

# Myeloid leukemia vulnerabilities at CTCF-enriched long noncoding RNA loci

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# Myeloid leukemia vulnerabilities at CTCF-enriched long noncoding RNA loci

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## 1 Abstract

2 The noncoding genome presents a largely untapped source of biological insights, including  
3 thousands of long noncoding RNA (lncRNA) loci. While some produce *bona fide* lncRNAs,  
4 others exert transcript-independent *cis*-regulatory effects, and the lack of predictive features  
5 renders mechanistic dissection challenging. Here, we describe CTCF-enriched lncRNA loci  
6 (C-LNC) as a subclass of functional genetic elements exemplified by *MYNRL15*, a pan-myeloid  
7 leukemia dependency identified by an lncRNA-based CRISPRi screen. *MYNRL15* per-  
8 turbation selectively impairs acute myeloid leukemia (AML) cells over hematopoietic stem /  
9 progenitor cells *in vitro*, and depletes AML xenografts *in vivo*. Mechanistically, we show that  
10 crucial DNA elements in the locus mediate its phenotype, triggering chromatin reorganization  
11 and downregulation of cancer dependency genes upon perturbation. Elevated CTCF density  
12 distinguishes *MYNRL15* and 531 other lncRNA loci in K562 cells, of which 43-54% associate  
13 with clinical aspects of AML and 18.4% are functionally required for leukemia maintenance.  
14 Curated C-LNC catalogs in other cell types will help refine the search for noncoding onco-  
15 genic vulnerabilities in AML and other malignancies.

## 16 Main

17 It becomes increasingly clear that the 98% of the human genome that does not encode pro-  
18 tein nonetheless contains a wide range of functional elements that are vital for cellular home-  
19 ostasis<sup>1,2</sup>. These include *cis*-regulatory elements such as enhancers and promoters, insula-  
20 tors and other determinants of genome topology, as well as a large number and variety of  
21 non-protein-coding transcripts. Long noncoding RNAs (lncRNAs) in particular comprise a  
22 substantial portion of the noncoding transcriptome<sup>3-5</sup> and in recent years, have emerged as  
23 important players in diverse cellular processes and contexts<sup>6-8</sup>. The hematopoietic system is  
24 no exception, where lncRNAs have been described to regulate cell programming and fate<sup>9</sup>,  
25 and where their dysregulation has been tied to malignancy<sup>10-16</sup>. lncRNAs present a signifi-  
26 cant opportunity to extend our understanding of human health and disease; however, the  
27 fact remains that the vast majority of lncRNA loci lack functional characterization, and may  
28 regulate cellular behaviour in ways yet unknown. Indeed, characterization is often a difficult  
29 process complicated by *cis*-regulatory mechanisms unrelated to the transcriptional product<sup>17-</sup>  
30 <sup>23</sup>. Improved functional classification systems are imperative for expediting investigations into  
31 lncRNA determinants of pathophysiology, including the search for noncoding oncogenic vul-  
32 nerabilities.

### 33 ***CRISPRi screens of HSPC/AML lncRNAs identify MYNRL15 as a leukemia dependency***

34 Aiming to identify lncRNAs that contribute to myeloid malignancy, we began by analyzing a  
35 noncoding RNA expression atlas of the human blood system encompassing hematopoietic  
36 stem cells (HSCs) and their differentiated progeny, as well as pediatric acute myeloid leuke-  
37 mia (AML) samples<sup>16</sup>. In addition to stem cell signatures reminiscent of those previously es-  
38 tablished for protein-coding genes<sup>24-27</sup>, we discovered progenitor- and AML subtype-associ-  
39 ated lncRNA profiles that could potentially serve as leukemia-specific targets, given their ab-  
40 sence in HSCs (Fig. 1a). To probe this resource for functionality and find novel AML vulnera-  
41 bilities, we conducted a CRISPRi-based dropout screen of 480 lncRNA genes from 8 distinct  
42 signatures in 6 human leukemia cell lines (Fig. 1b). Five were selected to represent relevant

43 cytogenetic subgroups of AML (ML-2, NOMO-1 [*KMT2A*-rearranged], SKNO-1, KASUMI-1  
44 [standard risk with t(8:21)], M-07E [high risk with inv(16)(p13.3q24.3)], and we also included  
45 the well-studied erythroleukemia line K562. One candidate emerged as crucial in all six cell  
46 lines – *AC068831.3* (ID: ENSG00000224441 in Ensembl v91 [release 12/2017]), which we  
47 renamed *MYNRL15* (myeloid leukemia noncoding regulatory locus on chromosome 15; Fig.  
48 1c-d, Extended Data Fig. 1).

49 *MYNRL15* is a low-abundance, nuclear-enriched transcript (Extended Data Fig. 2a-b) origi-  
50 nating from chromosome 15, where it is flanked by two protein-coding genes: *UNC45A* and  
51 *HDDC3* (Fig. 1c). Given the local effect of the CRISPRi system on nearby genes (Extended  
52 Data Fig. 2c), a range of gain- and loss-of-function approaches were necessary in order to  
53 delineate the source of the *MYNRL15* knockdown phenotype (Fig. 1e-f, Extended Data Fig.  
54 2d-g). While CRISPR mediated excision of *MYNRL15* recapitulated the effect produced by  
55 CRISPRi, repression of the transcript via RNAi and LNA-gapmeRs had little impact on cell  
56 viability (Fig. 1e, Extended Data Fig. 2d-f). Both protein-coding neighbors were also dispen-  
57 sable, as determined via CRISPR-Cas9 mediated knockout of *UNC45A* and *HDDC3*, and  
58 CRISPRi mediated knockdown of *HDDC3* (Fig. 1e, Extended Data Fig. 2d-g). In addition,  
59 overexpression of *MYNRL15* cDNAs failed to rescue the CRISPRi knockdown phenotype  
60 (Fig. 1f). Altogether, these results indicate that neither of the flanking protein-coding genes,  
61 nor the *MYNRL15* transcript, is responsible for the function of this locus in myeloid leukemia  
62 cells, and rather suggest *MYNRL15* as an expressed noncoding regulatory locus.

### 63 ***Functional dissection of the MYNRL15 locus reveals crucial regulatory regions***

64 Given the apparent dispensability of *UNC45A*, *HDDC3*, and the *MYNRL15* transcript itself in  
65 leukemia cells, we hypothesized that *MYNRL15* may harbor DNA regulatory elements which  
66 drive its leukemia dependency phenotype. To test this hypothesis, we functionally dissected  
67 the *MYNRL15* locus via complementary CRISPRi and CRISPR-Cas9 screens tiling a 15 kb  
68 area centered on *MYNRL15*. Notably, this area exhibits features characteristic of *cis*-regula-

69 tory elements (CREs), including H3K4Me1 and H3K27Ac histone marks, DNase hypersensi-  
70 tivity, and transcription factor occupancy (Fig. 2a). The screens uncovered two crucial DNA  
71 regions whose accessibility and integrity were required for the maintenance of leukemic cells  
72 (Fig. 2b, Extended Data Fig. 3a), and which enhanced reporter gene expression in dual lucif-  
73 erase assays (Extended Data Fig. 3b) – identifying the regions as functional sequences and  
74 candidate *cis*-regulatory elements (cCREs C1 and C2). We note that the parallel screening  
75 strategy using the dCas9 variant helps alleviate the risk of potential off-target DNA damage-  
76 driven phenotypes<sup>28</sup>, thus increasing the robustness of the screen. The CRISPR-Cas9 muta-  
77 genesis strategy also reiterates that leukemia cells do not appear particularly dependent on  
78 the *UNC45A* and *HDDC3* coding sequences, arguing against local enhancer functions on  
79 these genes underlying the anti-leukemic effect of *MYNRL15* perturbation.

80 Aiming to identify target genes and pathways controlled by the *MYNRL15* cCREs, we next  
81 performed RNA sequencing (RNA-seq) upon CRISPR-Cas9 mediated perturbation of the lo-  
82 cus. We opted for the CRISPR-Cas9 system in order to achieve a more targeted perturba-  
83 tion of *MYNRL15* and attenuate effects on *UNC45A* and *HDDC3* caused by CRISPRi. We  
84 selected two guides from each cCRE, all of which robustly depleted K562 and ML-2 leuke-  
85 mia cells (Fig. 2c). This phenotype was underpinned by global changes in gene expression  
86 (Fig. 2d, Extended Data Fig. 4a-d), including a dramatic suppression of cancer-essential sig-  
87 natures related to proliferation and metabolism (Fig. 2d, Extended Data Fig. 4d). While these  
88 results corroborated *MYNRL15*'s leukemia dependency phenotype – with the downregulated  
89 genes being enriched for members of key oncogenic pathways (Fig. 2d, Extended Data Fig.  
90 4c-d) – no clear target genes emerged, leading us to consider the possibility that *MYNRL15*  
91 may instead regulate multiple genes in a genomic neighborhood<sup>29</sup> in a more subtle manner.  
92 To explore this option, we applied a sliding window approach to gene set enrichment analy-  
93 sis using 1 Mb to 5 Mb sections of chromosome 15. This revealed positional gene sets that  
94 were coordinately deregulated upon *MYNRL15* perturbation, including the local region  
95 around *MYNRL15* and several distal regions (Fig. 2e, Extended Data Fig. 4e).

96 ***Altered chromosome 15 architecture underlies the MYNRL15 perturbation phenotype***

97 Given the deregulation of chromosome 15 neighborhoods upon *MYNRL15* perturbation, we  
98 explored whether *MYNRL15* may be involved in chromatin conformation via next generation  
99 Capture-C (NG Capture-C)<sup>30</sup>, using probes complementary to *MYNRL15* cCRE C1 to enrich  
100 for interactions involving the locus. This approach revealed extensive chromatin contacts be-  
101 tween *MYNRL15* and sequences within a 500 kb radius, with weaker contacts occurring up  
102 to 2 Mb away – a profile that was consistent across K562 and ML-2 cells (Fig. 3a-c). The lo-  
103 cal interaction peaks demarcate nearby contact domains (Fig. 3c, Extended Data Fig. 5a-b),  
104 implicating *MYNRL15* in the 3D organization of this region of chromosome 15. Interestingly,  
105 *MYNRL15* perturbation had little impact on this local interaction profile, instead causing cells  
106 to gain two long-range interactions 12 Mb and 15 Mb upstream of the locus at the base of a  
107 hierarchical loop (Fig. 3b, Extended Data Fig. 5a) – pointing to 3D chromatin reorganization  
108 upon *MYNRL15* perturbation that brings the locus into contact with this structure. We further  
109 note the presence of distal interactions in this region in CD34<sup>+</sup> hematopoietic stem / progeni-  
110 tor cells (HSPCs; Extended Data Fig. 6a), suggesting that *MYNRL15* perturbation in leuke-  
111 mic cells may re-establish the long-range connectivity of normal blood cells. Consistent with  
112 *MYNRL15*'s involvement in chromosome 15 conformation, CTCF – a fundamental determi-  
113 nant of genome architecture, which occupies three sites in the *MYNRL15* locus (Fig. 2a) –  
114 showed reduced binding at the locus and distal interaction sites following *MYNRL15* pertur-  
115 bation, among other changes (Fig. 3d, Extended Data Fig. 6b). This was accompanied by  
116 diffuse gains in chromatin accessibility across the distal interaction sites (Fig. 3e, Extended  
117 Data Fig. 6c).

118 Integrating our findings regarding altered chromatin conformation and gene expression with  
119 cancer dependency data, we eventually pinpointed the target genes of *MYNRL15* through a  
120 small-scale CRISPR-Cas9 screen of the 29 protein-coding genes located in the gained inter-  
121 action region. Combining these results with differential expression information, we identified

122 two downregulated protein-coding genes in the region that also score as leukemia depend-  
123 encies: *IMP3* and *WDR61* (Fig. 3f, Extended Data Fig. 6d-e). Notably, *WDR61* is a compo-  
124 nent of the PAF1 complex (PAF1c), which is involved in important transcriptional programs  
125 during hematopoiesis and leukemogenesis<sup>31,32</sup>. Gene expression changes induced by Paf1c  
126 inactivation<sup>33</sup> were also detected upon *MYNRL15* perturbation (Extended Data Fig. 6f). *IMP3*  
127 encodes a component of the 60-80S U3 small nucleolar ribonucleoprotein, which is required  
128 for cleavages in pre-18S ribosomal RNA processing<sup>34</sup>. It is a homolog of the yeast Imp3 pro-  
129 tein and has not been comprehensively studied in human cells to date. CRISPR-Cas9 medi-  
130 ated knockout of *WDR61* and *IMP3* robustly depleted K562 and ML-2 cells (Extended Data  
131 Fig. 6g), recapitulating the *MYNRL15* perturbation phenotype and positioning these genes  
132 as targets of *MYNRL15* (Fig. 3g).

### 133 ***AML specificity and potential therapeutic applicability of MYNRL15***

134 To evaluate whether *MYNRL15* dependency is specific to leukemic cells, we leveraged all-  
135 in-one lentiviral CRISPR-Cas9 constructs in primary human CD34<sup>+</sup> HSPCs and blasts de-  
136 rived from two AML patients (see Supplementary Table 1 for clinical characteristics). The  
137 transduced cells were sorted and seeded in methylcellulose-based colony-forming assays.  
138 While *MYNRL15* perturbation moderately attenuated colony formation in CD34<sup>+</sup> HSPCs, it  
139 had little effect on replating capacity and differentiation (Extended Data Fig. 7a). In contrast,  
140 AML colony-forming units were virtually eradicated (Extended Data Fig. 7b) – implying that  
141 *MYNRL15* perturbation selectively impacts AML cells, and outlining a possible therapeutic  
142 window (Fig. 4a).

143 To assess the therapeutic potential of *MYNRL15* perturbation, we applied CRISPRi-based  
144 two-color competitive xenotransplantation assays using AML cell lines and patient-derived  
145 xenografts (PDXs) (Fig. 4b). Importantly, *MYNRL15* perturbation significantly impaired the  
146 propagation of two AML cell lines and two PDXs (Fig. 4b, Extended Data Fig. 7c-d) in the  
147 hematopoietic organs of recipient mice, confirming its capacity to deplete leukemic cells *in*

148 *vivo*. Combined with the apparent AML-specific effect of *MYNRL15* perturbation, these re-  
149 sults provide a proof-of-principle of how *MYNRL15* perturbation may be leveraged as a ther-  
150 apeutic strategy.

