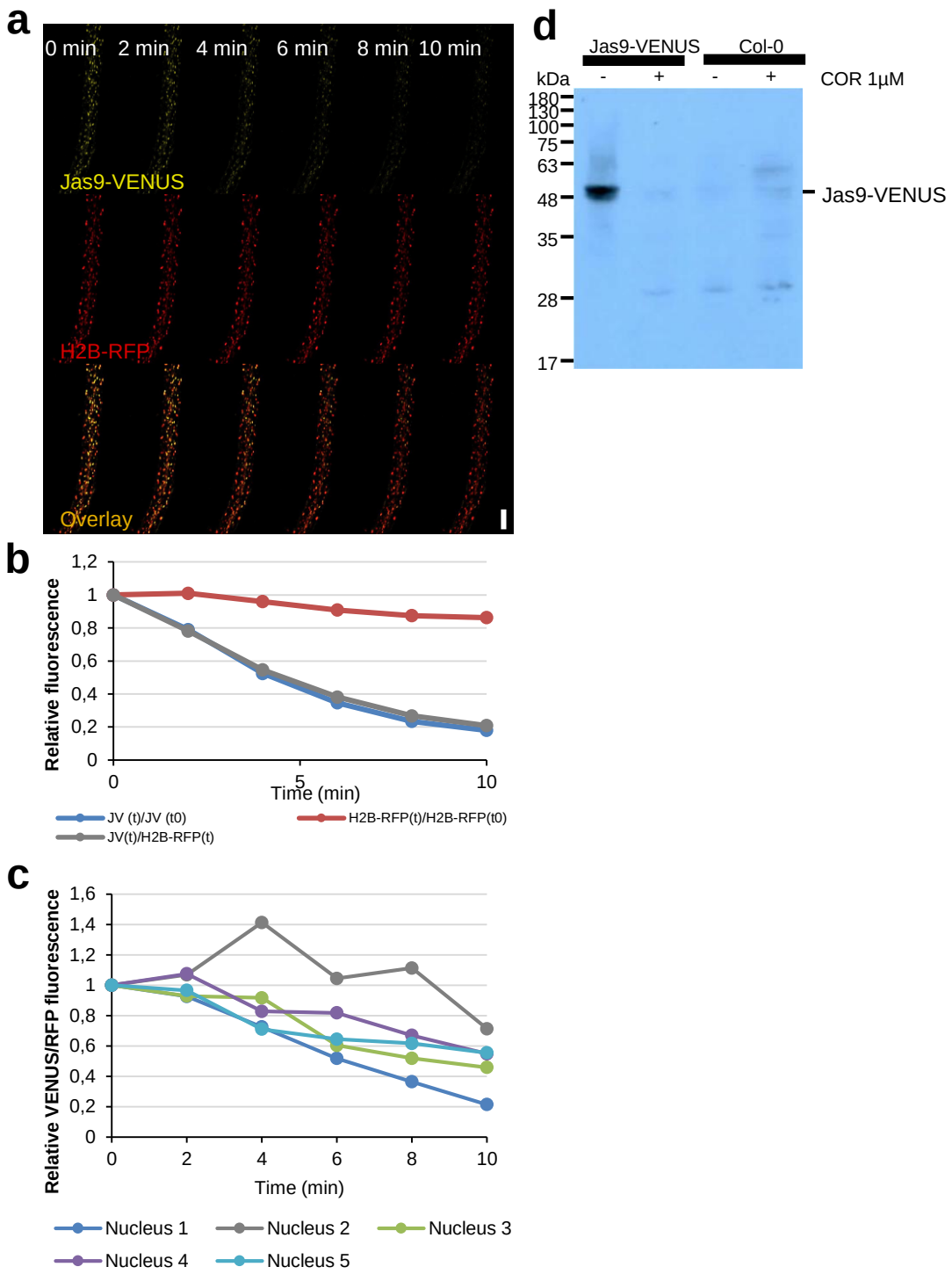
