Bifunctional Sphingosine for Cell-Based Analysis of Protein- Sphingolipid Interactions

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Authors

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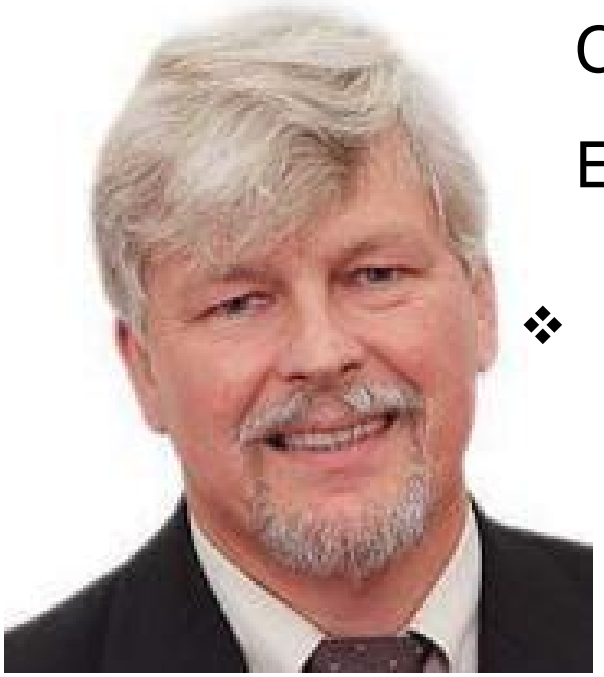


Carsten Schultz

EMBL Heidelberg/OHSU

- Developing tools to help understanding the cell biology of signaling networks

Per Haberkant

Senior Scientific Officer

at EMBL

Background

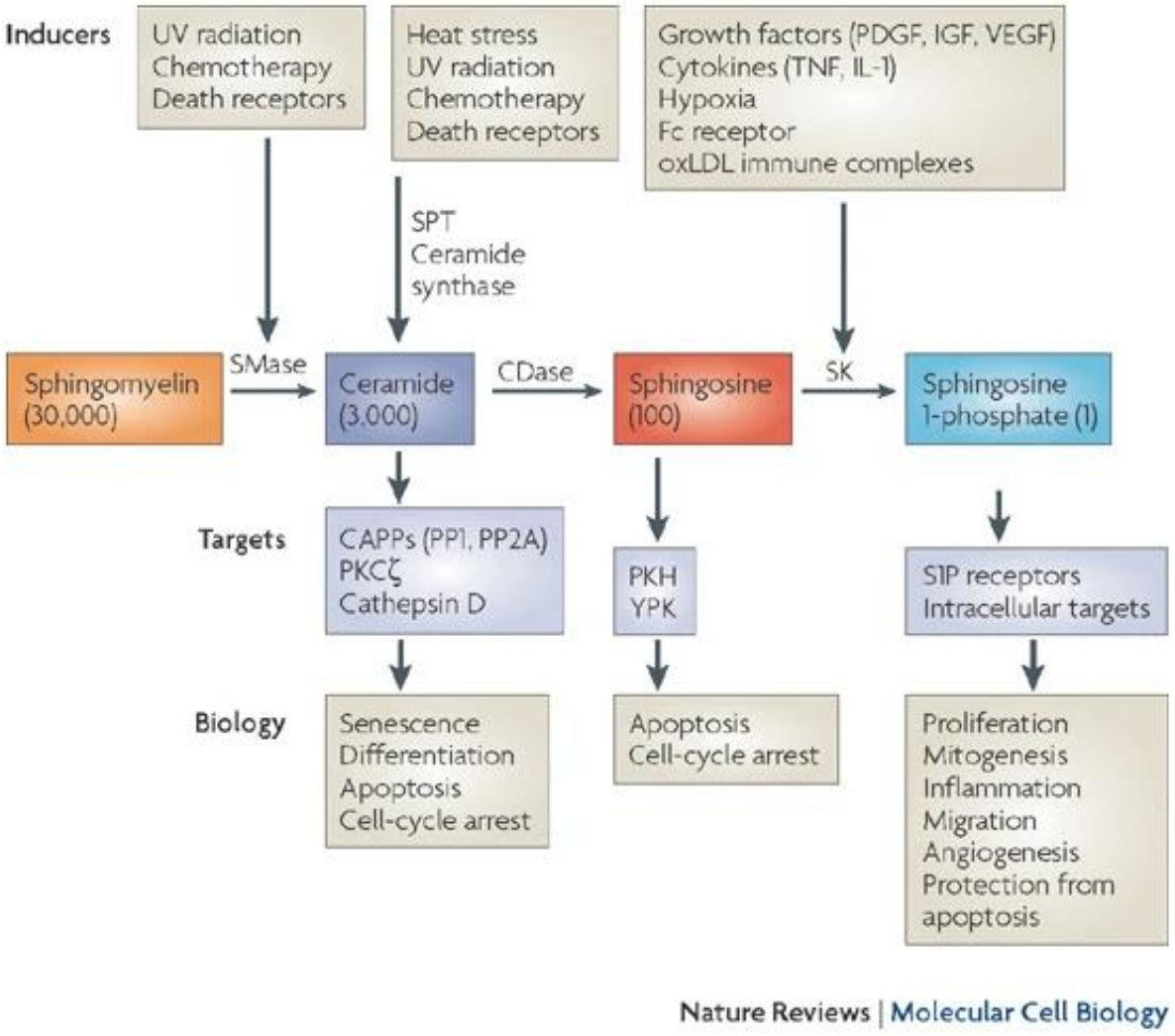
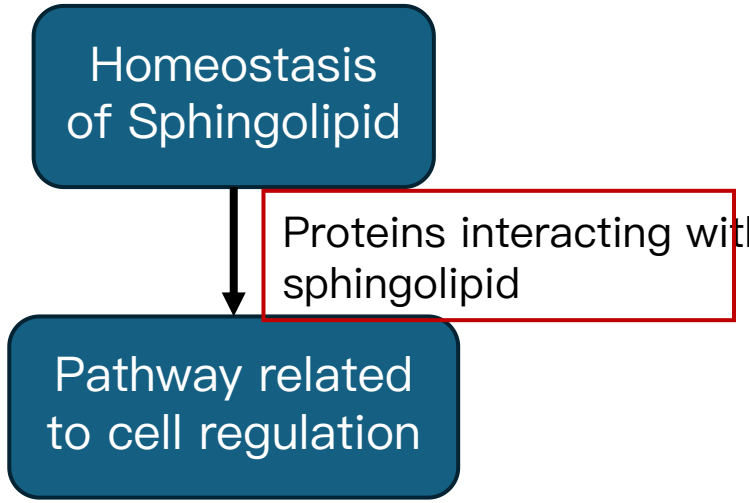


Fig1. The overview of sphingolipids in biology

- *"sphingolipids are involved in central cell-signaling pathways such as the **regulation of cell proliferation**, **apoptosis, senescence**, and **intracellular trafficking**."*

