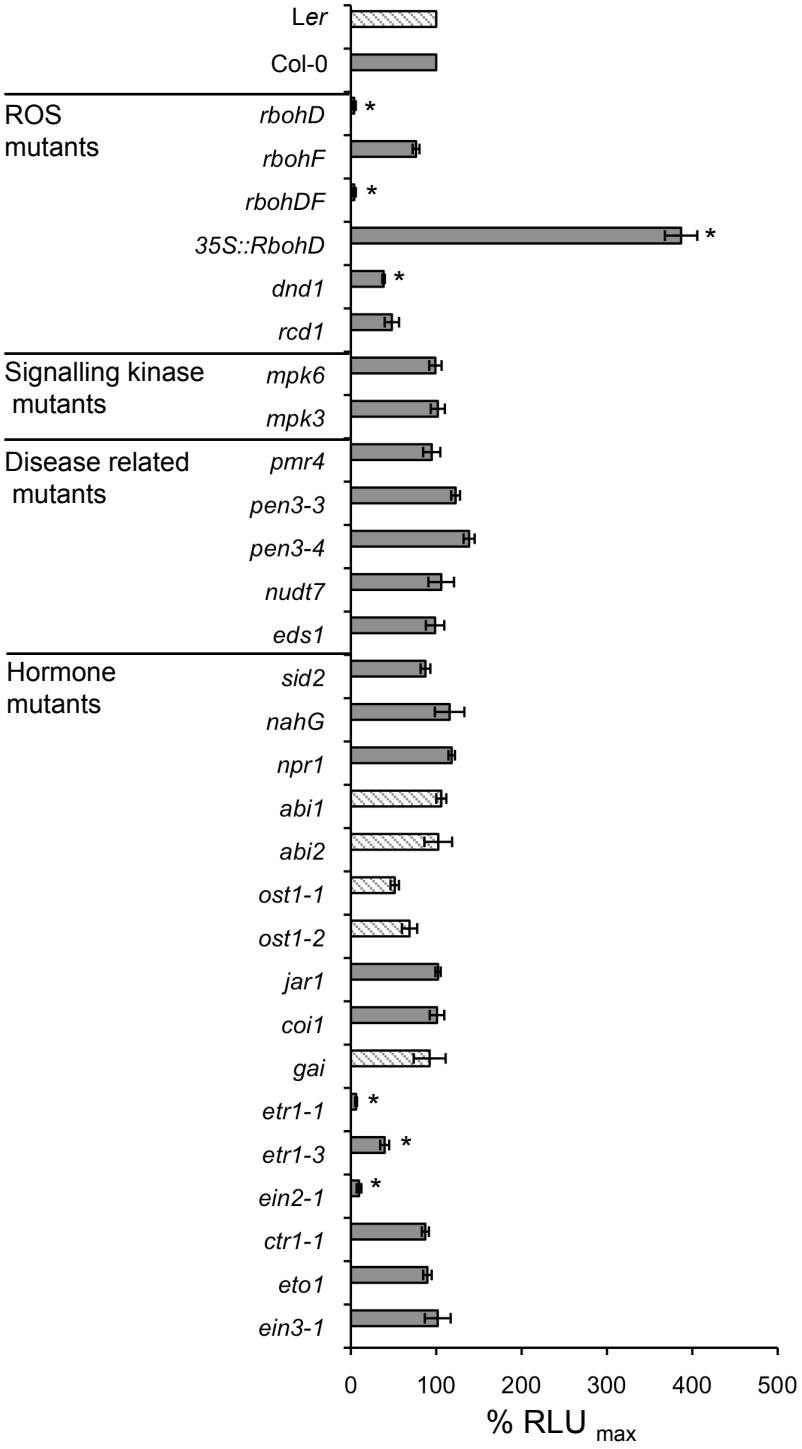
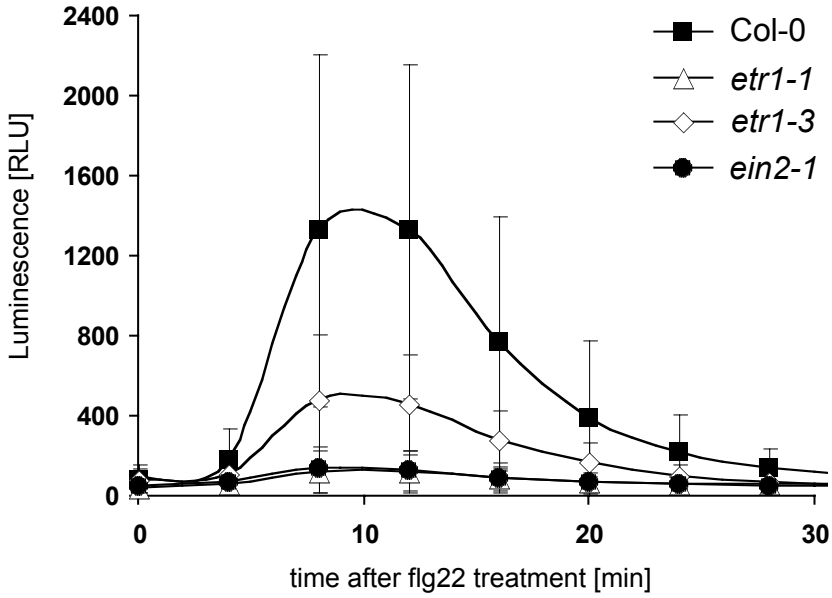
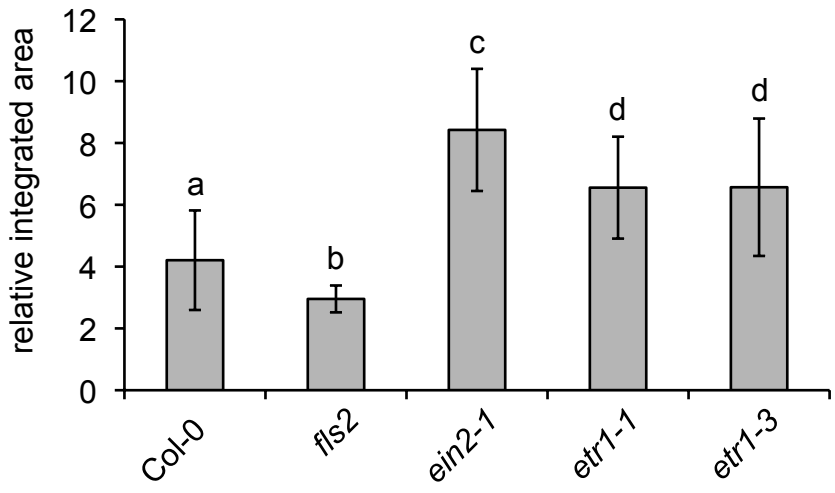


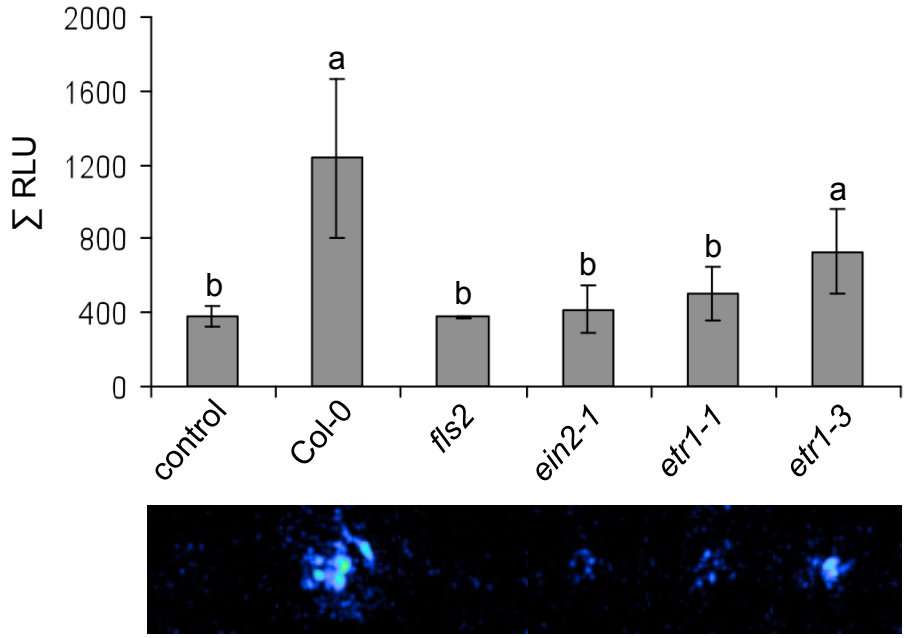
Suppl. Fig. 1. Overview of mutants with known roles in plant defence and stress signalling tested for flg22-induced ROS production. ROS production was monitored in seedlings of the indicated genotypes over time. Depicted are average values in percent (n=12) normalized to internal WT controls (L*er* or Col-0, respectively); bars represent +/- SD. Similar results were obtained in multiple independent experiments. RLU, relative light units. Asterisks indicate significant differences $p < 0.05$.



