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Supplementary Materials for

The receptor kinase FERONIA regulates phosphatidylserine localization at the cell surface to modulate ROP signaling

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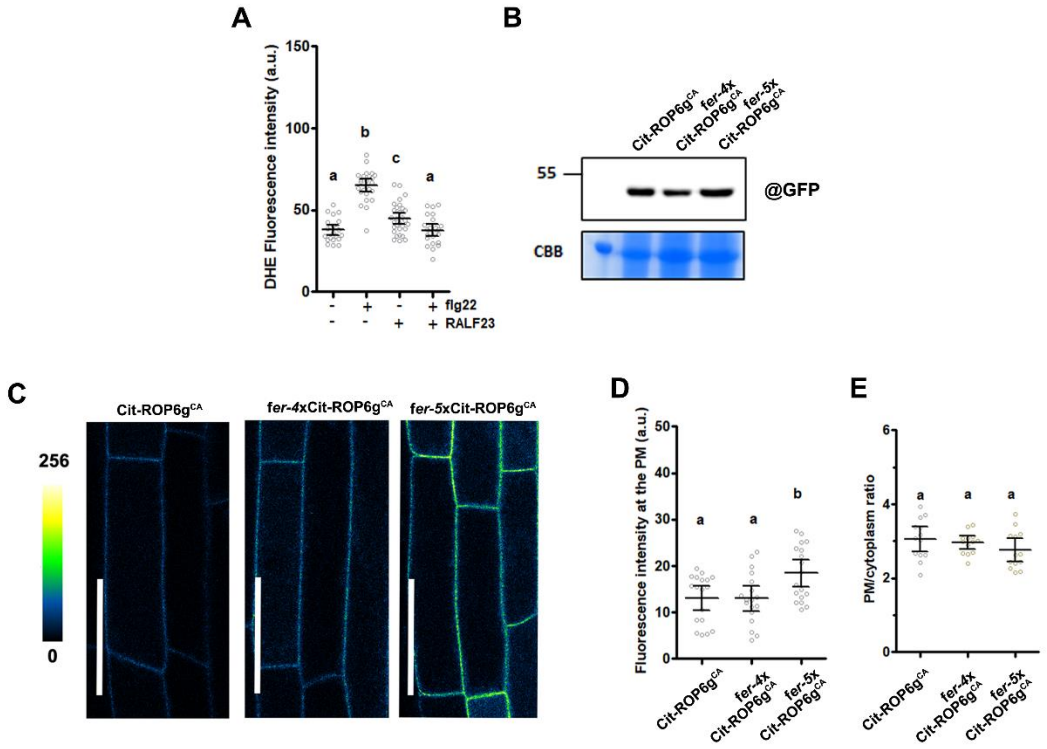
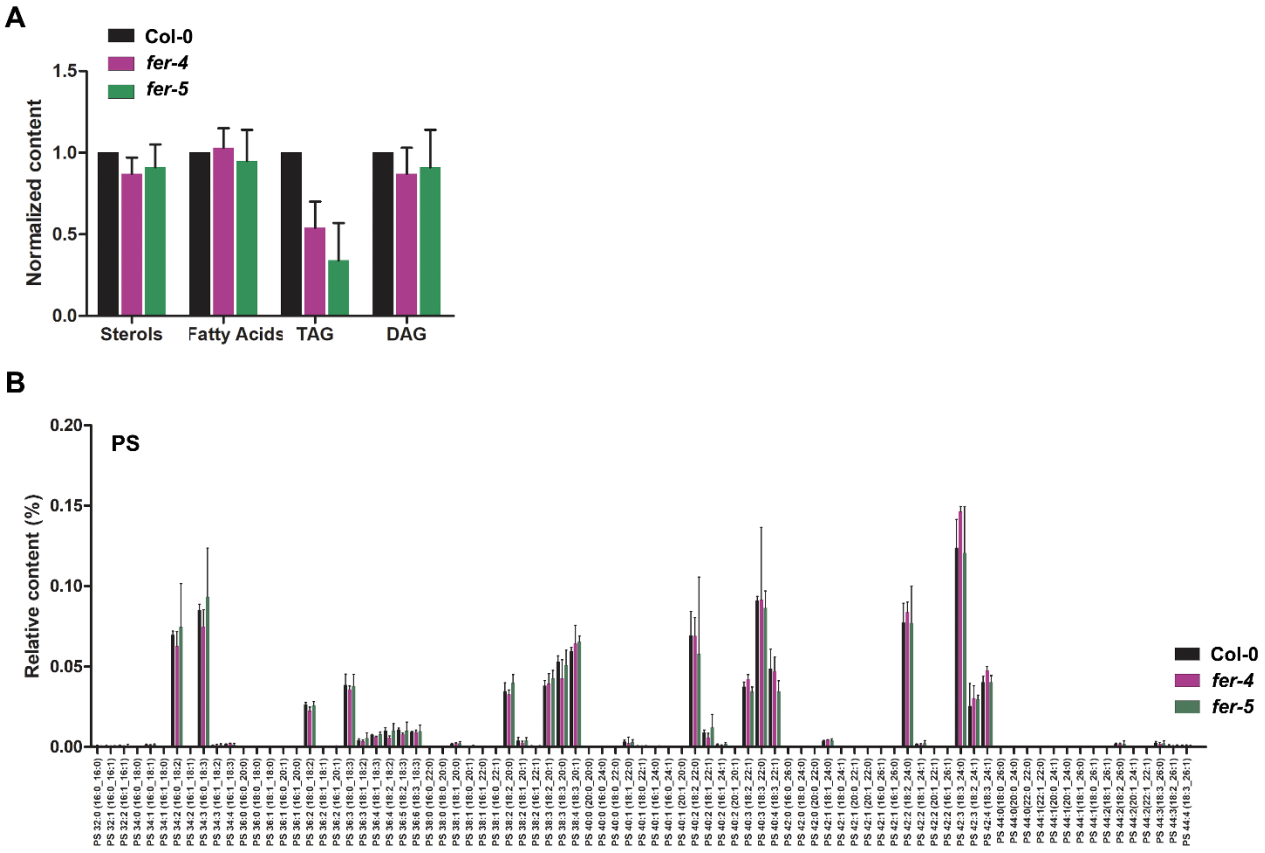
This PDF file includes:

