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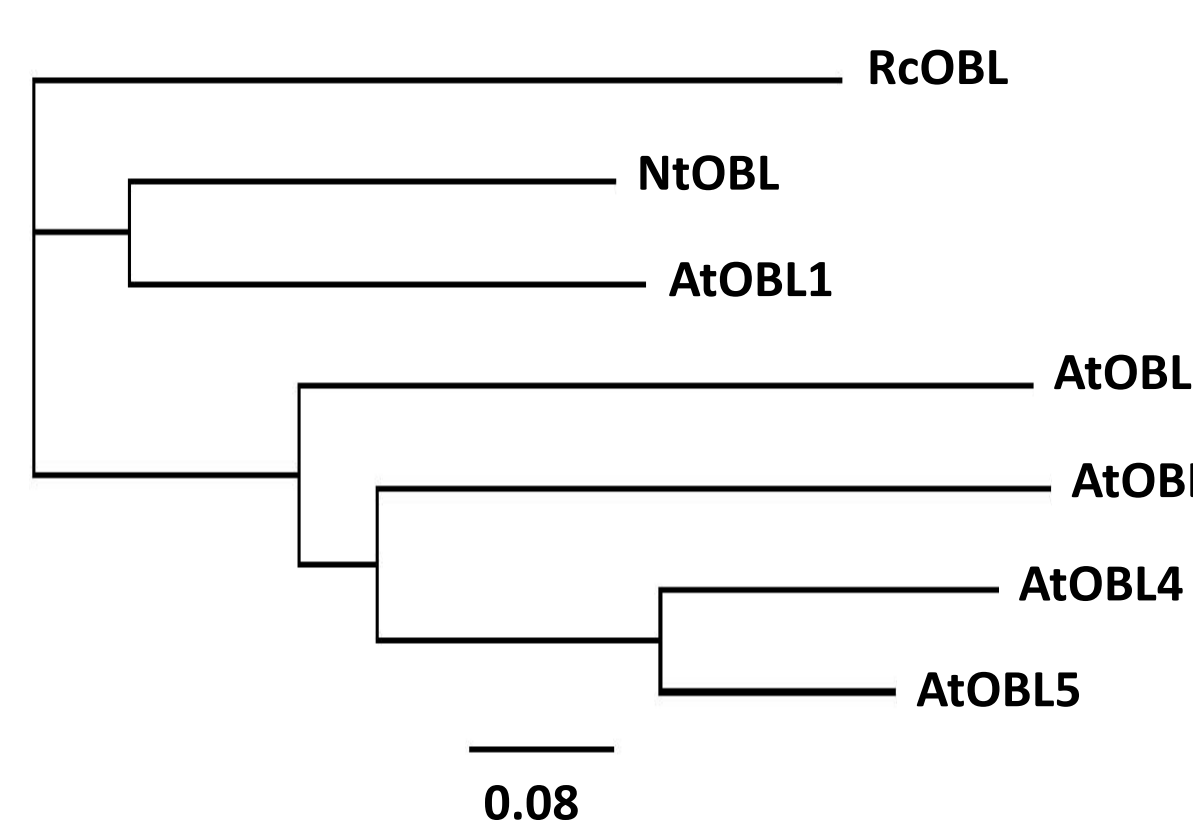
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## Introduction

Lipid droplets (LDs) are organelles consisting of a membrane lipid monolayer and a core of neutral lipids, most often triacylglycerols (TAGs) and fatty acid steryl esters (SE). They are also well described in yeast, drosophila and many mammalian cell types, especially adipocytes and hepatocytes. In plants LDs are most prominent in the seed embryo but are also formed in the pollen cytosol prior to desiccation and enter the pollen tube after germination.

