Appendix

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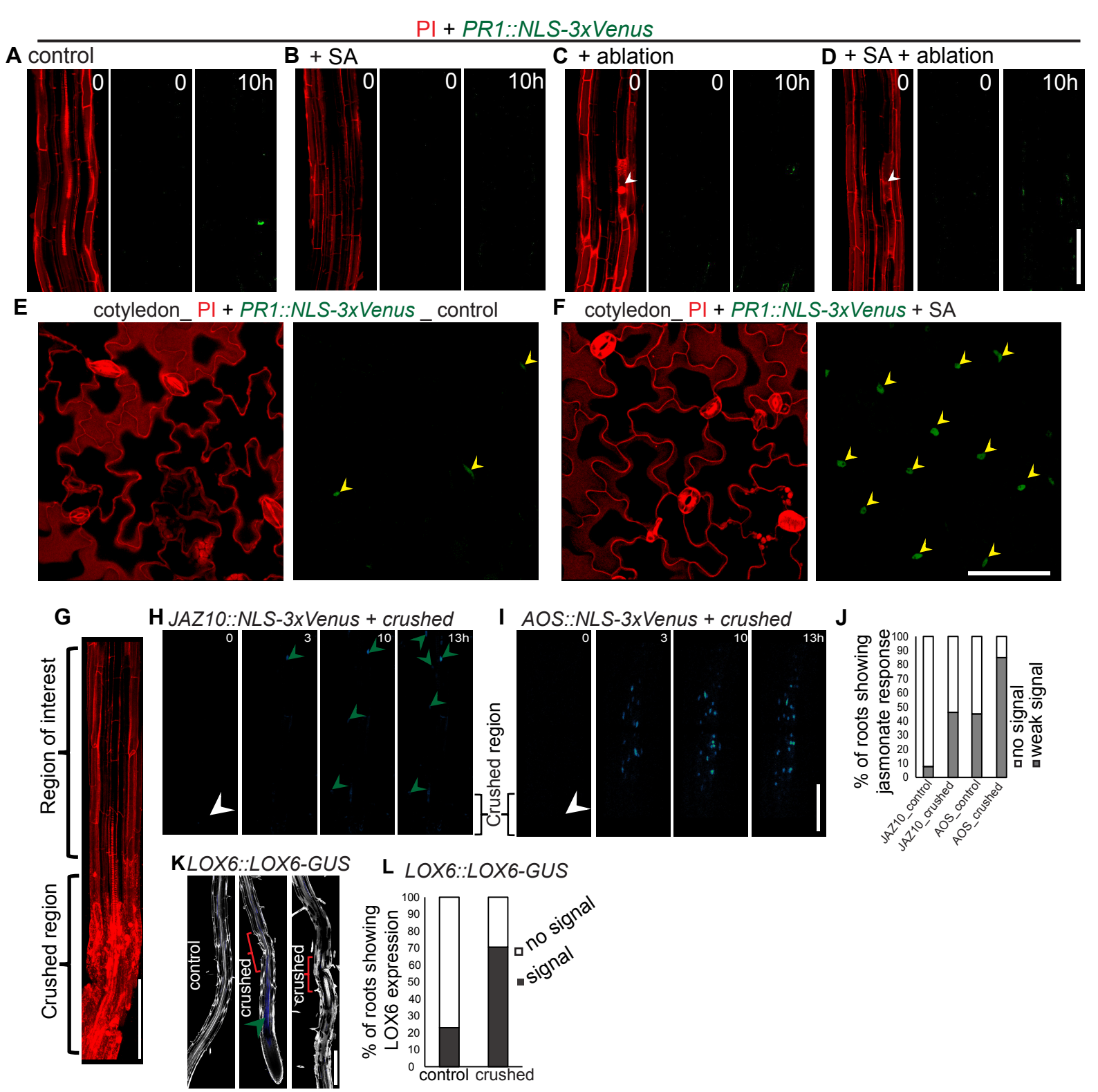
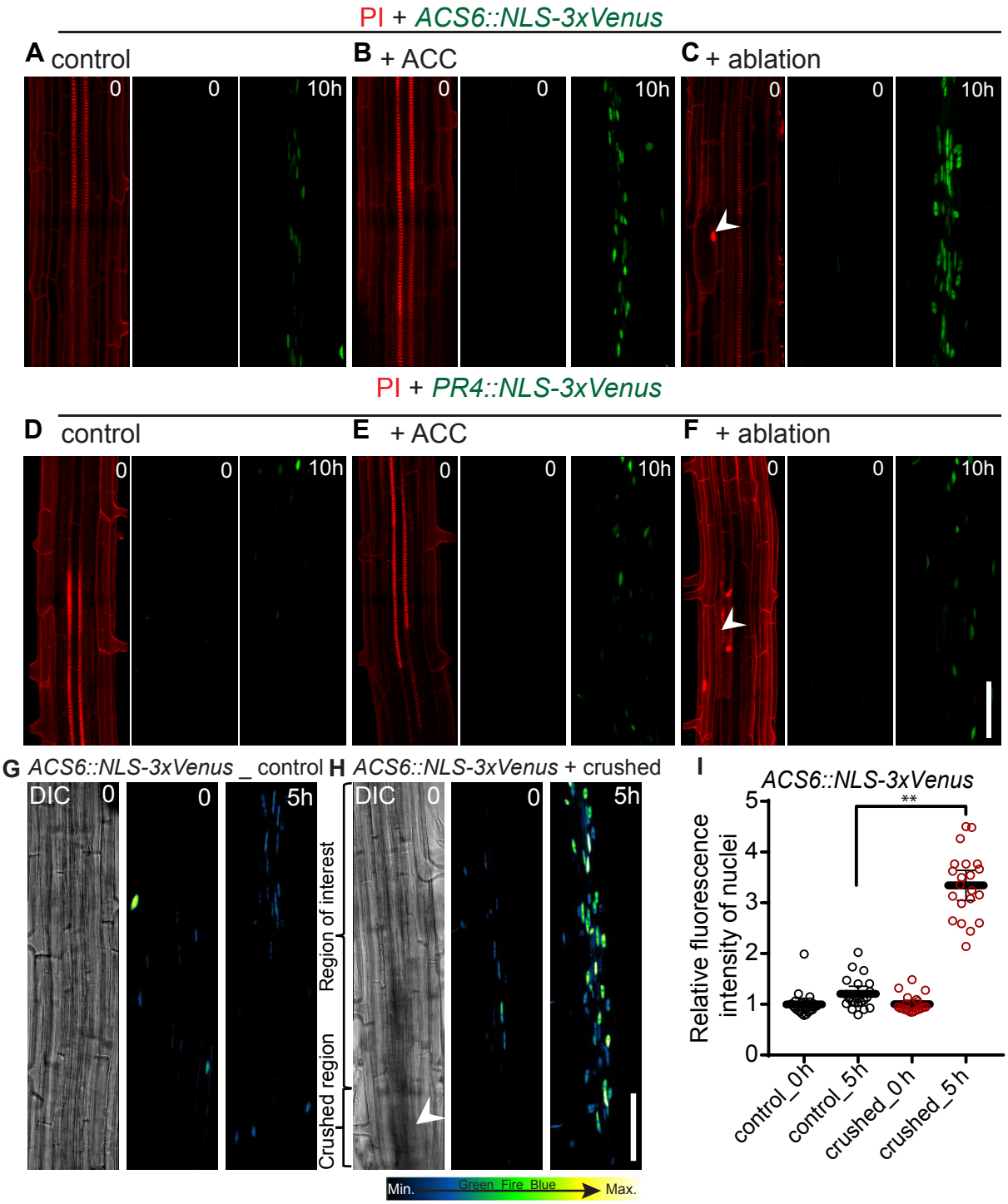


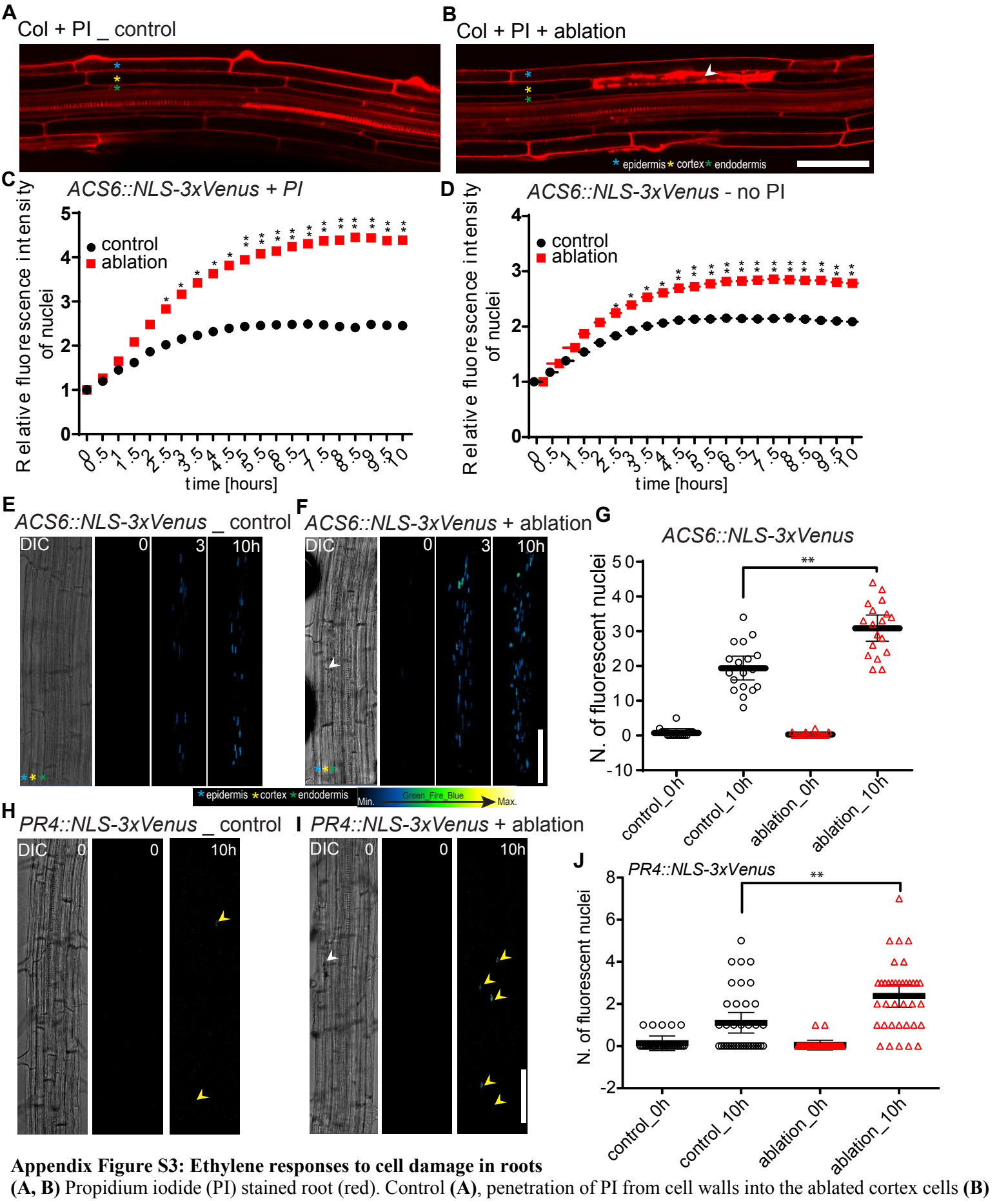
Appendix Figure S1: Laser ablation does not lead to salicylic acid response

(A - D) Time lapse, (xyz) maximum projection images of 4D tile-scan show expression of the salicylic acid response marker line *PR1::NLS-3xVenus* in the *Arabidopsis* root stained with PI (red). Laser ablation of cortex cell **(C)** does not induce expression of *PR1::NLS-3xVenus* neither in control media **(C)** nor in presence of 1µM SA **(D)**. **(E, F)** Maximum projection images show the expression of *PR1::NLS-3xVenus* in cotyledons stained with PI (red) **(E)** before and **(F)** after treatment with 1µM SA (n=10). **(G)** Crushed *Arabidopsis* root tip stained with propidium iodide (PI). **(H, J)** 4D (xyzt) maximum projection images monitoring expression of the jasmonate response marker *JAZ10::NLS-3xVenus* **(H)**, *AOS::NLS-3xVenus* **(I)** in root in response to crushing. **(J)** Percentage representation of roots of Non-damaged control root show no jasmonate response in 12 out of 13 roots in the *JAZ10::NLS-3xVenus line*. *AOS::NLS-3xVenus* control shows weak expression in 9 out of 20 roots. After manual damage of *JAZ10::NLS-3xVenus*, 14 roots showed no and 12 roots weak jasmonate response after crushing (n=26). The *AOS::NLS-3xVenus* shows jasmonate response in 17 roots (n=20). Example of weak response are shown in *JAZ10::NLS-3xVenus* **(H)** and in the *AOS::NLS-3xVenus* **(I)**. Live-imaging time points are indicated in the top right corner of each frame. **(K)** 3D (xyz) maximum projection images demonstrating jasmonate biosynthesis gene response *LOX6::LOX6-GUS* after crushing (n=15). **(L)** The *LOX6::LOX6-GUS* shows response in 5 out-of 15 roots after crushing. In non-crushed control roots *LOX6::LOX6-GUS* induction was observed in 3 out-of 15 roots. **(K)** After GUS (blue stain) reaction roots were stained in ClearSee solutions containing calcofluorwhite (white stain) to visualize cell walls. Representative images are shown (for control root without GUS is shown – on left side). **Scale bar**, **(A - F)** 50 µm **(G, H, I, K)** 100 µm.



