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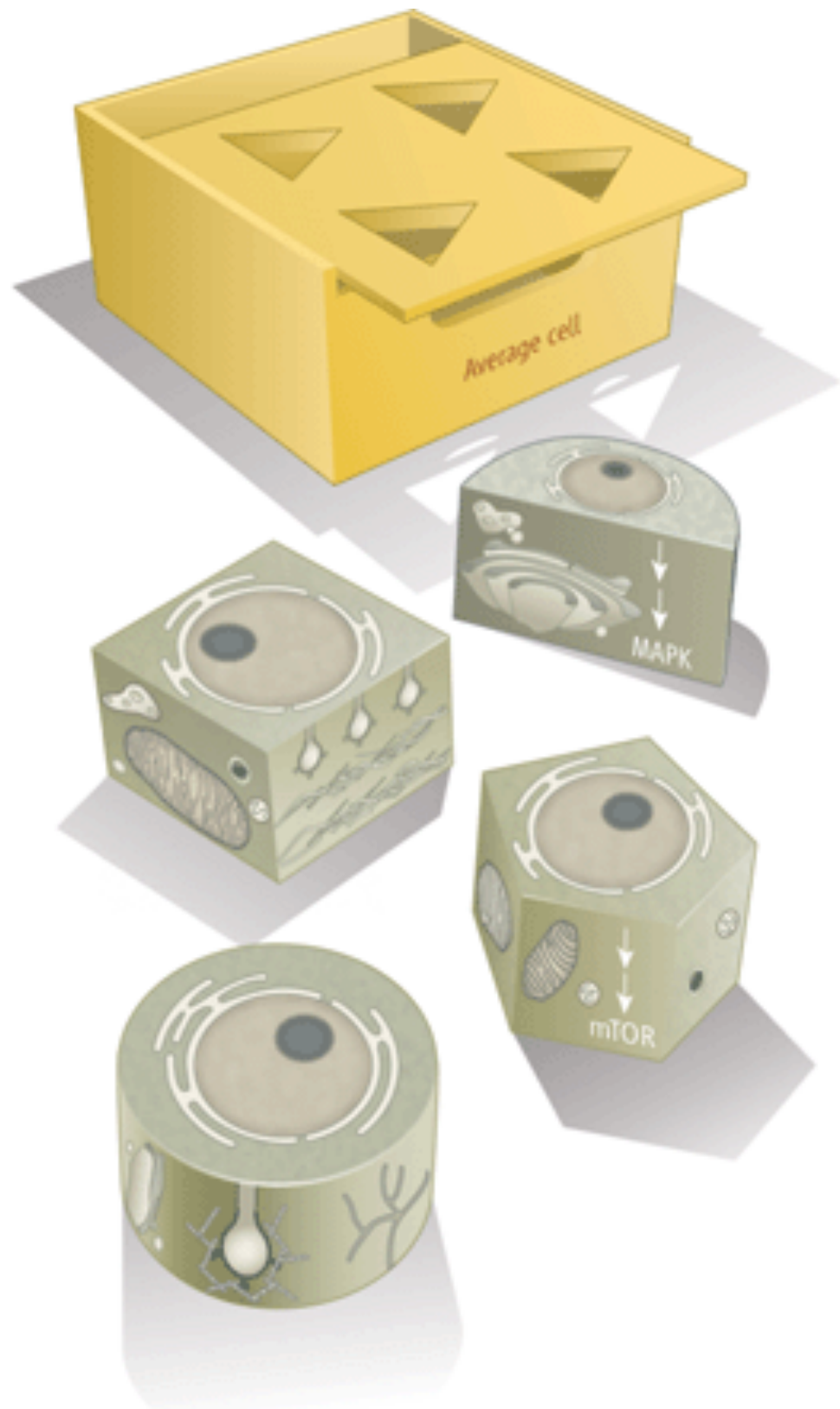
Chimie Biologique II

Biological Chemistry II

BIO-213

Teacher

Giovanni D'Angelo, IBI

# Lecture 12

# Single-Cell and Spatial Metabolomics

# Single-cell biology



A single cell can be regarded as the smallest functional biological unit and its chemical activities can provide unique insights into biological processes.

Different biological processes continuously occur in single cells as they move, divide, communicate, and respond to their individual chemical microenvironments.

Even cells with identical genotypes display different chemical phenotypes as a result of cellular dynamics and unique microenvironments.
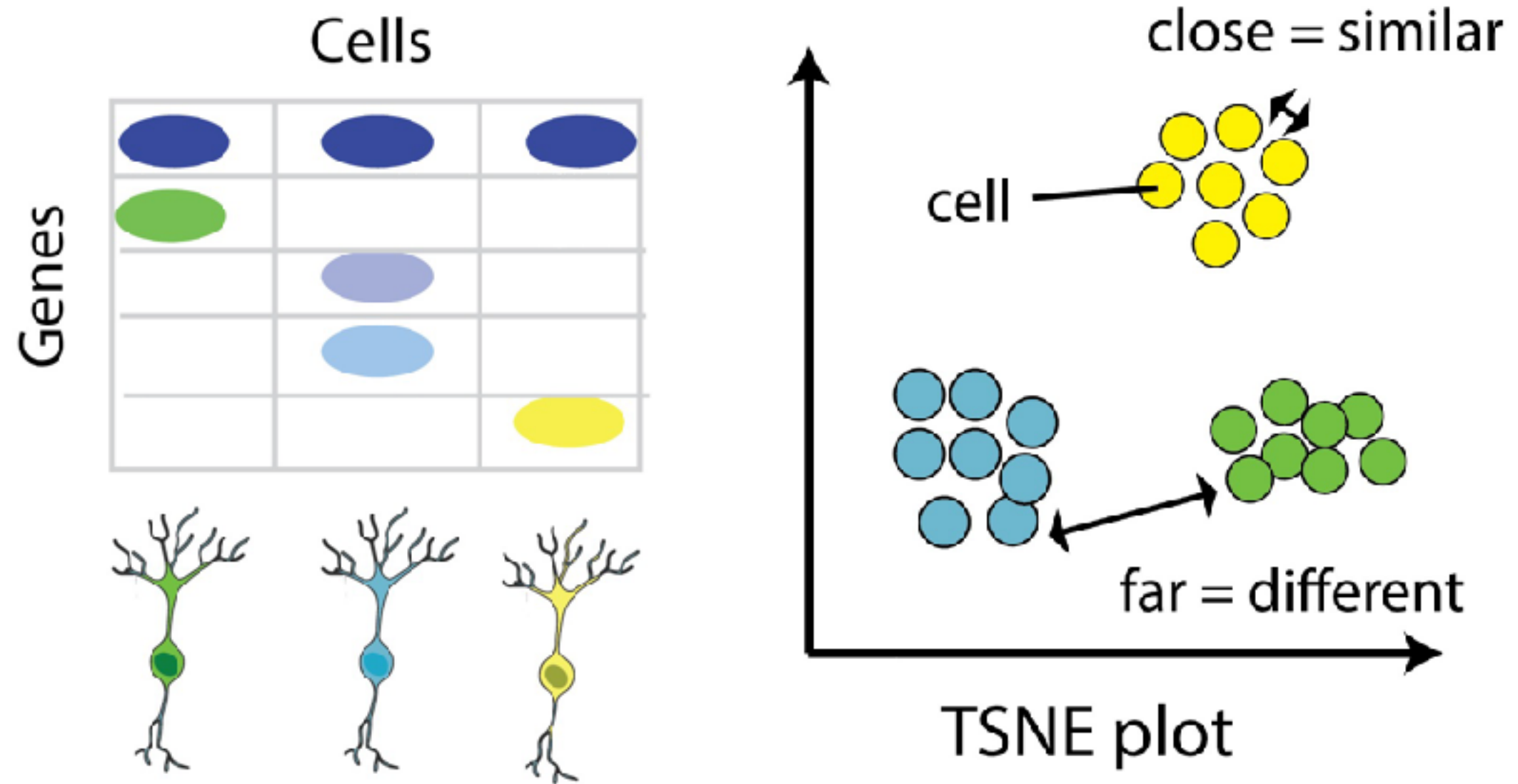
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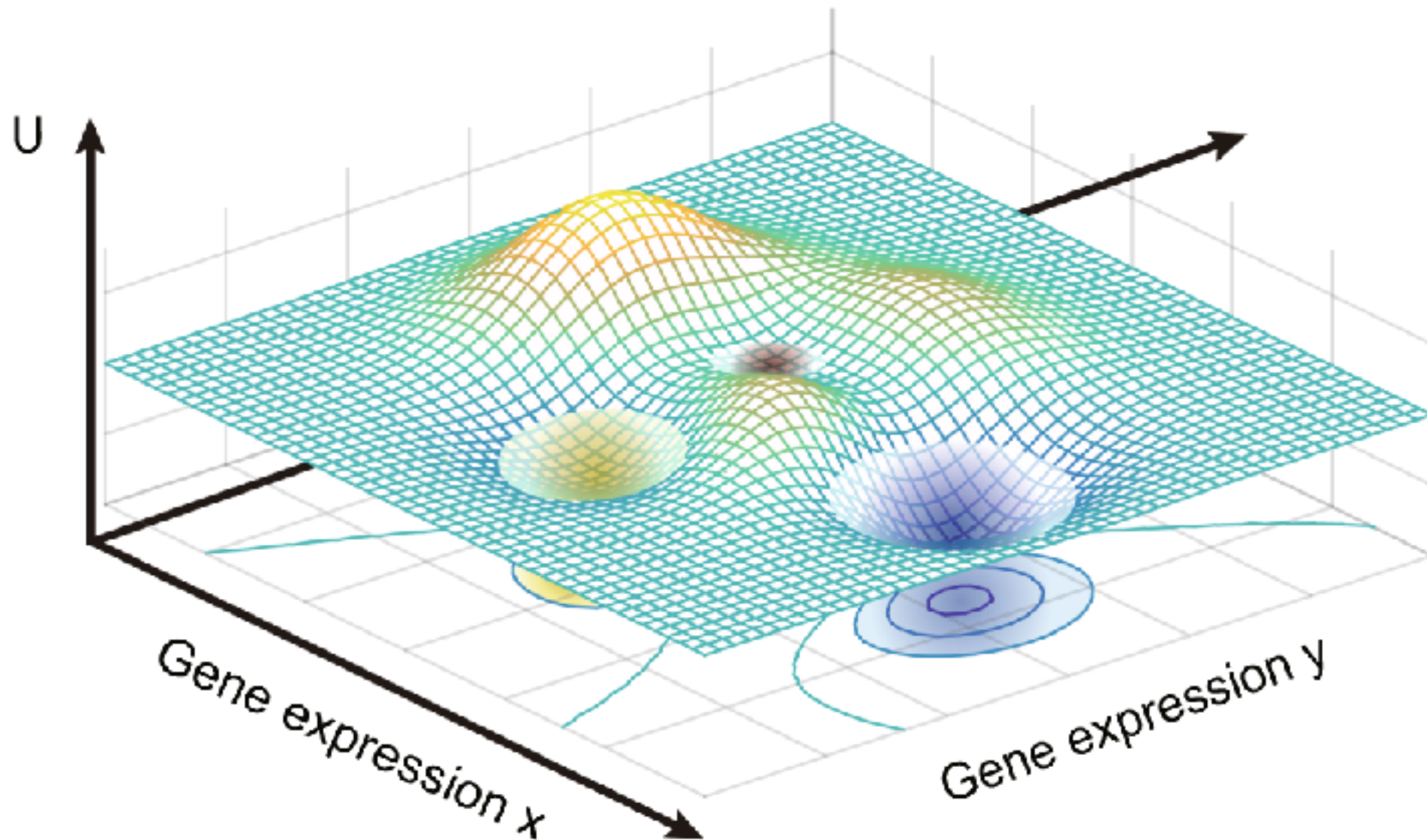


Regulatory circuits that generate stable phenotypic states have been selected by evolution to account for multicellularity
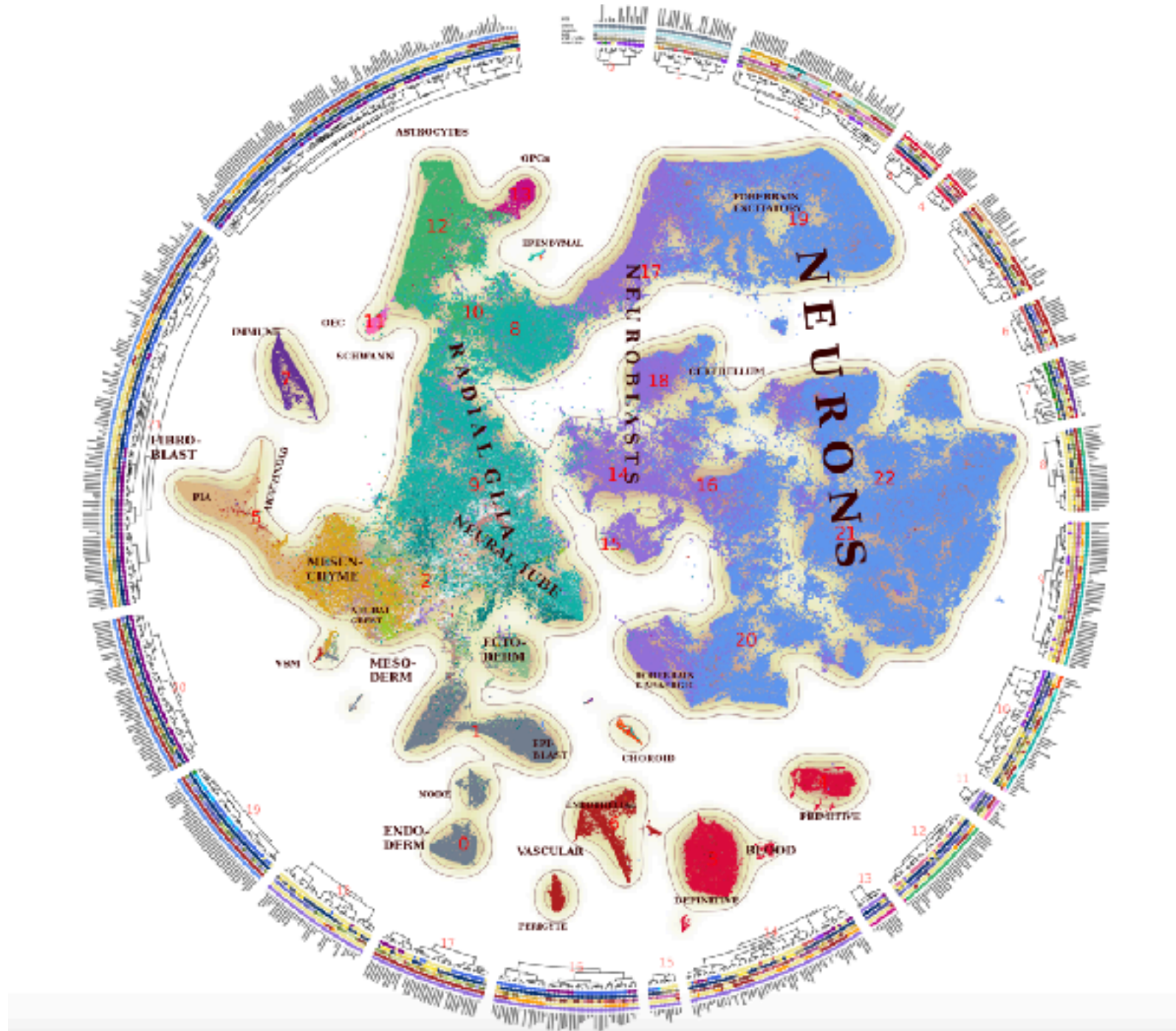
# Single-cell biology



Cells

Genes
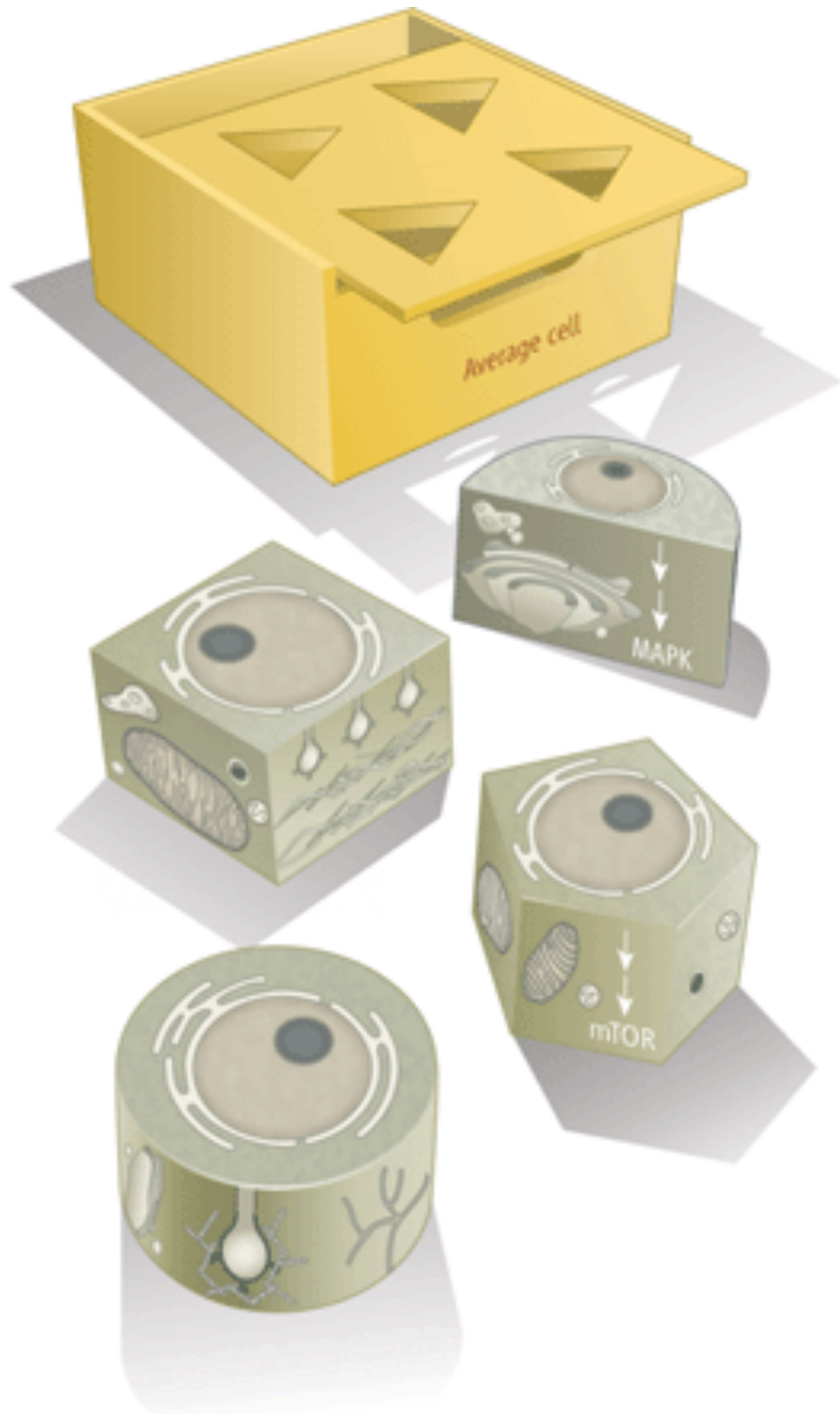
close = similar

cell

far = different

TSNE plot

t-distributed stochastic neighbor embedding

# Cell Atlases



Our current challenge is to understand how the different cells that compose our tissues emerge and get organised to mediate multicellular life.
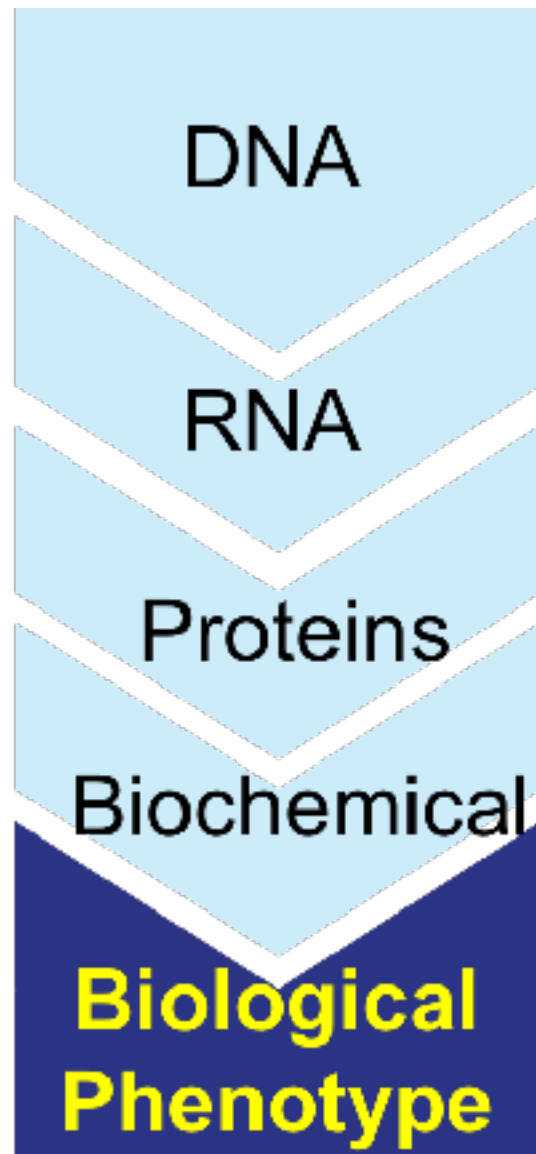
# Single-cell biology



Single cell Biology aims to reveal

• Cellular heterogeneity (Cell Types)
• Differentiation Trajectories
• Cell states
• Regulatory networks
• Rare cell States

What can we profile in single cells?

# Single-cell biology

DNA → Genomics

RNA → Transcriptomics

Proteins → Proteomics

Biochemical → **Metabolomics**

**Biological Phenotype**

**Metabolomics** is a field of study that aims to measure a large amount of metabolites at one time. Metabolites are small biomolecules, such as amino acids, sugars, and lipids, which constitute precursors, intermediates, and products in cellular processes

Can can we profile the metabolites in single cells?

# Single-cell biology

**Mass spectrometry** (**MS**) is an analytical technique that measures the mass-to-charge ratio of ions. The results are typically presented as a mass spectrum, a plot of intensity as a function of the mass-to-charge ratio. Mass spectrometry is used in many different fields and is applied to pure samples as well as complex mixtures. MS is used to identify and to quantify metabolites after optional separation. Identification leverages the distinct patterns in which analytes fragment which can be thought of as a mass spectral fingerprint. MS is both sensitive and can be very specific.
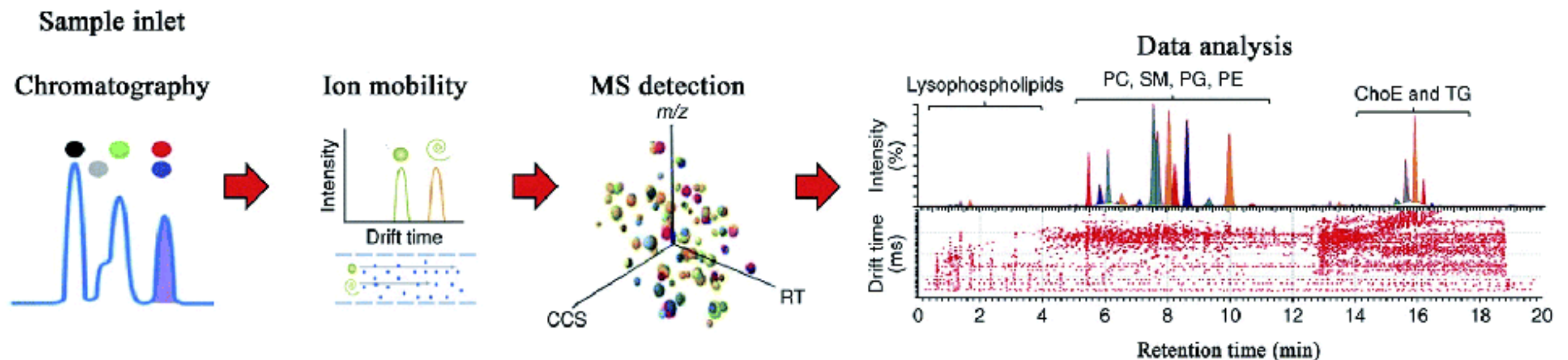


