

# 1 The receptor kinase SRF3 coordinates iron-level and flagellin dependent defense and 2 growth responses in plants

3  
4 **Matthieu P. Platre<sup>1</sup>, Santosh B. Satbhai<sup>1,2,3</sup>, Lukas Brent<sup>1</sup>, Matias F. Gleason<sup>1</sup>, Magali  
5 Grison<sup>4</sup>, Marie Glavier<sup>4</sup>, Ling Zhang<sup>1</sup>, Min Cao<sup>1</sup>, Christophe Gaillochet<sup>1</sup>, Christian Goeschl<sup>2</sup>,  
6 Marco Giovannetti<sup>2</sup>, Balaji Enugutti<sup>2</sup>, Marcel von Reth<sup>5</sup>, Ruben Alcázar<sup>5</sup>, Jane E. Parker<sup>5,6</sup>,  
7 Grégory Vert<sup>7</sup>, Emmanuelle Bayer<sup>4</sup>, Wolfgang Busch<sup>1,2\*</sup>**

8 <sup>1</sup> Salk Institute For Biological Studies, Plant Molecular And Cellular Biology Laboratory, 10010 N Torrey Pines Rd, La  
9 Jolla, CA 92037, USA.

10 <sup>2</sup> Gregor Mendel Institute (GMI), Austrian Academy of Sciences, Vienna Biocenter (VBC), Dr Bohr-Gasse 3, Vienna  
11 1030, Austria.

12 <sup>3</sup> Department of Biological Sciences, Indian Institute of Science Education and Research, Mohali, Sector 8, SAS Nagar,  
13 Punjab 14046, India

14 <sup>4</sup> University of Bordeaux, CNRS, Laboratoire de Biogenèse Membranaire, UMR 5200 CNRS, F-33140 Villenave  
15 d'Ornon, France

16 <sup>5</sup> Department of Plant-Microbe Interactions, Max Planck Institute for Plant Breeding Research, Cologne, Germany.

17 <sup>6</sup> Cologne-Düsseldorf Cluster of Excellence on Plant Sciences (CEPLAS), 50225 Düsseldorf, Germany

18 <sup>7</sup> Plant Science Research Laboratory (LRSV), UMR5546 CNRS/Université Toulouse 3, 24 chemin de Borde Rouge,  
19 31320 Auzerville-Tolosane, France.

20 \*Correspondence: [wbusch@salk.edu](mailto:wbusch@salk.edu)

## 21 Summary

22 Iron is critical for host-pathogen interactions. While pathogens seek to scavenge iron to spread,  
23 the host aims at decreasing iron availability to reduce pathogen virulence. Thus, iron sensing and  
24 homeostasis are of particular importance to prevent host infection and part of nutritional immunity.  
25 While the link between iron homeostasis and immunity pathways is well established in plants,  
26 how iron levels are sensed and integrated with immune response pathways remain unknown. We  
27 identified a receptor kinase, SRF3 coordinating root growth, iron homeostasis and immunity  
28 pathways via regulation of callose synthase activity. These processes are modulated by iron  
29 levels and rely on SRF3 extracellular and kinase domain which tune its accumulation and  
30 partitioning at the cell surface. Mimicking bacterial elicitation with the flagellin peptide flg22  
31 phenocopies SRF3 regulation upon low iron levels and subsequent SRF3-dependent  
32 responses. We propose that SRF3 is part of nutritional immunity responses involved in sensing  
33 external iron levels.

## 34 Introduction

35 Iron is a critical micronutrient for all living organisms. While iron is very abundant in the Earth's  
36 crust, its bioavailability is low. Organisms have evolved efficient iron uptake mechanisms that  
37 include a variety of membrane-associated uptake systems that absorb iron unbound or bound to  
38 iron-binding molecules. Mammals acquire iron mainly through the glycoprotein transferrin while  
39 bacteria, fungi and plants have evolved diverse systems that include siderophores, which are  
40 small, high-affinity diffusible secondary metabolites that chelate Fe<sup>3+</sup> from the surrounding  
41 environment (Kramer et al., 2020). In plants, Graminaceae species employ plant specific  
42 siderophores while non-Graminaceae such as *Arabidopsis thaliana* depend on an iron reduction-  
43 based uptake strategy (Kobayashi and Nishizawa, 2012).

44 During pathogen attack, iron is at the nexus of host-pathogen interaction as both organisms  
45 compete for this metal. Pathogens scavenge iron from the host through siderophore secretion  
46 while the host aims to sequester iron to prevent pathogen virulence. Thus, host external iron  
47 sensing and internal iron homeostasis regulation are of particular importance to prevent pathogen  
48

49 infection, and are part of the first line of defense called nutritional immunity (Cassat and Skaar,  
50 2013).

51 In mammals, two receptors, Transferrin Receptor 1 and 2 (TfR) which bind extracellular  
52 transferrin-associated iron, play a major role in regulating external iron sensing and homeostasis.  
53 Upon host-pathogen interaction, bacterial siderophores outcompete the host iron-bound to  
54 transferrin, which in turn leads to a loss of iron triggering independent local and systemic  
55 responses in the host (Ganz and Nemeth, 2015). Locally, the loss of iron induces TfR endocytosis  
56 and intracellular iron storage via ferritins. Systemically, TfR activation triggers stimulation of the  
57 BMPR complex to increase the expression of iron uptake genes (Ganz and Nemeth, 2015). The  
58 latter response is intertwined with defense pathway since the inflammatory Interleukine-6 pathway  
59 directly interacts with the BMPR complex to regulate iron uptake genes (Ganz and Nemeth, 2015).  
60 In *Drosophila melanogaster*, Transferrin-1 was recently shown to activate NF- $\kappa$ B, toll and  
61 immune deficiency immunity pathways, thereby mediating nutritional immunity through the  
62 control of intracellular iron partitioning (Iatsenko et al., 2020).

63 Although flowering plants do not contain TfR in their genomes (Bai et al., 2016), iron homeostasis  
64 and defense responses are linked (Verbon et al., 2017). Here, FERRETINS (FER) and NATURAL  
65 RESISTANCE-ASSOCIATED MACROPHAGE PROTEIN (NRAMPs) were shown to be involved  
66 in iron sequestration upon pathogen attack (Deák et al., 1999; Segond et al., 2009). Moreover,  
67 the metal transceptor IRON-REGULATED TRANSPORT 1 (IRT1) is critical to mount efficient  
68 defense responses (Aznar et al., 2014). Transcriptional signatures of *Pseudomonas simiae*  
69 WCS417 and long-term iron deficiency in leaves display an overlap of about 20%, among these  
70 genes, the transcription factor MYB DOMAIN PROTEIN 72 (MYB72) plays a role at the interface  
71 of both signaling pathways (Dinneny et al., 2008; Zamioudis et al., 2015). Recently, a protein  
72 effector from the foliar pathogen *Pseudomonas syringae* was shown to disable a key iron  
73 homeostasis regulator, the E3 ligase BRUTUS (BTS), to increase apoplastic iron content and  
74 promote colonization (Xing et al., 2021). Finally, the presence of the microbial siderophore,  
75 deferoxamine (DFO), affects the transcriptional landscape of iron homeostasis and immunity  
76 genes, suggesting a role for siderophores in mediating nutritional immunity (Aznar et al., 2014).  
77 While the link between iron deficiency and immunity is well documented in plants, the mechanism  
78 by which iron concentrations are sensed, and how they impinge on iron homeostasis, defense  
79 and growth pathways are unknown. Here, we identify the leucine-rich repeats receptor kinase  
80 STRUBBELIG RECEPTOR KINASE 3 (SRF3) through *Arabidopsis thaliana* natural root growth  
81 variation under low iron levels using a genome wide association study (GWAS). We find that root  
82 growth is rapidly reduced upon encountering low iron levels, that *SRF3* is required for this  
83 response, and at the same time modulates root iron homeostasis. The regulatory capacity of  
84 *SRF3* is dependent on its kinase and extracellular domains. Both domains are required for *SRF3*  
85 partitioning between the plasmodesmata and the so-called bulk PM where it acts as a negative  
86 regulator of callose synthases and is degraded upon low iron conditions in both sub-populations.  
87 We further establish that *SRF3* is a molecular link between responses to low external iron levels  
88 and bacterial defense responses, as *SRF3* is required to mediate root immune response to the  
89 flagellin peptide flg22 by the same mechanisms used for its response to low iron conditions. Our  
90 work uncovers a close coordination of responses to low iron levels and immunity pathways and  
91 indicates that *SRF3* is located at the nexus of both pathways, thereby constituting a key player in  
92 plant nutritional immunity.

## 93 94 **Results**

### 95 96 **SRF3 is a regulator of iron homeostasis genes and root growth under low iron levels**

97 Genome wide association studies (GWAS) for root growth rate under low iron levels revealed  
98 multiple significantly associated single nucleotide polymorphisms (SNPs) (Figure S1A-B and  
99 Spreadsheet S1). The most significant association was observed on chromosome 4 in close

100 proximity to the genes AT4G03390 (*STRUBBELIG-RECEPTOR FAMILY 3*, *SRF3*) and  
101 AT4G03400 (*DWARF IN LIGHT 2*, *DFL2*) (Figures 1A). To identify potential causal genes at this  
102 locus, we obtained Col-0 T-DNA mutant lines for these genes (Figures S1C-D) and quantified the  
103 root growth response after three days exposure to low iron levels. While the *df2* T-DNA mutant  
104 lines responded similarly to wildtype (WT) to low iron levels, *srf3* T-DNA lines displayed a  
105 significantly decreased root growth response compared to WT when exposed to different iron  
106 levels using the iron chelator Ferrozine (-FeFZ; 100, 50, 10 $\mu$ M; Figures 1B-C, S1E-G). Moreover,  
107 *srf3* mutants showed a slight reduction in their root growth rate in iron sufficient conditions  
108 compared to WT and responded similarly to WT to iron excess conditions (Figures 1C and S1G-  
109 H). Overall, our data show that *SRF3* is required for an appropriate root growth response to low  
110 iron levels.

111 To explore the function of *SRF3* on iron homeostasis genes, we performed RNAseq on roots from  
112 two independent *srf3* alleles and WT under iron sufficient growth conditions. Several key iron  
113 homeostasis regulators (*BTS*, *BHLH039*, *PYE*) and iron compartmentalization-related genes that  
114 are involved in iron transport to the vacuole (*ZIF1*) were upregulated in *srf3* mutants while a key  
115 iron distribution transporter involved in iron shoot-to-root partitioning was downregulated (*NAS4*;  
116 Figure 1D and Spreadsheet S2). Consistent with a mis-regulation of iron responsive genes, the  
117 transcriptional reporter line of the low iron inducible iron transporter *IRT1* (*pIRT1::NLS-2xYPet*) in  
118 *srf3-4* mutant showed a decreased activation after 24 hours under low iron (Figure 1E). In line  
119 with a misregulation of iron homeostasis, *srf3* mutants accumulate more iron compared to the  
120 WT, thereby phenocopying *bts-1* and *opt3-2*, two iron homeostasis mutants known to accumulate  
121 ectopic iron (Figures 1F and S2A-C; Hirayama, 2018; Mendoza-Cózatl et al., 2014; Selote et al.,  
122 2015). Importantly, the increased iron levels in *srf3-3* do not stem from increased iron content in  
123 the seeds since the iron localization was not altered in *srf3* mutant seeds compared to WT but  
124 different from *vit-1* embryos that are known for misdistribution of iron (Kim et al., 2006; Figure  
125 S2D). Taken together, these results indicate that *SRF3* is a post-embryonic regulator of iron  
126 homeostasis genes.

127 Next, we investigated the allelic variation at the *SRF3* locus and analyzed accessions according  
128 to the pattern of the four top marker polymorphisms associated with the growth response under  
129 low iron conditions. The four resulting groups of accessions were haplogroup A that grows slowly  
130 on low iron medium and the haplogroups B, C, D that grow faster (Figures S3A-C). While the  
131 haplogroup A and haplogroup B differed from several candidate polymorphisms including a larger  
132 deletion in the promoter region (Figure S3A), they do not show any significant differences in *SRF3*  
133 transcript level accumulation under low iron levels (Figure S3D-E). These results highlight that  
134 *SRF3* allelic variation does not lead to obvious changes in *SRF3* transcript levels in bulk root  
135 tissue, showing no correlation with the observed variation of root growth rates in low iron  
136 conditions. Taken together, our data show that *SRF3* is a negative root growth regulator under  
137 low iron levels and is involved in the post-embryonic regulation of iron homeostasis, a function  
138 which might be independent of its expression levels.

### 139 140 **The early growth response to low iron is dependent on SRF3 protein levels at the plasma** 141 **membrane**

142 *SRF3* encodes a gene belonging to the protein family of leucine-rich repeats receptor kinases  
143 (LRR-RKs) which are known to be involved in early signal transduction (Hohmann et al., 2017).  
144 We hypothesized that *SRF3* might mediate a novel, immediate root response to changes in  
145 external iron levels. Using live-light transmission microscopy for 12 hours, we found that low iron  
146 levels elicited a significant decrease of root growth after 4 hours and that this response was  
147 abolished in *srf3-3* (Figures 2A, S4A and Movie S1-2). The cause of this unresponsiveness in *srf3*  
148 is partly explained by a lack of cell elongation decrease upon low iron conditions (Figures S4B-  
149 F). To further investigate the role of *SRF3* in regulating root elongation under low iron, we  
150 monitored the response of *SRF3* transcription and *SRF3* protein abundance using transcriptional

151 and translational reporter lines. A SRF3-2xmCHERRY fusion construct driven by its own promoter  
152 fully complemented the *srf3-3* root growth defect under low iron levels, showing the functionality  
153 of the construct (Figure S1F and S4G). The transcriptional reporter line revealed that the *SRF3*  
154 promoter is active in the differentiation and elongation zones, and to a lesser extent in the  
155 transition zone (Figures 2B). Surprisingly, the SRF3 fluorescent protein fusion was detected  
156 mainly at the PM in the apical and basal meristem and to a lesser extent in the transition,  
157 elongation and differentiation zones (Figures 2B). We confirmed this finding in Landsberg *erecta*  
158 (Ler) WT background using a GFP tag fused to the respective full genomic fragment (Figure S4H-  
159 L). We reasoned that the SRF3 protein and/or transcript might be cell-to-cell mobile, or that the  
160 *SRF3* transcript might be expressed transiently in the meristematic cells. Analysis of numerous root  
161 tips showed that some roots expressed the *SRF3* transcriptional reporter in the meristematic  
162 zone, a finding backed up by single cell sequencing data, however, we could not exclude the  
163 alternative hypothesis (Figure S4M-P; Denyer et al., 2019). Overall, *SRF3* is constantly  
164 transcribed and translated in the transition-elongation zone and transiently or only in a subset of  
165 cells in the meristematic zone.

166 We next tested whether SRF3 protein abundance or *SRF3* transcription are altered in response  
167 to low iron conditions in the transition-elongation zone. While the signal intensity in the *SRF3*  
168 transcriptional reporter line did not differ between the two iron regimes, similarly to the control line  
169 H2B-mSCARLET, the fluorescent signal intensity in a reporter line in which SRF3 was driven by  
170 *UBIQUITIN10* promoter (*SRF3*<sup>WT</sup>) or its native promoter significantly decreased at the PM under  
171 low iron treatment compared to the Lti6b-GFP control line (Figures 2D and S5A-B). Time lapse  
172 analysis showed that a signal decrease was recorded after 50 minutes in *SRF3*<sup>WT</sup> but not in the  
173 other lines (Figures 2C, Movies S3, S4 and S5). We next set out to dissect the role of the functional  
174 domains of SRF3 for this process and generated a truncated version of SRF3 in which the  
175 extracellular domain had been removed (*SRF3*<sup>ΔExtraC</sup>) and a kinase dead version, containing a  
176 mis-sense mutation in a critical residue in the catalytic ATP binding pocket (*SRF3*<sup>KD</sup>, Figures S5C-  
177 D). While the functional SRF3 protein levels were decreased (*SRF3*<sup>WT</sup>) after two hours of  
178 exposure to low iron conditions, this was not observed for the *SRF3*<sup>ΔExtraC</sup> or *SRF3*<sup>KD</sup> lines (Figure  
179 2D). This shows that both, the extracellular cellular domain and kinase activity are required to  
180 mediate the decrease of SRF3 protein at the PM in response to low iron levels.

181 We then investigated whether SRF3 levels control early root growth rate under low iron conditions.  
182 Surprisingly, much like SRF3 loss of function, constitutive expression of SRF3 abolished the early  
183 root growth response to low iron levels (Figures 2E). However, we observed an opposite effect in  
184 *srf3* mutant and *SRF3*<sup>WT</sup> overexpressing plants during the late response to low iron (Figure S5E).  
185 Although this complex response is yet to be fully explained, we used this property to interrogate  
186 SRF3 domain functions. To do so, we investigated the early growth response of the  
187 overexpressing lines of *SRF3*<sup>ΔExtraC</sup> (*pUBQ10::SRF3*<sup>ΔExtraC</sup>-*mCITRINE*), *SRF3*<sup>KD</sup>  
188 (*pUBQ10::SRF3*<sup>KD</sup>-*mCITRINE*) to low iron conditions. For both early and late low iron growth  
189 responses, we observed that roots of *SRF3*<sup>ΔExtraC</sup> and *SRF3*<sup>KD</sup> presented a phenotype close to  
190 WT, while the *SRF3*<sup>WT</sup> version overexpressing line was hyposensitive or hypersensitive  
191 respectively (Figures 2E and S5E). Altogether, our results suggest that the root growth response  
192 to low iron conditions requires a fine regulation of SRF3 protein accumulation at the PM, which is  
193 dependent on the extracellular and kinase domains. These findings are consistent with a model  
194 in which SRF3 senses early apoplastic signals associated with iron depletion through its  
195 extracellular domain and transduces the signal(s) intracellularly to modulate root growth via its  
196 kinase activity.

197  
198 **SRF3 resides in two subpopulations at the plasma membrane which are both decreased**  
199 **under low iron conditions**

200 During the analysis of SRF3 expression, we had noticed its enrichment at the PM with a apical-  
201 basal localization in punctate foci but also along the entire PM, referred to as bulk PM (Figures



202 S6A and S6C). We tested the role of its extracellular domain and kinase activity for specifying its  
203 heterogenous distribution by calculating the standard deviation of the mean intensity (SDMI) along  
204 the apical-basal side of PM using SRF3 truncated and point mutant versions. We found that  
205 compared to the WT version, SRF3<sup>ΔExtraC</sup> only associates with the bulk PM since we observed a  
206 decrease of the SDMI (Figure 3A) while the removal of the kinase domain (SRF3<sup>ΔKinase</sup>) did not  
207 lead to SDMI changes (Figures S5C and S6B). This indicated that the extracellular domain is  
208 necessary and sufficient to drive SRF3 into the PM-associated foci. Surprisingly, the standard  
209 deviation of SRF3<sup>KD</sup> fluorescent signal was significantly lower compared to SRF3<sup>WT</sup> (Figures 3A),  
210 suggesting a role of the kinase activity in SRF3 partitioning. We then investigated the role of SRF3  
211 functional domains upon low iron levels. For SRF3<sup>WT</sup>, a decrease of SDMI and polarity upon  
212 exposure to low iron conditions were observed, indicating a loss of SRF3-associated punctate  
213 structures while such reduction was not observed in the control line, LTI6b-GFP (Figure 3A and  
214 S6C). Performing the same experiment with SRF3<sup>ΔExtraC</sup> and SRF3<sup>KD</sup> revealed no significant  
215 difference upon low iron levels compared to the control condition (Figure 3A). This points to a role  
216 of the extracellular domain and a requirement for an active SRF3 kinase in the removal of SRF3  
217 from the foci upon exposure to low iron levels. Taken together, our data show that SRF3 has a  
218 dual localization at the plasma membrane, in punctuated structures and the bulk PM that is  
219 controlled by the extracellular domain and the kinase activity. Finally, upon exposure to low iron  
220 levels, SRF3 seems to become less associated with the punctuated foci which also relies on its  
221 functional domains.

