 post-injection and mouse bone marrow (BM) and spleen cells (SPC) were studied for MLL-AF4 mRNA levels by quantitative RT-PCR. **(D)** Murine BCR-ABL1$^+$ leukemia cells were injected into three C57BL/6 mice and sacrificed on Day 3 post-injection. For engraftment studies, mouse BM and SPC tissue cells were screened for BCR-ABL1 mRNA levels by quantitative RT-PCR. Healthy NSG or C57BL/6 mice with no injection of leukemia cells were used as normal BM and SPC for negative (-) control. RS4;11 and murine BCR-ABL1$^+$ leukemia cells were used as positive (+) control.

Figure S12. *Combined integrin alpha4 blockade and chemotherapy eradicates primary ALL (LAX7R) in NSG recipient mice.*



Legend. Luciferase-labeled LAX7R cells were injected into sublethally irradiated NSG mice ($5x10^4$ cells/mouse). Recipient mice were treated with Immunoglobulin (Ig) or anti-alpha4 mAb, Natalizumab (NZM) (5 mg/kg), intraperitoneally once per week for 4 weeks, with or without chemotherapy treatment (Tx), VDL (V: Vincristine 0.8 mg/kg, once per week; D: Dexamethasone 10.5 mg/kg, 5 days/week; L: L-Asparaginase 800 IU/kg, 5 days/week) 3 days after leukemia injection. **(A)** Bioluminescent imaging of experimental groups on Day 29 and 57 post-cell injection and background control mouse (Ctrl) with no leukemia injection. **(B)** Kaplan-Meier Survival analysis: The Median Survival time (MST) for each group (n=4) is: Ig 30 days, NZM 37 days, VDL+Ig 62 days. The animals treated with combined VDL+NZM survived until the experiment was terminated on Day 151 post-cell injection. *p=0.03 between Ig and NZM treated groups, **p=0.007 between VDL+Ig and VDL+NZM treated groups, Log-rank Test. **(C)** The absence of human leukemia cells in the VDL+NZM group determined by flow cytometry (human CD45) in spleen cells (SPC) and bone marrow (BM) cells on the day of sacrificing mice.

Figure S13. *Combined integrin alpha4 blockade and chemotherapy eradicate pre-B ALL (RS4;11).*

