SUPPLEMENTAL DATA

Supplemental Methods

Characterization of T-ALL and B-ALL patient samples

T-ALL and B-ALL samples obtained at diagnosis were classified according to EGIL. Phenotypic characterization was performed by flow cytometry using the following mAbs: CD1a-biotin (AbD Serotec), CD3-PE, CD5-FITC, CD7-FITC, CD8-PECy7, CD19-PECy7 (BD Biosciences), CD4-PECy5, CD13-PECy5, CD56-PECy5, CD34-FITC (Beckman Coulter), CD10-FITC (Invitrogen) and streptavidin-APC (Biolegend). B-ALL patient samples were characterized by conventional karyotyping and molecular studies using standard procedures.

IL7R and *NOTCH1* mutational analysis

For *IL7R* sequencing, total RNA (1-2 µg) isolated from primary T-ALL samples using TRIzol Reagent (Thermo Fisher Scientific) was reverse transcribed to complementary DNA (cDNA) using Expand Reverse Transcriptase (Sigma-Aldrich) and oligo dT (Biotools). The complete coding sequence (CDS) of *IL7R* was amplified by RT-PCR using *PfuTurbo* DNA polymerase (Agilent Technologies) and specific primers indicated in Supplemental Table 3. PCR products were cloned into pGEM-T Easy vector (Promega) after dATP addition using AmpliTaq DNA Polymerase (Applied Biosystems). After screening, plasmid DNA was extracted by Wizard Plus SV Minipreps DNA Purification System (Promega) and subjected to Sanger sequencing with the specific primers shown in Supplemental Table 3.

Where indicated, genomic DNA obtained from T-ALL patients at the time of diagnosis was analyzed by Next Generation Sequencing (NGS) to determine *IL7R*

and *NOTCH1* mutational status, compared to DP thymocytes from healthy donors. Briefly, DNA samples were screened for mutations in *IL7R* and *NOTCH1* using a custom panel of genes (https://design.nimblegen.com, Roche). Libraries were generated and amplified using KAPA Library Preparation Kit (Illumina) and NimbleGen SeqCap EZ Library SR (Roche). The captured samples were read on the NextSeq 550 platform (Illumina) following the manufacturer's instructions. The resulting sequence reads were mapped on the human reference sequence hg19 using Bowtie2. PCR duplicates were removed using Picard and realignment and recalibration of the reads was performed using GATK. SNPs and indels were detected using UnifiedGenotyper and HaplotypeCaller. Variants were annotated with gene names, predicted functional effect, protein positions and amino-acid changes, conservation scores and population frequency data.

In vivo tumor progression assay of B-ALL cell lines

In vivo tumor progression of NALM-6 or REH B-ALL cell lines transduced with either shsc or shIL-7R was assessed after left-flank subcutaneous injection of 6-10 wks-old RAG-$2^{-/-}$ x $\gamma c^{-/-}$ mice (10^6 cells/mice). Tumor volume based on digital caliper measurements was calculated by the modified ellipsoidal formula: $Tumor\ volume = 1/2(length \times width^2)$. Mice were sacrificed when tumors reached a diameter >17 mm. Tumors were isolated, mechanically disaggregated and analyzed by flow cytometry for the presence of transduced cells.

