

SUPPLEMENTAL MATERIALS AND METHODS

Cell lines and patients' cells

The **human pre-B Nalm6** cell line (ATCC® CRL-3273™) was initiated from BCP-ALL relapse (1) and presents a near diploid karyotype with a translocation t(5;12)(q33.2 ; p13.2) (2). The human **REH** cell line (ATCC® CRL-8286™) is a pre-B ALL cell line initiated from the peripheral blood of a patient with pre-B acute lymphoid leukemia ALL in first relapse (3). REH cells carry the chromosomal translocation t(12;21) and chromosomal deletion del(12) producing respective *ETV6-RUNX1* (previously known as *TEL-AML1*) fusion gene and deletion of residual *ETV6* gene (previously known as *TEL*)(2). The human **BJAB** cells was initiated from human Burkitt lymphoma (EBV-negative). Nalm6, REH and BJAB cells were maintained in RPMI 1640 medium (Gibco, Thermo Fisher Scientific) containing 10% heat-inactivated fetal calf serum (FCS) (Eurobio) that was supplemented with antibiotics (100 U/mL penicillin-G and 100 U/mL streptomycin, Gibco). The **human embryonic kidney cells 293 (HEK293)** (ATCC® CRL-1573™) were maintained in DMEM/10% FCS/1% antibiotics. The cells were maintained at 37°C in a humidified incubator under a 5% CO₂ atmosphere.

Bone marrow mononuclear cells from pre-B leukemic patients were collected at diagnosis, after informed consent had been obtained, in accordance with the declaration of Helsinki. The protocol was approved by the ethic committee of Rennes Hospital (Rennes, France). Vital mononuclear cells were isolated from bone marrow by successive centrifugations through lymphocytes separation medium (Eurobio) (4).

Rapid immunoprecipitation mass spectrometry of endogenous proteins (RIME)

RIME was conducted with Nalm6 cells as previously described (5). The lysates were incubated with 10 µg of anti-RUNX1 (ab23980, Abcam), 5 µg of anti-RUNX1 (HPA004176, Sigma Aldrich) or with 10 µg of normal rabbit IgG (sc2027, Santa Cruz Biotechnology) antibodies. Peptides were visualized using Scaffold 4 software (<http://www.proteomesoftware.com/products/scaffold/>). We selected proteins where the sum of peptide count for both RUNX1 antibodies is at least 3 and higher than 2-fold IgG background. Proteins identified by only one RUNX1 antibody were excluded.

Proximity Ligation Assay (PLA)

PLA was carried out with Duolink® In Situ Detection Reagents Orange (DUO92007, Sigma Aldrich) and Duolink® In Situ PLA® Probes (DUO92002 and DUO92004, Sigma Aldrich). The protocol was followed as described by Debaize et al. (6). Quantification of PLA dots per nucleus was performed by automatic counting with ImageJ. Values below the assay cut-off (set to two standard deviations over the background signal) (7) were considered negative for the interaction of interest while samples with values higher than the threshold are positive.

Sorting of human hematopoietic stem and progenitor populations

Human mobilized CD34⁺ cells (German Red Cross Blood Donor Service Baden-Württemberg/Hessen, Frankfurt/Main, Germany) received after ethical vote consent were resuspended in FACS-buffer (PBS + 10% FCS, 0,2% EDTA, 1% NaN₃) for staining with the following antibodies: CD123 BV421 (BD Biosciences, Clone 9F5), CD45RA BV570 (BioLegend, Clone HI100), Thy1/CD90 PerCP-Cy5.5 (eBioscience, Clone 5E10), CD34 APC (BD Biosciences, Clone 8G12), CD38 APC-H7 (BD Biosciences, Clone HB7), CD2 biotin (eBioscience, Clone RPA-2.10), CD3 biotin (eBioscience, Clone OKT3), CD14 biotin (eBioscience, Clone 61D3), CD16 biotin (eBioscience, Clone CB16), CD56 biotin (eBioscience, Clone CMSSB), CD235a eBioscience, Clone HIR2), Streptavidin-PE-Dazzle 594 conjugate (BioLegend), Fixable Viability Dye eFluor 506 (eBioscience).

Hematopoietic stem/progenitor cell populations were identified by their immunophenotype (HSC, Lin-CD34⁺CD38⁻CD45RA⁻CD90⁺ ; MPP, Lin-CD34⁺CD38⁻CD45RA⁻CD90⁻ ; GMP, Lin-CD34⁺CD38⁺CD10⁺CD132⁺CD45RA⁺ ; MLP, Lin-CD34⁺CD38⁻CD90⁻/loCD45RA⁺) and sorted on a FACS Aria III (BD Biosciences) for the PLA assay.

Co-Immunoprecipitation (Co-IP) assay

Flag-FUBP1 was immunoprecipitated with beads coupled with anti-M2 Flag antibody (M8823, Sigma), according to the manufacturer's instructions. Briefly, 20.10⁶ HEK293 cells were collected and lysed with cell lysis buffer (20 mM Tris-HCl, pH8.0, 150 mM NaCl, 0.5 mM EDTA) for 30 min on ice. The supernatant was incubated with 20 µL of anti-M2 Flag magnetic beads overnight at 4°C under agitation. Beads were washed with TBS and eluted with 30 µL of Laemmli (Sigma Aldrich). Immunoprecipitates were analyzed by western blotting with anti-M2 FLAG (F1804, Sigma-Aldrich) antibody. HaloTag-RUNX1 was immunoprecipitated with the HaloTag® Mammalian Pull-Down System (G6504, Promega) according to the manufacturer's instructions, or with RUNX1 ab23980 antibody (**Table S1**).