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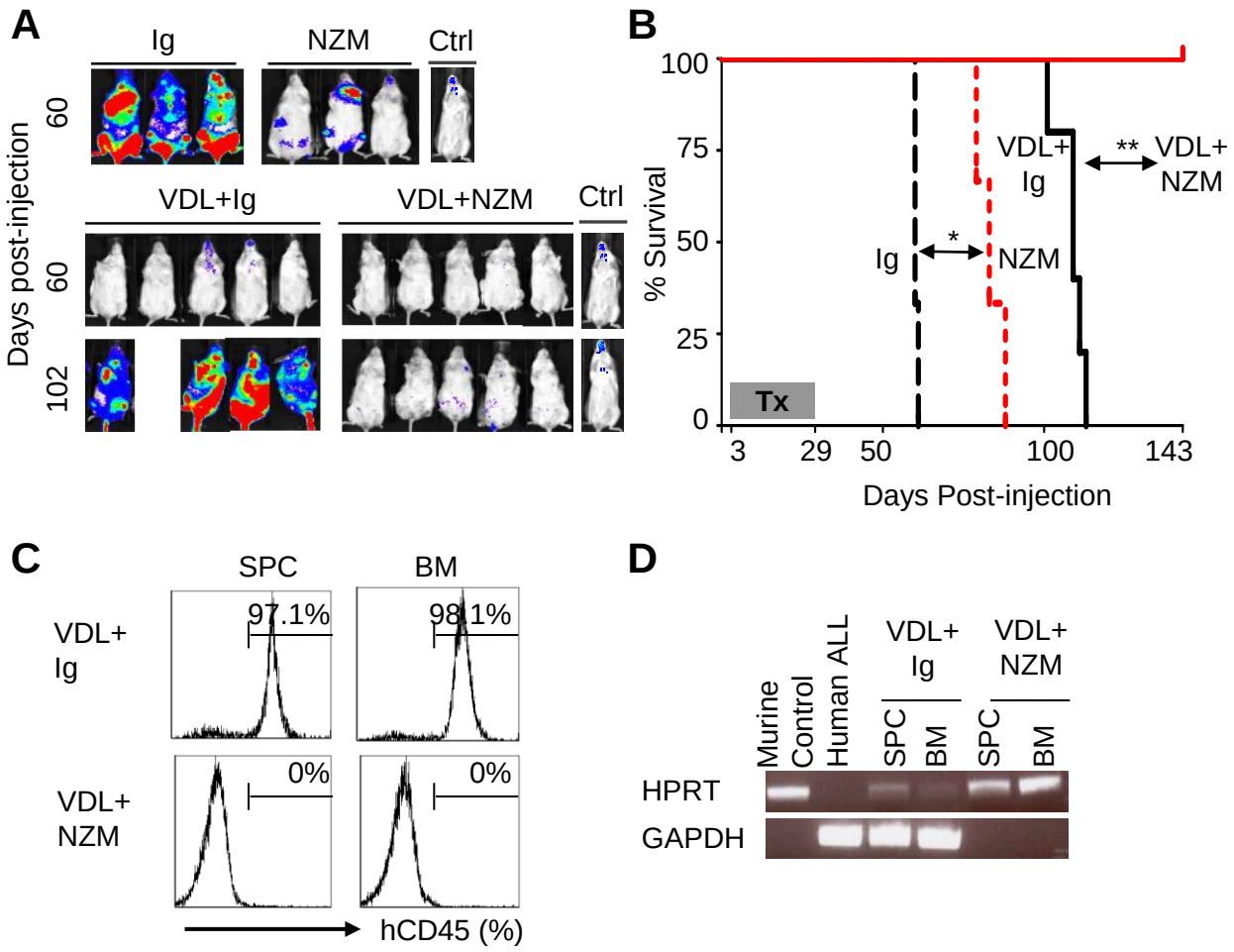
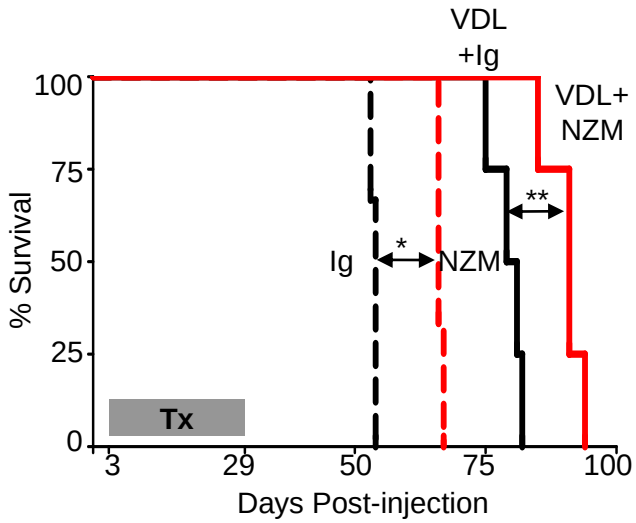


