FERONIA regulates FLS2 plasma membrane nanoscale dynamics to modulate plant immune signaling

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ABSTRACT

Cell surface receptors survey and relay information to ensure the development and survival of multicellular organisms. In the model plant *Arabidopsis thaliana*, the *Catharanthus roseus* RLK1-like receptor kinase FERONIA (FER) regulates myriad of biological processes to coordinate development, growth and responses to the environment. We recently showed that FER positively regulates immune signaling by controlling the ligand-induced complex formation between the leucine-rich repeat receptor kinase (LRR-RK) FLAGELLIN SENSING 2 (FLS2) and its co-receptor BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED KINASE 1/SOMATIC EMBRYOGENESIS RECEPTOR KINASE 3 (BAK1/SERK3). In this context, FER function is inhibited by binding of its peptide ligand RAPID ALKALINIZATION FACTOR 23 (RALF23). However, the mechanisms by which FER regulates FLS2-BAK1 complex formation remain unclear. Here, we show that FER-dependent regulation of immune signaling is independent of its kinase activity, indicating that FER rather plays a structural role. FER has been proposed to bind directly to the plant cell wall, but we found that a FER mutant unable to bind pectin is still functional in regulating immune signaling. Instead, FER- and cell wall-associated LEUCINE RICH REPEAT-EXTENSIN proteins are required for this regulation. Using high-resolution live-imaging and single-particle tracking, we observed that FER regulates FLS2 plasma membrane nanoscale dynamics, which may explain its role in controlling ligand-induced FLS2-BAK1 association. We propose that FER acts as an anchoring point connecting cell wall and plasma membrane nano-environments to enable the nucleation of pre-formed receptor/co-receptor complexes at the cell surface.

INTRODUCTION

Multicellular organisms evolved sophisticated surveillance systems to monitor changes in their environment. In plants, receptor kinases (RKs) and receptor proteins (RPs) are main ligand-binding cell-surface receptors perceiving self, non-self and modified-self molecules (Hohmann et al., 2017). For example, recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs) initiates signaling events leading to PRR-triggered immunity (PTI) (Couto & Zipfel, 2016; X. Yu et al., 2017). The Arabidopsis thaliana (hereafter Arabidopsis) leucine-rich repeat receptor kinases (LRR-RKs) FLAGELLIN SENSING 2 (FLS2) and EF-TU RECEPTOR (EFR) recognize the bacterial PAMPs flagellin (or its derived epitope flg22) and elongation factor-Tu (or its derived epitope elf18), respectively (Gómez-Gómez & Boller, 2000; Zipfel et al., 2006). Both FLS2 and EFR form ligand-induced complexes with the co-receptor LRR-RK BRASSINOSTEROID INSENSITIVE 1-ASSOCIATED KINASE 1/SOMATIC EMBRYOGENESIS RECEPTOR KINASE 3 (BAK1/SERK3, hereafter BAK1) to activate immune signaling (Chinchilla et al., 2007; Heese et al., 2007; Roux et al., 2011; Schulze et al., 2010; Schwessinger et al., 2011; Sun et al., 2013). This initiates a series of downstream responses, such as changes in ion fluxes, reactive oxygen species (ROS) production and activation of mitogen-activated protein kinase (MAPK) cascades, which ultimately lead to antimicrobial responses (Couto & Zipfel 2016, Yu et al., 2017).

RKs can also function as accessory proteins regulating the formation of ligand-binding RK complexes. For example, the pseudokinase LRR-RKs BAK1-INTERACTING RECEPTOR-LIKE KINASE 2 (BIR2) and BIR3 interact with BAK1 to negatively regulate FLS2-BAK1 complex formation (Halter et al., 2014; Imkampe et al., 2017; Ma et al., 2016). Similarly, the short LRR-RKs APEX and NUCLEAR SHUTTLE PROTEIN (NSP)-INTERACTING KINASE 1 (NIK1) also negatively regulate FLS2-BAK1 association (Li et al., 2019; Smakowska-Luzan et al., 2018). In contrast, the malectin-like/LRR-RK IMPAIRED OOMYCETE SUSCEPTIBILITY 1 (IOS1) associates with FLS2 and EFR, and positively regulates FLS2-BAK1 complex formation (Yeh et al., 2016). The FLS2-INTERACTING RECEPTOR (FIR) also positively regulates flg22-induced FLS2-BAK1 association (Smakowska-Luzan et al., 2018)

Recently, in a forward-genetics suppressor screen for restoration of reactive oxygen species (ROS) production in a *bak1-5* mutant background (Monaghan et al., 2014), we identified the subtilase S1P/SBT6.1 as a negative regulator of PTI (Stegmann et al., 2017). Upon PAMP perception, S1P cleaves the endogenous PRO-RAPID ALKALINIZATION FACTOR 23 (PRORALF23) protein into mature RALF23 that is perceived by a complex composed of the *Catharanthus roseus* RLK1-like (CrRLK1L) FERONIA (FER) and the LORELEI-LIKE-GPI ANCHORED PROTEIN 1 (LLG1) (Stegmann et al., 2017; Xiao et al.,

2019). RALF23 perception negatively regulates flg22-induced FLS2-BAK1 complex formation; thus, acting in a potential negative feedback loop dampening immune responses (Stegmann et al., 2017). In addition, *FER* and *LLG1* are genetically required for flg22-induced FLS2-BAK1 complex formation (Stegmann et al., 2017; Xiao et al., 2019). LLG1 associates with FLS2 (Shen et al., 2017) and FER associates with FLS2 and BAK1, suggesting that FER may scaffold the FLS2-BAK1 complex (Stegmann et al., 2017).

FER is composed of two extracellular malectin-like domains, a single-pass transmembrane domain and a catalytically-active cytoplasmic kinase domain (Escobar-Restrepo et al., 2007; Haruta et al., 2018; Boisson-Dernier et al., 2011; Minkoff et al., 2017). FER was initially identified for its role in fertilization where it plays a role in pollen tube reception by the synergid cells (Escobar-Restrepo et al., 2007; Huck et al., 2003; Rotman et al., 2003). Subsequently, FER has been implicated in multiple cellular processes (Franck et al., 2018), including root hair growth (Duan et al., 2010), hormone signaling (Guo et al., 2018; Li et al., 2018; Deslauriers et al., 2010; Yu et al., 2012), cell elongation (Dünser et al., 2019; Guo et al., 2009) salt stress tolerance (Feng et al., 2018; Zhao et al., 2018), proton pump regulation (Du et al., 2016; Haruta et al., 2014), and cell wall integrity sensing (Dünser et al., 2019; Feng et al., 2018). Recently, it has become evident that multiple RALF peptides, such as RALF1 and RALF23, are ligands for FER (Abarca et al., 2020; Haruta et al., 2014; Stegmann et al., 2017), and that RALF perception by FER involves additional proteins, such as the GPI-anchored LLGs (Li et al., 2015; Xiao et al., 2019) and LRR-EXTENSINs (LRXs) (Dünser et al., 2019; Herger et al., 2020; Zhao et al., 2018). In this study, we investigate the molecular determinants underlying FER function in regulating immune signaling mediated by PRRs.

RESULTS

FER kinase activity is dispensable to support PTI signaling

The FER intracellular domain contains a catalytically active kinase domain (Escobar-Restrepo et al., 2007; Haruta et al., 2018; Minkoff et al., 2017), and its activity has been proposed to regulate abscisic acid and jasmonic acid signaling (Guo et al., 2018; Li et al., 2018; Yu et al., 2012), proton pump activity (Du et al., 2016; Haruta et al., 2014), response to mechanical forces (Shih et al., 2014), and cell wall integrity sensing (Dünser et al., 2019). To determine if FER-mediated signaling events play a role in potentiating immune receptor complex formation, we tested the requirement of FER kinase activity for the signaling events triggered by the perception of flg22 by FLS2. Expression of a kinase-dead mutant allele FER^{K565R} C-terminally fused to GFP (FER^{KD}-GFP; Chakravorty et al., 2018) rescued the decreased flg22-induced FLS2-BAK1 complex formation observed in the loss-of-function mutant *fer-4* (Fig. 1A). FLS2-BAK1 complex formation leads to BAK1 auto-phosphorylation

on serine (Ser) residues located at the BAK1 C-terminal tail, which are required for the initiation of immune signaling (Perraki et al., 2018). Immunoblotting using a phospho-specific antibody for C-terminal tail BAK1-pSer612 (Perraki et al., 2018) suggested that the activation of the FLS2-BAK1 complex does not require FER kinase activity (Fig. 1A). In good agreement, FER^{KD}-GFP fully complemented ROS production in response to flg22 (Fig. 1B). FER also regulates complex formation between EFR and BAK1 upon elf18 perception (Stegmann et al., 2017). The defect in the elf18-induced ROS burst observed in *fer-4* was complemented by FER^{KD}-GFP (Fig. 1C), suggesting that, as for FLS2-BAK1 complex formation, FER kinase activity is not required for complex formation between EFR and BAK1. Together, these results indicate that FER kinase activity is dispensable to support PTI signaling.

Direct association of FER with pectin is dispensable to support PTI signaling

Cell wall integrity sensing modulates plant immune responses (Engelsdorf et al., 2018; Malinovsky et al., 2014; Vaahtera et al., 2019). The ectodomain of FER contains tandem malectin-like domains A and B (MalA and MalB), which share homology with malectin, a carbohydrate binding protein from *Xenopus laevis* (Boisson-Dernier et al., 2011). While the carbohydrate-binding site of malectin is not strictly conserved in MalA and MalB (Moussu et al., 2018; Xiao et al., 2019), MalA has been shown to bind pectin *in vitro* (Feng et al., 2018; Lin et al., 2018). Pectin sensing by FER was proposed to regulate acclimation to salt stress (Feng et al., 2018), cell shape (Lin et al., 2018) and sexual reproduction (Duan et al., 2020). To investigate if pectin sensing underlies FER function in regulating PTI, we used a FER deletion mutant lacking the MalA domain C-terminally fused to YFP ($FER^{\Delta MalA}$-YFP) in the null *FER* allele *fer-4* (Lin et al., 2018). Immuno-precipitation assays showed that expression of $FER^{\Delta MalA}$-YFP was able to fully complement complex formation between endogenous FLS2 and BAK1 upon flg22 perception (Fig. 2A). $FER^{\Delta MalA}$-YFP also complemented ROS production in response to flg22 and elf18 (Fig. 2B,C). Overall, these results suggest that direct association of FER to pectin *via* MalA is dispensable for FER function in promoting the formation of signaling-competent PRR complexes.

LRX3, LRX4 and LRX5 are required for FER-mediated regulation of PTI signaling

The fact that FER kinase activity and pectin binding are dispensable for the positive regulation of PTI signaling suggests that FER plays a structural role in regulating FLS2-BAK1 complex formation. LRX proteins are cell wall-associated proteins proposed to regulate cell wall integrity sensing in CrRLK1L-dependent pathways (Baumberger et al., 2001; Dünser et al., 2019; Herger et al., 2019; Herger et al., 2020; Mecchia et al., 2017). Among the 11-member LRX family, *LRX3*, *LRX4*, and *LRX5* are the most abundantly expressed in

vegetative tissues, and a *lrx3 lrx4 lrx5* triple mutant (hereafter *lrx3/4/5*) showed stunted growth and salt hypersensitivity phenotypes reminiscent of the *fer-4* mutant (Dünser et al., 2019; Zhao et al., 2018). We therefore hypothesized that LRXs also regulate immune signaling. Co-immunoprecipitation experiments showed that *lrx3/4/5* is defective in flg22-induced FLS2-BAK1 complex formation (Fig. 3A). Consistently, both flg22- and elf18-induced ROS production were reduced in *lrx3/4/5* similar to the levels observed in *fer-4* (Fig. 3B,C). These data therefore suggest that, like FER, LRX3/4/5 are positive regulators of PRR signaling. RALF17 and RALF23 are perceived by FER and oppositely regulate PTI (Stegmann et al., 2017). As LRXs have been previously shown to bind RALF peptides (Dünser et al., 2019; Mecchia et al., 2017; Moussu et al., 2020; Zhao et al., 2018), we hypothesized that LRX3, LRX4 and LRX5 might be required for RALF17 and RALF23 responsiveness. Indeed, *LRX3, LRX4* and *LRX5* were genetically required for both RALF17-induced ROS burst (Fig. 3D) and RALF23-induced inhibition of elf18-induced ROS burst (Fig. 3E), suggesting that these LRXs are part of a FER receptor complex. In agreement, using a transgenic Arabidopsis line expressing the LRR region of LRX4 C-terminally tagged with FLAG (LRR4-FLAG, Herger et al., 2020), we found that LRR4-FLAG co-immunoprecipitated with the endogenous FER protein and that FER-LLR4 association was insensitive to RALF23 treatment (Fig. 3F). Collectively, these data suggest that LRX3, LRX4 and LRX5 work with FER to regulate PRR-mediated immune signaling.

