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1 **Anti-müllerian hormone induces autophagy to preserve the primordial follicle pool in mice**

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23 **Abstract**

24 The reserve pool of primordial follicles (PMF) is finely regulated by molecules implicated in follicular
25 growth or PMF survival. Anti-müllerian hormone (AMH), produced by granulosa cells of growing
26 follicles, is known for its inhibitory role in the initiation of PMF growth. We observed in a recent *in vivo*
27 study that injection of AMH into mice seemed to induce an activation of autophagy. Furthermore,
28 injection of AMH into mice activates the transcription factor FOXO3A which is also known for its
29 implication in autophagy regulation. Many studies highlighted the key role of autophagy in the ovary at
30 different stages of folliculogenesis and particularly in PMF survival.

31 Through an *in vitro* approach with organotypic cultures of prepubertal mouse ovaries, treated or not
32 with AMH, we aimed to understand the link between AMH, autophagy and FOXO3A transcription
33 factor. Autophagy and FOXO3A phosphorylation were analyzed by western blot. The expression of
34 genes involved in autophagy was quantified by RT-qPCR. We confirmed in our *in vitro* model the
35 FOXO3A phosphorylation decrease and the induction of autophagy in ovaries incubated with AMH.
36 AMH also induces the expression of genes involved in autophagy. Interestingly, most of these genes
37 are known to be FOXO3A target genes. In conclusion, we have identified a new role for AMH namely
38 the induction of autophagy, probably through FOXO3A activation. Thus, AMH protects the ovarian
39 reserve by inhibiting the growth of PMF, but also by enabling their survival through activation of
40 autophagy.

41

42 **Keywords** : AMH, autophagy, FOXO3A, primordial follicle, ovarian reserve.

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50 **Introduction**

51 The ovarian reserve is built up during fetal life in women (1). The reserve pool is composed of
52 quiescent primordial follicles (PMF) resulting from the association of diplotene oocytes surrounded
53 by pregranulosa cells (1). PMF are activated to begin follicular growth from fetal life in humans or
54 during neonatal period in rodents and this recruitment persists throughout reproductive life (until
55 menopause in women) (2). The reserve pool is finely regulated by molecules implicated in follicular
56 activation or PMF survival (3).

57 Phosphatidylinositol 3-kinase (PI3K) signaling pathway plays a crucial role in PMF recruitment (4).
58 Its activation by different ligands such as Kit results in a phosphorylation cascade within the oocyte
59 leading to follicular growth. Among this cascade, the transcription factor FOXO3A is phosphorylated,
60 transported from the nucleus to the cytoplasm, utilizing the 14-3-3 molecular chaperone, and
61 degraded by proteasomes after polyubiquitination (5). Anti-Müllerian hormone (AMH) is a
62 glycoprotein of the TGF- β family expressed by granulosa cells and produced by growing follicles,
63 ranging from the primary stage of development to reach its maximum at preantral and small antral
64 stage (6). AMH is known to inhibit the initial PMF recruitment (6–8).

65 The maintenance of the reserve pool is therefore dependent on the survival of PMF. Atresia is
66 the fate of 99% of follicles, including PMF (9). Several studies have highlighted the key role of
67 autophagy in this survival (9–11). Autophagy is an intracellular lysosomal-dependent degradative
68 process of cytoplasmic components and defective organelles, phylogenetically conserved from yeast
69 to mammals (9, 12). This process is observed in all cells at basal state and is induced in case of stress
70 like starvation (13). Autophagy is tightly regulated at multiple levels and autophagy-related (ATG)
71 proteins are key players in this process. Autophagy proceeds in five successive steps, namely
72 initiation, nucleation, expansion, maturation, and degradation (14). Autophagy induction results in
73 the formation of a double membrane, the phagophore, which sequesters part of the cytoplasmic

74 content. This phagophore expands and closes to form a vacuole called autophagosome. The
75 autophagosome then fuses with a lysosome to form an autolysosome in which acid hydrolases
76 degrades the sequestered material. In the ovary, autophagy is also involved at different stages of
77 folliculogenesis from the formation of reserve pool to follicular atresia and corpus luteum regression
78 (15–17).

79 Although autophagy plays a key role in the PMF resting pool, the underpinning mechanisms are
80 not clearly understood. In a recent *in vivo* study, we obtained data suggesting that injection of AMH
81 into mouse induces autophagy and we showed that AMH activated the FOXO3A transcription factor
82 (18). This factor is also known to be a regulator of autophagy (19). Based on these observations, we
83 hypothesized that AMH protects the PMF pool by both inhibiting follicular activation and inducing
84 autophagy. Using a mouse ovary organotypic culture, we studied the link between AMH, FOXO3A
85 and autophagy.

86

87 **Materials and Methods**

88 **Animals**

89 Three-week-old C57BL6 weaned female mice (Janvier Labs) were acclimated during 5 days under
90 conditions of constant temperature ($21 \pm 2^\circ\text{C}$), humidity (min. 50%), and lighting (12-h light/dark,
91 lights on at 7 a.m) with *ad libitum* access to food and water (deionized).

92 The animal facility was granted approval (No. D94-043-12) by the French administration
93 (Ministère de l'Agriculture). All procedures were approved by the local ethic committee Consortium
94 des Animaleries Paris Sud (CAPSud). For organotypic culture, mice were euthanized at 26 post-natal
95 days and ovaries were removed.

96

97 **Organotypic culture**

98 Ovaries were incubated in 5% CO₂ at 37°C into cell culture inserts (12 mm diameter, polycarbonate
99 membrane, 0.4 μm pore size, Transwell, Corning) with Dulbecco's Modified Eagle Medium

100 (DMEM/F12) supplemented with 1% glutamine, 1% sodium pyruvate (100 mM), 0.3% bovine serum
101 albumin, 1% penicillin/streptomycin and 5.6 µg/mL transferrin. Murine recombinant AMH (1 µg/mL)
102 was added when necessary (*M. musculus* recombinant AMH, CliniSciences, Signalway Antibody).

