Characterization of upland cotton gene *GhLag1* encoding ceramide synthase

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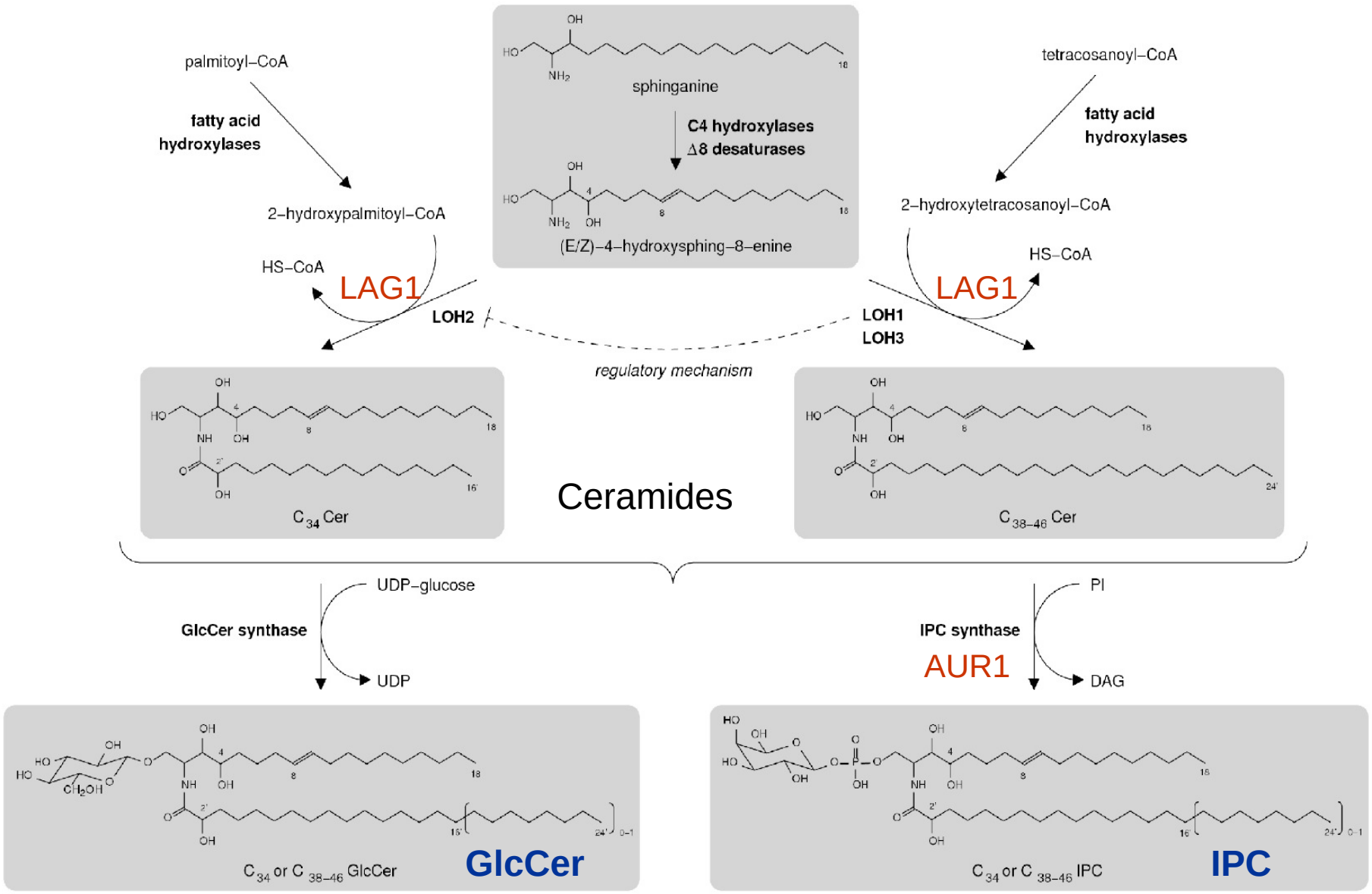
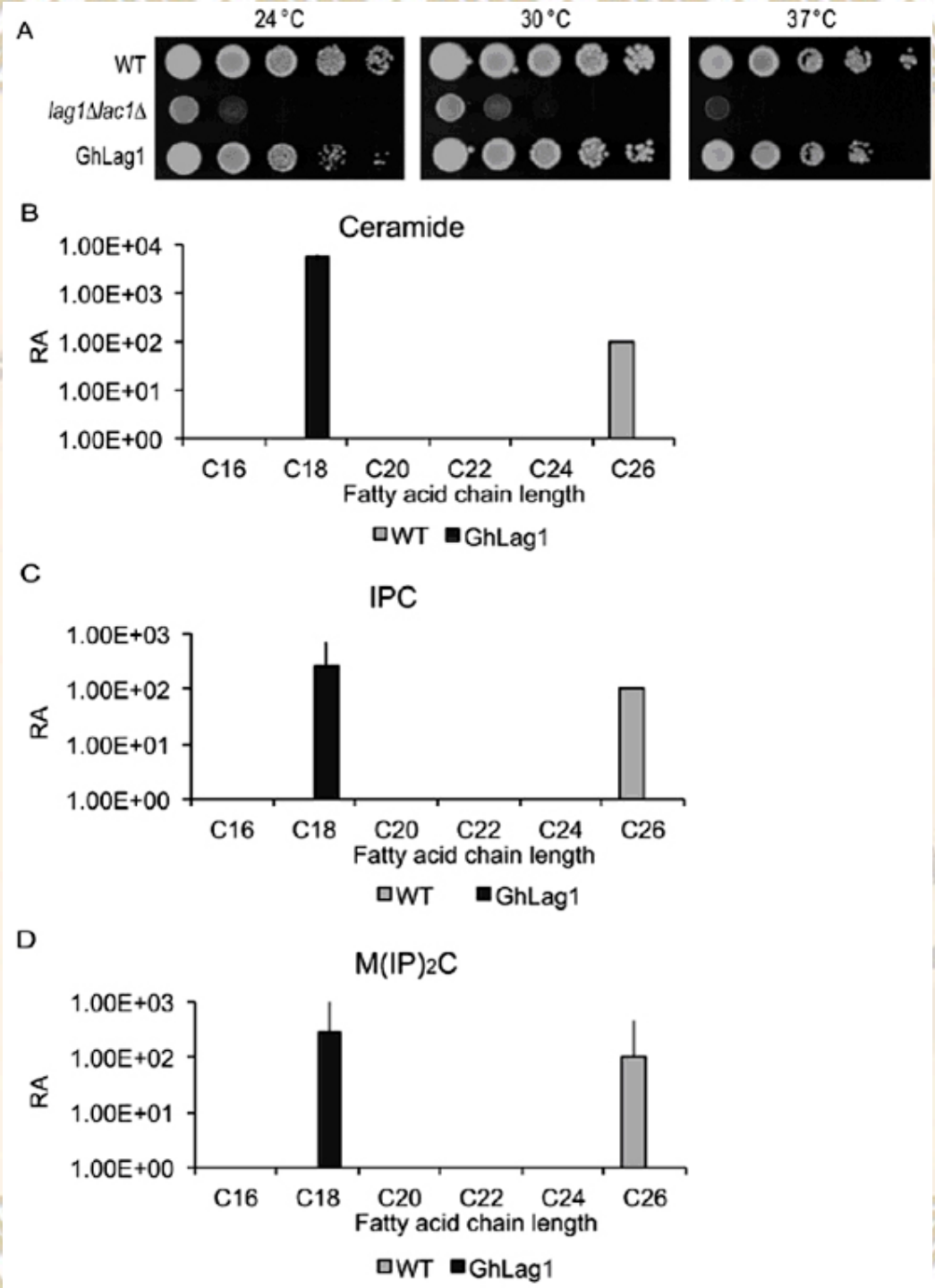
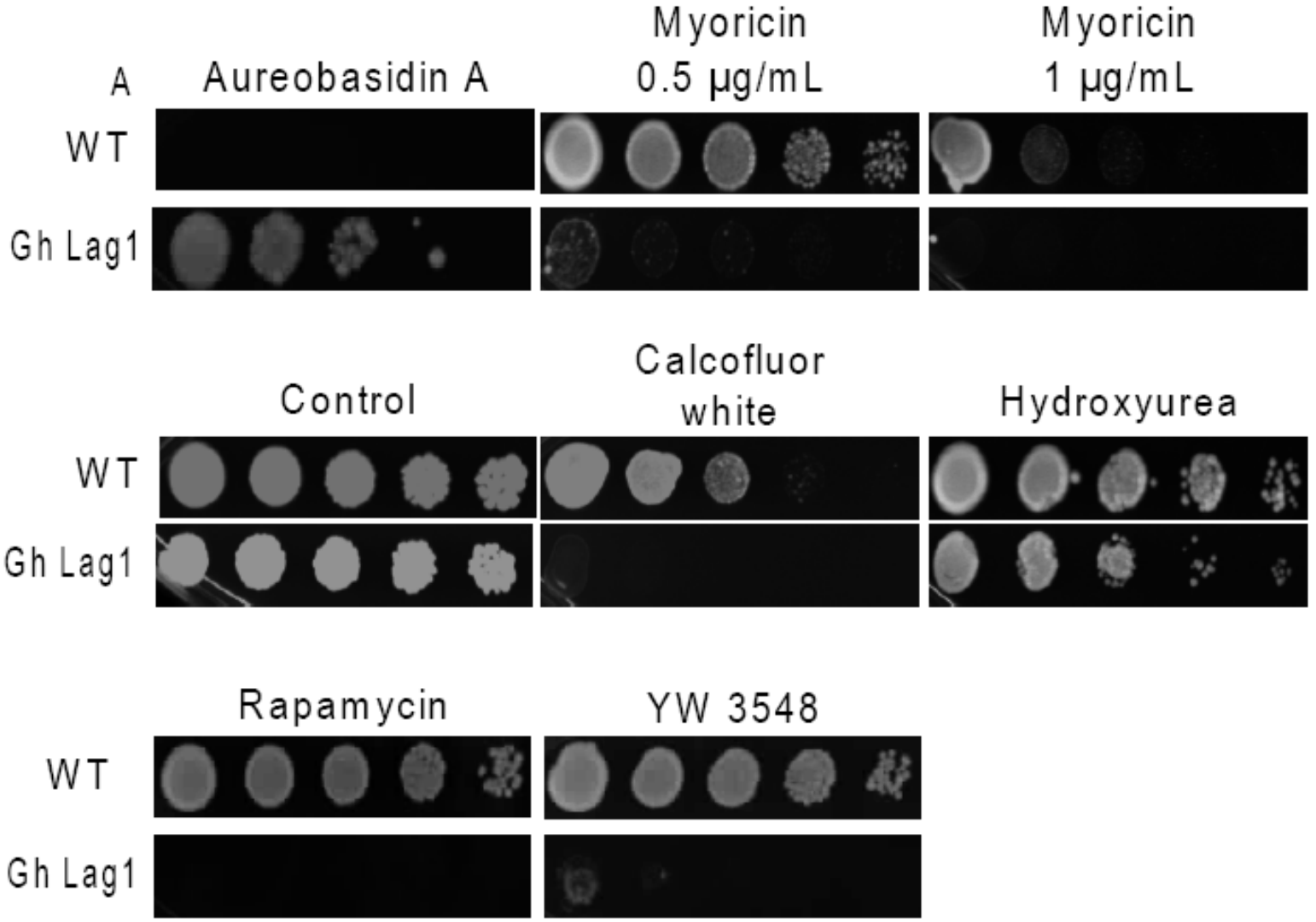
Sphingolipid biosynthesis pathway in plant





