

17 **Abstract**

18 LINE-1 (L1) are autonomous retroelements that have retained their ability to mobilize.
19 Mechanisms regulating L1 mobility include DNA methylation in somatic cells and the
20 Piwi-interacting RNA pathway in the germline. During pre-implantation stages of
21 mouse embryonic development, however, both pathways are inactivated leading to a
22 critical window necessitating alternate means of L1 regulation. We previously reported
23 an increase in L1 levels in *Dicer_KO* mouse embryonic stem cells (mESCs).
24 Intriguingly this was accompanied by only a marginal increase in retrotransposition,
25 suggestive of additional mechanisms suppressing L1 mobility. Here, we demonstrate
26 that L1 Ribonucleoprotein complexes (L1 RNP) accumulate as aggregates in
27 *Dicer_KO* cytoplasm along with the RNA helicase MOV10. The combined
28 overexpression of L1 RNAs and MOV10 is sufficient to create L1 RNP aggregates in
29 stem cells. In *Dicer_KO* mESCs, MOV10 is upregulated due to the loss of its direct
30 regulation by miRNAs. The newly discovered post-transcriptional regulation of *Mov10*
31 expression, and its role in preventing L1 retrotransposition by driving novel cytosolic
32 aggregation affords alternate routes to explore for therapy and disease progression.

33 **Introduction**

34 Approximately 17-20% of human and mouse genomes are composed of Long
35 Interspersed Nucleotide Elements 1 (LINE-1 or L1) ^{1,2}. These elements, ranging from
36 6 to 7 kb in length, encode enzymatic activities necessary for retrotransposition. In
37 mouse, L1s are composed of a 5' untranslated region (UTR) harboring an RNA
38 Polymerase II (Pol II) promoter encoding a bicistronic transcript. The two open reading
39 frames (ORF) encode for L1 ORF1 protein that is speculated to function as an RNA
40 chaperone and L1 ORF2 protein that has endonuclease and reverse transcriptase
41 activities. The transcript harbors a 3'UTR and a poly adenylation (poly(A)) signal. Only
42 a full-length poly(A) transcript is capable of transposing. Upon export from the nucleus,
43 L1 RNA is translated in the cytoplasm. L1 RNA, ORF1 and ORF2 proteins associate
44 to form ribonucleoprotein particles (L1 RNPs), which are imported back together into
45 the nucleus. Once in the nucleus, the L1 RNA is reverse transcribed and integrated
46 into a new genomic location by a coupled reverse transcription. During this
47 mobilization mechanism, the retrotransposon sequence is prone to truncations and
48 inversions, resulting in the insertion of mutated copies unable to jump a second time
49 ^{3,4}. Nevertheless, 100 ⁵ and 3000 ⁶ full length L1 elements in human and mouse
50 genomes, respectively, retain the ability to encode the machinery necessary for
51 production of the RNA intermediate, its reverse transcription, and consequent
52 integration into a new genomic location. In mouse, active L1s are divided into three
53 subfamilies: Tf, Gf and A, which are defined by the variable sequence and numbers of
54 monomers (tandem repeat units of 200 bp) contained in their 5'UTR ⁷⁻⁹.

55 While transposable elements are indispensable for genome variation and evolution,
56 rogue and/or rampant transposition leads to disease ³. Elucidating mechanisms that
57 regulate L1 transcription and mobility have been an active area of research since their
58 discovery. DNA methylation in somatic cells and Piwi-interacting RNA (piRNA)
59 pathway in the germline are well established regulators of L1 retrotransposition ¹⁰⁻¹².
60 At the blastocyst stage of embryonic development however, both the above mentioned
61 pathways are inactivated leading to a window necessitating alternate mechanisms of
62 L1 regulation. The microRNA (miRNA) effector protein DICER has been implicated in
63 modulating expression of L1 during this stage of development ¹³. MicroRNAs are 21-
64 24 nucleotide (nt) long Pol II transcripts that play a major role in fine-tuning gene
65 expression post-transcriptionally ^{14,15}. Briefly, miRNAs are transcribed as primary (pri)
66 miRNAs and processed into precursor (pre) miRNAs by DGCR8/DROSHA

67 microprocessor complex in the nucleus. Upon export into the cytoplasm DICER
68 cleaves pre-miRNAs to give rise to mature miRNAs. The mature miRNA duplex is
69 loaded onto ARGONAUTE (AGO) proteins, upon unwinding of the duplex, one of the
70 two strands is degraded. Along with accessory proteins, AGO loaded with the guide
71 miRNA strand forms the RNA-induced silencing complex (RISC) and acts as the
72 effector. Base pairing of miRNA at its seed sequence with complementary miRNA
73 response elements (MREs), typically found in the 3'UTR sequence of mRNAs induces
74 translational repression or mRNA degradation. Pre-implantation mouse embryos
75 deleted for *Dicer* present an upregulation of L1 elements ^{16,17}. In human cancer cells,
76 miR-128 was shown to regulate L1 transposition via two mechanisms. Firstly, miR-
77 128 repressed L1 expression directly by binding to a noncanonical binding site in L1
78 ORF2 RNA ¹⁸ and secondly, miR-128 bound to a canonical binding site in the 3'UTR
79 sequence of *Tnpo1* an import factor that regulates entry of L1 RNP complex into the
80 nucleus post translation ¹⁹. This mode of regulation via miR-128 however does not
81 appear to be conserved in mESCs ²⁰. Recently, the direct binding of miRNA let-7 to
82 L1 mRNA was shown to impair L1 ORF2 translation and consequently
83 retrotransposition ²¹. Since processing of pri-let7 miRNA to mature let-7 miRNA is
84 blocked in mESCs ²², this mechanism of fine-tuning L1 expression is also not
85 conserved in mESCs. To delve deeper into the role of *Dicer* in regulating L1 during
86 embryonic development our laboratory utilized mouse embryonic stem cells (mESCs)
87 as a model. In *Dicer*_Knockout (KO) mESCs, while a 6-8 fold increase in L1
88 transcription was observed, a concomitant increase in the rate of retrotransposition
89 was not uncovered ¹³. In this study, we demonstrate that miRNAs are involved in the
90 regulation of L1 retrotransposition in mESCs through the direct regulation of the RNA
91 helicase MOV10. Upon loss of miRNAs, MOV10 is strongly upregulated and
92 accumulates in the cytoplasm of mESCs, driving sequestration of L1 RNPs into novel
93 aggregates, thereby preventing L1 mobility.

94

95 **Results**

96 In order to better understand why the strong upregulation of L1 RNAs does not lead
97 to a subsequent retrotransposition in *Dicer*_KO mESCs ¹³, we looked at the
98 localization of L1 RNA and protein in Wild type (WT) and mutant cells. We probed for
99 L1 RNA derived from the Tf L1 family by RNA Fluorescent in Situ Hybridization (RNA
100 FISH) along with L1 ORF1 protein by indirect immunofluorescence (IF). While in WT

101 mESCs, we observed diffused signal for both L1 Tf RNA and ORF1 protein, they co-
102 localized as L1 ribonucleoprotein (L1 RNP) foci in cytoplasm of the two independent
103 *Dicer_KO* clones (Fig.1A). The median number of L1 RNP foci in the cytoplasm per
104 cell in *Dicer_KO1* and *Dicer_KO2* mESCs was 9 and 7 respectively as compared to 0
105 in WT cells. Additionally, in 30-35% of *Dicer_KO* clones, L1 RNP were observed to co-
106 localize in larger foci (Fig.1A). These observations led us to hypothesize that
107 sequestration of L1 RNP in the cytoplasm of *Dicer_KO* mESCs is preventing L1
108 retrotransposition.

109 To characterize L1 RNP foci we aimed to identify other cellular components that might
110 share their location with them. We therefore tested if known interactors of human L1
111 proteins might colocalize with L1 RNP cytoplasmic foci in *Dicer_KO* mESCs²³⁻²⁶.
112 Amongst the list of candidates interacting with both L1 ORF1 and L1 ORF2²⁶, we
113 looked at RNA helicases UPF1 and MOV10 by IF. While UPF1 was observed to have
114 diffused cytoplasmic staining (data not shown), MOV10 co-localized with L1 RNP in
115 the cytoplasm of *Dicer_KO* mESCs (Fig. 1B). Further analysis revealed MOV10 to co-
116 localize with L1 ORF1 protein in *Dicer_KO* cells with a median of 3 foci in WT cells
117 and 12 and 15 respectively in *Dicer_KO1* and *Dicer_KO2* mESCs. Percentage of cells
118 with large ORF1-MOV10 foci was 26-47% in the two *Dicer_KO* lines (Fig. 1B). The
119 higher frequency of ORF1-MOV10 foci as compared to Tf-ORF1 foci in *Dicer_KO* cells
120 is most likely due to the lower sensitivity for detecting Tf RNA by RNA FISH. Since
121 MOV10 co-localization with L1 ORF1 foci in *Dicer_KO* mESCs was high and due to
122 the absence of good antibodies available for L1 proteins raised in hosts other than
123 rabbit for co-staining IF experiments, we further used MOV10 as a proxy for L1 RNP
124 localization.

125 Localization of L1 RNP as cytoplasmic foci was previously reported for human L1
126 proteins upon their ectopic overexpression in HEK293T cells²⁷. L1 ORF1 foci were
127 furthermore shown to co-localize with stress granules and RNA-binding proteins
128 including components of the RISC complex²⁷. To assess the nature of the observed
129 mouse L1 RNP foci, we co-stained WT and *Dicer_KO* mESCs for G3BP1, a marker
130 for stress granules²⁸, along with MOV10. The signal for G3BP1 was mainly diffused
131 cytoplasmic in both WT and *Dicer_KO* mESCs, indicating that unlike human cancer
132 cells, mouse L1 ORF1-MOV10 foci are not stress granules (Fig. 2A). However,
133 treatment with 0.5mM Sodium Arsenite for 20 minutes to induce stress caused MOV10
134 to co-localize with G3BP1 as cytoplasmic bodies in *Dicer_KO* cells (Fig. 2A). These

135 data led us to hypothesize that L1 RNP foci in *Dicer*_KO mESCs might be poised but
136 are not as yet mature stress granules.

137 Partitioning of stress granule proteins as liquid-liquid phase separation (LLPS) is
138 emerging as a main driver for shifting dynamics from being near soluble to condensate
139 formation thereby impacting their biological function²⁹. RNA and RNA binding proteins
140 (RBPs) are key components of these cytoplasmic condensates³⁰. Recently, by
141 microscopy and NMR spectroscopy, human L1 ORF1 protein was shown to form liquid
142 droplets *in vitro* in a salt dependent manner³¹. To test whether L1 ORF1 foci in mESCs
143 undergo similar LLPS, we treated *Dicer*_KO mESCs with 3% 1,6 Hexanediol for 15
144 minutes, a concentration at which proteins undergoing LLPS have been previously
145 observed to change solubility from being in foci to becoming diffused in mESCs³². No
146 overt change in L1 ORF1-MOV10 foci was observed in cells treated with 1,6
147 Hexanediol (Extended Data Fig.1A), suggesting that L1 ORF1-MOV10 foci are not
148 LLPS condensates.

149 Human L1 ORF1 protein are also known to associate with Processing Body (P-body)
150 enriched mRNAs³³. While elucidation of the functional relevance of P-Bodies is an
151 active area of research, it is well established that these cytoplasmic granules also
152 undergo LLPS³⁴. Since the L1 RNP foci are not sensitive to 1,6 Hexanediol treatment
153 and most likely not undergoing LLPS, our data argues against L1 RNP foci being
154 components of P-body in mutant mESCs. Additionally, the protein ARGONAUTE2
155 (AGO2), a known component of P-bodies³⁵ and an effector of the miRNA biogenesis
156 pathway, is required for P-body formation^{36,37}. In *Dicer*_KO mESCs due to the
157 absence of miRNAs, AGO2 protein levels are reduced and the protein destabilized¹³
158 (Fig. 2B). However protein levels of DDX6, another known constituent of P-bodies³⁸,
159 were unchanged as compared to WT cells (Fig. 2B). We therefore looked at the cellular
160 localization of DDX6 to assess P-body integrity and association with L1 RNP foci.
161 Unlike WT cells where DDX6 formed droplet like foci characteristic of P-bodies in the
162 cytoplasm, in *Dicer*_KO cells, DDX6 was more diffusely localized in the cytoplasm. In
163 26-32% of *Dicer*_KO mESCs, multiple small DDX6 foci were observed co-localizing
164 with larger L1 Tf RNA foci (Fig. 2B). The partial co-localization with DDX6 in cells with
165 low AGO2 levels suggest that L1 RNP foci are not canonical P-bodies, corroborating
166 earlier studies enumerating the requirement of intact miRNA biogenesis in P-body
167 fidelity^{36,37}.

