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Supplemental Material

CRISPR/Cas9-Induced *fad2* and *rod1* Mutations Stacked with *fae1* Confer High Oleic Acid Seed Oil in Pennycress (*Thlaspi arvense* L.)

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Table S1. Seed TAG fatty acid compositions of the different pennycress CRISPR-induced oilseed mutants. Values represent means plus/minus standard deviations. Asterisks represent significant differences compared to wild type based on Student t-test analysis where $p < 0.05$. n = 3. These data are graphed in Figure 2.

Genotype	16:0	16:1	18:0	18:1	18:2	18:3	20:1	22:1	24:1
WT Spring 32-10	3.0±0.1	0.3±0.0	0.4±0.0	12.1±0.8	18.1±0.5	11.7±0.4	12.0±0.5	34.9±0.9	3.3±0.1
fad2-4 (-2bp)	2.1±0.1**	0.1±0.1**	0.4±0.0	34.9±0.7**	0.5±0.2**	1.7±0.1**	16.6±0.4**	39.3±0.7**	3.4±0.1**
fad2-5 (+A)	2.4	0.1	0.4	35.0	0.5	2.5	14.9	39.8	3.8
fad2-6 (-29bp)	2.5	0.1	0.4	34.0	0.5	2.7	14.2	41.4	3.6
rod1-3 (-18bp)	3.4±0.4*	0.4±0.0**	0.6±0.0**	22.7±1.0**	8.5±0.6**	11.8±0.6	14.0±0.3**	35.3±2.0	2.8±0.2**
rod1-4 (+A)	3.2±0.3	0.4±0.0**	0.5±0.0**	22.4±0.7**	9.1±0.8**	11.2±0.2	14.4±0.2**	35.7±1.5	3.0±0.3
rod1-5 (+T)	4.3	0.0	0.5	23.1	10.5	12.5	14.3	31.7	2.8
fae1-3 (-4bp)	3.5±0.1**	0.3±0.0	0.8±0.0**	47.8±1.9**	28.5±1.4**	17.8±0.6**	0.9±0.1**	0.0±0.0**	0.3±0.0**
fad2-4 fae1-3	2.5±0.2**	0.2±0.1	0.6±0.0**	90.6±0.6**	0.5±0.1**	2.6±0.3**	1.5±0.1**	0.0±0.0**	0.2±0.2**
rod1-4 fae1-3	3.4±0.0*	0.0±0.0**	0.6±0.0**	59.7±0.3**	17.1±0.2*	19.3±0.3**	0.0±0.0**	0.0±0.0**	0.0±0.0**

Table S2. Total seed germination on agar growth media plates of the different pennycress CRISPR-induced oilseed mutants. Values represent cumulative germination over a 10-day period (total means of 50). Standard deviations represent plus/minus the average daily new germination. Asterisks represent significant differences compared to wild type based on one-way ANOVA; Tukey test analysis versus wild type where $p < 0.05$* and $p < 0.01$**. n=3 biological reps of 50 seeds each. These data are graphed in Figure 4.

	Day 1	**Day 2**	**Day 3**	**Day 4**	**Day 5**	**Day 6**	**Day 7**	**Day 8**	**Day 9**	**Day 10**
WT Spring32-10	0.0±0.0	13.7±4.0	44.3±5.7	46.0±1.5	46.7±0.6	47.0±0.6	47.0±0.0	47.0±0.0	47.3±0.6	47.3±0.0
fae1-3 (-4bp)	0.0±0.0	24.0±4.4*	49.0±5.2	49.3±0.6	49.7±0.6	49.7±0.0	49.7±0.0	49.7±0.0	49.7±0.0	49.7±0.0
rod1-4 (+A)	0.0±0.0	14.3±4.2	44.3±5.6	46.7±0.6	47.3±0.6	48.0±1.2	48.0±0.0	48.0±0.0	48.3±0.6	48.3±0.0
fad2-5 (+A)	0.0±0.0	1.7±1.5**	10.3±7.1**	25.3±0.6**	33.7±3.1*	37.3±1.7**	43.0±1.0	44.7±0.0	45.3±0.0	45.3±0.0
fad2-4 (-2bp)	0.0±0.0	1.3±1.5**	31.0±4.2**	42.3±7.0	46.0±1.5	48.0±2.5	49.0±6.4	49.0±1.5	49.0±1.2	49.0±0.0
rod1-3 fae1-3	0.0±0.0	36.7±4.9**	49.7±4.4	49.7±0.0	50.0±0.6	50.0±0.0	50.0±0.0	50.0±0.0	50.0±0.0	50.0±0.0
fad2-4 fae1-3	0.0±0.0	0.7±0.6**	22.7±1.7**	38.3±0.6	45.0±1.2	46.7±1.5*	46.7±0.0	47.3±0.6	48.3±1.0	48.3±0.0
fad2-5 fae1-3	0.0±0.0	0.0±0.0**	10.0±0.0**	24.0±6.2**	35.0±4.0*	42.0±5.0**	44.0±2.0	45.3±1.5	45.7±0.6	45.7±0.0

