# ­­Online Methods

### Protein purification

cDNA encoding human bromodomains were cloned, expressed and purified as previously described[1](#_ENREF_1). For purification of *in vivo* biotinylated protein expression the same construct boundaries (e.g. CBP residues R1081-G1198) were bromodomain subcloned into pNIC-BIO1 vector, a derivative from pNIC28-Bsa4 vector (Gene Bank: EF198106), containing a 10 His-tag and TEV protease cleavage site at the N-terminus and an in frame biotinylation sequence (SSKGGYGLNDIFEAQKIEWHE) inserted at the C-terminus. The constructs were transformed into BL21 (DE3)-R3-BirA cell line (BL21 derivative co-expressing BirA using a pACYC co-expression vector).Cells were grown overnight at 37 ºC in 10 mL of Luria-Bertani medium with 50 μg/mL kanamycin and 34 μg/ml chloramphenicol (start-up culture). The start-up culture was diluted 1:1000 in fresh medium and cell growth was allowed at 37 ºC to an optical density of about ~1.0 (OD600) before the temperature was decreased to 25 ºC. d-Biotine was dissolved into 10 mM bicine pH8.3 and added to the culture at 500 μM final. The protein expression was induced for 8 h at 25 ºC with 50 µM isopropyl-β-D-thiogalactopyranoside (IPTG). Proteins were purified using Ni-affinity chromatography and size exclusion chromatography.

### Bio-Layer Interferometry (BLI)

Kinetic ligand binding measurements were done using an OctetRed384 instrument (ForteBio). Biotinylated protein was immobilized on Super Streptavidin Biosensors using a concentration of 0.05 mg/mL. Association and dissociation measurements were done in 25 mM HEPES pH 7.4, 100 mM NaCl, 0.01 % Tween at 25 °C with association and dissociation times of 240 sec. Compounds were prepared as 1:2.5 dilutions starting from 12 M. Binding to the reference sensors (no protein attached) was subtracted before calculations. Binding constants were calculated using the ForteBio Analysis software provided by manufacturer.

### Isothermal Titration Calorimetry (ITC)

Experiments were carried out on a VP-ITC microcalorimeter (MicroCal™). All experiments were performed at 15 °C in 50 mM HEPES pH 7.5, 150 mM NaCl. The titrations were conducted using an initial injection of 2 µl followed by 34 identical injections of 8 µl. The dilution heats were measured on separate experiments and were subtracted from the titration data. Thermodynamic parameters were calculated using **∆***G* = **∆***H* - T**∆***S* = -RTln*K*B, where **∆***G*, **∆***H* and **∆***S* are the changes in free energy, enthalpy and entropy of binding respectively. In all cases a single binding site model was employed.

### Thermal shift assay

Thermal melting experiments were carried out using an Mx3005p Real Time PCR machine (Stratagene). Proteins were buffered in 10 mM HEPES pH 7.5, 500 mM NaCl and assayed in a 96-well plate at a final concentration of 2 μM in 20 μL volume. Compounds were added at a final concentration of 10 μM. SYPRO Orange (Molecular Probes) was added as a fluorescence probe at a dilution of 1:1000. Excitation and emission filters for the SYPRO-Orange dye were set to 465 nm and 590 nm, respectively. The temperature was raised with a step of 3 °C per minute from 25 °C to 96 °C and fluorescence readings were taken at each interval. Data was analysed as previously described[1](#_ENREF_1).