168 Finally, we ascertained that L1 RNP foci were not autophagosomes³⁹ as LC3B a
169 marker for autophagosomes did not co-localize with MOV10 in mESCs by IF
170 (Extended Data Fig. 1B). We therefore called L1 RNP present in cytoplasmic foci of
171 *Dicer*_KO mESCs, aggregates as they contain an assembly of RNA and proteins
172 without undergoing phase separation.

173 L1 upregulation is amongst the many changes in gene expression observed upon
174 deleting *Dicer* in mESCs¹³. To parse out whether as observed in human cultured cells
175 overexpression of L1s was sufficient for cytoplasmic sequestration^{23,27}, we
176 engineered WT mESCs to endogenously upregulate L1 using CRISPRa (L1^{UP}) (Fig.
177 3A, Extended Data Fig. 2A). We designed single guide RNAs (sgRNAs) to target
178 dCas9 fused with VP160 to the 5'UTR sequence of the L1 Tf family (Extended Data
179 Fig. 2B). For the generation of independent clones (CI), L1^{UP} CI1 cells were transfected
180 with one sgRNA, while two sgRNA pairs were used to upregulate L1 in L1^{UP} CI2. A
181 2.5-fold increase in L1 Tf transcript levels as compared to the control cell line (Ctrl)
182 transfected with an empty sgRNA vector was observed (Extended Data Fig. 2C) in
183 L1^{UP} clones. Given the sequence homology of the three L1 families, we also observed
184 a 3-fold increase in transcript levels of L1 A family, while the increased expression of
185 L1 Gf family was found to be statistically significant for only CI1 (Extended Data Fig.
186 2C). While L1 transcript levels in L1^{UP} cells was lower than in *Dicer*_KO (Extended
187 Data Fig. 2C), expression of L1 ORF1 protein in L1^{UP} was similar to that observed in
188 *Dicer*_KO cells (Fig 3A).

189 To assess if L1 elements upregulated with CRISPRa were competent for
190 retrotransposition, we primarily performed Northern Blot analysis and observed that
191 like in *Dicer*_KO mESCs, full length L1 transcripts were being overexpressed¹³
192 (Extended Data Fig. 2D). Importantly, this level of upregulation of L1 RNA was not
193 sufficient to cause L1 RNP accumulation in cytoplasmic aggregates in L1^{UP} mESCs
194 (Fig. 3B). Using a plasmid based retrotransposition assay⁴⁰, we tested if in the
195 absence of L1 RNP cytosolic sequestration there was an enhanced rate of L1
196 retrotransposition in the engineered L1^{UP} mESCs. We transfected Ctrl, L1^{UP} CI1 and
197 L1^{UP} CI2 with either wild type JJ-L1SM (L1WT) or a plasmid with mutation in ORF2
198 rendering it incompetent for jumping (L1N21A) that carried *Blasticidin* resistance
199 (BlastR) as a reporter gene and *Hygromycin* (HygR) as a selection marker⁴¹. Unlike
200 in *Dicer*_KO cells¹³, L1 upregulation was accompanied by an increase in the rate of
201 mobility depicted by the higher number of BlastR colonies observed in L1^{UP} CI1 and

202 CI2 as compared to Ctrl mESCs (Fig. 3C). BlastR colonies observed in the two L1^{UP}
203 cell lines transfected with L1N21A reporter confirm previous observation of
204 mobilization of mutant L1s aided by endogenous full length L1s in the cell, but at
205 relatively low frequencies ⁴². To conclude, forced endogenous upregulation of L1
206 active elements in WT mESCs is not sufficient to create L1 RNP cytoplasmic
207 aggregates and leads to an increase in retrotransposition.

208 Given that upregulation of L1 in mESCs was not sufficient to induce L1 RNP
209 aggregation in the cytoplasm (Fig. 3B), and our finding that MOV10 co-localized with
210 L1 RNP in *Dicer_KO* cells (Fig. 1B), we speculated that cytosolic aggregation of L1
211 RNP might be driven by the upregulation of MOV10 observed in *Dicer_KO* mESCs at
212 RNA and protein levels (Extended Data Fig. 2E, 3A). MOV10 upregulation in *Dicer_KO*
213 mESCs was confirmed by RTqPCR analysis (Extended Data Fig. 2F). In addition, no
214 changes in MOV10 expression were observed either at RNA (Extended Data Fig.2F)
215 or protein levels in L1^{UP} mESCs (Fig. 3A). We therefore ruled out L1 overexpression
216 as the driver for MOV10 upregulation and investigated the role of miRNAs in post-
217 transcriptional regulation of *Mov10* as miRNA biogenesis is impaired in *Dicer_KO*
218 mESCs.

219 Using TargetScan software ⁴³, we identified multiple miRNAs (miR-138-5p, miR-30-
220 5p, miR-16-5p and miR-153-5p) as predicted to target the 3'UTR sequence of *Mov10*
221 (Fig. 4A). The relative expression of each miRNA in WT cells was determined using
222 previously published small RNA sequencing data from our laboratory ⁴⁴ (Extended
223 Data Fig. 3A). MiR-16-5p and miR-30-5p are highly expressed in WT mESCs
224 compared to the intermediate expression of miR-138-5p, and the low expression of
225 miR-153-3p (Extended Data Fig. 3A). We tested whether the predicted miRNAs might
226 directly regulate *Mov10* expression by performing a luciferase reporter assay ⁴⁵. We
227 subcloned the 3'UTR sequence of *Mov10* downstream of the *Renilla luciferase*
228 reporter gene in a plasmid that also encoded *Firefly luciferase* as a normalizer.
229 Transient transfection of this plasmid along with the respective miRNAs into HEK293T
230 followed by measurement of the respective luminescence showed that for the tested
231 mimics, RENILLA expression was significantly sensitive to transfection with miR-16-
232 5p and miR-153-3p (Fig. 4B). To corroborate that the upregulation of MOV10 in *Dicer_*
233 *KO* cells is indeed mediated by miRNAs and is not a consequence of noncanonical
234 function of *Dicer*, we tested whether a similar upregulation of MOV10 is present in
235 *Drosha_KO* cells where the canonical miRNA biogenesis pathway is also impaired ⁴⁶.

236 Western blot (WB) analysis on *Drosha_KO* cells revealed that MOV10 is indeed
237 upregulated in these cells (Extended Data Fig. 3B). Finally, to confirm miRNA
238 mediated regulation of *Mov10* expression, we transiently transfected *Drosha_KO*
239 mESCs with the respective miRNA mimics either singly or in pairs and measured
240 MOV10 expression. Unlike previously observed with the luciferase assay, expression
241 of MOV10 was downregulated upon transfection with each of the four tested miRNA
242 mimics (Fig. 4C). Interestingly, only paired transfection of miR-16-5p with miR-138-5p
243 or miR-153-3p acted synergistically to reduce MOV10 protein levels down to WT levels
244 (Fig. 4C). Collectively, these data reveal a role for miRNAs in fine-tuning MOV10
245 expression in mESCs, explaining the observed MOV10 upregulation in *Dicer_KO* and
246 *Drosha_KO* mESCs (Fig. 3A, Extended Data Fig. 3B).

247 Given the upregulation of MOV10 and L1 ORF1 in *Drosha_KO* cells as compared to
248 WT (Extended Data Fig. 3B), we next assessed if L1 RNP correspondingly also
249 aggregate in the cytoplasm of these miRNA mutants. We performed IF with L1 ORF1
250 and MOV10 antibodies in two independent *Drosha_KO* clones and observed MOV10
251 co-localizing with L1 RNP in the cytoplasm of *Drosha_KO* mESCs (Extended Data Fig.
252 3C). The median ORF1-MOV10 aggregates per cell were 21 and 12 in *Drosha_KO1*
253 and *Drosha_KO2* mESCs respectively (Extended Data Fig. 3C). Percentage of cells
254 with large ORF1-MOV10 foci was 31-44% in the two *Drosha_KO* lines (Extended Data
255 Fig. 3C), similar to that observed in *Dicer_KO* cells (Fig. 1B).

256 To confirm our hypothesis that aggregation of L1 RNP driven by MOV10
257 overexpression was preventing L1 retrotransposition, we examined whether restoring
258 MOV10 expression in *Drosha_KO* cells would allow L1 mobilization. We used a
259 plasmid based retrotransposition assay⁴⁰ and transiently co-transfected *Drosha_KO1*
260 and *Drosha_KO2* with pCEP-L1WT reporter plasmid that carried *Neomycin* resistance
261 (NeoR)⁴¹ as a reporter along with either Ctrl mimic or mimics for miR-16-5p and miR-
262 153-3p together to downregulate MOV10 expression. 500,000 cells were plated for
263 each condition for the colony forming assay and media was supplemented with G418
264 39 hours post transfection. The mean NeoR colonies obtained 15 days post selection
265 were 25 and 23 in the two *Drosha_KO* clones transfected with Ctrl mimics from 3
266 independent experiments. A statistically significant increase in NeoR colonies in cells
267 transfected with miRNA mimics was observed with the mean increasing to 178 and
268 226 in the two clones respectively (Fig. 4D). Our results are in line with data from
269 human cancer cells supporting the role for *Mov10* as a negative regulator of

270 retrotransposition^{23,47–50}, and to our knowledge, the first to report a role for miRNAs in
271 fine-tuning *Mov10* expression.

272 Mature miRNAs might regulate multiple mRNAs and an mRNA can be targeted by
273 several miRNAs⁵¹. While we show that transfection with miR-16-5p and miR-153-3p
274 mimics downregulates MOV10 expression leading to increased L1 mobility, we cannot
275 unequivocally rule out that changes in expression of another gene targeted by these
276 miRNAs might be responsible for the observed increase in transposition. To assess if
277 MOV10 expression is sufficient to induce L1 RNP aggregation in the cytosol, we
278 transiently transfected Ctrl, L1^{UP} CI1, L1^{UP} CI2 mESCs with a plasmid encoding HA
279 tagged human MOV10 (HA-MOV10). In IF experiments with an antibody against HA
280 to detect exogenously expressed HA-MOV10 along with anti-L1 ORF1 antibody, we
281 detected HA-MOV10-ORF1 aggregates in the cytoplasm of L1^{UP} CI1 and L1^{UP} CI2
282 significantly more than in Ctrl cell line (P-val < 0.001). The median number of foci
283 observed in Ctrl was 6 per cell while in the two L1^{UP} clones this was 15 (Fig. 5A).
284 Additionally, the morphology of the larger HA-MOV10-ORF1 aggregates observed in
285 L1^{UP} clones was reminiscent of those observed in *Dicer*_{KO} mESCs (Fig. 5A, Fig. 1B).
286 To prove that MOV10 induced L1 RNP aggregation restricts L1 mobility, we then
287 transiently co-transfected Ctrl, L1^{UP}CI1 and L1^{UP}CI2 mESCs with JJ-L1WT reporter
288 plasmid that carries *BlastR reporter*⁴¹ along with either Empty Vector (EV) or HA-
289 MOV10 plasmids. The mean BlastR colonies was 35 and 29 for the two L1^{UP} clones
290 and 2 in Ctrl cells, corroborating our earlier observation of increased L1 mobility in
291 L1^{UP} cells as compared to Ctrl (Fig. 5B, Fig 3C). Importantly, a statistically significant
292 decrease in BlastR colonies was observed in L1^{UP} clones transfected with HA-MOV10
293 when compared to EV with a mean of 1 BlastR colony obtained from the transfection
294 in both the clones (Fig. 5B). Together, our data implicate that MOV10 is playing a
295 direct role in cytosolic sequestration of L1 RNP thereby restricting retrotransposition
296 and maintaining genome integrity in mESCs (Fig. 5C).