103

104 **Antibodies**

105 Antibodies directed against actin (clone C4, Millipore, 1/5000 PBST-1% milk), Smad 1 (#6944, Cell
106 Signaling, 1/500-TBST-1% milk), Phospho-Smad1/5 (Ser463/465, #9516, Cell Signaling, 1/1000-TBST-
107 5% BSA), Phospho-FOXO3A (Ser318/321, #9465, Cell Signaling, 1/1000-TBST-5% BSA), FOXO3A
108 (#2497, Cell Signaling, 1/500-TBST-5% milk), LC3 (L7543, Sigma-Aldrich, 1/2000-PBST-1% milk), Ulk1
109 (D8H5, Cell Signaling 8054, 1/500, TBST 5% BSA), Wipi2 (Sigma SAB4200399, 1/500, PBST 1% Lait),
110 Atg16L1 (MBL PM040, 1/1000, PBST 1% lait), Atg5 (Cell Signaling 12994, 1/1000, TBST5% BSA) were
111 used for western-blot analysis. Antibodies directed against FOXO3A (#2497, Cell Signaling, 1/50 PBS
112 0.5%BSA) and LC3 (PM036, MBL, 1/1000-20mM Hepes/135mM NaCl/1% BSA) were used for
113 immunofluorescence analysis. Horseradish peroxidase-linked secondary antibodies (Jackson
114 ImmunoResearch) or immunofluorescent antibodies anti-mouse or anti-rabbit (Invitrogen) were used
115 for protein gel blot analysis.

116

117 **Immunofluorescence**

118 Ovaries were dissected and fixed in 4% paraformaldehyde, washed, embedded in paraffin, and
119 sectioned. The ovaries were cut 4 µm thick and mounted on slides. Sections were deparaffinized and
120 rehydrated. After epitope retrieval treatment (at 90°C for 30min in citrate buffer solution 1X, pH 6.0;
121 Dako, Glostrup, Denmark), nonspecific binding sites were blocked for 1 h and the sections were
122 incubated with appropriate primary antibodies overnight at 4°C. The sections were washed in PBS
123 and then incubated for 1 h with appropriate secondary antibodies. After washing, the sections were
124 finally incubated with DAPI for 5 min for fluorescent detection of nuclei morphology. All sections
125 were rinsed in PBS for 5 min, air dried, and coverslipped using fluorescence mounting medium

126 (Dako). The tissue sections were then examined by confocal microscopy (SP5 Leica Confocal
127 Microscope, Leica Microsystems, Deerfield, IL, USA) or digital whole-slide high-resolution images was
128 capture using The Panoramic 250 Flash Slide Scanner system (3DHitech, Budapest, Hungary with a
129 340 Plan-Apochromat objective and a 31.6 camera adapter magnification).

130

131 **Western blot analysis**

132 Ovaries were homogenized in 65 mM Tris, pH 6.8, 4% SDS, 1.3% β -mercapto-ethanol, phosphatase
133 and protease inhibitors (Sigma-Aldrich) using a TissueLyser LT homogenizer (Qiagen). Samples were
134 held at 100°C for 8 min. After SDS-PAGE, the proteins were electrotransferred onto a PVDF or a
135 nitrocellulose membrane. For immunoblotting, the membranes were probed overnight with relevant
136 antibodies and then incubated with appropriated fluorescent or horseradish peroxidase–linked
137 secondary antibodies. Bound immunoglobulins were revealed by fluorescence or chemiluminescent
138 detection (Immobilon™ Western, Millipore). Membranes were scanned with Odyssey imaging system
139 (Li-Cor) and quantification was conducted using Image Studio 5.2 software (Li-Cor). All western blots
140 shown are representative of 3 to 8 experiments.

141

142 **Real-time reverse-transcription quantitative polymerase chain reaction (RT-qPCR)**

143 Total RNA were extracted from ovaries using the TRI Reagent® (Molecular Research Center, Inc),
144 according to the manufacturer's recommendations. First strand cDNA was synthesized using the High
145 Capacity cDNA Reverse Transcription kit (Applied Biosystems). RT-qPCR was performed on a
146 QuantStudio 6 Flex System (Applied Biosystems). Threshold cycle (Ct) values were calculated using
147 the second derivative maximum algorithm provided by the QuantStudio 6 software. To compensate
148 for variations in the RNA input and in the efficiency of the RT-qPCR, we used a normalization strategy
149 based on the housekeeping genes GAPDH and 36B4. Primer sequences are shown in Table 1. *Atg2a*,
150 *Atg5*, *Becn1*, *Bnip3*, *Ulk1*, *Wipi2* and *Zfyve1* sequences are extracted from Audesse *et al* (20). The

151 results are expressed as means \pm standard error of the mean (SEM) of 2 independent experiments
152 with quadruplicate.

153

154 **Statistical analysis**

155 All results are expressed as means \pm standard error of the mean (SEM). Statistical analyses
156 (nonparametric t test (Mann-Whitney test) for 2-group comparisons and One-way ANOVA with
157 Kruskal-Wallis posttest for multiple comparisons) were performed by GraphPadPrism (GraphPad
158 Software Inc).

159

160 **Results**

161 **AMH decreases FOXO3A phosphorylation**

162 Organotypic ovarian cultures were used to study *in vitro* AMH signalling. We treated organotypic
163 cultures with and without AMH. First, we confirmed AMH activity, since the Smad phosphorylation
164 was induced after 15 min and 30 min incubation with AMH (Fig 1A). To highlight the transcription
165 factor FOXO3A activation, we studied the expression of FOXO3A and its phosphorylated form
166 (inactive) after 3 and 6 hours AMH treatment. We observed a decreased phosphorylation of FOXO3A
167 (Fig 1B-C) and a significant decrease of P-FOXO3A/FOXO3A ratio (Fig 1B-D).

168 In oocytes, phosphorylated FOXO3A is located in the cytoplasm, then translocated in the
169 nucleus after dephosphorylation to induce the expression of target genes. To study FOXO3A
170 localization after AMH treatment, we performed immunofluorescence analysis on ovarian sections
171 (Fig. 1E). In PMF oocyte, FOXO3A is located mainly in the cytoplasm in absence of AMH, while after
172 3h AMH incubation FOXO3A is both cytoplasmic and nuclear.

