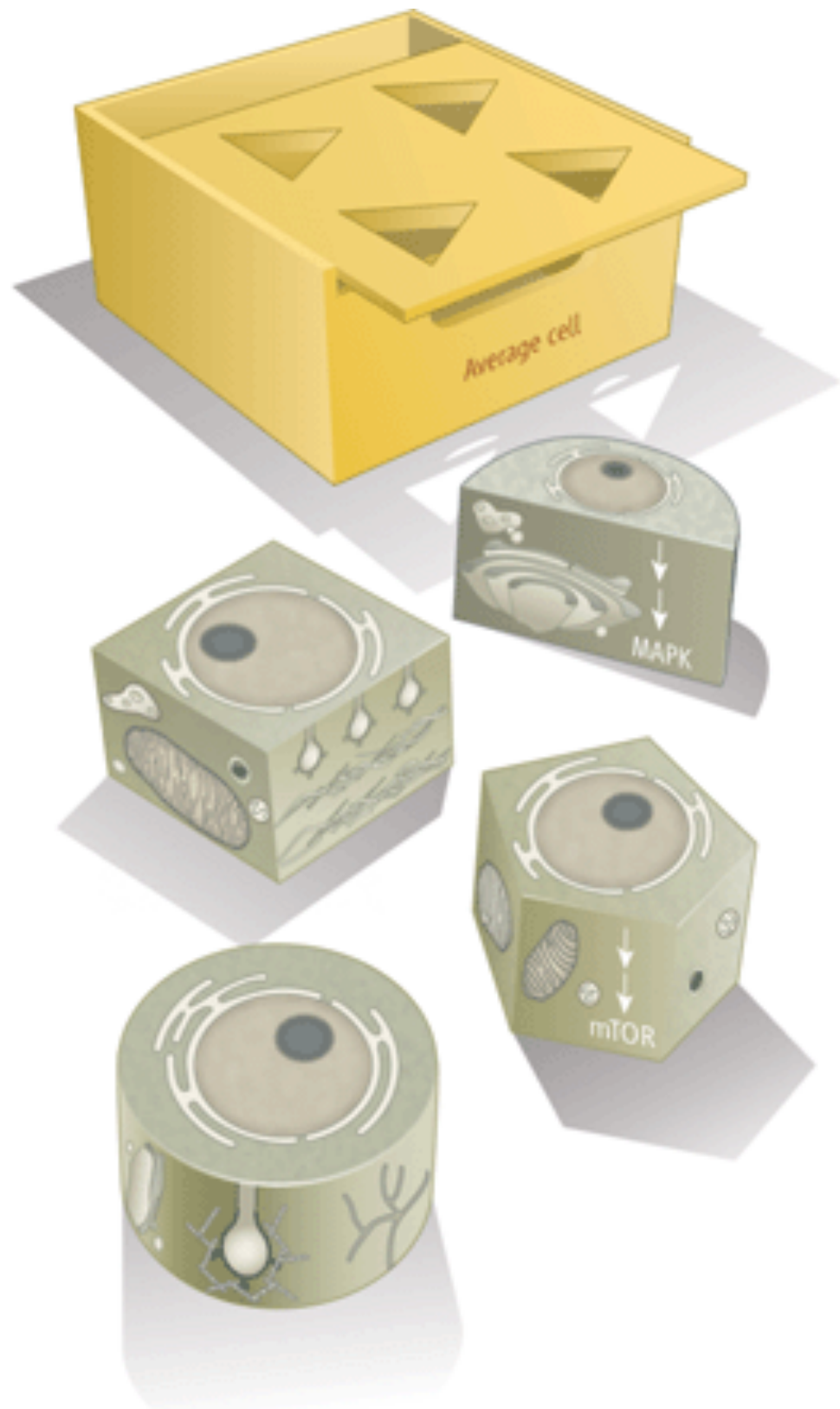


Single-cell biology

Single-cell Lipidomics

Single-cell biology

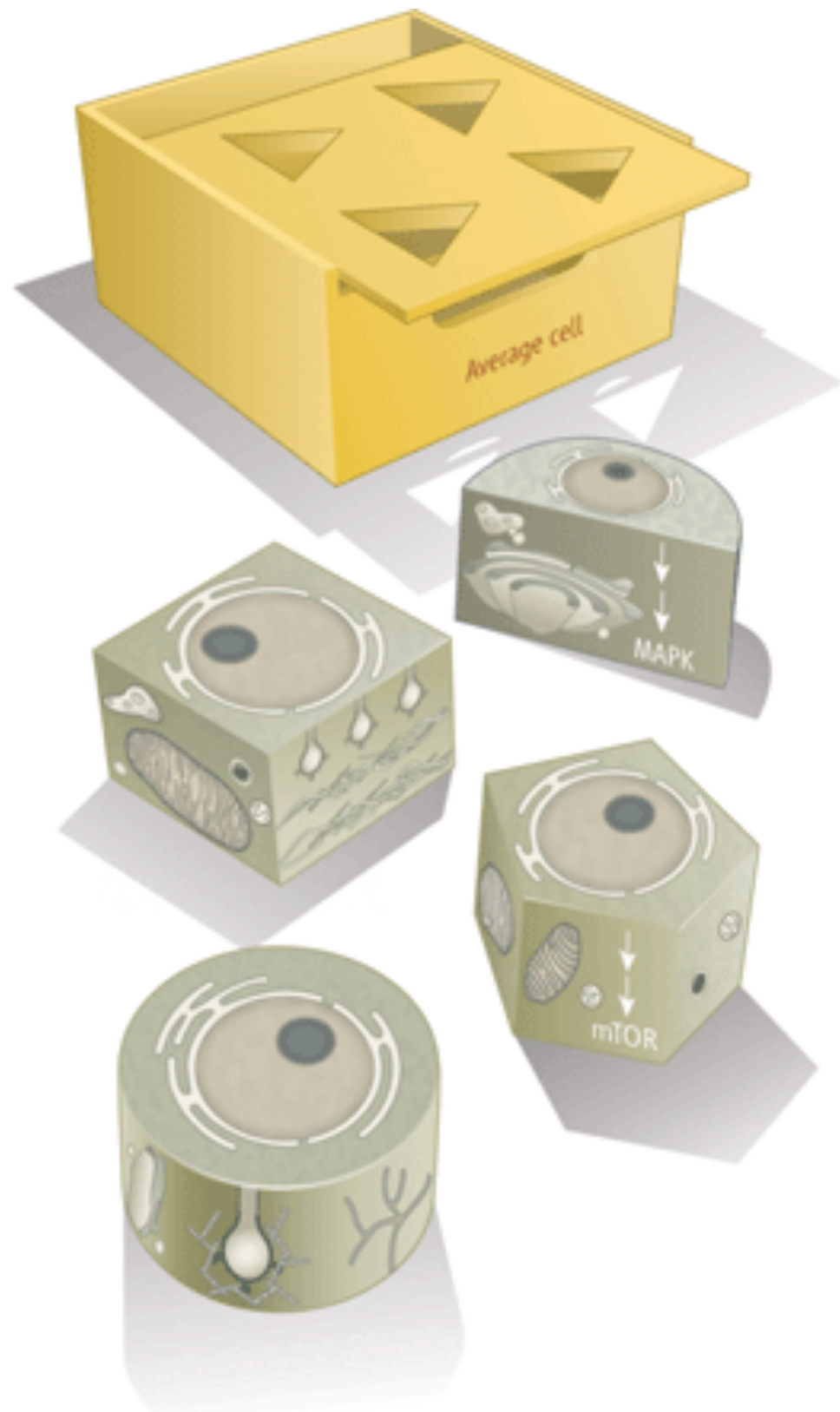


A single cell can be regarded as the smallest functional biological unit and its chemical activities can provide unique insights into biological processes.

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.

Single-cell biology

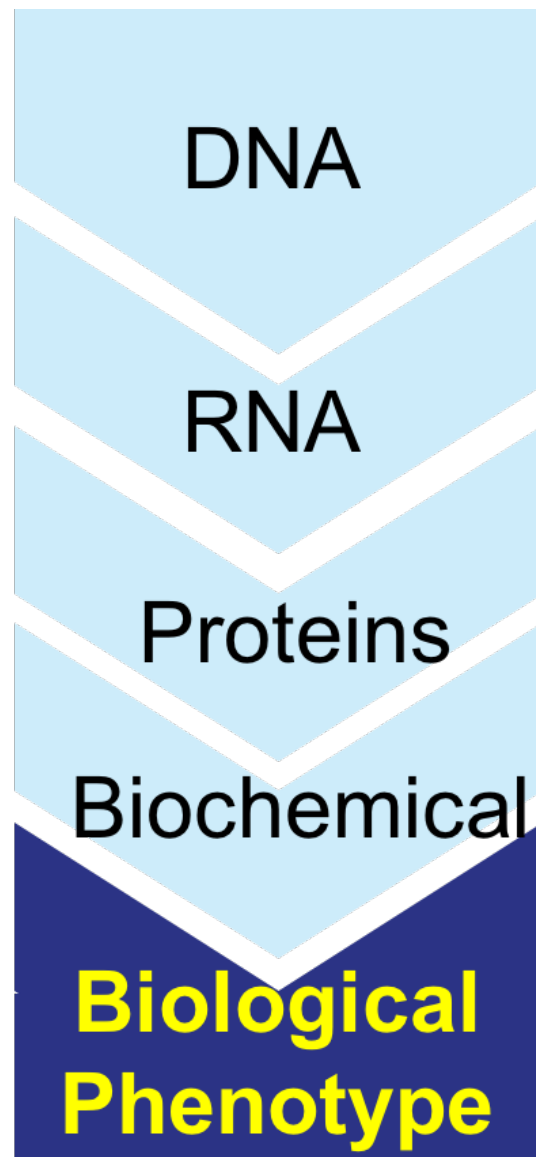


Single cell Biology aims to reveal

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

What can we profile
in single cells?

Single-cell biology



Genomics

Transcriptomics

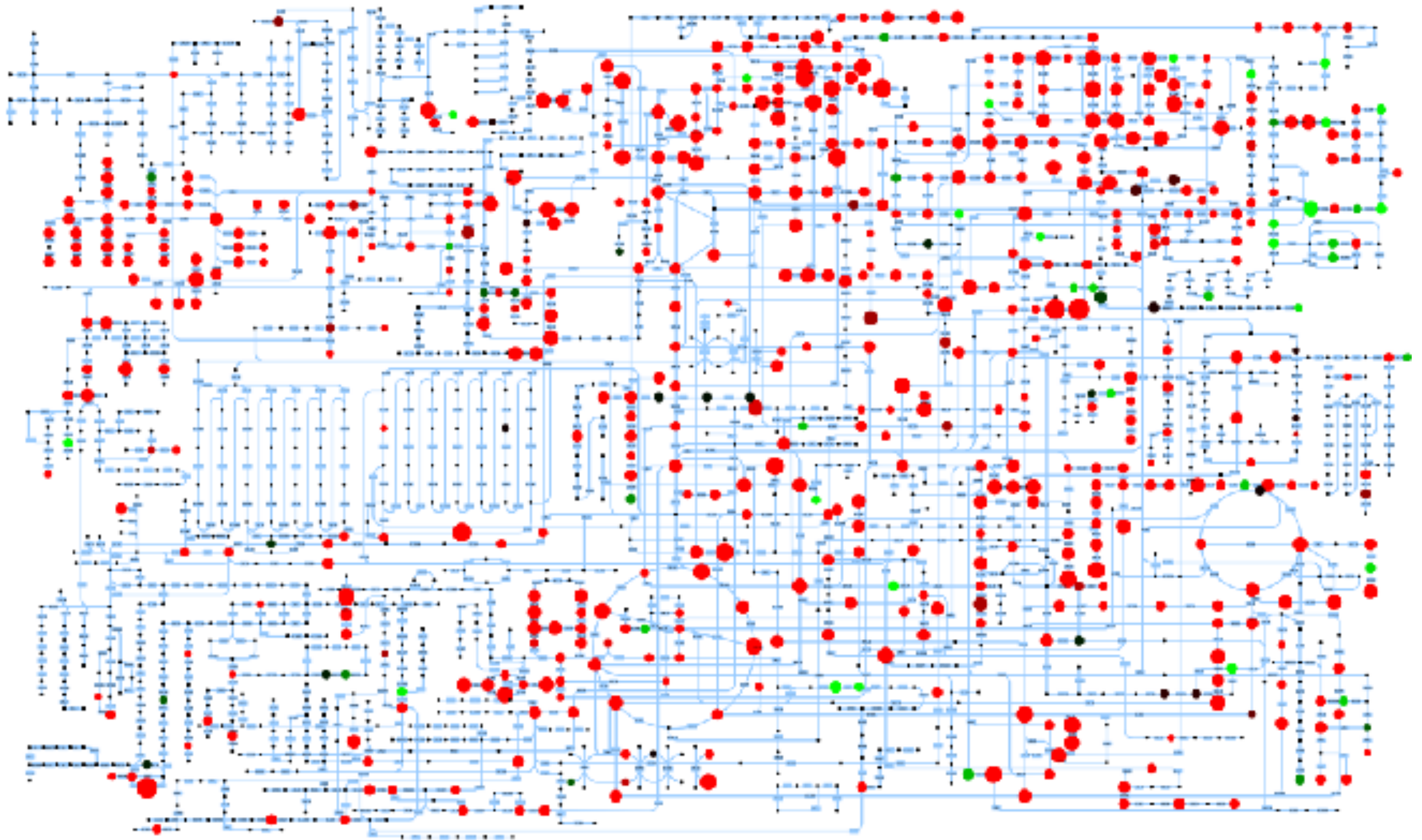
Proteomics

Metabolomics

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

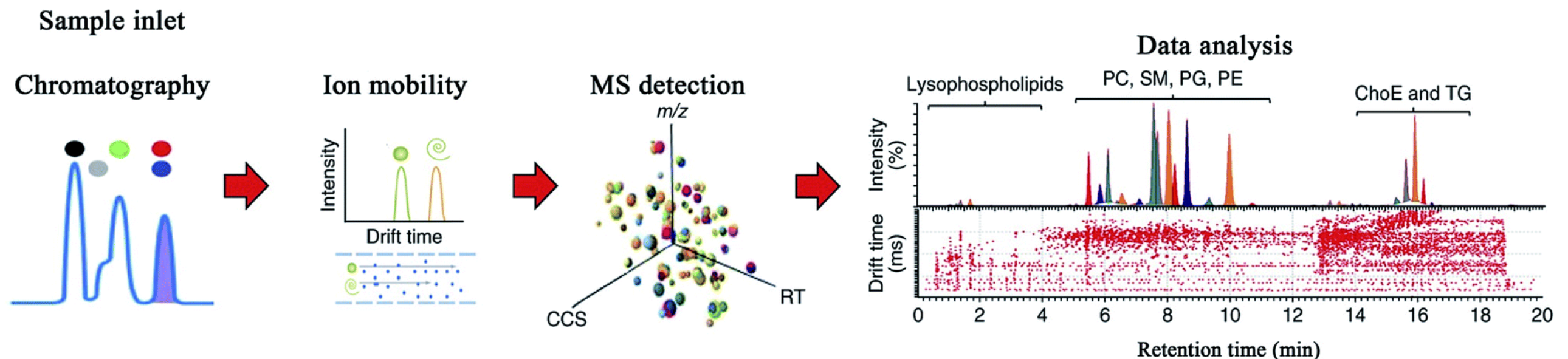
Can we profile the metabolites in single cells?

Single-cell biology



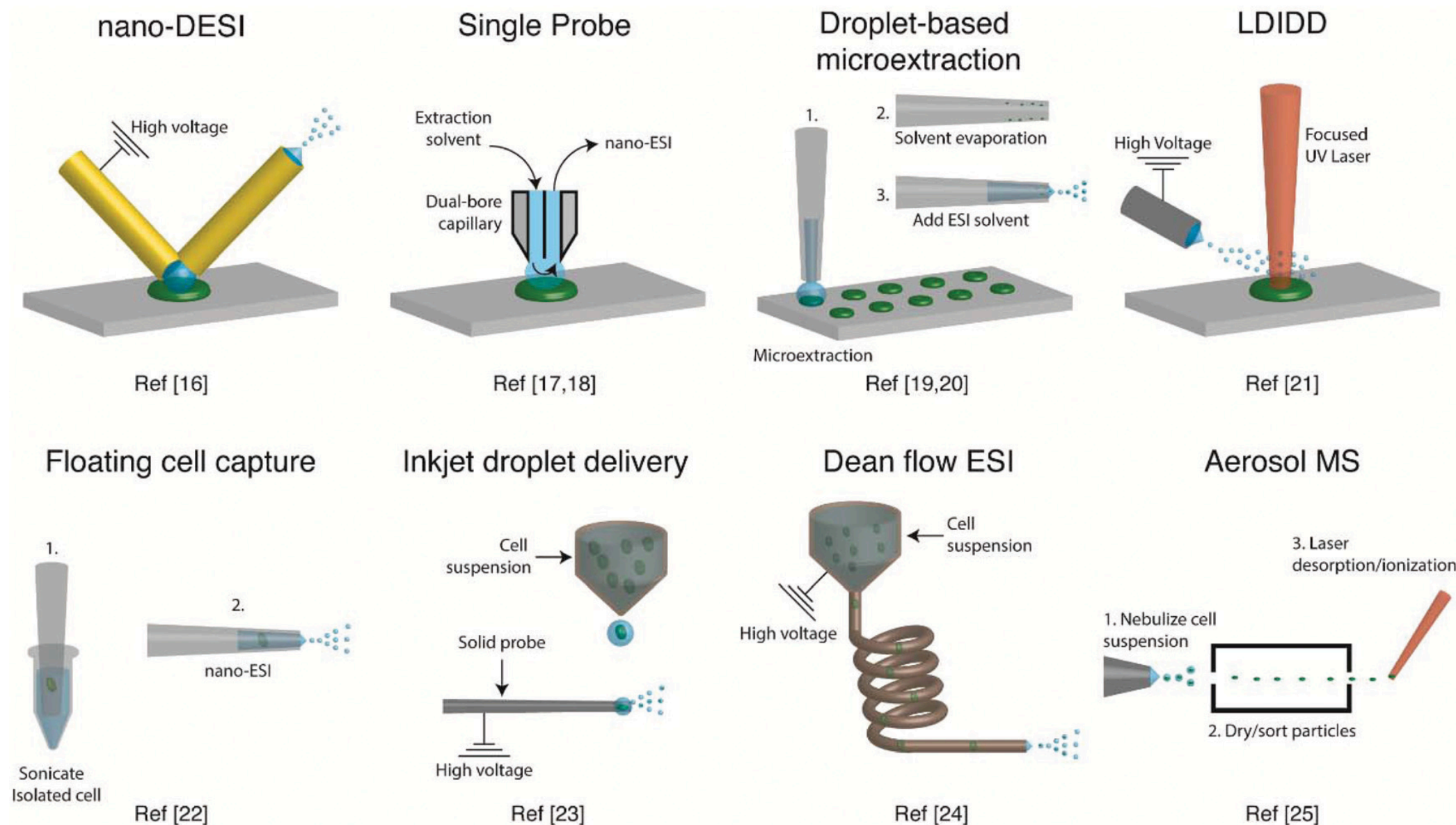
Single-cell biology

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.

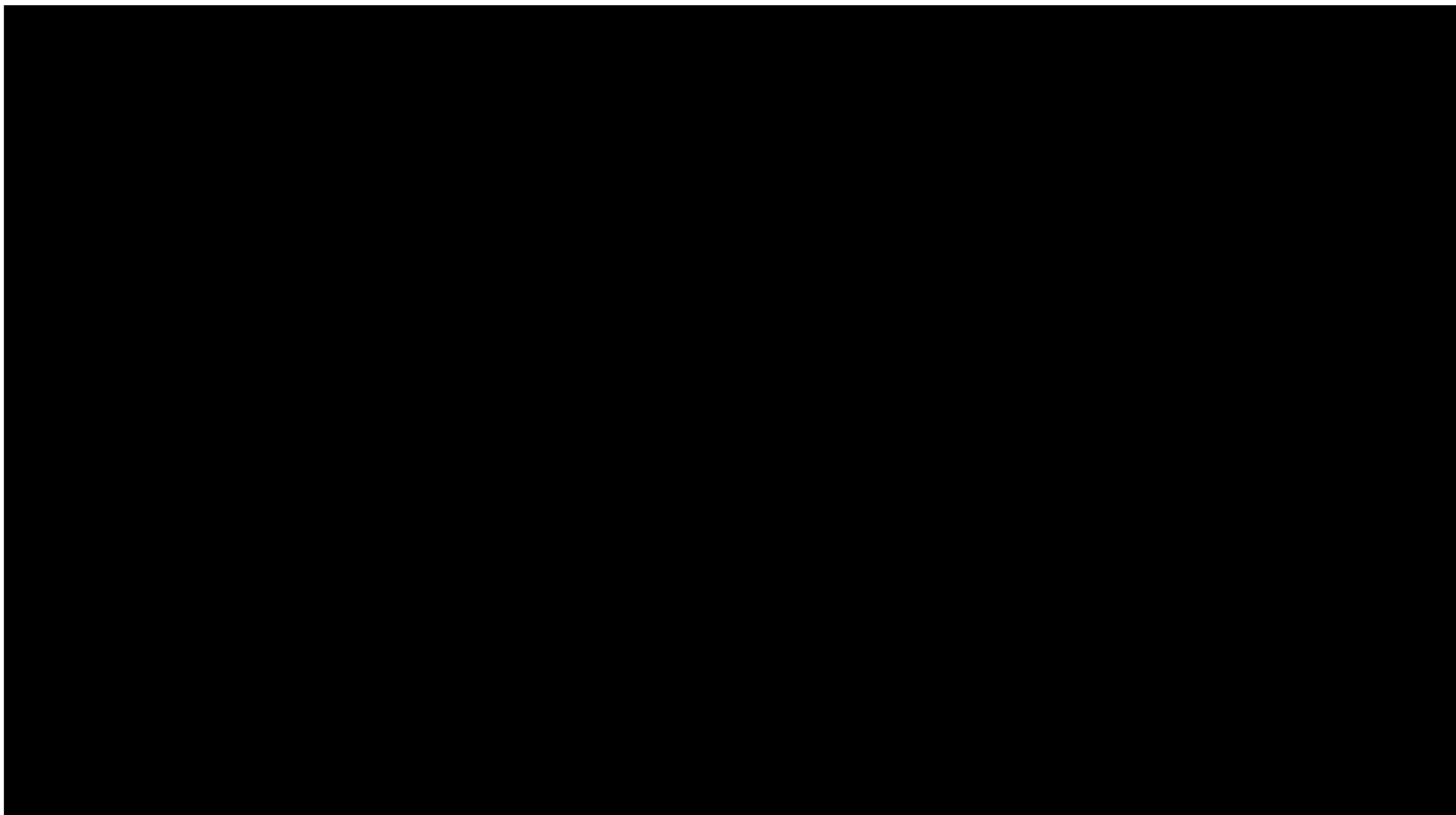


With high sensitivity and specificity, wide molecular coverage, relative quantitation, and structural identification capabilities, MS is becoming an important tool for **Single-cell metabolomics**. This, by the way, presents many challenges due to the limited sample volume, low analyte amounts, and rapid 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.

Single-cell Metabolomics

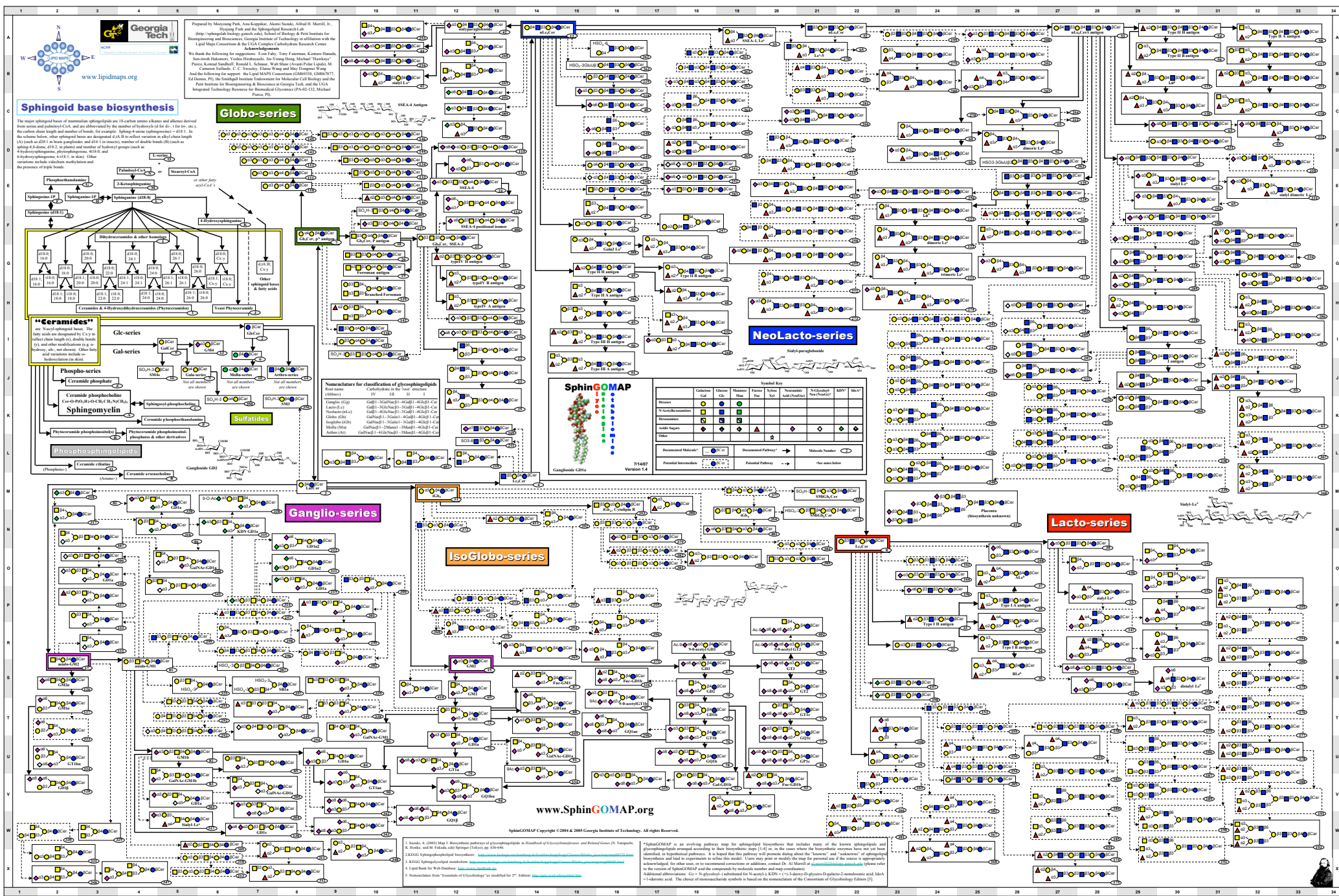


Single-cell Metabolomics



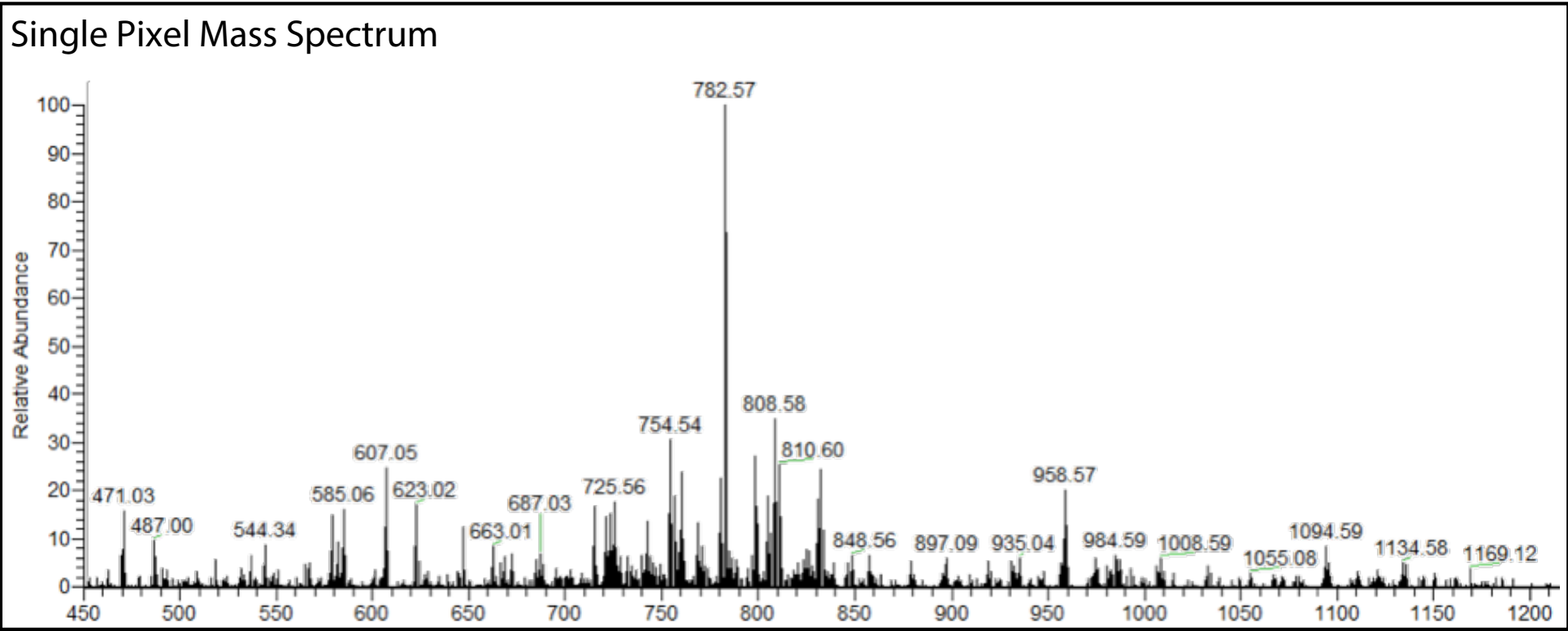
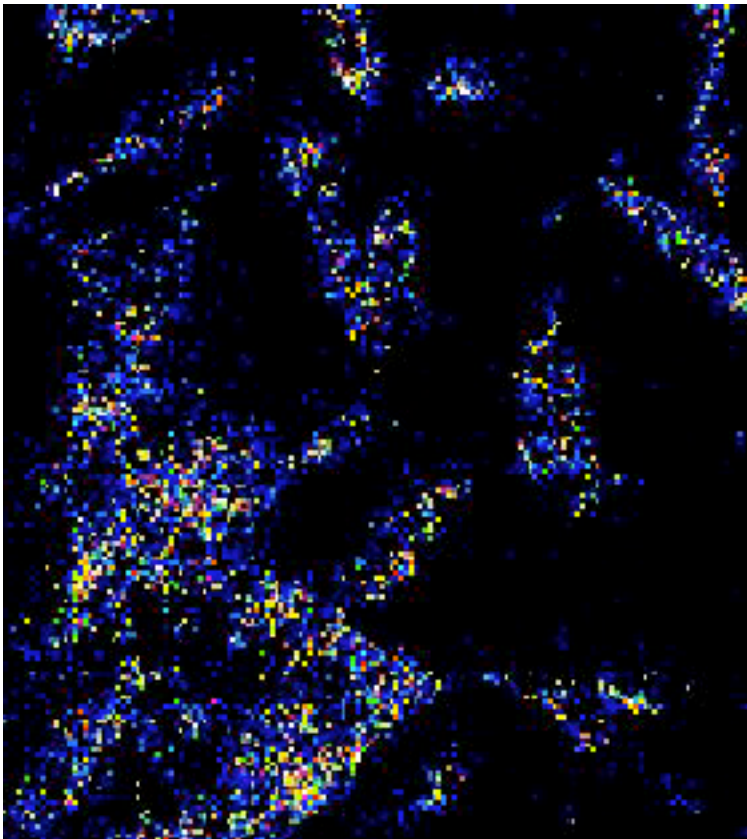
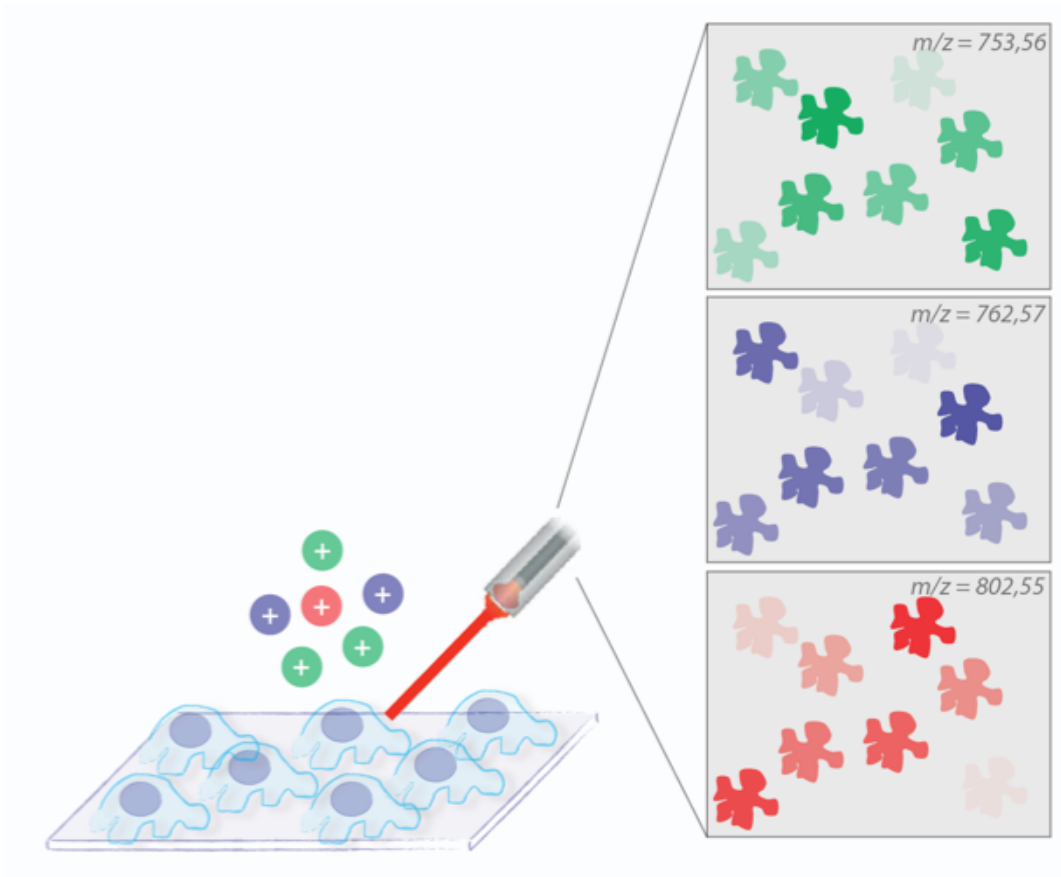
Single-cell Lipidomics

Lipids are the most abundant and functionally relevant metabolites



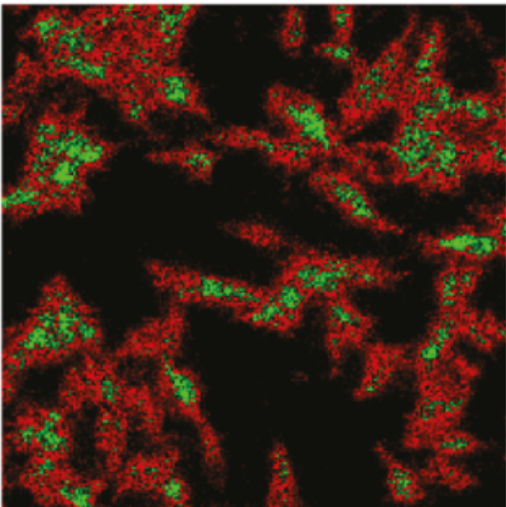
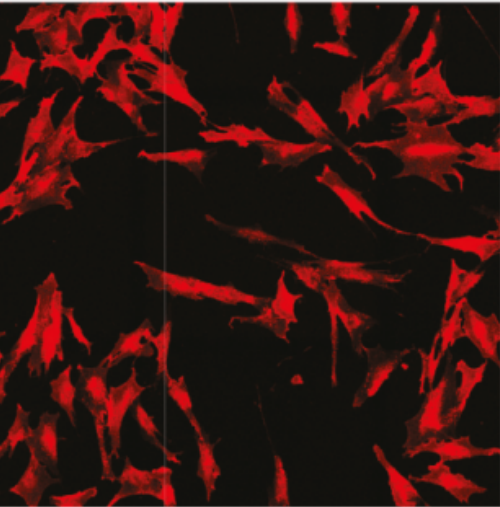
Why do we have so many membrane lipids?
What is their role in cell fate determination?

