Halo-LC3B processing assay to assess autophagy

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Buffers and reagents:

- Growth media: DMEM medium with GlutaMAX (Gibco #10566016) containing 10% FBS (Gibco #A5256801) and 10% Pen-Strep (Gibco #15140122).
- EBSS buffer (Gibco #24010043)
- JF646 HaloTag ligand (Promega # GA1120)
- Trypsin-EDTA (0.05%) (Gibco #25300120)
- 1x PBS
- Pierce Protease Inhibitor Tablets (Thermo Scientific #A32963)
- Lysis buffer: 25 mM HEPES pH 7.5, 200 mM NaCl, 2 mM MgCl2, 10% glycerol, 1 mM TCEP, 0.2% *n*-dodecyl-β-D-maltoside (GoldBio #DDM25) with pierce protease inhibitors and Benzonase (Millipore #70746)
- 4-12% Bis-Tris NuPAGE gels (Invitrogen #NP0322)
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Procedures:

1. Generating HeLa cells expressing HaloTag-LC3B using pMRX-IP-HaloTag7-LC3 from Mizushima lab (Addgene #184899; DOI: 10.7554/eLife.78923).
2. Seed HeLa cells at 100-150K cells/well in 12-well plate one day before.
3. Next day, incubate cells with complete DMEM medium and 50 nM JF646 HaloTag ligand (Promega) for 1 hour, and then wash twice with 1xPBS. The non-starved samples can be harvested immediately by trypsinization (step 5).
4. To induce autophagy by starvation, treat cells with EBSS buffer (Gibco) for desired period.
5. After the treatment, harvest the cells by trypsinization.
    - Wash the wells with 1x PBS
    - Incubate the wells with 0.5 ml trypsin at 37 °C for 5 minutes
    - Add 0.5ml complete medium into each well.
    - Transfer cells into pre-chilled Eppendorf tube, spin 2000x g for 5 minutes.
    - Aspirate off the liquid.
6. Resuspend the cell pellets in 30 µl of lysis buffer and incubate on ice for 30 minutes.
7. Centrifuge the cell lysate at 21,000xg for 10 minutes.
   Transfer the cleared lysate into another tube, and measure protein concentration nanodrop spectrophotometer (Thermo Fisher).
8. For each sample, load 20 µg clarified lysates onto NuPAGE 4-12% Bis-Tris Gel (Thermo Fisher).
9. For in-gel fluorescence imaging, the gel was immediately visualized with ChemiDoc MP imaging system (Bio-Rad) after SDS PAGE. Band intensities are acquired by exciting samples at 546 nm (mCherry signal) and 647 nm (JF646 HaloTag ligand signal).