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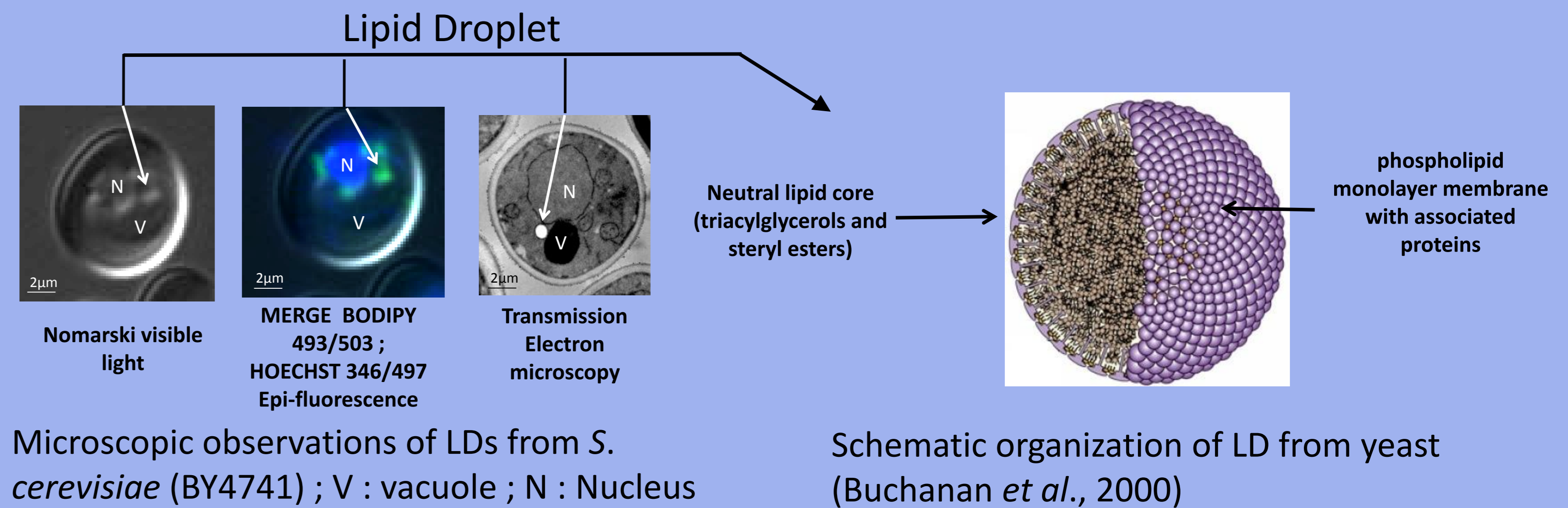
# Involvement of the Rab GTPase Ypt7p, the HOPS complex and the V1-ATPase in the dynamics of *Saccharomyces cerevisiae* lipid droplets

