Supplementary Methods:

Patient samples and human cell lines

98 BCP-ALL (mixed cytogenetics) and 61 E2A-PBX1$^+$ BCP-ALL patients were treated according to ALL-Berlin-Frankfurt-Münster (BFM) 2000 or 2009 protocols. Informed consent was obtained in accordance with the Declaration of Helsinki. 697 and HL-60 cell lines were purchased from DSMZ. Quantitative-PCR analyses were performed as published previously[1]. For knockdown experiments, a short hairpin RNA (shRNA) against IL7Rα (TRCN0000289766) or shRNA against GFP (control) were used as published previously[1-3].

Gene expression datasets for re-evaluation

The dataset from van der Velden et al. [4] contains gene expression data (Affymetrix U133 Plus 2.0 array) from ALL cells retrieved from the CSF of 8 children with CNS-relapse of BCP-ALL as well as from the BM of 22 patients at diagnosis, and cells from the BM of 20 patients at the time of isolated BM-relapse. The data was deposited on the NCBI GEO Data-Sets as GSE60926. The second dataset, "TARGET phase 1 ALL Project", is generated by the Therapeutically Applicable Research to Generate Effective Treatments (TARGET) initiative, phs000218, managed by the United States National Cancer Institute (NCI). The data used for this analysis are available on [ftp://caftpd.nci.nih.gov/pub/OCG-DCC/TARGET/ALL/clinical/Phase1/; microarray GEO accession GSE11877, accessed March 2016]. Information about TARGET can be found at http://ocg.cancer.gov/programs/target. Microarray data from diagnostic BM (n=131) or peripheral blood (n=76) samples of children with high risk ALL annotated with clinical follow-up data including CNS-relapse were thereby analysed[5,6]. We are extremely grateful for the contribution of the patients and families involved, as well as the clinical and research teams, for making this data available.

BCP-ALL xenografts

NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice were purchased from Charles River, bred and maintained in accordance with governmental animal care and use committees protocols. Xenograft mice were generated as described previously using 697 or E2A-PBX1$^+$ patient BM (>90% blasts) cells with high IL7R expression[1-3].

Western Blot

Western blotting was performed as described[1]. IL7R and isotype antibodies were purchased from R&D. p-ERK, p-AKT, Caspase 8 and GAPDH antibodies were obtained from Cell Signaling Technology.

Statistical analysis

Statistical tests are indicated in the figure legends. Results were analyzed for statistical significance with GraphPad, SPSS or R version 3.3.3. A P-value of <0.05 was considered significant. Pre-published datasets[4,6] were analyzed using the z-score approach. The association between gene expression and CNS status was

examined by multivariate analyses to calculate odds ratios (ORs) and 95% confidence intervals (CIs) or by using Cox proportional Hazards Model.

Supplementary Figure Legends:

Supplementary Figure 1: Correlation analyses of IL7Rα expression with clinical parameters of E2A-PBX1 patients.
Correlation analyses of IL7R expression in 61 E2A-PBX1 positive pediatric patients with sex (A), age (B), prednisone response (C) and minimal residual disease (D). SR: standard risk, IR: intermediate response and HR: high risk. Definitions of patient MRD risk groups and prednisone response are described in Supplementary Table 1.

Supplementary Figure 2: Correlation analyses of IL7R and ZAP70 expression in pediatric BCP-ALL cohort.
(A) Correlation analysis of both IL7R and ZAP70 in 98 pediatric BCP-ALL patients of mixed cytogenetics. (B) The patient cohort was sub-grouped into four quartiles according to expression levels. The first quartile (Lo) and the fourth quartile (Hi) were determined for each marker and correlated with the CNS status (left and middle). In order to investigate if a combination of both markers yields superior correlations, patients were sub-grouped into $IL7R^{Lo}ZAP70^{Lo}$ or $IL7R^{Hi}ZAP70^{Hi}$ depending on expression levels of both markers and the correlation analysis was repeated (right). Chi-square test.

Supplementary Figure 3: IL7R expression is associated with iCNS-relapse but not BM-relapse.
Kaplan-Meier curves showing (A/B) isolated CNS-relapse-free probability (A) and overall survival (B) for multiple z-score ranges for IL7R determined in BM/peripheral blood at diagnosis, <0 to ≥1.5. Note that the curves represent successively smaller groups of patients with successively higher z-score cut-offs. Gradually decreasing slopes of the curves for increasing z-scores (>=0, >=0.5, >=1.0, >=1.2) imply relevance of the z-score to relapse-free survival outcome. The gradual pattern breaks off at z >=1.5, where sample numbers become critically low (11 patients, two events). Z-score cut-off for the remainder of this study was chosen as z>=1.2. (C) isolated BM relapse-free probability; (D) any BM-relapse; (E) overall survival. Upregulation: z-scores ≥ 1.2, and No upregulation: z-scores < 1.2. TARGET phase 1 dataset.