------Yusuf A. Hannun



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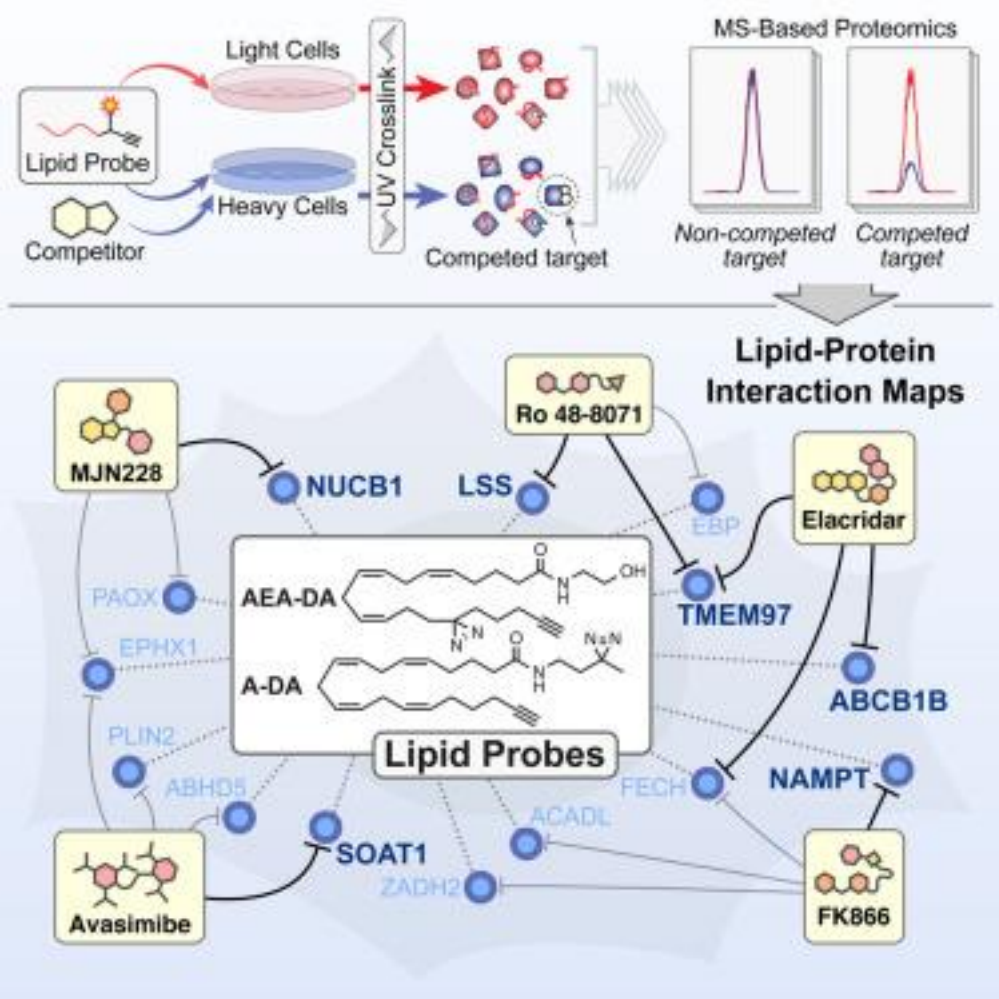

Fig1. Graphical abstract of methods for mapping of the protein interacting with Lipid

- *"We expand this technology to the class of **sphingolipids**. Aiming for **the visualization and proteomic profiling** of proteins-phingolipid complexes in living cells"*

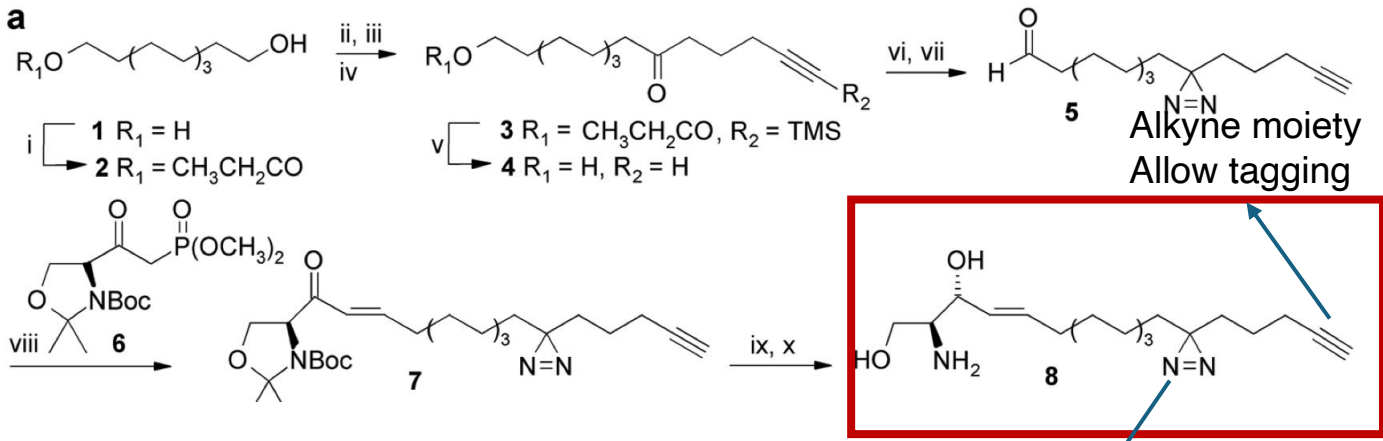

Fig2.Synthesizing process of the pacSph

Validation of pacSph contributing to the synthesis of other Sphingolipid in cellular environment