### 151 ***MYNRL15 exemplifies a putative new class of CTCF-bound long noncoding RNA loci***

152 Having implicated *MYNRL15* in 3D genome organization and demonstrated its therapeutic  
153 potential, we explored whether *MYNRL15* may belong to a sub-category of biologically rele-  
154 vant lncRNA loci that have thus far been overlooked due to their lack of transcript-specific  
155 functions. Given the effect of *MYNRL15* on chromatin architecture and the multiple CTCF  
156 binding sites in the locus, we explored CTCF density as a predictive metric for identifying  
157 noncoding regulatory loci like *MYNRL15* (Fig. 5a, Extended Data Fig. 8a-c). Interestingly,  
158  $\log_{10}$ -transformed values of this metric followed a near-normal distribution, and a cut-off of  
159 two standard deviations from the median identified 654 genes with elevated CTCF density  
160 which were highly enriched for lncRNAs (>80%, n=531 using K562 ChIP-seq data) (Fig. 5a,  
161 Extended Data Fig. 8a-b). Inversely, the remainder comprised mainly of protein-coding loci  
162 (Fig. 5a, Extended Data Fig. 8b), with *bona fide* lncRNAs such as *PVT1* and *XIST* also fea-  
163 turing in the lower part of the ranked list; others do not appear at all, due to an absence of  
164 CTCF binding sites. These observations support CTCF density as a relevant metric for dis-  
165 tinguishing a potential subset of lncRNA loci with transcript-independent functions, hereafter  
166 referred to as CTCF-enriched lncRNA loci, or C-LNC. Besides elevated CTCF density, these  
167 loci also tended to display low gene expression and short genomic length (median 1 kb; Ex-  
168 tended Data Fig. 8c) – mirroring *MYNRL15*, which produces a low-abundance transcript and  
169 spans 2544 bp on chromosome 15, and providing additional predictive features.

170 As a first step in determining the relevance of C-LNCs to myeloid leukemia, we tested their  
171 association with clinical aspects in two AML patient cohorts<sup>35,36</sup>. This revealed that 43% and  
172 54% of the identified C-LNCs associated with genetically-defined AML subgroups or patient  
173 survival in the two AML cohorts, respectively (Fig. 5a-b, Extended Data Fig. 8d-e), suggest-

174 ing that activity at these loci could underpin aberrant transcription factor programs and/or in-  
175 fluence patient outcomes. Furthermore, 18.4% functionally validated as essential for myeloid  
176 leukemia maintenance in CRISPR-Cas9 screens tiling the CTCF sites in the loci (Fig. 5c, Ex-  
177 tended Data Fig 8f) – a hit identification rate that is substantially higher than what is typically  
178 reported for lncRNA essentiality screens<sup>37-39</sup> (ranging from 2-6%), including our own (4.6%;  
179 Fig. 5c, Extended Data Fig. 8f). Taken together, these data illustrate the effectiveness of  
180 CTCF density metrics in refining functional lncRNA candidate lists, and underline the rele-  
181 vance such loci hold for AML and cancer pathophysiology in general. We provide a catalog  
182 of C-LNCs across 18 cell lines and primary cell types (Fig. 5d, Extended Data Fig. 9, Supple-  
183 mentary Table 11) – [www.C-LNC.org](http://www.C-LNC.org) – as a basis for advancing the discovery of both novel  
184 oncogenic vulnerabilities and functional lncRNA loci in other contexts.

## 185 Discussion

186 There is general agreement that the current lncRNA classification system leaves much to be  
187 desired, necessitating extensive experimental labor in order to discern between the possible  
188 modes of action for any given lncRNA<sup>22</sup>. Through the functional and molecular dissection of  
189 *MYNRL15*, we provide evidence for myeloid leukemia vulnerabilities at noncoding regulatory  
190 loci involved in chromatin architecture, and demonstrate pathophysiological as well as poten-  
191 tial therapeutic relevance. We moreover present predictive metrics based on distinct features  
192 of *MYNRL15* – namely, elevated CTCF density, low expression, and short span – and estab-  
193 lish their value in distinguishing a functionally and clinically relevant subclass of lncRNA loci  
194 (CTCF-enriched lncRNA loci, or C-LNC). A catalog of C-LNCs covering 18 cell lines and pri-  
195 mary cell types can be found at [www.C-LNC.org](http://www.C-LNC.org) or in Supplementary Table 11, providing a  
196 basis for extending investigations of C-LNCs into other cellular contexts. These and other ef-  
197 forts aimed at improving the functional classification of lncRNAs<sup>19,22,40,41</sup> will expedite the de-  
198 velopment of precise and comprehensive annotations, and facilitate the process of discrimi-  
199 nating transcript-dependent from -independent functions.

200 In our study, *MYNRL15* perturbation resulted in the formation of a long-range chromatin in-  
201 teraction, leading to the downregulation of *WDR61* and *IMP3* and tumor suppression. Given  
202 the accompanying reduction of CTCF occupancy, we expect this to occur through a mecha-  
203 nism similar to topologically associating domain (TAD) fusion<sup>42-44</sup>, although on a larger scale  
204 than typically observed for TADs<sup>45</sup>. Alternatively, or perhaps concurrently, the attenuation of  
205 CTCF binding upon *MYNRL15* perturbation may strengthen compartmentalization<sup>46</sup> and pro-  
206 mote longer-range, higher-order architecture. We note that, while there is substantial overlap  
207 between enhancer RNA (eRNA) and lncRNA annotations<sup>47</sup>, and while some of our data sup-  
208 port a local enhancer-like function for *MYNRL15*, we did not find evidence for locally-driven  
209 phenotypes or RNA function, and the long-range architectural changes upon perturbation of  
210 the locus especially separate *MYNRL15* from classical eRNAs.

211 Given the attenuated impact of *MYNRL15* perturbation on normal HSPCs compared to AML  
212 cells, we surmise that distal connectivity may be the native conformation of the locus that is  
213 lost during leukemic transformation; thus re-introducing it would selectively impair leukemic  
214 cells. The oncogenic rewiring of 3D chromatin architecture through mutations and structural  
215 variants has been reported in cancer<sup>44,48-51</sup>. However, it is unlikely that genetic alteration un-  
216 derlies *MYNRL15*'s role in leukemia, since the locus is required by cells from varied cytoge-  
217 netic and mutational backgrounds, and its perturbation drives matching chromatin changes  
218 in two divergent cell lines. We speculate instead that *MYNRL15* may be involved in unifying  
219 leukemic genome organization signatures – a phenomenon that has long been established  
220 for stemness-related expression and epigenetic signatures<sup>24,25,39</sup>. Recent works have begun  
221 to implicate aspects of chromatin architecture in cell state transitions during hematopoiesis<sup>52-</sup>  
222 <sup>55</sup> and in the maintenance of leukemic transcription programs<sup>56-58</sup>. We expect future studies  
223 will further reveal leukemic 3D genome organization signatures that underpin general onco-  
224 genic behaviors, irrespective of mutational drivers.

225 An important future direction will be to ascertain whether C-LNCs, like *MYNRL15*, contribute  
226 to cancer-related chromatin architecture as a class. Based on their elevated CTCF densities

227 and other shared features, we hypothesize that many may function through similar mecha-  
228 nisms as *MYNRL15*. With our preliminary catalog of C-LNCs spanning various human cell  
229 types and tissue contexts ([www.C-LNC.org](http://www.C-LNC.org)), we provide a resource that will help catalyze  
230 future research and lay a foundation for unravelling principles of C-LNC function in healthy  
231 and malignant cells. Given the high essentiality rate that we observed in myeloid leukemia,  
232 C-LNCs could symbolize a major refinement in the search for both functional lncRNA loci  
233 and noncoding oncogenic vulnerabilities across all types of cancer.

## 234 **Materials and methods**

### 235 ***Cells and cell culture***

236 HEK293T cells and the human leukemia cell lines K562, ML-2, NOMO-1, KASUMI-1, SKNO-  
237 1, and M-07E were obtained from the German National Resource Center for Biological Mate-  
238 rial (DSMZ, Braunschweig, Germany) and cultured according to their recommendations. All  
239 cell lines were routinely tested for *Mycoplasma* contamination. Human CD34<sup>+</sup> hematopoietic  
240 stem and progenitor cells (HSPCs) were isolated from mobilized peripheral blood from anon-  
241 ymous healthy donors, and enriched using anti-CD34 immunomagnetic microbeads (Miltenyi  
242 Biotech). Acute myeloid leukemia (AML) patient samples were provided by the Berlin-Frank-  
243 furt-Münster Study Group (AML-BFM-SG, Essen, Germany), and expanded via serial xeno-  
244 transplantation in immunocompromised humanized mice. Clinical information for the patient-  
245 derived xenografts (PDXs) used in this study can be found in Supplementary Table 1. CD34<sup>+</sup>  
246 HSPC and PDX maintenance are described below. Informed consent was obtained from all  
247 human participants or custodians. All investigations were approved by the local ethics com-  
248 mittee of the Martin Luther University Halle-Wittenberg.

### 249 ***Lentiviral vectors***

250 Individual single guide RNAs (sgRNAs) were designed using CCTop<sup>59</sup> ([https://cctop.cos.uni-  
251 heidelberg.de/](https://cctop.cos.uni-heidelberg.de/)), and cloned via BsmBI into the SGL40C.EFS.dTomato (Addgene 89395) or  
252 SGL40C.EFS.E2Crimson (100894) backbone. Short hairpin RNAs (shRNAs) were designed

253 using the Adams *et al.* miR-N tool<sup>60</sup> (<https://felixfadams.shinyapps.io/miRN/>) and cloned via  
254 BsmBI into the SIN40C.SFFV.GFP.miR30n (169278) backbone. Non-targeting control sgR-  
255 NAs and shRNAs were designed against firefly luciferase. *MYNRL15* cDNAs (transcript IDs:  
256 ENST00000448987.1 and ENST00000438890.1; long and short isoforms respectively) were  
257 expressed from the bidirectional LBid.Inc.GFP<sup>61,62</sup>. The L40C-CRISPR.EFS.mNeon (170483)  
258 all-in-one system was used on primary cells for *in vitro* assays. Stable cell lines were gener-  
259 ated using either pLKO5d.SFFV.dCas9-KRAB.P2A.BSD or pLKO5d.EFS.SpCas9.P2A.BSD  
260 (90332 and 57821 respectively). Stable PDXs were generated using SIN40C.SFFV.dCas9-  
261 KRAB.P2A.mNeon (170482). The sgRNA libraries used in this study were expressed from  
262 the following backbones: SGL40C.EFS.dTomato (89395; CRISPRi lncRNA and *MYNRL15*  
263 tiling), SGL.EFS.tBFP (173915; gained chromatin interaction region protein-coding), and  
264 SGL.EFS.dTomato.P2A.PAC (173914; CTCF-enriched loci).

265 Lentiviral particles were produced by co-transfecting the expression vector and the packag-  
266 ing plasmids pMD2.G and psPAX2 (Addgene 12259 and 12260 respectively) into HEK293T  
267 cells using polyethylenimine (PEI). Viral particles were concentrated via ultracentrifugation,  
268 and in the case of all-in-one constructs, were further concentrated using Lenti-X™ Concen-  
269 trator reagent (TaKaRa). Transductions were performed in normal cell culture media, in the  
270 presence of Polybrene (Sigma-Aldrich).

271 Individual sgRNA and shRNA sequences are listed in Supplementary Tables 2-3. All plas-  
272 mids have been deposited in Addgene.

### 273 **LNA-GapmeRs**

274 Custom- antisense LNA-GapmeRs targeting the *MYNRL15* transcript were obtained from Qi-  
275 agen through their in-house design tool. Negative control B (Qiagen 339515) was used as a  
276 non-targeting control. Cells were cultured in media containing 2.5  $\mu$ M LNA-GapmeR for de-  
277 livery by unassisted uptake<sup>63</sup>. Fresh LNA-GapmeR was added every 2 days to maintain its

278 concentration in the culture media. LNA-GapmeR sequences can be found in Supplemen-  
279 tary Table 4.

### 280 **CRISPR library sgRNA design**

281 Guides for the CRISPRi-based targeting of HSPC/AML lncRNAs were designed using the  
282 standalone version of CCTop<sup>59</sup> (<https://cctop.cos.uni-heidelberg.de/>). In brief, the lncRNA  
283 genes were annotated using GENCODE v25 (release 03/2016)<sup>64</sup>, LNCipedia 4.0 (release  
284 05/2016)<sup>65</sup>, and NONCODE v4 (release 01/2014)<sup>66</sup> as previously described<sup>16</sup>, and sgRNAs  
285 were selected 0 bp to 250 bp downstream of transcription start sites (TSSs)<sup>67</sup>. Three to nine  
286 sgRNAs were selected per gene, depending on the number of different TSSs present in the  
287 transcript isoforms and the distance between them. Genes with a single TSS, or with multi-  
288 ple TSSs with high transcript-level support (TSL 1 or 2, according to Ensembl annotations)  
289 spaced more than 300 bp apart, were targeted using three sgRNAs per TSS in a 0-150 bp  
290 window downstream of the respective TSS. Genes with multiple TSSs in close proximity to  
291 each other (spaced  $\leq 150$  bp apart) were targeted using five sgRNAs in a 0-250 bp window  
292 downstream of the first TSS. Guides were prioritized for low off-target binding – a criterion  
293 that is built-in to the CCTop tool.

294 Guides tiling the *MYNRL15* locus were designed by inputting 15 kb of DNA sequence (hg38)  
295 symmetrically centered on *MYNRL15* into the CRISPOR<sup>68</sup> (<http://crispor.tefor.net/>) saturating  
296 mutagenesis assistant. To maintain dense tiling of the region (mean coverage: 0.11 sgRNAs  
297 per bp), only guides with an MIT specificity score of 0 were excluded.

298 Guides targeting the 29 protein-coding genes located in the gained distal chromatin interac-  
299 tion region were designed using CCTop<sup>59</sup> (<https://cctop.cos.uni-heidelberg.de/>). Coding se-  
300 quences (CDS) from Ensembl v102 (release 11/2020) were used as inputs, and where pos-  
301 sible, sgRNAs were selected to target most, if not all, protein-coding isoforms. Guides were  
302 prioritized for low off-target binding, and those with low predicted on-target efficacies (CRIS-  
303 PRater<sup>69</sup> score  $< 0.4$ ) were excluded.

304 Guides targeting CTCF sites in CTCF-enriched gene loci were selected using GuideScan<sup>70</sup>  
305 (<http://www.guidescan.com/>) and CRISPick<sup>71,72</sup> (formerly the Broad GPP sgRNA design tool;  
306 <https://portals.broadinstitute.org/gppx/crispick/public>). CTCF binding sites were determined  
307 using ENCODE ChIP-seq peak calling data, and sgRNAs were selected to tile CTCF motifs  
308 and/or point-source(s) within the peaks. If both features were within 50 bp of each other, the  
309 target region was defined as a 150 bp region centered on the midpoint between the two. If  
310 the CTCF motif and point-source were within 100 bp of each other, a 300 bp target region  
311 was used. Otherwise, two 80 bp target regions were used for sgRNA selection, centered on  
312 the motif and point-source, respectively. Guides located in these target regions were first se-  
313 lected from GuideScan, then topped up from CRISPick in cases where a coverage of 0.15  
314 sgRNAs per bp was not met.