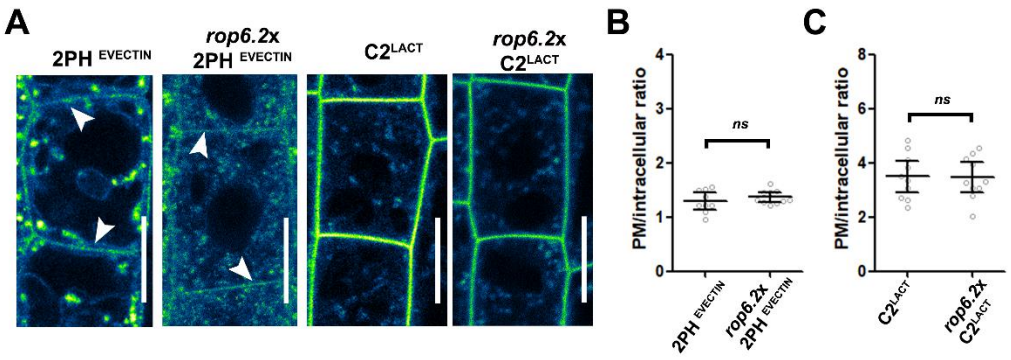
Figs. S1 to S6



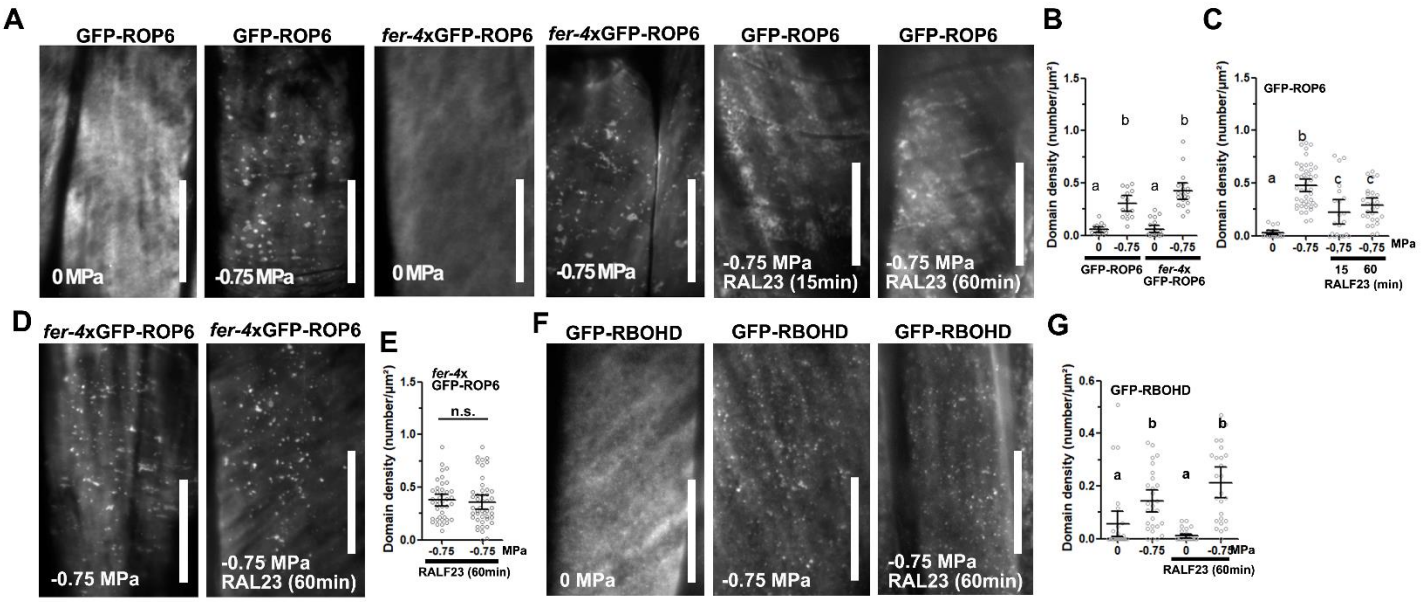
Supplemental figure 1: RALF23 can inhibit the flg22-induced ROS in roots and FER does not affect mCit-ROP6gCA protein quantity or localization. (A) DHE fluorescence quantification in Col-0 in control conditions or after 30 min of a treatment with 1µM flg22, with or without a pretreatment with 1µM RALF23 (1h). (B) Western blot analysis with an anti-GFP antibody of plant protein extracts from mCit-ROP6gCA, *fer-5*xmCit-ROP6gCA and *fer-4*xmCit-ROP6gCA. (D) Confocal micrographs showing mCit-ROP6gCA localization in Col-0, *fer-4* or *fer-5* background. PM localization of the mCitrine