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Supplemental information

Development of small fluorescent probes for the analysis of autophagy kinetics

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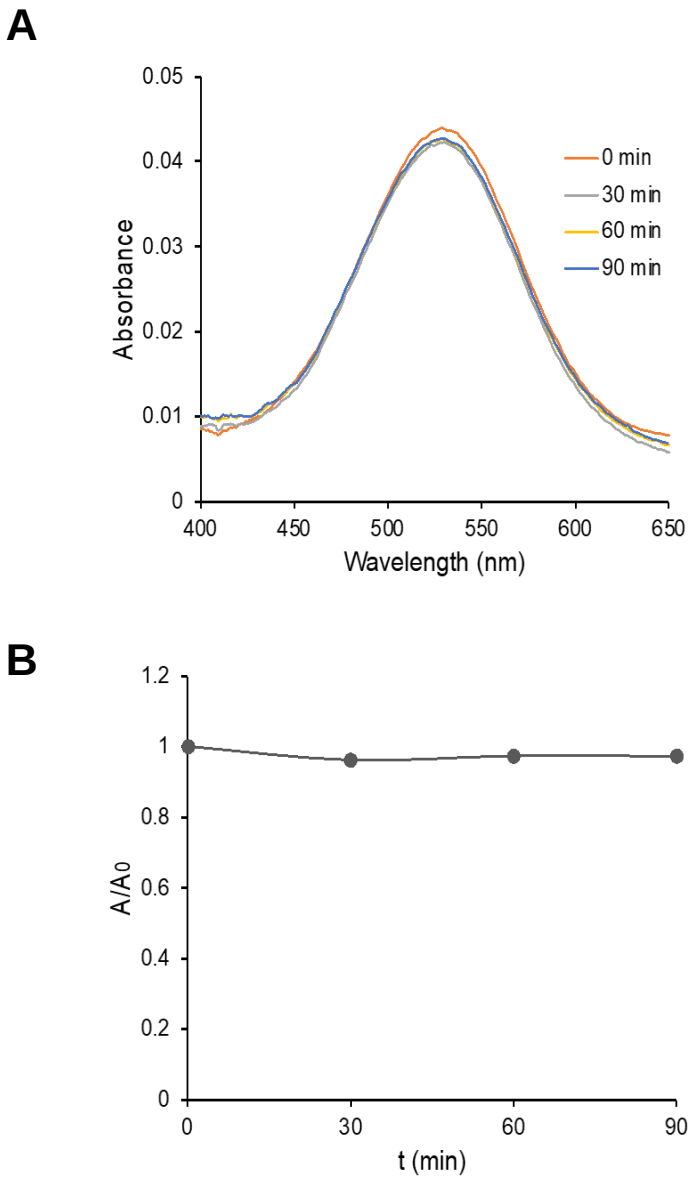


Figure S1. Photostability of DAPRed *in vitro*, related to Figure 1
Photostability of DAPRed was analyzed by irradiating a methanol solution of DAPRed (1 μM) with a 150 W xenon lamp. **(A)** The absorption spectra of DAPRed after irradiation for 0, 30, 60, and 90 min. **(B)** The relative absorbance at 528 nm was plotted at each time point.

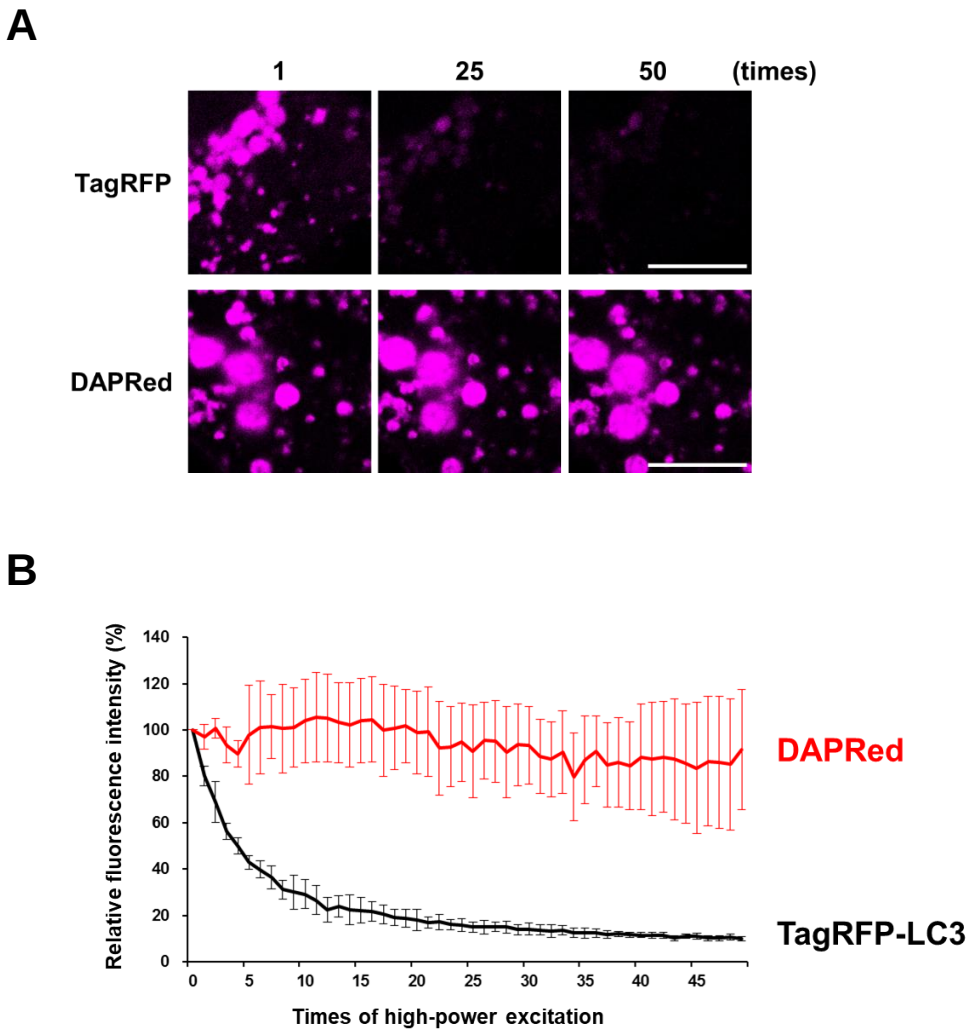


Figure S2. Photostability of DAPRed in cells, related to Figure 1

WT MEFs were preincubated with DAPRed (0.1 μM) for 30 min, or transiently expressed TagRFP-LC3, and these cells were starved for 6 hr in the presence of CQ (60 μM). Then, cells were exposed 50 times to a high-power laser (543 nm, 50%, interval: 0 sec) using confocal microscopy, and the relative intensity of each fluorescence (TagRFP-LC3 or DAPRed) was analyzed. Representative images are shown in **(A)**. Bar = 10 μm. Quantification results from six images are shown in **(B)**. Data are shown as the mean ± S.D from 6 ROIs (n = 6), respectively. All DAPRed intensities were significantly higher than that of TagRFP-LC3 after photobleaching (p < 0.001, Student t-test).

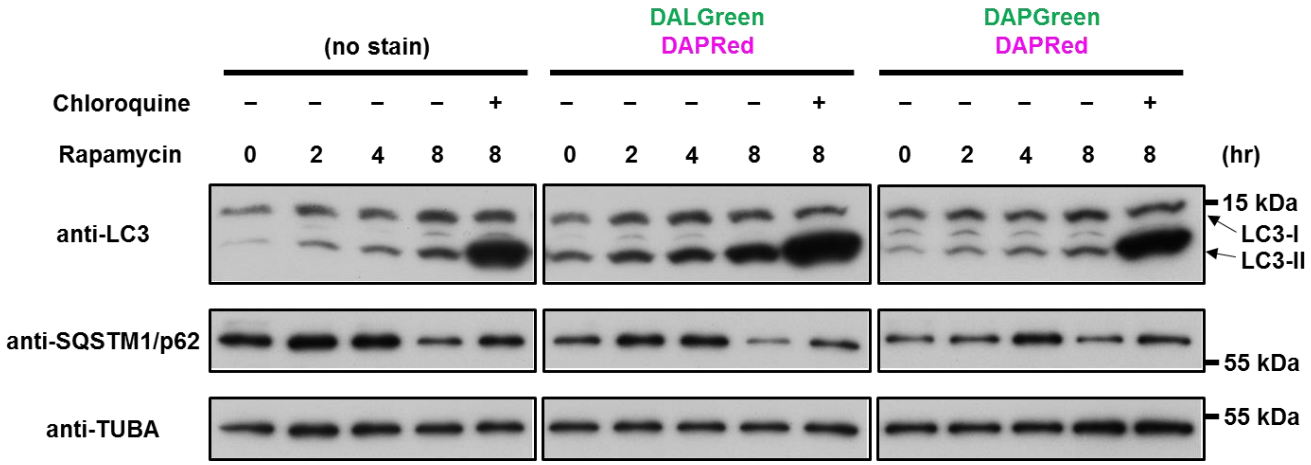


Figure S3. DALGreen, DAPGreen, and DAPRed do not affect autophagic flux, related to Figure 1
WT MEFs were preincubated with each probe for 30 min, and treated with rapamycin (0.5 μM) in the presence or absence of CQ (60 μM) for the indicated hours. Expression of the indicated proteins was analyzed by western blotting. α-Tubulin (TUBA) was included as a loading control. Autophagic flux was calculated as the difference in LC3-II and SQSTM1/p62 levels between the presence and absence of CQ.

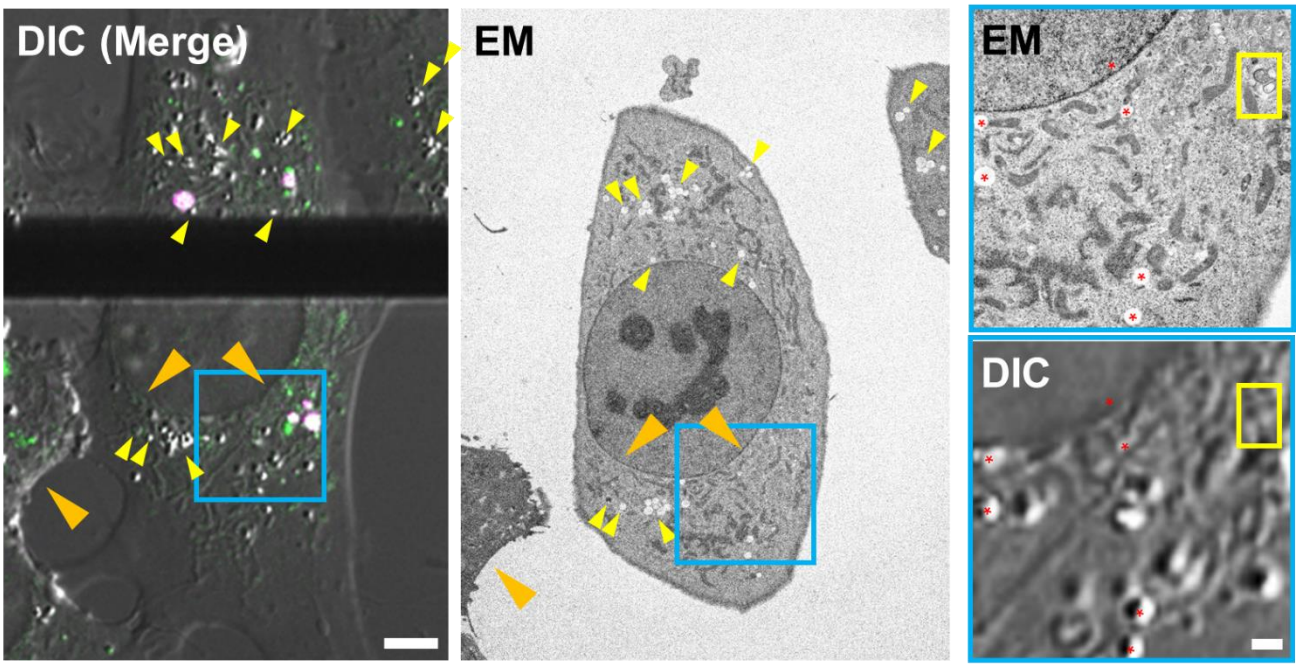


Figure S4. Low-magnification images of the cell in Figure 1H
TagRFP-LC3-transfected WT MEFs were preincubated with DAPGreen (0.25 µM), and then treated with 0.5 µM rapamycin for 4 hr. DIC and EM images were adjusted for the edge of the plasma membrane, nuclear membranes (orange arrowheads), and lipid droplets (yellow arrowheads). Bar = 5 µm. ROIs are indicated by the blue squares, and magnified images of the ROIs are shown in the right panels. Red asterisks indicate vacuoles. Bar = 1 µm. ROIs are indicated by the yellow squares, and high-magnification images are shown in Figure 1H.

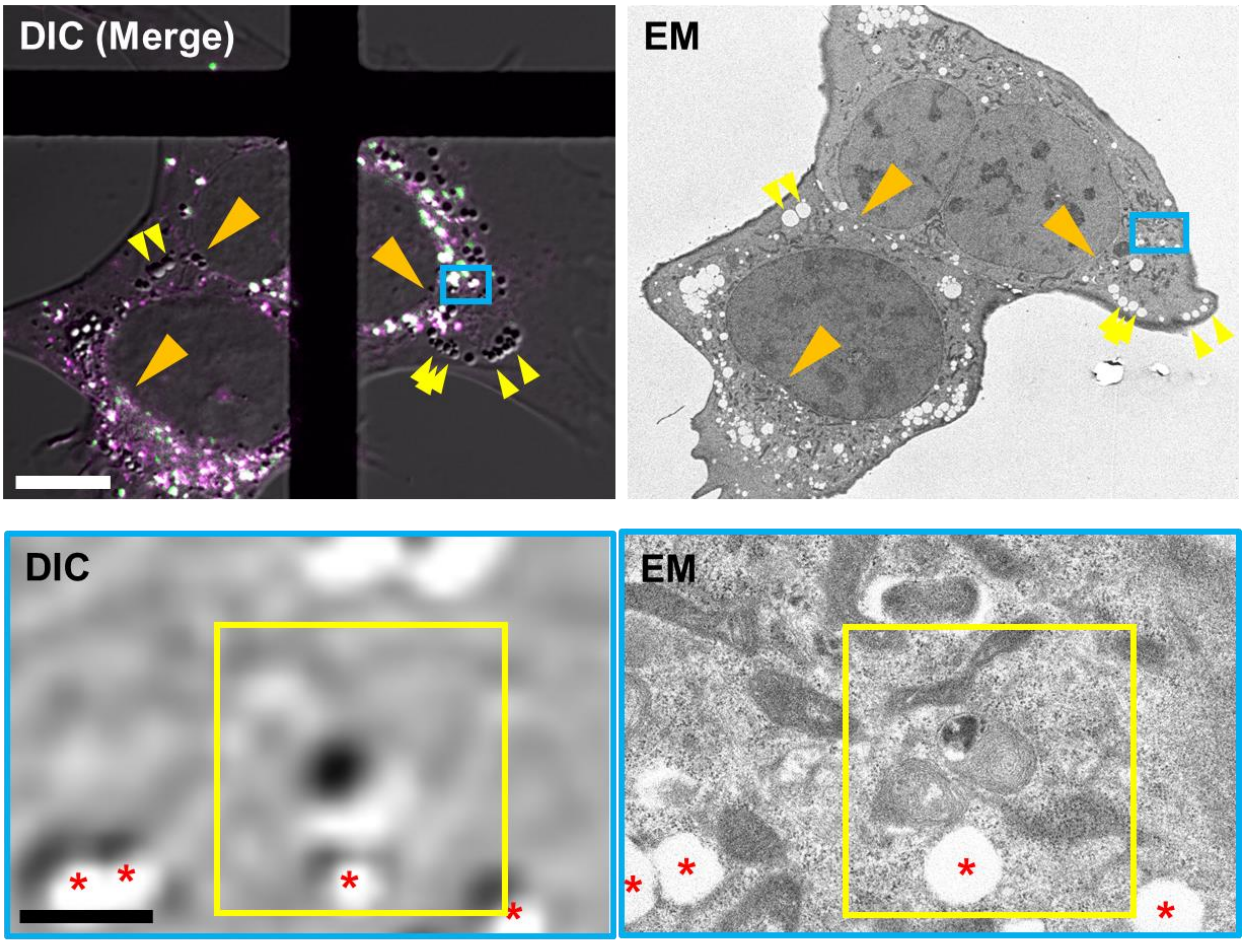


Figure S5. Low-magnification images of the cells in Figure 1I
WT MEFs were preincubated with DAPGreen (0.25 μM) and DAPRed (0.1 μM), and then treated with 0.5 μM rapamycin for 2 hr. DIC and EM images were adjusted for the edge of the plasma membrane, nuclear membranes (orange arrowheads), and lipid droplets (yellow arrowheads). Bar = 10 μm. The ROI is indicated by the blue squares, and magnified images are shown in the lower panels. Red asterisks indicate vacuoles. Bar = 1 μm. ROIs are indicated by the yellow squares, and high-magnification images are shown in Figure 1I.

