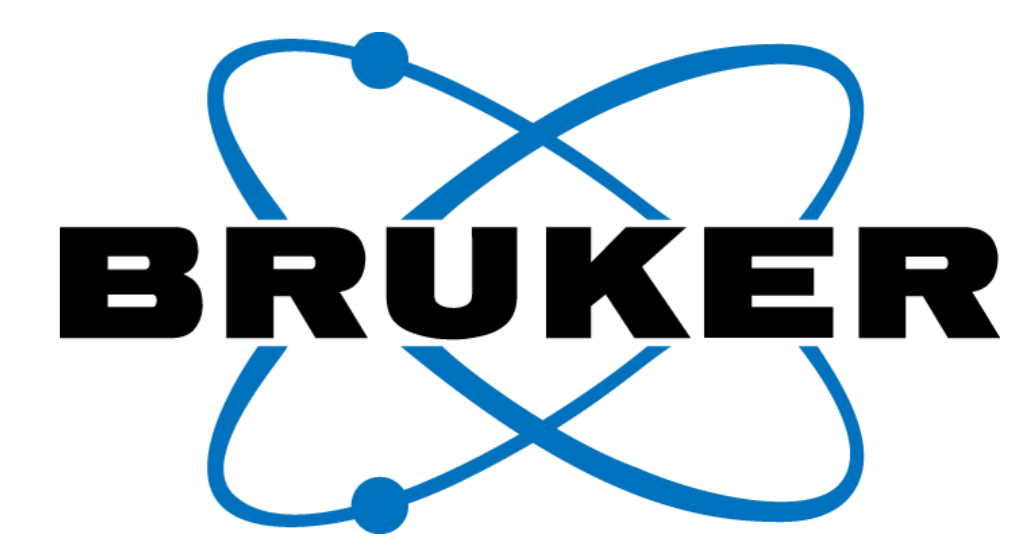


Using multi-omics to explore the uncharted realm of mitochondrial diversity



University of Bremen



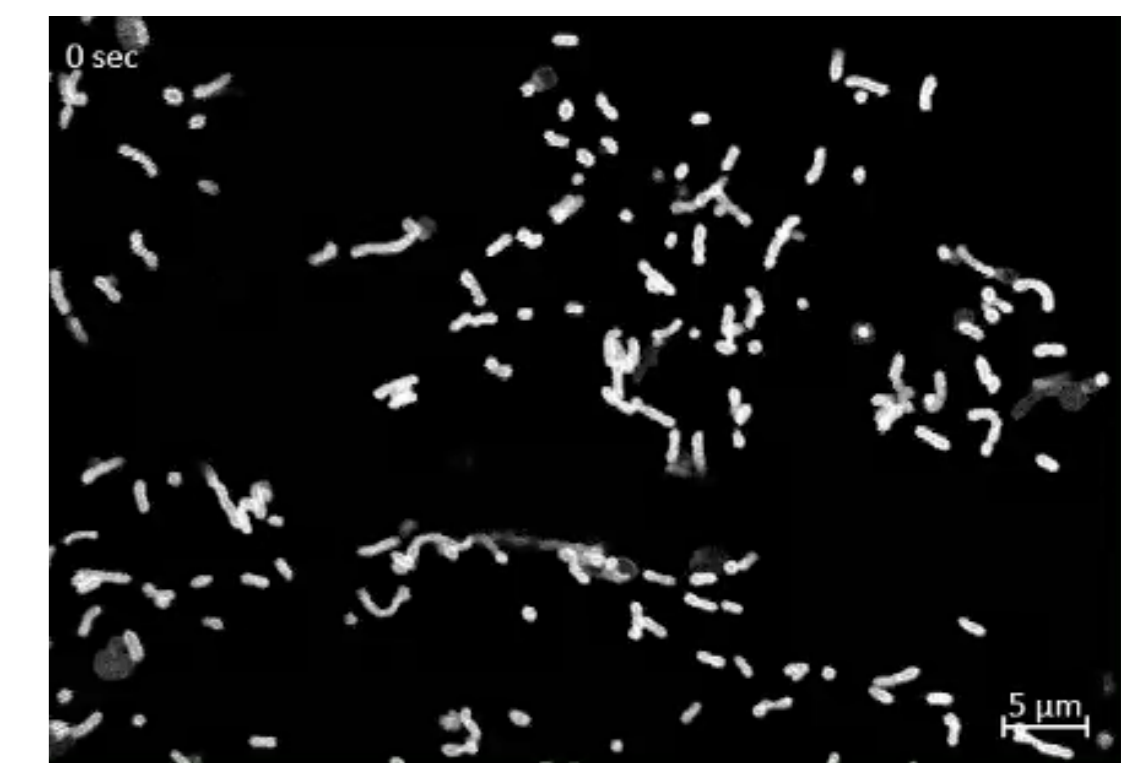
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Introduction