173

174 **AMH induces autophagy in ovary**

175 We thus showed that AMH leads to an increase of dephosphorylated FOXO3A active form which is
176 subsequently translocated in the nucleus to exert its transcription activity. As FOXO3A is known to be

177 an actor of autophagy regulation in different tissues (20–23), we next analyzed autophagy
178 modulation by AMH in our *in vitro* model.

179 First, the localization of LC3 a widely-used marker protein that is recruited to the
180 autophagosomal membrane, was analyzed by immunofluorescence staining of tissue sections of
181 ovaries treated or not with AMH. It reveals the appearance of a large number of autophagic puncta
182 in oocytes within primordial follicles of AMH-treated ovaries compared to control ovaries (Fig. 2A).

183 To better quantify autophagosomes, LC3 protein was analyzed by western blot in total
184 extracts of ovaries incubated with or without AMH and 100 μ M chloroquine (CQ) for 13 hours (Fig.
185 2B). This protein exists in two molecular forms, LC3-I and LC3-II. When autophagy is induced, the LC3-
186 I form is cleaved and conjugated to phosphatidyl-ethanolamine (PE) to give the LC3-II form which is
187 associated with the autophagosome membrane. The expression level of the LC3-II protein is
188 therefore correlated with the autophagosome number. CQ is a lysosomotropic agent which inhibits
189 fusion between autophagosomes and lysosomes and allows to evaluate autophagosome
190 biosynthesis.

191 After 13 hours of treatment with AMH and CQ, we observed a 80% increase of LC3-II
192 compared to ovaries treated with CQ alone, indicating an increase in autophagosome biosynthesis
193 (Fig. 2B and C). To assess the autophagic flux, which reflects the entire process of autophagy
194 including both biosynthesis and degradation of autophagosomes, we calculate the ratio LC3-II
195 CQ+/LC3-II CQ- in the AMH-treated and untreated groups. The flux is increased by about 40% in the
196 ovaries treated with AMH for 13 hours, as compared to the control ovary cultures (Fig. 2D).

197 We also measured by western-blot the expression of the polyubiquitin-binding protein p62
198 (sequestosome 1), a common autophagy substrate (Fig.2B). p62/ SQSTM1 protein level is reduced by
199 about 25% in AMH-treated ovaries compared to control ovaries (Fig. 2B and C).

200 Taken together, these results indicate that AMH induces autophagy in ovarian culture.

201

202 **AMH increases the expression of genes involved in autophagy**

203 To decipher the underlying mechanisms by which AMH induces autophagy, we studied the
204 expression of FOXO3A target autophagy genes involved in different stages of autophagy by RT-qPCR.

205 After 3h of AMH treatment, we observed a significant increase of *Atg16l1*, *Gabarapl2*, *Wipi2*,
206 *Atg2a*, *Ulk1*, *Zfyve1* and *Atg5* genes expression, ranging from 25% to 50% as compared to the control
207 group (Fig. 3). This significantly increase persists after 6h of incubation for *Atg16l1*, *Gabarapl2* and
208 *Wipi2*. In contrast, the expression of *Atg2a*, *Zfyve1*, *Atg5* and *Ulk1* tends to return to the level of the
209 control group after 6h of incubation with AMH. The expression of *Atg2b* is stagnant at 3h and
210 increases significantly at 6h.

211 We studied the protein expression of ULK1, Wipi2, Atg16L1 and Atg5 in protein extracts from
212 ovaries treated or not with AMH for 6h. We thus showed that increased expression of their genes
213 correlated well with an increase in their protein expression (Fig. 4).

214 Taken together, AMH induces autophagy in ovary by increasing the expression of a number
215 of genes involved in this process.

216

217

218 Discussion

219 AMH is a well-known key regulator of ovarian follicular growth. This role was highlighted in AMH *null*
220 female mice which exhibited an increased number of growing follicles (pre-antral follicles and small
221 antral follicles) and a reduced number of PMF at 4 months of life compared to the control population
222 (7). In the same manner, disruption of AMHR2 signaling leads to a phenotype similar to *Amh*-
223 deficient mice with persistence of the Müllerian ducts and premature depletion of the follicular pool
224 (6, 24). Moreover in women, mutation of *AMHR2* is responsible for clinical presentation of primary
225 ovarian insufficiency (POI) (25–27). To date, the mechanism by which AMH inhibits follicle growth
226 remains unknown. In the present study, we show that AMH decreases FOXO3A phosphorylation in
227 organotypic ovarian cultures and induced its nuclear translocation, confirming our previous *in vivo*
228 observation in pubescent mice injected with exogenous recombinant AMH (18).

229 In adult female, most PMF are quiescent. Foxo3A plays a crucial role in maintaining this
230 dormancy, which is necessary to preserve the ovarian reserve and maintain the reproductive capacity
231 of mammals (28). *Foxo3a null* female mice are sterile from 15 weeks of life due to complete follicular
232 loss (29). Conversely, *Foxo3*-transgenic mice, with FOXO3 overexpression, present a larger PMF
233 count than wild-type mice (30). Thus, FOXO3A signaling inhibits PMF activation, maintaining them
234 into their initial state, preserving thereby the follicular reserve. The decreased FOXO3A
235 phosphorylation and its nuclear translocation after AMH treatment suggest that inhibition of
236 follicular growth by AMH may involve FOXO3A signaling.

237 FOXO3A is also involved in autophagy regulation. FOXO3A induces expression of autophagy
238 genes in several cell models. In neural stem cells, Audesse *et al* identified FOXO3A target genes based
239 on ChIP-seq analysis (20). Through inactivation or overexpression of FOXO3A, they confirmed *Wipi2*,
240 *Atg2a*, *Atg5*, *Bnip3*, *Ulk1*, *Zfyve1*, *Atg10* and *Pink1* as FOXO3A target genes in this cellular model.
241 *Bnip3* as target gene was confirmed in hematopoietic stem cells (23). Zhao *et al* and Mammucari *et*
242 *al* studied FOXO3A target genes in muscle cells and identified two additional genes : *LC3* and *Becn1*
243 (21, 22). In the current study we demonstrate that AMH induces an increase in autophagosome
244 biosynthesis and autophagic flux in ovaries. Moreover, expression of *Ulk1*, *Atg2a*, *Atg2b*, *Atg5*,
245 *Becn1*, *Gabarapl2*, *Atg16l1*, *Wipi2* and *Zfyve1*, autophagy genes involved at different stages of
246 autophagy and controlled by FOXO3 at the transcriptional level (20, 21, 31, 32), was significantly
247 increased in AMH treated ovaries. These results strongly suggest that AMH induces autophagy in the
248 ovary through FOXO3A signaling.

