

**Plant Communications, Volume 4**

**Supplemental information**

**The PPR protein RARE1-mediated editing of chloroplast *accD* transcripts is required for fatty acid biosynthesis and heat tolerance in *Arabidopsis***

**Chao Huang, Dan Liu, Zi-Ang Li, David P. Molloy, Zhou-Fei Luo, Yi Su, Hai-Ou Li, Qing Liu, Ruo-Zhong Wang, and Lang-Tao Xiao**

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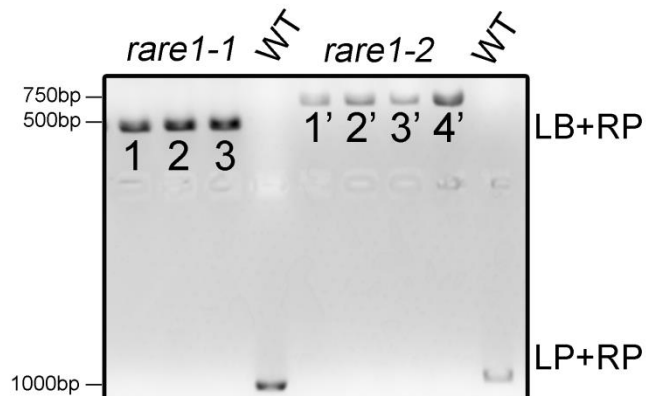
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43 Supplemental Figures

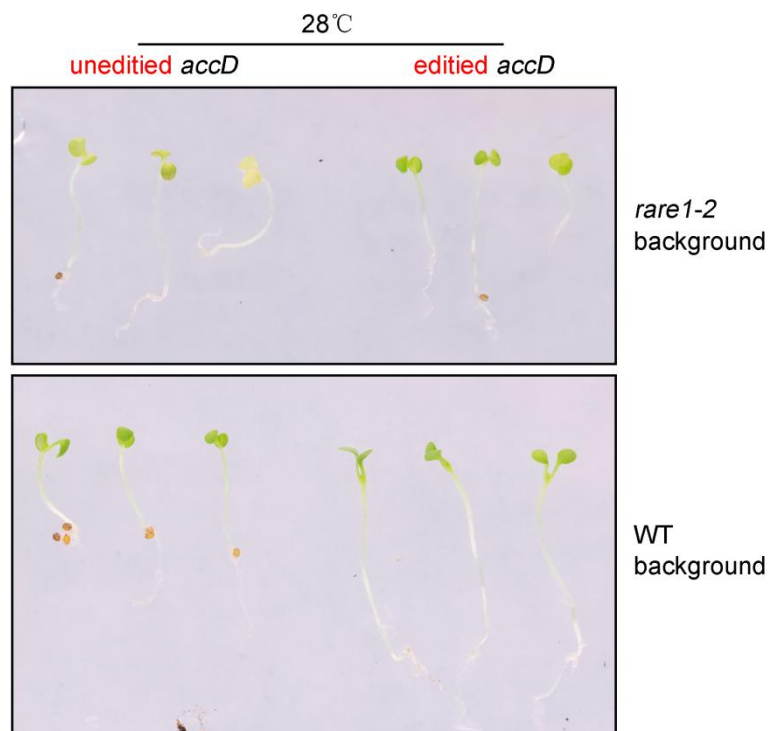
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46 **Figure 1.** PCR genotyping for analysis of *rare1* mutant plants. LP: Left primer; RP:

47 Right primer and LB: the left T-DNA border primer.

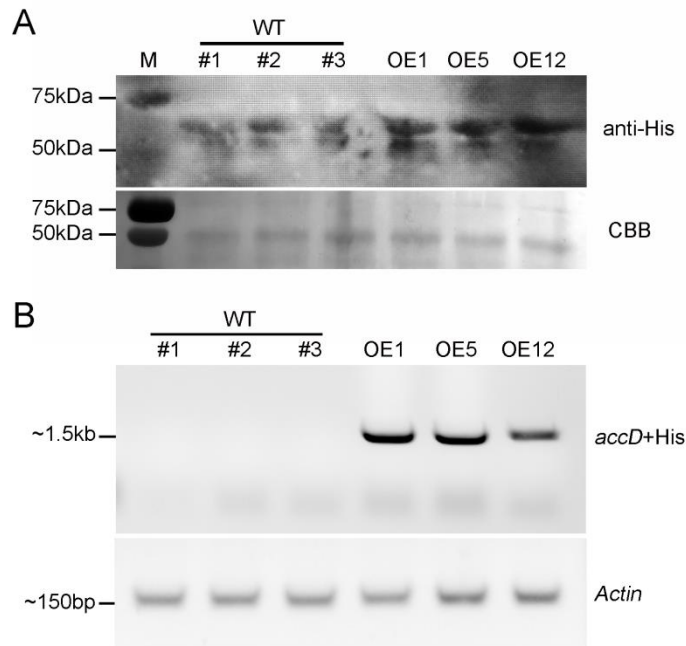


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49 **Figure 2.** Phenotype analysis of transgenic plants overexpressing unedited *accD* or

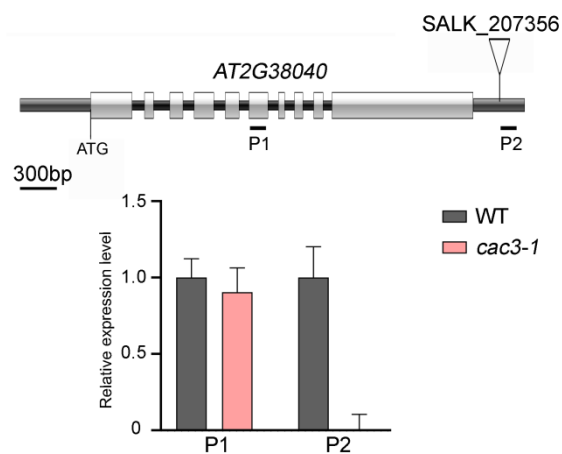
50 edited *accD* in *rare1* and WT background under heat stress conditions.

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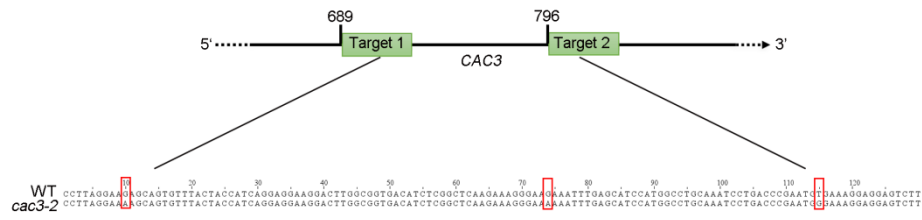
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53 **Figure 3.** Expression analysis of *accD-His* in C794-edited *accD* OE plants. (A)  
 54 Western blot using anti-His antibody confirmed *accD* overexpression in transgenic  
 55 plants. Coomassie Brilliant Blue (CBB) staining of the large rubisco subunit (RbcL) is  
 56 used as a loading control. (B) RT-PCR analysis of *accD-His* fusion transcripts.



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58 **Figure 4.** Identification of the *cac3-1* T-DNA insertion mutant. P1 and P2 represent  
 59 the detection area designed for qRT-PCR.



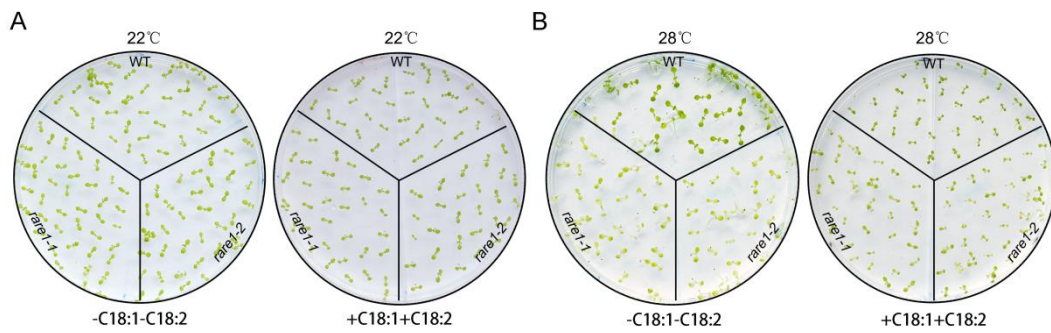
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61 **Figure 5.** Schematic representation of the CRISPR/Cas9 target sites in *CAC3* and  
62 mutation detection in T0 transgenic plants.