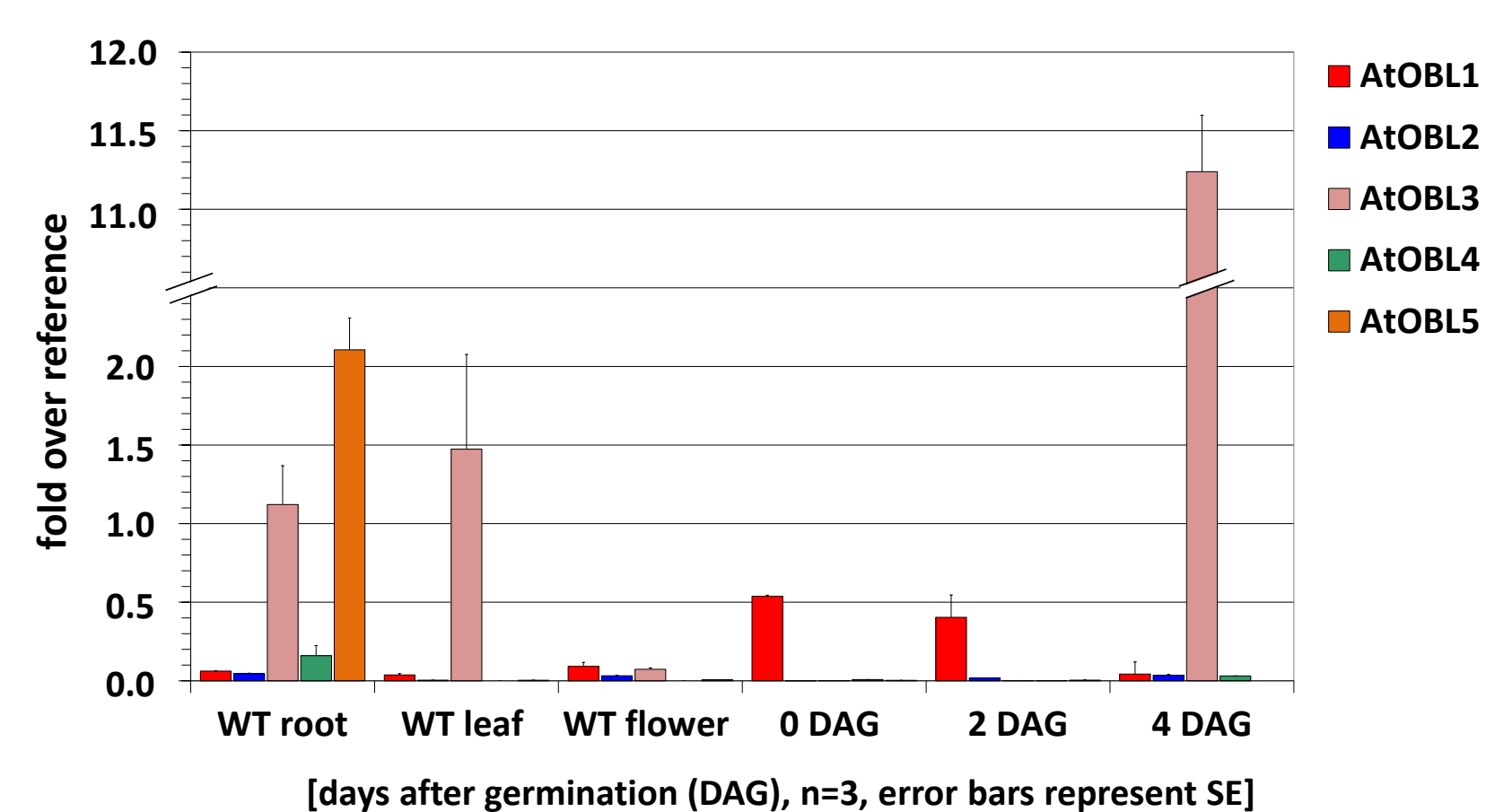
In a proteomic survey of tobacco pollen tube LDs, we identified a protein, NtOBL, which is homologous to an acidic TAG-lipase, RcOBL, from castor bean (Eastmond, 2004). NtOBL, like RcOBL, was shown to localize to lipid droplets in tobacco pollen tubes (Ischebeck *et al.*, unpublished). In Arabidopsis exists a five member gene family, which shares sequence homologies to RcOBL and NtOBL. This project aims to understand the physiological