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Ablation of ZC3H11A causes early embryonic lethality and dysregulation of metabolic processes

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15 **Abstract**

16 ZC3H11A is a stress-induced mRNA binding protein required for efficient growth of nuclear-
17 replicating viruses, while being dispensable for the viability of cultured human cells. The cellular
18 functions of ZC3H11A during embryo development are unknown. Here we report the generation
19 and phenotypic characterization of *Zc3h11a* knock-out mice. Heterozygous null *Zc3h11a* mice
20 were born at the expected frequency without distinguishable phenotypic differences compared
21 with wild-type. In contrast, homozygous null *Zc3h11a* mice were missing, indicating that *Zc3h11a*
22 is crucial for embryonic viability and survival. *Zc3h11a*^{-/-} embryos were detected at the expected
23 Mendelian ratios up to late preimplantation stage (E4.5). However, phenotypic characterization at
24 E6.5 revealed degeneration of *Zc3h11a*^{-/-} embryos, indicating developmental defects around the
25 time of implantation. Transcriptomic analyses documented a dysregulation of glycolysis and fatty
26 acid metabolic pathways in *Zc3h11a*^{-/-} embryos at E4.5. Proteomic analysis indicated a tight
27 interaction between ZC3H11A and mRNA-export proteins in embryonic stem cells. Furthermore,
28 CLIP-seq analysis demonstrated that ZC3H11A binds a subset of mRNA transcripts that are
29 critical for metabolic regulation of embryonic cells. Altogether, the results show that ZC3H11A is
30 participating in export and post-transcriptional regulation of selected mRNA transcripts required
31 to maintain metabolic processes in embryonic cells. While ZC3H11A is essential for the viability
32 of the early mouse embryo, inactivation of *Zc3h11a* expression in adult tissues using a conditional
33 knock-out did not lead to obvious phenotypic defects.

34 **Introduction**

35 The zinc finger CCCH domain-containing protein 11A (ZC3H11A) is a stress-induced mRNA-
36 binding protein that is required for the efficient growth of several human nuclear replicating
37 viruses, including human immunodeficiency virus (HIV-1), influenza A virus (IAV), human
38 adenovirus (HAdV) and herpes simplex virus 1 (HSV-1) [1]. Proteomic studies on human cells
39 have indicated that ZC3H11A is a component of the transcription-export (TREX) complex [2].
40 Functional studies indicated that ZC3H11A selectively export newly transcribed viral mRNAs to
41 the cytoplasm during virus infection [1, 3]. Thereby, inactivation of ZC3H11A in human cells
42 impaired the export of a subset of viral mRNA transcripts and resulted in a dramatic reduction in
43 virus growth [1]. These important functions of ZC3H11A in the growth cycle of several human
44 viruses makes ZC3H11A a potential target for development of an anti-viral therapy. The aim of
45 the present study was to develop an animal model to study the molecular functions of ZC3H11A
46 in prenatal and postnatal development.

47 The TREX complex serves a key function in nuclear mRNA export and consists of multiple
48 conserved core subunits including ALYREF (RNA binding adaptor of TREX), UAP56 (DEAD-
49 box type RNA helicase) and a stable subcomplex called THO, which in turn consists of at least six
50 subunits [4, 5]. Proteomic studies using human cells have indicated that ZC3H11A is an auxiliary
51 component of the TREX complex, but did not consider it as a core subunit of the TREX complex
52 [6, 7]. THO proteins are conserved from yeast to human and play pivotal roles during embryo
53 development, cell differentiation and cellular response to stimuli [8, 9]. It has been reported that
54 the disruption of THO proteins, such as THOC1, THOC2 or THOC5, leads to early embryonic
55 lethality [9–11]. The TREX complex controls the mRNA export in a selective manner, where
56 individual TREX components appear to be required for export of distinct subsets of mRNAs [12].

57 For instance, THOC2 or THOC5 are required for the export of mRNAs essential for pluripotency
58 such as *Nanog*, *Sox2* and *Klf4* in mouse embryonic stem cells [9]. Despite several reports
59 characterizing the role of THO proteins during embryogenesis, the cellular function of ZC3H11A
60 during embryo development is unknown.

61 In the current study, we established *Zc3h11a* knock-out (KO) mouse models to study the
62 effect of *Zc3h11a* loss of function on embryo development. Our results identify ZC3H11A as a
63 fundamental protein required for early embryo growth. Disruption of ZC3H11A is homozygous
64 lethal and leads to complete failure of embryo development and survival. Using proteomic and
65 RNA-seq analyses, we show that the ZC3H11A protein interacts with TREX complex core
66 proteins in mouse embryonic stem cells. ZC3H11A is apparently an auxiliary factor participating
67 in export and post-transcription coordination of selected mRNA transcripts required to maintain
68 the metabolic processes in embryonic cells. Interestingly, *Zc3h11a* inactivation in adult mouse
69 tissues using an inducible mouse model showed that the ZC3H11A protein is dispensable for
70 postnatal tissue growth.

71 72 **Results**

73 74 ***Zc3h11a* inactivation in mice is lethal in the homozygous condition**

75 *Zc3h11a* is located on chromosome 1 in both human and mouse genomes and harbors the coding
76 sequence of another gene encoding the DNA-binding zinc-finger protein ZBED6 [13–18] (Figure
77 1A). We used two strategies to target the *Zc3h11a* coding exons without affecting *Zbed6*. The first
78 *Zc3h11a* mouse model was developed by targeting exon 3 using the CRISPR/cas9 system with
79 two guide RNAs flanking the targeted sequences. This resulted in both a deletion of 567 bp
80 including the entire exon 3 and a frameshift (Figure 1B). The second mouse model was developed
81 by inserting loxP sites flanking the coding sequence of exon 2 using homologous recombination

82 (Figure 1C). These loxP mice were crossed with mice expressing Cre recombinase in germ-line
83 (PGK-Cre), which resulted in a deletion of 1.5 kb containing exon 2 and removal of the zinc finger
84 domains of the encoded ZC3H11A protein (Figure 1C). For each model, heterozygous mice were
85 crossed and the offspring were genotyped. No *Zc3h11a*^{-/-} mice were obtained from heterozygous
86 matings (Figure 1D and E), with the exception of one single homozygous *Zc3h11a*^{-/-} female from
87 the loxP mouse model (1 out of 204 mice). When we crossed this KO female with *Zc3h11a*^{+/-}
88 males, 10 out of 10 progeny were heterozygous *Zc3h11a*^{+/-}. The probability to get this outcome if
89 both parents are heterozygous is $P=0.5^{10}=0.001$. The result confirms our interpretation that one
90 single homozygous KO survived and were fertile.

91

92 ***Zc3h11a* deletion results in embryonic degeneration**

93

94 In order to explore at what point ZC3H11A is essential for embryo survival, we collected and
95 genotyped embryos at different time points post *Zc3h11a*^{+/-} X *Zc3h11a*^{+/-} mating (Figure 2A).
96 The genotyping of embryos at embryonic day E4.5 prior to implantation revealed expected
97 Mendelian proportions (Figure 2A). However, a clear deviation from expected Mendelian
98 proportions was observed after implantation (Figure 2A, bottom panel). Remarkably, phenotyping
99 at E6.5 showed dramatic changes in the morphology of the *Zc3h11a*^{-/-} embryos with a large degree
100 of tissue degeneration, whereas *Zc3h11a*^{+/-} heterozygotes appeared morphologically
101 indistinguishable from the WT embryos (Figure 2B).

102

103 **ZC3H11A is highly expressed at early stages of embryonic development**

104 The lethal effect of *Zc3h11a* inactivation in mouse embryos encouraged us to explore the cellular
105 localization of ZC3H11A at early embryonic stages. We used immunofluorescence (IF) staining

106 to visualize the ZC3H11A protein and the nuclear speckles marker SRSF2 (SC35) for expression
107 profiling in mouse 2-cell and blastocyst stages. The IF analysis indicated that ZC3H11A was
108 expressed at a detectable level as early as the 2-cell stage, with clear nuclear localization (Figure
109 3A, top panel). The z-stack imaging of the blastocysts showed that ZC3H11A was expressed in
110 trophectoderm (Figure 3A, middle panel) as well as in inner cell mass (ICM) (Figure 3A, bottom
111 panel). The localization pattern of ZC3H11A in ICM was overlapping with the nuclear speckles
112 as indicated using the anti-SC35 antibody (Figure 3A and B). This subcellular localization of
113 ZC3H11A in mouse embryonic cells is similar to the ZC3H11A localization in human cell lines
114 [1]. Re-analyzing single cell RNA-seq data from Deng *et. al.* [19] revealed that *Zc3h11a* mRNA
115 is highly expressed in mouse embryos as early as the zygotic stage, indicating maternal
116 contribution (Figure 3C).

117

118 **Disrupted metabolic pathways in the *Zc3h11a*^{-/-} embryos**

119 The degeneration of *Zc3h11a*^{-/-} embryos during early embryo development (E6.5) encouraged us
120 to perform whole transcriptome analysis of stage E4.5 embryos to reveal the dysregulated
121 pathways that led to the degeneration of *Zc3h11a*^{-/-} embryos at E6.5. We collected embryos from
122 *Zc3h11a*^{+/-} X *Zc3h11a*^{+/-} matings and extracted the RNA from the embryonic part for sequencing
123 (Figure 4A, left). Principle component analysis (PCA) of RNA-seq data showed that Het
124 (*Zc3h11a*^{+/-}) and WT (*Zc3h11a*^{+/+}) embryos clustered together and apart from the KO (*Zc3h11a*^{-/-})
125 embryos (Figure 4A). This result is in agreement with the observed morphological similarity
126 between WT and Het embryos (Figure 2B). Furthermore, the differential expression (DE) analysis
127 between WT and Het did not detect any significant DE genes with FDR <0.05. Therefore, we
128 performed the DE analysis between KO embryos vs. WT and Het embryos that revealed 660 DE

129 genes (FDR <0.05) out of ~11,000 expressed genes (Table S1). Among these DE genes, 419 were
130 up-regulated and 241 were down-regulated in KO embryos (FDR <0.05). Next, we performed a
131 gene set enrichment analysis (GSEA) using the DE genes in KO embryos in order to further
132 explore the function of ZC3H11A. The GSEA of ranked DE genes in KO embryos using the
133 hallmark gene sets revealed a significant negative enrichment (FDR <0.05) of genes involved in
134 glycolysis, fatty acid metabolism pathways and epithelial-mesenchymal transition (EMT)
135 processes (Figures 4B-4D). The heatmaps present the expression of the subset of genes that
136 contributed the most to the indicated pathway enrichment among significantly down-regulated
137 genes in KO embryos (Figures 4B-4D). Among the key down-regulated genes, contributing to the
138 significant GSEA result, are lactate dehydrogenase A (*Ldha*), which has an essential role in
139 glycolysis, and disruption of *Ldha* causes congenital disorders of carbohydrate metabolism [20,
140 21]; enoyl-CoA hydratase and 3-hydroxyacyl CoA dehydrogenase (*Ehhadh*), which is involved in
141 fatty acid beta-oxidation using acyl-CoA oxidase [22, 23]; and dickkopf WNT signaling pathway
142 inhibitor 1 (*Dkk1*), which is involved in several processes including cell fate determination and
143 cell differentiation processes during embryogenesis [24]. On the other hand, the positively
144 enriched gene sets among up-regulated genes in the KO mice included genes in the P53 pathway
145 and autophagy process-related genes (Figures 5A, 5B and S1). This includes the up-regulation of
146 autophagy related 12 (*Atg12*) and microtubule-associated proteins 1A/1B light chain 3A
147 (*Map1lc3a*) genes (Figure 5E). MAP1LC3A is known as LC3A protein and is required for
148 autophagosome formation [25].