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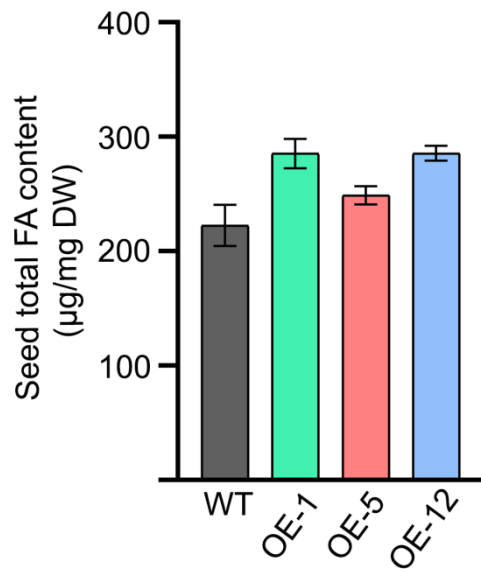
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67 **Figure 6.** Phenotypic observation of WT and the *rare1* mutants grown with C18:1 and  
68 C18:2 supplement at 22 (A) or 28°C (B).



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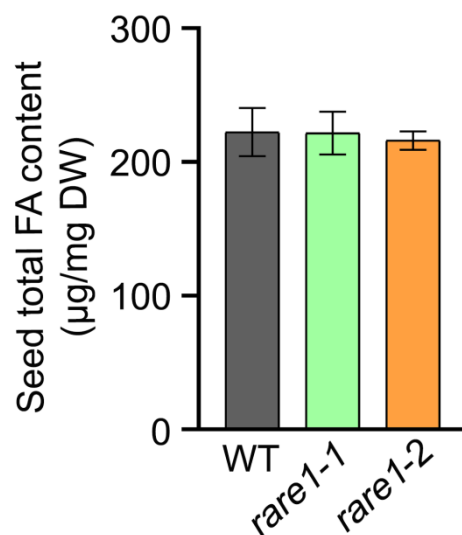
70 **Figure 7.** Comparison of seed total FA content (µg/mg) between WT and the *accD*

71 OE plants. Values are means  $\pm$  SD ( $n \geq 3$ ). DW, dry weight. Error bars denote SD.

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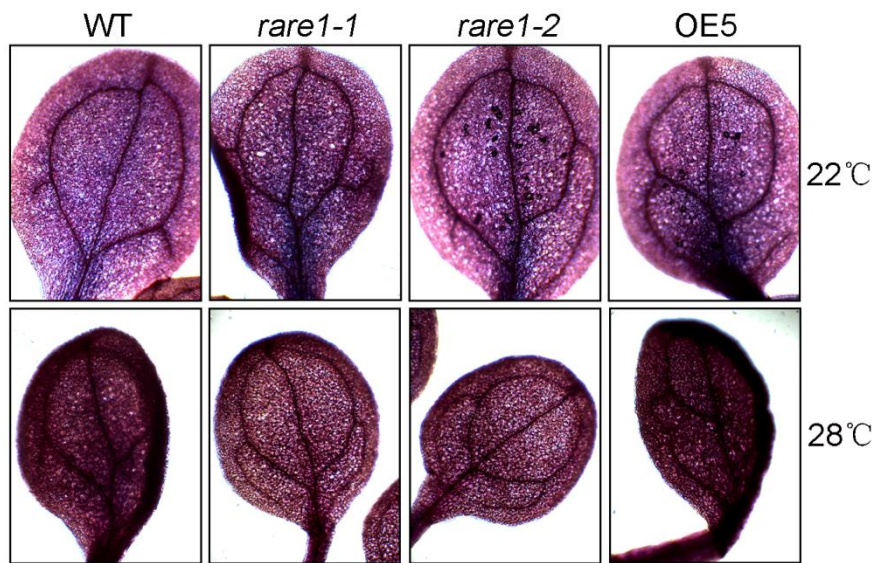
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76 **Figure 8.** Comparison of seed total FA content (µg/mg) between WT and *rare1*

