Potent, p53-independent Induction of NOXA Sensitizes MLL-rearranged B Cell Acute Lymphoblastic Leukemia Cells to Venetoclax

Klaudyna Fidyń1,2, Agata Pastorczak3, Julia Cyran1, Nicholas T. Crump4, Agnieszka Goral1, Joanna Madzio3, Angelika Muchowicz1, Martyna Poprzeczko1,5, Krzysztof Domka1,5, Lukasz Komorowski1,2, Magdalena Winiarska1, Joe R. Harman4, Karolina Siudakowska1, Agnieszka Graczyk-Jarzynka1,5, Elżbieta Patkowska6, Ewa Lech-Maranda6, Wojciech Młynarski3, Jakub Golab1, Thomas A. Milne4, and Małgorzata Firczuk1,6

1Department of Immunology, Medical University of Warsaw, Warsaw, Poland
2Postgraduate School of Molecular Medicine, Medical University of Warsaw, Warsaw, Poland
3Department of Pediatrics, Oncology and Hematology, Medical University of Lodz, Lodz, Poland
4Medical Research Council Molecular Haematology Unit at the University of Oxford, Oxford, United Kingdom
5Mossakowski Medical Research Institute, Polish Academy of Sciences, Warsaw, Poland
6Department of Hematology, Institute of Hematology and Transfusion Medicine, Warsaw, Poland

*Corresponding author: Małgorzata Firczuk, email: mfirczuk@wum.edu.pl, phone: +48 22 599 21 98, Department of Immunology, Medical University of Warsaw, Nielubowicza 5 Street, 02-097 Warsaw, Poland

Description: This file contains Supplementary Figures, Supplementary Materials and Methods, and Supplementary Tables.
Supplementary Fig. 1. Sensitivity of primary and primograft (PDX) BCP-ALL cells to VEN. Primary cells derived from MLLr (n=7) and non-MLLr (n=13) BCP-ALL patients' bone marrow and PDX samples were incubated with VEN in monoculture for 3 days. Following drug exposure, numbers of viable cells (CD19^+ 7AAD^-) were detected by flow cytometry using CountBright™ Absolute Counting Beads. EC50 value of VEN for each PDX sample was calculated by nonlinear regression dose-response analysis using GraphPad7. For non-MLLr samples, colors indicate: black – adult and pediatric BCP-ALL assigned to a standard-risk group, blue – Ph-positive or Ph-like, green – adult BCP-ALL assigned to a high-risk group with no recurrent genetic aberrations detected. Ns – not significant difference by unpaired t-test with Welch’s correction.
Supplementary Fig. 2. Long-term in vivo exposure of MLLr PDX cells to VEN induces global changes in gene expression and dysregulation of the p53 pathway genes. A, MLLr PDX cells PDX#4, PDX#5, and PDX#6 were injected into NSG mice and leukemia progression was monitored as described in Fig. 1B. Once the engraftment was confirmed in murine peripheral blood, 100 mg/kg VEN was administered 5 days per week for a total of 12 doses (PDX#4), 20 doses (PDX#5), and 15 doses (PDX#6). Graphs present the mean percentage of human blasts relative to murine mCD45^+ cells ± SD (n=3 mice per group for PDX#4 and PDX#6, n=7 mice per group for PDX#5). Statistical significance of the interaction effect was estimated by a 2-way ANOVA test, ***P < 0.001. B, MA plot visualizing the interaction between logFC and logCPM in RNA-seq from PDX#1 cells treated with vehicle or VEN in vivo with a total of 13 VEN doses (in cycles 5 days treatment/2 days off). Red dots represent differentially expressed genes (DEGs). C, Volcano plot showing DEGs in PDX#1 cells treated with VEN or vehicle as described in B. Green dots represent genes with |logFC| > 1 and FDR < 0.05; orange dots show genes with |logFC| > 1 and FDR > 0.05, whereas red dots show genes with |logFC| < 1 and FDR < 0.05. D, PDX#1, PDX#2, and PDX#3 were treated in vivo with VEN as described in Fig. 1B. RNA was isolated from hCD19^-enriched cells. The graphs show the relative mRNA levels (2^-ΔCt) for the p53 pathway genes, as defined in Fig. 1D, assessed by RT-qPCR using gene-specific TaqMan probes, in VEN-treated and control groups. Each dot represents a separate mouse and the horizontal lines indicate the median values.
Supplementary Fig. 3. AUR kills BCP-ALL cells independently of their p53 status. A, MLLr BCP-ALL (SEM, RS4;11) and hypodiploid (NALM-16) cell lines were exposed to increasing concentration of AUR for 48 h and subjected to MTT viability assay. The graph shows mean value ± SEM from 2 independent experiments for all cell lines. Nonlinear regression dose–response analysis (GraphPad7) was used to calculate EC50 values. B, Sanger sequencing chromatogram depicts heterozygous TP53 point mutation (R288Q) in SEM cell line. C, The basal p53 protein level was detected by immunoblotting in a panel of BCP-ALL cell lines. Equal protein loading was assessed by determination of β-actin levels. D, Basal TP53 mRNA expression in BCP-ALL cell lines as determined by RT-qPCR. For each cell line mean relative expression (2^−ΔΔCt) + SD is presented from 3 biological repeats, n=3. B2M and ACTB were used as reference genes.
Supplementary Fig. 4. Synergistic interaction between VEN and AUR against MLLr BCP-ALL PDX cells co-cultured with BM-MSC. A, Gating strategy used for determination of viable (CD19+ 7AAD-) cells in response to VEN, AUR, or both drugs in co-culture with BM-MSC. CountBright™ Absolute Counting Beads were used to assess the total number of leukemic cells. B, MLLr BCP-ALL PDXs derived from 5 diagnostic and 3 relapsed patient samples (samples derived from children marked in red and from adults marked in green) were co-cultured with primary BM-MSC cells and exposed to indicated concentrations of VEN, AUR or combination of both for 4-5 days. After that time the total number of live cells was assessed by flow cytometry. The graphs show the mean percentage of viable cells relative to control + SD from 2 wells of independently treated cells. C, Primary BM-MSC cells were exposed to a particular concentration of VEN, AUR, or mixture of both for 5 days. The assessment of dead cells following drug exposure was performed by flow cytometry and 7AAD staining. The bars indicate the mean percentage of 7AAD-negative cells + SEM, n=4. D, Human CD19+ cells were isolated from buffy coats of 3 different healthy donors and exposed to VEN, AUR, or mixture of both for 2 days. The viability was assessed by flow cytometry as the % of propidium iodide (PI)-negative cells. The bars indicate the mean percentage of viable cells (PI-negative cells) relative to controls + SEM, n=3.
Supplementary Fig. 5. AUR potentiates VEN anti-leukemic activity against non-MLLr BCP-ALL cell lines and PDXs. A, BCP-ALL cell lines BV-173 (Philadelphia-positive, Ph+) and NALM-16 (hypodiploid) were treated with EC20 and EC50 concentrations of AUR or VEN (calculated by nonlinear regression dose-response analysis using GraphPad7), as well as with a combination of both drugs. After 48 h incubation, cells were subjected to MTT viability assay. Survival in all groups was calculated as the percentage of the controls treated with vehicle only (DMSO). Bars represent means + SEM. B, CI matrices representing particular VEN+AUR CI values are shown for each cell line. The combination index (CI) was calculated using CompuSyn software employing the Chou-Talalay algorithm. CI below 1 indicates synergistic interactions. The intensity of the color determines the potency of the synergistic effects. C, Gating strategy applied for the evaluation of the viability of BCP-ALL PDX cells co-cultured with GFP-positive OP9 stromal cells. D, Adult (marked in green color) and pediatric (marked in red color) Philadelphia-positive (Ph+) and Philadelphia-like (Ph-like) PDXs were co-cultured with OP9-GFP stromal cell line and treated with single drugs or VEN+AUR combination for 3 days. Cell viability was determined by 7AAD staining and flow cytometry. The bars
represent the mean percentage of 7AAD-negative cells, from 2 wells of independently treated cells + SD. E, Matrices illustrating VEN+AUR CI for indicated PDXs. CI below 1 indicates synergistic interactions. Colour intensity corresponds to the potency of the synergistic effects, according to legends. F, GFP-positive OP9 cells were exposed to VEN, AUR, or a mixture of both for 3 days. The assessment of dead cells following drugs exposure was performed by flow cytometry and 7AAD staining. The bars indicate the mean percentage of 7AAD-negative cells + SEM, n=2-4.
Supplementary Fig. 6. Effects of p53 downregulation on VEN+AUR efficacy and p21 induction upon AUR and VEN+AUR co-treatment. A, RS4;11 cells, either wild type (WT, n=1), siNTC (n=2) or with downregulation of p53 (siTP53, n=2) were treated with indicated concentrations of VEN, AUR, and a combination of both for 24 h. The number of dead cells was evaluated by assessment of PI-positive cells in flow cytometry. The matrices present CI which was calculated in CompuSyn software. CI < 1 indicates synergistic interaction between tested drugs. Colour intensity determines the potency of the synergistic effects, according to legends. B, Immunoblotting showing p53 and p21 protein levels in RS4;11 cells, either with siRNA mediated knockdown of TP53 (siTP53) or control (siNTC), after 6 h exposure to 10 μM nutlin-3, 1.5 μM AUR, 50 nM VEN and combination of VEN and AUR. All blots are representative of 2 independent knockdown experiments.
Suppl. Fig. 7

