A mechanistic rationale for targeting the unfolded protein response in pre-B acute lymphoblastic leukemia

Behzad Kharabi Masouleh[1,3], Huimin Geng[1], Christian Hurtz[1], Lai N. Chan[1] Aaron C Logan[2], Mi Sook Chang[4], Chuanxin Huang[5], , Srividya Swaminathan[1], Haibo Sun[6], Valeria Cazzaniga[1,7], Giovanni Cazzaniga[7], Elisabeth Paietta[8], Ari Melnick[5], H. Phillip Koeffler[6], Markus Müschen[1,2]

- **Supplemental Material and Methods**
- **Figures S1-S10**
- **Tables S1-S5**

SUPPLEMENTAL MATERIAL AND METHODS

Primary human samples and human cell lines

We obtained primary cases (Table S1) in compliance with the IRB of the University of California San Francisco. The human Ph^+ ALL, lymphoma or multiple myeloma cell lines were obtained from DSMZ, Braunschweig, Germany. We maintained human leukemia cells in Roswell Park Memorial Institute medium (RPMI-1640, Invitrogen, Carlsbad, CA) with GlutaMAX containing 20% fetal bovine serum, 100 IU ml^{-1} penicillin and 100 µg ml^{-1} streptomycin at 37°C in a humidified incubator with 5% CO_2. We cultured all primary human ALL cells on OP9 stromal cells.

Extraction of bone marrow cells from mice

Bone marrow cells were extracted from young age-matched mice with different genetic backgrounds (Table S2). We obtained the bone marrow cells by flushing cavities of femur and tibia with PBS. After filtration through a 70 µm filter and depletion of erythrocytes using a lysis buffer (BD PharmLyse, BD Biosciences), washed cells were either frozen for storage or subjected to further experiments.

Mouse model of human Ph^+ ALL

We collected bone marrow cells from the above mentioned mice and retrovirally transformed them using *BCR–ABL1 or* $NRAS^{G12V}$ in the presence of 10 ng ml^{-1} interleukin-7 (Peprotech) in RetroNectin- (Takara) coated Petri dishes as described below. We maintained all *BCR–ABL1*-transformed ALL cells derived from bone marrow of mice in Iscove's modified Dulbecco's medium (IMDM, Invitrogen) with GlutaMAX containing 20% fetal bovine serum, 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin and 50 µM 2-mercaptoethanol. *BCR–ABL1* or *NRAS*-^{G12V}-transformed ALL cells were propagated only for short periods of time and usually not longer than for 2 months to avoid acquisition of additional genetic lesions during long-term cell culture. BCR-ABL1 transformed pre-B cells were transduced with different empty vector controls (EV) and either (4-OHT)-inducible or non-inducible Cre and deleted by Cre-mediated activation. A detailed overview of the used plasmids is shown in Table S5, For *in vivo* experiments ALL cells were then labeled with lentiviral firefly luciferase (D.B. Kohn, University of California Los Angeles, Los Angeles, CA) and injected via tail into sublethally irradiated (250 cGy) NOD/SCID recipient mice. Engraftment was monitored using luciferase bioimaging (VIS 100 bioluminescence/optical imaging system; Xenogen). D-Luciferin (Xenogen) dissolved in PBS was injected intraperitoneally at a dose of 2.5 mg per mouse 15 min before measuring the light

emission. Seven mice per group were injected via tail vein injection. When a mouse became terminally sick, it was sacrificed and bone marrow and spleen were collected for flow cytometry analysis. All mouse experiments were subject to approval by the Children's Hospital Los Angeles Institutional Animal Care and Use Committee.

Retroviral transduction

We performed transfections of retroviral constructs and their corresponding empty vector controls (Table S5) using Lipofectamine 2000 (Invitrogen) with Opti-MEM media (Invitrogen). We produced retroviral supernatants to infect murine cells by cotransfecting 293FT cells with the plasmids pHIT60 (gag-pol) and pHIT123 (ecotropic env; provided by D.B. Kohn, University of California, Los Angeles, Los Angeles, CA). To infect human cells we replaced pHIT123 with pHIT456 (amphotropic env; provided by D.B. Kohn, University of California, Los Angeles, Los Angeles, CA). Cultivation was performed in high glucose DMEM (Invitrogen) with GlutaMAX containing 10% fetal bovine serum, 100 IU ml^{-1} penicillin, 100 µg ml^{-1} streptomycin, 25 mM Hepes, 1 mM sodium pyruvate, and 0.1 mM of nonessential amino acids. We replaced regular media after 16 h with growth media containing 10 mM sodium butyrate. After 8 h of incubation, the media was changed back to regular growth media. 24 h later, the virus supernatants were harvested, filtered through a 0.45 µm filter, and loaded by centrifugation (2,000 g for 90 min at 32°C) two times on 50 µg ml^{-1} RetroNectin-coated non-tissue 6-well plates. $2–3 \times 10^6$ pre–B cells were transduced per well by centrifugation at 500 g for 30 min and maintained overnight at 37°C with 5% CO_2 before transferring into culture flasks.

Senescence-associated β-galactosidase assay

Senescence-associated β-galactosidase activity was performed on cytospin preparations as described ***(1)***. Briefly, a fixative solution (0.25% glutaraldehyde, 2% paraformaldehyde in PBS pH 5.5 for mouse cells) was freshly generated. To this end, 1 g paraformaldehyde was dissolved in 50 ml PBS at pH 5.5 by heating followed by addition of 250 µl of a 50% stock glutaraldehyde solution. 1× X-gal staining solution (10 ml) was prepared as follows: 9.3 ml PBS/$MgCl_2$, 0.5 ml 20× KC solution (that is, 820 mg $K_3Fe(CN)_6$ and 1,050 mg $K_4Fe(CN)_6 \times 3H_2O$ in 25 ml PBS) and 0.25 ml 40× X-gal (that is, 40 mg 5-bromo-4-chloro-3-indolyl β-D-galactoside per milliliter of N,N-dimethylformamide) solutions were mixed. For BCR–ABL1-transformed ALL cells, 100,000 cells per cytospin were used (700 r.p.m., 8 min). The fixative solution was pipetted onto cytospins and incubated for 10 min at room temperature, then washed twice for 5 min in

PBS/$MgCl_2$. Cytospin preparations were submerged in 1× X-gal solution, incubated overnight at 37 °C in a humidified chamber and washed twice in PBS.

Single-locus quantitative ChIP, ChIP-on-chip and ChIP-seq analysis

Cells were cross-linked with 1% formaldehyde for 10 minutes at room temperature, lysed, and DNA was sonicated to a fragment size of 150-350bp. After sonication, immunoprecipitations were performed with the following antibodies: anti-BCL6 (N3, Santa Cruz), anti-BACH2, or rabbit control IgG (ChIP-grade, Abcam). DNA fragments enriched by ChIP were identified by quantitatively PCR (ChIP-QPCR) using the Fast SYBR green kit. The GAPDH gene, which is not a BCL6/BACH2 target, was used as negative control. The enrichment for each antibody at examined loci is expressed as percentage of input. Primers are listed in Table S3. ChIP-seq libraries were prepared using Illumina ChIP-seq Library preparation Kit according to the manufacturer's instructions with 10ng of purified ChIP DNA and sequenced for 36 cycles using Illumina GAIIx sequencer. Peak calling and downstream analysis was performed using the ChIPseeqer package (2). The ChIPseq data have been uploaded to GEO under accession number GSE44420. ChIP-on-Chip was performed in three Ph^+ ALL cell lines in duplicates as described in (1) (GSE24404). Briefly, to identify target genes of BCL6 from each array, the log-ratio between the probe intensities of the ChIP product and input was computed by taking moving windows of three neighboring probes for the average log-ratio and determining the maximum value of those windows as the signal for each probeset. Random permutation probe log-ratios were used as background control. The peak cutoff was established as higher than the 99^{th} percentile of the 24,175 log-ratio values generated from random permutation probes on the array. A locus with maximum moving average above the cut-offs in both replicates, or in one replicate but the Pearson correlation coefficient of the probes signal of the promoter between the two replicates higher than 0.8 (a rescue procedure), is considered a binding target.

Gene expression - microarray and RNAseq analysis

Microarray. Total RNA from cells used for microarray was isolated by RNeasy purification. Biotinylated cRNA was generated and fragmented according to the Affymetrix protocol and hybridized to mouse 430A 2.0 Array (Affymetrix). After scanning (GeneChip Scanner 3000 7G; Affymetrix), the generated .cel files were imported to Expression Console software (Affymetrix) and processed using the RMA average algorithm for normalization and summarization. RNAseq. Total RNA were extracted and subjected to high-throughput sequencing. Illumina multiplexing library construction, HiSeq 2000 SR50 sequencing, and data generation were performed by

Epigenomics Core Facility at Weill Cornell Medical College (New York, NY) according to manufacturer's instructions. Raw image data were converted into base calls and fastq files via the Illumina pipeline CASAVA version 1.8 with default parameters. All 50-bp-long reads were mapped to the reference mouse genome sequence, mm9, using Tophat with the default parameters (Trapnell et al., 2009). The mRNA expression level for each gene was represented as log2 RPKM (reads per kilobase per million reads), called by Seqgene (Deng X, BMC Bioinformatics 2011).

