

1 Targeting acute myeloid leukemia by drug-induced c-MYB degradation

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23 **ABSTRACT**

24 Despite advances in our understanding of the molecular basis for particular subtypes of acute
25 myeloid leukemia (AML), effective therapy remains a challenge for many individuals
26 suffering from this disease. A significant proportion of both pediatric and adult AML patients
27 cannot be cured and since the upper limits of chemotherapy intensification have been
28 reached, there is an urgent need for novel therapeutic approaches. The transcription factor c-
29 MYB has been shown to play a central role in the development and progression of AML
30 driven by several different oncogenes, including mixed lineage leukemia (*MLL*)-fusion genes.
31 Here, we have used a *c-MYB* gene expression signature from *MLL*-rearranged AML to probe
32 the Connectivity Map database and identified mebendazole as a c-MYB targeting drug.
33 Mebendazole induces c-MYB degradation via the proteasome by interfering with the heat
34 shock protein 70 (HSP70) chaperone system. Transient exposure to mebendazole is sufficient
35 to inhibit colony formation by AML cells, but not normal cord blood-derived cells.
36 Furthermore, mebendazole is effective at impairing AML progression *in vivo* in mouse
37 xenotransplantation experiments. In context of the widespread human use of mebendazole,
38 our data indicate that mebendazole induced c-MYB degradation represents a safe and novel
39 therapeutic approach for AML.

40 INTRODUCTION

41 Rational combination of intensive chemotherapy with risk stratification has revolutionized
42 the treatment of acute leukemia in children. However, progress has not been uniform across
43 all subtypes of disease and many pediatric¹ and adult² Acute Myeloid Leukemia (AML)
44 patients cannot be cured by current therapies. It is widely accepted that further intensification
45 of chemotherapy is unlikely to improve outcomes,^{3, 4} but that this may be achieved by
46 developing novel therapeutics targeting specific leukemia drug-susceptibilities.

47 Rearrangements of the *MLL* gene are often associated with AML in children, and less
48 frequently in adults.⁵ These abnormalities result in generation of *MLL* fusion proteins, which
49 are responsible for driving development of the disease. These proteins hijack the normal
50 epigenetic machinery in developing hematopoietic cells, enforcing transcriptional
51 dysregulation of target genes.⁶⁻⁹ Identification of components of the *MLL* fusion-associated
52 complexes led to a number of drug discovery initiatives.^{10, 11} Recent studies indicate that one
53 transcriptional target in particular, encoding the transcription factor c-MYB, is responsible
54 for maintaining aberrant hematopoietic self-renewal programs necessary for initiation and
55 progression of *MLL*-rearranged AML.¹²⁻¹⁵

56 c-MYB is highly expressed in immature hematopoietic progenitor cells and is required for
57 definitive hematopoiesis,¹⁶ normal myelopoiesis¹⁷ and maintenance of adult hematopoietic
58 stem cell (HSC) self-renewal.¹⁸ *c-MYB* is rarely mutated in human leukemia, but has long
59 been associated with hematopoietic malignancies.^{19, 20} Some leukemia cells have been shown
60 to be more sensitive to c-MYB inhibition than normal hematopoietic cells.^{21, 22} This led to the
61 hypothesis that although it may not be an oncogenic driver itself, certain cancers are
62 nevertheless 'addicted' transcriptional dysregulation by c-MYB.²³ In contrast to absolute c-
63 MYB deficiency, low levels of c-MYB expression are compatible with limited

64 hematopoiesis, especially myelopoiesis,^{15, 24} suggesting that a window may exist for
65 therapeutic targeting of c-MYB in AML. However, difficulties associated with developing
66 small molecule inhibitors of transcription factor activity suggest that alternative approaches
67 may be required to target c-MYB.¹⁹ For example, the interaction between c-MYB and the
68 transcriptional co-activator p300 was recently shown to be essential for AML induction²⁵ and
69 to have promising potential as a target for pharmacological inhibition.²⁶

70 In order to identify bioactive compounds capable of inhibiting c-MYB transcriptional
71 activity in AML, we screened the Connectivity Map (CMAP) database with a c-MYB gene
72 expression signature derived from integrating MLL-fusion protein specific gene expression
73 changes with a list of previously published direct c-MYB target genes.^{27, 28} This analysis
74 identified the anti-helminth drug mebendazole as the top hit. We demonstrate that
75 mebendazole induces proteasomal degradation of c-MYB, inhibits AML cell self-renewal
76 and impairs AML progression *in vivo*. This work demonstrates that mebendazole has
77 excellent potential for repurposing in novel AML therapies.

78

79 MATERIALS AND METHODS

80 Mice

81 Mice were maintained in the UCL GOSICH animal facilities and experiments were
82 performed according to and approved by the United Kingdom Home Office regulations and
83 followed UCL GOSICH institutional guidelines.

84

85 Human samples

86 For human AML samples (Supplementary Table 1), approval by the Institutional Review
87 Board of the Erasmus MC for use of excess diagnostic material was obtained according to
88 laws and regulations of the Netherlands, DB AML 01 (MEC-2010-370), AML NOPHO DBH
89 2012 (MEC-2014-024).

90

91 Global gene expression, SPIEDw and Gene set enrichment (GSEA) analyses

92 Analysis of MLL-ENL/MLL-AF9 gene expression changes (Geo repository: GSE59236) was
93 performed using Affymetrix arrays (Affymetrix UK, High Wycombe, UK) and conditionally
94 immortalized MLL-ENL and MLL-AF9 mouse myeloid cells.²⁹⁻³² Gene expression changes
95 resulting from 6 hours exposure of THP1 cells to 10 μ M mebendazole or DMSO were
96 analysed by RNA-sequencing (Geo repository: GSE96544). A list (Supplementary Table 2)
97 of human orthologs of MLL-ENL/MLL-AF9 gene expression changes, for genes also
98 contained in the list of 1063 genes bound by c-MYB in mouse myeloid ERMVYB cells²⁷ and
99 deregulated in THP1 cells following siRNA-mediated c-MYB silencing,²⁸ were used to
100 interrogate the CMAP database (<https://portals.broadinstitute.org/cmap/>)³³ using the SPIEDw
101 web tool³⁴ (<http://www.spied.org.uk/>). For GSEA, c-MYB signatures were derived from
102 Zhao et al.²⁷ and LSC signatures from Somervaille *et al.*¹³

103

104

105 Cell culture and reagents

106 Human AML cell lines were purchased from the ATCC (THP1) and DSMZ (OCI-AML3,
107 NOMO-1, KCL22, U937, MV4;11, KASUMI-1 and SHI-1), authenticated by short tandem
108 repeat profiling using the PowerPlex 16 system (Promega, Southampton, UK) and
109 mycoplasma negative status confirmed using the MycoAlert Mycoplasma Detection Kit
110 (Lonza, Verviers, Belgium). 293FT cells were from ThermoFisher Scientific (ThermoFisher
111 Scientific, Hemel Hempstead, UK) and immortalized mouse myeloid cells were cultured as
112 previously described.²⁹⁻³²

113

114 Colony formation assays

115 AML cell lines were plated in HSC002 (Bio Techne, Abingdon, UK), normal CD34⁺ cord
116 blood-derived cells (ZenBio, NC, USA) in HSC005 (Bio Techne) and primary AML cells in
117 HSC005 methylcellulose medium supplemented with 50 ng/ml TPO and FLT3L.

118

119 Nematic protein organisation technique (NPOT) analysis

120 NPOT analysis was performed by INOVIEM Scientific (INOVIEM Scientific, Strasbourg,
121 France).

122

123 *In vivo* transplantation

124 Luciferase expressing THP1 cells were transplanted into non-irradiated NOD-SCID- $\gamma^{-/-}$
125 (NSG; The Jackson Laboratory, Bar Harbor, ME, USA) mice. Recipient mice were imaged
126 using the IVIS® Lumina Series III (PerkinElmer, Beaconsfield, UK) and randomly allocated
127 to control or mebendazole-treated groups. Mebendazole (200 mg/kg of diet) was
128 administered *ad libitum* in regular powdered diet, changed daily.

129

130 Lentivirus vector cloning

131 The Δ MYB cDNA³⁵ was cloned into the pCSGW-PIG vector, made by replacing the GFP
132 cDNA from pCSGW³⁶ with a puro-IRES-GFP cassette. Lentiviral MISSION shRNA
133 constructs targeting c-MYB (Clone ID:NM_005375.2-927s21c1), HSPA1A (sh1:Clone
134 ID:NM_005345.4-1539s1c1; sh2:Clone ID:NM_005345.4-566s1c1) and the scramble (SCR)
135 non-silencing control (SHC002) were purchased from Sigma-Aldrich (Gillingham, UK).

136

137 Western blot analysis

138 Antibodies against c-MYB (H-141, catalogue number sc-7874, Santa Cruz Biotechnology,
139 Dallas, TX, USA; clone 1-1, catalogue number 05-175, Merck Millipore, Watford, UK;
140 EPR718(2), catalogue number ab109127 , Abcam, Cambridge, UK), HSP70 (clone 242707,
141 catalogue number MAB1663, Bio Techne), HSP70/HSC70 (and H-300, catalogue number sc-
142 33575, and W27, catalogue number sc-24, Santa Cruz Biotechnology), Actin (I-19, catalogue
143 number sc-1616, Santa Cruz Biotechnology), β -Actin (C4, catalogue number sc-47778, Santa
144 Cruz Biotechnology), α Tubulin (YL1/2, catalogue number sc-53029, Santa Cruz
145 Biotechnology), SP1 (PEP 2, catalogue number sc-59, Santa Cruz Biotechnology) .

146

147 Microtubule depolymerization assay

148 Microtubule depolymerization was analysed as published previously.³⁷

149

150 Quantitative RT-PCR analysis

151 Quantitative RT-PCR (qPCR) was performed on isolated mRNA using TaqMan probe based
152 chemistry and an ABI Prism 7900HT fast Sequence Detection System (Life Technologies,
153 Paisley, UK). All primer/probe sets were from Applied Biosystems, Life Technologies.

154

155 Statistics

156 Statistical significance was determined using Prism (GraphPad) software. Statistical analysis
157 of survival curves was performed using the Mantel-Haenszel log-rank test. Statistical analysis
158 of means was performed using the one sample *t* test or unpaired Student's *t* test, two-tailed *P*
159 values < 0.05 being considered statistically significant. Variance was similar between groups.

160

161 Further details are provided in Supplementary Materials and Methods.

162

163 **RESULTS**

164 To identify candidate anti-leukemia drugs, we generated a therapeutic signature
165 (Supplementary Table 2) by integrating gene expression changes due to MLL fusion
166 silencing²⁹⁻³² with transcriptional regulation by c-MYB in acute myeloid leukemia.^{27, 28} This
167 signature was then used to interrogate drug-induced gene expression profiles in the CMAP
168 database³³ using the SPIEDw web tool³⁴ (Figure 1a). The top hit resulting from this analysis
169 was the anti-helminth agent mebendazole (Figure 1b).

170 In order to determine whether mebendazole was able to interfere with c-MYB regulated
171 transcriptional pathways in human AML cells, RNASeq was performed on the MLL-AF9
172 expressing cell line THP1, following short-term exposure to the drug. GSEA of mebendazole
173 induced gene expression changes confirmed that this drug was able to reverse both activation
174 and repression by c-MYB of its target genes (Figure 1c and Supplementary Figure 1).
175 Mebendazole exhibited anti-leukemia activity against *MLL* rearranged and non-rearranged
176 human AML cell lines (Figure 2a and Supplementary Figure 2) and was effective at blocking
177 their colony forming activity (Figure 2b and Supplementary Figure 3). This is not surprising,
178 since c-MYB has also been shown to play a critical function in non-rearranged subtypes of
179 AML.^{25, 26, 38-41} Indeed, shRNA mediated silencing of c-MYB expression had a severe impact
180 on colony formation (Supplementary Figure 4).

181 We then examined whether mebendazole interfered with c-MYB regulated gene
182 expression by targeting the transcription factor directly, by examining RNA and protein
183 expression 6 hours after exposure of AML cells to the drug. Interestingly, mebendazole
184 inhibited c-MYB protein expression in all cell lines examined, and RNA expression in some
185 (Figure 3a). c-MYB protein expression was affected at lower concentrations of mebendazole
186 than those required to inhibit RNA expression, and occurred without any change in RNA

187 expression in some cells, suggesting that mebendazole targets c-MYB expression primarily at
188 the protein level. Indeed, reversal of c-MYB protein loss by proteasomal inhibition indicates
189 that mebendazole targets c-MYB for proteasomal degradation (Figure 3b and Supplementary
190 Figure 5).

191 There has been considerable interest recently in repurposing mebendazole for cancer
192 therapy.⁴² However, the mechanism for its anti-cancer activity has remained elusive. Initially
193 this was thought to derive from its microtubule destabilizing activity, mebendazole binding
194 tubulin at the colchicine-site,⁴³ although recent experiments have suggested alternative
195 mechanisms.⁴⁴ We compared microtubule depolymerization and c-MYB degradation induced
196 by mebendazole in AML cells to that resulting from treatment of the cells with the
197 microtubule disrupting agent colcemid (Figure 4a and Supplementary Figure 6). Equivalent
198 concentrations of colcemid induced both microtubule depolymerization and loss of c-MYB
199 protein. In contrast, mebendazole induced c-MYB degradation at concentrations that do not
200 cause microtubule depolymerization, indicating that the former is not a result of the latter *per*
201 *se*.

202 Exactly how mebendazole induces c-MYB degradation is unclear. It does not appear to be
203 a secondary consequence of a cell cycle block, since c-MYB loss is already evident in THP1
204 cells after 4 hours mebendazole exposure, prior to drug-induced changes in cell cycle profiles
205 (Supplementary Figure 7). In order to address this question, nematic protein organisation
206 technique (NPOT) analysis (Inoviem Scientific) of mebendazole was performed using cell
207 lysates from a primary AML patient sample and THP1 cells (Supplementary Figures 8a and
208 8b). DAVID analysis of the proteins identified in the AML patient sample hetero-assemblies,
209 induced by mebendazole, highlighted 'protein folding' as the second most significant
210 functional category, with 12 of the 16 proteins in this category also identified in THP1
211 hetero-assemblies (Supplementary Figure 8c). This suggests that mebendazole disrupts the

212 cellular protein folding machinery leading to the proteasomal targeting of c-MYB (Figure
213 3b). Of particular interest were a number of proteins of the HSP70/HSP90 chaperone
214 complexes. c-MYB has previously been shown to be an HSC70 client protein in prostate
215 cancer cells.^{45, 46} Indeed, we found that the HSP70/HSC70 chaperone complex is also
216 associated with c-MYB in AML cells (Figure 4b and Supplementary Figure 9a), an
217 association that was lost upon exposure to mebendazole (Figure 4c). Furthermore, *HSPA1A*
218 knockdown and pharmacological HSP70 inhibition resulted in reduced c-MYB protein levels
219 (Figure 4d and Supplementary Figure 9b and 9c). However, mebendazole did not induce any
220 changes in expression levels or subcellular re-localization of the HSP70/HSC70 complex
221 (Supplementary Figure 9d).

