

# Promoter-bound METTL3 maintains myeloid leukaemia via m6A-dependent translation control

Isaia Barbieri<sup>1\*</sup>, Konstantinos Tzelepis<sup>2\*</sup>, Luca Pandolfini<sup>1\*</sup>, Junwei Shi<sup>3#</sup>, Gonzalo Millán-Zambrano<sup>1</sup>, Samuel C. Robson<sup>1¶</sup>, Demetrios Aspris<sup>2</sup>, Valentina Migliori<sup>1</sup>, Andrew J. Bannister<sup>1</sup>, Namshik Han<sup>1</sup>, Etienne De Braekeleer<sup>2</sup>, Hannes Ponstingl<sup>2</sup>, Alan Hendrick<sup>4</sup>, Christopher R. Vakoc<sup>3</sup>, George S. Vassiliou<sup>2§</sup> & Tony Kouzarides<sup>1§</sup>.

<sup>1</sup>The Gurdon Institute and Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QN, UK.

<sup>2</sup>Haematological Cancer Genetics, Wellcome Trust Sanger Institute, Cambridge, CB10 1SA, UK.

<sup>3</sup> Cold Spring Harbor Laboratory, 1 Bungtown Road, Cold Spring Harbor, NY 11724, USA.

<sup>4</sup> Storm Therapeutics Ltd, Moneta building (B280), Babraham Research Campus, Cambridge CB22 3AT, UK.

<sup>#</sup> Present address: Department of Cancer Biology, Abramson Family Cancer Research Institute, Perelman School of Medicine, University of Pennsylvania, 421 Curie Boulevard, Philadelphia, Pennsylvania 19104, USA.

<sup>¶</sup> Present address: School of Pharmacy & Biomedical Science, St Michael's Building, University of Portsmouth, White Swan Road, Portsmouth, UK"

\*These authors contributed equally to the work.

<sup>§</sup>Corresponding authors (t.kouzarides@gurdon.cam.ac.uk; gsv20@sanger.ac.uk)

## Abstract

N6-methyladenosine (m6A) is an abundant internal RNA modification in both coding<sup>1</sup> and non-coding RNAs<sup>2,3</sup>, catalysed by the METTL3/METTL14 methyltransferase complex<sup>4</sup>. Here we define a novel pathway specific for METTL3, implicated in the maintenance of the leukaemic state. We identify *METTL3* as an essential gene for growth of acute myeloid leukaemia (AML) cells in two distinct genetic screens. Down-regulation of METTL3 results in cell cycle arrest, differentiation of leukaemic cells and failure to establish leukaemia in immunodeficient mice. We show that METTL3, independently of METTL14, associates with chromatin and localizes to transcriptional start site (TSS) of active genes. The vast majority of these genes have the CAATT-box binding protein CEBPZ present at the TSS<sup>5</sup>, which is required for recruitment of METTL3 to chromatin. Promoter bound METTL3 induces m6A modification within the coding region of the associated mRNA transcript, and enhances its translation by relieving ribosome stalling. We show that genes regulated by METTL3 in this way are necessary for AML. Together, these data define METTL3 as a regulator of a novel chromatin-based pathway necessary for maintenance of the leukaemic state and identify this enzyme as a novel therapeutic target for AML.

45 **Main text**

46  
47

48 To identify RNA modifying enzymes necessary for survival and proliferation of AML cells,  
49 we performed two independent CRISPR screens. Firstly, we performed an *ex vivo* genome  
50 wide CRISPR dropout screen (Screen 1) using Cas9-expressing mouse primary  
51 leukaemia cells driven by an MLL-AF9 fusion gene and a FLT3 internal tandem  
52 duplication<sup>6</sup> (Fig. 1a). This identified 1550 dropout targets with a false discovery rate (FDR)  
53 of 0.25 (Supplementary Table 1), including 75 genes encoding possible RNA modifying  
54 enzymes whose expression is necessary for growth of primary leukaemia cells (see  
55 Methods; Supplementary Table 2).

56

57 Cas9-induced indel mutations induce stronger negative selection in dropout screens when  
58 critical functional domains are targeted<sup>7</sup>. Therefore, we constructed a custom domain-  
59 focused gRNA library including the 75 RNA modifying enzymes identified above (Screen  
60 2). This identified 46 high-confidence potential RNA modifying enzymes whose catalytic  
61 activity is required for leukaemia cell growth (Fig. 1b and Supplementary Table 2). Despite  
62 showing high overall correlation, comparison between the two screens highlights targets  
63 whose catalytic activity is specifically required for leukaemia cell growth (Extended Data  
64 Fig. 1a).

65

66 Two *Mettl* family members, *Mettl3* and *Mettl16*, scored very highly, whilst *Mettl14* and  
67 *Mettl1* showed significant but lower negative selection. METTL3 and METTL14 form a  
68 complex that catalyses RNA adenosine N6-methylation (m6A)<sup>4</sup>. METTL16 is also an m6A  
69 methyltransferase<sup>8</sup>. This modification is present in mRNAs<sup>1</sup>, pre-miRNA<sup>2</sup> and long non-  
70 coding RNAs<sup>3</sup>, and it affects mRNA stability<sup>9,10</sup> and translation<sup>11</sup>. Interestingly, an m6A  
71 demethylase, FTO, which is required for human leukaemia cell growth<sup>12</sup> was not identified

72 in our Screen 1, which may be explained by the heterogeneous genetic background of  
73 human AML cell lines.

74

75 We validated our results using growth competition assays with individual gRNAs targeting  
76 the catalytic domain of *Mettl3* and *Mettl16* (like in Screen 2) in mouse AML cells  
77 (Extended Data Fig. 1b). Furthermore, negative selection of gRNAs targeting either early  
78 exons (like Screen 1) or the catalytic domain of *METTL3* was validated in different mouse  
79 primary leukaemia cell lines (Extended Data Fig. 1c). Finally, disruption of *Mettl3*'s catalytic  
80 domain strongly suppresses primary murine AML cell colony formation (Fig. 1c and  
81 Extended Data Fig. 1d). In contrast, targeting *Mettl3* in non-transformed NIH3T3 and  
82 primary haematopoietic cells had no significant effect (Extended Data Fig. 1e and 1f). Our  
83 findings indicate that these genes are specifically essential for AML cell survival and not  
84 for general cellular viability.

85

86 We next targeted *METTLs* 1, 3, 14 and 16 in ten different human AML cell lines and 10 cell  
87 lines from heterogeneous cancer types. All four *METTLs* show negative selection in all  
88 AML cell lines tested (Extended Data Fig. 1g), but display varying degrees of negative  
89 selection in non-AML tumours (Extended Data Fig. 2a). These differences are not due to  
90 variable editing levels across cell lines (Extended Data Fig. 2b).

91

92 *METTL3* disruption reverses the myeloid differentiation block characteristic of AML, in both  
93 mouse and human AML cells (Fig. 1d and Extended Data Fig. 2c and d). Increased  
94 expression of CD11b, a granulocytic differentiation marker<sup>13</sup>, occurred in all *METTL3*-  
95 domain-knockout (KO) cells analysed, consistent with *METTL3* loss promoting AML cell  
96 differentiation. Strikingly, targeting *METTL3*'s methyltransferase domain markedly impairs  
97 human leukaemic cell engraftment into immunocompromised mice (Fig. 1e and Extended

98 Data Fig. 2e), with animals surviving significantly longer than controls (Fig. 1f). An  
99 independent genetic approach, using human MOLM13 cells harbouring inducible METTL3-  
100 specific shRNAs, was used to validate our findings. These cells showed near-complete  
101 loss of METTL3 mRNA and protein upon tetracycline induction of shRNAs (Extended Data  
102 Fig. 3a and b) and markedly reduced proliferation (Fig. 1g). Similar results were obtained  
103 using human AML cell line THP1 (Extended Data Fig. 3c). Importantly, ectopic expression  
104 of METTL3 (Extended Data Fig. 3d) fully rescued the proliferation defect, whilst a  
105 catalytically inactive mutant failed to do so (Fig. 1h), confirming that loss of growth was  
106 due to lack of METTL3's catalytic activity.

107

108 RNA-seq of METTL3 knock-down (KD) cells showed altered expression of transcripts,  
109 both upregulated (n=167) and downregulated (n=180; Extended Data Fig. 3e and  
110 Supplementary Table 3). Gene ontology analysis of differentially expressed genes  
111 revealed down-regulation of cell cycle and up-regulation of haematopoietic cell  
112 differentiation pathways, mirroring our findings obtained using CRISPR-Cas9 and flow  
113 cytometry analyses (Extended Data Fig. 3f and Supplementary Table 4). These data  
114 demonstrate that METTL3 is required for leukaemic cell growth and that its inactivation  
115 induces differentiation of MLL-AF9-driven AML cells.

116

117 Next we interrogated METTL3 expression levels across different cancer samples from the  
118 TCGA dataset<sup>14</sup>. Interestingly, highest expression of METTL3 (but not METTL14) was  
119 identified in AML cells, diffuse large B-cell lymphoma and prostate adenocarcinoma cells  
120 (Extended Data Fig. 4a). Strikingly, these three cancer types correspond to the cell lines  
121 sensitive to *METTL3* targeting (Extended Data Fig. 1g and 2a). Furthermore, METTL3  
122 over-expression in human AML cell lines increases their proliferation (Extended Data Fig.  
123 4b). These observations are consistent with METTL3 playing an oncogenic role in AML.

124

125 We previously described that cytoplasmic signalling enzymes can directly regulate genes  
126 by binding chromatin<sup>15</sup>. We therefore tested whether METTL3 binds chromatin. We  
127 detected both METTL3 and its partner METTL14 associated with chromatin fractions from  
128 MOLM13 cells (Extended Data Fig. 4c). To identify genomic loci bound by METTL3 and/or  
129 METTL14, we performed chromatin immunoprecipitation (ChIP) using antibodies against  
130 METTL3, METTL14 and H3K4me3. This identified 126 METTL3 and 119 METTL14  
131 genomic peaks (Fig. 2a, Extended Data Fig. 4d and Supplementary Table 5). Both  
132 METTL3 and METTL14 localized mainly to TSSs of coding genes characterized by  
133 bimodal H3K4me3 peaks (e.g. Fig. 2a, b and Extended Data Fig. 4e and f). Surprisingly,  
134 the two METTLs did not bind the same TSSs (Extended Data Fig. 4g and 5a), suggesting  
135 they have distinct roles on chromatin. Here, we focus on METTL3 and its functions. We  
136 validated several METTL3 peaks using two different METTL3-specific antibodies by ChIP-  
137 qPCR (Extended Data Fig. 5b and c) and confirmed reduction of METTL3 ChIP signal at  
138 specific promoters upon METTL3 KD (Fig. 2c and Extended Data Fig. 5d).

139

140 We interrogated sequences under METTL3 peaks for enriched motifs and identified the  
141 CCAAT-box as the top hit (Extended Data Fig. 6a). This motif binds the NFY complex<sup>16</sup>,  
142 which associates with H3R2me2s and WDR5 on active promoters<sup>17</sup>. Another significantly  
143 enriched METTL3 peak motif is that for transcription factor KLF9. Using existing ChIP-seq  
144 datasets<sup>5</sup> we observed high correlation between METTL3 binding sites and all of these  
145 factors. METTL14 peaks showed no correlation with the same factors except WDR5  
146 (Extended Data Fig. 6b). Co-occupancy by all of these factors on 447 promoters overlaps  
147 with METTL3 binding (Extended Data Fig. 6c and d), but their combined predictive power  
148 for defining METTL3 bound sites is limited.

149 We then selected genes most tightly co-expressed with METTL3 (top 2.5 percentile) in  
150 normal and cancer cell lines<sup>18</sup>. 11 AML Encode CHIP-seq datasets are available for these  
151 co-expressed factors<sup>5</sup>, including WDR5 (Extended Data Fig. 7a). Amongst them, CEBPZ  
152 shows high co-expression (Extended Data Fig. 7b) and CHIP co-localisation with METTL3  
153 (Fig. 2d, e and Extended Data Fig. 7c and d), but not with METTL14 (Extended Data Fig.  
154 7e). Interestingly, CEBPZ is a CCAAT-box binding factor and is amongst the top dropouts  
155 in the genomic CRISPR-Cas9 screen (Supplementary Table 1 and Extended Data Fig. 7f).  
156 CEBPZ KD in MOLM13 cells (Extended Data Fig. 7g) impairs cell proliferation (Extended  
157 Data Fig. 7h), as shown for METTL3.

158

159 To test whether CEBPZ is involved in recruiting METTL3 to TSSs, we performed METTL3  
160 CHIP-qPCR experiments upon CEBPZ KD. Figure 2f and Extended Data Figure 7i show  
161 reduction of chromatin-bound METTL3 at several promoters in CEBPZ KD cells. This  
162 shows for the first time that METTL3 and METTL14 are recruited to highly specific  
163 chromatin sites, with METTL3 localizing to TSSs with a well-defined signature of  
164 transcription factors and histone modifications. This suggested METTL3 regulates gene  
165 expression of mRNAs derived from its chromatin target genes.

166

167 Transcriptional profiling of METTL3-depleted cells showed that levels of mRNAs  
168 transcribed from METTL3-bound genes were not affected (Extended Data Fig. 7j and  
169 Supplementary Table 3; OR=0.75; p=1). Given that METTL3's catalytic activity is essential  
170 for AML cell growth (Fig. 1h), we investigated post-transcriptional events that could be  
171 regulated by METTL3-mediated RNA methylation.

172

173 To identify RNAs methylated by METTL3 in AML, we performed RNA-immunoprecipitation  
174 linked to high throughput sequencing using an m6A-specific antibody (m6A-IP). We used

175 RNA isolated from MOLM13 cells (CTRL) and from METTL3-depleted cells. This identified  
176 4085 METTL3-dependent m6A peaks on poly-A<sup>+</sup> enriched RNA (Supplementary Table 6,  
177 Extended Data Fig. 8a and b). As expected, we observed widespread reduction of m6A  
178 upon METTL3 KD (Extended Data Fig. 8c).

179

180 We then considered the distribution of m6A in transcripts derived specifically from  
181 METTL3-bound genes, 72.4% of which contained m6A peaks, compared to 38.4% of all  
182 transcripts (OR=4.2; p=5e-04). The m6A levels on mRNA encoding transcription factor  
183 SP1 are shown as an example (Fig. 3a). The majority of METTL3-dependent m6A occurs  
184 within the coding region of transcripts of METTL3-bound genes, in contrast to its  
185 distribution in the general transcriptome, where it is enriched within mRNA 3'UTRs (Fig. 3b  
186 and Extended Data Fig. 8d).

187

188 To investigate whether promoter-bound METTL3 is required for m6A modification of  
189 associated transcripts, we measured m6A modification in these mRNAs following CEBPZ  
190 KD, since this leads to loss of METTL3 at TSSs (Fig. 2f). This demonstrated reduced m6A  
191 in relevant mRNAs (Fig. 3c and Extended Data Fig. 8e). In contrast, no changes were  
192 observed in control mRNAs such as *GAPDH*. As expected, CEBPZ KD did not affect  
193 mRNA levels of METTL3 chromatin targets or levels of METTL3 itself (Extended Data Fig.  
194 8f).

195

196 Analysis of the whole coding sequences of these transcripts revealed enrichment of a  
197 [GAG]<sub>n</sub> motif (Fig. 3d). This motif, whilst common throughout coding transcripts, is  
198 significantly over-represented amongst transcripts derived from METTL3-bound genes  
199 (Extended Data Fig. 8g). Interestingly, the motif's reading frame (+2) is preserved  
200 throughout transcripts derived from METTL3-bound genes (Fig. 3e). Although the

201 significance of this motif remains unclear, its presence suggests these GAG rich  
202 transcripts require m6A modification for translational efficiency (TE). We therefore  
203 performed ribosome footprinting (RFP) of CTRL and METTL3 KD MOLM13 cells, to  
204 evaluate their translational output (Extended Data Fig. 9a, b and c). Intriguingly, while  
205 mRNAs marked by m6A generally tended to have an increased TE upon METTL3 KD (as  
206 seen by others<sup>19</sup>), the transcripts derived from genes harbouring METTL3 on their  
207 promoter were translated less efficiently (Fig. 3f and Supplementary Table 7). We  
208 therefore mapped ribosomal pausing sites on mRNAs produced from METTL3-bound  
209 genes. P-site codon occupancy in these transcripts indicated that four codons, GAG, GAT,  
210 GAC, GAA (GAN codons), are more occupied by ribosomes in METTL3 KD cells  
211 compared to CTRL cells (Fig. 3g and Extended Data Fig. 9d). The same was not observed  
212 as a general feature throughout the transcriptome (Extended Data Fig. 9e and f) and is not  
213 due to general over-representation of GAN codons throughout transcripts (Extended Data  
214 Fig. 9g).

215

216 To better characterise how METTL3 regulates translation, we focused on two genes  
217 expressing the transcription factors SP1 and SP2, which have promoters occupied by  
218 METTL3. The levels of SP1 and SP2 proteins are reduced upon METTL3 depletion  
219 (Figure 4a), but their mRNA levels are unaffected (Extended Data Fig. 10a). Indeed, a  
220 similar reduction in SP1 protein levels is observed upon CEBPZ KD (Extended Data Fig.  
221 10b). We next asked how METTL3 KD affects SP1 and SP2 mRNA association with  
222 polysomes (Extended Data Fig. 10c). Upon METTL3 depletion, there is a specific shift of  
223 SP1 and SP2 transcripts towards lower molecular weight polysomes (Figure 4b), indicating  
224 less efficient translation, whilst there are no differences for control mRNAs (Extended Data  
225 Fig. 10d).

226



227 The above results demonstrate that METTL3 affects translation of the mRNAs whose  
228 promoters it occupies. We next aimed to prove that recruitment of METTL3 to promoters is  
229 sufficient for this effect. For this, we used a reporter system (Fig. 4c) consisting of a  
230 plasmid harbouring GAL4 binding sites upstream of a constitutive promoter that expresses  
231 an SP2 (m6A peak)-luciferase mRNA fusion. We expressed METTL3 wild type catalytic  
232 domain, or an inactive point mutant<sup>20</sup>, in-frame with GAL4 DNA-binding domain.  
233 Consistently, only recruitment of wild type METTL3-GAL4 enhanced luciferase activity (Fig.  
234 4d and Extended Data Fig. 10e), without affecting luciferase mRNA levels (Extended Data  
235 Fig. 10f). These data corroborate the model that promoter-bound METTL3 augments  
236 translation of its target genes.

237

238 Consistent with SP1 and SP2 protein depletion in METTL3 KD cells, genes directly bound  
239 and regulated by these factors<sup>5</sup> were also generally down-regulated in METTL3 depleted  
240 MOLM13 cells (Extended Data Fig. 10g). Amongst these is the *c-MYC* oncogene, whose  
241 promoter is bound by both SP1 and SP2 (Extended Data Fig. 10h and Supplementary  
242 Table 3). We then tested whether loss of SP1 could explain the proliferation defect caused  
243 by METTL3 depletion. Figure 4e shows that ectopic over-expression of SP1 in METTL3  
244 KD cells (Extended Data Fig. 10i) rescues cell growth. Strikingly, CRISPR targeting of  
245 SP1 is lethal in cell lines, and only in cell lines, that show sensitivity to METTL3  
246 inactivation (Fig. 4f). These observations suggest that the sensitivity of leukaemic cells to  
247 the loss of METTL3 is dictated by the requirement of SP1 for cell growth.

248

249 Here we define a set of RNA modifying enzymes necessary for AML leukaemia and we  
250 identify a new leukaemic pathway for the METTL3 RNA methyltransferase. In this  
251 pathway, METTL3 is stably recruited by CEBPZ to promoters of a specific set of active  
252 genes, resulting in m6A methylation of the respective mRNAs and increased translation.

253 One important target is *SP1*, an oncogene in several cancers<sup>21</sup>, which regulates *c-MYC*  
254 expression<sup>22</sup>. Consistent with these findings, it was recently reported that METTL3 can  
255 methylate its targets co-transcriptionally<sup>23</sup>.

