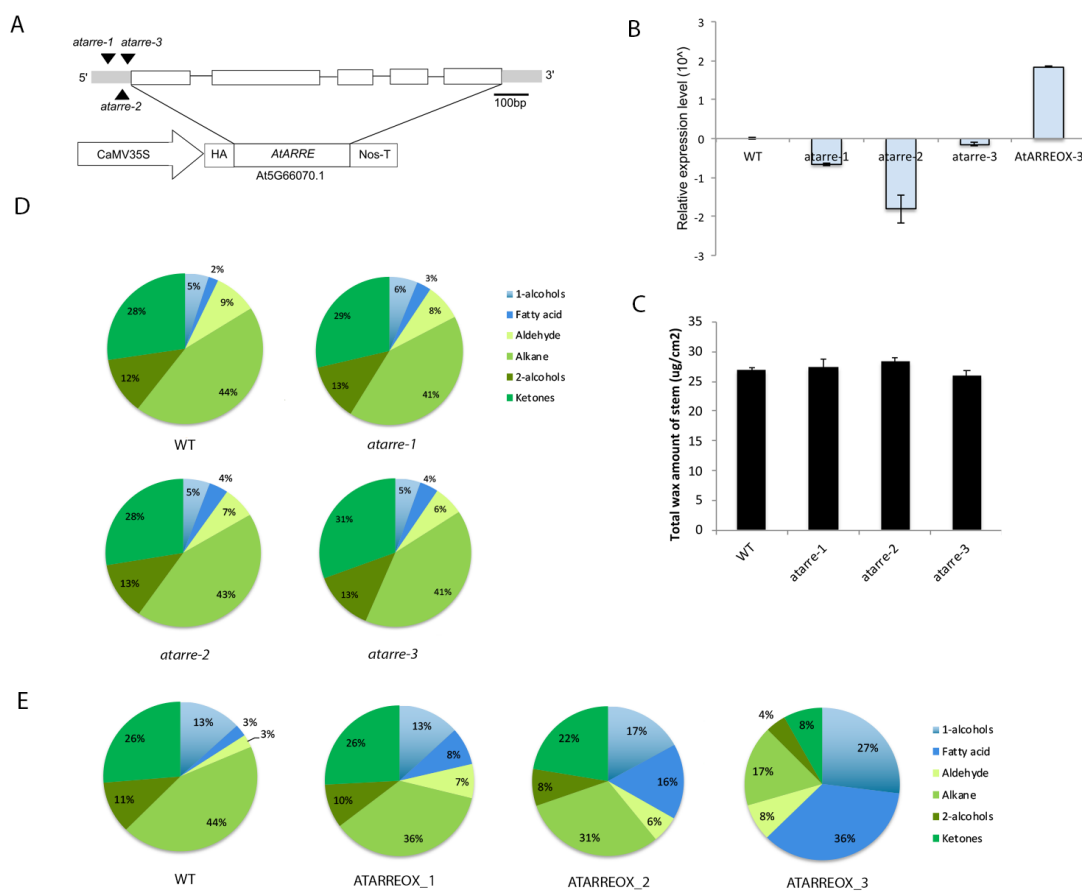


## Supplementary Material



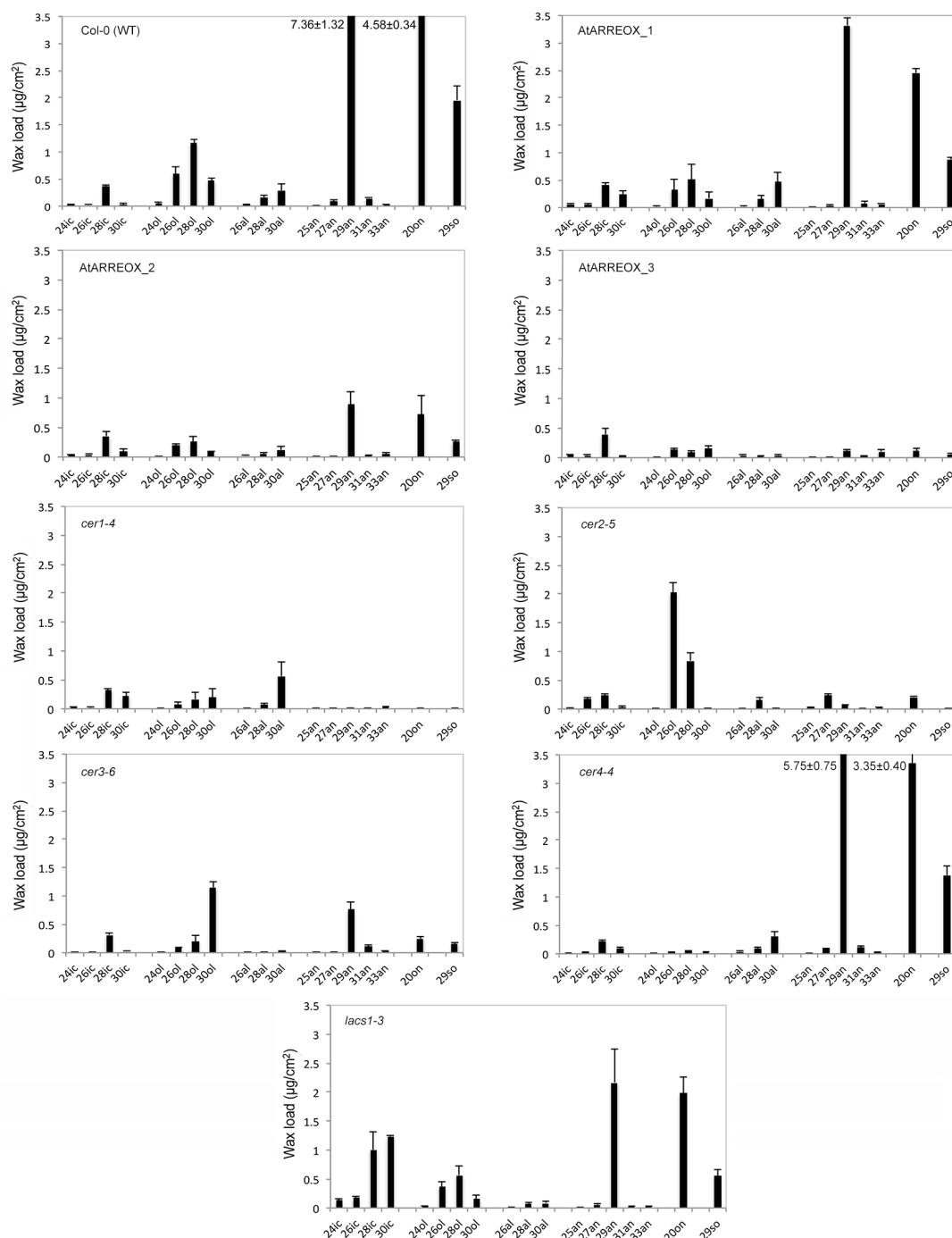
**Supplementary Figure 1.** Analysis of AtARREOX lines and *atarre* mutants.

**(A)** Schematic diagram of T-DNA insertion locations in *atarre* mutants and the original construct for generating AtARREOX lines. **(B)** *AtARRE* gene expression level in stems of the WT, *atarre* mutants, and a representative AtARREOX line, determined by qPCR. *ACTIN* was used as an internal control. The y-axis is shown in the logarithmic scale. Student's *t*-test was applied to identify statistically significant differences ( $p < 0.05$ ). Error bars represent means  $\pm$  SD (n=3). **(C)** Stem wax loads of *atarre* T-DNA mutants compared to the WT. **(D,E)** Stem wax composition of *atarre* mutant alleles (D) and AtARREOX lines (E) compared to the WT. Alkane pathway-derived compounds were labelled in shades of green color. Primary alcohol and fatty acids are labelled in shades of blue color.



