Supplemental Materials and Methods

Cell Culture

All media were supplemented with 10% FCS and 2 mM glutamine, 100 U/ml penicillin and 100 µg/ml streptomycin (all Gibco, Life technologies, Carlsbad, CA, USA) unless stated otherwise. All cytokines were purchased from Peprotech (Rocky Hill, NJ, USA). HEK293T and Plat-E cells were maintained in DMEM (Gibco). Lenti-X 293T were cultivated in DMEM with 4 mM L-glutamine and 1 mM sodium pyruvate (Sigma-Aldrich, St. Louis, MO, USA). The Cebpap30/C-mut. cell line was established by isolating single cell clones after six rounds of replating in methylcellulose (MethoCult M3434, Stem Cell Technologies, Vancouver, BC, Canada) and continuous liquid culture in the presence of murine stem cell factor (100 ng/ml), murine interleukin 3 (mIL-3, 10 ng/ml) and murine interleukin 6 (mIL-6, 10 ng/ml) in RPMI 1640 (Gibco) for 4 weeks. Established Cebpap30/p30 and Cebpap30/C-mut. cell lines were maintained in RPMI 1640 with 5 ng/ml mIL-3. For proliferation curves of Cebpap30/p30 cells stably expressing pMSCV-rtTA3-IRES-EcoR-PGK-Puro and inducible shRNA constructs, Doxycycline (Sigma-Aldrich) was added every 3-4 days (1 µg/ml). For RNA isolation, biological duplicates of cells were collected 9 days after Doxycycline treatment. MLL-AF9/NrasG12D cells were cultivated in RPMI 1640 supplemented with 10 mM HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, Sigma-Aldrich). Myc/NrasG12D/p53+ cells were cultivated in the same medium as above, supplemented with 50 µM β-MeOH (Life technologies). AML1-ETO9a/NrasG12D/p53- cells were cultivated in RPMI 1640 with 10 mM HEPES, 10 ng/ml mIL-3 and 150 ng/ml SCF. MV4-11, MOLM-13, HL-60 and K562 cells were cultivated in RPMI 1640 and THP-1 and Mono-Mac-6 cells in RPMI 1640 supplemented with 20% FCS. Primary human leukemia cells were obtained from eight patients with de novo AML and two with BCR-ABL1+ CML. The patients’ characteristics are shown in Supplemental Table S1. Cells were stored in a biobank until use. Bulk hematopoietic cells were used for sensitivity assays. All patients provided written informed consent before cells were
collected. The study was approved by the local ethics committee of the Medical University of Vienna. Primary leukemia cells were cultured in IMDM (Gibco) supplemented with 15% BIT (Bovine serum albumin, Insulin, and Transferrin, Stem Cell Technologies), 200 ng/ml SCF, 50 ng/ml FLT3L, 20 ng/ml IL-3, 20 ng/ml granulocyte-colony stimulating factor (G-CSF), 10^{-4} M β-MeOH, 50 µg/ml Gentamicin (Thermo Fisher Scientific, Waltham, MA, USA) and 10 µg/ml Ciprofloxacin (Thermo Fisher Scientific) plus 500 nM StemRegenin1 (SR1, APExBio, Houston, TX, USA) and 1 µM UM729\textsuperscript{1} (APExBIO). For proliferation curves, cells were seeded at low densities in biological triplicates and cell numbers were determined in regular intervals with a cell counter (CASY Model TT, Bremen, Germany). Cells were treated with MI-463 or MI-503 at indicated concentrations in 24- or 48-hour intervals. RNA was isolated 48 or 72 hours after the first treatment. For qPCR analyses biological duplicates and triplicates were measured in technical triplicates. Biological duplicates of SpCas9-Cebpap30/p30 transduced with LentiGuide-Puro-IRES-GFP constructs expressing sgRNAs against Renilla luciferase (sgRen, Control) or Mll1 (sgMll1\textsubscript{#2} and sgMll1\textsubscript{#4}) were selected with 2 µg/ml Puromycin (Sigma-Aldrich) and total RNA was isolated on day 14. Mycoplasma contamination was tested and excluded using the Venor GeM Classic Mycoplasma Detection Kit (Lonza, Basel, Switzerland).

