



A lipid droplet – plasma membrane contact site is conserved across the angiosperm lineage

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Abstract

In *Arabidopsis thaliana*, a tethering complex of three proteins, SEED LIPID DROPLET PROTEIN 1 and 2 (SLDP1 and 2) and LIPID DROPLET PLASMA MEMBRANE ADAPTOR (LIPA), binds lipid droplets (LDs) to the plasma membrane (PM). While the physiological function remains unknown, it seems to be conserved across seed plants, as we here observed this tethering of LDs in seedlings of a variety of angiosperms. The analysis of LIPA and SLDP homologs indicated that these proteins emerged in the early period of land plant evolution before seed plants became dominant. LIPA and SLDP are, however, more strongly conserved among seed plants and share distinct amino acid motifs that could be involved in their interaction. We cloned LIPA and SLDP homologs from *Arachis hypogaea*, *Glycine max* and *Zea mays* and transiently expressed them in tobacco pollen tubes, a suitable model system. Here, the LIPAs and SLDPs localised to the PM and LDs, respectively, like their *A. thaliana* homologs. When coexpressed in pollen tubes, where LDs normally move freely in the cytosol, the LIPAs and SLDPs can tether LDs to the PM indicating that they can interact and constitute a LD-PM contact site. This interaction was even functional across taxon boundaries.

Keywords Lipid droplet · Seedling · Plasma membrane · Molecular contact site · Angiosperms · Pollen tubes

Introduction

Lipid droplets (LDs) are ubiquitous organelles in eukaryotic cells and especially abundant in storage tissues such as plant seeds (Zhao et al. 2025). Derived from the endoplasmic reticulum (ER), they consist of a hydrophobic core surrounded by a phospholipid monolayer and proteins. While the major content is triacylglycerol (TAG) in seeds, LDs can

also store other hydrophobic compounds like esters of sterols and other triterpenes, with the ratios depending on the tissue (Guzha et al. 2023; Scholz et al. 2026). LDs have been discussed in the context of biotic and abiotic stress responses like drought or heat stress. Under abiotic stress, mounting evidence points towards an implication as a sink for acyl chains during membrane lipid remodeling and degradation (Higashi et al. 2015; Krawczyk et al. 2022a, b; Mueller et al. 2015; Scholz et al. 2025). Furthermore, LDs appear to be hubs for metabolic pathways such as the synthesis of triterpenes in roots (Scholz et al. 2026) and to generate precursors for wax synthesis (Rett-Cadman et al. 2019). Moreover, during the colonisation with lipid-auxotrophic mycorrhiza fungi, LDs increase in the surrounding plant tissue, suggesting an involvement of LDs in symbiotic interactions (Kobae et al. 2014).

Another main LD function lies in the capacity to store energy and carbon in form of TAG that can be degraded by lipases to deliver precursors for different metabolic pathways (Ischebeck et al. 2020). In this context, seed embryos can accumulate TAG during maturation which serves as a source for energy and carbon during seedling establishment.

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Regarding this increasing number of functions, it is likely that LDs do not act on their own but in concert with other subcellular structures for example through membrane contact sites (Scholz et al. 2022). Such membrane contact sites are generally formed between two membranes without membrane fusion, usually requiring multiple proteins (Eisenberg-Bord et al. 2016; Guzha et al. 2023). They facilitate substance exchange between organelles, enable signalling and can be highly important for cellular functioning (Voeltz et al. 2024).

An LD plasma membrane (PM) contact site has been described in *Arabidopsis thaliana* seedlings that is constituted by the proteins SEED LIPID DROPLET PROTEIN (SLDP) 1 and 2 in combination with LIPID DROPLET PLASMA MEMBRANE ADAPTOR (LIPA) (Krawczyk et al. 2022a, b; Kretschmar et al. 2020). This complex that tethers most LDs to the plasma membrane is realised by the LD association of SLDPs via an N-terminal hydrophobic region and a putative interaction domain at the C-terminus, allowing them to bind to LIPA proteins. LIPA proteins, on the other hand, target to the PM via a polybasic C-terminal stretch and might interact with SLDPs via their N-terminus. While this complex is essential to tether LDs to the plasma membrane in *A. thaliana* seedlings, its physiological function is unknown, as *A. thaliana sldp1 sldp2* mutant lines missing this contact site are not affected in TAG degradation or seedling establishment. It is also not known if this SLDP and LIPA-mediated contact site generally occurs in angiosperms or even beyond, or if it is restricted to a narrower clade that includes *A. thaliana*.

In this study, we could show that LDs localised in close proximity to the PM in seedlings of several angiosperms. Furthermore, we phylogenetically analysed SLDP and LIPA homologs in all vascular plants and studied three homologs from two dicotyledonous and one monocotyledonous plant species through a cell biological approach. Through this approach, we describe their cellular localisation and interaction capabilities with their partner proteins from the same species or from *A. thaliana*.

Materials and methods

LD documentation in seedlings

PM association of LDs in angiosperm seedlings was analysed in each species at a time point when cellular LD density was similar to that observed in 36 h old *A. thaliana* seedlings: *Arachis hypogaea*, 96 h old hypocotyl; *A. thaliana*, 36 h old hypocotyl; *Glycine max*, 48 h old cotyledon; *Helianthus annuus*, 96 h old hypocotyl; *Hypericum perforatum*, 48 h old hypocotyl; *Lactuca sativa*, 24 h old

hypocotyls; *Linum usitatissimum*, 24 h old hypocotyls; *Theobroma cacao*, 24 h old hypocotyls; *Zea mays*, 36 h old seedling. Seed germination was carried out in conditions needed for the respective species: *A. hypogaea* was germinated directly in soil in the dark at 22 °C; *Z. mays*, *L. sativa*, *L. usitatissimum*, *G. max*, *H. perforatum*, *T. cacao* were germinated on wet filter paper in a closed petri dish at 22 °C with long day conditions (16 h/8 h photo period, 120 µmol/m²/s). Seedling sections were stained using either Nile red or bodipy493/503 to visualise LDs. Images were recorded using a ZEISS LSM 980 (Carl Zeiss Microscopy Germany GmbH, Oberkochen, Germany) with settings described in Supplementary Table 1.