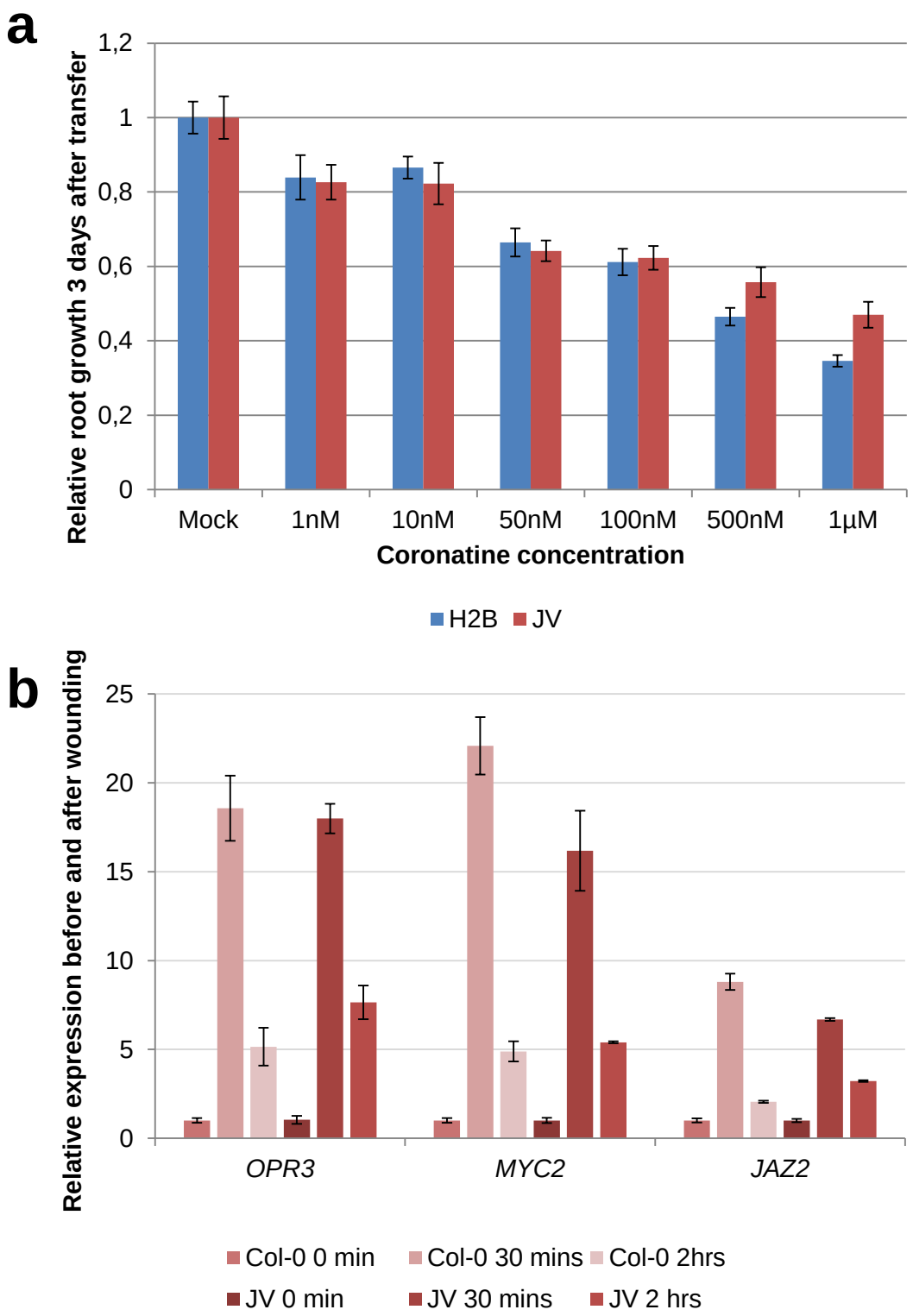