**Chromatin Immunoprecipitation (ChIP)**

Cebpap30 cells were crosslinked for 10 minutes with 11% formaldehyde (Thermo Fisher Scientific) alone (C/EBPα ChIP) or 11% formaldehyde following a 30-minute incubation with 2 mM disuccinimidyl glutarate (DSG, THP, Vienna, Austria) (MLL ChIP). The reaction was quenched with glycine (Sigma-Aldrich) for 5 minutes and cells were harvested and lysed in SDS lysis buffer (1% SDS, 10 mM EDTA, 50 mM Tris pH 8.0 (2-Amino-2-((hydroxymethyl)propane-1,3-diol), all Sigma-Aldrich) for 30 minutes at 4 °C with continuous rotation. The released chromatin was sonicated to obtain fragments of 150 bp (Bioruptor®, Diagenode SE, Seraing, Belgium). Samples were diluted in 0.01% SDS, 1.1% Triton X-100 (Sigma-Aldrich), 1.2 mM
EDTA, 16.7 mM Tris pH 8.0, 167 mM NaCl (Sigma-Aldrich). For solubilization of the sheared DNA, 0.5% Triton X-100 was added before spinning the samples for 10 minutes at 4 °C to clear the chromatin. The supernatant was incubated with 5 µg antibody overnight at continuous rotation. Antibodies used were: anti-MLL1 (Bethyl Laboratories, Montgomery, TX, USA, A300-086A) and anti-C/EBPα (Santa Cruz, Dallas, Texas, USA, sc-9314). Antibody-bound material was pulled down using protein G-coupled magnetic beads (Dynabeads Protein G, Invitrogen, Camarillo, CA, USA) for 3.5 hours at 4 °C with continuous rotation. Samples were washed 5 times with washing buffer (50 mM HEPES-KOH pH 7.4, 500 mM LiCl (Sigma-Aldrich), 1 mM EDTA, 1% NP-40 (Sigma-Aldrich) and 0.7% Na-Deoxycholate (Sigma-Aldrich), and DNA-protein complexes were subsequently released using elution buffer (50 mM Tris-HCl pH 8.0, 10 mM EDTA and 1% SDS) at 65 °C. To revert DNA-protein crosslinks, 200 mM NaCl was added and samples were incubated overnight at 65 °C. DNA was treated with RNase A (Thermo Fisher Scientific, 0.2 mg/ml, 1 hour) and Proteinase K (Thermo Fisher Scientific, 0.2 mg/ml, 2 hours). PCR-cleanup kit (Qiagen, Venlo, Netherlands) was used to purify the DNA. Enrichment of genomic regions was measured in technical duplicates by qPCR (supplemental Table S3) using the SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, Hercules, CA, USA).

ChIP-seq Analyses

The quality of raw sequencing reads was checked with FastQC² (version 0.11.4) and subsequent quality-filtering and -trimming was done with PRINSEQ-lite³ (version 0.20.4). Remaining high-quality reads were mapped against the Mus musculus (GRCm38) reference genome via BWA⁴ (version 0.7.15). SAMtools⁵ (version 1.4) was used for post-processing. RPKM normalization and conversion to BigWig files was carried out with Deeptools⁶ (version 2.5.0.1). Profile plots and heatmaps were also generated with Deeptools. Peak calling for the C/EBPα p30 ChIP-seq dataset of two biological replicates was done with MACS2⁷ using default parameters (version 2.1.1.20160309). For peak calling in MLL ChIP-seq data Sicer³ (version
1.1) was used with window 200, gap 400, fragment size 110, effective genome fraction 0.842028291805 and an FDR of 0.01. Overlapping regions were checked with intervene⁹ (version 0.6.2) and IGV¹⁰ (version 2.4.4) was used for manual inspection and visualization of data. Called peaks were annotated with Homer¹¹ (version 4.10). ChIPseeker¹² was applied on midpoint data of peaks for visualizing the distribution of binding loci.