With high sensitivity and specificity, wide molecular coverage, relative quantitation, and structural identification capabilities, MS is becoming an important tool for **Single-cell metabolomics.** This, by the way, presents many challenges due to the limited sample volume, low analyte amounts, and rapid turnover rates of the cellular metabolome. Most single cell metabolomics studies are, thus performed using MS in a shotgun-like approach, preferably with high mass resolution.
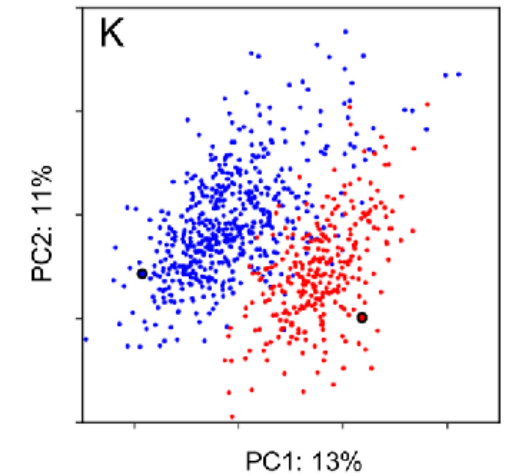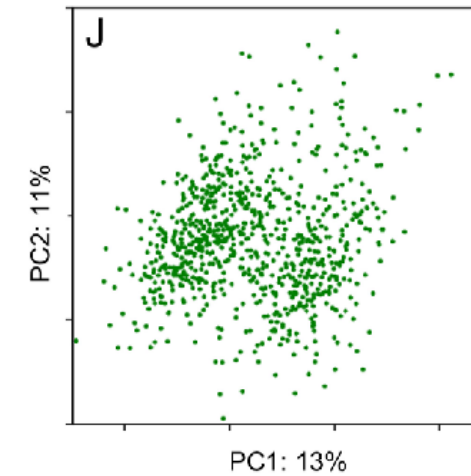
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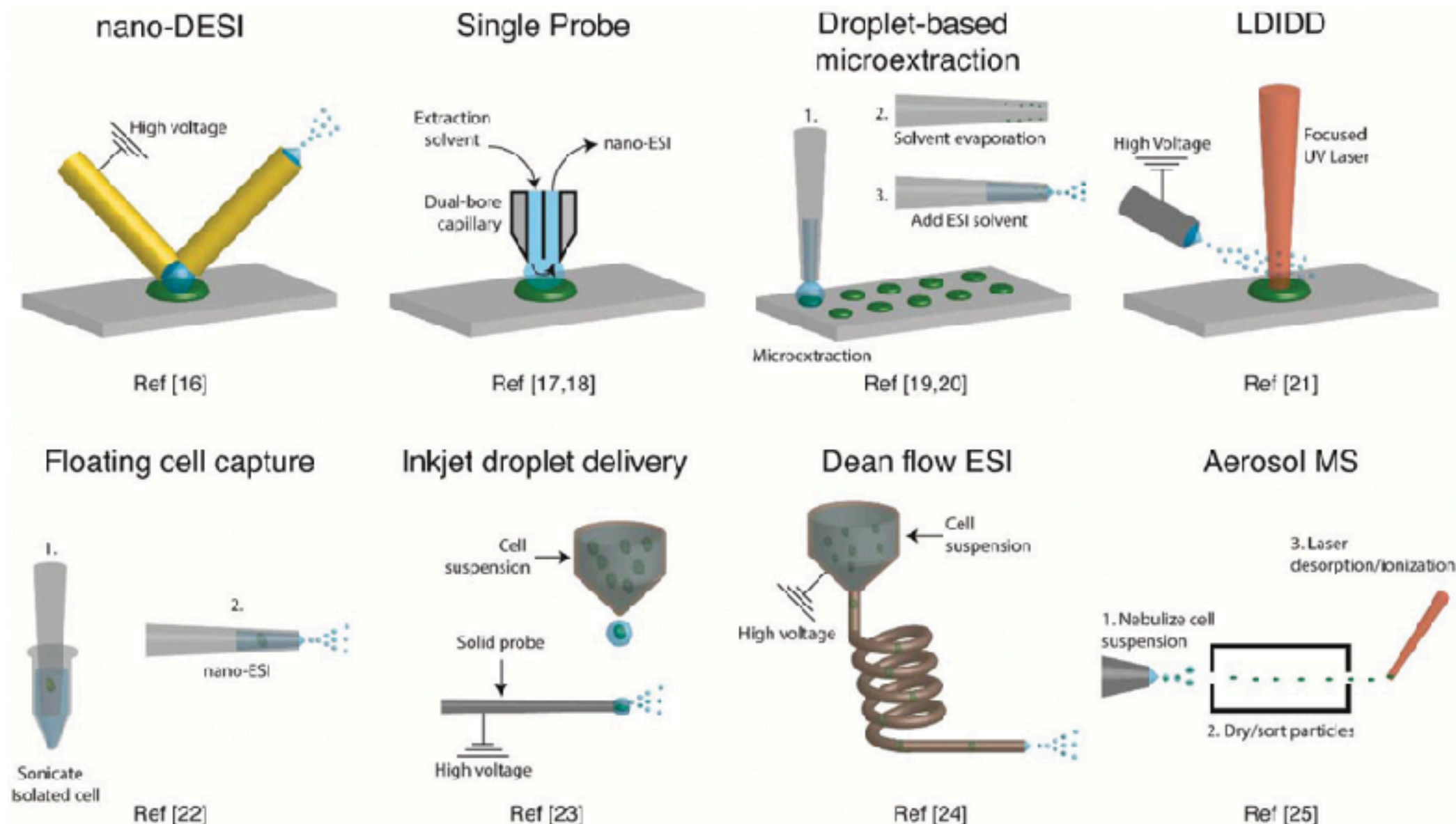
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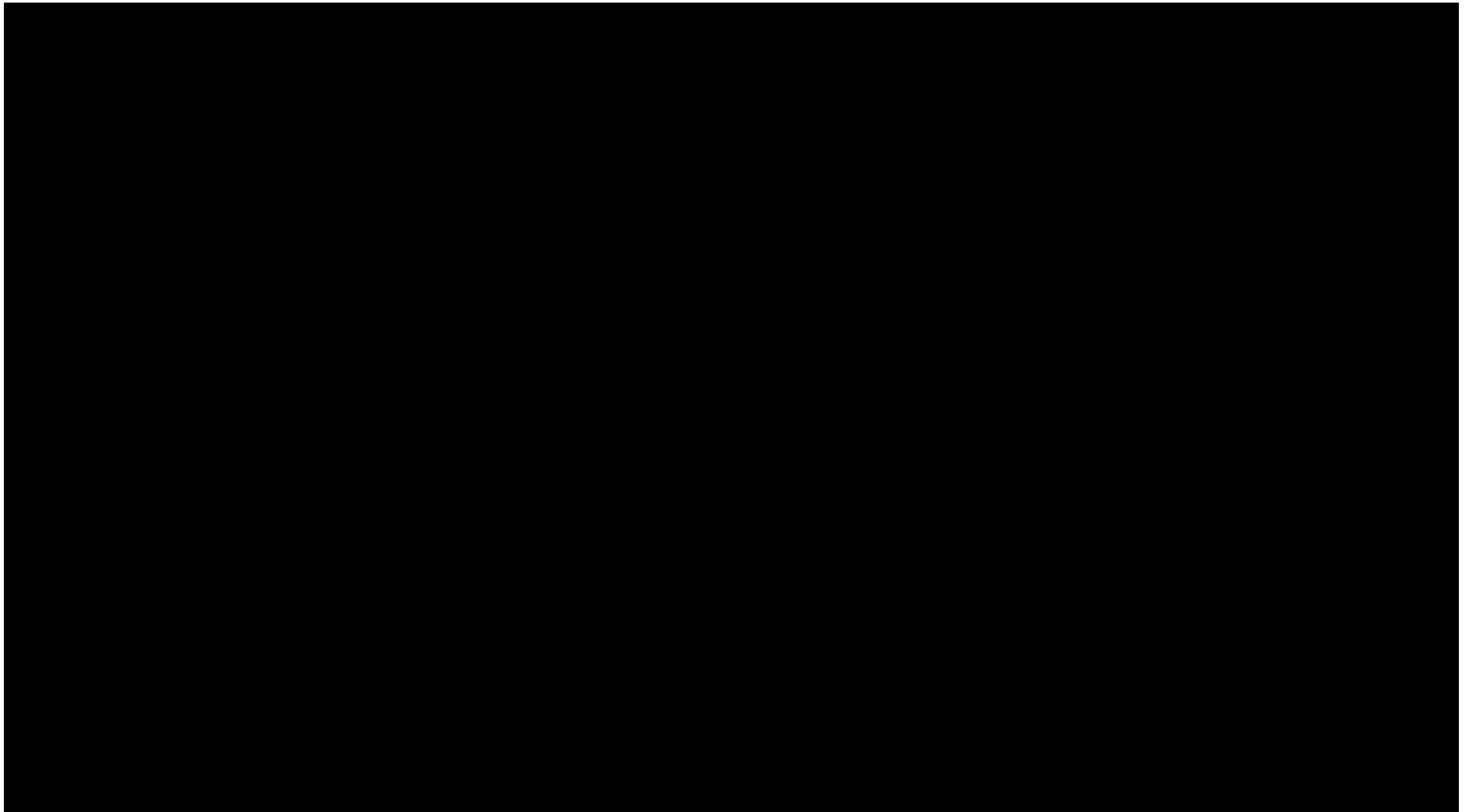
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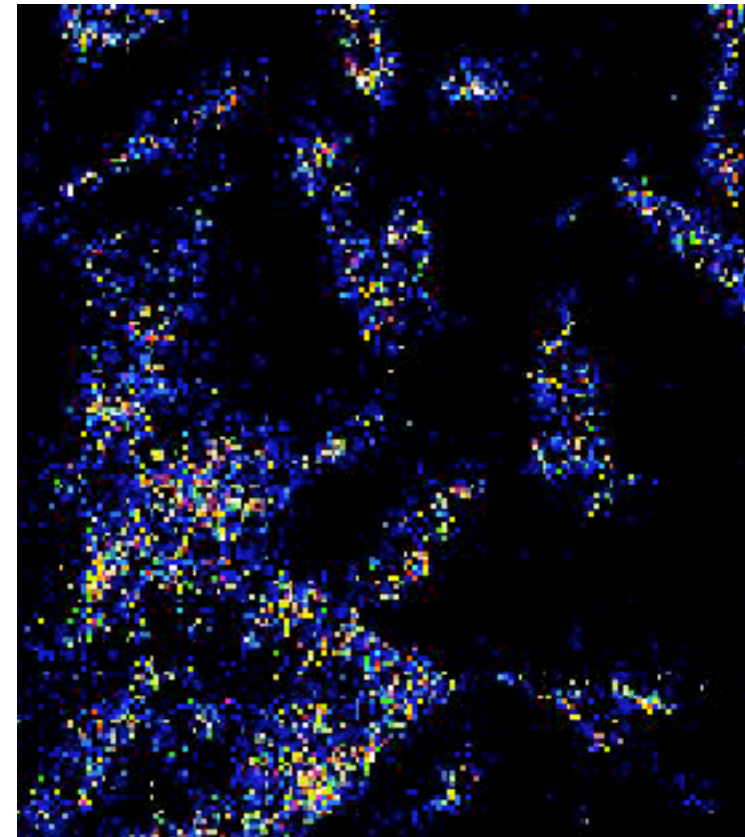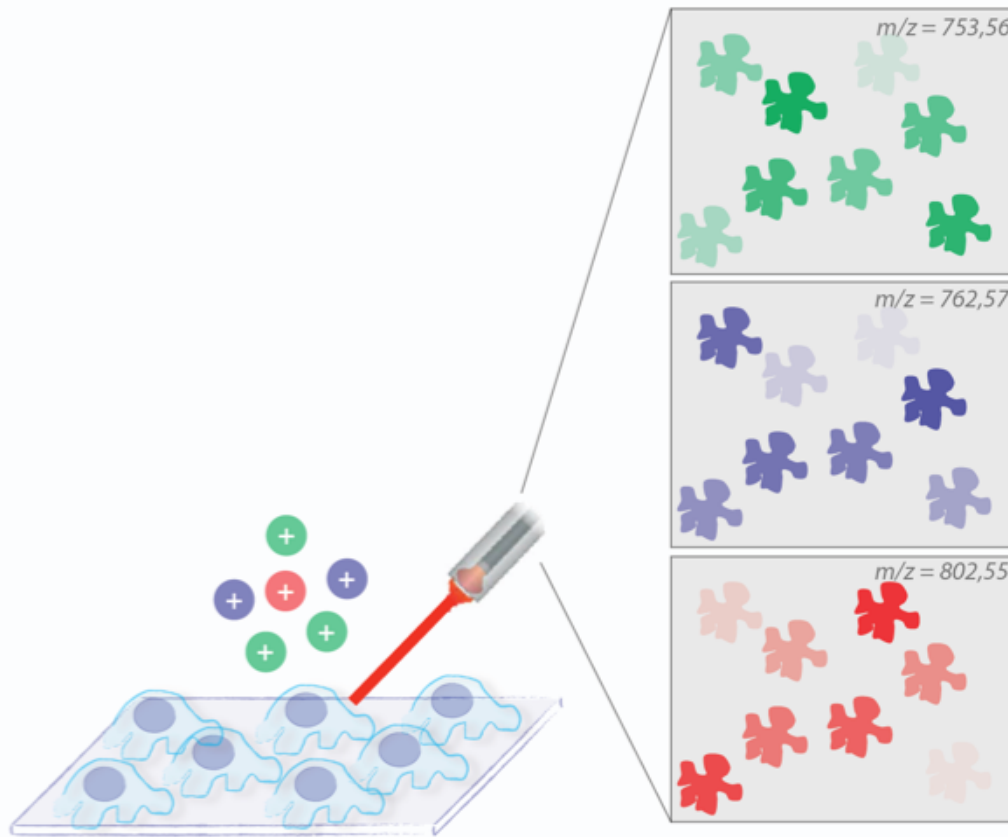


Single Pixel Mass Spectrum
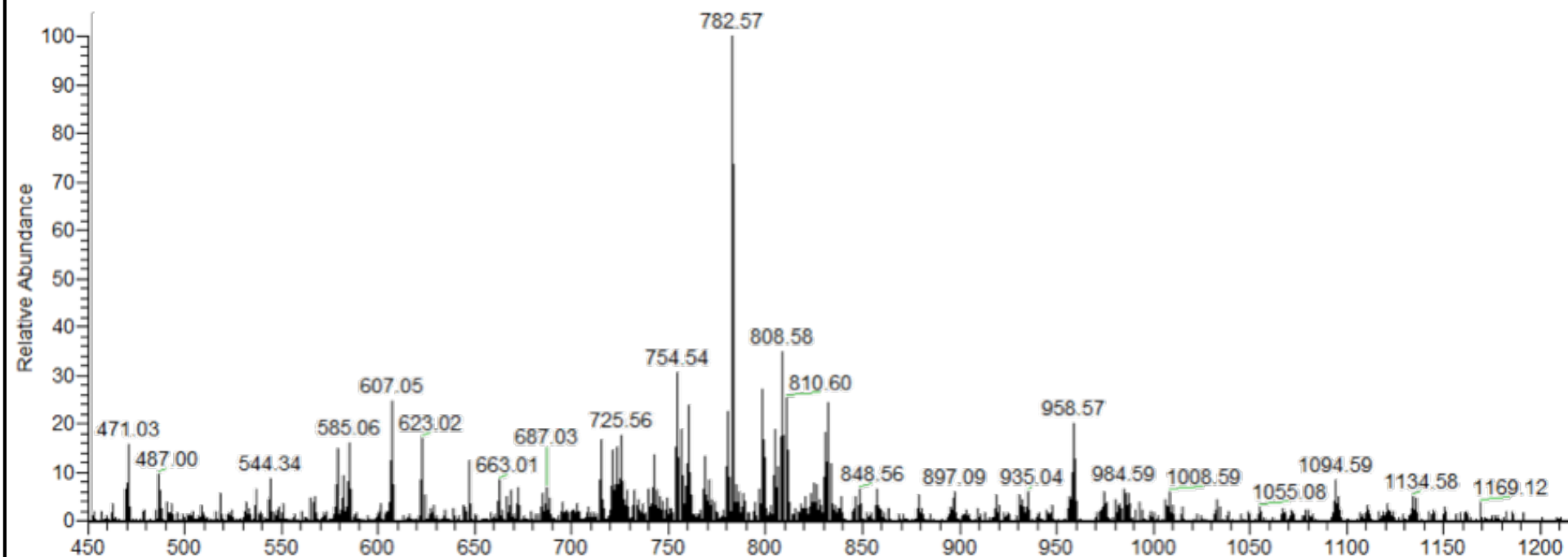
# Single-cell Metabolomics

## 2012

### Single Cell Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging

Numerous compounds including small metabolites such as adenine, guanine, and cholesterol as well as different lipid classes such as phosphatidylcholine, sphingomyelin, diglycerides, and triglycerides were detected and identified based on a mass spectrum acquired from an individual spot of 7 μm in diameter. These measurements provide molecularly specific images of larger metabolites (phospholipids) in native single cells. The developed method can be used for a wide range of detailed investigations of metabolic changes in single cells.
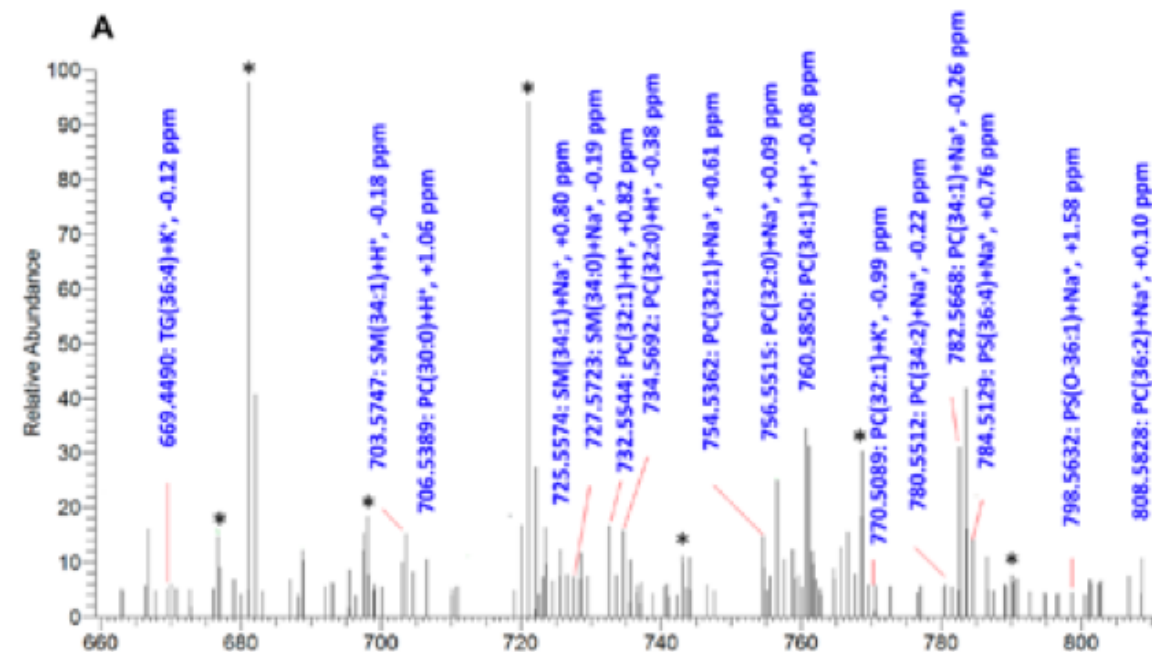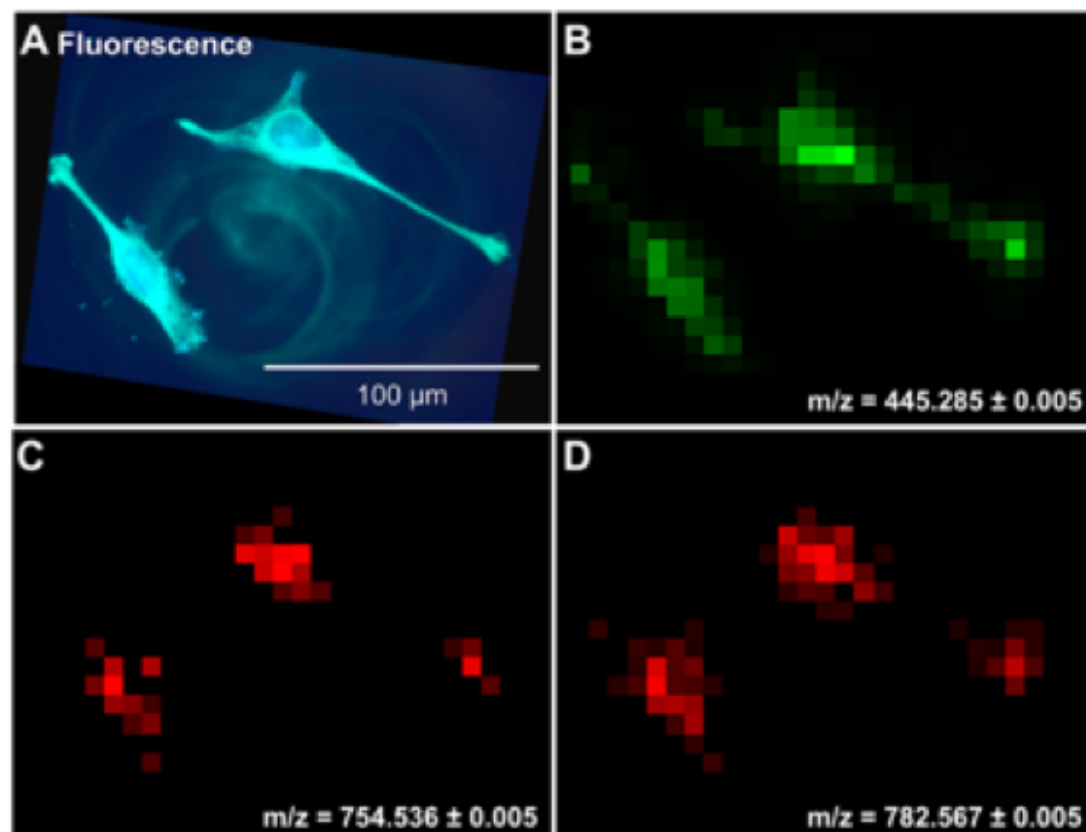
# Single-cell Metabolomics
## 2021
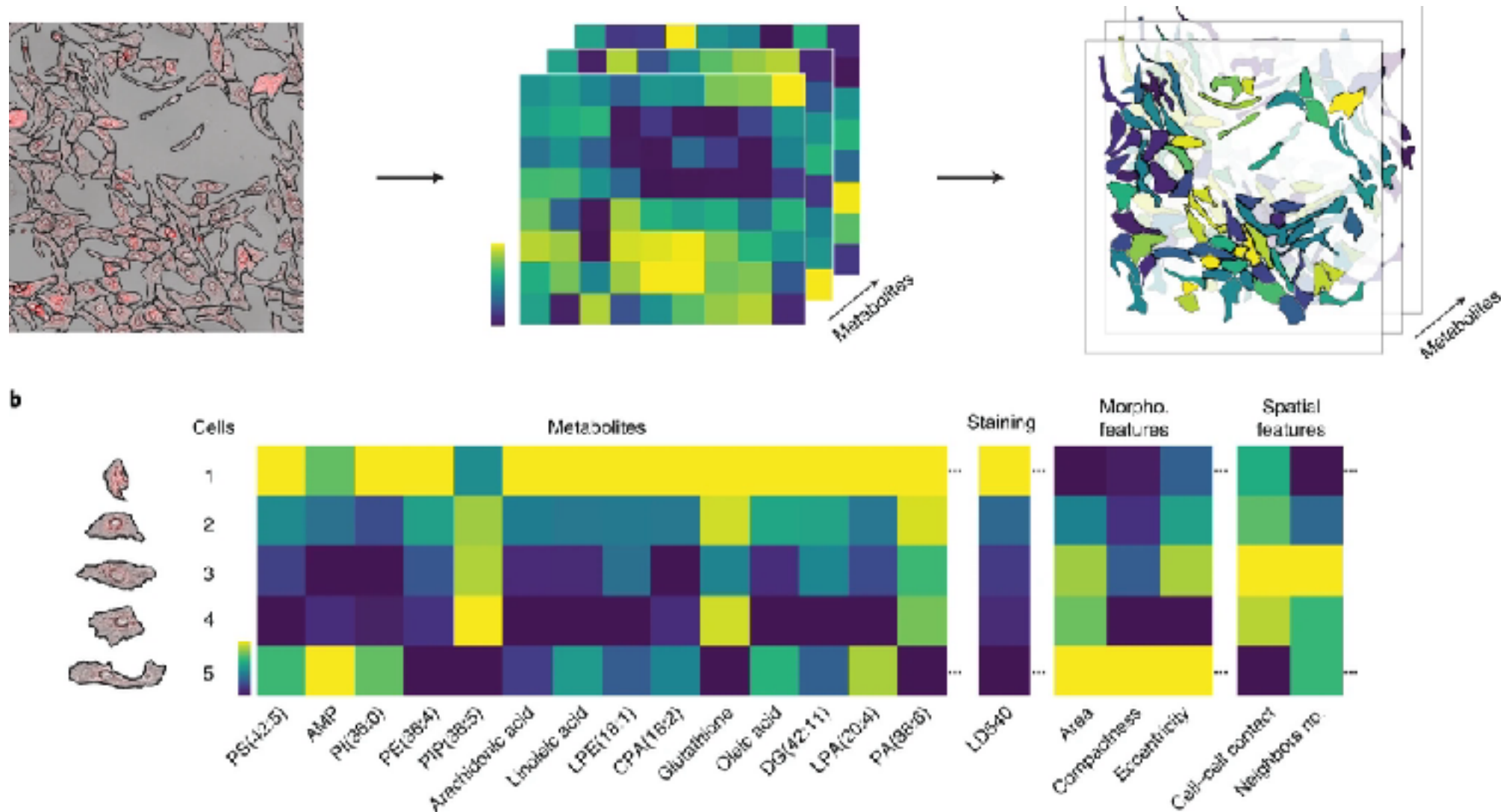


**SpaceM reveals metabolic states of single cells**

We present SpaceM, an open-source method for in situ single-cell metabolomics that detects >100 metabolites from >1,000 individual cells per hour, together with a fluorescence-based readout and retention of morpho-spatial features. We validated SpaceM by predicting the cell types of cocultured human epithelial cells and mouse fibroblasts.