222 We then addressed the nature of the PM-associated punctuated structures. Analysis of the  
223 intensity distribution profile at the PM apical-basal sides of SRF3 fluorescent reporter in the  
224 background of PM structure marker lines revealed a specific co-localization of SRF3 with  
225 plasmodesmata-associated proteins CALS3 and PDLP3 but not the general PM marker Lti6b  
226 (Figure 3B). Moreover, SRF3 co-localized with signals from Aniline blue staining that stains β-1,3-  
227 glucan, which are particularly enriched in plasmodesmata (Figure S6D). This strongly indicated  
228 that SRF3 localizes or is in close vicinity to the plasmodesma. To characterize and confirm SRF3  
229 subcellular dynamics at higher resolution, we conducted immunogold labeling electron  
230 microscopy of the *pSRF3::SRF3-GFP* line using an anti-GFP antibody. In standard conditions,  
231 SRF3 signal was localized at the bulk PM and to the plasmodesmata and more specifically to the  
232 plasmodesmatal neck region, and was removed not only from plasmodesmata but also the bulk  
233 PM under low iron conditions (Figure 3C). As a recent report had shown that some  
234 plasmodesmata-associated receptor kinases have a fast and reversible association between bulk  
235 PM and plasmodesmata under abiotic stress, which alters their diffusion rates within the PM  
236 (Grison et al., 2019), we estimated SRF3 diffusion via fluorescence recovery after photobleaching  
237 (FRAP). We found that a decrease of iron levels did not change SRF3 diffusion (Figure S6E),  
238 indicating that the decrease of SRF3 is not accompanied by a change in its partitioning. Taken  
239 together, our data indicate that SRF3 is associated with the bulk PM but also highly enriched at  
240 the neck of the plasmodesmata, in an extracellular domain- and kinase activity-dependent  
241 manner. Under low iron, SRF3 becomes depleted from these two subpopulations, a process  
242 which is dependent on both SRF3 functional domains.

### 243 244 **Early lack of iron mediates SRF3-dependent callose deposition without affecting cell-to-** 245 **cell movement**

246 Immunogold-labeling electron microscopy suggested that SRF3 is particularly concentrated at the  
247 plasmodesmata neck. This region is highly enriched in sterols, which are required for protein  
248 targeting to this specific subregion to regulate plasmodesmata function (Grison et al., 2015).  
249 Depleting plants expressing SRF3<sup>WT</sup> of sterols using sterol inhibitors, Fenpropimorph (Fen) and  
250 Lovastatin (Lova), showed that SRF3 localization is sterols-dependent since a decrease of SRF3  
251 polarity was observed (Figures S7A), suggesting that SRF3 might have a functional role in this  
252 plasmodesmata region. The plasmodesmatal neck is critical for regulating cell-to-cell trafficking,

253 as it is where callose turnover is thought to be regulated to determine plasmodesmata  
254 permeability (Sager and Lee, 2018). Iron homeostasis depends on long- and local-distance  
255 signaling relying on cell-to-cell movement to activate *IRT1* (Durrett et al., 2007; García et al.,  
256 2013; Grillet et al., 2018; Khan et al., 2018; Kumar et al., 2017; Vert et al., 2003). We therefore  
257 hypothesized that SRF3 might regulate cell-to-cell communication through callose turnover to  
258 properly activate *IRT1*. Analysis of signals from immunostaining with a callose antibody indicated  
259 that low iron levels trigger callose deposition in the epidermis and cortex cells of WT root tips  
260 (Figure 4A). This shows that iron levels influence callose deposition. In *srf3* mutants, we observed  
261 an increase of callose even in the basal condition while callose levels were not responsive to iron  
262 depleted media compared to WT (Figure 4A). Our data therefore show that early responses to  
263 low iron include an increased callose deposition and that SRF3 negatively regulates this process.  
264 To corroborate this finding, we used aniline blue to quantify the signal in the epidermis of the root  
265 transition-elongation zone. The positive control, a *CALLOSE SYNTHASE 3 (CALS3)*  
266 overexpressing line, which is known to accumulate ectopic callose showed higher signal intensity  
267 compared to WT. In agreement with the antibody based findings, low iron rapidly enhanced  
268 callose deposition in WT, however, increased callose was not observed in WT when adding the  
269 2-deoxy-d-glucose (DDG), a well-characterized callose synthase inhibitor (Figures 4B and S7B;  
270 Han et al., 2014; Huang et al., 2019; Jaffe and Leopold, 1984; Shikanai et al., 2020; Vatén et al.,  
271 2011). In *srf3* mutants, no difference in aniline blue signal intensity was observed under iron  
272 sufficient conditions while an increase was observed under low iron compared to WT in the same  
273 condition (Figure 4B). Although callose immunostaining and aniline blue slightly differed, both  
274 experiments suggest that callose is synthesized by callose synthases shortly after exposure to  
275 iron deficiency in an SRF3-dependent manner. Consistent with this conclusion, *srf3-2* and *srf3-3*  
276 displayed fused LR and a higher LR density than WT, both of which are traits associated with  
277 higher callose deposition (Benitez-Alfonso et al., 2013; Figures S7C-D).  
278 We next investigated whether callose deposition upon low iron levels modifies cell-to-cell protein  
279 movement in a SRF3-dependent manner. We first monitored the ability of GFP expressed in  
280 companion cells using *pSUC2::GFP* to diffuse to the surrounding cells through the  
281 plasmodesmata, as previously established (Benitez-Alfonso et al., 2013; Nicolas et al., 2017;  
282 Vatén et al., 2011a). Surprisingly, no difference in the GFP signal distribution between WT and  
283 *srf3-3* root tips from plants grown on iron sufficient and low iron containing media was observed  
284 (Figure 4C). To corroborate this observation, we photoactivated DRONPA-s fluorescent protein  
285 in a single root epidermis cell and monitored its spread to the upper and lower surrounding cells  
286 (Gerlitz et al., 2018). We noticed a decrease of signal in the activated cell and a concomitant  
287 increase in the surrounding cells, resulting from cell-to-cell movement. However and consistent  
288 with our *pSUC2::GFP* observations, no difference between conditions and/or genotypes was  
289 observed (Figure 4D). Altogether, our results suggest that a decrease of iron levels swiftly leads  
290 to SRF3- and callose synthase-dependent modulation of callose deposition. However, this does  
291 not generally impede cell-to-cell movement.

## 292 293 **Iron homeostasis and root growth are steered by SRF3-dependent callose synthases** 294 **signaling**

295 While *IRT1* activation is dependent on *SRF3*, this appears not to rely on a restriction of cell-to-  
296 cell movement via callose synthases-mediated callose deposition during the early responses to  
297 low iron conditions. We therefore reasoned that *IRT1* regulation might rely on early signaling  
298 events that are dependent on callose synthases, or that *IRT1* regulation only occurs at a later  
299 stage of the response. We first tested whether *IRT1* is regulated at the time during which SRF3-  
300 dependent callose deposition occurs. A 16-hour time lapse analysis of *IRT1* promoter activity  
301 indicated that the *IRT1* promoter becomes active during the first hours of low iron conditions while  
302 no or little activity was observed in iron sufficient media (Figures 5A, Movie S6 and S7). In *srf3-4*

303 mutant roots, we observed a lower expression of the *IRT1* reporter line upon low iron conditions  
304 compared to WT, indicating that early *IRT1* transcriptional activation depends on SRF3 (Figure  
305 5B). Next, we tested whether callose synthases activity was important to activate *IRT1*  
306 transcription by inhibiting callose synthases with DDG. The addition of DDG in low iron conditions  
307 strongly reduced *IRT1* promoter activation in WT, which was not observed in the *srf3-4* mutant  
308 compared to mock conditions (Figure 5B). All together, these observations indicate that *SRF3*  
309 likely acts upstream of callose synthases-mediated signaling to ultimately tune the expression of  
310 the major root iron transporter IRT1.

311 We next investigated the regulatory interaction of SRF3 and callose synthases by colocalization  
312 analysis in roots using dual-color total internal reflection fluorescence (TIRF) revealing that SRF3  
313 was organized in microdomains that partially colocalized with CALS3 but not with the  $\beta$ -1,3-  
314 glucanases reporter PdBG1 known to negatively regulate callose deposition (Figure 5C). To test  
315 these interactions genetically, we crossed *SRF3-OX*, which does not present any root growth  
316 defects, with a mutated version of CALS3 (*cals3-3d*) whose activity is up to 50% higher and  
317 subsequently accumulates more callose, resulting in shorter roots than in WT (Vatén et al.,  
318 2011a). The double homozygous lines of *SRF3-OXxcal3-3d* showed a further decrease of root  
319 growth compared to the *cals3-3d* single mutant (Figures 5D and S7E), indicating a genetic  
320 interaction of SRF3 and CALS3. To test whether the observed phenotype was due to a specific  
321 genetic interaction or a more general interaction of increased callose levels and SRF3  
322 overexpression, we used the *35s::GFP-PDLP5 (PDLP5-OX)* line, which presents similar callose  
323 and root growth phenotypes as observed in *cals3-3d* (Lee et al., 2011a; Sager et al., 2020). In  
324 this *SRF3-OXxPDLP5-OX* line, the root growth phenotype was indistinguishable from the *PDLP5-*  
325 *OX* line, therefore highlighting the specific genetic interaction of *SRF3* and *CALS3* (Figures 5D  
326 and S7E). Next, we set out to further test whether *SRF3* is acting upstream or downstream of  
327 CALSs. We reasoned that if CALSs were upstream of SRF3, the inhibition of callose synthase  
328 activity would impact SRF3 PM levels. Co-treatment of low iron with DDG did not modify PM-  
329 associated SRF3 levels and therefore suggested that callose synthase is downstream of SRF3  
330 (Figure S7F). This finding was corroborated by monitoring the early and late root growth rate of  
331 WT and *srf3-3* during the application of DDG and low iron levels as a partial complementation of  
332 *srf3-3* root growth phenotype was observed in that condition (Figures 5E and S7G). Overall, our  
333 data suggest that SRF3 acts upstream of the callose synthase early-on upon low iron levels to  
334 regulate iron homeostasis and root growth.

335

### 336 **SRF3 coordinates iron homeostasis and bacteria elicited immune responses**

337 *SRF3* was originally identified as a genetic locus underlying immune-related hybrid incompatibility  
338 in *Arabidopsis* and shown to be involved in bacterial defense-related pathways in leaves (Alcazar  
339 et al., 2010). Gene ontology (GO) analysis of root RNAseq data in standard condition and the  
340 analysis of the root specific *pCYP71A12::GUS* immune reporter upon treatment with the bacterial  
341 elicitor flg22 showed that SRF3 has a similar role in roots (Figure 6A and S8A; Millet et al., 2010).  
342 We then investigated the specificity of SRF3's role by assessing the late root growth responses  
343 to different pathogen-associated molecular patterns (PAMPs) and plant-derived damage-  
344 associated molecular patterns (DAMPs). *srf3* roots were only impaired in their response to flg22  
345 but not to chitin or AtPep1 compared to WT (Figures S8B-C). Similar to the low iron level  
346 response, the flg22 response was already apparent early-on in WT and absent in *srf3* mutants  
347 (Figures 6B, S8D-E and Movies S8, S9). To test whether the increased iron content of *srf3* might  
348 be related to this response, we analyzed the early and late root growth responses to flg22 of *bts-*  
349 *1* and *opt3-2*. They both responded like WT, indicating that higher iron root content does not  
350 generally affect the root growth regulation upon immune response elicitation (Figures S8F-H;  
351 Mendoza-Cózatl et al., 2014; Selote et al., 2015). We therefore concluded that the role of *SRF3*  
352 in controlling early root growth upon bacterial elicitation is specific and related to its signaling  
353 activity.

354 Because of the similar growth response to low iron levels and flg22 treatment, we hypothesized  
355 that the SRF3-dependent root growth regulation to these two stresses might rely on the similar  
356 molecular mechanism. Consistent with this idea, we found that upon flg22 treatment, the SRF3  
357 protein displays similar cellular dynamics as observed under low iron conditions (Figure 6C and  
358 S8I) while no significant changes of SRF3 transcriptional regulation were observed (Figure S8J).  
359 Therefore, SRF3 appears to be a point of convergence between iron and flg22-dependent  
360 signaling mediating root growth regulation.

361 We reasoned that one model explaining this convergence is that flg22 might trigger a transient  
362 decrease of cellular iron levels, thus promoting SRF3 degradation. We therefore performed  
363 RhoNox-1 staining after 1 hour of flg22 treatment and observed a decrease of fluorescence  
364 compared to mock treatment (Figure 6D). This indicates a swift decrease of local iron  
365 concentration in roots upon flg22 stimulus. Consistent with a rapid decrease of cellular iron, flg22  
366 treatment rapidly enhanced *IRT1* expression (Figure 6E, S9A and Movie S10). Moreover, mining  
367 of publicly available root RNAseq data revealed a broad impact of short-term flg22 treatment on  
368 the expression of iron homeostasis genes (Spreadsheet S3; Stringlis et al., 2018). We then  
369 wondered whether the flg22-triggered iron deficiency responses rely on SRF3-dependent callose  
370 synthase activity. Co-treatment of WT roots with DDG and flg22 led to a decrease of *IRT1*  
371 promoter activity compared to flg22 treatment alone, while in *srf3-4* this activation was decreased  
372 under flg22 compared to WT and insensitive upon co-treatment (Figure 6E). Overall, these data  
373 indicate that flg22-dependent *IRT1* activation relies on SRF3-mediated callose synthase signaling  
374 as observed for low iron conditions.

375 Finally, to investigate the extent of *SRF3*-dependent coordination of bacterial immune responses  
376 and iron homeostasis, we performed an RNAseq analysis after two hours of exposure to low iron  
377 levels or flg22 in *srf3* mutant and WT roots. Strikingly, 90% of the differentially expressed genes  
378 (DEGs) in these two conditions overlapped and were up or down regulated in the same manner  
379 in WT. Importantly, these DEGs were not associated with a general stress response since none  
380 of these common iron and flg22 DEGs were overlapping with those in cold, NaCl and mannitol  
381 datasets (Figure 6F, S9B and Spreadsheet S4 and S5; Kreps et al., 2002). To further confirm that  
382 low iron levels trigger immunity genes, we conducted qPCR for two early markers of flg22-  
383 triggered immunity, *FRK1* and *MYB51* that showed a transient activation of the two genes within  
384 four hours (Figure S9C; He et al., 2006). To determine how much of this common transcriptional  
385 program is coordinated by SRF3, we analyzed the *srf3* transcriptome datasets. DEGs in flg22 and  
386 iron deficiency in *srf3* mutant roots only overlapped by 24% demonstrating that *SRF3* coordinates  
387 a large part of the common transcriptional program that is triggered in response to early response  
388 to low iron and to flg22 (Figure 6F). Overall, our work establishes SRF3 as a major coordinator of  
389 bacterial immune response and iron deficiency signaling pathways which relies on callose  
390 synthase signaling.

## 391 392 **Discussion**

393 Based on a GWAS approach, we have identified an LRR-RK, *SRF3* as a regulator of early root  
394 growth responses to low iron conditions. We show that SRF3 transduces signals that lead to a  
395 coordinated response of root growth regulation, iron homeostasis and bacterial immunity through  
396 its modulation of callose synthase-dependent signaling. Because this is highly reminiscent of  
397 nutritional immunity conferred by the TfR mammalian and *Drosophila* systems that sense iron  
398 levels and control iron and immune responses, we propose that *SRF3* is instrumental in mediating  
399 plant nutritional immunity (Cassat and Skaar, 2013; Iatsenko et al., 2020).

400  
401 **The root responses to low iron are triggered rapidly and mediated by SRF3 signal**  
402 **transduction**



403 We discovered that root growth is modulated within the first four hours upon exposure to low iron  
404 levels looking at earlier time points than usually considered (Figure 2A; Durrett et al., 2007; Hindt  
405 et al., 2017; Mendoza-Cózatl et al., 2014; Satbhai et al., 2017)). This early response is SRF3-  
406 dependent, exposing this LRR-RK as being a key part of the genetically encoded ability of roots  
407 to perceive and transduce low environmental iron levels. A comprehensive SRF3 domain  
408 characterization showed that the LRR and the kinase activity are critical not only for its  
409 organization at the PM but also to mediate SRF3 decrease-dependent root growth arrest under  
410 low iron (Figure 2). In light of other LRR-RK signalling transduction mechanisms, such as those  
411 for BRI1 and FLS2 (Belkhadir and Jaillais, 2015; Hohmann et al., 2017; Jaillais and Vert, 2016;  
412 Tang et al., 2017), our results lead towards the following model for SRF3 1) the LRR extracellular  
413 domains senses a signal that is informative of the early lack of iron, 2) which in turn activates the  
414 kinase activity 3) which then triggers decrease of its level at the PM, 4) to regulate early root  
415 growth. We also found that the role of SRF3 in transducing low iron levels at an early stage is not  
416 restricted to the root growth regulation according to the RNAseq analysis (Figure 1E, 5B and 6).  
417 However, we did not provide direct evidence of the involvement of SRF3 kinase activity and LRR  
418 in transducing signals to regulate iron homeostasis and bacterial immune pathways. However,  
419 this is very likely since SRF3 is known to be part of the phosphorelay upon PAMP immune  
420 response (Benschop et al., 2007). Much of the *SRF3* function is tied to the SRF3 signal  
421 transduction because no obvious changes in SRF3 transcriptional level in *flg22* or low iron  
422 treatments were observed (Figures S5A and S8J), and no correlation between the expression  
423 level of *SRF3* in accessions that displayed contrasting root growth responses to low iron levels  
424 were observed (Figure S3E). Yet, an early or cell-type specific *SRF3* transcriptional regulation  
425 cannot be excluded. Altogether, our data indicate that roots perceive external variation of iron  
426 rapidly through SRF3-dependent signal transduction to coordinate root signaling pathways.  
427

#### 428 **Early responses to low iron are mediated by SRF3-dependent callose synthases regulation**

429 We have found that SRF3 acts upstream of iron-induced callose synthases activity to mediate  
430 proper signaling. Aniline blue and immunostaining showed that the early low iron response goes  
431 along with callose synthase-dependent callose deposition. Even though, these two techniques  
432 indicated conflicting results for callose deposition levels in the basal condition in WT and *srf3-3*,  
433 which might be explained by technical reasons, both approaches pinpointed SRF3 acts as  
434 negative regulator of this process (Figures 4A and 4B). The role of SRF3 acts as an upstream  
435 negative regulator of callose synthases is further strengthened by several lines of evidence: SRF3  
436 and CALS3 colocalize in both SRF3 PM subpopulations (Figure 3B and 5C), genetically act in the  
437 same pathway and the root growth response to low iron levels of *srf3* mutants is partially  
438 complemented upon inhibition of callose synthases (Figure 5B-E). Surprisingly, cell-to-cell  
439 movement of proteins were not affected early-on upon low iron levels, despite callose synthases  
440 activation and increased callose deposition in the plasmodesmata (Figure 4C-D). However, it is  
441 possible that callose deposition might impact later responses since callose deposition-mediated  
442 plasmodesmata closure can take hours to days to occur (Cheval et al., 2020; Lee et al., 2011a;  
443 Lim et al., 2016; Rutschow et al., 2011; Stonebloom et al., 2012). Another possibility is that callose  
444 deposition might have a different function early-on, for instance early ROS signaling which is  
445 thought to mediate callose deposition, actually increases cell-to-cell communication in leaves  
446 (Fichman et al., 2021). Thus, even though root growth, iron homeostasis and defense signaling  
447 can be controlled by cell-to-cell movement of signaling molecules movement, SRF3 dependent  
448 regulation of these pathways doesn't rely on impeding cell-to-cell movement thereby putting the  
449 spotlight onto a signaling function of callose synthases. In line with this idea, the double mutant  
450 *SRF3-OX/cals3-3d* displayed shorter roots compared to *cals3-3d* which should in fact show longer  
451 roots if SRF3 was strictly restricted to its repressive role on callose deposition (Figures 5D). Taken  
452 together, we have found that SRF3-dependent callose synthase activity is required to regulate  
453 early root growth, iron homeostasis and defense signaling pathways under low iron levels, which

454 might dependent directly on callose synthase-mediated signaling rather than impeding cell-to-cell  
455 movement.