Phylogenetic analysis

To understand the evolutionary history of SLDP and LIPA, we used manually selected homologs from *A. thaliana*, *Z. mays*, *G. max* and *A. hypogaea* as queries in a BLASTp search against a protein database inferred from the genomes of: *Arabidopsis thaliana* (Lamesch et al. 2012), *Arabidopsis lyrata* (V2.1; Hu et al. 2011; Rawat et al. 2015), *Arachis hypogaea* (Bertioli et al. 2019), *Brassica oleracea* (Liu et al. 2014), *Brassica rapa* (FPsc v1.3; Goodstein et al. 2012), *Capsella grandiflora* (Slotte et al. 2013), *Triticum aestivum* (Hernández Molina et al. 2018), *Carica papaya* (Ming et al. 2008), *Oryza sativa* (v7.0; Kawahara et al. 2013), *Brachipodium distachyon* (v3.2; International Brachypodium Initiative 2010), *Amborella trichopoda* (v1.0; Amborella Genome Project 2013), *Gossypium hirsutum* (Li et al. 2015), *Theobroma cacao* (Argout et al. 2011), *Solanum lycopersicum* (Tomato Genome Consortium 2012), *Nicotiana tabacum* (Sierro et al. 2014), *Zea mays* V4 (Jiao et al. 2017), *Gnetum montanum* (Wan et al. 2018), *Glycine max* (Valliyodan et al. 2019), *Picea abies* (Nystedt et al. 2013), *Azolla filiculoides* (Li et al. 2018), *Isoetes taiwaniensis* (Wickell et al. 2021), *Selaginella moellendorffii* (Banks et al. 2011), *Physcomitrium patens* (Lang et al. 2018), *Sphagnum fallax* (Healey et al. 2023), *Marchantia polymorpha* (Bowman et al. 2017), *Anthoceros punctatus* (Li et al. 2020), *Anthoceros agrestis* (Li et al. 2020), leveraging the diverse streptophyte algal genomes (Kunz et al. 2025) including, *Mesotaenium endlicherianum* (v1; Cheng et al. 2019; and v2; Dadras et al. 2023), *Penium margaritaceum* (Jiao et al. 2020), *Spirogloea muscicola* (Cheng et al. 2019), *Chara braunii* (Nishiyama et al. 2018), *Klebsormidium nitens* (v1.1; Hori et al. 2014), *Chlorokybus melkonianii* (Wang et al. 2020) — for naming, see Irisarri et al. 2021, *Mesostigma viride* (Wang et al. 2020), *Zygnema circumcarinatum* SAG 698-1b, UTEX 1559 and UTEX 1560 as well as *Zygnema cylindricum* SAG 698-1a (Feng et al. 2024), *Spirogyra pratensis* MZCH10213 (Goldbecker et al. 2025), *Micromonas*

pusilla (v3.0; (Worden et al. 2009), *Ostreococcus lucimarinus* (v2.0; Palenik et al. 2007), *Ulva mutabilis* De Clerck et al. 2018), *Chlamydomonas reinhardtii* (v5.6; Merchant et al. 2007), and *Chlorella variabilis* (Blanc et al. 2010). All significant hits (e value cutoff of 10^{-5} ; in case of larger gene families, we applied a bit score cutoff of 100) were aligned with MAFFT v7.490 (Kato and Standley 2013) (L-INS-I). We computed maximum likelihood trees using IQ-Tree v. 1.5.5 (Nguyen et al. 2015) with 1000 ultrafast bootstrap pseudo-replicates (Minh et al. 2013). The best models for protein evolution were determined by ModelFinder (Kalyaanamoorthy et al. 2017) and the best model according to Bayesian Information Criterion was used.

Structure models

Structure models of the SLDP2.1 and LIPA interaction (Supplementary Data 1) were predicted through alphafold3 (<https://alphafoldserver.com>; Abramson et al. 2024) using full peptide sequences of the respective gene loci. In the structure, regions are given local distance difference test (pIDDT) scores (Mariani et al. 2013) that indicate the probability that this part of the structure is predicted correctly. Polar contacts were predicted and highlighted using Pymol version 3.1.5.1 (The PyMOL Molecular Graphics System, Version 3.0 Schrödinger, LLC.) plugin “show_contacts” (https://pymolwiki.org/Show_contacts).

Plasmid construction and particle bombardment

Homologs of SLDP2.1 and LIPA were identified using the PHYTOZOME databank (Goodstein et al. 2012, <https://phytozome-next.jgi.doe.gov/>). The most consistent homologs were selected, and specific primers (Supplementary Table 2) were designed to amplify the open reading frames (ORFs). ORFs of SLDP2.1 and LIPA homologs were then amplified from seedling RNA from the respective species or synthesised by the company BioCat (Heidelberg, Germany) and cloned into pLAT52MVC (SLDP) or pLAT52MCN (LIPA) (Müller et al. 2017) by use of the Gateway cloning method (Müller et al. 2017) or the GIBSON assembly (Gibson et al. 2009).

Particle bombardment and pollen tube growth was carried out according to Müller et al. (2017). In brief, pollen for particle bombardment was collected from flowers of fully grown *Nicotiana tabacum* Samsun-NN plants, grown in the greenhouse under long day conditions with an adjusted light intensity of 230–250 $\mu\text{mol}/\text{m}^2/\text{s}$ at 22 °C. Pollen tubes were grown for 6 h at room temperature before analysis. For pollen tube imaging, monodansylpentane (MDH) was used as a LD stain and pollen tubes were fixated using a final concentration of 1.5% formaldehyde in pollen tube growth media.

Images were obtained using a ZEISS LSM 980 confocal microscope (Carl Zeiss Microscopy Germany GmbH, Oberkochen, Germany) with settings described in Supplementary Table 1.

Image analysis and statistical evaluation

For statistical analysis of LD distribution, images were taken at the median cross-sections of the cells, and cell spanning plot profiles were created. Fluorescence intensity in the first and last 20% of these plots were counted as peripheral (i.e., near the PM). The data plotted represents the % of fluorescence intensity at the periphery.

LD-PM tethering in transformed *N. tabacum* pollen tubes was determined using ImageJ 2.16.0/1.54p. The image section size was randomly chosen including two membranes and excluding pollen tube tips and regions with large vacuoles. The LD channel was segmented using an individual threshold for each picture, where LDs are clearly differentiated from the background, and “Analyze Particles” function was used to obtain the total LD count. PM localisation was determined visually for each particle and counted by hand by a person blind to the genetic background of the pollen tubes and after the order of images was scrambled. The proportion of PM located LDs of each pollen tube was calculated (number of PM associated LDs divided by number of total LDs) and used for statistical analysis in R version 4.5.2 (R Core Team 2021, www.R-project.org). A Kruskal-Wallis Test for not-normally distributed data was performed followed by a post-hoc Dunn Test with Bonferroni adjusted p-values to compare individual groups.

Results

LDs localise at the PM in seedlings of various plants

In *A. thaliana*, the SLDP- and LIPA-mediated LD-PM contact site is characterised by a close proximity of LDs to the PM during early seedling establishment (Krawczyk et al. 2022a, b). Through this interaction, LDs often form pearl-string-like structures along the lateral PM. When either of the constituents of the contact site is missing, LDs are no longer aligned at the plasma membrane but are clustered in the cell. To test whether an LD distribution comparable to wildtype *A. thaliana* seedlings occurs in other seed plants, LD localisation was analysed in young seedlings of several angiosperm species using confocal laser scanning microscopy. In all analysed species, namely *A. hypogaea*, *G. max*, *H. annuus*, *H. perforatum*, *L. sativa*, *L. usitatissimum*, *T. cacao*, and *Z. mays*, LDs were observed in close proximity to the lateral PM (Fig. 1).

