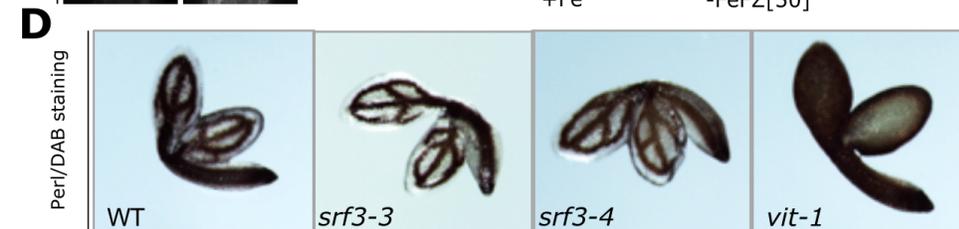
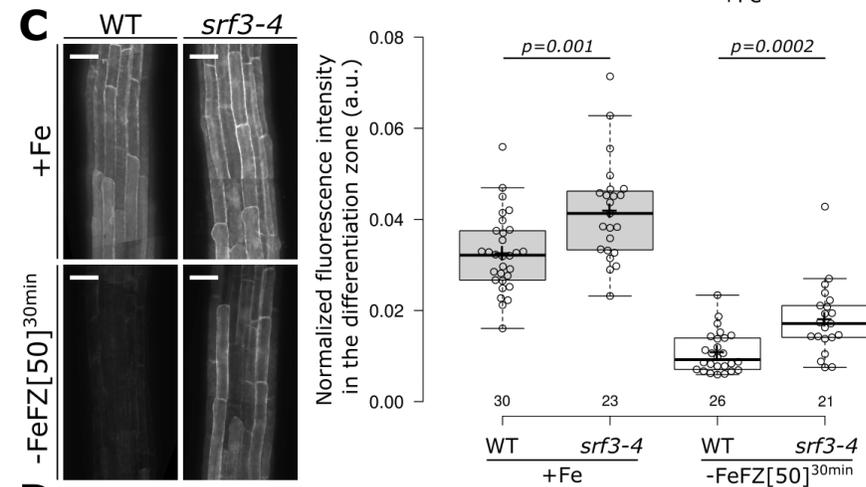
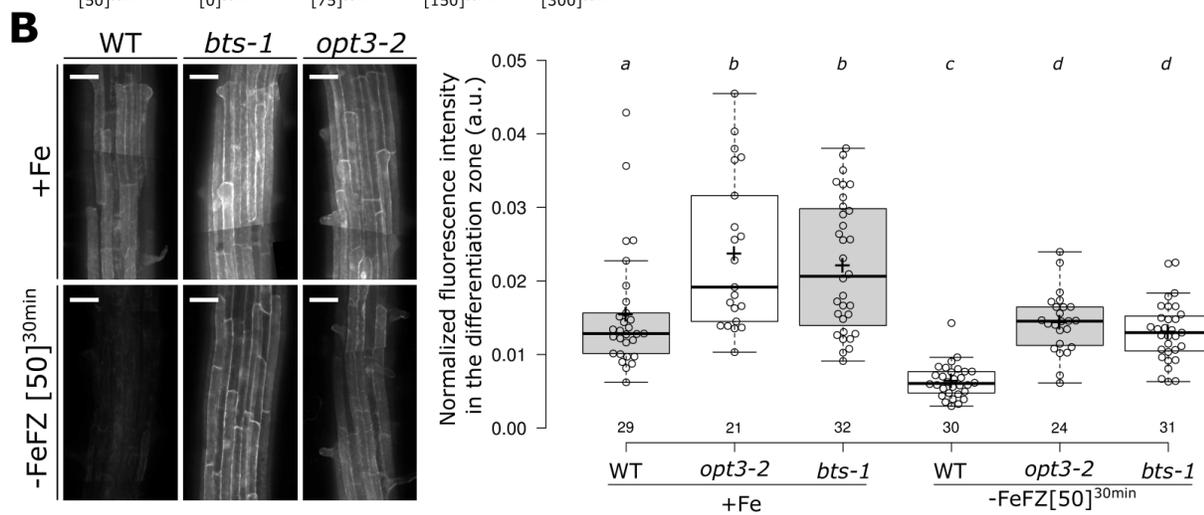
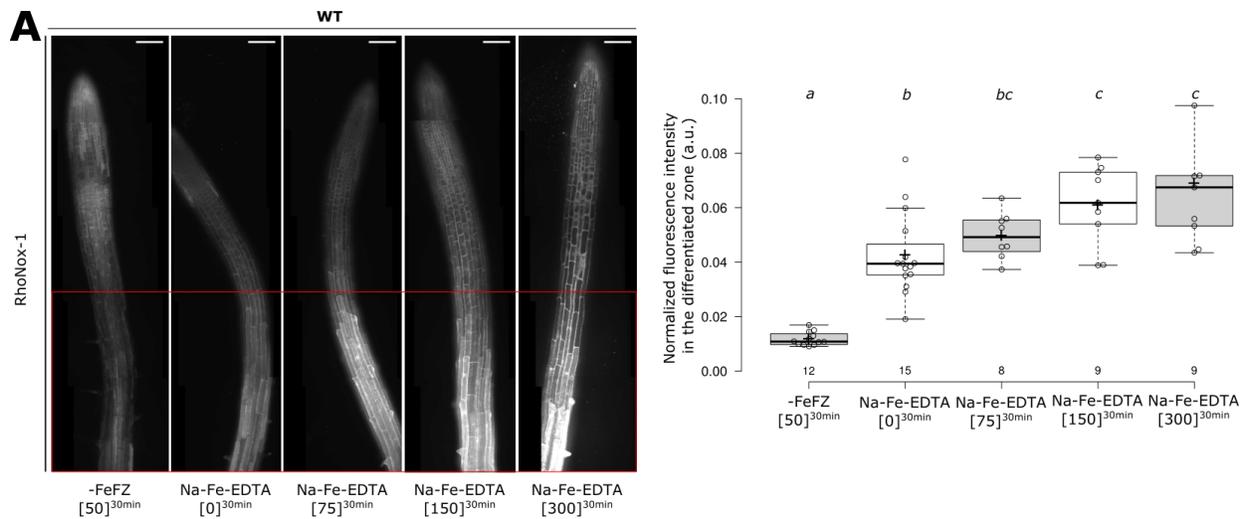
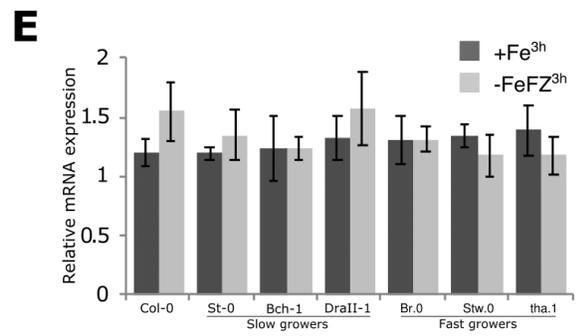
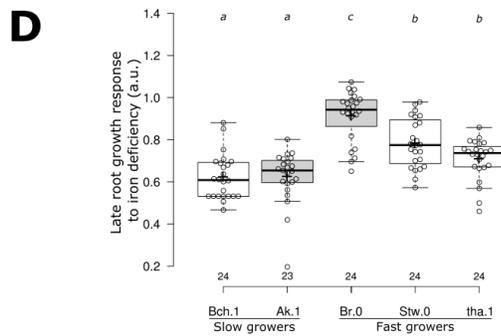
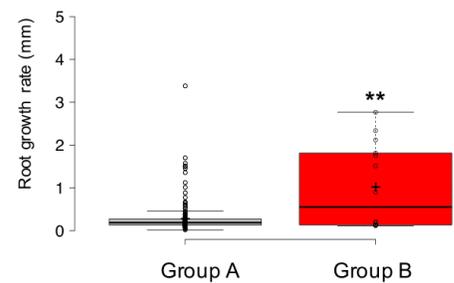
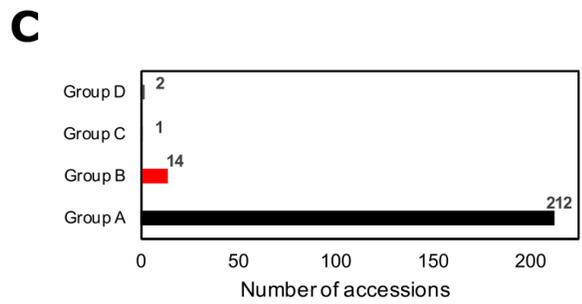
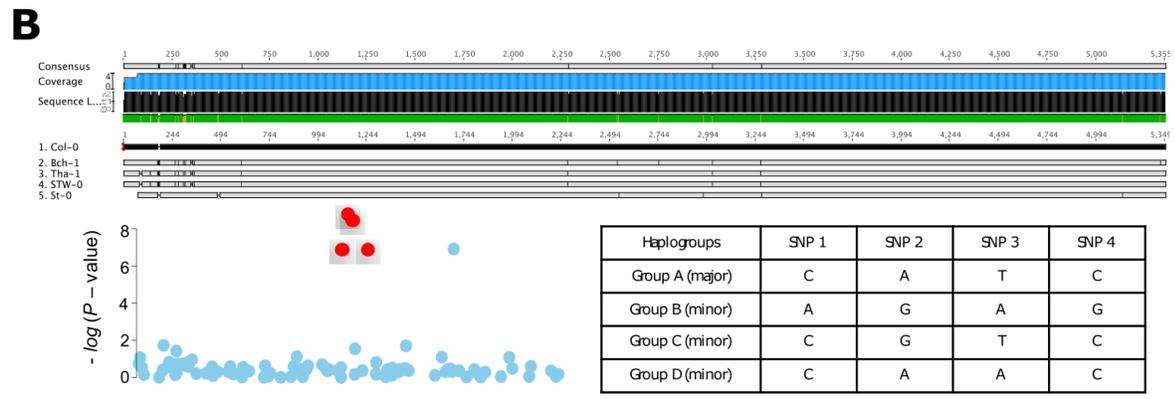
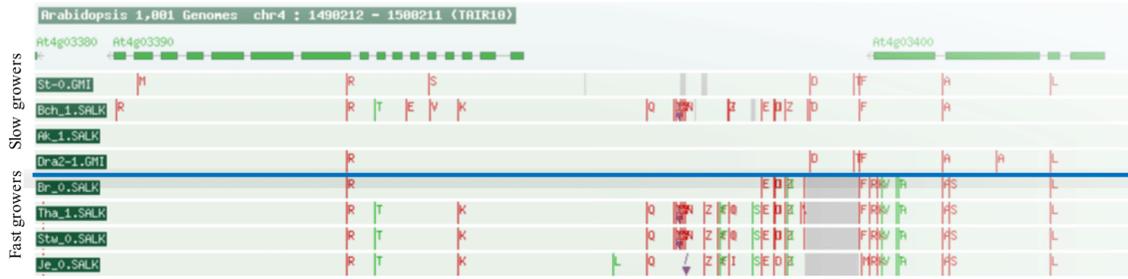
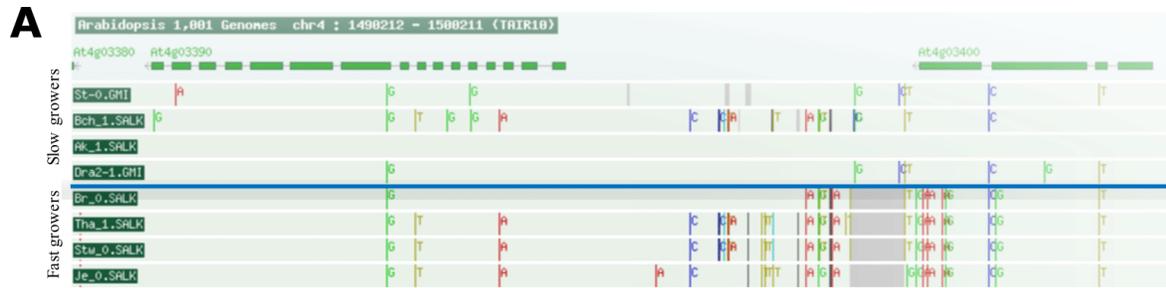


2 **Supplementary Figure 1 (Related to Fig1). Root growth rate distribution GWAS for**
3 **Arabidopsis accessions grown under–Fe used in this study and characterization of *srf3***
4 **mutants with *srf3*-neighbouring gene under low iron. (A)** Histograms of median root growth
5 rates (mm) of accessions grown on Fe deficient growth conditions (root growth rate day 1-2, root
6 growth rate day 2-3, root growth rate day 3-4, root growth rate day 4-5). x-axis: median root growth
7 rate in mm; y-axis: frequency. **(B)** Manhattan plots depicting genome wide SNP associations for
8 median root growth rate on Fe deficient growth conditions. The chromosomes are depicted in
9 different colors. The horizontal blue dash-dot line corresponds to a nominal 0.05 significance
10 threshold after Benjamini-Hochberg Correction. Black box indicates the significantly associated
11 region in close proximity to *SRF3* gene. x-axis: chromosomal position of SNP; y-axis: $-\log_{10}(p$ -
12 value). **(C)** Cartoon showing the genomic structures of (a) AT4G03390 and (b) AT4G03400 and
13 the T-DNA insertion sites. Black boxes indicate exons. Scale bar: 100 bp. Gene models were
14 generated by Exon-Intron graphic maker (<http://wormweb.org/exonintron>). **(D)** Transcript analysis
15 of AT4G03390 in *srf3-2*, *srf3-3* and *srf3-4* and transcript analysis of AT4G03400 in *at4g03400*.