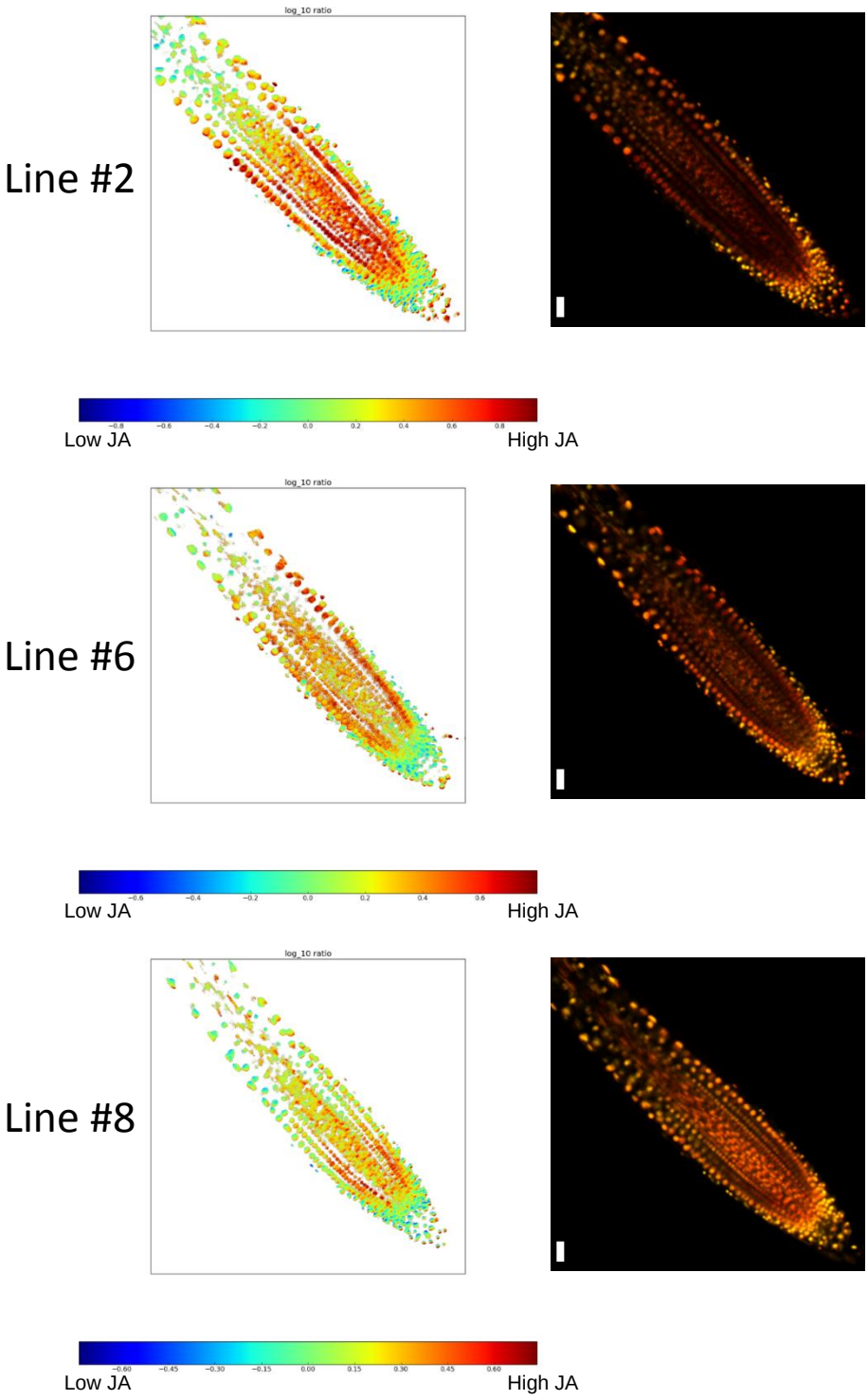