249 Autophagy is known to be crucial in PMF pool preservation. In fact, germ cell-specific *Atg7*
250 knockout in female mice lead to severely subfertility with a significant decline of PMF pool at 6
251 months of age (10). In the same manner, compromised autophagy within the perinatal ovary,
252 through the loss of *Becn1* or *Atg7*, also results in the premature loss of female germ cells (11). Along
253 this line we have demonstrated that *ATG7* and *ATG9* loss-of-function variants were involved in the
254 pathogenesis of POI in women (33).

255 In our previous study (18), we showed that *in vivo* AMH injection to mice, results in an
256 increase in PMF number which is in accordance with studies showing an increased number of PMF
257 after caloric restriction or rapamycin treatment which both induce autophagy (34, 35).

258 Thus, we can conclude that AMH plays a crucial role in follicular pool preservation. We
259 propose that AMH has two combined actions both mediated by FOXO3A signaling, the first one by
260 inhibiting follicular activation and second one by activating autophagy, both actions contributing to
261 the preservation of the ovarian reserve (Cf graphical abstract).

262

263 **Data Availability statement**

264 All study data are included in the article

265

266 **Conflict of interest**

267 The authors declare to have no competing interest.

268

269 **Authors contributions**

270 Isabelle Beau and Charlotte Sonigo designed research; Tatiana Lecot-Connan, Yasmine Boumerdassi,
271 Françoise Magnin and Isabelle Beau performed the experiments; Tatiana Lecot-Connan, Yasmine
272 Boumerdassi and Isabelle Beau analyzed data; Isabelle Beau, Charlotte Sonigo, Nadine Binart and
273 Peter Kamenický discussed the results and Tatiana Lecot-Connan, Charlotte Sonigo and Isabelle Beau
274 wrote the paper.

275

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284

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384

385 **Figure Legends**

386

387 **Figure 1. AMH decreases FOXO3A phosphorylated form.** Ovaries were incubated in the presence or
388 absence of 1 µg/mL AMH for 3 and 6 hours. A) Representative western-blot analysis of P-Smad1/5,
389 Smad1 and Actin proteins in the control group and after 15 and 30 min incubation with AMH and P-
390 Smad/Smad ratio. B) Representative western blot analysis of P-FOXO3A, FOXO3A and Actin proteins
391 in the control group and after 3 and 6 hours incubation with AMH. C) and D) The graphs represent
392 the P-FOXO3a/actin and P-FOXO3A/FOXO3 ratio at the different time points. Data represent the
393 mean ± SEM of at least 3 independent experiments (*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001
394 Kuskal-Wallis test). E) Histologic analysis of FOXO3A localization in PMF from ovaries incubated or
395 not with AMH for 3 hours. Ovarian sections were immunostained with an antibody against FOXO3A
396 (red). Nuclei were labelled with Dapi (blue). Images were taken on a Leica SP5 confocal microscope.
397 Bar scale : 10µm.

398

399 **Figure 2. AMH induces autophagy.** Ovaries were incubated in the presence or absence of 1 µg/mL
400 AMH and 100 µM CQ for 13 hours. A) Immunofluorescence analysis of autophagosomes in ovaries
401 incubated or not with AMH for 13 hours. Ovarian sections were immunostained with an antibody
402 against LC3 (red). Nuclei were labelled with Dapi (blue). Images were taken from scanned slides. Bar
403 scale : 10µm. B) Ovary lysates were subjected to immunoblotting analyses using anti-p62, anti-LC3
404 and anti-actin antibodies. C) and D) The graph represents the p62/Actin, LC3-II/Actin ratio and the
405 autophagic flux. Data represent the mean ± SEM of 8 independent experiments. *p<0.05, ** p < 0,01,
406 by Mann-Whitney test. D)

407

408 **Figure 3. AMH increases the expression of genes involved in autophagy.** Ovaries were incubated in
409 the presence or absence of 1 µg/mL AMH for 3 and 6 hours. Total RNA were extracted and quantified

410 by RT-qPCR. Results are expressed as the relative amount of ΔCt , between control and treated
411 ovaries, normalized to the mean ΔCt of two housekeeping genes, GAPDH and 36B4. These results are
412 from two experiments, in which each condition was represented in quadruplicate. Error bars
413 represent SEM and dots represent values distribution. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p <$
414 0.0001 , by Kruskal-Wallis test.

415

416 **Figure 4: AMH increases the expression of Ulk1, Wipi2, Atg16L1 and Atg5.** Ovaries were incubated
417 in the presence or absence of 1 $\mu\text{g}/\text{mL}$ AMH for 6 hours. Ovary lysates were subjected to
418 immunoblotting analyses using anti-Ulk1, anti-Wipi2, anti-Atg16L1 anti-Atg5 and anti-actin
419 antibodies. The band corresponding to each Atg protein was quantified and normalized against actin
420 used as a loading control (Atg/actin).