Mitochondria are vital, multifunctional organelles, and their dysfunction can impair development and survival across species. Traditionally viewed as uniform, **mitochondria actually exhibit variation across tissues, cells, and even within single cells**. Despite this, their **molecular diversity** remains largely uncharted, limiting our understanding of potential biomarkers. We developed **mRACE**, a novel method for isolating mitochondrial subpopulations—“**mitotypes**”—by targeting specific cell types or mitochondrial proteins. This technique enables high-resolution exploration of mitochondrial molecular diversity, offering new insights into the organelle’s specialized roles between and within cell.



Mitochondria in leaf cells of *A. thaliana*.

Methods

We developed **mRACE**, a method for **cell-type-specific mitochondrial isolation** using a chimeric protein (MTF) that enables mitochondrial **targeting, visualization, and**

biotinylation. By co-expressing MTF and a biotin ligase, mitochondria are selectively isolated via biotin-streptavidin interaction. Mitochondria samples were subjected to non-targeted **proteomics and lipidomics**. Lipidome analysis was based on C8 separation and 4D-TIMS-PASEF on a novel **timsMetabo** (Bruker Daltonics, Germany). Proteom was analysed in dia-PASEF mode on a timsTOF Ultra 2 coupled to the nanoElute 2 (Bruker Daltonics, Germany). Data were analyzed using MetaboScape 2025b and Spectronaut-19, respectively.