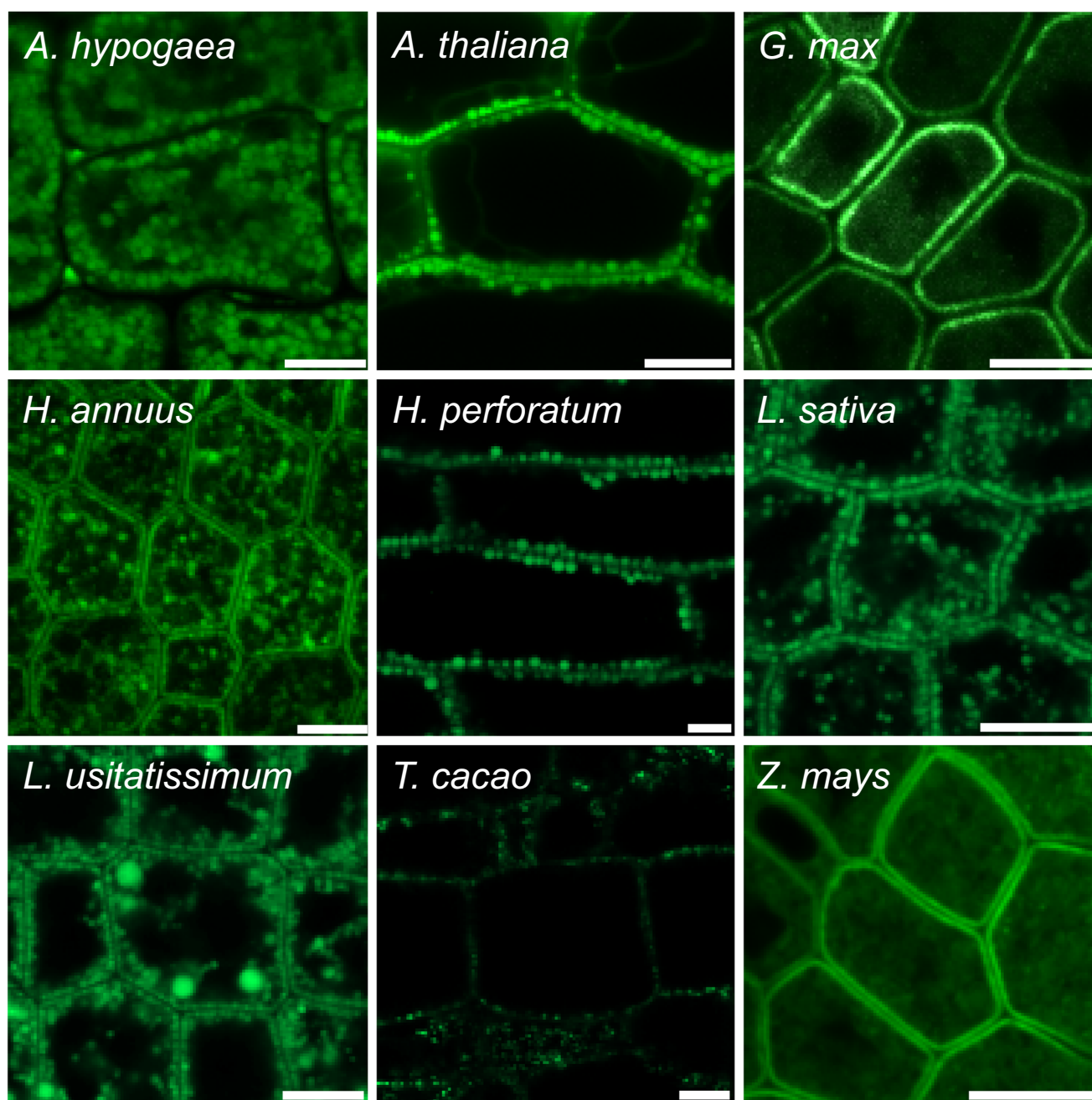


Fig. 1 LDs are localised in close proximity to the plasma membrane in seedlings across angiosperms. Lipid droplets were stained with BODIPY or Nile red and imaged as single planes by confocal laser scanning microscopy. *Arachis hypogaea*, 96 h old hypocotyl; *Arabidopsis thaliana*, 36 h old hypocotyl; *Glycine max*, 48 h old cotyledon;

Helianthus annuus, 96 h old hypocotyl; *Hypericum perforatum*, 48 h old hypocotyl; *Lactuca sativa*, 24 h old hypocotyls; *Linum usitatissimum*, 24 h old hypocotyls; *Theobroma cacao*, 24 h old hypocotyls; *Zea mays*, 36 h old hypocotyl. Bars, 10 μ m

We quantified this distribution by taking cell spanning fluorescence plot profiles of median cross-sections as previously performed (Krawczyk et al. 2022a, b). In most species, more than 70% of the fluorescence was found in the first and last 20% of the plots (Fig. 2).

By average, depending on the species 58–98% of the fluorescence were found in the periphery, which is higher than

the 40% expected from a random distribution and higher than the ~30–40% observed in *A. thaliana sldp1 sldp2* or *lipa* mutant seedlings, where the levels are decreased from ~80% in the wild type, as previously published (Krawczyk et al. 2022a, b).

The micrographs of the different plant species observed here do not prove that there is a contact site between the

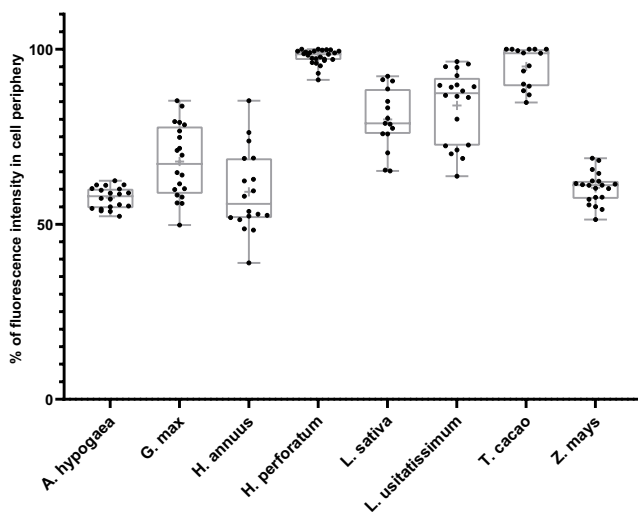


Fig. 2 The fluorescent signal of stained LDs is localised in close proximity to the plasma membrane in seedlings across angiosperms. Lipid droplets were stained with BODIPY or Nile red and imaged as single planes by confocal laser scanning microscopy. *Arachis hypogaea*, 96 h old hypocotyl; *Glycine max*, 48 h old cotyledon; *Helianthus annuus*, 96 h old hypocotyl ($n=18$); *Hypericum perforatum*, 48 h old hypocotyl; *Lactuca sativa*, 24 h old hypocotyl ($n=15$); *Linum usitatissimum*, 24 h old hypocotyl ($n=20$); *Theobroma cacao*, 24 h old hypocotyl ($n=15$); *Zea mays*, 36 h old hypocotyl ($n=20$). Dots indicate individual data points, the horizontal bar the median, the + the mean, and the boxes the quartiles around the median. Whiskers display the full range of all values. $n=15$ –25 cells from 3–8 seedlings

LDs and the PM in all species and that SLDP and LIPA are involved in its constitution. Nevertheless, these observations indicate that PM-associated LD distribution during seedling establishment is conserved among angiosperms.