16 **(E, F)** Root growth response doing transfer assay under low iron levels supplemented with 100µM
17 ferrozine with WT, SALK_202843 and SAIL811_C06 **(E)** and WT, *srf3-3*, *srf3-3* complementation
18 line **(F)**. One-way ANOVA follows by a post-hoc Tukey HSD test, letters indicate statistical
19 differences ($p < 0.05$). **(G)** Graph of the late root growth response to low iron levels after 3 days
20 provided by 100 µM, 50 µM or 10 µM of ferrozine and 300 µM of Na-Fe-EDTA on wild type (WT)
21 and *srf3-2* and *srf3-4*. Independent two ways student test ($p < 0.05$), n.s. non-significant. **(H)** Late
22 root growth after transfer under standard condition in WT, *srf3-2*, *srf3-3* and *srf3-4*. One-way
23 ANOVA follows by a post-hoc Tukey HSD test, letters indicate statistical differences ($p < 0.05$). For
24 boxplots, circles indicate a single biological replicate and the number below each box specifies
25 the number of replicates, horizontal black bars indicate the median, the black cross represents
26 the mean, box represents the interquartile range and the hinges the min and max whiskers.\

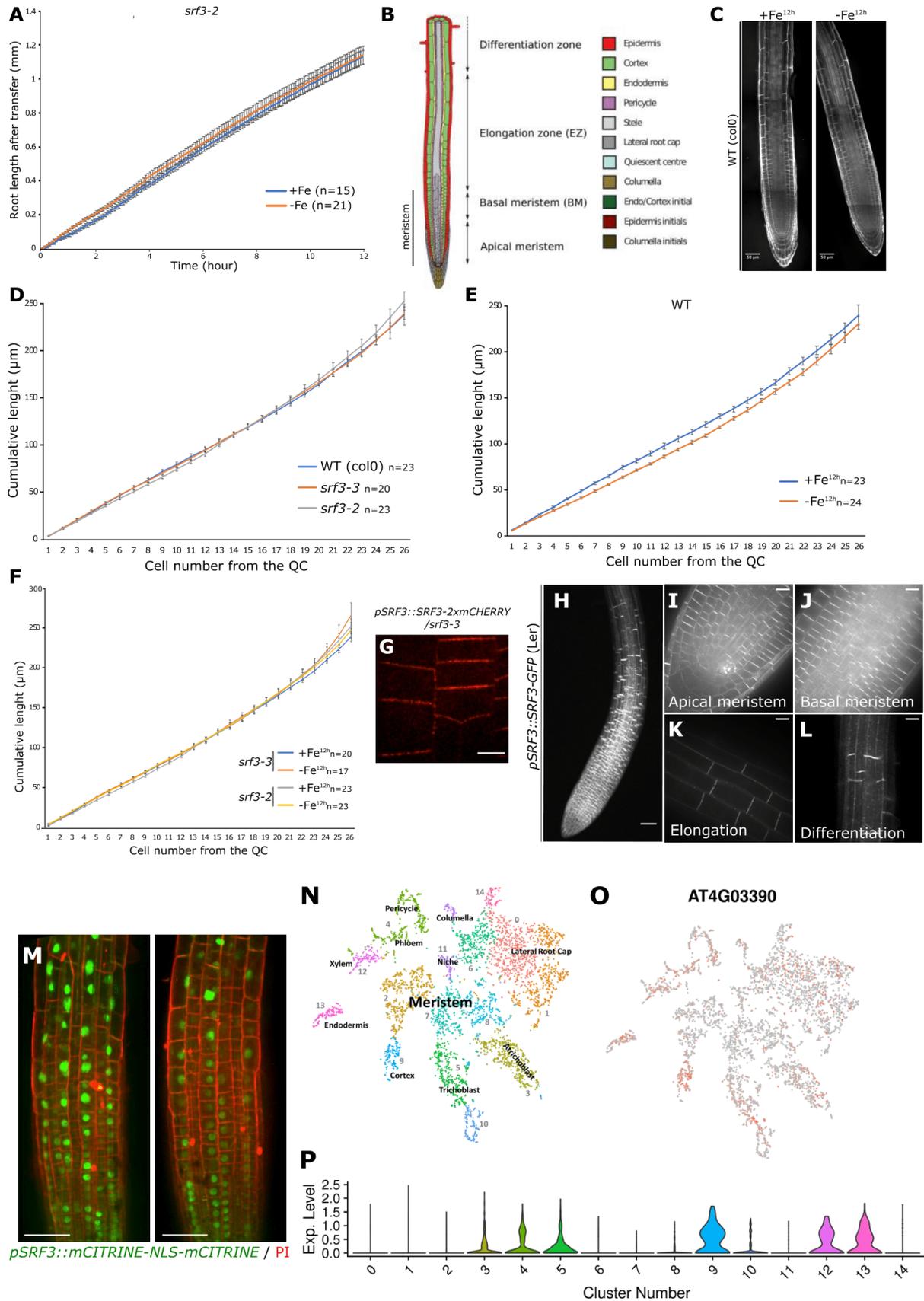


28 **Supplementary Figure 2 (Related to Fig1). Characterization of RhoNox-1 staining and**
29 **evaluation of iron content in embryo of WT, *srf3* mutants and *vit-1*. (A)** Left panel: confocal
30 images of 5 days old seedling of WT epidermal root cells stained with RhoNox-1 pretreated with
31 50µM, 0 µM of FerroZine (FZ) and 75, 150 and 300 µM of Na-Fe-EDTA for 30 minutes. Red box
32 indicates the region where the fluorescence intensity has been quantified. Scale bars, 100µm.
33 Right panel: the related fluorescence intensity quantification. One-way ANOVA, follows by a post-
34 hoc Tukey HSD test, letters indicate statistical differences ($p < 0.05$). **(B)** Left panel: confocal
35 images of differentiated epidermal root cells stained with RhoNox-1, WT, *bts-1* and *opt3-2* in
36 sufficient (+Fe), upper panel, and in low iron levels (-Fe) with 50µM of ferrozine for 30 minutes.
37 Scale bars, 50µm. Right panel: the related quantification of the normalized fluorescence intensity.
38 One-way ANOVA, follows by a post-hoc Tukey HSD test, letters indicate statistical differences
39 ($p < 0.05$). **(C)** Left panel: confocal images of differentiated epidermal root cells stained with
40 RhoNox-1, WT and *srf3-4* in sufficient (+Fe) and in low iron levels (-Fe) supplemented with 50µM
41 of ferrozine for 30 minutes. Scale bars, 50µm. Right panel: related quantification of the normalized
42 fluorescence intensity. One-way ANOVA followed by a post-hoc Tukey HSD test, letters indicate
43 statistical differences ($p < 0.05$). **(D)** Wild-type *srf3-3*, *srf3-4* and *vit-1* (positive control) dry seed
44 embryos were dissected and stained with Perls/DAB method. For boxplots, circles indicate a
45 single biological replicate and the number below each box specifies the number of replicates,
46 horizontal black bars indicate the median, the black cross represents the mean, box represents
47 the interquartile range and the hinges the min and max whiskers.