# Single-cell Metabolomics

HeLa and NIH3T3 coculture

mCherry  GFP
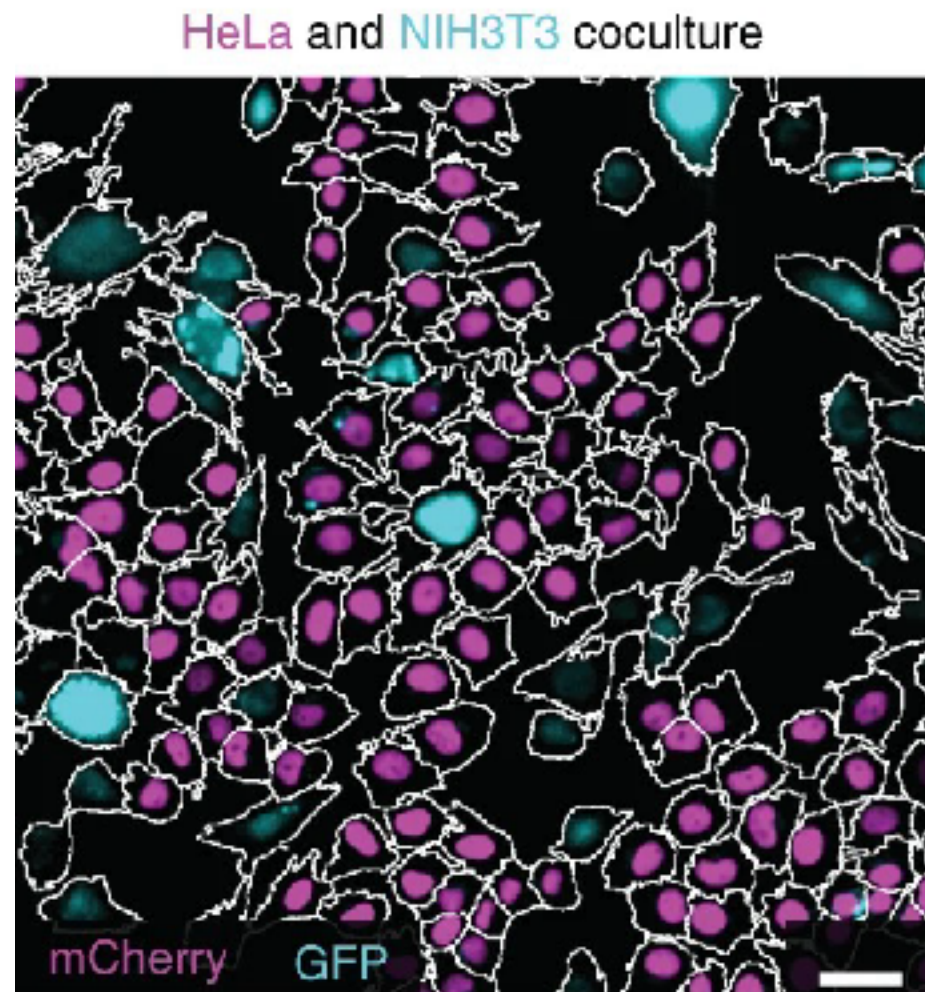
91.3% accuracy
846 cells
88 metabolites

UMAP 2

UMAP 1
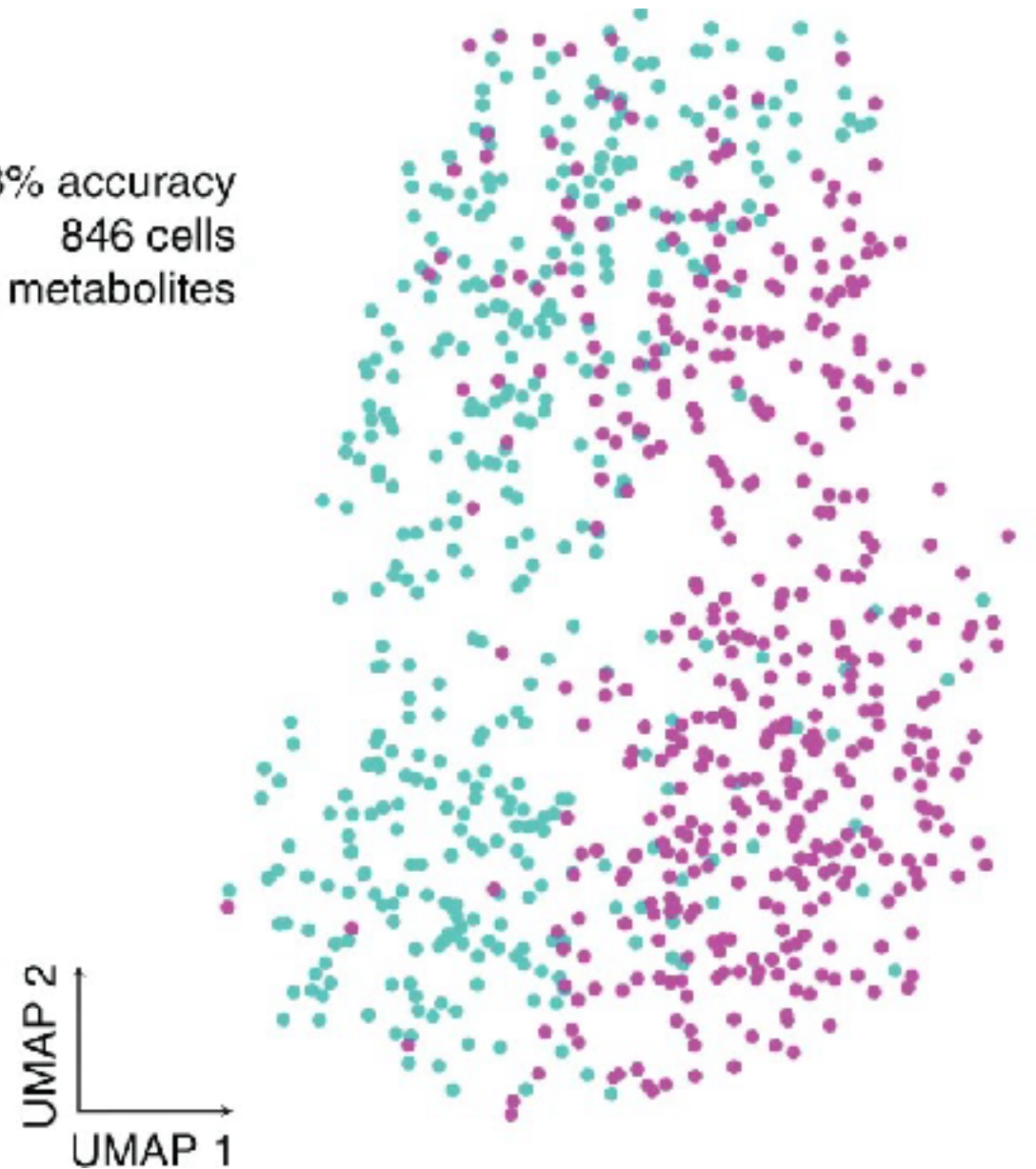
# Single-cell Metabolomics

**Mass spectrometry imaging to explore molecular heterogeneity in cell culture**
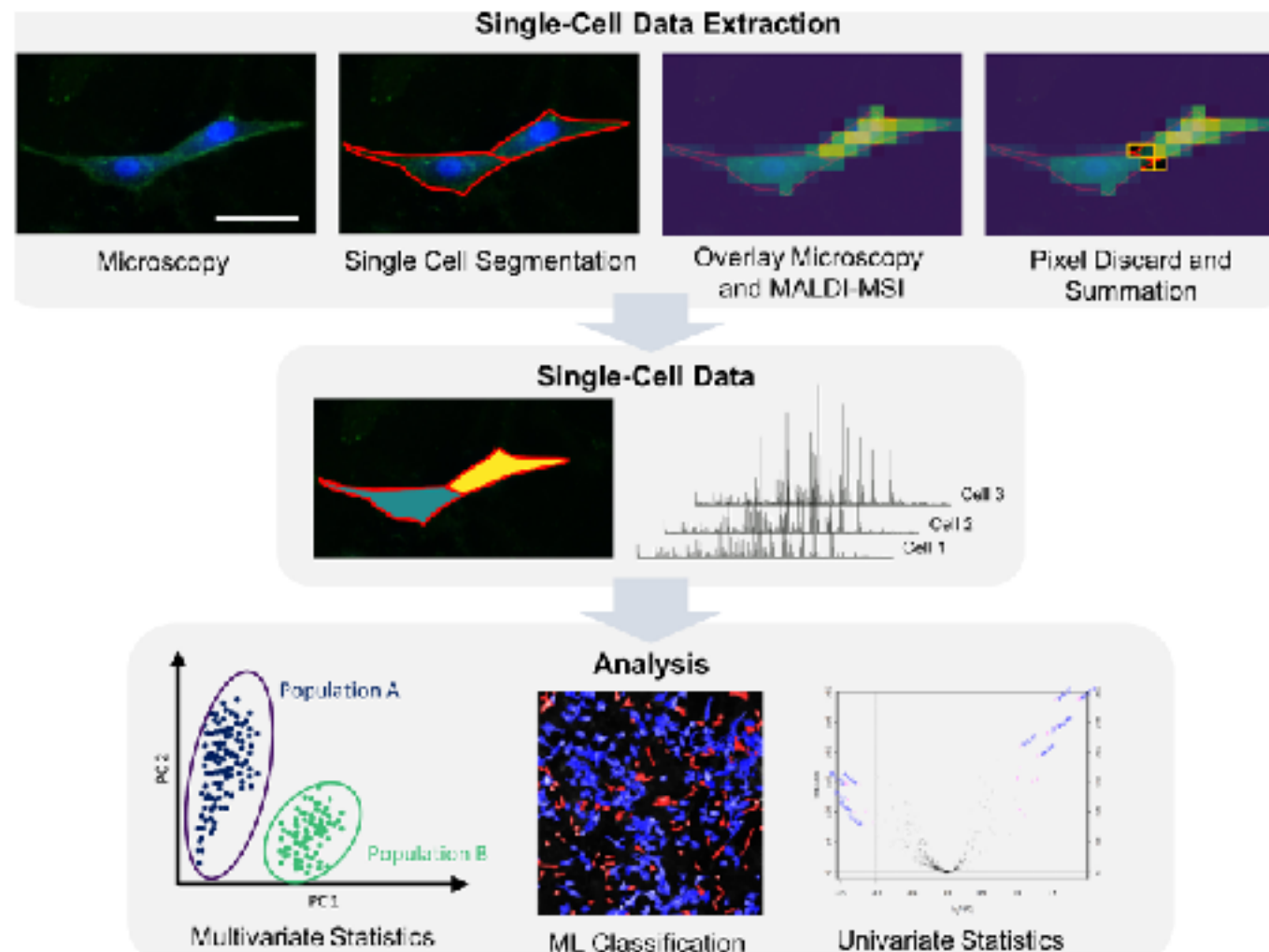


Here, we present a sensitive approach to single-cell MS based on high-resolution MALDI-2-MS imaging in combination with MALDI-compatible staining and use of optical microscopy. Our approach allowed analyzing large amounts of unperturbed cells directly from the growth chamber. Confident coregistration of both modalities enabled a reliable compilation of single-cell mass spectra and a straightforward inclusion of optical as well as mass spectrometric features in the interpretation of data. The resulting multimodal datasets permit the use of various statistical methods like machine learning-driven classification and multivariate analysis based on molecular profile and establish a direct connection of MS data with microscopy information of individual cells.

2022

# Single-cell Metabolomics
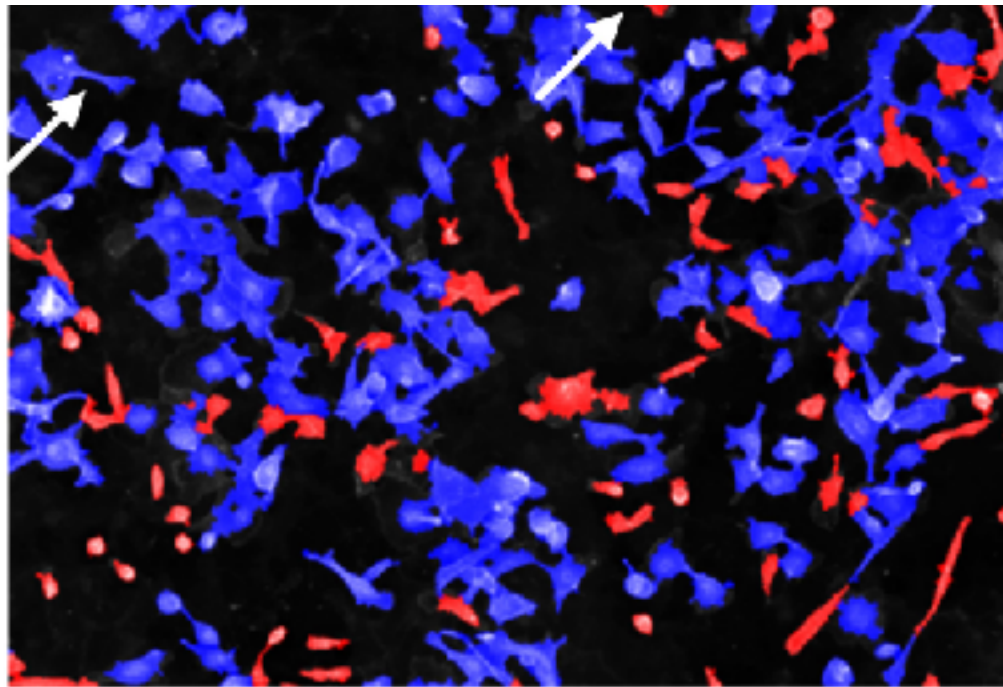


| G | Vero-B4 | Caki-2 | Total |
|---|---|---|---|
| Classified Vero-B4 | 275 | 2 | 277 |
| Classified Caki-2 | 92 | 74 | 166 |
| Classified Correctly | 74.93 % | 97.37 % | 78.78 % |

