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nMOST-LSD Protocol Collection V.1

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Protocol status: In development
We are still developing and optimizing this collection

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Keywords: ASAPCRN, cholesterol trafficking, lysosomal storage disease, dozen diverse lsd mutant cell line, lipid, lysosome, systematic lipid, autophagy receptor, aberrant accumulation of autophagy receptor, extracellular iron, associated defects in lysosomal function, lipidome, lsd mutant, using lsd mutant, proteome phenotyping, phosphatidylcholine species, simultaneous quantification of proteome, electron transport chain complex, ferritinophagy defect, lysosomal function, lsd protocol collection, containing electron transport chain complex, lsd, proteome, ferritinophagy cargo, sensitive nanoflow, mutant cell line, mitochondrial cristae

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Abstract

Lysosomal storage diseases (LSDs) comprise ~50 monogenic diseases display accumulation of cellular material in lysosomes and associated defects in lysosomal function, but systematic lipid-proteome phenotyping remains challenging. Here, we report a sensitive nanoflow-based multi-omic single-shot technology (nMOST) workflow allowing simultaneous quantification of proteomes and lipidomes. Benchmarking nMOST using LSD mutants linked with cholesterol trafficking revealed aberrant accumulation of autophagy receptors and ferritinophagy cargo, which correlated with accumulation of lyso-phosphatidylcholine species and multi-lamellar membrane structures visualized by cryo-electron-tomography, especially in *NPC2*$^{-/-}$ cells. Ferritinophagy defects correlated with loss of mitochondrial cristae and iron-sulfur cluster-containing electron transport chain complexes that could be rescued by extracellular iron. To further demonstrate the value of nMOST, we profiled more than two dozen diverse LSD mutant cell lines. We provide an accompanying web-portal resource of lipid-protein correlations across the entire dataset and demonstrate how the resource can be used to reveal common and distinct molecular phenotypes.

Troubleshooting

Files

SEARCH

Protocol

NAME

Sample preparation and vitrification of cell culture cells for PFIB cryo-ET

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cryo-Plasma Focused Ion Beam (PFIB) milling

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Cryo-ET data acquisition, tomogram reconstruction, analysis and segmentation

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Multiplexed TMTpro proteomic sample preparation of whole cell HeLa lysates from various growth conditions ± FAC

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General Protocol for Immunocytochemical analysis of adherent cell culture cells

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Fixed-cell, microscopy-based evaluation of Ferritin accumulation in lysosomes

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Evaluation of GFP-SopF expression on ATGlyation using spinning disk microscopy

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Live-cell microscopy for mitochondrial membrane potential measurements

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Sample preparation and live-cell 3D-SIM imaging of PKmitoRed

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Sample preparation and live-cell 3D-SIM imaging of LysoTrackerRed and Dextran647 fusion assay

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Sample Preparation and 3D-SIM fixed-cell imaging of FTH1 and Filipin in NPC1 and NPC2 mutants

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