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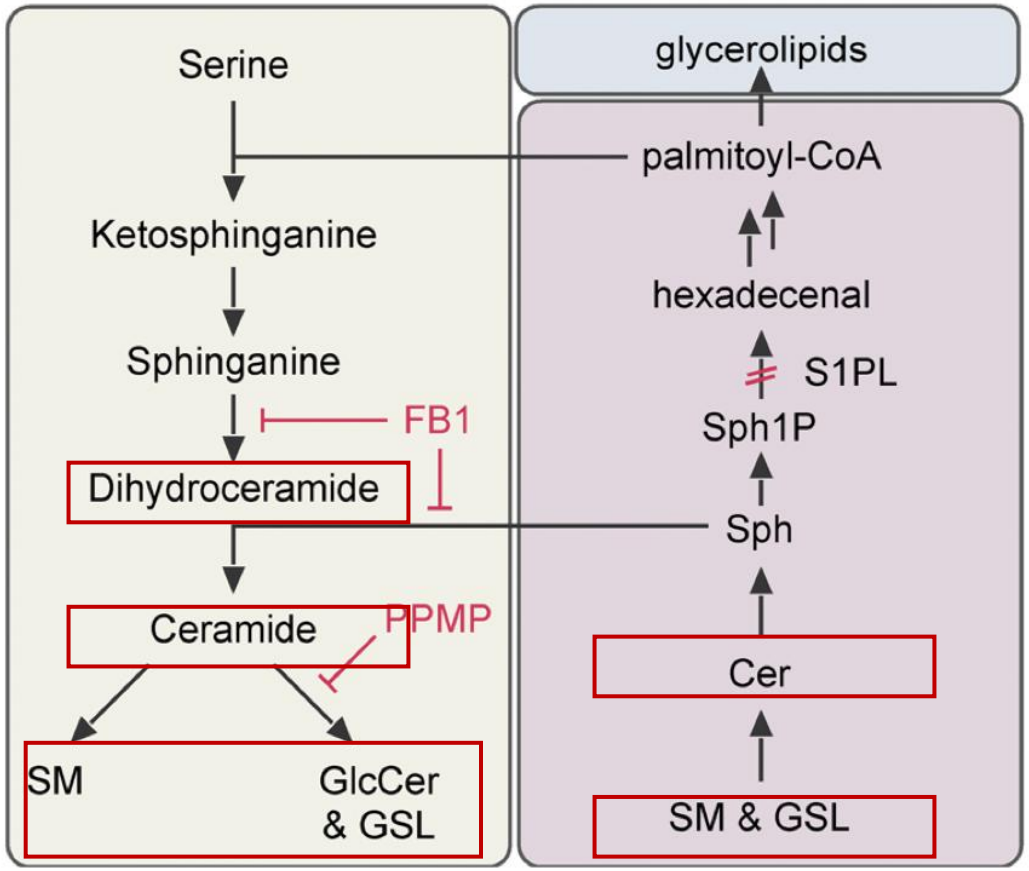

Fig1. Simplified representation of the biosynthesis (highlighted in yellow) and degradation (highlighted in red) of sphingolipids.

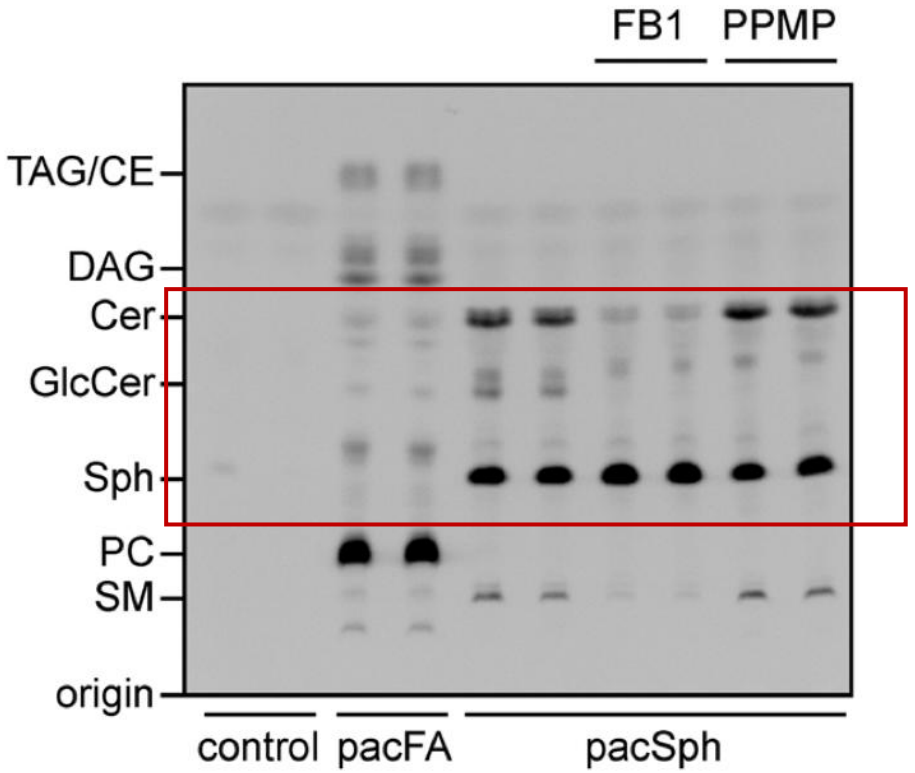

Fig2. Analysis of lipid extracts from S1PL–/– cells that were fed with pacFA or pacSph

- Feeding pacSph to S1PL$^{-/-}$ MEFs yielded **bifunctional sphingolipid species**, while pacFA gave rise to **bifunctional glycerolipids**.

The dynamic metabolic pathway of pacSph in cells

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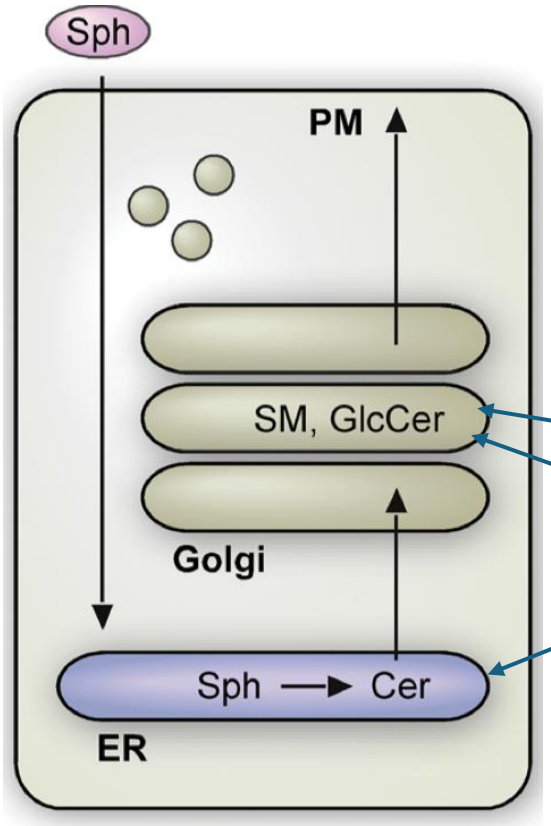

Fig1. Schematic representation of sphingolipid biosynthesis

- Rapid conversion of pacSph in the **endoplasmic reticulum** and subsequent processing in the **Golgi apparatus**
- There is a **time-dependent** metabolism of pacSph into various sphingolipids