| H | Vero-B4 | Caki-2 | Total |
|---|---|---|---|
| Classified Vero-B4 | 340 | 7 | 347 |
| Classified Caki-2 | 24 | 69 | 93 |
| Classified Correctly | 92.64 % | 90.79 % | 92.33 % |
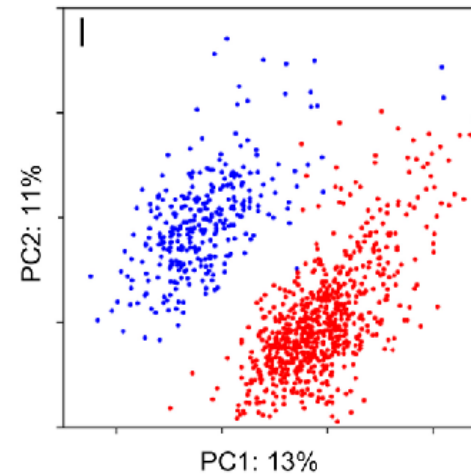
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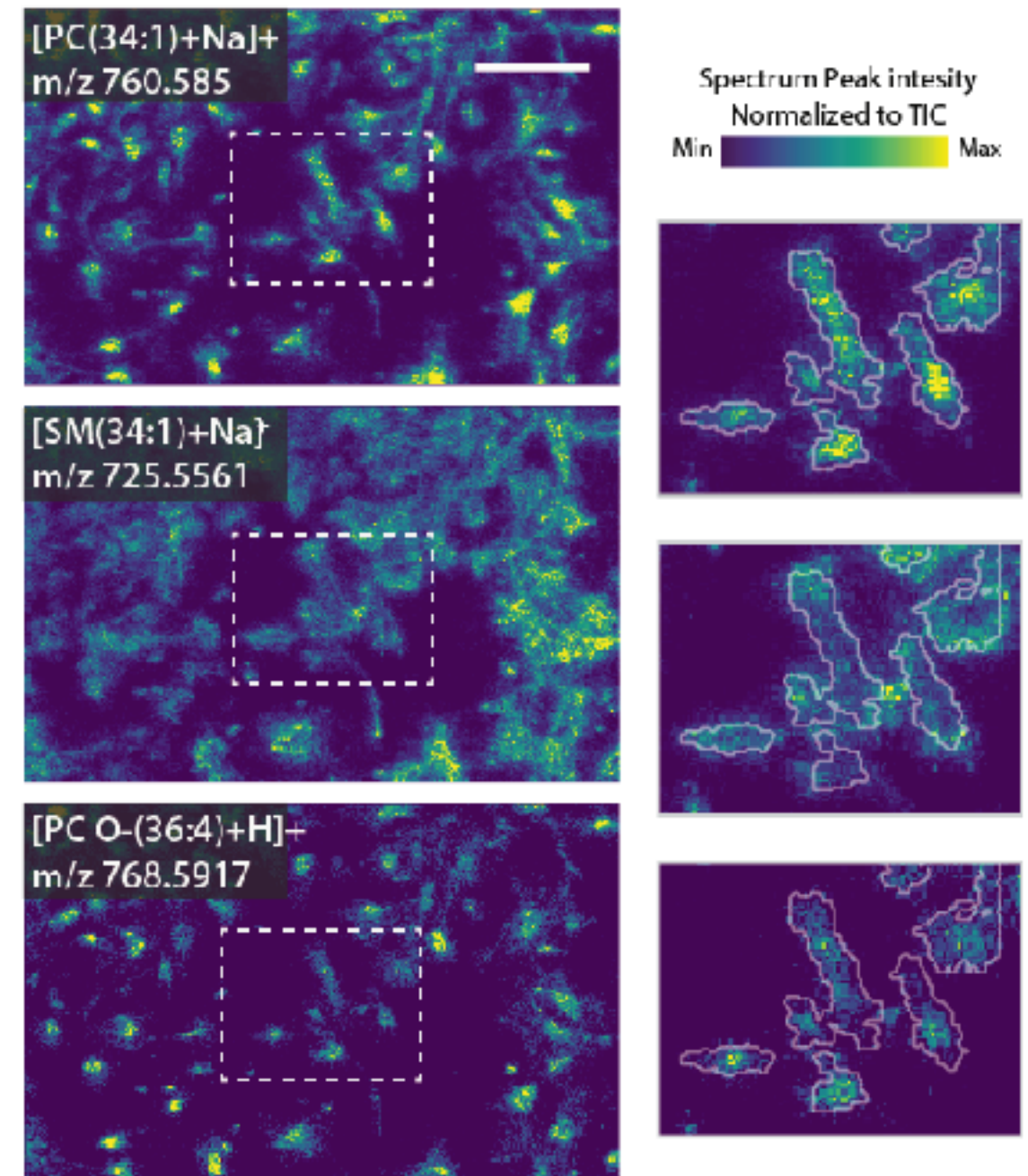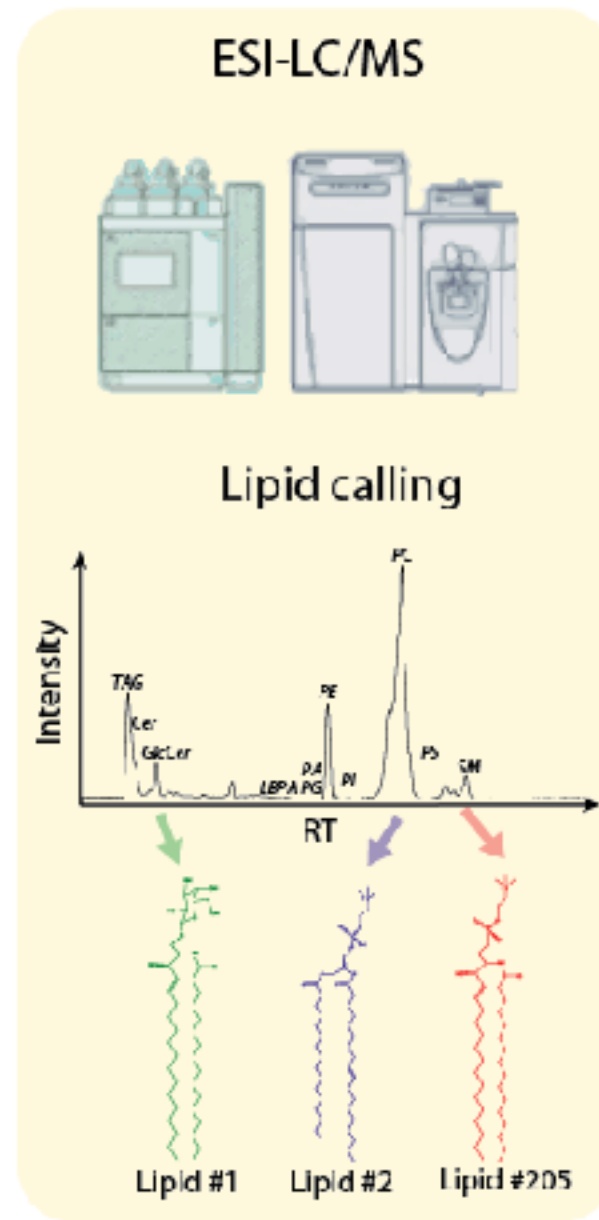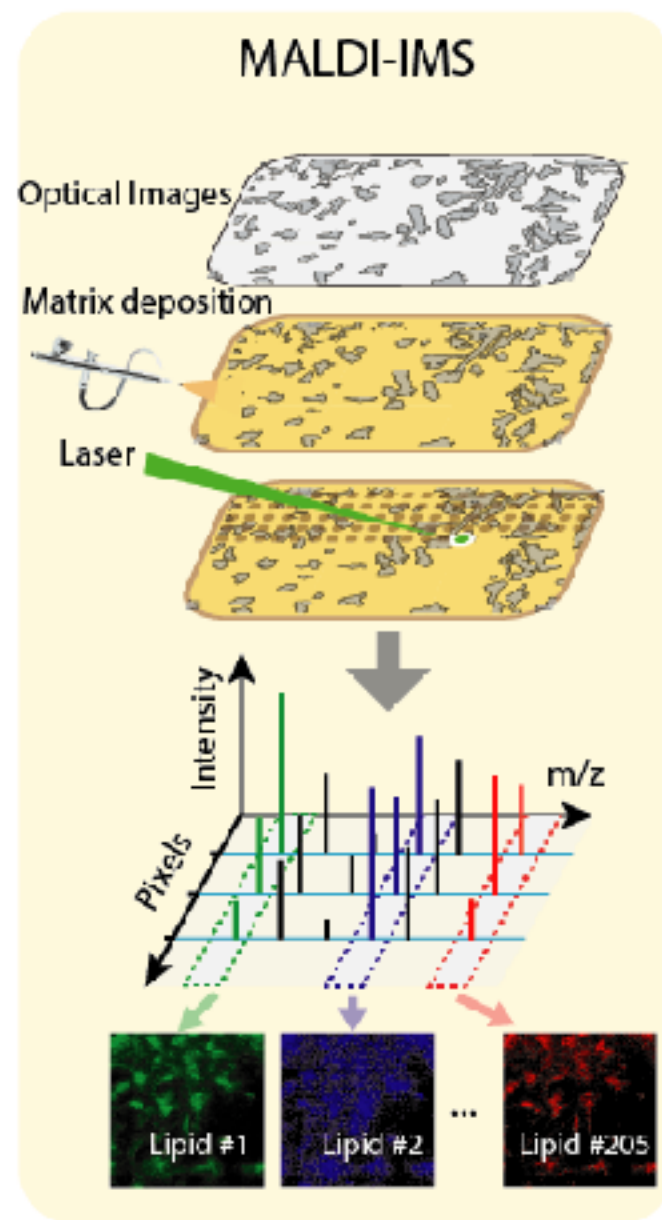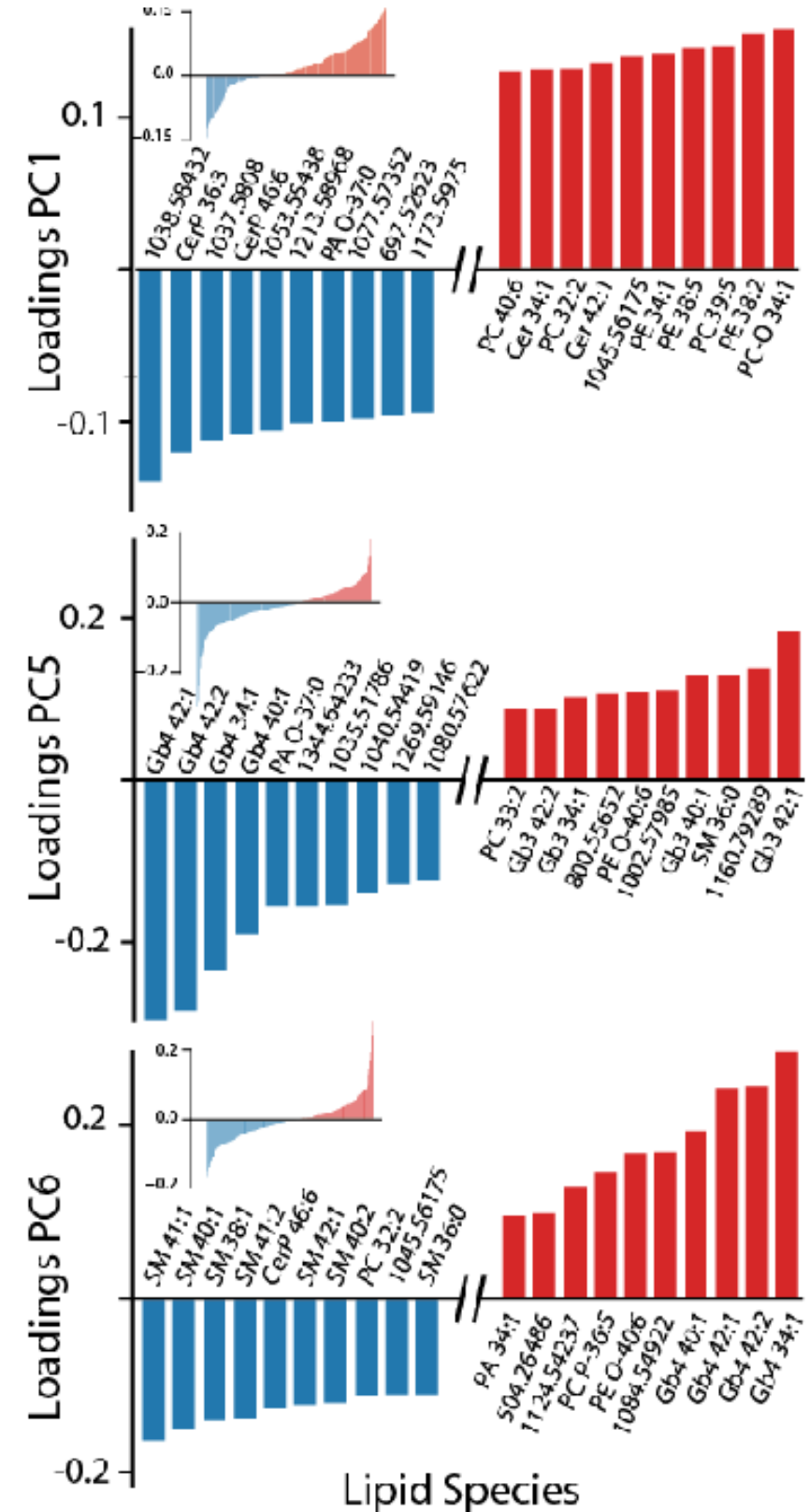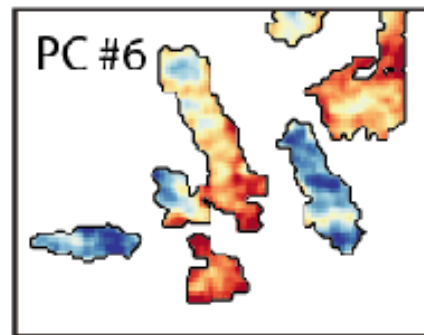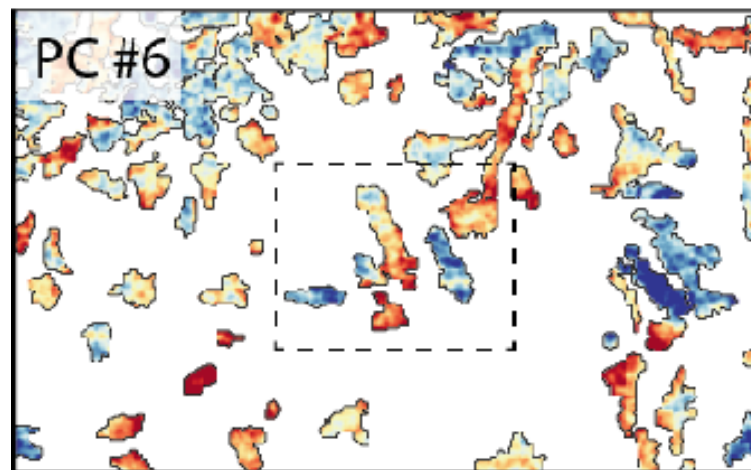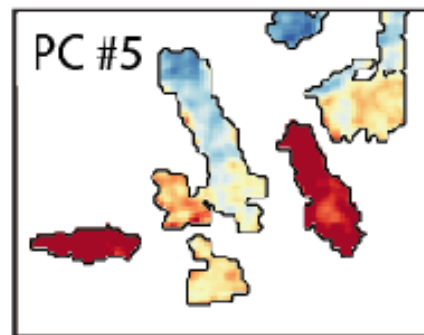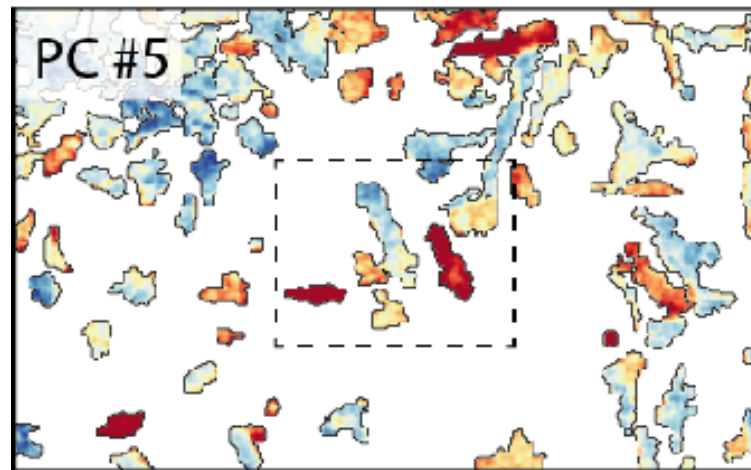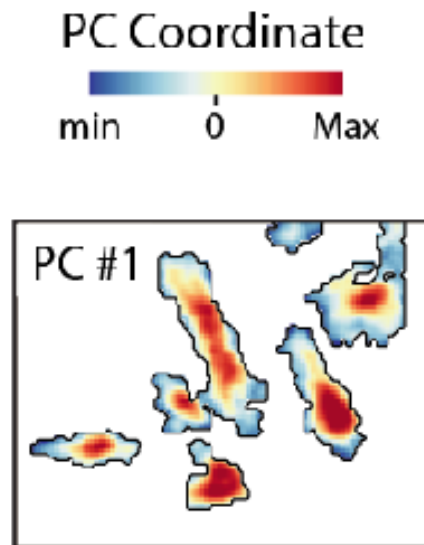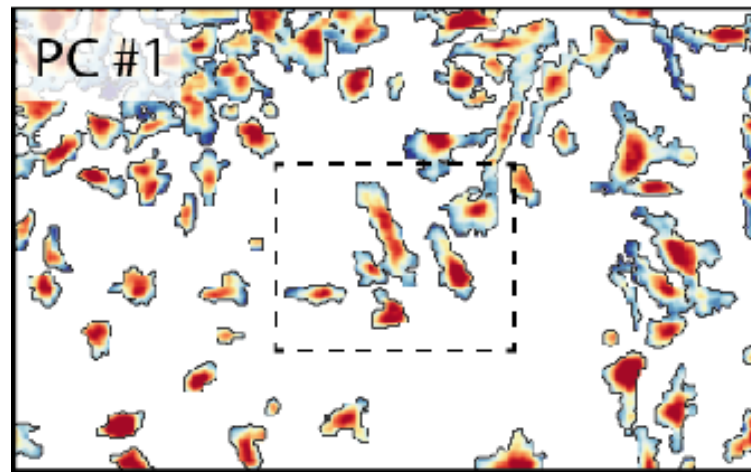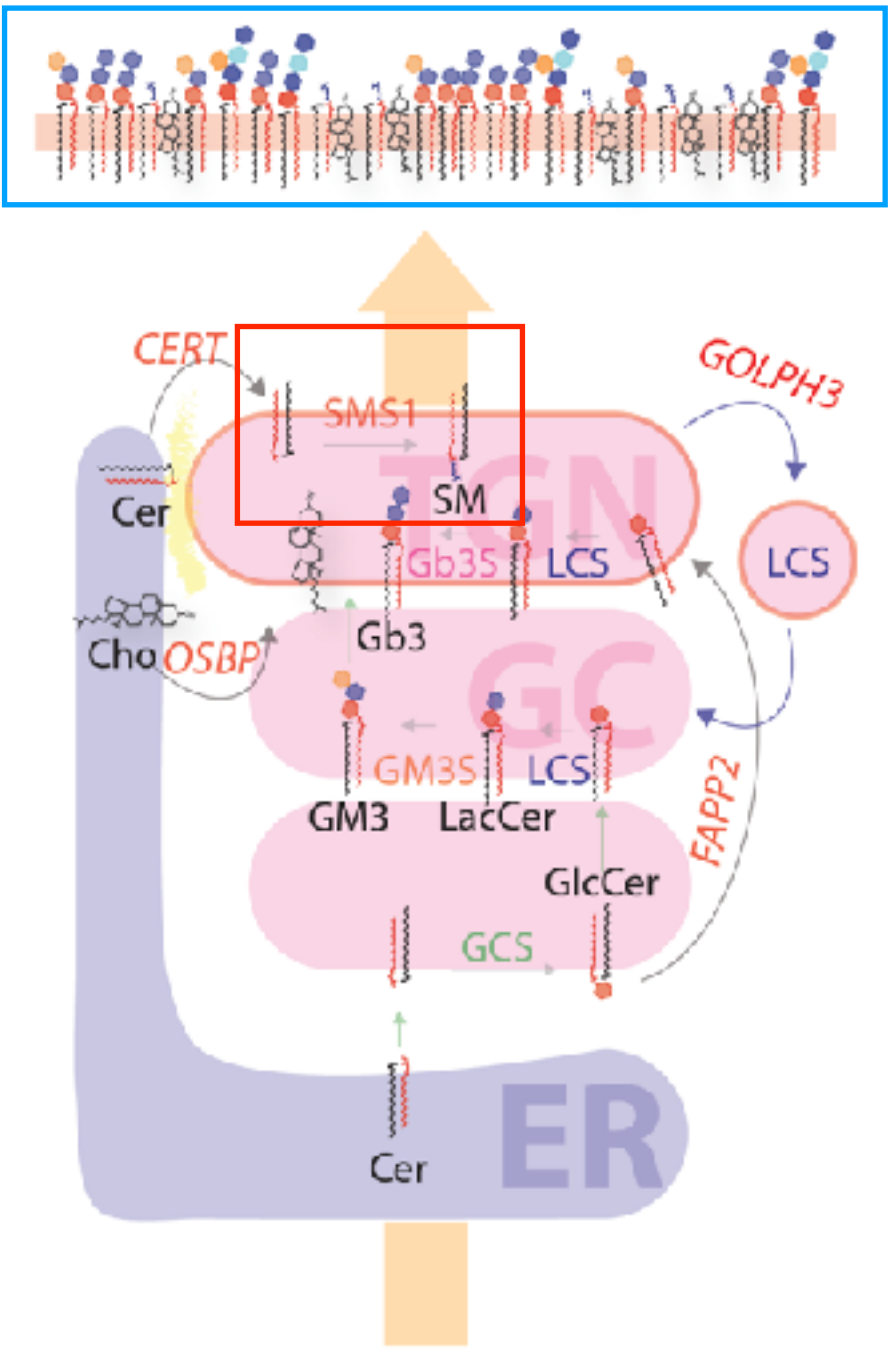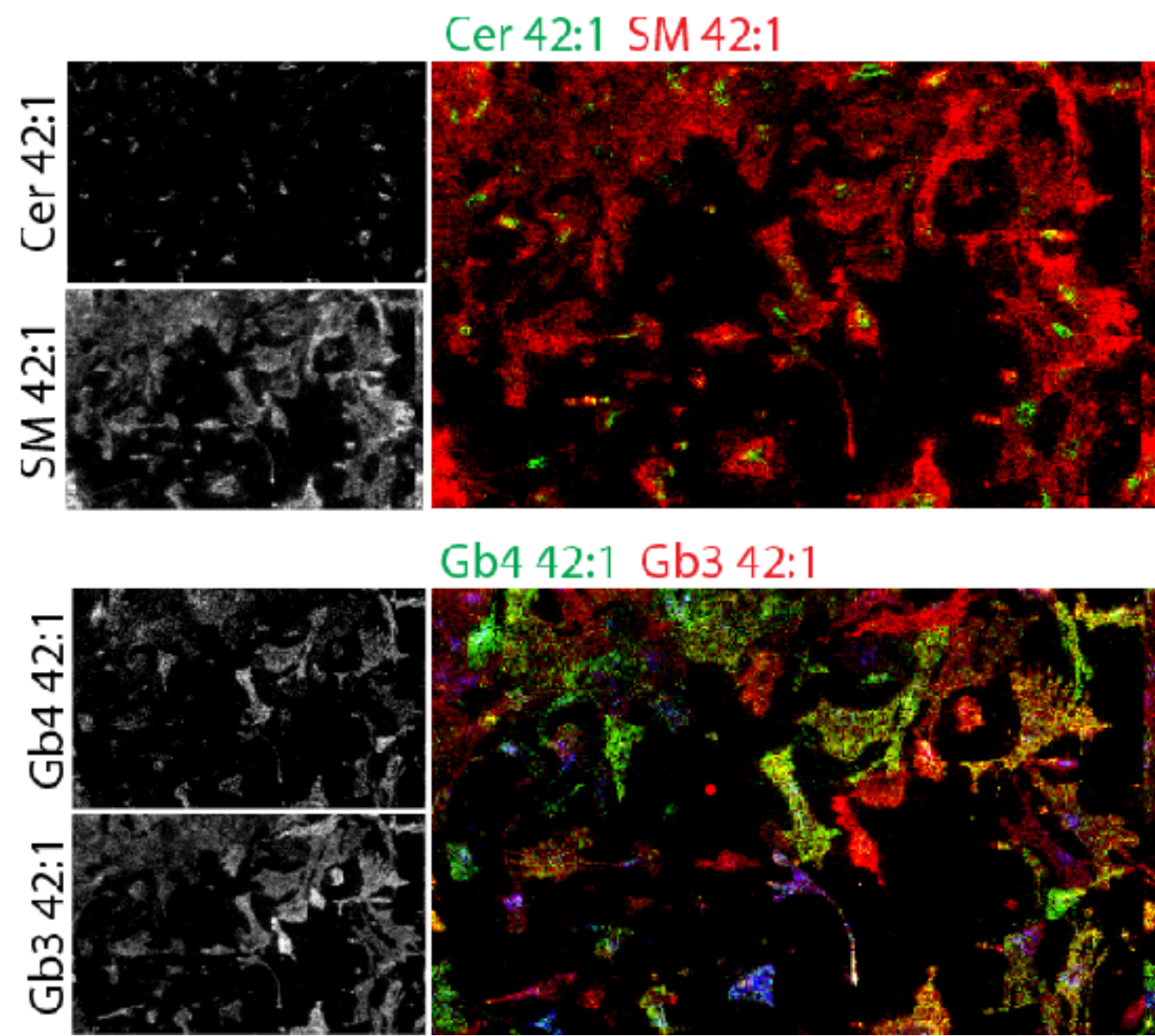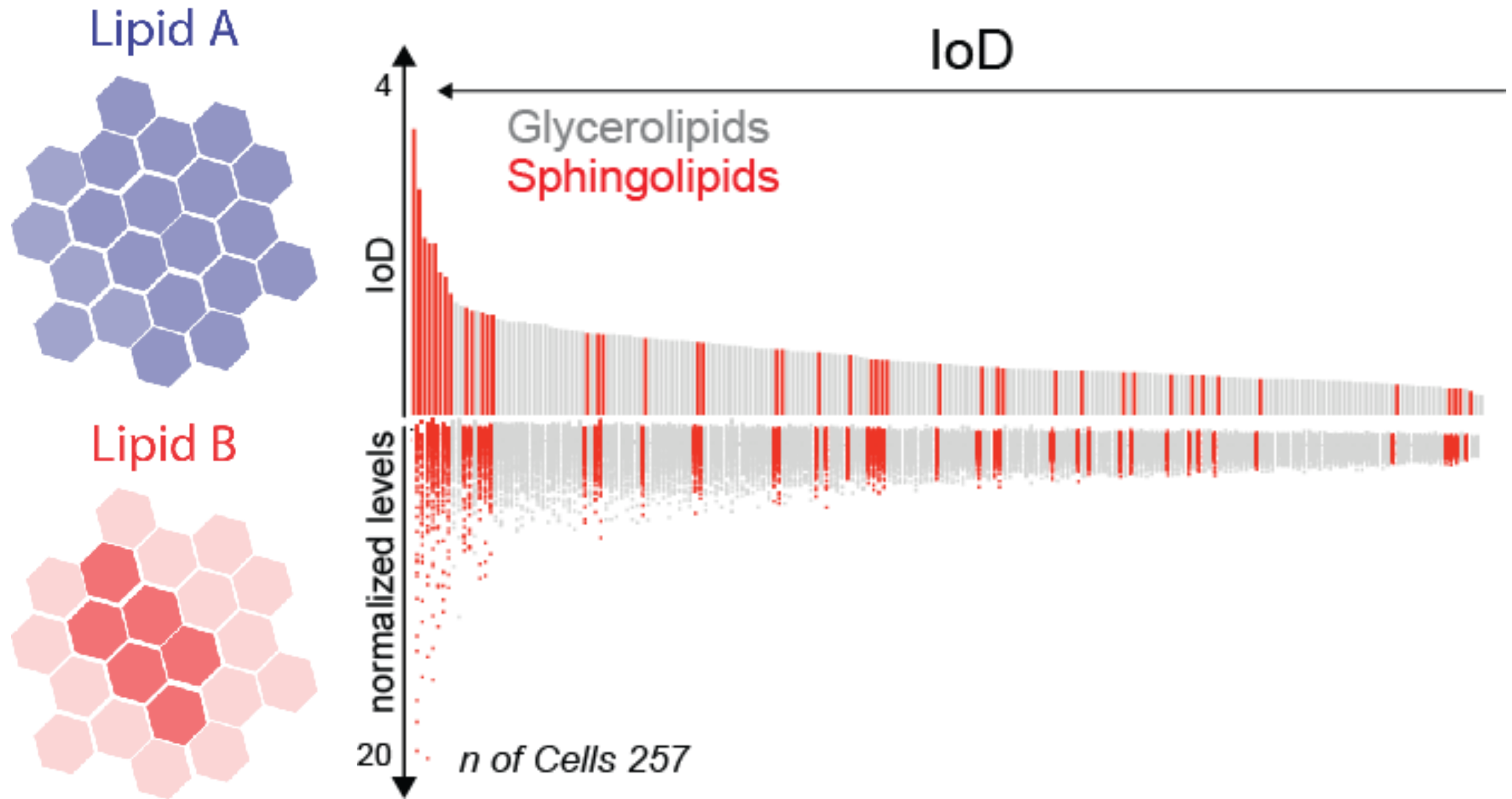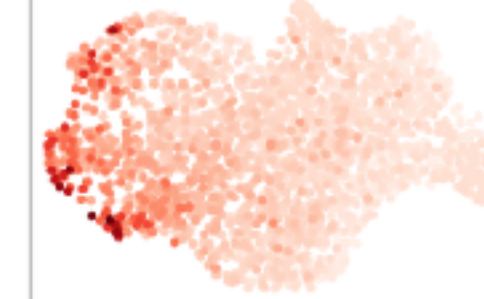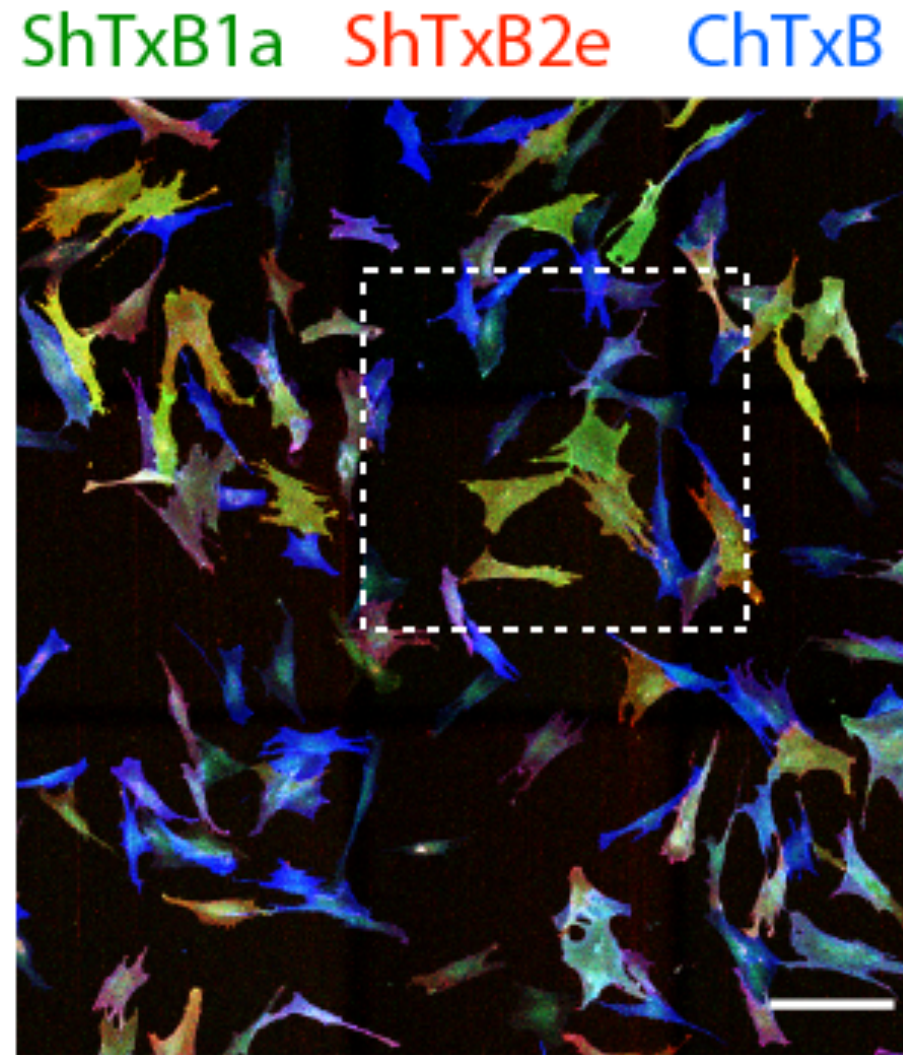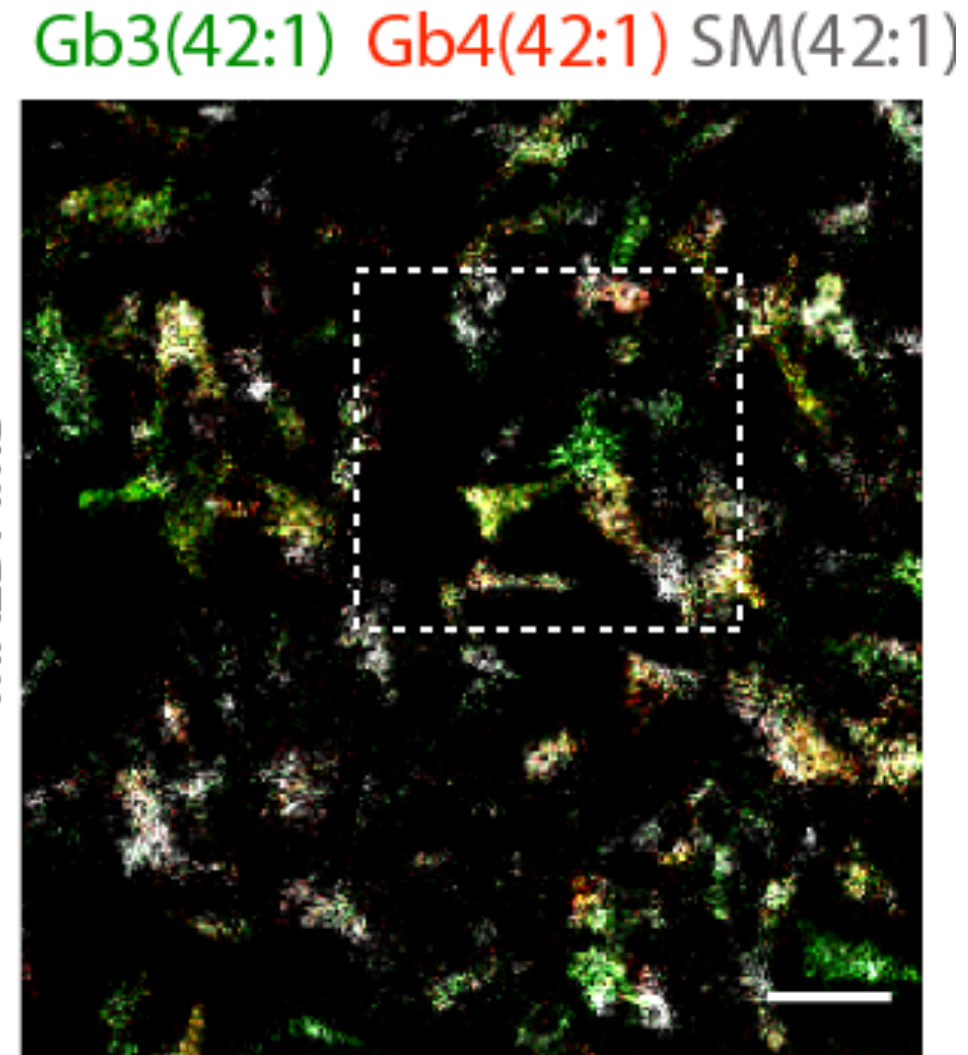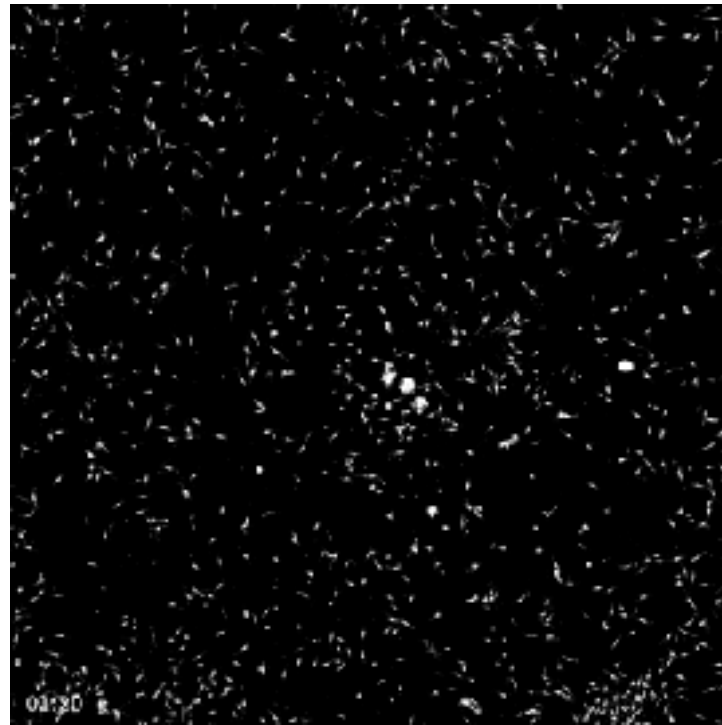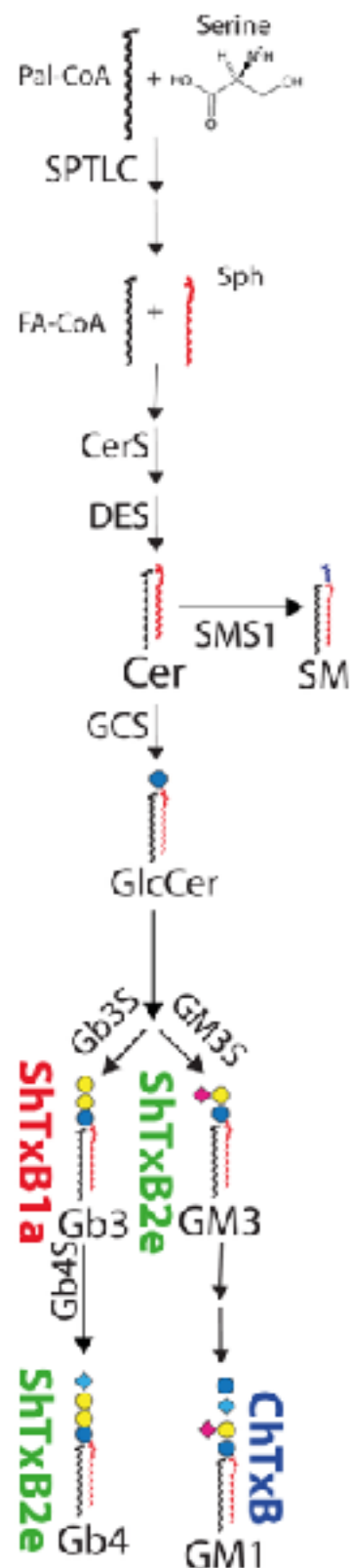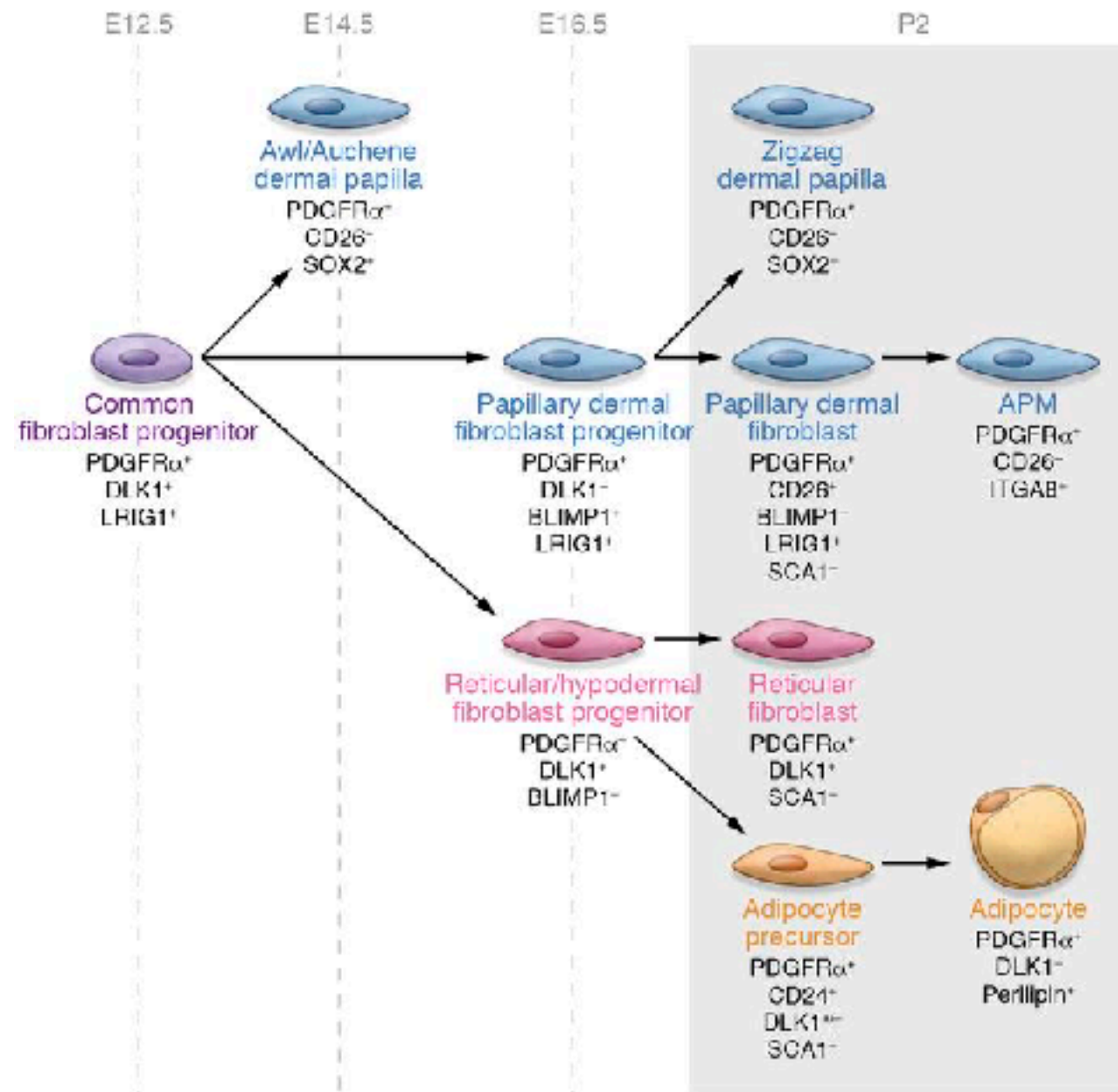
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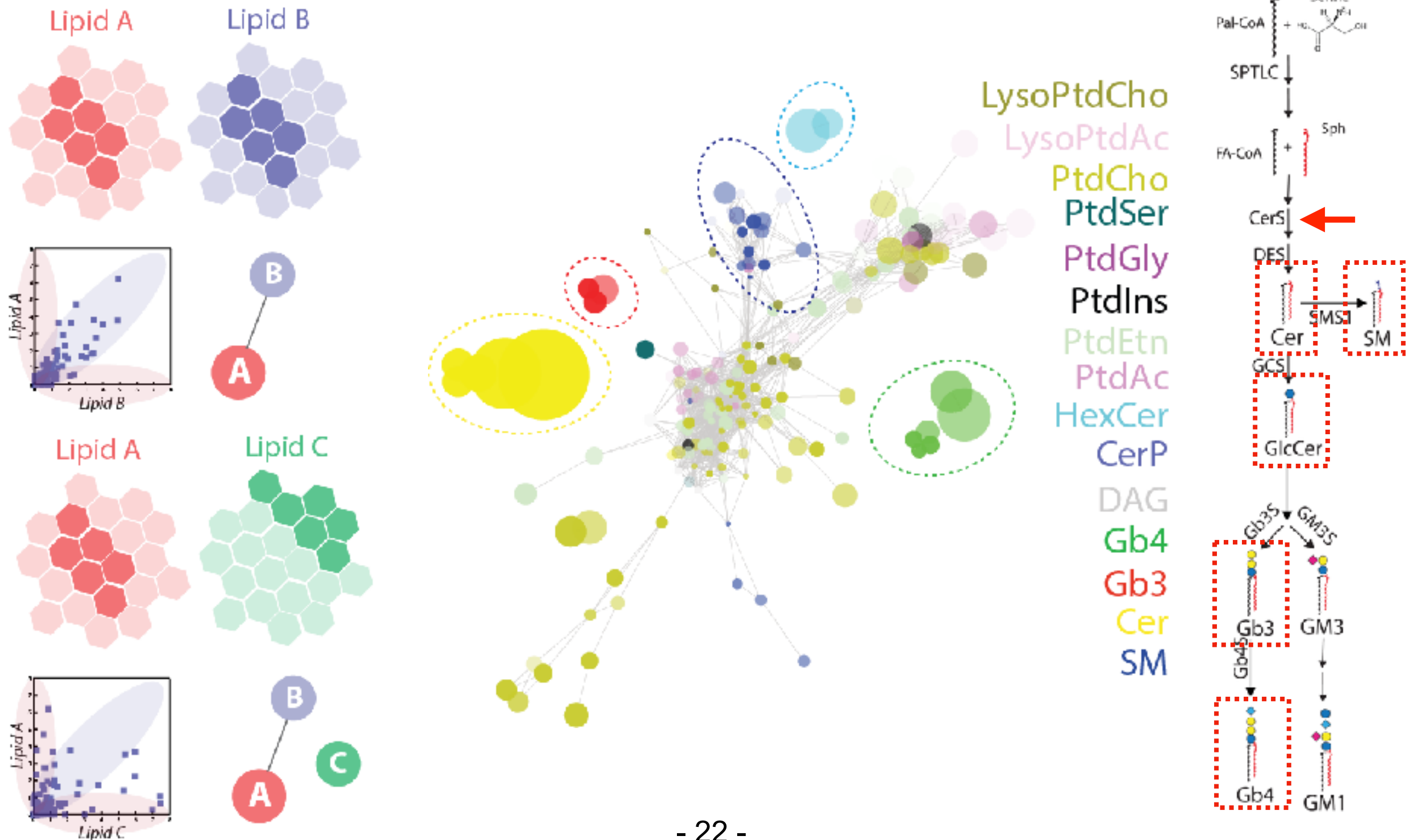
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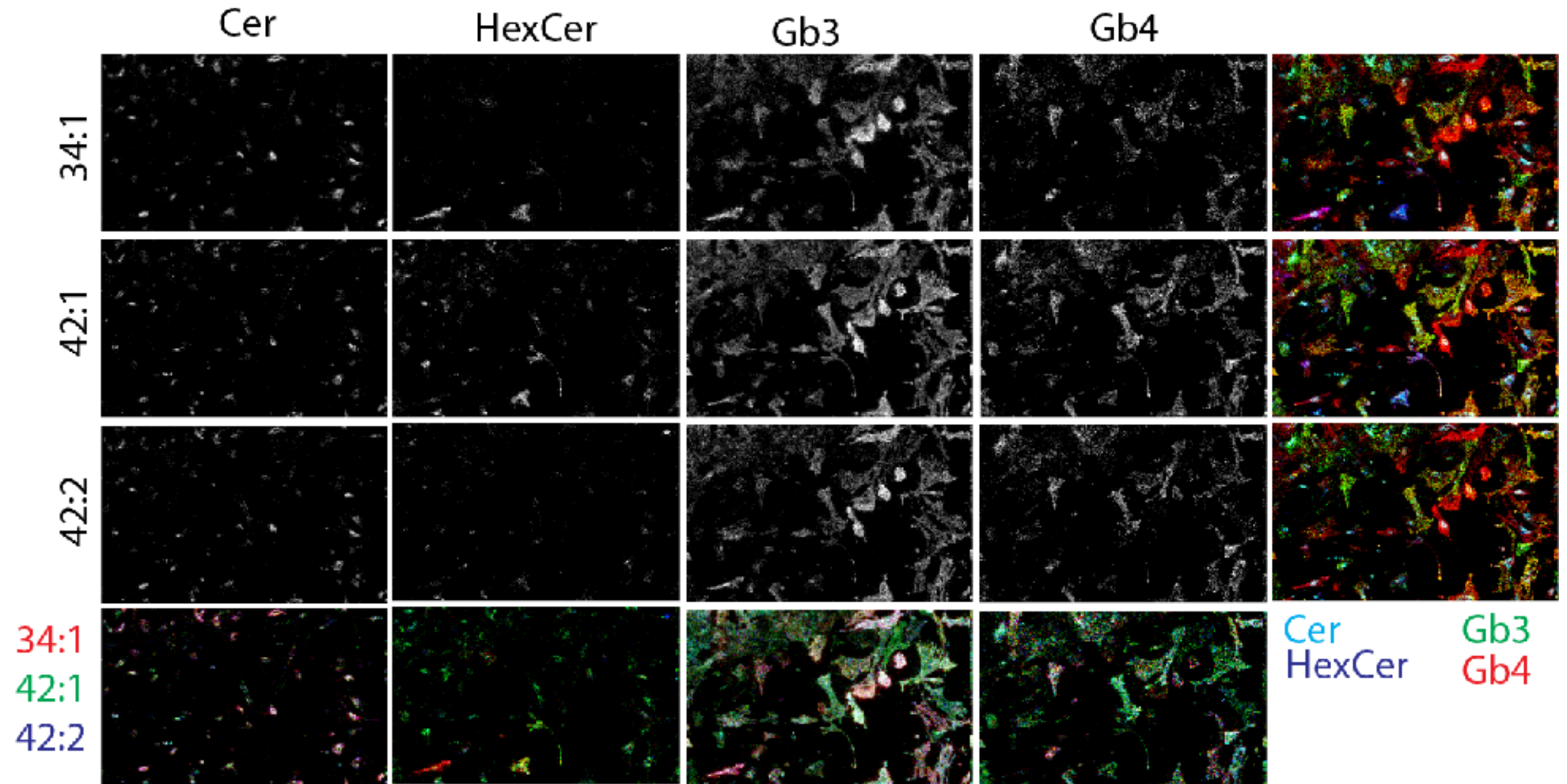
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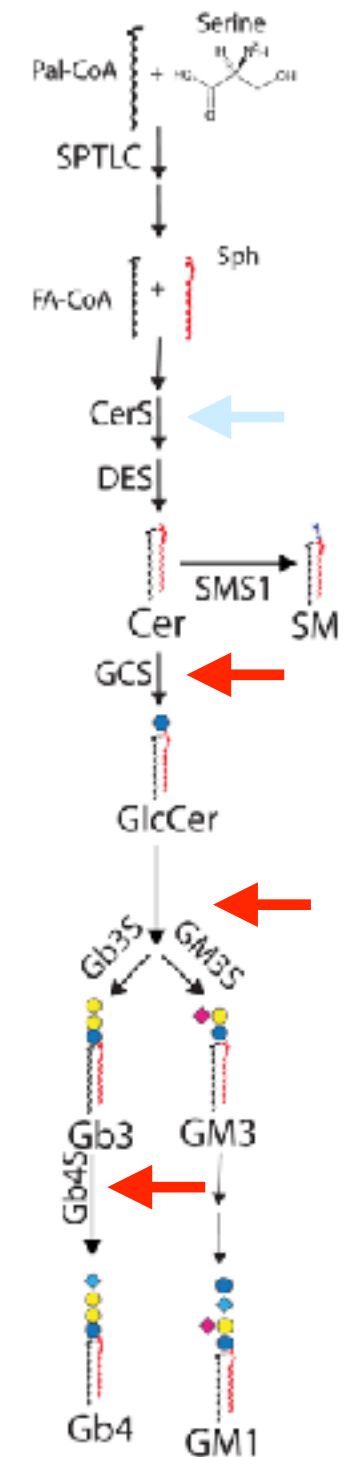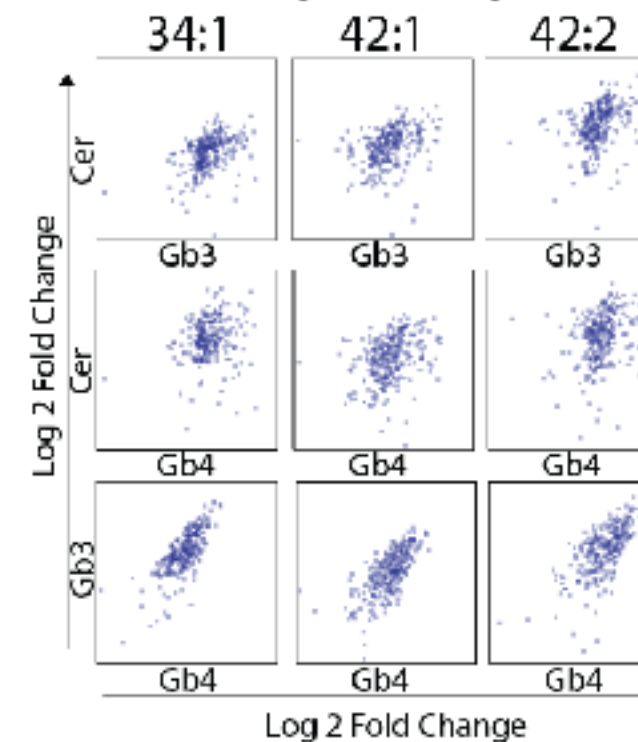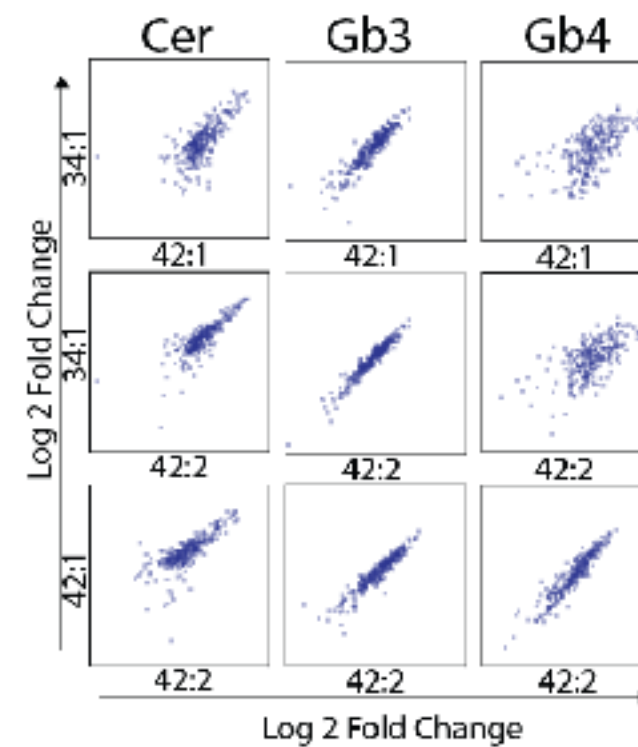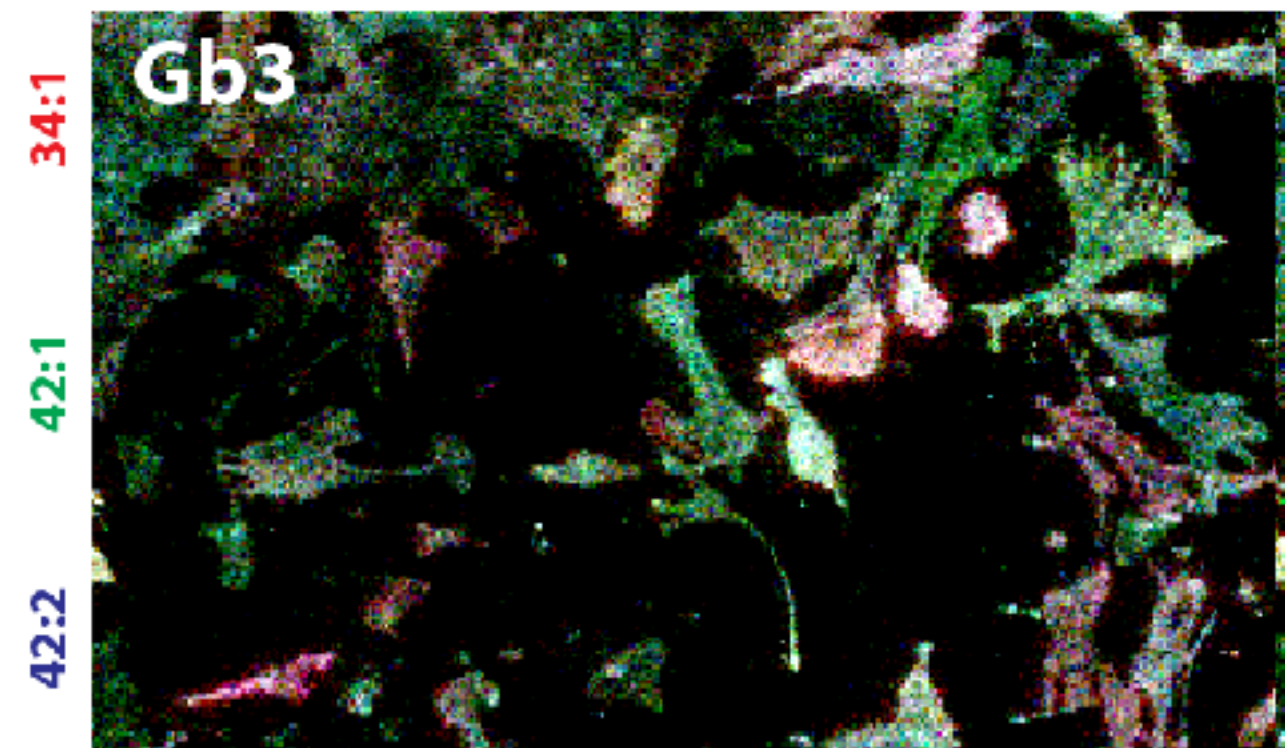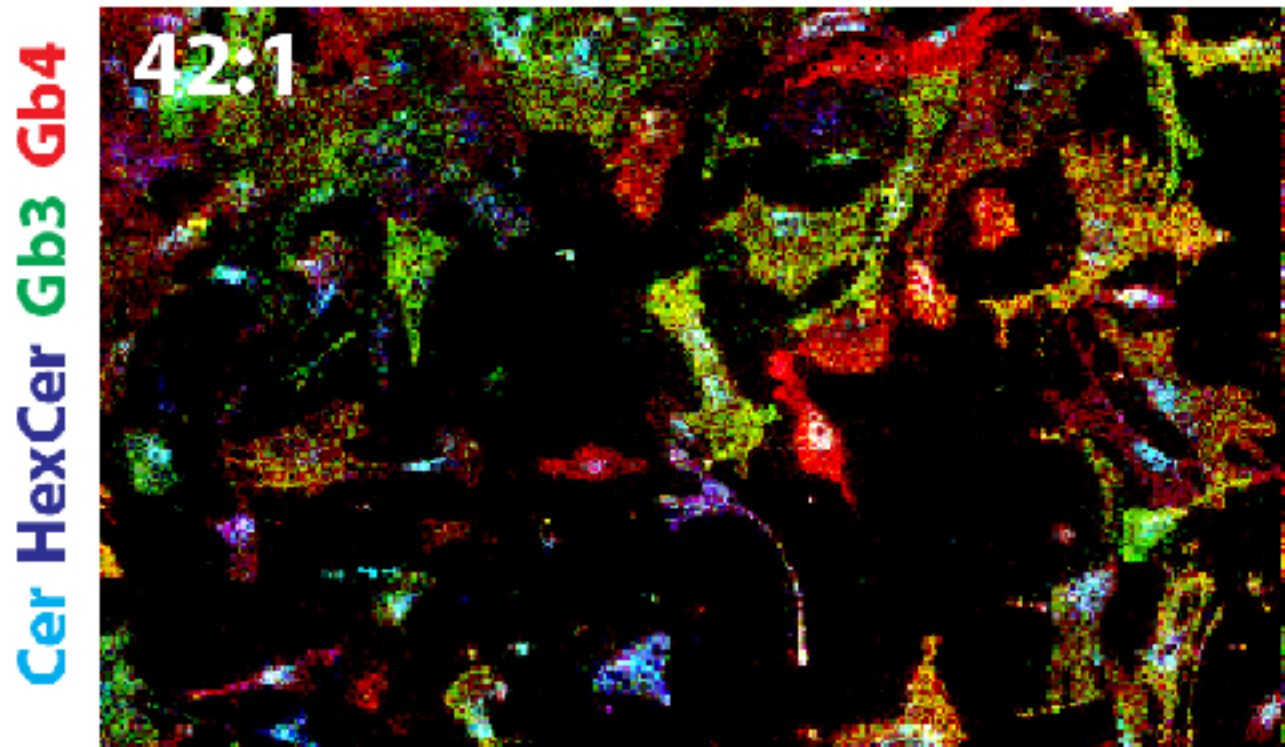
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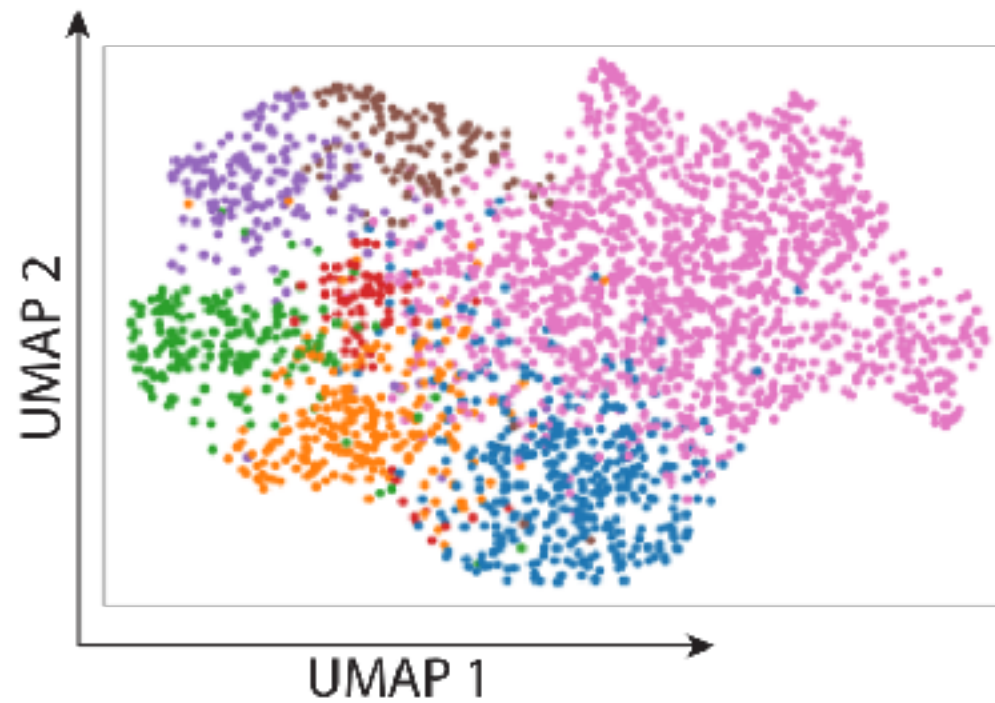
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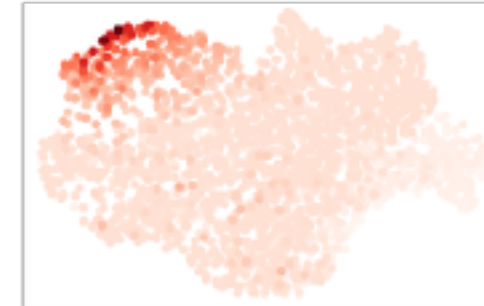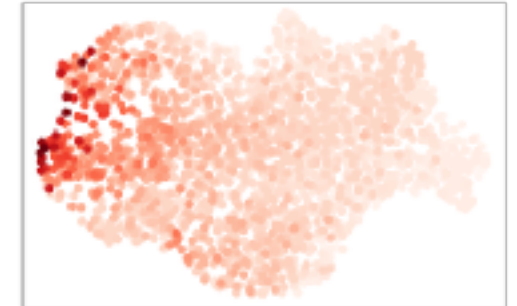
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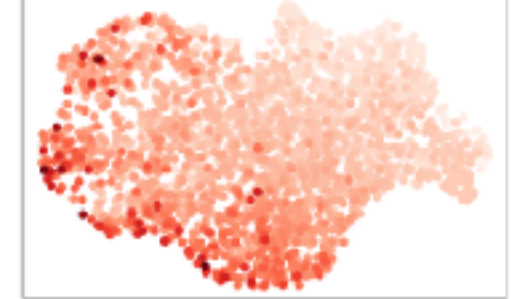
# Single-cell Lipidomics

