

Iron-dependent reprogramming of damage-associated peptide receptor signaling coordinates immunity and phosphate stress adaptation

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Abstract

Plant adaptation to inorganic phosphate (Pi) limitation entails extensive developmental and metabolic reprogramming, collectively termed the phosphate starvation response (PSR). How plants balance nutrient acquisition with immune competence remains unresolved. Here, we show that PSR reconfigures the immune receptor landscape in *Arabidopsis thaliana*, selectively retaining multifunctional damage-associated molecular pattern (DAMP) receptors. Membrane proteomics revealed increased abundance of the Pep receptor PEPR1 under Pi deficiency, whereas most receptor kinases, including microbial pattern-recognition receptors such as FLS2 and CERK1, are reduced. Consistently, Pep-triggered responses are strongly potentiated under Pi depletion, while flagellin- and chitin-induced responses are not, indicating receptor-level immune pathway prioritization rather than global suppression. Mechanistically, Pep sensitization requires the PSR-related ferroxidases *LPR1/LPR2*, NADPH oxidases *RBOHD/RBOHF*, and transcription factor *WRKY33*, linking iron-dependent redox remodeling to selective immune amplification. Beyond inducible defense, PEPRs promote basal PSR transcriptional reprogramming and growth under Pi limitation. Loss of *PEPR1/PEPR2* compromises bacterial resistance and alters root-associated microbiota composition under nutrient deficiency. Together, our findings indicate that specific DAMP receptors exhibit context-dependent dual functionality, promoting immunity or nutrient stress adaptation under Pi depletion, sustaining immune vigilance while optimizing nutrient acquisition. This receptor-level prioritization provides a framework for how nutritional status reshapes plant innate immune architecture.

Introduction

Plants dynamically adjust the immune system to maintain nutrient homeostasis and accommodate beneficial microbial associations. Activation of defense responses often constrains growth and nutrient acquisition, reflecting a fundamental growth–defense trade-off (Huot et al., 2014; Lozano-Durán and Zipfel, 2015; Karasov et al., 2017; He et al., 2022; Inoue et al., 2024). This trade-off becomes particularly acute under nutrient limitation, where plants must optimize resource allocation for nutrient acquisition while preserving protection against pathogens. How immune competence is sustained without compromising nutrient stress adaptation remains a central unresolved question in plant biology.

Phosphorus (P) is an essential macronutrient that often limits plant productivity, as inorganic phosphate (Pi) readily forms insoluble complexes with metals such as iron (Fe) in soils. In response to Pi scarcity, plants activate the phosphate starvation response (PSR), largely governed by the MYB transcription factors PHOSPHATE RESPONSE1 (PHR1) and PHR1-LIKE1 (PHL1) (Rubio et al., 2001; Bustos et al., 2010; Paz-Ares et al., 2022). PSR encompasses transcriptional induction of high-affinity PHOSPHATEPHOSPHATE TRANSPORTER1 (PHT1) transporters, remodeling of root system architecture, and secretion of organic acids and phosphatases to mobilize Pi from the soil (Peret et al., 2014; Lambers et al., 2015). In parallel, primary root growth is inhibited through *PHR1*-independent, Fe-dependent redox processes in the root meristem. The ferroxidases LOW PHOSPHATE ROOT1 and 2 (LPR1/LPR2) oxidize Fe²⁺ to Fe³⁺, promoting Fe³⁺ accumulation, reactive oxygen species (ROS) production, and callose deposition. These processes restrict cell-to-cell movement of the transcription factor SHORT-ROOT (SHR), thereby impairing radial patterning and stem cell maintenance (Svistonoff et al., 2007; Müller et al., 2015; Gutiérrez-Alanís et al., 2017). PSR also entails extensive rhizosphere remodeling to enhance P mobilization. Carbohydrate secretion and proton extrusion lead to rhizosphere acidification, which increases the solubility and availability of both Pi and Fe at the root-soil interface (Ding et al., 2026). Such acidification suppresses immune signaling and bacterial resistance in the root (Yu K et al., 2019). Given that Fe availability and ROS are key modulators of immune signaling (Verbon et al., 2017; Herlihy et al., 2020), these observations suggest that PSR-driven changes in rhizosphere chemistry may reshape immune signaling capacity.

Beyond intrinsic adaptive responses, plants engage beneficial microbes to improve nutrient acquisition. Although *Arabidopsis thaliana* (hereafter *Arabidopsis*) does not establish arbuscular mycorrhizal symbioses, it associates with beneficial endophytic fungi such as *Colletotrichum tofieldiae*, which promotes Pi uptake under P limitation (Hiruma et al., 2016). During beneficial interactions with *C. tofieldiae*, defense-related gene expression is attenuated, whereas a pathogenic relative triggers defense activation even under Pi deficiency (Hacquard et al., 2016). Thus, immune outputs are dynamically modulated according to nutritional status and microbial lifestyle. However,

the molecular mechanisms that preserve pathogen resistance under nutrient stress remain unclear.

Pattern-triggered immunity (PTI) in plants is initiated by cell-surface pattern-recognition receptors (PRRs) that detect microbe- or damage-associated molecular patterns (MAMPs/DAMPs) (Saijo et al., 2018; Snoeck et al., 2025; Zhang et al., 2024). Canonical MAMP receptors in Arabidopsis include the leucine-rich repeat (LRR) receptor kinase (RK) FLAGELLIN-SENSITIVE2 (FLS2), which detects bacterial flagellin (Gómez-Gómez & Boller, 2000), and the lysin-motif (LysM) RK CHITIN ELICITOR RECEPTOR KINASE1 (CERK1), which mediates perception of fungal chitin and bacterial peptidoglycan (Miya et al., 2007; Willmann et al., 2011). Activation of these receptors triggers conserved signaling cascades, involving reactive oxygen species (ROS) burst, mitogen-activated protein kinase (MAPK) activation, and extensive transcriptional reprogramming. Endogenous danger signals also elicit comparable PTI outputs through phyto cytokine receptors (Rzemieniewski and Stegmann, 2022; Jian Y et al., 2025). The LRR-RKs PEP1 RECEPTOR1 (PEPR1) and PEP2 perceive plant elicitor peptides (Peps) derived from PROPEP precursors, while RECEPTOR-LIKE KINASE7 (RLK7) recognizes PAMP-induced peptides (PIPs) derived from PREPIP precursors (Yamaguchi et al., 2010; Krol et al., 2010; Hou et al., 2014). These receptors amplify and propagate defense signaling downstream of primary MAMP perception and effector-triggered immunity under nutrient sufficiency (Ma et al., 2012; Tintor et al., 2013; Ross et al., 2014; Wang et al., 2026). PEPR signaling can compensate when other LRR-containing PRR pathways are compromised through depletion of the shared coreceptor BRI1-ASSOCIATED RECEPTOR KINASE1 (BAK1) (Yamada et al., 2016). In addition to immune amplification, PEPR2 has been implicated in root meristem differentiation under low Pi via CLAVATA3/EMBRYO SURROUNDING REGION-RELATED 14 peptide signaling in conjunction with the LRR-RK CLAVATA2 (Gutiérrez-Alanís et al., 2017), suggesting that PEPR signaling can intersect with nutrient-responsive developmental pathways.

Recent studies established that PSR antagonizes PTI, showing that FLS2-mediated signaling is attenuated under Pi starvation in a *PHR1*- or *PHT1*-dependent manner (Castrillo et al., 2017 Nature; Dindas et al., 2022; Tang et al., 2022). These findings have been interpreted as evidence for a global nutrient-immunity trade-off in which Pi starvation suppresses immune signaling. However, whether Pi limitation uniformly dampens PRR pathways or instead selectively reconfigures immune receptor composition and signaling capacity has remained unresolved.

Here, we demonstrate that Pi deficiency does not globally suppress immunity but instead remodels the immune receptor landscape. Quantitative membrane proteomics reveals preferential retention and increased abundance of a defined subset of RKs, including the DAMP receptors PEPR1 and RLK7, whereas canonical MAMP receptors are comparatively attenuated. Moreover, Pi limitation enhances

specific downstream defense outputs of retained receptors, indicating output-selective potentiation rather than uniform amplification of PRR signaling. Genetic and transcriptomic analyses further indicate that PEPR signaling contributes not only to inducible defense but also to basal PSR-associated transcriptional reprogramming and plant growth. Together, our findings support a model of receptor-level immune prioritization during nutrient stress, refining the prevailing trade-off paradigm by showing that Pi limitation selectively biases immune architecture toward multifunctional, context-integrating receptors.

Results

Pi deficiency selectively alters the plasma membrane receptor landscape

To determine whether Pi starvation affects the abundance of PRRs, we performed quantitative proteomic analysis of endogenous plasma membrane proteins from Arabidopsis seedlings grown under Pi-sufficient and Pi-deficient conditions (Fig. 1a). Across conditions, 116 plasma membrane-localized receptor proteins were identified, spanning multiple receptor classes, including LRR-RKs, cysteine-rich repeat receptor kinases (CRKs), LysM-RKs, lectin RKs, CrRLK1L family members, and receptor-like proteins (RLPs) (Fig. 1b; Supplementary Fig. 1; Supplementary Data 1). Among these were previously described PRRs such as PEPR1, RLK7, CERK1, and FLS2, whereas PEPR2 was not detected above the analytical threshold.

Comparative quantification revealed a non-uniform response to Pi deprivation. The abundance of PEPR1 and RLK7 was maintained or increased under Pi-deficient conditions, whereas numerous other receptors, including CERK1 and FLS2, were reduced relative to Pi sufficiency (Fig. 1c). These findings indicate that Pi starvation does not globally suppress membrane-resident immune receptors but instead reconfigures the receptor landscape, selectively preserving a defined subset of receptors under nutrient limitation.

Interestingly, transcript levels of *PEPR1*, *RLK7*, *FLS2*, and *CERK1* were all elevated under low Pi conditions (Supplementary Fig. 2a), despite their divergent protein abundance profiles. Thus, selective receptor retention or reduction under Pi deficiency cannot be explained solely at the mRNA level, indicating substantial post-transcriptional regulation or differential protein stability. Among these genes, only *PEPR1* harbors a canonical PHR1-binding site (P1BS; Rubio et al., 2001) in its regulatory DNA sequences (Supplementary Fig. 2b). Consistently, *PEPR1* expression was induced under low Pi partly in a *PHR1/PHL1*-dependent manner (Supplementary Fig. 2c), indicating direct integration of *PEPR1* transcription into the core PSR regulatory network.

Pi deficiency selectively potentiates PEPR signaling outputs

To determine how Pi availability influences PRR signaling competence, we compared immune responses mediated through PEPR1/PEPR2 and CERK1 pathways under Pi deficiency. Given that FLS2 signaling is attenuated under low Pi (Tang et al., 2022), comparison with CERK1 allowed us to assess whether Pi limitation broadly suppresses PRR pathways across RK classes or instead selectively reprograms specific receptor subsets.

