Supplemental methods

Cell culture

MUTZ-3 cells were cultured in 60% MEMalpha (Thermo Fisher Scientific, Waltham, MA, USA), 20% fetal bovine serum (FBS) (Biochrom AG, Berlin, Germany), 20% conditioned medium of 5637 cells, and 10 ng/mL GM-CSF (BIOZOL GmbH, Eching, Germany). UCSD-AML1 cells were cultured in 60% RPMI 1640 (Thermo Fisher Scientific) including the same supplements as MUTZ-3. HNT-34 and MOLM-1 were cultured in 80% RPMI 1640 and 20% FBS. U-937 and K-562 were cultured in 90% RPMI 1640 and 10% FBS. All cultures were supplemented with 1x penicillin/streptomycin (Thermo Fisher Scientific). Metabolic activity was measured by CellTiterGlo assay (Promega, Madison, WI, USA). Cell identity was confirmed by a Multiplex human Cell line Authentication Test and tested for mycoplasma contamination regularly.

Lentiviral transduction

Lentiviral packaging vectors pMD2.G and psPAX2 were a gift from D. Trono (Addgene plasmid #12259 and #12260). Lentiviruses were produced according to standard procedures in Lenti-X 293T cells (Takara Bio, Kusatsu, Japan). For lentiviral transduction, 6-well plates coated with RetroNectin (Takara Bio, Kusatsu, Japan) were used. 500 µL of viral soup was added into a well of a 6-well plate with 2 mL medium and spun down for 45 min at 1 350 g and room temperature (RT). $1x10^6$ cells in 1 mL and 3 µL polybrene (Merck KGaA, Darmstadt, Germany) were added and spun down for 5 min at 314 g and RT. Cells were transduced twice on consecutive days. Puromycin (InvivoGen, San Diego, CA, USA) was added at a concentration of 1 µg/mL two days after the last transduction. Cells were selected for 3 days before samples were harvested.

Western blot

Cells were lysed in lysis buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM EDTA pH 8.0, 1% (v/v) Triton X-100, 1x complete protease inhibitor (Roche, Basel, Switzerland)) for 20 min

27 on ice. Debris was pelleted for 30 min at 16 000 g and 4°C, and the supernatant was
28 transferred into a fresh tube. SDS-Page and western blot were carried out according to
29 standard procedures. The following antibodies were used for protein detection: α-EVI1
30 (#2265; Cell Signaling, Beverly, MA, USA), α-CEBPA D56F10 (#8178; Cell Signaling), α-
31 RUNX1 (sc-365644 X; Santa Cruz Biotechnology, Dallas, TX, USA), α-IKZF1 D10E5
32 (#9034S; Cell Signaling, Beverly), α-PARP1 (#9542S; Cell Signaling), α-β-Actin (AM1829B;
33 Biomol, Hamburg, Germany), and α-GAPDH (sc-25778; Santa Cruz Biotechnologies).
34 Western blots were imaged on an Odyssey CLx Imaging System (LI-COR Biosciences,
35 Lincoln, NB, USA) using the Image Studio software (LI-COR Biosciences).

36 **Co-Immunoprecipitation**

37 Lysates were prepared as described previously in the western blot section. 75 µg of total
38 protein lysate were used as input control. Per IP, 3 mg of protein was used and diluted with
39 500 µL IP buffer (1% (v/v) Triton X-100, 0.5% (v/v) NP40, 50 mM Tris-HCl pH8.0, 5 mM
40 EDTA). Triton X-100 was added to a final concentration of 2% (v/v). 10 µL PARP1 antibody
41 (ab227244, Abcam, Cambridge, UK) or 2 µL control IgG (#2729S; Cell Signaling) were
42 added to the lysates and the samples were incubated overnight on a thermoshaker at 4°C
43 and 750 rpm. Per sample, 60 µL Dynabeads Protein G for immunoprecipitation (Thermo
44 Fisher Scientific) were washed with IP buffer and 60 µL beads were added to each IP.
45 Samples were rotated for 3 h at 4°C. Beads were placed on a magnet and washed seven
46 times with wash buffer (20 mM Tris-HCl pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5% (v/v) NP-
47 40) and two times with LiCl buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0, 250 mM LiCl,
48 0.1 % (w/v) Na-deoxycholate, 0.5 % (v/v) NP-40). After washing, the beads were
49 resuspended in 25 µL 1x SDS sample loading buffer with reducing agent and boiled for 10
50 min at 95°C. Beads were removed from the solution and the eluate was used for western blot
51 analysis.

52 ## DNA streptavidin pull-down

53 Biotinylated DNA probes were generated with PCR using *Taq* DNA polymerase (Qiagen,
54 Venlo, Netherlands) according to the manufacturer's protocol with the primers shown in
55 **Table S1**. The enhancer-containing pGL3 plasmid was used as template for the *G2DHE*
56 probe and MUTZ-3 genomic DNA for the chromosome Y control region. Nuclear lysate of
57 non-fixed MUTZ-3 cells was prepared as described in the ChIP-SICAP methods section
58 using 500 µL lysis buffer (LB) 1, 1 mL LB2, and 100 µL LB3 per 10^7 cells. NaCl was added to
59 a final concentration of 450 mM. The chromatin in the nuclear lysate was sheared
60 mechanically by pulling it at least six times through a 21G and six times through a 23G
61 syringe. Lysates were centrifuged for 30 min at 16 000 g and 4°C. The supernatant was
62 transferred into a new tube and the pellet discarded. 30 µg of the nuclear lysate was used as
63 input control. 1.2 mg of nuclear lysate was used per sample. Each sample was diluted 1:3
64 with Buffer C (20 mM HEPES-KOH pH 7.9, 1.5 mM $MgCl_2$, 0.2 mM EDTA, 0.5 mM DTT, 1x
65 complete Protease Inhibitor (Roche)) to reach a NaCl concentration of 150 mM. Sonicated
66 salmon sperm DNA was added to the lysate at a final concentration of 100 µg/mL to block
67 unspecific protein-DNA interactions. Samples were rotated for 20 min at 4°C and 25 rpm. 1.2
68 µg of biotinylated DNA probe was added per sample, and samples were rotated for 30 min at
69 4°C and 25 rpm. 60 µL streptavidin beads (S1420S; New England Biolabs, Frankfurt am
70 Main, Germany) were added to each sample and samples were rotated for 30 min at RT and
71 25 rpm. Supernatant was discarded and beads were transferred into a fresh tube with 400 µL
72 Buffer C with 150 mM NaCl. Beads were washed seven times with 400 µL Buffer C with 150
73 mM NaCl. 30 µL of the last wash step were kept as control. Beads were resuspended in 30
74 µL 1x NuPAGE LDS Sample Buffer with 1x NuPAGE Sample Reducing Agent (Thermo
75 Fisher Scientific, Waltham, MA, USA) and heated to 95°C for 10 min. Beads were discarded
76 and the supernatant was used for SDS-PAGE and western blot analysis.

77

78 # Next generation sequencing

79 ## ChIP-Seq

80 Cells were fixed with 1% (v/v) methanol-free formaldehyde (Thermo Fisher Scientific) for
81 10 min. The reaction was stopped by adding glycine to a final concentration of 125 mM and
82 incubation at RT for 5 min. Nuclear lysates were prepared as described in the ChIP-SICAP
83 section by using 250 µL LB1, 500 µL LB2, and 475 µL LB3 per $5x10^6$ cells. Samples were
84 split in sonication tubes with 150 µL volume per tube. Shearing of the chromatin was
85 performed by sonication in a Bioruptor Pico device (Diagenode, Seraing, Belgium). 220 µL of
86 RIPA buffer I (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0, 140 mM NaCl, 0.1% (w/v) Na-
87 deoxycholate) were added to each tube. Samples were spun for 10 min at 12 000 g at 4°C.
88 The supernatant was pooled. The pellet was resuspended in 370 µL of RIPA buffer I and
89 spun again. The supernatant was pooled with the previous supernatant. 50 µL of input were
90 taken aside. Dynabeads Protein G for Immunoprecipitation (Thermo Fisher Scientific) were
91 coupled to α-CEBPA D56F10 (#8178; Cell Signaling) antibody and rotated with the sheared
92 chromatin at 4°C overnight. The supernatant was discarded and the beads were transferred
93 into a PCR tube with 180 µL of RIPA buffer with 0.1% (w/v) SDS. The beads were washed
94 five times with RIPA buffer with 0.1% (w/v) SDS, twice with RIPA-500 (10 mM Tris-HCl pH
95 8.0, 1 mM EDTA pH 8.0, 500 mM NaCl, 1% Triton X-100, 0.1% SDS; 0.1% (w/v) Na-
96 deoxycholate), twice with LiCl wash buffer (10 mM Tris-HCl pH 8.0, 1 mM EDTA pH 8.0,
97 250 mM LiCl, 0.1% (w/v) Na-deoxycholate, 0.5% NP-40), and twice with 1x TE. The beads
98 were air-dried and eluted in 50 µL direct elution buffer (10 mM Tris-HCl pH 8.0; 10 mM EDTA
99 pH 8.0, 300 mM NaCl, 0.5% (w/v) SDS). Eluates and the input were digested with RNase A
100 (10 mg/ml, Thermo Fisher Scientific), proteinase K (20 mg/mL, Thermo Fisher Scientific), and
101 decrosslinked. Samples were purified with AMPure XP beads (Beckman Coulter, Brea, CA,
102 USA). Library preparation was performed using the NEBNext system for Illumina (New
103 England Biolabs, Frankfurt am Main, Germany) according to the manufacturer's protocol.
104 Samples were sequenced on a HiSeq 2000 v4 platform (Illumina, San Diego, CA, USA) with