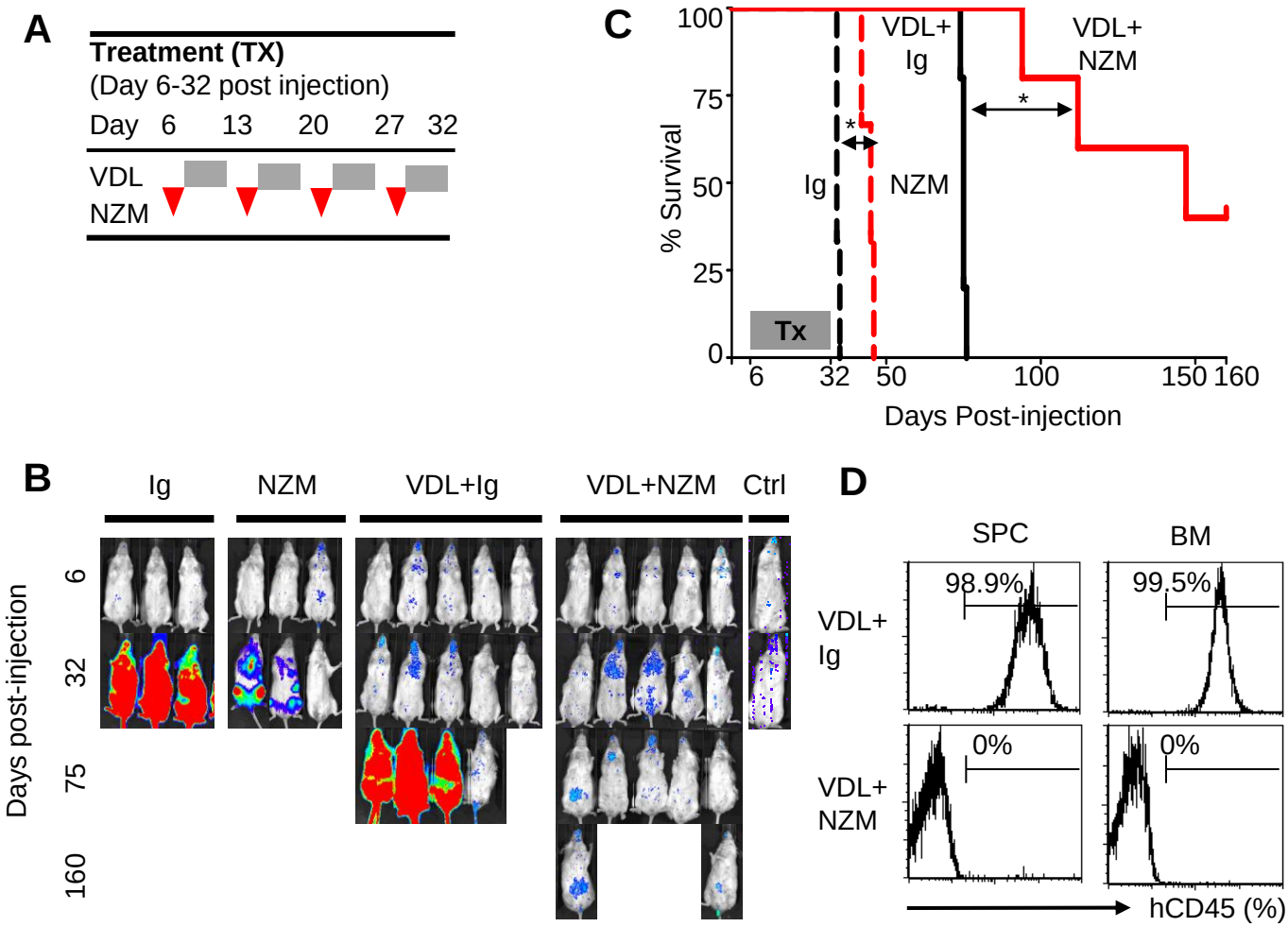
Legend. (A) Bioluminescent imaging of mice receiving Ig (n=3), Natalizumab (NZM) (n=3), chemotherapy treatment (Tx) VDL (V: Vincristine 0.8 mg/kg, once per week; D: Dexamethasone 10.5 mg/kg, 5 days/week; L: L-Asparaginase 800 IU/kg, 5 days/week)+Ig (n=5) and VDL+Natalizumab (NZM) (n=5) treatments on Day 60 and Day 102 after leukemia cell transfer. Bio-imaging control mouse (Ctrl) with no leukemia injection. **(B)** Kaplan-Meier survival curve was analyzed and MST was calculated for each group: Ig:0 days, NZM:83 days, VDL+Ig:109 days. VDL+Natalizumab was sacrificed at end of follow-up, Day 143 post leukemia injection. *p=0.02, **p=0.002, Log-rank Test. **(C)** The absence of human RS4;11 cells in spleen cells (SPC) and bone marrow (BM) of the VDL+Natalizumab group was determined by flow cytometry using an anti-human CD45 Ab on the day of sacrificing mice. **(D)** The presence of murine and human cells in spleen (SPC) and bone marrow (BM) was evaluated using PCR for murine HPRT and human GAPDH, respectively on the day sacrificing mice. The decreasing mouse HPRT expression indicates less mouse cells remaining in bone marrow of the mice treated with VDL+Ig. Pre-B ALL cells are known to initiate leukemia in the bone marrow replacing resident hematopoiesis, and then spread to other organs.

