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MLSA5 and VPS34 inhibitor treatments and immunoblotting of whole-cell lysates from cell culture systems

In 1 collection

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We use this protocol and it's working

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Abstract

This is a protocol for assessing ATG8 lipidation after CASM induction by Western blotting from whole-cell lysates derived from HeLa cell culture systems.

Troubleshooting

Safety warnings

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Cell Culture and Treatments

5m

1 Seed HeLa TMEM192-3xHA cells of desired genotypes into 6-well plates.

2 Upon reading desired confluency (~70%), treat cells according to the following four treatments:
1. Fed
2. 3 h with the VPS34 inhibitor SAR405 (1 µM)
3. 2 h with MLSA5 (10 µM)
4. 2 h VPS34 inhibitor + MLSA5

3 Aspirate growth media, wash twice with 1xPBS and harvest cells in ice-cold PBS on ice by scraping cells from the wells.

SDS-PAGE and immunoblotting

5m

4 For whole-cell lysates, prepare samples following standard protocols, and final samples should be in LDS buffer with DTT or similar. Incubate samples at 80 °C for 00:05:00 . 5m

5 Load samples into a NuPAGE Novex® 4-12% Bis-Tris Midi Protein Gels and separate by electrophoresis in 1xTris/Glycine/SDS buffer.

6 Transfer proteins to PVDF or nitrocellulose membranes by standard wet transfer in 20% methanol Tis/Glycine buffer.

7 Block membrane in blocking buffer (5% non-fat dry milk or 3% BSA in TBST) at Room temperature for 01:00:00 . 1h

8 Incubate membrane in primary antibody solution (blocking solution plus primary antibody at 1:500-1:1,000, depending on the primary antibody) at 4 °C for 12:00:00 to 16:00:00 . 1d 4h

Primary antibodies:
LC3B
panGARBARAP
Actin

9 Wash membrane six times with TBST for 00:05:00 each wash. 5m

10 Incubate membrane in secondary antibody solution (blocking solution plus secondary antibody conjugated to HRP at 1:5,000-1:10,000) at Room temperature for 01:00:00 . 1h

11 Wash membrane four times with TBST for 00:05:00 each wash. 5m

12 Apply Western Lightning Plus Chemiluminescence substrate (Revvity) to membrane and acquire blot images using a ChemiDoc MP imager.

13 Process raw image files with Image Lab software (Bio-Rad).