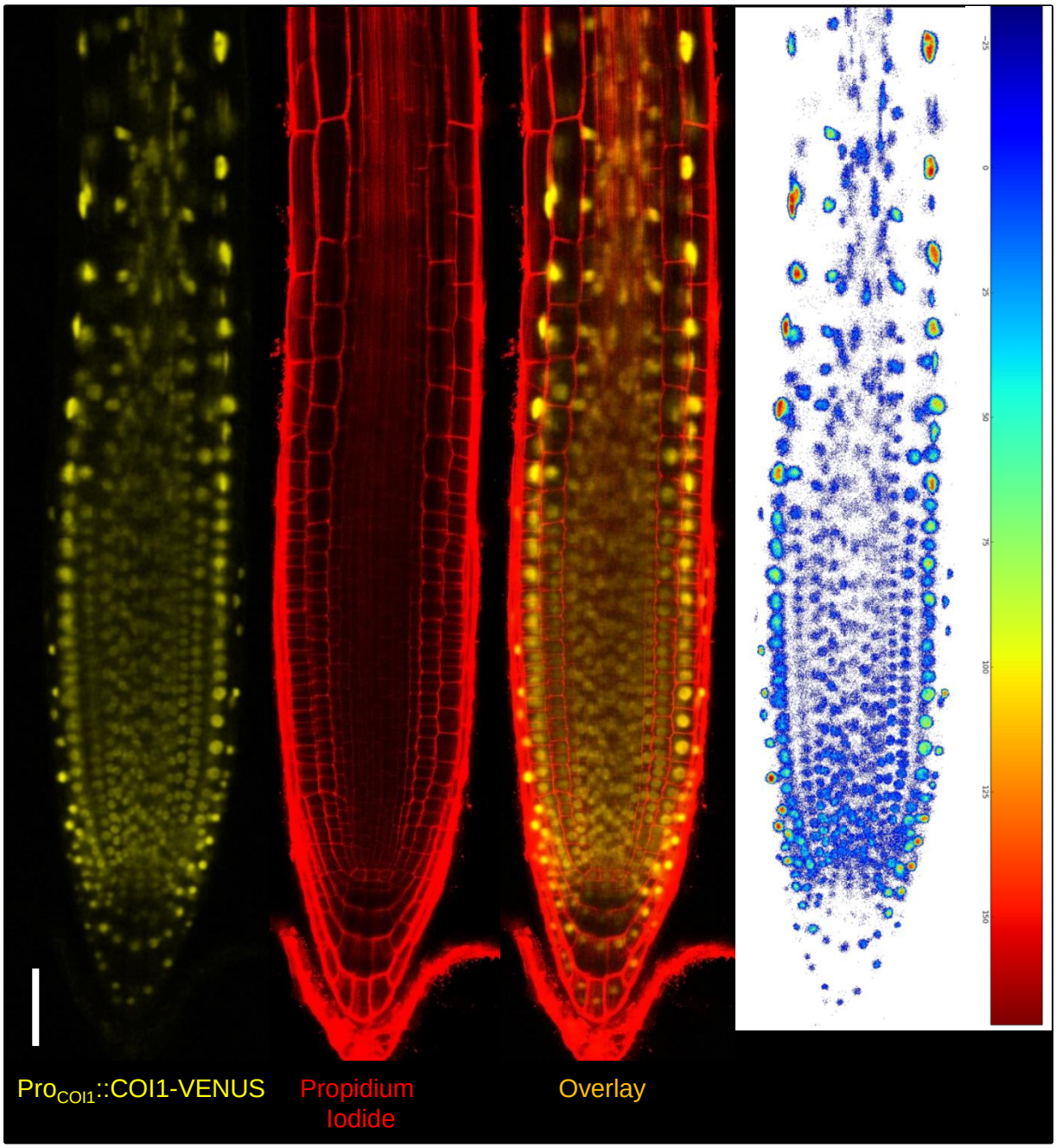

**Supplementary Figure 1. Jas9-VENUS but not H2B-RFP is degraded in response to coronatine.** (**a**) Time course confocal laser scanning micrographs of Jas9-VENUS, H2B-RFP and the overlay of both signals at the indicated time points after treatment with 1 µM coronatine (scale bar = 100µm). (**b** and **c**) Time course quantification of Jas9-VENUS fluorescence, H2B-RFP fluorescence and of Jas9-VENUS fluorescence normalised to H2B-RFP fluorescence, either measured from all the nuclei visible on the image (**b**) or from randomly selected individual nuclei (**c**). (**d**) Uncropped version of the western blot shown in Figure 1c that shows the analysis of total protein extracts of Jas9-VENUS and Col-0 seedlings treated for 30 minutes with or without 1µM coronatine and probed with an anti-GFP antibody. JV: Jas9-VENUS.