Supplementary Figure 4: IL7R expression is associated with CNS infiltration in xenografted mice. (A) 11 primary samples of pediatric BCP-ALL patients were xenografted into duplicate NSG-mice. Mice were sacrificed when leukemic symptoms were visible and semi-quantitative scoring of the CNS was performed. Surface IL7R expression was measured in xenograft samples using flow cytometry. Xenografts were sub-grouped according to IL7R MFI ($IL7R^{Hi}$: MFI higher or equal than the median; $IL7R^{Lo}$: MFI lower than the median). Patient and xenograft characteristics are depicted in Supplementary Table 6. (B) Western blot analysis of splenic cells from xenografts for p-AKT and p-ERK (left) and the quantification of normalized AKT, p-AKT, ERK and p-ERK for each group (right). Quantification was performed using

ImageJ. Normalized AKT= ratio of total AKT/GAPDH, normalized p-AKT= ratio of p-AKT/ normalized AKT, normalized ERK= ratio of total ERK/GAPDH, normalized p-ERK= ratio of p-ERK/ normalized ERK. AU: arbitrary unit.

Supplementary Figure 5: *In vitro* effect of IL7R blockade by the antibody. (A) Extracellular flow cytometry staining for basal IL7R in 697 BCP-ALL and HL-60 AML cell lines. (B) Cells were treated with different concentrations of anti-IL7R antibody for 30 minutes and then stained for intracellular p-AKT. Pervanadate stimulation (25μM; 5 minutes) was used as a positive control. (C) 697 cells or E2A-PBX1^{+} xenograft cells were serum-starved for 2 hours and then treated with 5 or 10μg of anti-IL7R antibody for 30 minutes. Cell lysates were subjected to western blotting and Caspase-8 levels were detected. Apoptosis activates caspase-8 cleavage and leads to the release of the caspase-8 active fragment p18.

Supplementary Table Legends:

Supplementary Table 1: Characteristics of 98 BCP-ALL patients at initial diagnosis.

Supplementary Table 2: Univariate and multivariate associations for IL7R expression quartiles and CNS status in 98 childhood BCP-Acute Lymphoblastic Leukemia patients.

Supplementary Table 3: Number of patients in the z-score groups of patients from the TARGET database.

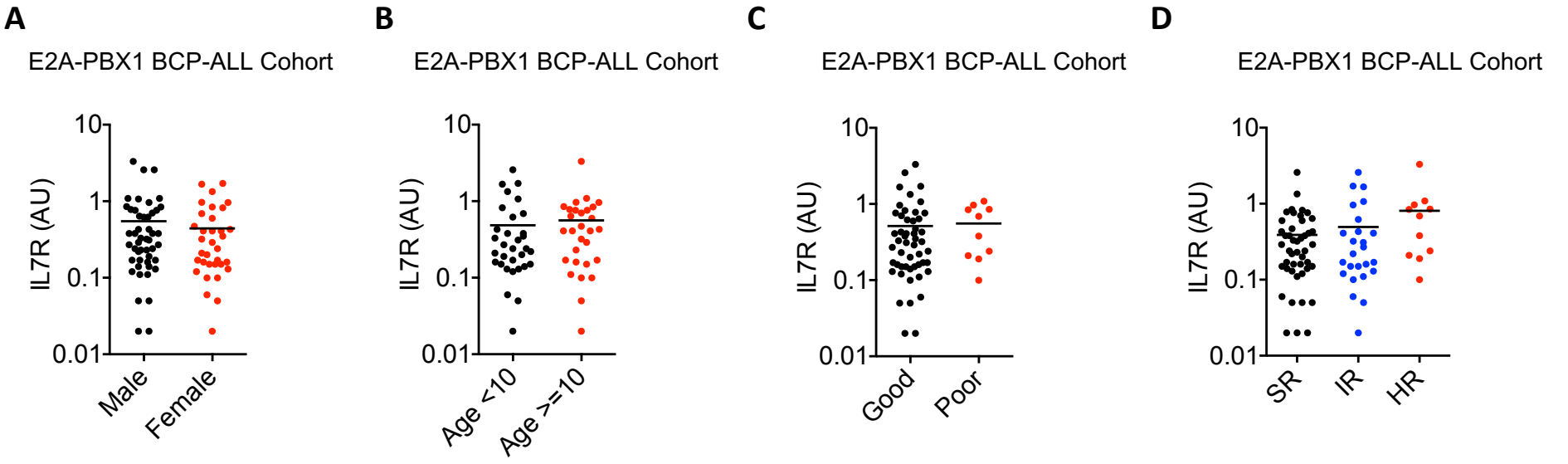
Supplementary Table 4: Multivariate analysis for the TARGET phase 1 dataset using Cox Proportional Hazards Model of risk factors for isolated CNS relapse.

