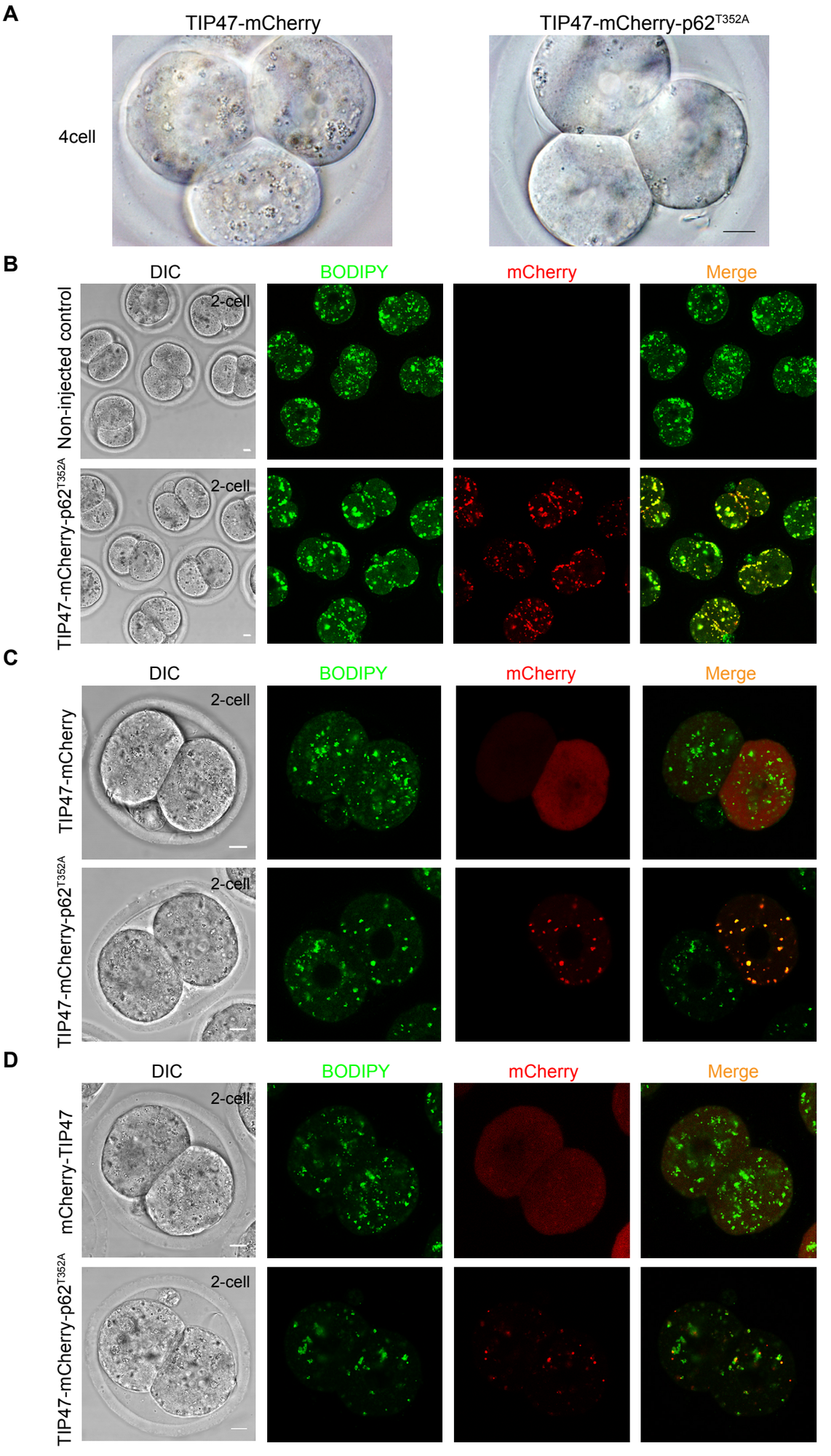


Fig. S1. Forced lipophagy in fertilized mouse embryos.

(A) Morphology of LDs in four-cell embryos expressing either TIP47-mCherry or TIP47-mCherry-p62^{T352A}. Scale bars, 10 μm.