Patient outcome and gene expression microarray data

Gene expression microarray and patient outcome data were obtained from the GEO database accession numbers GSE5314 (3) and GSE34941 (Geng et al., 2012) of the Eastern Cooperative Oncology Group (ECOG) Clinical Trial E2993 for adult B-ALL, from GSE11877 and GSE28460 (4) of the Children's Oncology Group (COG) Clinical Trial P9906 for children B-ALL (the National Cancer Institute TARGET Data Matrix, http://target nci.nih.gov/dataMatrix/TARGET_DataMatrix.html), from the St. Jude Research Hospital pediatric ALL clinical trial (raw data can be downloaded from http://www.stjuderesearch.org/site/data/ALL3/). The microarray raw data were normalized using the RMA method with Expression ConsoleTM software HG-U133 A and B (St Jude data, n=132) or NimbleScan software (version 2.5, Roche NimbleGen, Madison, WI) for the NimbleGen arrays HG18 60mer expression 385K platform (ECOG data). Expression level of a gene in a sample was determined by the average of expression values from multiple probe sets on the array representing this gene. The *P*-values of differential expression of a gene between BCR-ABL1 ALL and normal pre-B samples and other ALL subtypes were determined by two-sided Wilcoxon test. The end points of the clinical data include overall survival (OS), relapse-free survival (RFS), risk, time to relapse, and expression levels in samples at the time of relapse vs. expression levels at diagnosis.

Array-based methylation analysis using HELP

The HELP (HpaII tiny fragment enrichment by ligation mediated PCR) assay was performed as previously published (Geng et al., 2012). One microgram of high molecular weight DNA was digested overnight with isoschizomer enzymes HpaII and MspI (NEB, Ipswich, MA). DNA fragments were purified using phenol/chloroform, resuspended in 10mM Tris-HCl pH 8.0, and used immediately to set up the ligation reaction with MspI/HpaII-compatible adapters and T4 DNA ligase. Ligation-mediated PCR was performed with enrichment for the 200 to 2000 base pair (bp) products and then submitted for labeling and hybridization onto a human HG_17 promoter custom-designed oligonucleotide array covering 25,626 HpaII amplifiable fragments within the promoters

of the genes. HELP data analysis was performed as described previously (5), using R software and Bioconductor package (http://www.bioconductor.org/). Basically, raw data (.pair) files were generated using NimbleScan software. Signal intensities at each HpaII amplifiable fragment were calculated as a robust (25% trimmed) mean of their component probe-level signal intensities. Any fragments found within the level of background MspI signal intensity, measured as 2.5 mean-absolute- deviation (MAD) above the median of random probe signals, were considered as "failed" probes and removed. A median normalization was performed on each array by subtracting the median log-ratio (HpaII/MspI) of that array (resulting in median log-ratio of 0 for each array).

References:

1. Duy C et al. (2011) BCL6 enables Ph+ acute lymphoblastic leukaemia cells to survive BCR-ABL1 kinase inhibition. *Nature* 473:384–388.

2. Giannopoulou EG, Elemento O (2011) An integrated ChIP-seq analysis platform with customizable workflows. *BMC Bioinformatics* 12:277.

3. Juric D et al. (2007) Differential gene expression patterns and interaction networks in BCR-ABL-positive and -negative adult acute lymphoblastic leukemias. *J Clin Oncol Off J Am Soc Clin Oncol* 25:1341–1349.

4. Hogan LE et al. (2011) Integrated genomic analysis of relapsed childhood acute lymphoblastic leukemia reveals therapeutic strategies. *Blood* 118:5218–5226.

5. Geng H et al. (2012) Integrative epigenomic analysis identifies biomarkers and therapeutic targets in adult B-acute lymphoblastic leukemia. *Cancer Discov* 2:1004–1023.

Fig. S1: *Validation of Hspa5 (Ig heavy chain binding protein) deletion*

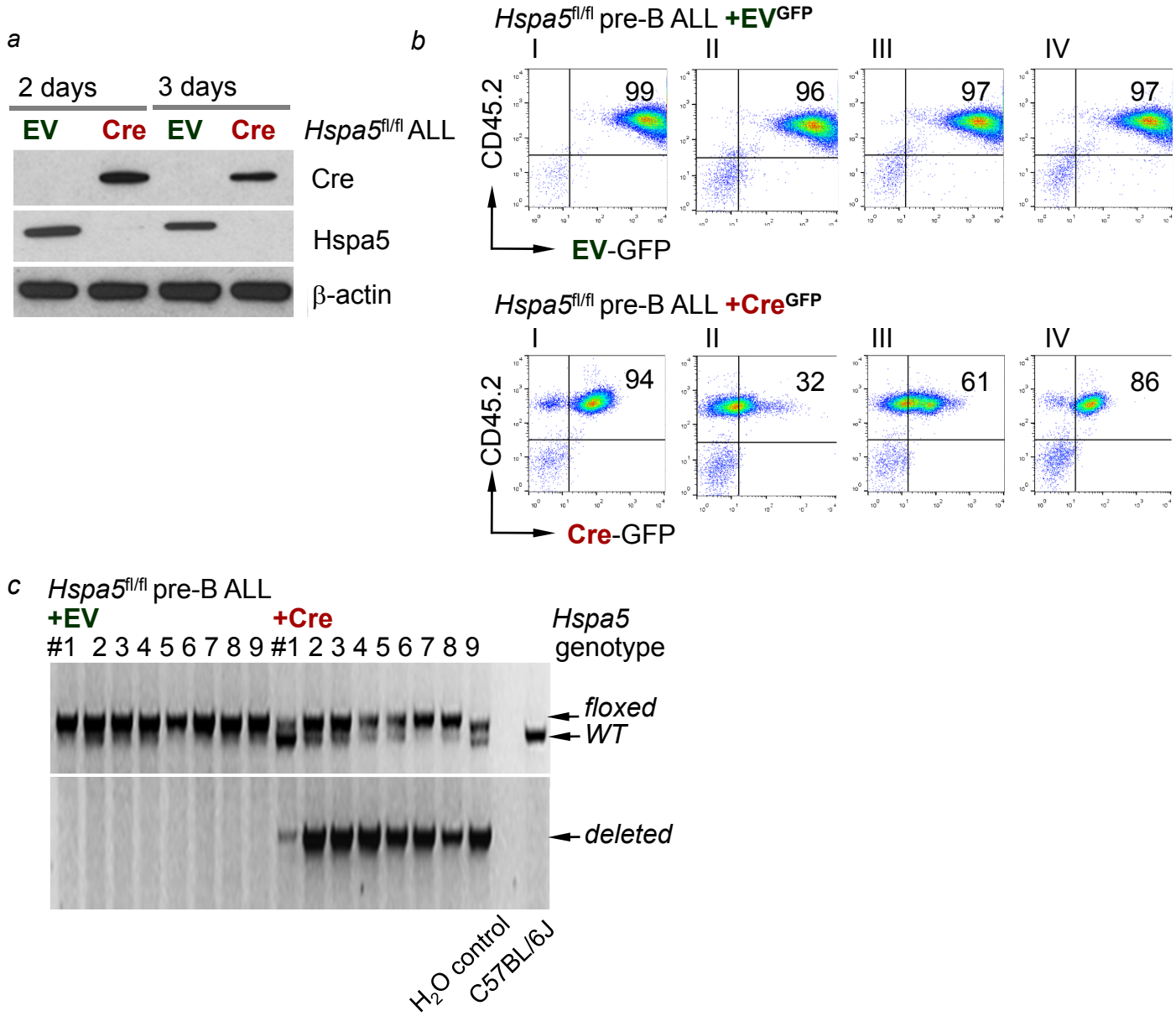


Figure S1 legend: The protein levels of Hspa5 and Cre are measured by Western Blot analyses in *Hspa5*$^{fl/fl}$ pre-B ALL cells with EV and Cre after two and three days of 4-OHT treatment using β-actin as loading control **(a)**. Bone marrows and spleens from transplant recipient mice carrying *Hspa5*$^{fl/fl}$ pre-B ALL with either Cre^{GFP} or EV^{GFP} were stained for the surface markers CD45.1^{-} and CD45.2^{+}. A representative analysis is shown (n=4) (**b**). A genomic PCR amplifying either the floxed allele (floxed), wildtype (WT) or after deletion (deleted) using a wildtype (C57BL6J), H_2O and *in vitro* deletion controls is shown for NOD/SCID mice (n=9) transplanted with *Hspa5*$^{fl/fl}$ pre-B ALL with either Cre^{GFP} or EV^{GFP} (**c**).