222 Since c-MYB has been placed at the center of a leukemia stem cell (LSC) transcriptional
223 self-renewal program in *MLL*-rearranged leukemia,¹³ we hypothesized that mebendazole
224 treatment of *MLL*-rearranged AML cells would inhibit this program. Indeed, GSEA analysis
225 of gene expression changes following treatment of THP1 cells with mebendazole
226 demonstrated negative enrichment of the LSC self-renewal signature (Figure 5a). In order to
227 examine whether short-term exposure to mebendazole would indeed compromise AML cell
228 self-renewal, we treated THP1 cells with 10 μ M mebendazole for 16 hours, washed them and
229 examined their colony forming potential *in vitro*. Consistent with the observed inhibition of
230 the LSC self-renewal program, transient exposure to mebendazole resulted in a more than
231 80% reduction in colony formation by THP1 cells (Figure 5b). Our hypothesis predicts that
232 stabilization of the c-MYB protein would have the potential to rescue this loss of self-renewal
233 induced by mebendazole. In order to examine this possibility, we generated a C-terminal
234 deletion mutant of c-MYB (Δ MYB), previously shown to result in enhanced protein
235 stability,³⁵ and expressed this mutant in THP1 cells. Δ MYB was partially protected from
236 mebendazole-induced degradation (Figure 5c). Since the mutant contained the unaltered

237 DNA-binding domain, which is necessary for the transcriptional activity of c-MYB, but
238 which contains residues known to be targeted by ubiquitin-mediated proteasomal
239 degradation,⁴⁷ it is not surprising that it was not completely resistant to mebendazole.
240 However, Δ MYB expressing THP1 cells were found to express higher levels of total c-MYB
241 protein than control THP1 cells, following 6 hours treatment with mebendazole (Figure 5c).
242 Importantly, this partial rescue of c-MYB protein expression correlated with increased colony
243 forming potential of Δ MYB expressing THP1 cells following transient exposure to
244 mebendazole (Figure 5d). These data indicate that mebendazole inhibits AML colony
245 formation by disrupting the c-MYB regulated LSC self-renewal program. Interestingly,
246 transient mebendazole exposure also inhibited *in vitro* colony formation by two independent
247 MLL-AF9 expressing primary AML patient samples, but had no significant effect on colony
248 formation by normal CD34⁺ cord blood cells (Figure 5e and Supplementary Figure 10).
249 Interestingly, mebendazole only caused partial loss of c-MYB in the latter (Supplementary
250 Figure 11).

251 Having shown the anti-leukemia activity of mebendazole *in vitro*, we decided to test
252 whether it could make an impact on AML disease progression *in vivo*. Oral administration of
253 mebendazole, by simply mixing the drug in the diet, was found to have significant activity
254 against disease in THP1 transplanted NSG mice, inhibiting leukemia progression (Figures 6a
255 and b) and prolonging survival of treated mice (Figure 6c). Interestingly, THP-1 cells isolated
256 from mebendazole-treated mice were found to express increased c-MYB protein levels and to
257 be more resistant to drug treatment (Supplementary Figure 12). These data indicate that oral
258 administration of mebendazole is sufficient to significantly impair AML progression *in vivo*.

259

260

261 **DISCUSSION**

262 Our data show that mebendazole induces degradation of the transcription factor c-MYB. c-
263 MYB is essential for survival and self-renewal of multiple AML subtypes. c-MYB
264 degradation results in loss of AML cell viability, colony formation and impaired *in vivo*
265 leukemia progression. The rescue of AML cells from the inhibition of colony formation,
266 following transient exposure to mebendazole, by over-expression of the Δ MYB mutant
267 highlights the significance of c-MYB degradation in the anti-AML effects of this drug.
268 Although, the Δ MYB mutant exhibits enhanced protein stability,³⁵ it is not completely
269 resistant to mebendazole induced degradation, potentially explaining why the rescue of
270 colony formation is only partial. It is likely that mebendazole interferes with multiple
271 pathways in cancer cells⁴⁴ and it has been suggested to be a promising candidate for anti-
272 cancer drug repurposing.⁴² However, the induction of c-MYB degradation by mebendazole
273 makes it particularly suitable to repurposing into AML therapy.

274 Some solid cancers are also addicted to continued and relatively high expression of the
275 oncoprotein c-MYB. This is particularly evident in colorectal cancer where c-MYB is over-
276 expressed and essential to continued proliferation and tumor cell survival.¹⁹ In a recent study,
277 mebendazole showed activity in the majority of the colon cancer cell lines tested.⁴⁴ It is
278 noteworthy that a patient with refractory metastatic colon cancer treated with mebendazole
279 showed near complete remission of the metastases in the lungs and lymph nodes and a good
280 partial remission in the liver.⁴⁸ This suggests that mebendazole induced proteolysis of c-MYB
281 may also have major clinical implications outside of AML therapy, in the treatment of a
282 variety of cancers.

283 Induction of c-MYB degradation by mebendazole is blocked by proteasomal inhibition
284 and occurs at much lower concentrations than those necessary to achieve microtubule

285 depolymerization in AML cells. We present evidence for the involvement of the
286 HSP70/HSC70 chaperone complex in c-MYB targeting by mebendazole. The complex was
287 found to bind c-MYB in AML cells, an association that was lost upon exposure of cells to
288 mebendazole. Pharmacological or shRNA directed inhibition of HSP70 also resulted in loss
289 of c-MYB protein. Interestingly, the HSP70/HSC70 complex has previously been shown to
290 interact with c-MYB in prostate cancer cells, its displacement from the complex by glioma
291 pathogenesis-related protein 1 (GLIPR1) over-expression resulting in c-MYB protein
292 destabilization.^{45, 46}

293 In summary, we have used Connectivity Map analysis to identify mebendazole as a novel
294 candidate anti-AML therapeutic. Our data highlight a hitherto unappreciated link between
295 microtubule interacting agents and the regulation of c-MYB protein degradation. Importantly,
296 widespread use of mebendazole is tolerated in children and adults across the world,⁴⁹
297 suggesting that this drug has real potential for safe use in the treatment of human AML.

298

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448

449 **FIGURE LEGENDS**

450 **Figure 1.** Identification of mebendazole as a c-MYB targeting drug in AML. (a) Diagram
451 summarizing the generation of a c-MYB signature used to interrogate the CMAP database³³
452 using SPIEDw³⁴. (b) The 1,309 CMAP drugs are ranked based on the significance of
453 regression scores between their transcriptional profiles and that of the query. The Z-score
454 corresponds to the number of standard deviations of the score away from the mean. Inset is
455 the structure of mebendazole (rank 1). (c) GSEA of the c-MYB activated (top) and repressed
456 (bottom) gene sets in global gene expression changes in THP1 cells following 6 hours
457 exposure to 10 μ M mebendazole (MBZ) versus DMSO.

458 **Figure 2.** Mebendazole inhibits AML growth and colony formation. (a) Viability, normalized
459 to DMSO controls, of AML cell lines treated for 72 hours with indicated mebendazole
460 concentrations. Bars and error bars are means and SD of three independent experiments, each
461 in triplicate. (b) Examples of AML cell line colony formation in methylcellulose cultures in
462 the presence of DMSO or 1.25 μ M mebendazole (quantification in Supplementary Figure 1).

463 **Figure 3.** Mebendazole induces proteasomal degradation of c-MYB. (a) Protein and RNA
464 expression in AML cells after 6 hours treatment with DMSO or indicated mebendazole
465 concentrations, normalized to DMSO controls. Bars and error bars are means and SD of three
466 independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant (relative
467 to DMSO controls), one sample t test. Western blots below graphs show examples of c-MYB
468 protein expression. (b) Western blot analysis of c-MYB protein expression in AML cells after
469 6 hours treatment with DMSO, 10 μ M mebendazole or 10 μ M mebendazole and 10 μ M
470 MG132 (quantification in Supplementary Figure 3).

471 **Figure 4.** Mebendazole destabilizes c-MYB protein by interfering with the HSP70/HSC70
472 chaperone pathway. (a) Percent polymerized tubulin in THP1 cells following 6 hours

473 treatment with indicated concentrations of mebendazole (top left) or colcemid (top right), and
474 western blot analysis of corresponding c-MYB protein expression (bottom). Tubulin
475 stabilization by 5 μ M paclitaxel (Pax) is also shown. Bars and error bars are means and SD of
476 three independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant,
477 unpaired Student's t-test. (b) Western blot analysis of mouse IgG and anti-HSP70/HSC70
478 immunoprecipitates from THP1 cells, stained with anti-c-MYB (top) and anti-HSP70/HSC70
479 (bottom). Representative data from one of three independent experiments. (c) Western blot
480 analysis of anti-HSP70/HSC70 immunoprecipitates from THP1 cells following 6 hours
481 treatment with DMSO or 10 μ M mebendazole, stained with anti-c-MYB (top) and anti-
482 HSP70/HSC70 (bottom). Representative data from one of three independent experiments. (d)
483 Western blot analysis of HSP70 (top) and c-MYB (middle) expression in THP1 cells 7 days
484 after transduction with control scramble (shSCR) shRNA or two independent shRNA
485 targeting *HSPA1A* (sh1 and sh2) (quantification in Supplementary Figure 7b).

486 **Figure 5.** Transient exposure to mebendazole inhibits colony formation by AML but not
487 normal cord blood-derived hematopoietic cells. (a) GSEA of the gene expression signatures
488 positively (top) and negatively (bottom) correlating with leukemia stem cell frequency¹³ in
489 global gene expression changes in THP1 cells following 6 hours exposure to 10 μ M
490 mebendazole versus DMSO. (b) Example of THP1 colony formation after pre-treatment with
491 DMSO or 10 μ M mebendazole. Cells were treated with vehicle or drug for 16 hours, washed
492 and placed into methylcellulose culture. The mean (\pm SD) fold change in colony formation is
493 shown from five independent experiments, normalized to DMSO controls. *** $P < 0.001$
494 (relative to DMSO controls), one sample t test. (c) c-MYB protein expression in empty vector
495 (Con) or c-MYB deletion mutant (Δ MYB) transduced THP1 cells, 6 hours after treatment
496 with DMSO or 10 μ M mebendazole. (d) Fold change in colony formation by Con or Δ MYB
497 transduced THP1 cells, following 16 hours pre-treatment with DMSO or 10 μ M

498 mebendazole, normalized to Con or Δ MYB DMSO controls. Bars and error bars are means
499 and SD of seven independent experiments. *** $P < 0.001$, unpaired Student's t-test. (e)
500 Colony formation frequency by two independent primary AML patient samples (AML1547,
501 AML1497) and two independent normal CD34⁺ cord blood samples (CB1, CB2) after pre-
502 treatment with DMSO or 10 μ M mebendazole. Cells were treated with vehicle or drug for 20
503 hours, washed and placed into methylcellulose culture.

504 **Figure 6.** Mebendazole impairs AML progression *in vivo*. (a) Bioluminescence imaging of
505 NSG recipient mice 10 days after injection with THP1-LUC2 cells, and before drug
506 treatment, (day 0, top), and 22 days after treatment with normal or mebendazole-containing
507 diet (bottom). Bars for luminescence signal represent photons/s/cm²/steradian. (b)
508 Luminescence signal in treatment groups, 10 days after THP1-LUC2 cell injection and before
509 drug treatment (left), and fold increase in luminescence signal in the groups 7 and 17 days
510 after treatment with normal or mebendazole-containing diet (right). Bars and error bars are
511 means and SD of values from control (n = 5) and mebendazole-treated (n = 9) groups. *** $P <$
512 0.001; n.s. not significant, unpaired Student's t-test. (c) Survival curve for control (n = 9) and
513 mebendazole-treated (n = 12) mice, $P < 0.0001$, Mantel-Haenszel log-rank test.