421

Figure 1

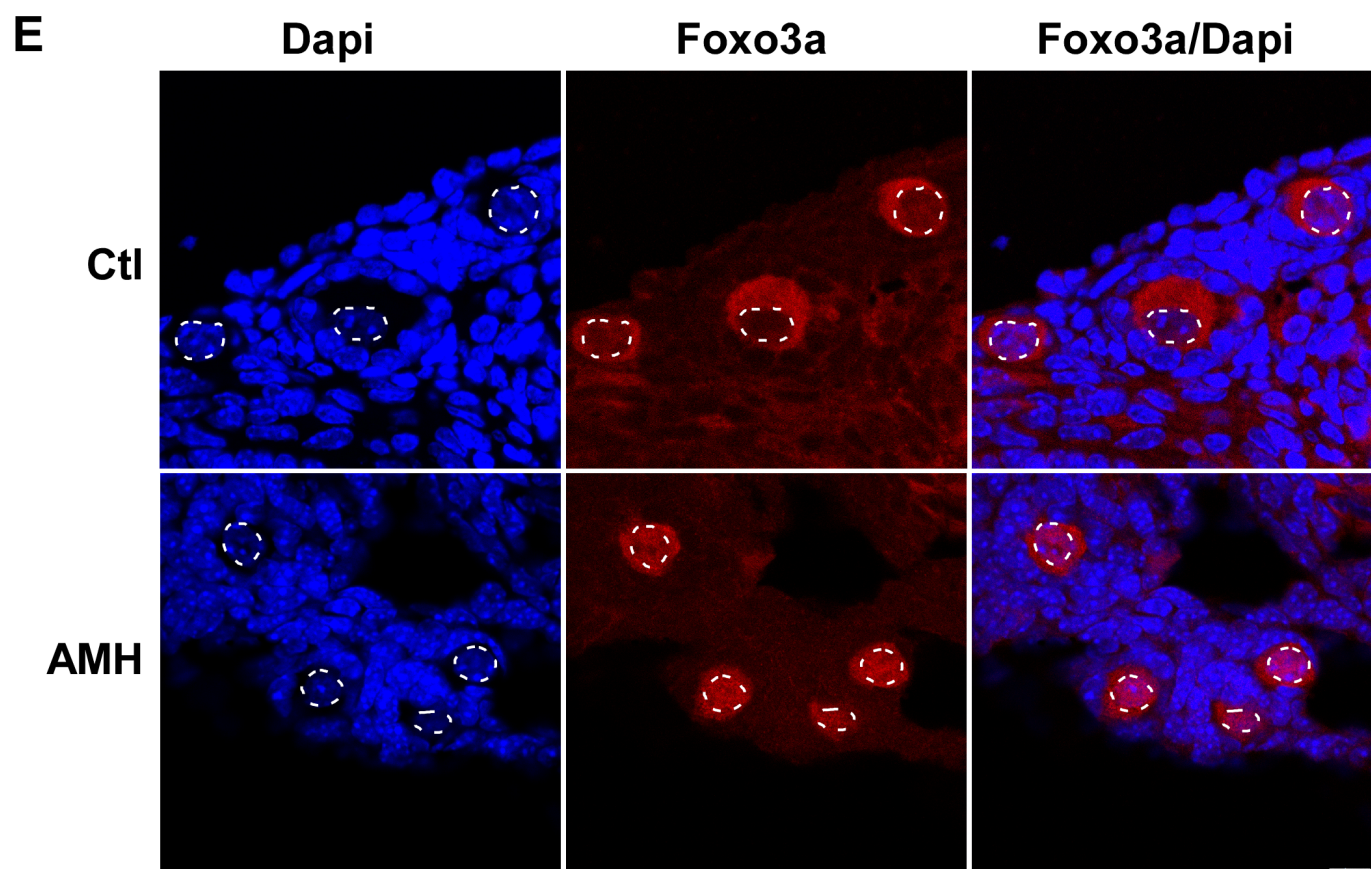
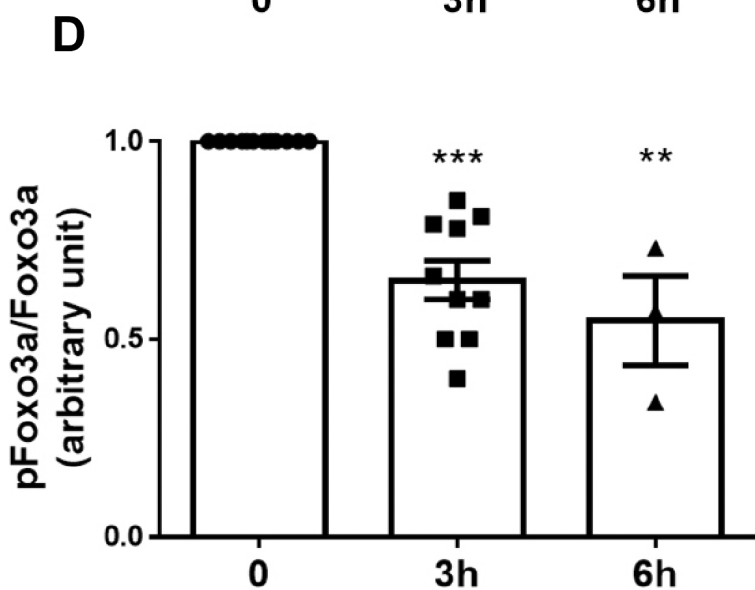
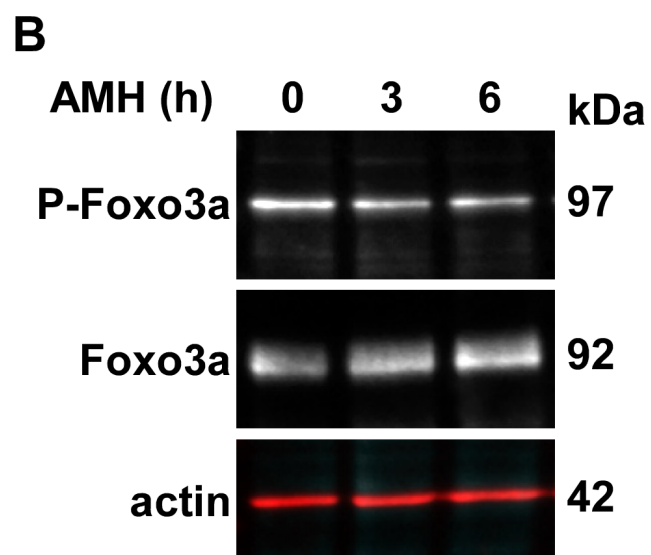
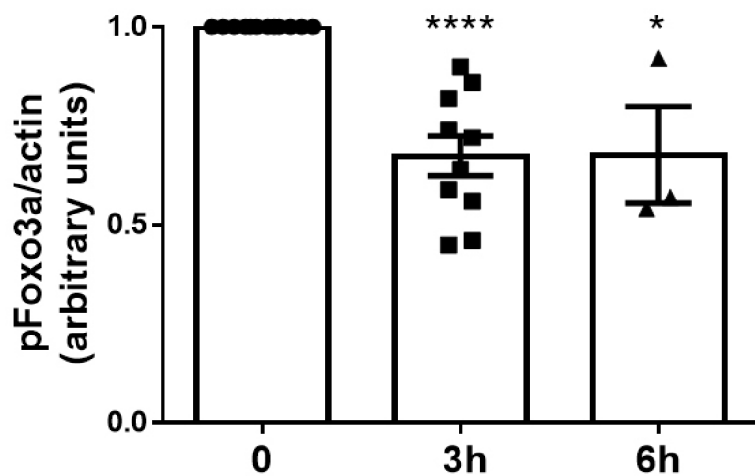
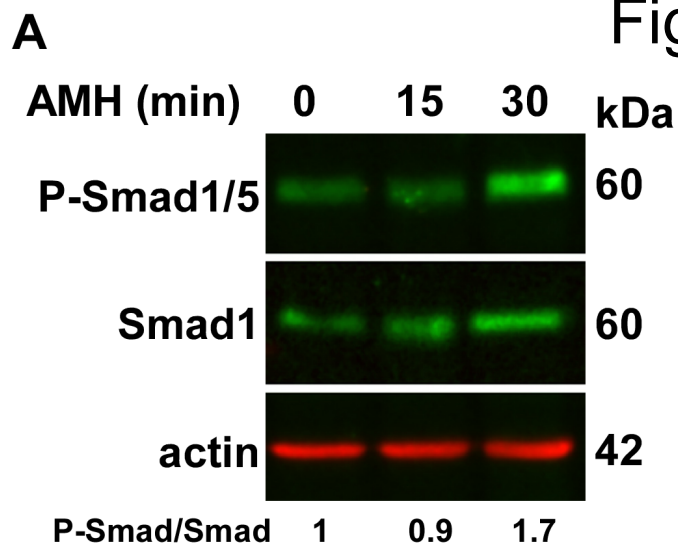


