Supplementary Methods:

Ethical statements

Written informed consent was obtained from all patients and from parents/carers in the cases where the patients were minors. The study was performed in accordance with the ethical standards of the responsible committee on human experimentation (written approval by Ethikkommission des Klinikums der Ludwig-Maximilians-Universität München, Ethikkommission@med.unimuenchen.de, April 2008, number 068-08 and October 2010, number 222-10) and with the Helsinki Declaration of 1975, as revised in 2000.

All animal trials were performed in accordance with the current ethical standards of the official committee on animal experimentation (written approval by Regierung von Oberbayern, poststelle@reg-ob.bayern.de, September 2010, number 55.2-1-54-2531-95-10, October 2012, number 55.2-1-54-2531.6-10-10 and August 2016, number 55.2-1-54-2532.0-56-2016).

Primary sample sources

Fresh bone marrow (BM) or peripheral blood (PB) aspirates from adult patients with acute lymphoblastic leukemia (ALL) were obtained from the Department of Medicine III, University Hospital, LMU Munich, Germany or Department of Medicine III – Hematology, Oncology and Palliative Care, Rostock University Medical Center, Rostock, Germany.

Acute myeloid leukemia (AML) aspirates were obtained from the Department of Medicine III, University Hospital, LMU Munich, Germany.

Pediatric ALL samples were obtained from the ALL-BFM study group in Kiel, from the Dr. von Haunersches Kinderspital, Ludwig-Maximilian Universität, Munich, Germany.

Figure S1: Overall engraftment and time required for passage 0 of primary adult ALL samples

Means of the data shown in printed Figure 1A or B.

Figure S2: Generation of GEPDX cells *in vivo* in mice

GEPDX cells were generated by repetitive passaging of PDX cells in immunodeficient NSG mice. First, primary cells of acute leukemia patients were engrafted in mice. After reisolation from bone marrow and spleen, cells were genetically engineered using lentiviruses. After another passage in mice, positively transduced cells were enriched by fluorescent-activated cell sorting. AL, acute leukemia; PDX, patient derived xenograft; GEPDX, genetically engineered PDX.

Figure S3: Lentiviral transduction allows bioluminescent *in vivo* imaging of GEPDX cells

After lentiviral transduction of PDX cells, expression of a luciferase, e.g., Gaussia Luciferase, enables leukemia growth monitoring *in vivo* over time. PDX, patient derived xenograft; GEPDX, genetically engineered PDX.

Figure S4: Cell viability after 5 days *in vitro* in culture.

Cell viability was analyzed using forward scatter against side scatter (FSC/SSC). Analysis shown representatively for two samples, PDX ALL-707 and PDX ALL-763, comparing

frozen/thawed and freshly isolated samples after 5 days *in vitro* in culture. PDX, patient derived xenograft; ALL, acute lymphoblastic leukemia.

Figure S5: Lentiviral transduction of acute leukemia PDX cells

A Schematic representation of the lentiviral construct used for PDX cell transduction.

B, C Gating strategy to enrich genetically engineered PDX (GEPDX) cells shown representatively for PDX ALL-223 and AML-393. (**B:** dot plot and **C:** histogram) Cells were always pregated on living cells using forward scatter against side scatter (FCS/SSC) (data not shown). EF1α, Elongation factor 1 alpha; Gluc, Gaussia Luciferase; mtagBFP, monomeric tag blue fluorescent; PDX, patient derived xenograft; GEPDX, genetically engineered PDX.

Table S1: Patients´ clinical data and sample characteristics

BM, bone marrow; PB, peripheral blood; UPN, unique patient number; f, female; m, male; n.d., not determined.