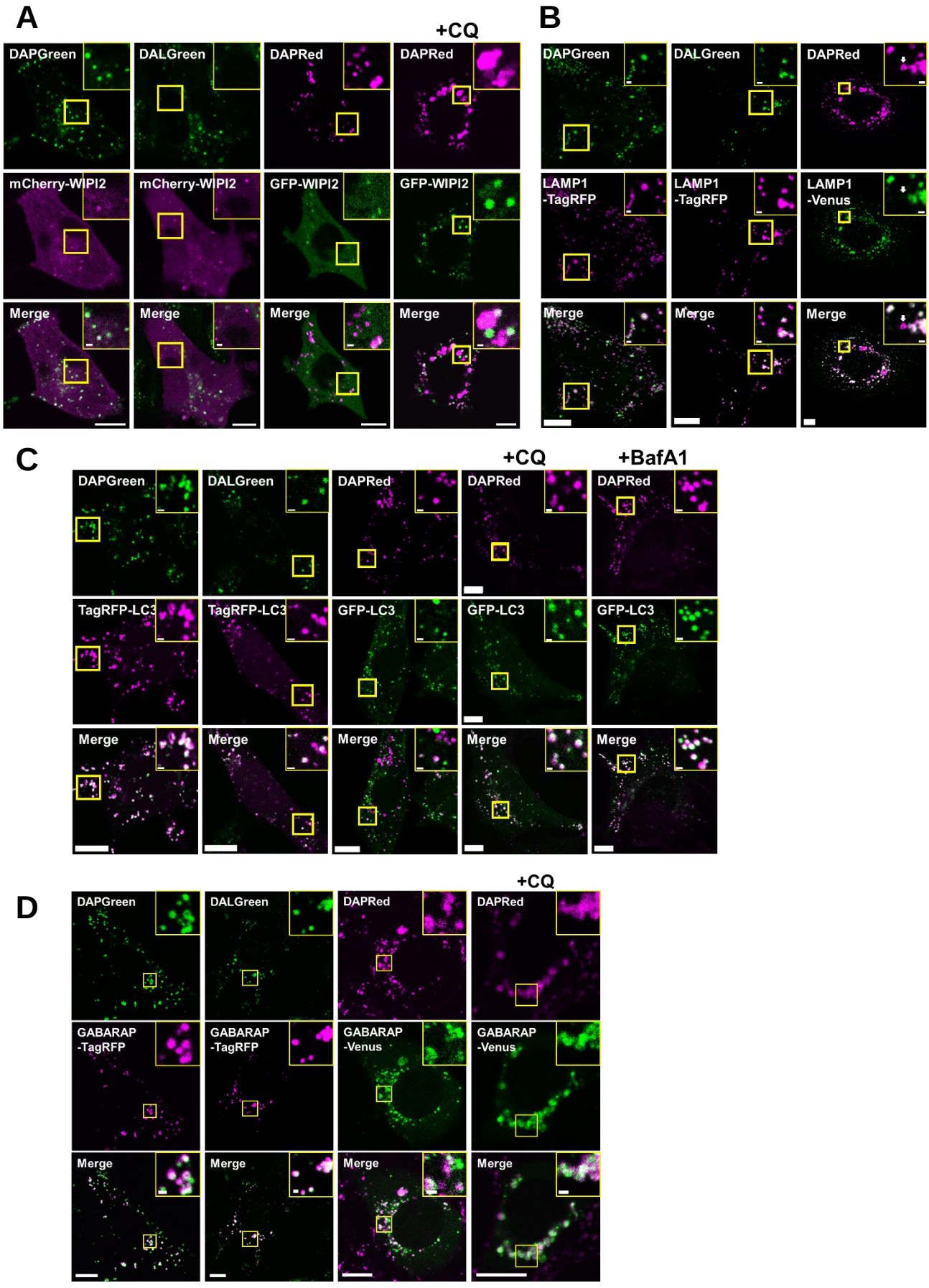

A
+CQ
DAPGreen
DALGreen
DAPRed
DAPRed
mCherry-WIPI2
mCherry-WIPI2
GFP-WIPI2
GFP-WIPI2
Merge
Merge
Merge
Merge
B
DAPGreen
DALGreen
DAPRed
LAMP1
-TagRFP
LAMP1
-TagRFP
LAMP1
-Venus
Merge
Merge
Merge
C
+CQ
+BafA1
DAPGreen
DALGreen
DAPRed
DAPRed
DAPRed
TagRFP-LC3
TagRFP-LC3
GFP-LC3
GFP-LC3
GFP-LC3
Merge
Merge
Merge
Merge
Merge
D
+CQ
DAPGreen
DALGreen
DAPRed
DAPRed
GABARAP
-TagRFP
GABARAP
-TagRFP
GABARAP
-Venus
GABARAP
-Venus
Merge
Merge
Merge
Merge

Figure S6. Colocalization of DAPGreen, DALGreen, and DAPRed with various autophagy-associated molecules, related to Figure 2

(A-C) Single-color images of Figure 2 are shown. **(A)** WIPI2 (Fig. 2A), **(B)** LAMP1 (Fig. 2C), and **(C)** LC3 (Fig. 2E). **(D)** Colocalization analysis of the three probes with GABARAP. WT MEFs were transiently transfected with *TagRFP-GABARAP* (for DAPGreen and DALGreen) or *Venus-GABARAP* (for DAPRed). Then, cells were preincubated with each probe for 30 min, and treated with rapamycin (0.5 μM) in the presence or absence of CQ (60 μM) for 4 hr. Cells were then analyzed by confocal microscopy. Bars = 10 μm. ROIs are indicated by the yellow squares and magnified images are shown in the insets. Bars = 1 μm.

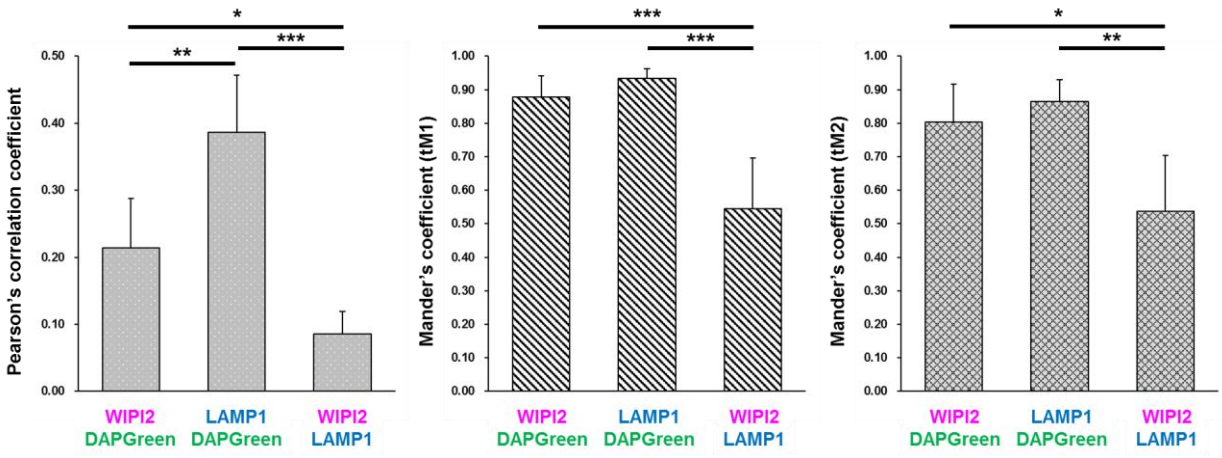


Figure S7. Colocalization of DAPGreen with various autophagic structures, related to Figure 3
LAMP1-SNAP and *mCherry-WIPI2* were transfected into WT MEFs. The cells were preincubated with DAPGreen (20 nM) and SNAP-Cell430 (3 μM) for 30 min, stimulated by nutrient depletion, and spectral imaging was performed using the ZEN application. Several coefficient values within each signal were quantified. "WIPI2/Lamp1" is a negative control. Data are shown as the mean ± S.D from 5 cells (n = 5). *p < 0.05, **p < 0.01, ***p < 0.001 (one-way ANOVA followed by the Tukey post hoc test).

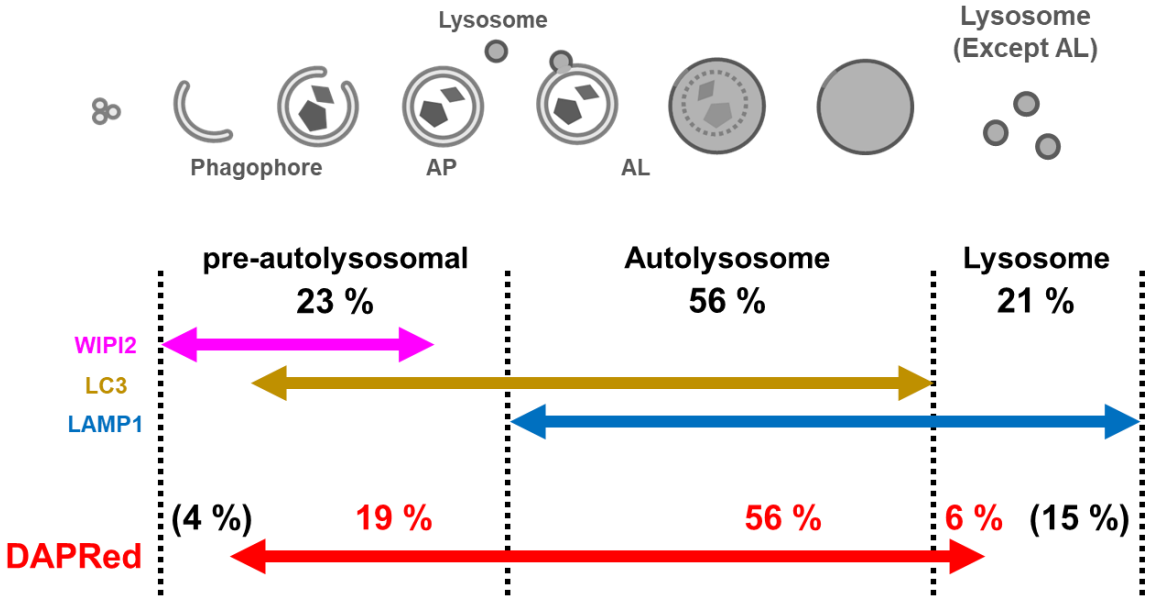


Figure S8. Summary of the colocalization of DAPRed with the various autophagic structures from Figure 3B

The number of each type of puncta from Fig. 3B was counted and classified into preautolysosomes (WIPI2 or LC3-positive/Lamp1-negative puncta: 23%), autolysosomes (LC3/Lamp1-positive puncta: 56%), and simple lysosomes (LC3-negative/Lamp1-positive puncta: 21%). The populations of DAPRed puncta colocalized with sequential autophagic structures (positive puncta/ total puncta) are shown.

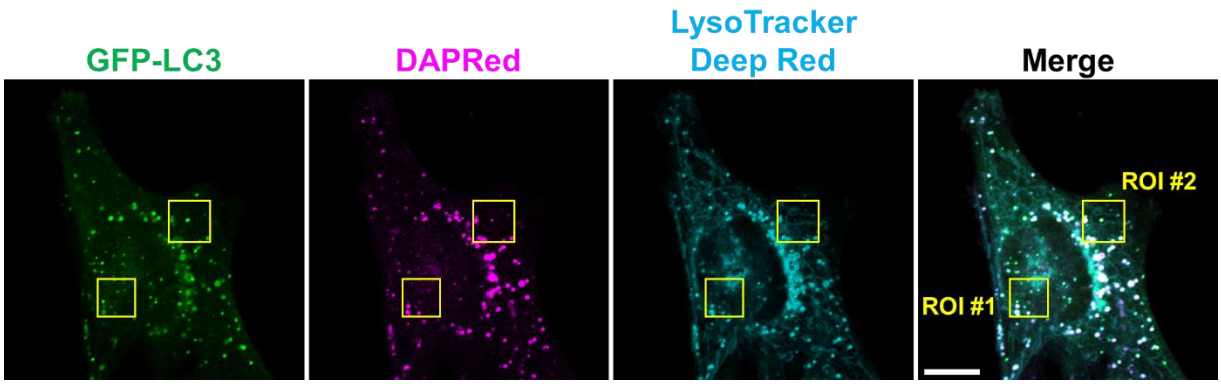


Figure S9. Low-magnification images of the cells in Figure 4A
GFP-LC3-transfected WT MEFs were preincubated with DAPRed (0.1 μM) and LysoTracker Deep Red (0.5 μM), treated with 1 μM rapamycin for 4 hr, and time-lapse three-color images were acquired. ROIs are indicated by the yellow squares and the magnified time-lapse images are shown in Figure 4A. Bars = 10 μm.

