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Supplementary Materials for

MAcro Plant Projection Imaging (MAPPI): An open, scalable platform for whole-plant fluorescence real-time imaging

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

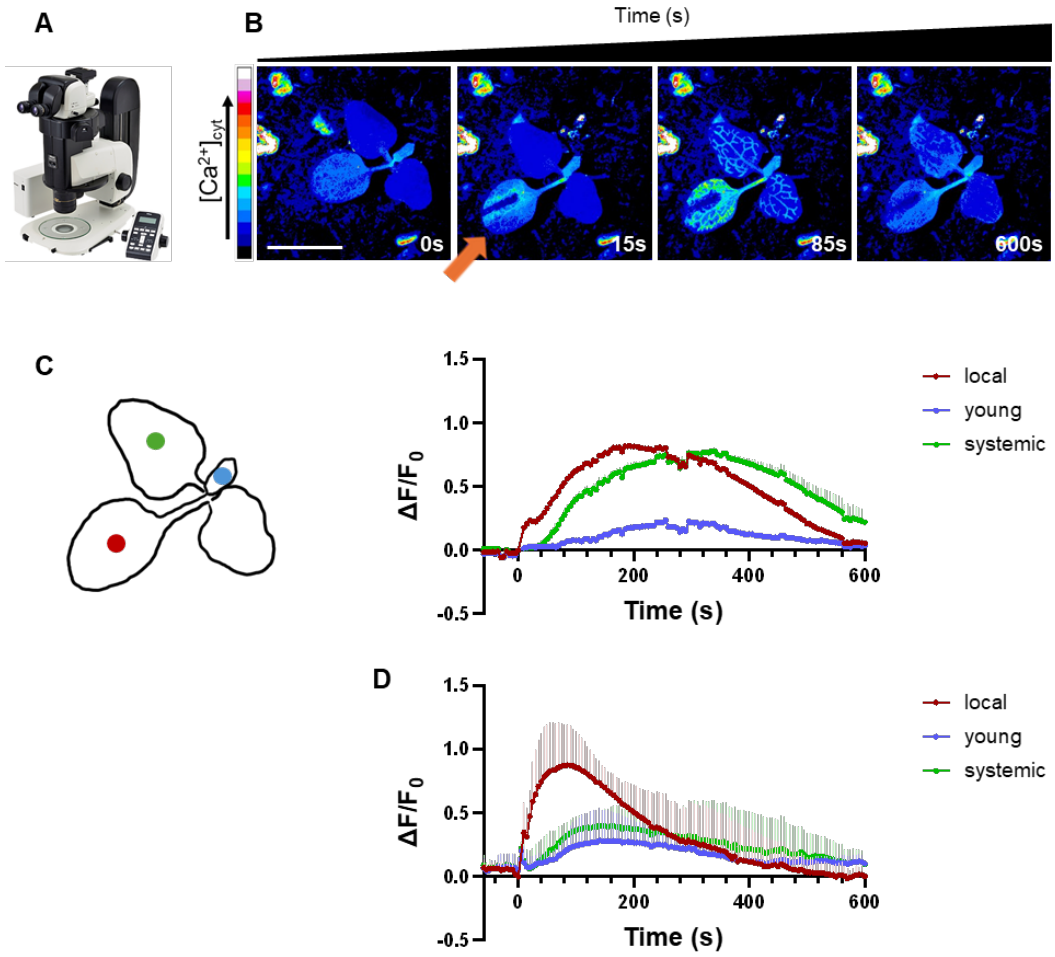

Fig. S1.

Propagation of cytosolic Ca^{2+} signals in seedlings of *N. benthamiana* in response to press wounding at commercial stereomicroscope. (**A**) Photograph of a commercial stereomicroscope. (**B**) Representative false-color images (16-color lookup table) of *N. benthamiana* plants expressing GCaMP3, before and after wounding, scale bar: 1 cm. Arrow indicates the wounded leaf. (**C**) Left: schematic representation of plants at the developmental stage used in the experiment. Colored dots indicate the leaves where GCaMP3 fluorescence was quantified: wounded leaves (red), young leaves (blue), systemic leaves (green). Right: normalized GCaMP3 fluorescence signals ($\Delta F/F_0$) over time from leaves of one representative plant. (**D**) Quantification of normalized ($\Delta F/F_0$) GCaMP3 signals in areas indicated in panel (**B**) on the left. Averages of four plants are shown. Error bars = SD. Exposure time: 500 ms. Sampling interval: 5 seconds. Total acquisition time: 30 minutes. Wounding was applied 3 minutes after the acquisition started.

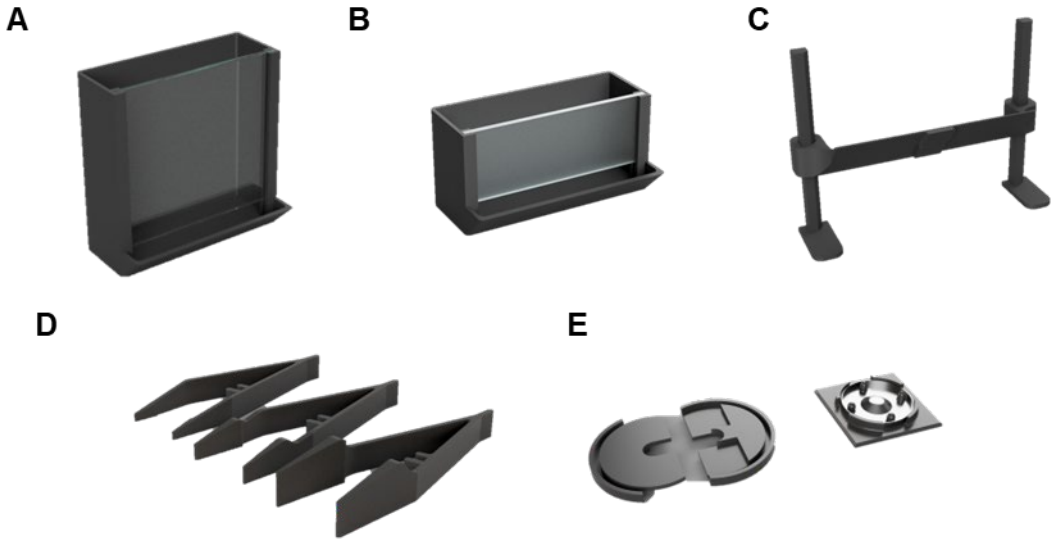

Fig. S2.