Single-cell Lipidomics



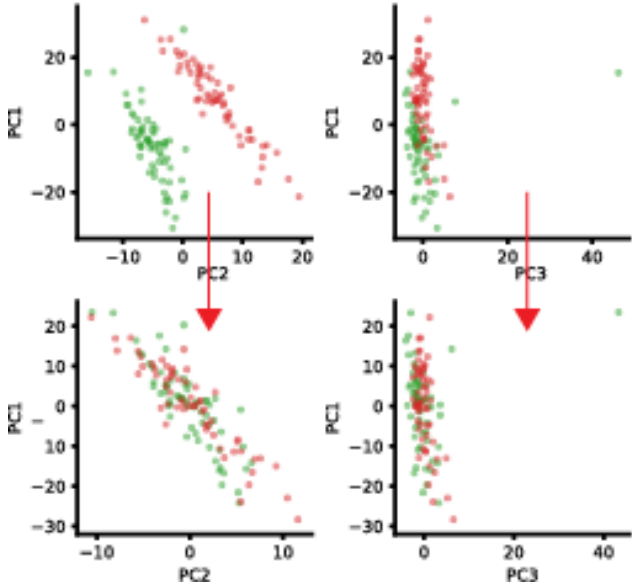
Single-cell Lipidomics

Optical Image → Mass Image → Segmentation → Single-cell Data –

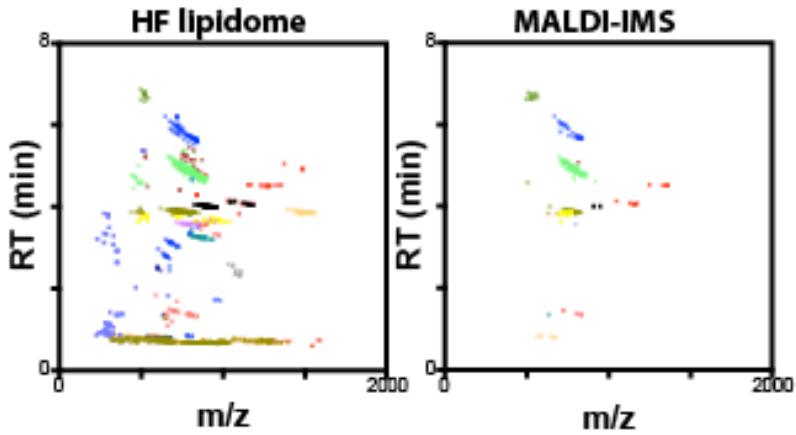


	Cell 1	Cell 2	Cell 3	Cell i
peak 1	0.123	0.473	12.87	0.028
peak 2	6.378	6.598	5.988	6.003
peak 3	4.765	4.033	1.021	4.184
peak 4	156.9	230.4	145.8	149.3
peak i	0.473	0.023	0.510	0.772

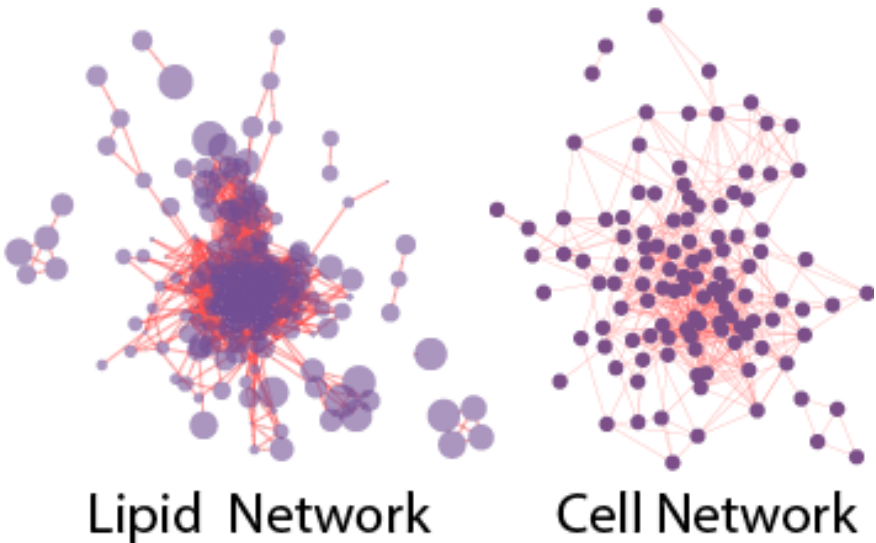
Normalization



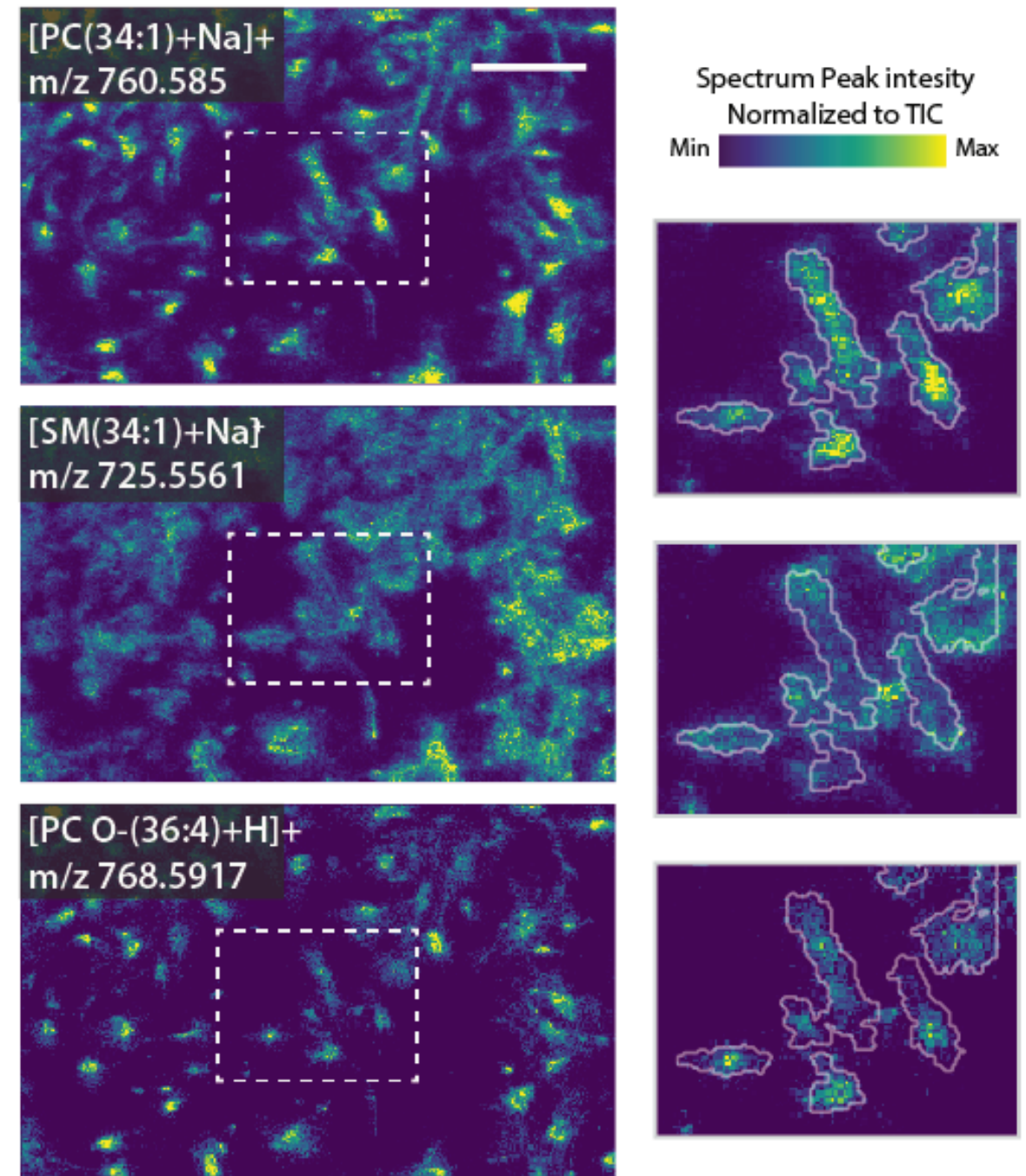
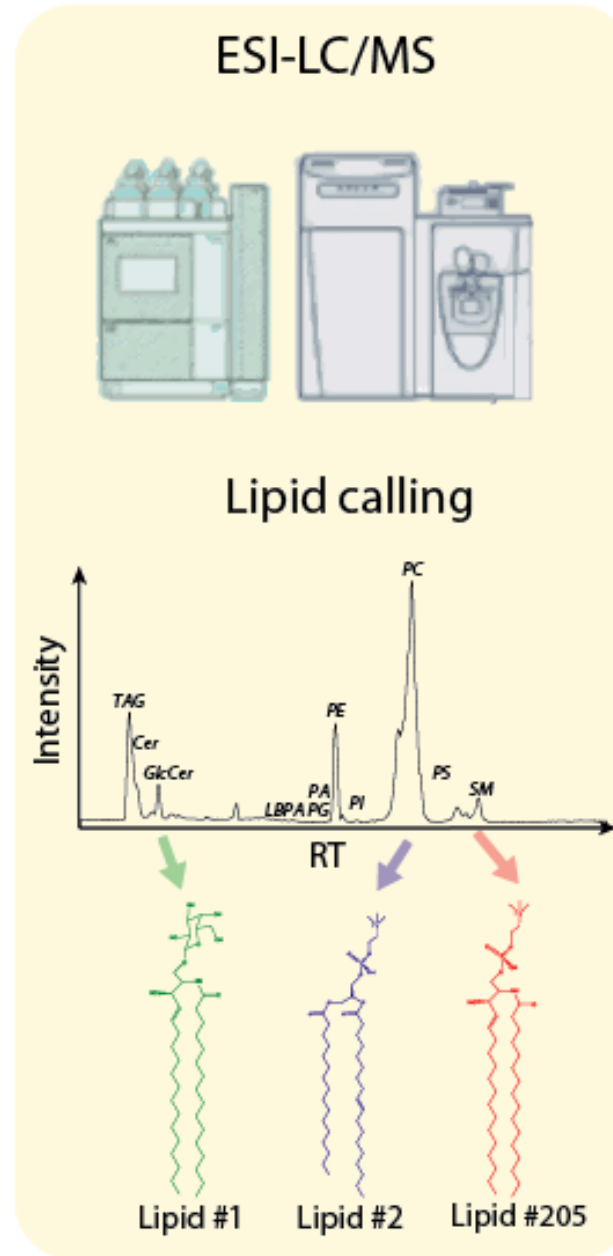
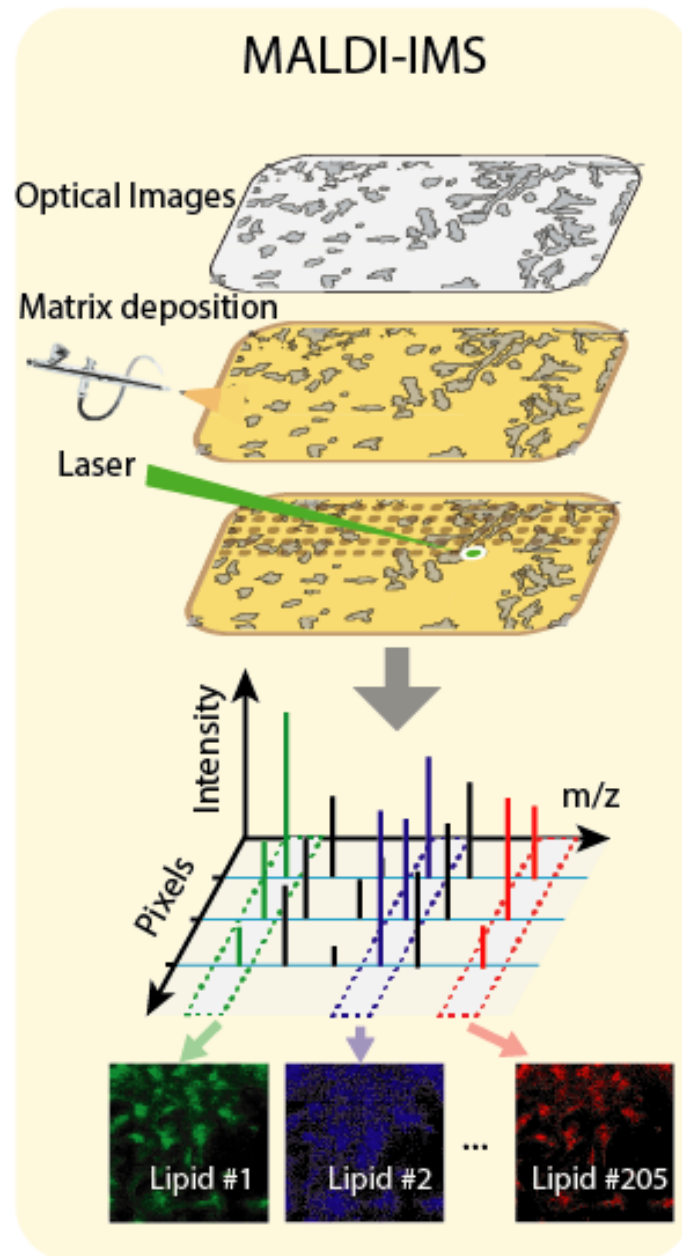
Peak identification



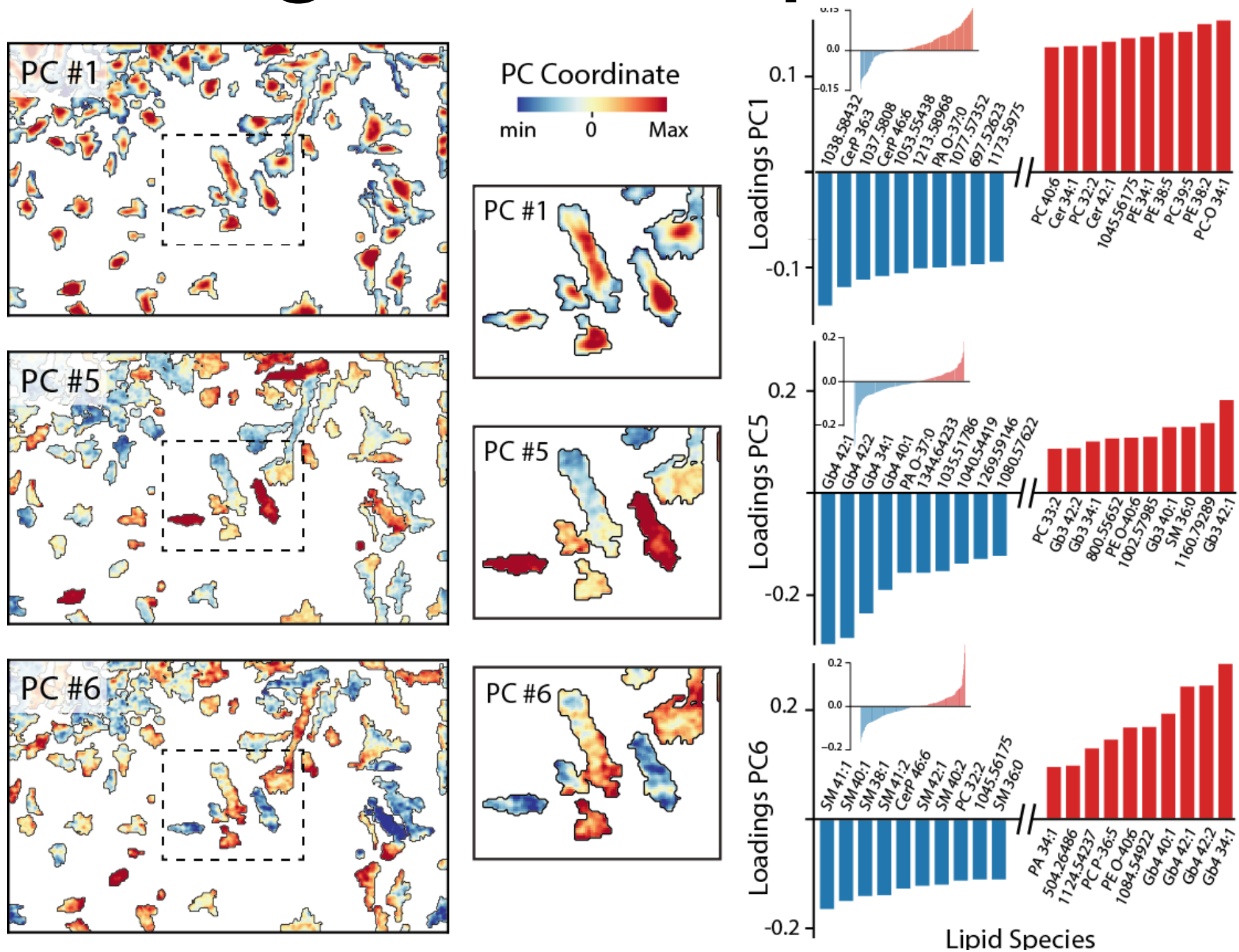
Data analysis



Single-cell Lipidomics

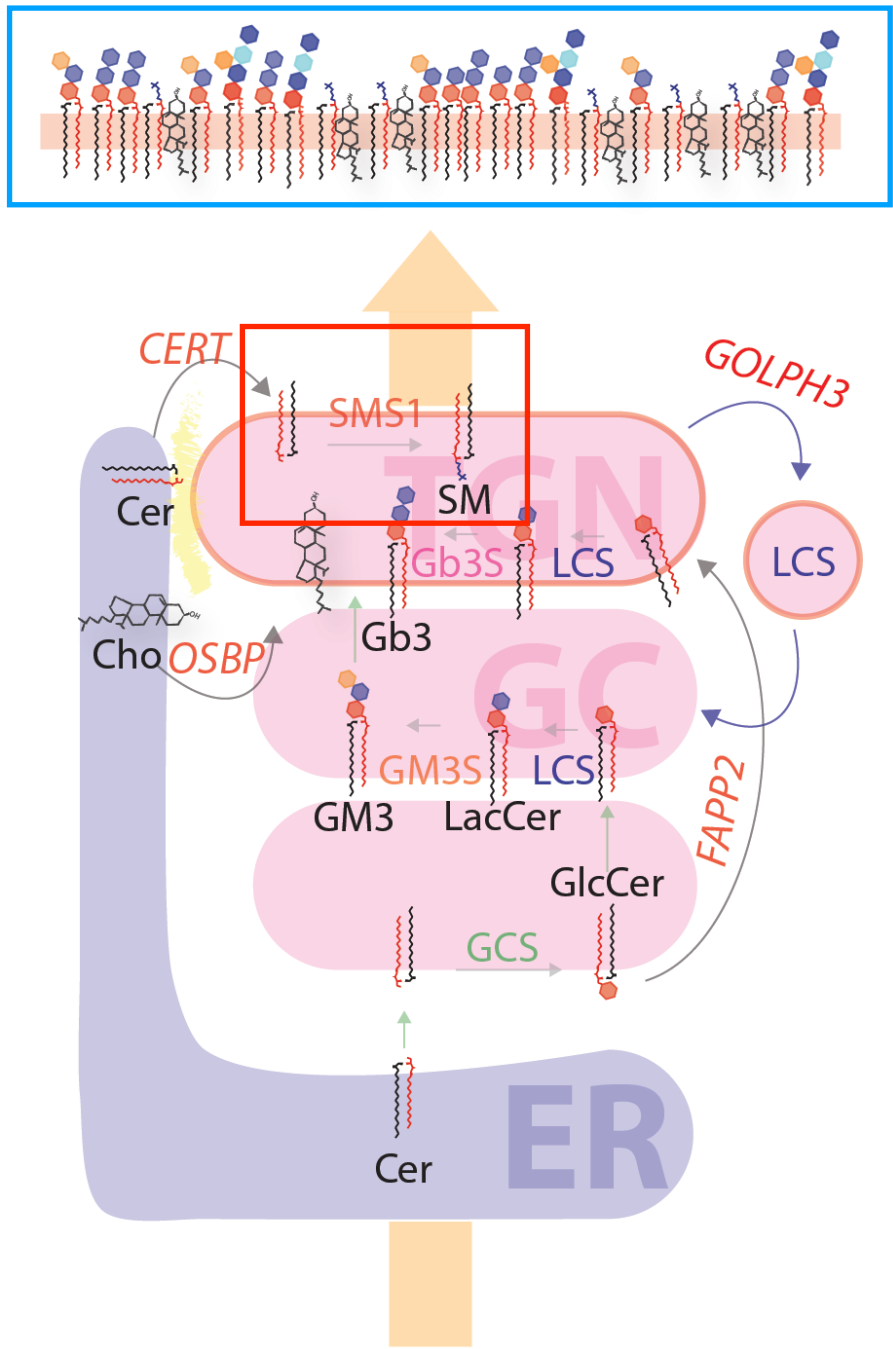
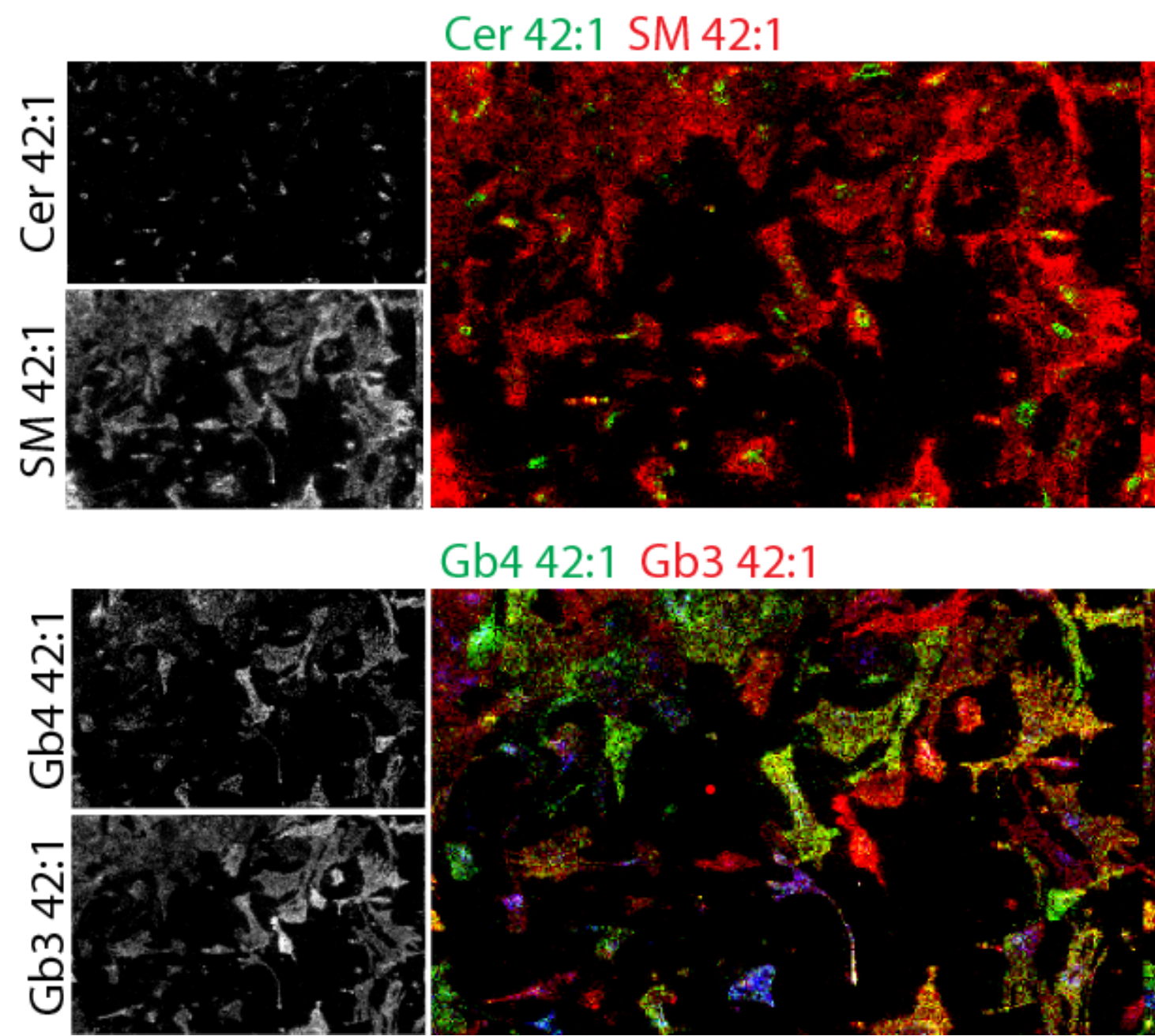


Single-cell Lipidomics



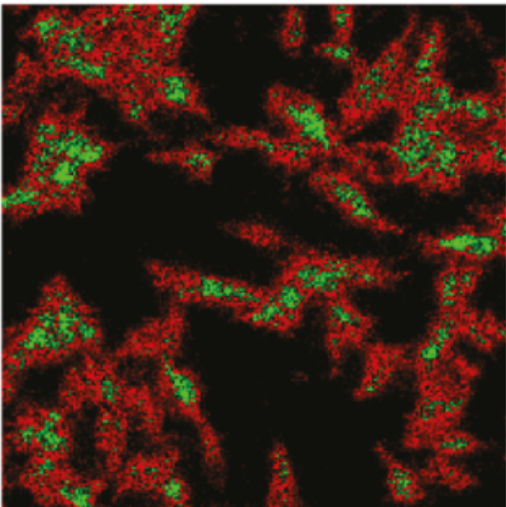
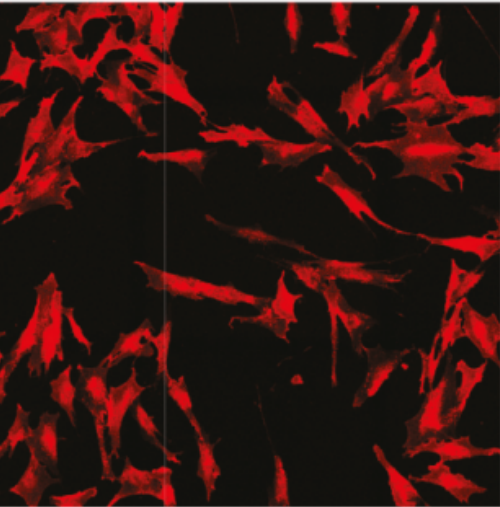
Two major variation axes exist in lipid composition:
one intracellular and one intercellular

Single-cell Lipidomics



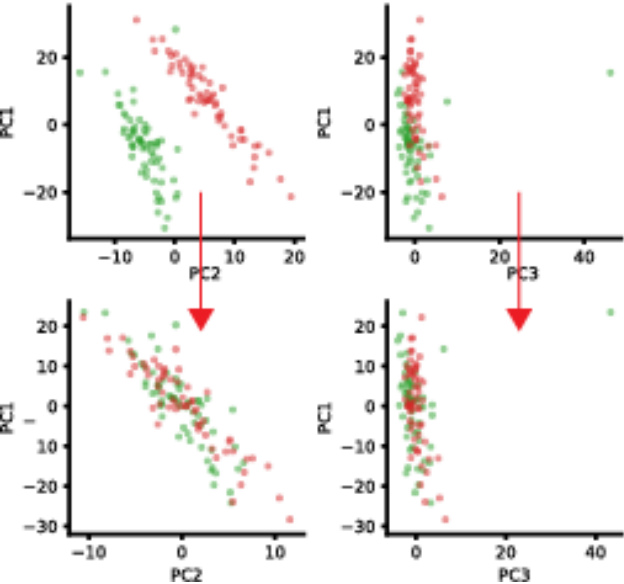
Single-cell Lipidomics

Optical Image → Mass Image → Segmentation → Single-cell Data –

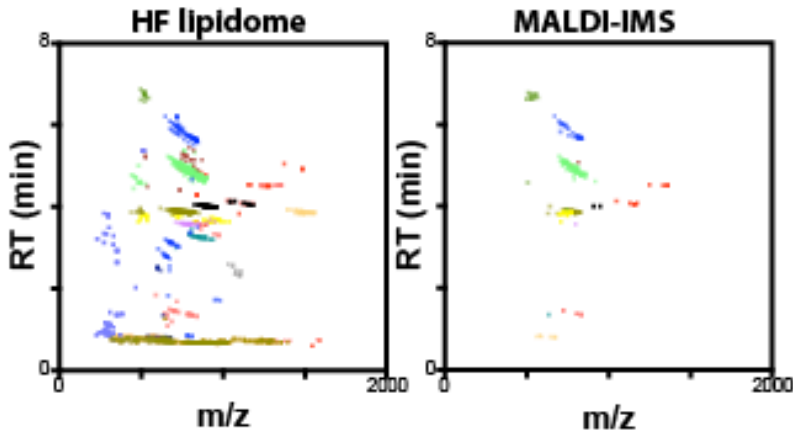


	Cell 1	Cell 2	Cell 3	Cell i
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peak i	0.473	0.023	0.510	0.772