Supplementary Table 5: Association between IL7R expression and chromosomal abnormalities in the TARGET phase 1 dataset.

Supplementary Table 6: Basic characteristics of 13 patients injected into NSG mice.

Supplementary References:

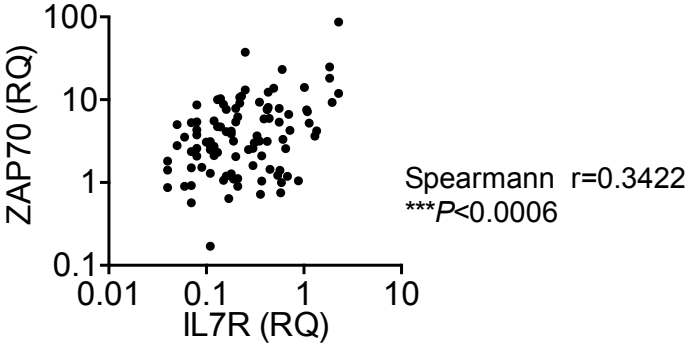
1. Alsadeq A, Fedders H, Vokuhl C, et al. The role of ZAP70 kinase in acute lymphoblastic leukemia infiltration into the central nervous system. *Haematologica*. 2017;102(2):346-355.
2. Alsadeq A, Strube S, Krause S, et al. Effects of p38alpha/beta inhibition on acute lymphoblastic leukemia proliferation and survival in vivo. *Leukemia*. 2015.
3. Krause S, Pfeiffer C, Strube S, et al. Mer tyrosine kinase promotes the survival of t(1;19)-positive acute lymphoblastic leukemia (ALL) in the central nervous system (CNS). *Blood*. 2015;125(5):820-830.
4. van der Velden VH, de Launaij D, de Vries JF, et al. New cellular markers at diagnosis are associated with isolated central nervous system relapse in paediatric B-cell precursor acute lymphoblastic leukaemia. *Br J Haematol*. 2016;172(5):769-781.
5. Bowman WP, Larsen EL, Devidas M, et al. Augmented therapy improves outcome for pediatric high risk acute lymphocytic leukemia: results of Children's Oncology Group trial P9906. *Pediatr Blood Cancer*. 2011;57(4):569-577.
6. Borowitz MJ, Pullen DJ, Shuster JJ, et al. Minimal residual disease detection in childhood precursor-B-cell acute lymphoblastic leukemia: relation to other risk factors. A Children's Oncology Group study. *Leukemia*. 2003;17(8):1566-1572.



Supplementary Figure 1

A

Extended BCP-ALL Cohort



B

Extended BCP-ALL Cohort

	IL7R^{Lo}	IL7R^{Hi}
CNS1/2	22 (92%)	16 (67%)
CNS3	2 (8%)	8 (33%)
Total	24	24

** P= 0.0330*

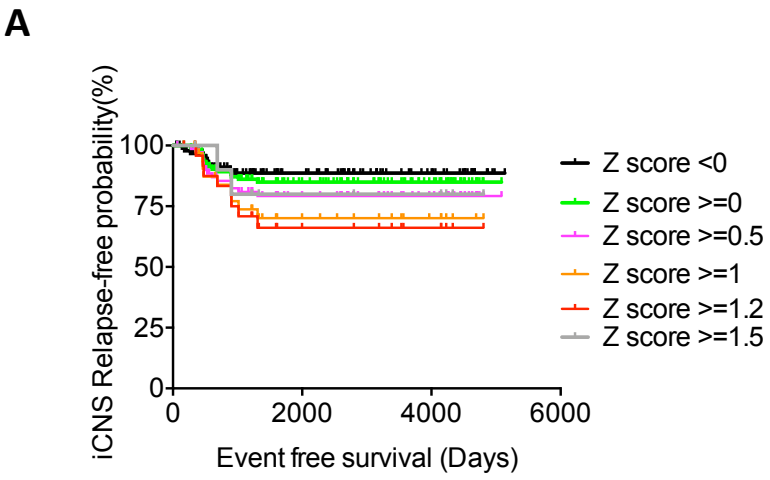
	ZAP70^{Lo}	ZAP70^{Hi}
CNS1/2	24 (92%)	14 (61%)
CNS3	2 (8%)	9 (39%)
Total	26	23

