ENL links histone acetylation to oncogenic gene expression in acute myeloid leukaemia

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Cancer cells are characterized by aberrant epigenetic landscapes and often exploit chromatin machinery to activate oncogenic gene expression programs1. Recognition of modified histones by ‘reader’ proteins constitutes a key mechanism underlying these processes; therefore, targeting such pathways holds clinical promise, as exemplified by the development of bromodomain and extra-terminal (BET) inhibitors2,3. We recently identified the YEATS domain as an acetyl-lysine-binding module4, but its functional importance in human cancer remains unknown. Here we show that the YEATS domain–containing protein ENL, but not its parologue AF9, is required for disease maintenance in acute myeloid leukaemia. CRISPR–Cas9-mediated depletion of ENL led to anti-leukaemic effects, including increased terminal myeloid differentiation and suppression of leukaemia growth in vitro and in vivo. Biochemical and crystal structural studies and chromatin-immunoprecipitation followed by sequencing analyses revealed that ENL binds to acetylated histone H3, and co-localizes with H3K27ac and H3K9ac on the promoters of actively transcribed genes that are essential for leukaemia. Disrupting the interaction between the YEATS domain and histone acetylation via structure-based mutagenesis reduced the recruitment of RNA polymerase II to ENL-target genes, leading to the suppression of oncogenic gene expression programs. Notably, disrupting the functionality of ENL further sensitized leukaemia cells to BET inhibitors. Together, our data identify ENL as a histone acetylation reader that regulates oncogenic transcriptional programs in acute myeloid leukaemia, and suggest that displacement of ENL from chromatin may be a promising epigenetic therapy, alone or in combination with BET inhibitors, for aggressive leukaemia.

The YEATS (Ya9, ENL, AF9, Taf14, Sas5) domain–containing proteins AF9 and ENL are frequently fused with the mixed lineage leukaemia (MLL) protein as a result of MLL1 (also known as KMT2A) chromosomal translocations. The resultant fusion proteins are oncogenic drivers in acute myeloid leukaemia (AML) and acute lymphoid leukaemia (ALL)4. To assess the functional importance of the wild-type alleles of AF9 (MLLT3) and ENL (MLLT1) in MLL-rearranged leukaemias, we used a CRISPR–Cas9-mediated gene-editing and negative-selection strategy (Fig. 1a). Cells expressing ENL single-guide RNAs (sgRNAs) were outcompeted by non-transduced cells in various MLL-rearranged leukaemia cell lines (Fig. 1a and Extended Data Fig. 1a–d). Using a clonal MOLM-13 cell line in which Cas9 expression can be induced by doxycycline (Dox) administration (iCas9-MOLM-13), we observed a consistent effect of ENL sgRNAs on cell growth after Dox treatment (Extended Data Fig. 1e–g). CRISPR–Cas9- or short hairpin RNA (shRNA)-mediated depletion of ENL also impaired the clonogenic potential of MOLM-13 (Fig. 1b) and MV4;11 (Extended Data Fig. 1h, i) cells grown in cytokine-supplemented methylcellulose. ENL depletion increased the surface expression of the myeloid differentiation marker CD11b (also known as ITGAM) (Fig. 1c) and induced a morphological change to a macrophage-like appearance (Extended Data Fig. 1j). Notably, ENL sgRNA-induced growth inhibition and differentiation were rescued by the presence of a mouse Enl (Mllt1) cDNA that contains several mismatches to the human ENL sgRNA (Fig. 1d and Extended Data Fig. 1k). In addition to MLL-rearranged leukaemias, ENL depletion suppressed the growth of non MLL-rearranged leukaemia cell lines such as U-937 and K562, but not human cervical adenocarcinoma HeLa or osteosarcoma U2OS cells (Extended Data Fig. 1l–m). ENL silencing also had minimal effect on the growth, colony-forming ability or differentiation potential of sorted Lin−/Sca−1−/c-Kit+/LSK cells that are enriched for haematopoietic stem cells (Extended Data Fig. 1p–r).

To examine the relevance of ENL and AF9 to leukaemia progression in vivo, MOLM-13 cells were transduced with different sgRNAs and transplanted into immunodeficient recipient mice. The expression of ENL sgRNA resulted in a marked delay in leukaemia progression (Fig. 1e and Extended Data Fig. 2a) and prolonged survival of the recipient mice (Fig. 1f). Although the terminal leukaemia in the ENL sgRNA group was composed of predominantly sgRNA-positive cells, these leukaemia cells had similar protein levels of ENL to those from the control group (Extended Data Fig. 2b, c), indicating that the mice succumbed to an outgrowth of cells that escaped ENL depletion. Together, these results suggest a critical requirement of ENL, but not AF9, for AML maintenance in vitro and in vivo.