297

298 Discussion

299 The role of MOV10 in inhibiting retrotransposition in human tissue culture was
300 discovered almost ten years ago²³. Since then, multiple reports have corroborated
301 this seminal function, where it participates either directly or along with protein partners
302 in curbing retrotransposition^{47–50,52,53}. Here, we discover cytosolic-body formation
303 induced by MOV10 as a novel line of defense for sequestration of L1 RNP particles to

304 prevent deleterious L1 retrotransposition. It appears that L1 RNP aggregates in
305 miRNA mutant mESCs are different from those observed upon ectopic overexpression
306 of MOV10 and L1 in human cancer cells as the latter unlike in our study were found to
307 be stress granules.

308 MOV10 is a known interactor of proteins that are a part of the miRNA induced silencing
309 complex (RISC) and plays an important role in mRNA decay⁵⁴. It also localizes with
310 AGO and TNRC6 proteins in P-bodies⁵⁵. L1 ORF1 protein has been previously
311 reported to interact with P-body enriched proteins and RNA^{27,33}. We hypothesize that
312 the absence of AGO2 and mature miRNAs in the miRNA mutant mESCs prevent P-
313 body formation and hinders similarly L1 ORF1 partitioning and LLPS. We think that
314 the observed aggregates in mESCs have evolved as a specialized compartment
315 where diverse activities for L1 RNP metabolism are brought together, which will
316 require further dissection. MOV10 is a 5' to 3' RNA helicase⁵⁶ and its catalytic activity
317 is essential for inhibiting human L1 retrotransposition²³. Whether this activity is
318 essential for inducing L1 RNP aggregate formation could provide further mechanistic
319 insight.

320 Given the plethora of functions MOV10 has been implicated in, it is not surprising that
321 mechanisms have evolved to regulate its expression and activity⁵³. Post-translational
322 modification of MOV10 occurs via ubiquitination in neuron cultures derived from rat
323 hippocampus resulting in its degradation⁵⁷. Moreover, phosphorylation and
324 acetylation of MOV10 have been observed to occur in human cancer cell lines and
325 speculated to regulate its activity and levels⁵³. Data presented here, to the best of our
326 knowledge, is a first to unveil miRNA mediated post-transcriptional regulation of
327 *Mov10* expression. Since MOV10 expression levels observed in *Dicer_KO* were
328 higher than those in *Drosha_KO* mESCs (Extended Data Fig. 3B) it is possible that
329 expression of MOV10 might also be modulated by microprocessor independent
330 miRNAs. While transient transfection with all four tested miRNAs resulted in
331 downregulation of MOV10, the absence of synergistic effect for miR-16-5p and miR-
332 30-5p may rise from the inherent closeness of the two MREs in the 3'UTR of *Mov10*
333 causing steric hindrance and preventing the large RISC complex from binding the two
334 simultaneously. MREs in *Mov10* for all four tested miRNAs miR-138-5p, miR-30-5p,
335 miR-16-5p and miR-153-3p in mESCs are conserved in the 3'UTR sequence of
336 hMOV10, raising the possibility that this mechanism regulating MOV10 expression
337 may also be conserved in humans. Of note miR-138-5p and miR-153-3p are highly

338 expressed in the human brain ⁵⁸ and both miRNAs are downregulated in brain
339 pathologies from Alzheimer's Disease patients ^{59,60}. Activation of expression and
340 mobility of transposable elements has been reported in a majority of neurological
341 disorders ⁶¹ and certain cancers ⁶². In case the mode of L1 regulation uncovered here
342 in mESCs is conserved, fine-tuning MOV10 expression in disease conditions using
343 miRNA mimics to downregulate or conversely Antagomirs to upregulate MOV10
344 expression can afford novel means of therapy.

345

346 **Material and Methods**

347 **Cell culture**

348 E14TG2a mESC (ATCC CRL-1821) were used as wild type cells. *Dicer_KO* ¹³ and
349 *Drosha_KO* ⁴⁶ were previously generated from E14TG2a in our laboratory using a
350 paired CRISPR-Cas9 approach ⁶³. Cells were cultured in Dulbecco's Modified Eagle's
351 Medium (DMEM) (Invitrogen) supplemented with 15% pre-selected batch of FBS
352 (GIBCO) tested for optimal mESCs growth, 1000 U/mL of LIF (Millipore), 0.1 mM of 2-
353 β -mercapto-ethanol (Life Technologies), 0.05 mg/mL of streptomycin, and 50 U/mL of
354 penicillin (Sigma). For routine culturing cells were grown on 0.2% gelatin-coated cell
355 culture grade plastic vessels in the absence of feeder cells. For microscopy coverslips
356 were coated with 10 μ g/ml Fibronectin (Sigma, FC010) for at least 2 hours at 37°C,
357 coverslips were washed three times with 1x PBS and cells were seeded 16-18 hours
358 before processing them for microscopy. HEK293T cells were grown in Dulbecco's
359 Modified Eagle's Medium (DMEM) (Invitrogen) supplemented with 10% FBS (GIBCO),
360 0.05 mg/mL of streptomycin, and 50 U/mL of penicillin (Sigma). Concentration of
361 various antibiotics used were as follows 1 μ g/ml Puromycin (Sigma), 100 μ g/ml
362 Hygromycin (Invitrogen), 250 μ g/ml G418 (Sigma), 50 μ g/ml Blastidin (Invitrogen).

363

364 **Plasmids**

365 3'UTR sequence of mouse MOV10 transcript ENSMUST00000168015.8 was PCR
366 amplified using Fwd 5'-taggcgatcgctcgaggccacagccgccgctt-3' and Rev 5'-
367 ttgcggccagcggccttttgcataaacagcattttgt-3' primers using cDNA generated with
368 random primers from mESCs as template. The PCR product was subcloned into
369 plasmid psiCHECK2 (Promega) previously digested with NotI using the In-Fusion
370 cloning kit (Takara Bio) giving rise to plasmid psiCHECK2-mMov10-3'UTR (addgene
371 178905). Human MOV10 was PCR amplified with primers Fwd 5'-

372 ggtcggaggcggatccatgccagtaagttcagctgc-3' and Rev 5'-
373 gatatctgcagaattctcagagctcattcctccactc-3' using plasmid pFLAG/HA-MOV10 (addgene
374 10976)⁶⁴ as template and subcloned into BamH1 and Xho1 digested pCDNA3-T11-
375 HA plasmid⁶⁵ a kind gift from Prof. Polymenidou using In-Fusion cloning kit (Takara)
376 to yield plasmid pCDNA3-T11HA-hMOV10-WT (addgene 178907) for transient
377 transfections to over express MOV10 in L1^{UP} and Ctrl cells. Plasmids used for the
378 retrotransposition assay with mneo1 cassette as reporter was pCEP-L1SM (hygro)
379 and with mblast1 cassette was JJ-L1SM WT and JJ-L1SM N21A (hygro), all gifts from
380 Prof. Garcia-Perez.

381

382 **Generation of L1^{UP} mESCs using CRISPRa**

383 L1^{UP} mESCs were generated from E14TG2a mESCs using the CRISPRa approach
384 ⁶⁶. Single gRNAs (sgRNAs) were designed using the L1 Tf consensus sequences
385 (Extended Data Fig. 2B)⁶⁷. Sequence alignments^{6,67,68} were performed using T-
386 Coffee⁶⁹. SgRNAs to upregulate L1 Tf were individually sub-cloned into the plasmid
387 pKLV-U6gRNA(BbsI)-PGKpuro2ABFP a gift from Prof. Yusa (addgene 50946),⁷⁰,
388 using the BbsI restriction site. Guide sequence used for generating Cl1 was 5'-
389 caccgccagagaacctgacagcttc-3' (addgene nb pending) For Cl2 two guide pairs were
390 used 5'-caccgccagagaacctgacagcttc-3' (addgene nb pending, same as for Cl1) and
391 5'-caccagaggacaggtgcccgcctt-3' (addgene nb pending). AC95-pmax-dCas9VP160-
392 2A-neo was a gift from Prof. Jaenish (addgene 48227)⁶⁶. Cells were transfected with
393 1 µg of each plasmid and 24h hours post transfection they were cultured in presence
394 of puromycin (1 µg/mL) and G418 (250 µg/mL). Single clones were picked one week
395 post transfection. The first screening for selection of L1^{UP} candidates was performed
396 at the protein level for ORF1 expression by immunoblot analysis.

397

398 **Ectopic protein expression**

399 L1^{UP} and Ctrl mESC lines were transiently transfected with 2 µg T11HA-hMOV10
400 plasmid (addgene nb pending) for ectopic expression of hMOV10 or T11HA-EV⁶⁵ as
401 empty vector control using Lipofectamine 3000 (Invitrogen). Transfection complex was
402 removed 6 hours post transfection. Cells were trypsinized 32 hours post transfection
403 and plated on fibronectin coated cover slips. Samples were processed 48 hours post
404 transfection for Indirect Immunofluorescence (IF).

405

406 **MiRNA mimic transfections in mESCs**

407 100,000 *Drosha*_KO mESCs were seeded per well in a 6 well plate in duplicate for
408 respective miRNA mimic transfections. Cells were grown in antibiotic free media and
409 transfected with 20 nM mimic when transfected singly or 10 nM respective mimic for
410 dual transfections using RNAimax reagent (Invitrogen). Cells were harvested 39 hours
411 post transfection and duplicate samples were pooled for protein extraction and
412 subsequent western blot analysis. The following miRNA mimics (Dharmacon, A
413 horizon discovery Group company) were used:

414 mmu-miR-16-5p 5'-UAGCAGCACGUAAAUUUGGCG-3' (C-310511-05-05)

415 mmu-miR-30e-5p 5'-UGUAAACAUCUUGACUGGAAG-3' (C-310466-07-0002)

416 mmu-miR-138-5p 5'-AGCUGGUGUUGUGAAUCAGGCCG-3' (C310414-07-0002)

417 mmu-miR-153-3p 5'-UUGCAUUAGUCACAAAAGUGAUC-3'(C310428-05-0002)

418 miRIDIAN microRNA negative control 1 (CN-001000-01-05)

419

420 **Indirect Immunofluorescence (IF)**

421 Cells grown on coverslips were washed with 1x PBS, fixed with 3.7% formaldehyde
422 (Sigma) in 1x PBS for 10 minutes at room temperature. Post fixation cells were washed
423 three times in 1x PBS and permeabilized with CSK buffer (100 mM NaCl, 300 mM
424 sucrose, 3 mM MgCl₂, 10 mM PIPES pH 6.8, 0.5% Triton-X) for 4 minutes on ice. After
425 three further washes with 1x PBS, blocking was initiated in 1x PBS supplemented with
426 1% BSA and 0.1% Tween-20 for 30 minutes at room temperature. Samples were
427 incubated with primary antibody diluted in blocking buffer for 1 hour at room
428 temperature, there after washed three times with 1x PBS-0.1% Tween-20, incubated
429 with secondary antibody diluted in blocking solution for 1 hour and counterstained with
430 100ng/ml DAPI (Sigma) in 1x PBS for 4 minutes before mounting on slides in
431 Vectashield (Vector labs). The following primary antibodies diluted in blocking buffer
432 were used: rabbit polyclonal anti-ORF1p (1:1000 kind gift from Donal O'Carroll),
433 mouse monoclonal 15C1BB anti-MOV10 (1:500, A500-009A-T Bethyl Laboratories
434 Inc), rabbit polyclonal anti-G3BP1 (1:500 A302-033A, Bethyl Laboratories Inc), rabbit
435 polyclonal anti-LC3B antibody (1:250, 2775, Cell Signaling Technology), rabbit
436 polyclonal anti-DDX6 (1:500, GTX102795, GeneTex), rat monoclonal anti-HA (1:500,
437 3F10, Roche). Secondary antibody used were Alexa fluor 488 goat anti-rat IgG
438 (1:4000, 11006, life Technologies), Alexa fluor 488 donkey anti-mouse IgG (1:4000,
439 A21202, Life technologies), Alexa fluor 546 donkey anti-rabbit IgG (1:4000, A10040,

440 Life technologies), Alexa fluor 647 donkey anti-mouse IgG (1:4000, A31571, Life
441 technologies). Images were acquired using the Deltavision multiplex system equipped
442 with an Olympus 1X71 (inverse) microscope, pco.edge 5.5 camera and 60x 1.4NA
443 DIC Oil PlanApoN objective. Z stacks were taken 0.2 μm apart, images de-convolved
444 using Softworx software. Further image analysis and processing were performed using
445 ImageJ. Excel (Microsoft) and Prism 9 (Graphpad) were used for data analysis and
446 statistical testing.

