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GFP-ATG3 GUV Assay

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We use this protocol and it's working

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Abstract

LC3 lipidation on GUVs

1 GUV Preparation

1.1 Clean the coverglass

1.2 Coat cleaned coverslips with 100 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 Spread a lipid mixture at 1 mg/ml with a molar composition of 70% DOPC, 20% DOPE, 5% DO-PI(3)P, 5% DOPS, 0.3% Atto647N DOPE uniformly onto the PVA film.

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

1.5 Use 100 µL 330 mOsm sucrose solution for swelling for 1 h at room temperature

1.6 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 1 mg/ml β casein for 10 min.

2.3 Wash the coated chamber with reaction buffer (25 mM HEPES at pH 7.4, 150 mM NaCl and 1 mM TCEP).

2.4 Make a 120 µL reaction mixtures with the proteins and 5 mM ATP and 1 mM MgCl2. The final concentration of WIPI2d 200 nM, E3 complex is 50 nM, ATG7 is 100 nM, GFP-ATG3 or Mutant is 100 nM, and mCherry-LC3B is 500 nM.

2.5 Add 3 µL GUVs to initiate the reaction.

2.6 Pick random views for imaging within 5 min.