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Inducible depletion of PI(4,5)P$_2$ by the synthetic iDePP system in Arabidopsis

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Phosphatidylinositol 4,5-bisphosphate (PI(4,5)P$_2$) is a low-abundance membrane lipid essential for plasma membrane function$^{1,2}$. In plants, mutations in phosphatidylinositol 4-phosphate (PI4P) 5-kinases (PIPSK) suggest that PI(4,5)P$_2$ production is involved in development, immunity and reproduction$^{3-5}$. However, phospholipid synthesis is highly intricate$^6$. It is thus likely that steady-state depletion of PI(4,5)P$_2$ triggers confounding indirect effects. Furthermore, inducible tools available in plants allow PI(4,5)P$_2$ to increase$^{7-9}$ but not decrease, and no PIP5K inhibitors are available. Here, we introduce iDePP (inducible depletion of PI(4,5)P$_2$ in plants), a system for the inducible and tunable depletion of PI(4,5)P$_2$ in plants in less than three hours. Using this strategy, we confirm that PI(4,5)P$_2$ is critical for various aspects of plant development, including root growth, root-hair elongation and organ initiation. We show that PI(4,5)P$_2$ is required to recruit various endocytic proteins, including AP2-µ, to the plasma membrane, and thus to regulate clathrin-mediated endocytosis. Finally, we find that inducible PI(4,5)P$_2$ perturbation impacts the dynamics of the actin cytoskeleton as well as microtubule anisotropy. Together, we propose that iDePP is a simple and efficient genetic tool to test the importance of PI(4,5)P$_2$ in given cellular or developmental responses, and also to evaluate the importance of this lipid in protein localization.

The synthetic inducible system iDePP (inducible depletion of PI(4,5)P$_2$ in plants) is designed to specifically dephosphorylate PI(4,5)P$_2$ at the plasma membrane at ~20 °C (Extended Data Fig. 1). It consists of the isolated phosphatase domain of the Drosophila melanogaster inositol polyphosphate 5-phosphatase OCRL protein (dOCRL), which is artificially targeted to the plasma membrane by a myristoylation and palmitoylation (MAP) sequence (Extended Data Fig. 2). iDePP is fused with the fluorescent protein mCherry (mCH) for visualization, and its expression is driven by a dexamethasone (dex)-inducible promoter (Extended Data Fig. 3). We included two controls: (1) MAP-3xmCH, which serves as a general control to test for the possible side effects of dex/glucoctocoid-inducible system/MAP, and (2) a catalytic-dead version of dOCRL (MAP-3xmCH-dOCRL$^{40sv}$), which controls that phenotypes are indeed caused by 5-phosphatase activity and not by other parts of the dOCRL protein (Extended Data Fig. 2).

We validated that induction of either MAP-mCH-dOCRL$^{40sv}$ or MAP-3xmCH controls had no effect on the total levels of phosphatidylinositol bisphosphate (PIP$_2$), which refers to the combined levels of PI(4,5)P$_2$ and PI(3,5)P$_2$ in Arabidopsis seedlings (Fig. 1a,b and Extended Data Fig. 4). In contrast, induction of the active phosphatase MAP-mCH-dOCRL resulted in a dramatic decrease of $^{32}$P incorporation into PI(4,5)P$_2$ ($^{32}$P-PIP$_2$ levels; that is, ~90%, Fig. 1a,b and Extended Data Fig. 4). In addition, dex induction did not perturb the levels of other phospholipids in any of the lines tested, confirming the specificity of the iDePP system and its controls (Fig. 1a and Extended Data Fig. 4). At the subcellular level we showed that, in the absence of dex, MAP-mCH-dOCRL was not expressed (Fig. 1c, left panel), while the yellow fluorescent mCITRINE-tagged (mCIT) PI(4,5)P$_2$ biosensor mCIT-2xPH$_{PLC}$ (ref. $^9$) localized to the plasma membrane (Fig. 1c, right panel). Induction of MAP-mCH-dOCRL resulted in the release of the mCIT-2xPH$_{PLC}$ fluorescence from the plasma membrane (Fig. 1c,d and Extended Data Fig. 5), confirming the efficient depletion of PI(4,5)P$_2$ from this membrane. The localization of the mCIT-2xFHYVE$_{IR}$ biosensor was not affected in the control lines (Fig. 1d–f and Extended Data Fig. 5). We further confirmed the impact of MAP-mCH-dOCRL expression on PI(4,5)P$_2$ using an independent PI(4,5)P$_2$ biosensor (mCIT-TUBBY-C$^8$), Fig. 1d,g and Extended Data Fig. 5). In agreement with the results obtained from the biochemical quantification, we did not observe any effect on the localization of the biosensors for PI4P (mCIT-P4M$_{IR}$), phosphatidylinositol 3-phosphate (PI3P) (mCIT-2xFHYVE$_{IR}$) or phosphatidylserine (PS) (mCIT-C2$_{IR}$)$^{10}$ after MAP-mCH-dOCRL induction (Fig. 1d,b–j and Extended Data Fig. 5). Overall, our results show that MAP-mCH-dOCRL efficiently, specifically and directly hydrolyzes the PI(4,5)P$_2$ pool at the plasma membrane in living plant cells.