# Break
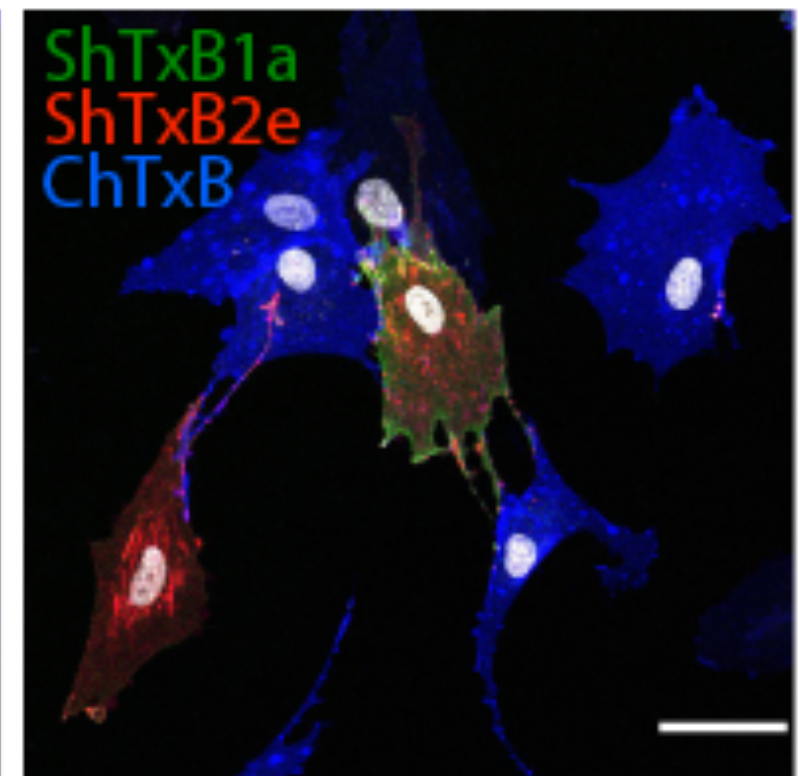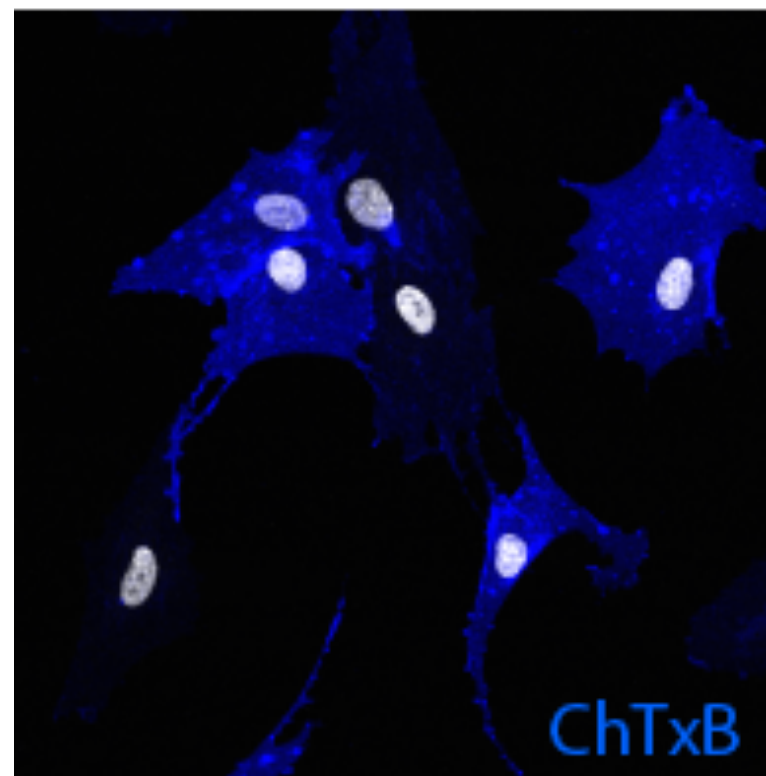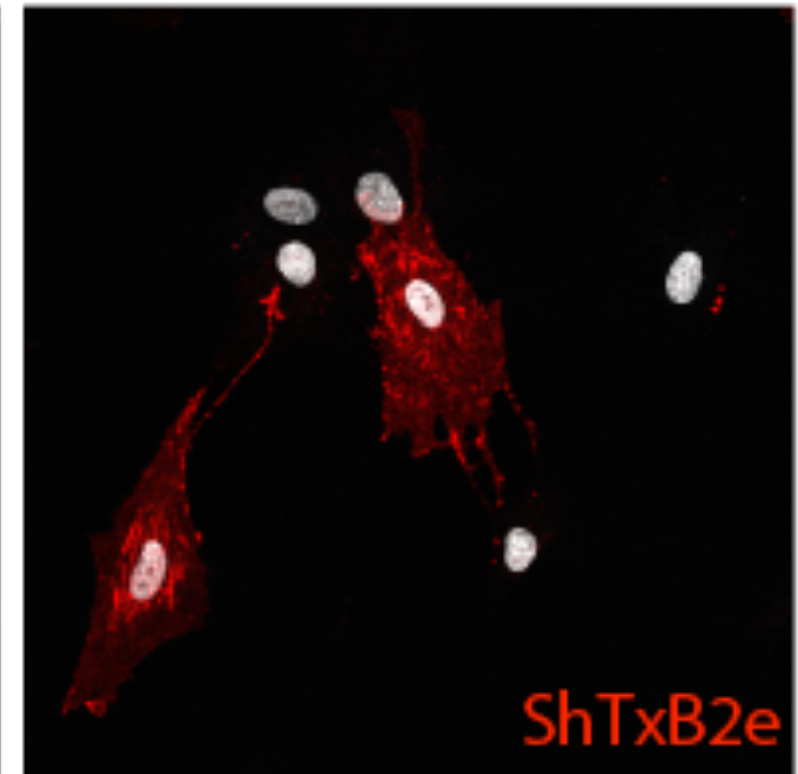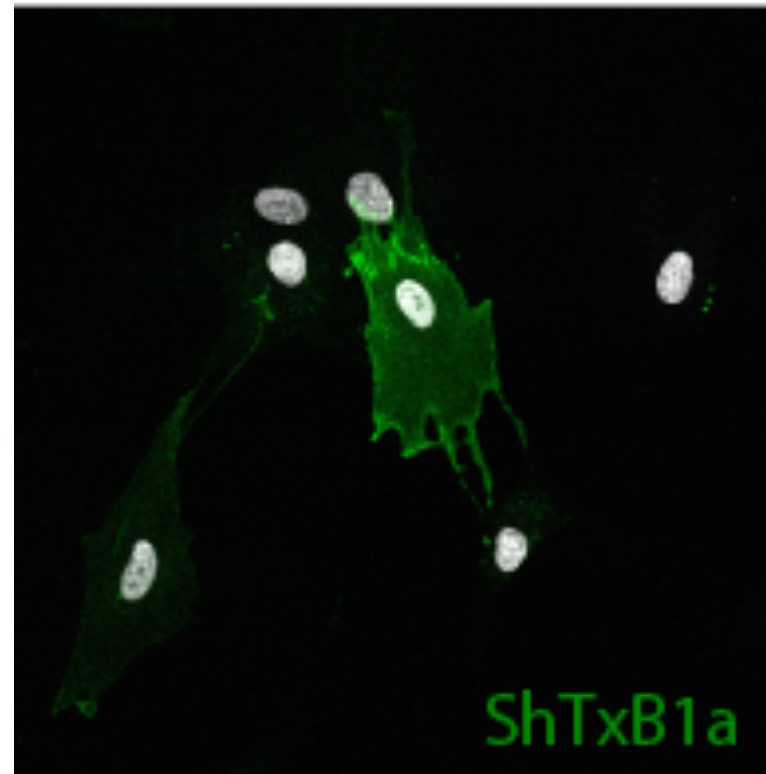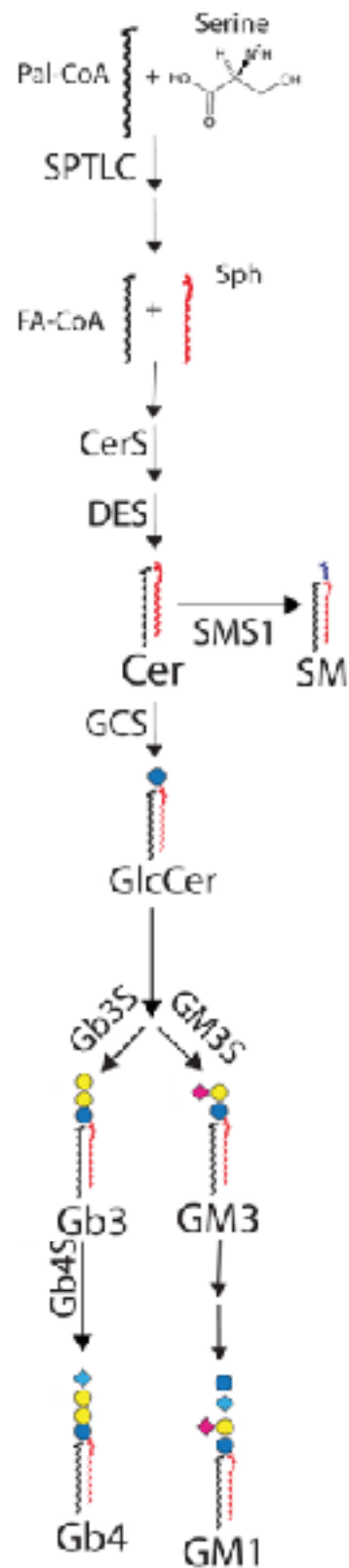
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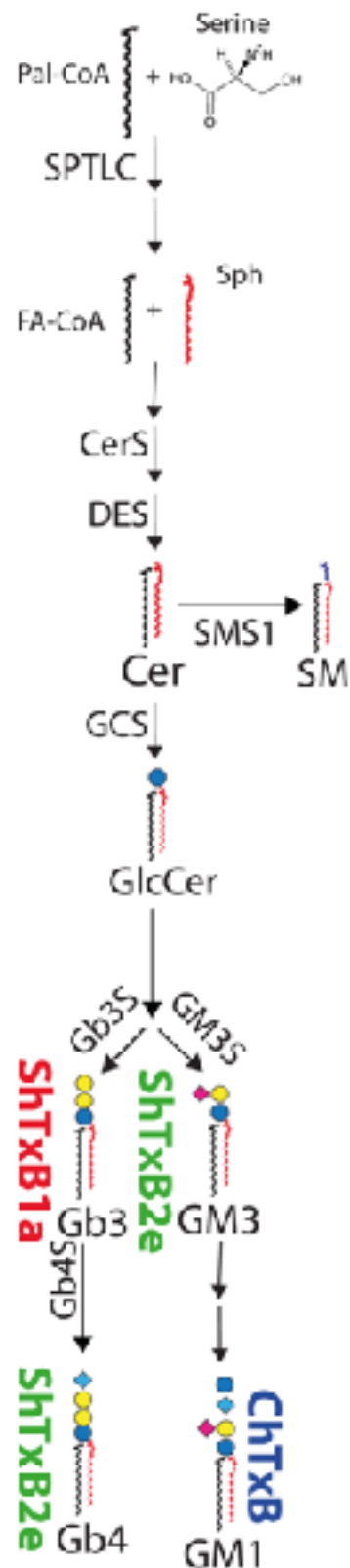
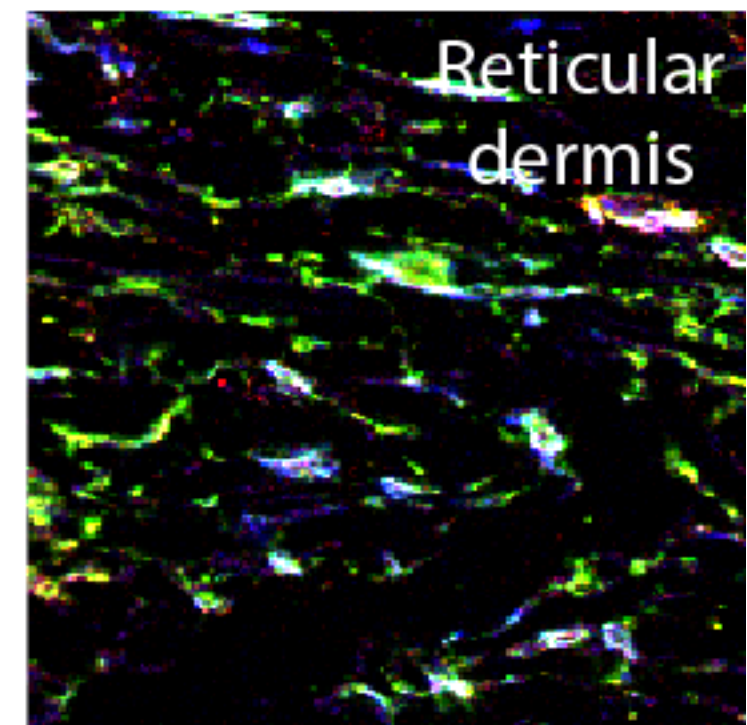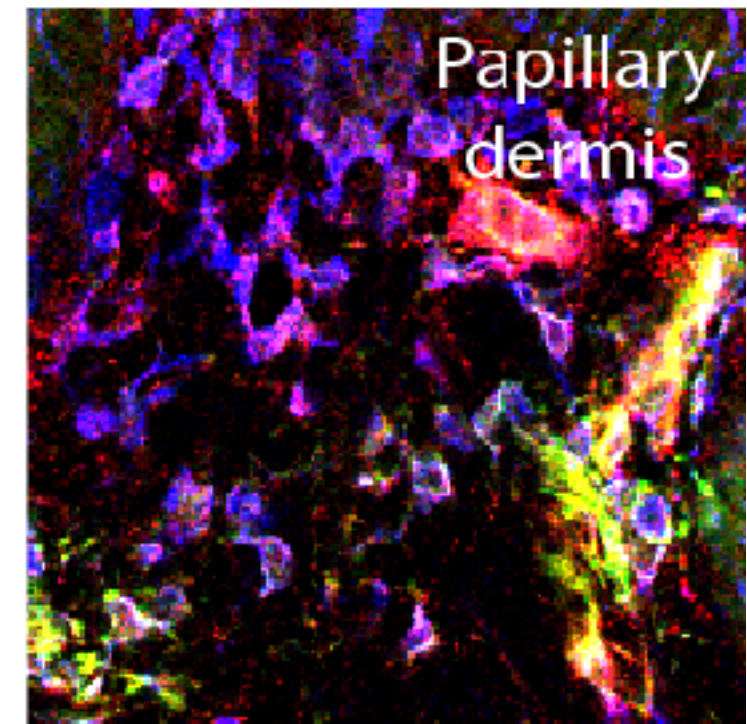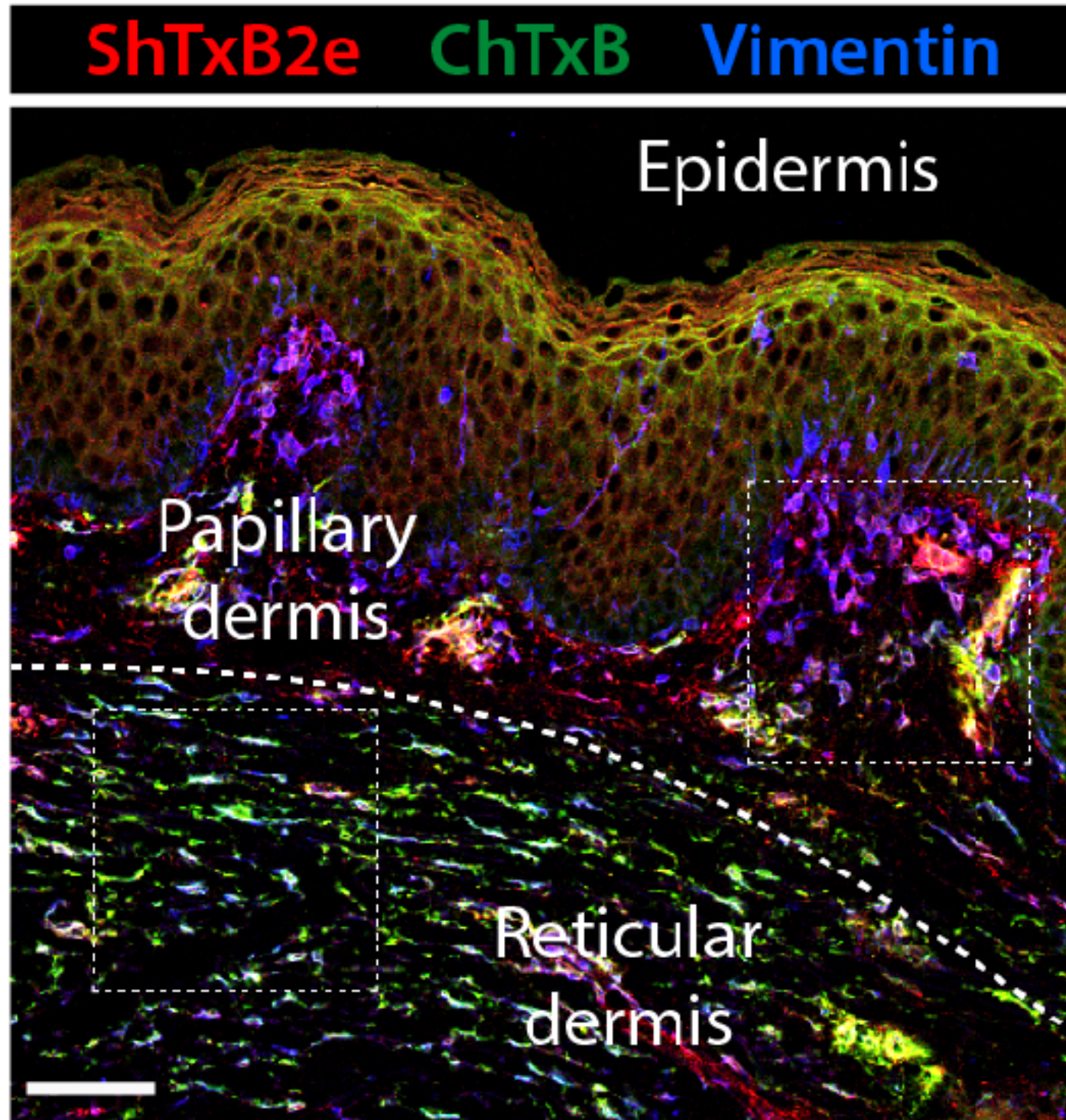# Single-cell Lipidomics

