Metabolomics

MDA-MB-231 cells or HEK293T cells were transfected with ΔATG1+2 or Flag-hSPAR for 48 hours. The metabolites were extracted for metabolite quantification by LC/MS analysis at PTMBio (HangZhou, CHINA).

1. Chemicals and reagents

HPLC-grade acetonitrile (ACN) and methanol (MeOH) were purchased from Merck (Darmstadt, Germany). MilliQ water (Millipore, Bradford, USA) was used in all experiments. All of the standards were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ammonium acetate and formic acid were bought from Sigma-Aldrich (St. Louis, MO, USA). The stock solutions of standards were prepared at the concentration of 1 mg/mL in MeOH. All stock solutions were stored at -20°C. The stock solutions were diluted with MeOH to working solutions before analysis.

2. Sample preparation and extraction

The sample was thawed on ice, 100 μL of ultrapure water extract (containing protease inhibitors, PMSF and EDTA) was added to resuspend the cell pellet. Divide 50 μL cell suspension and add 200 μL of methanol (precooled at -20°C) and vortexed for 2 min under the condition of 2500 r/min. The sample was frozen in liquid nitrogen for 5 min, removed on ice for 5 min, after that, the sample was vortexed for 2 min.The previous step was repeated for 3 times. The sample was centrifuged at 12000 r/min for 10 min at 4° C. Take 200 μL of supernatant into a new centrifuge tube and place the supernatant in -20°C refrigerator for 30 min. Then the supernatant was centrifuged at 12000 r/min for 10 min at 4°C. After centrifugation, transfer 180 μL of supernatant through Protein Precipitation Plate for further LC-MS analysis. The left 50 μL cell suspension was frozen and thawed for 3 times, centrifuged at 12,000 r/min for 10 min, and the supernatant was taken to determine the protein concentration by BCA Protein Assay kit.

3. UPLC Conditions

The sample extracts were analyzed using an LC-ESI-MS/MS system (UPLC, ExionLC AD, https://sciex.com.cn/; MS, QTRAP® 6500+ System, https://sciex.com /). The analytical conditions were as follows, HPLC: column, ACQUITY BEH Amide (i.d.2.1×100 mm, 1.7 μm); solvent system, water with 2 mM ammonium acetate and 0.04% formic acid (A), acetonitrile with 2 mM ammonium acetate and 0.04% formic acid (B); The gradient was started at 90% B (0-1.2 min), decreased to 60% B (9 min), 40% B (10-11 min), finaly ramped back to 90% B (11.01-15 min); flow rate, 0.4 mL/min; temperature, 40°C; injection volume: 2 μL.

4. ESI-MS/MS

AB 6500+ QTRAP® LC-MS/MS System, equipped with an ESI Turbo Ion-Spray interface, operating in both positive and negative ion modes and controlled by Analyst 1.6 software (AB Sciex). The ESI source operation parameters were as follows: ion source, turbo spray; source temperature 550°C; ion spray voltage (IS) 5500 V(Positive) ,-4500 V(Negative); curtain gas (CUR) were set at 35.0 psi; DP and CE for individual MRM transitions was done with further DP and CE optimization. A specific set of MRM transitions were monitored for each period according to the amino acid eluted within this period.

5. Detection of Amino acid and its metabolites

Amino acid and its metabolites were detected by (http://www.metware.cn/) based on the AB Sciex QTRAP 6500 LC-MS/MS platform.