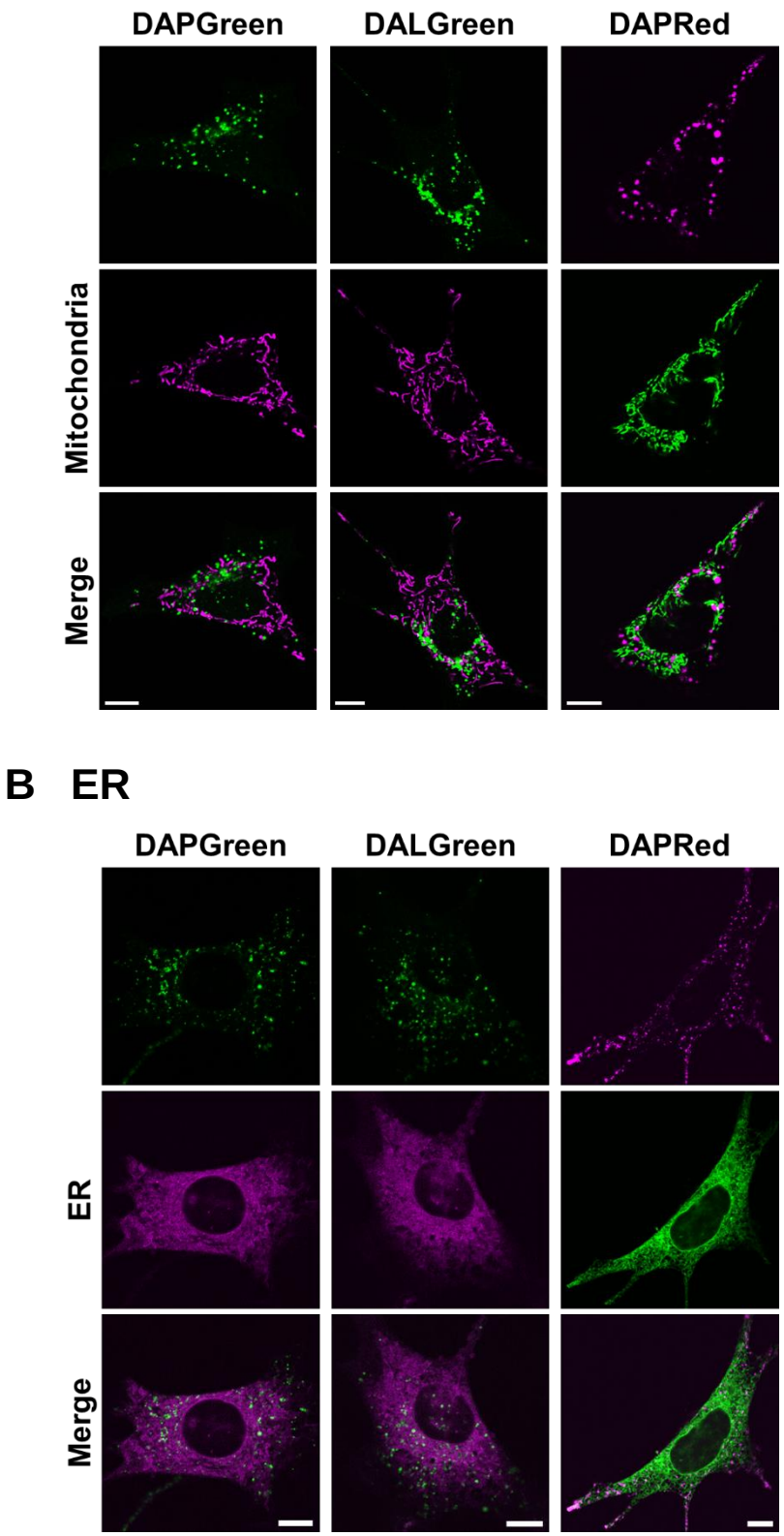
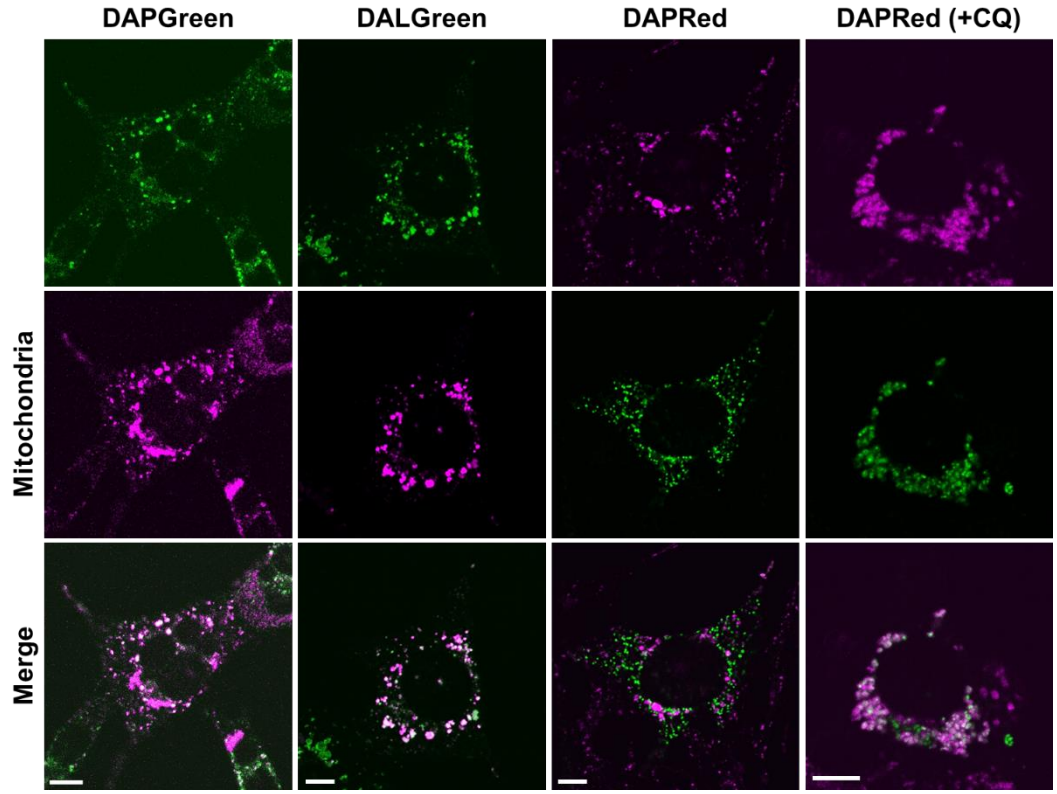


Figure S10. No colocalization of the probes with mitochondria or the ER, related to Figure 1H and 1I
WT MEFs were incubated with the indicated probes for 30 min, and costained with MitoTracker Deep Red FM (for DAPGreen and DALGreen) or MitoTracker Green FM (for DAPRed) **(A)** or ER-Tracker Red (for DAPGreen and DALGreen) **(B)**. *GFP-Cyb5*$^{104\text{-}134}$ was transiently expressed in WT MEFs as an ER marker for DAPRed **(B)**. Representative images are shown. Bars = 10 μm.

A Mitophagy



B ER-phagy

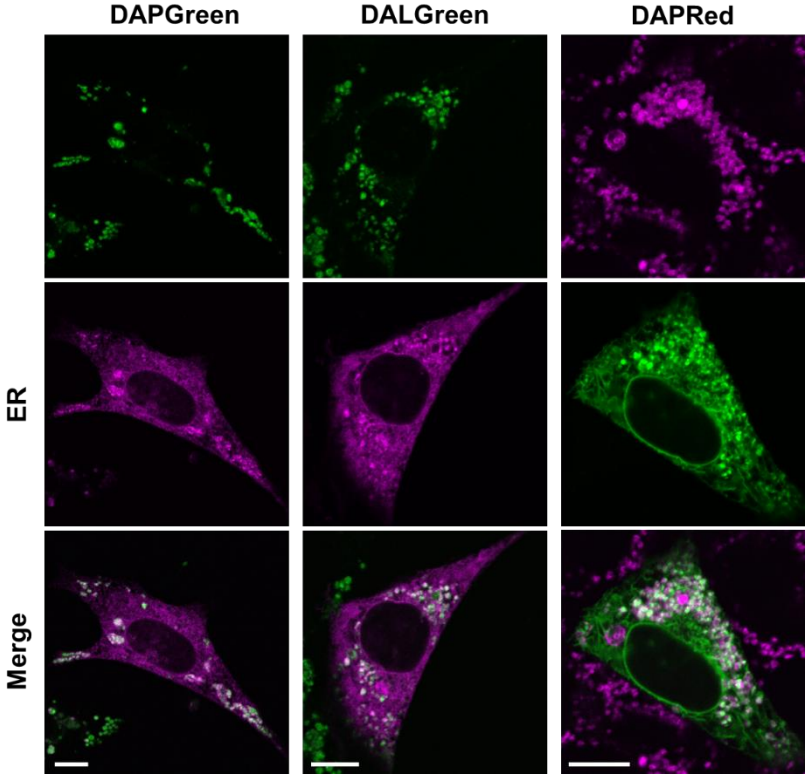
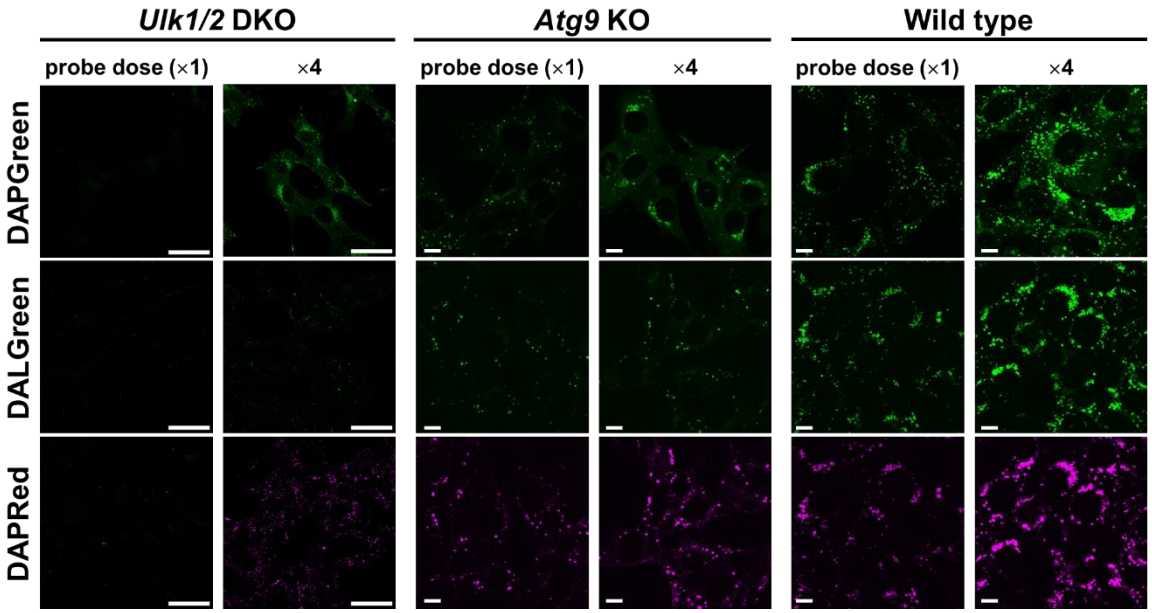


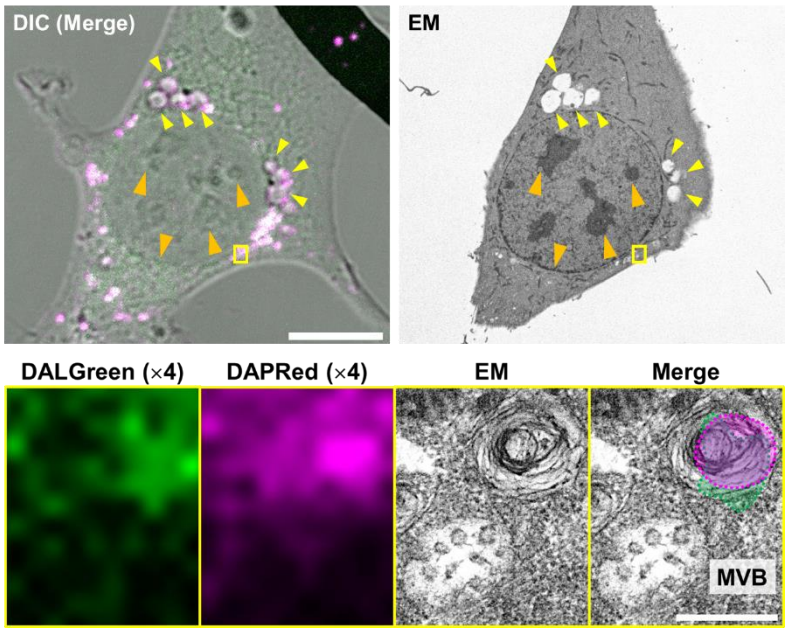
Figure S11. Colocalization of the probes with mitophagy or ER-phagy structures, related to Figure 1H and 1I

(A) *Tom20-mRFP* (for DAPGreen and DALGreen) or *Tom20-GFP* (for DAPRed) were cotransfected together with *Flag-Parkin* into WT MEFs. The cells were then incubated with the indicated probes for 30 min, and treated with CCCP (20 μM) in the presence or absence of CQ (60 μM) for 20 hr to induce mitophagy. Representative images are shown. Bars = 10 μm. **(B)** *TagRFP-Cyb5*$^{104\text{-}134}$ (for DAPGreen and DALGreen) or *GFP-Cyb5*$^{104\text{-}134}$ (for DAPRed) were transfected into WT MEFs. The cells were then treated with rapamaycin (1 μM) and CQ (60 μM) for 24 hr to induce ER-phagy. Representative images are shown. Bars = 10 μm.