Interestingly, in two species, *H. annuus* and *L. usitatissimum*, we also observed a close proximity of LDs and the storage vacuole (Fig. 3). While the mechanisms resulting in this proximity need to be resolved, these results could point towards another so far unresolved membrane contact site.

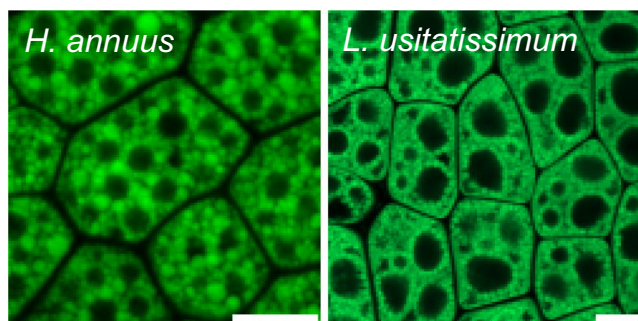


Fig. 3 LDs are arranged around vacuoles in seedlings of *H. annuus* and *L. usitatissimum*. Lipid droplets were stained with Nile red and Bodipy493/503 and imaged as single planes by confocal laser scanning microscopy in cotyledons of 72 h old seedlings of *H. annuus* and 24 h old cotyledons of *L. usitatissimum*. Bars, 10 μ m

SLDPs and LIPAs may have evolved around the devonian age

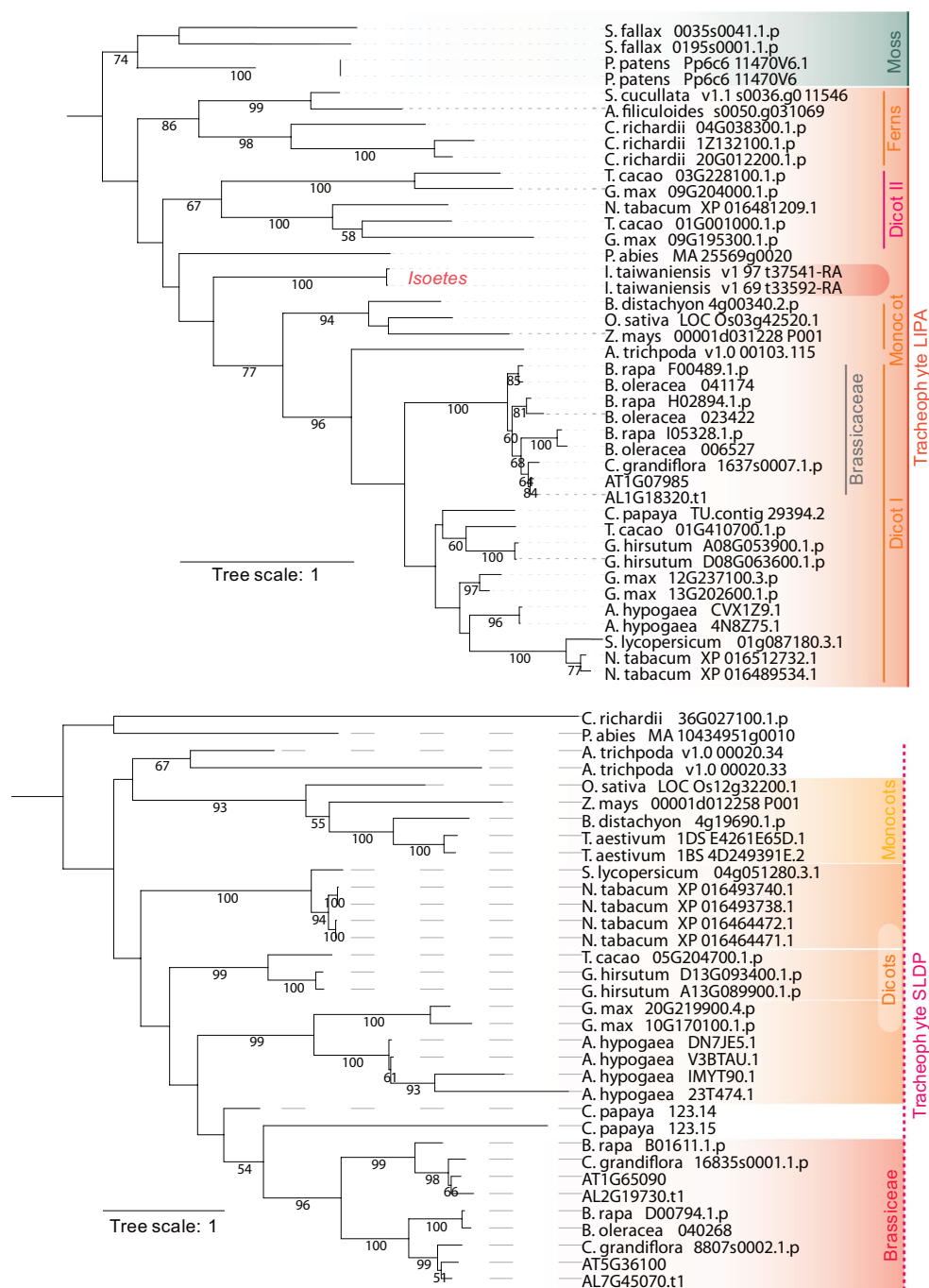
Based on the subcellular distribution of LDs in several species, we reasoned that also here the SLDP/LIPA complex might play a role in constituting this contact site. As of this, we checked in which species homologs of LIPA and SLDP are present. Using angiosperm sequences of LIPA and SLDP as query, we performed BLASTp searches against the predicted proteomes of phylodiverse *Chloroplastida* with a focus on *Streptophyta*. For both proteins, we recovered phylogenies with a low backbone support of their deep genetic structure but well-defined (based on support level and meaningful distribution of the taxonomic affiliation of the sequences) and informative subclades (Fig. 4).

LIPA homologs were detected in mosses, where they formed a medium-supported sub-clade (bootstrap 74). Among tracheophytes, several well supported subclades occurred for ferns (86), the lycophyte *Isoetes* (100), one monocot and two dicot clades to one of which a single *Amborella* sequences branched sister. A single sequence of the gymnosperm *Picea abies* with uncertain phylogenetic placement was detected. SLDP homologs were limited to tracheophytes, with most sequences scattered over largely family-specific sub-clades with high support. Also for the SLDP phylogeny, the backbone support was overall low. Owing to this, the single SLDP detected each for *Picea abies* and the fern *Ceratopteris richardii* had an uncertain placement.

Overall, LIPA and SLDP appear to have emerged early during the evolution of embryophytes and, as far as we can glean from the current data, they occur ubiquitously in seed plants.

Generally, the homologs within the two protein families each follow the same basic predicted architecture (Fig. 5, Supplementary Figs. 1 and 2). In the LIPA proteins, three distinct sequence patterns appear to be conserved. Based on the model, two motifs on the N-terminal region might be part of interaction sites with SLDP according to the AlphaFold 3 model of the SLDP-LIPA dimer and a C-terminal poly-basic region might mitigate the PM targeting. The SLDP models contain a N-terminal hydrophobic region, likely to contain the LD targeting site (Krawczyk et al. 2022a, b). The underlying sequence motif here contains a collection of amino acids with large hydrophobic side chains such as leucine, but also four prolines are strongly conserved as part of a consistent globular structure in AlphaFold 3 models. The AlphaFold 3 model of the dimer indicates that indeed the conserved motifs in the C-terminal part of the protein are involved in the interaction. While these predictions could guide mutagenesis studies in future efforts, in this work we assessed first, if the targeting and the interaction of the SLDP and LIPA homologs from other species are conserved.