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## BACKGROUND

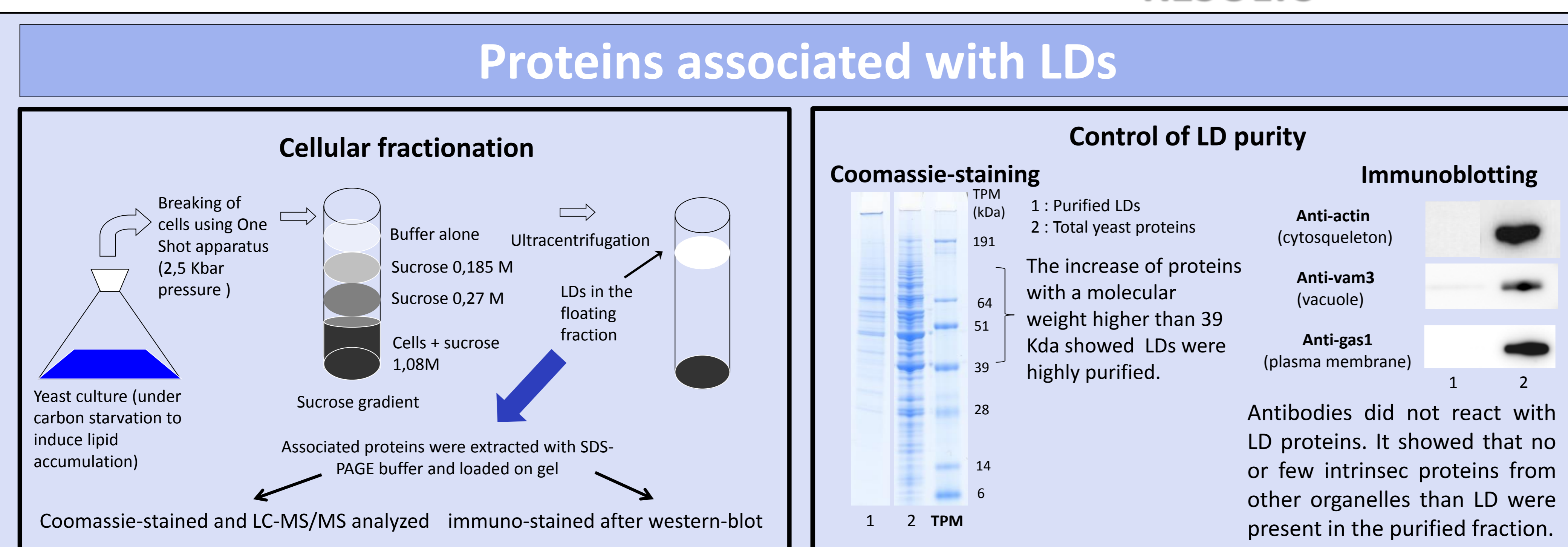
In yeast, storage lipids are maintained in the cytoplasm in specialized organelles called lipid droplets (LDs) (Murphy *et al.*, 2012). These structures consist mainly of a core of neutral lipids (triacylglycerols and/or steryl esters) enclosed in a monolayer of phospholipids. They contain a number of associated proteins, involved in many metabolisms, signaling and trafficking. Indeed, LD is now known to be a complex dynamic organelle. Many studies have reported interactions between LDs and intracellular organelles, like endoplasmic reticulum, early endosome, peroxisome and mitochondria (Zehmer *et al.*, 2009). We have previously shown, in *S. cerevisiae*, the relationship between vacuole and LDs, and described first evidences of the involvement of the Rab GTPase Yp7p in the dynamics of LDs.