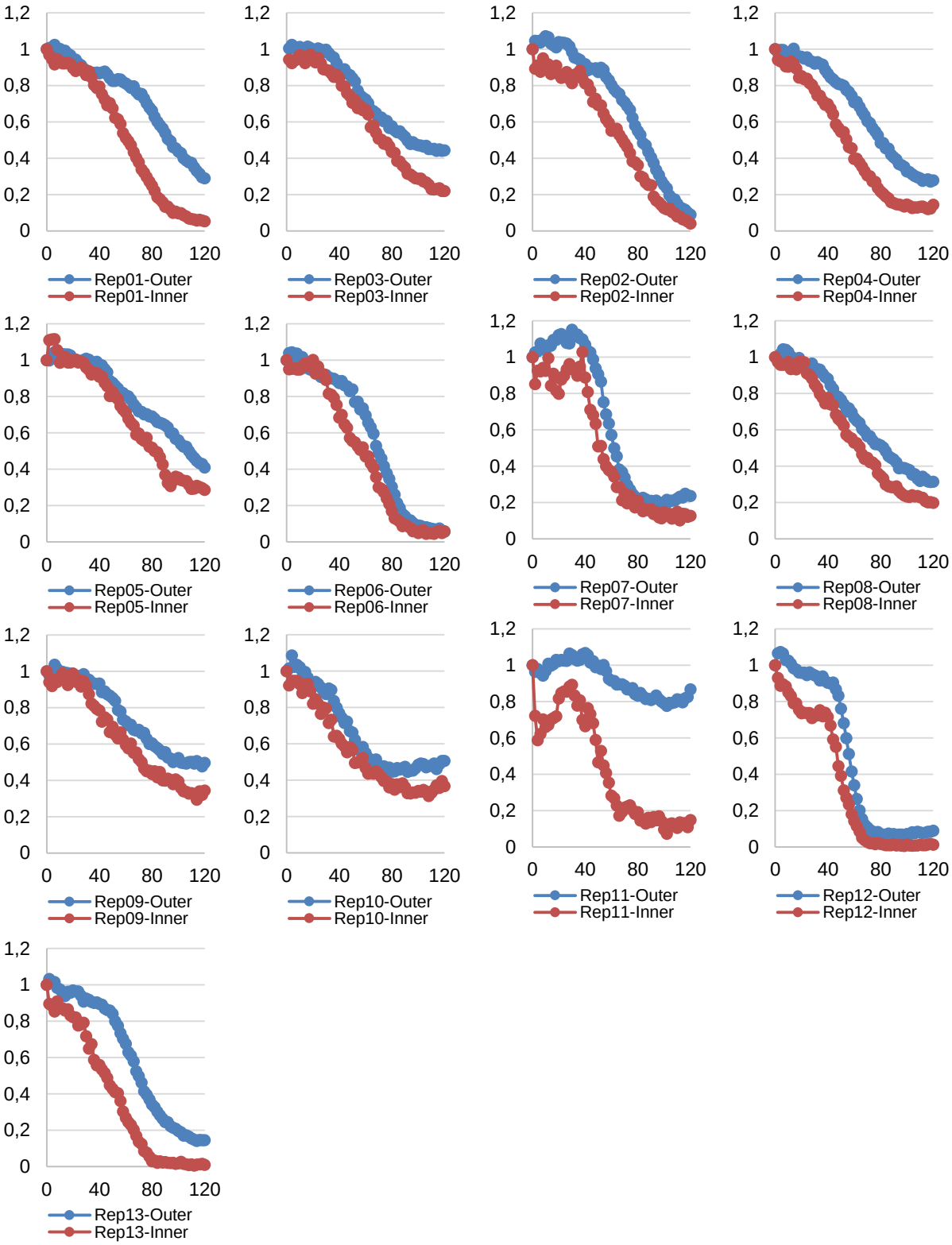

**Supplementary Figure 2. The Jas9-VENUS sensor does not affect physiological or transcriptional responses to JA.** (**a**) 1-week old H2B-RFP (parental line, control) and Jas9-VENUS seedlings show a similar inhibition of root growth by coronatine. Error bars represent the SEM. (**b**) Shoots and roots of wounded Col-0 and Jas9-VENUS seedlings were harvested 30 minutes and 2 hours after wounding by cutting a cotyledon and the expression of wound inducible genes were investigated using RT qPCR. The genes display a similar dynamic expression pattern in both genetic backgrounds. The experiment was repeated twice and the results show a representative dataset. Error bars represent the standard deviation of four technical replicates. JV: Jas9-VENUS.



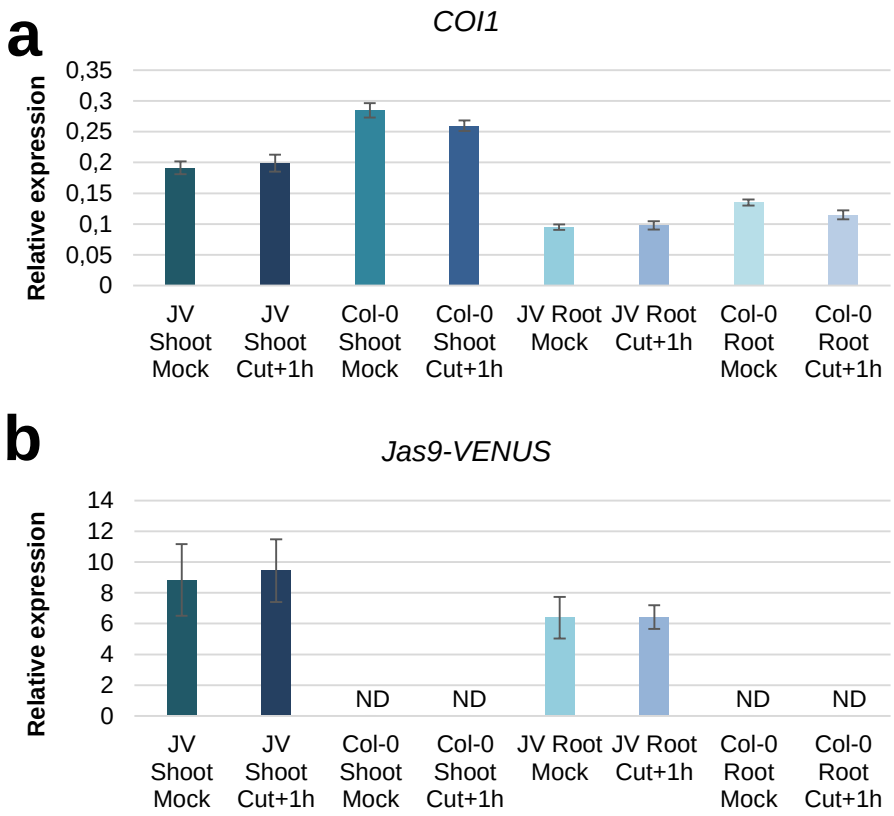

**Supplementary Figure 3. Jas9-VENUS root maps of three independent lines.** Distribution map of bioactive JA in the root apical meristem based on the ratio of Jas9-VENUS fluorescence to H2B-RFP fluorescence of a representative seedling for three independent transgenic lines imaged using settings chosen to avoid pixels saturation (see Methods for computing details). Similar patterns were observed in the different Jas9-VENUS lines. As the three lines were generated from the same H2B-RFP line, the differences observed between the 3 lines are likely to represent the impact of the transgene insertion site on its expression. The overlay of the Jas9-VENUS and H2B-RFP channels used for generating the root map is shown (scale bar = 30µm).



