

Supplemental Figure 1. Protein alignment of ABD1 from other model organisms.

The alignment was performed with *H. sapiens* DCAF8, *M. musculus* DCAF8 and *O. sativa* Os10g0544500. The WD40 domains are underlined. The red triangles indicate the WDxR motif within the WD40 domain and "x" stands for an undefined amino acid. The shading mode indicates the level of conservation, with red letters in black shading corresponding to a high level of conservation (100%), blue letters in dark gray shading corresponding to a moderate level of conservation (80%) and green letters in light gray shading corresponding to a low level of conservation (60%).





7-day-old Col-0 wt, *abd1-1* and *abd1-2* seeds were grown in the absence or presence of 0.5 μ M ABA or 100 mM NaCI. mRNA levels were determined by quantitative real-time PCR analysis. Relative amounts of transcripts were normalized to the levels of *ACTIN2* within the same sample. (A) *RD22* after ABA treatment. (B) *RD22* after NaCI treatment. (C) *RD20* after ABA treatment. (D) *RD20* after NaCI treatment. (E) *ABI3* after ABA treatment. Results are from three biological replicates and values represent mean \pm SD (n=9). N.S., not significant.



Supplemental Figure 3. ABD1 does not directly interact with DWA proteins, ABI5 homologs or ABI3 by yeast two hybrid assays.

Assays were performed with ABD1 protein as prey and various ABA responsive proteins as bait to monitor their interactions. Empty vector was used as a negative control. Yeast were grown in the presence of X-gal for 15 hours, after which images were taken. (A) Interaction between ABD1 and DWA1, DWA2, or DWA3. (B) Interaction between ABD1 and the five most closely related ABI5 homologs: ABF1, DPBF2, AREB1, AREB3 and AREB1. (C) Interaction between ABD1 and ABI3. (D) Immunoblots of the target fusion proteins in the yeast two hybrid assays in (B) and (C). The LexA fusions were detected by anti-LexA antibodies and the expression of these proteins are indicated by the arrows. Empty LexA vector was used as a negative control. Ponceau S staining was used as a loading control. A total of 15 μ g of protein was loaded in each lane.



Supplemental Figure 4. Quantification of ABI5 protein degradation after ABA removal.

Immunoblot assays shown in Figure 9 were quantified showing relative amounts of ABI5 protein in Col-0 wt and *abd1-1* seeds that were treated with 5 μ M ABA in white light for 3 days, and then harvested at the indicated times after the removal of ABA with either being washed out with **(A)** liquid media, **(B)** liquid media supplemented with the proteasome inhibitor MG132 (50 μ M) or **(C)** the protein synthesis inhibitor CHX (100 μ M). Relative amounts of ABI5 protein were normalized to the levels of RPN6 within the same sample. Values are means ± SD (n=3). Significant difference was determined by a Student's t-test; single or double stars indicate a P values of P<0.03 or P<0.004, respectively.

Supplemental Tables

Supplemental Table 1. List of primers used for genotyping and RT-PCR analysis.

| Name | T-DNA Line | Forward Primer (5'-3') | Reverse Primer (5'-3') | Genotyping ^a | RT-PCR |
|---------------------|-----------------------------------|--|------------------------|-------------------------|---------|
| ABD1 (At4g38480) | SALK_051074 (<i>abd1-1</i>) | GAAAAGGCCGAGGACCGACCA T | CTGTCATCAGAACCAGAAAG | FP1+RP1 (F/LBb1.3) | FP1+RP1 |
| ABD1 (At4g38480) | SAIL_648_G02 (<i>abd1-2</i>) | CTTTCTGGTTCTGATGACAGA | ATGTCTGTCTGCTTCCATGG | FP2+RP2 (F/LB3) | FP2+RP2 |
| | | GAAAAGGCCGAGGACCGACCA T | TATTATTGAAGTGGCCGGAA | | FP1+RP3 |
| LBb1.3 | | ATTTTGCCGATTTCGGAAC | | | |
| LB3 | | TAGCATCTGAATTTCATAACCA ATCTCGATACAC | | | |
| RPN6 | | AAGGCACGATAGATCTGCAGA | TCGAGGGTAGCCGAGTAGAT | | |

^a primer sets for confirmation of homo- or heterozygous lines

| Name | Forward Primer (5'-3') | Reverse Primer (5'-3') |
|------------|--------------------------------|--------------------------------|
| ABD1 | TGGGTCTCTTACCCAATCGCAGTT | CAGAAGCAGTCTGCCAATCCCAA A |
| RD29A | GGAGGAGAAGAAGAAGAAGAA | TAAAGCTCCTTCTGCACCGG |
| RD29B | ACGGAAACATCGGACTGG | TCCGTTGACCACCGAGAT |
| RD22 | TTCGGAAGAAGCGGAGAT | CAGTGGAAACAGCCCTGA |
| RD20 | CGTTTCAAACAAAGTTGAATGGAT AC | TTAGTGCTTGTTTGCGAGAATTGG CC |
| ABI5 | AACATGCATTGGCGGAGT | TTGTGCCCTTGACTTCAAACT |
| ABI3 | TCTTGAATGGGTCCAAAC | AGGGTTTTGAAATGGATC |
| ACTIN 2 | CAAGGCCGAGTATGATGAGG | GAAACGCAGACGTAAGTAAAAAC |

Supplemental Table 2. List of primers used in quantitative real-time PCR analysis.