FER regulates FLS2 nanodomains dynamics at the plasma membrane

We previously showed that FLS2 localizes to static membrane nanodomains (Bücherl et al., 2017) which are nanoscale membrane compartments or proteo-lipidic molecular assemblies proposed to orchestrate signaling (Gronnier et al., 2018; Jaillais & Ott, 2020). The cell wall has been proposed to modulate protein diffusion in the plane of the plasma membrane (Feraru et al., 2011; Martinière et al., 2012). Notably, inhibition of pectin methylesterases, which are regulators of cell wall chemistry (Micheli, 2001), alters the organization of FLS2-GFP within plasma membrane nanodomains (McKenna et al., 2019). Interestingly, observation of FER-GFP by total internal reflection fluorescence microscopy (TIRFM) showed that FER is organized in nanodomains (Fig. S1). FER was previously shown to be enriched in detergent-resistant membrane fractions (Keinath et al., 2010), suggesting that FER associates with sterol- and sphingolipid-enriched nanodomains. Because FER associates with the cell wall *via* LRX proteins (Dünser et al., 2019; Herger et al., 2019; Herger et al., 2020, Fig. 3F) and associates with nanodomains (Fig. S1), we hypothesized that FER regulates FLS2 plasma membrane nanodomain organization. To test this hypothesis, we combined TIRFM and single-particle tracking (SPT) to study the lateral mobility of FLS2-GFP proteins with high temporal and spatial resolution in transgenic

Arabidopsis lines expressing FLS2-GFP (Movie S1). Super-resolved trajectories showed that FLS2-GFP exhibits a confined mobility behavior (Fig. 4). Analysis of the diffusion coefficient (D), which describes the diffusion properties of a molecule, showed that FLS2-GFP is more mobile in *fer-2* than in Col-0 (Fig. 4). Consistently, we observed an increase in FLS2-GFP nanodomain size but not density in *fer-2* compared to Col-0 (Fig. 4, Fig. S2,3). Thus, we conclude that *FER* is genetically required to control FLS2-GFP mobility and nanodomain organization.

Nanodomains have been proposed to provide scaffolds for protein-protein interaction (Hutten et al., 2017; Jarsch et al., 2014; Somssich et al., 2015), and recent studies have linked altered plasma membrane organization and mobility with defects in protein functionality Gronnier et al., 2017; Liang et al., 2018; Platre et al., 2019; Pan et al., 2019). Thus, an alteration of FLS2 mobility might explain the defect in flg22-induced FLS2-BAK1 complex formation observed in *FER* null mutants (Fig. 1, Stegmann et al., 2017). We then used the fact that RALF23 inhibits FER function in regulating immune signaling to pharmacologically test whether FER directly regulates FLS2 nanoscale plasma membrane dynamics. Indeed, we observed an increase of FLS2-GFP mobility and an alteration of FLS2-GFP nanodomain size within minutes of RALF23 treatment, and this, in a FER-dependent manner (Fig. 4).

In *Medicago truncatula* and in yeast, alteration of nanodomain localization has been linked to impaired protein accumulation at the plasma membrane due to increase protein endocytosis (Grossman et al., 2008; Liang et al., 2018). To inquire for potential defect in FLS2 plasma membrane accumulation, we observed FLS2-GFP by confocal microscopy. Sub-cellular localization studies revealed a decrease of FLS2-GFP accumulation in *fer-2* compared to Col-0 (Fig. S4). Together, our data suggest that FER and its cognate ligand RALF23 modulates FLS2 mobility and organization in plasma membrane nanodomains to regulate its function.

DISCUSSION

FER and related CrRLK1Ls have been implicated in numerous biological processes, including development, immunity and reproduction (Franck et al., 2018). Several reports propose that these functions are directly related to cell wall integrity sensing, as these CrRLK1Ls possess two malectin-like domains in their ectodomains which were predicted to bind cell -wall carbohydrates. Recent structural studies however showed that the residues normally involved in carbohydrate-binding in animal malectin are not conserved in the ectodomains of FER and the related CrRLK1Ls ANXUR (ANX)1 and ANX2 (Moussu et al., 2018; Xiao et al., 2019). Yet, the ectodomain of FER, BUDDHA'S PAPER SEAL (BUPS)1, ANX1 and ANX2 have been shown to bind pectin *in vitro* (Feng et al., 2018; Lin et al., 2018)

and pectin sensing by FER has been proposed to regulate acclimation to salt stress (Feng et al., 2018), cell shape (Lin et al., 2018) and sexual reproduction (Duan et al., 2020). Here, we show that in the context of PTI, FER function does not rely on direct binding to pectin (Fig. 2) but requires the cell wall binding proteins LRXs (Fig. 3). It has recently become clear that RALF peptides are *bona fide* ligands for CrRLK1Ls (Haruta et al., 2014; Stegmann et al., 2017; Xiao et al., 2019). FER binds to RALF1 and RALF23 to regulate root elongation and immunity respectively (Haruta et al., 2014, Dünser et al., 2019, Stegmann et al., 2017), while THESEUS1 binds RALF34 to regulate cell expansion (Gonneau et al., 2018). In addition, ANX1/2 and BUPS1/2 have been proposed to bind RALF4/19 as part of a heteromeric complex to regulate pollen tube growth and integrity (Ge et al., 2017; Mecchia et al., 2017). Interestingly, RALF perception by FER and BUPSs/ANXs engages the GPI-anchored proteins LLGs as part of a ligand-induced complex (Ge et al., 2019; Xiao et al., 2019), and RALFs were recently shown to bind LRXs (Dünser et al., 2019; Herger et al., 2020; Mecchia et al., 2017; Moussu et al., 2020; Zhao et al., 2018). However, the potential inter-relationship between the RALF-LRX and RALF-LLG-CrRLK1L complexes remains unclear (Herger et al., 2019). While some genetic data suggest that LRXs and ANX1/2 may function as part of distinct complexes perceiving RALF4/19 (Herger et al., 2019; Mecchia et al., 2017; Moussu et al., 2020), LRX and FER have been suggested to work genetically together in the context of RALF1-mediated regulation of root growth (Haruta et al., 2014; Dünser, et al., 2019). Consistently, we now report that the shoot-expressed LRX3, 4 and 5 play a similar role as FER in regulating immune signaling, and in mediating RALF23- and RALF17 effects in this context (Fig. 3). Also, as previously reported for the LRR domain of LRX1 (Herger et al., 2020), we could show that the LRR domain of LRX4 can associate with FER (Fig. 3). Together, these data illustrate the potential roles of interconnected heteromeric LRX-LLG-CrRLK1L modules in RALF perception. Yet, the exact mechanistic details of such a heteromeric complex remain to be elucidated. CrRLK1Ls also form heteromeric complexes with additional CrRLK1Ls, as shown in the context of plant reproduction (Galindo-Trigo et al., 2020; Ge et al., 2017). Whether FER associates with additional CrRLK1Ls to modulate PTI remains an open question, but ANX1/2 have been shown for example to inhibit PTI (Mang et al., 2017), suggesting additional layers of complexity. Perception of RALF23 by FER-LLG1-LRXs dampens PTI signaling (Fig. 3, Stegmann et al., 2017; Xiao et al., 2019), but the mechanism by which this occurs remains unclear. Recently, RALF22 and RALF23 have been proposed to induce FER endocytosis to inhibit FER function in cell wall integrity sensing (Zhao et al., 2018). In this context, LRX3, LRX4 and LRX5 have been proposed to titrate out RALF peptides from binding to FER and thus preventing inhibition of FER function (Zhao et al., 2018). We however observed that both RALF17 and RALF23, which positively and negatively regulate PTI, respectively (Stegmann et al., 2017), induce FER internalization

(Fig. S5). Thus, in the context of immunity, RALF-induced FER endocytosis does not explain the function of RALFs. Interestingly, RALF23-inhibition of FLS2 signaling requires FER kinase activity (Fig. S6) suggesting that RALF23 inhibits FLS2-BAK1 complex formation via active FER signaling. Further experiments will be required to uncover molecular events following RALF23 perception, and to identify the RALF(s) peptide(s) that conversely supports FLS2-BAK1 complex formation.

Sensing of cell wall integrity by CrRLK1L-dependent pathways has emerged as a cornerstone of cell homeostasis regulating plant reproduction, growth and immunity (Franck et al., 2018). Cell wall integrity regulates membrane dynamics (Daněk et al., 2020; Feraru et al., 2011; Martinière et al., 2012; McKenna et al., 2019); yet, the underlying molecular mechanisms remain poorly understood. Here, we show that the cell wall integrity sensor FER regulates FLS2 nanoscale dynamics (Fig. 4). FER associates with LRXs (Fig. 3, Dünser et al., 2019; Herger et al., 2020) and FLS2 (Stegmann et al., 2017), is enriched in detergent-resistant fractions (Keinath et al., 2010), and localizes to plasma membrane nanodomains (Fig. S1). Nanodomains have been proposed to provide scaffolds for protein-protein interactions (Hutten et al., 2017; Jarsch et al., 2014; Somssich et al., 2015). We propose that, by bridging specific membrane environments to the cell wall (potentially via LRX proteins), FER acts as a physical anchor regulating FLS2 mobility and nanodomain organization. The fact that FER kinase activity is not required to support FLS2-BAK1 complex formation (Fig.1) and that FER constitutively associates with both FLS2 and LRXs (Fig. 3; Stegmann et al., 2017; Dünser et al., 2019; Herger et al., 2020) supports this model. Interestingly, using super resolution microscopy, Haas and co-authors recently showed that pectin forms nanofilaments in the cell wall (Haas et al., 2020). It would be particularly interesting to determine if FER-LRX complexes link specific cell wall and plasma membrane nano-environments. In light of recent reports associating defects in nanodomain organization and protein mis-functionality (Gronnier et al., 2017; Liang et al., 2018; Platre et al., 2019), we propose that regulation of FLS2 mobility is required for optimal ligand-induced complex formation between FLS2 and BAK1, and thus initiation of PTI.

Plants have evolved coordinated RK protein-protein interaction networks to process extracellular signals into specific responses (Smakowska-Luzan et al., 2018), and thus may have co-evolved mechanisms to regulate these interactions in both space and time. Nanodomains represent common organizational units for RKs (Bücherl et al., 2017; Burkart & Stahl, 2017.; Gronnier et al., 2018; McKenna et al., 2019; Ott, 2017; Jaillais & Ott 2020) and provide a conceptual framework for the regulation of RK interactions. Our study unravels the regulation of FLS2 nanodomain dynamics by FER and its peptide ligand to modulate PTI signaling. Importantly, our proposal that FER acts a nucleating scaffold connecting the cell

wall and the plasma membrane regulating the formation of pre-formed RK complexes to allow them to form signaling-active units upon ligand perception could explain some of the many functions reported for FER (and potentially additional CrRLK1Ls). Notably, FER kinase activity is clearly required for some FER functions (Dünser et al., 2019; Shih et al., 2014; Zhu et al., 2020), but is dispensable for others (*e.g.* reproduction and immunity, Haruta et al., 2018; Kessler et al., 2015; Fig. 1). Therefore, the function of FER in other processes might similarly rely on the regulation of RK nanoscale dynamics, and the identification of such FER-associated RKs in these processes is an exciting prospect.

ACKNOWLEDGMENTS

We thank all present and past members of the Zipfel laboratory for fruitful discussions and comments on the manuscript. We thank the members of the Grossniklaus, Sanchez-Rodriguez and Keller laboratories for sharing results and comments during our stimulating informal meetings. This research was funded by the Gatsby Charitable Foundation (C.Z.), the University of Zürich (C.Z.), the European Research Council under the Grant Agreements 309858 and 773153 (grants PHOSPHinnATE and IMMUNO-PEPTALK to C.Z.), the Swiss National Science Foundation (grant no. 31003A_182625 to C.Z. and 31003A_166577/1 to C.R.), and the European Molecular Biology Organization (EMBO Long-Term Fellowships 438-2018 to J.G, 512-2019 to C.M.F and 100-2017 to T.A.D). M.S. was supported by a post-doctoral fellowship (STE 2448/1) from the Deutsche Forschungsgemeinschaft (DFG). The Austrian Academy of Sciences (ÖAW) (DOC fellowship to K.D.), Austrian science fund (FWF; P 33044), and the European Research Council (ERC; 639478-AuxinER) supported work in the lab of J.K-V.