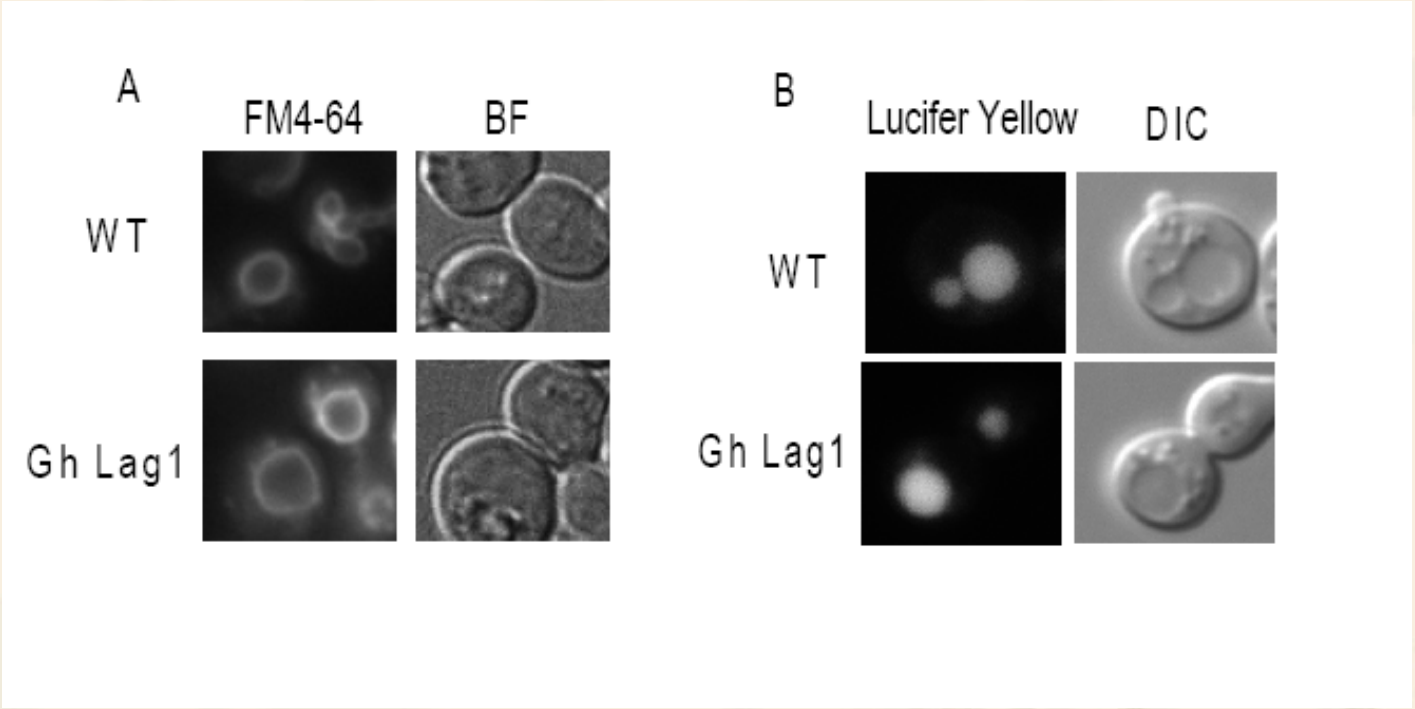
The cotton ceramide synthase GhLag1p complements the yeast ceramide synthase mutant strain *lag1Δlac1Δ* and leads to production of ceramides and sphingolipids with C18 acyl chains

The GhLag1 mutant showed decreased sensitivity to Aurobasidin A and increased sensitivity to myriocin, rapamycin, YW3548 and Calcofluor white



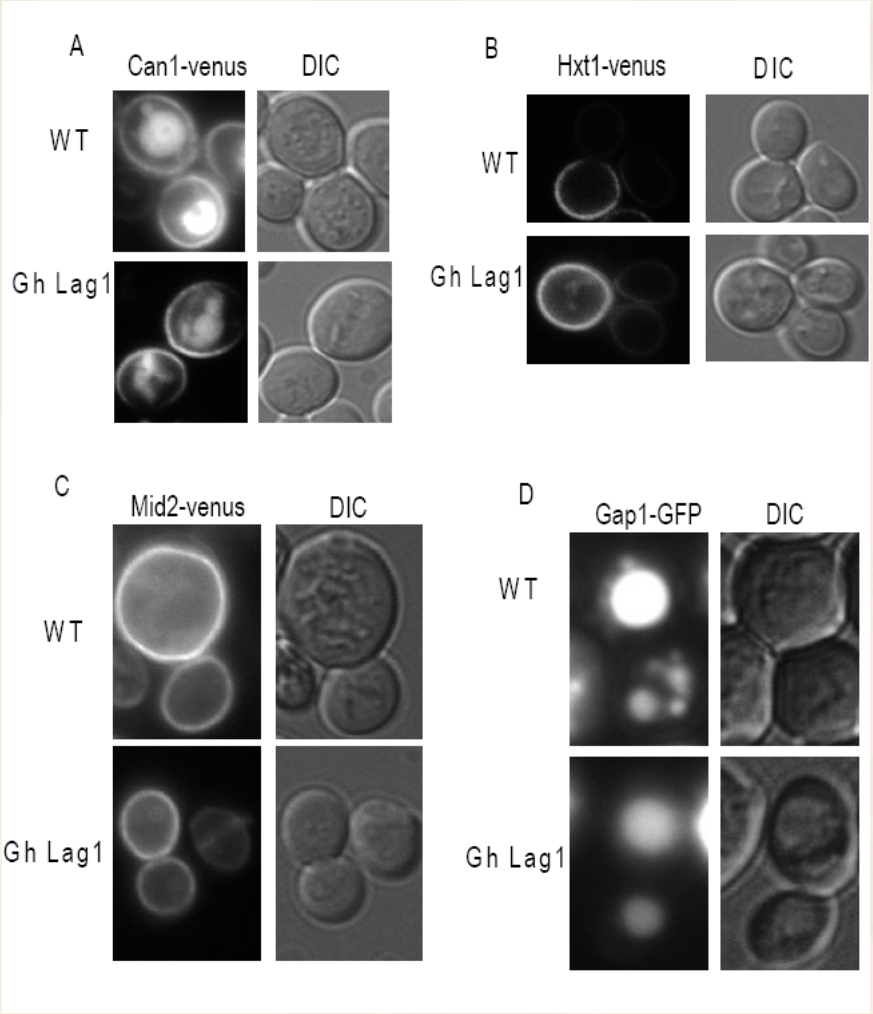
YW3548 : glycosylphosphatidylinositol synthesis inhibitor