105 50 bp single reads. Reads were aligned to human genome version hg19, and the input was
106 subtracted as background using an in-house pipeline. Integrative Genome Viewer version
107 2.6.3 (Broad Institute, Cambridge, MA, USA) was used for the visualization. RUNX-1 ChIP-
108 Seq data from *de novo* AML cell patients were published previously and online available
109 (GEO Accession number GSM3734708).[1]

110 **RNA-Seq**

111 Cells were treated with PARP inhibitors or DMSO and harvested at various time points or
112 they were lentivirally transduced with *EVI1* shRNAs and harvested after selection. RNA was
113 extracted using the RNeasy Plus Mini Kit (Qiagen, Venlo, Netherlands), and extracts with
114 RIN values >8 were sent for sequencing. Library preparation was performed according to
115 standard procedures by the DKFZ Genomics and Proteomics Core Facility. Samples were
116 sequenced on an Illumina HiSeq 4000 with paired-end 100 bp reads. Bioinformatic analysis
117 was performed as described below. Published RNA-Seq data from AML samples and cell
118 lines were reanalyzed as described previously.[2,3]

119 **Processing of PARPi and *EVI1* knockdown RNA-Seq data**

120 Reads were aligned to the genome in a 2-pass alignment procedure using STAR aligner (v.
121 2.7.3a). First, the genome index for the Genome Reference Consortium Human Build version
122 38 was built with STAR, then reads were aligned, a new index was created using splice
123 junction information from the first pass, and finally, reads were re-aligned to yield the final
124 alignment files in sorted BAM format. Duplicate reads were marked using PICARD
125 MarkDuplicates (v. 2.21.2), and BAM files from multiple sequencing lanes that belong to the
126 same samples were merged using samtools merge (v. 1.9). Gene-level read counting was
127 performed using the Rsubread package (Bioconductor version 3.10). TPM values were then
128 calculated by first normalizing the raw counts per gene length and then scaling them per
129 million.

130 **Determination of differential genes**

131 Top most variable 1 500 genes in the anti-PARP treatment were selected, and clustered into
132 4 profile clusters using k-means. Differential genes were determined using DESeq2, using
133 the following design: (1) for the anti-PARP treatment experiment, time and treatment type
134 were used as variables, including an interaction term. We selected genes for which the
135 interaction term was significant (2) for the shEVI1 experiments, we considered the time-
136 points (day 3 and day 6) as replicates, and determined the differential genes between shRNA
137 treatment and control.

138 **4C-Seq**

139 4C experiments were conducted as described previously.[2,4] DpnII was used as first cutter
140 and Csp6I as second cutter. The primers shown in **Table S5** were used for library generation.
141 Sequencing was performed on a HiSeq 2000 v4 platform (Illumina) with 50 bp single reads.
142 4C-Seq fastq files were demultiplexed using the viewpoint specific barcodes. Reads were
143 trimmed using TrimGalore, and aligned using BWA on the hg19 genome. Aligned reads were
144 processed using a custom made pipeline implementing methods inspired by the FourCSeq
145 package in order to produce smoothed bigwig files obtained by smoothing the signal over a
146 number of neighbouring fragments (typically 21).[5] Integrative Genome Viewer version 2.6.3
147 (Broad Institute) was used for the visualization.

148 ChIP-SICAP

149 Cells were fixed with 1.5% (v/v) methanol-free formaldehyde for 15 min, and the reaction was
150 stopped with 125 mM glycine. Instead of permeabilizing the fixed cells with Triton X-100 in
151 TE buffer, a two-step lysis protocol was used. For this, the outer cell membrane was lysed for
152 10 min in 1 mL of LB1 (50 mM HEPES-KOH pH 7.5, 140 mM NaCl, 1 mM EDTA, 10% (v/v)
153 glycerol, 0.5% (v/v) NP40, 0.25% (v/v) Triton X-100, 1x complete protease inhibitor; Roche)
154 per $25x10^6$ cells and then centrifuged for 2 min at 1 000 g and 4°C. The nuclear pellet was
155 incubated with 2 mL LB2 (10 mM Tris-HCl pH 8.0, 200 mM NaCl, 1 mM EDTA, 0.5 mM
156 EGTA, 1x complete protease inhibitor) for 5 min, centrifuged again and the pellet was

157 resuspended in 600 µL LB3 (10 mM Tris-HCl pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 mM
158 EGTA, 0.1% (w/v) Na-deoxycholate, 0.5% (w/v) N-lauroylsarcosine, 1x complete protease
159 inhibitor) to extract the nuclear fraction. Sonication was performed in tubes with 300 µL
160 volume on a Bioruptor Pico (Diagenode, Seraing, Belgium) device with 30 s ON/ 30 s OFF
161 intervals for 20-30 cycles. Triton X-100 was added to a final concentration of 1% (v/v), and 1-
162 5% of total sample volume was taken as input control. The respective amounts of antibodies
163 were added to each sample: 14 µL of α-CEBPA D56F10 (#8178; Cell Signaling, Beverly, MA,
164 USA), 5 µg of α-RUNX1 (ab23980; Abcam, Cambridge, UK), and 5 µg of control IgG
165 (#2729S; Cell Signaling). Samples were vortexed and incubated overnight at 750 rpm and
166 4°C in a thermomixer. 500 µL IP buffer (50 mM Tris-HCl pH 7.5-8, 5 mM EDTA, 1% (v/v)
167 Triton X-100, 0.5% (v/v) NP40) and 60 µL magnetic Dynabeads Protein G for
168 immunoprecipitation (Thermo Fisher Scientific) were added per sample, and the sample was
169 head-to-tail rotated for 3 h at 4°C. Beads were washed and sheared chromatin was
170 biotinylated, followed by an additional wash step as published previously.[6] Elution of the
171 complexes from the protein G beads was done for 30 min instead of 15 min with 7.5% (w/v)
172 SDS and 200 mM 1,4-dithiothreitol (DTT). The supernatant was diluted with 1 mL IP buffer
173 with 150 mM NaCl. LysC-resistant magnetic streptavidin beads (S1420S; New England
174 Biolabs, Frankfurt am Main, Germany) were generated as described previously and 100 µL
175 beads were added to the supernatant.[7] DNA-bound protein complexes were purified as
176 described before, followed by one washing step with isopropanol and one with acetonitrile
177 wash buffer, respectively.[6] Digestion of the eluted proteins was performed using 300 ng of
178 LysC (12505061; Wako Chemicals, Neuss, Germany) for 16 h at 37°C. Digested peptides
179 and the DNA fraction were cleaned up according to the SP3 protocol as described
180 previously.[6]

181 Mass spectrometry

182 Cleaned up peptides were loaded on a trap column (PepMap100 C18 Nano-Trap 100 µm x
183 2 cm) and separated over a 25 cm analytical column (Waters nanoEase BEH, 75 µm x

184 250 mm, C18, 1.7 µm, 130 Å,) using the Thermo Easy nLC 1200 nanospray source (Thermo
185 EasynLC 1200; Thermo Fisher Scientific). Solvent A was water with 0.1% formic acid and
186 solvent B was 80% acetonitrile, 0.1% formic acid. During the elution step, the percentage of
187 solvent B increased in a linear fashion from 3% to 8% in 4 min, then increased to 10% in 2
188 min, to 32% in 68 min, to 50% in 12 min, and finally to 100% in a further 1 min and went
189 down to 3% for the last 11 min. Peptides were analyzed on a Tri-Hybrid Orbitrap Fusion
190 mass spectrometer (Thermo Fisher Scientific) operated in positive (+2 kV) data dependent
191 acquisition mode with HCD fragmentation. The MS1 and MS2 scans were acquired in the
192 Orbitrap and ion trap, respectively with a total cycle time of 3 s. MS1 detection occurred at
193 120 000 resolution, AGC target 1E6, maximal injection time 50 ms and a scan range of 375-1
194 500 m/z. Peptides with charge states 2 to 4 were selected for fragmentation with an
195 exclusion duration of 40 s. MS2 occurred with CE 33%, detection in topN mode, and scan
196 rate was set to Rapid. AGC target was 1E4 and maximal injection time allowed of 50 ms.
197 Data were recorded in centroid mode.