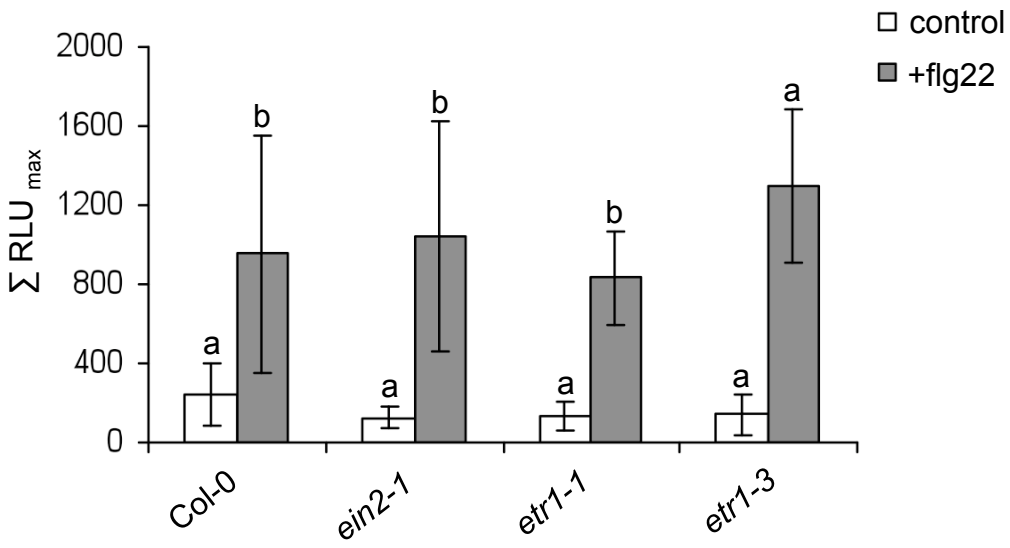
Suppl. Fig. 2. Oxidative burst in ethylene-insensitive mutants. Flg22-triggered ROS production was monitored in seedlings of the indicated genotypes over time. Depicted are average values (n=18); bars represent +/- SD. Similar results were obtained in multiple independent experiments. RLU, relative light units.



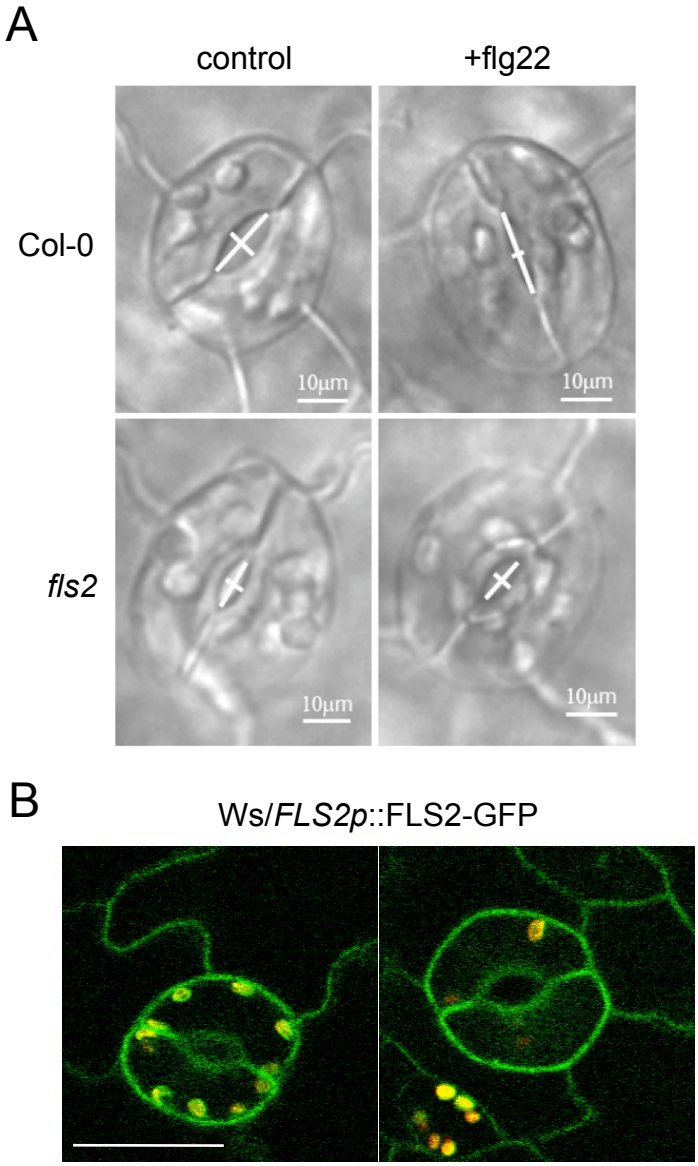
Suppl. Fig. 3. Steady-state accumulation of ethylene. Ethylene levels of intact Col-0, *fls2*, *etr1-1*, *etr1-3*, *ein2-1* seedlings were determined by GC-MS. Depicted are average values of relative integrated areas obtained in at least three independent experiments; bars represent +/-SD. Letters indicate significant differences $p < 0.05$.