A

Supplementary Fig. 7. Effects of TXNRD1 and TXNRD2 downregulation on VEN cytotoxicity and cytoplasmic ROS levels in cells incubated with VEN, AUR, and VEN+AUR. A, B, Control SEM cells (siNTC) and cells with siRNA-mediated knockdown of TXNRD1 (siTXNRD1) or TXNRD2 (siTXNRD2) were treated with increasing concentrations of VEN for 48 h, and then the viability was determined by MTT assay. The data are presented as means from 2 independent knockdown experiments + SEM. *P < 0.05, ns – not significant by 2-way ANOVA test (left panels) for the interaction presented, n=2. The efficiency of siRNA-mediated knockdown was determined by RT-qPCR and immunoblotting. The graphs in the middle panel show mean mRNA expression of either TXNRD1 or TXNRD2 relative to control genes (GUSB, RPL29) + error. Representative immunoblots show TXNRD1 and TXNRD2 protein levels from 2 independent knockdown experiments. β-actin serves as a loading control.

C

Cytoplasmic ROS

SEM cells were treated with indicated doses of VEN, AUR, or both drugs for 1, 2, and 4 h. After the treatment cytoplasmic ROS levels were detected by CM-H2-DCFDA dye using flow cytometry. The graph shows mean fold change in mean fluorescence intensity (MFI) over untreated control (DMSO) + SEM, n=4. *P < 0.05, **P < 0.01, ***P < 0.001 by 1-way ANOVA with Dunnett’s post-hoc test.
Supplementary Fig. 8. AUR enhances VEN-induced apoptosis. A, SEM cells were treated with VEN, AUR, and both drugs for 16h and subjected to MultiCaspase assay. Activation of caspases and cell death was determined by staining of the cells with a fluorescently-labeled inhibitor of caspases (FLICA) and 7AAD dye and analyzed using Muse Cell Analyzer. Plots present percentages of viable and dead cells with activated caspases. B, SEM cells were treated with 0.8 μM AUR, 400 nM VEN, or a combination of both drugs for 8 h and 24 h. The level of cleaved PARP protein in response to the drugs was determined by immunoblotting. An equal sample loading was assessed by detection of
α-tubulin, n=3. C, SEM cells were exposed to 0.8 μM AUR, 400 nM VEN, and a combination of both drugs for 4, 8, and 24 h. The levels of members of selected pro-apoptotic BCL-2 family proteins were determined by immunoblotting. Representative blots of at least 2 independent experiments are presented.
Supplementary Fig. 9. Levels of NOXA protein do not significantly affect sensitivity to vincristine and doxorubicin. SEM cells with PMAI/P1 KO [sgNOXA(2)] and control cells (sgNTC) were exposed to chemotherapeutics, vincristine or doxorubicin, at indicated concentration range for 48 h. The viability of the cells was evaluated by MTT assay. The graphs show the mean percentage of the controls (treated with DMSO) ± SEM, from 8 biological replicates (n=8). *P < 0.05 for the interaction by 2-way ANOVA.
Supplementary Fig. 10. AUR strongly induces NOXA at mRNA and protein levels in various BCP-ALL cell lines. A, The levels of PMAIP1 mRNA in BCP-ALL cell lines (BV-173, REH, 697) exposed to EC50 concentration of AUR for 1 and 3 h were assessed by RT-qPCR. Data are presented as a mean expression of the target gene relative to RPL29 and GUSB control genes. Bars show mean values + SEM, n=2. *\( P < 0.05 \), **\( P < 0.01 \) by 1-way ANOVA with Dunnett’s post-hoc test. B, Same BCP-ALL cell lines as in (A) plus NALM-16 were treated with EC50 or EC80 of AUR and collected after 8 h. NOXA protein levels were assessed by immunoblotting. Representative blots of 2 independent experiments are shown. C, Human primary PBMC, PDX#4, and PDX#5 cells were incubated with 1.5 \( \mu \)M AUR and collected after 4 h. NOXA protein levels were assessed by immunoblotting.
**Supplementary Fig. 11.** ATF4 only partially contributes to AUR-mediated NOXA upregulation.