# Single-cell Lipidomics
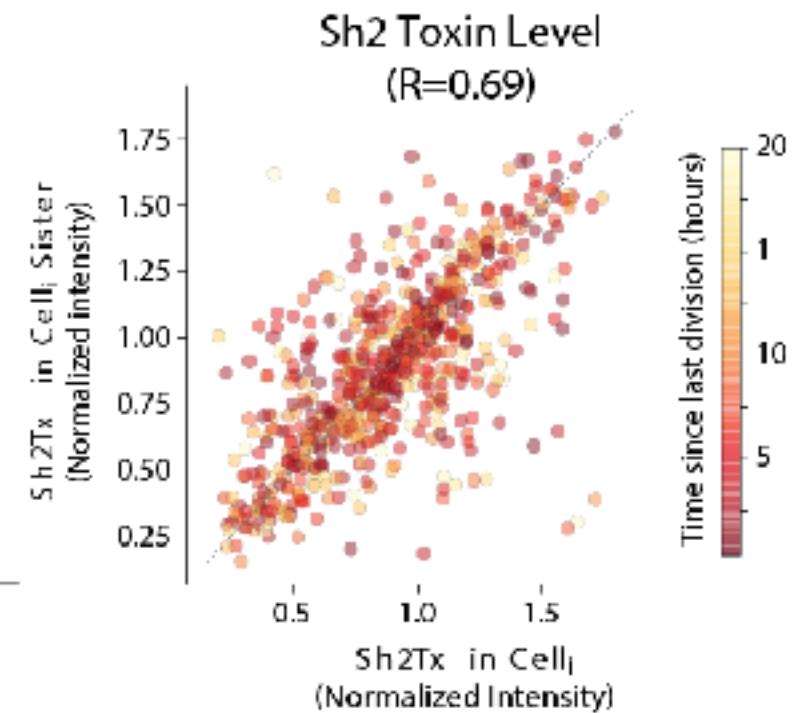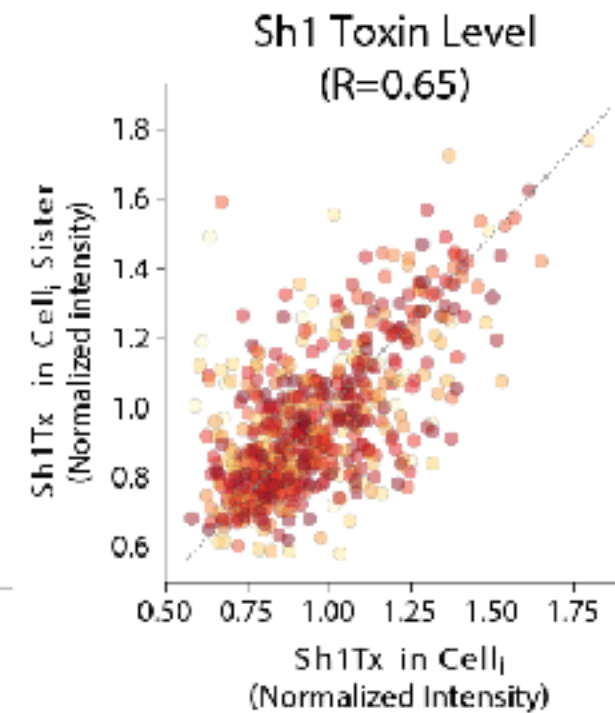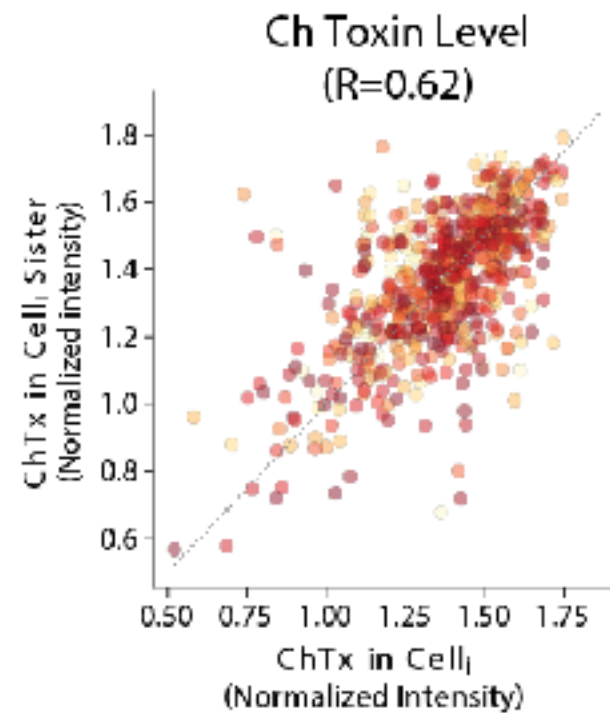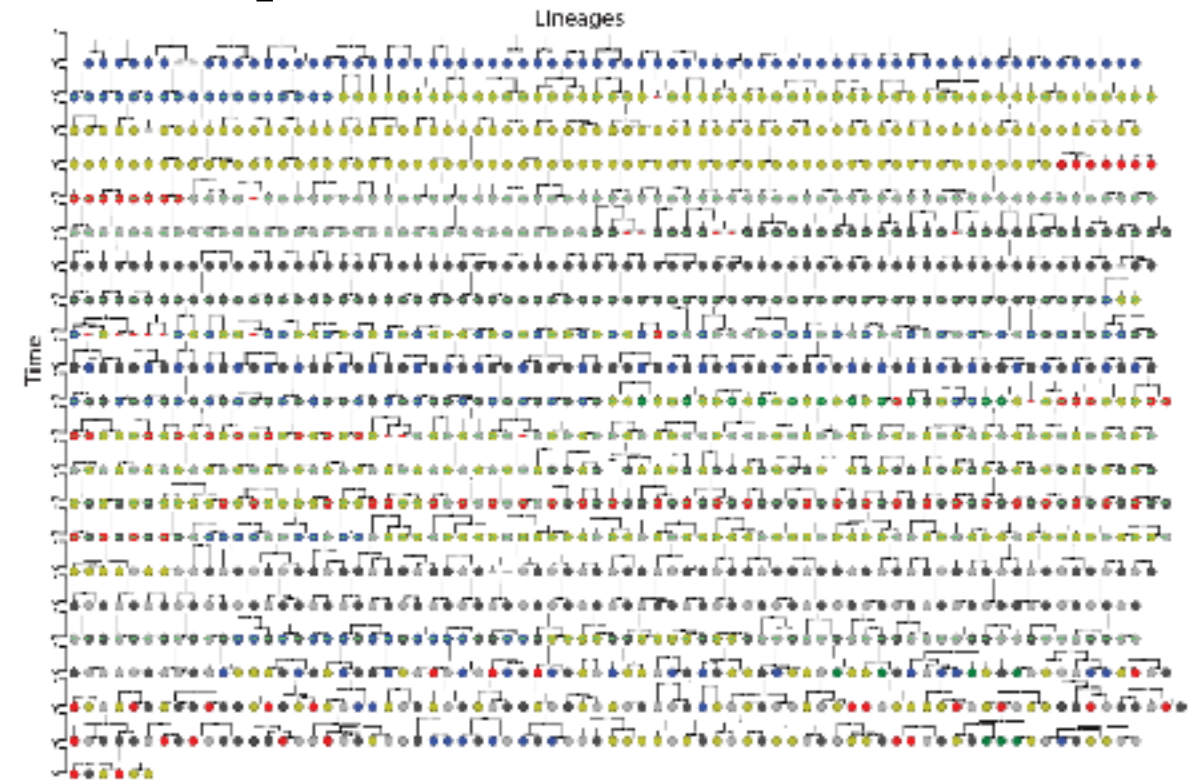
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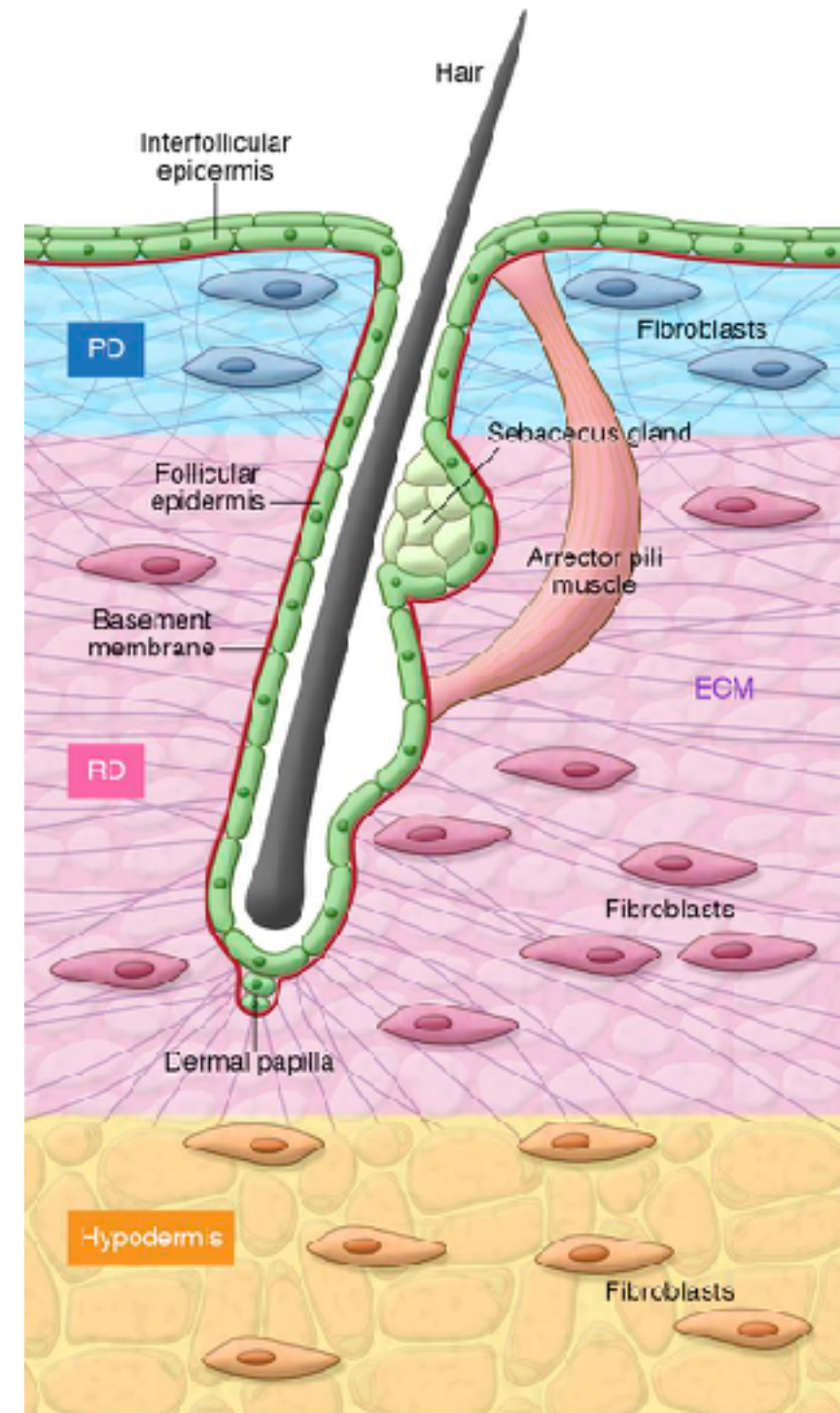
# Single-cell Lipidomics

# The Lipotype Hypothesis

*Lipotypes* represent local energetic minima of the lipid compositional landscape.

*Lipotypes* are both a consequence and a component of differentiation programs that lead to cell state emergence.

