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GUV preparation and assay

Forked from GUV assay

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ABSTRACT

LC3 lipidation on GUVs

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1 GUV Preparation

1.1 Clean the coverslips of 25 mm diameter.

1.2 Coat cleaned coverslips with 60 µL 5% (w/w) polyvinyl alcohol (PVA) with a molecular weight of 145,000 (Millipore).

Place the coated coverslip in a heating incubator at 60 °C to dry the PVA film for 30 min.

1.3

1.4 Spread a lipid mixture with a molar composition of 64.8% DOPC, 20% DOPE, 10% POPI, 5% DOPS and 0.2% Atto647N DOPE at 1 mg/ml uniformly onto the PVA film.

1.5 Put the lipid-coated coverslip under vacuum overnight to evaporate the solvent.

1.6 Use 400 µL 400 mOsm sucrose solution for swelling for 1 h at room temperature

1.7 Harvest the GUVs and use them with 12 h.

2 GUV Assay

2.1 Set up the reaction in an eight-well observation chamber (Lab Tek) at room temperature.

2.2 Coat the chamber with 5 mg/ml β casein for 30 min.

2.3 Wash the coated chamber three times with reaction buffer (20 mM HEPES at pH 8.0, 190 mM NaCl and 1 mM TCEP).

2.4 Make a 120 µL reaction mixtures with the proteins and 50 µM ATP. The final concentration the each protein is 5 µM GST-4xUb, 500 nM cargo receptors, 25 nM ULK1 complex, 25 nM PI3KC3-C1 complex, 100 nM WIPI2d, 50 nM ATG12–ATG5-ATG16 complex, 100 nM ATG7, 100 nM ATG3, and 500 nM mCherry-LC3B.

2.5 Add 10 µL GUVs to initiate the reaction.

2.6 Pick random views for imaging within 5 min.

2.7 Acquire time-lapse images in multitracking mode on a Nikon A1 confocal microscope.

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