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Evolutionary *PTEN* gene divergence underpins the remodeling of plant vacuolar compartments

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Summary

Membrane fusion and fission are fundamental processes in sustaining cellular compartmentalization. Fission of a lipid bilayer requires a furrow formation that brings membranes in close proximity prior to a contiguous membrane cleavage. Although plant ancestors abandoned cleavage furrow-mediated cytokinesis more than 500 million years ago, here we show that plants still employ this mechanical principle to divide embryonic vacuoles. The evolutionary divergence in PHOSPHATASE AND TENSIN HOMOLOG DELETED ON CHROMOSOME TEN (*PTEN*) enzymes was required to coordinate this process, as *Arabidopsis* loss-of-function *pten2a pten2b* mutants contain hyper compartmentalized embryonic vacuoles. In contrast, *PTEN2* overexpression hinders lytic and secretion cellular pathways downstream of TGN in xylem cells. These processes are critical for the formation of secondary cell walls in xylem cells and depend on a poorly characterized and evolutionarily novel N-terminal domain in *PTEN2*s. The *PTEN2* subfamily appeared with the emergence of the *Phragmoplastophyta* clade, when vacuolar compartments enlarged and cleavage furrow-mediated cytokinesis became extinct. Together, our work suggests that the evolutionary innovation of the *PTEN* family is conserved across terrestrial plants and central to vacuolar remodelling.

Keywords: Vacuole division, cleavage furrow, xylem, cell differentiation, *PTEN*

INTRODUCTION

Cell compartmentalisation is a basic organisational principle of eukaryotic life that separates variety of partitions within the cell to generate multiple different metabolic environments. Communication between endomembrane compartments occurs by a tightly regulated interplay of membrane fission and fusion^{1,2}. Although the molecular players involved in membrane fission can vary across living kingdoms, a common mechanistic requirement is to bring two membranes into close proximity^{1,3}. Generally, this energetically costly membrane bending is followed by a furrow formation at the cleavage site that progresses centripetally until a neck structure is generated on which cleavage proteins can act^{3,4}. The membrane bending requires dynamic changes in its physiochemical properties. For example, the activation of phosphatidylinositol 3-kinase (PtdIns3-kinase) to produce phosphatidylinositol 3,4,5-trisphosphate (PtdIns[3,4,5]P₃) was shown to be a prerequisite for membrane ruffling in mammalian cells as well as for the leading membrane bending during neutrophil cells or amoeba chemotaxis^{5,6}. Membrane relaxation is achieved by PtdIns[3,4,5]P₃ dephosphorylation at the 3' position by PHOSPHATASE AND TENSIN HOMOLOG DELETED ON CHROMOSOME TEN (PTEN) activity from the rear sides of the motile membrane⁷. Interestingly, the phosphoinositides species phosphorylated at 3' positions are scarce in plants, while PtdIns[3,4,5]P₃ has never been detected^{8,9}. Yet, the *Arabidopsis thaliana* (*Arabidopsis*) genome encodes three *PTEN* homologs split in two subfamilies: *PTEN1*, and *PTEN2* comprised of the paralogs *PTEN2a* and *PTEN2b* (*PTEN2s*)¹⁰. The need for expansion of *PTEN* genes in plants is unclear to date.

In the green lineage, cytokinesis mechanism evolved from ancestral centripetal cleavage, still occurring in algal *Streptophyta* clade, to centrifugal fusion model where vesicles fuse to the growing septum membrane called cell plate^{11,12}. Membrane donors here are guided by the evolutionary novel structure – phragmoplast, thus all organisms using this model of cytokinesis are collectively termed *Phragmoplastophyta*, including algal division Charophyta as well as all land plants¹². In addition to discontinuation of a cleavage furrow for cytokinesis, plant cells enlarged their vacuolar compartment in comparison to unicellular green algae in the protist clade¹³. These large compartments became essential for land plant cells' viability as they provide plant hydrostatic skeleton in a form of turgor pressure¹⁴. Additionally, vacuoles display a vast array of cellular functions¹³. Vegetative tissues contain lytic vacuoles, which are acidic

compartments abundant in hydrolases critical for ion homeostasis and lytic degradation¹³. In contrast, protein storage vacuoles (PSVs) accumulate protein reserves that fuel plant development during germination and are characterized by a neutral pH¹³. Recently, *PTEN2a* was implicated in vacuolar trafficking in Arabidopsis¹⁵ - consistent with the reported PTEN2s substrate preference to be PtdIns3P, typically residing in membranes of lytic compartments^{9,10,15}. The latter poses the question whether plant *PTEN2s* may have a role in remodelling vacuolar membrane (tonoplast) instead of the plasma membrane as reported in other eukaryotes. However, how voluminous vacuolar compartments divide in plants is still a matter of debate¹⁶. Recent 3D models of vacuoles suggested that the compartments that appear fragmented in 2D images are actually not physically separated but rather form a tubular network¹⁷⁻¹⁹. Thus, many previously reported examples of a vacuole fragmentation have to be revalidated in order to gain better understanding of vacuolar membrane dynamics. Here, we followed the stepwise conversion of large embryonic vacuoles (EVs) into smaller PSVs in Arabidopsis. Our 3D reconstructions of EV remodelling revealed vacuolar division by a process morphologically resembling the progressing cleavage furrow division. Moreover, we show that PTEN2 enzymes are essential to coordinate membrane tubularization at EV division initiation sites and cleavage furrow progression. In *pten2a pten2b* double mutants EVs become hyper-compartmentalized instead of fragmented. On the contrary, overexpression of *PTEN2s* prevents the fusion of trans-Golgi network (TGN)-derived small vacuoles to the central vacuole that does not enlarge but stays tubular. This phenomenon was cell type specific and predominantly occurring in xylem tissues. Aberrant cell trafficking affected both vacuolar and secretory pathways, essential for xylem tissue maturation. Notably, *PTEN2* function in remodelling vacuolar architecture depends on their poorly characterized N-terminal domain, that evolutionarily appeared in the *Phragmoplastophyta* clade, coinciding with vacuolar enlargement and loss of cleavage furrow-mediated cytokinesis²⁰. Thus, it seems plausible that *PTEN2s* evolved to provide molecular support to preserve this ancient model of membrane fission to modulate the biggest plant cell compartment - the vacuole.

RESULTS

Embryonic vacuole division involves cleavage furrow formation and requires PTEN2 activity

During plant embryogenesis, EV become transformed into numerous small PSVs prior seed desiccation^{21,22}. To assess whether this process entails vacuolar fragmentation, we followed the dynamics of previously described vacuole marker *TONOPLAST INTRINSIC PROTEIN (TIP) 3;2* during Arabidopsis embryo development. Expressed from a native *TIP3;2* promoter fragment, this marker is detectable from the late heart or early torpedo stage of Arabidopsis embryogenesis onwards. *TIP3;2*-GFP first accumulates in the endoplasmic reticulum (ER) around the nucleus and near the plasma membrane, similar to the V-PPase VHP1/AVP1 tonoplast marker at the same stage (stage I in Fig. 1a Extended Data Fig. 1a). In succeeding stages of embryo development, the first pre-EV can be observed in addition to the ER signal (stage II). Next, the *TIP3;2* accumulation becomes restricted to the tonoplast of pre-EVs that undergo fusion, evident by the hollowed spaces bridging two vacuoles at the fusion site (stage III in Fig. 1a and Extended Data Fig. 1b). As a result of these homotypic fusions, larger EVs are generated (stage IV in Fig. 1a), followed by the onset of the typical PSV autofluorescence in the successive stages. Next, the large EV starts dividing, evidenced by the symmetrical tonoplast invaginations towards the vacuolar lumen (stage V in Fig. 1a). By performing 3D reconstructions based on maximal projections and surface rendering we could observe formation of a cleavage furrow-like structure in the region where the tonoplast is contracting around the incipient separation site (Fig. 1b). As one EV splits in multiple PSVs, various cleavage progression stages can be observed in a single EV. Stage VI is characterized by the final fragmentation of EV into multiple PSVs (Fig. 1a). During the process of EV division in some samples we noticed the presence of small vacuoles that may be the membrane source necessary for tonoplast invagination during furrowing or suggest an additional alternative pathway of vacuole fragmentation (Extended Data Fig. 1c).

To determine the molecular mechanisms underpinning embryonic vacuolar division in plants, we decided first to explore whether PtdIns3P metabolic enzymes are involved in the regulation of this process. In budding yeast, a local enrichment of PtdIns3P at the neck occurs before membrane fission and it is required to stabilize membrane

invaginations²³. Detailed examination of *pten2a pten2b* mutants revealed a crucial difference in the progression of EV division in stage V in comparison to wild type embryos (Fig. 1a). In *pten2a pten2b* embryos the tonoplast inward invaginations are not coordinated, and often asymmetric unilateral. The invaginated membrane can be seen in 3D as individual sheets growing inwards; and even when these sheets meet the other side of the vacuole, a ring-shaped cleavage furrow-like structure will be absent or incomplete (Fig. 1b). The membrane sheet would in later stages roll in on itself and form a cylindrical shape, or sometimes a sphere that may eventually pinch off. At the end, instead of fragmented, EVs of *pten2a pten2b* mutants appear hyper-compartmentalized with their vacuolar lumen crisscrossed with several membranes (stage VI in Fig. 1a). During seedling germination, the homotypic fusion of PSVs (stage VII in Fig. 1a and 1c) generates the central lytic vacuolar compartment, a process that can be scored by the gradual disappearance of the typical PSV autofluorescence by 48h after seed imbibition. Surprisingly, lytic vacuole formation appeared unaffected in *pten2a pten2b* double mutants, although mutant seedlings germinated slightly faster than wild type (Fig. 1d and Extended Data Fig. 1d). Yet, this process does not translate in a faster post-embryonic growth, as manifested by a similar root growth and underlying meristematic activity (Fig. 1e-f).

PTEN2s overexpression prevents xylem cell differentiation

To further elucidate the potential role of PTEN2s in vacuolar remodeling and its effects on plant development, we decided to analyze the impact of altered *PTEN2* levels. As, the constitutive *PTEN2b* overexpression from UBQ10 promoter was lethal, we employed the estradiol inducible system to assess its short-term overexpression effects in relevant cell types (Extended Data Fig. 1e). During germination, the consumption of PSV reserves was proposed to occur primarily in vascular cells, most likely to allow for the development of a functional vascular system before the amino acids are mobilized from other parts of the plant body^{24,25}. Hence, we focused on xylem cells. To become conductive units, xylem cells undergo a complex developmental process that encompasses the reinforcement of the cell wall (SCW) and a vacuolar-driven programmed cell death (PCD)^{26,27}. The formation of fully differentiated xylem cells with lignin-reinforced SCWs can already be observed 48h after seed imbibition (Extended Data Fig. 1f). While *pten2a pten2b* double mutants showed neither

acceleration nor delay in xylem vessel differentiation, seedlings overexpressing *PTEN2b* showed significant defects in xylem maturation (Extended Data Fig. 1f). Interestingly, the xylem differentiation inhibitory effect had only overexpression of *PTEN2* paralogs but not *PTEN1* isoform (Fig 2a-b), suggesting a functional evolutionary divergence between orthologs. In the further text we will mainly focus on *PTEN2b* overexpressing lines (*PTEN2b_{ox}*), as the results obtained in either *PTEN2a* or *PTEN2b* overexpressing lines were very redundant. The phenotypical effects of *PTEN2_{ox}* were dosage dependent and sensitive to the duration of induction (Extended Data Fig. 1e and 1g-h). The induction with a lower estradiol concentration (0.2 μ M) for shorter time (24-48h) did not affect much the overall root growth, but inhibited xylem differentiation completely or allowed only individual cells to differentiate (xylem islands) (Fig 2b). Higher expression detected in independent transgenic lines or achieved by estradiol induction in higher concentrations (2 μ M) significantly shortened the root length but reverted xylem phenotypes almost to normal (Extended Data Fig. 1e and 1g-h). The latter was scored mainly in distal root parts where the tissue was longer exposed to *PTEN2b* induction agent prior differentiation.

Next, we assessed if xylem differentiation in *PTEN2b_{ox}* was only delayed or lastingly inhibited by analyzing the expression of known xylem markers associated with xylem maturation. The expression of the protoxylem master regulator *VASCULAR RELATED NAC-DOMAIN PROTEIN 7 (VND7)* and its downstream target gene *MYB DOMAIN PROTEIN 46 (MYB46)* in seemingly non-differentiated xylem cells in *PTEN2b_{ox}* validated that these cells committed to the xylem cell fate (Fig. 2c). Moreover, the expression of secondary cellulose synthetic machinery subunits as well as PCD-associated enzymes as a hallmark of xylem cell maturation can be detected in *PTEN2b_{ox}* (Fig.2c and Extended Data Fig. 2a). These findings demonstrated that although the xylem cells reached their transcriptional maturity, they failed to lay down SCWs as confirmed by transmission electron microscopy (Fig. 2d). Notably, xylem cells failing to form SCWs contained vacuoles of altered morphology compared to wild type (Fig. 2d), implying correlation between the SCW formation and vacuolar morphology in xylem cells.

PTEN2b overexpression modulates vacuolar and secretory vesicular trafficking of xylem cells

Despite its key role in protoxylem differentiation²⁷, very little is known about xylem vacuolar biogenesis, mostly due to its relatively deep positioning within a root. Hence, we decided first to reassess vacuolar biogenesis in protoxylem developing cells by monitoring VHP1-GFP dynamics. Live-cell imaging, followed by 3D image reconstruction revealed the formation of elongated tubular structures in dividing meristematic cells (Fig. 3a). The subsequent enlargement of tubular compartments results from the fusion of small rounded vacuoles, giving rise to two large vacuolar compartments connected by a narrow tubular connection. *PTEN2_{s_{ox}}* effectively abolished the fusion of small vacuoles to the elongated tubular vacuole (Fig. 3b-c). Surprisingly, neither *PTEN2_{a_{ox}}* nor *PTEN2_{b_{ox}}* affected vacuolar morphology in root ground tissues (Fig. 3c). The latter suggested that xylem cells require a cell type specific vacuolar regulation. To corroborate the prevention of the xylem tubular vacuole enlargement and its globularization in *PTEN2_{s_{ox}}*, we assessed the trafficking pathways summarized in Figure 4a. Similar to VHP1, VHA-a3 was present on the membranes of both tubular and small vacuoles in *PTEN2_{b_{ox}}* suggesting that the direct ER-to-vacuole trafficking pathway is functional (Fig. 4b). Next, we aimed to assess TGN-dependent vesicle delivery to the vacuole by analyzing an artificial cargo ToIM²⁸ comprising a soluble vacuolar sorted part RFP_{AFVY} and GFP retained in the cytoplasm. Overexpression of *PTEN2b* precluded the loading of RFP_{AFVY} into the vacuole, as well as other cargos known to be transported to the vacuole (Fig. 4c-e and Extended Data Fig. 4a). Together, these observations indicated that *PTEN2b* regulates vesicle trafficking from TGN to the vacuole. This result was consistent with the previously reported *PTEN2a* localization at TGN¹⁵. Similarly, *PTEN2b* exhibits an early colocalization with membrane tracer FM4-64 and TGN marker VHA-a1 (Extended Data Fig. 4b-c). However, failed vacuolar delivery of these cargos cannot directly explain the inability of xylem cells to build a SCW. With the onset of SCW synthesis, the primary cell wall CELLULOSE SYNTHASE (CESA) enzyme complexes ceased to be delivered to the plasma membrane and are gradually removed²⁹. As CESA6 is a primary cell wall CESA subunit, it may be plausible to hypothesize that the sequestering of this subunit in the vacuole is critical for the secondary cellulose synthase complex to assemble and/or be active. Although CESA6 colocalized with

PTEN2b in discrete punctae, *PTEN2b* overexpression still abolished the xylem SCW formation in *cesa6* genetic background (Extended Data Fig. 4d-e), invalidating our hypothesis. SCW formation however also depends on the cellular secretion pathway as hemicellulose, lignin monomers and biosynthetic enzymes must be delivered to the apoplast before crosslinking³⁰⁻³². For example, LACCASE 17 (LAC17) is an enzyme essential for lignin biosynthesis that in wild type plants can be found in the apoplast following the SCW spiral pattern³³. Surprisingly, in *PTEN2b_{ox}* LAC17 was not secreted, but rather retained inside the cell forming aggregates between the vacuoles (Fig. 4f-g), explaining the lack of lignin formation as a part of xylem SCW in *PTEN2b_{ox}*. Although the vacuolar and secretion trafficking pathways may converge at multi-vesicular-bodies (MVBs), CESA6 and LAC17 aggregates created in *PTEN2b_{ox}* did not always colocalize (Extended Data Fig. 4f). The latter suggests that *PTEN2b* can affect MVBs (as confirmed by Rha1 marker line) but also it may affect TGN downstream pathways at different levels (Extended Data Fig. 4g). In stronger *PTEN2b_{ox}* we even occasionally noticed in epidermal cells RABG3f (RAB7 GTPase HOMOLOG) positive aggregates in a grape-like structures seemingly unable to fuse (Extended Data Fig. 4h), further supporting our findings in xylem tissue.

Since vacuolar-driven PCD is the final step of xylem tissue maturation, we evaluated PCD execution in xylem cells upon *PTEN2b* overexpression. Remarkably, PCD execution can still be detected in cells incapable of forming SCW, as manifested by the absence of organelles such as the nuclei (Fig. 4h). Xylem cells incapable to form SCW ultimately collapse, as seen in orthogonal sections stained with toluidine blue and transmission electron microscopy images (Fig. 4i-j).

Interestingly, a sharp quenching of YFP signal in comparison to mCHERRY could be detected prior to PCD execution (Fig. 4k-l). The different pH fluorescence of YFP and mCHERRY proteins³⁴ may be accounted for this phenomenon, suggesting timed vacuolar acidification only in the last steps of xylem differentiation. The visibility of YFP fluorophore inside xylem vacuoles suggests that these vacuoles have a milder pH³⁵, similar to storage vacuoles. Hence, this phenomenon may explain the cell-specificity of *PTEN2s* action on vacuoles in xylem tissues but not in other cell types (Fig.3c).

Together, our results showed that *PTEN2s* overexpression restricts tubular xylem vacuoles from enlarging. This phenotype is opposite to the phenomenon occurring during EV division, where even membrane tubularization is essential to create a furrow surrounding the incipient cleavage site (Extended Data Fig. 4i).

***PTEN2* function was conserved through evolution before vascular plants appearance**

Contrary to *PTEN2*s, overexpression of *PTEN1* did not affect vacuolar formation nor xylem differentiation (Figs. 2a-b and 3b-c). This observation raised the question whether the duplication of PTEN enzymes and their vacuolar remodeling function was an evolutionary prerequisite that contributed to the emergence of vascular plants (Fig. 5a). To answer this question, we applied a phylogenomic strategy for identifying orthologs in the green lineage. We blasted Arabidopsis *PTEN2b* full-length amino acid sequence against proteome assemblies of 142 plant species from chlorophytes to angiosperms (Fig. 5b, Supplementary Table 1). Detailed analysis revealed that PTEN enzymes from the green lineage can be divided into 3 subfamilies: algal PTEN, PTEN1 and PTEN2 (Fig. 5b). Algal PTEN subfamily includes *Chlamydomonas reinhardtii* PTEN (CrPTEN) that interestingly clusters with the referent human (HsPTEN) isoform. PTEN1 subfamily contains previously mentioned Arabidopsis PTEN1 (AtPTEN1), while PTEN2 subfamily clusters Arabidopsis isoforms (AtPTEN2a and AtPTEN2b) with *Marchantia polymorpha* PTEN2 isoform (MpPTEN2). The divergence of the *PTEN2* gene subfamily could be traced back to the origin of the *Phragmoplastophyta* clade (Fig. 5a) as confirmed by the absence of xylem and vacuolar phenotypes when overexpressing *CrPTEN* in Arabidopsis seedlings (Fig. 5c-d). Moreover, we observed that *PTEN2* genes conserved their functions even in the non-vascular plant *Marchantia* as the effect of overexpressing *MpPTEN2* mimicked *AtPTEN2b* overexpression. These observations suggest that vacuolar remodeling and xylem differentiation are *PTEN2*-specific functions that remained highly conserved across land plants despite hundreds of millions of years of evolution³⁶.

PTEN2 function lies in its N-terminal domain that determines its subcellular localization

Cross-examination of PTEN sequences from the three subfamilies revealed extended N-terminal and C-terminal sequences in PTEN2s compared to other two subfamilies (Fig. 6a). To test whether these sequences may explain PTEN2s functional divergence from other PTEN subfamilies, we overexpressed N-terminal and C-terminal truncated versions of AtPTEN2b (*PTEN2b*^{ΔNter} and *PTEN2b*^{ΔCter}, respectively). Deletion of

PTEN2b C-terminal sequence did not alter PTEN2b -dependent suppression of xylem continuity, whereas the lack of N-terminal sequence (*PTEN2b ΔNter*) inhibits this enzyme's ability to impair xylem development (Fig. 6b) or vacuolar biogenesis (Fig. 6c). Furthermore, we were able to pinpoint the functional necessity of 57 AA of PTEN2 N-terminal domain (*PTEN2b^{Δ1-131}*) upstream of its phosphatase catalytic domain (Fig. 6a-b). This conserved N-terminal part of PTEN2s makes it less hydrophobic in comparison to N-terminal of PTEN1, supporting the functional divergence between orthologues (Extended Data Fig. 6a-b). As expected, the N-terminals swapping from PTEN1 to PTEN2b (N1-PTEN2b) abolished PTEN2b function (Fig. 6b), confirming the functional specificity of PTEN2 N-terminus. Further *in silico* mining pointed out an enrichment in intrinsically disordered domains in the PTEN2 N-terminus suggesting the importance of macromolecular interaction partners in achieving stable PTEN2 three-dimensional structure (Extended Data Fig. 6c). Notably, the PTEN2b variants lacking complete (*PTEN2b^{ΔNter}*) or partial N-terminus (*PTEN2b^{Δ1-131}*) could not properly localize to TGN (Fig. 6d and Extended Data Fig. 6d-f), where in addition to the cytosol PTEN2s normally accumulate¹⁵(Extended Data Fig 4c). PTEN1 isoform, shown to be restricted to pollen grains³⁷, when expressed from the *PTEN2b* promoter cannot be detected in *PTEN2b* expressing tissues. Here it could be speculated that the long PTEN2 N-terminus (not present in PTEN1) is important not only for TGN localization but also for protein stabilization (Fig. 6d). Hence, it appears possible that PTEN2b Nter-mediated TGN anchoring is critical for PTEN2 functionality in vacuolar remodeling and contribute to the evolution of land plants

DISCUSSION

Eukaryotic cell compartmentalization occurred evolutionary concomitant with cell enlargement as the plasma membrane surface was not sufficient to provide all membrane-dependent functions³⁸. There are different hypotheses explaining the origin of different membrane-bound organelles such as the endosymbiotic origin of mitochondria and chloroplasts, *de novo* formation of peroxisomes or transformation of existing endomembrane structures into new ones³⁹⁻⁴¹. PSV formation represents an example when an existing compartment is remodeled into an organelle with a different function²². Yet, very little is known about the mechanisms underpinning this functional reprogramming process. Here we showed a PTEN2-mediated mechanisms by which numerous PSVs are formed by the fragmentation of EVs following a cleavage furrow-resembling mechanism (Fig. 1). Without PTEN2 enzymes the EV becomes hyper compartmentalized instead of fragmented. Interestingly, the observed EV membrane invaginations in *pten2a pten2b* mutants resemble formation of the mitochondrial cristae or chloroplast thylakoid membranes especially when the membrane is seen as pinched off (Fig. 1b)^{38,41,42}.