To identify the transcriptional pathways controlled by ENL, we performed RNA sequencing (RNA-seq) analyses on iCas9–MOLM-13 cells expressing different sgRNAs. Whereas AF9 sgRNA caused minimal changes in gene expression, ENL sgRNA induction led to differential expression of a subset of genes, but not to global transcriptional dysregulation (Fig. 2a and Extended Data Fig. 3a). The transcriptional programs altered by the two ENL sgRNAs were highly correlated (Extended Data Fig. 3b). Gene set enrichment analyses (GSEA)

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ENL-bound genes in leukaemia cell lines were significantly more prominent on ENL-occupied genes than on non-ENL-bound genes (Fig. 2e). After ENL depletion, Pol II ChIP–seq signals decreased more prominently on ENL-occupied genes than on non-ENL-bound genes (P < 0.0001), including two key AML transcription factors MYC and H2O-like homeobox (HLX)4. The reduction of Pol II occupancy spanned from the promoter proximal region to the 3' end of gene body, suggesting reduced Pol II activities in both transcriptional initiation and elongation. Indeed, ChIP–seq analyses of the elongation-specific Pol II that is phosphorylated at serine 2 (S2P)11 showed a markedly decreased occupancy of the elongating Pol II on ENL target genes in ENL-depleted cells (Fig. 2f and Extended Data Fig. 4i). In addition, ENL depletion also resulted in a decrease in the occupancy of CDK9, a SEC component12 that phosphorylates Pol II at the serine 2 site13, and the DOT1L-mediated H3K79me2 and H3K79me3 on ENL-target genes (Fig. 2g and Extended Data Fig. 4j, k). Together, these data suggest that ENL regulates gene expression by modulating the recruitment of Pol II through both the SEC and DOT1L complexes.

In light of the AF9 YEATS domain being a reader for histone acetylation4, we proposed that ENL might link the Pol II transcriptional machinery to chromatin through its YEATS domain. To test this hypothesis, we performed histone peptide array screening and found that the ENL YEATS domain bound specifically to a subset of acetylated histone H3 peptides (Extended Data Fig. 5a). Preferential binding to the H3K27ac, H3K9ac and H3K18ac peptides was further confirmed by peptide pull-down (Fig. 3a) and quantitative isothermal titration calorimetry (ITC) assays (Fig. 3b), with binding dissociation constants (Kd) of 30.5, 32.2 and 50.0 μM for H3K27ac, H3K9ac and H3K18ac, respectively.

To explore the underlying molecular basis of this binding specificity, we explored the underlying molecular basis of this binding specificity, we

revealed a marked upregulation of the myeloid lineage differentiation signature along with a downregulation of a leukaemia stem cell and MYC-associated gene expression signature6 in ENL sgRNA- or shRNA-expressing MOLM-13 and MV4;11 cells (Fig. 2b and Extended Data Fig. 3c–h), suggesting that ENL is required to sustain the oncogenic gene expression programs crucial for leukaemia maintenance.

ENL resides in large protein complexes termed the super elongation complex (SEC)3, elongation assisting proteins (EAP)8 or AF4–ENL–P–TEFb complex (APF)9 that contain overlapping subunits such as AFF1–AFF4, ELL, EAF1–EAF2, ENL–AF9 and P–TEFb (for simplicity, we refer to SEC hereafter). To determine the genomic distribution of ENL, we generated stable MOLM-13 and MV4;11 cells ectopically expressing Flag-tagged ENL at levels equivalent to the endogenous ENL proteins, and performed chromatin-immunoprecipitation following by sequencing (ChIP–seq) experiments. ENL bound to a larger cohort of genes than that of MLL-fusion proteins in both cell lines, suggesting a broader role of ENL than MLL-fusion proteins in transcriptional regulation (Extended Data Fig. 4a, b). There was a greater overlap of ENL-bound genes between the leukaemia cell lines than their overlaps with ENL occupancy in the non-leukaemia HeLa cells (Extended Data Fig. 4c). Notably, the ENL-bound genes in leukaemia cell lines were significantly enriched in pathways implicated in cancer and haematological disease (Extended Data Fig. 4d).

ENL ChIP–seq peaks showed a strong enrichment at promoter regions (transcription start site (TSS) ± 3 kb) (Fig. 2c and Extended Data Fig. 4e), which overlapped with the promoter-proximal RNA polymerase II (Pol II) peak (Fig. 2d and Extended Data Fig. 4f). ENL-bound genes showed a much higher Pol II occupancy than non-ENL-bound genes (Fig. 2e). After ENL depletion, Pol II ChIP–seq signals decreased more prominently on ENL-occupied genes than on non-ENL-bound genes (P < 0.0001), including two key AML transcription factors MYC and H2O-like homeobox (HLX)4. The reduction of Pol II occupancy spanned from the promoter proximal region to the 3' end of gene body, suggesting reduced Pol II activities in both transcriptional initiation and elongation. Indeed, ChIP–seq analyses of the elongation-specific Pol II that is phosphorylated at serine 2 (S2P)11 showed a markedly decreased occupancy of the elongating Pol II on ENL target genes in ENL-depleted cells (Fig. 2f and Extended Data Fig. 4i). In addition, ENL depletion also resulted in a decrease in the occupancy of CDK9, a SEC component12 that phosphorylates Pol II at the serine 2 site13, and the DOT1L-mediated H3K79me2 and H3K79me3 on ENL-target genes (Fig. 2g and Extended Data Fig. 4j, k). Together, these data suggest that ENL regulates gene expression by modulating the recruitment of Pol II through both the SEC and DOT1L complexes.