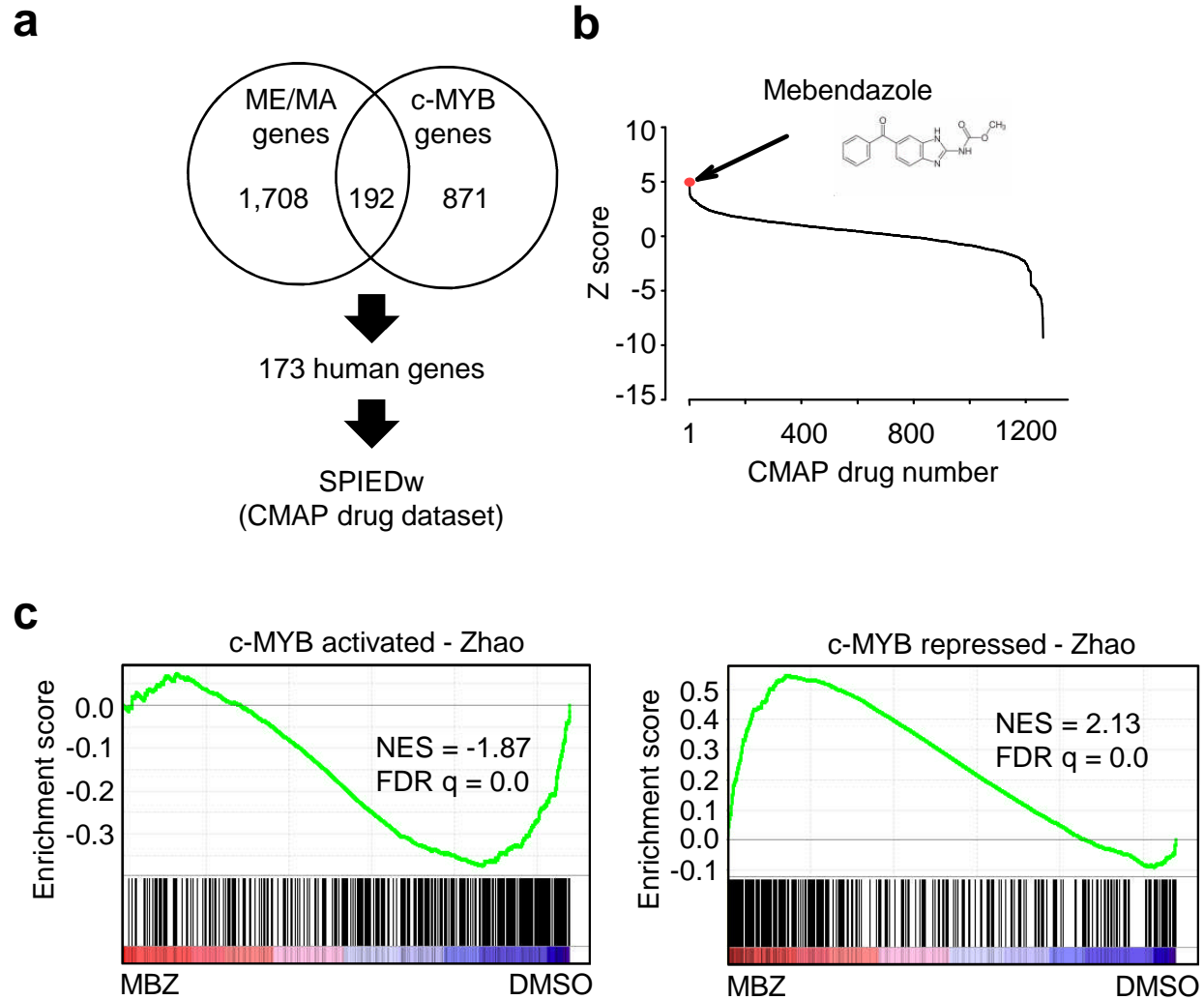


FIGURE 1

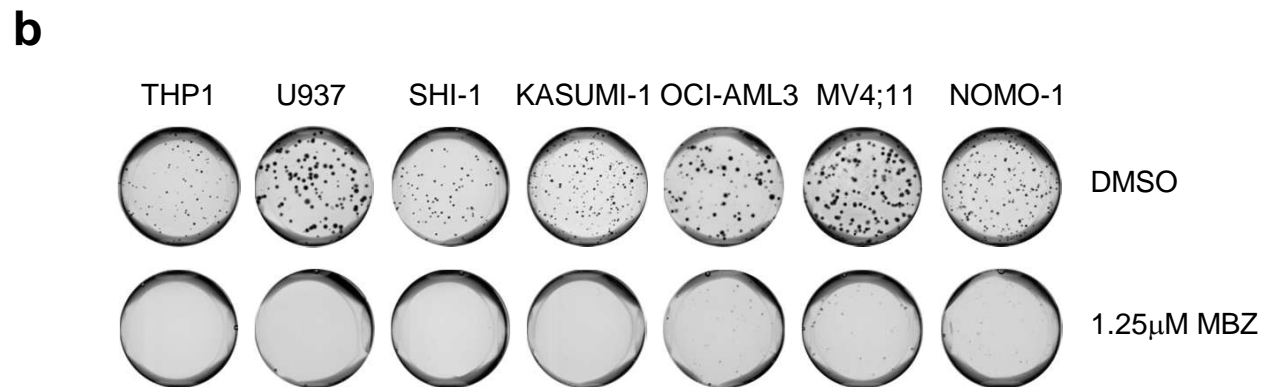
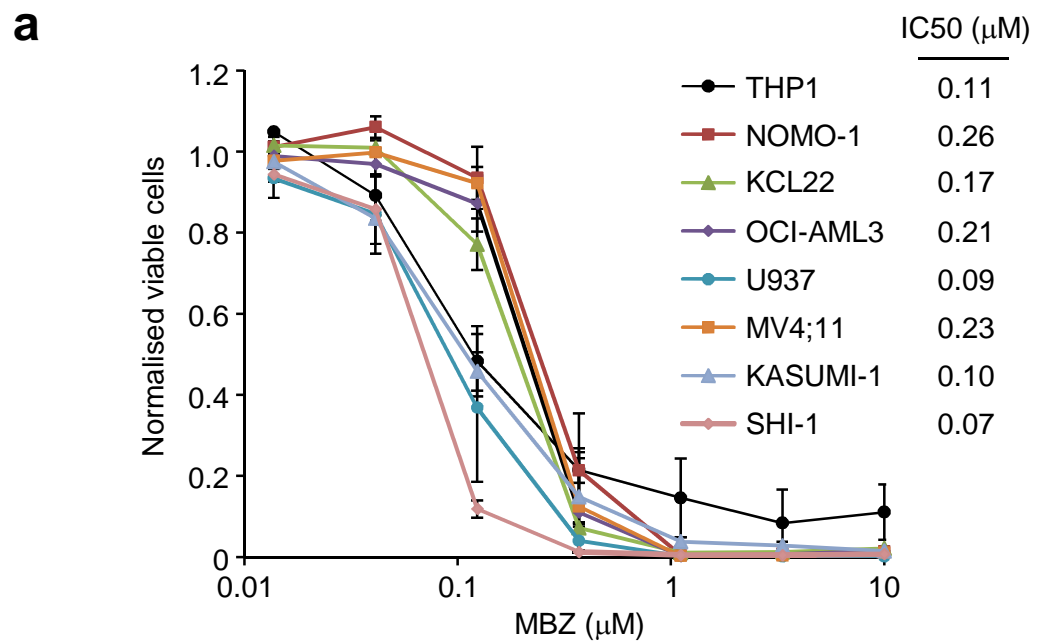


FIGURE 2

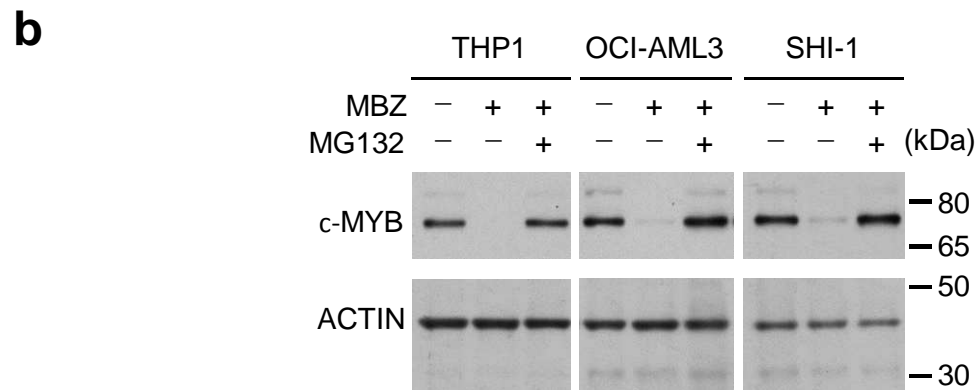
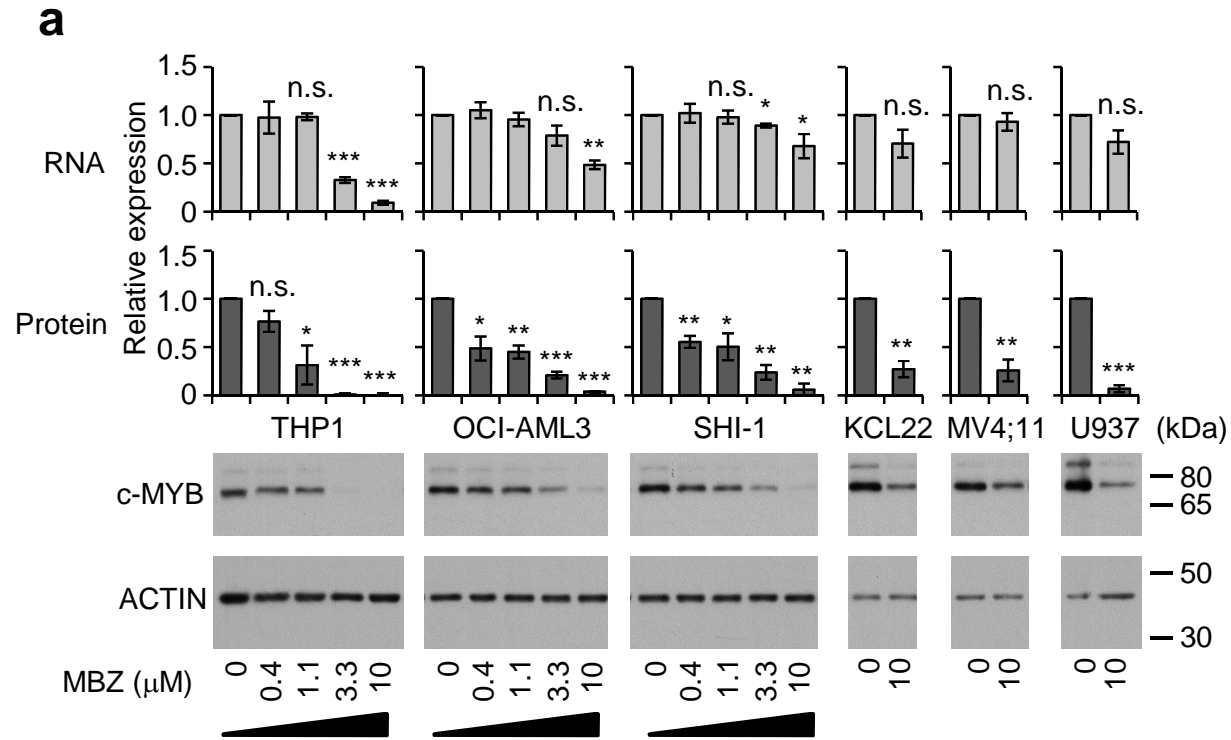
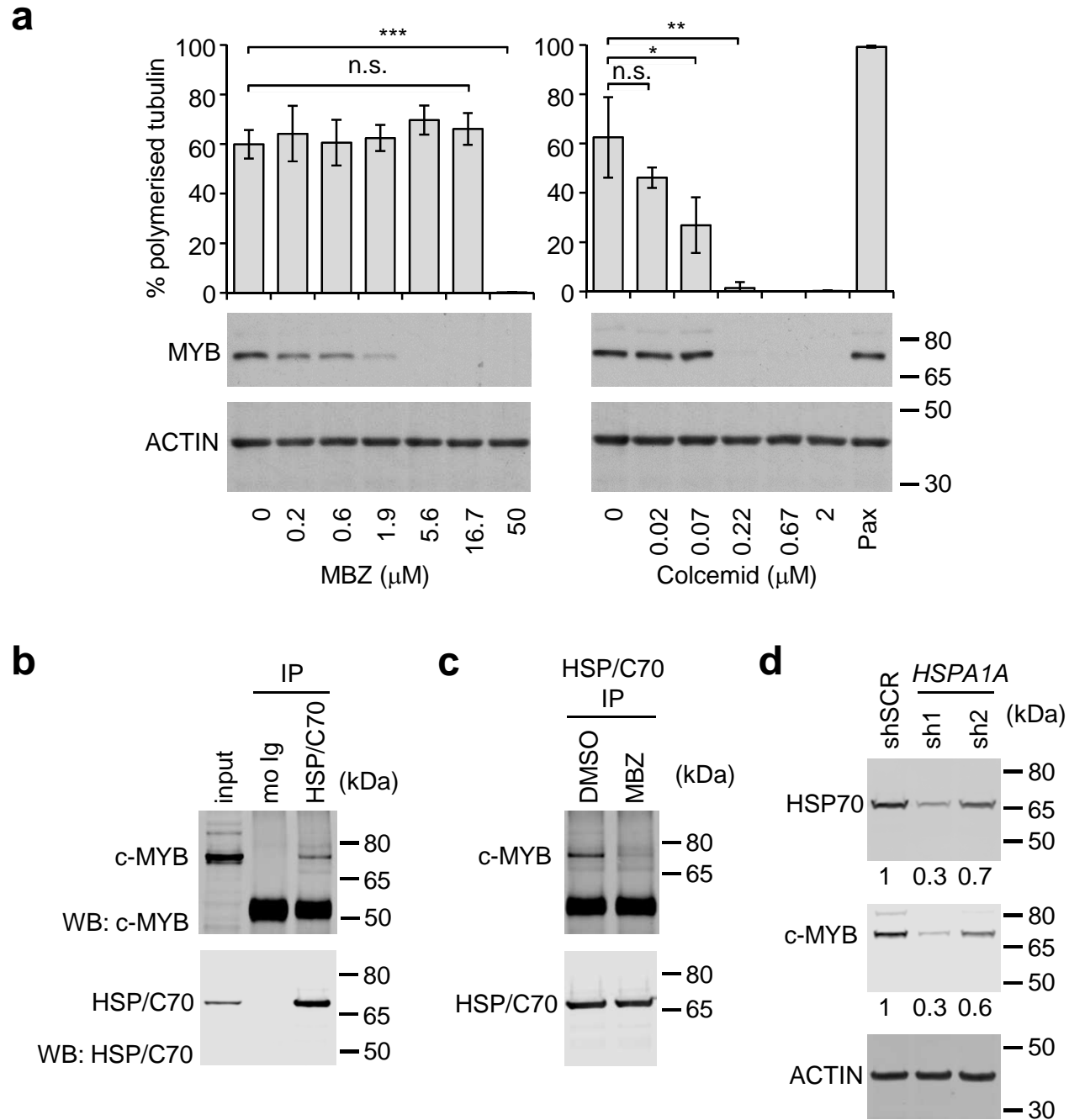