Cell type specific isolation of mitochondria

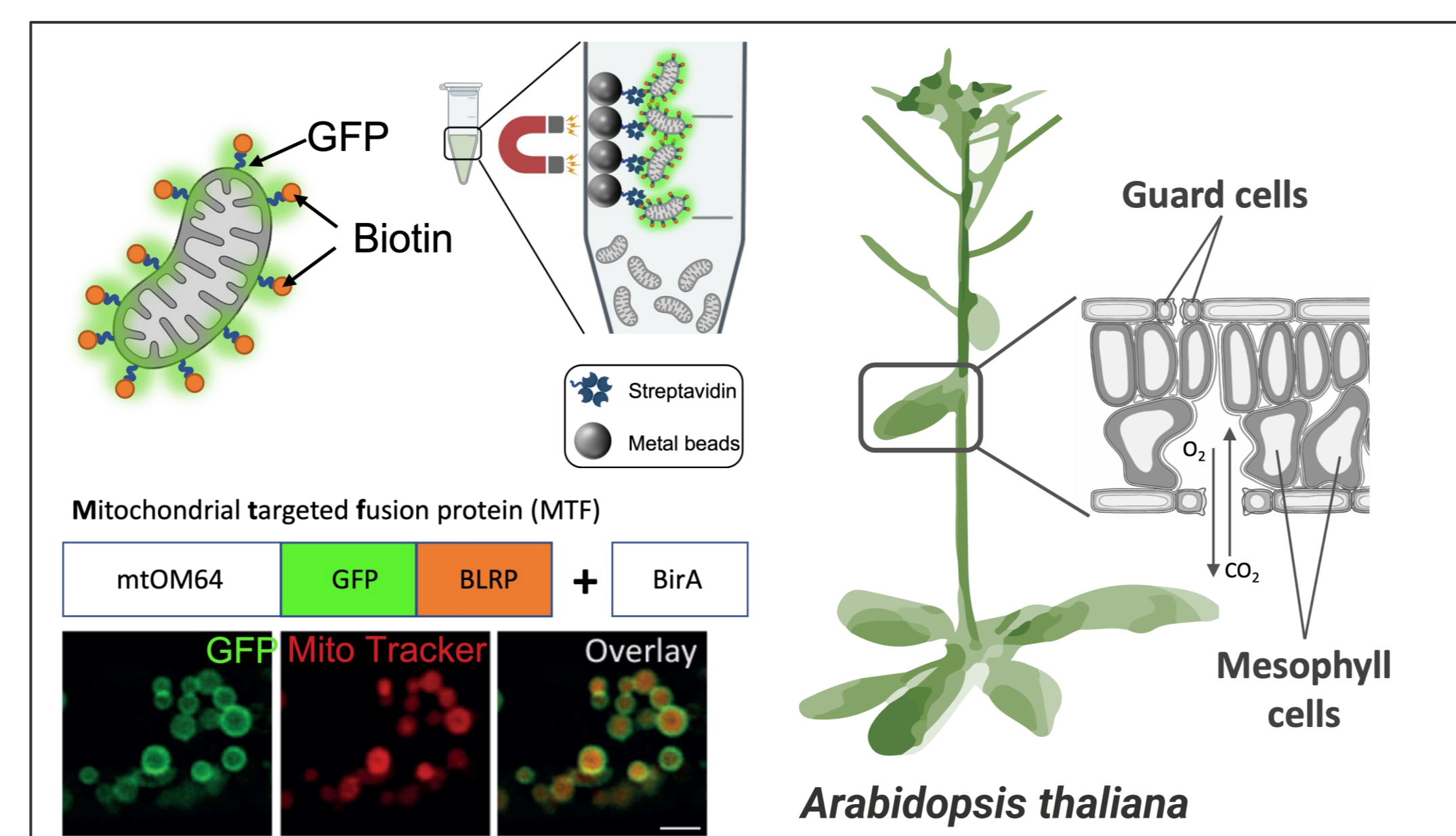


Fig. 1 Schematic illustration of the **Mitochondrial Rapid Cell-Type specific Isolation (mRACE)**.

