



Supplemental Figure 1. Effect of BTH, acd6-1, Water Treatment on PAMP (co-) Receptor Levels. (A) to (E) Chemical treatment schemes for the indicated panels: (A) for (F); (B) for (G); (C) for (H to I); (D) for (J); (E) for (K). In (A) and (C), "on water" indicates that tissue was excised and floated on water to facilitate flg22 uptake. (F) to (G) ROS accumulation after 1 µM flg22 treatment in the indicated plants (*n*>8). Graph; total ROS accumulation after 1 μ M flg22 treatment. (H) to (I) FLS2 and BAK1 protein levels after BTH treatment of wild type (Col). Leaves were collected 48 h (H) or 24 h (I) after spray treatment with 100 µM BTH or mock-treatment. Total and microsomal fraction (MF) proteins isolated from plants were analyzed by immunoblotting with FLS2 and BAK1 antibodies. Full coomassie-stained membrane are shown in (H) as examples of equal loading of the Figure 2. See graphs in Figure 2C for quantification. (J) High levels of FLS2 and BAK1 in microsomal fraction of acd6-1 (a6-1) with or without water treatment. Total (top) or microsomal (bottom) proteins isolated from wild-type (Col) and acd6-1 plants with or without water treatment were analyzed by immunoblotting with FLS2 and BAK1 antibodies. N: no water treatment, W; leaves floated on water for 4 h. a-FLS2 (Short); short exposure, a-FLS2 (Long); long exposure. Numbers below Western blots show the average fold change in receptor levels (normalized to total protein (1), rubisco only (2), or all proteins except rubisco (3)) of the indicated plants relative to no water-treated Col guantified from three independent experiments. Graphs show means of the water-treated samples guantified from three independent experiments. Bars indicate standard error. * P<0.05, indicates the acd6-1 values were different from wild-type (Col) values. (K) FLS2 accumulation by water treatment. FLS2 and BAK1 protein levels 4 h after water or BTH treatment of Col. Leaves were collected 4 h after spray (no wounding) or infiltration treatment with 100 μ M BTH or water-treatment. Total proteins were extracted and analyzed as in (H). In (H) to (K), C: coomassie blue stained.



Supplemental Figure 2. Verification That *acd6-1* Has Elevated MPK3 Levels. Analysis of MPK and active MPK levels in *acd6-1* (a6) and *mpk3* double mutants. Total proteins were isolated from leaves of different plants and subjected to SDS-PAGE and immuno-detected by MPK3, MPK6 and phospho-p44/42 MPK antibodies, respectively. C: coomassie blue stained. This experiments was repeated three with similar results.



Supplemental Figure 3. FLS2-BAK1 Complexes Induced by flg22 Are Less Stable in *acd6-1*. Top panel: Chemical treatment scheme. Bottom: total protein levels from extracts of leaves of the indicated plants; top: BAK1-containing complexes immunoprecipitated with BAK1 antibody, separated by SDS-PAGE and subjected to Western blot analysis with FLS2 antibody. Leaves from Col plants were collected 10 min after treatment with 1 μ M flg22. Note the lack of FLS2 in immunoprecipitated BAK1 complexes of *acd6-1* without flg22 treatment and reduced complex formation after flg22 relative to that seen in Col. Input is 2% of the extract used for the IP. This experiments was repeated three with similar results.



Supplemental Figure 4. MPK Activity and ROS Accumulation Are Not Induced by BTH. (A) MPK activity analysis using wild-type (Col) plants 10 min after water, 1 μ M flg22 or 100 μ M BTH infiltration immuno-detected by phospho-p44/42 MPK antibodies with total protein extracts. C: coomassie blue stained. (B) Total ROS accumulation after water, 1 μ M flg22 or 100 μ M BTH treatment (as in Figure. 1 (A) and (E)) in wild-type (Col) plant. * P<0.05, indicates the sample value was different from mock treatment. Bars show standard deviation from a representative experiment (n=12). These experiments were repeated three times with similar results.



Supplemental Figure 5. BTH Has No Effect on ROS Measurements. **(A)** Total ROS (measured during 30 min.) from 0.3% hydrogen peroxide added to water pretreated with BTH for 4 h (n>10). **(B)** Total ROS accumulation in wild type (Col) after mixed solution treatment that included 1 μ M flg22 and water or 100 μ M BTH (n>10) (as in **Figure 1 (A)** and **(E)**). Bars show standard deviations of data from a representative experiment. These experiments were repeated three times with similar results.



Supplemental Figure 6. Confirmation that plants that express ACD6-HA show normal regulation of CERK1 after BTH treatment. Leaves were collected 4 or 24 h after spray treatment of the same set of plants with 100 μ M BTH or mock-treatment. Microsomal fraction proteins isolated from ACD6-HA transgenic plants were analyzed by immunoblotting with CERK1 antibodies. C, coomassie-stained membrane. Blots are from one continuous membrane.