New Phytologist Supporting Information

Article title: **Chloroplast redox state changes mark cell-to-cell signaling in the hypersensitive response**

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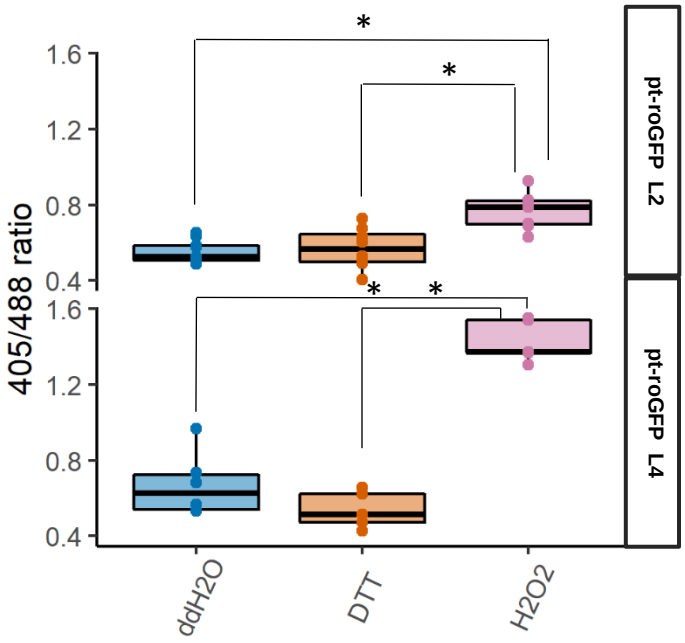
Article acceptance date: 26th July 2022

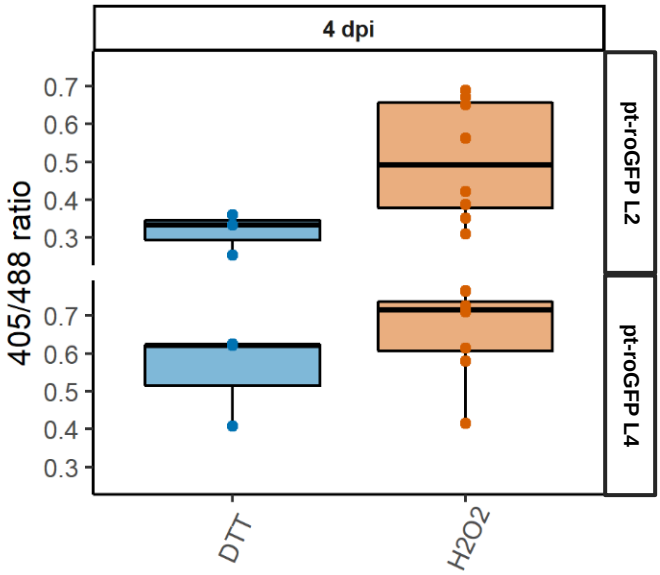
Supporting Information Fig. S1: Relative chloroplast redox state after PVY and mock inoculation and H_2O_2 and DTT treatments. Relative chloroplast redox state was measured in redox state sensor plants (pt-roGFP and SA-deficient pt-roGFP-NahG) with redox sensitive GFP targeted to chloroplasts following PVY inoculation. Chloroplast redox state was measured in different transgenic lines L2, L4 and L15 of pt-roGFP (a) and L2 and L7 of pt-roGFP-NahG (b) in the area adjacent to the lesion (ROI1), adjacent to ROI1 (ROI2) and distant from the lesion (CTR) (upper panel, see Fig. 1a for sampling scheme). As a control, chloroplast redox state was also measured in mock-inoculated plants at 3, 5 and 7 days post inoculation To confirm that roGFP targeted to potato chloroplasts can be oxidized and reduced, we treated the leaves with DTT and H_2O_2 at 4 dpi. Chloroplast redox state was determined as the relative proportion of oxidized and reduced roGFP (405/488 ratio) in above mentioned leaf areas in transgenic lines. Results are presented as boxplots with 405/488 ratios of each measured ROI shown as dots (Supporting Information Table S1). Grey lines connect ROI1 and ROI2 pairs for each lesion. Results of independent experiment are presented on separate graphs and named with consecutive numbers (e.g. Exp2, Exp3,…). Asterisks denote statistically significant differences between the marked regions (ROI2, CTR or MOCK) and ROI1 for upper panels or differences between treatments for bottom panels (see Supporting Information Table S2 for the p-values). The title of each graph is linked with the Supporting Information Table S1 by the experiment name. See Supporting Information Table S2 for statistically significant differences between all regions and the p-values. Moderate difference in expression level between transgenic lines in different experiments had no impact on spatial redox state.