signal (D) and PM/intracellular mCitrine (E) signal was quantified. Error bars correspond to a confidence interval at 95%. ANOVA followed by Tukey test, with letters indicating significant differences among means (p-value<0.01). n>10 from at least 2 biological replicates. Scale bar 20 µm



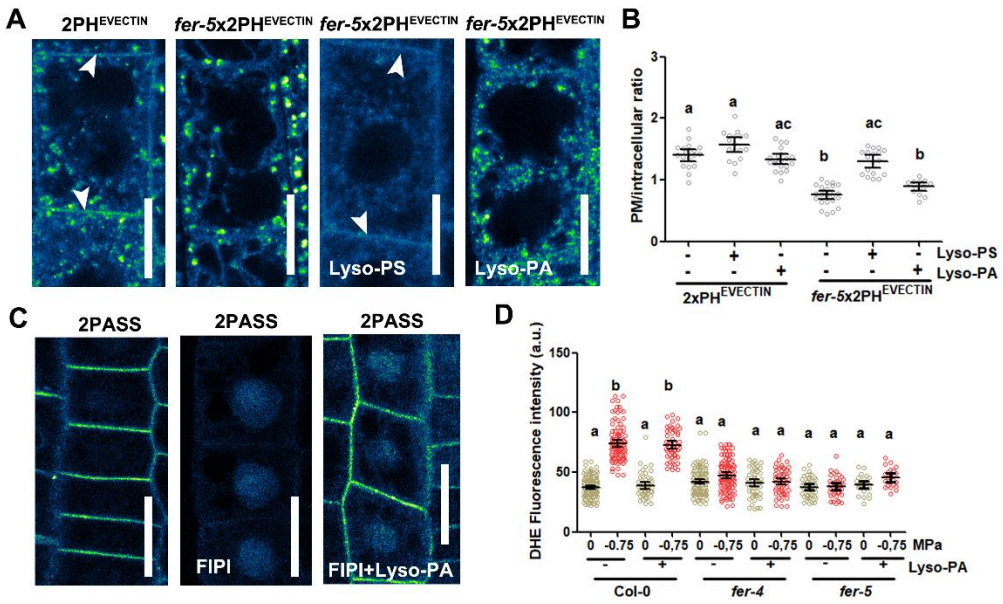
Supplemental figure 2: Lipid quantification of *fer-4* and *fer-5.* (A) Relative content in sterols, fatty acids, triacylglycerols (TAG), diacylglycerols (DAG) as quantified by high performance thin layer chromatography (HPTLC). (B) Analysis and relative quantification of the PS molecular species were performed by LC-MS/MS. (A) N=7 et (B and C) N=3.