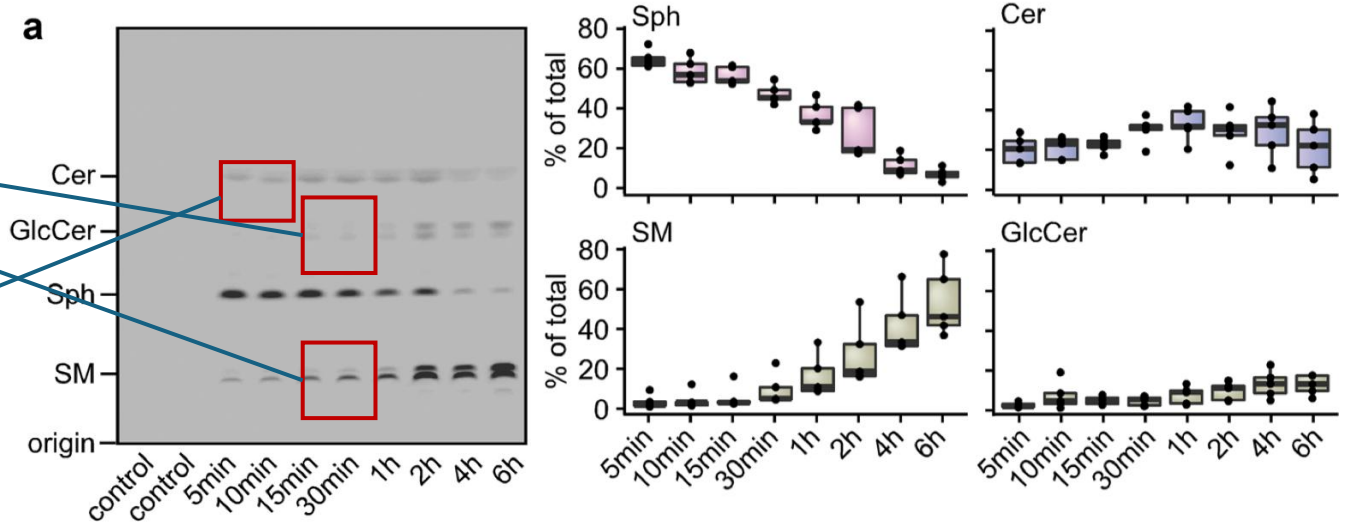

Fig2. Quantification of pacSph labeled lipids five independent experiments

Imaging of Protein-Lipid complex

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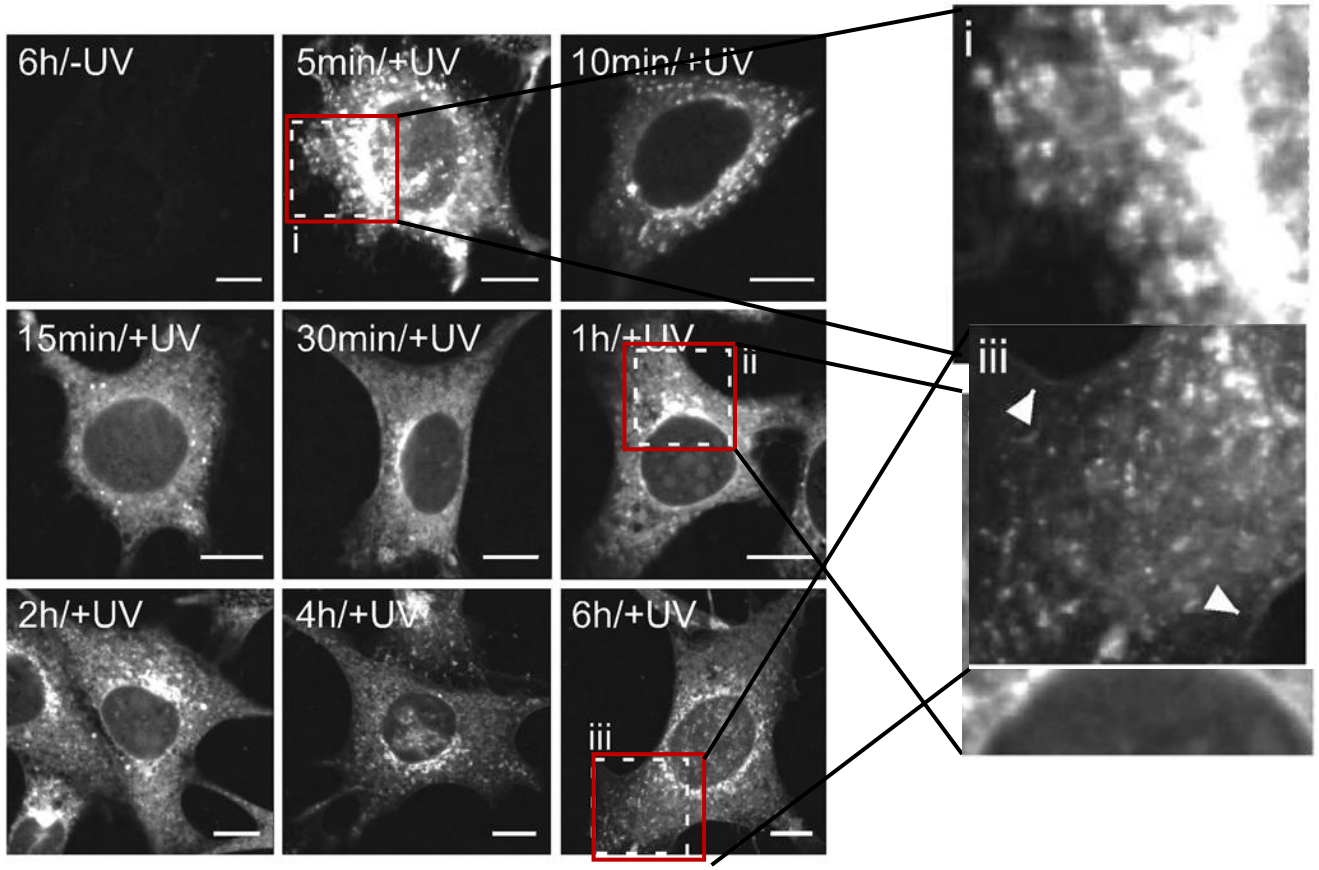

Fig1. Protein–sphingolipid complexes were captured by UV-light and then visualized by click reactions with Alexa 488 azide