Generation of stable cell lines

The **Nalm6**^{+FUBP1} (or **REH**^{+FUBP1}) and **Nalm6**^{+RUNX1} stable cell lines were obtained from lentiviral transduction. Flag-FUBP1 human ORF from *pCDNA3-FLAG-FUBP1* and Halotag-RUNX1 human ORF from *pFN21A* (#FHC01784, Kazusa collection, Promega) respectively were cloned into a lentivirus *pLenti-CMV-Puro-DEST* by Gateway technology. *pLenti-CMV-Puro-DEST* (w118-1) was a gift from Eric Campeau (Addgene plasmid #17452) (8). To produce lentivirus, HEK293 cells were co-transfected with *pLenti-CMV-Puro-DEST* bearing Flag-FUBP1 or Halotag-RUNX1, *pSPAX2* and *pCMV-VSV-G* for packaging using Lipofectamin 3000 transfection reagent (Thermo Fisher Scientific). The plasmid *psPAX2* was a gift from Didier Trono (Addgene plasmid # 12260) and *pCMV-VSV-G* was a gift from Bob Weinberg (Addgene plasmid # 8454) (9). After 48h, supernatant was harvested, filtered and added to Nalm6 cells with 4µg/mL polybrene, and transduced cells were selected in medium containing 0.25 µg/mL puromycin (Invivogen).

To produce the **Nalm6**^{+RUNX1+FUBP1} cells, Nalm6 were infected first with lentivirus *pLenti-CMV-Puro-DEST* carrying Flag-FUBP1 and selected in medium containing 0.25 µg/mL puromycin. Second, cells were infected with *pLenti-CMV-Puro-DEST* caring Halotag-RUNX1. Cells carrying Halotag-RUNX1 were labelled with cell permeant HaloTag® Oregon Green® Ligand (496_{Ex}/516_{Em}) (Promega) according to the manufacturer's instructions and selected by flow cytometry.

To produce the **Nalm6**^{shFUBP1} cells, HEK293 cells were co-transfected with *pSEW-shFBP1*, *pMDG2* and *p8.91* (kindly given by Dr. Zornig(10)) using Lipofectamine 3000 transfection reagent. After 48h, supernatant was harvested, filtered, and added to Nalm6 cells with 4 µg/mL of polybrene. Transduced cells were selected with GFP fluorescence using flow cytometry.

To produce the **HEK293**^{shFUBP1} cells, HEK293 cells were co-transfected with pLKO1-shFUBP1 targeting FUBP1 3'UTR (TRCN0000230197, Sigma Aldrich), *pSPAX2* and *pCMV-VSV-G* for packaging using Lipofectamin 3000. Transduced cells were selected in medium containing 1.1 µg/mL puromycin.

Immunoblotting

Cells were lysed for 30 min in 20 mM Tris-HCl, PH8.0, 150 mM NaCl, 0.5 mM EDTA and 1% Triton supplemented with protease inhibitors (04693113001, Roche) with addition of

phosphatase inhibitors (30 mM Sodium fluoride, 40 mM Glycero Phosphate, 1 mM Sodium orthovanadate and 1 mM PMSF) for the study of phospho-proteins. The samples were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), transferred to nitrocellulose membranes, blocked in TBS/0.05% Tween-20 (TBST)/5% BSA and probed with an appropriate antibody (listed in the **Table S1**).

The membranes were washed and incubated 1 h at room temperature in TBST/5%BSA containing the secondary antibody. The immunoblots were visualized with enhanced chemiluminescence Western blotting detection system (WBKLS0500, Merck Millipore) according to the manufacturer's instructions.

Chromatin immunoprecipitation (ChIP) and binding site analysis

The procedure was adapted from a previous study (11). Approximately 1.10^8 of Nalm6, REH or blasts from BCP-ALL patient were fixed in 1 % formaldehyde (648336, Polysciences) for ChIP sequencing (or 50.10^6 for ChIP-qPCR) at room temperature for 10 min and then quenched in 100 mM glycine for 1 min. Cells were washed twice in PBS and lysed 15 min in 50 mM Tris-HCl pH8.1/10 mM EDTA/0.5 % Empigen BB/1 % SDS. Lysates were sonicated to shear DNA to lengths between 200 and 600 base pairs and spun at 10,000 g for 10 min at 4°C. The sonicated cell supernatants were diluted 6-fold in ChIP Dilution Buffer (20 mM tris-HCl pH 8.1/150 mM NaCl/0.1% triton) and incubated or with 5 µg of antibody for ChIP-qPCR or 10 µg for ChIP sequencing for overnight at 4°C. Antibodies are listed in **Table S1**. Then, 100 µl of magnetic bead protein G (10004D, Invitrogen) and yeast tRNA (R5636, Sigma Aldrich) were added to the lysate for 4 h à 4°C under agitation. After four washings, immunoprecipitated DNA was eluted with elution buffer containing 1% SDS and 0.1 M NaHCO₃. The protein–DNA crosslinks were reversed by heating at 65°C overnight, and chromatin was cleaned up using PCR purification kit (Qiagen). For ChIP-qPCR, primers are provided in supplemental **Table S2**. For sequencing, DNA quality was assessed with the Agilent Bioanalyzer with a High Sensitivity Chip.

ChIP-Seq libraries were generated using TruSeq® ChIP Library Preparation Kit (Illumina) according to the manufacturer's protocol. High-throughput sequencing was conducted on a Genome Analyzer II (Illumina, San Diego, CA) at the Human and Environmental Genomics' platform of Rennes (Biogenouest génomique, Rennes, France). ChIP-Seq reads were aligned to the reference human genome version GRCh37 (hg19) and peak calling were carried out as described previously(11). We performed ChIP-Seq of histones H3K4me1, H3K4me3 and

H3K27ac in Nalm6 cells. We performed also RUNX1 ChIP-Seq: 2 with Nalm6 cells, 3 with bone marrow leukemia cells isolated from three BCP-ALL patients. Common peaks between both RUNX1 ChIP-Seq from Nalm6 cells were selected. The correlation coefficient for RUNX1 ChIP-Seq experiments from Nalm6 was 0.69 allowing us to identify common binding regions. This coefficient was calculated using the multiple wiggle files correlation tool (Cistrome) (12) with a 1kb window.