REFERENCES

Abarca, A., Franck, C. M., & Zipfel, C. (2020). Family-wide evaluation of RALF peptides in Arabidopsis thaliana. *BioRxiv*, 2020.06.26.174169. https://doi.org/10.1101/2020.06.26.174169

Baumberger, N., Ringli, C., & Keller, B. (2001). The chimeric leucine-rich repeat/extensin cell wall protein LRX1 is required for root hair morphogenesis in Arabidopsis thaliana. *Genes and Development*, *15*(9), 1128–1139. https://doi.org/10.1101/gad.200201

Boisson-Dernier, A., Kessler, S. A., & Grossniklaus, U. (2011). The walls have ears: The role of plant CrRLK1Ls in sensing and transducing extracellular signals. In *Journal of Experimental Botany* (Vol. 62, Issue 5, pp. 1581–1591). https://doi.org/10.1093/jxb/erq445

Bücherl Christoph A Jarsch, I. K., Schudoma, C., Robatzek, S., Maclean, D., Ott, T., Zipfel, C., Genome, P., National, S., & Biology, C. (2017). *Plant immune and growth receptors share common signalling components but localise to distinct plasma membrane nanodomains*. 1–28. https://doi.org/10.7554/eLife.25114

Burkart, R. C., & Stahl, Y. (2017). Dynamic complexity: plant receptor complexes at the plasma membrane. In *Current Opinion in Plant Biology* (Vol. 40, pp. 15–21). https://doi.org/10.1016/j.pbi.2017.06.016

Chakravorty, D., Yu, Y., & Assmann, S. M. (2018). A kinase-dead version of FERONIA receptor-like kinase has dose-dependent impacts on rosette morphology and RALF1-mediated stomatal movements. *FEBS Letters*, *592*(20), 3429–3437. https://doi.org/10.1002/1873-3468.13249

Chinchilla, D., Zipfel, C., Robatzek, S., Kemmerling, B., Nürnberger, T., Jones, J. D. G., Felix, G., & Boller, T. (2007). A flagellin-induced complex of the receptor FLS2 and BAK1 initiates plant defence. *Nature*, *448*(7152), 497–500. https://doi.org/10.1038/nature05999

Couto, D., & Zipfel, C. (2016). Regulation of pattern recognition receptor signalling in plants. *Nature Reviews Immunology*, *16*, 537–552. https://doi.org/10.1038/nri.2016.77

Daněk, M., Angelini, J., Malínská, K., Andrejch, J., Amlerová, Z., Kocourková, D., Brouzdová, J., Valentová, O., Martinec, J., & Petrášek, J. (2020). Cell wall contributes to the stability of plasma membrane nanodomain organization of *Arabidopsis thaliana* FLOTILLIN2 and HYPERSENSITIVE INDUCED REACTION1 proteins. *The Plant Journal*, *101*(3), 619–636. https://doi.org/10.1111/tpj.14566

Deslauriers, S. D., & Larsen, P. B. (2010). FERONIA is a key modulator of brassinosteroid and ethylene responsiveness in arabidopsis hypocotyls. *Molecular Plant*, *3*(3), 626–640. https://doi.org/10.1093/mp/ssq015

Duan, Q., Kita, D., Li, C., Cheung, A. Y., & Wu, H. M. (2010). FERONIA receptor-like kinase regulates RHO GTPase signaling of root hair development. *Proceedings of the National Academy of Sciences of the United States of America*, *107*(41), 17821–17826. https://doi.org/10.1073/pnas.1005366107

Duan, Q., Liu, M. C. J., Kita, D., Jordan, S. S., Yeh, F. L. J., Yvon, R., Carpenter, H., Federico, A. N., Garcia-Valencia, L. E., Eyles, S. J., Wang, C. S., Wu, H. M., & Cheung, A. Y. (2020). FERONIA controls pectin- and nitric oxide-mediated male–female interaction. *Nature*, *579*(7800), 561–566. https://doi.org/10.1038/s41586-020-2106-2

Du, C., Li, X., Chen, J., Chen, W., Li, B., Li, C., Wang, L., Li, J., Zhao, X., Lin, J., Liu, X., Luan, S., & Yu, F. (2016). Receptor kinase complex transmits RALF peptide signal to inhibit root growth in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(51), E8326–E8334. https://doi.org/10.1073/pnas.1609626113

Dünser, K., Gupta, S., Herger, A., Feraru, M. I., Ringli, C., & Kleine-Vehn, J. (2019). Extracellular matrix sensing by <scp>FERONIA</scp> and Leucine-Rich Repeat Extensins controls vacuolar expansion during cellular elongation in *Arabidopsis thaliana*. *The EMBO Journal*, *38*(7). https://doi.org/10.15252/embj.2018100353

Engelsdorf, T., Gigli-Bisceglia, N., Veerabagu, M., McKenna, J. F., Vaahtera, L., Augstein, F., van der Does, D., Zipfel, C., & Hamann, T. (2018). The plant cell wall integrity maintenance and immune signaling systems cooperate to control stress responses in Arabidopsis thaliana. *Science Signaling*, *11*(536). https://doi.org/10.1126/scisignal.aao3070

Escobar-Restrepo, J. M., Huck, N., Kessler, S., Gagliardini, V., Gheyselinck, J., Yang, W. C., & Grossniklaus, U. (2007). The Feronia receptor-like kinase mediates male-female interactions during pollen tube reception. *Science*, *317*(5838), 656–660. https://doi.org/10.1126/science.1143562

Feng, W., Kita, D., Peaucelle, A., Wu, H.-M., Cheung, A. Y., & Dinneny, R. (2018). The FERONIA Receptor Kinase Maintains Cell-Wall Integrity during Salt Stress through Ca 2+ Signaling. *Current Biology*, *28*, 666-675.e5. https://doi.org/10.1016/j.cub.2018.01.023

Feraru, E., Feraru, M. I., Kleine-Vehn, J., Martinière, A., Mouille, G., Vanneste, S., Vernhettes, S., Runions, J., & Friml, J. (2011). PIN polarity maintenance by the cell wall in Arabidopsis. *Current Biology*, *21*(4), 338–343. https://doi.org/10.1016/j.cub.2011.01.036

Franck, C. M., Westermann, J., & Boisson-Dernier, A. (2018). Plant Malectin-Like Receptor Kinases: From Cell Wall Integrity to Immunity and Beyond. *Annual Review of Plant Biology*, *69*(1). https://doi.org/10.1146/annurev-arplant-042817-040557

Galindo-Trigo, S., Blanco-Touriñán, N., DeFalco, T. A., Wells, E. S., Gray, J. E., Zipfel, C., & Smith, L. M. (2020). CrRLK1L receptor-like kinases HERK1 and ANJEA are female determinants of pollen tube reception. *EMBO Reports*, *21*(2). https://doi.org/10.15252/embr.201948466

Ge, Z., Bergonci, T., Zhao, Y., Zou, Y., Du, S., Liu, M. C., Luo, X., Ruan, H., García-Valencia, L. E., Zhong, S., Hou, S., Huang, Q., Lai, L., Moura, D. S., Gu, H., Dong, J., Wu, H. M., Dresselhaus, T., Xiao, J., … Qu, L. J. (2017). Arabidopsis pollen tube integrity and sperm release are regulated by RALF-mediated signaling. *Science*, *358*(6370), 1596–1600. https://doi.org/10.1126/science.aao3642

Ge, Z., Zhao, Y., Liu, M. C., Zhou, L. Z., Wang, L., Zhong, S., Hou, S., Jiang, J., Liu, T., Huang, Q., Xiao, J., Gu, H., Wu, H. M., Dong, J., Dresselhaus, T., Cheung, A. Y., & Qu, L. J. (2019). LLG2/3 Are Co-receptors in BUPS/ANX-RALF Signaling to Regulate Arabidopsis Pollen Tube Integrity. *Current Biology*, *29*(19), 3256-3265.e5. https://doi.org/10.1016/j.cub.2019.08.032

Göhre, V., Spallek, T., Häweker, H., Mersmann, S., Mentzel, T., Boller, T., de Torres, M., Mansfield, J. W., & Robatzek, S. (2008). Plant Pattern-Recognition Receptor FLS2 Is Directed for Degradation by the Bacterial Ubiquitin Ligase AvrPtoB. *Current Biology*, *18*(23), 1824–1832. https://doi.org/10.1016/j.cub.2008.10.063

Gómez-Gómez, L., & Boller, T. (2000). FLS2: An LRR receptor-like kinase involved in the perception of the bacterial elicitor flagellin in Arabidopsis. *Molecular Cell*, *5*(6), 1003–1011. https://doi.org/10.1016/S1097-2765(00)80265-8

Gronnier, J., Gerbeau-Pissot, P., Germain, V., Mongrand, S., & Simon-Plas, F. (2018). Divide and Rule: Plant Plasma Membrane Organization. *Trends in Plant Science*, *23*(10). https://doi.org/10.1016/j.tplants.2018.07.007

Gronnier, Julien, Crowet, J.-M., Habenstein, B., Nasir, M. N., Bayle, V., Hosy, E., Platre, M. P., Gouguet, P., Raffaele, S., Martinez, D., Grelard, A., Loquet, A., Simon-Plas, F., Gerbeau-Pissot, P., Der, C., Bayer, E. M., Jaillais, Y., Deleu, M., Germain, V., Mongrand, S. (2017). Structural basis for plant plasma membrane protein dynamics and organization into functional nanodomains. *ELife*, *6*, 1–24. https://doi.org/10.7554/eLife.26404

Guo, H., Li, L., Ye, H., Yu, X., Algreen, A., & Yin, Y. (2009). Three related receptor-like kinases are required for optimal cell elongation in Arabidopsis thaliana. *Proceedings of the National Academy of Sciences of the United States of America*, *106*(18), 7648–7653. https://doi.org/10.1073/pnas.0812346106

Guo, H., Nolan, T. M., Schnable, P. S., Walley, J. W., & Yin, Y. (2018). FERONIA Receptor Kinase Contributes to Plant Immunity by Suppressing Jasmonic Acid Signaling in Arabidopsis thaliana. *Current Biology*, *28*, 3316-3324.e6. https://doi.org/10.1016/j.cub.2018.07.078

Haas, K. T., Wightman, R., Meyerowitz, E. M., & Peaucelle, A. (2020). Pectin homogalacturonan nanofilament expansion drives morphogenesis in plant epidermal cells. *Science*, *367*(6481), 1003–1007. https://doi.org/10.1126/science.aaz5103

Halter, T., Imkampe, J., Mazzotta, S., Wierzba, M., Postel, S., Bücherl, C., Kiefer, C., Stahl, M., Chinchilla, D., Wang, X., Nürnberger, T., Zipfel, C., Clouse, S., Borst, J. W., Boeren, S., de Vries, S. C., Tax, F., & Kemmerling, B. (2014). The leucine-rich repeat receptor kinase BIR2 is a negative regulator of BAK1 in plant immunity. *Current Biology*, *24*(2), 134–143. https://doi.org/10.1016/j.cub.2013.11.047

Haruta, M., Gaddameedi, V., Burch, H., Fernandez, D., & Sussman, M. R. (2018). Comparison of the effects of a kinase-dead mutation of FERONIA on ovule fertilization and root growth of Arabidopsis. *FEBS Letters*, *592*(14), 2395–2402. https://doi.org/10.1002/1873-3468.13157

Haruta, M., Sabat, G., Stecker, K., Minkoff, B. B., & Sussman, M. R. (2014). A peptide hormone and its receptor protein kinase regulate plant cell expansion. *Science*, *343*(6169), 408–411. https://doi.org/10.1126/science.1244454

Heese, A., Hann, D. R., Gimenez-Ibanez, S., Jones, A. M. E., He, K., Li, J., Schroeder, J. I., Peck, S. C., & Rathjen, J. P. (2007). The receptor-like kinase SERK3/BAK1 is a central regulator of innate immunity in plants. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(29), 12217–12222. https://doi.org/10.1073/pnas.0705306104

Herger, A., Dünser, K., Kleine-Vehn, J., & Ringli, C. (2019). Leucine-Rich Repeat Extensin Proteins and Their Role in Cell Wall Sensing. In *Current Biology* (Vol. 29, Issue 17, pp. R851–R858). Cell Press. https://doi.org/10.1016/j.cub.2019.07.039

Herger, A., Gupta, S., Kadler, G., Franck, C. M., Boisson-Dernier, A., & Ringli, C. (2020). Overlapping functions and protein-protein interactions of LRR-extensins in Arabidopsis. *PLOS Genetics*, *16*(6), e1008847. https://doi.org/10.1371/journal.pgen.1008847

Hohmann, U., Lau, K., & Hothorn, M. (2017). The Structural Basis of Ligand Perception and Signal Activation by Receptor Kinases. *Annual Review of Plant Biology*, *68*(1), 109–137. https://doi.org/10.1146/annurev-arplant-042916-040957

Huck, N., Moore, J. M., Federer, M., & Grossniklaus, U. (2003). The Arabidopsis mutant feronia disrupts the female gametophytic control of pollen tube receptor. In *Development* (Vol. 130, Issue 10, pp. 2149–2159). Development. https://doi.org/10.1242/dev.00458

Hutten, S. J., Hamers, D. S., Aan den Toorn, M., van Esse, W., Nolles, A., Bücherl, C. A., de Vries, S. C., Hohlbein, J., Borst, J. W., Clouse, S., Jaillais, Y., Hothorn, M., Belkhadir, Y., Dabi, T., Nimchuk, Z., Meyerowitz, E., Wang, X., Chory, J., Wang, X., … Förster, T. (2017). Visualization of BRI1 and SERK3/BAK1 Nanoclusters in Arabidopsis Roots. *Plos One*, *12*(1), e0169905. https://doi.org/10.1371/journal.pone.0169905