function of the gene family in Arabidopsis, suggesting that they are involved in TAG degradation within LDs. By TAG-degradation, TAG-stored fatty acid can be released and turned into new compounds and/or energy necessary e.g. for pollen tube growth or seed germination.

### The OBL family



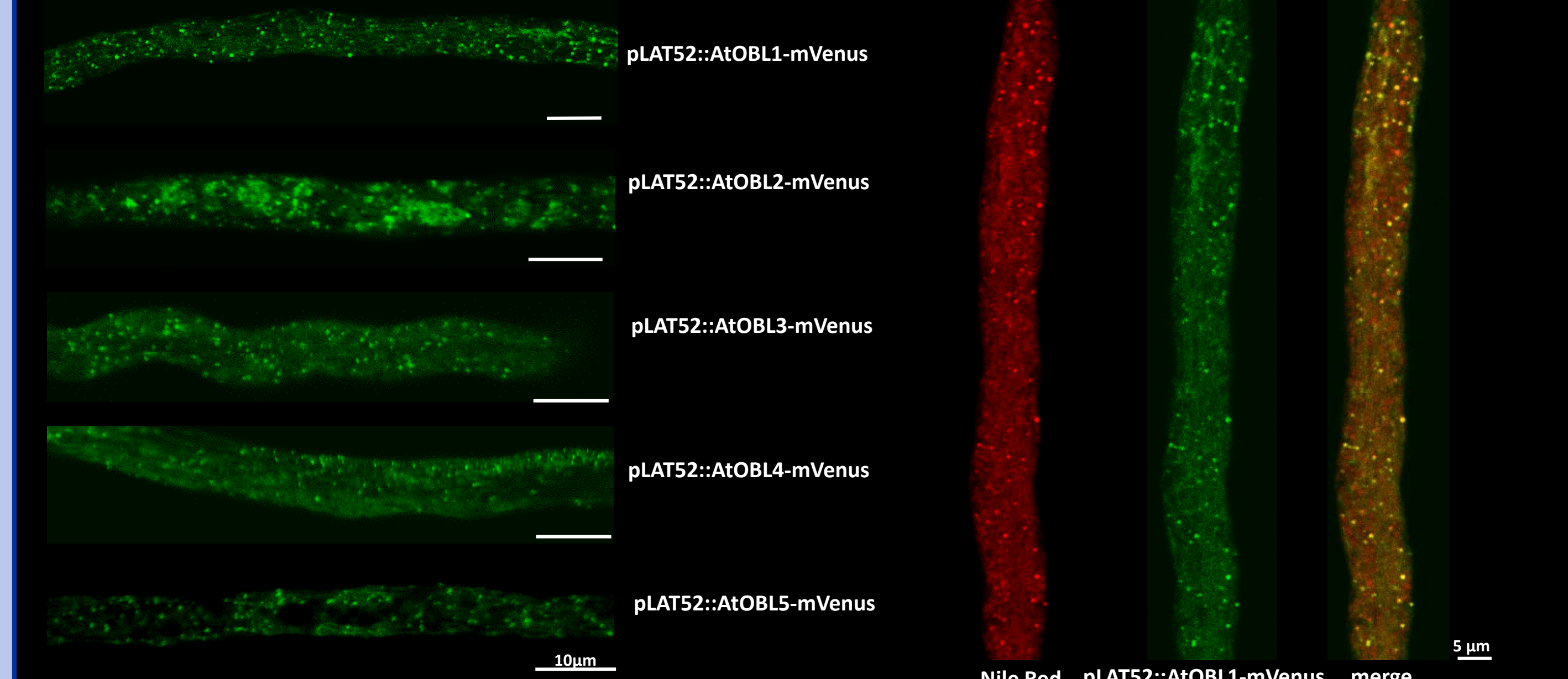
Phylogenetic tree of the oil body lipases family from Arabidopsis (AtOBLs) including the homologues from tobacco (NtOBL) and castor bean (RcOBL).