256

257 METTL3 affects mRNA translation in numerous ways, including by promoting RNA loading  
258 onto ribosomes<sup>24</sup> and recruiting specific m6A reader proteins (e.g. YTHFD1)<sup>25</sup>. The  
259 findings presented here provide significant insight into the mechanisms through which  
260 METTL3 promotes post-transcriptional regulation of gene expression. They identify a new  
261 paradigm for selecting RNAs to be modified, namely the stable recruitment of the RNA  
262 modifying enzyme to specific genomic loci. They also demonstrate a new mechanism by  
263 which m6A can affect translation, namely relief of ribosome stalling at GAN codons of  
264 specific transcripts.

265

266 The new pathway described here is critical for AML leukaemia since three of its  
267 components are required for AML cell growth: (i) the m6A RNA methyltransferases  
268 METTL3, (ii) the transcription factor CEBPZ, which targets this enzyme to promoters and  
269 (iii) SP1, whose translation is dependent upon the m6A modification by METTL3. Together,  
270 these observations define METTL3 enzymatic activity as a new candidate target for the  
271 treatment of AML.

272

273

## 274 **Acknowledgements**

275 We thank Dr. Ka Hing Che for help generating the RNA enzyme list. The Kouzarides  
276 laboratory is supported by grants from Cancer Research UK (Grant Reference RG17001)  
277 and ERC (Project number 268569), in addition to benefiting from core support from the  
278 Wellcome Trust (Core Grant reference 092096) and Cancer Research UK (Grant  
279 Reference C6946/A14492). I.B. is funded by a Kay Kendall Leukaemia Fund project grant  
280 (Grant Reference RG88664). G.M.Z. is funded by an EMBO fellowship (ALTF907-2014).  
281 G.S.V. is funded by a Wellcome Trust Senior Fellowship in Clinical Science (Grant  
282 Reference WT095663MA) and work in his laboratory is funded by Bloodwise. C.R.V. and  
283 J.S. are funded by a translational research grant from Northwell Health.

284

285

286 **Competing financial interests**

287 T.K. is a co-founder of Abcam Plc and Storm Therapeutics Ltd, Cambridge, UK.

288 A.H. is an employee of Storm Therapeutics Ltd, Cambridge, UK.

289

290 **Author contributions**

291

292 I.B., K.T and J.S. designed, performed and validated the CRISPR screens. K.T., J.P. and

293 E.D.B. performed the phenotypic analysis of human mouse targeted cells. I.B. and L.P.

294 generated the conditional KD cells, performed and validated the RNA-seq, ChIP-seq,

295 RNA-IP and riboprofiling experiments. L.P., S.C.R. and N.H. performed bioinformatic

296 analyses of datasets. N.H. generated the expression profiles from the TCGA dataset

297 G.M.Z. performed and analysed the polysome fractionation experiments. I.B., L.P, K.T and

298 D.A. performed the rescue experiments and the luciferase assays. V.M. A.J.B and A.H.

299 took part in the validation of ChIP-seq and RNA-IP experiments. I.B., K.T. and L.P.

300 designed experiments and interpreted results. C.R.V., G.S.V., and T.K. devised and

301 supervised the project. A.J.B., G.S.V. and T.K. wrote the manuscript with contributions

302 from all authors.

303

304

305 **References**

- 306 1. Dominissini, D. *et al.* Topology of the human and mouse m6A RNA methylomes  
307 revealed by m6A-seq. *Nature* **485**, 201–206 (2012).
- 308 2. Alarcón, C. R., Lee, H., Goodarzi, H., Halberg, N. & Tavazoie, S. F. N6-  
309 methyladenosine marks primary microRNAs for processing. *Nature* **519**, 482–485  
310 (2015).
- 311 3. Patil, D. P. *et al.* m6A RNA methylation promotes XIST-mediated transcriptional  
312 repression. *Nature* **537**, 369–373 (2016).
- 313 4. Liu, J. *et al.* A METTL3-METTL14 complex mediates mammalian nuclear RNA N6-  
314 adenosine methylation. *Nat. Chem. Biol.* **10**, 93–5 (2014).
- 315 5. Dunham, I. *et al.* An integrated encyclopedia of DNA elements in the human genome.  
316 *Nature* **489**, 57–74 (2012).
- 317 6. Tzelepis, K. *et al.* A CRISPR Dropout Screen Identifies Genetic Vulnerabilities and  
318 Therapeutic Targets in Acute Myeloid Leukemia. *Cell Rep.* **17**, 1193–1205 (2016).
- 319 7. Shi, J. *et al.* Discovery of cancer drug targets by CRISPR-Cas9 screening of protein  
320 domains. *Nat. Biotechnol.* **33**, 661–667 (2015).
- 321 8. Pendleton, K. E. *et al.* The U6 snRNA m6A Methyltransferase METTL16 Regulates  
322 SAM Synthetase Intron Retention. *Cell* **169**, 824–835.e14 (2017).
- 323 9. Batista, P. J. *et al.* m(6)A RNA modification controls cell fate transition in mammalian  
324 embryonic stem cells. *Cell Stem Cell* **15**, 707–19 (2014).
- 325 10. Wang, Y. *et al.* N6-methyladenosine modification destabilizes developmental  
326 regulators in embryonic stem cells. *Nat. Cell Biol.* **16**, 191–198 (2014).
- 327 11. Meyer, K. D. *et al.* 5' UTR m6A Promotes Cap-Independent Translation. *Cell* **163**,  
328 999–1010 (2015).
- 329 12. Li, Z. *et al.* FTO Plays an Oncogenic Role in Acute Myeloid Leukemia as a N6-  
330 Methyladenosine RNA Demethylase. *Cancer Cell* **31**, 127–141 (2017).
- 331 13. Ripperger, T. *et al.* The heteromeric transcription factor GABP activates the  
332 ITGAM/CD11b promoter and induces myeloid differentiation. *Biochim. Biophys. Acta*  
333 - *Gene Regul. Mech.* **1849**, 1145–1154 (2015).
- 334 14. Cancer Genome Atlas Research Network, J. N. *et al.* The Cancer Genome Atlas  
335 Pan-Cancer analysis project. *Nat. Genet.* **45**, 1113–20 (2013).
- 336 15. Dawson, M. A. *et al.* JAK2 phosphorylates histone H3Y41 and excludes HP1alpha  
337 from chromatin. *Nature* **461**, 819–22 (2009).
- 338 16. Ronchi, A. E., Bottardi, S., Mazzucchelli, C., Ottolenghi, S. & Santoro, C. Differential  
339 binding of the NFE3 and CP1/NFY transcription factors to the human gamma- and

- 340 epsilon-globin CCAAT boxes. *J. Biol. Chem.* **270**, 21934–41 (1995).
- 341 17. Migliori, V. *et al.* Symmetric dimethylation of H3R2 is a newly identified histone mark  
342 that supports euchromatin maintenance. *Nat. Struct. Mol. Biol.* **19**, 136–144 (2012).
- 343 18. Uhlen, M. *et al.* Tissue-based map of the human proteome. *Science (80-. )*. **347**,  
344 1260419–1260419 (2015).
- 345 19. Slobodin, B. *et al.* Transcription Impacts the Efficiency of mRNA Translation via Co-  
346 transcriptional N6-adenosine Methylation. *Cell* **169**, 326–337.e12 (2017).
- 347 20. Fustin, J. M. *et al.* XRNA-methylation-dependent RNA processing controls the speed  
348 of the circadian clock. *Cell* **155**, (2013).
- 349 21. O'Connor, L., Gilmour, J. & Bonifer, C. The Role of the Ubiquitously Expressed  
350 Transcription Factor Sp1 in Tissue-specific Transcriptional Regulation and in  
351 Disease. *Yale J. Biol. Med.* **89**, 513–525 (2016).
- 352 22. Geltinger, C., Hörtnagel, K. & Polack, A. TATA box and Sp1 sites mediate the  
353 activation of c-myc promoter P1 by immunoglobulin kappa enhancers. *Gene Expr.* **6**,  
354 113–27 (1996).
- 355 23. Knuckles, P. *et al.* RNA fate determination through cotranscriptional adenosine  
356 methylation and microprocessor binding. *Nat. Struct. Mol. Biol.* **24**, 561–569 (2017).
- 357 24. Lin, S., Choe, J., Du, P., Triboulet, R. & Gregory, R. I. The m6A Methyltransferase  
358 METTL3 Promotes Translation in Human Cancer Cells. *Mol. Cell* **62**, 335–345  
359 (2016).
- 360 25. Wang, X. *et al.* N<sup>6</sup>-methyladenosine modulates messenger RNA translation  
361 efficiency. *Cell* **161**, 1388–1399 (2015).
- 362

363  
364  
365  
366  
367  
368  
369  
370  
371  
372  
373  
374  
375  
376  
377  
378  
379  
380  
381  
382  
383  
384  
385  
386  
387  
388  
389  
390  
391  
392  
393  
394  
395  
396  
397  
398  
399  
400  
401  
402  
403  
404  
405  
406  
407  
408  
409  
410  
411  
412  
413  
414  
415  
416  
417  
418  
419  
420  
421  
422  
423  
424  
425

## Figure 1

### **METTL3 is essential for AML cells both *in vivo* and *in vitro*.**

**a)** Dropout p-values of the genome-wide screen in MLL-AF9/FLT-ITD cells are displayed. Discontinuous blue line shows the 25%FDR threshold. RNA enzymes are shown as red dots. **b)** CRISPR score for the 75 RNA enzymes (black circles) or the METTL family members (red/blue) as controls in AML-AF9 RN2C cells. **c)** Colony forming assay of MLL-AF9/FLT3-ITD-Cas9 cells targeted for Mettl3 (catalytic domain-specific) or CTRL showing decreased replating ability. Mean+S.D. of three independent replicates is shown; CFU: colony forming units. (\*\*p< 0.001; t-test). **d)** CD11b expression in METTL3 (catalytic domain-specific) targeted cells (MLL-AF9/FLT-ITD mouse cells and MOLM13 human cells) was measured by flow cytometry 8 days (mouse) and 6 days (human) after infection. **e)** Bioluminescence imaging of mice transplanted with luciferase-expressing MOLM13 cells transduced with the indicated gRNAs. **f)** Kaplan-Meier plot showing the survival time of mice from Fig. 1e. A log rank test was performed. **g)** Proliferation assay of METTL3 KD or CTRL cells measured between day 4 and day 8 after tetracycline induction. Mean+S.D. of three independent replicates is shown. **h)** Proliferation assay of MPN1c/Flt3<sup>ltd/+</sup>/Rosa26<sup>Cas9/+</sup> mouse Leukaemia cells transduced with gRNA targeting the catalytic domain of METTL3 and plasmids expressing wild type METTL3 or a catalytically inactive mutant. Mean+S.D. of three independent replicates is shown. (\*\*p< 0.01; t-test).

## Figure 2

### **METTL3 localises on specific TSSs on chromatin.**

**a)** Genomic visualisation of METTL3 and H3K4me3 ChIP-seq dataset at the SP2 locus. **b)** Distribution of METTL3 ChIP-seq reads centred on TSSs (upper panel) and histogram of ChIP-seq reads distribution relative to TSSs (lower panel). **c)** METTL3 ChIP-seq validation by ChIP-qPCR on the SP2 TSS in CTRL or METTL3 KD MOLM13 cells, showing a specific reduction of METTL3 binding in KD cells. Mean+S.D. of three technical replicates is shown. The experiment was performed independently three times. **d)** Venn diagram showing the overlap between CEBPZ and METTL3 ChIP-seq peaks. **e)** Distribution and heatmaps of normalised ChIP-seq reads for CEBPZ centred on METTL3 peaks. **f)** ChIP-qPCR of METTL3 binding on target TSSs in and MOLM13 cells, expressing a control shRNA or two independent shRNAs against CEBPZ, showing a specific reduction of METTL3 binding in CEBPZ KD cells. Mean+S.D. of three technical replicates is shown. The experiment was performed independently three times.

## Figure 3

### **Transcripts derived from METTL3-bound promoters harbour m6A within their CDS.**

**a)** Genomic visualisation of the m6A-IP normalised signal in METTL3 KD or CTRL MOLM13 cells on the SP1 transcript (upper tracks), along with the genomic visualisation of the METTL3 ChIP-seq. **b)** Pie charts of the distribution of METTL3-dependent m6A peaks within the whole transcriptome or METTL3 chromatin targets mRNAs. **c)** m6A-IP followed by qPCR for M6A peaks of SP1, SP2 and HNRNPL or GAPDH as a control. The plot show the m6A-IP signal over total input in MOLM 13 cells expressing a control shRNA or shRNAs targeting CEBPZ. Mean+S.D. of three technical replicates are shown; the experiment has been performed independently twice. **d)** Motif enriched in mRNAs from METTL3-bound TSSs. **e)** Reading frame distribution of the [GAG]<sub>n</sub> motif on the transcripts produced at METTL3-bound TSSs. Significance was obtained by multinomial test. **f)** Box plot showing the difference in translational efficiency (TE) between METTL3 KD and CTRL cells. The distributions of log<sub>2</sub>FC(TE) for all coding genes, mRNAs harbouring METTL3-dependent m6A and mRNAs originated from METTL3-bound or METTL14-bound TSSs are shown (\*p<0.05; Wilcoxon test). **g)** Frequency of P-site occupancy of GAN codons in METTL3 KD or CTRL MOLM13 cells (\*p<0.05; t-test).

## Figure 4

### **Negative effect of METTL3 depletion on the translation efficiency of genes necessary for AML growth.**

**a)** Western blot showing METTL3, SP1, SP2 and ACTIN protein levels in MOLM13 cells infected with METTL3-specific or CTRL TET-inducible shRNAs 8 days after doxycycline treatment. Two independent biological replicates are shown. For gel source data see Supplementary Information. **b)** SP1 and SP2 mRNAs in each ribosome fraction were quantified through qPCR and plotted as a percentage of the total. Data are from two independent polysome-profiling experiments. Mean ±SEM are shown. **c)** Schematic representation of the engineered reporter system. **d)** Firefly luciferase activity from UAS or scrambled (SCR) sequence carrying plasmid in presence of GAL4 either alone or fused with METTL3 wild type (CD) or inactive (CD DW/AA) catalytic domain (\*p<0.05; t-test). The mean +S.D. of three independent transfections is shown, for two different cell lines (HT-29 and FADU) **e)** Proliferation assay of MOLM13 cells infected with METTL3-specific or CTRL TET-inducible shRNAs and with an SP1 expression vector between day 3 and

426 day 6 after doxycycline treatment. Mean+S.D. of three independent replicates is shown. **f)** Competitive co-  
427 culture assay showing negative selection of BFP+ human tumour cell lines upon targeting of *METTL3* or *SP1*  
428 by CRISPR-Cas9. Results were normalized to day 4 for each gRNA. Mean+S.D. of two independent  
429 infections is shown.

## Materials and Methods (Online only)

### Cell culture

MOLM13, THP-1, MV4-11, NOMO-1, HL-60, EOL-1, KG-1, RN2c, HEL, JURKAT, LOUCY, K562 cells were cultured in RPMI1640 (Invitrogen) supplemented with 10% FBS and 1% penicillin/streptomycin/glutamine. OCI-AML2 and OCI-AML3 cells were cultured in Alpha-MEM supplemented with 20% FBS and 1% penicillin/streptomycin/glutamine. 293T, mouse immortalized fibroblasts NIH-3T3, SU-DHL-4, HT1080, SBC-3, DETROIT-562, FADU, SH-SY5Y and HT-29 cells were cultured in DMEM (Invitrogen), supplemented with 10% FBS and 1% penicillin/streptomycin/glutamine. 293T, FADU and HT-29 cells were transfected using the Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer instructions. All human cancer cell lines were obtained from the Sanger Institute Cancer Cell Collection and tested to be negative for mycoplasma contamination. Human cell lines employed are not listed in the cross-contaminated or misidentified cell lines database curated by the International Cell Line Authentication Committee (ICLAC).

### Isolation of haematopoietic progenitors

*Flt3*<sup>ITD/+</sup> mice<sup>26</sup> were kindly provided by Gary Gilliland and crossed with *Rosa26*<sup>Cas9/+</sup> mice<sup>6</sup>. Freshly isolated bone marrow from 6- to 10-week-old female *Rosa26*<sup>Cas9/+</sup>, *Flt3*<sup>ITD/+</sup>; *Rosa26*<sup>Cas9/+</sup> or moribund *Npm1*<sup>flox-CA/+</sup>; *Flt3*<sup>ITD/+</sup> mice was used. Bone marrow cells were exposed to erythrocyte lysis (BD PharmLyse, BD Bioscience), followed by magnetic bead selection of Lin<sup>-</sup> cells using the Lineage Cell Depletion Kit (Miltenyi Biotec) according to the manufacturer's instructions. Lin<sup>-</sup> cells were cultured in X-VIVO 20 (Lonza) supplemented with 5% BIT serum substitute (Stem Cell Technologies), 10ng ml<sup>-1</sup> IL3 (Peprotech), 10ng ml<sup>-1</sup> IL6 (Peprotech) and 50ng ml<sup>-1</sup> of SCF (Peprotech). Retrovirus constructs pMSCV-MLL-AF9-IRES-YFP and pMSCV-MLL-ENL-IRES-Neo were used with package plasmid psi-Eco to produce retrovirus. 293T cells (Life Technologies) were cultured and prepared for transduction in 10cm plates as described above. For virus production, 5 µg of the above plasmids and 5 µg psi-Eco packaging vector were transfected drop wise into the 293T cells using 47.5 µl TransIT LT1 (Mirus) and 600 µl Opti-MEM (Invitrogen). Transduction of primary mouse cells was performed in 6-well plates as mentioned above. After transduction, cells were sorted for YFP (for MLL-AF9) or selected with neomycin (for MLL-ENL).

### Generation of genome-wide mutant libraries and screening

Ex-vivo CRISPR screens were performed using the previously reported WT Sanger genome-wide CRISPR library<sup>6</sup>, which employs 5 gRNA for each of the 18424 targeted genes.  $3.0 \times 10^7$  cells were infected with a pre-determined volume of the genome-wide gRNA lentiviral supernatant that gave rise to 30% transduction efficiency measured by BFP expression. Two independent infections were conducted for the murine primary AML cells. Two days after transduction, the cells were selected with puromycin at 1.5 µg ml<sup>-1</sup>. Genomic DNA extraction and Illumina sequencing of gRNAs were conducted as described previously<sup>6</sup>. For the primary murine AML screen, 19-bp single-end sequencing was performed with the custom sequencing primer 5'-TCTTCCGATCTCTTGTGGAAAGGACGAAACACCG-3'. The number of reads for each guide was counted with an in-house script. Enrichment and depletion of guides and genes

were analyzed using MAGeCK statistical package<sup>27</sup> by comparing read counts from each cell line with counts from matching plasmid as the initial population.

The 296 RNA enzyme list which has been interrogated for dropouts was compiled as follows: 171 SAM-binding proteins without known histone methyltransferase activity; 65 ATP-dependent helicases with reported RNA interaction in at least 3 out of 4 CLIP datasets<sup>28-31</sup>; 12 Pseudouridylases; 48 annotated RNA enzymes not included in the previous lists (Supplementary Table 2)

### **Lentiviral vectors production and infection.**

For virus production 293T cells were transfected with the appropriate Lentiviral vector (PLKO.1 for shRNA, LRG and LentiCRISPR-v2 for CRISPR-Cas9) together with the packaging plasmids PAX2 and VSVg at a 1:1.5:0.5 ratio. Supernatant was harvested 48 and 72 hours after transfection.

$1 \times 10^6$  cells and viral supernatant were mixed in 2 ml of culture medium supplemented with  $8 \mu\text{g ml}^{-1}$  (human) or  $4 \mu\text{g ml}^{-1}$  (mouse) polybrene (Millipore), followed by spinfection (60 min, 900 g, 32 °C) and further incubated overnight at 37 °C. The medium was refreshed on the following day and the transduced cells were cultured further.