**A,** SEM cells were exposed to either 1.5 μM AUR or 5 μg/ml tunicamycin (TUNI) for the indicated period of time. ATF4 and NOXA proteins levels were determined by immunoblotting. GAPDH served as a loading control. **B,** SEM cells were treated with 1.5 μM AUR, 5 μg/ml TUNI or DMSO (control) for 4 h, and ATF4 transcription factor binding within PMAIP1 loci was determined by ChIP-qPCR. SCL7A1 is a known ATF4 target and served as a positive control. The bars indicate mean + SEM. *P < 0.05 by paired t-test, n=4. **C, D,** 24 h following siRNA-mediated ATF4 downregulation, SEM cells were exposed to 1.5 μM AUR for 4 h. ATF4 and PMAIP1 mRNA expression (C), as well as their protein levels (D), were determined in control (siNTC) and in ATF4 siRNA-treated SEM cells (siATF4). The bars indicate the mean relative expression of the target to reference genes (RPL29, GUSB) + SEM. **P < 0.01 by paired t-test, n=3. **E,** SEM cells were exposed to 1.5 μM AUR or 5 μg/ml tunicamycin (TUNI) for 1, 2, and 4 h. The graph shows mean PMAIP1 gene expression relative to GAPDH over control + SEM, n=2. Mean expression values were calculated using cycle threshold (Ct) values and presented as 2^(-ΔΔCt). ***P < 0.001 by 1-way ANOVA with Dunnett’s post-hoc test; ns – not significant.
Supplementary Materials and Methods

Cell culture

Human BCP-ALL cell lines representing various genetic subtypes of the disease were purchased from DSMZ (SEM, RS4;11, BV-173, SUP-B15, SD-1, MUTZ-5, NALM-16) and from ATCC (697, REH), and include hypodiploid cells (NALM-16) as well as cells with specific genetic translocations, such as MLL-AF4 (SEM, RS4;11), BCR-ABL1 (BV-173, SUP-B15), E2A-PBX1 (697), TEL-AML1 (REH). MUTZ-5 represents a Philadelphia-like subtype carrying IGH-CRLF2 translocation and a JAK2 R683G mutation. All cell lines were maintained in RPMI 1640 medium (Gibco, Paisley, UK) supplemented with 10% FBS (HyClone, Logan, UT, USA) and 1% penicillin/streptomycin solution (Sigma-Aldrich, St. Louis, MO, USA). Acute myeloid leukemia cell line, HL-60, was purchased from DSMZ and cultured in IMDM medium (Gibco) with 10% FBS and 1% penicillin/streptomycin solution (Sigma-Aldrich). TP53 deletion in HL-60 was confirmed as described in (1). GFP-expressing OP9 murine bone marrow stromal cell line was kindly provided by Prof. Meinrad Busslinger from The Research Institute of Molecular Pathology (IMP), Vienna, Austria. OP9-GFP cells were cultured in α-MEM medium (Gibco) with 20% FBS (Gibco) and 1% penicillin/streptomycin solution (Sigma-Aldrich). All cell lines were maintained for the maximum time of 4 weeks at 37°C and 5% CO2 conditions and tested for Mycoplasma contamination once a week.

Reagents

Venetoclax (DC Chemicals, Shanghai, China), auranofin (Sigma-Aldrich), nutlin-3 (Sigma-Aldrich), and tunicamycin (MP Biomedicals, Santa Ana, CA, USA), were dissolved in DMSO. Drug aliquots were stored at -20°C or -80°C. In all experimental procedures control groups were treated with DMSO (Sigma-Aldrich).

MTT assay

The cytostatic/cytotoxic effects of single drugs and drugs combinations in BCP-ALL cell lines were tested using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The assay was performed as described in (2) and the cells were exposed to particular drugs for 48 h. Based on the results from single drug treatment (at least 2 independent experiments), EC20 and EC50 values were determined by nonlinear regression dose–response analysis using GraphPad Prism 7. EC20 and EC50 values of each drug were further used for combinatorial drug testing.

Combination index (CI) calculation

In order to determine the potency of drug combinations, combination index (CI) was calculated using CompuSyn software. The software employs Chou-Talalay method as described in (3). The algorithm
defines synergistic (CI < 1), additive (CI = 1) and antagonistic (CI > 1) effects of drug combinations.

**Animal studies**

All animal procedures were in line with EU Directive 2010/63/EU and the Polish legislation for animal experiments of the Polish Ministry of Science and Higher Education (February 26, 2015) and with ARRIVE guidelines. For animal experiments the sample size was calculated by power analysis or resource equation approach as described in (4). The exclusion criteria were pre-established and aligned with ARRIVE guidelines. The experiments were carried out in SPF animal facility with IVC systems.

*Primografts generation*

For propagation of primografts, also referred as patient-derived xenografts (PDXs), primary material isolated from BCP-ALL patients was used. The blasts were isolated as described in (2) following the patients’ written consent and approval of Bioethics Committee of Medical University of Warsaw and the Medical University of Lodz (KB/44/2015, RNN/51/19/KE). 6-12 weeks old severely immunodeficient NOD.Cg-Prkdc<sup>scid</sup> Il2rg<sup-tm1Wjl</sup>/SzJ (NSG) mice (female and male) (Charles River Laboratories, Wilmington, MA, USA) were used for the propagation of BCP-ALL PDXs. All performed animal procedures were approved by the Ethics Committee of the Warsaw University and the Warsaw University of Life Sciences (287/2016, WAW2/095/2019). The detailed experimental procedure and monitoring of PDXs engraftment were previously described (2). See Sup Table 1 and Sup Table 2 for detailed information about generated PDX samples and for selected antibodies.

**Drug testing in BCP-ALL PDXs in monoculture**

BCP-ALL PDXs were thawed and their viability was determined using trypan blue exclusion method. When the percentage of dead cells exceeded 20%, the viable cell population was enriched using Lymphoprep™ density gradient medium (STEMCELL Technologies, Vancouver, BC, Canada). For that purpose, cells were centrifuged at 550 × g for 25 min and their viability determined once again in a hemocytometer. Successfully enriched cell population was seeded onto 96-well plate, 1.0 × 10<sup>5</sup> cells per well in 100 µl of SFEM II medium (STEMCELL Technologies) supplemented with 20% of FBS (Gibco), 20 ng/mL of recombinant IL-3 (R&D Systems, Minneapolis, MN, USA), and 10 ng/mL of recombinant IL-7 (R&D Systems). The same day VEN was added to each well at the concentration range between 1 nM – 200 nM in a total volume of 200 µl in 2 repeats. Cells were exposed to the drug for 3 days. In order to determine the absolute number of viable cells after treatment, the cells were stained with human anti-CD19 antibody (BD Biosciences, Franklin Lakes, NJ, USA), and 7AAD (BD Biosciences) following the addition of CountBright™ Absolute Counting Beads (ThermoFisher...
Scientific, Waltham, MA, USA) and analysis on CANTO II flow cytometer (BD Biosciences). The number of CD19+ 7AAD− cells was calculated based on the equation provided by the beads’ manufacturer. Finally, for each PDX EC50 value of VEN was calculated as described above for BCP-ALL cell lines.