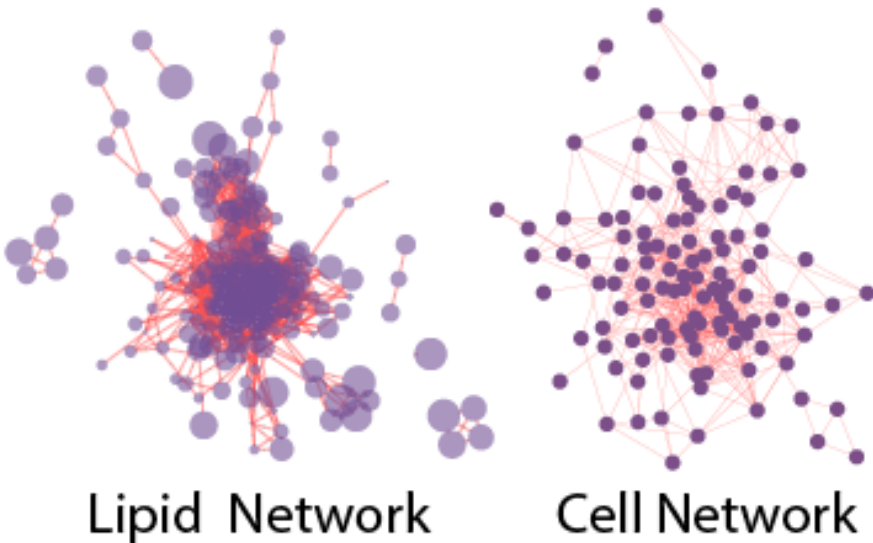
Normalization



Peak identification

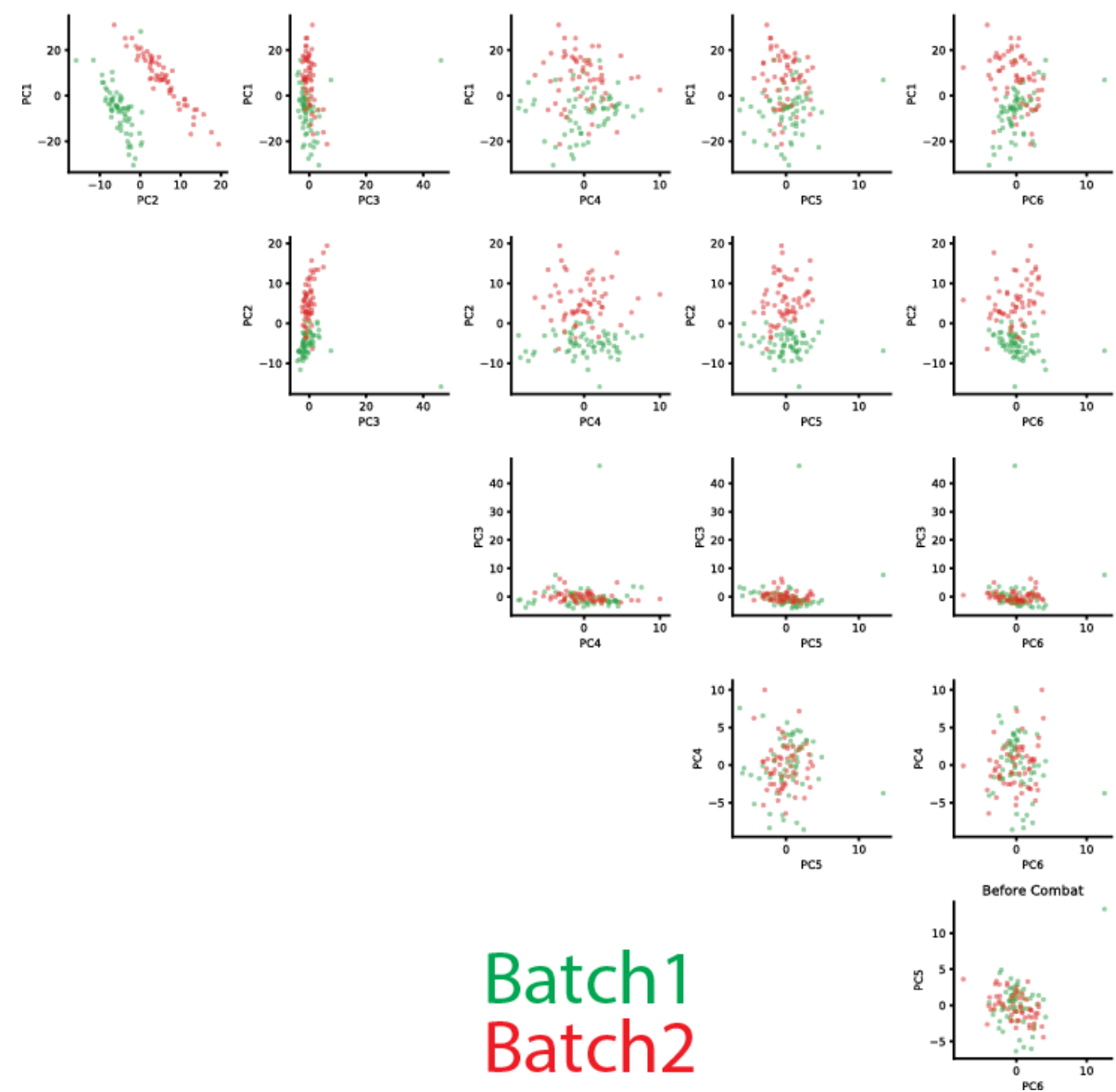


Data analysis

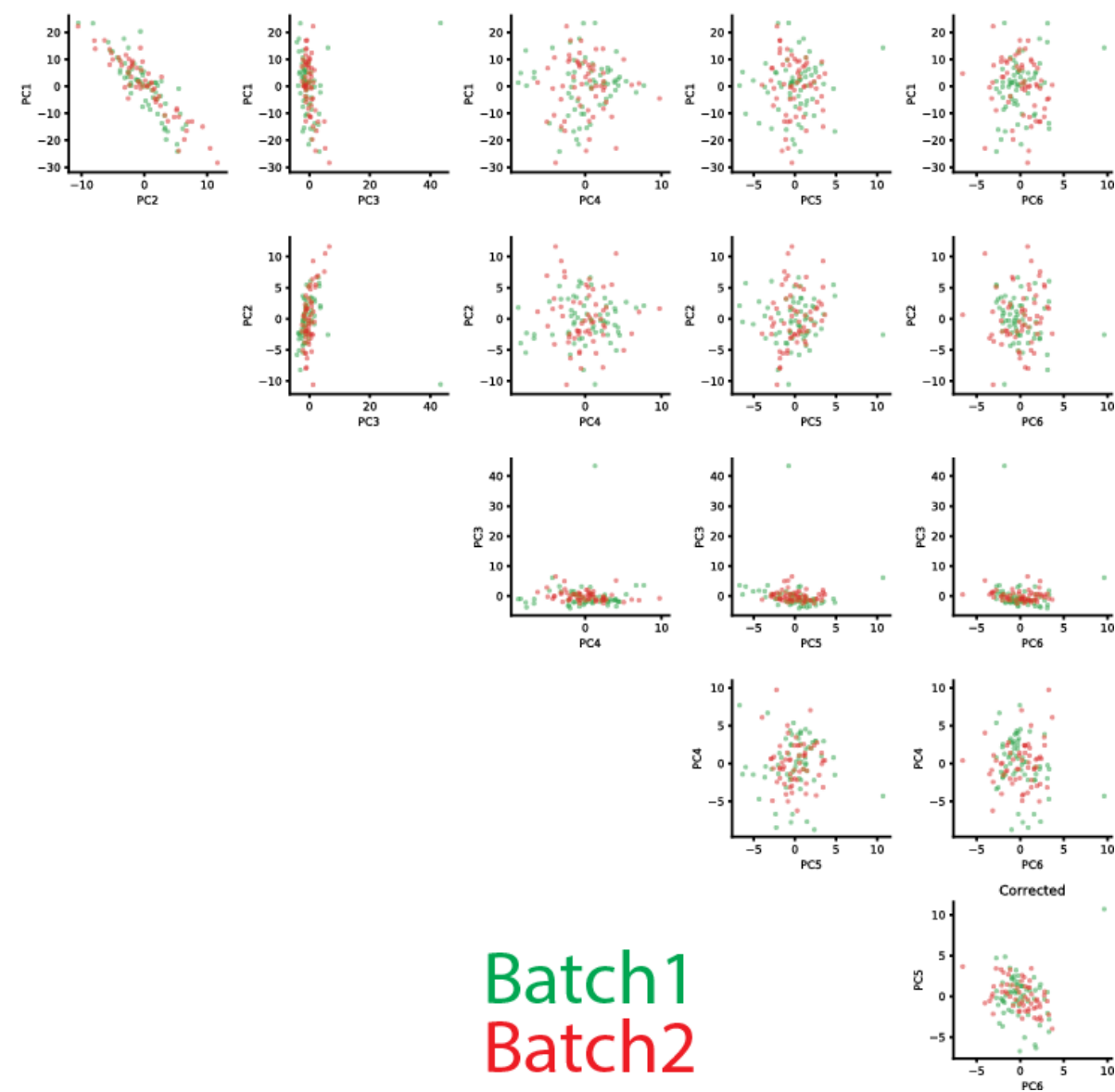


Normalization

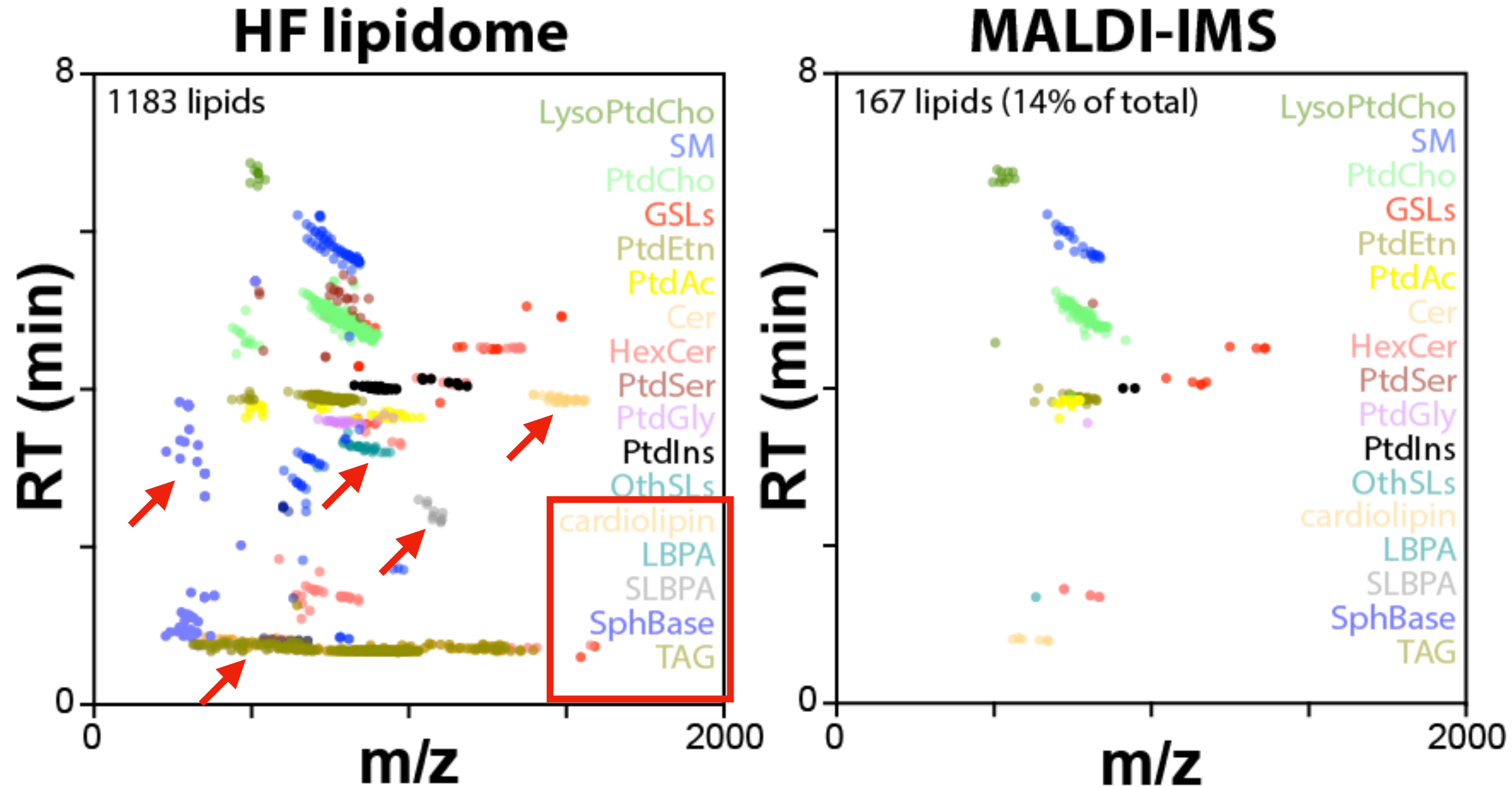
Concatenated



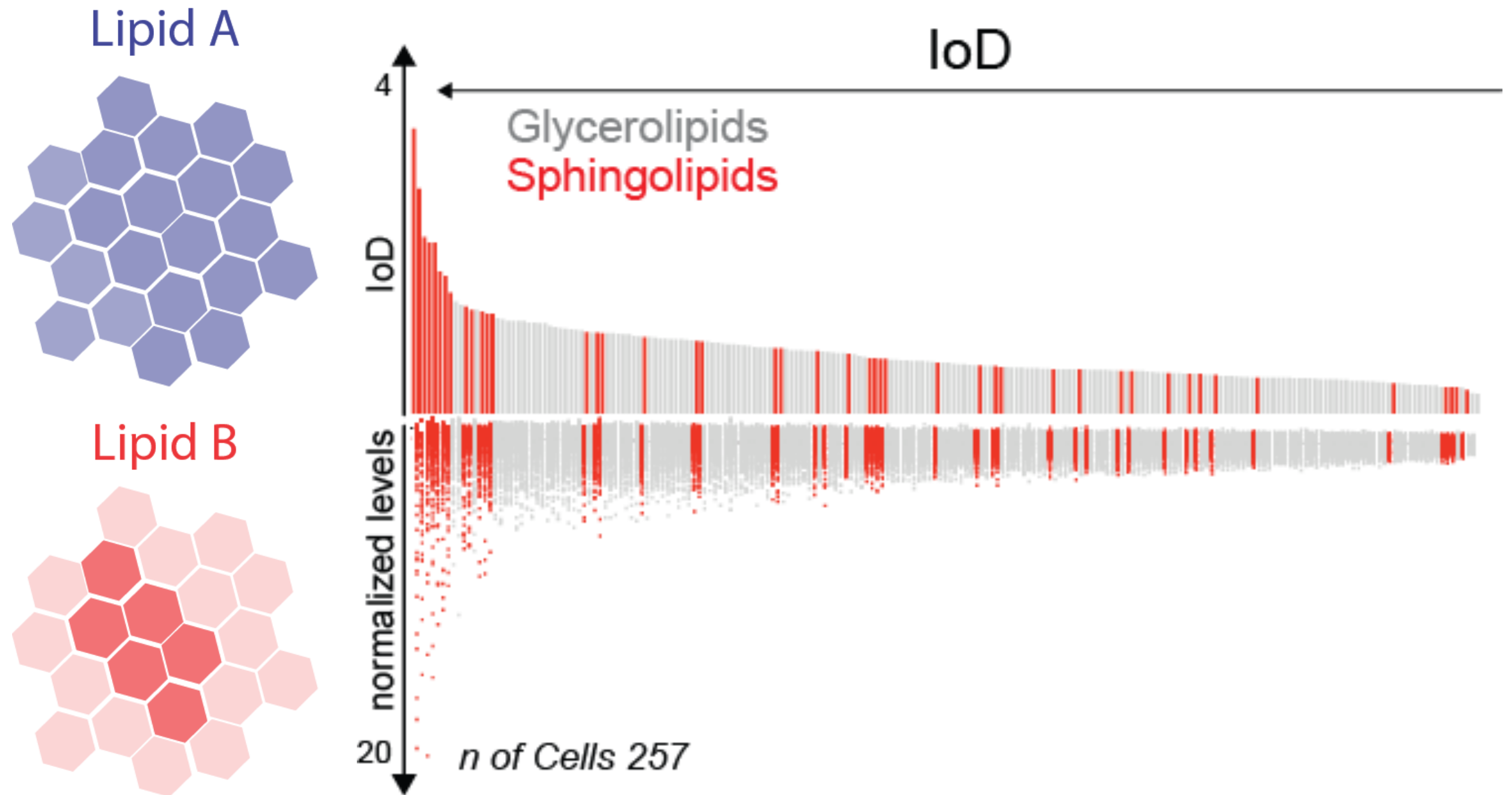
Concatenated and Integrated



Peak calling/ coverage

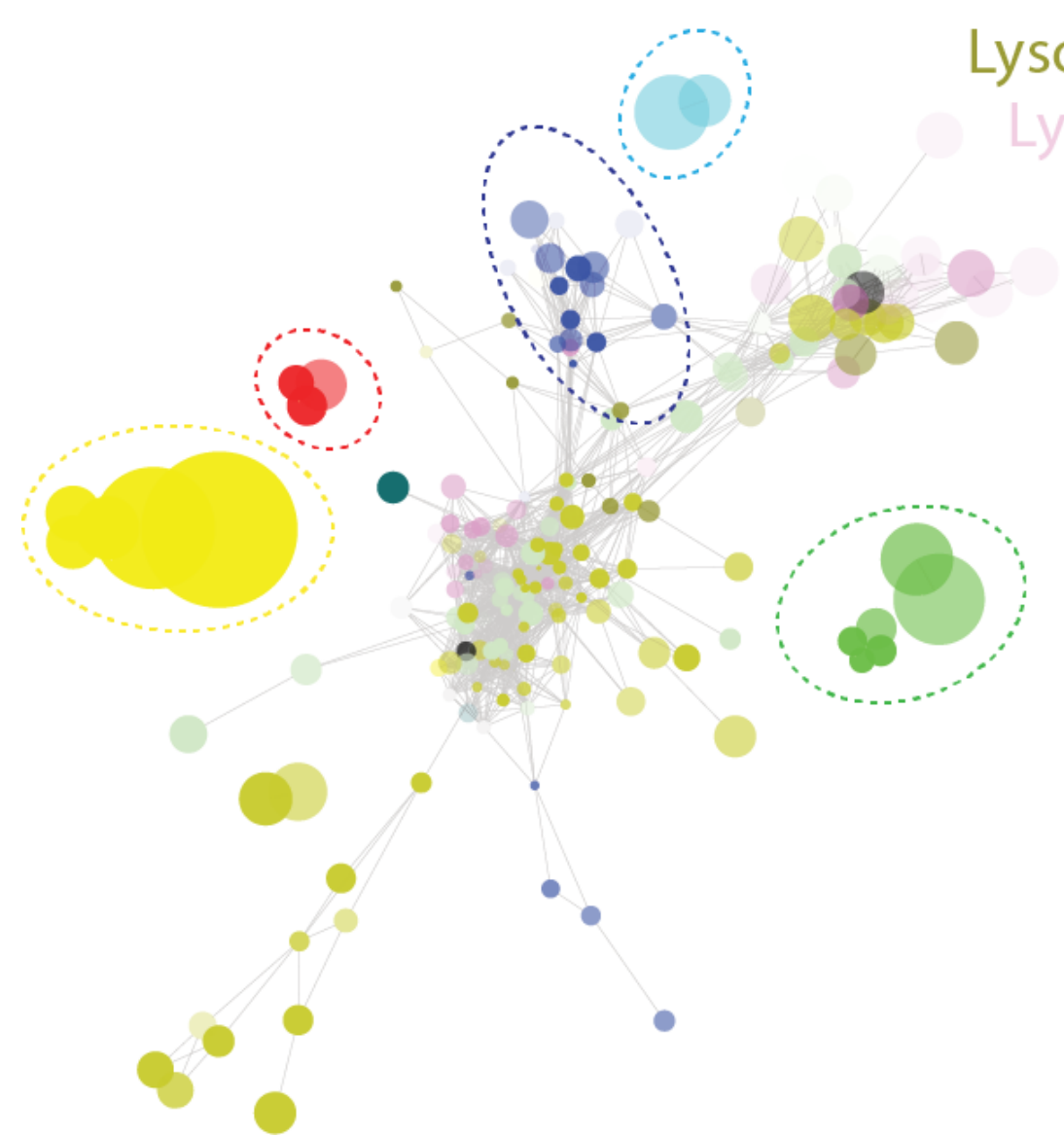
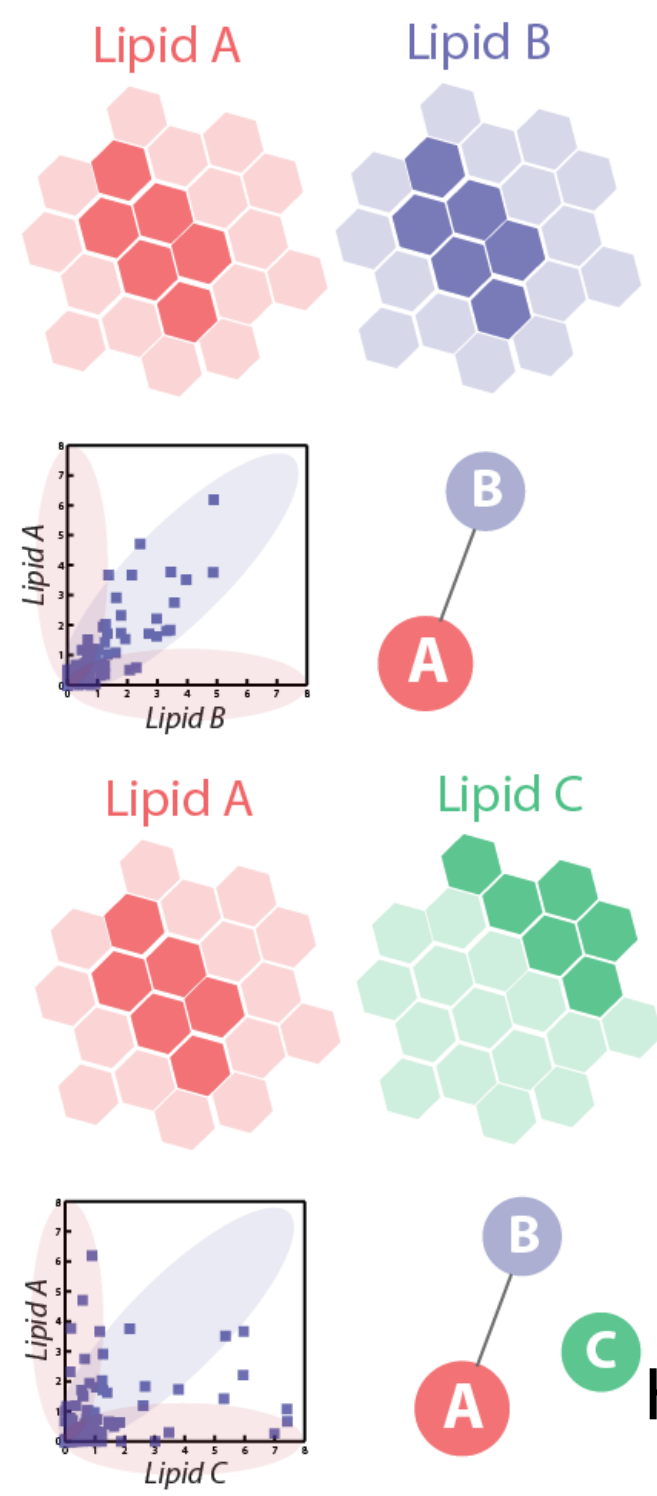


Single-cell Lipidomics

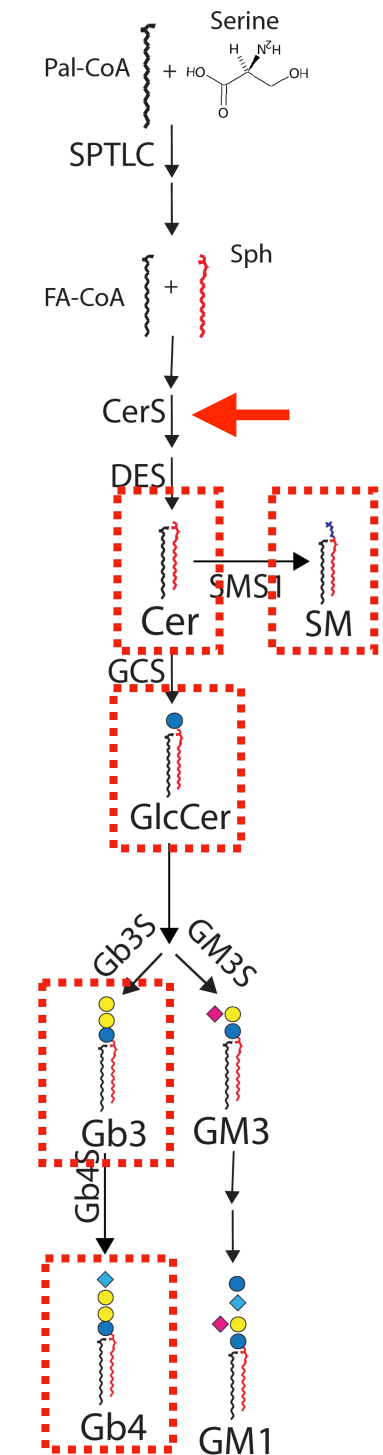


Sphingolipids are the most variable lipid class in single cells

Single-cell Lipidomics

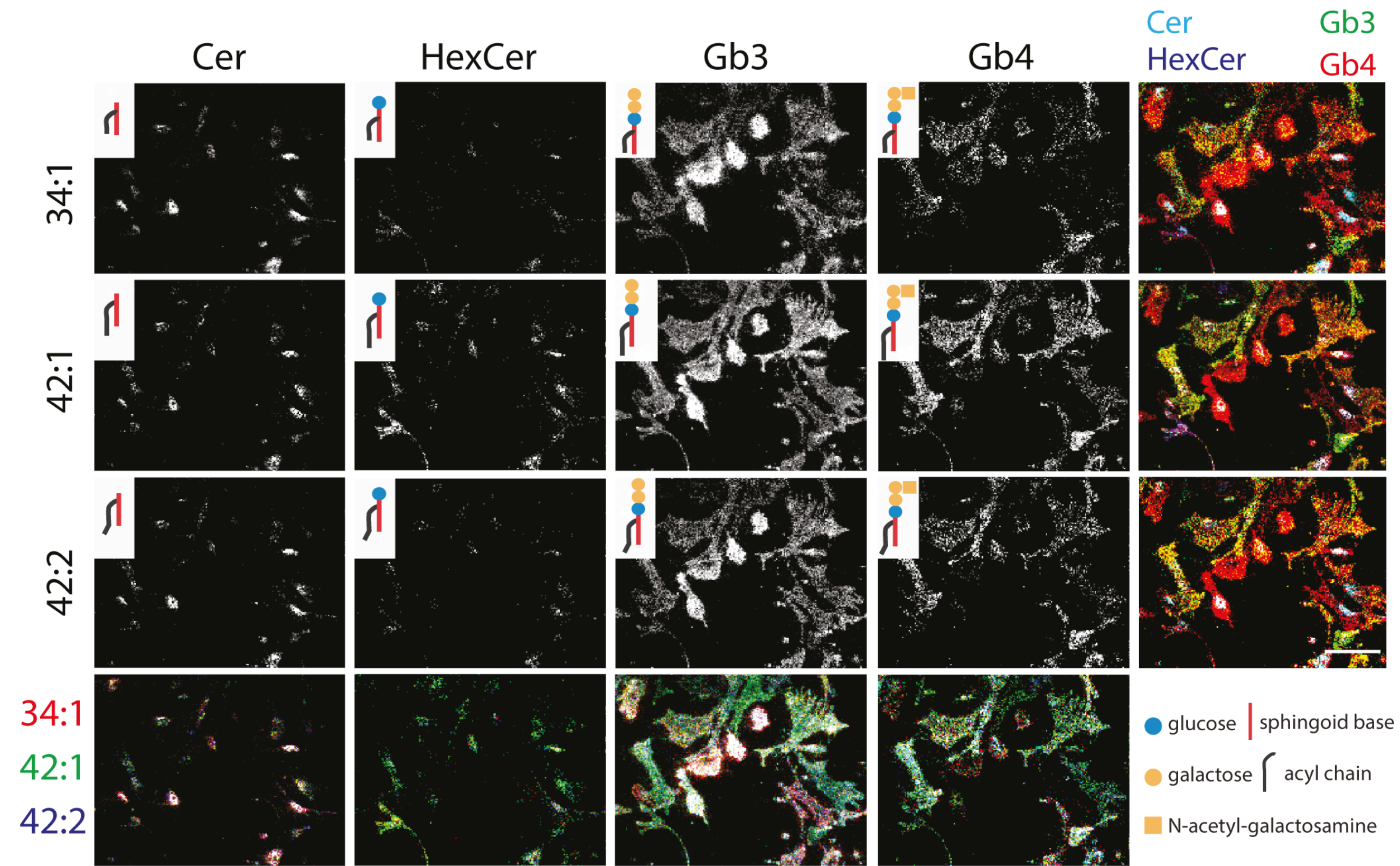


- LysoPtdCho
- LysoPtdAc
- PtdCho
- PtdSer
- PtdGly
- PtdIns
- PtdEtn
- PtdAc
- HexCer
- CerP
- DAG
- Gb4
- Gb3
- Cer
- SM

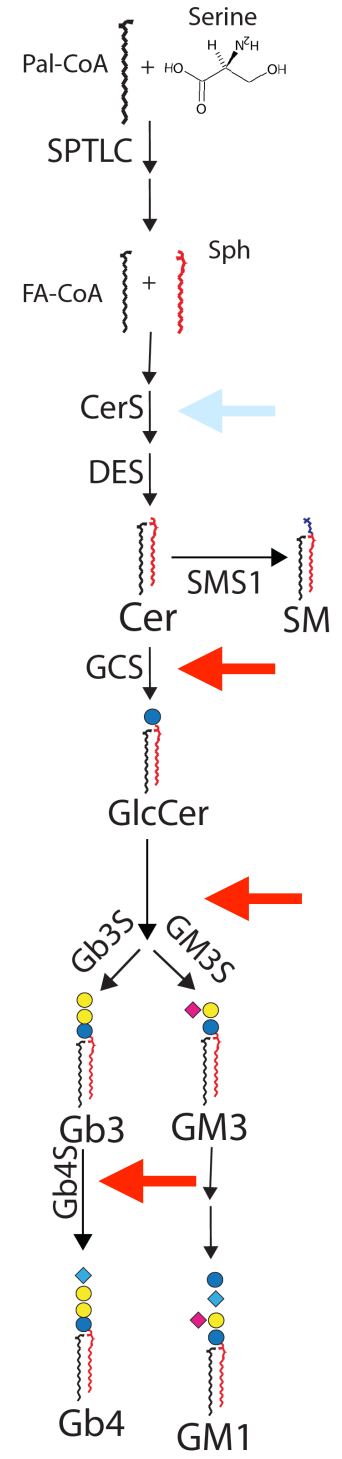
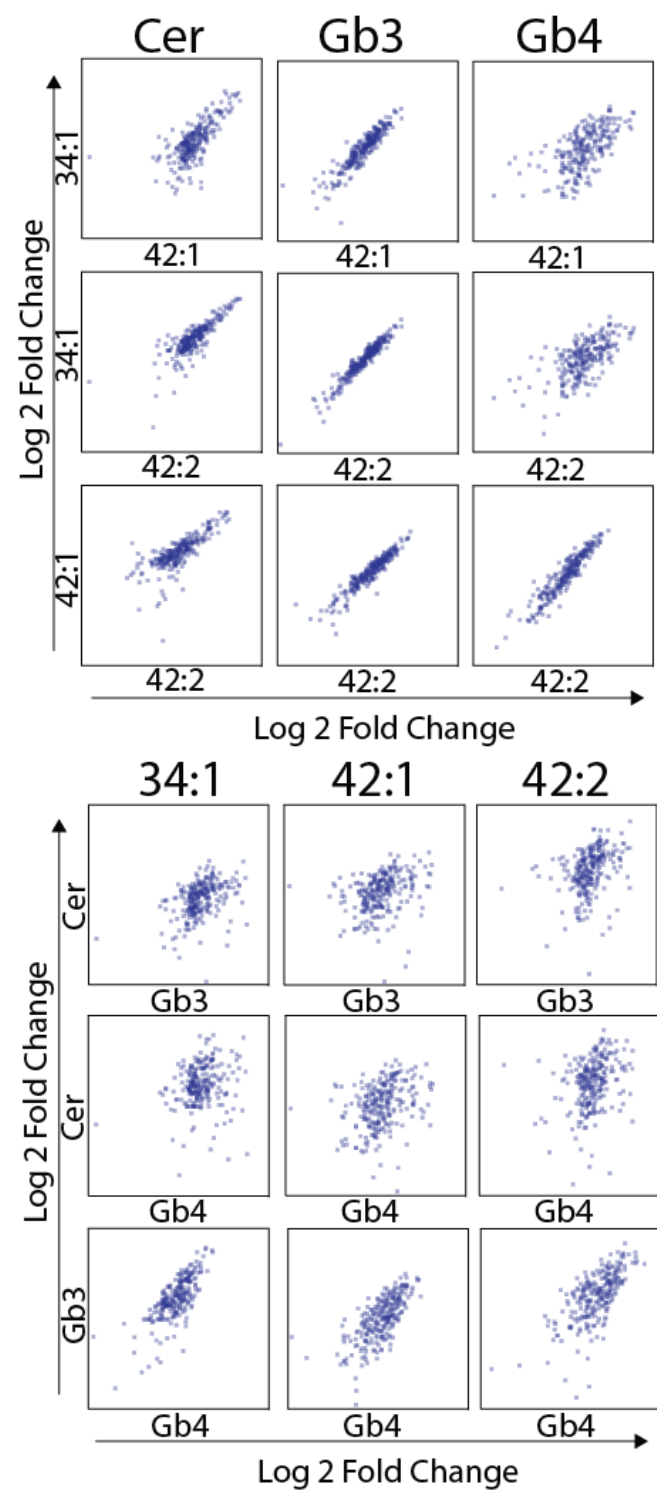
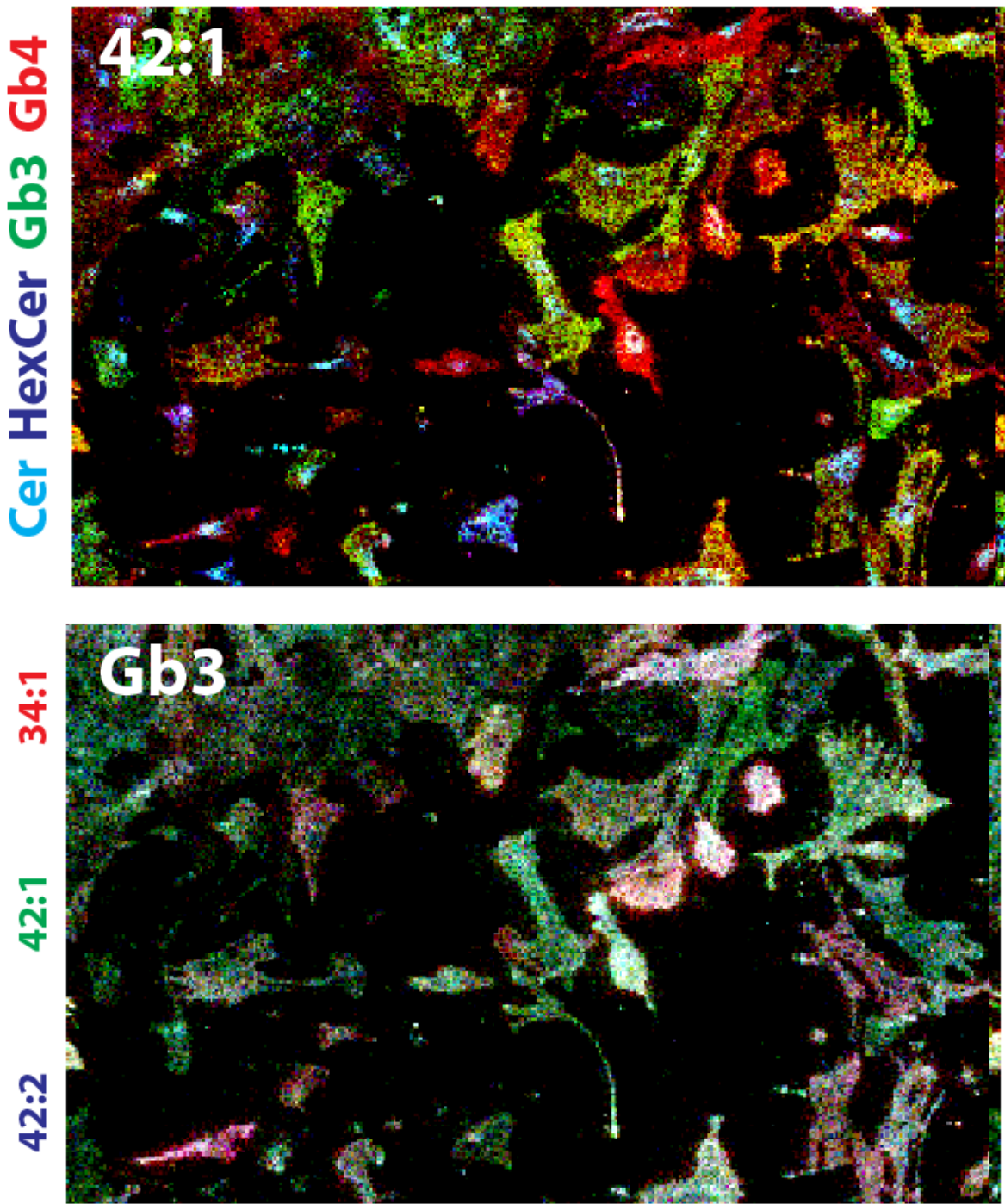


Highly variable sphingolipids form discrete co-variation groups that are not connected among them

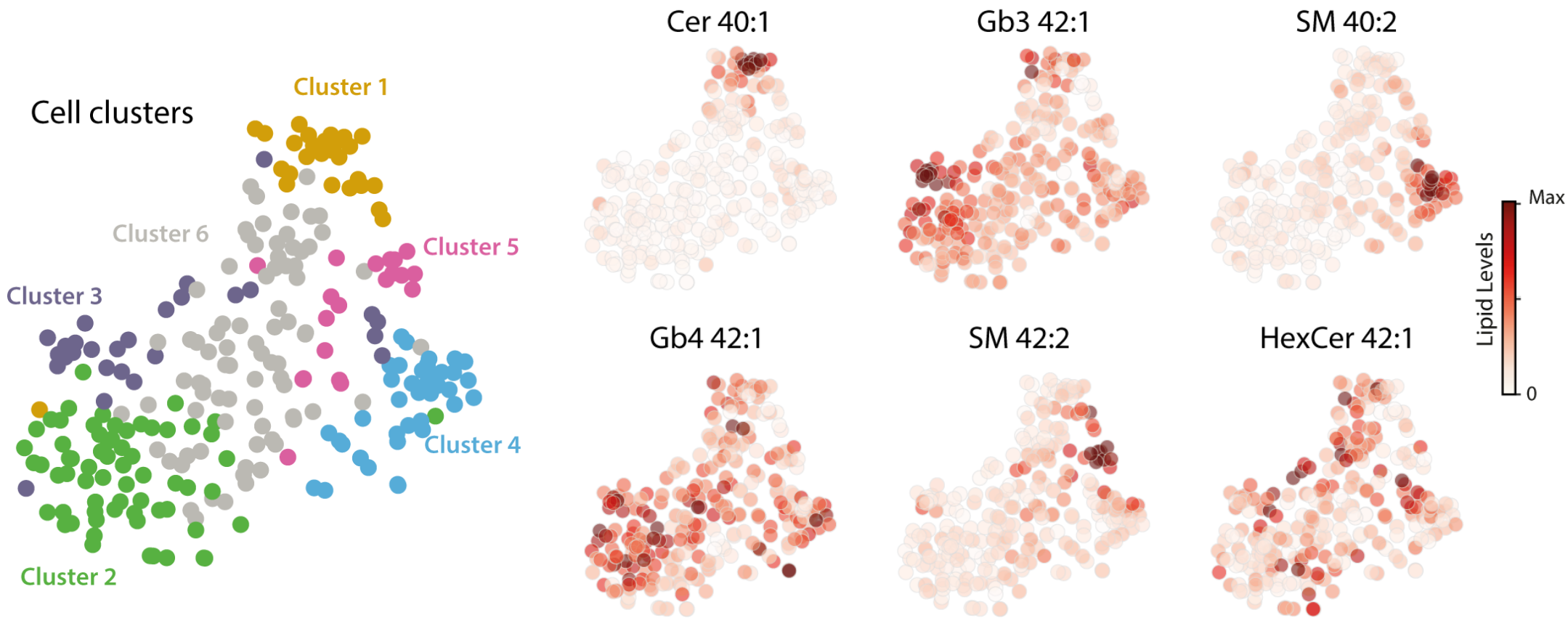
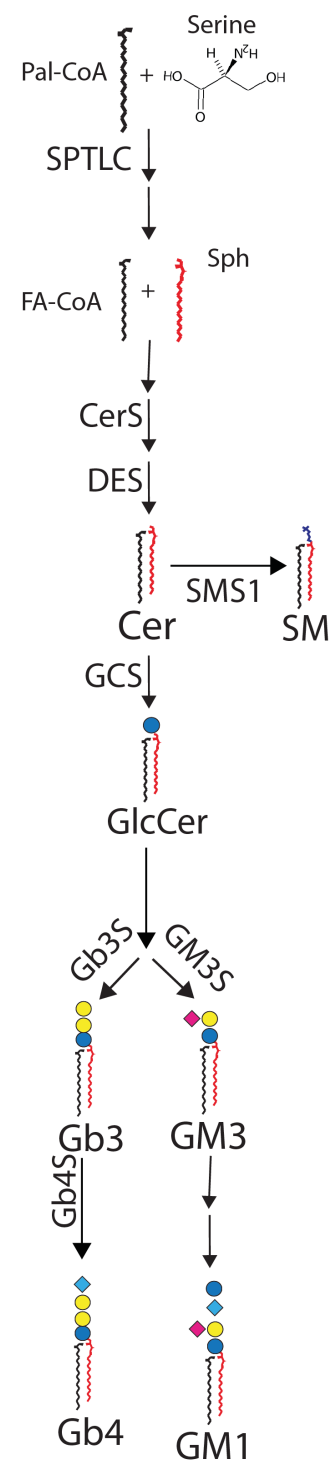
Single-cell Lipidomics



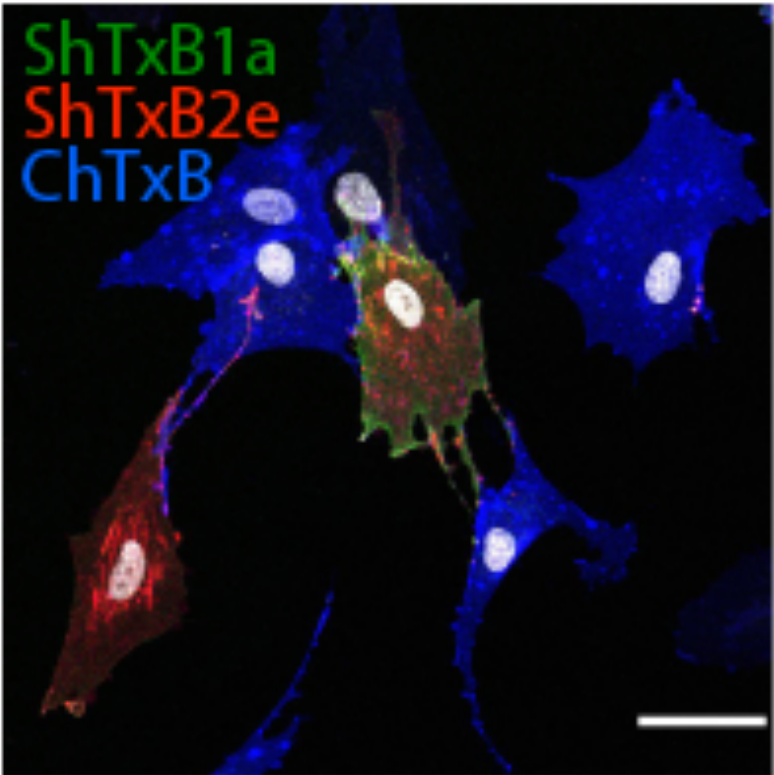
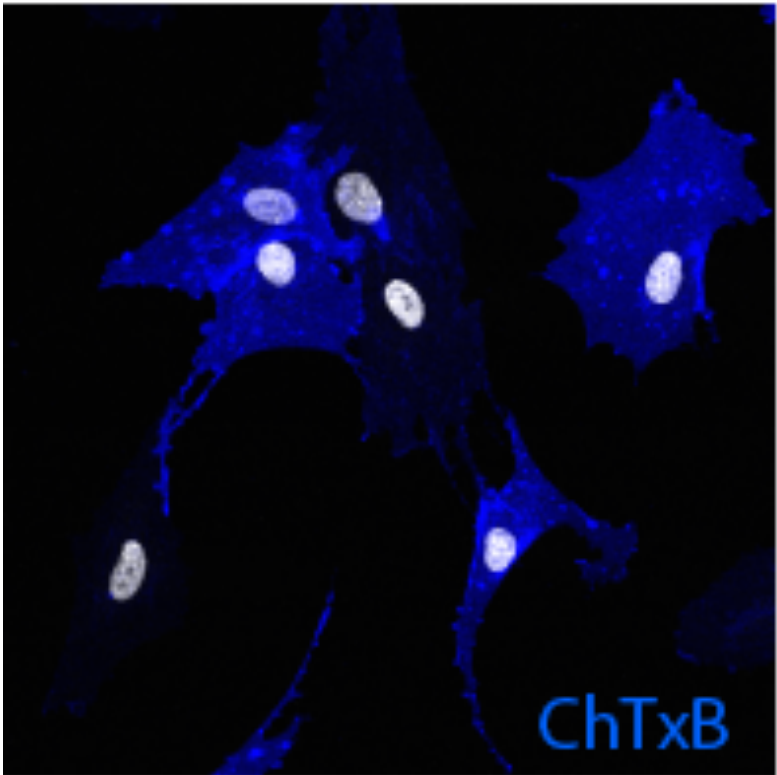
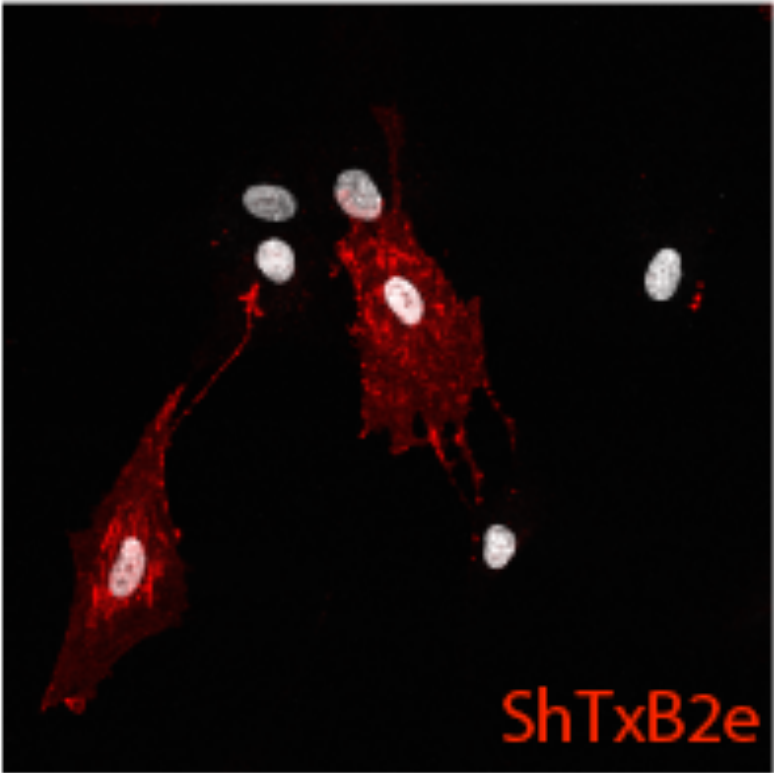
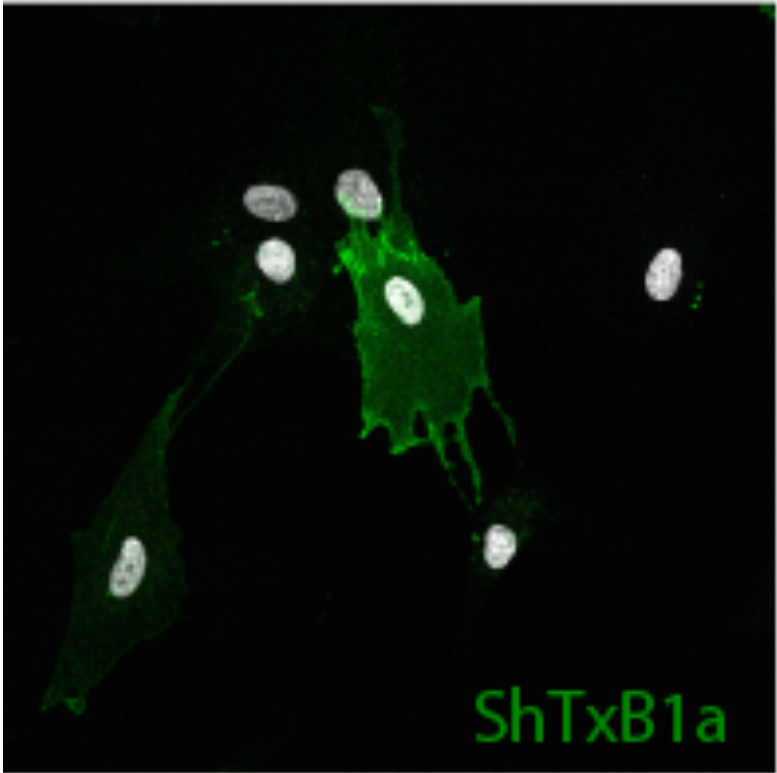
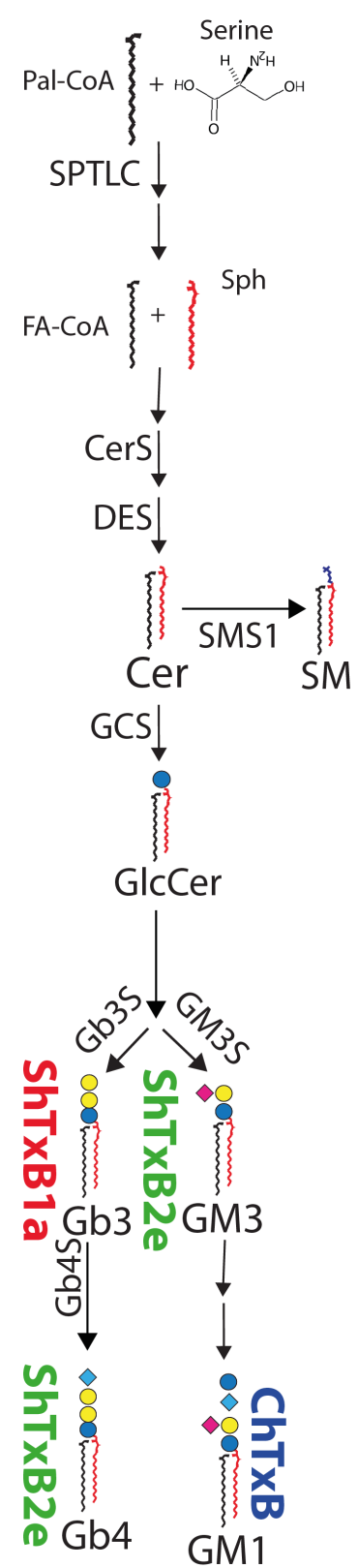
Single-cell Lipidomics