Cells populating different regions in tissues and organs are likely to belong to different *lipotypes* and *lipotypes* to mark different anatomical structures
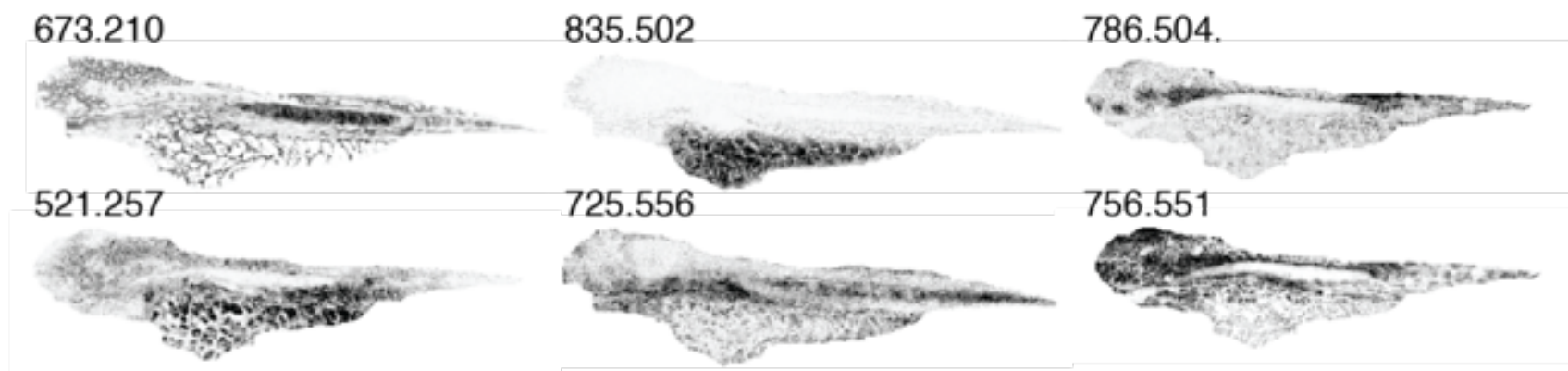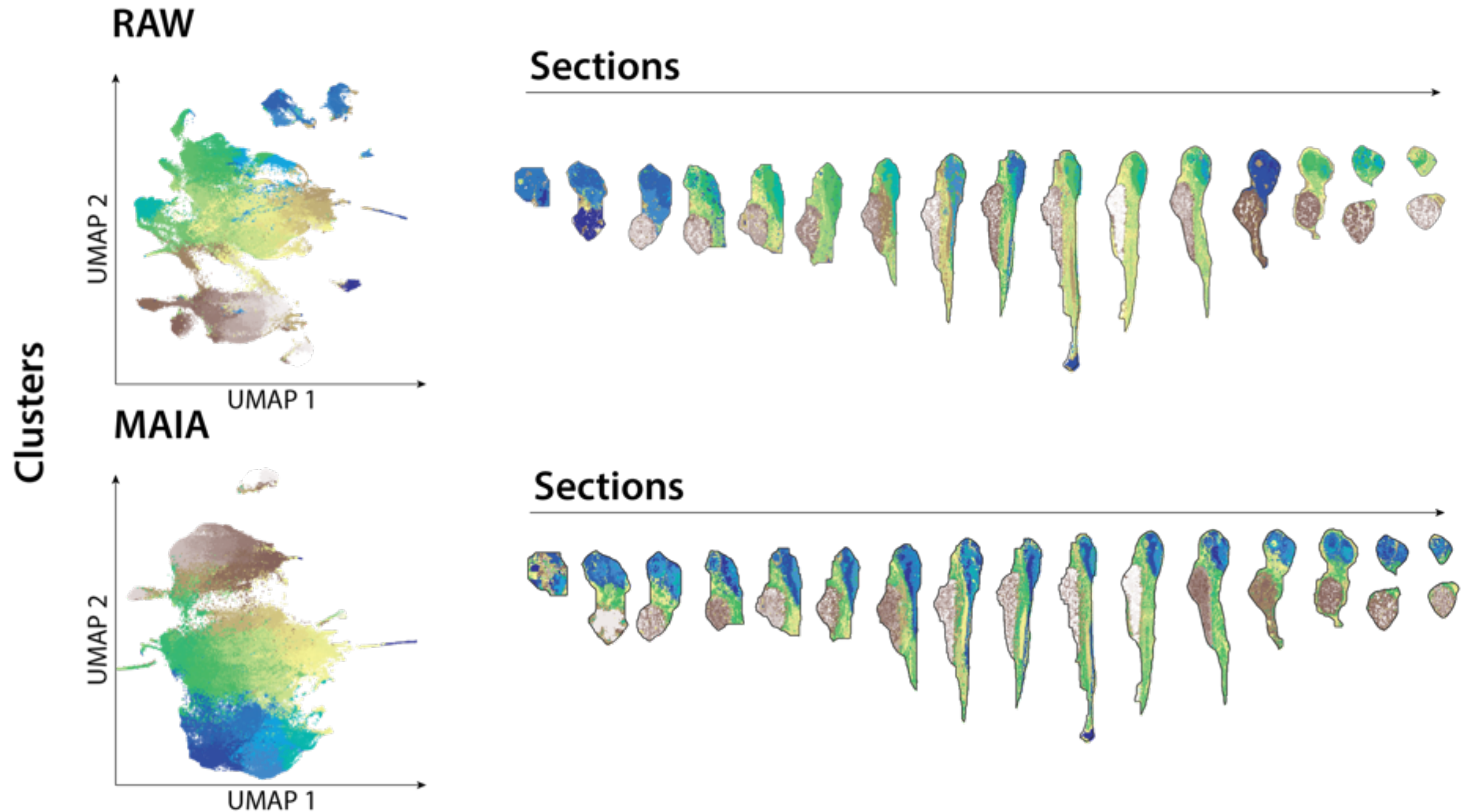
Lipid composition is a major driver for the establishment of cell identity
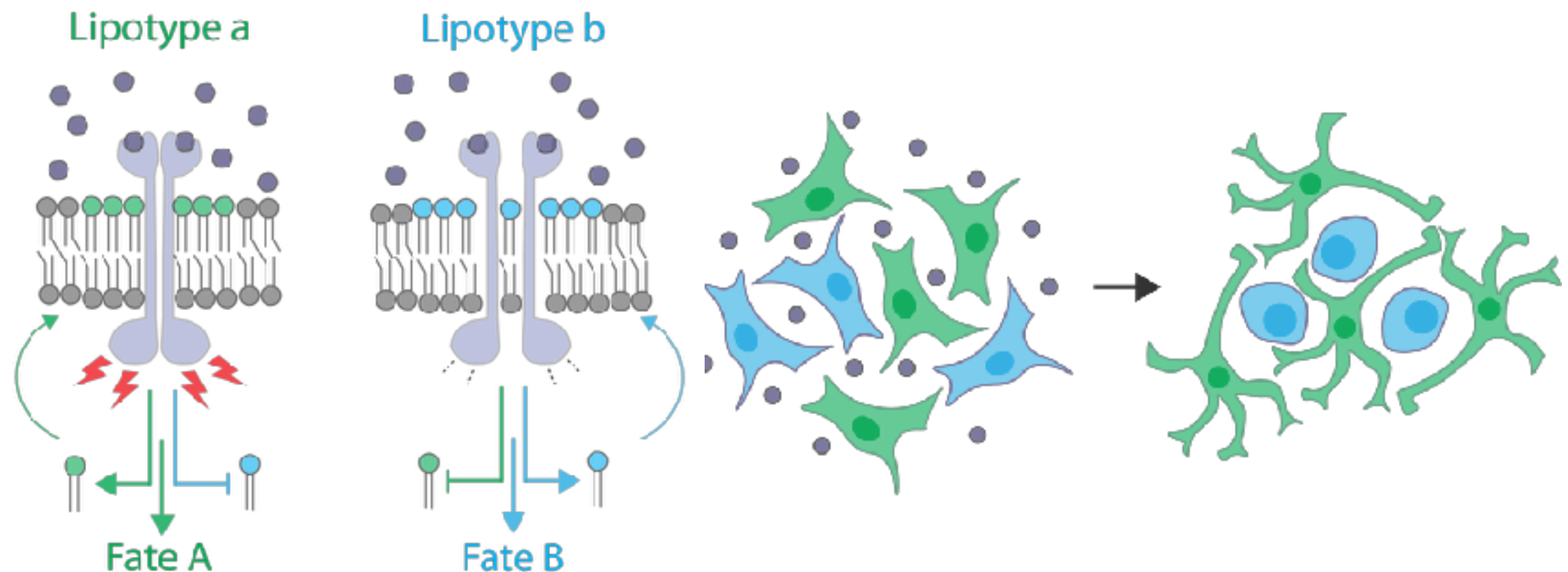
# Towards Metabolic Anatomy
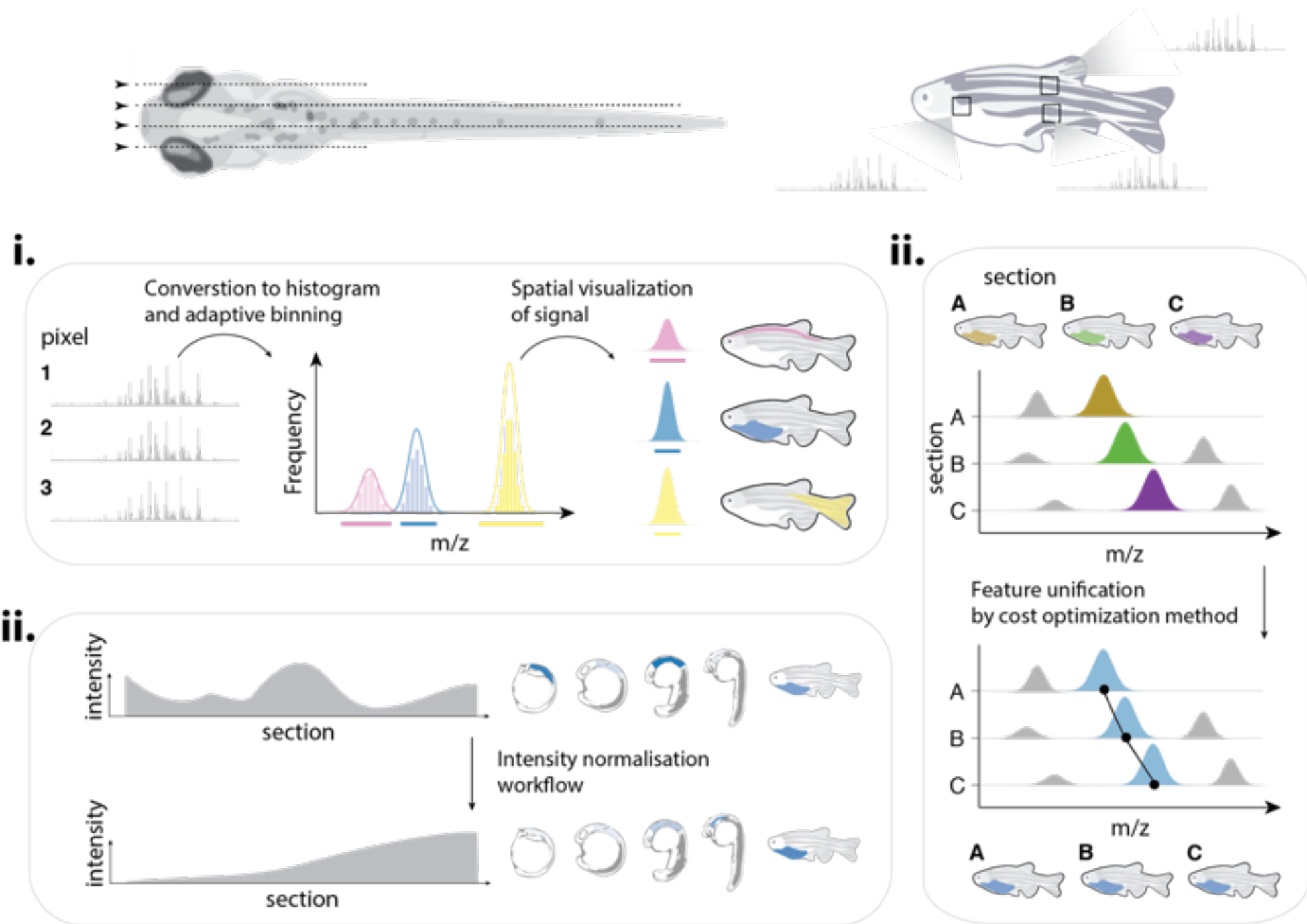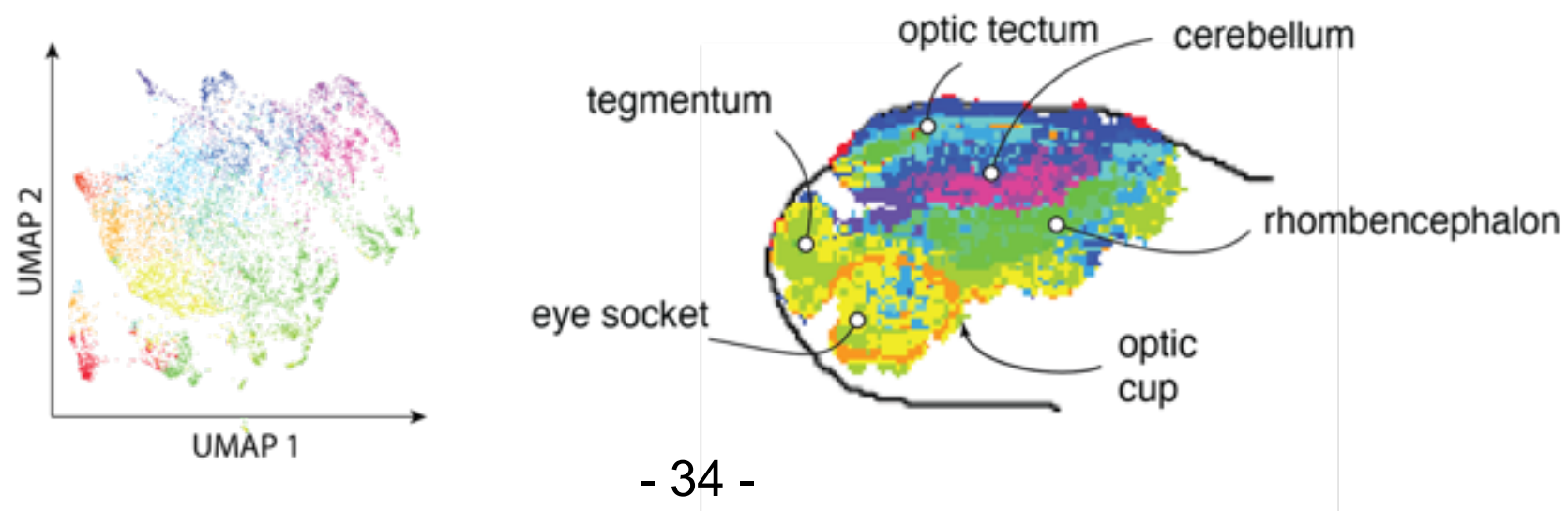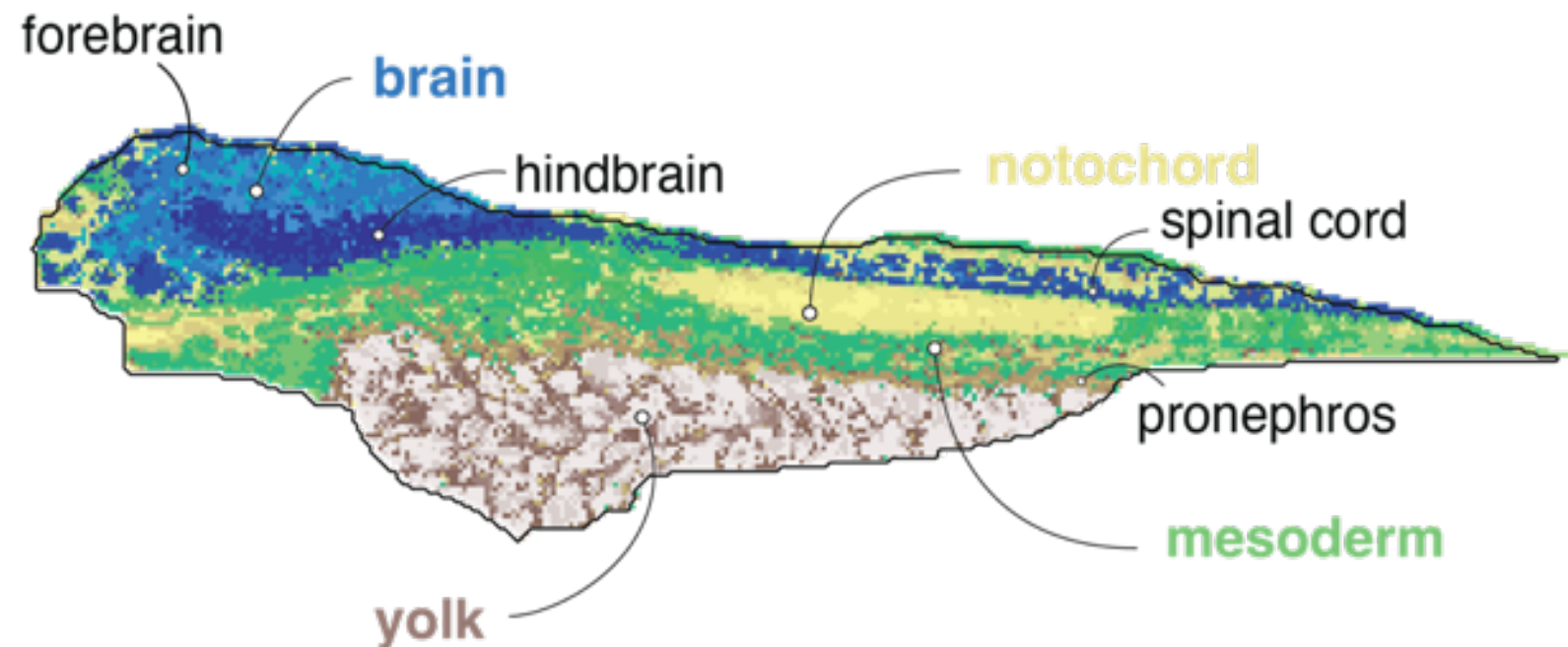
# Towards Metabolic Anatomy