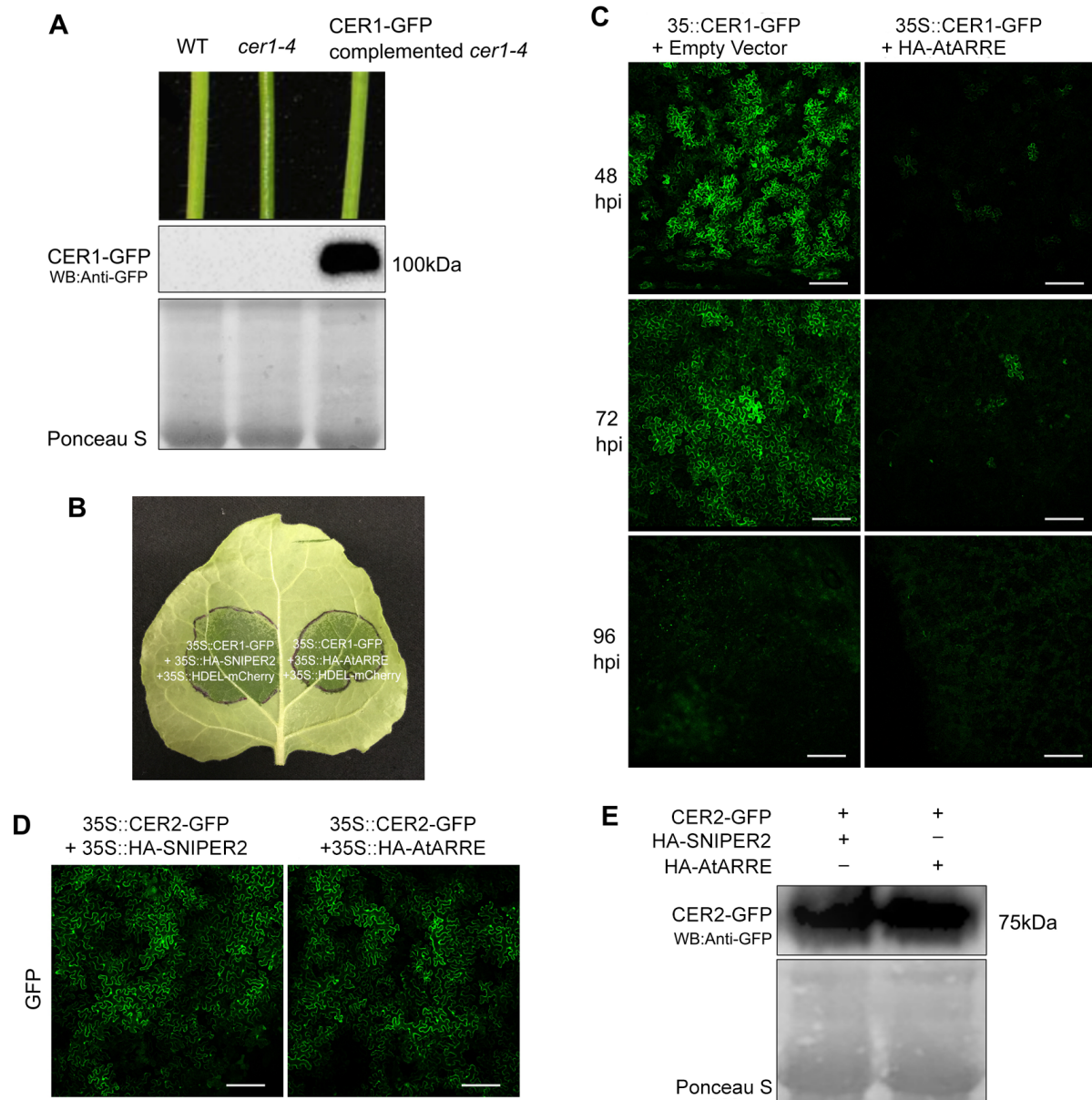
**Supplementary Figure 2.** Fertility of AtARREOX lines can be restored by high humidity.

Dry stems of WT Col-0 (left), AtARREOX\_2 (middle), and AtARREOX\_3 (right). WT was grown under normal humidity conditions; AtARREOX lines were grown under normal (below dashed line) and high-humidity conditions (above dashed line). High-humidity conditions were created by covering the plant with a plastic bag. AtARREOX\_3 line showed a severe organ fusion phenotype and fertility could not be fully recovered by growth in high humidity.



**Supplementary Figure 3.** Stem wax load and composition of WT Col-0, AtARREOX lines, *cer1-4*, *cer2-5*, *cer3-6*, *cer4-4*, and *lacs1-3* mutants.