Imkampe, J., Halter, T., Huang, S., Schulze, S., Mazzotta, S., Schmidt, N., Manstretta, R., Postel, S., Wierzba, M., Yang, Y., van Dongen, W. M. A. M., Stahl, M., Zipfel, C., Goshe, M. B., Clouse, S., de Vries, S. C., Tax, F., Wang, X., & Kemmerling, B. (2017). The Arabidopsis Leucine-Rich Repeat Receptor Kinase BIR3 Negatively Regulates BAK1 Receptor Complex Formation and Stabilizes BAK1. *The Plant Cell*, *29*(9), 2285–2303. https://doi.org/10.1105/tpc.17.00376

Jaillais, Y., & Ott, T. (2020). The Nanoscale Organization of the Plasma Membrane and Its Importance in Signaling: A Proteolipid Perspective. *Plant Physiology*, *182*(4), 1682–1696. https://doi.org/10.1104/pp.19.01349

Jaqaman, K., Loerke, D., Mettlen, M., Kuwata, H., Grinstein, S., Schmid, S. L., & Danuser, G. (2008). Robust single-particle tracking in live-cell time-lapse sequences. *Nature Methods*, *5*(8), 695–702. https://doi.org/10.1038/nmeth.1237

Jarsch, I. K., Konrad, S. S. a, Stratil, T. F., Urbanus, S. L., Szymanski, W., Braun, P., Braun, K.-H., & Ott, T. (2014). Plasma Membranes Are Subcompartmentalized into a Plethora of Coexisting and Diverse Microdomains in Arabidopsis and Nicotiana benthamiana. *The Plant Cell*, *26*, 1698–1711. https://doi.org/10.1105/tpc.114.124446

Kadota, Y., Sklenar, J., Derbyshire, P., Stransfeld, L., Asai, S., Ntoukakis, V., Jones, J. D., Shirasu, K., Menke, F., Jones, A., & Zipfel, C. (2014). Direct Regulation of the NADPH Oxidase RBOHD by the PRR-Associated Kinase BIK1 during Plant Immunity. *Molecular Cell*, *54*, 43–55. https://doi.org/10.1016/j.molcel.2014.02.021

Keinath, N. F., Kierszniowska, S., Lorek, J., Bourdais, G., Kessler, S. A., Shimosato-Asano, H., Grossniklaus, U., Schulze, W. X., Robatzek, S., & Panstruga, R. (2010). PAMP (Pathogen-associated Molecular Pattern)-induced changes in plasma membrane compartmentalization reveal novel components of plant immunity. *Journal of Biological Chemistry*, *285*(50), 39140–39149. https://doi.org/10.1074/jbc.M110.160531

Kessler, S. A., Lindner, H., Jones, D. S., & Grossniklaus, U. (2015). Functional analysis of related CrRLK1L receptor-like kinases in pollen tube reception. *EMBO Reports*, *16*, 107–115. https://doi.org/10.15252/embr.201438801

Liang, P., Stratil, T. F., Popp, C., Marín, M., Folgmann, J., Mysore, K. S., Wen, J., & Ott, T. (2018). Symbiotic root infections in Medicago truncatula require remorin-mediated receptor stabilization in membrane nanodomains. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(20), 5289–5294. https://doi.org/10.1073/pnas.1721868115

Li, B., Ferreira, M. A., Huang, M., Camargos, L. F., Yu, X., Teixeira, R. M., Carpinetti, P. A., Mendes, G. C., Gouveia-Mageste, B. C., Liu, C., Pontes, C. S. L., Brustolini, O. J. B., Martins, L. G. C., Melo, B. P., Duarte, C. E. M., Shan, L., He, P., & Fontes, E. P. B. (2019). The receptor-like kinase NIK1 targets FLS2/BAK1 immune complex and inversely modulates antiviral and antibacterial immunity. *Nature Communications*, *10*(1), 1–14. https://doi.org/10.1038/s41467-019-12847-6

Li, Chao, Yeh, F. L., Cheung, A. Y., Duan, Q., Kita, D., Liu, M. C., Maman, J., Luu, E. J., Wu, B. W., Gates, L., Jalal, M., Kwong, A., Carpenter, H., & Wu, H. M. (2015). Glycosylphosphatidylinositol-anchored proteins as chaperones and co-receptors for FERONIA receptor kinase signaling in Arabidopsis. *ELife*, *4*(JUNE), 1–21. https://doi.org/10.7554/eLife.06587

Li, Chiyu, Liu, X., Qiang, X., Li, X., Li, X., Zhu, S., Wang, L., Wang, Y., Liao, H., Luan, S., & Yu, F. (2018). EBP1 nuclear accumulation negatively feeds back on FERONIA-mediated RALF1 signaling. *PLOS Biology*, *16*(10), e2006340. https://doi.org/10.1371/journal.pbio.2006340

Lin, W., Tang, W., Anderson, C., & Yang, Z. (2018). FERONIA's sensing of cell wall pectin activates ROP GTPase signaling in Arabidopsis. *BioRxiv*, *35*(June), 269647. https://doi.org/10.1101/269647

Malinovsky, F. G., Fangel, J. U., & Willats, W. G. T. (2014). The role of the cell wall in plant immunity. In *Frontiers in Plant Science* (Vol. 5, Issue MAY, p. 178). Frontiers Research Foundation. https://doi.org/10.3389/fpls.2014.00178

Mang, H., Feng, B., Hu, Z., Boisson-Dernier, A., Franck, C. M., Meng, X., Huang, Y., Zhou, J., Xu, G., Wang, T., Shan, L., & He, P. (2017). Differential regulation of two-tiered plant immunity and sexual reproduction by ANXUR receptor-like kinases. *Plant Cell*, *29*(12), 3140–3156. https://doi.org/10.1105/tpc.17.00464

Martinière, A., Lavagi, I., Nageswaran, G., Rolfe, D. J., Maneta-Peyret, L., Luu, D.-T., Botchway, S. W., Webb, S. E. D., Mongrand, S., Maurel, C., Martin-Fernandez, M. L., Kleine-Vehn, J., Friml, J., Moreau, P., & Runions, J. (2012). Cell wall constrains lateral diffusion of plant plasma-membrane proteins. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(31), 12805–12810. https://doi.org/10.1073/pnas.1202040109

Ma, X., Xu, G., He, P., & Shan, L. (2016). SERKing Coreceptors for Receptors. *Trends in Plant Science*, *21*(12), 1017–1033. https://doi.org/10.1016/j.tplants.2016.08.014

McKenna, J. F., Rolfe, D. J., Webb, S. E. D., Tolmie, A. F., Botchway, S. W., Martin-Fernandez, M. L., Hawes, C., & Runions, J. (2019). The cell wall regulates dynamics and size of plasma-membrane nanodomains in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, *116*(26), 12857–12862. https://doi.org/10.1073/pnas.1819077116

Mecchia, M. A., Santos-Fernandez, G., Duss, N. N., Somoza, S. C., Boisson-Dernier, A., Gagliardini, V., Martínez-Bernardini, A., Fabrice, T. N., Ringli, C., Muschietti, J. P., & Grossniklaus, U. (2017). RALF4/19 peptides interact with LRX proteins to control pollen tube growth in Arabidopsis. *Science*, *358*(6370), 1600–1603. https://doi.org/10.1126/science.aao5467

Micheli, F. (2001). Pectin methylesterases: Cell wall enzymes with important roles in plant physiology. In *Trends in Plant Science* (Vol. 6, Issue 9, pp. 414–419). Trends Plant Sci. https://doi.org/10.1016/S1360-1385(01)02045-3

Minkoff, B. B., Makino, S. I., Haruta, M., Beebe, E. T., Wrobel, R. L., Fox, B. G., & Sussman, M. R. (2017a). A cell-free method for expressing and reconstituting membrane proteins enables functional characterization of the plant receptor-like protein kinase FERONIA. *Journal of Biological Chemistry*, *292*(14), 5932–5942. https://doi.org/10.1074/jbc.M116.761981

Minkoff, B. B., Makino, S. I., Haruta, M., Beebe, E. T., Wrobel, R. L., Fox, B. G., & Sussman, M. R. (2017b). A cell-free method for expressing and reconstituting membrane proteins enables functional characterization of the plant receptor-like protein kinase FERONIA. *Journal of Biological Chemistry*, *292*(14), 5932–5942. https://doi.org/10.1074/jbc.M116.761981

Monaghan, J., Matschi, S., Shorinola, O., Rovenich, H., Matei, A., Segonzac, C., Malinovsky, F. G., Rathjen, J. P., Maclean, D., Romeis, T., & Zipfel, C. (2014). The calcium-dependent protein kinase CPK28 buffers plant immunity and regulates BIK1 turnover. *Cell Host and Microbe*, *16*(5), 605–615. https://doi.org/10.1016/j.chom.2014.10.007

Moussu, S., Augustin, S., Roman, A.-O., Broyart, C., & Santiago, J. (2018a). Crystal structures of two tandem malectin-like receptor kinases involved in plant reproduction. *Research Papers Acta Cryst*, *74*, 671–680. https://doi.org/10.1107/S205979831800774X

Moussu, S., Augustin, S., Roman, A.-O., Broyart, C., & Santiago, J. (2018b). Crystal structures of two tandem malectin-like receptor kinases involved in plant reproduction. *Acta Crystallographica Section D Structural Biology*, *74*(7), 671–680. https://doi.org/10.1107/S205979831800774X

Moussu, S., Broyart, C., Santos-Fernandez, G., Augustin, S., Wehrle, S., Grossniklaus, U., & Santiago, J. (2020). Structural basis for recognition of RALF peptides by LRX proteins during pollen tube growth. *Proceedings of the National Academy of Sciences of the United States of America*, *117*(13), 7494–7503. https://doi.org/10.1073/pnas.2000100117

Ott, T. (2017). Membrane nanodomains and microdomains in plant–microbe interactions. In *Current Opinion in Plant Biology* (Vol. 40, pp. 82–88). Elsevier Ltd. https://doi.org/10.1016/j.pbi.2017.08.008

Pan, X., fang, L., Liu, J., Senay-Aras, B., Lin, W., Zheng, S., Zhang, T., Manor, U., Chen, W., & Yang, Z. (2019). Auxin-induced nanoclustering of membrane signaling complexes underlies cell polarity establishment in Arabidopsis. *BioRxiv*, 734665. https://doi.org/10.1101/734665

Perraki, A., DeFalco, T. A., Derbyshire, P., Avila, J., Séré, D., Sklenar, J., Qi, X., Stransfeld, L., Schwessinger, B., Kadota, Y., Macho, A. P., Jiang, S., Couto, D., Torii, K. U., Menke, F. L. H., & Zipfel, C. (2018). Phosphocode-dependent functional dichotomy of a common co-receptor in plant signalling. *Nature*, *561*(7722), 248–252. https://doi.org/10.1038/s41586-018-0471-x

Platre, M. P., Bayle, V., Armengot, L., Bareille, J., del Mar Marquès-Bueno, M., Creff, A., Maneta-Peyret, L., Fiche, J. B., Nollmann, M., Miège, C., Moreau, P., Martinière, A., & Jaillais, Y. (2019). Developmental control of plant Rho GTPase nano-organization by the lipid phosphatidylserine. *Science*, *364*(6435), 57–62. https://doi.org/10.1126/science.aav9959

Rotman, N., Rozier, F., Boavida, L., Dumas, C., Berger, F., & Faure, J. E. (2003). Female control of male gamete delivery during fertilization in Arabidopsis thaliana. *Current Biology*, *13*(5), 432–436. https://doi.org/10.1016/S0960-9822(03)00093-9

Roux, M., Schwessinger, B., Albrecht, C., Chinchilla, D., Jones, A., Holton, N., Malinovsky, F. G., Tör, M., de Vries, S., & Zipfel, C. (2011). The Arabidopsis leucine-rich repeat receptor-like kinases BAK1/SERK3 and BKK1/SERK4 are required for innate immunity to hemibiotrophic and biotrophic pathogens. *Plant Cell*, *23*(6), 2440–2455. https://doi.org/10.1105/tpc.111.084301

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., Tinevez, J. Y., White, D. J., Hartenstein, V., Eliceiri, K., Tomancak, P., & Cardona, A. (2012). Fiji: An open-source platform for biological-image analysis. In *Nature Methods* (Vol. 9, Issue 7, pp. 676–682). Nature Publishing Group. https://doi.org/10.1038/nmeth.2019

Schulze, B., Mentzel, T., Jehle, A. K., Mueller, K., Beeler, S., Boller, T., Felix, G., & Chinchilla, D. (2010). Rapid heteromerization and phosphorylation of ligand-activated plant transmembrane receptors and their associated kinase BAK1. *Journal of Biological Chemistry*, *285*(13), 9444–9451. https://doi.org/10.1074/jbc.M109.096842

