Figure S1

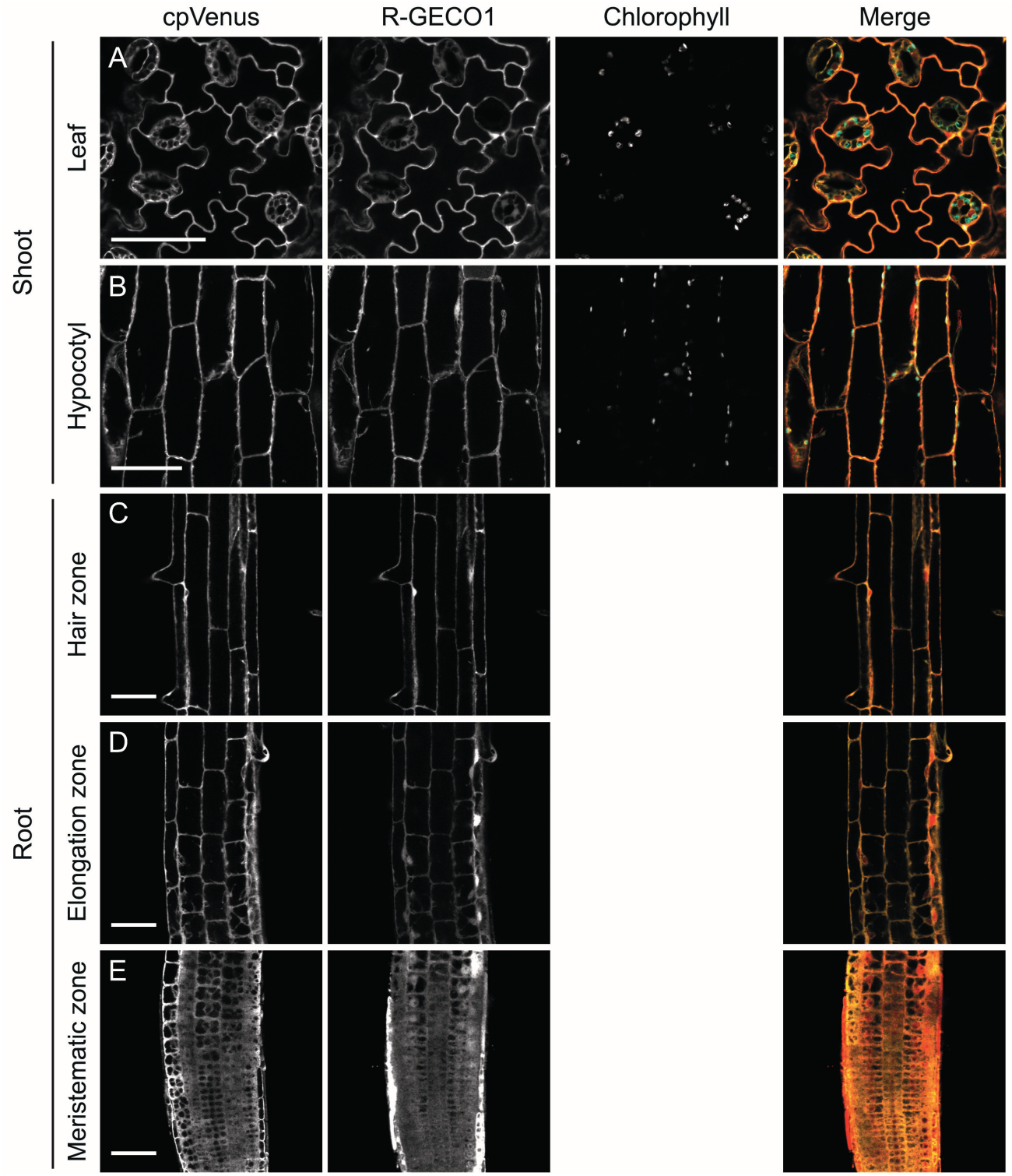

Figure S1. Dual expression of NES-YC3.6 and R-GECO1 in Arabidopsis. Arabidopsis lines expressing the Ca^{2+} sensors NES-YC3.6 and R-GECO1 under control of the *UBQ10* promoter were examined for fluorescence in different tissues. Fluorescence of cpVenus (NES-YC3.6), R-GECO1 and chlorophyll was investigated in (A) leaves, (B) the hypocotyl and (C) – (E) in different developmental zones of the primary root. The merged images indicate chlorophyll (cyan), NES-YC3.6 (yellow) and R-GECO1 (red) fluorescence. Co-localization of R-GECO1 and NES-YC3.6 is observed in the cytosol. Scale bars represent 50 µm.