A Starvation 7 hr



B *Ulk1/2* DKO MEF, Starvation 5 hr



C WT MEF, Starvation 7 hr

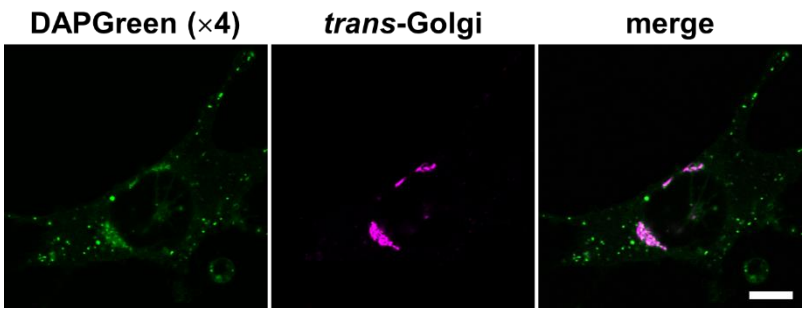
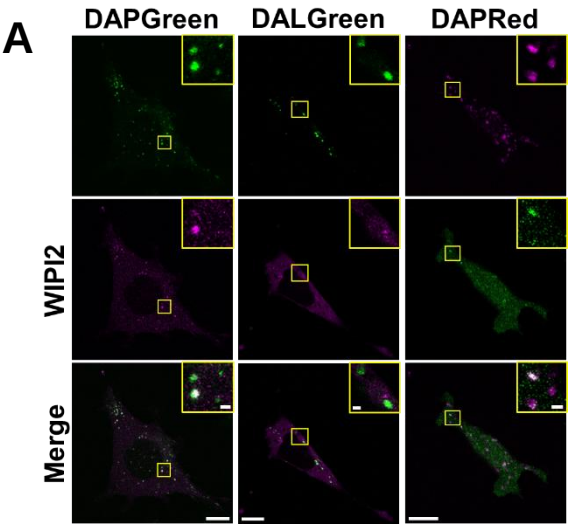
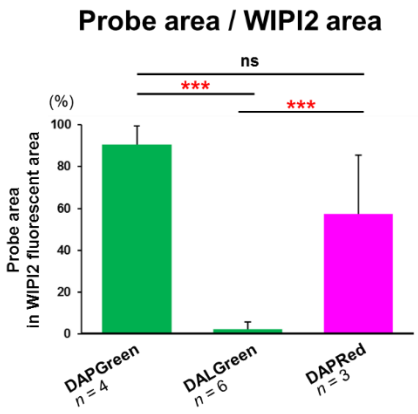


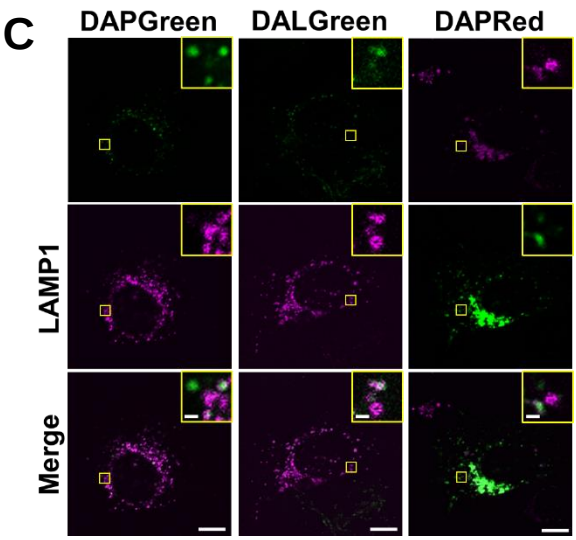
Figure S12. Nonspecific staining of the Golgi apparatus by excess doses of DAPGreen, but not DAPRed and DALGreen, related to Figure 4

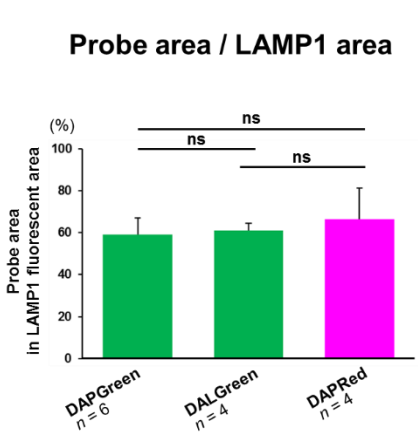
(A) WT, *Atg9*-deficient, and *Ulk1/2*-deficient MEFs were preincubated with DAPGreen (0.25 and 1 μM), DALGreen (1 and 4 μM), or DAPRed (0.1 and 0.4 μM) for 30 min, and then starved for 7 hr. Cells were then analyzed by confocal microscopy. Bars = 10 μm. DAPGreen signals appeared as a Golgi-like pattern even in the cells lacking *Atg9* or *Ulk1/2* by the addition of a four-fold higher dose than normal. **(B)** *Ulk1/2*-deficient MEFs were preincubated with DALGreen (4 μM) and DAPRed (0.4 μM) for 30 min, starved for 5 hr, and performed CLEM analysis. DIC and EM images were adjusted for the edge of the plasma membrane, nucleoli (orange arrowheads), and lipid droplets (yellow arrowheads). Bar = 10 μm. The ROI is indicated by the yellow squares and magnified images are shown in the lower panels. Fluorescent signals are indicated by the dashed circles in the merged image. Bar = 500 nm. A DALGreen/DAPRed punctum merged with an autophagic structure. The multivesicular body (MVB) was not labeled by these probes. **(C)** *ST6GAL1-TagRFP*-expressing WT MEFs were preincubated with DAPGreen (1 μM), and then starved for 7 hr. Cells were then analyzed by confocal microscopy, and representative images are shown. Bar = 10 μm.

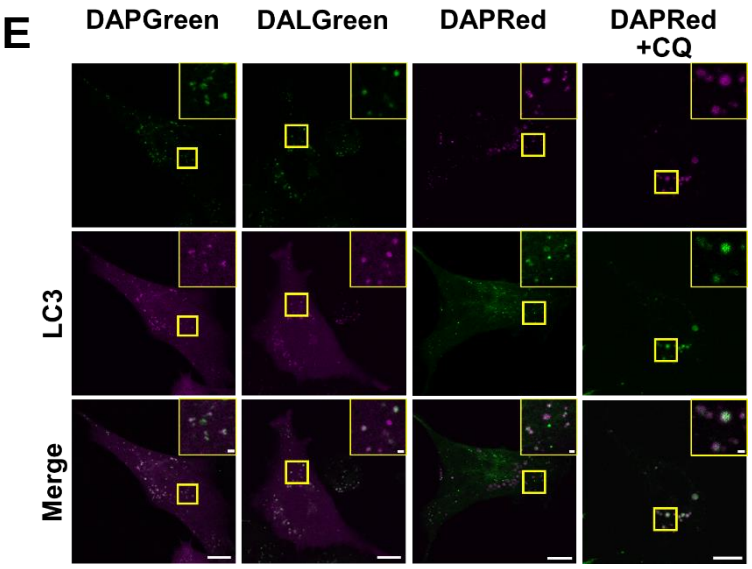
WT MEF, No treatment











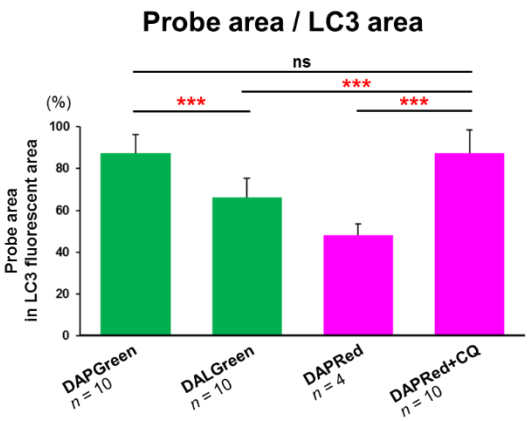
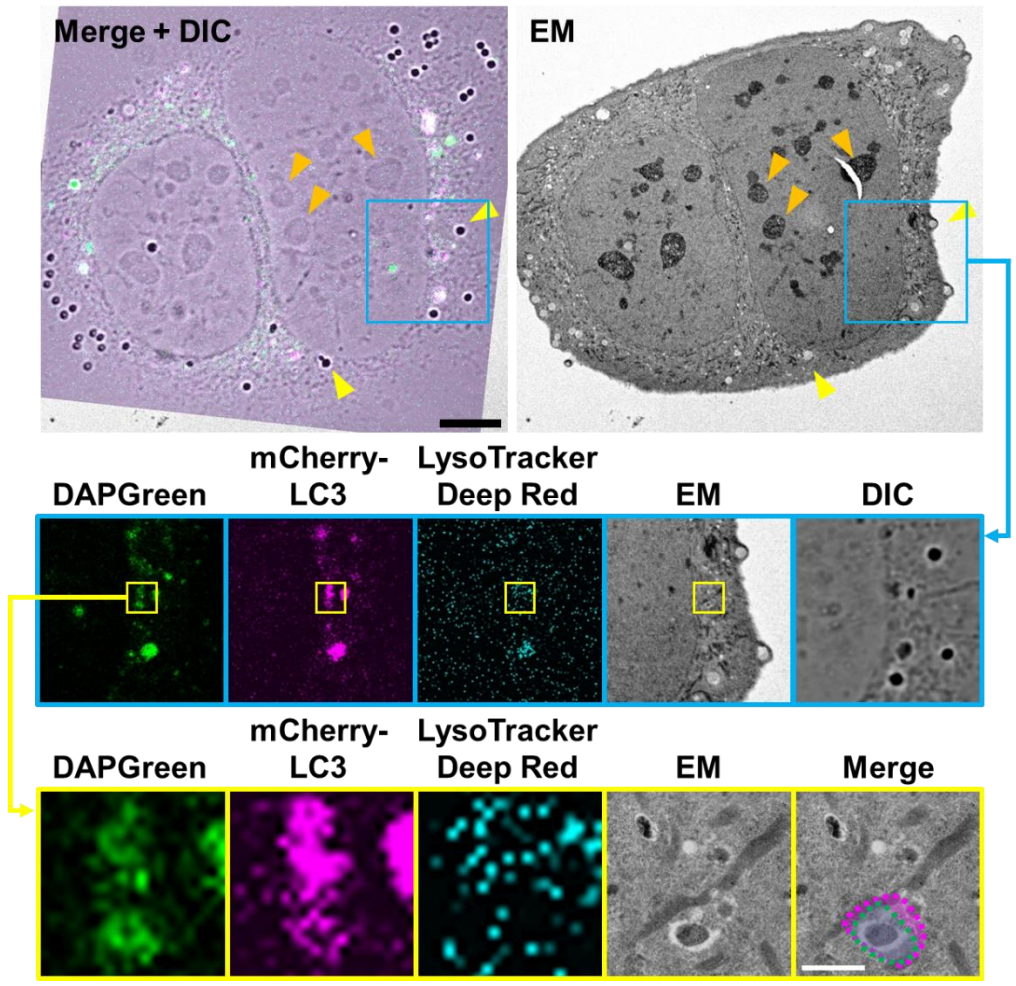


Figure S13. Colocalization of DAPGreen, DALGreen, and DAPRed with various autophagic structures of basal autophagy, related to Figure 2

(A, B) WT MEFs were transiently transfected with *mCherry-WIPI2* (for DAPGreen and DALGreen) or *GFP-WIPI2* (for DAPRed). Then, cells were preincubated with each probe for 30 min and incubated for a further 2 hr without any stimuli. Cells were then analyzed by confocal microscopy. Bars = 10 μm. ROIs are indicated by the squares and their magnified images are shown in the insets. Bars = 1 μm. In **(B)**, the colocalization rate of each probe with the WIPI2 puncta was quantified. **(C, D)** Colocalization analysis of the three probes with LAMP1. Similar experiments to **(A, B)** were performed using *LAMP1-TagRFP* and *LAMP1-Venus*. **(E, F)** Colocalization analysis of the three probes with LC3. Similar experiments to **(A, B)** were performed using *mCherry-LC3* and *GFP-LC3*. In addition, WT MEFs were incubated with complete medium in the presence or absence of CQ (60 μM) for 2 hr. Data are shown as the mean ± S.D. The total cell numbers are given as the *n*. **p* < 0.05, ***p* < 0.01 (one-way ANOVA followed by the Tukey post hoc test). ns: no significant difference.

A WT MEF, No treatment



B WT MEF, No treatment

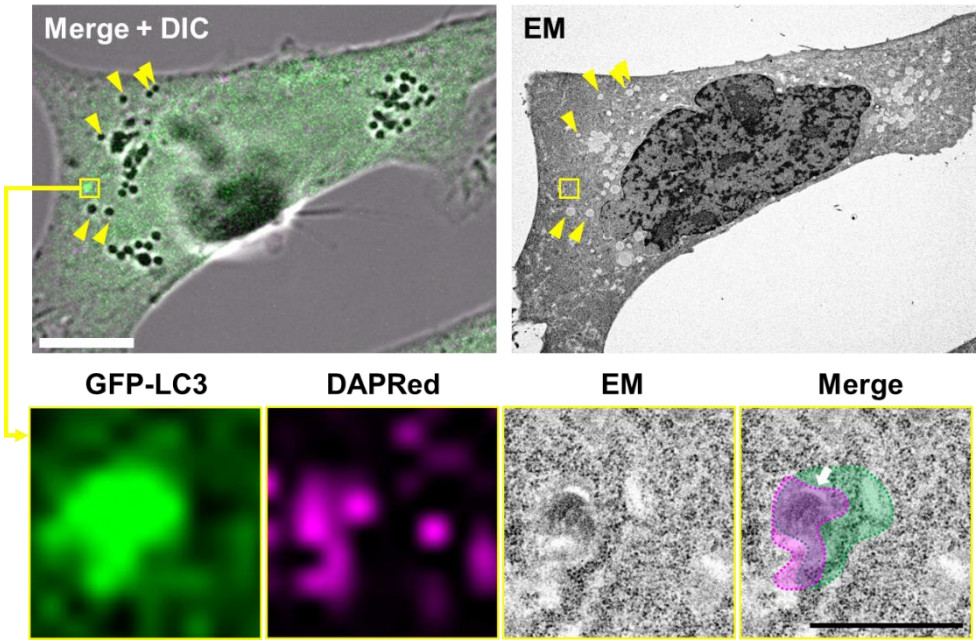


Figure S14. Colocalization of DAPGreen and DAPRed with autophagic structures in basal autophagy, related to Figure 3C

(A) Identification of DAPGreen/mCherry-LC3 puncta (LysoTracker Deep Red-negative) as basal autophagic structures by CLEM analysis. *mCherry-LC3*-transfected WT MEFs were incubated with DAPGreen (0.25 μM) and LysoTracker Deep Red (0.5 μM) for 0.5 hr. DIC and EM images were adjusted for the edge of the plasma membrane, nucleoli (orange arrows), and lipid droplets (yellow arrowheads). Bar = 10 μm. ROIs are indicated by the blue and yellow squares, and magnified images are shown in the middle and bottom panels, respectively. Fluorescent signals are indicated by the dashed circles in the merged image. A LysoTracker-negative DAPGreen/mCherry-LC3 punctum merged with an autophagosome is shown. **(B)** Identification of DAPRed/GFP-LC3 puncta as basal autophagic structures detected by CLEM analysis. *GFP-LC3*-transfected WT MEFs were incubated with DAPRed (0.1 μM) for 0.5 hr. DIC and EM images were adjusted for the edge of the plasma membrane and lipid droplets (yellow arrowheads). The ROI is indicated by the squares and magnified images are shown in the lower panels. Fluorescent signals are indicated by the dashed circles in the merged image. Arrow indicates a phagophore. Bar = 10 μm (top) and 1 μm (bottom). A DAPRed/ GFP-LC3 punctum was merged with an autophagic vacuole.