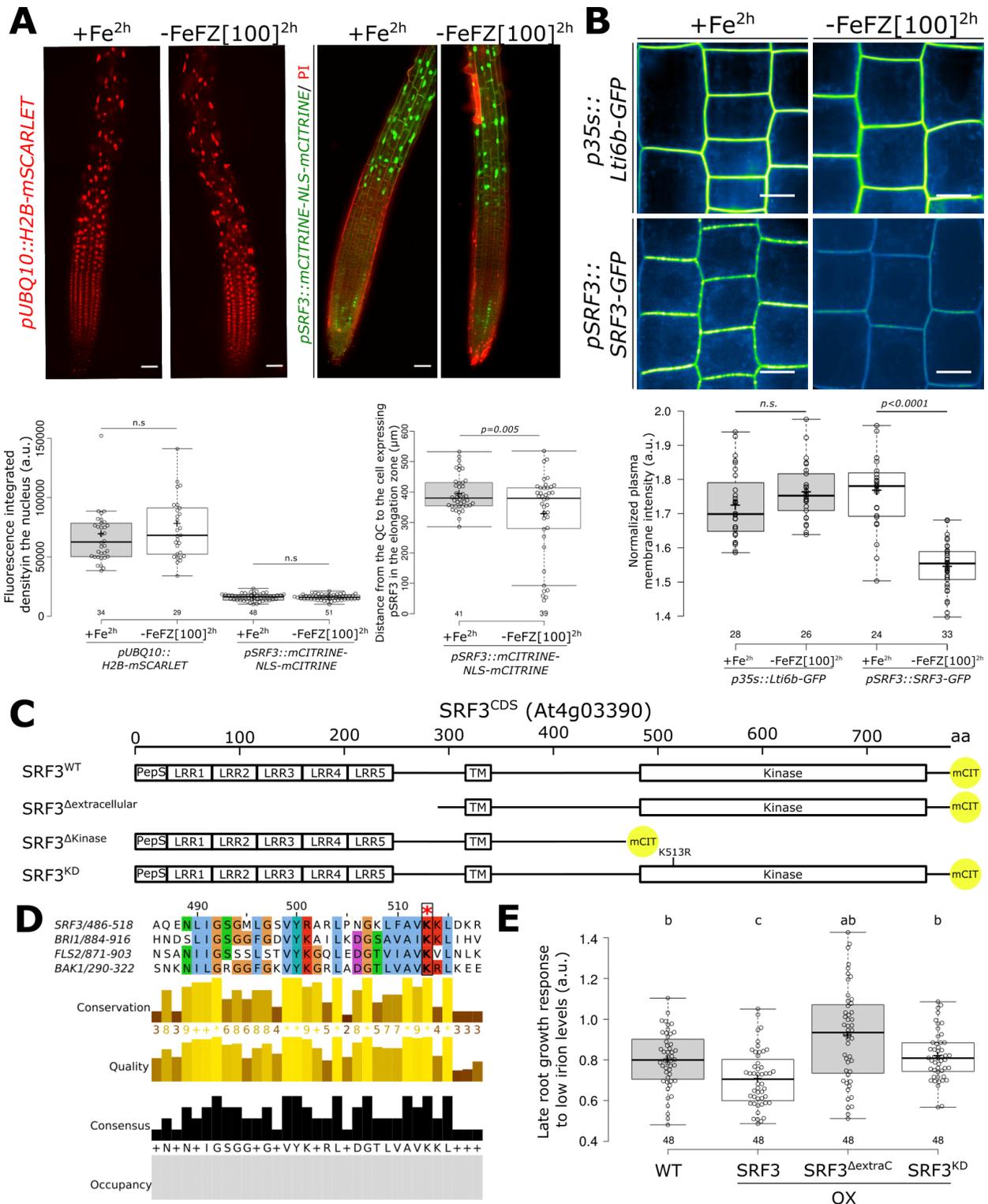


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Supplementary Figure 3 (Related to Fig1). SNP polymorphism around the SRF3 locus and qPCR in extreme accessions. (A) SNP polymorphism, top, and amino acid, bottom, changes surrounding the SRF3 locus in four representative accessions (<http://signal.salk.edu/atg1001/3.0/gebrowser.php>). **(B)** SNP polymorphisms in regulatory and coding regions of SRF3 gene in extreme accessions as confirmed by Sanger sequencing. SRF3 genomic DNA sequences from Bch-1, St-0, STW-0 and Tha-1 accessions were obtained by Sanger sequencing and aligned to Col-0 sequence and SNP changes (compared to Col-0 reference) from extreme accessions are shown in vertical lines. **(C)** Left panel: distribution of marker SNPs highlighted in red color in haplogroup A (major), B (minor), C (minor) and D (minor). Bar plot shows division of haplogroups A, B, C and D in 231 accessions. Right panel: box plots for root growth rate in Group A (major) and Group B (minor) accessions. Asterisks indicate significant difference with Tukey's HSD comparison (p -value < 0.05). **(D)** Late root growth response to low iron levels after transfer with extreme accession found in the GWAS. One-way ANOVA follows by a post-hoc Tukey HSD test, letters indicate statistical differences ($p < 0.05$). **(E)** Relative expression level of SRF3 in Col-0, St-0, Bch-1, Drall-1, Br-0, Stw-0 and Tha-1 at T0, in mock treatment after 3 hours in low iron with 100 μ M of ferrozine. For boxplots, circles indicate a single biological replicate and the number below each box specifies the number of replicates, horizontal black bars indicate the median, the black cross represents the mean, box represents the interquartile range and the hinges the min and max whiskers.



71 **Supplementary Figure 4 (Related to Fig2). SRF3 is transcription and translation co-exist in**
72 **the transition-elongation zone and low iron triggers a root growth decrease in the**
73 **elongation zone in an *SRF3*-dependent manner. (A)** Graph showing time lapse of the root
74 length of WT and *srf3-2* under sufficient and low iron levels for 12 hours. Error bars indicated
75 standard deviation of the mean (SEM). **(A)** Scheme of root tip showing the different zone of the
76 root and the cell types. **(C)** Confocal images of 5 days old seedling stained with propidium iodide
77 in WT under sufficient and low iron levels for 12 hours. Scale bars, 50µm. **(D)** Cumulative cell
78 length from the quiescent center in WT under sufficient and low iron levels for 12 hours. Error
79 bars indicated standard deviation of the mean (SEM). **(E)** Cumulative cell length from the
80 quiescent center in WT under sufficient and low iron levels for 12 hours. Error bars indicated
81 standard deviation of the mean (SEM). **(F)** Cumulative cell length from the quiescent center in
82 *srf3* mutants under sufficient and low iron levels for 12 hours. Error bars indicated standard
83 deviation of the mean (SEM). We observed that under low iron medium the roots present shorter
84 cell length while the steepness of the curve representing the cumulative cell length was identical
85 in both conditions in WT. However, no difference was noted in *srf3* mutants. This shows that the
86 cell division process is not affected under low iron levels and that the decreased of the root growth
87 is due to SRF3-dependent reduction of cell elongation. **(G)** Confocal image of root epidermal cells
88 of plant expressing *pSRF3::SRF3-2xmCHERRY-4xmyc* in *srf3-3* (*srf3-3* comp). Scale bar, 10µm.
89 **(H, I, J, K,L)** Confocal images of 5 day-old seedlings expressing *pSRF3::SRF3-GFP* in root **(H)**
90 (scale bar, 50µm), apical meristem **(I)**, basal meristem **(J)**, elongation **(K)** and differentiated tissue
91 **(L)**. (Scale bars, 10µm). **(M)** Confocal images of 5 day-old seedlings expressing
92 *pSRF3::mCITRINE-NLS-mCITRINE* and stain with propidium iodide (PI). Scale bars, 50µm.
93 **(N)** Scheme of t-SNE plot represent gene expression across clusters in the different cell types.
94 **(O)** t-SNE plots represent *SRF3* AT4G03390 gene expression across clusters in the different cell
95 types. **(P)** Violin-plot depicting the distribution of expression levels for cells in the cluster. Y-axis
96 (length) - gene expression level across each cluster. X-axis - proportion of cells showing a given
97 expression value.