Root transcriptomes were analyzed following Pep1 or chitin treatment under Pi-sufficient and Pi-deficient conditions. Among the top 150 genes inducible under Pi sufficiency, a substantially larger proportion of Pep1-responsive genes exhibited further enhancement under Pi deficiency, whereas only a minority of chitin-induced genes retained comparable induction (Fig. 2a, b). Thus, Pi limitation

preferentially amplifies PEPR-dependent transcriptional outputs.

Quantitative RT-PCR (qRT-PCR) of defense markers *CYP71A12* and *PDF1.2a* (Millet et al., 2010; Penninckx et al., 1996) confirmed that Pep1-triggered gene expression was significantly enhanced in Pi-depleted roots, whereas responses to flg22 or chitin were unchanged or attenuated (Fig. 2c), consistent with reduced FLS2 signaling under low Pi (Tang et al., 2022). Pep3-triggered responses, mediated specifically by PEPR1 (Yamaguchi et al., 2010), were similarly enhanced (Supplementary Fig. 3a). Moreover, Pep1-induced expression of *CYP71A12*, but not *PDF1.2a*, was further elevated in plants constitutively expressing PEPR1 under the Cauliflower mosaic virus 35S promoter (Supplementary Fig. 3b). These results indicate that increased receptor abundance contributes to Pep sensitization under low Pi, while full potentiation reflects downstream signaling reinforcement.

Time-course analyses revealed sustained amplification of Pep1-induced marker gene expression from 2 h to 10 h after treatment (Supplementary Fig. 3c), accompanied by stronger MAPK activation (Fig. 2d) and increased extracellular release of PROPEP3-derived peptides (Supplementary Fig. 3d) under Pi deficiency. Enhanced Pep1 responsiveness persisted in *bak1-4* plants (Supplementary Fig. 3e), and BAK1 protein levels were unchanged across Pi conditions (Supplementary Fig. 1, 3f), indicating that PEPR sensitization under Pi limitation is mechanistically distinct from the reinforced PEPR signaling described upon BAK1 depletion (Yamada et al., 2016). In contrast, Pep1-induced PEPR1-BAK1 complex formation and the early ROS burst were unaffected by Pi status (Supplementary Fig. 3f, g), suggesting that Pi deficiency selectively potentiates downstream signaling branches rather than globally enhancing receptor activation.

Importantly, Pep1 pretreatment conferred enhanced resistance to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pto*) DC3000 in nutrient-deficient soil in a *PEPR1/PEPR2*-dependent manner (Fig. 2e), demonstrating that amplified PEPR signaling translates into effective pathogen resistance under Pi limitation.

Consistent with increased receptor abundance, RLK7-mediated *CYP71A12* induction triggered by PIP2 was likewise enhanced under Pi deficiency (Supplementary Fig. 3h). Collectively, these data demonstrate that Pi limitation does not globally suppress PRR signaling but selectively prioritizes DAMP receptor outputs mediated by PEPRs and RLK7, while canonical MAMP receptor pathways remain attenuated. This selective immune potentiation prompted us to investigate how distinct Pi sensing modules coordinate immune receptor pathway reprogramming under nutrient stress.

External Pi sensing via LPR1/LPR2 drives PEPR-dependent immune potentiation under Pi deficiency

To dissect the regulatory basis of this selective immune sensitization, we examined the contributions of the major external and internal Pi sensing modules, LPR1/LPR2 and PHR1/PHL1, respectively (Rubio et al., 2001; Svistoonoff et al., 2007; Paz-Ares et al., 2022; Yang et al., 2024). LPR1/LPR2 mediate local external Pi sensing through Fe-dependent redox processes in the root meristem, whereas PHR1/PHL1 function as central regulators of systemic internal Pi status.

We compared root transcriptomic responses to Pep1 in the wild type (WT), *phr1 phl1*, *lpr1 lpr2*, and *pepr1 pepr2* seedlings after 2 h and 10 h treatment under Pi-sufficient and Pi-deficient conditions. Differential expression analysis ($\log_2$ fold change > 1, FDR < 0.05) identified 1,511 and 1,288 Pep1-inducible genes under Pi-sufficient and Pi-deficient conditions, respectively, defined as transcripts significantly upregulated in WT compared to *pepr1 pepr2* (Fig. 3a). Although the number of inducible genes was not increased under low Pi (Fig. 3a), Pep1-induced transcriptional responses were stronger under Pi deficiency (Fig. 3b), indicating quantitative amplification rather than expansion of PEPR target gene sets.

At 2 h, enhanced Pep1 responses under low Pi were compromised in both *lpr1 lpr2* and *phr1 phl1* (Fig. 3c, Supplementary Fig. 4). By 10 h, however, this impairment persisted predominantly in *lpr1 lpr2*, whereas the Pep1-inducible transcriptional profile in *phr1 phl1* largely resembled that of WT, with many genes exhibiting comparable or even stronger induction (Fig. 3c, Supplementary Fig. 4). These results reveal a temporal divergence between external and internal Pi sensing pathways in coordinating Pep-triggered transcriptional reprogramming.

Focused analysis of genes exhibiting enhanced Pep1 responsiveness under Pi deficiency ($\Delta\log_2FC$ > 0.5, FDR < 0.05) showed that loss of *LPR1/LPR2* resulted in a substantially broader reduction of immune potentiation than loss of *PHR1/PHL1*. The affected genes were enriched for jasmonic acid (JA) biosynthesis and signaling, and defense responses (Fig. 3d), indicating that the LPR1/LPR2 module promotes JA-associated immune outputs during PEPR signaling under Pi deficiency. Conversely, global Pep1-induced transcriptional amplification at 10 h was largely WT-like or enhanced in *phr1 phl1*, consistent with a broad tempering role of internal Pi status on PEPR-dependent defense amplification. Together, these data support a framework in which external Pi sensing via LPR1/LPR2 preferentially promotes PEPR-dependent immune sensitization, whereas internal Pi status exerts a counterbalancing regulatory influence.

Iron-dependent ROS signaling amplifies PEPR-mediated immunity under Pi deficiency

Because iron availability modulates LPR1/LPR2-dependent root responses to low Pi (Naumann et al., 2022), we examined whether iron levels influence PEPR-mediated defense activation under Pi deficiency. Under low Pi, Pep1-induced defense gene expression and MAPK activation were further enhanced by excess iron, whereas both responses were attenuated under iron-deficient conditions (Fig. 4a, b). In contrast, flg22- and chitin-triggered responses were not similarly potentiated by excess iron (Supplementary Fig. 5a, b), indicating that iron-dependent sensitization preferentially reinforces PEPR signaling. Despite this signaling amplification, Pep1-induced primary root growth inhibition occurred irrespective of iron or Pi status (Supplementary Fig. 5c, d), suggesting that PEPR-mediated defense activation and growth arrest are separable outputs.

Because iron solubility increases under acidic conditions (Karimian et al., 2018), we tested whether pH modulates PEPR signaling under low Pi. Although alkaline pH enhances Pep1–PEPR binding *in vitro* (Liu et al., 2022), Pep1-induced defense gene expression in our system was elevated under acidic conditions (Fig. 4c), consistent with increased iron availability promoting immune potentiation *in vivo*.

Under Pi deficiency, Fe³⁺ accumulation in the root meristem is strongly reduced in *lpr1 lpr2* mutants (Naumann et al., 2022). Consistently, enhanced Pep1-induced gene expression under low Pi was lowered in *lpr1 lpr2* even in the presence of excess iron (Fig. 4d), indicating that LPR1/LPR2-mediated redox regulation is required for iron-dependent amplification of PEPR outputs.

Excess iron can elevate ROS and reinforce defense responses, while increasing oxidative stress (Li et al., 2019; Herlihy et al., 2020; Jwa and Hwang, 2025). Under low Pi with excess iron, ROS accumulation increased in root tips (Supplementary Fig. 5e), as previously described (Naumann et al., 2022). In *rbohD rbohF* mutants defective in apoplastic ROS production (Torres et al., 2001; Galletti et al., 2008), Pep1-induced defense gene expression was reduced under low Pi (Fig. 4e), demonstrating that ROS production is required for iron-dependent enhancement of PEPR signaling.

Finally, several defense regulators, including *FLAVIN-DEPENDENT MONOOXYGENASE1* (*FMO1*), *ENHANCED DISEASE SUSCEPTIBILITY5* (*EDS5*), and *PHYTOALEXIN DEFICIENT3* (*PAD3*) (Zhang et al., 2008; Bernsdorff et al., 2016; Rawat et al., 2023), were induced by low Pi or excess iron alone, even without exogenous Pep1 application (Supplementary Fig. 5f). RNA-seq analysis confirmed that these genes were upregulated under Pi deficiency (Supplementary Fig. 5g, h). However, their induction was diminished in *pepr1 pepr2*, indicating that endogenous Pep–PEPR

signaling contributes to iron-associated defense activation under Pi limitation.

WRKY33 mediates PEPR-dependent immune amplification under Pi limitation

Cis-regulatory motif enrichment analysis of Pep1-inducible genes further upregulated under low Pi revealed significant enrichment of WRKY33-related motifs, including those recognized by its closest paralogue WRKY25, at both 2 h and 10 h after Pep1 treatment (Fig. 5a). WRKY33 is a central regulator of camalexin biosynthesis and defense-associated transcriptional reprogramming (Qiu et al., 2008; Mao et al., 2011; Birkenbihl et al., 2017). Beyond immunity, WRKY33 has been implicated in nutrient stress responses, including Pi starvation, as a potential integrator of nutrition and defense signaling (Shen et al., 2021; Zhang et al., 2023; Tao et al., 2024).

Consistent with this notion, *WRKY33* expression was induced by Pep1 under low Pi and excess iron conditions (Fig. 5b). In *wrky33* mutants, Pep1 induction of *CYP71A12* and *PDF1.2a* expression under low Pi was reduced relative to WT (Fig. 5c), indicating that WRKY33 contributes to amplification of PEPR-mediated transcriptional outputs during Pi limitation.

PEPR1/PEPR2 promote PSR and shape root microbiota under Pi limitation partly via PHR1/PHL1

Our RNA-seq analysis revealed that Pep1 application to Pi-deprived seedlings led to suppression of phosphate starvation-inducible (PSI) genes (Fig. 6a), confirming antagonism between acute immune activation and PSR transcriptional outputs (Castrillo et al., 2017; Dindas et al., 2022). Notably, however, in the absence of exogenous Pep1, PSI gene induction was reduced in *pepr1 pepr2* under Pi deficiency (Fig. 6a). These suppressed genes included canonical PHR1/PHL1 targets involved in Pi uptake (Fig. 6b), indicating that basal PEPR signaling contributes positively to PSR-associated transcription in non-elicited plants. Consistently, constitutive expression of PEPR1-FLAG or PEPR2-FLAG increased shoot biomass under sterile low Pi conditions (Fig. 6c, d), supporting a role for PEPR signaling in nutrient stress adaptation.

A 16S rRNA amplicon sequencing analysis revealed shifts in root microbiota composition in *phr1 phl1*, *pepr1 pepr2*, and *lpr1 lpr2* compared with WT, in nutrient-deficient soil, indicated by PERMANOVA analysis (Fig. 6e; Supplementary Fig. 6). Microbiota alterations in *pepr1 pepr2* and *lpr1 lpr2* were partially attenuated in the *phr1 phl1* background, and the magnitude of divergence (pseudo-F) was reduced when *pepr1 pepr2* mutations were introduced into the *phr1 phl1* background (Supplementary Fig. 6). These results indicate that PEPR1 and PEPR2 influence root microbiota assembly under nutrient limitation, acting partly through PHR1/PHL1-dependent networks.