149 To get further insight on which cell type in blastocysts was most affected by *Zc3h11a*
150 inactivation, we explored the expression profile of the DE genes in *Zc3h11a*^{-/-} embryos in
151 embryonic lineages (ICM and epiblast) and trophoctoderm (TE). Using previously published

152 datasets of mouse gene expression (GSE76505 [26]), the ICM/TE ratio of expression was
153 computed for genes down-regulated and up-regulated in the KO embryos ($FDR \leq 0.05$, fold change
154 ≥ 2). We also explored the expression of DE genes at earlier stages using gene expression dataset
155 (E-MTAB-2950) [27]. This showed that down-regulated genes in *Zc3h11a*^{-/-} embryos are
156 primarily expressed in the ICM/early epiblast rather than trophoctoderm (Figure 5C), while the
157 expression of the up-regulated genes is nearly equally present in ICM/early epiblast and TE (Figure
158 S2). The down-regulated genes in *Zc3h11a*^{-/-} embryos with high expression in the ICM include:
159 *Ldha*, teratocarcinoma-derived growth factor (*Tdgfl*, *Cripto*), growth differentiation factor 3
160 (*Gfd3*), phosphofructokinase (*Pfkp*) (Figure 5D). GDF3 is an analog of NODAL and uses TDGF1
161 as co-factor [28]. Both *Ldha*, *Pfkp*, *Pfkm* and *Pdk2* are down-regulated in *Zc3h11a*^{-/-} embryos and
162 are involved in glycolysis and lactate production, as indicated in the GSEA (Figure 4). At peri-
163 implantation stages, there is a major metabolic switch from oxidative phosphorylation to anaerobic
164 glycolysis, with increased lactate production [29, 30]. *Cripto/Tdgfl* has been reported as an
165 essential factor regulating this metabolic switch [31]. That explains the GSEA results that show
166 that the down-regulated pathways mostly concern metabolic regulation processes. Altogether, this
167 strongly suggests that the primary consequence of ZC3H11A deficiency is in the ICM, due to
168 perturbed metabolic regulation. The enrichment of genes associated with autophagy and apoptosis-
169 related pathways (Figure 5A-5B) among the up-regulated genes in *Zc3h11a*^{-/-} embryos could be
170 secondary effect caused by the metabolic stress encountered by the ICM cells [32, 33].

171

172 **ZC3H11A is associated with the RNA export machinery in embryonic stem cells**

173 In human somatic cells, ZC3H11A has been recently characterized as an RNA-export protein that
174 functions through its interaction with TREX complex proteins [1]. In order to identify its

175 interacting partners in embryonic cells and to investigate if ZC3H11A maintains its association
176 with the TREX complex in mouse embryonic stem cells (mESCs), we performed co-
177 immunoprecipitations (co-IPs) using anti-ZC3H11A, anti-THOC2 and anti-IgG antibodies
178 followed by mass spectrometry (MS) analyses (Figure 6A). Statistical analyses of detected MS
179 intensities from the biological replicates (n=4) revealed a number of proteins with statistically
180 significant interaction with ZC3H11A and THOC2 (Figure 6B). Proteins belonging to the TREX
181 complex and RNA export machinery are highlighted in bold. The log-fold change in protein
182 intensities in the ZC3H11A co-IP relative to the IgG co-IP is presented along with the adjusted *P*-
183 values (Figure 6C). The interaction between ZC3H11A and THOC2 was validated by a reciprocal
184 co-IP and western blot using mESCs (Figure 6D). The majority of the significant partners
185 interacting with ZC3H11A are part of the TREX complex and also showed significant enrichment
186 in the THOC2 co-IP, including THOC5, THOC7 (Figure 6E), THOC1 and THOC6 (Figure 6C).
187 ZC3H11A also interacts with other RNA-binding proteins that are required for RNA maturation,
188 such as polyadenylate-binding nuclear protein 1 (PABPN1) [34]; FYTDD1, which acts as an
189 adaptor for RNA helicase UAP56 [35]; and the RNA export adaptor ALYREF/THOC4 [36]
190 (Figure 6D and F). Notably, almost half of the ZC3H11A partners detected by co-IP were also
191 found in the THOC2 co-IP (Figure S3). These data indicate that ZC3H11A is an essential
192 component of the TREX complex that is known to play pivotal roles during embryogenesis and
193 for maintaining pluripotency of ESCs [9, 10]. Furthermore, the proteomic analysis identified
194 additional interacting partners, independent of the TREX complex, such as the RNA-binding
195 protein DDX18; the PRC2 components SUZ12 and JARID2; and the two zinc finger proteins
196 ZNF638 and ZFP57 (Figure 6G and S3B). DDX18 is an RNA binding protein that plays a crucial
197 role in pluripotency and self-renewal of embryonic stem cells [37].

198

199 **ZC3H11A selectively binds mRNA transcripts in mESCs**

200 Previous studies using human somatic cells indicated that ZC3H11A is an RNA-binding protein
201 that selectively binds subsets of mRNA upon stress or viral infection [1]. To study the RNA-
202 binding properties of ZC3H11A in embryonic cells, we performed UV-crosslinking of mESCs
203 followed by ZC3H11A immunoprecipitation (CLIP) and RNaseI treatment to isolate the RNA
204 protected by ZC3H11A. We used two anti-ZC3H11A antibodies to minimize any artifact caused
205 by antibodies, and anti-ALYREF and anti-IgG as positive and negative controls, respectively.
206 High-throughput sequencing of the RNA isolated by CLIP (CLIP-seq) revealed an almost
207 exclusive interaction between ZC3H11A and protein-coding mRNAs in mESCs (Figure S4A),
208 with a preference to bind 3'UTRs over the 5'UTRs (Figure 7A). The analysis of ZC3H11A CLIP-
209 seq peaks from the two ZC3H11A antibodies revealed a significant enrichment of short purine-
210 rich motifs (Figure 7B, top panel). Moreover, ZC3H11A exhibited strong binding to the
211 paraspeckle *Neat1* transcript (Figure S4B), similar to what has been observed in human somatic
212 cells [1]. Comparing the CLIP-seq ZC3H11A mRNA targets with genes that were significantly
213 down-regulated in RNA-seq data, we identified subsets of genes as putative direct targets of
214 ZC3H11A in mESCs (Figure 7B, bottom panel). The gene ontology analysis of these genes
215 suggested that they are involved in germ cell development and metabolic processes (Figure 7C).
216 These 29 genes were dramatically down-regulated in *Zc3h11a*^{-/-} embryos (Figure 7D) and are
217 involved in cellular processes vital for embryonic development [38–41]. Putative direct targets
218 included the *Tdgfl*, nucleoporin 85 (*Nup85*), proliferation-associated protein 2G4 (*Pa2g4*) and gap
219 junction protein beta 3 (*Gjb3*) genes. The CLIP-seq analysis detected ZC3H11A binding sites at
220 the 3'UTR of these genes that were either overlapped with ALYREF binding sites (for *Tdgfl* and

221 *Pa2g4*) or distinct from them in *Nup85* (Figure 7E). These results suggest a crucial role of
222 ZC3H11A in post-transcriptional processing and mRNA export of key genes in embryonic cells.

223

224 **ZC3H11A is required for *in vitro* derivation of ESCs.**

225 To further understand the role of ZC3H11A in the peri-implantation development and especially
226 its role in the pluripotent epiblast, twenty-five E3.5 blastocysts were recovered from matings
227 between heterozygous mice, and cultured *in vitro*. From these, 14 ESC lines were obtained but
228 none were homozygous KO ($X^2=4.7$, d.f.=1; $P<0.05$). This suggests that ZC3H11A is required for
229 establishing ESC *in vitro*.

230

231 **Mice with postnatal *Zc3h11a*-ablation are healthy and viable**

232 We developed an inducible *Zc3h11a*-KO model to assess the effect of *Zc3h11a* ablation
233 postnatally. *Loxp-Zc3h11a* mice were crossed with mice containing fusion of a mutated estrogen
234 receptor T2 and Cre recombinase (Cre-ER), allowing temporal control of floxed gene deletion
235 upon tamoxifen induction *in vivo* [42]. We generated a strain that is homozygous *Zc3h11a-loxp*
236 (*Zc3^{loxP/loxP}*) with one copy of Cre-ER (CRE.ER⁺ *Zc3^{loxP/loxP}*) and crossed it with the original strain
237 (*Zc3^{loxP/loxP}*) lacking Cre-ER. The offspring were injected with tamoxifen at week 3-4 after birth
238 (Figure 8A). Genotyping of the tamoxifen-injected mice at week 6 using genomic DNA from tail
239 biopsies revealed a balanced ratio between WT and induced KO (iKO) due to the presence/absence
240 of Cre-ER (Figure 8B). By injecting CRE.ER⁺ *Zc3^{loxP/loxP}* mice with tamoxifen postnatally we
241 succeeded in achieving >90% reduction of *Zc3h11a* expression in multiple adult tissues including
242 bone marrow, liver and spleen (Figure 8C and D). The examination of tamoxifen-injected mice
243 were carried out at week 12 and involved histology staining of multiple organs including stomach,

244 pancreas, small and large intestine tissues. The histology phenotyping did not exhibit obvious
245 defects between the floxed (WT) and iKO adult mice (Figure 8E and S5). Furthermore, the
246 measurement of body weight, dissected kidney and spleen tissues from WT and inducible ZC3-
247 KO adult mice did not show significant differences (Figure 8F).

248

249 **Discussion**

250 ZC3H11A is important for the growth of nuclear replicating viruses, where viruses take advantage
251 of the ZC3H11A protein to facilitate the export of their mRNA transcripts into cytoplasm.
252 Thereby, ZC3H11A is considered a possible target for development of anti-viral therapy. Hence,
253 we developed ZC3H11A mouse models to study its physiological functions across developmental
254 stages. The current study reports that ZC3H11A is an essential protein required for the viability of
255 mouse embryos. Loss of function of ZC3H11A leads to developmental defects and embryo
256 degeneration at peri-implantation stages associated with dysregulation of metabolic pathways such
257 as glycolysis and fatty acid metabolic processes. Interestingly, the defects mainly originate from
258 the epiblast, as most of the down-regulated genes are expressed predominantly in this lineage.
259 Moreover, even though ZC3H11A is expressed in all cells of the blastocyst, *Tdgfl*, one of its key
260 down-regulated target genes is expressed specifically in the epiblast cells [31]. TDGF1 (also called
261 *Cripto*) is a membrane-bound protein, co-receptor for NODAL/GDF3 [43]. TDGF1 and NODAL
262 signaling play important roles during specification of the early lineages and maintenance of the
263 pluripotent epiblast at early post-implantation stages [43]. Interestingly it also controls the
264 metabolic switch occurring at the time of implantation in the mouse, when cells transit from a
265 OXPHOS based metabolism to a glycolytic one [29, 31, 44]. Our CLIP-seq analysis detected two
266 strong peaks for ZC3H11A binding at the 3' end of the *Tdgfl* mRNA in mESCs (Figure 7E).

267 Furthermore, ZC3H11A binds the 3' end of *Nup85* and *Pa2g4* mRNA transcripts (Figure 7E).
268 Both *Nup85* and *Pa2g4* were down-regulated in the KO embryos and play crucial roles in
269 embryonic development [38–41]. For instance, NUP85 is a core component of the nuclear pore
270 complex (NPC) proteins and is required for mRNA export and maintenance and assembly of the
271 NPC [40, 41, 45]. Loss of function studies showed that inactivation of the NPC proteins in mouse
272 models resulted in early embryonic lethality [46–48]. Recent phenotypic characterization of the
273 *Nup85* knock-out mouse model from the International Mouse Phenotyping Consortium
274 (www.mousephenotype.org, accessed 22 August 2022) [49] has indicated complete preweaning
275 lethality of *Nup85*^{-/-} mice. Furthermore, the ErbB3 binding protein-1 gene (*Ebp1/Pa2g4*) is
276 implicated in regulating the proliferation and differentiation during developmental stages. The
277 *Pa2g4* knock-out mice exhibited growth retardation and were 30% smaller than wild-type mice
278 [50]. A recent study has reported more severe phenotypes in *Pa2g4*-deficient mice with death
279 between E13.5 and 15.5, massive apoptosis, and cessation of cell proliferation [38]. These putative
280 ZC3H11A targets identified by CLIP-seq are known to be critical for embryonic viability and
281 implicated in diverse cellular functions, disruption of their expression leads to embryonic
282 degeneration.

283 Another key down-regulated gene in KO embryos is *Ldha*, the enzyme that controls the
284 level of anaerobic glycolysis by catalyzing the transformation of pyruvate into lactate. Hence, in
285 KO embryos, the establishment of a more anaerobic glycolysis is impaired, which compromises
286 survival when the environment becomes more hypoxic as embryos implant. Upregulation of
287 autophagy as observed in KO embryos can be viewed as reaction to a suboptimal metabolic
288 environment [33]. Although KO embryos can survive up to E6.5, they have already undergone a
289 process of degeneration, as suggested by the upregulation of P53 mediated apoptotic pathway

290 already at E4.5. The transcriptomic analysis also indicated a significant dysregulation in the EMT
291 process (Figure 4C). The EMT process is fundamental for embryo development and takes place
292 during implantation of the embryo into the uterus and during early gastrulation, where embryo is
293 transformed from a single layer to three germ layers. Defects in EMT and subsequently in
294 gastrulation usually lead to a failure in embryonic development [51, 52].

