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Supplemental information

Lack of ethylene does not affect reproductive success and synergid cell death in *Arabidopsis*

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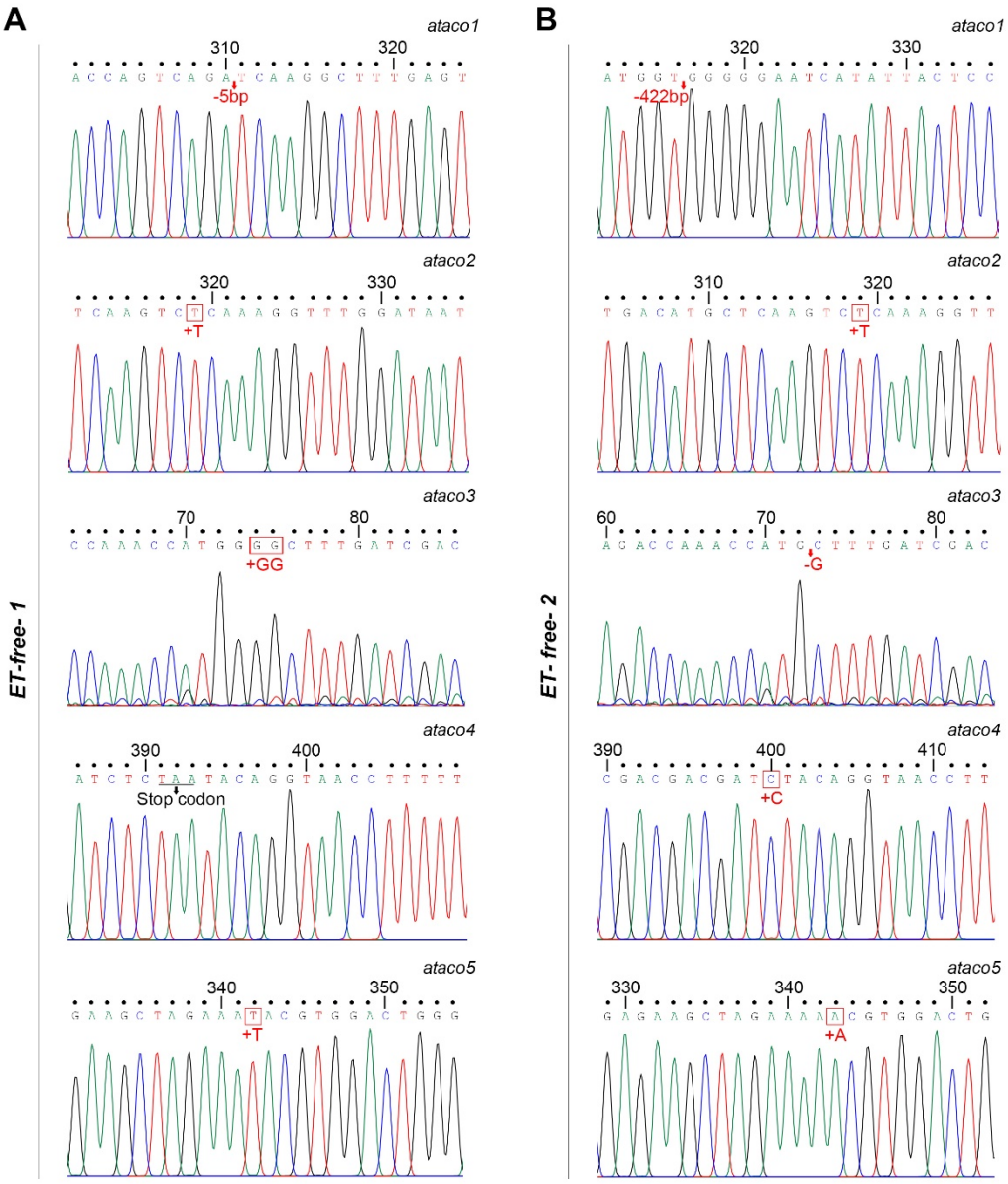
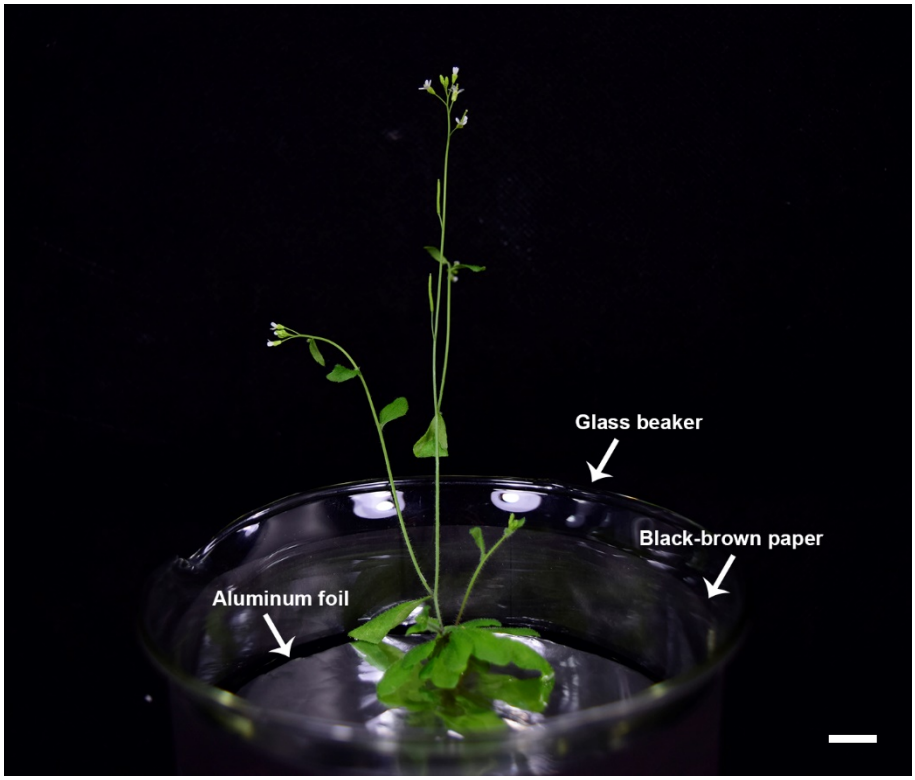
SUPPLEMENTAL FIGURES