Fig, S2: *Validation of microarray gene expression analysis of Hspa5-deletion*

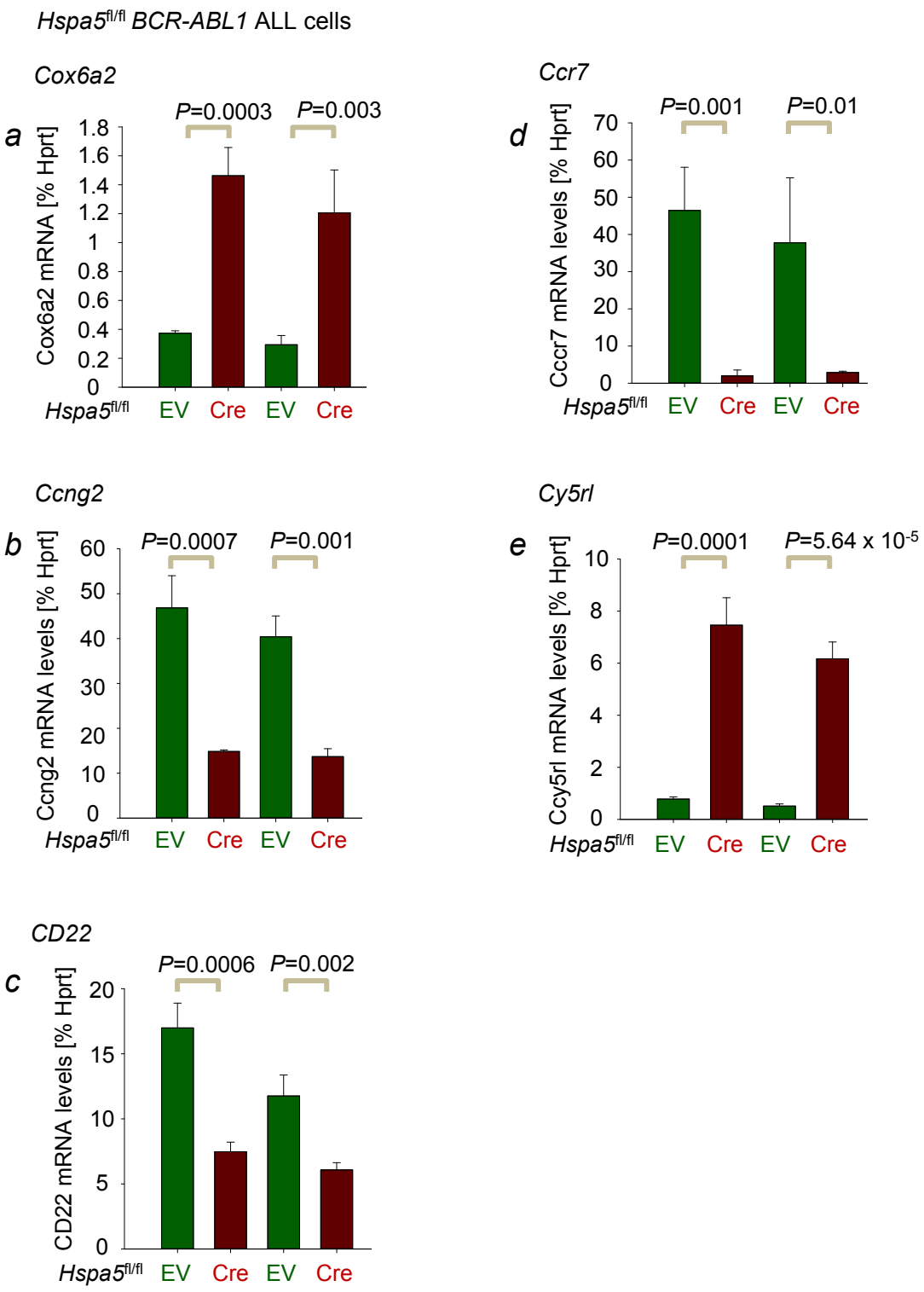


Fig. S2 legend: The mRNA levels of *Cox6a2*, *Ccng2*, *CD22*, *Ccr7* and *Cy5rl* were measured by qRT-PCR in *Hspa5*$^{fl/fl}$ pre-B ALL cells transduced with empty vector control (EV) and 4-OHT-inducible retroviral Cre (Cre) after two days of 4-OHT treatment using *Hprt* as a reference gene (n=2; **a-e**)

Fig. S3: *Expression of plasma cell-specific unfolded protein response molecules ERN1, HSPA5, PRDM1 and XBP1 in normal pre-B cells and pre-B ALL*

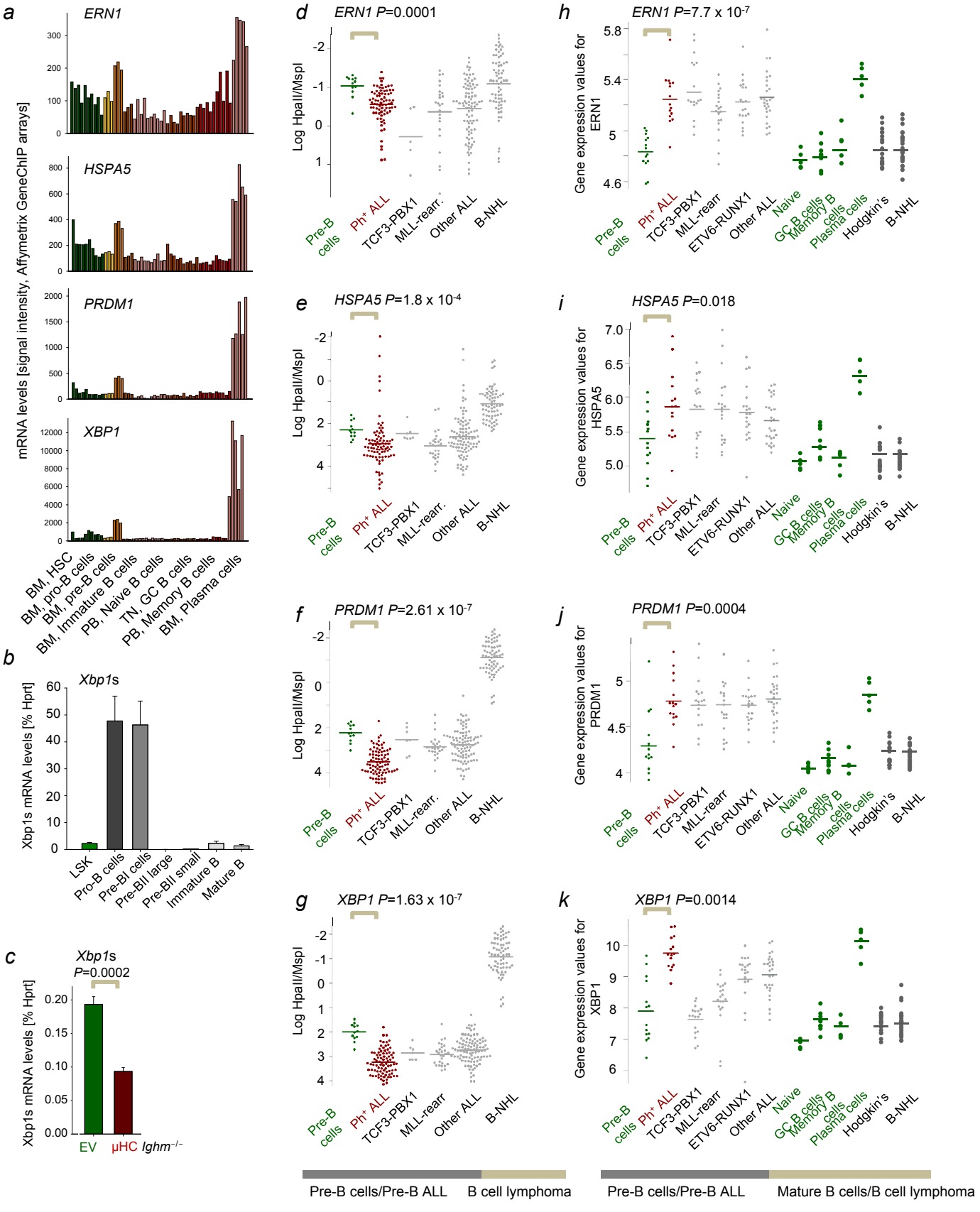
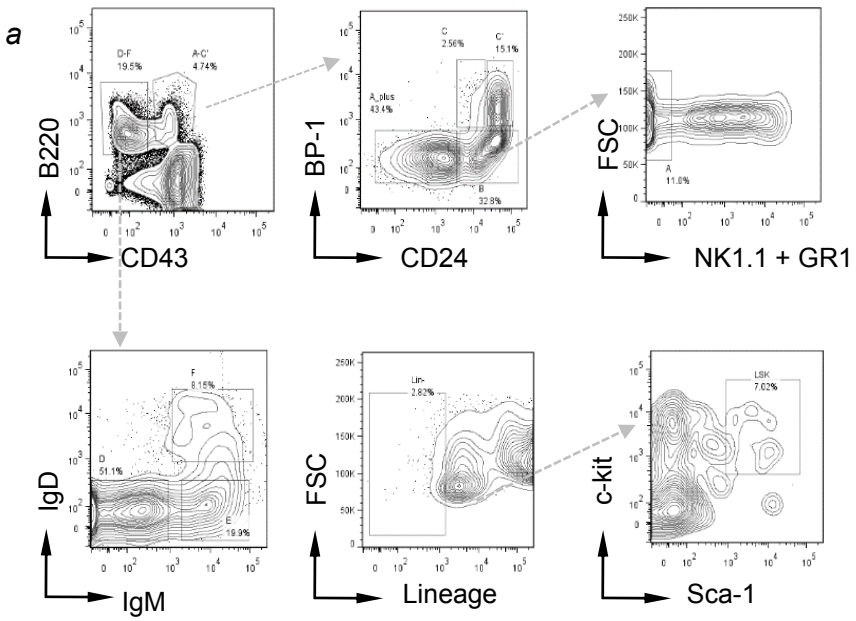