3D-printed chambers and equipment employed in the study. (**A**-**B**) Rhizoboxes for (**A**) *N. benthamiana* and (**B**) *A. thaliana* plants. Rhizoboxes have a transparent surface for root imaging and a plant saucer for sub-irrigation. (**C**) Partition to avoid double illumination of the sample during acquisition with the orthogonal system. (**D**) Tweezers of varying sizes for leaf wounding on plants at different developmental stages. (**E**) Pot mask and base for flooding experiment.

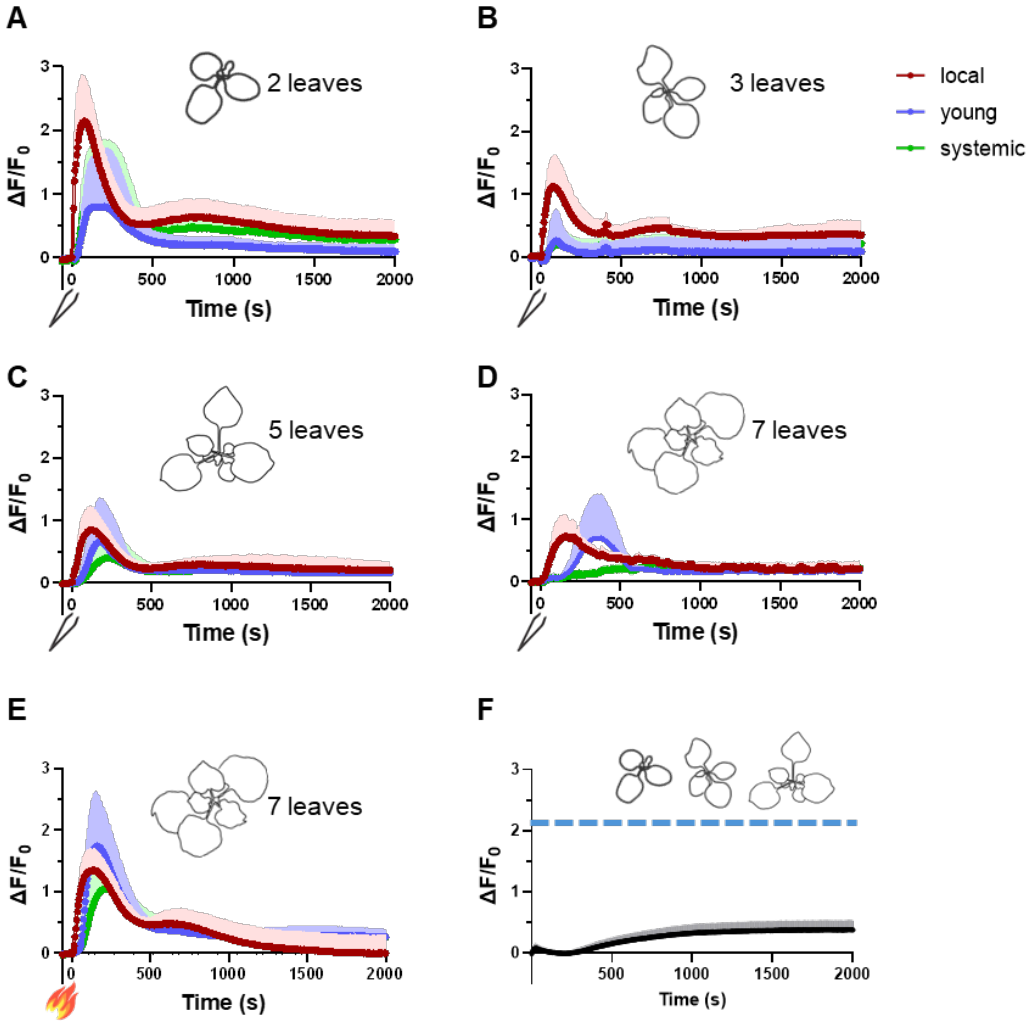

Fig. S3.

Propagation of cytosolic Ca^{2+} signals in GCaMP3 *N. benthamiana* plants in response to press wounding and leaf burning. (**A-D**) Normalized GCaMP3 fluorescence signals ($\Delta F/F_0$) over time from leaves of plants ad different developmental stages treated with press wounding. (**A**) Plants at 2-leaf stage. (**B**) Plants at 3-leaf stage. (**C**) Plants at 5-leaf stage and (**D**) plants at ≥7-leaf stage. (**E**) Normalized GCaMP3 fluorescence signals ($\Delta F/F_0$) over time from leaves of ≥7-leaf stage plants treated with burning. Averages of n ≥ 7 plants are shown. Wounding and burning were applied 3 minutes after acquisition started. (**F**) Normalized GCaMP3 fluorescence signals ($\Delta F/F_0$) over time from leaves of plants ad different developmental stages (2, 3 and 5 leaf stages) not treated and only illuminated with pulsed blue light. Averages of n ≥ 20 plants are shown. Error bars = SD. Exposure time: 500 ms. Sampling interval: 5 s. Total acquisition time: 35 min.