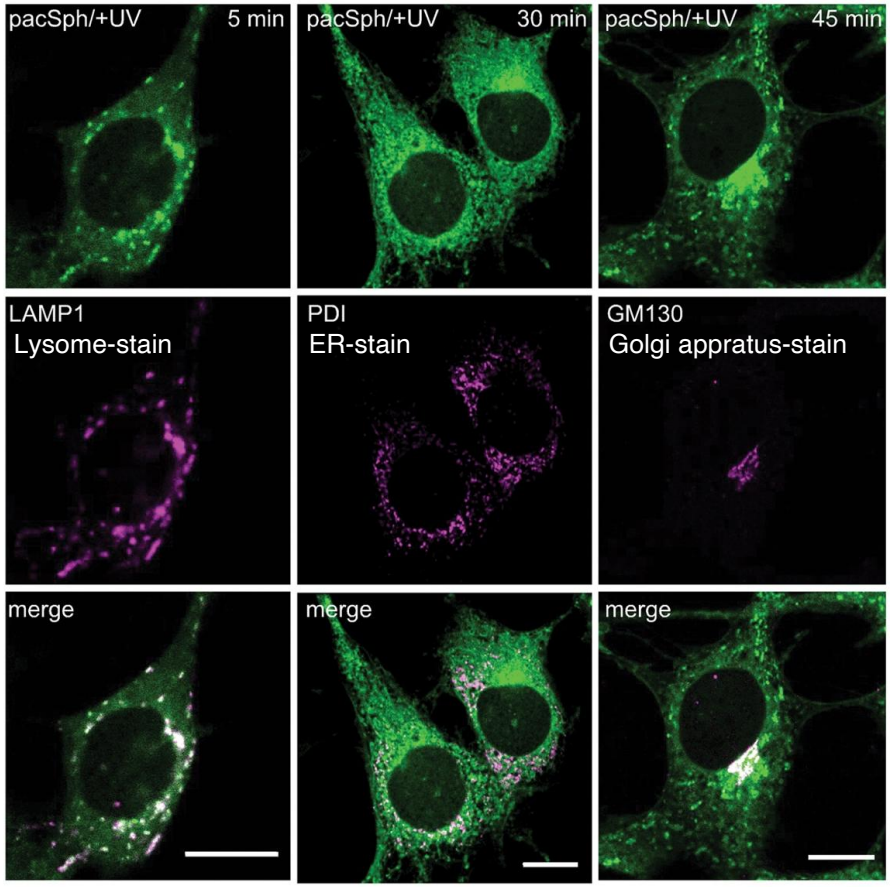

Fig2. representative images of protein-sphingolipid complexes (green) and the respective organelle marker (magenta)

Imaging of Protein-Lipid complex

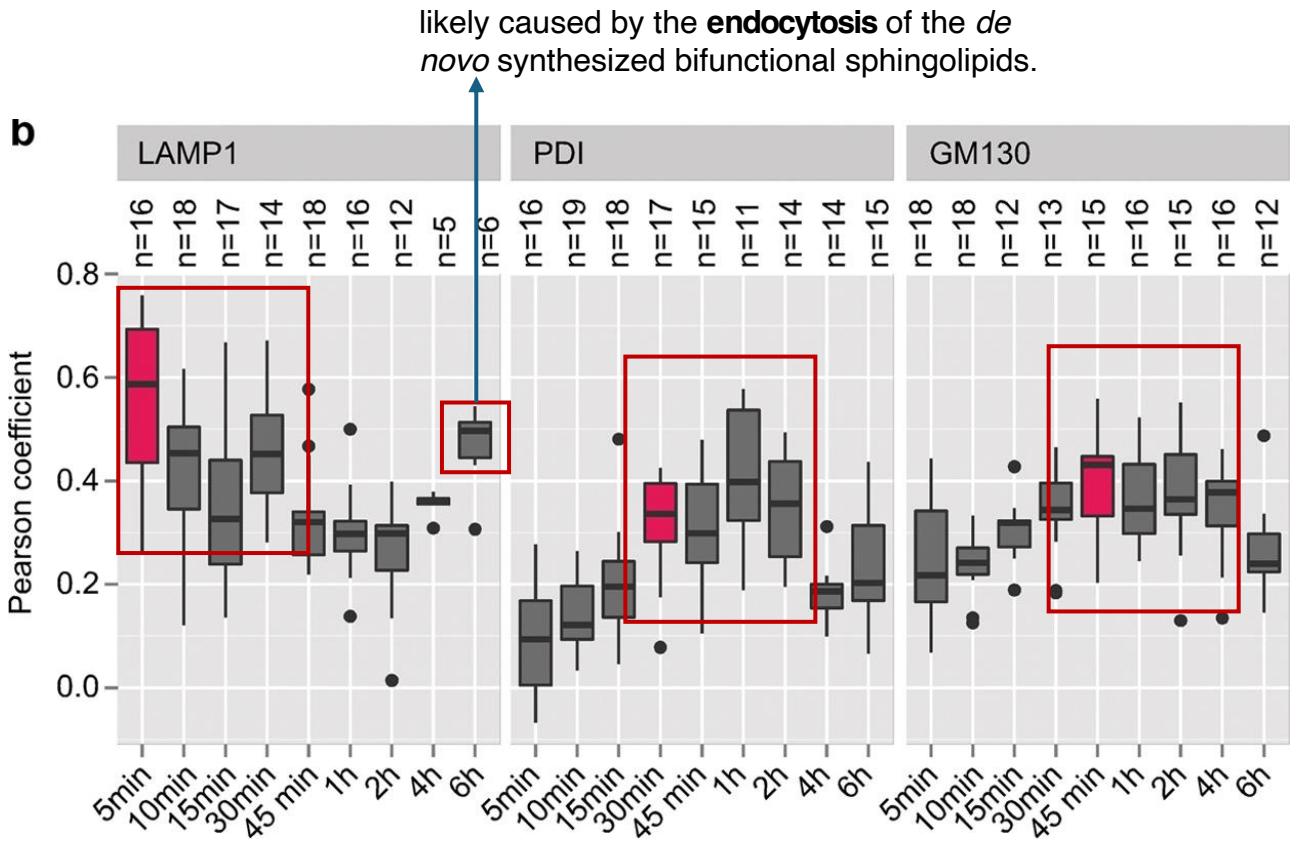

Fig1. Measure for the colocalization of protein–sphingolipid complexes with LAMP1, PDI, and GM130

- The coefficients for LAMP1, PDI, and GM130 change over time, reflecting the dynamic movement of pacSph from **lysosomes to the ER and then to the Golgi apparatus**.

Identification of protein-lipid complex

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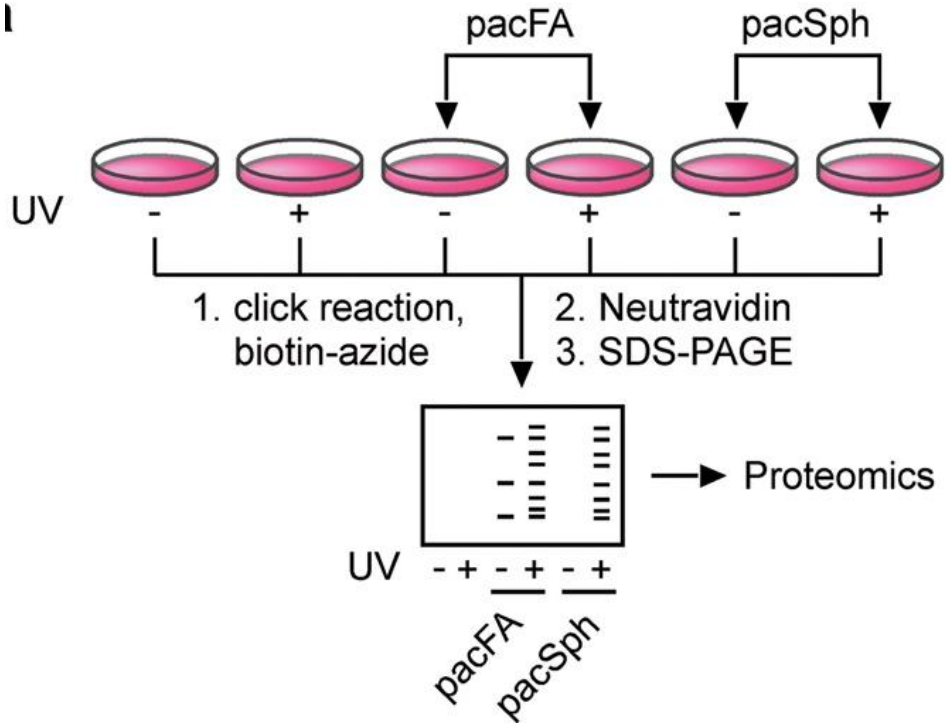

