



American Society of Hematology
 2021 L Street NW, Suite 900,
 Washington, DC 20036
 Phone: 202-776-0544 | Fax 202-776-0545
 editorial@hematology.org

A MIR17HG-derived Long Noncoding RNA Provides an Essential Chromatin Scaffold for Protein Interaction and Myeloma Growth

Tracking no: BLD-2022-016892R2

Eugenio Morelli (Dana Farber Cancer Institute, United States) Mariateresa Fulciniti (Dana Farber Cancer Institute, United States) Mehmet Samur (Dana-Farber Cancer Institute and Harvard School of Public Health, United States) Caroline Ribeiro (Weill Cornell Medical College, United States) Leon Wert-Lamas (Dana Farber Cancer Institute, United States) Jonathan Henninger (Whitehead Institute of Biomedical Research, United States) Annamaria Gulla (Dana-Farber Cancer Institute, United States) Anil Aktas Samur (Dana Farber Cancer Institution, United States) Katia Todoerti (Fondazione Cà Granda IRCCS Policlinico, Italy) Srikanth Talluri (DFCI, United States) Woojun Park (Baylor College of Medicine, United States) Cinzia Federico (ASST DEGLI SPEDALI CIVILI DI BRESCIA, Italy) Francesca Scionti (Institute for Biomedical Research and Innovation (IRIB), National Research Council of Italy (CNR), 98164 Messina, Italy, Italy) Nicola Amodio (University Magna Graecia of Catanzaro, Italy) Giada Bianchi (Brigham and Women's Hospital, United States) Megan Johnstone (Dana Farber Cancer Institute, United States) Na Liu (Dana Farber Cancer Institute, United States) Doriane Gramegna (Dana Farber Cancer Institute, United States) Domenico Maisano (Dana-Farber Cancer Institute, United States) Nicola Russo (Biogem s.c.ar.l., Italy) Charles Lin (Baylor College of Medicine, United States) Yu-Tzu Tai (Dana Farber Cancer Institute, United States) Antonino Neri (University of Milan, Italy) Dharminder Chauhan (Dana Farber Cancer Institute, United States) Teru Hideshima (Dana-Farber Cancer Institute, United States) Masood Shammas (Dana Farber Cancer Institute, United States) Pierfrancesco Tassone (Magna Graecia University, Italy) Sergei Gryaznov (MAIA Therapeutics, United States) Richard Young (Whitehead Institute, United States) Kenneth Anderson (Dana Farber Cancer Institute, United States) Carl Novina (Harvard Medical School, United States) Massimo Loda (Weill Cornell Medicine, United States) Nikhil Munshi (V/A healthcare system, United States)

Abstract:

Long noncoding RNAs (lncRNA) can drive tumorigenesis and are susceptible to therapeutic intervention. Here, we used a large-scale CRISPR interference viability screen to interrogate cell growth dependency to lncRNA genes in multiple myeloma (MM), and identified a prominent role for the *miR-17-92 cluster host gene* (MIR17HG). We show that a MIR17HG-derived lncRNA, named lnc-17-92, is the main mediator of cell growth dependency acting in a microRNA- and DROSHA- independent manner. Lnc-17-92 provides a chromatin scaffold for the functional interaction between c-MYC and WDR82, thus promoting the expression of ACACA, which encodes the rate-limiting enzyme of *de novo* lipogenesis acetyl-coA carboxylase 1 (ACC1). Targeting MIR17HG pre-RNA with clinically applicable antisense molecules disrupts the transcriptional and functional activities of lnc-17-92, causing potent anti-tumor effects both *in vitro* and *in vivo* in three pre-clinical animal models, including a clinically relevant PDX-NSG mouse model. This study establishes a novel oncogenic function of MIR17HG and provides potent inhibitors for translation to clinical trials.

Conflict of interest: COI declared - see note

COI notes: N.C.M. serves on advisory boards/consultant to Takeda, BMS, Celgene, Janssen, Amgen, AbbVie, Oncopep, Karyopharm, Adaptive Biotechnology, and Novartis and holds equity ownership in Oncopep. K.C.A. serves on advisory boards to Janssen, Pfizer, AstraZeneca, Amgen, Precision Biosciences, Mana, Starton, and Raquia, and is a Scientific Founder of OncoPep and C4 Therapeutics. R.A.Y. is a founder and shareholder of Syros Pharmaceuticals, Camp4 Therapeutics, Omega Therapeutics, and Dewpoint Therapeutics. E.M., S.G. and N.C.M filed a provisional patent on MIR17HG as a target for cancer therapy. No potential conflicts of interest were disclosed by the other authors. D.C. reports other support from Stemline Therapeutics, Oncopeptides, and C4 Therapeutics outside the submitted work.

Preprint server: Yes; Biorxiv <https://doi.org/10.1101/2021.12.08.471297>

Author contributions and disclosures: E.M. and N.C.M. conceived and designed the research studies. E.M., M.F. and N.C.M. wrote the manuscript. M.K.S., A.A.S. and K.T. performed in silico analysis of transcriptomic data. C.F.R. performed lipidomic studies. L.W.L. performed yeast-3-hybrid experiments. J.E.H. performed RNA FISH, dual RNA FISH and Co-IF/dual RNA FISH. S.T. provided MM cells expressing CAS9. W.D.P. analyzed ChIP-seq data. S.G. designed t-ASOs. C.F., N.R. and F.S. provided support for the identification of RROL isoforms. M.F., A.G., N.A., M.J., G.B., C.L., Y.T.T., A.N., D.C., T.H., M.A.S., P.T., R.A.Y., K.C.A., C.D.N. and M.L. contributed to the design and interpretation of key experiments. M.L. supervised lipidomic studies. C.D.N. supervised Y3H experiments.

Non-author contributions and disclosures: Yes; We thank Dr. Christina Usher (Dana-Farber Cancer Institute) for editing the manuscript and insightful comments.

Agreement to Share Publication-Related Data and Data Sharing Statement: RNA-seq data after depletion of lnc-17-92 have been deposited and are publicly available.

Clinical trial registration information (if any):

1 **A MIR17HG-derived Long Noncoding RNA Provides an Essential Chromatin**
2 **Scaffold for Protein Interaction and Myeloma Growth**

3 *Short title:* A long noncoding RNA supporting myeloma.

4 Eugenio Morelli^{1,2,*}, Mariateresa Fulciniti^{1,2}, Mehmet K. Samur^{1,2}, Caroline F. Ribeiro³, Leon
5 Wert-Lamas⁴, Jon E. Henninger⁵, Annamaria Gullà^{1,2}, Anil Aktas-Samur^{1,2}, Katia Todoerti⁶,
6 Srikanth Talluri^{1,2,7}, Woojun D. Park⁸, Cinzia Federico⁹, Francesca Scionti¹⁴, Nicola Amodio⁹,
7 Giada Bianchi^{1,2}, Megan Johnstone¹, Na Liu¹, Doriana Gramegna^{1,2}, Domenico Maisano^{1,2},
8 Nicola A. Russo¹⁰, Charles Lin⁸, Yu-Tzu Tai^{1,2}, Antonino Neri^{6,11,12}, Dharminder Chauhan^{1,2}, Teru
9 Hideshima^{1,2}, Masood A. Shammas^{1,2,7}, Pierfrancesco Tassone⁹, Sergei Gryaznov¹³, Richard A.
10 Young⁵, Kenneth C. Anderson^{1,2}, Carl D. Novina⁴, Massimo Loda³, and Nikhil C. Munshi^{1,2,7,*}.

11
12 ¹Department of Medical Oncology, Jerome Lipper Multiple Myeloma Center; Dana-Farber Cancer
13 Institute; Boston, MA, 02215; USA.

14 ²Harvard Medical School, Boston, MA, 02215; USA.

15 ³Department of Pathology and Laboratory Medicine; Weill Cornell Medical College; New York, NY, 10065;
16 USA.

17 ⁴Department of Cancer Immunology and Virology; Dana-Farber Cancer Institute; Boston, MA, 02215;
18 USA.

19 ⁵Whitehead Institute of Biomedical Research; Massachusetts Institute of Technology; Cambridge, MA,
20 02142; USA.

21 ⁶Department of Hematology; Fondazione Cà Granda IRCCS Policlinico; Milan, 20122; Italy.

22 ⁷VA Boston Healthcare System; Boston, MA, 02132; USA.

23 ⁸Department of Molecular and Human Genetics; Baylor College of Medicine; Houston, TX, 77030; USA.

24 ⁹ASST DEGLI SPEDALI CIVILI DI BRESCIA, Italy.

25 ¹⁰Istituto di Ricerche Genetiche "G. Salvatore"; Biogem s.c.ar.l.; Avellino, 83031; Italy.

26 ¹¹Department of Oncology and Hemato-oncology; University of Milan; Milan, 20122; Italy.

27 ¹²Scientific Directorate, Azienda USL-IRCCS Reggio Emilia, 42123, Italy

28 ¹³Maia Biotechnology Inc, 444 W. Lake St., STE 1700, Chicago, IL 60606.

29 ¹⁴Institute for Biomedical Research and Innovation (IRIB)

30

31 *Correspondence: Nikhil_munshi@dfci.harvard.edu; Eugenio_morelli@dfci.harvard.edu.

32 Word counts (text): 3887

33 Word counts (abstract): 152

34 Figures: 6
35 References: 67
36

- 37 **KEY POINTS**
- 38 • MIR17HG produces a long noncoding RNA that acts as a chromatin scaffold for
39 protein interaction and tumor cell growth.
- 40 • Targeting this long noncoding RNA with optimized antisense oligonucleotides
41 has potent anti-myeloma activity in pre-clinical models.
- 42

43 **ABSTRACT**

44 Long noncoding RNAs (lncRNA) can drive tumorigenesis and are susceptible to
45 therapeutic intervention. Here, we used a large-scale CRISPR *interference* viability
46 screen to interrogate cell growth dependency to lncRNA genes in multiple myeloma
47 (MM), and identified a prominent role for the *miR-17-92 cluster host gene* (MIR17HG).
48 We show that a MIR17HG-derived lncRNA, named lnc-17-92, is the main mediator of
49 cell growth dependency acting in a microRNA- and DROSHA- independent manner.
50 Lnc-17-92 provides a chromatin scaffold for the functional interaction between c-MYC
51 and WDR82, thus promoting the expression of ACACA, which encodes the rate-limiting
52 enzyme of *de novo* lipogenesis acetyl-coA carboxylase 1 (ACC1). Targeting MIR17HG
53 pre-RNA with clinically applicable antisense molecules disrupts the transcriptional and
54 functional activities of lnc-17-92, causing potent anti-tumor effects both *in vitro* and *in*
55 *vivo* in three pre-clinical animal models, including a clinically relevant PDX-NSG mouse
56 model. This study establishes a novel oncogenic function of MIR17HG and provides
57 potent inhibitors for translation to clinical trials.

58 **INTRODUCTION**

59 Multiple myeloma (MM) is a genetically complex malignancy of plasma cells that
60 accounts for about 10% of hematologic cancers and remains largely incurable¹. A
61 growing body of evidence points to a key role played by noncoding RNA (ncRNA)
62 networks in MM², suggesting that MM cells can become significantly addicted to and
63 therapeutically susceptible to the modulation of oncogenic ncRNAs³⁻⁸. In particular, long
64 ncRNAs (lncRNAs) outnumber protein-coding genes in humans and are susceptible to
65 the same oncogenic pathogenetic events^{9,10}. These RNA molecules, defined as
66 transcripts greater than 200nt with no protein-coding potential, have a diverse array of
67 functional roles, ranging from being precursor molecules for the biogenesis of mature
68 microRNAs (miRNAs) to direct interactions with proteins and nucleic acids to regulate
69 protein function and/or stability¹¹⁻¹³. With the plethora of biological functions that
70 lncRNAs modulate to control cellular processes at multiple levels, it is not surprising that
71 their aberrant expression and function have been implicated in the progressive gain of a
72 malignant phenotype by tumor cells¹⁴. Indeed, the expression of 14 lncRNAs in newly
73 diagnosed MM patients is correlated (or anti-correlated) with progression-free survival
74 independent of cytogenetic, international staging system (ISS), or minimal residual
75 disease (MRD) status¹⁵. Other lncRNAs, including SMILO, also independently predict
76 MM progression and response to therapy^{16,17}.

77 To find lncRNAs that have a direct impact on MM proliferation and survival, thus
78 providing cell growth dependency, we conducted a lncRNA-targeted large-scale
79 CRISPR *interference* (CRISPRi) viability screen. CRISPRi makes use of a catalitically
80 inactive Cas9 (dCas9)-KRAB fusion protein to repress the expression of endogenous

81 lncRNA genes^{18,19}. From this screen, we identified MIR17HG as essential in MM and *i*)
82 characterized its novel function as lncRNA mediating protein-protein and protein-DNA
83 interactions, *ii*) developed potent inhibitors for translation to clinical trials.

84 **METHODS**

85 **Cells**

86 Human cell lines and primary cells were grown at 37°C, 5% CO₂. Detailed information is
87 included in Supplementary Methods section.

88

89 **RNA-seq, microarray-based gene expression analysis and miRNA profiling of MM**
90 **patients and cell lines.**

91 These analyses were performed in purified CD138+ cells. Detailed information can be
92 found in Supplementary Methods section.