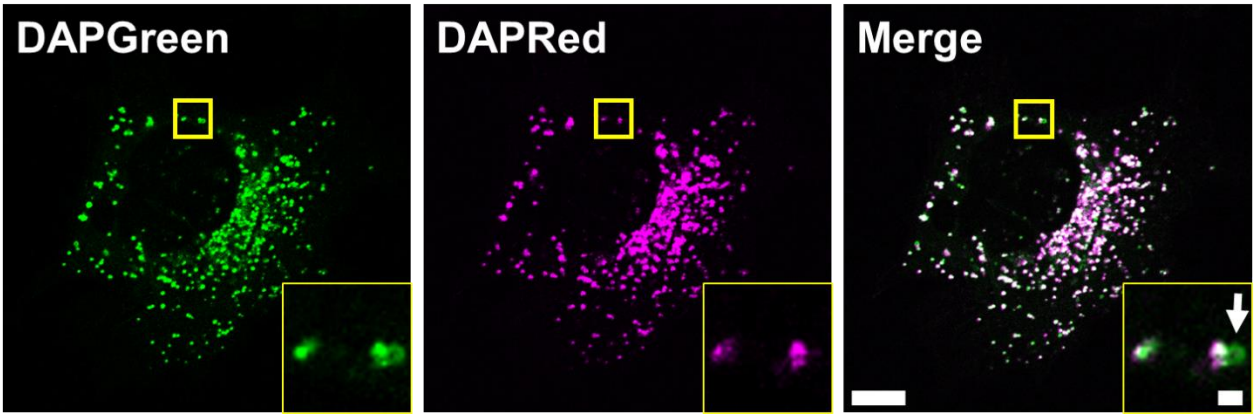


Figure S15. Analysis of phagophore generation dynamics using DAPGreen and DAPRed, related to Figure 5B

WT MEFs were preincubated with DAPGreen (0.25 μM) and DAPRed (0.1 μM), and treated with rapamycin (0.5 μM) for 2 hr, and cells were observed by confocal microscopy. Representative images are shown. Bar = 10 μm. ROIs are indicated by the squares and magnified images are shown in the insets. Bars = 1 μm. Time-lapse images of this experiment are shown in Figure 5B. DAPGreen signals preceded DAPRed signals (arrow).

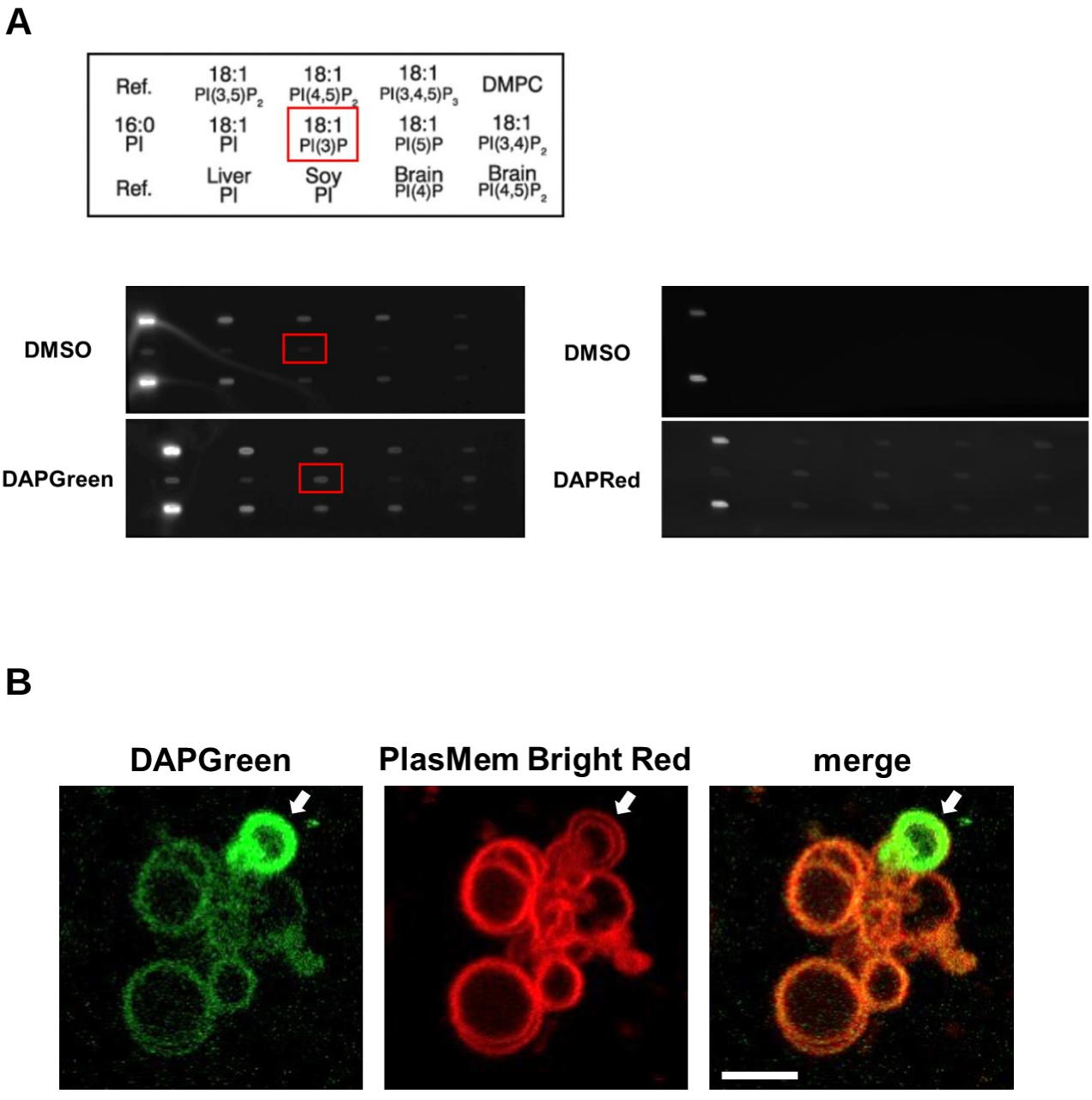


Figure S16. Lipid-binding specificity of DAPGreen and DAPRed, related to Figure 5
(A) Membranes blotted with 14 different lipid species (top panel) were incubated with DAPGreen (0.25 µM) or DAPRed (0.1 µM) for 30 min. After washing, the membranes were excited and visualized by 470 nm or 525 nm fluorescence. Note that DAPGreen showed PI3P-specific binding (lower left panels, red rectangles). **(B)** Egg yolk phosphatidylcholine (EYPC) liposomes containing PI3P (EYPC:PI3P = 5:1) were prepared in the presence of PlasMem Bright Red (1% v/v), and incubated with DAPGreen (0.25 mM) for 30 min. The liposome membranes were all stained with PlasMem Bright Red. DAPGreen fluorescence was observed only on the double-membrane structures (white arrows). Bar = 5 µm.

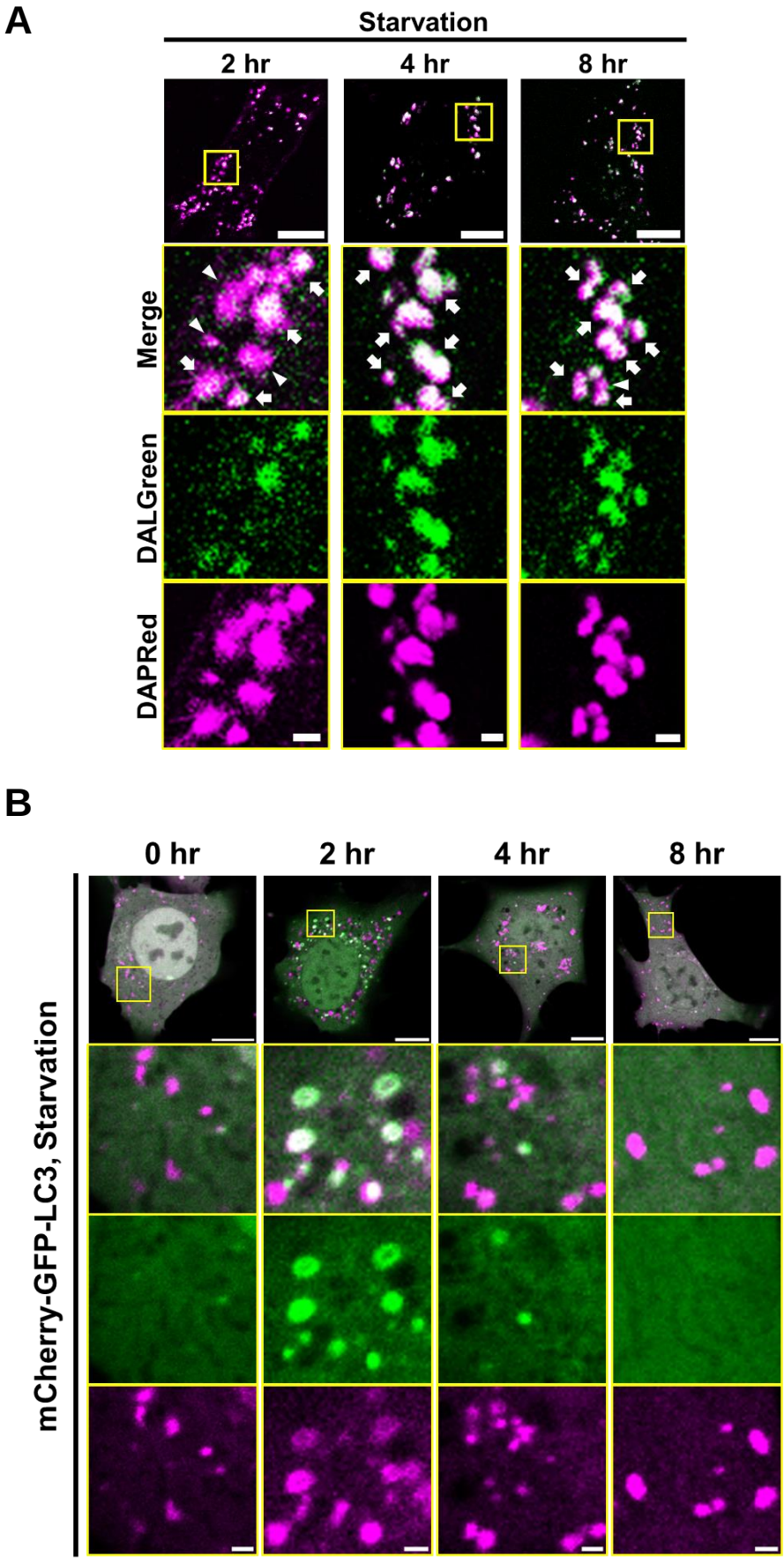

A
Starvation
2 hr
4 hr
8 hr
Merge
DALGreen
DAPRed
B
0 hr
2 hr
4 hr
8 hr
mCherry-GFP-LC3, Starvation

Figure S17. Comparison between labeling by DAPRed/DALGreen and mCherry-GFP-LC3, related to Figure 5F and 5G

(A) WT MEFs were preincubated with DAPRed (0.1 μM) and DALGreen (1 μM) and starved for 8 hr. Cells were observed at the indicated times by confocal microscopy. Representative low-magnification images are shown at the top. Bars = 10 μm. ROIs are indicated by the squares and magnified images are shown in the lower panels. Bars = 1 μm. Arrows and arrowheads indicate DAPRed/DALGreen-merged puncta (autolysosomes) and single DAPRed puncta (phagophores/autophagosomes), respectively. Quantitative data are shown in Figure 5F. **(B)** WT MEFs were transiently transfected with the *mCherry-GFP-LC3* plasmid, and starved for the indicated hours. Then, cells were observed by confocal microscopy. Representative low-magnification images are shown at the top. Bars = 10 μm. ROIs are indicated by the squares and magnified images are shown in the lower panels. Bars = 1 μm. Quantitative data are shown in Figure 5G.

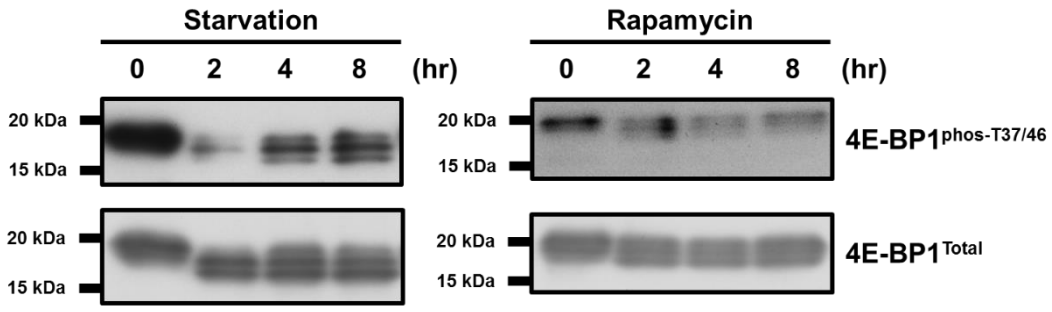


Figure S18. Distinct regulation of 4E-BP1 phosphorylation in starvation and rapamycin treatment, related to Figure 5

WT MEFs were stimulated with HBSS (starvation) or rapamycin (2 μM) for the indicated hours. The phosphorylation status of 4E-BP1 was analyzed by western blotting.