Table S3. Amounts of seed germination in constant light at different temperatures over a 16-day period. Data can be found in graph form in Figure S5. Values are the means of the number of seeds that germinated on the given day plus/minus standard deviations. 3 biological reps of 25 seeds each rep. Student T-test versus wild type, $p < 0.05$*, $p < 0.01$**.

WT Spring32-10	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16
4 ºC	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.7±0.6	0.7±1.2	1.3±2.3	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0
12 ºC	0.0±0.0	0.0±0.0	0.0±0.0	14.3±8.5	8.3±6.7	1.7±1.5	0.0±0.0	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
22 ºC	0.0±0.0	11.3±6.0	9.7±6.7	2.7±0.6	1.00±1.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
28 ºC	0.0±0.0	14.7±3.1	7.0±2.6	1.0±1.0	0.0±0.0	0.3±0.6	0.3±0.6	0.0±0.0	0.0±0.0	0.67±1.2	0.33±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
fae1-3																
4 ºC	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.3±0.6	0.0±0.0	0.3±0.0	1.0±1.7	0.3±0.6	0.0±0.0	0.3±0.6	0.3±0.6
12 ºC	0.0±0.0	0.0±0.0	1.7±2.9	9.7±6.1	10.0±6.6	2.7±2.3	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
22 ºC	0.0±0.0	16.0±0.0	7.7±0.6	1.00±1.0*	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.33±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
28 ºC	0.0±0.0	12.0±5.6	7.3±3.5	4.7±4.0	0.7±1.2	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
rod1-3																
4 ºC	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.6	0.7±1.2	0.7±1.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
12 ºC	0.0±0.0	0.0±0.0	3.3±5.8	7.3±7.1	10.3±9.6	3.7±3.2	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
22 ºC	0.0±0.0	17.7±3.2	5.0±3.6	1.3±2.3	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
28 ºC	0.0±0.0	10.3±5.5	10.3±4.2	3.00±1.0	1.00±1.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
fad2-5																
4 ºC	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.7±1.2	0.0±0.0	0.3±0.6	0.0±0.0	0.3±0.6	0.3±0.6	0.0±0.0
12 ºC	0.0±0.0	0.0±0.0	0.0±0.0	2.7±2.5*	6.0±3.0	5.7±1.5	3.0±2.0	1.0±0.0	0.3±0.6	1.0±1.0	0.7±0.6	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
22 ºC	0.0±0.0	3.3±2.1	10.0±6.0	7.0±1.0*	1.3±1.5	0.7±1.2	2.0±1.0*	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
28 ºC	0.0±0.0	11.3±0.6	6.3±3.8	4.3±3.2	0.7±0.6	0.7±1.2	0.3±0.6	0.3±0.6	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
rod1-4 fae1-3																
4 ºC	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.6	0.0±0.0	0.3±0.6	1.0±1.7	0.7±1.2	0.7±1.2	0.0±0.0	0.0±0.0	0.0±0.0
12 ºC	0.0±0.0	0.0±0.0	2.7±4.6	11.3±2.3	10.3±7.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
22 ºC	0.0±0.0	16.3±2.3	8.0±2.0	0.7±1.2*	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
28 ºC	0.0±0.0	1.3±0.6*	5.3±3.2	10.7±4.5*	4.0±3.0	2.0±1.0*	1.0±1.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
fad2-4 fae1-3																
4 ºC	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.6	0.7±1.2	0.7±1.2	1.3±1.2	1.7±2.1	0.3±0.6	0.0±0.0
12 ºC	0.0±0.0	0.0±0.0	3.0±5.2	8.7±2.1	8.0±4.0	1.7±2.1	1.3±1.5	0.7±0.6	1.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
22 ºC	0.0±0.0	14.7±0.6	6.0±3.5	3.3±3.1	1.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
28 ºC	0.0±0.0	16.3±4.7	4.0±2.0	2.7±1.2	0.3±0.6	0.7±1.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

Table S4. Amounts of seed germination in constant darkness at different temperatures over a 16-day period. Data can be found in graph form in Figure S5. Values are the means of the number of seeds that germinated on the given day plus/minus standard deviations. 3 biological reps of 25 seeds each rep. Student T-test versus wild type, $p<0.05$*, $p<0.01$**.