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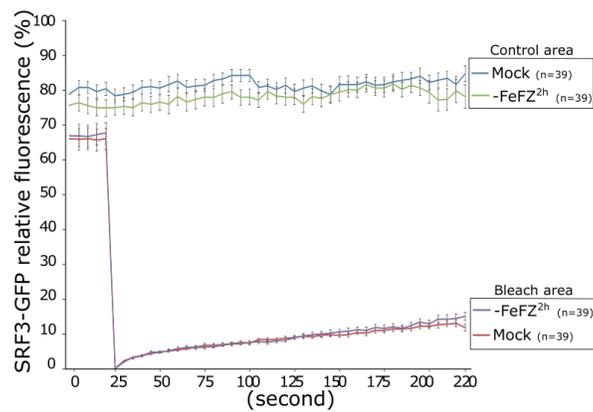
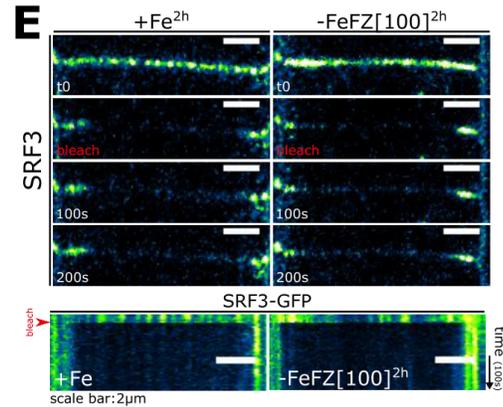
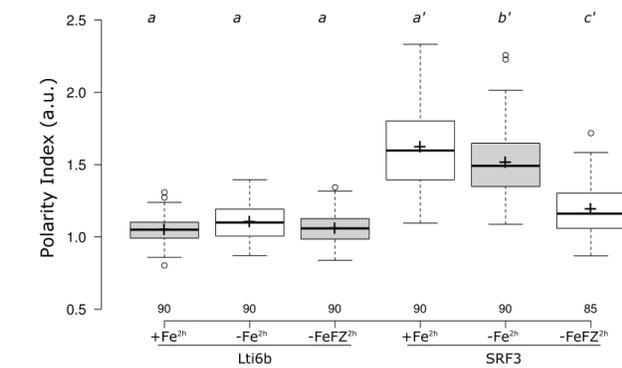
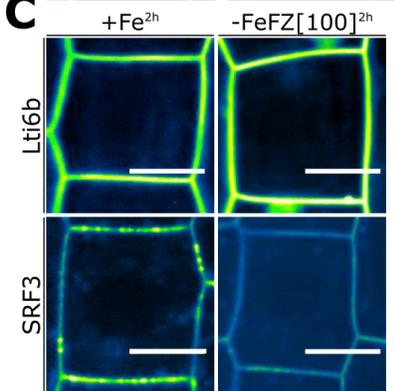
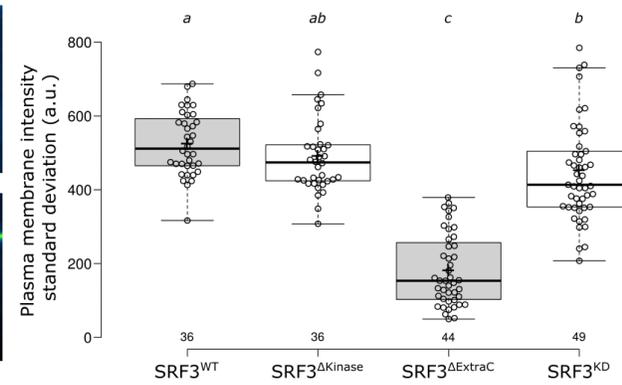
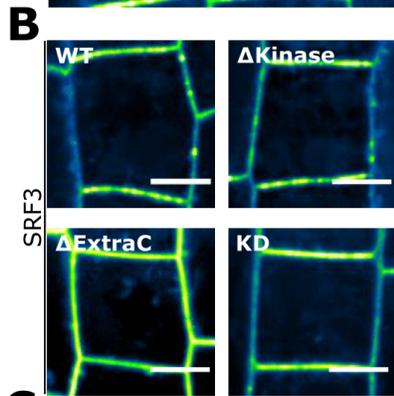
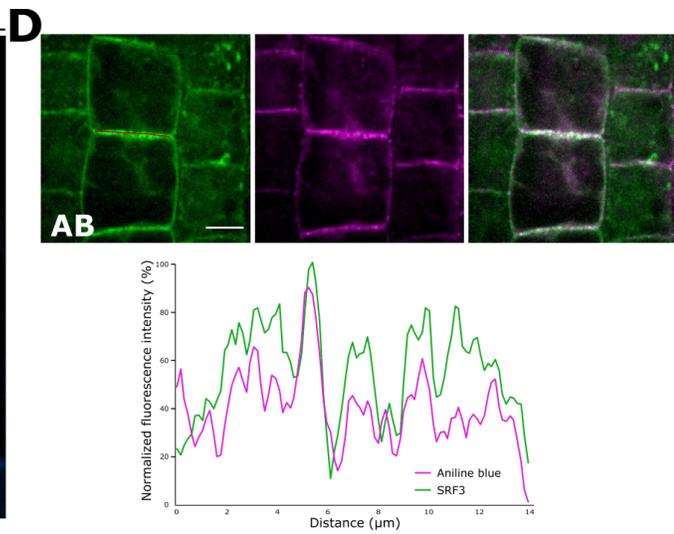
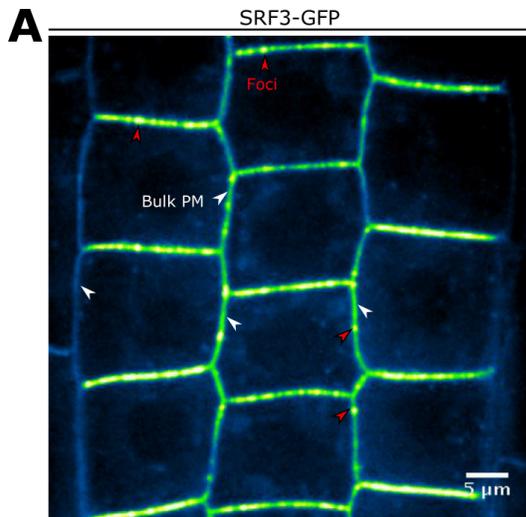
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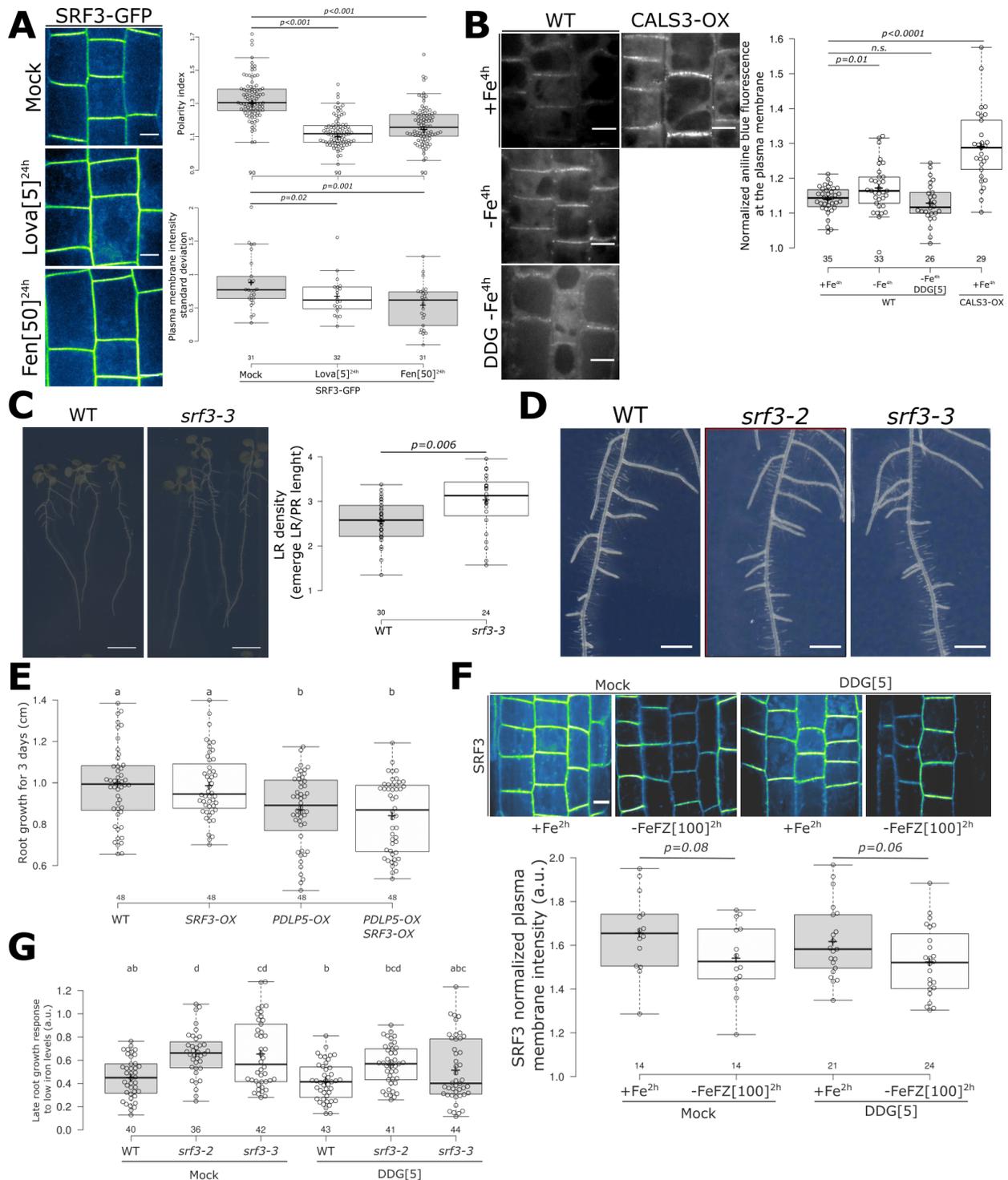
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Supplementary Figure 5 (Related to Fig2). The early lack of iron affects SRF3 protein levels at the plasma membrane which is dependent on the extracellular domain and kinase activity to regulate root growth. (A) Confocal images of root tip of 5 days old seedling expressing *pUBQ10::H2B-mSCARLET* (Left) and *pSRF3::mCITRINE-NLS-mCITRINE* (right) with

103 the plasma membrane stained by propidium iodide (PI) under mock (+Fe) and under low iron
104 levels provided with 100 μ M of ferrozine (-FeFZ[100]) for 2 hours. Scale bars, 50 μ m. Below, the
105 related quantification of the nuclear fluorescence integrated density (lower left) and of the distance
106 from the QC to the cell expressing pSRF3 in the elongation zone in μ m (lower right). Independent
107 two-ways student test ($p < 0.05$), n.s. non-significant. **(B)** Left panel: Confocal images of root
108 epidermis of 5 days old seedling expressing *p35s::Lti6b-GFP* and *pSRF3::SRF3-GFP* under mock
109 (+Fe) and under low iron levels provided by 100 μ M of ferrozine (FZ) for two hours. Scale bar,
110 10 μ m. Right panel: the related quantification of the normalized plasma membrane intensity.