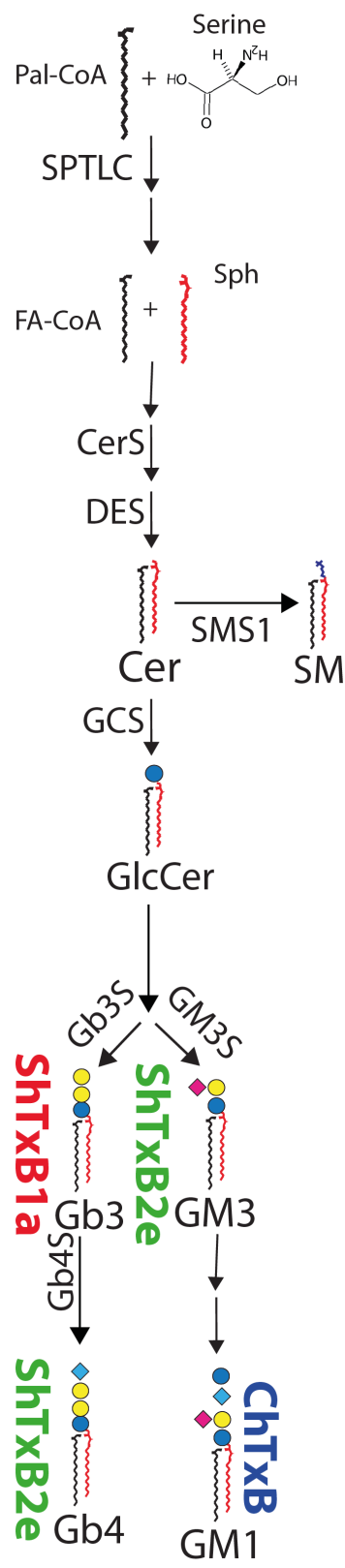
Single-cell Lipidomics



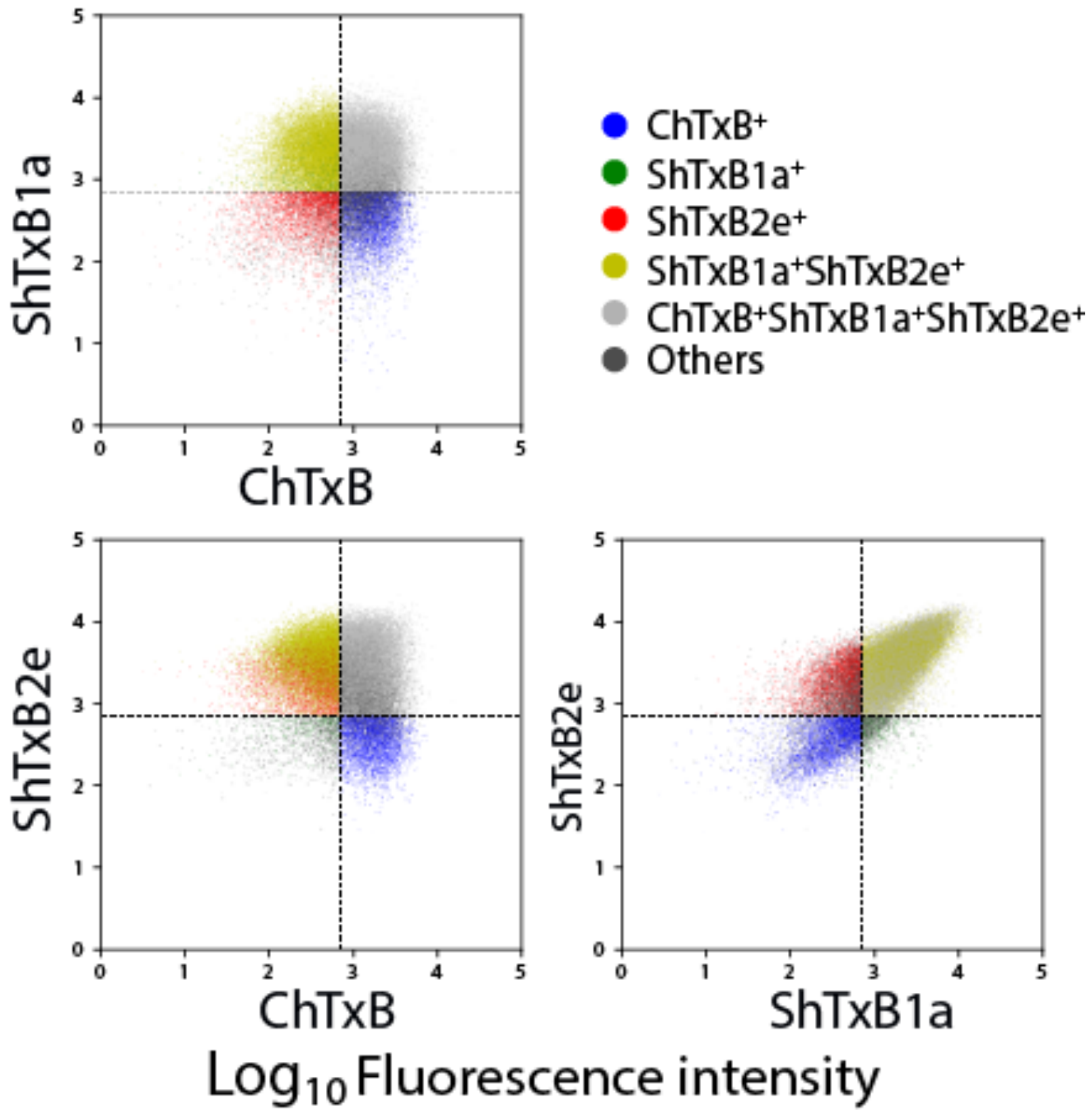
Validation



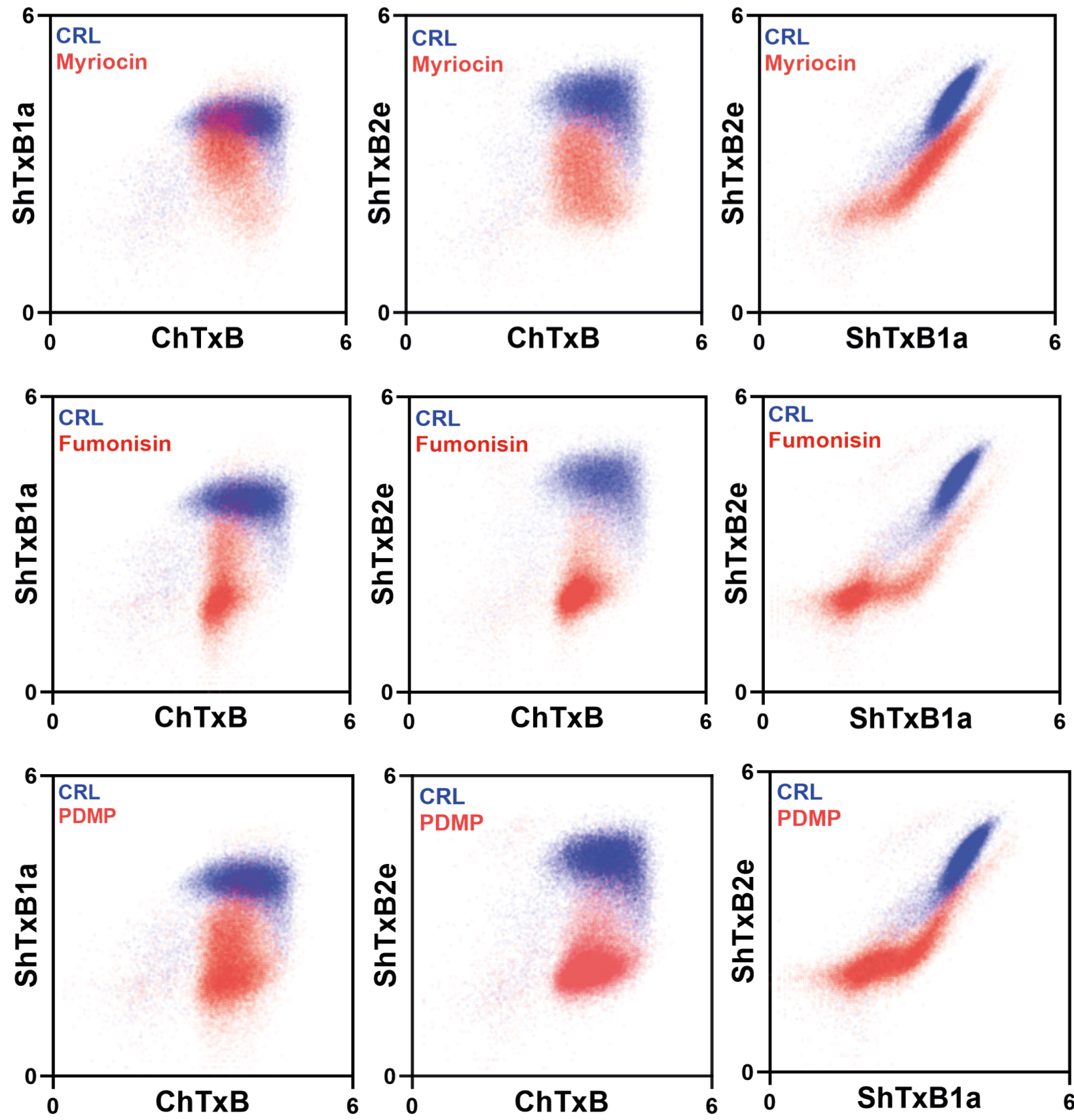
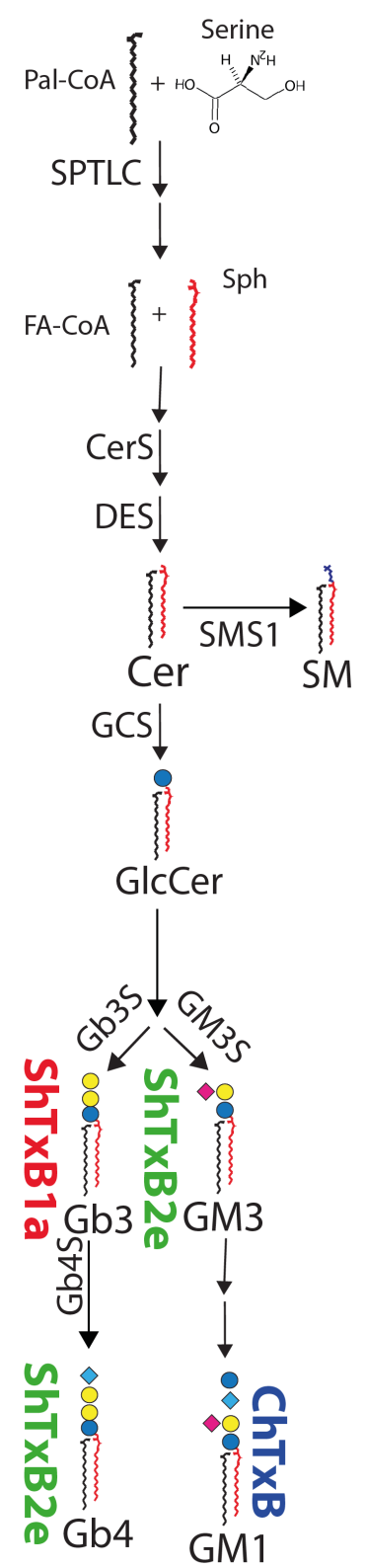
Validation



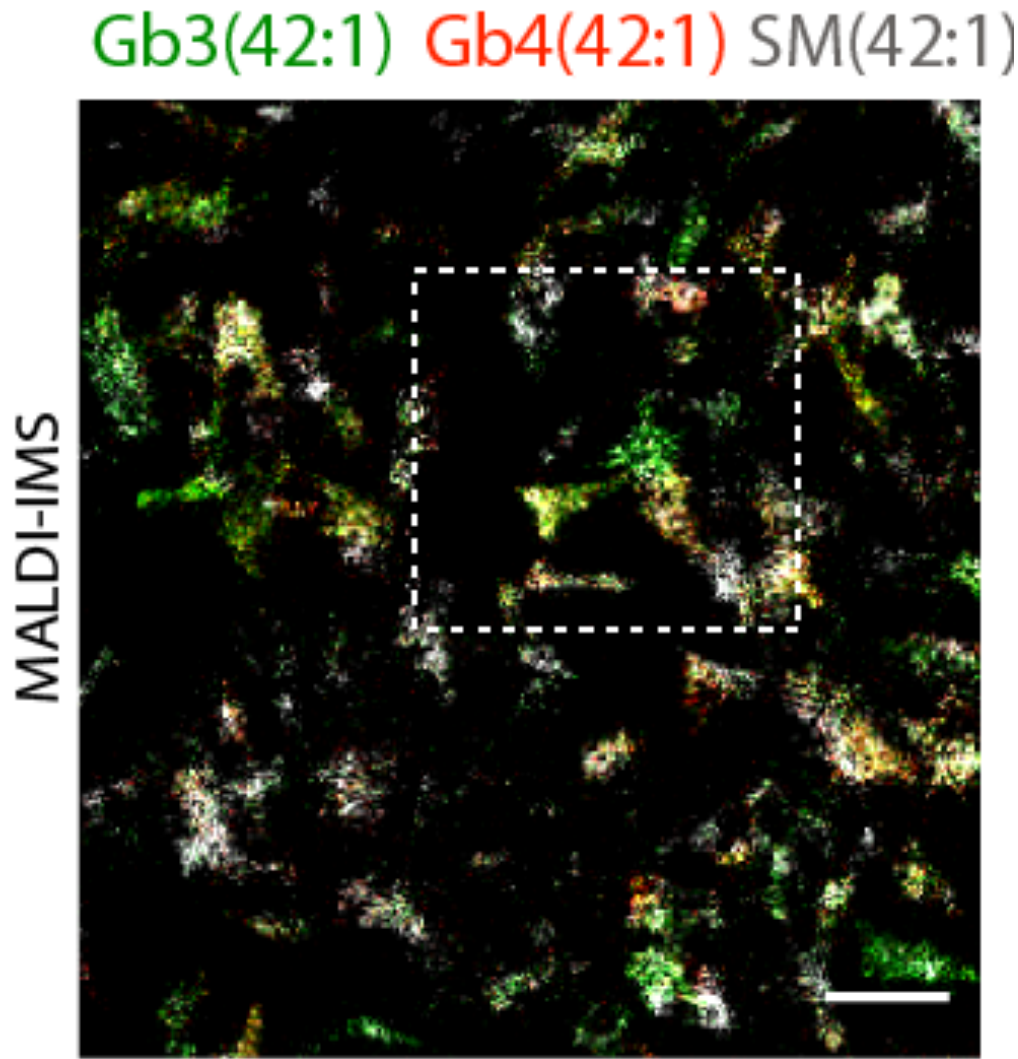
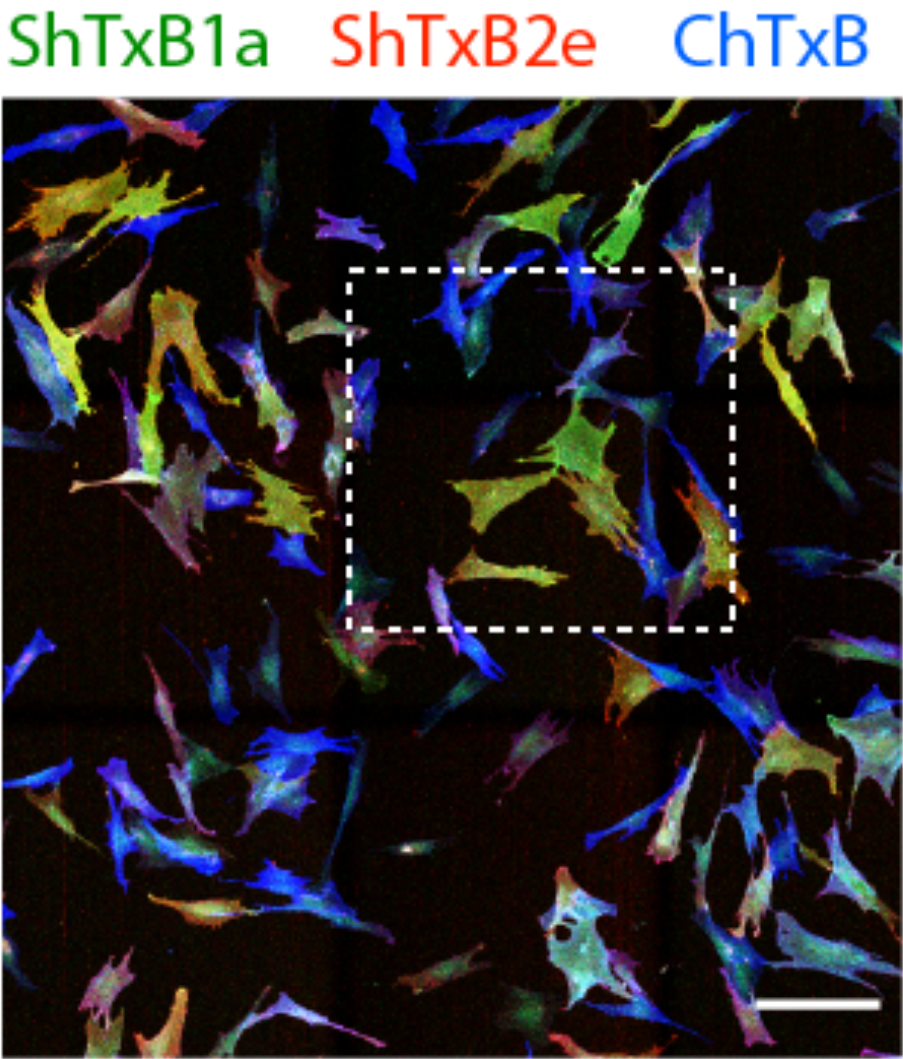
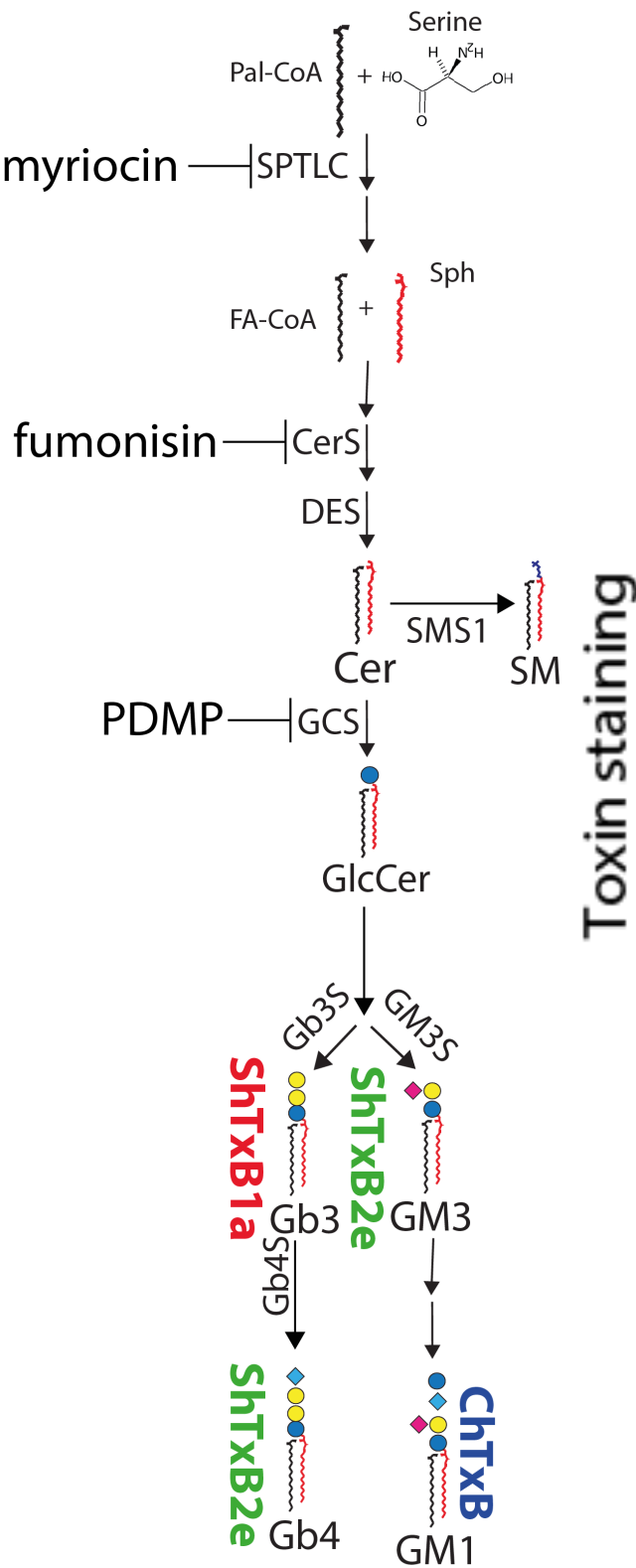
Log₁₀ Fluorescence intensity



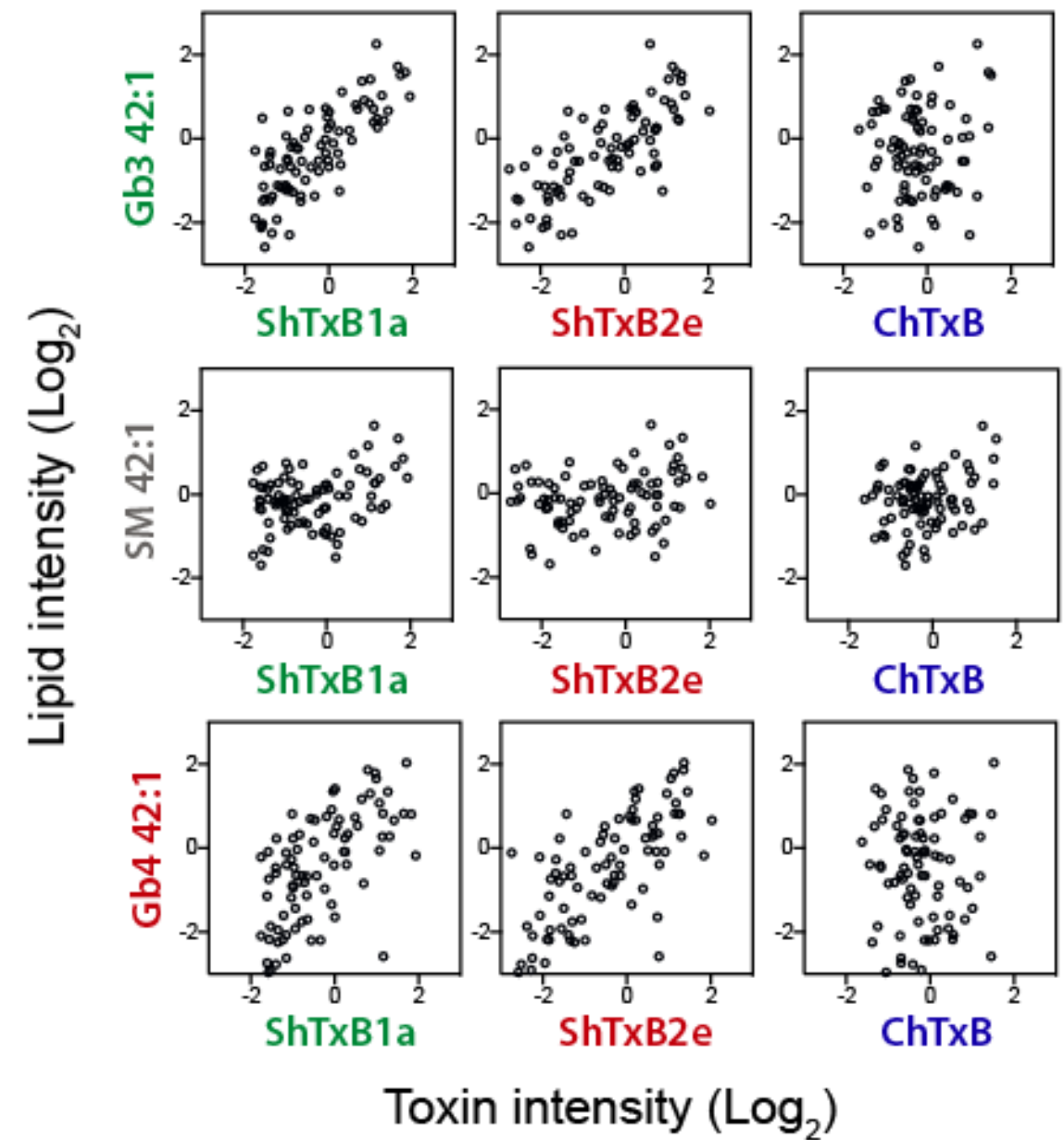
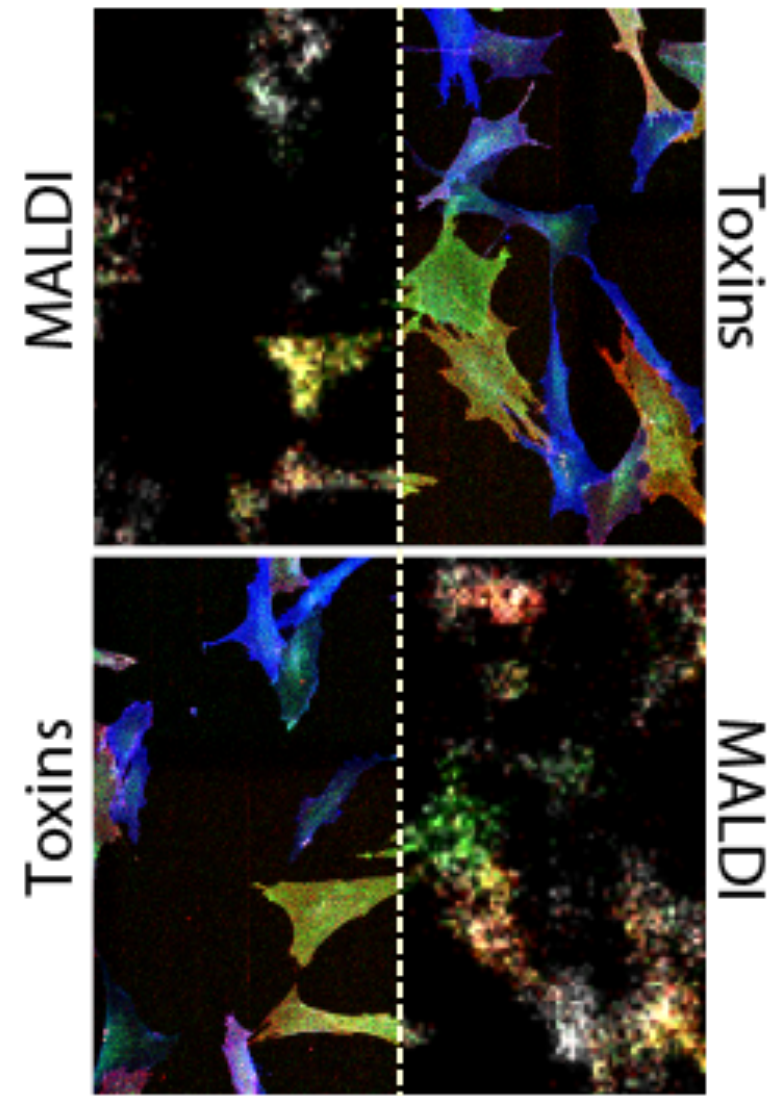
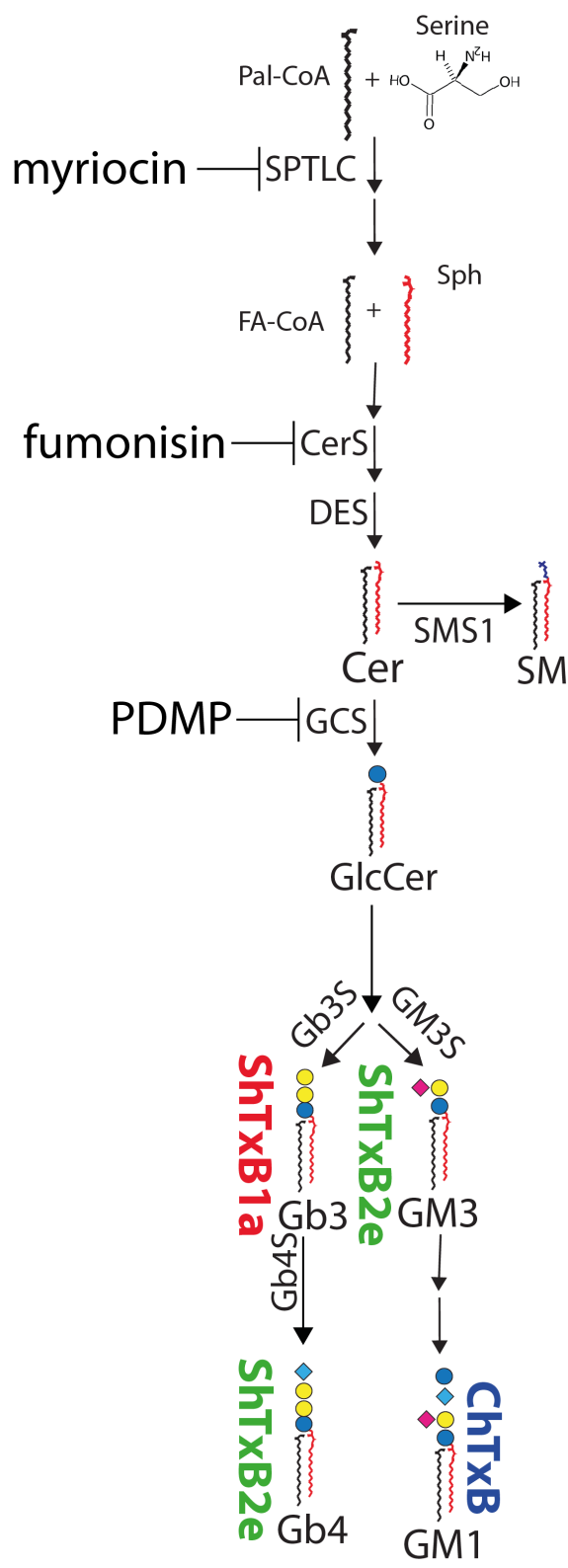
Validation