198 Bioinformatic analysis of ChIP-SICAP data

199 Raw files were analyzed using MaxQuant version 1.5.3.30 (Max-Planck-Institute of
200 Biochemistry, Martinsried, Germany) including the Andromeda search engine.[8,9] Peptides
201 were identified using the Uniprot *Homo sapiens* database concatenated to a database
202 containing protein sequences of contaminants. Default MaxQuant parameters were used
203 with the following modifications: digestion with LysC, default variable modification
204 (methionine oxidation and N-terminal acetylation), cytosine carbamidomethylation as fixed
205 modification, and minimum peptide length 5. FDR was set to 1% at both protein and peptide
206 level. Match between runs option was enabled, Label Free Quantification (LFQ) and iBAQ
207 calculated. Further bioinformatics analyses were performed using an in-house pipeline as
208 follows: known contaminants were removed and fold change of the bait sample intensities
209 over the IgG control intensities was computed. The maximum ratio was limited to 20 and the
210 minimum ratio to 0.5. P-values were calculated with the Limma package and adjusted to

211 Benjamini-Hochberg (adjusted p-value).[10] Identified proteins with an adj. p-value≤0.10 were
212 considered relevant. Pathway enrichment analysis was performed using DAVID 6.8
213 (https://david.ncifcrf.gov/home.jsp).[11,12]

214 Quantitative PCR

215 The DNA fractions of ChIP-SICAP experiments were purified with AMPure XP beads
216 (Beckman Coulter). ChIP-SICAP qPCR was performed using the TB Green Premix
217 DimerEraser master mix (Takara Bio, Kusatsu, Japan) according to the manufacturer's
218 protocol with the primers shown in **Table S1**. Samples were analyzed with a Light Cycler 480
219 system (Roche).

220 For RT-qPCR, mRNA was extracted using the RNeasy Plus Kit (Qiagen, Venlo, Netherlands)
221 and transcribed into cDNA using the SuperScript III First-Strand Synthesis System (Thermo
222 Fisher Scientific) according to the manufacturer's protocols. iTaq Universal SYBR Green
223 Supermix (Bio-Rad Laboratories, Hercules, CA, USA) was used for qPCR according to the
224 manufacturer's protocol with the primers shown in **Table S1**. Samples were run on a CFX96
225 system (Bio-Rad Laboratories) and analyzed using the CFX Maestro Software (Bio-Rad
226 Laboratories).

227 May-Grünwald-Giemsa staining

228 $8x10^4$ cells were resuspended in 150 µL PBS and fixed on a slide using a Shandon CytoSpin
229 3 device (Thermo Fisher Scientifc) for 5 min at 500 rpm. Slides were stained with May-
230 Grünwald (Sigma-Aldrich, St. Louis, MO, USA) for 3 min, washed three times for 1 min with
231 water, stained with Giemsa (Sigma-Aldrich, St. Louis, MO, USA) for 10 s and washed four
232 times for 1 min with water. The slides were mounted with a drop of Entellan (Merck Millipore,
233 Burlington, MA, USA) and dried for 24 h. Samples were imaged on a Cell Observer (Zeiss,
234 Oberkochen, Germany) with 40x oil objective using the software ZEN blue (Zeiss).

235 ## Flow cytometry

236 Apoptosis staining was carried out according to the FITC Annexin V Apoptosis Detection Kit I
237 (BD Bioscience, San Jose, CA, USA) manufacturer's protocol with the following
238 modifications: 2.5 µL Annexin-V antibody were used, 7-aminoactinomycin (7-AAD) was used
239 instead of propidium iodide.

240 For staining with differentiation markers, $1x10^6$ cells were resuspended in 50 µL FACS Buffer
241 (PBS with 2% FBS). Antibodies against cell surface markers were added (CD34-PerCPCy5.5,
242 #343611; Biolegend, San Diego, CA, USA), CD11b-APC (#301309; BioLegend), CD14-
243 APCH7 (MϕP9; BD Biosciences), and the samples were incubated for 15 min at 4°C in the
244 dark. 150 µL of 1 µg/mL DAPI (4',6-diamidino-2-phenylindole) solution (BSBTAR1176; VWR,
245 Darmstadt, Germany) was added to 50 µL of cells, and the samples were incubated for 5 min
246 at 4°C in the dark. Cells were washed with 3 mL PBS with 5% FBS and resuspended in
247 100 µL fixation medium (FIX & PERM Reagent A; Thermo Fisher Scientific). The samples
248 were incubated for 15 min at RT in the dark and washed once with 3 mL PBS with 5% FBS.
249 The cells were resuspended in 100 µL permeabilization medium (FIX & PERM Reagent B,
250 Thermo Fisher Scientific), and the intracellular antibody (cMPO-FITC, sc-51741 FITC; Santa
251 Cruz Biotechnology, Dallas, TX, USA or γH2AX-Alexa488, 560445, BD Biosciences) was
252 added. The samples were incubated for 20 min at RT in the dark and washed with 3 mL PBS
253 with 5% FBS. Cells were resuspended in 300 µL FACS buffer. All samples were acquired on
254 a FACSCelesta device (BD Biosciences) using FACSDiva software (BD Biosciences).

255 ## Statistical analysis and data presentation

256 Unless otherwise stated, statistical significance was calculated using two-sided two-sample t-
257 tests or two-sided one-sample t-tests. Values were assumed to be normally distributed.
258 Exact replicate numbers for the DLA assays are shown in **Table S6**.

259 Plots were generated using the ggplot2 R package version 3.3.2.[13] Boxplots show the
260 median. The lower and upper hinges of the boxplots correspond to the first and third quartiles

261 and the whiskers extend to the smallest and largest value as long as their distance from the
262 hinge is smaller than 1.5× inter-quartile range.

263 Sequence logos were generated using seqLogo R package version 1.56.0.[14] FACS plots
264 were generated with the FACSDiva or FlowJo software. The Integrative Genomics Viewer
265 (IGV) was used for visualization of ChIP-Seq and 4C-Seq tracks. Affinity Designer was used
266 for illustrations.

267 Supplemental tables

268 **Table S1.** List of oligonucleotides and primers with name, sequences, and applied method.
269 PD = pull-down

Name	Sequence	Method
EnhCore-for	tttGGATCCACTCCAAGCACCTGCCAAGGC	cloning pGL3
EnhCore-rev	tttGTCGACCTCCAGGTGTCCAGAGCCCG	cloning pGL3
EnhRight-for	tttGGATCCGAAGGCCTGGGGATGGTGAGG	cloning pGL3
EnhLeft-rev	tttGTCGACCCTCACCATCCCCAGGCCTTC	cloning pGL3
5´mirE-XhoI	TACAATACTCGAGAAGGTATATTGCTGTTGACAGTGAGCG	miR-E cloning
3´mirE-EcoRI	TTAGATGAATTCTAGCCCCTTGAAGTCC GAGGCAGTAGGCA	miR-E cloning
SGEP-SEQ	TGTTTGAATGAGGCTTCAGTAC	sequencing
pGL3-p300-for	GATGAGTTTGGACAAACCAC	sequencing
pGL3-p300-rev	GAGCTGACTGGGTTGAAG	sequencing
qPCR-Enh-for	CTCCCTGCATCCGTTACTTG	ChIP-qPCR
qPCR-Enh-rev	CAGCCTGCTTTACCACATCA	ChIP-qPCR
ChIP-CTRL1-for	TACTAACTTCGTGGTGGCGT	ChIP-qPCR
ChIP-CTRL1-rev	TGGAGTGAAAGGCCCTGAAA	ChIP-qPCR
ChIP-CTRL2-for	TAGGTAGCGTGCTGTTTGGG	ChIP-qPCR
ChIP-CTRL2-rev	TGCTCTCAATGCAACCCTGA	ChIP-qPCR
qPCR-EVI1-for	AGTGCCCTGGAGATGAGTTG	RT-qPCR
qPCR-EVI1-rev	CTGCTTAAGTTCCTCTGGCAC	RT-qPCR
qPCR-PBGD-for	GGCAATGCGGCTGCAA	RT-qPCR
qPCR-PBGD-rev	GGGTACCCACGCGAATCAC	RT-qPCR
PD-Enh-for	CCACAGGCAGTGGACGG	PD PCR
PD-Enh-rev	[Biotin]-ATCCCCAGGCCTTCACATC	PD PCR
PD-ChrY-For	TGTAGCCACTTCAAGGACTCT	PD PCR
PD-ChrY-Rev	[Biotin]-ACGAGATGTGTCCACCTACT	PD PCR

270

Table S2. List of mutagenesis primers with mutant number (Mut) and sequences. Mutations are marked in lowercase letters.