Supplemental Table 1. Primary human T-ALL samples analyzed

Name	Classification	Surface IL-7R	*IL7R* mutations	IL-7Rα predicted amino acid alterations	*NOTCH1* mutations	NOTCH1 predicted amino acid alterations
T-ALL2	Cortical	+	c.741_742insTGTCTTCTCAAGAGTTCC [a]	p.Ile247_Leu248insCysLeuLeuLysSerSer	Wild type [a]	Wild type
T-ALL3	Pre-T	+	Wild type [a]	N/A	c.4721T>C [a]	p.Leu1574Pro
T-ALL5	Cortical	+	Wild type [a]	N/A	c.4729_4734delGTGGTG [a]	p.Val1577_Val1578del
T-ALL6	Cortical	+	Wild type [b]	N/A	nd	nd
T-ALL8	Mature	+	Wild type [a]	N/A	Wild type [a]	N/A
T-ALL9	Cortical	-/lo	Wild type [b]	N/A	nd	nd
T-ALL10	Pre-T	+	Wild type [b]	N/A	nd	nd
T-ALL12	Pre-T	+	Wild type [b]	N/A	nd	nd
T-ALL13	Mature	+	Wild type [b]	N/A	nd	nd
T-ALL14	Pro-T	+	Wild type [b]	N/A	nd	nd
T-ALL15	Mature	+	c.755_757delTinsGGGTCCCCTC [b]	p.Ser252_Val253insGlyProLeu	nd	nd
T-ALL16	Mature	+	Wild type [b]	N/A	nd	nd
T-ALL18	Cortical	+	Wild type [a]	N/A	c.5165A>C [a]	p.Gln1722Pro
T-ALL26	Pre-T	+	Wild type [a]	N/A	c.4721T>C c.5033T>C [a]	p.Leu1574Pro p.Leu1678Pro
T-ALL30	ETP-ALL	+	Wild type [b]	N/A	nd	nd
T-ALL31	Pro-T	+	nd	nd	nd	nd

T-ALL samples from adult (T-ALL15) or pediatric (all the rest) patients were obtained at diagnosis and classification was established according to EGIL. Cells analyzed for surface IL-7R expression by flow cytometry were characterized as positive (+), negative (-) or low (-/lo). ([a]) *IL7R* and *NOTCH1* mutations were characterized by NGS. ([b]) *IL7R* mutations were also analyzed by conventional Sanger sequencing of full length *IL7R* coding sequences. N/A, not applicable; nd, not determined.

Supplemental Table 2. Primary human B-ALL samples analyzed

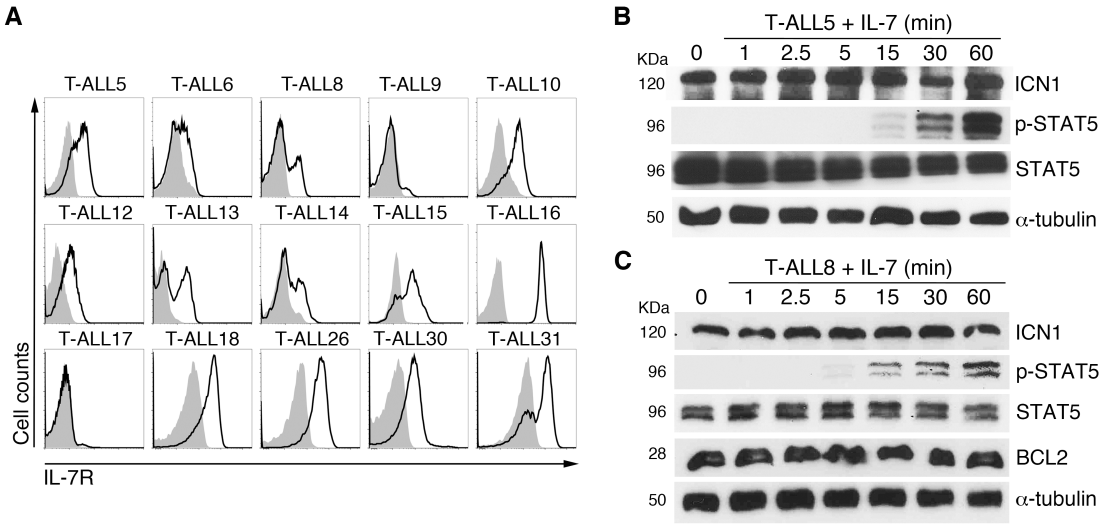
Name	Origin	Disease status	Source	IL-7R expression	Cytogenetics/Risk status	Classification
B-ALL1	Pediatric	Diagnosis	BM	+	nd/Standard	Common B-ALL
B-ALL3	Pediatric	Diagnosis	BM	nd	nd/Intermediate	Common B-ALL
B-ALL4	Pediatric	Diagnosis	BM	+	nd/Intermediate	Common B-ALL
B-ALL5	Pediatric	Diagnosis	BM	nd	TEL/AML1/Standard	Common B-ALL
B-ALL6	Pediatric	Diagnosis	BM	+	MLL/AF4/High	Pro-B-ALL
B-ALL7	Pediatric	Diagnosis	BM	+	Hyperdiploidy/Intermediate	Common B-ALL
B-ALL8	Pediatric	Diagnosis	BM	+	t(1,19)/Intermediate	Common B-ALL
B-ALL9	Pediatric	Diagnosis	BM	+	t(1/19)/Intermediate	Common B-ALL
B-ALL10	Pediatric	Diagnosis	BM	+	Hyperdiploidy/Intermediate	Common B-ALL
B-ALL11	Pediatric	Diagnosis	BM	-	t(1,14)/High	Pro-B-ALL