**Primary BM-MSC isolation and culture conditions**

Before sample collection appropriate individual written consent was obtained and all the procedures were approved by Bioethical Committee of Medical University of Warsaw. The cells were isolated and maintained in culture for the maximum of 5 passages as described in (5). Prior to use in *ex vivo* experiments BM-MSC cells were characterized and tested for the expression of particular positive (CD73, CD105, CD90) and negative (CD45, CD34, CD19) surface markers by flow cytometry and their differentiation capacity as presented in our previous work (2).

**Drug testing in BCP-ALL and BM-MSC stromal cells co-culture model**

To assess cytotoxic/cytostatic effects of the drugs on MLLr BCP-ALL PDXs in the presence of stromal cells, we employed the co-culture model described in (2, 5), with some modifications. Specifically, bone marrow-derived mesenchymal stem cells (BM-MSC) were seeded onto 96-well plate at 0.2 × 10^4 cells per well. The next day, after removal of the medium, PDX cells processed as described above for monoculture conditions were seeded onto BM-MSC (1.0 × 10^5 cells per well in 100 µl of their medium). Next, single drugs or VEN+AUR combination were added (total volume of 200 µl), in 2 independent wells. After additional 4-5 days, the total amount of viable cells was assessed by flow cytometry using CountBright™ Absolute Counting Beads (ThermoFisher Scientific, Waltham, MA, USA). The results are presented as the percentage of untreated control (DMSO). To assess drug interaction, combination index (CI) was calculated using CompuSyn software.

**Drug testing in BCP-ALL-OP9 cells co-culture model**

The activity of single drugs and VEN+AUR combination on Philadelphia (Ph)+ and Ph-like BCP-ALL cells was tested in the presence of OP9 stromal cells. For this purpose, GFP-expressing OP9 cells were seeded at the density of 0.3 × 10^4 cell per well onto 96-well plate in 100 µl of their medium. After 6 h leukemic cells were thawed and seeded as depicted for PDXs grown in monoculture. The same day cells were exposed to single and VEN+AUR drugs combination and following 3 days of the treatment they were stained with 7AAD dye. The viable PDX cells were determined by flow cytometry using CANTO II (BD Biosciences) and presented as the percentage of GFP+/7ADD− cells. The type and potency of drug interactions was presented as CI, which was calculated as described above.
**CD19' cells isolation and drug testing**

Peripheral blood of healthy donors was obtained from Regional Blood and Hemotherapy Center in Warsaw. Normal peripheral blood mononuclear cells (PBMC) were isolated from the blood using density gradient medium – Lymphoprep™ (1.077 g/ml, STEMCELL Technologies). Next, normal CD19+ cells were isolated from PBMC using EasySep™ Human CD19 Positive Selection Kit II (STEMCELL Technologies). For the viability assay, the enriched cells (more than 75% of CD19+ detected by flow cytometry) were seeded onto 96-well plate, 2.0 × 10^5 cells per well in 100 μl of SFEM II medium containing 20% FBS and 1% penicillin/streptomycin. The same day, the cells were treated with VEN, AUR or both (final volume per well - 200 μl) and following 48 h the viable cells were detected by flow cytometry (Fortessa X-20, BD Biosciences) using propidium iodide (PI) dye (final concentration 1 μg/ml, Sigma-Aldrich). The viable cells are presented as the percentage of PI-negative cells, normalized to DMSO-treated control cells.

**DNA and RNA isolation**

Genomic DNA was extracted from leukemic cells using the Sherlock AX (A&A Biotechnology, Gdansk, Poland). For RT-qPCR analysis of TP53, MDM2 and CDKN2A RNA was extracted from BCP-ALL cell lines and PDX cells using RNeasy Mini Kit (QIAGEN, Hilden, Germany) with an additional step of DNase digestion with an RNase-Free DNase Set (QIAGEN), according to manufacturer’s protocol. The concentration and quality of isolates were determined by ultraviolet spectrophotometry (NanoDrop 8000, Thermo Scientific, Waltham, MA, USA). RNA integrity (RIN value) was measured by 2200 TapeStation System (Agilent Technologies, Waldbronn, Germany).

**Panel-based and Sanger DNA sequencing**

Targeted NGS sequencing of leukemic genome was performed using custom designed SureSelect XT-HS2 DNA System panel (Agilent Technologies) comprising 217 genes involved in the pathogenesis of pediatric malignancies. 10 ng DNA was processed according to the manufacturers' protocol. The DNA libraries were prepared according to manufacturer’s protocols and sequenced on a NextSeq 550 system (Illumina, San Diego, CA, USA) in the process of 300 bp paired-end run. The raw data analyses were performed using SureCall v. 4.1 software and mapped to the GRCh37/hg19 reference sequence. The presence of the TP53 mutation in SEM cell line was confirmed using direct Sanger sequencing with the following primers:

For_exon7: 5’-TCCATCTCAAAAAAAAAAAAAAAGG-3’
Rev_exon7: 5’-CCCAAGGCGAGAAAGAAAAC-3’.
The analysis of chromatograms was conducted using Sequencher DNA Sequence Analysis Software v. 5.2.0 (GeneCodes Corporation, Ann Arbor, MI, USA).

**RT-qPCR**

**Cell lines:**

For RT-qPCR analysis (*PMAIP1, TXNRD1, TXNRD2, ATF4*) RNA was extracted using RNeasy Mini Kit (QIAGEN). In order to remove genomic DNA contamination, 0.5 µg RNA was incubated with DNase (Sigma-Aldrich) and then reverse transcribed with either Transcriptor First Strand cDNA Synthesis Kit (Roche, Mannheim, Germany) or with Superscript III (ThermoFisher Scientific) and random hexamer primers (ThermoFisher Scientific). mRNA levels of *PMAIP1, TXNRD1, TXNRD2, ATF4* and housekeeping genes, *RPL29, GUSB or GAPDH* were assessed using LightCycler® 480 SYBR Green I Master (Roche) or Fast SYBR™ Green Master Mix (ThermoFisher Scientific). The relative gene expressions were analysed using LightCycler 480 Software 1.5 (Roche) as described in (6) or calculated based on the cycle threshold (Ct) values as detected in QuantStudio 3 Real-Time PCR System (ThermoFisher Scientific). Primer sequences are listed in Sup Table 3.