Mut	Sequence
1-for	GGGGACATGCCACCCCgatgGGCAGTGGACGGGGCC
1-rev	GGCCCCGTCCACTGCCcatcGGGGTGGCATGTCCCC
2-for	CCCACAGGCAGTGcAtGGGGCCTCCCTGC
2-rev	GCAGGGAGGCCCCaTgCACTGCCTGTGGG
3-for	CCAGACTCTGCCCACCGCACAGgAGctccAGATAGTTTGTGAAATACCCTG
3-rev	CAGGGTATTTCACAAACTATCTggagCTcCTGTGCGGTGGGCAGAGTCTGG
4-for	CGCACAGCAGTGATAGATAGTaacTGAcATACCCTGAGCTTGCGGTTGA
4-rev	TCAACCGCAAGCTCAGGGTATgTCAgttACTATCTATCACTGCTGTGCG
5-for	GATAGATAGTTTGTGAAATACCCTGAGCacGCttTTGACCAGAATTCTGAAAT AATTCCATT
5-rev	AATGGAATTATTTCAGAATTCTGGTCAAaaGCgtGCTCAGGGTATTTCACAAA CTATCTATC
6-for	CCTGAGCTTGCGGTTGACCAGAAccCTGAgATAATTCCATTGAGACCGGAATTC
6-rev	GAATTCCGGTCTCAATGGAATTATgTCAGggTTCTGGTCAACCGCAAGCTCAGG
7-for	CCATTGAGACgtacATTCATGCAATGTTCTGTATCCTGATGTGGTAAAGCAGGCTGCCC
7-rev	GCATGAATgtacGTCTCAATGGAATTATTTCAGAATTCTGGTCAACCGCAAGCTCAGGGT
8-for	CCGGAATTCATGCAATGTTCTGTATtgcGcaGTaGTAAAGCAGGCTGCCCGCGGGGG
8-rev	CCCCCGCGGGCAGCCTGCTTTACtACtgCgcaATACAGAACATTGCATGAATTCCGG
9-for	GGGGCTTCTGCCACCCtttCTCGCatACGGGCTCCGGAAGGC
9-rev	GCCTTCCGGAGCCCGTatGCGAGaaaGGGTGGCAGAAGCCCC
10-for	CGCCCACGGGCTCatGAAGGCCCATCTG
10-rev	CAGATGGGCCTTCatGAGCCCGTGGGCG
11-for	CGGGCTCCGGAAGGCCCATCTagtAGGAGCAGGGC
11-rev	GCCCTGCTCCTactAGATGGGCCTTCCGGAGCCCG
12-for	TCTGACAGGAGCAGGGCCCTtggccGCACCACCTGACCAGGAG
12-rev	CTCCTGGTCAGGTGGTGCggccaAGGGCCCTGCTCCTGTCAGA
13-for	GGAGCAGGGCCCTGATAAGggtatgCTGACCAGGAGGCCCCCGG
13-rev	CCGGGGGCCTCCTGGTCAGcataccCTTATCAGGGCCCTGCTCC
14-for	CCCCCGGCAGAGAGATTAATGCACAAATTacgcACAACCTCCCCATGC
14-rev	GCATGGGGAGGTTGTgcgtAATTTGTGCATTAATCTCTCTGCCGGGGG
15-for	CCGCAGAGGAGCAGGaCaaGGGGGCACTGGGAAA
15-rev	TTTCCCAGTGCCCCCttGtCCTGCTCCTCTGCGG
16-for	GCACTGGGAAAGGAAGtttAGAGGAGGCCTCACCC
16-rev	GGGTGAGGCCTCCTCTaaaCTTCCTTTCCCAGTGC
17-for	GGCCTGGCCTCCCGaccCCTCTCCTAAGCCC
17-rev	GGGCTTAGGAGAGGggtCGGGAGGCCAGGCC
18-for	GCAGGGGACACGCCATgcaTCTGCAGAGGCAGG
18-rev	CCTGCCTCTGCAGAtgcATGGCGTGTCCCCTGC
19-for	GCCACCCCCACAGGtgaTGGACGGGGCCTCC
19-rev	GGAGGCCCCGTCCAtcaCCTGTGGGGGTGGC
20-for	GCCCACCGCACAGCAGTGATAGATccaTTGTGAAATACCCTGA
20-rev	TCAGGGTATTTCACAAtggATCTATCACTGCTGTGCGGTGGGC

21-for	TTGTGAAATACCCTGAGCTTGCGccgGACCAGAATTCTGAAATAATTCC
21-rev	GGAATTATTTCAGAATTCTGGTCcggCGCAAGCTCAGGGTATTTCACAA

273

274 **Table S3.** List of 97-mer oligonucleotides used for miR-E cloning. Sequences were extracted
275 from the Genome-wide Sensor-Based shRNA Prediction by Fellmann *et al.* (see ID).[15] The
276 targeted protein and the sequence are given. SCR = Scrambled control.

shRNA ID	Target	Sequence
CEBPA.2538	CEBPA	TGCTGTTGACAGTGAGCGATCACTCTAGATGTTTTATGTATAGTGAAGC CACAGATGTATACATAAAACATCTAGAGTGAGTGCCTACTGCCTCGGA
CEBPA.1471	CEBPA	TGCTGTTGACAGTGAGCGATGCCTTGGAAATGCAAACTCATAGTGAAGCC ACAGATGTATGAGTTTGCATTTCCAAGGCACTGCCTACTGCCTCGGA
RUNX1.524	RUNX1	TGCTGTTGACAGTGAGCGCTCGGCAGAAACTAGATGATCATAGTGAAGC CACAGATGTATGATCATCTAGTTTCTGCCGATTGCCTACTGCCTCGGA
RUNX1.468	RUNX1	TGCTGTTGACAGTGAGCGAACCTACCACAGAGCCATCAAATAGTGAAGC CACAGATGTATTTGATGGCTCTGTGGTAGGTGTGCCTACTGCCTCGGA
/	SCR	TGCTGTTGACAGTGAGCGCCACTTCAGATCGTAAGGAGAATAGTGAAGC CACAGATGTATTCTCCTTACGATCTGAAGTGATGCCTACTGCCTCGGA
Ren.713	*Renilla* (REN)	Already present in SGEP empty vector

277

278 **Table S4.** Known CEBPA and RUNX1 interactors identified by ChIP-SICAP with references
279 of previous reports.

Protein	Interactor	References
CEBPA	CEBPB	Cao *et al.* (1991)[16], Lin *et al.* (2005)[17], Tomaru *et al.* (2009)[18]
	CEBPG	Reinke *et al.* (2013)[19]
	EP300	Rodríguez-Ubreva *et al.* (2012)[20]
	PARP1	Yin *et al.* (2006)[21]
	SMARCD2	Grebien *et al.* (2015)[22]
RUNX1	ARID1A	Huber *et al.* (2017)[23]
	CBFB	Kitabayashi *et al.* (2001)[24], Shia *et al.* (2012)[25], Nguygen *et al.* (2005)[26], Philipot *et al.* (2010)[27], Imai *et al.* (2004)[28], Yamaguchi *et al.* (2004)[29], Kim *et al.* (2013)[30], Leong *et al.* (2016)[31], Warren *et al.* (2000)[32], Yonezawa *et al.* (2017)[33], Luck *et al.* (2020)[34], Yu *et al.* (2012)[35]
	DPF2	Huber *et al.* (2017)[23]
	EP300	Yamaguchi *et al.* (2004)[29], Aikawa *et al.* (2006)[36]
	FUBP1	Huber *et al.* (2017)[23]
	HNRNPR	Huber *et al.* (2017)[23]
	HNRNPU	Huber *et al.* (2017)[23]
	IKZF1	Zhou *et al.* (2019)[37]
	KHSRP	Huber *et al.* (2017)[23]
	NONO	Huber *et al.* (2017)[23]
	RBBP4	Yu *et al.* (2012)[35]
	RPA1	Huber *et al.* (2017)[23]
	SET	Yu *et al.* (2012)[35]
	SMARCC1	Huber *et al.* (2017)[23], Bakshi *et al.* (2010)[38], Yu *et al.* (2012)[35]
	SMARCE1	Yu *et al.* (2012)[35]
	TAL1	Hu *et al.* (2009)[39], Yu *et al.* (2012)[35]
	TKT	Huber *et al.* (2017)[23]

280

281

282

283 **Table S5.** List of primers used for 4C library preparation. The same reverse primer was used
284 for all samples.