Figure S2

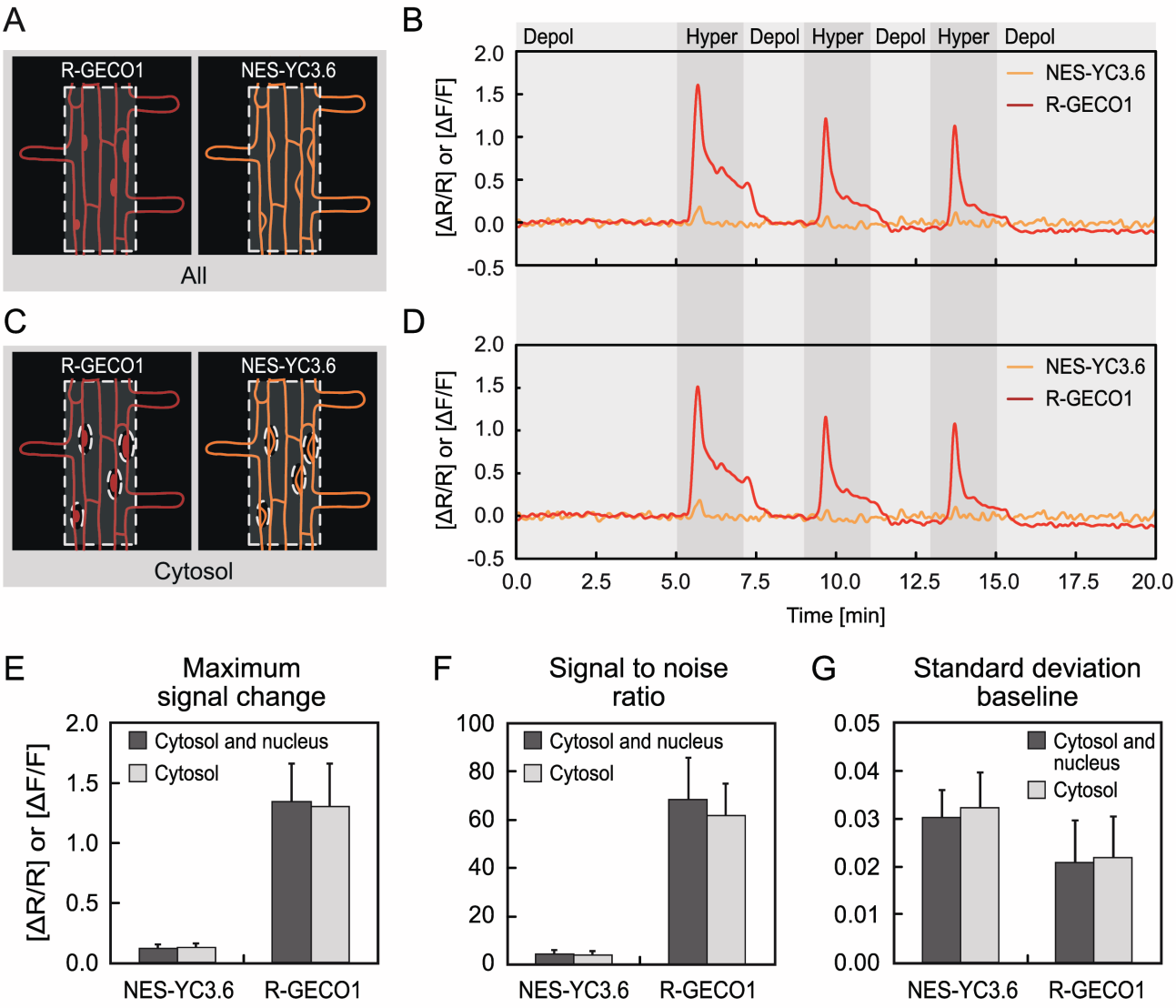

Figure S2. R-GECO1 exhibits enhanced Ca^{2+}-dependent signal change in response to plasma membrane hyperpolarisation and signal change of R-GECO1 is not influenced by nuclear exclusion. Ca^{2+}-dependent signal changes in response to plasma membrane hyperpolarisation in roots of 6 – 8-day-old seedlings expressing NES-YC3.6 and R-GECO1. A series of Ca^{2+} transients was induced by alternate applications of de- (Depol) and hyperpolarisation buffer (Hyper) at indicated time points. The contribution of nuclear [Ca^{2+}] increase to the total cellular [Ca^{2+}] increase was analysed by drawing different ROIs. In (A) and (B) the ROI covers the cytosol and nuclei (Cytosol and nucleus) and in (C) and (D) nuclei were excluded from data analyses (Cytosol). (A) and (C) Schematic drawings of roots expressing NES-YC3.6 and R-GECO1 and corresponding ROIs used for data analyses. (B) and (D) Time-dependent normalised NES-YC3.6 emission ratios (ΔR/R) and normalised R-GECO1 fluorescence intensities (ΔF/F). (D) Maximum signal change, (E) signal-to-noise ratios and (F) standard deviation of the baseline. Error bars represent SD of three independent experiments.

