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WIPI2d puncta formation assay

In 1 collection

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ABSTRACT

Labels sites of autophagosome generation.

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1 Target cells are cultured until they reach 80% confluence on cell-culture treated, chambered confocal slide, and then transfect cells with a WIPI2d-Halo mammalian expression plasmid using lipofectamine 3000 (Thermo Fisher). Calculate DNA amount to use via the manufacturer instructions, scale DNA according to culture area.

2 Wash cells into antibiotic free medium prior to transfecting. Incubate the cells for 24 H post transfection, then wash cells into fresh medium. Incubate for an additional 24 H before imaging.

3 On day of experiment, replace medium with growth medium supplemented with 10 uM Oligomycin A, 5 uM Antimycin A (OA). Incubate for 45 min.

4 Add 1x cell permeable, fluorescent Halo Ligand diluted in OA medium to label. Incubate for 15 min before washing cells twice with PBS and fixing in 4% PFA. Wash once more with PBS. Image cells immediately on a confocal microscope.

5 To analyze open images in FIJI, find a cell with largely cytosolic WIPI signal, and measure. Subtract this value from the image as a whole using the math > subtract function.

6 Define ROIs around target cells and measure total signal using FIJI's native measure function. Inspect cells to make sure cytosolic WIPI signal is being reliably eliminated, and most of the fluorescence intensity comes from punctate WIPI.