Schwessinger, B., Roux, M., Kadota, Y., Ntoukakis, V., Sklenar, J., Jones, A., & Zipfel, C. (2011). Phosphorylation-Dependent Differential Regulation of Plant Growth, Cell Death, and Innate Immunity by the Regulatory Receptor-Like Kinase BAK1. *PLoS Genetics*, *7*(4), e1002046. https://doi.org/10.1371/journal.pgen.1002046

Shen, Q., Bourdais, G., Pan, H., Robatzek, S., & Tang, Di. (2017). Arabidopsis glycosylphosphatidylinositol-anchored protein LLG1 associates with and modulates FLS2 to regulate innate immunity. *Proceedings of the National Academy of Sciences of the United States of America*, *114*(22), 5749–5754. https://doi.org/10.1073/pnas.1614468114

Shih, H. W., Miller, N. D., Dai, C., Spalding, E. P., & Monshausen, G. B. (2014). The receptor-like kinase FERONIA is required for mechanical signal transduction in Arabidopsis seedlings. *Current Biology*, *24*(16), 1887–1892. https://doi.org/10.1016/j.cub.2014.06.064

Smakowska-Luzan, E., Mott, G. A., Parys, K., Stegmann, M., Howton, T. C., Layeghifard, M., Neuhold, J., Lehner, A., Kong, J., Grünwald, K., Weinberger, N., Satbhai, S. B., Mayer, D., Busch, W., Madalinski, M., Stolt-Bergner, P., Provart, N. J., Mukhtar, M. S., Zipfel, C., … Belkhadir, Y. (2018). An extracellular network of Arabidopsis leucine-rich repeat receptor kinases. *Nature*, *553*(7688), 342–346. https://doi.org/10.1038/nature25184

Somssich, M., Ma, Q., Weidtkamp-Peters, S., Stahl, Y., Felekyan, S., Bleckmann, A., Seidel, C. A. M., & Simon, R. (2015). Real-time dynamics of peptide ligand–dependent receptor complex formation in planta. *Sci. Signal*, *8*. https://doi.org/10.1126/scisignal.aab0598

Stegmann, M., Monaghan, J., Smakowska-Luzan, E., Rovenich, H., Lehner, A., Holton, N., Belkhadir, Y., & Zipfel, C. (2017). The receptor kinase FER is a RALF-regulated scaffold controlling plant immune signaling. *Science*, *355*(6322), 287–289. https://doi.org/10.1126/science.aal2541

Sun, Y., Li, L., Macho, A. P., Han, Z., Hu, Z., Zipfel, C., Zhou, J. M., & Chai, J. (2013). Structural basis for flg22-induced activation of the Arabidopsis FLS2-BAK1 immune complex. *Science*, *342*(6158), 624–628. https://doi.org/10.1126/science.1243825

Tinevez, J. Y., Perry, N., Schindelin, J., Hoopes, G. M., Reynolds, G. D., Laplantine, E., Bednarek, S. Y., Shorte, S. L., & Eliceiri, K. W. (2017). TrackMate: An open and extensible platform for single-particle tracking. *Methods*, *115*, 80–90. https://doi.org/10.1016/j.ymeth.2016.09.016

Vaahtera, L., Schulz, J., & Hamann, T. (2019). Cell wall integrity maintenance during plant development and interaction with the environment. In *Nature Plants* (Vol. 5, Issue 9, pp. 924–932). Palgrave Macmillan Ltd. https://doi.org/10.1038/s41477-019-0502-0

Wagner, T., Kroll, A., Haramagatti, C. R., Lipinski, H.-G., & Wiemann, M. (2017). Classification and Segmentation of Nanoparticle Diffusion Trajectories in Cellular Micro Environments. *PLOS ONE*, *12*(1), e0170165. https://doi.org/10.1371/journal.pone.0170165

Xiao, Y., Stegmann, M., Han, Z., DeFalco, T. A., Parys, K., Xu, L., Belkhadir, Y., Zipfel, C., & Chai, J. (2019). Mechanisms of RALF peptide perception by a heterotypic receptor complex. *Nature*, *572*(7768), 270–274. https://doi.org/10.1038/s41586-019-1409-7

Yeh, Y. H., Panzeri, D., Kadot, Y., Huang, Y. C., Huang, P. Y., Tao, C. N., Roux, M., Chien, H. C., Chin, T. C., Chu, P. W., Zipfel, C., & Zimmerli, L. (2016). The arabidopsis malectin-like/LRR-RLK IOS1 is critical for BAK1-dependent and BAK1-independent pattern-triggered immunity. *Plant Cell*, *28*(7), 1701–1721. https://doi.org/10.1105/tpc.16.00313

Yu, F., Qian, L., Nibau, C., Duan, Q., Kita, D., Levasseur, K., Li, X., Lu, C., Li, H., Hou, C., Li, L., Buchanan, B. B., Chen, L., Cheung, A. Y., Li, D., & Luan, S. (2012). FERONIA receptor kinase pathway suppresses abscisic acid signaling in Arabidopsis by activating ABI2 phosphatase. *Proceedings of the National Academy of Sciences of the United States of America*, *109*(36), 14693–14698. https://doi.org/10.1073/pnas.1212547109

Yu, X., Feng, B., He, P., & Shan, L. (2017). From Chaos to Harmony: Responses and Signaling upon Microbial Pattern Recognition. *Annual Review of Phytopathology*, *55*(1), 109–137. https://doi.org/10.1146/annurev-phyto-080516-035649

Zhao, C., Zayed, O., Yu, Z., Jiang, W., Zhu, P., Hsu, C. C., Zhang, L., Andy Tao, W., Lozano-Durán, R., & Zhu, J. K. (2018). Leucine-rich repeat extensin proteins regulate plant salt tolerance in Arabidopsis. *Proceedings of the National Academy of Sciences of the United States of America*, *115*(51), 13123–13128. https://doi.org/10.1073/pnas.1816991115

Zhu, S., Martínez Pacheco, J., Estevez, J. M., & Yu, F. (2020). Autocrine regulation of root hair size by the RALF-FERONIA-RSL4 signaling pathway. In *New Phytologist* (Vol. 227, Issue 1, pp. 45–49). Blackwell Publishing Ltd. https://doi.org/10.1111/nph.16497

Zipfel, C., Kunze, G., Chinchilla, D., Caniard, A., Jones, J. D. G., Boller, T., & Felix, G. (2006). Perception of the Bacterial PAMP EF-Tu by the Receptor EFR Restricts Agrobacterium-Mediated Transformation. *Cell*, *125*(4), 749–760. https://doi.org/10.1016/j.cell.2006.03.037

FIGURE LEGENDS

Fig. 1 ¦ FER kinase activity is dispensable to support PTI signaling.

A. flg22-induced FLS2-BAK1 complex formation. Immunoprecipitation of FLS2 in Arabidopsis Col-0, *fer-4*, and *fer-4*/pFER::FER^{KD}-GFP seedlings that were either untreated or treated with 100 nM flg22 for 10 min. Blots stained with Coomassie brilliant blue (CBB) is presented to show equal loading. Western blots were probed with α-FLS2, α-BAK1, α-BAK1-pS612 or α-FER antibodies. Similar results were obtained in at least three independent experiments.

B-C. ROS production after elicitation with 100 nM flg22 (**A**), or 100 nM elf18 (**B**). Values are means of total photon counts over 40 min, *n* = 16. Red crosses and red horizontal lines denote mean and SEM, respectively. Conditions which do not share a letter are significantly different in Dunn's multiple comparison test (p< 0,0001).

Fig. 2 ¦ FER Malectin A domain is dispensable to support PTI signaling.

A. flg22-induced FLS2-BAK1 complex formation. Immunoprecipitation of FLS2 in Arabidopsis Col-0, *fer-4*, and *fer-4*/p35S::FER$^{\Delta MalA}$-YFP seedlings that were either untreated or treated with 100 nM flg22 for 10 min. Blot stained with Coomassie brilliant blue (CBB) is presented to show equal loading. Western blots were probed with α-FLS2, α-BAK1, or α-FER antibodies. Similar results were obtained in at least three independent experiments.

B-C. ROS production after elicitation with 100 nM flg22 (**A**), or 100 nM elf18 (**B**). Values are means of total photon counts over 40 min, *n* = 8. Red crosses and red horizontal lines denote mean and SEM, respectively. Conditions which do not share a letter are significantly different in Dunn's multiple comparison test (p< 0,0001).

Fig. 3 ¦ LRX3, LRX4 and LRX5 are required for FER-mediated regulation of PTI signaling.

A. flg22-induced FLS2-BAK1 complex formation. Immunoprecipitation of FLS2 in Arabidopsis Col-0 and *lr3/4/5* seedlings either untreated or treated with 100 nM flg22 for 10 min. Blot stained with CBB is presented to show equal loading. Western blots were probed with α-FLS2, α-BAK1, or α-FER antibodies. Similar results were obtained in at least three independent experiments.

B-C. ROS production after elicitation with 100 nM elf18 (**B**), or 100 nM flg22 (**C**). Values are means of total photon counts over 40 min. Red crosses and red horizontal lines denote mean

and SEM, *n* = 32. Conditions which do not share a letter are significantly different in Dunn's multiple comparison test (p< 0,0001).

D-E. ROS production in Col-0 and *lr3/4/5* leaf discs treated with1 µM RALF17 (**D**), or 100 nM elf18 with or without 1 µM RALF23 co-treatment (**E**); all in 2 mM MES–KOH pH 5.8. Values are means of total photon counts over 40 min. Red crosses and red horizontal lines show mean and SEM, n = 12. Conditions which do not share a letter are significantly different in Dunn's multiple comparison test (p< 0,0001) and *** indicate p< 0,001.

F. FER-LRX4 association. Immunoprecipitation of LRX4-FLAG in Arabidopsis seedlings untreated or treated with 1 µM RALF23 for 10 min. Western blots were probed with α-GFP or α-FER antibodies. Blot stained with CBB is presented to show equal loading. Two independent transgenic LRX4-FLAG lines (#1 and #7) were used. Similar results were obtained in at least three independent experiments.

Fig. 4 ¦ FER regulates dynamics and size of FLS2-GFP nanodomains.

A-B. Representative super-resolved images of FLS2-GFP single-particles tracked in Col-0 and *fer-2* cotyledon epidermal cells of 5-day-old seedlings with or without 1 µM RALF23 treatment (**A**) and quantification of FLS2-GFP diffusion coefficient (D) in Col-0 and *fer-2* with or without 1 µM RALF23 treatment (**B**). Data represent analysis of *ca* 1800 to 2900 single particles observed across 14 to 18 cells. Individual data point represents the mean diffusion coefficient for each cell. Red crosses and red horizontal lines show mean and SEM. Conditions which do not share a letter are significantly different in Dunn's multiple comparison test (p< 0,0001). Similar results were obtained in at least three independent experiments.

C-D. FLS2-GFP nanodomain organization. Pictures are maximum projection images (20 TIRFM images obtained at 20 frames per seconds) of FLS2-GFP in Col-0 and *fer-2* cotyledon epidermal cells with or without 1 µM RALF23 treatment (**C**) and quantification of FLS2-GFP nanodomains area (**D**). Red crosses and red horizontal lines show mean and SEM. Conditions which do not share a letter are significantly different in Dunn's multiple comparison test (p< 0,0001). Similar results were obtained in at least three independent experiments.

MovieS1 ¦ TIRFM single particle imaging of FLS2-GFP.

Representative stream images acquisition of FLS2-GFP particles observed at the surface of five-days old cotyledon epidermal cell by TIRF microscopy.

Fig. S1 ¦ FER-GFP localizes to plasma membrane nanodomains.

Pictures are maximum projection images (20 TIRFM images obtained at 20 frames per seconds) of LTI6B-GFP and FER-GFP in cotyledon epidermal cells of 5-day-old seedlings.

Fig. S2 ¦ Segmentation and quantification of FLS2-GFP nanodomains.

Twenty time-series images obtained at 50 frames per second in TIRFM (**A**) were used to generate maximum projections of fluorescence intensity (**B**). Subsequently, we applied a background subtraction with a rolling ball radius of 30 pixels (**C**) and a smooth filter (**D**) to facilitate the segmentation of nanodomains. Regions of interest (ROI) of 8,5 μm^2 presenting homogenous illumination were selected for each cell. Nanodomains were segmented by applying auto-threshold, watershed and analyse particle in Fiji. Obtained masks were then reported to maximum projection images for quantification (**E**).

Fig. S3 ¦ FER does not regulate FLS2-GFP nanodomain density.

Related to Figure 4. Quantification of FLS2-GFP nanodomains density expressed as number (#) per μm^2. Red crosses and red horizontal lines show mean and SEM. Conditions which share a letter are not significantly different in Dunn's multiple comparison test (p>0,05). Similar results were obtained in three independent experiments.