**Co-Immunoprecipitation**

HEK293T or Lenti-X 293T cells were transfected using polyethylenimine (PEI, Polysciences Inc., Warrington, PA, USA) with a total of 5 µg DNA of indicated constructs. Cell lysates were prepared in IP-lysis buffer (50 mM Tris (2-Amino-2-(hydroxymethyl)propane-1,3-diol), pH 7.5, 150 mM NaCl, 1% NP-40, 5 mM EDTA, 5 mM EGTA (all Sigma-Aldrich)) supplemented with protease and phosphatase inhibitors (Sigma-Aldrich). For immunoprecipitation, 8 mg of total protein were incubated with anti-FLAG M2 affinity gel (Sigma-Aldrich) overnight at 4 °C. Beads were washed 5 times with lysis buffer and proteins were eluted in Lämmli-sample-buffer.

**Western Blot Analysis**

Western blotting was performed according to standard laboratory protocols. The following antibodies were used: anti-FLAG (Sigma-Aldrich, F1804), anti-V5-tag (Cell Signaling, Danvers, MA, USA, 13202), anti-b-Actin (Cell Signaling, 4967S), anti-C/EBPα (Santa Cruz, Dallas, Texas, USA, sc-61) and anti-HSC70 (Santa Cruz, sc-7298); anti-rabbit IgG, HRP-linked (Cell Signaling, 7074P2) and anti-Mouse IgG, HRP-linked (GE Healthcare, Chicago, IL, USA, NA931).

**Vector Construction and Lentiviral Transduction**

Short hairpin RNAs (supplemental Table S2) were cloned into the RT3REVIN backbone. Single guide RNAs (supplemental Table S2) were designed using CHOPCHOP²⁰,²¹ and cloned into a lentiviral expression vector containing a puromycin resistance cassette coupled to GFP.
(LentiGuide-Puro-IRES-GFP). For lentiviral transduction, Lenti-X cells were transfected with indicated constructs together with psPAX2 and pMD2.G (Addgene, Cambridge, MA, USA). Target cells were transduced via spinoculation with lentiviral supernatants supplemented with polybrene.

**Flow Cytometry**

Cells were stained with fluorescence-labelled antibodies against Mac-1 (CD11b M1/70, eBioscience, Thermo Fisher Scientific) and Gr-1 (Ly6G/Ly6C-biotinylated, Cat. No. 553124 and Streptavidin-PE/Cy7 Cat. No. 557598, BD Biosciences, Franklin Lakes, NJ, USA). Apoptosis analysis was performed with Annexin V Apoptosis Detection Kit PE (eBioscience, Thermo Fisher Scientific) according to the manufacturer’s instructions. For cell cycle analysis, cells were fixed in 70% Ethanol and stored at -20 °C until staining with propidium iodide solution. Data for biological triplicates were recorded on a BD FACS Canto II flow cytometer (BD Biosciences) and analyzed with FlowJo software (FlowJo, LLC, Ashland, OR, USA).

**RNA-seq Analyses**

The quality of raw sequencing reads was checked with FastQC\(^2\) (version 0.11.4) followed by quality-filtering and -trimming with PRINSEQ-lite\(^3\) (version 0.20.4). Remaining reads were mapped against the *Mus musculus* (GRCm38) reference genome with STAR\(^13\) (version 2.5.0b). Mapped reads per gene were counted with featureCounts\(^14\) (version 1.6.0). Normalization and differential expression analysis was carried out with DESeq2\(^15\). Gene Set Enrichment Analysis (GSEA)\(^16\) was done with the complete normalized gene-sets. For the visualization of gene expression and unsupervised hierarchical clustering (Pearson correlation and ward.D clustering) of the samples, the rlog normalization in DESeq2 was used as input for the R\(^17\) library heatmap.2 from the gplots\(^18\) package.
Real-time PCR Analysis

Total RNA was isolated using the RNeasy Mini Kit (Qiagen) and reverse transcribed into cDNA using oligo(dT)\textsubscript{18} primers and RevertAid H Minus First Strand cDNA Synthesis Kit (Thermo Fisher Scientific). The iTaq Universal Green Supermix (Bio-Rad) was used for qPCR, which was performed on a Bio-Rad CFX-Connect Real-Time PCR Detection System. Data of technical triplicates were analyzed as relative enrichment to the housekeeping gene \textit{Gapdh} (supplemental Table S3).