315 Due to our usage of SGL40C vectors for lentiviral sgRNA delivery, in which sgRNA transcrip-  
316 tion is driven from a human U6 promoter, guides containing polyT stretches (4 or more) were  
317 excluded from all libraries, to avoid premature termination of sgRNA transcription mediated  
318 by RNA polymerase III. Guides directed against luciferase and the neomycin resistance cas-  
319 sette were used as non-targeting controls; guides targeting *PPP1R12C* and *SLC22A13* were  
320 used as nonessential cutting controls; guides against *MYC*, *MYB*, *ACTB*, *U2AF1*, *RPL9*, and  
321 *POL2RA* were used as positive depletion controls. The sgRNA spacer sequences of the four  
322 CRISPR/Cas9 libraries used in this study can be found in Supplementary Tables 7-10.

### 323 ***CRISPR library cloning and screening***

324 Library spacer sequences were purchased from Integrated DNA Technologies, pooled, and  
325 cloned via BsmBI into one of the following vectors: SGL40C.EFS.dTomato (Addgene 89395;  
326 CRISPRi lncRNA and *MYNRL15* tiling), SGL.EFS.tBFP (173915; gained chromatin interac-  
327 tion region protein-coding), or SGL.EFS.dTomato.P2A.PAC (173914; CTCF-enriched loci).  
328 XL1-Blue supercompetent cells (Agilent 200236) were used for transformation, and subse-

329 quently plated on 15 cm LB agar plates containing ampicillin. Colonies were counted to en-  
330 sure sufficient library representation, and then harvested and prepped for plasmid DNA us-  
331 ing the QIAGEN Plasmid Maxi Kit. Lentiviral particles were produced as outlined above.

332 Stable dCas9-KRAB- or Cas9-expressing cell lines were transduced with the sgRNA librar-  
333 ies at an MOI of 0.3, and maintained at 1000-fold representation of the library for 16-18 pop-  
334 ulation doublings. Genomic DNA was isolated from cells via the QIAmp DNA Blood Mini Kit  
335 (Qiagen) at the beginning and end of the screen, and the sgRNA cassettes were PCR ampli-  
336 fied using NEBNext® High-Fidelity 2x PCR Master Mix (New England Biolabs) and primers  
337 containing the Illumina P5 and P7 adapter sequences as overhangs. The amplicons (~300  
338 bp) were gel purified using the GeneJET Gel Extraction Kit (Thermo Fisher Scientific) and  
339 sequenced on an Illumina HiSeq 2000 (50 bp single-end reads).

340 We applied the MAGeCK (model-based analysis of genome-wide CRISPR-Cas9 knockout)<sup>73</sup>  
341 pipeline to process raw reads and call AML dependency genes from the CRISPRi IncRNA,  
342 gained chromatin interaction region, and CTCF-enriched loci screens. The *MYNRL15* tiling  
343 screens were analysed in R, using DESeq2<sup>74</sup> (Bioconductor) to combine replicates and per-  
344 form pan-cell line analysis.

#### 345 ***Hematopoietic assays with primary cells***

346 CD34<sup>+</sup> HSPCs were thawed and expanded in StemSpan SFEM (STEMCELL Technologies)  
347 containing 1% penicillin/streptomycin (Gibco™), 100 ng/ml SCF, 100 ng/ml FLT3L, 20 ng/ml  
348 IL6, 50 ng/ml TPO (cytokines from Peprotech), and 750nM SR1 (STEMCELL Technologies)  
349 for 2 days prior to transduction. Cells were transduced in the presence of 4 µg/ml Polybrene  
350 (Sigma-Aldrich) on RetroNectin®-coated plates (TaKaRa), using two consecutive rounds of  
351 super-concentrated virus ~4 hours apart. Four days post-transduction, HSPCs were sorted  
352 and plated in human methylcellulose complete medium HSC003 (R&D Systems) for colony-  
353 forming assays. Fifteen thousand cells were initially plated across two 6 mm dishes. The col-  
354 onies were counted once they had reached a sufficient size (10-14 days).

355 For assays using patient-derived AML blasts, *in vivo* expanded PDXs were thawed and pre-  
356 cultured in StemSpan SFEM (STEMCELL Technologies) containing 1% penicillin/streptomycin  
357 (Gibco™), 50 ng/ml SCF, 50 ng/ml FLT3L, 10 ng/ml IL6, 2.5 ng/ml IL3, 10 ng/ml TPO  
358 (cytokines from Peprotech), 750 nM SR1 (STEMCELL Technologies), and 35 nM UM171  
359 (STEMCELL Technologies) for 24 to 48 hours. Transductions were carried out in the pres-  
360 ence of 2 µg/ml Polybrene (Sigma-Aldrich). The cells were harvested 48 hours post-trans-  
361 duction for xenotransplantation into mice or for colony-forming assays.

### 362 ***Mice and transplantation experiments***

363 Two-color *in vivo* competition experiments were performed in murine xenograft models of  
364 AML as previously described<sup>61,75</sup>. In brief, stable dCas9-KRAB cell lines or *in vivo* expanded  
365 patient-derived AML cells (PDXs) were transduced with E2Crimson or dTomato sgRNA vec-  
366 tors, mixed 1:1, and injected via tail vein into irradiated (2.5 Gy), 8-10 week old NOD.Cg-Prk-  
367 dc<sup>scid</sup> Il2rgtm<sup>1Wjl</sup>/SzJ (NSG) recipients. One to two million cells were injected per mouse, and  
368 tracked via peripheral blood every 4 weeks. The mice were sacrificed upon leukemia onset,  
369 and cells harvested from the hematopoietic organs (bone marrow, spleen, and liver) were  
370 analyzed by flow cytometry. All mice were housed in a specific pathogen-free environment.  
371 All animal procedures were approved by the local state authorities (Landesverwaltungsamt  
372 Niedersachsen/Sachsen-Anhalt).

### 373 ***Flow cytometry and cell sorting***

374 Flow cytometry analyses were performed on a CytoFLEX B4-R3-V5 or CytoFLEX S V4-B2-  
375 Y4-R3 (Beckman Coulter). Cell sorting was performed on a FACSAria™ II or FACSMelody™  
376 (BD Biosciences). An anti-human CD45 FITC (Beckman Coulter) antibody was used to ana-  
377 lyze xenotransplantation experiments. Kaluza 2.1 (Beckman Coulter) or FlowJo™ v10.6 (BD  
378 Biosciences) software was used for data analysis.

379 **Gene expression analyses**

380 RNA was isolated from cells using the Quick-RNA™ Microprep or Miniprep Kits (Zymo Re-  
381 search). RNA fractionation was performed as previously described<sup>76</sup>, except that we lysed  
382 the nuclear pellet directly rather than isolating the nuclear-soluble and chromatin-associated  
383 fractions separately. The TURBO DNA-free™ Kit (Invitrogen) was used for DNase treatment.  
384 cDNA was synthesized using the High-Capacity cDNA Reverse Transcription Kit, and gene  
385 expression was quantified via real-time PCR using SYBR™ Select Master Mix and specific  
386 primers on a StepOnePlus™ Real-Time PCR cycler (all products from Applied Biosystems).  
387 *B2M* was used as a housekeeping control. Primer sequences for qRT-PCR can be found in  
388 Supplementary Table 5. To detect *WDR61* and *IMP3*, we used QuantiTect® primer assays  
389 (Qiagen QT00083776 and QT00232330 respectively).

390 RNA sequencing was performed by Novogene Company, Ltd. on an Illumina NovaSeq 6000  
391 (150 bp paired-end reads) using polyA-enriched total cellular RNA. The raw sequence data  
392 were processed by Novogene using a standard pipeline. In brief, reads were filtered using  
393 in-house scripts and mapped to human reference genome hg38 using HISAT2<sup>77</sup>, and gene  
394 expression was quantified using featureCounts<sup>78</sup> in R. The processed count data were sub-  
395 sequently analyzed in R using DESeq2<sup>74</sup> (Bioconductor). Gene sets from MSigDB v7.2 (H1,  
396 C2, C3, C6), custom hematopoietic<sup>16</sup> and chromosome 15 gene sets, and PAF1c-knockout  
397 gene expression signatures<sup>33</sup> were checked for enrichment via the Broad GSEA software<sup>79</sup>.  
398 The custom positional gene sets were generated by walking a 1 Mb or 5 Mb window along  
399 chromosome 15 and taking expressed genes within the windows.

400 **NG Capture-C**

401 Chromatin conformation capture with selective enrichment for *MYNRL15*-interacting se-  
402 quences was performed using next generation (NG) Capture-C as previously described<sup>30</sup>,  
403 with the following modifications: (1) Five to ten million transduced cells were harvested per  
404 sample and the DpnII digestion reactions were scaled down accordingly. Transduced K562  
405 or ML-2 cells were used to assess the effects of *MYNRL15* perturbation. *In vitro* expanded

406 CD34<sup>+</sup> HSPCs (day 3) were used to assess the native conformation of the locus. (2) DNA  
407 was sheared to 200 bp fragments using a Branson 450 Digital Sonifier (Marshall Scientific)  
408 (time 18 s, amplitude 20%, pulse 0.5 s, pause 1.5 s; repeat 5x). (3) All material from the first  
409 capture was used as input for the second capture. (4) The libraries were sequenced by No-  
410 vogene Company, Ltd. on an Illumina NovaSeq 6000 (150 bp paired-end reads).

411 Biotinylated oligonucleotides for library capture were designed using CapSequm2<sup>80</sup> (refer to  
412 Supplementary Table 6 for sequences) and purchased from Integrated DNA Technologies.  
413 These probes capture a viewpoint corresponding to the candidate *cis*-regulatory region C1.  
414 Two biological replicates were prepared per sample and pooled for oligonucleotide capture  
415 (multiplexed library capture<sup>30</sup>). The raw sequence data were processed with the capC-MAP  
416 package<sup>81</sup> using default settings. Normalized pileups (RPMs; binstep=3000, window=6000)  
417 were capped at the 99<sup>th</sup> percentile and scaled to the highest signal within the sample, such  
418 that cross-sample comparisons could be made on a 0 to 1 scale. The tracks were viewed in  
419 the UCSC Genome Browser<sup>82</sup> using a smoothing window of 2 pixels, alongside CTCF ChIP-  
420 seq data from K562 cells (ENCODE accession no. ENCFF519CXF) and Knight-Ruiz matrix-  
421 balanced<sup>83</sup> Micro-C<sup>84</sup> data from H1-hESC cells. Hi-C data from Rao *et al.*<sup>85</sup> were also used to  
422 confirm the presence of specific 3D chromatin structures in other cell lines.

### 423 **ATAC-seq**

424 We performed assay for transposase accessible chromatin sequencing (ATAC-seq) as pre-  
425 viously described<sup>86,87</sup>. On day 3 post-transduction, 50,000 cells were sorted and processed  
426 using the Illumina Tagment DNA Enzyme and Buffer Kit (20034197). The resulting libraries  
427 were sequenced by Novogene Company, Ltd. on an Illumina NovaSeq 6000 (150 bp paired-  
428 end reads). The data processing was also performed by Novogene: In brief, raw reads were  
429 trimmed and filtered using Skewer<sup>88</sup> and clean reads were aligned to hg19 with BWA<sup>89</sup>. Mito-  
430 chondrial reads were removed prior to subsequent analysis. Normalized pileups were gener-  
431 ated using deepTools<sup>90</sup> and viewed in the Integrated Genomics Viewer (IGV)<sup>91</sup>.

432 **CTCF CUT&RUN**

433 We performed CUT&RUN as previously described<sup>92,93</sup>. On day 3 post-transduction, 400,000  
434 cells were sorted and incubated with the following antibodies: rabbit anti-human CTCF (1:50;  
435 Diagenode C15410210), and rabbit IgG (Diagenode C15410206). The pAG/MNase nuclease  
436 (Addgene 123461) was produced and purified as previously described<sup>93</sup>, after removal of the  
437 HA tag. Illumina libraries were constructed from cleaved DNA and sequenced by Novogene  
438 Company, Ltd. on an Illumina NovaSeq 6000 (150 bp paired-end reads). For processing the  
439 raw data, we used Trimmomatic<sup>94</sup> to remove adapter sequences, followed by Kseq<sup>95</sup> to trim  
440 reads containing  $\leq 6$  bp of adapter sequence, which are not effectively handled by Trimmo-  
441 matic. Trimmed reads were aligned to hg38 using bowtie2<sup>96</sup>. The resulting SAM files were  
442 converted into BAM format and sorted and indexed using Samtools<sup>97</sup>. Normalized bigWig  
443 tracks were generated using bamCoverage from deepTools<sup>90</sup>. The processed data were  
444 viewed in the Integrated Genomics Viewer (IGV)<sup>91</sup>.

445 **ENCODE datasets**

446 The following K562 ChIP-seq data were used in this study: CTCF (ENCFF519CXF), SMC3  
447 (ENCFF175UEE), H3K27Ac (ENCFF469JMR), H3K4Me1 (ENCFF100FDI), and H3K4Me3  
448 (ENCFF767UON). In addition, the following CTCF ChIP-seq data from other cell lines and  
449 primary cells were also used: GM12878 (ENCFF960ZGP), H1-hESC (ENCFF821AQO),  
450 A549 (ENCFF535MZG), HeLa-S3 (ENCFF502CZS), IMR90 (ENCFF307XFM), MCF-7  
451 (ENCFF867BUQ), HCT-116 (ENCFF171SNH), HEK293 (ENCFF285QVL), HL-60  
452 (ENCFF432AMS), NB4 (ENCFF456PDQ), CD14+ monocyte (ENCFF300XXC), B cell  
453 (ENCFF910TER), neutrophil (ENCFF122IMV), fibroblast lung (ENCFF777ODE), cardiac  
454 muscle cell (ENCFF301YXM), kidney epithelial cell (ENCFF674KUN), and osteoblast  
455 (ENCFF744PXO).

456 **Dual luciferase assays**

457 Dual luciferase assays were performed using the Dual-Luciferase® Reporter Assay System  
458 (Promega). The candidate *cis*-regulatory regions C1 and C2 were cloned alone or in combi-  
459 nation upstream of the minimal promoter in the pGL4.23 firefly luciferase reporter construct  
460 (Promega E8411). A pGL4.7 *Renilla* luciferase reporter construct (Promega E6881) driven  
461 from the EF1 $\alpha$  promoter was used as a background control. The firefly and *Renilla* vectors  
462 were co-transfected into K562 cells at a 20:1 ratio via nucleoporation, using the Lonza 4D-  
463 Nucleofector™ and SF Cell Line X Kit S. 24 h post-transfection, cells were harvested and  
464 measured on a GloMax® 96 Luminometer (Promega).

