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Development of a New Autophagosome Sensor With an LC3-interacting Region (LIR) Motif and a Hydrophobic Domain

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Macroautophagy, hereafter referred to as autophagy, is an evolutionarily conserved lysosomal degradation pathway for long-lived proteins, organelles, or certain cytosolic components such as RNA, or lipids [1]. Although autophagy is primarily considered a kind of a cellular defense mechanism for the provision of nutrients via self-digestion and protection of cells during starvation [2, 3], a growing body of evidence points to autophagy's important roles in many other physiological processes, such as regulation of cellular homeostasis, differentiation, or metabolism [4]. Formation or degradation of the autophagosome, a hallmark of autophagy, is necessary for the whole autophagic process. Therefore, monitoring of autophagosomes is the most important part of autophagy research. To date, overexpression of green fluorescent protein (GFP)/red fluorescent protein (RFP)-light chain 3 (LC3), with specific localization to the autophagosome, has been commonly used for research in this field, but has some limitations due to artifacts related to overexpression of LC3. Here, we developed a new probe for autophagosomes that detects endogenous LC3 in the autophagosome using an LC3-interacting region (LIR(Fy)) motif from FYVE and coiled-coil domain containing 1 (FYCO1) and an N-terminal hydrophobic domain (S(N20)) from *Aplysia* phosphodiesterase 4 (ApPDE4) short-form. Although either LIR(Fy)-EGFP or S(N20)-EGFP alone was barely localized to the autophagosome, when S(N20) was fused to LIR(Fy)-EGFP, the resulting S(N20)-LIR(Fy)-EGFP was efficiently localized to endogenous LC3-positive autophagosomes induced by either starvation or rapamycin treatment in wild-type (WT) mouse embryonic fibroblasts (MEFs) but not in *atg5*$^{-/-}$ MEFs. Our immunogold-electron-microscopic experiments confirmed that S(N20)-LIR(Fy)-EGFP was preferentially localized to the autophagosome in WT MEFs but not *atg5*$^{-/-}$ MEFs. Finally, we demonstrated that S(N20)-LIR(Fy)-EGFP could detect autophagosomes accumulated in poly(Q)-expanded-mutant-huntingtin–expressing neurons; this finding points to possible applications to disease models based on autophagy. Thus, we propose a novel autophagosome sensor for live cells, which is expected to be widely used in autophagy research and practical applications [5].

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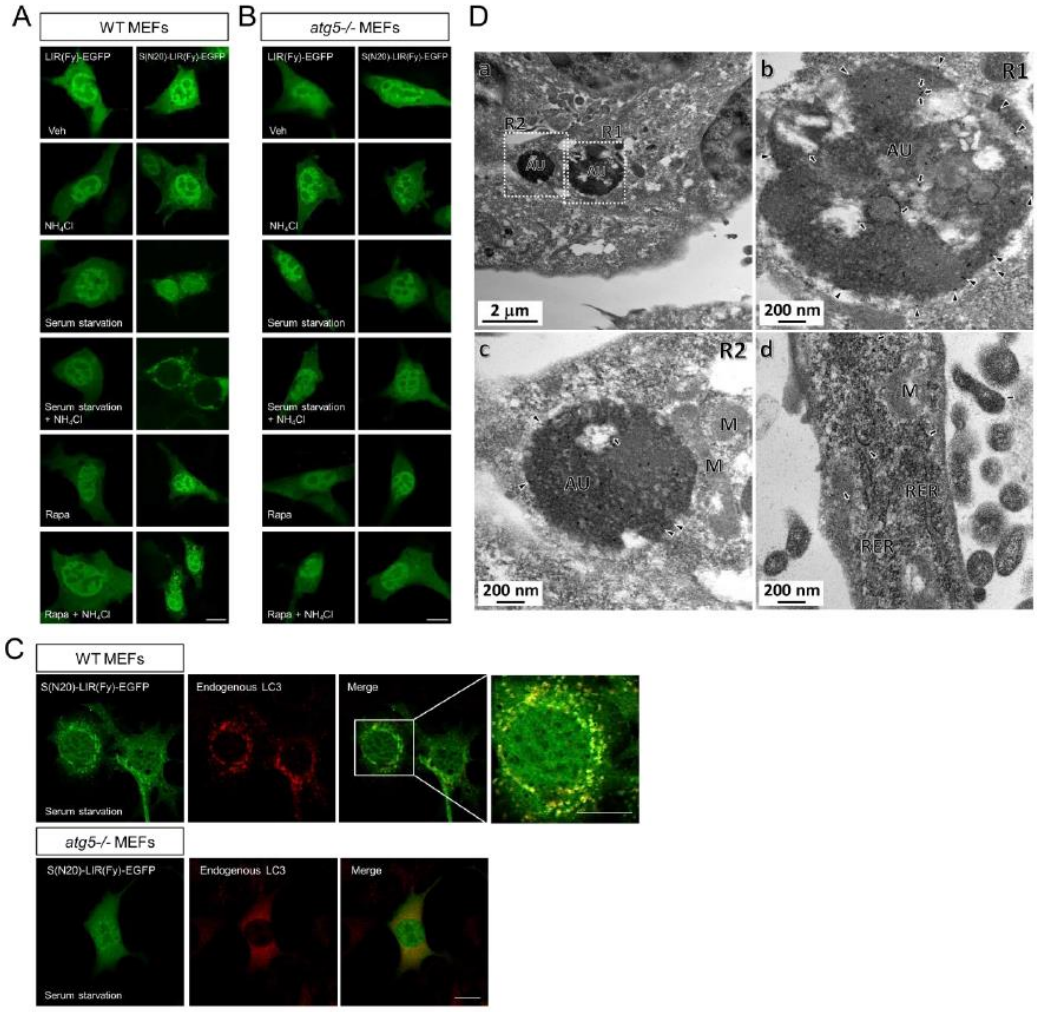

Figure 1. Effective localization of S(N20)-LIR(Fy)-EGFP to autophagosome structures during autophagy induction with serum starvation or rapamycin in an autophagy-dependent manner. A vector encoding LC3-interacting region (LIR(Fy))-EGFP or S(N20)-LIR(Fy)-EGFP was transfected into WT or *atg5*$^{-/-}$ MEFs. For autophagy induction, the cells were incubated with 10 nM rapamycin for 4 h or starved (serum deprivation) for 24 h in the presence or absence of NH_4Cl (10 mM). **(A-B)** Confocal images showing the localization of S(N20)-LIR(Fy)-EGFP to autophagic vacuoles during autophagy induction in WT MEFs (A) or *atg5*$^{-/-}$ MEFs (B). **(C)** Confocal images showing the localization of S(N20)-LIR(Fy)-EGFP to endogenous LC3-positive autophagosome detected by anti-LC3 antibody during autophagy induction in WT MEFs (upper) or *atg5*$^{-/-}$ MEFs (lower). Scale bar, 10 μm. **(D)** Cell images showing immunogold labeling of LC3-bound GFP in autophagic vacuoles (AU) in WT MEFs (a–c: an enlarged view of R1 and R2) or in *atg5*$^{-/-}$ MEFs (d) during autophagy induction with serum starvation. Arrowheads indicate immunogold labeling of LC3-bound GFP in the autophagosomal membrane, whereas the arrows show LC3 inside autophagosomes. R: region, **M:** mitochondrion, **RER:** rough endoplasmic reticulum. **Veh,** vehicle; **Rapa,** rapamycin.

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