As a typical cleavage furrow requires cytoskeleton involvement, it is tempting to speculate the importance of the cytoskeleton during vacuole division. Although actin was reported to be important in lytic vacuole tubularization, it is not clear if actin prevents vacuole expansion by physical constriction or by preventing actin-dependent membrane delivery to the vacuole⁴³. Notably, vacuole invaginations can occur in a cytoskeleton independent fashion as reported during microautophagy in yeast⁴⁴. Moreover, the dynamics of contractile vacuoles present in protists depends rather on membrane tethering complexes than on the activity of cytoskeletal elements^{45,46}. Consistent with the coupled occurrence of fusion and fission events in membrane homeostasis, our work revealed a *PTEN2*-mediated effect on vesicle fusion in xylem cells (Fig. 4). Inducible overexpression of *PTEN2*s potentiated tubular vacuole structures by preventing small vacuoles to fuse with it (arrows in Fig. 3b). Vacuole tubularization (as the most extreme form of membrane bending) is actually a core phenomenon necessary to form a cleavage furrow-like structure during EV division (Extended Data Fig. 4i). In *pten2a pten2b* mutants, vacuolar fission is hindered by the failure to form a symmetric ring of tonoplast invaginations at the division site (Fig. 1b). Coincidentally or not, plant *PTEN2*s appeared exactly in the

Phragmoplastophyta clade and diverged their function from ancestral PTENs with the loss of cleavage furrow as cytokinesis mechanism⁴⁷.

PTEN2s overexpressing lines provided a critical genetic tool to research the cell type specificities of vesicle trafficking in a developmental context. In this study we focused on the cell trafficking during xylem cell differentiation into a water conducting unit. We showed that *PTEN2b_{ox}* prevents SCW formation in xylem cells, partially by inhibiting LAC17 secretion to the apoplast. As mutations in hemicellulose biosynthetic genes translate into xylem phenotypes, a potential suppressed delivery of hemicellulose to the apoplast may explain the lack of SCW cellulose in xylem cells upon *PTEN2b_{ox}* induction⁴⁸⁻⁵⁰. Furthermore, our current knowledge about xylem formation indicates that concomitant with SCW formation, hydrolytic enzymes necessary for PCD execution are loaded into the vacuole. Vacuoles store these enzymes in an inactive form until SCW formation is completed, ensuring the correct timing of PCD execution⁵¹. Our observations indicated that vacuolar acidification occurs just prior to PCD execution (Fig. 4k), suggesting a mechanism for the activation of hydrolytic enzymes. Subsequently, the vacuole swells, the tonoplast's permeability changes and finally the vacuole collapses releasing its content into the cytoplasm^{52,53}. This process is thought to trigger a rapid cytoplasmic content degradation⁵⁴. However, we showed that PCD-associated genes are correctly expressed and that protoxylem cells undergo cell death in the absence of SCW formation and without the formation of a large central vacuole, contrary to the expected sequence of xylem differentiation events⁵⁵. Remarkably, previous studies reported autophagy as responsible for the gradual cellular content hydrolysis and reduced cytoplasmic density observed during the SCW biosynthesis^{53,56}. Thus, autophagy may be an alternative mechanism for xylem cell clearance when vacuole-mediated pathway is inhibited. Indeed, we observed the creation of multiple cup-shaped vesicular structures resembling phagophore upon high *PTEN2a* induction (Fig. 4d) as well as that RabG3f positive small vacuoles creating grape-like aggregates (Extended Data Fig. 4h). It has been reported that another member of the same RabG3 subfamily, RabG3b can either stimulate or inhibit both autophagy and xylem formation, depending on its activation status⁵⁷. Interestingly, the autophagy resembling pathway occurs also when massive amounts of synthesized proteins have to be delivered to the vacuole, as it occurs for seed storage proteins^{58,59}. Similarly, some proteases necessary to mobilize the PSV content during germination was shown to also skip Golgi/TGN and directly from ER translocate

to the vacuole^{60,61}. Utilization of this direct ER-to-vacuole pathway may explain the absence of germination defects in *pten2a pten2b* seedlings incapable to form conventional PSVs. This notion is supported by our result that the direct ER-to-vacuole trafficking route remains unaffected in *PTEN2b_{ox}*, evident by the presence of VHP1 and VHA-a3 in xylem vacuolar membranes (Fig. 3b and 4b)¹⁸. Further investigation is needed to elucidate the exact downstream players in PTEN2s signaling cascade, and distinguish the biological importance of its dual phosphatase activity¹⁰, especially in the new light of its evolutionarily novel N-terminal. However, it is tempting to speculate that the *PTEN2* gene family diverged in green lineage to control vacuolar morphology and dynamics as their emergence coincided with vacuole enlargement during evolution.

METHODS

Plant materials and growth conditions

Arabidopsis thaliana ecotype *Columbia-0* (*Col-0*) was used as wild-type control in all cases. Seeds of *pten2a* (SALK_114721), *pten2b* (SALK_120020) and *cesa6* (SALK_004587) were obtained from the Nottingham Arabidopsis Stock Centre and combined by crossing. Homozygous lines were selected by genotyping using the primers listed in Supplementary Table 2. The following transgenic lines used in this study were described elsewhere: *MYB46::GFP*⁶², *DMP4::H2A-GFP*⁶³, *EXI1::H2A-GFP*⁶³, *PASPA3::H2A-GFP*⁶³, *RNS3::H2A-GFP*⁶³, *SCPL48::H2A-GFP*⁶³, *PASPA3::ToIM*²⁸, *VHA-a3::VHA-a3-GFP*¹⁸, *VHP1::VHP1-GFP*⁶⁴, *VHA-a1::VHA-a1-RFP*⁶⁵, *CESA6::YFP-CESA6*⁶⁶, *LAC17::LAC17-mCHERRY*³³, *BRI1::BRI1-mCITRINE*⁶⁷, *UBQ::Rha1-YFP (W7Y)*⁶⁸, *UBQ::RabG3f-YFP (W5Y)*⁶⁸. These lines were combined with mutants or other transgenics by crossing. Following constructs (detailed information in Supplementary Table 3) were generated in this study: *TIP3;2::TIP3;2-GFP*, *UBQ::XVE::PTEN1*, *UBQ::XVE::PTEN2a*, *UBQ::XVE::PTEN2b*, *VND7::NLS-3xVENUS*, *CESA7::NLS-3xVENUS*, *CESA4::NLS-3xVENUS*, *CESA8::NLS-3xVENUS*, *XCP1::XCP1-mCHERRY*, *BFN1::NLS-dtTOMATO*, *PTEN2b::PTEN2b-CITRINE*, *PTEN2b::PTEN2b-mCHERRY*, *UBQ::XVE::CrPTEN*, *UBQ::XVE::MpPTEN2*, *UBQ::XVE::PTEN2b^{ΔCter}*, *UBQ::XVE::PTEN2b^{ΔNter}*, *UBQ::XVE::PTEN2b^{Δ1-131}*, *UBQ::XVE::N1-PTEN2b*, *PTEN2b::PTEN2b^{ΔNter} - CITRINE*, *PTEN2b::PTEN2b^{Δ1-131} -CITRINE}*, *PTEN2b::PTEN1-CITRINE*. Constructs were transformed into *Col-0*, *pten2a* *pten2b* and marker lines (unless indicated) using *Agrobacterium*-mediated floral dip transformation according to standard procedures. For *in vitro* growth, seeds were surfaced sterilized, stratified 2 days at 4°C and grown on 0.5 x Murashige and Skoog (MS, Duchefa) medium with MES buffer, pH 5.7, 0.7% agar, and 1% sucrose. Seedlings were grown in vertical plates under continuous light conditions. The estradiol inducible lines were either germinated or transferred to identical media (48h treatments) containing 0.2 μM or 2μM estradiol (ES, Sigma Aldrich). Seedlings were analyzed six days after germination unless specified otherwise.

Cloning procedures

To generate *pPROMOTER::NLS-3xVENUS* reporter lines, the genomic region upstream the ATG of *VND7* (1596bp), *CESA7* (1153bp), *CESA4* (1939bp), *CESA8* (1949bp), or *BFN1* (1975bp) was PCR-amplified using the primers listed in Supplementary Table 2. The resulting fragments were cloned into *pDONRPr-P1r* (Gateway) and subsequently recombined together with a *pENzeo-NLS-3xVENUS*⁶⁹ plasmid into *EDO097pFR7m24G*⁷⁰. Entry clone with *BFN1* promoter was recombined together with *pEN-L1-NLS-tdTOMATO-L2* (Gateway) into destination vector *pK7m24GW2* (Gateway) plasmid following the manufacturer instructions (Gateway, Invitrogen).

TIP3;2::TIP3;2-GFP line was generated by synthesizing *TIP3;2* coding genomic sequence together with 845bp of promoter region upstream of ATG (as found in The Arabidopsis Information Resource Platform) and cloned into *pDONRP4-P1r*. Obtained entry clone was recombined with *pEN-L1-GFP-L2* into *EDO097pFR7m24G*⁷⁰.

Protein overexpression was achieved by estradiol XVE system (Gateway plasmid *pMDC7*⁷¹). Coding sequences of *PTEN1*, *PTEN2a* and *PTEN2b* were amplified from Arabidopsis genomic DNA. *MpPTEN2* (Mapoly0016s0179) was PCR amplified from Marchantia cDNA. *CrPTEN* (Cre06.g308400) was in vitro synthesized (Invitrogen). DNA amplicons containing attB1-B2 sites were and recombined into *pMDC7* via *pEN207* (*PTEN1* and *PTEN2b*) or *pEN221* (*MpPTEN2* and *CrPTEN*) Gateway plasmids. *PTEN2a* was firstly cloned into *p17ACCD2P*, a plasmid created in this study. *p17ACCD2P* contains multicloning restriction sites: 5'- GAA TTC GAA GCT CGG TAC CCG GGG ATC CTC TAG AGT CGA CCT GCA GGC CCA TGG TGA CTA GTC AAG CTT – 3' between attL1 and attL2 Gateway recombination sites, thus providing direct creation of an entry clone without BP reaction.

Translational reporters were created by amplifying promoter regions of: *PTEN2a* (1116bp), *PTEN2b* (1173bp) and *XCP1* (1601bp) and cloned into *pDONRPr-P1r*. Coding regions were amplified from whole seedling cDNA (for *PTEN2a*, *PTEN2b* and *XCP1*) or pollen enriched cDNA (*PTEN1*). Entry clones were made using *pDONR207* except for *PTEN2a*, where *p17ACCD2P* was used. Final constructs were generated by recombining the entry clones into *pH7m34GW*. Similarly, the distinct *PTEN2b* protein variants (*PTEN2b*^{ΔNter}, *PTEN2b*^{ΔCter}, *PTEN2b*^{Δ1-131} and *N1-PTEN2b*) were cloned in frame with CITRINE by LR recombination into *pH7m34GW* or for overexpression into *pMDC7*. Primers used for cloning can be found in Supplementary Table 2.

Histological analysis

PSV biogenesis was visualized in epidermal cells of Arabidopsis embryos extracted from green siliques (stages I-III), yellow siliques (stages IV-V), dry seeds after 4h imbibition in water (stage VI) or 24-48h after imbibition in constant light conditions (stages VII-VIII). Cellulose (by Calcofluor White from Sigma-Aldrich) and lignin staining (Fuchsin from Sigma-Aldrich) were performed after seedlings clearing following ClearSee protocol as previously described⁷². DAPI (4',6-diamidino-2-phenylindole, Sigma-Aldrich) staining used to verify PCD status of xylem cells was performed after fuchsin staining when seedlings were exposed to 50 µg/ml DAPI in 1x PBS (phosphate-buffered saline) with 1% TRITON-X for 1h with gentle shaking. After thorough washes in 1x PBS, seedlings were visualized with a confocal microscope. Live imaging of green fluorophores was performed upon propidium iodide (Sigma-Aldrich) or FM4-64 (Invitrogen) staining according to standard procedures⁶⁵. Chemical treatment with Brefeldin A (BFA, Sigma-Aldrich) was performed for duration of 90 minutes in concentration of 50 µM in liquid 0.5 MS media. Transverse plastic sections of roots were performed and visualized as previously described⁷³.

Confocal microscopy and image analysis

Confocal laser-scanning microscopy images were obtained using either a Leica SP8 (in Fig. 4, Fig. 5 [b-g, k], Fig. 6d, Fig. 7c-d and Extended Data Fig. 5 [a, f-h]) or Zeiss LSM 780 microscopes. Blue dyes such as Calcofluor White and DAPI were excited at 405nm and detected at 430-485nm as well as PSV autofluorescence. Green fluorophores (GFP, CITRINE, Venus, YFP) were excited at 488nm and detected between 500-550nm. Red fluorophores and dyes (RFP, mCHERRY, tdTOMATO, propidium iodide, FM4-64 and fuchsin) were excited at 561nm and detected at 590-650nm. 63x Oil Plan-Apochromat DIC M27 objective was used to visualize Arabidopsis embryos, otherwise the 40x water LD C-Apochromat M27 objective was used on Zeiss LSM 780 microscope. For two photon fluorescence excitation, Mai Tai XF (Spectra-Physics) laser at 980nm was used to excite GFP, YFP and CITRINE, while InSight DeepSee (Spectra-Physics) at 1060nm was used to excite RFP and mCHERRY fluorophores. Here, 40x water HC PL IRAPO objective was used. Signal detection was collected with external detectors based on FITC (525/50nm) / TexasRed (617/70nm) filters. Images were processed in ImageJ or Imaris image processing

software. When image colors were inverted and/or adjusted, all images belonging to one experiment were processed simultaneously. Scale bars were added in ImageJ.

Transmission electron microscopy

The root of 7-day-old seedlings was mounted in 1-hexadecene (Sigma) on a carrier with a 2 mm diameter and high-pressure frozen using the Leica EM HPM100. Then the samples were substituted in 1% OsO₄ for 6h at -90°C, followed by 3h at -60°C, 3h at -30°C, and 1h at 0°C. After one hour incubation, samples were rinsed twice with anhydrous acetone and incubated for 2h in 33% Epon/Araldite (Epon 812-Sigma, Durcupam ACM-Sigma, Dibutylphtalat-Sigma) in anhydrous acetone at 4°C. Next, samples were incubated in 66% Epon/Araldite in anhydrous acetone at 4°C overnight, and finally embedded in 100% Epon/Araldite. Samples were then trimmed using a glass knife and 70nm sections were cut with a diamond knife (DiATOME) using a ultramicrotome (Leica Ultracut UCT). Sections were assembled on a grid (2mmx1mm slit diaphragm, PLANO), coated with formvar (0.85% formvar in 1,2-dichlorethane). Contrast of the samples on the grids were enhanced with lead citrate. Samples were examined using the FEI Tecnai G2 Spirit transmission electron microscope with two digital CCD cameras (Gatan Orius 1000, FEI Eagle).

RNA-extraction, RT-qPCR and Western Blot analysis

Total RNA was extracted from 7-day-old seedlings using RNeasy® Plant Mini-Kit (QIAGEN) and treated with RNase-Free DNase (QIAGEN). cDNA synthesis was performed using RevertAid First Strand cDNA synthesis kit (Thermoscientific). RT-qPCR was performed using KAPA SYBR® FAST (KAPA BIOSYSTEMS), primers listed in Table S1 and 2µL of 1:10 dilution of cDNA. All reactions were performed in triplicates. Expression data derived from Cp values calculated according to the second derivative maximum method (LightCycler®LC480 II, Roche) and normalized to the expression of *PDF2* (At4g04890). To detect PTEN2-CITRINE protein in the transgenic lines, total proteins of 7-day-old seedlings were extracted using Laemmli buffer (v/v), separated in 12.5% (w/v) sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to an Amersham™ Hybond™ 0.45µm polyvinylidene difluoride (PVDF) membrane (Merck). CITRINE-fusion proteins were detected with anti-GFP antibody (JL-8, Takara Bio Clontech, dilution: 1:5000, overnight incubation) and anti-Mouse IgG (Fc-specific)-Peroxydase (Sigma, dilution: 1/10000, 1.5hr

incubation). Anti-Hsp90 (Agrisera, dilution: 1:2500, overnight incubation) and anti-Rabbit IgG (whole molecule)–HRP (Sigma A0545, dilution: 1:5000, 1.5hr incubation) were used as loading control. Chemiluminescence was revealed using Roti®-Lumin (ROTH) as substrate and imaged with a ChemiDoc Touch Imaging System (Bio-Rad).

Phylogenetic analysis

Protein sequences of PTEN2b homologous from 142 plant species from Phytozome, National Center for Biotechnology Information and Plaza 4.0 databases were analyzed using the following criteria: E value cut off 10^{-10} (Phytozome), total score cut off 100 (NCBI) and score cut off 100 (Plaza). Next, we removed manually the sequences whose identity was higher than 99% within the same species as well as the incomplete sequences. We only represented one splice variant for each locus and remove miss-aligned sequences. The resulting sequences were aligned using CLUSTAL OMEGA algorithm and the tree was generated by using FigTree version 1.4.3 software (<http://tree.bio.edu.ac.uk/software/figtree/>) and color-coded edited manually.

Bioinformatic analysis of physicochemical protein properties

The coding sequences of the N-terminal domain of *CrPTEN*, *AtPTEN1*, *AtPTEN2a*, *AtPTEN2b* and *MpPTEN2* were aligned (CLUSTAL OMEGA) and the presence of membrane binding domains was predicted using a BH score above 0.6 as described by Brzeska et al⁷⁴. By using IUPred2A score^{75,76}, domains with values above 0.5 were assigned as highly probably intrinsic disorder domains.

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FIGURE LEGENDS

Figure 1: Embryonic vacuole division involves cleavage furrow formation and requires PTEN2 activity. a-h, Representative confocal images of vacuoles in epidermal cells in *Arabidopsis* embryos extracted from green siliques (stages I-III), yellow siliques (stages IV-V), dry seeds (VI), germinated for 24h (stage VII) or 48h (stage VIII). Tonoplast (vacuolar membrane) decorated by *TIP3;2-GFP* (in green). **a,** Comparative seven stages of *TIP3;2* dynamics between wild type and *pten2a pten2b*. At the onset of its expression, *TIP3;2* accumulates in endoplasmic reticulum close to plasma membrane (orange arrows), or follow nuclear shape (yellow arrows). First tonoplast is visible in stage II marked with a white arrow. Small pre-embryonic vacuoles fuse in stage III (fusion sites are marked with blue arrows). Please note that image of EVs in *pten2a pten2b* in stage IV is slightly advanced than in wild type. In stages V-VII PSV exhibit autofluorescence in blue part of the spectrum (represented blue in images). Magenta shows autofluorescence in red part of the spectrum. **b,** EV division starts by tonoplast invagination at division site visible as a circle (white dashed arrows). In addition to the lack of synchronicity in tonoplast invaginations, in *pten2a pten2b* double mutants, membrane unilaterally ingress (red dashed arrow) and can reach the other side of the vacuole, however the division does not occur. Possible cause is the absence of membrane bending into hourglass shape as visible in WT (yellow asterisk). The ingrown membrane possibly rolls into a cylindrical shape as visible in 3D reconstructions of the late-stage V in double mutants. **c,** Representative confocal images of storage vacuoles' autofluorescence in epidermal cells of *Arabidopsis* embryos extracted from dry seeds and germinating seedlings in wild type and *pten2a pten2b* mutants. Scale bars represent 20 μ m. **d,** Germination 24h after imbibition in indicated genotypes. Error bars represent SE. n>500. **e-f,** Quantification of root length (**e**) and meristem size (**f**) in 6 days old seedlings of the indicated genotypes. n>30 (**e**) or n=10 (**f**). All error bars represent standard error. n.s., not significant; **p < 0.01.

Figure 2: PTEN2s prevent xylem cell differentiation. a, Representative confocal images of protoxylem cells in the indicated genotypes stained with Calcofluor White (cellulose in cyan) and Fuchsin (lignin in magenta). Secondary cell wall (SCW) was also visualised by transmission light (TM). Scale bars represent 20 μ m. **b,**

Quantification of xylem phenotypes observed in the roots displayed in a. The two phenotypes observed and scored were the total absence of xylem strands (without xylem) or the appearance of several protoxylem cells with SCW (islands). **c**, Representative images of indicated xylem differentiation markers in wildtype (WT) and seedlings incubated in 0.2 μ M estradiol for 48h to trigger *PTEN2b_{ox}*. Roots were stained with Calcofluor White (cyan) and fuchsin (magenta). Marker lines: *VND7* (*VASCULAR RELATED NAC-DOMAIN PROTEIN 7*), *MYB46* (*MYB DOMAIN PROTEIN 46*), *CESA7* (*CELLULOSE SYNTHASE CATALYTIC SUBUNIT 7*), *CESA4* (*CELLULOSE SYNTHASE A4*), *CESA8* (*CELLULOSE SYNTHASE 8*), *DMP4* (*DUF679 DOMAIN MEMBRANE PROTEIN 4*), *EX11* (*EXITUS 1*). Scale bars represent 20 μ m. **d**, Transmission electron microscopy images of differentiating proto- (px) and metaxylem (mx) cells in WT and *PTEN2b_{ox}*. Xylem cells in WT formed thick secondary cell wall (SCW), vacuoles are enlarging in mx while px underwent programmed cell death. *PTEN2b* overexpression prevents SCW formation while mx cells contain multiple small vacuoles. Here px cell also underwent clearance.

Figure 3: Regulation of xylem vacuolar biogenesis regulation by PTEN2s. **a**, 3D reconstruction of VHP1-GFP decorated vacuolar compartments in protoxylem cells in wild type plants at progressive developmental stages counterstained with propidium iodide (PI) to label cell wall. Yellow arrows mark small vacuole-like compartments and black arrows mark tubular connecting membranes. For easier visualization, protoxylem cells margins were squared by a white dashed line. Scale bars represent 20 μ m. **b**, Representative images of mature protoxylem cells in the indicated genotypes, visualized as in a. Scale bars represent 20 μ m. Black arrows mark tubular connecting membranes. **c**, Comparison of vacuolar morphology in mature epidermis (ep), cortex (co), endodermis (en), pericycle (pc) and protoxylem (asterisk) between indicated genotypes. Scale bars represent 20 μ m.