Fig. S4 ¦ FLS2-GFP accumulation at the PM is altered in *fer-2*.

A. Representative confocal microscopy pictures of FLS2-GFP driven by its native promoter in Col-0 and *fer-2* cotyledon epidermal cells of 5-day-old seedlings. Scale bar indicate 2 µm.

B. Quantification of FLS2-GFP fluorescence intensity at plasma membrane level in Col-0 and *fer-2* cotyledon epidermal cells of 5-day-old seedlings. Two-way student's t test. Similar results were obtained in at least three independent experiments.

Fig. S5 ¦ RALF17 and RALF23 induces FER endocytosis.

Representative confocal microscopy pictures of FER-GFP driven by its native promoter in *fer-4* root epidermal cells of 5-day-old seedlings with or without 10 µM RALF23 or 10 µM RALF17. Seedlings were pre-treated with 100 µM cycloheximide (CHX) and 2 µM

Concanamycin A (ConA) 1 h before RALF treatment. Similar results were obtained in at least three independent experiments. Scale bar indicate 10 µm.

Fig. S6 ¦ Inhibition of PTI-signaling by RALF23 requires FER kinase activity.

A. flg22-induced FLS2-BAK1 complex formation. Immunoprecipitation of FLS2 in Arabidopsis *fer-2*, and *fer-2*/pFER::FER^{KD}-GFP seedlings that were either untreated or treated with 100 nM of flg22 and 1 µM RALF23 for 10 min. Western blots were probed with α-FLS2, α-BAK1 antibodies.

B. ROS production after elicitation with 100 nM elf18, with and without 1 µM RALF23 co-treatment. Values are means of total photon counts over 40 min, n = 10. Red crosses and red horizontal lines denote mean and SEM, respectively. Conditions which do not share a letter are significantly different in Dunn's multiple comparison test ($p < 0,05$). Similar results were obtained in at least three independent experiments.

MATERIALS AND METHODS

Plant materials and growth

Arabidopsis thaliana ecotype Columbia (Col-0) was used as WT control. The *fer-4, fer-4/FER-GFP* (Fig. S6; (Duan et al., 2010), *fer-4/FER^{KD}-GFP* (Fig. 1; (Chakravorty et al., 2018), *fer-4/FER$^{\Delta MalA}$-GFP* (Fig.2 (Lin et al., 2018), Col- 0/pFLS2::FLS2-GFP (Fig. 4, Fig. S2-4; (Göhre et al., 2008), *fer-2*/pFLS2::FLS2-GFP (Fig. 4; (Stegmann et al., 2017), *lrx3/4/5* (Fig. 3; (Dünser et al., 2019) lines were previously published. For ROS burst assays, plants were grown in individual pots at 20-21 °C with a 10-h photoperiod in environmentally controlled growth rooms. For seedling-based assays, seeds were surface-sterilized using chlorine gas for 5 h and grown at 22□°C and a 16-h photoperiod on Murashige and Skoog (MS) medium supplemented with vitamins, 1 % sucrose and 0.8 % agar.

ROS burst measurement

ROS burst measurements were performed as previously documented (Kadota et al., 2014). At least eight leaf discs (4 mm in diameter) per individual genotype were collected in 96-well plates containing sterile water and incubated overnight. The next day, the water was replaced by a solution containing 17 µg/mL luminol (Sigma Aldrich), 20 µg/mL horseradish peroxidase (HRP, Sigma Aldrich) and the peptides in the appropriate concentration. Luminescence was measured for the indicated time period using a charge-coupled device camera (Photek Ltd., East Sussex UK). The effect of RALF23 on elf18-triggered ROS production was performed as previously described (Stegmann et al., 2017). Eight-to-ten leaf discs per treatment and/or genotype were collected in 96-well plates containing water and incubated overnight. The following day, the water was replaced by 75 µL of 2 mM MES-KOH pH 5.8 to mimic the apoplastic pH. Leaf discs were incubated further for 4-5 h before adding 75 µL of a solution containing 40 µg/mL HRP, 1 µM L-O12 (Wako Chemicals, Germany) and 2X elicitor RALF peptide solution (final concentration 20 µg/mL HRP, 0.5 µM L-O12, 1x elicitors). ROS production is displayed as the integration of total photon counts.

Live cell imaging

For confocal microscopy and TIRF microscopy experiments, surface-sterilized seeds were individually placed in line on square Petri dishes containing 1/2 MS 1 % sucrose, 0.8 % phytoagar, stratified 2 d in the dark at 4 °C, then placed in a growth chamber at 22□°C and a 16-h photoperiod for 5 d. Seedlings were mounted between a glass slide and a cover slip in liquid 1/2 MS, 1 % sucrose medium. To test the effect of RALF23 on FLS2-GFP dynamics

and nanodomain organization, seedlings were pre-incubated in 2 mM MES-KOH pH 5.8 for 3 to 4h prior treatment. Seedlings were image 2-30 min after treatment.

Confocal laser scanning microscopy (CLSM)

Confocal microscopy was performed using a Leica SP5 CLSM system (Leica, Wetzlar, Germany) equipped with Argon, DPSS, He-Ne lasers, hybrid detectors and using a 63X 1.2 NA oil immersion objective. GFP was excited using 488 nm argon laser and emission wavelengths were collected between 495 and 550 nm. In order to obtain quantitative data, experiments were performed using strictly identical confocal acquisition parameters (*e.g.* laser power, gain, zoom factor, resolution, and emission wavelengths reception), with detector settings optimized for low background and no pixel saturation. Pseudo-color images were obtained using look-up-table (LUT) of Fiji software (Schindelin et al., 2012).

Total Internal Reflection Fluorescence (TIRF) microscopy

TIRF microscopy was performed using an inverted Leica GSD equipped with a 160x objective (NA = 1.43, oil immersion), and an Andor iXon Ultra 897 EMCCD camera. Images were acquired by illuminating samples with a 488 nm solid state diode laser set at 15 mW, using a cube filter with an excitation filter 488/10 and an emission filter 535/50. The optimum critical angle was determined as giving the best signal-to-noise. Images time series were recorded at 20 frames per second (50 ms exposure time).

Single particle tracking analysis

To analyse single particle tracking experiments, we used the plugin TrackMate 2.7.4 (Tinevez et al., 2017) in Fiji (Schindelin et al., 2012). Single particles were segmented frame-by-frame by applying a LoG (Laplacian of Gaussian) filter and estimated particle size of 0.4 µm. Individual single particle were localized with sub-pixel resolution using a built-in quadratic fitting scheme. Then, single particle trajectories were reconstructed using a simple linear assignment problem (Jaqaman et al., 2008) with a maximal linking distance of 0.4 µm and without gap-closing. Only tracks with at least ten successive points (tracked for 500 ms) were selected for further analysis. Diffusion coefficients of individual particles were determined using TraJClassifier (Wagner et al., 2017). For each particle, the slope of the first four time points of their mean square displacement (MSD) plot was used to calculate their diffusion coefficient according to the following equation: $MSD= (x-x_0)^2+(y-y_0)^2$ and $D=MSD/4t$,

where x0 and y0 are the initial coordinates, and x and y are the coordinates at any given time, and *t* is the time frame.

Quantification of nanodomain density and size

See Fig. S2. Twenty time-series images acquired at 50 frames per second were used to obtain a maximum projection of fluorescence intensity. Subsequently, we applied a background subtraction with a rolling ball radius of 30 pixels and a smooth filter to facilitate the segmentation of nanodomains. Regions of interest (ROI) of 8.5 μm^2 presenting homogenous illumination were selected for each cell. Nanodomains were segmented by applying auto-threshold, watershed and analysed particle in Fiji (Schindelin et al., 2012). Obtained masks were then reported to original images for quantification. Nanodomain density was calculated by dividing the number of nanodomain segmented by the area of each ROI.

Co-immunoprecipitation experiments

Twenty to thirty seedlings per plate were grown in wells of a 6-well plate for 2 weeks, transferred to 2 mM MES-KOH, pH 5.8 and incubated overnight. The next day, flg22, (final concentration 100 nM) and/or RALF23 (final concentration 1 µM) were added and incubated for 10 min. Seedlings were then frozen in liquid N2 and subjected to protein extraction. To analyse FLS2-BAK1 receptor complex formation, proteins were isolated in 50 mM Tris-HCl pH 7.5, 150 mM NaCl, 10 % glycerol, 5 mM dithiothreitol, 1 % protease inhibitor cocktail (Sigma Aldrich), 2 mM Na2MoO4, 2.5 mM NaF, 1.5 mM activated Na3VO4, 1 mM phenylmethanesulfonyl fluoride and 0.5 % IGEPAL. For immunoprecipitations, α- rabbit Trueblot agarose beads (eBioscience) coupled with α-FLS2 antibodies (Chinchilla et al., 2007) or GFP-Trap agarose beads (ChromoTek) were used and incubated with the crude extract for 3-4 h at 4 °C. Subsequently, beads were washed 3 times with wash buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM phenylmethanesulfonyl fluoride, 0,1 % IGEPAL) before adding Laemmli sample buffer and incubating for 10 min at 95 °C. Analysis was carried out by SDS-PAGE and immunoblotting.

To test association between Flag-LRX4 and FER, total protein from 60-90 seedlings per treatment per genotype was extracted as previously described. For immunoprecipitations, M2 anti-Flag affinity gel (Sigma A2220-5ML) was used and incubated with the crude extract for 2-3 h at 4 °C. Subsequently, beads were washed 3 times with wash buffer (50 mM Tris-HCl pH 7.5, 150 mM NaCl, 1 mM phenylmethanesulfonyl fluoride, 0,1 % IGEPAL) before

adding Laemmli sample buffer and incubating for 10 min at 95 °C. Analysis was carried out by SDS-PAGE and immunoblotting.

Immunoblotting

Protein samples were separated in 10 % bisacrylamide gels at 150 V for approximately 2 h and transferred into activated PVDF membranes at 100 V for 90 min. Immunoblotting was performed with antibodies diluted in blocking solution (5 % fat-free milk in TBS with 0.1 % (v/v) Tween-20). Antibodies used in this study: α-BAK1 (1:5 000; (Roux et al., 2011); α-FLS2 (1:1000; (Chinchilla et al., 2007); α-FER (1:2000; (Xiao et al., 2019); α-BAK1 pS612 (1:3000; (Perraki et al., 2018));), α-FLAG-HRP (Sigma Aldrich, A8592, dilution 1:4000); α -GFP (sc-9996, Santa Cruz, used at 1:5000). Blots were developed with Pierce ECL/ ECL Femto Western Blotting Substrate (Thermo Scientific). The following secondary antibodies were used: anti-rabbit IgG-HRP Trueblot (Rockland, 18-8816-31, dilution 1:10000) for detection of FLS2-BAK1 co-immunoprecipitation or anti-rabbit IgG (whole molecule)–HRP (A0545, Sigma, dilution 1:10000) for all other western blots.

Synthetic peptides and chemicals

The flg22, elf18, RALF17 and RALF23 peptides were synthesized by EZBiolab (United States) with a purity of >95 %. All peptides were dissolved in sterile pure water.

Statistical analysis

Statistical analyses were carried out using Prism 6.0 software (GraphPad). As mentioned in the figure legend, statistical significances were assessed using non-parametric Kruskal-Wallis bilateral tests combined with post-hoc Dunn's multiple pairwise comparisons, or using a two-way student's t test.

Accession numbers

FER (AT3G51550), LRX3 (AT4G13340), LRX4 (AT2G23780), LRX5 (AT4G18670), RALF23 (AT3G16570), FLS2 (AT5G46330), BAK1 (AT4G33430).