465 **CTCF-enriched lncRNA loci**

466 To identify CTCF-bound genic loci, we overlapped ENCODE CTCF ChIP-seq peaks with  
467 gene annotations from GENCODE v23 (release 07/2015)<sup>64</sup> using the findOverlaps function  
468 from the IRanges package in R (Bioconductor). We confirmed the presence of CTCF motifs  
469 in the ChIP-seq peaks using GimmeMotifs<sup>98</sup>. Within each gene, the number of CTCF binding  
470 sites was counted and normalized by gene length. Log10-transformed values of this metric  
471 followed an approximately normal distribution; thus, we defined elevated CTCF density as  
472 over two standard deviations above the median. Our analysis focused on loci that produce  
473 long coding or noncoding transcripts (>200 nt) and included the following biotypes: protein  
474 coding, lncRNA, lincRNA, processed transcript, and pseudogene. Refer to Supplementary  
475 Table 11 for a catalog of CTCF-enriched lncRNA loci (C-LNC) across 18 different cell lines  
476 and primary cell types.

477 For the analysis of C-LNCs in the context of AML, gene expression values were obtained  
478 from the TCGA<sup>36</sup> and NCI-TARGET<sup>35</sup> AML patient cohorts. C-LNCs were deemed clinically  
479 significant if 1) stratifying patients based on their expression yielded a significant difference  
480 in event-free or overall survival ( $P < 0.05$ , log-rank test), or 2) their expression significantly dif-  
481 fered in cases harboring any of the following genetic abnormalities compared to cases with-

482 out: complex karyotype, t(8:21), inv(16), PML-RARA or BCR-ABL translocation, *KMT2A* re-  
483 arrangement, *FLT3*-ITD, or mutations in *CEBPA*, *NPM1*, *DNMT3A*, *TP53*, *cKIT*, or *WT1*  
484 ( $P < 0.05$ , two-sided t-test).

#### 485 ***TCGA/TARGET survival analysis***

486 Event-free survival was defined as the time elapsed between diagnosis and the first event or  
487 last follow-up. An event was defined as death from any cause, failure to achieve remission,  
488 relapse, and secondary malignancy. Failure to achieve remission was considered an event  
489 on day 0. Overall survival was defined as time elapsed between diagnosis and death from  
490 any cause or last follow-up. We used the Kaplan-Meier method of estimating survival rates  
491 and two-sided log-rank tests to compare differences in survival, as implemented in the sur-  
492 vival<sup>99</sup> and survminer<sup>100</sup> packages (base R). DESeq2 (Bioconductor) was used to normalize  
493 and variance-stabilize read count data<sup>74</sup> from the TCGA<sup>36</sup> and NCI-TARGET<sup>35</sup> AML cohorts.  
494 The NCI-TARGET dataset also required batch correction, for which we used sva<sup>101</sup> (Biocon-  
495 ductor). Normalized (and batch corrected) gene expression values were used for all subse-  
496 quent analyses. For patient stratification, optimal cut-offs were determined via maximally se-  
497 lected log-rank statistics as implemented in the maxstat package<sup>102</sup> (base R).

#### 498 ***Statistical analyses and definitions***

499 Statistical evaluations of experimental data were carried out in GraphPad Prism 8 using un-  
500 paired, two-tailed t tests. Data are presented as mean  $\pm$  s.d. or s.e.m. as indicated in the fig-  
501 ure legends. Statistical analysis of gene expression data (RNA-seq) was performed in R us-  
502 ing DESeq2 (Bioconductor). Survival analysis was also done in R using the survival and sur-  
503 vminer packages (base R). CRISPR-Cas9 screening data were analyzed with the MAGeCK  
504 suite, with the exception of the tiling screens, which were analyzed in R using DESeq2. In all  
505 cases, measurements were taken on at least two biological replicates and  $P < 0.05$  was con-  
506 sidered significant. Sample sizes are indicated in the figure legends. No statistical methods  
507 were used to predetermine sample size.

508 **Data availability**

509 All RNA-seq, Capture-C, CUT&RUN, and ATAC-seq data have been deposited in the Gene  
510 Expression Omnibus (GEO) under the accession number GSE172240. The CRISPR-Cas9  
511 screening data have been deposited in the European Nucleotide Archive (ENA) at EMBL-  
512 EBI under the accession numbers PRJEB44308 and PRJEB44320.

513 **Code availability**

514 All computational tools used in this study are publically available. Please refer to the corre-  
515 sponding Methods sections for links or references to the relevant publications. R scripts are  
516 available from the authors upon request.

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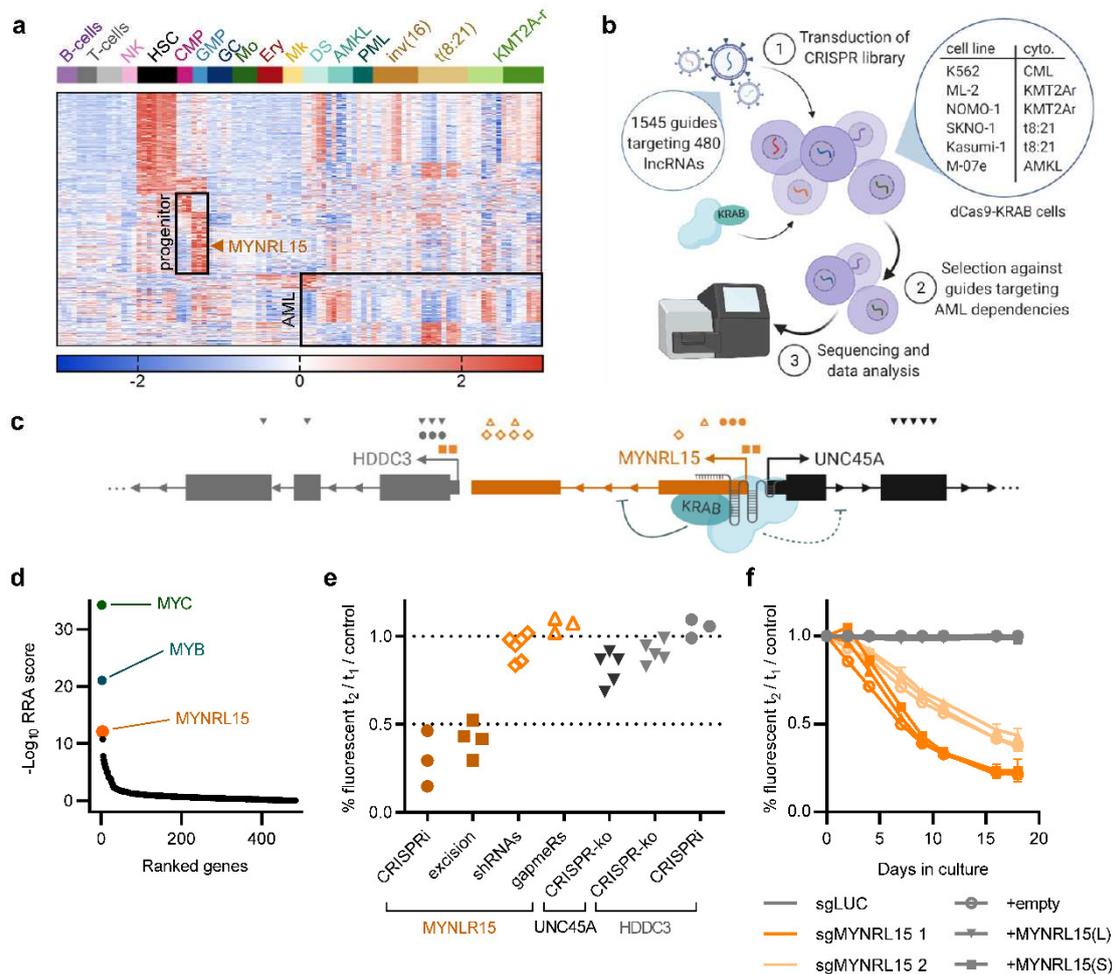
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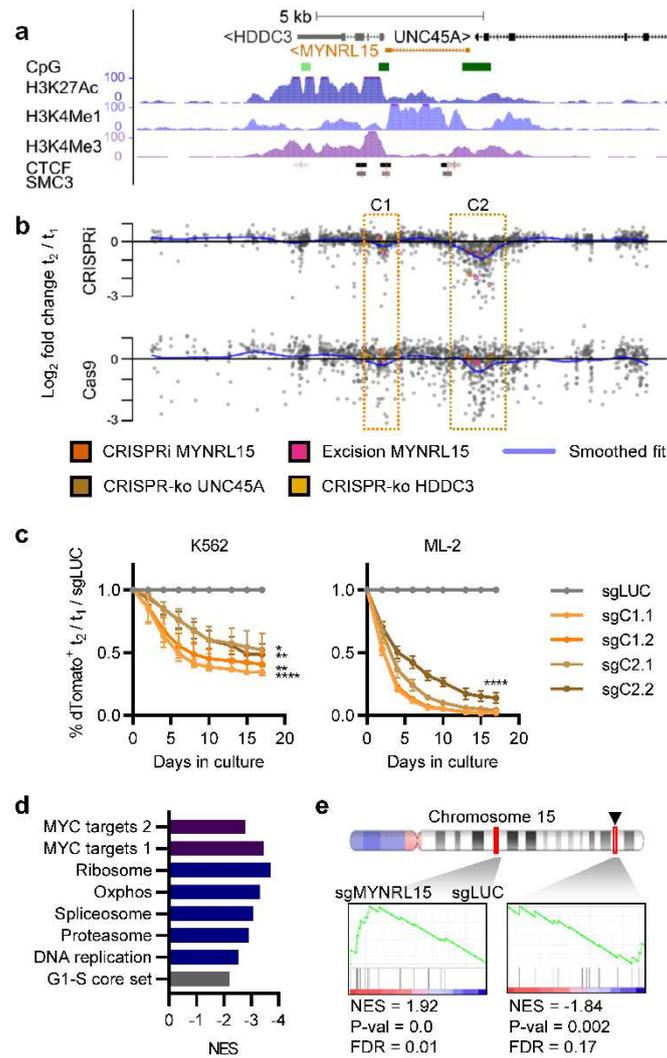
## Figures

**Fig. 1: CRISPRi screen of HSPC/AML IncRNA signatures identifies *MYNRL15* as a myeloid leukemia dependency.**



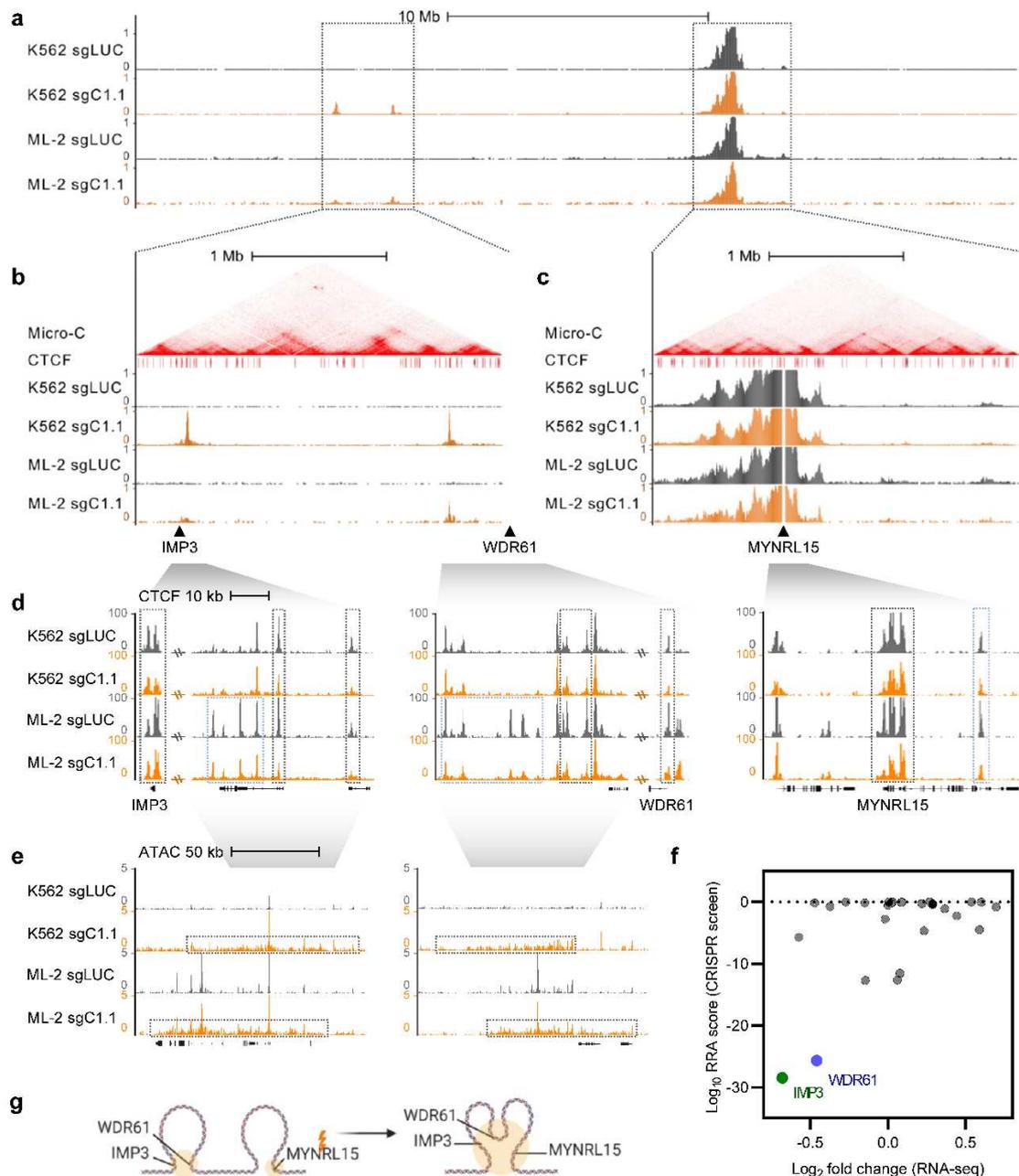
**a**, Expression of HSPC/AML IncRNAs across 12 normal blood cell populations and 46 pediatric AML samples<sup>16</sup>. Signatures of particular therapeutic interest are outlined. Natural killer cell (NK), hematopoietic stem cell (HSC), common myeloid progenitor (CMP), granulocyte-monocyte progenitor (GMP), granulocyte (GC), monocyte (Mo), erythroid precursor (Ery), megakaryocyte (Mk), myeloid leukemia of Down syndrome (DS), non-DS megakaryoblastic leukemia (AMKL), promyelocytic leukemia (PML), *KMT2A*-rearranged leukemia (*KMT2A*-r). **b**, Workflow for screening HSPC/AML IncRNAs. **c**, Schematic of the *MYNRL15* locus (not to scale), including target sites of the different perturbation approaches used. Target gene: *MYNRL15* (orange), *UNC45A* (black), *HDDC3* (grey). Perturbation strategy: CRISPRi (filled circle), dual sgRNA mediated excision (filled square), RNAi (empty diamond), LNA-gapmeR (empty triangle), CRISPR-Cas9 knockout (filled triangle). **d**, Pan-cell line analysis of the CRISPRi screen identifies *MYNRL15* as the top hit behind the positive controls *MYC* and *MYB*. **e**, Endpoint depletion values from proliferation assays using different perturbation strategies in ML-2 cells. Each point corresponds to a vector used for perturbation (mean of n=3 shown). **f**, Proliferation assays using cDNA overexpression to rescue the *MYNRL15* CRISPRi depletion phenotype (n=2; mean  $\pm$  s.e.m.). Long isoform (L), short isoform (S).