FIGURE 3



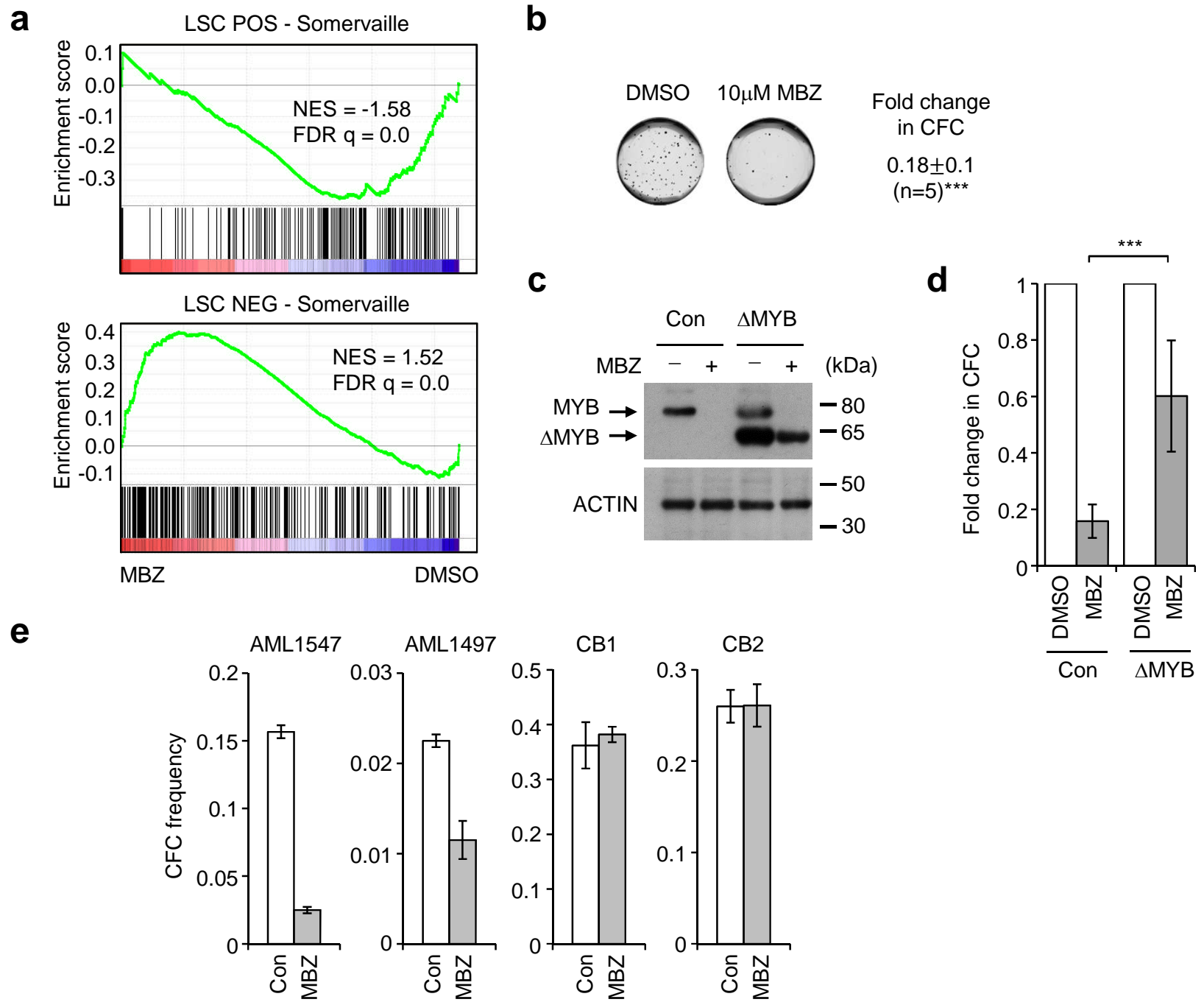


FIGURE 5

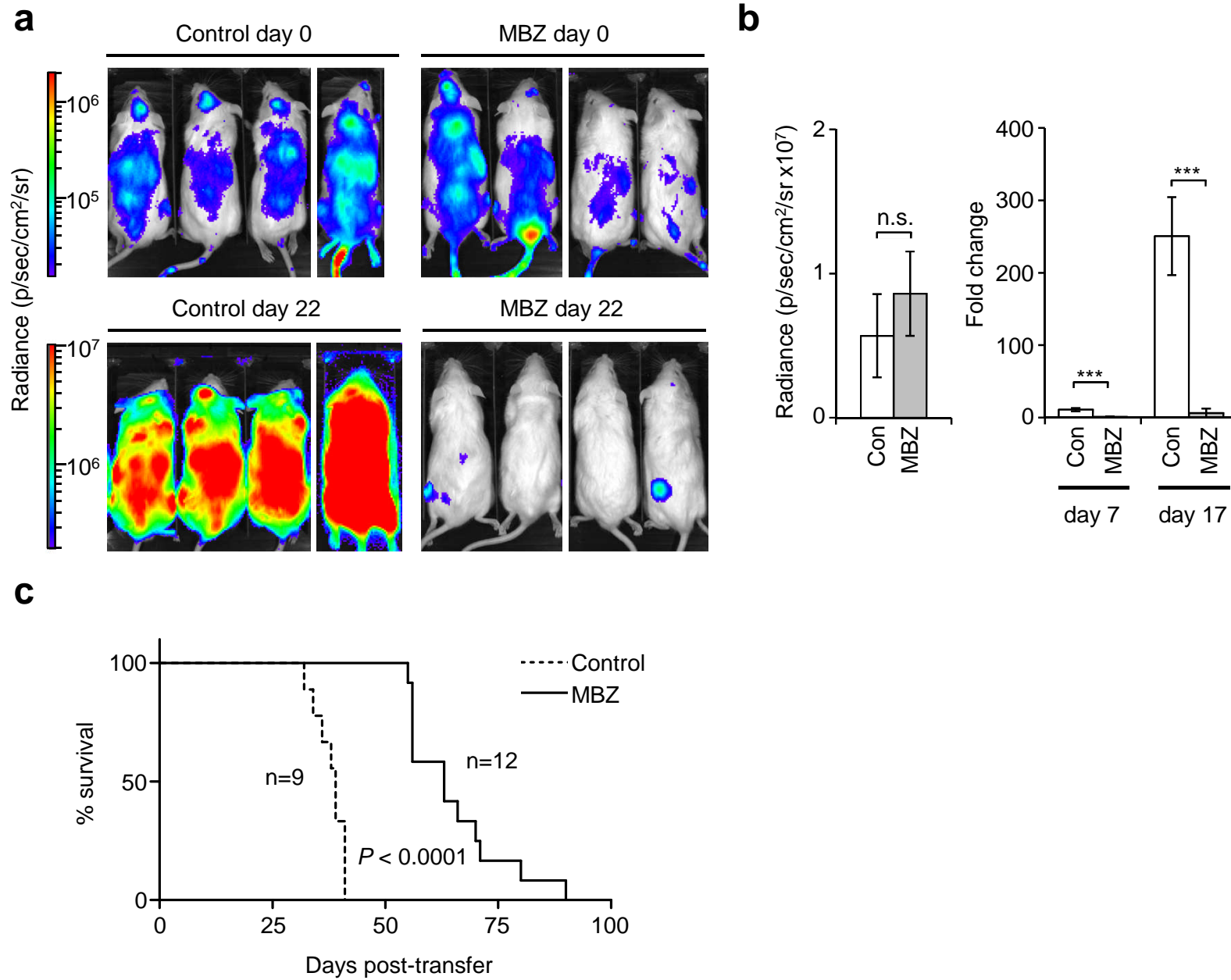


FIGURE 6

SUPPLEMENTARY MATERIALS AND METHODS

Mice

All mice were maintained in the animal facilities of the UCL Great Ormond Street Institute of Child Health, London. All mouse experiments were performed according to and approved by the United Kingdom Home Office regulations and followed UCL Great Ormond Street Institute of Child Health institutional guidelines.

Human samples

Primary AML mononuclear cells were isolated by sucrose gradient centrifugation (Lymphoprep, 1.077 g/ml density; Nycomed Pharma).

Global gene expression analysis

Conditionally immortalized MLL-ENL and MLL-AF9 mouse myeloid cells have been previously described.¹⁻⁴ Control constitutive and conditionally immortalized cells were treated with 2 µg/ml doxycycline for 48 hours, RNA extracted using TRIzol reagent (ThermoFisher Scientific) and quality verified using an Agilent 2100 Bioanalyzer (Agilent Technologies, Wokingham, UK). RNA was hybridized to GeneChip Mouse Genome 430.2 arrays (Affymetrix). Data was analyzed using GeneSpring 7.3.1 software (Agilent Technologies). Gene expression changes of more than 2-fold, with a defined *P*-value cut-off ≤ 0.05 , upon treatment with doxycycline were selected. Genes whose expression changed more than 2-fold in constitutive cells, upon doxycycline treatment, were excluded. Duplicate probe sets were removed, keeping those with the highest fold change. THP1 cells were cultured at 0.5×10^6 cells/ml and treated with 10 µM mebendazole or DMSO control, for 6 hours. RNA was purified and submitted to UCL Genomics for RNA-sequencing. Samples were processed using an Illumina TruSeq RNA sample prep kit Version 2 (RS-122-2001)

according to manufacturer's instructions (Illumina, Cambridge, UK) and sequenced on an Illumina NextSeq 500 (Illumina). Differential expression was obtained using the RNA Express BaseSpace® app (Illumina). Low expressed genes were removed from the output file. This was used for GSEA analysis.

Gene expression changes for SPIEDw analysis

MLL-ENL/MLL-AF9 gene expression changes, for genes also contained in the list of 1063 genes bound by c-MYB in mouse myeloid ERMYP cells⁵ and deregulated in THP1 cells following siRNA-mediated c-MYB silencing,⁶ were selected. These genes were converted into human gene names using the HGNC Comparison of Orthology Predictions (HCOP) search tool,⁷ using the HGNC orthologs. This list (Supplementary Table 2) was then used to interrogate the CMAP database (<https://portals.broadinstitute.org/cmap/>)⁸ using the SPIEDw web tool⁹ (<http://www.spied.org.uk/>), which allows queries to be entered as gene names rather than probe sets.

Gene set enrichment analysis

c-MYB signatures⁵ were genes bound by c-MYB in mouse myeloid ERMYP cells and deregulated in THP1 cells following siRNA-mediated c-MYB silencing,⁶ either decreasing in expression (MYB activated) or increasing in expression more than 1.5-fold (MYB repressed). LSC signatures¹⁰ consisted of probe sets positively correlated with LSC frequency (LSC POS) and negatively correlated with LSC frequency (LSC NEG). Mouse gene names were converted into human gene names using the HCOP using the HGNC orthologs, as above.

Cell culture and reagents

Human AML cell lines were cultured in RPMI or IMDM (SHI-1) supplemented with 10–20% FCS, L-glutamine and penicillin/streptomycin. 293FT (ThermoFisher Scientific) cells were maintained in DMEM with 10% FCS, L-glutamine, penicillin/streptomycin and 500 µg/ml G418. For some experiments, CD34⁺ cord blood-derived cells (ZenBio) were expanded in StemSpan SFEM II medium (StemCell Technologies, Grenoble, France) supplemented with 100 ng/ml human SCF, TPO and FLT3L (all from Peprotech, London, UK).

Colony formation assays

AML cell lines were plated out in HSC002 methylcellulose medium (Bio Techne) at 150-500 cells/well in 24-well plates. Colonies were grown for 7-14 days and visualized by staining with 1 mg/ml p-iodonitrotetrazolium. For pre-stimulation, primary AML cells and normal CD34⁺ cord blood-derived cells (ZenBio) were cultured with DMSO or 10 µM mebendazole for 20 hours in IMDM with 10% FCS, L-glutamine, penicillin/streptomycin. AML cells were supplemented with 100 ng/ml human SCF, TPO and FLT3L, 10 ng/ml IL3, IL6 and IL11, and CD34⁺ cord blood-derived cells with 100 ng/ml human SCF, TPO and FLT3L (all growth factors were from Peprotech). Cells were plated out into HSC005 methylcellulose medium (Bio Techne) containing 50 ng/ml human SCF, 20 ng/ml IL3, IL6, G-CSFD, GM-CSF and 3 IU/ml EPO at 300-1000 cells/well in 35 mm plates. Primary AML methylcellulose cultures were further supplemented with 50 ng/ml TPO and FLT3L. Cultures were counted and morphology scored 14 days later.

Growth, apoptosis and cell cycle assays

The effect of mebendazole on growth of AML cell lines was examined by exposing them to different concentrations of mebendazole in liquid culture, starting at 1.25-2.5 x 10⁵ cells/ml,

and 72 hours later were stained with TO-PRO®-3 (ThermoFisher Scientific) stain and total viable cells were determined by flow cytometry analysis on a BD FACSAArray™ Bioanalyzer, using Summit 4.3 software (Beckman Coulter, High Wycombe, UK). Apoptosis was detected using the Annexin V Apoptosis detection kit (eBioscience, Hatfield, UK). Cell cycle analysis was performed using the Click-iT EdU Alexa Flour 647 Flow Cytometry Assay Kit (Invitrogen, Life Technologies). Cells were analyzed on an LSR II (BD Bioscience, San Jose, CA, USA), and the data were analyzed with Summit 4.3 software (Beckman Coulter).

Nematic protein organisation technique (NPOT) analysis

NPOT is a label free proprietary technology offered by INOVIEM Scientific, used to isolate and identify specific macromolecular scaffolds implemented in basic or pathological situations directly from human tissue. Specifically, primary AML sample AML4298 and THP1 cell homogenates were prepared under low temperature (4°C) in the absence of any detergent, reducing agent or protease or phosphatase inhibitors. All dilutions and washes were performed in HBSS with osmolality, trace elements, vitamins and salts concentrations as close as possible to those of the interstitial medium or cellular cytoplasm. Mebendazole (1µM) was added to the total tissue material. The macromolecular assemblies related to the specific ligands were then separated using a differential microdialysis system, based on liquid transitory pH gradient (pH5-10) where the macromolecules (protein groups) can migrate in the liquid phase to their mean molecular zwitterion positions.¹¹⁻¹⁴ The gradually growing and migrating macromolecules will form nematic crystals to macromolecular hetero-assemblies thanks to the molecular interactions between the ligand(s) and their targets. The hetero-assemblies are then trapped in mineral oil and isolated and identified by mass spectroscopy directly in liquid. Formation of hetero-assemblies and crystals were induced by addition of mebendazole and did not form in its absence.

In vivo transplantation

THP1 cells were transduced with a lentivirus vector containing a luciferase (LUC2)-IRES-EGFP cassette, and EGFP⁺ cells purified by flow cytometric sorting. $1-2 \times 10^6$ THP1-LUC2 cells were transplanted into non-irradiated 6-12 week old male NOD-SCID- $\gamma^{-/-}$ (NSG; The Jackson Laboratory) mice by lateral tail vein injection. Recipient mice were imaged 10 minutes following subcutaneous injection of 5 mg/mouse D-luciferin (Cayman Chemical Company, Ann Arbor, MI, USA) using the IVIS® Lumina Series III pre-clinical *in vivo* imaging system (PerkinElmer) and randomly allocated to control or mebendazole-treated groups by flipping a coin. Mebendazole (200 mg/kg of diet) was administered *ad libitum* in regular powdered diet, changed daily. Group sizes were chosen based on previous estimates of disease latency in THP1 transplanted mice and experiments in the literature performing similar studies. No mice were excluded from the analysis. No blinding was used in group allocation or analysis of data.

Lentivirus vector cloning, production and transduction of cell lines

The Δ MYB cDNA¹⁵ was cloned into the pCSGW-PIG vector, made by replacing the GFP cDNA from pCSGW¹⁶ with a puro-IRES-GFP cassette, and the resulting vector used to transduce THP1 cells. The luciferase (LUC2) cDNA was cloned into pCSGW. Lentiviral MISSION shRNA constructs targeting c-MYB (Clone ID:NM_005375.2-927s21c1), HSPA1A (sh1:Clone ID:NM_005345.4-1539s1c1; sh2:Clone ID:NM_005345.4-566s1c1) and the scramble (SCR) non-silencing control (SHC002) were purchased from Sigma-Aldrich. The 293FT packaging cells (ThermoFisher Scientific) were transiently cotransfected with the lentiviral expression vectors, the pCMV-PAX2 construct and the pVSV-G envelope construct (kind gifts of D. Trono, Lausanne, Switzerland). Human leukemia cells were

transduced with lentiviral supernatant by spinoculation at 700g, 25°C for 45 minutes in the presence of 5 µg/mL polybrene. Transduced cells were selected in puromycin for 72 hours.

Western blot analysis

Cells were lysed in reducing sample buffer (100 mM dithiothreitol, 2% sodium dodecyl sulfate, 10 % glycerol, 0.002 % bromophenol blue, 62.5 mM Tris-HCL pH 6.8). Nuclear and cytoplasmic lysates were prepared using the Nuclear Extract kit (Active Motif, La Hulpe, Belgium). Protein samples were resolved on gels 10 % polyacrylamide (0.36 M bis-Tris, 10 % acrylamide/bis) in MOPS-SDS running buffer (50 mM Tris, 50 mM MOPS, 1 mM EDTA, 0.1 % SDS). Gels were transferred onto a PVDF (Merck Millipore) or nitrocellulose (LI-COR Biosciences, Cambridge, UK) membranes. Proteins were detected using appropriate secondary horseradish peroxidase-conjugated antibodies and visualized using a chemiluminescence reagent (GE Healthcare, Little Chalfont, UK) or IRDye 800CW and IRDye 680RD labelled secondary antibodies (LI-COR Biosciences). Quantification was performed on scanned unsaturated bands using the GS800 Imaging densitometer and Quantity One software (Bio-Rad Laboratories, Hemel Hempstead, UK) or on fluorescent images using the Odyssey® CLx and Image Studio software (LI-COR Biosciences).

Microtubule depolymerization assay

AML cells were treated for 6 hours with mebendazole, colcemid (ThermoFisher Scientific) or paclitaxel (Cayman Chemical Company). 1×10^6 cells were washed twice in PBS and resuspended in 100 µl depolymerization buffer (20mM Tris-HCl pH6.8, 0.14 M NaCl, 0.5% NP-40, 1 mM MgCl₂, 2 mM EGTA, 10 µM paclitaxel, protease inhibitors)¹⁷ and vortexed on maximum setting. Lysis of cells with this microtubule-stabilizing buffer preserves the proportions of polymerized and soluble microtubules present *in vivo* in cells, following the

period of drug treatment. Cells were then centrifuged at 12,000 x g for 10 min at 4⁰C. The supernatant was decanted and 100 µl 2x reducing sample buffer added (S, soluble tubulin). The pellet was resuspended in 100 µl 1 mM CaCl₂ (with protease inhibitors), incubated at room temperature for 15 minutes, with brief vortexing and 100 µl 2x reducing sample buffer added (P, lysate pellet). Percent polymerized tubulin was calculated from the formula $P/(P + S)100$ from quantified western blot bands.