Figure 4: PTEN2s inhibit vacuolar and secretion trafficking pathway in xylem cells but not PCD. **a**, Schematic representation of analysed trafficking pathways important for xylem cell differentiation. **b-g**, Representative images of the corresponding xylem cells in wild type (WT) and seedlings with 0.2 μ M estradiol-mediated *PTEN2b* induction for 48h visualizing different trafficking markers: tonoplast

marker VHA-a3 (VACUOLAR PROTON ATPASE A3) (**b**), xylem specific promoter *PASPA3* (*PUTATIVE ASPARTIC PROTEINASE A3*) driving expression of *ToIM* (tonoplast integrity marker) showing GFP in ER and a vacuolar targeted mRFP (**c**), vacuolar cargos CESA6 (CELLULOSE SYNTHASE SUBUNIT A6) (**d**) and XCP1 (XYLEM CYSTEINE PEPTIDASE 1) (**e**), secreted cargo LAC17 (LACCASE 17) (**f**). **g**, non-secreted LAC17 is not delivered into VHP1-labeled vacuoles. **h-j**, PCD execution occurs even without SCW formation in *PTEN2b_{ox}*. **h**, DAPI-stained nuclei are absent in the cells where the xylem-specific expression of MYB46 ceased due to the PCD execution (white arrows). Scale bars represent 20 μ m. **i**, Toluidine-stained root cross-sections of the indicated genotypes. Xylem secondary cell wall stains bright blue as visible in WT but absent in *PTEN2b_{ox}* overexpression where xylem cells appear collapsed as in transmission electron microscopy images (**j**). **j**, Transmission electron microscopy images of the indicated genotypes. Notice a high number of small vacuoles and aggregates in *PTEN2b_{ox}*. Yellow dashed circle highlights a cup-shaped phagophore. Scale bars represent 2 μ m. **k**, Xylem vacuole acidification in wild type prior PCD. Note faster fading of pH-sensitive YFP in comparison to pH-tolerant mCHERRY. The cell where acidification occurs is encircled with a white dashed line. Scale bars represent 10 μ m. **l**, continuation cell from **k**, where PCD is executed and mCHERRY signal disappears too. Scale bars represent 10 μ m

Figure 5: PTEN2s functions in vacuolar fusion and xylem differentiation were conserved through evolution. **a**, Schematic tree of the evolution of plant PTEN subfamilies. **b**, Phylogenetic tree of 418 plant PTENs from 142 plant species. For simplification, only the isoforms of species of interest have been represented. Details about all the sequences and the complete distribution of the isoforms in the three subfamilies (ancestor-like PTENs, PTEN1s, PTEN2s) can be found in Supplementary Table 2. **c**, Representative confocal microscopy images of fuchsin-stained protoxylem strands from roots grown on mock conditions or upon 2 μ M estradiol-mediated induction for 48h of *Chlamydomonas reinhardtii* PTEN (*CrPTEN*), *Arabidopsis thaliana* *PTEN1* (*AtPTEN1*), *PTEN2a* (*AtPTEN2a*), and *PTEN2b* (*AtPTEN2b*) and *Marchantia polymorpha* *PTEN2* (*MpPTEN2*). **d**, Representative confocal microscopy images of vacuolar morphology in mature xylem cells (VHP1-GFP labels tonoplast, propidium iodide stains cell wall) of inducible over-expressor lines of the different PTEN isoforms

mentioned above. *PTEN* over-expression was induced by 2 μ M estradiol for 48h. Protoxylem gaps are highlighted with white dashed lines. Scale bars represent 20 μ m.

Figure 6: A conserved domain within PTEN2s N-terminal sequence is critical for their functionality and TGN anchoring. **a**, Schematic representation of the PTEN enzymes from *Homo Sapiens* (*Hs*), *Arabidopsis* (*At*), *Marchantia* (*Mp*) and *Chlamydomonas* (*Cr*) analysed in this study. On the right are represented the truncated version of PTEN2b without the entire C- (PTEN2b Δ Cter) or N-terminal (PTEN2b Δ Nter) sequences, PTEN2b with a partial N-terminal sequences (PTEN2b Δ 1-131) and the hybrid version with PTEN1 N-terminal (N1-PTEN2b). Colour filled boxes represent phosphatase catalytic domains whereas empty squared boxes represent C2 domains. **b**, Representative confocal microscopy images of fuchsin-stained protoxylem strands after 2 μ M estradiol-mediated induction for 48h of indicated PTEN2b versions. Protoxylem gap cells are highlighted with white dashed lines. **c**, Representative images of vacuole morphology in mature xylem cell upon 48h overexpression of indicated PTEN2b variants. VHP1-GFP labels tonoplast, while propidium iodide labels cell wall. Protoxylem gap cells are highlighted with white dashed lines. **d**, Representative confocal images of 6-day-old plants harbouring indicated constructs illustrating the dependence of PTEN2b localization at TGN to its N-terminal. Scale bars represent 20 μ m.

Extended Data Figure 1: Aberrant EV division does not affect post-embryonic development. **a-c**, Representative confocal images of aquaporin *TIP3;2-GFP* (green) distribution during embryogenesis. **a**, Similar to *TIP3;2*, VHP1 tonoplast marker also labels ER. Magenta shows autofluorescence from chloroplasts visible in embryos isolated from green siliques. **b**, 3D maximal projection of a stage III vacuole corresponding to 2D image shown in Figure 1a. **c**, Small vacuoles labelled with *TIP3;2* close to EV tonoplast preceding the cleavage furrow division. Magenta shows autofluorescence detected in red part of the spectrum. Scale bars represent 20 μ m. **d**, Germination rate between indicated genotypes. Error bars represent SE. n>500. **e**, Analysis of normalized, relative *PTEN2b* overexpression by qRT-PCR in two independent transgenic lines induced with 2 μ M estradiol for the duration of 6 days. Error bars represent SE among three independent biological replicates. **f**,

Representative confocal images of embryos dissected from dry seeds, 24h and 48h after imbibition of the indicated genotypes, stained with Calcofluor White (cellulose in cyan) and fuchsin (lignin in magenta). Yellow arrows mark xylem discontinuities in seedlings with induced *PTEN2b* overexpression from imbibition (2 μ M estradiol). Note the appearance of differentiated xylem cells (magenta) only 48h after germination. Scale bars 200 μ m. **g**, Root length quantification of 6-days-old seedlings illustrate dose dependant effect of *PTEN2b* overexpression after 48h of estradiol induction. Error bars represent SE. $n > 40$ **h**, *PTEN2b* overexpression prevents xylem differentiation in T3.4.40 line in both proximal and distal part of the root. Higher overexpression in the line T3.13.41 dramatically shortens the root length (**g**) but does not prevent xylem differentiation in younger distal root parts. Undifferentiated xylem is only labelled with MYB46 marker in yellow, while differentiated xylem is labelled with fuchsin staining for lignin in magenta, or white (overlap between yellow and magenta). Asterisk labels xylem position within vascular cylinder. White arrows label ectopic lignification in endodermis. Scale bars represent 100 μ m. n.s., not significant; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Extended Data Figure 2: *PTEN2b* overexpression does not alter the expression of genes associated with xylem PCD execution. **a**, Representative confocal microscopy images of the mature protoxylem cells stained with Calcofluor White for cellulose (cyan) and fuchsin for lignin (magenta). *PTEN2b* was induced for 48h with 0.2 μ M estradiol. Note the expression of genes associated with PCD such as the *PUTATIVE ASPARTIC PROTEINASE A3* (*PASPA3*), *RIBONUCLEASE 3* (*RNS3*), *SERINE CARBOXYPEPTIDASE-LIKE 48* (*SCPL48*) and *BIFUNCTIONAL NUCLEASE 1* (*BFN1*) can still be detected in seedlings with *PTEN2b* upregulation. Asterisks mark protoxylem strands. Scale bars represent 20 μ m.

Extended Data Figure 4: *PTEN2b* colocalize to TGN and impinges on vacuolar and cell secretion pathways. **a**, Brassinosteroid receptor BRI1 (BRASSINOSTEROID INSENSITIVE 1) cannot be delivered to xylem vacuoles upon *PTEN2b* overexpression. Seedlings were counterstained with propidium iodide (PI). **b**, *PTEN2b* colocalize with cellular compartments early labelled with FM4-64 (magenta). **c**, *PTEN2b* partially colocalize with PROTON ATPASE A1 (*VHA-a1*) in

TGN (arrows). **d**, PTEN2b partially colocalize with CELLULOSE SYNTHASE SUBUNIT A6 (CESA6). **e**, *cesa6* mutant cannot rescue secondary cellulose building upon *PTEN2b* overexpression. **f**, Aggregates of vesicles carrying vacuolar destined cargo (CESA6 in green) and secretion cargo (LAC17 in red) do not colocalize. Arrows' color corresponds to fluorophores and points the aggregates where proteins do not colocalize **g**, Multivesicular body (MVB) marker Rha1 (ARABIDOPSIS RAB HOMOLOG F2A) creates aggregates in xylem cells upon PTEN2b upregulation. **h**, Prevacuolar compartment and tonoplast marker RabG3f (RAB GTPASE HOMOLOG G3F) upon prolonged *PTEN2b* overexpression creates grape like structures in vicinity of the central vacuole in mature epidermal cells. Asterisk labels xylem strands. Scale bars represent 20 μ m. **i**, Schematic representation of membrane phenomena regulated by PTEN2s.

Extended Data Figure 6: The N-terminal domains of PTEN1 and PTEN2s exhibit different biochemical properties that determines their subcellular localization.

a, Alignment of N-terminal PTEN sequences from: human (*HsPTEN*), Chlamydomonas (*CrPTEN*), Arabidopsis (*AtPTEN1*, *AtPTEN2a* and *AtPTEN2b*), and *Marchantia* (*MpPTEN2*) proteome assemblies obtained using CLUSTAL OMEGA. Identical amino acids are represented in green while similar amino acids are represented in magenta. **b**, Prediction of membrane binding domain in PTEN N-terminal sequences of indicated isoform using BH score⁷⁴. Domains with values above 0.6 are predicted to be membrane binding domain. **c**, Prediction of intrinsic disordered region in N-terminal sequences of indicated isoform using IUPred2A score^{75,76}. Regions with values above 0.5 are supposed to be enriched in intrinsic disorders. The grey areas highlight the domain identified in PTEN2b (amino acid 132-188) as responsible for its functionality. **d**, Representative confocal images of 6 day-old plants expressing the TGN marker *VHAa1-RFP* together with *PTEN2b::PTEN2b-citrine* or *PTEN2b::PTEN2b Δ 1-131-CITRINE*. Arrows indicate the position of some of the dotted structures observed for *PTEN2b::PTEN2b Δ 1-131-CITRINE*. **e**, Representative confocal images of 6 day-old plants expressing *PTEN2b::PTEN2b Δ 1-131-CITRINE* with *PTEN2b::PTEN2b-mCHERRY*. Please note the co-localization of both constructs. **f**, Confocal images of 6 day-old plants expressing *PTEN2b::PTEN2b Δ 1-131-citrine* and treated with DMSO or 50 μ M BFA for 1.5h. Scale bars represent 10 μ m.

Supplementary Figure 1: Transgenic lines validation (Supporting data for Figures 6 and 7). **a**, qPCR analyses confirmed the over-expression of PTEN in different inducible lines described in Fig. 6 and Fig. 7. RNA was extracted from roots of 7day-old plants treated with DMSO or 2 μ M estradiol for 48hrs. Expression values of the different genes of interest in estradiol-treated plants were normalized by the corresponding expression values measured in DMSO-treated plants. Values represent the mean of two biological replicates (both including three technical replicates), error bars indicate the standard deviation. **b**, qPCR analyses revealed the presence of *CITRINE* mRNA in independent *PTEN2b::PTEN1-CITRINE* that do not exhibit any fluorescence signal in root cells (Fig 6). Values represent the mean of three technical replicates, error bars indicate the standard deviation. **c**, The expression of the different Nter-truncated versions of PTEN2b tagged with citrine (Fig 6) was confirmed by Western blot using anti-GFP antibody. Anti-Hsp90 was used as a loading control. The star indicates the presence of an unspecific band.

Supplementary Table 1: Protein sequences used to build the phylogenetic tree.

Supplementary Table 2: Primers used in this study.

Supplementary Table 3: Constructs generated in this study.

REFERENCES

- 1 Peters, C., Baars, T. L., Buhler, S. & Mayer, A. Mutual control of membrane fission and fusion proteins. *Cell* **119**, 667-678, doi:10.1016/j.cell.2004.11.023 (2004).
- 2 Martens, S. & McMahon, H. T. Mechanisms of membrane fusion: disparate players and common principles. *Nat Rev Mol Cell Biol* **9**, 543-556, doi:10.1038/nrm2417 (2008).
- 3 Lenz, M., Morlot, S. & Roux, A. Mechanical requirements for membrane fission: common facts from various examples. *FEBS Lett* **583**, 3839-3846, doi:10.1016/j.febslet.2009.11.012 (2009).
- 4 Schmid, S. L. & Frolov, V. A. Dynammin: functional design of a membrane fission catalyst. *Annu Rev Cell Dev Biol* **27**, 79-105, doi:10.1146/annurev-cellbio-100109-104016 (2011).
- 5 Iijima, M., Huang, Y. E., Luo, H. R., Vazquez, F. & Devreotes, P. N. Novel mechanism of PTEN regulation by its phosphatidylinositol 4,5-bisphosphate binding motif is critical for chemotaxis. *J Biol Chem* **279**, 16606-16613, doi:10.1074/jbc.M312098200 (2004).
- 6 Derman, M. P. *et al.* The lipid products of phosphoinositide 3-kinase increase cell motility through protein kinase C. *J Biol Chem* **272**, 6465-6470, doi:10.1074/jbc.272.10.6465 (1997).
- 7 Funamoto, S., Meili, R., Lee, S., Parry, L. & Firtel, R. A. Spatial and temporal regulation of 3-phosphoinositides by PI 3-kinase and PTEN mediates chemotaxis. *Cell* **109**, 611-623, doi:10.1016/s0092-8674(02)00755-9 (2002).

- 8 Munnik, T. & Testerink, C. Plant phospholipid signaling: "in a nutshell". *J Lipid Res* **50 Suppl**, S260-265, doi:10.1194/jlr.R800098-JLR200 (2009).
- 9 Vermeer, J. E. *et al.* Visualization of PtdIns3P dynamics in living plant cells. *Plant J* **47**, 687-700, doi:10.1111/j.1365-313X.2006.02830.x (2006).
- 10 Pribat, A. *et al.* A novel class of PTEN protein in Arabidopsis displays unusual phosphoinositide phosphatase activity and efficiently binds phosphatidic acid. *Biochem J* **441**, 161-171, doi:10.1042/BJ20110776 (2012).
- 11 Jurgens, G. Plant cytokinesis: fission by fusion. *Trends Cell Biol* **15**, 277-283, doi:10.1016/j.tcb.2005.03.005 (2005).
- 12 Buschmann, H. & Zachgo, S. The Evolution of Cell Division: From Streptophyte Algae to Land Plants. *Trends Plant Sci* **21**, 872-883, doi:10.1016/j.tplants.2016.07.004 (2016).
- 13 Becker, B. Function and evolution of the vacuolar compartment in green algae and land plants (Viridiplantae). *Int Rev Cytol* **264**, 1-24, doi:10.1016/S0074-7696(07)64001-7 (2007).
- 14 Beauzamy, L., Derr, J. & Boudaoud, A. Quantifying Hydrostatic Pressure in Plant Cells by Using Indentation with an Atomic Force Microscope. *Biophys J* **108**, 2448-2456, doi:10.1016/j.bpj.2015.03.035 (2015).
- 15 Delgadillo, M. O. *et al.* MTV proteins unveil ER- and microtubule-associated compartments in the plant vacuolar trafficking pathway. *Proc Natl Acad Sci U S A* **117**, 9884-9895, doi:10.1073/pnas.1919820117 (2020).
- 16 Cui, Y. *et al.* A whole-cell electron tomography model of vacuole biogenesis in Arabidopsis root cells. *Nature plants* **5**, 95-105, doi:10.1038/s41477-018-0328-1 (2019).
- 17 Kruger, F. & Schumacher, K. Pumping up the volume - vacuole biogenesis in Arabidopsis thaliana. *Semin Cell Dev Biol* **80**, 106-112, doi:10.1016/j.semcd.2017.07.008 (2018).
- 18 Viotti, C. *et al.* The endoplasmic reticulum is the main membrane source for biogenesis of the lytic vacuole in Arabidopsis. *Plant Cell* **25**, 3434-3449, doi:10.1105/tpc.113.114827 (2013).
- 19 Tanaka, Y. *et al.* Intra-vacuolar reserves of membranes during stomatal closure: the possible role of guard cell vacuoles estimated by 3-D reconstruction. *Plant Cell Physiol* **48**, 1159-1169, doi:10.1093/pcp/pcm085 (2007).
- 20 Beilby, M. J. Chara braunii genome: a new resource for plant electrophysiology. *Biophys Rev* **11**, 235-239, doi:10.1007/s12551-019-00512-7 (2019).
- 21 Cui, Y., Zhao, Q., Hu, S. & Jiang, L. Vacuole Biogenesis in Plants: How Many Vacuoles, How Many Models? *Trends Plant Sci* **25**, 538-548, doi:10.1016/j.tplants.2020.01.008 (2020).
- 22 Feeney, M., Kittelmann, M., Menassa, R., Hawes, C. & Frigerio, L. Protein Storage Vacuoles Originate from Remodeled Preexisting Vacuoles in Arabidopsis thaliana. *Plant Physiol* **177**, 241-254, doi:10.1104/pp.18.00010 (2018).
- 23 Gopaldass, N., Fauvet, B., Lashuel, H., Roux, A. & Mayer, A. Membrane scission driven by the PROPPIN Atg18. *EMBO J* **36**, 3274-3291, doi:10.15252/embj.201796859 (2017).
- 24 Tiedemann, J., Schlereth, A. & Muntz, K. Differential tissue-specific expression of cysteine proteinases forms the basis for the fine-tuned mobilization of storage globulin during and after germination in legume seeds. *Planta* **212**, 728-738, doi:10.1007/s004250000435 (2001).
- 25 Zheng, H. & Staehelin, L. A. Protein storage vacuoles are transformed into lytic vacuoles in root meristematic cells of germinating seedlings by multiple, cell type-specific mechanisms. *Plant Physiol* **155**, 2023-2035, doi:10.1104/pp.110.170159 (2011).
- 26 Gujas, B. *et al.* Perturbing phosphoinositide homeostasis oppositely affects vascular differentiation in Arabidopsis thaliana roots. *Development* **144**, 3578-3589, doi:10.1242/dev.155788 (2017).
- 27 Lucas, W. J. *et al.* The plant vascular system: evolution, development and functions. *J Integr Plant Biol* **55**, 294-388, doi:10.1111/jipb.12041 (2013).
- 28 Fendrych, M. *et al.* Programmed cell death controlled by ANAC033/SOMBRERO determines root cap organ size in Arabidopsis. *Curr Biol* **24**, 931-940, doi:10.1016/j.cub.2014.03.025 (2014).

- 29 Watanabe, Y. *et al.* Cellulose synthase complexes display distinct dynamic behaviors during xylem transdifferentiation. *Proc Natl Acad Sci U S A* **115**, E6366-E6374, doi:10.1073/pnas.1802113115 (2018).
- 30 Mottiar, Y., Vanholme, R., Boerjan, W., Ralph, J. & Mansfield, S. D. Designer lignins: harnessing the plasticity of lignification. *Curr Opin Biotechnol* **37**, 190-200, doi:10.1016/j.copbio.2015.10.009 (2016).
- 31 Takenaka, Y. *et al.* Patterned Deposition of Xylan and Lignin is Independent from that of the Secondary Wall Cellulose of Arabidopsis Xylem Vessels. *Plant Cell* **30**, 2663-2676, doi:10.1105/tpc.18.00292 (2018).
- 32 Northcote, D. H., Davey, R. & Lay, J. Use of antisera to localize callose, xylan and arabinogalactan in the cell-plate, primary and secondary walls of plant cells. *Planta* **178**, 353-366, doi:10.1007/BF00391863 (1989).
- 33 Schuetz, M. *et al.* Laccases Direct Lignification in the Discrete Secondary Cell Wall Domains of Protoxylem. *Plant Physiology* **166**, 798-U489, doi:10.1104/pp.114.245597 (2014).
- 34 Young, B., Wightman, R., Blanvillain, R., Purcel, S. B. & Gallois, P. pH-sensitivity of YFP provides an intracellular indicator of programmed cell death. *Plant Methods* **6**, 27, doi:10.1186/1746-4811-6-27 (2010).
- 35 Kleine-Vehn, J. *et al.* Differential degradation of PIN2 auxin efflux carrier by retromer-dependent vacuolar targeting. *Proc Natl Acad Sci U S A* **105**, 17812-17817, doi:10.1073/pnas.0808073105 0808073105 [pii] (2008).
- 36 Han, X. *et al.* Origin and Evolution of Core Components Responsible for Monitoring Light Environment Changes during Plant Terrestrialization. *Mol Plant* **12**, 847-862, doi:10.1016/j.molp.2019.04.006 (2019).
- 37 Gupta, R., Ting, J. T., Sokolov, L. N., Johnson, S. A. & Luan, S. A tumor suppressor homolog, AtPTEN1, is essential for pollen development in Arabidopsis. *Plant Cell* **14**, 2495-2507 (2002).
- 38 Alberts, B. *Molecular biology of the cell*. 5th ed. edn, (Garland Science ; [London : Taylor & Francis, distributor], 2008).
- 39 Joshi, A. S., Zhang, H. & Prinz, W. A. Organelle biogenesis in the endoplasmic reticulum. *Nat Cell Biol* **19**, 876-882, doi:10.1038/ncb3579 (2017).
- 40 Schrader, M. & Pellegrini, L. The making of a mammalian peroxisome, version 2.0: mitochondria get into the mix. *Cell death and differentiation* **24**, 1148-1152, doi:10.1038/cdd.2017.23 (2017).
- 41 Munoz-Gomez, S. A. *et al.* Ancient homology of the mitochondrial contact site and cristae organizing system points to an endosymbiotic origin of mitochondrial cristae. *Curr Biol* **25**, 1489-1495, doi:10.1016/j.cub.2015.04.006 (2015).
- 42 Vothknecht, U. C. & Westhoff, P. Biogenesis and origin of thylakoid membranes. *Biochim Biophys Acta* **1541**, 91-101, doi:10.1016/s0167-4889(01)00153-7 (2001).
- 43 Scheuring, D. *et al.* Actin-dependent vacuolar occupancy of the cell determines auxin-induced growth repression. *Proc Natl Acad Sci U S A* **113**, 452-457, doi:10.1073/pnas.1517445113 (2016).
- 44 Muller, O. *et al.* Autophagic tubes: vacuolar invaginations involved in lateral membrane sorting and inverse vesicle budding. *J Cell Biol* **151**, 519-528, doi:10.1083/jcb.151.3.519 (2000).
- 45 Allen, R. D. & Naitoh, Y. Osmoregulation and contractile vacuoles of protozoa. *Int Rev Cytol* **215**, 351-394, doi:10.1016/s0074-7696(02)15015-7 (2002).
- 46 Komsic-Buchmann, K., Stephan, L. M. & Becker, B. The SEC6 protein is required for contractile vacuole function in *Chlamydomonas reinhardtii*. *J Cell Sci* **125**, 2885-2895, doi:10.1242/jcs.099184 (2012).
- 47 Nishiyama, T. *et al.* The Chara Genome: Secondary Complexity and Implications for Plant Terrestrialization. *Cell* **174**, 448-464 e424, doi:10.1016/j.cell.2018.06.033 (2018).