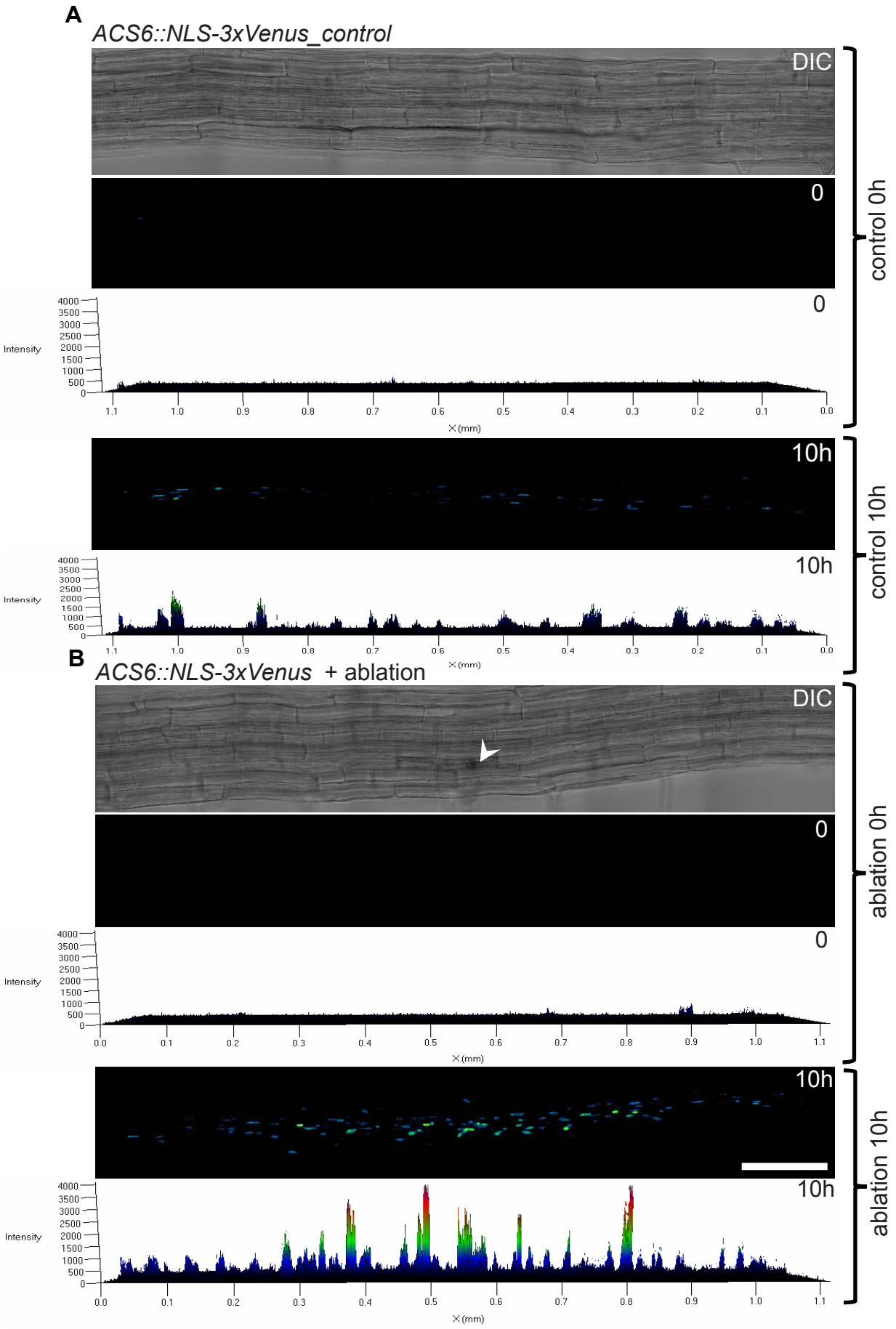
Appendix Figure S2: Mechanically crushing roots leads to ethylene response

(A - F) Real time monitoring 4D (xyzt) maximum projection images of ethylene response marker lines *ACS6::NLS-3xVenus* **(A - C)**, *PR4::NLS-3xVenus* **(D - F)** in the root with/without 2µM 1-aminocyclopropanecarboxylic acid (ACC) before/after laser ablation of cortex cells. *ACS6::NLS-3xVenus* **(C)**, *PR4::NLS-3xVenus* **(F)** biosynthesis and response markers and enhances differences upon laser ablation of single cortex cell as well as upon 2µM ACC treatment regardless of ablation *ACS6::NLS-3xVenus* **(B, C)**, *PR4::NLS-3xVenus* **(E, F)**. Live-imaging time points are indicated in the top right corner of each frame. **(A - F)** n=10 roots repeated two times; time-lapse of representative movies are shown. **(G - I)** 4D (xyzt) maximum projection images monitoring expression and **(I)** quantification of the ethylene response marker *ACS6::NLS-3xVenus* in the root in response to crushing (**p <0.005, n= 20 - 21 roots, pooled from two independent experiments (n=10 - 11 roots), error bars indicate mean value with 95% CI, representative pictures are shown) . **Scale bar**, **(A - F)** 50 µm, **(G, H)** 100 µm.