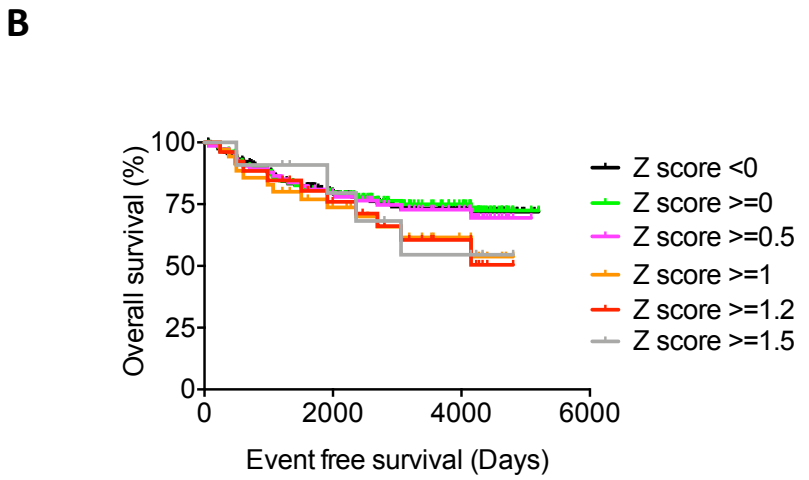
*** P= 0.0085*

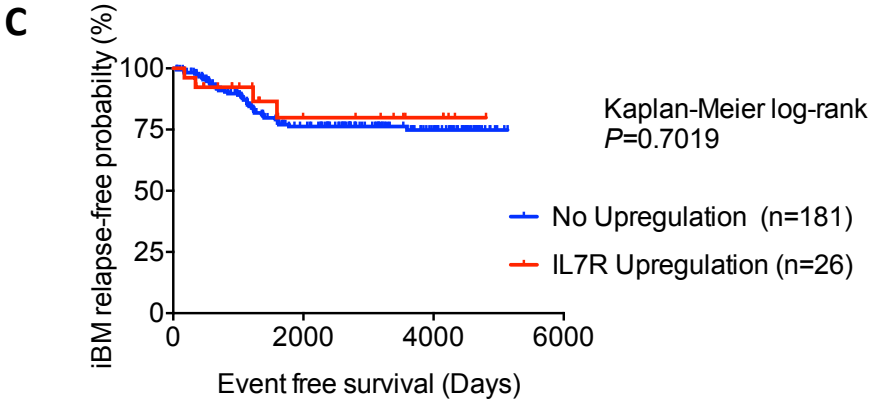
	IL7R^{Lo}ZAP70^{Lo}	IL7R^{Hi}ZAP70^{Hi}
CNS1/2	13 (93%)	4 (57%)
CNS3	1 (7%)	3 (43%)
Total	14	7