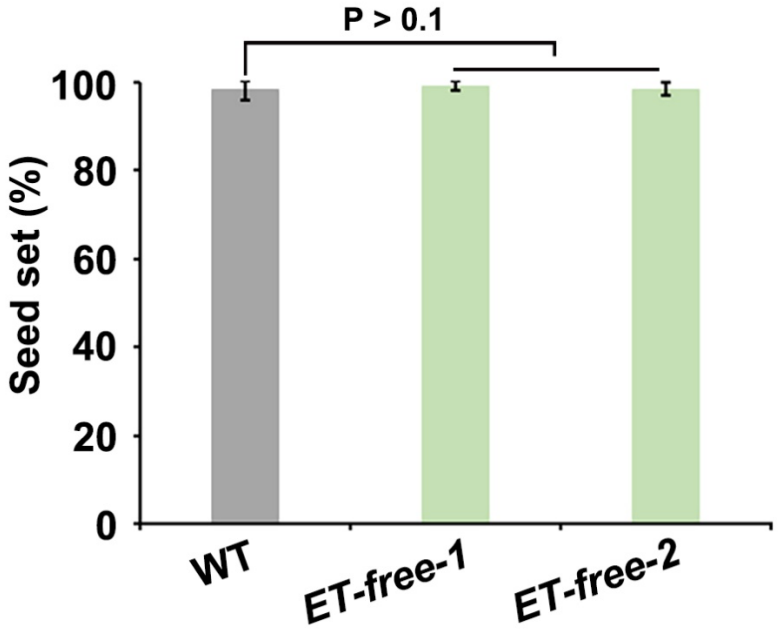
Supplemental Figure 1: CRISPR/Cas9-mediated mutation sites in Arabidopsis *ACOs* genes of the two *ET-free* mutants.