295 The ZC3H11A protein exhibited strong interactions with members of the RNA-export
296 machinery in ESCs and the top interacting partners with ZC3H11A are members of the TREX
297 complex, including THO proteins (Figure 6). The enrichment analysis of interacting partners with
298 ZC3H11A showed significant enrichment of proteins involved in metabolism of RNA, mRNA 3'-
299 end processing and transport of mature transcript to cytoplasm (Figure S3A). These proteomics
300 results are in agreement with the analysis of the CLIP-seq of ZC3H1A in mESCs that revealed
301 preferential binding at 3'UTRs over the 5'UTRs of target transcripts (Figure 7A). It also supports
302 the model of action that ZC3H11A interacts with TREX-complex proteins and contribute to
303 efficient mRNA maturation and export of the target transcripts. In agreement with this model,
304 several studies have described the pivotal roles of the TREX-complex in the embryonic
305 development [9–11]. THO proteins such as THOC1, THOC2 and THOC5 play essential roles
306 during early development but in a different way than ZC3H11A, as their depletion affects
307 pluripotency establishment and maintenance [9, 10]. In contrast, ZC3H11A depletion does not
308 directly affect pluripotency maintenance. The fact that *Zc3h11a*^{-/-} blastocysts did not give rise to
309 ESC lines in the present study may be due to the metabolic impairment rather than a defect in
310 pluripotency maintenance, as they all form outgrowth, in contrast to *Thoc1*^{-/-} embryos [10].

311 Our results provide evidence that ZC3H11A is required for the post-transcriptional
312 regulation of genes that are crucial for the embryonic cell. In contrast to the severe phenotypes in

313 *Zc3h11a* germline KO embryos, *Zc3h11a* inactivation in the adult tissues did not cause obvious
314 defects. The phenotypic characterization of the inducible ZC3-KO adult mice indicated a
315 dispensable role for ZC3H11A in adult tissues and a single surviving *Zc3h11a*^{-/-} female showed
316 no pathological conditions, were fertile and gave birth to 10 progeny from three litters.
317 Furthermore, complete inactivation of *Zc3h11a* in human and mouse cell lines did not lead to
318 significant effects on cell growth or viability [1, 3].

319

320

321 **Methods**

322 **Animal models**

323 All mice were group-housed with free access to food and water in the pathogen-free facilities of
324 Uppsala University and INRAE. All procedures described in this study were approved by the
325 Uppsala Ethical Committee on Animal Research (#17346/2017), following the rules and
326 regulations of the Swedish Animal Welfare Agency, and were in compliance with the European
327 Communities Council Directive of 22 September 2010 (2010/63/EU). All efforts were made to
328 minimize animal suffering and to reduce the number of animals used. The loxP *Zc3h11a* mouse
329 model was generated by homologous recombination in mouse C57BL/6 ES cells (Cyagen, USA).
330 The PGK-Cre mice expressing the Cre recombinase in the germ line [53] was obtained as gift from
331 Klas Kullander's lab (Uppsala University). The CRISPR/cas9 *Zc3h11a* mouse model was
332 purchased from the Mutant Mouse Resource & Research Centers (MMRRC, USA, Strain No:
333 043457-UCD). For inducible knock-out model, the mice containing fusion of a mutated estrogen
334 receptor T2 and Cre recombinase (Cre-ER) was ordered from The Jackson Laboratory (USA,
335 Strain No: 008463). Mice were genotyped (Tables S2) based on tail biopsies.

336

337 **Collection of mouse embryos**

338 The *Zc3h11a* heterozygous males and females were mated and the following day, the presence of
339 a vaginal plug was recorded. To determine the time of developmental lethality, females were
340 sacrificed at E6.5 and embryos dissected out from the decidual swellings. Their morphology was
341 recorded and each of them was then processed for genotyping. Samples for RNA-sequencing were
342 collected at peri-implantation stage (E4). These embryos were bisected using glass needles and
343 both parts were individually snap-frozen. The abembryonic part (mural TE) was used for
344 genotyping and the embryonic part (ICM and polar TE) for subsequent RNA extraction.

345

346 **RNA sequencing**

347 The collected embryonic ICM and polar TE (as described above) were used for RNA-seq library
348 preparation using the SMART-Seq HT Kit (Takara Bio USA, Inc.) following the manufacturer's
349 instructions. Briefly, cDNA was generated using the oligo-dT primer to enrich for mRNA,
350 followed by the tagmentation of the cDNA (Illumina Nextera XT) to generate Illumina-compatible
351 RNA-seq libraries. The libraries were amplified by 12 PCR cycles and size-selected for an average
352 insert size of 150 bp and sequenced as 100 bp paired-end reads using Illumina Nova-Seq. Sequence
353 reads were mapped to the reference mouse genome (mm10) using STAR 2.5.1b [54] with
354 parameter --quantMode GeneCounts to generate read counts. The edgeR (Bioconductor package)
355 [55] was used to identify differentially expressed (DE) genes using gene models for mm10
356 downloaded from UCSC (www.genome.ucsc.edu). The abundance of gene expression was
357 calculated as count-per-million (CPM) reads. Genes with less than one CPM in at least three
358 samples were filtered out. The filtered libraries were normalized using the trimmed mean of M-

359 values (TMM) normalization method [56]. *P*-values were corrected for multiple testing using the
360 False Discovery Rate (FDR) approach. Gene set enrichment analyses (GSEA) were performed
361 using the fgsea R package [57]. Genes were ranked based on the fold-change and the datasets were
362 downloaded from the GSEA website (<https://www.gsea-msigdb.org/gsea/>).

363

364 **Immunoprecipitation**

365 Mouse embryonic stem cell line (mESC) was cultured on gelatin-coated plates and maintained in
366 Dulbecco's Modified Eagle Medium (DMEM) complemented with 10% heat-inactivated fetal
367 bovine serum, penicillin (0.2 U/mL), streptomycin (0.2 µg/mL) and L-glutamine (0.2 µg/mL)
368 (Gibco, Waltham, Massachusetts, United States) and supplemented with recombinant mouse
369 Leukemia Inhibitory Factor (LIF, 20 U/ml, Millipore). Cultured mESCs at 60% confluency were
370 washed with PBS twice before the preparation of total lysate. Total protein lysates were prepared
371 using Pierce IP lysis buffer (Thermo Fisher Scientific) supplemented with protease inhibitors
372 (Complete Ultra Tablets, Roche) and Pierce Universal Nuclease (Thermo Fisher Scientific). Lysate
373 was cleared by centrifugation at 20 x *g* for 10 min at 4°C, and incubated rotating end-over-end at
374 4°C with anti-IgG, anti-ZC3H11A or anti-THOC2 antibodies in Protein LoBind 2-ml tubes
375 (Eppendorf). Thereafter, 30 µg of Dynabeads Protein G (Thermo Fisher Scientific) was added to
376 each tube and incubated for 30 min at room temperature, followed by washing three times with
377 Pierce IP lysis buffer. The co-IP proteins were eluted from the magnetic beads by adding 50 µg of
378 elution buffer (5% SDS, 50mM TEAB, pH 7.55) and heat denaturation for 5 min at 90 °C. The
379 eluted co-IP proteins were used for western blot and Mass spectrometric analysis. co-IP
380 experiments were performed in four replicates.

381

382 **Immunoblot analysis**

383 Equal volumes (5 μ g) of the prepared co-IPs were separated by SDS-PAGE (4–15%, Bio-Rad)
384 and transferred to PVDF membranes (Millipore). StartingBlock buffer (Thermo Fisher Scientific)
385 was used to block the membrane before the primary anti-ZC3H11A, anti-THOC2 or anti-ALYREF
386 antibodies (1:1000) were added. Proteins were visualized and detected by the Odyssey system (LI-
387 COR).

388

389 **Protein clean-up and digestion**

390 The co-IPs were cleaned up and prepared for mass spectrometry quantification using the S-Trap
391 column method [58]. First, the eluted co-IPs were treated by TCEP (5 mM) to reduce disulfide
392 bonds, followed by adding methyl methanethiosulfonate (MMTS) to a final concentration of 15
393 mM to alkylate cysteines. Thereafter, the lysate was acidified by adding phosphoric acid to a final
394 concentration of 1.2%. The acidified lysate was added to an S-Trap microcolumn (Protifi,
395 Huntington, NY) containing 300 μ l of S-Trap buffer (90% MeOH, 100 mM TEAB, pH 7.5) and
396 centrifuged at $4000 \times g$ for 2 min. The S-Trap microcolumn was washed twice with S-Trap buffer.
397 The columns were transferred to new tubes and incubated with 10 ng/ μ L sequencing-grade trypsin
398 (Promega) overnight at 37°C. The digested proteins were eluted by centrifugation at $4000 \times g$ for
399 1 min with 50 mM TEAB, 0.2% formic acid (FA), followed by 50% acetonitrile (ACN)/0.2% FA,
400 and finally 80% ACN/0.1% FA. The eluted peptides were dried down in a vacuum centrifuge
401 (ThermoSavant SPD SpeedVac, Thermo Fisher Scientific), and finally dissolved in 1% FA.
402 Digested peptides were thereafter desalted by StageTips (Thermo Fisher Scientific) according to
403 the manufacturer's instructions, and subsequently dissolved in 0.1% FA.

404 **Liquid chromatography and mass spectrometry**

405 The dissolved peptides were quantified by mass spectrometry as previously described [17].
406 Briefly, a Thermo Scientific EASY-nLC 1000 liquid chromatography system coupled with an
407 Acclaim PepMap 100 (2 cm x 75 μ m, 3 μ m particles, Thermo Fisher Scientific) pre-column in line
408 with an EASY-Spray PepMap RSLC C18 reversed phase column (50 cm x 75 μ m, 2 μ m particles,
409 Thermo Fisher Scientific) was utilized to fractionate the peptide samples. The eluted peptides were
410 analyzed on a Thermo Scientific Orbitrap Fusion Tribrid mass spectrometer, operated at a Top
411 Speed data-dependent acquisition scan mode, ion-transfer tube temperature of 275°C, and a spray
412 voltage of 2.0 kV.

413

414 **Mass spectrometric data analysis**

415 Data analysis of raw files was performed using MaxQuant software (version 1.6.4) and the
416 Andromeda search engine [59, 60], with the following parameters: cysteine methyl
417 methanethiosulfonate (MMTS) as a static modification and methionine oxidation and protein N-
418 terminal acetylation as variable modifications. First search peptide MS1 Orbitrap tolerance was
419 set to 20 ppm, and iontrap MS/MS tolerance was set to 0.5 Da. Peak lists were searched against
420 the UniProtKB/Swiss-Prot *Mus musculus* proteome database (UP000000589, version 2019-04-01)
421 with a maximum of two trypsin miscleavages per peptide. The MaxQuant contaminants database
422 was also utilized. A decoy search was made against the reversed database, with the peptide and
423 protein false discovery rates both set to 1%. Only proteins identified with at least two peptides of
424 at least 7 amino acids in length were considered reliable. The peptide output from MaxQuant was
425 filtered by removing reverse database hits, potential contaminants, and proteins only identified by
426 site (PTMs).

427 Intensity values were used to determine the protein abundance. First, proteins with missing
428 values in more than one replicate in at least one group were filtered out. Thereafter, the filtered
429 intensities were normalized using the variance stabilizing normalization (vsn) method [61] and
430 followed by the imputation of missing values with the deterministic minimal value approach
431 (MinDet) [62] to replace the missing values in the normalized intensities. The normalized
432 intensities were fitted to a linear model and the empirical Bayes moderated t-statistics and their
433 associated *P*-values were used to calculate the significance of differential enriched proteins [63,
434 64]. The *P*-values were adjusted for multiple testing using the Benjamini–Hochberg procedure
435 [65]. Proteomics data was visualized using the ggplot R-package and Cytoscape v3.8.2.