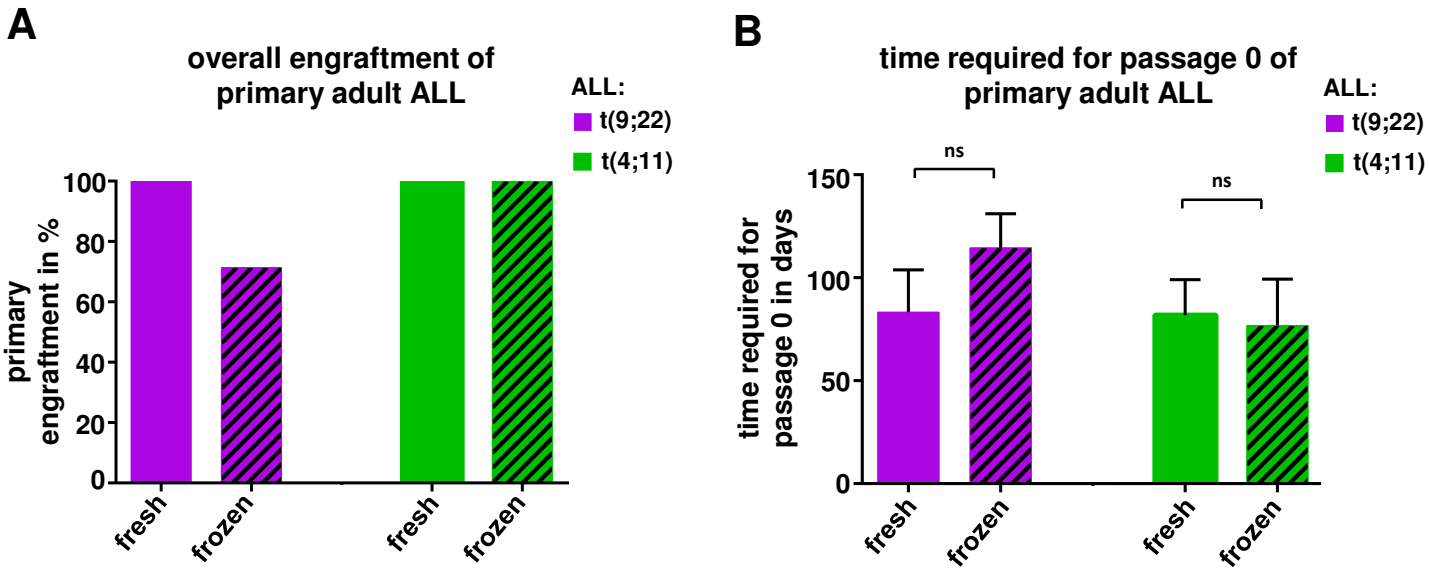
Table S2: Sample source from BM or PB and time required for P0

BM, bone marrow; PB, peripheral blood; P0, passage 0.

Table S3: Engraftment of primary adult rearranged ALL samples

Engraftment data of all samples; raw data to Figures 1A, 1B and S1.

Figure S1



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Means of the data shown in printed Figure 1A or B.