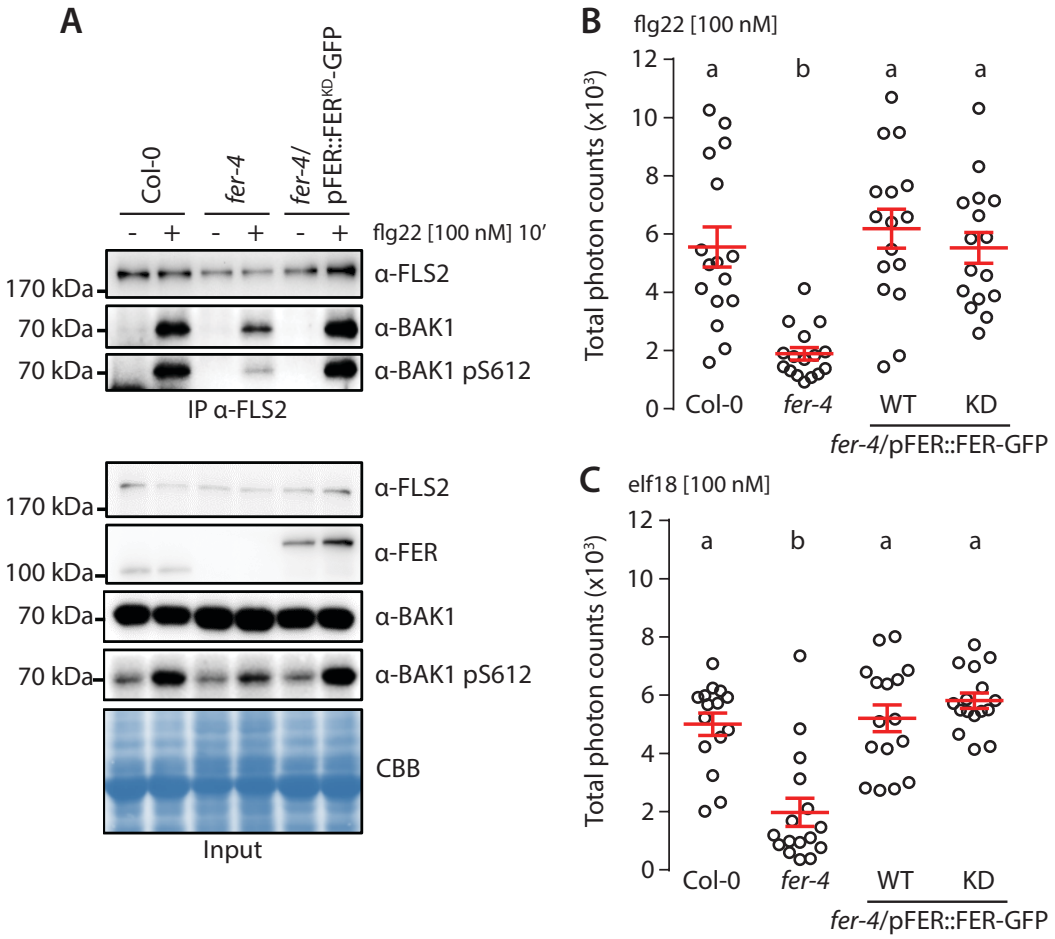

Fig. 1 ¦ FER kinase activity is dispensable to support PTI signaling.

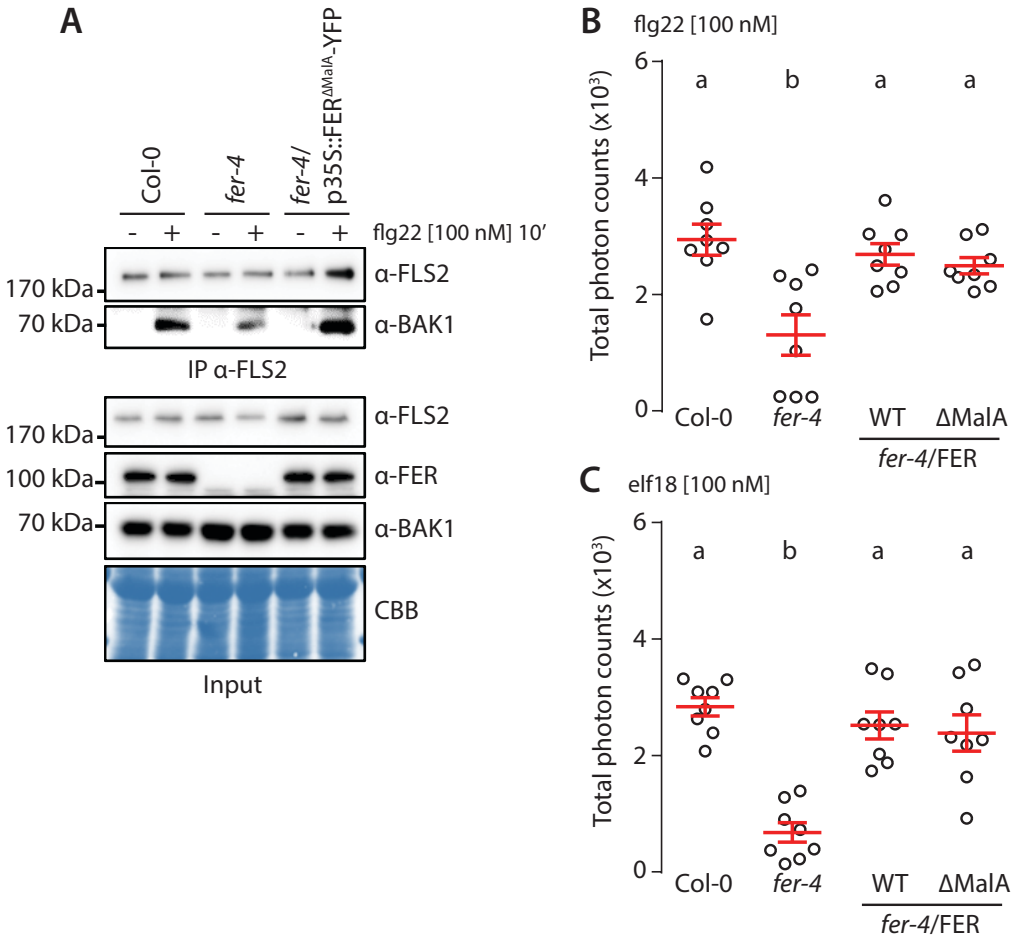

Fig. 2 ¦ FER Malectin A domain is dispensable to support PTI signaling.

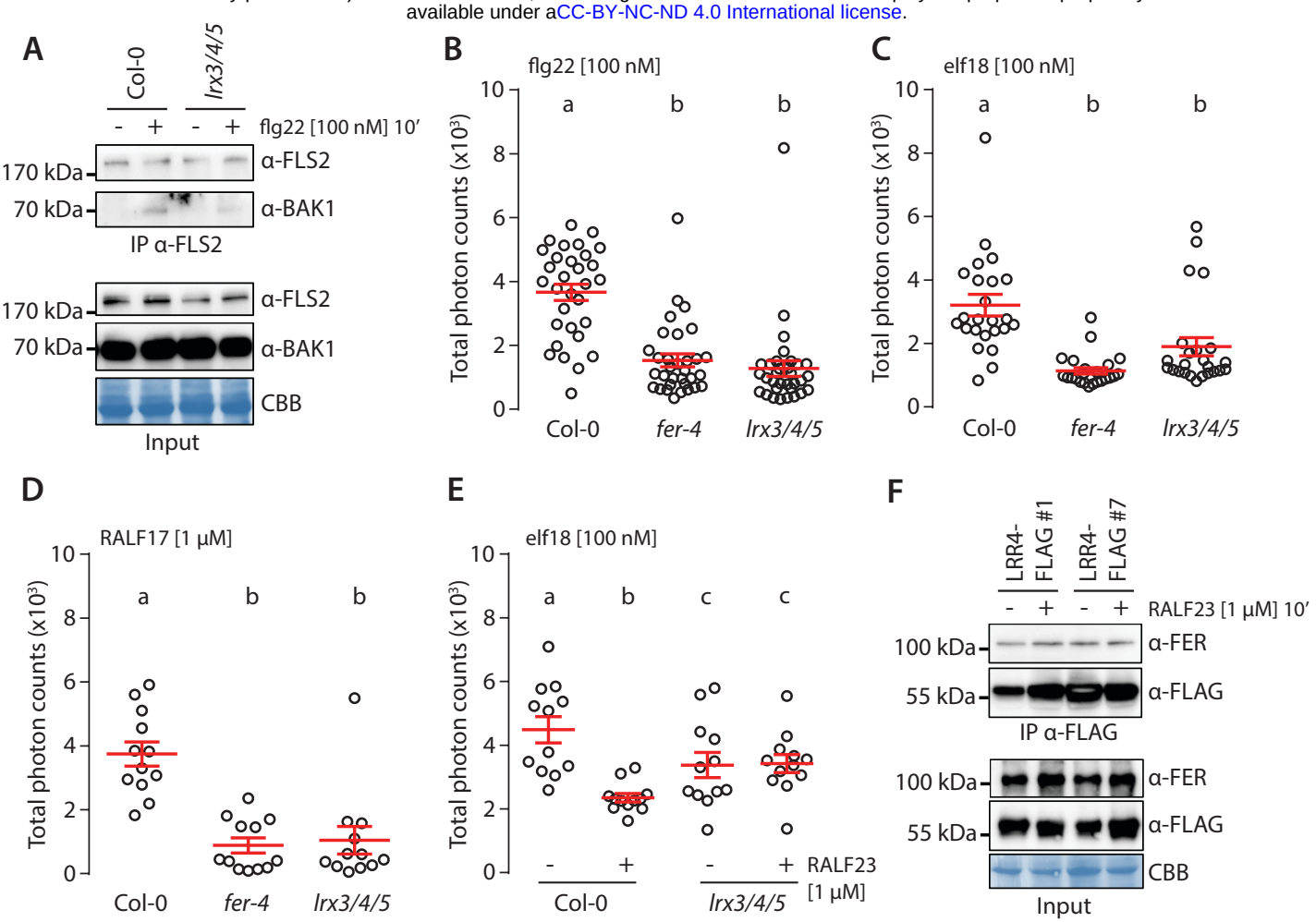

Fig. 3 ¦ LRX3, LRX4 and LRX5 are required for FER-mediated regulation of PTI signaling.

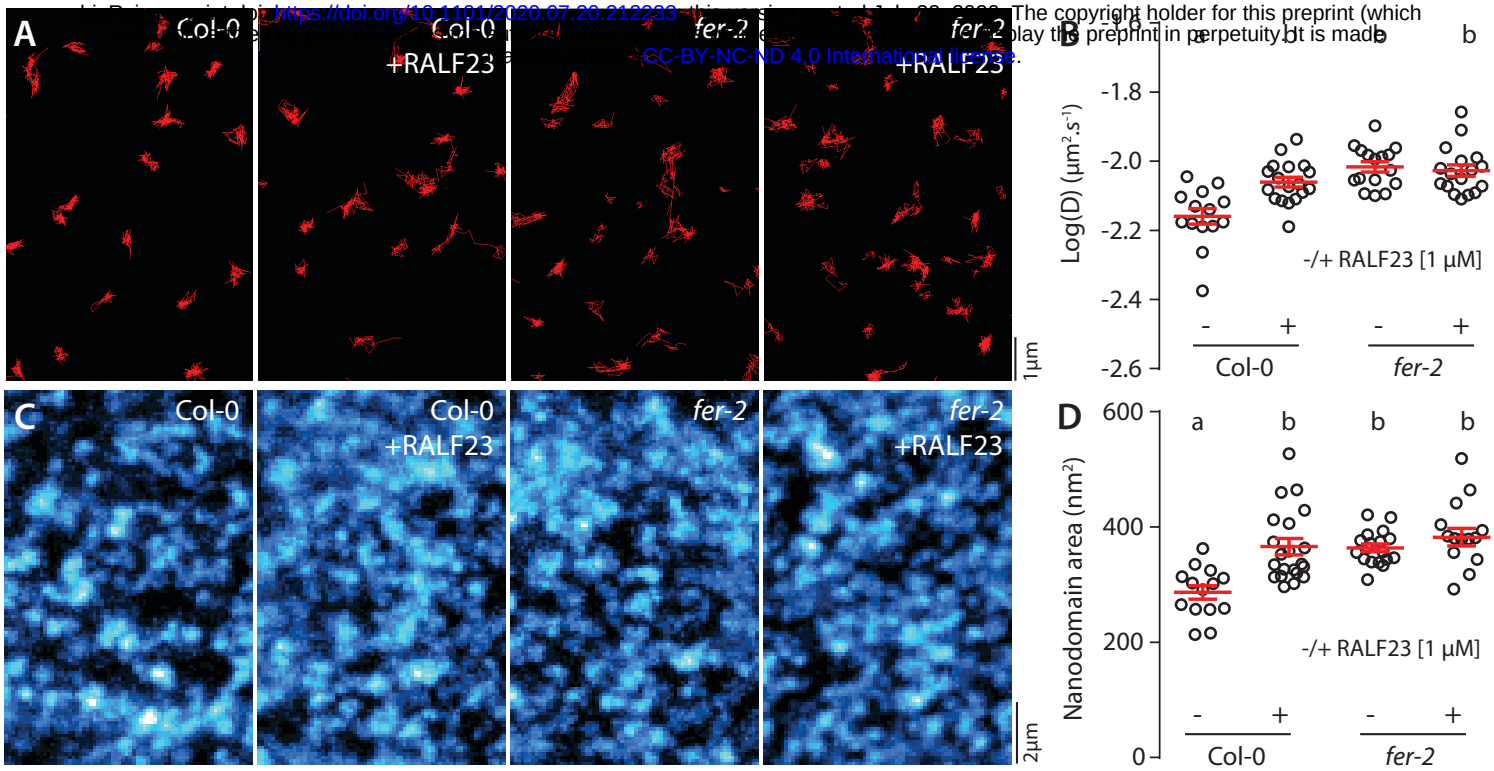

Fig. 4 ¦ FER regulates dynamics and size of FLS2-GFP nanodomains.