Validation



Validation



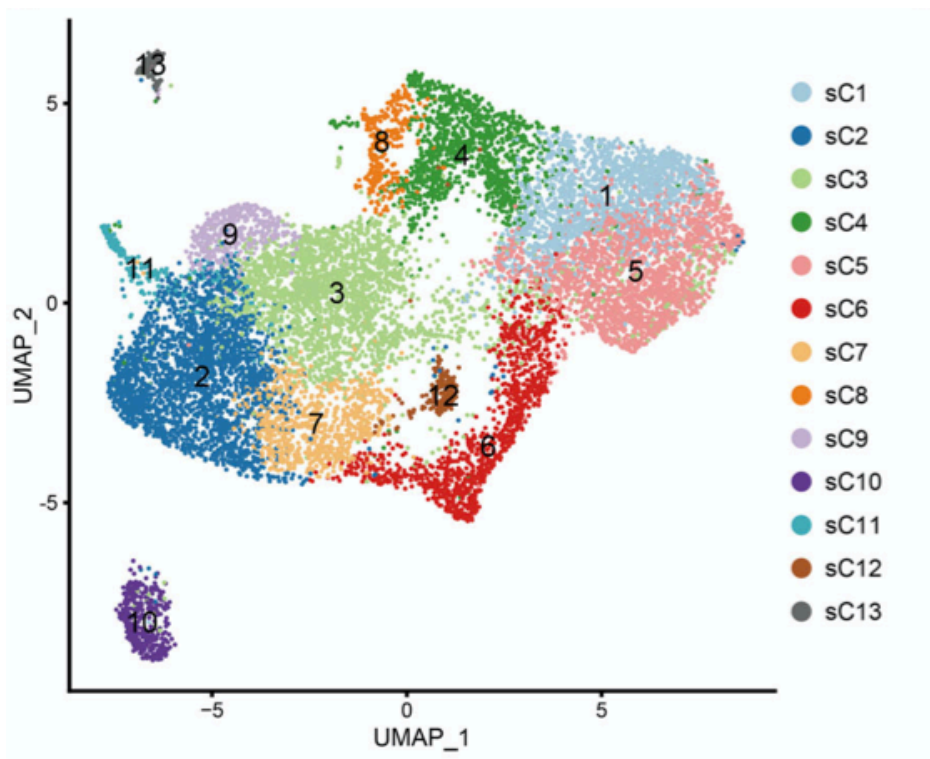
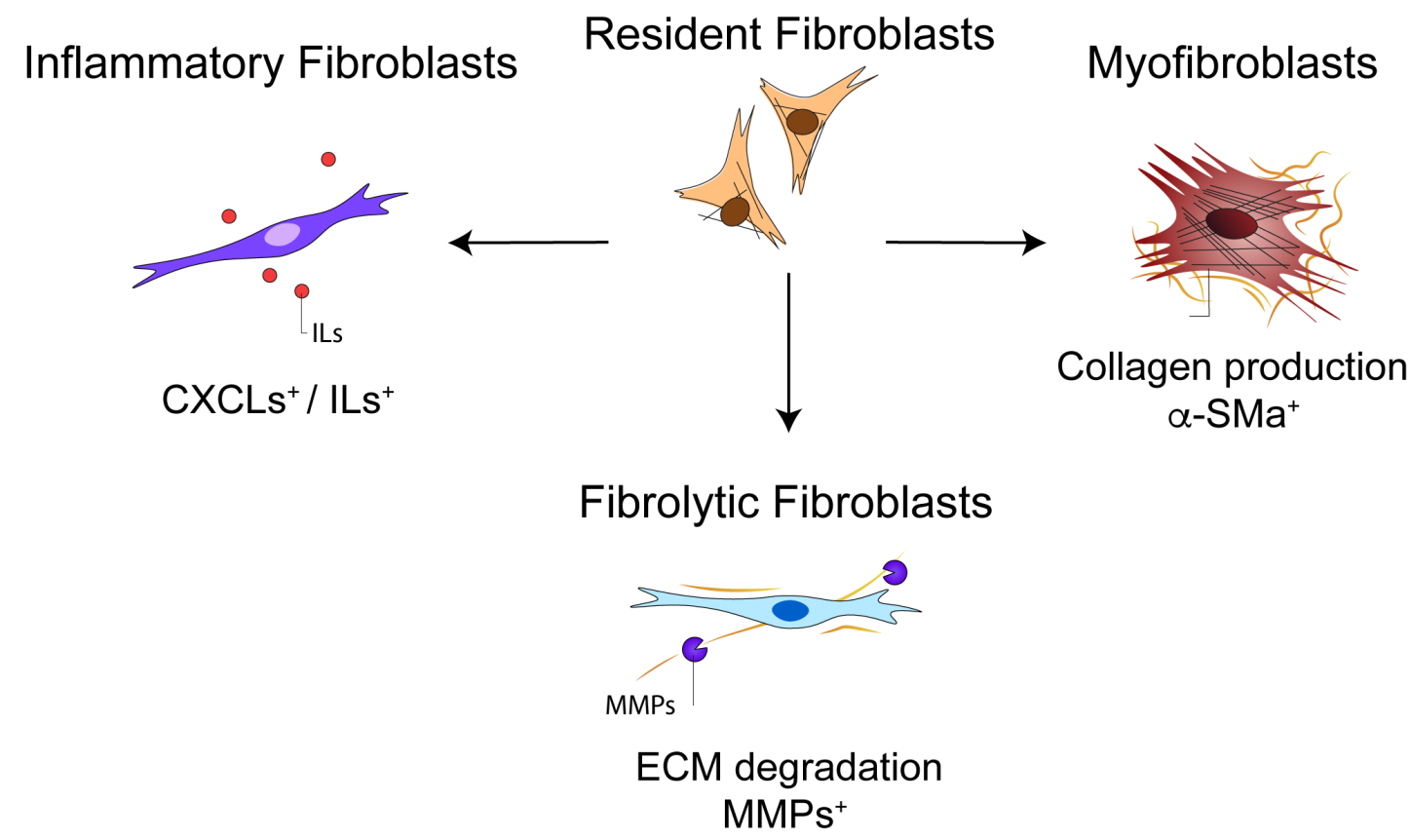
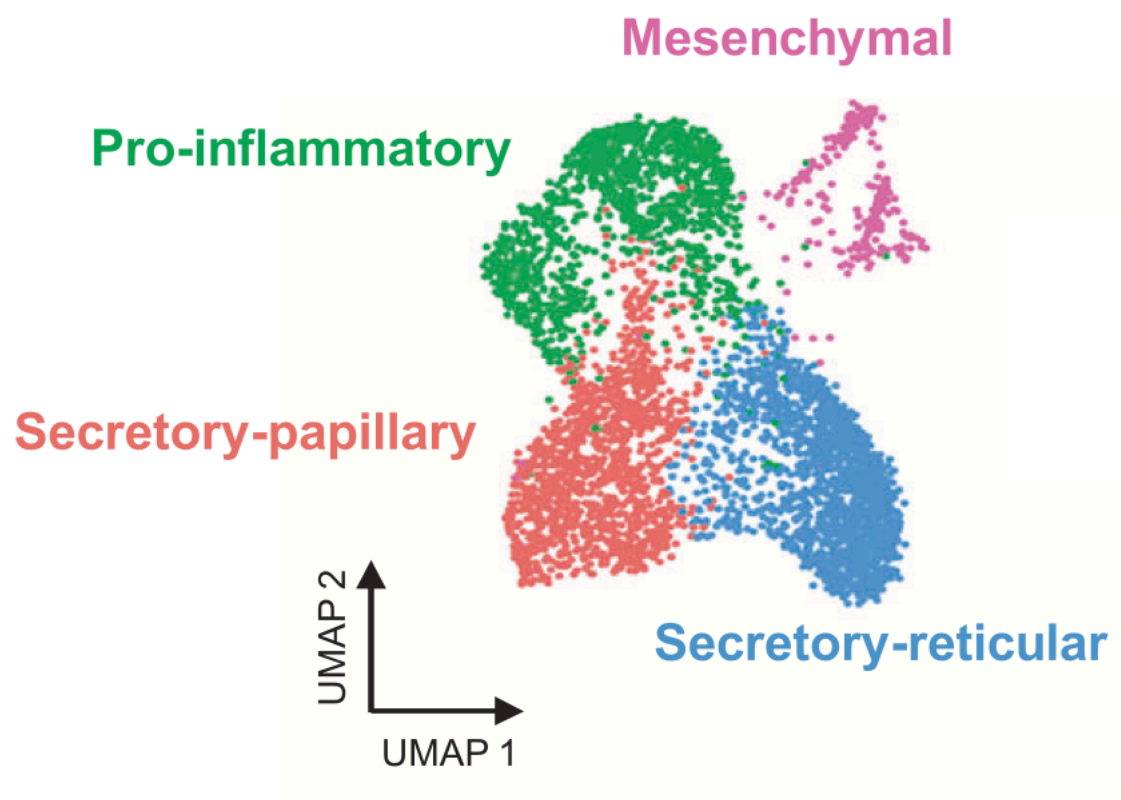
Lipotypes are relatively stable cell states and exist under physiological conditions

Conclusions_1

- We have measured lipidomes (parts of) at the single cell level
- Specific lipid metabolic segments (sphingolipids) are cell-to-cell variable
- sphingolipid variability is accounted majorly by variations in the head-group processing (by Golgi Complex localised enzymes)
- Cell-to-cell lipid variability is sufficient to describe cell subpopulations (lipotypes)

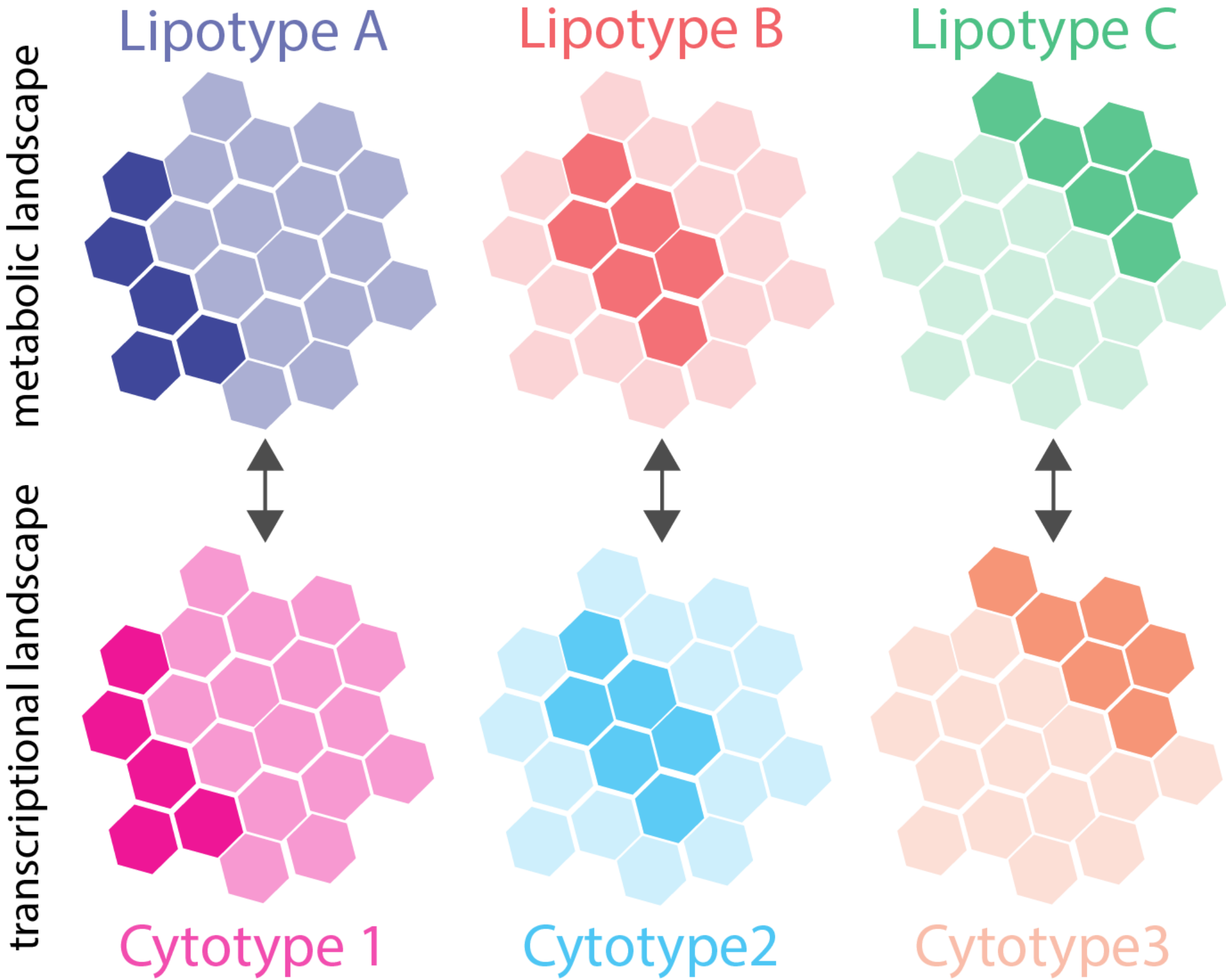
What these lipid-based cell populations correspond to?

Physiology

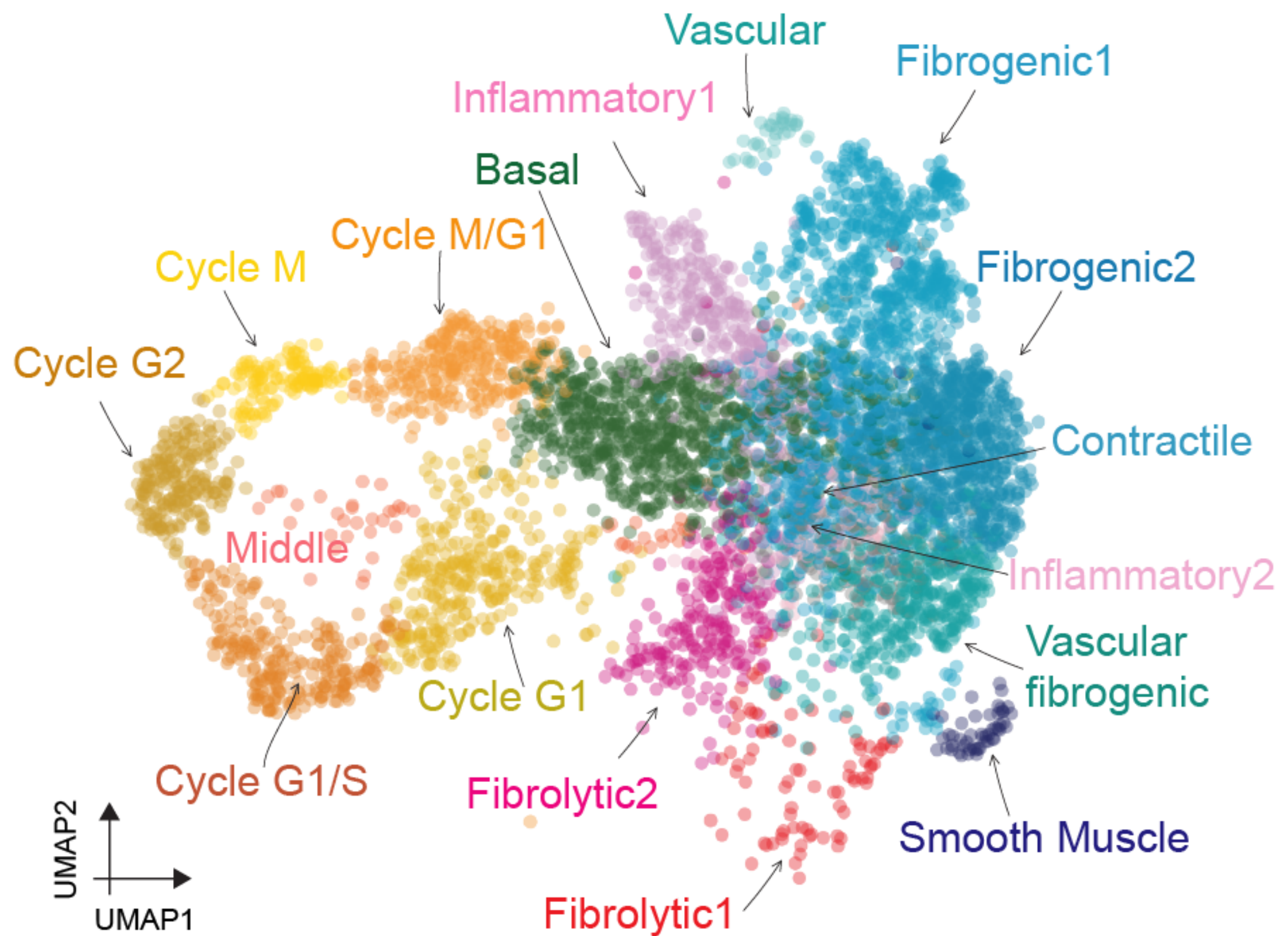


Fibroblasts are a heterogenous cell population
What drives dHFs heterogeneity?
Are lipids involved?

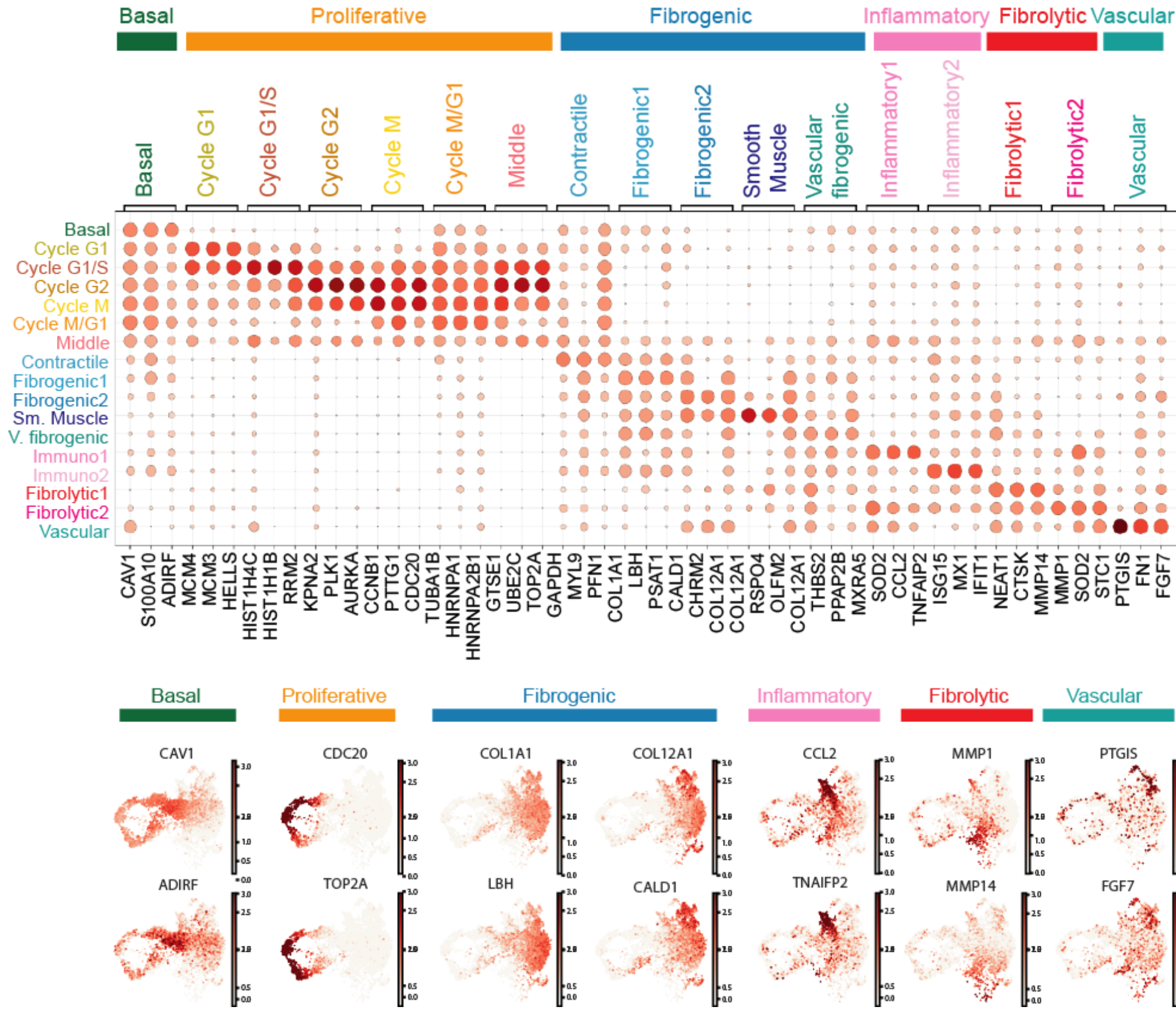
Physiology



Physiology

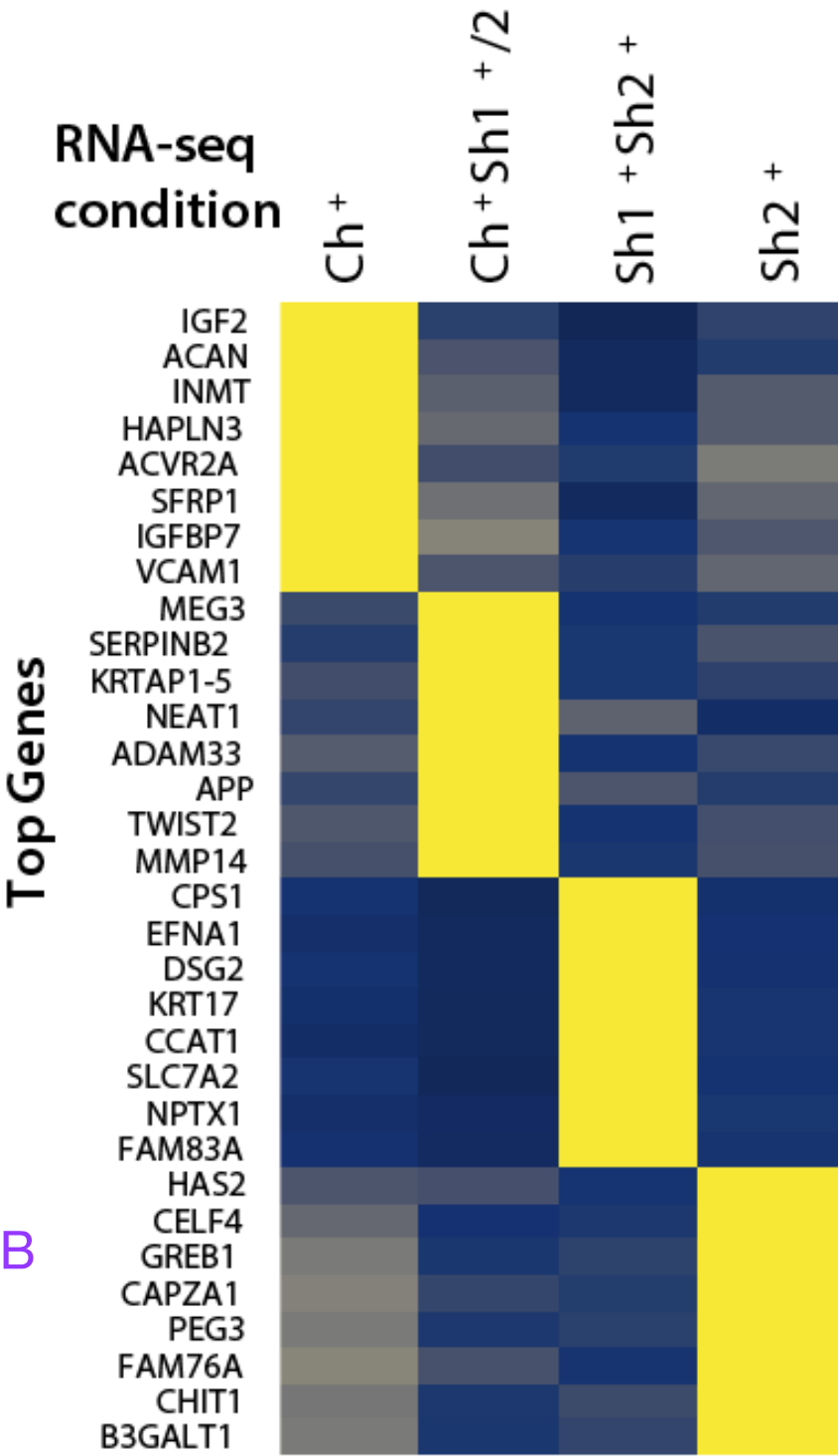
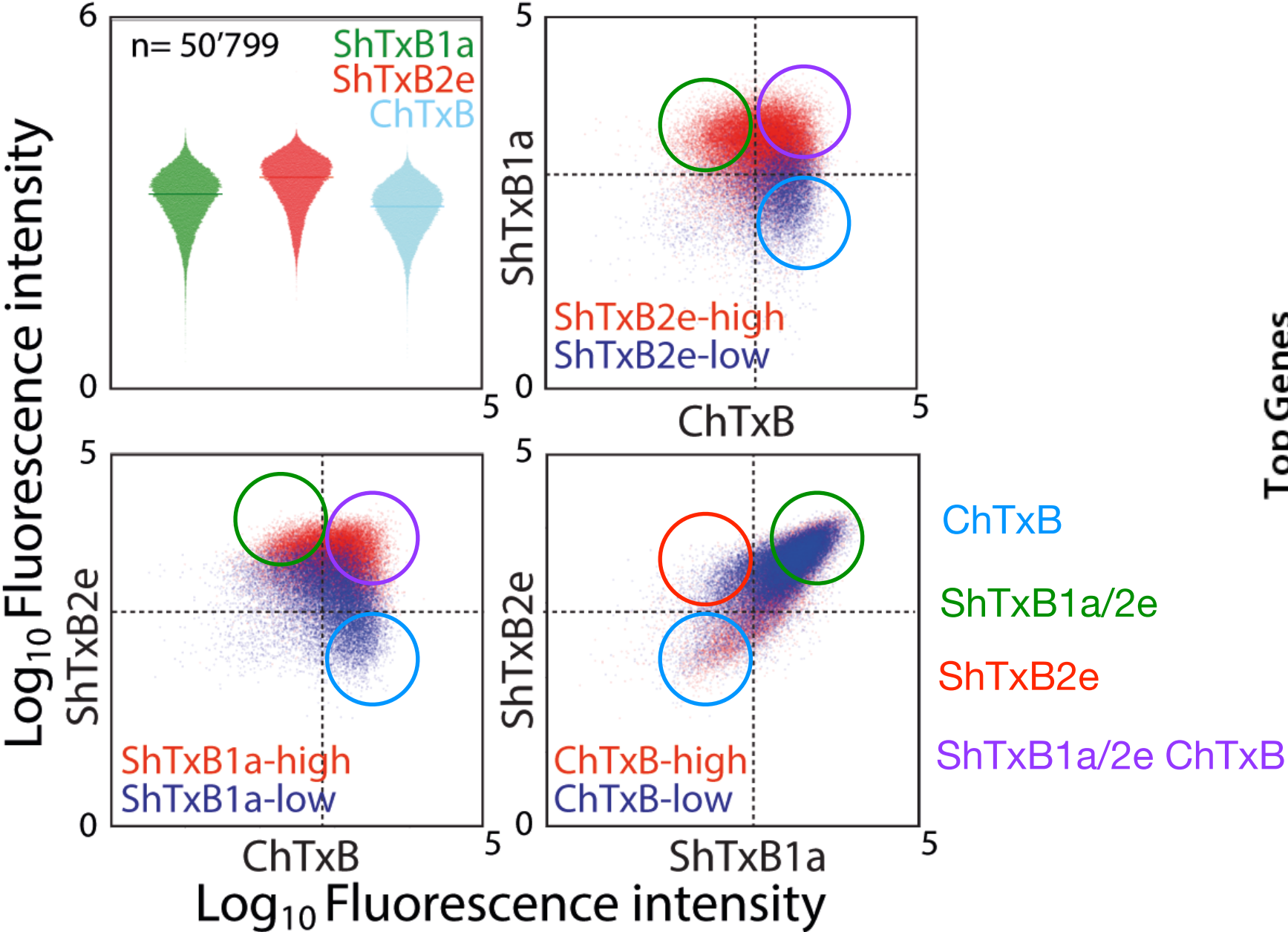


Physiology

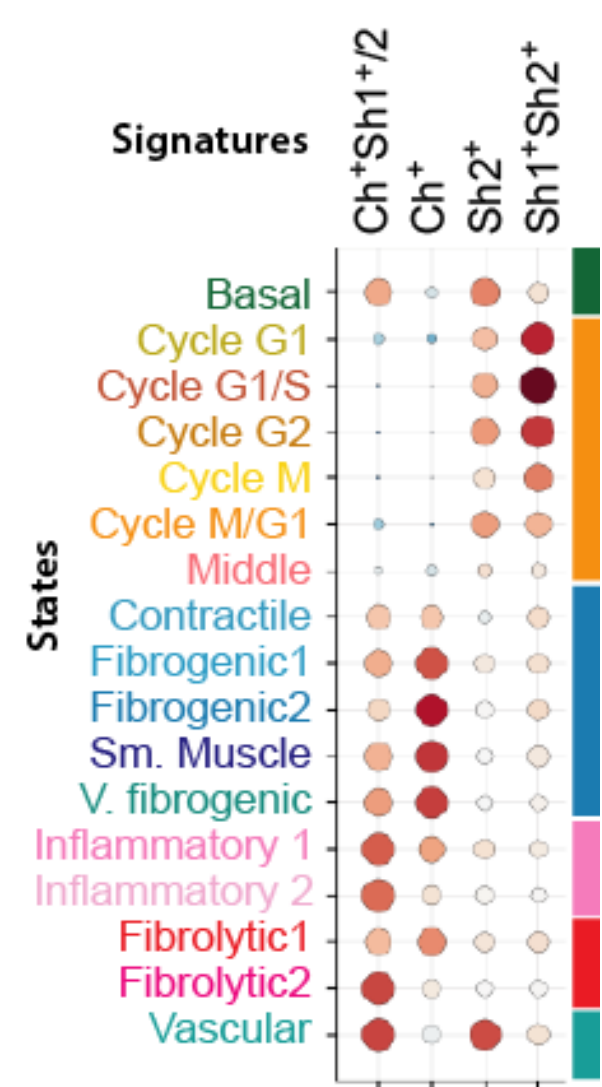
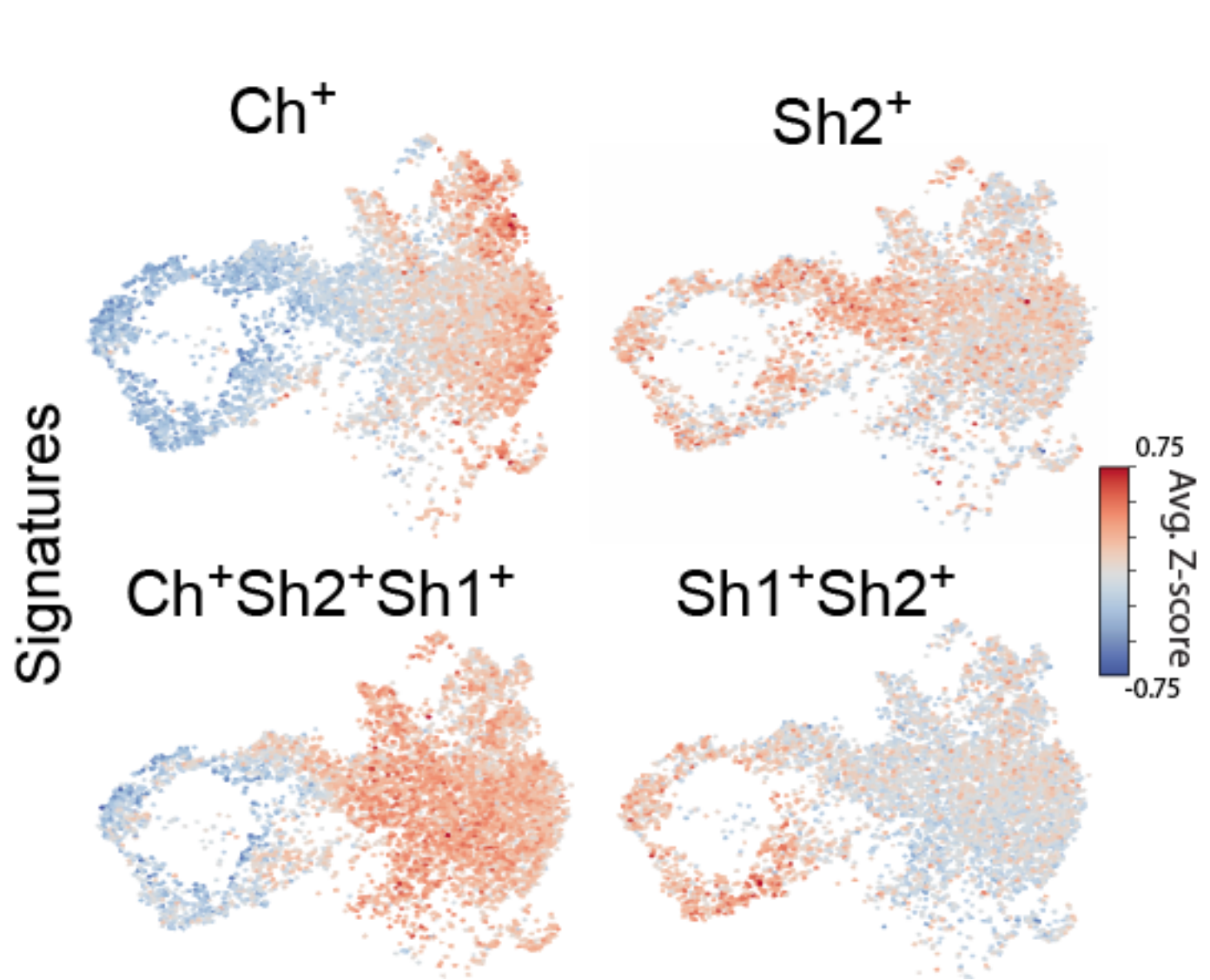
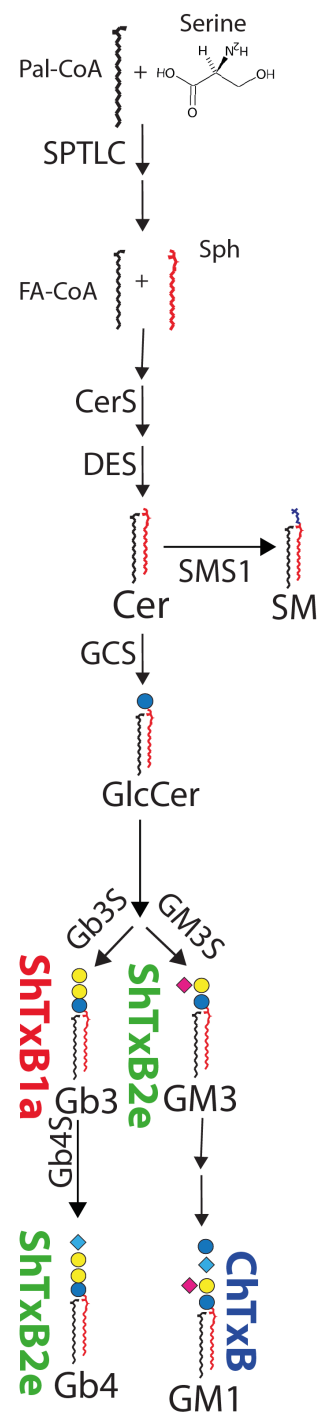


Physiology

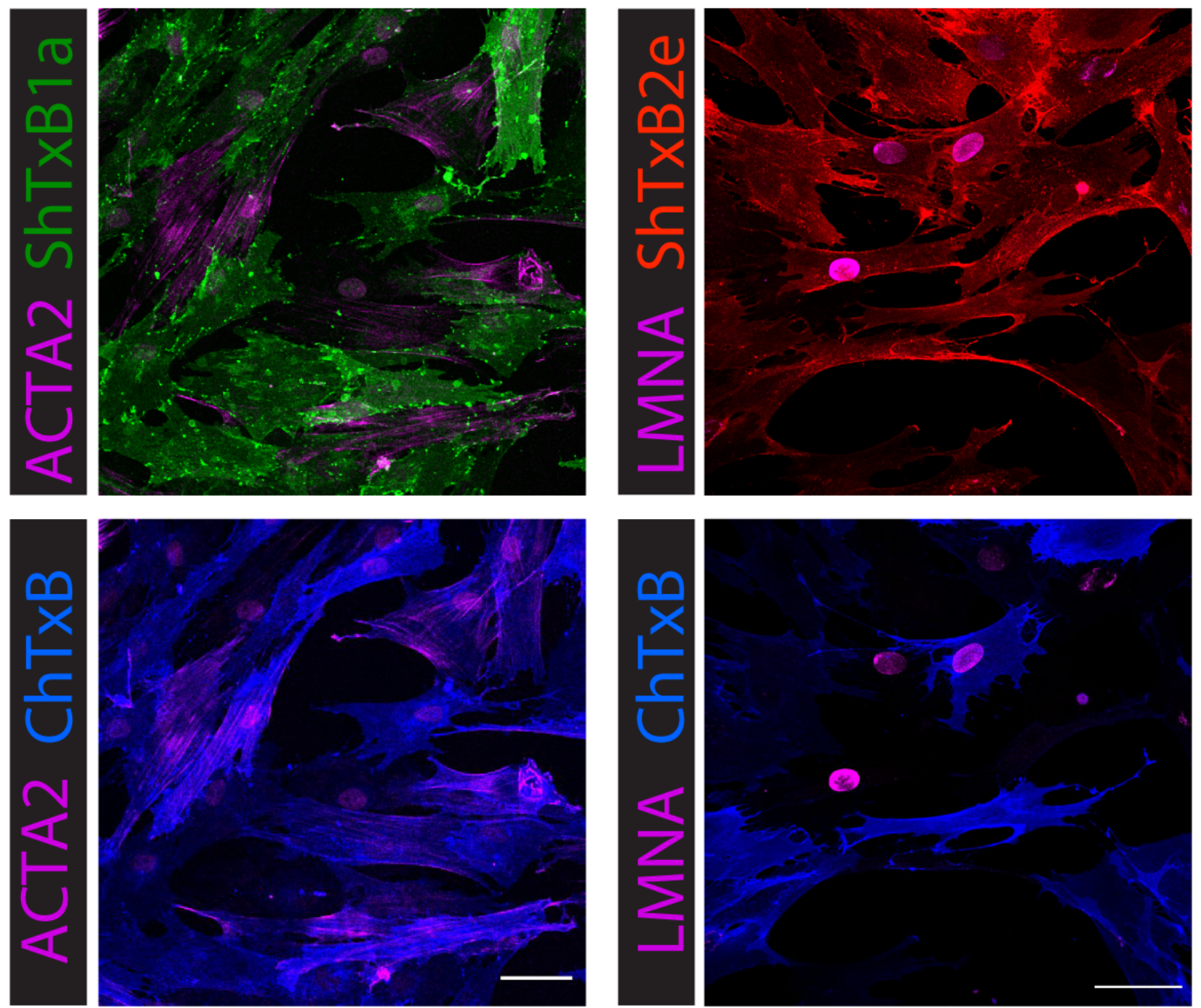
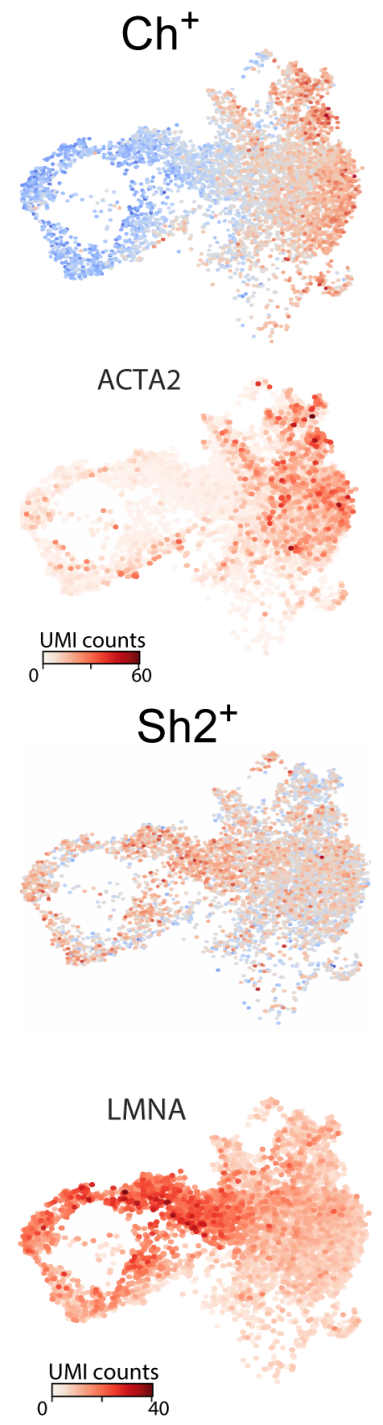
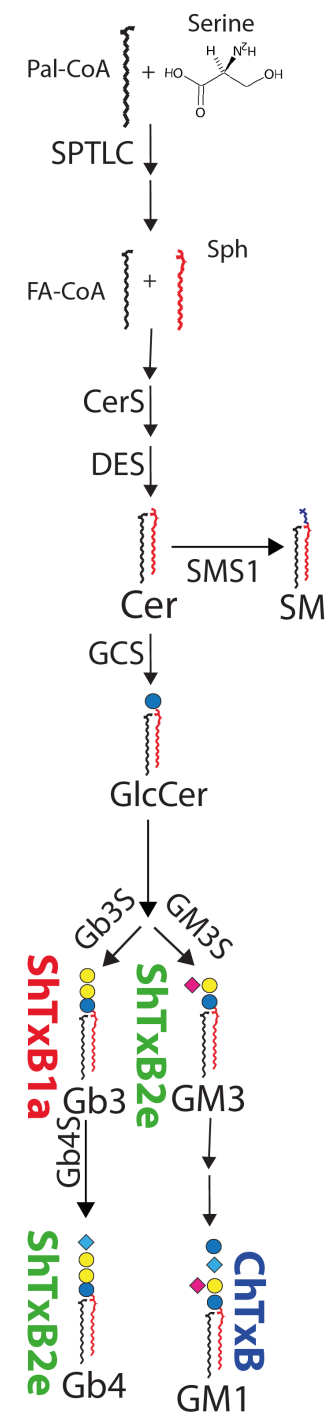
Flow Cytometry