To assess the dynamics of PI(4,5)P$_2$ depletion after iDePP induction, we followed mCIT-2xFPH$_{PLC}$ and MAP-mCH-dOCRL localization upon dex treatment. After 15 min of dex treatment (first time point), mCIT-2xFPH$_{PLC}$ labelled the plasma membrane while no signal corresponding to MAP-mCH-dOCRL fluorescence was observed (Fig. 2a,b and Supplementary Videos 1 and 2). After 90 min, a fraction of mCIT-2xFPH$_{PLC}$ started to be partially released from the plasma membrane in the cytosol of some cells, indicating that PI(4,5)P$_2$ had started to be depleted from the targeted membrane (Fig. 2a,b and Supplementary Videos 1 and 2). After 180 min, mCIT-2xFPH$_{PLC}$ was cytosolic in most cells, and over the next few hours mCIT-2xFPH$_{PLC}$ remained cytosolic while MAP-mCH-dOCRL fluorescence increased (Fig. 2a,b). Biochemical monitoring of the PI$_2$ levels using $^{32}$P-prelabelled seedlings ± dex demonstrated that within 2 hours of induction over 60% of the $^{32}$P incorporation into
PIP₂ was gone in MAP-mCH-dOCRL seedlings, while the levels of the other anionic lipids remained stable (Fig. 2c–e and Extended Data Fig. 6). Together, these results confirm the impact on either mCIT-2xPH PLC localization (Supplementary Data Fig. 4). In addition, controls without dex treatment had no dissociation indexes of lipid biosensors upon expression of MAP-mCH-dOCRL or negative controls. Dissociation index is the ratio of (1) plasma membrane to cytosol fluorescence ratio without dex treatment, to (2) plasma membrane to cytosol fluorescence ratio after dex treatment. Statistical analysis with lmer (Type-II Wald χ² test) and post-hoc. Representative images of the fluorescent signal corresponding to MAP-mCH-dOCRL (left panel) and PI(4,5)P₂ sensor mCIT-2xPH (right panel) in the same root cells, without dex treatment. Dissociation indexes of lipid biosensors upon expression of MAP-mCH-dOCRL in development (Extended Data Fig. 7). To bypass the strong lethality associated with severe PI(4,5)P₂ depletion, we first took advantage of the dose-dependent activation of iDePP upon treatment with different concentrations of dex. At low dex concentrations (0.05 μM), root-growth arrest was mild, while at higher concentrations a complete arrest in root growth was observed (from 0.5 to 5 μM dex, Fig. 3a,b). As has been performed for the inducible production of PI(4,5)P₂ upon human PIP5K induction by estradiol, we also performed dex treatments later during root development. In five-day-old seedlings transferred to 5 μM dex medium for 22 hours (±2 hours), root-hair growth was abolished, specifically after induction of MAP-mCH-dOCRL but not MAP-mCH-dOCRLind (Fig. 3c–f and Supplementary Video 4).
Accordingly, expression of MAP-mCH-dOCRL released mCIT-2xPH
PI(4,5)P₂ fluorescence from the plasma membrane into the cytoplasm of already-formed root-hair cells, indicating efficient PI(4,5)P₂ depletion (Fig. 3g–i). These experiments show that dose response and temporal activation of the iDePP system can be used to perturb plant development at a range of intensities and at fine-tuned temporal resolution. Here by inducing PI(4,5)P₂ perturbation at different times of root epidermis differentiation...
we demonstrate that iDePP can be used to dissect the manifold function of this lipid in root-hair development, that is, initiation, growth and polarity.

During our experiments, we regularly observed circular plasma membrane protuberances (Extended Data Fig. 8) that could be the result of altered endocytosis, as observed upon PIP5K6 overexpression in pollen tubes11. Indeed, long-term treatment of the non-induced MAP-mCh-dOCLR line with ES9-17, a clathrin-mediated endocytosis inhibitor12, led to similar phenotypes (Extended Data Fig. 8). We thus assessed the localization of the proteins implicated in endocytosis in the lines expressing the iDePP system. MAP-mCh-dOCLR induction caused a decrease in both clathrin-adaptor protein AP-μ2 (AP-μ2–green fluorescent protein (AP-μ2–GFP); Fig. 4a–c) and Src homology 3 (SH3)-domain-containing protein 2 at the plasma membrane (SH3P2–GFPs; Extended Data Fig. 8), while the localization of clathrin light chain 2 (CLC2–GFP) was not affected at the plasma membrane, nor at the trans-Golgi network (TGN) (Extended Data Fig. 8). These results suggest that CLC2 is likely recruited to the plasma membrane not only by AP-2 but also by additional adaptor proteins whose localizations are not fully dependent on PI(4,5)P2, such as the TPLATE complex13. The amphiphilic dye FM 4-64 uptake experiments confirmed that bulk endocytosis was reduced in the line expressing MAP-mCh-dOCLR compared with the controls, with a reduction of vacuolar staining after one hour of FM 4-64 incubation (Fig. 4d,e). These results are fully in line with previous findings that clathrin-mediated endocytosis and related trafficking to the vacuole is partially impaired upon PIP5K5 SK loss-of-function or gain-of-function14,15,16. Thus, our results confirmed the importance of PI(4,5)P2 in regulating endocytosis in plants by further investigating some of the endocytic proteins that rely on PI(4,5)P2 plasma membrane localization. However, unlike animal systems, where PI(4,5)P2 is absolutely required for clathrin-mediated endocytosis17, our results suggest that internalization still occurs upon iDePP induction in plants, albeit at a reduced rate. Because the plant plasma membrane strongly accumulates PI4P18, it is possible that this lipid partially substitutes for PI(4,5)P2 function in endocytosis. It also highlights the fact that clathrin-mediated endocytosis is very different in plant and animal systems19,20.

Since phospholipids have been involved in the control of cytoskeleton anchoring and dynamics in plants and animals21,22, we investigated whether PI(4,5)P2 depletion using iDePP might affect the cytoskeleton organization in Arabidopsis. Expression of MAP-mCh-dOCLR in the root epidermis did not led to a reduction in polymerized F-actin (Extended Data Fig. 9), as has been reported in animal cells22,23, but did cause a clear reduction in its dynamics as shown with the Lifeact reporter fused to the fluorescent protein YFP-venus (Lifeact-YFPv) (Fig. 4f and Supplementary Video 5).