B-ALL BM samples were obtained from pediatric patients at diagnosis. Classification was established according to EGIL. Conventional karyotyping and molecular studies were performed using standard procedures. Cells analyzed for surface IL-7R expression by flow cytometry were characterized as positive (+) or negative (-). (nd), not determined.

Supplemental Table 3. Primers used for PCR amplification, cloning, sequencing, mutagenesis and ChIP assays

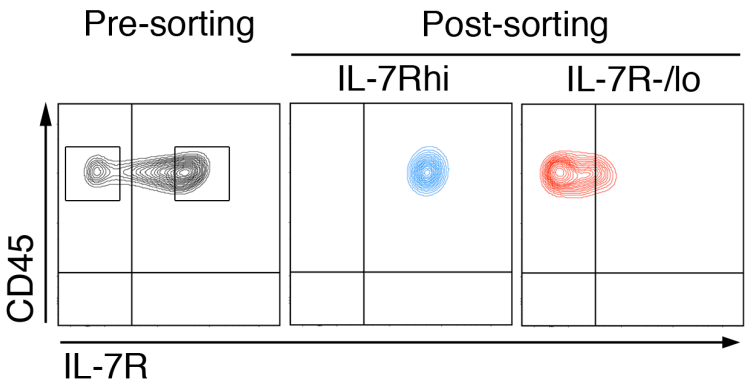
Primer	Assay	Sequence 5'-3'
441	*IL7R* CDS cloning EcoRI	cggaattccttatgacaattctaggtacaact
442	*IL7R* CDS cloning EcoRI	cggaattctcactggttttggtagaagct
SP6	*IL7R* sequencing	atttaggtgacactatag
T7	*IL7R* sequencing	taatacgactcactataggg
176	*IL7R* sequencing	gccctcgtggaggtaaagtgc
177	*IL7R* sequencing	ggggagactgggccatacgatagg
178	*IL7R* sequencing	gcagagaaagctccaaccggcagc
635	*Il7r* promoter cloning (F) MluI	cgacgcgtaatttgccaacacttgcattt
636	*Il7r* promoter cloning (R) BglII	cgagatctagaagcacgcttgtatgtgc
913	RBP-Jk site mutagenesis (F)	accctcatagactccGTACCgttttcattgcccttg
914	RBP-Jk site mutagenesis (R)	caagggcaatgaaaacGGTACggagtctatgagggt
639	*Il7r* 5'UTR sequencing	aacaaatgcagtgacccaca
642	*Il7r* 5'UTR sequencing	ctttctcccttccctccaac
644	*Il7r* 5'UTR sequencing	acgatgctcctcagttggtt
637	RBP-Jk site ChIP assay (F)	ccctcctgtcttccctacct
638	RBP-Jk site ChIP assay (R)	gtgtcccagttcctccattg

Cloning restriction sites are underlined. Nucleotide changes for site-directed mutagenesis are noted in capital letters. (F) Forward primer, (R) reverse primer.