- 48 Persson, S. *et al.* The Arabidopsis irregular xylem8 mutant is deficient in glucuronoxylan and homogalacturonan, which are essential for secondary cell wall integrity. *Plant Cell* **19**, 237-255, doi:10.1105/tpc.106.047720 (2007).
- 49 Chiniquy, D. *et al.* Three Novel Rice Genes Closely Related to the Arabidopsis IRX9, IRX9L, and IRX14 Genes and Their Roles in Xylan Biosynthesis. *Front Plant Sci* **4**, 83, doi:10.3389/fpls.2013.00083 (2013).
- 50 Zhang, B., Gao, Y., Zhang, L. & Zhou, Y. The plant cell wall: Biosynthesis, construction, and functions. *J Integr Plant Biol* **63**, 251-272, doi:10.1111/jipb.13055 (2021).
- 51 Avci, U., Earl Petzold, H., Ismail, I. O., Beers, E. P. & Haigler, C. H. Cysteine proteases XCP1 and XCP2 aid micro-autolysis within the intact central vacuole during xylogenesis in Arabidopsis roots. *Plant J* **56**, 303-315, doi:10.1111/j.1365-313X.2008.03592.x (2008).
- 52 Kuriyama, H. Loss of Tonoplast Integrity Programmed in Tracheary Element Differentiation. *Plant Physiol* **121**, 763-774 (1999).
- 53 Groover, A., DeWitt, N., Heidel, A. & Jones, A. Programmed cell death of plant tracheary elements differentiating in vitro. *Protoplasma* **Protoplasma**, 197-211, doi:https://doi.org/10.1007/BF01279568 (1997).
- 54 Hara-Nishimura, I. & Hatsugai, N. The role of vacuole in plant cell death. *Cell death and differentiation* **18**, 1298-1304, doi:10.1038/cdd.2011.70 (2011).
- 55 Groover, A. & Jones, A. M. Tracheary element differentiation uses a novel mechanism coordinating programmed cell death and secondary cell wall synthesis. *Plant Physiol* **119**, 375-384, doi:10.1104/pp.119.2.375 (1999).
- 56 Courtois-Moreau, C. L. *et al.* A unique program for cell death in xylem fibers of Populus stem. *Plant J* **58**, 260-274, doi:10.1111/j.1365-313X.2008.03777.x (2009).
- 57 Kwon, S. I. *et al.* The Rab GTPase RabG3b functions in autophagy and contributes to tracheary element differentiation in Arabidopsis. *Plant J* **64**, 151-164, doi:10.1111/j.1365-313X.2010.04315.x (2010).
- 58 Herman, E. & Schmidt, M. Endoplasmic reticulum to vacuole trafficking of endoplasmic reticulum bodies provides an alternate pathway for protein transfer to the vacuole. *Plant Physiol* **136**, 3440-3446, doi:10.1104/pp.104.051722 (2004).
- 59 Michaeli, S., Avin-Wittenberg, T. & Galili, G. Involvement of autophagy in the direct ER to vacuole protein trafficking route in plants. *Front Plant Sci* **5**, 134, doi:10.3389/fpls.2014.00134 (2014).
- 60 Toyooka, K., Okamoto, T. & Minamikawa, T. Mass transport of proform of a KDEL-tailed cysteine proteinase (SH-EP) to protein storage vacuoles by endoplasmic reticulum-derived vesicle is involved in protein mobilization in germinating seeds. *J Cell Biol* **148**, 453-464, doi:10.1083/jcb.148.3.453 (2000).
- 61 Okamoto, T., Shimada, T., Hara-Nishimura, I., Nishimura, M. & Minamikawa, T. C-terminal KDEL sequence of a KDEL-tailed cysteine proteinase (sulfhydryl-endopeptidase) is involved in formation of KDEL vesicle and in efficient vacuolar transport of sulfhydryl-endopeptidase. *Plant Physiol* **132**, 1892-1900, doi:10.1104/pp.103.021147 (2003).
- 62 Lee, J. Y. *et al.* Transcriptional and posttranscriptional regulation of transcription factor expression in Arabidopsis roots. *Proc Natl Acad Sci U S A* **103**, 6055-6060, doi:10.1073/pnas.0510607103 (2006).
- 63 Olvera-Carrillo, Y. *et al.* A Conserved Core of Programmed Cell Death Indicator Genes Discriminates Developmentally and Environmentally Induced Programmed Cell Death in Plants. *Plant Physiol* **169**, 2684-2699, doi:10.1104/pp.15.00769 (2015).
- 64 Segami, S., Makino, S., Miyake, A., Asaoka, M. & Maeshima, M. Dynamics of vacuoles and H⁺-pyrophosphatase visualized by monomeric green fluorescent protein in Arabidopsis: artifactual bulbs and native intravacuolar spherical structures. *Plant Cell* **26**, 3416-3434, doi:10.1105/tpc.114.127571 (2014).
- 65 Dettmer, J., Hong-Hermesdorf, A., Stierhof, Y. D. & Schumacher, K. Vacuolar H⁺-ATPase activity is required for endocytic and secretory trafficking in Arabidopsis. *Plant Cell* **18**, 715-730, doi:10.1105/tpc.105.037978 (2006).

- 66 Paredez, A. R., Somerville, C. R. & Ehrhardt, D. W. Visualization of cellulose synthase demonstrates functional association with microtubules. *Science* **312**, 1491-1495, doi:10.1126/science.1126551 (2006).
- 67 Jaillais, Y., Belkadir, Y., Balsemao-Pires, E., Dangl, J. L. & Chory, J. Extracellular leucine-rich repeats as a platform for receptor/coreceptor complex formation. *Proc Natl Acad Sci U S A* **108**, 8503-8507, doi:10.1073/pnas.1103556108 (2011).
- 68 Geldner, N. *et al.* Rapid, combinatorial analysis of membrane compartments in intact plants with a multicolor marker set. *Plant J* **59**, 169-178, doi:10.1111/j.1365-313X.2009.03851.x TPJ3851 [pii] (2009).
- 69 Vermeer, J. E. M. *et al.* A Spatial Accommodation by Neighboring Cells Is Required for Organ Initiation in Arabidopsis. *Science* **343**, 178-183, doi:10.1126/science.1245871 (2014).
- 70 Chauvin, A., Caldelari, D., Wolfender, J. L. & Farmer, E. E. Four 13-lipoxygenases contribute to rapid jasmonate synthesis in wounded Arabidopsis thaliana leaves: a role for lipoxygenase 6 in responses to long-distance wound signals. *New Phytol* **197**, 566-575, doi:10.1111/nph.12029 (2013).
- 71 Curtis, M. D. & Grossniklaus, U. A gateway cloning vector set for high-throughput functional analysis of genes in planta. *Plant Physiol* **133**, 462-469, doi:10.1104/pp.103.027979 (2003).
- 72 Ursache, R., Andersen, T. G., Marhavy, P. & Geldner, N. A protocol for combining fluorescent proteins with histological stains for diverse cell wall components. *Plant J* **93**, 399-412, doi:10.1111/tpj.13784 (2018).
- 73 Mahonen, A. P. *et al.* A novel two-component hybrid molecule regulates vascular morphogenesis of the Arabidopsis root. *Genes Dev* **14**, 2938-2943 (2000).
- 74 Brzeska, H., Guag, J., Remmert, K., Chacko, S. & Korn, E. D. An experimentally based computer search identifies unstructured membrane-binding sites in proteins: application to class I myosins, PAKS, and CARMIL. *J Biol Chem* **285**, 5738-5747, doi:10.1074/jbc.M109.066910 (2010).
- 75 Erdos, G. & Dosztanyi, Z. Analyzing Protein Disorder with IUPred2A. *Curr Protoc Bioinformatics* **70**, e99, doi:10.1002/cpbi.99 (2020).
- 76 Meszaros, B., Erdos, G. & Dosztanyi, Z. IUPred2A: context-dependent prediction of protein disorder as a function of redox state and protein binding. *Nucleic Acids Res* **46**, W329-W337, doi:10.1093/nar/gky384 (2018).

FIGURE 1

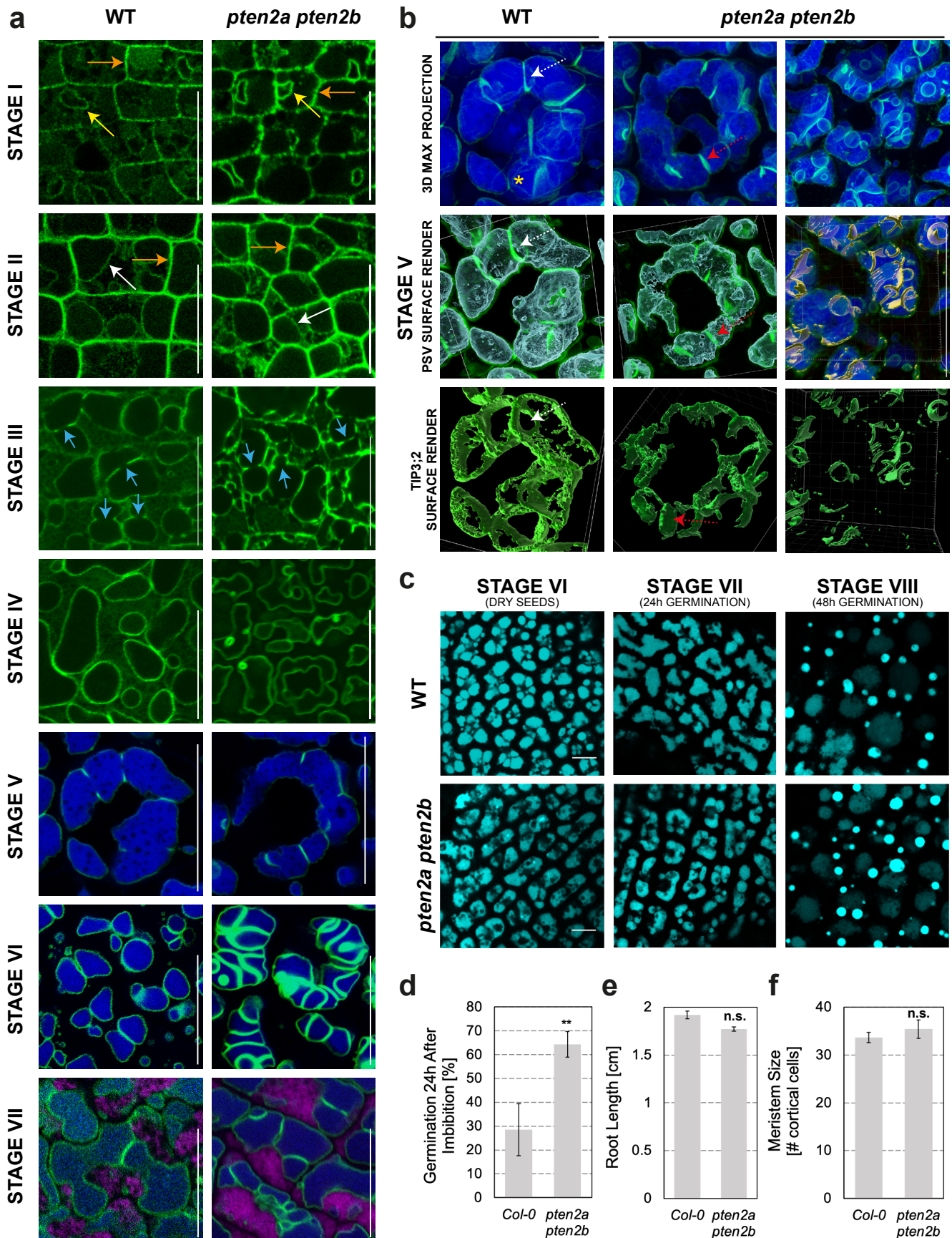
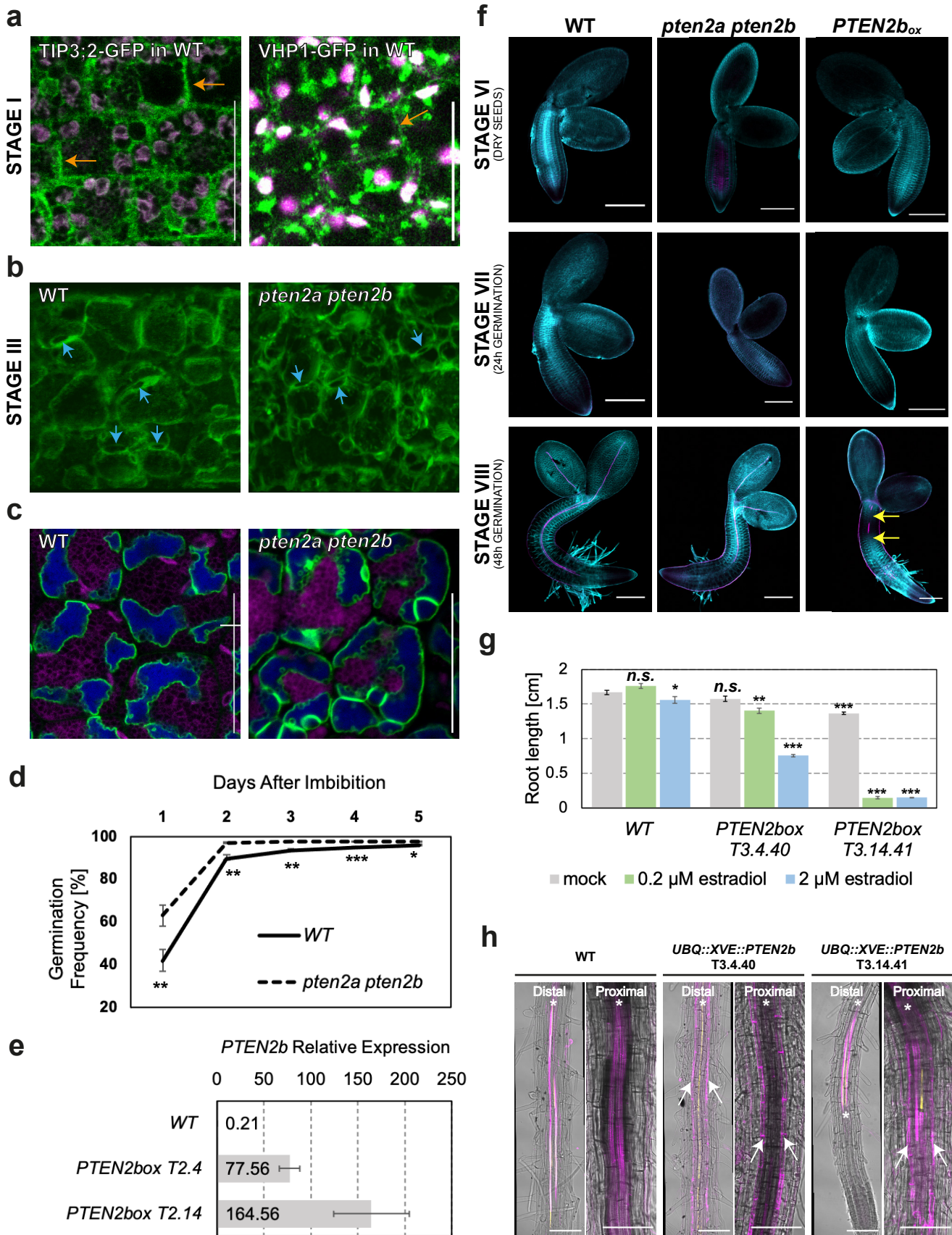


Figure 1: Embryonic vacuole division involves cleavage furrow formation and requires PTEN2 activity. a-h, Representative confocal images of vacuoles in epidermal cells in Arabidopsis embryos extracted from green siliques (stages I-III), yellow siliques (stages IV-V), dry seeds (VI), germinated for 24h (stage VII) or 48h (stage VIII). Tonoplast (vacuolar membrane) decorated by TIP3;2-GFP (in green). a, Comparative seven stages of TIP3;2 dynamics between wild type and *pten2a pten2b*. At the onset of its expression, TIP3;2 accumulates in endoplasmic reticulum close to plasma membrane (orange arrows), or follow nuclear shape (yellow arrows). First tonoplast is visible in stage II marked with a white arrow. Small pre-embryonic vacuoles fuse in stage III (fusion sites are marked with blue arrows). Please note that image of EVs in *pten2a pten2b* in stage IV is slightly advanced than in wild type. In stages V-VII PSV autofluorescence in blue part of the spectrum (represented blue in images). Magenta shows autofluorescence in red part of the spectrum. b, EV division starts by tonoplast invagination at division site visible as a circle (white dashed arrows). In addition to the lack of synchronicity in tonoplast invaginations, in *pten2a pten2b* double mutants, membrane unilaterally ingress (red dashed arrow) and can reach the other side of the vacuole, however the division does not occur. Possible cause is the absence of membrane bending into hourglass shape as visible in WT (yellow asterisk). The ingrown membrane possibly rolls into a cylindrical shape as visible in 3D reconstructions of the late-stage V in double mutants. c, Representative confocal images of storage vacuoles' autofluorescence in epidermal cells of Arabidopsis embryos extracted from dry seeds and germinating seedlings in wild type and *pten2a pten2b* mutants. Scale bars represent 20µm. d, Germination 24h after imbibition in indicated genotypes, n>500. e-f, Quantification of root length (e) and meristem size (f) in 6 days old seedlings of the indicated genotypes. n>30 (e) and n=10 (f). All error bars represent standard error. n.s., not significant; **p < 0.01.

EXTENDED DATA FIGURE



Extended Data Figure 1: Aberrant EV division does not affect post-embryonic development. **a-c**, Representative confocal images of aquaporin TIP3;2-GFP (green) distribution during embryogenesis. **a**, Similar to TIP3;2, VHP1 tonoplast marker also labels ER. Magenta shows autofluorescence from chloroplasts visible in embryos isolated from green siliques. **b**, 3D maximal projection of a stage III vacuole corresponding to 2D image shown in Figure 1a. **c**, Small vacuoles labelled with TIP3;2 close to EV tonoplast preceding the cleavage furrow division. Magenta shows autofluorescence detected in red part of the spectrum. Scale bars represent 20µm. **d**, Germination rate between indicated genotypes. Error bars represent SE. n>500. **e**, Analysis of normalized, relative *PTEN2b* overexpression by qRT-PCR in two independent transgenic lines induced with 2µM estradiol for the duration of 6 days. Error bars represent SE among three independent biological replicates. **f**, Representative confocal images of embryos dissected from dry seeds, 24h and 48h after imbibition of the indicated genotypes, stained with Calcofluor White (cellulose in cyan) and fuchsin (lignin in magenta). Yellow arrows mark xylem discontinuities in seedlings with induced *PTEN2b* overexpression from imbibition (2µM estradiol). Note the appearance of differentiated xylem cells (magenta) only 48h after germination. Scale bars 200 µm. **g**, Root length quantification of 6-days-old seedlings illustrate dose dependant effect of *PTEN2b* overexpression after 48h of estradiol induction. Error bars represent SE. n>40 h, *PTEN2b* overexpression prevents xylem differentiation in T3.4.40 line in both proximal and distal part of the root. Higher overexpression in the line T3.13.41 dramatically shortens the root length (g) but does not prevent xylem differentiation in younger distal root parts. Undifferentiated xylem is only labelled with MYB46 marker in yellow, while differentiated xylem is labelled with fuchsin staining for lignin in magenta, or white (overlap between yellow and magenta). Asterisk labels xylem position within vascular cylinder. White arrows label ectopic lignification in endodermis. Scale bars represent 100µm. n.s., not significant; *p<0.05; **p<0.01; ***p<0.001.

FIGURE 2

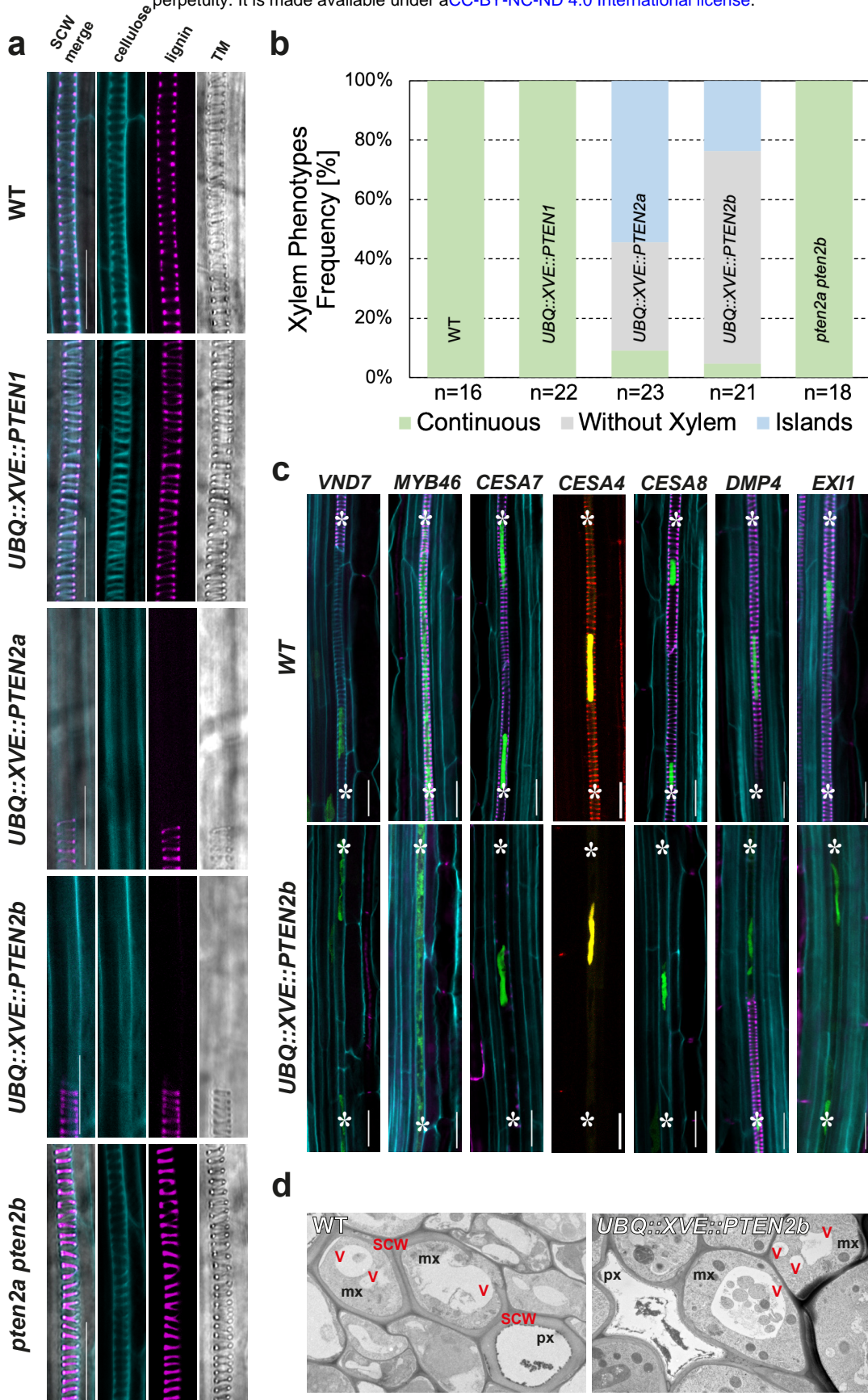
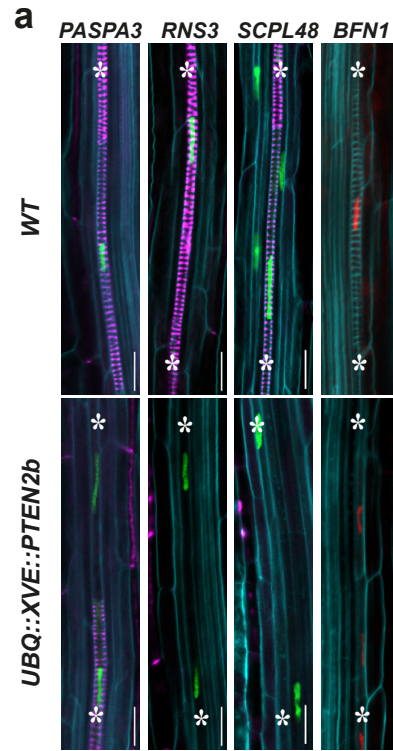


Figure 2: PTEN2s prevent xylem cell differentiation. **a**, Representative confocal images of protoxylem cells in the indicated genotypes stained with Calcofluor White (cellulose in cyan) and Fuchsin (lignin in magenta). Secondary cell wall (SCW) was also visualised by transmission light (TM). Scale bars represent 20µm. **b**, Quantification of xylem phenotypes observed in the roots displayed in **a**. The two phenotypes observed and scored were the total absence of xylem strands (without xylem) or the appearance of several protoxylem cells with SCW (islands). **c**, Representative images of indicated xylem differentiation markers in wildtype (WT) and seedlings incubated in 0.2µM estradiol for 48h to trigger *PTEN2box*. Roots were stained with Calcofluor White (cyan) and fuchsin (magenta). Marker lines: *VND7* (VASCULAR RELATED NAC-DOMAIN PROTEIN 7), *MYB46* (MYB DOMAIN PROTEIN 46), *CESA7* (CELLULOSE SYNTHASE CATALYTIC SUBUNIT 7), *CESA4* (CELLULOSE SYNTHASE A4), *CESA8* (CELLULOSE SYNTHASE 8), *DMP4* (DUF679 DOMAIN MEMBRANE PROTEIN 4), *EX11* (EXITUS 1). Scale bars represent 20µm. **d**, Transmission electron microscopy images of differentiating proto- (px) and metaxylem (mx) cells in WT and *PTEN2box*. Xylem cells in WT formed thick secondary cell wall (SCW), vacuoles are enlarging in mx while px underwent programmed cell death. *PTEN2b* overexpression prevents SCW formation while mx cells contain multiple small vacuoles. Here px cell also underwent clearance.