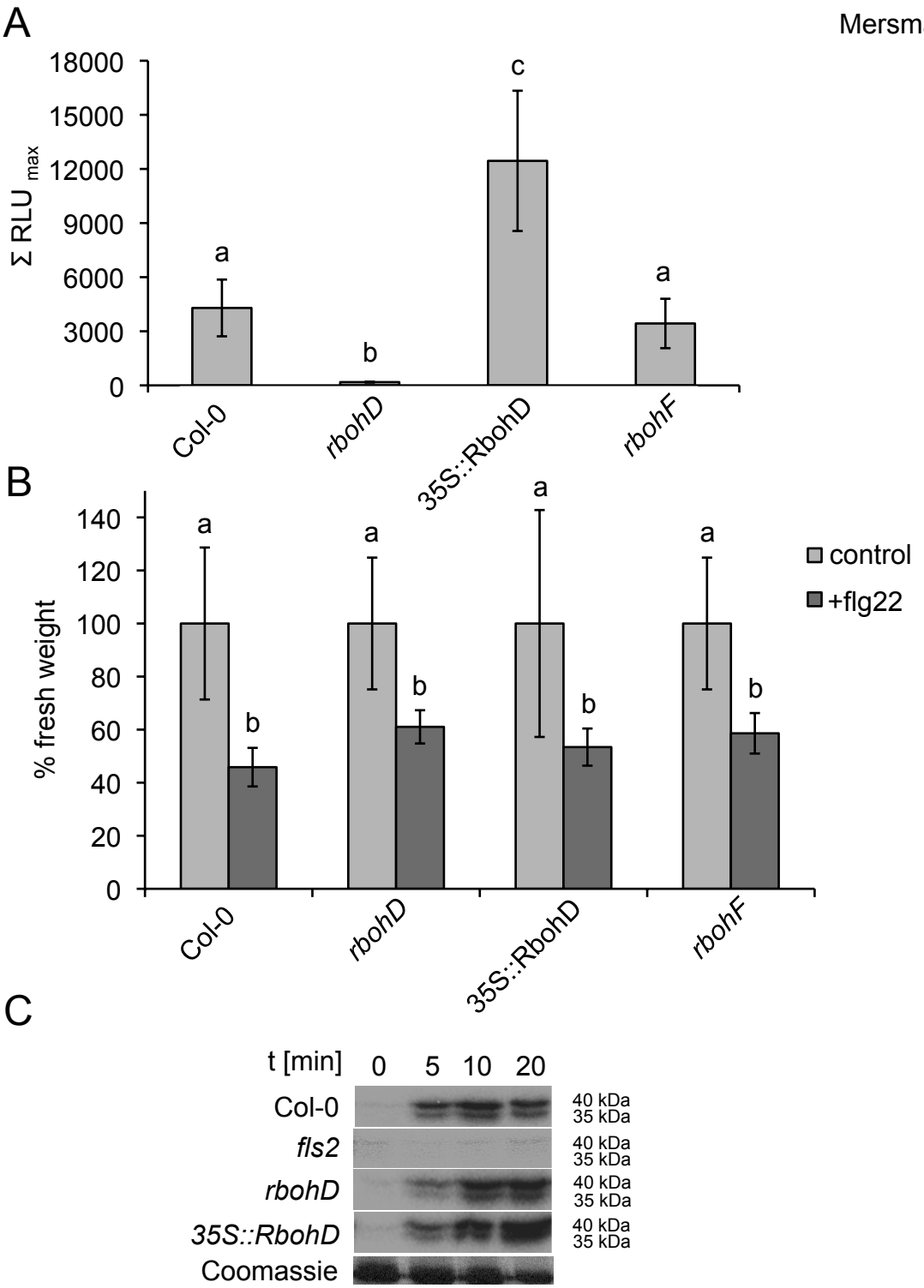
Suppl. Fig. 4. Oxidative burst in ethylene signalling mutants at adult stage. Flg22-triggered ROS production was monitored in 4 weeks old plants of the indicated genotypes over time. Upper panel: Depicted are average values (n=6); bars represent +/-SD. Similar results were obtained in two independent experiments. RLU, relative light units. Letters indicate significant differences $p < 0.05$. Lower panel: representative images of emitted luminescence by the indicated genotypes upon flg22 elicitation. Control, Col-0 wild type without flg22.