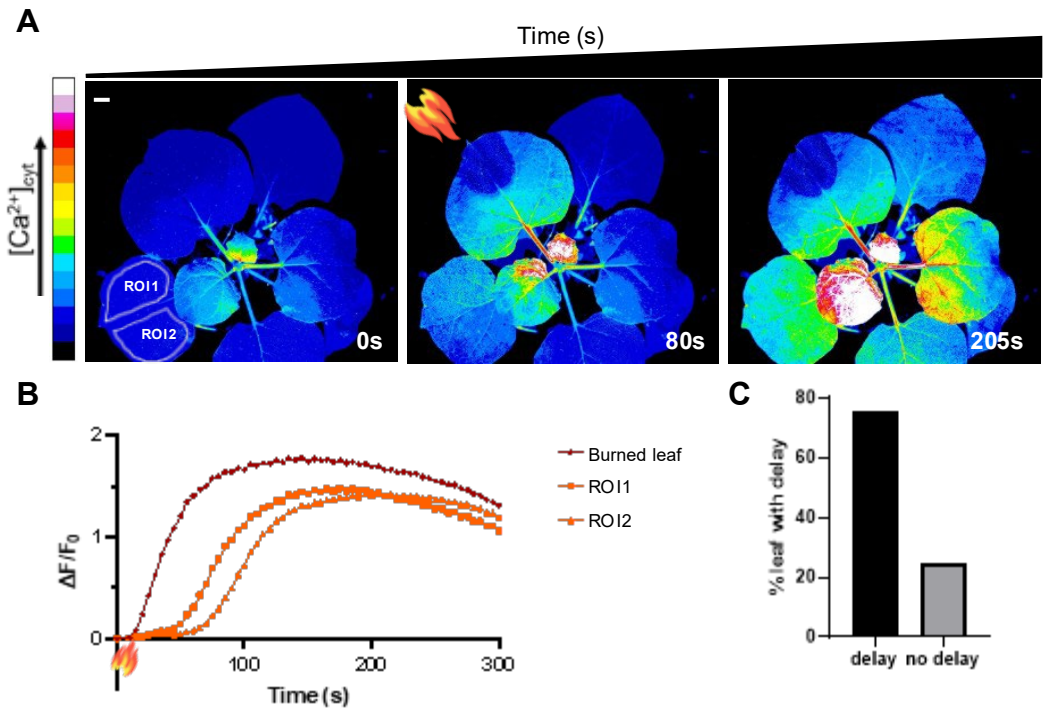

Fig. S4.

Propagation of cytosolic Ca^{2+} waves in adult *N. benthamiana* plants in response to leaf burning. (**A**) Representative false-color images (16-color lookup table) of adult (≥ 7-leaf stage) *N. benthamiana* plants expressing GCaMP3, before and after burning. Scale bar: 1 cm. Flame indicates the burned leaf. In the first panel are shown the two halves of the leaf (ROI1 and ROI2) where GCaMP3 fluorescence was quantified for the analysis reported in panels (B) and (C). Note: The same representative plant shown in Fig. 1D is used here to highlight intra-leaf signal dynamics. (**B**) Normalized GCaMP3 fluorescence signals ($\Delta F/F_0$) over time from burned leaf (red), ROI1 (orange squares) and ROI2 (orange triangles) of a representative plant. (**C**) Percentage of leaves where a delay in fluorescence in between the two halves of the leaves was reported: 31 leaves over 41 (75.6%) reported a delay with a mean of 24.3 s while 10 leaves over 41 (24.4%) did not show any delay. Exposure time: 500 ms. Sampling interval: 5 s. Total acquisition time: 35 min. Burning was applied 3 min after the acquisition started.