To investigate whether PI(4,5)P2 was also affecting the organization of the microtubule cytoskeleton, the cortical microtubules in the root elongation zone were studied where they are transversely aligned24, reflecting the unidirectional anisotropic cell expansion (Fig. 4h–j and Extended Data Fig. 8). In the absence of dex in the MAP-mCh-dOCLR line, or upon expression of MAP-3xmCh, cortical microtubules of elongating epidermal cells labelled by the microtubule binding domain of MAP4 fused to a green fluorescent protein (MBD–GFP) or the α tubulin 6 fused to GFP (TU6–GFP) were observed to form a network of transversely aligned microtubules, orthogonal to the elongation axis of the cells (Fig. 4h–j and Extended Data Fig. 8). Depletion of PI(4,5)P2 led to solubilization of the microtubule reporters (Extended Data Fig. 8), suggesting that PI(4,5)P2 is required for the polymerization of microtubules. Moreover, depletion of PI(4,5)P2 led to a clear loss of microtubule anisotropy (Fig. 4h–j and Extended Data Fig. 8), implying that its organization requires PI(4,5)P2 at the plasma membrane, at least during the elongation process of epidermal root cells.

Here we describe iDePP, a genetically encoded system that allows for depletion of PI(4,5)P2 from the plasma membrane within a few hours in planta. This system bypasses the long-term effects of chronic PI(4,5)P2 depletion, for example, because of a constitutive overexpression

![Image](image-url)
of a plasma-membrane-targeted 5-phosphatase or genetic elimination of a key enzyme responsible for PI(4,5)P₂ homeostasis (for example, PIP5Ks). The use of adequate negative controls demonstrates that the observed effect on PI(4,5)P₂ only depends on dOCRL enzymatic activity and is not caused by side effects of the dex-inducible system or the recruitment of an endogenous PI(4,5)P₂ phosphatase. We could have used a phospholipase C (PLC) activity to deplete PI(4,5)P₂ from the plasma membrane, but we believe a 5-phosphatase produces a specific effect and might be a key enzyme responsible for PI(4,5)P₂ homoeostasis (for example, PIP5Ks).
minimal side effects\textsuperscript{30}. Indeed, PLC activity would increase the production of inositol polyphosphates and diacylglycerol (and possibly PA, via the subsequent action of diacylglycerol kinases), which act as second messengers in plants\textsuperscript{23,31}. Furthermore, PLCs are often acting on PI4P, which is highly present at plant plasma membranes\textsuperscript{4,11}. By contrast, a 5-phosphatase such as dOCRL specifically dephosphorylates PI4P. Indeed, PLC activity would increase the production of inositol polyphosphates and diacylglycerol (and possibly PA, via the subsequent action of diacylglycerol kinases), which act as second messengers in plants\textsuperscript{23,31}. Furthermore, PLCs are often acting on PI4P, which is highly present at plant plasma membranes\textsuperscript{4,11}. By contrast, a 5-phosphatase such as dOCRL specifically dephosphorylates PI4P. 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PI(4,5)P₂ to produce PI4P. Because PI4P is present in much greater amounts at the plasma membrane than PI(4,5)P₂ (ref. 32), the additional PI4P produced by the dephosphorylation reaction is unlikely to have a functional impact. This is validated by the absence of any measurable effects on the quantity of various other membrane lipids, including total phosphatidylinositol monophosphate (PIP) and phosphatic acid (PA) levels and the localization of PI4P, PS and PI3P sensors. Thus, iDePP specifically targets PI(4,5)P₂, without massively affecting other anionic lipids, although, depending on the assays, a contribution from the produced PI4P to the observed phenotypes cannot be formally excluded.

Importantly, the release of the fluorescence from the mCIT-2xPHPLC PI(4,5)P₂ sensor from the plasma membrane was effective in both root and shoot tissues. Here we mostly focused our analyses on epidermal tissues because they are easier to image. However, MAP-mCH-dOCRL is induced in the broad expression domain of the UBQ10 promoter and thus iDePP may be used in additional tissues and organs. Indeed, the dex-inducible system that we have used has previously been shown to be active in many different tissues. As a testament to the extensive effect of iDePP in seedlings, a reduction of up to 90% of the total PiP, levels was observed, indicating the system was active beyond epidermal tissues. Alternatively, MAP-mCH-dOCRL can easily be expressed in an inducible and tissue-specific manner32, as has been done with human PI5PK5. Because the iDePP system is tunable, we predict that the spatial control of PI(4,5)P₂ depletion will be of great interest to elucidate additional PI(4,5)P₂ functions in cell differentiation and plant development and will complement the already-available tools designed to increase PI(4,5)P₂ in an inducible manner. iDePP can also be used to assess the importance of PI(4,5)P₂ in targeting specific proteins at the plasma membrane, as exemplified for AP-µ2 and SH3P2. While loss-of-function approaches for PI5PK5s have previously suggested a general impact of PI(4,5)P₂ on clathrin-mediated endocytosis16,17, our strategy now allows for specifically pinpointing which proteins within this pathway rely on PI(4,5)P₂ for localization.

Overall, we believe that the iDePP system will have the potential to be useful for numerous research groups working in the fields of plant cell biology, plant development, immunity33 and reproduction, and will serve as a template to develop additional tools aimed at perturbing membrane lipids in an inducible manner.

**Methods**

**Sequence alignment.** The human HsOCRL 234–539 and the Drosophila dOCRL amino acid sequences were aligned using T-Coffee software (http://tcoffee.crg.cat/apps/tcoffee/doRegular) and the fasta_all files obtained were then treated with BoxShade (https://embnet.vital-it.ch/software/BOX_form.html) and Inkscape (https://inkscape.org) programs.