Colony Formation Assay

\textit{Cebpap30/p30} and \textit{Cebpap30/C-mut} fetal liver cells were seeded in methylcellulose (MethoCult M3434, Stem Cell Technologies) with either DMSO, MI-463 or MI-503 at a final concentration of 4 µM. On day 7, colonies of 8 technical replicates were scored and imaged (model eclipse Ts2R-FL, Nikon, Minato, Japan). Images were processed using Adobe Photoshop (Adobe; San José, CA, USA).

Cytospin Analysis

Cells were cytocentrifuged onto glass slides and stained with the Rapid-Chrome Kwik-Diff Staining System (Thermo Fisher Scientific) before microscopic analysis (Axio Image.Z1, Zeiss, Jena, Germany). Images were processed using Adobe Photoshop (Adobe).

Chemistry

Chemical synthesis and chemical characterization of MI-463 and MI-503 compounds have been described previously.\textsuperscript{19}

Accession Number

The GEO accession ID for aligned and raw data is GSE117780 (www.ncbi.nlm.nih.gov/geo/).
# Supplemental Tables

Table S1. List of shRNAs and sgRNAs used in this study

| Target       | Sequence                                                                 |
|--------------|---------------------------------------------------------------------------|
| shRNAs       |                                                                           |
| shRen        | TGCTGTTGACAGTGAGCGCAGGAATTATAATGCTTATCTATAGTGAAGCCAAGATGTATAGATAAGCATTATAATTCTATGCTACTGCGTCCCGGA |
| shCebpa      | TGCTGTTGACAGTGAGCGCAGACAGTGAGACGCAACAGTGGAGACGCAACAGAATAGTGAAGCACAGATGTATAGATAAGCATTATAATTCTATGCTACTGCGTCCCGGA |
| Control sgRNAs|                                                                             |
| sgRen        | GGATGATAAACTGGTCCGCAG                                                     |
| sgRpa3       | GCTGGGCCTTGGACGCGCGGCTT                                                   |
| sgRNAs against Cebpa |                                         |
| 1            | GCAGGAGAAACAGCAACAACATCG                                                   |
| 2            | TGACCCGCTGCGCAAGCGGG                                                       |
| 3            | GCTGTTCACCACCGCTTGCG                                                       |
| 4            | CGTGAACCTGGACACGCTGCG                                                       |
| sgRNAs against Gata2 |                                         |
| 1            | GTGCTGCACATTCAATACCG                                                      |
| 2            | GCCATAGTCATGAGCTGCG                                                        |
| sgRNAs against Sox4 |                                         |
| 1            | GGTACAACAGACAAACAACAG                                                    |
| 2            | CGCCCAAGCTGGCCACATCAAG                                                    |
| 3            | ACAACGCCAGATCTCAAG                                                        |
| 4            | CGAACAAGATTCCGTTCAATCC                                                     |
| Target | Sequence |
|--------|----------|
| 1      | GGCGCTTCCCCGCCCGACCC |
| 2      | AAGGCCCAGGACCGTGGCCG |
| 3      | GGGCCGCCACCGCAGGCTG |
| 4      | GGCTGGGGTTCCAGGGGGAG |
| 5      | GGATCATCAAGACTCCCGGG |
| 6      | AGAAAGGGCGGCAGTCAAGG |
| 7      | CGCAGCTGGAATGGAGACCCAG |
| 8      | GCAGCCGTTAGACCTCGGAAG |
| 9      | GAATGAGGCGCGATCTGGCGA |
| 10     | GACGGACGAGACCGATGCCA |
| 11     | AGGGTTTCAAGGAGGGTTTCGT |
| 12     | GAGCCGTGAGGGAGTTCAGGG |
| 13     | GAACCCTCACCGAGCTAGCCA |
| 14     | AAAAGACCCCGGACCATGGAT |
| 15     | TACAGAAAGACCCCGACCA |
| 16     | GAGATGGGTGATTGAATACGC |
| 17     | GCTTGTCTGTCTGGAGGAG |
| 18     | GTTCCGAATGGATGACTCGG |
| 19     | CATGCCTAGATCTACCGGG |
| 20     | GCCATCGTGAAAGATCTACCG |
Table S2. List of qPCR and ChIP-qPCR primers used in this study

