Supplemental Information

Fluorescence-based visualization of autophagic activity predicts mouse embryo viability

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Supplementary Figure 1

Embryos, microinjected at the 1-cell stage with GFP-LC3 mRNA, were cultured with or without $BafA_1$ until the 4-cell stage, and then observed under confocal microscopy. Massive accumulation of GFP-LC3 puncta was observed in $BafA_1$-treated embryos. DIC, differential interference contrast. Scale bar, 10 μm.

Supplementary Figure 2

GFP-LC3 degradation occurs in an autophagy-dependent manner.

(**A**) Inhibition of autophagy or lysosomal functions reduces the degradation of GFP-LC3. Embryos injected with a mixture of GFP-LC3 and control RFP mRNAs were cultured with inhibitors of either autophagy (wortmannin) or the lysosome (E64d+pepstatin A), and GFP-LC3 fluorescence was observed throughout embryo development. (**B**) Graph showing the total fluorescence intensities of GFP-LC3 and control RFP during embryonic development; values are expressed relative to the fluorescence at the 2-cell stage. Values are means ± s.e.m. of three different experiments, with at least 10 embryos analyzed per group. $^{*}P < 0.05$; $^{**}P < 0.01$ (Student's *t*-test). DIC, differential interference contrast. Scale bars, 100 μm.

Supplementary Figure 3

Parameters of fertility with advancing maternal age.

(**A**) The number of ovulated oocytes is significantly lower in aged mice than in young mice. These females were superovulated by hormone administration. Error bars, s.e.m.; $^{**}P < 0.01$ (Student's *t*-test). (**B**) Fertilization rate in aged mice is comparable to that in young mice. Oocyte collected from either young or aged females were *in vitro* fertilized

with wild-type sperm, and the resultant fertilized zygotes were scored. $P = 0.0511$ (**C**) Comparison of development between embryos from young and aged mothers. *In vitro* fertilized embryos were cultured and scored at the 4-cell stage (50–51 h after IVF). *n* represents the number of mice (**A**) or embryos (**B, C**) analyzed.

Supplementary Figure 4

Decline of lysosomal enzyme activity during maternal aging.

(**A**) DQ-Red BSA staining of 4-cell embryos from young or aged mothers. (**B**) Comparison of the number of DQ-Red BSA dots per blastomere between embryos from young and aged mothers. Numbers of embryos analyzed are shown above the bars. Error bars, s.e.m.; $^{*}P < 0.05$ (Student's *t*-test).

Supplementary Table 1

Comparison between autophagic activity and embryonic developmental ability (related to Figure 3).

Supplementary Videos 1

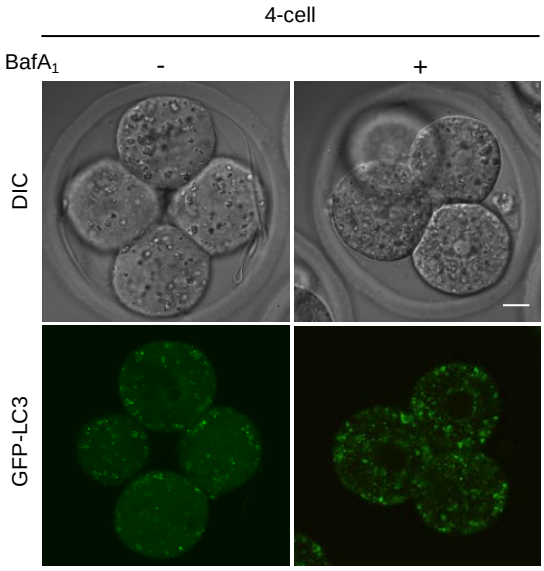
GFP-LC3 degradation in developing embryos. Embryos, microinjected with GFP-LC3 mRNA at the 1-cell stage, were cultured and imaged at 1-h intervals from the 1-cell to morula stages. The GFP-LC3 fluorescence rapidly disappeared between the 4- and 8-cell stages. (AVI; 4 MB).