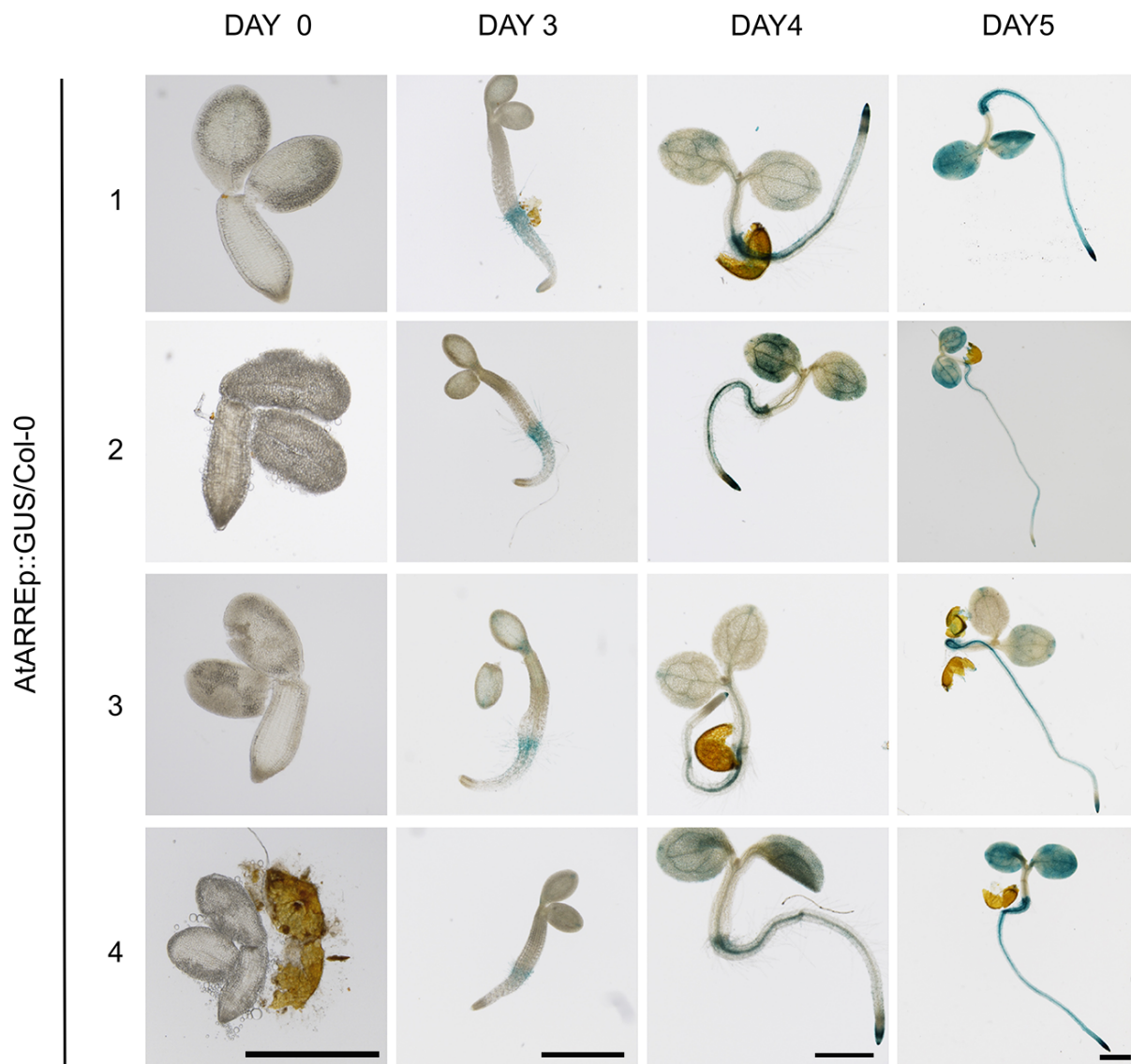
Total stem wax load of 6-week-old plants were determined by GC-FID. Values are means of four biological replicates and error bars represent SD.



**Supplementary Figure 4.** AtARRE specifically targets CER1 for degradation in *N. benthamiana* leaves.

(A) Stems of the WT, *cer1-4*, and *cer1-4* complemented with *CER6pro:CER1-GFP* transgene (top). CER1-GFP protein level examined by immunoblotting using anti-GFP antibody (middle). Ponceau S staining indicates equal protein loading (bottom). (B) An infiltrated *N. benthamiana* leaf showing the combination of constructs used for co-infiltration. (C) Leaf samples were collected at the indicated time after infiltration and the GFP fluorescence for each sample was examined by confocal microscopy. hpi, hours post infiltration. *35Spro:CER1-GFP* was co-expressed with a *35Spro:HA* empty vector (left panels) or *35Spro:HA-AtARRE* (right panels) (D) *35Spro:CER2-GFP* was co-expressed with *35Spro:HA-SNIPER2* (left) or *35S:HA-AtARRE* (right) and GFP fluorescence was examined by confocal microscopy. Scale bars = 100µm. (E) CER2-GFP protein levels from the protein extracts derived from D were determined by immunoblotting using anti-GFP antibody. Ponceau S staining indicates equal protein loading.