(B) C3H mouse two-cell embryos, microinjected with or without TIP47-mCherry- p62^{T352A} mRNA, were stained with BODIPY 493/503 and observed by laser confocal fluorescence microscopy. DIC: differential interference contrast. Scale bars, 10 μm.

(C) mRNA for either TIP47-mCherry or TIP47-mCherry-p62^{T352A} was microinjected into one blastomere of two-cell embryos, which were then cultured for several hours, stained with BODIPY 493/503, and observed by laser confocal fluorescence microscopy.
DIC: differential interference contrast. Scale bars, 10 μm.

(D) Two-cell embryos microinjected at the one-cell stage with mRNA encoding either mCherry-TIP47 or mCherry-TIP47-p62^{T352A} (instead of TIP47-mCherry or TIP47-mCherry-p62^{T352A}) were stained with BODIPY 493/503 and observed by laser confocal fluorescence microscopy.
DIC: differential interference contrast. Scale bars, 10 μm.

Note that mRNAs for either TIP47-mCherry or mCherry-TIP47 were not detected at the LD surface, and instead remained diffusely distributed in the cytoplasm, whereas mRNAs encoding either TIP47-mCherry-p62^{T352A} or mCherry-TIP47-p62^{T352A} could induce lipophagy, suggesting that p62 expression at the LD surface stabilizes TIP47 or its interaction with LDs.