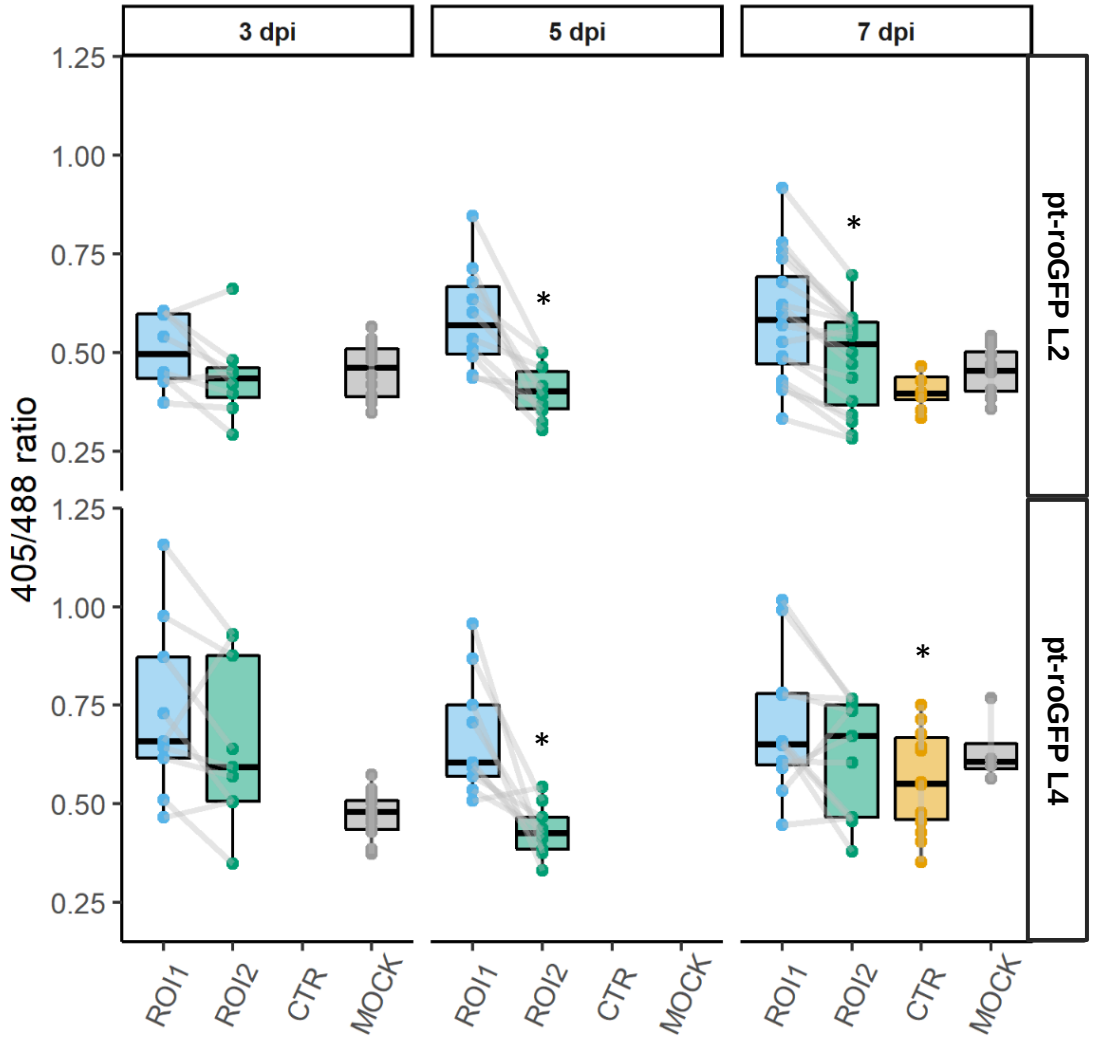
a) pt-roGFP

Redox Exp1TNT

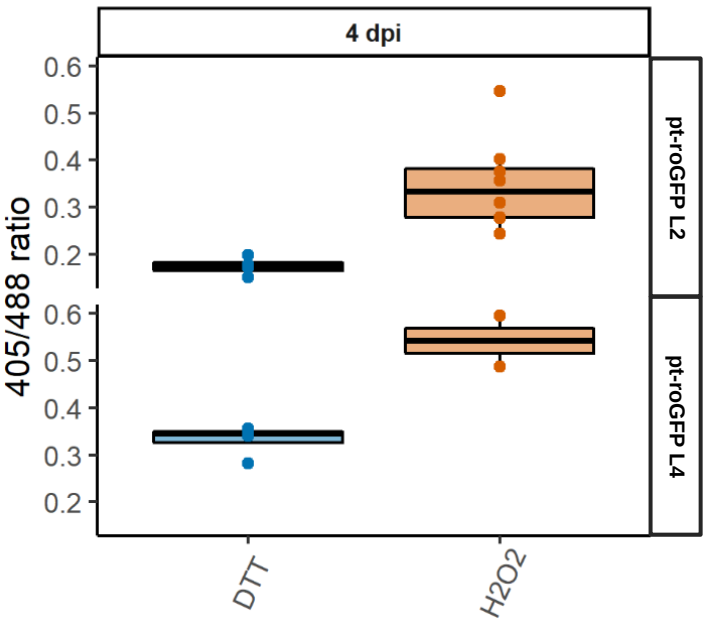


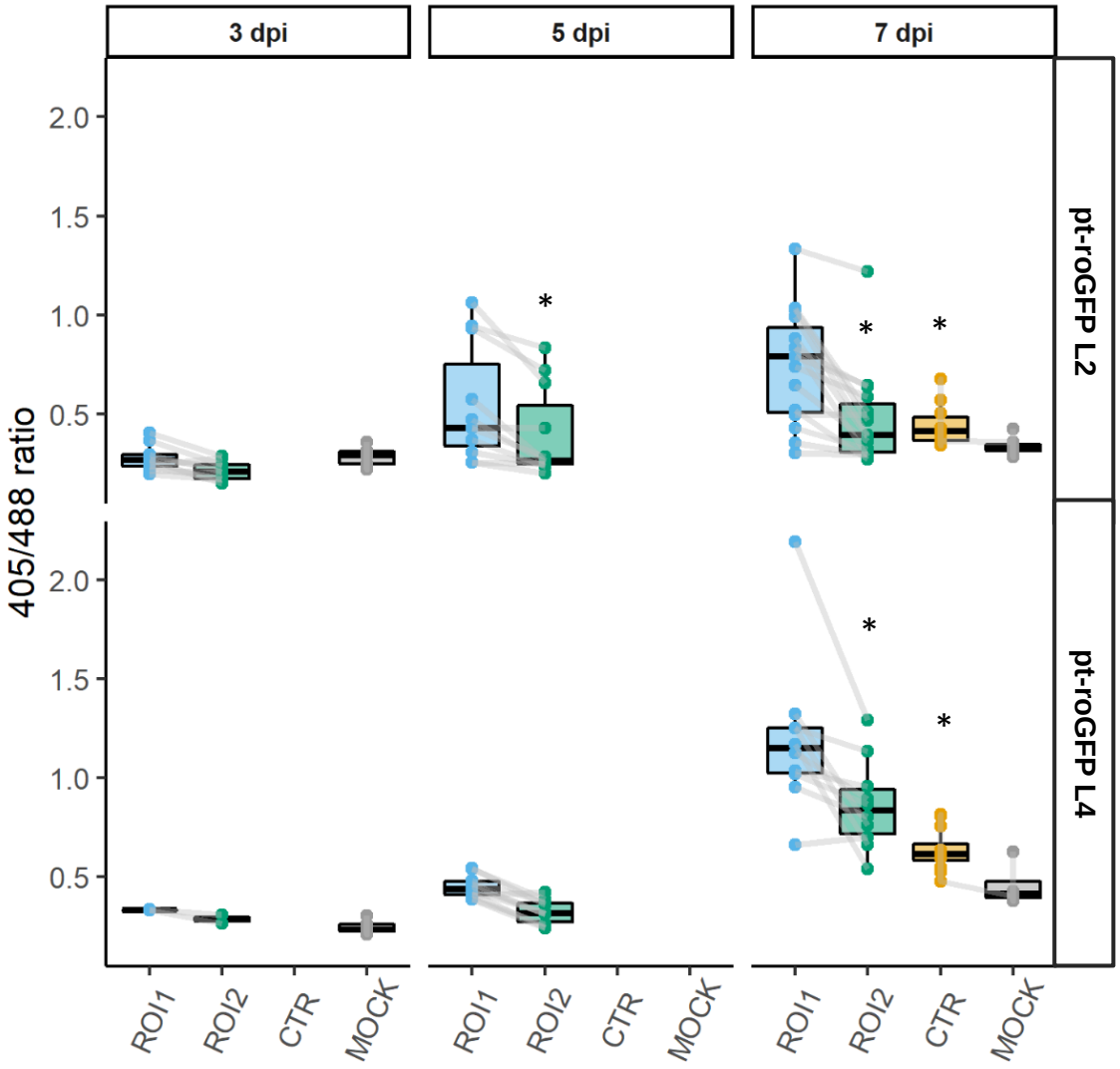
Redox Exp4NT



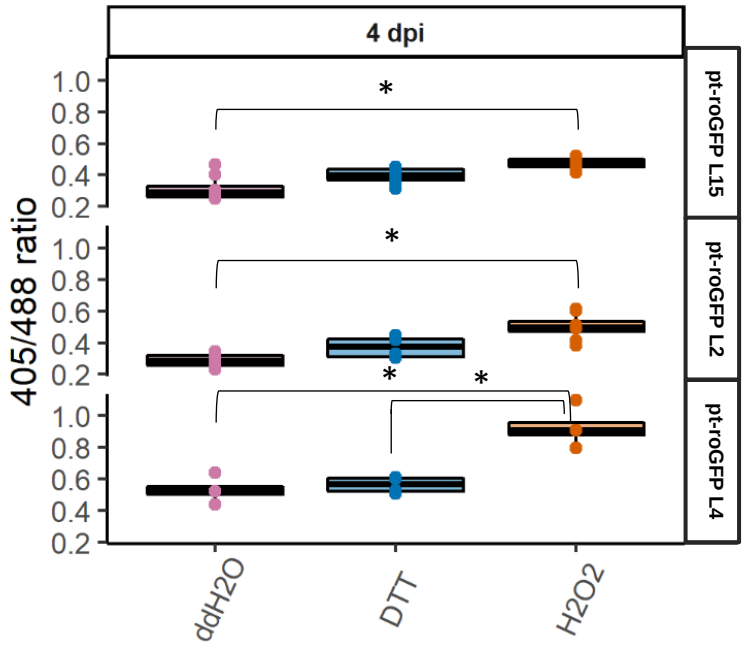


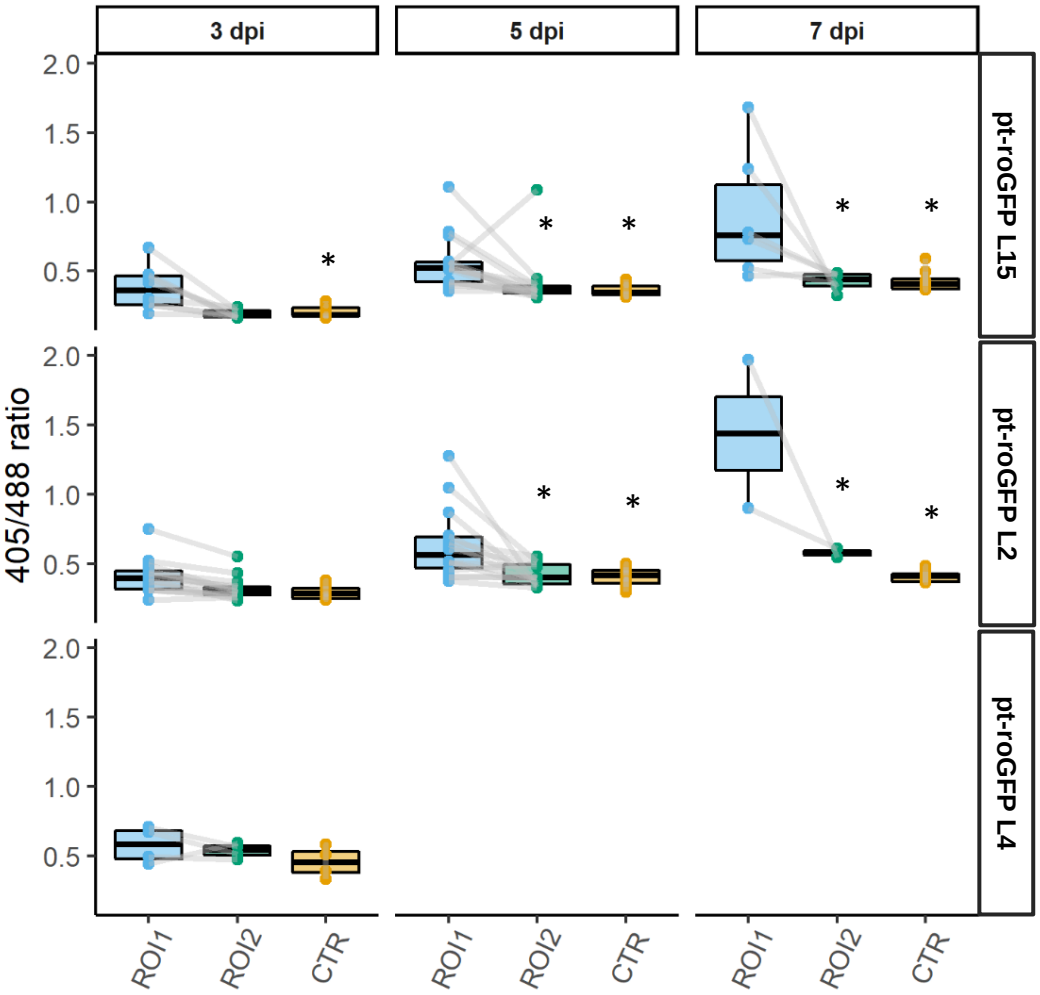