**Primary samples:**

1 µg RNA was used for cDNA synthesis using the High-Capacity cDNA Reverse Transcription kit (ThermoFisher Scientific) and 60 ng cDNA was used for each RT-qPCR reaction. The expression of *TP53, MDM2* and *CDKN2A* target genes and *ACTB* and *B2M* as internal control genes was measured in duplicate by a fluorescence-based kinetic RT-qPCR using the following TaqMan™Gene Expression Assays: Hs01034249_m1, Hs00923894_m1, Hs01066930_m1, Hs01060665_g1, Hs00187842_m1 (Applied Biosystems, Foster City, CA, USA) and TaqMan™ Gene Expression Master Mix (Applied Biosystems). The reaction was performed using AriaMx Real-time PCR System (Agilent Technologies) in accordance with the manufacturer's instructions and data processing was performed using Agilent Aria1.71 software. For the analysis of RT-qPCR data, the relative gene expression (FC – fold change) was calculated using cycle threshold (Ct) values and following the formula: FC = $2^{-\Delta Ct}$; \(\Delta Ct = Ct(\text{target}) - Ct(\text{ref})\).

**Downregulation of selected genes with siRNA in MLLr BCP-ALL cell lines**

SEM or RS4;11 cells were washed twice in Opti-MEM medium (ThermoFisher Scientific), and then $1.0 \times 10^7$ cells were subjected to electroporation in Gene Pulser Xcell Electroporation System (Bio-Rad Laboratories, Hercules, CA, USA), under following conditions: 330V, 10ms, 1 pulse, program Square Wave. The electroporation was performed in 400 µl of Opti-MEM medium in Bio-Rad Cuvettes with
For all knockdown experiments ON-TARGETplus Human siRNA, SMARTpool (Dharmacon, Lafayette, CO, USA) were used. The sequences of SMARTpool siRNAs are presented in Sup Table 4.

Following electroporation cells were cultured in their standard medium (RPMI + 10% FBS + antibiotics) at the density of \(1.0 \times 10^6\) cells/ml, until further experimental procedures. The viability of electroporated cells exceeded 90% in all knockdown experiments.

**Lentiviral modifications of SEM cell line**

For the genomic knockout of *PMAIP1* (encoding NOXA), specific sgRNA sequences were designed and cloned into pLentiCRISPRv2 plasmid (Addgene plasmid #52961). These sgRNA sequences are as follows: **sgNOXA(1):** TCGAGTGTGCTACTCAACTC, **sgNOXA(2):** ACGCTCAACCGAGCCCCGCG, **sgNOXA(3):** TTCTTGCAGCGCCTTCTTCCC. The construct encoding sgRNA against the non-mammalian targeting control (NTC, sequence: ACGGAGGCTAAGCGTCGCAA) served as a control.

Transfection of human HEK293T cells and lentivirus production was performed as described in (7), however in this study plasmid delivery was performed using polyethylenimine (31.5 µg PEI per 10.5 µg DNA). Supernatant containing lentiviral particles was added to 0.5 \(\times\) \(10^6\) of SEM cells and then cells underwent spinoculation for 1 h (750 × g at 32°C) in the presence of 5 µg/ml polybrene. Following transduction, clonal lines were selected using 2 µg/ml puromycin.

**ROS detection**

Cytosolic ROS levels were assessed using CM-H2-DCFDA dye (Molecular Probes, Eugene, OR, USA) as described in (2) and mitochondrial ROS using MitoSOX Red dye (Molecular Probes). For MitoSOX assay cells were seeded at the density of 0.2 \(\times\) \(10^6\) cells per 1 ml and stained with 5 µM of the dye for 10 min at 37°C. In both assays the cells were treated with indicated concentrations of single drugs and VEN+AUR combination for 1, 2 and 4 h.

**Caspase activity**

0.2 \(\times\) \(10^6\) of SEM cells were seeded onto 24-well plate in their medium and treated with AUR, VEN and combination of both for 16 h. The activity of particular caspases (-1, -3, -4, -5, -6, -7, -8, -9) was performed using Muse MultiCaspase Assay Kit (Merck Millipore, Burlington, MA, USA) following the manufacturer’s instructions and analysed in Muse Cell Analyzer (Merck Millipore).

**Immunoblotting**

Whole cell extracts from BCP-ALL cell lines were prepared in Triton X-100-based buffer and subjected to immunoblotting as previously described in (2). Extraction and detection of histone proteins was performed as in (8). Antibodies used for detection of particular proteins are listed in Sup Table 2.
Detection of dead cells by propidium iodide staining

Genetically modified SEM and RS4;11 cells were seeded onto 96-well plates (0.2 × 10^6 cells/ml density) in their medium and treated with VEN, AUR or combination of both for 24 or 48 h. The cells were then stained with propidium iodide (PI, final concentration of 1 µg/ml) (Sigma-Aldrich) and analysed in Canto II or Fortessa X-20 flow cytometer (BD Biosciences).

ChIP-qPCR

ChIP assay was performed as described in (8, 9). SEM cells were exposed to 1.5 µM AUR for 4 h and 1.0 × 10^7 cells were fixed [1% formaldehyde for 10 min (histone ChIP), or 2 mM disuccinimidyl glutarate for 30 min followed by 1% formaldehyde for 30 min (ATF4 ChIP)] and then DNA was fragmented on Covaris ME220 sonicator according to the manufacturer’s protocol. Next, magnetic isolation of antibody-chromatin complexes (for antibodies used see Sup Table 2) was performed using Protein A and G Dynabeads (ThermoFisher Scientific) following a wash step with a solution of 50 mM HEPES-KOH, pH 7.6, 500 mM LiCl, 1 mM EDTA, 1% NP-40 and 0.7% Na-deoxycholate, which was repeated three times. Finally, the beads were washed once with Tris-EDTA, eluted, and incubated with RNase A and Proteinase K and crosslinks were reversed at 65°C overnight. DNA was then isolated using PCR Purification Kit (QIAGEN) and analysed by qPCR (primer sequences are listed in Sup Table 5). The enriched genomic DNA was normalized to inputs as described in (10). SEM H3K27ac ChIP-seq is taken from GSE74812.

ATAC-seq

SEM cells were exposed to AUR or DMSO for 4 h and then 5 × 10^4 cells were harvested. For ATAC-seq the samples were processed as described in (8) using the Nextera Tn5 transposase (Illumina). Paired-end sequencing was performed on DNA libraries using NextSeq 500 (Illumina). Quality control of FASTQ reads, alignments, PCR duplicate filtering, blacklisted region filtering and UCSC data hub generation were performed using the NGseqBasic pipeline (11).