436

437 **Crosslinking immunoprecipitation sequencing (CLIP-seq)**

438 Cultured mESCs were cross-linked using a 254 nM UV crosslinker with an energy setting of 400
439 mJ/cm². The cross-linked cells were collected in ice-cold PBS with a cell scraper and aliquoted in
440 1.5 ml tubes (25 million cells/ml). The CLIP-seq library preparation was performed as indicated
441 [66]. Briefly, the total lysate was digested with RNase-I and immunoprecipitated with 10 µg of the
442 following antibodies: anti-ZC3H11A (HPA028526 and HPA028490, Atlas Antibodies), anti-
443 ALYREF (ab202894, Abcam) or anti-IgG (ab37415, Abcam). The IP-RNA complexes were
444 loaded on a 4–12% Bis-Tris (Bio-Rad) gel, transferred to a nitrocellulose membrane, and the bands
445 above 75 kDa for each lane were cut. Extracted RNA molecules from the membrane were used for
446 Illumina library construction as indicated [66]. CLIP-seq reads were trimmed out using
447 *trim_galore* with the criteria to remove reads with low quality and shorter than 15 bp. The trimmed
448 reads were aligned to the mouse reference genome mm10 using STAR aligner with end-to-end
449 options --alignEndsType EndToEnd. The CLAM workflow were used for peak calling and

450 counting the fold enrichment of IP vs IgG control [67]. The identified peaks with adjusted *P*-
451 value <0.01 were annotated to the mouse mm10 genome using the *peak_annotator* function from
452 CLAM. HOMER software was used for motif finding using the findMotifsGenome.pl script with
453 default parameters for RNA motifs [68].

454

455 **Quantitative RT-PCR**

456 Total RNA was extracted using the RNeasy Mini kit (Qiagen) and the samples were treated with
457 DNase I to eliminate genomic DNA. The High Capacity cDNA Reverse Transcription Kit
458 (Applied Biosystems) was used to generate cDNA from RNA. Quantitative PCR analysis was
459 performed in ABI MicroAmp Optical 384-well Reaction plates on an ABI 7900 real-time PCR
460 instrument using SYBR gene expression reagents (Applied Biosystems). The amplification and
461 detection of each gene was performed using forward and reverse primers for *Zc3h11a*, F:
462 TGCCTAATCAGGGAGAAGACTG, R: AGCTTCACAGTGACGGAATG and *Actb* as a
463 housekeeping gene F: CTAAGGCCAACCGTGAAAAG, R: ATCACAATGCCTGTGGTACG.

464

465 **Derivation of ESCs**

466 E3.5 blastocysts were collected from heterozygous matings. They were plated individually on a
467 layer of Mitomycin C inactivated mouse embryonic fibroblasts (feeder layer) in 4-well plates, in
468 naïve ESC medium. This medium was composed of Chemically Defined Medium (CDM)
469 supplemented with LIF (700 U/ml), PD0332552 (1 µM) and CHIR99201 (final 3 µM) (2i/Lif
470 CDM) [69]. After 4-5 days, the blastocysts have attached and outgrowths formed. Individual
471 outgrowths were dislodged from the feeders, dissociated in Tryple Select (Invitrogen) and the
472 single cell suspension was plated in 4-well plates on fresh feeders. ESC colonies appeared within

473 the following days and were individually picked, dissociated and replated on feeders in 2i/Lif
474 CDM. The procedure was repeated a few times, until stable expansion of the ESCs that allows
475 passaging using trypsin and removal of feeders, replaced by plate coating with gelatin 0.2% and
476 serum.

477

478 **Immunostaining of ZC3H11A on pre-implantation embryos**

479 Mouse CD1 embryos were collected at 4-cell (E1.5) and blastocyst (E3.5) stage in M2 medium
480 (Sigma) by oviduct and uterine flushing, respectively. They were fixed in 2% PFA for 20 min,
481 followed by permeabilization by 1% Triton-X100, for 30 min. Permeabilized samples were
482 blocked with 1% BSA in PBS for 40 min, followed by incubation with primary antibodies in 1%
483 BSA overnight at 4°C. The day after, samples were washed by PBS and incubated with the
484 fluorophore conjugated secondary antibodies (Jackson ImmunoResearch) for an hour at room
485 temperature. Samples were then washed and stained by DAPI for nuclei staining. Samples were
486 mounted in Vectashield mounting agent (Vectorlabs, H1000). Embryos were imaged by a Zeiss
487 LSM710 confocal microscope. Antibodies used were anti-SC35 (ab11826, Abcam, 1:250) and
488 anti-ZC3H11A (HPA028526, Atlas Antibodies, 1:300)

489

490 **Author contributions**

491 SY and LA conceived the study. SY performed experimental and bioinformatic analysis. AJ, VB
492 and JO performed experiments on embryos and ESC derivation with contribution from SY. SY
493 and ML performed mass spectrometry analysis. SY, AJ and LA wrote the paper with input from
494 ML. All authors approved the final version before submission.

495

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505 Research Infrastructure for the Control of Animal and Zoonotic Emerging Infectious Diseases
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507 images were acquired in the French ISC MIMA2 (Microscopy and Imaging Facility for Microbes,
508 Animals and Foods, doi: 10.15454/1.5572348210007727E12).

509

510 **Additional information**

511 **Competing interests**

512 The authors declare no competing interests.

513 **Ethics statement**

514 Animal procedures were carried out according to the rules and regulations of the Swedish Animal Welfare
515 Agency and French national rules on Ethics and Animal Welfare in the Animal Facility; and were in
516 compliance with the European Communities Council Directive of 22 September 2010 (2010/63/EU).
517 This work was approved by the French Ministry of Higher Education, Research, and Innovation (n°15-55
518 &21-01) and the local Ethical Committee (INRAE Jouy-en-Josas Centre). The study was carried out in
519 compliance with the ARRIVE guidelines.

520

521 **Data availability**

522 The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the
523 PRIDE partner repository with the accession numbers (to be added). The RNA-seq reads have been
524 submitted to the sequence read archive (<http://www.ncbi.nlm.nih.gov/sra>) with the accession numbers (to
525 be added).

526

527 **Figure legends**

528 **Figure 1. Development of *Zc3h11a*^{-/-} mouse models.** (A) The *Zc3h11a* locus showing the
529 targeted exons for generating *Zc3h11a*^{-/-} mouse models. (B) Two CRISPR/cas9 guide RNAs were
530 used to delete exon 3 of *Zc3h11a*. Scissors indicate the location of the gRNAs and the length of
531 deleted sequences. (C) Two homology arms were used to insert loxP sites flanking exon 2. The
532 conditional knock-out mice were crossed with mice expressing Cre in germ line to eliminate the
533 sequences between the loxP sites resulting in the elimination of the entire exon 2 coding sequences.
534 (D and E) Genotyping of the offspring of *Zc3h11a* heterozygous matings (*Zc3*^{+/-} X *Zc3*^{+/-}) using
535 the CRISPR/cas9-based KO mouse model (D) and the loxP/Cre-based KO mouse model (E). The
536 total numbers of genotyped mice at week 4 are indicated.

537

538 **Figure 2. Ablation of *Zc3h11a* leads to early embryo degeneration.** (A, top) Schematic
539 illustration showing embryo stages and time points of collecting embryos for genotyping of
540 *Zc3h11a*. (A, low) PCR genotyping of collected embryos at the above time points. (B) Morphology
541 of collected embryos at E6.5 from *Zc3h11a* heterozygous mating (*Zc3*^{+/-} X *Zc3*^{+/-}).

542

543 **Figure 3. Cellular localization of ZC3H11A in early embryonic cells.** (A) Immunofluorescence
544 staining of mouse embryos using anti-ZC3H11A and anti-SRSF2 (SC35) antibodies at 2-cell (top)
545 and blastocyst stage (middle and bottom). Middle panel shows a z-projection of the whole
546 blastocyst while the bottom panel is a mid-section through the ICM. (Scale bar: 20 μm). (B)
547 Fluorescent intensity profile of the ZC3H11A signal and paraspeckle marker SRSF2 signal across
548 paraspeckles in an ICM nucleus showing the co-localization of ZC3H11 to paraspeckles. (Scale
549 bar: 5 μm.). (C) Expression of *Zc3h11a* and *Srsf2* measured by smartseq2 single cell RNA-seq.
550 Re-analysis of data from Deng *et. al.* [19].

551
552 **Figure 4. Transcriptome analysis reveals dysregulated pathways in *Zc3h11a*^{-/-} embryos.** (A,
553 left) Dissected inner cell mass (ICM) cells were used for RNA-sequencing. (A, right) Principle
554 component analysis (PCA) of RNAseq data from embryonic parts at E4.5. Dots represent
555 individual embryos and colors represent different genotypes. (B-D) Gene set enrichment analysis
556 (GSEA) of ranked DE genes in *Zc3h11a*^{-/-} embryos using hallmark gene sets. (B-C, below)
557 Heatmaps showing the expression of the genes contributing to the above pathways and found
558 significantly down-regulated in *Zc3h11a*^{-/-} embryos (FDR <0.05). (E) Heatmap showing the
559 expression of the genes contributing to fatty acid metabolism pathway and significantly down-
560 regulated in *Zc3h11a*^{-/-} embryos (FDR <0.05). FDR: false discovery rate, NES: normalized
561 enrichment score.

562
563 **Figure 5. Down-regulated genes in *Zc3h11a*^{-/-} embryos are ICM-related.** (A-B) GSEA of
564 ranked DE genes in *Zc3h11a*^{-/-} embryos with positive enrichment for the P53 pathway (A) and
565 autophagy-related genes (B) among the up-regulated genes in *Zc3h11a*^{-/-} embryos (FDR <0.05).
566 (C) Heatmap of down-regulated genes in *Zc3h11a*^{-/-} embryos (FDR <0.05) and their expression
567 profile during embryonic stages as indicated. Re-analyzed data from GSE76505 and E-MTAB-
568 2950. (D) Expression level of the indicated genes as count per millions (CPM). *, ** and ***
569 correspond to *FDR* < 0.05, 0.01 and 0.001, respectively.

570
571 **Figure 6. ZC3H11A binds RNA-export TREX complex proteins in mESC.** (A) Schematic
572 illustration of co-immunoprecipitation (co-IP) mass-spectrometry experiments using anti-
573 ZC3H11A, anti-THOC2 and anti-IgG antibodies and mouse embryonic stem cells (mESCs). (B)
574 Heatmap of the interacting partners to ZC3H11A (adjusted *P* <0.05). Data presented as log

575 intensities of four replicates. Proteins associated with the TREX complex and mRNA export are
576 in bold. (C) Volcano plot showing the enrichment of co-IP proteins from anti-ZC3H11A/anti-IgG.
577 (D) Western blot of reciprocal co-IP using anti-ZC3H11A, anti-THOC2 and anti-IgG antibodies
578 and probed with the indicated antibodies. Asterisk indicates a cut in the western blot membrane.
579 (E) Log intensities of the ZC3H11A and THOC proteins. (F) Log intensities of FYTDD1 (UAP56)
580 and the polyadenylation factor PABPN1. (G) Log intensities of proteins interacting with
581 ZC3H11A independent of THOC2 and the TREX complex. *, **, *** and **** correspond to
582 adjusted $P < 0.05$, 0.01, 0.001 and 0.0001, respectively. ns: not significant.

583
584 **Figure 7. CLIP-seq analysis of ZC3H11A RNA targets in mESCs.** (A) Distribution of the
585 proportion of ZC3H11A CLIP-seq mapped reads over the various elements of a gene in mESC
586 using two anti-ZC3H11A antibodies and an anti-IgG control antibody. (B, top) Predicted motifs
587 for ZC3H11A binding. (B, bottom) The overlap between differential down-regulated genes in KO
588 embryos and predicted ZC3H11A CLIP-seq targets. (C) Gene ontology analysis of the down-
589 regulated genes with ZC3H11A binding sites. (D) Heatmap of the down-regulated genes with
590 ZC3H11A binding sites. (E) The visualization of CLIP-seq reads and their distribution over the
591 indicated genes. Black arrows indicate the direction of transcription from 5' UTR to 3' UTR.

592
593 **Figure 8. Phenotype characterization of conditional *Zc3h11a*-KO mice.** (A) The loxP-*Zc3h11a*
594 mouse model was crossed with mice containing a fusion of a mutated estrogen receptor and Cre
595 recombinase (Cre-ER). The mice were bred to obtain two genotypes of homozygous loxP-*Zc3h11a*
596 mice ($Zc3^{loxP/loxP}$), one with one copy of Cre-ER ($CRE.ER^+ Zc3^{loxP/loxP}$) and the other with null
597 Cre-ER ($CRE.ER^- Zc3^{loxP/loxP}$). These mice were crossed and the offspring were injected with
598 tamoxifen at week 3-4 after birth. The time line indicates the time points of injection and sample

599 collection for genotyping and phenotyping. (B) Genotyping of the Cre-ER $Zc3^{loxP}$ mice. (C) qPCR
600 analysis of *Zc3h11a* mRNA expression in bone marrow, liver and spleen tissues from WT and
601 induced *Zc3*-KO (iKO) mice both injected with tamoxifen. ** and *** correspond to *t-test* P
602 <0.01 and 0.001 , respectively. (D) Western blot analysis of spleen tissues dissected from WT and
603 iKO adult mice. (E) Histology (H&E staining) of small intestine from WT and induced iKO adult
604 mice. (F) Body weight in grams (g), weight of dissected kidney and spleen in milligrams (mg)
605 from WT and induced iKO adult mice. Results are means \pm SEM.