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solved the co-crystal structure of human ENL YEATS bound to the H3K27ac peptide at 2.7 Å (Extended Data Table 1). The ENL YEATS domain adopts an eight-stranded β-sandwich fold (Fig. 3c) with the H3K27ac peptide snugly attached to an acidic surface at the top (Fig. 3d) and the flat acetylamide group of K27ac sandwiched by aromatic residues F59 and Y78 (Fig. 3e). Recognition of the H3K27ac peptide is further stabilized by a network of hydrogen bonds in addition to hydrophobic contacts (Extended Data Fig. 5b). Acetylated histone H3 at K9, K18 and K27 share a common ‘R-Kac’ recognition signature, in which the ‘−1’ arginine forms a charge-stabilized hydrogen bond with D103 of ENL (Fig. 3e). Structure-based mutagenesis followed by isothermal titration calorimetry (ITC) and peptide pull-down assays revealed a 2.3-fold to more than 17-fold affinity drop or binding elimination upon alanine mutation of the R-Kac-binding residues (Fig. 3f, g).

ChIP-seq analyses of both MOLM-13 and MV4;11 cells revealed that ENL-bound peaks were associated with regions of H3K9ac or H3K27ac modifications (Fig. 3h and Extended Data Fig. 6a). Consistently, the

Figure 3 | ENL binds and colocalizes with acetylated histone H3 genome-wide via its YEATS domain. a, Peptide pull-down assay of indicated histone peptides and ENL YEATS domain. b, ITC titration and fitting curves of human ENL YEATS domain titrated with H3(17–28)K27ac, H3(1–10)K9ac, H3(1–25)K18ac or unmodified H3(1–34) peptides. c, Overall structure of the ENL YEATS domain bound to H3K27ac peptide. ENL YEATS is depicted as blue ribbons with key residues highlighted in green sticks. Histone H3K27ac peptide is shown as a yellow ribbon, with side chains highlighted by sticks. Red dashes, hydrogen bonds; red sphere, water molecule. d, Electrostatic potential surface of the ENL YEATS domain ranging from −10 to 10 kT e−1. Histone H3 peptide is depicted as space-filling spheres. e, Top, hydrogen bonding network between H3K27ac peptide and ENL. Hydrogen bonds are shown as red dashes. Key residues of ENL are depicted as green sticks and labelled black; the H3 peptide is shown as yellow sticks and labelled red. Grey meshes, F1–F5, omit map countered at 2.0σ level. Bottom, sequence alignment of histone H3 sequences flanking residues K9, K18 and K27. Conserved residues are highlighted in pink. f, ITC titration fitting curves of ENL YEATS mutants with H3(17–28)K27ac peptide. g, Peptide pull-downs of ENL YEATS mutants and indicated histone H3 peptides. See Supplementary Fig. 1 for gel source data. h, Venn diagram showing the overlap of Flag–ENL–, H3K9ac– and H3K27ac ChIP–seq peaks in MOLM-13 cells. P < 1 × 10−300 (three-way Fisher’s exact test). See Supplementary Tables 4 and 5. i, Average occupancy of ENL-bound genes along the transcription unit. j, Average genome-wide occupancies of wild-type (blue) and mutant ENL (F59A in cyan or Y78A in purple) on ENL-bound genes along the transcription unit.
Figure 4 | Disrupting the YEATS-histone acetylation interaction inhibits the functionality of ENL and sensitizes leukaemia cells to BET inhibitors. a. Heat map representation of genes differentially expressed in iCas9-MOLM-13 cells expressing sgRNAs targeting luciferase control or ENL (fold change > 1.5 and adjusted P < 0.05) in the indicated rescue conditions. b, GSEA plots evaluating the changes in monocyte differentiation (top) and leukaemia stem-cell gene signatures (bottom) in the indicated comparisons. c, Negative-selection competition assay that plots the relative percentage of RFP+ sgRNA+ cells after transduction of leukaemia cells with indicated constructs. n = 3. d, FACS analysis of CD11b surface expression in iCas9-MOLM-13 cells expressing ENL sgRNA and indicated mouse Enl rescue constructs after 4 days of Dox treatment. See Extended Data Fig. 7b for quantification. n = 3. e, Kaplan–Meier survival curves of mice (n = 10 per group) transplanted with MOLM-13 cells transduced with indicated sgRNAs and rescue constructs. P < 0.0001 using a log-rank test. f, Effect of JQ1 on the proliferation (normalized to DMSO control) of MOLM-13 cells transduced with indicated sgRNAs and rescue constructs. n = 5. g, h, Leukaemia burden (g) and survival curves (h) of mice transplanted with iCas9-MOLM-13 cells expressing indicated sgRNAs. Treatment with JQ1 (or vehicle control) and doxycycline was initiated at day 10 after transplantation. Percentage of human CD45+ cells in the peripheral blood of mice (n = 5) at 40 days after transplantation. P < 0.01 (ENL sgRNA plus JQ1 versus all other groups) using Mann–Whitney test. h, Kaplan–Meier survival curves of JQ1-treated mice and vehicle controls (n = 10 per group). P < 0.0001 (ENL sgRNA plus JQ1 versus all other groups) using a log-rank test. i, j, RNA for RNA-seq experiments was obtained from sorted RFP+ sgRNA+ iCas9-MOLM-13 cells treated with DMSO or 50 nM JQ1 for 24 h. Genes found to be more than twofold downregulated (i) or upregulated (j) after JQ1 treatment in ENL sgRNA-expressing cells were examined. Left, box plots comparing the JQ1-induced fold changes of these genes in either control (red) or ENL sgRNA-expressing (blue) cells. Error bars indicate minimum and maximum, lines denote median, and top and bottom of boxes denote first and third quartile, respectively. P = 2.29 × 10⁻⁷ (i) and P = 2.44 × 10⁻⁷ (j) by two-tailed paired Student’s t-test. Right, pie charts showing the categorization of these genes based on the relationship to ENL depletion. Genes in which the absolute JQ1-induced fold change was more than 1.2-fold higher or lower in ENL sgRNA-expressing cells compared to control were classified as enhanced (blue) or decreased (green) by ENL sgRNA, respectively. There are significantly more genes in the enhanced than the decreased by ENL sgRNA group (P < 0.0001 by Fisher’s exact test). See Supplementary Table 10. All error bars represent mean ± s.d. (n = 3) unless noted otherwise.