PROPEP6 antagonizes Pep-triggered immunity and redirects PEPR signaling under phosphate limitation

Although Pep-triggered PEPR activation typically inhibits plant growth (Liu et al., 2013; Ross et al., 2014), our data indicate that, in the absence of exogenous elicitation, PEPR1/PEPR2 promote PSR and biomass accumulation under low Pi. Immunoprecipitation–mass spectrometry (IP–MS) identified PROPEP6 as a PEPR1-associated protein, and this association was diminished upon Pep1 treatment (Supplementary Fig. 7a). This dynamic interaction suggests that PROPEP-derived ligands distinct from canonical immunogenic Peps may occupy PEPR1 under non-elicited conditions, biasing signaling toward nutrient adaptation rather than defense activation. Consistently, transcripts of *PROPEP1–PROPEP8* were detected in non-elicited plants, with *PROPEP1/2/3/4* up-regulated under Pi deficiency relative to Pi sufficiency (Supplementary Fig. 8a, b), prompting us to examine whether specific PROPEPs functionally modulate PEPR signaling outputs.

Transient expression of Arabidopsis PROPEPs in *Nicotiana benthamiana* revealed that, unlike other family members, PROPEP6 was not processed into a detectable mature Pep peptide (Supplementary Fig. 7b), consistent with its reported resistance to cleavage by the metacaspase METACASPASE4 (Shen et al., 2019). We further confirmed extracellular release of PROPEP6-derived peptides, including an apparently full-length form (~39 kDa), in Arabidopsis (Supplementary Fig. 7c). Functionally, PROPEP6 strongly suppressed Pep1-induced ROS burst and MAPK activation (Fig. 7a, b; Supplementary Fig. 7d) and selectively inhibited Pep–PEPR1 signaling without affecting PEPR2 or other PRR pathways (Fig. 7b; Supplementary Fig. 7d). Co-immunoprecipitation in *N. benthamiana* and *in vitro* binding assays confirmed physical association between PROPEP6 and PEPR1 via both extracellular and cytoplasmic domains (Supplementary Fig. 7e, f). PROPEP6 attenuated Pep1-induced PEPR1–BAK1 complex formation but did not affect PEPR2–BAK1 association (Fig. 7c). Notably, suppression was observed with non-tagged PROPEP6 (Fig. 7b, c), excluding artefacts caused by fluorescent protein fusions. Because ligand-induced LRR–RK–BAK1 complex formation was independent of kinase activity or trans-phosphorylation (Schulze et al., 2010), these results support a model in which PROPEP6 interferes with receptor complex assembly rather than directly inhibiting PEPR1 kinase activity.

To assess the contribution of endogenous PROPEPs, we generated CRISPR/Cas9 mutants targeting *PROPEP1*, *PROPEP2*, *PROPEP3*, and *PROPEP6*. Higher-order *propep* mutants displayed enhanced Pep1-induced defense gene expression, particularly under low Pi (Supplementary Fig. 7g), supporting a role for endogenous PROPEPs in constraining PEPR-mediated immune outputs.

Given the positive role of PEPR1/PEPR2 in PSR and growth under low Pi, we next examined the impact of PROPEP6 on plant growth. Constitutive PROPEP6-mCitrine expression increased shoot biomass under low Pi in a *PEPR*-dependent manner (Fig. 7d–f). Consistently, *propep* octuple mutants

exhibited alleviated inhibition of primary root growth under low Pi (Fig. 7g) and reduced *PHT11;4* induction (Fig. 7h).

Collectively, these findings demonstrate that PROPEPs, particularly PROPEP6, act as antagonistic modulators that attenuate PEPR-mediated immune activation while permitting or even facilitating PEPR-dependent promotion of PSR and growth.

Discussion

Pi limitation imposes a fundamental trade-off between immune activation and nutrient acquisition. Our study identifies PEPR signaling as a central regulatory node within this balance, capable of biasing signaling outputs toward defense or nutrient adaptation depending on context (Fig. 8). Quantitative proteomics revealed that Pi deficiency selectively preserves or increases the abundance of specific DAMP receptors, including PEPR1 and RLK7, while reducing several canonical MAMP receptors (Fig. 1). Although this receptor-level reconfiguration provides a framework for altered immune responsiveness, our data demonstrate that immune receptor pathway reprogramming at the post-receptor level also plays a decisive role in the nutritional regulation of immunity. Under Pi limitation, PEPR-mediated immunity is reinforced in a pathway-selective manner rather than through uniform amplification of receptor activation: early receptor-proximal events, including PEPR–BAK1 complex formation and ROS burst amplitude, remain largely unchanged, whereas downstream transcriptional outputs, partly via WRKY33, are potentiated. This uncoupling indicates that Pi deficiency biases signal propagation both at the level of receptor abundance and along downstream signaling cascades. Integration of external Pi sensing with the LPR1/LPR2–iron–ROS axis likely primes MAPK cascades and transcriptional regulators, enabling selective amplification of immune gene expression without globally increasing receptor activation. This receptor-specific potentiation contrasts with other PRRs. For example, FLS2 signaling is attenuated under Pi deficiency and is not enhanced by elevated iron availability, despite transcriptomic output similarity to iron deficiency responses (Platre et al., 2022). Such divergence indicates that nutrient status does not impose uniform regulation across PRR systems but instead differentially modulates distinct receptor modules. The molecular basis for this specificity, potentially involving differential co-receptor usage, phosphorylation dynamics, scaffold recruitment, membrane compartmentalization, or connectivity to downstream steps, remains an important area for future investigation.

Beyond immune potentiation, PEPR signaling exerts a positive role in PSR when immunogenic Pep ligands remain at low levels. In non-elicited plants, PEPR promotes PSR-associated transcription and growth-related processes, partly through PHR1/PHL1-dependent pathways, indicating that basal PEPR activity is biased toward nutrient adaptation in the absence of strong damage cues. The identification of PROPEPs, particularly PROPEP6, as ligand-state-dependent modulators further supports this dual functionality. Rather than acting solely as precursors of canonical agonistic Peps, PROPEPs may generate distinct ligand states that attenuate high-amplitude Pep-triggered immune activation while permitting adaptive nutrient-associated outputs. Such ligand-dependent signal bias provides a mechanistic framework for how a single receptor system can differentially route signaling toward immunity or nutrient adaptation. How PEPR promotes PSR in the apparent absence of canonical Pep elicitation, whether through low-level constitutive signaling, ligand-independent basal activity, or alternative PROPEP processing states, remains to be clarified.

Recent studies illustrate ligand-dependent dual or antagonistic receptor functions. PSY peptide receptors activate stress-responsive programs upon ligand depletion, enabling localized sensing of metabolic dysfunction (Ogawa-Onishi et al., 2023). The tomato systemin receptors SYR1 and SYR2 form a high- and low-affinity pair that respectively initiate and attenuate defense signaling, establishing intrinsic feedback control (Zhou 2025 Dev Cell). In maize, ZmPep5a peptide competitively antagonizes immune responses induced by immunogenic ZmPeps (Poretsky et al., 2020), and tomato antiSYS peptides function as antagonists required for immune homeostasis (Wang et al., 2025). Scaffold receptors such as FERONIA further demonstrate how distinct classes of RALF peptide ligands can direct signaling toward immune activation or suppression (Stegmann, 2017). Together, these studies support an emerging view that endogenous peptide receptor systems frequently operate through ligand-context-dependent signal partitioning rather than binary on–off activation.

The dual functionality of PEPR aligns with an emerging paradigm in plant receptor biology: certain endogenous peptide receptors function as integrative hubs that coordinate immunity and development. In Arabidopsis, RLK7 perceives PIP peptides derived from PREPIP precursors to amplify immune signaling downstream of MAMP perception, while also regulating lateral root spacing through auxin-dependent developmental pathways (Toyokura et al., 2019). RLK7 additionally perceives CEP4 peptide, linking immune signaling with nitrogen starvation responses (Rzemieniewski et al, 2024). Similarly, Pep–PEPR signaling extends beyond inducible immunity. In addition to reinforcing defense outputs, PEPR signaling promotes root hair development (Okada et al., 2021; Jing et al., 2024) and contributes positively to PSR under non-Pep-elicited conditions (Fig. 6). These observations indicate that specific DAMP receptors act as nodes integrating immune and growth-related pathways, distinguishing them from canonical PRRs that are largely confined to microbial pattern recognition and defense activation. The preferential retention of PEPR1 and RLK7 under Pi deficiency raises the possibility that receptors endowed with dual immune–developmental functions are selectively maintained when plants must reconcile resource limitation with environmental threat sensing and adaptation.

Iron further anchors this integration between nutrition and immunity. As an essential cofactor and catalyst of ROS generation, iron availability profoundly influences plant–microbe interactions (Verbon et al., 2017; Herlihy et al., 2020; Jwa and Hwang, 2025). Plants can restrict iron accessibility to pathogens or locally accumulate iron to enhance antimicrobial ROS production, whereas pathogens deploy siderophores to capture or scavenge host iron. Accordingly, iron homeostasis and immune signaling are tightly interconnected: iron deficiency activates defense-associated pathways, while PTI signaling negatively regulates iron uptake programs (Platre et al, 2022; Cao et al, 2024; Shikha et al., 2026).

Recent studies show that a PROPEP2–PEPR2 module down-regulates iron uptake, rhizosphere

acidification, and primary root growth during iron deficiency (Shikha et al., 2026), consistent with our findings that Pep1-induced root growth inhibition occurs independently of iron availability (Supplementary Fig 5). Together, these observations indicate that distinct PEPR outputs differ in their reliance on iron-associated redox processes: iron is dispensable for Pep-mediated growth inhibition but required for immune potentiation under low Pi. Our data further show that external Pi sensing, iron-dependent redox remodeling, and PEPR signaling converge to reinforce immune outputs. We propose that the LPR1/LPR2-dependent iron-redox module couples external Pi depletion to quantitative tuning of PEPR-mediated immune outputs. How PEPR mechanistically intersects with established iron sensing and signaling regulators remains an important question for future investigation.

PEPR activity under Pi limitation may also have ecological consequences at the root-microbiota interface. PSR profoundly influences root exudation patterns, architecture, and microbial community composition (Svistoonoff et al., 2007; Castrillo et al., 2017; Tang et al., 2022; Liu et al., 2024). Because PEPR contributes to PSR-associated transcription and growth in non-elicited plants, it is conceivable that PEPR shapes microbiota assembly through nutrient-driven effects rather than immune activation.

Together, our results redefine PEPR signaling as a context-responsive regulatory interface coordinating immune activation and nutrient acquisition. Rather than being uniformly amplified or attenuated by nutrient stress, PEPR outputs are selectively biased through integration of ligand state, redox cues, and transcriptional circuitry. This immune receptor pathway reprogramming model provides a mechanistic framework for how plants dynamically allocate signaling capacity between immunity or nutrient stress adaptation in fluctuating environments.