WT Spring32-10	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Day 11	Day 12	Day 13	Day 14	Day 15	Day 16
4 ºC	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.0±0.0	0.7±1.2	4.0±2.6	3.7±4.0	1.7±2.9	4.3±3.1	0.7±0.6	1.0±1.7	0.7±1.2
12 ºC	0.0±0.0	0.0±0.0	2.7±4.6	5.7±9.0	8.0±6.2	7.0±6.1	1.0±1.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
22 ºC	0.0±0.0	16.0±3.5	6.7±1.5	1.0±0.0	0.0±0.0	0.3±0.6	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.33±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
28 ºC	0.0±0.0	15.3±4.2	6.7±5.9	0.7±1.2	0.0±0.0	0.00±0.0	0.0±0.0	0.3±0.6	0.0±0.0	1.0±1.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
fae1-3																
4 ºC	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	1.3±0.6	0.3±0.0	4.7±3.2	9.0±2.0	0.7±1.2	4.3±2.9	0.7±0.6	0.7±0.6	0.0±0.0
12 ºC	0.0±0.0	0.0±0.0	6.7±11.5	4.0±3.0	7.7±5.8	6.3±6.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
22 ºC	0.0±0.0	18.3±2.3	5.3±2.1	0.7±0.6	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
28 ºC	0.0±0.0	15.7±3.8	5.0±2.6	1.0±1.0	1.3±1.2	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
rod1-3																
4 ºC	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.7±0.6	2.0±2.6	3.3±3.5	2.7±4.6	0.3±0.6	0.7±0.6	0.7±0.6	0.0±0.0
12 ºC	0.0±0.0	0.0±0.0	4.7±8.1	3.3±5.8	7.3±5.7	5.7±5.5	2.0±2.6	1.3±2.3	0.0±0.0	0.7±1.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
22 ºC	0.0±0.0	18.7±5.0	5.7±5.1	0.3±0.6	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
28 ºC	0.0±0.0	14.0±6.2	9.3±5.5	0.7±0.6	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
fad2-5																
4 ºC	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.6	0.0±0.0	0.0±0.0	1.0±1.0	0.0±0.0	0.7±0.6	0.7±1.2	0.0±0.0	0.0±0.0
12 ºC	0.0±0.0	0.0±0.0	0.0±0.0	3.3±4.9	2.3±2.1	5.3±2.1	4.33±1.5**	1.7±1.5	2.7±0.6**	0.3±0.6	1.0±1.0	1.0±1.7	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
22 ºC	0.0±0.0	4.0±4.4*	9.3±2.1	6.7±3.8	1.7±1.2	1.0±1.0	0.7±1.2	0.7±1.2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
28 ºC	0.0±0.0	17.7±2.1	3.0±0.0	1.7±2.1	1.0±1.0	0.0±0.0	0.0±0.0	0.7±0.6	0.0±0.0	0.7±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
rod1-4 fae1-3																
4 ºC	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.7±1.2	4.0±1.7	6.3±2.3	1.3±2.3	6.7±5.0	2.0±1.0	0.7±0.6	0.7±1.2
12 ºC	0.0±0.0	0.0±0.0	7.0±12.1	1.3±2.3	6.0±5.3*	9.7±8.7	0.7±1.2	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
22 ºC	0.0±0.0	15.7±1.2	6.7±1.2	2.3±0.6	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
28 ºC	0.0±0.0	6.0±2.6*	10.3±2.9	5.3±5.9	1.0±1.0	1.7±1.2	0.3±0.6	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
fad2-4 fae1-3																
4 ºC	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.3±0.0	2.0±0.0	0.7±0.0	1.3±0.0	1.3±0.0	0.7±0.0	1.0±0.0	4.3±0.0	2.0±0.0	0.0±0.0	1.0±0.0
12 ºC	0.0±0.0	0.0±0.0	5.3±0.0	2.7±0.0	5.3±0.0	7.7±0.0	1.0±0.0	1.0±0.0	0.3±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
22 ºC	0.0±0.0	16.3±0.0	6.0±0.0	2.0±0.0	0.7±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
28 ºC	0.0±0.0	22.7±0.0*	1.3±0.0	0.3±0.0	0.0±0.0	0.3±0.0	0.0±0.0	0.3±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0