111 Independent two-ways student test ($p < 0.05$), n.s. non-significant. **(C)** Scheme representing the
112 SRF3 CDS full length as well as truncated version and mutated version. **(D)** Alignment done with
113 Jalview of SRF3, BRI1, FLS2 and BAK1 ATP binding pocket. **(E)** Graph showing the quantification
114 of late root growth response to low iron levels for WT and overexpressing lines of SRF3 CDS full
115 length as well as truncated version and mutated version. One-way ANOVA followed by a post-
116 hoc Tukey HSD test, letters indicate statistical differences ($p < 0.05$). For boxplots, circles indicate
117 a single biological replicate and the number below each box specifies the number of replicates,
118 horizontal black bars indicate the median, the black cross represents the mean, box represents
119 the interquartile range and the hinges the min and max whiskers.



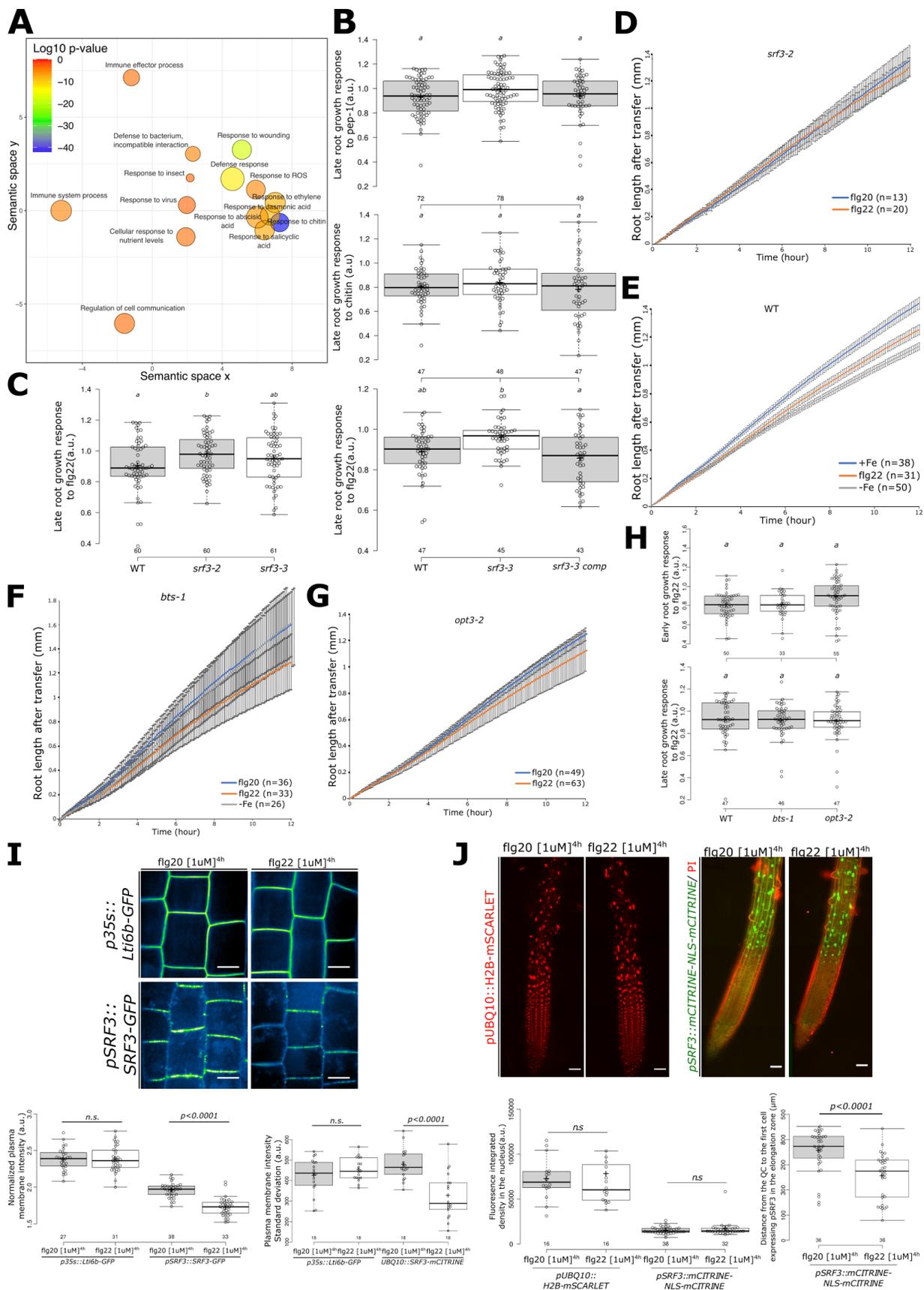
121 **Supplementary Figure 6 (Related to Fig3). SRF3 is removed from the two PM-associated**
122 **subpopulations under low iron. (A)** Confocal images of root epidermal cells in the elongation
123 zone of 5 days old seedling expressing *pSRF3::SRF3-GFP*. Red arrows indicate foci and white
124 arrows bulk plasma membrane (Bulk PM). Scale bar, 5 μ m. **(B)** Left panel: confocal images of root
125 epidermis of 5 days old seedling expressing *pUBQ10::SRF3^{WT}-mCITRINE*, *pUBQ10::SRF3 ^{Δ Kinase}-*
126 *mCITRINE*, *pUBQ10::SRF3 ^{Δ ExtraC}-mCITRINE*, *pUBQ10::SRF3^{KD}-mCITRINE* (SRF3^{WT},
127 SRF3 ^{Δ Kinase}, SRF3 ^{Δ ExtraC} and SRF3^{KD}). Scale bars, 10 μ m. Right panel: related quantification of
128 the mean standard deviation of the intensity mean at the apical-basal side of the cell. One-way