### **Pooled domain-focused CRISPR gRNA construction**

For the RNA enzyme domain-focused CRISPR screen, 5 gRNAs were designed to target the catalytic domain of each protein based on the NCBI database annotation. gRNAs were synthesized in a pooled format on an array platform (Twist Bioscience) and PCR-cloned into a lentiviral gRNA expression vector (LRG, Addgene: #65656). To check the representation and identity of individual gRNAs within the lentiviral pool, a deep-sequence analysis was performed. This confirmed that 100% of the designed gRNAs were cloned in the LRG vector and the abundance of >95% of the gRNA constructs was within 5-folds of the mean.

### **Pooled gRNA screening, Miseq library construction, and data analysis**

Virus containing the domain-focused gRNA library against RNA modifying enzymes was generated as described above. A serial dilution of this virus in correlation with the GFP+ cell population was used to estimate the viral titer multiplicity of infection (MOI). In the initial infected cell population, the total number of RN2c cells corresponded to an approximately one thousand-fold representation of each gRNA. To ensure that a single gRNA was transduced per cell, the viral volume for infection corresponded to an MOI of 0.4~0.5. At 2 days post-infection, a portion of the RN2c cells was harvested and used as a reference time point. At the end point of the negative selection experiment, a portion of the RN2c cells was harvested again and saved for gRNA abundance quantification through deep sequencing analysis.

The gRNA cassette MiSeq deep sequencing library was constructed using a similar method as previously described<sup>7</sup>. Genomic DNA was extracted using QiAamp DNA mini kit (Qiagen #51304), following the manufacturer's protocol. In order to maintain approximately one thousand-fold gRNA library representation, 25 parallel PCR reactions were performed to amplify the gRNA cassette using the 2X High Fidelity Phusion Master Mix (Thermo Scientific #F-548). PCR products were subjected to Illumina MiSeq library construction and sequencing. First, the PCR product was end repaired with T4 DNA polymerase (New England BioLabs, NEB), DNA polymerase I (NEB), and T4 polynucleotide kinase (NEB). Then, an A overhang was added to the end-repaired DNA using Klenow DNA Pol Exo- (NEB). The A-overhang DNA fragment was ligated with diversity-increased barcoded Illumina adaptors followed by seven pre-capture PCR cycles. The barcoded libraries were pooled at an equal molar ratio and subjected to massively



parallel sequencing through a MiSeq instrument (Illumina) using paired-end 150-bp sequencing (MiSeq Reagent Kit v2; Illumina MS-102-2002).

The sequence data were de-barcoded and trimmed to contain only the gRNA sequence, and subsequently mapped to the reference gRNA library without allowing any mismatches. The read counts were calculated for each individual gRNA. To calculate the relative abundance of each gRNA, the read counts of each gRNA were divided by the total read counts of the pooled gRNA library.

### **Validation of the Catalytic domain-specific screen**

RN2C cells or NIH3t3 mouse fibroblast cells were infected with LRG lentiviral vectors expressing GFP and a single gRNA targeting the catalytic domain of the indicated RNA enzymes and controls. The % of GFP+ cells was measured at day 2 after infection as a baseline by flow cytometry. The % of GFP+ cells was measured again at day 10 and day 12 for RN2C or NIH3T3 cells, respectively. Three gRNAs from Screen 2 were used for each target and one gRNA for the *rosa26* locus as a negative control. The gRNA sequences are listed in the supplementary material table. The data were analysed using Flowjo software.

### **gRNA competition**

gRNA competition assays were performed using single and dual gRNA vectors as described previously<sup>2</sup>. For the validation of individual target genes, one gRNA was derived from the CRISPR library used in the screens and another gRNA was designed using WTSI Genome Editing website (<http://www.sanger.ac.uk/htgt/wge/>). Viral supernatants were collected 48 h after transfection. All transfections and viral collections were performed in 24-well plates and transduction was performed as mentioned above. For gRNA/BFP competition assays, flow cytometry analysis was performed on 96-well plates using a LSRFortessa instrument (BD). Gating was performed on live cells using forward and side scatter, before measuring of BFP+ cells. The gRNA sequences are listed in the supplementary material table.

Efficiency of genome editing in the pool of sgRNA-targeted cells was evaluated by Tracking of Indels by Decomposition (TIDE)<sup>32</sup>.

### **Replating Assays**

For re-plating assays, 5,000 lineage negative cells and primary murine AML cells expressing Cas9 were plated in three wells of 6-well-plate of M3434 methylcellulose (Stem Cell Technologies) after infection with lentiviral vectors expressing gRNAs targeting the catalytic domain of METTL3 or empty vectors. The colonies were counted 7 days later and further 5,000 cells re-seeded and re-counted after a week until no colonies were observed (for WT) or until the 3rd replating (for primary murine AMLs).

### **Flow cytometry analyses of AML cells**

Cas9 expressing cells were transduced with gRNA vectors targeting the catalytic domain of METTL3 or empty vectors and stained with anti-mouse CD11b PE/Cy5 (Biolegend, cat. no. 101210) and anti-human CD11b PE (eBiosciences, cat. no. 9012-0118). Data were analysed by using LSRFortessa (BD) and FlowJo.

### **Mouse whole-body bioluminescent imaging**

For in vivo experiments, MOLM13 cells expressing Cas9 were transduced with a firefly luciferase-expressing plasmid (System Biosciences). After propagation, the cells were transduced with a lentivirus either empty or expressing Mettl3 gRNA (day 0) and selected with puromycin from day 2 to day 5. At day 5 post transduction, the cells were suspended in fresh medium without puromycin. At day 6,  $1 \times 10^5$  cells were transplanted into female

6-week old Rag2<sup>-/-</sup> IL2RG<sup>-/-</sup> mice by tail-vein injection. At day 14 post-transplant, the tumour burdens of the animals were detected using IVIS Lumina II (Caliper) with Living Image version 4.3.1 software (PerkinElmer). Briefly, 100 µl of 30 mg/ml D-luciferin (BioVision) was injected into the animals intraperitoneally. Ten min after injection, the animals were maintained in general anaesthesia by isoflurane and put into the IVIS chamber for imaging. The detected tumour burdens were measured and quantified by the same software. Animals were culled when the tumour burden was 10<sup>8</sup> photons per second or higher. Diseased mice welfare was assessed blindly by qualified animal technicians from the Sanger mouse facility. All animal studies were carried out in accordance with the Animals (Scientific Procedures) Act 1986, UK and approved by the Ethics Committee at the Sanger Institute. Randomisation and blinding were not applied.

### **Conditional Knock-Down cells generation.**

5\*10<sup>5</sup> MOLM13 or THP1 cells were infected as described above using PLKO-TETon-Puro lentiviral vectors expressing shRNAs against the coding sequence of human METTL3, CEBPZ or a scramble control. 24 hours after spinfection the cells were replated in fresh medium containing 1 µg/ml of puromycin and kept in selection medium for 7 days. ShRNA was induced by 200 ng/ml doxycycline treatment for the indicated times. The shRNA sequences are listed in the supplementary material table.

### **Proliferation assays**

5\*10<sup>4</sup> MOLM13 or THP1 CTRL or METTL3 KD cells (4 days after doxycycline induction) were seeded in 2 ml of complete RPMI medium and counted 4 days after plating using the Countess II cell counter. For SP1 rescue experiments the number of doxycycline treated cells were normalised to their untreated counterparts.

### **Rescue Experiments**

cDNA was obtained by reverse transcription of MOLM13 RNA with Superscript III (ThermoFisher Scientific), then SP1 full length coding sequence was amplified by PCR and cloned into pHIV-ZsGreen plasmid (Addgene #18121) by Gibson assembly (Gibson Assembly® Cloning Kit, NEB), using the HpaI site.

MOLM13 cells were transduced with the lentiviral constructs, then GFP+ cells were isolated by flow cytometry sorting after 5 days and employed in proliferation assays as described in the previous section.

For rescue experiments with either active or catalytically mutant METTL3, pcDNA3/Flag-METTL3 plasmid (Addgene #53739) was mutagenized at the positions D394A (GAC / GCC) and W397A (TGG / GCG) as described by Fustin et al<sup>33</sup> with QuikChange II Site-Directed Mutagenesis Kit (Stratagene).

NPM1c<sup>Flt3ItD/+</sup>/Rosa26<sup>Cas9/+</sup> cells were transduced with an empty or domain-specific Mettl3 gRNA lentiviral vector and selected with puromycin until day 5 post-transduction. Then cells were electroporated in Buffer R (Invitrogen) with the plasmids encoding WT METTL3, catalytically inactive METTL3 or an empty vector as a control. In each replicate 150,000 cells were electroporated with 500 ng of each plasmid and plated in triplicate at 50,000 cells per well in a 6-well plate. Electroporation was performed using the Neon Transfection System (Thermo Fisher Scientific) as previously described<sup>34</sup>. 3 days after electroporation (day 8), live cells were measured per well/condition.

### **METTL3 overexpression/proliferation experiments**

AML cells were electroporated in Buffer R (Invitrogen) with plasmids encoding the WT METTL3 or an empty vector as a control. In each replicate 200,000 cells were electroporated with 500 ng of each plasmid and plated in triplicate at 50,000 cells per well

in a 6-well plate. 2 days after electroporation (day 3), live cells were measured per well/condition.

### **Chromatin Immunoprecipitation**

Chromatin immunoprecipitation was performed as previously described<sup>35</sup>. Firstly MOLM13 cells were cross-linked with 0.5% formaldehyde for 10 minutes.  $20 \times 10^6$  cells were used for each immunoprecipitation with 3  $\mu$ g of specific antibodies or IgG. Immunoprecipitated DNA was purified with ChIP DNA Clean & Concentrator Columns (Zymo) and either amplified for massive parallel sequencing or analysed on an ABI 7900 real-time PCR machine, Fast SybrGreen PCR mastermix according to the manufacturer's instructions. Primer sequences are listed in the supplementary material table.

### **ChIP-sequencing**

For the METTL3, METTL14, H3K4me3 and IgG ChIP-Seq experiment 5, 5, 2 and 3 independent biological replicates were used, respectively. Single end 50bp libraries were prepared using the Bioo Scientific NEXTflex ChIP-Seq kit following manufacturer's recommendations. Reads were sequenced using HiSeq 1500 and multiplexed reads were split based on their barcodes using Illumina Basespace. Reads were trimmed to remove the TRUseq adapter using trim\_galore ([http://www.bioinformatics.babraham.ac.uk/projects/trim\\_galore](http://www.bioinformatics.babraham.ac.uk/projects/trim_galore)) with parameters '-q 0 -a AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC --phred33 --fastqc'. Read quality was assessed using FastQC. Ribosomal contamination was removed by first mapping the reads to hg38 rDNA sequences using bwa<sup>36</sup> (default parameters), and removing all reads that mapped. Trimmed non-ribosomal reads were mapped to the hg38 genome using bwa with parameters '-n 3 -k 2 -R 300 -t 4'. Multiple reads mapping to a single genomic locus were treated as PCR duplicates, and were removed using samtools rmdup<sup>37</sup>. Mapped reads were filtered to remove reads mapping to more than one unique genomic locus (multihits) by keeping only reads with flag XT:A:U in the output bam file from bwa. Reads were further filtered to remove those with mapping quality less than 20 using samtools. Genome coverage bedgraph files were generated using genomeCoverageBed from the bedtools<sup>38</sup> suite of tools. Coverage files were converted to bigwig format using bedGraphToBigWig. Peaks were called against input sample using MACS2 using default parameters<sup>39</sup>. Peaks from all replicates were merged to give a master list of potential binding loci per condition, and read count (normalised by overall read depth of the library) for each replicate was calculated using the GenomicRanges package in R<sup>40</sup>. Peaks were treated as potential binding loci if all replicates showed normalised score greater than 1 and did not overlap a peak called in the IgG. Genomic annotation of ChIP peaks and reads and gene set operations were performed taking advantage of the R packages ChIPseeker<sup>41</sup> and VennDiagram<sup>42</sup>, respectively. HOMER tool suite<sup>43</sup> was used for DNA motif discovery coupled with the hypergeometric enrichment calculations (or binomial) to determine motif enrichment.

### **m6A RNA IP-sequencing**

Total RNA was isolated from MOLM13 CTRL cells or METTL3KD cells (2 independent biological replicate for each shRNA) 8 days after doxycycline administration using the RNAeasy midi Kit (Quiagen). Successively polyA+ RNA was purified from 300 $\mu$ g of total RNA using the NEBNext® Poly(A) mRNA Magnetic Isolation Module (New England Biolabs). 500 ng of polyA+ purified RNA was used for each immunoprecipitation reaction. m6A RNA immunoprecipitation was performed using the Magna MeRIP™ m6A kit (Millipore) according with the manufacturer instructions.

Single end 50bp stranded libraries were prepared using the Bioo Scientific NEXTflex Rapid Directional RNA kit following manufacturer's recommendations. Reads were sequenced using HiSeq 4000 and multiplexed reads were split based on their barcodes using Illumina Basespace. Reads were trimmed to remove the TRUseq adapter using trim\_galore with parameters:

```
'-q 0 -a AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC --phred33 --fastqc'
```

Read quality was assessed using FastQC. Ribosomal contamination was removed by first mapping the reads to hg38 rDNA sequences using bwa (default parameters), and removing all reads that mapped. Trimmed non-ribosomal reads were mapped to the hg38 genome using Tophat2 with parameters '--no-coverage-search --max-multihits 300 --report-secondary-alignments --read-mismatches 2'. Gene annotations from Ensembl v86 were used to direct transcript alignment. Mapped reads were filtered to remove those mapping to more than one unique genomic locus (multihits) by keeping only reads with flag NH:i:1 in the output bam file from Tophat. Reads were further filtered to remove the ones with mapping quality less than 20 using samtools. Genome coverage bedgraph files were generated for forward and reverse strand reads using genomeCoverageBed from the bedtools suite of tools. Coverage files were converted to bigwig format using bedGraphToBigWig. Transcript assembly was performed using cufflinks2 and a single transcript database was generated using cuffmerge<sup>44</sup>. Statistical analysis of differentially methylated peaks in CTRL and METTL3 KD cells was performed using the R package MeTDiff<sup>45</sup>. Metagene plots were generated by RNAModR package (<https://github.com/mevers/RNAModR>), while m6A motifs were analysed according to published protocols<sup>46</sup>. For evaluating statistical significance of [GAG]<sub>n</sub> motif enrichment, individual motif occurrences were searched throughout human transcriptome with FIMO program (MEME suite<sup>47</sup>).

### **Ribosome profiling**

Ribosome profiling was obtained using the Illumina TruSeq® Ribo Profiler (Mammalian) kit. 5\*10<sup>7</sup> MOLM13 CTRL (2 pooled independent biological replicates) or METTL3 KD cells (2 pooled independent biological replicates for each shRNA) 5 or 8 days after doxycycline induction were treated with 0.1 mg/ml of cycloheximide for 1 minute and the RPF fraction of mRNA was isolated following the manufacturer instruction. Ribosomal RNA was removed using the Illumina Ribozero Kit.

Single end 50 bp stranded libraries were prepared following manufacturer's recommendations. Reads were sequenced using HiSeq 1500 and multiplexed reads were split based on their barcodes using Illumina Basespace. Reads were trimmed to remove the TRUseq adapter using trim\_galore with parameters '-q 0 -a AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC --phred33 --fastqc'. Read quality was assessed using FastQC. Ribosomal contamination was removed by first mapping the reads to hg38 rDNA sequences using bwa (default parameters), and removing all reads that mapped. Trimmed non-ribosomal reads were mapped to the hg38 genome using Tophat2 with parameters '--no-coverage-search --max-multihits 300 --report-secondary-alignments --read-mismatches 2'. Gene annotations from Ensembl v86 were used to direct transcript alignment. Mapped reads were filtered to remove those mapping to more than one unique genomic locus (multihits) by keeping only reads with flag NH:i:1 in the output bam file from Tophat. Reads were further filtered to remove the ones with mapping quality less than 20 using samtools. Genome coverage bedgraph files were generated for forward and reverse strand reads using genomeCoverageBed from the bedtools suite of tools. Coverage files were converted to bigwig format using bedGraphToBigWig. Transcript assembly was performed using cufflinks and a single transcript database was generated using cuffmerge. Statistical analysis of differentially translated genes in CTRL and METTL3 KD cells was performed using the R package xtail<sup>48</sup>. In order to estimate the

offset value relative to the read start required to localize the position of P-sites (in our case ~12nt, as already described in the literature), we employed the function “psite” of the *plastid* Python library<sup>49</sup>.

### **Polysome fractionation**

5\*10<sup>7</sup> MOLM13 CTRL (2 pooled independent biological replicates) or METTL3 KD cells (2 pooled independent biological replicates) 8 days after doxycycline induction were treated with 0.1 mg/ml of cycloheximide for 5 minutes at 37°C, then they were lysed and polysomes were fractionated on a sucrose gradient as described before<sup>50</sup>. Relative RNA abundance in each fraction was then quantified by RT-qPCR.

### **RNA-sequencing**

Input samples from the m6A RNA IP and Riboprofiling experiments were combined to give a set of transcriptional profiling samples to treat as an RNA seq experiment (CTRL n=4, METTL3 KD n=6). Gene counts were calculated at the transcript level for the combined transcript database from cuffmerge using *summarizeOverlaps* from the GenomicAlignments<sup>40</sup> package in R using a generalized linear model fit which takes into account possible batch effects of the two distinct experiments. Differential gene expression analysis was conducted using DESeq2<sup>ref.51</sup>. Differentially expressed genes were identified as those with RPKM greater than 1 for WT or KD showing differential expression greater than 2 fold (up or down) with a Benjamini and Hochberg corrected p value less than 0.05 unless stated otherwise.

Gene set enrichment analysis was performed using R package GAGE<sup>52</sup>.

### **Construction of the engineered translation reporter system**

A DNA sequence carrying 10 GAL4 recognition motifs (or a scrambled version of the same sequence) was in vitro synthesized and cloned into pMirGlo plasmid (Promega) upstream to the constitutive PGK promoter by cutting with BglII and ligating with T4 DNA ligase (NEB). The sequence of SP2 known to harbor the m6A peak and the [GAN]<sub>n</sub> motif was amplified by PCR and subcloned in frame at the N-terminus of Firefly luciferase by Gibson assembly (Gibson Assembly® Cloning Kit, NEB), using the Apal site.

To generate GAL4-METTL3(CD) construct, The CREB coding sequence in plasmid pcDNA1-GAL4-CREB-S133A (Addgene #46770) was swapped with METTL3 catalytic domain obtained by plasmid pcDNA3/Flag-METTL3 (Addgene #53739) using restriction enzymes XbaI and EcoRI. Wild type METTL3, was mutagenized at the positions D394A (GAC / GCC) and W397A (TGG / GCG) as described by Fustin et al<sup>33</sup> with QuikChange II Site-Directed Mutagenesis Kit (Stratagene). Cloning primer sequences are listed in the supplementary material table.

### **Luciferase assay**

293T cells were co-transfected with the GAL4-METTL3 construct and UAS-SP2-Luc or SCR-SP2-Luc vectors expressing the Renilla luciferase as transfection control. 24 hours after transfection the Firefly and Renilla luciferases activities were measured using the Dual-Luciferase Reporter Assay System (Promega) on CLARIOstar microplate reader (BMG Labtech).

Alternatively, transfected cells were lysed and total RNA extracted using the Qiagen RNAeasy Mini kit according to the manufacturer instructions.

### **Western blotting**

Western blotting was performed as previously described<sup>53</sup>. For the isolation of nuclei from the cytoplasmic fraction, cells were incubated in hypotonic buffer (20mM Tris-HCl, pH 7.4, 10mM NaCl, 3mM MgCl<sub>2</sub>,) and the cytoplasmic membranes disrupted by adding 0.5%

NP40. Pelleted nuclei were successively lysed in IPH buffer (50mM Tris-HCl pH=8.0, 150mM NaCl, 5mM EDTA, 0.5% NP-40) for 10 minutes on ice. Nucleoplasmic and chromatin fractions were separated by 15 minutes centrifugation at 4°C. The chromatin fraction was resuspended and sonicated in Laemmli buffer before loading on SDS-PAGE.