### AtOBLs are differently expressed



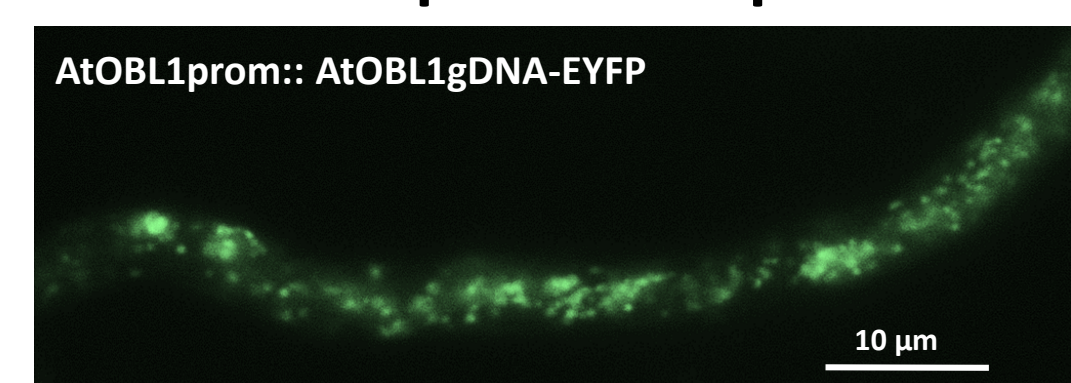
qRT-PCR analysis revealed large differences in the tissue-specific expression of the different isoforms. According to RNA seq data (Loraine *et al.*, 2013), AtOBL1 is the only isoform expressed in mature pollen.

### AtOBLs localize to LDs in tobacco pollen tubes

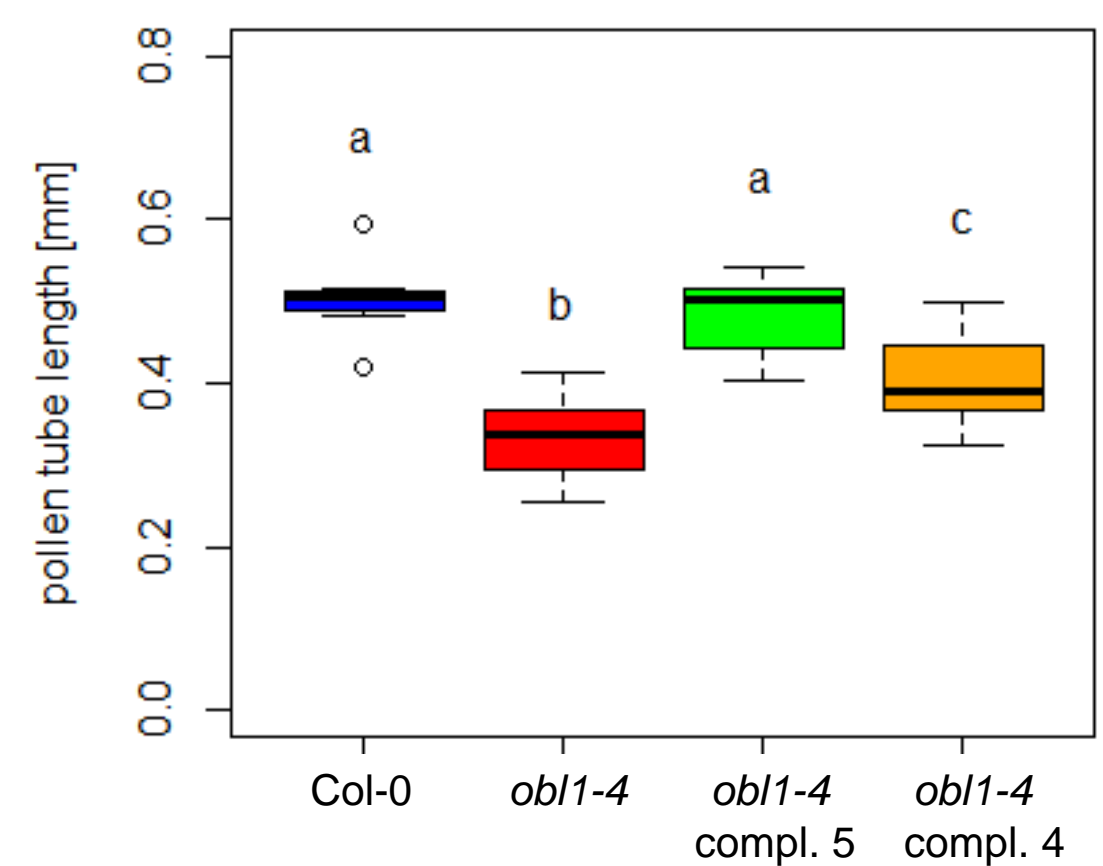


AtOBLs were transiently expressed in tobacco pollen tubes. Colocalization of AtOBLs with LDs were confirmed by Nile Red staining of the LDs shown as an example for OBL1.