All sequencing data are available at NCBI GEO (GSE109377). Tracks were visualized with Integrated Genome Browser 9.0.0 (13). Cis-regulatory Element Annotation System (CEAS) analysis (14) provides summary statistics on ChIP enrichment in different genomic regions. Overlapping regions among RUNX1 ChIP-Seq from Nalm6 replicates and at least 2 out of 3 pre-B leukemic patients were then assigned to gene loci with Genomic Regions Enrichment of Annotations Tool (GREAT) software (<http://bejerano.stanford.edu/great/public/html/>) (15) within ± 50 kb of protein coding genes and analyzed to uncover enriched annotated pathways with The database for annotation, visualization and integrated discovery (DAVID) (<http://david.abcc.ncifcrf.gov/>) (16).

Identification of RUNX1 potential binding sites was obtained using the Jaspar software tool (<http://jaspar.genereg.net/>) (17). KIT fragments were scanned with the Jaspar's human RUNX1 matrix (MA0002.1). Predicted sites with high sequence conservation (more than 80%) have been selected.

RNA extraction, cDNA synthesis, and RT-qPCR

RNA was extracted using the NucleoSpin RNA II (Macherey Nagel). cDNA was synthesized using High capacity cDNA RT kit (Life Technologies) according to the manufacturer's protocol. Real-time PCR was carried out in sealed 384-well microtiter plates using the SYBRTM Green PCR Master Mix (Applied Biosystems), according to Applied Biosystems gene amplification specifications (40 cycles of 15 sec at 95°C and 1 min at 60°C). The forward (F) and reverse (R) primers (synthesized by Eurogentec) were described in **Table S2**. Data analysis was performed using the $\Delta\Delta$ CT-method (18). The housekeeping gene GAPDH was used to normalize the data. The log2 fold change of all genes of interest compared to Nalm6 control cells was calculated.

Transient transfections and luciferase assays

Genomic DNA fragments derived from the human *KIT* proximal region (chr4: 55,524,722-55,524,952 from GRCh37/hg19), the +30 kb enhancer region (chr4:55,554,102-55,554,596) and the +30 kb enhancer region deleted for the 29-nucleotides long FUSE-like sequence were cloned with a minimal promoter (5'-AGACACTAGAGGGTATATAATGGAAGCTCGACTTCCAG-3') into a luciferase reporter vector (pGL4.10-*luc*; Promega) by Gibson Assembly® Cloning Kit (NEB) according to the manufacturer's instructions. Genomic DNA fragments were synthesized by Integrated DNA Technologies, Inc. Mutagenesis of the predicted RUNX1 binding sites were performed following the instructions of QuikChange II XL Site-Directed Mutagenesis Kit (Agilent) protocol. All constructs were checked by sequencing.

Biological replicates of HEK293^{shFUBP1} cells were plated into 12-well plates and co-transfected with 0.25 µg of pGL4.10-*luc* plasmid DNA, together with 0.25 µg of pFN-Halotag-RUNX1 and/or pCDNA-Flag-FUBP1 and supplemented up to 1 µg/condition with empty pCDNA vector, using lipofectamine 3000 (L3000015, Invitrogen) following manufacturer's indications. *pCMV-renilla* luciferase vector was also transfected as an internal control for transfection efficiency. Forty-eight hours after transfection, cells were lysed and assayed for luciferase activity using the dual luciferase reporter system (Promega) according to the manufacturer's protocol and a LB 960 Centro luminometer (Berthold technologies).

In the same way, for luciferase assays in Nalm6 cells, the +30 kb enhancer region was cloned with the minimal promoter into a MSCV Luciferase PGK-hygro vector (a gift from Scott Lowe, Addgene plasmid #18782) by the Gibson Assembly® Cloning Kit. To produce the +30 kb enhancer-MSCV luc retrovirus, HEK293 cells were co-transfected with 3.91 µg of +30 kb enhancer-MSCV luc together with 1.23 µg *pCMV-VSV-G* and with 3.91 µg pGag-pol (19) using Lipofectamine 3000 transfection reagent. After 48h, supernatant was harvested, filtered, and added to Nalm6^{control}, Nalm6^{+RUNX1}, Nalm6^{+FUBP1} and Nalm6^{+RUNX1+FUBP1} cells with 4 µg/mL of polybrene. Transduced cells were selected in medium containing 300 µg/mL hygromycin (Invivogen). Ten days after, 1 million of cells were seed in duplicate in 100 µL of PBS into a MW96. Luciferase activities were determined upon addition of D-Luciferin (Cayman Chemicals) (1 mg/1 million cells) to the cells with a PhotonIMAGER™ Optima (Biospace Lab).

FUBP1 truncated expression vector (FUBP1^{del-CEN}) was kindly provided by W. Keith Miskimins(20). Mutated RUNX1 (RUNX1^{R174Q}, RUNX1^{R174Q-T161A}) expression vectors were generated by site-directed mutagenesis.

Molecular simulation

The models of the KH3 FUBP1 subdomain complexed to 7 bp DNA were all based on the NMR solution structure 1J4W from the Protein Data Bank. Oligonucleotide sequences of interest (*KIT* gene) were substituted to the experimentally associated MYC sequence by using the mutation (swap residue) tool of YASARA software (<http://www.yasara.org>). Models of the complex involving KH3 subdomain and short single-stranded DNA were all solvated, neutralized and submitted to an energy minimization using the Amber03 forcefield and standard protocols (21). Final binding energies were computed for the protein by the method implemented in YASARA. The reference value found for the KH4 subdomain complex (PDB structure) was 579.2 kcal.mol⁻¹.

Immunophenotyping

Flow cytometry analysis of the intracellular c-KIT protein (CD117) was performed with transcription factor buffer set (BD Biosciences) according the manufacturer's instructions. The APC Mouse Anti-Human CD117 antibody (Clone YB5.B8, BD Biosciences) and the APC Mouse IgG1 κ Isotype Control (Clone MOPC-21, BD Biosciences) were used at the dilution of 1:100 in PBS/1%d-FCS. For the surface staining, cells were incubated 5 min with 100 ng/mL of SCF when indicated, washed in PBS/1%d-FCS, and incubated with CD117 or IgG1 antibodies during 30 min at 37°C. After two washes, cells were analyzed on a BD FACS Fortessa cytometer (Biosit, SFR UMS CNRS 3480 - INSERM 018, Rennes, France).