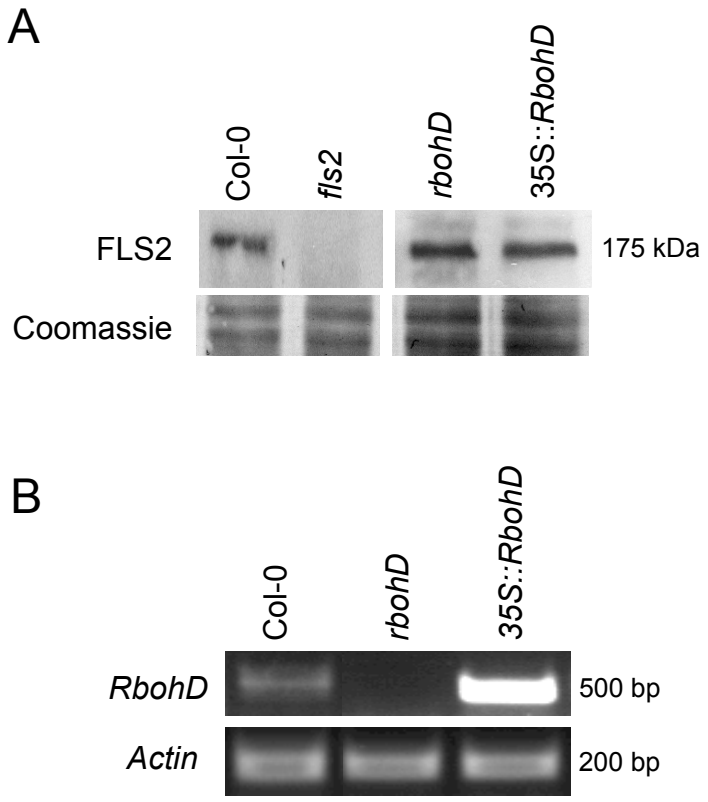
Suppl. Fig. 5. Flg22-induced ROS in wounded seedling material. Leaf discs of Col-0, *etr1-1* and *ein2-1* seedlings were monitored for ROS production upon flg22 treatment. Depicted are average values (n=6); bars represent +/-SD. Letters indicate significant differences $p < 0.05$. Similar results were obtained in three independent experiments. RLU, relative light units.



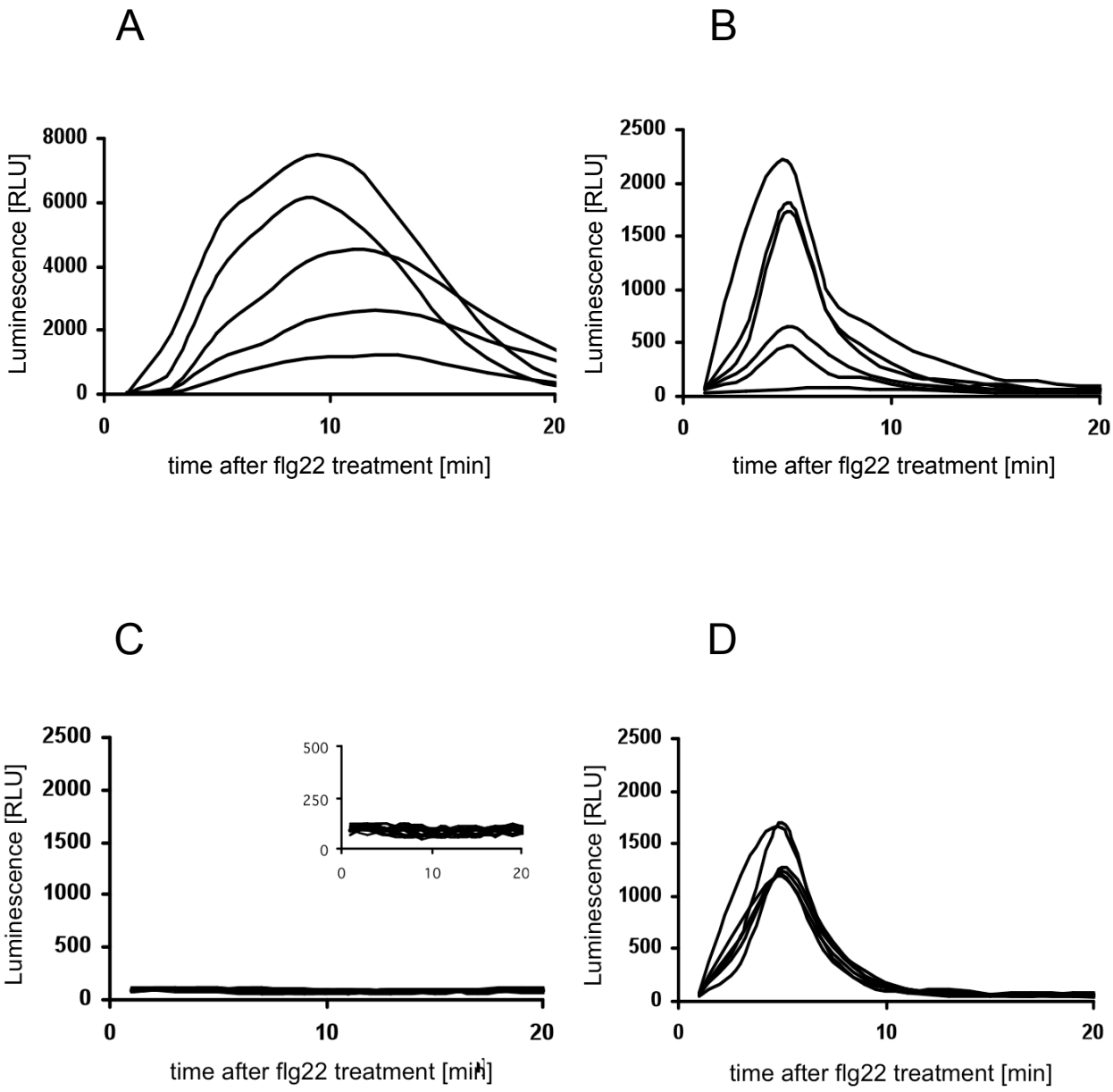
Suppl. Fig. 6. Imaging of stomata. A) Measurement of stomatal aperture. Microscopic images of stomata are taken with focal planes at inner sites of guard cells. The width and length of stomata is determent within the inner area of guard cells (white bars), and its ratio is calculated. Depicted are representative images of mock and flg22 treated Col-0 and *fls2* mutant leaves showing open (top left and bottom panels) and closed (top right panel) stomata; Scale bar = 10 µm. B) Micrographs show optical cross sections of membrane-resident FLS2-GFP in stomata of leaf epidermal tissue. Scale bar = 20 µM.



Suppl. Fig. 7. Flg22-stimulated responses in lines with altered ROS production. A) ROS production was monitored in seedlings lacking or overexpressing the indicated NADPH oxidases over time. Depicted are average values (n=12); bars represent +/-SD. Letters indicate significant differences $p < 0.05$. Similar results were obtained in multiple independent experiments. RLU, relative light units. B) Seedling growth of the indicated genotypes was measured in the absence or presence of 100 nM flg22. Bars represent +/-SD. Letters indicate significant differences $p < 0.05$. C) Flg22-induced MAP kinase activation was determined at the indicated time points and seedling genotypes.



Suppl. Fig. 8. FLS2 accumulation in lines with altered ROS production. A) FLS2 immunoblot analysis. Coomassie staining is shown for equal loading. B) *AtRbohD* transcript accumulation was analyzed by RT-PCR in the indicated genotypes. *Actin* is shown as a control. Results were obtained in three independent experiments.



Suppl. Fig. 9. Flg22-stimulated oxidative burst. ROS production was provoked with 100 nM flg22 and monitored in individual leaf discs (A), individual seedlings (B), and seedlings with pre-incubation of 10 nM flg22 (C) over time. Pre-incubation with 10 nM flg22 did not elicit an oxidative burst in seedlings (D) but reduced variation between individual seedlings (C). Similar results were obtained in multiple independent experiments. RLU, relative light units.