129 ANOVA followed by a post-hoc Tukey HSD test, letters indicate statistical differences ($p < 0.05$).
130 **(C)** Left panel: Confocal images of root epidermal cells in the elongation zone of 5 days old
131 seedling expressing *p35s::Lti6b-GFP* (Lti6b) and *pSRF3::SRF3-GFP* (SRF3) under mock (+Fe)
132 and under low iron media provided or not by 100 μ M of ferrozine (-FeZ[100]) for two hours. Scale
133 bar, 5 μ m. Right panel related quantification of polarity index. One-way ANOVA followed by a post-
134 hoc Tukey HSD test, letters indicate statistical differences ($p < 0.05$). **(D)** Plant stained with the
135 callose maker Aniline Blue (AB; left) and SRF3 (middle) and the relative merge (right), graph at
136 the bottom indicates the signal intensity in both channel on the apical basal part of the cell. Red
137 line on the left image indicates where the scan line has been traced. Scale bars, 10 μ m. **(E)** Left
138 panel: confocal images of *pSRF3::SRF3-GFP* (SRF3) of 5 days old seedling during FRAP
139 experiment in WT in the mock and under low iron media supplemented with 100 μ M of ferrozine
140 and the related kymograph (time scale 15 seconds) on the bottom. Scale bar, 2 μ m. Right panel:
141 traces of *pSRF3::SRF3-GFP* fluorescence intensity at the plasma membrane during FRAP
142 analyses in the different conditions. For boxplots, circles indicate a single biological replicate and
143 the number below each box specifies the number of replicates, horizontal black bars indicate the
144 median, the black cross represents the mean, box represents the interquartile range and the
145 hinges the min and max whiskers.



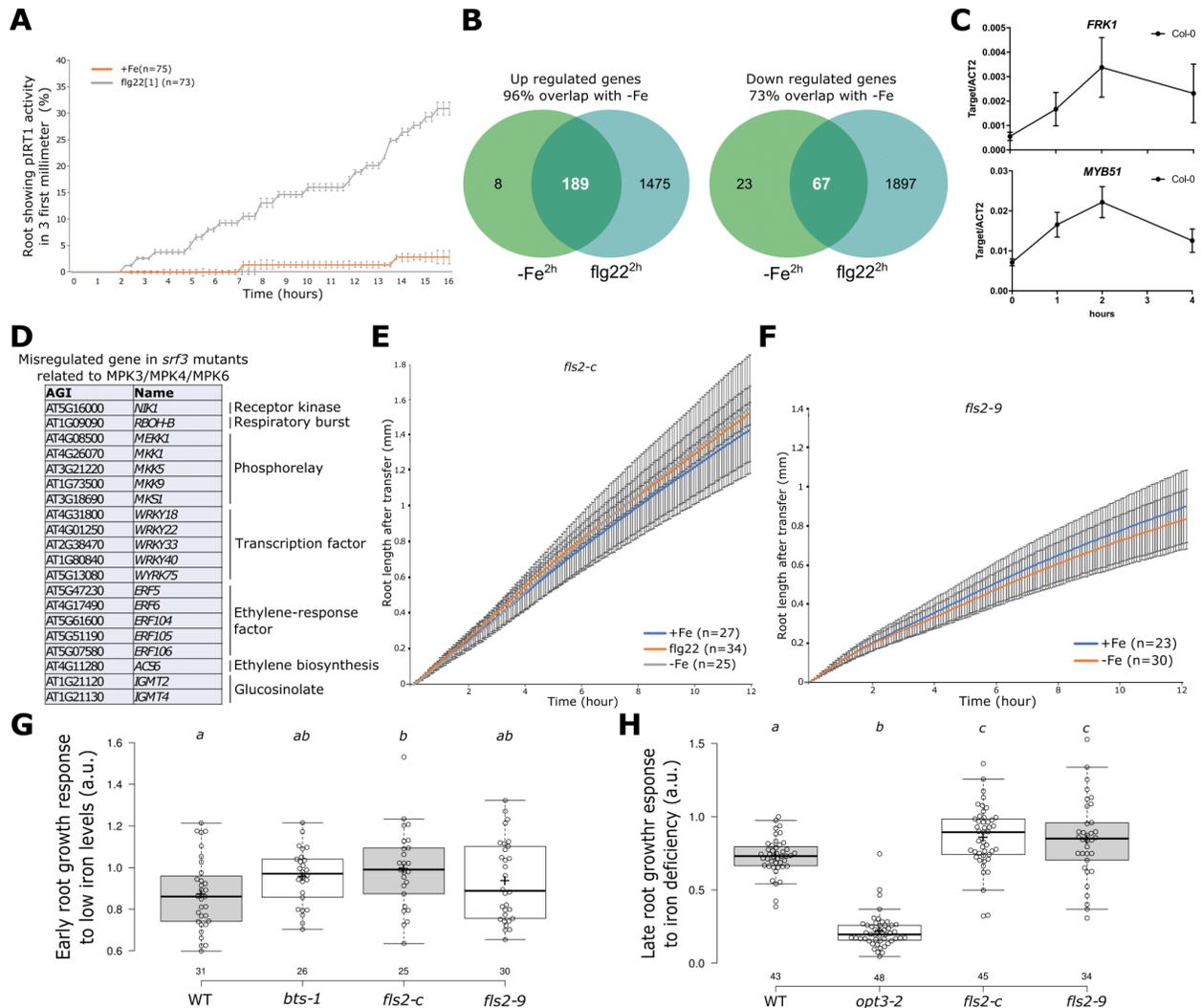
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Supplementary Figure 7 (Related to Fig4 & 5). SRF3 is an upstream negative regulator of callose synthases. (A) Upper panel: confocal images of root epidermis of 5 days old seedling expressing *pSRF3::SRF3-GFP* under mock and treated for 24 hours with Lovastatin (Lova) and Fenpropimorph (Fen). Scale bar, 10 μ m. Lower panel: related quantification of the polarity index and the standard deviation of the mean intensity at the apical basal part of the cell. **(B)** Left panel: confocal images of root epidermal cells in the elongation zone of 5 days old seedling stained with aniline blue (AB) in the indicated genotypes, WT and 35s::GFP-CALS3 under mock (+Fe) and

154 under low iron media for four hours with or without 2-deoxy-d-glucose (DDG) Scale bar, 10 μ m.