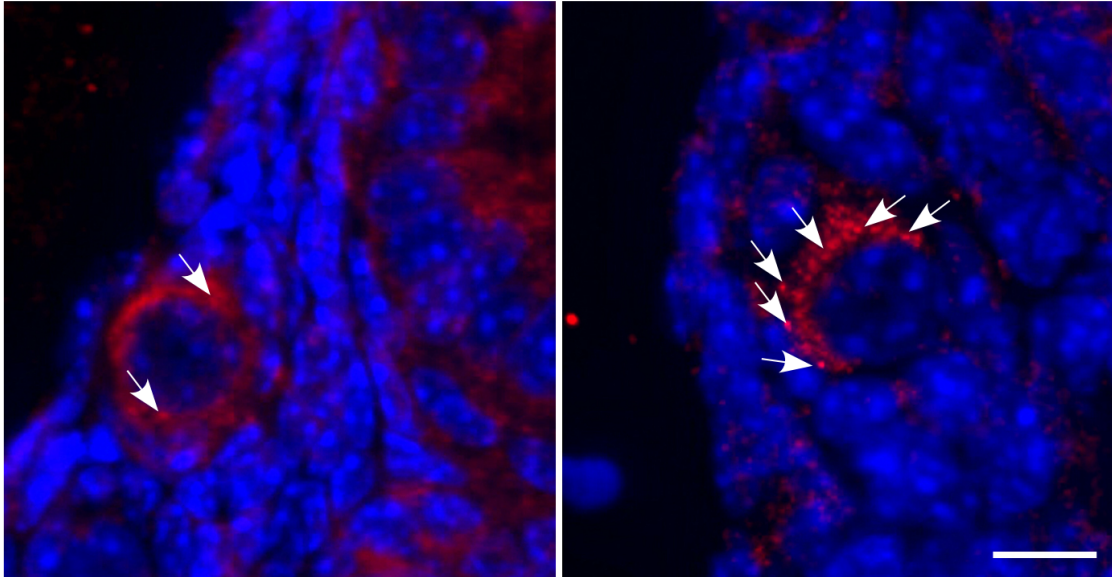
Figure 2

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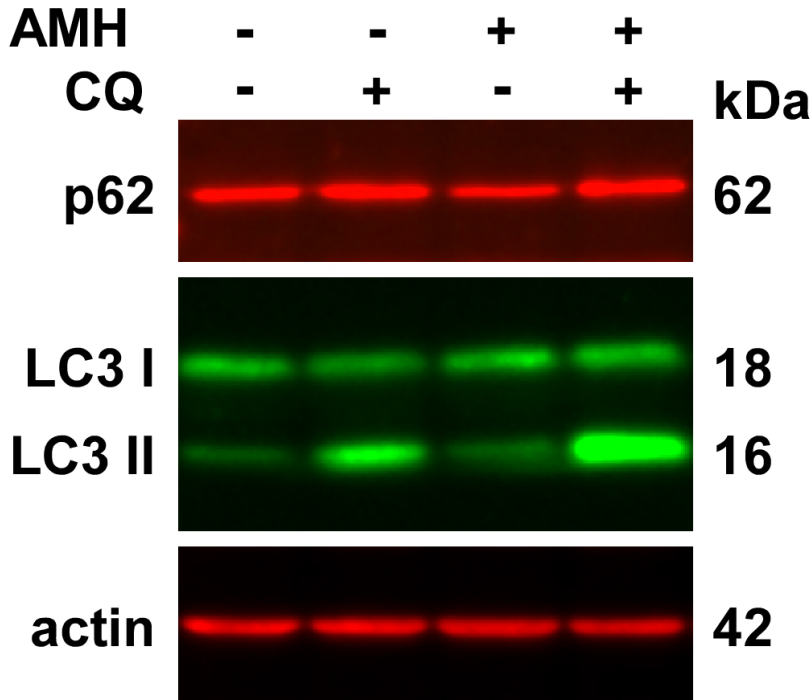
Ctl