Heat stress treatment & LC-MS analysis

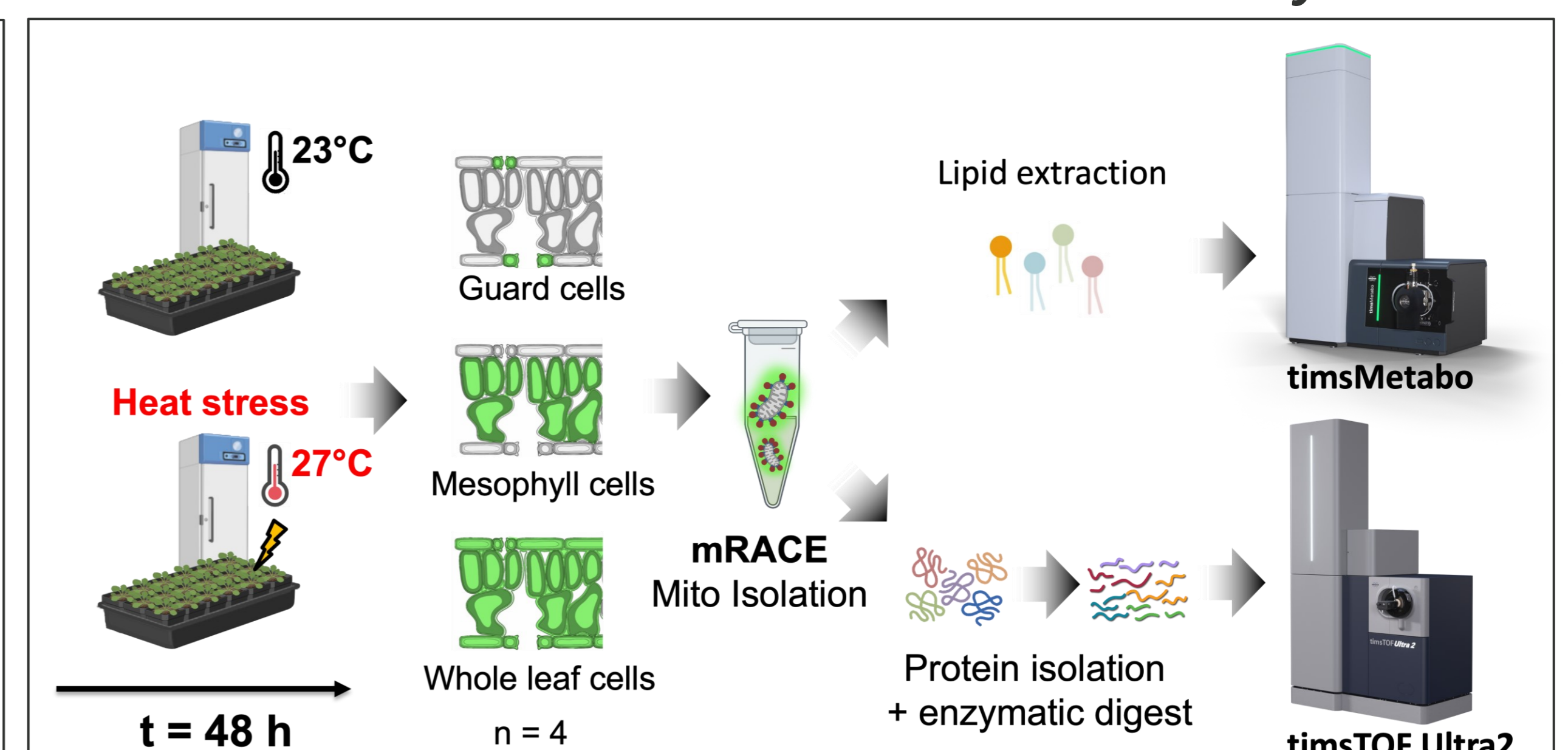


Fig. 2 Schematic workflow of the **heat stress treatment** experiment.

Results

We used **mRACE** and **LC-MS/MS** to isolate mitochondria from mesophyll and guard cells, revealing cell type and treatment-specific differences in protein and lipid profiles (Fig. 3 and 4). **Differentially enriched mitochondrial proteins** (Fig. 3) and diverse lipid classes (Fig. 4) reflected functional specialization. Multivariate analysis highlighted **distinct mitochondrial lipidomes**, while functional enrichment showed regulation of metabolism, respiration, and translation (Fig. 3). Integrating proteomic and lipidomic data enables advanced organelle-level insights. This multi-omics approach offers a powerful tool for exploring **mitochondrial diversity**, with broad relevance for plant and animal biology, biomarker discovery, and stress response analysis.