Fig. 4 Phylogenetic trees of LIPA and SLDP homolog in vascular plants. Phylogenies were generated using homologs of Angiosperm species for a BLASTp search and significant hits (e value cut-off of 10^{-5} ; in case of larger gene families, we applied a bit score cut-off of 100) were aligned using MAFFT v7.490. Maximum likelihood trees were computed using IQ-Tree v. 1.5.5 with 1000 ultrafast bootstrap pseudo-replicates



SLDP and LIPA homologs show distinct conserved subcellular localisation

To analyse whether the targeting mechanisms of SLDP and LIPA homologs are conserved across species, we made use of the tobacco (*Nicotiana tabacum*) pollen tube system. There is a chance that findings of protein localisation and interaction in this system could differ from protein behaviour in their native surrounding considering the high expression level and different cell type. However, transient

and stable expression systems in seedlings are not always available for all species and the pollen tube system has a number of advantages. This system allows a clear distinction between PM, ER and cytosolic localisation and contains lipid droplets in high number (Müller et al. 2017). In addition, SLDP and LIPA proteins were not detected in the proteome of tobacco pollen tubes (Kretzschmar et al. 2018), enabling the analysis of their localisation following ectopic expression without interference from endogenous interaction partners. Finally, AtLIPA and AtSLDP behaved similar

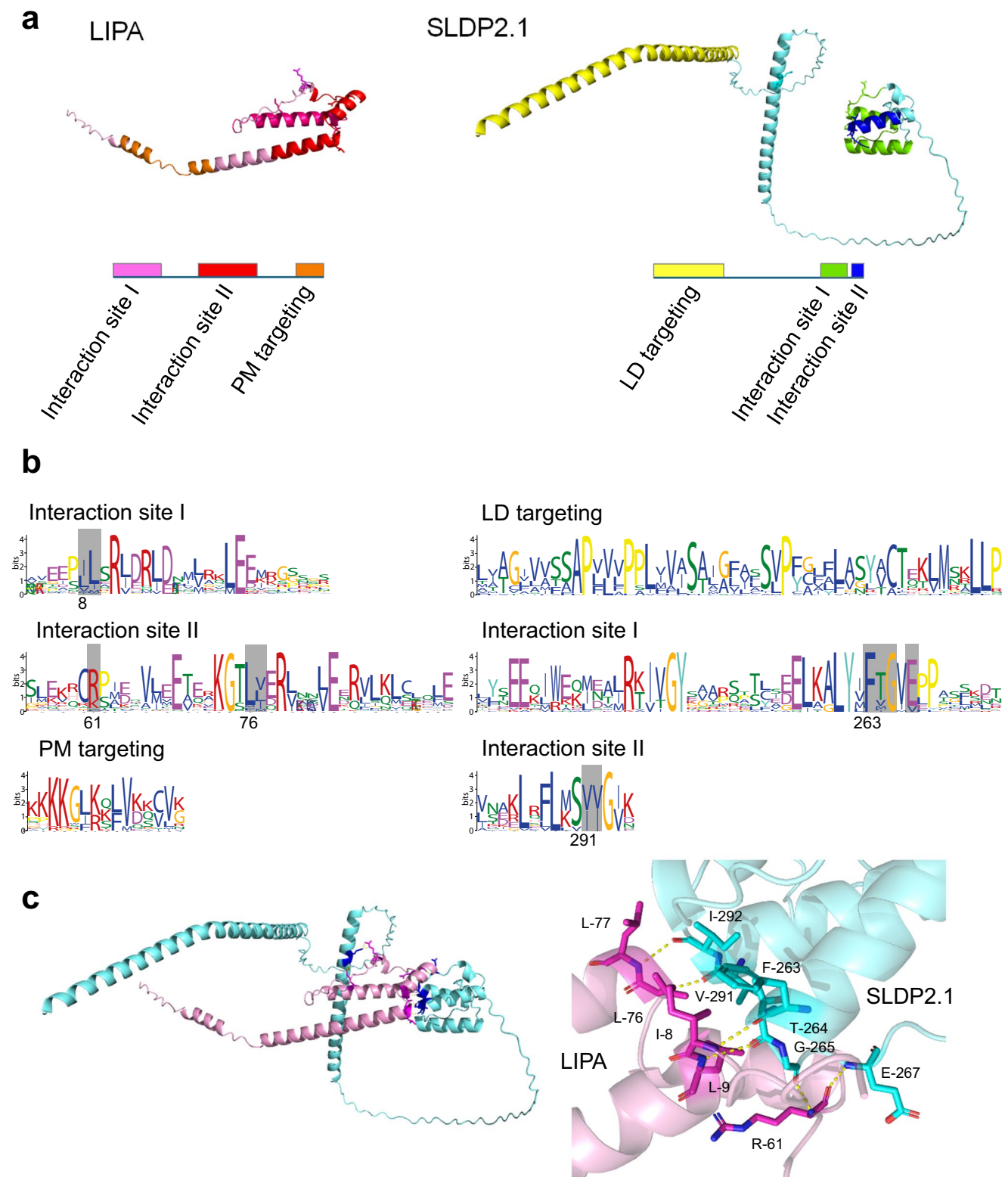


Fig. 5 SLDP and LIPA interaction might be facilitated through conserved residues. AlphaFold models of *AtSLDP2.1* and *AtLIPA* are shown in **(a)**. **(b)** shows the conserved domains of SLDP and LIPA homologs in all vascular plants inferred from the multiple sequence alignment. Higher letters indicate stronger conservation of the amino acid. Numbering in **(b)** refers to numbering in **(c)**. The interaction of

AtSLDP2.1 and *AtLIPA* as predicted by alphaFold is depicted in **(c)** with the polar contact sites of the backbone highlighted with dashed yellow lines. Residues found as polar contacts are underlined in gray in **(b)**. Their in part hydrophobic side chains might also contribute to the interaction

in regard to localisation and interaction in this system in comparison to *A. thaliana* seedlings. Based on these prerequisites, we expressed LIPA and SLDP2.1 homologs from the species *Arachis hypogaea*, *Glycine max* and *Zea mays* in tobacco pollen tubes and compared their localisation to the proteins from *A. thaliana* that were previously explored (Krawczyk et al. 2022a, b).

One of the highly conserved regions in the LIPA homologs in our phylogenetic analysis was the C-terminal polybasic stretch, previously shown to be important for PM association (Krawczyk et al. 2022a, b). Consistent with this, the four tested LIPA homologs all harbour this region and targeted predominantly to the PM as N-terminal fusions to mVenus (Fig. 6). Also, the SLDP targeting appears to be conserved in the different homologs, as all tested SLDP2.1 homologs localised to LDs similar to their *A. thaliana* counterpart (Fig. 6).