### AtOBL1 is expressed in pollen tubes

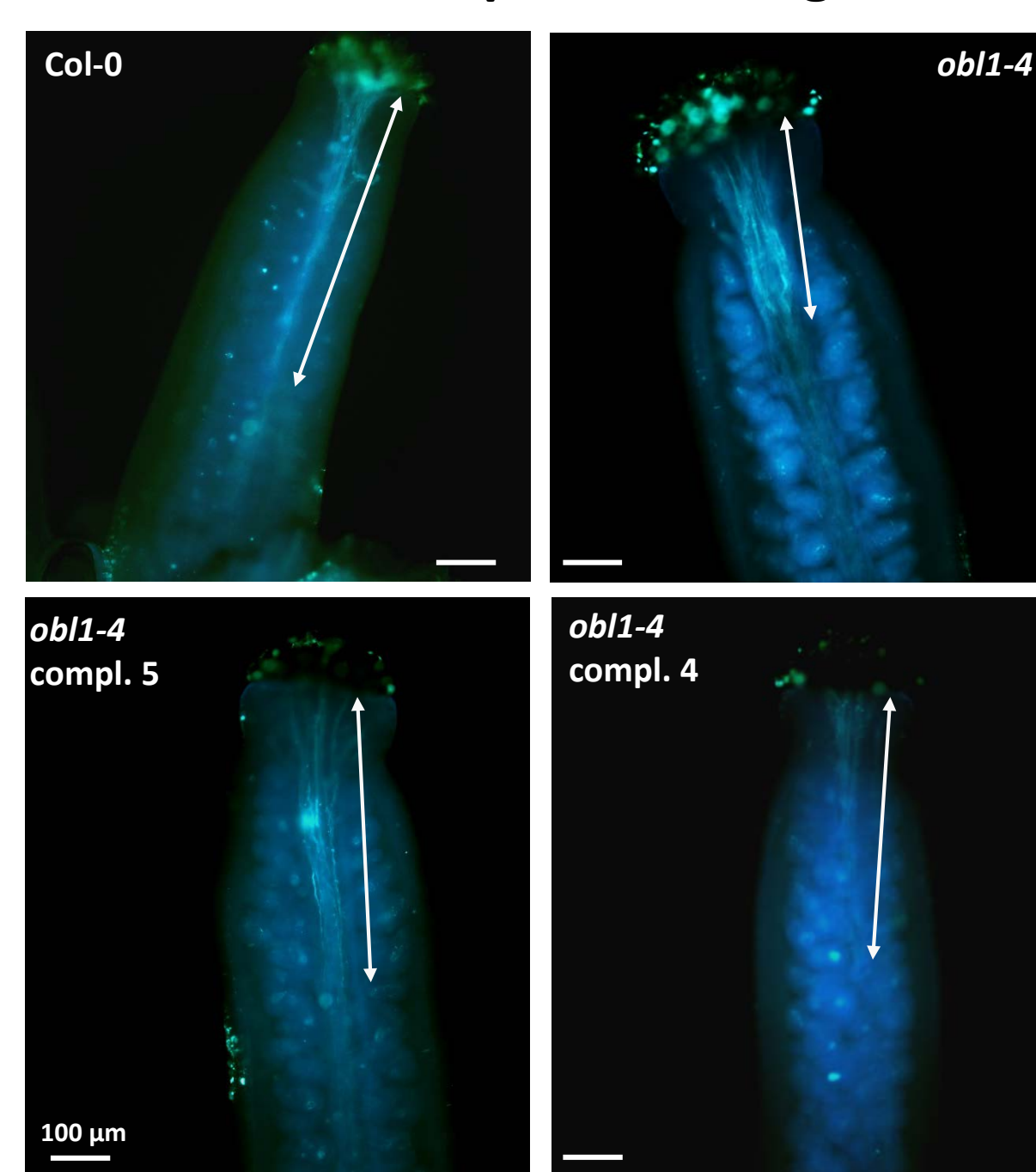


### In vivo pollen tube growth assay

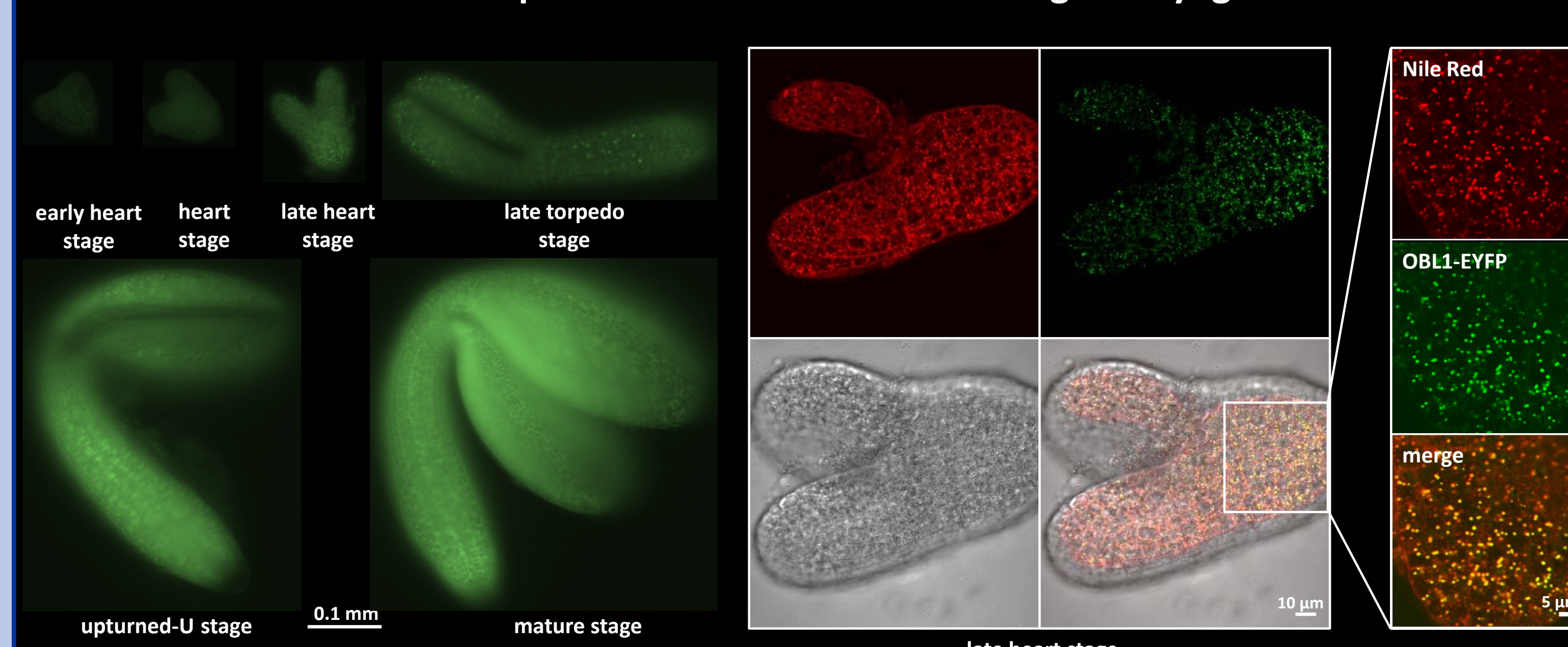


OBL1 expressed under its native promoter localizes to LDs in pollen tubes (upper left). A possible reduction of Arabidopsis *obl1-4* mutant pollen tube growth was assayed *in vivo*. Pistils of Col-0 plants were pollinated and the pistils were fixed after 3 h. They were stained with Aniline Blue and analyzed via fluorescence microscopy (right). n = 8-12. According to a Tukey's test (lower left), *obl1-4* mutant pollen tubes showed a slight but significant ( $p < 0.01$ ) reduction in length compared to pollen tubes from wild type and the complemented lines.

