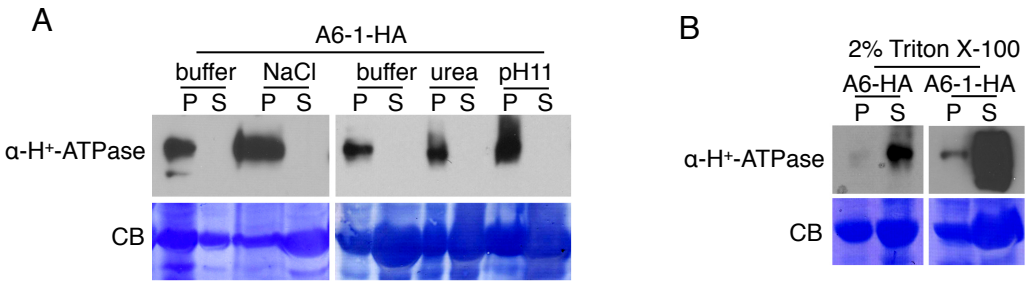
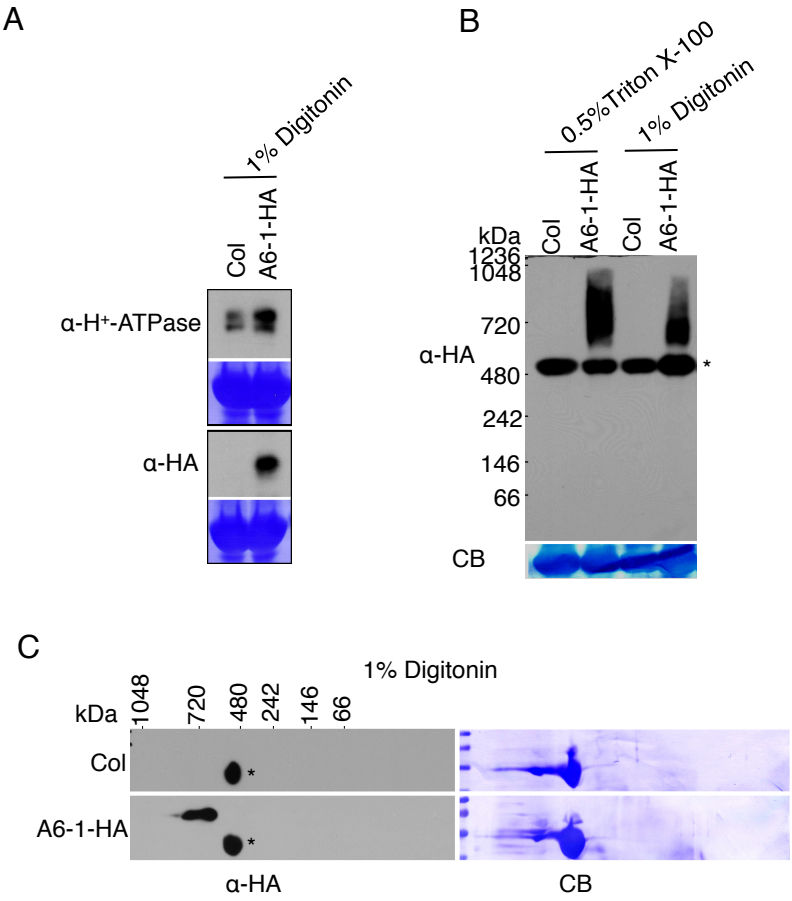
Supplemental Figure 1.