GhLag1 strain is not defective in endocytosis

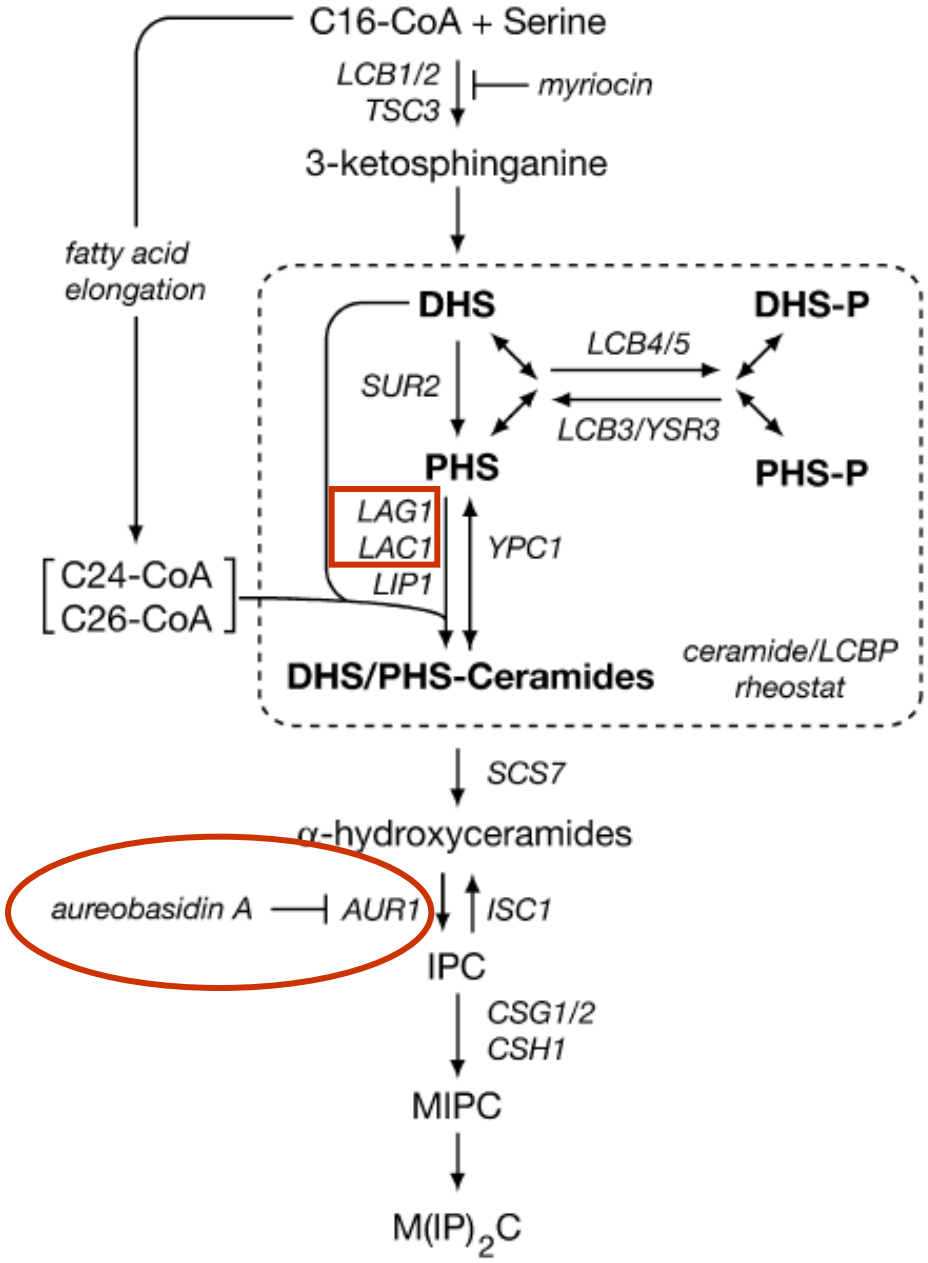


FM4-64 : a marker for membrane lipids;
LY : a control for fluid phase endocytosis;

C18 sphingolpidis do not alter membrane protein localizations



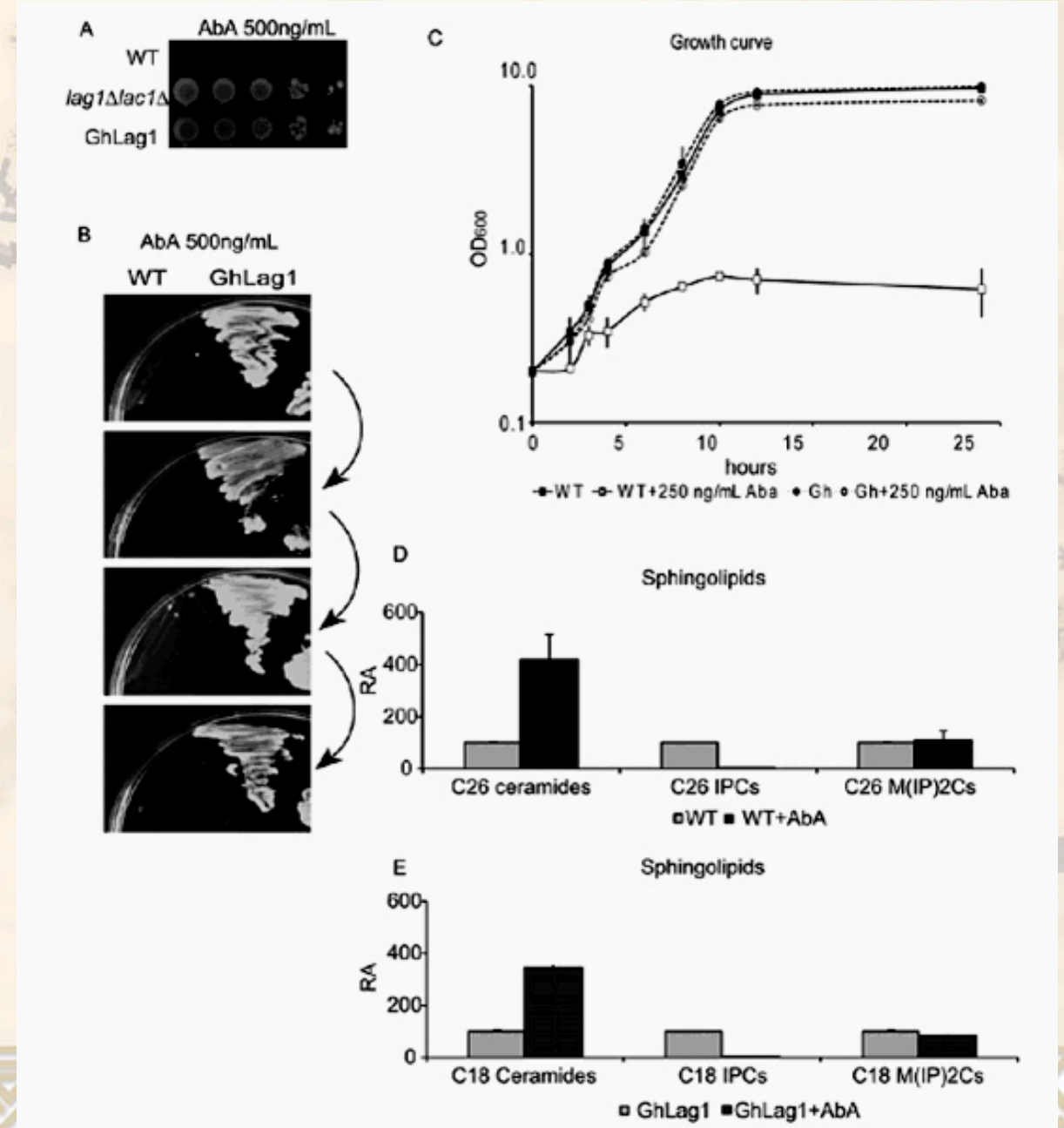
Can1p: canavanine transporter;
Hxt1p: hexose transporter;
Mid2p: cell wall integrity sensor;
Gap1p: amino acid transporter.