93

94 **CRISPRi viability screen and validation**

95 Cell lines expressing the dCas9-KRAB fusion protein were generated as previously
96 described¹⁹. Detailed information on library design, gRNA pool library production,
97 titering of virus, primary and secondary screenings, validation study, as well as on data
98 analysis can be found in Supplementary Methods section.

99

100 **Antisense oligonucleotides (ASO), synthetic miRNA mimics and inhibitors,**
101 **siRNAs**

102 Long Non-Coding LNA gapmerRs were custom-designed and purchased from Exiqon
103 (Vedbaek, Denmark). Sequences can be found in Supplementary Methods section.

104 Synthetic miRNA mimics and inhibitors, as well as silencer select siRNAs, were
105 purchased from Ambion (Applied Biosystems, CA, US). SiRNA pool targeting WDR82
106 was purchased from Horizon Discovery (Waterbeach, U.K.). Design of clinically
107 applicable ASOs is described in **Supplementary Table 8**.

108

109 **Gymnosis**

110 Gymnotic experiments were performed as previously described²⁰.

111

112 **Transient and stable transfection of cells**

113 Cell transfection and transduction was performed as previously described⁵. Detailed
114 information can be found in Supplementary Methods section.

115

116 **Detection of cell proliferation and apoptosis.**

117 Cell viability was evaluated by Cell Counting Kit-8 (CCK-8) assay (Dojindo Molecular
118 Technologies), according to the manufacturer's instructions. Apoptosis was investigated
119 by an Annexin V/7-AAD flow cytometry assay using FACS CANTO II (BD Biosciences).

120

121 **Reverse transcription (RT) and quantitative real-time amplification (qRT-PCR)**

122 RNA extraction, reverse transcription (RT) and quantitative real-time amplification (qRT-
123 PCR) were performed as previously described⁵. Detailed information can be found in
124 Supplementary Methods section.

125

126 **Western blot analysis**

127 Protein extraction and western blot analysis were performed as previously described.
128 Detailed information can be found in Supplementary Methods section.

129

130 **RNA FISH and Co-immunofluorescence with RNA FISH (Co-IF/FISH)**

131 These experiments were conducted according to established protocols^{21,22}. Detailed
132 information can be found in Supplementary Methods section.

133

134 **Luciferase reporter assay**

135 Promoter reporter clones for human ACACA (NM_198834), ANO6 (NM_001025356),
136 CCDC91 (NM_018318), EPT1 (NM_033505), EXT1 (NM_000127), FER
137 (NM_001308028) and ZYG11A (NM_001004339) were cloned into the GLuc-ON™
138 Promoter Reporter Vector (GeneCopoeia, Rockville, MD). A luciferase reporter assay
139 was performed according to the manufacturer's instructions.

140

141 **ChIRP**

142 Lnc-17-92 and LacZ antisense DNA probes were designed using the online probe
143 designer at singlomoleculefish.com. Oligonucleotides were biotinylated at the 3' end
144 with an 18-carbon spacer arm. AMO1 cells were collected and subjected to ChIRP
145 using the EZ- Magna ChIRP RNA Interactome Kit (Millipore Sigma, Bedford, MA),
146 according to the manufacturer's instructions and established protocols²³.

147

148 ***De novo* lipogenesis assay**

149 These experiments were conducted as previously described²⁴. Detailed information can
150 be found in Supplementary Methods section.

151

152 **ChIP-qPCR**

153 ChIP-qPCR was performed as previously described²⁵. Detailed information can be
154 found in Supplementary Methods section.

155

156 **RNA-Protein Pull-Down**

157 Lnc-17-92 transcripts and truncated versions were cloned into a pBlueScript vector and
158 sequence verified. In vitro transcription and biotinylation was performed using
159 AmpliScribe™ T7-Flash™ Biotin-RNA Transcription Kit (Lucigen, cat. no. #ASB71110),
160 according to the manufacturer's instructions. Cell nuclear lysates (from 1x10⁷ AMO1
161 cells) were incubated with biotinylated RNA and streptavidin beads for RNA pull-down
162 incubation, using Pierce™ Magnetic RNA-Protein Pull-Down Kit (Thermo Fisher
163 Scientific, cat. no. #20164), according to the manufacturer's instructions. RNA-
164 associated proteins were eluted and analyzed by western blotting.

165

166 **RNA Yeast 3 Hybrid**

167 These experiments were conducted according to established protocols^{21,22}. Detailed
168 information can be found in Supplementary Methods section.

169

170 **RIP-qPCR**

171 RNA immunoprecipitation (RIP) experiments were performed using the Magna RIP
172 RNA-binding Protein Immunoprecipitation Kit (Millipore Sigma, cat. no. 17-701),
173 according to the manufacturer's instructions. The anti-MYC antibody [Y69] used for RIP
174 was purchased from Abcam (ab32072). Normal Rabbit IgG was purchased from Cell

175 Signaling Technology (cat. no. #2729). The primers used for detecting Inc-17-92 are
176 listed in Supplementary Methods section.

177

178 **Co-immunoprecipitation (Co-IP)**

179 Protein lysates were obtained from 1×10^7 cells (AMO1, H929 and U266^{MYC+}, with
180 corresponding treatments). Coimmunoprecipitation was performed using the Pierce™
181 Co-Immunoprecipitation Kit (Thermo Fisher Scientific, cat. no. 26149), according to the
182 manufacturer's instructions. IP antibodies are listed in Supplementary Methods section.

183

184 **Proximity-dependent biotin identification (BioID)**

185 BioID was performed as described by Kalkat *et al.*²⁶. Detailed information can be found
186 in Supplementary Methods section.

187

188 **Mass Spectrometry**

189 Mass Spectrometry analysis of Co-IP and BioID samples was performed at the Taplin
190 Mass Spectrometry Facility (Harvard Medical School, Boston, MA).

191

192 **Animal study**

193 6-week-old female immunodeficient NOD.CB17-Prkdcscid/NCrCrl (NOD/SCID) mice
194 (Charles River) or NSG mice (Jackson Laboratory) were housed in our animal facility at
195 Dana-Farber Cancer Institute (DFCI). All experiments were performed after approval by
196 the Animal Ethics Committee of the DFCI and performed using institutional guidelines.
197 Detailed information can be found in Supplementary Methods section.

198

199 **Statistical Analysis**

200 All *in vitro* experiments were repeated at least three times and performed in triplicate. A
201 representative experiment was shown in the figures. Statistical significances of
202 differences were determined using Student's t test (unless otherwise specified), with the
203 minimal level of significance specified as p<0.05. Kaplan-Meier survival curves were
204 compared by log-rank test. Statistical analyses were determined using GraphPad
205 software (<http://www.graphpad.com>). Graphs were obtained using GraphPad software
206 (unless otherwise specified).

207

208 **Data availability**

209 The authors declare that all data supporting the findings of this study are available
210 within the article and its Supplementary Information. Files or reagents are available from
211 the corresponding authors on request. Data is stored at accession number GSE208599.

212 **RESULTS**

213 **CRISPRi viability screens identify MIR17HG as a leading cell growth dependency
214 in MM.**

215 We analyzed RNA-seq data from 360 newly diagnosed MM patients and identified 913
216 lncRNA transcripts expressed in primary MM cells (**Fig. 1A, I.**) and in a panel of 70 MM
217 cell lines (data not shown). To systematically interrogate the role of these lncRNAs in
218 MM cell growth, we transduced 3 MM cell lines (H929, KMS-11 and KMS-12-BM)
219 engineered to express a dCAS9-KRAB fusion protein with a pooled library consisting of
220 7 sgRNAs against each of the 913 transcription start sites (TSS) of the identified
221 lncRNAs and 576 negative control sgRNAs (**Fig. 1A, II.** and **Supplementary Table 1**).
222 After 3 weeks, we tested for sgRNAs that were relatively depleted or enriched in the MM
223 cell population using deep sequencing and the Model-based Analysis of Genome-wide
224 CRISPR-Cas9 Knockout (MAGECK) robust rank aggregation (RRA) algorithm²⁷.

225 The most enriched or depleted sgRNAs were further tested in secondary screens
226 using a pooled library targeting the TSS of 224 lncRNAs, the TSS of known protein-
227 coding oncogenes (MYC, IRF4)^{28,29} or tumor suppressors (TP53)³⁰ as positive controls,
228 and 2245 non-targeting sgRNAs as negative controls (**Fig. 1A; III.** and **Supplementary**
229 **Table 2**). In the secondary screens, 4 MM cell lines (H929, KMS11, KMS12BM and
230 AMO1) were used to detect and rank significantly depleted or enriched sgRNAs. As
231 expected, sgRNAs targeting IRF4 and MYC were significantly depleted in three (MYC)
232 or all (IRF4) cell lines, while sgRNAs targeting TP53 were significantly enriched in both
233 TP53 wild-type cell lines (AMO1 and H929)³¹.

234 Focusing on depleted sgRNAs, we identified lncRNA dependencies in MM cells
235 that were either cell-line specific (54%) or shared (46%) (**Supplementary Fig. 1A** and
236 **Supplementary Table 3**). A ranked analysis of sgRNA depletion identified MIR17HG as
237 the leading dependency, with RRA scores equal or superior to those obtained by
238 targeting MYC or IRF4 in all cell lines tested (**Fig. 1B**). To validate this data further, we
239 next transduced MM cell lines expressing dCAS9-KRAB fusion protein with the top four
240 sgRNAs targeting MIR17HG under the regulation of a tetracycline-inducible promoter
241 and observed reduced cell growth compared to cells infected with non-targeting
242 sgRNAs after continued exposure to doxycycline (**Fig. 1C** and **Supplementary Fig.**
243 **1B**). Moreover, we used 2 different locked nucleic acid (LNA) gapmeR ASOs (simply
244 referred to as ASO), which target the MIR17HG nascent RNA (pre-RNA) for RNase H–
245 mediated degradation^{32,33}, to transfet 11 MM cell lines including those resistant to
246 conventional anti-MM agents (AMO1-ABZB resistant to bortezomib; AMO1-ACFZ
247 resistant to carfilzomib; MM.1R resistant to dexamethasone) and confirmed a significant
248 impact on MM cell viability independent of the genetic and molecular background (**Fig.**
249 **1D** and **Supplementary Fig. 1C**).

250

251 **MIR17HG-derived lnc-17-92 mediates cell growth dependency in a miRNA- and**
252 **DROSHA-independent manner.**

253 MIR17HG is the locus of the miRNA cluster miR-17-92 and a lncRNA^{34,35}, named here
254 lnc-17-92. Lnc-17-92 has two isoforms, one that is ~5,000 nt long (lnc-17-92^{TV1}) and
255 one that is ~900 nt (lnc-17-92^{TV2})^{34,35}, and both have yet to be functionally explored
256 (**Fig. 2A**). In MM cells, both RNA-seq (**Supplementary Fig. 2A**) and qRT-PCR

257 (Supplementary Fig. 2B) indicated preferential expression of Inc-17-92^{TV1}, which is the
258 isoform further investigated in this study and hereafter referred to as Inc-17-92. Using
259 RNA-seq, we confirmed its expression in CD138+ cells from an additional large cohort
260 of MM patients (MMRF/CoMMpass, n=720) and in MM cell lines (n=60)
261 (Supplementary Fig. 2C-D). In MM cell lines, we also demonstrated nuclear
262 enrichment of Inc-17-92 using single molecule RNA-FISH (Fig. 2B) and subcellular
263 qRT-PCR (Supplementary Fig. 2E). We observed that Inc-17-92 expression was
264 higher during disease progression in two independent datasets from MM patients
265 analyzed at diagnosis and/or relapse (Supplementary Fig. 2F-G) and that higher
266 expression of Inc-17-92 was associated with shorter event-free survival (EFS) and
267 overall (OS) survival in 3 large cohorts of newly diagnosed MM patients (Fig. 2C).
268 Expression of Inc-17-92 did not significantly correlate with the expression of miR-17-92
269 miRNAs in CD138+ MM cells from 140 patients (average Spearman $r = 0.16$),
270 suggesting that Inc-17-92 and miR-17-92 are under independent regulatory control and
271 function in distinct molecular pathways mediating cell growth dependency to MIR17HG
272 (Supplementary Fig. 2H).

273 To test independent activity of Inc-17-92, we first established two MM cell lines
274 over-expressing miR-17-92 via ectopic expression of the primary precursor pri-mir-17-
275 92 (Supplementary Fig. 3A). We specifically depleted Inc-17-92 in these cell lines with
276 ASOs targeting the 5'-end of MIR17HG pre-RNA, a region not covered by pri-mir-17-92,
277 and observed a significant inhibition of cell growth that was not rescued by ectopic pri-
278 mir-17-92 (Fig. 2D). Next, we established two DROSHA knockout (DR-KO) MM cell
279 lines (AMO1^{DR-KO} and H929^{DR-KO}), which are unable to enzymatically digest pri-mir-17-

92 and produce miR-17-92s (**Supplementary Fig. 3B**)³⁶, and still observed strong anti-
proliferative activity in both DR-WT and DR-KO cell systems after Inc-17-92 depletion
using gymnotic treatment with ASO1 (**Fig. 2E**) or after transfection with 3 different ASOs
(-1/-2/-3) (**Supplementary Fig. 3C**). Importantly, exposure to gymnotic ASO1 (**Fig. 2E-F**) or transfection with ASO2 (**Supplementary Fig. 3D**) abrogated the ability of AMO1^{DR-KO} cells to establish tumors in NOD SCID mice, resulting in prolonged animal survival. Next, using the easy-to-transfect colorectal cancer cell line HCT-116, which is driven by MIR17HG³⁴, we found that ectopic expression of Inc-17-92^{TV1} significantly rescued the anti-proliferative activity of ASOs targeting MIR17HG pre-RNA more effectively than ectopic Inc-17-92^{TV2} or pri-mir-17-92 (**Supplementary Fig. 3E-F**). Finally, we confirmed the independent activity of Inc-17-92 in the HCT-116 and DLD-1 colorectal cancer cell lines carrying a mutant Dicer, which confers a hypo-morphic phenotype preventing the cells from enzymatically processing mature miRNAs³⁷ (**Supplementary Fig. 3G**).