### AlphaScreen Assay

Assays were performed as described previously[2](#_ENREF_2) with minor modifications from the manufacturer’s protocol (PerkinElmer, USA). All reagents were diluted in 25 mM HEPES, 100 mM NaCl, 0.1 % BSA, pH 7.4 supplemented with 0.05 % CHAPS and allowed to equilibrate to room temperature prior to addition to plates. A 11-point 1:2.5 serial dilution of the ligands was prepared over the range of 5000 – 0 μM and 0.1 µL transferred to low-volume 384-well plates filled with 5 uL of the assay buffer (ProxiPlateTM-384 Plus, PerkinElmer, USA), followed by 7 uL of biotinylated peptide H-ALREIRRYQK(ac)STELLIRKLK(biotin)-OH and His-tagged protein to achieve final assay concentrations of 50 nM. Plates were sealed and incubated for a further 30 minutes, before the addition of 8 μl of the mixture of streptavidin-coated donor beads (12.5 μg/ml) and nickel chelate acceptor beads (12.5 μg/ml) under low light conditions. Plates were foil-sealed to protect from light, incubated at room temperature for 60 minutes and read on a PHERAstar FS plate reader (BMG Labtech, Germany) using an AlphaScreen 680 excitation/570 emission filter set. IC50 values were calculated in Prism 5 (GraphPad Software, USA) after normalization against corresponding DMSO controls and are given as the final concentration of compound in the 20 μl reaction volume.

### SPOT analysis

SPOT analysis was performed as described[1](#_ENREF_1),[3](#_ENREF_3). Briefly, membranes were washed three times with PBST (3.2 mM Na2HPO4, 0.5 mM KH2PO4, 1.3 mM KCl, 135 mM NaCl and 0.1 % Tween 20, pH 7.4) and were subsequently blocked over night with 5 % BSAin PBST at 4 °C. After 2 washes with PBST followed by a single wash with PBS (3.2 mM Na2HPO4, 0.5 mM KH2PO4, 1.3 mM KCl, 135 mM NaCl pH 7.4) for 5 min, His-6 tagged CBP was added to a final concentration of 1 μM and the membranes were incubated over night at 4 °C in PBS. Each membrane was washed 3 times in PBST, blocked for 1 h with 5 % BSA in PBST, and washed again 3 x 5 min with PBST. HPR-conjugated anti-His-tag antibodies (Novagen #71841) were added in 1.5 % BSA/PBST solution at a dilution of 1:2000. After 1 h incubation, membranes were washed 3 x 20 min in PBST. The assay was developed using an ECL kit (Pierce ECL Western Blotting Substrate, Thermo Scientific) following the manufacturer’s protocol. Chem-illuminescence was detected with an Image reader (Fujifilm LAS-4000 ver.2.0) with an incremental exposure time of 5 min for a total of 80 min.

### Fluorescence Recovery After Photobleaching (FRAP)

FRAP studies were performed using a protocol modified from previous studies[4](#_ENREF_4). In brief, U2OS cells were transfected (Fugene HD; Roche) with mammalian over-expression constructs encoding GFP fused to the N-terminus of full length BRD4. The FRAP and imaging system consisted of a Zeiss LSM 710 laser-scanning and control system (Zeiss) coupled to an inverted Zeiss Axio Observer.Z1 microscope equipped with a high-numerical-aperture (N. A. 1.3) 40 x oil immersion objective (Zeiss). Samples were placed in an incubator chamber capable of maintaining temperature and humidity. FRAP and GFP fluorescence imaging were both carried out with an argon-ion laser (488 nm) and with a PMT detector set to detect fluorescence between 500-550 nm. Once an initial scan had been taken, a region of interest corresponding to approximately 50 % of the entire GFP positive nucleus was empirically selected for bleaching. A time lapse series was then taken to record GFP recovery using 1% of the power used for bleaching. The image datasets and fluorescence recovery data were exported from ZEN 2009, the microscope control software, into Microsoft Excel to determine the average half-time for full recovery for 10-20 cells per treatment point.

### Cell culture and reagents

Human cell lines (MOLM13, MONOMAC, THP1, KOCL45, RS4;11, SEM, MV4;11, KOCL44, KOPN8, KASUMI-1, MUTZ5, HL60, K562, PL21, REH, U937) were obtained from ATCC and the Leibnitz Institute DSMZ-German Collection of Microorganisms and cell cultures (www.dsmz.de). Cell lines were cultured in RPMI-1640 medium (Sigma) containing 10% fetal bovine serum (FBS), 100 U/ml penicillin and 100 U/ml streptomycin (Gibco). Synthesis of I-CBP112 and related analogues will be described elsewhere. The inhibitor was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 10 mM and diluted further in culture medium RPMI-1640 immediately before use.