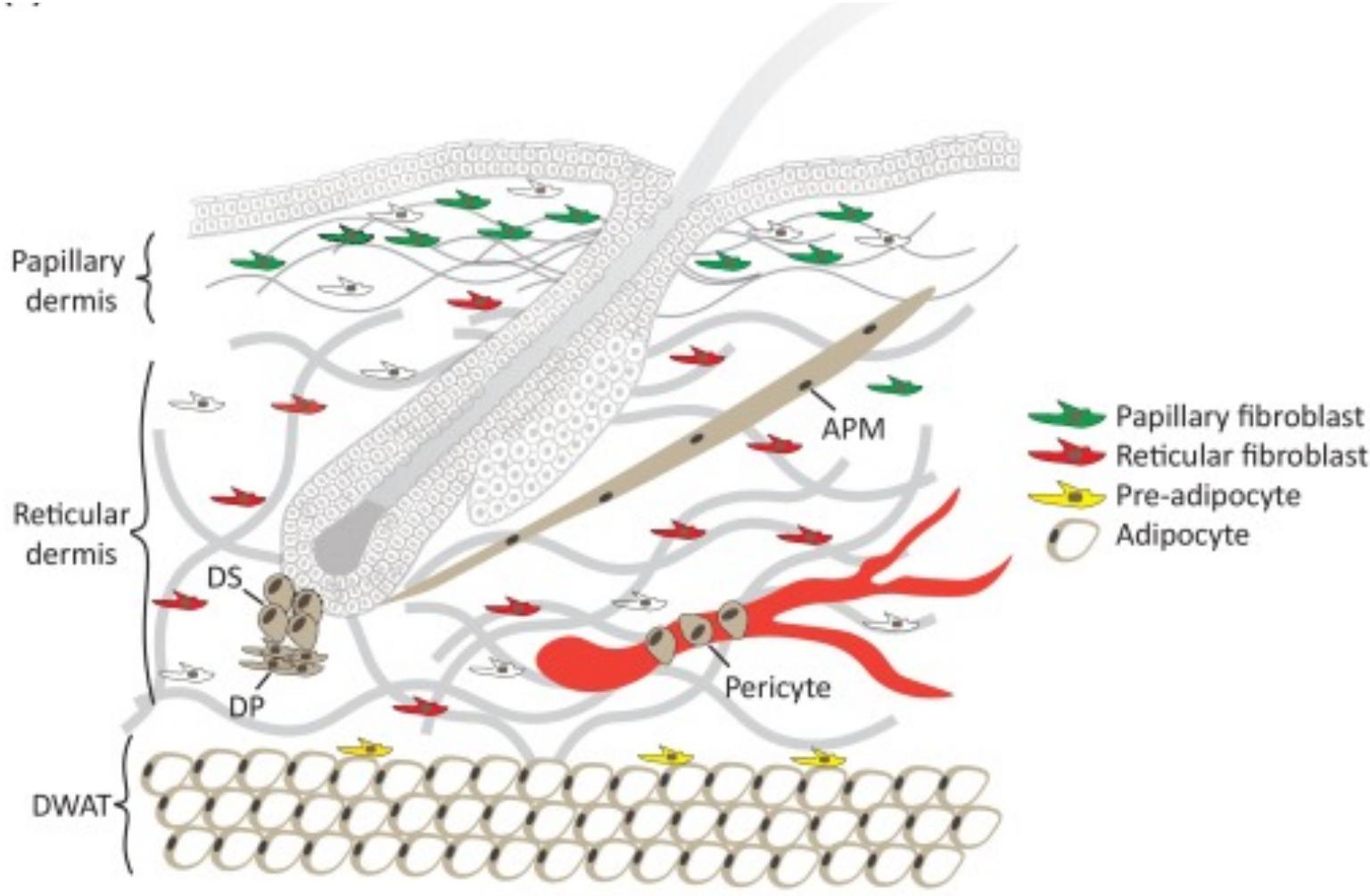
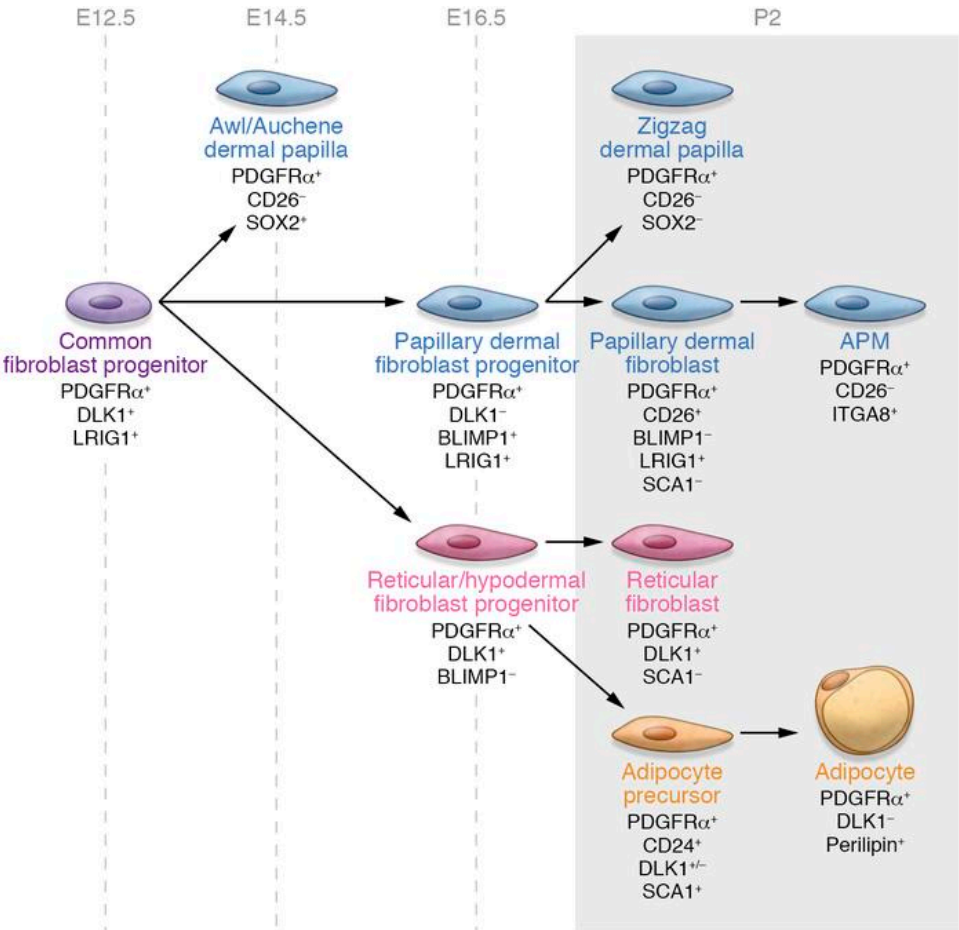
Physiology



Physiology

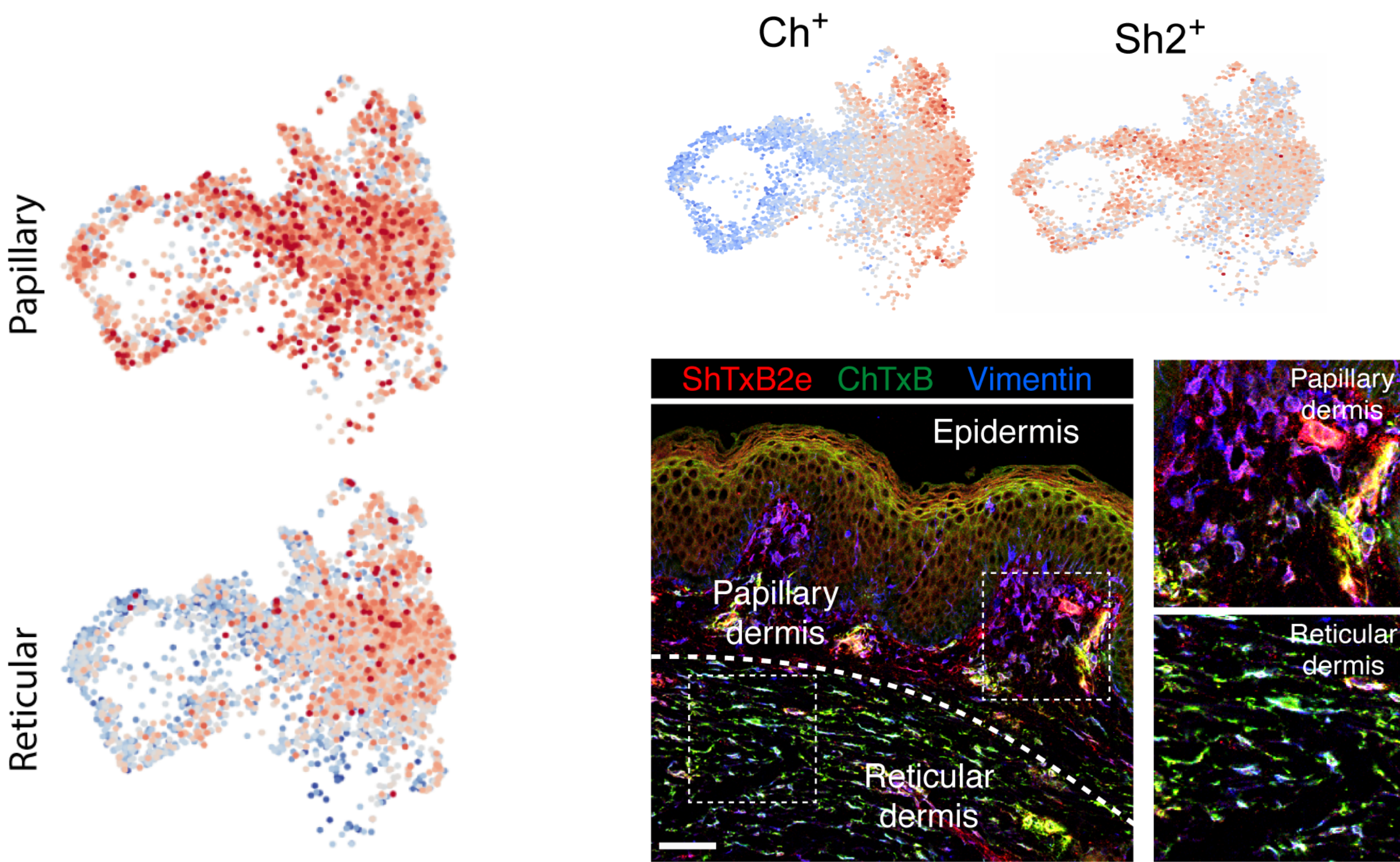


Physiology



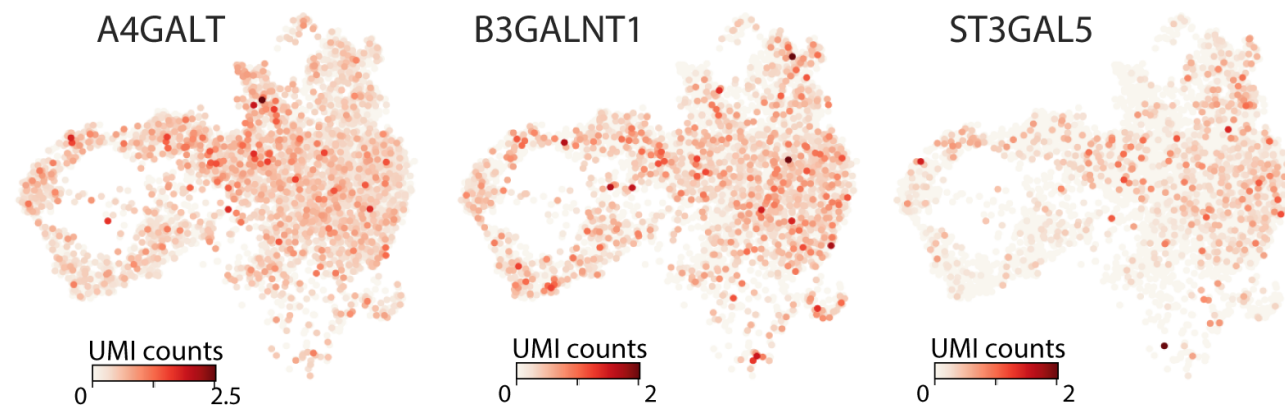
Physiology

Do different lipotypes populate distinct region of the skin?

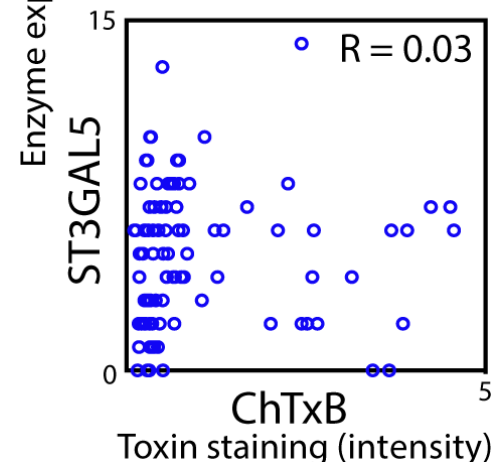
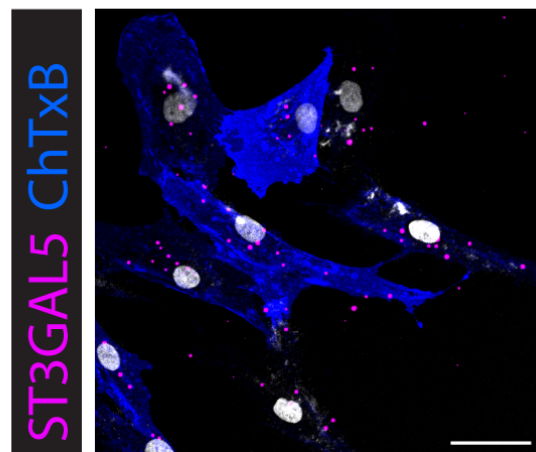
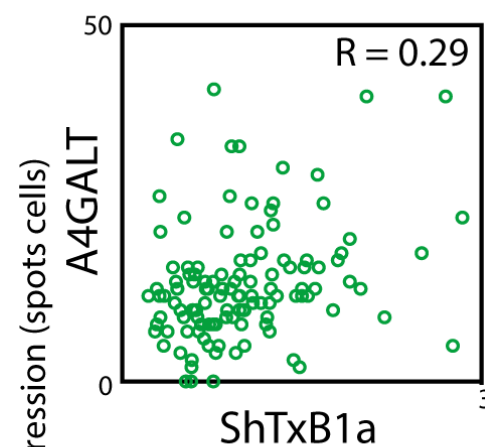
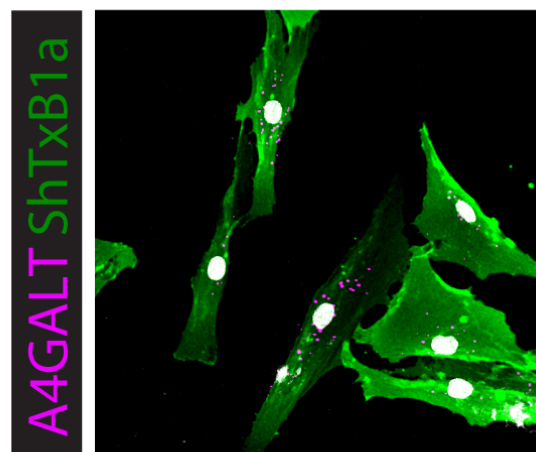


Physiology

Are *lipotypes* the result of specific transcriptional programs?



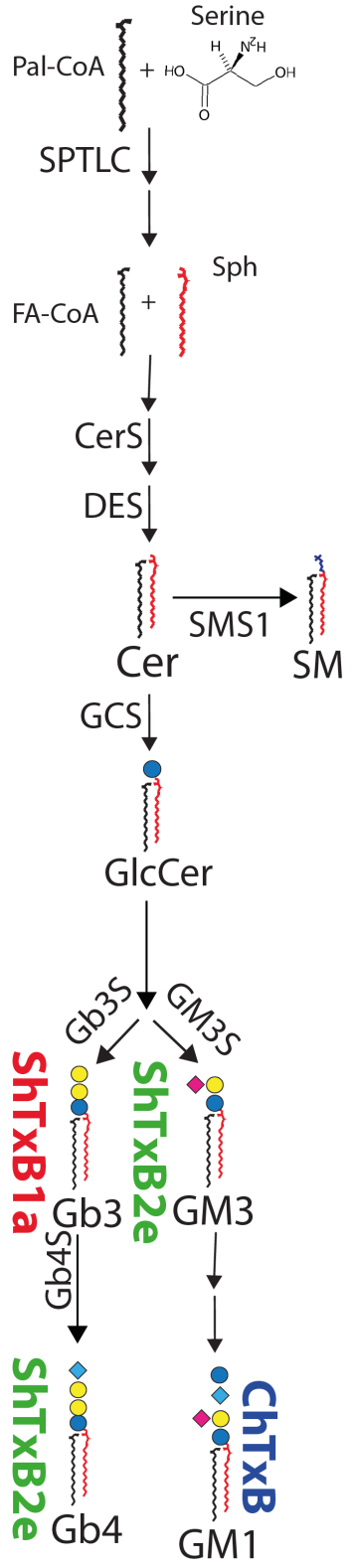
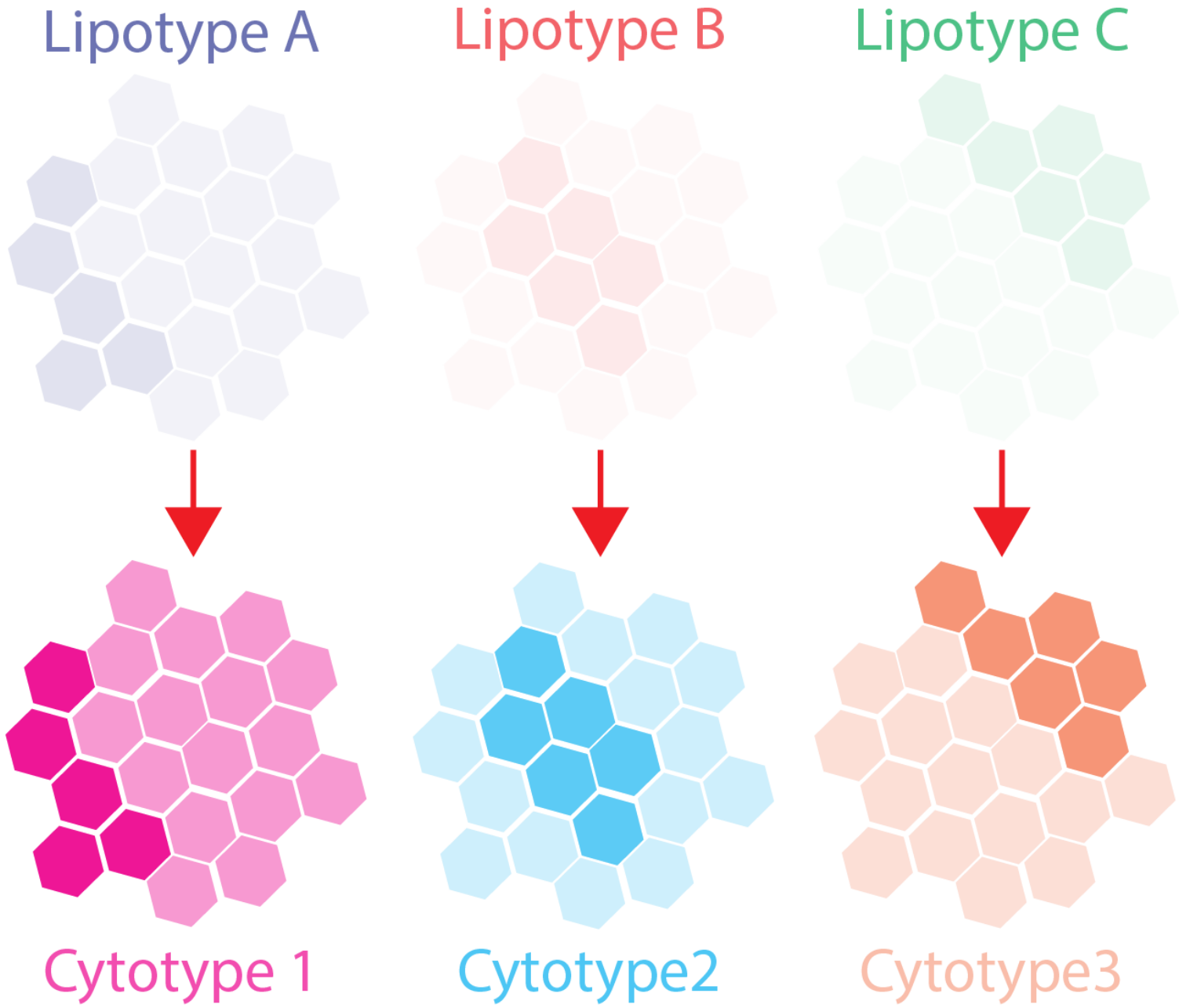
Lipid metabolising enzymes don't show a cell-state specific localisation



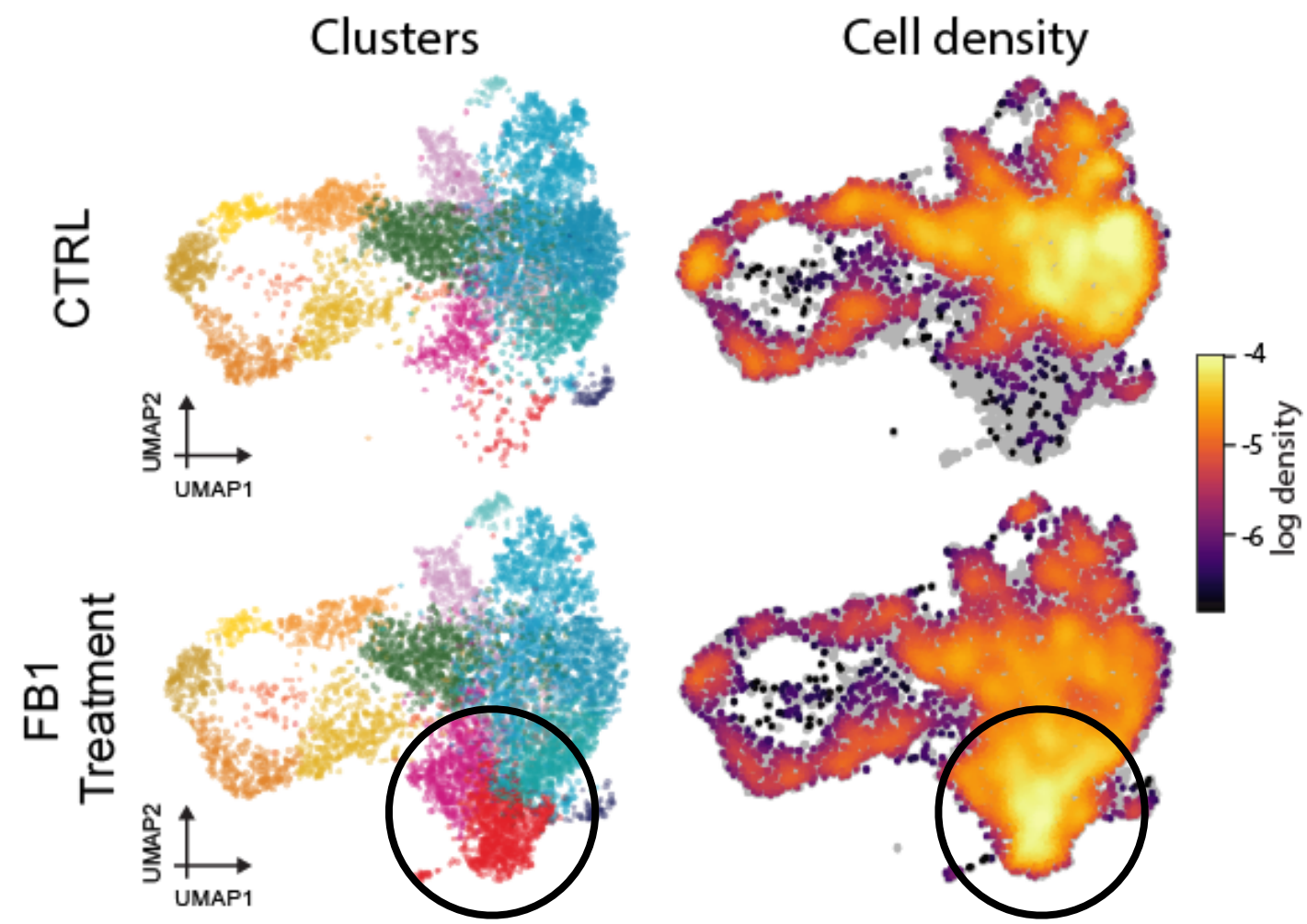
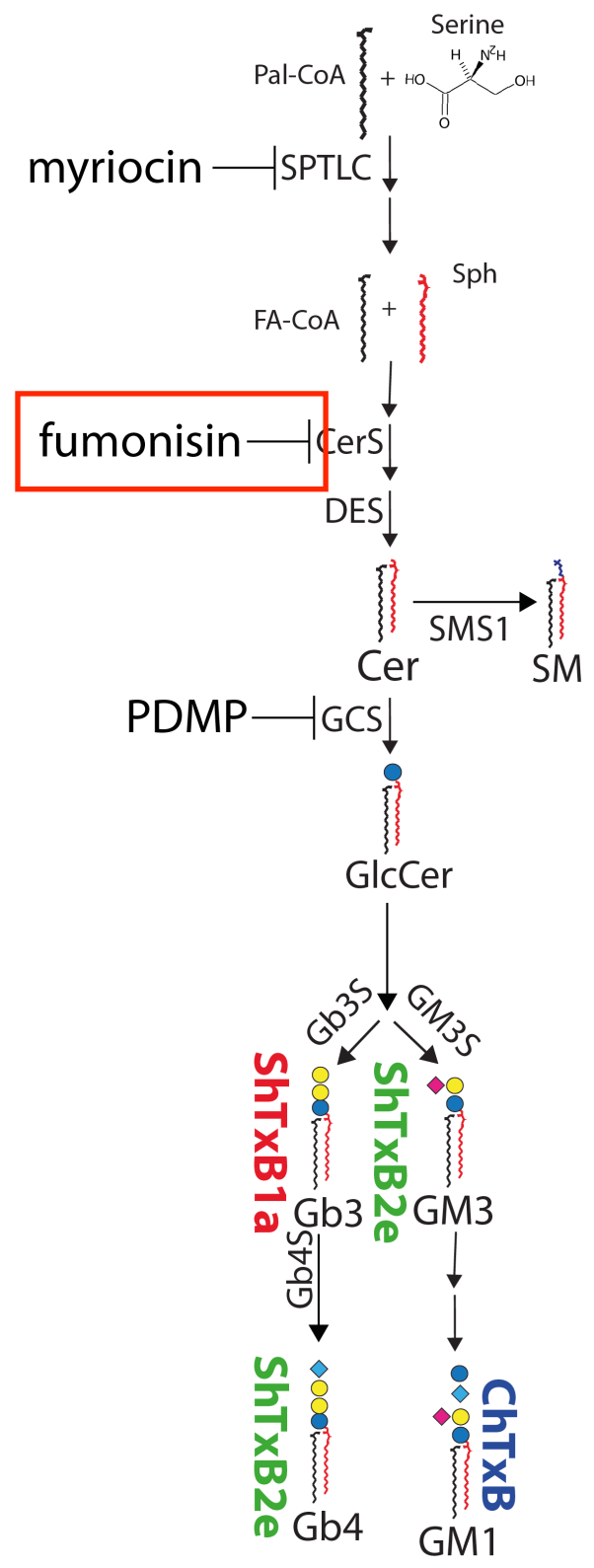
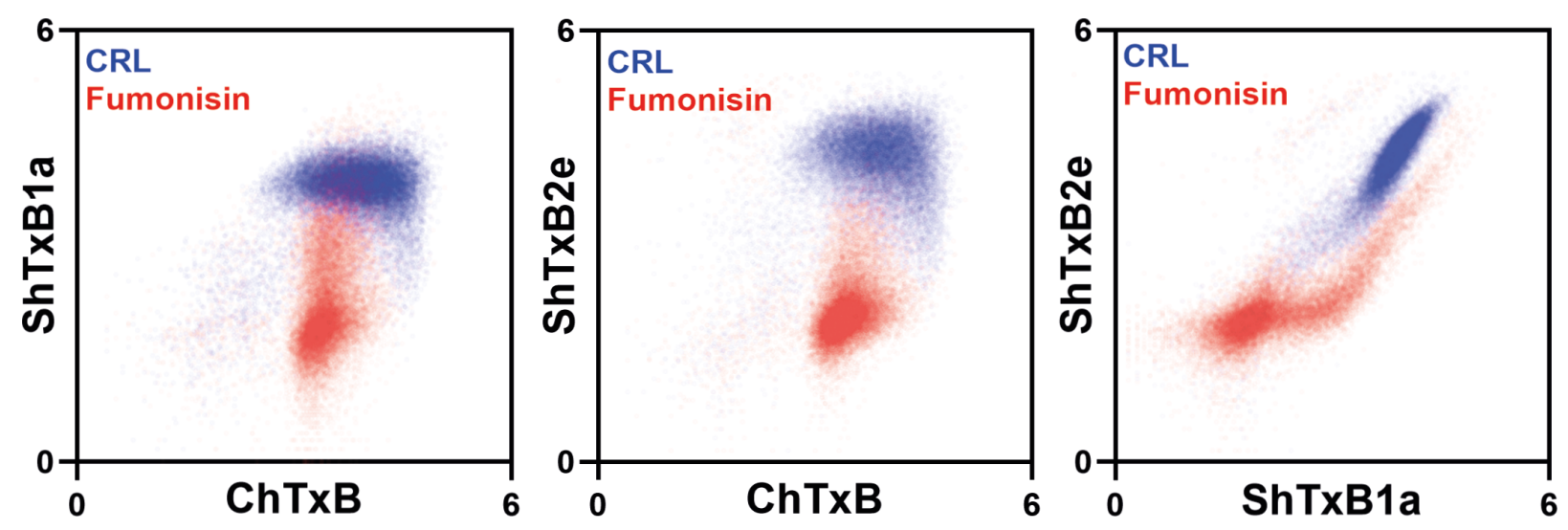
Are lipotypes causally upstream of cell states and influence cell-to-cell transcriptional heterogeneity?

Physiology

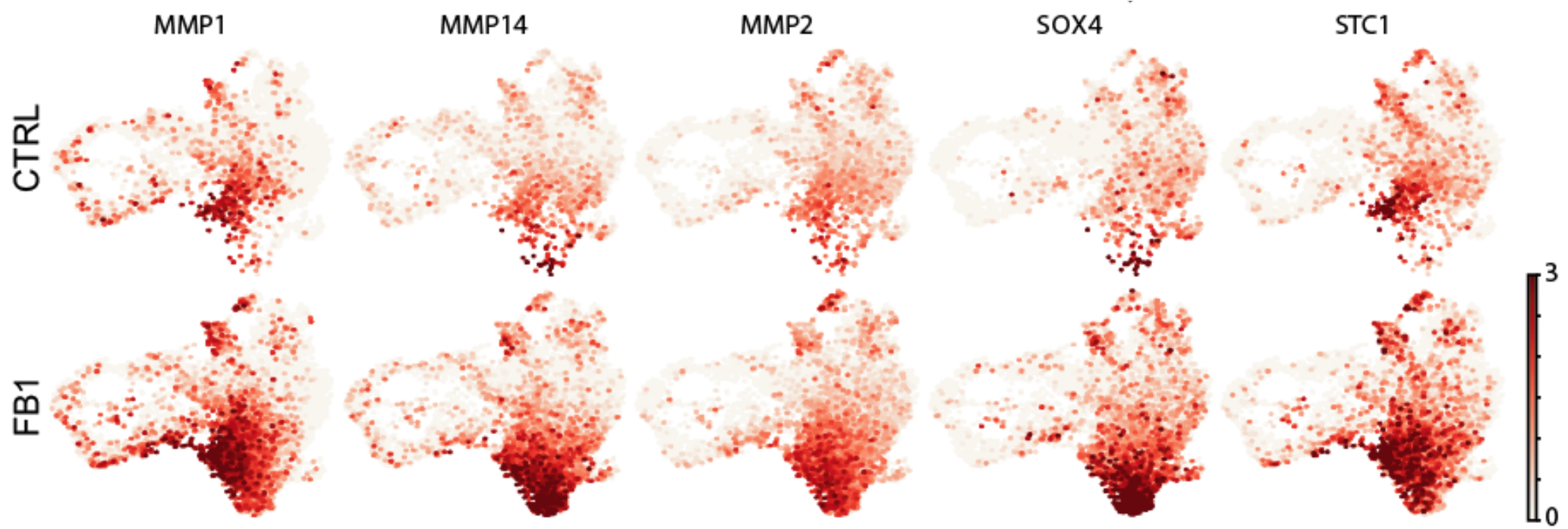
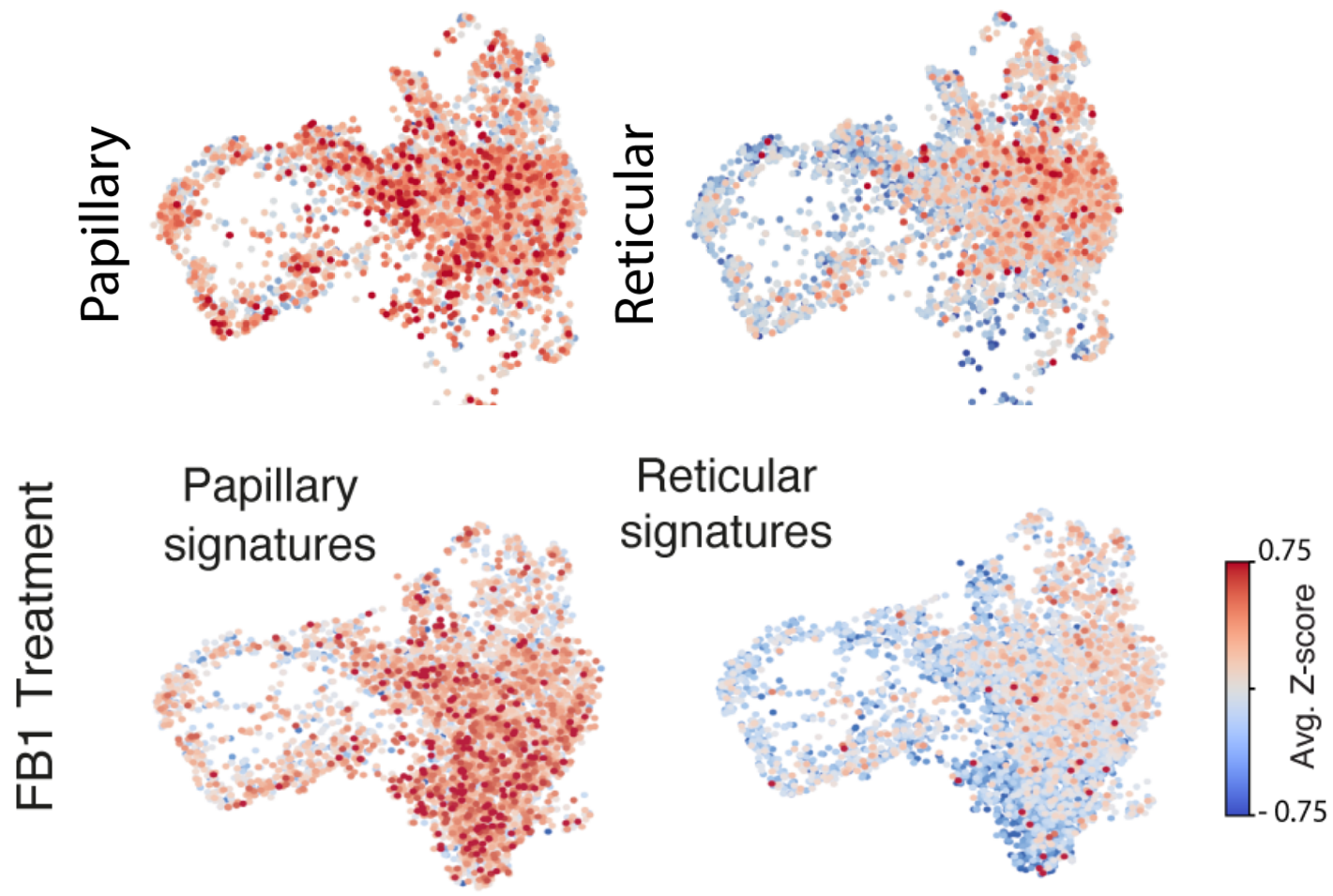
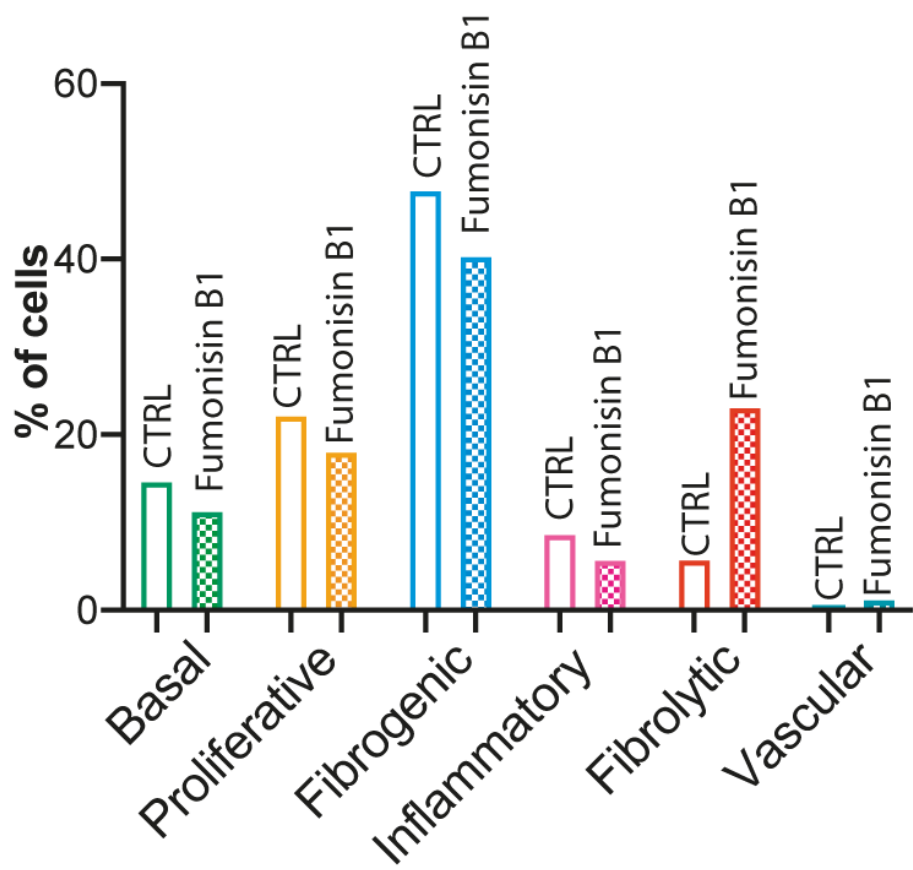
transcriptional landscape metabolic landscape