(A and B) Sequencing results of the mutated sites of *ACO* genes as indicated. Red boxes show inserted bases and arrows indicate deleted bases.



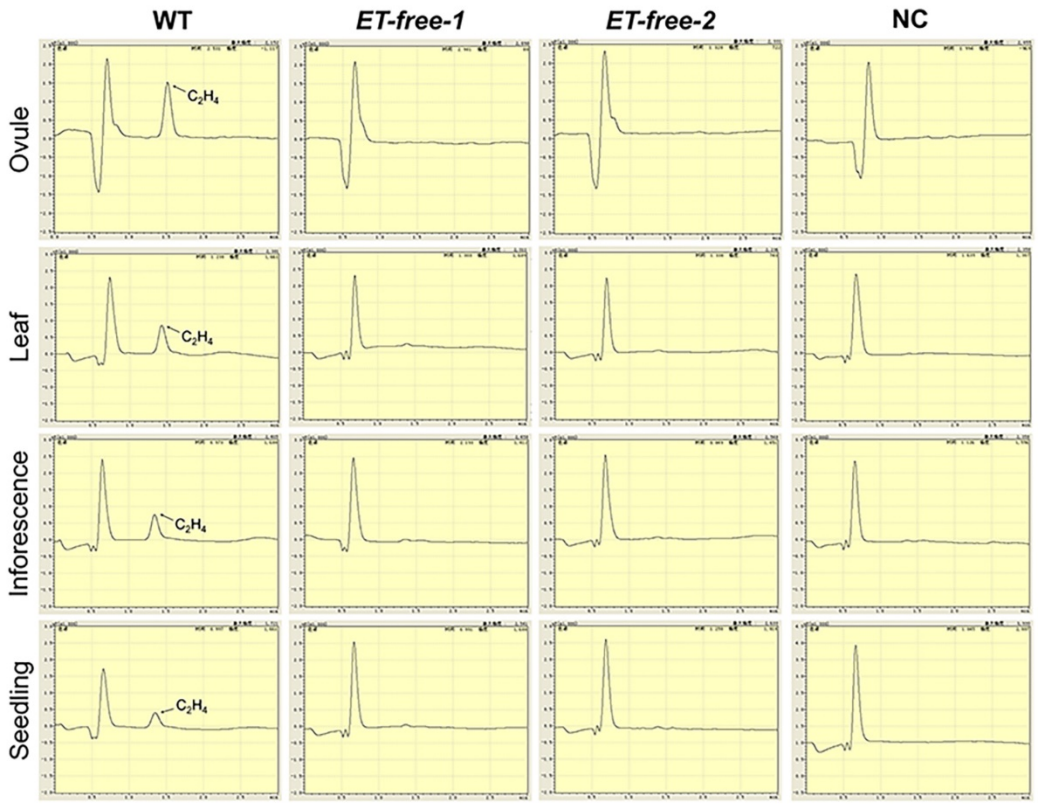
Supplemental Figure 2: Representative image of a hydroponic-grown Arabidopsis plant.

The plant was placed in liquid medium in a glass beaker that was covered by aluminum foil and was shaded by black-brown paper. Scale bar, 1 cm.