### RT-qPCR

Total RNA from MOLM13, THP1 or 293T cells was purified using the RNAeasy mini kit according to the manufacturer instructions. 1µg of purified total RNA was retrotranscribed using the High capacity cDNA reverse transcription kit (Applied Biosystems). The levels of specific RNAs were measured using the ABI 7900 real-time PCR machine and the Fast SybrGreen PCR mastermix according to the manufacturer's instructions. Firefly luciferase levels were normalised on Renilla luciferase levels while METTL3 mRNA levels were normalised on β-ACTIN mRNA. For quantification of polysome fractionation and m6A-RIP experiments, probes from Universal ProbeLibrary (Roche) were used with TaqMan® Fast Advanced Master Mix (ThermoFisher). Primer sequences are listed in the Supplementary Material tables.

### Antibodies

For the ChIP experiments the following antibodies were used: anti-METTL3 from Bethyl Laboratories (A301-568A), anti-METTL3 from Bethyl Laboratories (A301-567A), rabbit polyclonal anti-METTL14 from Abcam (ab98166), anti H3k4me3 from Abcam (ab8580) and IgG Isotype Control (ab171870).

Western blot experiment were performed using the following antibodies: anti-METTL3 from Bethyl Laboratories (A301-568A), anti-METTL14 from Abcam (ab98166), anti-Histone H3 from Active motif (39763), anti-WDR5 from Abcam (ab178410), anti-CEBPZ from Abcam (ab176579), anti-SP1 from Abcam (ab13370) and anti-SP2 from Abcam (ab137238), anti-ACTIN from Abcam (ab8227).

### Statistical analysis

All general statistical analyses were performed using either a two-tailed Student's t-test or a Wilcoxon test (when distributions were assessed not to be normal and homoscedastic) at a confidence interval of 95%, unless differently specified.

### Availability of data and material

Raw sequencing data have been deposited in the Gene Expression Omnibus database with accession code: GSE94613 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE94613>).

## Methods References

430  
431

- 432 26. Lee, B. H. *et al.* FLT3 Mutations Confer Enhanced Proliferation and Survival  
433 Properties to Multipotent Progenitors in a Murine Model of Chronic Myelomonocytic  
434 Leukemia. *Cancer Cell* **12**, 367–380 (2007).  
435 27. Li, W. *et al.* MAGeCK enables robust identification of essential genes from genome-

- 436 scale CRISPR/Cas9 knockout screens. *Genome Biol.* **15**, 554 (2014).
- 437 28. Castello, A. *et al.* Insights into RNA Biology from an Atlas of Mammalian mRNA-  
438 Binding Proteins. *Cell* **149**, 1393–1406 (2012).
- 439 29. Kwon, S. C. *et al.* The RNA-binding protein repertoire of embryonic stem cells. *Nat.*  
440 *Struct. Mol. Biol.* **20**, 1122 (2013).
- 441 30. Gerstberger, S., Hafner, M. & Tuschl, T. A census of human RNA-binding proteins.  
442 *Nat. Rev. Genet.* **15**, 829–845 (2014).
- 443 31. Baltz, A. G. *et al.* The mRNA-Bound Proteome and Its Global Occupancy Profile on  
444 Protein-Coding Transcripts. *Mol. Cell* **46**, 674–690 (2012).
- 445 32. Brinkman, E. K., Chen, T., Amendola, M. & van Steensel, B. Easy quantitative  
446 assessment of genome editing by sequence trace decomposition. *Nucleic Acids Res.*  
447 **42**, e168 (2014).
- 448 33. Fustin, J.-M. *et al.* RNA-Methylation-Dependent RNA Processing Controls the Speed  
449 of the Circadian Clock. *Cell* **155**, 793–806 (2013).
- 450 34. Gundry, M. C. *et al.* Highly Efficient Genome Editing of Murine and Human  
451 Hematopoietic Progenitor Cells by CRISPR/Cas9. *Cell Rep.* **17**, 1453–1461 (2016).
- 452 35. Dawson, M. A. *et al.* Inhibition of BET recruitment to chromatin as an effective  
453 treatment for MLL-fusion leukaemia. *Nature* **478**, 529–533 (2011).
- 454 36. Li, H. & Durbin, R. Fast and accurate long-read alignment with Burrows–Wheeler  
455 transform. *Bioinformatics* **26**, 589–595 (2010).
- 456 37. Li, H. *et al.* The Sequence Alignment/Map format and SAMtools. *Bioinformatics* **25**,  
457 2078–9 (2009).
- 458 38. Quinlan, A. R. & Hall, I. M. BEDTools: a flexible suite of utilities for comparing  
459 genomic features. *Bioinforma. Appl. NOTE* **26**, 841–84210 (2010).
- 460 39. Zhang, Y. *et al.* Model-based Analysis of ChIP-Seq (MACS). *Genome Biol.* **9**, R137  
461 (2008).
- 462 40. Lawrence, M. *et al.* Software for Computing and Annotating Genomic Ranges. *PLoS*  
463 *Comput. Biol.* **9**, e1003118 (2013).
- 464 41. Yu, G., Wang, L.-G. & He, Q.-Y. ChIPseeker: an R/Bioconductor package for ChIP  
465 peak annotation, comparison and visualization. *Bioinformatics* **31**, 2382–2383 (2015).
- 466 42. Chen, H. & Boutros, P. C. VennDiagram: a package for the generation of highly-  
467 customizable Venn and Euler diagrams in R. *BMC Bioinformatics* **12**, 35 (2011).
- 468 43. Heinz, S. *et al.* Simple Combinations of Lineage-Determining Transcription Factors  
469 Prime cis-Regulatory Elements Required for Macrophage and B Cell Identities. *Mol.*  
470 *Cell* **38**, 576–589 (2010).

- 471 44. Trapnell, C. *et al.* Transcript assembly and quantification by RNA-Seq reveals  
472 unannotated transcripts and isoform switching during cell differentiation. *Nat.*  
473 *Biotechnol.* **28**, 511–515 (2010).
- 474 45. Cui, X. *et al.* MeTDiff: a Novel Differential RNA Methylation Analysis for MeRIP-Seq  
475 Data. *IEEE/ACM Trans. Comput. Biol. Bioinforma.* 1–1 (2015).  
476 doi:10.1109/TCBB.2015.2403355
- 477 46. Dominissini, D., Moshitch-Moshkovitz, S., Salmon-Divon, M., Amariglio, N. &  
478 Rechavi, G. Transcriptome-wide mapping of N6-methyladenosine by m6A-seq  
479 based on immunocapturing and massively parallel sequencing. *Nat. Protoc.* **8**, 176–  
480 189 (2013).
- 481 47. Bailey, T. L. *et al.* MEME SUITE: tools for motif discovery and searching. *Nucleic*  
482 *Acids Res.* **37**, W202-8 (2009).
- 483 48. Xiao, Z., Zou, Q., Liu, Y. & Yang, X. Genome-wide assessment of differential  
484 translations with ribosome profiling data. *Nat. Commun.* **7**, 11194 (2016).
- 485 49. Dunn, J. G. & Weissman, J. S. Plastid: nucleotide-resolution analysis of next-  
486 generation sequencing and genomics data. *BMC Genomics* **17**, 958 (2016).
- 487 50. Panda, A. C., Martindale, J. L. & Gorospe, M. Polysome Fractionation to Analyze  
488 mRNA Distribution Profiles. *Bio-protocol* **7**, (2017).
- 489 51. Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and  
490 dispersion for RNA-seq data with DESeq2. *Genome Biol.* **15**, 550 (2014).
- 491 52. Luo, W., Friedman, M. S., Shedden, K., Hankenson, K. D. & Woolf, P. J. GAGE:  
492 generally applicable gene set enrichment for pathway analysis. *BMC Bioinformatics*  
493 **10**, 161 (2009).
- 494 53. Wyspiańska, B. S. *et al.* BET protein inhibition shows efficacy against JAK2V617F-  
495 driven neoplasms. *Leukemia* **28**, 88–97 (2014).
- 496  
497



498 **Extended Data Figure Legends**

499

500

501

502 **Extended Data Figure 1 (Related to Figure 1)**

503 **Validation of CRISPR screens.**

504 **a)** Correlation between gene rankings from the two independent CRISPR-Cas9 screens.  
505 Goodness of fit is calculated as Pearson Correlation Coefficient. **b)** Average ratio of the  
506 percentage of GFP positive RN2C cells between day 2 and day 10 after infection with  
507 lentiviral vectors expressing GFP and individual gRNAs against the indicated targets. The  
508 mean  $\pm$ S.E.M. depletion of 3 different gRNAs against the catalytic domain of the targets is  
509 shown. gRNA targeting the Rosa26 locus was a negative control. Rpa (replication protein  
510 A) is a positive control. **c)** Competitive co-culture assay showing negative selection of  
511 BFP+ MOLM13 or MLL-AF9 primary mouse cells upon targeting of METTL3 by CRISPR-  
512 Cas9. Cells were transduced with lentiviruses expressing four different gRNAs targeting  
513 the 5' exons or the catalytic domain of METTL3 and the BFP-positive fraction was  
514 compared with the non-transduced population. Results were normalized to those at day 4  
515 for each gRNA. The mean  $\pm$ S.D. of two independent infections is shown. **d)** Colony  
516 forming assay of MLL-ENL/FLT3-ITD Cas9-expressing cells targeting METTL3 (catalytic  
517 domain-specific) or CTRL showing decreased replating ability. CFU: colony forming units  
518 ( $***p < 0.001$ ; t-test). The mean  $\pm$ S.D. of three independent experiments is shown. **e)**  
519 Average ratio of the percentage of GFP positive NIH-3T3 mouse fibroblasts between day 2  
520 and day 12 after infection with lentiviral vectors expressing GFP and individual gRNAs  
521 against the indicated targets. The mean  $\pm$ S.E.M. depletion of 3 different gRNAs against  
522 the catalytic domain of the targets is shown. Rpa (replication protein A) is a positive control.  
523 **f)** Colony forming assay of lineage negative haematopoietic Cas9-expressing cells  
524 targeting METTL3 (catalytic domain-specific; right panel) or CTRL. CFU: colony forming  
525 units ( $***p < 0.001$ ; t-test). The mean  $\pm$ S.D. of three independent experiments is shown. **g)**  
526 Competitive co-culture assay showing negative selection of BFP+ AML cell lines upon  
527 targeting of METTL3, METTL1, METTL14 and METTL16 by CRISPR-Cas9 using two  
528 independent gRNAs for each target. Cells were transduced with lentiviruses expressing  
529 BFP and four different gRNAs targeting the 5' exons or the catalytic domain of each target  
530 and the BFP-positive fraction was compared with the non-transduced population. Results  
531 were normalized to those at day 4 for each gRNA. The mean  $\pm$ S.D. of two independent  
532 infections is shown.

533

534 **Extended Data Figure 2 (Related to Figure 1)**

535 **Effects of targeting METTL factors in human cancer cell lines.**

536 **a)** Competitive co-culture assay showing negative selection of BFP+ human cancer cell  
537 lines upon targeting of METTL3, METTL1, METTL14 and METTL16 by CRISPR-Cas9  
538 using two independent gRNAs for each target. The experiment was performed as  
539 described above. **b)** Efficiency of genome editing for gRNAs targeting METTL3, METTL1,  
540 METTL14 and METTL14 was measured across the indicated 20 human cell lines through  
541 TIDE analysis. Efficiency of targeting was also measured in mouse primary cell lines for  
542 gRNAs targeting Mettl3. **c)** CD11b expression in METTL3 (catalytic domain-specific)  
543 targeted cells (THP1 human cell line) was measured by flow cytometry 6 days after  
544 infection. **d)** Haematoxylin eosin staining of human and mouse AML cell lines infected with  
545 a control gRNA or gRNAs targeting the catalytic domain of METTL3. **e)** Time course  
546 quantification of luminescence from mice transplanted with luciferase-labelled MOLM13  
547 cells targeting METTL3 using gRNAs specific for the catalytic domain or CTRL ( $***p <$   
548  $0.001$ ).

549

550 **Extended Data Figure 3 (Related to Figure 1)**

551 **METTL3 depletion in AML human cell lines leads to cell cycle arrest.**

552 **a)** METTL3 mRNA levels detected by RT-qPCR 4 days after shRNAs induction with  
553 doxycycline in MOLM13 cells. The mean  $\pm$ S.E.M. of four independent cultures is shown. **b)**  
554 Western blot showing METTL3 and H3 levels in MOLM13 cells infected with specific or  
555 CTRL TET-inducible shRNAs 5 days after doxycycline treatment. For gel source data see  
556 Supplementary Information. **c)** METTL3 mRNA levels detected by RT-qPCR 4 days after  
557 shRNA induction with doxycycline in THP1 cells (left panel). The mean  $\pm$ S.E.M. of three  
558 independent cultures is shown. A proliferation assay of the cells was then performed with  
559 cell numbers measured between day 0 (4d post doxycycline) and day 4 (8d post  
560 doxycycline) (right panel). The mean  $\pm$ S.D. of two independent replicates is shown. **d)**  
561 Western blot for METTL3 and actin in mouse AML cells. MPN1c/Flt3<sup>ltd/+</sup>/Rosa26<sup>Cas9/+</sup>  
562 mouse AML cells were transduced with gRNAs targeting the catalytic domain of Mettl3 and  
563 plasmids expressing either wild type METTL3 or a catalytically inactive mutant (DW/AA).  
564 For gel source data see Supplementary Information. **e)** Volcano plots for CTRL vs  
565 METTL3 KD samples, showing the significance p-value (log<sub>10</sub>) versus. Fold Change (log<sub>2</sub>)  
566 of gene expression. Significantly upregulated and downregulated transcripts are shown in  
567 red ( $|\log_{2}FC| > 1$ ,  $p < 0.001$ ,  $FDR < 0.01$ ). **f)** Graphical representation of KEGG pathways  
568 regulation showing cell cycle down-regulation (upper panel) and haematopoietic  
569 differentiation up-regulation (lower panel) as obtained by comparing RNA-seq derived from  
570 METTL3 KD and CTRL MOLM13 cells (up-regulated genes: red; down-regulated genes:  
571 green).

572

573 **Extended Data Figure 4 (Related to Figure 1 and 2)**

574 **METTL3 is overexpressed in human AML and it is recruited on chromatin.**

575 **a)** METTL3 (top panel) and METTL14 (lower panel) mRNA expression levels across  
576 cancer types from the TCGA database. **b)** Proliferation assay of human AML cell lines  
577 upon transduction with a vector expressing METTL3. Cell numbers were measured  
578 between day 1 and day 3 after electroporation. The mean  $\pm$ S.D. of three independent  
579 replicates is shown. **c)** Western blot for METTL3 and METTL14, GAPDH and histone H3  
580 on cytoplasmic, nucleoplasmic and chromatin fractions from MOLM13 cells. For gel source  
581 data see Supplementary Information. **d)** Genomic browser screenshot of METTL14 and  
582 H3K4me3 normalised ChIP-seq datasets on the human *SP2* gene locus from MOLM13  
583 cells. **e)** Pie charts of genomic regions associated with METTL14 (top) and METTL3  
584 (bottom) ChIP-seq peaks. **f)** Distribution of METTL14 ChIP-seq reads centred on TSSs  
585 (upper panel) and histogram of ChIP-seq reads distribution relative to TSSs (lower panel).  
586 **g)** Top: Venn diagram showing the overlap between METTL3 and METTL14 peak  
587 datasets (statistical significance was evaluated by a  $\chi^2$ -test). Bottom: Distribution of  
588 METTL3 and METTL14 ChIP-seq reads centred on METTL14 (left panel) or METTL3  
589 (right panel) peaks.

590

591 **Extended Data Figure 5 (Related to Figure 2)**

592 **Validation of METTL3 ChIP-seq.**

593 **a)** ChIP-seq validation by ChIP-qPCR of METTL3 and METTL14 binding on the *SP2* and  
594 *RFX1* loci. The mean of six technical replicates  $\pm$ S.D. is shown. The experiment was  
595 performed independently three times. **b)** METTL3 ChIP-seq validation by ChIP-qPCR on  
596 the indicated loci. The *LMO2* promoter was used as a negative control. The mean of three  
597 technical replicates  $\pm$ S.D. is shown. The experiment was performed independently three  
598 times. **c)** METTL3 ChIP-seq validation by ChIP-qPCR on the indicated TSSs using two  
599 independent METTL3 antibodies in MOLM13 cells. The mean of six technical replicates  
600  $\pm$ S.D. is shown. The experiment was performed independently three times. **d)** METTL3  
601 ChIP-seq validation by ChIP-qPCR on the indicated TSS in CTRL or METTL3 KD

602 MOLM13 cells, showing a specific reduction of METTL3 binding in KD cells. The mean of  
603 three technical replicates  $\pm$ S.D. is shown. The experiment was performed independently  
604 three times.

605

#### 606 **Extended Data Figure 6 (Related to Figure 2)**

##### 607 **METTL3 colocalise with a defined set of chromatin factors.**

608 **a)** Motif discovery analysis of the genomic sequences under METTL3 ChIP-seq peaks  
609 using HOMER. Significance was obtained using a hypergeometric test. **b)** Distribution of  
610 ChIP-seq reads for the indicated factors or histone modifications, centred on METTL3  
611 (green) and METTL14 (blue) ChIP peaks. Statistical significance of the binary overlap was  
612 evaluated by a  $\chi^2$ -test. **c)** Venn diagram showing the overlap of H3R2me2s, WDR5, KLF9,  
613 NFYA and NFYB ChIP-seq peaks after filtering for H3K4me3 promoters. **d)** Venn diagram  
614 showing significant overlap between METTL3 but not METTL14 peaks with the 447 loci  
615 carrying all five factors as in panel above. Statistical significance of the binary overlap was  
616 evaluated by a  $\chi^2$ -test.

617

#### 618 **Extended Data Figure 7 (Related to Figure 2)**

##### 619 **CEBPZ recruits METTL3 on chromatin.**

620 **a)** Histogram representing the positive predictive power of the combined 5 factors  
621 compared with the predictive power of the ENCODE factors whose expression levels are  
622 tightly correlated with METTL3 expression. **b)** Correlation between CEBPZ and METTL3  
623 mRNA expression levels in the Human Protein Atlas RNA-seq datasets, including non-  
624 transformed (blue) and cancer (pink) cell lines. ( $\rho$ = Spearman correlation coefficient). **c)**  
625 Genomic plot of METTL3 and CEBPZ normalised ChIP-seq datasets on the human *SP1*  
626 and *SP2* gene loci in MOLM13 and K562 cells, respectively. **d)** Distribution and heatmaps  
627 of normalised ChIP-seq reads for METTL3 centred on CEBPZ peaks. **e)** Distribution and  
628 heatmaps of normalised ChIP-seq reads of METTL14 and CEBPZ centred on METTL14  
629 (left panel) and CEBPZ (right panel) peaks. **f)** Competitive co-culture assay showing  
630 negative selection of BFP+ AML cell lines upon targeting of CEBPZ by CRISPR-Cas9  
631 gRNAs. Cells were transduced with lentiviruses expressing a gRNA targeting the first exon  
632 of CEBPZ and the BFP-positive fraction was compared with the non-transduced  
633 population. Results were normalized to those at day 4. The mean  $\pm$ S.D. of two  
634 independent infections is shown. **g)** CEBPZ mRNA levels detected by RT-qPCR 4 days  
635 after shRNA induction with doxycycline in MOLM13 cells. The mean  $\pm$ S.D. of three  
636 independent cultures is shown.