Redox Exp5NT





Redox Exp6NT





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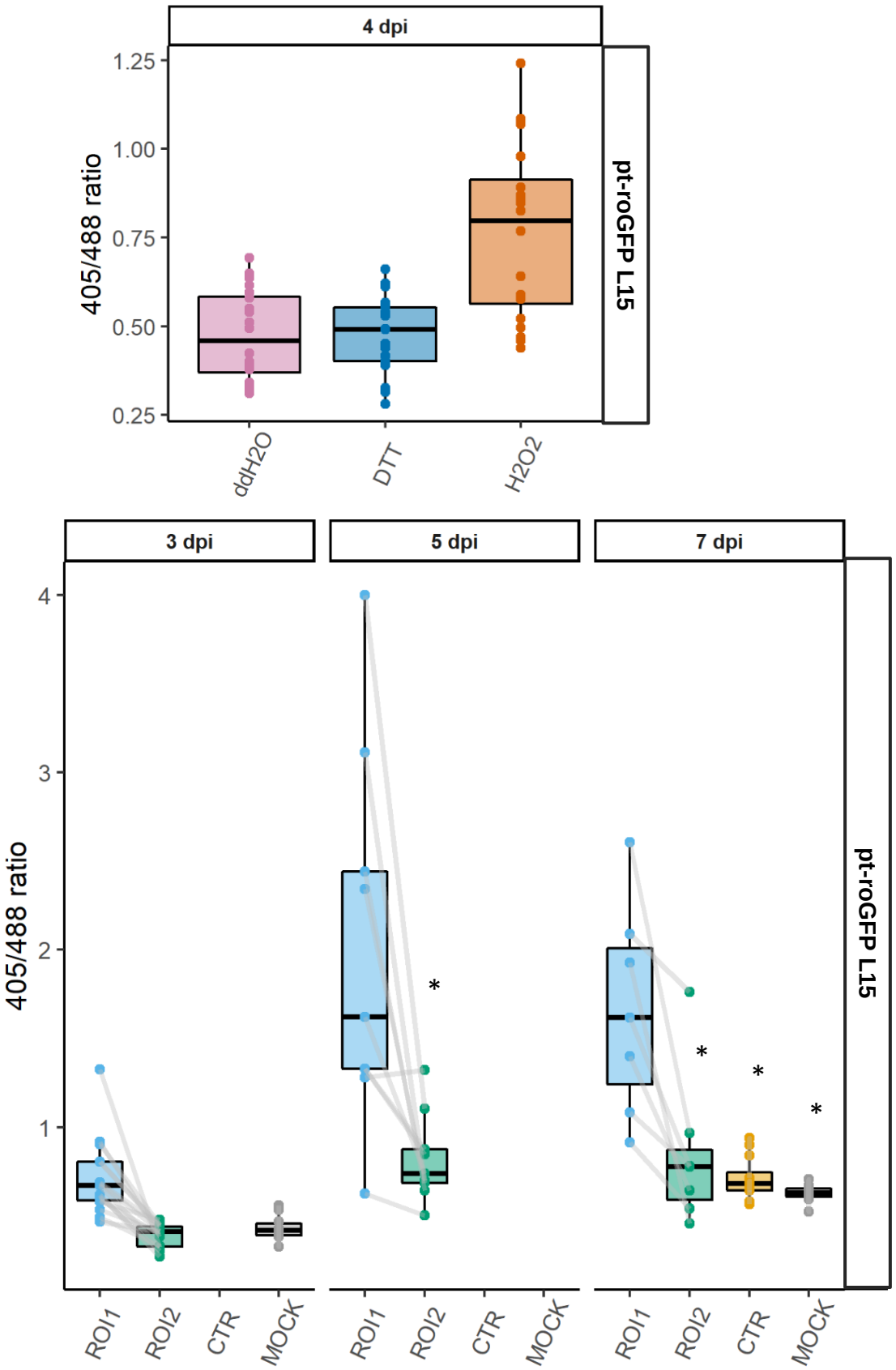
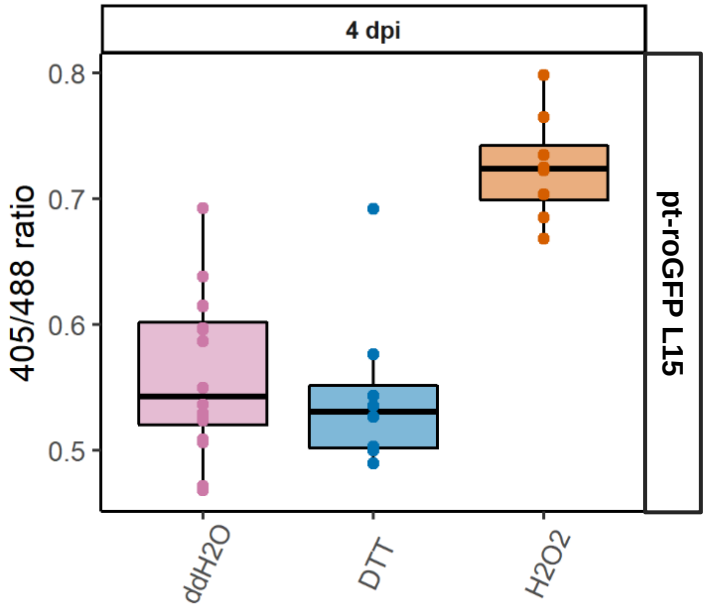
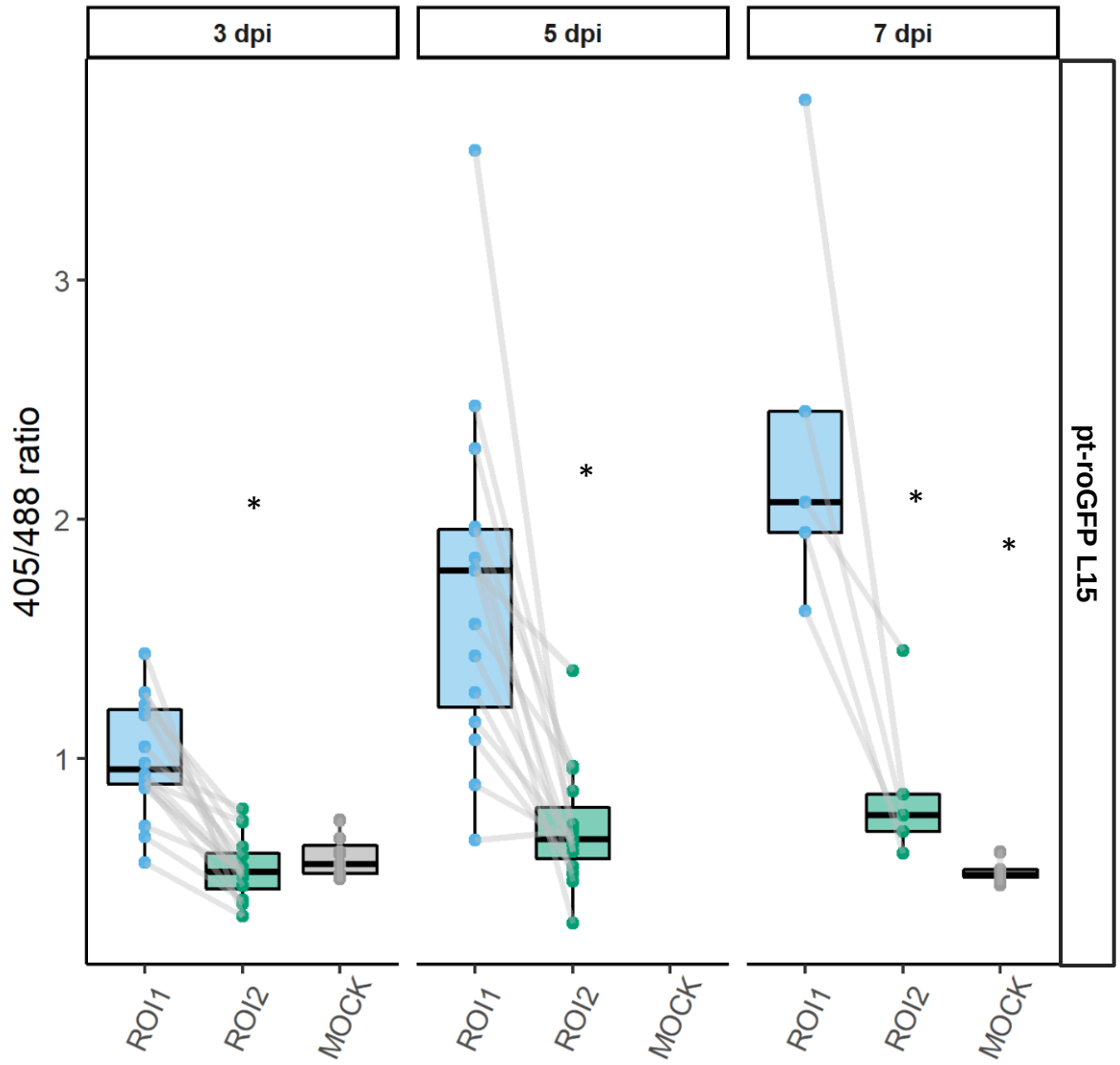
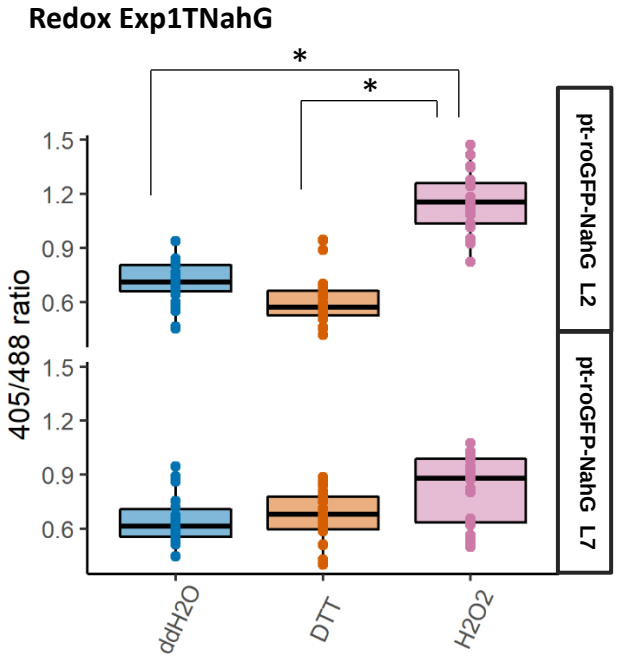