606

607

608 **Supplementary information**

609

610 **Figure S1.** Heatmaps of the normalized expression of genes involved in the P53 pathway (A) and
611 in positive regulation of autophagy (B).

612

613 **Figure S2.** (A) Heatmap of up-regulated genes (A, left) and down-regulated genes (A, right) in
614 *Zc3h11a*^{-/-} embryos (FDR <0.05) and their expression profile during embryonic stages as
615 indicated. Re-analyzed data from GSE76505 and E-MTAB-2950. (B) Violin plot of the relative
616 expression of inner cell mass (ICM)-related genes and trophectoderm (TE)-related genes
617 (ICM/TE) as detected among the down-regulated and up-regulated genes in *Zc3h11a*^{-/-} embryos
618 (FDR <0.05).

619

620 **Figure S3.** (A, top) Chord diagram illustrating the overlap between the interacting partners
621 detected with ZC3H11A and THOC2 co-IPs. (A, bottom) GO analysis of the identified interacting

622 partners with ZC3H11A and THOC2 co-IPs in mESCs. (B) Network analysis of the identified
623 interacting partners with ZC3H11A and THOC2 based on co-IPs in mESCs. Blue lines represent
624 the interaction detected by our proteomics analysis, and gray lines represent the predicted
625 interaction from the STRING database.

626

627 **Figure S4.** (A) Genome-wide distribution of the ZC3H11A CLIP-seq peaks from mESCs across
628 different types of transcripts. (B) Visualization of CLIP-seq reads and their distribution over the
629 *Neat1* gene.

630

631 **Figure S5.** Histology staining of intestine, colon and stomach tissues from three WT and three
632 induced ZC3-KO adult mice.

633

634 **Table S1.** Differentially expressed genes in *Zc3h11a* knock-out (KO) E4.5 embryos.

635

636 **Table S2.** Sequences of PCR primers used for mouse genotyping.

637

638

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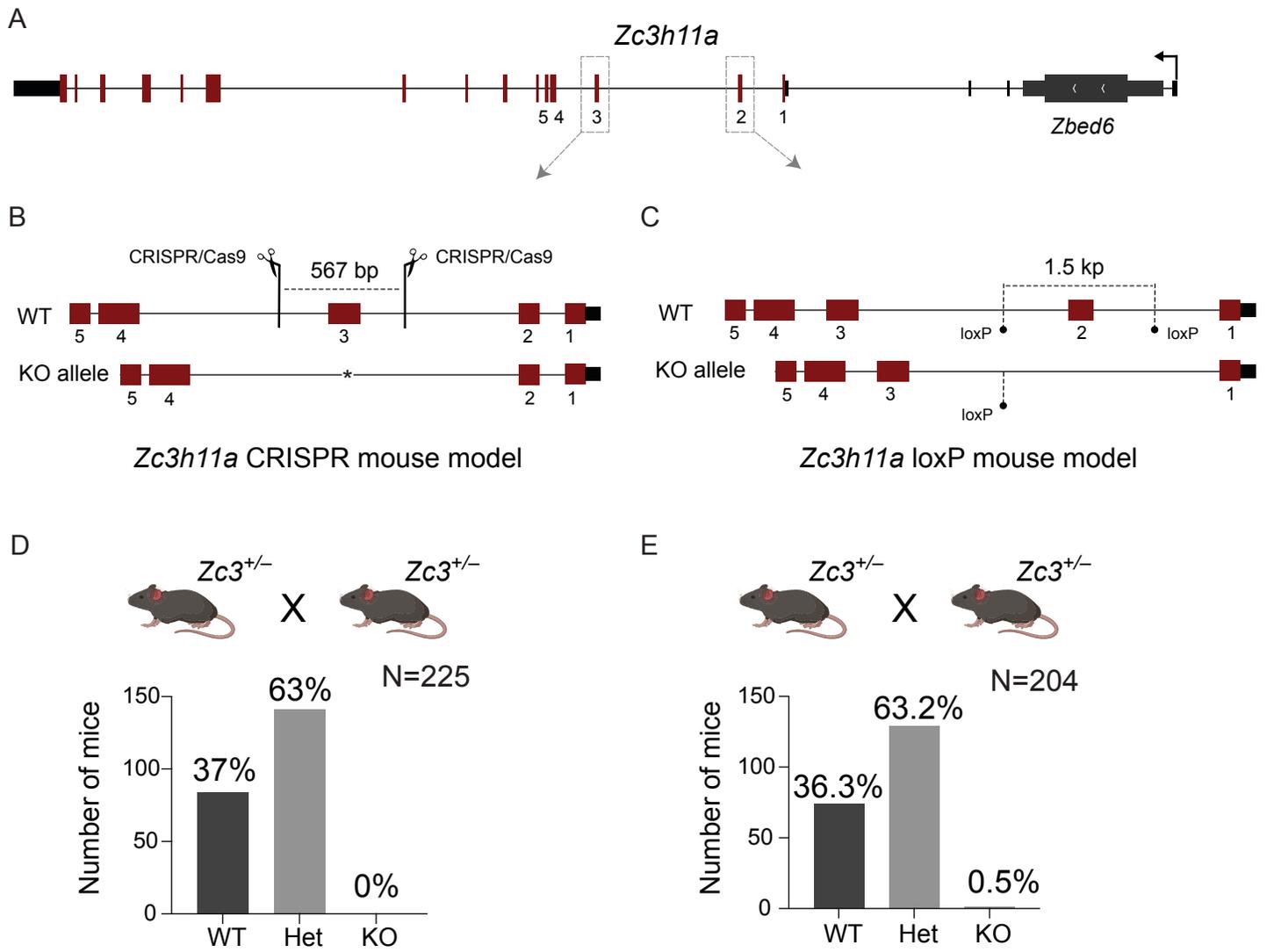


Figure 1

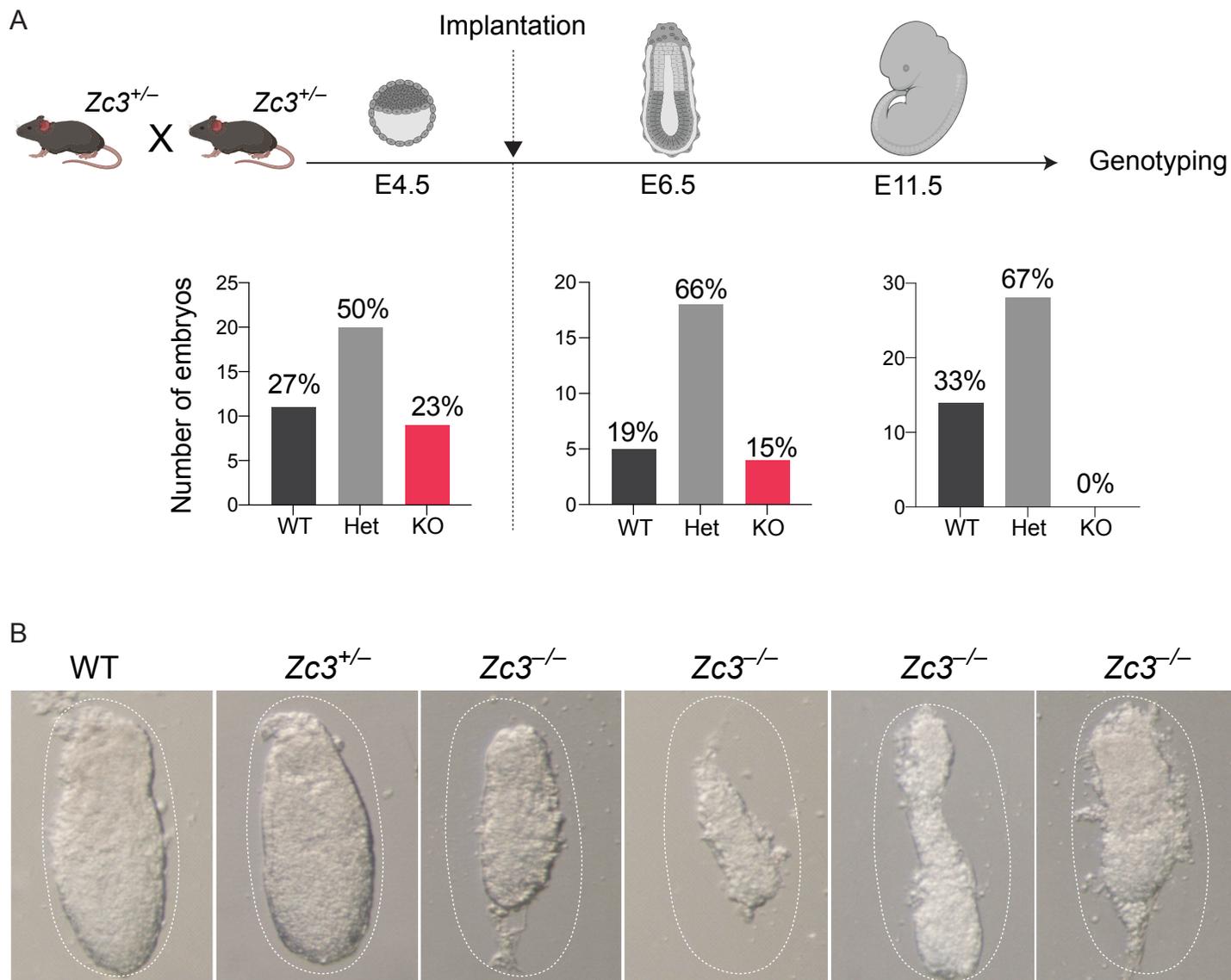


Figure 2

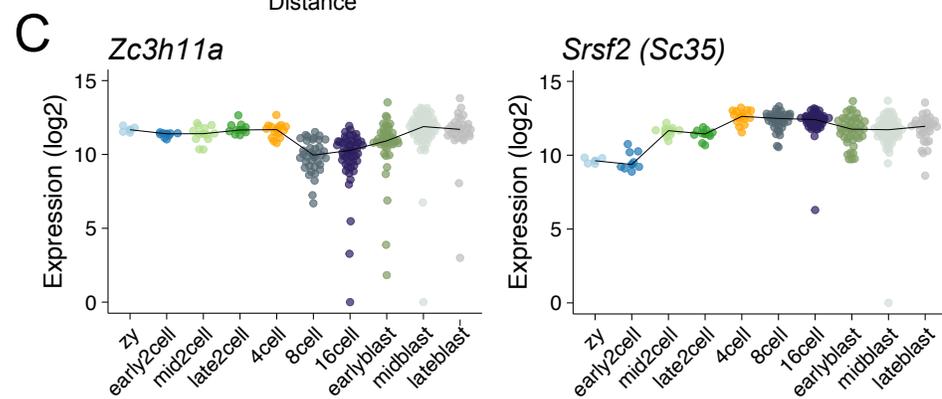
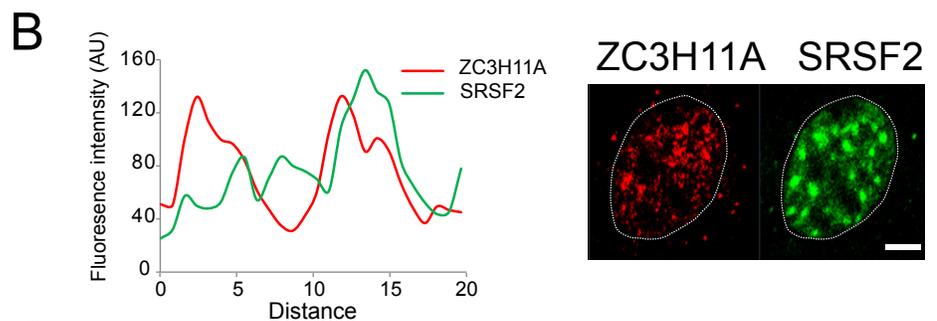
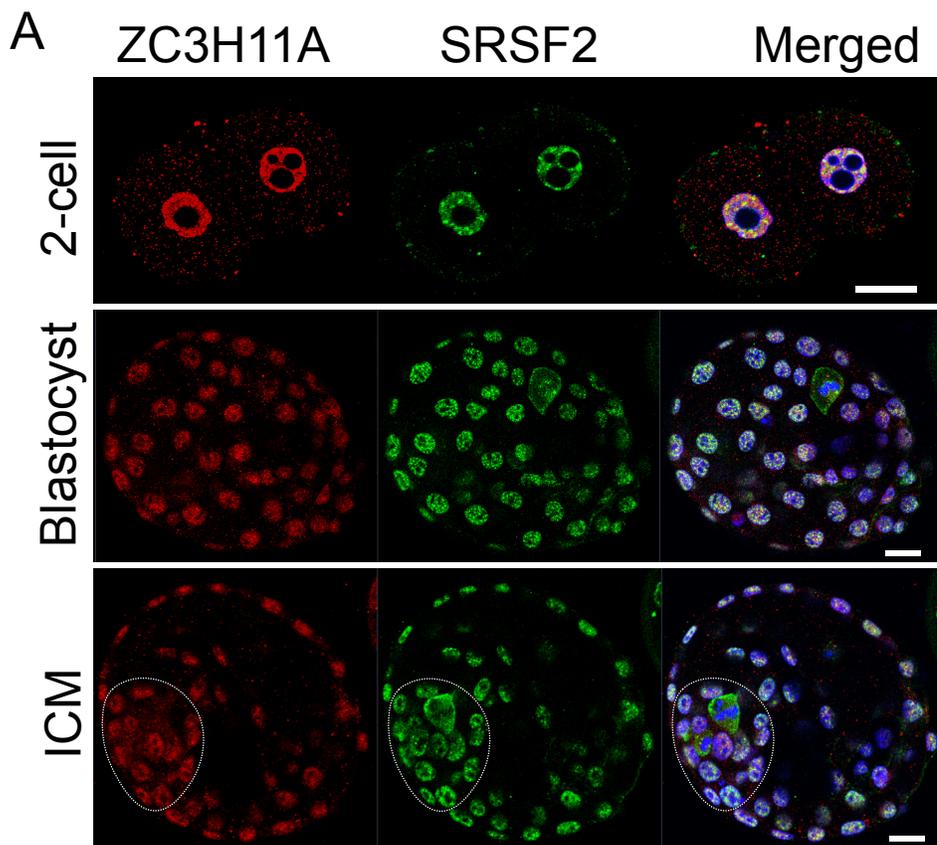