Name	Sequence	sample
EVI1 prom_R1	CAAGCAGAAGACGGCATACGAATGCAATGAATTCTCCATTC	all
EVI1 prom_GC_F6	AATGATACGGCGACCACCGAACACTCTTTCCCTACACG ACGCTCTTCCGATCTgcATTATTTAGAAGACCTGATC	DMSO
EVI1 prom_CG_F4	AATGATACGGCGACCACCGAACACTCTTTCCCTACACG ACGCTCTTCCGATCTcgATTATTTAGAAGACCTGATC	tala
EVI1 prom_GT_F3	AATGATACGGCGACCACCGAACACTCTTTCCCTACACG ACGCTCTTCCGATCTgtATTATTTAGAAGACCTGATC	ola

285

286 **Table S6.** List of exact number of biological replicates (n) for each DLA condition.

Experiment	Figure	Condition	n
DLA *G2DHE* fragments	1b	all	4
DLA *G2DHE* mutants	1c	pGL3, Prom, wildtype *G2DHE*	13
		Mut 1, 5	5
		Mut 2, 7, 9, 10, 11, 12, 15, 17, 19, 20, 21	3
		Mut 3, 8	4
		Mut 4, 6	8
		Mut 13	9
		Mut 14, 16, 18	6
	S1b	HNT-34:	
		pGL3, Prom, wildtype *G2DHE*	13
		Mut 1, 5	5
		Mut 4, 6	8
		Mut 1+5, 4+6	3
		Mut 8	4
		MUTZ-3:	
		All conditions	5
		MOLM-1:	
		pGL3, Prom, wildtype G2DHE, Mut 1, 4, 5, 6, 8	6
		Mut 1+5, 4+6	5

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409

410 ## Supplemental figures

411 **Fig. S1. *G2DHE* mutants used for dual luciferase assay. a** *In silico* analysis of the *G2DHE*
412 element revealed several putative TF binding sites (TFBS) as identified with the
413 JASPAR2016 and the Alggen Promo (TRANSFAC) tool. The names of the TFs are indicated
414 in bold and the respective database identifier is written in parentheses. Position weight
415 matrices of the human proteins were used for the analysis except for IKZF1 where the
416 murine (m) matrix was used for prediction. Sequence logos are shown for those sites
417 identified with the JASPAR2016 database. The wildtype (WT) and mutant (M) enhancer
418 sequences are shown for each predicted site. For the JASPAR2016 analysis a relative
419 similarity score ≥ 0.80 was considered relevant and for the TRANSFAC database a relative
420 dissimilarity score of < 15% was used. **b** Luciferase reporter assay in three different leukemic
421 cell lines with 3q-rearrangements for chosen mutations (Mut) and their respective TFBS
422 (n≥3). TFBS in parentheses are in close proximity to the mutations without being affected by
423 them. Cells were co-transfected with the luciferase reporter plasmid pGL3 and the *Renilla*
424 control plasmid. pGL3 empty vector (pGL3), pGL3 vector containing the *EVI1* promoter
425 (Prom), and pGL3 vector containing the *EVI1* promoter and the wildtype *G2DHE* were used
426 as controls (CTRL). Luciferase signal was normalized to *Renilla* signal. The relative
427 luciferase signal was further normalized to the signal of the wildtype *G2DHE* (red line).
428 Statistical significance was calculated with two-sided one-sample t-tests.

429

430 **Fig. S2. CEBPA and RUNX1 expression, association with *G2DHE*, and ChIP-SICAP**
431 **results. a** Western blot to show the expression levels of CEBPA and RUNX1 in different 3q-
432 rearranged and non-3q-rearranged cell lines. **b** Quantification of CEBPA and RUNX1 protein
433 expression of the cell lines shown in a. **c** ChIP-Seq binding profiles of CEBPA and RUNX1 in
434 leukemic cell lines. Light blue track: CEBPA ChIP-Seq peaks in 3q-rearranged MOLM-1.
435 Dark blue track: RUNX1 ChIP-Seq peaks of *de novo* AML cell patient samples (dataset
436 GSM3734708 by Gerritsen *et al.*).[1] Numbers show the data range. Dark grey: Conservation

437 (SiPhy rate 10 mer).[40] The core enhancer sequence, the qPCR product used for detection in
438 ChIP-SICAP-qPCR, and the pull-down (PD) probe are annotated in black, the predicted
439 CEBPA TFBS in light blue, and the predicted RUNX1 TFBS in dark blue. **d-f** ChIP-SICAP
440 was performed in three different 3q-rearranged cell lines using antibodies against CEBPA
441 and RUNX1, or an unspecific IgG control. Experiments were carried out in duplicates (n=2).
442 **d** The DNA fraction of the ChIP-SICAP experiment was used for enrichment quantification of
443 the *G2DHE* region by qPCR compared to two unrelated control regions (CTRL). **e-f** The
444 protein fraction of the ChIP-SICAP experiments was analyzed by mass spectrometry to
445 identify chromatin-bound interactors of RUNX1 and CEBPA. **e** The fold change of the
446 intensity of CEBPA or RUNX1 samples over the IgG control was calculated for both
447 replicates (R1 and R2). A cut-off of 20 was used for all proteins with a fold change >20. The
448 graph shows the correlation between replicate 1 and 2. Marked in red is the threshold of fold
449 change = 2. **f** Proteins enriched in the CEBPA- or RUNX1-captured samples over the IgG
450 controls were ranked according to their iBAQ intensity.

451

452 **Fig. S3. GOTERM and BioGRID analysis of proteins identified with ChIP-SICAP.**
453 Proteins identified in at least two cell lines per bait protein were fed into the DAVID online
454 tool and analyzed according to their cellular component (CC), biological pathway (BP), and
455 molecular function (MF) according to GOTERM. Known human and murine interactors of
456 CEBPA and RUNX1 were extracted from BioGRID and annotated accordingly. **a** Analysis of
457 CEBPA interactors. **b** Analysis of RUNX1 interactors. **c** Potential true positive (PTP) and
458 potential false positive (PFP) hits. Relative protein intensities (iBAQ) are shown for the
459 average of RUNX1 and CEBPA ChIP-SICAP assays. Proteins were categorized into PTP if
460 they are interactors of CEBPA and RUNX1, chromatin or DNA-binders or if they have nuclear
461 localization. Proteins were categorized as PFP if they are ribosomal proteins or cytoplasmic
462 proteins. Please note that the categories are exclusive meaning that if a protein falls into a
463 category, it is not considered in the next categories.

464 **Fig. S4. Western blot quantification of MUTZ-3 treated with PARPi or *PARP1* shRNAs**
465 **shows reduction in EVI1 protein levels. a** MUTZ-3 cells were treated with 10 μM olaparib
466 (ola), 1 μM talazoparib (tala), or DMSO or were left untreated for 24 h (n=3). Proteins were
467 extracted from cells treated with PARPi and EVI1 was measured by western blot. β-Actin was
468 used as loading control. Protein bands were quantified and normalized to the loading control
469 and DMSO. Statistical significance was calculated using two-sided two-sample t-tests. **b**
470 MUTZ-3 cells were lentivirally transduced with constructs encoding shRNAs against *PARP1*
471 or *Renilla* (REN) and selected with puromycin. Samples were harvested on day 3 of
472 puromycin selection, and protein levels were analyzed by western blot using antibodies
473 against PARP1 and EVI1. β-Actin served as a loading control. A miR-E construct targeting
474 Renilla (REN) and untreated parental cells served as non-targeting controls.

475

476 **Fig. S5. PARPi sensitivity in different AML cell lines.** Cells were treated with the indicated
477 amounts of olaparib or talazoparib (n=3). Metabolic activity was measured as an indicator of
478 cell viability by CellTiter-Glo assay. The values were normalized to those of the 0 h time point
479 and to the DMSO control of each time point.