**Strategy.** We initially tested four systems that were previously published in mammalian literature and had used the following phosphatases: Inp54 (from yeast)34, Pseudojanin (combined yeast and human phosphatases), INPP5E and OCRL (from humans), and none of these published phosphatases worked efficiently in plants. We then switched to a new uncharacterized phosphatase from drosophila (dOCRL, which works at the same temperature in plants as in its native organism; characterization in Supplementary Table 1). Note that we chose to use an animal enzyme rather than a plant 5-phosphatase because we wanted to be as orthogonal as possible; that is, to avoid our synthetic enzyme being recognized by endogenous plant phosphatases. In addition, we did not simply express a full-length phosphatase, but an engineered chimeric enzyme. To this end, we used only the catalytic domain of the enzyme to get rid of all the endogenous targeting sequences from the full-length phosphatase, and then used a plasma-membrane-targeting sequence to artificially re-target the catalytic activity in a specific subcellular localization of choice. For example, OCRL is known to interact with multiple small GTPases of the Rab family (RABs) and with clathrin. Using the full-length OCRL proteins would clearly have had an impact on its localization and would have interfered with our approach. Unfortunately, our structure–function knowledge of plant 5-phosphatases is fragmented compared animal ones. For example, their catalytic domains have not been well delineated and, protein importantly, the internal sequences responsible for their membrane targeting are largely unknown. This lack of knowledge of plant 5-phosphatases guided our choice toward animal ones. The second module that we had to optimize was the synthetic targeting signal for the plasma membrane localization of the isolated phosphatase domain. In the mammalian field, the most-used targeting sequence is the so-called Lin11 peptide (used in previous PI(4,5)P₂ depletion systems in human cells). We initially used this sequence, but we later realized that it destabilized the fusion protein when expressed in stable transgenic plants. We then switched to new targeting sequences, including L16b (which did not work efficiently because of rerouting of the chimeric enzyme to the vacuole) and the MAP-seq vector. To our knowledge, such a strategy has not been previously used to manipulate the levels of phosphoinositides in plants. However, overexpression of a human type I inositol–polyphosphate 5-phosphatase was reported in tobacco cells15. Importantly, this enzyme specifically hydrolyses inositol 1,4,5-trisphosphate (InsP₃), not phosphoinositides. This is therefore a fundamentally different type of experiment since InsP₃ is soluble, like the described enzyme. In our case, we wanted to target PI(4,5)P₂ at the plasma membrane, while most known PI(4,5)P₂–phosphatases actually localize in different membrane compartments.

**Cloning.** A synthetic gene (generated by Integrated DNA Technologies; https://eu.idtdna.com/page) corresponding to the dOCRL 168–509 codon optimized for expression in Arabidopsis thaliana (sequence below) was amplified and flanked with Ncol and Xhol restriction sites by polymerase chain reaction (PCR) and cloned into pmHIS-SUMO vectors35 by restriction and ligation (corresponding primers in Supplementary Table 1).

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168-509: gtaaagcaagagttaaagaagcgagagagtgagtatatcgtctataaggacattatcatttttgagtgacgcacagacatatctacgaagctggcgccagcattatacgttgtcgacccaaatccgtggcccgtggaatattcaacacgctcggaaataagggaggcattatacgttgtcgacccaaatccgtggcccgtggaatattcaacagcctcaatagtacccaagtccaagcaattgaaaaacagtggatagataagatgatggacagtgtgcatcctgacgta
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To induce cultures, 45 ml of glycerol were added and temperature was lowered by transferring the bacterial pellets to ice for 15 min. The induced culture was then grown for 18 hours at 18 °C with 200 rpm shaking. Bacteria were then centrifuged for 30 min at 6,000 g and 4 °C and the bacterial pellets scooped, resuspended in lysis buffer (200 mM NaCl; 20 mM Tris-HCl pH 7.5, 4 °C). Bacterial lysate was run through the resin twice. Then elution fractions were directly sown on ½ MS medium on line and allowed to grow vertically up to seven days in vitro on ½ MS basal medium supplemented with 0.8% plant agar (pH 7.2) supplemented with antibiotics (spectinomycin, gentamycin and rifampicin). After two days of growth at 28 °C, bacteria were scooped and resuspended in roughly 200 ml of transformation buffer (10 mM MgCl₂; 5% sucrose; 0.25% Silwet) and Col-0 Arabidopsis were transformed by dippling. Plants were then inoculated in vitro with the appropriate antibiotic/herbicide (glufosinate for mCIT, hygromycin for mCh-tagged proteins).

Approximately 20 independent T1s were selected for each line. In the second (T2) generation, at least two independent transgenic lines were selected using the following criteria when possible: (1) good expression level in the root for detection by confocal microscopy upon dexamethasone induction, (2) relatively uniform expression pattern and absence of abnormal developmental defects for development in vitro and in soil.

Lines were rescanned in the third generation (T3) using similar criteria as in T2 with the exception that we selected homozygous lines (100% resistant). At this step, we selected one transgenic line for each marker that was used for further analyses and crosses. The following lines have been donated to NASC and can be ordered as a set (NASC no. N110797) or as individual lines: MAP-mCh-dOCRL (NASC no. N2110785), MAP-mCh-dOCRL (NASC no. N2110786), MAP-mCh-dOCRL (NASC no. N2110787), MAP-mCh-dOCRL × mCIT-2xFyc (NASC no. N2110788), MAP-mCh-dOCRL × mCIT-2xFyc (NASC no. N2110789), MAP-mCh-dOCRL × mCIT-TUBBY (NASC no. N2110791), MAP-mCh-dOCRL × mCIT-TUBBY (NASC no. N2110792), MAP-mCh-dOCRL × mCIT-TUBBY (NASC no. N2110793), MAP-mCh-dOCRL × mCIT-PAM (NASC no. N2110794), MAP-mCh-dOCRL × mCIT-PAM (NASC no. N2110795) and MAP-mCh-dOCRL × mCIT-PAM (NASC no. N2110796). The following lines have been transformed by dipping: N.1-Naphthylphthalamic Acid (NPA) treatments were carried out as previously described. Plants were then kept on NPA-containing medium for the duration of the experiments.