Quantitative RT-PCR analysis

Total RNA was isolated from cells using RNeasy Mini Kit (Qiagen, Manchester, UK) according to the manufacturer's instructions. RNA was converted into cDNA using a cDNA synthesis kit (Invitrogen, Life Technologies) according to the manufacturer's instructions. Samples were treated with DNase I (Invitrogen, Life Technologies) prior to reverse transcription using the Moloney murine leukemia virus reverse transcriptase (Invitrogen, Life Technologies).

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Supplementary Figure Legends

Supplementary Figure 1. Mebendazole inhibits expression of c-MYB targets in AML cells. QPCR analysis of selected c-MYB target gene expression in THP1 cells after 6 hours treatment with DMSO or 10 μ M mebendazole, normalized to DMSO controls. Bars and error bars are means and SD of three independent experiments. ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$; n.s. not significant (relative to DMSO controls), one sample t test.

Supplementary Figure 2. Mebendazole inhibits growth and induces apoptosis in AML cells. (a) The chart shows the fold change in viable cell number of AML cell lines treated with DMSO or 10 μ M mebendazole, over 72 hours. Symbols and error bars are means and SD of three independent experiments, each in triplicate. (b) Examples of Annexin V/PI staining of THP1 cells exposed to DMSO or 10 μ M mebendazole for 48 hours. (c) Apoptotic analysis of AML cell lines exposed to DMSO or 10 μ M mebendazole, over 72 hours. Symbols, representing the percentage of Annexin V/PI cells, and error bars are means and SD of three independent experiments.

Supplementary Figure 3. Mebendazole inhibits colony formation by AML cells. Colony formation, normalized to DMSO controls, of AML cell lines in the presence of indicated mebendazole concentrations. Bars and error bars are means and SD of three independent experiments, each in quadruplicate.

Supplementary Figure 4. *c-MYB* silencing inhibits colony formation by AML cells. (a) Western blot analysis of c-MYB protein expression in AML cells transduced with control scramble (SCR) shRNA or shRNA targeting c-MYB (MYB). (b) Example of colony formation in methylcellulose cultures of shSCR or shMYB transduced THP1 cells. (c) Colony formation, normalized to DMSO controls, of shSCR or shMYB transduced AML cell lines. Bars and error bars are means and SD of quadruplicate cultures.

Supplementary Figure 5. Proteasomal inhibition blocks mebendazole induced c-MYB degradation. Quantification of c-MYB protein expression in indicated AML cells lines following 6 hours treatment with DMSO, 10 μ M MBZ or 10 μ M MBZ/10 μ M MG132. Bars and error bars are means and SD of four (THP1) and three (OCI-AML3, SHI-1) independent experiments. Data are normalized to DMSO controls. *** $P < 0.001$; n.s. not significant (relative to DMSO controls), one sample t test.

Supplementary Figure 6. Mebendazole induces c-MYB degradation at lower concentrations than those required for tubulin depolymerization. (a) Example of western blot analysis of tubulin depolymerization assay. Polymerized tubulin, present in the lysate pellet (P), and soluble tubulin (S) bands are shown for THP1 cells treated for 6 hours with indicated concentrations of mebendazole (top) or colcemid and 5 μ M paclitaxel (Pax) (bottom). (b) Percent polymerized tubulin in OCI-AML3 cells following 6 hours treatment with indicated concentrations of mebendazole (top left) or colcemid (top right), and western blot analysis of corresponding c-MYB protein expression (bottom). Tubulin stabilization by 5 μ M paclitaxel (Pax) is also shown. Bars and error bars are means and SD, respectively, of four independent experiments. * $P < 0.05$; *** $P < 0.001$, n.s. not significant, unpaired Student's t -test.

Supplementary Figure 7. Mebendazole induced c-MYB loss is not due to cell cycle inhibition. (a) Quantification of c-MYB protein expression in THP1 cells following 4 hours treatment with DMSO or indicated concentrations of mebendazole. Bars and error bars are means and SD of four independent experiments. Data are normalized to DMSO controls. (b) Apoptotic analysis of THP1 cells following 4 hours exposure to DMSO or 10 μ M mebendazole. Bars, representing the percentage of Annexin V/PI cells, and error bars are means and SD of three independent experiments. (c) Examples of flow cell cycle cytometry plots of THP1 cells treated for 4 hours with DMSO or the indicated concentrations of

mebendazole. Numbers inside plots are percentages of cells in Go/G1 (bottom left), S (top) and G2/M (bottom right) phases of the cell cycle. **(d and e)** Bars and error bars are means and SD of percentages of THP1 cells in the Go/G1, S and G2/M phases of the cell cycle following 4 **(d)** and 24 **(e)** hours of treatment with DMSO or the indicated concentrations of mebendazole, from four **(d)** and three **(e)** independent experiments. *** $P < 0.001$; n.s. not significant, unpaired Student's t-test.

Supplementary Figure 8. Nematic protein organisation technique (NPOT) analysis of mebendazole in AML cell lysates. **(a)** Pictures of three independent hetero-assemblies (circled in red) induced by mebendazole in primary AML sample AML4298 and THP1 cell lysates. **(b)** Venn diagrams for the number of proteins identified in AML4298 and THP1 lysates, and in both lysates. **(c)** DAVID analysis and the annotation sources GOTERM_BP2 (Biological process) were used to identify the functional categories identified in AML4298 lysates. Shown in bold are the proteins comprising the 'protein folding' category also identified in THP1 lysates.

Supplementary Figure 9. The HSP70/HSC70 chaperone complex binds c-MYB and is required for maintenance of c-MYB protein expression. **(a)** Western blot analysis of mouse IgG and anti-c-MYB immunoprecipitates from THP1 cells, stained with anti-HSP70/HSC70 (top) and anti-c-MYB (bottom). Representative data from one of three independent experiments. **(b)** Quantification of HSP70 (left) and c-MYB (right) protein expression in THP1 cells 7 days after transduction with control scramble (shSCR) shRNA or two independent shRNA targeting *HSPA1A* (sh1 and sh2). Bars and error bars are means and SD of three independent experiments. **(c)** Representative data and quantification of c-MYB protein expression in THP-1 cells after 6 hours treatment with DMSO or the indicated concentrations of the HSP70 inhibitors, KNK437 and VER-155008. Bars and error bars are means and SD of three independent experiments. **(d)** Representative data and quantification

of HSP70/HSC70 protein expression in total (total), cytoplasmic (cyto) and nuclear (nuc) extracts from THP-1 cells treated with DMSO or 10 μ M mebendazole for 6 hours. The extracts were validated using antibodies against α Tubulin (cytoplasmic) and SP1 (nuclear), and nuclear expression of c-MYB is also shown. Bars and error bars represent relative HSP70/HSC70 expression in total (using α Tubulin for loading), cytoplasmic (using α Tubulin for loading) and nuclear extracts (using SP1 for loading), and are means and SD of three independent experiments. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant (relative to DMSO controls), one sample t test.

Supplementary Figure 10. Mebendazole pre-treatment inhibits colony formation by primary AML cells but not CD34⁺ CB cells. **(a)** Example of colony formation by a primary AML patient sample (AML1547) and a normal CD34⁺ cord blood sample (CB1), after pre-treatment with DMSO or 10 μ M mebendazole. Cells were treated with vehicle or drug for 20 hours, washed and placed into methylcellulose culture. Colonies were grown for 14 days and visualized by staining with 1 mg/ml p-iodonitrotetrazolium. **(b)** CFU-G, CFU-GM, BFU-E, CFU-M, CFU-GEMM and total colony numbers of two independent normal CD34⁺ cord blood samples (CB1, CB2) after pre-treatment with DMSO or 10 μ M mebendazole. Cells were treated with vehicle or drug for 20 hours, washed and placed into methylcellulose culture, and colony formation scored 14 days later.

Supplementary Figure 11. Mebendazole induced c-MYB loss in CB cells is less acute than in AML cells. **(a)** Representative data and **(b)** quantification of c-MYB protein expression in CB cells following treatment with DMSO or 10 μ M mebendazole for 6 hours. Prior to drug treatment, CD34⁺ CB cells were expanded for 7 days. Bars and error bars are means and SD of data from three independent CB samples. * $P < 0.05$ (relative to DMSO controls), one sample t test.

Supplementary Figure 12. *In vivo* drug treatment selects for increased c-MYB expression and reduced sensitivity to mebendazole. **(a)** Quantification of c-MYB protein expression in THP1 cells *ex vivo* (T-RES1), isolated from NSG mice following treatment with mebendazole *in vivo*, compared to control THP1 cells. **(b)** Increased c-MYB expression is still exhibited after two weeks *in vitro* culture in the absence of mebendazole. c-MYB protein expression in THP1 cells isolated from mebendazole-treated NSG mice (T-RES1 and T-RES2) following 2 weeks *in vitro* culture (*in vitro*). **(c)** c-MYB protein expression in T-RES1 cells, after 6 hours *ex vivo* exposure to the indicated concentrations of mebendazole, compared to control THP1 cells. **(d)** Viability, normalized to DMSO controls, of control THP1 and T-RES1 cells treated for 72 hours with indicated mebendazole concentrations. Bars and error bars are means and SD of triplicate cultures.

Supplementary Table 1. Patient characteristics.

Patient id	Sex	Age (yrs)	FAB	Karyotype
1497	M	1.6	4	46, XY, t(9;11;9)(p22;p23;q34)
1547	F	8.8	5	46, XX, t(9;11)(p22;q23)(low% 47, idem, +8)
4298	M	11.2	5	46, XY, t(9;11)

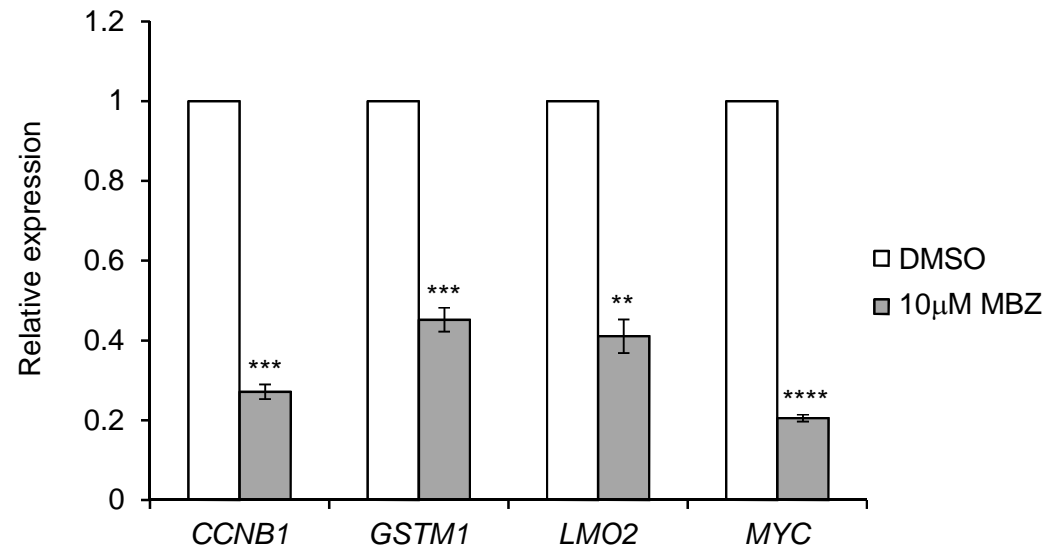
Supplementary Table 2. Gene expression data for SPIEDw analysis.

Gene	Fold change upon loss of MLL-ENL/MLL-AF9
ANTXR1	-14.040
SIX4	-9.696
P2RY2	-7.474
FRAT2	-6.627
IRAK1BP1	-5.853
IPO11	-5.278
LRIG1	-4.860
SIX1	-3.968
TASP1	-3.809
CABLES1	-3.734
MYC	-3.724
MMACHC	-3.586
PDCD4	-3.571
NT5DC2	-3.562
PDXK	-3.490
ST7	-3.435
MID1	-3.373
SPRED1	-3.043
PFKL	-3.026
HSPA2	-2.994
MRPS27	-2.957
PRMT6	-2.933
SLC39A8	-2.867
MRPS6	-2.862
MSI2	-2.786
GATA2	-2.744
UCK2	-2.714
STEAP3	-2.714
PRPF19	-2.664
CRTAP	-2.642
EXOSC6	-2.631
GPR180	-2.629
LIPT1	-2.617
PAPSS2	-2.613
AHCY	-2.607
EBNA1BP2	-2.563
TMEM97	-2.558
ABI2	-2.554
BAMBI	-2.548
PCDH7	-2.547
TRAP1	-2.518
MINPP1	-2.490
PRPS1	-2.452