637 **h)** A proliferation assay of the CEBPZ CTRL and KD cells was performed with cell  
638 numbers measured between day 0 (4d post doxycycline) and day 4 (8d post doxycycline).  
639 The mean  $\pm$ S.D. of six independent replicates is shown. **i)** ChIP-qPCR of METTL3 binding  
640 on target TSSs in and MOLM13 cells, expressing a control shRNA or two independent  
641 shRNAs against CEBPZ, showing a specific reduction of METTL3 binding in CEBPZ KD  
642 cells. The mean of three technical replicates  $\pm$ S.D. is shown. The experiment was  
643 performed independently three times. **j)** Box plot representing the expression levels of  
644 METTL3 targets upon METTL3 KD from the dataset shown in Extended Data Figure 3e.

645

#### 646 **Extended Data Figure 8 (Related to Figure 3)**

##### 647 **Validation of the m6A RNA-IP upon METTL3 depletion.**

648 **a)** Motif analysis under the identified m6A-IP peaks showing enrichment of the expected  
649 UGCAG and GGACU sequences and their central distribution throughout the m6A-IP  
650 peaks, as obtained by MEME and CentriMo. **b)** Distribution of m6A-IP reads throughout  
651 the mRNA metatranscript, showing the expected enrichment around the STOP codon in  
652 MOML13 cells. **c)** Scatter plots and density plot showing the general down-regulation of  
653 m6A-IP signal upon METTL3 knock-down in MOLM13 cells. **d)** Histogram showing

654 METTL3–dependent m6A-IP read coverage in mRNAs from METTL3-bound TSSs (ChIP),  
655 whole transcriptome (All) or the permutation of random sets of genes (Rand). **e)** m6A-IP  
656 followed by qPCR for m6A peaks of HNRNPL or GAPDH as a control. The plot show the  
657 m6A-IP signal over total input in MOLM 13 cells expressing a control shRNA or shRNAs  
658 targeting CEBPZ. Mean  $\pm$ S.D. of three technical replicates are shown; the experiment has  
659 been performed independently twice. **f)** SP1, SP2, HNRNPL and METTL3 mRNA levels  
660 detected by RT-qPCR 8 days after doxycycline induction in MOLM13 CTRL or CEBPZ KD  
661 cells. The mean  $\pm$ S.D. of three independent cultures is shown. **g)** Histogram showing the  
662 enrichment of the [GAG]<sub>n</sub> motif within the transcript sequences of METTL3 ChIP-targets  
663 compared with random permutations of genes.  
664

### 665 **Extended Data Figure 9 (Related to Figure 3)**

#### 666 **Ribosome profiling analysis.**

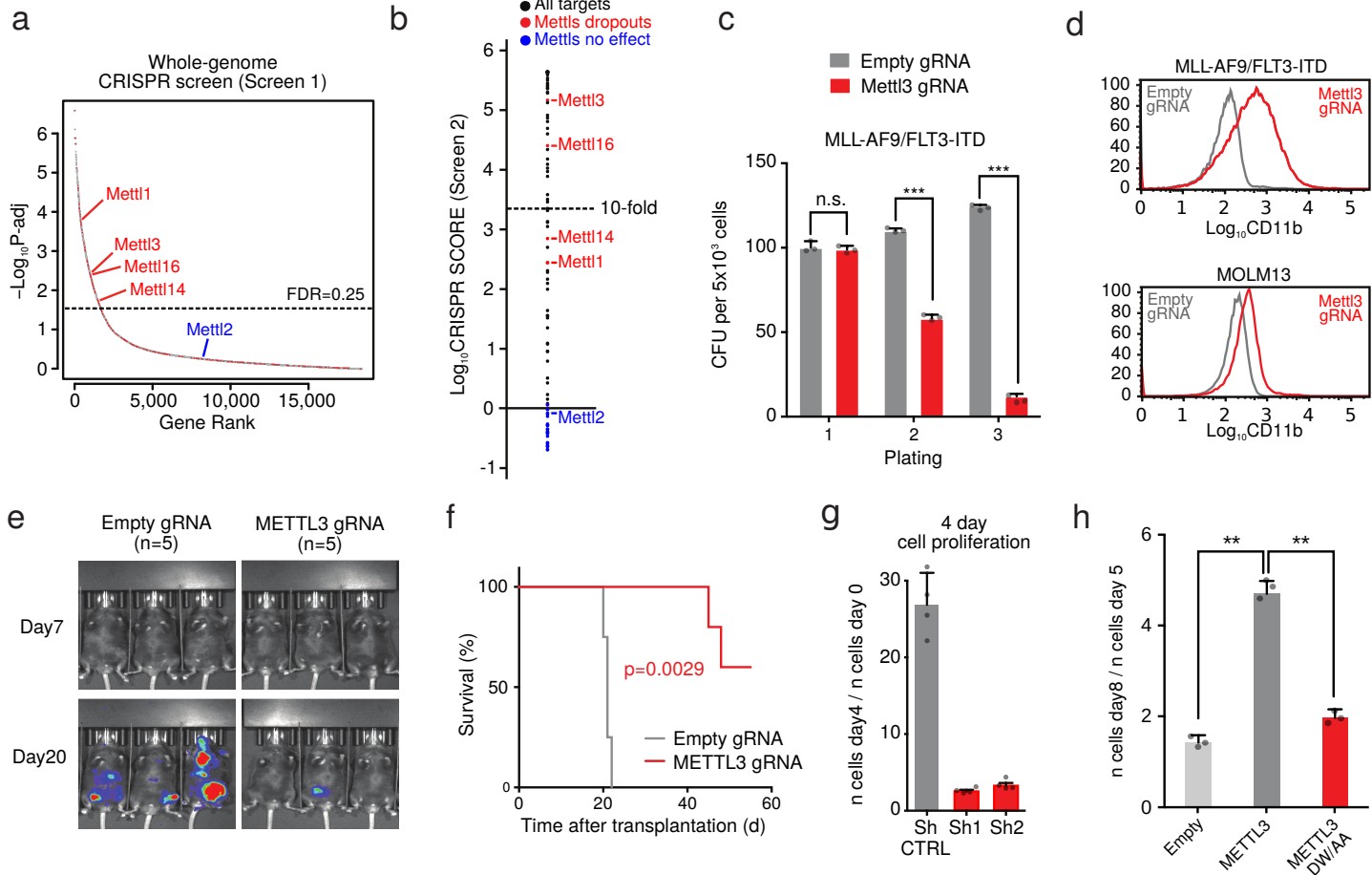
667 **a)** Distribution of ribosome profiling reads throughout the mRNA metatranscript from RNA  
668 inputs or ribosome-protected fragments (RPF) showing absence of 3'UTR specifically in  
669 the RPF dataset. **b)** Reading frame analysis of ribosome profiling reads from RNA inputs  
670 and RPF in MOLM13 cells showing enrichment of the 0 reading frame specifically in the  
671 RPF reads. **c)** Average read alignments to 5' and 3' ends of coding sequences in RNA  
672 inputs (upper panel) or RPF (lower panel) showing triplet periodicity and accumulation of  
673 reads on the start site typical of cycloheximide pre-treatment. **d)** Principal component  
674 analysis of P-site codon distribution on mRNAs from METTL3-bound TSSs as obtained by  
675 ribosome footprinting, 5 or 8 days after doxycycline administration, of METTL3 KD (KD5,  
676 KD8) or CTRL (WT5, WT8) MOLM 13 cells. **e)** Principal component analysis of P-site  
677 codon distribution on all mRNAs, as above. **f)** Frequency of P-site occupancy of codons in  
678 METTL3 KD or CTRL MOLM13 cells for either all coding genes or genes harbouring a  
679 METTL3 ChIP peak on their promoter (\* $p < 0.05$ ; t-test). **g)** Frequency of codons within the  
680 coding sequence of METTL3 chromatin targets compared with the general frequency  
681 throughout the coding transcripts. The plot shows no significant overrepresentation of GAN  
682 codons in the METTL3 chromatin targets.  
683

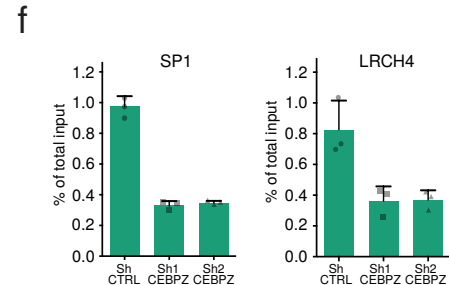
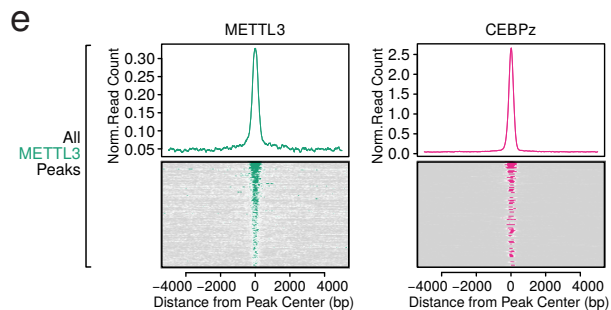
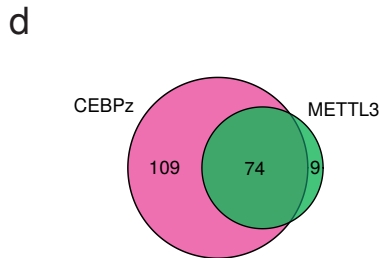
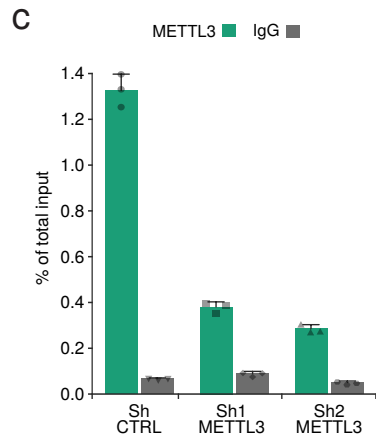
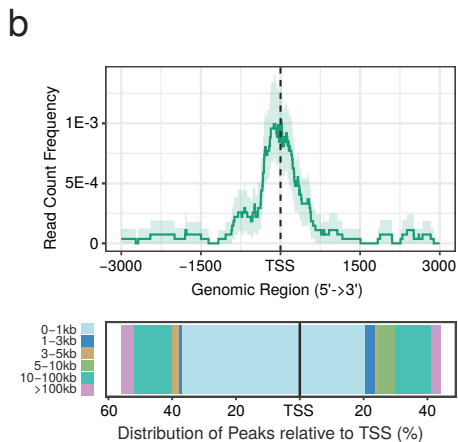
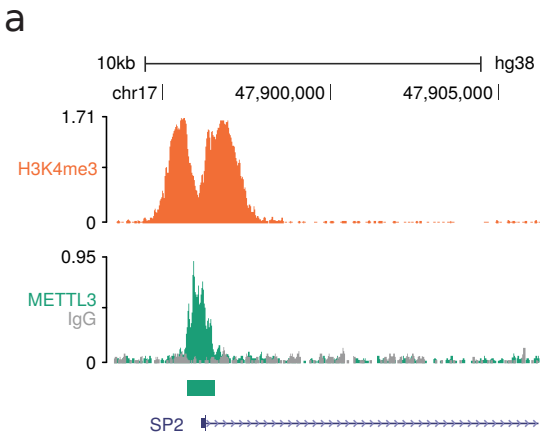
### 684 **Extended Data Figure 10 (Related to Figure 4)**

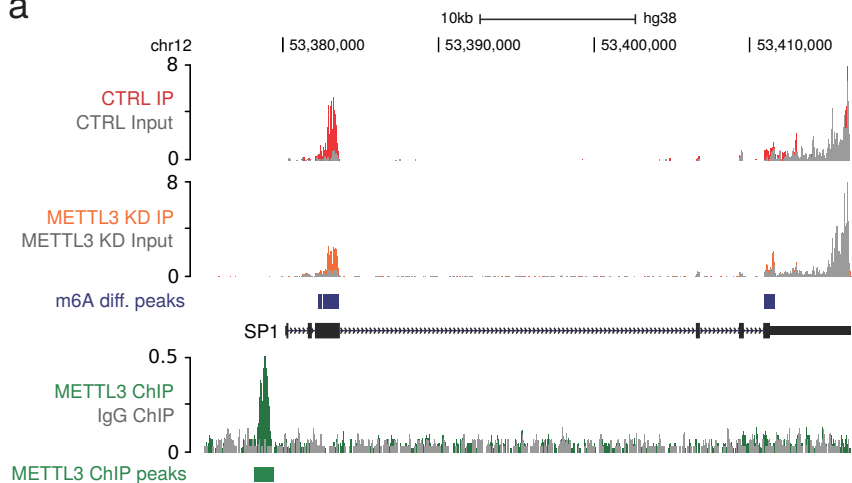
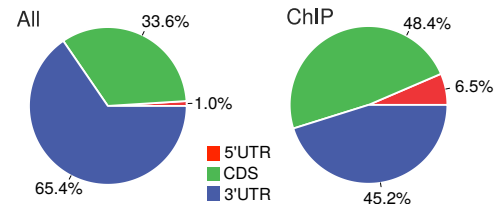
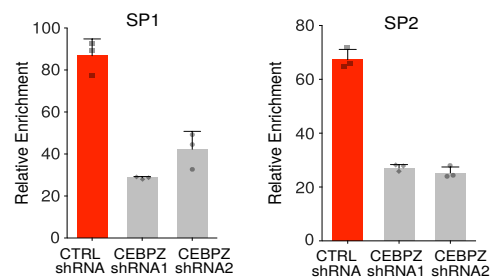
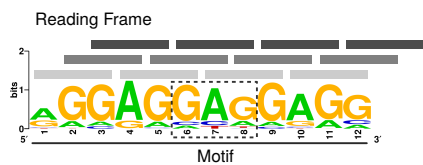
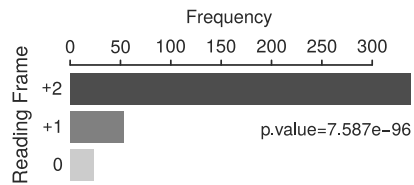
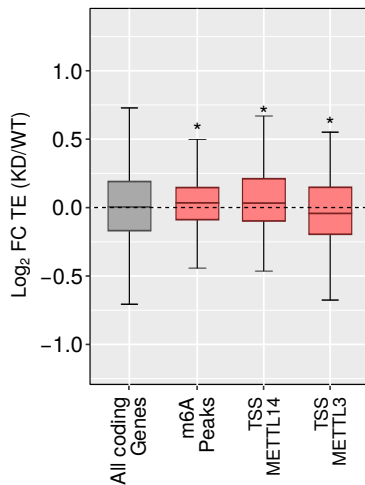
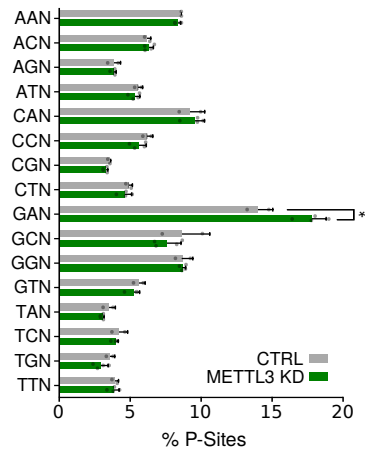
#### 685 **METTL3 controls the translation of SP1 and SP2.**

686 **a)** RNA-seq normalised counts of SP1 and SP2 mRNAs from CTRL or METTL3 KD  
687 MOLM13 cells at day 8 after doxycycline induction. Mean +S.D. of at least three biological  
688 replicates are shown; **b)** Western blot showing CEBPZ, SP1 and GAPDH levels in CTRL  
689 and CEBPZ KD cells. For gel source data see Supplementary Information. **c)** Polysome  
690 fractionation analysis. Cell extracts from CTRL or METTL3 KD cells were prepared and  
691 resolved in 5 to 50 % sucrose gradient. The absorbance at 254 was continuously  
692 measured. The peaks corresponding to free 40S and 60S subunits, 80S and polysomes  
693 are indicated. **d)** DICER1 and ACTIN mRNAs in each ribosome fraction were quantified  
694 through qPCR and plotted as a percentage of the total. Data are from two independent  
695 polysome-profiling experiments. Mean  $\pm$ S.E.M. are shown. **e)** Firefly luciferase activity in  
696 FADU cell line from UAS or scrambled (SCR) sequence carrying plasmid in presence of  
697 GAL4 either alone or fused with METTL3 wild type (CD) or inactive (CD DW/AA) catalytic  
698 domain (\* $p < 0.05$ ; t-test). The mean +S.D. of three independent transfections is shown. **f)**  
699 Firefly luciferase mRNA from plasmids carrying UAS or scrambled sequence in presence  
700 of GAL4 either alone or fused with METTL3 wild type (CD) or inactive (CD DW/AA)  
701 catalytic domain, as evaluated by qPCR. The mean  $\pm$ S.D. of three replicates is shown. **g)**  
702 Box plot showing transcriptional modulation of genes bound by SP1, SP2 or both between  
703 METTL3 KD and CTRL MOLM13 cells (\* $p < 0.05$ ; Wilcoxon test). **h)** Genomic browser  
704 screenshot of SP1 and SP2 normalised ChIP-seq dataset on the human *c-MYC* gene  
705 locus in K562 cells (from ENCODE). **i)** Western blot showing METTL3, SP1 and ACTIN

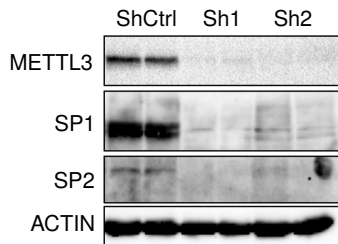
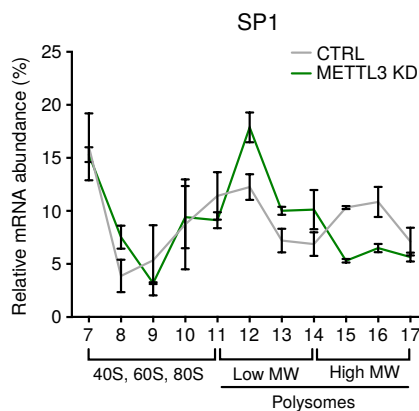
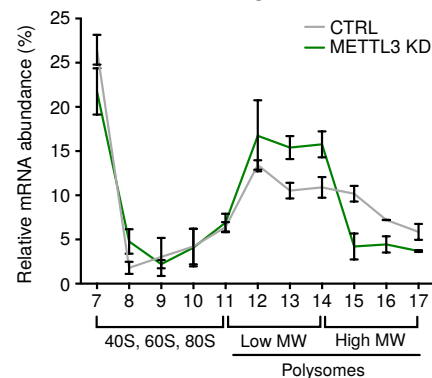
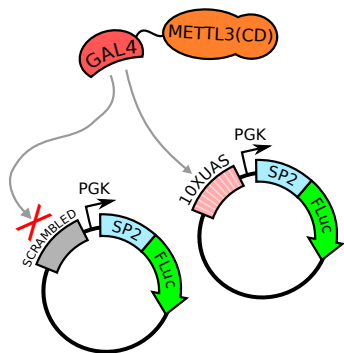
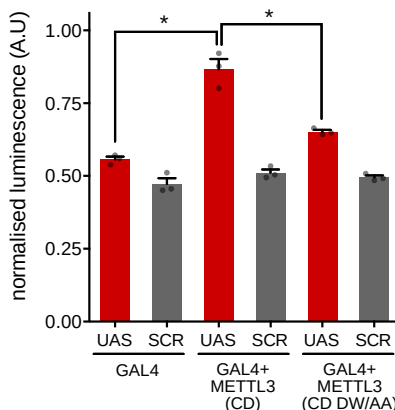
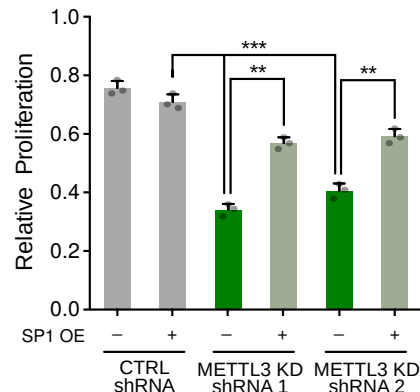
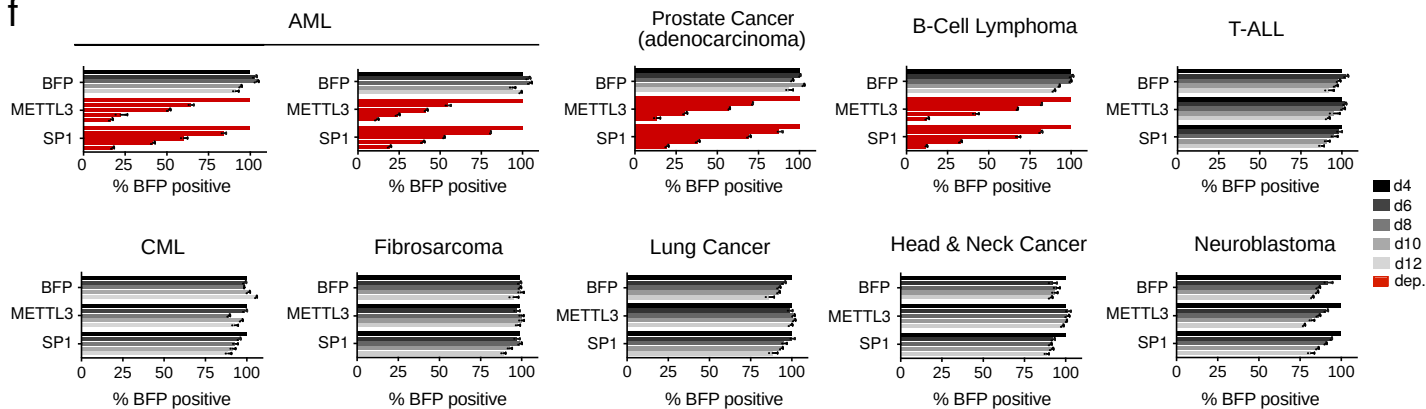
706 protein levels in MOLM13 cells infected with METTL3-specific or CTRL TET-inducible  
707 shRNAs and with an SP1 expression vector 5 days after doxycycline treatment. For gel  
708 source data see Supplementary Information.  
709