## OBJECTIVES

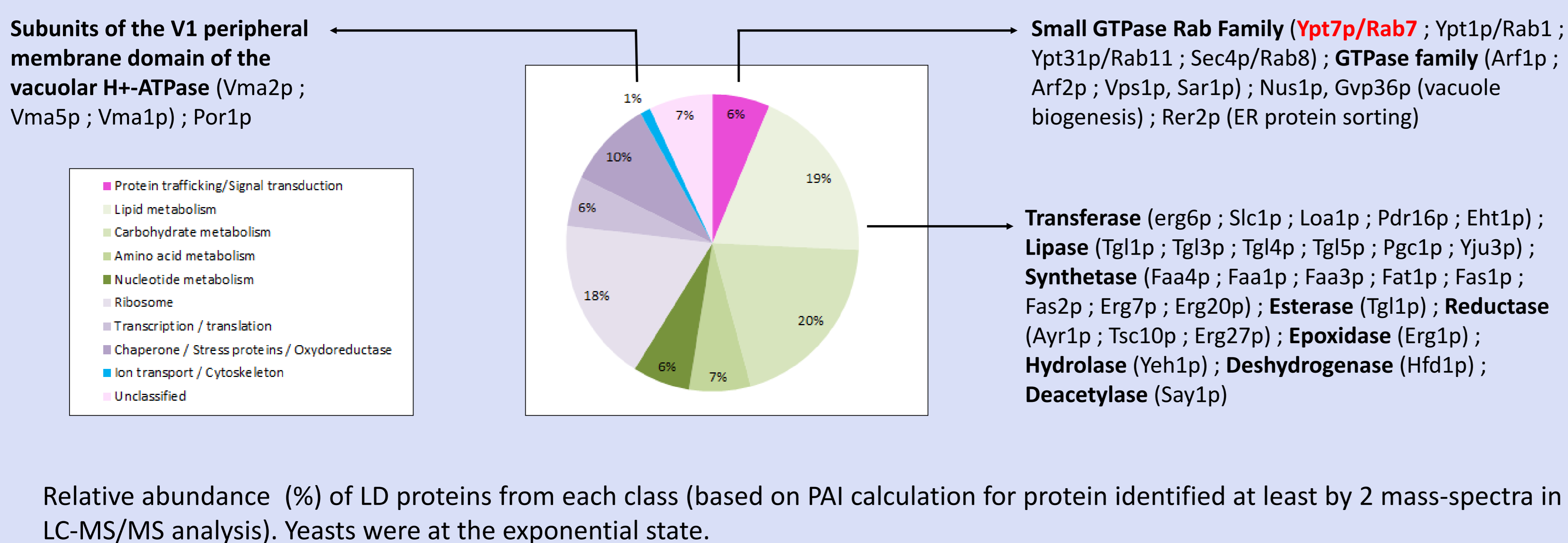
The aim of this study was to investigate the involvement of Ypt7p in LD dynamics by identifying its potential partners on LDs and by exploring the trafficking pathways which could be involved in these dynamics.