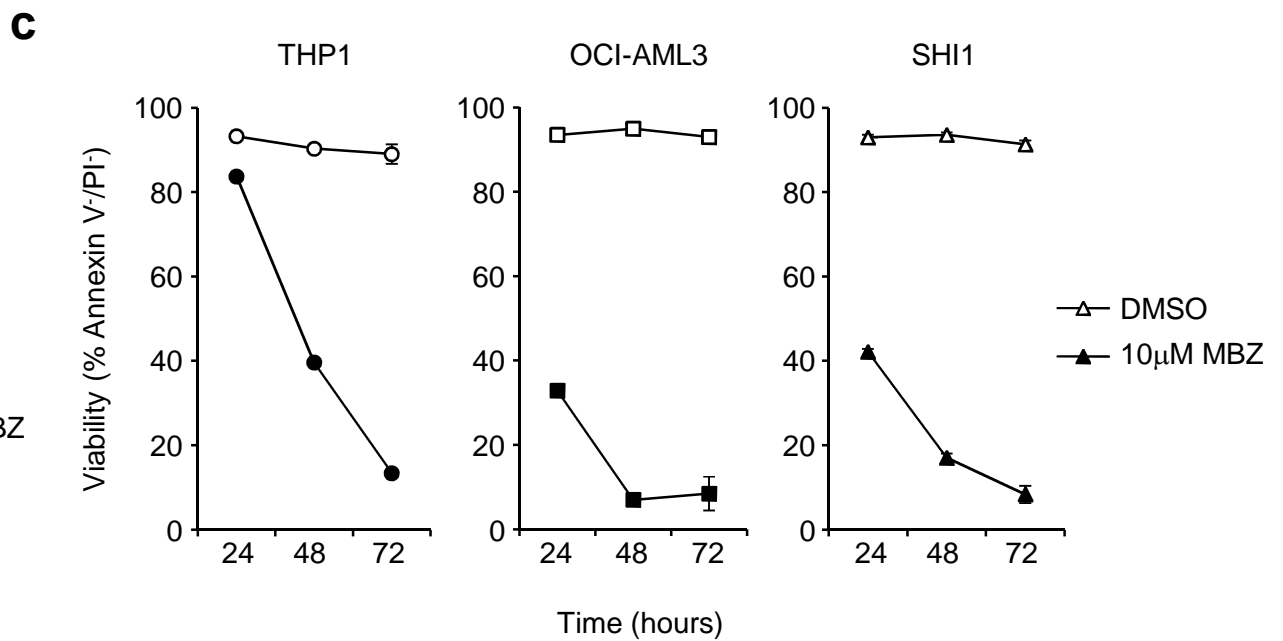
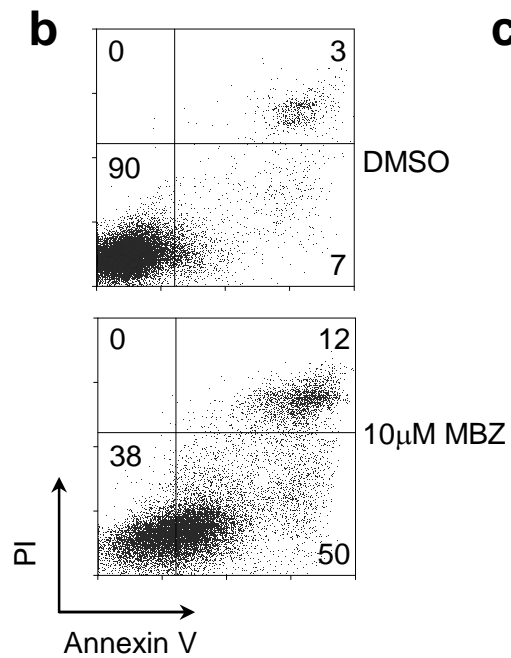
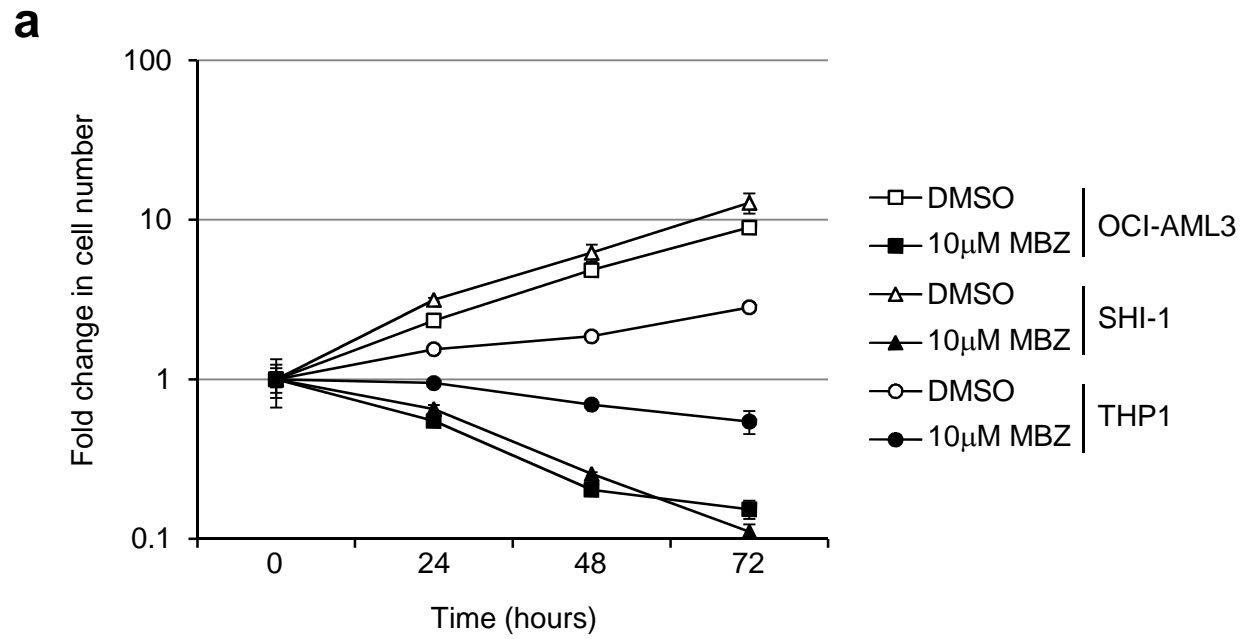
SPRED2	-2.409
POLR1E	-2.275
SPRY2	-2.226
OLA1	-2.198
LIMK1	-2.182
COMMD9	-2.172
NUP35	-2.151
ACSL3	-2.149
KLHL5	-2.147
LPAR1	-2.132
DTYMK	-2.103
EPRS	-2.099
IDH2	-2.095
TMEM107	-2.080
TRIM45	-2.057
SASH1	-2.045
CXorf21	-2.029
BCLAF1	-2.005
NIT2	-1.992
SNRPF	-1.988
IL13RA1	1.992
ECM1	2.004
AGTRAP	2.004
RUNX1	2.020
CENPJ	2.024
PSAP	2.037
PTPRC	2.041
DACH1	2.053
FMNL1	2.053
SULF2	2.058
SLA	2.070
SSH1	2.070
PTK2B	2.070
ABCG1	2.088
PLEK	2.092
INTS12	2.096
TDRD7	2.119
PLEKHO2	2.123
CDC42EP3	2.132
RBMS1	2.132
IQSEC1	2.132
FHOD1	2.141
LST1	2.146
H2AFX	2.151
NUMB	2.160
ACVR1B	2.165
ABCA13	2.165
IQGAP1	2.174

TOB1	2.174
WDR26	2.174
PLEKHA2	2.179
DMXL2	2.217
AKAP13	2.222
MAPK3	2.227
PRKCD	2.252
IFT122	2.257
PIK3CB	2.262
HBP1	2.304
SSH2	2.309
PTPRE	2.326
LPP	2.342
SH3KBP1	2.347
PYGL	2.347
MBP	2.381
WIP1	2.381
HEXB	2.410
RTKN2	2.427
TNFAIP3	2.451
PHF21A	2.457
KLF13	2.457
GLRX	2.481
FLNA	2.488
NCF4	2.494
PSTPIP2	2.513
FLOT1	2.525
SMARCA2	2.538
NEU1	2.558
SNX10	2.558
RAC2	2.591
SIRPA	2.660
VCAN	2.667
SEMA4D	2.695
ITGAL	2.710
JAZF1	2.747
KLF6	2.762
IQGAP2	2.778
BMX	2.786
MYO1F	2.874
SORL1	2.882
LTA4H	2.890
PELI2	2.907
ACSL1	2.933
MYH9	2.933
SPSB1	2.976
SNAI3	3.012
CD9	3.012

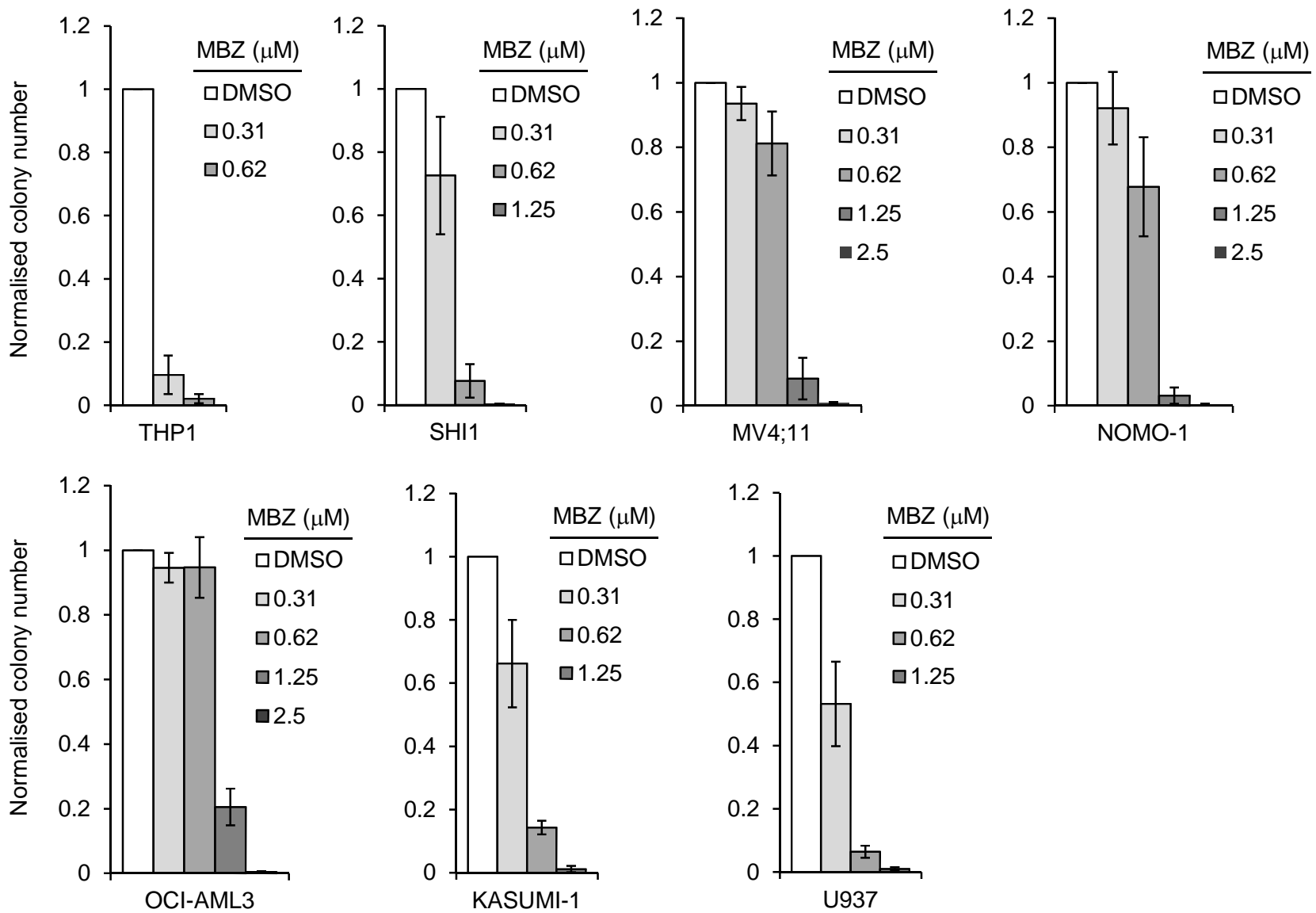
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CCND3	3.040
THBS1	3.077
DOCK5	3.125
LGALS3	3.226
PLAUR	3.268
ZFP36	3.390
ZYX	3.390
ZFP36L1	3.509
GLIPR2	3.636
DHRS3	3.650
NOTCH1	3.676
ST3GAL2	3.690
NFKBIZ	3.831
PLAU	3.906
LTB4R	3.922
NCF1	4.000
FGR	4.219
LYST	4.237
DAPK2	4.274
PHLDA1	4.367
BCL6	4.425
SQRDL	4.785
GPAT3	5.181
GCNT2	5.435
HLX	6.024
SERPINB2	6.452
S100A4	8.130
EGR2	9.259
CAMP	9.434
PLP2	9.615
HCK	9.709
PRAM1	12.422
C5AR1	13.889



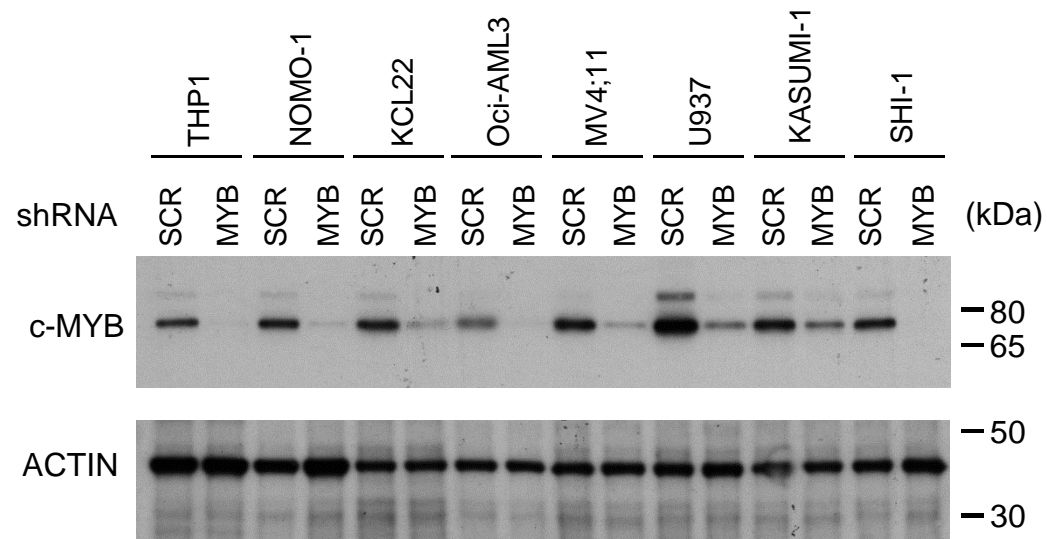
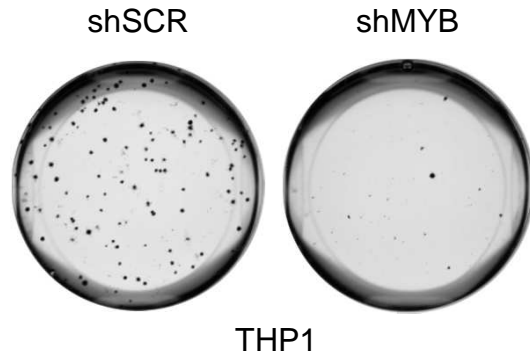
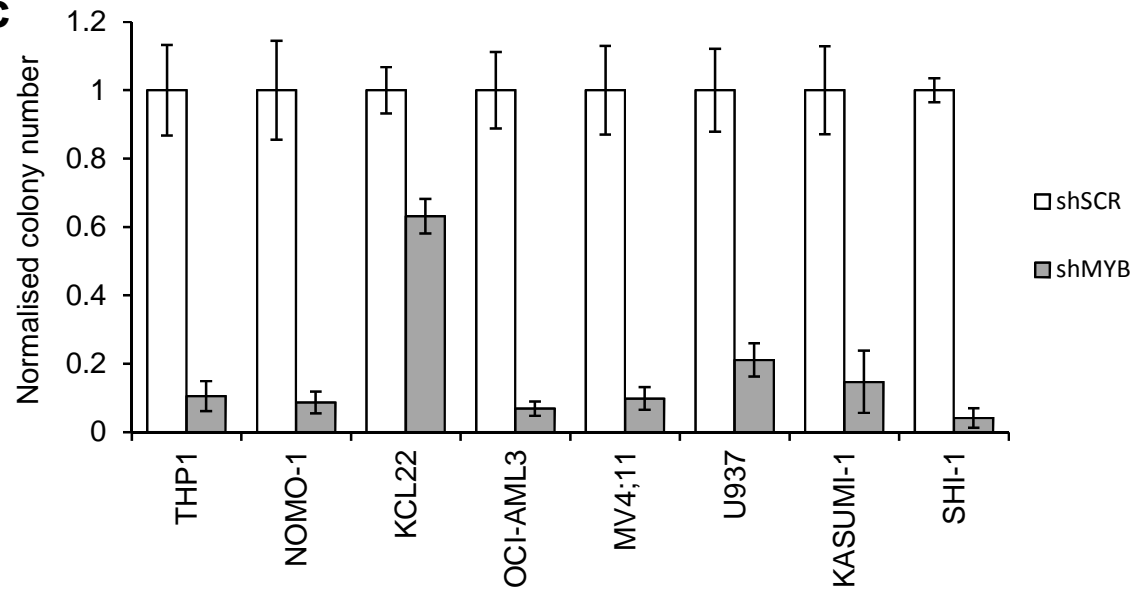
SUPPLEMENTARY FIGURE 1