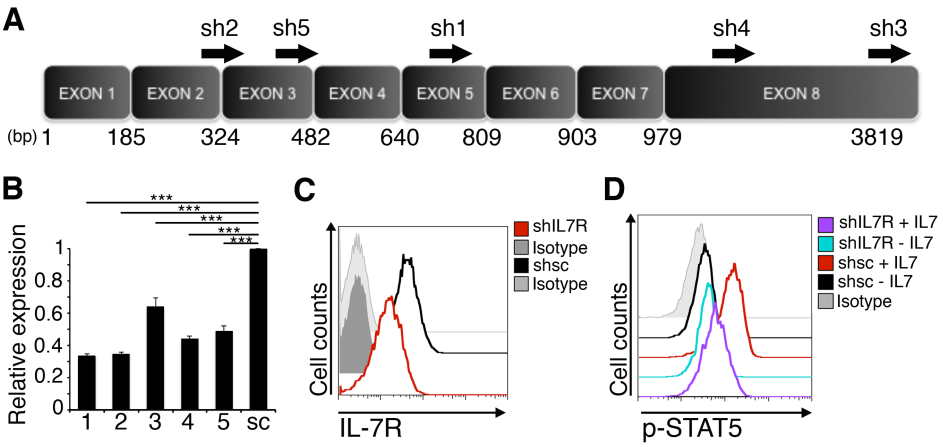
Supplemental Figure 1

Supplemental Figure 1. **Primary human T-ALL cells express functional receptors for IL-7.** (A) IL-7R expression analyzed by flow cytometry in a panel of primary human T-ALL samples shown in Supplemental Table 1. Shaded histograms represent background staining with irrelevant isotype-matched antibodies. (B, C) Western blotting of intracellular NOTCH1 (ICN1), total STAT5, activated STAT5 (p-STAT5) and BCL2 expression in human T-ALL cells either stimulated with rhIL-7 (200IU/ml) for the indicated times or non-stimulated. T-ALL5 (B) and T-ALL8 (C) cells were obtained from PB patient-derived xenografts isolated from the spleen of transplanted NSG mice. α-tubulin was used as loading control. Molecular weight in kilodaltons (KDa) is indicated on the left.