The interaction of SLDP and LIPA is conserved in several seed plants

To assess functional interaction, i.e. tethering of LDs to the PM, between SLDP2.1 and LIPA homologs from the three investigated plants, SLDP2.1 and LIPA homologs of each species were co-expressed in tobacco (*N. tabacum*) pollen tubes (Fig. 7). In this system, lipid droplets are highly mobile and are not naturally tethered to the PM, as the endogenous SLDP–LIPA complex is absent (Kretzschmar et al. 2018). This allows direct evaluation of LD tethering and therefore interaction of SLDP and LIPA upon co-expression of the respective proteins (Krawczyk et al. 2022a, b).

In pollen tubes expressing either of these SLDP2.1 or LIPA proteins alone, only around 10% of LDs appear close to the PM. When co-expressing SLDP2.1 homologs from the different species each with the LIPA of the same species, the ratio of LDs close to the PM increased to an average of 20–40% depending on the species.

The next question we asked is if the interaction of SLDP2.1 and LIPA homologs is conserved, and if therefore the proteins from *A. thaliana* can interact with their counterparts from the three other species. Again, we assayed this interaction in the pollen tube system to test if LDs could be localised in higher numbers to the PM (Fig. 8, Supplementary Figs. 3 and 4). In all cases, the expression of a LIPA protein in addition to *At*SLDP2.1 led to a significant increase of LDs close to the PM in comparison to the expression of the *A. thaliana* counterpart alone (Fig. 8a). For the SLDP2.1 homologs when coexpressed with *At*LIPA all but *Zm*SLDP showed a significant increase in PM associated LDs in comparison to the expression of *At*LIPA alone. However, also *Zm*SLDP combined with *At*LIPA showed a trend with a higher maximum rate of LD association than *At*LIPA alone (Fig. 8b).

Discussion

The emergence of seeds has been one of the most defining steps in land plant evolution. The high density of lipid droplets present in these structures may have driven the evolutionary manifestation of the SLDP–LIPA complex and its prominent role in the subcellular organisation of LDs that was described in *A. thaliana* seedling establishment. While we collected evidence here that the SLDP–LIPA complex might also be present in seedlings of other species, evidence for a role outside this tissue are limited. Whereas SLDP homologs are also found in the LD fraction of the oil-rich tubers of yellow nutsedge (Niemeyer et al. 2022), they are not detectable in proteomic data of other high oil tissues like olive mesocarp (Karagiannis et al. 2021). Also, studies on *A. thaliana* leaves and roots, and tobacco pollen tubes did not detect either SLDP or LIPA in the LD fractions. (Brocard et al. 2017; Kretzschmar et al. 2018; Scholz et al. 2025, 2026). In addition, transcript data indicates that in several seed plants such as *Glycine max* or *Zea mays* expression is almost limited to embryos, seeds and seedlings (<https://bar.utoronto.ca/efpsoybean/cgi-bin/efpWeb.cgi>; Libault et al. 2010 and https://bar.utoronto.ca/efp_maize/cgi-bin/efpWeb.cgi; Li et al. 2010). The reason for the more prominent abundance of SLDP and LIPA in seed and seedling tissues remains so far unknown and is linked to the elucidation of the physiological role of the SLDP–LIPA complex.

Furthermore, the presence of LIPA and SLDP proteins or their respective transcripts and genes in certain tissues and species is not a proof of a functional LD–PM contacts site involving these proteins. Nevertheless, it is interesting to probe for the evolutionary roots of these proteins. The overall lack of conservation in most of the peptide sequence makes the search for homologs in non-seed plants more difficult. Especially for SLDP homologs, the size of the peptide increases drastically outside of the seed plant clade, making the small, conserved interaction domain hard to recognise. Still, when specifically searching for the potential interaction domain of SLDP, putative homologs could be identified in *Selaginella moellendorffii*, *Physcomitrium patens* and *Azolla filiculoides*. These putative homologs indeed share striking similarities in their tertiary structure to their seed plant homologs (Supplementary Figs. 1 and 2). This would indicate that SLDP and LIPA may have first emerged around the time when plants started to colonize land. When now looking at the expression levels amongst these homologs in the respective databases PEATmoss (<https://peatmoss.p.lantcode.cup.uni-freiburg.de/>; Fernandez-Pozo et al. 2020), and Selaginella EFP browse (https://bar.utoronto.ca/efp_selaginella/cgi-bin/efpWeb.cgi; Ferrari et al. 2020), expression levels are highest in spore tissues in *Physcomitrium patens* and *Selaginella moellendorffii*. Spores are paralogous to