Figure S14. *Integrin alpha4 blockade prolongs survival of murine recipients of primary pre-B ALL cells (ICN12).*



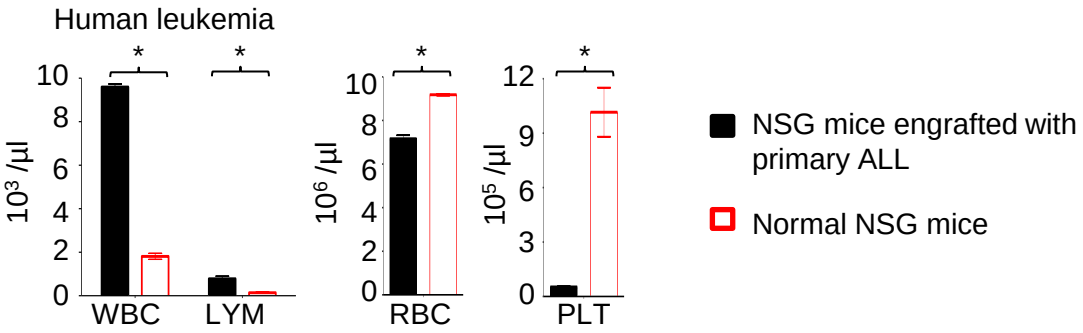
Legend. Pre-B ALL cells, ICN12, were injected into NOD/SCID mice ($5x10^4$ cells/mouse). 3 days after leukemia injection, mice were treated for 4 weeks with Ig, Natalizumab (NZM), Ig+VDL (V: Vincristine 0.8 mg/kg/d, once per week; D: Dexamethasone 10.5 mg/kg/d, 5 days/week; L: L-Asparaginase 800 IU/kg/d, 5 days/week), or NZM+VDL. Kaplan-Meier survival curves were analyzed and median survival time (MST) was calculated for each group: Ig:54 days (n=3); NZM:66 days (n=3) , VDL+Ig: 80 days (n=4); VDL+NZM:91 days (n=4). *p=0.03; **p=0.007, Log-rank Test.

Figure S15. *Late alpha4 blockade (Day 6 post-injection of LAX7R) prolongs survival.*