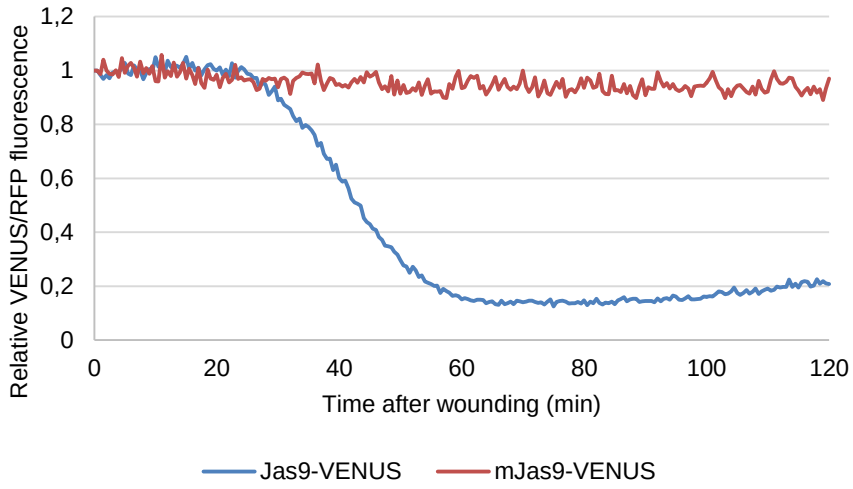

**Supplementary Figure 4. COI1-VENUS expression map.** Distribution map of proCOI1::COI1-VENUS in the root apical meristem based on the COI1-VENUS fluorescence of a representative seedling of a representative transgenic line. The original image used for generating the root map is shown (scale bar = 30µm) (see Methods for computing details).



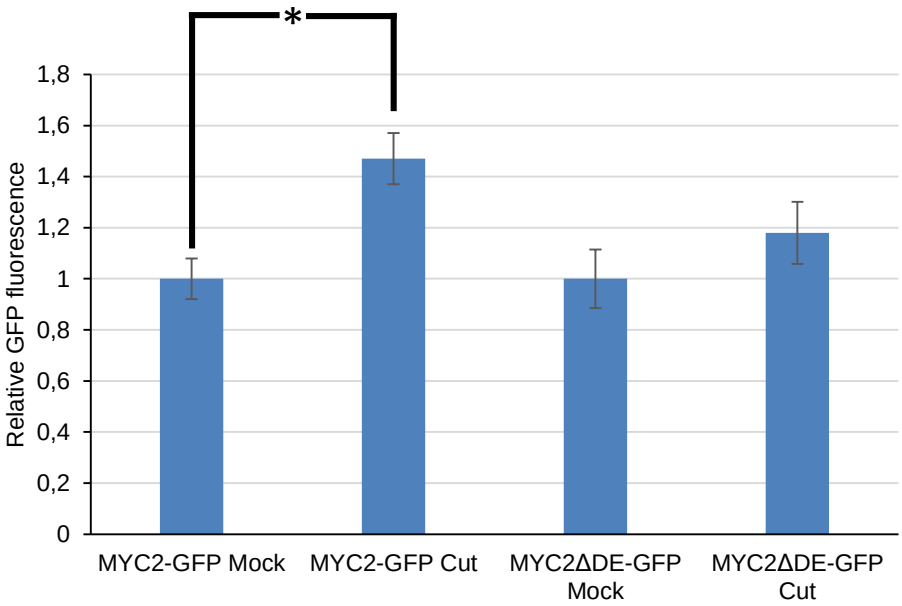

**Supplementary Figure 5. Jas9-VENUS degradation in response to wounding is faster in the inner tissues compared to the outer tissues.** Seedlings were wounded by cutting a cotyledon using dissection scissors. Time course quantification of Jas9-VENUS fluorescence, normalised to H2B-RFP fluorescence, in the inner and outer tissues for the 13 replicates that were used to generate the averaged quantification shown on Figure 3a. The x-axis shows time in minutes after wounding and the y-axis shows the relative fluorescence.