## RESULTS

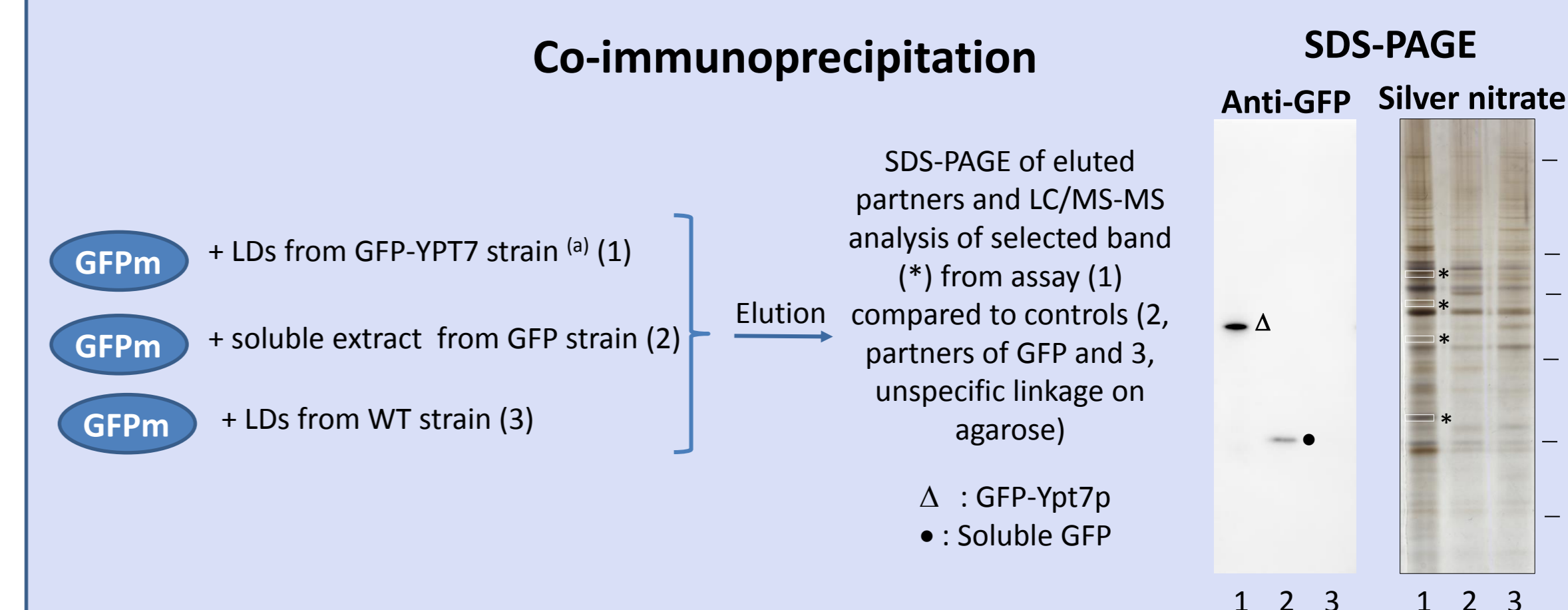


### Proteomic of purified LDs from WT *S. cerevisiae*

The 151 LD-associated proteins were classified according to their functionality (SGB database : <http://www.yeastgenome.org>). Many metabolism enzymes, especially lipid metabolism, were found showing important biological processes were carried at its surface. Several membrane-trafficking proteins were also identified showing that these proteins were functionally associated with LDs.



### Potential partners of Ypt7p



### Selected list of potential Ypt7p partners identified by LC/MS-MS

Potential partners	Function	Main localization
Lsp1p	BAR-domain protein ; Lipid binding ; Endocytose	Eisosome
Pet10p	Unknown	LD
Vma13p	Subunit H of the V1 peripheral membrane domain of the vacuolar H <sup>+</sup> -ATPase	Vacuole, mb
Kes1p	Ergosterol biosynthesis	Golgi, cytoplasm
Gdi1p	GDP dissociating inhibitor ; known partner of Ypt7p	Secretory pathway

The identification of **Gdi1p**, a known partner of Ypt7p, validated the experiment.

### Functional validation with mutants

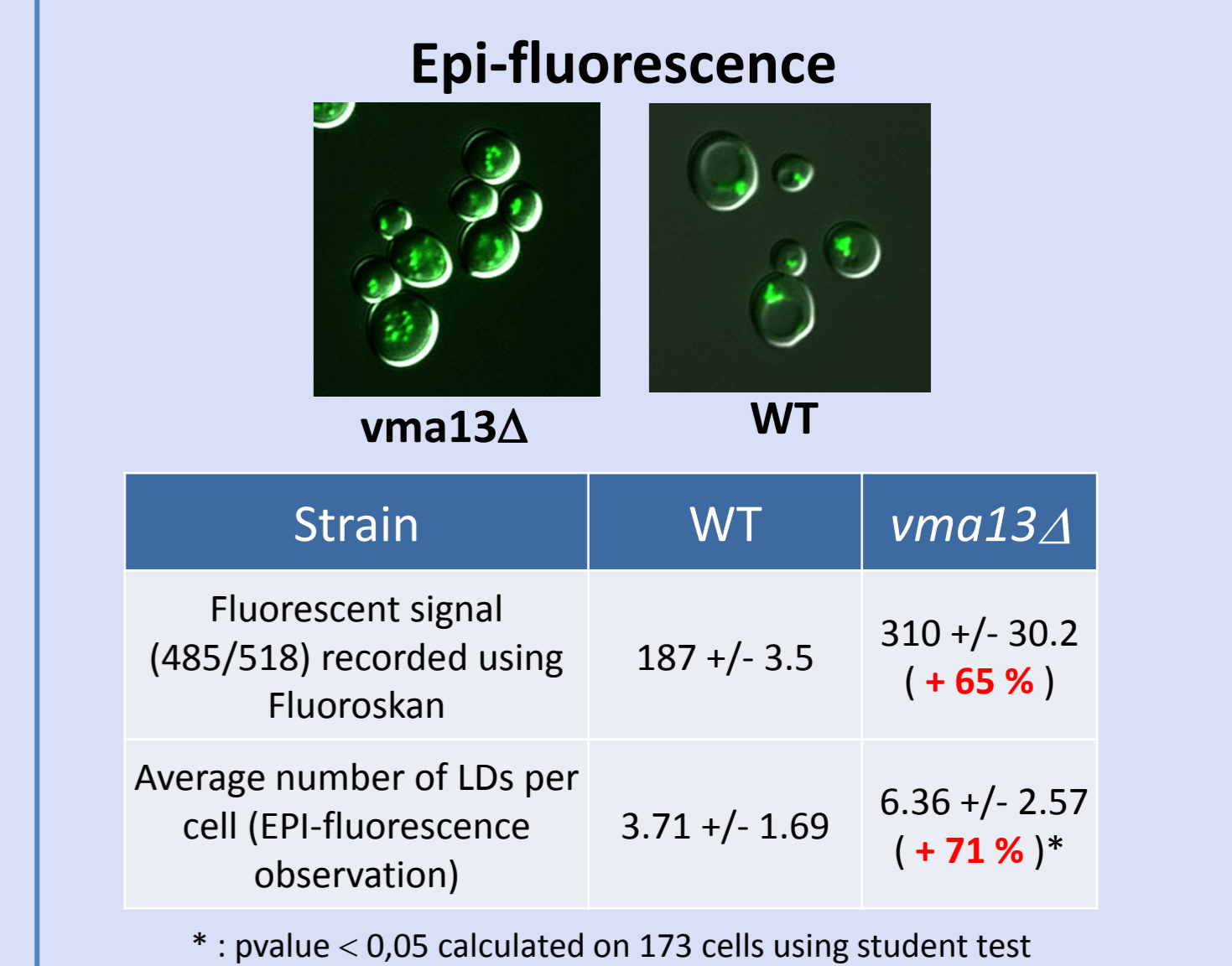
#### Fatty-acid (FA) analysis of mutant strains of selected Ypt7p partners