Figure S2

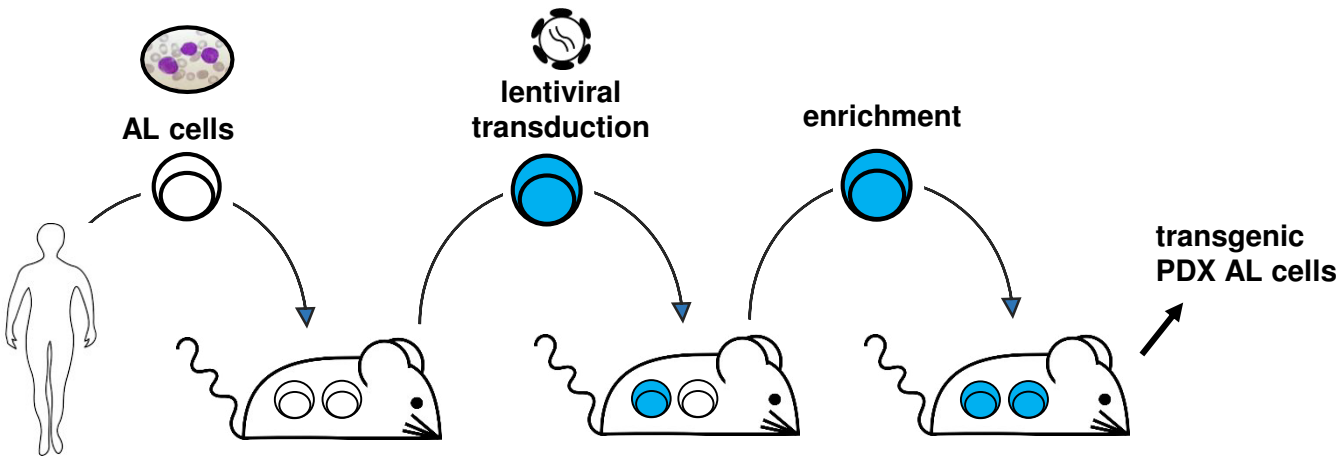

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Figure S3

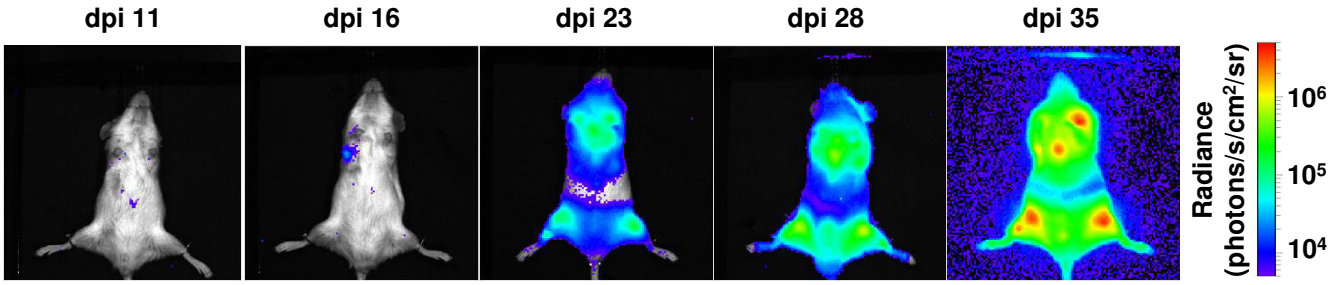

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Figure S4

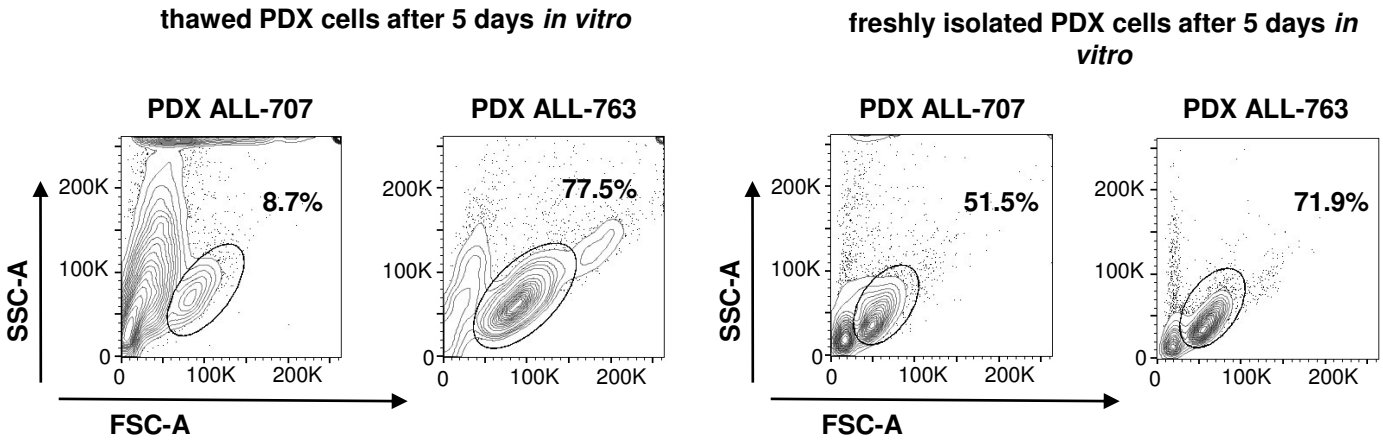

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Figure S5

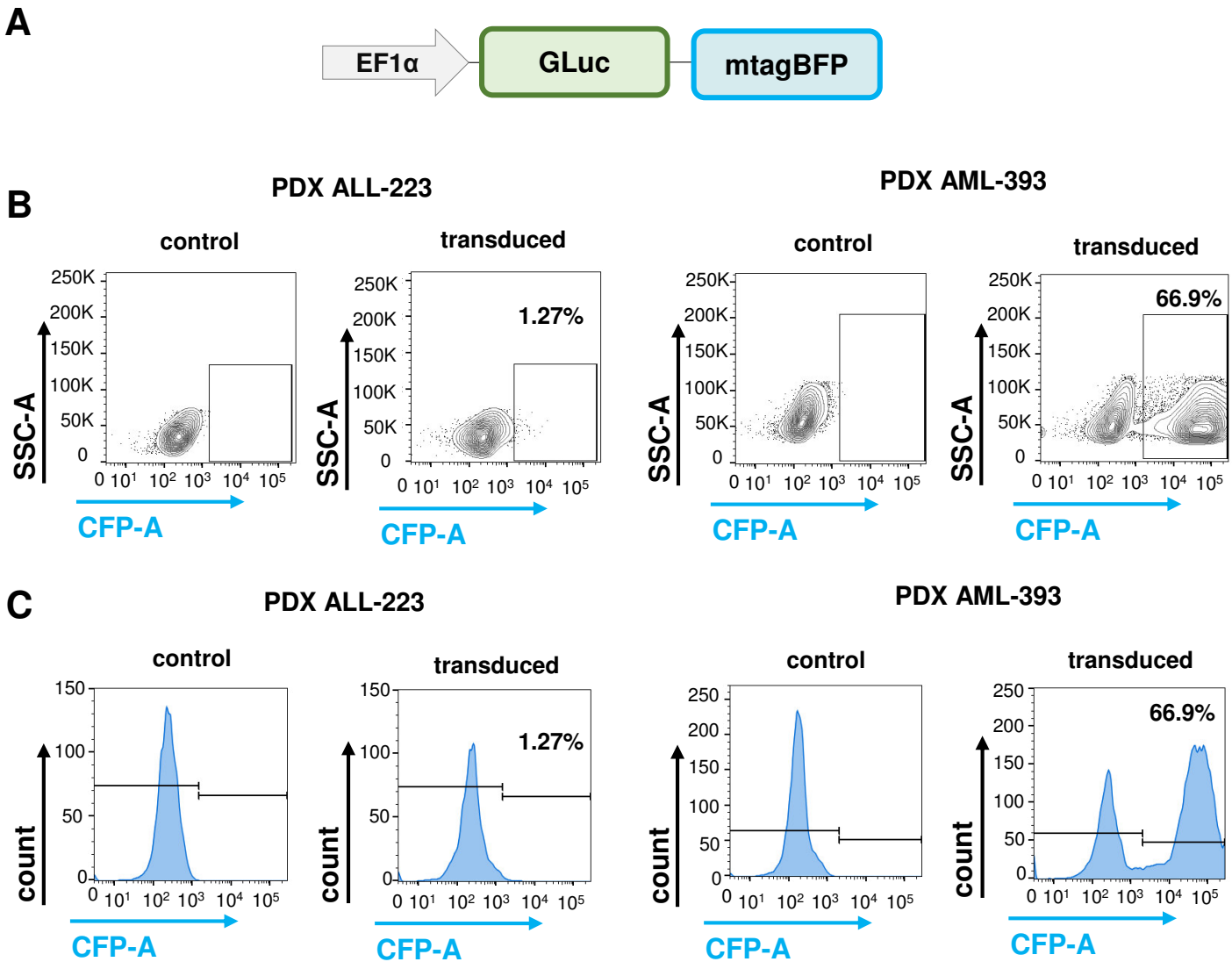

Figure S5: Lentiviral transduction of acute leukemia PDX cells
A: Schematic representation of the lentiviral construct used for PDX cell transduction. **B and C:** Gating strategy to enrich GEPDX cells shown representatively for PDX ALL-223 and AML-393. (**B:** dot plot and **C:** histogram) Cells were always pregated on living cells using forward scatter against side scatter (FCS/SSC) (data not shown). EF1α, Elongation factor 1 alpha; Gluc, Gaussia Luciferase; mtagBFP, monomeric tag blue fluorescent; PDX, patient derived xenograft; GEPDX, genetically engineered PDX.