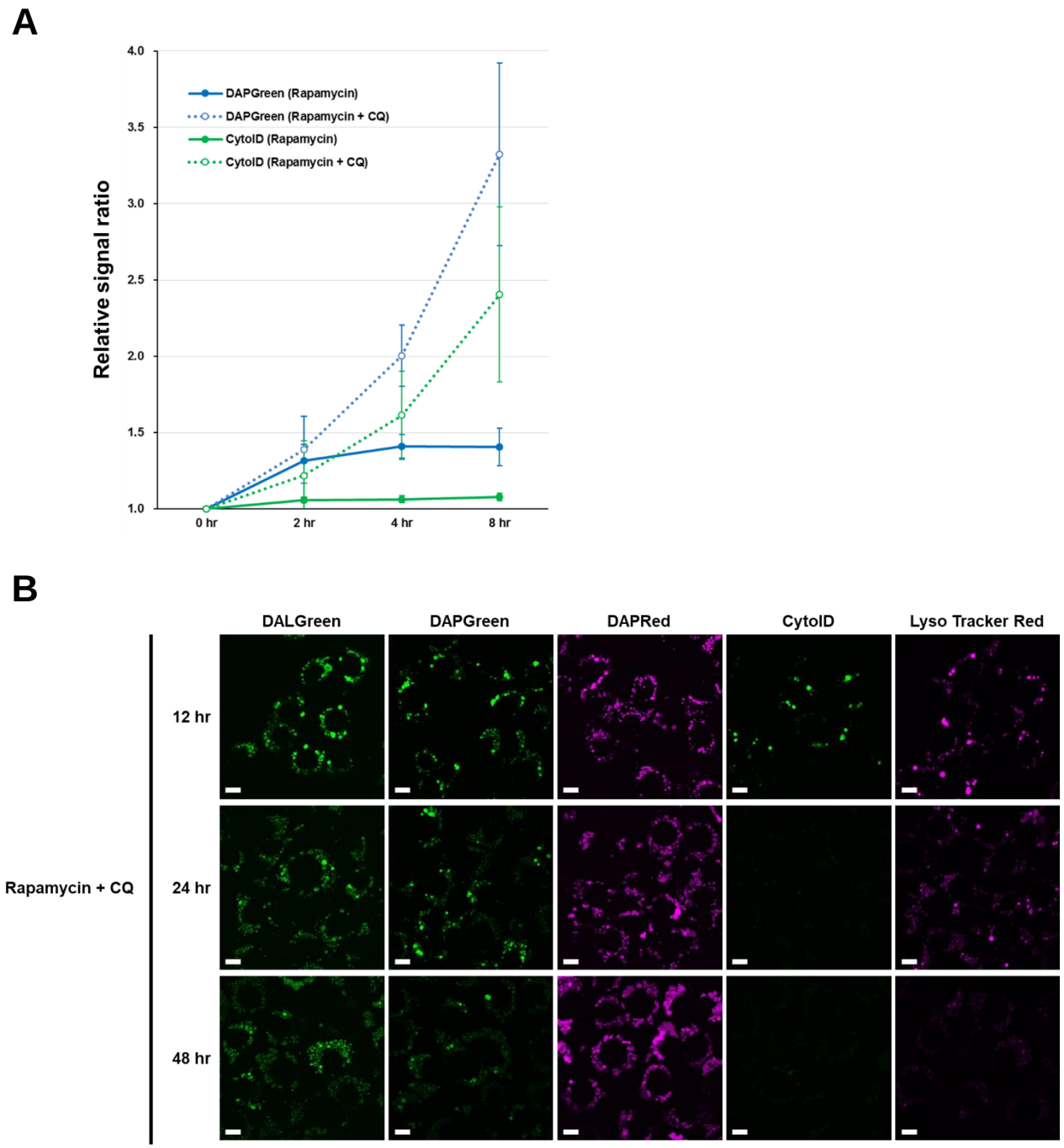


Figure S19. Comparison between DAPGreen and CytoID, related to Figure 4 and 5

(A) For the analysis of DAPGreen (blue lines), WT MEFs were preincubated with DAPGreen (0.5 μM), and then treated with rapamycin (0.5 μM) in the presence (dashed line) or absence (solid line) of CQ (60 μM). CytoID (green lines) was analyzed according to the manufacturer's instructions, as follows. WT MEFs were treated with rapamycin (0.5 μM) in the presence (dashed line) or absence (solid line) of CQ (60 μM). At the indicated hours, CytoID (1/2,000 for 2×10^5 cells) was added, and incubated for 30 min. Then, cells were collected, and their fluorescence was measured (each mean value of FITC intensity) using a flow cytometer. The fold signal changes from non-treated cells at each time point were calculated as relative signal ratios. **(B)** WT MEFs were incubated with DALGreen (1 μM), DAPGreen (0.25 μM), or DAPRed (0.1 μM) for 30 min, and then treated with rapamycin (1 μM) and CQ (60 μM) for the indicated times. For CytoID (0.2% v/v) and Lyso Tracker Red (1 μM) staining, WT MEFs were first treated with rapamycin (1 μM) and CQ (60 μM) for 12 hr, and then stained with each probe for 30 min. After staining, the medium containing rapamycin and CQ was returned to the dishes, and the cells were analyzed after further treatment for 12 hr (labeled for 24 hr) or 36 hr (labeled for 48 hr). Bars = 10 μm.

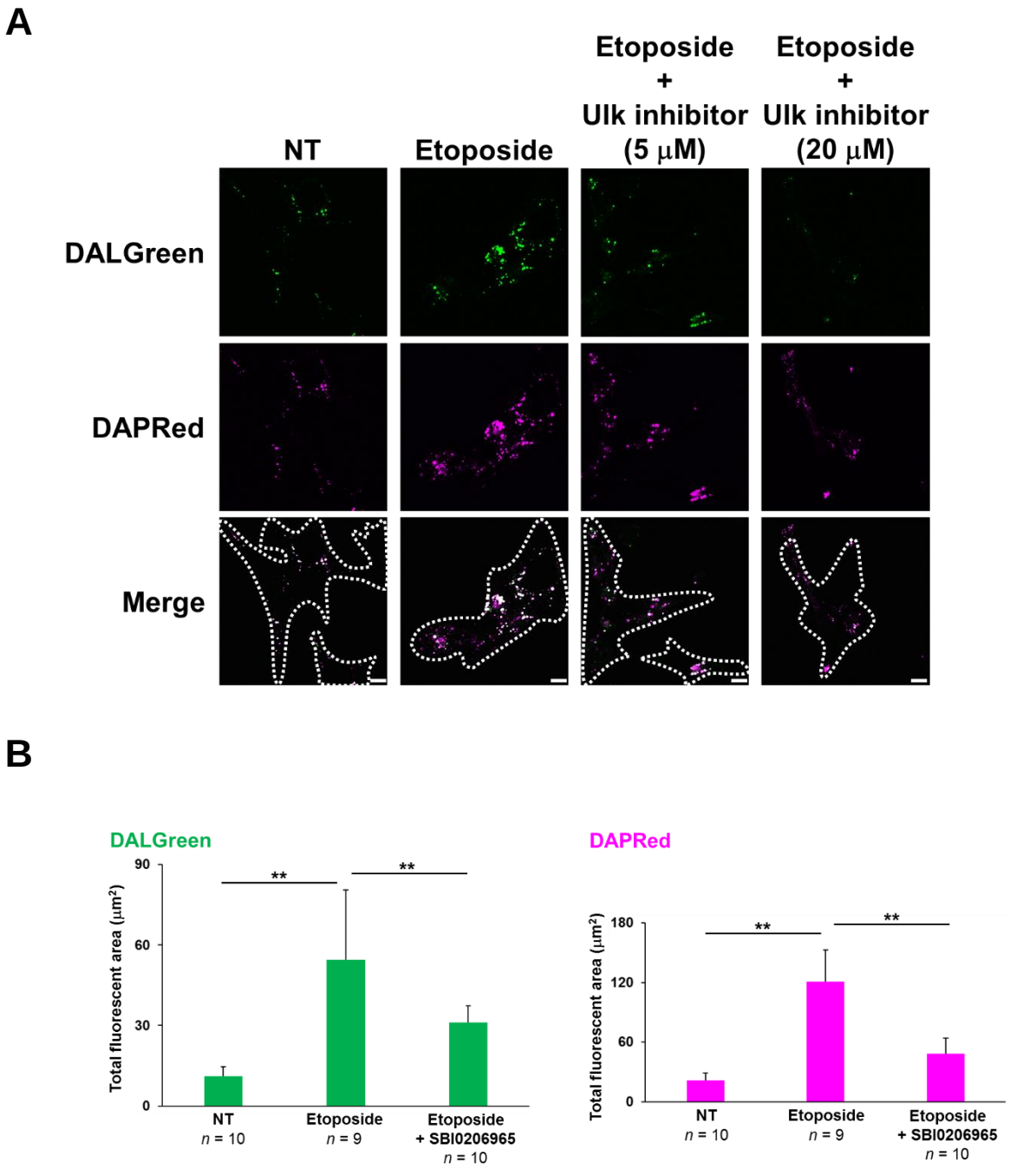


Figure S20. Alternative autophagic structures are stained with DAPRed, DAPGreen, and DALGreen, related to Figure 6A and 6B

(A, B) *Ulk1/2*-dependent staining of DALGreen/DAPRed signals in etoposide-treated *Atg5*-deficient MEFs. *Atg5*-deficient MEFs were stained with DAPRed (0.1 μM) and DALGreen (1 μM), and were treated with etoposide (10 μM) for 15 hr with or without SBI-0206965 (a ULK1/2 inhibitor, 5 or 20 μM). Then, cells were analyzed by fluorescence microscopy **(A)**. Dotted lines indicate the cell shape. Bars = 10 μm. Fluorescence areas per cell were calculated from the images **(B)**. Data are shown as the mean ± S.D. The total number of images used for analysis is given as the *n*. $^{**}p < 0.01$ (Mann-Whitney *U* test).

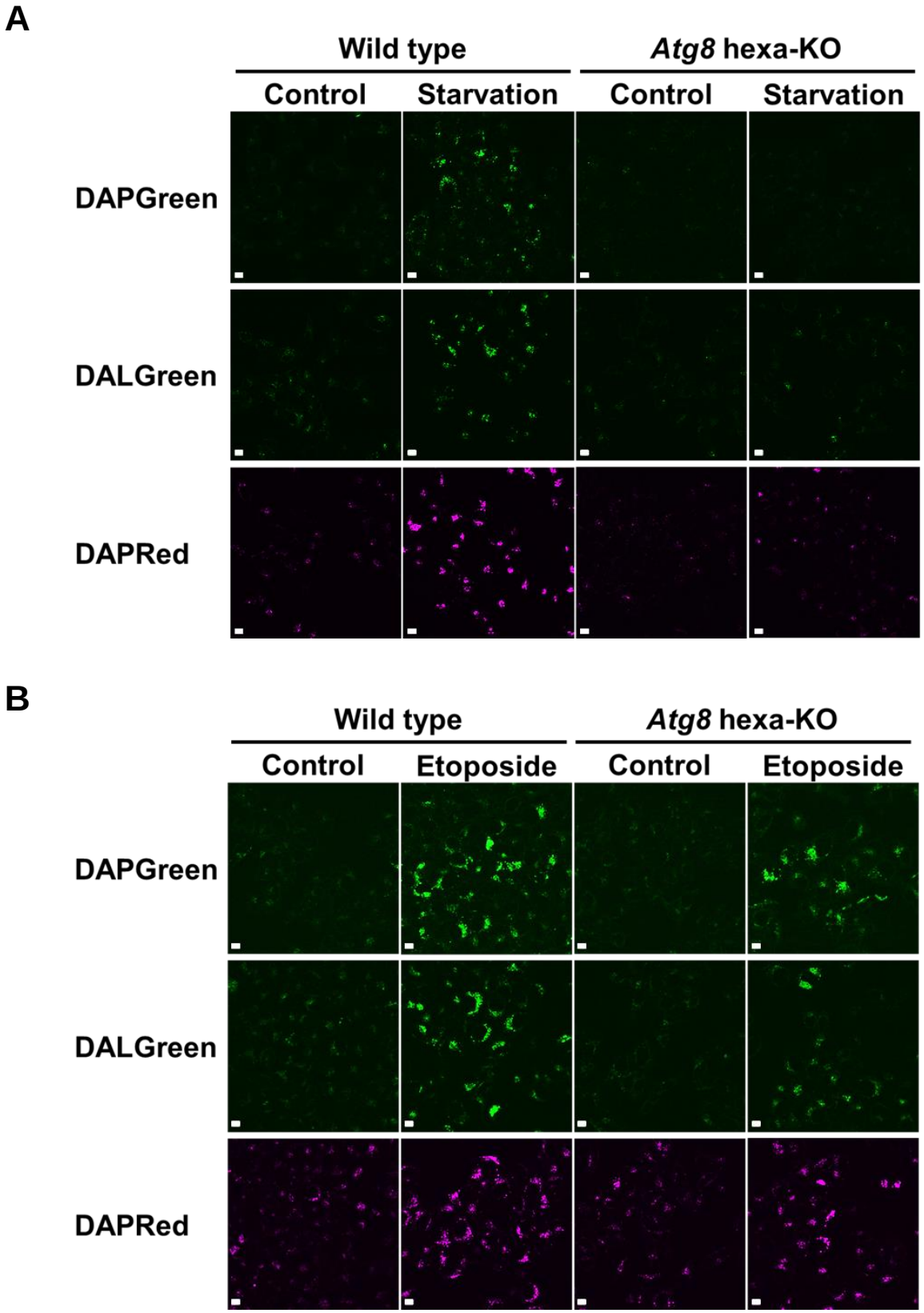


Figure S21. Detection of canonical and alternative autophagy with DAPGreen, DALGreen, and DAPRed in *Atg8* hexa-KO HeLa cells, related to Figure 6A and 6B

WT and *Atg8* hexa-KO HeLa cells were incubated with DAPGreen (0.2 μM), DALGreen (1 μM), and DAPRed (0.1 μM) for 30 min. The cells were untreated or starved for 5 hr to induce canonical autophagy **(A)**, and also treated with or without etoposide (100 μM) for 10 hr to induce alternative autophagy **(B)**. Bars = 10 μm.

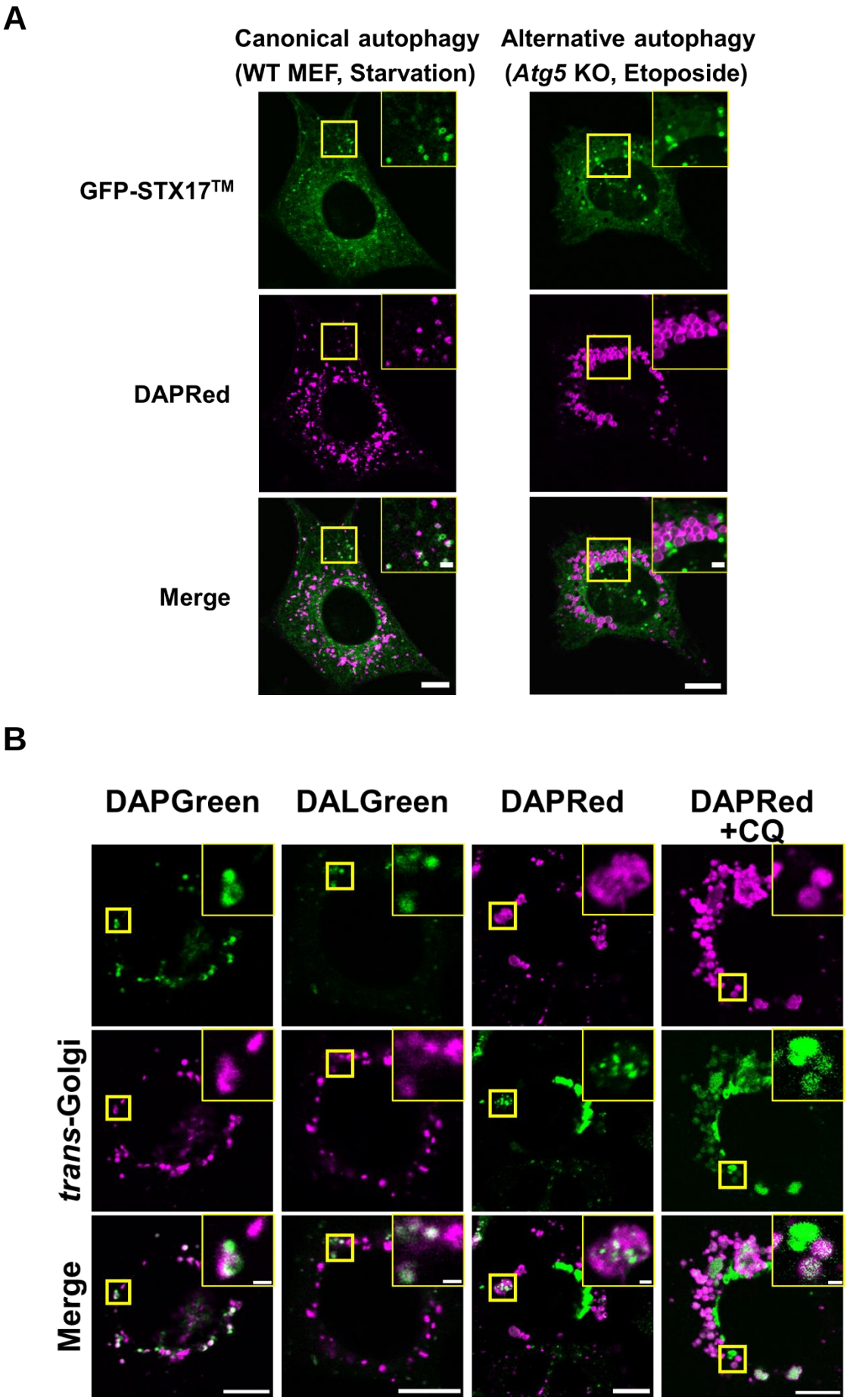

A
Canonical autophagy
(WT MEF, Starvation)
Alternative autophagy
(Atg5 KO, Etoposide)
GFP-STX17TM
DAPRed
Merge
B
DAPGreen
DALGreen
DAPRed
DAPRed
+CQ
trans-Golgi
Merge

Figure S22. Alternative autophagic structures are labeled with DAPRed, DAPGreen, and DALGreen, related to Figure 6C

(A) WT MEFs (left) and *Atg5*-deficient MEFs (right) stably expressing *GFP-Stx17^{TM}* were prestained with DAPRed (0.1 μM). Then, cells were starved for 2 hr (left) or treated with etoposide for 10 hr (right), and were analyzed using fluorescence microscopy. ROIs are indicated by the squares and magnified images are shown in the inset. Bars = 10 μm (2 μm in insets). DAPRed signals merged with STX17 in starved WT MEFs, but not in etoposide-treated *Atg5*-deficient MEFs. **(B)** Colocalization analysis of the three probes with the *trans*-Golgi during alternative autophagy. *Atg5*-deficient MEFs transiently expressing *ST6GAL1-TagRFP* (for DAPGreen and DALGreen) or *ST6GAL1-GFP* (for DAPRed) were preincubated with each probe for 30 min, and treated with etoposide (10 μM) in the presence or absence of CQ (60 μM) for 10 hr. Cells were then analyzed by confocal microscopy. Representative images are shown. Bars = 10 μm. ROIs are indicated by the squares and their magnified images are shown in the insets. Bars = 1 μm.