456

### 457 **SRF3 mediates early root responses to low iron levels and a bacterial PAMP**

458 We have found that *SRF3*-mediated signaling is at the nexus of the early root responses to low  
459 iron and bacterial-derived signal. In fact, RNAseq analysis revealed that early responses to low  
460 iron and flg22 are highly similar and largely coordinated by *SRF3* (Figure 6F). The axis of *SRF3*  
461 and callose synthases is of particular importance for the regulation iron homeostasis genes in  
462 both conditions as revealed by monitoring *IRT1* promoter activity (Figure 5B and 6E). The local  
463 and swift decrease of iron in the root upon flg22 treatment might be the mechanism that underlies  
464 the flg22 dependent activation of iron homeostasis genes (Figure 6D-C). Conversely, the early  
465 lack of iron is able to activate the PTI signaling pathways, which is also mediated by *SRF3*  
466 (Figures 6F and S9C). This activation of PTI signaling upon low iron levels is likely due to *SRF3*'s  
467 role to modulate iron homeostasis which is important to coordinate immune responses (Figures  
468 1D-E and 5B Spreadsheet S2; Palmer et al., 2013; Verbon et al., 2017). However, there is an  
469 alternative model that cannot be excluded. In this model *SRF3* regulates flg22-mediated PTI  
470 signaling pathways, which in turn modulates iron homeostasis. This model is in line with the  
471 specific *SRF3*-dependent root growth regulation under flg22, the RNAseq data from *srf3* mutant  
472 roots in which PTI-dependent genes are misregulated (Spreadheet S2, S4 and Figure S9D) and  
473 experimental data provided in Smakowska-Luzan et al., 2018, based on the extracellular network.  
474 Further supporting this hypothesis, we found that the early and late root growth responses of *fls2*  
475 mutants, which are impaired in PTI-triggering immunity are decreased under low iron levels  
476 (Figure S9E-H).

477 Altogether, our observations lead to a model in which *SRF3* perceives an early lack of iron to  
478 modulate iron homeostasis and PTI signaling pathways, however it remains to be investigated  
479 which pathway is upstream of the other.

480

### 481 **The interaction of low iron levels and pathogens**

482 During host-pathogen interactions, an early host line of defense is to withhold iron to limit  
483 pathogen virulence, which is part of the nutritional immune responses as previously reported in  
484 vertebrates and invertebrates (Ganz and Nemeth, 2015; Iatsenko et al., 2020). Eliciting bacterial  
485 immune responses triggers a *SRF3*-dependent decrease of cellular iron levels, showing a  
486 conserved principle of this nutritional immune response being present in plants (Figures 1F and  
487 6D). In line with this idea, we have found that the lack of *SRF3* impedes mechanisms relating to  
488 the ability of root tissues to withhold iron. For instance, *ZIF1* that is involved in iron storage in the  
489 vacuole is upregulated in *srf3* mutants, while *NAS4* that is involved in root-to-shoot iron transport  
490 is downregulated (Figure 1D; Spreadsheet S2; Haydon et al., 2012; Klatte et al., 2009). In parallel,  
491 *NAS4* modulates ferritin accumulation, which is another way for the plant to withhold iron (Koen  
492 et al., 2013). Moreover, similar to the nutritional immunity systems described in mammals and  
493 *Drosophila melanogaster* that are based on TfR, *SRF3* senses the immediate lack of iron which  
494 is also relayed to a common signaling pathway linking iron deficiency and immunity responses  
495 (e.g. BMPR; Figure 6F; Cassat and Skaar, 2013). Altogether, we therefore propose that *SRF3* is  
496 a central player in a mechanism that embodies a fundamental principle of nutritional immunity by  
497 coordinating bacterial immunity and iron signaling pathways via sensing iron levels.

498

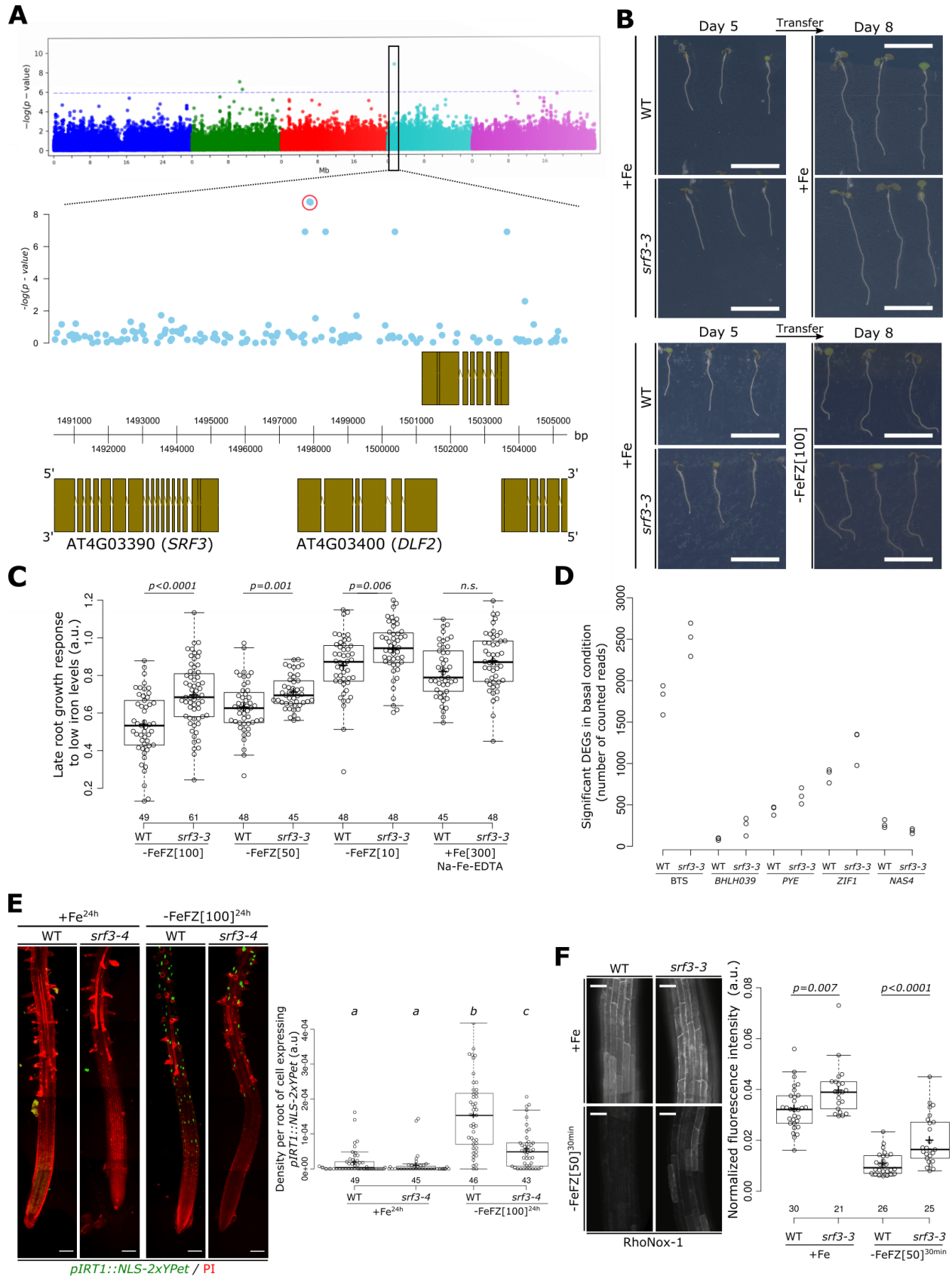
499

500 **Acknowledgements:** We thank Y. Belkhadir, B. Lacombe, I. Helariutta and all the Busch lab  
501 members for critical discussions. Y. Jaillais for sharing cloning materials, F. Berger for providing  
502 H2B in PDONR P1P2, J.B.D Long for providing SV40, Y. Benitez-Alfonso for providing  
503 35s:PdBG1-mCITRINE line, I. Helariutta for providing feedback on the manuscript and sharing

504 35s::GFP-CALS3 and cal3-3d as well as R. Stadler for providing 35s::DRONPA-s line. J.Y. Lee  
505 for providing 35s::PDLP5-GFP line. E. Bayer for providing pPDLP3::PDLP3-YFP and  
506 pSUC2::GFP lines. pCYP72A::GUS line was kindly provided by Y. Belkhadir. We thank, T. Zhang  
507 and the Salk Biophotonics core team for microscopy advance and assistance in quantification.  
508 We thank as well Salk peptide synthesis core especially Jill Meisenhelder and finally Br. Moussu  
509 for thoughtful discussion. This study was funded by the National Institute of General Medical  
510 Sciences of the National Institutes of Health (grant number R01GM127759 to W. Busch), a grant  
511 from the Austrian Science Fund (FWF I2377-B25 to W. Busch), funds from the Austrian Academy  
512 of Sciences through the Gregor Mendel Institute (W. Busch), and start-up funds from the Salk  
513 Institute for Biological Studies (W. Busch). M.P. Platre was supported by a long-term postdoctoral  
514 fellowship (LT000340/2019 L) by the Human Frontier Science Program Organization. R.A. and  
515 J.E.P. were supported by The Max-Planck Society and Germany's Excellence Strategy CEPLAS  
516 (EXC-2048/1, Project 390686111). M.v.R. was funded by an IMPRS PhD fellowship. The  
517 European Research Council (ERC) under the European Union's Horizon 2020 research and  
518 innovation program (grant agreement No 772103-BRIDGING) to E. Bayer with the EMBO Young  
519 Investigator Program to E. Bayer.

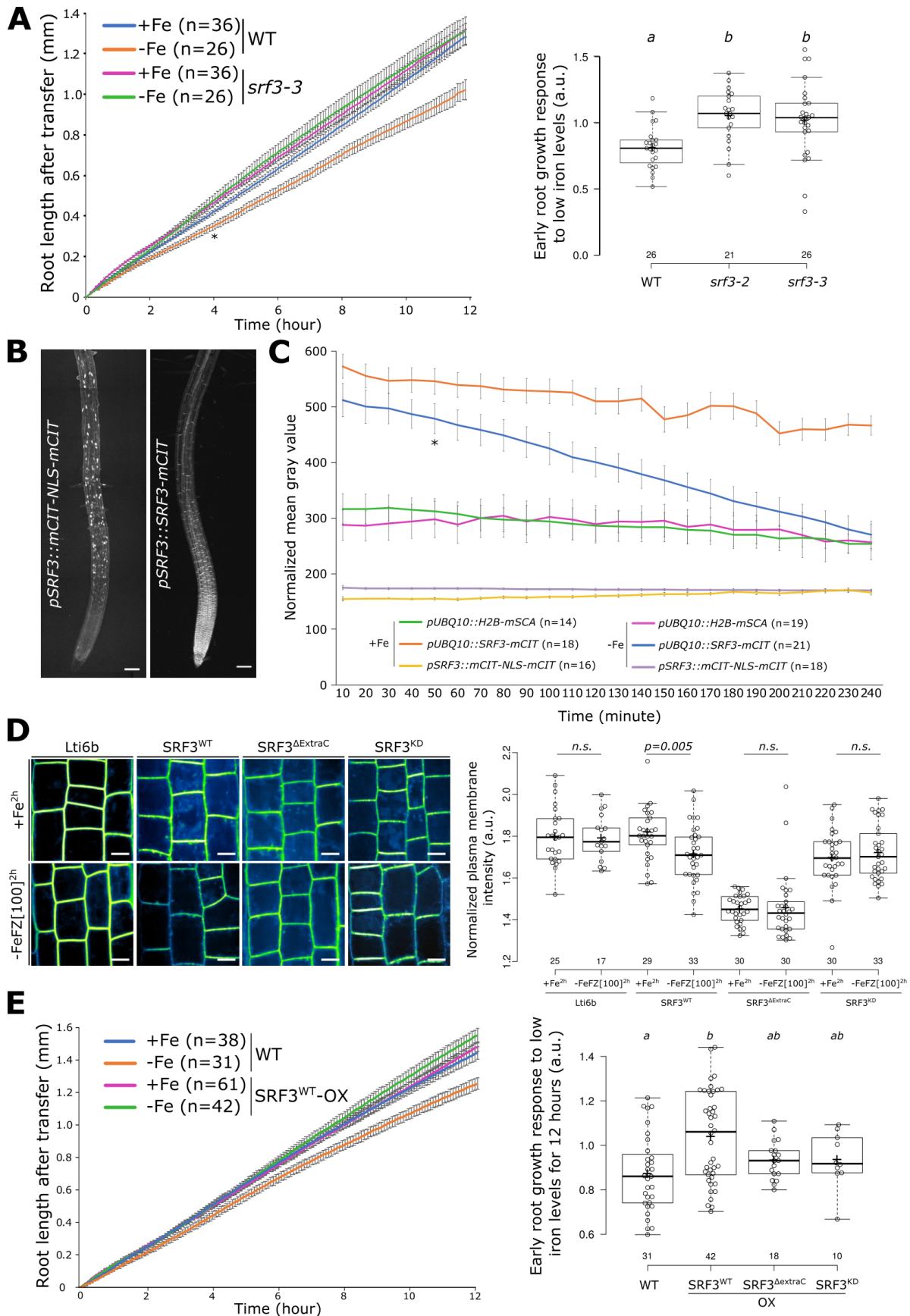
520  
521 **Author contributions:** M.P. Platre was responsible of all experiments described in the  
522 manuscript except for : qRT-PCR from extreme accessions performed by M. Giovannetti, B.  
523 Enugutti did dry seed embryo dissection, Marcel von Reth, R. Alcazar and Jane E. Parker  
524 generated and characterized the *pSRF3-SRF3-GFP* line, G. Vert provided pIRT1-NLS-2xYPET  
525 line, S.B. Satbhai was involved in phenotyping, GWAS data processing and analysis, and  
526 performing *pCYP72A11::GUS* experiment. C. Goeschl performed GWAS data plotting and GUS  
527 signal quantification, L. Brent performed the selection and generation of transgenic lines, M.F.  
528 Gleason imaged SRF3 reporter lines, M. Cao conducted qRT-PCR for immune genes under iron  
529 deficiency, C. Gaillochet and L. Zhang performed RNAseq data analysis, M. Glavier performed  
530 SRF3 immuno-gold electron microscopy and M. Grison performed callose immuno-localization.  
531 M.P. Platre, S.B. Satbhai and W. Busch conceived the study and designed experiments. M.P.  
532 Platre, W. Busch and E. Bayer wrote the manuscript, and all the authors discussed the results and  
533 commented on the manuscript.

534  
535 **Declaration of interest:** Authors declare no conflict of interest.

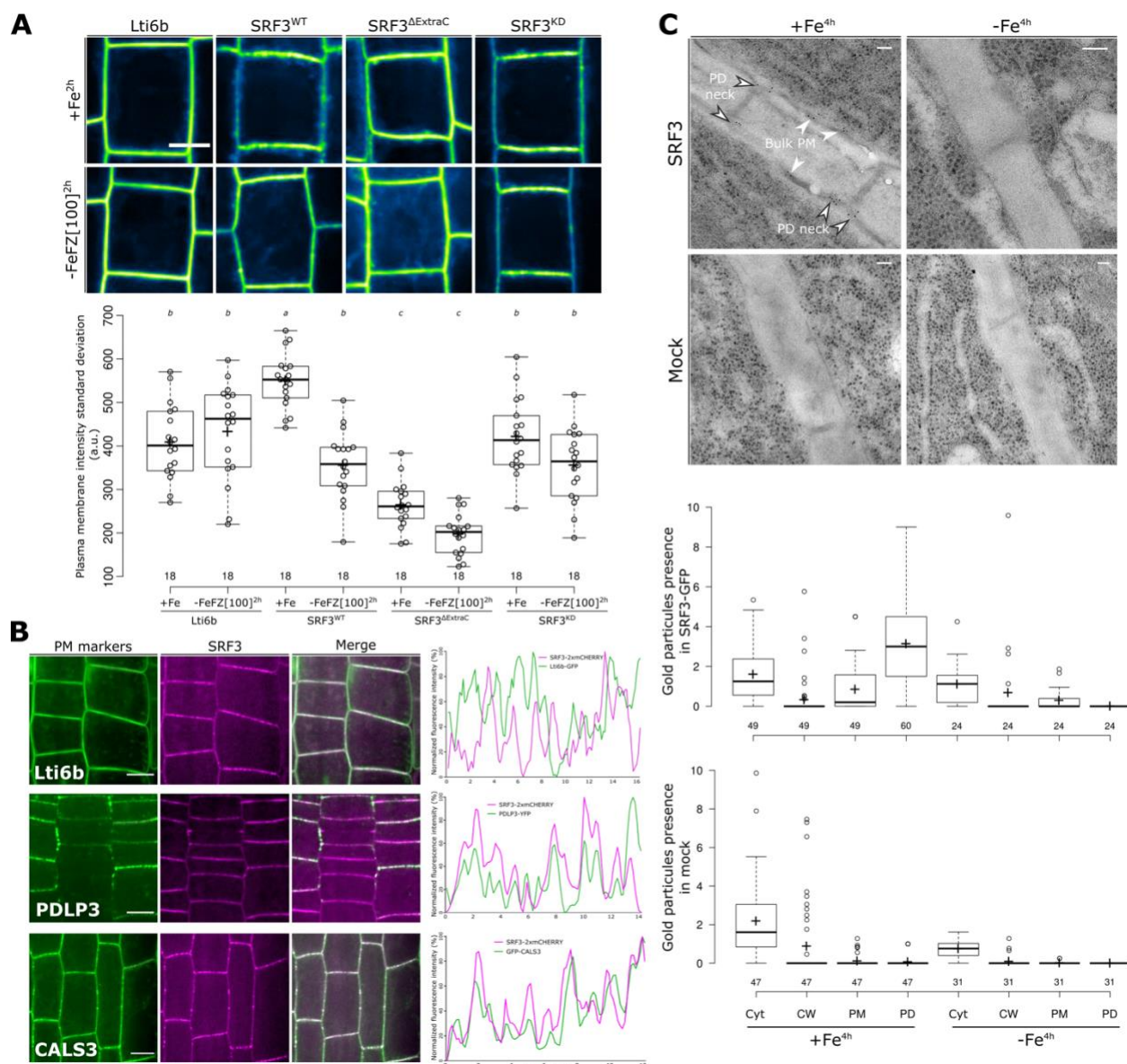




538 **Figure 1. SRF3 regulates root growth and iron homeostasis upon low iron conditions. (A)**  
539 Upper panel: Manhattan plot for GWA mapping of the root growth rate day 4-5 of natural  
540 accessions grown under low iron conditions. The horizontal dash dot line corresponds to a 5%  
541 false discovery rate threshold. Black box indicates the significantly associated SNP that is in  
542 proximity to *SRF3*. Lower panel: Magnified associations in the *SRF3* region with gene models.  
543 **(B)** Representative images of 5 days old seedlings of WT and *srf3-3* under iron sufficient medium  
544 for 5 days (left panel) and then transferred to iron sufficient media (+Fe; upper right panel), or to  
545 low iron medium (-FeZ 100 $\mu$ M, lower right panel) and grown for 3 days. Scale bars, 1 cm. **(C)**  
546 Boxplots of late root growth response to different iron levels (-FeZ 10,50,100 $\mu$ M or Na-Fe-EDTA  
547 300 $\mu$ M) in WT and *srf3-3* seedlings [two-ways student test ( $p < 0.05$ ), n.s. non-significant]. **(D)**  
548 RNAseq read counts of differentially expressed iron homeostasis genes in roots of WT and *srf3-*  
549 *3* in iron sufficient conditions. **(E)** 5 days old seedlings stained with propidium iodide (PI; red  
550 channel) expressing *pIRT1::NLS-2xYPet* (green channel) in WT and *srf3-4* on sufficient (+Fe) or  
551 low (-Fe) iron medium and the related quantification [one-way ANOVA follows by a post-hoc  
552 Tukey HSD test, letters indicate statistical differences ( $p < 0.05$ )]. Scale bars, 100 $\mu$ m. **(F)** Confocal  
553 images of 5 days old seedlings stained with RhoNox-1 in WT and *srf3-3* on sufficient medium  
554 (+Fe; upper panel) or low iron medium (ferrozine 50 $\mu$ M, 30min; lower panel) and related  
555 quantification [Independent two ways student test ( $p < 0.05$ )]. Scale bars 50 $\mu$ m.