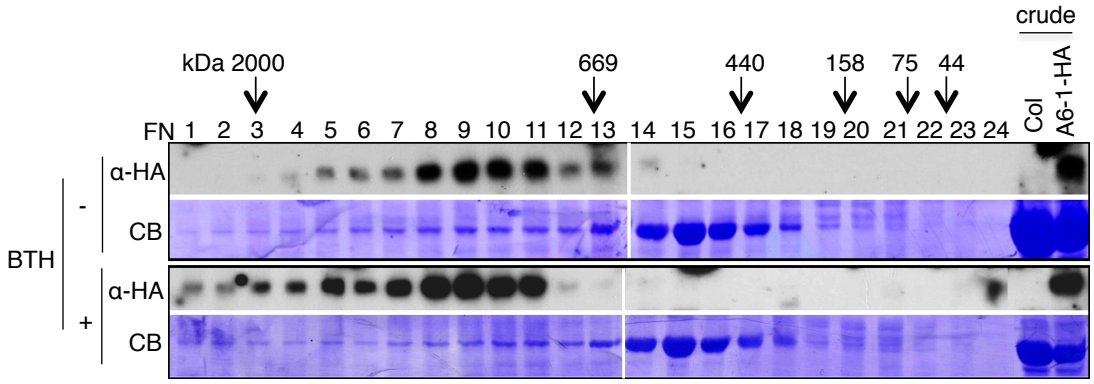
Supplemental Figure 1. Figure S1. ACD6-1 and ACD6 behave like the integral membrane protein H^+-ATPase. Samples from Figure 1 (A) and (B) were separated by SDS-PAGE and analyzed by immunoblotting with ATPase antibody to show that ACD6 behaves like another integral membrane protein, which can only be solubilized by detergent.

Supplemental Figure 2.



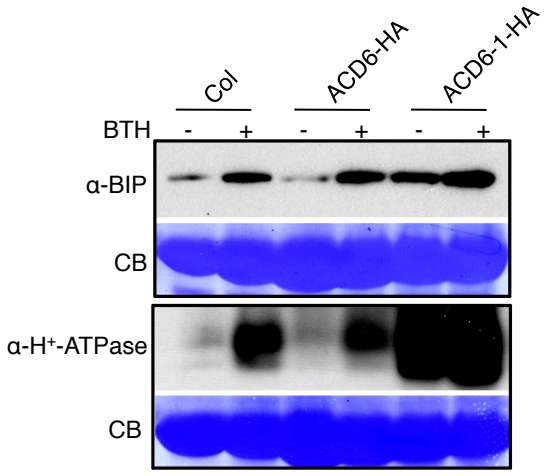
Supplemental Figure 2. Characterization of ACD6-1 Complex After Digitonin Solubilization. Microsomal proteins from leaves of ACD6-1-HA (A6-1-HA) plants were solubilized with 0.5% Triton X-100 or 1% digitonin. Solublized protein were separated using SDS-PAGE **(A)**, the first dimension of BN-PAGE **(B)**, or SDS-PAGE following BN-PAGE **(C)**, and analyzed by immunoblotting with ATPase- and HA-antibody, respectively. CB: coomassie-blue stained membrane. * Non-specific band

Supplemental Figure 3.



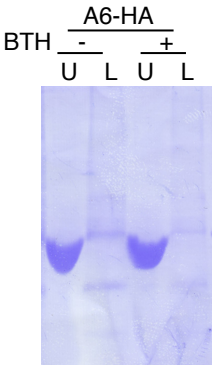
Supplemental Figure 3. Characterization of ACD6-1-HA Complexes by Fast Protein Liquid Chromatography Gel Filtration (FPLC). Microsomal proteins were isolated from untreated (top blot) and BTH-treated (bottom blot) plants that express ACD6-1-HA (A6-1-HA). Triton X-100-solubilized microsomal proteins were separated by FPLC and an equal volume of each fraction was subjected to SDS-PAGE, and immunoblotted with HA antibody. Numbers on the top show the size of markers. Note the increase in the molecular weight of complexes in the extract from BTH-treated plants. CB: coomassie blue stained membrane. White lines separate two membranes. All samples were processed, run on two gels blotted to two membranes, probed and developed at the same time.

Supplemental Figure 4.