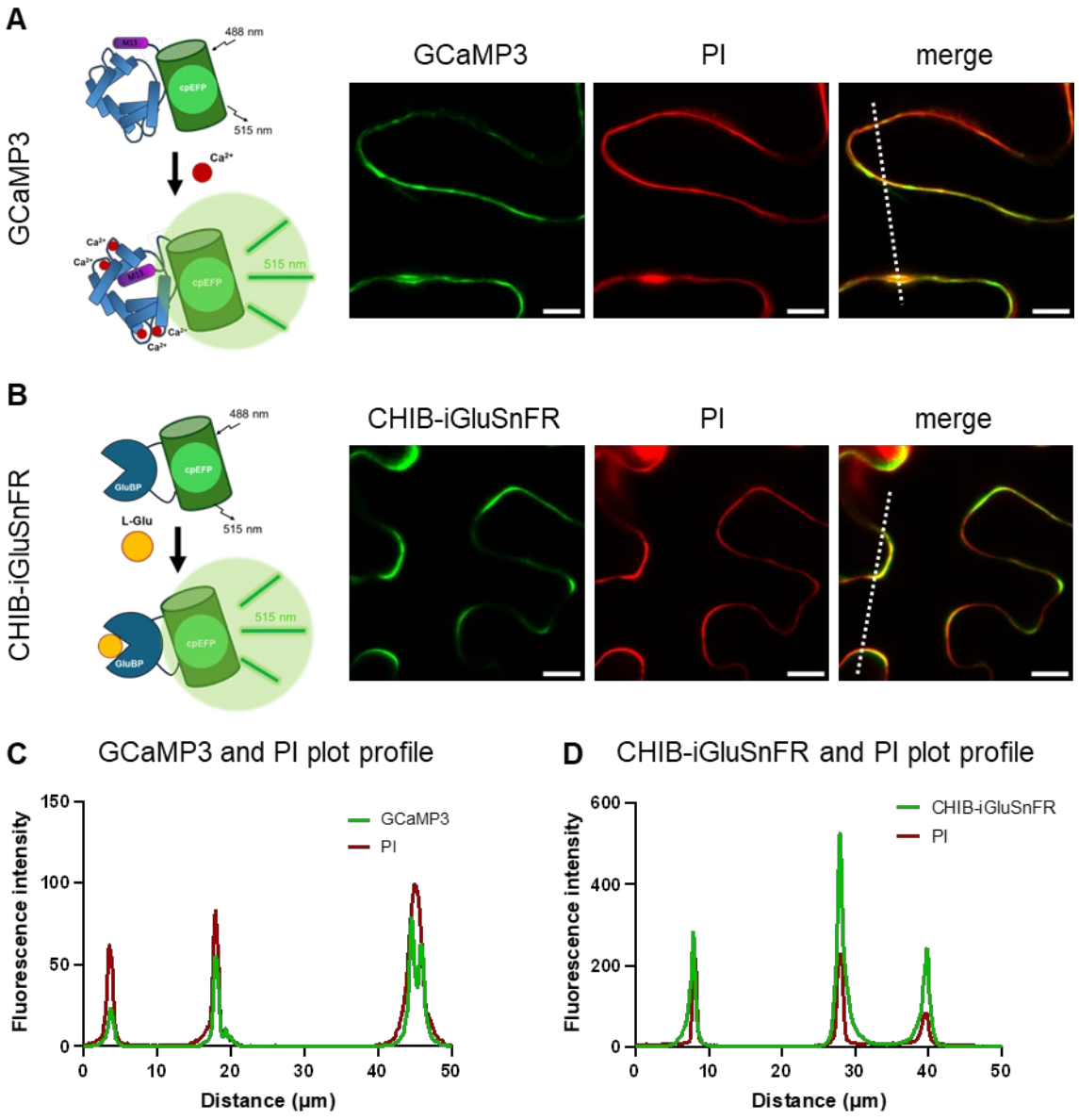

Fig. S5.

Subcellular localizations of GCaMP3 and CHIB-iGluSnFR indicators in transgenic *N. benthamiana* plants. (**A**) Left: schematic representation of the cytosolic green-shifted GCaMP3 Ca^{2+} indicator architecture. Right: confocal microscope images of a representative leaf epidermal cell from a GCaMP3 *N. benthamiana* plant stained with Propidium Iodide (PI, cell wall staining). Green: GCaMP3 fluorescence. Red: PI fluorescence. Merge: overlay of GCaMP3 and PI fluorescences. Scale bar 10 µm. (**B**) Left: schematic representation of the apoplastic green-shifted CHIB-iGluSnFR glutamate indicator architecture. Right: confocal microscope images of a representative leaf epidermal cell from CHIB-iGluSnFR *N. benthamiana* plant stained with PI. Green: CHIB-iGluSnFR fluorescence. Red: PI fluorescence. Merge: overlay of CHIB-iGluSnFR and PI fluorescences. Scale bar 10 µm. (**C**) Analysis of GCaMP3 (green curves) and PI (red curves) fluorescence distribution. The fluorescence intensity profile shows the distribution of fluorescence

across the dotted line (x-axis) in panel (**A**), merge. The fluorescence intensities are plotted along the y-axis. (**D**) Analysis of CHIB-iGluSnFR (green curves) and PI (red curves) fluorescence distribution. The fluorescence intensity profile shows the distribution of fluorescence across the dotted line (x-axis) in panel (**B**), merge. The fluorescence intensities are plotted along the y-axis.