### It functions in pollen tube growth

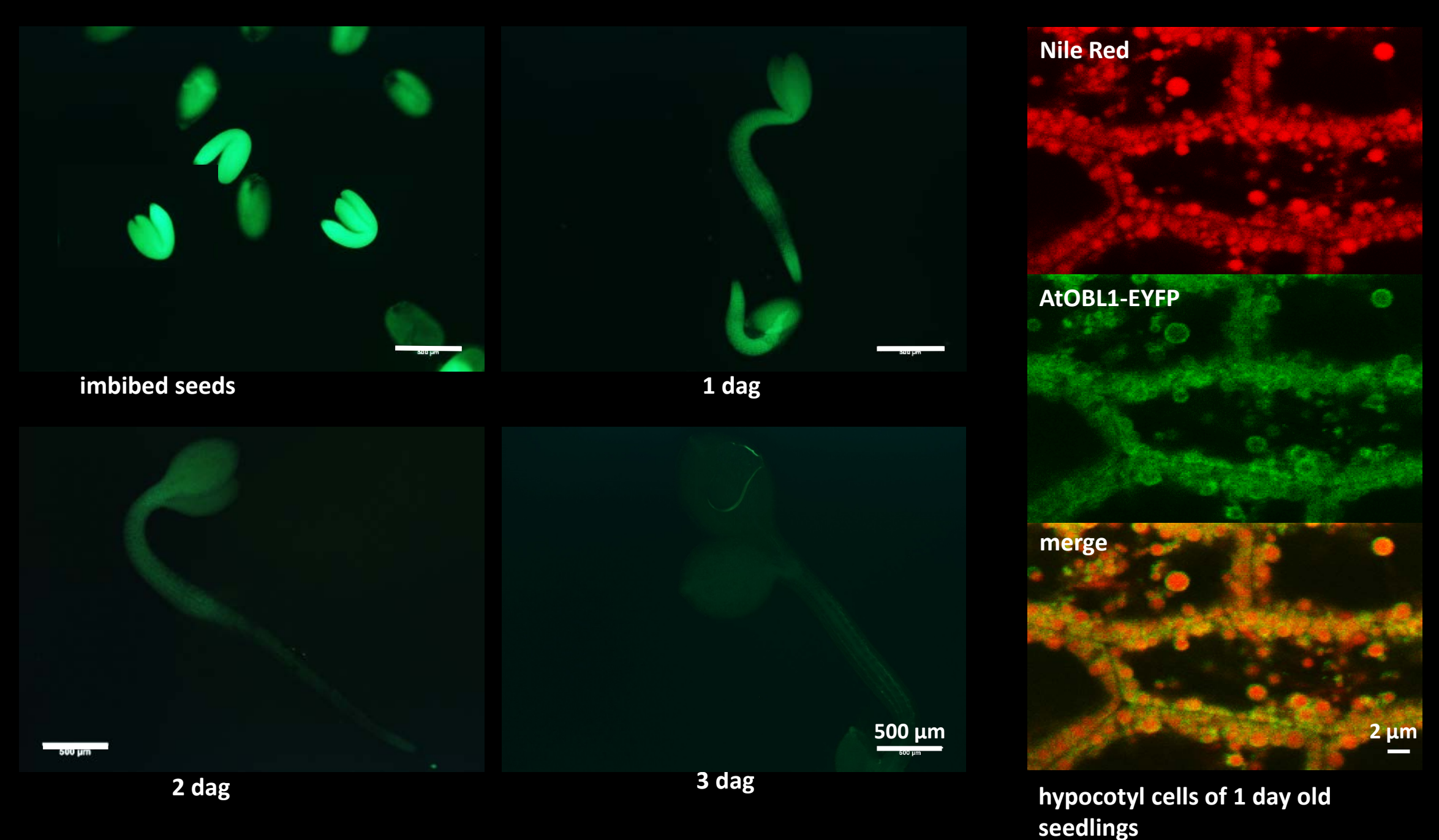


### Expression of AtOBL1 starts during embryogenesis



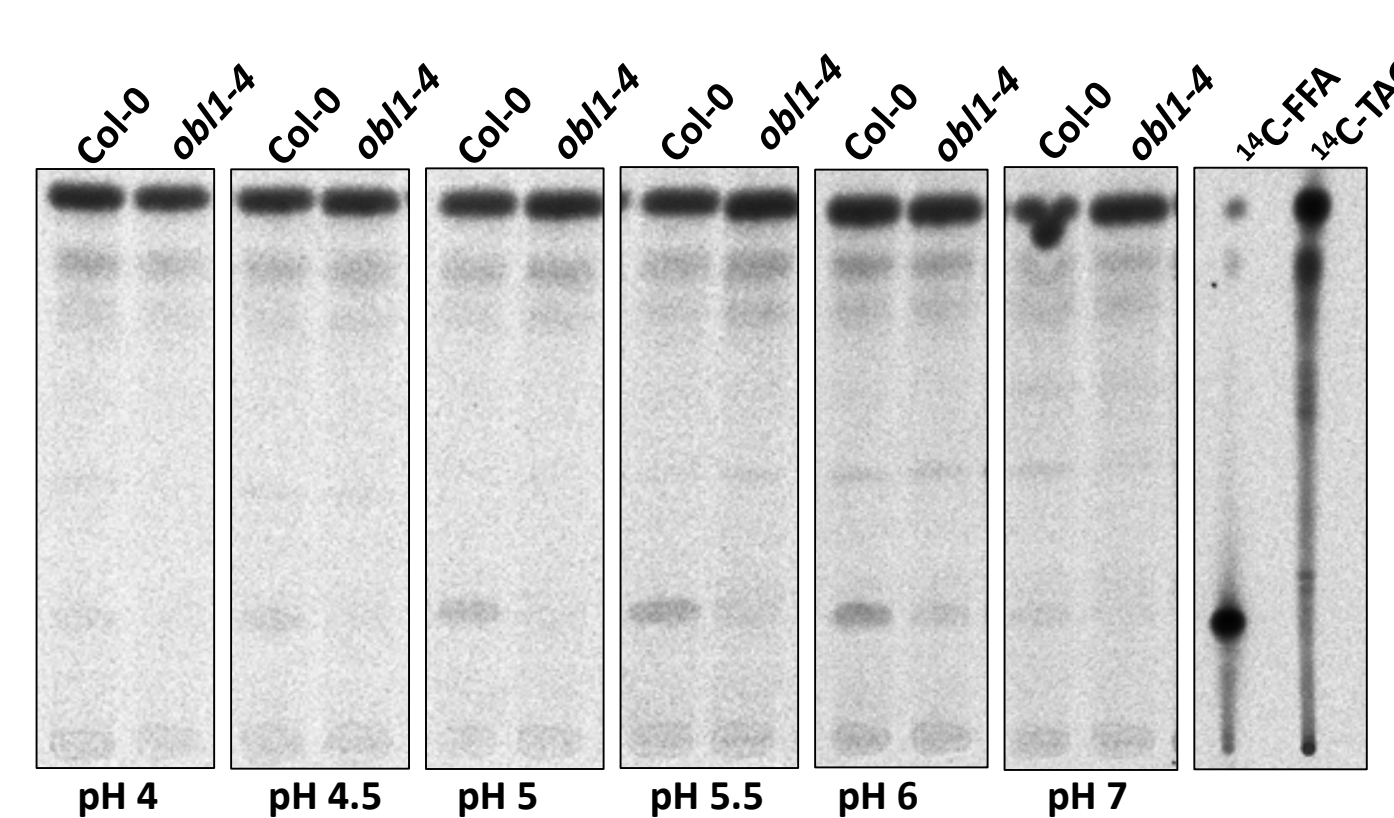
Epifluorescence (left) and confocal microscopy (right) revealed that AtOBL1 is abundant throughout embryogenesis. AtOBL1 localizes to the emerging LDs already in the heart stage (stained with Nile Red; upper right) and encircles them (lower right). This early expression hints to a role for AtOBL1 in TAG-remodelling or turnover prior to germination.