### In vitro cytotoxicity assays

Cytotoxicity activity of I-CBP112 on cell lines was assessed using two different colorimetric assays. Cell viability was assessed using Trypan blue (Sigma). Cells were harvested from exponential phase cultures and plated in 96 well opaque flat-bottom plates at the cell density of 4 x 104 cells per well (50 μl). After 2 to 4 h recovery, 50 μl of medium containing DMSO (vehicle) or the test compound was added to the wells. For each concentration (0, 0.1 to 10 μM) cells were plated in quadruplicate. Cells were exposed to the compound for 48h and 72h before 10ul of WST-1 reagent (Roche) was added to every well. After 30sec on an orbital shaker and further incubation for two hours, absorbance of the samples was measured with an ELISA plate reader (Synergy H1 Hybrid Multi-Mode Microplate Reader) at a wavelength of 450nm versus 650nm (background). Samples were blanked with a control well and the percentage of surviving cells compared to controls.

### Clonogenic and re-plating assays in Methylcellulose

The impact of I-CBP112 on the clonogenic potential of the cells was assessed in methylcellulose cultures in presence or I-CBP112 at different concentrations (1, 3, 5 and 10μM) or DMSO (vehicle control). KASUMI-1 cells were plated in methylcellulose, supplemented with human cytokines (MethoCult H4535, Stemcell Technologies) whereas SEM and MOLM13 cells were plated in methylcellulose without additional cytokines (5510, StemAlpha) at 2-3 x 103 cells per plate. Primary murine bone marrow cells were plated in methylcellulose (MethoCult M3534) supplemented with mouse cytokines at a cell dose of 0.3- 1 x 104 per plate. CD34+ human hematopoietic stem cells from healthy donors, and mononuclear cells from 5 AML patients, harvested from peripheral blood, were plated in methylcellulose supplemented with human cytokines (H4535, Stem Cell Technologies) at a cell dose of 4 x 104 or 2 x 104 per plate respectively. All plates were incubated at 37 °C, 5 % CO2 for 8-10 days before counting the number of colonies and harvesting viable cells.

NanoBRET assays:

HEK293T cells (8 x 105) were plated in each well of a 6-well plate and co-transfected with Histone H3.3-HaloTag (NM\_002107) and NanoLuc-CBP full-length (Q92793) or NanoLuc-CBP BRD domain (amino acids 1081-1211). Twenty hours post-transfection, 1 x 104 cells were trypsinized, washed with PBS and exchanged into media containing phenol red-free DMEM, 10% FBS in the absence (control sample) or the presence (experimental sample) of 100nM NanoBRET 618 fluorescent ligand (Promega). They were then re-plated in a 96-well assay white plate (Corning Costar #3917). After incubation for 18-24 h at 37oC in the presence of 5% CO2, inhibitor I-CBP112 was added at final concentration spanning 0 to 20µM and the plates were incubated for 4h at 37oC in the presence of 5% CO2. NanoBRET substrate (Promega) was added to both control and experimental samples at a final concentration of 10µM. Readings were performed within 5 minutes using the Varioskan Flash spectral scanning multimode reader (Thermo Scientific) equipped with 450/80 nm bandpass and 610 nm longpass filters. A corrected BRET ratio was calculated and is defined as the ratio of the emission at 610 nm/450 nm for experimental samples (i.e. those treated with NanoBRET fluorescent ligand) subtracted by and the emission at 610 nm/450 nm for control samples (not treated with NanoBRET fluorescent). BRET ratios are expressed as milliBRET units (mBU), where 1 mBU corresponds to the corrected BRET ratio multiplied by 1000.