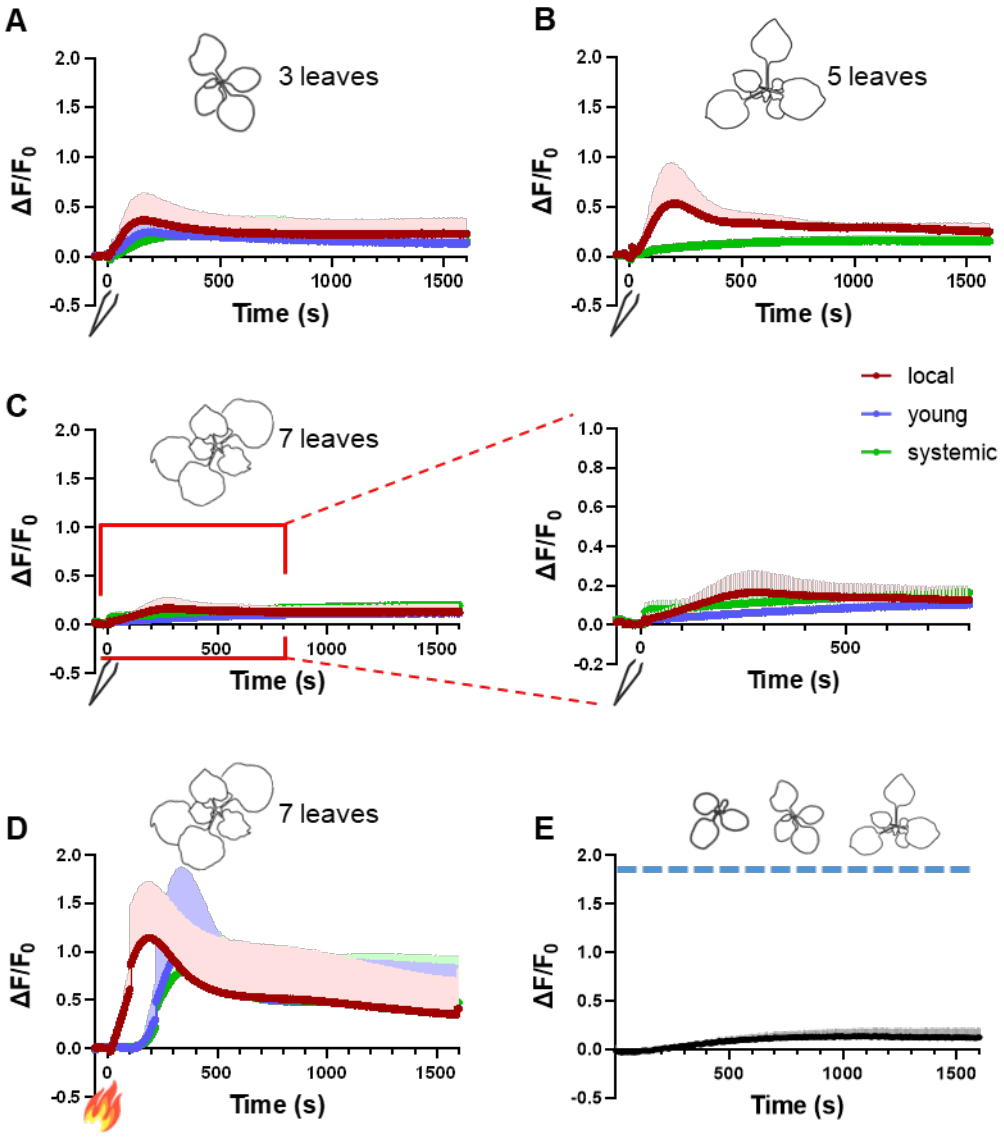

Fig. S6.

Apoplastic glutamate levels in local and systemic leaves of iGluSnFR *N. benthamiana* plants in response to press wounding and leaf burning. (**A-C**) Normalized iGluSnFR fluorescence signals ($\Delta F/F_0$) over time from leaves of plants ad different developmental stages treated with press wounding. (**A**) Plants at 3-leaf stage. (**B**) Plants at 5-leaf stage. (**C**) Left: plants at ≥ 7-leaf stage. Right: enlargement of the left panel. (**D**) Normalized iGluSnFR fluorescence signals ($\Delta F/F_0$) over time from leaves of ≥ 7-leaf stage plants treated with burning. Averages of n ≥ 3 plants are shown. (**E**) Normalized GCaMP3 fluorescence signals ($\Delta F/F_0$) over time from leaves of plants at different developmental stages (2, 3 and 5-leaf stages) not treated and only illuminated with pulsed blue light. Averages of n = 14 plants are shown. Error bars = SD. Exposure time: 500 ms. Sampling interval: 5 s. Total acquisition time: 35 min. Wounding and burning were applied 3 min after the acquisition started.

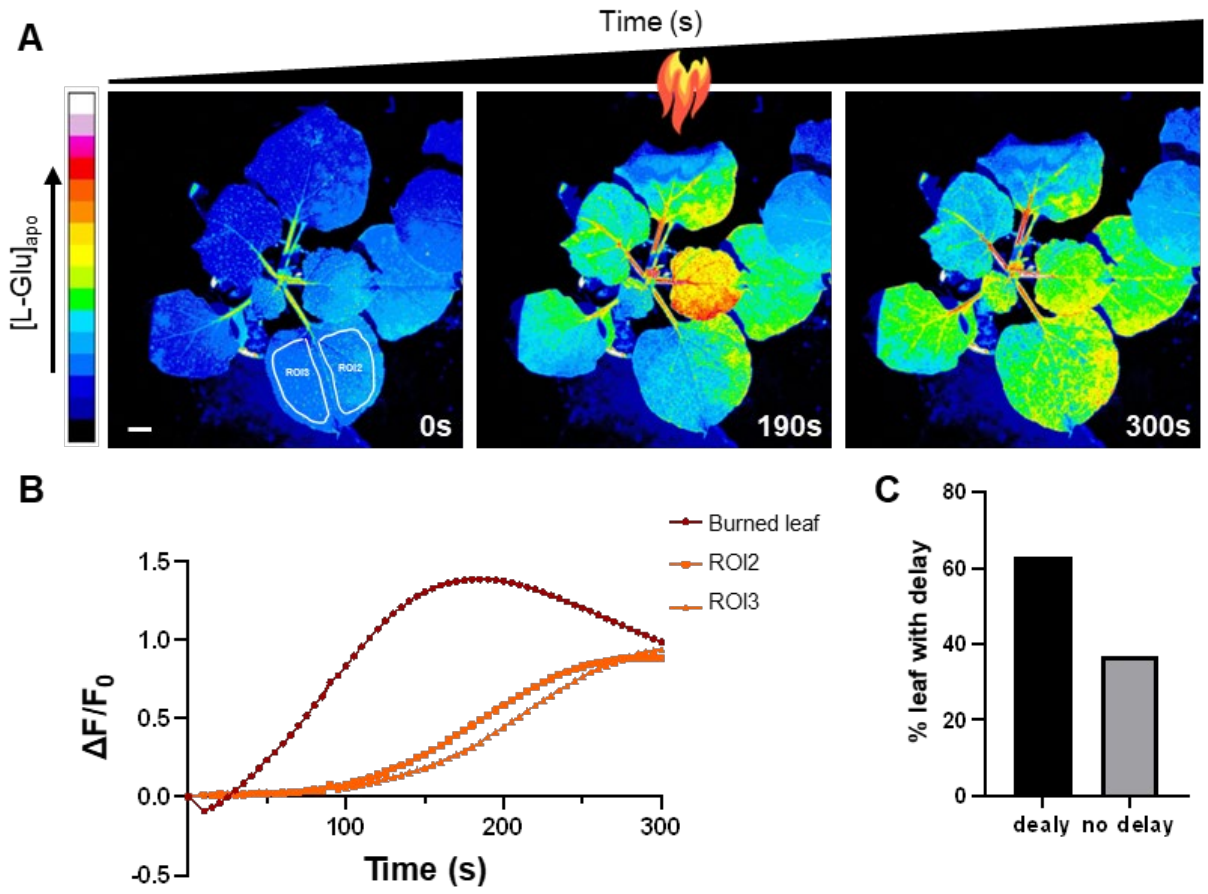

Fig. S7.