EXTENDED DATA FIGURE 2



Extended Data Figure 2: *PTEN2b* overexpression does not alter the expression of genes associated with xylem PCD execution. **a**, Representative confocal microscopy images of the mature protoxylem cells stained with Calcofluor White for cellulose (cyan) and fuchsin for lignin (magenta). *PTEN2b* was induced for 48h with 0.2 μM estradiol. Note the expression of genes associated with PCD such as the *PUTATIVE ASPARTIC PROTEINASE A3* (*PASPA3*), *RIBONUCLEASE 3* (*RNS3*), *SERINE CARBOXYPEPTIDASE-LIKE 48* (*SCPL48*) and *BIFUNCTIONAL NUCLEASE 1* (*BFN1*) can still be detected in seedlings with *PTEN2b* upregulation. Asterisks mark protoxylem strands. Scale bars represent 20 μm.

FIGURE 3

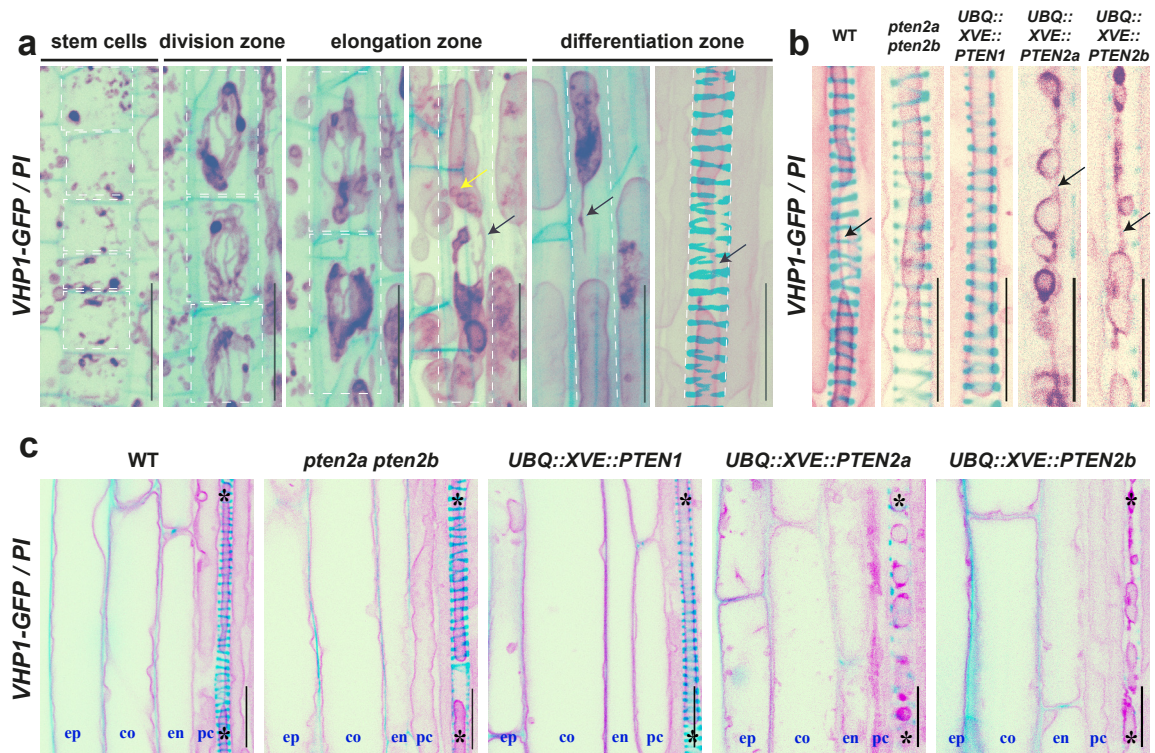


Figure 3: Regulation of xylem vacuolar biogenesis regulation by PTEN2s. **a**, 3D reconstruction of VHP1-GFP decorated vacuolar compartments in protoxylem cells in wild type plants at progressive developmental stages counterstained with propidium iodide (PI) to label cell wall. Yellow arrows mark small vacuole-like compartments and black arrows mark tubular connecting membranes. For easier visualization, protoxylem cells margins were squared by a white dashed line. Scale bars represent 20 μ m. **b**, Representative images of mature protoxylem cells in the indicated genotypes, visualized as in **a**. Scale bars represent 20 μ m. Black arrows mark tubular connecting membranes. **c**, Comparison of vacuolar morphology in mature epidermis (ep), cortex (co), endodermis (en), pericycle (pc) and protoxylem (asterisk) between indicated genotypes. Scale bars represent 20 μ m.

FIGURE 4

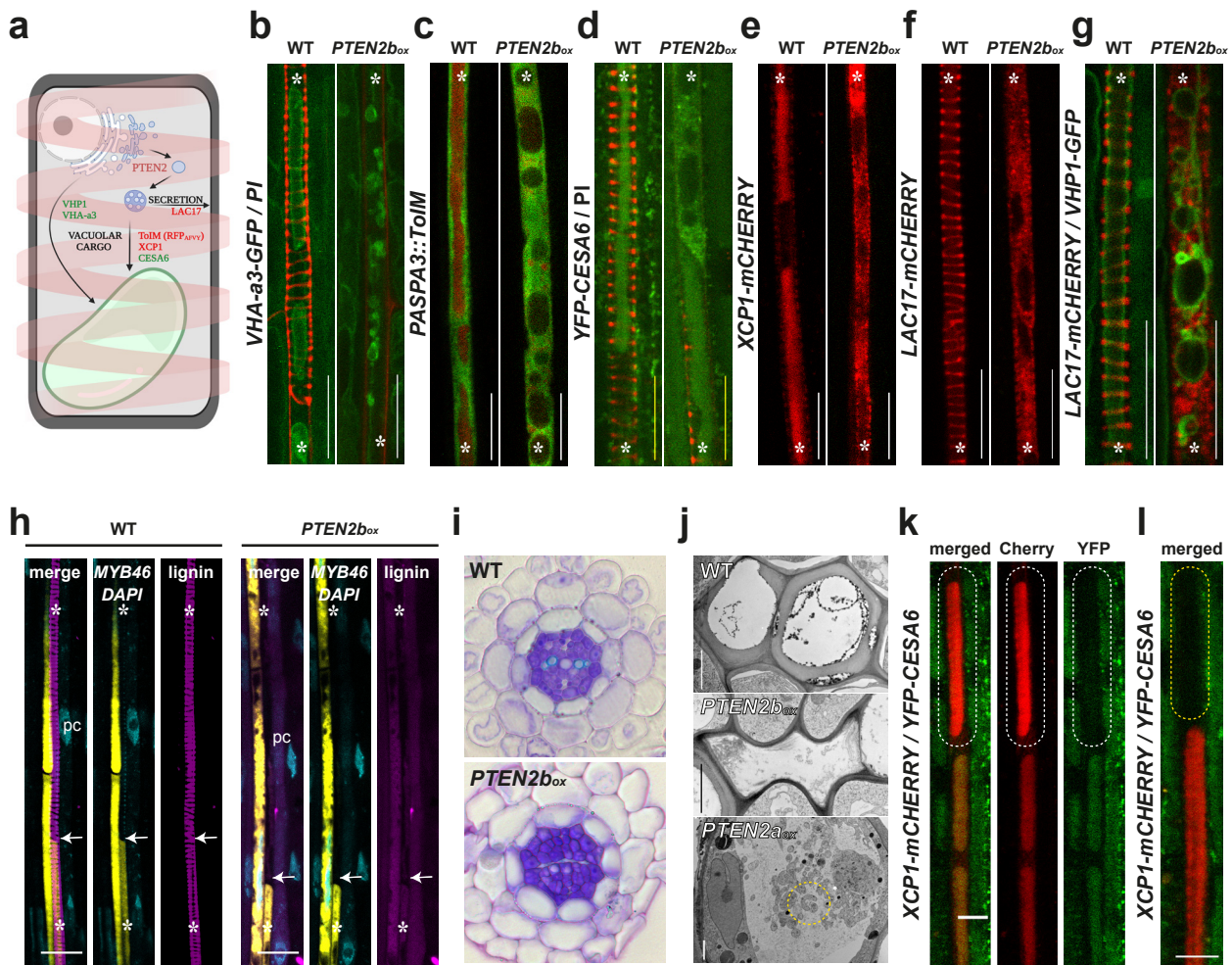
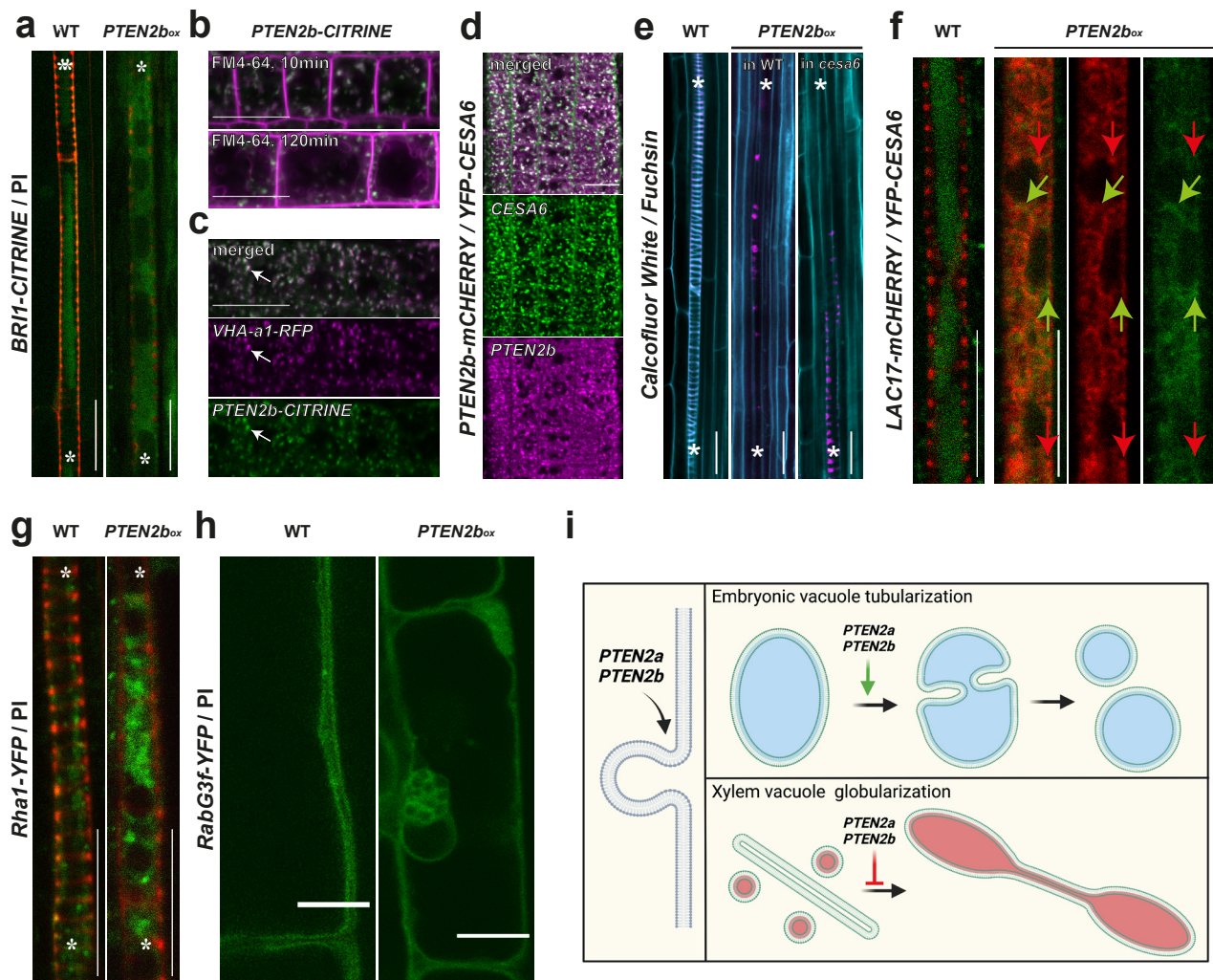


Figure 4: PTEN2s inhibit vacuolar and secretion trafficking pathway in xylem cells but not PCD. **a**, Schematic representation of analysed trafficking pathways important for xylem cell differentiation. **b-g**, Representative images of the corresponding xylem cells in wild type (WT) and seedlings with 0.2 μ M estradiol-mediated *PTEN2b* induction for 48h visualizing different trafficking markers: tonoplast marker VHA-a3 (VACUOLAR PROTON ATPASE A3) (**b**), xylem specific promoter *PAsPA3* (*PUTATIVE ASPARTIC PROTEINASE A3*) driving expression of ToIM (tonoplast integrity marker) showing GFP in cytosol and a vacuolar targeted mRFP (**c**), vacuolar cargos CESA6 (CELLULOSE SYNTHASE SUBUNIT A6) (**d**) and XCP1 (XYLEM CYSTEINE PEPTIDASE 1) (**e**), secreted cargo LAC17 (LACCASE 17) (**f**). **g**, non-secreted LAC17 is not delivered into VHP1-labeled vacuoles. **h-j**, PCD execution occurs even without SCW formation in *PTEN2box*. **h**, DAPI-stained nuclei are absent in the cells where the xylem-specific expression of *MYB46* ceased due to the PCD execution (white arrows). Scale bars represent 20 μ m. **i**, Toluidine-stained root cross-sections of the indicated genotypes. Xylem secondary cell wall stains bright blue as visible in WT but absent in *PTEN2box* overexpression where xylem cells appear collapsed as in transmission electron microscopy images (**j**). **j**, Transmission electron microscopy images of the indicated genotypes. Notice a high number of small vacuoles and aggregates in *PTEN2box*. Yellow dashed circle highlights a cup-shaped phagophore. Scale bars represent 2 μ m. **k**, Xylem vacuole acidification in wild type prior PCD. Note faster fading of pH-sensitive YFP in comparison to pH-tolerant mCherry. The cell where acidification occurs is encircled with a white dashed line. Scale bars represent 10 μ m. **l**, continuation cell from **k**, where PCD is executed and mCherry signal disappears too. Scale bars represent 10 μ m.

EXTENDED DATA FIGURE 4



Extended Data Figure 4: PTEN2b colocalize to TGN and impinges on vacuolar and cellular secretion pathways. **a**, Brassinosteroid receptor BRI1 (BRASSINOSTEROID INSENSITIVE 1) cannot be delivered to xylem vacuoles upon *PTEN2b* overexpression. Seedlings were counterstained with propidium iodide (PI). **b**, *PTEN2b* colocalize with cellular compartments early labelled with FM4-64 (magenta). **c**, *PTEN2b* partially colocalize with PROTON ATPASE A1 (VHA-a1) in TGN (arrows). **d**, *PTEN2b* partially colocalize with CELLULOSE SYNTHASE SUBUNIT A6 (CESA6). **e**, *cesa6* mutant cannot rescue secondary cellulose building upon *PTEN2b* overexpression. **f**, Aggregates of vesicles carrying vacuolar destined cargo (CESA6 in green) and secretion cargo (LAC17 in red) do not colocalize. Arrows' color corresponds to fluorophores and points the aggregates where proteins do not colocalize. **g**, Multivesicular body (MVB) marker Rha1 (ARABIDOPSIS RAB HOMOLOG F2A) creates aggregates in xylem cells upon *PTEN2b* upregulation. **h**, Prevacuolar compartment and tonoplast marker RabG3f (RAB GTPASE HOMOLOG G3F) upon prolonged *PTEN2b* overexpression creates grape like structures in vicinity of the central vacuole in mature epidermal cells. Asterisk labels xylem strands. Scale bars represent 20µm. **i**, Schematic representation of membrane phenomena regulated by PTEN2s.

FIGURE 5

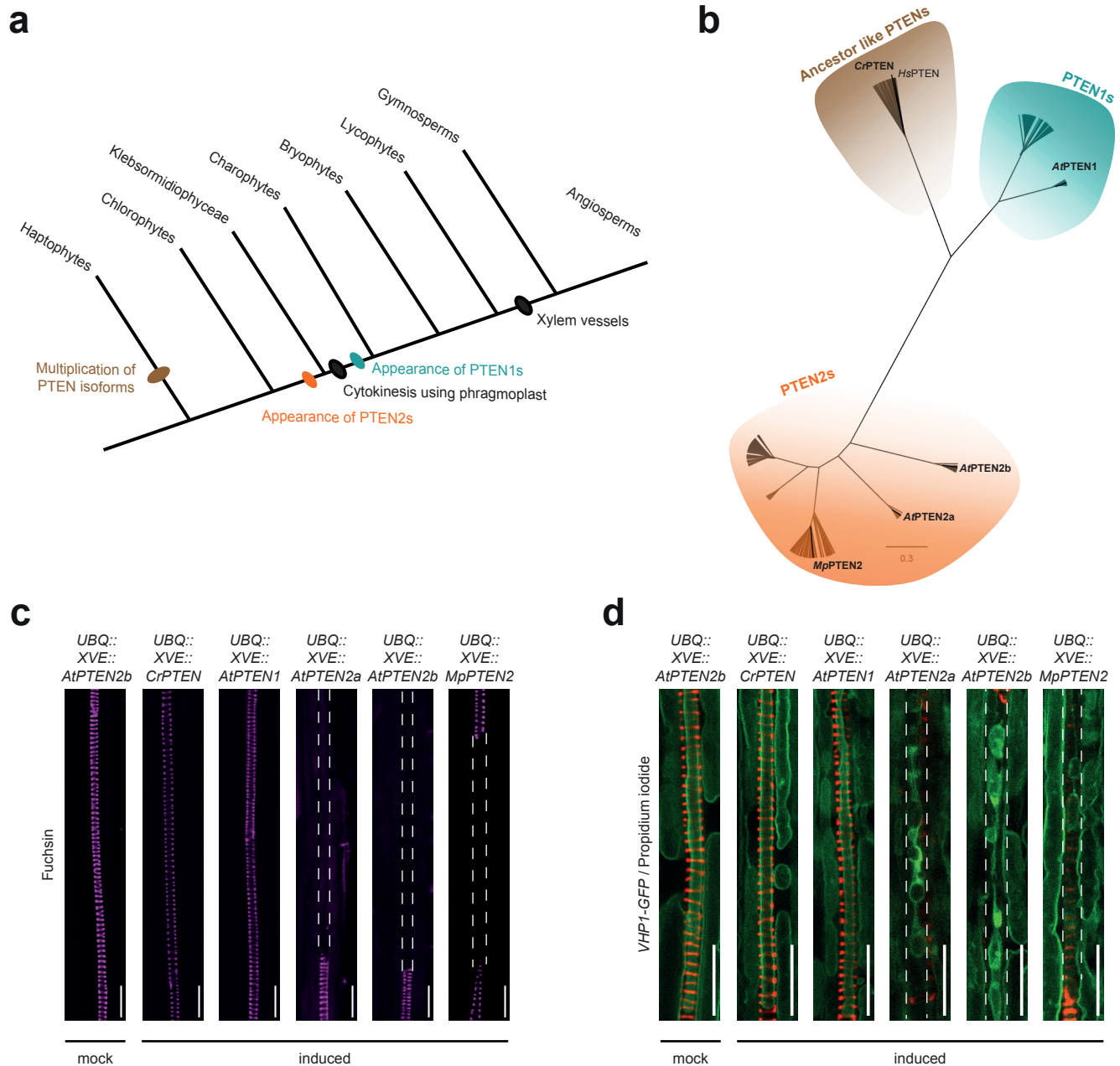


Figure 5: *PTEN2s* functions in vacuolar fusion and xylem differentiation were conserved through evolution. **a**, Schematic tree of the evolution of plant *PTEN* subfamilies. **b**, Phylogenetic tree of 418 plant *PTENs* from 142 plant species. For simplification, only the isoforms of species of interest have been represented. Details about all the sequences and the complete distribution of the isoforms in the three sub-families (ancestor-like *PTENs*, *PTEN1s*, *PTEN2s*) can be found in Supplementary Table 1. **c**, Representative confocal microscopy images of fuchsin-stained protoxylem strands from roots grown on mock conditions or upon 2 μ M estradiol-mediated induction for 48h of *Chlamydomonas reinhardtii* *PTEN* (*CrPTEN*), *Arabidopsis thaliana* *PTEN1* (*AtPTEN1*), *PTEN2a* (*AtPTEN2a*), and *PTEN2b* (*AtPTEN2b*), *Marchantia polymorpha* *PTEN2* (*MpPTEN2*). **d**, Representative confocal microscopy images of vacuolar morphology in mature xylem cells (VHP1-GFP labels tonoplast, propidium iodide stains cell wall) of inducible over-expressor lines of the different *PTEN* isoforms mentioned above. *PTEN* overexpression was induced for 48h with 2 μ M estradiol. Protoxylem gaps are highlighted with white dashed lines. Scale bars represent 20 μ m.