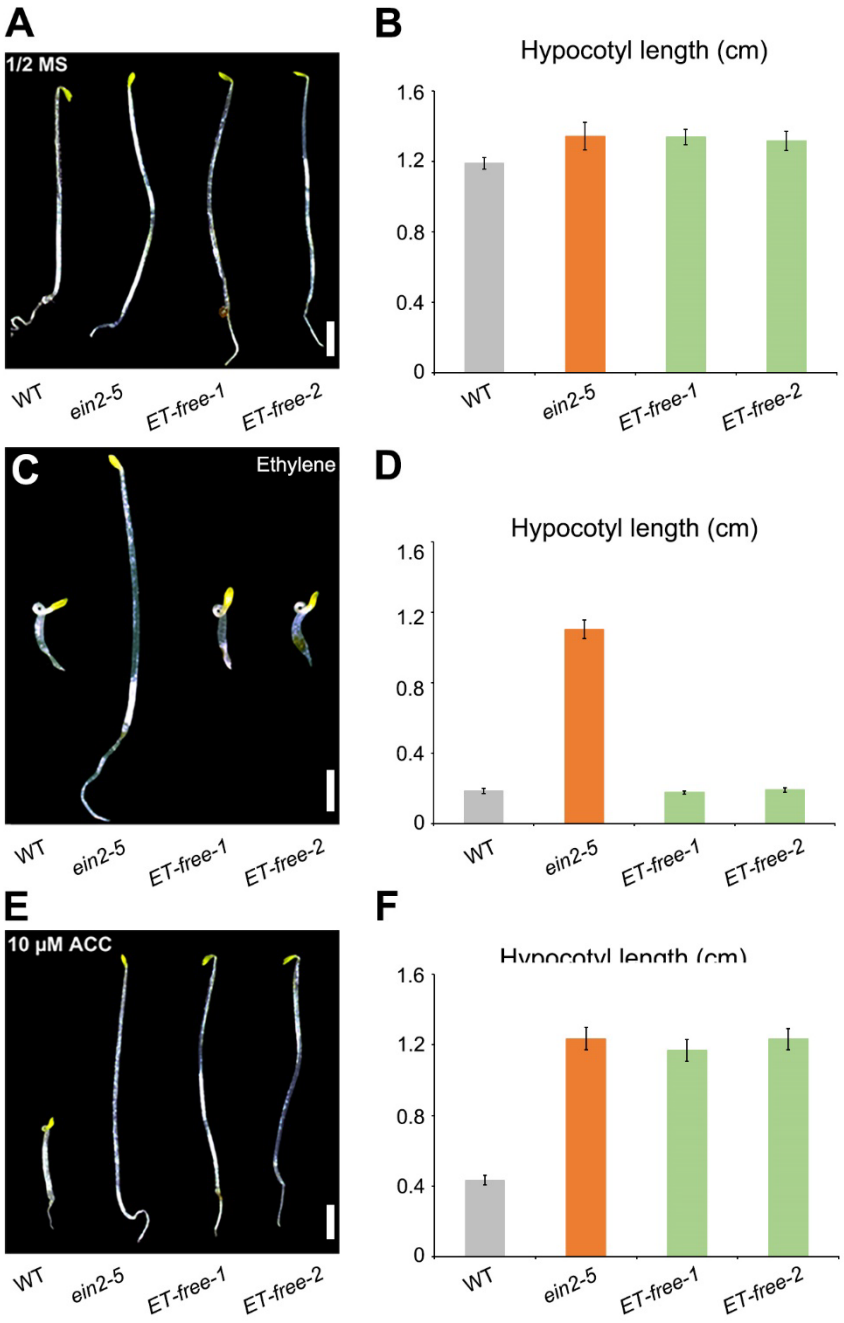
Supplemental Figure 3: Fertility and seed set are not affected in *ET-free* Arabidopsis mutants.

Statistical analysis of seed set in selfed wild type (WT) and *ET-free* mutants. Data are mean values ± SD; P-values are > 0.05 (Student's *t* test).



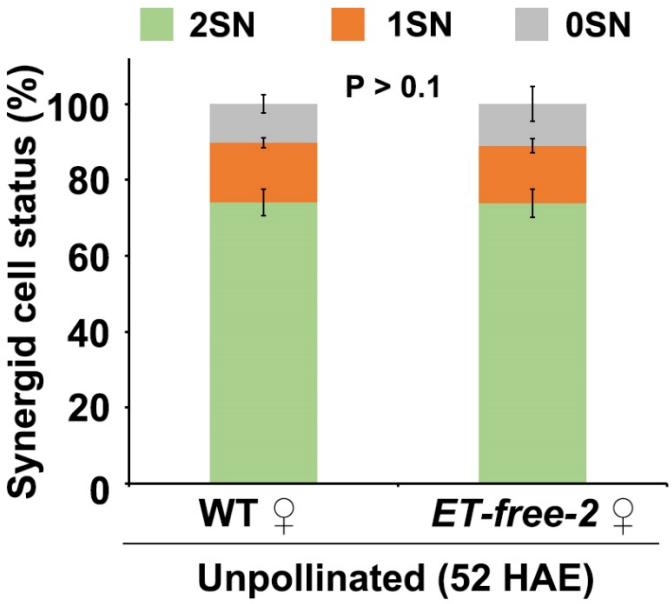
Supplemental Figure 4: Representative images of gas chromatograph (GC) results shown in Figure 1.