Redox Exp8NT

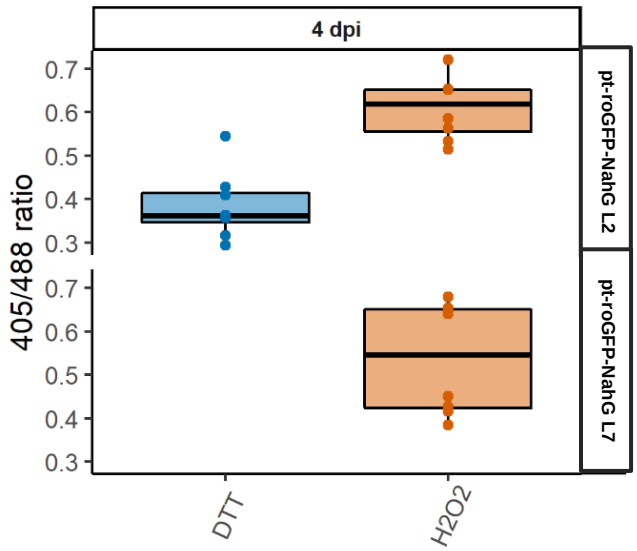


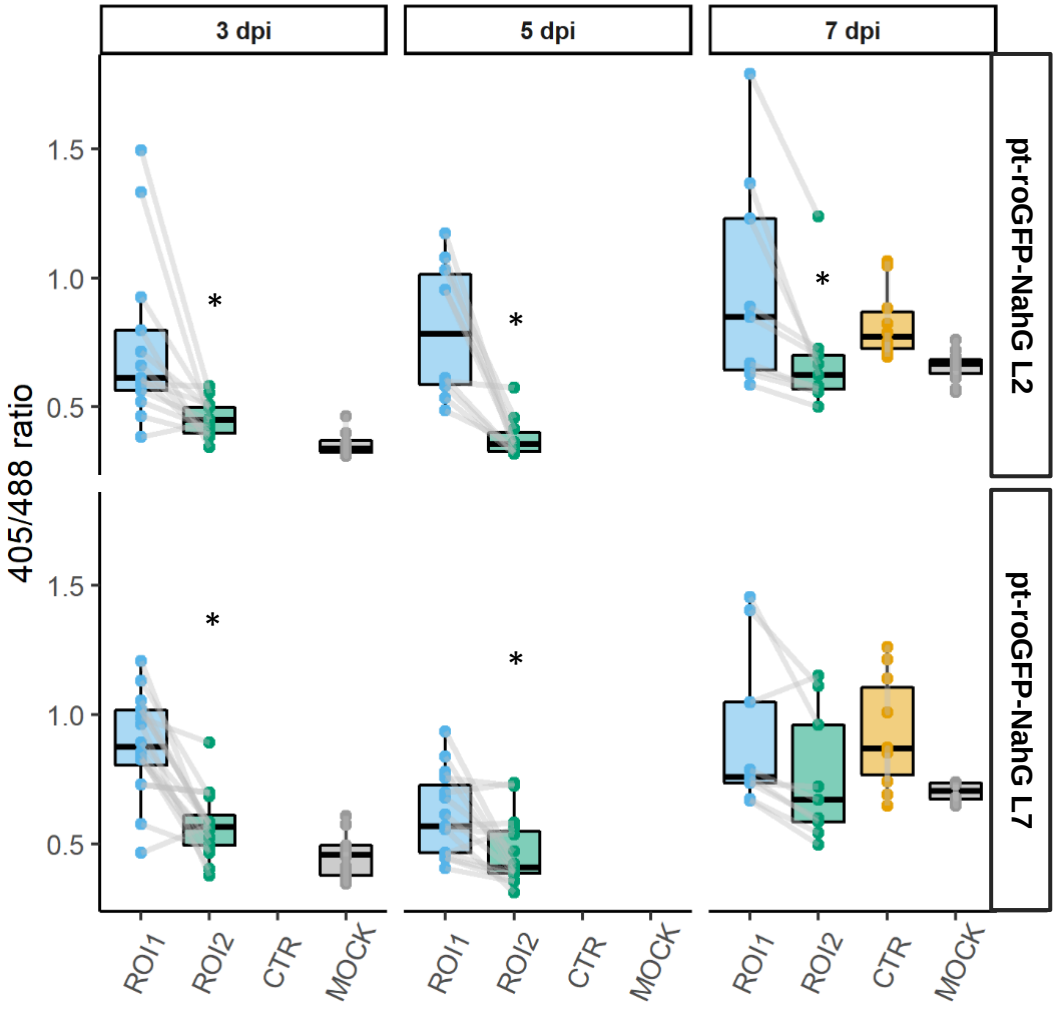


b) pt-roGFP-NahG

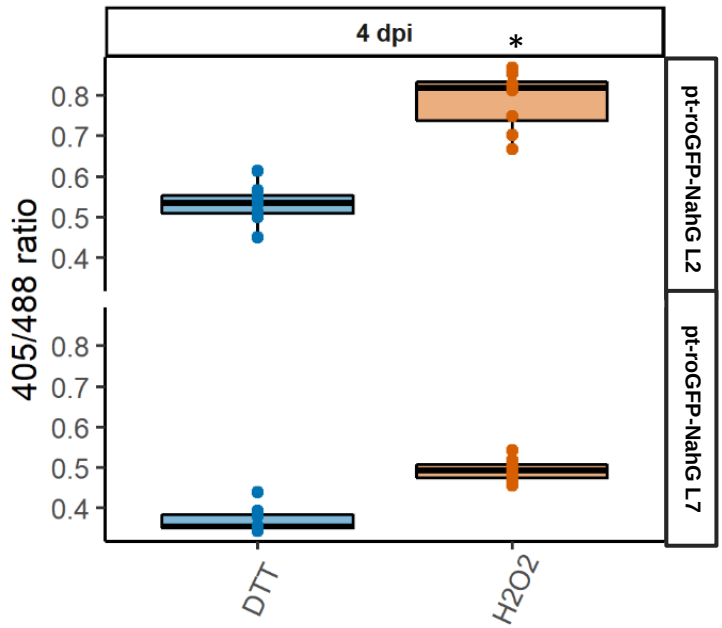


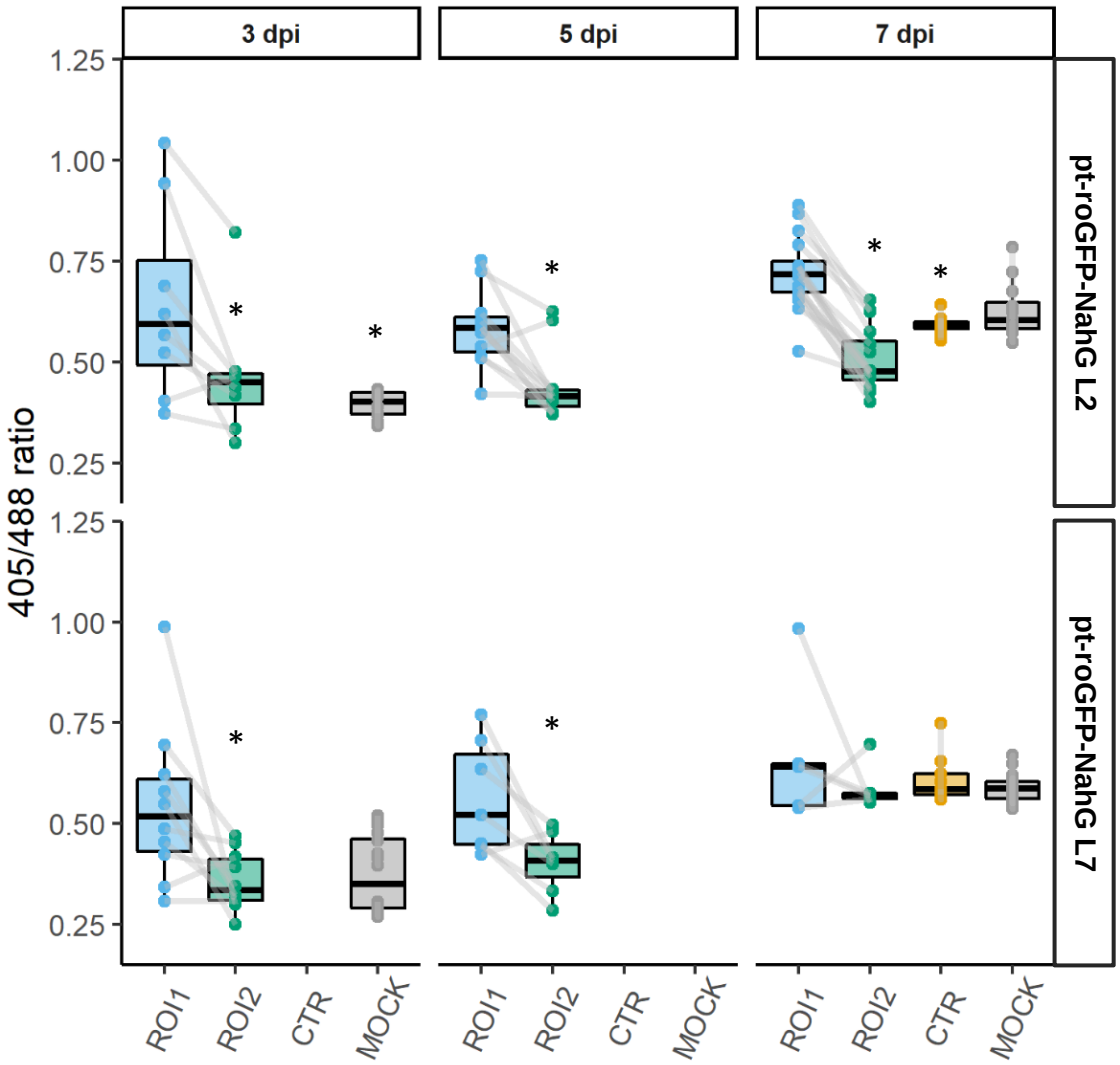
Redox Exp2NahG





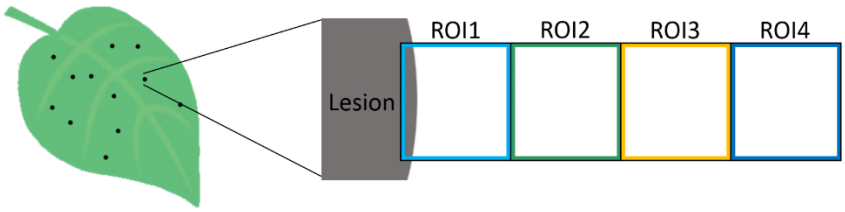
Redox Exp3NahG



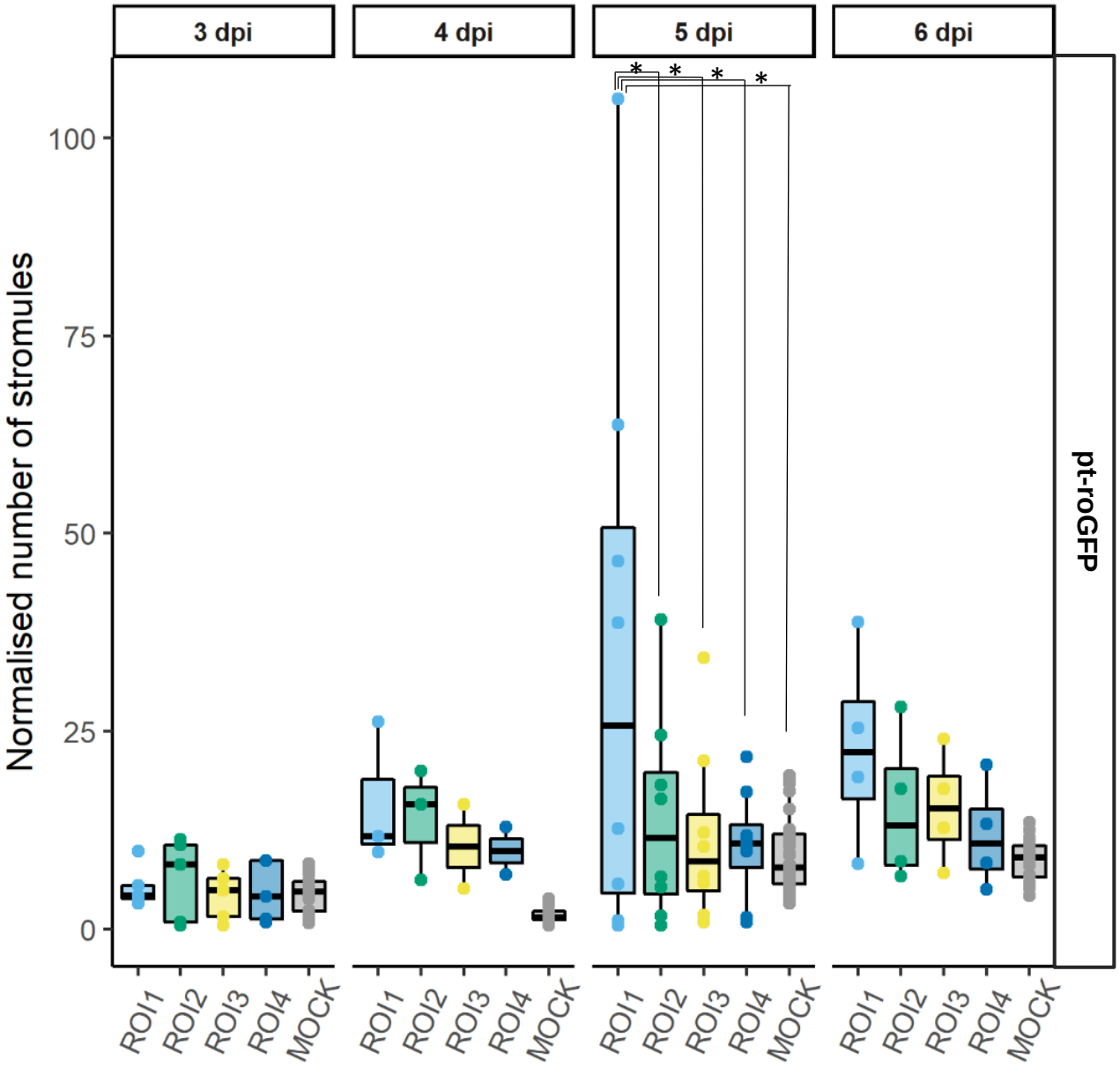


Supporting Information Fig. S2: Stromule formation around the cell death zone is differently spatiotemporally regulated between potato genotypes. a) Stromule formation was followed in redox state sensor plants (pt-roGFP and SA-deficient pt-roGFP-NahG) with redox sensitive GFP targeted to chloroplasts following PVY inoculation in two (ROI1, ROI2) or four (ROI1-ROI4) regions around the cell death zone in four experiments (see Supporting Information Table S3 for details). As a control, stromule formation was also followed in MOCK-inoculated plants. Normalised number of stromules was calculated by dividing the number of stromules by the number of chloroplasts counted in the above mentioned regions to obtain normalized number of stromules at different days post inoculation (dpi) (Supporting Information Table S3). Results are presented as boxplots with normalised numbers of stromules for each ROI shown as dots (Supporting Information Table S3). Asterisks denote statistically significant differences between regions (see Supporting Information Table S7 for p-values). b) Normalised number of stromules in pt-roGFP L2 in four consecutive regions adjacent to the cell death zone (ROI1-ROI4), at each dpi. c) Normalised number of stromules in pt-roGFP L2 in two consecutive regions adjacent to the cell death zone (ROI1 and ROI2) at 4 and 5 dpi. d) Normalised number of stromules in pt-roGFP-NahG L2 in four consecutive regions adjacent to the cell death zone (ROI1-ROI4) at each dpi. e) Normalised number of stromules in pt-roGFP-NahG L2 in two consecutive regions adjacent to the cell death zone (ROI1 and ROI2), averaged across the four dpis (see Fig. 4d for the normalized number of stromules on the particular dpi). The title of each graph is linked with the Supporting Information Table S3 by the experiment name. Note different scales on the ordinate axes.

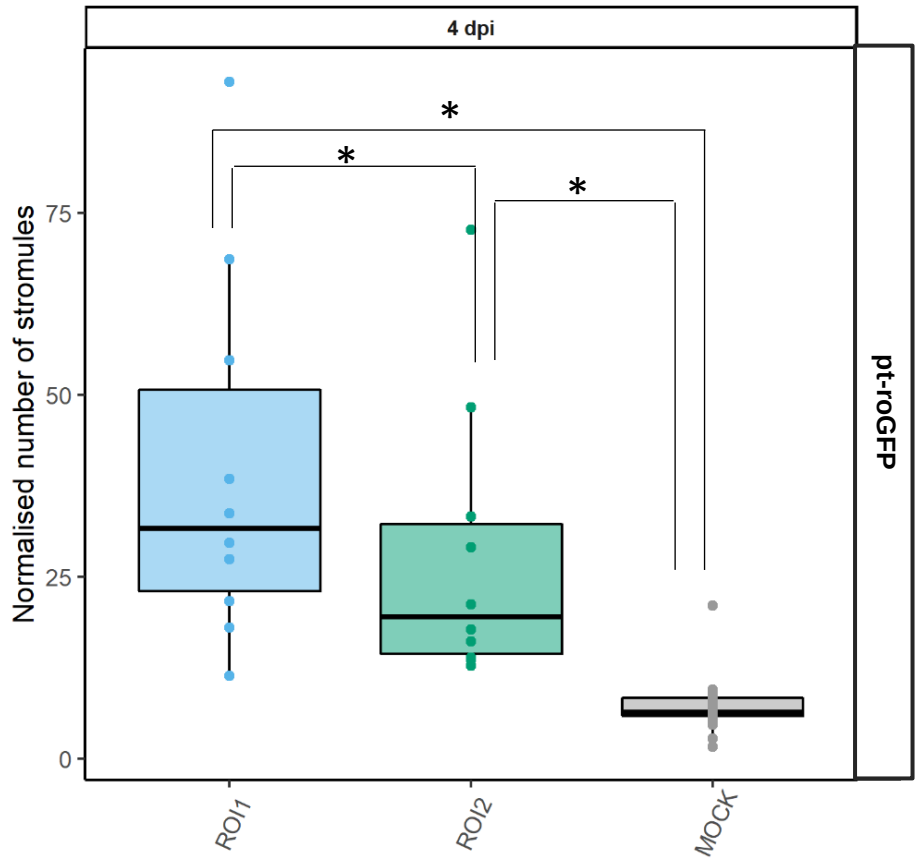
a)