Confocal Imaging:

HEK293T cells were transfected with Histone H3.3-HaloTag using FuGENE HD (Promega). Twenty-four hours post-transfection cells were labelled with 5$µ$M HaloTag TMR ligand (Promega) in complete media (DMEM and 10%FBS) for 15 minutes at 37˚C and 5% CO2. Media containing HaloTag-TMR ligand was then washed twice with fresh complete media, cells were placed back at 37˚C and 5% CO2 for 30 minutes, and then imaged. Images were acquired on an Olympus Fluoview FV500 confocal microscope (Olympus, Center Valley, PA, USA) containing a 37˚C and CO2 environmental chamber (Solent Scientific Ltd., Segensworth, UK) using appropriate filter sets.

### Fixation, immunostaining and confocal microscopy

Cytospots were prepared by centrifugating 105 cells at 300 rpm for 3 min using a Shandon Cytospin 3 centrifuge. Cytospots were stained with Wright-Giemsa and analyzed with a Olympus BX62 or Nikon TI microscope at 60x magnification.

The amount of blasts and differentiated (granulocyte monocyte, macrophage) cells was counted by a trained pathologist and normalized to the total number counted cells of 20 fields. Percentages were normalized to vehicle- treated cells.

For H2Ax staining the cells were fixed in 4% paraformaldehyde solution in PBS. Incubation was conducted for 16 hours at 4°C in 0.3% Triton X-100, 0.5% BSA, and the phospho-H2AX (Ser139) (05-636 Millpore) primary antibody. After washing, the cells were incubated with the secondary antibody for 2 hours. The cells were washed and a coverslip was placed on top by adding a droplet of mounting solution (Clear-Mount, Invitrogen). Confocal fluorescence images were obtained by a Ti NIKON microscope

### Generation of MLL-CBP and MLL-AF9 *in vitro* and *in vivo* immortalized cells

### Lineage surface marker negative bone (Lin-) marrow cells (MagCellect Kit, R&D systems, Minneapolis, MN, USA) were extracted from the bones of C57/BL6 or Balb/C mice and retrovirally transduced with *pMSCV-MLL-AF9*, *pMSCV-MLL-CBP* expression vectors by spinoculation (2000x*g*, 90min at 33°C) in the presence of polybrene 4μg/ml on two consecutive days. To establish *in vitro* immortalized cells, retrovirally transduced cells were selected in neomycin (0.8mg/ml) for 1 week followed by serial plating in methylcellulose for three times (M3534, Stem cell technologies, Vancouver, British Columbia, Canada). Cells were harvested from the semi-solid medium and further kept in liquid culture in RPMI-1640 (10% FCS, 1% penicillin/streptomycin) containing 10ng/ml of human interleukin-6 (hIL-6), 6ng/ml of murine interleukin-3 (mIL-3) and 100ng/ml of murine stem cell factor (mSCF). *In vivo* immortalized cells *were* generated by transplantation of the *in vitro* immortalized cells into syngeneic sublethally irradiated recipient mice. Leukemic blasts from diseased mice were harvested andexpanded in the medium containing growth factors indicated above.

### I-CBP112 in an in vivo model of MLL-AF9+ leukaemia

Leukemic blasts expressing MLL-AF9 were treated in liquid culture with 5 µM of I-CBP112 for 3 days. Control cells were exposed to the corresponding concentration of the DMSO vehicle.

 50 x 103 treated cells were then transplanted into sublethally irradiated (600Rad) syngeneic mice via tail vein injection. Upon the development of signs of disease the mice were sacrificed and analysed. All experiments were done according to Swiss laws for animal welfare and approved by the Swiss Cantonal Veterinary Office of Basel (Switzerland).

### Flow cytometry analysis (Apoptosis)

The ratios of apoptotic cells were determined with the Annexin V-APC apoptosis detection kit (Becton Dickinson). Briefly, after treatment, the cells were collected and washed twice with cold PBS buffer, resuspended in 100 µL of 1x binding buffer, incubated with 5 µL of Annexin V conjugated to APC and 5 µL 7-AAD for 15 min at room temperature, and analyzed by flow cytometry. Cells treated with DMSO were used as the negative control.