Methods

Plant materials and growth conditions

The *Arabidopsis thaliana* accession Columbia-0 (Col-0) was used as the wild type (WT) throughout this study. A complete list of plant materials is provided in Supplementary Data 2. Seeds were surface-sterilized in 5% sodium hypochlorite containing 0.05% Triton X-100 for 5 min, followed by three rinses with sterile distilled water. After stratification at 4°C for 2 days, seeds were sown on half-strength Murashige and Skoog (1/2 MS) medium containing 2.3 g/L MS basal salts (FUJIFILM Wako Pure Chemical), 1% (w/v) sucrose, and 1% (w/v) agar (Sigma-Aldrich, MO, USA). Plants were grown at 23°C under long-day conditions (16 h light/8 h dark) with 65% relative humidity. For phosphate deficiency assays, seedlings were initially cultivated on 1/2 MS medium for 4 days and then transferred either to control medium supplemented with 625 µM phosphate (+Pi) or to low-phosphate medium containing 10 µM phosphate (−Pi) for an additional 4 days. The +Pi medium consisted of 1/2 MS supplemented with 1% (w/v) sucrose and 1% (w/v) agarose (Biowest Regular Agarose G-10). For −Pi conditions, KH₂PO₄ in the standard medium was replaced with KCl. The pH of both media was adjusted to 5.7 using 1 M KOH. For bacterial inoculation experiments, two-week-old seedlings were transplanted into soil and maintained in growth chambers at 22°C, 60% relative humidity, under short-day conditions (8 h light/16 h dark). Nutrient-deficient soil consisted of organic black soil (Maruishi Engei) mixed with Ezo sand (Kobayashi Sangyo) at a 2:1 (w/w) ratio. Plants were watered with distilled water. *N. benthamiana* plants were grown on 1/2 MS medium for 12 days under long-day conditions and subsequently transferred to the soil consisted of 1:1 (v/v) mixture of Super Mix A (Sakata Seed) and Vermiculite G20 (Maruishi Engei). Leaves from 4- to 5-week-old plants were used for transient expression assays.

Plasmid DNA construction

The genomic region of *PROPEPs*, extending from the start codon to just before the stop codon, was amplified from wild-type *Arabidopsis* genomic DNA. PCR amplification was performed using PrimeSTAR GXL DNA Polymerase (Takara Bio, Cat# R050A). The amplified products were cloned into the pENTR/D-TOPO vector using the In-Fusion Snap Assembly Master Mix (Takara Bio, Cat# 638948). Proper insertion of the entry constructs was verified by Sanger sequencing with the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Cat# 4337455). Confirmed entry clones were then recombined into the destination vector pGWB502 (C-mCitrine) via the Gateway LR Clonase II Enzyme Mix (Invitrogen, Cat# 11791020), whereas entry clones containing the stop codon were transferred into the no-tag version of pGWB502 via the same Gateway LR reaction. To generate constructs for recombinant proteins for in vitro binding assays, PROPEP6 CDS was cloned into pCold GST DNA (Takara). The coding sequence of PEPR1, PEPR2, FLS2, and EFR extending from the start

codon to just before the stop codon, and ectoPEPR1 (1-792 bp) and cytoPEPR1 (793-1123) were cloned into the pGWB502 (C-FLAG). The primer sequences used for cloning are listed in Supplementary Data 3.

Generation of transgenic plants and *propep1/2/3/6* mutants

The p35S::PROPEP6-mCitrine construct described above were transformed into Col-0 plants by *Agrobacterium tumefaciens*-mediated transformation using the floral dip method (Zhang et al. 2006). For multiple sgRNA expression, complementary oligonucleotide pairs comprising the target sequence of *PROPEPs* (Supplementary Data 4) were annealed and then inserted into BsaI site and AarI site in pUC-AtU6-26_gtg (accession number: LC906469). For the construction of all-in-one CRISPR-Cas9 vectors, sgRNA expression cassettes from pUC-AtU6-26_gtg were transferred to pGHO-YC9 (accession number: LC906470).

Plasma membrane enrichment

Plasma membrane fractions were enriched with minor modifications to a published protocol (Collins et al., 2017). Briefly, 8-day-old seedlings (~200 mg fresh weight) grown under +Pi or -Pi conditions were frozen in liquid nitrogen, ground to a fine powder, and homogenized in 500 µl extraction buffer (250 mM sucrose, 50 mM Tris-HCl pH 7.5, 5% glycerol, 1 mM sodium molybdate, 25 mM NaF, 2 mM sodium orthovanadate, 10 mM EDTA, 3 mM DTT, and 1 mM PMSF). The homogenate was centrifuged at 8,000 × g for 10 min at 4°C, and the supernatant was transferred to ultracentrifuge tubes for subsequent fractionation according to the published protocol.

Sample preparation for proteomic analysis

Proteins present in the supernatant were precipitated using pre-chilled acetone (-30°C), and the resulting pellets were resuspended in a digestion buffer containing 100 mM Tris-HCl (pH 9.0), 12 mM sodium lauryl sulfate (SLS), and 12 mM sodium deoxycholate (SDC). For each sample, 200 µg of protein was subjected to reduction and alkylation in a solution consisting of 10 mM TCEP, 40 mM chloroacetamide (CAA), and 50 mM ammonium bicarbonate for 30 min at 24°C in the dark. The samples were then diluted fivefold with 50 mM ammonium bicarbonate and digested overnight at 37°C with 2 µg of trypsin (Promega). Surfactants, including SLS and SDC, were removed using a phase-transfer method as previously described (Masuda et al., 2008). The resulting peptides were desalted using an in-house StageTip packed with SDB Empore disks (CDS), dried, and stored at -20°C until LC-MS/MS analysis.

LC-MS/MS analysis and raw data processing

Peptide samples were analyzed using an Easy-nLC 1200 nanoLC system (Thermo Scientific) coupled to an Orbitrap Exploris 480 mass spectrometer (Thermo Scientific) equipped with a FAIMS Pro interface. Dried peptides were reconstituted in 2% acetonitrile (ACN) with 0.1% formic acid (FA) and loaded onto a C18 capillary column (75 $\mu\text{m} \times 15\text{ cm}$; Nikkyo Technos). Peptides were separated at 300 nL/min and 60°C using nonlinear gradients of 0.1% FA in water (Buffer A) and 0.1% FA in 80% ACN (Buffer B). A 140-min gradient was applied for global and phosphoproteomic analyses, and a 105-min gradient for IP-MS samples. Eluted peptides were ionized at 2.2 kV and analyzed in positive mode using data-dependent acquisition. MS1 spectra (m/z 375–1500) were acquired at 120,000 resolution, and MS2 spectra (m/z >120) at 30,000 resolution. FAIMS compensation voltages of –40/–60 V or –50/–70 V were used under standard resolution settings (IT 100°C, OT 100°C). Peptide identification and MS1-based label-free quantification (LFQ) were performed with Proteome Discoverer 2.5 (Thermo Scientific) using the SEQUEST HT algorithm against the Araport11 database. Search parameters included trypsin digestion (≤ 2 missed cleavages), precursor tolerance of 10 ppm, fragment tolerance of 0.02 Da, carbamidomethylation of cysteine as a fixed modification, and oxidation (M), N-terminal acetylation, and phosphorylation (S/T/Y) as variable modifications (≤ 3 per peptide). Peptide validation was conducted with Percolator, and only high-confidence identifications (FDR <1%) were used for protein inference and LFQ. Total ion chromatograms were normalized across biological conditions. Phosphorylation site localization probabilities were calculated using the IMP-ptmRS node in Proteome Discoverer 2.5.

qRT-PCR

Total RNA was isolated from root tissues using Sepasol-RNA I Super G (Nacalai Tesque). Following DNase treatment, cDNA was synthesized with the PrimeScript RT Reagent Kit with gDNA Eraser (Takara) according to the manufacturer's protocol. qRT-PCR was conducted using Power SYBR Green PCR Master Mix (Applied Biosystems) on a Thermal Cycler Dice Real Time System III (Takara). Relative transcript levels were calculated by the $\Delta\Delta\text{Ct}$ method and normalized to ACTIN2. Primer sequences are provided in Supplementary Data 3.

RNA-sequencing and data analysis

Total RNA was extracted from plant root tissue, followed by DNase treatment using NucleoSpin RNA Plant (Macherey-Nagel, Cat# 740949.50) according to the manufacturer's instruction. Each raw read data stored in DDBJ with accession numbers DRR to DRR was generated and analyzed as follows. Each RNA library was prepared from 1000 ng of 669 total RNA using the NEBNext Ultra II RNA

Library Prep Kit for Illumina (New England 670 Biolabs, Cat# E7770) according to the manufacturer's instruction. PCR for library amplification was performed for 7 cycles. The average length of the library was 350 bp, 672 as measured by a bioanalyzer. Concentrations were measured by Kapa Library

***Pto* DC3000 infection assay**

Pto DC3000 was grown overnight at 28°C with shaking (180 rpm) in King's B medium (2% Bacto Proteose Peptone No. 3, 0.15% K₂HPO₄, 1% glycerol, 5 mM MgSO₄) supplemented with 50 µg/mL rifampicin. Bacterial cells were harvested and resuspended in sterile water to an OD₆₀₀ of 0.2. For inoculation, the suspension was diluted 1 × 10⁻³ and infiltrated into three leaves per plant using a needleless syringe. Excess liquid was gently blotted from the leaf surface. Plants were maintained under standard growth conditions for the indicated times, with high humidity (~95% RH) ensured by covering with a transparent plastic container.

ROS measurement

For total ROS measurements, 7-day-old seedlings were placed in 96-well plates containing 100 µL sterile water, covered, and incubated in the dark overnight. After 24 h, the water was replaced with 100 µL of assay solution containing 20 µM luminol L-012 (Sigma-Aldrich), 1 µg/mL horseradish peroxidase (HRP; Sigma-Aldrich), and 1 µM Pep1. Luminescence was recorded using a Fluoroskan Ascent FL microplate reader (Thermo Scientific), and ROS production was quantified as the cumulative photon counts.

For H2DCFDA staining, seedlings were grown on 1/2 MS medium for 4 days and then transferred to either +Pi or -Pi conditions for an additional 4 days. H2DCFDA was dissolved in DMSO (10 mg/mL), diluted to a 10× stock (500 µM) in 0.1 M phosphate buffer (pH 7.0), and stored at 4°C. Prior to use, aliquots were thawed in the dark, kept on ice, and diluted to 1× in phosphate buffer. Whole seedlings were incubated in 2 mL staining solution per well (12-well plate) for 10 min at room temperature in the dark. Fluorescence images were acquired with FV1000 confocal laser scanning biological microscope using a 10×/0.4 NA objective. H2DCFDA was excited at 488 nm, and emission was detected between 500–550 nm.

Western blotting analysis

Frozen plant tissues were ground to a fine powder and homogenized in protein extraction buffer containing 62.5 mM Tris-HCl (pH 6.8), 10% glycerol, 2% SDS, 5% (v/v) 2-mercaptoethanol, and 0.02% bromophenol blue, supplemented with 1 mM EDTA (pH 8.0), phosphatase inhibitors, and a protease inhibitor cocktail (Roche). The buffer was added at a ratio of 3 µL per mg fresh weight.