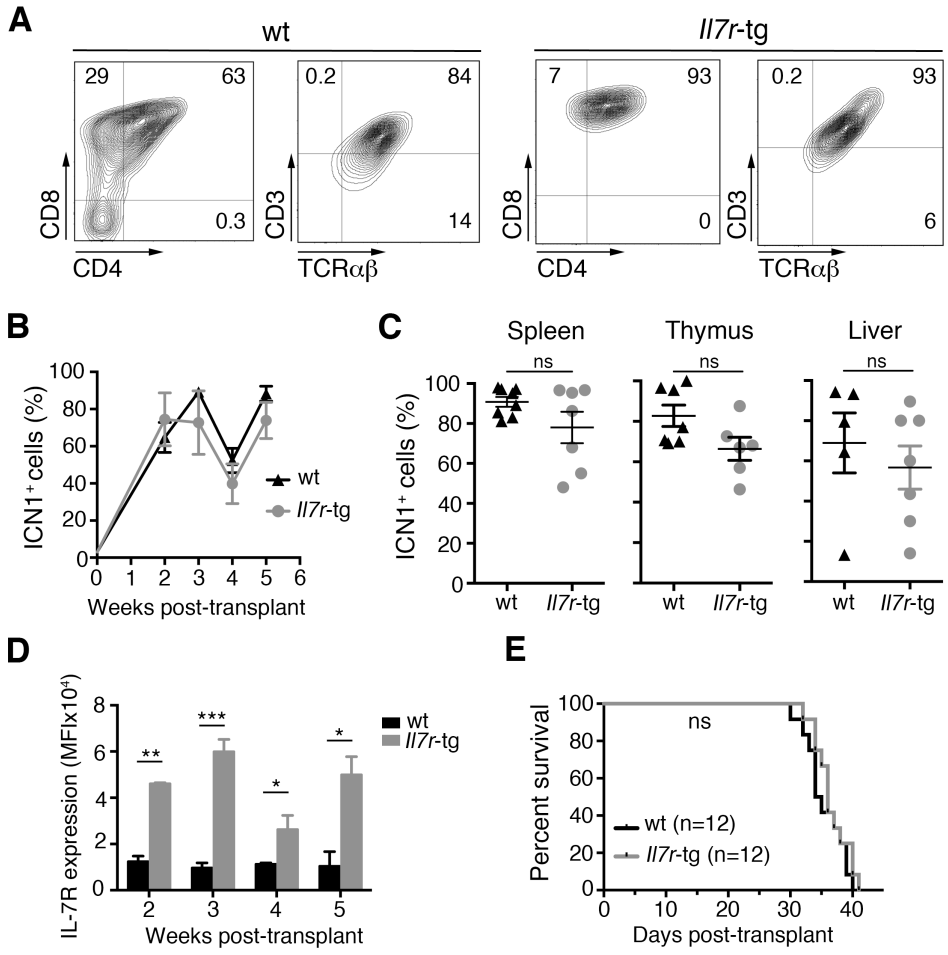
Supplemental Figure 2

Supplemental Figure 2. Sorting strategy used to isolate IL-7R$^{-/lo}$ and IL-7R^{hi} primary human T-ALL8 cells. Flow cytometry analysis of CD45 and IL-7R expression on T-ALL8 cells obtained from PB patient-derived xenografts isolated from the spleen of transplanted NSG mice (pre-sorting) is shown in the left panel. Phenotype of IL-7R^{hi} and IL-7R$^{-/lo}$ T-ALL8 cells FACS-sorted according to gates in the left is shown in the right.



Supplemental Figure 3

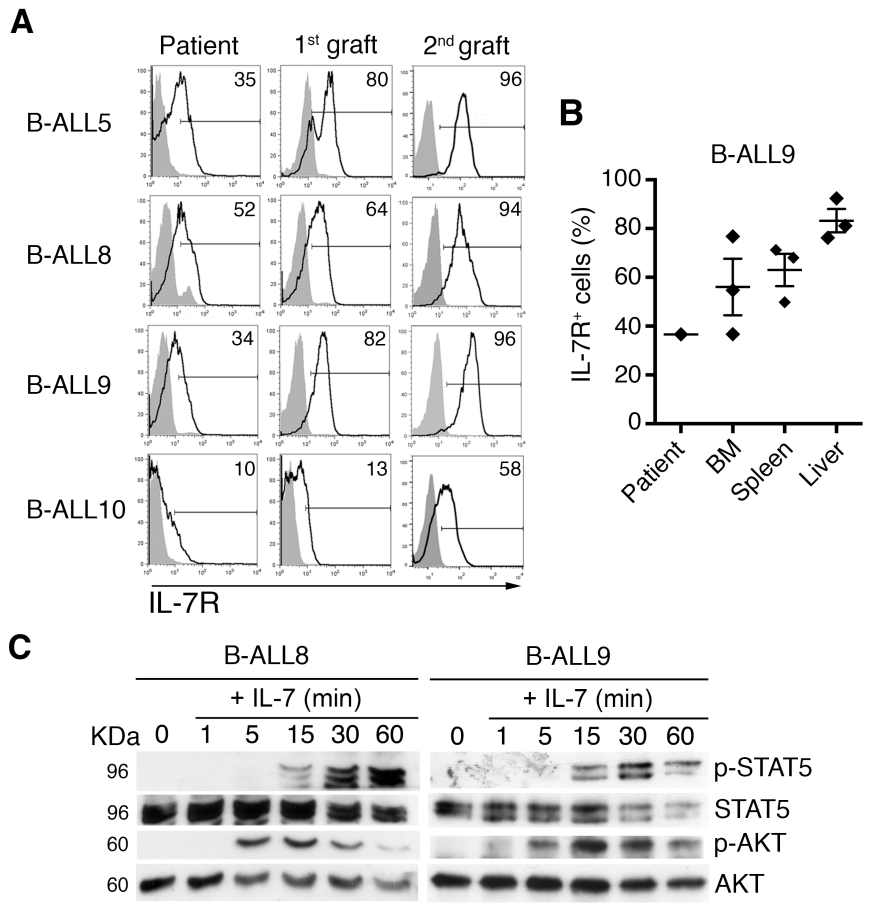
Supplemental Figure 3. *IL7R* silencing by shRNA impairs IL-7R/IL-7 signaling. (A) Schematic representation of human *IL7R* mRNA (NM_002185.3) showing exon distribution and regions recognized by different short hairpin RNAs (sh) designed against *IL7R*. Numbers indicate base pairs (bp) from the transcription initiation site. (B) Relative *IL7R* mRNA expression analyzed by quantitative PCR in HPB-ALL T-ALL cells transduced with lentiviral vectors encoding either shRNA (1-5) against *IL-7R* shown in (A) or scramble shRNA (shsc), and selected for 1 wk in cultures supplemented with puromycin. Values were normalized to expression levels of sc-transduced cells and are shown as mean ± SEM (n=3). (C) Flow cytometry of surface IL-7R expression on HPB-ALL cells transduced with shIL7R5 or shsc. Background staining with an isotype-matched irrelevant Ab is shown. Results are representative of one out of three experiments. (D) Flow cytometry of p-STAT5 expression of HPB-ALL cells transduced with shIL7R5 or shsc and stimulated for 30 min with IL-7 (200IU/ml) at 37ºC. CytoFix/CytoPerm-treated cells were labeled with Alexa-647-labeled anti-phospho-Tyr694 STAT5 Ab (BD Biosciences). Shaded histograms show background staining with irrelevant isotype-matched Abs. Representative results from one out of three experiments are shown.