For H2Ax analysis, the cells were fixed with 4% paraformaldehyde solution in PBS and stained with the phospho-H2AX (Ser139) (05-636, Millpore) primary antibody for 1 hour and then with the conjugated secondary (AlexaFluor 488, Invitrogen Molecular Probes).

### RNA preparation and gene expression profiling

### KASUMI-1, SEM and MOLM13 cells were treated 3 μM of I-CBP112 or the DMSO vehicle for 4 days. On the third day of treatment, the cells were replated in fresh medium with or without the inhibitor. Total RNA was extracted using the RNA extraction kit (Macherey-Nagel, GmbH & Co, Duren, Germany) according to the manufacturer’s protocol.

### Quantitative reverse-transcriptase (Q-PCR) analysis

KASUMI-1, SEM and MOLM13 cells were treated with 3 μM of I-CBP112 and DMSO vehicle for the indicated time points. On the third day of treatment cells were re-plated in fresh medium with or without the inhibitor. cDNA synthesis was carried out using the high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). Gene expression was assessed in duplicates by quantitative real-time PCR using SYBR Green on an ABI prism 7500 Sequence Detection System (Applied Biosystems). Results were normalized to *Gapdh* expression. The sequences of the used oligoprimers are compiled in the following table:

|  |  |  |
| --- | --- | --- |
|  | Forward | Reverse |
| ID1 | 5’-AATCCGAAGTTGGAACCCCC-3’ | 5’-AACGCATGCCGCCTCG-3’ |
| ID2 | 5’-GACCACCCTCAACACGGATA-3’ | 5’-TGAACACCGCTTATTCAGCC-3’ |
| CDKN1A | 5’-AGAGGAGGCGCCATGTCAG-3’ | 5’-CATTAGCGCATCACAGTCGC-3’ |
| CCND1 | 5’-ATGCCAACCTCCTCAACGAC-3’ | 5’-CGCAGACCTCCAGCATCC-3’ |
| EGR1 | 5’-CACCTGACCGCAGAGTCTTT-3’ | 5’-GCGGCCAGTATAGGTGATGG-3’ |
| ERG | 5’-CTGAAGACCAGCGTCCTCAGT-3’ | 5’-CTGTCCGACAGGAGCTCCA-3’ |
| MDM2 | 5’-AGGAGATTTGTTTGGCGTGC-3’ | 5’-TGAGTCCGATGATTCCTGCTG-3’ |
| FCGR1b | 5’-CTGCTCCTTTGGGTTCCAGT-3’ | 5’-TCCTCTTGGAACACGCTGAC-3’ |
| TRH | 5’-GGCCTGGATGACTTCCTGC-3’ | 5’-GAAAGATCTGGGACGCGGAG-3’ |
| TNFSF13B | 5’-CACCGCGGGACTGAAAATCT-3’ | 5’-TGCAAGCAGTCTTGAGTGAC-3’ |
| GAPDH | 5’-GTGGTCTCCCTGACTTTCAACAGC-3’ | 5’-ATGAGGTCCACCTGCTTGCTG-3’ |
| BCL-2 | 5’-GGATAACGGAGGCTGGGATG-3’ | 5’-GGGCCAAACTGAGCAGAGTC-3’ |
| CBP | 5’-GCCCATTGTCGATCTTCACG-3’ | 5’-ACCCCAGGATGGTTTGTTGG-3’ |
| EP300 | 5’-CCAGCGATGGCACAGATTTT-3’ | 5’-TCACCACCATTGGTTAGTCCC-3’ |
| MPO | 5’-TGGCGAAGGCCATTCAATGT-3’ | 5’-AGCCGCTTGACTTGGACAAC-3’ |
| HOXA9 | 5’-GAAGGGGCCTTCTCTGAA-3’ | 5’-ACTCTTTCTCCAGTTCCAGG-3’ |
| CCNE1 | 5’-CCCATCATGCCGAGGGAG-3’ | 5’-TATTGTCCCAAGGCTGGCTC-3’ |
| CDK2 | 5’-GCATCTTTGCTGAGATGGTGAC-3’ | 5’-CCACTTGGGGAAACTTGGCT-3’ |