These results indicate that Inc-17-92^{TV1} is the main mediator of MIR17HG cancer dependency, separate from the actions and biogenesis pathway of miR-17-92.

Lnc-17-92 forms a transcriptional axis with ACACA to promote MM cell growth.
The nuclear enrichment of Inc-17-92 suggests a possible role in the regulation of gene expression. We therefore depleted Inc-17-92 in DR-WT (AMO1 and H929) and DR-KO (AMO1^{DR-KO}) MM cell lines using early exposure to gymnotic ASO1 to avoid modulation of miR-17-92 (**Supplementary Fig. 4A**) and miR-17-92's canonical targets in DROSHA WT cells (**Supplementary Fig. 4B-C**) and identified 7 genes rapidly downregulated

303 after depletion of Inc-17-92 in all the cell lines tested (**Fig. 3A**). We validated these
304 findings in CD138+ cells from 3 MM patients treated *ex-vivo* with ASO1 (**Fig. 3B**) and in
305 the lymphoma cell lines Raji and Daudi (**Supplementary Fig. 4D**). Conversely, the
306 expression of these genes was not affected by modulating individual members of miR-
307 17-92 using synthetic mimics or inhibitors (**Supplementary Fig. 4E-F**). Moreover, we
308 observed significant positive correlation (Spearman $r > 0.3$; $p < 0.001$) between Inc-17-92
309 and its target genes in at least 1 out of 2 large RNA-seq MM patient datasets (IFM/DFCI
310 and MMRF/CoMMpass) (**Fig. 3C**).

311 Using a luciferase reporter assay, performed in 293T^{DR-KO} cells in the presence
312 or absence of Inc-17-92 depletion, we demonstrated that the regulatory control of Inc-
313 17-92 over these genes, except ANO6, occurs at the promoter level (**Fig. 3D**).
314 Consistently, we confirmed Inc-17-92 interaction at the promoter region of the top target,
315 ACACA, by a chromatin isolation by RNA precipitation (ChIRP) assay followed by qRT-
316 PCR analysis (**Fig. 3E** and **Supplementary Fig. 4G-H**) and showed frequent
317 localization of Inc-17-92 to the ACACA locus by single-molecule dual RNA FISH
318 analysis of Inc-17-92 and ACACA pre-mRNA (<300nm to nearest Inc-17-92 spot in
319 ~50% of ACACA pre-RNA spots analyzed ($n=60$)) (**Fig. 3F**). Proximal localization of Inc-
320 17-92 to the ACACA gene locus was significantly more frequent compared to random
321 spots (**Fig. 3F**).

322 Among the identified Inc-17-92 targets, ACACA had the largest impact on the
323 proliferation and survival of MM cells (**Fig. 3G**). ACACA encodes the rate-limiting
324 enzyme for the *de novo* lipogenesis pathway ACC1, which supports tumorigenesis in
325 different cancer contexts³⁸. To confirm that Inc-17-92's control over ACACA expression

326 has a functional effect, we depleted Inc-17-92 and found that the incorporation of C¹⁴-
327 radiolabeled glucose into the lipid pool was significantly reduced, indicating a reduced
328 amount of *de novo* lipogenesis²⁴, both in MM cell lines and CD138+ MM patient cells
329 (**Supplementary Fig. 4I**). This was not observed after transfection of MM cells with
330 synthetic inhibitors of miR-17-92s (**Supplementary Fig. 4J**). Moreover, supplementing
331 palmitate, which is the main downstream product of ACC1 activity, significantly rescued
332 the anti-proliferative and pro-apoptotic effects of Inc-17-92 depletion in MM cells
333 (**Supplementary Fig. 4K-L**).

334 Altogether, these data indicate Inc-17-92 is a chromatin-interacting lncRNA with
335 transcriptional regulatory functions. We next sought to determine how it can promote
336 transcription by searching for its protein-binding partners.

337

338 **Lnc-17-92 directly interacts with c-MYC and promotes its occupancy at the**
339 **ACACA promoter.**

340 The targeting of MIR17HG primarily kills c-MYC positive (MYC+) tumor cells, including
341 in MM^{5,34,39,40}. Intriguingly, MYC is known to reactivate ACACA expression and *de novo*
342 lipogenesis in tumor cells⁴¹, with MYC+ tumor cells becoming addicted to this metabolic
343 pathway, findings that we validated in MM cells (**Supplementary Fig. 5A-D**). Therefore,
344 we hypothesized that Inc-17-92 mediates the functional interplay between MYC and
345 MIR17HG by directly interacting with MYC protein to promote gene expression.

346 We performed an RNA Protein pull-down (RPPD) experiment and found that
347 MYC forms a complex with Inc-17-92^{TV1} (**Fig. 4A**). RNA immunoprecipitation (RIP)
348 assay with MYC antibody confirmed the enrichment of Inc-17-92^{TV1} (**Fig. 4B**).

349 Moreover, an RNA yeast-3-hybrid (Y3H) assay confirmed the Inc-17-92^{TV1}-MYC
350 interaction in an *in vivo* cellular model⁴², as shown by yeast colony growth (**Fig. 4C**). An
351 analysis using truncated versions of Inc-17-92^{TV1} further indicated the 3'-end regions,
352 which do not include miR-17-92, as particularly relevant for the interaction with MYC in
353 MM cells (**Supplementary Fig. 5E**).

354 Next, we evaluated if MYC and Inc-17-92 cooperate to promote ACACA
355 expression in MM cells. Depletion of Inc-17-92 in MM cells indeed abrogated MYC
356 occupancy at the ACACA promoter while not affecting MYC expression (**Fig. 4D**) and
357 reduced the expression of ACACA in the conditional MYC Tet-Off cell line P493-6⁴³ only
358 in presence of high MYC levels (**Fig. 4E**). Moreover, by coupling RNA FISH analysis of
359 Inc-17-92^{TV1} and ACACA pre-RNA with immunofluorescence analysis of MYC protein
360 (FISH/IF), we captured the co-localization of Inc-17-92^{TV1} and MYC at the ACACA gene
361 locus (**Supplementary Fig. 5F**).

362 These data demonstrate that Inc-17-92^{TV1} forms an RNA-protein complex with
363 the transcription factor MYC to promote its chromatin occupancy and transcriptional
364 activity at the ACACA promoter.

365

366 **Lnc-17-92 mediates the assembly of a MYC-WDR82 transcriptional complex,
367 leading to transcriptional and epigenetic activation of ACACA.**

368 MYC transcriptional activity is modulated through the interaction with transcriptional and
369 epigenetic co-regulators⁴¹. To determine if Inc-17-92 affects these protein-protein
370 interactions, we integrated the results of a proximity-dependent biotin identification
371 (BiOID) analysis (**Supplementary Fig. 6A**) with a co-immunoprecipitation assay

372 followed by mass-spectrometry analysis (Co-IP/MS) in three MM cell lines (AMO1,
373 H929 and U266^{MYC+}), in the presence and absence of depletion of Inc-17-92. This
374 analysis highlighted WDR82 as a very high-confidence Inc-17-92-dependent MYC
375 interactor (**Fig. 5A** and **Supplementary Tables 4-7**). A direct RNA-protein interaction
376 between Inc-17-92^{TV1} and WDR82 was further confirmed by both RPPD (**Fig. 5B**) and
377 RNA Y3H (**Fig. 5C**) assays. Analysis using the truncated versions of Inc-17-92^{TV1}
378 indicated that this interaction may involve different domains across Inc-17-92
379 (**Supplementary Fig. 6B**).

380 WDR82 is a regulatory component of the SET1 methyltransferase complex,
381 which catalyzes histone H3 Lys-4 (H3K4) methylation (mono-, di-, tri-) at the
382 transcriptional start sites of active loci^{44,45}, a prerequisite for MYC binding to chromatin
383 and transactivation⁴⁶. We confirmed a global effect of silencing of WDR82 on H3K4
384 methylation in MM cells (**Supplementary Fig. 6C**). Consistently, depletion of WDR82
385 reduced the occupancy of H3K4me3 (**Fig. 5D** and **Supplementary Fig. 6D**) and MYC
386 (**Fig. 5E** and **Supplementary Fig. 6E**) at the ACACA promoter, and decreased ACACA
387 mRNA expression (**Fig. 5F** and **Supplementary Fig. 6F**) in MM cells. Furthermore,
388 using MM cells expressing an ectopic WDR82-GFP fusion protein (**Supplementary Fig.**
389 **6G**), we demonstrated that Inc-17-92 expression is essential for WDR82 occupancy at
390 the ACACA promoter (**Fig. 5G**). Additionally, Inc-17-92 depletion resulted in reduced
391 levels of H3K4me3 at the ACACA promoter (**Fig. 5H**), without globally impacting the
392 H3K4 methylation status (**Fig. 5I**).

393 These findings suggest Inc-17-92^{TV1} is a chromatin scaffold mediating the
394 assembly of the MYC-WDR82 multiprotein transcriptional complex to control the
395 expression of ACACA and likely other genes.

396

397 **Therapeutic inhibitors of MIR17HG exert potent anti-tumor activity *in vitro* and *in***
398 **vivo in animal models of human MM.**

399 We next explored MIR17HG as a therapeutic target, which includes both the lncRNA
400 and miRNA factors. To develop clinically applicable inhibitors, we screened >80 fully
401 phosphorothioated (PS), 2'-O-methoxyethyl (2'-MOE)-modified, lipid-conjugated ASOs
402 that could either trigger RNase H-mediated degradation of MIR17HG pre-RNA
403 (gapmeRs) or exert function via an RNase H-independent mechanism (blockmeRs)⁴⁷
404 (**Supplementary Fig. 7A-B**). This procedure identified an 18-mer tocopherol (T)-
405 conjugated gapmeR G2-15b-T ("G") and an 18-mer tocopherol (T)-conjugated steric
406 blocker SB9-19-T ("B") as both having strong anti-proliferative effects (cell growth
407 inhibition, CGI > 50%) in a large panel of MM cell lines as well as CD138+ primary MM
408 cells, while sparing (CGI < 50%) non-malignant cell lines (THLE-2, HK-2, HS-5 and
409 293T) and PBMCs from 3 healthy donors (**Supplementary Fig. 7C**).

410 To assess the *in vivo* anti-tumor activity of both compounds, we first used a
411 AMO1-based plasmacytoma xenograft model in immunocompromised NOD SCID mice.
412 Here, we observed a significant reduction of tumor growth after a treatment cycle with
413 either G2-15b-T (tumor growth inhibition, TGI=76%) or B9-19-T (TGI=69%) (**Fig. 6A**).
414 Analysis of tumors retrieved from mice following this treatment confirmed reduced
415 expression of Inc-17-92 (**Fig. 6B**) and miR-17-92s (**Supplementary Fig. 7D**), as well as

416 modulation of Inc-17-92's targets (ACACA, *EPT1*, *EXT1*, *CCDC91*, *ANO6*, *FER* and
417 *ZYG11A*) (**Fig. 6C**) and miR-17-92's target BIM (aka *BCL2L11*) (**Supplementary Fig.**
418 **7E**). We also observed reduced levels of tripalmitin (**Supplementary Fig. 7F**), a
419 surrogate for the *de novo* lipogenesis product palmitate⁴⁸. This demonstrates efficient
420 uptake of G2-15b-T and B9-19-T by tumor cells *in vivo*. We observed no overt toxicity in
421 the mice after treatment, as shown by blood cell count, clinical biochemistry
422 (**Supplementary Tables 9-10**), and body weight analysis (not shown).

423 We next confirmed the significant anti-MM activity of G2-15b-T and B9-19-T in an
424 aggressive model of diffused myeloma, in which tumor growth of MOLP8-luc+ MM cells
425 is assessed by bioluminescence imaging (BLI). In this model, tumor growth was
426 significantly antagonized after a treatment cycle with either G2-15b-T (TGI=84%) or B9-
427 19-T (TGI=52%). Treatment with G2-15b-T resulted in tumor clearance in 2 out of 8
428 mice (25%) (**Fig. 6D**). Importantly, both inhibitors significantly prolonged animal survival
429 (**Fig. 6E**).

430 Finally, we established a clinically relevant PDX-NSG mouse model by tail-vein
431 injection of CD138+ MM cells obtained from an advanced-stage patient (PDX-NSG). In
432 this model, tumor growth was monitored in serum samples using human kappa light
433 chain as a surrogate. Remarkably, we observed a regression of tumor growth after a
434 treatment cycle with G2-15b-T, whose effects were comparable to bortezomib (a
435 positive control) (**Fig. 6F**).