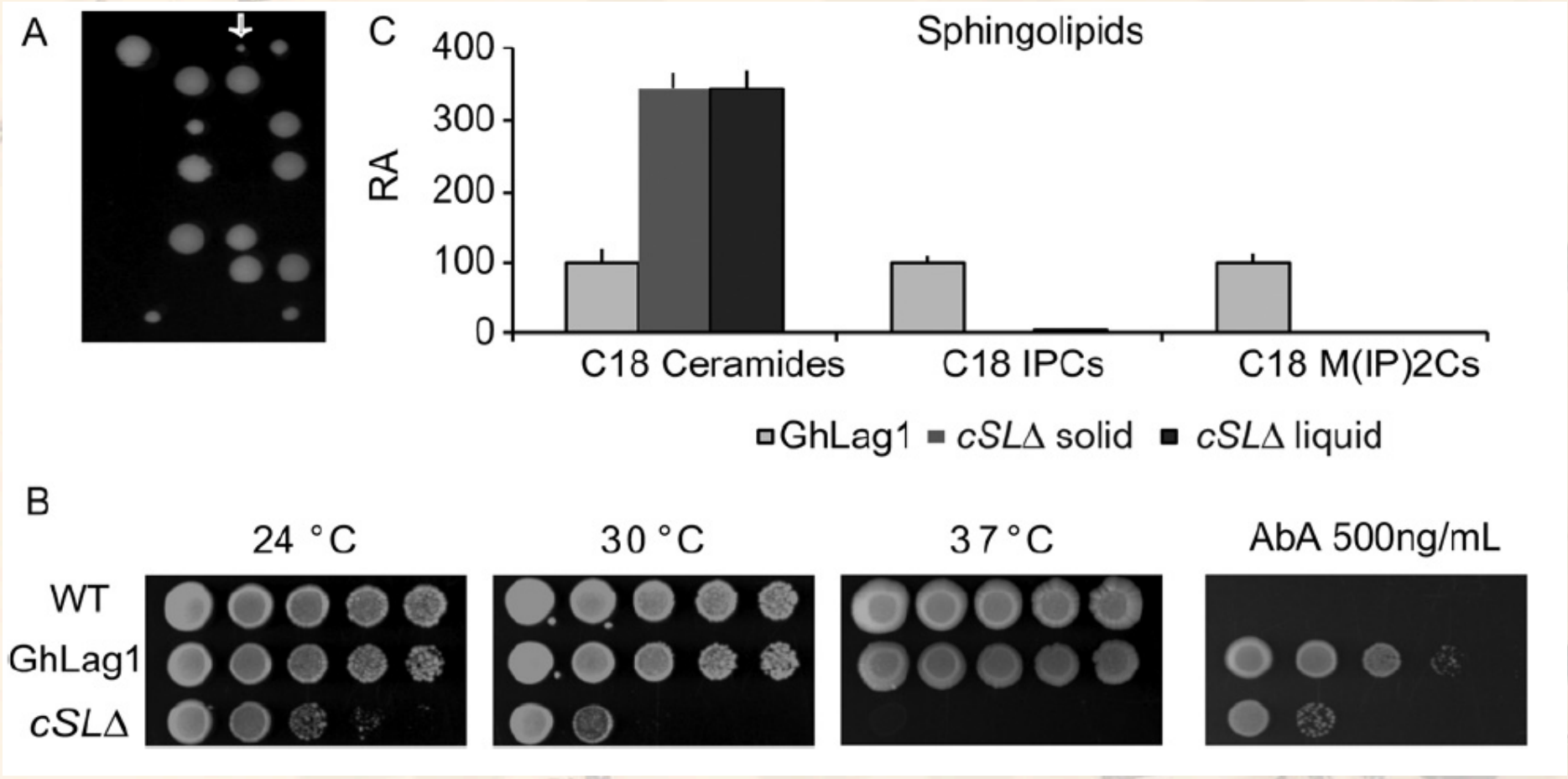
Yeast sphingolipid biosynthesis pathway

Aronova et al, 2008,
Cell Metabolism 7, 148-158

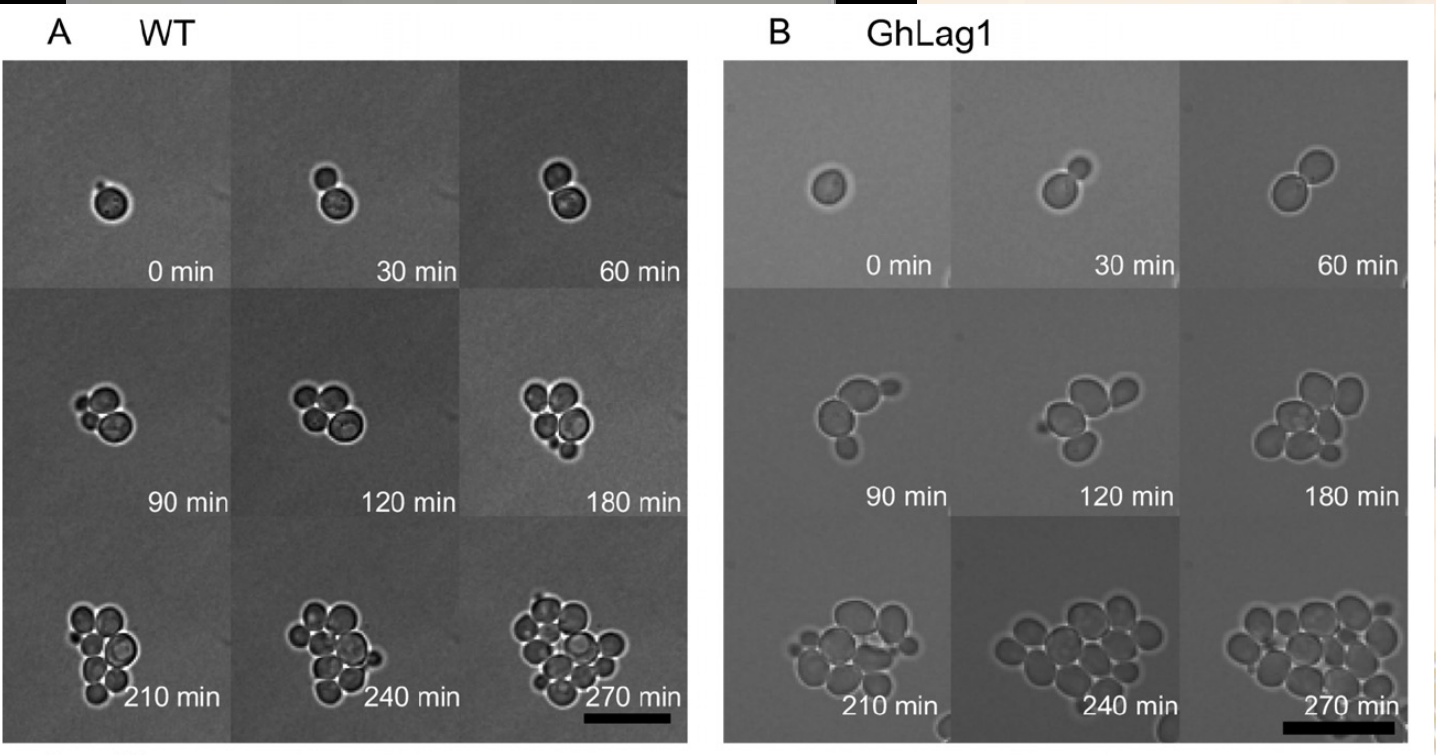
The GhLag1 strain is resistant to Aureobasidin A