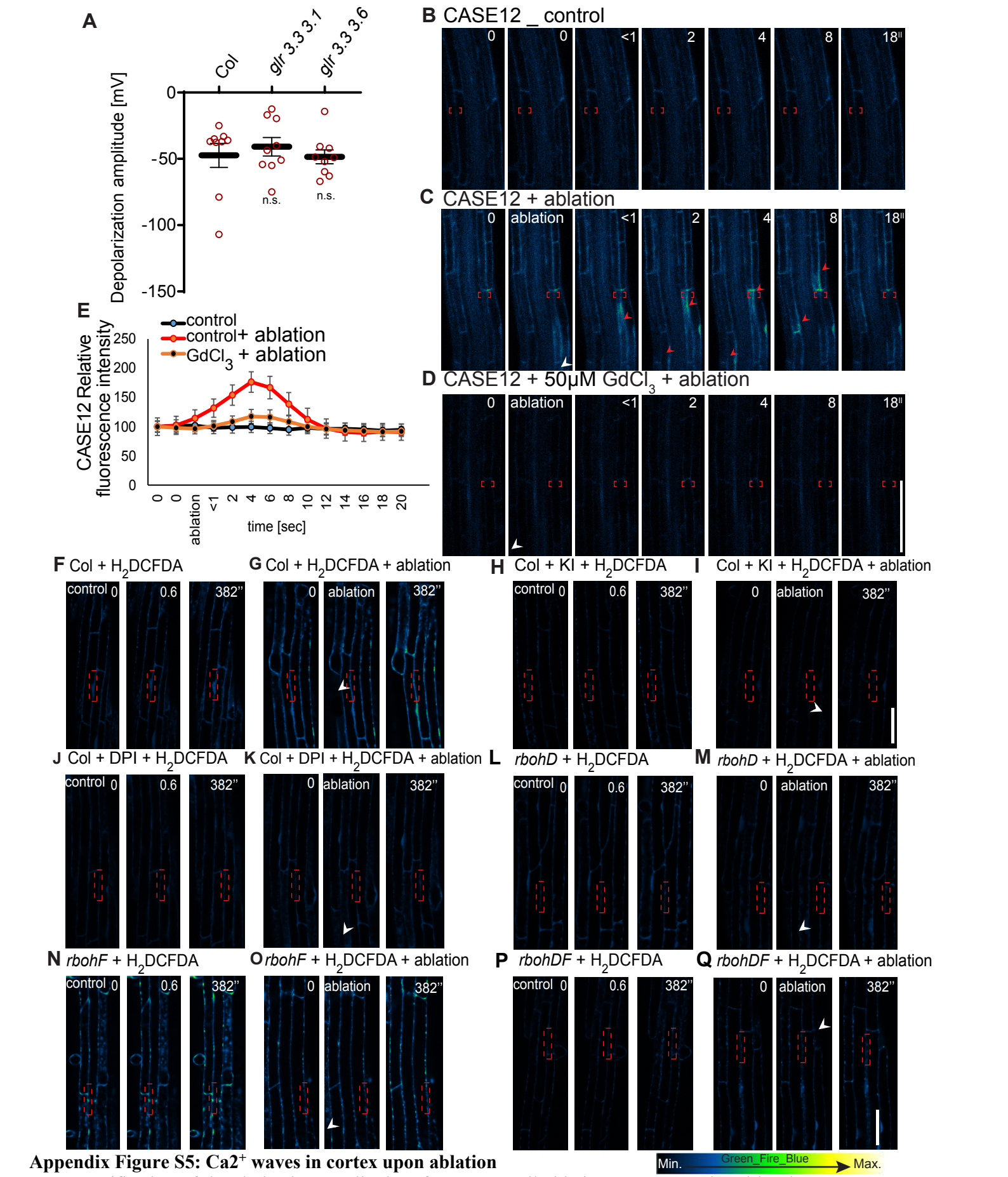
Appendix Figure S3: Ethylene responses to cell damage in roots

(A, B) Propidium iodide (PI) stained root (red). Control **(A)**, penetration of PI from cell walls into the ablated cortex cells **(B)** visualizes cell death (white arrowhead). **(C)** Roots stained with PI show attenuated background induction during time-lapse observations of the *ACS6* response marker and enhanced differences upon laser ablation of single cortex cell, as compared to **(D)**. Quantification of average signal intensity (*p<0.05, **p<0.01, n=10 roots, repeated three times). **(D - G)** Despite background induction, time lapse imaging after laser ablation of single cortex cell without PI still shows induced expression of ethylene response marker line *ACS6::NLS-3xVenus*, quantified both as increases in average signal intensity (D) and number of cells with positive nuclear signal **(G)** (**p<0.005, n=6 roots, repeated three times, error bars indicate mean value with 95% CI). Representative time lapse images shown in **(E, F)**. **(H, I)** Cortex cell ablation also induces expression of *PR4::NLS-3xVenus* in the root. Representative pictures of real time monitoring 4D (xyzt) maximum projections shown. **(J)** After laser