447

448 **Combined RNA FISH and IF**

449 Cells grown on coverslips were first processed for IF following the protocol described
450 above except all buffers and solution other than the fixative were also supplemented
451 with 10 mM Ribonucleoside Vanadyl Complex (NEB). After incubation with the
452 secondary antibody, cells were fixed with 3.7% formaldehyde in 1x PBS for 10 minutes
453 at room temperature and blocked in 1x PBS supplemented with 1% BSA, 0.1% Tween-
454 20, 2 mM Glycine and 10 mM RVC for 15 minutes. Cells were next washed and
455 incubated in 2x SSC (0.03 M Sodium citrate in 0.3 M Sodium chloride) for 5 minutes.
456 Probe specific for Tf L1 family was labeled with Red-dUTP (Enzo Life sciences) using
457 a nick translation kit (Abbot). 2 μg TFkan plasmid kind gift from Prof. Heard^{71,72} as
458 incubated with 0.2 mM labelled dUTP, 0.1 mM dTTP, 0.1 mM dNTP mix and 2.5 μl
459 nick translation enzyme in a 50 μl final volume as per guidelines from the kit. The
460 reaction was incubated at 15°C for 15 hours. A PCR purification column (zymogen)
461 was used to clean the probe which was eluted in 50 μl water. The volume of the probe
462 was decreased down to 5 μl using a speed vac, and the probe was diluted in 100 μl
463 hybridization solution (1 part 20x SSC, 2 parts 10 mg/ml BSA, 2 parts 50% Dextran
464 sulfate and 5 parts deionized formamide). The probe solution was denatured at 78°C
465 for 5 minutes, placed on ice for 5 minutes and 7 μl probe was spotted on a pre-baked
466 slide for each sample. During the overnight hybridization at 37°C in a humid chamber
467 the overturned coverslips were sealed using rubber cement. Post hybridization
468 washes were performed with 50% formamide in 2x SSC thrice for five minutes followed
469 by 3 washes with 2x SSC. DNA was counterstained with 100 ng/ml DAPI in 2x SSC
470 and mounted on slides with Vectashield. Image acquisition and analysis was as for IF.

471

472 **Western blot analysis**

473 Total cellular protein was extracted from mESC pellets using a NP40 based lysis buffer
474 (1% NP40, 137 mM NaCl, 20 mM Tris-HCl, 1 mM EDTA) complemented with EDTA-
475 free protease inhibitor cocktail (Roche). Protein concentrations were determined by
476 Bradford Assay (Bio-Rad). 10-20 µg of total cellular protein were separated in 8% or
477 10% SDS-PAGE gels and transferred on PVDF membranes. The following antibodies
478 were used: rabbit polyclonal anti L1 ORF1p (1:5000, gift from Donal O'Carroll), rabbit
479 polyclonal anti-Dicer (1:2000, SAB42000087, Sigma), rabbit polyclonal anti-
480 Argonaute2 (1:2000 C34C6 Cell Signaling Technologies), rabbit anti-Drosha (1:2000,
481 D28B1 Cell Signaling Technology), rat monoclonal anti-HA (1:500, 3F10, Roche),
482 Mouse anti-Tubulin antibody (1:10000, A01410, GenScript), rabbit anti-LaminB1
483 (1:5000, ab16048, Abcam), rabbit anti-DDX6 (1:2000, GTX102795 GeneTex), anti-
484 rabbit IgG HRP-linked (1:10000 7074, Cell Signaling Technologies), anti-mouse IgG
485 HRP-linked (1:10000, 7076, Cell Signaling Technologies), anti-rat IgG HRP-linked
486 antibody (1:10000, 7077, Cell Signaling Technologies). Immunoblot blot were
487 developed using the Clarify™ Western ECL substrate (BioRad) kit or SuperSignal™
488 West Femto Maximum Sensitivity Substrate (Thermo Scientific) and detected using
489 ChemiDoc™ MP imaging system (BioRad). All membranes were stained with
490 coomassie to ensure equal loading.

491

492 **RT qPCR analysis**

493 Total cellular RNA was extracted from cell pellets using TRizol® Reagent (Life
494 Technologies). Extract quality was verified by loading 1 µg of total cellular RNA on a
495 1% Agarose gel. 1 µg cellular RNA was treated with DNase (RQ1 Rnase-Free DNase
496 kit Promega) and reverse-transcribed following the GoScript™ Reverse Transcriptase
497 Kit (Promega) manufacturer's instructions. The produced cDNAs were diluted five-fold
498 in distilled water. For each extract, PCR on the *Rrm2* gene were performed, with and
499 without reverse transcriptase treatment, to insure absence of genomic DNA
500 contamination. The quality-controlled cDNAs were diluted two times in distilled water.
501 Amplifications were performed on the Light Cycler® 480 (Roche) using 2 µL of the
502 diluted cDNAs and the KAPA SYBR® FAST qPCR Kit Optimized for Light Cycler®
503 480 (KAPA biosystems). Differences between samples and controls were calculated
504 based on the $2^{-\Delta CT}$ method. RT-qPCR assays were performed in biological triplicate.
505 Primers utilized for the RT-qPCR assays are as follows: *Rrm2* fwd 5'-
506 ccgagctggaaagtaaagcg-3', *Rrm2* rev 5'-atgggaaagacaacgaagcg-3', *Mov10* fwd 5'-

507 gacgatttacaaccacgacttca-3', Mov10rev 5'-gccagatttgcgatcttcattcc-3', Dicerfwd 5'-
508 ccgatgatgcagcctctaataag-3' Dicerrev 5'-tccatctcgagcaattctctca-3', L1-Tffwd 5'-
509 cagcggtcgccatcttg-3', L1-Tfrev 5'-caccctctcacctgttcagactaa-3',
510 L1-Afwd 5'-ggattccacacgtgatcctaa-3', L1-Arev 5'-tcctctatgagcagacctgga-3', L1-Gffwd
511 5'-ctccttggtccgggact-3', L1-Gfrev 5'-caggaaggtggccggttg-3', L1-ORF1fwd 5'-
512 actcaaagcaggcaacact-3' L1-ORF1rev 5'-ctttgattgttgccgatg-3', L1-ORF2fwd 5'-
513 ggagggacatttcattctcatca-3', L1-ORF2rev 5'-gctgctctgtattggagcataga-3'.

514

515 **Northern Blot analysis**

516 Northern blot analysis was performed as previously described^{13,73}. 30 µg of total RNAs
517 extracted using Trizol were run on a denaturing 1% Agarose gel with 1%
518 Formaldehyde. Following capillary transfer to nylon membranes overnight the
519 membrane was cross-linked by UV radiation. PerfectHybTM Plus was used for pre
520 hybridization blocking and hybridization at 42°C. Post hybridization washes were
521 performed in 2x SSC + 0.1% SDS. For detection of full-length L1 transcripts, random
522 primer extension labeling was carried out. DNA used for the reaction was PCR
523 amplified using E14TG2a mESCs genomic DNA as template and L1specific primers
524 Fwd 5'-gagttttgagtctgtatcc-3' and Rev 5'-ctctccttagtttcagtgg-3'.

525

526 **Dual luciferase reporter assay**

527 70,000 HEK293T cells were plated per well in a 24 well plate 16 hours prior to
528 transfection with Lipofectamine 2000 (Invitrogen). 0.5 µg of plasmid psiCHECK2-
529 3'UTR-WT-Mov10'UTR was co-transfected with 50 nM indicated miRNA mimics or
530 control mimic. Transfection complexes were removed 6 hours post transfection.
531 Luciferase activity was measured on a GloMax[®] Discover Multimode Microplate
532 Reader (Promega, USA) after processing cells using the Dual-Glow Luciferase Assay
533 kit (E2920 Promega, USA) 48 hours post transfection. Results are means and error
534 bars are standard deviation (SD) from three to four independent experiments.

535

536 **Retrotransposition reporter and colony forming assays**

537 1×10^6 L1^{UP} and Ctrl mESCs were seeded in 10 cm dish 16 hours prior to transfection
538 with 6 µg of JJ-L1SM (WT and L1N21A) plasmid using Lipofectamine 3000
539 (Invitrogen). Media exchange was initiated 6 hours post transfection and hygromycin

540 supplemented media was added 48 hours post transfection to select for stably
541 transfected cells. Once the mock transfected cells were dead, 150,000 hygromycin
542 resistant cells were seeded per well in a 6 well plate in triplicate and grown in media
543 *sans* hygromycin for 16 hours after which the media was supplemented with
544 Blastidicin. Media exchange with fresh antibiotics was performed every 48 hours for
545 approximately 15 days, when individual Blastidicin resistant colonies were visible with
546 the naked eye. Cells were washed with 1x PBS and stained with 1% crystal violet blue,
547 1% formaldehyde, 1% methanol for 20 minutes at room temperature, followed by
548 washes with tap water. Plates were air dried and imaged using the ChemiDoc™ MP
549 system (BioRad). Individual colonies were counted using ImageJ. Results are means
550 and error bars are SD from three independent transfections.

551 Transient transfections of reporter plasmids were carried out using Lipofectamine
552 3000 (Invitrogen) when co-transfections with miRNA mimics or plasmids for ectopic
553 expression of hMOV10 were assayed for retrotransposition. 500,000 cells were
554 seeded for transient transfection with 6 µg of reporter plasmid and either 10 nM mimic
555 for mmu-miR-16-5p + 10 nM mmu-miR-153-3p mimic or 6 µg of plasmid T11HA-EV or
556 T11HA-hMOV10. Media exchange was initiated 6 hours post transfection. 39 hours
557 post transfection cells were grown in media supplemented with antibiotic resistance
558 encoded by the respective cassette. Subsequent media exchanges, staining and
559 counting of colonies, was the same as stated for stably transfected cells. Results are
560 means and error bars are SD from three independent transfections.

561

562 **Acknowledgments**

563 We would like to thank the members of the Ciaudo lab and Dr Tobias Beyer for fruitful
564 discussions and the critical reading of this manuscript. This work was supported by
565 the Swiss National Science Foundation (grants 31003A_173120 and 310030_196861)
566 and Novartis Foundation for medical-biological Research (grant 19A018) to C.C. In
567 addition, R.A and C.C. were supported by the NCCR RNA and Disease. We also want
568 to thank the ScopeM facility at ETH Zürich for their support with microscopy and image
569 analysis.

570

571 **Author Contributions**

572 Conceptualization, RA, MB and CC, laboratory experiments RA, MB, LP, CH; writing
573 original draft preparation, RA and CC; writing, review and editing, CC; visualization,
574 RA, MB and CC; supervision, CC; funding acquisition, CC. All authors have read and
575 agreed to the published version of the manuscript.