Supplementary Videos 2

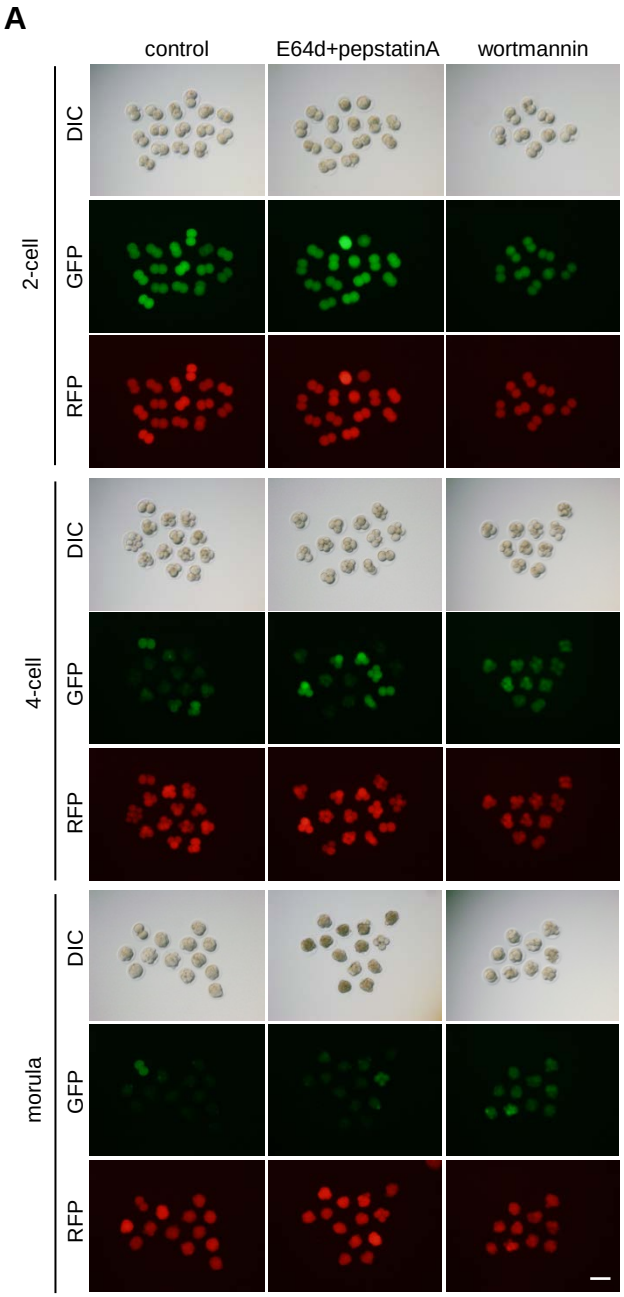
Inhibition of GFP-LC3 turnover in developing embryos. Embryos, microinjected with

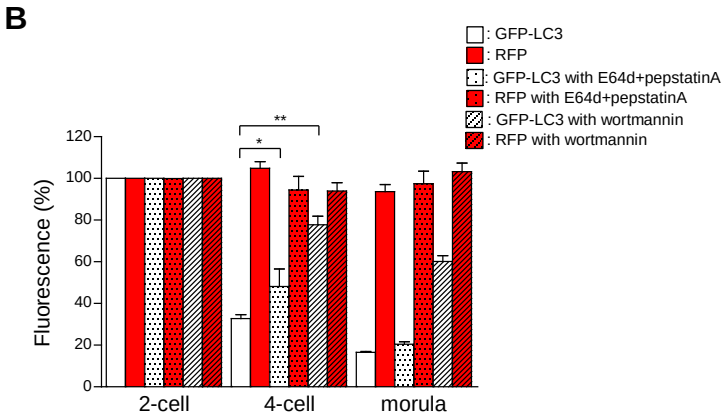
GFP-LC3 mRNA at the 1-cell stage, were co-cultured with BafA1 and imaged at 1-h intervals from the 2-cell to morula stages. In the presence of BafA1, GFP-LC3 fluorescence remained visible even beyond the 4-cell stage. (AVI; 4 MB).