| Target | Sequence                        |
|--------|---------------------------------|
| **qPCR primer** |                             |
| Gapdh_fwd | AGAAGGTGGTGTAAGCAGGCAT         |
| Gapdh_rev | CGGCATCGGAAGGTGGAAGAGT         |
| Lyz2_fwd  | AATGGCTGGCTACTATGGAG          |
| Lyz2_rev  | CTCTTTGCACATTGTATGGCT         |
| She_fwd   | GGACTACGCTGACCCGTATG          |
| She_rev   | AACCACGGGCCTGCTTCTTCTTCT     |
| Frzb_fwd  | AAGCAGCAAGCTTGCAAAT           |
| Frzb_rev  | TGACGGTGTCTCCTTGGAAATG        |
| Gata2_fwd | ACAGGCGCTGACCCCTGAGA          |
| Gata2_rev | AAGGGCGGTGACTTCTTCTTGA        |

| Target | Sequence                        |
|--------|---------------------------------|
| **ChIP-qPCR primer** |                     |
| neg. region_fwd | TGCCTGCACTGAGTAATCG         |
| neg. region_rev | AGCTCGTGCCATTGTGCTAT        |
| Cyp51_fwd  | TGATGGCTGACAAATGATCG         |
| Cyp51_rev  | GCTGTCCGCCACCAATCTG          |
| Kit_fwd    | TAAAAGTTGCGCGTGGTGGA         |
| Kit_rev    | CAAGCAAGCCCGAGATTCTGA        |
| Gata2_fwd  | GCTTTCCACCCCTCCTTGGATT      |
| Gata2_rev  | GGCAGGATTTGACAGTCCGTA       |
Table S3. Clinical characteristics of patient samples used in this study. n.d., not detectable; n.t., not tested; BM, bone marrow; PB, peripheral blood.