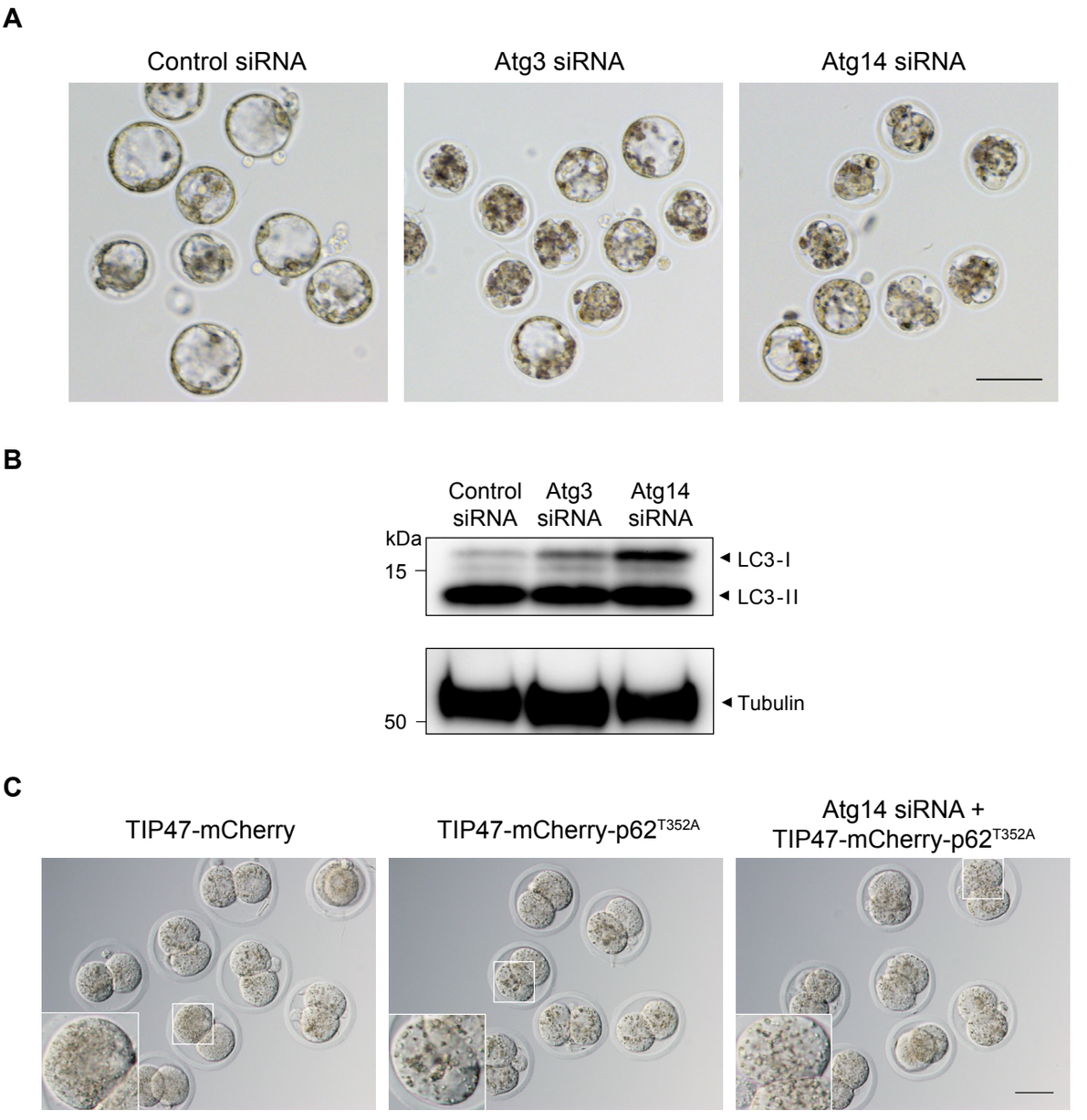


Fig. S2. Blocking of embryonic autophagic activity by siRNA injection.

(A) Representative images of embryos microinjected with control siRNA or siRNA targeting either Atg3 or Atg14, and cultured to the blastocyst stage. Scale bars, 100 µm.

(B) Immunoblotting of LC3 in embryos microinjected with control siRNA or siRNA targeting either Atg3 (Atg3 siRNA) or Atg14 (Atg14 siRNA), and cultured to the 8-cell stage. Total cell extracts (35 embryos per lane) were subjected to immunoblot analysis with anti-LC3 antibody. The membrane was re-probed with anti-tubulin antibody (as a loading control). Note that LC3 lipidation (LC3-II form) was detected even in Atg14 siRNA-injected embryos, even though autophagic activity was profoundly inhibited (Fig. 4C; LC3 dots [representing autophagosome] were almost absent in these embryos). A similar phenomenon was observed upon RNAi-mediated suppression of Atg14 in HeLa cells (Itakura et al., 2008), as well as in Atg14-deficient mouse embryonic fibroblasts (Matsunaga et al., 2009).

(C) Morphology of LDs in embryos microinjected with either TIP47-mCherry (left) or TIP47-mCherry-p62^{T352A} (middle) or a mixture of TIP47-mCherry-p62^{T352A} and Atg14 siRNA, and cultured to the 2-cell stage. Inset, higher-magnification image. Scale bars, 50 µm.