FIGURE 6

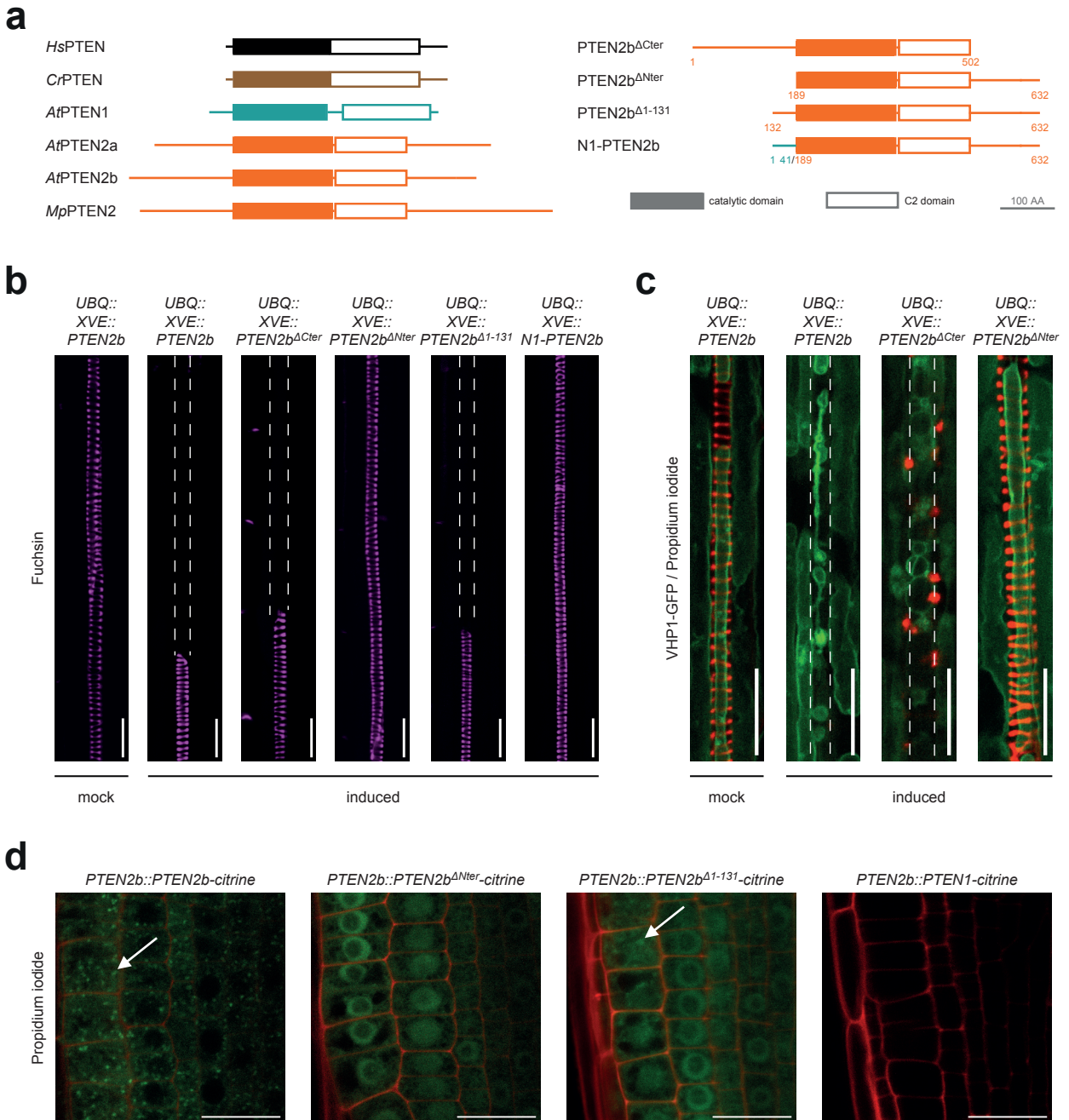
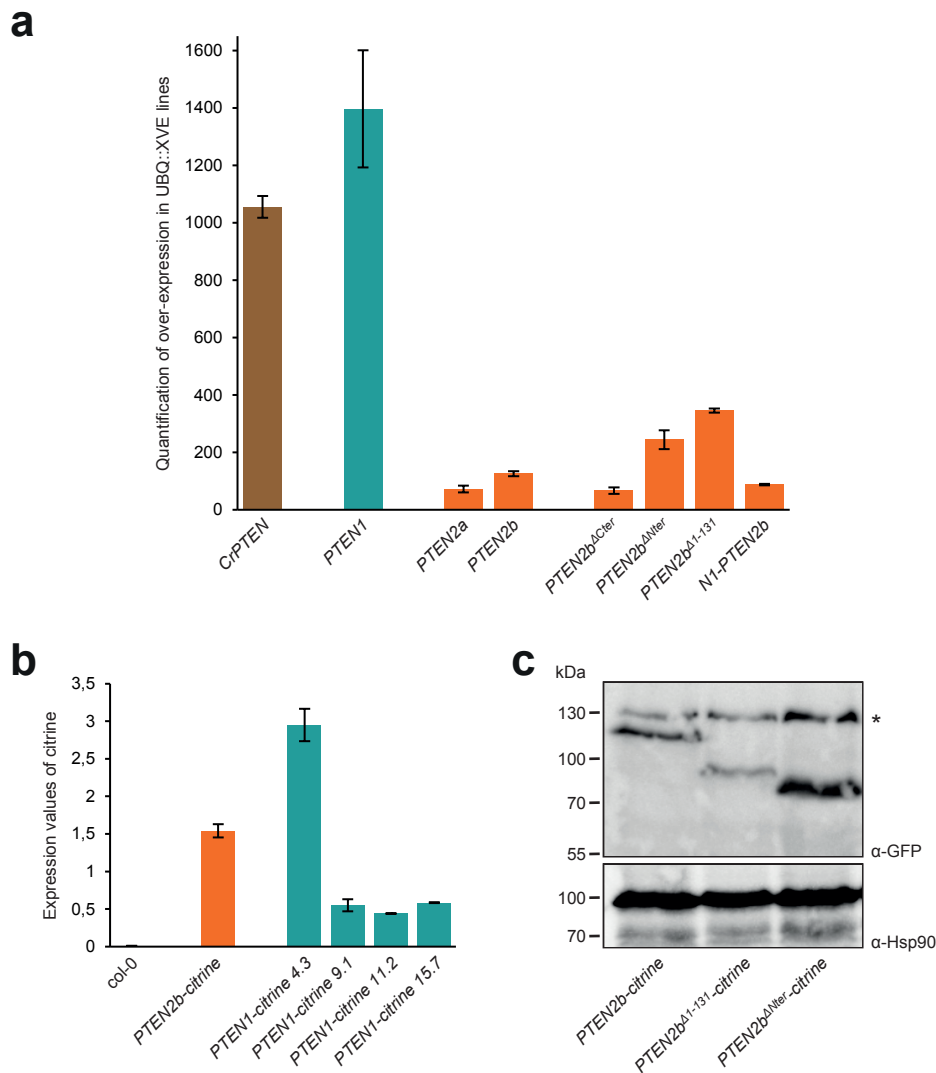


Figure 6: A conserved domain within PTEN2s N-terminal sequence is critical for their functionality and TGN anchoring. a. Schematic representation of the PTEN enzymes from *Homo Sapiens* (Hs), *Arabidopsis* (At), *Marchantia* (Mp) and *Chlamydomonas* (Cr) analysed in this study. On the right are represented the truncated version of PTEN2b without the entire C- (PTEN2b^{ΔCter}) or N-terminal (PTEN2b^{ΔNter}) sequences, PTEN2b with a partial N-terminal sequences (PTEN2b^{Δ1-131}) and the hybrid version with PTEN1 N-terminal (N1-PTEN2b). Colour filled boxes represent phosphatase catalytic domains whereas empty squared boxes represent C2 domains. **b.** Representative confocal microscopy images of fuchsin-stained protoxylem strands after 2 μ M estradiol-mediated induction for 48h of indicated PTEN2b versions. Protoxylem gap cells are highlighted with white dashed lines. **c.** Representative images of vacuole morphology in mature xylem cell upon 48h overexpression of indicated PTEN2b variants. VHP1-GFP labels tonoplast, while propidium iodide labels cell wall. Protoxylem gap cells are highlighted with white dashed lines. **d.** Representative confocal images of 6-day-old plants harbouring indicated constructs illustrating the dependence of PTEN2b localization at TGN to its N-terminal. Scale bars represent 20 μ m.

FIGURE S1



Supplementary Figure 1: Transgenic lines validation (Supporting data for Figures 6 and 7). **a**, qPCR analyses confirmed the over-expression of *PTEN* in different inducible lines described in Fig. 6 and Fig. 7. RNA was extracted from roots of 7day-old plants treated with DMSO or 2 μ M estradiol for 48hrs. Expression values of the different genes of interest in estradiol-treated plants were normalized by the corresponding expression values measured in DMSO-treated plants. Values represent the mean of two biological replicates (both including three technical replicates). Error bars indicate the standard deviation. **b**, qPCR analyses revealed the presence of CITRINE mRNA in independent *PTEN2b::PTEN1-CITRINE* that do not exhibit any fluorescence signal in root cells (Fig 6). Values represent the mean of three technical replicates, error bars indicate the standard deviation. **c**, The expression of the different Nter-truncated versions of PTEN2b tagged with citrine (Fig 6) was confirmed by Western blot using anti-GFP antibody. Anti-Hsp90 was used as a loading control. The star indicates the presence of an unspecific band.

Supplementary Table 1: Protein sequences used to build the phylogenetic tree

Organism	Protein name	Order	Clade	Database
<i>Acorus americanus</i>	Acora.04G145300	Acorales	Monocotyledones	phytozome
<i>Acorus americanus</i>	Acora.11G167200	Acorales	Monocotyledones	phytozome
<i>Aegilops tauschii</i>	LOC109747383	Poales	Monocotyledones	NCBI
<i>Alyssum linifolium</i>	Alyli.0051s0214	Brassicales	Eudicotyledones	phytozome
<i>Alyssum linifolium</i>	Alyli.0020s0220	Brassicales	Eudicotyledones	phytozome
<i>Alyssum linifolium</i>	Alyli.0204s0005	Brassicales	Eudicotyledones	phytozome
<i>Alyssum linifolium</i>	Alyli.0031s0213	Brassicales	Eudicotyledones	phytozome
<i>Alyssum linifolium</i>	Alyli.0216s0006	Brassicales	Eudicotyledones	phytozome
<i>Amaranthus hypochondriacus</i>	AH006150	Caryophyllales	Eudicotyledones	phytozome
<i>Amaranthus hypochondriacus</i>	AH001650	Caryophyllales	Eudicotyledones	phytozome
<i>Amborella trichopoda</i>	evm_27.model.AmTr_v1.0_scaff old00177.28	Amborellales	Amborellales	phytozome
<i>Amborella trichopoda</i>	evm_27.model.AmTr_v1.0_scaff old00051.97	Amborellales	Amborellales	phytozome
<i>Anacardium occidentale</i>	Anaoc.0018s0955	Sapindales	Eudicotyledones	phytozome
<i>Anacardium occidentale</i>	Anaoc.0017s0442	Sapindales	Eudicotyledones	phytozome
<i>Anacardium occidentale</i>	Anaoc.1291s0006	Sapindales	Eudicotyledones	phytozome
<i>Anacardium occidentale</i>	Anaoc.0003s1165	Sapindales	Eudicotyledones	phytozome
<i>Ananas comosus</i>	Aco005843	Poales	Monocotyledones	phytozome
<i>Ananas comosus</i>	Aco024739	Poales	Monocotyledones	phytozome
<i>Aquilegia coerulea</i>	Aqcoe3G057800	Ranunculales	Eudicotyledones	phytozome
<i>Aquilegia coerulea</i>	Aqcoe5G142900	Ranunculales	Eudicotyledones	phytozome
<i>Aquilegia coerulea</i>	Aqcoe6G311500	Ranunculales	Eudicotyledones	phytozome
<i>Arabidopsis halleri</i>	Araha.12280s0002	Brassicales	Eudicotyledones	phytozome
<i>Arabidopsis halleri</i>	Araha.2717s0004	Brassicales	Eudicotyledones	phytozome
<i>Arabidopsis lyrata</i>	AL7G50930	Brassicales	Eudicotyledones	phytozome
<i>Arabidopsis lyrata</i>	AL3G32830	Brassicales	Eudicotyledones	phytozome
<i>Arabidopsis lyrata</i>	AL5G30230	Brassicales	Eudicotyledones	phytozome
<i>Arabidopsis thaliana</i>	At5g39400	Brassicales	Eudicotyledones	phytozome
<i>Arabidopsis thaliana</i>	At3g19420	Brassicales	Eudicotyledones	phytozome
<i>Arabidopsis thaliana</i>	At3g50110	Brassicales	Eudicotyledones	phytozome
<i>Arachis hypogaea</i>	arahy.Tifrunner.gnm1.ann1.854 N7M	Fabales	Eudicotyledones	phytozome
<i>Arachis hypogaea</i>	arahy.Tifrunner.gnm1.ann1.PFF 9RU	Fabales	Eudicotyledones	phytozome
<i>Arachis hypogaea</i>	arahy.Tifrunner.gnm1.ann1.132 VHE	Fabales	Eudicotyledones	phytozome
<i>Arachis hypogaea</i>	arahy.Tifrunner.gnm1.ann1.69L NNJ	Fabales	Eudicotyledones	phytozome
<i>Arachis hypogaea</i>	arahy.Tifrunner.gnm1.ann1.AK4 RCU	Fabales	Eudicotyledones	phytozome
<i>Asparagus officinalis</i>	evm.model.AsparagusV1_08.13 99	Asparagales	Monocotyledones	phytozome
<i>Asparagus officinalis</i>	evm.model.AsparagusV1_07.85 7	Asparagales	Monocotyledones	phytozome
<i>Asparagus officinalis</i>	evm.model.AsparagusV1_08.32 54	Asparagales	Monocotyledones	phytozome
<i>Aureococcus anophagefferens</i>	AURANDRAFT_26423	Pelagomonadales	Heterokonts	NCBI
<i>Aureococcus anophagefferens</i>	AURANDRAFT_71576	Pelagomonadales	Heterokonts	NCBI
<i>Aureococcus anophagefferens</i>	AURANDRAFT_70790	Pelagomonadales	Heterokonts	NCBI
<i>Beta vulgaris</i>	EL10Ac9g22309	Caryophyllales	Eudicotyledones	phytozome
<i>Beta vulgaris</i>	EL10Ac6g15699	Caryophyllales	Eudicotyledones	phytozome

Organism	Protein name	Order	Clade	Database
<i>Betula platyphylla</i>	BPChr11G05686	Fagales	Eudicotyledones	phytozome
<i>Betula platyphylla</i>	BPChr06G22535	Fagales	Eudicotyledones	phytozome
<i>Boechea stricta</i>	Bostr.25849s0003	Brassicales	Eudicotyledones	phytozome
<i>Boechea stricta</i>	Bostr.19424s0720	Brassicales	Eudicotyledones	phytozome
<i>Boechea stricta</i>	Bostr.6864s0231	Brassicales	Eudicotyledones	phytozome
<i>Brachypodium distachyon</i>	Bradi4g08080	Poales	Monocotyledones	phytozome
<i>Brachypodium hybridum</i>	Brahy.D04G0113800	Poales	Monocotyledones	phytozome
<i>Brachypodium hybridum</i>	Brahy.S10G0102100	Poales	Monocotyledones	phytozome
<i>Brachypodium sylvaticum</i>	Brasy5G104500	Poales	Monocotyledones	phytozome
<i>Brassica oleracea capitata</i>	Bol040114	Brassicales	Eudicotyledones	phytozome
<i>Brassica oleracea capitata</i>	Bol018098	Brassicales	Eudicotyledones	phytozome
<i>Brassica oleracea capitata</i>	Bol031011	Brassicales	Eudicotyledones	phytozome
<i>Brassica oleracea capitata</i>	Bol016970	Brassicales	Eudicotyledones	phytozome
<i>Brassica rapa</i>	Brara.A02880	Brassicales	Eudicotyledones	phytozome
<i>Brassica rapa</i>	Brara.E02262	Brassicales	Eudicotyledones	phytozome
<i>Brassica rapa</i>	Brara.C04308	Brassicales	Eudicotyledones	phytozome
<i>Cakile maritima</i>	Camar.1507s0005	Brassicales	Eudicotyledones	phytozome
<i>Cakile maritima</i>	Camar.0166s0020	Brassicales	Eudicotyledones	phytozome
<i>Cakile maritima</i>	Camar.0140s0022	Brassicales	Eudicotyledones	phytozome
<i>Cakile maritima</i>	Camar.0046s0044	Brassicales	Eudicotyledones	phytozome
<i>Capsella grandiflora</i>	Cagra.12117s0008	Brassicales	Eudicotyledones	phytozome
<i>Capsella grandiflora</i>	Cagra.1211s0012	Brassicales	Eudicotyledones	phytozome
<i>Capsella grandiflora</i>	Cagra.26626s0001	Brassicales	Eudicotyledones	phytozome
<i>Capsella rubella</i>	Carub.0007s3654	Brassicales	Eudicotyledones	phytozome
<i>Capsella rubella</i>	Carub.0003s1923	Brassicales	Eudicotyledones	phytozome
<i>Capsella rubella</i>	Carub.0005s1645	Brassicales	Eudicotyledones	phytozome
<i>Carex littledalei</i>	FCM35_KLT03997	Cyperales	Monocotyledones	NCBI
<i>Carex littledalei</i>	FCM35_KLT07398	Cyperales	Monocotyledones	NCBI
<i>Carica papaya</i>	evm.model.supercontig_14.140	Brassicales	Eudicotyledones	phytozome
<i>Carica papaya</i>	evm.model.supercontig_200.15	Brassicales	Eudicotyledones	phytozome
<i>Carya illinoensis</i>	Caril.03G085200	Juglandales		phytozome
<i>Carya illinoensis</i>	Caril.04G059000	Juglandales		phytozome
<i>Carya illinoensis</i>	Caril.01G012500	Juglandales		phytozome
<i>Carya illinoensis</i>	Caril.06G171800	Juglandales		phytozome
<i>Carya illinoensis</i>	Caril.05G009200	Juglandales		phytozome
<i>Castanea dentata</i>	Caden.01G186200	Fagales	Eudicotyledones	phytozome
<i>Castanea dentata</i>	Caden.02G186200	Fagales	Eudicotyledones	phytozome
<i>Castanea dentata</i>	Caden.07G119900	Fagales	Eudicotyledones	phytozome
<i>Caulanthus amplexicaulis</i>	Caamp.0044s0232	Brassicales	Eudicotyledones	phytozome
<i>Caulanthus amplexicaulis</i>	Caamp.1041s0897	Brassicales	Eudicotyledones	phytozome
<i>Caulanthus amplexicaulis</i>	Caamp.1039s1127	Brassicales	Eudicotyledones	phytozome
<i>Caulanthus amplexicaulis</i>	Caamp.0051s0388	Brassicales	Eudicotyledones	phytozome
<i>Caulanthus amplexicaulis</i>	Caamp.0078s0093	Brassicales	Eudicotyledones	phytozome
<i>Caulanthus amplexicaulis</i>	Caamp.0026s0499	Brassicales	Eudicotyledones	phytozome
<i>Ceratodon purpureus</i>	CepurGG1.8G115300	Dicranales	Bryophytes	phytozome

Organism	Protein name	Order	Clade	Database
<i>Ceratodon purpureus</i>	CepurGG1.11G093000	Dicranales	Bryophytes	phytozome
<i>Ceratopteris richardii</i>	Ceric.25G072600	Polypodiales	Monilophytes	phytozome
<i>Ceratopteris richardii</i>	Ceric.16G021900	Polypodiales	Monilophytes	phytozome
<i>Chara braunii</i>	CBR_g29865	Charales	Charophytes	NCBI
<i>Chara braunii</i>	CBR_g30221	Charales	Charophytes	NCBI
<i>Chenopodium quinoa</i>	AUR62013248	Caryophyllales	Eudicotyledones	phytozome
<i>Chenopodium quinoa</i>	AUR62010267	Caryophyllales	Eudicotyledones	phytozome
<i>Chlamydomonas reinhardtii</i>	Cre06.g308400	Chlamydomonales	Chlorophytes	phytozome
<i>Chromochloris zofigiensis</i>	Cz03g22130.t1	Sphaeropleales	Chlorophytes	phytozome
<i>Chrysochromulina tobinii</i>	Ctob_005637	Prymnesiales	Haptophytes	NCBI
<i>Chrysochromulina tobinii</i>	Ctob_015421	Prymnesiales	Haptophytes	NCBI
<i>Chrysochromulina tobinii</i>	Ctob_003434	Prymnesiales	Haptophytes	NCBI
<i>Chrysochromulina tobinii</i>	Ctob_004562	Prymnesiales	Haptophytes	NCBI
<i>Chrysochromulina tobinii</i>	Ctob_005565	Prymnesiales	Haptophytes	NCBI
<i>Cicer arietinum</i>	Ca_02178	Fabales	Eudicotyledones	phytozome
<i>Cicer arietinum</i>	Ca_05590	Fabales	Eudicotyledones	phytozome
<i>Cinnamomum kanehirae</i>	CKAN_02766500	Laurales	Magnoliides	phytozome
<i>Cinnamomum kanehirae</i>	CKAN_02500000	Laurales	Magnoliides	phytozome
<i>Cinnamomum kanehirae</i>	CKAN_01173600	Laurales	Magnoliides	phytozome
<i>Cinnamomum kanehirae</i>	CKAN_00608500	Laurales	Magnoliides	phytozome
<i>Citrus clementina</i>	Ciclev10011769m	Sapindales	Eudicotyledones	phytozome
<i>Citrus clementina</i>	Ciclev10025539m	Sapindales	Eudicotyledones	phytozome
<i>Citrus clementina</i>	Ciclev10028042m	Sapindales	Eudicotyledones	phytozome
<i>Citrus sinensis</i>	orange1.1g012952m	Sapindales	Eudicotyledones	phytozome
<i>Citrus sinensis</i>	orange1.1g014325m	Sapindales	Eudicotyledones	phytozome
<i>Citrus sinensis</i>	orange1.1g037030m	Sapindales	Eudicotyledones	phytozome
<i>Cleome violacea</i>	Clevi.0004s2083	Brassicales	Eudicotyledones	phytozome
<i>Cleome violacea</i>	Clevi.0032s0574	Brassicales	Eudicotyledones	phytozome
<i>Cleome violacea</i>	Clevi.0005s2503	Brassicales	Eudicotyledones	phytozome
<i>Coccomyxa subellipsoidea</i>	66502		Chlorophytes	phytozome
<i>Coffea arabica</i>	evm.model.Scaffold_952.463	Gentianales	Eudicotyledones	phytozome
<i>Coffea arabica</i>	evm.model.Scaffold_612.518	Gentianales	Eudicotyledones	phytozome
<i>Coffea arabica</i>	evm.model.Scaffold_634.624	Gentianales	Eudicotyledones	phytozome
<i>Coffea arabica</i>	evm.model.Scaffold_952.172	Gentianales	Eudicotyledones	phytozome
<i>Corymbia citriodora</i>	Cocit.A0156	Myrtales	Eudicotyledones	phytozome
<i>Corymbia citriodora</i>	Cocit.L5056	Myrtales	Eudicotyledones	phytozome
<i>Corymbia citriodora</i>	Cocit.G0778	Myrtales	Eudicotyledones	phytozome
<i>Crambe hispanica</i>	Crahi.1829s0005	Brassicales	Eudicotyledones	phytozome
<i>Crambe hispanica</i>	Crahi.0455s0005	Brassicales	Eudicotyledones	phytozome
<i>Crambe hispanica</i>	Crahi.0412s0031	Brassicales	Eudicotyledones	phytozome
<i>Crambe hispanica</i>	Crahi.0943s0003	Brassicales	Eudicotyledones	phytozome
<i>Crambe hispanica</i>	Crahi.0276s0014	Brassicales	Eudicotyledones	phytozome
<i>Cucumis sativus</i>	Cucsa.338540	Cucurbitales	Eudicotyledones	phytozome
<i>Daucus carota</i>	DCAR_001632	Apiales	Eudicotyledones	phytozome
<i>Daucus carota</i>	DCAR_029077	Apiales	Eudicotyledones	phytozome