Black arrow indicates the peak of C_2H_4 in the wild-type (WT) ovules and vegetative tissues like leaf, inflorescence and seedling.



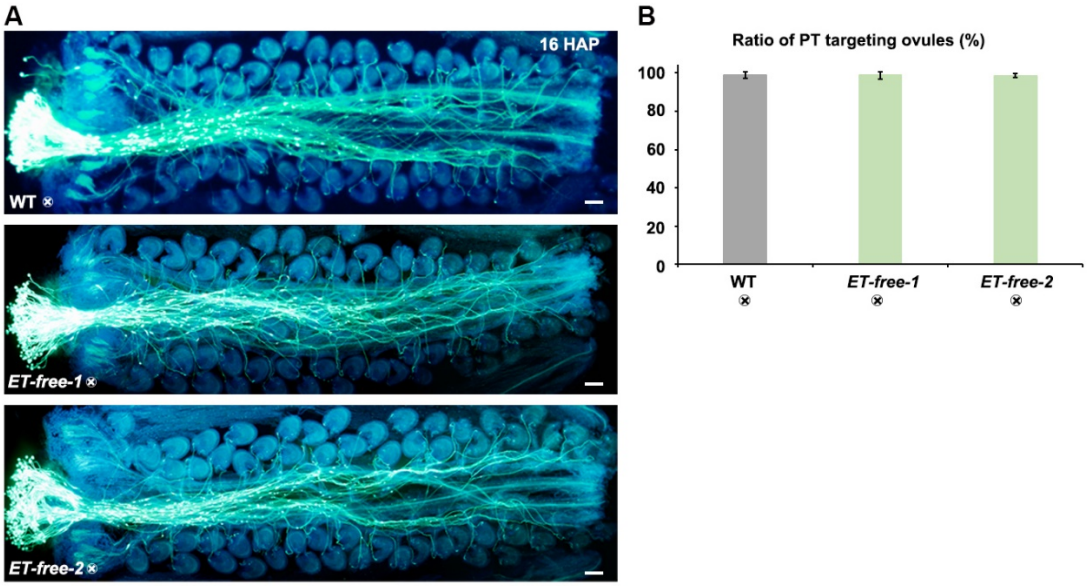
Supplemental Figure 5: Triple response of WT, *ein2-5* and *ET-free* mutants.

(A and B) Seedlings grown on 1/2MS medium and statistical analysis of hypocotyl length. (C and D) Seedlings grown on 1/2MS medium with 10 ppm ethylene treatment in darkness for four days and statistical analysis of hypocotyl length. (E and F) Seedlings grown on 1/2MS medium in the darkness for four days treated with 10 µM of ACC and corresponding statistical analysis of hypocotyl length. Gray boxes indicate WT, orange boxes indicate *ein2-5* mutant and green boxes indicate *ET-free* mutants. Data are mean values ± SD. Scale bars, 1 mm.