Fig1. Schematic representation of the identification of protein–lipid complexes

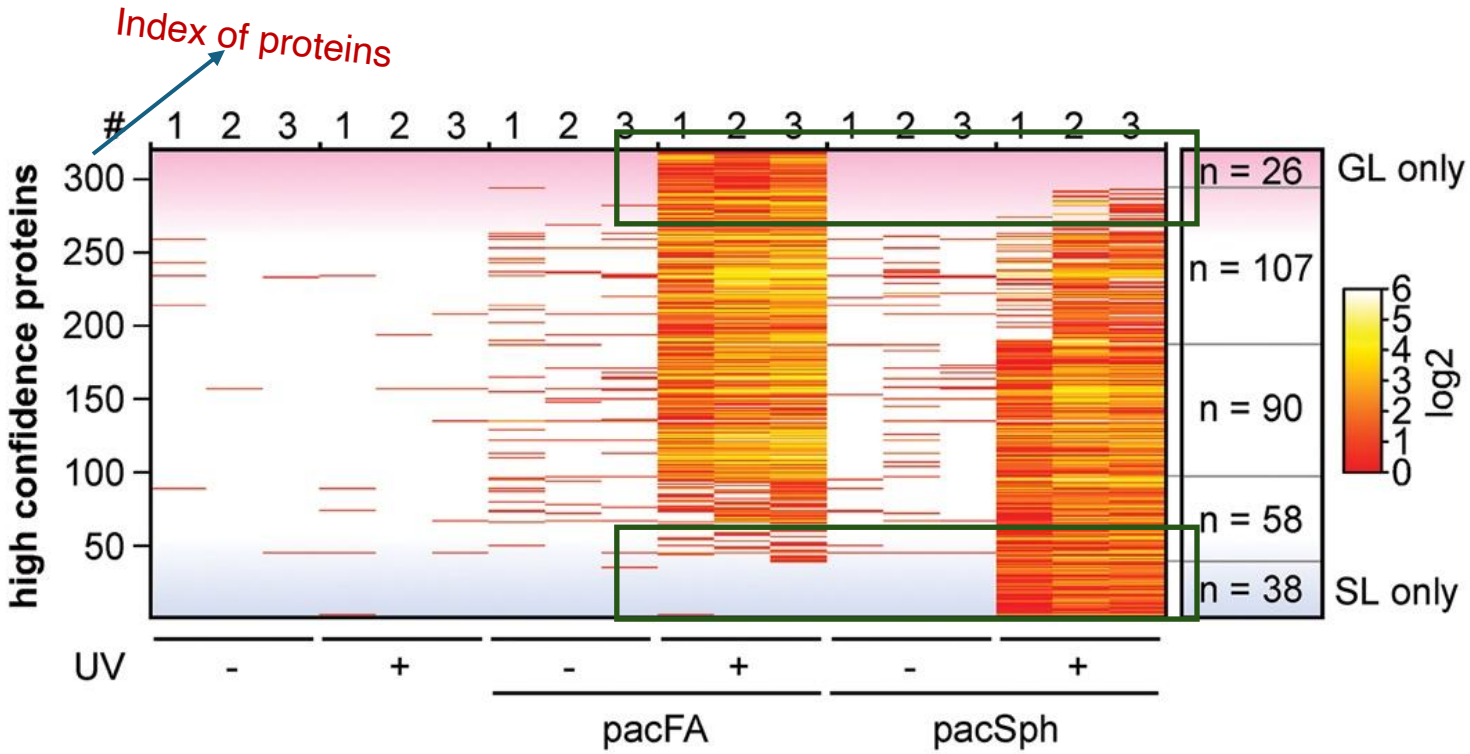

Fig2. The abundance of the proteins identified by pacFA/+UV and pacSph/+UV treated cells are displayed as a heat map.

- The proteins forms different complex with pacSph and pacFA, and **pacSph selectively** captured the protein that can interact with the Sphringolipid

The localization of the interaction

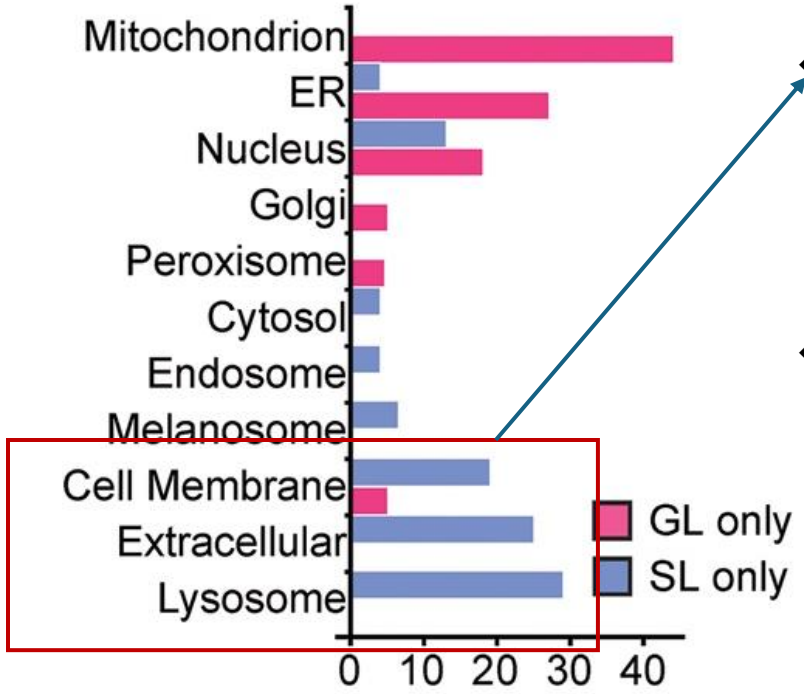

Fig1. Localizations of high confidence proteins according to GO-annotations of proteins

- Proteins identified in pacSph/+UV treated cells were enriched in **lysosomal and plasma membrane**
- The pacSph particularly targets cellular membranes and organelles **involved in pacSph metabolism**