average distribution of ENL correlated extremely well with that of H3K9ac and H3K27ac in the region immediately downstream of the TSS (Fig. 3i and Extended Data Fig. 6b, c). Notably, compared to wild-type ENL, the YEATS domain mutants deficient in acetyl-binding exhibited markedly reduced occupancy at ENL-bound genes (Fig. 3j) and Extended Data Fig. 6d–f), whereas their interactions with other SEC components were not affected (Extended Data Fig. 6g, h). The ENL YEATS domain is also required for Pol II recruitment, as cells expressing the YEATS mutants exhibited reduced Pol II occupancy on ENL target genes such as MYC (Extended Data Fig. 6i). Together, these findings revealed an important ‘reader’ function of the YEATS domain in mediating the chromatin localization of ENL and the recruitment of the Pol II transcriptional machinery.

To test whether the YEATS domain is required for ENL-dependent maintenance of oncogenic gene expression in leukaemia, we performed rescue experiments with ectopically expressed mouse wild-type or mutant ENL proteins in cells expressing a human ENL sgRNA. Transcriptional profiling analyses revealed that wild-type ENL, but not the F59A or Y78A mutant, restored the transcriptional changes caused by the ENL sgRNA (Fig. 4a). GSEA analyses further demonstrated the incompetency of these ENL mutants to revert the effect of ENL depletion on the expression of genes involved in crucial cellular processes (Fig. 4b and Extended Data Fig. 7a). Consequently, the mutants were unable to rescue the growth and differentiation defects caused by ENL inhibition (Fig. 4c, d and Extended Data Fig. 7b). In line with these observations, replacement of the ENL YEATS domain with that of AF9 retained the full functionality of ENL (Extended Data Fig. 7c, d). Finally, mice transplanted with ENL sgRNA-transduced cells expressing mutant ENL proteins exhibited a marked decrease in leukaemia burden and prolonged survival as compared to the wild-type counterpart (Fig. 4e and Extended Data Fig. 7e). Taken together, these findings demonstrate a crucial role for the ENL YEATS-histone acetylation interaction in regulating oncogenic gene expression and leukaemia maintenance.

Strategies to disrupt the interaction between acetylated histones and their reader proteins, such as the BET family of bromodomain-containing proteins, have been shown to be effective in clinical trials for treatment of a variety of cancers, including haematological malignancies. Interestingly, similar to ENL, BRD4 also associates with P-TEFb and modulates the Pol II transcriptional machinery to regulate key oncogenes such as MYC. This finding raised the possibility that ENL and BRD4 might cooperate to sustain optimal oncogenic gene expression programs crucial for leukaemias. In line with this speculation, depletion...
of ENL or other SEC components sensitized MOLM-13 cells to the BET bromodomain inhibitor JQ1 (ref. 2; Fig. 4f and Extended Data Fig. 8a). Point mutations disrupting the interaction between the ENL YEATS domain and histone acetyltransferase were sufficient to confer cells with greater sensitivity to JQ1 (Fig. 4f). Increased JQ1 sensitivity by ENL silencing was also observed in a panel of leukemia cell lines, but not in HeLa or U2OS cells (Extended Data Fig. 8b–d). Furthermore, ENL depletion coupled with JQ1 treatment resulted in a significant survival advantage compared with single treatments in vivo (Fig. 4g, h and Extended Data Fig. 8e). Mechanistically, ENL depletion markedly enhanced JQ1-induced transcriptional changes in MOLM-13 cells, including MYC (Fig. 4i, j and Extended Data Fig. 8f–i), the inhibition of which has been shown to contribute to the sensitivity to BET inhibitors16,17. Together, these findings suggest that inhibition of ENL functionality is a potential therapeutic strategy to synergize with BET inhibitors.