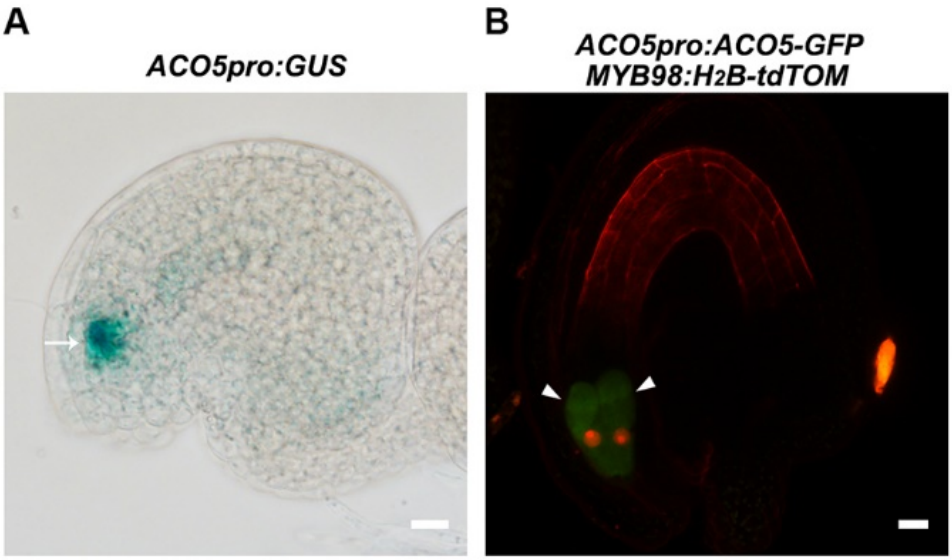
Supplemental Figure 6: Synergid cell status in unpollinated WT and *ET-free* mutant.

Statistical analysis of synergid cell status in unpollinated WT (n=235) and *ET-free-2* (n=271). HAE, hours after emasculation. Data are mean values ± SD; P-values are > 0.05 (Student's *t* test). Gray box represents the percentage of ovules with no synergid cell nucleus, while orange boxes and green boxes show the percentage of ovules with one synergid cell nucleus and two synergid cell nuclei, respectively.



Supplemental Figure 7: Pollen tube targeting ratio at 16 HAP is not affected in *ET-free* mutants.

(A and B) Aniline blue staining and statistical analysis of pollen tube targeting ratio in self-crossed wild-type (WT) and *ET-free* mutants at 16 HAP. (A) Representative images of aniline blue staining of self-crossed WT and *ET-free* mutants at 16 HAP. (B) Statistical analysis of pollen tube targeting ratio in self-crossed WT and *ET-free* mutants at 16 HAP. Data are mean values ± SD; P values > 0.05 (Student's *t* test). Scale bar, 100 μm.



Supplemental Figure 8: *AtACO5* is transcribed and translated in synergid cells.

(A) GUS signal from the *AtACO5* promoter was predominantly concentrated in synergid cells. White arrow indicates GUS signals detected in synergid cells. (B) Image of an ovule showing AtACO5-GFP fusion proteins expressed from the endogenous promoter co-localized with the synergid cell marker *MYB98pro:H$_2$B-tdTOM*. White arrowheads indicate GFP signal detected in the two synergid cells. Scale bars, 10 μm.

Supplemental Table 1: Primers used in this study.