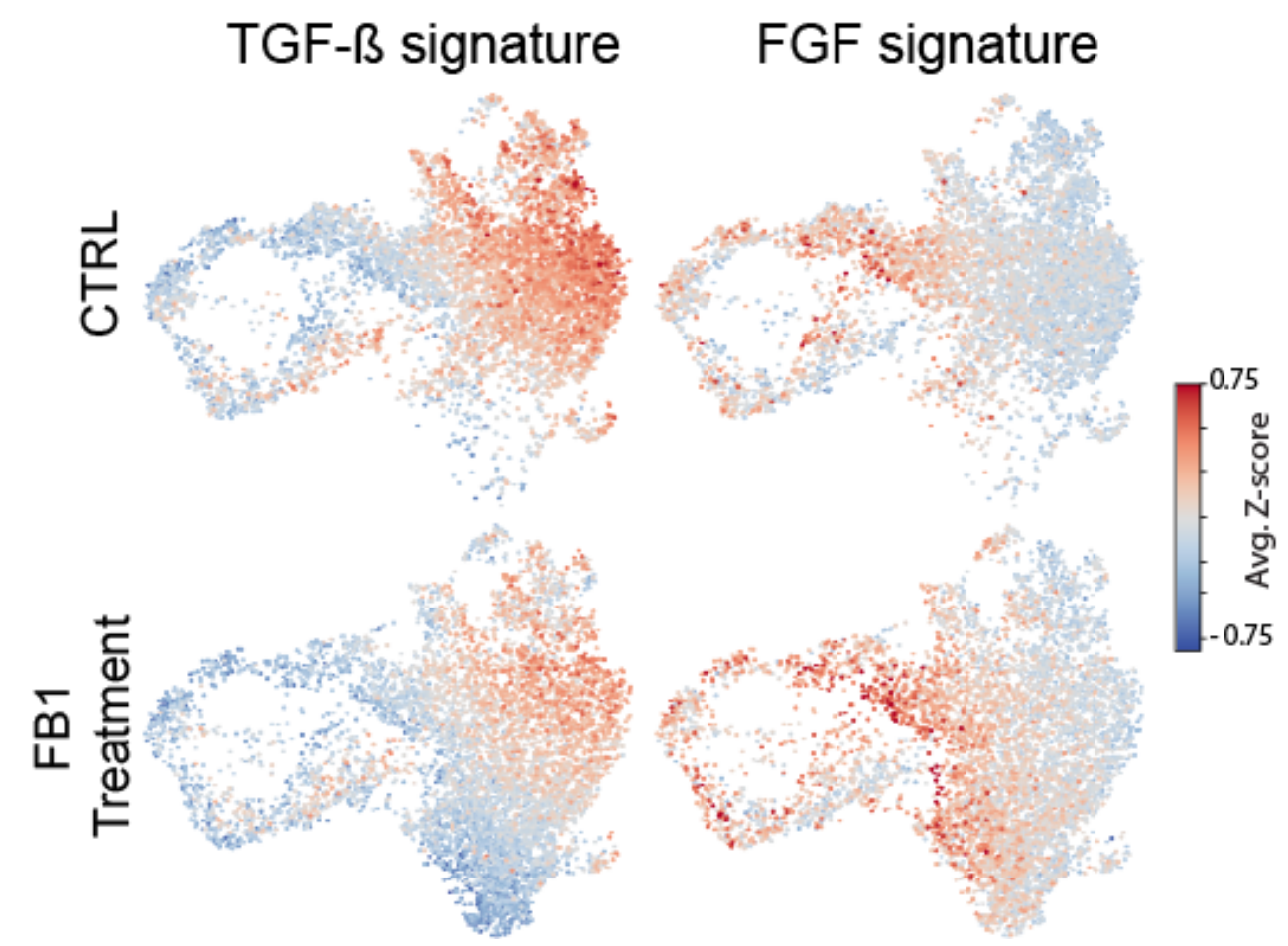
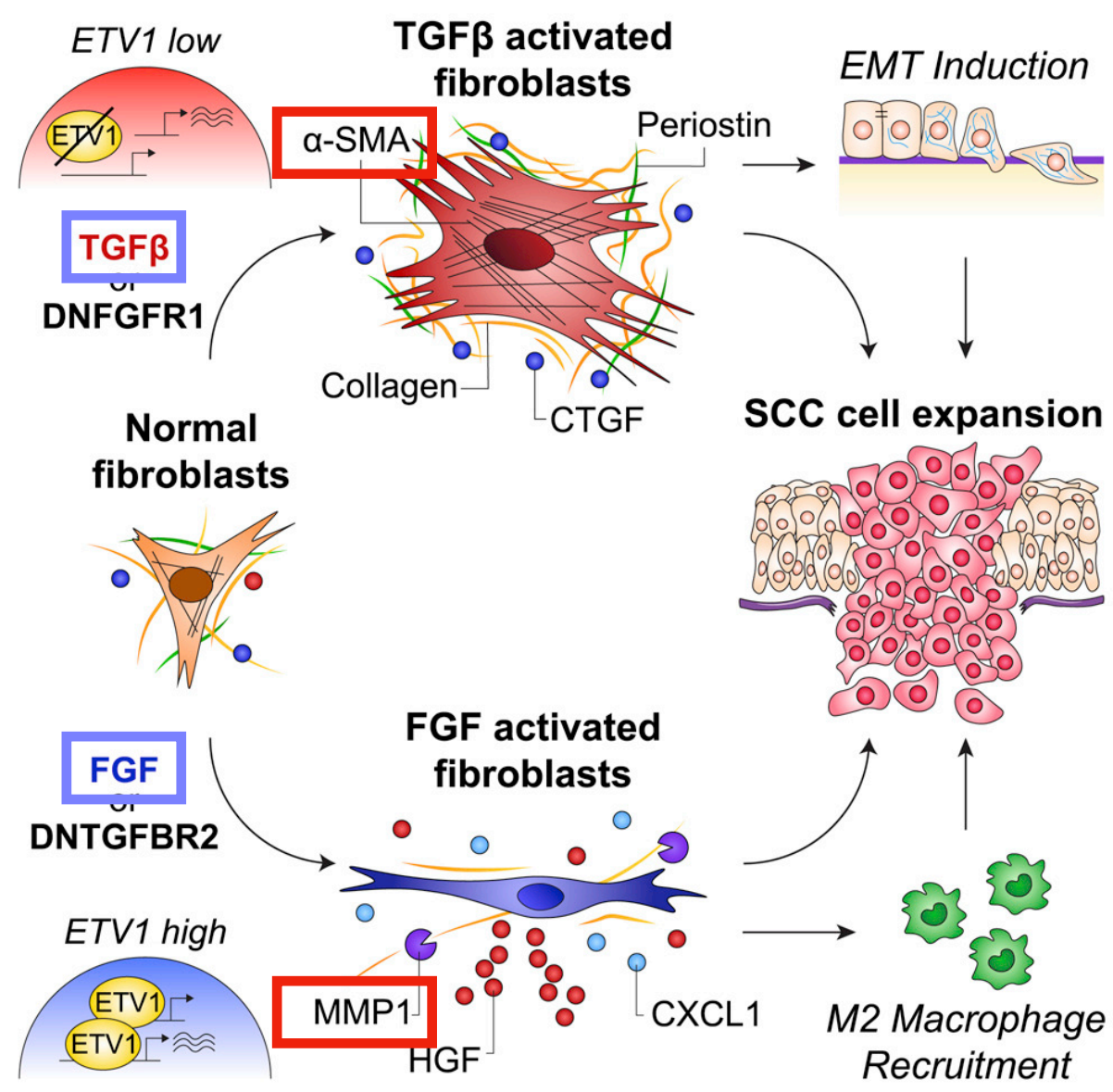
Physiology



Physiology

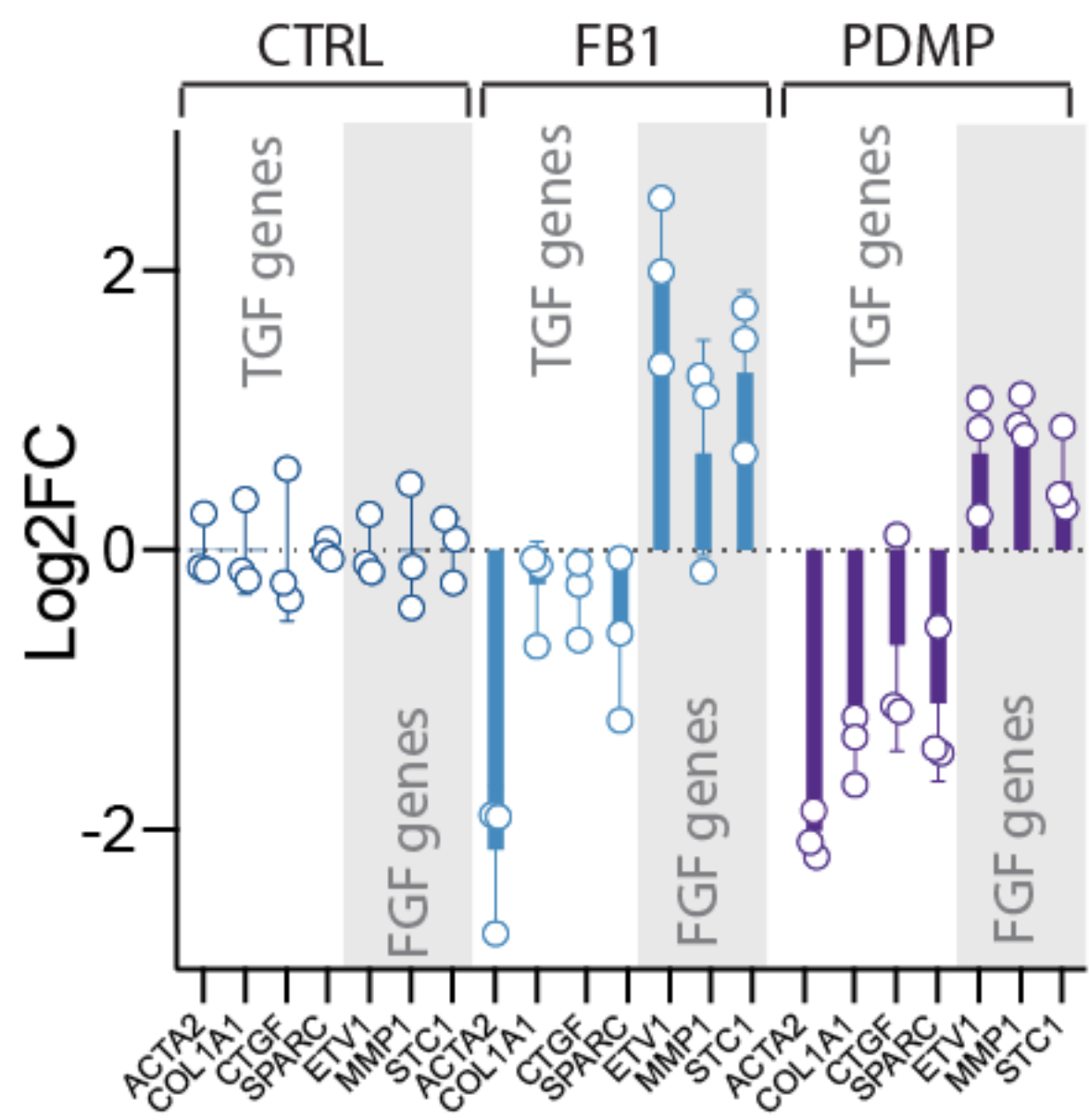
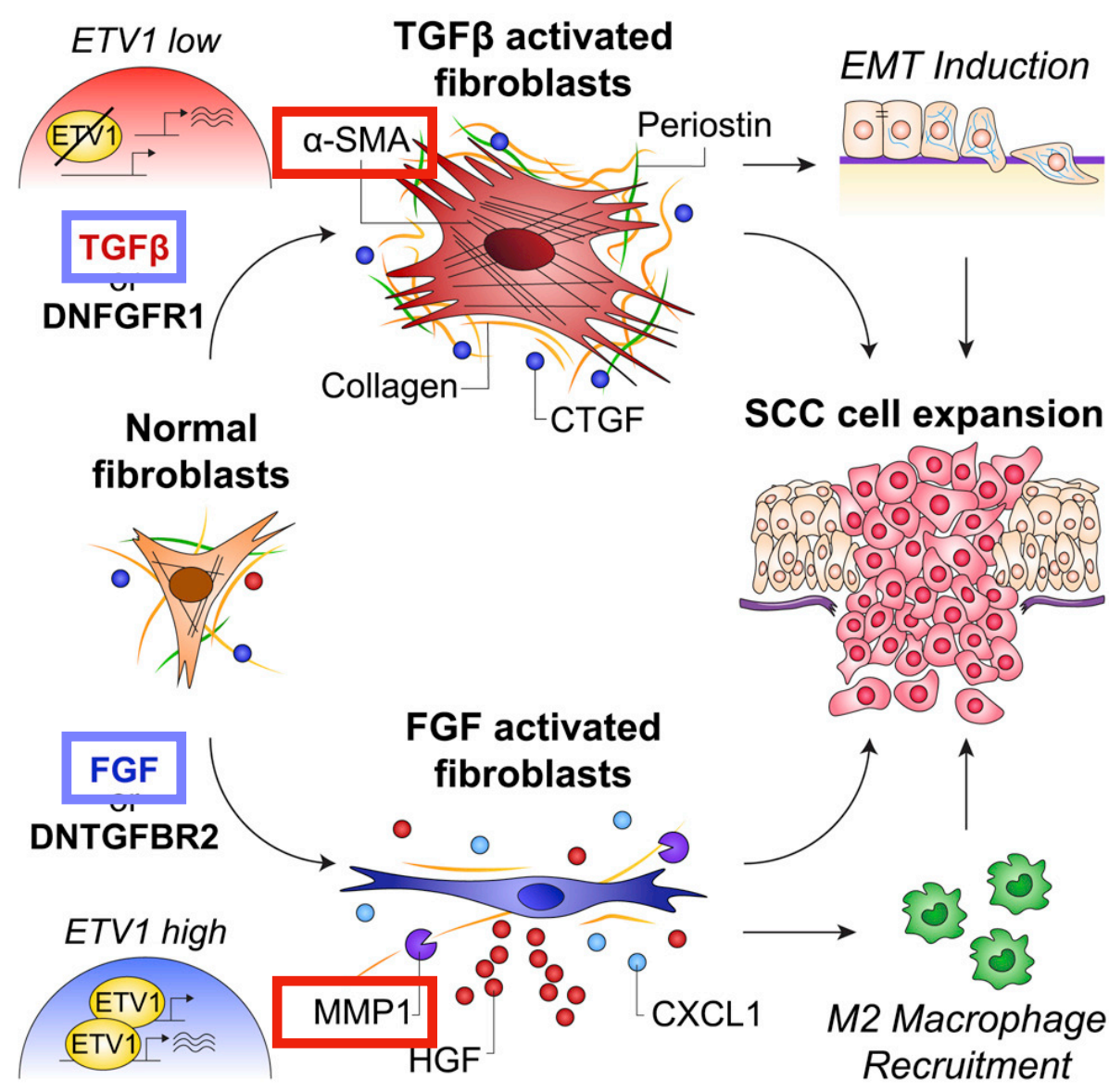


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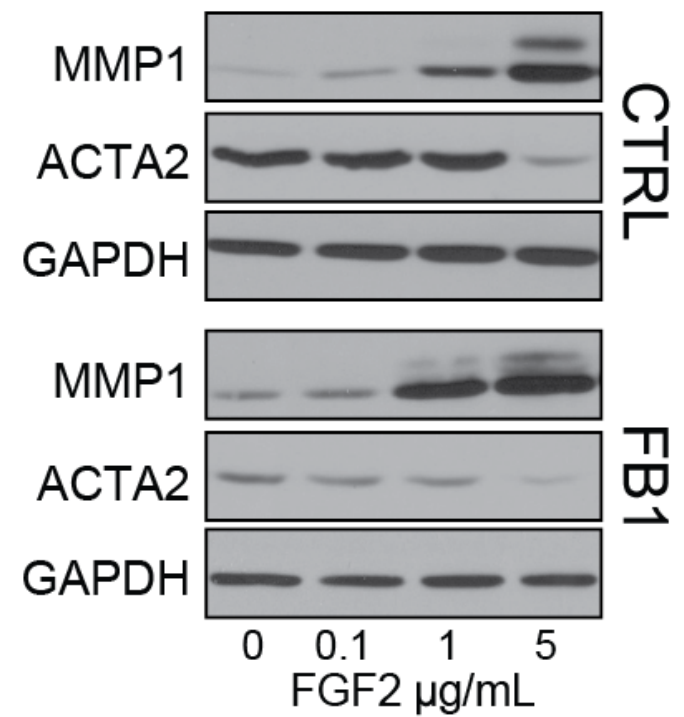
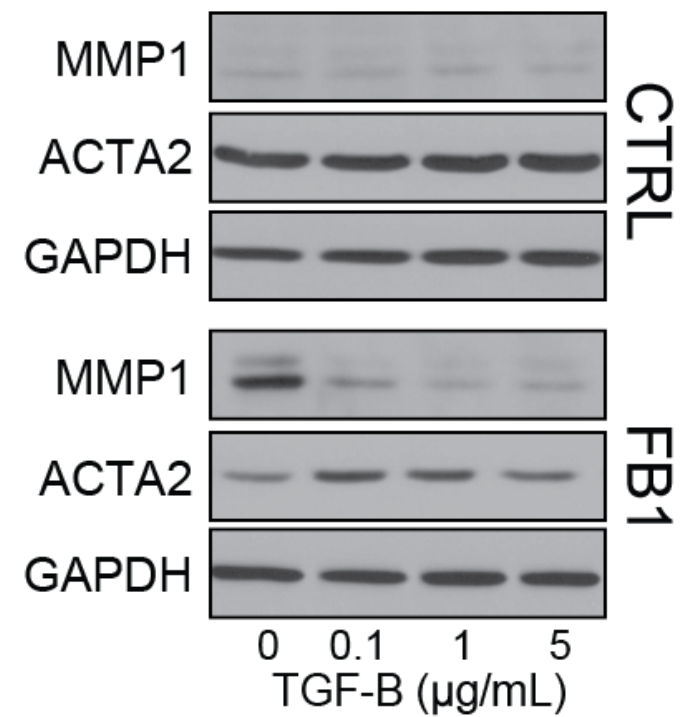
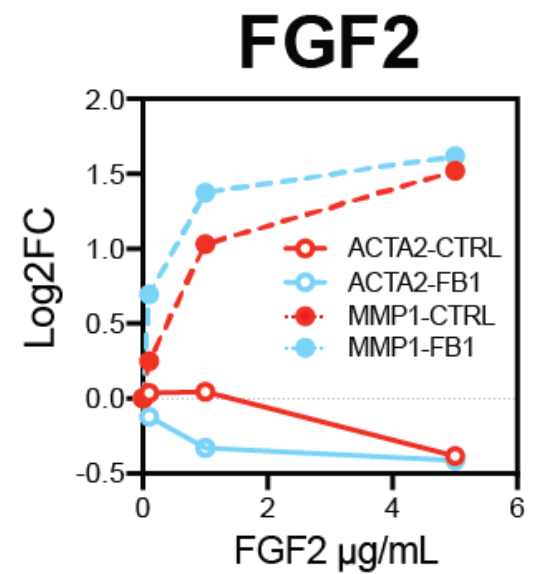
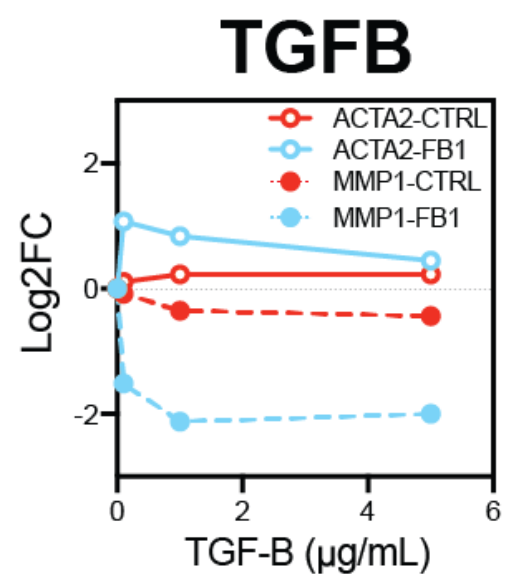
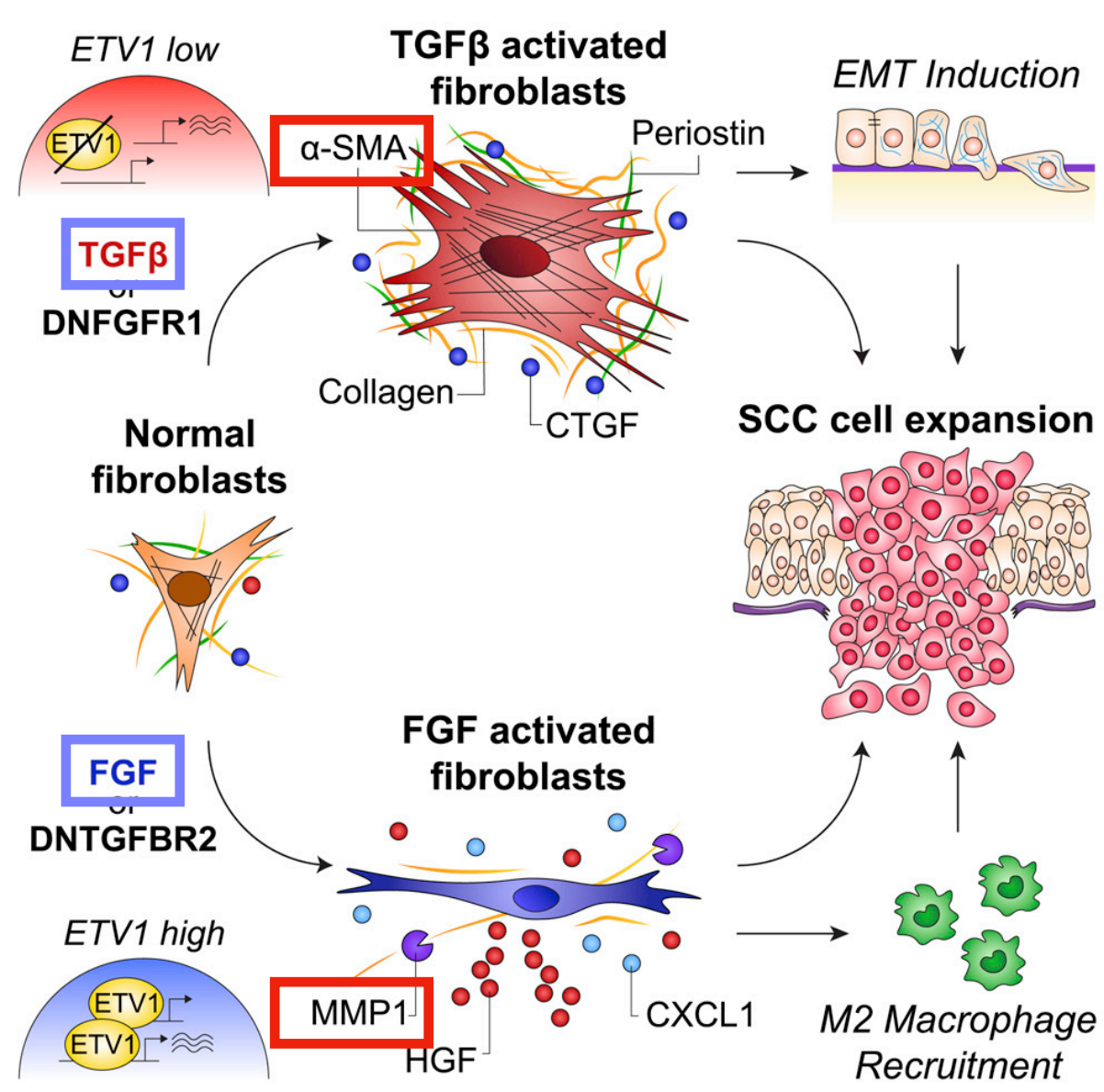
Bordignon, Pino, et al. "Dualism of FGF and TGF-β Signaling in Heterogeneous Cancer-Associated Fibroblast Activation with ETV1 as a Critical Determinant." *Cell reports* 28.9 (2019): 2358-2372.

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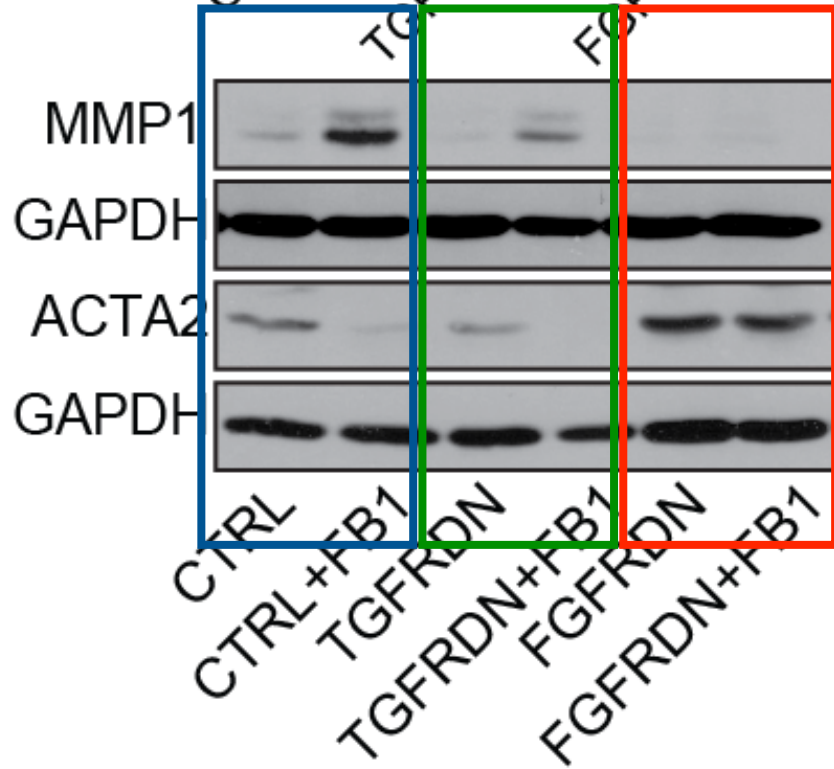
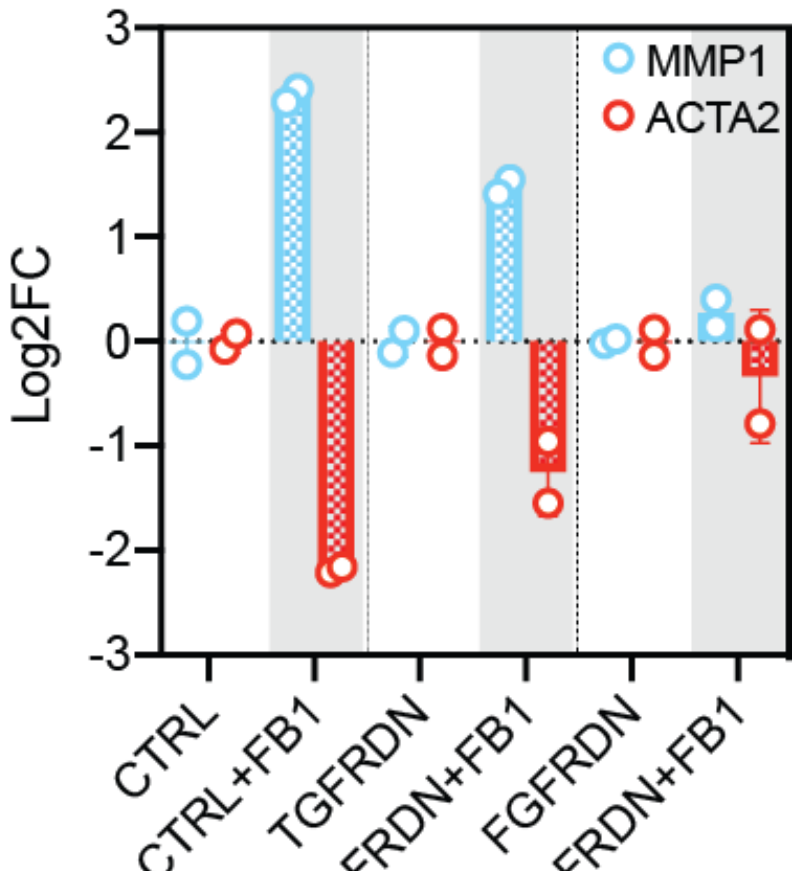
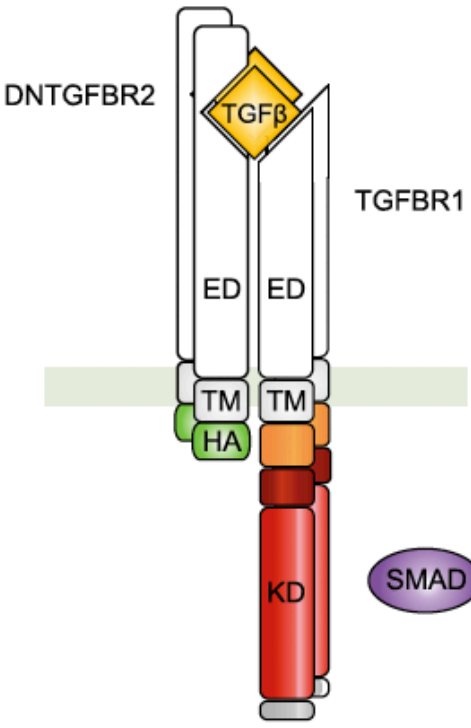
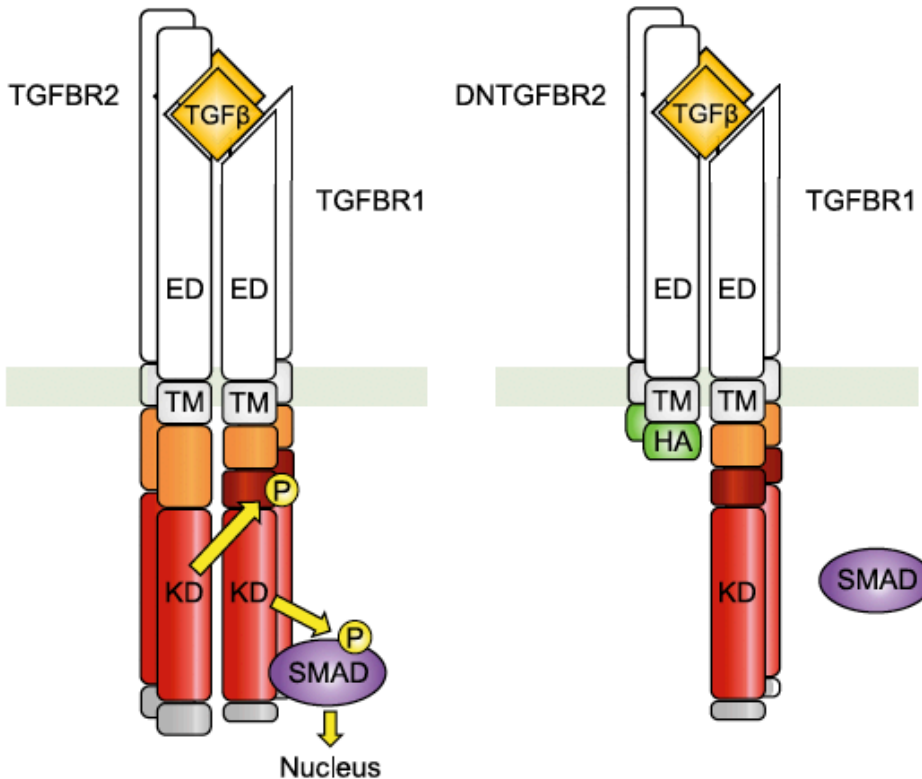
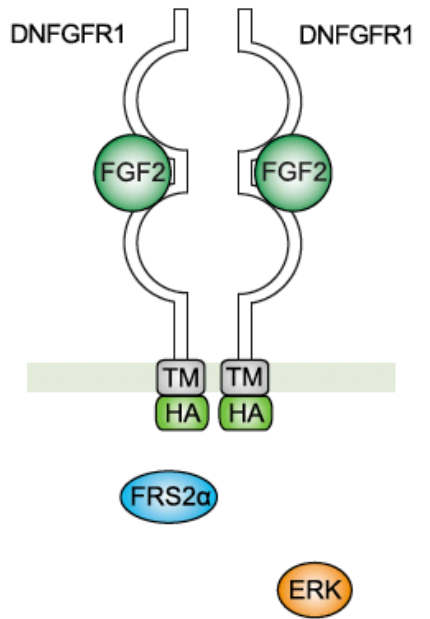
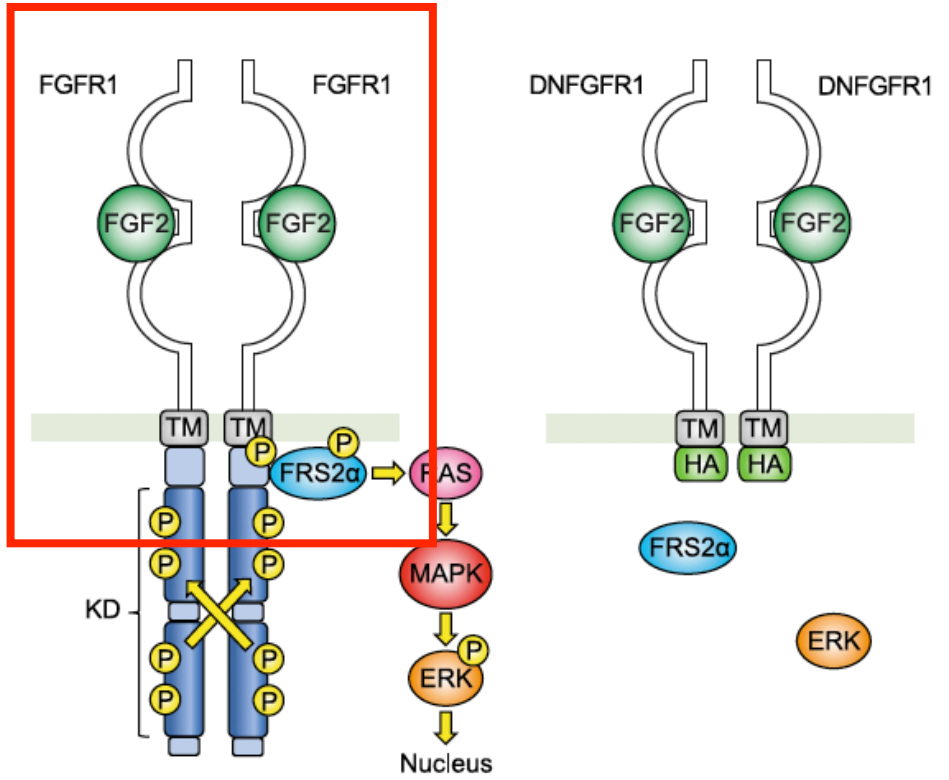
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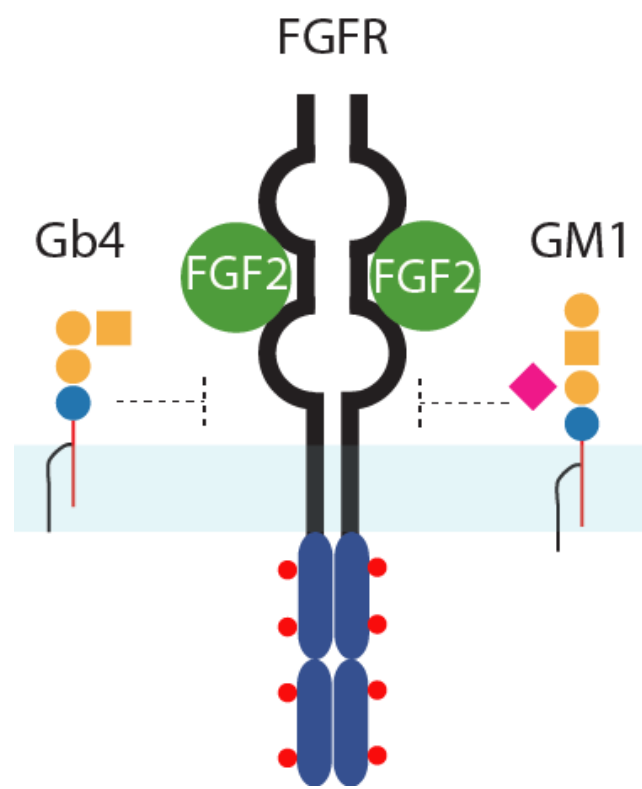
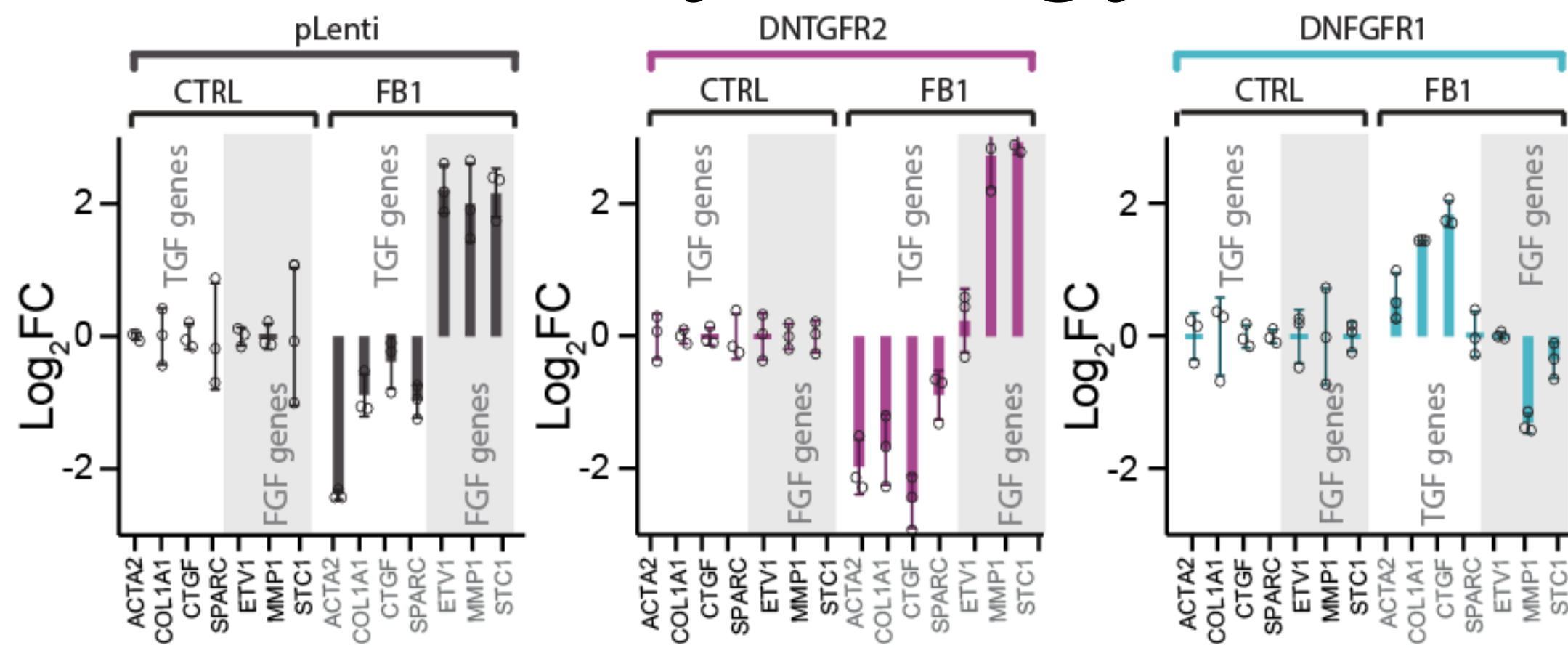