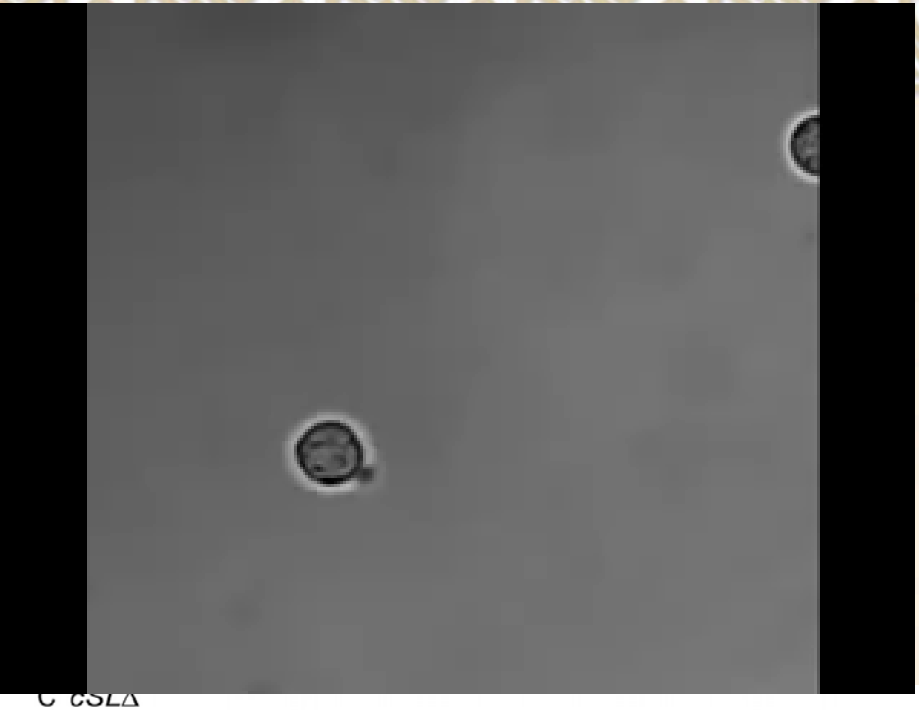


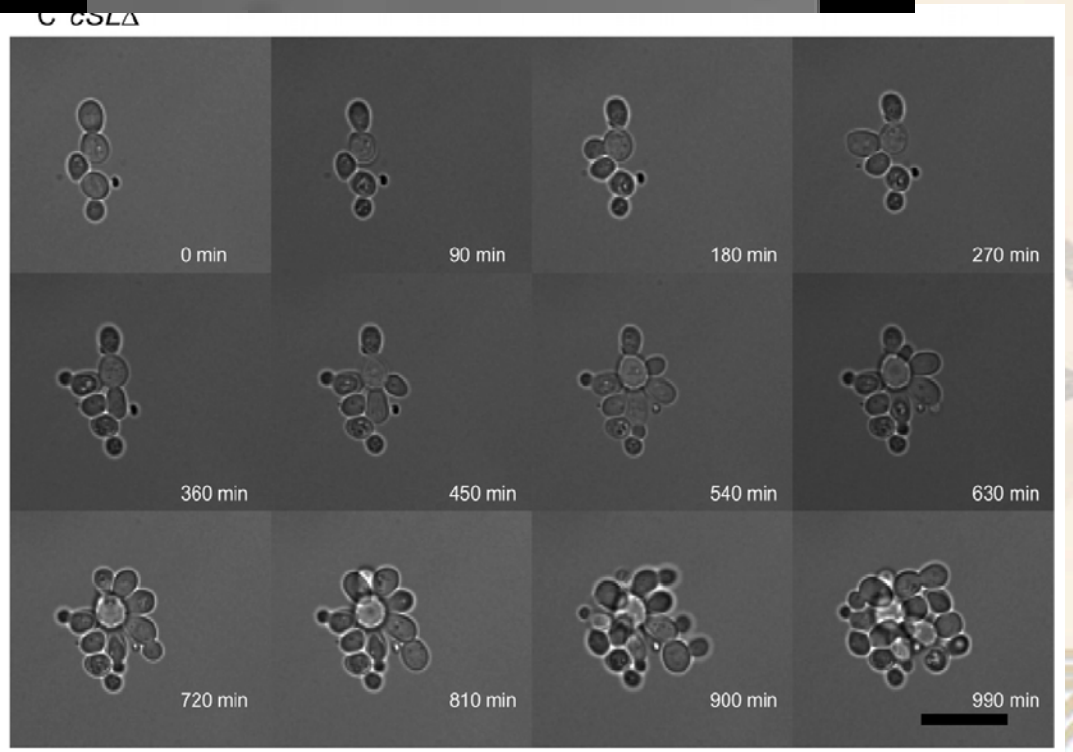
Depletion of *AUR1* reduces growth and depletes IPCs