Figure S3

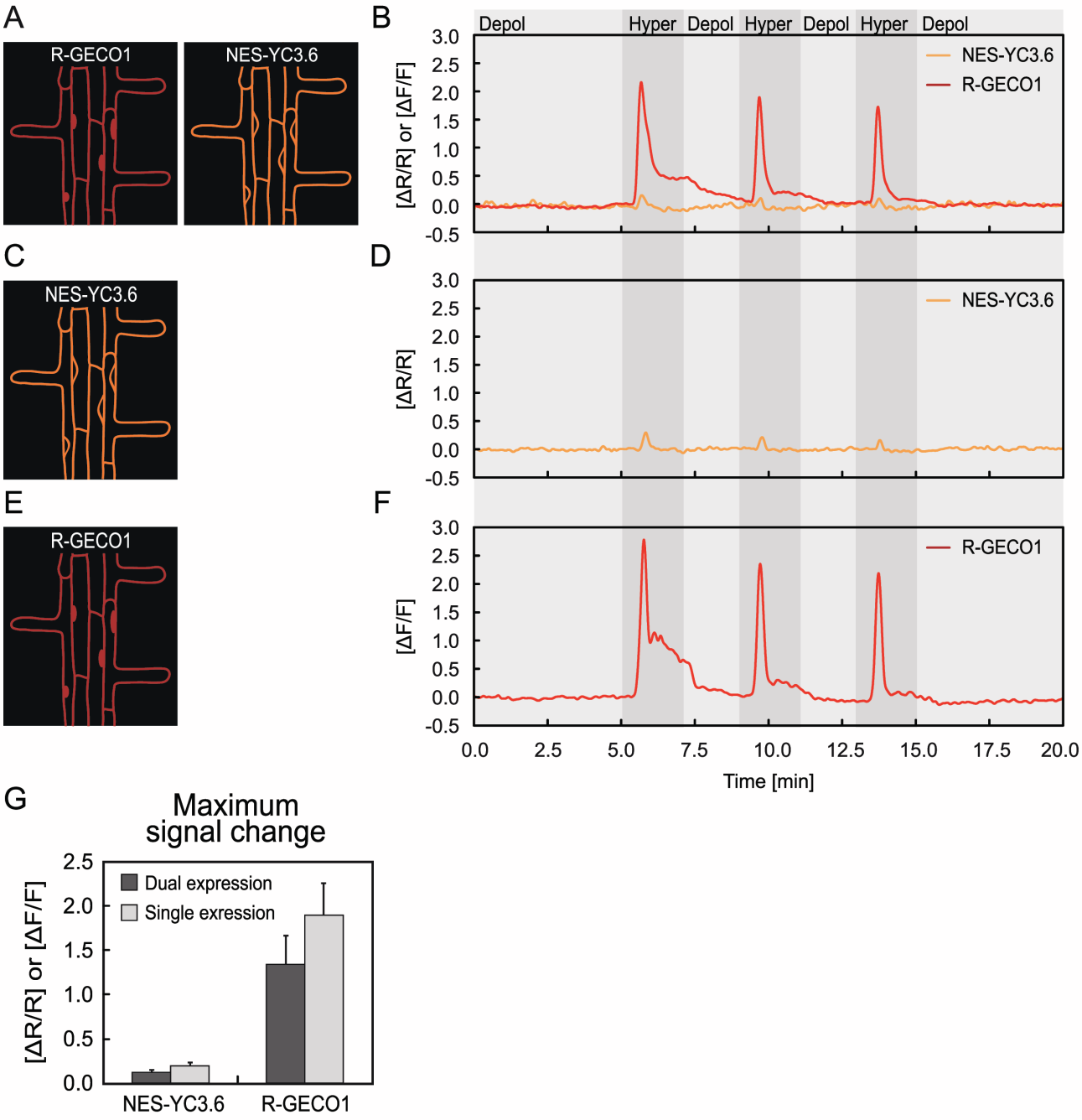

Figure S3. Individual sensor performance of NES-YC3.6 and R-GECO1 is not impaired in dual expression lines. Ca^{2+}-dependent signal changes in response to plasma membrane hyperpolarisation in roots of 6 – 8-day-old seedlings were compared between dual expression lines of NES-YC3.6 and R-GECO1 (A, B) and single expression lines of NES-YC3.6 (C, D) and R-GECO1 (E, F). A series of Ca^{2+} transients was induced by alternate applications of de- (Depol) and hyperpolarisation buffer (Hyper) at indicated time points. (A, C and E) Schematic drawings of roots expressing NES-YC3.6 and R-GECO1. (B, D and F) Time-dependent normalised NES-YC3.6 emission ratios (ΔR/R) and normalised R-GECO1 fluorescence intensities (ΔF/F). (G) Maximum signal change. Student's *t*-test revealed no significant differences for NES-YC3.6 (*p*-value 0.118, n=3) and R-GECO1 (*p*-value 0.133, n=3) between dual and single expression lines. Error bars represent SD of three independent experiments.