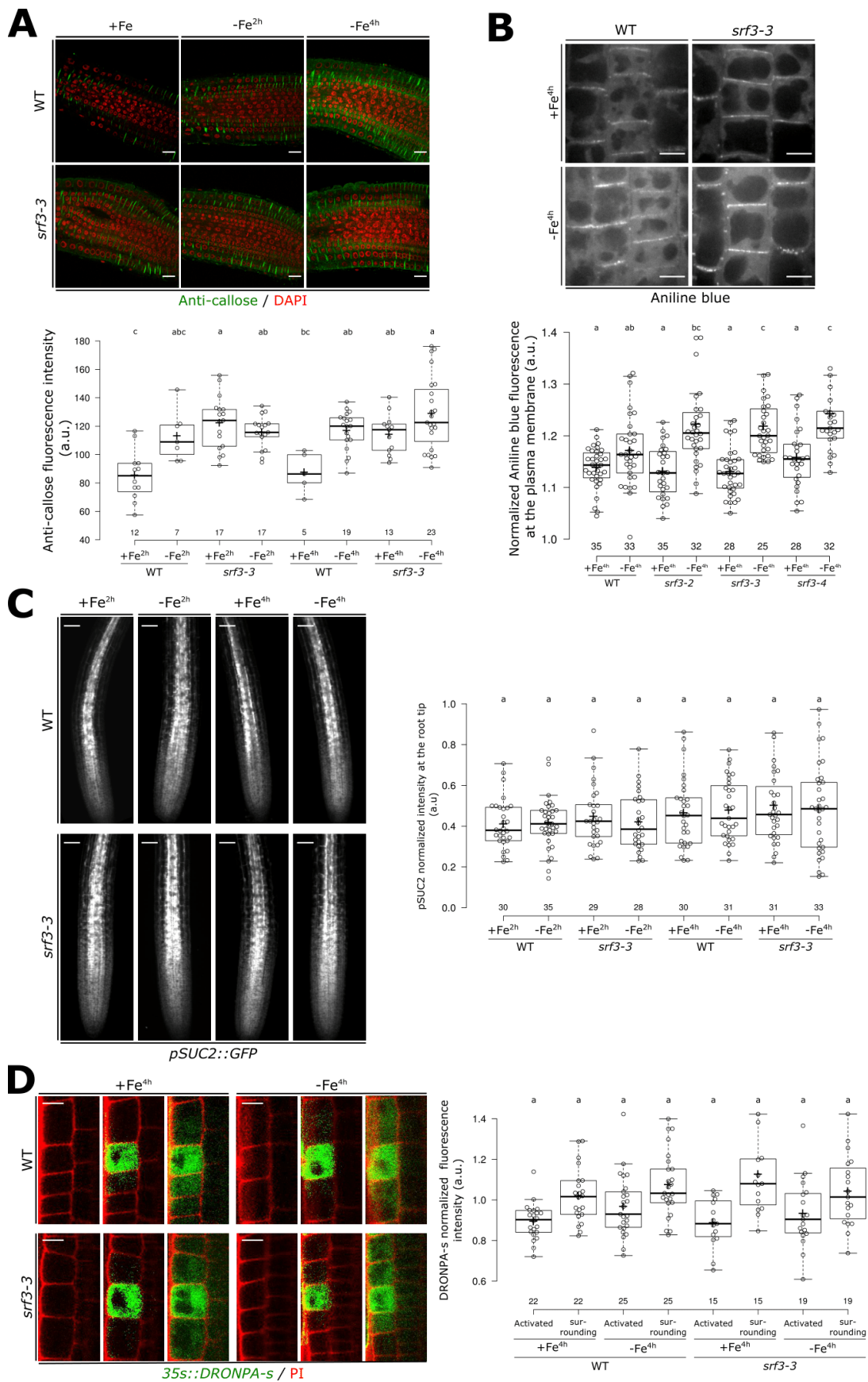


557 **Figure 2. SRF3 regulates early root growth response and undergoes for degradation**  
558 **through its extracellular domain and kinase activity under iron deficiency. (A)** Graph  
559 showing time lapse of the root length of WT and *srf3-3* under sufficient (+Fe) and low (-Fe) iron  
560 medias [error bars: SEM; Asterix: significant difference between WT in +Fe and -Fe conditions  
561 according to a mixed effect model ( $p < 0.05$ )] and the related quantification including the *srf3-2*  
562 mutant [ANOVA with post-hoc Tukey test; Letters: statistical differences ( $p < 0.05$ )]. **(B)** Confocal  
563 images of root tips of 5 days old seedlings expressing *pSRF3::mCITRINE-NLS-mCITRINE* and  
564 *pSRF3::SRF3-mCITRINE*. Scale bars, 100 $\mu$ m. **(C)** Graph representing the fluorescence intensity  
565 in the root tip of the indicated protein fusions under sufficient (+Fe) and low (-Fe) iron medias  
566 [Asterix: significant difference between +Fe and -Fe for *pUBQ10::SRF3-mCITRINE* according to  
567 a mixed effect model ( $p < 0.05$ )]. **(D)** Confocal images of 5 days old seedling expressing  
568 *p35s::Lti6b-GFP*, *pUBQ10::SRF3<sup>WT</sup>-mCITRINE*, *pUBQ10::SRF3<sup>ΔExtraC</sup>-mCITRINE*,  
569 *pUBQ10::SRF3<sup>KD</sup>-mCITRINE* under sufficient (+Fe, 2h) and low iron levels (-FeZ 100 $\mu$ M, 2h)  
570 and the related quantification [two-ways student test ( $p < 0.05$ ), n.s.: non-significant]. Scale bars  
571 10 $\mu$ m. **(E)** Graph showing time lapse of the root length of WT and *SRF3<sup>WT</sup>-OX* under sufficient  
572 (+Fe) and low (-Fe) iron medias [error bars: SEM] and related quantification including *SRF3<sup>ΔExtraC</sup>*  
573 and *SRF3<sup>KD</sup>* [ANOVA with post-hoc Tukey test; Letters: statistical differences ( $p < 0.05$ )].

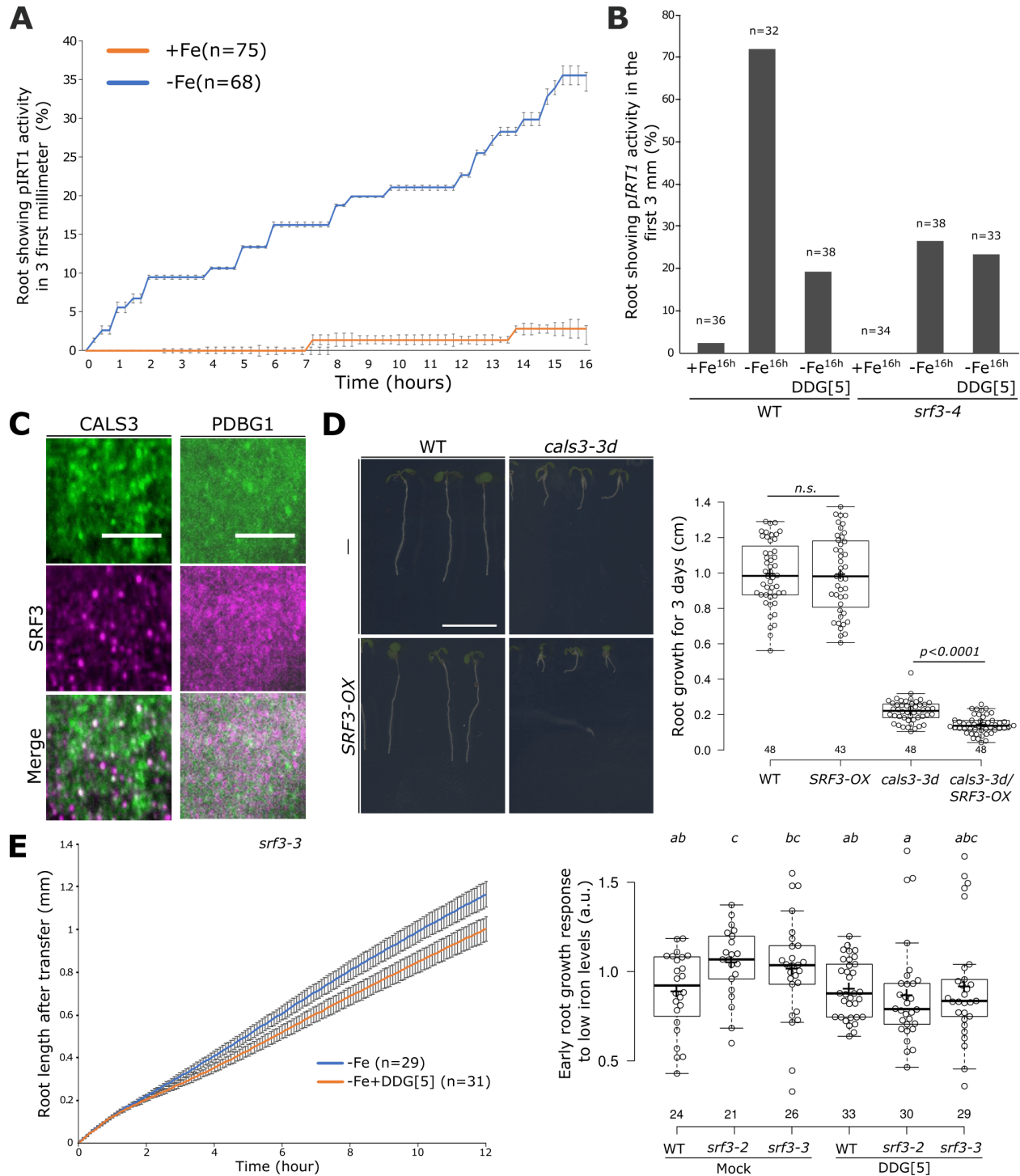


574  
 575 **Figure 3. SRF3 co-exists in two subpopulations at the plasma membrane which decrease**  
 576 **under low iron levels. (A)** Confocal images of 5 days old seedling expressing *p35s::Lti6b-GFP*,  
 577 *pUBQ10::SRF3-mCITRINE*, *pUBQ10::SRF3<sup>WT</sup>-mCITRINE*, *pUBQ10::SRF3<sup>ΔExtraC</sup>-mCITRINE*,  
 578 *pUBQ10::SRF3<sup>KD</sup>-mCITRINE* under iron sufficient (+Fe, 2h) or low iron (-FeZ 100μM, 2h) and  
 579 the related quantification [ANOVA with post-hoc Tukey test; Letters: statistical differences  
 580 (p<0.05)]. Note that the pictures have been pseudo-colored to emphasize changes in polarity and  
 581 localization in the punctuated foci which does not reflect the proper fluorescence intensity. Scale  
 582 bars, 10μm **(B)** Confocal images of 5 days-old seedlings co-expressing, *p35s::Lti6b-GFP*,  
 583 *pPDLF3-PDLF3-YFP*, *35s::CALS3-GFP*, left, with *pUBQ10::SRF3-2xmCHERRY*, middle and  
 584 the relative merge. Red line on the left image indicates where the scan line has been traced.  
 585 Scale bars, 10μm. Right panel: graphs showing the signal intensity in both channel on the apical  
 586 basal part of the cell. **(C)** Micrograph of immune gold with plant expressing *pSRF3::SRF3-GFP*  
 587 (SRF3) and the relative control in *Ler* background under sufficient (+Fe, 4h) and low (-Fe, 4h) iron  
 588 medias and the related quantification. Cyt, cytosol; CW, cell wall; PM, plasma membrane; PD,  
 589 plasmodesmata. Scale bars, 100nm





591 **Figure 4. SRF3 is a negative regulator of callose deposition but does not regulate cell-to-**  
592 **cell signaling. (A)** Confocal images of root of 5 days old seedling stained with callose antibody  
593 (green) and DAPI to stain the nucleus (red) under sufficient (+Fe, 2h and 4h) and low (-Fe, 2h  
594 and 4h) iron medias and the related quantification [ANOVA with post-hoc Tukey test; Letters:  
595 statistical differences ( $p < 0.05$ )]. Scale bars, 10 $\mu$ m. **(B)** Confocal images of 5 days old seedling  
596 stained with aniline blue under sufficient (+Fe, 4h) and low (-Fe, 4h) iron medias in WT and *srf3-*  
597 *3* and the related quantification [ANOVA with post-hoc Tukey test; Letters: statistical differences  
598 ( $p < 0.05$ )]. Scale bars, 10 $\mu$ m. **(C)** Confocal images of 5 days old seedling expressing *pSUC2::GFP*  
599 in WT and *srf3-3* under sufficient (+Fe) and low (-Fe) iron medias and the related quantification  
600 [ANOVA with post-hoc Tukey test; Letters: statistical differences ( $p < 0.05$ )]. Scale bars, 50 $\mu$ m **(D)**  
601 Confocal images of 5 days old seedling expressing *p35s::DRONPA-s* in WT and *srf3-3* under  
602 sufficient (+Fe) and low (-Fe) iron medias and the related quantification [ANOVA with post-hoc  
603 Tukey test; Letters: statistical differences ( $p < 0.05$ )]. Scale bars, 10 $\mu$ m.

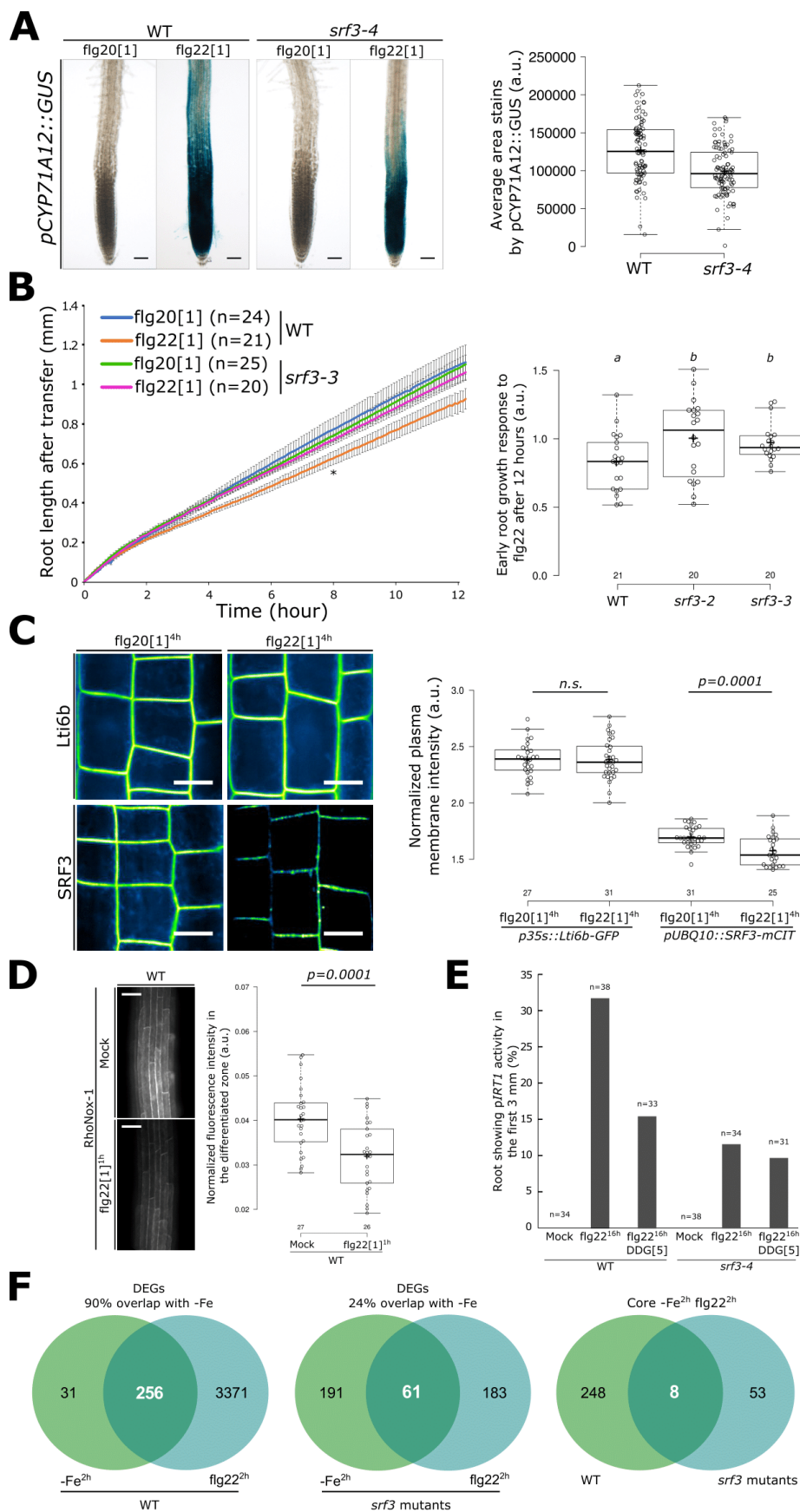


604  
605  
606  
607  
608  
609  
610  
611  
612

**Figure 5. Regulation of *IRT1* and root growth by SRF3-dependent callose synthase activity under low iron levels.** (A) Graph representing the quantification of *pIRT1::NLS-2xYPet* time lapse analysis under mock (+Fe) and low iron levels (-Fe) [error bars indicate SEM]. (B) Graph representing the percentage of root showing *IRT1* promoter activation under sufficient (+Fe, 16h) and low (-Fe, 16h) iron medias in WT and *srf3-4* in presence or absence of DDG. (C) Micrographs of 5 days old seedling expressing *35s::GFP-CALS3* (upper) and *UBQ10::SRF3-2xmCHERRY* (middle) and merge channel (lower) in TIRF. Scale bars, 5µm. (D) Picture of 9 days old seedling of WT, *cals3-3d*, *pUBQ10::SRF3-mCITRINE* (*SRF3-OX*) and *cals3-3d**xSRF3-OX* and the related

613 quantification [two-ways student test ( $p < 0.05$ ), n.s. non-significant]. Scale bar, 1cm. **(E)** Graph  
614 showing time lapse of the root length of *srf3-3* under low iron (-Fe) medias in presence or absence  
615 of DDG and the related quantification including the *srf3-2* mutant [ANOVA with post-hoc Tukey  
616 test; Letters: statistical differences ( $p < 0.05$ ); Error bars: SEM].