436 **DISCUSSION**

437 MIR17HG is often amplified and/or overexpressed in human cancer and has a driver
438 role^{34,39,49}. One of its transcriptional products, pri-mir-17-92, is enzymatically digested by
439 DROSHA into six precursor transcripts (pre-mir-17 / -18a / -19a / -20a / -19b1 / -92a)
440 that are further processed by DICER to generate the miR-17-92 mature miRNAs (miR-
441 17 / -18a / -19a / -20a / -19b1 / -92a). These miRNAs post-transcriptionally repress
442 relevant tumor-suppressive mRNAs^{34,39,40,49}, such as the pro-apoptotic factor BIM⁵⁰.
443 Their impact on tumorigenesis is particularly relevant when co-expressed with MYC^{34,39},
444 as there's a well-documented interplay between miR-17-92, especially of miR-19b^{51,52},
445 and MYC transcriptional targets in maintaining cancer cell homeostasis^{5,39,40}. We have
446 previously demonstrated that these miRNAs play a relevant role in MM by forming
447 homeostatic feed-forward loops with MYC and BIM⁵. Interestingly, our previous work
448 also showed that depletion of mature miRNAs does not phenocopy the inhibition of
449 MIR17HG pre-RNA, suggesting other tumor-promoting functions for this transcript⁵. An
450 alternative mechanism to explain the oncogenic role of MIR17HG has been recently
451 identified and involves the overload of DROSHA by an overexpressed pri-mir-17-92 in B
452 cell lymphomas⁵³. Our description in this study of the miRNA-, DROSHA- and DICER-
453 independent function of MIR17HG, via Lnc-17-92, establishes this gene as having both
454 *short* (miR-17-92) and *long* (Lnc-17-92) noncoding RNA activities, with the latter
455 mediating tumor-promoting activity in MM and likely other cancer contexts (e.g.,
456 colorectal cancer).

457 We described Lnc-17-92 as a specific regulator of gene expression via chromatin
458 occupancy and interaction with MYC and WDR82. Lnc-17-92 directly reduces the

459 expression of a small subset of genes and prevents the accumulation of H3K4me3 at
460 the ACACA promoter. These effects are in contrast to what is observed by depleting c-
461 MYC (i.e., global effect on gene expression) or WDR82 (i.e., global effect on
462 methylation of H3K4) in cancer cells, as reported in this and other studies^{44,54}. Our data
463 support the emerging paradigm whereby chromatin occupancy by transcription factors
464 like MYC may be determined through interacting with specific lncRNAs⁵⁵, in addition to
465 protein partners²⁶. In a broader perspective, our observations on lnc-17-92 suggest
466 lncRNAs are key mediators of the epigenetic and transcriptional reprogramming of MM
467 cells. In this molecular scenario, while proteins act as catalytic effectors, the intrinsic
468 structural flexibility of lncRNAs makes them good modular scaffolds able to mediate
469 both protein-protein and protein-DNA interactions at specific chromatin regions.

470 We further showed that the lnc-17-92-MYC-WDR82 complex impacts tumor cell
471 metabolism by activating the *de novo* lipogenesis pathway via regulation of ACACA.
472 This anabolic pathway is primarily restricted to liver and adipose tissue in normal adults
473 but is reactivated in cancer cells via mechanisms yet to be fully described^{38,56}. Notably,
474 MYC has been implicated in the reprogramming of tumor cell metabolism by activating
475 that pathway via ACACA and other genes⁵⁷. In turn, lipogenesis has emerged as an
476 essential pathway for the onset and progression of MYC-driven cancers, which are
477 susceptible to pharmacologic inhibition of ACC1⁴¹. This seems particularly relevant in
478 MM, where tumor cells need to adapt their metabolic pathways to meet the high
479 bioenergetic and biosynthetic demand posed by the malignant cell growth coupled with
480 unceasing production of monoclonal immunoglobulin^{58,59}. Nevertheless, we
481 acknowledge that the oncogenic roles of lnc-17-92 are likely not limited to the

482 transcriptional axis with ACACA and will require further investigation to be
483 comprehensively elucidated.

484 Deletion of MIR17HG is tolerated in adult mice⁶⁰, and its haploinsufficiency is
485 compatible with life in humans⁶¹. The physiological role of MIR17HG seems particularly
486 relevant only for the hematopoietic stem cell compartment⁶⁰, which continuously
487 renews. These observations support the development of MIR17HG as a target. Thus,
488 for translational purposes, we developed two therapeutic ASOs that target the
489 MIR17HG pre-RNA via different mechanisms of action (i.e., RNase H-dependent or -
490 independent). With the recent advances in RNA medicine⁶²⁻⁶⁴, the use of ASOs to
491 therapeutically antagonize disease-driver genes is becoming increasing possible^{47,65},
492 including in MM therapy^{5,66}. Our optimization of design and chemistry has helped to
493 overcome the major obstacles to the clinical use of ASOs, such as poor bioavailability⁶⁵,
494 while limiting off-target toxicity. The inhibitors described here bear state-of-the-art
495 chemical modifications (2'MOE, PS-backbone, lipid conjugation) and have sufficient
496 nucleotide length (18mer) to ensure high specificity for MIR17HG. Inhibitors of this kind
497 have already been tested within clinical trials and a few are already FDA approved for
498 use in different human diseases⁶⁵. Our optimized ASOs targeting MIR17HG with
499 demonstrated activity in 3 different murine models of human MM provide the rationale to
500 now consider clinical application in MM.

501 Different questions remain open about the dual nature of MIR17HG and its
502 therapeutic targeting. Important future directions will be to uncover how the splicing of
503 MIR17HG is alternatively regulated to produce Inc-17-92 or miR-17-92 and to address
504 the relative contribution of Inc-17-92 and miR-17-92 to the oncogenic activity of

505 MIR17HG in other cancer models. Whether the host genes of the two paralogs of miR-
506 17-92, miR-106a-363 and miR-25-106b, also retain miRNA-independent function will be
507 an important line of investigation.

508 Overall, this study establishes MIR17HG with a unique lncRNA function of
509 facilitating protein-protein and protein-DNA interactions, mediating tumor-promoting
510 activity with therapeutic implications.

511 **ACKNOWLEDGEMENTS**

512 This work is supported by NIH/NCI grants SPORE-P50CA100707, R01-CA050947,
513 R01CA207237, P01CA155258, R01-CA178264 (N.C.M., K.C.A.); by VA Healthcare
514 System grant No. 5I01BX001584 (N.C.M.); by the Paula and Roger Riney Foundation
515 grant (N.C.M., K.C.A.) by NIH/NCI grants RO1CA131945, R01CA187918, P50
516 CA211024 and Department of Defense grants DoD PC160357 and DoD PC180582
517 (M.L.); by the Sheldon and Miriam Medical Research Foundation (K.C.A.); by the Italian
518 Association for Cancer Research (AIRC) with “Special Program for Molecular Clinical
519 Oncology–5 per mille”, 2010/15 and its Extension Program” No. 9980, 2016/18 (P.T.).
520 E.M. is supported by a Brian D. Novis Junior Grant from the International Myeloma
521 Foundation, by a Career Enhancement Award from Dana Farber/Harvard Cancer
522 Center SPORE in Multiple Myeloma (SPORE-P50CA100707), by a Special Fellow grant
523 from The Leukemia & Lymphoma Society, and by a Scholar Award from the American
524 Society of Hematology. A.G. is supported by a Fellow grant from The Leukemia &
525 Lymphoma Society and by a Scholar Award from the American Society of Hematology.
526 JEH is supported by a NIH F32 fellowship (NCI 1F32CA254216-01). A.N. is supported
527 by a grant from the Italian Association for Cancer Research (AIRC, IG24365). N.A. is
528 supported by a grant from the Italian Association for Cancer Research (AIRC, IG24449).
529 K.C.A. is an American Cancer Society Clinical Research Professor,
530
531 We gratefully acknowledge the members of our laboratories for technical advice and
532 critical discussions. We thank Dr. Christina Usher (Dana-Farber Cancer Institute) for
533 editing the manuscript and insightful comments. We thank Dr. Dirk Eick (Helmholtz-

534 Zentrum München, Molecular Epigenetics), Dr. Christoph Driessen (Cantonal Hospital
535 St Gallen, St Gallen, Switzerland) and Dr. Linda Penn (Princess Margaret Cancer
536 Centre, University Health Network, Toronto, Ontario, Canada) for providing us relevant
537 cellular models. We thank Dr. Piersandro Tagliaferri (Magna Graecia University,
538 Catanzaro, Italy) for insightful discussions on the dual long and short nature of
539 MIR17HG. We thank Dr. Benjamin Izar and Dr. Johannes Melms (Columbia University,
540 New York, NY, USA.) for insightful discussions on CRISPR viability screen
541 methodology. We thank the “8th Annual Miracles for Myeloma 5K Virtual Run/Walk”
542 organizers and attendees for the fundraising in support to this research project.

543

544 **AUTHOR CONTRIBUTIONS**

545 E.M. and N.C.M. conceived and designed the research studies. E.M., M.F. and N.C.M.
546 wrote the manuscript. M.K.S., A.A.S. and K.T. performed in silico analysis of
547 transcriptomic data. C.F.R. performed lipidomic studies. L.W.L. performed yeast-3-
548 hybrid experiments. J.E.H. performed RNA FISH, dual RNA FISH and Co-IF/dual RNA
549 FISH. S.T. generated MM cells expressing CAS9. W.D.P. analyzed ChIP-seq data. S.G.
550 designed t-ASOs. C.F., N.R., D.M. and F.S. provided support for the identification of Inc-
551 17-92 isoforms. M.F., A.G., N.A., M.J., G.B., C.L., Y.T.T., A.N., D.C., T.H., M.A.S., P.T.,
552 R.A.Y., K.C.A., C.D.N. and M.L. contributed to the design and interpretation of key
553 experiments. M.L. supervised lipidomic studies. C.D.N. supervised Y3H experiments.

554

555 **COMPETING INTERESTS STATEMENT**

556 N.C.M. serves on advisory boards/consultant to Takeda, BMS, Celgene, Janssen,
557 Amgen, AbbVie, Oncopep, Karyopharm, Adaptive Biotechnology, and Novartis and
558 holds equity ownership in Oncopep. K.C.A. serves on advisory boards to Janssen,
559 Pfizer, Astrazeneca, Amgen, Precision Biosciences, Mana, Starton, and Raquia, and is a
560 Scientific Founder of OncoPep and C4 Therapeutics. R.A.Y. is a founder and
561 shareholder of Syros Pharmaceuticals, Camp4 Therapeutics, Omega Therapeutics, and
562 Dewpoint Therapeutics. E.M., S.G. and N.C.M filed a provisional patent on MIR17HG as
563 a target for cancer therapy. D.C. reports other support from Stemline Therapeutics,
564 Oncopeptides, and C4 Therapeutics outside the submitted work. No potential conflicts
565 of interest were disclosed by the other authors.

566 **FIGURE LEGENDS**

567 **Figure 1. CRISPRi viability screens identify MIR17HG as a leading cell growth**
568 **dependency in MM.** **A)** Schematic of CRISPRi viability screens. **B)** RRA-based ranked
569 analysis of lncRNA dependencies in the secondary screen, considering 4 MM cell lines
570 either together or individually. The top lncRNA dependency, MIR17HG, is highlighted,
571 along with the protein-coding genes IRF4 and MYC used as positive controls. **C)** CCK-8
572 proliferation assay of MM cell lines (AMO1, H929, KMS11 and KMS12BM) stably
573 expressing KRAB-dCAS9 fusion protein and transduced with lentivectors to
574 conditionally express anti-MIR17HG sgRNAs. CCK-8 assay was performed at indicated
575 time points after exposure to doxycycline (0.5 μ g/mL). Cell proliferation is calculated
576 compared to parental cells infected with the empty sgRNA vector and exposed to
577 doxycycline under the same conditions. **D)** CCK-8 proliferation assay of MM cell lines
578 (n=11) transfected with 2 different ASOs targeting the MIR17HG pre-RNA or a non-
579 targeting ASO (NC). ASOs were used at a concentration of 25nM. Cell viability was
580 measured 2 and 4 days after electroporation, and it is represented as % viability
581 compared to cells transfected with NC-ASO. Data from 1 out of 3 independent
582 experiments is shown in panel D and E. Data present mean \pm s.d.in D and E. *p<0.05
583 by Student's *t* test.

584

585 **Figure 2. MIR17HG-derived Inc-17-92 mediates cell growth dependency in a**
586 **miRNA- and DROSHA- independent manner.** **A)** Overview of MIR17HG locus,
587 including both lncRNA (lnc-17-92) and miRNA (miR-17-92)-derived transcripts. **B)**
588 Single molecule RNA FISH analysis of subcellular localization of lnc-17-92 in AMO1.