Cell proliferation and cell analysis

For the cell proliferation assays, cells were seeded at $3 \cdot 10^5$ cells/ml into a MW24 plate and culture in growth medium supplemented with 0 or 100 ng/mL of SCF the first day (130-096-695, Miltenyi Biotech). Cells were maintained at 37°C in a humidified incubator under a 5% CO₂ atmosphere for 7 days and counted every 2 days by automatic counting with a Cellometer Auto 1000 Cell Viability Counter (Nexcelom).

Cell cycle analysis was performed using the APC BrdU Flow Kit (552598, BD Biosciences) according to the manufacturer's instructions. In the pulse-chase time course experiment, cells

were pulsed with BrdU for 45 min followed by washes with PBS then cultured for an additional 24 hours in growth medium. Cells were fixed and stained using the APC BrdU flow kit after 0, 8, and 24 hours post pulse, and the data for each time point was acquired by flow cytometry.

For the apoptosis necrosis and the inhibition curve assays, 30,000 cells were seeded in triplicate into a MW96 plate and treated with 100 ng/mL of SCF combined with growing concentration of imatinib mesylate (sc202180, Santa Cruz Biotechnology): 0 to 10 μ M in the growth medium for two days. Imatinib mesylate was dissolved in sterile water and stored as 25 mM stock solution. Cells were stained for 30 min with Hoechst (bisbenzimidazole H 33258, B1155, Sigma Aldrich), propidium iodide (P3566, Thermo Fisher Scientific) and Yo-Pro-1 (Y3603, Thermo Fisher Scientific) and 2,000 to 6,000 cells per condition were automatically counted and analyzed by ImpACcell core facility (Biosit, SFR UMS CNRS 3480 - INSERM 018, Rennes, France). Untreated cells served also as cell death quantification. Necrotic cells were stained red-fluorescent with propidium iodide while apoptotic cells were stained green-fluorescent with Yo-Pro-1.

Xenograft transplantation and survival analysis

NOD/*scid* IL2 Rg^{null} mice (Charles River Laboratories) were maintained in the ARCHE Animal Center (Biosit, SFR UMS CNRS 3480 - INSERM 018, Rennes, France) at Rennes 1 University. Animal experiments are performed after authorization from French Research Ministry, and according to European regulation. Four-week-old mice received 2 intraperitoneal injections of 20 μ g/g busulfan (Busilvex; Pierre Fabre) on 2 days. They were then allowed to rest for 2 days before the retro-orbital injection of 100,000 cells as previously described (4).

Statistical analysis

Statistical analyses were performed with GraphPad Prism 6.0 software. Statistical significance was analyzed using nonparametric tests (Wilcoxon or Mann-Whitney), with values of $p < 0.05$ considered significant. Correlation were analyzed with Pearson test. For survival studies, Kaplan-Meier curves were generated with Prism 6 software and analyzed with Mantel-Cox tests.

SUPPLEMENTAL TABLES

Table S1: List of all antibodies used.

Table S2: List of primers used in qPCR or ChIP-qPCR.

Table S3: List of the shared 2,651 potential target genes of RUNX1 in Nalm6 cells and pre-B leukemic patient cells (related to Figure 1).

Table S4: Functional analyses (biological process and disease ontology) of the 2,651 potential target genes of RUNX1 (related to Figure 1).

Table S5: Peptides enriched at RUNX1 binding sites in chromatin identified RIME.