Fig. S3 legend: The gene expression profiling of UPR-related genes (*ERN1* (IRE1), *HSPA5* (BiP), *PRDM1* (Blimp1) and *XBP1* microarray data (GEO accession numbers GSE45460, GSE19599, GSM488979, GSE13411, GSE12453 is shown **(a)**. *Xbp1s* mRNA levels were measured by qRT-PCR using specific primers in Pro-B and Pre-BI cells using sorted Lin^- $Sca\text{-}1^+$ $c\text{-}Kit^+$ (LSK) multilineage progenitors, and bone marrow B cell precursor populations as well as in mature B cells from spleens of C57BL/6 mice (n=3; **b**). *Xbp1s* mRNA levels were measured by qRT-PCR after reconstitution of BCR-ABL1 $Ighm^{-/-}$ pre-B ALL cells with an Ig µ-heavy chain expression vector (µHC) and empty vector control (EV; n=3; **c**). The methylation of the promoter regions of *ERN1*, *HSPA5*, *PRDM1* and *XBP1* genes in ALL subsets compared to mature B cell Non-Hodgkin lymphomas and pre-B cells was assessed by HELP assay (GEO number GSE34937) and the p-values were calculated from Wilcoxon test (**d-g**). the mRNA levels of *ERN1*, *HSPA5*, *PRDM1* and *XBP1* genes from gene expression microarray data in different ALL subsets in comparison to B-cell subsets, Hodgkin and B-NHL and the p-value was calculated by Wilcoxon test (St Jude http://www.stjuderesearch.org/data/ALL3; **h-k**)

Fig. S4: *Sorting strategies of different human and murine B-cell fractions*



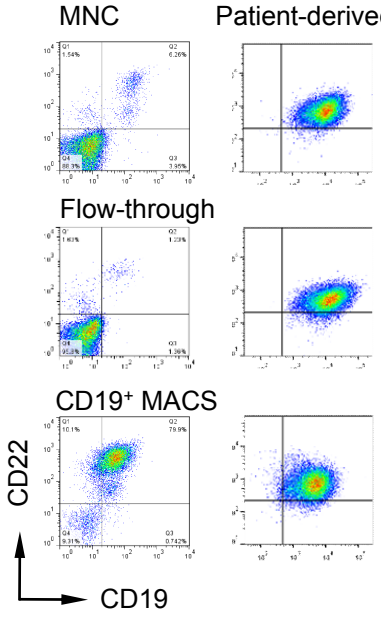


Fig. S4 legend: Multilineage progenitors, and bone marrow B cell precursor populations as well as mature B cells from spleens of C57BL/6 mice and healthy human donors were sorted according to Hardy fractions A-F using multiple surface markers including B220, CD43, BP-1, CD24, NK1.1/Gr1, IgD, IgM, Lineage, c-kit and Sca-1 with a detailed gating strategy as shown in the figure (n=5.**a**). The different fractions of MACS-enriched $CD19^+$ pre-B cells (original mononuclear cells: MNC; flow-through and positively selected $CD19^+$ cells: $CD19^+$ MACS) were stained with the surface markers CD22 and CD19 with three patient-derived pre-B ALL cases (ALL-X2; ALL-Q5; ALL-O2) as a positive control (n=3; **b**).

Fig. S5: *Prdm1 (Blimp1) is required for proliferation and survival of pre-B ALL cells*

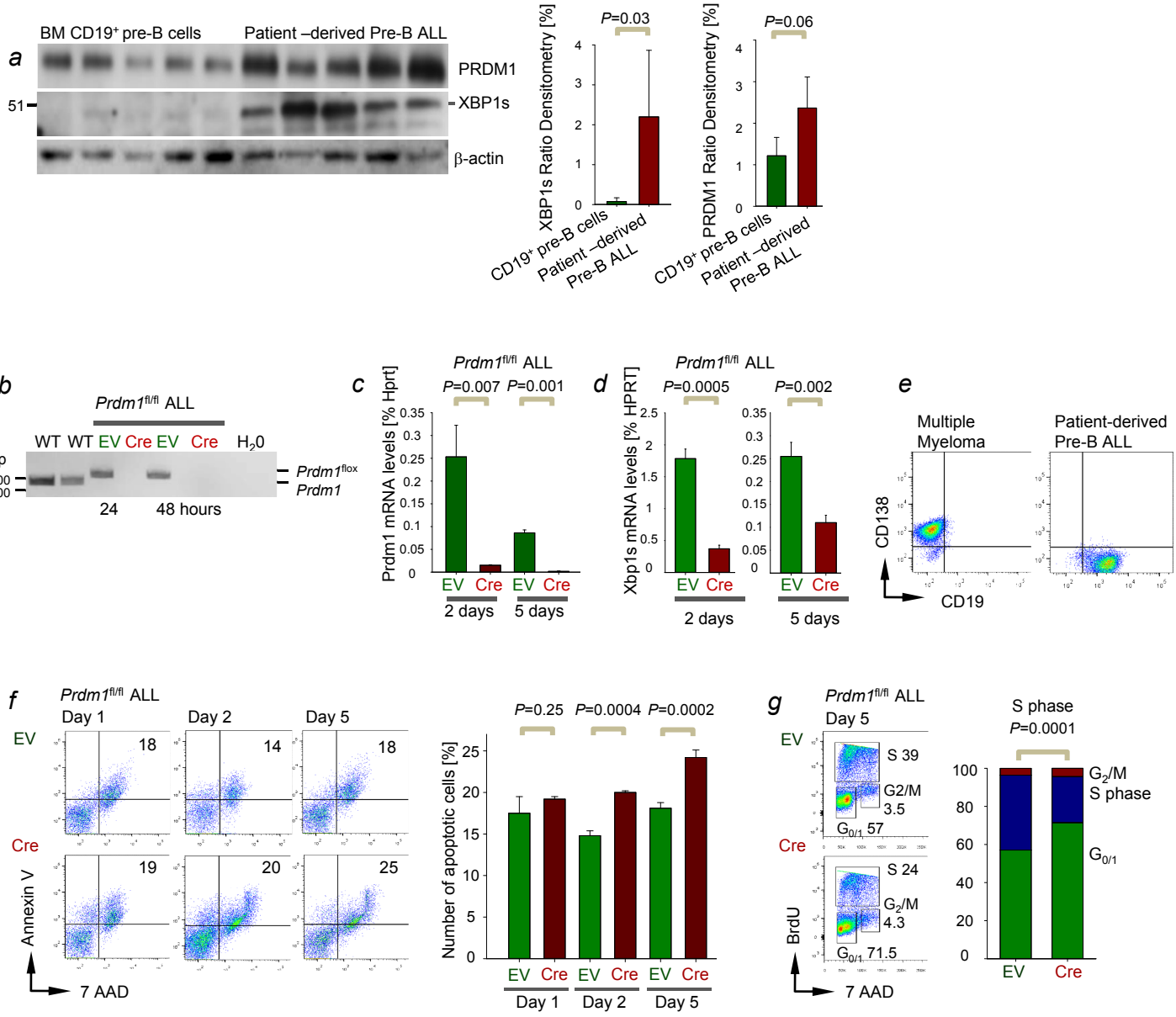


Figure S5 legend: The protein levels of PRDM1 and spliced XBP1 (XBP1s) were measured by Western blot analysis in MACS-enriched normal human CD19^{+} pre-B cells compared to patient-derived pre-B ALL cells and analyzed by densitometry (ALL-Q5, ALL-LAX9, ALL-X2, ALL-BLQ11, ALL-O2) using β-actin as loading control (n=3; **a**). Genomic deletion was assessed after 24 hours treatment with 4-OHT using specific primers to amplify the floxed allele in *Prdm1*$^{fl/fl}$ ALL cells transduced with empty vector control (EV) and 4-OHT-inducible retroviral Cre (Cre) using a wildtype (WT) and H_2O control (n=2) (**b**). *Prdm1* mRNA levels were assessed in *Prdm1*$^{fl/fl}$ ALL cells with EV and Cre at 2 and 5 days after treatment with 4-OHT (n=3) (**c**). Likewise, the *Xbp1s* mRNA levels were measured in *Prdm1*$^{fl/f}$ ALL cells with EV and Cre by qRT-PCR after 2 and 5 days post 4-OHT treatment (n=3; **d**). A representative FACS blot is shown for a patient-derived Pre-B ALL sample (ALL-ICN1) and a multiple myeloma cell line (JJN3) stained for the surface markers CD138 and CD19 (**e**). Apoptosis was assessed by Annexin-V and 7AAD FACS staining in *Prdm1*$^{fl/f}$ ALL cells with EV and Cre after 1, 2 and 5 days treatment with 4-OHT (n=3; **f**). *Prdm1*$^{fl/f}$ ALL cells with EV and Cre were stained with BrdU and 7AAD and the percentages of cells in the $G_{0/1}$, S and G_2/M cell cycles are indicated after 5 days of 4-OHT treatment and shown in bar graph (n=3, p=0.0001 for the S-Phase; **g**).