Induction of gene expression with dexamethasone. To study the effect of the iDeP system in roots, seedlings of each genotype were grown vertically for five to seven days in vitro on ½ MS basal medium supplemented with 0.8% plant agar (pH 7.2) which had been induced by 1 mg l⁻¹ dexamethasone. Cotyledons were placed at the surface of the medium, ½ MS medium and imaged by confocal microscopy. For growth experiments, seedlings were directly sown on ½ MS medium on line and allowed to grow vertically up to 7 days. For root-hair phenotyping, five-day-old plants grown on root-hair medium, ½ MS medium, 2% sucrose, 0.6% phytogel, 0.01% myo-inositol and vitamins (0.5 mg l⁻¹ of nicotinic acid and vitamin B6, 0.1 mg l⁻¹ vitamin B1 and 2 mg l⁻¹ glycine), were transferred to 5 ml dexamethasone-containing root-hair medium. Roots were imaged with a manual solenoid-operated mCIT-TUBBY confocal microscope (magnification: ×7.5 to ×135, zoom ratio 18:1, LED diaciscopic base, LED fluorescence light source (CoolLED PE-300 Lite)), equipped with a Hamamatsu ORCA-Flash4.0 LT camera (Nikon NIS acquisition software) and controlled by a Dell Precision 3630 computer.

For cotyledon analysis, seeds were sterilized and sown on Arabidopsis medium (MS medium without sugars and vitamins) for seven days. Seedlings were then carefully transferred to ½ MS medium plates with 0.8% agar (pH 7.2) containing 5 mg l⁻¹ dexamethasone. Both non-treated seedlings and d-extracted seedlings were, all together, grown back in continuous daylight (see previous). Sixteen hours (±0 min) after transfer to dexamethasone plates, seedlings were mounted in ½ MS medium and imaged by confocal microscopy. For growth experiments, seedlings were directly sown on ½ MS medium on line and allowed to grow vertically up to 7 days. For root-hair phenotyping, five-day-old plants grown on root-hair medium, ½ MS medium, 2% sucrose, 0.6% phytogel, 0.01% myo-inositol and vitamins (0.5 mg l⁻¹ of nicotinic acid and vitamin B6, 0.1 mg l⁻¹ vitamin B1 and 2 mg l⁻¹ glycine), were transferred to 5 ml dexamethasone-containing root-hair medium. Roots were imaged with a manual solenoid-operated mCIT-TUBBY confocal microscope (magnification: ×7.5 to ×135, zoom ratio 18:1, LED diaciscopic base, LED fluorescence light source (CoolLED PE-300 Lite)), equipped with a Hamamatsu ORCA-Flash4.0 LT camera (Nikon NIS acquisition software) and controlled by a Dell Precision 3630 computer.

For cotyledon analysis, seeds were sterilized and sown on Arabidopsis medium (MS medium without sugars and vitamins) for seven days. Seedlings were then carefully transferred to MS medium (without sugars and vitamins) supplemented with 5 µM dexamethasone. Cotyledons were placed at the surface of the medium, as flat as possible, while the rest of the plant was immersed in the medium.
We observed a strong correlation between Map-mCH-dOCLR expression and its impact on mCIT-2xPHPC localization. Indeed, in the centre of the shoot meristem, Map-mCH-dOCLR was not expressed and mCIT-2xPHPC labelling was clearly localized at the cell periphery, probably representing the plasma membrane. In contrast, in the boundary zone, where Map-mCH-dOCLR was expressed mCIT-2xPHPC was not localized sharply at the cell edge and instead filled the entire volume of the cell except the nucleus. This suggested that like in the root epidermis, expression of Map-mCH-dOCLR in the shoot epidermis displaced mCIT-2xPHPC from the plasma membrane into the cytosol, and thus efficiently erased PI(4,5)P₂ from the plasma membrane.

Because UBQ10-driven Map-mCH-dOCLR induction was not uniform at the shoot apical meristem, we decided to test the iDePP system on NPA-grown shoot meristems. NPA is a polar auxin transport inhibitor, which induces naked shoot apical meristems without any organ. In this condition, the UBQ10 promoter is more uniformly expressed throughout the meristem, including the central and peripheral zones, as exemplified by the homogenous expression of mCIT-2xPHPC. To generate naked meristems in vitro, seeds were first directly sown on Arabidopsis medium supplemented with 10 μM NPA. Seedlings with naked meristems were then selected and transferred to a shoot apex medium without NPA and supplemented with 5 μM exogenous auxin, which was taken at 18 hours after induction. To test organ initiation on NPA-treated meristems, seedlings with naked meristems were selected and then transferred to shoot apex medium without NPA and supplemented with 10 μM dexamethasone. These seedlings were then placed for 4 days in long-day conditions (16 hours of light, 8 hours of dark; 21°C).

To quantify their PIP2 levels, seedlings were metabolically labelled with radioactive phosphate by incubating them overnight for ~16–20 hours with 10 μl (5–10 μCi) carrier-free [³²P]-PO₄⁻⁻⁻⁻⁻⁻ (³²P; PerkinElmer) in labelling buffer. Incubations were performed with 0.5 mM dexamethasone and either 1 mM KCl and 5 μM exochitinase, or with radioactive phosphate by incubating them overnight for ~16–20 hours with 10 μl (5–10 μCi) carrier-free [³²P]-PO₄⁻⁻⁻⁻⁻⁻ (³²P; PerkinElmer) in labelling buffer. Incubations were stopped by adding 50 μl of 50% weight per volume perchloric acid and lipids extracted. PIP2 was separated from the rest of the phospholipids by thin-layer chromatography (TLC) using K-oxalate-impregnated and heat-activated silica gel 60/250 μm (Merck) and an alkaline TLC solvent, containing chloroform/methanol/25% ammonia/water (90:70:4:16). Each lane contained one-fifth of the extract. Radioactivity was visualized by autoradiography and quantified by phosphoimaging (Typhoon FLA 7000, GE Healthcare). PIP2 levels were quantified as percentage of total [³²P]-labelled phospholipids. Experiments were performed in duplicate or triplicate and repeated 2–5 times, depending on the genotype.

