SD-NSC-HETEROGENEITY OF AGING CORE

MASS SPECTROMETRY CORE FOR PROTEOMICS AND METABOLOMICS

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SAN DIEGO NATHAN SHOCK CENTER 2022 WORKSHOP









Mass Spectrometers





Prebvs



Success comes from working together

- We meet with you to discuss project details and goals, ٠ determine the best experimental strategy
- You prepare and submit samples
- We run samples, generate data and send to you ٠
- We meet again with you to discuss the data, plan future • experiments
- We assist with incorporating data into papers / grants ٠





Protein Identification: Gel bands or IPs

Protein Identification: Binding Partners by Co-Immunoprecipitation







Metabolic Labeling Improves Quantitation but Increases Complexity

Pulsed SILAC can be used to measure protein turnover



SILAC



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Prebys





Tandem Mass Tags (TMT) Enable Quantitation Without Increasing Complexity





Amine reactive tags



Sanford Burnhan Prebys



Proximity Labeling to Identify Protein- Protein Interactors of Mapping of Organelles

Many types of proximity labeling

- BirA
- BioID
- BioID2
- Turbo ID
- APEX
- HRP



Qian Chu,¹ Thomas F. Martinez,¹ Sammy Weiser Novak,² Cynthia J. Donaldson,¹ Dan Tan,¹ Joan M. Vaughan,¹ Tina Chang,¹ Jolene K. Diedrich,¹ Leo Andrade,² Andrew Kim,¹ Tong Zhang,² Uri Manor,¹² and Alan Saghatelian¹³¹



Mass spectrometry and imaging using the same constructs

REPORT

Proteomic Mapping of Mitochondria in Living Cells via Spatially Restricted Enzymatic Tagging

Hyun-Woo Rhee^{1,*,+}, Peng Zou^{1,*}, Namrata D. Udeshi², Jeffrey D. Martell¹, Vamsi K. Mootha^{2,3,4}, Steven A. Carr², Alice Y. Ti... + See all authors and affiliations



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Developmental Cell Supports open access

ARTICLE | VOLUME 56, ISSUE 21, P2952-2965.E9, NOVEMBER 08, 2021

Identification of long-lived proteins in the mitochondria reveals increased stability of the electron transport chain

Shefali Krishna • Rafael Arrojo e Drigo 4 • Juliana S. Capitanio • Ranjan Ramachandra • Mark Ellisman • Martin W. Hetzer A 5 🖂 • Show footnotes

Published: October 28, 2021 • DOI: https://doi.org/10.1016/j.devcel.2021.10.008 • 🖲 Check for updates



ETC complexes and supercomplexes, turnover rates measured by native gel bands and SILAC



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Various Proteomic Approaches Available to Investigate Aging Systems





LIPIDOMICS

- Instrument: Q-Exactive •
- Lipid profile (positive/negative ionization modes) ٠
- Database-like search (LipidSearch) ullet
- **Relative Quantitation** •
 - Internal Standards, Heavy-Labeled Lipids



Prebvs



Prebys

TARGETED METABOLOMICS

- Several Targeted Methods Available
 - Bile Acids
 - Polar Metabolites (TCA Cycle)
 - Amino Acids
 - Short Chain Fatty Acids
 - FAHFAs
- Method Development ٠
 - Customized methods





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JCI insight

Inhibition of ceramide accumulation in AdipoR1^{-/-} mice increases photoreceptor survival and improves vision

Dominik Lewandowski,¹ Andrzej T. Foik,² Roman Smidak,¹ Elliot H. Choi,^{1,3} Jianye Zhang,¹ Thanh Hoang,⁴ Aleksander Tworak,¹ Susie Suh,^{1,3} Henri Leinonen,^{1,5} Zhigian Dong,⁶ Antonio F.M. Pinto,⁷ Emily Tom,¹ Jennings Luu,^{1,3} Joan Lee,⁸ Xiuli Ma,⁶ Erhard Bieberich,⁹ Seth Blackshaw,⁴ Alan Saghatelian,⁷ David C. Lyon,¹⁰ Dorota Skowronska-Krawczyk,^{1,11} Marcin Tabaka,² and Krzysztof Palczewski^{1,11,12,13}



AdipoR1-ko causes gradual thinning of the outer nuclear layer

Reference information: *[CI Insight.*

2022;7(4):e156301. https://doi.org/10.1172/jci. insight.156301.

Adiponectin Receptor 1 (ADIPOR1): lipid/glucose metabolism regulator with ceramidase activity.

Mutations have been associated with advanced age-related macular degeneration in Finnish people.

Prebvs

ADIPOR1-ko causes accumulation of ceramides in the retina. leading to inflammation and cell death



Ceramides accumulate in the retina and retinal pigment epithelium/eyecup. Some ceramide species showed 1.5- to 2.5-fold increase in the retina and RPE/eycup of 4-month old AdipoR1-Ko (red) mice.

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Thanks!