Figure S1

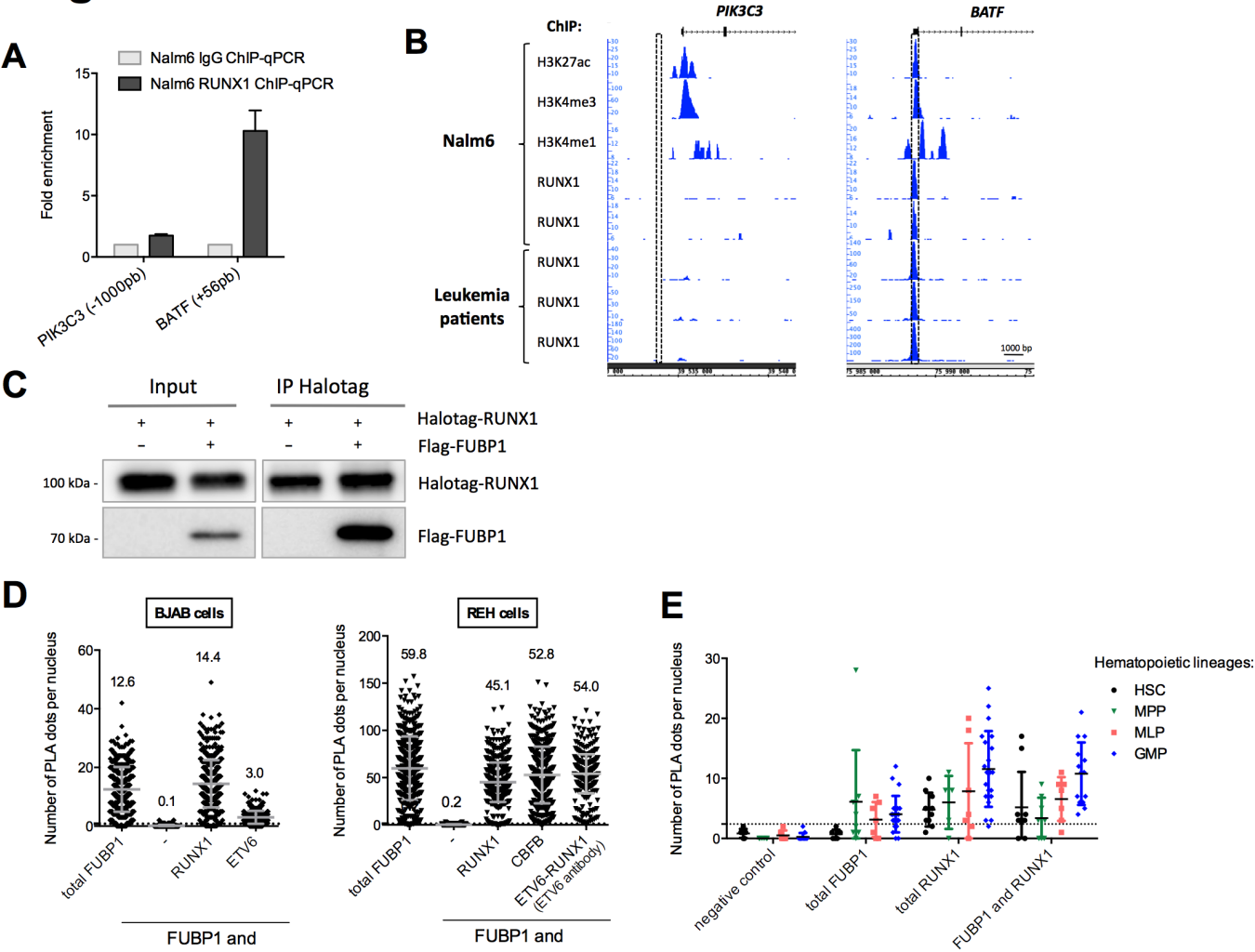


Figure S2

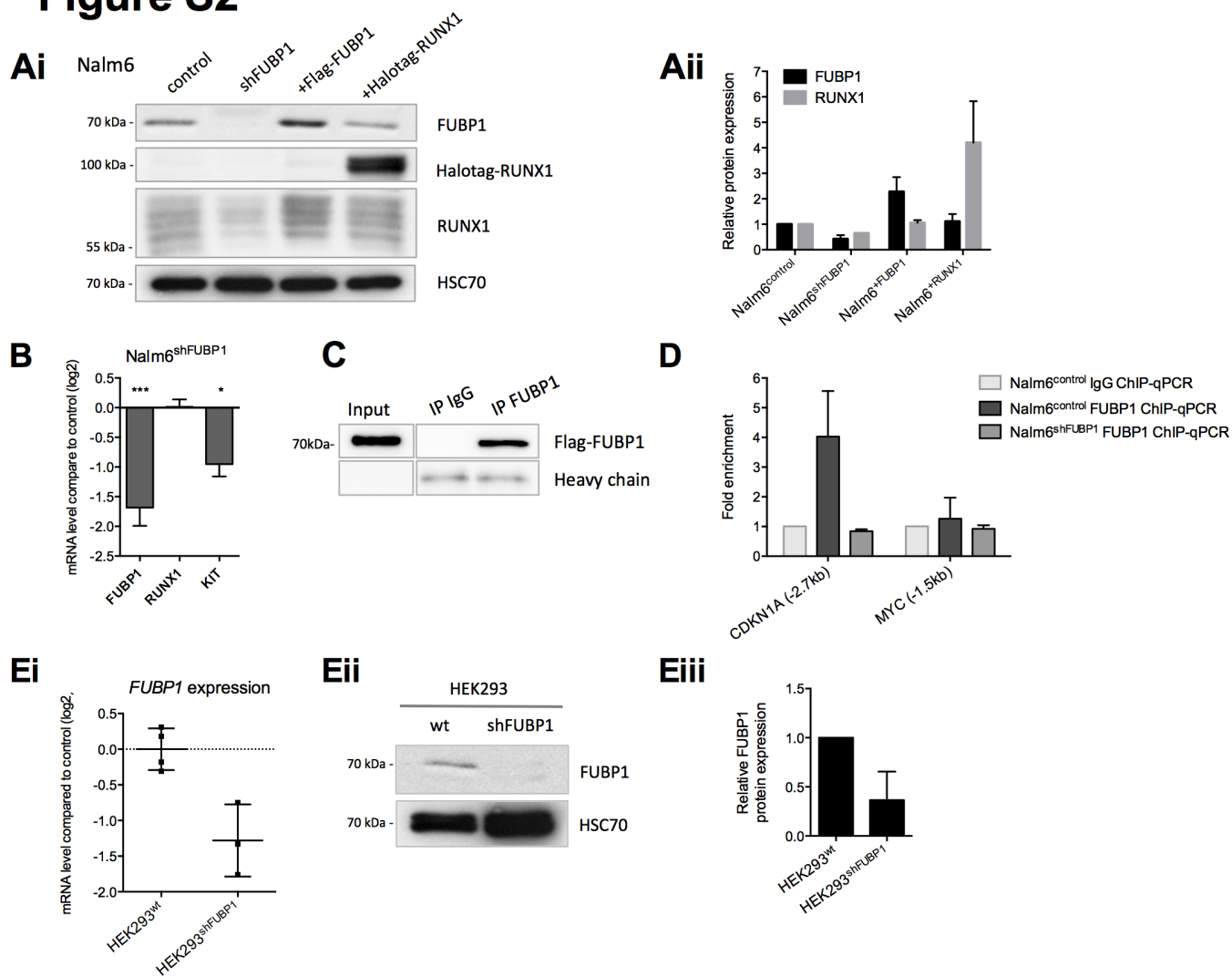
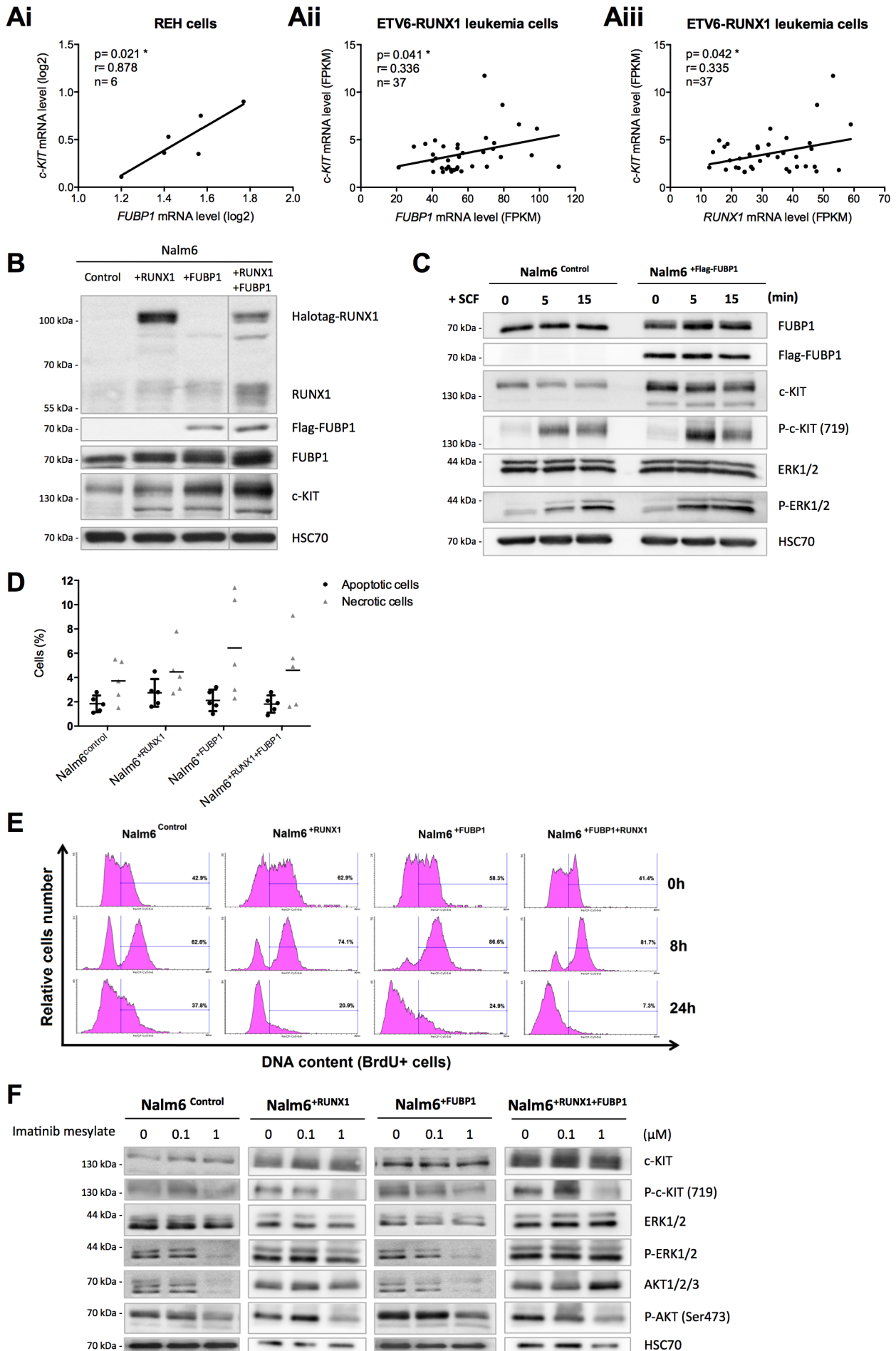