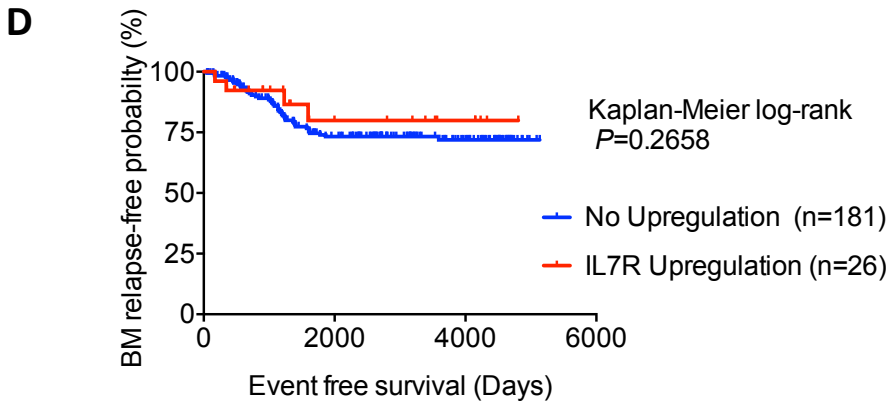
** P= 0.0494*

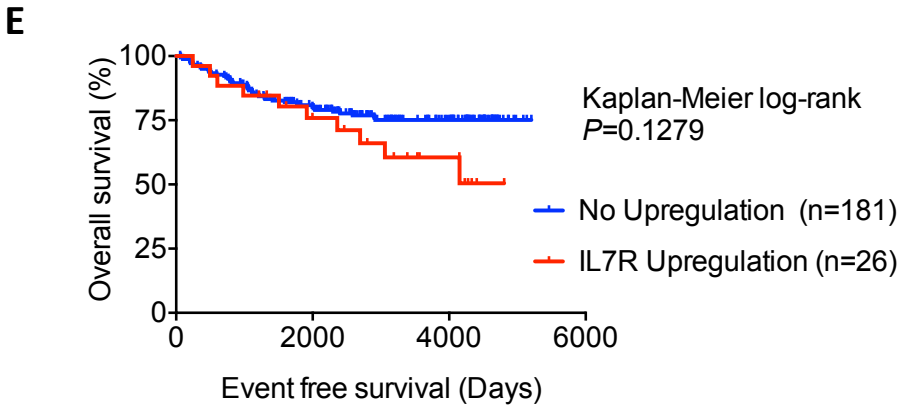
Supplementary Figure 2



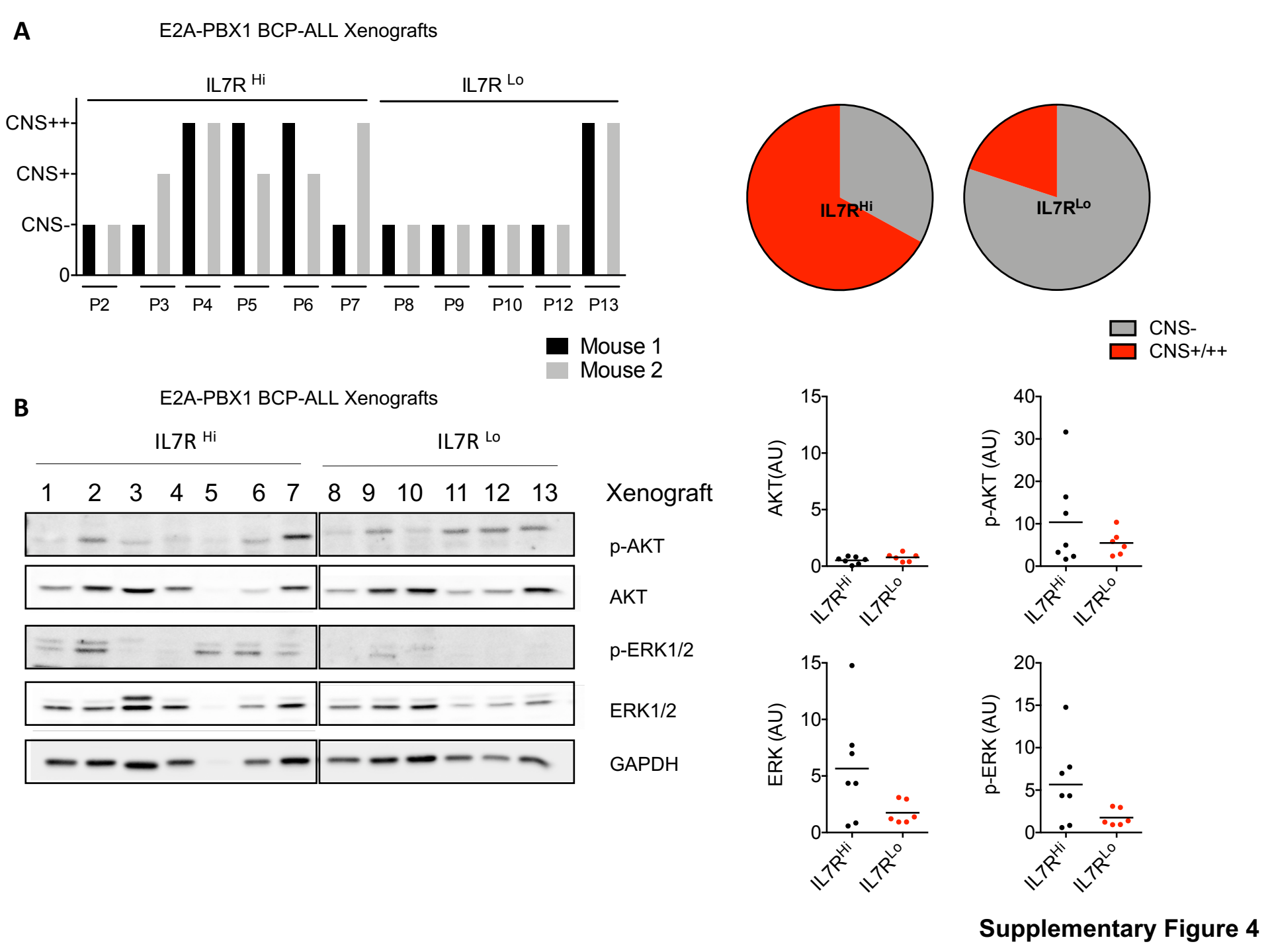






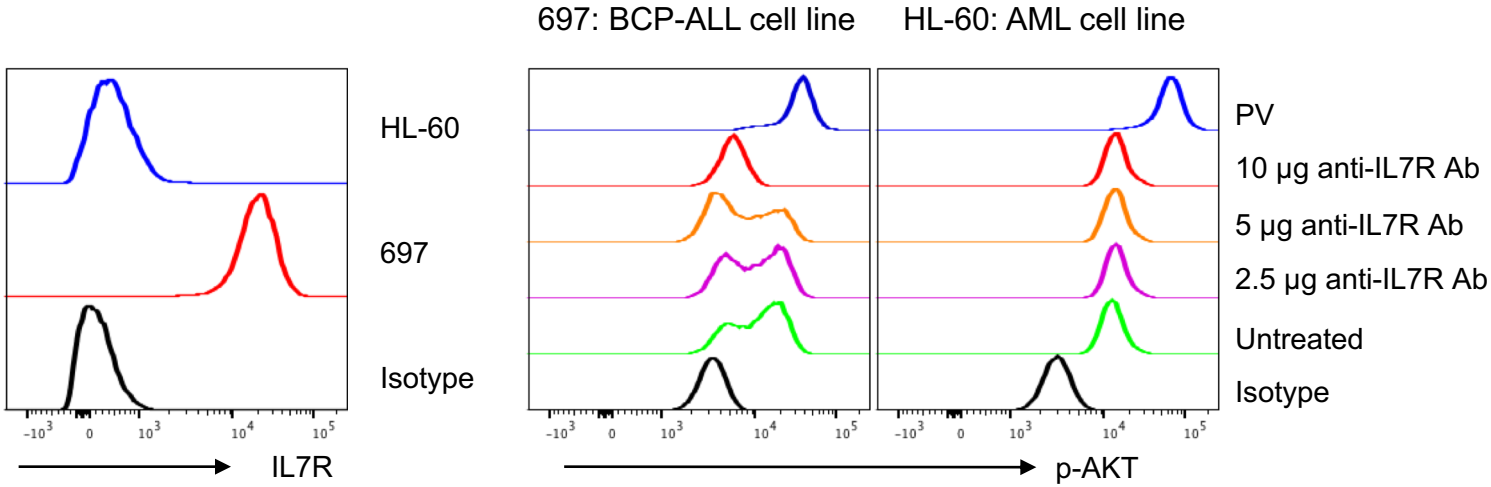


Supplementary Figure 3