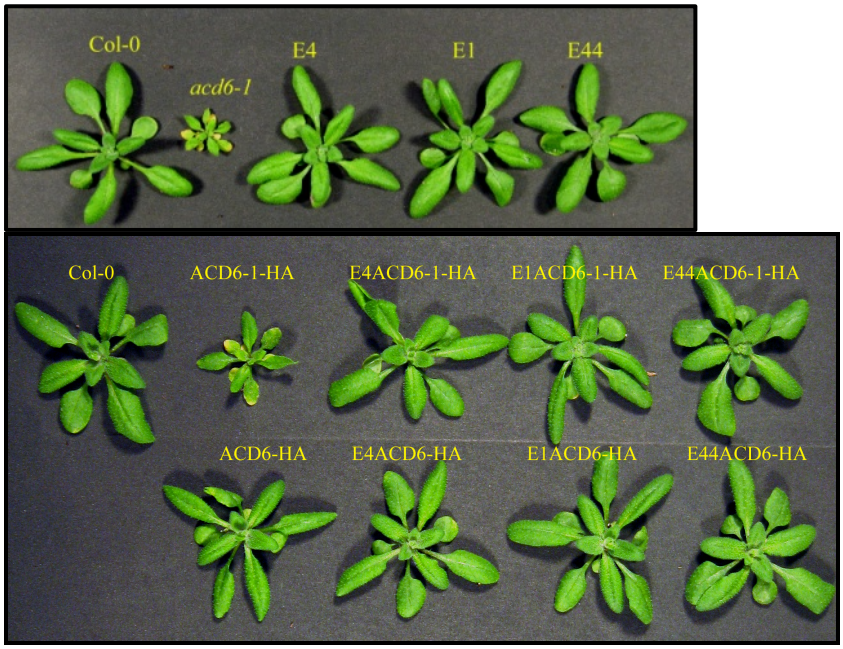
Supplemental Figure 4. Levels of Marker Proteins ATPase and BIP Increase in the Membrane After BTH Treatment. Microsomal membrane protein extracts from the indicated plants treated with BTH (+) or mock treated (-) as in Figure 3A were subjected to SDS-PAGE and immunoblotting with indicated antibodies. CB: coomassie-blue stained membrane. These experiments were repeated three times with similar results.

Supplemental Figure 5.