Figure S4

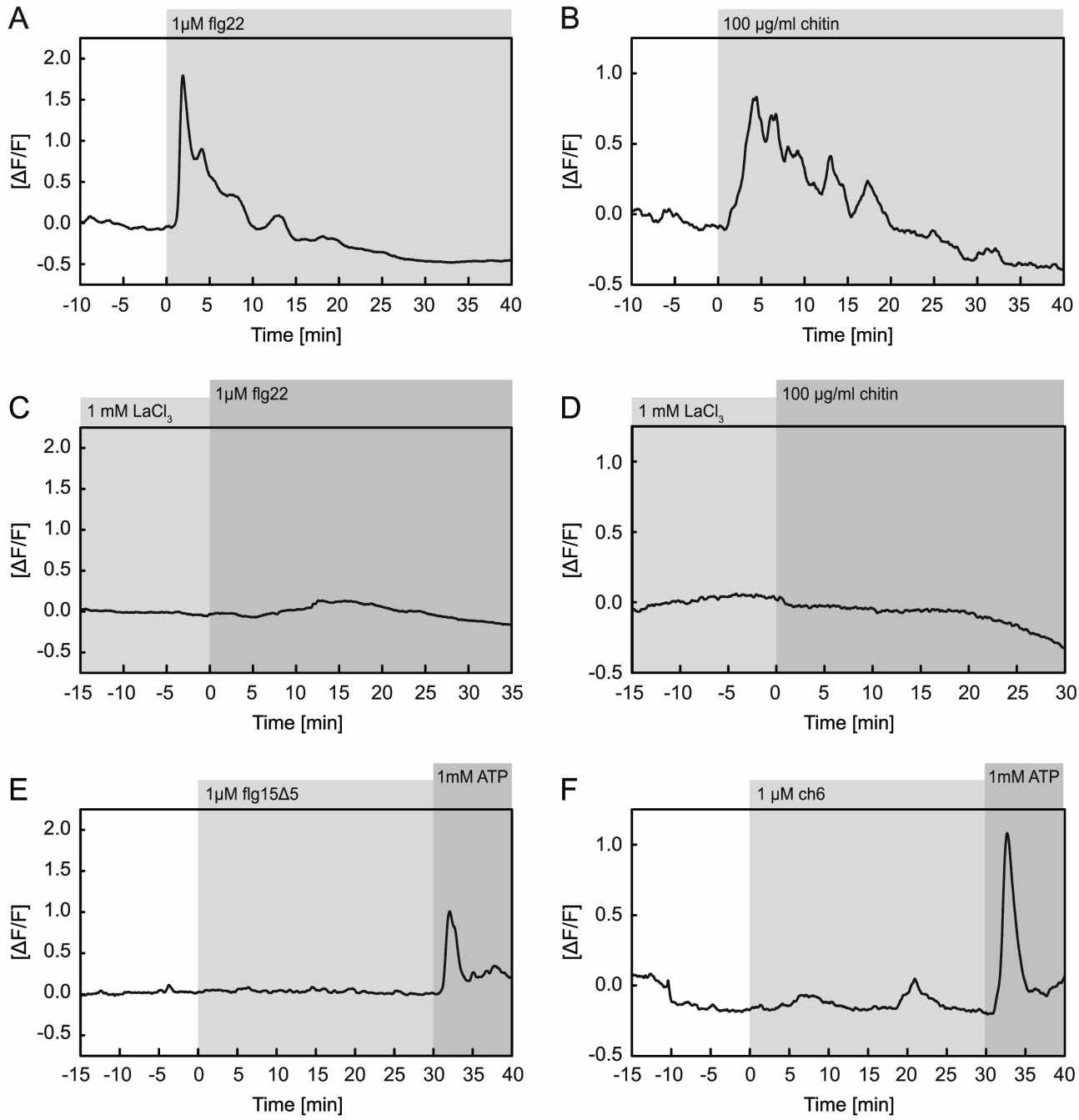

Figure S4. PAMP-induced signal changes of R-GECO1 are Ca^{2+}-dependent and specific for flg22 and chitin. To prove that PAMP-induced signal changes of R-GECO1 are Ca^{2+}-dependent, samples were pre-treated for 30 min with the plasma membrane Ca^{2+} channel blocker $LaCl_3$ (C, D). To test whether changes in $[Ca^{2+}]_{cyt}$ are specific for flg22 and chitin, samples were treated with inactive flg15Δ5 and inactive ch6 (E, F). All experiments were carried out with true leaves of 14 – 16-day-old seedlings. Graphs show time-dependent normalised R-GECO1 fluorescence intensities (ΔF/F) in response to different treatments at time points indicated by grey boxed areas. (A) 1 µM flg22. (B) 100 µg/ml chitin. (C) 30 min pre-treatment with 1 mM $LaCl_3$ followed by 1 µM flg22. (D) 30 min pre-treatment with 1 mM $LaCl_3$ followed by 100 µg/ml chitin. (E) 1 µM flg15Δ5 followed by 1 mM ATP. (F) 1 µM ch6 followed by 1 mM ATP. Shown are representative experiments with n ≥ 3.