Figure S3



SUPPLEMENTAL FIGURE LEGEND

Supplemental Figure S1: FUBP1 protein interacts with RUNX1 in pre-B cell lines and in human CD34+ cells.

(A) The antibody against RUNX1 was selected according to the literature and was validated in Nalm6 cells by ChIP-qPCR. Histogram represents fold enrichment obtained by ChIP-qPCR for *PIK3C3* (negative control) and *BATF* (positive control) promoters with RUNX1 ChIP compared to normal IgG ChIP in Nalm6 cells. The error bars are the S.D. of three biological repeats.

(B) Genomic tracks display ChIP-Seq data for histones H3K27ac, H3K4me3, H3K4me1 and RUNX1 in Nalm6 and ChIP-Seq data for RUNX1 in bone marrow mononuclear cells isolated from three pre-B acute lymphoblastic leukemia patient over human *PIK3C3* and *BATF* genes. Dotted vertical lines represent regions studied in ChIP-qPCR (panel A).

(C) Co-immunoprecipitation (IP) using anti-HaloTag antibody in HEK293 cells expressing HaloTag-RUNX1 and/or Flag-FUBP1 vectors. Western blot was revealed with M2-Flag and HaloTag antibodies.

(D) Quantitation of protein colocalization per nucleus -visualized by Proximity Ligation Assay (PLA) dots- in the B lymphoblastic cell line BJAB, and the pre-B lymphoblastic cell line REH, presented with the mean values \pm S.D. REH cells carry the chromosomal translocation t(12;21) and chromosomal deletion del(12) producing respective *ETV6-RUNX1* (previously known as *TEL-AML1*) fusion gene and deletion of residual *ETV6* gene (previously known as *TEL*). This fusion protein is present in 25% of childhood BCP-ALL (22). Here, the positive threshold values represented by dotted lines (set at two S.D over the background signal) are 0.8 dots for BJAB cells and 1.2 dots for REH cells.

(E) Quantitation of protein colocalization per nucleus -visualized by PLA dots- in human mobilized CD34+ cells. Hematopoietic stem/progenitor cell populations were identified by their immunophenotyped and sorted by FACS as followed: Hematopoietic Stem Cell (HSC) were Lin-CD34+CD38-CD45RA-CD90+, Multipotent Progenitor (MPP) were Lin-CD34+CD38-CD45RA-CD90-, Granulocyte Macrophage Progenitor (GMP) were Lin-CD34+CD38+CD10-CD132+CD45RA+ and Multipotent Lymphoid Progenitor (MLP) were Lin-CD34+CD38-CD90-/loCD45RA+. Here, the positive threshold value represented by dotted line is 2.4 dots.

Supplemental Figure S2: Validation of modified Nalm6 and HEK293 stable cell lines.

(Ai) and (Aii) Validation of modified Nalm6 stable cell lines. Relative protein levels of FUBP1, RUNX1 and HSC70 in Nalm6^{control}, Nalm6^{shFUBP1}, Nalm6^{+FUBP1}, Nalm6^{+RUNX1}. Representative images of western blot **(Ai)** and densitometric analysis **(Aii)** showing the quantification of FUBP1 and RUNX1 protein level normalized to HSC70.

