

## 1 **Supporting Information Legends**

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3 **Figure S1. Fatty acid composition in leaves of *ar21* and wild type.** 16:1 includes *cis* and  
4 *trans*-16:1 fatty acid. Values are expressed as means  $\pm$  SD ( $n = 3$ ).

5 **Figure S2. Confirmation of the point mutation in *ar21* by Sanger sequencing.** *AR21/AR21*,  
6 wild type; *ar21/AR21*, heterozygous line; *ar21/ar21*, mutant line.

7 **Figure S3. Seed fatty acid analysis with the *ar21* x Col F2 population.** Re-sequencing of the  
8 point mutation in *ar21* with 107 lines from F2 population revealed that there were 26 wild type  
9 (*AR21/AR21*), 58 heterozygous lines (*AR21/ar21*) and 23 mutant lines (*ar21/ar21*) (Table S3). 10  
10 lines of each genotype were randomly selected for fatty acid composition analysis in mature  
11 seeds. (a) Fatty acid composition presented as average values of 10 lines with three replicates for  
12 each line carried out on batches of 20 seeds. (b) 18:3 fatty acid composition from individual lines.  
13 (c) 20:1 fatty acid composition from individual lines. *AR21/AR21*, wild type; *ar21/AR21*,  
14 heterozygous lines; *ar21/ar21*, mutant lines. Values are expressed as means  $\pm$  SD ( $n = 3$ ).

15 **Figure S4. Quantitative RT-PCR (qRT-PCR) analysis of *accD* and *ACCI*.** Quantitative RT-  
16 PCR (qRT-PCR) was conducted with 13 DAF seeds from *ar21*, *i4g1* and wild type (WT).  
17 Relative expression in mutants was presented as fold change against WT. Values are expressed  
18 as mean  $\pm$  SD ( $n=3$ ). \*,  $p$  value  $< 0.05$  (by Student's t-test).

19 **Figure S5. Fatty acid composition in mature seeds of wild type and *i4g2*.** (a) PCR  
20 confirmation of T-DNA insertion in *i4g2* mutant. (b) Fatty acid composition in mature seeds of  
21 wild type and *i4g2*. 16:1 includes *cis* and *trans*-16:1 fatty acid. Values are expressed as means  $\pm$   
22 SD ( $n = 3$ ).

23 **Figure S6. Growth phenotype of wild type, *i4g1*, *i4g2* and *i4g1/i4g2* mutants.** The top panel  
24 showed the seedlings at three-weeks after germination. The middle panel showed the

25 representative primary inflorescences. The bottom panel showed the representative siliques.

26 Scale bar, 1 cm.

27 **Figure S7. Fatty acid composition in seeds and leaves of wild type and mutants.** (a) Fatty  
28 acid composition in mature seeds of wild type (WT) and *i4g1/i4g2* double mutants. (b) Fatty acid  
29 composition in three-week old leaves of wild type (WT), *ar21*, *i4g1*, *i4g2* and *i4g1/i4g2*. 16:1  
30 includes *cis* and *trans*-16:1 fatty acid. Values are expressed as means  $\pm$  SD ( $n = 3$ ). \*, p value <  
31 0.05; \*\*, p < 0.01; \*\*\*, p < 0.001 (by Student's t-test).

32 **Table S1. Summary of genome sequencing data from *ar21*.** SNV, single nucleotide variant.

33 **Table S2. Summary of premature stop mutations detected in *ar21*.** SNV, single nucleotide  
34 variant.

35 **Table S3. Genotyping with the *ar21* x Col F2 population.** Genomic DNA was extracted from  
36 107 lines individually. PCR products were sequenced to identify the point mutation in  
37 *At5g57870*. T, mutation; C, wild type; H, heterozygous.

38 **Table S4. Primer pairs used in this study.**

39 **Data S1. Differentially expressed genes in the developing seeds of *ar21* and *i4g1*.**

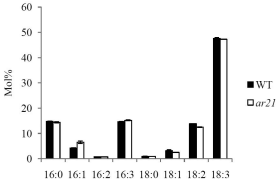
40 **Data S2. Plastidic gene expression in the developing seeds of *ar21* and *i4g1*.**

41 **Data S3. Differentially expressed ribosomal genes in the developing seeds of *ar21* and *i4g1*.**

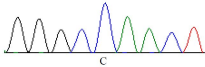
42 Ribosomal genes were compiled according to Sormani et al., 2011.

43 **Data S4. Differentially expressed genes pertinent to lipid metabolism in the developing  
44 seeds of *ar21* and *i4g1*.**

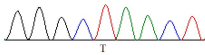
45 **Data S5. Lipidomics analysis of developing seeds from *ar21* and wild type.** Values are means  
46 of five replicates. Statistically significant differences (two-tailed Student t-test) were calculated  
47 between *ar21* and wild type. SD, standard deviation.



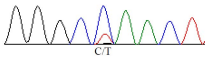
*AR21/AR21*

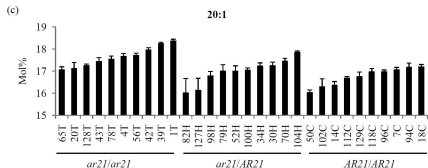
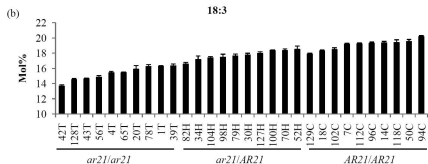
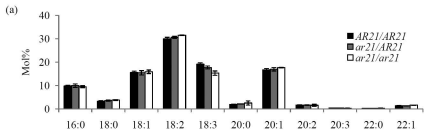


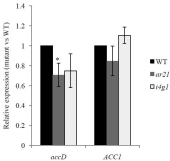
*ar21/ar21*



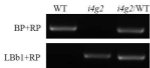
*AR21/ar21*



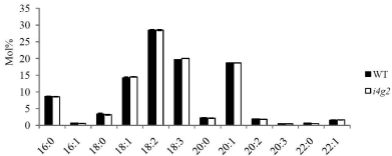




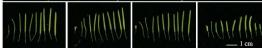
(a)



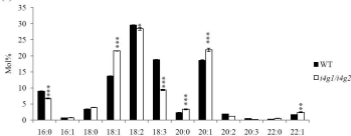
(b)



WT

*i4g1**i4g2**i4g1/i4g2*



**(a)****(b)**