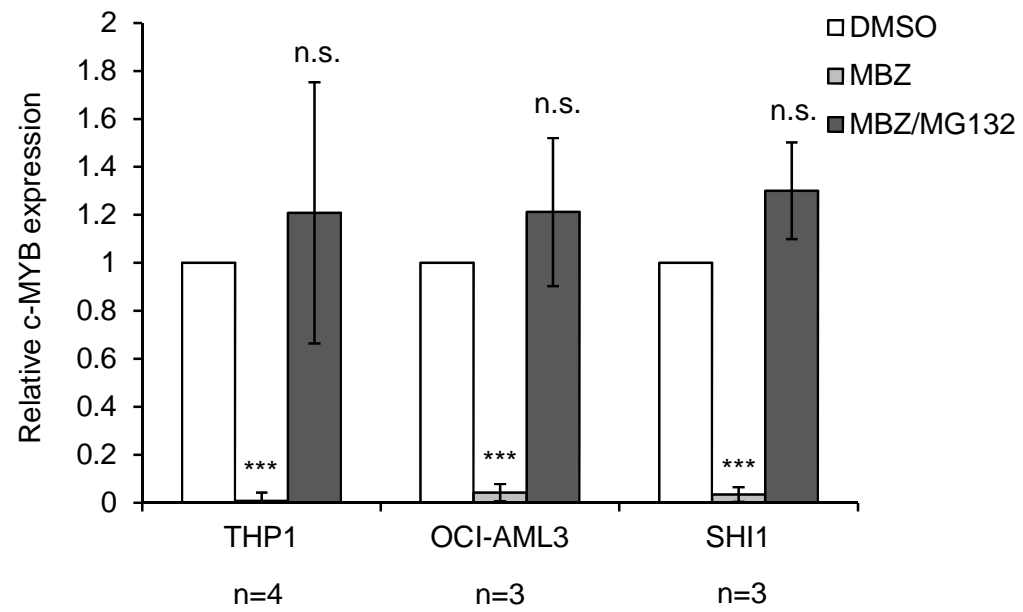


SUPPLEMENTARY FIGURE 2



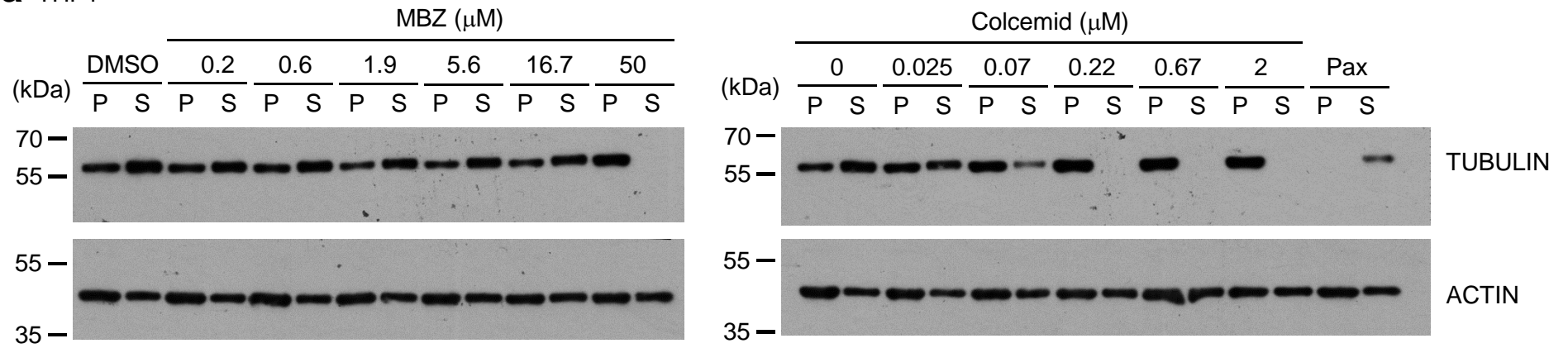
SUPPLEMENTARY FIGURE 3

a**b****c**

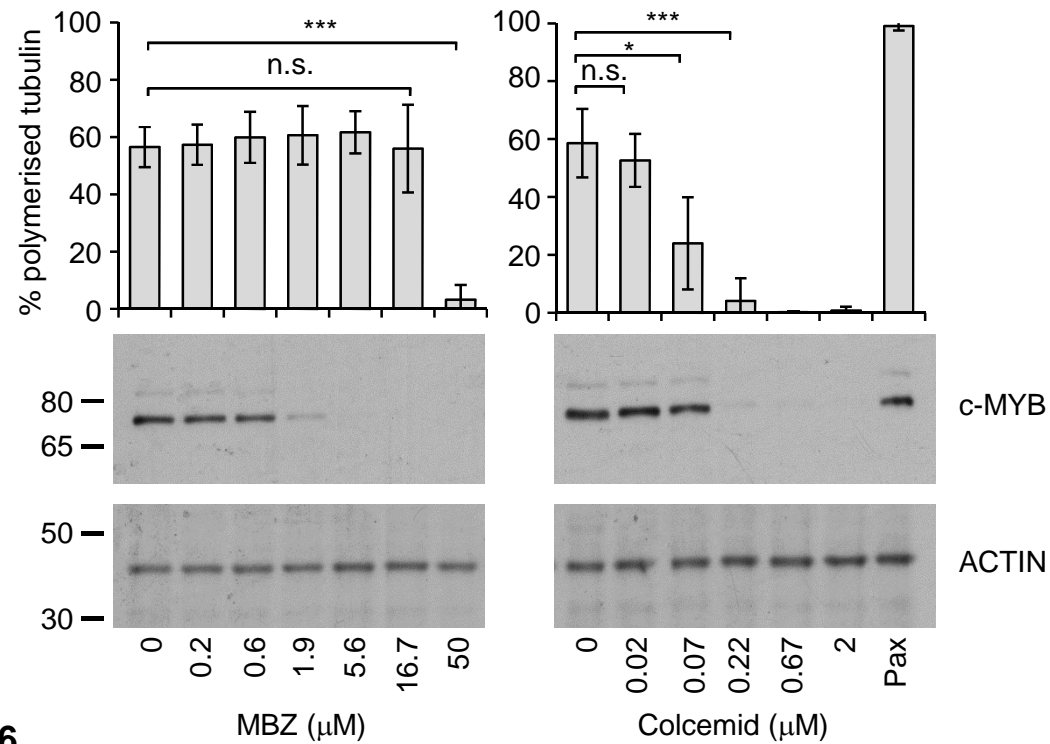


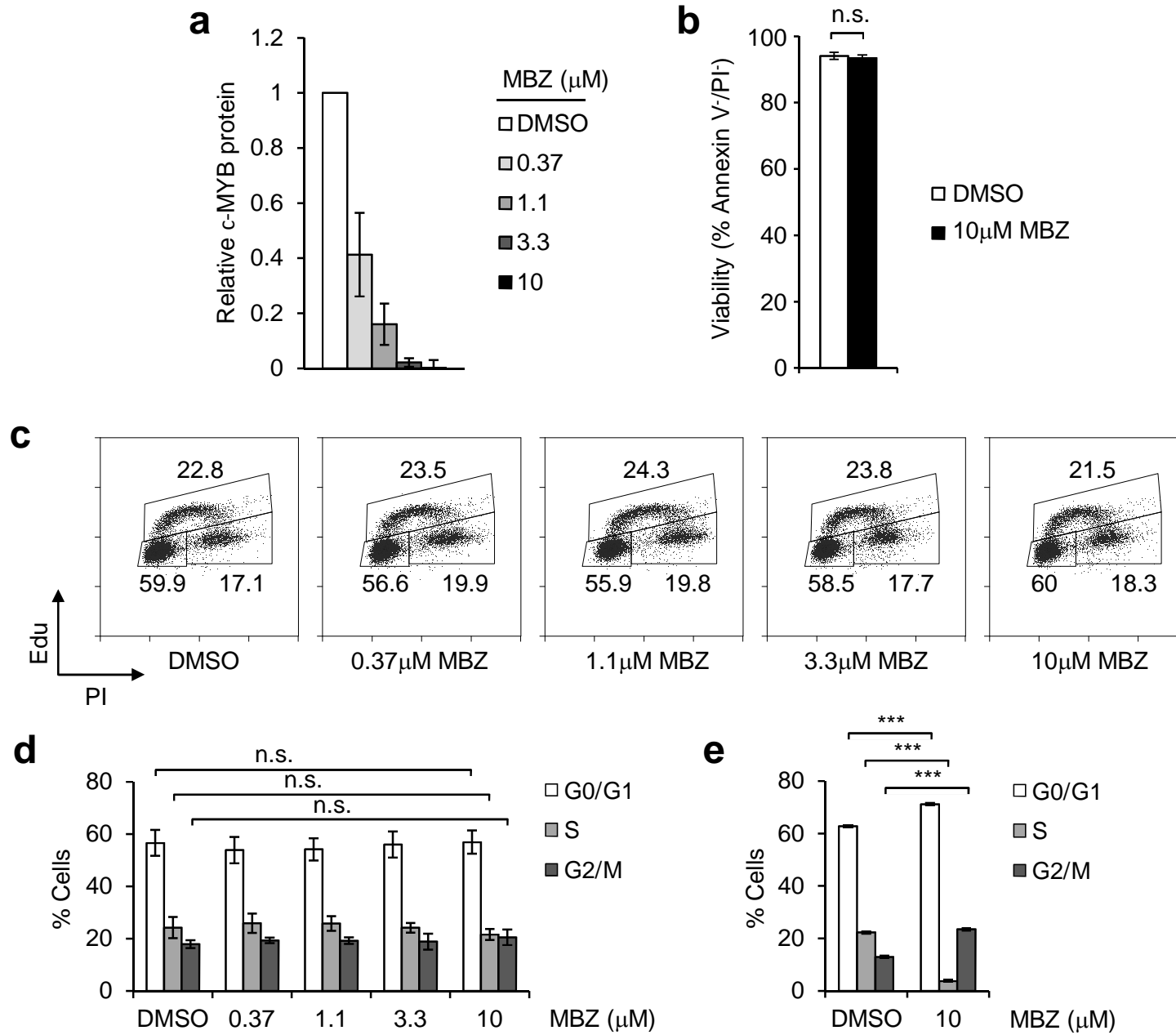
SUPPLEMENTARY FIGURE 5

a THP1

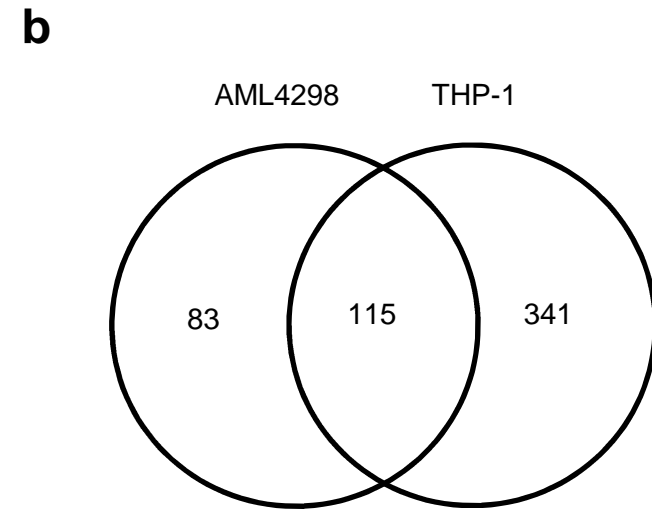
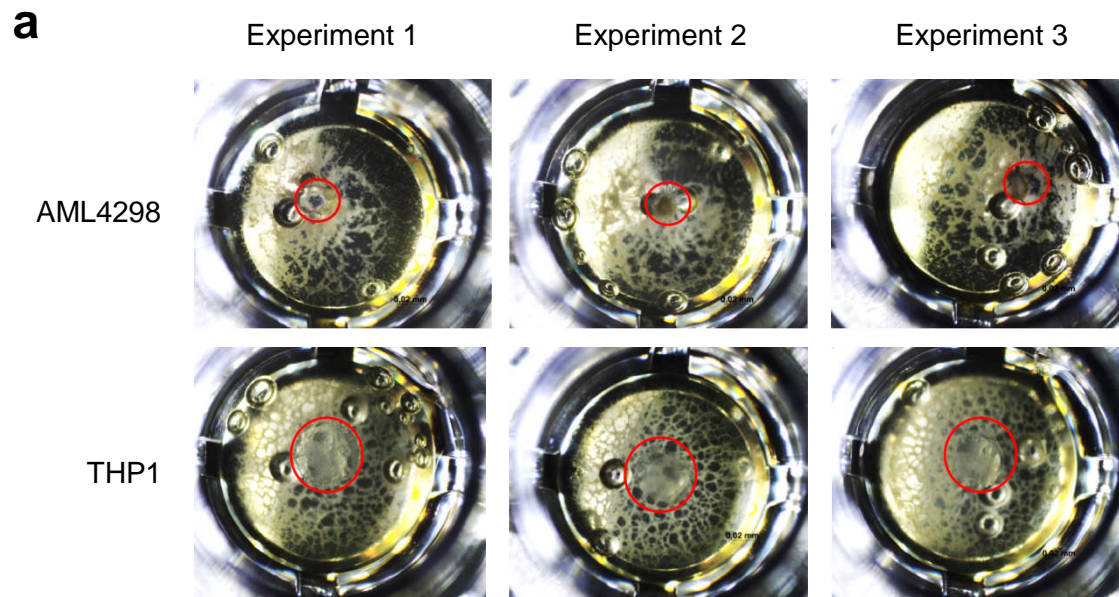