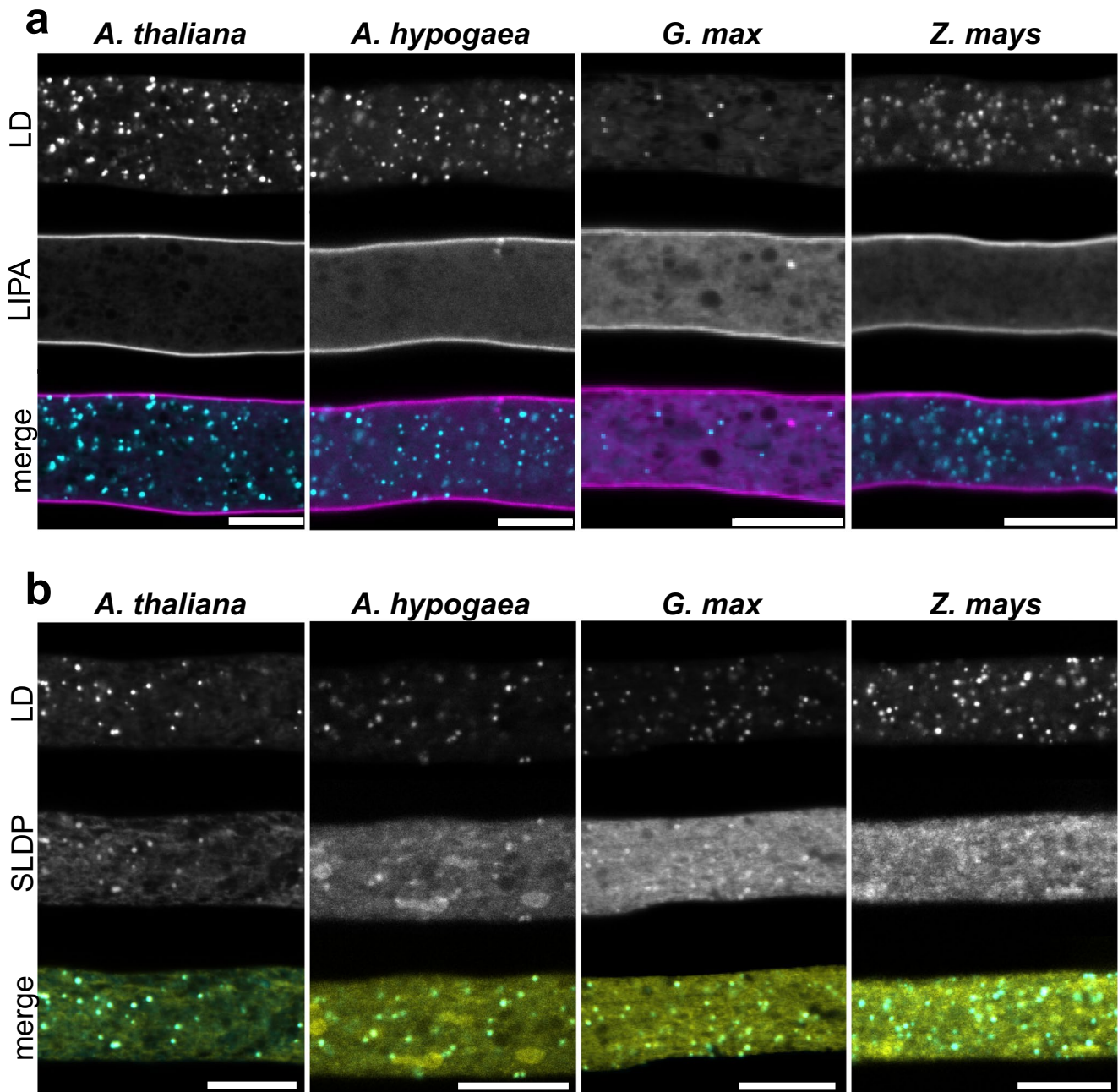


Fig. 6 Ectopically expressed SLDP and LIPA homologs localise at LDs and PM, respectively, in pollen tube cells. N-terminally mVenus-tagged (*A. thaliana* homolog) or mCherry-tagged (all other homologs) LIPA homologs (**a**) and C-terminally mVenus-tagged SLDP homologs (**b**) were expressed in *N. tabacum* pollen tubes. LDs were stained with MDH or BODIPY 493/503 in 6-h-old pollen tubes fixated with form-

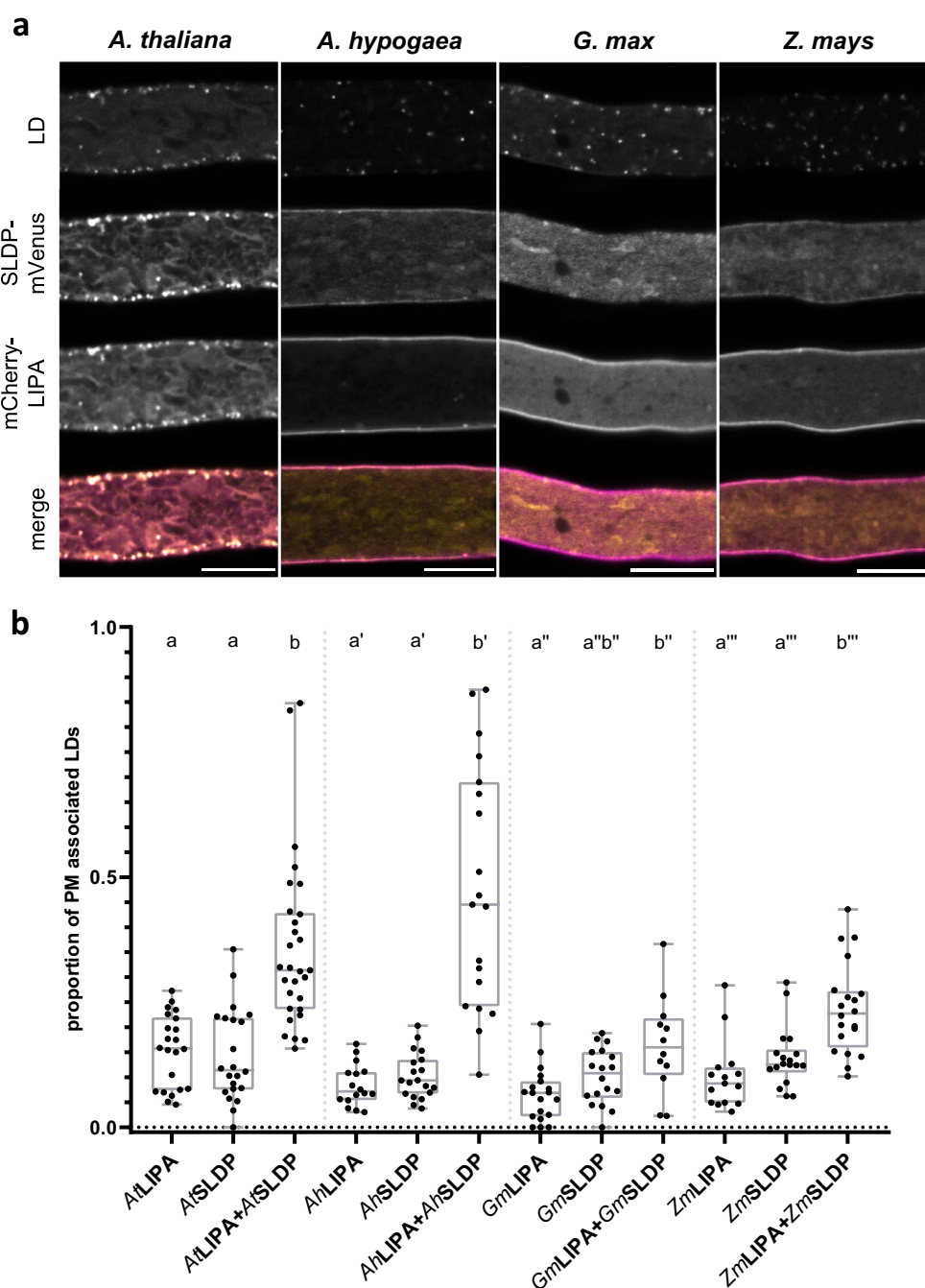
aldehyde prior to confocal microscopy. All SLDP variants (yellow in merge) showed at least partial colocalisation with LDs (cyan in merge) and all LIPA variants (magenta in merge) showed at least partial colocalisation with the plasma membrane but not LDs (cyan in merge). Bars, 10 μm

seeds and do not share the same developmental ancestor, but they still share many features associated with seeds and a similar proteome (Hembach et al. 2024). While these SLDP and LIPA initially may have had a different purpose, the co-occurrence in high-oil perseverance structures strengthens the assumption of an involvement in LD-related processes.

The homologs analysed in this study share a strong conservation in the relevant domains for interaction and targeting, respectively, but have a largely variable region in between (Figs. 4 and 5). The structural conservation is also underscored by the high degree of robustness in the cell biological function, where homologs of different clades of seed

Fig. 7 LIPA and SLDP homologs colocalise, and tether LDs to the plasma membrane of tobacco pollen tubes when co-expressed.