Supplementary Fig. S1

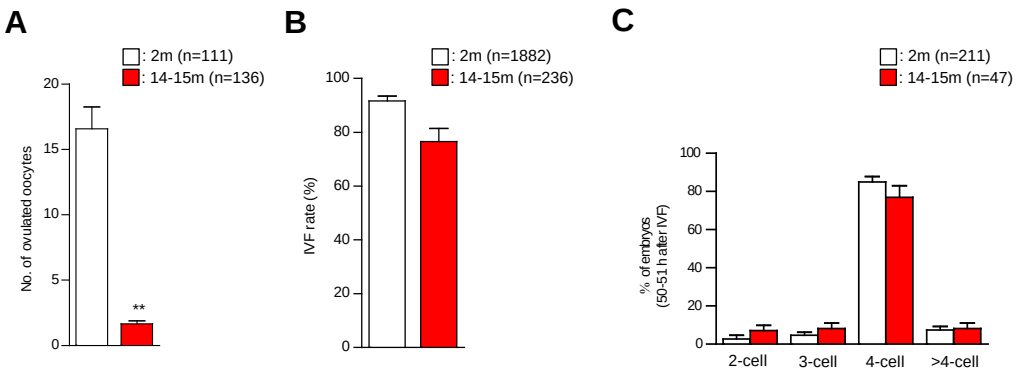


Supplementary Fig. S2

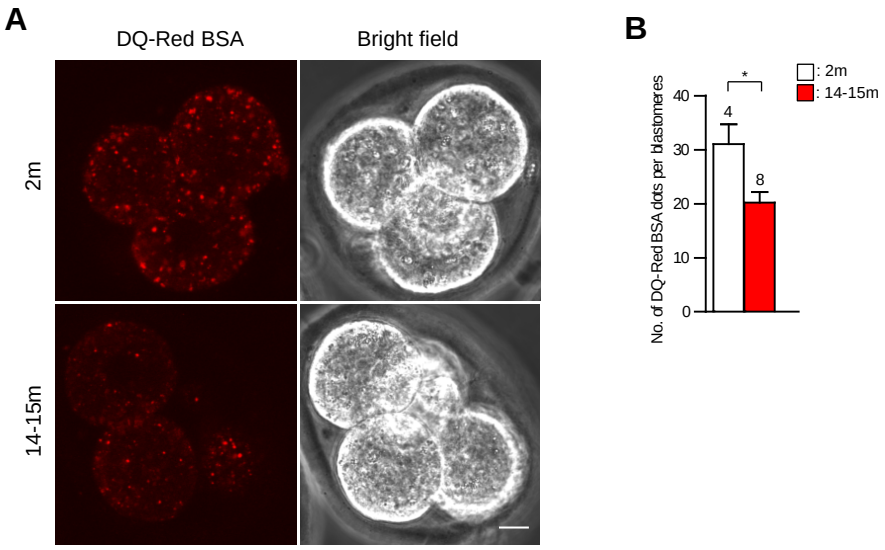




Supplementary Fig. S3



Supplementary Fig. S4



Supplementary Table S1 | Comparison between autophagic activity and embryonic developmental ability (related to Fig. 3).

Experimental No.	No. of embryos examined*	Fluorescence levels of GFP-LC3	No. of embryos categorized (%)	Average of GFP-LC3 fluorescence intensity (mean ± s.d.)	Estimated embryonic developmental potential	Hatched Blastocyst (%)	No. of 4-cell embryos transferred (recipients)	No. of implantation sites (%)[#]	Pups (%)[#]
1	9	Low	5 (44.4)	16.76 ± 2.4	Good	100			
		High	4 (38.9)	25.45 ± 1.6	Poor	60			
2	18	Low	11 (61.1)	11.51 ± 3.0	Good	81.2			
		High	7 (38.9)	25.47 ± 3.0	Poor	28.6			
3	43	Low	27 (62.8)	14.38 ± 3.5	Good	77.8			
		High	16 (37.2)	30.26 ± 4.1	Poor	31.2			
4	19	Low	13 (68.4)	9.95 ± 3.1	Good	53.8			
		High	6 (31.6)	25.55 ± 6.2	Poor	33.3			
5	66	Low	55 (83.3)	11.98 ± 3.6	Good	78.2	11 (1)	11 (100)	8 (72.7)
		High	11 (15.2)	23.49 ± 4.2	Poor	45.5	11 (1)	9 (81.8)	5 (45.5)
6	53	Low	34 (64.2)	11.9 ± 3.6	Good		22 (2)	21 (95.5)	14 (63.6)
		High	19 (35.8)	23.4 ± 4.1	Poor		19 (2)	12 (63.2)	7 (36.8)
7	56	Low	42 (75.0)	14.1 ± 2.7	Good		35 (3)	33 (94.3)	27 (77.1)
		High	14 (25.0)	24.9 ± 5.2	Poor		14 (1)	10 (71.4)	5 (35.7)

*Only embryos that normally developed to the 4-cell stage were used in this analysis.

[#]The number of 4-cell embryos transferred was taken as 100% for the calculation of the percentage of the implantation sites or pups.