RNA-seq

Male and female NOD.Cg-Prkdc<sup>scid</sup> Ii2rg<sup>tm1Wjl</sup>/SzJ (NSG) mice (Charles River Laboratories) were injected intravenously with 0.5 × 10^6 PDX#1 cells (derived from adult MLL-AF4+ BCP-ALL patient). Leukemia engraftment was detected once a week and determined as percentage of human blasts in murine peripheral blood using specific antibodies and flow cytometry. Once the percentage of human CD45+/CD19+ cells among murine CD45+ cells exceeded 1% (26 days post engraftment), the mice were randomly selected and treated with either control (60% Phosal 50PG; 30% PEG400;
10% ethanol), or 100mg/kg of VEN (oral gavage). The drugs were given 5 times per week for a total of 13 doses and the mice were sacrificed at the last day of the treatment. The spleens were harvested from all mice and leukemic cells were isolated using EasySep™ Human CD19 Positive Selection Kit II (STEMCELL Technologies). After the magnetic separation, the total amount of viable CD19+ cells was evaluated in trypan blue (>90% viable cells). The cells were washed in PBS and dissolved in Trizol for RNA isolation. RNA was isolated from leukemic cells according to manufacturer’s protocol (Ambion, Life Technologies, Carlsbad, CA) with an additional step of DNase digestion with an RNase-Free DNase Set (QIAGEN). The concentration and quality of isolates were determined by ultraviolet spectrophotometry (NanoDrop 8000, ThermoFisher Scientific). RNA integrity (RIN value) was measured by 2200 TapeStation System (Agilent Technologies).

RNA sequencing was performed using TruSeq Stranded mRNA (Illumina). 1 μg RNA was processed according to the manufacturers' protocol. Briefly, polyA containing mRNA molecules were purified using oligo-dT attached magnetic beads in two rounds of purification. Then, RNA was fragmented and primed for cDNA synthesis followed by the second strand synthesis resulting in blunt-ended cDNA. Secondly, adapter ligation to the ends of the ds cDNA fragments was performed and samples were amplified with PPC (PCR Primer Cocktail) that anneals to the ends of the adapters, and cleaned-up. In order to optimize cluster densities across every lane of the flow cell, quantification of DNA libraries using DNA 1000 chip on an Agilent Technologies 2200 TapeStation System was performed. Indexed DNA libraries were normalized to 4 nM and then pooled in equal volumes in the PDP plate. Library was sequenced on a NextSeq 550 system (Illumina) using NextSeq® Reagent Kit v3 (150 cycles) with a TG NextSeq 500/550 High Output Flow Cell. Images from the instrument were processed to generate FASTQ sequence files.

FASTQ files were quality checked using FastQC (v0.11.4). Adapters and poor-quality bases were then trimmed from the reads using trim_galore (v0.4.1). Paired-end reads were mapped to the human genome assembly (hg19) using the STAR aligner (v2.4.2). PCR duplicates were removed using picard-tools MarkDuplicates (v1.83). Mapped reads were then quantified over gene exons using subread featureCounts (v1.6.2) to measure gene expression levels.

Statistical analysis was performed in R using the edgeR package (12), and P values were corrected using the Benjamini & Hochberg method (13) to acquire False Discovery Rates (FDR). Genes were considered differentially expressed if they had an FDR of less than 0.05.

Differentially expressed genes were analysed for enriched pathways using PANTHER (v15) (14). p53 pathway genes for visualisation/exploration were extracted from the PANTHER database (14), and the KEGG database (hsa04115). Data visualisations were performed in R. Heatmaps were
visualised using log_{2} CPM expression values that were Z-score transformed, such that the data are represented by the standard deviations from mean expression.

**VEN-treatment in vivo and RT-qPCR**

Male and female NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ (NSG) mice (Charles River Laboratories) were injected intravenously with MLLr BCP-ALL PDX samples as follows: PDX#2 (0.5 × 10^6 cells/mouse), PDX#3 (5.0 × 10^6 cells/mouse), PDX#4 (5.0 × 10^6 cells/mouse), PDX#5 (5.0 × 10^6 cells/mouse), and PDX#6 (2.0 × 10^6 cells/mouse). Leukemia engraftment was detected by flow cytometry using specific antibodies as described above. When the percentage of human blasts in the murine blood reached around 1%, the mice were randomly selected and treated with either vehicle control (60% Phosal 50PG; 30% PEG400; 10% ethanol), or 100mg/kg of VEN (oral gavage). The mice received the drug for a total of 11 doses (PDX#2), 12 doses (PDX#3, PDX#4), 15 doses (PDX#6), or 20 doses (PDX#5) and were sacrificed at the last day of the treatment. Next, human CD19^+ leukemic cells were isolated from the spleens of control and VEN-treated mice with EasySep™ Human CD19 Positive Selection Kit II (STEMCELL Technologies). These include mice with limited VEN efficacy (from PDX#2 and PDX#3). PBS-washed cell pellets were then dissolved in Trizol for RNA isolation. RNA isolation and quality check steps were performed as described in “RNA-seq” methods section. The expressions of selected p53 pathway-related genes were assessed by RT-qPCR using gene-specific TaqMan probes (TaqMan™Gene Expression Assays, Applied Biosystems) (Sup Table 3). The reaction was performed using AriaMx Real-time PCR System (Agilent Technologies) in accordance with the manufacturer’s instructions and data processing was performed using Agilent Aria1.71 software. For the analysis of RT-qPCR data, the relative gene expression (FC – fold change) was calculated using cycle threshold (Ct) values and following the formula: FC = 2^{-\Delta \text{Ct}}; \Delta \text{Ct} = \text{Ct(target)} - \text{Ct(ref)}. RT-qPCR analysis of genes involved in p53 pathway was performed on three PDXs including the one previously subjected to RNA-seq analysis (PDX#1).

**Data availability**

RNA-seq and ATAC-seq data have been deposited in the Gene Expression Omnibus (GEO) under accession number GSE163229.

**Statistical Analysis**

The n numbers provided in all figure legends state the number of independent biological replicates (cell lines) or the number of individual samples (patients and animal data). Statistical analyses were performed using GraphPad Prism 7 Software (La Jolla, CA, USA), and p values were considered
statistically significant when lower than 0.05.

The type of statistical test used is described in Figure legends. All tests were two-sided and for multiple comparisons appropriate corrections were adjusted, as indicated in Figure legends. The exclusion criteria were pre-established. Samples/experiments exclusion from a given analysis was performed only when positive or negative controls did not give appropriate results.
References:

1. Deregowska A, Pepek M, Pruszczyk K, Machnicki MM, Wnuk M, Stoklosa T. Differential Regulation of Telomeric Complex by BCR-ABL1 Kinase in Human Cellular Models of Chronic Myeloid Leukemia—From Single Cell Analysis to Next-Generation Sequencing. Genes (Basel). 2020; 11(10):1145.