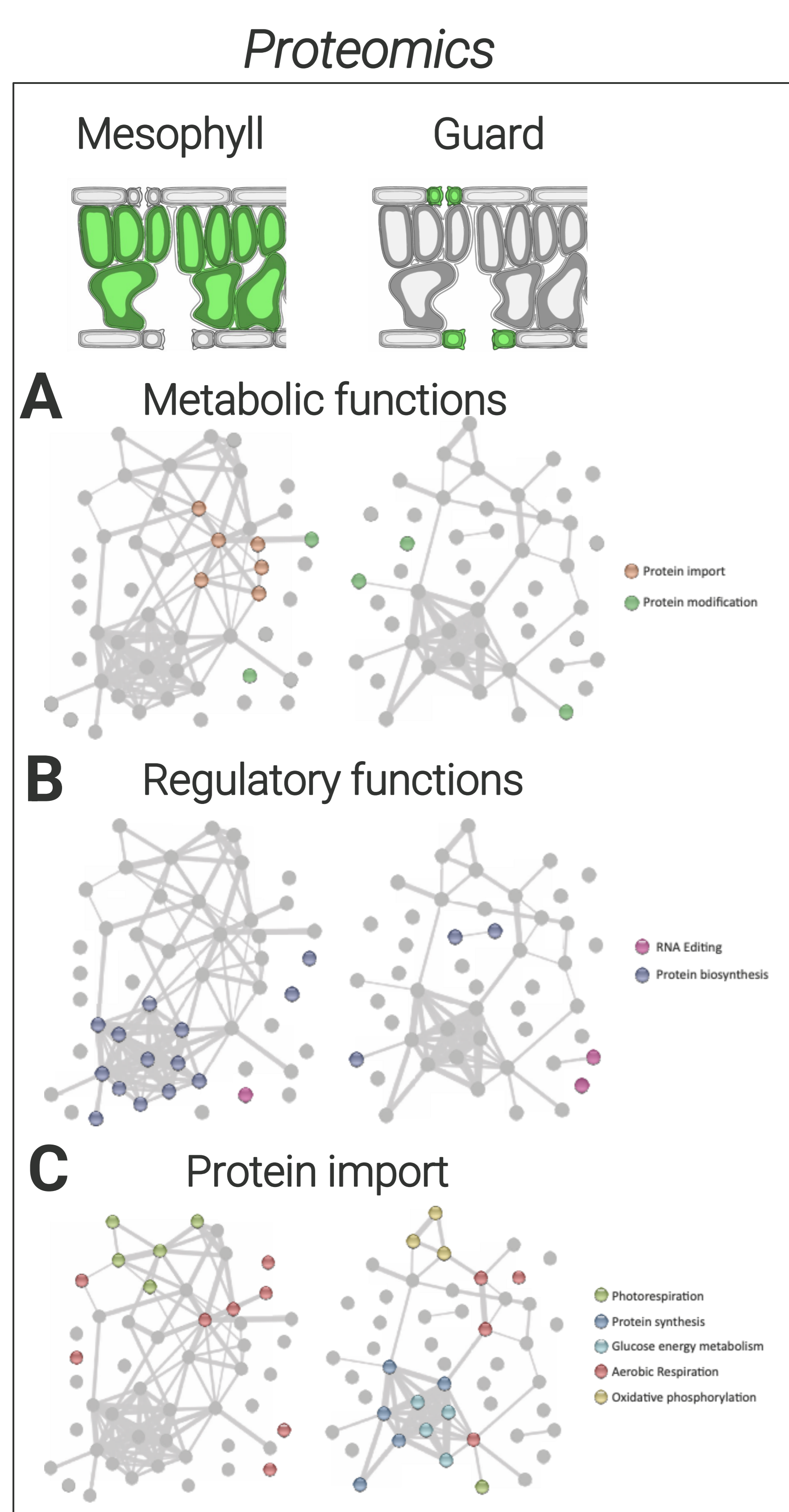


Fig. 3 **Protein-protein interaction networks analysis of mitochondrial proteins isolated from mesophyll and guard cells**. Networks were calculated using STRING data base.