Supplemental Figure 4

Supplemental Figure 4. ICN1 overexpression induces T-ALL generation from *Il7r*-transgenic and wild type Lin$^-$ c-kit$^+$ bone marrow progenitors with similar efficiencies. (A) Flow cytometry analysis of BM HPCs isolated from wild type (wt) or *Il7r*-transgenic mice (*Il7r*-tg) and transduced with active Notch1 (ICN1), which engrafted the BM of transplanted mice at 5-wks post-transplant. Representative results from one out of three experiments are shown. (B, C) Percentages of ICN1-transduced cells in (A) engrafting the BM at the indicated times (B), or recovered from the spleen, thymus and liver of host mice at 5-wks post-transplant (n=5-8 mice/group) (C). (D) Mean fluorescence intensity (MFI) of IL-7R expression in ICN1-transduced cells engrafting the spleen of host mice in (A) at 5-wks post-transplant. Data are shown as mean ± SEM values of at

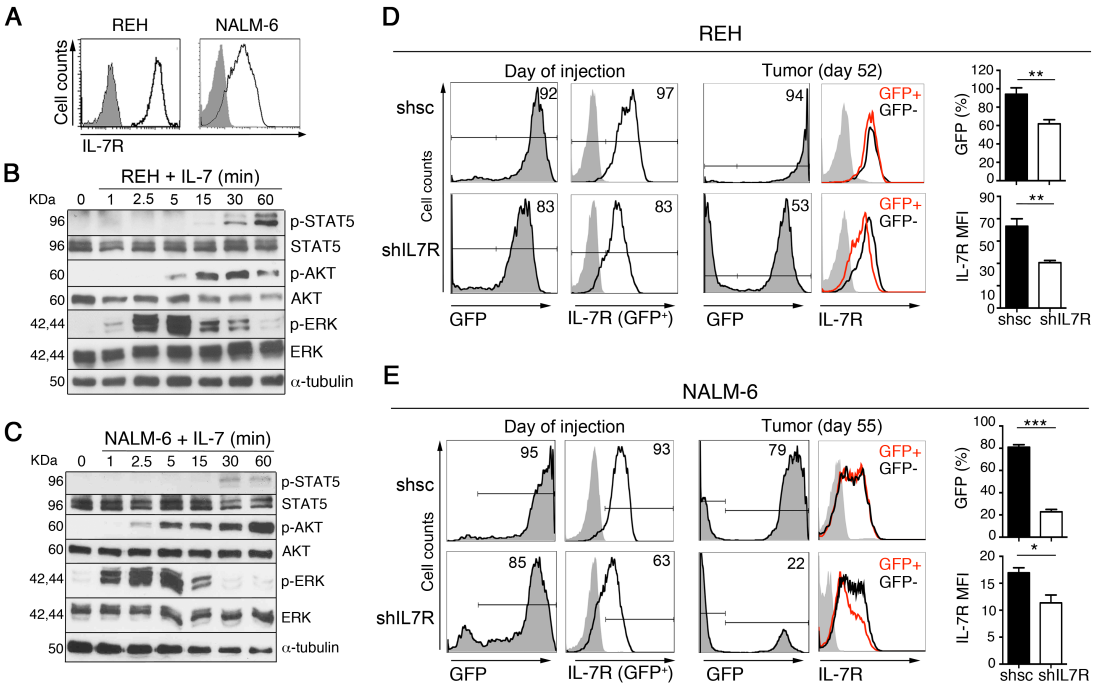
least 3 mice/group. (E) Kaplan-Meyer survival curves of mice transplanted with ICN1-transduced BM HPCs derived from either wt or *Il7r*-tg mice as in (A).