618 **Figure 6. Coordination of bacterial immunity and iron homeostasis signaling pathways by**  
 619 **SRF3. (A)** Pictures of plants expressing *pCYP71A12::GUS* in WT and *srf3-4* under flg20 and  
 620 flg22 treatment (1μM, 24h) and the related quantification. Scale bars, 50μm. **(B)** Graph showing  
 621 time lapse of the root length of WT and *srf3-3* under flg20 and flg22 (1μM) [Error bars: SEM;  
 622 Asterix: significant difference between +Fe and -Fe for the WT according to a mixed effect model  
 623 ( $p < 0.05$ )] and the related quantification including the *srf3-2* mutant [ANOVA with post-hoc Tukey  
 624 test; Letters: statistical differences ( $p < 0.05$ )]. **(C)** Confocal images of 5 days old seedling  
 625 expressing *p35s::Lti6b-GFP* and *pUBQ10::SRF3-mCITRINE* in flg20 and flg22 (1μM, 4h) and the  
 626 related quantification [two-ways student test ( $p < 0.05$ ); n.s.: non-significant]. Scale bars, 10μm. **(D)**  
 627 Confocal images of 5 days old seedling stained with RhoNox-1 in WT in mock or flg22 (1μM, 1h)  
 628 and the related quantification [two-ways student test ( $p < 0.05$ )]. Scale bars, 50μm. **(E)** Graph  
 629 representing the percentage of root showing *IRT1* promotor activation under mock and flg22  
 630 (1μM, 16h) treatment in WT and *srf3-4* in presence or absence of DDG. **(F)** Venn diagram of  
 631 differentially expressed genes under low iron levels (-Fe, 2h) and flg22 (1μM, 2h) in WT (left) in  
 632 *srf3* (middle) and DEGs in both condition between WT and *srf3*.  
 633

## 634 STAR METHODS

## 635 KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-callose	Bio-supply	CAT #400-2
anti-GFP	Thermo Fisher Scientific	CAT #A11122
anti-rabbit IgG	Tebu-Bio	CAT #NA.41
Chemicals, peptides, and recombinant proteins		
DDG	Tocris	CAT #4515
Flg20	Salk peptide synthesis core	N/A
Flg22	Salk peptide synthesis core	N/A
Ferrozine	Acros Organics	CAT #AC410570010
Na-Fe-EDTA	PhytoTechnology Lab	CAT #E349
Aniline blue	Sigma	CAT #28631-66-5
RhoNox-1	Goryo Chemical	CAT #GC901
Experimental models: organisms/strains		
<i>A. thaliana</i> : <i>Col-0</i>	NASC	NASC# N1092
<i>A. thaliana</i> : <i>srf3-2</i>	Alcazar et al., 2010	NASC #557621
<i>A. thaliana</i> : <i>srf3-3</i>	NASC	NASC #501389
<i>A. thaliana</i> : <i>srf3-4</i>	NASC	NASC #878396

A. thaliana: <i>cals3-3d</i>	Vatén et al., 2011	N/A
A. thaliana: <i>pCYP71A12::GUS</i>	Millet et al., 2010	N/A
A. thaliana: <i>pIRT1::NLS-2xYPet</i>	This study	N/A
A. thaliana: <i>p35s::DRONPA-s</i>	Gerlitz et al., 2018	N/A
A. thaliana: <i>pUBQ10::H2B-mSCARLET</i>	This study	N/A
A. thaliana: <i>pSRF3::mCITRINE-NLS-mCITRINE</i>	This study	N/A
A. thaliana <i>pSRF3::SRF3-GFP/Ler</i>	This study	N/A
A. thaliana: <i>pSRF3::SRF3-2xmCHERRY/srf3-3</i>	This study	N/A
A. thaliana: <i>pSRF3::SRF3-mCITRINE/srf3-3</i>	This study	N/A
A. thaliana: <i>pUBQ10::SRF3-mCITRINE</i>	This study	N/A
A. thaliana: <i>pUBQ10::SRF3<sup>KD</sup>-mCITRINE</i>	This study	N/A
A. thaliana: <i>pUBQ10::SRF3<sup>ΔKinase</sup>-mCITRINE</i>	This study	N/A
A. thaliana: <i>pUBQ10::SRF3<sup>ΔExtraC</sup>-mCITRINE</i>	This study	N/A
A. thaliana: <i>35s::mCITRINE-PdBG1</i>	Benitez-Alfonso et al., 2013	N/A
A. thaliana: <i>35s::GFP-CALS3</i>	Vatén et al., 2011	N/A
A. thaliana: <i>35s::PDLP5-GFP</i>	Lee et al., 2011	N/A
A. thaliana: <i>pPDLP3::PDLP3-YFP</i>	Thomas et al., 2008	N/A
<b>Oligonucleotides</b>		
For primers used in genotyping, RT-PCR and cloning see Spreadheet S6, related to STAR Methods	N/A	N/A
<b>Recombinant DNA</b>		
Empty gateway entry vector: pDONR221	thermofisher	CAT# 12536017
Empty gateway entry vector: pDONR P2RP3	thermofisher	CAT# 12537023
Empty gateway destination vector: pB7m34GW	(Karimi et al., 2007)	N/A
Empty gateway destination vector: pH7m34GW	(Karimi et al., 2007)	N/A
Gateway entry vector (promoter): UBQ10prom/ pDONR P4P1R	(Jaillais et al., 2011)	NASC# N2106315
Gateway entry vector (promoter): pSRF3/ pDONR P4P1R	This study	N/A
Gateway entry vector (protein): SRF3/ pDONR221	This study	N/A
Gateway entry vector (protein): SRF3 <sup>ΔKinase</sup> / pDONR221	This study	N/A

Gateway entry vector (protein): SRF3ΔExtraC/ pDONR221	This study	N/A
Gateway entry vector (protein): SRF3KD/ pDONR221	This study	N/A
Gateway entry vector (fluorescent protein): H2BnoSTOP/pDONR221	This study	N/A
Gateway entry vector (fluorescent protein): 2xmCHERRYnoSTOP/ pDONR P2RP3	(Platre et al., 2018)	N/A
Gateway entry vector (fluorescent protein): mCITRINEnoSTOP/pDONR P2RP3	(Jaillais et al., 2011)	NASC# N2106287
Gateway entry vector (fluorescent protein): mSCARLET/pDONR P2RP3	This study	N/A
Gateway destination vector (for plant transformation): pUBQ10::H2B-mSCARLET/ pH7m34GW	This study	N/A
Gateway destination vector (for plant transformation): pSRF3::SRF3-2xmCHERRY pH7m34GW	This study	N/A
Gateway destination vector (for plant transformation): pSRF3::SRF3- mCITRINE/pB7m34GW	This study	N/A
Gateway destination vector (for plant transformation): pUBQ10::SRF3- mCITRINE/pB7m34GW	This study	N/A
Gateway destination vector (for plant transformation): pUBQ10::SRF3KD- mCITRINE/pB7m34GW	This study	N/A
Gateway destination vector (for plant transformation): pUBQ10::SRF3ΔKinase- mCITRINE/pB7m34GW	This study	N/A
Gateway destination vector (for plant transformation): pUBQ10::SRF3ΔExtraC- mCITRINE/pB7m34GW	This study	N/A
Gateway destination vector (for plant transformation): pUBQ10::SRF3- 2xmCHERRY/pH7m34GW	This study	N/A
Software and algorithms		
BRAT	(Slovak et al., 2014)	N/A
Fiji	(Schindelin et al., 2012)	<a href="https://fiji.sc/">https://fiji.sc/</a>
Matlab_RootWalker	Busch's lab	N/A
Macro_PM_intensity	Busch's lab	N/A
Macro_RhoNox-1	Busch's lab	N/A
Macro_Match_Align	Busch's lab	N/A
Macro_Nuclear_signal_Intensity	Busch's lab	N/A
Macro_Nuclear_density	Busch's lab	N/A

636  
637  
638

**Plant materials and growth conditions.** For surface sterilization, *Arabidopsis thaliana* seeds of 231 accessions from the Regmap panel (Spreadheet S1) that had been produced under uniform



639 growth conditions were placed for 1 h in opened 1.5-mL Eppendorf tubes in a sealed box  
640 containing chlorine gas generated from 130 mL of 10% sodium hypochlorite and 3.5 mL of 37%  
641 hydrochloric acid. For stratification, seeds were imbibed in water and stratified in the dark at 4 °C  
642 for 3 days. Seeds were then put on the surface of 1X MS agar plates, pH 5.7, containing 1%  
643 (w/v) sucrose and 0.8% (w/v) agar (Duchefa Biochemie) using 12-cm x 12-cm square plates. The  
644 iron-sufficient medium contained 100 µM Na-Fe-EDTA and the iron-deficient (1XMS iron free)  
645 medium contained 300 µM Ferrozine, a strong iron chelator [3-(2-pyridyl)-5,6-diphenyl-1,2,4-  
646 triazinesulfonate, Sigma Aldrich](Dinneny et al., 2008). This condition was only used for GWAS.  
647 For further experimentation, we used the Fe -sufficient or -free media described in Gruber et al.,  
648 2013, with no or a decrease level of Ferrozine, 100, 50 and 10 µM. Using the Gruber et al., 2013  
649 iron-free medium, we add 300 µM of Na-Fe-EDTA to test *srf3* phenotype under iron  
650 excess(Gruber et al., 2013). The *srf3-2*, *srf3-3*, *bts-1*, *opt3-2*, *fls2-c*, *fls2-9*, *vit-1* and *cals3-3d*  
651 mutant lines are in Col-0 background and were described and characterized(Alcázar et al., 2010;  
652 Groen et al., 2013; Higashi et al., 2008; Kim et al., 2006; Mendoza-Cózatl et al., 2014; Selote et  
653 al., 2015; Vatén et al., 2011b). The reporter lines, *35s::PdBG1-mCITRINE*, *35s::GFP-CALS3*,  
654 *pPDLP3::PDLP3-YFP*, *35s::GFP-PDLP5*, *35s::Lti6b-GFP*, *35s::DRONPA-s*, *pSUC2::GFP* and  
655 *pCYP71A12::GUS* are in Col-0 background and were described and characterized(Benitez-  
656 Alfonso et al., 2013; Cutler et al., 2000; Lee et al., 2011b; Millet et al., 2010; Thomas et al., 2008b;  
657 Vatén et al., 2011b). The T-DNA insertion lines for SRF3, SAIL1176\_B01 (*srf3-4*) and  
658 SALK\_202843, as well as for *at4g03400*, SAIL\_811\_C06 (*at4g03400*) were purchased from  
659 Nottingham Arabidopsis Stock Center (NASC, Nottingham, United Kingdom). The primers used  
660 for genotyping the T-DNA lines are listed below (List of primers, Spreadsheet S6). Plants were  
661 grown in long day conditions (16/8h) in walk in growth chambers at 21°C, 50µM light intensity,  
662 60% humidity. During nighttime, temperature was decreased to 15°C.

663  
664 **Plant transformation and selection.** Each construct (see below: “Construction of plant  
665 transformation vectors (destination vectors) and plant transformation”), was transformed into C58  
666 GV3101 *Agrobacterium tumefaciens* strain and selected on YEB media (5g/L beef extract; 1g/L  
667 yeast extract; 5g/L peptone; 5g/L sucrose; 15g/L bactoagar; pH 7.2) supplemented with antibiotics  
668 (Spectinomycin, Gentamycin). After two days of growth at 28C, bacteria were collected using a  
669 single-use cell scraper, re-suspended in about 200 mL of transformation buffer (10mM MgCl<sub>2</sub>;  
670 5% sucrose; 0.25% silwet) and plants were transformed by the floral dipping method(Clough and  
671 Bent, 1998). Plants from the Columbia-0 (Col0) accession were used for transformation. Primary  
672 transformants (T1) were selected *in vitro* on the appropriate antibiotic/herbicide (glufosinate for  
673 mCITRINE, hygromycin for mCHERRY and mSCARLET tagged proteins). Approximately 20  
674 independent T1s were selected for each line. In the T2 generation at least 3 independent  
675 transgenic lines were selected using the following criteria when possible: i) good expression level  
676 in the root for detection by confocal microscopy, ii) uniform expression pattern, iii) single insertion  
677 line (1 sensitive to 3 resistant segregation ratio) and, iv) line with no obvious abnormal  
678 developmental phenotypes. Lines were rescreened in T3 using similar criteria as in T2 with the  
679 exception that we selected homozygous lines (100% resistant). At this step, we selected one  
680 transgenic line for each marker that was used for further analyses and crosses.

681  
682  
683  
684 **GWA mapping.** 231 natural accessions (12 plants/accession were planted) were grown on 1 ×  
685 MS agar plates containing 300 µM Ferrozine under long day conditions (16 hours light) at 21°C.  
686 Plant images were acquired by EPSON flatbed scanners (Perfection V600 Photo, Seiko Epson  
687 CO., Nagano, Japan) every 24 hours for 5 days (2 DAG – 6 DAG). Root image analyses and trait  
688 quantification were performed using the BRAT software(Slovak et al., 2014). Median root growth  
689 rate (n ≥3) values between 4 to 5 days were used for GWA study. For more accuracy, the roots

690 not detected or not germinated were not included in the analyses. GWA mapping was conducted  
691 using a mixed model algorithm which has been used previously to correct population structure  
692 confounding and SNP data from the 250K SNP chip (Atwell et al., 2010; Brachi et al., 2010; Horton  
693 et al., 2012; Kang et al., 2008; Seren et al., 2012). SNPs with minor allele counts equal or greater  
694 to 10 were taken into account. The significance of SNP associations was determined at 5% FDR  
695 threshold calculated by the Benjamini-Hochberg-Yekutieli method to correct for multiple  
696 testing (Benjamini and Yekutieli, 2001). The GWAS peak in proximity of *SRF3* (Figure 1a)  
697 contained 4 significant SNPs. By analyzing the unique combinations of these 4 SNPs in the 231  
698 accessions, four groups of haplotypes were defined as Group A, Group B, Group C and Group  
699 D.  
700

701 **Phenotyping of early root growth responses.** Seeds were sowed in +Fe media described in  
702 Gruber et al., 2013 and stratified for 2-3 days at 4°C. Five days after planting, about 15 seedlings  
703 were transferred to a culture chamber (Lab-Tek, Chamberes #1.0 Borosilicate Coverglass  
704 System, catalog number: 155361) filled with – Fe or +Fe medium described in Gruber et al., 2013  
705 or +Fe medium containing flg20 or flg22. Note that the transfer took about 45-60 seconds. Images  
706 were acquired every 5 minutes for 12 hours representing 145 images per root in brightfield  
707 conditions using a Keyence microscope model BZ-X810 with a BZ NIKON Objective Lens (2X)  
708 CFI Plan-Apo Lambda.  
709

710 **Phenotyping of late root growth responses.** Seeds were sowed in +Fe media described in  
711 Gruber et al., 2013 and stratified for 2-3 days at 4°C. Five days after planting, 6 plants per  
712 genotype were transferred to four 12x12cm plates in a pattern in which the positions of the  
713 genotypes were alternating in a block design (top left, top right, bottom left and bottom right). After  
714 transfer, the plates were scanned every 24 hours for 3 days using the BRAT software (Slovak et  
715 al., 2014).  
716

717 **Quantitative real time PCR.** For *SRF3* expression analysis seedlings were grown initially on iron  
718 sufficient media (1xMS, 1% w/v Caisson Agar) for 5 days and then shifted to either iron sufficient  
719 or low iron (100 uM FerroZine) 1xMS liquid medium. Nylon mesh (Nitex Cat 03-100/44; Sefar)  
720 was placed on top of the solidified media to facilitate transfer. Root tissues were collected for RNA  
721 extraction 3 hours post transfer by excision with fine scissors. Each biological replicate was  
722 constituted by RNA extraction from 30-40 whole roots. Samples were immediately frozen in liquid  
723 nitrogen, ground, and total RNA was extracted using the RNeasy Plant Mini kit (QIAGEN GmbH,  
724 Hilden, Germany). qRT-PCR reactions were prepared using 2x SensiMix SYBR & Fluorescein Kit  
725 (PEQLAB LLC, Wilmington, DE, USA) and PCR was conducted with a Roche Lightcycler 96  
726 (Roche) instrument. Relative quantifications were performed for all genes with the  $\beta$ -tubulin gene  
727 (AT5G62690) used as an internal reference. The primers used for qRT-PCR are shown in list of  
728 primers (Spreadsheet S6).  
729

730 **RNAseq.** Total RNA was extracted from roots of plants 5 days after germination using RNA  
731 protein purification kit (Macherey-Nagel). Next generation sequencing (NGS) libraries were  
732 generated using the TruSeq Stranded mRNA library prep kits (Illumina, San Diego, CA, USA).  
733 Libraries were sequenced on a HiSeq2500 (Illumina, San Diego, CA, USA) instrument as single  
734 read 50bases. NGS analysis was performed using Tophat2 for mapping reads onto the  
735 Arabidopsis genome (TAIR10) (Kim et al., 2013), HT-seq for counting reads and EdgeR for  
736 quantifying differential expression (Robinson et al., 2009). We set a threshold for differentially  
737 expressed genes (Fold change (FC) >2 or FC <-2, FDR < 0.01). Genotype x Environment  
738 interaction analysis was performed using linear model and type II Anova analyses in R (codes are  
739 available upon request). Gene ontology analysis was performed using the AgriGOv2 online

740 tool(Tian et al., 2017). Venn diagrams were generated with the VIB online tool  
741 (<http://bioinformatics.psb.ugent.be/webtools/Venn/>). The plot in figure 3a was generated using the  
742 online Revigo software (<http://revigo.irb.hr/>).

743  
744  
745 **Microscopy setup.** All imaging experiments except when indicated below, were performed with  
746 the following spinning disk confocal microscope set up: inverted Zeiss microscope equipped with  
747 a spinning disk module (CSU-X1, Yokogawa, <https://www.yokogawa.com>) and the prime 95B  
748 Scientific CMOS camera (<https://www.photometrics.com>) using a 63x Plan- Apochromat objective  
749 (numerical aperture 1.4, oil immersion) or low resolution 10x lens for time lapse imaging. GFP,  
750 mCITRINE, Aniline blue and RhoNox-1 staining were excited with a 488 nm laser (150mW) and  
751 fluorescence emission was filtered by a 525/50 nm BrightLine® single-band bandpass filter.  
752 mSCARLET, mCHERRY and propidium iodide dyes were excited with a 561 nm laser (80 mW)  
753 and fluorescence emission was filtered by a 609/54 nm BrightLine® single-band bandpass filter  
754 (Semrock, <http://www.semrock.com/>). 405 nm laser was used to excite aniline blue and emission  
755 was recorded at 480–520 nm with 40x objectives. For propidium iodide, 488nm for excitation and  
756 around 600nm was used with 40x objectives. For quantitative imaging, pictures of root cells were  
757 taken with detector settings optimized for low background and no pixel saturation. Care was taken  
758 to use similar confocal settings when comparing fluorescence intensity or for quantification.

759 **FRAP experiment.** Fluorescence in a rectangle ROI (50  $\mu\text{m}^2$ , 15  $\mu\text{m}$  long), in the plasma  
760 membrane region, was bleached in the root optical section by four successive scans at full laser  
761 power (150 W) using the FRAP module available on the Zeiss LSM 980 Airyscan 2. Fluorescence  
762 recovery was subsequently analysed in the bleached ROIs and in controlled ROIs (rectangle with  
763 the same dimension in unbleached area). FRAP was recorded continuously during 90 s with a  
764 delay of 0.3 s between frames. Fluorescence intensity data were normalized as previously  
765 described (Platre et al, 2019). For visualization, kymographs were obtained using kymograph  
766 function in Fiji.