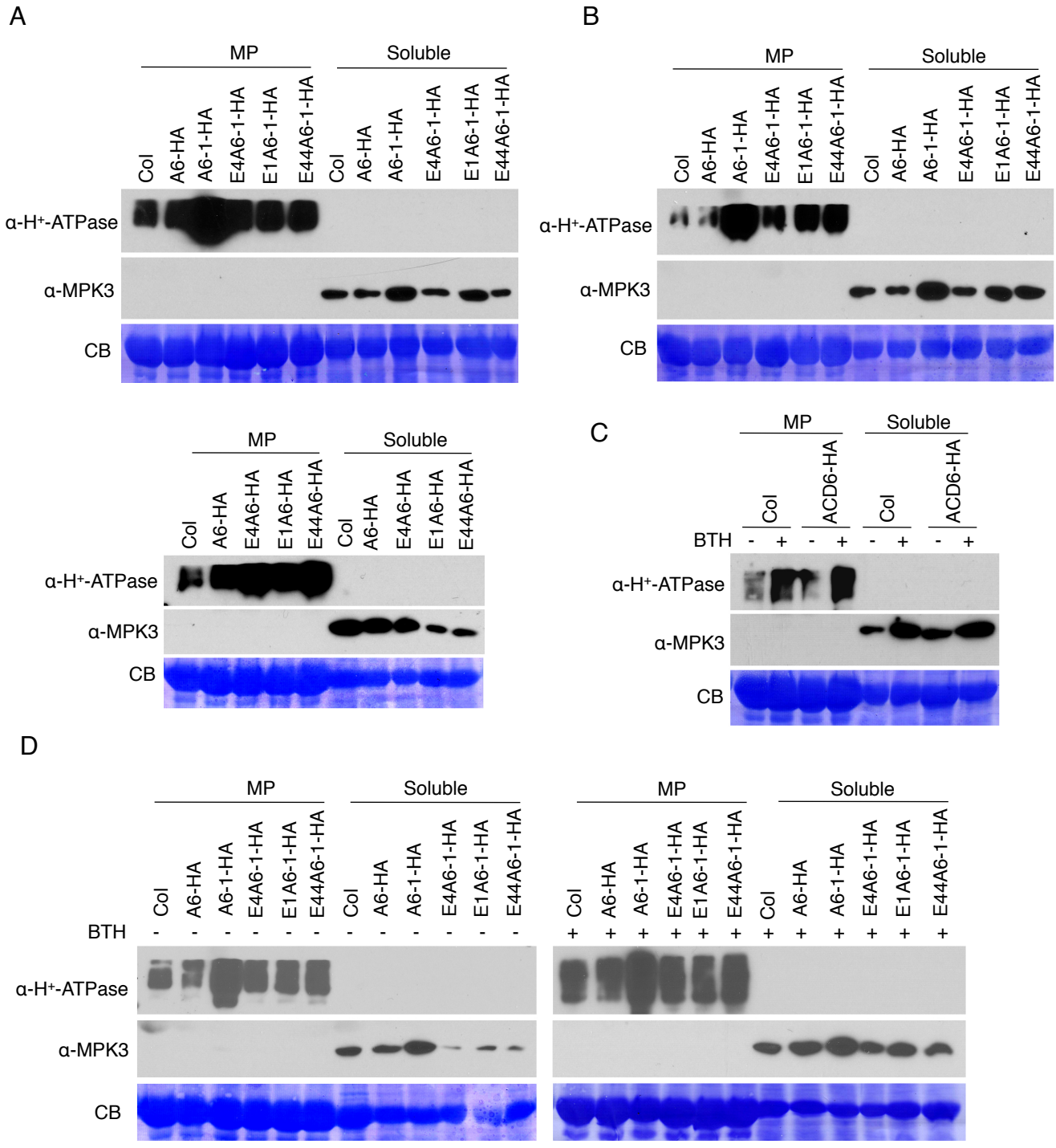
Supplemental Figure 5. Coomassie-blue stained membrane for Figure 3 (C) showing that BTH treatment does not cause changes in the overall protein levels in the respective fractions.

Supplemental Figure 6.