**Figure 2: Functional dissection of the *MYNRL15* locus reveals crucial regulatory regions.**



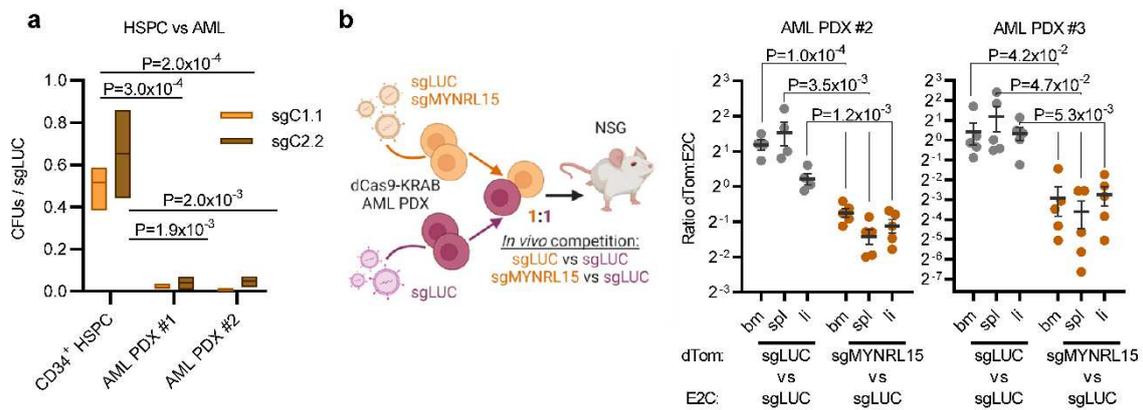
**a**, Tracks from the UCSC Genome Browser showing from top to bottom: gene annotations, CpG islands, histone marks, and CTCF and cohesin occupancy (ChIP-seq from ENCODE). **b**, Tiling screens of the *MYNRL15* locus using complementary CRISPRi (top) and CRISPR-Cas9 based (bottom) strategies (mean of 4 cell lines; n=2 per cell line). Pre-tested sgRNAs are depicted in color. A smoothed fit curve is shown in blue. The two identified leukemia-essential cCREs are outlined. **c**, Individual proliferation assays using sgRNAs from C1 and C2 for CRISPR-Cas9 based perturbation of the *MYNRL15* locus (n=3, mean  $\pm$  s.e.m.; 2 guides per cCRE). \*  $P < 0.05$ ; \*\*  $P < 0.01$ ; \*\*\*\*  $P < 0.0001$ ; where only one set of asterisks is shown, all conditions shared the same  $P$ -value. **d-e**, GSEA comparing *MYNRL15* perturbation (using 4 guides, 2 per cCRE; “sgMYNRL15”) to the non-targeting control (n=2 per guide; “sgLUC”) in combined analyses of K562 and ML-2 cells. **d**, Normalized enrichment scores (NES) of cancer dependency gene sets upon *MYNRL15* perturbation. **e**, Two chromosome 15 gene sets that are downregulated upon *MYNRL15* perturbation.

**Figure 3: *MYNRL15* perturbation alters genome architecture on chromosome 15, leading to downregulation of *WDR61* and *IMP3*.**



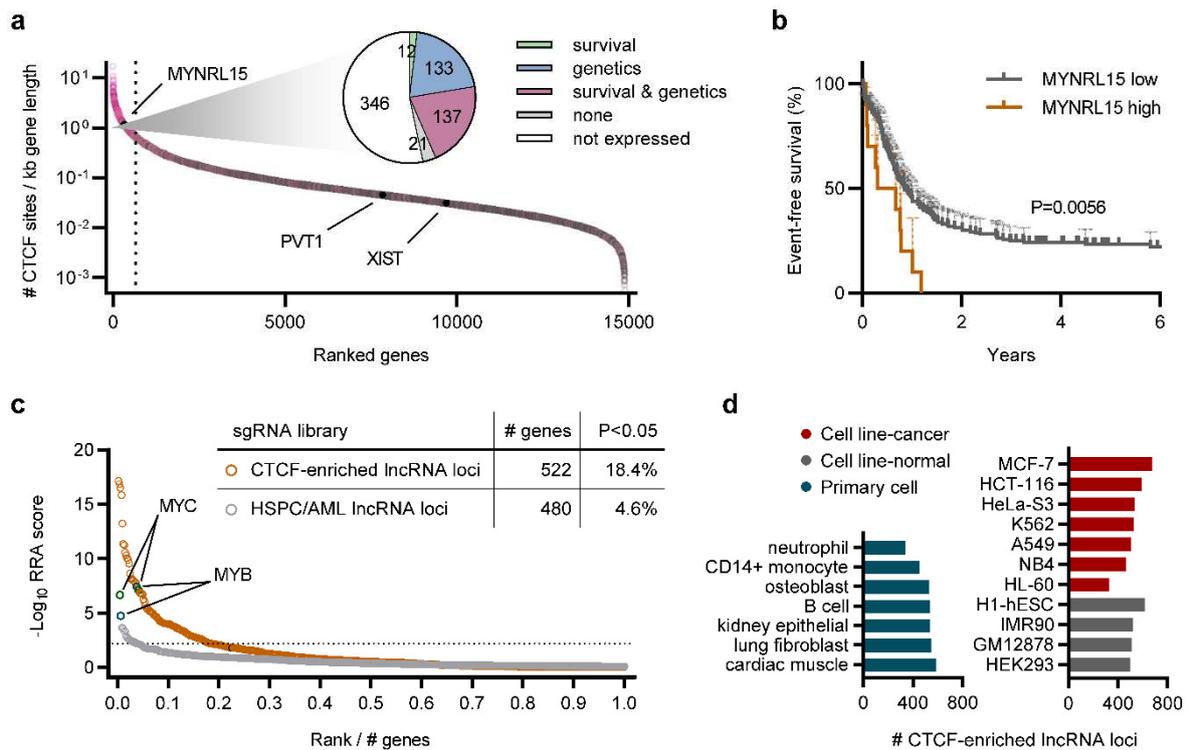
**a**, NG Capture-C interaction profiles on chromosome 15 in K562 and ML-2 cells, using one guide targeting *MYNRL15* (sgC1.1) and a non-targeting control (sgLUC) (n=2; viewpoint in C1; smoothing window 2 pixels). **b-c**, Close-ups of the gained interaction region and the region surrounding *MYNRL15*, with ENCODE CTCF ChIP-Seq and Micro-C<sup>103</sup> tracks from the UCSC Genome Browser. **d**, CTCF occupancy at the *MYNRL15* locus and distal interaction sites, as determined via CUT&RUN. The *MYNRL15*, *IMP3*, and *WDR61* loci, and gained interaction sites are outlined with black boxes. Note the track discontinuity in the left and center views. **e**, Chromatin accessibility at the gained interaction sites, as determined via ATAC-seq. **f**, Integrative analysis depicting CRISPR-Cas9 screening scores and differential expression upon *MYNRL15* perturbation for the 29 coding genes in the gained interaction region. **g**, Model of chromosome 15 reorganization following *MYNRL15* perturbation.

**Figure 4: AML specificity and therapeutic potential of *MYNRL15*.**



**a**, Direct comparison of the impact of *MYNRL15* perturbation on colony-forming capacity in CD34<sup>+</sup> HSPCs and two AML PDXs (HSPC, n=3; PDXs, n=4 each; data presented as mean  $\pm$  range). **b**, Setup (left) and results (right) of direct two-color *in vivo* competition assays testing CRISPRi mediated perturbation of *MYNRL15* in AML PDXs. The data are presented as ratios of dTomato<sup>+</sup> (dTom) to E2Crimson<sup>+</sup> (E2C) cells in the bone marrow (bm), spleen (spl), and liver (li) of transplanted mice at the experimental endpoint (n=4 in the AML PDX #2 control group; otherwise n=5).

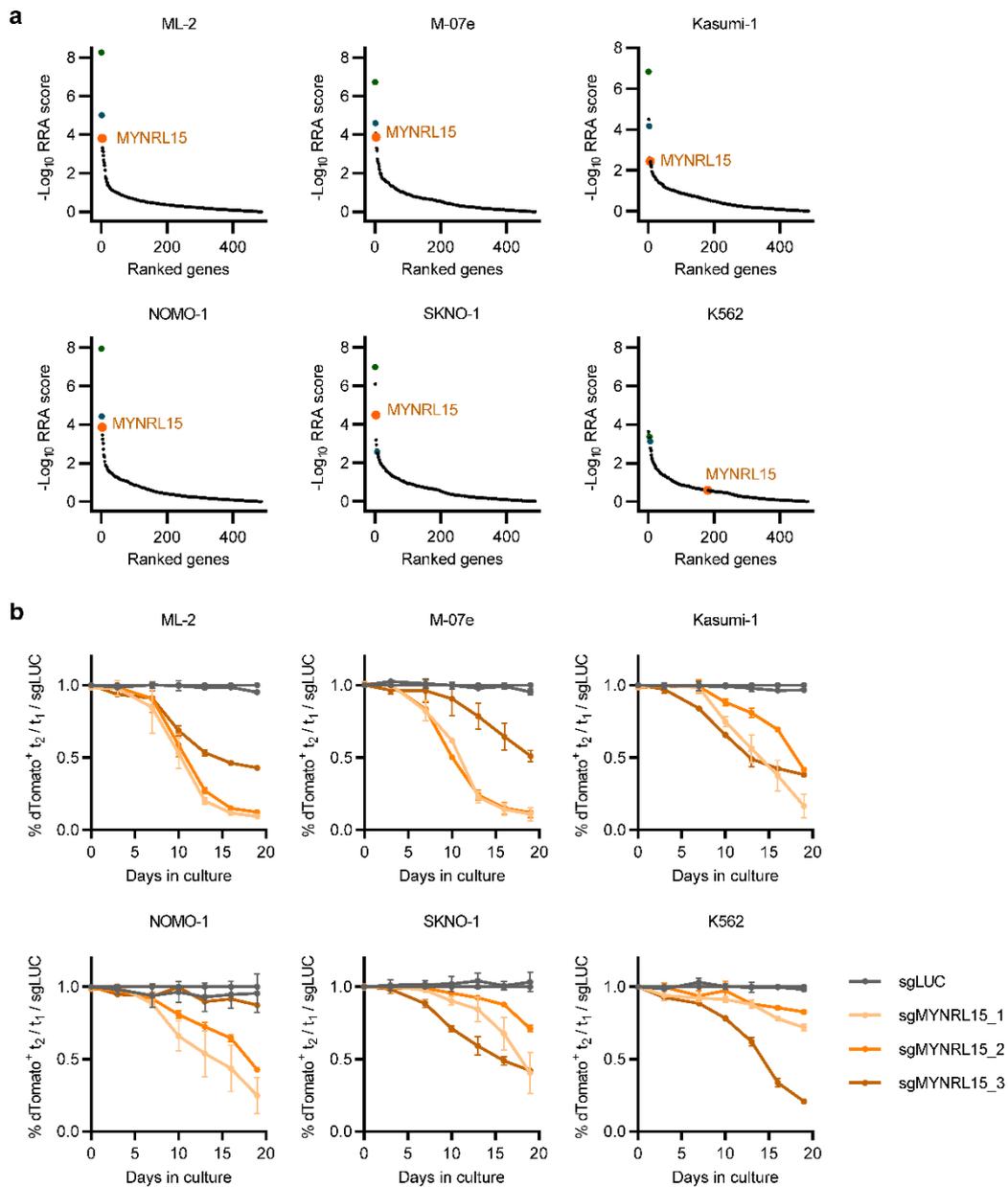
**Figure 5: A subclass of clinically relevant, functionally validated CTCF-enriched lncRNA loci.**



**a**, Ranked list of protein-coding and lncRNA loci ordered by CTCF density. The positions of *MYNRL15*, and the *bona fide* lncRNAs *PVT1* and *XIST* are marked. Non-CTCF-bound loci are not shown. The dashed line indicates the cut-off defined for elevated CTCF binding (i.e. median + 2 s.d. of log<sub>10</sub>-transformed values). Inset: breakdown of CTCF-enriched loci based on their association with clinical characteristics like cytogenetics, mutations, and survival in the TCGA AML cohort. **b**, Kaplan-Meier survival curves of patients with high (n=10) versus low (n=171) expression of *MYNRL15* in the TCGA AML cohort<sup>36</sup> (survival probability ± 95% C.I.). Five-year event-free survival: 23.2% vs 0.0%. **c**, Ranked list of CTCF-enriched lncRNA loci ordered by essentiality, as determined via CRISPR-Cas9 screening and MAGeCK analysis (orange). Results from our initial CRISPRi lncRNA library are displayed alongside (grey) for comparison. Gene ranks are normalized to library size. The positive controls *MYC* and *MYB* are indicated. **d**, Numbers of CTCF-enriched lncRNA loci (C-LNC) in 18 different cell lines and primary cell types.