Apoplastic L-Glutamate levels increase asymmetrically in systemic leaves of *N. benthamiana* plants in response to leaf burning. (**A**) Representative false-color images (16-color lookup table) of adult *N. benthamiana* plants expressing iGluSnFR, before and after burning. Scale bar: 1 cm. Flame indicates the burned leaf. In the first panel are shown the two halves of the leaf (ROI1 and ROI2) where iGluSnFR fluorescence was quantified for the analysis reported in panels (**B)** and (**C**). (**B**) Normalized iGluSnFR fluorescence signals ($\Delta F/F_0$) over time from burned leaf (red), ROI1 (orange square) and ROI2 (orange triangles) of a representative plant. (**C**) Percentage of leaves where a delay in fluorescence in between the two halves of the leaves was reported: 12 leaves over 19 (63.2%) reported a delay with a mean of 18.3 s while 7 leaves over 19 (36.8%) did not show any delay. Exposure time: 500 ms. Sampling interval: 5 s. Total acquisition time: 35 min. Burning was applied 3 min after the acquisition started.

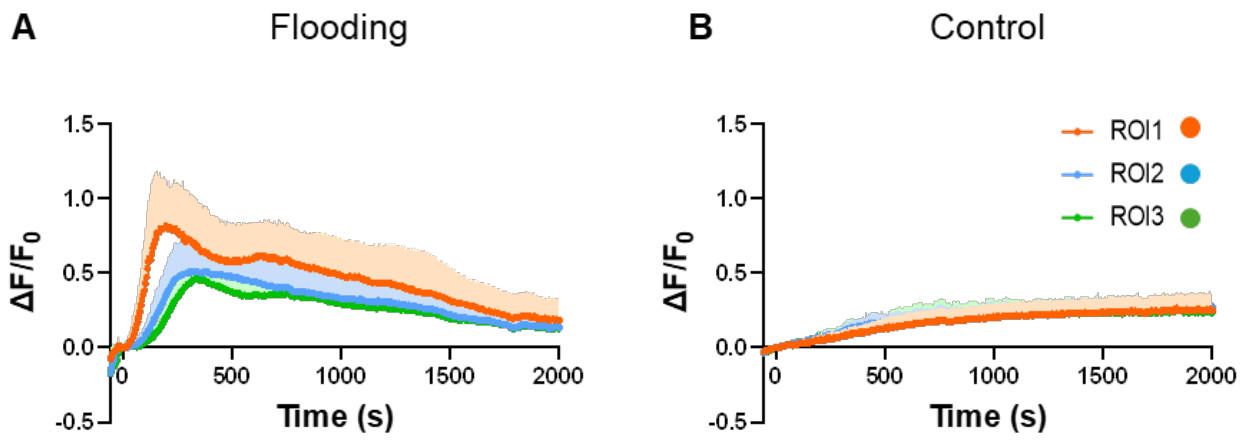

Fig. S8.

Submergence of *N. benthamiana* plants triggers a rapid increase in cytosolic Ca^{2+} levels in leaves. (**A**-**B**) Normalized GCaMP3 fluorescence signals ($\Delta F/F_0$) over time, from leaves of (**A**) submerged and (**B**) control (non-submerged) plants. Averages of n = 10 plants per treatment are shown. Error bars = SD. Exposure time: 500 ms. Sampling interval: 5 s. Total acquisition time: 35 min. Flooding treatment was applied 3 min after the acquisition started.