Cancer cells often co-opt chromatin regulatory pathways and the general transcriptional machinery to sustain the oncogenic state; hence chromatin and transcriptional regulators are being explored as promising candidate drug targets. Targeting epigenetic readers represents a class of anti-cancer therapy that holds clinical promise. Our study reveals ENL as a chromatin reader that regulates oncogenic programs by linking histone acetylation and the transcription apparatus in AML, thus establishing the ENL YEATS domain as a potential drug target, either alone or in combination with BET inhibitors. Notably, recurrent mutations in the ENL YEATS domain were recently reported in Wilms’ tumours18, suggesting potentially crucial and broad roles of the ‘reader’ function of ENL in other cancer types that warrant further investigations.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

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**METHODS**

Plasmids. Mouse wild-type or mutant Enl were cloned into pMSCV-ires-GFP. Human ENL, DOT1L, AFF4, ELL2 and CDK9 cDNA in pENTR or pDONR were purchased from Open Biosystems. The coding sequences in pENTR were subsequently cloned into p3FLAG, pCDH-Flag, or pCAG-Myc destination vectors using Gateway techniques (Invitrogen). Constitutive (pLKO5d.EFS.SpCas9.P2A.BSD, Addgene 57821, a gift from B. Ebert) and inducible (pCW-CAS9, Addgene 50661, a gift from E. Lander and D. Sabatini) Cas9 vectors were used to generate Cas9-expressing cell lines. All shRNA targeting human and mouse ENL were obtained from Sigma. sgRNAs were cloned into pKOS.sgRNA.EFS.RFP657 vector (Addgene 57824, a gift from B. Ebert). All shRNA and sgRNA sequences are used are provided in Supplementary Table 11.

Cell culture, virus transduction and functional assays. Human leukemia cell lines MOLM-13, MV4;11, ML-2, SEMK2, U-937 and K562 were maintained in RPMI 10% FBS supplemented with 2mM L-glutamine and 100 U ml⁻¹ penicillin/streptomycin. HeLa and U2OS cells lines were maintained in DMEM 10% FBS supplemented with 2mM L-glutamine and 100 U/ml penicillin/streptomycin. Lin − Sca-1 − c-Kit (LSK) cells were freshly sorted from the bone marrow of 6–8-week-old C57BL/6J male mice and cultured in IMDM plus 15% FBS supplemented with 20 ng ml⁻¹ mouse SCF (PeproTech), 10 ng ml⁻¹ mouse IL-3 (PeproTech) and 10 ng ml⁻¹ mouse IL-6 (PeproTech). All cell culture medium contained l-glutamine (2mM; Gibco), penicillin (100 U/ml; Gibco), streptomycin (100µg ml⁻¹; Gibco) and plasmocin (5µg ml⁻¹; InvivoGen). All human cell lines were mycoplasma-negative and were tested for authenticity by short tandem repeat (STR) profiling performed by ATCC or by the MDACC CCGS-funded Characterized Cell Line Core, NCI CA016672.

Lentivirus and retrovirus packaging were performed in HEK293T and Platnum-E cells (Cell Biologics), respectively, using Lipofectamine 2000 reagent (Invitrogen) in accordance with the manufacturer’s instructions. Medium containing virus was concentrated using PEG-virus Precipitation Solution (System Biosciences). Spin infection was performed at 1,500 rcf at 35 °C for 90 min and transduced cell populations were usually selected or sorted 48 h after infection. Competitive proliferation assays using sgRNAs for constitutive Cas9 expression, cells were analysed for RFP⁺ expression 3 days after infection. For Dox-regulated Cas9 expression cells, cells were analysed for RFP⁺ expression before and after Dox treatment (1 µg ml⁻¹). The percentage of sgRNA-expressing cells (RFP⁺) was measured over time using flow cytometry and normalized to the starting time point. Colony-forming assays were performed in Methocult (StemCell Technologies, no. H4435 for human leukaemia cells and M3234 supplemented with 20 ng ml⁻¹ mouse SCF, 10 ng ml⁻¹ mouse IL-3 and 10 ng ml⁻¹ mouse IL-6 for mouse LSKs). Indicated number of cells was resuspended in 100 µl PBS and added to 900 µl Methocult. After 7 days, different types of colony were counted. Cell viability assays for JQ1 treatment were performed using CellTiter-Glo Luminescent Cell Viability Assay (promega/VWR) according to manufacturer’s guidelines.

For flow cytometry immunophenotyping, cultured or freshly dissociated cells were collected and stained using the indicated antibodies. Stained samples were analysed on an LSR Fortessa (BD) flow cytometer. Data analysis was performed using FlowJo software.

Antibodies. All antibodies used are provided in Supplementary Table 12.

qRT–PCR analyses. Total RNA was isolated using the RNeasy kit (Qiagen) and reverse transcribed with the high capacity cDNA reverse transcription kit (Applied Biosystems) in accordance with the manufacturer’s instructions. Quantitative PCR was performed using the SYBR Green PCR Master Mix (Applied Biosystems) with the StepOne System (Applied Biosystems). qPCR primers used are provided in Supplementary Table 11.

DNA sequencing. DNA was isolated from human leukaemia cells using the RNeasy Mini Kit (Qiagen) and then samples were prepared as instructed using the FlowMax DNAクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームクレームク레
weeks \((n=10\) per group). Dox-containing chow and water were supplied during the course of the experiments. For JQ1 \textit{ex vivo} treatment trials, leukaemia cells were treated with 1 \(\mu\)g ml\(^{-1}\) doxycycline for 2 days and then treated with 100 nM JQ1 and 1 \(\mu\)g ml\(^{-1}\) doxycycline for the next 2 days before equal number of viable cells were transplanted. For JQ1 \textit{in vivo} treatment trials, JQ1 was resuspended in DMSO at 200 mg ml\(^{-1}\) (for 50 mg kg\(^{-1}\) dosing) and then diluted 1:20 with 10.5\% (2-hydroxypropyl)-\(\beta\)-cyclodextrin in 0.9\% sterile saline. The solution was sonicated until homogenous. Vehicle was prepared and administered the same with the exception that no JQ1 was added. 100 \(\mu\)l doses were administered by intraperitoneal injection for 5 consecutive days with 2-day rest for 30 days starting from day 10 after transplantation. The animals were randomly assigned to the experimental groups. Kaplan–Meier survival curves of mice and the log-rank test were performed using the Prism 6 software program (GraphPad). No animal was excluded from any of the analyses. The investigators were not blinded to allocation during experiments and outcome assessment.