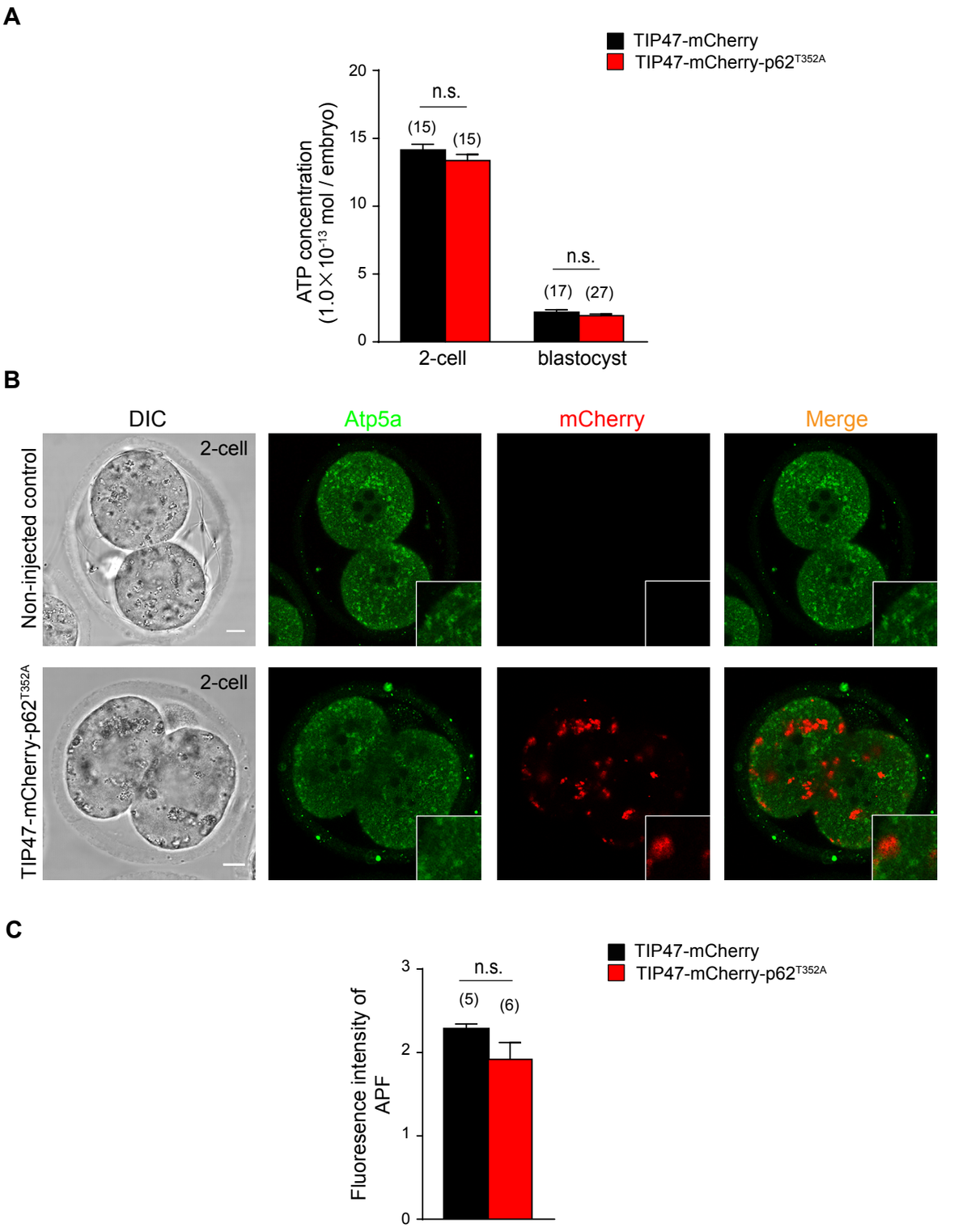


Fig. S3. Intracellular energy level does not change in lipophagy-induced embryos.

(A) ATP content of embryos expressing or not expressing TIP47-mCherry-p62^{T352A} was analyzed at the indicated stage. The number of embryos analyzed is shown above each error bar. Error bars, S.E.M.; n.s., not significant.

(B) Immunofluorescence imaging of LD colocalization with Atp5a (mitochondria marker) in two-cell embryos either injected or not injected with TIP47-mCherry-p62^{T352A} mRNA. Inset, higher-magnification image. Scale bars, 10 μm.

(C) ROS levels did not change between control and lipophagy-induced embryos. Embryos expressing either TIP47-mCherry or TIP47-mCherry-p62^{T352A} were cultured until the blastocyst stage, and then stained with aminophenyl fluorescein. Total cellular fluorescence was estimate by CCM. Error bars, S.E.M.; n.s., not significant.