Strain	% of variation of total FA / WT (GC)
kes1Δ	+ 6 %
lsp1Δ	- 6 %
pet10Δ	- 8 %
vma13Δ	+ 25 %

Vma13p, good candidate?

Vma13p : Potential partner of Ypt7p, involves in LD dynamics

#### Staining of neutral lipids with Bodipy 493/503



## CONCLUSION and ACKNOWLEDGMENT

The proteome of purified LDs from WT *S. cerevisiae* showed several membrane-trafficking proteins, whom Ypt7p, and many subunits of the V1 peripheral membrane domain of the vacuolar H<sup>+</sup> ATPase. One other V1 subunit of this pump, Vma13p, was identified by Co-IP as a potential partner of Ypt7p. Its deletion had a great effect on LD dynamics. As its deletion is also known to abolish the homeostasis and the acidification of the vacuole, the effect on LDs could be a consequence of these dysfunctions. Anyway, this showed a tight link between LD dynamics and vacuole. Thus, we hypothesized that accumulation of LDs in the cytosol of VMA13 mutants could be due to modifications of interactions between LDs and vacuole, involving Ypt7p and Vma13p, in the context of defects in vacuole-linked homeostasis. This remains to be confirmed by other interacting tools, like yeast double-hybrid.

We also showed that deletion of subunits of the HOPS complex lead to accumulation of LDs in the cells. Thus, this showed this tethering complex, known to be involved in fusion processes, is involved in LD dynamics.

Altogether, these results lead us to hypothesize that LDs could interact with vacuole, via a hemi-fusion mechanism involving Ypt7p, as Rab effector, the HOPS complex and the V-ATPase.

We greatly acknowledge C. Ungermann<sup>(a)</sup> for the gift of the GFP-MYC-YPT7 strain, H. Riezman (Univ basel, Biozentrum, CH-4056 Basel, Switzerland) for the gift of Anti-Gas1 antibodies and A. Guillot, from PAPPSO (Jouy-en-Josas, France), for the quality of the LC-MS/MS analysis.