NameForward Primer (5'-3')Reverse Primer (5'-3')ABD1_KpnI_GGGGTACCATGAGTGGAAGGCCGGGGTACCTCATCCCTCATCcLUCGGCGCCCATCCCCAB15_KpnI_nGGGGTACCATGGTAACTAGAGAAGGGGTACCGAGTGGACAACTLUCACGAAGTTGCGGGTTC

Supplemental Table 3. List of primers used for LCI analysis.

| Name | Sequence (5'-3') |
|------------------------------|--|
| ABD1_EcoRI_Forward | GGGAATTCTCATCCCTCATCCCCATC |
| ABD1_EcoRI_Reverse | CGGAATTCATGAGGGAAGGCCGGC |
| <i>AREB1_Eco</i> RI_Forwar d | CCGGAATTCATGGATGGTAGTATGAATTTGGGG |
| AREB1_NotI_Reverse | AAGGAAAAAAGCGGCCGCTCACCAAGGTCCCGACTC |
| <i>AREB2_Eco</i> RI_Forwar d | CCGGAATTCATGGGAACTCACATCAATTTCAAC |
| AREB2_NotI_Reverse | AAGGAAAAAAGCGGCCGCTCACCATGGTCCGGTTAATGT CC |
| DWA1_EcoRI_Forward | CGGAATTCATGTACGGAGACGCTACAAAC |
| DWA1_EcoRI_Reverse | CGGAATTCTTACAATGAACTGCTACGAA |
| DWA2_XhoI_Forward | CCGCTCGAGATGCAAGGAGGATCATCGGG |
| DWA2_XhoI_Reverse | CCGCTCGAGCTATCTTCTTGGCAGGAAAG |
| DWA3_EcoRI_Forward | CGGAATTCATGGCGAAGCGTGGTTATAA |
| DWA3_EcoRI_Reverse | CGGAATTCCTATGGTGTTTGAAGAACTA |
| ABI5_EcoRI_Forward | CGGAATTCGGATGGTAACTAGAGAAACGAAG |
| ABI5_EcoRI_Reverse | CGGAATTCTTAGAGTGGACAACTCGGGT |
| ABF1_BamHI_Forward | CGGGATCCCGATGGGTACTCACATTGATATCAAC |
| ABF1_NotI_Reverse | AAGGAAAAAAGCGGCCGCTCACCTTCTTACCACGGACC |
| <i>DPBF2_Bam</i> HI_Forwa rd | CGGGATCCCGATGTCGGTTTTCGAATCGGAGACTTCG |
| DPBF2_NotI_Reverse | AAGGAAAAAAGCGGCCGCTTACCACCCGGCACTGGCCA |
| <i>AREB3_Bam</i> HI_Forwa rd | CGGGATCCCGATGGATTCTCAGAGGGGTATTG |
| AREB3_NotI_Reverse | AAGGAAAAAAGCGGCCGCTCAGAAAGGAGCCGAGCTTG |
| ABI3_BamHI_Forward | CGGGATCCCGATGAAAAGCTTGCATGTGGCG |
| ABI3_NotI_Reverse | AAGGAAAAAAGCGGCCGCTCATTTAACAGTTTGAGAAGT TGG |

Supplemental Table 4. List of primers used for yeast two-hybrid analysis.

| Name | Sequence (5'-3') |
|---------------------------------------|-------------------------------------|
| ABD1_NotI_pSY728_Forward | TTGCGGCCGCAAATGAGTGGAAGGCCGGCGAAA |
| ABD1_NotI_pSY728_Reverse | TTGCGGCCGCTTTCCCTCATCCCCATCCCCAT |
| <i>ABI5_Bam</i> HI_ pSY735_Forward | CGGGATCCCGATGGTAACTAGAGAAACGAAGTTG |
| <i>ABI5_Bam</i> HI_ pSY735_Reverse | CGGGATCCTTAGAGTGGACAACTCGGGTTC |
| DDB1a-NotI_pSY738_Forward | TTGCGGCCGCAAATGAGCTCATGGAACTACGTTG |
| DDB1a-NotI_pSY738_Reverse | TTGCGGCCGCTTGTGAAGCCTAGTGAGTTCTTCAA |

Supplemental Table 5. List of primers used for BiFC assay.