576

577 Declaration of Interests

578 The authors declare no financial and non-financial competing interests.

579

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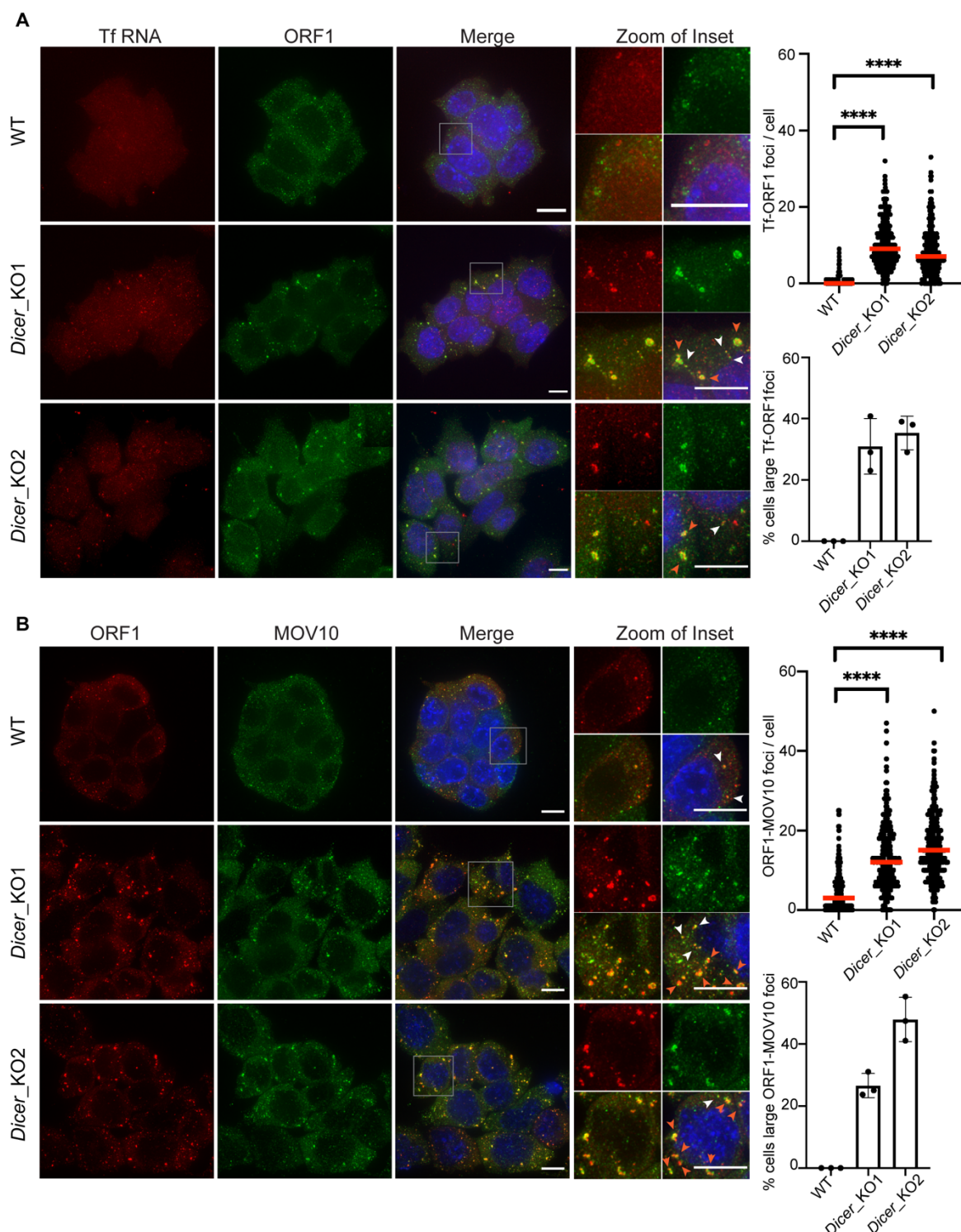
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732 **Figure 1**



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734 **Figure 1. L1 RNP accumulate as cytoplasmic foci in *Dicer_KO* mESCs** (A) Maximum intensity
 735 projections across Z stacks of example images from indicated mESCs stained for L1 Tf RNA (red)
 736 combined with immunostaining for L1 ORF1 protein (green) and nuclei stained with DAPI (blue). The
 737 grey square marks position of the inset. White arrow heads point to cytoplasmic foci where L1 RNA and
 738 ORF1 protein co-localize. Red arrow heads point to relatively larger sized L1 RNP foci. Data collected
 739 from 275 WT, 304 *Dicer_KO1*, 311 *Dicer_KO2* cells from three independent experiments are depicted

740 as scatter plots where circles are single data points representing number of co-localized L1 Tf-ORF1
741 foci in the cytoplasm per cell, red bar is median for the distribution. P-value was determined using Mann-
742 Whitney *U* test and **** represent p-value < 0.0001. In *Dicer_KO* cells, L1 RNA and protein co-localize
743 in variably sized foci in the cytoplasm. Bar graphs are mean values of percentage of cells with large L1
744 Tf-ORF1 foci co-localizing in the cytoplasm. Dots represent data from three independent experiments,
745 error bars are standard deviations. Scale bar 5 μ m. **(B)** Maximum intensity projections across Z stacks
746 of example images from indicated mESCs immunostained for L1 ORF1 (red), MOV10 (green) and
747 nuclei stained with DAPI (blue). The grey square marks position of inset in the zoomed image. White
748 arrow heads point to cytoplasmic foci where L1 ORF1 and MOV10 co-localize. Red arrow heads point
749 to relatively larger sized L1 ORF1-MOV10 foci. Data collected from 293 WT, 295 *Dicer_KO1*, 295
750 *Dicer_KO2* cells from three independent experiments are depicted as scatter plots where circles are
751 single data points representing number of co-localized L1 ORF1-MOV10 foci in the cytoplasm per cell,
752 red bar is median for the distribution. In *Dicer_KO* cells, L1 ORF1 and MOV10 proteins co-localize in
753 the cytoplasm. P-value was determined using Mann-Whitney *U* test and **** represent p-value < 0.0001.
754 Bar graphs are mean values of percentage of cells with large L1 ORF1-MOV10 foci co-localizing in the
755 cytoplasm. Dots represent data from three independent experiments, error bars are standard
756 deviations. Scale bar 5 μ m.

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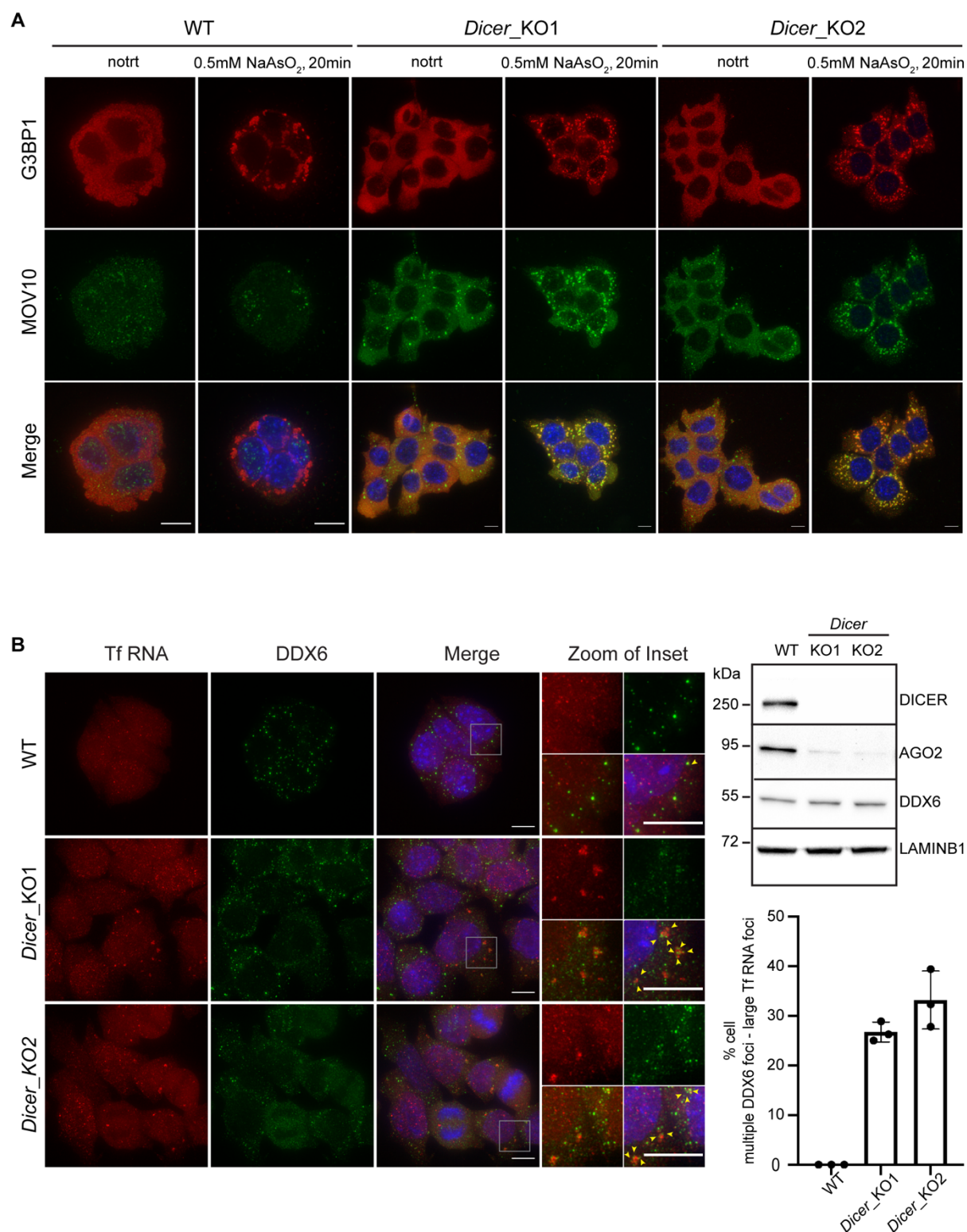
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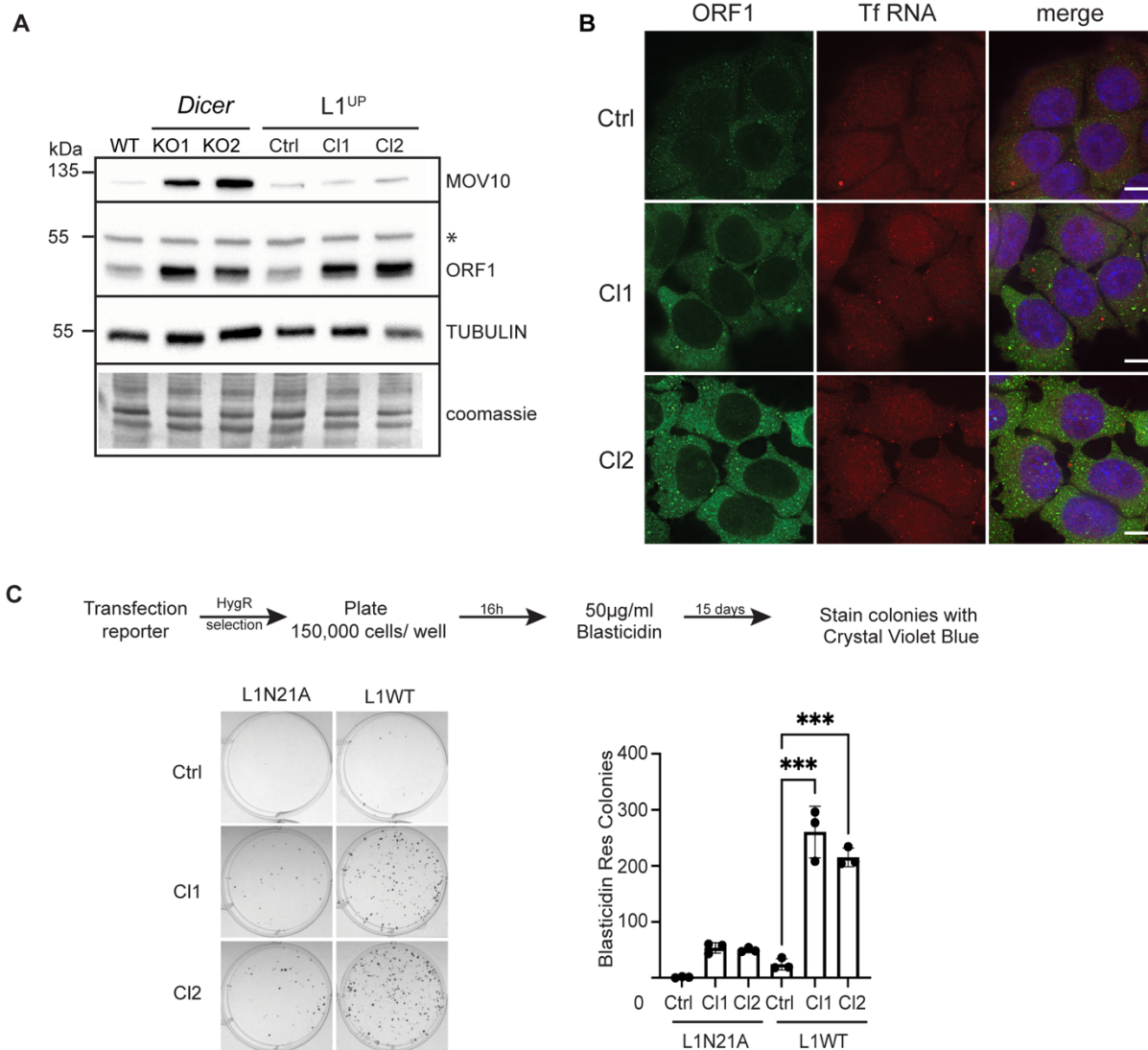
776 **Figure 2**