Supplementary Figure 4

A



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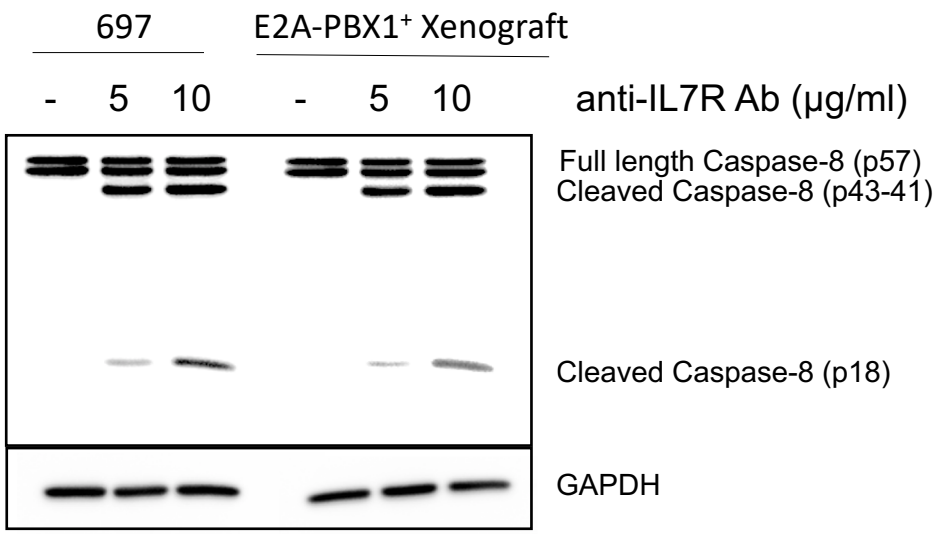

Supplementary Figure 5

Supplementary Table 1: Characteristics of 98 BCP-ALL patients at initial diagnosis.