Cytokinesis occurs in yeast cells

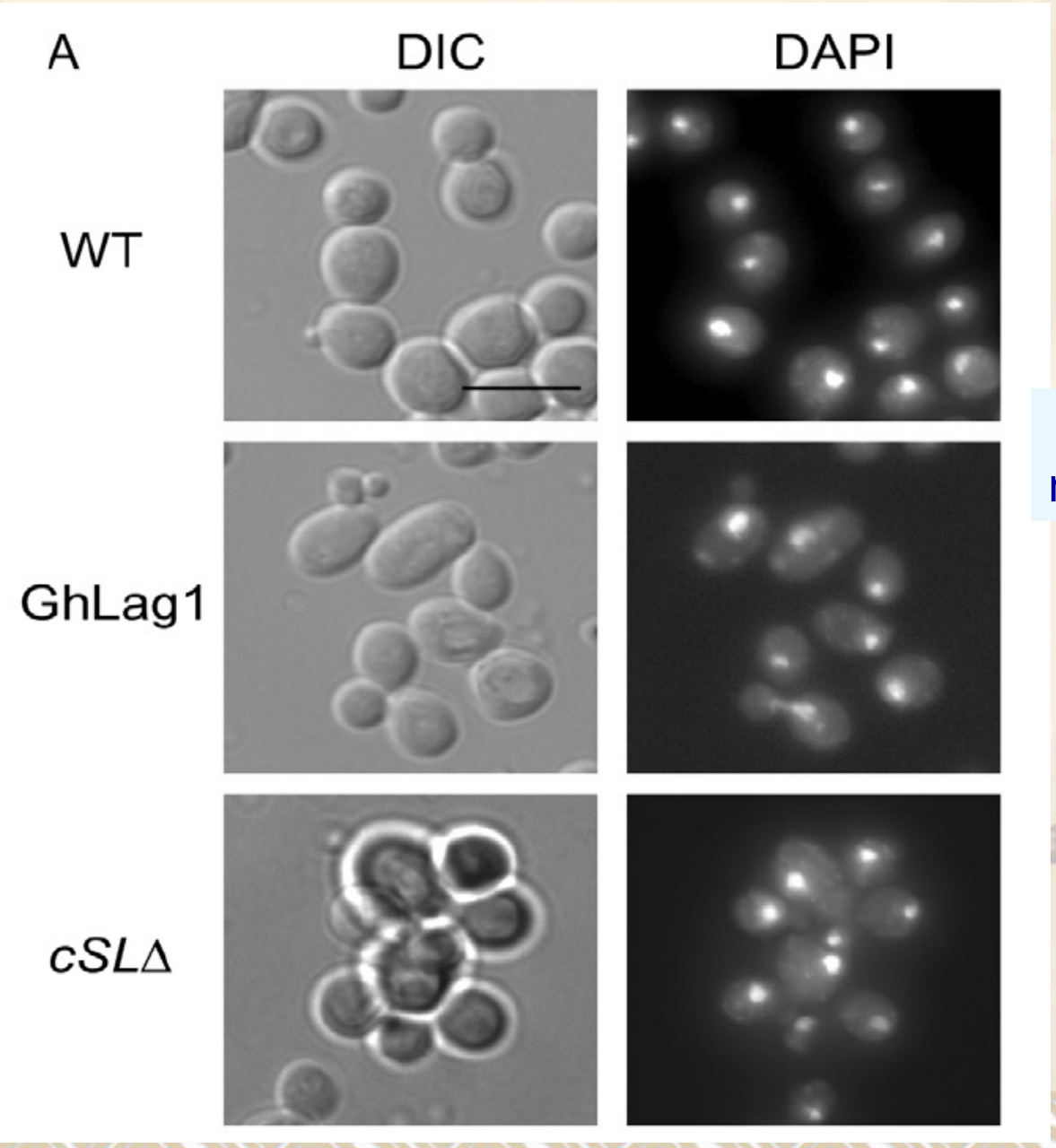






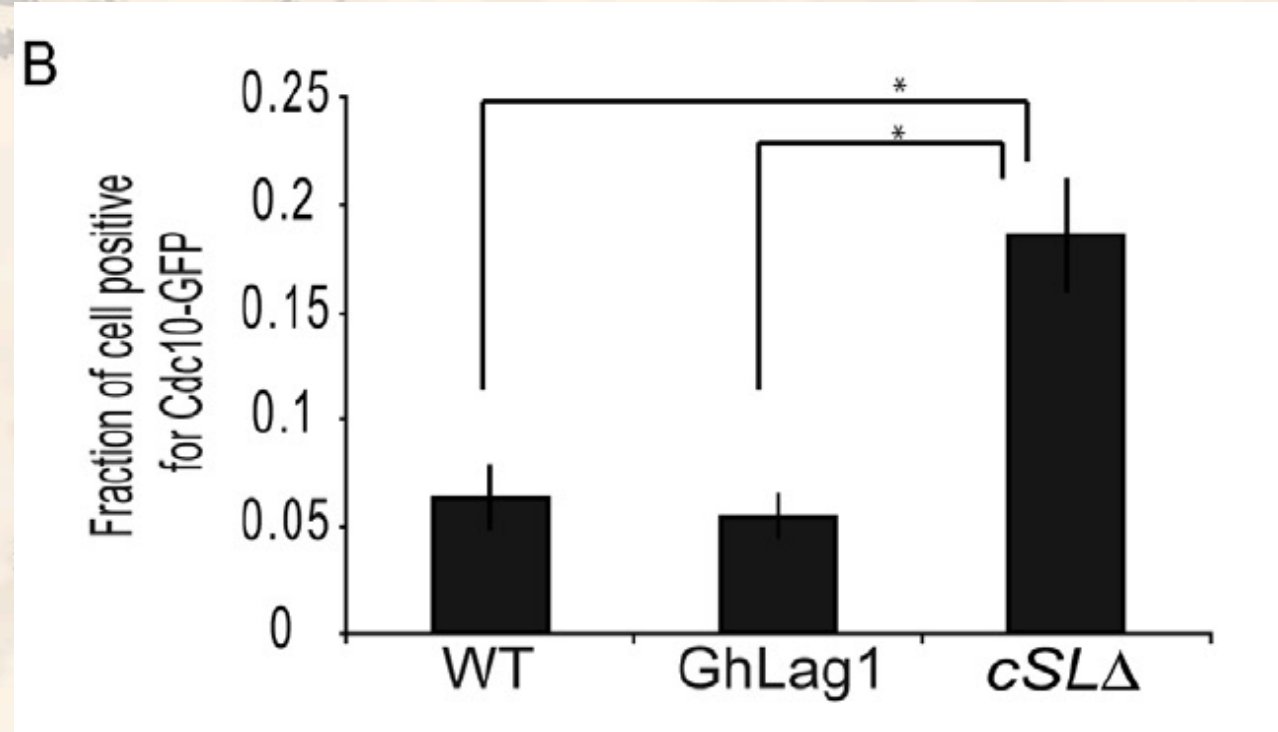
Depletion of IPCs leads to a defect in cytokinesis

Nuclear division is normal in the mutant cells

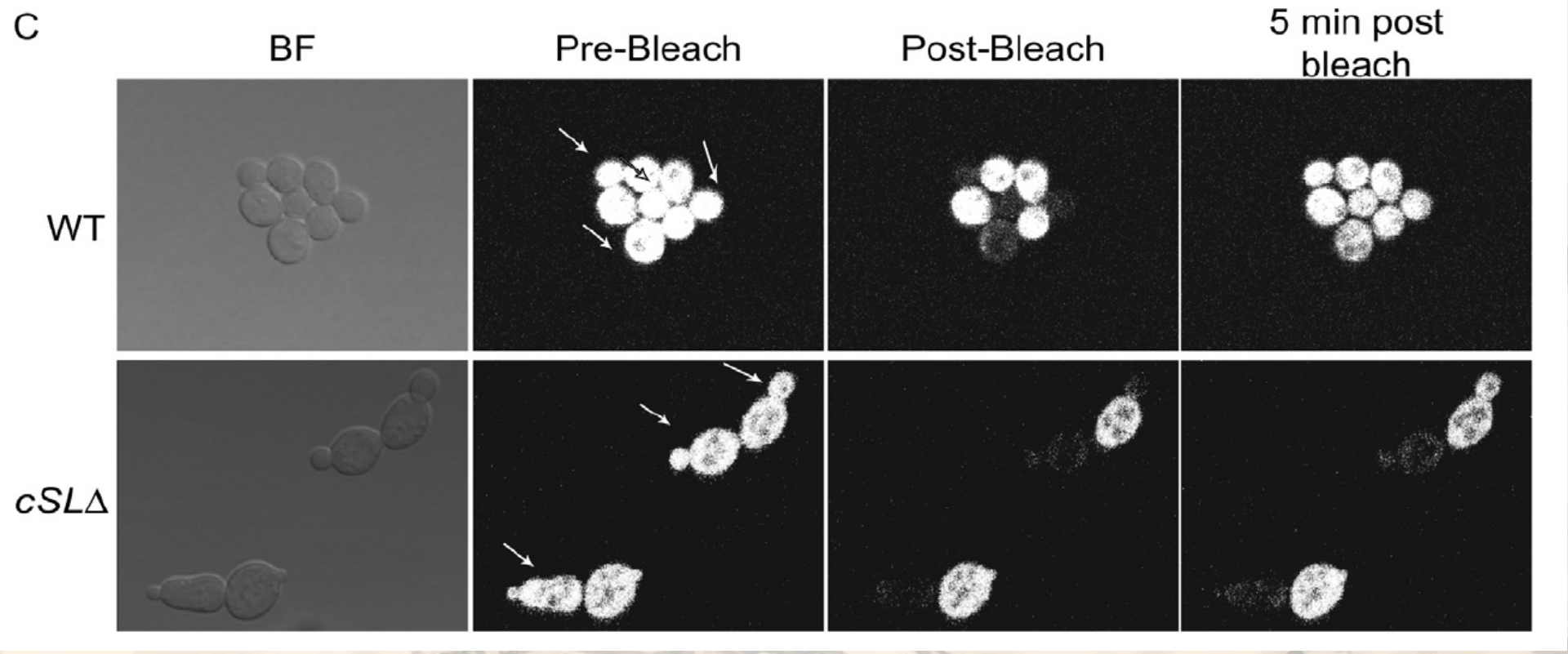


Cells labeled with septin marker protein, Cdc10-GFP

The fraction of cells labeled with Cdc10-GFP at the bud neck was significantly higher than WT cells