| Patient No | Material | Diagnosis | Sex | Age (diagnosis) | Age (sampling) | WBC, g/L | Hb, g/dL | Plt, g/L | Blasts (BM), % | Blasts (PB), % |
|------------|----------|-----------|-----|----------------|---------------|----------|----------|----------|----------------|----------------|
| #1         | PB       | AML       | f   | 51             | 51            | 17.11    | 8.3      | 20       | 95             | 67             |
| #2         | PB       | AML       | f   | 61             | 61            | 2.18     | 9.2      | 40       | 24             | <1             |
| #3         | PB       | AML       | f   | 64             | 64            | 25.84    | 9.7      | 14       | 80             | 89             |
| #4         | PB       | AML       | m   | 28             | 28            | 57.25    | 11.1     | 19       | 90             | 20             |
| #5         | PB       | AML       | m   | 38             | 38            | 166      | 8.7      | 122      | 47             | 49             |
| #6         | PB       | AML       | m   | 53             | 53            | 106.75   | 7.7      | 30       | 35             | 71             |
| #7         | PB       | AML       | m   | 72             | 72            | 25.63    | 11.40    | 121      | 66             | 88             |
| #8         | BM       | AML       | m   | 71             | 71            | 46.73    | 10.80    | 116      | 72             | 90             |
| #9         | PB       | CML       | m   | 50             | 50            | 146.2    | 16.2     | 163      | 1              | 1              |
| #10        | PB       | CML       | m   | 34             | 34            | 97.55    | 14.8     | 281      | <1             | <1             |
| Patient No | Karyotyp | FLT3 ITD | FLT3 D835 | mDx HemaVision multiplex RT-PCR system | CEBPA sequence analysis | Insertion in exon 12 of NPM1 gene | BCR-ABL |
|------------|----------|----------|-----------|----------------------------------------|--------------------------|----------------------------------|---------|
| #1         | 46, XX   | n.d.     | n.d.      | no translocations or chromosomal aberration detectable | c.584-589 dupACCCGC      | no insertions detected            | n.t.    |
| #2         | 46, XX   | n.d.     | n.d.      | no translocations or chromosomal aberration detectable | nt.1066_1071del; N356_C357del | n.d.                             | n.t.    |
| #3         | 46,XX    | n.d.     | n.d.      | no translocations or chromosomal aberration detectable | c.180_183dupGTCC c.928_933delACGCAG | n.t.                             | n.t.    |
| #4         | 49, XY, +21 | n.d.     | n.t.      | MLL1-AF6, t(6;11)(q27;q23) | no mutation detected | n.d.                             | n.t.    |
| #5         | 46,XY, t(11;17)(q23;q12-21)/46,XY | n.d.     | n.t.      | no translocations or chromosomal aberration detectable | no mutation detected | n.d.                             | n.t.    |
| #6         | 46, XY   | n.d.     | n.t.      | no translocations or chromosomal aberration detectable | no mutation detected | CTTG (heterozygous manifestation) | n.t.    |
| #7         | 45,XY,-7 | n.d.     | n.t.      | no translocations or chromosomal aberration detectable | no mutation detected | n.d.                             | n.t.    |
| #8         | 47,XY,+mar | n.d.     | n.t.      | no translocations or chromosomal aberration detectable | no mutation detected | n.d.                             | n.t.    |
| #9         | 46, XY, t(9;22)(q34;q11) | n.t.     | n.t.      | n.t. | n.t. | n.t. | detectable |
| #10        | 46, XY, t(9;22)(q34;q11) | n.t.     | n.t.      | n.t. | n.t. | n.t. | detectable |
Figure S1. shRNA-mediated knock-down of C/EBPa p30 in $\text{Cebp}^{\text{p30/p30}}$ cells induces growth arrest. (A) Growth curves of $\text{Cebp}^{\text{p30/p30}}$ cells treated with indicated cytokines (IL-3: 10 ng/ml, IL-6: 10 ng/ml, SCF: 100 ng/ml) or no cytokines (none). (B) Histological staining (Rapid-Chrome Kwik-Diff Staining System) of cytospin preparations of $\text{Cebp}^{\text{p30/p30}}$ cells. (C) Flow cytometric analysis of Mac-1 and c-Kit levels in $\text{Cebp}^{\text{p30/p30}}$ cells. (D) Growth curves of
Cebpap30/p30 rtTA3 cells transduced with Doxycycline (Dox)-inducible shRNAs against Renilla luciferase (shRen, control) or C/EBPα (shCebpα) upon Dox administration. (E) Western blot analysis for C/EBPα p30 (C/EBPα) and HSC70 expression in lysates of Cebpap30/p30 cells expressing indicated shRNA constructs 48 h after Dox administration. (F) Genome-wide distribution of p30 and MLL1 ChIP-seq peaks relative to TSS in Cebpap30/p30 cells. (G) Western blot (WB) analysis of lysates and FLAG-purifications from extracts of HEK293 cells expressing indicated constructs using indicated antibodies. Mw, molecular weight.