Samples were heated at 95°C for 5 min and centrifuged at 20,000 × g for 10 min at 25°C. The supernatants were collected for subsequent analysis. Proteins were resolved on 10% polyacrylamide gels and transferred onto PVDF membranes (Millipore). Membranes were blocked with 2% (w/v) skim milk (Nacalai Tesque), in TBS-T (25 mM Tris, 192 mM glycine, 0.1% [v/v] Tween-20) for 1 h at room temperature with agitation. After blocking, membranes were briefly rinsed with deionized water and TBS-T, followed by overnight incubation at 4°C with primary antibodies in TBS-T. Membranes were washed five times with deionized water and once in TBS-T for 5 min at room temperature, then incubated with secondary antibodies for 1 h at room temperature with agitation. After secondary antibody incubation, membranes were washed as described above. Signals were detected using Chemi-Lumi One L (Nacalai Tesque) and visualized with a FUSION FX imaging system (Vilber). The following antibodies were used: anti-pERK antibody (Cell Signaling, Cat#4370), anti-PROPEP3 (Yamada et al., 2016), using anti-BAK1 (Stegmann et al., 2017), anti-GFP (MBL, Cat#598), anti-FLAG (Sigma-Aldrich, Cat# F1804), HRP-conjugated anti-rabbit IgG (Cell Signaling Technology, Cat# 7074) and HRP-conjugated anti-mouse IgG (Cell Signaling Technology, Cat# 7076). All antibodies were diluted in TBS-T.

Co-immunoprecipitation (Co-IP) assays

Arabidopsis seedlings were grown on either +Pi or −Pi medium for 7 days, treated with 1 μM Pep1 for 15 min, and then immediately frozen in liquid nitrogen. Roots were lysed with protein extraction buffer (50 mM pH7.5 Tris-HCl, 150 mM NaCl, 10% [v/v] glycerol, 0.5% [v/v] TritonX-100, supplemented with 1 mM EDTA (pH 8.0) and a protease inhibitor cocktail (Roche). Immunoprecipitation was performed using Anti-FLAG M2 Magnetic Beads (sigma), which were incubated with crude protein extracts for 1 h at 4°C. The beads were subsequently washed five times with protein extraction buffer, resuspended in SDS sample buffer, and incubated at 95°C for 5 min. Proteins were analyzed by SDS-PAGE followed by immunoblotting

Extracellular PROPEP detection assays

Seven-day-old seedlings grown under either +Pi or −Pi conditions were transferred to liquid MS medium. After 24 h, the medium was replaced with fresh medium prior to Pep1 treatment. The following day, seedlings were treated with 1 μM Pep1 for the indicated time periods. Culture media were collected, and proteins were concentrated using Strataclean resin (Agilent Technologies). After centrifugation, the resin was boiled in SDS sample buffer, and proteins were separated by SDS-PAGE. Seedling tissues were also harvested and subjected to immunoblot analysis.

DNA extraction and 16S rRNA gene amplicon sequencing

Root tissues were homogenized using a Mixer Mill (Verder Scientific Co., Ltd., Tokyo, Japan), and total genomic DNA was isolated with the NucleoSpin® Soil kit (MACHEREY-NAGEL GmbH & Co. KG, Germany) in accordance with the manufacturer's protocol. The V4 region of the 16S rRNA gene was amplified by touchdown PCR using KOD FX Neo polymerase (TOYOBO Co., Ltd., Osaka, Japan) together with Illumina adapter-linked barcoded primers 515F (TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGCCAGCMGCCGCGGTA-A) and 806R (GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGGACTACHVGGGTWTCTAAT), as previously described (Edwards et al., 2015). PCR products of the expected size were separated on a 1% agarose gel, excised, and purified using the FastGene Gel/PCR Extraction Kit (Nippon Genetics Co., Ltd., Tokyo, Japan) according to the manufacturer's instructions. Amplicon libraries were subsequently constructed using Nextera XT Index kits (Illumina, Inc., CA, USA), and 300-bp paired-end sequencing was carried out on an Illumina MiSeq platform with the MiSeq Reagent Kit v3.

Sequence data processing

Raw sequencing reads were demultiplexed using the `clsplitseq` command implemented in `Claident` (Tanabe and Toju, 2013), applying a minimum quality threshold of 30. Amplicon sequence variants (ASVs) were inferred using the DADA2 pipeline (Callahan et al., 2016) in the R package `dada2`. For the `filterAndTrim()` function, parameters were specified as follows: `truncQ = 2`; `truncLen = 220` (forward) and 150 (reverse); `trimLeft = 19` (forward) and 20 (reverse); `maxN = 0`; and `maxEE = 2` for both forward and reverse reads. Chimeric sequences were detected and eliminated using default settings. Taxonomic assignment of ASVs was conducted with `SINA v1.2.11` (Pruesse et al. 2012) against the `SILVA SSURF NR99` database release 132 (Quast et al., 2012), applying a minimum similarity cutoff of 85%. Sequences classified as Eukarya, Archaea, mitochondrial or chloroplast in origin, as well as those that could not be aligned to reference sequences, were excluded from further analyses.

Statistical analyses

Beta diversity analyses were performed on the Galaxy Europe platform using the `QIIME2` wrapper developed by the European Galaxy Team. Bray–Curtis dissimilarity matrices were generated, and statistical differences among groups were evaluated by `PERMANOVA` (beta-group-significance, `method = permanova`) with pairwise comparisons and 999 permutations. Differences between groups were determined based on the separation of multivariate centroids in the distance matrix space.

Statistical significance was assessed using false discovery rate (FDR)-adjusted q-values provided by Galaxy. Data visualization was carried out in R using the ggplot2 package (Wickham 2016).

Agrobacterium-mediated transient expression

Agrobacterium strains (GV3101::pMP90) carrying the relevant expression vectors were cultured overnight at 28°C with shaking (180 rpm) in 2×YT liquid medium [1% (w/v) yeast extract, 1.6% (w/v) tryptone, 0.5% (w/v) NaCl] supplemented with 50 µg/mL gentamycin and 100 µg/mL spectinomycin. Bacterial cells were collected by centrifugation at 2,000 g for 10 min at 25°C, washed once with sterilized water, and resuspended in sterilized water to an OD600 of 0.5. Acetosyringone was added to the suspension to a final concentration of 500 µM. The bacterial suspensions were infiltrated into the leaves of *N. benthamiana* using a needleless syringe. The infiltrated plants were maintained under their growth conditions for approximately 48 hours.

In vitro protein interaction assays

GST-tagged recombinant proteins were expressed in *E. coli* BL21 and purified using Glutathione Sepharose 4B (GE Healthcare) according to the manufacturer's instructions. Beads were washed with wash buffer (50 mM Tris-HCl, pH 8.0, 100 mM NaCl), and proteins were eluted with elution buffer (50 mM Tris-HCl, pH 8.0, 10 mM reduced glutathione). Protein concentrations were determined by the Bradford assay. Expression constructs encoding ectoPEPR1-FLAG and cytoPEPR1-FLAG were transformed into *A. tumefaciens* GV3101 and transiently expressed in *N. benthamiana* leaves. Two days after infiltration, leaf tissue was homogenized in extraction buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10% [v/v] glycerol, 0.5% [v/v] Triton X-100) supplemented with 1 mM EDTA (pH 8.0) and protease inhibitor cocktail (Roche). Extracts were incubated with anti-FLAG M2 magnetic beads (Sigma) for 1 h at 4°C, washed with extraction buffer, and eluted with TBS containing 500 µg/mL FLAG (DYKDDDDK) peptide. For pull-down assays, 2 µL of eluted ectoPEPR1 or cytoPEPR1 were incubated with 150 nM purified PROPEP6-GST in pull-down buffer (50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 10% glycerol) for 2 h at 4°C. GFP-FLAG was used as control. Glutathione beads were then added and incubated for 2 h at 4°C. Beads were washed with buffer and boiled in SDS sample buffer prior to analysis.

Data availability

The raw RNA-sequencing data have been deposited in the DDJB database under accession code PRJDB37679 and PRJDB35626.

LC-MS/MS raw data have been deposited in Japan Proteome Standard Repository/ Database

(jPOSTP) <https://repository.jpostdb.org/preview/1230333443699fda85f12b0> Access key 7188.
The 16S rRNA gene amplicon and metagenome sequencing data have been deposited in NCBI under the accession number DRA012727.

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Author contributions (under partial revision)

Y.S. conceived the study. N.T., T.H.L., K.H., and Y.S. designed the experiments. N.T., T.H.L., M.L., K.Y., K.H., T.H., S.Y., Y.D.U., M.F., and T.U. performed the experiments and analyzed the data. H.A. developed the materials. K.Y., Y.D.U., K.O., S.C., and T.U. advised on the data analyses. N.T. and Y.S. wrote the manuscript with contributions from the other authors.

Competing interests

The authors declare no competing interests.

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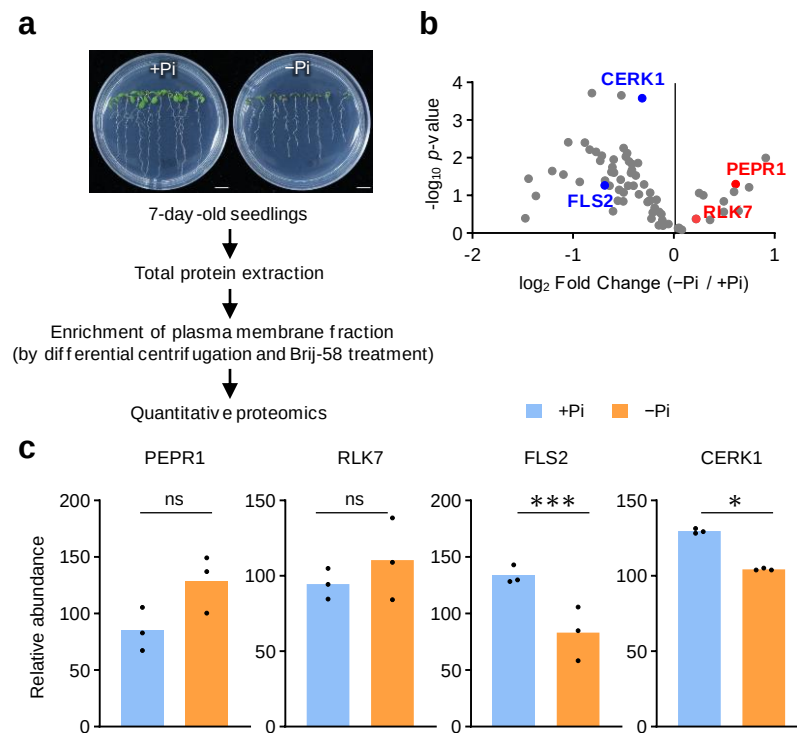


Fig. 1: Abundance changes of plasma membrane-localized receptors under low Pi

a, Workflow for proteome analysis with plasma membrane enriched fraction. Plants grown under Pi-sufficient conditions (+Pi, 625 μM) or Pi-deficient conditions (-Pi, 10 μM) are shown.

b, Volcano plots show changes in the receptor protein abundance according to the average ratio ($\log_2$) and p value ($-\log_{10}$ adjusted p value) in WT seedlings.

c, Relative abundance of PRR proteins under +Pi and -Pi conditions. Data are means, $n = 3$ biological replicates. Asterisks indicate significant differences between +Pi and -Pi; * $p < 0.05$, *** $p < 0.0001$ (student's t -test); ns, not significant.