2. Fidyt K, Pastorczak A, Goral A, Szczypiels K, Fendler W, Muchowicz A, et al. Targeting the thioredoxin system as a novel strategy against B-cell acute lymphoblastic leukemia. Mol Oncol. 2019; 13(5):1180–95.

3. Chou T. Drug Combination Studies and Their Synergy Quantification Using the Chou-Talalay Method. Cancer Res. 2010; 70(2):440–6.

4. Charan J, Kantharia ND. How to calculate sample size in animal studies? J Pharmacol Pharmacother. 2013; 4(4):303–6.

5. Pal D, Blair HJ, Elder A, Dormon K, Rennie KJ, Coleman DJL, et al. Long-term in vitro maintenance of clonal abundance and leukaemia-initiating potential in acute lymphoblastic leukaemia. Leukemia. 2016; 30(8):1691–700.

6. Muchowicz A, Firczuk M, Wachowska M, Kujawa M, Jankowska-Steifer E, Gabrysiak M, et al. SK053 triggers tumor cells apoptosis by oxidative stress-mediated endoplasmic reticulum stress. Biochem Pharmacol. 2015; 93(4):418–27.

7. Bajor M, Zych AO, Graczyk-Jarzynka A, Muchowicz A, Firczuk M, Trzeciak L, et al. Targeting peroxiredoxin 1 impairs growth of breast cancer cells and potently sensitises these cells to prooxidant agents. Br J Cancer. 2018; 119(7):873–84.

8. Godfrey L, Crump NT, Thorne R, Lau IJ, Repapi E, Dimou D, et al. DOT1L inhibition reveals a distinct subset of enhancers dependent on H3K79 methylation. Nat Commun. 2019; 10(1):1–15.

9. Benito JM, Godfrey L, Kojima K, Hogdal L, Wunderlich M, Geng H, et al. MLL-Rearranged Acute Lymphoblastic Leukemias Activate BCL-2 through H3K79 Methylation and Are Sensitive to the BCL-2-Specific Antagonist ABT-199. Cell Rep. 2015; 13(12):2715–27.

10. Milne TA, Zhao K, Hess JL. Chromatin Immunoprecipitation (ChIP) for Analysis of Histone Modifications and Chromatin-Associated Proteins. In: Eric So CW, editor. Leukemia: Methods and Protocols. Totowa, NJ: Humana Press; 2009. p. 409–23.
11. Telenius J, Hughes JR. NGseqBasic - a single-command UNIX tool for ATAC-seq, DNasel-seq, Cut-and-Run, and ChIP-seq data mapping, high-resolution visualisation, and quality control. bioRxiv. 2018; 393413. Available from: http://biorxiv.org/content/early/2018/08/16/393413.abstract

12. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential expression analysis of digital gene expression data. Bioinformatics. 2010; 26(1):139–40.

13. Benjamini Y, Hochberg Y. Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. J R Stat Soc Ser B. 1995; 57(1):289–300.

14. Thomas PD, Campbell MJ, Kejariwal A, Mi H, Karlak B, Daverman R, et al. PANTHER: a library of protein families and subfamilies indexed by function. Genome Res. 2003; 13(9):2129–41.
# Supplementary Tables

## Supplementary Table 1. Primary (ALL) and primograft (PDX) BCP-ALL

| Sample no | BCP-ALL subtype | Risk group  | Age at diagnosis [years] | Sex   | Diagnosis – Relapse |
|-----------|-----------------|-------------|--------------------------|-------|--------------------|
| ALL#1     | undefined       | HR          | 55                       | female| D                  |
| ALL#2     | undefined       | HR          | 20                       | male  | D                  |
| ALL#3     | ETV6-RUNX1      | SR          | 3                        | female| D                  |
| ALL#4     | ETV6-RUNX1      | SR          | 5                        | male  | D                  |
| ALL#5     | undefined       | SR          | 4                        | male  | D                  |
| ALL#6     | ETV6-RUNX1      | SR          | 3.5                      | male  | D                  |
| PDX#1     | MLL-AF4         | HR          | 48                       | female| D                  |
| PDX#2     | MLL-ENL         | HR          | 3 months                 | female| R                  |
| PDX#3     | MLL-AF4         | HR          | 1.5                      | female| R                  |
| PDX#4     | MLL-AF4         | HR          | 4                        | female| D                  |
| PDX#5     | MLL-AF4         | HR          | 3.5                      | male  | R                  |
| PDX#6     | MLL-AF4         | HR          | 1.5 month                | male  | D                  |
| PDX#7     | MLL-AF4         | HR          | 11                       | female| D                  |
| PDX#8     | MLL-AF4         | HR          | 29                       | female| D                  |
| PDX#9     | Ph+             | HR          | 41                       | male  | D                  |
| PDX#10    | Ph+             | HR          | 69                       | female| R                  |
| PDX#11    | Ph-like         | HR          | 9                        | male  | D                  |
| PDX#12    | Ph-like         | HR          | 14                       | male  | R                  |
| PDX#13    | Ph-like         | HR          | 11.5                     | male  | R                  |
| PDX#14    | undefined       | SR          | 72                       | male  | D                  |
| PDX#15    | undefined       | SR          | 40                       | female| D                  |
Supplementary Table 2. Antibodies; abbreviations: m - mouse, h - human

| Antibody                  | Clone    | Catalog no | Company         |
|---------------------------|----------|------------|-----------------|
| **Antibodies for flow cytometry** |          |            |                 |
| anti-mCD45 APC            | 30-F11   | 17-0451-83 | eBioscience     |
| anti-hCD45 PE             | HI30     | 12-0459-42 | eBioscience     |
| anti-hCD34 APC            | 4H11     | 17-0349-42 | eBioscience     |
| anti-hCD19 FITC           | HIB19    | 11-0199-42 | eBioscience     |
| anti-hCD19 APC            | HIB19    | 555415     | BD Biosciences  |
| anti-hCD10 PE             | CB-CALLA | 12-0106-42 | eBioscience     |
| **Antibodies for immunoblotting and/or ChIP-qPCR** |          |            |                 |
| anti-NOXA                 | D8L7U    | 14766      | Cell Signaling  |
| anti-MCL1                 | D35A5    | 5453       | Cell Signaling  |
| anti-BCL2                 | D55G8    | 4223       | Cell Signaling  |
| anti-BCL-XL               | 54H6     | 2764       | Cell Signaling  |
| anti-BIM                  | C34C5    | 2933       | Cell Signaling  |
| anti-BID                  | polyclonal | 2002     | Cell Signaling  |
| anti-BAK                  | D4E4     | 12105      | Cell Signaling  |
| anti-BAX                  | D2E11    | 5023       | Cell Signaling  |
| anti-TXNRD1               | 19A1     | ab16847    | Abcam           |
| anti-TXNRD2               | 3F2-E12-F10 | 12029   | Cell Signaling  |
| anti-p53                  | 1C12     | 2524       | Cell Signaling  |
| anti-p21 Waf1/Cip1        | 12D1     | 2947       | Cell Signaling  |
| anti-cleaved PARP         | D64E10   | 5625       | Cell Signaling  |
| anti-H2AK119ub            | D27C4    | 8240       | Cell Signaling  |
| anti-H3K27ac              | polyclonal | C15410196 | Diagenode       |
| anti-ATF4                 | D4B8     | 11815      | Cell Signaling  |
| anti-α-tubulin            | DM1A     | CP06       | Calbiochem      |
| anti-β-actin              | AC-15    | A3854      | Sigma-Aldrich   |
| anti-GAPDH                | polyclonal | A300-641A | Bethyl Laboratories |
Supplementary Table 3. List of primers and TaqMan probes used for RT-qPCR