767 **TIRF microscopy.** Total Internal Reflection Fluorescence (TIRF) Microscopy was done using the  
768 inverted ONI Nanoimager from Oxford microscope with 100x Plan-Apochromat objective  
769 (numerical aperture 1.50, oil immersion). The optimum critical angle was determined as giving the  
770 best signal-to-noise ratio. Images were acquired with about 15% excitation (1W laser power) and  
771 taking images every 100ms for 500-time steps.

772 **DRONPA-s bleaching and activation.** 5 day-old seedlings were transferred to a culture chamber  
773 (Lab-Tek, Chamberes #1.0 Borosilicate Coverglass System, catalog number: 155361) filled with  
774 – Fe or +Fe medium described in Gruber et al., 2013(Gruber et al., 2013) for 4 hours. After 4  
775 hours, the cell wall was counter-stained by placing one drop of propidium iodide 15  $\mu\text{M}$  (10  $\mu\text{g}/\text{mL}$   
776 in distilled water) on the root tip for 1 minutes. A coverslip was placed on the surface of the root  
777 for further imaging. DRONPA-s was bleached using the full laser power (150 W) of the 488nm  
778 laser for 10 seconds. Then 2-4 regions of interest (ROIs) were drawn on the external lateral side  
779 of the epidermal root cells and DRONPA-s was activated in this region using the 405 nm laser  
780 doing 8 cycles at 15W using the FRAP module available on the Zeiss LSM 980 Airyscan 2. Right  
781 after activation and then again 6 minutes later, images were acquired in both channel, PI and  
782 DRONPA-s.

783 **IRT1 reporter lines after 24h.** About 24 of 5-day-old seedlings grown on iron sufficient medium  
784 were transferred to agar plate filled with +Fe or – Fe supplemented with 100 Ferrozine for 24  
785 hours. 15 seedlings were transferred to a culture chamber (Lab-Tek, Chamberes #1.0 Borosilicate

786 Coverglass System, catalog number: 155361) filled with +Fe described in Gruber et al.,  
787 2013(Gruber et al., 2013). The cell wall was counter-stained by placing one drop of propidium  
788 iodide 15  $\mu\text{M}$  (10  $\mu\text{g}/\text{mL}$  in distilled water) on the root tip for 1 minutes. A coverslip was placed on  
789 the surface of the root for further imaging. Images were acquired using the spinning disc set up  
790 described above using stitching and z-stack modes.

791  
792 **Time lapse imaging of IRT1 reporter lines.** 5-day-old seedlings were grown on iron sufficient  
793 medium and then about 15 seedlings were transferred to a culture chamber (Lab-Tek, Chamberes  
794 #1.0 Borosilicate Coverglass System, catalog number: 155361) filled with +Fe or – Fe medium  
795 described in Gruber et al., 2013(Gruber et al., 2013) or +Fe medium containing flg22. Note that  
796 the transfer took about 45-60 seconds. Images were acquired every 20 minutes for 16 hours  
797 representing 80 images per root using a Keyence microscope model BZ-X810 with BZ NIKON  
798 Objective Lens (2X) CFI Plan-Apo Lambda in brightfield, green (ET470/40x ET525/50m T495lpxr-  
799 UF1) or red (ET560/40x ET630/75m T585lpxr-UF1) channels.

800  
801 **Time lapse imaging of SRF3 transcriptional and translational reporter and control lines**  
802 About 15 5-day-old seedlings grown on iron sufficient medium were transferred to a culture  
803 chamber (Lab-Tek, Chamberes #1.0 Borosilicate Coverglass System, catalog number: 155361)  
804 filled with +Fe or – Fe medium described in Gruber et al., 2013. Note that the transfer took about  
805 45-60 seconds. Images were acquired every 10 minutes for 4 hours using the spinning disc set  
806 up described above and assembled using the stitching mode, z-satck and definite focus options  
807 to keep track of the root and be localized at the same z-stage a long time, respectively.

808  
809 **Cryofixation and freeze-substitution.** 5-day-old seedlings of pSRF3::SRF3-GFP line  
810 (*Landsberg erecta* background) and LER were grown vertically on Caisson media complemented  
811 in iron. The seedlings were incubated for 4 hours in liquid Caisson media which were  
812 complemented or deficient in iron. Root tips were taken and cryofixed in 20% BSA filled copper  
813 platelets (100 nm deep and 1.5 mm wide) with EM PACT1 high pressure freezer (Leica). The  
814 samples were transferred for freeze-substitution in AFS2 (Leica) at  $-90^{\circ}\text{C}$  in cryosubstitution mix:  
815 uranyl acetate 0.36%, in pure acetone, for 24 hours. The temperature was raised stepwise by  $3^{\circ}\text{C}$   
816  $\text{h}^{-1}$  until reaching  $-50^{\circ}\text{C}$  and maintained for 3 hours. The cryosubstitution mix was removed and  
817 replaced by pure acetone and then pure ethanol, for each of them 3 washes of 10 minutes were  
818 performed. The copper platelets were not removed in order to avoid sample loss. HM20 Lowicryl  
819 resin (Electron Microscopy Science) solutions of increasing concentrations were used for  
820 infiltration: 25% and 50% (1 hour each), 75% (2 hours), 100% (overnight, 4 hours, 48 hours- each  
821 bath was performed with new resin). The samples were then polymerized under ultraviolet light  
822 for 24 hours at  $-50^{\circ}\text{C}$  before raising the temperature stepwise by  $3^{\circ}\text{C h}^{-1}$  until reaching  $20^{\circ}\text{C}$  and  
823 maintained for 6 hours.

824  
825  
826 **Immunogold labelling.**

827 The samples were recovered by removing exceeding resin on the top and edges of the copper  
828 platelets. The latter were removed by applying alternatively heat shocks with liquid nitrogen and  
829 on a  $40^{\circ}\text{C}$  heated knife to dissociate copper platelet from the resin. Ultrathin sections of 90 nm  
830 thickness were trimmed at a speed of  $1 \text{ mm s}^{-1}$  (EM UC7 ultramicrotome, Leica) and recovered  
831 on electron microscopy grids (T 300mesh copper grids, Electron Microscopy Science) covered  
832 by 2% parlodion film. Once the grids were dry immunogold labelling was performed. The grids



833 were successively incubated in 10  $\mu$ l droplets of different reagents (0.22  $\mu$ m filtered). The grids  
834 were first incubated in PHEM Tween 0.2% BSA 1% buffer (pH6.9) for 1 minute of rinsing before  
835 30 minutes of blocking. The primary antibody anti-GFP rabbit polyclonal antibody (A11122,  
836 Thermo Fisher Scientific) and secondary antibody 10 nm colloidal gold-conjugated goat anti-rabbit  
837 IgG (Tebu-Bio) were diluted in PHEM Tween 0.2% BSA 1% buffer (pH6.9) to 1/200 and 1/40  
838 respectively and grids were incubated for 1 hour. 3 rinsing steps of 5 minutes each were  
839 performed between the primary and secondary antibody incubation and after the secondary  
840 incubation. The grids were rinsed on filtered milliQ water droplets before drying and imaging.  
841 Image acquisition was performed at 42000x magnification on a FEI Tecnai G2 Spirit TWIN TEM  
842 with axial Eagle 4K camera.

843

#### 844 **Immunolocalization of callose.**

845 Arabidopsis seedlings were grown on  $\frac{1}{2}$  MS 1% sucrose agar plate for 6 days and then incubated  
846 for 3 hours in  $\frac{1}{2}$  MS 1% sucrose liquid medium for control condition or  $\frac{1}{2}$  MS 1% sucrose liquid  
847 medium containing 0.4 M mannitol, prior to fixation. The immunolocalization procedure was done  
848 according to Boutté *et al.* 2014 (Boutté and Grebe, 2014). The callose antibody (Australia  
849 Biosupplies) was diluted to 1/300 in MTSB (Microtubule Stabilizing Buffer) containing 5% of  
850 neutral donkey serum. The secondary anti-mouse antibody coupled to TRITC  
851 (tetramethylrhodamine) was diluted to 1/300 in MTSB buffer containing 5% of neutral donkey  
852 serum. The nuclei were stained using DAPI (4',6-diamidino-2-phénylindole) diluted to 1/200 in  
853 MTSB buffer for 20 minutes. Samples were then imaged with a Zeiss LSM 880 using X40 oil lens.  
854 DAPI excitation was performed using 0,5% of 405 laser power and fluorescence collected at 420-  
855 480 nm; GFP excitation was performed using 5% of 488 nm laser power and fluorescence  
856 emission collected at 505-550 nm; TRITC excitation was performed with 5% of 561 nm power  
857 and fluorescence collected at 569-590 nm. All the parameters were kept the same between  
858 experiments to allow quantifications.

859

860 **Short-term iron deficiency, flg20 and flg22 treatments.** Seeds were sowed in +Fe medium  
861 described in Gruber *et al.*, 2013 and stratified for 2-3 days at 4°C. 5-day-old seedlings were  
862 treated for 4 hours with low iron medium or for 2 hours adding 100uM of FerroZine or sufficiency  
863 in liquid medium described in Gruber *et al.*, 2013 using 12-well plates. Note that after the addition  
864 of FerroZine the pH was adjusted to the same pH=5.7 as the control medium +Fe. However, no  
865 change in the pH was detected in agar adjusted with MES as described earlier and in Gruber *et*  
866 *al.*, 2013. For flg22 treatment, Seeds were sowed in +Fe medium described in Gruber *et al.*, 2013  
867 and stratified for 2-3 days at 4°C. 5-day-old seedlings were treated for 4 hours in iron sufficient  
868 media described in Gruber *et al.*, 2013 supplemented or not with flg22 or flg20.

869

870 **RhoNox-1 staining.** 5-day-old seedlings were treated in ultra-pure distilled water (Fisher  
871 Scientific Invitrogen UltraPure Distilled Water 500 mL Plastic Container – 10977015) called +Fe  
872 condition in order to get rid of any iron trace in water, 50uM of FerroZine was added for 30 minutes.  
873 Then, the plants were transferred to ultra-pure distilled water supplemented with 2.5uM of  
874 RhoNox-1 for 15 minutes (stock solution 5mM; <https://goryochemical.com/>).

875

876 **Perls staining and DAB/H<sub>2</sub>O<sub>2</sub> intensification.** Perls staining and DAB/H<sub>2</sub>O<sub>2</sub> intensification was  
877 performed as described previously (Roschztardt *et al.*, 2009). The embryos were dissected and  
878 isolated from dry seeds previously imbibed in distilled water for 3-4 h. The embryos were then  
879 vacuum infiltrated with Perls stain solution (equal volumes of 4% (v/v) HCl and 4% (w/v) K-  
880 ferrocyanide) for 15 min and incubated for 30 min at room temperature (Stacey *et al.*, 2008). The  
881 DAB intensification was performed as described in Meguro *et al.*, 2007. After washing with

882 distilled water, the embryos were incubated in a methanol solution containing 0.01 M NaN<sub>3</sub> and  
883 0.3% (v/v) H<sub>2</sub>O<sub>2</sub> for 1 h, and then washed with 0.1 M phosphate buffer (pH 7.4). For the  
884 intensification reaction the embryos were incubated between 10 to 30 min in a 0.1 M phosphate  
885 buffer (pH 7.4) solution containing 0.025% (w/v) DAB (Sigma), 0.005% (v/v) H<sub>2</sub>O<sub>2</sub>, and 0.005%  
886 (w/v) CoCl<sub>2</sub> (intensification solution). The reaction was terminated by rinsing with distilled water.  
887

### 888 **GUS Histochemical Assay**

889 Transgenic seedlings carrying *pCYP71A12:GUS* were grown on ½ MS media for 4 days and  
890 seedlings were then grown in 6 well plates containing ½ MS (+Fe or -Fe) liquid media for 16  
891 hours. Seedlings were then treated with 1 µM Flg22 or 1 µM Flg20 for 16 hours. After treatment  
892 with peptides plants were washed with 50 mM sodium phosphate buffer, pH 7. One milliliter of  
893 GUS substrate solution (50 mM sodium phosphate, pH 7, 10 mM EDTA, 0.5 mM K<sub>4</sub>[Fe(CN)<sub>6</sub>],  
894 0.5 mM K<sub>3</sub>[Fe(CN)<sub>6</sub>], 0.5 mM X-Gluc, and 0.01% Silwet L-77) was poured in each well. The plants  
895 were vacuum infiltrated for 5 min and then incubated at 37°C for 4 h. Tissues were observed  
896 using a Discovery V8 microscope (Zeiss). Quantification of GUS signal in root tips of the stained  
897 seedlings was done using Fiji.  
898  
899

900 **Aniline blue staining.** 5 day-old seedlings were incubated for 2h in iron deficient or sufficient  
901 medium(Gruber et al., 2013) with or without DDG and then transferred for 2 hours to 150 mM  
902 K<sub>2</sub>HPO<sub>4</sub> and 0.01% aniline blue in 12-well plates wrapped in aluminum foil for light protection.  
903 Then imaging of the root epidermis in the elongation zone was performed.  
904

905 **Sterol treatments.** For inhibitor experiments, 5-day-old seedlings were transferred to MS agar  
906 plates containing 50 µg/mL Fenpropimorph (<https://www.caymanchem.com/>; stock solution 50  
907 µg/µL in DMSO) or 1 µM Lovastatin ([https://www.tocris.com/products/lovastatin\\_1530](https://www.tocris.com/products/lovastatin_1530); stock  
908 solution 1 mM in DMSO) for 24 hours.  
909

910 **2-deoxy-d-glucose (DDG) treatment.** Seedlings were grown on iron sufficient medium and after  
911 5 days transferred to iron sufficient or low iron medium or flg22 containing medium with or without  
912 DDG (diluted in H<sub>2</sub>O, stock 50mM used at 50µM; [https://www.tocris.com/products/2-deoxy-d-](https://www.tocris.com/products/2-deoxy-d-glucose_4515)  
913 [glucose\\_4515](https://www.tocris.com/products/2-deoxy-d-glucose_4515)).  
914

### 915 **QUANTIFICATION**

916 **Late root growth response.** Plates containing seedlings were scanned from days 5 to 9 after  
917 transfer to different media in order to acquire images for further quantification of the root growth  
918 rate per conditions. Plates were scanned using BRAT software(Slovak et al., 2014) each day and  
919 were stacked together using a macro in Fiji (Macro\_Match\_Align). We then calculated the root  
920 length for every day per genotype in each condition to evaluate the root growth rate in Fiji using  
921 the segmented line. We first calculated the mean of the root growth rate for each days 5 to 6, 6  
922 to 7, 7 to 8, 8 to 9. These values were used to calculate the mean of root growth rate for 3 days  
923 . Then, we divided the mean of root growth rate for 3 days to a given media for each plant by the  
924 mean of root growth rate for 3 days after transfer to the control media for the entire related  
925 genotype. This ratio was used as the late root growth response to low iron levels Every experiment  
926 was repeated twice.  
927

928 **Early root growth response.** Root length for each seedling was recorded for 12 hours taking a  
929 picture every 5 minutes and quantified using a Matlab script (Matlab\_RootWalker). From these  
930 measurements, we plotted the root length from T0 to T12 after transfer. We obtain a curve  
931 representing the root length after transfer from which we calculated the area under the curve using  
932 the following formula “(Root length T1+Root length T2)/2\*(Time T2-Time T1)”. Then, we divided  
933 the value of the area under the curve after transfer for each plant in a given condition by the area  
934 under the curve after transfer to the control media for the entire related genotype. This ratio was  
935 used as the early root growth response to low iron levels. Every experiment has been repeated  
936 three times.

937  
938 **Lateral root density.** 12-day-old seedlings were used for quantification for the lateral root assays.  
939 Plates were scanned using BRAT software(Slovak et al., 2014). A ratio of the number of lateral  
940 roots divided by the root length was applied in order to calculate the lateral root density. This final  
941 value was used for further analysis. This experiment has been repeated twice.

942  
943 **Root meristem size.** 5 days old seedlings were transferred to iron sufficient or deficient medium  
944 (as described in Gruber et al., 2013) that was contained in small chambers used for the early root  
945 growth response (Lab-Tek, Chamberes #1.0 Borosilicate Coverglass System, catalog number:  
946 155361). After 12 hours, the cell wall was stained by placing one drop of propidium iodide 15  $\mu$ M  
947 (10  $\mu$ g/mL in distilled water) on the root tip for 5 minutes. Images were acquired using the stitching  
948 mode on the microscope. The cell size was determined using the Cell-O-Type software in the  
949 cortex cells(French et al., 2012). Every experiment has been repeated three times.

950  
951 **Measuring signal intensities at the plasma membrane.** Confocal images were first denoised  
952 using an auto local threshold applying the Otsu method with a radius of 25 and a median filter  
953 with a radius of 2 in Fiji(Schindelin et al., 2012). In order to remove every single bright pixel on  
954 the generated-binary image the despeckle function was applied. In order to obtain plasma  
955 membrane skeleton, we detected and removed every intracellular dot using the “Analyze  
956 Particles” plugin with the following parameter, size between 0.0001 and 35 000  $\mu$ m<sup>2</sup> and a  
957 circularity between 0.18 and 1. Then, we selected and cropped a zone which only showed a  
958 proper plasma membrane skeleton. We created a selection from the generated-plasma  
959 membrane skeleton and transposed it to the original image to calculate the plasma membrane  
960 intensity. This process has been automatized in a Macro (Macro\_PM\_Intensity). The plasma  
961 membrane intensity value was then divided by the total intensity of the image to normalize the  
962 plasma membrane intensity. An average of 45 cells were used for quantification per root. Every  
963 experiment was repeated three times.

964  
965 **Calculating standard deviation measures of the intensities at the plasma membrane.** The  
966 standard deviation of the apical-basal plasma membrane was calculated using the segmented  
967 lines in Fiji toolbox with a width of 3 pixels. 5 plasma membranes were used per root and the  
968 mean was calculated per root. Every experiment has been repeated three times.

969  
970 **Fluorescence intensity in the root tip during time lapse experiments.** To acquire images, z-  
971 stacks with a stepsize of 50  $\mu$ m were performed coupled with the stitching mode. To determine  
972 the variation of our translational and transcriptional reporters under different condition, we  
973 measured the signal intensity in the root tip over time using Fiji. Prior analysis, confocal images  
974 were stitched, and we generate the maximum intensity projection. We drew a region of interest of  
975 the same size in the x and y dimensions, corresponding to the width of the root and in length  
976 corresponding to the basal meristem, transition and elongation zones. In this region, for each time  
977 point we determined the mean grey value. Note that this value is normalized since for each root

978 the same area has been kept between conditions and genotypes. Every experiment was repeated  
979 three times.