**Microarray analysis**

Cells were treated with I-CBP112 and DMSO at the indicated concentration and time point. Total RNA was extracted as described above. Quality analysis of total RNA, hybridisation and data captured were performed at the Cambridge Genomic Services. HumanHT-12 v4 beadchips were used for gene expression screening. Results were analysed using the Lumi Bioconductor package in R[5](#_ENREF_5),[6](#_ENREF_6). Differentially expressed genes were identified in each cell line using limma[7](#_ENREF_7), and all resulting p-values were corrected for multiple testing by using the Benjamini and Hochberg False Discovery Rate correction[8](#_ENREF_8). Genes were defined as being significantly altered following I-CBP112 treatment in each cell line if they showed a fold change greater than 1.5 (up or down compared to non-treated) with an adjusted p-value lower than 0.01. Heatmaps were generated for all genes identified as significant in both Kasumi and MOLM, or in all three of KASUMI-1, MOLM13 and SEM. Genes were clustered using hierarchical clustering on the magnitude of the fold change across the three cell lines.

**ChIP analysis**

Kasumi-1 cells were seeded at a cell density of 5 x E05 cells/ml in 6 wp and treated with 3 µM iCBP-112 or DMSO. After 8 h (A) or 4 days (B) of treatment, cells were processed according to the manufactures’ instructions of “LowCell ChipKit” (Diagenode). Briefly, cells were cross-linked with paraformaldehyde and following washes the chromatin was sheared using a Diagenode Bioruptor® sonicator for 3 cycles of 30s sonication plus 30 s of a break in 4°C water bath. In parallel, protein G beads were incubated with the IgG (Diagenode), p300 (Santa Cruz, c-20), H3K36Ac (Diagenode, C15410307) and H3K56Ac (Diagenode, C15410213) antibodies for 6 hrs at 4°C. Chromatin was added to the antibody-bead complexes and incubated over night at 4 °C. After washes next day, DNA was isolated with DNA-isolation buffer and Proteinase K according to the manufacturer’s instructions. ChIP qPCR was performed with SYBR green and Primers covering the gene regions of the potential p300 binding sites of ID2 and TNFSF13B, respectively (table x). (Primers have been designed following the consensus sequence of p300 and ChIPseq data from UCSC genome browser for the potential binding sites of p300). Relative binding of p300 was calculated by the ratio of IgG-normalized sample to input DNA for the ChIP.