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 778 **Figure 2. Cytosolic L1 RNP foci are poised to be stress granules that co-localize with multiple**
 779 **small DDX6 foci (A)** WT and *Dicer_KO* mESCs were treated with 0.5mM Sodium Arsenite (NaAsO₂)
 780 for 20 minutes or left untreated prior to fixation with formaldehyde. Maximum intensity projections across
 781 Z stacks of example images from indicated mESCs immunostained for G3BP1 (red) and MOV10
 782 (green) with nuclei stained with DAPI (blue). Diffused cytoplasmic staining of G3BP1 was observed in

783 all untreated samples, while MOV10 was found to localize in cytoplasmic foci in *Dicer*_KO cells.
784 Treatment with Sodium Arsenite resulted in co-localization of G3BP1 and MOV10 in stress granules in
785 *Dicer*_KO cells. Images are representative of 3 independent experiments. **(B)** Representative Western
786 Blots out of 3 independent experiments showing low AGO2 protein levels in *Dicer*_KO as compared to
787 WT mESCs (right side). No change in protein levels for DDX6 was observed, LAMINB1 served as
788 loading control. Immunoblotting with Anti-DICER antibody was performed to confirm the fidelity of the
789 KO clones. On the left side, maximum intensity projections across Z stacks of example images from
790 indicated mESCs stained for L1 Tf RNA FISH (red) combined with immunostaining for a resident protein
791 of P-bodies, DDX6 (green) and nuclei stained with DAPI (blue). The grey square marks position of the
792 inset. Yellow arrow heads point to cytoplasmic foci where L1 RNA and DDX6 protein co-localize.
793 Multiple small DDX6 foci were observed to co-localize with large L1 Tf RNA foci in the cytoplasm of
794 *Dicer*_KO but not in WT mESCs as depicted in the bar graph. Dots represent data from three
795 independent experiments with percentage computed from at least 94-150 cells per cell line per
796 experiment, error bars are standard deviations. Scale bar 5 μ m.
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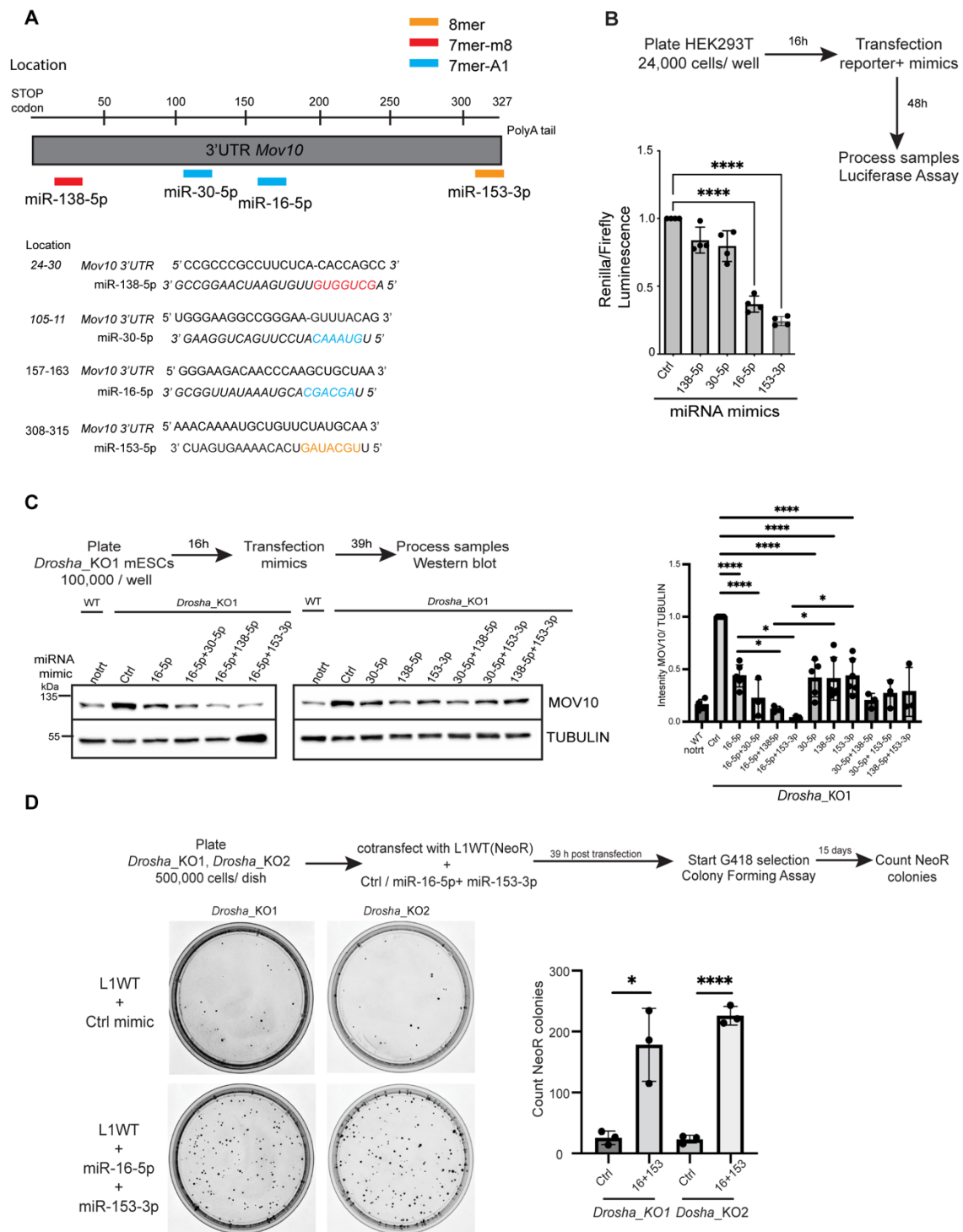
798 **Figure 3**



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800 **Figure 3. Endogenous L1 upregulation leads to L1 retrotransposition (A)** Representative Western
 801 Blots out of 3 independent experiments showing L1 ORF1 and MOV10 protein levels in the indicated
 802 cell lines. Immunoblot with antibody recognizing TUBULIN and coomassie stained membranes depict
 803 the loading, asterisk marks position of non-specific band in the ORF1 immunoblot. While higher L1
 804 ORF1 levels are observed in *Dicer*_{KO} and *L1^{UP}* cells compared to WT and Ctrl cells respectively,
 805 MOV10 overexpression is only observed in *Dicer*_{KO} cells. **(B)** Maximum intensity projections across
 806 Z stacks of example images from indicated mESCs immunostained for L1 ORF1 protein (green)
 807 combined with RNA FISH for L1 Tf RNA (red) and nuclei stained with DAPI (blue). No aggregation of
 808 L1 RNP in the cytoplasm is observed in *L1^{UP}* cells. Scale bar 5 μ m. **(C)** Representative images of BlastR
 809 colonies stained with crystal violet blue of indicated cell lines is shown on the left. Cells were transfected
 810 with either mutant reporter plasmid (*L1N21A*) or retrotransposition competent reporter (*L1WT*) as
 811 shown in the scheme with timeline for the experiment on the top. Bar graphs on the right depict the
 812 average number of BlastR colonies, dots are mean values obtained from 3 independent experiments,
 813 error bars are standard deviations. P-value was determined using unpaired student t-test and ***
 814 represent p-value < 0.001.

815 **Figure 4**



816

817 **Figure 4. Multiple miRNAs regulate MOV10 expression and L1 retrotransposition in mESCs (A)**

818 Schematic of 3'UTR sequence of *Mov10* RNA helicase. Location of miRNA response elements (MREs)

819 for mouse miR-138-5p (red), miR-30-5p (blue) miR-16-5p (blue) and miR-153-3p (orange) predicted to

820 target *Mov10* (ENSMUST00000168015.8) are color coded based on seed type matching for respective

821 miRNAs. (B) Schematic showing design and timeline of luciferase assay performed in human Hek293T

822 cells to assay direct miRNA mediated repression of *Mov10*. Bar graphs show the average relative

823 luminescence of reporter gene *Renilla* to which the 3'UTR sequence of *Mov10* was fused, normalized

824 by Firefly luminescence, where relative ratio observed for transfection with control (Ctrl) mimic was set
825 to 1. Each dot on the bar graph is the mean from 4 independent experiments, errors are standard
826 deviation. P-values were calculated using an unpaired t-test and **** are p-values < 0.0001. Renilla
827 expression was sensitive to transfection with miR-16-5p and miR-153-3p. **(C)** Schematic showing
828 design and timeline for processing samples for WB analysis in *Drosha_KO1* cells. *Drosha_KO1* were
829 transfected either with Ctrl mimic or with indicated miRNA mimics either singly or in pairs. For
830 comparison protein from untreated (notrt) WT cells was also run on the same blot. Blots were probed
831 with anti-MOV10 and anti-TUBULIN antibodies. Bar graphs show mean intensity of MOV10 normalized
832 by TUBULIN from 3 independent experiments relative to transfection for the Ctrl mimic that was set to
833 1. P-values were computed using ordinary one-way ANOVA test comparing the mean of each sample
834 to the mean of Ctrl, and the mean of doubly transfected mimic with its singly transfected counterpart. *
835 depict p-value < 0.05, and **** depict p-value < 0.0001. MOV10 expression was found to be sensitive
836 to transfection with all four transfected mimics. MiR-16-5p was found to down-regulate expression of
837 MOV10 synergistically with both miR-138-5p and miR-153-3p, to levels similar to those observed in WT
838 mESCs. **(D)** Schematic summarizing the experiment and timeline followed for colony forming assay in
839 *Drosha_KO* mESCs transfected with either Ctrl mimic or miRNA mimics for miR-16-5p + miR-153-3p
840 along with L1WT plasmid bearing NeoR gene as reporter. Representative images of NeoR colonies
841 stained with crystal violet blue of indicated cell lines are shown on the left. Bar graphs on the right depict
842 the average number of NeoR colonies, dots are mean values obtained from 3 independent experiments,
843 error bars are standard deviations. P-value was determined using unpaired student t-test and *
844 represent p-value < 0.05, **** represent p-value < 0.0001. Downregulation of *Mov10* expression due to
845 transfection with indicated miRNA mimics in *Drosha_KO* cells resulted in an increase in the rate of
846 mobility of L1 elements.