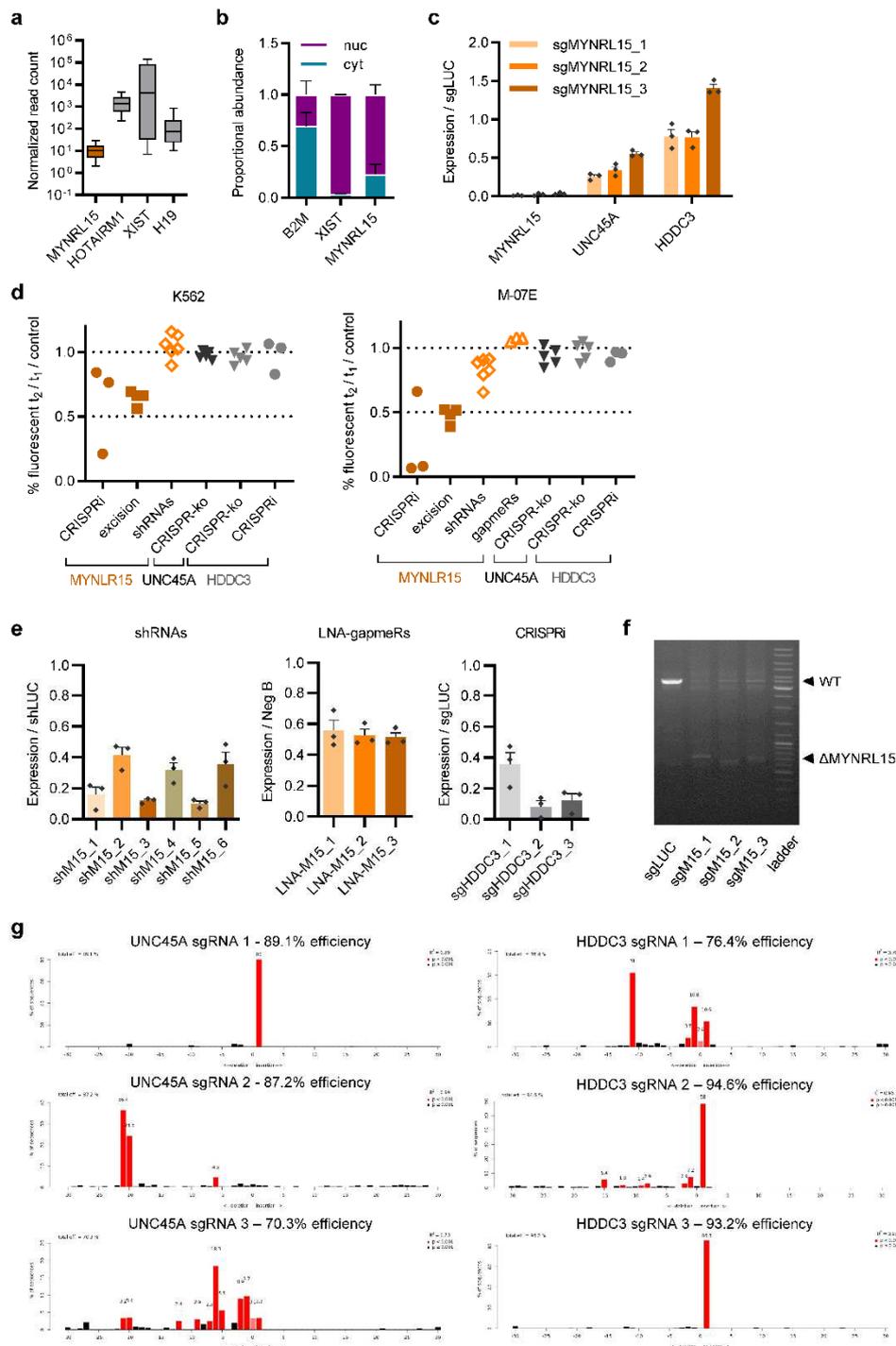
## Extended data figures

### Extended Data Figure 1: Identification and validation of *MYNRL15* in six myeloid leukemia cell lines.



**a**, Ranked lists from MAGeCK analysis identifying *MYNRL15* (orange) among the top hits from the CRISPRi screen in all cell lines. The positive control genes, *MYC* (green) and *MYB* (turquoise), are also colored. **b**, Individual validation of guides targeting *MYNRL15* via proliferation assays ( $n=2$ ; mean  $\pm$  s.e.m.).

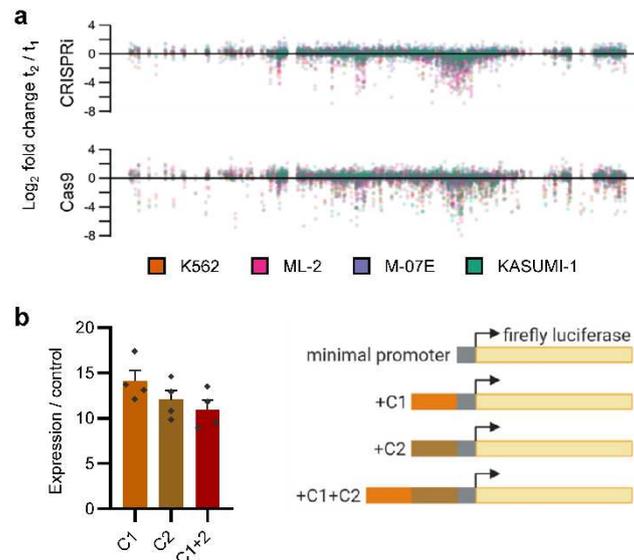
## Extended Data Figure 2: Further validation of *MYNRL15* and perturbation approaches.



**a**, Expression of *MYNRL15* compared to *bona fide* lncRNAs in the NCI-TARGET pediatric AML cohort<sup>35</sup> (n=258; zeros omitted). Midline, median; box limits, lower and upper quartiles; whiskers, 10% and 90% quantiles. **b**, Subcellular localization of *MYNRL15* compared to control transcripts *XIST* (nuclear) and *B2M* (cytoplasmic), determined via fractionated qRT-PCR (n=2). **c**, Targeting the CRISPRi system to the *MYNRL15* TSS causes concurrent *UNC45A* knockdown, as determined by qRT-PCR (n=3). **d**, Endpoint depletion values from proliferation assays using different perturbation strategies in K562 (left) or M-07E (right) cells. Each point corresponds to one vector that was used for perturbation (mean of n=3). **e**, qRT-PCR validations of *MYNRL15* knockdown using RNAi (left; n=3) and LNA-gapmeRs (center; n=3),

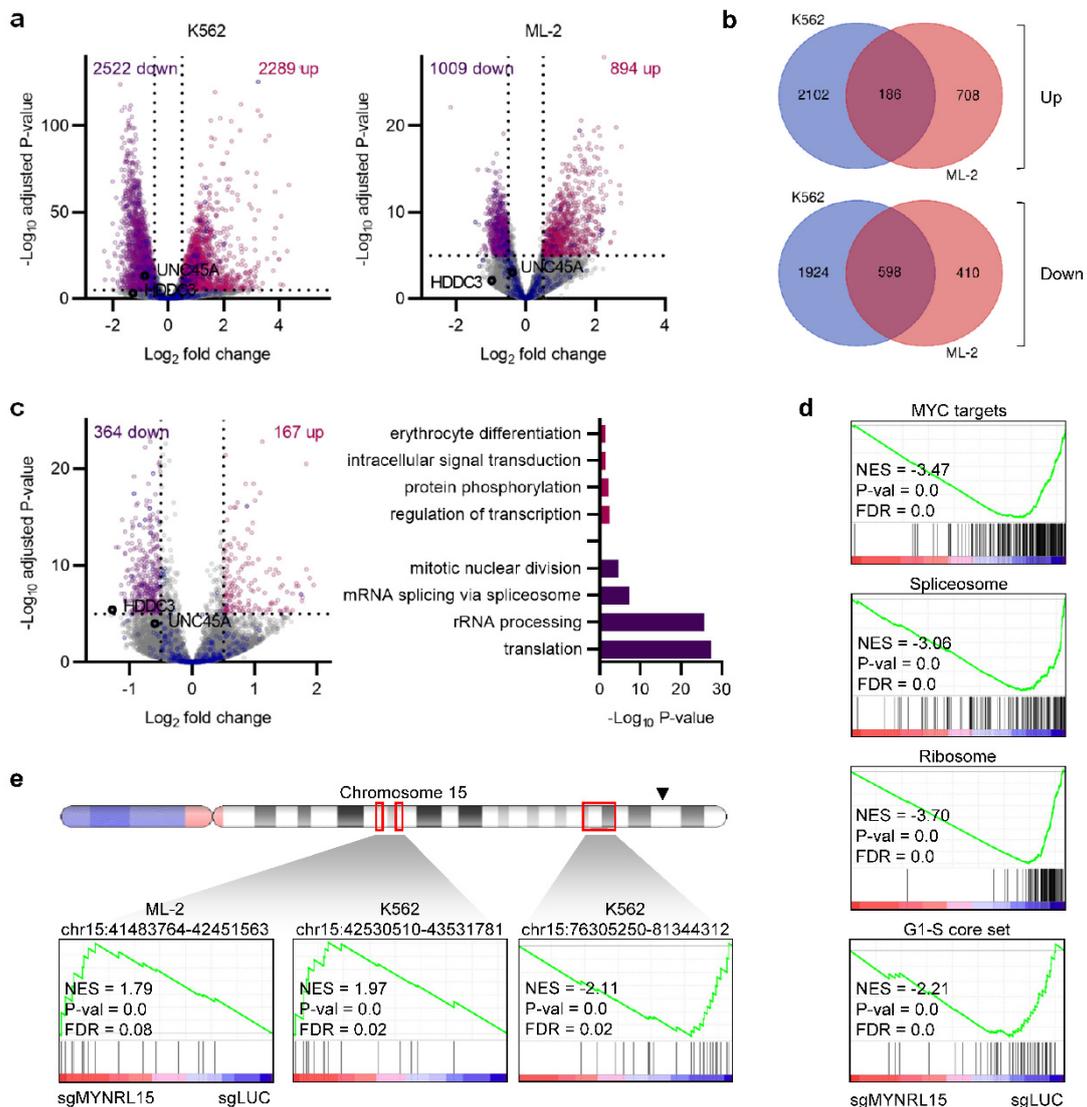
and of *HDCC3* knockdown via CRISPRi (right; n=3) **f**, PCR validation of *MYNRL15* excision, using bulk genomic DNA isolated from cells transduced with dual sgRNA vectors. **g**, Representative TIDE analyses showing the cutting efficiencies of guides targeting *UNC45A* and *HDCC3*. Where error bars are shown, data are presented as mean  $\pm$  s.e.m.

**Extended Data Figure 3: Extended data from the *MYNRL15* tiling screen.**



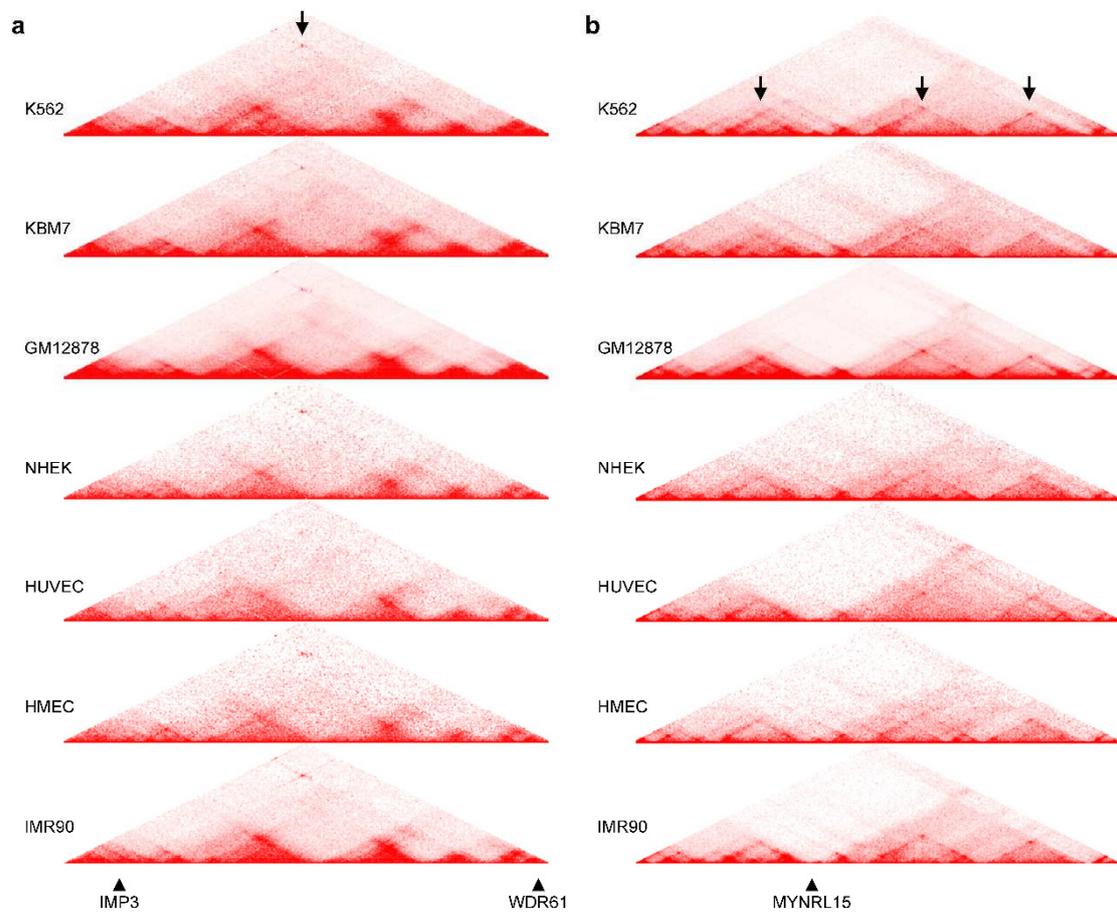
**a**, Tiling screens of the *MYNRL15* locus using CRISPRi (top) and CRISPR-Cas9 (bottom). The four tested cell lines are shown in different colors (mean of n=2 per cell line). **b**, Dual luciferase assays in K562 cells, using reporter constructs containing C1 and/or C2 upstream of a minimal promoter (n=4; mean  $\pm$  s.e.m.).