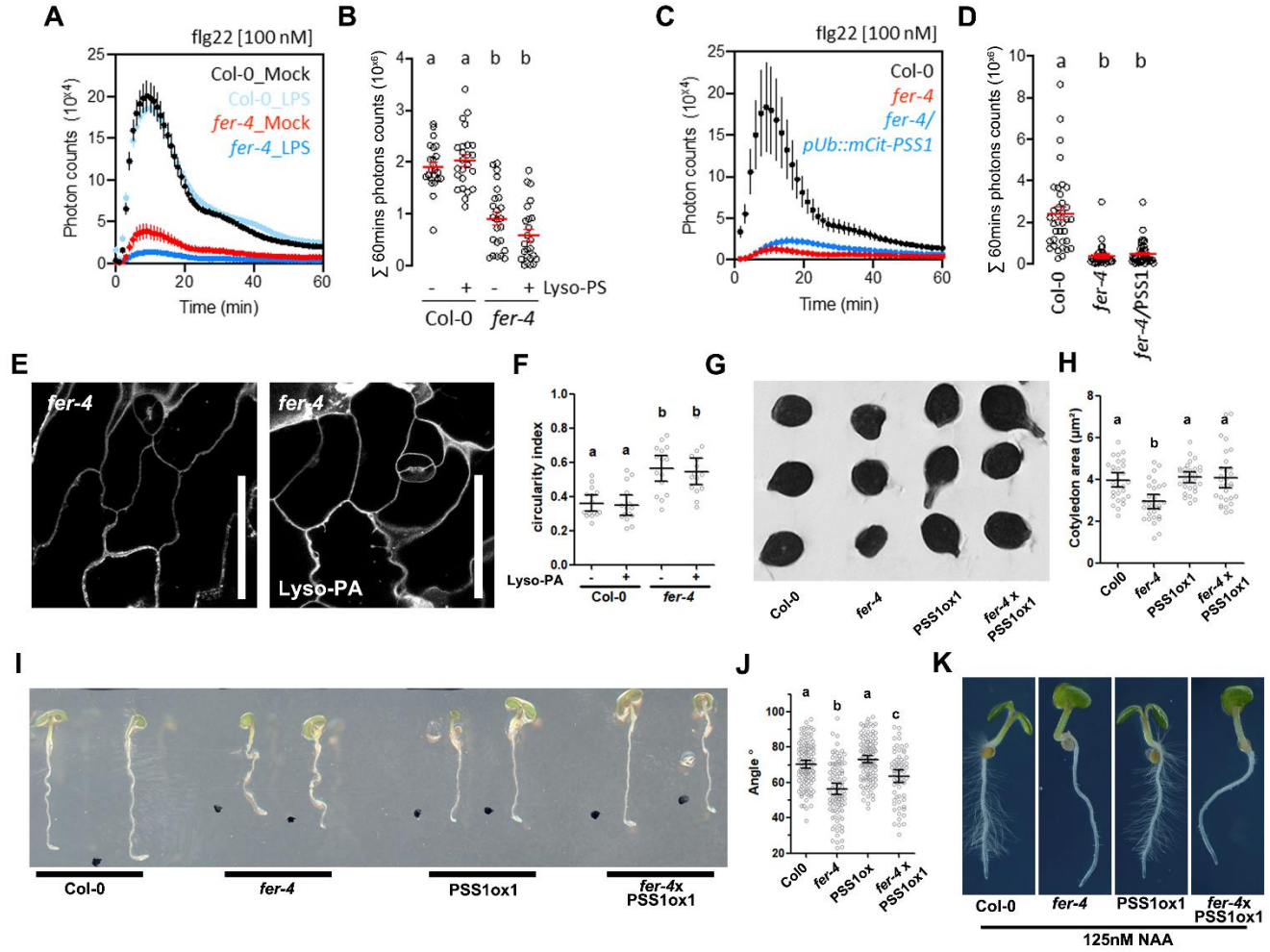
Supplemental figure 3: Effect of *ROP6* loss-of-function on PM PS localization. (A) Confocal micrographs of 2PH^{EVECTIN}, *rop6-2*x2PH^{EVECTIN}, C2^{LACT} and *rop6-2x*C2^{LACT} with their quantification (B and C). t-tests indicate that value differences are non significant. n>10 from at least 2 independent biological replicates. Scale bar 20µM.