	CNS-*		CNS+*		Statistics
	No.	%	No.	%	*P*
BCP-ALL	**72**	**100**	**26**	**100**	
Sex[†]					0.1693
Male	45	62.5	12	46.2	
Female	27	37.5	14	53.8	
Age, years[†]					0.7984
< 10 years	53	73.6	18	69.2	
≥ 10 years	19	26.4	8	30.8	
WBC count[‡]					0.7716
>50,000/µl	42	58.3	12	50.0	
50,000<100,000/µl	13	18.1	5	20.8	
≥ 100,000/µl	17	23.6	7	29.2	
Risk group[‡*]					0.7354
SR	23	32.0	6	25.0	
IR	27	37.5	11	45.8	
HR	22	30.5	7	29.2	
Prednisone response[†**]					0.7723
Good	58	82.9	21	80.8	
Poor	12	17.1	5	19.2	
Cytogenetics[‡]					0.240
BCR-ABL (and MLL)	3 (2)	06.9	1(1)	07.7	
TEL-AML1 (and E2A-PBX1)	9(3)	12.5	5(1)	20.8	
Others/None	55	76.4	18	69.2	

SR – standard risk, IR – intermediate risk, HR – high risk

WBC – white blood cell count in the peripheral blood at initial diagnosis

RBC – red blood cells

***CNS status is defined as follows:**

CNS-:

CNS1: neither clinical nor radiological signs of CNS involvement AND no blasts in the cerebrospinal fluid (CSF) cytospin.

CNS2: neither clinical nor radiological signs of CNS involvement AND CNS2a: <10 per microliter RBC and no macroscopic blood; ≤ 5 per microliter WBC; positive blasts in cytospin.

CNS2b: macroscopic blood and/or ≥ 10 per microliter RBC; ≤ 5 per microliter WBC; positive blasts in cytospin.

CNS2c: macroscopic blood and/or ≥ 10 per microliter RBC; >5 per microliter WBC; positive blasts in cytospin; negative according to algorithm $(WBC_L/RBC_L)/(WBC_B/RBC_B)$ >2.

CNS+:

CNS3-CNS3a: <10 per microliter RBC and no macroscopic blood; >5 per microliter WBC; positive blasts in cytospin.

CNS3b: macroscopic blood and/or ≥ 10 per microliter RBC; >5 per microliter WBC; positive according to algorithm $(WBC_L/RBC_L)/(WBC_B/RBC_B)$ >2.

CNS3c: clinical signs of CNS involvement, radiologically detectable cerebral lesion, retinal infiltrations.

†Fisher´s exact test, 2-sided *P*-value, ‡Chi-squared test was performed, 2-sided *P*-value.

*Risk stratification according to minimal residual disease (MRD) risk groups: MRD-SR: TP1+2 negative, MRD-IR: TP1 and/or TP2 $<10^{-3}$, MRD-HR: TP2 ≥ 10^{-3}. MRD risk group was missing for 2 patients in the CNS pos. Prednisone poor responders were stratified into the HR treatment group.

**Prednisone response was missing for 2 patients in the CNS- group.

Supplementary Table 2: Univariate and multivariate associations for *IL7R* expression quartiles and CNS status in 98 childhood BCP-Acute Lymphoblastic Leukemia patients.

BCP-ALL	CNS- (n=72)		CNS+ (n=26)		Univariate			Multivariate		
IL7R Quartile*	**No.**	**%**	**No.**	**%**	**OR†**	**95% CI**	***P***	**OR‡**	**95% CI**	***P***
I	22	30.6	2	07.6	1.000[3]			1.000[3]		
II	17	23.6	8	30.8	5.176	0.971-27.602	0.054	5.373	0.996-28.993	0.051
III	17	23.6	8	30.8	5.176	0.971-27.602	0.054	5.392	0.997-29.117	0.050
IV	16	22.2	8	30.8	5.500	1.027-29.451	0.046	5.617	1.023-30.842	0.047

Abbreviations: OR: odds ratio, CI: Confidence interval.

*Based on expression as measured by RT-PCR of IL7R in all 98 BCP-ALL patients.

†Multivariate OR controlled for age and WBC count at diagnosis.

‡Reference category.

Supplementary Table 3: Number of patients in the z-score groups of patients from the TARGET database.

Z-score	z-score < 0	z-score ≥ 0	z-score ≥ 0.5	z-score ≥ 1	z-score ≥ 1.2	z-score ≥ 1.5
Total Number of Patients	207	207	207	207	207	207
Number of Patients in group	97	110	74	35	26	11
Number of censored subjects	87	95	60	26	18	9
Number of events	10	15	14	9	8	2
Rate of CNS-relapse (%)	10.3	13.6	18.9	25.7	30.8	18.2

Supplementary Table 4: Multivariate analysis of TARGET phase 1 dataset using Cox Proportional Hazards Model of risk factors for isolated CNS relapse.