ablation, the number of cells with positive nuclear *PR4::NLS-3xVenus* signal increased (**p<0.005, n=37 roots, error bars indicate mean value with 95% CI). Time points are indicated in the top right corner of each frame. **(E, F, H, I)** movies were recorded within 10 hours with 30 minutes interval per frame. **Scale bar**: **(A, B)** 50 µm **(E, F, H, I)** 100 µm.



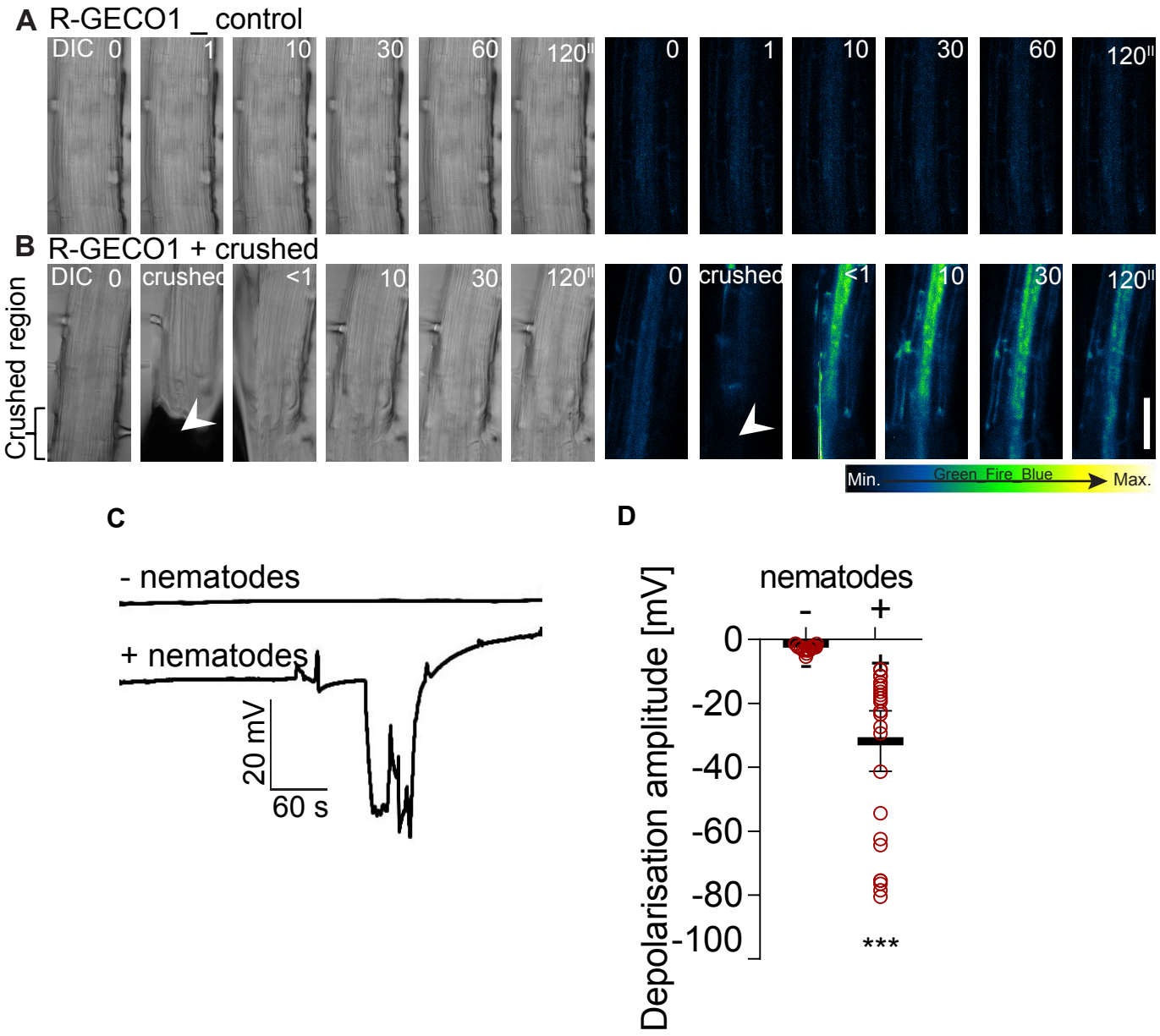
Appendix Figure S4: Ethylene response to cell ablation is a localized, non-systemic response

(A, B) 3D projection of a tile scan showing *ACS6::NLS-3xVenus* expression and intensity plots, visualising the spatially restricted increase of *ACS6::NLS-3xVenus* expression upon laser ablation after 10h **(B)** when compared to control **(A)**. Representative pictures are shown (n=7 roots). Time points are indicated in the top right corner of each frame. White arrow indicates ablated cell. **Scale bar**: **(A, B)** 100 μm.



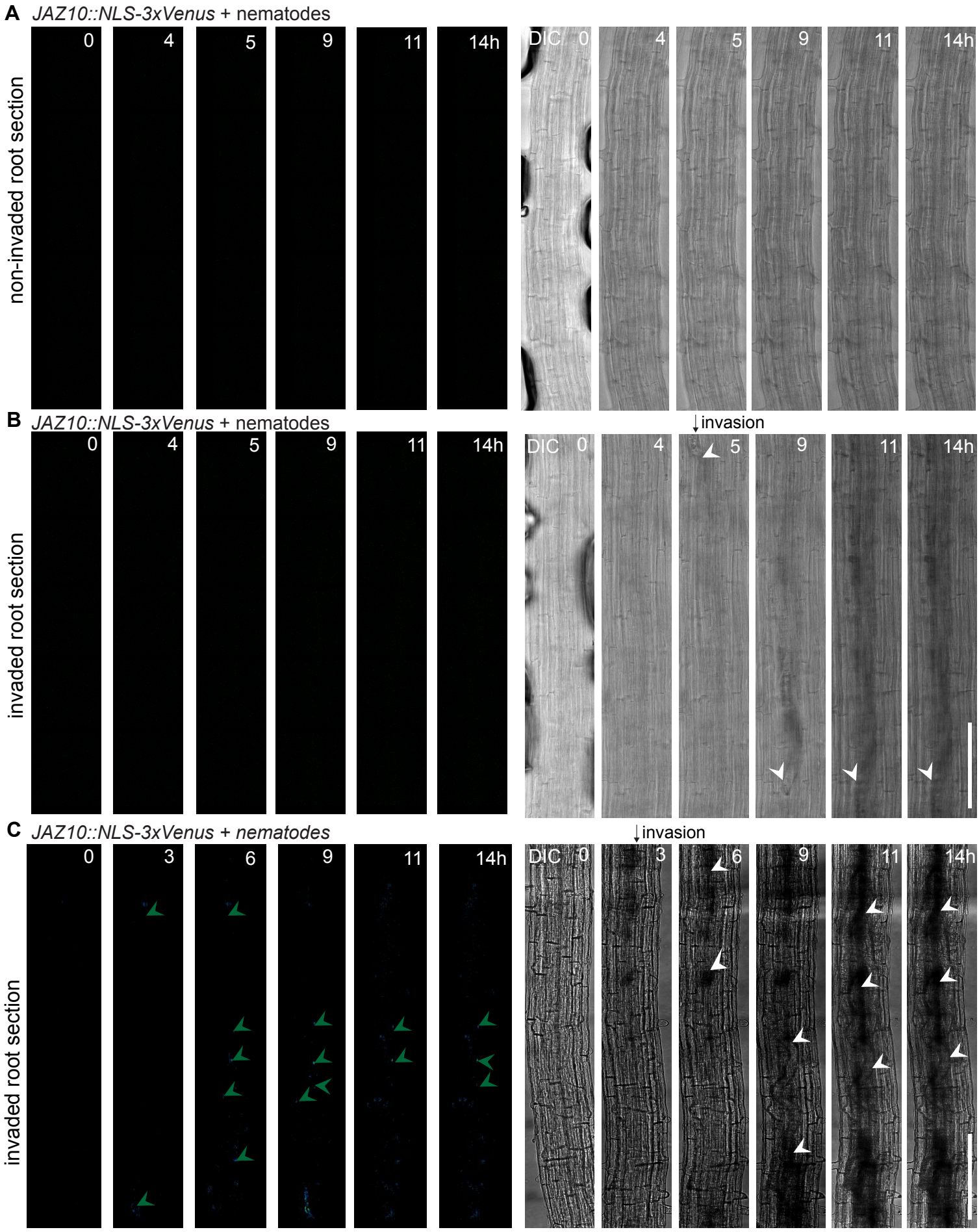
Appendix Figure S5: $Ca2^{+}$ waves in cortex upon ablation

(A) Quantification of depolarization amplitudes after cortex cell ablation were not reduced in glutamate receptor mutants (*glr3.3 3.1* and *glr3.3 3.6*) when compared to the wt (col) (n=10 roots, error bars indicate