	Primers Name	Sequences(5'-3')
Spacers cloning	AtACO3 sgRNA5-BsF	ATATATGGTCTCGATTGGCATCGTCGATCAAAGCCAGT
(For ataco1-5 quintuple mutants)	AtACO3 sgRNA5-F0	TGGCATCGTCGATCAAAGCCAGTTTTAGAGCTAGAAATAG
	AtACO3 sgRNA6-R0	AACGTTGTCCCATGAAACACCTCAATCTCTTAGTCGACTCTA
	AtACO3 sgRNA6-BsR	ATTATTGGTCTCGAAACGTTGTCCCATGAAACACCT
	AtACO2 sgRNA3-BsF	ATATATGGTCTCGATTGATGACATGCTCAAGTCCAAGT
	AtACO2 sgRNA3-F0	TGATGACATGCTCAAGTCCAAGTTTTAGAGCTAGAAATAG
	AtACO2 sgRNA4-R0	AACTGTGGTGACTCAACAAGAACAATCTCTTAGTCGACTCTA
	AtACO2 sgRNA4-BsR	ATTATTGGTCTCGAAACTGTGGTGACTCAACAAGAA
	AtACO4 sgRNA7-BsF	ATATATGGTCTCGATTGCCTAATCCGGACCTAGTCAGT
	AtACO4 sgRNA7-F0	TGCCTAATCCGGACCTAGTCAGTTTTAGAGCTAGAAATAG
	AtACO4 sgRNA8-R0	AACGTAATCGTCGTCGAGATCACAATCTCTTAGTCGACTCTA
	AtACO4 sgRNA8-BsR	ATTATTGGTCTCGAAACGTAATCGTCGTCGAGATCA
	AtACO5 sgRNA9-BsF	ATATATGGTCTCGATTGCTCAGGATGAGGACAAGGAGT
	AtACO5 sgRNA9-F0	TGCTCAGGATGAGGACAAGGAGTTTTAGAGCTAGAAATAG
	AtACO5 sgRNA10-R0	AACCGTTTTCTAGCTTCTCGCCCAATCTCTTAGTCGACTCTA
	AtACO5 sgRNA10-BsR	ATTATTGGTCTCGAAACCGTTTTCTAGCTTCTCGCC
	AtACO1 sgRNA1-BsF	ATATATGGTCTCGATTGGAGAACATACGGATGCTGGGT
	AtACO1 sgRNA1-F0	TGGAGAACATACGGATGCTGGGTTTTAGAGCTAGAAATAG
	AtACO1 sgRNA2-R0	AACTGACCATCTCTGACTGGTACAATCTCTTAGTCGACTCTA
	AtACO1 sgRNA2-BsR	ATTATTGGTCTCGAAACTGACCATCTCTGACTGGTA
Genotyping analysis	ACO3JDF	AGCTAGCGACCCTCTCTCAA
(For ataco1-5 quintuple mutants)	ACO3JDR	AGAACCGGATTCTGCTATTTGT
	ACO2JDF	AGCTCTTAGTGGTAAGGTTGATCT
	ACO2JDR	CTTCATTGCTGCGAACCGTG
	ACO4JDF	CTGGGGCTTCTTTGAGGTACT
	ACO4JDR	GATCGCCGAGATTAACGACC
	ACO5JDF	GCTTGCGAAGAGTGGGGATT
	ACO5JDR	TAGTGTTGTCGTCGTGTCACC
	ACO1JDF	ATGCACAAGGCAGTGTTTTC
	ACO1JDR	ACAAGAGCTTTGGAGCTGGA
Genotyping analysis	EIN3JDF	GGATGTGGAGAGACAAAATGC
(For *ein3eil1* mutants)	EIN3JDR	GAGGTGGACATGACTCGGG
	EIL1JDF	GATGGGAATGTATGGAAACATGG
	EIL1JDR	CACAATGTTGCATCAAAGCCG
Genotyping analysis	EIN2JDF	TGGAGCAGGTTTGTTCTGACGG

(For *ein2* mutants)	EIN2JDR	ACTCGCCAACCTGAGGGATTTT
CAS9 identification	Hyg-IDF	CAAAGATCGTTATGTTTATCGGCACT
(For ataco1-5 quintuple mutants)	Hyg-IDR	AAGAAGATGTTGGCGACCTCGTATT
Transcription and protein expression pattern	AtACO5p-bpF	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAAACGAGACATCACCTGCTC
	AtACO5p-bpR	GGGGACCACTTTGTACAAGAAAGCTGGGTATTCAGATCCGCAAAGAGAGAC
	AtACO5pg-topoF	CACCAACGAGACATCACCTGCTC
	AtACO5pg-topoR	GAGAGACTTTACAGCTAGAAAACC
Synergid nuclear maker	MYB98pro-B4F	GGGGACAACTTTGTATAGAAAAGTTGTAAGTAACGGTAACGACGGAG
	MYB98pro-B1rR	GGGGACTGCTTTTTTGTACAAACTTGAGTTTTTTTTTTCTCCTTTCCAAAAC
	H2B-bpF	GGGGACAAGTTTGTACAAAAAAGCAGGCTTAATGGCGAAGGCAGATAAGAAA
	tdTOM-bpR	GGGGACCACTTTGTACAAGAAAGCTGGGTATTACTTGTACAACTCGTCCATAC