Organism	Protein name	Order	Clade	Database
<i>Daucus carota</i>	DCAR_010857	Apiales	Eudicotyledones	phytozome
<i>Descurainia sophioides</i>	Desop.0231s0613	Brassicales	Eudicotyledones	phytozome
<i>Descurainia sophioides</i>	Desop.0248s0666	Brassicales	Eudicotyledones	phytozome
<i>Descurainia sophioides</i>	Desop.0207s0242	Brassicales	Eudicotyledones	phytozome
<i>Dichantheium oligosanthes</i>	BAE44_0008647	Poales	Monocotyledones	NCBI
<i>Dioscorea alata</i>	Dioal.19G152700	Dioscoreales	Monocotyledones	phytozome
<i>Diptychocarpus strictus</i>	Distr.0012s22625	Brassicales	Eudicotyledones	phytozome
<i>Diptychocarpus strictus</i>	Distr.0005s223300	Brassicales	Eudicotyledones	phytozome
<i>Diptychocarpus strictus</i>	Distr.0006s170500	Brassicales	Eudicotyledones	phytozome
<i>Ectocarpus siliculosus</i>	Esi_0301_0020	Ectocarpales	Heterokonts	NCBI
<i>Ectocarpus siliculosus</i>	Esi_0147_0077	Ectocarpales	Heterokonts	NCBI
<i>Ectocarpus siliculosus</i>	Esi_0147_0079	Ectocarpales	Heterokonts	NCBI
<i>Elaeis guineensis</i>	LOC105056003	Arecales	Monocotyledones	NCBI
<i>Elaeis guineensis</i>	LOC105045483	Arecales	Monocotyledones	NCBI
<i>Elaeis guineensis</i>	LOC105035125	Arecales	Monocotyledones	NCBI
<i>Eleusine coracana</i>	ELECO.r07.5BG0436130	Poales	Monocotyledones	phytozome
<i>Eleusine coracana</i>	ELECO.r07.5AG0388310	Poales	Monocotyledones	phytozome
<i>Emiliania huxleyi</i>	EMIHUDDRAFT_195717	Isochrysidales	Haptophytes	NCBI
<i>Emiliania huxleyi</i>	EMIHUDDRAFT_437207	Isochrysidales	Haptophytes	NCBI
<i>Eragrostis curvula</i>	EJB05_06262	Cyperales	Monocotyledones	NCBI
<i>Eragrostis curvula</i>	EJB05_01954	Cyperales	Monocotyledones	NCBI
<i>Eruca vesicaria</i>	Eruve.3288s0001	Brassicales	Eudicotyledones	phytozome
<i>Eruca vesicaria</i>	Eruve.2621s0007	Brassicales	Eudicotyledones	phytozome
<i>Eruca vesicaria</i>	Eruve.2403s0005	Brassicales	Eudicotyledones	phytozome
<i>Eruca vesicaria</i>	Eruve.0429s0037	Brassicales	Eudicotyledones	phytozome
<i>Eruca vesicaria</i>	Eruve.1019s0020	Brassicales	Eudicotyledones	phytozome
<i>Eruca vesicaria</i>	Eruve.1393s0007	Brassicales	Eudicotyledones	phytozome
<i>Eucalyptus grandis</i>	Eucgr.J01367	Myrtales	Eudicotyledones	phytozome
<i>Eucalyptus grandis</i>	Eucgr.G01374	Myrtales	Eudicotyledones	phytozome
<i>Eucalyptus grandis</i>	Eucgr.I00804	Myrtales	Eudicotyledones	phytozome
<i>Euclidium syriacum</i>	Eusyr.0002s0385	Brassicales	Eudicotyledones	phytozome
<i>Euclidium syriacum</i>	Eusyr.0017s0515	Brassicales	Eudicotyledones	phytozome
<i>Euclidium syriacum</i>	Eusyr.0120s0222	Brassicales	Eudicotyledones	phytozome
<i>Eutrema salsugineum</i>	Thhalv10027783m	Brassicales	Eudicotyledones	phytozome
<i>Eutrema salsugineum</i>	Thhalv10020332m	Brassicales	Eudicotyledones	phytozome
<i>Eutrema salsugineum</i>	Thhalv10010193m	Brassicales	Eudicotyledones	phytozome
<i>Fragaria vesca</i>	FvH4_6g47530	Rosales	Eudicotyledones	phytozome
<i>Fragaria vesca</i>	FvH4_2g05490	Rosales	Eudicotyledones	phytozome
<i>Fragaria vesca</i>	FvH4_1g16580	Rosales	Eudicotyledones	phytozome
<i>Ginkgo biloba</i>	GBI00005494	Ginkgoales	Acrogymnosperms	Gymno plaza 1.0
<i>Glycine max</i>	GlysoPI483463.08G218300	Fabales	Eudicotyledones	phytozome
<i>Glycine max</i>	GlysoPI483463.01G151800	Fabales	Eudicotyledones	phytozome
<i>Glycine max</i>	GlysoPI483463.11G048000	Fabales	Eudicotyledones	phytozome
<i>Glycine max</i>	GlysoPI483463.10G224200	Fabales	Eudicotyledones	phytozome
<i>Glycine max</i>	GlysoPI483463.20G094900	Fabales	Eudicotyledones	phytozome

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<i>Gnetum montanum</i>	GMO00031901	Gnetales	Acrogymnosperms	Gymno plaza 1.0
<i>Gossypium bardadense</i>	Gobar.A11G078400	Malvales	Eudicotyledones	phytozome
<i>Gossypium bardadense</i>	Gobar.D11G079100	Malvales	Eudicotyledones	phytozome
<i>Gossypium bardadense</i>	Gobar.D11G053600	Malvales	Eudicotyledones	phytozome
<i>Gossypium bardadense</i>	Gobar.A11G053700	Malvales	Eudicotyledones	phytozome
<i>Gossypium bardadense</i>	Gobar.D02G241200	Malvales	Eudicotyledones	phytozome
<i>Gossypium bardadense</i>	Gobar.A03G202000	Malvales	Eudicotyledones	phytozome
<i>Gossypium bardadense</i>	Gobar.A12G022900	Malvales	Eudicotyledones	phytozome
<i>Gossypium bardadense</i>	Gobar.D12G024700	Malvales	Eudicotyledones	phytozome
<i>Gossypium bardadense</i>	Gobar.D01G007700	Malvales	Eudicotyledones	phytozome
<i>Gossypium bardadense</i>	Gobar.A01G007300	Malvales	Eudicotyledones	phytozome
<i>Gossypium hirsutum</i>	Gohir.A11G071400	Malvales	Eudicotyledones	phytozome
<i>Gossypium hirsutum</i>	Gohir.D11G050950	Malvales	Eudicotyledones	phytozome
<i>Gossypium hirsutum</i>	Gohir.A11G047500	Malvales	Eudicotyledones	phytozome
<i>Gossypium hirsutum</i>	Gohir.D11G051100	Malvales	Eudicotyledones	phytozome
<i>Guillardia theta</i>	GUITHDRAFT_146552	Pyrenomonadales	Cryptomonades	NCBI
<i>Guillardia theta</i>	GUITHDRAFT_158972	Pyrenomonadales	Cryptomonades	NCBI
<i>Guillardia theta</i>	GUITHDRAFT_136489	Pyrenomonadales	Cryptomonades	NCBI
<i>Helianthus annuus</i>	HanXRQChr15g0473081	Asterales	Eudicotyledones	phytozome
<i>Helianthus annuus</i>	HanXRQChr03g0070181	Asterales	Eudicotyledones	phytozome
<i>Helianthus annuus</i>	HanXRQChr04g0128111	Asterales	Eudicotyledones	phytozome
<i>Helianthus annuus</i>	HanXRQChr11g0348401	Asterales	Eudicotyledones	phytozome
<i>Hydrangea quercifolia</i>	Hyque.05G148600	Rosales	Eudicotyledones	phytozome
<i>Hydrangea quercifolia</i>	Hyque.03G170500	Rosales	Eudicotyledones	phytozome
<i>Hydrangea quercifolia</i>	Hyque.13G030400	Rosales	Eudicotyledones	phytozome
<i>Iberis amara</i>	lbeam.3529s0003	Brassicales	Eudicotyledones	phytozome
<i>Iberis amara</i>	lbeam.1232s0005	Brassicales	Eudicotyledones	phytozome
<i>Iberis amara</i>	lbeam.6756s0002	Brassicales	Eudicotyledones	phytozome
<i>Iberis amara</i>	lbeam.3246s0002	Brassicales	Eudicotyledones	phytozome
<i>Isatis tinctoria</i>	Isati.8565s0004	Brassicales	Eudicotyledones	phytozome
<i>Isatis tinctoria</i>	Isati.0832s0027	Brassicales	Eudicotyledones	phytozome
<i>Isatis tinctoria</i>	Isati.0178s0011	Brassicales	Eudicotyledones	phytozome
<i>Isatis tinctoria</i>	Isati.1336s0016	Brassicales	Eudicotyledones	phytozome
<i>Isatis tinctoria</i>	Isati.1514s0004	Brassicales	Eudicotyledones	phytozome
<i>Joinvillea ascendens</i>	Joasc.14G102000	Poales	Monocotyledones	phytozome
<i>Kalanchoe fedtschenkoi</i>	Kaladp0026s0043	Saxifragales	Eudicotyledones	phytozome
<i>Kalanchoe fedtschenkoi</i>	Kaladp0015s0211	Saxifragales	Eudicotyledones	phytozome
<i>Kalanchoe fedtschenkoi</i>	Kaladp0058s0422	Saxifragales	Eudicotyledones	phytozome
<i>Kalanchoe fedtschenkoi</i>	Kaladp0028s0094	Saxifragales	Eudicotyledones	phytozome
<i>Klebsormidium nitens</i>	GAQ85806	Klebsormidiales	Charophytes	NCBI
<i>Klebsormidium nitens</i>	GAQ91864	Klebsormidiales	Charophytes	NCBI
<i>Klebsormidium nitens</i>	GAQ91212	Klebsormidiales	Charophytes	NCBI
<i>Klebsormidium nitens</i>	GAQ79654	Klebsormidiales	Charophytes	NCBI
<i>Lactuca sativa</i>	Lsat_1_v5_gn_3_98660	Asterales	Eudicotyledones	phytozome
<i>Lactuca sativa</i>	Lsat_1_v5_gn_3_98880	Asterales	Eudicotyledones	phytozome

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<i>Lactuca sativa</i>	Lsat_1_v5_gn_7_89141	Asterales	Eudicotyledones	phytozome
<i>Lactuca sativa</i>	Lsat_1_v5_gn_3_98761	Asterales	Eudicotyledones	phytozome
<i>Lactuca sativa</i>	Lsat_1_v5_gn_3_98721	Asterales	Eudicotyledones	phytozome
<i>Lactuca sativa</i>	Lsat_1_v5_gn_3_90360	Asterales	Eudicotyledones	phytozome
<i>Lactuca sativa</i>	Lsat_1_v5_gn_9_111380	Asterales	Eudicotyledones	phytozome
<i>Lactuca sativa</i>	Lsat_1_v5_gn_5_70961	Asterales	Eudicotyledones	phytozome
<i>Lepidium sativum</i>	Lesat.0070s0837	Brassicales	Eudicotyledones	phytozome
<i>Lepidium sativum</i>	Lesat.0041s0230	Brassicales	Eudicotyledones	phytozome
<i>Lepidium sativum</i>	Lesat.0086s0034	Brassicales	Eudicotyledones	phytozome
<i>Lepidium sativum</i>	Lesat.0019s0255	Brassicales	Eudicotyledones	phytozome
<i>Lepidium sativum</i>	Lesat.0013s0306	Brassicales	Eudicotyledones	phytozome
<i>Lepidium sativum</i>	Lesat.0024s0016	Brassicales	Eudicotyledones	phytozome
<i>Lindenbergia philippensis</i>	Liphi.09G031000	Lamiales	Eudicotyledones	phytozome
<i>Lindenbergia philippensis</i>	Liphi.02G151000	Lamiales	Eudicotyledones	phytozome
<i>Lindenbergia philippensis</i>	Liphi.09G060300	Lamiales	Eudicotyledones	phytozome
<i>Linum usitatissimum</i>	Lus10027876	Malpighiales	Eudicotyledones	phytozome
<i>Linum usitatissimum</i>	Lus10002826	Malpighiales	Eudicotyledones	phytozome
<i>Lotus japonicus</i>	Lj1g0022114	Fabales	Eudicotyledones	phytozome
<i>Lotus japonicus</i>	Lj2g0026106	Fabales	Eudicotyledones	phytozome
<i>Lotus japonicus</i>	Lj5g0017669	Fabales	Eudicotyledones	phytozome
<i>Lunaria annua</i>	Luann.0281s0031	Brassicales	Eudicotyledones	phytozome
<i>Lunaria annua</i>	Luann.0007s0091	Brassicales	Eudicotyledones	phytozome
<i>Lunaria annua</i>	Luann.0026s0012	Brassicales	Eudicotyledones	phytozome
<i>Lunaria annua</i>	Luann.0006s0072	Brassicales	Eudicotyledones	phytozome
<i>Lupinus albus</i>	Lalb_Ch17g0337091	Fabales	Eudicotyledones	phytozome
<i>Lupinus albus</i>	Lalb_Ch04g0258551	Fabales	Eudicotyledones	phytozome
<i>Lupinus albus</i>	Lalb_Ch16g0378681	Fabales	Eudicotyledones	phytozome
<i>Lupinus albus</i>	Lalb_Ch21g0308431	Fabales	Eudicotyledones	phytozome
<i>Malcomia maritima</i>	Mamar.0082s0142	Brassicales	Eudicotyledones	phytozome
<i>Malcomia maritima</i>	Mamar.0029s0743	Brassicales	Eudicotyledones	phytozome
<i>Malcomia maritima</i>	Mamar.0003s0492	Brassicales	Eudicotyledones	phytozome
<i>Malus domestica</i>	MD17G1056300	Rosales	Eudicotyledones	phytozome
<i>Malus domestica</i>	MD09G1060900	Rosales	Eudicotyledones	phytozome
<i>Malus domestica</i>	MD02G1177900	Rosales	Eudicotyledones	phytozome
<i>Malus domestica</i>	MD15G1287800	Rosales	Eudicotyledones	phytozome
<i>Malus domestica</i>	MD05G1087100	Rosales	Eudicotyledones	phytozome
<i>Manihot esculenta</i>	Manes.02G050801	Malpighiales	Eudicotyledones	phytozome
<i>Manihot esculenta</i>	Manes.12G108000	Malpighiales	Eudicotyledones	phytozome
<i>Manihot esculenta</i>	Manes.10G077300	Malpighiales	Eudicotyledones	phytozome
<i>Marchantia polymorpha</i>	Mapoly0058s0093	Marchantiales	Marchantiophytes	phytozome
<i>Marchantia polymorpha</i>	Mapoly0016s0179	Marchantiales	Marchantiophytes	phytozome
<i>Marchantia polymorpha</i>	Mapoly0117s0012	Marchantiales	Marchantiophytes	phytozome
<i>Medicago truncatula</i>	Medtr7g012250	Fabales	Eudicotyledones	phytozome
<i>Medicago truncatula</i>	Medtr5g016550	Fabales	Eudicotyledones	phytozome
<i>Medicago truncatula</i>	Medtr1g107540	Fabales	Eudicotyledones	phytozome

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<i>Mimulus guttatus</i>	Migut.H00656	Lamiales	Eudicotyledones	phytozome
<i>Mimulus guttatus</i>	Migut.H00402	Lamiales	Eudicotyledones	phytozome
<i>Mimulus guttatus</i>	Migut.B00375	Lamiales	Eudicotyledones	phytozome
<i>Miscanthus sinensis</i>	Misin03G029900	Cyperales	Monocotyledones	phytozome
<i>Miscanthus sinensis</i>	Misin04G012900	Cyperales	Monocotyledones	phytozome
<i>Musa acuminata</i>	GSMUA_Achr10P29220_001	Zingiberales	Monocotyledones	phytozome
<i>Musa acuminata</i>	GSMUA_Achr7P21640_001	Zingiberales	Monocotyledones	phytozome
<i>Musa acuminata</i>	GSMUA_Achr5P07740_001	Zingiberales	Monocotyledones	phytozome
<i>Musa balbisiana</i>	C4D60_Mb05t09990	Zingiberales	Monocotyledones	NCBI
<i>Musa balbisiana</i>	C4D60_Mb07t05750	Zingiberales	Monocotyledones	NCBI
<i>Musa balbisiana</i>	C4D60_Mb10t02290	Zingiberales	Monocotyledones	NCBI
<i>Myagrum perfoliatum</i>	Myper.0019s0558	Brassicales	Eudicotyledones	phytozome
<i>Myagrum perfoliatum</i>	Myper.0009s1500	Brassicales	Eudicotyledones	phytozome
<i>Myagrum perfoliatum</i>	Myper.0005s1433	Brassicales	Eudicotyledones	phytozome
<i>Nelumbo nucifera</i>	LOC104598017	Proteales	Eudicotyledones	NCBI
<i>Nelumbo nucifera</i>	LOC104589843	Proteales	Eudicotyledones	NCBI
<i>Nelumbo nucifera</i>	LOC104605416	Proteales	Eudicotyledones	NCBI
<i>Nymphaea colorata</i>	Nycol.I01022	Nymphaeales	Nymphaeales	phytozome
<i>Nymphaea colorata</i>	Nycol.C00833	Nymphaeales	Nymphaeales	phytozome
<i>Olea europaea</i>	Oeu062142	Lamiales	Eudicotyledones	phytozome
<i>Olea europaea</i>	Oeu014788	Lamiales	Eudicotyledones	phytozome
<i>Olea europaea</i>	Oeu001763	Lamiales	Eudicotyledones	phytozome
<i>Olea europaea</i>	Oeu044292	Lamiales	Eudicotyledones	phytozome
<i>Oryza sativa</i>	LOC_Os12g21890	Poales	Monocotyledones	phytozome
<i>Panicum hallii</i>	Pahal.2G380700	Poales	Monocotyledones	phytozome
<i>Panicum virgatum</i>	Pavir.2KG446900	Poales	Monocotyledones	phytozome
<i>Panicum virgatum</i>	Pavir.2NG500600	Poales	Monocotyledones	phytozome
<i>Paspalum vaginatum</i>	Pavag02G285600	Poales	Monocotyledones	phytozome
<i>Pharus latifolius</i>	Phala.10G061700	Poales	Monocotyledones	phytozome
<i>Phaseolus vulgaris</i>	Phvul.008G036300	Fabales	Eudicotyledones	phytozome
<i>Phaseolus vulgaris</i>	Phvul.002G015700	Fabales	Eudicotyledones	phytozome
<i>Phaseolus vulgaris</i>	Phvul.007G038300	Fabales	Eudicotyledones	phytozome
<i>Phoenix dactylifera</i>	LOC103711183	Arecales	Monocotyledones	NCBI
<i>Phoenix dactylifera</i>	LOC103706227	Arecales	Monocotyledones	NCBI
<i>Phoenix dactylifera</i>	LOC103705030	Arecales	Monocotyledones	NCBI
<i>Physcomitrella patens</i>	Pp3c19_18320V3	Funariales	Bryophytes	phytozome
<i>Physcomitrella patens</i>	Pp3c22_10340V3	Funariales	Bryophytes	phytozome
<i>Physcomitrella patens</i>	Pp3c21_8410V3	Funariales	Bryophytes	phytozome
<i>Physcomitrella patens</i>	Pp3c22_14420V3	Funariales	Bryophytes	phytozome
<i>Picea glauca</i>	PGL00015278	Pinales	Acrogymnosperms	Gymno plaza 1.0
<i>Pinus pinaster</i>	PPI00036745	Pinales	Acrogymnosperms	Gymno plaza 1.0
<i>Pinus pinaster</i>	PPI00005815	Pinales	Acrogymnosperms	Gymno plaza 1.0
<i>Pinus pinaster</i>	PPI00034805	Pinales	Acrogymnosperms	Gymno plaza 1.0
<i>Pinus sylvestris</i>	PSY00021791	Pinales	Acrogymnosperms	Gymno plaza 1.0

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<i>Pinus sylvestris</i>	PSY00016716	Pinales	Acrogymnosperms	Gymno plaza 1.0
<i>Pinus taeda</i>	PTA00064026	Pinales	Acrogymnosperms	Gymno plaza 1.0
<i>Pinus taeda</i>	PTA00032593	Pinales	Acrogymnosperms	Gymno plaza 1.0
<i>Pinus taeda</i>	PTA00009584	Pinales	Acrogymnosperms	Gymno plaza 1.0
<i>Poncirus trifoliata</i>	Ptrif.0006s2220	Sapindales	Eudicotyledones	phytozome
<i>Poncirus trifoliata</i>	Ptrif.0007s0475	Sapindales	Eudicotyledones	phytozome
<i>Poncirus trifoliata</i>	Ptrif.0008s0442	Sapindales	Eudicotyledones	phytozome
<i>Populus deltoides</i>	Podel.17G093700	Malpighiales	Eudicotyledones	phytozome
<i>Populus deltoides</i>	Podel.07G056300	Malpighiales	Eudicotyledones	phytozome
<i>Populus deltoides</i>	Podel.01G321200	Malpighiales	Eudicotyledones	phytozome
<i>Populus deltoides</i>	Podel.09G100700	Malpighiales	Eudicotyledones	phytozome
<i>Populus trichocarpa</i>	Potri.017G089700	Malpighiales	Eudicotyledones	phytozome
<i>Populus trichocarpa</i>	Potri.007G047900	Malpighiales	Eudicotyledones	phytozome
<i>Populus trichocarpa</i>	Potri.001G302000	Malpighiales	Eudicotyledones	phytozome
<i>Populus trichocarpa</i>	Potri.009G098000	Malpighiales	Eudicotyledones	phytozome
<i>Portulaca amilis</i>	FUN_051737	Caryophyllales	Eudicotyledones	phytozome
<i>Portulaca amilis</i>	FUN_052803	Caryophyllales	Eudicotyledones	phytozome
<i>Portulaca amilis</i>	FUN_047201	Caryophyllales	Eudicotyledones	phytozome
<i>Prunus persica</i>	Prupe.3G259200	Rosales	Eudicotyledones	phytozome
<i>Prunus persica</i>	Prupe.6G230100	Rosales	Eudicotyledones	phytozome
<i>Prunus persica</i>	Prupe.8G131700	Rosales	Eudicotyledones	phytozome
<i>Pseudotsuga menziesii</i>	PME00026064	Pinales	Acrogymnosperms	Gymno plaza 1.0
<i>Pseudotsuga menziesii</i>	PME00001365	Pinales	Acrogymnosperms	Gymno plaza 1.0
<i>Pseudotsuga menziesii</i>	PME00077364	Pinales	Acrogymnosperms	Gymno plaza 1.0
<i>Quercus rubra</i>	Qurub.02G186400	Fagales	Eudicotyledones	phytozome
<i>Quercus rubra</i>	Qurub.11G098700	Fagales	Eudicotyledones	phytozome
<i>Quercus rubra</i>	Qurub.06G075200	Fagales	Eudicotyledones	phytozome
<i>Ricinus communis</i>	29970.m000976	Malpighiales	Eudicotyledones	phytozome
<i>Ricinus communis</i>	29801.m003123	Malpighiales	Eudicotyledones	phytozome
<i>Rorippa islandica</i>	Roisl.0070s0018	Brassicales	Eudicotyledones	phytozome
<i>Rorippa islandica</i>	Roisl.0046s0950	Brassicales	Eudicotyledones	phytozome
<i>Salix purpurea</i>	Sapur.017G073600	Salicales	Eudicotyledones	phytozome
<i>Salix purpurea</i>	Sapur.007G045200	Salicales	Eudicotyledones	phytozome
<i>Salix purpurea</i>	Sapur.016G181600	Salicales	Eudicotyledones	phytozome
<i>Salix purpurea</i>	Sapur.009G076600	Salicales	Eudicotyledones	phytozome
<i>Schrenkiella parvula</i>	Sp7g02580	Brassicales	Eudicotyledones	phytozome
<i>Schrenkiella parvula</i>	Sp3g17480	Brassicales	Eudicotyledones	phytozome
<i>Schrenkiella parvula</i>	Sp5g12010	Brassicales	Eudicotyledones	phytozome
<i>Selaginella moellendorffii</i>	91219	Selaginellales	Lycophytes	phytozome
<i>Selaginella moellendorffii</i>	165134	Selaginellales	Lycophytes	phytozome
<i>Setaria italica</i>	Seita.2G322700	Cyperales	Monocotyledones	phytozome
<i>Setaria viridis</i>	Sevir.2G334300	Cyperales	Monocotyledones	phytozome
<i>Sinapis alba</i>	Sialb.0606s0023	Brassicales	Eudicotyledones	phytozome