Figure S5

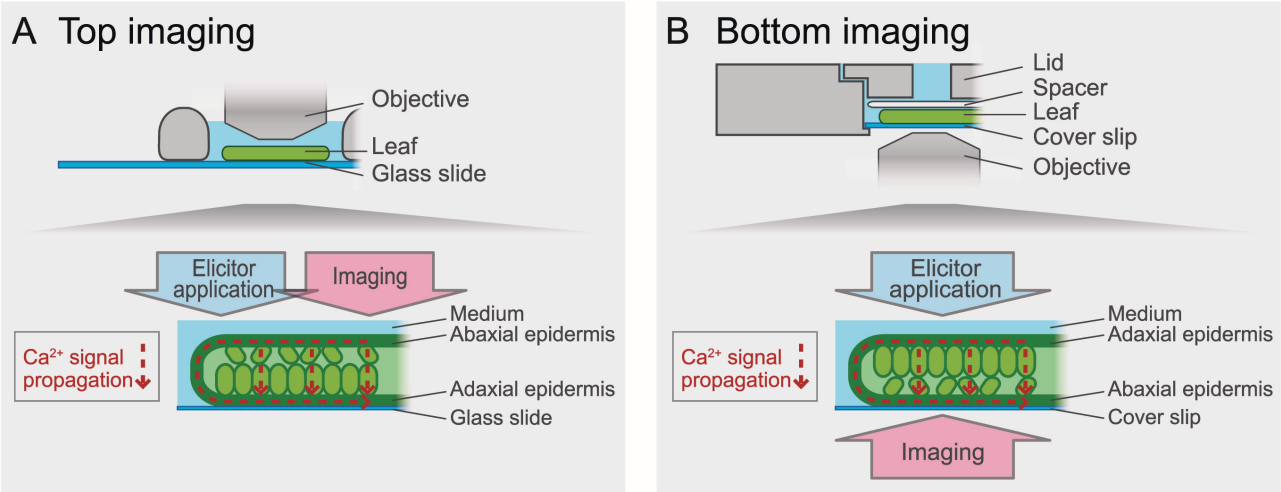

Figure S5. Experimental setup for top and bottom imaging. Detached first true leaves were mounted for top (A) or bottom imaging (B). In (A) the adaxial site of the leaf is facing the glass slide, whereas the abaxial site is oriented towards the objective and the site of PAMP application. In (B) the abaxial site of the leaf is facing the cover slip and oriented towards the objective, whereas the adaxial site is oriented towards the site of PAMP application.

Movie legends

Movie S1. $[Ca^{2+}]_{cyt}$ oscillations in growing root hair tip. Time laps of R-GECO1-based Ca^{2+} imaging in roots. Movie corresponds to measurements shown in Figure 2C and 2D. Scale bar, 20 µm. Time format, sss.

Movie S2. Flg22-induced $[Ca^{2+}]_{cyt}$ transients in Arabidopsis leaves. Time laps of R-GECO1-based Ca^{2+} imaging in leaf epidermal cells after stimulation with 100 nM flg22. Movie corresponds to measurements shown in Figure 4A, 4B. Scale bar, 50 µm. Time format, mm:ss.

Movie S3. Chitin-induced $[Ca^{2+}]_{cyt}$ transients in Arabidopsis leaves. Time laps of R-GECO1-based Ca^{2+} imaging in leaf epidermal cells after stimulation with 100 µg/ml chitin. Movie corresponds to measurements shown in Figure 4C, 4D. Scale bar, 50 µm. Time format, mm:ss.