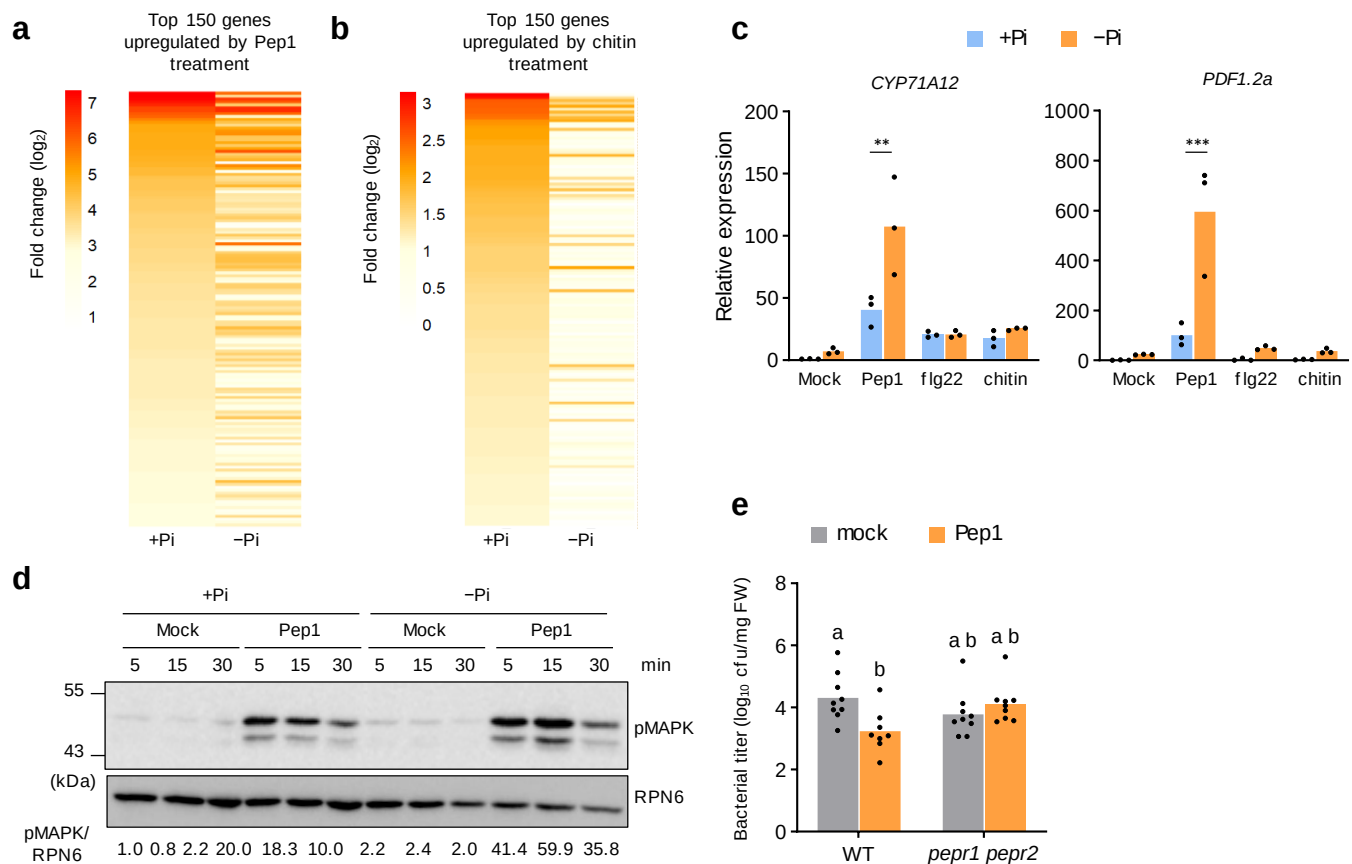


Fig. 2: Pi deficiency selectively potentiates PEPR signaling outputs

a, Fold-change of the top 150 genes significantly induced by Pep1 treatment under Pi-sufficient conditions (+Pi, 625 μ M). Heatmap is shown as log₂ fold change compared with mock-treated plants under Pi-sufficient conditions (+Pi, 625 μ M) or Pi-deficient conditions (-Pi, 10 μ M).

b, Fold-change of the top 150 genes significantly induced by chitin treatment under +Pi conditions. Heatmap is shown as log₂ fold change compared with mock-treated plants in +Pi or -Pi conditions.

c, Transcript levels of *CYP71A12* and *PDF1.2a* in the WT roots grown under +Pi or -Pi conditions and treated with water, 1 μ M Pep1, 1 μ M flg22, or 1 mg / ml chitin for 6 h. Data are means, $n = 3$ biological replicates. Asterisks indicate statistically significant differences between +Pi and -Pi; ** $p < 0.01$, *** $p < 0.0001$ (Two-way ANOVA followed by Sidak's multiple comparisons test).

d, Western blot analysis of Pep1-induced MAPK phosphorylation in WT roots grown under +Pi or -Pi conditions and treated with water or 1 μ M Pep1 for the indicated times. RPN6 loading controls are shown (bottom).

e, Growth of *Pto* DC3000 in WT and *pepr1 pepr2* grown on nutrient-deficient soil. Data are means, $n \geq 8$ biological replicates. Different letters indicate significant difference; $p < 0.05$ (Two-way ANOVA followed by Tukey's multiple comparisons test).

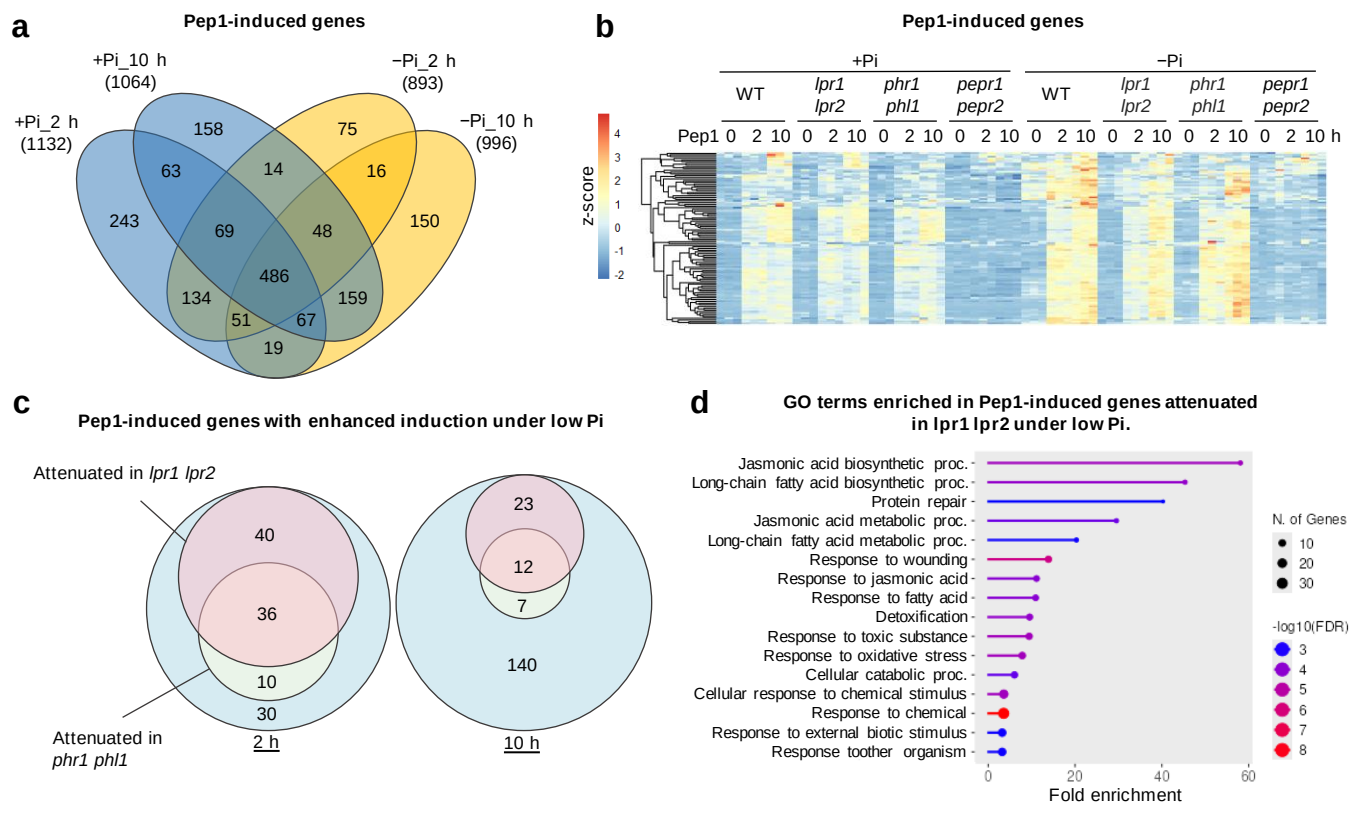


Fig. 3: External Pi sensing via LPR1/LPR2 drives PEPR-dependent immune potentiation under Pi deficiency

a, Venn diagram showing the Pep1-induced genes (log₂FC > 1, FDR < 0.05).

b, Transcript profiling of Pep-induced genes whose expression levels are significantly up-regulated (log₂FC > 1, FDR < 0.05) upon Pep1 treatment in WT roots at least one time point and up-regulated (log₂FC > 1, FDR < 0.05) in WT than *pepr1 pepr2*. The genes which classified under GO terms “defense response”, “response to biotic stimulus”, “response to wounding”, response to stimulus”, and “response to stress” were selected for visualization. The transcript profiling is shown as a heatmap. The log₂(TPM + 1) scores were normalized as Z score.

c, Venn diagram showing the Pep1-induced genes which upregulated in low Pi condition, compared with normal Pi condition (log₂FC > 0.5, FDR < 0.05) and downregulated in *lpr1 lpr2* or *phr1 phl1* compared with WT (log₂FC < 0.5, FDR < 0.05) after Pep1 treatment.

d, Representative GO terms which downregulated in *lpr1 lpr2* compared with WT, 2 h after Pep1 treatment under low Pi conditions.