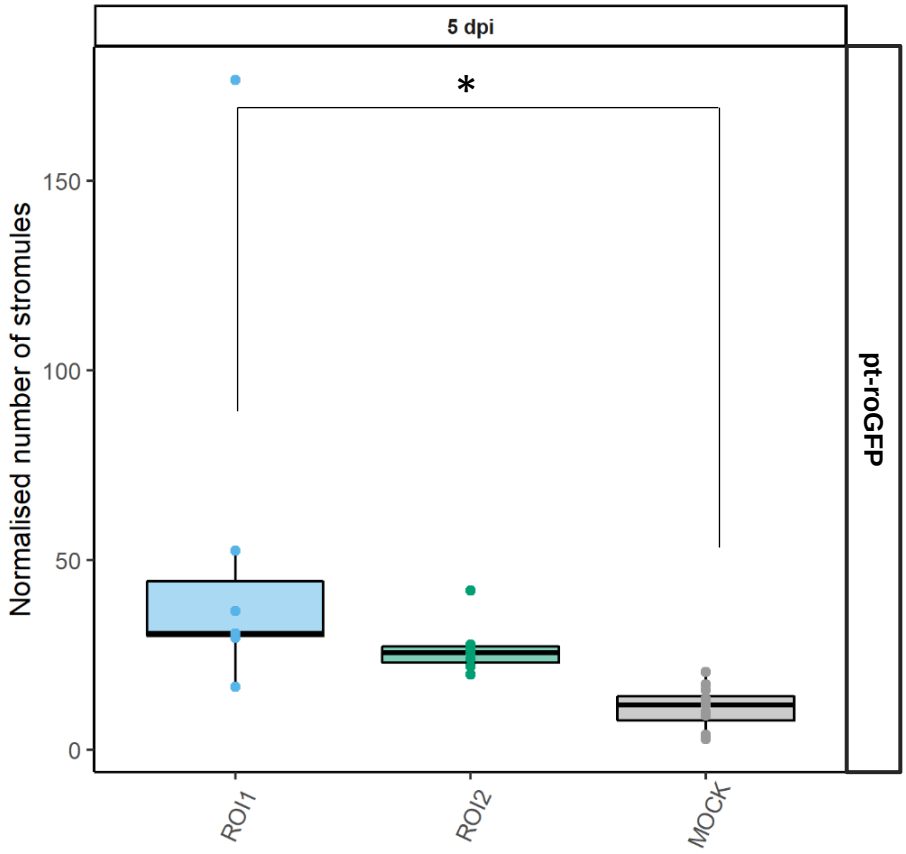


b) Stromules Exp3NT

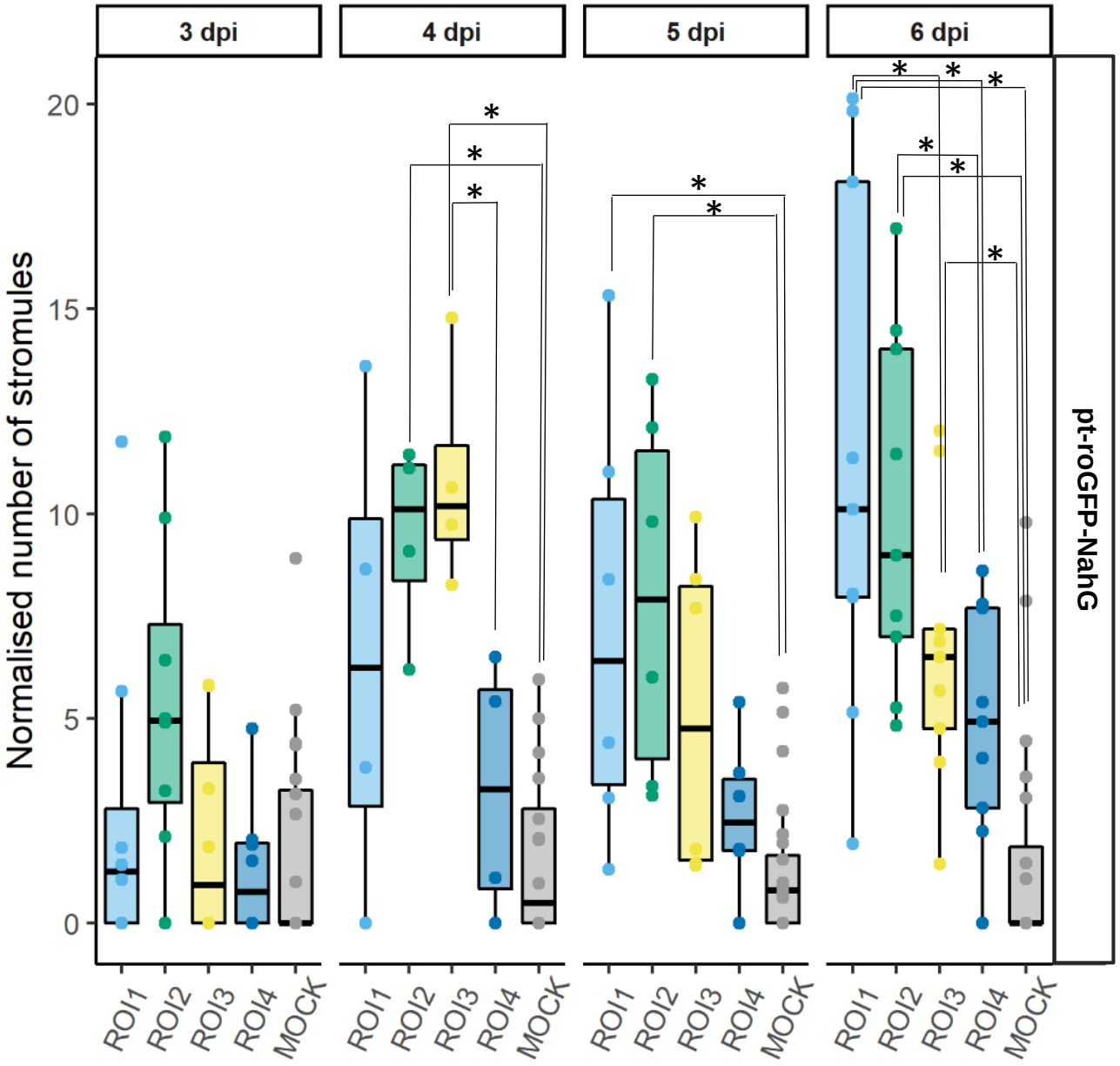


c) Stromules Exp4NT

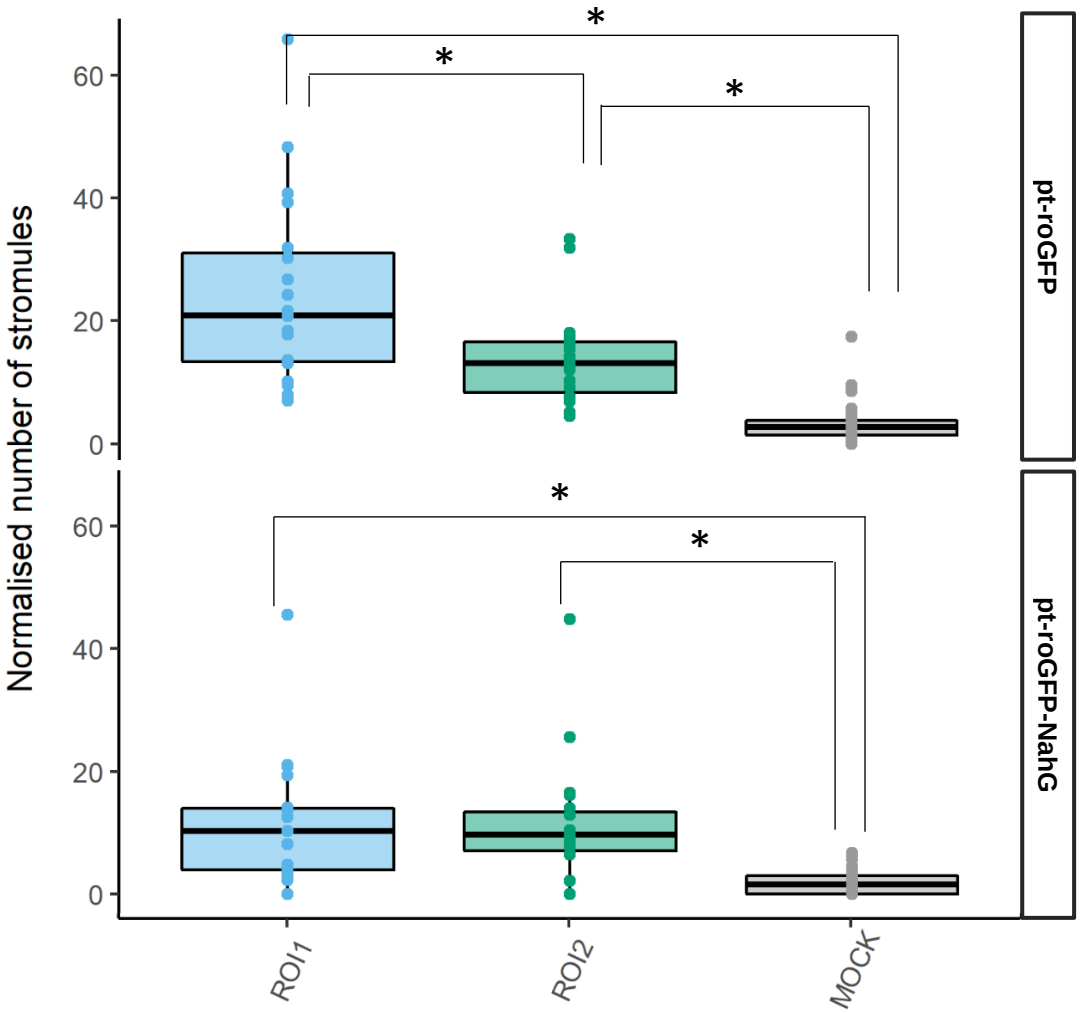




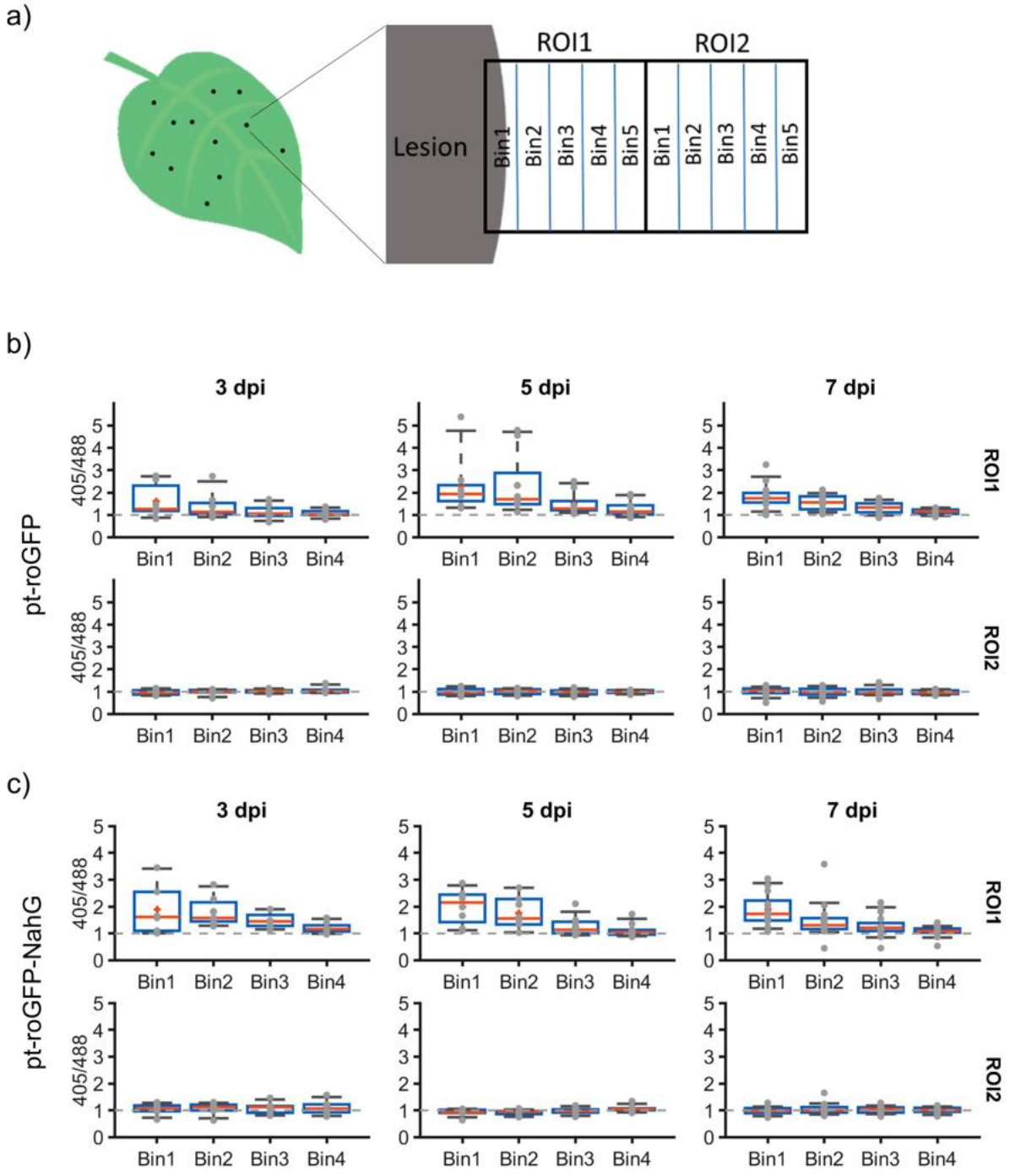
d) Stromules Exp5NahG



e) Stromules Exp6NTNahG



Supporting Information Fig. S3: Chloroplast redox state is highly oxidized around the cell death zone. a) Chloroplast redox state was measured in pt-roGFP and pt-roGFP-NahG sensor plants s following PVY inoculation in the area adjacent to the lesion (ROI1) and adjacent to ROI1 (ROI2), separated into five regions (Bins) according to the distance from the cell death zone. b, c) The normalized 405/488 ratio in five Bins of ROI1 (top panel) and ROI2 (bottom panel) in pt-roGFP L2 (b) and pt-roGFP-NahG L2 (c). The 405/488 ratios in each of the first four Bins were normalized to the 405/488 ratio from Bin 5, which was set to 1 (dotted line) in both ROIs. 405/488 ratio in Bin 5 of ROI1 was similar to 405/488 ratio in Bin 5 of ROI2, allowing for comparisons within and between ROIs. Results are presented as boxplots, with normalized 405/488 ratio for each Bin shown as dots (Redox Exp3NahG and Exp5NT in the Supporting Information Table S1 and Supporting Information Table S5). See Supporting Information Table S2 for the comparison between genotypes determined by statistical analysis (mixed effects model (ANOVA)).

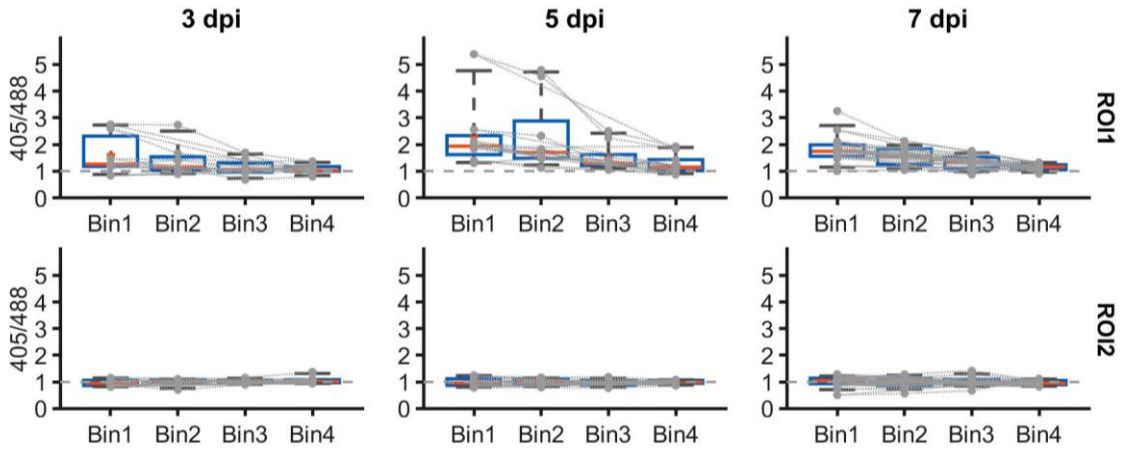

a)
ROI1
ROI2
Lesion
Bin1
Bin2
Bin3
Bin4
Bin5
b)
3 dpi
5 dpi
7 dpi
pt-roGFP
405/488
ROI1
ROI2
c)
pt-roGFP-NahG

Supporting Information Figure S4: Detailed spatial analysis of relative chloroplast redox state around the cell death zone. Chloroplast redox state, measured in redox state sensor plants (pt-roGFP L2, L4, L15 (a) and SA-deficient pt-roGFP-NahG L2, L7 (b)) with redox state sensitive GFP (roGFP) targeted to chloroplasts following PVY inoculation in the area adjacent to the lesion (ROI1) and adjacent to ROI1 (ROI2), each separated into five regions (Bins) according to the distance from the cell death zone (see Supporting Information Fig. S3a). Relative chloroplast redox state was determined as the ratio of fluorescence intensities of roGFP after excitation with 405 and 488 nm laser. The 405/488 ratios in each of the first four Bins were normalized to the 405/488 ratio from Bin 5, which was set to 1 (dotted line) in both ROIs. 405/488 ratio in Bin 5 of ROI1 was similar to 405/488 ratio in Bin 5 of ROI2, allowing for comparisons within and between ROIs. Results are presented as boxplots, with normalized 405/488 ratio for each Bin shown as dots (Supporting Information Table S1 and Supporting Information Table S5). L – transgenic line. For each image, the respective experiment and image names are shown. The title of each Figure is linked with the Supporting Information Table S1 by the experiment name. See Supporting Information Table S1 for the details regarding presented images.