980  
981 **Polarity Index.** 5 days old seedlings of transgenic lines were analyzed to determine the “Polarity  
982 index” in root tip epidermis. “Polarity index” is the ratio between the fluorescence intensity (Mean  
983 Grey Value function of Fiji software) measured at the PM apical/basal side and PM lateral sides  
984 (Line width=3). We selected only cells for which the PM at each pole (apical, basal and laterals)  
985 were easily viewable and we selected cells that were entering elongation (at least as long as wide,  
986 but no more than twice as long as wide). Quantification was conducted in 100 cells over more  
987 than 15 independent plants. This Polarity index reveals the degree of polarity of the fluorescent  
988 reporters between the apical/basal side and lateral sides of the PM. Every experiment was  
989 repeated three times

990  
991 **Integrated Nuclear and fluorescence signal density of transcriptional reporter lines.** To  
992 acquire images, z-stacks with a stepsize of 50  $\mu\text{m}$  were performed coupled with the stitching  
993 mode. Then, we generated the maximum intensity projection for the z-dimension and then  
994 binarized the images using the auto local threshold Bernsen with a radius of 15. The despeckle  
995 and erode functions were subsequently used to remove background artefacts. The nucleus in this  
996 region were selected using the analyze particles function with the settings for size of 15 and 700  
997  $\mu\text{m}^2$  and for circularity 0.25 to 1.00. The regions selected were used on the original picture for  
998 determining the fluorescence intensity in each nucleus. Then the average nuclear integrated  
999 density was calculated per root in order to normalize the intensity by the total root area. This  
1000 process has been automatized in a macro in Fiji (Macro\_Nuclear\_Signal\_Intensity). From the  
1001 same images, the number of nuclei was calculated and the root area was detected using the  
1002 plugin “Wavelet a trou” (<http://www.ens-lyon.fr/RDP/SiCE/METHODS.html>)(Bayle et al., 2017).  
1003 The number of nuclei detected was then divided by the area of the respective root to determine  
1004 the nuclear density per root. This process was automatized using a Fiji macro  
1005 (Macro\_Nuclear\_Density). Every experiment was repeated three times.

1006  
1007 **IRT1 promotor activation in time lapse serie.** At each time point, roots showing fluorescence  
1008 signal in the apical 3 mm of the root were counted. Roots that were already showing activation in  
1009 this zone or showed slight signals at timepoint 0 were removed from the analysis. Then the  
1010 number of roots that started to show a fluorescent signal after timepoint 0, was divided by the  
1011 number of total roots observed in this experiment and multiplied by 100 to obtain the percentage  
1012 of root showing pIRT1 activation in the apical 3 mm of the root. Every experiment was repeated  
1013 three times.

1014 **Distance of signals from the Quiescent Center in the SRF3 transcriptional reporter line.**  
1015 Based on the propidium iodide staining, the Quiescent Center (QC) region was determined by its  
1016 morphology. Then, using the straight-line option in Fiji, a line was traced from the QC to the first  
1017 appearance of a clear, bright signal that reported pSRF3 activity. The distance along this line was  
1018 calculated to determine the distance from the QC to the first cell expressing  $pSRF3$  in the  
1019 elongation zone. Every experiment was repeated three times.

1020 **DRONPA-s diffusion.** After activation of the DRONPA-s reporter, the signal intensity using the  
1021 integrated density of the signal was calculated in the activated cells as well as the adjacent upper  
1022 and lower cells using Fiji. The signal intensity once again was calculated in the same regions 6  
1023 minutes after the activation. To account for photo bleaching, these values were normalized by  
1024 dividing the DRONPA-s signal intensity after and post bleach in a zone where DRONPA-s was  
1025 visible in both images. The average of the normalized integrated density in the surrounding cells  
1026 was calculated, averaging the values obtained in upper and lower cells. Finally, the ratio of the



1027 normalized integrated density after and post bleach was calculated by dividing both values to  
1028 obtain the DRONPA-s normalized fluorescence intensity. Every experiment was repeated three  
1029 times.

1030  
1031 **pSUC2::GFP diffusion.** In order to evaluate the diffusion of the GFP protein, a ROI of about 1800  
1032  $\mu\text{m}^2$  was drawn at the root tip of each root using Fiji. The mean gray value was calculated and  
1033 divided by the corresponding area to normalize the value. Every experiment was repeated three  
1034 times.

1035  
1036 **RhoNOx-1 signal intensity.** The root area was detected using the plugin “Wavelet a trou”  
1037 (<http://www.ens-lyon.fr/RDP/SiCE/METHODS.html>)(Bayle et al., 2017) and then in this area the  
1038 mean gray value was calculated and divided by the size of the size of the area to obtain the  
1039 normalized signal intensity. This process has been automatize on a Fiji Macro, Macro\_RhoNox-  
1040 1

1041  
1042 **Aniline blue fluorescence intensity.** Using Fiji, the mean gray value of 10 plasma membranes  
1043 in the apical-basal side of the epidermis in the transition-elongation zone was calculated with the  
1044 segmented line option with 3 pixels wide. The mean of these values was then divided by the mean  
1045 gray value in the total area where the plasma membrane signal has been calculated in order to  
1046 normalize the value due to differential strength of the staining. This final value was used for further  
1047 analysis. Every experiment was repeated three times.

1048  
1049  
1050 **Anti-callose antibody fluorescence intensity**  
1051 Callose deposition was quantified using the Fiji software. Callose fluorescence intensity was  
1052 measured at the apico-basal cell walls of root epidermal and cortex cells using the segmented  
1053 line with a width of 3 pixels. A total of 8 to 10 cell walls were measured per roots and used to  
1054 calculate the average of anti-callose fluorescence intensity per root. Between 5 to 20 roots per  
1055 transgenic lines and conditions were used in two independent biological replicates were used.

1056  
1057 **Immunogold**  
1058 The number of gold particles in the EM micrographs were quantified using Fiji software. The  
1059 number of particles were counted manually in each compartment; e.g. Cytosol, Cell wall, PM and  
1060 PD; and then reported relative to the surface of cytosol or cell wall ( $/\mu\text{m}^2$ ), to the length of PM  
1061 ( $/\mu\text{m}$ ) and to individual PD. A total of 25 to 50 micrographs were analyzed for each line and  
1062 conditions. Two biological replicates were used.

## 1063 1064 **STATISTICS**

1065  
1066 Each experiment has been repeated independently at least twice, as in every cases the same  
1067 trend has been recorded for independent experiment, the data the different has been pooled for  
1068 further statistical analysis. Each sample were subjected to four different normality tests (Jarque-  
1069 Bera, Lilliefors, Anderson-Darling and Shapiro-Wilk), sample were considered as a Gaussian  
1070 distribution when at least one test was significant ( $p=0.05$ ) using Xlstat.

1071  
1072 - As a normal distribution was observed a one-way ANOVA coupled with post hoc Tukey  
1073 honestly significant difference (HSD) test was performed ( $p=0.05$ ) using R software or  
1074 Xlstat. Figures: 1F, 2A, 3A, 4A, 4B, 4C, 4D, 5E, 6B, S2C, S2D, S2F, S3C, S4A, S4B, S8C,  
1075 S9D, S11A, S11B, S12B, S12C, S12D, S12E, S13D, S13E, S17C, S17D.

1076

- 1077 - As a normal distribution was observed at one-way ANOVA coupled with post hoc Lowest  
1078 significant difference (LSD) test was performed ( $p=0.05$ ) using Xlstat: 5E.  
1079  
1080 - As a normal distribution was observed an independent two-ways student test was  
1081 performed ( $p=0.05$ ) using Xlstat. Figures: 1C, 1D, 2D, 5D, 6C, 6D, S2E, S4C, S7B (left  
1082 panel), S7C, S10A, S10B, S10C, S11C, S14A, S14C (both panel).  
1083  
1084 - As a normal distribution was not observed at two-ways Kruskal-Wallis coupled with post  
1085 hoc Steel-Dwass-Critchlow-Fligner procedure was performed ( $p=0.05$ ) using Xlstat.  
1086 Figure: S9C.  
1087  
1088 - As a normal distribution was not observed a two-ways Mann-Whitney test was performed  
1089 ( $p=0.05$ ) using Xlstat. Figures: S7B (right panel)  
1090

1091 For time lapse analysis SAS software was used based on a mixed effect model ( $p<0.05$ ) to test  
1092 the statistical significance. Figures: 2C  
1093

## 1094 CLONING

### 1095 **pIRT1 transcriptional reporter line:**

1096 To generate the transcriptional pIRT1::NLS-2xYPet reporter line, the *IRT1* promoter (2.6 kb) was  
1097 cloned at *SalI* and *BamHI* restriction sites using the primers pIRT1 Sal\_F and pIRT1 Bam\_R in  
1098 the pBJ36 vector carrying two in frame copies of the YPet yellow fluorescent protein fused to  
1099 SV40 nuclear localization signal (kind gift of Dr. Jeff D.B. Long, UCLA). The *pIRT1::2xYPet-NLS*  
1100 cassette was digested with *NotI* and cloned in the pART27 binary vector (Gleave, 1992). Note that  
1101 About 20 independent T1 lines were isolated and between three to six representative mono-  
1102 insertion lines showing strong activation of *IRT1* promoter in the root epidermis upon low iron, as  
1103 previously described (Vert et al., 2002), were selected in T2.  
1104  
1105

### 1106 **SRF3 constructs in Ler**

1107 To generate pSRF3::SRF3g-GFP from *Ler* background, the SRF3 gene and its native promoter  
1108 (-1492 nt from the transcription start) was amplified by PCR using genomic DNA as template and  
1109 the primers attB1-SRF3Ler\_F and attB2-SRF3Ler\_R. The resulting amplicon was purified,  
1110 sequenced and subcloned into pDONR221 by Gateway BP recombination, following  
1111 manufacturer's instructions. To generate the C-terminus GFP fusion, the pSRF3::SRF3g fragment  
1112 was cloned into the binary vector pGWB450 (Nakagawa et al., 2007) by Gateway LR  
1113 recombination.  
1114  
1115

### 1116 **SRF3 constructs (entry vectors):**

1117 The full-length coding sequence of SRF3 (At4g03390) was amplified by RT-PCR using 7-day old  
1118 Arabidopsis seedlings cDNA as template and the SRF3\_CDS\_p221\_F and  
1119 PSRF3\_CDS\_p221\_noSTOP\_R primers. The corresponding PCR product was recombined into  
1120 pDONR221 vector by BP reaction to give SRF3cds-noSTOP/pDONR221. To remove the SRF3  
1121 extracellular domain the primers SRF3\_kinase\_p221\_F and SRF3\_kinase\_p221\_R. The  
1122 corresponding PCR product was recombined into pDONR221 vector by BP reaction to give  
1123 SRF3cds $\Delta$ extraC\_p221.  
1124 To remove the SRF3 kinase domain 5' phosphorylated primers were used SRF3\_ $\Delta$ Kinase2-5'\_F  
1125 and SRF3\_ $\Delta$ Kinase-5'\_R followed by a ligation to give SRF3cds $\Delta$ Kinase\_pDONR221.

1126 SRF3 mutant impaired in the kinase activity was obtained by site directed mutagenesis using  
1127 SRF3-cds\_mutKD\_p221\_F and SRF3-cds\_mutKD\_p221\_R to give SRF3cds  
1128 KDmut\_pDONR221.

1129

1130

1131 **Promoters and fluorescent proteins (entry vectors):**

1132 The *SRF3* promoter (5078bp upstream of 5'UTR until the 3'UTR of the previous gene) was cloned  
1133 using the gibson cloning method (<https://www.neb.com/applications/cloning-and-synthetic-biology/dna-assembly-and-cloning/gibson-assembly#tabselect3>) with the following primers,  
1134 Insert\_pSRF3\_F, Insert\_pSRF3\_R and Backbone\_pSRF3\_F and Backbone\_pSRF3\_R  
1135 introduced into the P4P1R vector (life technologies [www.lifetechnologies.com/](http://www.lifetechnologies.com/)) to give  
1136 SRF3prom/pDONR4P1R.

1137

1138  
1139 The fluorescent mSCARLET protein was synthesized (GeneArt, [www.thermofischer.com](http://www.thermofischer.com)),  
1140 amplified with attB2r and attB3 gateway sites using the mSCARLET\_F and  
1141 mSCARLETwSTOP\_R primers, and then recombined into pDONRP2R-P3 by BP reaction to yield  
1142 the mSCARLET/pDONRP2R-P3 entry vector.

1143

1144

1145 **References:**

1146

1147 Alcázar, R., García, A.V., Kronholm, I., Meaux, J. de, Koornneef, M., Parker, J.E., and  
1148 Reymond, M. (2010). Natural variation at Strubbelig Receptor Kinase 3 drives immune-triggered  
1149 incompatibilities between *Arabidopsis thaliana* accessions. *Nature Genetics* *42*, 1135–1139.

1150 Anders, S., Pyl, P.T., and Huber, W. (2014). HTSeq—a Python framework to work with high-  
1151 throughput sequencing data. *Bioinformatics* *31*, 166–169.

1152 Atwell, S., Huang, Y.S., Vilhjálmsson, B.J., Willems, G., Horton, M., Li, Y., Meng, D., Platt, A.,  
1153 Tarone, A.M., Hu, T.T., et al. (2010). Genome-wide association study of 107 phenotypes in  
1154 *Arabidopsis thaliana* inbred lines. *Nature* *465*, 627–631.

1155 Aznar, A., Chen, N.W.G., Rigault, M., Riache, N., Joseph, D., Desmaële, D., Mouille, G., Boutet,  
1156 S., Soubigou-Taconnat, L., Renou, J.-P., et al. (2014). Scavenging Iron: A Novel Mechanism of  
1157 Plant Immunity Activation by Microbial Siderophores1[C][W]. *Plant Physiol* *164*, 2167–2183.

1158 Bai, L., Qiao, M., Zheng, R., Deng, C., Mei, S., and Chen, W. (2016). Phylogenomic analysis of  
1159 transferrin family from animals and plants. *Comp Biochem Physiol Part D Genomics Proteomics*  
1160 *17*, 1–8.

1161 Bayle, V., Platre, M.P., and Jaillais, Y. (2017). Automatic Quantification of the Number of  
1162 Intracellular Compartments in *Arabidopsis thaliana* Root Cells. *BIO-PROTOCOL* *7*.

1163 Belkhadir, Y., and Jaillais, Y. (2015). The molecular circuitry of brassinosteroid signaling. *New*  
1164 *Phytologist* *206*, 522–540.

1165 Benitez-Alfonso, Y., Faulkner, C., Pendle, A., Miyashima, S., Helariutta, Y., and Maule, A.  
1166 (2013). Symplastic Intercellular Connectivity Regulates Lateral Root Patterning. *Developmental*  
1167 *Cell* *26*, 136–147.

- 1168 Benjamini, Y., and Yekutieli, D. (2001). The control of the false discovery rate in multiple testing  
1169 under dependency. *Ann. Statist.* *29*, 1165–1188.
- 1170 Benschop, J.J., Mohammed, S., and Slijper, M. (2007). Quantitative Phosphoproteomics of  
1171 Early Elicitor Signaling in Arabidopsis. *Mol Cell Proteomics* *17*.
- 1172 Boutté, Y., and Grebe, M. (2014). Immunocytochemical fluorescent in situ visualization of  
1173 proteins in arabidopsis. *Methods in Molecular Biology* *1062*, 453–472.
- 1174 Brachi, B., Faure, N., Horton, M., Flahauw, E., Vazquez, A., Nordborg, M., Bergelson, J.,  
1175 Cuguen, J., and Roux, F. (2010). Linkage and Association Mapping of Arabidopsis thaliana  
1176 Flowering Time in Nature. *PLOS Genetics* *6*, e1000940.
- 1177 Cassat, J.E., and Skaar, E.P. (2013). Iron in Infection and Immunity. *Cell Host & Microbe* *13*,  
1178 509–519.
- 1179 Cheval, C., Samwald, S., Johnston, M.G., Keijzer, J. de, Breakspear, A., Liu, X., Bellandi, A.,  
1180 Kadota, Y., Zipfel, C., and Faulkner, C. (2020). Chitin perception in plasmodesmata  
1181 characterizes submembrane immune-signaling specificity in plants. *PNAS* *117*, 9621–9629.
- 1182 Clough, S.J., and Bent, A.F. (1998). Floral dip: a simplified method for Agrobacterium-mediated  
1183 transformation of Arabidopsis thaliana. *Plant J* *16*, 735–743.
- 1184 Cutler, S.R., Ehrhardt, D.W., Griffitts, J.S., and Somerville, C.R. (2000). Random GFP::cDNA  
1185 fusions enable visualization of subcellular structures in cells of Arabidopsis at a high frequency.  
1186 *PNAS* *97*, 3718–3723.
- 1187 Deák, M., Horváth, G.V., Davletova, S., Török, K., Sass, L., Vass, I., Barna, B., Király, Z., and  
1188 Dudits, D. (1999). Plants ectopically expressing the iron-binding protein, ferritin, are tolerant to  
1189 oxidative damage and pathogens. *Nat. Biotechnol.* *17*, 192–196.
- 1190 Denyer, T., Xiaoli, M., Klesen, S., Scacchi, E., Nieselt, K., and Timmermans, M.C.P. (2019).  
1191 Spatiotemporal Developmental Trajectories in the Arabidopsis Root Revealed Using High-  
1192 Throughput Single-Cell RNA Sequencing. *Developmental Cell* *49*, 19.
- 1193 Dinneny, J.R., Long, T.A., Wang, J.Y., Jung, J.W., Mace, D., Pointer, S., Barron, C., Brady,  
1194 S.M., Schiefelbein, J., and Benfey, P.N. (2008). Cell identity mediates the response of  
1195 Arabidopsis roots to abiotic stress. *Science* *320*, 942–945.
- 1196 Durrett, T.P., Gassmann, W., and Rogers, E.E. (2007). The FRD3-mediated efflux of citrate into  
1197 the root vasculature is necessary for efficient iron translocation. *Plant Physiol* *144*, 197–205.
- 1198 Fichman, Y., Myers, R.J., Grant, D.G., and Mittler, R. (2021). Plasmodesmata-localized proteins  
1199 and ROS orchestrate light-induced rapid systemic signaling in Arabidopsis. *Sci. Signal.* *14*.
- 1200 French, A.P., Wilson, M.H., Kenobi, K., Dietrich, D., Voss, U., Ubeda-Tomas, S., Pridmore, T.P.,  
1201 and Wells, D.M. (2012). Identifying biological landmarks using a novel cell measuring image  
1202 analysis tool: Cell-o-Tape. *Plant Methods* *8*, 7.
- 1203 Ganz, T., and Nemeth, E. (2015). Iron homeostasis in host defence and inflammation. *Nat Rev*  
1204 *Immunol* *15*, 500–510.