**Extended Data Figure 4: Global gene expression profiling upon *MYNRL15* perturbation.**



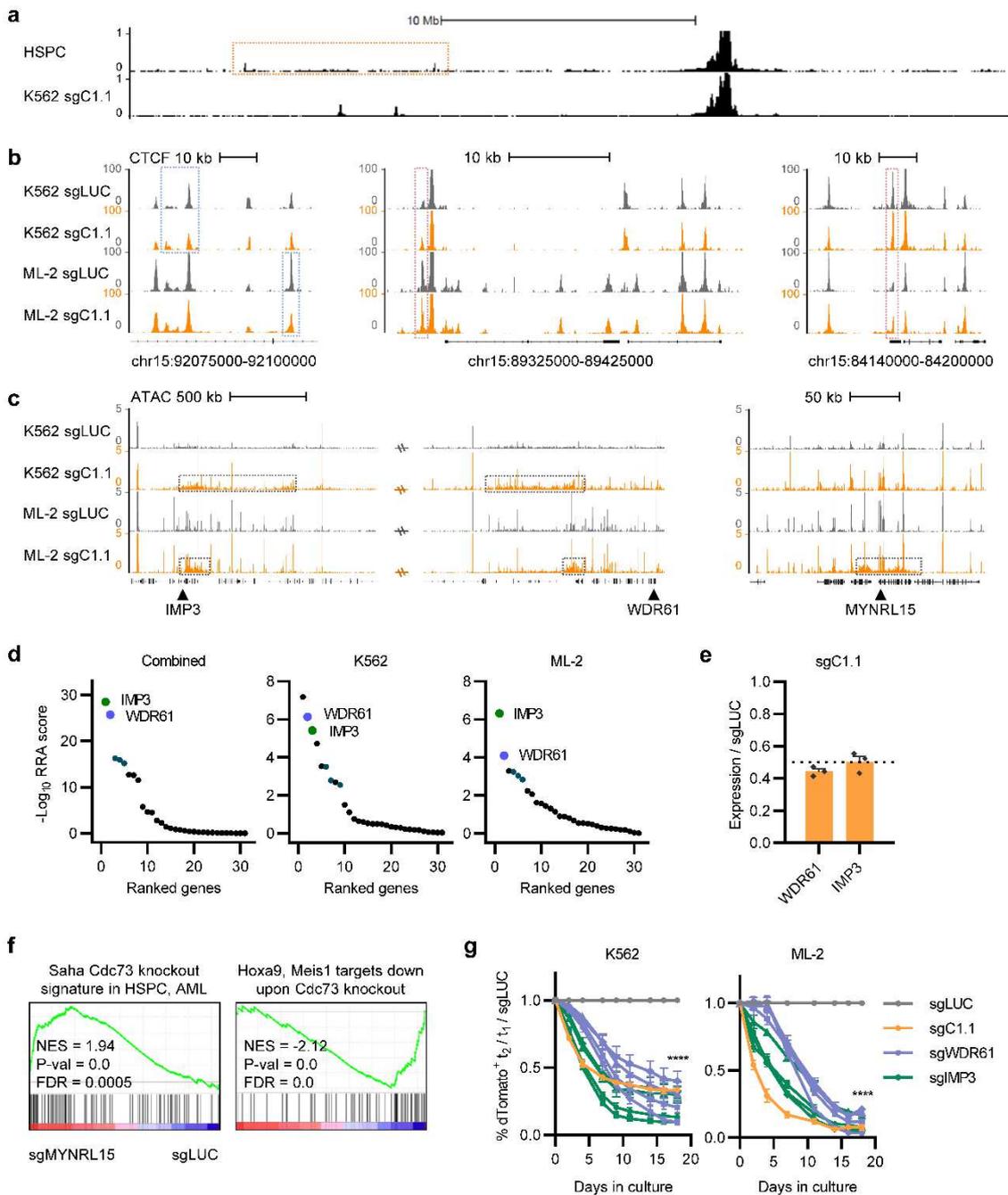
**a**, Differentially expressed genes following *MYNRL15* perturbation in K562 (left) and ML-2 (right) cells. Up- (pink) and downregulated (purple), and chromosome 15 genes (navy) are shown in color. **b**, Overlap of up- and downregulated genes in K562 and ML-2 cells. **c**, Combined differential expression analysis of K562 and ML-2 cells (left), and enriched gene ontology terms in the differentially expressed gene lists (right). **d**, A selection of the most significantly dysregulated gene sets from GSEA. A combined analysis of K562 and ML-2 cells is shown. **e**, Chromosome 15 gene sets that are not commonly deregulated across K562 and ML-2 cells. The locus is indicated by a black arrow. All analyses compare *MYNRL15* perturbation (4 guides, two per cCRE; “sgMYNRL15”) to the non-targeting control (n=2 per guide; “sgLUC”).

**Extended Data Figure 5: Hi-C maps of the *MYNRL15* locus and distal interaction region in multiple cell types.**



**a-b**, Hi-C maps from 7 different cell lines<sup>85</sup> including K562s. Knight-Ruiz matrix-balanced values are shown. **a**, Chromatin contacts in the gained distal interaction region. The interaction denoting the hierarchical loop is indicated with a black arrow. **b**, Local chromatin interactions around the *MYNRL15* locus. Contact domains are indicated with black arrows.

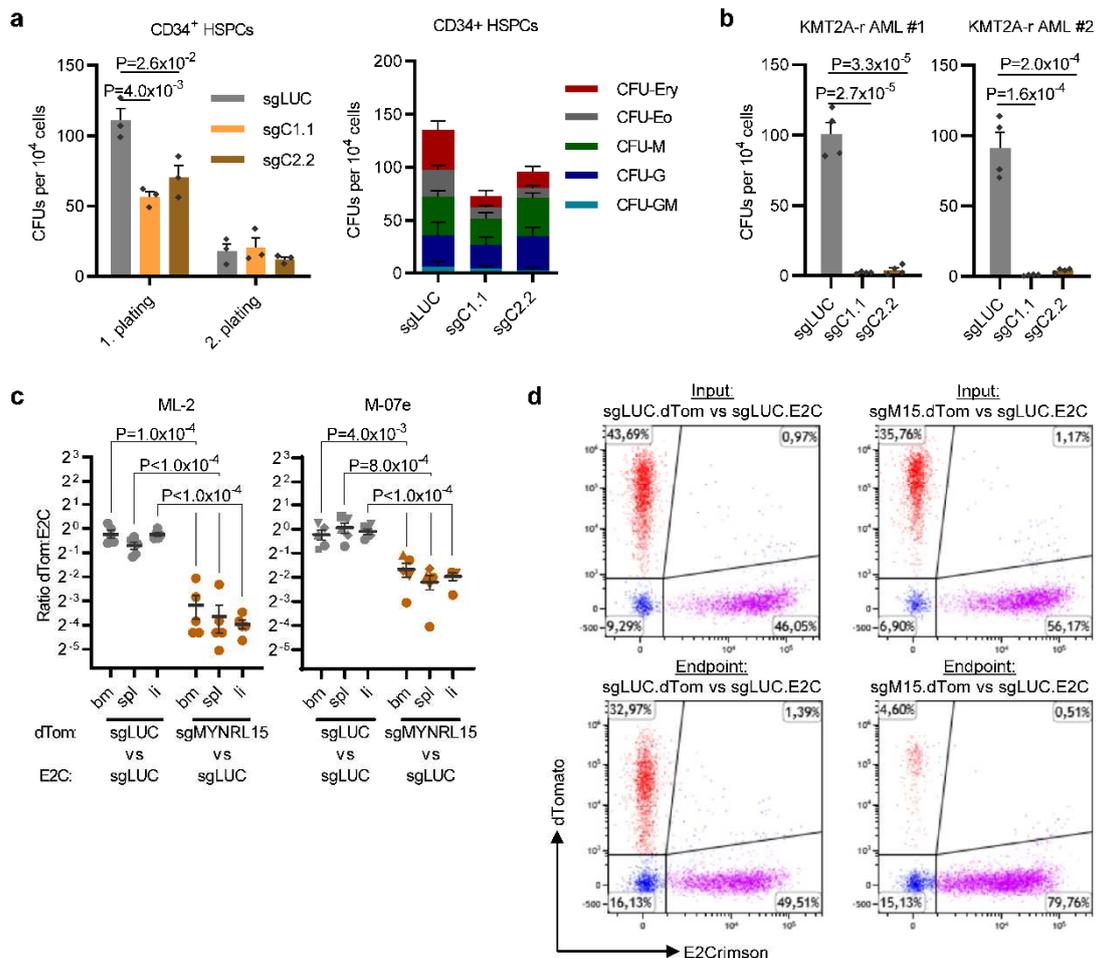
**Extended Data Figure 6: Extended data from the mechanistic delineation of *MYNRL15* perturbation.**



**a**, Chromosome 15 NG Capture-C interaction profiles from HSPCs (n=2; viewpoint in C1; smoothing window 2 pixels). The K562 sgC1.1 track is shown for reference. **b**, CUT&RUN tracks showing other examples of altered CTCF occupancy downstream of *MYNRL15* (left), and in the intervening space between the gained distal interactions and the *MYNRL15* locus (center and right). Decreases upon *MYNRL15* perturbation are outlined in blue; increases in pink. **c**, ATAC-seq tracks showing a wide view of chromatin accessibility in the gained distal interaction region (left; note the track discontinuity), and around the *MYNRL15* locus (right). **d**, MAGeCK analysis of CRISPR-Cas9 screens of the 29 coding genes from the gained distal interaction region (n=3). *WDR61* and *IMP3* are depicted in color, as are the positive controls *U2AF1*, *POL2RA*, and *RPL9* (turquoise). **e**, qRT-PCR validation of *WDR61* and *IMP3*

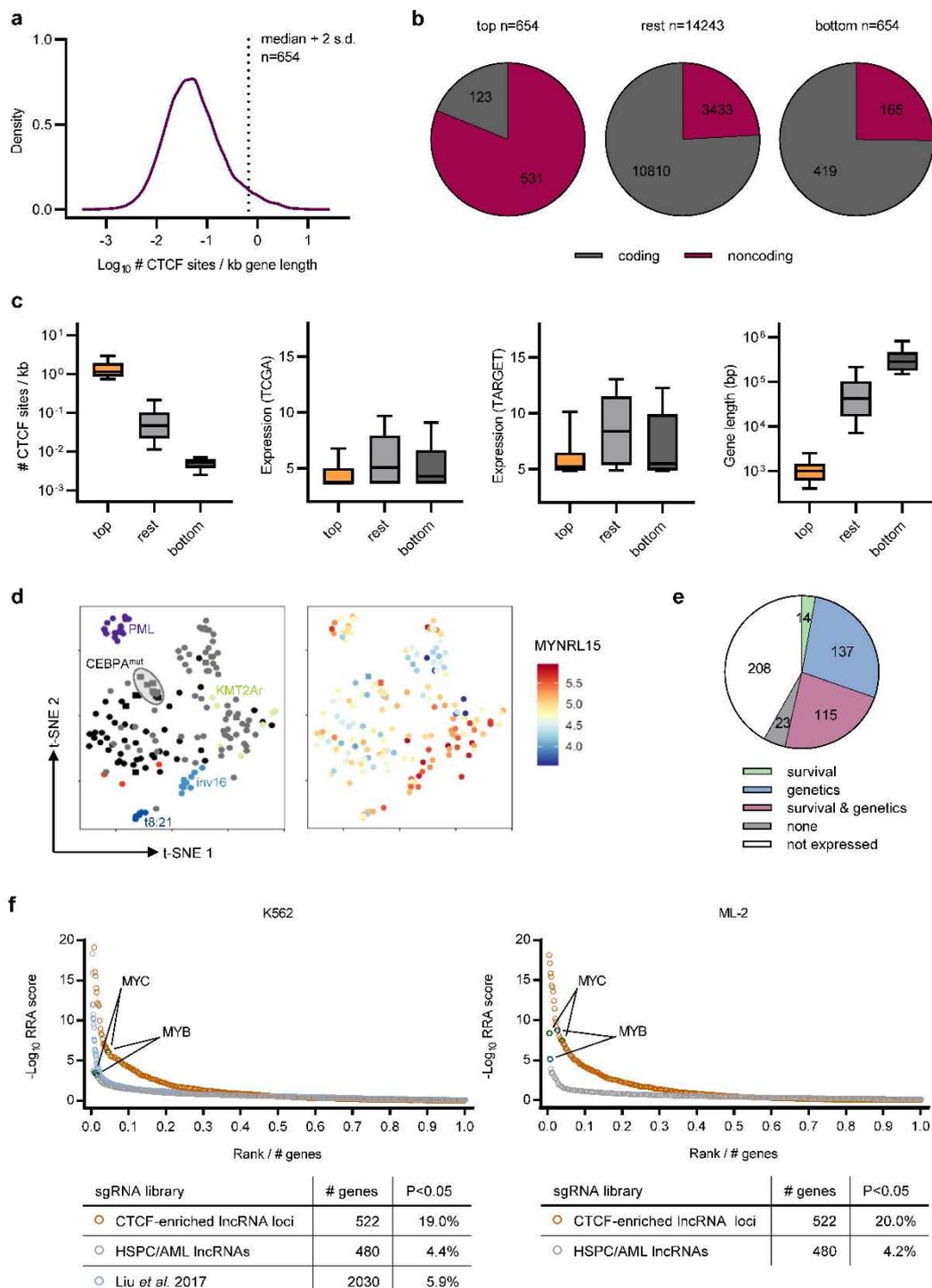
downregulation upon *MYNRL15* perturbation using sgRNA C1.1. **f**, Retrieval of PAF1c loss-associated gene sets upon *MYNRL15* perturbation in our RNA-seq data. **g**, Individual proliferation assays validating depletion of K562 and ML-2 cells upon *WDR61* and *IMP3* knock-out, compared to *MYNRL15* perturbation using sgRNA C1.1 (n=4, mean  $\pm$  s.e.m.; 4 guides each targeting *WDR61* and *IMP3*). \*\*\*\* $P < 0.0001$ ; all conditions shared the same  $P$ -value.

**Extended Data Figure 7: Extended data from *MYNRL15* perturbation experiments in primary and patient-derived cells.**



**a**, Colony counts upon *MYNRL15* perturbation in CD34<sup>+</sup> HSPCs from healthy donors (n=3; mean  $\pm$  s.e.m.). Replating (left) and differentiation (right) assays were performed in parallel. **b**, Colony counts following *MYNRL15* perturbation in two patient-derived AML samples (n=4; mean  $\pm$  s.e.m.). **c**, Representative flow cytometry data from two-color competitive xenotransplantation assays.