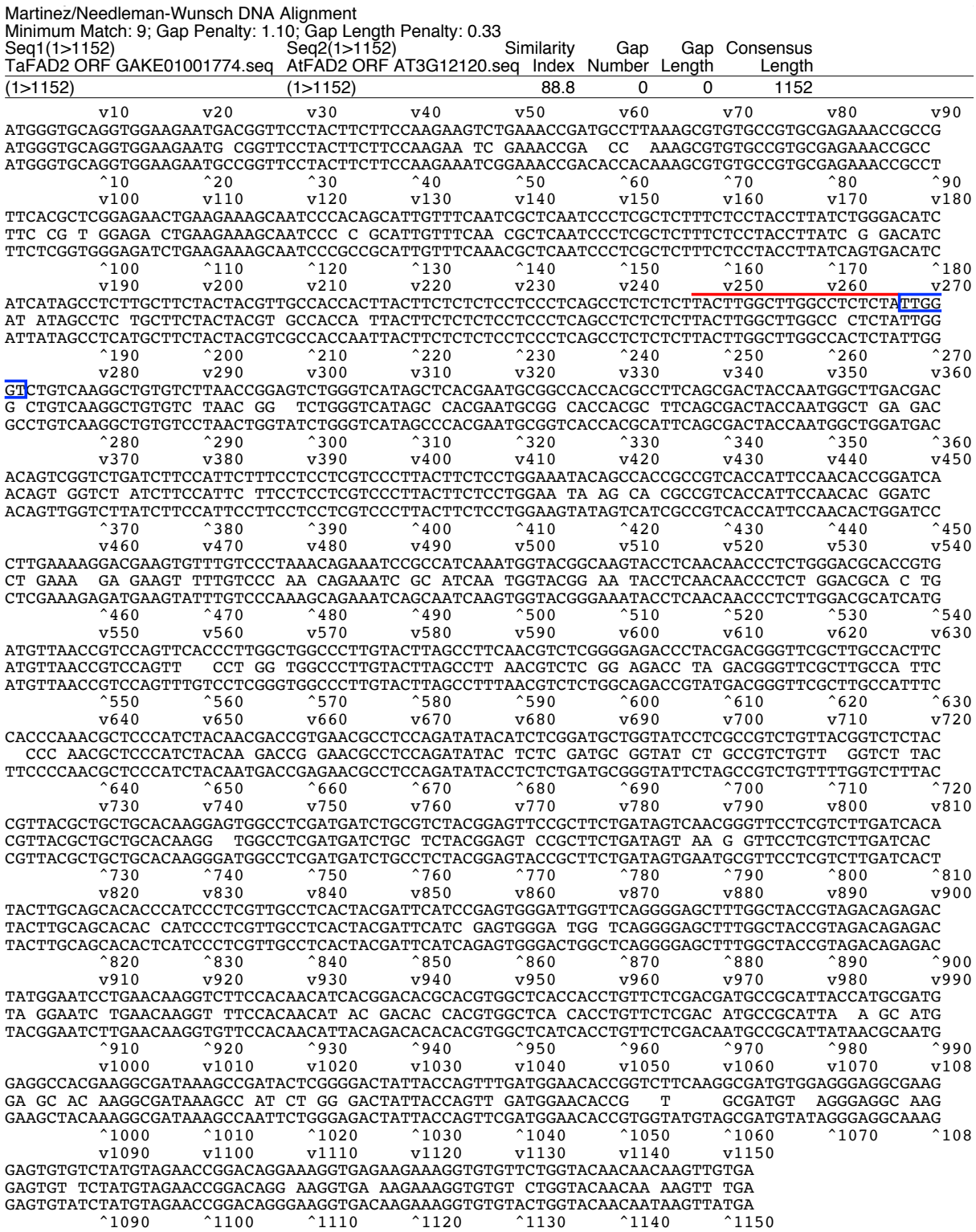

Figure S1. Nucleotide sequence alignment of the *Thlaspi arvense TaFAD2* ORF (top sequence) versus the *Arabidopsis thaliana AtFAD2* ORF (AT3G12120.1). The *TaFAD2* ORF sequence is derived from transcriptome assembly contig GAKE01001774.1 (Dorn et al., 2014), which shares 100% identity with *Thlaspi arvense* MN106 reference genome sequences. The red line delineates the 20 nucleotide protospacer sequence used in the CRISPR/SaCas9 construct. Outlined in blue is the NNGGGT protospacer adjacent motif (PAM) recognized by SaCas9.

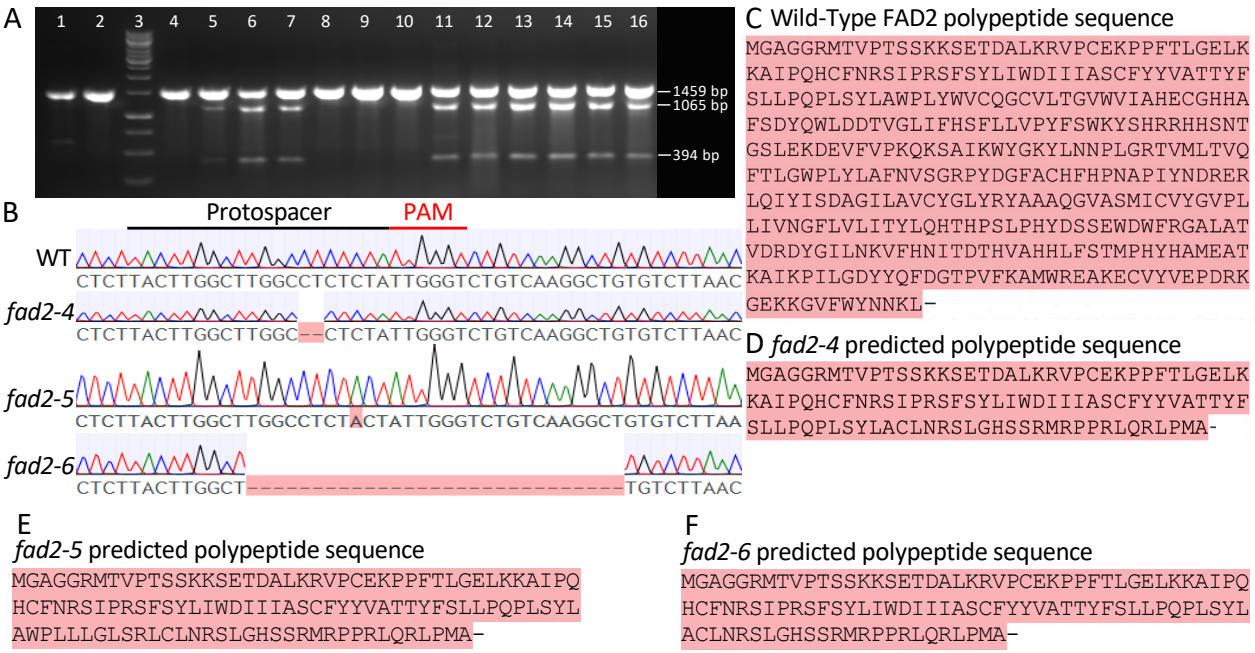

Figure S2. Characterization of the CRISPR-induced *Thlaspi arvense fad2* mutants' sequences. (**A**) Electrophoresed T7 endonuclease I-digested PCR products scoring wild type (WT) *FAD2* (1459 bp band) versus *fad2* mutations (partial digestion of 1459 bp band producing 1065 and 394 bp products). Lanes 1, 2, 4, 8-10 are WT segregants, whereas lanes 5-7 and 11-16 harbor the *fad2-3* mutation. Lane 3: Fermentas 1kb GeneRuler. (**B**) DNA sequence chromatograms. WT *TaFAD2* (top); *fad2-4* allele (2 bp deletion); *fad2-5* allele (A insertion); *fad2-6* allele (29 bp deletion). All three mutations are located as expected within the protospacer binding site of the CRISPR/*Sa*Cas9 guide RNA. (**C, D**) Predicted FAD2 polypeptide sequence in (**C**) WT pennycress versus the (**D**) *fad2-4* **(E)** *fad2-5*, and **(F)** *fad2-6* mutants. Note the predicted polypeptides encoded by each mutant are predicted to be truncated due to the frameshift mutations in the open reading frame (ORF).