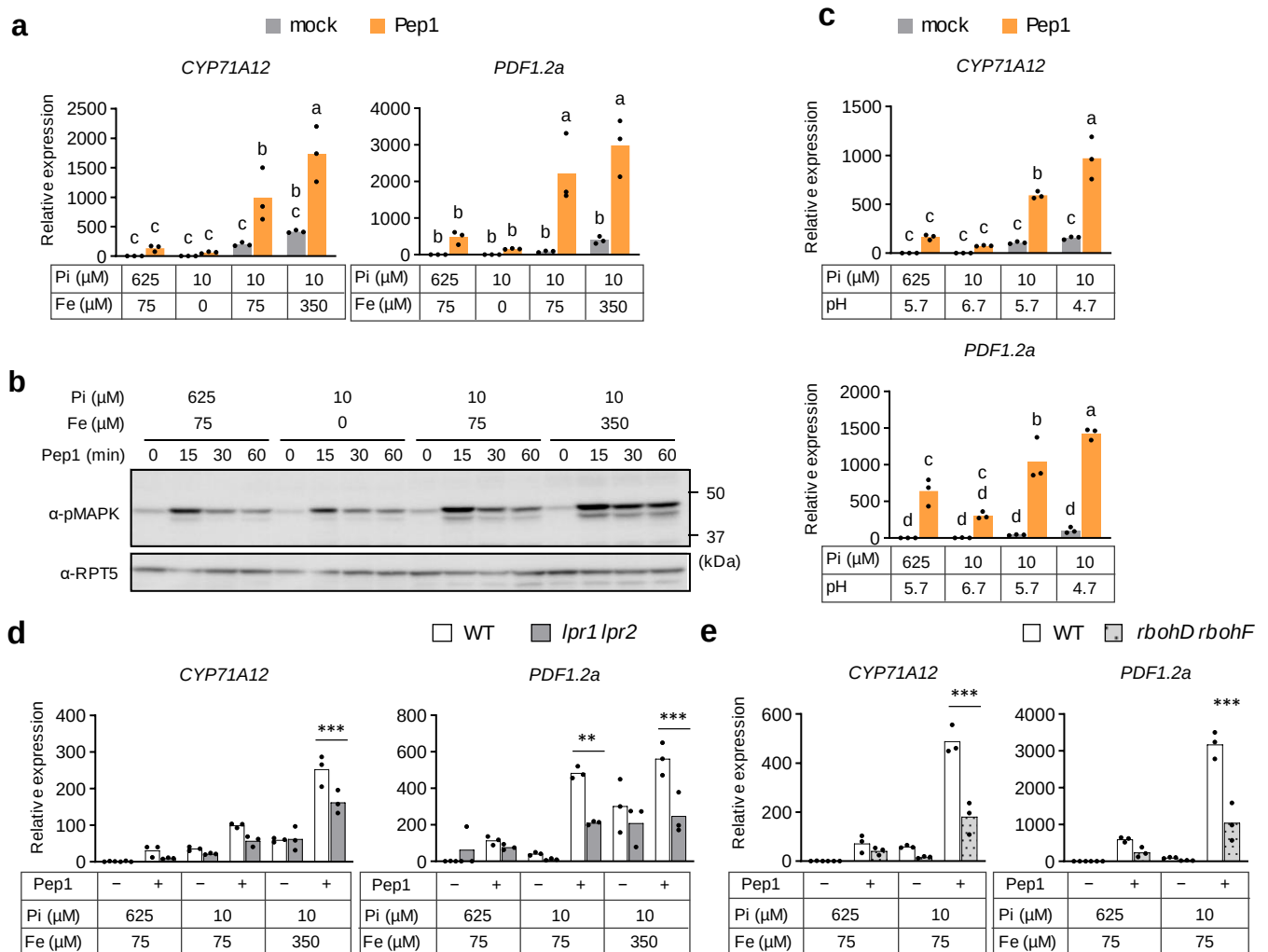


Fig. 4: Iron-dependent ROS signaling amplifies PEPR-mediated immunity under Pi deficiency

a, Transcript levels of *CYP71A12* and *PDF1.2a* in the WT roots grown under indicated Pi and Fe conditions and treated with 1 μ M Pep1 for 6 h. Data are mean, $n = 3$ biological replicates. Different letters indicate significant difference; $p < 0.05$ (Two-way ANOVA followed by Tukey's multiple comparisons test).

b, Western blot analysis of Pep1-induced MAPK phosphorylation in WT roots grown under indicated Pi and Fe concentration and treated with water or 1 μ M Pep1 for the indicated times. RPT5 loading controls are shown (bottom).

c, Transcript levels of *CYP71A12* and *PDF1.2a* in the WT roots grown under indicated Pi and pH conditions and treated with 1 μ M Pep1 for 6 h. Data are mean, $n = 3$ biological replicates. Different letters indicate significant difference; $p < 0.05$ (Two-way ANOVA followed by Tukey's multiple comparisons test).

d, Transcript levels of *CYP71A12* and *PDF1.2a* in the WT and *lpr1 lpr2* roots grown under indicated Pi and Fe conditions and treated with 1 μ M Pep1 for 6 h. Data are mean, $n = 3$ biological replicates. Asterisks indicate significant differences; $**p < 0.01$, $***p < 0.0001$ (Two-way ANOVA followed by Sidak's multiple comparisons test).

e, Transcript levels of *CYP71A12* and *PDF1.2a* in the WT and *rbohD rbohF* roots grown under indicated Pi and Fe conditions and treated with 1 μ M Pep1 for 6 h. Data are mean, $n = 3$ biological replicates. Asterisks indicate significant differences; $***p < 0.0001$ (Two-way ANOVA followed by Sidak's multiple comparisons test).

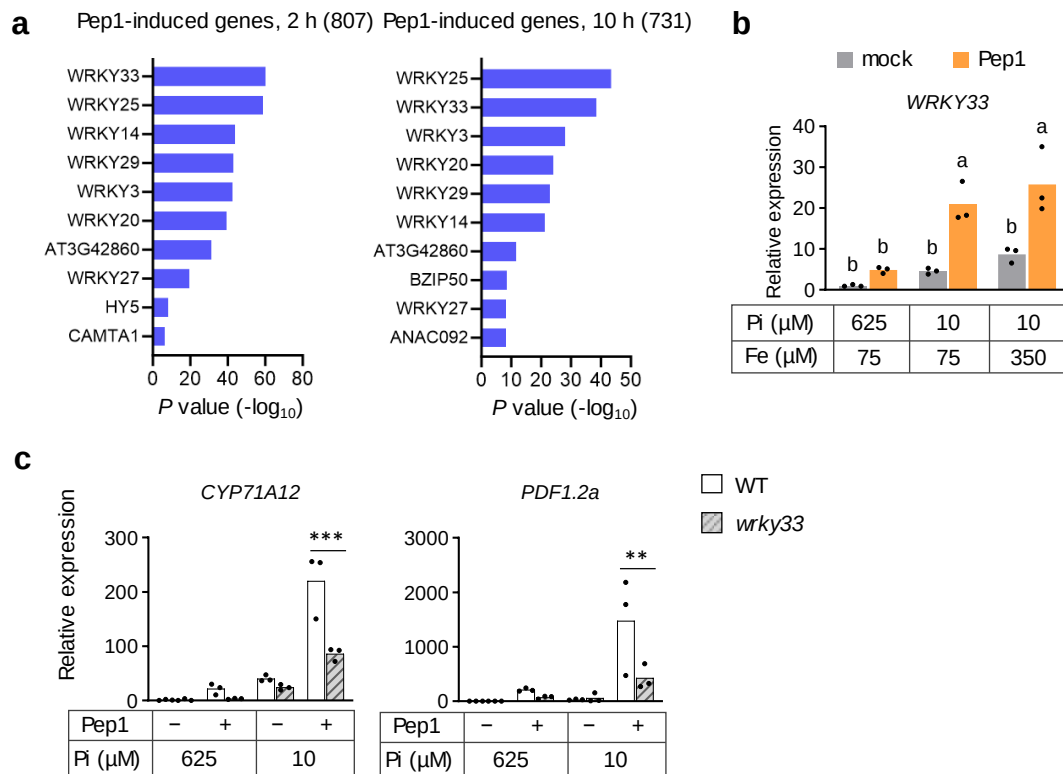


Fig. 5: WRKY33 mediates PEPR-dependent immune amplification under Pi limitation.

a, Top 10 enriched *cis*-regulatory elements among the Pep1-induced genes ($\log_2FC > 1$, FDR < 0.05).

b, Transcript levels of *WRKY33* expression in WT roots grown under indicated Pi and Fe conditions were treated with 1 μM Pep1 for 6 h. Data are mean, $n = 3$ biological replicates. Different letters indicate significant difference; $p < 0.05$ (Two-way ANOVA followed by Tukey's multiple comparisons test).

c, Transcript levels of *CYP71A12* and *PDF1.2a* in the WT and *wrky33* roots grown under Pi-sufficient conditions (+Pi, 625 μM) or Pi-deficient conditions (-Pi, 10 μM) and treated with 1 μM Pep1 for 6 h. Data are mean, $n = 3$ biological replicates. Asterisks indicate significant differences; ** $p < 0.01$, *** $p < 0.0001$ (Two-way ANOVA followed by Sidak's multiple comparisons test).

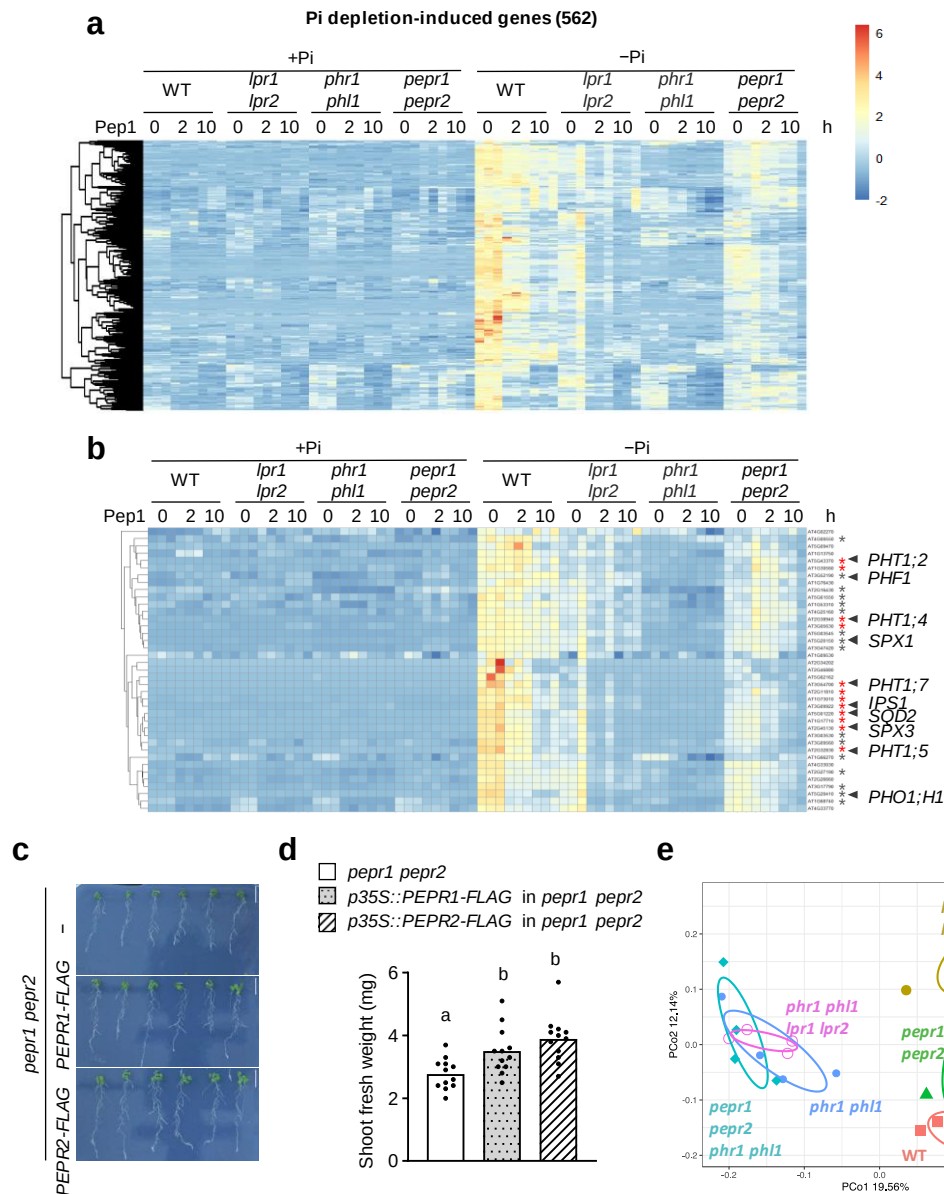


Fig. 6: PEPR1/PEPR2 promote PSR and shape root microbiota under Pi limitation partly via PHR1/PHL1.