Lipidomics

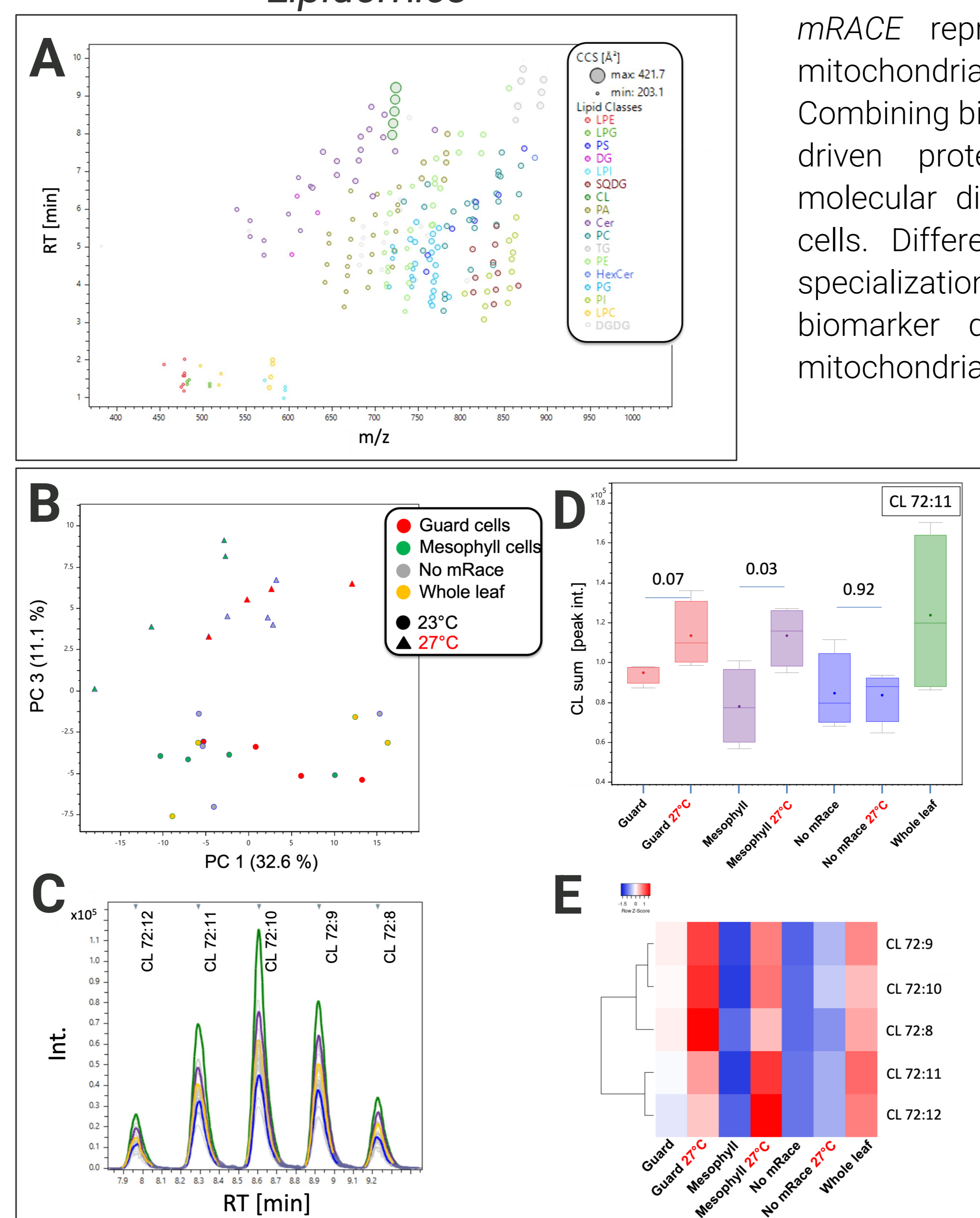
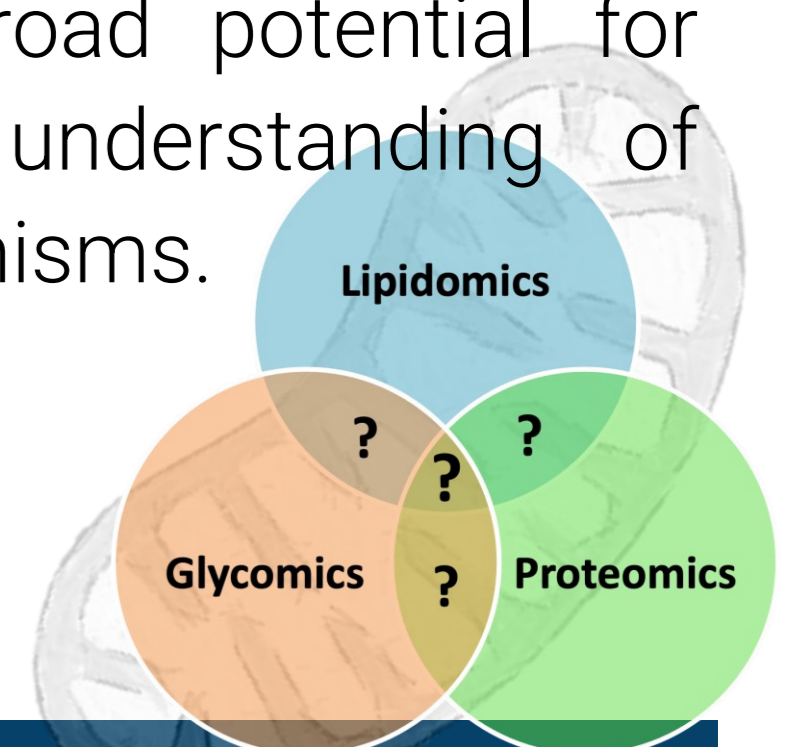


Fig. 4 **Lipidomics of heat-treated mitochondria from guard and mesophyll cells** revealed 229 lipids across 16 subclasses (A) in total. Five cardiolipin species were identified (C), showing overall enrichment under heat stress (D) and cell-type-specific distribution, (E).

Summary

mRACE represents a novel tool enabling cell-type-specific mitochondrial isolation and molecular characterization. Combining biotin-based targeting with high resolution LC-MS/MS-driven proteomics and lipidomics, distinct mitochondrial molecular diversity pattern were identified in *Arabidopsis* leaf cells. Differential protein and lipid profiles reflect functional specialization. This approach is offering broad potential for biomarker discovery and advancing our understanding of mitochondrial roles across cell types and organisms.



Conclusion

- **mRACE** enables the cell type-specific isolation of mitochondrial subtypes
- Mitochondrial **proteome** and **lipidome** profiles vary between mesophyll and guard cells, reflecting their specialized functions
- Integrated **multi-omics** uncovers mitochondrial regulation at multiple levels (e.g., metabolism, respiration, translation)