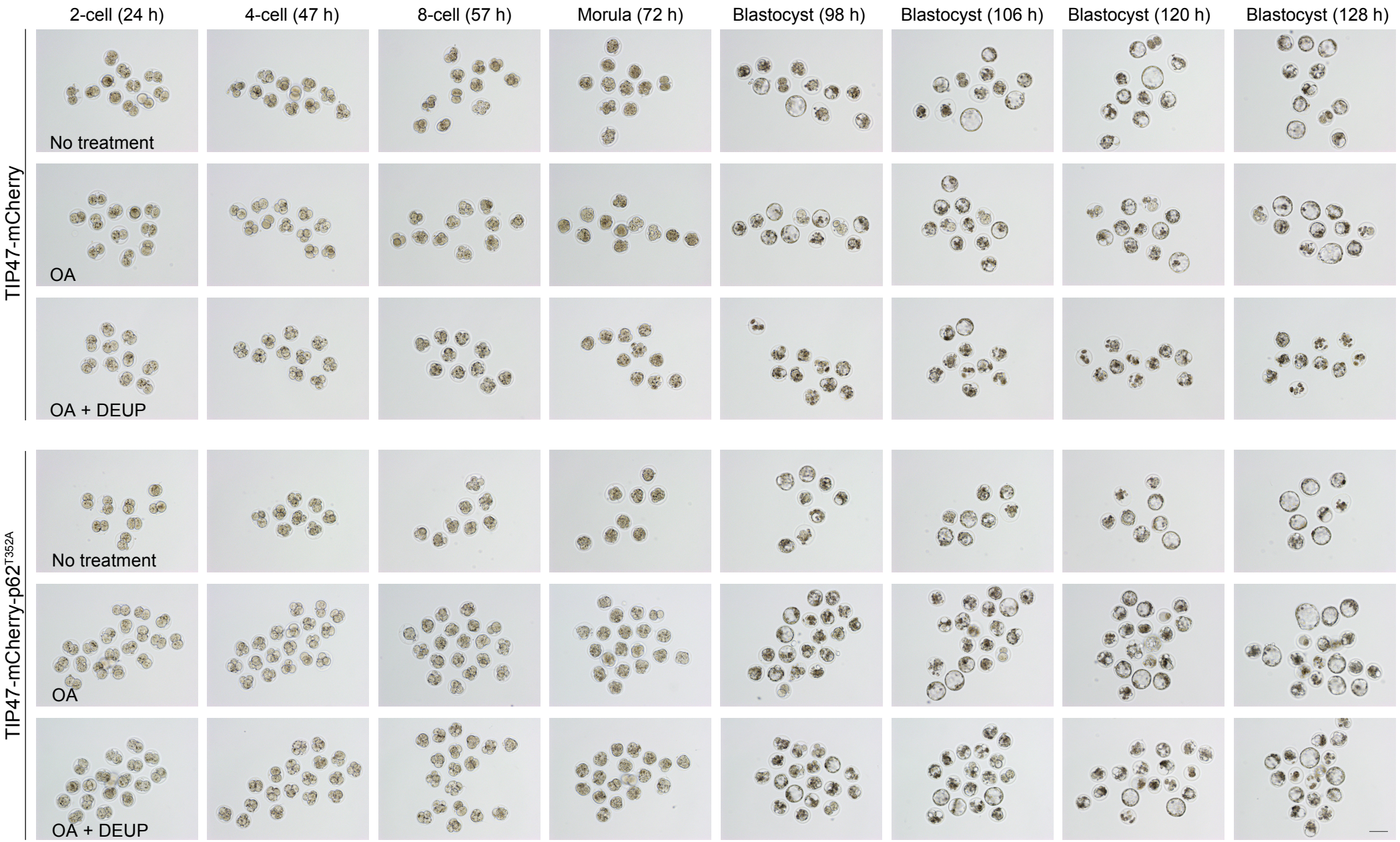


Fig. S4. Preimplantation development of lipophagy-induced embryos.

Representative examples of embryos expressing TIP47-mCherry or TIP47-mCherry-p62^{T352A}, cultured under the indicated conditions.
Parentheses, time after IVF. Scale bars, 100 μm.