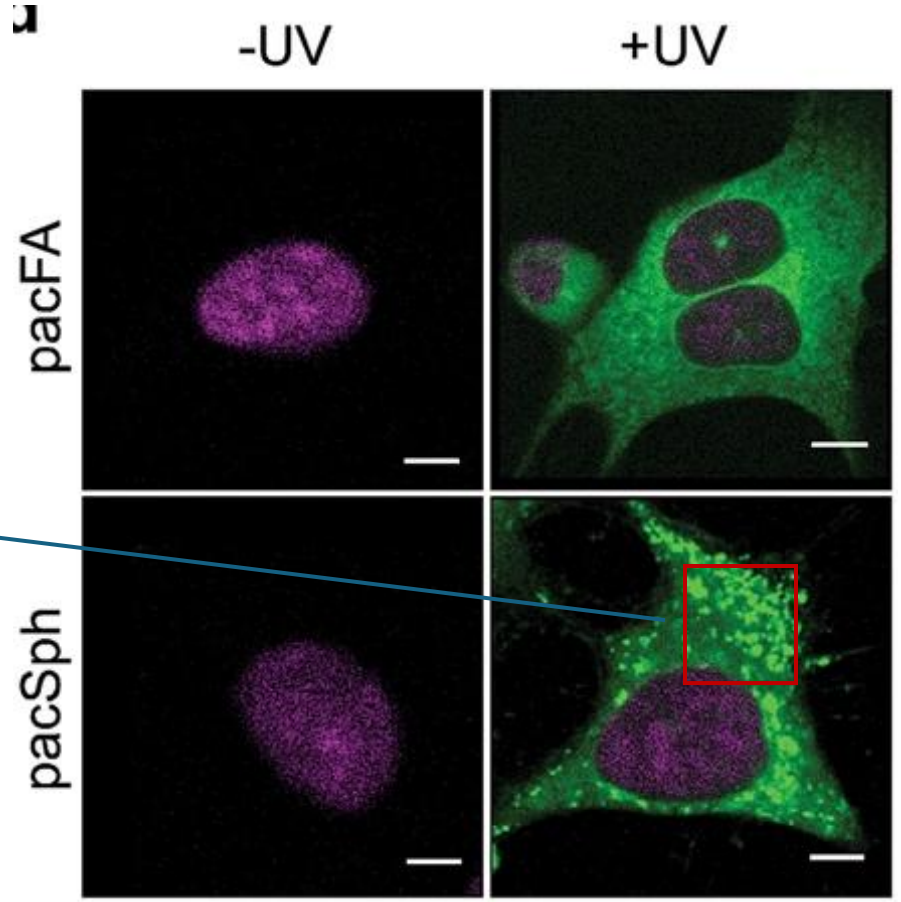

Fig2. Observation of the Colocalization

Identification of noval Sphingolipid-handling machinery

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- *"Many of the complexes identified from pacSph/+UV treated cells were found to have a **functional relationship to sphingolipids**"*

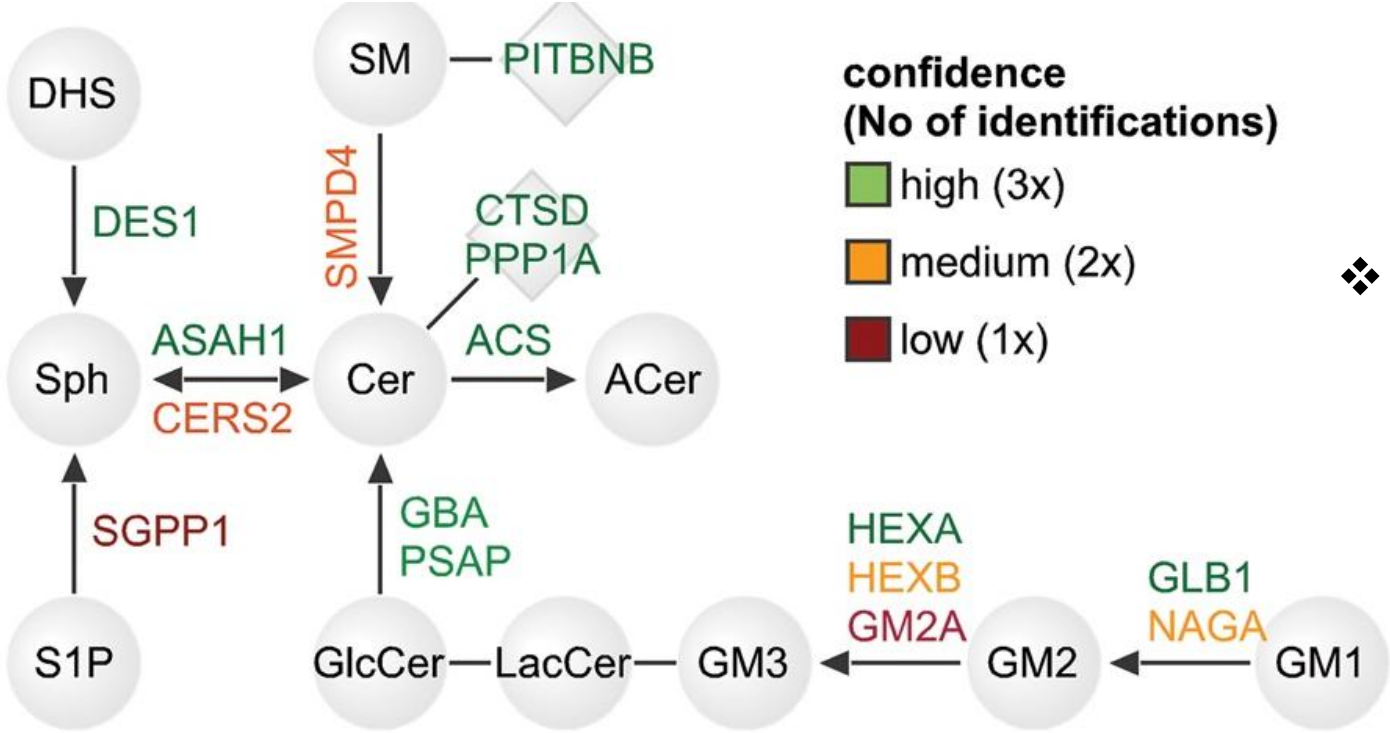

Fig1. Overview of the sphingolipid biosynthetic pathway and sphingolipid handling machinery that was identified from pacSph/+UV treated cells

- The pacSph also has the potential to track down **novel sphingolipid-handling machinery**