Organism	Protein name	Order	Clade	Database
<i>Sinapis alba</i>	Sialb.0540s0029	Brassicales	Eudicotyledones	phytozome
<i>Sinapis alba</i>	Sialb.0766s0022	Brassicales	Eudicotyledones	phytozome
<i>Sinapis alba</i>	Sialb.0054s0274	Brassicales	Eudicotyledones	phytozome
<i>Sinapis alba</i>	Sialb.0001s0595	Brassicales	Eudicotyledones	phytozome
<i>Sinapis alba</i>	Sialb.0672s0053	Brassicales	Eudicotyledones	phytozome
<i>Solanum lycopersicum</i>	Solyc03g013310	Solanales	Eudicotyledones	phytozome
<i>Solanum lycopersicum</i>	Solyc01g107750	Solanales	Eudicotyledones	phytozome
<i>Solanum lycopersicum</i>	Solyc02g093000	Solanales	Eudicotyledones	phytozome
<i>Solanum tuberosum</i>	Soltu.DM.02G028260	Solanales	Eudicotyledones	phytozome
<i>Solanum tuberosum</i>	Soltu.DM.03G007160	Solanales	Eudicotyledones	phytozome
<i>Solanum tuberosum</i>	Soltu.DM.01G047110	Solanales	Eudicotyledones	phytozome
<i>Sorghum bicolor</i>	Sobic.002G008800	Poales	Monocotyledones	phytozome
<i>Sphagnum fallax</i>	Sphfalx16G057000	Sphagnales	Bryophytes	phytozome
<i>Sphagnum fallax</i>	Sphfalx17G006600	Sphagnales	Bryophytes	phytozome
<i>Sphagnum fallax</i>	Sphfalx18G079700	Sphagnales	Bryophytes	phytozome
<i>Sphagnum fallax</i>	Sphfalx13G023500	Sphagnales	Bryophytes	phytozome
<i>Sphagnum fallax</i>	Sphfalx14G024100	Sphagnales	Bryophytes	phytozome
<i>Sphagnum fallax</i>	Sphfalx06G107500	Sphagnales	Bryophytes	phytozome
<i>Sphagnum magellanicum</i>	Sphmag16G056400	Sphagnages	Bryophytes	phytozome
<i>Sphagnum magellanicum</i>	Sphmag17G006400	Sphagnages	Bryophytes	phytozome
<i>Sphagnum magellanicum</i>	Sphmag06G111300	Sphagnages	Bryophytes	phytozome
<i>Sphagnum magellanicum</i>	Sphmag18G019000	Sphagnages	Bryophytes	phytozome
<i>Sphagnum magellanicum</i>	Sphmag13G020000	Sphagnages	Bryophytes	phytozome
<i>Sphagnum magellanicum</i>	Sphmag14G023700	Sphagnages	Bryophytes	phytozome
<i>Spinacia oleracea</i>	Spov3_C0007.00101	Caryophyllales	Eudicotyledones	phytozome
<i>Spinacia oleracea</i>	Spov3_chr1.02493	Caryophyllales	Eudicotyledones	phytozome
<i>Spirodela polyrhiza</i>	Spipo2G0093300	Alismatales	Monocotyledones	phytozome
<i>Spirodela polyrhiza</i>	Spipo4G0005500	Alismatales	Monocotyledones	phytozome
<i>Stanleya pinnata</i>	Stapi.1453s0002	Brassicales	Eudicotyledones	phytozome
<i>Stanleya pinnata</i>	Stapi.1874s0008	Brassicales	Eudicotyledones	phytozome
<i>Stanleya pinnata</i>	Stapi.1161s0006	Brassicales	Eudicotyledones	phytozome
<i>Stanleya pinnata</i>	Stapi.0737s0004	Brassicales	Eudicotyledones	phytozome
<i>Stanleya pinnata</i>	Stapi.0692s0011	Brassicales	Eudicotyledones	phytozome
<i>Taxus baccata</i>	TBA00001628	Taxales	Acrogymnosperms	Gymno plaza 1.0
<i>Taxus baccata</i>	TBA00011682	Taxales	Acrogymnosperms	Gymno plaza 1.0
<i>Theobroma cacao</i>	Thecc.04G090400	Malvales	Eudicotyledones	phytozome
<i>Theobroma cacao</i>	Thecc.01G062500	Malvales	Eudicotyledones	phytozome
<i>Theobroma cacao</i>	Thecc.02G105700	Malvales	Eudicotyledones	phytozome
<i>Thinopyrum intermedium</i>	Thint.15G0279400	Poales	Monocotyledones	phytozome
<i>Thinopyrum intermedium</i>	Thint.13G0199200	Poales	Monocotyledones	phytozome
<i>Thinopyrum intermedium</i>	Thint.14G0237600	Poales	Monocotyledones	phytozome
<i>Thlaspi arvense</i>	Thlar.0083s0010	Brassicales	Eudicotyledones	phytozome
<i>Thlaspi arvense</i>	Thlar.0014s0526	Brassicales	Eudicotyledones	phytozome
<i>Thlaspi arvense</i>	Thlar.0021s1378	Brassicales	Eudicotyledones	phytozome
<i>Thuja plicata</i>	Thupl.29382416s0045	Pinales	Acrogymnosperms	phytozome

Organism	Protein name	Order	Clade	Database
<i>Thuja plicata</i>	Thupl.29377609s0023	Pinales	Acrogymnosperms	phytozome
<i>Trifolium pratense</i>	Tp57577_TGAC_v2_mRNA345	Fabales	Eudicotyledones	phytozome
<i>Trifolium pratense</i>	Tp57577_TGAC_v2_mRNA278	Fabales	Eudicotyledones	phytozome
<i>Trifolium pratense</i>	Tp57577_TGAC_v2_mRNA143	Fabales	Eudicotyledones	phytozome
<i>Urochloa fusca</i>	Urofu.1G032300	Poales	Monocotyledones	phytozome
<i>Urochloa fusca</i>	Urofu.2G334900	Poales	Monocotyledones	phytozome
<i>Vigna unguiculata</i>	Vigun05g037300	Fabales	Eudicotyledones	phytozome
<i>Vigna unguiculata</i>	Vigun07g259200	Fabales	Eudicotyledones	phytozome
<i>Vigna unguiculata</i>	Vigun02g146800	Fabales	Eudicotyledones	phytozome
<i>Vitis vinifera</i>	VIT_214s0108g01410	Vitales	Eudicotyledones	phytozome
<i>Vitis vinifera</i>	VIT_203s0180g00020	Vitales	Eudicotyledones	phytozome
<i>Vitis vinifera</i>	VIT_207s0031g00020	Vitales	Eudicotyledones	phytozome
<i>Volvox carteri</i>	Vocar.0030s0065	Chlamydomonadales	Chlorophytes	phytozome
<i>Zea mays</i>	Zm00001d007942	Cyperales	Monocotyledones	phytozome
<i>Zostera marina</i>	Zosma06g28190	Alismatales	Monocotyledones	phytozome
<i>Zostera marina</i>	Zosma01g09840	Alismatales	Monocotyledones	phytozome

Supplementary Table 2: Primers used in this study

Primer Name	Primer Sequence	Objective
<i>pVND7_FW_attB4</i>	5'- GGGGACAACCTTTGTATAGAAAAAGTTGTCCTGCCGGTAAAGTGAGAGAAG -3'	<i>VND7</i> promoter
<i>pVND7_RV_attB1r</i>	5'- GGGGACTGCTTTTTTGTACAAAACCTTGCCACGATGATCCTATAAACG -3'	<i>VND7</i> promoter
<i>pXCP1_FW_attB4</i>	5'- GGGGACAACCTTTGTATAGAAAAAGTTGTCGCATTGCTGTGTCGATGG -3'	<i>XCP1</i> promoter
<i>pXCP1_RV_attB1r</i>	5'- GGGGACTGCTTTTTTGTACAAAACCTGTAGCCAAATTTGTTCACTG -3'	<i>XCP1</i> promoter
<i>XCP1_FW_attB1</i>	5'- GGGGACAAGTTTGTACAAAAAAGCAGGCTTCATGGCTTTTTCTGCACCATCAC -3'	<i>XCP1</i> coding
<i>XCP1_NS_attB2</i>	5'- GGGGACCACTTTGTACAAGAAAGCTGGGTACTTGGTCTTGGTAGGATATG -3'	<i>XCP1</i> coding
<i>pBFN1-1975_FW_attB4</i>	5'- GGGGACAACCTTTGTATAGAAAAAGTTGGAAATTAAGTATTTACCTGCCAAAAG -3'	<i>BFN1</i> promoter
<i>pBFN1_RV_attB1r</i>	5'- GGGGACTGCTTTTTTGTACAAAACCTGATCTTCAAAGTTTGAACTTATATAATG -3'	<i>BFN1</i> promoter
<i>pCESA4_FW_attB4</i>	5'- GGGGACAACCTTTGTATAGAAAAAGTTGGACATGCGATGGCATGGATGC -3'	<i>CESA4</i> promoter
<i>pCESA4_RV_attB1r</i>	5'- GGGGACTGCTTTTTTGTACAAAACCTGGGCGAGGTACTGAGCTCTC -3'	<i>CESA4</i> promoter
<i>pCESA7_FW_attB4</i>	5'- GGGGACAACCTTTGTATAGAAAAAGTTGCCAGTTTGAACGACACTTAGAAAAATAAG -3'	<i>CESA7</i> promoter
<i>pCESA7_RV_attB1r</i>	5'- GGGGACTGCTTTTTTGTACAAAACCTGGAGGGACGGCCGGAGATTAG -3'	<i>CESA7</i> promoter
<i>pCESA8_FW_attB4</i>	5'- GGGGACAACCTTTGTATAGAAAAAGTTGCGCCTCACAATGTGTTCTTGC -3'	<i>CESA8</i> promoter
<i>pCESA8_RV_attB1r</i>	5'- GGGGACTGCTTTTTTGTACAAAACCTGCTTCGAATCCCCTGTTGGAG -3'	<i>CESA8</i> promoter
<i>pPTEN2a_FW_attB4</i>	5'- GGGGACAACCTTTGTATAGAAAAAGTTGTGAATAAACATGTAATCTCCATTTTTTGTCTC -3'	<i>PTEN2a</i> promoter
<i>pPTEN2a_RV_attB1r</i>	5'- GGGGACTGCTTTTTTGTACAAAACCTGCGTTTCTATCTTAATCCAAAATGTGAATTCTC -3'	<i>PTEN2a</i> promoter
<i>pPTEN2b_FW_attB4</i>	5'- GGGGACAACCTTTGTATAGAAAAAGTTGCTAGATTTAACTTGTTGGTATACCGC -3'	<i>PTEN2b</i> promoter
<i>pPTEN2b_RV_attB1r</i>	5'- GGGGACTGCTTTTTTGTACAAAACCTGTTTAGCAATCCAACGCTAGCTC -3'	<i>PTEN2b</i> promoter
<i>PTEN1_FW_attB1</i>	5'- GGGGACAAGTTTGTACAAAAAAGCAGGCTCC ATGGGTCTCAAGCTCTCACGAG -3'	<i>PTEN1</i> coding
<i>PTEN1_NS_RV_attB2</i>	5'- GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAGAGAGAGAAAGGTCATCGCGG -3'	<i>PTEN1</i> coding
<i>PTEN1_RV_attB2</i>	5'- GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAAGAGAGAGAAAGGTCATCGCG -3'	<i>PTEN1</i> _{STOP} coding
<i>PTEN2a_FW_BstBI</i>	5'- ATTTTCGAATGTCGTCTGAGTCACCGAATTTG -3'	<i>PTEN2a</i> coding
<i>PTEN2a_NS_RV_SpeI</i>	5'- ATTACTAGTATCGCTTTCAAAGTCGTCTTCATCTC -3'	<i>PTEN2a</i> coding
<i>PTEN2a_RV_SpeI</i>	5'- ATTACTAGTTCAATCGCTTTCAAAGTCGTCTTCATC -3'	<i>PTEN2a</i> _{STOP} coding
<i>PTEN2b_FW_attB1</i>	5'- GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGGAACTGATCCTGCTAACTCTTC -3'	<i>PTEN2b</i> coding
<i>PTEN2b_NS_RV_attB2</i>	5'- GGGGACCACTTTGTACAAGAAAGCTGGGTCTCGCTTTTCATAGTCTTCTTCTC -3'	<i>PTEN2b</i> coding
<i>PTEN2b_RV_attB2</i>	5'- GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAGTCTGCTTTTCATAGTCTTCTTCC -3'	<i>PTEN2b</i> _{STOP} coding
<i>PTEN2bΔNter_FW_attB1</i>	5'- GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGAGAAGATACCAGGTATGG -3'	<i>PTEN2bΔNter</i> gDNA
<i>PTEN2bΔNter_FW_attB1</i>	5'- GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGAGAAGATACCAGGAGGGGG -3'	<i>PTEN2bΔNter</i> cDNA
<i>PTEN2bΔCter_RV_attB2</i>	5'- GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAGGGTCTATCATTACGATCTCG -3'	<i>PTEN2bΔCter</i> coding
<i>PTEN2bΔ1-131_FW_attB1</i>	5'- GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGCTTGGTCTGCATTTGCCAACG -3'	<i>PTEN2bΔ1-131</i> coding
<i>PTEN1^{Nterm}_RV</i>	5'- GGTATCTTCTTCGTTTCTTGACACCAAGTTACG -3'	<i>PTEN1</i> Nter coding
<i>PTEN2b-Nter_FW</i>	5'- CCAAGAAACGAATGAGAAGATACCAGGTATGG -3'	<i>PTEN2b-Nter</i> gDNA

Supplementary Table 2: Primers used in this study (continuation)

Primer Name	Primer Sequence	Objective
<i>PTEN2b-Nter_FW</i>	5'- CCAAGAAACGAAGAAGATACCAGGAGGGGG -3'	<i>PTEN2b-Nter</i> cDNA
<i>MpPTEN2_attB1_FW</i>	5'- GGGGACAAGTTTGTACAAAAAAGCAGGCTCCATGGACGACTCAGCCAACAG -3'	<i>MpPTEN2</i> coding
<i>MpPTEN2_attB1_RV</i>	5'- GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAATCTTCATCACTCTCGAAATCC -3'	<i>MpPTEN2</i> coding
<i>qPTEN1_FW</i>	5'- GTCCGTGCTCGATATGCGACATC -3'	qPCR <i>PTEN1</i>
<i>qPTEN1_RV</i>	5'- GTACGCCGATACCATTAGCCCTG -3'	qPCR <i>PTEN1</i>
<i>qPTEN2a_FW</i>	5'-CAACCAGGCCGTAGGTGTATGC-3'	qPCR <i>PTEN2a</i>
<i>qPTEN2a_RV</i>	5'-CCCCTTTCTTTGGGGCACTGAAC-3'	qPCR <i>PTEN2a</i>
<i>qPTEN2b_FW</i>	5'-GCTGAAGAGGCTATTGATTACT-3'	qPCR <i>PTEN2b</i>
<i>qPTEN2b_RV</i>	5'-AAATCCTCTGAGCATGCATCTTCGC-3'	qPCR <i>PTEN2b</i>
<i>qCrPTEN_FW</i>	5'-AGAGGTGGTGGGAAAGATG-3'	qPCR <i>CrPTEN</i>
<i>qCrPTEN_RV</i>	5'-GAGGTTGAAATAAGGAAGAGG-3'	qPCR <i>CrPTEN</i>
<i>qCITRINE_FW</i>	5'-GAAGTTCATCTGCACCACC-3'	qPCR <i>CITRINE</i>
<i>qCITRINE_RV</i>	5'-TTGTA CTCCAGCTTGTGCC-3'	qPCR <i>CITRINE</i>
<i>qPDF2_FW</i>	5'- TAACGTGGCCAAAATGATGC-3'	qPCR <i>PDF2</i>
<i>qPDF2_RV</i>	5'-GTTCTCCACAACCGCTTGGT -3'	qPCR <i>PDF2</i>
<i>pten2a_LP</i>	5'-CGGCAATATGTCATTATGCAG-3'	genotyping <i>pten2a</i>
<i>pten2a_RP</i>	5'-TTTTTCCTGATCTGAATTCGAG-3'	genotyping <i>pten2a</i>
<i>pten2b_LP</i>	5'-CTCGAAAAATCCGAAAAGACC-3'	genotyping <i>pten2b</i>
<i>pten2b_RP</i>	5'-GAAGCGAATTTAGCCAAAACC-3'	genotyping <i>pten2b</i>
<i>cesa6_LP</i>	5'-GCTTGCAGCTGAATCAATACC-3'	genotyping <i>cesa6</i>
<i>cesa6_RP</i>	5'-AACCTGATCAAATCCAATCCC-3'	genotyping <i>cesa6</i>

Supplementary Table 3: Constructs made in this study

Transient Construct Name	DNA Origin	Transient Vector	Final Construct Name	Final Vector
<i>attL4-pTIP3;2::TIP3;2_{NOSTOP}-attR1</i>	<i>in vitro</i> synthesis	pEN L4-R1	TIP3;2::TIP3;2-GFP	EDO097pFR7m24G
<i>attL1-PTEN1_{STOP}-attL2</i>	<i>Arabidopsis</i> gDNA	pEN 207	UBQ::XVE::PTEN1	pMDC7
<i>attL1-PTEN2a_{STOP}-attL2</i>	<i>Arabidopsis</i> gDNA	p17ACCD2P*	UBQ::XVE::PTEN2a	pMDC7
<i>attL1-PTEN2b_{STOP}-attL2</i>	<i>Arabidopsis</i> gDNA	pEN 207	UBQ::XVE::PTEN2b	pMDC7
<i>attL4-pVND7-attR1</i>	<i>Arabidopsis</i> gDNA	pEN L4-R1	VND7::NLS-3xVENUS	EDO097pFR7m24G
<i>attL4-pCESA7-attR1</i>	<i>Arabidopsis</i> gDNA	pEN L4-R1	CESA7::NLS-3xVENUS	EDO097pFR7m24G
<i>attL4-pCESA4-attR1</i>	<i>Arabidopsis</i> gDNA	pEN L4-R1	CESA4::NLS-3xVENUS	EDO097pFR7m24G
<i>attL4-pCESA8-attR1</i>	<i>Arabidopsis</i> gDNA	pEN L4-R1	CESA8::NLS-3xVENUS	EDO097pFR7m24G
<i>attL4-pXCP1-attR1</i>	<i>Arabidopsis</i> gDNA	pEN L4-R1	XCP1::XCP1-mCherry	pH7m34GW
<i>attL1-XCP1_{NOSTOP}-attL2</i>	<i>Arabidopsis</i> gDNA	pEN 207		
<i>attL4-BFN1-attR1</i>	<i>Arabidopsis</i> gDNA	pEN L4-R1	BFN1::NLS-dtTOMATO	pK7m24GW2
<i>attL4-pPTEN2b-attR1</i>	<i>Arabidopsis</i> gDNA	pEN L4-R1	PTEN2b::PTEN2b-CITRINE	
<i>attL1-PTEN2b_{NOSTOP}-attL2</i>	<i>Arabidopsis</i> cDNA	pEN 207	PTEN2b::PTEN2b-mCherry	pH7m34GW
<i>attL1-CrPTEN_{STOP}-attL2</i>	<i>in vitro</i> synthesis	pEN 221	UBQ::XVE::CrPTEN	pMDC7
<i>attL1-MpPTEN2_{STOP}-attL2</i>	<i>Marchantia</i> cDNA	pEN 221	UBQ::XVE::MpPTEN2	pMDC7
<i>attL1-PTEN2b^{ΔCter}_{STOP}-attL2</i>	<i>Arabidopsis</i> gDNA	pEN 221	UBQ::XVE::PTEN2b^{ΔCter}	pMDC7
<i>attL1-PTEN2b^{ΔNter}_{STOP}-attL2</i>	<i>Arabidopsis</i> gDNA	pEN 221	UBQ::XVE::PTEN2b^{ΔNter}	pMDC7
<i>attL1-PTEN2b^{Δ1-131}_{STOP}-attL2</i>	<i>Arabidopsis</i> gDNA	pEN 221	UBQ::XVE::PTEN2b^{Δ1-131}	pMDC7
<i>attL1-N1-PTEN2b_{STOP}-attL2</i>	<i>Arabidopsis</i> gDNA	pEN 221	UBQ::XVE::N1-PTEN2b	pMDC7
<i>attL1-PTEN2b^{ΔCter}_{NOSTOP}-attL2</i>	<i>Arabidopsis</i> cDNA	pEN 221	PTEN2b:: PTEN2b^{ΔNter} -CITRINE	pH7m34GW
<i>attL1-PTEN2b^{Δ1-131}_{NOSTOP} -attL2</i>	<i>Arabidopsis</i> cDNA	pEN 221	PTEN2b:: PTEN2b^{Δ1-131} -CITRINE	pH7m34GW
<i>attL1-PTEN1_{NOSTOP}-attL2</i>	<i>Arabidopsis</i> cDNA	pEN 207	PTEN2b::PTEN1-CITRINE	pH7m34GW

* p17ACCD2P was *in vitro* synthesized in pMk-RQ plasmid (Invitrogen) and contain attL1-MCS**-attL2 sequences.

**MCS: 5' – GAA TTC GAA GCT CGG TAC CCG GGG ATC CTC TAG AGT CGA CCT GCA GGC CCA TGG TGA CTA GTC AAG CTT-3'