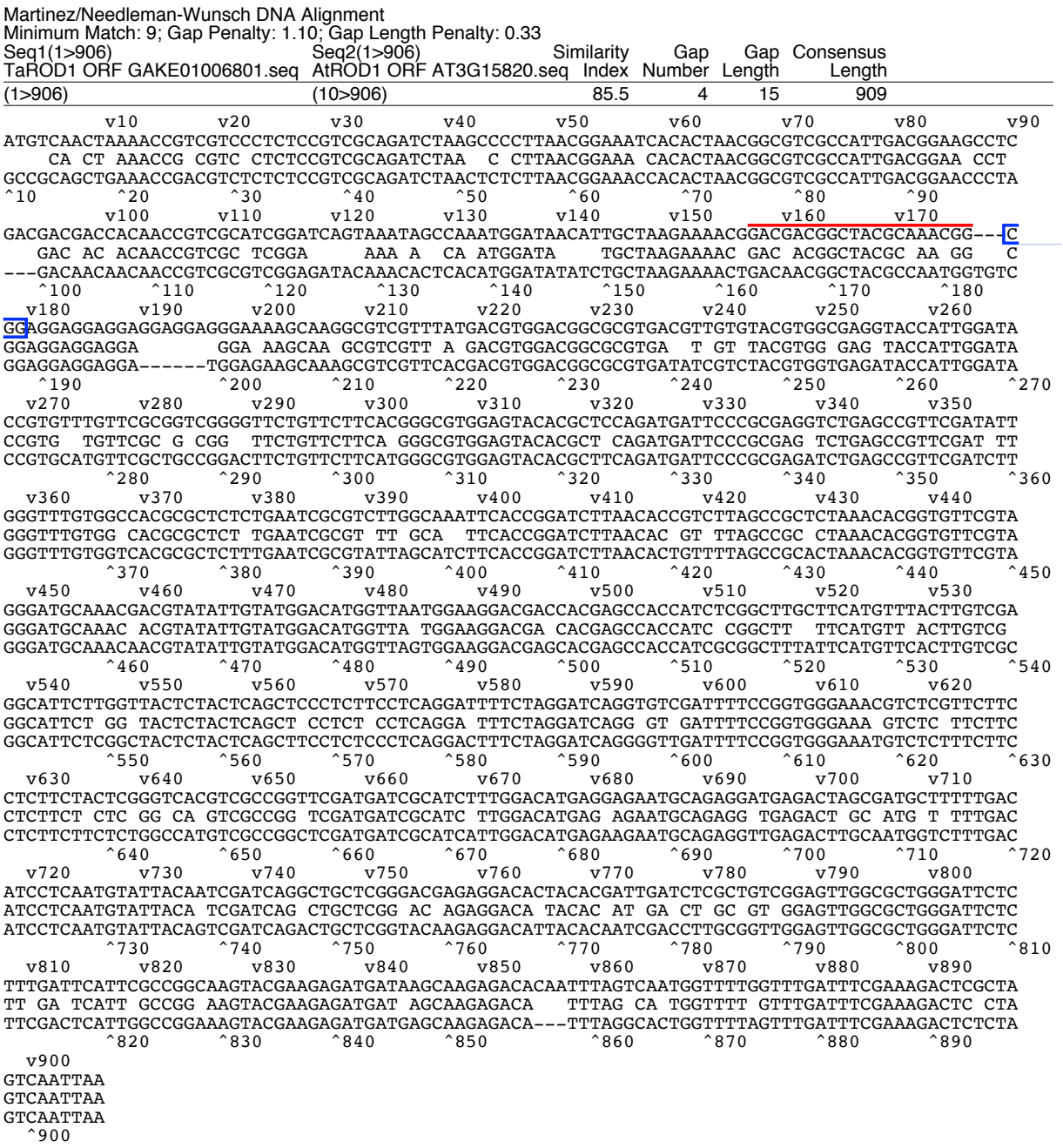

Figure S3. Nucleotide sequence alignment of the *Thlaspi arvense TaROD1* ORF (top sequence) versus the *Arabidopsis thaliana AtROD1* ORF (AT3G15820.1). The *TaROD1* ORF sequence was derived from transcriptome assembly contig GAKE01006801.1 (Dorn et al., 2014), which shares 100% identity with *Thlaspi arvense* MN106 reference genome sequences. The red line delineates the 20 nucleotide protospacer sequence used in the CRISPR/SpCas9 construct. Outlined in blue is the NGG protospacer adjacent motif (PAM) recognized by SpCas9.