b OCI-AML3





SUPPLEMENTARY FIGURE 7



c

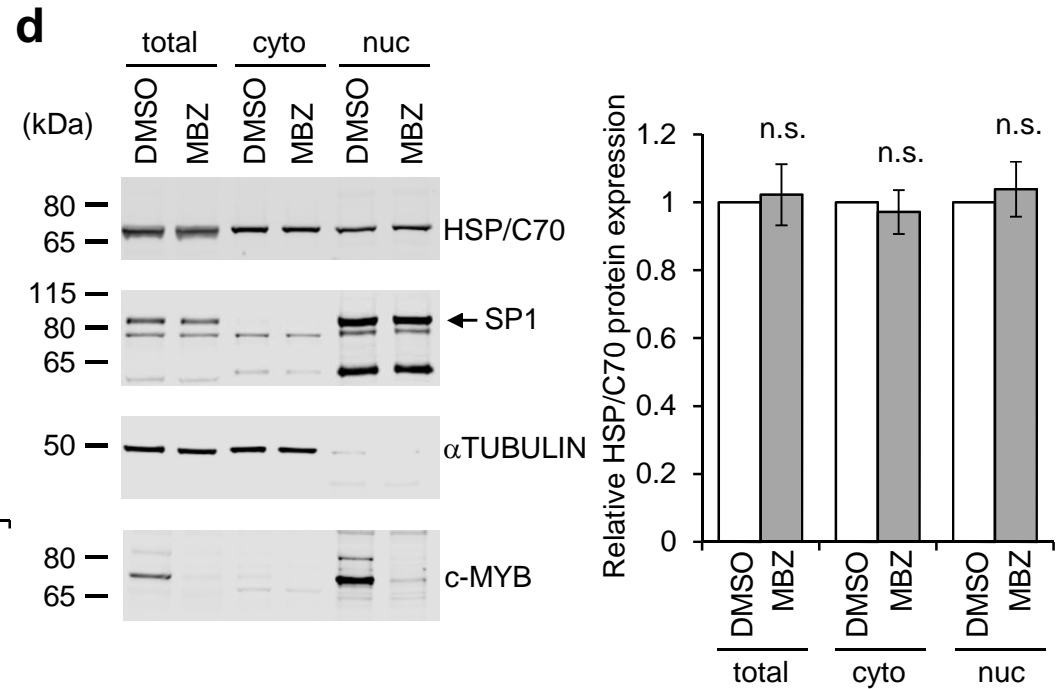
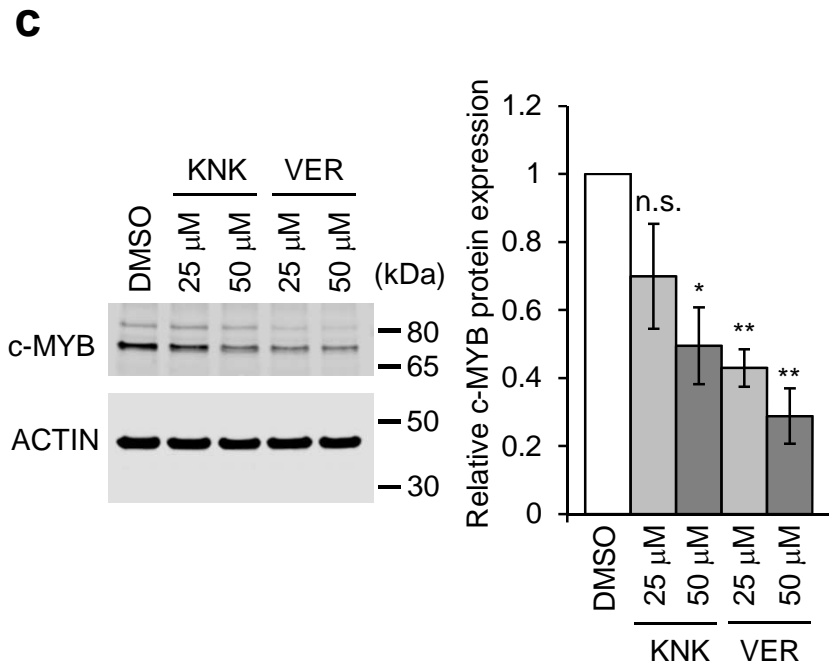
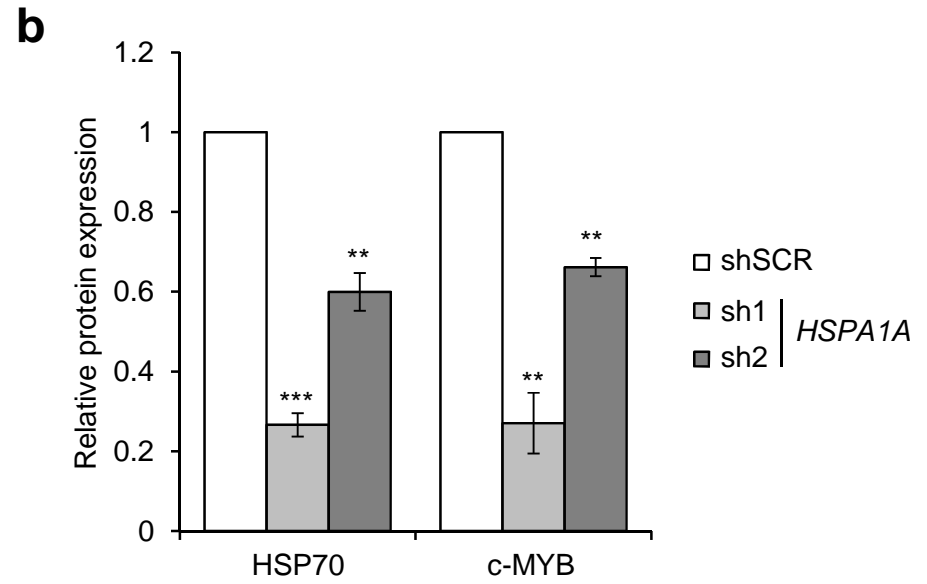
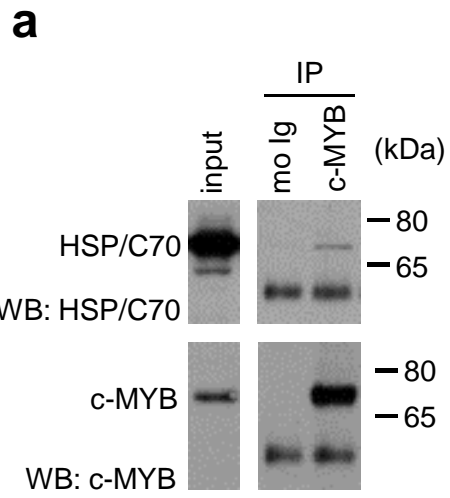
David analysis patient sample AML4298 with MBZ

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catabolic process	48	1.10E-06
cellular component biogenesis	59	8.10E-06

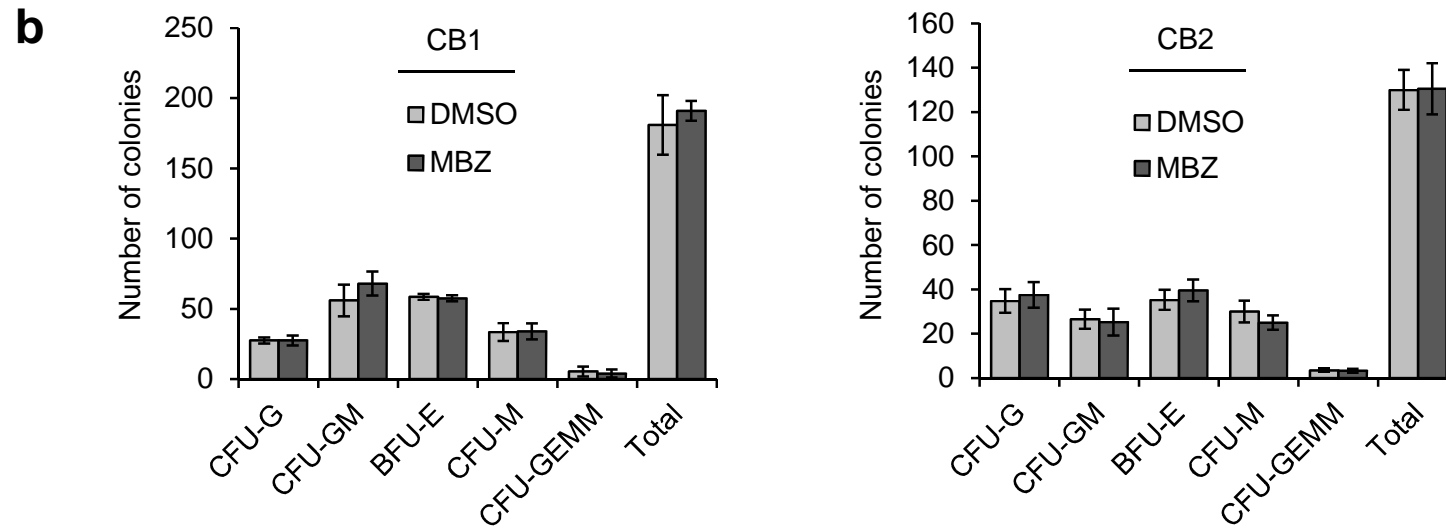
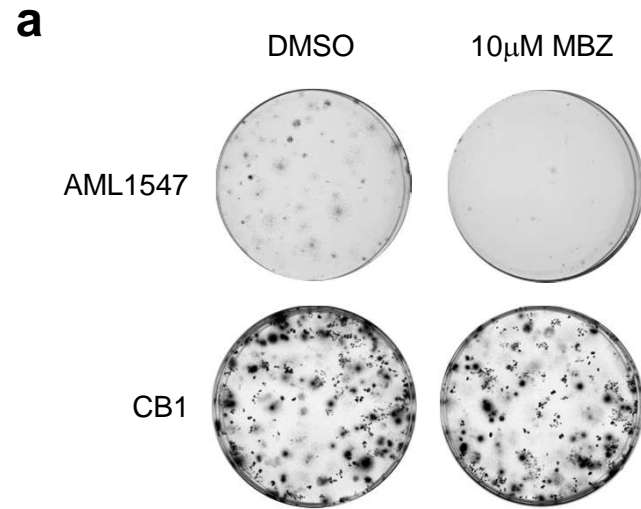
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glucosidase II alpha subunit(GANAB)
heat shock protein 90 alpha family class A member 1(HSP90AA1)
heat shock protein 90 alpha family class B member 1(HSP90AB1)
heat shock protein family A (Hsp70) member 1A(HSPA1A)
heat shock protein family A (Hsp70) member 6(HSPA6)
heat shock protein family A (Hsp70) member 8(HSPA8)
heat shock protein family A (Hsp70) member 9(HSPA9)
heat shock protein family D (Hsp60) member 1(HSPD1)
peptidylprolyl isomerase A(PPIA)
peptidylprolyl isomerase B(PPIB)
protein disulfide isomerase family A member 3(PDIA3)
protein disulfide isomerase family A member 6(PDIA6)
protein kinase C substrate 80K-H(PRKCSH)
RuvB like AAA ATPase 2(RUVBL2)
TNF receptor associated protein 1(TRAP1)

SUPPLEMENTARY FIGURE 8

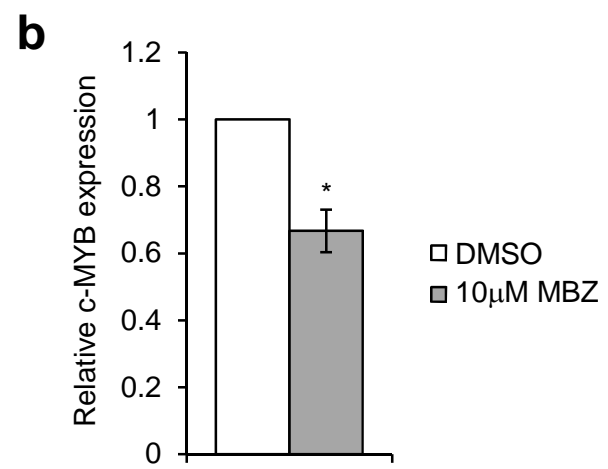
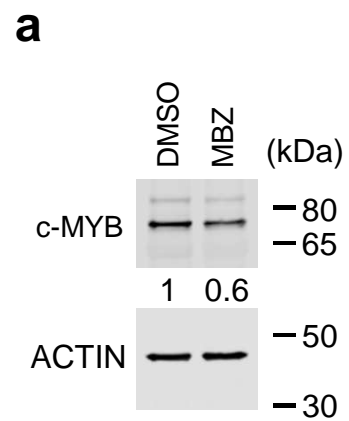
*In bold confirmed in THP-1 cells.



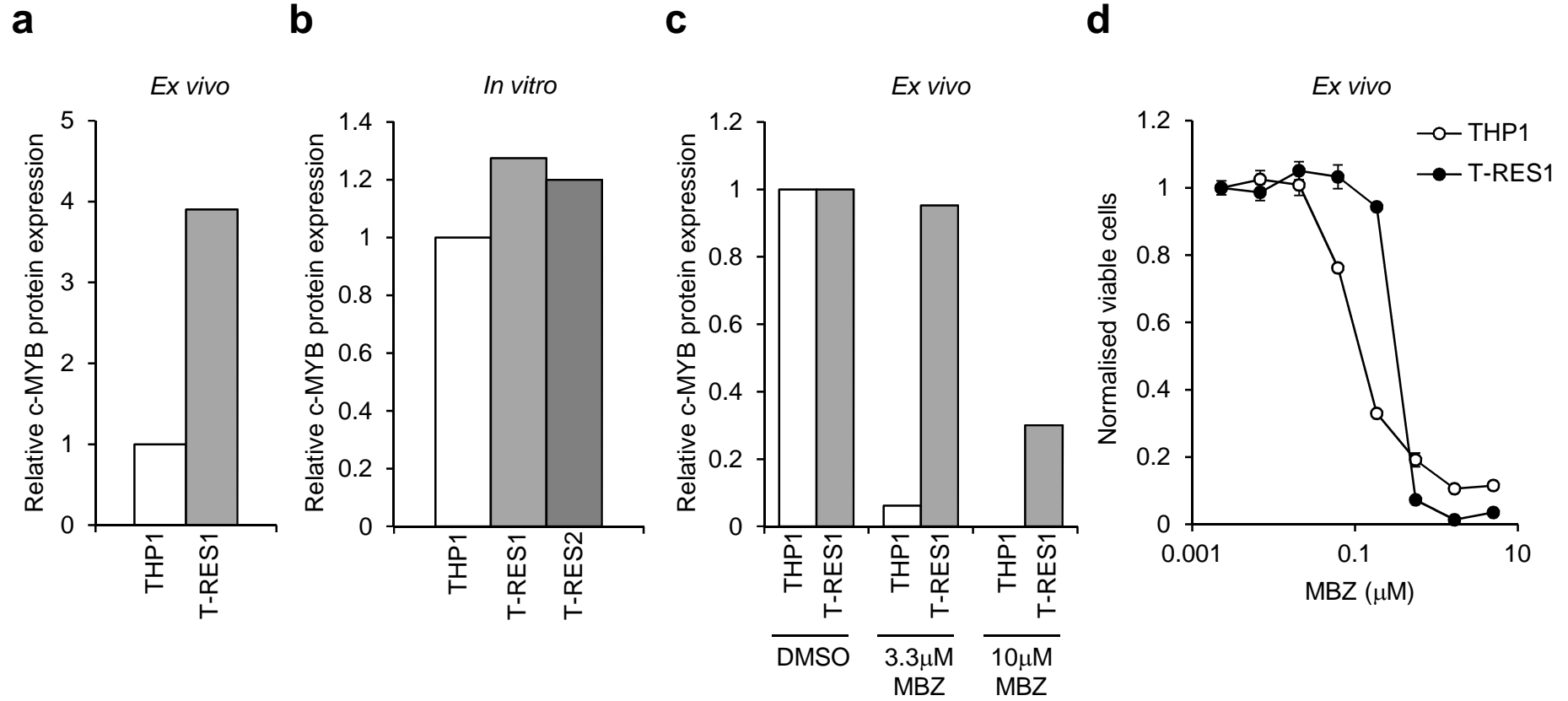
SUPPLEMENTARY FIGURE 9



SUPPLEMENTARY FIGURE 10



SUPPLEMENTARY FIGURE 11



SUPPLEMENTARY FIGURE 12