**Statistical analyses.** Experimental data are presented as mean ± s.e.m. unless stated otherwise. Statistical significance was calculated by two-tailed unpaired \(t\)-test on two experimental conditions with \(P<0.05\) considered statistically significant unless stated otherwise. Statistical significance levels are denoted as follows: \(*P<0.05\); \(*P<0.01\); \(**P<0.001\); \(***P<0.0001\). No statistical methods were used to predetermine sample size.

**Data availability.** Structure data has been deposited in Protein Data Bank (PDB) under accession number 5J9S. The ChIP–seq and RNA-seq data have been deposited in the Gene Expression Omnibus (GEO) database under accession numbers GSE80779 and GSE80774, respectively. All other raw data generated or analysed during this study are included in this published article (and its Supplementary Information files).

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Extended Data Figure 1 | See next page for caption.
Extended Data Figure 1 | Depletion of ENL impairs the growth of AML. a, Western blot demonstrating the knockdown efficiency of five independent sgRNAs (sg1–sg5) targeting ENL. b, Competition assay that plots the relative percentage of RFP<sup>+</sup>sgRNA<sup>+</sup> cells after transduction of leukaemia cells with indicated sgRNAs. n = 3. c, Western blot demonstrating the knockdown efficiency of five independent sgRNAs targeting AF9. d, Competition assay that plots the relative percentage of RFP<sup>+</sup>sgRNA<sup>+</sup> cells after transduction of leukaemia cells with indicated sgRNAs. n = 3. e, Western blot demonstrating the induction of Cas9 expression after Dox treatment in iCas9-MOLM-13 cells. f, Western blot demonstrating the decrease in ENL protein levels upon Dox treatment in iCas9-MOLM-13 cells. g, Competition assay that plots the relative percentage of RFP<sup>+</sup>sgRNA<sup>+</sup> cells after Dox treatment in iCas9-MOLM-13 cells. n = 3. h, Relative ENL mRNA levels determined by quantitative PCR after reverse transcription (qRT–PCR) in MV4;11 cells transduced with non-targeting (NT) control or ENL shRNAs (shENL-1, shENL-2). i, Representative images (left) and quantification (right) of colonies formed by MV4;11 cells transduced with indicated shRNAs. j, Light microscopy of May–Grünwald/Giemsa-stained MV4;11 leukaemia cells transduced with control or ENL shRNAs. k, FACS analysis of CD11b surface expression after 4 days of Dox-induced Cas9 expression (left) and quantification of CD11b median intensity (right) in iCas9-MOLM-13 cells transduced with indicated sgRNAs and rescue constructs. n = 3. l–o, Negative-selection competition assay that plots the relative percentage of RFP<sup>+</sup>sgRNA<sup>+</sup> cells after Dox treatment in iCas9-U-937 (l), iCas9-K562 (m), iCas9-HeLa (n) and iCas9-U2OS (o) cells. n = 3. p–r, LSK cells were sorted from bone marrow of C57BL/6 mice and transduced with luciferase (shLuc) or Enl shRNA (shEnl). p, Relative Enl mRNA levels determined by qRT–PCR quantification after 3 days of drug selection. q, Relative proliferation of control (shLuc) or Enl-knockout (shEnl) LSKs. n = 4. r, Quantification of colonies formed by LSK cells cultured for 7 days. n = 4. G, granulocyte; GM, colony-forming unit containing granulocyte and macrophage; M, macrophage. All error bars represent mean ± s.d. See Supplementary Fig. 1 for western blot gel source data.
Extended Data Figure 2 | ENL is required for AML growth in vivo.

**a**, Representative flow cytometry plots of donor-derived (human CD45+) peripheral blood cells 27 or 32 days after transplantation. The gate shown includes RFP⁺ sgRNA⁺ human leukaemia cells. **b**, Representative flow cytometry plots of bone marrow cells in terminally diseased mice receiving cells transduced with ENL sgRNA. Most outgrowing leukaemia cells were RFP⁺ sgRNA⁺. **c**, Western blot of sorted RFP⁺ sgRNA⁺ leukaemia cells from terminally diseased mice (n = 3) receiving cells transduced with control or ENL sgRNA. See Supplementary Fig. 1 for western blot gel source data.
Extended Data Figure 3 | Depletion of ENL deregulates core cellular processes and oncogenic pathways that are required for AML maintenance. a–f, RNA for RNA-seq experiments was obtained from sorted RFP−sgRNA−iCas9-MOLM-13 cells after 3 or 5 days of Dox treatment. a, Venn diagram showing the number and overlap of genes for which expression is significantly changed (adjusted P < 0.05) upon expression of indicated sgRNAs as compared to GFP control. b, Dot plots showing a strong correlation of transcriptional changes (log2 fold change over GFP control) caused by two independent sgRNAs targeting ENL. r, correlation coefficient. c, Heat map representation of genes differentially expressed between iCas9-MOLM-13 cells expressing sgRNAs targeting GFP control, ENL or AF9 (fold change > 1.5 and adjusted P < 0.05) after 3 days of Dox induction. d, e, GSEA plots evaluating the changes in monocyte differentiation and leukaemia stem cell gene signatures (d) and the MYC pathways (e) upon ENL depletion. f, Gene Ontology (GO) term analyses of downregulated (ENL-KO-DN, top) or upregulated (ENL-KO-UP, bottom) genes in ENL sgRNA-expressing cells. The top five biological processes that each group of genes were enriched in were shown (details in Supplementary Table 2). Fold enrichment and P values are shown.