**Used primer:**

**TNFSF13B:**

Promoter: 5’ Primer: ATGCAGAAAGGCAGAAAGGA

 3’ Primer: AGGCAAGAAGTAAGGCGTGA

Promoter/exon 1: 5’ Primer: TCCACAGAAAGGGAGCAGTC

 3’ Primer: GGCCACCTGGTAGAAAGACA

Upstream promoter 5’ 5’ Primer: CTGGAGGCAAGGCTGATTCT

 3’ Primer: TTTCCTTCTGGGACTCATCAC

Intron 2 5’ Primer: GAGCACCTGGAACGTATCGTA

 3’ Primer: TGATAGAAGGGTCCCTGGTC

**ID2**

Enhancer 5’ Primer: ATGCAGAAAGGCAGAAAGGA

 3’ Primer: AGGCAAGAAGTAAGGCGTGA

Promoter/exon 1 5’ Primer: CGTGAGGTCCGTTAGGAAAA

 3’ Primer: GCAGGATTTCCATCTTGCTC

Exon 2 5’ Primer: TGCTGGTCTGTGGACTACAA

 3’ Primer: CACACAGTGCTTTGCTGTCA

Intron 2 5’ Primer: TGGGCTAGTTGAGGATTGCT

 3’ Primer: ACTTCCCAACCCAATCTCCT

Exon 3UTR 5’ Primer: AAATGCCCTTTCTGCAGTTG

 3’ Primer: CAGCATTCAGTAGGCTTGTGTC

### Cell cycle analysis

Cells were treated in liquid culture with increasing concentrations of I-CBP112. At indicated time points, cells were washed twice with PBS, fixed with ice-cold 70% ethanol and stained with propidium iodide (PI). DNA content was measured on CyAn (Beckman-Coulter) and data were analyzed using FlowJo and ModFit software. The experiments were repeated at least three times.

### Primary human blasts

Peripheral blood samples were collected with informed consent from patients with newly diagnosed or relapse acute myeloid leukeamia (AML). Mononuclear cells were separated by Ficoll-Histopaque and frozen in 10% DMSO. Cells were then used for colony formation assays, as described above.

**Extreme Limiting Dilution Analysis (ELDA)**

Leukemic blasts expressing MLL-AF9 were treated in liquid culture with 5 µM of I-CBP112 for 3 days. Control cells were exposed to the corresponding concentration of the DMSO vehicle. The stem cell frequency of the cells was assessed in methylcellulose (MethoCult M3534) supplemented with mouse cytokines at a cell dose of 1, 2.5, 5 or 10 cells per well. Plates were incubated at 37 °C, 5 % CO2 for 8-10 days before counting the number of colonies and scoring colony formation. Presence of colonies was scored and stem cell frequency analyzed by ELDA software (<http://bioinf.wehi.edu.au/software/elda/>)[9](#_ENREF_9).

### Crystallization

Aliquots of the purified proteins were set up for crystallization using a mosquito® crystallization robot (TTP Labtech). Coarse screens were typically setup onto Greiner 3-well plates using three different drop ratios of precipitant to protein per condition (100+50 nL, 75+75 nL and 50+100 nL). All crystallizations were carried out using the sitting drop vapour diffusion method at 4°C. CBP crystals with I-CBP112 (2 mM final concentration) were grown by mixing 200 nL of the protein (8.6 mg/ml) with 100 μL of reservoir solution containing 0.10 M MgCl2, 0.1 M MES pH 6.0, 20 % PEG 6K and 10 % ethylene glycol.

### Data Collection and Structure solution

Crystals were cryo-protected using the well solution supplemented with additional ethylene glycol and were flash frozen in liquid nitrogen. Data were collected at diamond beamline I04 at a wavelength of 1.0121 Å. Indexing and integration was carried out using MOSFLM[10](#_ENREF_10) and scaling was performed with SCALA[11](#_ENREF_11). Initial phases were calculated by molecular replacement with PHASER[12](#_ENREF_12) using an ensemble of known bromodomain models (PDB IDs 2OSS, 2OUO, 2GRC, 2OO1, 3DAI, 3D7C, 3DWY). Initial models were built by ARP/wARP[13](#_ENREF_13) and building was completed manually with COOT[14](#_ENREF_14). Refinement was carried out in REFMAC5[15](#_ENREF_15). Thermal motions were analyzed using TLSMD[16](#_ENREF_16) and hydrogen atoms were included in late refinement cycles. Data collection and refinement statistics are compiled in Supplemental Table S5. The models and structure factors have been deposited with PDB accession codes: 4NR6.

**Drug synergism**

KASUMI-1, SEM and MOLM13 cells were plated in a 9 well plate and treated with iCBP112 combined with Doxorubicine or JQ1 in cytotoxicity assays. 8 different concentrations of iCBP112 and 15 of JQ1 and Doxorubicine were used in ratios from 8:1 to 1:16 (**Supplemental Figure 12A**). As 1X we set approximately the IC50 concentration. After excluding the concentrations that gave either no or extreme effect for the single treatment (**Supplemental Figure 13**), we ran the CompuSyn software and calculated the CI value using the Chou-Talalay method where CI<1 indicates synergism.

**Method References**

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