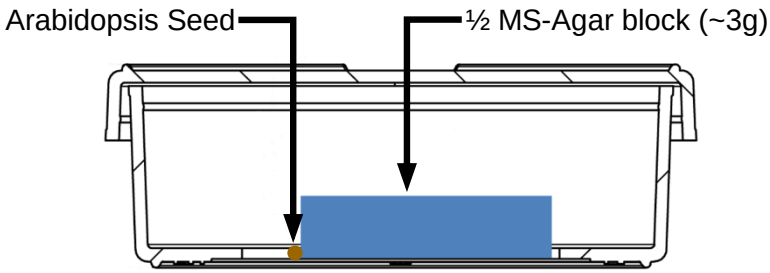

**Supplementary Figure 6. Wounding does not alter the expression levels of the JA receptor *COI1* or of the *Jas9-VENUS* sensor.** (**a** and **b**) Seedlings were wounded by cutting a cotyledon and shoot and root samples were harvested separately. The expression of *COI1* (**a**) and *Jas9-VENUS* (**b**) were investigated by RT qPCR 1 hour after wounding. The experiment was repeated twice and the results show a representative dataset. Expression of *COI1* and *Jas9-VENUS* were normalised to the expression of the control gene *At1G04850* (CTRL1). Error bars represent the standard deviation of four technical replicates. JV: Jas9-VENUS transgenic line, Col-0: control line, ND: Not Detected.




**Supplementary Figure 7. Jas9-VENUS degradation in response to wounding requires a functional Jas motif.** Seedlings were wounded by cutting a cotyledon using dissection scissors. Jas9-VENUS and Jas9m-VENUS fluorescence, normalised to H2B-RFP fluorescence were quantified on the whole root after wounding for 120 min. The experiment was repeated twice and the results show a representative dataset.




**Supplementary Figure 8. MYC2-GFP is stabilized in the root after wounding a cotyledon.** 35S:MYC2-GFP and 35S:MYC2ΔDE-GFP seedlings were wounded and the GFP fluorescence was measured two hours after wounding. The GFP fluorescence measured was statistically higher (t-test, α=0.05, P=0.0008) in wounded MYC2-GFP compared to unwounded MYC2-GFP, showing a stabilization of MYC2 protein. The stabilization of MYC2 is not apparent when the Destruction Element (DE) motif is absent since MYC2 is constitutively stabilized (t-test, α=0.05, P=0.27). Error bars represent the SEM.




**Supplementary Figure 9. Schematic diagram of glass-bottom Petri dishes (base diameter=35mm) used for time course experiments.** For treatments and wound response time courses, seeds were sown as shown on the diagram. Original drawing kindly provided by Greiner Bio-One GmbH, Frickenhausen, Germany.

**Supplementary Table 1. Primers used in this study.**

### *Cloning primers*

| | |
|---|---|
| qOPR3-F | aagattcgatctctctcatcgagt |
| qOPR3-R | ggagtggtccgttgagca |
| qMYC2-F | tccgagtccggttcattct |
| qMYC2-R | tctcgggagaaagtgttattgaa |
| qJAZ2-F | ctctttagcctgcgaactcc |
| qJAZ2-R | ttggtatggtgcctttgatg |
| qJas9-VENUS-F | tcttgatttgtcgaccggaga |
| qJas9-VENUS-F | cccggtgaacagctcctc |
| qCOI1-F | gtgtcctaatttggaagttctcg |
| qCOI1-R | ctccattccttgttcatctgc |
| qCTRL1-F | agtggagaggctgcagaaga |
| qCTRL1-R | ctcgggtagcacgagcttta |

### *qPCR primers*

| | |
|---|---|
| JAZ9-F2 | caccatgatcatgttatgcgccgggaacgg |
| JAZ9-R1 | tgtaggagaagtagaagagtaattc |
| JAZ9-F3m | cctcaagctgctgcggcatccttgg |
| JAZ9-R3m | ccaaggatgccgcagcagcttgagg |
| COI1-Fw | caccctcctcgagtgcatcatcaa |
| COI1-Rev-Full | tattggctccttcaggactc |