(B) RT-qPCR for *FUBP1*, *RUNX1* and *c-KIT* mRNA in Nalm6^{shFUBP1}. RT-qPCR were performed at least in biological triplicate and expression data are given compared to Nalm6^{control} cells after normalizing with *GAPDH* mRNA (error bars are S.D.). * p<0.05, **p<0.01, ***p<0.001 in Mann-Whitney tests.

(C) Validation of anti-FUBP1 antibody in HEK293 cells transfected with a Flag-FUBP1 lentivirus vector. Flag-FUBP1 was immunoprecipitated with an anti-FUBP1 antibody. Western blot was revealed with M2 Flag antibodies. IgG heavy chains appeared as control of loading.

(D) Histogram represents fold enrichment obtained by ChIP-qPCR for *CDKN1A* and *c-MYC* promoters with an anti-FUBP1 ChIP antibody compared to IgG ChIP, in Nalm6^{control} (n=3) and Nalm6^{shFUBP1} (n=3) cells. The error bars are the S.E.M. of the biological repeats.

(Ei), (Eii) and (Eiii) Relative transcript and protein levels of FUBP1 in HEK293^{hFUBP1} cells. RT-qPCR were performed in triplicate and expression data are given compared to the Nalm6 control cells after normalizing with *GAPDH* mRNA (error bars are S.D.) **(Ei)**. Representative images of western blot **(Eii)** and densitometric analysis **(Eiii)** showing the quantification of FUBP1 protein level normalized to HSC70.

Supplemental Figure S3: FUBP1 and RUNX1 exacerbate the c-KIT pathway and contribute to cell proliferation.

(Ai) Correlation between *FUBP1* and *c-KIT* mRNA levels in REH^{+FUBP1} cell line detected by RT-qPCR after normalizing with *GAPDH* mRNA (on the right). REH cells are originate from a BCP-ALL patient with t(12;21) producing ETV6-RUNX1 fusion protein. **(Aii)** and **(Aiii)**: Correlation between *FUBP1* or *RUNX1* and *c-KIT* relative mRNA levels (expressed in Fragments Per Kilobase Million FPKM) in ETV6-RUNX1 BCP-ALL cells from RNA-Seq analyses (data from St. Jude Children's Research Hospital - Pediatric Cancer Data Portal) (23). Samples with a minimal expression of *c-KIT* (1.5 FPKM) were selected (n=37). Pearson correlation coefficient (r), p-value (p), and number of biological replicates analyzed (n) are indicated.

(B) Representative western blot analysis of total Halotag-RUNX1, RUNX1, Flag-FUBP1, c-KIT, and HSC70 in Nalm6^{control}, Nalm6^{+RUNX1}, Nalm6^{+FUBP1}, and Nalm6^{+RUNX1+FUBP1}.

(C) Representative western blot analysis of total FUBP1, Flag-FUBP1, c-KIT, P-c-KIT(719), ERK1/2, P-ERK1/2, and HSC70 in Nalm6^{control} and Nalm6^{+FUBP1} exposed to 100 ng/ml of SCF over time (0, 5 and 15 minutes).

(D) Percentage of apoptotic and necrotic Nalm6^{control}, Nalm6^{+RUNX1}, Nalm6^{+FUBP1}, or Nalm6^{+RUNX1+FUBP1} cells. After two days of culture, cells were stained with Hoechst, Propidium iodine and Yo-Pro and quantified (n=5 experiments, 3,000-6,000 cells counted/experiment).

(E) BrdU pulse-chase time course showing progression of actively dividing BrdU-pulsed Nalm6^{control}, Nalm6^{+RUNX1}, Nalm6^{+FUBP1} or Nalm6^{+RUNX1+FUBP1} cells over a 24-hour time course. Cells were pulsed with BrdU for 45 minutes, washed and then cultured for an additional 24 hours. The DNA content of BrdU labeled cells at each time point (0, 8, 24h) is analyzed by FACS and displayed in a 7-AAD histogram overlay, which further shows cells went through the phases of the cell cycle (4N DNA to 2N DNA content). Histograms show the DNA content of the BrdU-positive cells (X-axis) and cell number (Y-axis). The percentage of cells in S phase (4N) is represented.

(F) Western blot analysis of total FUBP1, Flag-FUBP1, c-KIT, P-c-KIT(719), ERK1/2, P-ERK1/2, AKT1/2/3, P-AKT(473) and HSC70 in Nalm6^{control}, Nalm6^{+RUNX1}, Nalm6^{+FUBP1}, or Nalm6^{+RUNX1+FUBP1} cells exposed to 100 ng/ml SCF during 5 min and to 0, 0.1, 1 or 10 μ M of imatinib mesylate for 2h.