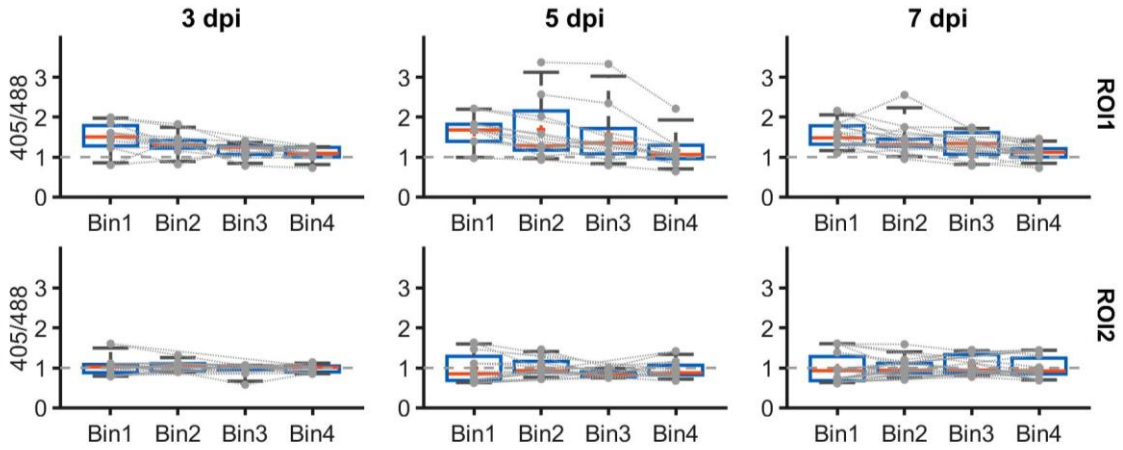
a) pt-roGFP

Redox Exp4NT

L2

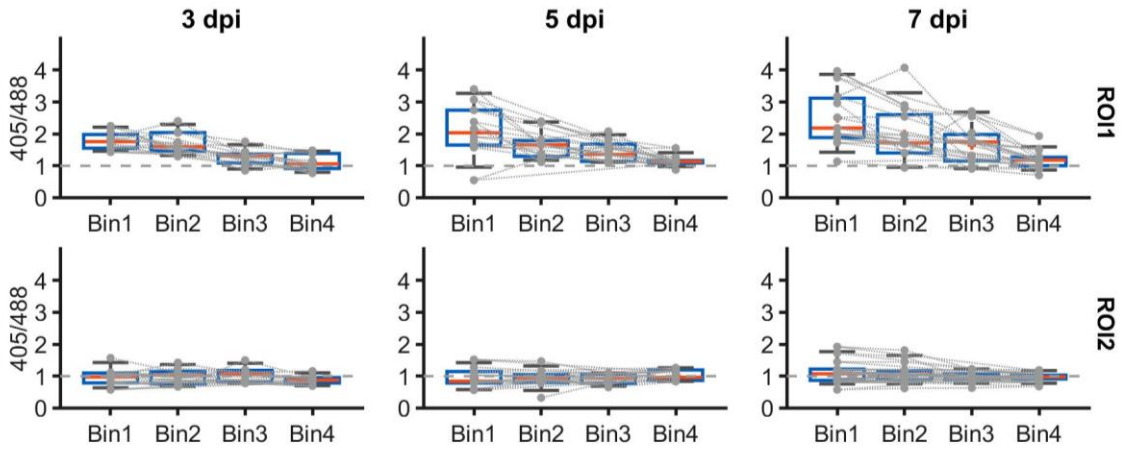


L4

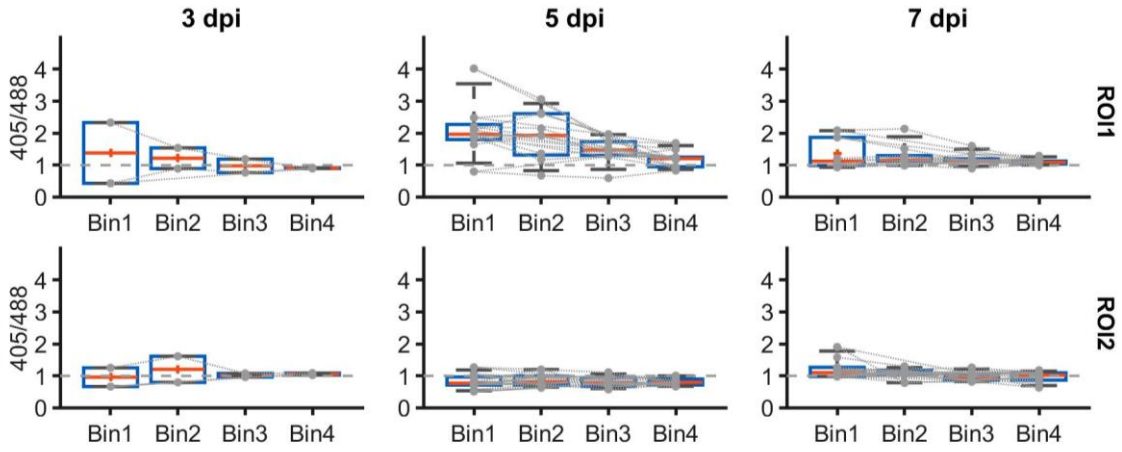


Redox Exp5NT

L2

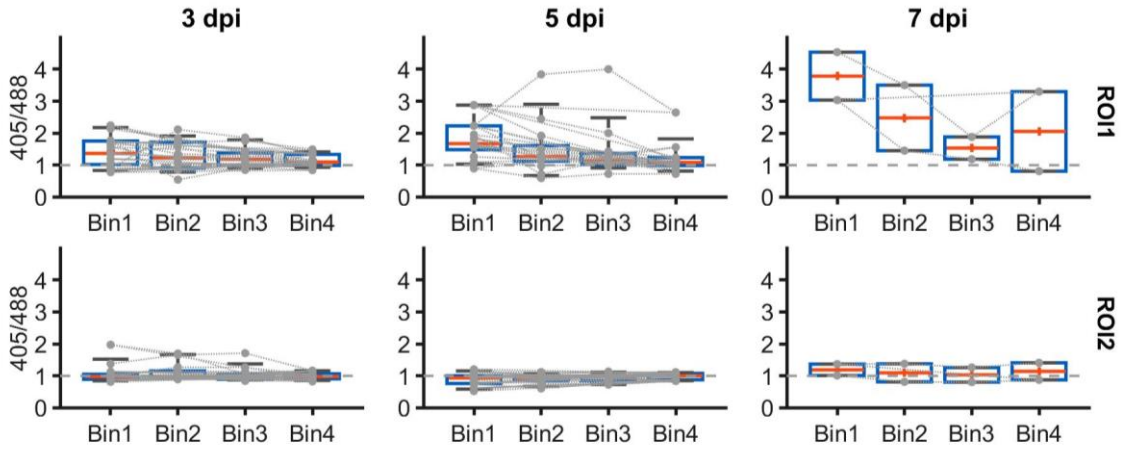


L4

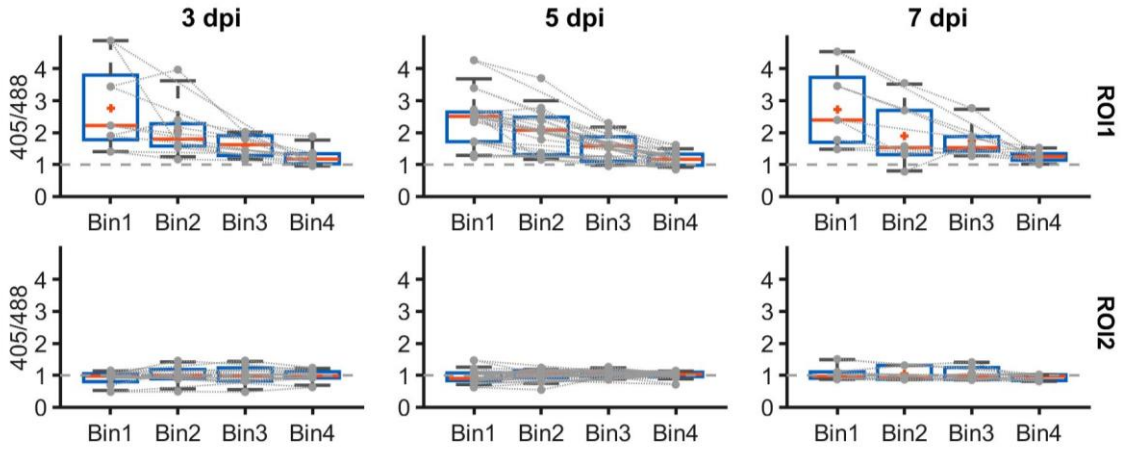


Redox Exp6NT

L2

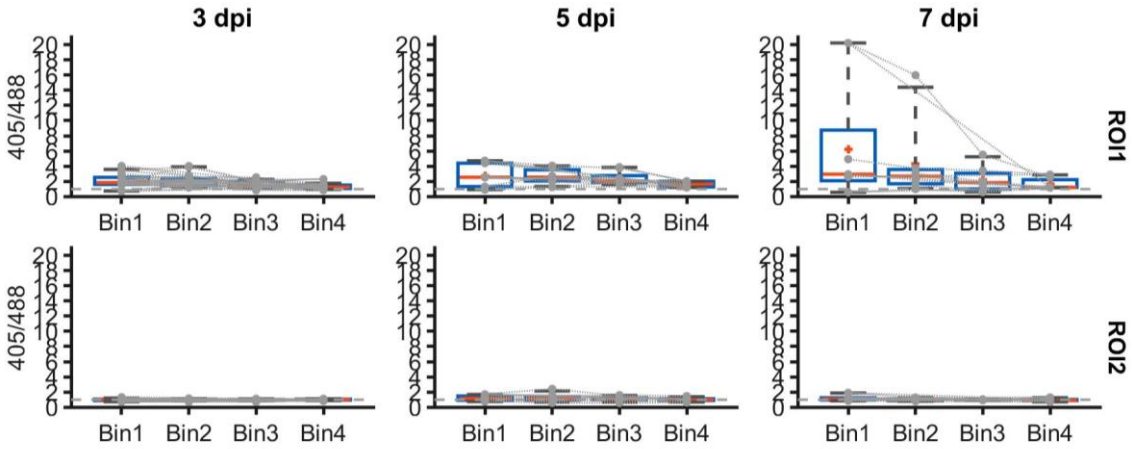


L15



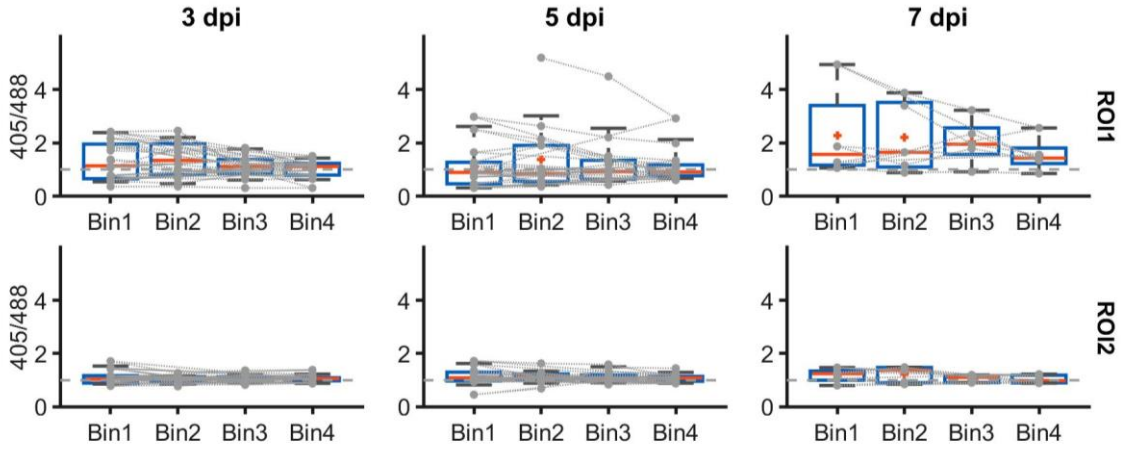
Redox Exp7NT

L15



Redox Exp8NT

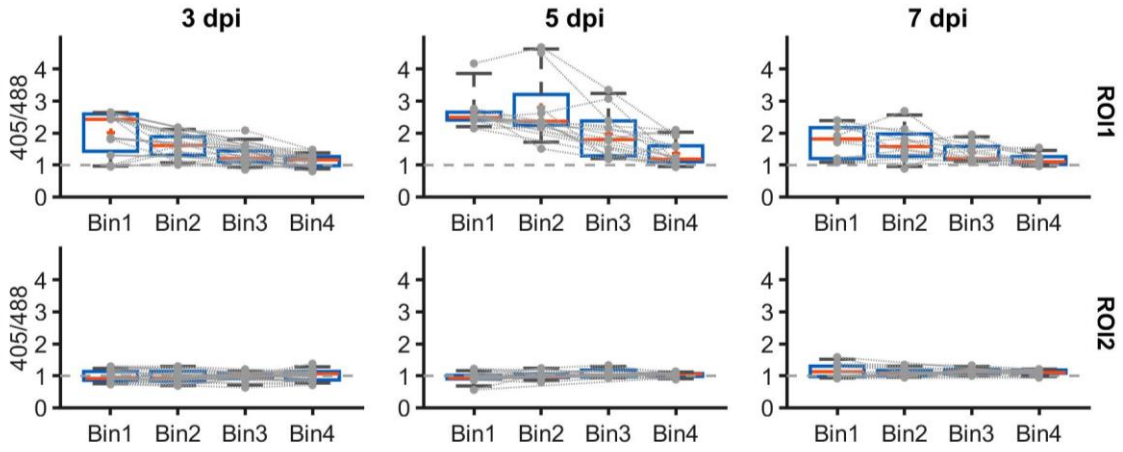
L15



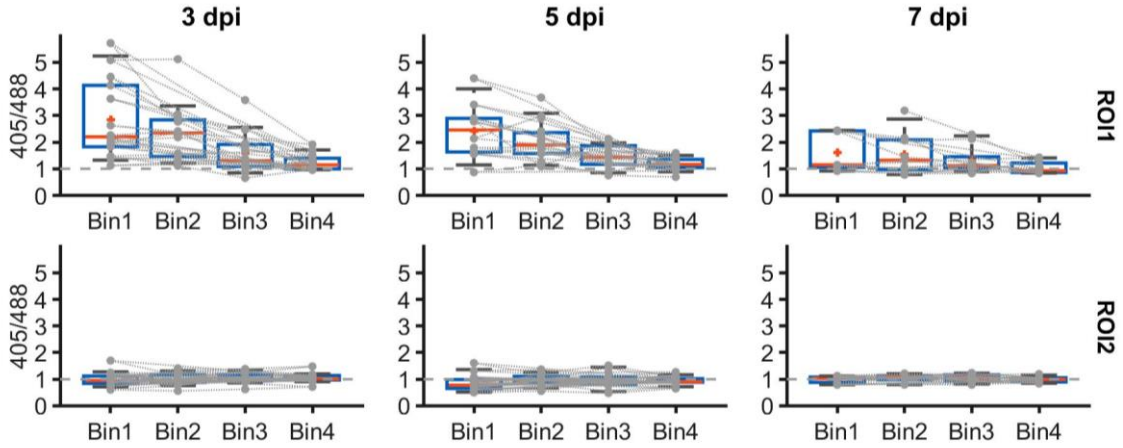
b) pt-roGFP-NahG

Redox Exp2NahG

L2

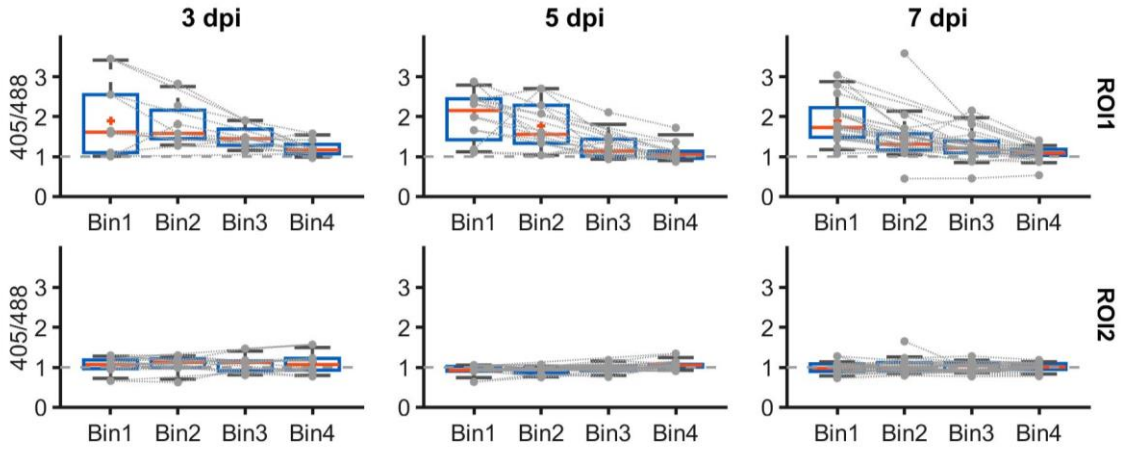


L7

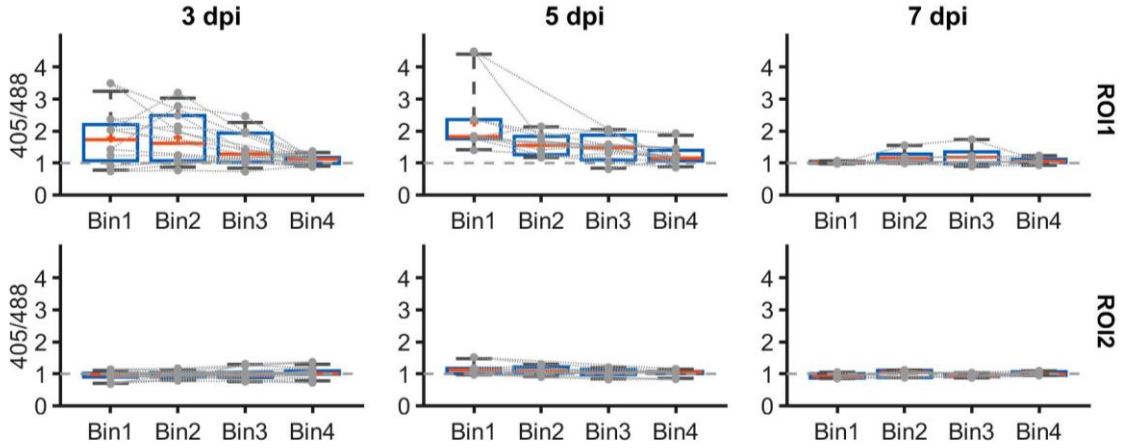


Redox Exp3NahG

L2



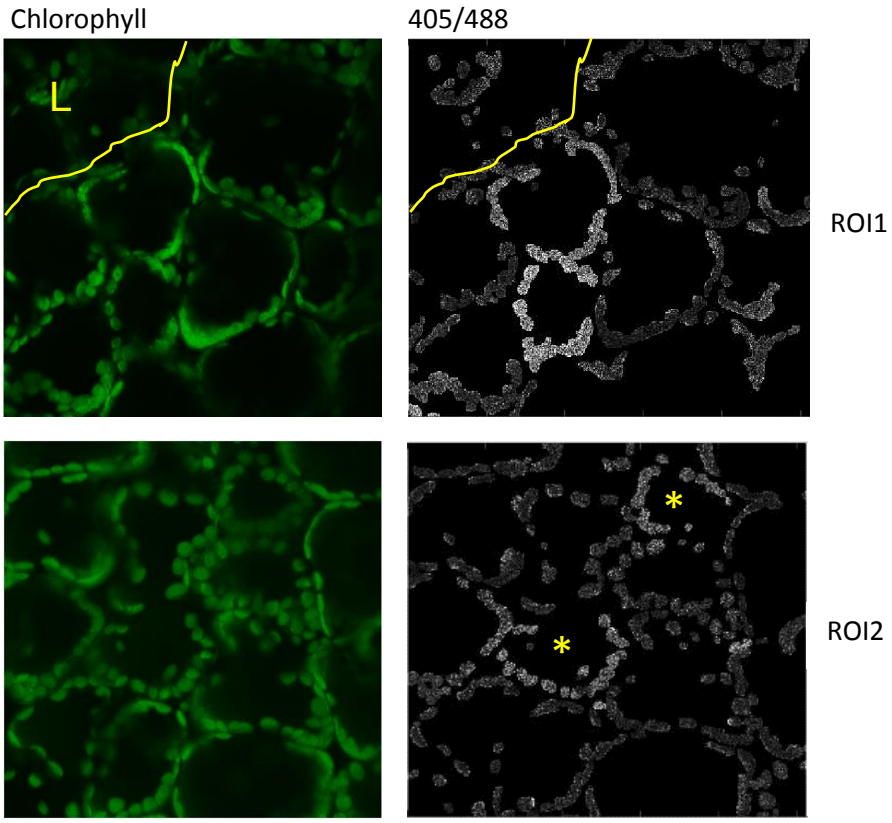
L7



Supporting Information Fig. S5: Individual cells with chloroplasts in oxidized redox state in ROI2 in PVY-inoculated redox state sensor plants (pt-roGFP (a) and SA-deficient pt-roGFP-NahG (b)). The cell death zone is marked by the yellow L and the edge of the cell death zone is marked by the yellow line. For each image, the respective experiment and image names are shown. See Supporting Information Table S1 for all images and the details regarding presented images. Left: chlorophyll fluorescence in ROI1 (top) and ROI2 (bottom), right: 405/488 ratio (the ratio of fluorescence intensities of roGFP after excitation with 405 and 488 nm laser) in ROI1 (top) and ROI2 (bottom). 405/488 ratio reflects relative chloroplast redox state (brighter chloroplasts are more oxidized). Cells with oxidized chloroplasts in ROI2 are marked with asterisks. The title of each graph is linked with the Supporting Information Table S1 by the experiment name.