589 Cell nuclei are stained by DAPI. **C**) Prognostic significance (PFS and OS) of high Inc-
590 17-92 expression (top quartile) in 3 large cohorts of MM patients. **D**) CCK-8 proliferation
591 assay in AMO1 and H929 cells stably transduced with either a lentivector carrying pri-
592 mir-17-92 (pri-miR) or a lentiviral vector carrying GFP as a control, and transfected with
593 2 different ASOs targeting the 5'end (5'-ASO) of MIR17HG pre-RNA or a scrambled
594 control (NC). Effects on cell proliferation were assessed 48h after transfection. **E**) CCK-
595 8 proliferation assay of DROSHA WT or KO AMO1 and H929 exposed to ASO1 (1µM
596 for AMO1 and 2.5µM for H929) for 6 days. Western blot analysis of DROSHA
597 expression in WT and KO cells. Vinculin was used as a protein-loading control. **F**)
598 Effects of lnc-17-92 depletion in a matrigel-based AMO1^{DR-KO} xenograft in NOD SCID
599 mice. Tumor growth of AMO1^{DR-KO} with (ASO-1) or without (NC) lnc-17-92 depletion. **G**)
600 Survival analysis of tumor-injected mice. *indicates p<0.05, ns indicates p>0.05 after
601 Student's t test.

602

603 **Figure 3. Lnc-17-92 forms a transcriptional axis with ACACA to promote**
604 **proliferation and survival of MM cells. A)** Transcriptomic analysis after lnc-17-92
605 depletion in MM cell lines that have either DROSHA WT (AMO1, H929) or KO (AMO1^{DR-}
606 ^{KO}). Venn diagram of commonly downregulated genes (adj p<0.05; log2FC<-1). Cells
607 were exposed to ASO1 for 24h. **B)** qRT-PCR analysis of lnc-17-92 targets in CD138+
608 cells from 3 MM patients exposed to ASO1 for 24h. The results shown are average
609 mRNA expression levels after normalization with GAPDH and ΔΔCt calculations. RNA
610 level in cells exposed to NC (vehicle) were set as an internal reference. **C)** Correlation
611 analysis between lnc-17-92 targets (mRNA) and lnc-17-92 in CD138+ MM patient cells

612 from 2 large RNA-seq cohorts (DFCI/IFM, n=360; MMRF/CoMMpass, n=720).
613 Spearman r obtained in DFCI/IFM (x axis) and MMRF/CoMMpass (y axis) dataset.
614 Dotted red lines indicate $r=0.3$. Individual correlation plots are shown below. **D)** GLuc/
615 SEAP dual reporter assay showing reduced activity of *ACACA*, *ANO6*, *CCDC91*, *EPT1*,
616 *EXT1*, *FER* and *KIAA1109* promoter activity after Inc-17-92 knockdown using ASO1.
617 The reporter vectors were co-transfected into 293T cells with either ASO1 or control
618 ASO. Cells were harvested for the luciferase activity assay 48h after transfection.
619 Results are shown as % of normalized Gluc activity in ASO1-transfected cells compared
620 to control. **E)** ChIP-qPCR analysis showing effective amplification of *ACACA* promoter
621 in chromatin purified using two Inc-17-92 antisense probe sets (ps1 and ps2), compared
622 to chromatin purified using LacZ antisense probes (negative control). **F**) (left) Snapshot
623 obtained by dual RNA-FISH analysis of *ACACA* pre-mRNA (green) and Inc-17-92
624 (purple) in a representative AMO1 cell; (right) box plot showing the distance (nm) of
625 *ACACA* pre-RNA spots to the nearest Inc-17-92 spots (n=57) or to the nearest random
626 spots (160). 300nm was used as a cut-off determining proximity. **G)** CCK-8 proliferation
627 assay in 5 MM cell lines after transfection with siRNAs against Inc-17-92 targets. Two
628 siRNAs were used for each target, plus a scramble siRNA (NC) as a control. Cell
629 viability was measured at the indicated time point and it is represented as % of NC-
630 transfected cells. *indicates p<0.05 after Student's t test in panels B, D, and G, or after
631 Fisher Exact Test in E and F.

632

633 **Figure 4. Lnc-17-92 directly interacts with c-MYC and promotes its occupancy at**
634 **the ACACA promoter. A)** Western blot analysis of MYC in RPPD material precipitated

635 with control RNA or Inc-17-92^{TV1} or Inc-17-92^{TV2}. 5% input is used as a reference. **B)**
636 qRT-PCR analysis of Inc-17-92 (detecting Inc-17-92^{TV1}) in RIP material precipitated
637 using an anti-MYC antibody (α -MYC) or IgG control. LncRNA PVT1 is used as a
638 positive control for its role as MYC interactor. **C)** RNA Y3H using MYC as hybrid protein
639 2 and, as hybrid RNAs, a negative control RNA (-) or Inc-17-92^{TV1} or Inc-17-92^{TV2}. **D)**
640 ChIP-qPCR analysis of MYC occupancy at the ACACA promoter in AMO1, H929 and
641 U266^{MYC+} exposed for 24h to ASO1 or NC (vehicle). MYC occupancy at ACACA
642 promoter is calculated as % of input chromatin. Below each histogram plot is the
643 western blot analysis of MYC from paired samples. GAPDH or α -tubulin were used as
644 protein loading controls. **E)** qRT-PCR analysis of ACACA mRNA in P493-6 cells
645 exposed for 2 days to either doxycycline or DMSO to knockdown MYC, and then
646 exposed for 2 additional days to either ASO1 or vehicle (NC) to deplete Inc-17-92.
647 ACACA expression levels in cells exposed to NC were set as an internal reference. *p <
648 0.05, Student's t test.

649

650 **Figure 5. Lnc-17-92 mediates the assembly of a MYC-WDR82 transcriptional**
651 **complex, leading to transcriptional and epigenetic activation of ACACA. A)**
652 Schematic of integrated BiOID and Co-IP/MS assays to explore the MYC-protein
653 interacting network in the presence or absence of Inc-17-92 depletion. **B)** Western blot
654 analysis of WDR82 in RPPD material precipitated with Inc-17-92^{TV1} or Inc-17-92^{TV2} or
655 with control RNA. 5% input is used as a reference. **C)** RNA Y3H using WDR82 as
656 hybrid protein 2 and, as hybrid RNAs, either a negative control RNA (-) or Inc-17-92^{TV1}
657 or Inc-17-92^{TV2}. Red arrows indicate yeast colony growth. **D)** ChIP-qPCR analysis of

658 H3K4me3 occupancy at ACACA promoter after silencing of WDR82 with a siRNA pool
659 (n=4) in H929 (24h time point). Data are represented as % of input chromatin. **E**) ChIP-
660 qPCR analysis of MYC occupancy at the ACACA promoter after silencing of WDR82
661 with a siRNA pool (n=4) (24h time point). Data are represented as % of input chromatin.
662 **F**) qRT-PCR analysis of ACACA mRNA after silencing of WDR82 with a siRNA pool (n-
663 4) (48h time point). Raw Ct values were normalized to GAPDH mRNA and expressed
664 as $\Delta\Delta Ct$ values calculated using the comparative cross threshold method. ACACA
665 expression levels in cells transfected with NC were set as an internal reference. **G**)
666 ChIP-qPCR analysis of WDR82-GFP occupancy at the ACACA promoter in AMO1
667 exposed for 24h to gymnotic ASO1. Data are represented as % of input chromatin.
668 Western blot analysis of WDR82-GFP from paired samples. α -tubulin was used as the
669 protein loading control. **H**) ChIP-qPCR analysis of H3K4me3 occupancy at the ACACA
670 promoter in AMO1 and H929 exposed for 24h to gymnotic ASO1. Data are represented
671 as % of input chromatin. **I**) Western blot analysis of H3, H3H3K4me1, H3H3K4me2, and
672 H3H3K4me3 in AMO1 and H929 exposed for 24h to gymnotic ASO1. Lamin A/C was
673 used as the protein loading controls (nuclear lysates). *p < 0.05, Student's t test.

674

675 **Figure 6. Therapeutic inhibitors of MIR17HG exert potent anti-tumor activity *in***
676 ***vitro* and *in vivo* in animal models of human MM. A)** Subcutaneous *in vivo* tumor
677 growth of AMO1 cells in NOD SCID mice, 21 days after treatment with G2-15b*-TO (G;
678 n=5) or B9-19-TO (B; n=5) or vehicle (NC; n=5). **B-C)** qRT-PCR analysis of Inc-17-92
679 (C) and Inc-17-92 targets (D) in AMO1 xenografts, retrieved from animals treated with
680 G2-15b*-TO (G; n=1) or B9-19-TO (B; n=1) or vehicle (NC; n=1) as a control. Raw Ct

681 values were normalized to *ACTB* mRNA and expressed as $\Delta\Delta Ct$ values calculated
682 using the comparative cross threshold method. Expression levels in NC were set as an
683 internal reference. **D)** BLI-based measurement of *in vivo* tumor growth of MOLP8-luc+ in
684 NSG mice, after treatment with G2-15b*-TO (G; n=8) or B9-19-TO (B; n=6) or vehicle
685 (NC; n=11). On the top, a scatter plot showing the analysis of bioluminescence intensity.
686 Red bars indicate median value. Bioluminescence was measured at the end of the
687 treatment cycle (day 15). Below, image acquisition. Mice removed from the study due to
688 failed I.V. injection of tumor cells are covered by a black rectangle. **E)** Survival analysis
689 from experiment in panel E. **F)** Human Kappa light chain ELISA-based measurement of
690 *in vivo* tumor growth of MM patient cells in NSG mice (PDX-NSG), after treatment with
691 G2-15b*-TO (G; n=2), bortezomib (BTZ; n=2) or vehicle (NC; n=3). Black arrows
692 indicate treatments. *indicates p<0.05; **means p<0.01; ***means p<0.001.

693 REFERENCES

- 694 1. Gulla A, Anderson KC. Multiple myeloma: the (r)evolution of current therapy and a
695 glance into future. *Haematologica*. 2020.
- 696 2. Morelli E, Gulla A, Rocca R, et al. The Non-Coding RNA Landscape of Plasma Cell
697 Dyscrasias. *Cancers (Basel)*. 2020;12(2).
- 698 3. Pichiorri F, Suh SS, Rocci A, et al. Downregulation of p53-inducible microRNAs 192, 194,
699 and 215 Impairs the p53/MDM2 Autoregulatory Loop in Multiple Myeloma Development.
700 *Cancer Cell*. 2016;30(2):349-351.
- 701 4. Amodio N, Stamato MA, Juli G, et al. Drugging the lncRNA MALAT1 via LNA gapmeR ASO
702 inhibits gene expression of proteasome subunits and triggers anti-multiple myeloma activity.
703 *Leukemia*. 2018;32(9):1948-1957.
- 704 5. Morelli E, Biamonte L, Federico C, et al. Therapeutic vulnerability of multiple myeloma
705 to MIR17PTi, a first-in-class inhibitor of pri-miR-17-92. *Blood*. 2018;132(10):1050-1063.
- 706 6. Hu Y, Lin J, Fang H, et al. Targeting the MALAT1/PARP1/LIG3 complex induces DNA
707 damage and apoptosis in multiple myeloma. *Leukemia*. 2018;32(10):2250-2262.
- 708 7. Morelli E, Leone E, Cantafio ME, et al. Selective targeting of IRF4 by synthetic microRNA-
709 125b-5p mimics induces anti-multiple myeloma activity in vitro and in vivo. *Leukemia*.
710 2015;29(11):2173-2183.
- 711 8. Leone E, Morelli E, Di Martino MT, et al. Targeting miR-21 inhibits in vitro and in vivo
712 multiple myeloma cell growth. *Clin Cancer Res*. 2013;19(8):2096-2106.
- 713 9. Wang Z, Yang B, Zhang M, et al. lncRNA Epigenetic Landscape Analysis Identifies EPIC1
714 as an Oncogenic lncRNA that Interacts with MYC and Promotes Cell-Cycle Progression in Cancer.
715 *Cancer Cell*. 2018;33(4):706-720 e709.
- 716 10. Hon CC, Ramilowski JA, Harshbarger J, et al. An atlas of human long non-coding RNAs
717 with accurate 5' ends. *Nature*. 2017;543(7644):199-204.
- 718 11. Ulitsky I, Bartel DP. lincRNAs: genomics, evolution, and mechanisms. *Cell*.
719 2013;154(1):26-46.
- 720 12. Lu Y, Zhao X, Liu Q, et al. lncRNA MIR100HG-derived miR-100 and miR-125b mediate
721 cetuximab resistance via Wnt/beta-catenin signaling. *Nat Med*. 2017;23(11):1331-1341.
- 722 13. Tseng YY, Moriarity BS, Gong W, et al. PVT1 dependence in cancer with MYC copy-
723 number increase. *Nature*. 2014;512(7512):82-86.
- 724 14. Gutschner T, Diederichs S. The hallmarks of cancer: a long non-coding RNA point of
725 view. *RNA Biol*. 2012;9(6):703-719.
- 726 15. Samur MK, Minvielle S, Gulla A, et al. Long intergenic non-coding RNAs have an
727 independent impact on survival in multiple myeloma. *Leukemia*. 2018;32(12):2626-2635.
- 728 16. Carrasco-Leon A, Ezponda T, Meydan C, et al. Characterization of complete lncRNAs
729 transcriptome reveals the functional and clinical impact of lncRNAs in multiple myeloma.
730 *Leukemia*. 2021.
- 731 17. Ronchetti D, Agnelli L, Taiana E, et al. Distinct lncRNA transcriptional fingerprints
732 characterize progressive stages of multiple myeloma. *Oncotarget*. 2016;7(12):14814-14830.
- 733 18. Liu SJ, Horbeck MA, Cho SW, et al. CRISPRi-based genome-scale identification of
734 functional long noncoding RNA loci in human cells. *Science*. 2017;355(6320).