# Towards Metabolic Anatomy
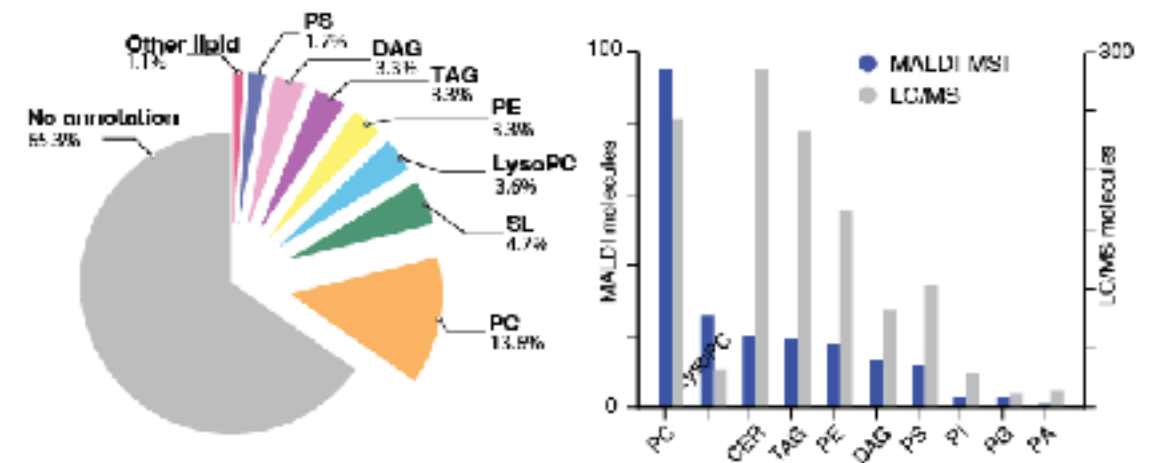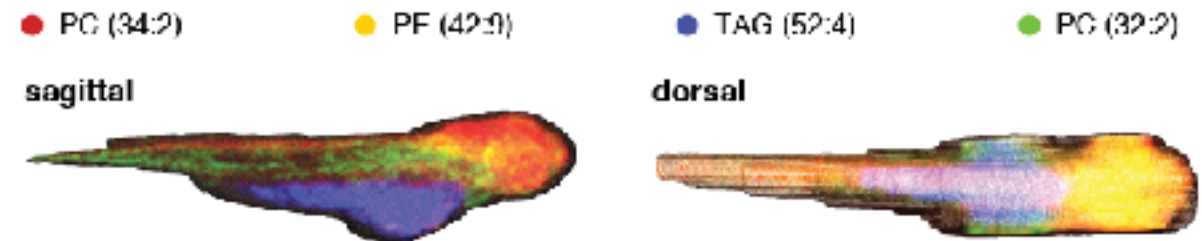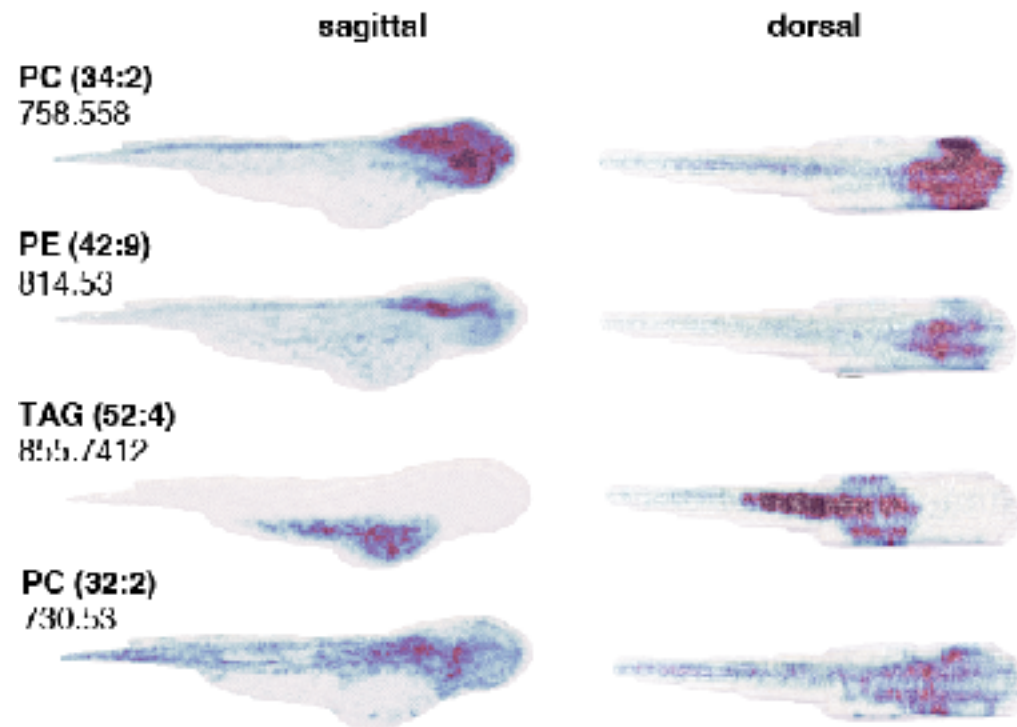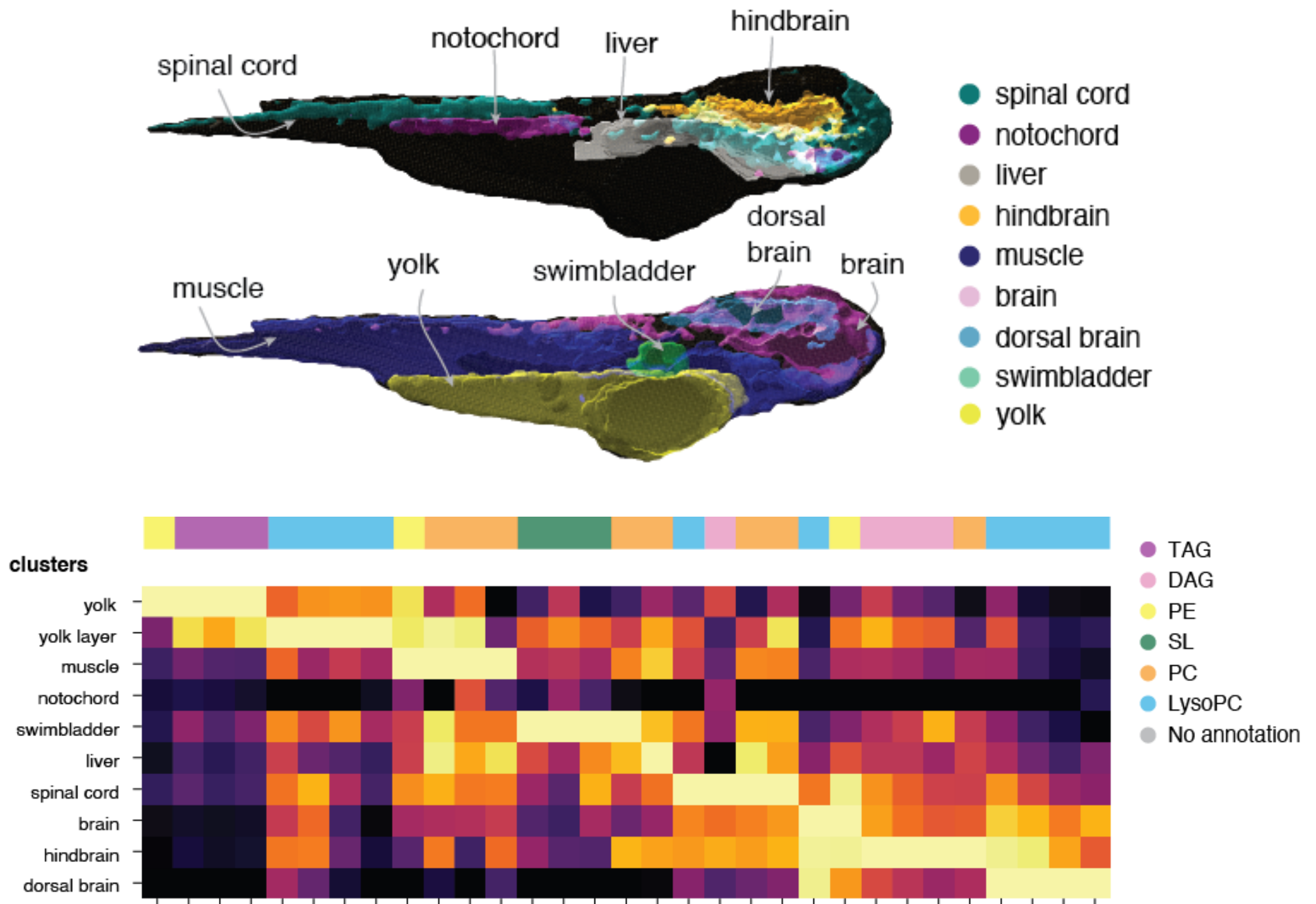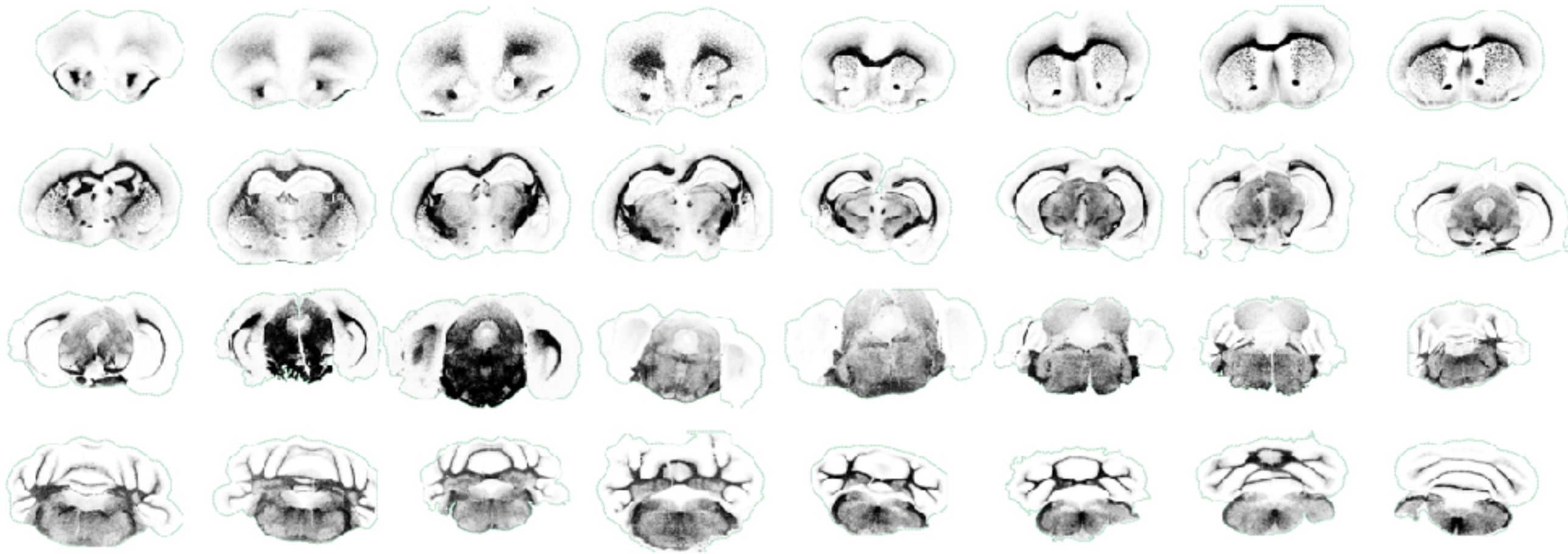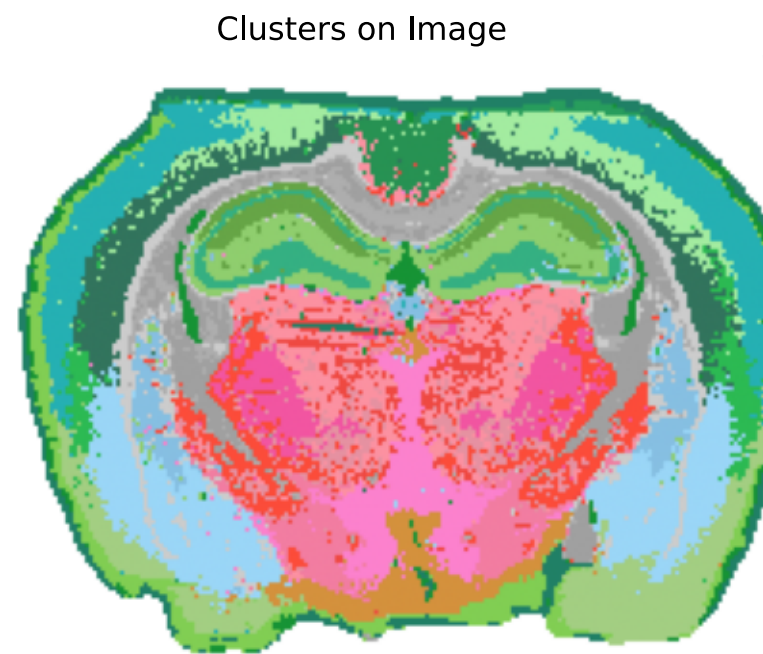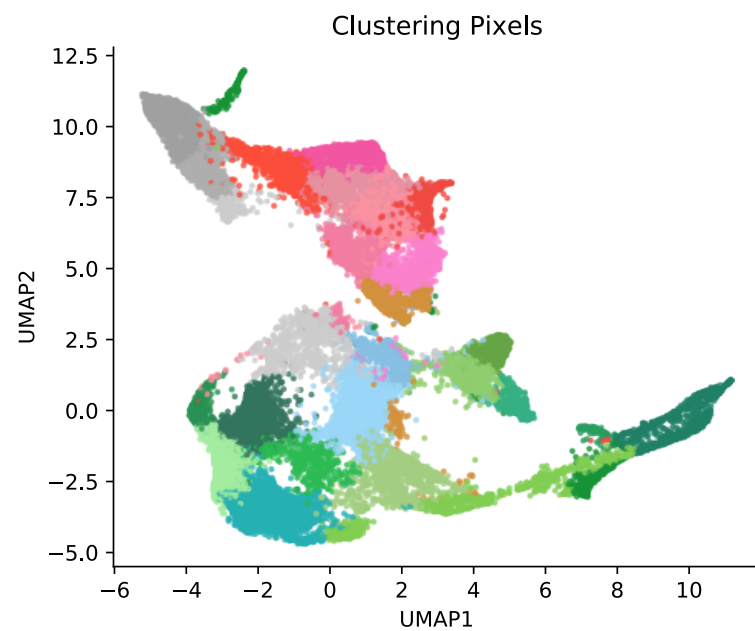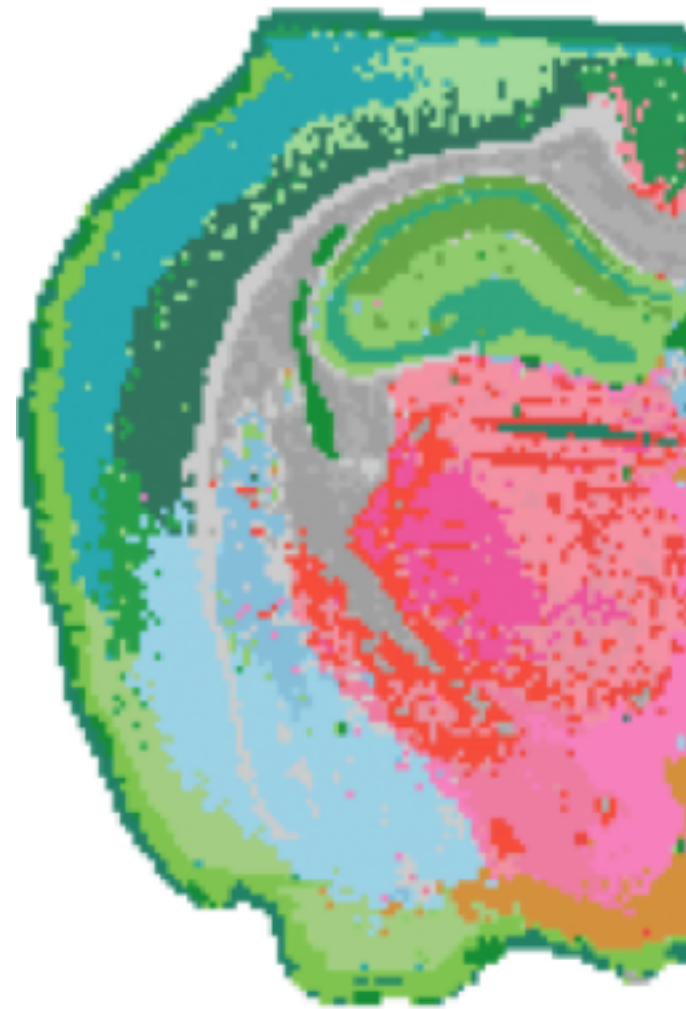
# Towards Metabolic Anatomy
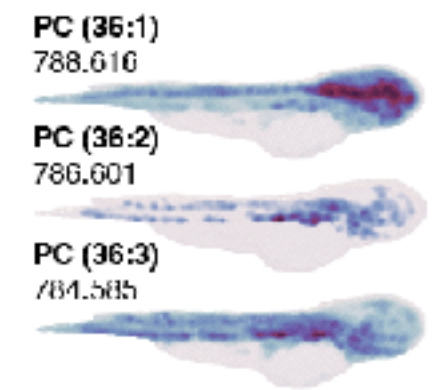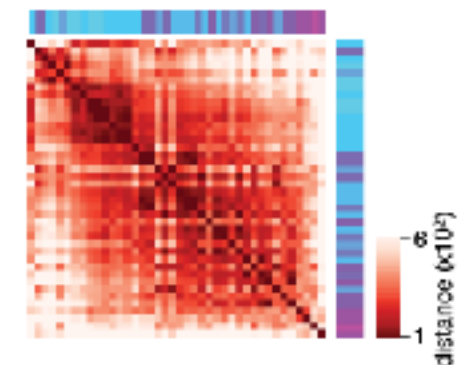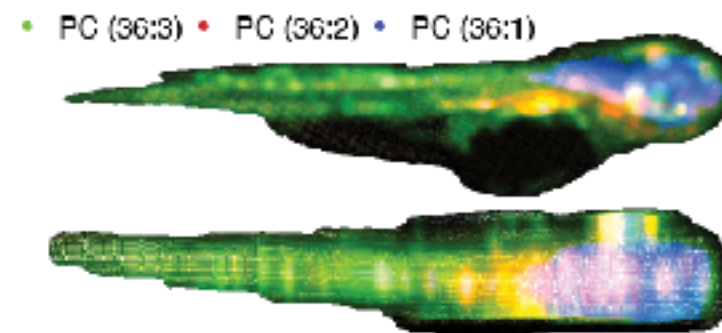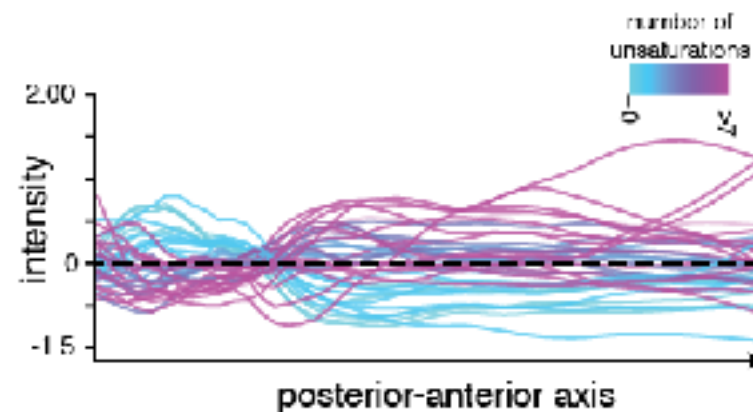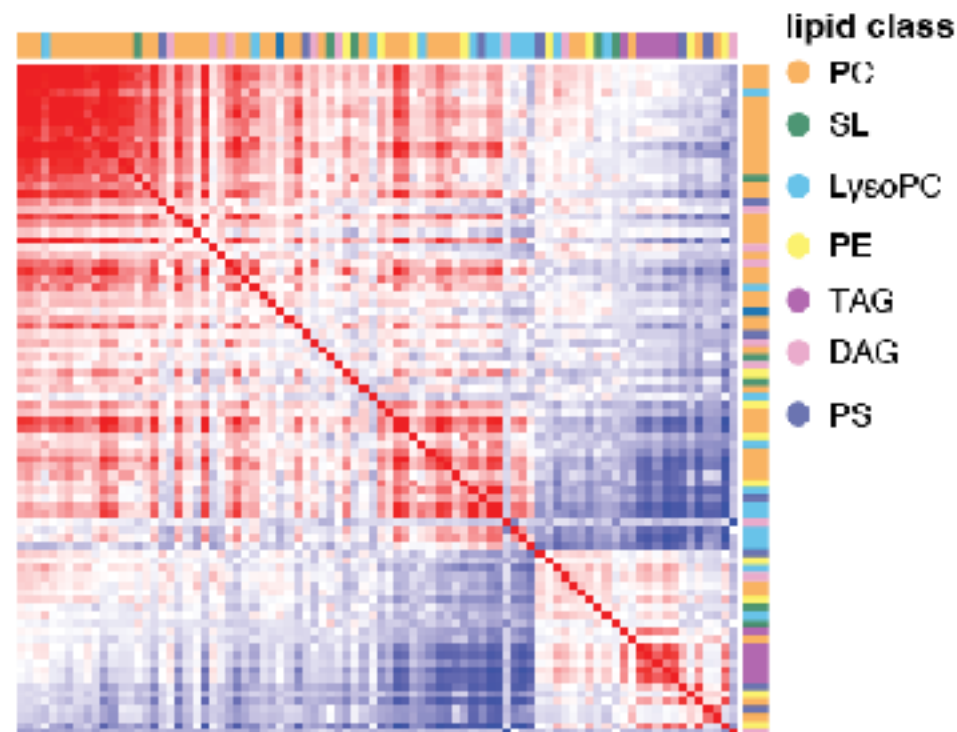
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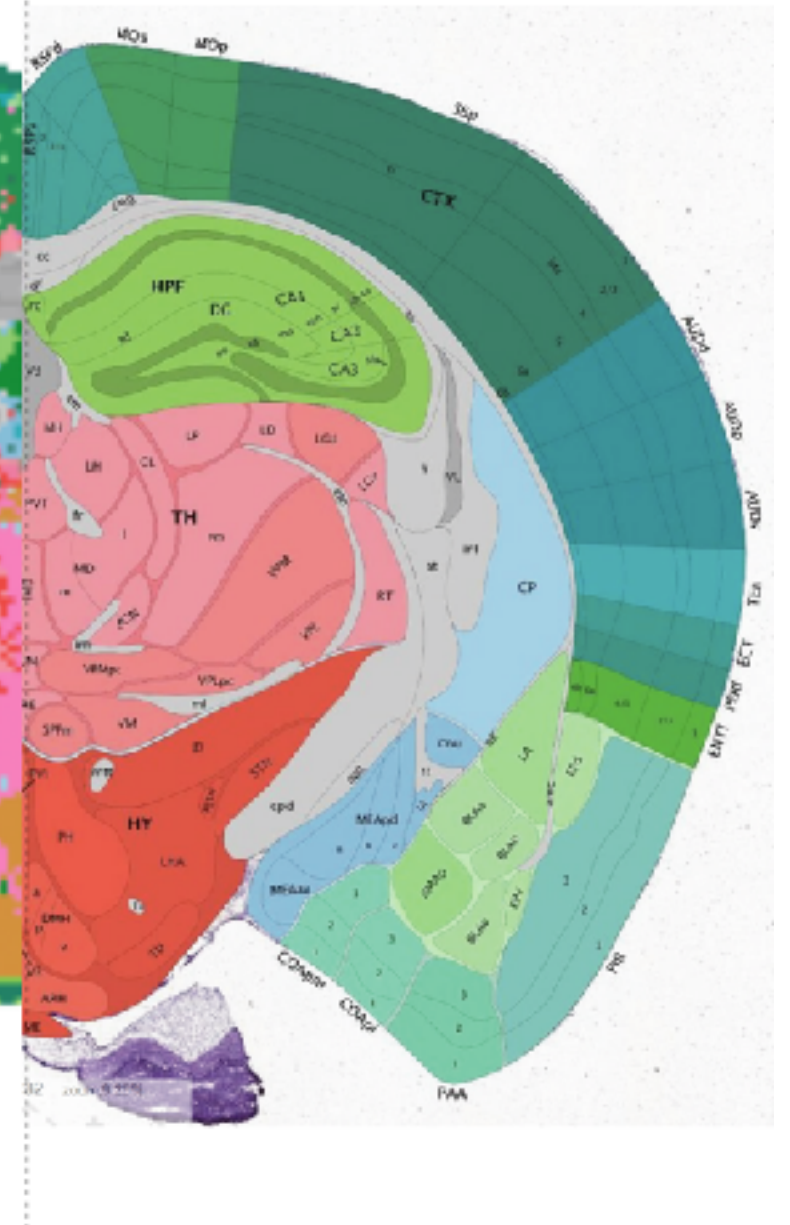
# Towards Metabolic Anatomy

## m/z 864.6322

# Towards Metabolic Anatomy



Clustering Pixels
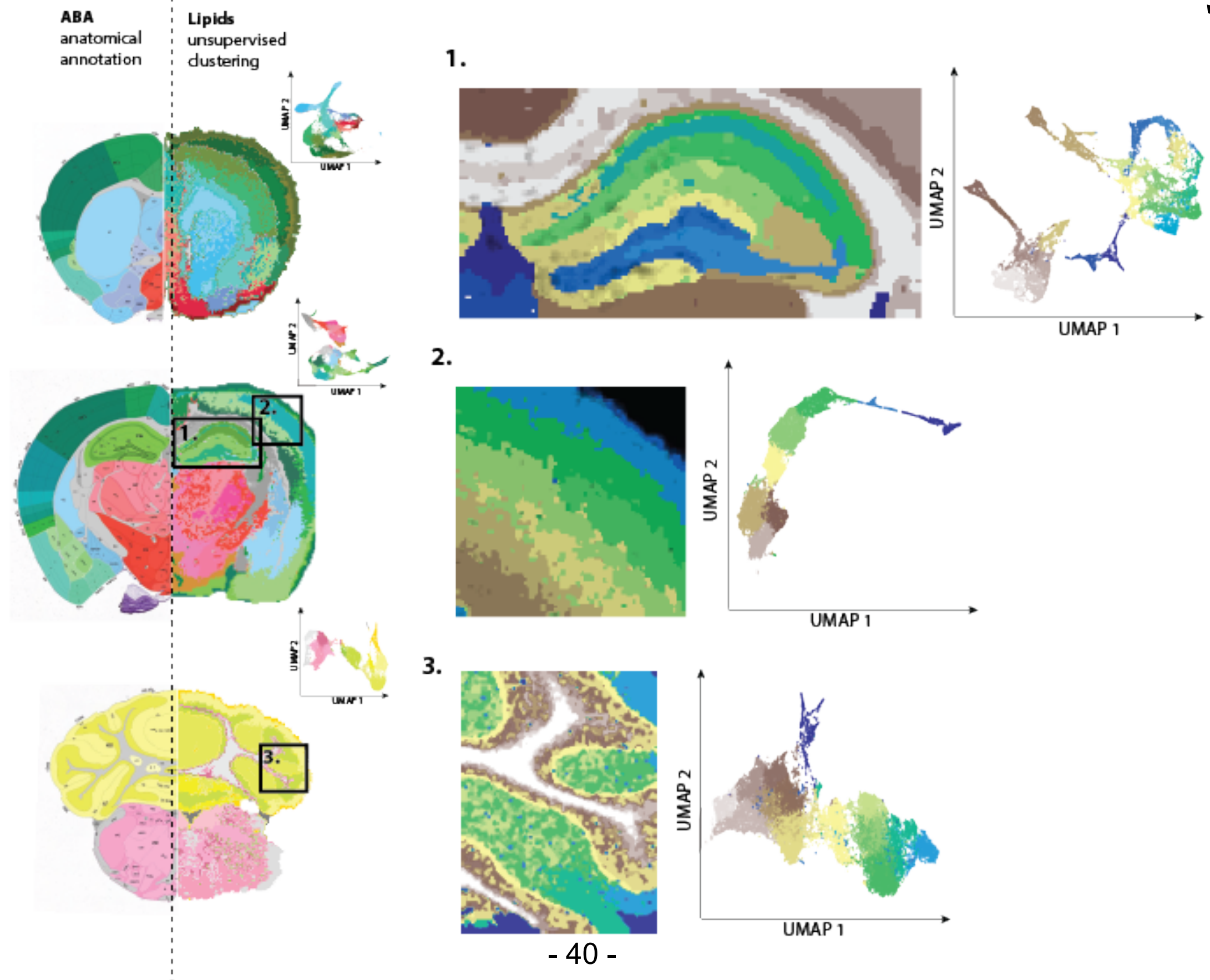
Clusters on Image

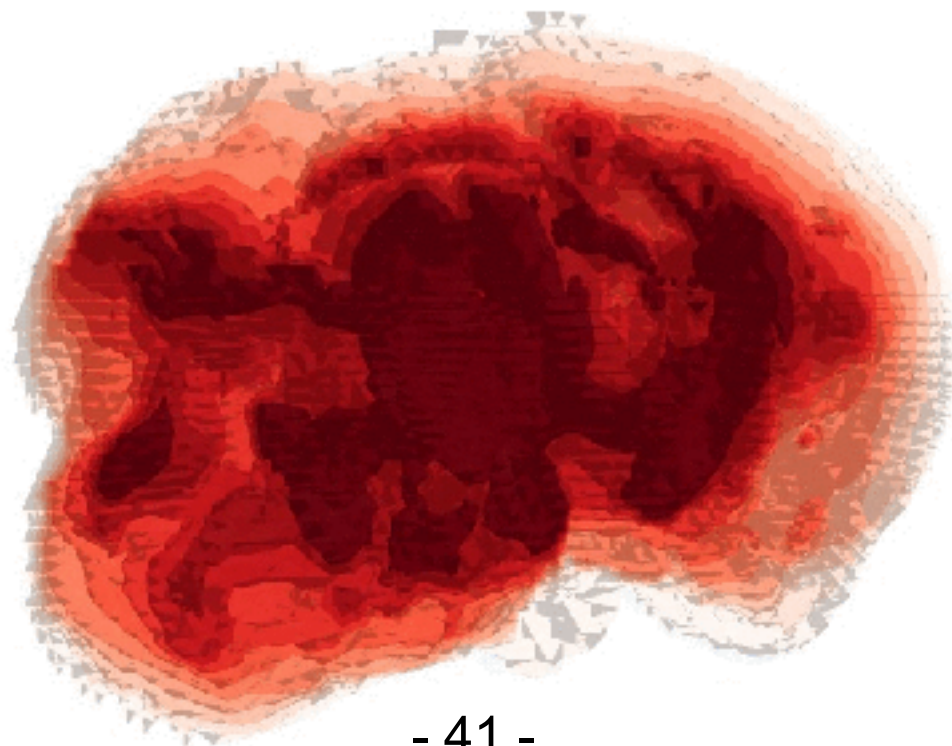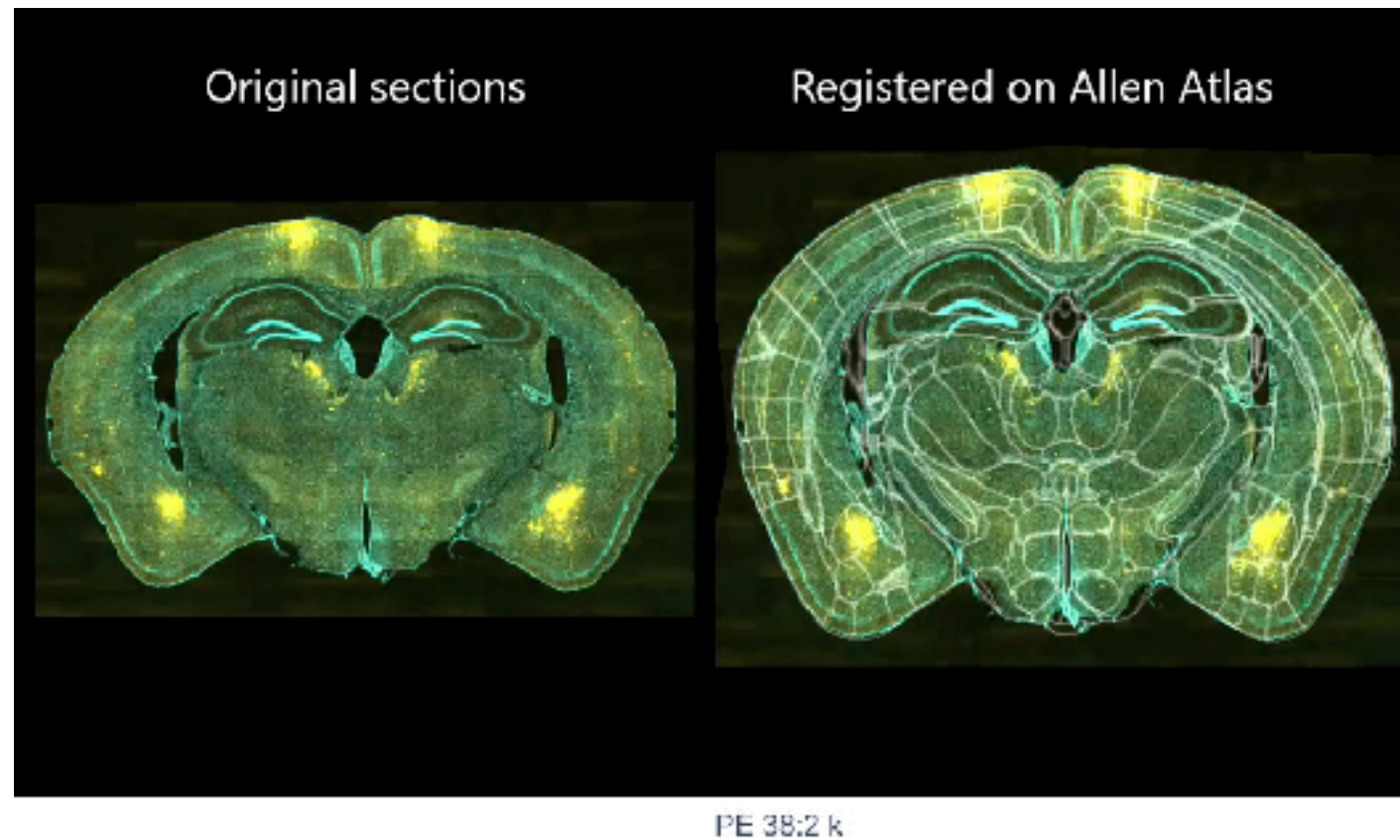Lipid Clusters

Reference Atlas

# Towards Metabolic Anatomy

# Towards Metabolic Anatomy



PE 38:2 k

# Take Home Messages

- Single cell metabolomics is possible (listed among the 7 technologies to watch in 2023 by *Nature*).

- Imaging Mass Spectrometry is a suitable way to perform single cell metabolomics.

- Sigle cell and spatial metabolomics provide informations about cell-to-cell heterogeneity and tissue patterning from a biochemical perspective.