Fig. S6: *Regulation of XBP1 in Ph⁺ ALL*

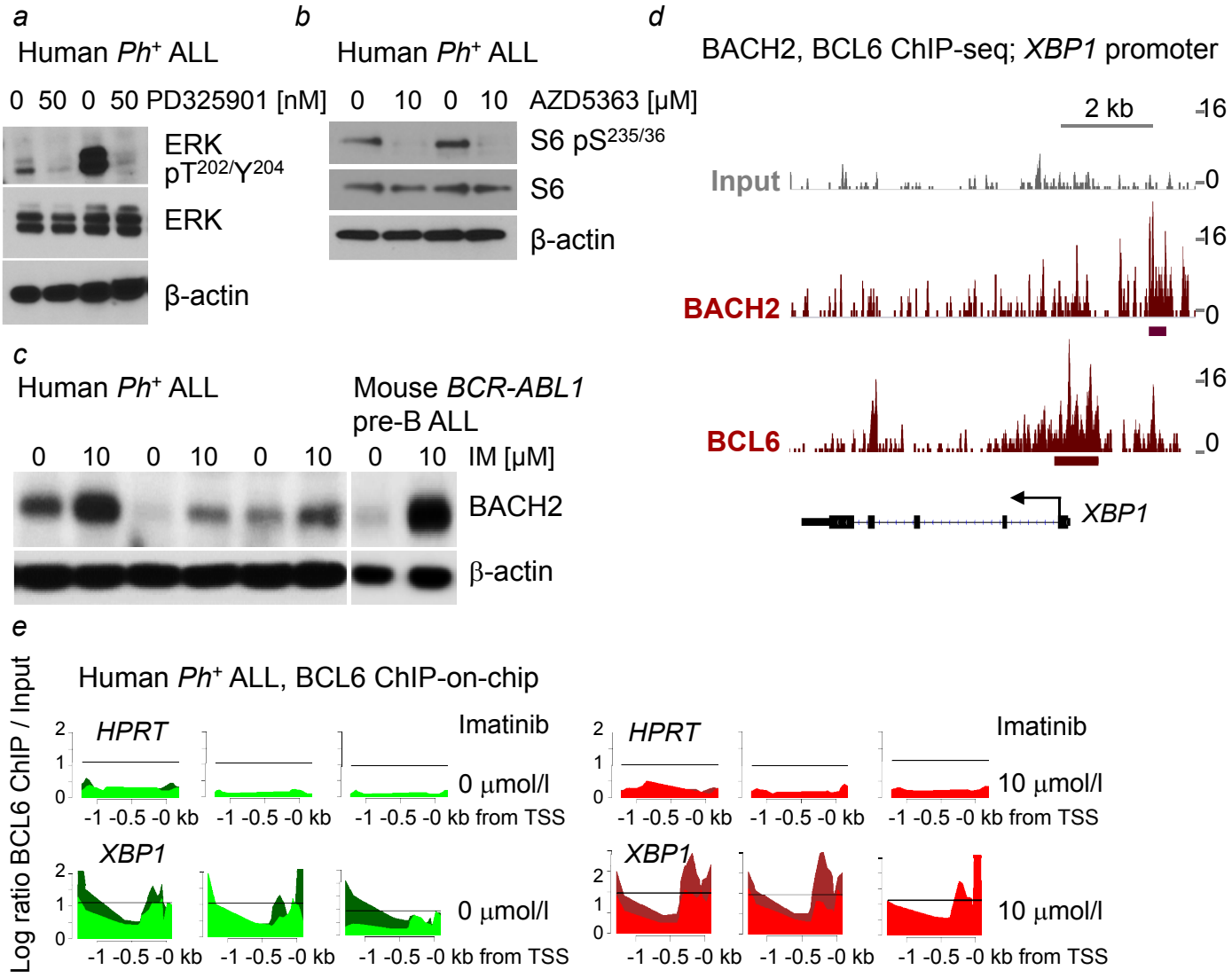


Fig S6 legends: Protein levels of phosphorylated and total ERK were measured by Western blot analysis in human *Ph*⁺ ALL cells (PDX2, LAX7R) treated with or without the MEK-inhibitor PD325901 for 5 hours (50 nM PD325901) using β-actin as loading control (**a**). Protein levels of phosphorylated and total S6 were measured by Western blot analysis in human *Ph*⁺ ALL cells (PDX2, LAX7R) treated with or without the AKT-inhibitor AZD5363 for 5 hours (10 μM AZD5363) using β-actin as loading control (**b**). Protein levels of BACH2 were measured by Western blot analysis in human *Ph*⁺ ALL cells (ICN1, BV-173, TOM1) and mouse BCR-ABL1 pre-B ALL cells treated with or without the TKI imatinib for 16 hours (human: 10 μM IM; mouse 2 μM IM) using β-actin as loading control (**c**). ChIP-seq analysis was performed using antibodies against BACH2 and BCL6 (GEO number GSE44420). Peaks with significant enrichment of ChIP-seq relative to input were identified by ChIP-seeqer (http://physiology.med.cornell.edu/faculty/elemento/lab/chipseq.shtml) and shown as bold underlines. The gene model in UCSC genome browser view (hg18) and the arrow for transcription start site were shown (**d**). BCL6 ChIP-on-chip analysis was performed on human *Ph*⁺ ALL cell lines (Tom1, Nalm1, BV-173) treated with and without imatinib (10 μmol^{-l}) for 24 h as previously described (26) (two representatives shown; GEO number GSE24404) (**e**).

Fig. S7: *Verification of Xbp1-deletion in pre-B cells and BCR-ABL1 ALL*

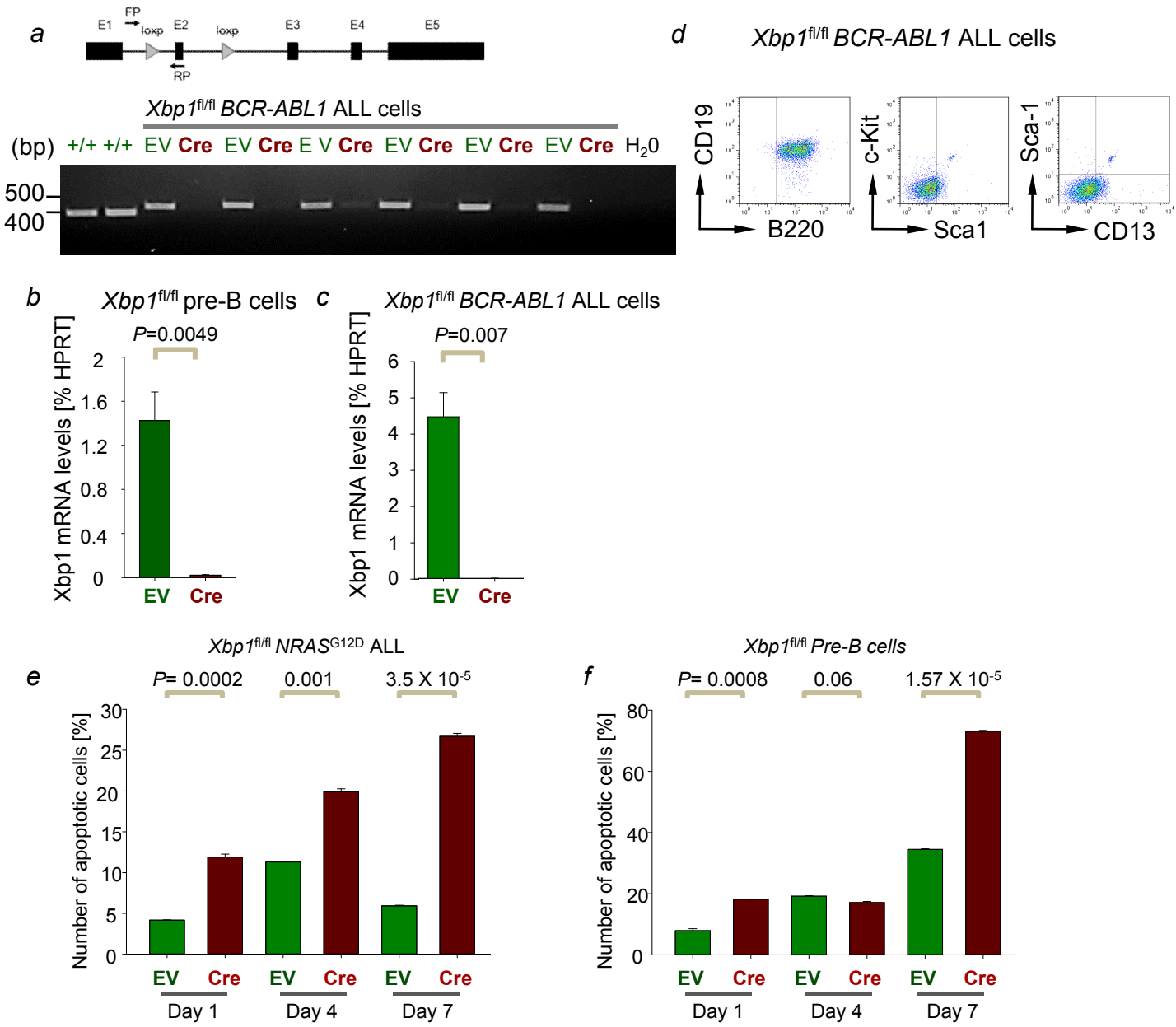
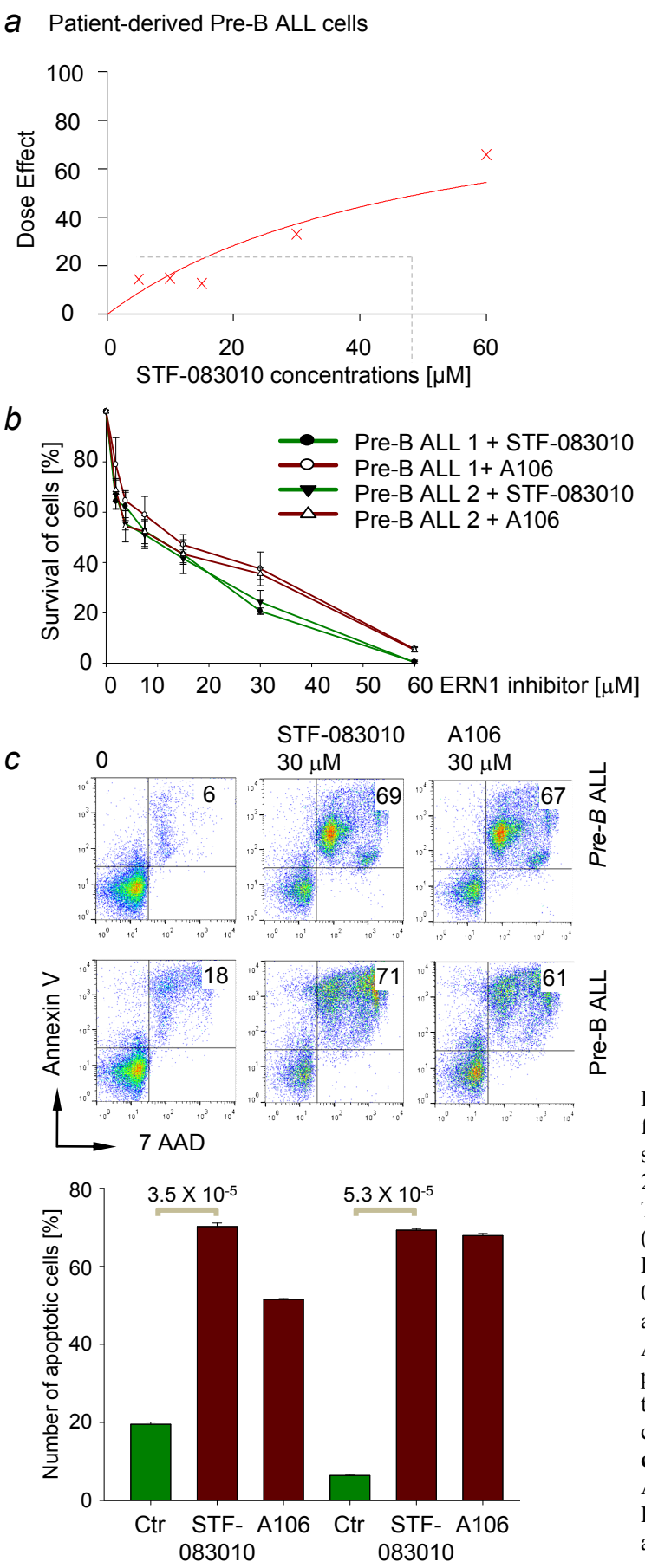