- 735 19. Morelli E, Gulla A, Amodio N, et al. CRISPR Interference (CRISPRi) and CRISPR Activation
736 (CRISPRa) to Explore the Oncogenic lncRNA Network. *Methods Mol Biol.* 2021;2348:189-204.
- 737 20. Taiana E, FavaSuli V, Ronchetti D, et al. In Vitro Silencing of lncRNAs Using LNA
738 GapmeRs. *Methods Mol Biol.* 2021;2348:157-166.
- 739 21. Shaffer SM, Wu MT, Levesque MJ, Raj A. Turbo FISH: a method for rapid single molecule
740 RNA FISH. *PLoS One.* 2013;8(9):e75120.
- 741 22. Raj A, van den Bogaard P, Rifkin SA, van Oudenaarden A, Tyagi S. Imaging individual
742 mRNA molecules using multiple singly labeled probes. *Nat Methods.* 2008;5(10):877-879.
- 743 23. Chu C, Quinn J, Chang HY. Chromatin isolation by RNA purification (ChIRP). *J Vis Exp.*
744 2012(61).
- 745 24. Zadra G, Ribeiro CF, Chetta P, et al. Inhibition of de novo lipogenesis targets androgen
746 receptor signaling in castration-resistant prostate cancer. *Proc Natl Acad Sci U S A.*
747 2019;116(2):631-640.
- 748 25. Fulciniti M, Lin CY, Samur MK, et al. Non-overlapping Control of Transcriptome by
749 Promoter- and Super-Enhancer-Associated Dependencies in Multiple Myeloma. *Cell Rep.*
750 2018;25(13):3693-3705 e3696.
- 751 26. Kalkat M, Resetca D, Lourenco C, et al. MYC Protein Interactome Profiling Reveals
752 Functionally Distinct Regions that Cooperate to Drive Tumorigenesis. *Mol Cell.* 2018;72(5):836-
753 848 e837.
- 754 27. Li W, Xu H, Xiao T, et al. MAGeCK enables robust identification of essential genes from
755 genome-scale CRISPR/Cas9 knockout screens. *Genome Biol.* 2014;15(12):554.
- 756 28. Shaffer AL, Emre NC, Lamy L, et al. IRF4 addiction in multiple myeloma. *Nature.*
757 2008;454(7201):226-231.
- 758 29. Chesi M, Robbiani DF, Sebag M, et al. AID-dependent activation of a MYC transgene
759 induces multiple myeloma in a conditional mouse model of post-germinal center malignancies.
760 *Cancer Cell.* 2008;13(2):167-180.
- 761 30. Jovanovic KK, Escure G, Demonchy J, et al. Dereulation and Targeting of TP53 Pathway
762 in Multiple Myeloma. *Front Oncol.* 2018;8:665.
- 763 31. Tessoulin B, Moreau-Aubry A, Descamps G, et al. Whole-exon sequencing of human
764 myeloma cell lines shows mutations related to myeloma patients at relapse with major hits in
765 the DNA regulation and repair pathways. *J Hematol Oncol.* 2018;11(1):137.
- 766 32. Lai F, Damle SS, Ling KK, Rigo F. Directed RNase H Cleavage of Nascent Transcripts
767 Causes Transcription Termination. *Mol Cell.* 2020;77(5):1032-1043 e1034.
- 768 33. Lee JS, Mendell JT. Antisense-Mediated Transcript Knockdown Triggers Premature
769 Transcription Termination. *Mol Cell.* 2020;77(5):1044-1054 e1043.
- 770 34. He L, Thomson JM, Hemann MT, et al. A microRNA polycistron as a potential human
771 oncogene. *Nature.* 2005;435(7043):828-833.
- 772 35. Ota A, Tagawa H, Karnan S, et al. Identification and characterization of a novel gene,
773 C13orf25, as a target for 13q31-q32 amplification in malignant lymphoma. *Cancer Res.*
774 2004;64(9):3087-3095.
- 775 36. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. *Cell.*
776 2004;116(2):281-297.
- 777 37. Cummins JM, He Y, Leary RJ, et al. The colorectal microRNAome. *Proc Natl Acad Sci U S*
778 *A.* 2006;103(10):3687-3692.

- 779 38. Rohrig F, Schulze A. The multifaceted roles of fatty acid synthesis in cancer. *Nat Rev Cancer*. 2016;16(11):732-749.
- 780 39. Li Y, Choi PS, Casey SC, Dill DL, Felsher DW. MYC through miR-17-92 suppresses specific target genes to maintain survival, autonomous proliferation, and a neoplastic state. *Cancer Cell*. 2014;26(2):262-272.
- 781 40. Izreig S, Samborska B, Johnson RM, et al. The miR-17 approximately 92 microRNA Cluster Is a Global Regulator of Tumor Metabolism. *Cell Rep*. 2016;16(7):1915-1928.
- 782 41. Gouw AM, Margulis K, Liu NS, et al. The MYC Oncogene Cooperates with Sterol-Regulated Element-Binding Protein to Regulate Lipogenesis Essential for Neoplastic Growth. *Cell Metab*. 2019;30(3):556-572 e555.
- 783 42. Hook B, Bernstein D, Zhang B, Wickens M. RNA-protein interactions in the yeast three-hybrid system: affinity, sensitivity, and enhanced library screening. *RNA*. 2005;11(2):227-233.
- 784 43. Schuhmacher M, Staeger MS, Pajic A, et al. Control of cell growth by c-Myc in the absence of cell division. *Curr Biol*. 1999;9(21):1255-1258.
- 785 44. Lee JH, Skalnik DG. Wdr82 is a C-terminal domain-binding protein that recruits the Setd1A Histone H3-Lys4 methyltransferase complex to transcription start sites of transcribed human genes. *Mol Cell Biol*. 2008;28(2):609-618.
- 786 45. Beurton F, Stempor P, Caron M, et al. Physical and functional interaction between SET1/COMPASS complex component CFP-1 and a Sin3S HDAC complex in *C. elegans*. *Nucleic Acids Res*. 2019;47(21):11164-11180.
- 787 46. Amento S, Lania L, Majello B. Epigenetic reprogramming of Myc target genes. *Am J Cancer Res*. 2011;1(3):413-418.
- 788 47. Puttaraju M, Jackson M, Klein S, et al. Systematic screening identifies therapeutic antisense oligonucleotides for Hutchinson-Gilford progeria syndrome. *Nat Med*. 2021.
- 789 48. Falchook G, Infante J, Arkenau HT, et al. First-in-human study of the safety, pharmacokinetics, and pharmacodynamics of first-in-class fatty acid synthase inhibitor TVB-2640 alone and with a taxane in advanced tumors. *EClinicalMedicine*. 2021;34:100797.
- 790 49. Mogilyansky E, Rigoutsos I. The miR-17/92 cluster: a comprehensive update on its genomics, genetics, functions and increasingly important and numerous roles in health and disease. *Cell Death Differ*. 2013;20(12):1603-1614.
- 791 50. Labi V, Peng S, Kliromanos F, et al. Context-specific regulation of cell survival by a miRNA-controlled BIM rheostat. *Genes Dev*. 2019;33(23-24):1673-1687.
- 792 51. Olive V, Bennett MJ, Walker JC, et al. miR-19 is a key oncogenic component of mir-17-92. *Genes Dev*. 2009;23(24):2839-2849.
- 793 52. Olive V, Sabio E, Bennett MJ, et al. A component of the mir-17-92 polycistronic oncomir promotes oncogene-dependent apoptosis. *Elife*. 2013;2:e00822.
- 794 53. Donayo AO, Johnson RM, Tseng HW, et al. Oncogenic Biogenesis of pri-miR-17 approximately 92 Reveals Hierarchy and Competition among Polycistronic MicroRNAs. *Mol Cell*. 2019;75(2):340-356 e310.
- 795 54. Lin CY, Loven J, Rahl PB, et al. Transcriptional amplification in tumor cells with elevated c-Myc. *Cell*. 2012;151(1):56-67.
- 796 55. Zhang B, Lu HY, Xia YH, Jiang AG, Lv YX. Long non-coding RNA EPIC1 promotes human lung cancer cell growth. *Biochem Biophys Res Commun*. 2018;503(3):1342-1348.

- 822 56. Beloribi-Djeafilia S, Vasseur S, Guillaumond F. Lipid metabolic reprogramming in cancer
823 cells. *Oncogenesis*. 2016;5:e189.
- 824 57. Stine ZE, Walton ZE, Altman BJ, Hsieh AL, Dang CV. MYC, Metabolism, and Cancer.
825 *Cancer Discov*. 2015;5(10):1024-1039.
- 826 58. Masarwi M, DeSchiffart A, Ham J, Reagan MR. Multiple Myeloma and Fatty Acid
827 Metabolism. *JBMR Plus*. 2019;3(3):e10173.
- 828 59. El Arfani C, De Veirman K, Maes K, De Bruyne E, Menu E. Metabolic Features of Multiple
829 Myeloma. *Int J Mol Sci*. 2018;19(4).
- 830 60. Brinkmann K, Ng AP, de Graaf CA, et al. miR17~92 restrains pro-apoptotic BIM to ensure
831 survival of haematopoietic stem and progenitor cells. *Cell Death Differ*. 2020;27(5):1475-1488.
- 832 61. Sirchia F, Di Gregorio E, Restagno G, et al. A case of Feingold type 2 syndrome associated
833 with keratoconus refines keratoconus type 7 locus on chromosome 13q. *Eur J Med Genet*.
834 2017;60(4):224-227.
- 835 62. Sullenger BA, Nair S. From the RNA world to the clinic. *Science*. 2016;352(6292):1417-
836 1420.
- 837 63. Crooke ST, Witztum JL, Bennett CF, Baker BF. RNA-Targeted Therapeutics. *Cell Metab*.
838 2018;27(4):714-739.
- 839 64. Damase TR, Sukhovershin R, Boada C, Taraballi F, Pettigrew RI, Cooke JP. The Limitless
840 Future of RNA Therapeutics. *Front Bioeng Biotechnol*. 2021;9:628137.
- 841 65. Dhuri K, Bechtold C, Quijano E, et al. Antisense Oligonucleotides: An Emerging Area in
842 Drug Discovery and Development. *J Clin Med*. 2020;9(6).
- 843 66. Mondala PK, Vora AA, Zhou T, et al. Selective antisense oligonucleotide inhibition of
844 human IRF4 prevents malignant myeloma regeneration via cell cycle disruption. *Cell Stem Cell*.
845 2021;28(4):623-636 e629.