Supplemental Figure 4: FER signaling pathway acts on ROP6 nanodomain density (A) TIRFM images of GFP-ROP6-expressing Col-0 or *fer-4* plants in control conditions or after a -0.75 MPa osmotic treatment and in the presence or absence of RALF23 pre-treatment. Quantification of GFP-ROP6 cluster density in different conditions (B and C). (D) TIRFM images of GFP-ROP6 expressing *fer-4* plants after a -0.75 MPa osmotic treatment and in the presence or absence of RALF23 pre-treatment; and its quantification (E). (F) TIRFM images of GFP-RBOHD expressing Col-0 plants in control conditions or after a -0.75 MPa osmotic treatment and in the presence or absence of RALF23 pre-treatment. (G) Quantification of GFP-ROP6 cluster density in different conditions. Error bars correspond to 95% confidence interval. ANOVA followed by Tukey test, with letters indicating significant differences among means (p-value<0.01). n>18 from at least 2 biological replicates. Scale bar 10 µm.



Supplemental figure 5: Exogenous application of Lyso-PS but not Lyso-PA can complement PS misregulation in *fer-5* mutant and osmotically induced ROS accumulation. (A) Confocal micrographs of 2PH^{EVECTIN} *fer-5*x2PH^{EVECTIN} with or without Lyso-PS and Lyso-PA supplementation. (B) Quantification of the PM/intracellular ratio, of 2PH^{EVECTIN} PS sensors in a *fer-5* background, with lyso lipid treatment. (C) Confocal images of phosphatidic acid (PA) sensor 2PASS localization with or without PLD inhibitor, 5-fluoro-2-indolyl des-chlorohalopemide (FIPI), and with exogenous application of Lyso-PA. (D) DHE fluorescence quantification in Col-0, *fer-4* and *fer-5* plants in control conditions or after 15 min of a -0.75MPa treatment with (+) or without (-) a Lyso-PA pretreatment. Arrowheads show PM labelling Error bars correspond to a confidence interval at 95%. ANOVA followed by Tukey test, with letters indicating significant differences among means (p-value<0.001). n>12 from at least 2 independent biological replicates. Scale bar 20µm.



Supplemental figure 6: Impact of PSS1 over-expression or Lyso-PS exogenous application on various *fer4* related phenotype. (A-D) ROS production in Col-0, *fer-4* or *fer-4*xPSS1ox1 leaf discs treated with flg22 (100nM), and pre-treated for an hour with or without Lyso-PS (A-B). Data are presented as kinetics (A, C) or total photon counts for 60 min (B, D). (E) Confocal images of *fer-4* that was PVC grown in media with or without Lyso-PA for three days and its corresponding cell circularity quantification (F). (G) Comparison of 6-day-old cotyledons from Col-0, *fer-4,* PSS1ox1 and *fer-4x*PSS1ox1. Quantification of cotyledon sizes (H). (I, J) Root angle after 6 hours of 90° gravistimulation. (K) Col-0, *fer-4,* PSS1ox1 and *fer-4x*PSS1ox1 grown for 5 days on 125nM NAA to induce root hair elongation. Error bars correspond to a confidence interval at 95%. ANOVA followed by Tukey test, with letters indicating significant differences among means (p-value<0.001). n>12 from at least 2 independent biological replicates.