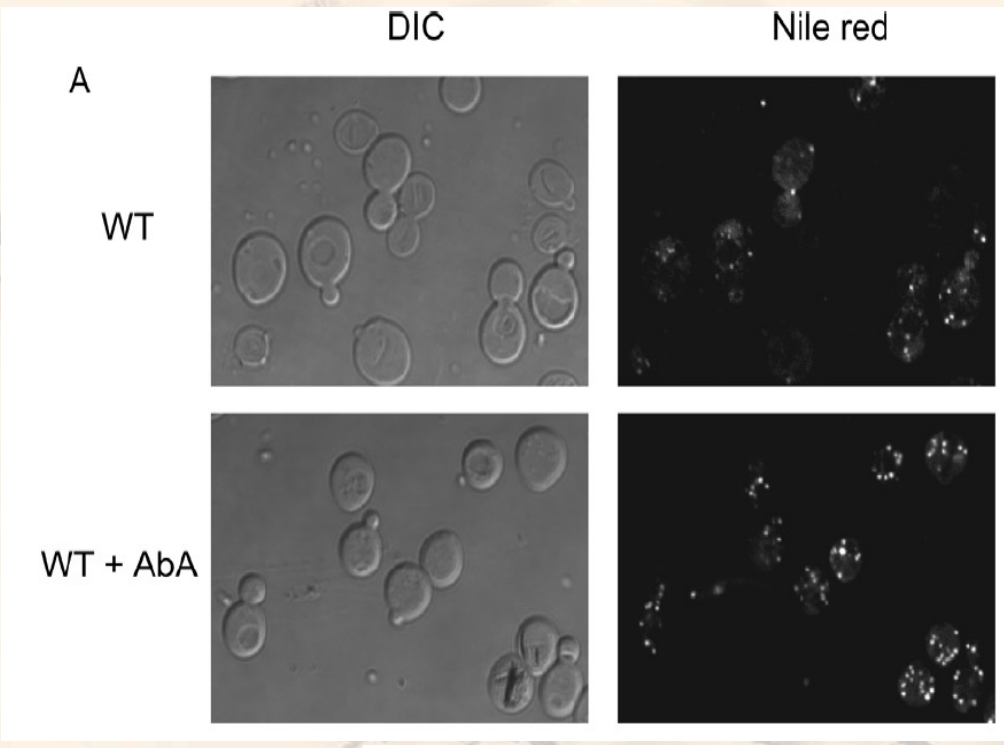


Photobleaching experiment



Cells labeled with cytoplasmic protein Rpl5-GFP

Lipid bodies contain ceramides except for neutral lipids



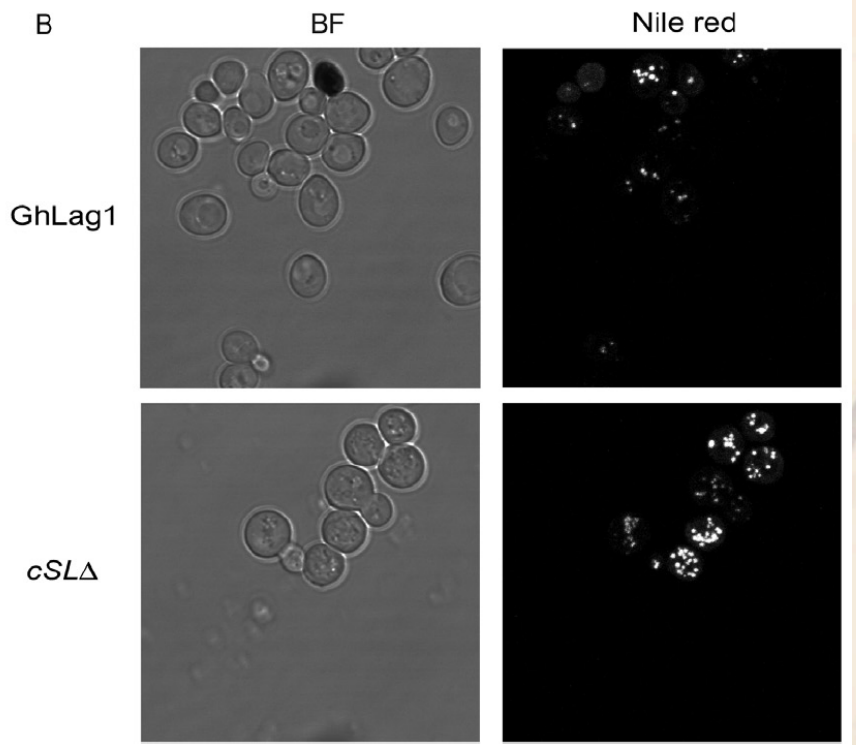


Table 1. Trace amounts of ceramides detected in isolated lipid bodies (given as percentage of detected ceramides).

	WT		GhLag1
Ceramide	Control	2 h AbA	Control
18:0/18:0 ceramide	ND	ND	ND
18:0/24:0 ceramide	ND	85.6	99
20:0/24:0 ceramide	ND	6.8	0.01
18:0/26:0 ceramide	ND	7.3	0.09

ND, not detected.

- Ceramides are bioactive lipids and precursors to sphingolipids.

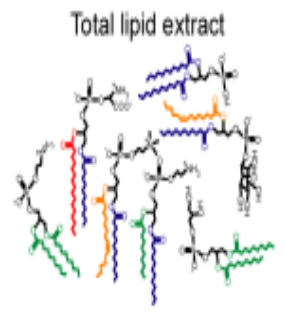
- A yeast strain in which the endogenous ceramide synthase was replaced by a cotton gene *GhLag1*, resulting in yeast cells producing C18 rather than C26 dihydroceramides.

- The data indicate that sphingolipids affect cytokinesis and cell separation, implying that sphingolipids are required for cell wall remodeling.

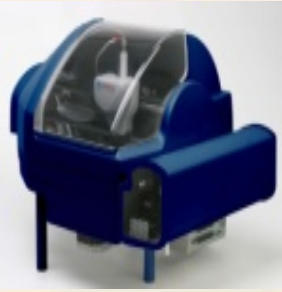
Large-scale analysis of cotton sphingolipid species by LC-MS/MS

Total lipid extracts → **Automated sample infusion** → **Multiple Precursor Ion Scanning** → **Lipid identification and quantification** → **Result reporting**

