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Autophagosome live cell imaging

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Protocol status: Working
We use this protocol and it's working

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Abstract

For quantification of PINK-PARKIN mitophagy

Cell Culture

1 Seed Parkin-expressing HeLa cells for ~80% confluence on day of transfection on tissue-culture coated glass slides, suitable for use on a confocal microscope.

2 On day of transfection, transfect pCIG2-LAMP1-mNeon Green, pHaloTag-LC3B, and an autophagy regulating protein tagged with mcherry via lipofectamine 3,000 (ThermoFisher). Briefly, dilute plasmids in Opti-mem reduced serum medium and add P3000 reagent in accordance with manufacturer instructions. Also dilute lipofectamine 3000 reagent in optimem, then mix the dilute plasmid and lipofectamine solutions together. Incubate for ~10 minutes, then add dropwise to adhered cells.

3 Allow cells to recover overnight

4 On day of experiment swap cells into growth medium containing 10 µM Oligomycin A and 5 µM Antimycin A. Return to incubator for 4 H

Imaging and Analysis

5 When ready to image, swap medium with Leibovitz's L-15 medium (Gibco 11415064) supplemented
with 10% fetal bovine serum and 1% Glutamax along with Antimycin A and Oligomycin A.

6 Calibrate laser power and exposure for best image quality. Keep these parameters consistent across treatments or conditions. Collect Z stacks of each target cells.

7 To analyze, threshold LC3 and LAMP1 channels, then binarize using Yen thresholding in
ImageJ. To quantify area of colocalization, use the 'AND' function from the Image Calculator function. Then calculate area of colocalization using Analyze Particles.