| Primers (SYBR Green) | Sequences                      |
|----------------------|--------------------------------|
| PMAIP1_F             | CAGGAGATTTGGGAGACAAAC          |
| PMAIP1_R             | CTCATGCAAGTTTTTGATGC          |
| TP53_F               | AGTCTACCTCCCGCCATAAA          |
| TP53_R               | GGGAAACAGAGAGTGAGAATGT         |
| TXNRD1_F             | TCACCCCCATGGCAATCC            |
| TXNRD1_R             | GGTGGAACATTTTCTAGTCACA        |
| TXNRD2_F             | CAGCGGACTATGATCTCCT           |
| TXNRD2_R             | AGGTCCACGGTAGTCACA            |
| ATF4_F               | CCTAGTGCTCTTTAGATGATTACC      |
| ATF4_R               | CAAAGTCAAATCCTCTCAAATC        |
| RPL_F                | CAGCTCAGGCTCCCAAAC            |
| RPL_R                | GCACCAGTCTTCTGTCCCT           |
| GUSB_F               | GAAAATATGTTGGTGGAGAATCATT     |
| GUSB_R               | CGGAGTGAAGATCCCCCTTTTTA       |
| GAPDH_F              | AACAGCGACACCCCATCCT           |
| GAPDH_R              | CATACCAGGAAATGAGCTTGACAA      |

TaqMan™Gene Expression Assays | Assay ID                  |
|-----------------------------|---------------------------|
| BBC3                        | Hs00248075.m1             |
| TNFRSF10B                   | Hs00366272.m1             |
| CDKN1A                      | Hs00355782.m1             |
| FAS                         | Hs00236330.m1             |
| ZMAT3                       | Hs00536976.m1             |
| DDB2                        | Hs00172068.m1             |
| SESN1                       | Hs00902787.m1             |
| BAX                         | Hs00180269.m1             |
| TP53I3                      | Hs00936520.m1             |
| TNFRSF10D                   | Hs00388742.m1             |
| SESN2                       | Hs00230241.m1             |
| CDC25A                      | Hs00947994.m1             |
| CHEK1                       | Hs00967506.m1             |
| GTSE1                       | Hs01126673.m1             |
| ACTB                        | Hs01060665.g1             |
### Supplementary Table 4. Sequences of ON-TARGETplus Human siRNA, SMARTpool

| Gene  | 5’ → 3’                      |
|-------|------------------------------|
| NTC   |                              |
|       | 1) UGGUUUACAUUGUCGACUAA      |
|       | 2) UGGUUUACAUUGUUGUGUGA      |
|       | 3) UGGUUUACAUUGUUCUCUGA      |
|       | 4) UGGUUUACAUUGUUUCUCA       |
| TXNRD1 |                             |
|       | 1) GCAUCAGUUUAUAAGACA        |
|       | 2) GCGAUAAUUGGAGGAUAA        |
|       | 3) CUAAGGAGGCGCCAUAUAU       |
|       | 4) GGACAGCACAUAUGGAAUC       |
| TXNRD2 |                             |
|       | 1) GCAUCCCAGUGUAUGUAUA       |
|       | 2) GCGAAGUUACUAGGAAUU        |
|       | 3) AGGAUCUUGUGAGGCUA         |
|       | 4) GCACACAGGUUGCGCGUU        |
| TP53  |                              |
|       | 1) GAAAUUUGCGUGUAGAGUA       |
|       | 2) GUGCAGCUGUGGGUGGAUU       |
|       | 3) GCAGUCAGAUCCUGCGUC        |
|       | 4) GGAGAAUAAUUCACCACUUC      |
| ATF4  |                              |
|       | 1) CAGAUUGGAUGUUGAGAA        |
|       | 2) CGACUUGGAUUCUGCUGUG       |
|       | 3) GAGAGACGAGCUCUAAAAA       |
|       | 4) GAGAUAGGAAAGCCAGACUA      |
Supplementary Table 5. Sequences of primers used for ChIP-qPCR

| Primers                     | Sequences                      |
|-----------------------------|--------------------------------|
| PMAIP1_F (H3K27ac ChIP)     | GGCGCCTCCAGAAAGTT              |
| PMAIP1_R (H3K27ac ChIP)     | CTGCATCCCAATCGCAAATC           |
| CDK6_F (H3K27ac ChIP)       | TCGAAGGGAAGTCTCTCAACA          |
| CDK6_R (H3K27ac ChIP)       | GCTTGGGCAGAGGCTATGTA           |
| PMAIP1_F (ATF4 ChIP)        | GCTGTTGCACTCAGACGATTATAC      |
| PMAIP1_R (ATF4 ChIP)        | CAACTTAGGCATGGTCACATTT        |
| SLC7A1_F (ATF4 ChIP)        | CACGCTTACTCACTCCTGTT          |
| SLC7A1_R (ATF4 ChIP)        | GCCTCCACGGAGTCACATTT          |
| PMAIP1_F (H2AK119ub ChIP)   | TTCCTCCTGGTTAAACCCTTC         |
| PMAIP1_R (H2AK119ub ChIP)   | GGCTTTGACCATCTGCAAAC          |
| HOXC8_F (H2AK119ub ChIP)    | CAGAAGGATAGGAGGAGCTGC         |
| HOXC8_R (H2AK119ub ChIP)    | TCAAAACAGGAAGAGGAAGGAGG       |
| Negative control region (neg_F) | GGCTCGTGGACCCAACCCTACC        |
| Negative control region (neg_R) | CCTCTGGGCTGGGCTTACC         |