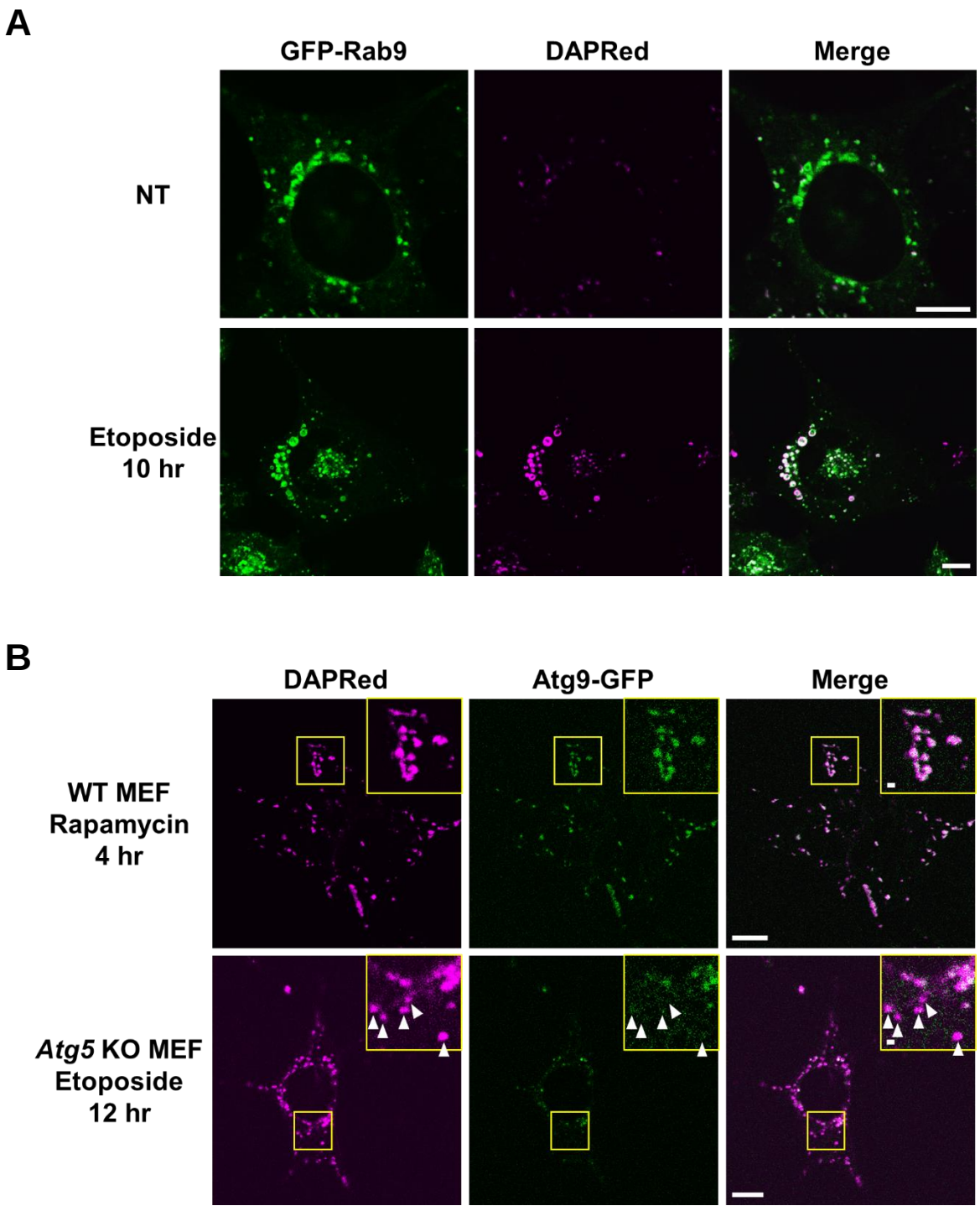


Figure S23. DAPRed colocalization analyses with proteins involved in alternative autophagy and canonical autophagy, related to Figure 6

(A) *Atg5*-deficient MEFs stably expressing GFP-Rab9 were incubated with DAPRed (0.1 μM), and treated with or without etoposide (10 μM) for 10 hr. DAPRed fluorescence was colocalized with GFP-Rab9. Bars = 10 μm. **(B)** *Atg9-GFP* was transfected into WT and *Atg5*-deficient MEFs, and treated with rapamycin (1 μM) for 4 hr to induce canonical autophagy. Almost all the DAPRed puncta were colocalized with Atg9-GFP in canonical autophagy (top panels). In contrast, multiple DAPRed puncta were not colocalized with Atg9-GFP in alternative autophagy induced by 10 μM etoposide treatment for 12 hr (bottom panels, white arrowheads). Bars = 10 μm. ROIs are indicated by the yellow squares, and magnified images are shown in the insets. Bars = 1 μm.

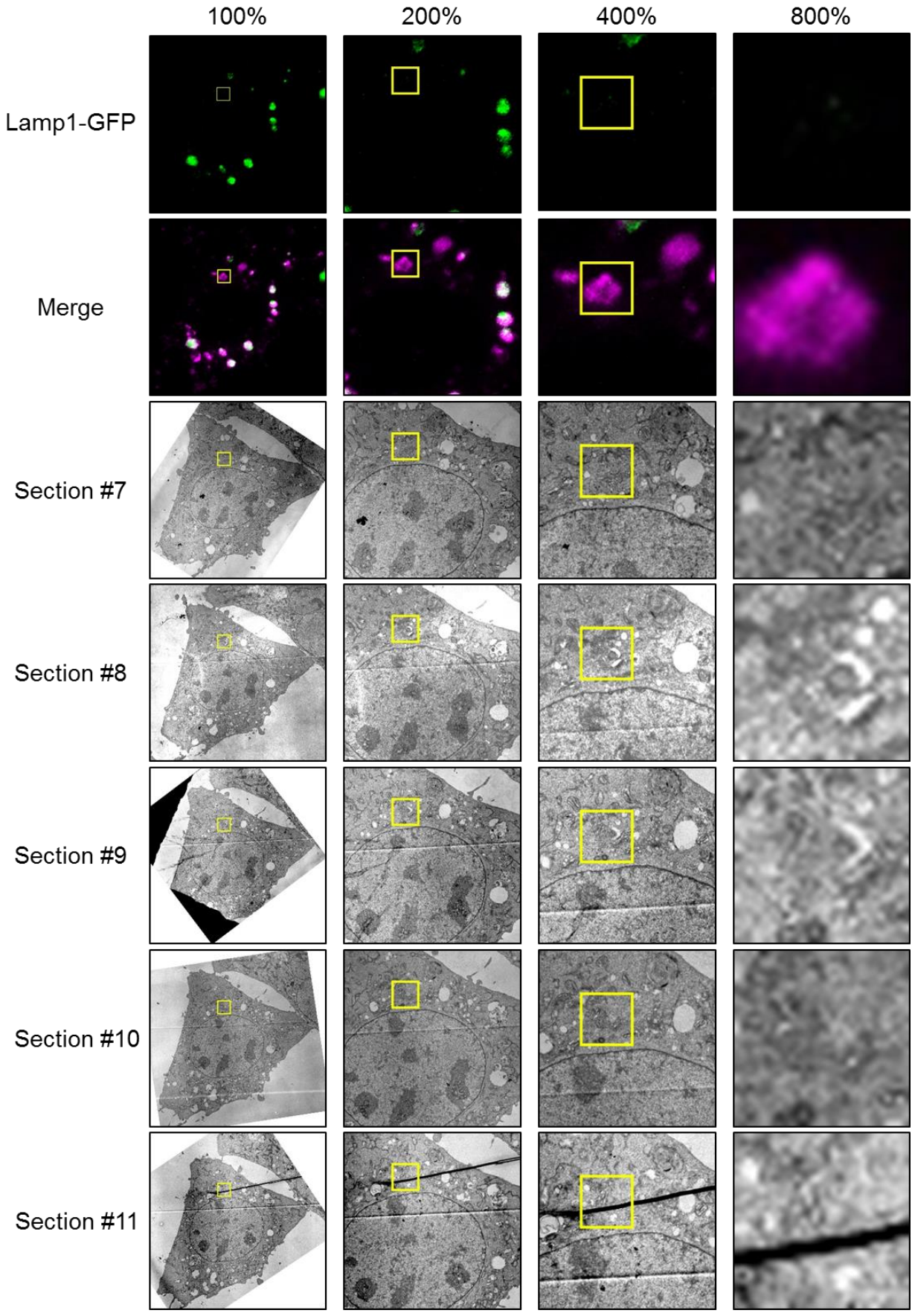

100%
200%
400%
800%
Lamp1-GFP
Merge
Section #7
Section #8
Section #9
Section #10
Section #11

Figure S24. Various magnifications and stepwise z-section images of the cells in Figure 6C
Atg5-deficient MEFs stably expressing LAMP1-GFP were preincubated with DAPRed (0.1 μM), and then treated with 10 μM etoposide for 10 hr. LAMP1-GFP-single-color images and LAMP1/DAPRed merged images are shown. The optical section thickness of confocal images were 456 nm. Regarding EM, a series of 80 nm ultrathin sections (#7 to #11) are also indicated in the lower panels. The ROIs are indicated by the squares, and magnified images are shown in the right panels. Section #8 is magnified in Fig. 6C.

Atg5-KO MEF, Etoposide 10 hr

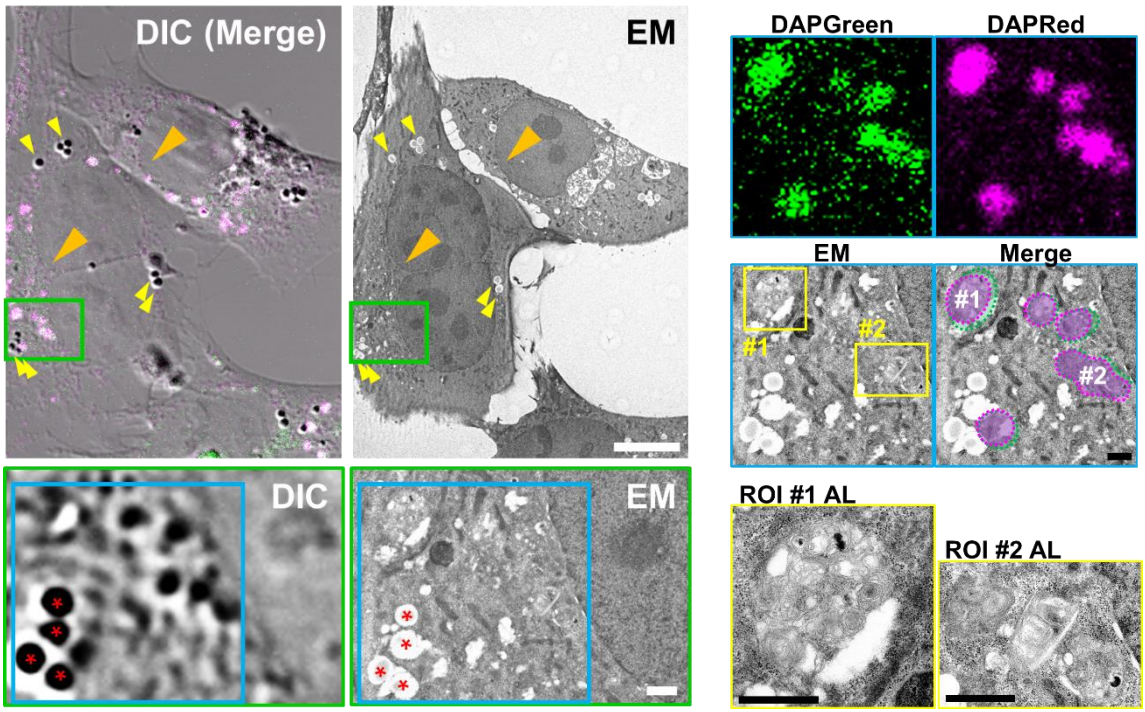


Figure S25. Alternative autophagic structures are labeled by DAPRed and DAPGreen, related to Figure 6
Atg5-deficient MEFs were preincubated with DAPGreen (0.25 μM) and DAPRed (0.1 μM), were treated with etoposide (10 μM) for 10 hr, and performed CLEM analysis. DIC and EM images were adjusted for the edge of the plasma membranes, nuclear membranes (orange arrowheads), and lipid droplets (yellow arrowheads). Bar = 10 μm. ROIs are indicated by the green square and magnified images are shown in the lower panels. Red asterisks indicate vacuoles. Bar = 1 μm. ROIs are indicated by the blue squares and images are shown in the upper right panels. Fluorescent signals are indicated by the dashed circles in the merged image. Bar = 1 μm. High-magnification images of the yellow squares are shown in the bottom right panels (ROI #1 and #2). Bar = 1 μm.