REFERENCES

1. Hurwitz,R., Hozier,J., LeBien,T., Minowada,J., Gajl-Peczalska,K., Kubonishi,I. and Kersey,J. (1979) Characterization of a leukemic cell line of the pre-B phenotype. *Int. J. Cancer*, **23**, 174–180.
2. Matsuo,Y. and Drexler,H.G. (1998) Establishment and characterization of human B cell precursor-leukemia cell lines. *Leuk. Res.*, **22**, 567–579.
3. Rosenfeld,C., Goutner,A., Venuat,A.M., Choquet,C., Pico,J.L., Dore,J.F., Liabeuf,A., Durandy,A., Desgrange,C. and The,G.D. (1977) An effective human leukaemic cell line: Reh. *Eur. J. Cancer* 1965, **13**, 377–379.
4. Arnaud,M.-P., Vallée,A., Robert,G., Bonneau,J., Leroy,C., Varin-Blank,N., Rio,A.-G., Troadec,M.-B., Galibert,M.-D. and Gandemer,V. (2015) CD9, a key actor in the dissemination of lymphoblastic leukemia, modulating CXCR4-mediated migration via RAC1 signaling. *Blood*, **126**, 1802–1812.
5. Mohammed,H., D’Santos,C., Serandour,A.A., Ali,H.R., Brown,G.D., Atkins,A., Rueda,O.M., Holmes,K.A., Theodorou,V., Robinson,J.L.L., *et al.* (2013) Endogenous Purification Reveals GREB1 as a Key Estrogen Receptor Regulatory Factor. *Cell Rep.*, **3**, 342–349.
6. Debaize,L., Jakobczyk,H., Rio,A.-G., Gandemer,V. and Troadec,M.-B. (2017) Optimization of proximity ligation assay (PLA) for detection of protein interactions and fusion proteins in non-adherent cells: application to pre-B lymphocytes. *Mol. Cytogenet.*, **10**, 27.
7. Nordengrahn,A., Gustafsdottir,S.M., Ebert,K., Reid,S.M., King,D.P., Ferris,N.P., Brocchi,E., Grazioli,S., Landegren,U. and Merza,M. (2008) Evaluation of a novel proximity ligation assay for the sensitive and rapid detection of foot-and-mouth disease virus. *Vet. Microbiol.*, **127**, 227–236.
8. Campeau,E., Ruhl,V.E., Rodier,F., Smith,C.L., Rahmberg,B.L., Fuss,J.O., Campisi,J., Yaswen,P., Cooper,P.K. and Kaufman,P.D. (2009) A Versatile Viral System for Expression and Depletion of Proteins in Mammalian Cells. *PLoS ONE*, **4**, e6529.
9. Stewart,S.A., Dykxhoorn,D.M., Palliser,D., Mizuno,H., Yu,E.Y., An,D.S., Sabatini,D.M., Chen,I.S.Y., Hahn,W.C., Sharp,P.A., *et al.* (2003) Lentivirus-delivered stable gene silencing by RNAi in primary cells. *RNA N. Y. N*, **9**, 493–501.
10. Rabenhorst,U., Beinoraviciute-Kellner,R., Brezniceanu,M.-L., Joos,S., Devens,F., Lichter,P., Rieker,R.J., Trojan,J., Chung,H.-J., Levens,D.L., *et al.* (2009) Overexpression of the far upstream element binding protein 1 in hepatocellular carcinoma is required for tumor growth. *Hepatol. Baltim. Md*, **50**, 1121–1129.
11. Sérandour,A.A., Avner,S., Oger,F., Bizot,M., Percevault,F., Lucchetti-Miganeh,C., Palierne,G., Gheeraert,C., Barloy-Hubler,F., Péron,C.L., *et al.* (2012) Dynamic hydroxymethylation of deoxyribonucleic acid marks differentiation-associated enhancers. *Nucleic Acids Res.*, **40**, 8255–8265.

12. Liu,T., Ortiz,J.A., Taing,L., Meyer,C.A., Lee,B., Zhang,Y., Shin,H., Wong,S.S., Ma,J., Lei,Y., *et al.* (2011) Cistrome: an integrative platform for transcriptional regulation studies. *Genome Biol.*, **12**, R83.
13. Nicol,J.W., Helt,G.A., Blanchard,S.G., Raja,A. and Loraine,A.E. (2009) The Integrated Genome Browser: free software for distribution and exploration of genome-scale datasets. *Bioinforma. Oxf. Engl.*, **25**, 2730–2731.
14. Shin,H., Liu,T., Manrai,A.K. and Liu,X.S. (2009) CEAS: cis-regulatory element annotation system. *Bioinforma. Oxf. Engl.*, **25**, 2605–2606.
15. McLean,C.Y., Bristor,D., Hiller,M., Clarke,S.L., Schaar,B.T., Lowe,C.B., Wenger,A.M. and Bejerano,G. (2010) GREAT improves functional interpretation of cis-regulatory regions. *Nat. Biotechnol.*, **28**, 495–501.
16. Jiao,X., Sherman,B.T., Huang,D.W., Stephens,R., Baseler,M.W., Lane,H.C. and Lempicki,R.A. (2012) DAVID-WS: a stateful web service to facilitate gene/protein list analysis. *Bioinformatics*, **28**, 1805–1806.
17. Sandelin,A., Alkema,W., Engström,P., Wasserman,W.W. and Lenhard,B. (2004) JASPAR: an open-access database for eukaryotic transcription factor binding profiles. *Nucleic Acids Res.*, **32**, D91–D94.
18. Schefe,J.H., Lehmann,K.E., Buschmann,I.R., Unger,T. and Funke-Kaiser,H. (2006) Quantitative real-time RT-PCR data analysis: current concepts and the novel ‘gene expression’s CT difference’ formula. *J. Mol. Med. Berl. Ger.*, **84**, 901–910.
19. Nègre,D., Mangeot,P.E., Duisit,G., Blanchard,S., Vidalain,P.O., Leissner,P., Winter,A.J., Rabourdin-Combe,C., Mehtali,M., Moullier,P., *et al.* (2000) Characterization of novel safe lentiviral vectors derived from simian immunodeficiency virus (SIVmac251) that efficiently transduce mature human dendritic cells. *Gene Ther.*, **7**, 1613–1623.
20. Zheng,Y. and Miskimins,W.K. (2011) Far upstream element binding protein 1 activates translation of p27Kip1 mRNA through its internal ribosomal entry site. *Int. J. Biochem. Cell Biol.*, **43**, 1641–1648.
21. Krieger,E. and Vriend,G. (2015) New ways to boost molecular dynamics simulations. *J. Comput. Chem.*, **36**, 996–1007.
22. Pui,C.-H., Carroll,W.L., Meshinchi,S. and Arceci,R.J. (2011) Biology, Risk Stratification, and Therapy of Pediatric Acute Leukemias: An Update. *J. Clin. Oncol.*, **29**, 551–565.
23. Zhou,X., Edmonson,M.N., Wilkinson,M.R., Patel,A., Wu,G., Liu,Y., Li,Y., Zhang,Z., Rusch,M.C., Parker,M., *et al.* (2016) Exploring genomic alteration in pediatric cancer using ProteinPaint. *Nat. Genet.*, **48**, 4–6.