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).

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

1.4 Spread a lipid mixture 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. For E3 membrane recruitment and LC3 lipidation experiment, the final concentration of PI3KC3-C1 complex is 50 nM, WIPI2d WT or mutant is 250 nM , E3-GFP complex is 50 nM, ATG7 is 100 nM, ATG3 is 100 nM, and mCherry-LC3B is 500 nM. For WIPI2d membrane binding in the presence of PI3KC3-C1, the final concentration of PI3KC3-C1 complex is 50 nM, the mCherry tagged WIPI2d WT or mutant is 400 nM. For WIPI2d membrane binding to PI(3)P GUVs, the final concentration of the mCherry tagged WIPI2d WT or mutant is 400 nM.

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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