Figure 1

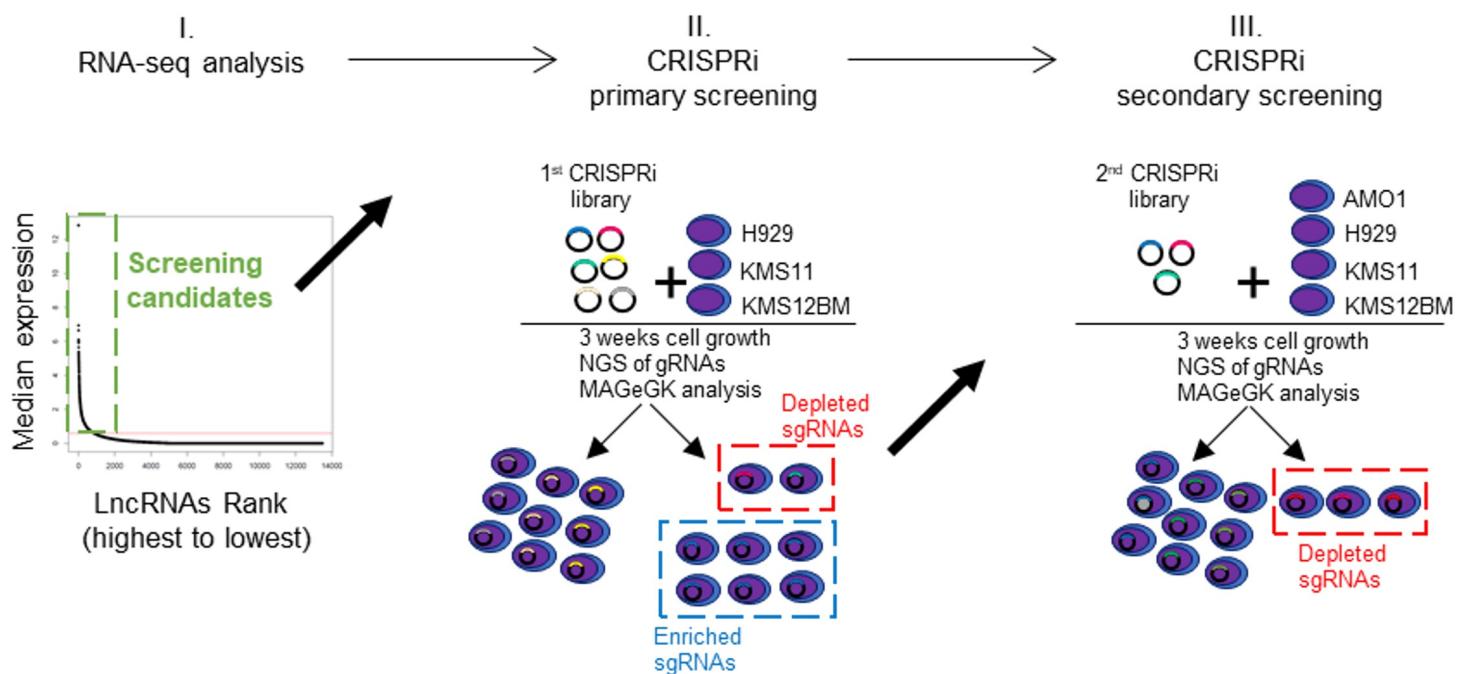
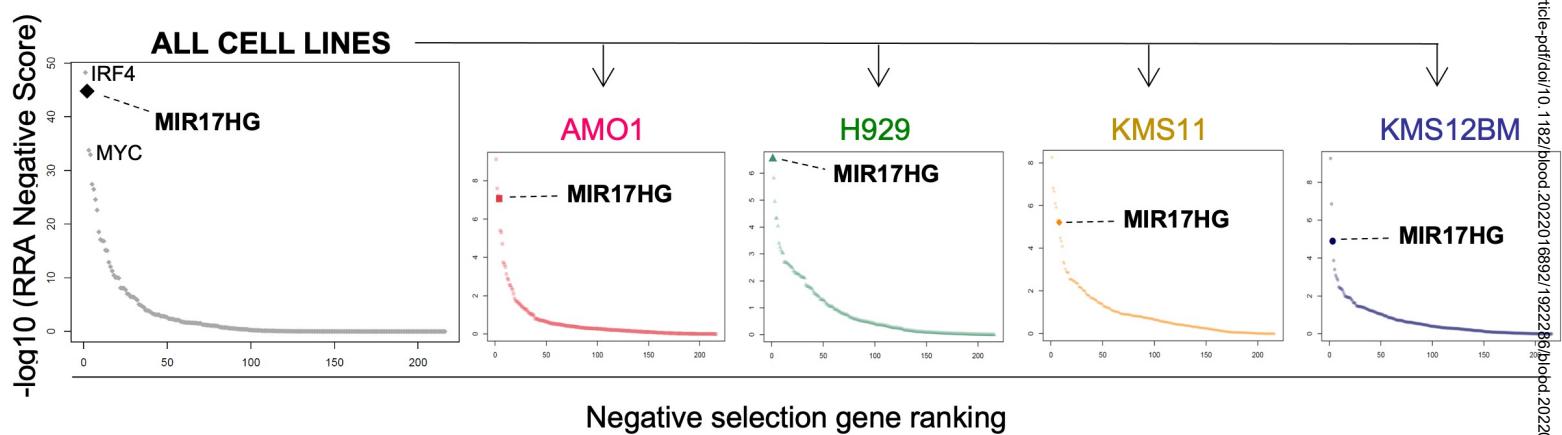
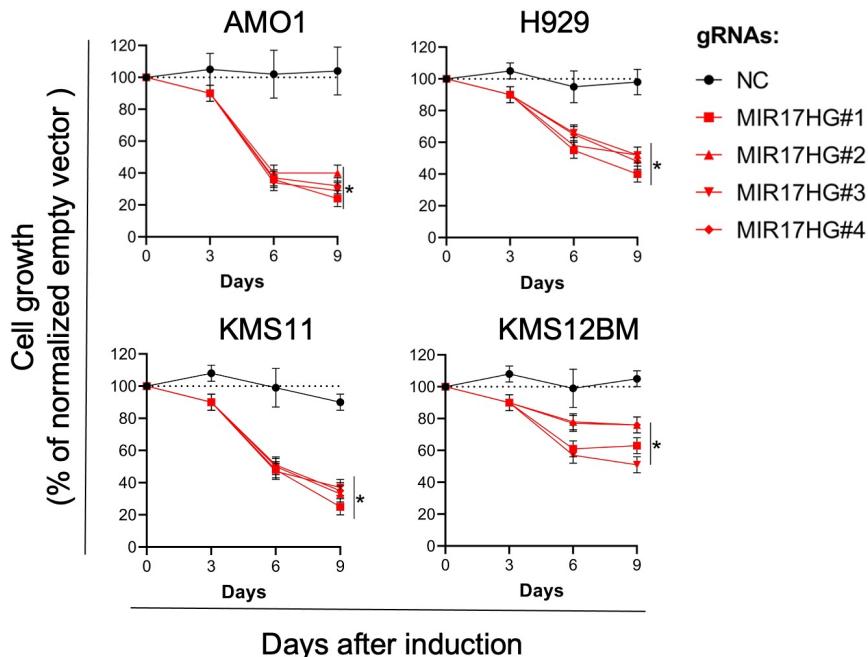
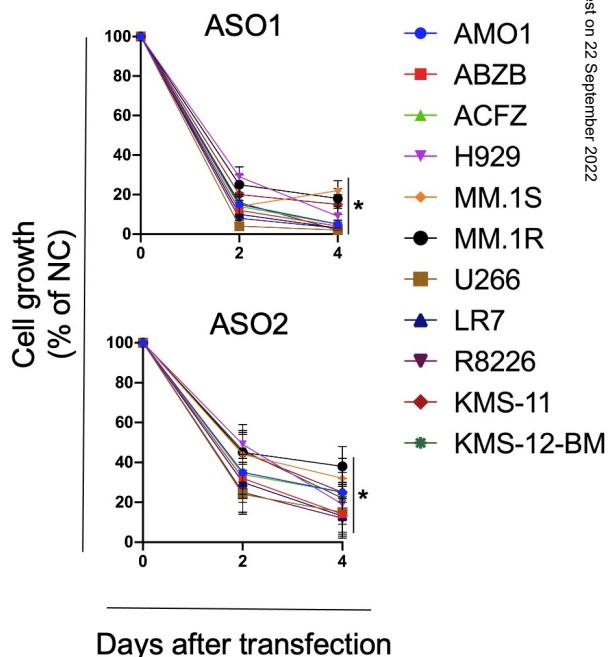
A

B

C

D


Figure 2

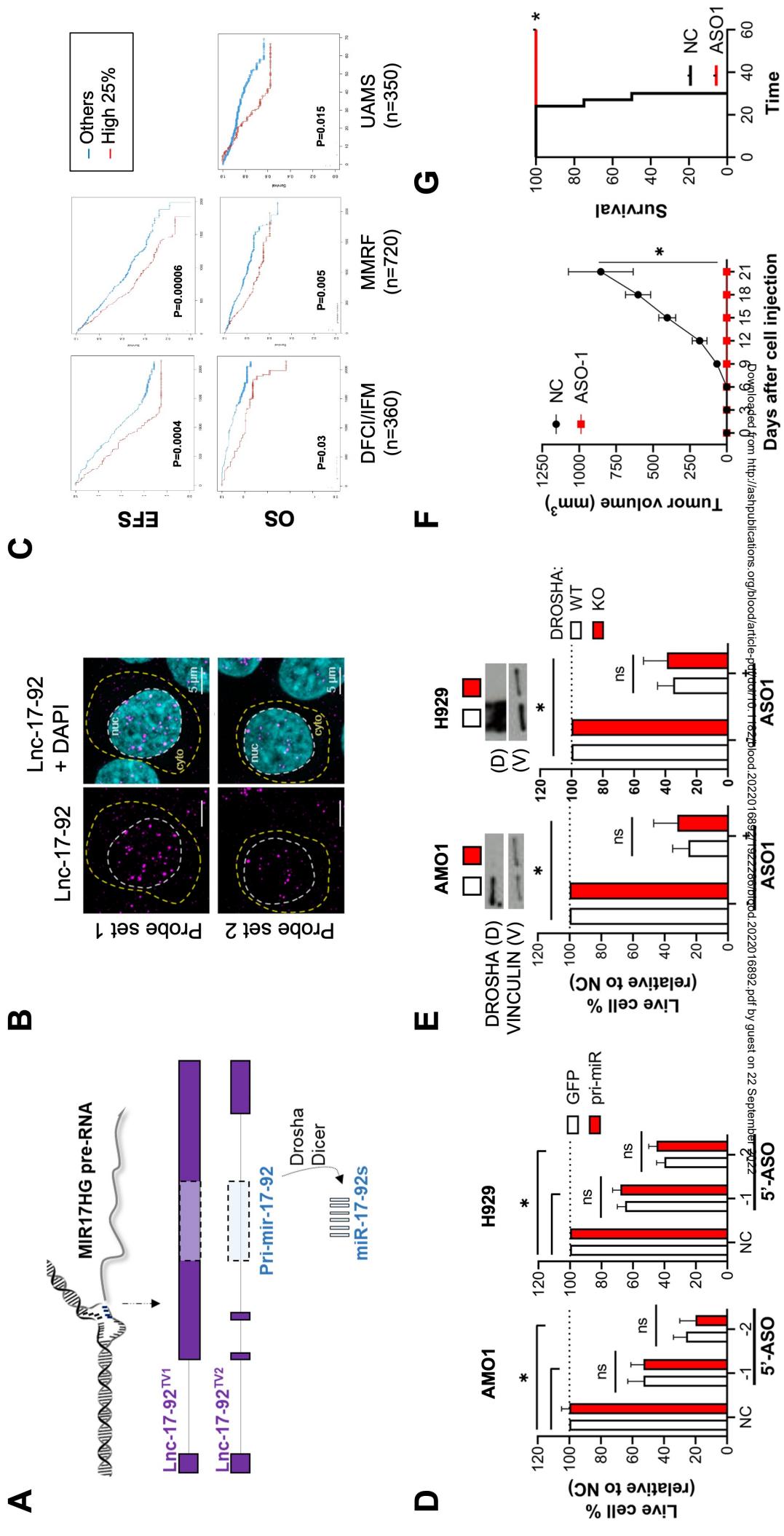
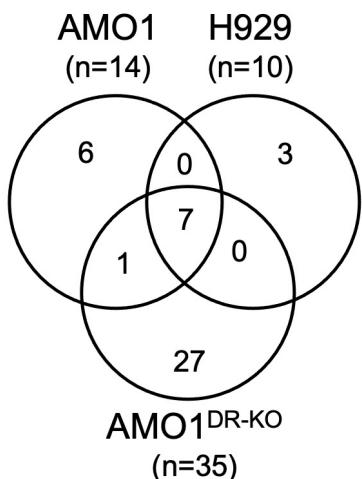
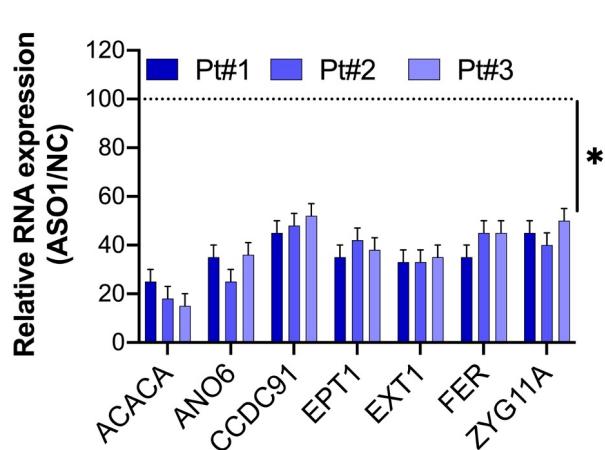
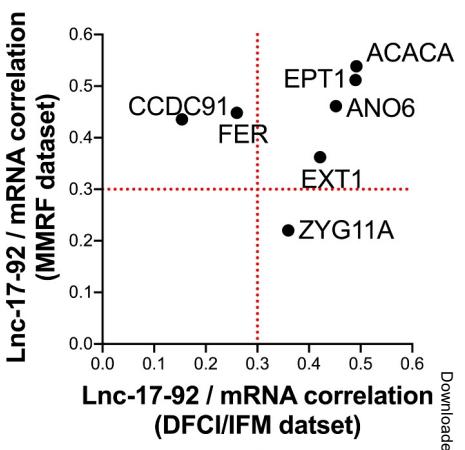


Figure 3

A**B****C**

Downloaded from https://academic.oup.com/molehr/article/10/182/921/6882265 by guest on 22 September 2022