Liver Biopsies



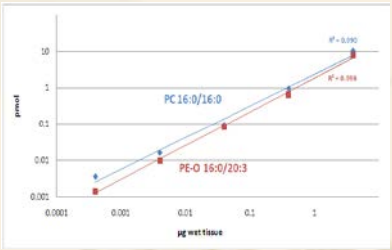
Advion NanoMate TriVersa



QTRAP® 5500 System

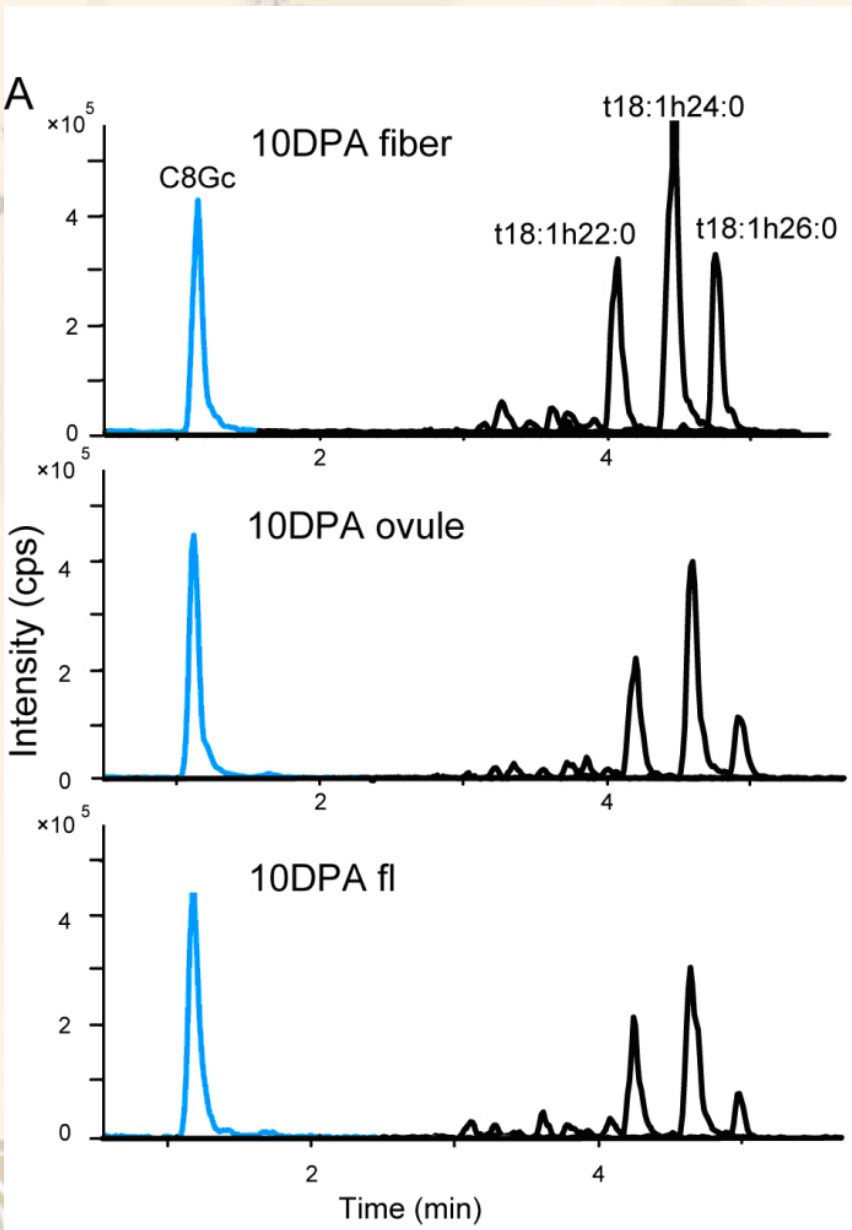
LipidView™ Software

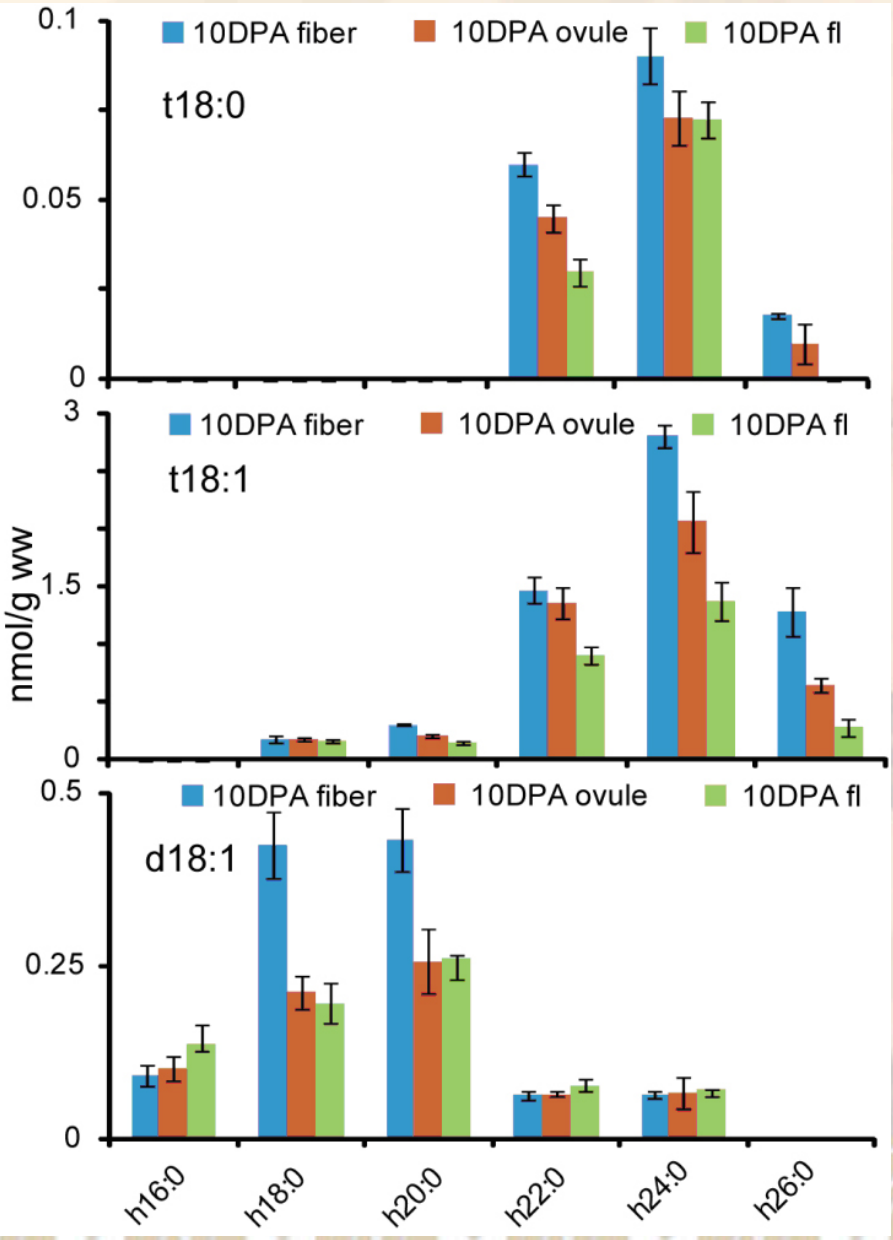
LipidView™ & MarkerView™ Softwares



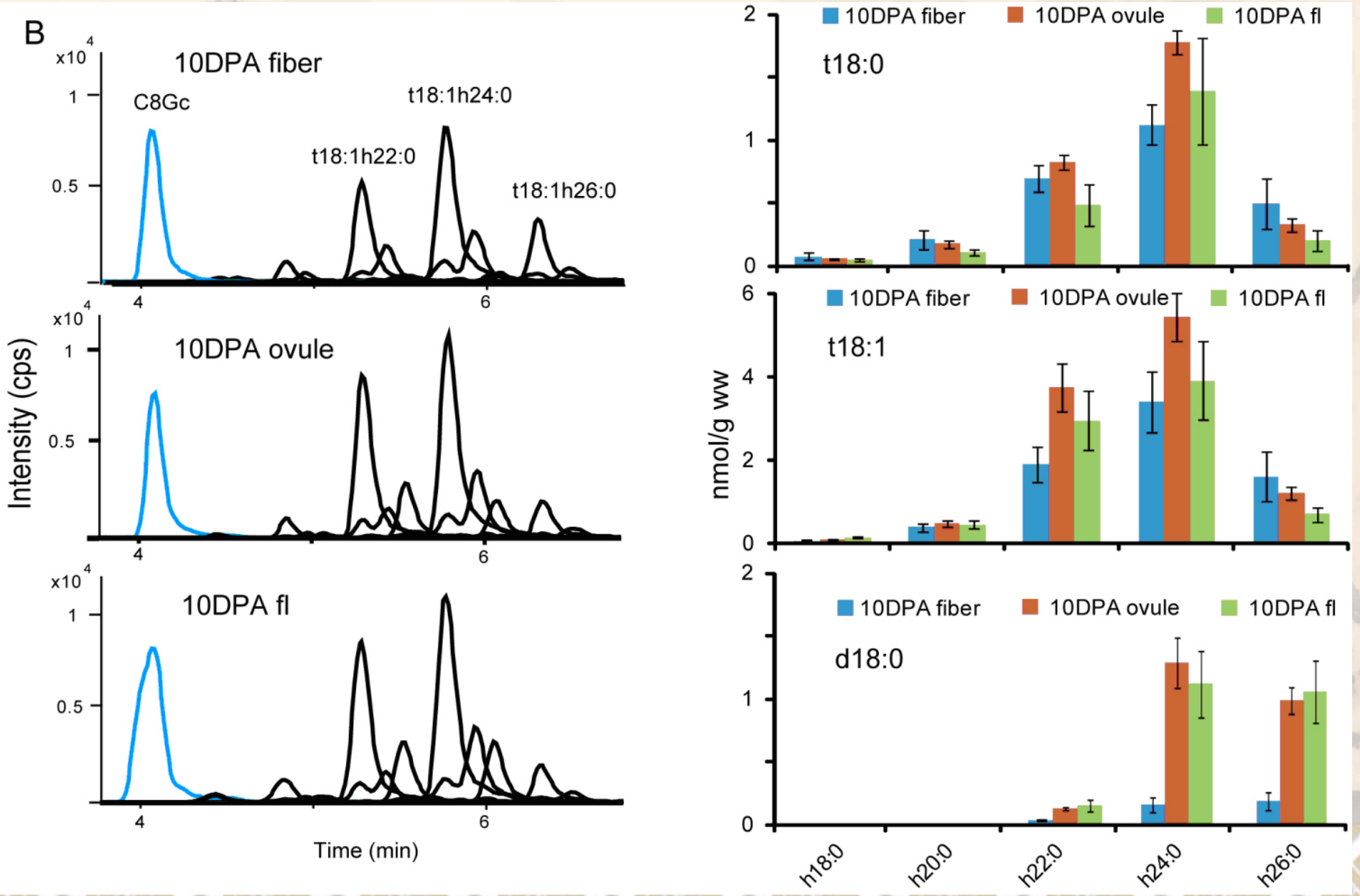
Ekroos, K et al., Methods in Pharmacology and Toxicology: Biomarker Methods in Drug Discovery and Development, Humana Press 2008
Ståhlman, M et al. High-throughput shotgun lipidomics by quadrupole time-of-flight mass spectrometry, J Chrom B 2009

Fast-elongating cotton fibers contain higher levels of glucosylceramides (GlcCer)





Cotton ovules (10 dpa) contain higher levels of glycosylinositolphosphoceramides (GIPCs)



Acknowledgements

Peking University, China

Prof. Yu-Xian Zhu

Msc. Gaojun Liu

University of Geneva, Switzerland

Prof. Howard Riezman

Dr. Sharon Epstein

Dr. Guillaume A. Castillon

Supported by Sino Swiss Science and Technology Cooperation Program, and the National "973" Program, Ministry Of Science and Technology.