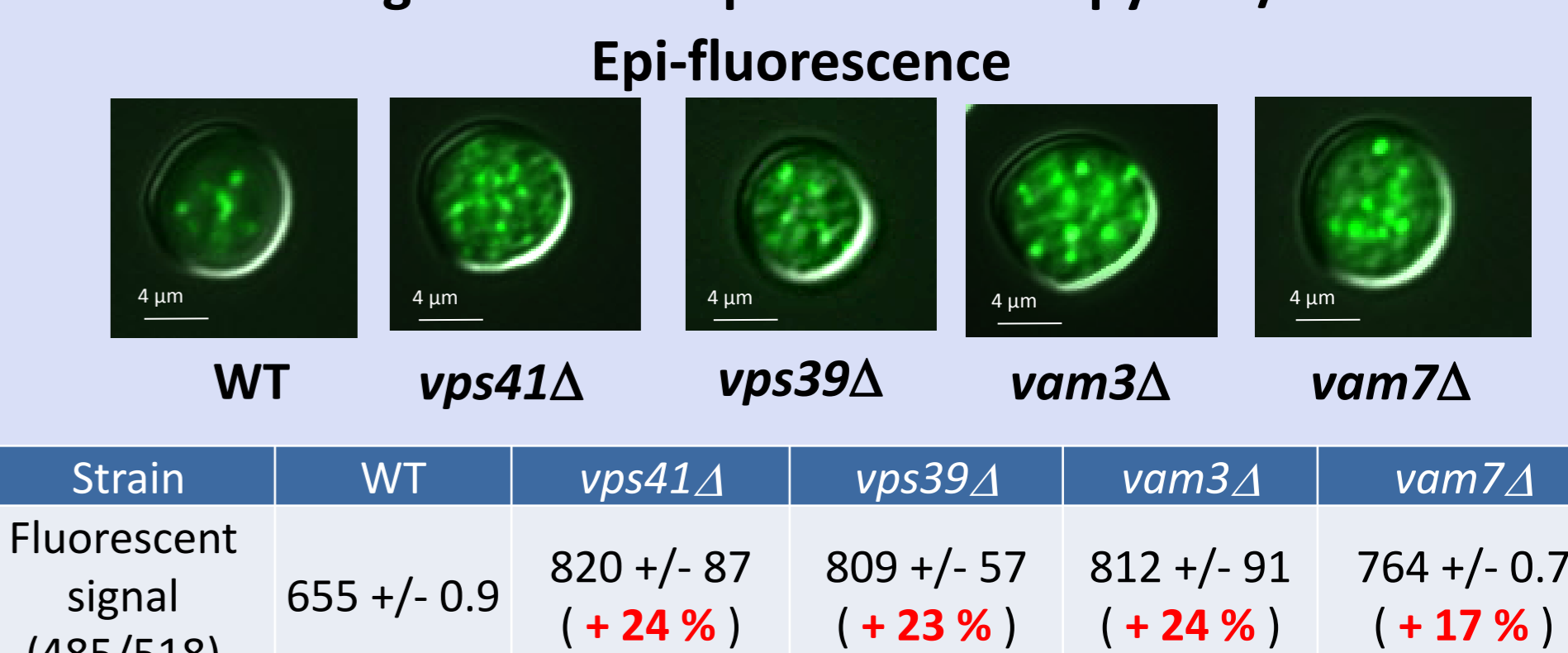
### Is HOPS complex involved in LD dynamics ?

Ypt7p is the Rab effector of the multisubunit tethering HOPS complex, which involves specifically Vps41p, Vps39p and the interacting SNAREs Vam3p and Vam7p. It acts in late endosome/vacuole or vacuole/vacuole membrane fusion.

#### FA analysis of mutant strains of HOPS pathway

Strain	% of variation of total FA / WT (GC)
vps41Δ	+ 10 %
vps39Δ	+ 27 %
vam3Δ	+ 30 %
vam7Δ	+ 19 %

#### Staining of neutral lipids with Bodipy 493/503



HOPS complex involves in LD dynamics