Fig. S7 legend: A representative schematic of the designed primers amplifying the loxP sites at exon 2 of the *Xbp1* gene is shown in the top panel. In the lower panel, genomic deletion by PCR was assessed in *Xbp1*$^{fl/fl}$ ALL cells with EV or Cre after 1 day administration of 4-OHT using a wildtype (WT) and H_2O control (n=6; **a**). *Xbp1* mRNA levels were measured in IL7-dependent *Xbp1*$^{fl/fl}$ pre-B cells with EV or Cre after 24 hours of 4-OHT treatment by qRT-PCR (n=3; **b**). *Xbp1* mRNA levels were measured in *Xbp1*$^{fl/fl}$ *BCR-ABL1* ALL cells with EV and Cre after 24 hours of 4-OHT treatment by qRT-PCR (n=3; **c**). *Xbp1*$^{fl/fl}$ *BCR-ABL1* ALL cells were stained for the surface markers CD19, B220, c-Kit, Sca-1 and CD13 (n=3; **d**). Apoptosis was quantified by Annexin-V and 7AAD staining in *Xbp1*$^{fl/fl}$ *NRAS*^{G12D} ALL cells with EV and Cre after 1, 4 and 7 days treatment with 4-OHT (n=3; **e**) Apoptosis was quantified by Annexin-V and 7AAD FACS staining in IL7-dependent *Xbp1*$^{fl/fl}$ pre-B cells with (Cre) or without (EV) deletion of *Xbp1* after 1, 2 and 5 days treatment with 4-OHT (n=3; **f**).

Fig. S8: *Pharmacological inhibition of ERN1 endoribonuclease activity causes cell death in patient-derived pre-B ALL cells*



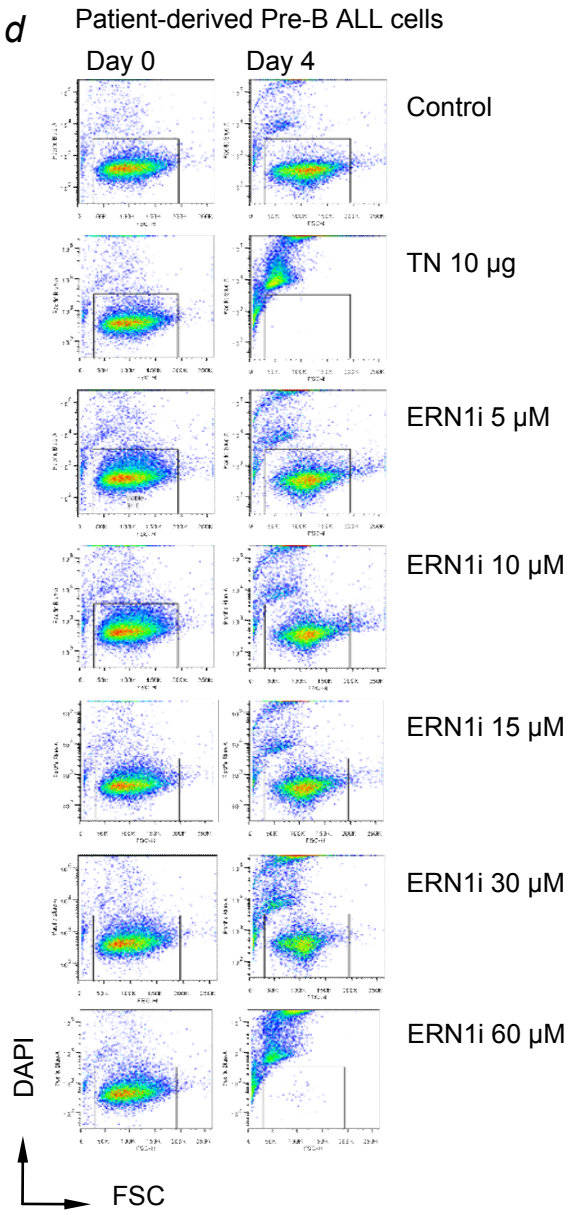


Fig. S8 legend: The dose effect was calculated as measured for mRNA levels of *XBP1s* by qRT-PCR with the CalcuSyn software in patient-derived pre-B ALL cells (ALL-Q5) after 24 hours treatment with either the ER-stress inducer Tunicamycin (TN; 10 µg) in combination with STF-083010 (0-60 µM) (ERN1i) for 24 hours (n=3; **a**). Patient-derived pre-B ALL cases (ALL-Q5 and ICN1) were treated with STF-083010 or A106 (0-60 µM) and the relative viability was assessed by CCK-8 assay (n=3; **b**). Apoptosis was assessed by Annexin-V and 7AAD FACS staining in two patient-derived pre-B ALL cases (ALL-Q5 and ALL-O2) after 3 days treatment with either STF-083010 and A106 at two concentrations (30 and 60 µM) or DMSO control (0 µM; n=3; **c**). Patient-derived pre-B ALL (ALL-Q5) were treated with A106 (0-60µM) and TN (10µg) and viability was assessed by DAPI staining up to four days. The representative FACS blots are shown at day 0 and 4 (n=3; **d**).