AMH

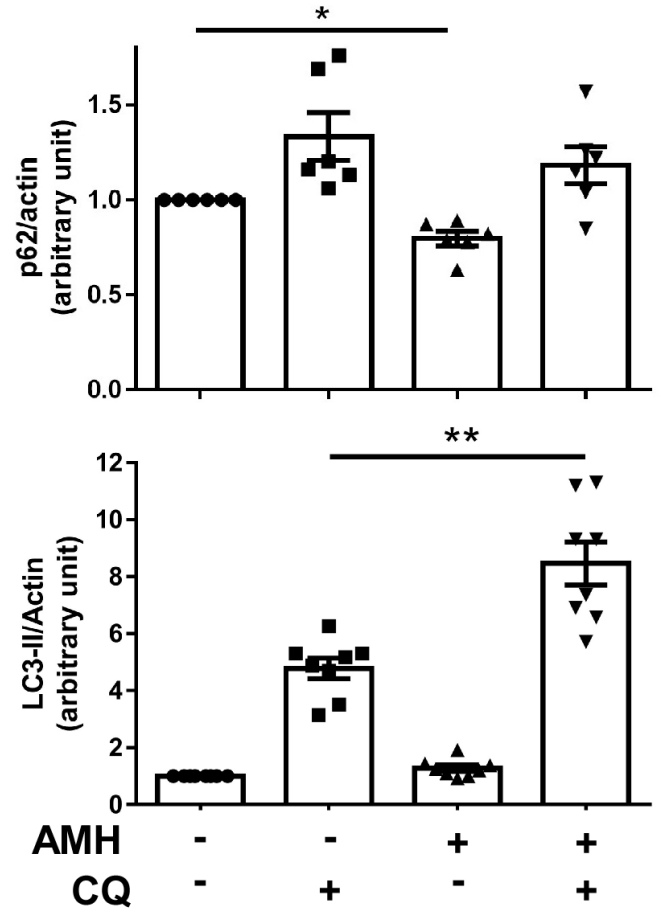
LC3/
Dapi



B



C



D

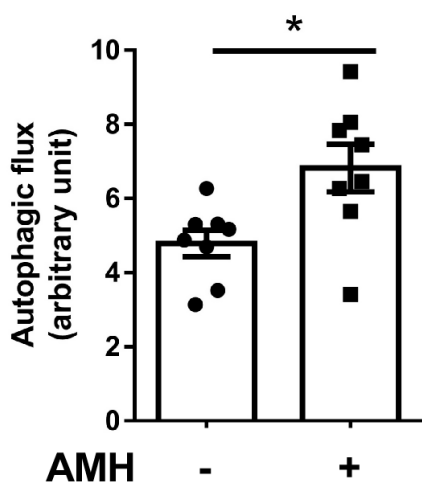


Figure 3

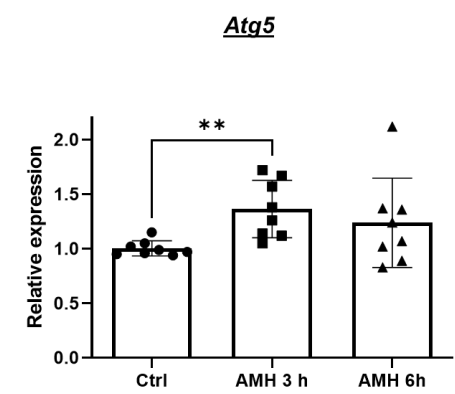
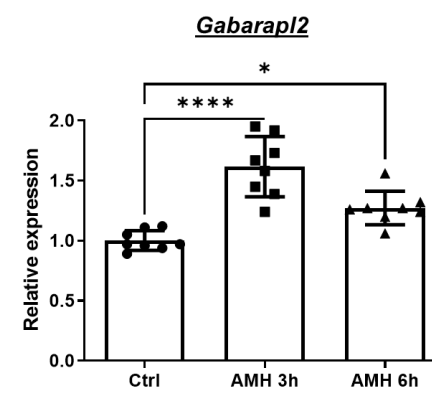
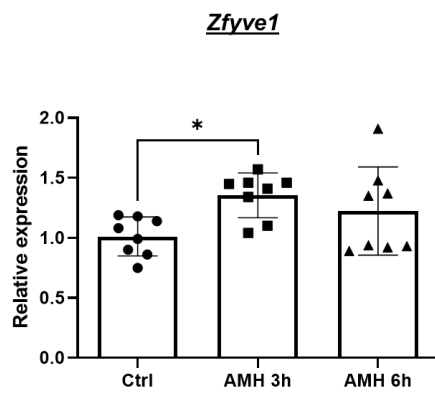
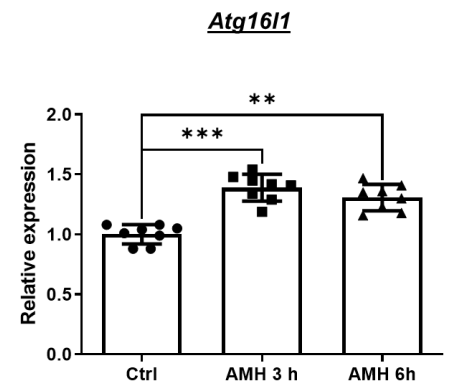
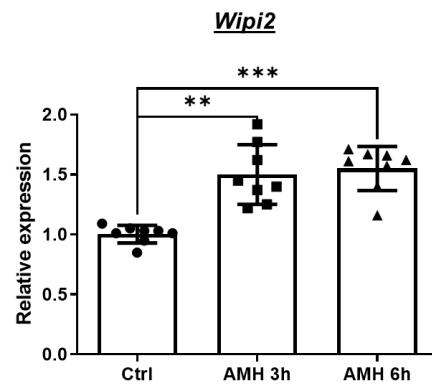
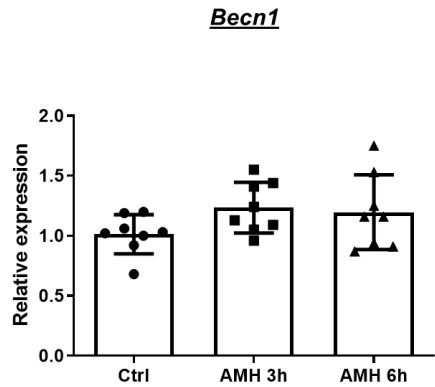
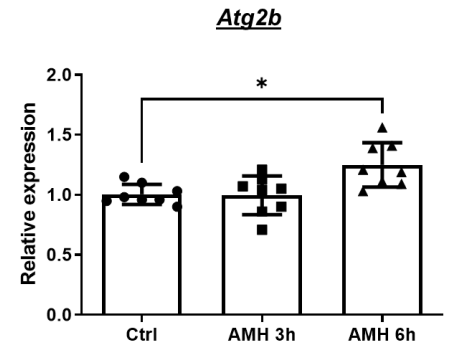
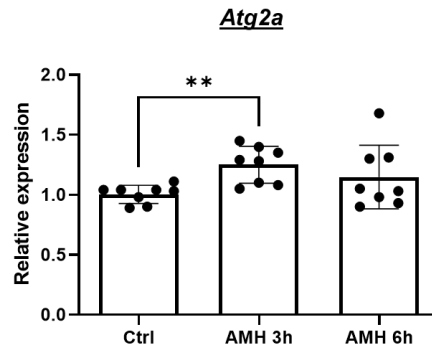
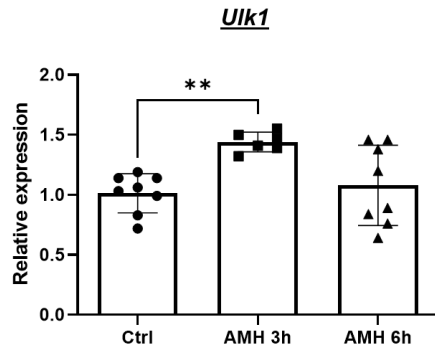