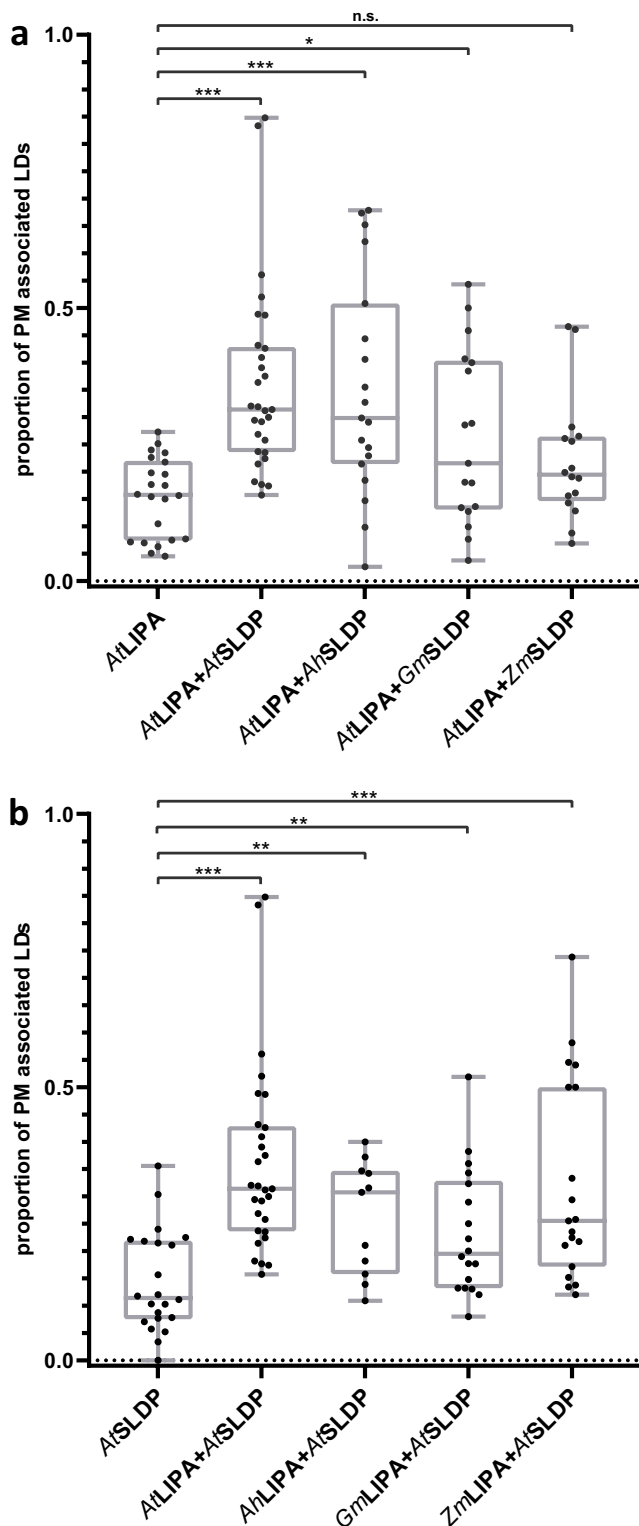
(a) CLSM of species-specific LIPA (magenta in merge) and SLDP (yellow in merge) in transiently transformed and fixated tobacco pollen tubes 6 h post transformation. LDs were stained with MDH (cyan in merge). Scale bars 10 μ m. **(b)** SLDPs and LIPAs of four different species were expressed either alone or together in tobacco pollen tubes. mCherry or mVenus alone were expressed as an additional control. Tobacco pollen tubes were transiently transformed and fixated, and tethering of LDs to the plasma membrane was determined. Dots indicate individual data points, the horizontal bar the median and the boxes the quartiles around the median. Whiskers display the full range of all values. $n = 15$ – 22 . Statistical significance was assessed by a Kruskal-Wallis test and Dunn post-hoc analysis. For each sub-experiment, different letters indicate a significant difference with a p-value smaller than 0.05. *At*, *Arabidopsis thaliana*; *Ah*, *Arachis hypogaea*; *Gm*, *Glycine max*; *Zm*, *Zea mays*



plants can still interact with each other despite the low total sequence identity apart from the interaction domains. Especially, the residues responsible for the interaction as well as the formation of the secondary structures of the interaction domains in AlphaFold 3 models for the *A. thaliana* homologs appear to be highly conserved, making the cross-species interaction possible.

While the subcellular function of the SLDP complex in the formation of the LD-PM is well explored in *A. thaliana*, its physiological role remains unknown, as TAG degradation and seedling establishment was not affected in the

slp double mutant lines under standard laboratory conditions. Our data (Figs. 1, 2, 4 and 5) indicate that SLDPs and LIPAs might contribute to a similar contact site in the seedlings of other species. This is especially interesting, as these species have very different seed architecture and are likely adapted to different environmental conditions during seedling establishment. A presumed existence of the SLDP facilitated LD-PM contact site across seed plants and maybe even non-seed plants therefore point to a fundamental albeit not essential role during seedling establishment or even spore germination. It has been speculated that LDs could



help to maintain plasma membrane integrity during seedling establishment under abiotic stress conditions by supporting membrane remodelling (Scholz et al. 2022). Another possibility could be that a better subcellular ordering of organelles such as LDs could give a small increase in seedling

Fig. 8 LIPA and SLDP homologs tether LDs to the plasma membrane of tobacco pollen tubes when co-expressed with the counterpart from Arabidopsis. **(a)** AtSLDP2.1 was expressed alone or with a LIPA homolog from one of four different species or **(b)** AtLIPA was expressed alone or with a SLDP homolog from one of four different species. Tobacco pollen tubes were transiently transformed and fixated, and tethering of LDs to the plasma membrane was determined. Dots indicate individual data points, the horizontal bar the median and the boxes the quartiles around the median. Whiskers display the full range of all values. $n=12-29$. Statistical significance was assessed using a Kruskal-Wallis test and pairwise Dunn post-hoc analysis. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$. At, *Arabidopsis thaliana*; Ah, *Arachis hypogaea*; Gm, *Glycine max*; Zm, *Zea mays*

fitness that is not measurable in the lab but high enough to keep the integrity of the genetic basis of the protein complex intact across seed plants.

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Author contributions J.D., J.d.V. and T.I. designed the research; P.P., J.D., S.B. and J.d.V. performed research; all authors analyzed data; T.I. wrote the paper with the help of all authors. P.P. and J.D. contributed equally.

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Accessions AtLIPA, At1G07985.1; AtSLDP2.1, At5G36100.1; AhSLDP, Ahy_A09g043970; AhLIPA, Ahy_A08g040645; AzfiLIPA, Azfi_s0050.g031069; AzfiSLDP, Azfi_s0343.g065744; GmSLDP, GLYMA_20G219900; GmLIPA, GLYMA_13G202600; SmLIPA, SELMODRAFT_441210; SmSLDP, SELMODRAFT_446671; ZmSLDP, Zm00001d012258; ZmLIPA, Zm00001d031228.

Declarations

Competing interests The authors declare no competing interests.

Accessions AtLIPA, At1G07985.1; AtSLDP2.1, At5G36100.1; AhSLDP, AhY_A09g043970; AhLIPA, AhY_A08g040645; AzfiLIPA, Azfi_s0050.g031069; AzfiSLDP, Azfi_s0343.g065744; GmSLDP, GLYMA_20G219900; GmLIPA, GLYMA_13G202600; SmLIPA, SELMODRAFT_441210; SmSLDP, SELMODRAFT_446671; ZmSLDP, Zm00001d012258; ZmLIPA, Zm00001d031228.

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