480

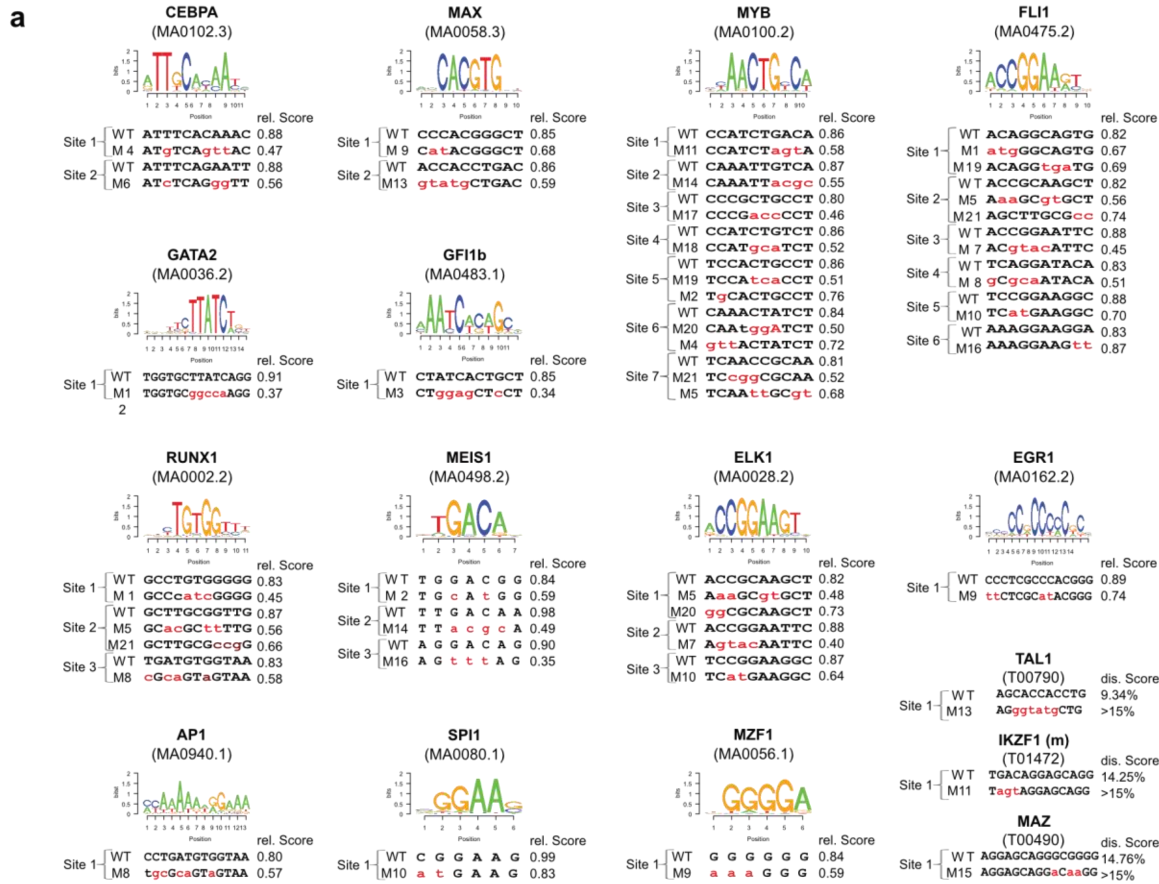
481 **Fig. S6. RNA-Seq following PARPi in inv(3) AML cells.** MUTZ-3 cells were treated with
482 10 μM olaparib, 1 μM talazoparib, or DMSO, and RNA was harvested for sequencing after 12
483 h, 24 h, and 48 h. **a** Heatmap of clusters identified. The top most variable 1 500 genes in the
484 samples treated with PARPi were selected, and clustered into 4 profile clusters using k-
485 means. Experiment, time, and treatment type were used as variables. **b-e** Pathway
486 enrichment analysis of the clusters identified in **a**. Shown is the number of genes of the
487 cluster assigned to the pathway (gene count), the proportion of genes from this cluster that
488 were assigned to the pathway (GeneRatio), and the adjusted p-value.

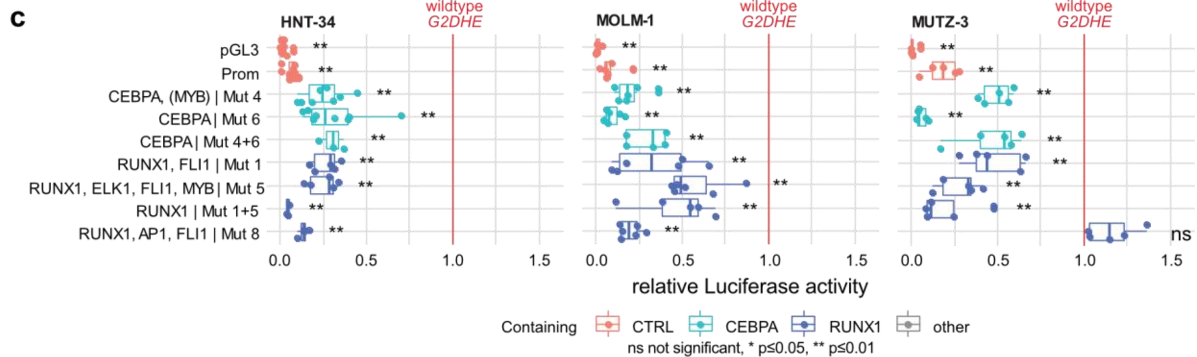
489

490 **Figure S7. Genes deregulated by PARPi and *EVI1* knockdown as identified by RNA-**
491 **Seq. a** and **c** Commonly downregulated (a) and upregulated (c) genes by PARPi and *EVI1*
492 knockdown as identified by RNA-Seq. Genes included in cluster 3 of the PARPi data were
493 compared to the genes deregulated by *EVI1* knockdown to determine the genes
494 downregulated by both conditions. The same was performed using cluster 4 of the PARPi
495 data for determining upregulated genes. Fold change of gene expression under treatment
496 conditions (PARPi or shEVI1, respectively) over the control (DMSO or non-targeting control,
497 respectively) is shown. **b** and **d** Pathway enrichment analysis of the commonly
498 downregulated (b) and upregulated (d) genes identified in **a** and **c**. Shown is the number of
499 genes assigned to the pathway (gene count), the proportion of genes from this cluster that
500 were assigned to the pathway (GeneRatio), and the adjusted p-value.