### Abundance of AtOBL1 decreases with germination

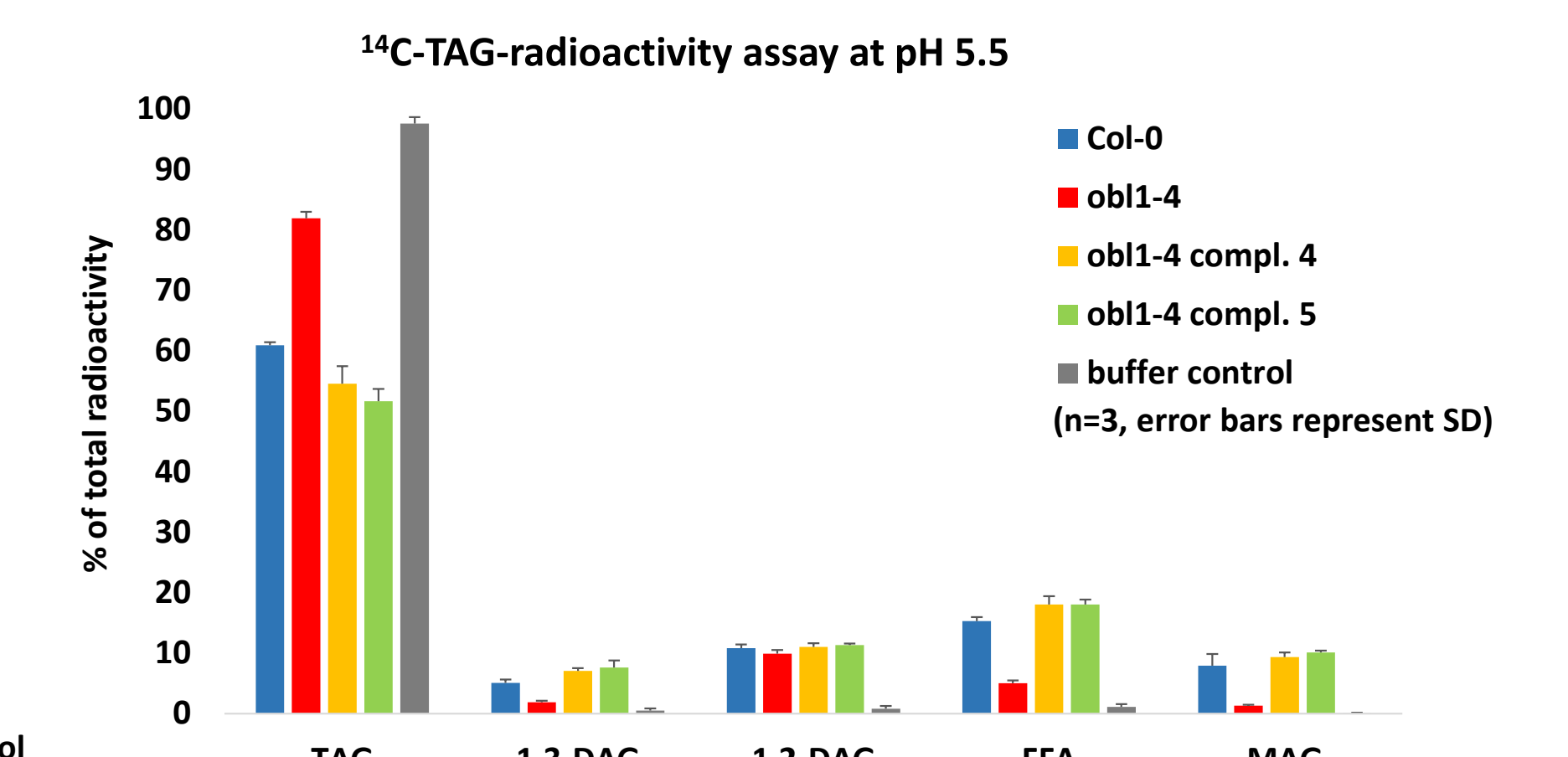
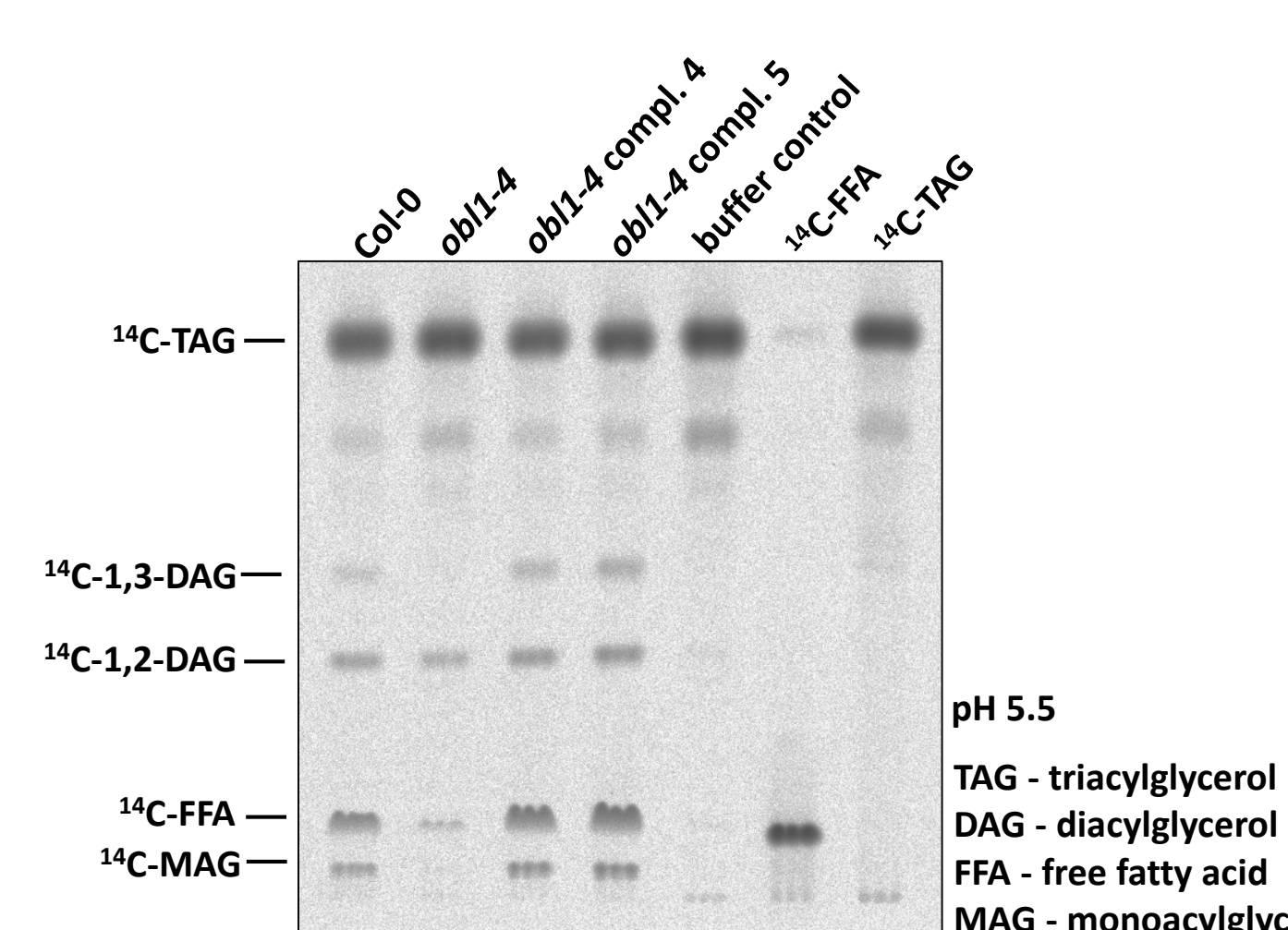