Supplemental Figure 5

Supplemental Figure 5. Human primary B-ALL cells express functional IL-7Rs that provide growth advantage *in vivo*. (A) IL-7R expression levels displayed by primary B-ALL cells from four patients analyzed by flow cytometry either at the time of diagnosis (patient), or after serial transplantation and spleen engraftment into consecutive immunodeficient mice (1st and 2nd graft). Numbers in quadrants indicate percentages of positive cells. Representative results from one out of three experiments are shown. (B) Percentages of B-ALL cells expressing IL-7R in patient-derived xenografts recovered from the indicated organs of transplanted NSG immunodeficient mice at 10-12 wks post-transplant (A). Data are shown as mean ± SEM of 3 mice. Percentages at the

time of diagnosis (patient) are shown for comparison. (C) Western blotting of total STAT5 and activated STAT5 (p-STAT5) and AKT (p-AKT) expressed in primary human B-ALL8 and B-ALL9 cells either stimulated with IL-7 (200IU/ml) for the indicated times or non-stimulated. Molecular weight in kilodaltons (KDa) is indicated on the left.



Supplemental Figure 6

Supplemental Figure 6. *IL7R* silencing impairs *in vivo* expansion of B-ALL cell lines. (A) Flow cytometry of IL-7R expression in human B-ALL cell lines REH and NALM-6. (B, C) Western blotting of total and activated STAT5 (STAT5, p-STAT5); AKT (AKT, p-AKT) and ERK (ERK, p-ERK) in REH (B) and NALM-6 (C) cell lines either stimulated with IL-7 (200IU/ml) for the indicated times or non-stimulated. α-tubulin was used as loading control. Molecular weight in kilodaltons (KDa) is indicated in the left. (D, E) FACS analysis of IL-7R expression in subcutaneous tumors recovered from immunodeficient NSG mice injected with REH (D) or NALM-6 (E) cells transduced with a lentiviral vector encoding either shRNA against IL7R (shIL7R) or scrambled shRNA (shsc) together with GFP. Percentages of transduced cells (GFP^+, shaded histograms) and proportions of transduced cells expressing IL-7R (empty histograms) at the day of injection are shown in the left. Middle histograms show percentages of transduced cells at the indicated days post-transplant (shaded) and IL-7R

expression levels on transduced (GFP^+, red empty histograms) and non-transduced cells (GFP^-, black empty histograms). Shaded histograms show background staining of transduced cells. Bar graphs in the right show percentages of transduced cells (upper panel) and mean of fluorescence intensity (MFI) of IL-7R expression (bottom panel) in subcutaneous tumors. Data are shown as mean ± SEM of values from at least 3 mice/group.