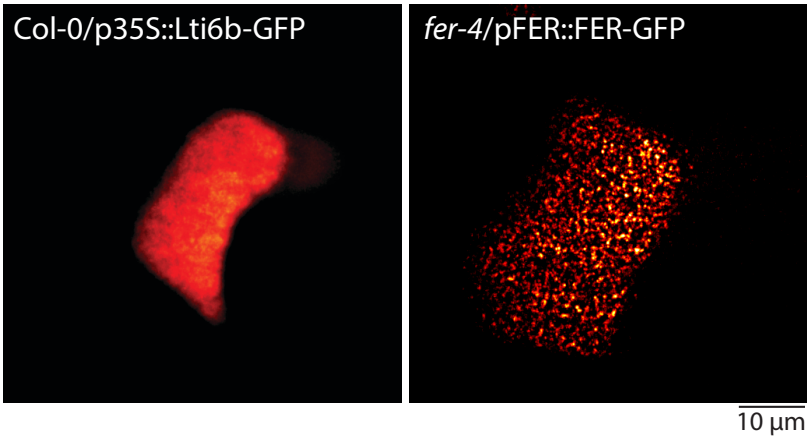

Fig. S1 ¦ FER-GFP localizes to nanodomain.

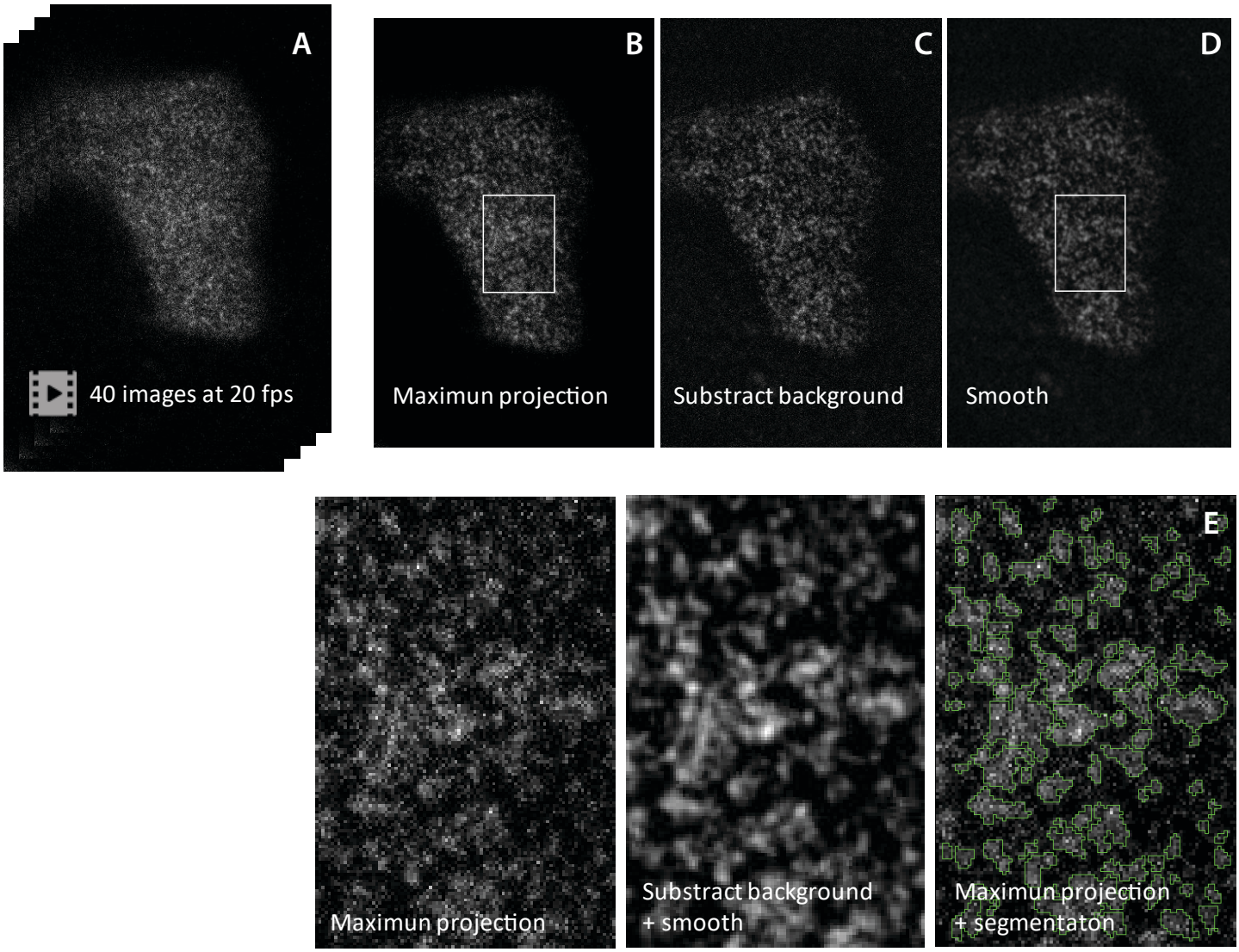

Fig. S2 ¦ Segmentation and quantification of FLS2-GFP nanodomains.

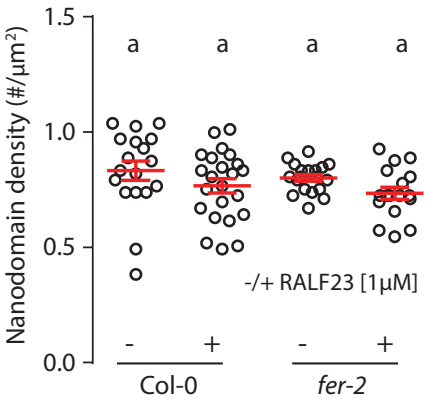

Fig. S3 ¦ FER does not regulate FLS2-GFP nanodomain density.

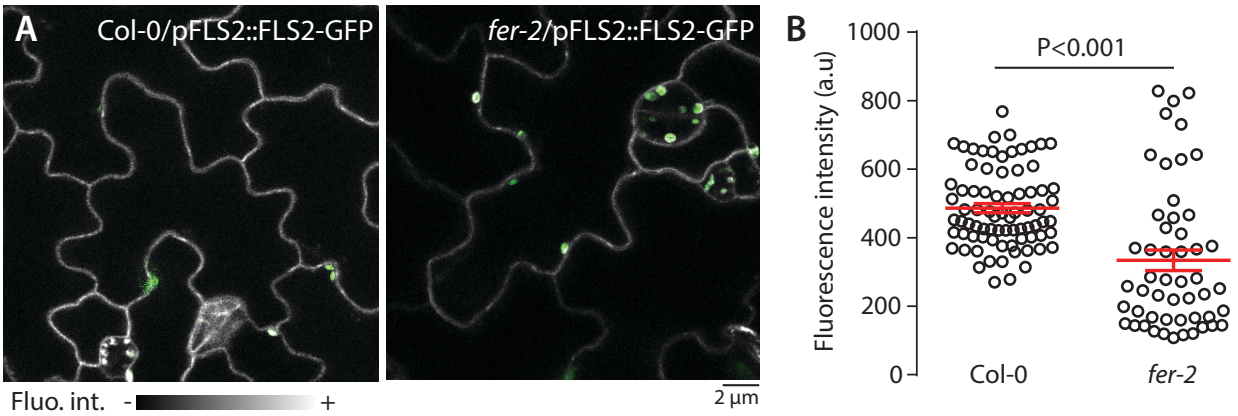

Fig. S4 ¦ FLS2-GFP accumulation at the PM is altered in *fer-2*.

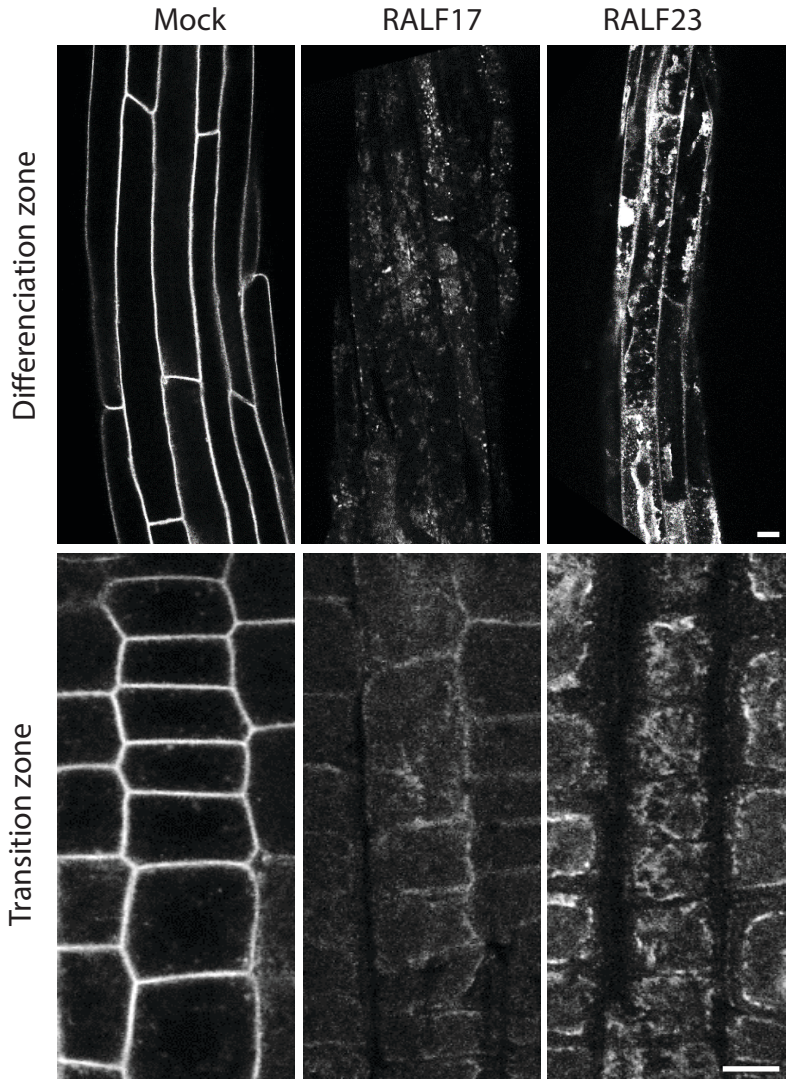

Fig. S5 ¦ RALF17 and RALF23 induces FER endocytosis.

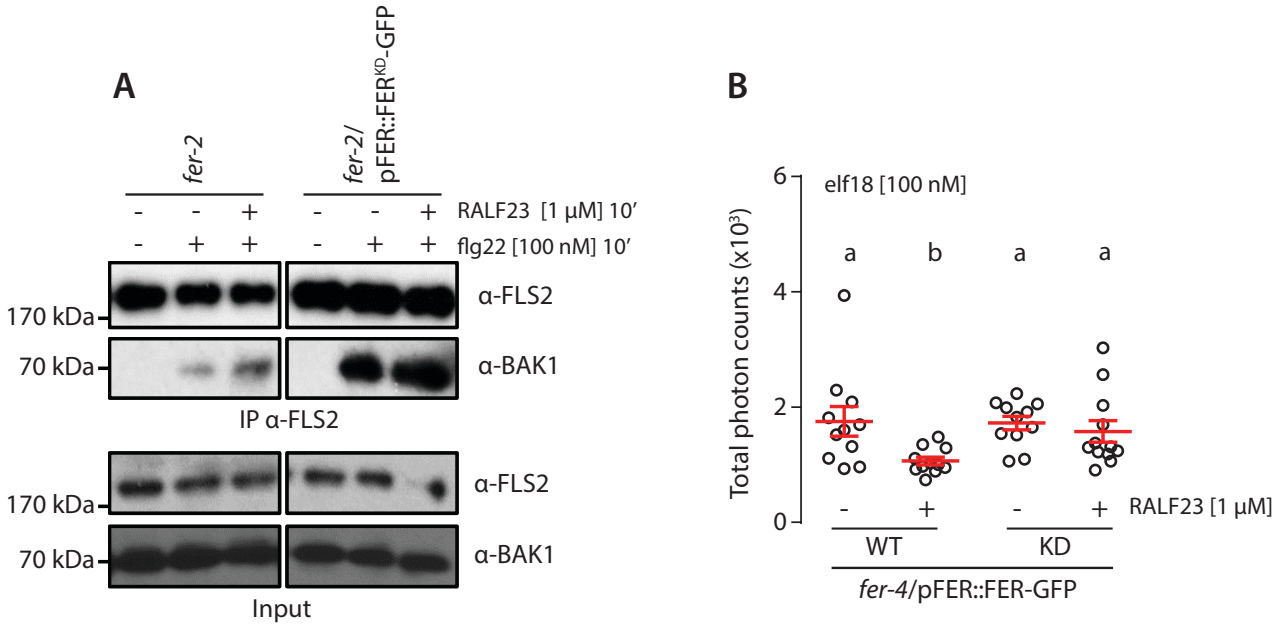

Fig. S6 ¦ Inhibition of PRR-signaling by RALF23 requires FER kinase activity.