77 mutants. Values are means  $\pm$  SD ( $n \geq 3$ ). DW, dry weight. Error bars denote SD.



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79 **Figure 9.** Staining of total lipid in leaves of 7-day-old WT, *rare1* mutants and  
80 *C794*-edited *accD* OE plants with Sudan Red under 22 and 28°C.

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84 **Supplemental Table 1. List of primers used in this study**

Gene	Primer name	Sequence (5'-3')
<i>RARE1</i>	T-DNA-P (415940)	ATATTGACCATCATACTCATTGC
	RARE1(415940) RP	AAGAGAAGCACACGCTTTAAGAAC
	RARE1(415940) LP	AACAACGAACCCTAGTAGCC
	RARE1(851454) RP	GGCCTTTCTCCATTCTCAAAG
	RARE1(851454) LP	TAATCTGTACACCTGGGCTGG
	T-DNA-P (851454)	AACGTCCGCAATGTGTTATTAAGTTGTC
<i>accD</i>	C794 RNA editing-F	TAGTGAAAGCGGAAAGATTC
	C794 RNA editing-R	AATACCGTTTAGTTGACCTG
	qPCR-F	TGTGGATTCAATGCGACAAT
	qPCR-R	TTTTGCGCAGAGTCAATACG
	35S-F	CTAGTCTAGAATGGAAAAATCGTGGTTCAAT
	35S-His-R	AAACTGCAGGTGATGGTGGTGTGATGATGATTT GTGTTCAAAGGAAAAAAGCATGGAGCT
<i>psaB</i>	qPCR-F	GGACCCCACTACTCGTCGTA
	qPCR-R	ATTGCTAATTGCCCGAAATG
<i>psbA</i>	qPCR-F	GAGCAGCAATGAATGCGATA
	qPCR-R	CCTATGGGGTCGCTTCTGTA
<i>psbB</i>	qPCR-F	CGTGCGACTTTGAAATCTGA
	qPCR-R	TAGCACCATGCCAAATGTGT
<i>rbcL</i>	qPCR-F	GTGTTGGGTTCAAAGCTGGT
	qPCR-R	CATCGGTCCACACAGTTGTC
<i>atpB</i>	qPCR-F	GAGCTCGTATGAGAGTTGGT
	qPCR-R	ACCCAATAAGGCGGATACCT
<i>atpE</i>	qPCR-F	TCCACAAGAAGCTCAGCAA
	qPCR-R	GTGTCCGAGCTCGTCTGAG
<i>rrn16</i>	qPCR-F	CGGTATCTGGGGAATAAGCA
	qPCR-R	GATTTGACGGCGGACTTAAA
<i>rpoA</i>	qPCR-F	CAAGCCGACACAATAGGCAT
	qPCR-R	AGCGCGTTGCGCGTTCCATA
PORA	CTP-F	TACGAGCTCATGGCCCTTCAAGCTGCTTCT
	CTP-R	CTAGTCTAGATCTCAATGAGGAAGAGACAA AG



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<i>CAC3</i>	<i>cac3</i> LP	GAGAGAGCAAAGGTCTAAAC
	<i>cac3</i> RP	GCACAACAAACTGTGTATTC
	LBb1.3	ATTTTGCCGATTTCGGAAC
	P1 qPCR-F	CAACAATCTGAGGACGATGTTC
	P1 qPCR-R	TCGAGCATCAGCATTATTCG
	P2 qPCR-F	TCTGGATTGGAACAAGCGTT
	P2 qPCR-R	AGGTTTTGACCAGAAGAGGAGT

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