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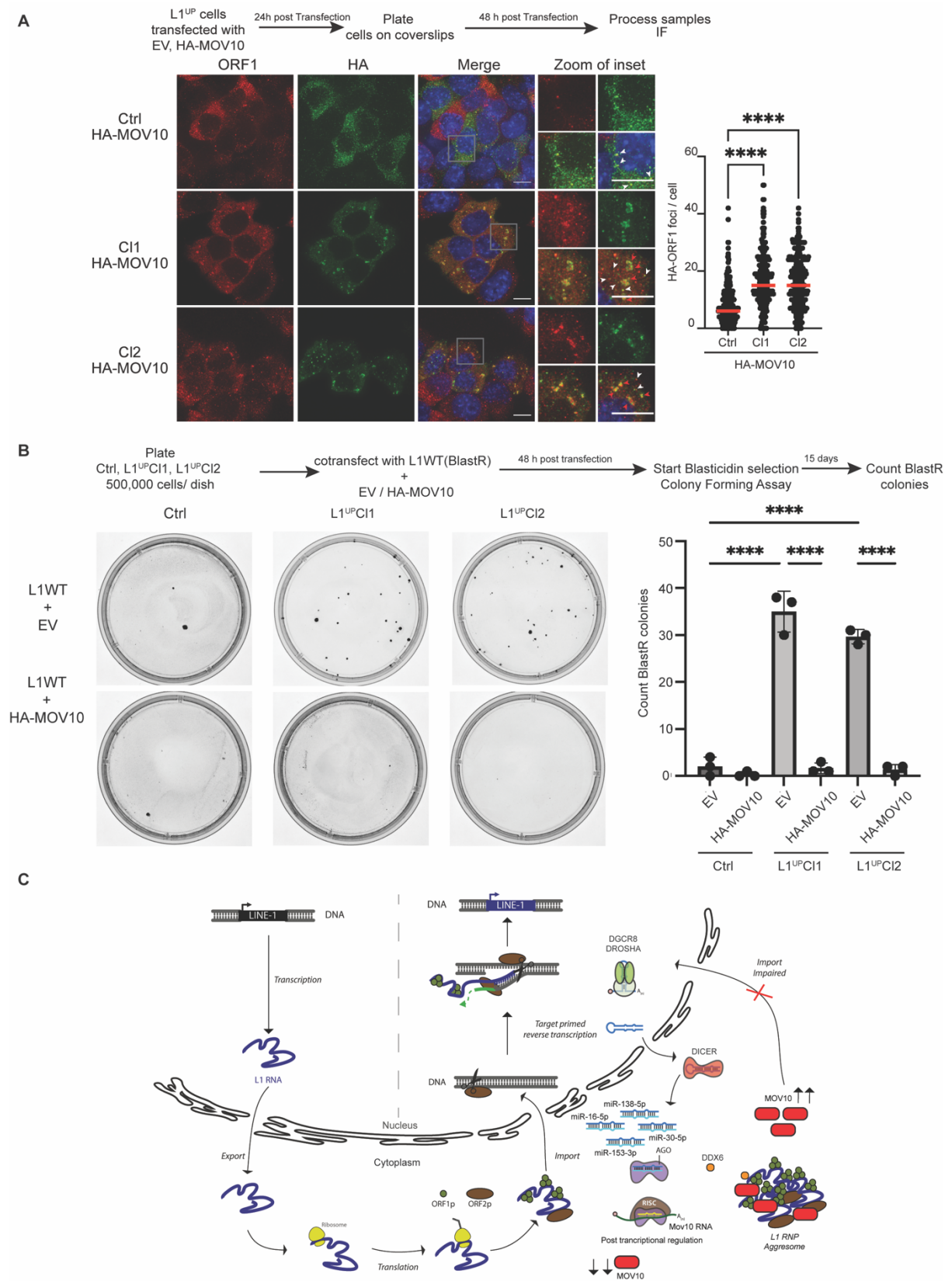
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861 **Figure 5**



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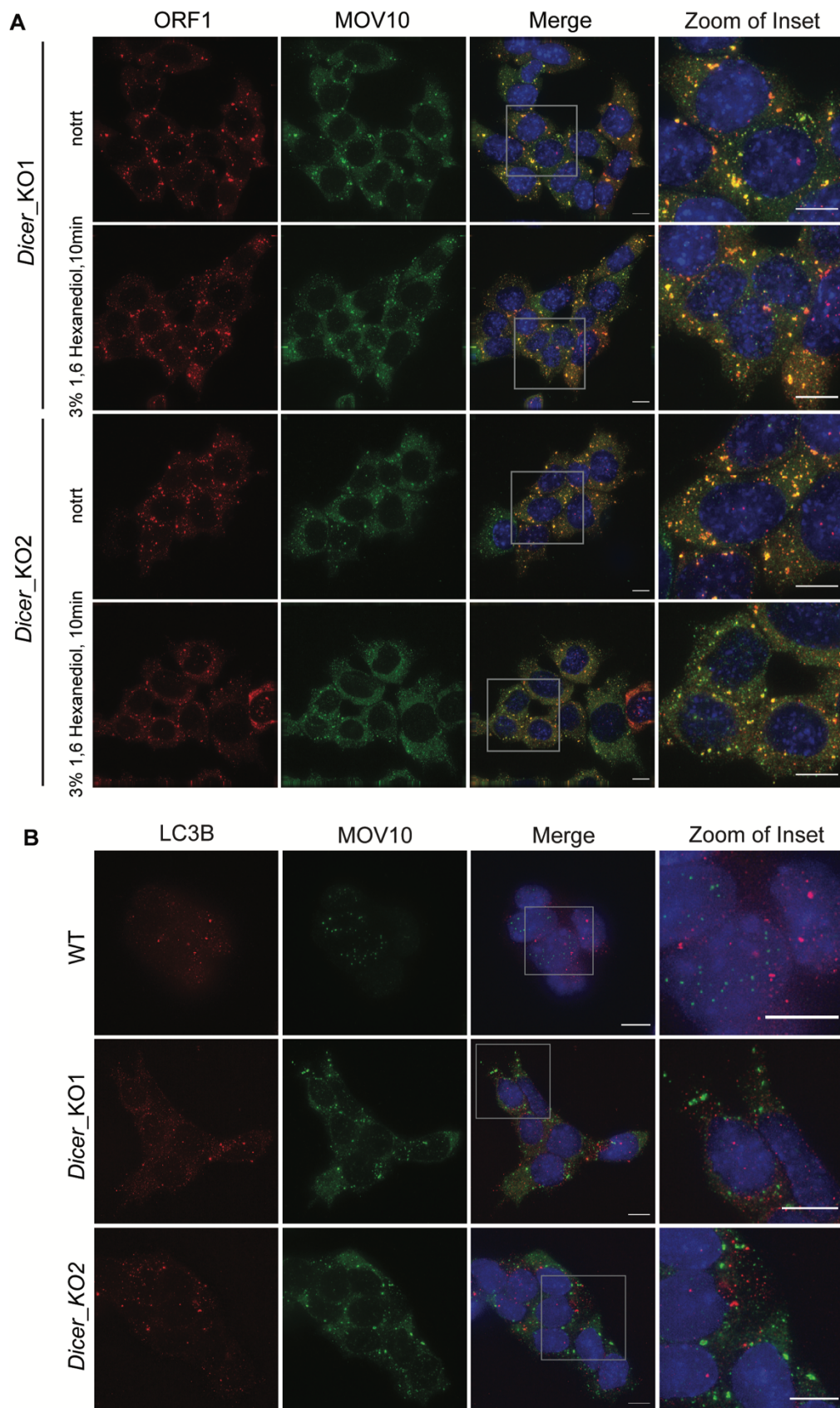
863 **Figure 5. MOV10 upregulation is sufficient to create L1 RNP aggregates in L1^{UP} mESCs**

864 **abrogating L1 retrotransposition. (A)** Scheme for transfection and timeline for processing samples

865 for IF. Maximum intensity projections across Z stacks of example images from indicated mESCs stained

866 for L1 ORF1 (red) combined with immunostaining for HA (green) to detect ectopically expressed MOV10
867 tagged with HA at the N-terminus, and nuclei stained with DAPI (blue). White arrow heads point to
868 cytoplasmic foci where L1 ORF1 and HA-MOV10 protein co-localize. Red arrow heads point to relatively
869 larger sized HA-ORF1 foci. Data collected from 289 Ctrl, 275 L1^{UP} CI1 and 296 L1^{UP} CI2 mESCs from
870 three independent experiments are depicted as scatter plots where circles are single data points
871 representing number of co-localized HA-ORF1 foci in the cytoplasm per cell. Red bar marks median for
872 the distribution. P-value was determined using Mann-Whitney *U* test and **** represent p-value <
873 0.0001. Statistically significant increase in cytoplasmic L1 ORF1 aggregates was observed upon
874 ectopic expression of HA-MOV10 in L1^{UP} as compared to Ctrl mESCs. **(B)** Schematic summarizing the
875 experiment and timeline followed for colony forming assay in Ctrl and L1^{UP} mESCs transfected with
876 either Empty Vector (EV) or HA-MOV10 plasmid along with L1WT plasmid bearing BlastR as reporter.
877 Representative images of BlastR colonies stained with crystal violet blue of indicated cell lines are
878 shown on the left. Bar graphs on the right depict the average number of BlastR colonies, dots are mean
879 values obtained from 3 independent experiments, error bars are standard deviations. P-value was
880 determined using unpaired student t-test and, **** represent p-value < 0.0001. Upregulation of MOV10
881 in L1^{UP} mESCs restricted L1 retrotransposition. **(C)** The life cycle of L1 retrotransposition is depicted.
882 Only full length L1 elements get transcribed driven by the promoter residing in its 5'UTR sequence. The
883 bicistronic L1 RNA is exported from the nucleus into the cytosol and translated to give rise to L1 ORF1
884 (ORF1p) and L1 ORF2 (ORF2p) proteins. The L1 RNA and proteins form a complex (L1 RNP) and are
885 imported back into the nucleus. Endonuclease activity of ORF2 nicks the target DNA and using a
886 mechanism referred to as Target primed reverse transcription a new copy of L1 element is inserted into
887 the genome via a copy past mechanism of mobilization^{3,12}. A key regulatory step for retrotransposition
888 is the import of L1RNP back into the nucleus. The canonical miRNA biogenesis pathway illustrates the
889 miRNAs discovered in this study that regulate expression of RNA helicase *Mov10* a known modulator
890 of L1 mobility. In the absence of miRNAs when either DICER or DROSHA proteins are deleted in
891 mESCs, both L1 and MOV10 expression are upregulated. Our data suggests that in microRNA mutant
892 mESCs MOV10 induces L1 RNP aggregate formation in the cytoplasm, the impaired import
893 consequently prevents L1 retrotransposition despite high L1 expression. While DDX6 was also found
894 to co-localize with the larger L1 RNP particles, identification of molecular partners and biochemical
895 activities intrinsic to the L1 RNP aggregates should unveil the bottle neck afforded to prevent import.
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906 **Extended Data Fig. 1**



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908 **Extended Data Fig. 1. L1 RNP cytosolic aggregates are not sensitive to treatment with 3% 1,6**

909 **Hexanediol (A)** WT and *Dicer_KO* mESCs were treated the 3% 1,6 Hexanediol for 10 minutes to

910 assess ability of L1 RNP to phase separate. Maximum intensity projections across Z stacks of example
911 images from indicated mESCs immunostained for L1 ORF1 (red) and MOV10 (green) with nuclei
912 stained with DAPI (blue). The lack of any discernible change in L1 ORF1-MOV10 foci formation
913 indicates absence of LLPS for L1 ORF1-MOV10 foci. Images are representative of 3 independent
914 experiments. Grey box mark position of the insets. **(B)** Maximum intensity projections across Z stacks
915 of example images from indicated mESCs immunostained for LC3B (red) and MOV10 (green) with
916 nuclei stained with DAPI (blue). The absence of any co-localization of LC3B with MOV10 in the tested
917 cell lines indicate that the L1 RNP foci are not autophagosomes. The grey square depicts position of
918 inset. Images are representative of 3 independent experiments. Scale bar 5 μ m.