501

502 **Figure S1**

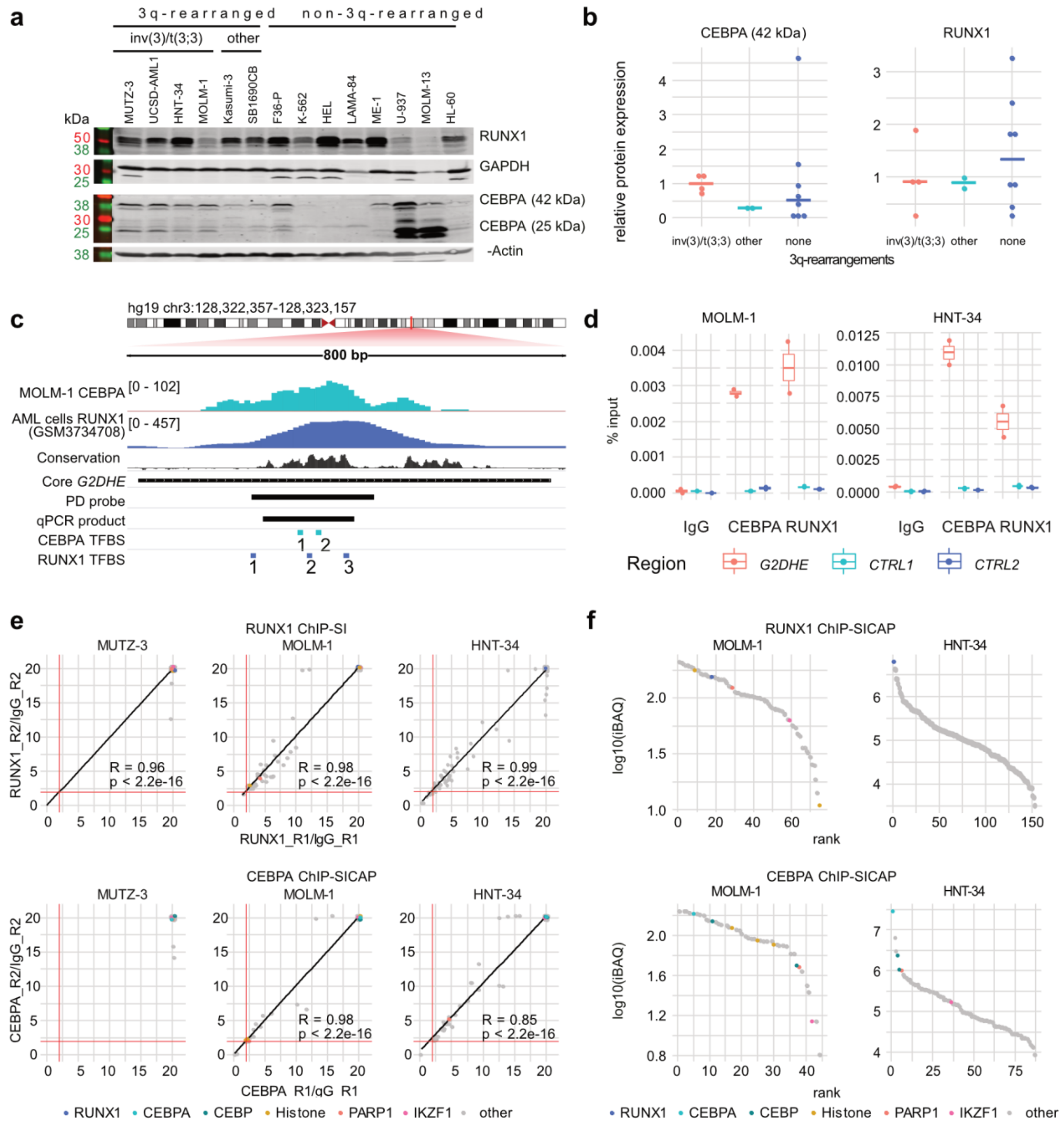




503

504

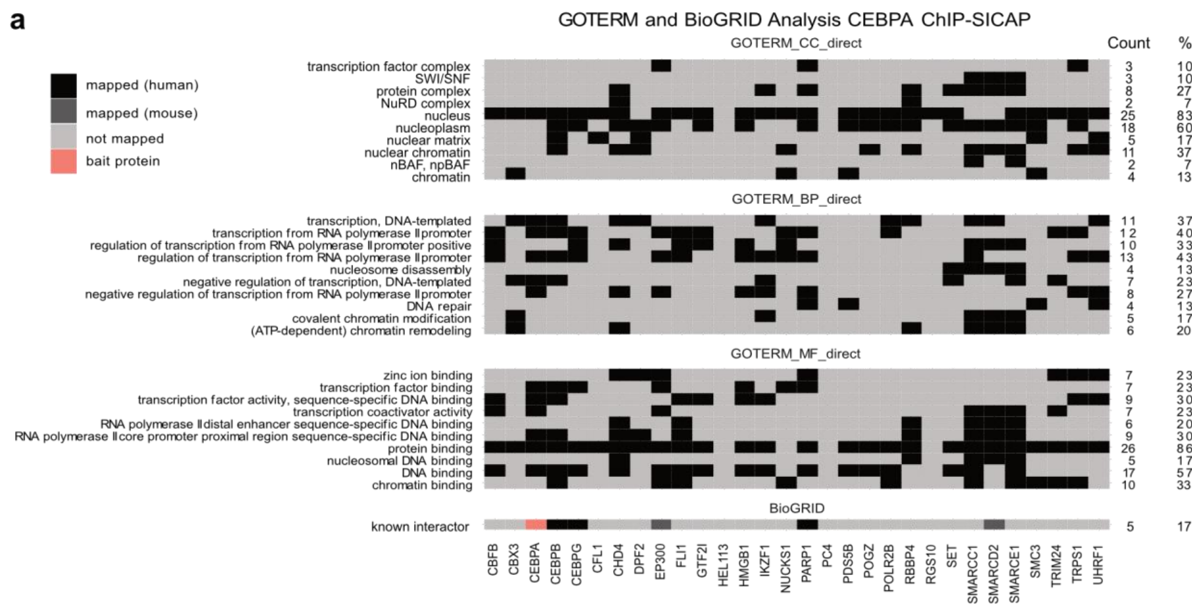
505 **Figure S2**



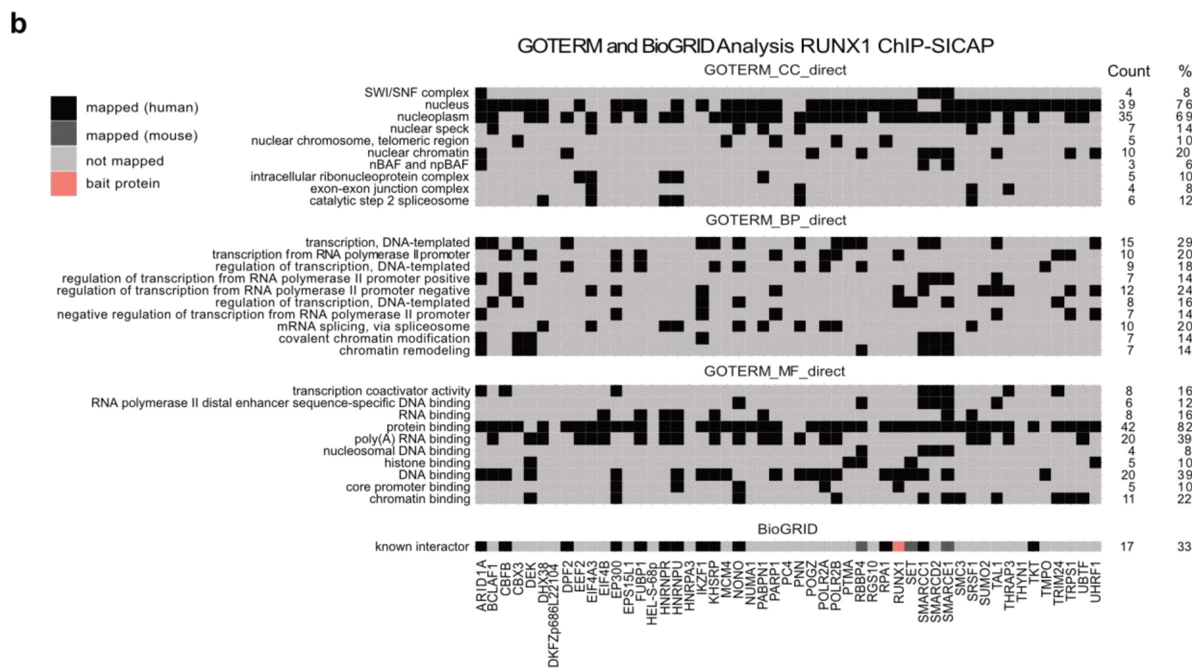
506

507 **Figure S3**

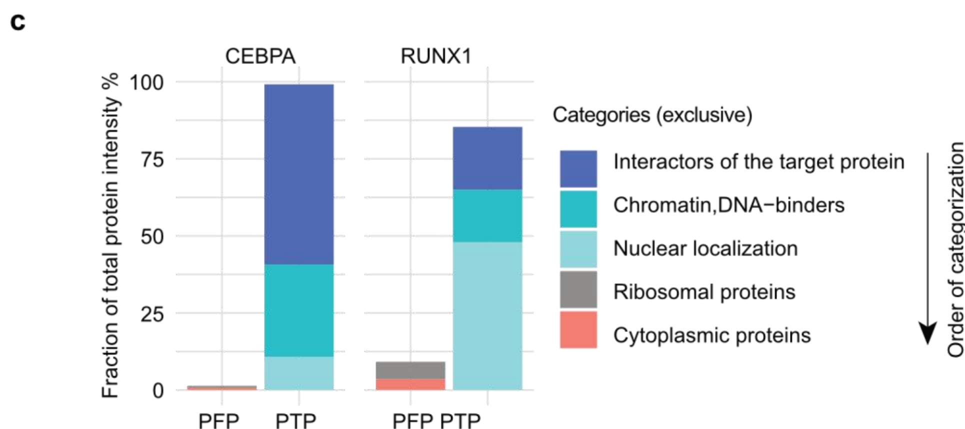
a



b



c



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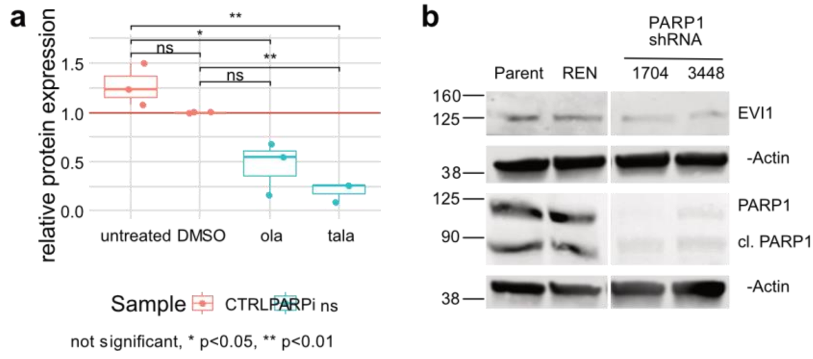
509 **Figure S4**