Figure 3

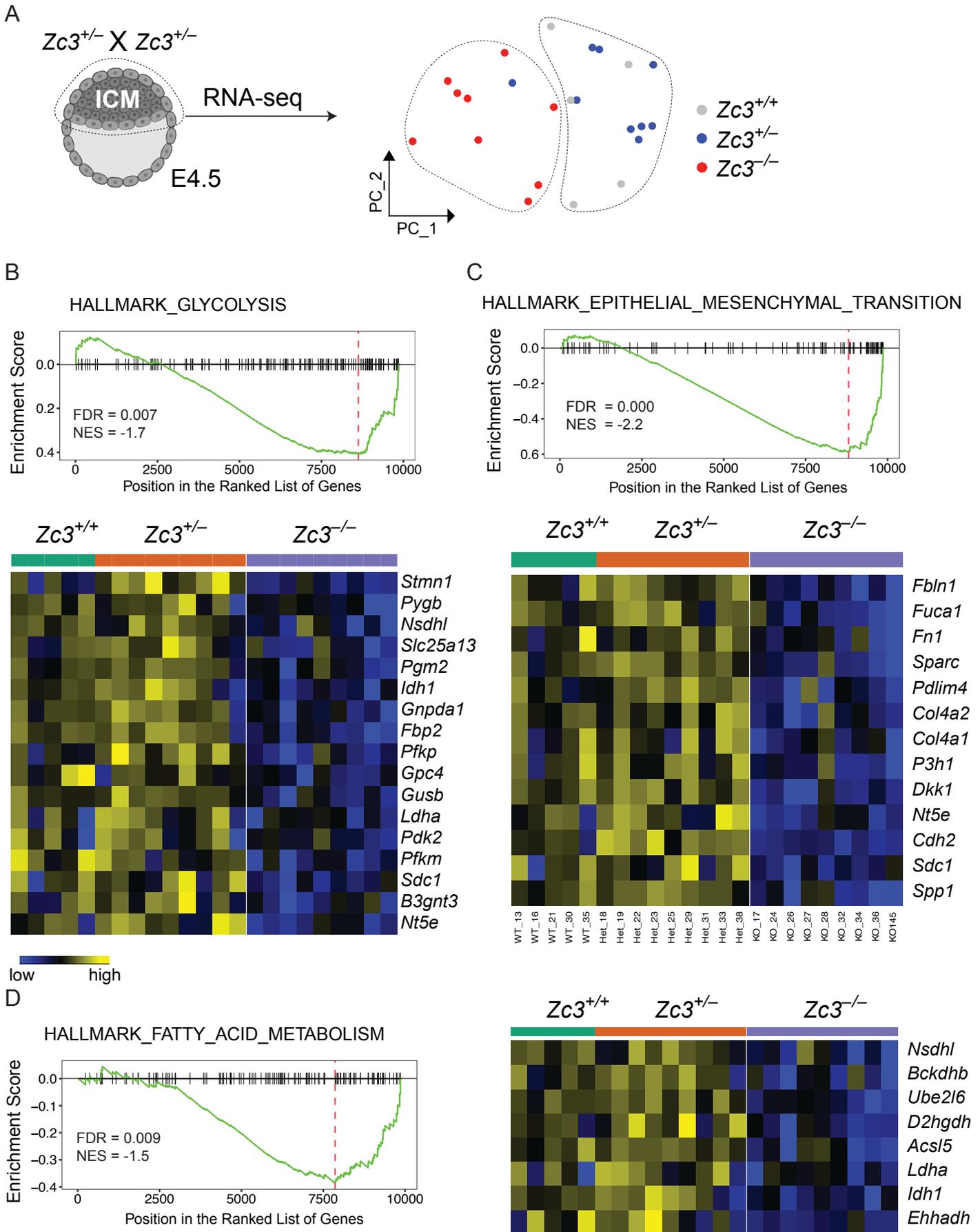


Figure 4

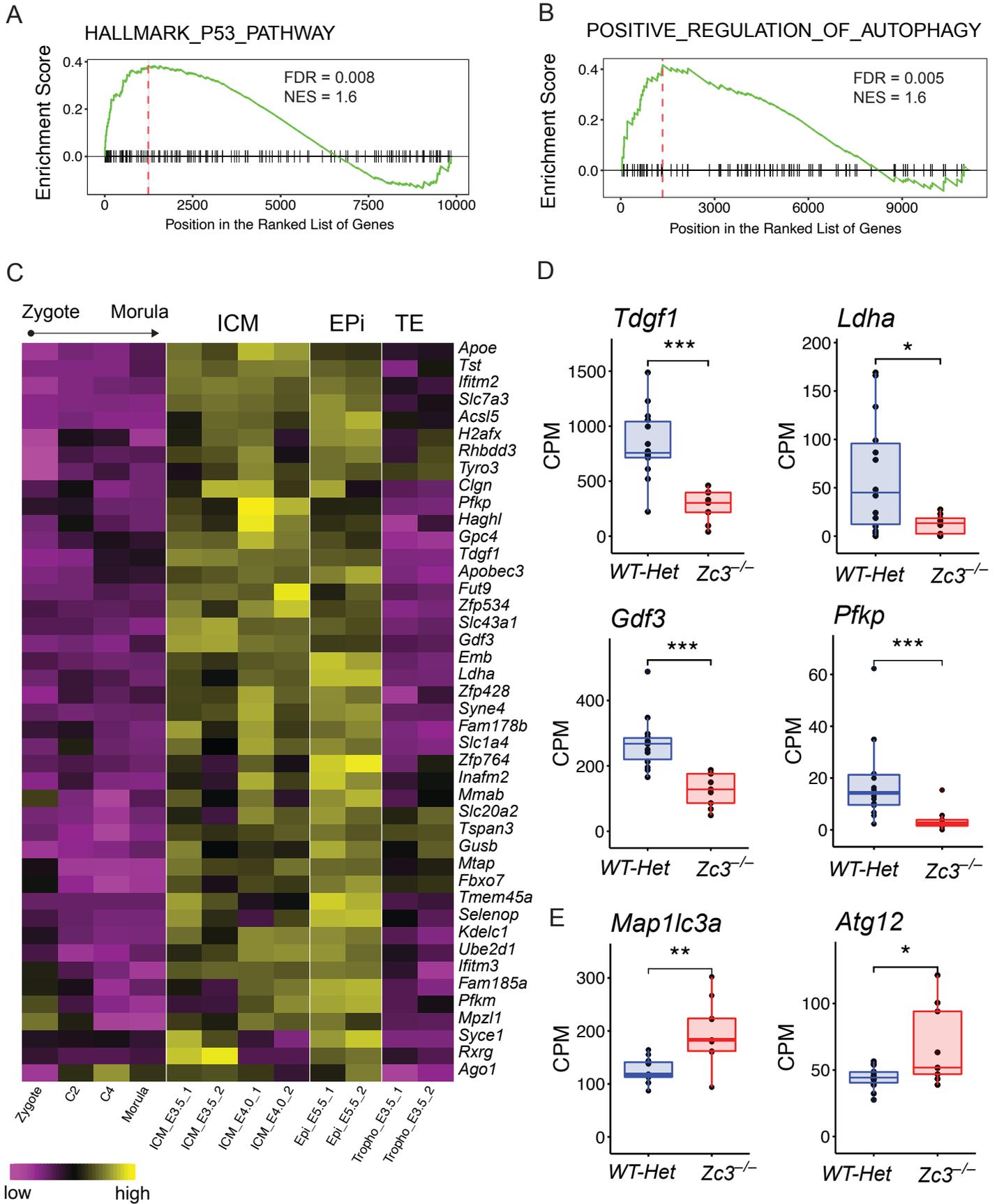


Figure 5

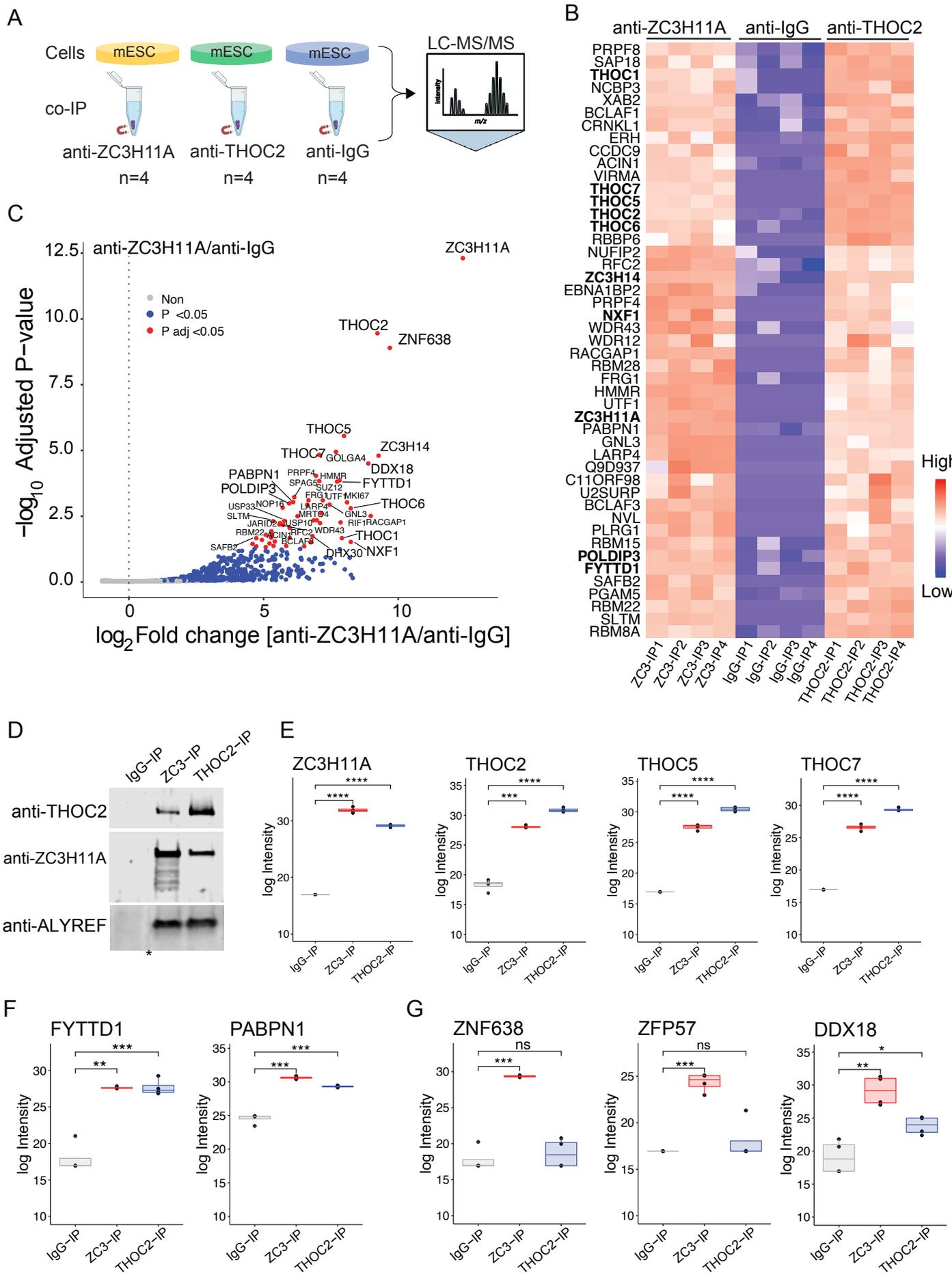