Risk factor	Hazard R	95% CI	*P*
IL7R upregulation*	3.12	**(1.28-7.60)**	0.01207
Day 29 MRD >0.01	1.21	(0.52-2.85)	0.65434
WBC (diagnosis) ≥ 50 $x10^9$/L	1.03	(0.38-2.74)	0.95822
CNS status 3	0.74	(0.15-3.55)	0.70778
Age (diagnosis) ≥ 10 yrs	0.43	(0.17-1.10)	0.07948
MLL Rearrangement	1.32	(0.37-4.66)	0.67135
ZAP70 upregulation*	0.7	(0.16-3.11)	0.64093

Abbreviations: Hazard R: Hazard ratio, CI: Confidence interval.

Analysis performed using R v 3.3.3

*Upregulated expression is defined as z-score ≥ 1.2 based on mean of probes in the TARGET database.

Supplementary Table 5: Association between IL7R expression and chromosomal abnormalities in the TARGET phase 1 dataset.

Cytogenetic subtype	ILR7 Upregulation*		No ILR7 upregulation		Statistics
	No.	%	No.	%	*P*
Total number of patients	26	13	181	87	
MLL	2	10	18	90	0.7162
ETV6/RUNX1 Fusion	0	0	4	100	0.4440
TRISOMY 4 or 10	2	29	5	71	0.1934
E2A-PBX1	**7**	**30**	**16**	**70**	**0.0060**

*Upregulated IL-7R expression is defined as z-score ≥ 1.2

†*P*-value determined using Chi-Squared test

Supplementary Table 6. Basic characteristics of 13 patients injected into NSG mice.

Patient Data								Xenograft Data					
Patient	**Age (y)**	**Sex**	**WBC**	**Patient CNS[1]**	**MRD Risk[1]**	**PR[2]**	**IL7R[3]**	**Engraftment[4]**	**% BM blasts**	**Survival (days)**	**IL7R[5] (MFI)**	**IL7R group[6]**	**Xenograft CNS[7]**
P1	3.0	F	≥50.000 <100.000	1	1	g	0.20	0.3, 1	94, N/A	97, N/A	4.81	Hi	N/A
P2	4.0	M	≥100.000	2b	2	g	0.62	2, 3	95, 86	71, 72	4.36	Hi	1,1
P3	3.7	M	≥100.000	3b	2	g	1.07	1, 3	86, 89	70, 71	4.17	Hi	1,2
P4	10.4	M	≥100.000	2a	1	g	0.78	3, 2	97, 94	103, 79	4.12	Hi	3,3
P5	10.3	M	<10.000	1	2	g	0.17	1, 1	94, 91	91, 91	4.08	Hi	3, 2
P6	14.6	M	<10.000	1	1	g	0.7	1, 2	80, 95	94, 104	3.75	Hi	3,2
P7	8.3	F	<10.000	2b	1	g	1.34	68, 46	100, 99	44, 55	3.23	Hi	1,3
P8	11.3	M	<10.000	3c	1	g	0.76	1, 3	91, 100	75, 75	3.05	Lo	1,1
P9	12.8	F	≥10.000 <50.000	1	1	g	0.17	21, 23	99, 99	45, 44	2.81	Lo	1,1
P10	14.5	F	≥10.000 <50.000	1	2	g	0.15	20, 19	93, 85	49, 49	2.51	Lo	1,1
P11	3.8	F	≥10.000 <50.000	1	2	g	0.12	1, 1	97, 96	102, 106	2.32	Lo	N/A
P12	12.3	F	≥100.000	3c	3	p	0.10	1, 1	96, N/A	76, 224	1.29	Lo	1,1
P13	17.5	M	<10.000	3c	3	g	3.31	3, 10	86, 42	40, 40	1.22	Lo	3/3

[1]Definitions of patient CNS status and MRD risk groups are described in Supplementary Table 1.

[2]PR: Prednison response; g: good (less than 1000 leukemic blasts/µl blood on treatment day 8); p: poor (more than 1000/µl on day 8)

[3]IL7R mRNA expression levels were normalized to 697 cells.

[4]Percentage of blasts in the peripheral blood 6 weeks after the injection.

[5]IL7R MFI as calculated form FACS MFI values after normalizing to isotype.

[6]IL7R groups were identified as Hi or Lo in correspondence to IL7R MFI values (Hi: >= median MFI; Lo:<median MFI).

[7]CNS status in xenografted animals as shown in Supplementary Figure 4a and defined in Supplementary Methods

N/A: Data not available. WBC: White blood cell count at initial diagnosis.