- 1205 García, M.J., Romera, F.J., Stacey, M.G., Stacey, G., Villar, E., Alcántara, E., and Pérez-  
1206 Vicente, R. (2013). Shoot to root communication is necessary to control the expression of iron-  
1207 acquisition genes in Strategy I plants. *Planta* 237, 65–75.
- 1208 Gerlitz, N., Gerum, R., Sauer, N., and Stadler, R. (2018). Photoinducible DRONPA-s: a new tool  
1209 for investigating cell-cell connectivity. *Plant J* 94, 751–766.
- 1210 Gleave, A.P. (1992). A versatile binary vector system with a T-DNA organisational structure  
1211 conducive to efficient integration of cloned DNA into the plant genome. *Plant Mol Biol* 20, 1203–  
1212 1207.
- 1213 Grillet, L., Lan, P., Li, W., Mokkapati, G., and Schmidt, W. (2018). IRON MAN is a ubiquitous  
1214 family of peptides that control iron transport in plants. *Nature Plants* 4, 953–963.
- 1215 Grison, M.S., Brocard, L., Fouillen, L., Nicolas, W., Wewer, V., Dörmann, P., Nacir, H., Benitez-  
1216 Alfonso, Y., Claverol, S., Germain, V., et al. (2015). Specific Membrane Lipid Composition Is  
1217 Important for Plasmodesmata Function in Arabidopsis. *The Plant Cell* 27, 1228–1250.
- 1218 Grison, M.S., Kirk, P., Brault, M.L., Wu, X.N., Schulze, W.X., Benitez-Alfonso, Y., Immel, F., and  
1219 Bayer, E.M. (2019). Plasma Membrane-Associated Receptor-like Kinases Relocalize to  
1220 Plasmodesmata in Response to Osmotic Stress. *Plant Physiology* 181, 142–160.
- 1221 Groen, S.C., Whiteman, N.K., Bahrami, A.K., Wilczek, A.M., Cui, J., Russell, J.A., Cibrian-  
1222 Jaramillo, A., Butler, I.A., Rana, J.D., Huang, G.-H., et al. (2013). Pathogen-Triggered Ethylene  
1223 Signaling Mediates Systemic-Induced Susceptibility to Herbivory in Arabidopsis[W]. *Plant Cell*  
1224 25, 4755–4766.
- 1225 Gruber, B.D., Giehl, R.F.H., Friedel, S., and von Wirén, N. (2013). Plasticity of the Arabidopsis  
1226 Root System under Nutrient Deficiencies. *Plant Physiol.* 163, 161–179.
- 1227 Han, X., Hyun, T.K., Zhang, M., Kumar, R., Koh, E., Kang, B.-H., Lucas, W.J., and Kim, J.-Y.  
1228 (2014). Auxin-Callose-Mediated Plasmodesmal Gating Is Essential for Tropic Auxin Gradient  
1229 Formation and Signaling. *Developmental Cell* 28, 132–146.
- 1230 Haydon, M.J., Kawachi, M., Wirtz, M., Hillmer, S., Hell, R., and Krämer, U. (2012). Vacuolar  
1231 nicotianamine has critical and distinct roles under iron deficiency and for zinc sequestration in  
1232 Arabidopsis. *Plant Cell* 24, 724–737.
- 1233 He, P., Shan, L., Lin, N.-C., Martin, G.B., Kemmerling, B., Nürnberger, T., and Sheen, J. (2006).  
1234 Specific Bacterial Suppressors of MAMP Signaling Upstream of MAPKKK in Arabidopsis Innate  
1235 Immunity. *Cell* 125, 563–575.
- 1236 Higashi, K., Ishiga, Y., Inagaki, Y., Toyoda, K., Shiraishi, T., and Ichinose, Y. (2008). Modulation  
1237 of defense signal transduction by flagellin-induced WRKY41 transcription factor in Arabidopsis  
1238 thaliana. *Mol Genet Genomics* 279, 303–312.
- 1239 Hindt, M.N., Akmakjian, G.Z., Pivarski, K.L., Punshon, T., Baxter, I., Salt, D.E., and Guerinot,  
1240 M.L. (2017). BRUTUS and its paralogs, BTS LIKE1 and BTS LIKE2, encode important negative  
1241 regulators of the iron deficiency response in Arabidopsis thaliana. *Metallomics* 9, 876–890.

- 1242 Hirayama, T. (2018). Development of Chemical Tools for Imaging of Fe(II) Ions in Living Cells: A  
1243 Review. *Acta Histochem Cytochem* 51, 137–143.
- 1244 Hohmann, U., Lau, K., and Hothorn, M. (2017). The Structural Basis of Ligand Perception and  
1245 Signal Activation by Receptor Kinases (Annual Reviews).
- 1246 Horton, M.W., Hancock, A.M., Huang, Y.S., Toomajian, C., Atwell, S., Auton, A., Mulyati, N.W.,  
1247 Platt, A., Sperone, F.G., Vilhjálmsón, B.J., et al. (2012). Genome-wide patterns of genetic  
1248 variation in worldwide *Arabidopsis thaliana* accessions from the RegMap panel. *Nature*  
1249 *Genetics* 44, 212–216.
- 1250 Huang, D., Sun, Y., Ma, Z., Ke, M., Cui, Y., Chen, Z., Chen, C., Ji, C., Tran, T.M., Yang, L., et  
1251 al. (2019). Salicylic acid-mediated plasmodesmal closure via Remorin-dependent lipid  
1252 organization. *PNAS* 116, 21274–21284.
- 1253 Iatsenko, I., Marra, A., Boquete, J.-P., Peña, J., and Lemaitre, B. (2020). Iron sequestration by  
1254 transferrin 1 mediates nutritional immunity in *Drosophila melanogaster*. *PNAS* 117, 7317–7325.
- 1255 Jaffe, M.J., and Leopold, A.C. (1984). Callose deposition during gravitropism of *Zea mays* and  
1256 *Pisum sativum* and its inhibition by 2-deoxy-D-glucose. *Planta* 161, 20–26.
- 1257 Jaillais, Y., and Vert, G. (2016). Brassinosteroid signaling and BRI1 dynamics went  
1258 underground. *Current Opinion in Plant Biology* 33, 92–100.
- 1259 Jaillais, Y., Hothorn, M., Belkhadir, Y., Dabi, T., Nimchuk, Z.L., Meyerowitz, E.M., and Chory, J.  
1260 (2011). Tyrosine phosphorylation controls brassinosteroid receptor activation by triggering  
1261 membrane release of its kinase inhibitor. *Genes Dev.* 25, 232–237.
- 1262 Kang, H.M., Zaitlen, N.A., Wade, C.M., Kirby, A., Heckerman, D., Daly, M.J., and Eskin, E.  
1263 (2008). Efficient Control of Population Structure in Model Organism Association Mapping.  
1264 *Genetics* 178, 1709–1723.
- 1265 Karimi, M., Depicker, A., and Hilson, P. (2007). Recombinational Cloning with Plant Gateway  
1266 Vectors. *Plant Physiology* 145, 1144–1154.
- 1267 Khan, M.A., Castro-Guerrero, N.A., McInturf, S.A., Nguyen, N.T., Dame, A.N., Wang, J.,  
1268 Bindbeutel, R.K., Joshi, T., Jurisson, S.S., Nusinow, D.A., et al. (2018). Changes in iron  
1269 availability in *Arabidopsis* are rapidly sensed in the leaf vasculature and impaired sensing leads  
1270 to opposite transcriptional programs in leaves and roots. *Plant Cell Environ* 41, 2263–2276.
- 1271 Kim, D., Pertea, G., Trapnell, C., Pimentel, H., Kelley, R., and Salzberg, S.L. (2013). TopHat2:  
1272 accurate alignment of transcriptomes in the presence of insertions, deletions and gene fusions.  
1273 *Genome Biology* 14, R36.
- 1274 Kim, S.A., Punshon, T., Lanzirrotti, A., Li, L., Alonso, J.M., Ecker, J.R., Kaplan, J., and Guerinot,  
1275 M.L. (2006). Localization of iron in *Arabidopsis* seed requires the vacuolar membrane  
1276 transporter VIT1. *Science* 314, 1295–1298.
- 1277 Klatte, M., Schuler, M., Wirtz, M., Fink-Straube, C., Hell, R., and Bauer, P. (2009). The Analysis  
1278 of *Arabidopsis* Nicotianamine Synthase Mutants Reveals Functions for Nicotianamine in Seed  
1279 Iron Loading and Iron Deficiency Responses. *Plant Physiology* 150, 257.

- 1280 Kobayashi, T., and Nishizawa, N.K. (2012). Iron Uptake, Translocation, and Regulation in  
1281 Higher Plants. *Annual Review of Plant Biology* 63, 131–152.
- 1282 Koen, E., Besson-Bard, A., Duc, C., Astier, J., Gravot, A., Richaud, P., Lamotte, O., Boucherez,  
1283 J., Gaymard, F., and Wendehenne, D. (2013). *Arabidopsis thaliana* nicotianamine synthase 4 is  
1284 required for proper response to iron deficiency and to cadmium exposure. *Plant Sci* 209, 1–11.
- 1285 Kramer, J., Özkaya, Ö., and Kümmerli, R. (2020). Bacterial siderophores in community and host  
1286 interactions. *Nature Reviews Microbiology* 18, 152–163.
- 1287 Kreps, J.A., Wu, Y., Chang, H.-S., Zhu, T., Wang, X., and Harper, J.F. (2002). Transcriptome  
1288 Changes for *Arabidopsis* in Response to Salt, Osmotic, and Cold Stress. *Plant Physiol* 130,  
1289 2129–2141.
- 1290 Kumar, R.K., Chu, H.-H., Abundis, C., Vasques, K., Rodriguez, D.C., Chia, J.-C., Huang, R.,  
1291 Vatamaniuk, O.K., and Walker, E.L. (2017). Iron-Nicotianamine Transporters Are Required for  
1292 Proper Long Distance Iron Signaling. *Plant Physiology* 175, 15.
- 1293 Lee, J.-Y., Wang, X., Cui, W., Sager, R., Modla, S., Czymmek, K., Zybaliov, B., Wijk, K. van,  
1294 Zhang, C., Lu, H., et al. (2011a). A Plasmodesmata-Localized Protein Mediates Crosstalk  
1295 between Cell-to-Cell Communication and Innate Immunity in *Arabidopsis*. *The Plant Cell Online*  
1296 23, 3353–3373.
- 1297 Lee, J.-Y., Wang, X., Cui, W., Sager, R., Modla, S., Czymmek, K., Zybaliov, B., van Wijk, K.,  
1298 Zhang, C., Lu, H., et al. (2011b). A Plasmodesmata-Localized Protein Mediates Crosstalk  
1299 between Cell-to-Cell Communication and Innate Immunity in *Arabidopsis*. *The Plant Cell* 23, 22.
- 1300 Lim, G.-H., Shine, M.B., de Lorenzo, L., Yu, K., Cui, W., Navarre, D., Hunt, A.G., Lee, J.-Y.,  
1301 Kachroo, A., and Kachroo, P. (2016). Plasmodesmata Localizing Proteins Regulate Transport  
1302 and Signaling during Systemic Acquired Immunity in Plants. *Cell Host & Microbe* 19, 541–549.
- 1303 Marquès-Bueno, M.M., Morao, A.K., Cayrel, A., Platre, M.P., Barberon, M., Caillieux, E., Colot,  
1304 V., Jaillais, Y., Roudier, F., and Vert, G. (2016). A versatile Multisite Gateway-compatible  
1305 promoter and transgenic line collection for cell type-specific functional genomics in *Arabidopsis*.  
1306 *The Plant Journal* 85, 320–333.
- 1307 Mendoza-Cózatl, D.G., Xie, Q., Akmakjian, G.Z., Jobe, T.O., Patel, A., Stacey, M.G., Song, L.,  
1308 Demoin, D.W., Jurisson, S.S., Stacey, G., et al. (2014). OPT3 Is a Component of the Iron-  
1309 Signaling Network between Leaves and Roots and Misregulation of OPT3 Leads to an Over-  
1310 Accumulation of Cadmium in Seeds. *Mol Plant* 7, 1455–1469.
- 1311 Millet, Y.A., Danna, C.H., Clay, N.K., Songnuan, W., and Simon, M.D. (2010). Innate Immune  
1312 Responses Activated in *Arabidopsis* Roots by Microbe-Associated Molecular Patterns W OA.  
1313 *The Plant Cell* 22, 18.
- 1314 Nemoto, K., Seto, T., Takahashi, H., Nozawa, A., Seki, M., Shinozaki, K., Endo, Y., and  
1315 Sawasaki, T. (2011). Autophosphorylation profiling of *Arabidopsis* protein kinases using the cell-  
1316 free system. *Phytochemistry* 72, 1136–1144.

- 1317 Nicolas, W.J., Grison, M.S., Trépout, S., Gaston, A., Fouché, M., Cordelières, F.P., Oparka, K.,  
1318 Tilsner, J., Brocard, L., and Bayer, E.M. (2017). Architecture and permeability of post-  
1319 cytokinesis plasmodesmata lacking cytoplasmic sleeves. *Nat Plants* 3, 17082.
- 1320 Palmer, C.M., Hindt, M.N., Schmidt, H., Clemens, S., and Guerinot, M.L. (2013). MYB10 and  
1321 MYB72 Are Required for Growth under Iron-Limiting Conditions. *PLOS Genetics* 9, e1003953.
- 1322 Perraki, A., DeFalco, T.A., Derbyshire, P., Avila, J., Séré, D., Sklenar, J., Qi, X., Stransfeld, L.,  
1323 Schwessinger, B., Kadota, Y., et al. (2018). Phosphocode-dependent functional dichotomy of a  
1324 common co-receptor in plant signalling. *Nature* 561, 248–252.
- 1325 Platre, M.P., Noack, L.C., Doumane, M., Bayle, V., Simon, M.L.A., Maneta-Peyret, L., Fouillen,  
1326 L., Stanislas, T., Armengot, L., Pejchar, P., et al. (2018). A Combinatorial Lipid Code Shapes  
1327 the Electrostatic Landscape of Plant Endomembranes. *Dev Cell* 45, 465-480.e11.
- 1328 Robinson, M.D., McCarthy, D.J., and Smyth, G.K. (2009). edgeR: a Bioconductor package for  
1329 differential expression analysis of digital gene expression data. *Bioinformatics* 26, 139–140.
- 1330 Rutschow, H.L., Baskin, T.I., and Kramer, E.M. (2011). Regulation of Solute Flux through  
1331 Plasmodesmata in the Root Meristem. *Plant Physiology* 155, 1817–1826.
- 1332 Sager, R.E., and Lee, J.-Y. (2018). Plasmodesmata at a glance. *J Cell Sci* 131.
- 1333 Sager, R., Wang, X., Hill, K., Yoo, B.-C., Caplan, J., Nedo, A., Tran, T., Bennett, M.J., and Lee,  
1334 J.-Y. (2020). Auxin-dependent control of a plasmodesmal regulator creates a negative feedback  
1335 loop modulating lateral root emergence. *Nature Communications* 11, 1–10.
- 1336 Satbhai, S.B., Setzer, C., Freynschlag, F., Slovak, R., Kerdaffrec, E., and Busch, W. (2017).  
1337 Natural allelic variation of FRO2 modulates Arabidopsis root growth under iron deficiency.  
1338 *Nature Communications* 8, 15603.
- 1339 Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch,  
1340 S., Rueden, C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-source platform for  
1341 biological-image analysis. *Nat Methods* 9, 676–682.
- 1342 Segond, D., Dellagi, A., Lanquar, V., Rigault, M., Patrit, O., Thomine, S., and Expert, D. (2009).  
1343 NRAMP genes function in Arabidopsis thaliana resistance to Erwinia chrysanthemi infection.  
1344 *The Plant Journal* 58, 195–207.
- 1345 Selote, D., Samira, R., Matthiadis, A., Gillikin, J.W., and Long, T.A. (2015). Iron-Binding E3  
1346 Ligase Mediates Iron Response in Plants by Targeting Basic Helix-Loop-Helix Transcription  
1347 Factors. *Plant Physiol.* 167, 273–286.
- 1348 Seren, Ü., Vilhjálmsson, B.J., Horton, M.W., Meng, D., Forai, P., Huang, Y.S., Long, Q., Segura,  
1349 V., and Nordborg, M. (2012). GWAPP: A Web Application for Genome-Wide Association  
1350 Mapping in Arabidopsis. *The Plant Cell* 24, 4793–4805.
- 1351 Shikanai, Y., Yoshida, R., Hirano, T., Enomoto, Y., Li, B., Asada, M., Yamagami, M.,  
1352 Yamaguchi, K., Shigenobu, S., Tabata, R., et al. (2020). Callose Synthesis Suppresses Cell  
1353 Death Induced by Low-Calcium Conditions in Leaves. *Plant Physiology* 182, 2199–2212.



- 1354 Simon, M.L.A., Platre, M.P., Assil, S., van Wijk, R., Chen, W.Y., Chory, J., Dreux, M., Munnik,  
1355 T., and Jaillais, Y. (2014). A multi-colour/multi-affinity marker set to visualize phosphoinositide  
1356 dynamics in Arabidopsis. *Plant J* 77, 322–337.
- 1357 Slovak, R., Goschl, C., Su, X., Shimotani, K., Shiina, T., and Busch, W. (2014). A Scalable  
1358 Open-Source Pipeline for Large-Scale Root Phenotyping of Arabidopsis. *The Plant Cell* 26,  
1359 2390–2403.
- 1360 Smakowska-Luzan, E., Mott, G.A., Parys, K., Stegmann, M., Howton, T.C., Layeghifard, M.,  
1361 Neuhold, J., Lehner, A., Kong, J., Grünwald, K., et al. (2018). An extracellular network of  
1362 Arabidopsis leucine-rich repeat receptor kinases. *Nature* 553, 342–346.
- 1363 Stonebloom, S., Brunkard, J.O., Cheung, A.C., Jiang, K., Feldman, L., and Zambryski, P.  
1364 (2012). Redox States of Plastids and Mitochondria Differentially Regulate Intercellular Transport  
1365 via Plasmodesmata. *Plant Physiology* 158, 190–199.
- 1366 Stringlis, I.A., Proietti, S., Hickman, R., Verk, M.C.V., Zamioudis, C., and Pieterse, C.M.J.  
1367 (2018). Root transcriptional dynamics induced by beneficial rhizobacteria and microbial immune  
1368 elicitors reveal signatures of adaptation to mutualists. *The Plant Journal* 93, 166–180.
- 1369 Tang, D., Wang, G., and Zhou, J.-M. (2017). Receptor Kinases in Plant-Pathogen Interactions:  
1370 More Than Pattern Recognition. *The Plant Cell* 29, 618–637.
- 1371 Thomas, C.L., Bayer, E.M., Ritzenthaler, C., Fernandez-Calvino, L., and Maule, A.J. (2008a).  
1372 Specific Targeting of a Plasmodesmal Protein Affecting Cell-to-Cell Communication. *PLOS*  
1373 *Biology* 6, e7.
- 1374 Thomas, C.L., Bayer, E.M., Ritzenthaler, C., Fernandez-Calvino, L., and Maule, A.J. (2008b).  
1375 Specific Targeting of a Plasmodesmal Protein Affecting Cell-to-Cell Communication. *PLoS*  
1376 *Biology* 6.
- 1377 Tian, T., Liu, Y., Yan, H., You, Q., Yi, X., Du, Z., Xu, W., and Su, Z. (2017). agriGO v2.0: a GO  
1378 analysis toolkit for the agricultural community, 2017 update. *Nucleic Acids Research* 45, W122–  
1379 W129.
- 1380 Vatén, A., Dettmer, J., Wu, S., Stierhof, Y.-D., Miyashima, S., Yadav, S.R., Roberts, C.J.,  
1381 Campilho, A., Bulone, V., Lichtenberger, R., et al. (2011a). Callose Biosynthesis Regulates  
1382 Symplastic Trafficking during Root Development. *Developmental Cell* 21, 1144–1155.
- 1383 Vatén, A., Dettmer, J., Wu, S., Stierhof, Y.-D., Miyashima, S., Yadav, S.R., Roberts, C.J.,  
1384 Campilho, A., Bulone, V., Lichtenberger, R., et al. (2011b). Callose biosynthesis regulates  
1385 symplastic trafficking during root development. *Dev Cell* 21, 1144–1155.
- 1386 Verbon, E.H., Trapet, P.L., Stringlis, I.A., and Kruijs, S. (2017). Iron and Immunity. *Annual*  
1387 *Review of Phytopathology* 55, 1–15.
- 1388 Vert, G.A., Briat, J.-F., and Curie, C. (2003). Dual Regulation of the Arabidopsis High-Affinity  
1389 Root Iron Uptake System by Local and Long-Distance Signals. *Plant Physiol* 132, 796–804.

1390 Xing, Y., Xu, N., Bhandari, D.D., Lapin, D., Sun, X., Luo, X., Cao, J., Wang, H., Coaker, G.,  
1391 Parker, J.E., et al. (2021). Bacterial effector targeting of a plant iron sensor facilitates iron  
1392 acquisition and pathogen. *The Plant Cell Online* 31.

1393 Zamioudis, C., Korteland, J., Van Pelt, J.A., van Hamersveld, M., Dombrowski, N., Bai, Y.,  
1394 Hanson, J., Van Verk, M.C., Ling, H.-Q., Schulze-Lefert, P., et al. (2015). Rhizobacterial  
1395 volatiles and photosynthesis-related signals coordinate MYB72 expression in Arabidopsis roots  
1396 during onset of induced systemic resistance and iron-deficiency responses. *Plant J.* 84, 309–  
1397 322.

1398

1399

1400