Figure 6

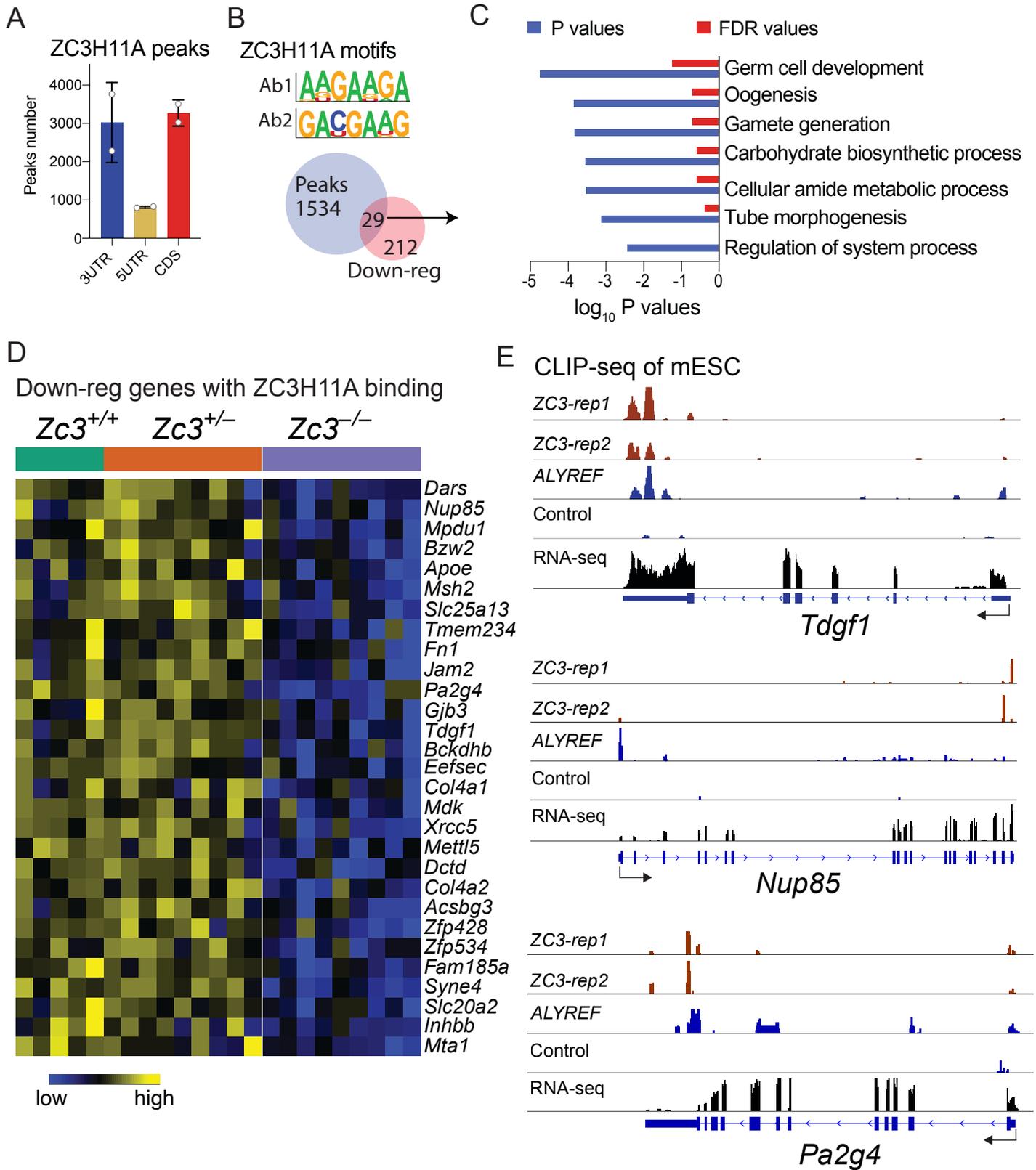


Figure 7

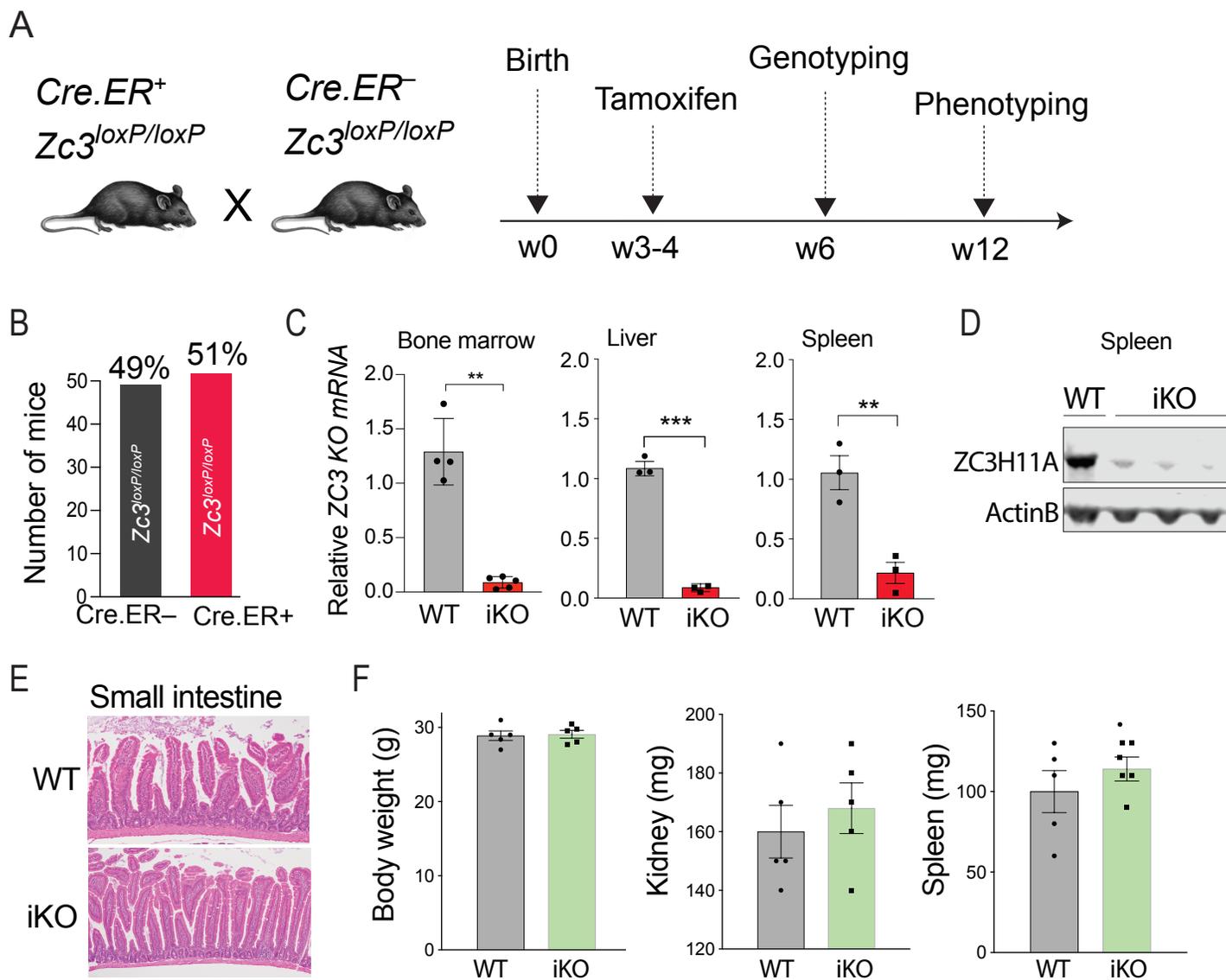
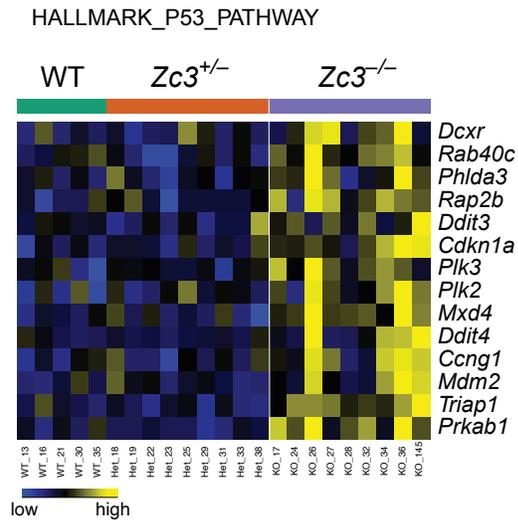