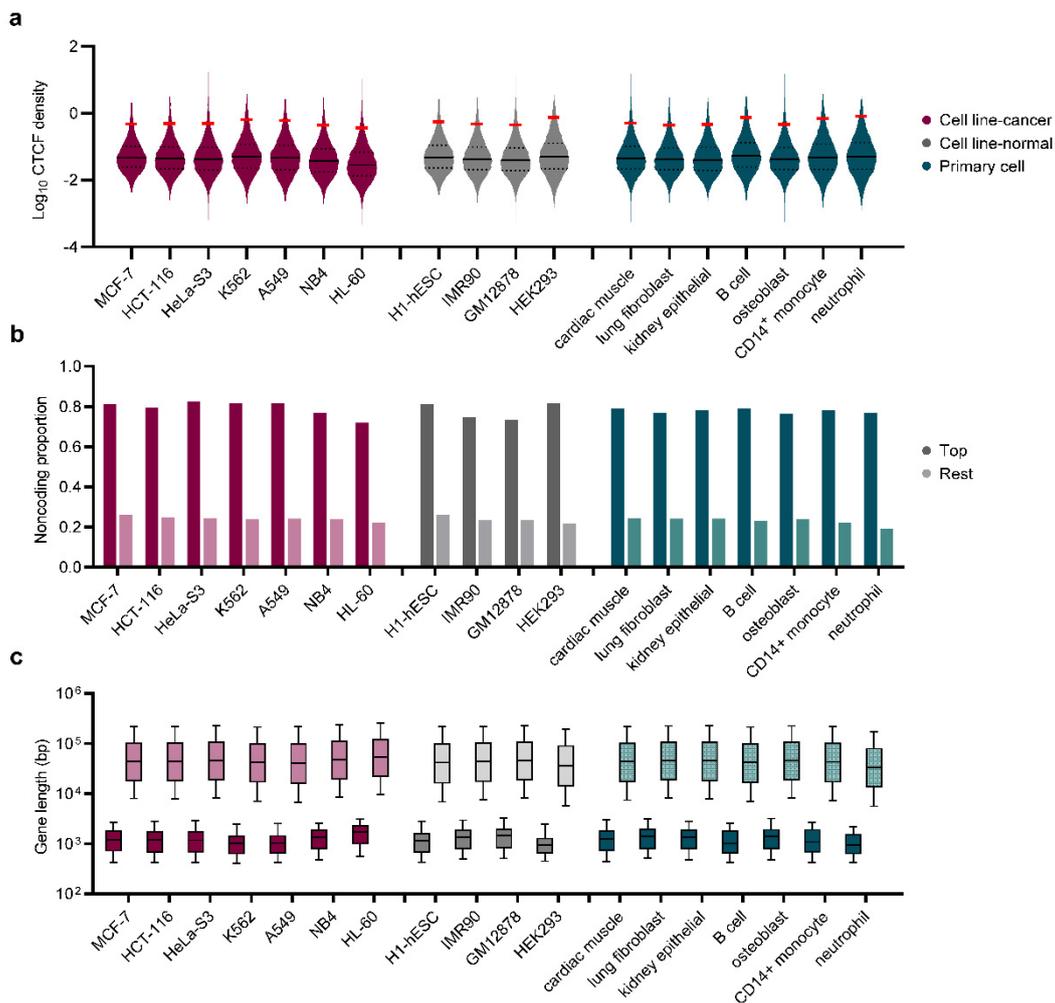
## Extended Data Figure 8: Defining a new subclass of CTCF-enriched lncRNA loci.



**a**, Distribution of  $\log_{10}$ -transformed values for the CTCF density metric (number of CTCF sites per kb of gene length). The dashed line indicates the cut-off defined (median + s.d.) for elevated CTCF binding. **b**, Proportions of coding and noncoding genes in different sections of the ranked list of CTCF-bound loci. **c**, Box plots illustrating predictive features for other loci like *MYNRL15*. From left to right: CTCF density, normalized expression in the TCGA AML cohort<sup>36</sup>, normalized expression in the NCI-TARGET AML cohort<sup>35</sup>, gene length. The top CTCF-bound loci are compared to the rest, or to the bottom of the ranked list in all plots. Midline, median; box limits, lower and upper quartiles; whiskers, 10% and 90% quantiles. **d**,

Unsupervised clustering of the TCGA cohort groups patients based on molecular subtypes (left), several of which are associated with *MYNRL15* expression (i.e. PML, inv16, *KMT2Ar*) (right). **e**, Breakdown of CTCF-enriched loci based on their association with clinical aspects such as cytogenetics, mutations, and survival in the NCI-TARGET cohort (left). **f**, Ranked lists of CTCF-enriched lncRNA loci ordered by essentiality, as determined using MAGECK on the results of the CRISPR-Cas9 screens in K562 (left) and ML2 (right) cells. The C-LNC screening results (orange) are displayed alongside those from the CRISPRi lncRNA library (grey) and Liu *et al.* 2017 (blue). Gene ranks are normalized to library size. The positive controls *MYC* and *MYB* are indicated.

**Extended Data Figure 9: Catalog of C-LNCs in 18 cell lines and primary cell types.**



**a**, Distributions of  $\log_{10}$ -transformed values for the CTCF density metric (number of CTCF sites per kb of gene length) across 18 cell lines and primary cell types. Midline, median; dotted lines, lower and upper quartiles. The red lines indicate the cut-off defined (median + s.d.) for elevated CTCF binding in each cell type. **b**, Proportion of noncoding genes in the top ( $\geq$  cut-off) and rest ( $<$  cut-off) of CTCF-bound loci. **c**, Box plots illustrating differences in gene length distributions between the top and rest of CTCF-bound loci. Midline, median; box limits, lower and upper quartiles; whiskers, 10% and 90% quantiles.

## Supplementary tables

**Supplementary Table 1: Patient sample information**

	<u>AML PDX #1</u>	<u>AML PDX #2</u>	<u>AML PDX #3</u>
Used in CFU assays	Yes	Yes	No
Used in transplants	No	Yes	Yes
Gender	Female	Male	Female
Age at diagnosis (years)	7	16	15
WBC ( $\times 10^9/L$ )	585.0	69.7	2.5
Hemoglobin (g/dl)	8.3	10.7	11
BM blasts (%)	84	93	90
CNS	No	No	No
SCT	Yes	No	No
Molecular genetics	Not determined	NRAS <sup>mut</sup>	Not determined
Cytogenetics	46,XX,t(9;11)(p22;q23)[8]/50,XX,i-dem,+3,+8,+18,+19[15]	42~44,XY,t(6;11)(q27;q23)[cp2]/51,i-dem,+X,+der(6)t(6;11)(q27;q23),+8,+19,+21[5]	47,XX,+19[12]/46,XX[5]
Response	CCR	NR	CCR
Relapse	No	No	Yes
Death	No	Yes	Yes

**Supplementary Table 2: sgRNA sequences**

<u>Target</u>	<u>System</u>	<u>Spacer</u>
<i>LUC</i>	All systems	CCGCTGGAAGATGGAACCGC
<i>LUC</i>	All systems	GGGCATTTTCGCAGCCTACCG
<i>MYNRL15</i> TSS	CRISPRi	GTGCACTTCTGCTGCGGTCG
<i>MYNRL15</i> TSS	CRISPRi	GCACGAGGTCTACGGTCATC
<i>MYNRL15</i> TSS	CRISPRi	GGAGCGCGCCCGGGCAGGGG
<i>MYNRL15</i> front	Excision	AGGTTGTCTCGTGCCCGCGC
<i>MYNRL15</i> front	Excision	TCCGACGCAAGAGTGGGGCG
<i>MYNRL15</i> back	Excision	GTCGGCCCCATCCGCGCGAT
<i>MYNRL15</i> back	Excision	TGATGTAGGGGGTCCCCTCG
<i>MYNRL15</i> C1	CRISPR-Cas9	TGCGGCCGCAGGGGCAGGGA
<i>MYNRL15</i> C1	CRISPR-Cas9	CCCTGGCGCCGGGGAGGCC
<i>MYNRL15</i> C2	CRISPR-Cas9	GGAGCGCGCCCGGGCAGGGG
<i>MYNRL15</i> C2	CRISPR-Cas9	GCCCCGGCCGCCACCCCCG
<i>UNC45A</i>	CRISPR-Cas9	GTTCAAATGTGGAGACTACG
<i>UNC45A</i>	CRISPR-Cas9	AGGCCGTTCTGCACCGGAAC
<i>UNC45A</i>	CRISPR-Cas9	GGGGCGTCGCGTCCAGACCC
<i>UNC45A</i>	CRISPR-Cas9	CGCCCTGGCGGCCTACTACTC
<i>UNC45A</i>	CRISPR-Cas9	GGTGGCAGGCGGCCCGGTTTC
<i>HDDC3</i>	CRISPR-Cas9, CRISPRi	TGATGTAGGGGGTCCCCTCG
<i>HDDC3</i>	CRISPR-Cas9, CRISPRi	GTGCTTGCGAGCCGCGAAGT

<i>HDDC3</i>	CRISPR-Cas9, CRISPRi	GCTGGAGGCTGCCGACTTCG
<i>HDDC3</i>	CRISPR-Cas9	TGATTCCCGCCTCGTGGGTC
<i>HDDC3</i>	CRISPR-Cas9	GGCCCTGCTCCATGACACGG
<i>IMP3</i>	CRISPR-Cas9	ACGCGGAAGTGGTCGCGTTC
<i>IMP3</i>	CRISPR-Cas9	GGAGCAAGGGCACGTACGCG
<i>IMP3</i>	CRISPR-Cas9	CGGGGTCCGTAACCACGTCA
<i>IMP3</i>	CRISPR-Cas9	GGAGCTCTGCGACTTCGTCA
<i>WDR61</i>	CRISPR-Cas9	GACCTTCACCAGGTCATCTA
<i>WDR61</i>	CRISPR-Cas9	CTGGCTAGGTATTTCCCATC
<i>WDR61</i>	CRISPR-Cas9	GCTCAGCGTGCCAGCCAAAT
<i>WDR61</i>	CRISPR-Cas9	GAATGCAACGTTTCAGCACCC

**Supplementary Table 3: shRNA sequences**

<u>Target</u>	<u>Sense strand</u>
<i>LUC</i>	TGGCTACATTCTGGAGACATA
<i>LUC</i>	CCGCCTGAAGTCTCTGATTAA
<i>MYNRL15</i>	CAGGCTTATGTTCTTCTTGCA
<i>MYNRL15</i>	AGCAGAAGTGCACGAGGTCTA
<i>MYNRL15</i>	CCGGTCAGCTCCAGAGGAATT
<i>MYNRL15</i>	CCGCTGAATTAGCCTCCACGA
<i>MYNRL15</i>	AAGAGCCAGGCTTATGTTCTT
<i>MYNRL15</i>	CGCACCTAAGCTGTCCCCGCA

**Supplementary Table 4: LNA-gapmerR sequences**

<u>Target</u>	<u>Sequence</u>
<i>Negative control B</i>	GCTCCCTTCAATCCAA
<i>MYNRL15</i>	TCGTGGAGGCTAATTC
<i>MYNRL15</i>	GGTGCAAGAAGAACAT
<i>MYNRL15</i>	GACAGCTTAGGTGCGC

**Supplementary Table 5: qRT-PCR primer sequences**

<u>Target</u>	<u>Direction</u>	<u>Sequence</u>
<i>MYNRL15</i>	Forward	CAGGCTTATGTTCTTCTTGCA
<i>MYNRL15</i>	Reverse	AGCAGAAGTGCACGAGGTCTA
<i>UNC45A</i>	Forward	CCGGTCAGCTCCAGAGGAATT
<i>UNC45A</i>	Reverse	CCGCTGAATTAGCCTCCACGA
<i>HDDC3</i>	Forward	AAGAGCCAGGCTTATGTTCTT
<i>HDDC3</i>	Reverse	CGCACCTAAGCTGTCCCCGCA
<i>B2M</i>	Forward	TCTCTTTTCTGGCCTGGAG
<i>B2M</i>	Reverse	AATGTCGGATGGATGAAACC

**Supplementary Table 6: Oligonucleotides for NG Capture-C**

<u>Target</u>	<u>Sequence</u>
<i>C1 front</i>	GATCATGCCTACCGTTCCAATTGTTATGAGGCTTAAACGGTATATCG

	CACTAAGCTTGGGACAAAACAGGTGCTCAATGAGGTGCACGCCCCCC ACGTTTCCTTCCCAGTCCCCACCACC
C1 back	CAGCCTCCAGCAGCTGCGCCGCTCAGAGCCCATCGCGGGATGGGG CCGACGACTGCGGCCGAGGGGCAGGGACGGAACGTTTACAGCGCCC CCTGGCGCCGGGGAGGCCCGGGGATC
C2 front	GATCCAGAGCCAAGCGCCCCGCCCTGCCCGGGCGCGCTCCCTCCTT AGCCCTGCCCTCTCTGACCCACCTCCGACGCAAGAGTGGGGCGGG GCAGCTGCCGGTGGCGTCCCGAACCC
C2 back	CCCCGAGCCCCGGCCGCCACCCCCGGGGTGCCTACCCAACCCCCG CGCCATCACCCCTTCGACCCGCCCTGACCATCCCTGGCCTCCTTCT CCCCATCCATGAGGCTCGCCCCGATC

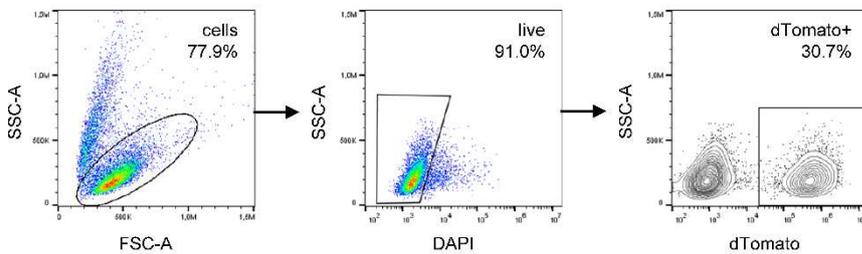
**Supplementary Table 7-11:**

Supplementary Tables 7-10 contain spacer sequences for the CRISPR libraries used in this study. Supplementary Table 11 contains a catalog of CTCF-enriched lncRNA loci (C-LNC) in 18 different cell lines and primary cell types. All four are large tables and are thus attached as separate files.

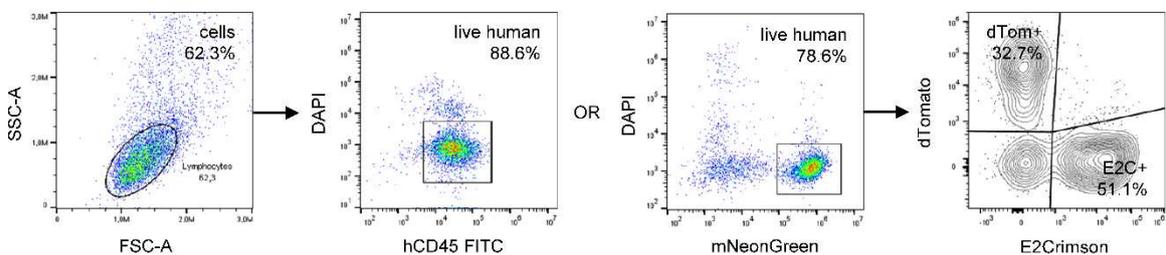
**Supplementary information**

**Gating strategies for flow cytometry:**

Simple fluorescence tracking



Two-colour xenotransplants



## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [SupplementaryTable7CRISPRiIncRNAlibrary.xlsx](#)
- [SupplementaryTable8MYNRL15tilinglibrary.xlsx](#)
- [SupplementaryTable9CaptureCgainedlibrary.xlsx](#)
- [SupplementaryTable10CLNClibrary.xlsx](#)
- [SupplementaryTable11CLNCcatalog.xlsx](#)