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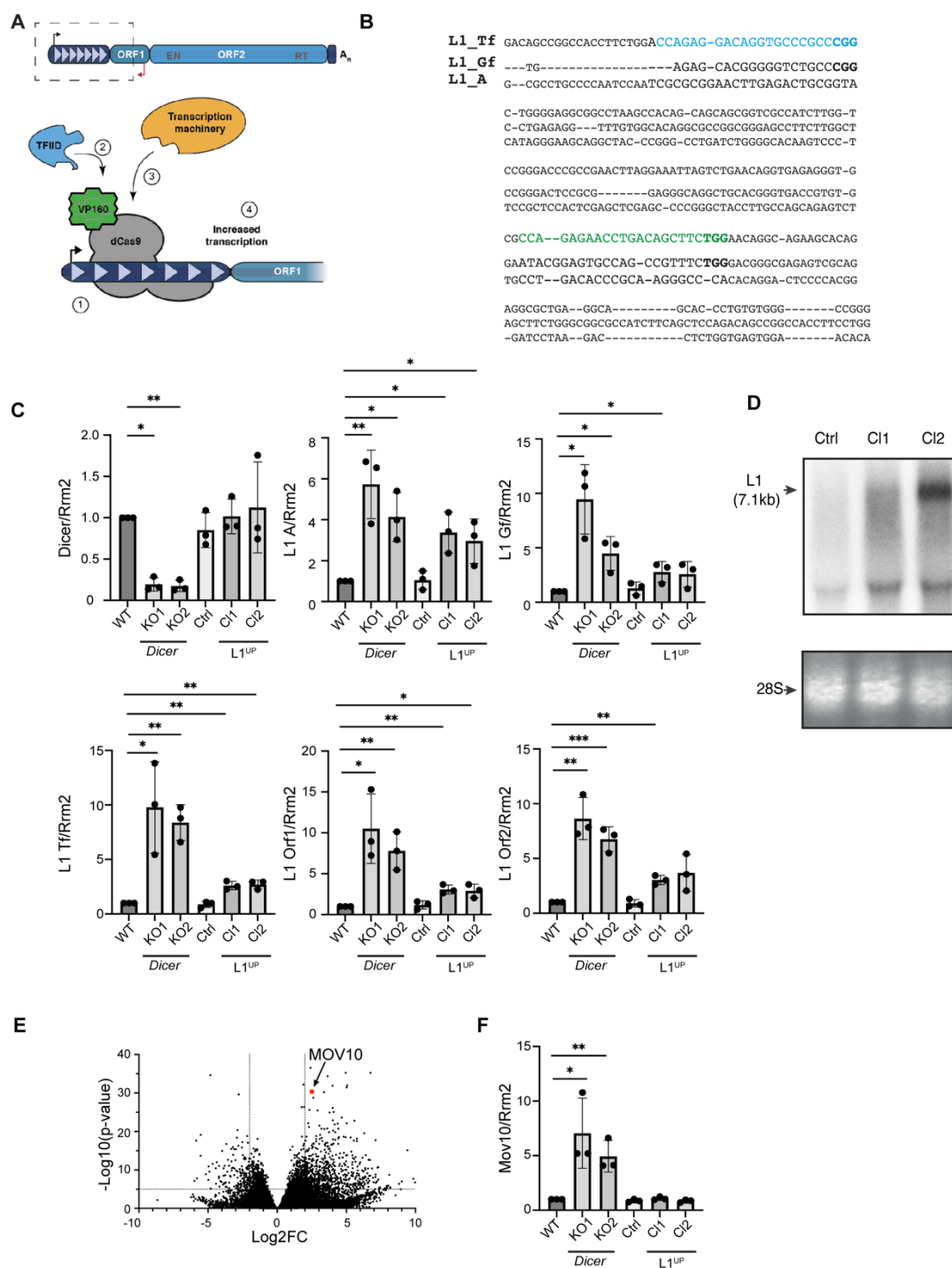
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945 **Extended Data Fig. 2**



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 947 **Extended Data Fig. 2. Generation of mESCs upregulating L1 expression using CRISPRa (A)**
 948 Schematic depicting full length L1 element and summary of CRISPRa. To generate L1^{UP} cells, mESCs
 949 were co-transfected with plasmid encoding catalytically dead Cas9 protein (dCas9) fused to VP160 and
 950 sgRNAs that (1) targeted the fusion protein to the 5'UTR sequence of Tf L1 family allowing (2)
 951 recruitment of transcription factors and (3) transcription machinery to (4) upregulate L1 transcription.

952 **(B)** Sequence alignment of 5'UTR sequences of murine L1 Tf, Gf and A subfamilies. The two sgRNA
953 sequences used to upregulate L1 expression are indicated in blue and green, with protospacer adjacent
954 motifs (PAM) in bold. **(C)** RT qPCR analysis to quantitate *Dicer*, and L1 RNA expression levels in the
955 depicted cell lines. *Rrm2* was utilized for normalization, and graphs depict fold change in transcript
956 levels in the indicated cell lines as compared to WT which was set to one. Bar graphs show means from
957 3 independent experiments, error bars are standard deviations, p-values were computed using unpaired
958 t-test comparing results from individual cells to WT mESCs. Asterisk are p-values * < 0.05, ** < 0.001,
959 *** < 0.0005. **(D)** Northern blot analysis probed for L1 RNA to assess L1 transcript length and expression
960 levels in the engineered L1^{UP} CI1, CI2 as compared to Ctrl cells. Arrow points to full length L1 transcript.
961 Ethidium bromide staining of 28S RNA was used to confirm equal loading. **(E)** Differential gene
962 expression from RNA-seq analysis of *Dicer*_KO vs. WT mESCs plotted using previously published data
963 ¹³. Each dot represents a single gene. Position for *Mov10* in the graph is marked. Values for Log2 fold
964 change (Log2FC) were plotted on the x-axis and Log10 of the p-value on the y-axis. **(F)** RT qPCR
965 analysis to confirm upregulation of *Mov10* in *Dicer*_KO cells. No change in *Mov10* transcript levels were
966 observed in Ctrl, L1^{UP} CI1, CI2 as compared to WT mESCs. *Rrm2* was utilized for normalization, and
967 graphs depict fold change in transcript levels in the indicated cell lines as compared to WT which was
968 set to one. Bar graphs show means from 3 independent experiments, error bars are standard
969 deviations, p-values were computed using unpaired t-test comparing results from individual cells to WT
970 mESCs. Asterisk are p-values * < 0.05, ** < 0.001.

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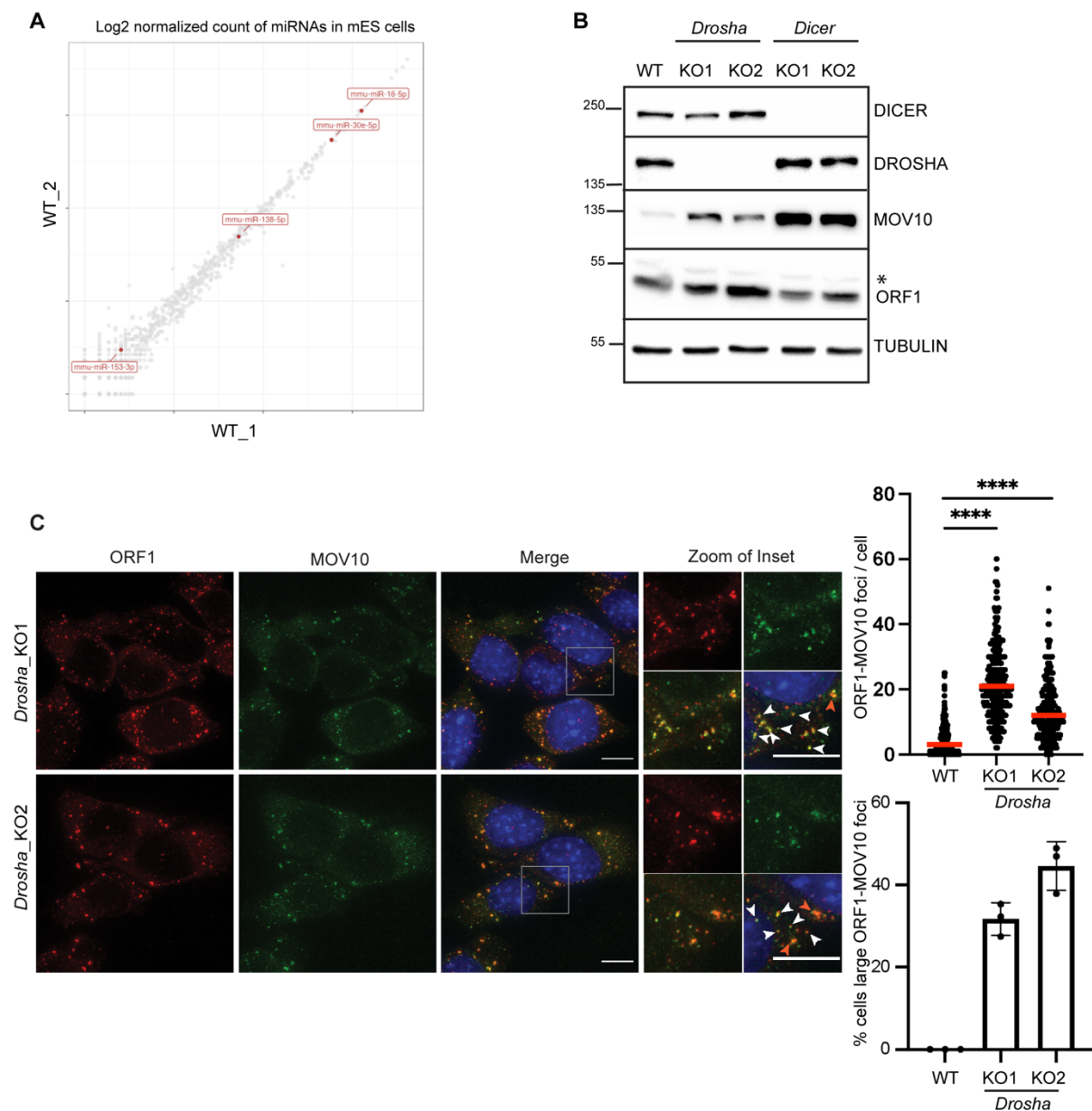
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989 **Extended Data Fig. 3**



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 991 **Extended Data Fig. 3. Upregulation of L1 ORF1 and MOV10 in the absence of miRNAs in**
 992 ***Drosha*_KO mESCs induces L1 RNP aggregation in the cytoplasm (A)** Log2 normalized count of
 993 miRNAs in WT mESCs from previously published small RNA-seq data ⁴⁴. Each dot depicts a single
 994 miRNA and miRNAs predicted using TargetScan ⁴³ to regulate *Mov10* expression are shown in red.
 995 **(B)**Western Blot analysis to assess expression of L1 ORF1 and MOV10 in the indicated cell lines,
 996 immunoblot with TUBULIN served to control for loading. Membranes were probed with anti-DICER and
 997 anti-DROSHA antibodies to confirm the deletion status of the cells. Upregulation of L1 ORF1 and of
 998 MOV10 was observed in *Drosha*_KO relative to WT mESCs. Asterisk marks non-specific band
 999 recognized by ORF1 antibody **(C)** Maximum intensity projections across Z stacks of example images
 1000 from *Drosha*_KO mESCs immunostained for L1 ORF1 (red), MOV10 (green) and nuclei stained with
 1001 DAPI (blue). White arrow heads point to cytoplasmic foci where L1 ORF1 and MOV10 co-localize. Red
 1002 arrow heads point to relatively larger sized L1 RNP foci. Data collected from 288 *Drosha*_KO1, 299

1003 *Drossha*_KO2 cells from three independent experiments are depicted as scatter plots where circles are
1004 single data points representing number of co-localized L1 ORF1-MOV10 foci in the cytoplasm per cell,
1005 red bar is median for the distribution. Data for WT cells for comparison is the same as in Figure 1B. P-
1006 value was determined using Mann-Whitney *U* test and **** represent p-value < 0.0001. Bar graphs are
1007 mean values of percentage of cells with large L1 ORF1-MOV10 foci co-localizing in the cytoplasm. Dots
1008 represent data from three independent experiments, error bars are standard deviations. Scale bar 5
1009 μm .
1010