Table S1

Table S1: Patientsúclinical data and sample characteristics

UPN	subtype	Age (years)	Gender	Disease status	Sample type	Cytogenetic	Major cytogenetic group
ALL-352	adult	67	m	Diagnosis	PB	46,XY,t(4;11)(q21;q23)[2]/46,XY[23]	t(4;11)
ALL-389	adult	43	f	Diagnosis	BM	45,XX,t(4;11)(q21;q23),i(7)(q10),del(9)(p1?3),dic(12;17)(p1?2;p1?2)[8]/ 44,XX,sl,der(X)t(X;9)(q2?;?),-9[3]/	t(4;11)
ALL-816	adult	52	f	Diagnosis	PB	46,XX,t(4;11)(q21;q23)	t(4;11)
ALL-817	adult	74	f	Diagnosis	PB	46,XX,t(4;11)(q21;q23)[19]/46,XX[6]FISH: MLL+	t(4;11)
ALL-818	adult	47	m	Diagnosis	PB	46,XY,t(4;11)(q21;q23)[13]/47,XY,+X,t(4;11)(q21;q23),-9,+21[1]/48,XY,+X,t(4;11)(q21;q23),+21[2]/46,XY[1]	t(4;11)
AML-388	adult	57	m	Diagnosis	BM	t(6;11); FLT3 -, NPM1 -, CEBPA -	t(6;11)
AML-669	adult	49	f	Relapse	PB	t(9;11); CEBPA ?, karyotype 46,XX,t(9;11)(p22;q23)[20]/46,XX[3]; myeloma, pancytopenia; FLT3 -, NPM1 -, FISH: MLL [74/114]	t(9;11)
AML-393	adult	47	f	Relapse	BM	t(10;11); CEBPA n.a., karyotype aberrant; FLT3 -, NPM1 -	t(10;11)
ALL-209	adult	61	f	Relapse	BM	45,XX,t(2;7)(p1?;p1?),del(3)(p12),-9,t(9;22)(q34;q11),del(15)(q1?)[7]/46,XX[6]	t(9;22)
ALL-210	adult	35	f	Diagnosis	BM	48,XX,+X,der(2)t(2;7)(p?;p?),+5,-7,der(9)t(9;13)(?;q11)t(2;9)(?;?),t(9;22)(q34;q11),der(13)t(9;13)(?;q11),+der(22)t(9;22)(q34;q11)[9]	t(9;22)
ALL-223	adult	48	f	Diagnosis	PB	n.d., FISH t(9;22)	t(9;22)
ALL-224	adult	70	f	Diagnosis	PB	46,XX,del(5)(q?31),del(5*)(q?31),del(7)(p12),t(9;22)(q34;q11),del(11)(q1?3) [9]	t(9;22)
ALL-256	adult	41	f	Diagnosis	PB	47,XX,+8,t(9;22)(q34;q11),der(9)t(9;22)(q34;q11). ish (ABL1x3),(BCRx4),(ABL1 con BCRx3)	t(9;22)
ALL-262	adult	42	m	Diagnosis	BM	48,XY,+der(5)t(5;?22),-22,+der(22)t(9;22)(q34;q11.2)x2[2]/46,XY[18]	t(9;22)
ALL-360	adult	33	f	Diagnosis	BM	n.d. , FISH t(9;22), del(9), del(17)	t(9;22)
ALL-363	adult	66	m	Diagnosis	PB	46,XY,t(9;22)(q34;q11)[2]/46,XY[5]	t(9;22)
ALL-589	adult	44	m	Diagnosis	BM	46,XY,t(9;22)(q34;q11.2),+21,der(22)t(9;22)(q34;q11.2)[6]/ 46,XY,t(9;22)(q34;q11.2)[21]	t(9;22)
ALL-590	adult	84	m	Diagnosis	PB	46,XY[13] FISH: bcr/abl positiv, del(9)(p21) (=p16)	t(9;22)
ALL-199	pediatric	8	f	Relapse	BM	trisomy 21; no detection of the following fusion transcripts: E2A-PBX1, MLL-AF4, MLL-ENL, p190 BCR-ABL, p210 BCR-ABL, TEL-AML147,XX,+21c. . . nuc ish 6q23 (MYBx2) /)oo7, 9,P2~ (P16x0), cen9 (CóP9x2) [19/100(, 9q34 (ABLx2), 22q11 (BcRxi) l).oo], 1	trisomy 21