Fig. S9: *Pharmacological inhibition of ERN1 endoribonuclease activity causes cell death in patient-derived pre-B ALL cells*

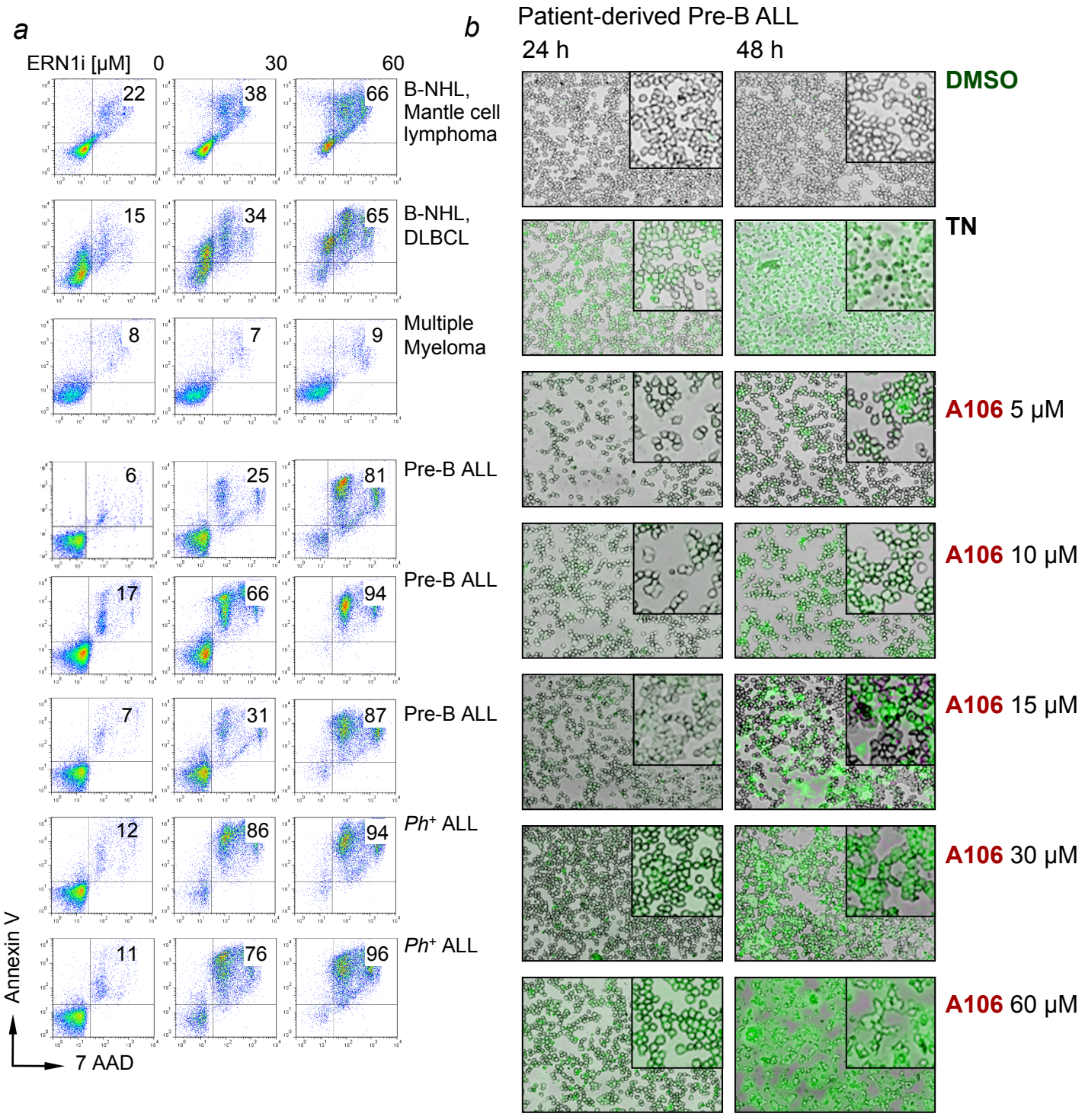


Fig. S9 legend: Apoptosis was assessed by Annexin-V and 7AAD FACS staining in a panel of human cell lines including B-NHL (Toledo, Jeko), multiple myeloma (JJN3) and patient-derived Pre-B and *Ph*$^+$ ALL cases (BLQ1, ICN1, ALL-O2, ALL-L3, ALL-Q5) after 3 days treatment with STF-083010 at two concentrations (30 and 60 μM) or DMSO control (n=3; **a**)). Patient-derived pre-B ALL cells (ALL-Q5) were treated with either TN (10μg) or STF-083010 (0-60 μM) and stained with ER-Tracker green after 24 hours and 48 hours (n=3; **b**).

Fig. S10: *High XBP1 mRNA levels at the time of diagnosis predict poor clinical outcome in patients with pre-B ALL*

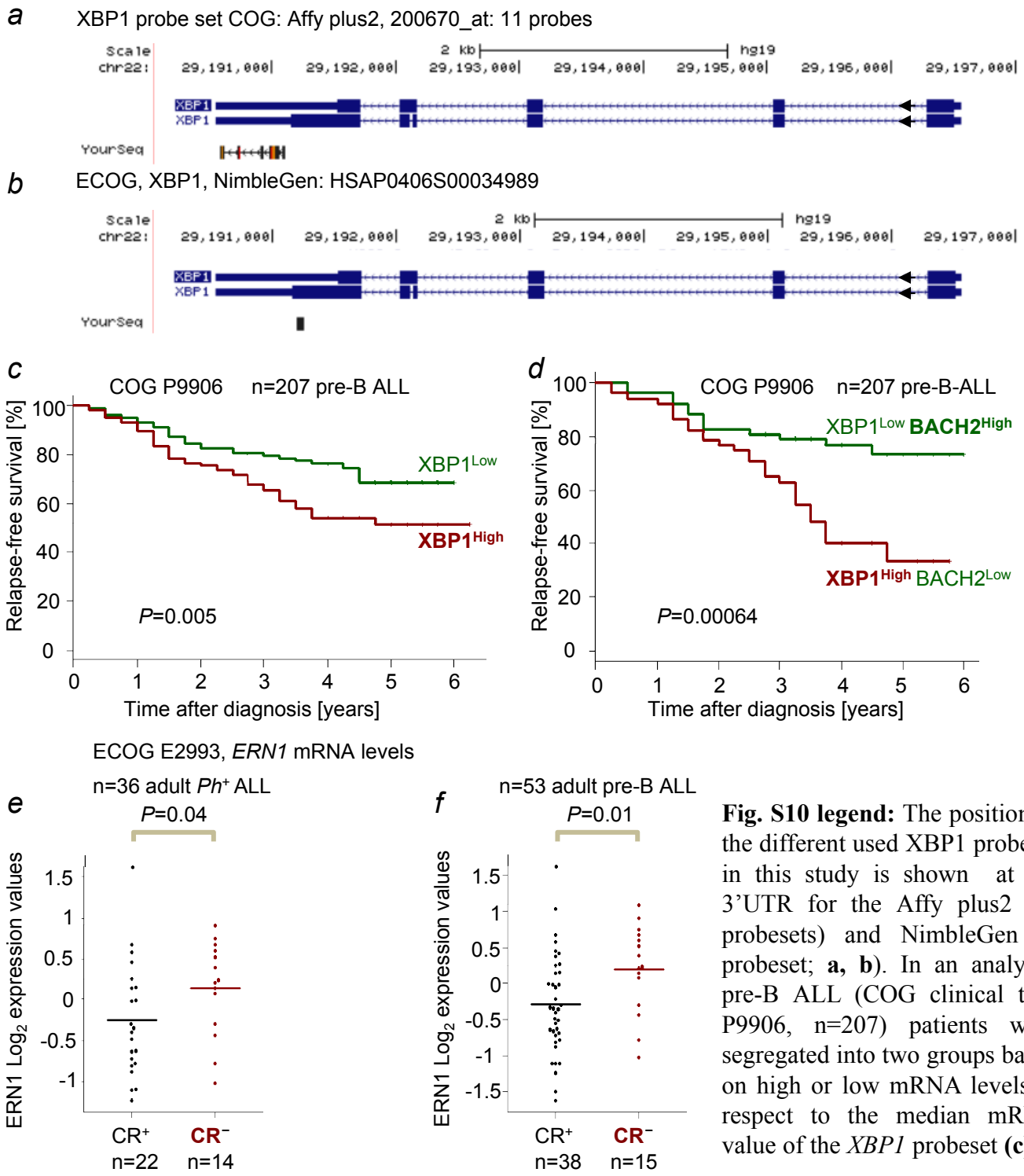


Fig. S10 legend: The position of the different used XBP1 probeset in this study is shown at the 3'UTR for the Affy plus2 (11 probesets) and NimbleGen (1 probeset; **a, b**). In an analysis, pre-B ALL (COG clinical trial P9906, n=207) patients were segregated into two groups based on high or low mRNA levels in respect to the median mRNA value of the *XBP1* probeset **(c)**.

In a multivariate analysis, pre-B ALL (COG clinical trial P9906, n=207) patients were segregated into two groups based on high or low mRNA levels of *XBP1* or *BACH2*, in respect to the median expression value of the *XBP1* probeset. **(d)**. *ERN1* expression was measured in patients with either a positive (CR^+) or negative complete response (CR^-) in both adult Ph^+ ALL (n=36) and adult pre-B ALL patients (n=53) **(e, f)**.