Validation of protein-lipid interaction

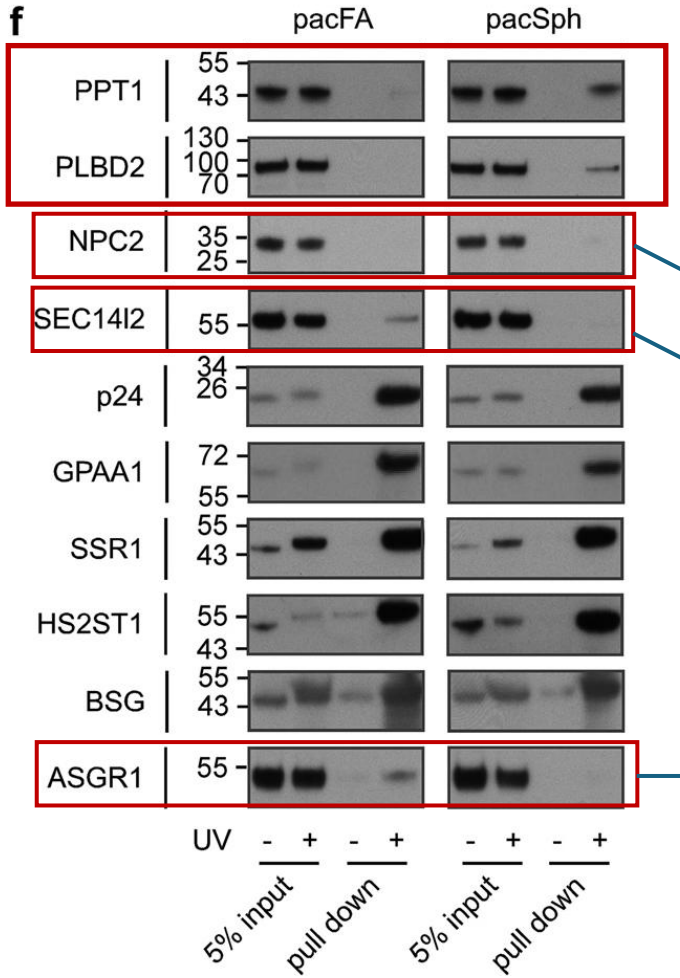

Fig1. Experimental validation of sphingolipid-binding proteins.

- *"In line with our proteomic identification, we observed exclusive binding of the **lysosomal candidate proteins PLBD2 and PPT1** to sphingolipids"*

A lysosomal cholesterol transfer protein

Can bind to the glycerophospholipids

- ***p24 and GPAA1** as well as **SSR1, HS2ST1, and BSG** gave rise to cross-linked products with both sphingolipids and glycerolipids*

Lack sphingolipid- binding motif of p24 and GPAA1

Validation of protein-lipid interaction

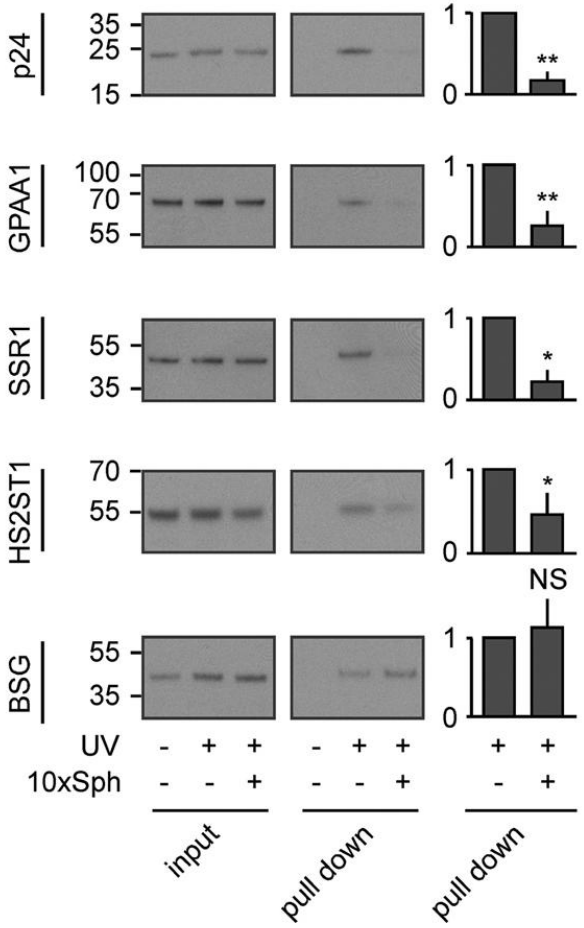

Fig1. Proteins tested for sphingolipid-binding ability under UV irradiation and in the presence of excess natural sphingosine

- Adding natural sphingosine can decrease the interaction between pacSph and proteins
- FB1 also decrease the interaction between pacSph and proteins because this FB1 can inhibit the synthesis of sphingolipid

P24, GPAA1, SSR1, HS2ST1 tend to bind the **intermediate** of sphingolipid synthesis

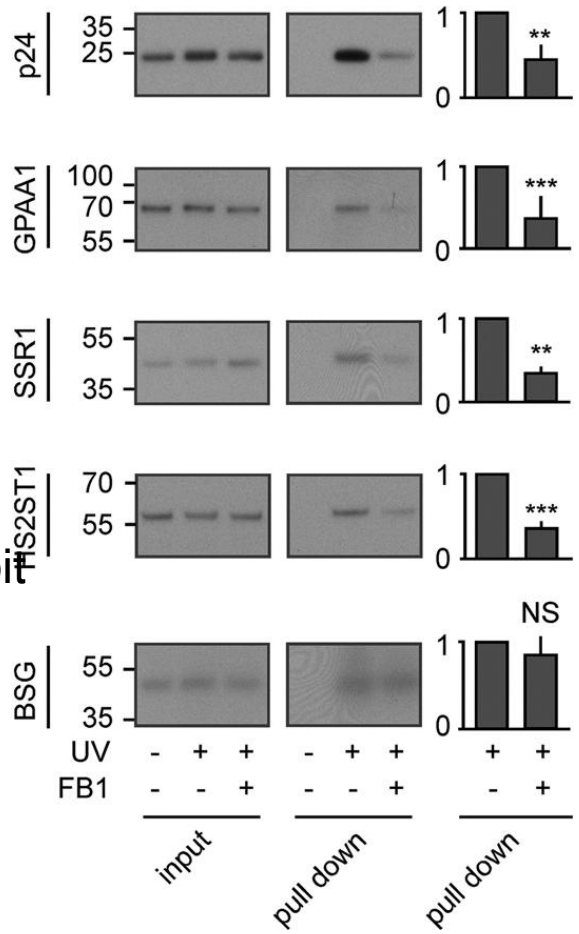

Fig2. Proteins tested for sphingolipid-binding ability under UV irradiation and in the presence of FB1

Conclusion and limitation

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Conclusion

- **Synthesis of the Bifunctional sphingosine**
- **The metabolic fate of pacSph**
  - The pacSph metabolism occurred in the **endoplasmic reticulum and Golgi apparatus**
  - There is a **time-dependent** metabolism of pacSph into various sphingolipids
- **Chemoproteomics profiling of protein-lipid complex**
  - pacSph can **selectively capture** the protein that can interact with the Sphringolipid
  - Proteins captured by pacSph are enriched in **lysosomal and plasma membrane**
- **Validation of protein lipid interaction**
  - The **lysosomal protein** binds to Sphingolipid
  - The P24, GPAA1, SSR1, HS2ST1 tend to **bind the intermediate** of sphingolipid synthesis

The number of the proteins is limited

Did not futher prove the function of the protein–lipid complex

The synthesis is not sterospecific

Questions

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If we want to see the function of the sphingolipid-protein complex in the cell regulation, What further research can we conduct?

In the last three weeks we read some paper that published on the *Cell, Science, Nature*. What do you think the reason this paper can not be published on these journal