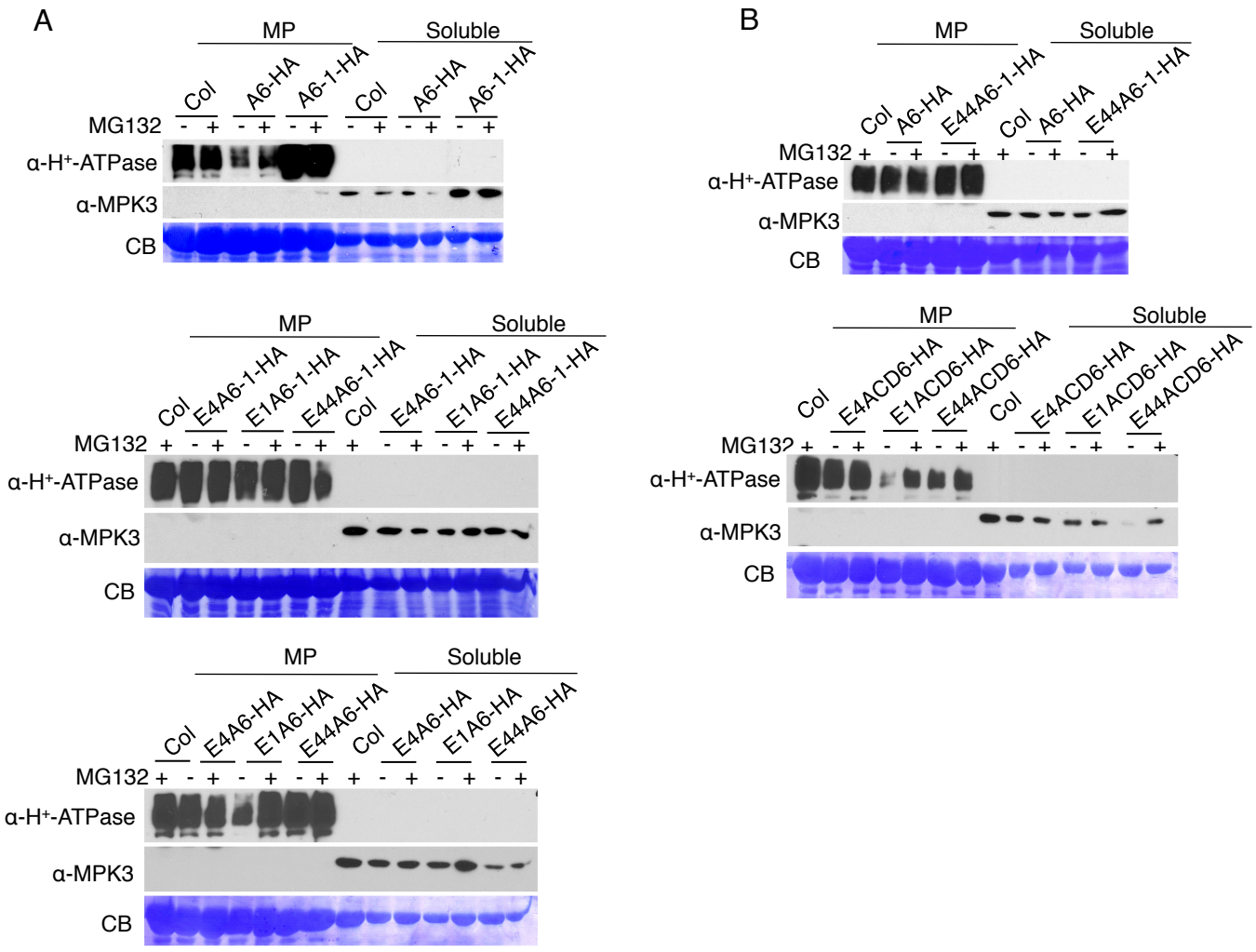
Supplemental Figure 6. Phenotypes of Transgenic Plants that Produce ACD6-1-HA or ACD6-HA Variants that Contain Ankyrin Domain Amino Acid Substitutions. All transgenic plants expressing ACD6-1-HA or ACD6-HA with one of the amino acid substitutions in the ankyrin domain (see legend to Figure 4) appeared morphologically similar to wild type (Col-0). Original intragenic ankyrin mutants, *acd6-1* (top panel) and transgenic plants that produce ACD6-1-HA or ACD6-HA are shown for comparison. Plants were 21 days old when photographed.

Supplemental Figure 7.



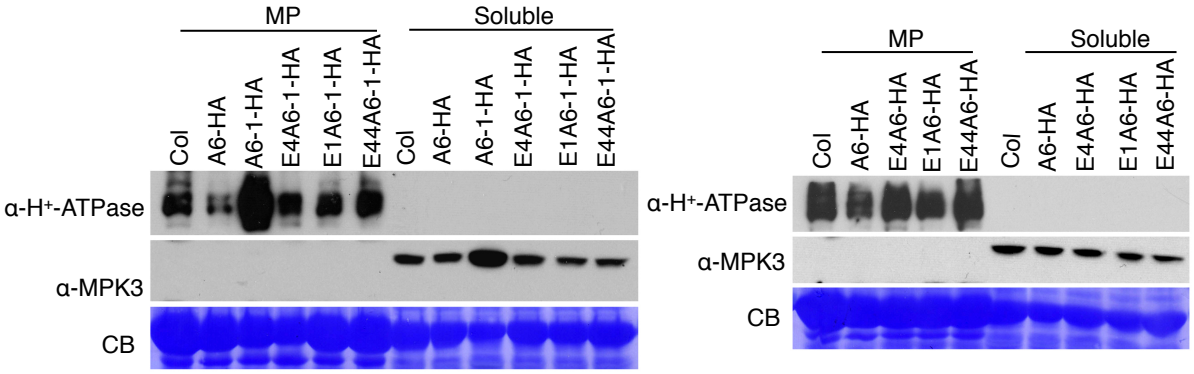
Supplemental Figure 7. Fractionation Controls for Samples from Figure 5 (A) to (D). Samples were separated by SDS-PAGE and analyzed by immunoblotting with ATPase and MPK3 antibodies, as membrane and cytosolic markers, respectively.