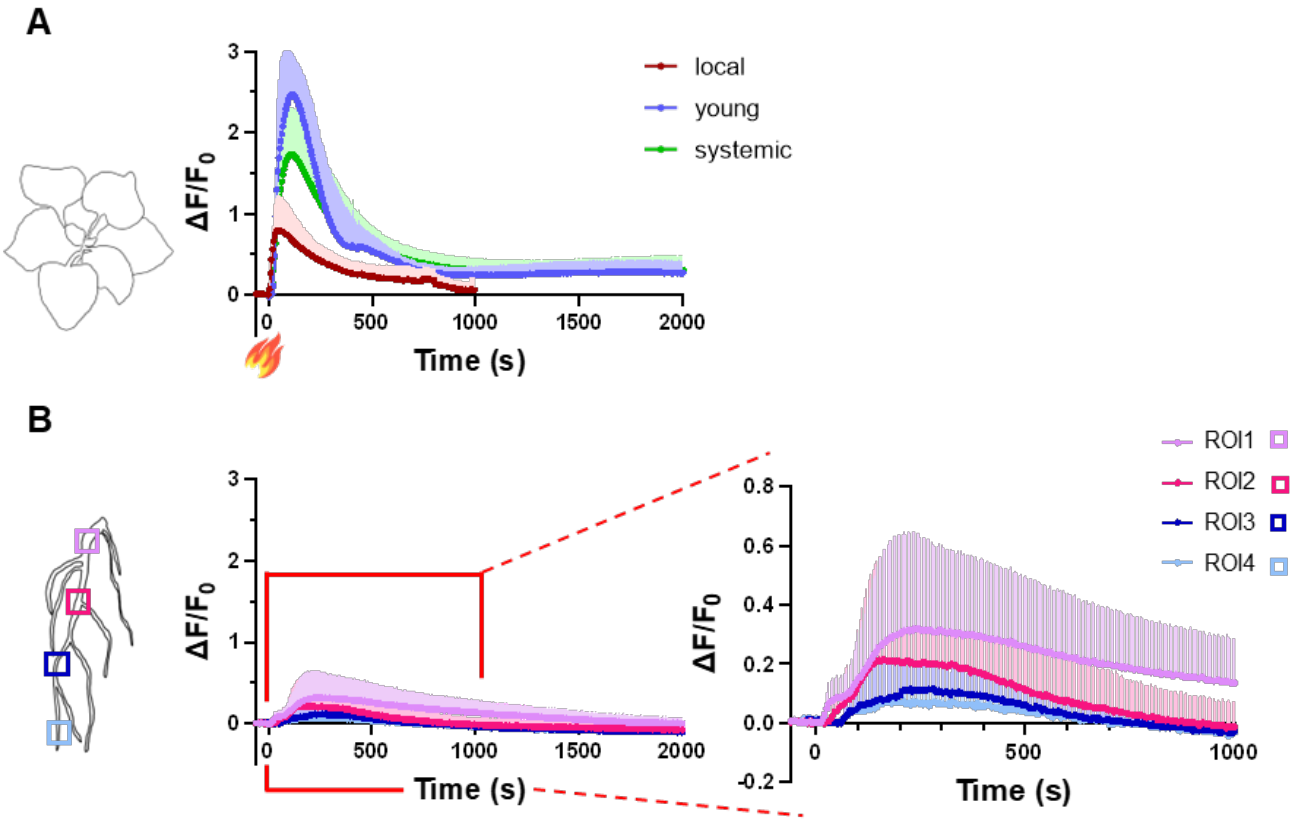

Fig. S9.

Shoot-to-root Ca^{2+} signal propagation in 7-leaf stage GCaMP3 *N. benthamiana* plants subjected to leaf burning. (**A**-**B**) Left: schematic representation of the shoot and the root of plants used in the experiment. (**A**) Right: normalized GCaMP3 fluorescence signals ($\Delta F/F_0$) over time of plants subjected to leaf burning. (**B**) Left: colored squares, indicate the root areas where GCaMP3 fluorescence was quantified. Center: normalized GCaMP3 fluorescence signals ($\Delta F/F_0$) over time. Right: enlargement of the central panel. Averages of n = 8 plants are shown. Error bars = SD. Exposure time: 500 ms. Sampling interval: 5 s. Total acquisition time: 35 min. Wounding and burning were applied 3 min after the acquisition started.

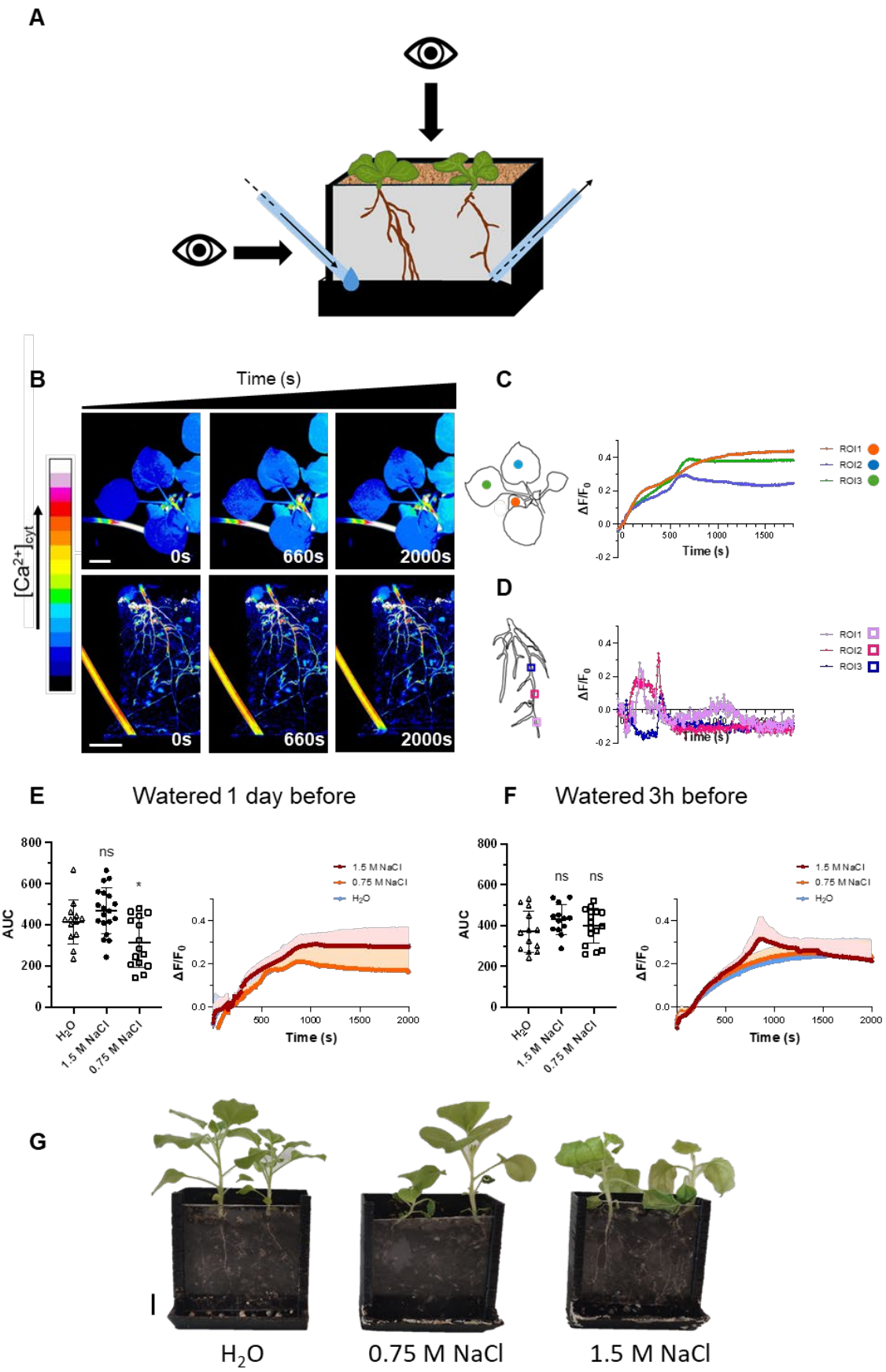
A
B
Time (s)
[Ca2+]cyt
0s
660s
2000s
0s
660s
2000s
C
ROI1
ROI2
ROI3
ΔF/F0
Time (s)
D
ROI1
ROI2
ROI3
ΔF/F0
Time (s)
E
Watered 1 day before
AUC
ns
*
H2O
1.5 M NaCl
0.75 M NaCl
1.5 M NaCl
0.75 M NaCl
H2O
ΔF/F0
Time (s)
F
Watered 3h before
AUC
ns
ns
H2O
1.5 M NaCl
0.75 M NaCl
1.5 M NaCl
0.75 M NaCl
H2O
ΔF/F0
Time (s)
G
H2O
0.75 M NaCl
1.5 M NaCl

Fig. S10.