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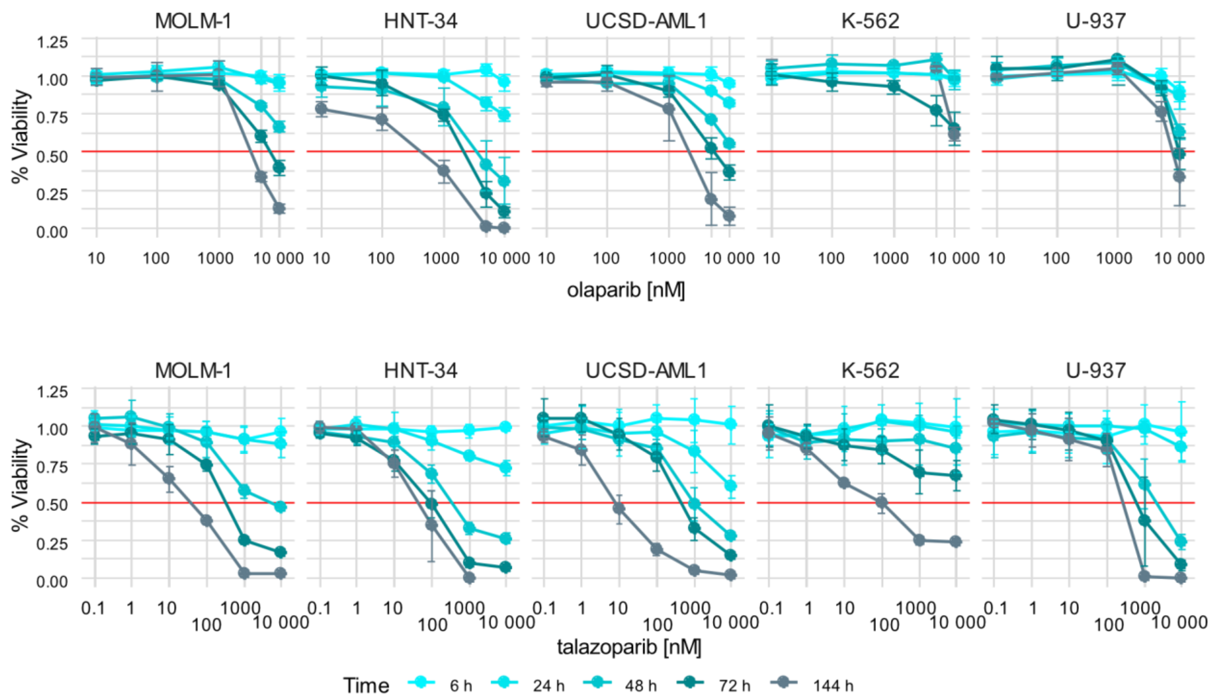
511

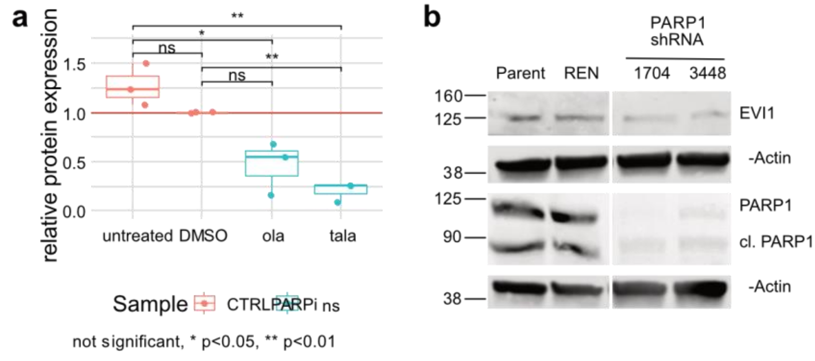
512 **Figure S5**



513

514

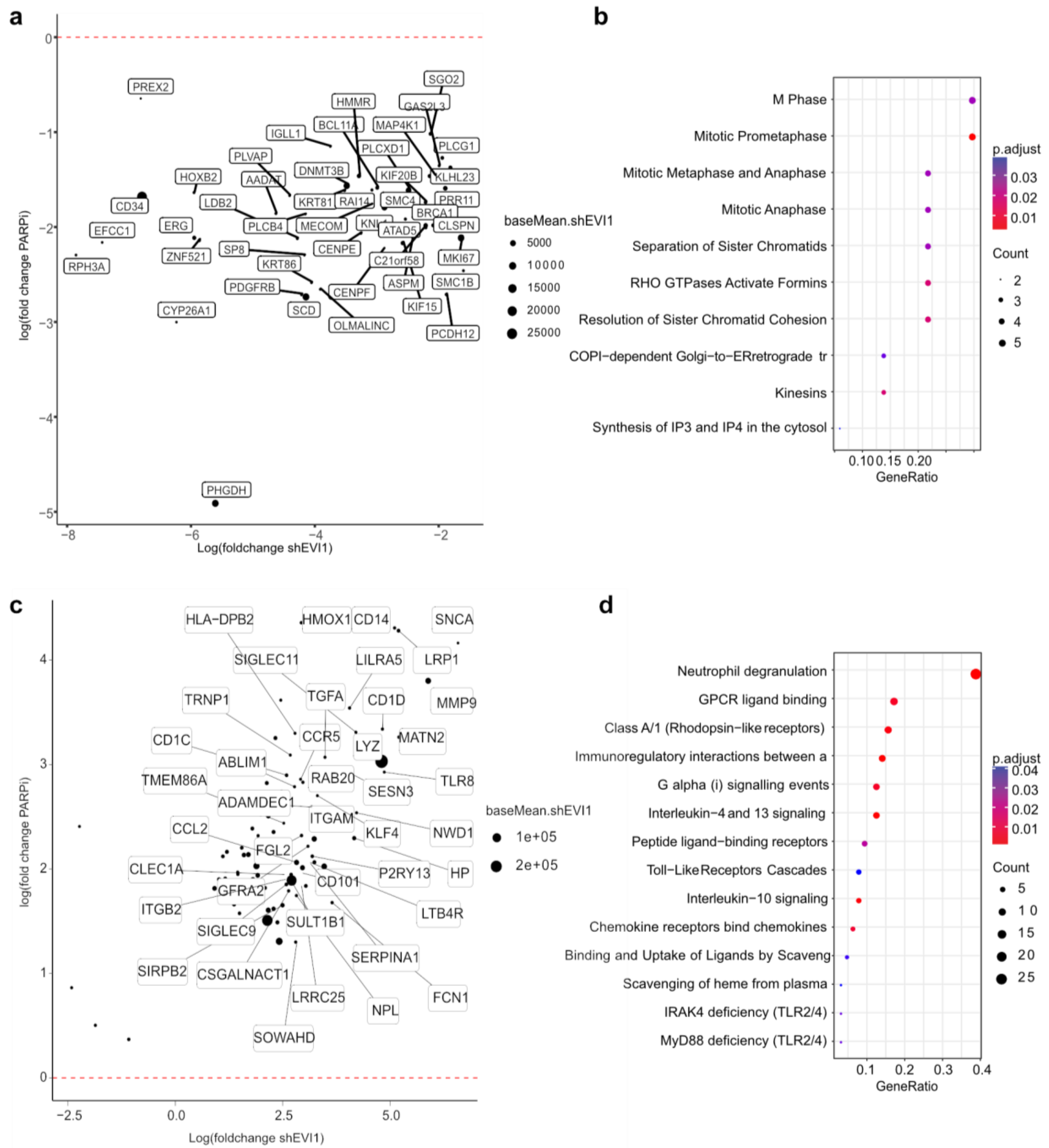
515 **Figure S6**



516 ns not significant, * p<0.05, ** p<0.01

517

518 **Figure S7**



519

520

521 ## Supplemental files

522 The supplementary File S1 “ChIP-SICAP results.xlsx” summarizes the proteins identified by
523 ChIP-SICAP using CEBPA and RUNX1 as baits in the cell lines MUTZ-3, MOLM-1 and HNT-
524 34. Given are the official gene names and Uniprot IDs, as well as the intensity ratios of
525 Bait/IgG for both replicates, the average log of the intensity ratios, the average log of the
526 iBAQ intensities, the p-values and the adjusted p-values (p.adj.).

527 The supplementary File S2 “TPM values.xlsx” summarizes the normalized transcript per
528 million (TPM) values of *EVI1* and *PARP1* transcripts from RNA-Seq data of primary human
529 AML patient samples (n=73) and cell lines (n=9) and states their karyotype. The occurrence
530 of inv(3)/t(3;3) is marked in an additional column.

531 The supplementary File S3 “diffExprGenes.xlsx” contains the RNA-Seq results and
532 summarizes the differentially expressed genes after 48 h PARPi treatment or *EVI1*
533 knockdown, respectively. Shown are the mean expression value (baseMean), the gene
534 expression foldchange over the control (DMSO in case of PARPi and non-targeting control in
535 case of *EVI1* Knockdown) as well as the p-values and adjusted p-values.