g, h, RNA for RNA-seq experiments was obtained from MV4;11 transduced with non-targeting (NT) or ENL shRNAs. GSEA plots evaluating the changes in monocyte differentiation and leukaemia stem-cell gene signatures (g) and the oncogenic pathways (h) after ENL knockdown.
Extended Data Figure 4 | See next page for caption.
Extended Data Figure 4 | ENL depletion decreases the occupancies of total Pol II and Pol II S2P on ENL-bound genes. a, b, Venn diagram showing overlaps of Flag–ENL-occupied genes with those of MLL-AF9 in MOLM-13 (ref. 26) (a) or MLL-AF4 in MV4;11 cells (ref. 27) (b), respectively. c, Venn diagram showing overlaps of Flag–ENL-occupied genes in MOLM13, MV4;11 and HeLa cells. See Supplementary Table 7. d, IPA analysis of ENL-bound genes overlapped among leukaemia cells but not HeLa cells. e, Genomic distribution of Flag–ENL ChIP–seq peaks in MV4;11 cells. The peaks are enriched in the promoter regions (TSS ± 3 kb). P < 1 × 10^-300 (binomial test). See Supplementary Table 6. f, Average occupancies of Flag–ENL (blue) and Pol II (black) on Flag–ENL-bound genes in MV4;11 cells along the transcription unit. g, Box plots showing the fold changes (normalized to GFP control) of Pol II occupancy at TSS (TSS – 30 bp to TSS +300 bp) or the rest of the gene body on ENL-bound and activated genes upon the expression of ENL sgRNA. The fold changes at both TSS and gene body were significantly lower than 1 (P < 0.0001 by one sample, two-tailed Student's t-test). h, The genome browser view of Pol II signals in a few of ENL-bound genes (MYC, HLX, SLC1A5) in cells expressing sgRNAs targeting GFP (red) or ENL (blue). TSS is indicated by an arrow. i, Western blot showing comparable cellular levels of Pol II S2P in MOLM-13 cells expressing sgRNAs targeting GFP or ENL. See Supplementary Fig. 1 for gel source data. j, k, Average H3K79me2 (j) and H3K79me3 (k) occupancy on Flag–ENL-bound or non-ENL-bound genes (others) in cells expressing sgRNAs targeting GFP control or ENL.
Extended Data Figure 5 | Binding specificity and detail of H3K27ac-bound ENL YEATS complex. 