Root-to-shoot Ca^{2+} signal propagation in 5-leaf stage GCaMP3 *N. benthamiana* plants treated with NaCl. (**A**) Schematic representation of the treatment: H_2O, 0.75 M NaCl and 1.5 M NaCl solutions were added to the bottom of the rhizobox chamber using a perfusion system connected to a peristaltic pump (3.3 mL/min constant rate). Both the x- and y-axis arms of the MAPPI system were used. (**B**) Representative false-color images (16-colors lookup table) of shoot and root of a 5 leaves *N. benthamiana* plants expressing the GCaMP3 Ca^{2+} indicator watered the day before the experiment and treated with 1.5 M NaCl. Exposure time: 500 ms. Sampling time: 5 s. Acquisition time: 30 min. Salt stress was applied 3 min after acquisition start. Scale bar: 1 cm. (**C**-**D**) Left: schematic representation of the shoot and the root of plants used in the experiment. Colored dots and squares, indicate the leaves and the root areas where GCaMP3 fluorescence was quantified. Right: Normalized GCaMP3 fluorescence signals ($\Delta F/F_0$) over time from the regions of interest selected in the shoot and the root of one representative plant. (**E**) Analysis of GCaMP3 fluorescence changes in the shoot of samples watered the day before the experiment and treated with H_2O, 0.75 M NaCl and 1.5 M NaCl solutions. Left: Area Under the Curve. Right: normalized GCaMP3 fluorescence changes ($\Delta F/F_0$). (**F**) Analysis of GCaMP3 fluorescence changes in the shoot of samples watered 3h before the experiment and treated with H_2O, 0.75M NaCl and 1.5M NaCl solution. Left: Area Under the Curve. Right: normalized GCaMP3 fluorescence changes ($\Delta F/F_0$). Error bars = SD, ns non-significant, * $p < 0.05$ (One-Way ANOVA); $n \geq 3$ plants. (**G**) Images acquired 2 days after the treatment of representative *N. benthamiana* GCaMP3 plants treated with H_2O, 0.75 M NaCl and 1.5 M NaCl solutions.

Species	**Stimulus**	**MAPPI imaging arms**	**LED used**
N. benthamiana	Wounding	y-axis	y-axis
N. benthamiana	Burning shoot	y-axis	y-axis
N. benthamiana	Flooding	y-axis	y-axis
N. benthamiana	Wounding and Burning shoot	x-axis, y-axis	y-axis
N. benthamiana	Shade Avoidance Syndrome	x-axis, y-axis	y-axis
N. benthamiana	Burning shoot-to-root	x-axis, y-axis	x-axis, y-axis
N. benthamiana	NaCl root-to-shoot	x-axis, y-axis	x-axis, y-axis
A. thaliana	NaCl root-to-shoot	x-axis, y-axis	x-axis, y-axis

Table S1.
List of samples and treatments.

Movie S1.
Propagation of cytosolic Ca^{2+} signals in *N. benthamiana* GCaMP3 leaves in response to press wounding.

Movie S2.
Propagation of cytosolic Ca^{2+} signals in *N. benthamiana* GCaMP3 leaves in response to burning and blue light.

Movie S3.
Propagation of cytosolic Ca^{2+} signals in *N. benthamiana* GCaMP3 leaves in response to burning.

Movie S4.
Chewing of *S. littoralis* larvae on ≥ 7-leaf stage *N. benthamiana* GCaMP3 plant.

Movie S5.
Propagation of apoplastic glutamate levels in local and systemic leaves of *N. benthamiana* iGluSnFR plants in response to press wounding and leaf burning.

Movie S6.
Propagation of apoplastic glutamate levels in *N. benthamiana* iGluSnFR plants in response to burning and blue light.

Movie S7.
Chewing of *S. littoralis* larvae on ≥ 7-leaf stage *N. benthamiana* iGluSnFR plant.

Movie S8.
Submergence of *N. benthamiana* GCaMP3 plants triggers a rapid increase in cytosolic Ca^{2+} levels in leaves.

Movie S9.
Simultaneous orthogonal imaging of ≥ 7-leaf stage *N. benthamiana* GCaMP3 plants in response to wounding or burning.

Movie S10.
Simultaneous orthogonal imaging of 5-leaf stage *N. benthamiana* GCaMP3 plants under overnight pulsed blue LED light illumination.

Movie S11.
Shoot-to-root Ca^{2+} signal propagation in ≥ 7-leaf stage *N. benthamiana* GCaMP3 plants subjected to leaf burning.

Movie S12.
Propagation of cytosolic Ca^{2+} signals in leaves and roots of 2-leaf stage *N. benthamiana* GCaMP3 plant in response to chewing of *S. littoralis* larvae.

Movie S13.

Propagation of cytosolic Ca^{2+} signals in leaves and roots of 5-leaf stage *N. benthamiana* GCaMP3 plants in response to chewing of *S. littoralis* larvae.

Movie S14.

Root-to-shoot Ca^{2+} signal propagation in 5-leaf stage *N. benthamiana* GCaMP3 plants treated with NaCl.

Movie S15.

Root-to-shoot Ca^{2+} signal propagation in adult plants of *A. thaliana* GCaMP3 treated with NaCl.