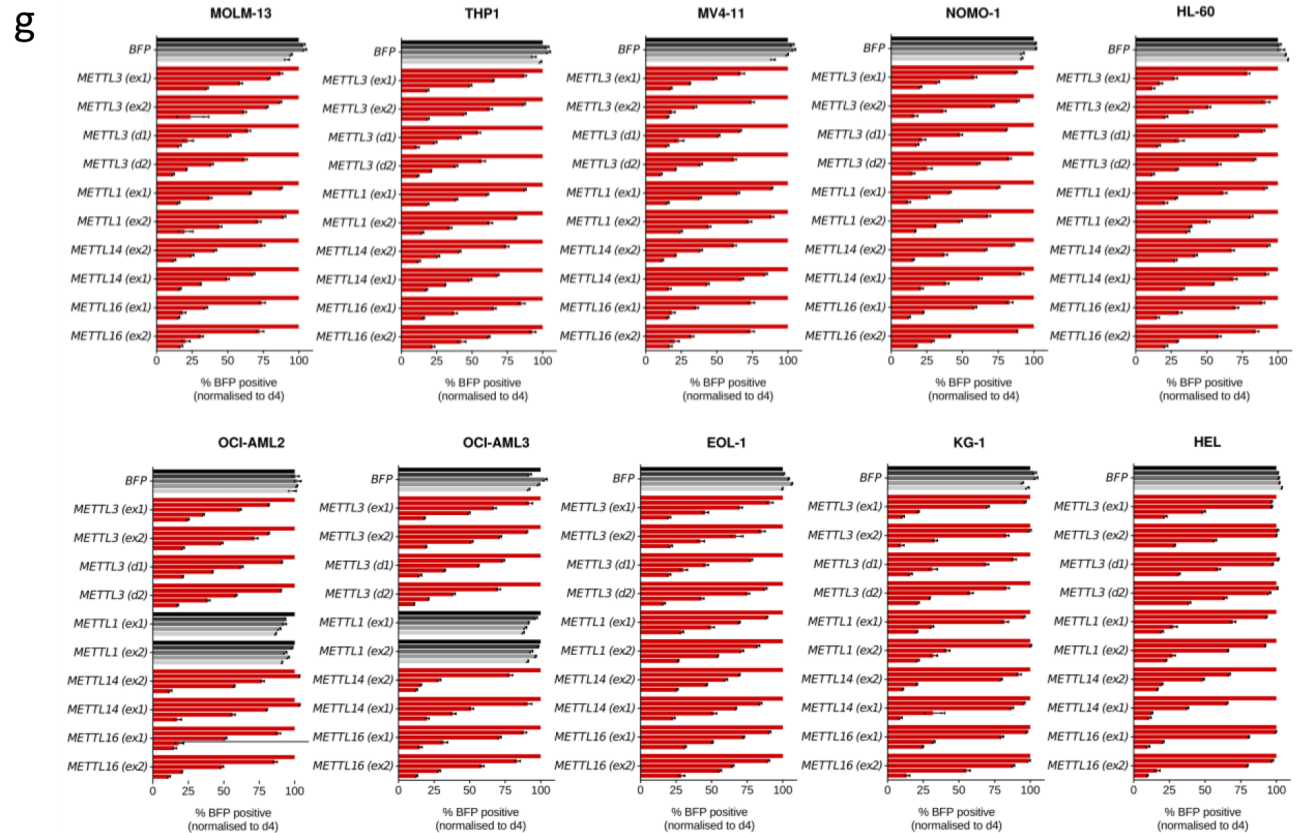
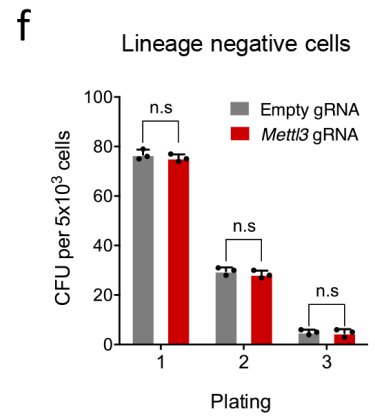
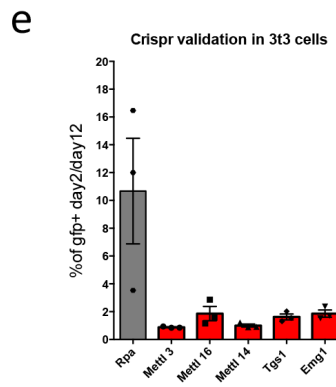
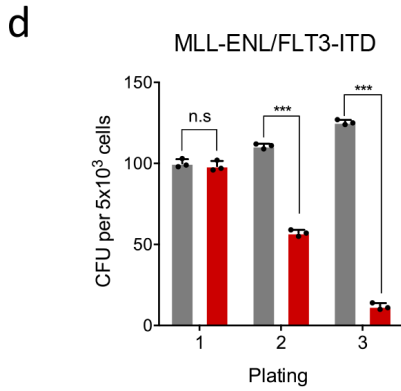
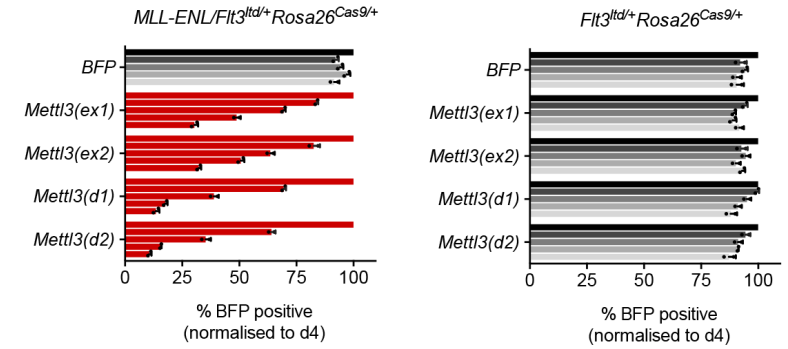
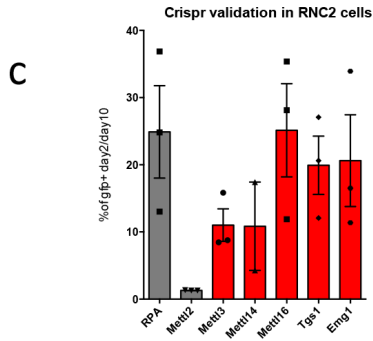
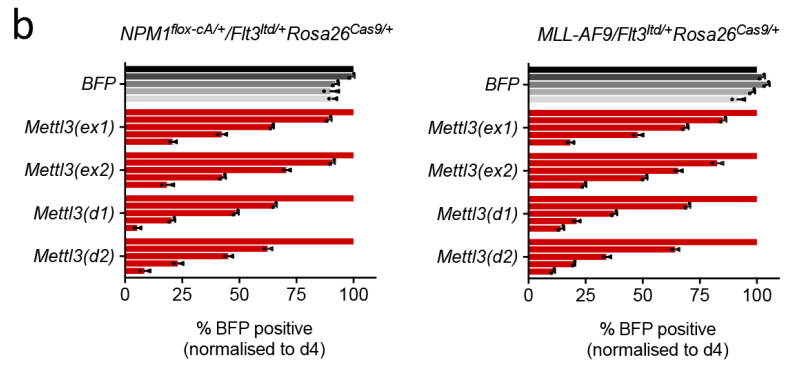
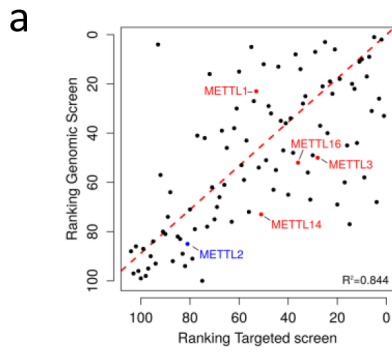


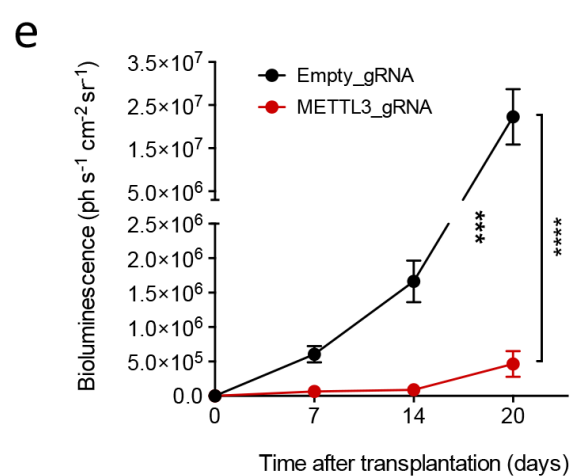
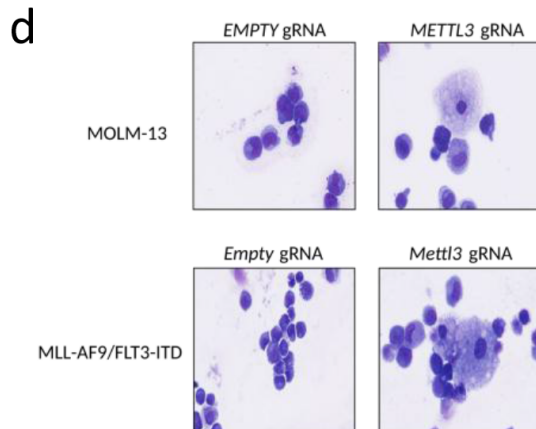
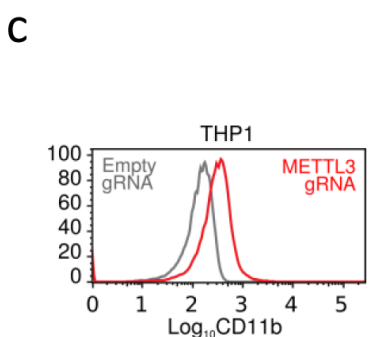
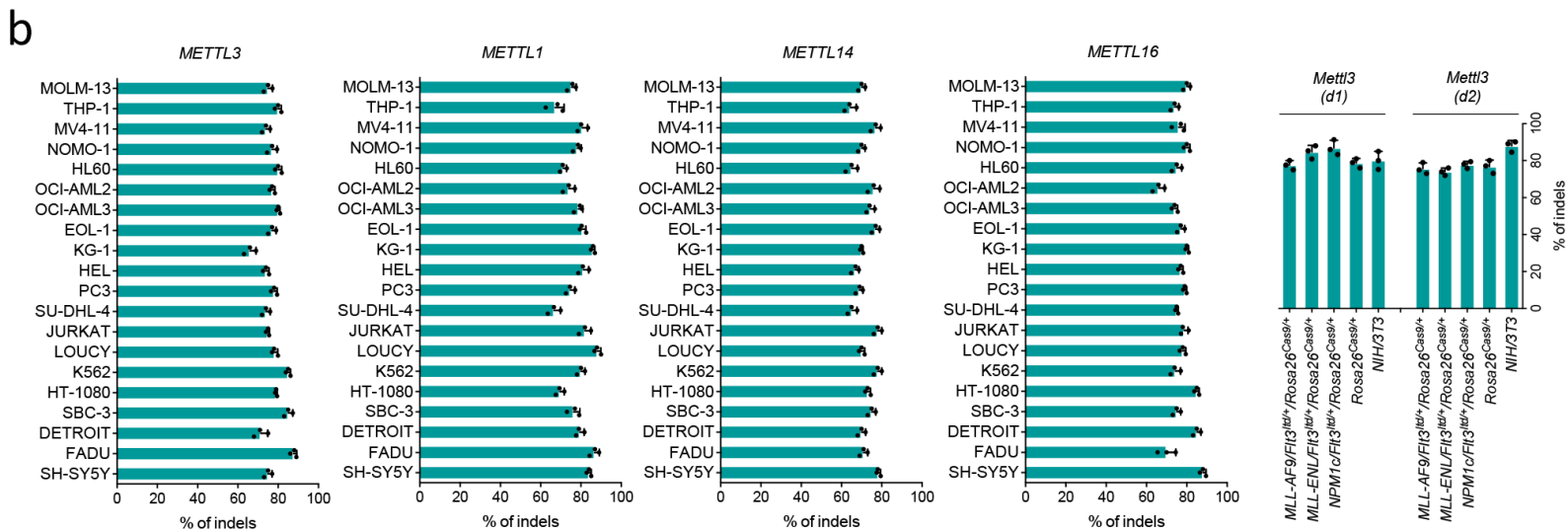
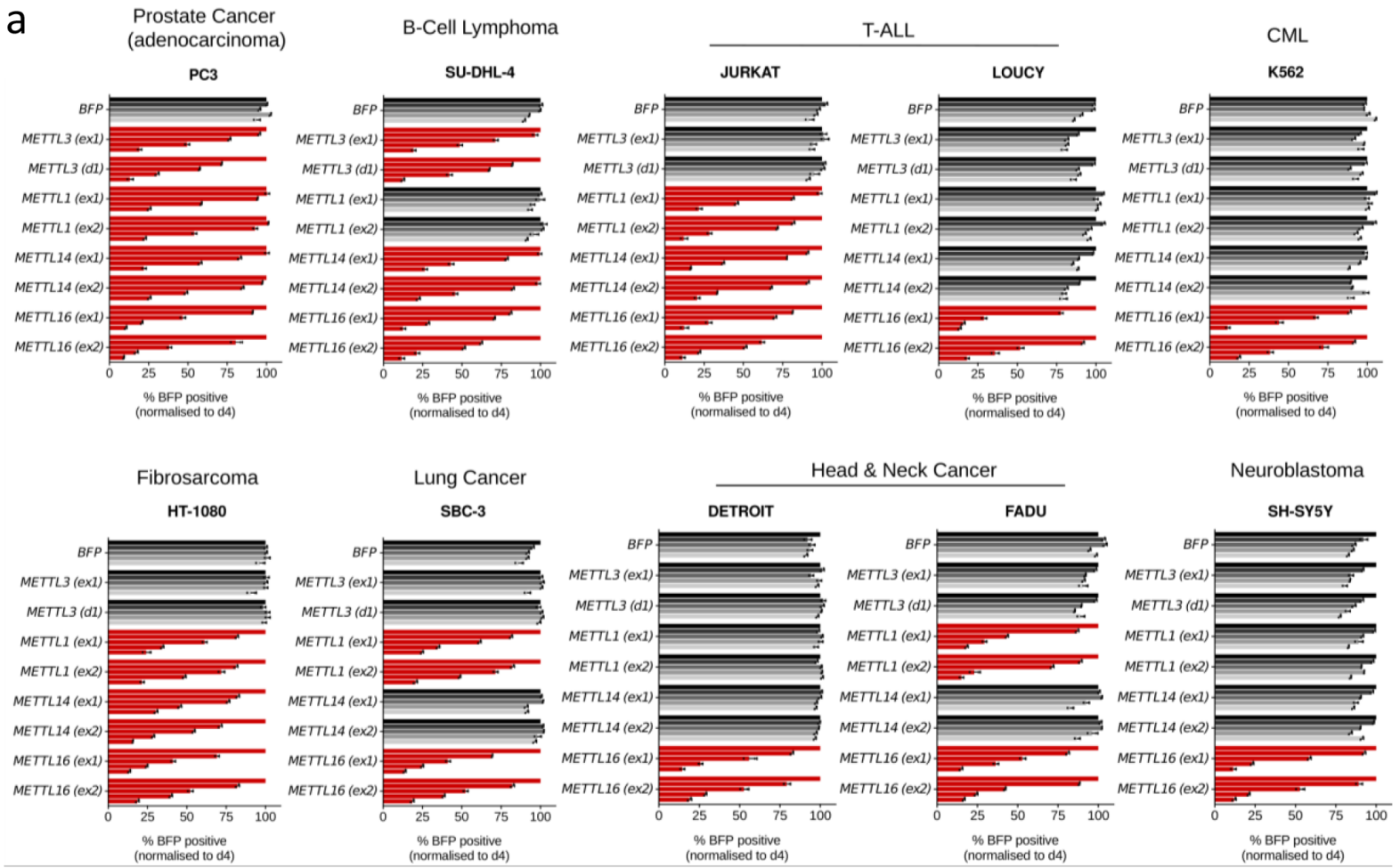