Movie S4. $[Ca^{2+}]_{cyt}$ dynamics in guard cells after flg22 treatment. Time laps of R-GECO1-based Ca^{2+}-imaging in epidermal and guard cells of a first true leaf after stimulation with 100 nM flg22. Movie corresponds to measurement shown in Figure 5C, 5F Scale bar, 15 µm. Time format, mm:ss.

Movie S5. $[Ca^{2+}]_{cyt}$ dynamics in guard cells after chitin treatment. Time laps of R-GECO1-based Ca^{2+}-imaging in epidermal and guard cells of a first true leaf after stimulation with 100 µg/ml chitin. Movie corresponds to measurement shown in Figure 5D, 5H. Scale bar, 15 µm. Time format, mm:ss.

Movie S6. Flg22-induced $[Ca^{2+}]_{cyt}$ transients in Arabidopsis roots. Time laps of R-GECO1-based Ca^{2+} imaging in 7-day-old roots was performed in the RootChip16. Signal is shown as normalised fluorescence intensity (ΔF/F). The growing root tip appears white as a result of image calculation. Shown are $[Ca^{2+}]_{cyt}$ dynamics in roots after stimulation with 1 µM flg22. Movie corresponds to measurement shown in Figure 6A, 6C. Scale bar, 200 µm. Time format: mm:ss.

Movie S7. Chitin-induced $[Ca^{2+}]_{cyt}$ transients in Arabidopsis roots. Time laps of R-GECO1-based Ca^{2+} imaging in 7-day-old roots was performed in the RootChip16. Signal is shown as normalised fluorescence intensity (ΔF/F). The growing root tip appears white as a result of image calculation. Shown are $[Ca^{2+}]_{cyt}$ dynamics in roots after stimulation with 100 µg/ml chitin. Movie corresponds to measurement shown in Figure 6B, 6D. Scale bar, 200 µm. Time format: mm:ss.