a, Transcript profiling of low Pi-induced gene cluster ($\log_2\text{FC} > 1$, $\text{FDR} < 0.05$). The $\log_2$ (TPM) scores were normalized as Z score.

b, Transcript profiling of representative low Pi-induced genes ($\log_2\text{FC} > 1$, $\text{FDR} < 0.05$). The $\log_2$ (TPM) scores were normalized as Z score. Asterisks represent a significant difference between WT and *pepr1 pepr2* under low Pi without Pep1 treatment (red: $\log_2\text{FC} > 1$, $\text{FDR} < 0.05$, gray: $\log_2\text{FC} > 0.5$, $\text{FDR} < 0.05$).

c, Three-day-old seedlings were exposed to Pi-deficient (50 μM Pi) conditions for 10 days.

d, Shoot fresh weight of c. Data are mean, $n = 12$ biological replicates. Different letters indicate significant difference; $p < 0.05$ (Two-way ANOVA followed by Tukey's multiple comparisons test).

e, PCoA with mutants of PSR regulator and *pepr1 pepr2* grown in nutrient-deficient soil ($n \geq 4$, biological replicates).

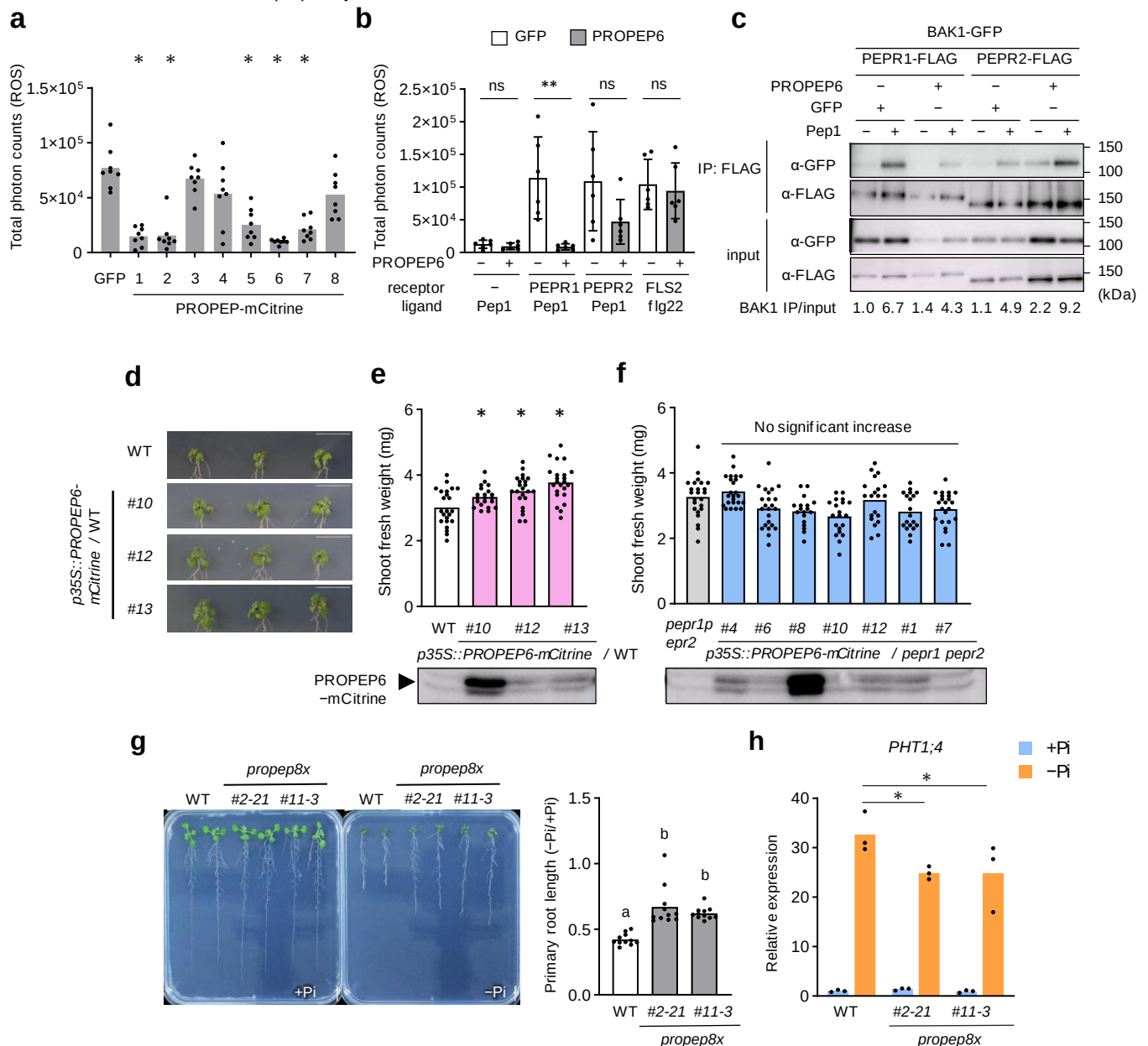


Fig. 7: PROPEP6 antagonizes Pep-triggered immunity and redirects PEPR signaling under phosphate limitation.

a, Total photon counts of Pep1-induced ROS production in *N. benthamiana* leaves which Arabidopsis PEPR1 and PROPEPs are transiently expressed. Data are mean, $n = 8$ biological replicates. Asterisks indicate significant differences compared with WT; * $p < 0.05$ (One-way ANOVA followed by Dunnett's multiple comparisons test).

b, Total photon counts of ROS production in *N. benthamiana* leaves which Arabidopsis PROPEP6 are transiently expressed. Data are mean, $n = 8$ biological replicates. Asterisks indicate significant differences; ** $p < 0.01$ (Two-tailed t -test).

c, Complex formation of PEPR1 and BAK1. *N. benthamiana* leaves transfected with indicated constructs were treated with water or 1 μ M Pep1 for 15 min before IP with anti-FLAG beads. BAK1 were detected by immunoblotting with anti-BAK1 antibody. Input proteins are shown with immunoblotting.

d, Three-day-old seedlings were exposed to Pi-deficient conditions (50 μ M Pi) for 10 days. Scale bar, 10 mm

e, Shoot fresh weight of d. Data are mean, $n \geq 21$ biological replicates. Asterisks indicate significant differences compared with WT; * $p < 0.05$ (One-way ANOVA followed by Dunnett's multiple comparisons test).

f, Shoot fresh weight of 3-day-old seedlings which were exposed to Pi-deficient conditions (50 μ M Pi) for 10 days. Data are mean, $n \geq 18$ biological replicates.

g, The primary root length of Arabidopsis WT and CRISPR: *propep8x* mutants grown under Pi-sufficient conditions (+Pi, 625 μ M) or Pi-deficient conditions (-Pi, 50 μ M), $n \geq 11$ biological replicates. Asterisks indicate significant differences; $p < 0.05$ (Two-way ANOVA followed by Tukey's multiple comparisons test).

h, *PHT1;4* gene expression in roots of 13-day-old seedlings grown under +Pi or -Pi conditions. Data are mean, $n = 3$ biological replicates. Asterisks indicate significant differences (Two-way ANOVA followed by Tukey's multiple comparisons test).

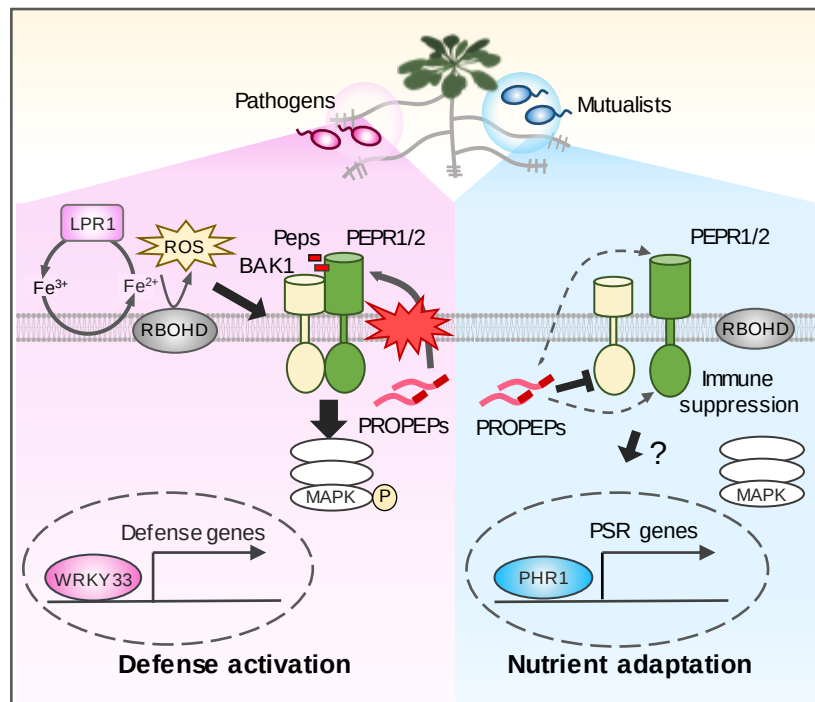


Fig. 8: Model of nutrient-dependent reprogramming of PEPR signaling during Pi limitation

Under low Pi, the immune receptor landscape is reconfigured. Damage-associated Pep–PEPR signaling is selectively potentiated through the LPR1-dependent iron–redox module, involving iron accumulation, ROS production and WRKY33-mediated transcriptional amplification. PEPR signaling is preferentially maintained and contributes to phosphate starvation responses (PSR). Distinct ligands derived from PROPEPs further modulate PEPR outputs, constraining Pep-triggered immune activation while permitting PSR-associated growth. This context-dependent dual functionality of PEPR likely enables plants to balance defense and nutrient adaptation during phosphate limitation.