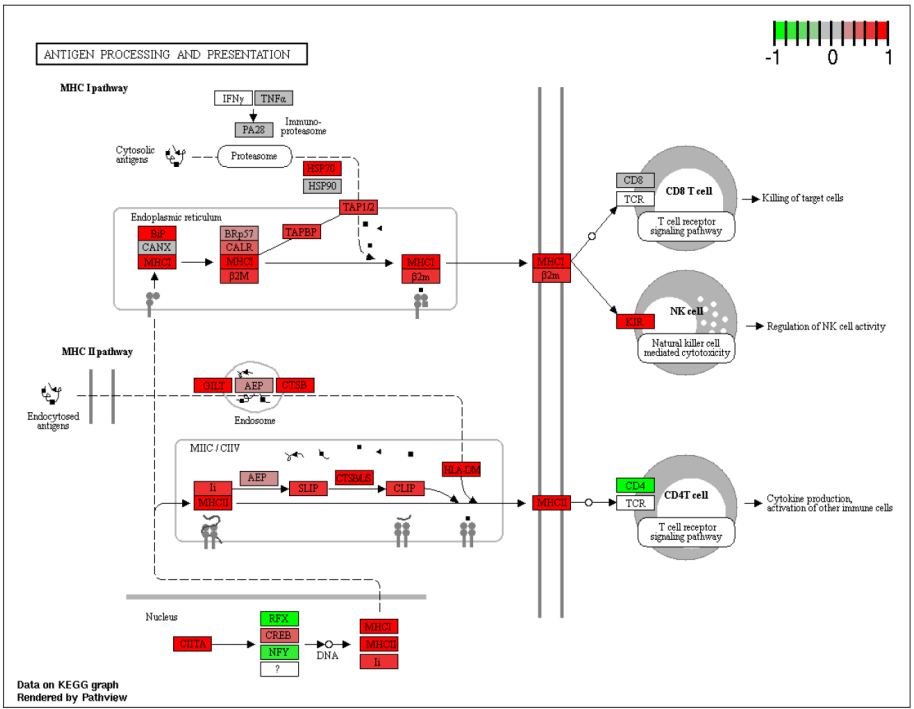
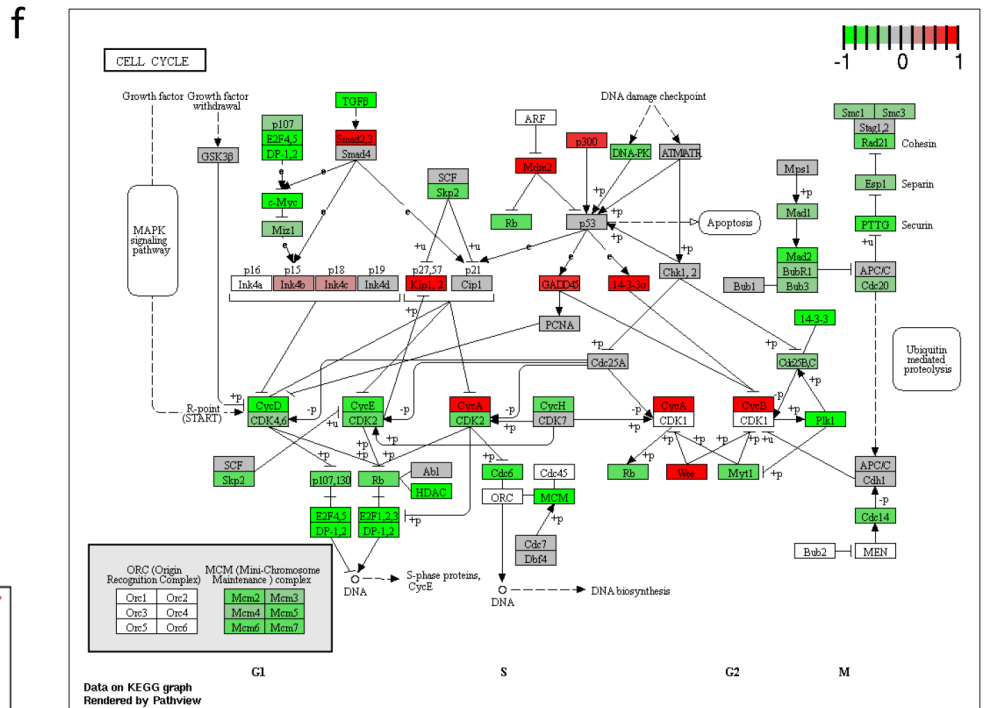
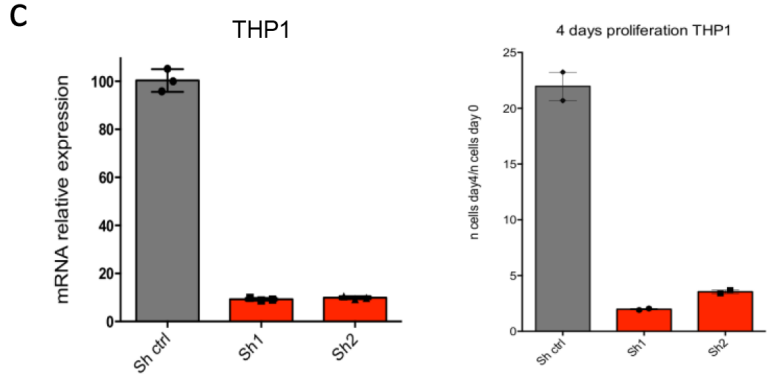
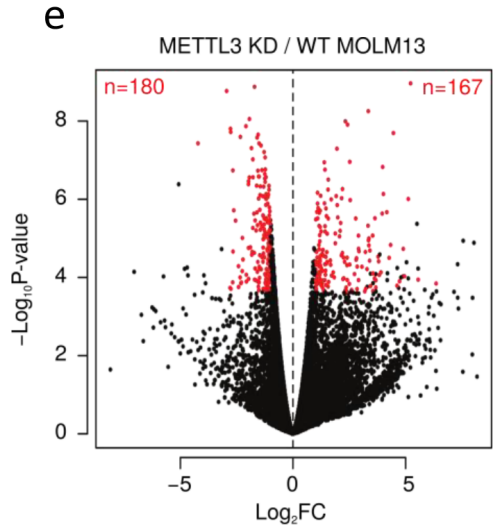
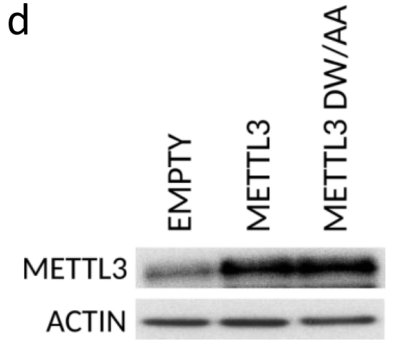
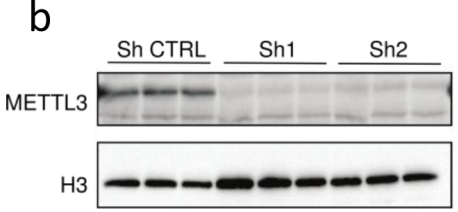
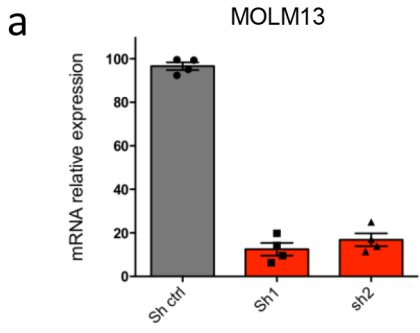
**a****b****c****d****e****f****g**

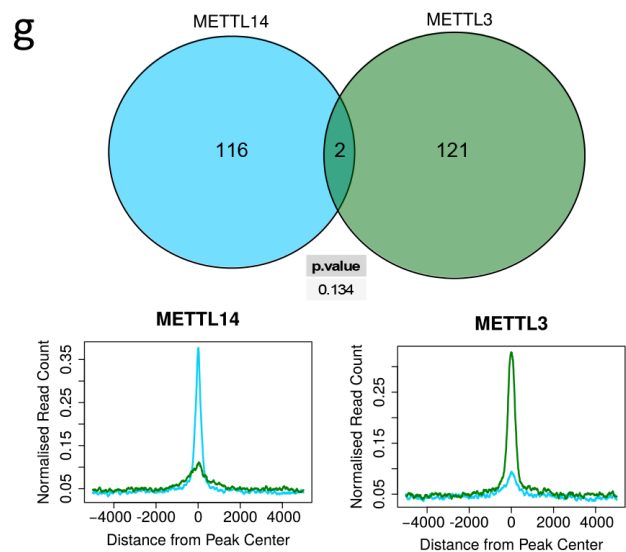
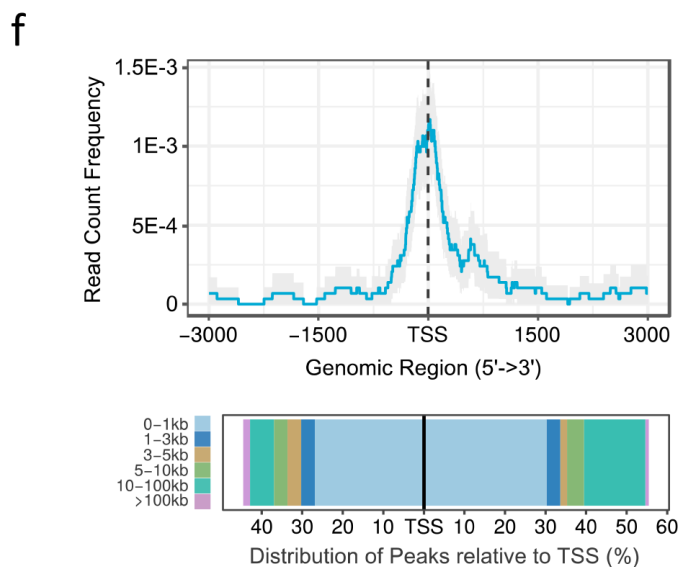
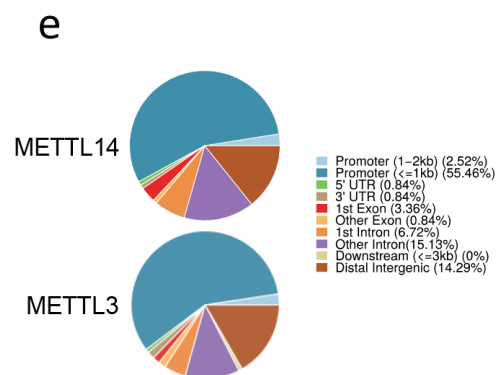
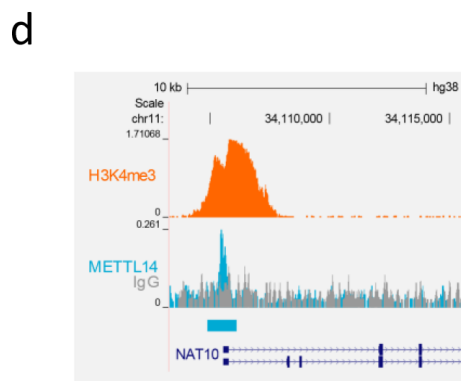
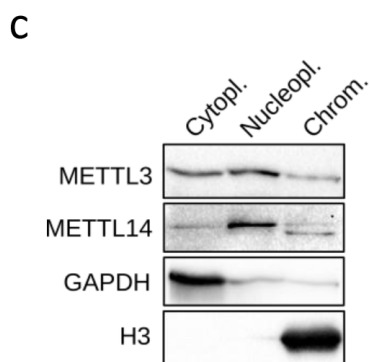
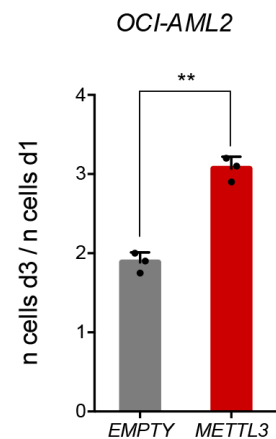
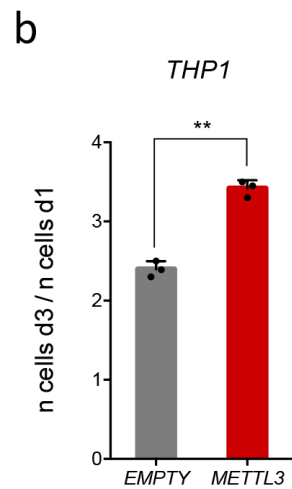
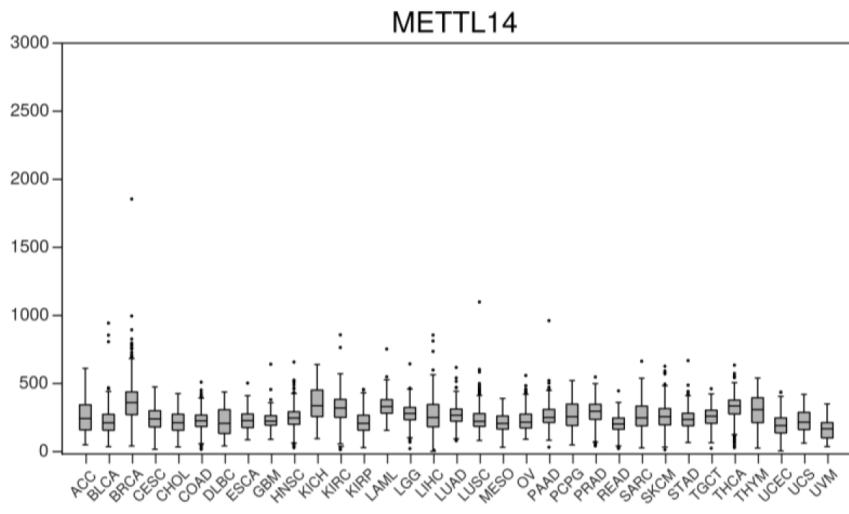
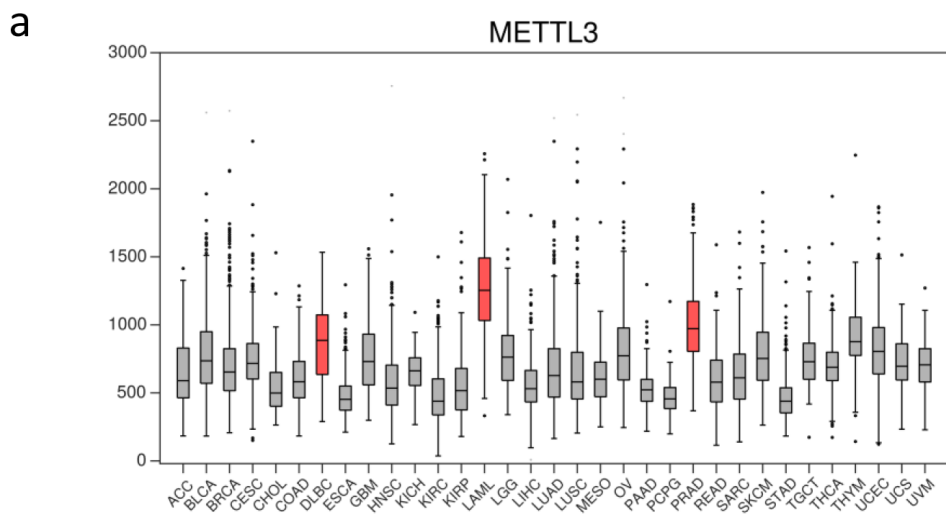