Figure 8

A



B

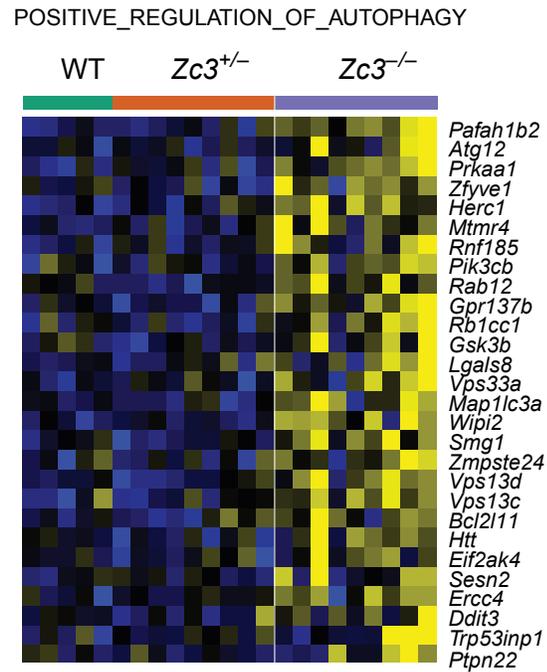


Figure S1

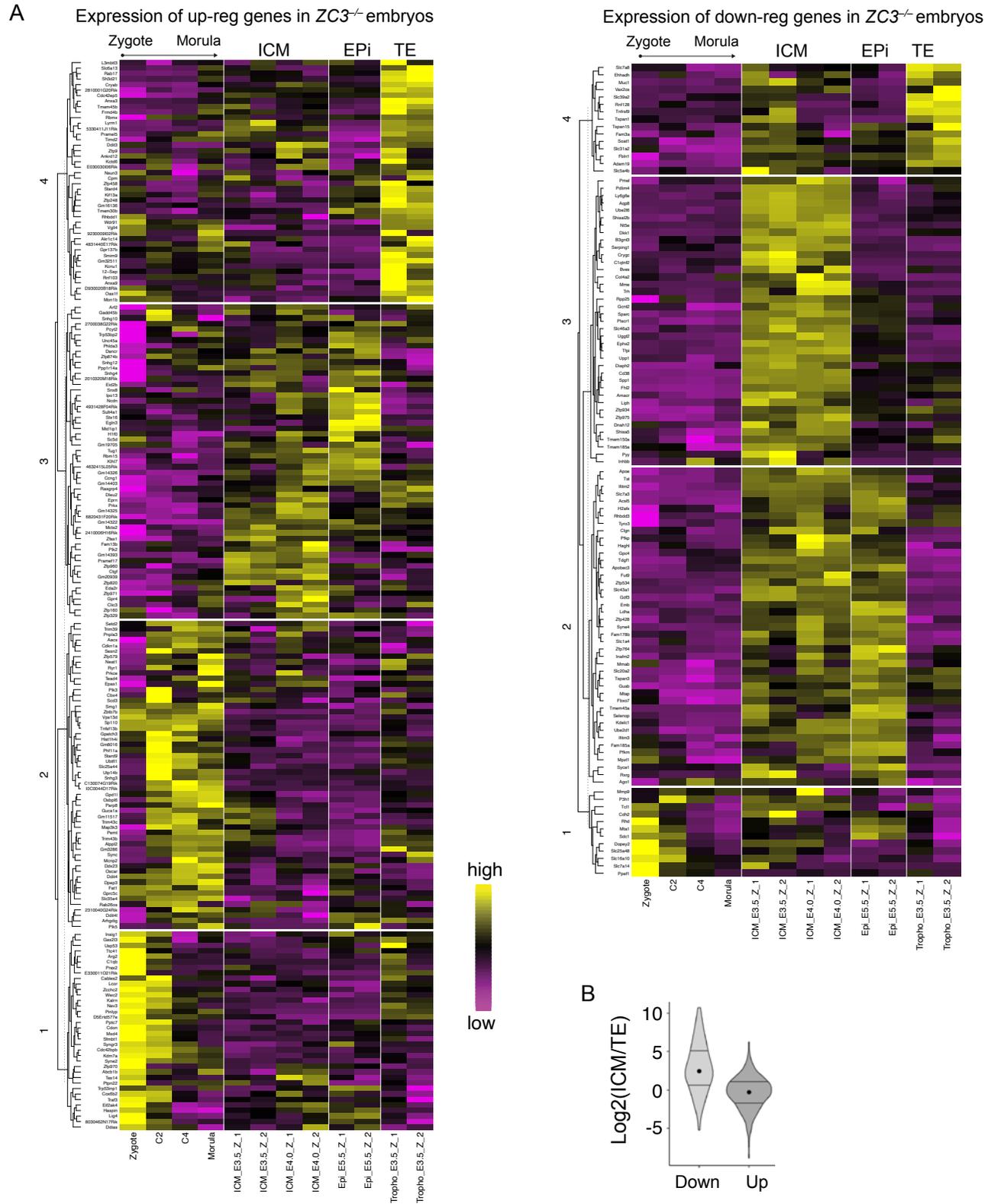


Figure S2

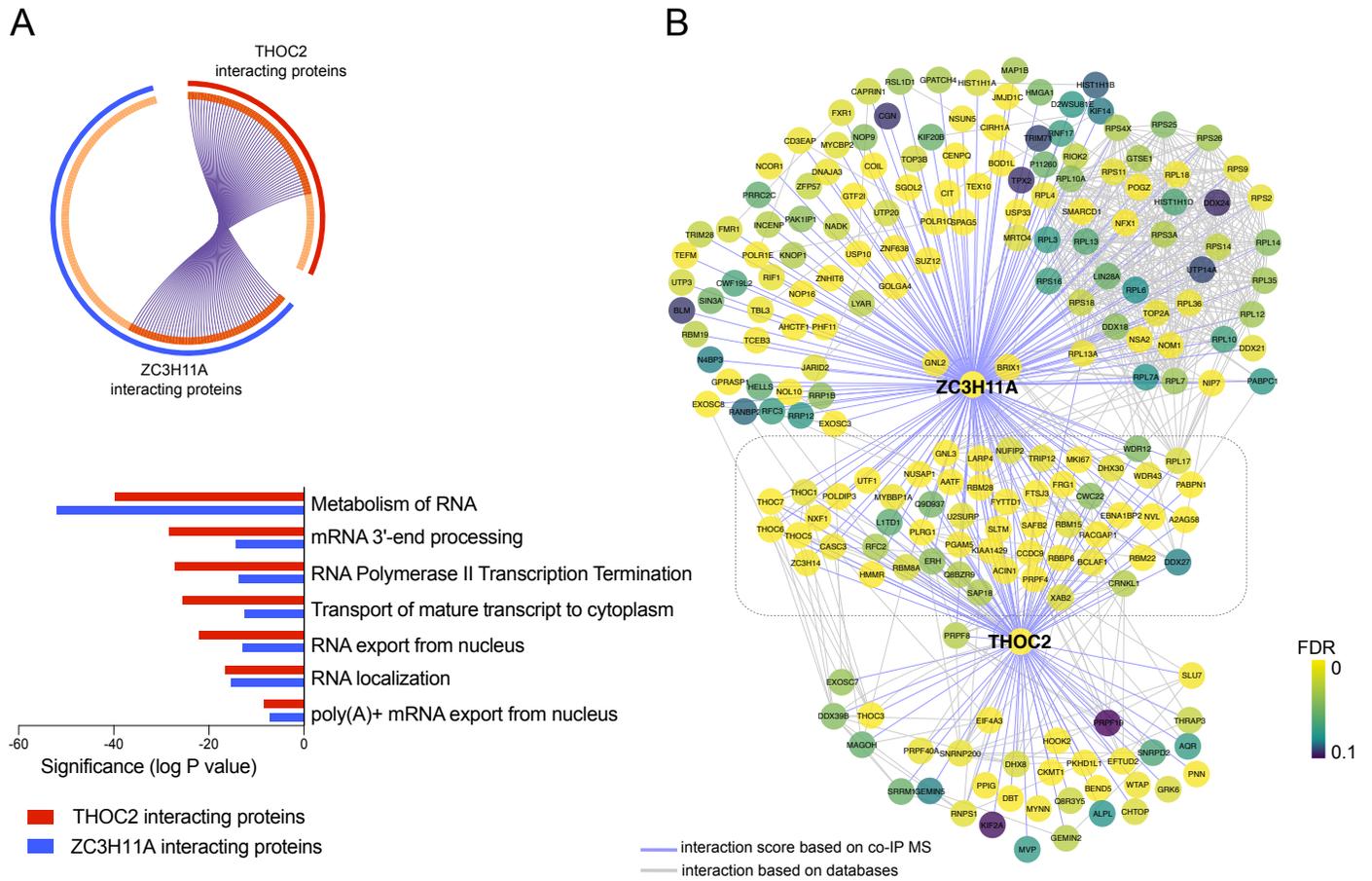
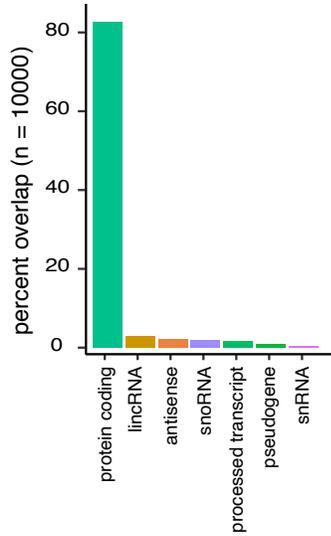


Figure S3

A

ZC3H11A target transcripts



B

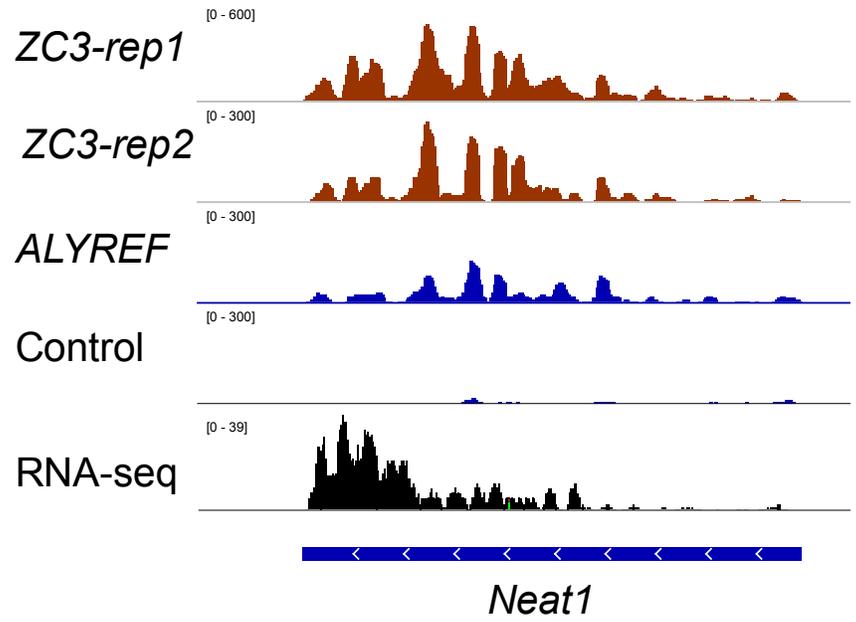


Figure S4

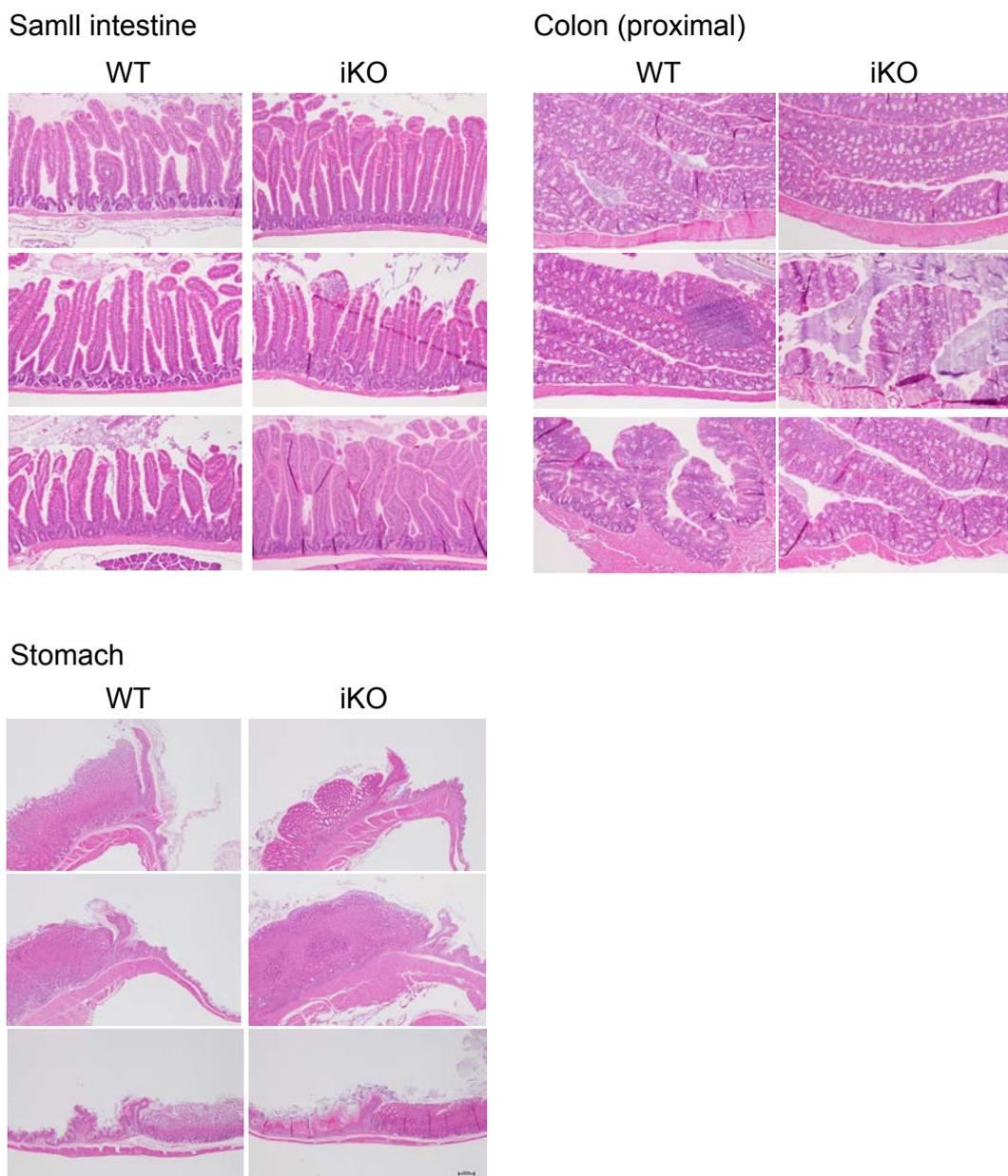


Figure S5