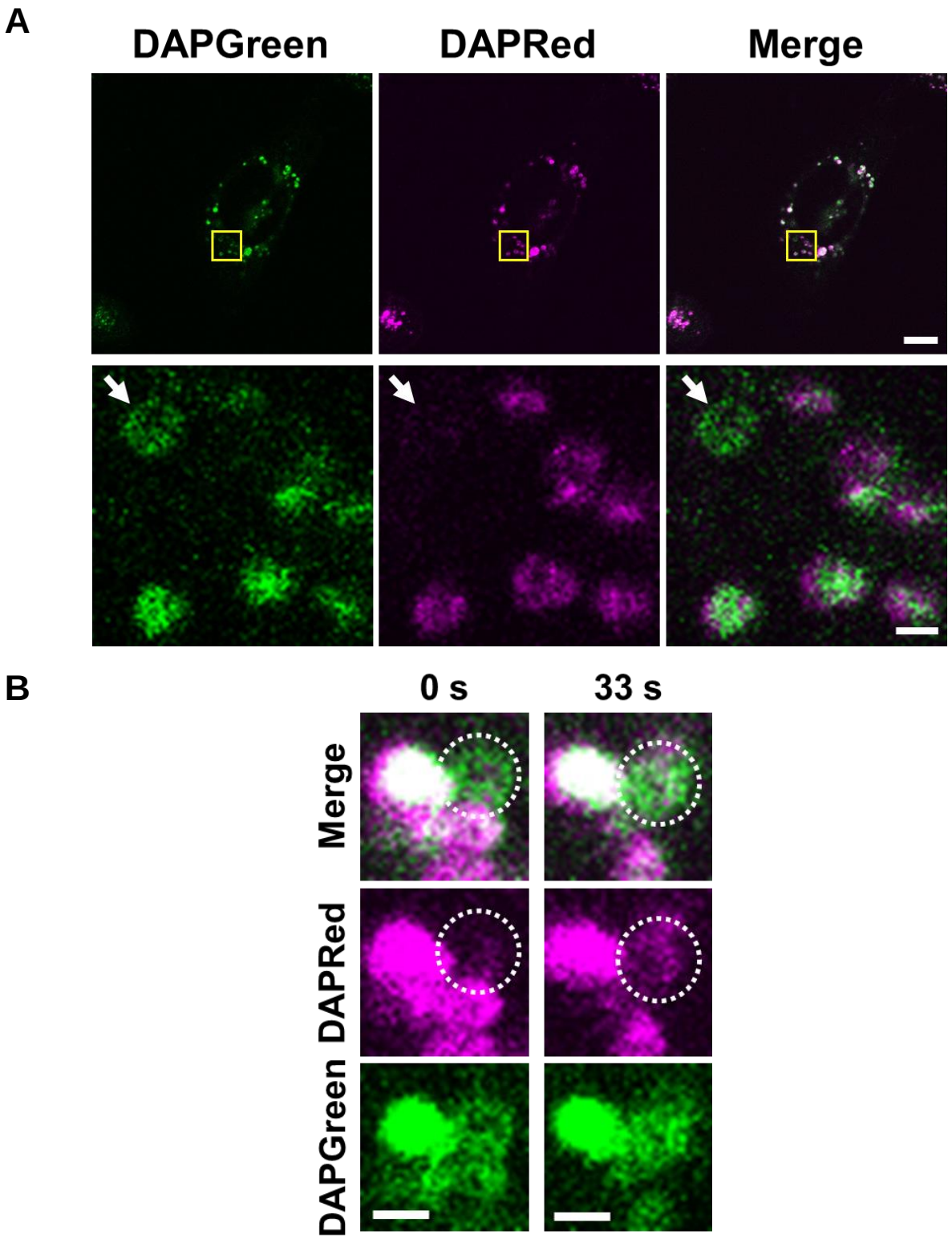


Figure S26. Analysis of alternative autophagy dynamics using DAPRed and DAPGreen, related to Figure 5

Atg5-deficient MEFs were incubated with DAPGreen (0.25 μM) and DAPRed (0.1 μM), and then treated with etoposide (10 μM) for 10 hr. Cells were observed by confocal microscopy, and representative images are shown in **(A)**. Magnified images of the yellow squares are shown in the lower panels. Bars = 10 μm (top), and 1 μm (bottom). White arrows indicate single DAPGreen puncta. **(B)** Time-lapse imaging of DAPGreen and DAPRed showed that the appearance of DAPGreen signals preceded that of DAPRed signals (dotted circles). Bars = 1 μm.

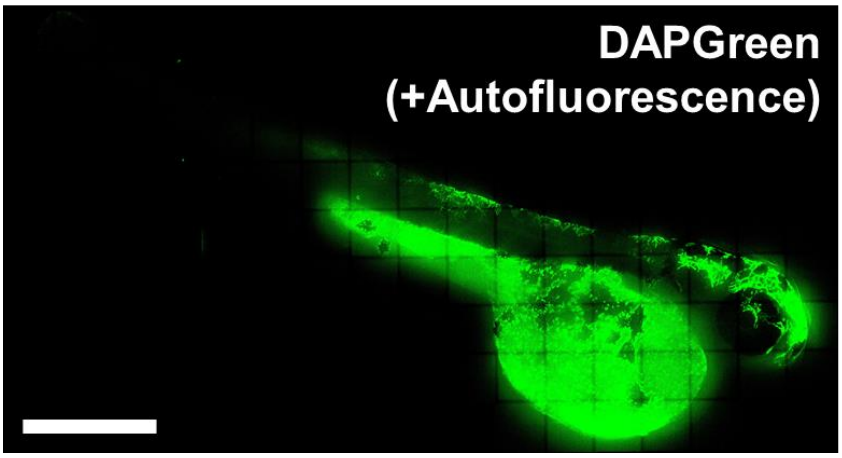


Figure S27. Zebrafish autofluorescence interferes with the detection of DAPGreen signals, related to Figure 7

DAPGreen-treated zebrafish larvae were observed by the whole-mount rapid 3D imaging system. Green fluorescence was visualized by excitation with a 488-nm laser. Autofluorescence of zebrafish larvae was detected at the same wavelength as DAPGreen. Bar = 500 µm.

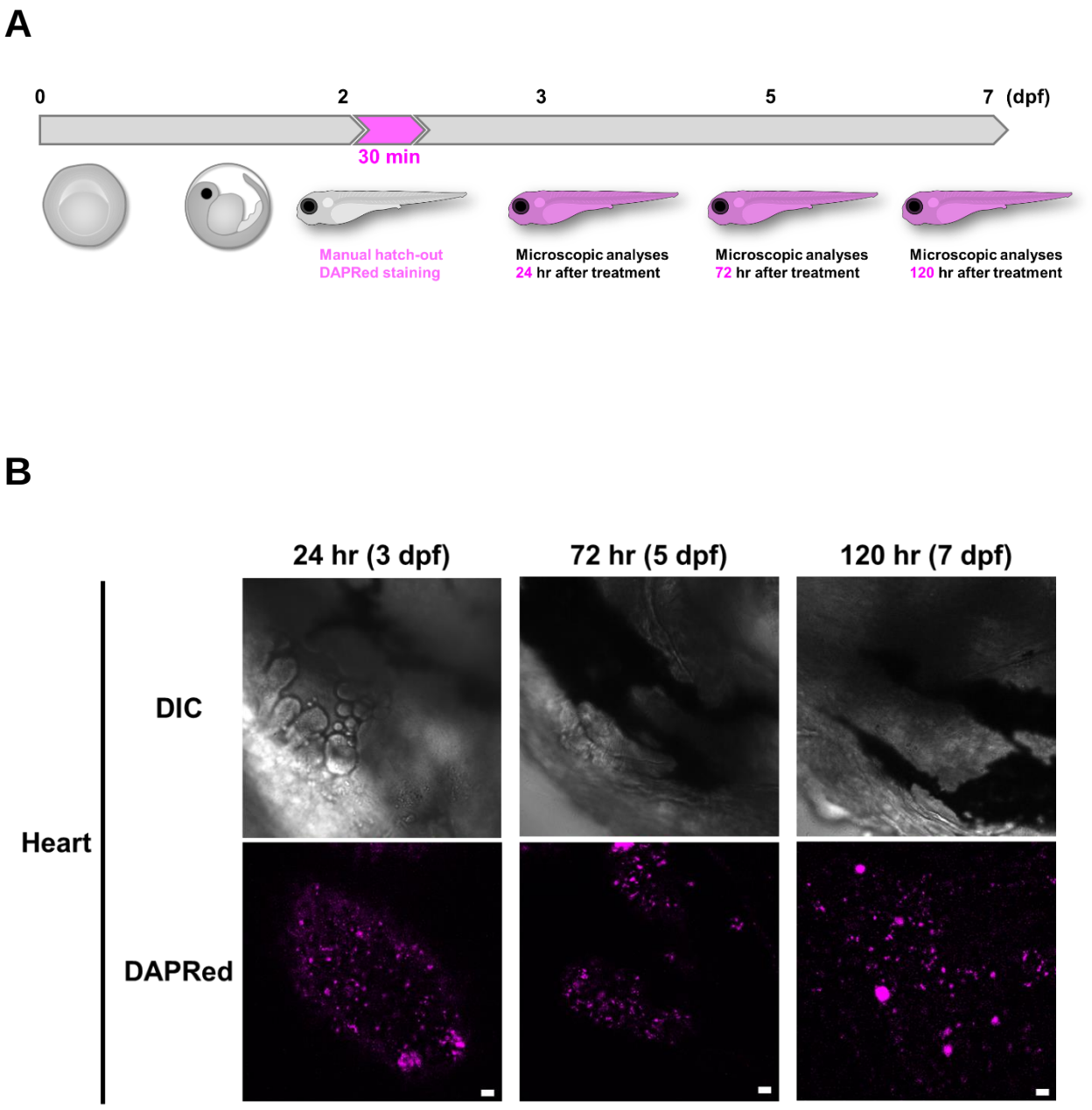


Figure S28. Analysis of DAPRed stability in zebrafish, related to Figure 7

(A) Schematic flowchart of DAPRed staining and observation in zebrafish. DAPRed (0.1 μM) was added to the water for 30 min at 2 dpf. **(B)** Zebrafish larvae were observed by confocal microscopy at the indicated times. DAPRed signals in the heart were stably observed at 120 hr after staining. Bar = 10 μm.

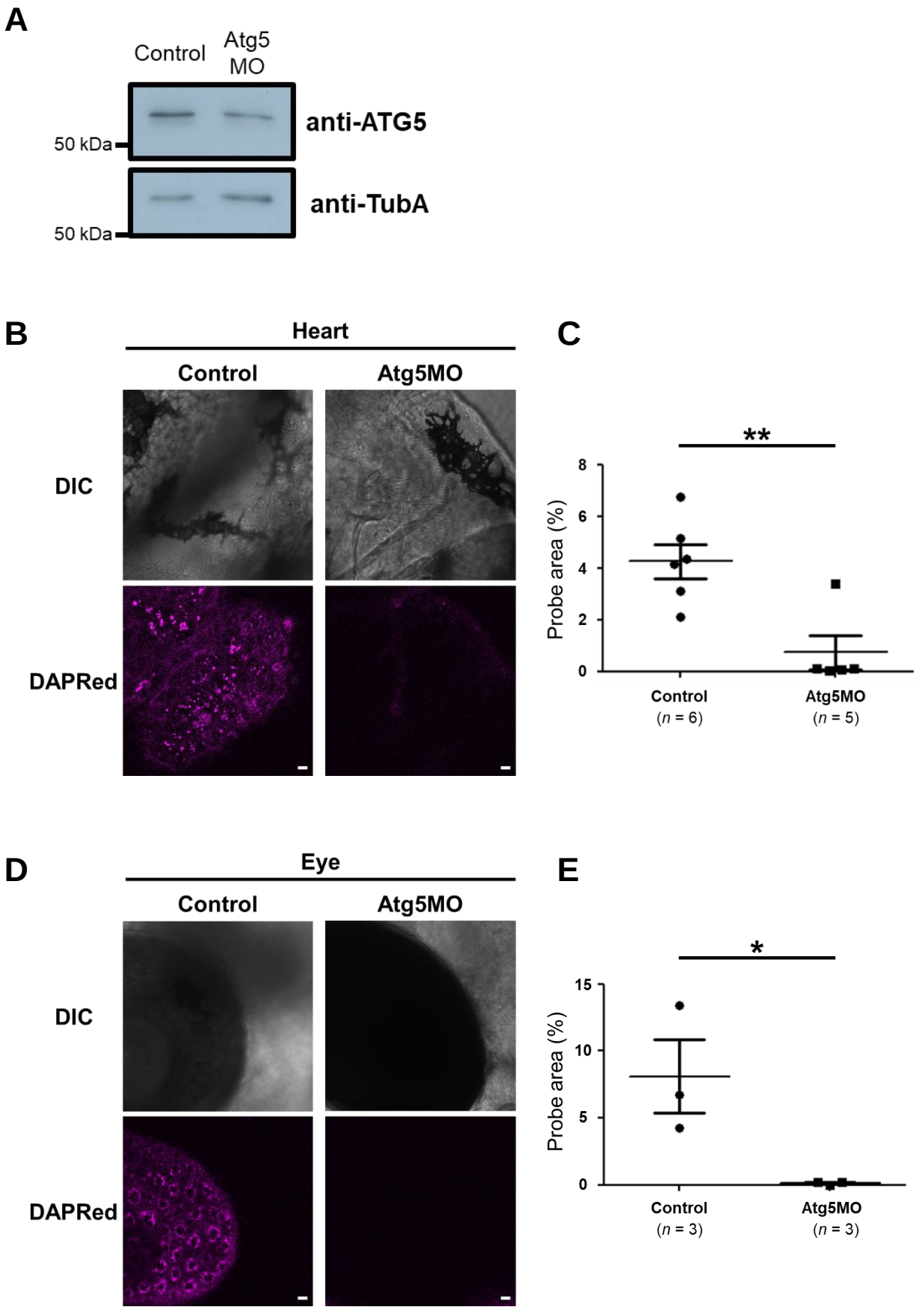

A
Control
Atg5 MO
anti-ATG5
50 kDa
anti-TubA
50 kDa
B
Heart
Control
Atg5MO
DIC
DAPRed
C
**
Probe area (%)
8
6
4
2
0
Control
(n = 6)
Atg5MO
(n = 5)
D
Eye
Control
Atg5MO
DIC
DAPRed
E
*
Probe area (%)
15
10
5
0
Control
(n = 3)
Atg5MO
(n = 3)

Figure S29. Inhibition of Atg5 translation using morpholino oligos in zebrafish, related to Figure 7
(A) The efficacy of Atg5 translation inhibition by Atg5MO was evaluated by immunoblotting using anti-Atg5 antibody which reacts with zebrafish Atg5. In 2 dpf embryos, the intensity of the band representing the Atg12-Atg5 complex (above 50 kDa) was decreased by Atg5MO treatment. **(B–E)** Atg5MO and a control oligo were injected into fertilized eggs, and then DAPRed (0.1 μM) was added to the water for 30 min at 2 dpf. The hearts and eyes of zebrafish larvae were observed by confocal microscopy at 3 dpf, and representative images are shown in **(B, D)**. In **(C, E)**, the ratio of DAPRed-positive area to total area of ROIs was quantified. Bars = 10 μm. Data are shown as the mean ± S.D. The total number of images used for analysis is given as the *n*. **p* < 0.05, ***p* < 0.01 (Student *t*-test).