155 Right panel: the related quantification of normalized plasma membrane intensity. Independent
156 two-ways student test ($p < 0.05$), n.s. non-significant. **(C)** Upper panel: images of 12 days old
157 seedling of WT and *srf3-3* under normal growth condition. Scale bar, 1cm. Lower panel: the
158 quantification of the lateral root (LR) density. **(D)** Images of 12 days old seedling of WT and *srf3-*
159 *2* and *srf3-3*. Scale bar, 2mm. **(E)** Graph representing the quantification of the mean root growth
160 rate for 3 days WT, *UBQ10::SRF3-mCITRINE* (*SRF3-OX*), *35s::PDLP5-GFP* (*PDLP5-OX*) and
161 *PDLP5-GFPxSRF3-OX*. One-way ANOVA followed by a post-hoc Tukey HSD test, letters indicate
162 statistical differences ($p < 0.05$). **(F)** Upper panel: confocal images of root epidermis in the
163 elongation zone of 5 days old seedling expressing *pUBQ10::SRF3-mCITRINE* in iron sufficient
164 and deficient media supplemented with 100 μ M of ferrozine for 2 hours in presence or absence of
165 DDG. Scale bar, 10 μ m. Lower panel: related quantification of the normalized plasma membrane
166 intensity. Independent two-ways student test ($p < 0.05$). **(G)** Graph representing the root growth
167 response to low iron levels for 3 days with or without 2-deoxy-d-glucose (DDG) in WT, *srf3-2* and
168 *srf3-3*. One-way ANOVA followed by a post-hoc Tukey HSD test, letters indicate statistical
169 differences ($p < 0.05$). For boxplots, circles indicate a single biological replicate and the number
170 below each box specifies the number of replicates, horizontal black bars indicate the median, the
171 black cross represents the mean, box represents the interquartile range and the hinges the min
172 and max whiskers.



174 **Supplementary Figure 8 (Related to Fig6). Specific regulation of flg22-induced bacterial**
175 **root innate immunity by SRF3. (A)** Gene ontology of differential expressed genes in *srf3*
176 mutants compare to the WT under normal growth condition. **(B)** Box plot showing the late root
177 growth response to pep-1, top panel, chitin, middle panel and flg22, bottom panel in WT, *srf3-3*,
178 *srf3-3* complementation. One-way ANOVA followed by a post-hoc Tukey HSD test, letters indicate
179 statistical differences ($p < 0.05$). **(C)** Box plot showing the late root growth response to flg22 in WT,
180 *srf3-2* and *srf3-3*. One-way ANOVA followed by a post-hoc Tukey HSD test, letters indicate
181 statistical differences ($p < 0.05$). **(D)** Graph showing time lapse of the root length of WT and *srf3-2*
182 under flg20 and flg22 for 12 hours. Error bars indicated standard deviation of the mean (SEM).
183 **(E)** Graph showing time lapse of the root length of WT under iron sufficient and low iron media
184 and media supplemented with flg22 at 1 μ M for 12 hours. Error bars indicated standard deviation
185 of the mean (SEM). **(F)** Graph showing time lapse of the root length of *bts-1* under flg20 and flg22
186 at 1 μ M for 12 hours. **(G)** Graph showing time lapse of the root length of *opt3-2* under iron
187 deficiency, flg20 and flg22 at 1 μ M for 12 hours. Error bars indicated standard deviation of the
188 mean (SEM). **(H)** Box plot of the early, top panel, and late, bottom panel, root growth response to
189 flg22 of WT, *fls2-c*, *bts-1* and *opt3-2* for 12 hours and 3 days. One-way ANOVA followed by a
190 post-hoc Tukey HSD test, letters indicate statistical differences ($p < 0.05$). **(I)** Top panel: Confocal
191 images of root epidermis of 5 days old seedling expressing *p35s::Lti6b-GFP* and *pSRF3::SRF3-*
192 *GFP* (*Ler* background) under flg20 and flg22 at 1 μ M for four hours. Scale bars, 10 μ m. Bottom
193 left panel: related quantification of the normalized plasma membrane intensity. Bottom right panel:
194 quantification of the standard deviation of the signal intensity at the apical basal side of the PM in
195 *p35s::Lti6b-GFP* and *pUBQ10::SRF3-mCITRINE* under flg20 and flg22 at 1 μ M for four hours.
196 Independent two-ways student test ($p < 0.05$), n.s. non-significant. **(J)** Upper panel: confocal
197 images of root tip of 5 days old seedling expressing *pUBQ10::H2B-mSCARLET* and
198 *pSRF3::mCITRINE-NLS-mCITRINE* with the plasma membrane stained by propidium iodide (PI)
199 under flg22 at 1 μ M for four hours, scale bars, 50 μ m, the related quantification of the nuclear
200 fluorescence integrated density (bottom left) and of the distance from the QC to the cell expressing
201 pSRF3 in the elongation zone in μ m (bottom right). Independent two-ways student test ($p < 0.05$),
202 n.s. non-significant. For boxplots, circles indicate a single biological replicate and the number
203 below each box specifies the number of replicates, horizontal black bars indicate the median, the
204 black cross represents the mean, box represents the interquartile range and the hinges the min
205 and max whiskers.



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Supplementary Figure 9 (Related to Fig6). SRF3 is involved in PTI signaling pathways regulation. (A) Quantification of time lapse analysis of pRT1::NLS-2xYPet under mock and flg22 at 1 μ M treatment for 16 hours. (B) Venn diagram of differentially expressed genes, up regulated and down regulated, under iron deficiency and flg22 for 2 hours. (C) Graph representing the expression level of immune-related genes, *FRK1* top and *MYB51* bottom under low iron levels according to time. Error bars indicate SEM. (D) List of PIT-dependent genes mis regulated in *srf3* mutants belonging to the early iron/defense core regulatory network. (E, F) Graph showing time lapse of the root length of WT under sufficient (+Fe) and low (-Fe) iron levels media and flg22 at 1 μ M for 12 hours in *fls2-c* (E) and *fls2-9* mutants (F), (G) the related quantification including *bts-1* mutant as a positive control. One-way ANOVA followed by a post-hoc Tukey HSD test, letters indicate statistical differences ($p < 0.05$). (H) Quantification of the late root growth response to iron deficiency media in *fls2-c* and *fls2-9* mutants and *opt3-2* used as a positive control. One-way ANOVA followed by a post-hoc Tukey HSD test, letters indicate statistical differences ($p < 0.05$). For boxplots, circles indicate a single biological replicate and the number below each box specifies the number of replicates, horizontal black bars indicate the median, the black cross represents the mean, box represents the interquartile range and the hinges the min and max whiskers.