Figure 4

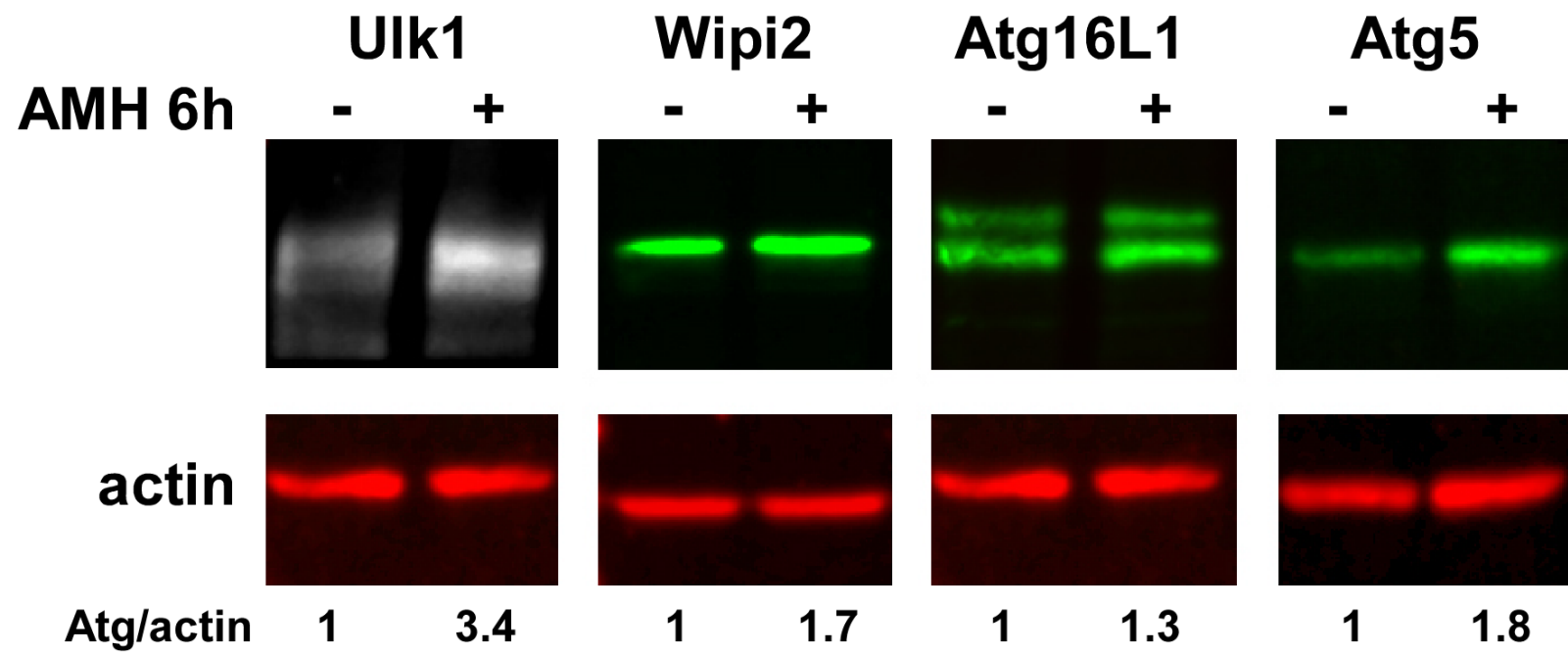


Table 1

Gene	Forward primer sequence	Reverse primer sequence
<i>Atg2a</i>	GGCATGGCTAAGGCTTATGA	TCAGTCCCTTCTGCTCATGG
<i>Atg2b</i>	CAAAATCTTCCCCGTGTGT	AGTTGTGGGTGTGGCTCATAAA
<i>Atg5</i>	GATGGACAGCTGCACACACT	TTGGCTCTATCCCGTGAATC
<i>Atg9b</i>	TCCTTCGCTGCGTGGATTAC	CGAGGGTAGAATGGCATCTGA
<i>Atg16l1</i>	TTTTCAGGGCTGGTGGTATGT	AGGAAGGGAGTTCAGGGTTCTG
<i>Becn1</i>	TGTGTGCAGCAGTTCAAAGA	CACTGCCTCCAGTGTCTTCA
<i>Bnip3</i>	TTCTGAAGGTTTTCTTCCATC	TTCATCAGAAGGTGCTAGTGGA
<i>Gabarapl2</i>	ACAGTCCCACAGTCCAGCCTAA	CTCTCCGCTGTAGGCCACAT
<i>Ulk1</i>	CCAGGCAGACATTGAGAACA	GTTGGCAGCAGGTAGTCAGG
<i>Wipi2</i>	GTGCCTATGTGCCCTCGT	ATCCTCTAGGCATGCACCAC
<i>Zfyve1</i>	CCGTGCAGAACACCTTGG	GTGCAGGATTCATGGTCAG