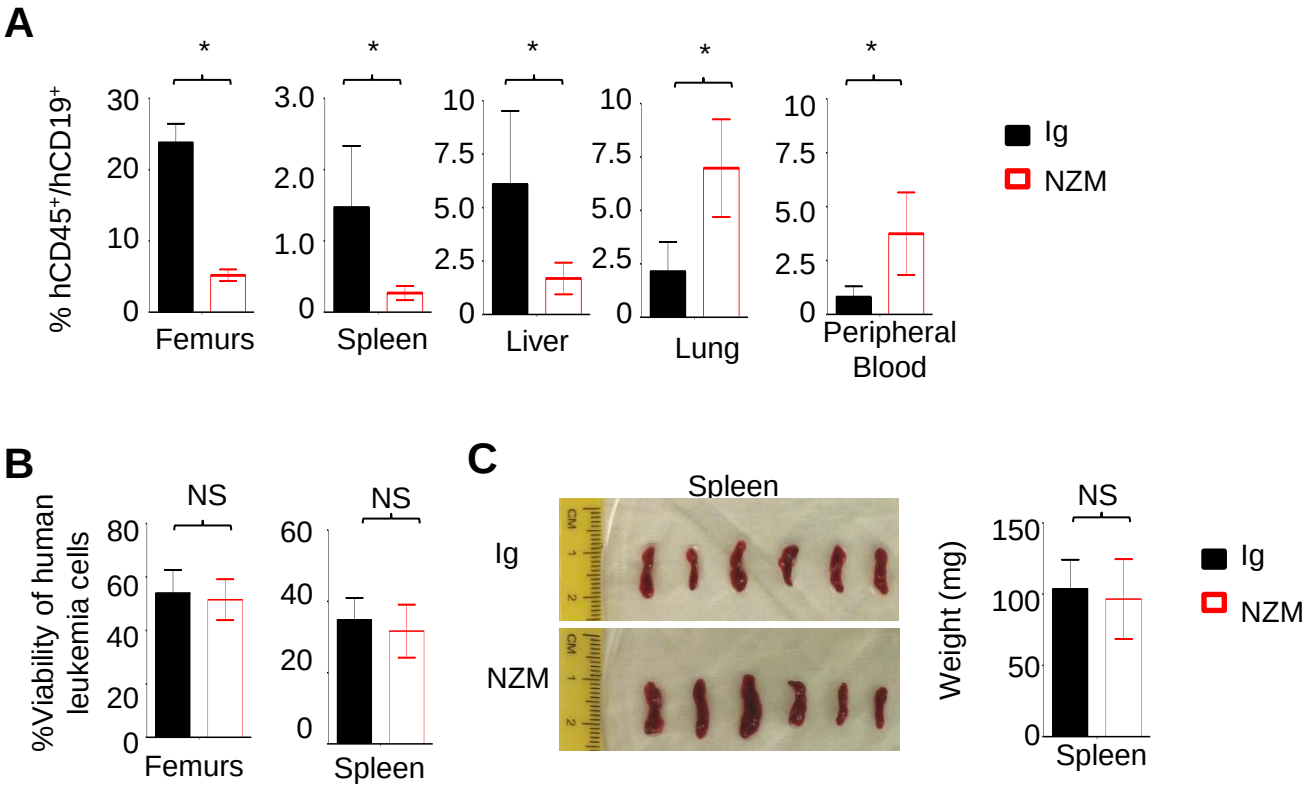
Legend. (A) (B) LAX7R NOD/SCID recipient mice ($5x10^4$ cells/mouse) were treated from Day 6 to Day 32 with Ig (n=3), Natalizumab (NZM) (n=3), VDL (V: Vincristine 0.8 mg/kg/d, once per week; D: Dexamethasone 10.5 mg/kg/d, 5 days/week; L: L-Asparaginase 800 IU/kg/d, 5 days/week) +Ig (n=5) or VDL+NZM (n=5) and the engraftment of leukemia cells was monitored by bioImaging on Day 6, 32, 75 and 160 post-cell injection. A mouse with no leukemia injection was used as background control (Ctrl). **(C)** Kaplan-Meier survival curve was analyzed and median survival time (MST) determined for each group: Ig: 34 days, NZM: 45 days, VDL+Ig: 75 days, VDL+NZM: 147 days. * $p<0.05$, Log-rank Test. **(D)** Spleen cells (SPC) and bone marrow (BM) cells were stained with human CD45 (hCD45) antibody for FACS analysis from the group treated with VDL+Ig (sacrificed day 75 post-leukemia injection) and VDL+NZM (Mice survived to day 160 and were sacrificed on day 160 post-cell injection).