Supplemental Figure 8.



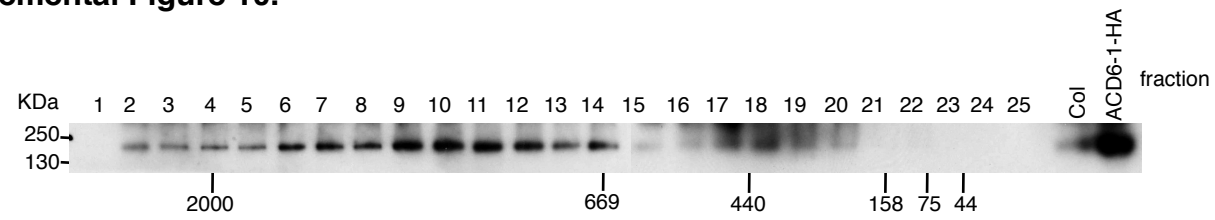
Supplemental Figure 8. Fractionation Controls for Samples from Figure 6 (A) and (B). Samples were separated by SDS-PAGE and analyzed by immunoblotting with ATPase and MPK3 antibodies, as membrane and cytosolic markers, respectively.

Supplemental Figure 9.



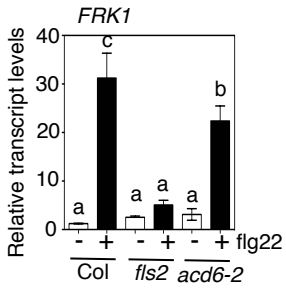
Supplemental Figure 9. Fractionation controls for samples from Figure 7 **(D)** Samples were separated by SDS-PAGE and analyzed by immunoblotting with ATPase- and MPK3- antibody, respectively.

Supplemental Figure 10.



Supplemental Figure 10. FLS2 Complexes In Plants that Express ACD6-1-HA Characterized Using FPLC. Solubilized microsomal proteins were separated by FPLC. Fractions were loaded on SDS-PAGE, and analyzed by immunoblotting with FLS2 antibodies. Results are similar to those obtained using 2D BN-gels, see Figure 8B. Additionally, note the overlap in the size of complexes with ACD6-1-HA (Supplemental Figure 3 online).

Supplemental Figure 11.



Supplemental Figure 11. Induction of the flg22-responsive gene FRK1 is partially ACD6-dependent.
Transcript levels of the flg22-responsive gene *FRK1* 1h after water or 1μM flg22 infiltration of WT (Col), *fls2* and *acd6-2* leaves determined by qRT-PCR. Each experiment to determine transcript level used leaves from four different plants per genotype and treatment. The graph shows the combined data of two biological repeats performed at different times. – or + indicates water (-) or flg22 (+). Letters above bars represent significance groups as determined by the Newman-Keuls test, $P<0.05$ or better. Bars indicate standard error. The *acd6-2* mutant also showed reduced FRK1 expression relative to wild type after flg22 treatment in two additional replicates using plants grown on sterile media.

Supplemental Table 1. Peptides recovered by LC-MS/MS from ACD6-1-HA Immunoprecipitates

Name	Peptide sequence	Sequest ΔCn score	# times peptide was recovered	Coverage
FLS2	Sample-1: RTRVILIILGSAAALLLVLLLVLILTCCK,	1	1	Sample-1: 31/1173, 3%
	VILIILGSAAALLLVLLLVLILTCCKKK	0.711	1	
	Sample-2: TRVILIILGSAAALLLVLLLVLILTCCK	1	1	Sample-2: 28/1173, 2%
	Sample-3: VILIILGSAAALLLVLLLVLILTCCKKK	1	1	Sample-3: 28/1173, 2%