ALL-265	pediatric	5	f	Relapse	BM	trisomy 21	trisomy 21
AML-346	pediatric	1	f	Relapse	unknown	interstitial 5q deletion and interstitial 13q deletion.; FLT3 -, NPM1 -, MLL -, CEBPA -,	normal
ALL-706	pediatric	5	f	Diagnosis	BM	46,XX,t(4;11)(q21;q23)[11]/46,XX[3]	t(4;11)
ALL-707	pediatric	2	m	Diagnosis	PB	46,XY,t(4;11)(q21;q23),del(17)(p12)[1]/46,XY[24] nuc ish 11q23(MLLx2),17p13,1(P53x2)[100] 46,XY,t(4;11)(q21;q23)[13]/46,idem,del(17)(p12)[5]/46,XY[3] nuc ish 11q23(MLLx2)(5'MLLsep3'MLLx1)[80/100],12p13(TELx2),21q22)(AML1x2)[100]17p13.1(P53x1)[20/100]	t(4;11)
ALL-762	pediatric	1	f	Diagnosis	PB	46,XX,t(4;11)(q21;q23),inc[11]/46,XX[1]	t(4;11)
ALL-763	pediatric	17	f	Diagnosis	BM	46,XX[12], nuc ish 4q21-22(AFF1x2),11q23(MLLx2)[100] 46,XX,t(2;4;11)(p22;q21;q23)7add(6)(q24)[17]/46,XX[3], nuc ish 4q21-22(AF4x3),11q23(MLLx3)(AF4conMLL2x2)[78/100]6q23(MYBx2),9q34(ABLx2),22q11(BCRx2),14q32(IGHx2), nuc ish 12q13(TELx2),21q22(AML1x2)[100],9p21(P16x1),cen9(CEP9x2)[52/100],11q23(MLLx2)(5'MLLsep3'MLLx1)[92/100]	t(4;11)
ALL-703	pediatric	1	f	Diagnosis	BM	46,XX,t(9;11)(p22;q23)[7]/46,XX[7] nuc ish 9q34(ABLx22),22q11(BCRx2)(100),12p13(TELx2),21q22(AML1x2)(100)	t(9;11)
ALL-704	pediatric	1	f	Diagnosis	BM	46,XX,t(9;11)(p22;q23)[6]/46,XX[7] nuc ish 9q34(ABLx2),22q11(BCRx2)[100],11q23(5'MLL,3'MLLx2)(5'MLLsep3'MLLx1)[77/100],12p13(TELx2),21q22(AML1x2)[100]	t(9;11)
ALL-705	pediatric	1	f	Diagnosis	BM	n.d. t(9;11)	t(9;11)

BM, bone marrow; PB, peripheral blood; UPN, unique patient number; f, female; m, male; n.d., not determined.

Table S2

Table S2: Sample source from BM or PB and time required for P0

UPN	Sample type	average time required for P0 in days
ALL-352	PB	99
ALL-389	BM	64,5
ALL-816	PB	120,5
ALL-817	PB	46
ALL-818	PB	63,5
ALL-210	BM	165,5
ALL-223	PB	90,5
ALL-224	PB	118
ALL-256	PB	83
ALL-360	BM	47
ALL-363	PB	84,5
ALL-589	BM	91
ALL-590	PB	142

[illegible]

Table S3

Table S3: Engraftment of primary adult rearranged ALL samples

	number of engrafted samples	in %
all	13\15	86.68
fresh only	5\5	100
frozen only	8\10	80
t(9;22) all	8\10	80
t(9;22) fresh only	3\3	100
t(9;22) frozen only	5\7	71.43
t(4;11) all	5\5	100
t(4;11) fresh only	2\2	100
t(4;11) frozen only	3\3	100

Engraftment data of all samples; raw data to Figures 1A, 1B and S1.