**Three-dimensional projections, dissociation indexes and anisotropy.** The fluorescence intensity in root hairs was obtained using the Fiji tool. A line of 66 pixels was drawn at the proximity of the root-hair tip and the intensity of grey fluorescence was plotted using the Plot Profil tool. The values were transferred to Excel to obtain the graph. Shoot apical meristem and NPA-induced meristem projected images were obtained using the MorphoGraphX software (https://www.mpipi.mpg.de/MorphoGraphX). The signal projections were generated by extracting the fluorescent signal at the surface of the meristem (between 2 and 5 μm from the meristem surface) and by projecting it onto the cellular mesh. Projections of root epidermal cells were obtained using the Fiji Maximum intensity projection tool on z-stacks of 21 slices 0.5 μm distant from each other.

Dissociation indexes of membrane lipid fluorescent biosensors were measured and calculated as previously described. Briefly, we calculated ‘indexNoDex’ in the mock condition, defined as the ratio between the fluorescence intensity (mean grey value function of Fiji software) measured in two elliptical regions of interest (ROIs) from the plasma membrane region (one in the apical/basal plasma membrane region and one in the lateral plasma membrane region) and two elliptical ROIs in the cytosol. Next, we measured a similar ratio after dexamethasone treatments (‘indexDex’). The dissociation index is the ratio of (indexNoDex)/(indexDex). This dissociation index reveals the degree of relocalization of the fluorescent reporters from the plasma membrane to the cytosol between the non-treated and perturbed conditions (pharmacological treatment or mutant). Dissociation indexes of mCIT-2xPHPC during time-lapse induction of Map-mCH-dOCLR were measured in 30 cells of the same root at each time point.

For quantification of the anisotropy of microtubule arrays in the different transgenic lines, maximal z projections of z-stacks of epidermal root cells in the elongation zone were obtained, using Fiji. In fibroblasts, the tool for ‘multiply line length by’ was set to 1 and ROIs were generated for individual cells (>100 cells per condition) using the built-in polygon tool. The data from the log file were used to extract the average anisotropy of microtubule arrays (a score between zero and one) and to run statistical analyses.

**Statistical analysis.** For dissociation index and anisotropy, we performed all our statistical analyses in R (version 3.6.1, R Core Team, 2019), using the RStudio interface and the packages ggplot2 (ref. 47), lme4 (ref. 48), car (ref. 49), multcomp (ref. 50) and the package ‘effects’ (ref. 51). For each model, assuming normality, we plotted residual values against the fitted values, and an ANOVA was performed. For each raw quantiles to confirm their normal distribution. Graphs were obtained with the R and RStudio software and customized with Inkscape. Details can be found in Supplementary Tables 2–9.

**Data availability.** The Arabidopsis lines and plasmids generated in this study are available from the corresponding authors upon request and the transgenic lines are also available at NASC as NASC stocks N2110785–N2110797. Source data are provided with this paper.

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Competing interests
The authors declare no competing interests.