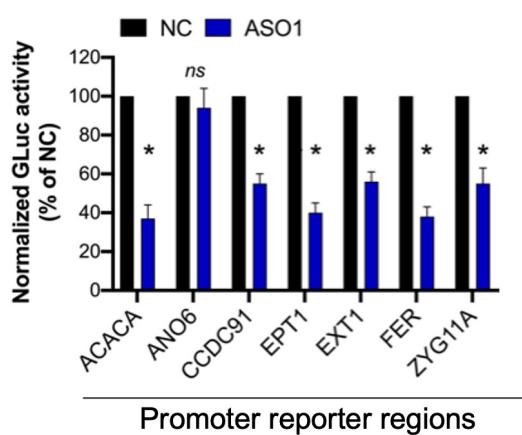
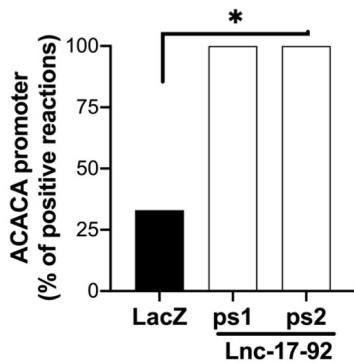
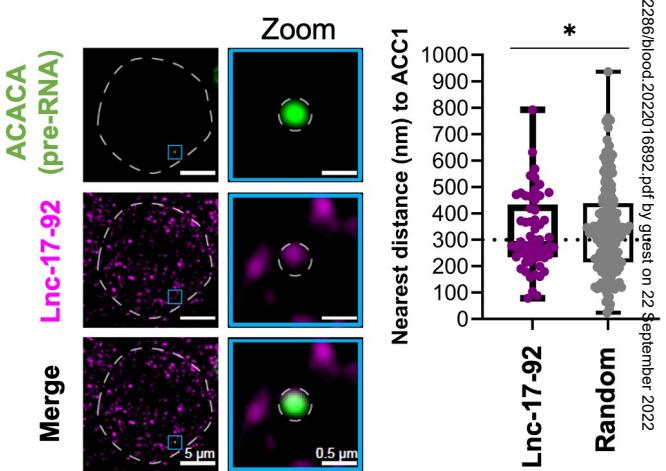
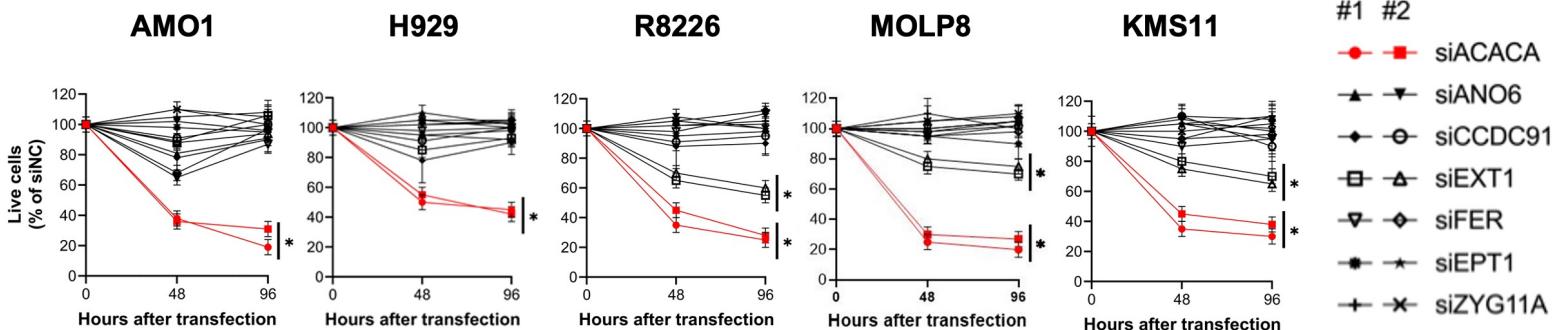
D**E****F****G**

Figure 4

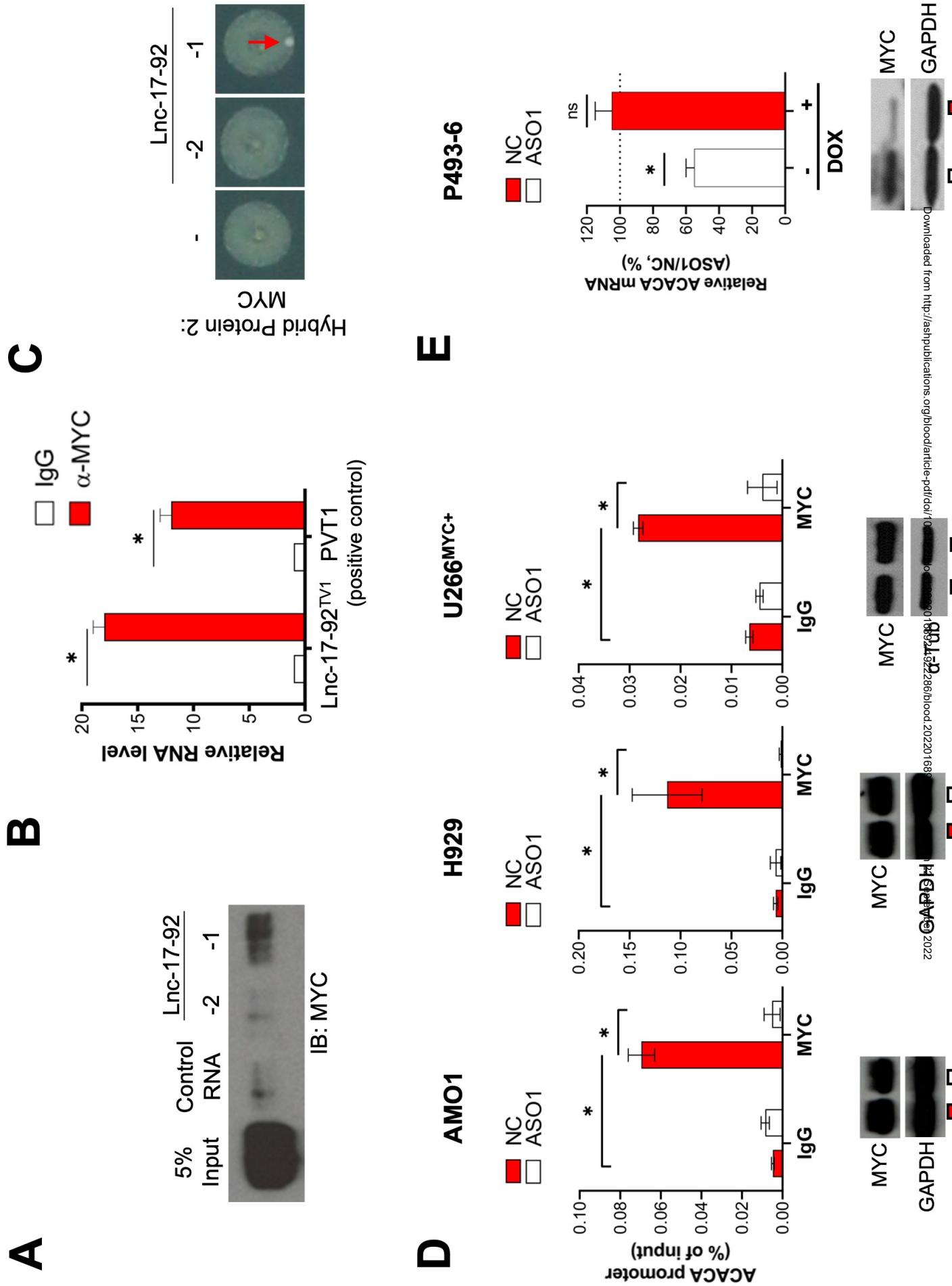
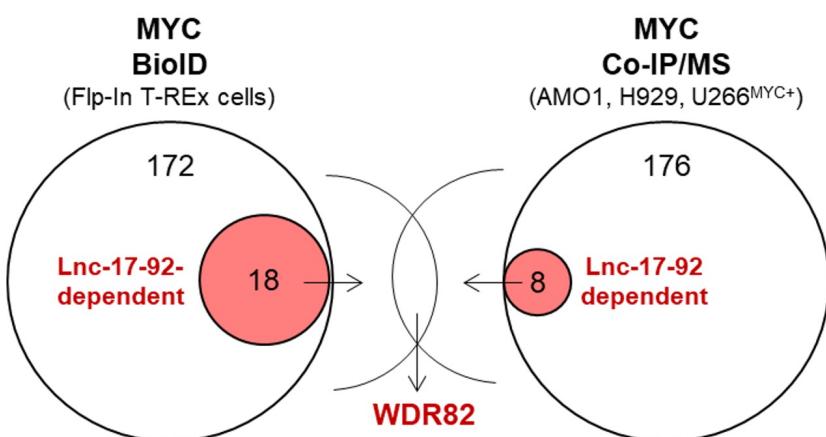
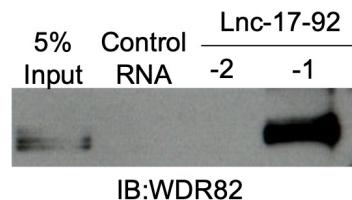
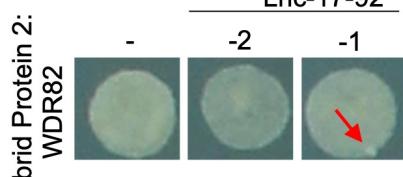
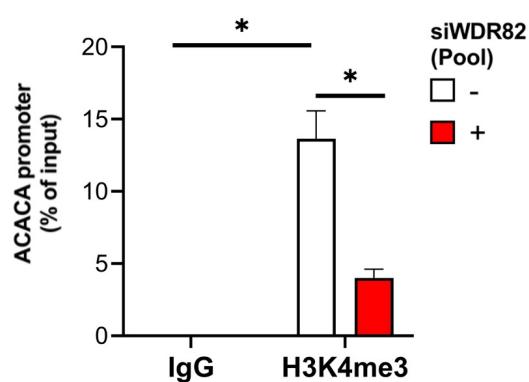
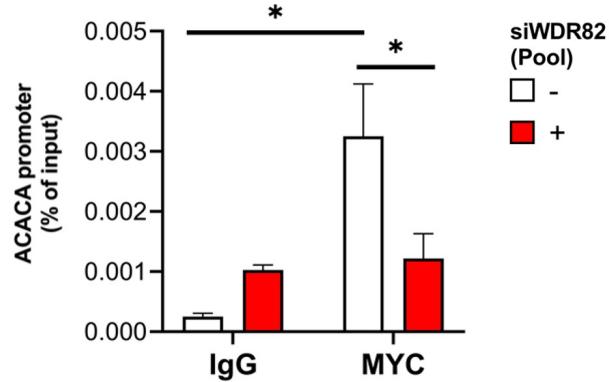
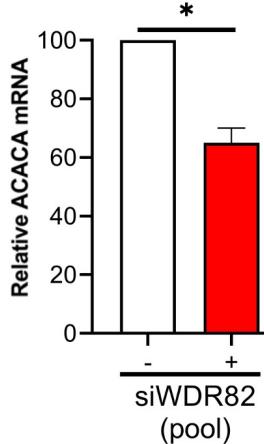
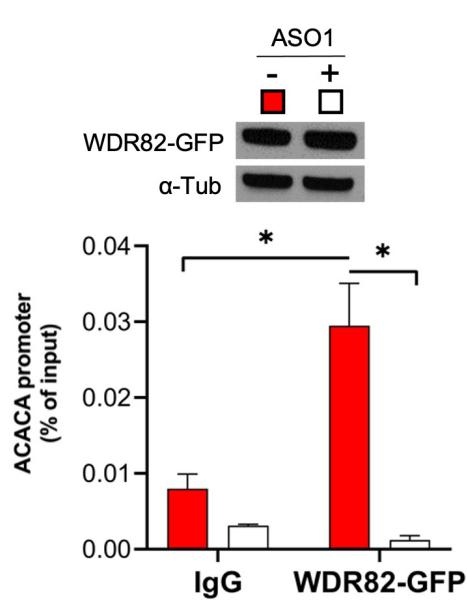
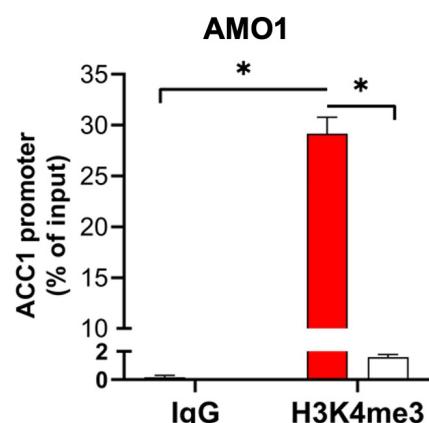


Figure 5

A**B**

IB:WDR82

C**D****E****F****G****H**

H929

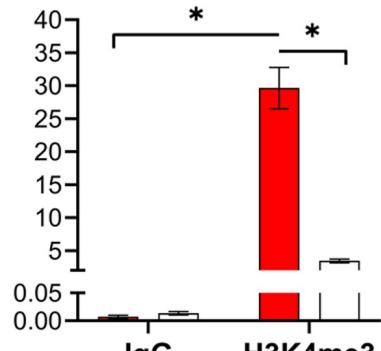
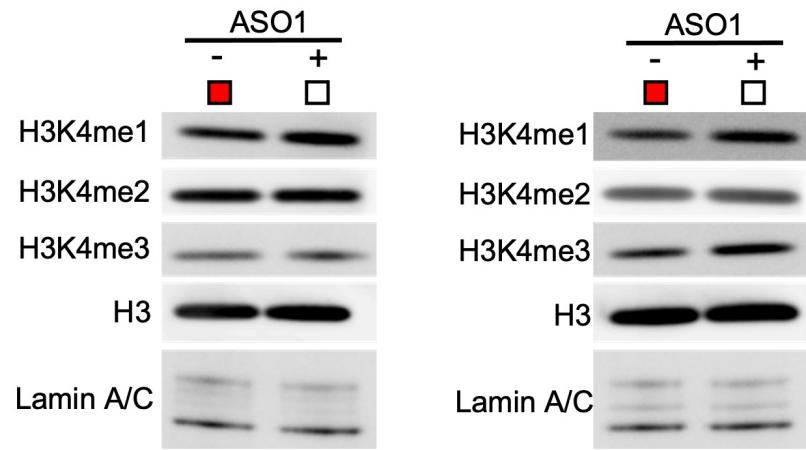
**I**

Figure 6