Figure S2. Impairment of C/EBPα p30 function in Cebpa<sup>p30/p30</sup> cells induces growth arrest and myeloid differentiation. (A) Growth curve of Cebpa<sup>p30/p30</sup> cells (green) and the Cebpa<sup>p30/p30</sup> SpCas9 clone (yellow). (B) Assessment of surface marker levels of Mac-1, Gr-1 and c-Kit in Cebpa<sup>p30/p30</sup> cells (green) and the Cebpa<sup>p30/p30</sup> SpCas9 clone (yellow). (C) Top, schematic structure of the p30 protein. Positions of sgRNAs are indicated by dashed grey lines and by red arrows. BM, basic-motif; LZ, leucine zipper. Bottom, heatmap showing survival of GFP-positive (GFP<sup>+</sup>) sgRNA-expressing cells over time. Ctrl, negative control; Rpa3, positive control. (D)
Scatter plot representing percentages of GFP$^+$ cells expressing indicated sgRNAs (day 21). (E) Flow cytometric analysis of Mac-1 and Gr-1 surface expression after Cebpα targeting with indicated sgRNAs (gated on GFP$^+$ cells).
Figure S3. *Cebpap*<sup>30p30</sup> cells are sensitive to pharmacological targeting of the MLL1 complex by small-molecule inhibitors. (A) Growth curves of *Cebpap*<sup>30p30</sup> cells treated with indicated doses of MI-503 or DMSO. (B) Quantification of cell cycle analysis of *Cebpap*<sup>30p30</sup> cells at indicated time points after treatment with MI-503. (C) Quantification of flow cytometric analysis of apoptosis as measured by Annexin V staining of *Cebpap*<sup>30p30</sup> cells at indicated time points after treatment with MI-503. (D-E) Heatmap representation of the half maximal effective concentrations (EC<sub>50</sub>) of MI-463 and MI-503 in *Cebpap*<sup>30p30</sup> cells and mouse (D) or human (E) leukemia cell lines.
Figure S4. *Cebpap30/C-mut* cells and CEBPA-mutated AML primary leukemia samples are sensitive to perturbation of the MLL1 complex. (A) Growth curves of *Cebpap30/C-mut* cells treated with indicated doses of MI-463 or DMSO. (B) Quantification of flow cytometric analysis of...
apoptosis as measured by Annexin V staining of Cebpa^{30/C-mut.} cells at indicated time points after treatment with MI-463. (C) Colony-formation assay of primary mouse Cebpa^{p30/p30} and Cebpa^{p30/C-mut.} cells in presence of MI-503 (4 µM) or DMSO. Counts were normalized to the colonies in the DMSO samples for each genotype. (D) Morphology of colonies of Cebpa^{p30/p30} and Cebpa^{p30/C-mut.} cells upon treatment with 4 µM MI-503 (original magnification ×10). (E) Cell viability of primary human leukemia cells with the indicated mutational status after 5 days of exposure to 1.5 µM MI-503. Viability was normalized to DMSO controls for each patient sample. Numbers #1-10 indicate patient IDs (supplemental Table 1). Mean viability of CEBPA-mutant AML samples indicated by grey dashed line. (F) Heatmap representation of the half maximal effective concentration (EC_{50}) of MI-503 in primary human leukemia cells. C-term, C-terminal; dm, double-mutated; MLLr, MLL-rearranged.
Figure S5. Global gene expression profiling in Cebpap30/p30 cells upon treatment with MI-463 and MI-503. (A) Bar chart showing numbers of up- and down-regulated genes upon treatment with MI-463 or MI-503 in Cebpap30/p30 cells. P(adj) < .01. (B) Venn diagram showing the overlap of differentially expressed genes (fold change > 2; P(adj) < .01) between MI-463- and MI-503-treated Cebpap30/p30 cells. (C) Heatmap representation of differentially regulated genes (fold change > 2; P(adj) < .01) in Cebpap30/p30 cells after 3 h treatment with MI-463 (3.0 µM) or MI-503 (2.5 µM). (D) Gene Set Enrichment Analysis showing global down-regulation of genes associated with self-renewal in Cebpap30/p30 cells upon treatment with inhibitors. NES, Normalized Enrichment Score. (E) qRT-PCR analysis of Lyz2 in Cebpap30/p30 cells treated with MI-463 (3.0 µM) or MI-503 (2.5 µM) for 3 days. (F) Flow cytometric analysis (top) and quantification (bottom) of Mac-1 and Gr-1 surface markers in Cebpap30/p30 cells upon treatment with 2.0 µM MI-503 at indicated time points. (G) qRT-PCR analysis of Frzb in Cebpap30/p30 cells upon inhibitor treatment, CRISPR/Cas9-mediated mutagenesis of Mll1 or shRNA-mediated knockdown of Cebpap relative to control. MI-463: 3.0 µM; MI-503: 2.5 µM; shRen/shCebpap, shRNA directed against Renilla luciferase/Cebpap; sgRen/sgMll1, sgRNAs directed against Renilla luciferase/Mll1.
Figure S6. Identification of GATA2 as an effector of the p30-MLL1 axis. (A) Filtering scheme to identify effectors of p30-MLL1-coordinated gene control. (B) List of 13 effector candidates of the p30-MLL1 axis. (C) **Top**, schematic structure of the Sox4 protein. Positions of sgRNAs are indicated by dashed grey lines and by red arrows. HMG Box, high mobility group box. **Bottom**, heatmap showing survival of GFP-positive (GFP⁺) sgRNA-expressing cells over time. Ctrl, negative control; Rpa3, positive control. (D) Scatterplot representing the percentage of GFP⁺ cells expressing indicated sgRNAs at day 21. (E) Ratio of Gata2 expression upon overexpression of C/EBPα wild-type (wt) or p30 vs. mock-transduced FDCP-1 myeloid cells.²⁰ (F) Schematic structure of the GATA2 protein. Positions of sgRNAs are indicated by dashed grey lines and by red arrows.
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