**a****b****SP2****c****d****e****f**

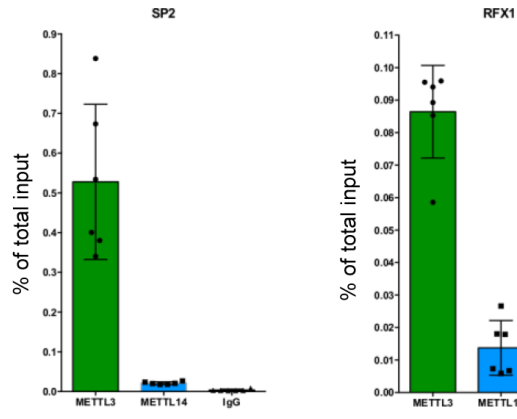




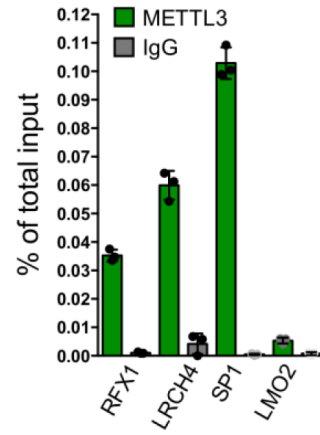




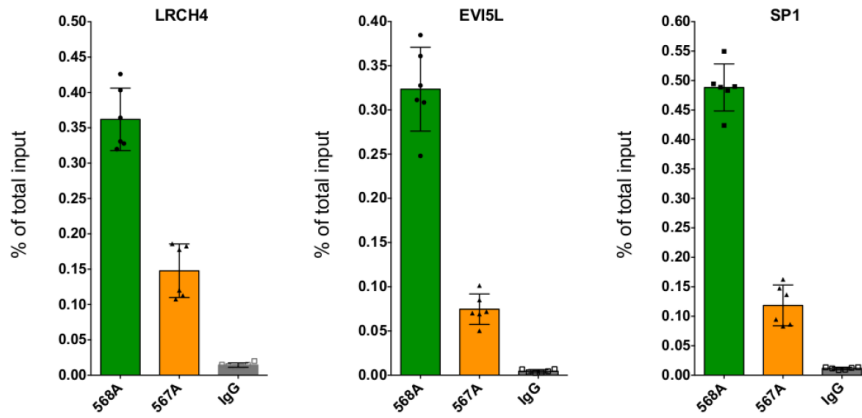
a



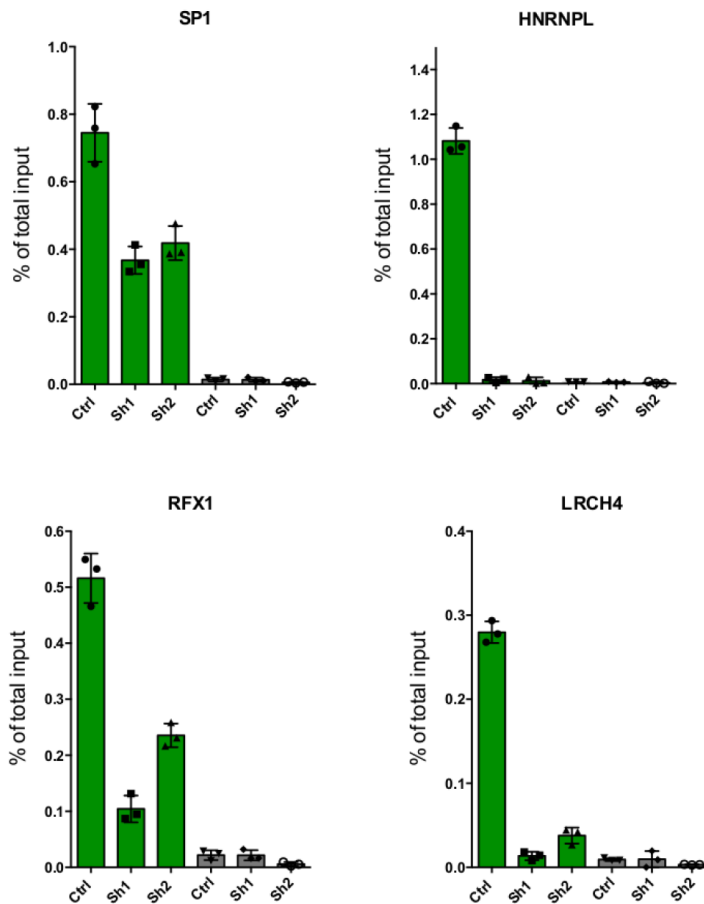
b



c



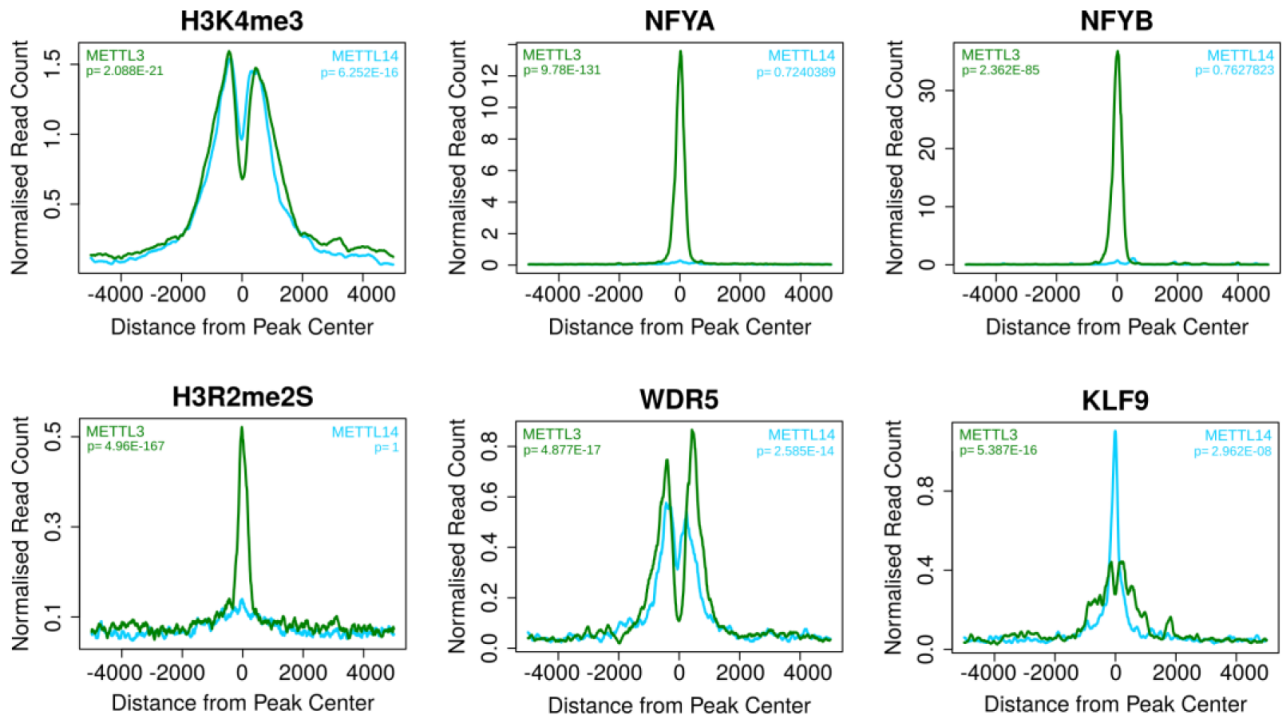
d



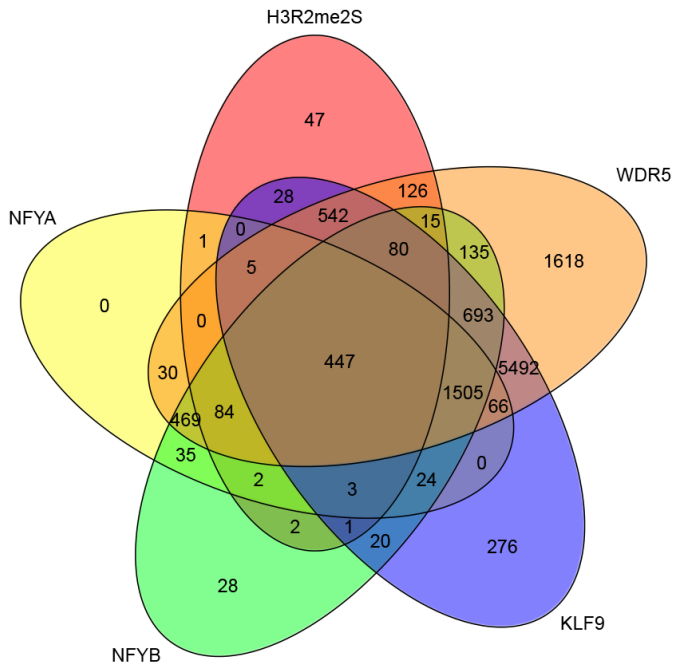
**a**

Rank	Motif	Name	P-value	log P-value	q-value (Benjamini)	# Target Sequences with Motif	% of Targets Sequences with Motif	# Background Sequences with Motif	% of Background Sequences with Motif
1		NFY(CCAAT)/Promoter/Homer	1e-13	-3.050e+01	0.0000	20.0	58.82%	3508.6	8.03%
2		Klf9(Zf)/GBM-Klf9-ChIP-Seq(GSE62211)/Homer	1e-4	-9.345e+00	0.0139	10.0	29.41%	3104.8	7.11%
3		KLF14(Zf)/HEK293-KLF14 GFP-ChIP-Seq(GSE58341)/Homer	1e-3	-8.413e+00	0.0236	21.0	61.76%	13600.1	31.12%
4		Pbx3(Homeobox)/GM12878-PBX3-ChIP-Seq(GSE32465)/Homer	1e-2	-6.489e+00	0.1212	4.0	11.76%	643.0	1.47%
5		Pknox1(Homeobox)/ES-Prep1-ChIP-Seq(GSE63282)/Homer	1e-2	-6.222e+00	0.1267	4.0	11.76%	691.6	1.58%
6		Lhx3(Homeobox)/Neuron-Lhx3-ChIP-Seq(GSE31456)/Homer	1e-2	-5.144e+00	0.3103	10.0	29.41%	5318.1	12.17%

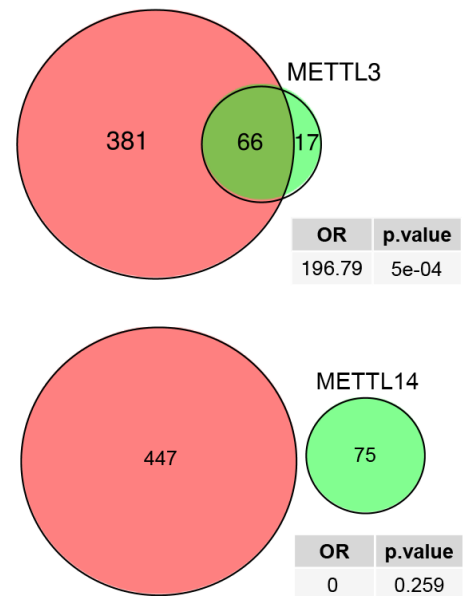
**b**

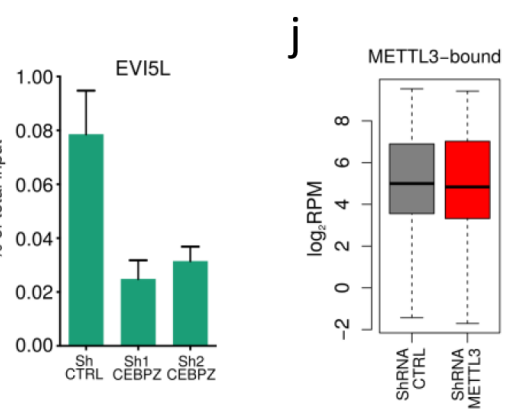
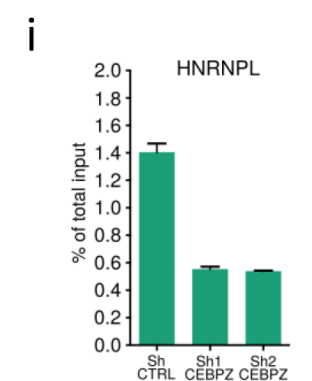
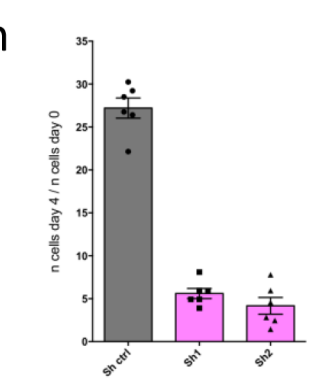
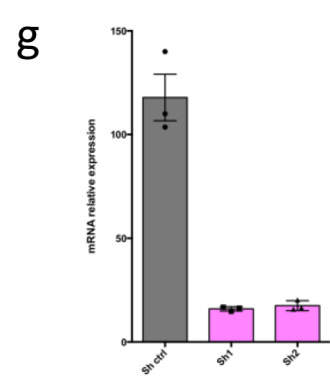
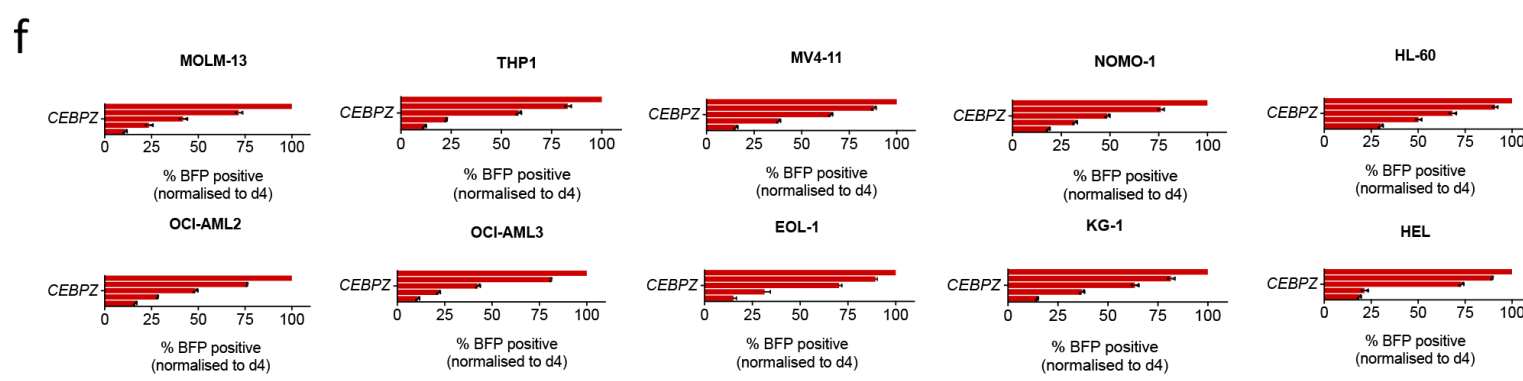
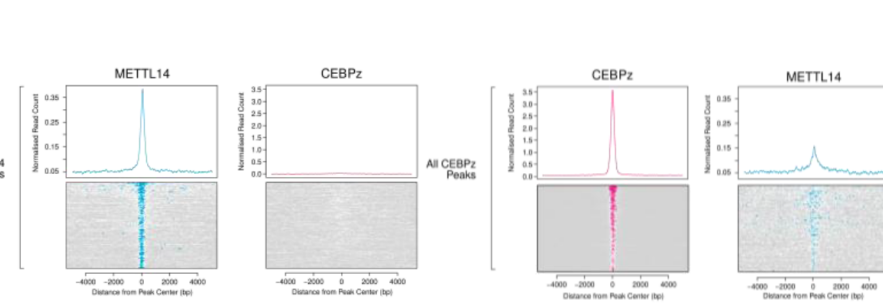
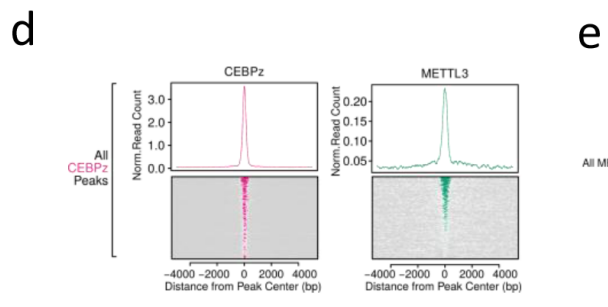
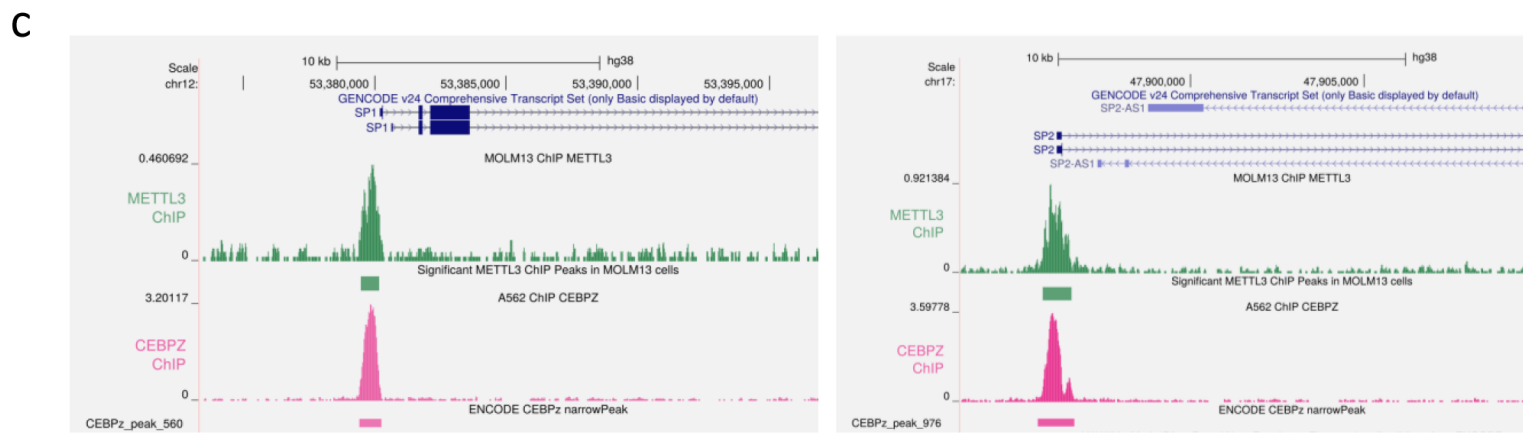
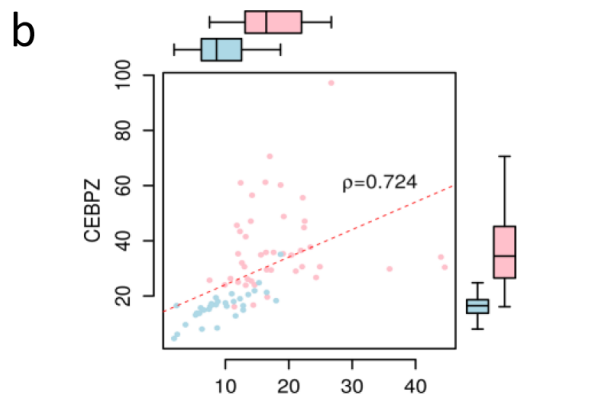
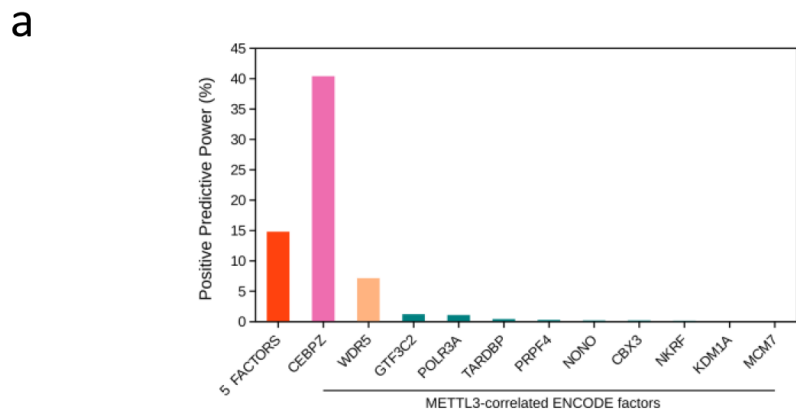


**c**

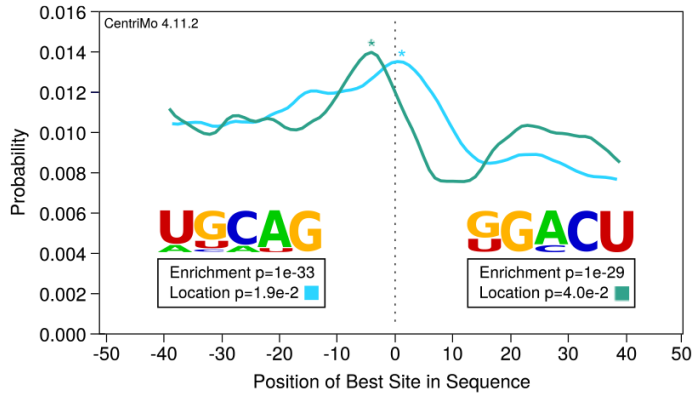
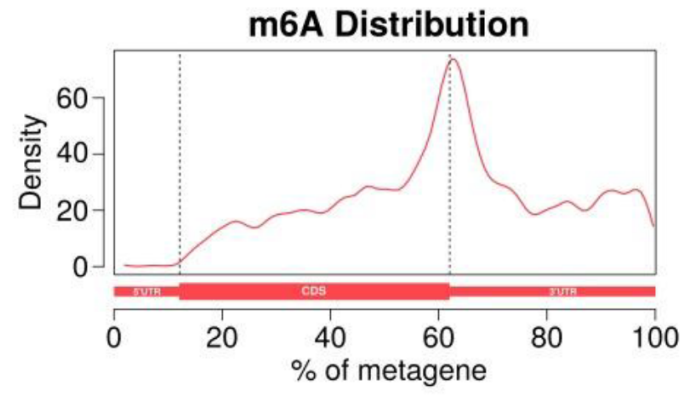
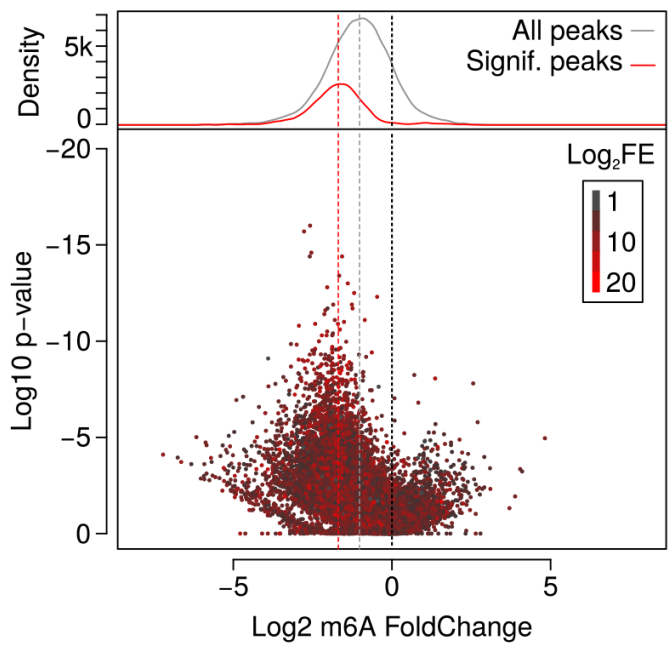
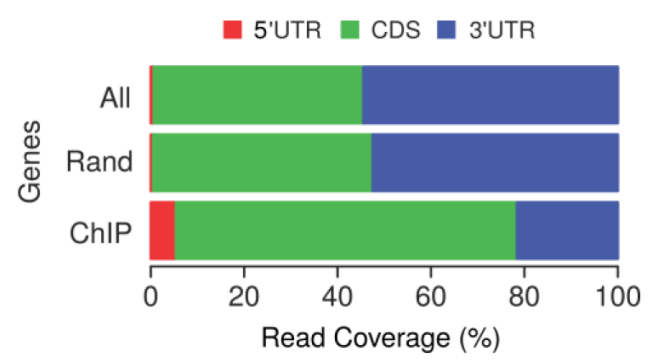
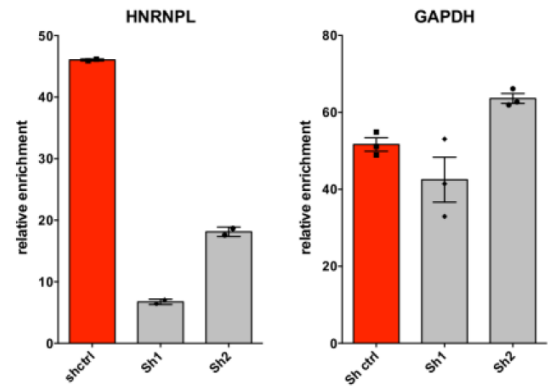
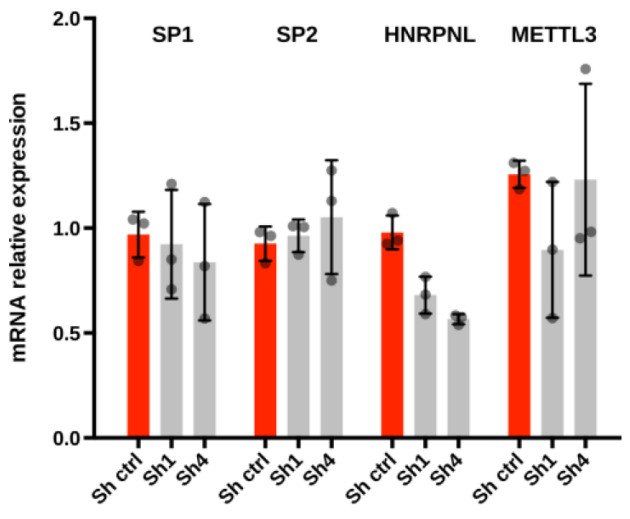


**d**







**a****b****c****d****e****f****g**