standard error). **(B - D)** Representative time-lapse images of calcium wave propagation using a CASE12 reporter line. Live-imaging time points are indicated in seconds at top right corner of each frame. Laser ablation of a cortex cell leads to signal increases immediately after ablation at the ablated side with slide delay on opposite side of the root in cortex cells **(C)** and was not observed in non-ablated controls **(B)**. Calcium transport inhibitor $GdCl_3$ (50 µM) lowered calcium wave propagation after ablation **(D)**. White arrowheads indicate ablation position, red arrowheads indicate calcium wave propagation. **(E)** Quantification of signal intensities of time lapses as shown in **(B - E)** (n=10 roots, repeated two times, error bars indicate standard error). **(F - Q)** Visualizations and of ROS production after single cell laser ablation. For ROS visualization, 20µM H_2DCFDA was used. Time points in seconds at top right corners of images. White arrowheads indicate ablated cells, red frames indicate regions of signal quantification used for graphs (see **Fig 5 C – E**). **Scale bar**: **(B – D** and **F - Q)** 100 µm.



Appendix Figure S6: Cyst nematode damage induced membrane depolarizations

(A, B) 3D (xyt) images monitoring calcium wave propagation **(B)** after crushing root tips. Ca^{2+} dynamics were not observed in control roots **(A)** (n=10). Live-imaging time points are indicated in the top right corner of each frame. White arrowheads indicate crushed region of the root. **(C, D)** surface potential changes **(C)** recording example and **(D)** quantification of recordings after nematode infection of roots (n=27 events) from 6 recordings, error bars indicate mean value with 95% CI). **Scale bar**: **(A, B)** 100 µm.



Appendix Figure S7: Cyst nematode damage leads to weak or no jasmonate response
(A, C) Tile-scan of XYZT stacks monitoring jasmonate response with *JAZ10::NLS-3xVenus* prior to cyst nematode (*Heterodera schachtii*) infection in 5-day-old roots. **(A)** Absence of *JAZ10::NLS-3xVenus* response in a non-invaded root section over 14h (n=10). **(B, C)** After nematodes invaded the root *JAZ10::NLS-3xVenus* we detected very weak (n=3) or no (n=5) response over 14h. Time points at top right corners of images. White arrows indicate nematode positions inside the root, green arrows indicate weak *JAZ10::NLS-3xVenus* response and the black arrow indicate time of nematode invasion in to the root. **Scale bar**: **(A, B)** 200µm (C) 150µm.