Epifluorescence (left) and confocal microscopy (right) revealed that AtOBL1 expression decreases during germination. AtOBL1 colocalizes with LDs stained with Nile Red and encircles them (right).

### AtOBL1 is an acidic TAG-lipase



Imbibed seeds (24 h, 4 °C) of Col-0 and *obl1-4* mutants were tested for TAG-lipase activity with <sup>14</sup>C-TAG as a substrate under different pH-conditions (upper left). The lipids were separated by TLC. Col-0 seeds showed high TAG-lipase activity at acidic pH from 5 to 6. In *obl1-4* mutants, the activity was diminished, supporting the idea that AtOBL1 is an acidic TAG-lipase and accounts for most of this in mature seeds. The assay was repeated at pH 5.5 using imbibed seeds of Col-0, *obl1-4* and two complemented lines in triplicates (lower left). The intensities of the different lipid species on the TLC plate were densitometrically evaluated and the percentage of the total radioactivity for each lipid class was calculated (lower right).



## Summary and conclusions

- Gene family of five lipases (AtOBLs) localize to lipid droplets in tobacco pollen tubes
- One isoform (AtOBL1) is expressed in pollen tubes, during embryogenesis and expression decrease during germination
- AtOBL1 functions in pollen tube growth
- AtOBL1 is an acidic TAG-lipase primarily active at pH 5 to 6
- It can probably use other neutral lipids like 1,3-DAG or MAG as substrates
- Acidic-TAG lipase might be necessary for TAG-remodelling during germination or the release of TAG-stored fatty acids as carbon and energy resource for pollen tube growth

## Outlook

- Determination of the fatty acid composition and total fatty acid content in seeds of wild type, *obl1-4* and complemented lines
- Analysis of the substrate preference of AtOBL1 by radioactivity assay with other lipid species
- Repetition of experiments with double and triple mutant of *obl1-4* with *sdp1*, *sdp1-like*, which are other TAG-lipases expressed in pollen and seedlings respectively
- Further analysis of the other four members of the gene family

## References

- Eastmond, P.J.: Cloning and characterization of the acid lipase from castor beans. *J. Biol. Chem.* 279, 45540-45545 (2004)  
Loraine, A.E., McCormick S., Estrada A., Patel K., Qin P.: RNA-seq of Arabidopsis pollen uncovers novel transcription and alternative splicing. *Plant Physiol.* 162(2),1092-109 (2013)