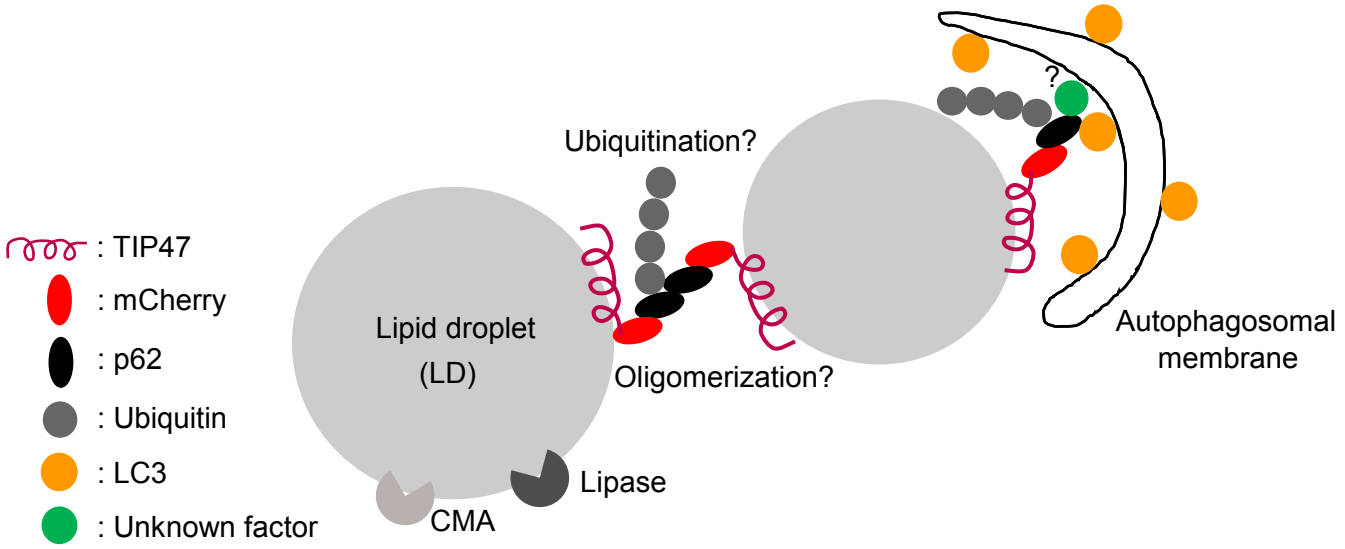
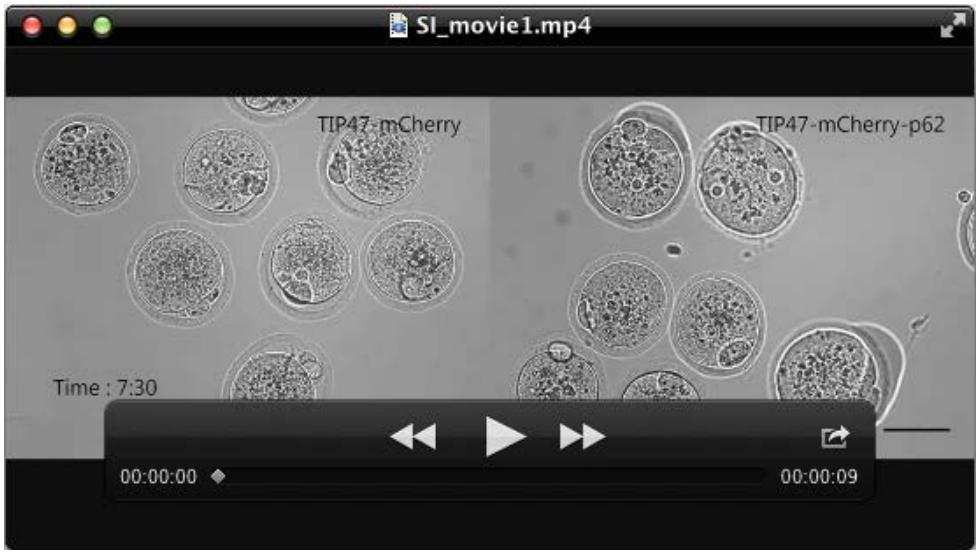


Fig. S5. Model of forced lipophagy.

p62 localized on the LD surface can interact with LC3. Because LC3 associates stably with autophagosomes, the fraction of LDs sequestered by the autophagosome is delivered to the lysosome for degradation. See details in the Discussion.



Movie 1. Morphology of LDs in lipophagy-induced embryos.

Live-cell imaging of developing mouse embryos injected with TIP47-mCherry (left) or TIP47-mCherry-p62 mRNA (right).
Time, post-IVF (hr:min). Scale bars, 100 µm. (mp4; 3.1 MB)

Supplemental references.

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