Table S1: *Overview of patient-derived samples of Ph$^+$ ALL and cell lines*

Case	Karyotype	Clinical course	Gender/Age
Primary cases, Pre-B ALL			
ALL-X2	uncharacterized	Unknown	m/38
LAX9	t(9;22)(q34;q11) del(12)(p12;p13)	At diganosis	m
ALL-O2	*NRAS*^{G12D}	At diagnosis	m/7
BLQ1	FISH der(9), der(22)	Unknown	
ALL-Q5	*KRAS*^{G12V}	Unknown	f
BLQ11	FISH der(9), der(22)	Relapse (Imatinib)	m
ALL-L3	*KRAS*^{G12V}	At diagnosis	
ICN1	der(9)(q10)t(9;22)(q34;q11)	At diagnosis	f
LAX7R	uncharacterized	Relapse	
PDX2	BCR-ABL1 p190	At diagnosis	f/52
Primary cases, Multiple myeloma			
MM1	n.d.	Newly diagnosed	m/44
MM2	n.d.	Relapsed	m/66
MM3	n.d.	Persistent /Relapsed	m/66
MM4	n.d.	Persistent /Relapsed	m/68
MM5	n.d.	Newly diagnosed	f/36
MM6	n.d.	Persistent, at diagnosis	m/65
Cell lines			
BV173	add(1)(q42), add(8)(p23), der(22)t(9;22)(q34;q11)		m/45
Nalm1	der(7)t(7;9;15)(q10;?;q15), t(9;9)(p24;q33-q34) t(9;22) (q34;q11), der(15)t(7;9;15)		f/3
SUP-B15	t(1;1)(p11;q31), add(3)(q27), add(10)(q25), t(9;22)(q34;q11)	Relapse	m/9
TOM1	del(7)(p14),der(9)del(9)(q13q34) der(22)t(9;22)(q34;q11)	Refractory	f/54
JJN-3	hypotriploid karyotype with 9% polyploidy	At diagnosis	f/57
JeKo-1	*ATM* amplification, *MYC* amplification *P16INK4A* deletion *SYK* amplification *P53* deletion/mutation		f/78
Toledo	Complex karyotype		f

Notes: All primary samples are bone marrow biopsies, blast content >80%

Table S2: *Overview of mouse strains used in this study*

Mouse strain	Source	Purpose
Bach2$^{-/-}$	Kazuhiko Igarashi, Tohoku University	Analysis of *Xbp1* as Bach2 target gene
BCL6$^{-/-}$	B. Hilda Ye, Albert Einstein College	Analysis of *Xbp1* as BCL6 target gene
[a]*Xbp1*$^{fl/fl}$	Laurie H. Glimcher, Cornell Medical School	Genetic loss-of-function experiments
[b]*Stat5*$^{fl/fl}$	Lothar Hennighausen, NIDDK	Analysis of *Xbp1* as STAT5 target gene
Prdm1$^{fl/fl}$	Jackson Laboratories	Genetic loss-of-function experiments
Hspa5 $^{fl/fl}$	Amy S Lee, USC	Genetic loss-of-function experiments
NOD/SCID	Jackson Laboratories	Xenograft recipient mice

Notes:

[a] Lee, A.-H., Scapa, E. F., Cohen, D. E. & Glimcher, L. H. Regulation of hepatic lipogenesis by the transcription factor XBP1. *Science* **320**, 1492–1496 (2008).

[b] Liu, X. *et al.* Stat5a is mandatory for adult mammary gland development and lactogenesis. *Genes Dev.* **11**, 179–186 (1997).

Table S3: *Sequences of oligonucleotide primers used*

Quantitative RT-PCR

Human

COX6B_F	5'-AACTACAAGACCGCCCCTTT-3'
COX6B_R	5'-GCAGCCAGTTCAGATCTTCC-3'
XBP1s_F	5'- TGCTGAGTCCGCAGCAGGTG -3'
XBP1s_R	5'- GCTGGCAGGCTCTGGGGAAG -3'
ERDJ4_F	5'- GCTACTCCCCAGTCAATTTTCA -3'
ERDJ4_R	5'- CCGATTTTGGCACACCTAAGAT -3'
p58IPK_F	5'- TGTGTTTGGGATGCAGAACTAC -3'
p58IPK_R	5'- TCTTCAACTTTGACGCAGCTT -3'
SEC61A1_F	5'- TGTCATCTCCCAAATGCTCTCA -3'
SEC61A1_R	5'- ACAGGTAATAGCAAAGGCCAC -3'

Mouse

Hprt_F	5'-GGGGGCTATAAGTTCTTTGC-3'
Hprt_R	5'-TCCAACACTTCGAGAGGTCC-3'
Xbp1 (ex2)_F	5'- CCTGAGCCCGGAGGAGAA -3'
Xbp1 (ex2)_R	5'- CTCGAGCAGTCTGCGCTG -3'
ERdj4_F	5'- GGATGGTTCTAGTAGACAAAGG-3'
ERdj4_R	5'- CTTCGTTGAGTGACAGTCCTGC-3'
p58IPK_F	5'- GCATCTTGAATTGGGGAAGA-3'
p58IPK_R	5'- CAAGCTTCCCTTGTTTGAGC-3'
Prdm1_F	5'- GAAATGGAAAGATCTATTCCAGAG-3'
Prdm1_R	5'- GGCATTCTTGGGAACTGTGT-3'
Cox6a2_F	5'- CGGTTATGAGCACCCTTGAT-3'
Cox6a2_R	5'- CTGTTCCCAAAGAGCCAGAG-3'
Ccng2_F	5'-TGATCCGCATCAGTCAGTGT-3'
Ccng2_R	5'- ACAATCGCGTGGTACAAGTG-3'
CD22_F	5'-GAGCAAGCTCACCTTCCAAC-3'
CD22_R	5'-CACCTCTGTGGGATTGACCT-3'
Ccr7_F	5'- GCCTTGATCACCATCCAAGT-3'
Ccr7_R	5'- GATCACCTTGATGGCCTTGT-3'
Cy5r1_F	5'- ATCCAACCCAGTGCTTTCTG-3'
Cy5rl_R	5'- CAAAGCCCTTGCTGTAGGTC-3'

Genomic PCR

Mouse

Xbpt_F	5'-TTTGGCTTGGGGAGGGACA-3'
Xbpt_R	5'-AGCAGTCTGCGCTGCTACTCT-3'
Prdm1_F	5'-CAATGCTTGTCTAGTGTC 3'
Prdm1_R	5'-AGTAGTTGAATGGGAGC-3'
Hspa5_F	5'-GATTTGAACTCAGGACCTTCGGAAGAGCAG-3'
Hspa5_R	5'-TTGTTAGGGGTCGTTCACCTAGA-3'

Table S3: *Sequences of oligonucleotide primers used* –continued

Single locus ChIP PCR

Human

BACH2_F	CCTACCTGGCAAAAACAAAAAC
BACH2_R	TCTTTTTGAGCAGTGGCATAGA
BCL6_F	5’-CCGAGAATTGAGCTCTGTTGA-3’
BCL6_R	5’- GGCAGCAACAGCAATAATCA-3
XBP1_F	AATGTTTTGGGAGAAGTCTGGA
XBP1_R	TAAAACGTAACAGGTGGCATGA
GAPDH_F	ACGTAGCTCAGGCCTCAAGA
GADPH_R	GCTGCGGGCTCAATTTATAG

Table S4: *Antibodies used for Western blotting and flow cytometry*

Western blot

Antigen	Clone ID	Source
ACTB	Polyclonal (ab8227)	Abcam
XBP1	Polyclonal (ab37152)	Abcam
ARF- Mo	Polyclonal (ab80)	Abcam
P21	C-19	Santa Cruz Biotechnology
P27	F-8	Santa Cruz Biotechnology
p38	5F11	Cell Signaling Technology
Tp53	IC12	Cell Signaling Technology
p-P38(Thr180/Tyr182)	Polyclonal (9211)	Cell Signaling Technology
JNK	Polyclonal (9252)	Cell Signaling Technology
p-JNK(Thr183/Tyr185)	Polyclonal (9251)	Cell Signaling Technology
CHOP	Polyclonal (5554)	Cell Signaling Technology
Prdm1	Monoclonal (9115)	Cell Signaling Technology
Cre	Polyclonal (ab40011)	Abcam
pERK(Thr202/Tyr)	Monoclonal (2211)	Cell Signaling Technology
ERK	Polyclonal (ab40011)	Abcam
pS6(Ser235/236)	Polyclonal (2211)	Cell Signaling Technology
S6	Monoclonal (2217)	Cell Signaling Technology

Flow cytometry

Surface Antigen	Source
CD19	BD Biosciences
Sca-1/Ly6a	BD Biosciences
CD117/cKIT	BD Biosciences
CD138/SDC1	BD Biosciences
B220/CD45R	BD Biosciences
CD43	Biolegend
BP-1	Biolegend
NK1.1 and GR1	Biolegend
IgD	Biolegend
IgM	Biolegend
Lin	Biolegend
CD24	BD Biosciences
Annexin-V	BD Biosciences
7AAD	Invitrogen
TruStain FxX block	BD Biosciences
Lin (CD3/33/56)	BD Biosciences
CD34	Biolegend
CD10	BD Biosciences
CD19	Invitrogen
CD22	BD Biosciences
Human Fc block	Miltenyi
BrdU	Invitrogen

Table S5: *Retroviral and lentiviral vectors used*

Constitutive expression	**Inducible activation**
MSCV BCR-ABL1-IRES-Neo	MSCV-Cre-ER^{T2}
MSCV-GFP-IRES	MSCV-ER^{T2}
MSCV-GFP-IRES-CRE	MSCV-Cre-ER^{T2}-GFP
MI µHC-CD8	MSCV-ER^{T2}-GFP
MI CD8	PAX5-ERT2-GFP
pCCL-c-MNDU3-Luc-SV40-Neo	pcl6-ERT2-GFP