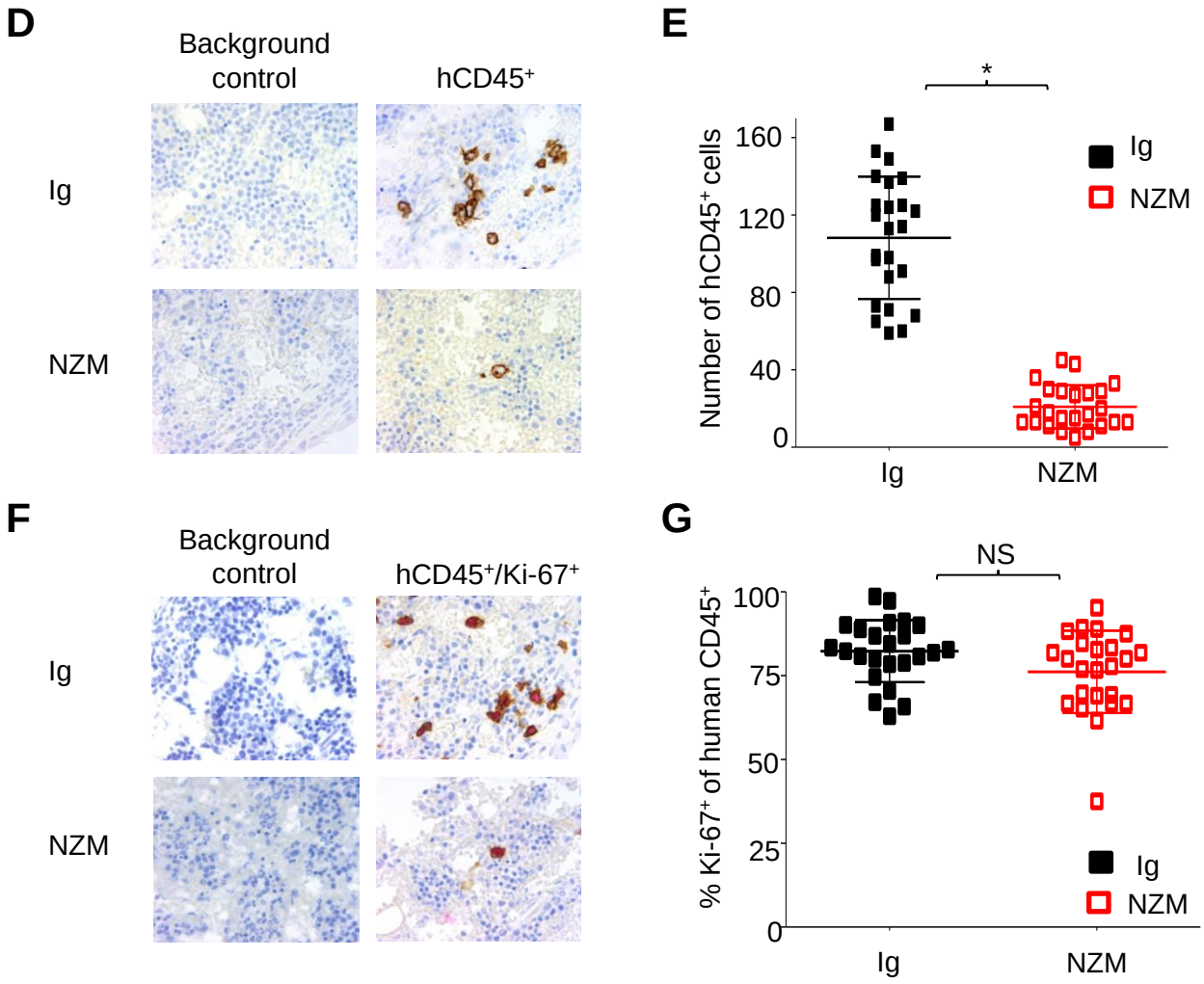
E



Legend. (E) *Blood counts of recipient mice of human pre-B ALL.* To determine the cause of death in mice, blood samples NSG mice engrafted with human pre-B ALL (LAX7R) were collected via tail vein bleeding for blood count analysis when mice were sacrificed. Blood samples (~100µl /mouse, 3 mice/group) were collected in BD microtainer tubes with EDTA (BD Biosciences) and analyzed by VetScan HM5 cell counter (Abaxis, Union City, CA). WBC = White blood cell, LYM = Lymphocyte, RBC = Red blood cell, PLT = Platelet. *$p<0.05$, mean±s.d. (unpaired two-tailed t-test). Experiments were performed in triplicates.

Figure S16. Mobilization of primary leukemia cells by Natalizumab.





Legend. To determine the mobilization effect, primary ALL (LAX7R) cells were injected into NOD/SCID mice ($5x10^4$ cells/mouse; n=6/group). Mice were treated with Natalizumab (NZM) or Ig on Day 14 post-leukemia injection. **(A)** White blood cells from femurs, spleen, lung, liver and peripheral blood were analyzed at 60 hours post-NZM treatment and stained with human CD19 and CD45 antibodies to distinguish human pre-B ALL cells from mouse recipient cells. Percentages of $hCD45^+/hCD19^+$ cells were analyzed by flow cytometry. **(B)** Viability of human pre-B ALL cells ($hCD45^+/hCD19^+$) in femurs and spleen was determined by PI staining and flow cytometry. **(C)** Spleens in Ig and NZM groups were photographed and the weights were measured (n=6/group). **(D)** Femur section with immunohistochemistry staining showed more $hCD45^+$ human leukemia cells (brown) in the Ig-treated group. Background control was the secondary antibody for hCD45 staining. 400x magnification **(E)** Numbers of $hCD45^+$ cells in each section was quantified. **(F)** $hCD45^+/Ki\text{-}67^+$ double staining was performed to visualize proliferative ($Ki\text{-}67^+$) human $hCD45^+$leukemia cells in femurs. **(G)** The % Ki-67 was defined by the percent of $Ki\text{-}67^+$ cells (red nucleus) per 500 human leukemia cells ($hCD45^+$)[25]. 20 sections /group. * $p<0.05$, mean±s.d. (unpaired two-tailed t-test). NS= non-significant ($p>0.05$)