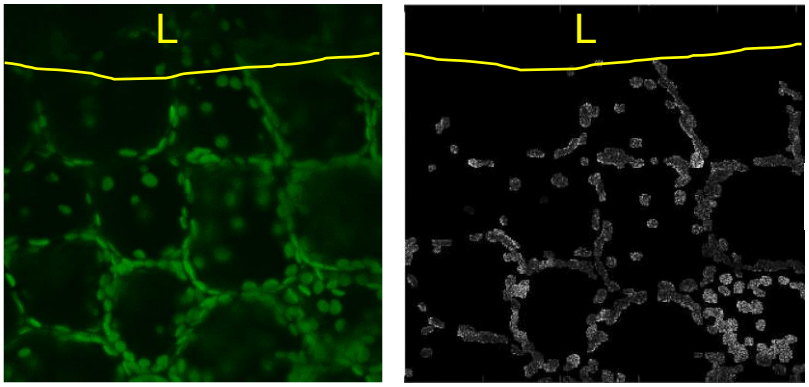
a) pt-roGFP

Redox Exp4NT 11052020_Rywal_pt-roGFP_L2_P1_L2_PVY-WILGA_3dpi.lif_lesion02

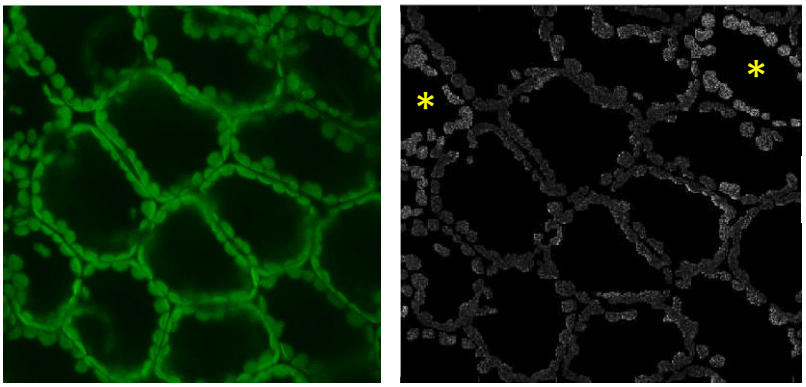


Redox Exp4NT 15052020_Rywal_pt-roGFP_L2_P5_L3_PVY-WILGA_7dpi.lif_lesion03

Chlorophyll 405/488



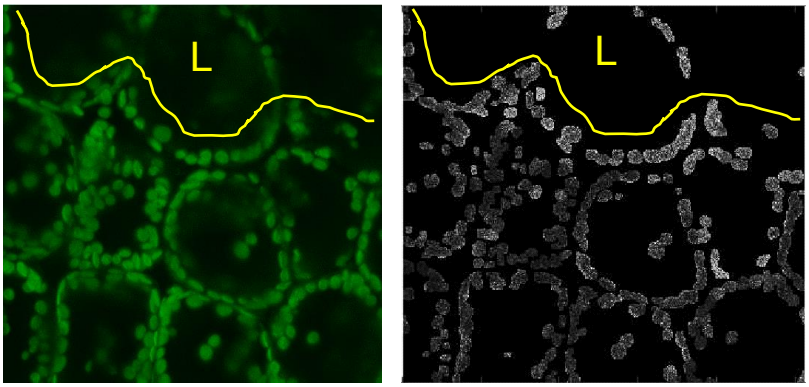
ROI1



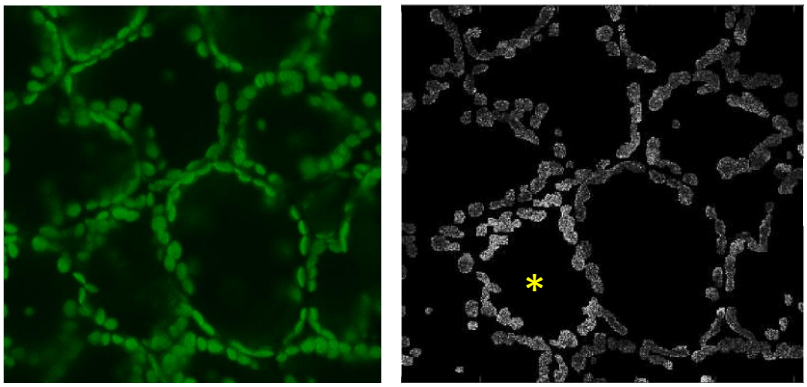
ROI2

Redox Exp4NT 15052020_Rywal_pt-roGFP_L2_P6_L2_PVY-WILGA_7dpi.lif_lesion02

Chlorophyll 405/488



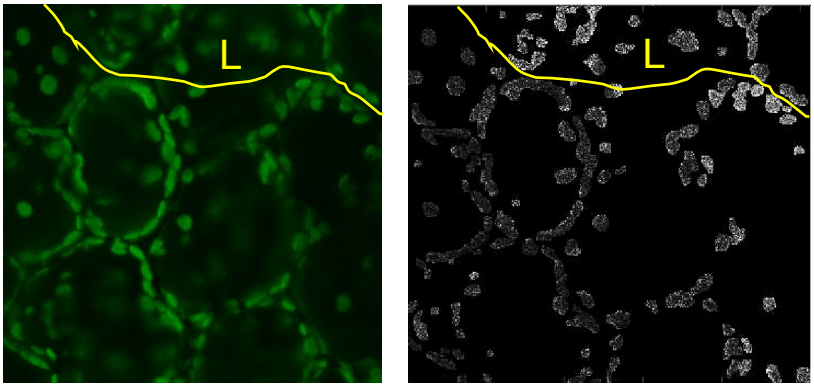
ROI1



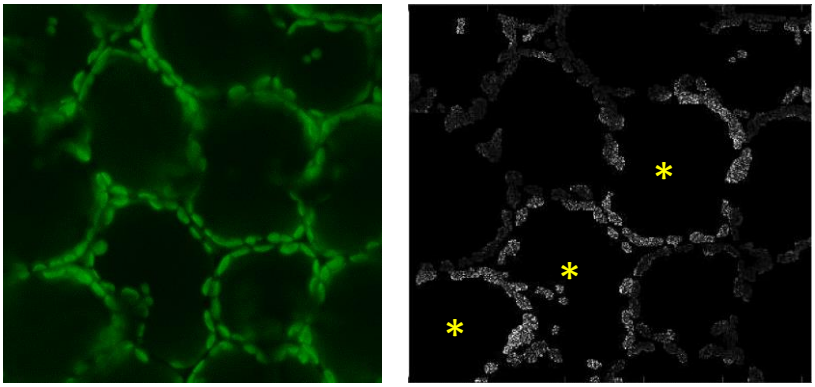
ROI2

Redox Exp5NT 20052020_Rywal_pt-roGFP_L4_P3_L2_PVY-WILGA_5dpi.lif_lesion01

Chlorophyll 405/488



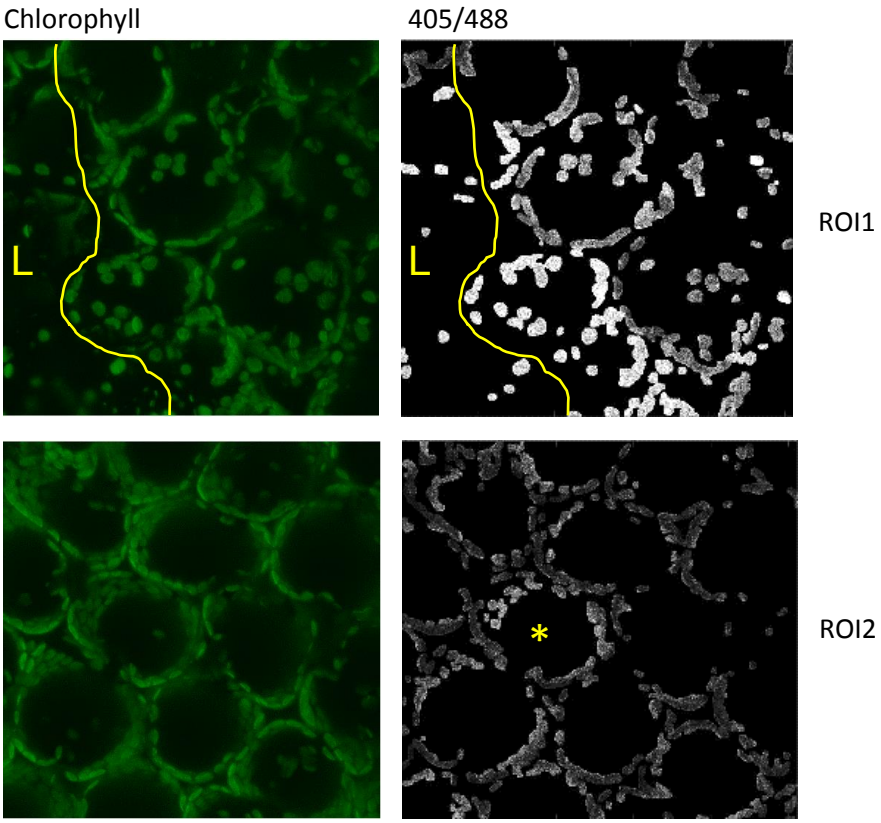
ROI1



ROI2

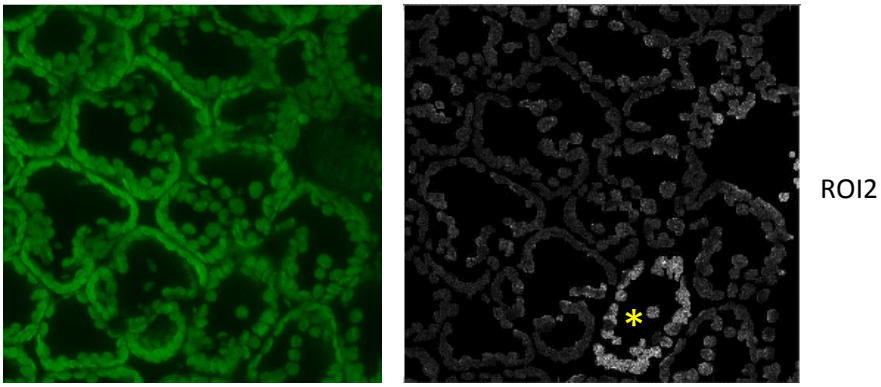
b) pt-roGFP-NahG

Redox Exp2NahG 17022020_Rywal_NahG_pt-roGFP_L2_P2_L2_PVY-WILGA_3dpi.lif_lesion03

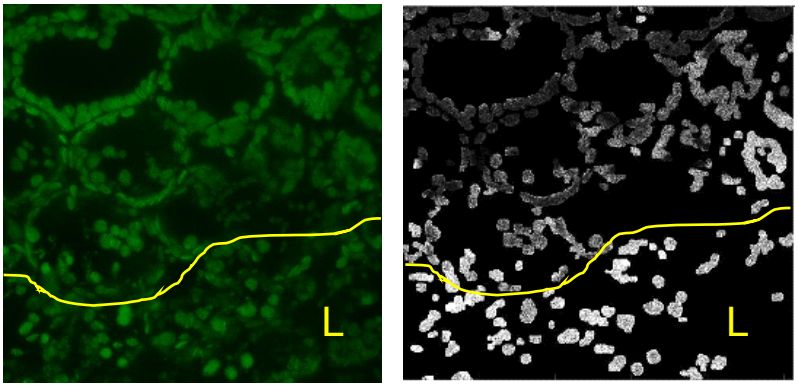


Redox Exp2NahG 19022020_Rywal_NahG_pt-roGFP_L2_P4_L3_PVY-WILGA_5dpi.lif_lesion02

Chlorophyll 405/488



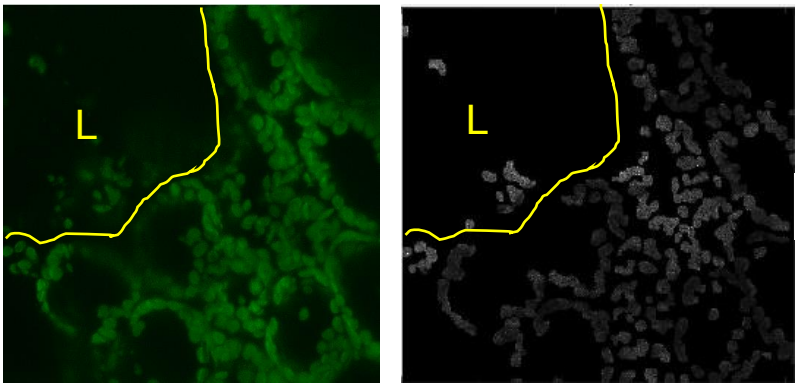
ROI2



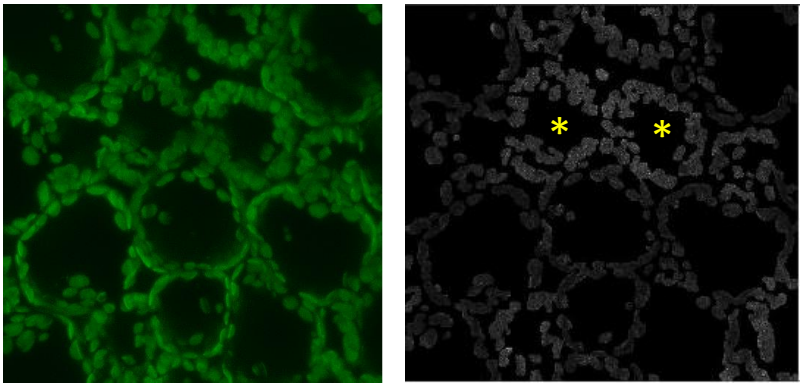
ROI1

Redox Exp2NahG 19022020_Rywal_NahG_pt-roGFP_L2_P3_L2_PVY-WILGA_5dpi.lif_lesion02

Chlorophyll 405/488

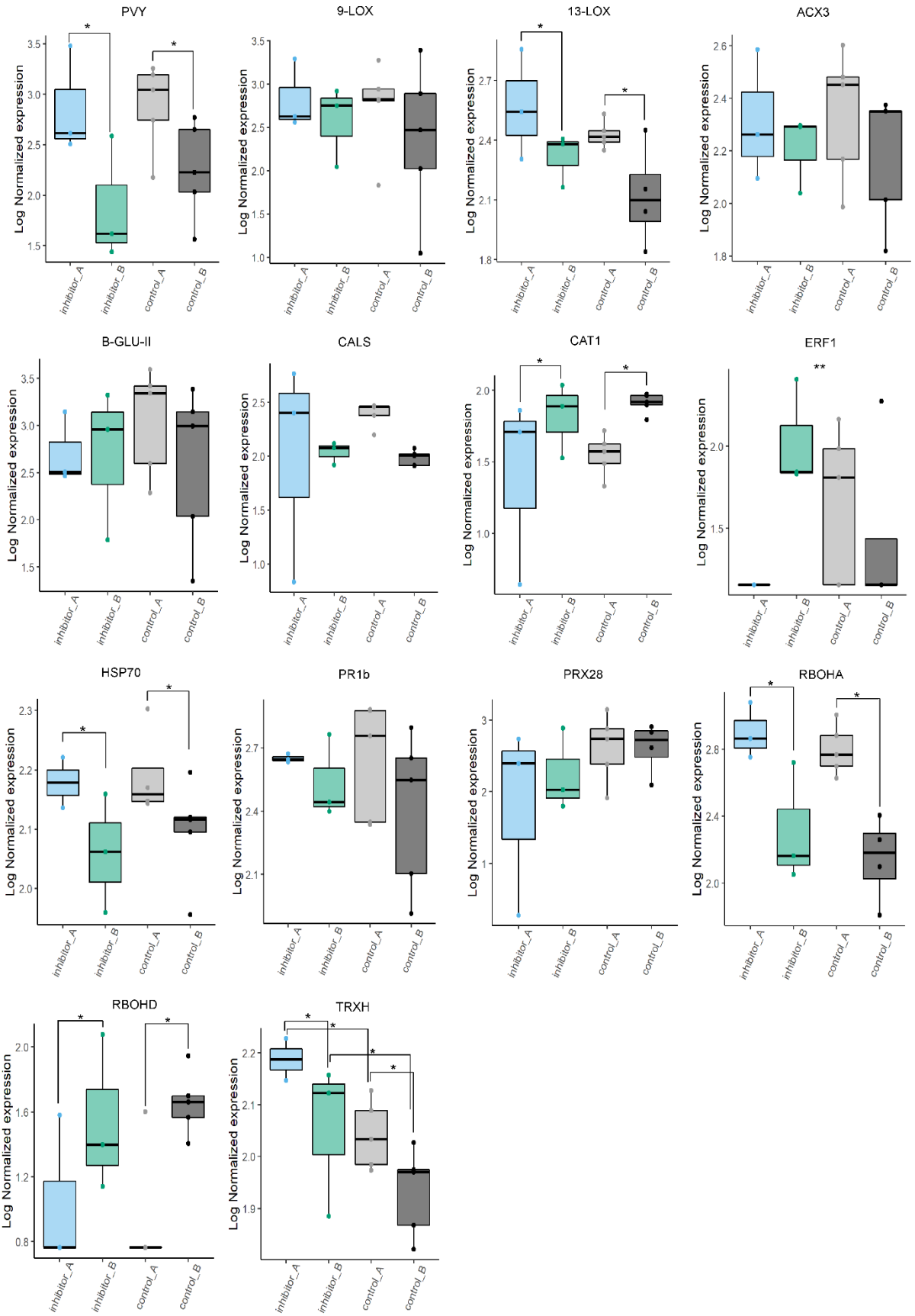


ROI1



ROI2

Supporting Information Fig. S6: Impact of chloroplast inhibitor on transcriptional response. PVY-N605(123)-GFP-inoculated leaves of pt-roGFP L2 plants were infiltrated 4 dpi. 5 dpi, two tissue sections were sampled: lesion (A) and 1 mm section adjacent to section A (B). As a control, tissue sections of the same size as A and B sections together were sampled further from the lesions on both inhibitor- and control-treated side of the leaf. Expression profiles of thirteen selected genes in two tissue sections are presented as boxplots with logarithmic normalized relative expression in sections A and B for chloroplastic ROS inhibitor-treated and control-treated lesions shown as dots. Expression is presented as logarithmic ratio between relative expression in each section and averaged relative expression in CTR sections (sections further from the lesion, see Fig. 6b). Selected genes were B-GLU_II, 1,3-β-glucosidase, HSP70, heat shock protein 70; RBOHD, potato respiratory burst oxidase homolog D; RBOHA, potato respiratory burst oxidase homolog A; PR1b, pathogenesis-related protein 1b; CAT1, catalase 1; 9-LOX, 9-lipoxygenase; ACX3, acyl-CoA oxidase; 13-LOX, 13-lipoxygenase; ERF1, potato ethylene responsive transcription factor 1a; TRXH, thioredoxin H; PRX28, peroxidase 28; CALS; callose synthase. In parallel with the gene expression of those genes, the relative quantity of PVY RNA was measured. Asterisks denotes statistically significant difference between the marked regions. See Supporting Information Table S4 and Supporting Information Table S6 for the details and number of analysed lesions and Supporting Information Table S8 for statistics.


PVY
9-LOX
13-LOX
ACX3
B-GLU-II
CALS
CAT1
ERF1
HSP70
PR1b
PRX28
RBOHA
RBOHD
TRXH
Log Normalized expression
inhibitor_A
inhibitor_B
control_A
control_B