a, Histone peptide microarray (detailed annotations on the left) probed with anti-GST antibody against GST–ENL YEATS domain. H3K9ac, H3K18ac and H3K27ac are highlighted in yellow boxes. 
b, LIGPLOT diagrams of H3K27ac-ENL YEATS complex, listing interactions between H3 peptide and ENL YEATS. H3 segments (orange) and key residues of ENL YEATS (blue) are depicted in ball-and-stick mode. Grey ball, carbon; blue ball, nitrogen; red ball, oxygen; large cyan sphere, water molecule. Hydrogen bonds are indicated as green dashed lines with bond length shown in ångströms. Hydrophobic contacts are represented by an arc with spokes radiating towards the ligand atoms they contact, and the contacted atoms are shown with spokes radiating back.
Extended Data Figure 6 | The YEATS domain is required for the chromatin localization of ENL. a, Box plots showing H3K9ac (red) and H3K27ac (green) occupancy in ENL-bound or unbound genes (others) in MOLM-13 cells. \( P < 8.1 \times 10^{-152} \) (H3K9ac) and \( P < 2.2 \times 10^{-136} \) (H3K27ac) by two-tailed unpaired Student’s t-test. b, Venn diagram showing the overlap of Flag–ENL (blue) and H3K27ac ChIP–seq peaks (green) at promoter or enhancer regions. Promoter H3K27ac is defined as H3K27ac peaks at TSS ± 3 kb regions co-occupied with H3K4me3; enhancer H3K27ac is defined as non-promoter H3K27ac peaks co-occupied with H3K4me1. There is a significant overlap between Flag–ENL and H3K27ac ChIP–seq peaks at TSS (\( P = 5.7 \times 10^{-105} \), two-way Fisher exact test) but not at enhancer (\( P = 1.0 \), two-way Fisher exact test). c. Average genome-wide occupancies of Flag–ENL (blue), H3K9ac (red), and H3K27ac (green) at Flag–ENL-bound genes along the transcription unit in MV4;11 cells. See Supplementary Tables 8 and 9. d, Western blot showing the protein levels of ectopically expressed wild-type or mutant Flag–ENL (marked by asterisk) and endogenous ENL (marked by double asterisk). e, The genome browser view of H3K27ac, H3K9ac, Flag–ENL signals in a few of ENL-bound genes (MYC, HLX). TSS is indicated by an arrow. f, Average occupancies of wild-type, F59A or Y78A mutant Flag–ENL on ENL-bound genes along the transcription unit in MV4;11 cells. g, Western blot analysis of co-immunoprecipitation using the M2 anti-Flag antibody in cells expressing Flag–ENL and Myc-tagged DOT1L, AFF4, CDK9 or ELL2 proteins. FL, full-length; ΔN, deletion of amino acids 1–113; ΔC, deletion of amino acids 430–559. h, Western blot analysis of immunoprecipitation using the M2 anti-Flag antibody in cells expressing wild-type or mutant Flag–ENL. Endogenous CDK9 and AFF4 were assessed. i, qPCR analysis of the Pol II ChIP signal in MYC gene in ENL sgRNA-expressing cells rescued by wild-type or mutant (F59A or Y78A) mouse ENL. Error bars represent mean ± s.e.m. *\( P < 0.5 \), **\( P < 0.001 \) (two-tailed unpaired Student’s t-test). See Supplementary Fig. 1 for gel source data.
Extended Data Figure 7 | The YEATS domain-histone acetylation interaction is required for the role of ENL in leukaemias. a, GSEA plots evaluating the enrichment of signatures related to stem cells, cell cycle or the MYC pathway in the indicated comparisons. b, Quantification of CD11b median intensity 4 days after Dox induction in iCas9-MOLM-13 cells transduced with indicated sgRNAs and rescue constructs. n = 3. ***P < 0.001 by two-tailed unpaired Student’s t-test. c, Negative-selection competition assay that plots the relative percentage of RFP⁺sgRNA⁺ cells after transduction of leukaemia cells with indicated constructs. n = 3. d, Quantification of CD11b median intensity 6 days after Dox induction in iCas9-MOLM-13 cells transduced with indicated sgRNAs and rescue constructs. n = 3. ***P < 0.001 by two-tailed unpaired Student’s t-test. e, Percentage of human CD45⁺ cells in peripheral blood of mice transplanted with MOLM-13 cells expressing indicated sgRNAs and rescue constructs 30 days after injection (n ≥ 8). ****P < 0.0001 by two-tailed unpaired Student’s t-test. All error bars represent mean ± s.d.
Extended Data Figure 8 | Depletion of ENL increases sensitivity to JQ1 by potentiating JQ1-induced transcriptional changes. a, Effect of JQ1 on the proliferation (normalized to DMSO control) of MOLM-13 cells transduced with indicated sgRNAs targeting SEC components. n = 5. b, Effect of JQ1 on the proliferation of indicated MLL-rearranged leukaemia cells transduced with shNT (red) or shENL (blue) shRNAs. (n = 3). c, d, Effect of JQ1 on the proliferation of indicated non-leukaemia cells (U2OS and HeLa) transduced with GFP, AF9 or ENL sgRNAs. n = 5. e, Kaplan-Meier survival curves of mice (n = 10 per group) transplanted with iCas9-MOLM-13 cells expressing indicated sgRNAs and pretreated with doxycycline for 4 days and JQ1 (or DMSO control) for 2 days ex vivo. P values were calculated using a log-rank test. f–i, RNA for RNA-seq experiments was obtained from sorted RFP+ sgRNA+ iCas9-MOLM-13 cells treated with DMSO (marked as '0') or 50 nM JQ1 for 24 h. Row-normalized heat map (f and h) and box plots of relative expression levels (z-scores, g and i) of genes found to be twofold downregulated (f and g) or upregulated (h and i) after JQ1 treatment in ENL sgRNA-expressing cells. All error bars represent mean ± s.e.m.
Extended Data Table 1 | Data collection and refinement statistics

|                       | ENL YEATS-H3K27ac† |
|-----------------------|--------------------|
|                       | (PDB code: 5J9S)   |
| **Data collection**   |                    |
| Space group           | P3,21              |
| Cell dimensions       |                    |
|   \(a, b, c\) (Å)     | 105.0, 105.0, 45.1 |
|   \(\alpha, \beta, \gamma\) (°) | 90, 90, 120 |
| Resolution (Å)        | 50.00-2.70(2.75-2.70)* |
| \(R_{\text{sym}}\)    | 19.5(53.3)         |
| \(I/\sigma(I)\)       | 8.5(2.3)           |
| Completeness (%)      | 98.5(97.7)         |
| Redundancy            | 4.2(4.0)           |
| **Refinement**        |                    |
| Resolution (Å)        | 40.4-2.7           |
| No. reflections       | 8,050              |
| \(R_{\text{work}} / R_{\text{free}}\) | 18.5/21.6 |
| No. atoms             |                    |
|   Protein             | 1,249              |
|   Peptide/ion(sulfate)| 44/20              |
|   Water               | 51                 |
| \(B\) factors (Å²)   |                    |
|   Protein             | 22.4               |
|   Peptide/ion(sulfate)| 34.9/42.4          |
|   Water               | 22.9               |
| R.m.s. deviations     |                    |
|   Bond lengths (Å)    | 0.003              |
|   Bond angles (°)     | 0.611              |

* Values in parentheses are for highest-resolution shell.
† One crystal was used for the data collection.

*a Values in parentheses are for highest-resolution shell.
*b One crystal was used for the data collection.