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Extended Data Fig. 1 | See next page for caption.
Extended Data Fig. 1 | *Drosophila* dOCRL displays a PI(4,5)P₂ phosphatase activity in vitro. **a**, Alignment of (i) *Homo sapiens* OCRL (HsOCRL or OCRL1) truncation (HsOCRL1234-539) successfully used in optogenetic systems in mammalian cells, and (ii) of *Drosophila melanogaster* dOCRL. We were concerned that this domain, that underwent natural selection in an endotherm organism with set-point temperature of 37 °C, would be less active in *Arabidopsis thaliana*, a plant of temperate climate which we usually grow at 21 °C. Therefore, we decided to use the phosphatase domain of dOCRL, *Drosophila melanogaster* homolog, as *Drosophila* are ectotherm and live at ~20 °C. Alignment of OCRL1234-539 and dOCRL allowed us to identify dOCRL168-509 as the homolog region of OCRL1 generally used in optogenetic systems. **b**, Coomassie blue staining monitoring purification of recombinant dOCRL phosphatase domain (His-SUMO-dOCRL168-509-His) from *Escherichia coli*. We first assessed dOCRL activity, which has not been reported so far, even though *in vivo* data suggest a conserved PI(4,5)P₂ 5-phosphatase activity. We ordered a synthetic gene corresponding to dOCRL168-509 and codon optimized for expression in *Arabidopsis*. We cloned a recombinant dOCRL protein (His-SUMO-dOCRL168-509-His) tagged with two 6-histidine tags and a SUMO tag, making it suitable for expression in *Escherichia coli* and purification. We purified His-SUMO-dOCRL168-509-His using a cobalt resin-based affinity chromatography and protein concentration IN: input, lysate of bacteria induced for the expression of His-SUMO-dOCRL168-509-His; FL: flow through; W: wash; E1 to E3: elution fraction 1 to 3; C1 and C2: concentrated fractions from pulled E1, E2 and E3. C1 and C2 were obtained separately on different days. His-SUMO-dOCRL168-509-His expected molecular weight is 54.8 kD; 3 Replicates (**c**) Malachite green phosphatase assay on His-SUMO-dOCRL168-509-His using short chain water-soluble phosphoinositides. We subjected the purified protein fractions C1 and C2 to Malachite Green Phosphatase assays, that detects phosphate released by dephosphorylations, in presence of short chain (diC:8) water-soluble phosphoinositides. Note that we only assessed dOCRL168-509 activity toward phosphoinositides reported in plant cells. Replicate 1 correspond to C1 fraction. Replicate 2 to C2 fractions. Mock: no phosphoinositide added.
Extended Data Fig. 2 | Rational behind the design of the iDePP system. a, Schematic drawing depicting constructs used to genetically-induced PI(4,5)P₂ pool modification at the PM. After dex treatment, MAP-mCh-dOCRL is anchored at the plasma membrane and dephosphorylates PI(4,5)P₂, causing the PI(4,5)P₂ biosensor to be released into the cytosol. b, Controls used in this study includes 3xmCh and a catalytic dead version of dOCRL. After dex treatment, both MAP-3xmCh and MAP-mCh-dOCRLdead are anchored at the PM, but do not affect the pool of PI(4,5)P₂. The PI(4,5)P₂ biosensor is found at the PM. To build the iDePP system, we had to engineer a synthetic enzyme, which consists of the isolated catalytic domain of a 5-phosphatase (which if expressed on its own would be soluble in the cytosol and likely inactive) and then target this module to the plasma membrane, for specific depletion of PI(4,5)P₂. Details can be found in the Methods section.
Extended Data Fig. 3 | Successful inducible expression and subcellular targeting of MAP-mCH-dOCRL and negative controls. a, Inducible system used in this study. We performed site-directed D468G mutation in dOCRL catalytic domain, similarly to D523G mutation already described in human OCRL1 that abolishes its phosphatase activity. We could therefore use MAP-mCH-dOCRL\textsuperscript{D468G} (hereafter named MAP-mCH-dOCRL\textsubscript{dead}) as negative control, together with a MAP-3xmCH recombinant protein. UBQ10 promoter is responsible for mild/strong and ubiquitous expression of GVG (GAL4 VP16 GR) synthetic gene. GR domain binds dexamethasone (dex) and subsequently induces GVG homodimerization and nuclear import. There, GAL4 domain binds UAS DNA elements and VP16 strongly activates the expression of downstream gene: MAP-mCH-dOCRL\textsubscript{D468G}, MAP-mCH-dOCRL\textsubscript{dead}, or MAP-3xmCH. MAP is myristoylation and palmytoylation sequence, responsible for plasma membrane targeting. The mCH corresponds to monomeric CHERRy fluorescent protein. dOCRL\textsubscript{D468G} is an inactive phosphatase domain (later on called dOCRL\textsubscript{dead}). 3xmCH correspond to three fused mCHERRY. b, We addressed the timing of expression and the localization of each construct in Arabidopsis lines stably transformed with UBQ10pro:GVG:MAP-mCH-dOCRL, UBQ10pro:GVG:MAP-mCH-dOCRL\textsubscript{dead}, or UBQ10pro:GVG:MAP-3xmCH. In root meristematic epidermal cells, without dex treatment, none of the recombinant-proteins were detected using confocal microscopy. A 16 h treatment with 0.5 µM dex led to the detection of mCH fluorescence, indicating that induction of the genetic construct had occurred. However, we observed a mosaic induction and a significant number of roots had no cells expressing the fluorescent reporter to detectable levels. To overcome these issues, we optimized the treatment to a 16 h induction with 5 µM dex. Using this set up, we robustly observed red fluorescence using confocal microscopy where MAP-mCH-dOCRL, MAP-mCH-dOCRL\textsubscript{dead} and MAP-3xmCH. Each of the synthetic protein was efficiently targeted to membranes, including the plasma membrane, where PI(4,5)P\textsubscript{2} accumulates, and intracellular compartments. Therefore, a 16 h 5 µM dex treatment is sufficient for an effective expression of dOCRL in Arabidopsis stable transgenic lines. For all three inducible constructs we monitored mCH fluorescence without dex treatment or after a 16h-treatment with either 0.5 µM or 5 µM dex. Signal intensity is color-coded (green fire blue scale); 2 Replicates. Scale bars, 20 µm.
Extended Data Fig. 4 | Biochemical quantification of the PIP2, PIP and PA after iDePP induction. The 32P-PIP levels in iDePP lines and controls ± dex of 3 independent replicates. a-c, Seedlings were labelled with 32P, and incubated ± dex O/N (16–20 hrs). Each sample contained the lipid extract of three seedlings, of which 1/5th was analyzed by TLC and quantified by phosphoimaging, of which 32P-PIP2 (a), PIP (b) and PA (c) were calculated as percentage of total 32P-lipids. d-f, Time-course analysis of 32P-PIP2 (d), PIP (e) and PA (f) levels in iDePP seedlings ± dex in MAP-mCH-dOCRL line. Seedlings of MAP-mCH-dOCRL line were labelled for 20 hrs with 32P, and co-incubated with or without dex for the times indicated (0–20 hrs). Each sample contained the lipid extract of three seedlings of which 1/5 was analyzed by TLC and quantified by phosphoimaging. First, the mean of control on all time points was calculated. The graphs represent the calculated mean of the sample reported to the mean of the control. In the plots, middle horizontal bars represent the median, while the bottom and top of each box represent the 25th and 75th percentiles, respectively. At most, the whiskers extend to 1.5 times the interquartile range, excluding data beyond. For range of value under 1.5 IQR, whiskers represent the range of maximum and minimum values. All statistical tests were two-sided.