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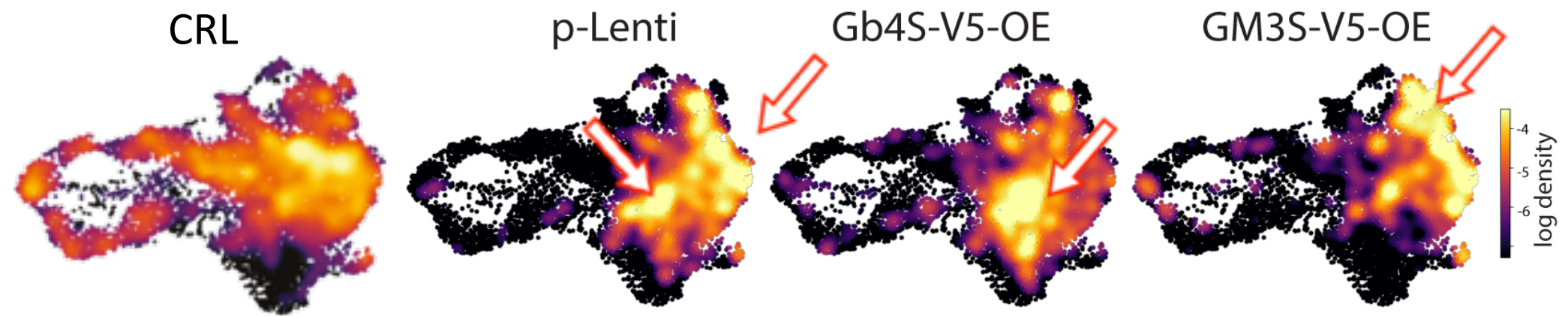
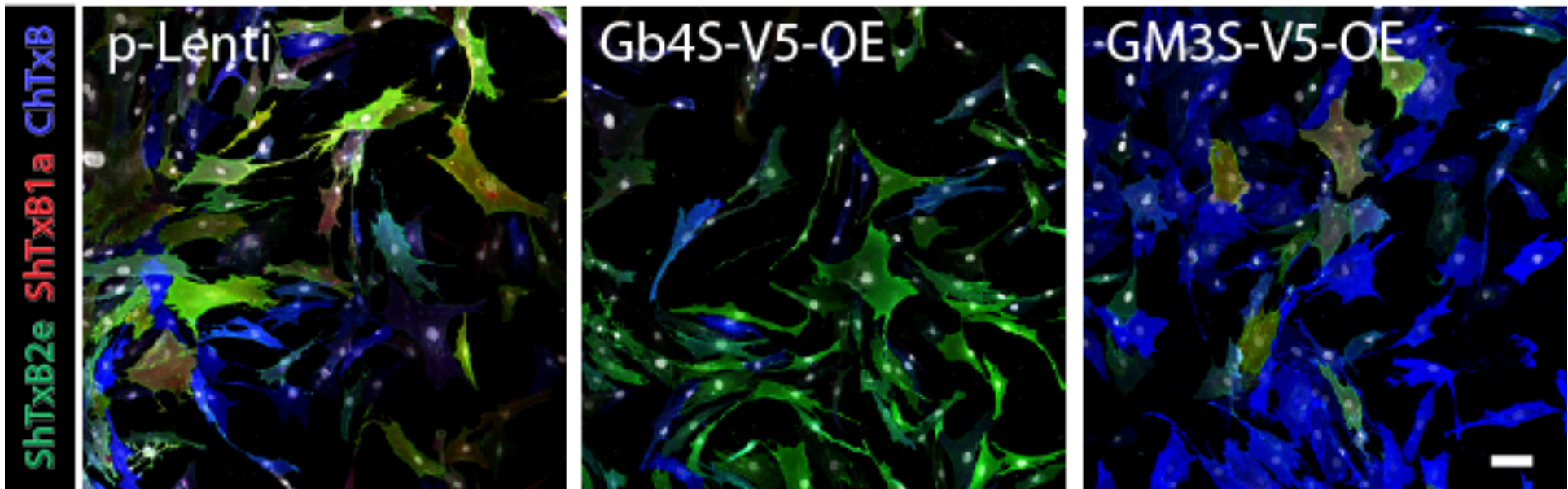
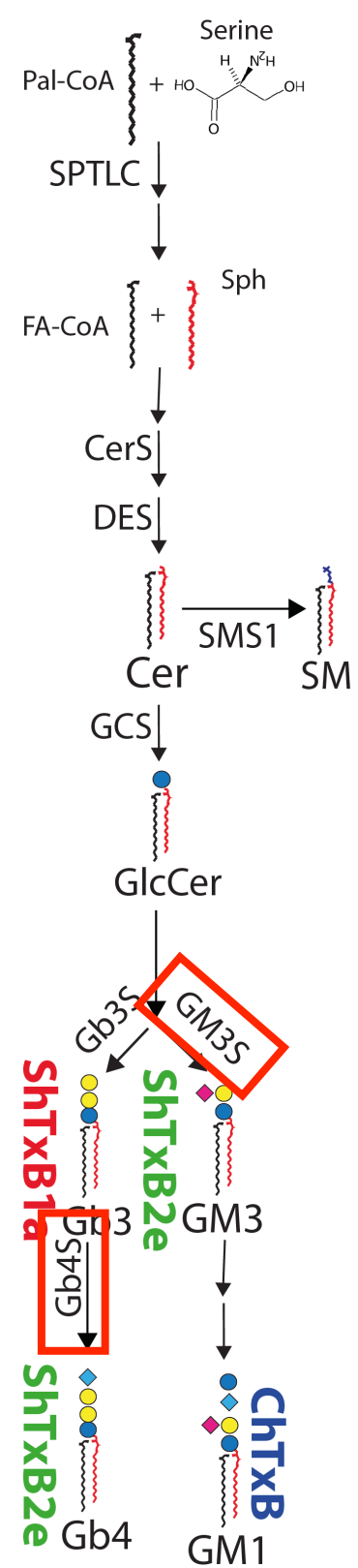


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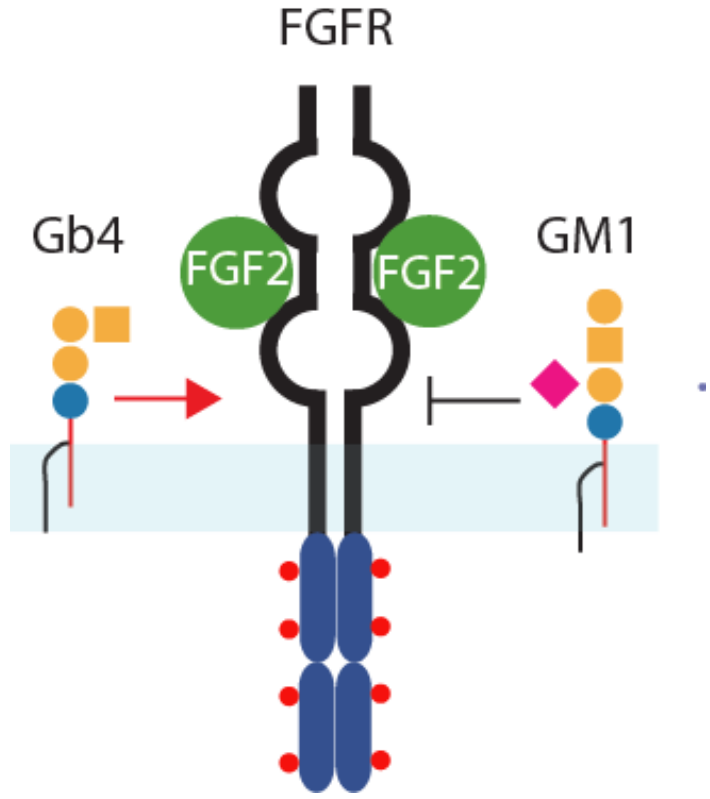
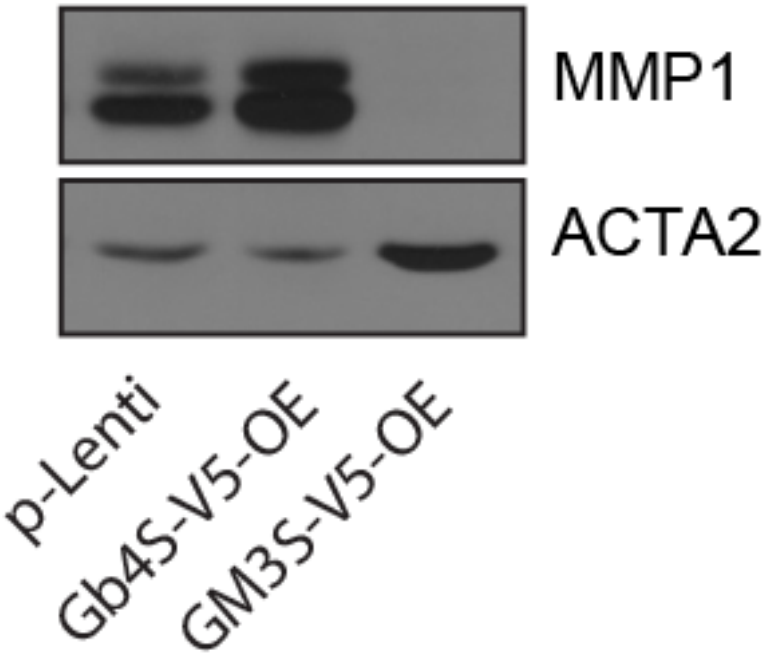
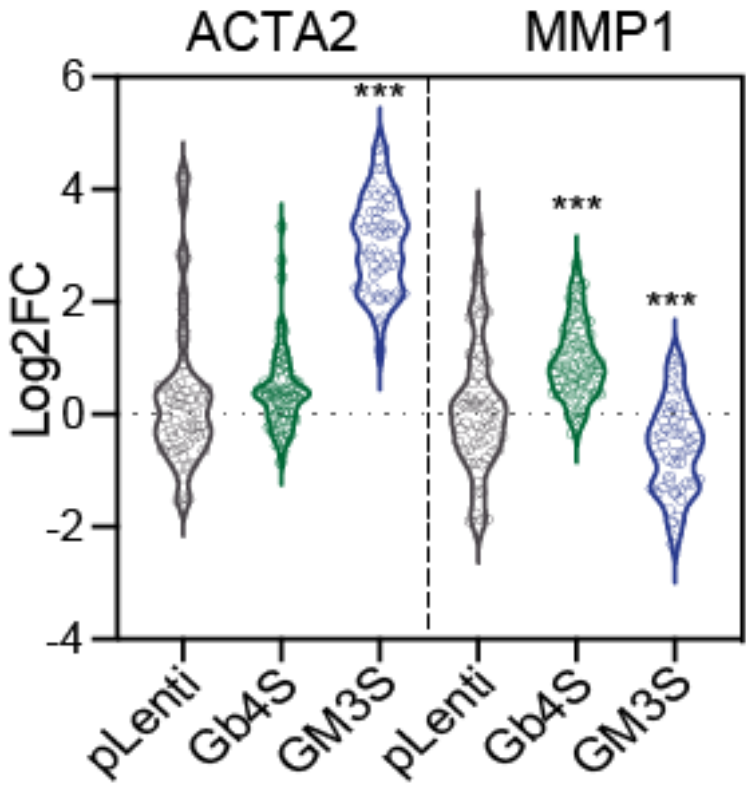
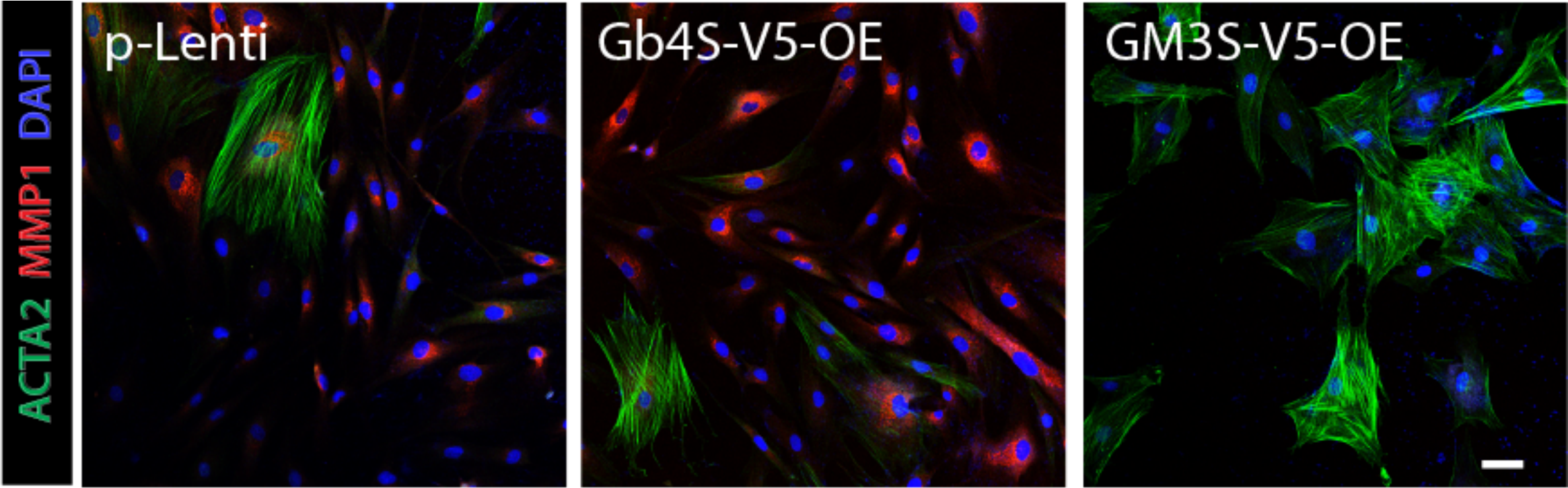


What is the role of specific lipids?

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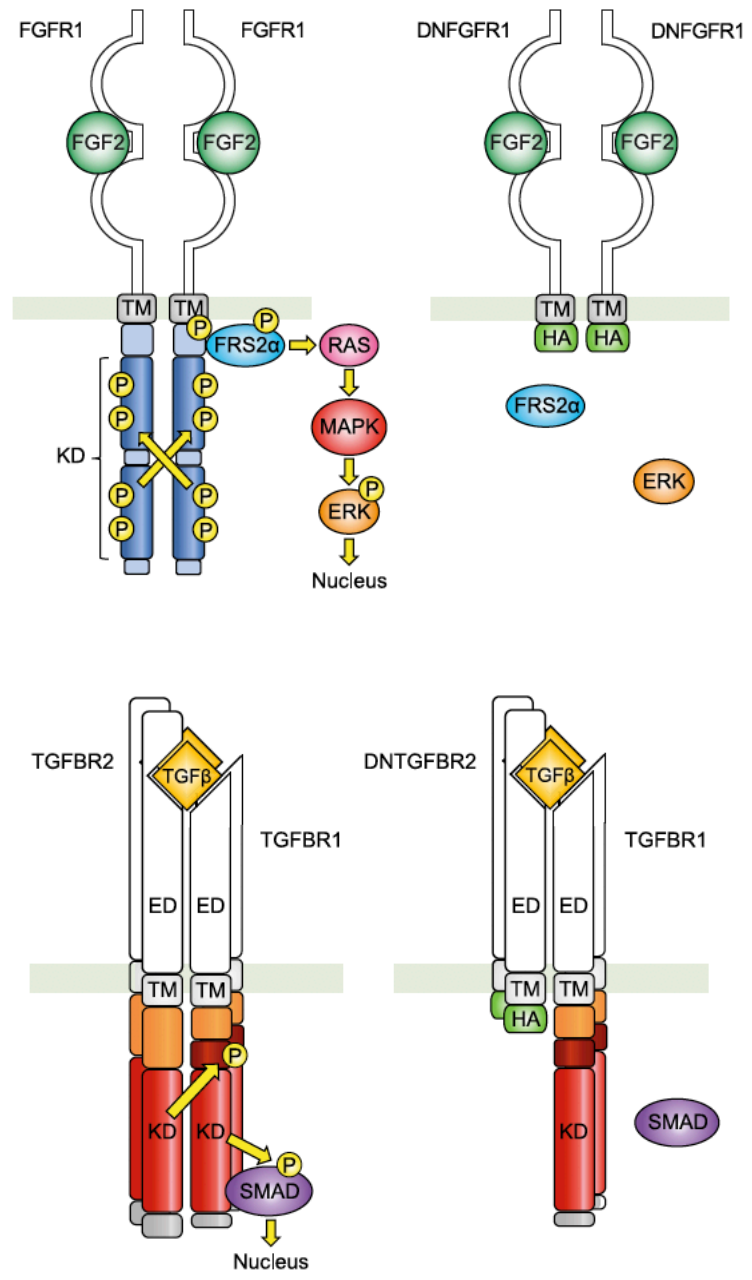
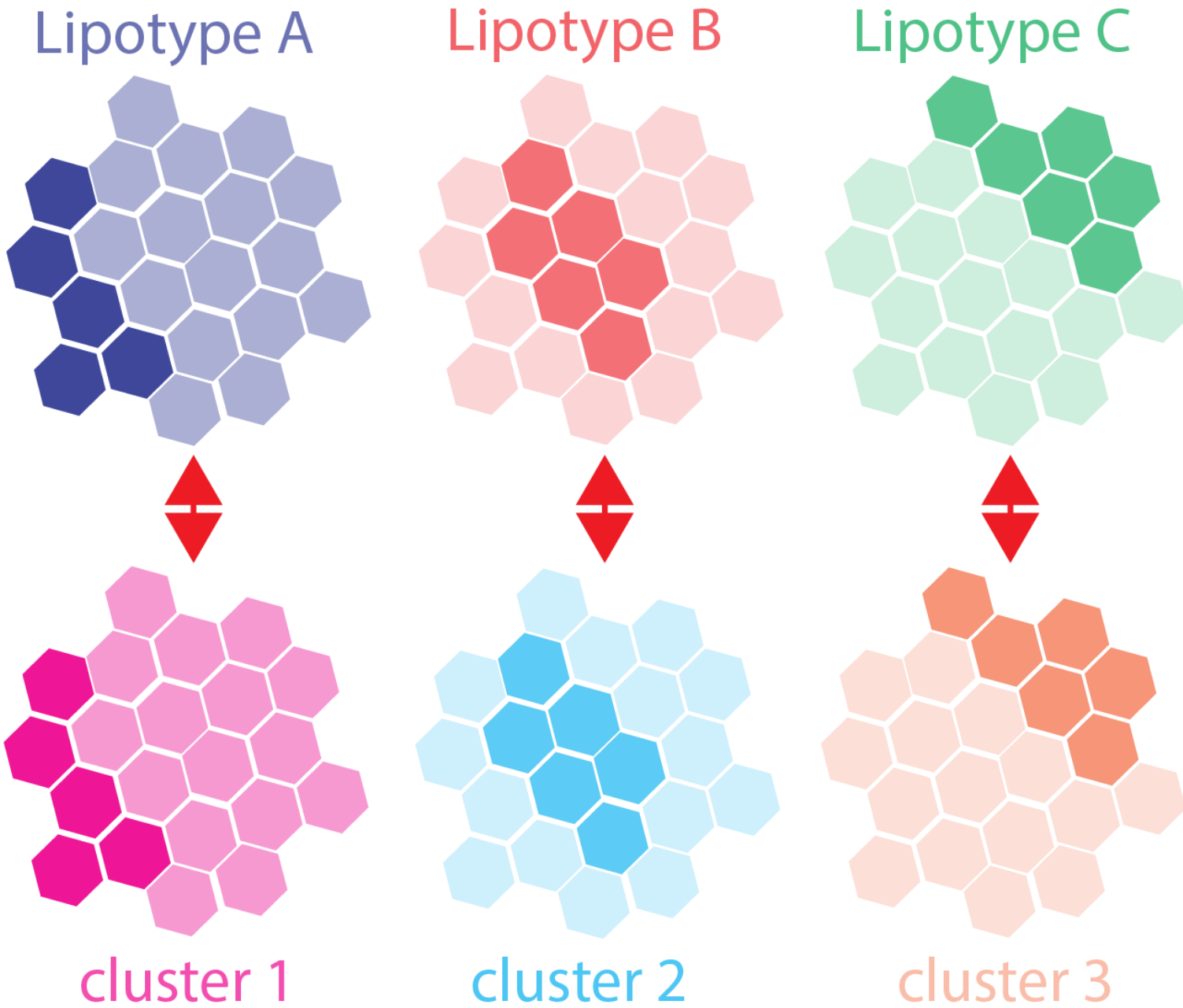


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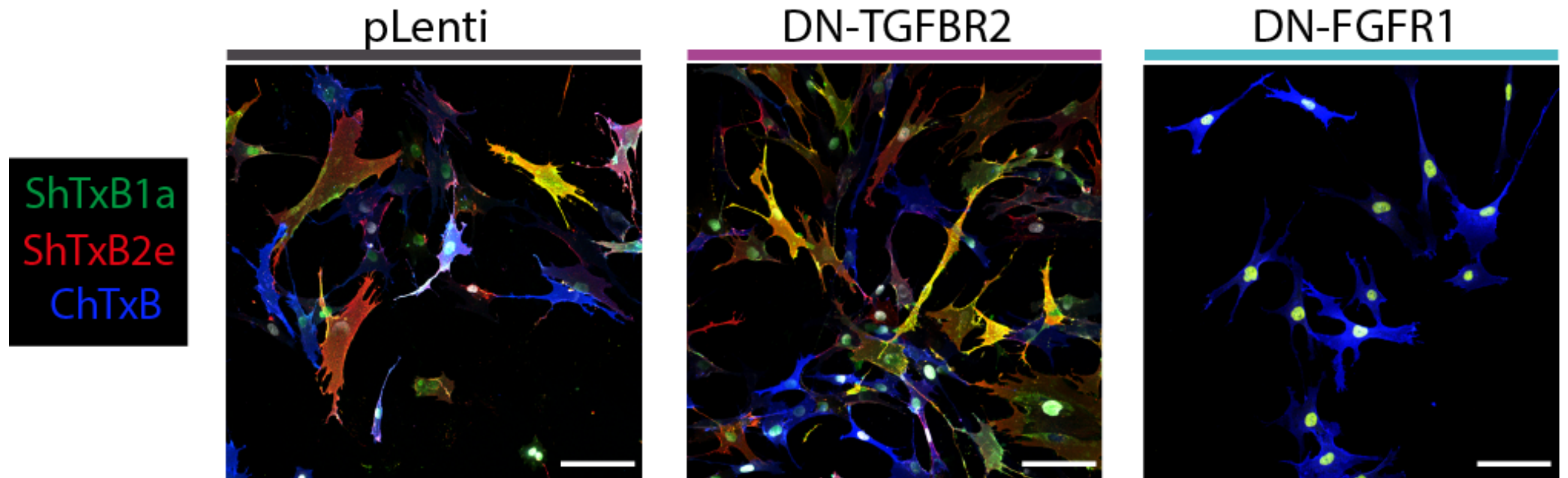


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transcriptional landscape metabolic landscape

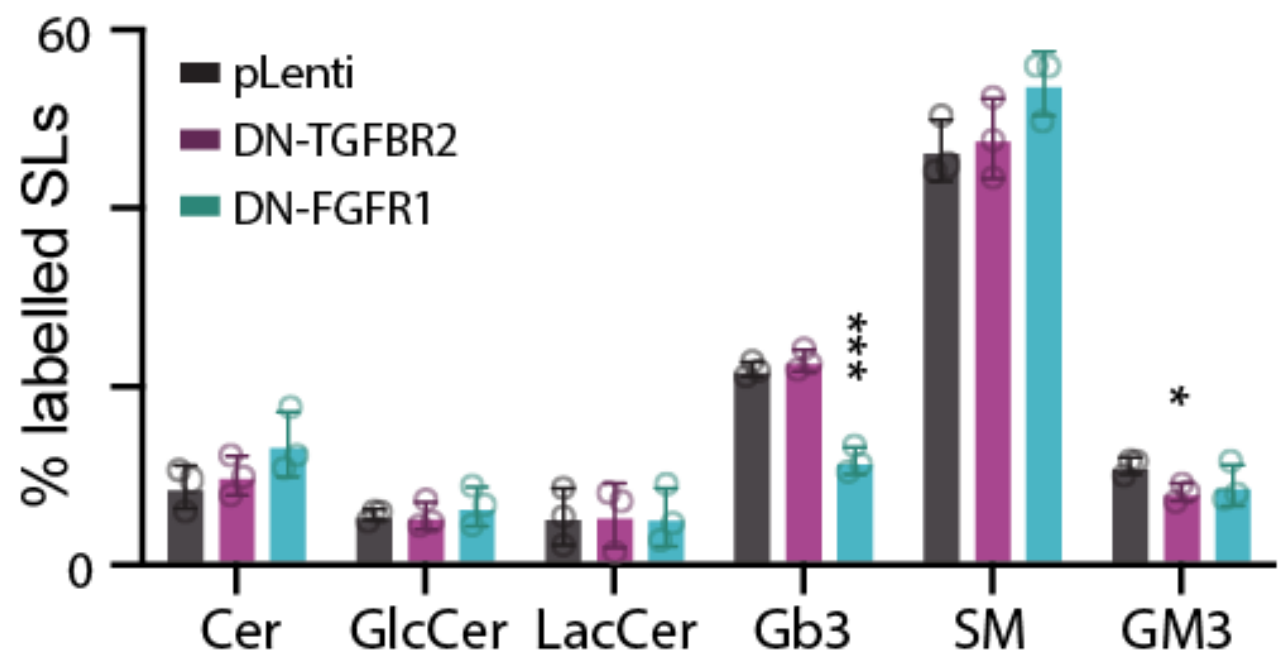
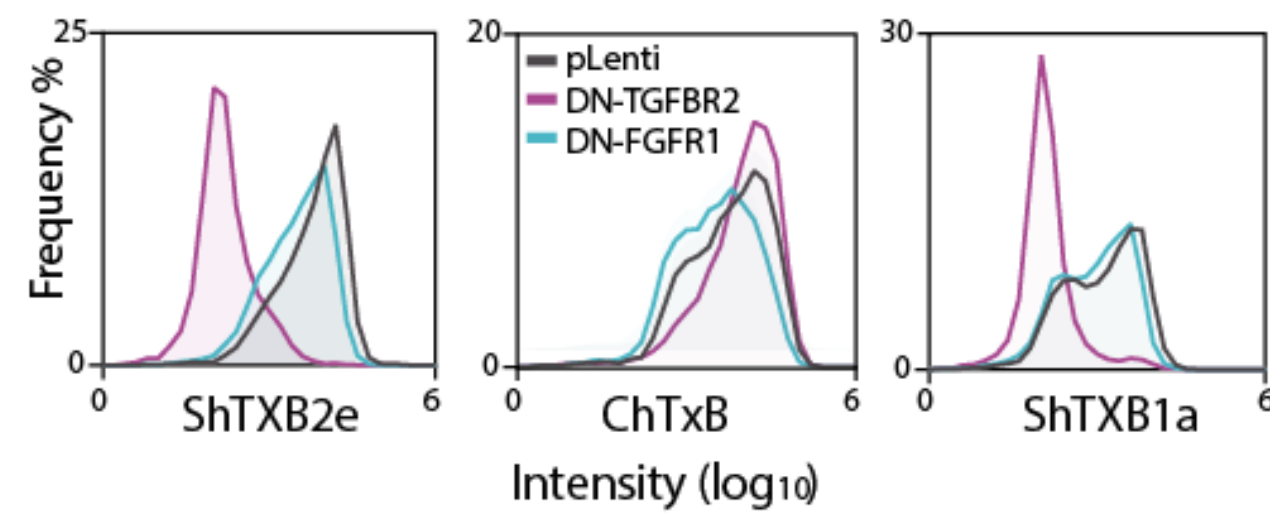
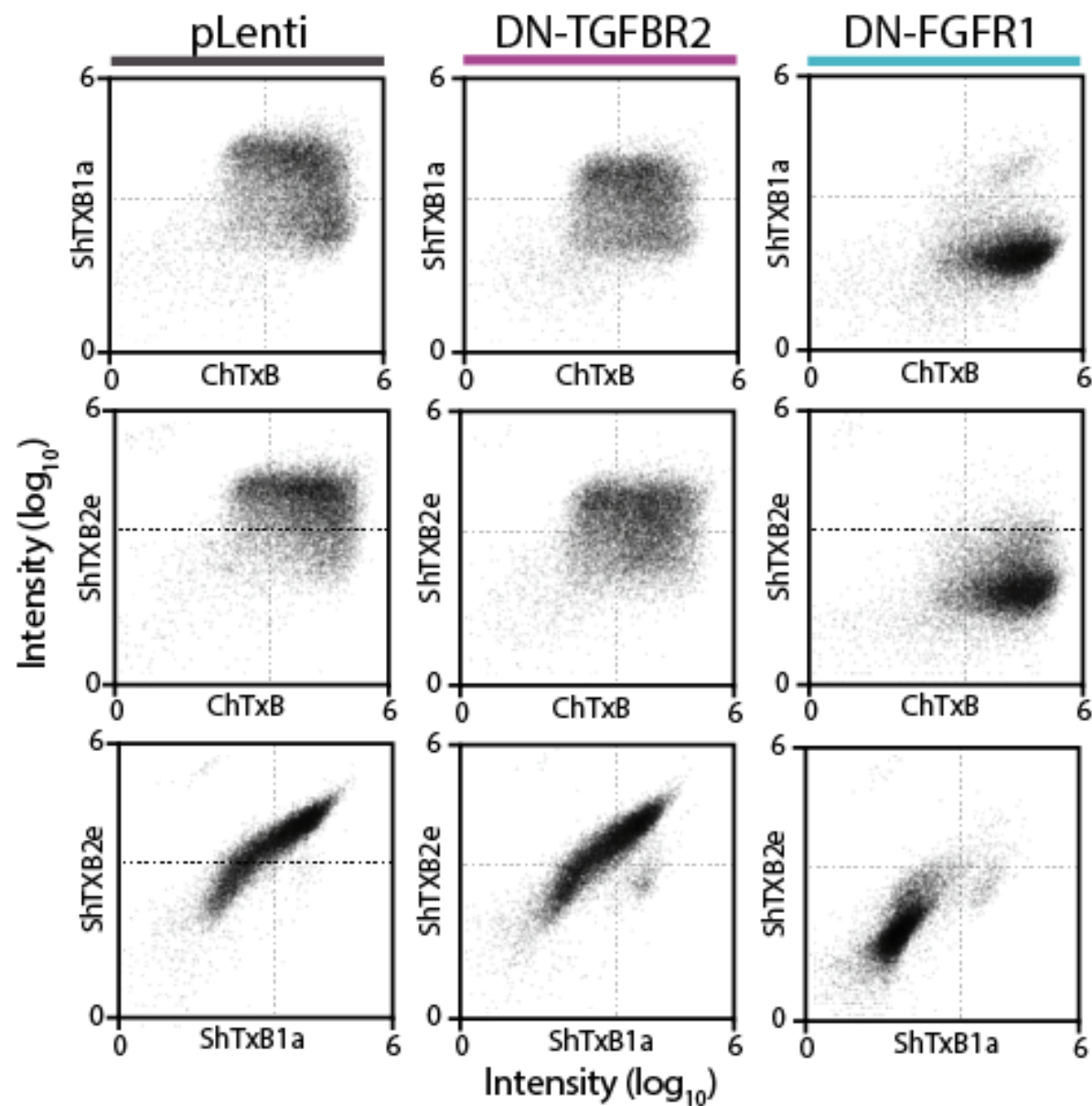


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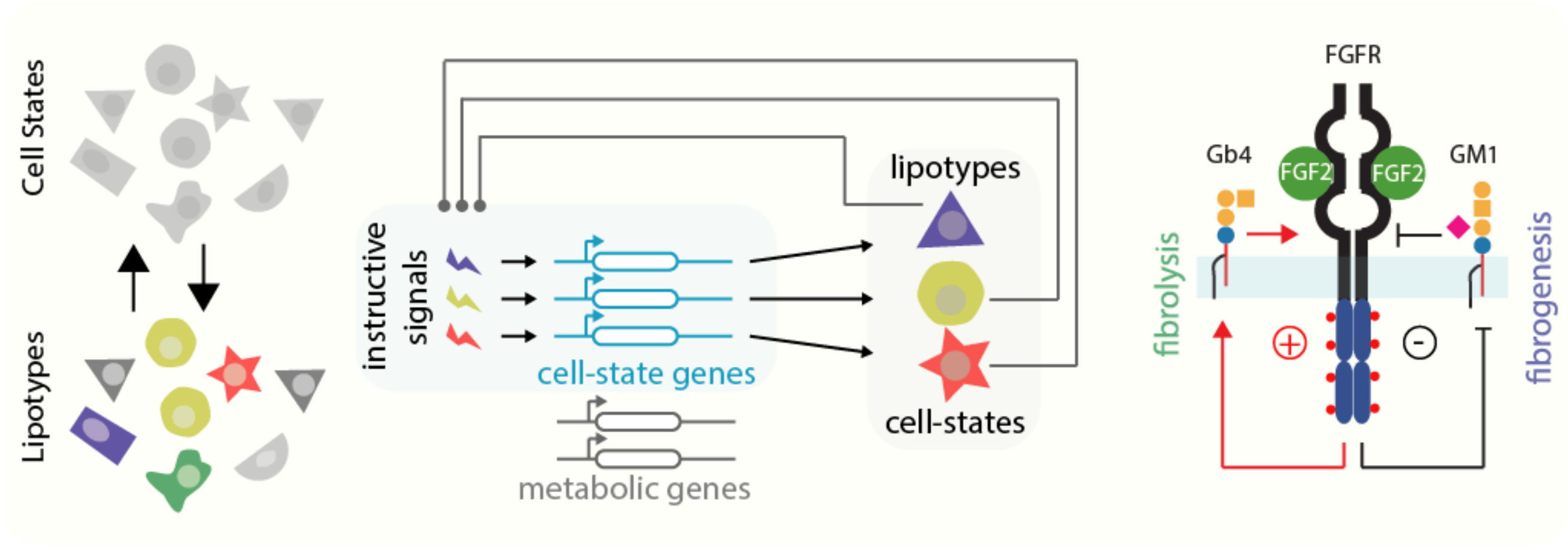


Lipids and receptors work together to
diversi the response of the cells

Physiology



Conclusion



Conclusion