**Supplementary Figure 5.** Cell-type specific expression of the *AtARRE* gene determined by GUS activity assays in transgenic lines expressing *AtARREpro:GUS* construct.

Tissue samples from ten independent transgenic lines were examined for GUS activity, with four of these lines analyzed in detail. Seedlings of four *AtARREpro:GUS* transgenic lines were harvested on the day indicated and stained in GUS staining solution. Columns indicate days after imbibition, with day 0 representing embryos from dry seed. Scale bar = 1mm.

**Supplementary Table 1.** Primers used in this study.

Primer	Sequences (5'-3')
<b>Primers used for genotyping T-DNA lines of mutants</b>	
atarre-1 LP	CTGACTCTGGACGGCACTTAG
atarre-1 RP	GTACAGGGGAAAGGTGAGGAGG
atarre-2 LP	AAGGCATCCAATTCCAGACTC
atarre-2 RP	GTACAGGGGAAAGGTGAGGAGG
atarre-3 LP	TGAGAAGTTGGGTGGTGTTC
atarre-3 RP	CAAACCTGAAGGCAGACAGAG
<b>Primers used for genotyping transgenic lines of AtARREOX</b>	
TransAtARREOX_F	TGTTCCAGACTACGCTGTCG
TransAtARREOX_R	TCCTCTGATGAAACCGCTCT
<b>Primers used for RT-PCR &amp; qPCR</b>	
ACT1-NF	CTCAGTACCTTCCAGCAGATGT
ACT1-NR	AAAAACCCGGCTTGAGAAAT
ATARRE_RT-PCR_F	TCTTGCTCTGTCTGCCTTCA
ATARRE_RT-PCR_R	GCACAAGGGACAAGAAGCAT
CER1F-RT	AGTAGATTAGCAGCAGCTGTTG
CER1R-RT	GCTCTTCTCTTGTTGTTTCCTT
CER3-qPCR-F	CTCATCTCCTGTTCCACATCC
CER3-qPCR-R	TCAATGGAACACCAGCTACG
<b>Primers used for site-directed mutagenesis of ATARREOX lines</b>	
H197200Y_F	TATATGTTCTACCTACCATGCATCGAC
H197200Y_R	GGTAGGTAGAACATATAATGGCAGTGCGG
ATARREmutate_Seq	AGGCTTGTTTCGTGAGCGTAT
<b>Primer used for CER3 transient expression in tobacco leaves</b>	
CER3cDNA_attbF	GGGGACAAGTTTGTACAAAAAAGCAGGCTCAATGGTT GCTTTTTTATCAGCTTGG
CER3cDNA_attbR_WSTOP	GGGGACCACTTTGTACAAGAAAGCTGGGTCTCAATTTG TGAGTGAAGAAACAGCA
CER3cDNA_attbR_NoSTOP	GGGGACCACTTTGTACAAGAAAGCTGGGTCATTTGTGA GTGAAGAAACAGCA
<b>Primers used for GUS assay</b>	
LP_attb1_AtARRE	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAGAGGGA TGATTGAGGTAAGTTTAAG
RP1_attb2_AtARRE	GGGGACCACTTTGTACAAGAAAGCTGGGTATCTGCAA AAGAGATGAAGAGGAGG
RP2_attb2_AtARRE	GGGGACCACTTTGTACAAGAAAGCTGGGTAAAGATGT CTTCTGCACAAGGGACAA
GUS_Rseq	CTGTGGAATTGATCAGCGTTGG

<b>Primers used for AtARRE and CER1 expression in bacteria</b>	
ATARRETMdel_F_EcoRI_28b	GACTGAATTCGAGGCTTGTTTCGTGAGC
ATARRETMdel_R_SalI	CTAGTCGACAAGATGTCTTCTGCACAAG
T7terminator_R_Seq	CTCAAGACCCGTTTAGAGGC
ATARRETMdel_BamHI	GAAGGATCCAGGCTTGTTTCGTGAG
ATARRETMdel_StuI	GTCAGGCCTAAGATGTCTTCTGCACAAG
CER1TMdel_EcoRI_F	GACTTGAATTCCTCTTTGTCGCTGAG