Extended Data Fig. 5 | Graphic representation of the fluorescent intensity observed for the different biosensors, used for the quantification of the dissociation index. Orange lines represent the means. In the plots, middle horizontal bars represent the median, while the bottom and top of each box represent the 25th and 75th percentiles, respectively. At most, the whiskers extend to 1.5 times the interquartile range, excluding data beyond. For range of value under 1.5 IQR, whiskers represent the range of maximum and minimum values.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | iDePP enables efficient depletion of PI(4,5)P2 in various tissues and organs. a–c, Subcellular localization of mCIT-2xPH\textsuperscript{PDE} and either MAP-mCH-dOCRL (a), or MAP-3xMCH; N = 12 (b), MAP-mCH-dOCRL\textsuperscript{4xMCH}; N = 12 (c), in cotyledon epidermal cells; N = 6. Fluorescence intensity is color-coded (green fire blue scale), cytoplasmic strands are indicated by the arrows. Scale bars, 10 µm. d–g, Induction of the iDePP system in the shoot apical meristem of Arabidopsis; N = 15 (Scale bar, 20 µm in (a–b), 10 µm in (c–d). Asterisks represent the position of the nucleus in the cell. (h) and (i) Subcellular localization of MAP-mCH-dOCRL (left panel) and mCIT-2xPH\textsuperscript{PDE} (right panel) in a naked shoot meristem from an NPA-treated seedling, as viewed from the top; N = 4. In the absence of dex induction, mCIT-2xPH\textsuperscript{PDE} was localized at the plasma membrane throughout the meristem, including both the central and peripheral zones (h, right panel). By contrast, upon induction of MAP-mCH-dOCRL expression, mCIT-2xPH\textsuperscript{PDE} was no longer sharply localizing at the cell edge (i, right panel). Instead, mCIT-2xPH\textsuperscript{PDE} accumulated in the cytosol, while still being excluded from the round central nuclei (i, right panel). Note that after dex induction, MAP-mCH-dOCRL was strongly expressed in the peripheral zone, and was only weekly expressed in the central zone (i, left panel). However, although its expression was weak in the central zone it appeared to be sufficient to impact mCIT-2xPH\textsuperscript{PDE} localization. Scale bar, 20 µm. (j) and (k) Effect of PI(4,5)P2 depletion on organ initiation in NPA-treated meristems; N = 8. Altogether, these observations suggest that PI(4,5)P2 depletion seems to affect organ initiation in NPA-treated meristems. Scale bars, 700 µm.
Extended Data Fig. 7 | Quantification of the growth phenotype obtained using the iDePP system. Quantification over time (at 3, 5- and 7-days post germination) of the root length (cm) in transgenic lines MAP-mCH-dOCRL (a) and MAP-mCH-dOCRL<sub>dead</sub> (b) grown on MS ½ plate with 5µM of dex. Details for the statistical analysis can be found in the Methods. In the plots, middle horizontal bars represent the median, while the bottom and top of each box represent the 25th and 75th percentiles, respectively. At most, the whiskers extend to 1.5 times the interquartile range, excluding data beyond. For range of value under 1.5 IQR, whiskers represent the range of maximum and minimum values.
Extended Data Fig. 8 | Effects on endocytosis of the depletion of P(4,5)P₂, using the iDePP system. a, b, Representative confocal images of Arabidopsis root cells coexpressing mCIT-P4M<sup>33</sup> with either MAP-mCH-dOCRL (a) or MAP-3xmCH (b). c, d, Confocal imaging showing the effect of the expression of MP-mCH-dOCRL on CLC2-GFP localization. e, Quantification of the effect of MAP-mCH-dOCRL expression on CLC2-GFP localization. The dissociation index is the ratio of (i) plasma membrane to cytosol fluorescence ratio without dex treatment, (ii) plasma membrane to cytosol fluorescence ratio after dex treatment. Statistical analysis with LMER (Type II Wald χ² test) and posthoc tests; 3 replicates (f–g) Confocal imaging showing the effect of the expression of MAP-mCH-dOCRL on SH3P2-sGFP line (h) Quantification of the effect of MAP-mCH-dOCRL expression on SH3P2-sGFP localization. Per root, cells showing a delocalization of SH3P2-sGFP were manually counted for untreated and dex treated plants using Fiji ‘multi-point’ tool and compared by a generalized linear model, Poisson family; 3 replicates. In the plots, middle horizontal bars represent the median, while the bottom and top of each box represent the 25th and 75th percentiles, respectively. At most, the whiskers extend to 1.5 times the interquartile range, excluding data beyond. For range of value under 1.5 IQR, whiskers represent the range of maximum and minimum values. All statistical tests were two-sided. Scale bar, 10 μm.
Extended Data Fig. 9 | Effects on the cytoskeleton of the depletion of PI(4,5)P₂ using the iDePP system. a, F-actin cytoskeleton labelled by LifeAct-YFPv in cells expressing MAP-mCh-dOCRL or MAP-3xMCh 16h after 5µM dex; 4 replicates. b, Cortical microtubules labelled by TUA6-GFP, in non-treated cells (no dex) or treated (5µM dex after 16 hours) to induce MAP-mCh-dOCRL or MAP-3xMCh expression; 3 replicates. All pictures are z-projections; no dex: no treatment; dex: dexamethasone treatment. Scale bars, 10 µm. c, Example of the images used for the quantification of the anisotropy using FibrilTool macro for ImageJ. In the plots, middle horizontal bars represent the median, while the bottom and top of each box represent the 25th and 75th percentiles, respectively. At most, the whiskers extend to 1.5 times the interquartile range, excluding data beyond. For range of value under 1.5 IQR, whiskers represent the range of maximum and minimum values. All statistical tests were two-sided.
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Software and code

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Data collection

Metamorph was used to pilote the spinning disk confocal.

Data analysis

Image analyses and quantifications and root tracking analysis were performed on ImageJ/Fiji and by using pertinent plugins and macro (as described in the methods section); Shoot apical meristem and NPA-induced meristem projected images were obtained by using the MorphoGraphX software (https://www.mpipz.mpg.de/MorphoGraphX).

Figure were prepared with inkscape and powerpoint

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- **Sample size**: Sample size was chosen empirically based on prior experience to provide statistically robust data with n typically 20-30 or higher. Expected "size-of-effect" was taken into account. The sample size is represented by each dot on the graph, details are presented in the legend of the figures and in the material and methods.

- **Data exclusions**: We performed no exclusion except from imaging healthy seedlings.

- **Replication**: Experiments were repeated 2 to 4 times. All attempts at replication were successful.

- **Randomization**: Seedlings used in the various experimental conditions were chosen randomly.

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