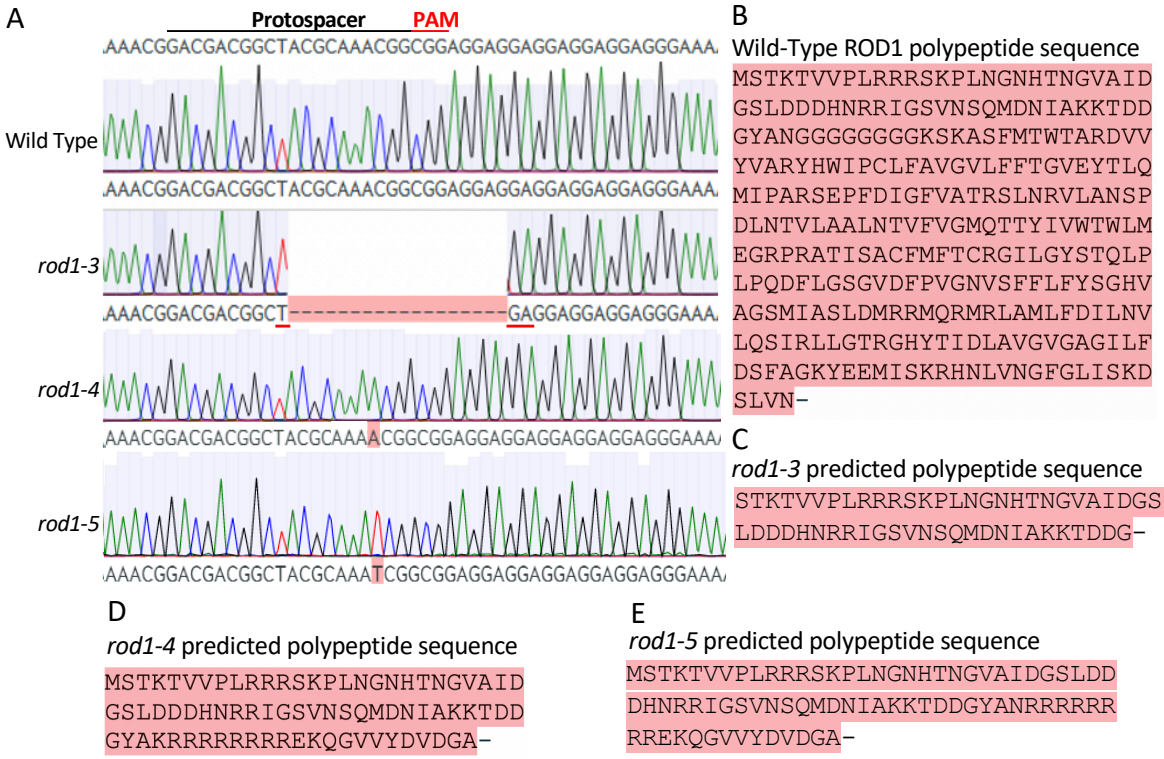

Figure S4. Characterization of the CRISPR-induced *Thlaspi arvense rod1* mutants' sequences. (**A**) *Tarod1* DNA sequence chromatograms showing the nature of each mutation. *TaROD1* coding sequences homozygous for wild type (top sequence); 18 bp deletion (*rod1-3*); +A insertion (*rod1-4*); and +T insertion (*rod1-5*). All three mutations are located as expected at the CRISPR/*Sp*Cas9 guide RNA binding site ("Protospacer" location delineated with a black line). (**B, C, D, E**) Predicted ROD1 polypeptide sequence in (**B**) wild-type pennycress versus the **(C)** *rod1-3* **(D)** *rod1-4*, and **(E)** *rod1-5* mutants. Note the predicted polypeptides encoded by *rod1-4* and *rod1-5* are truncated due to frameshifts in the open reading frame (ORF), whereas the 18 bp deletion in *rod1-4* introduces a premature stop codon (underlined in red).

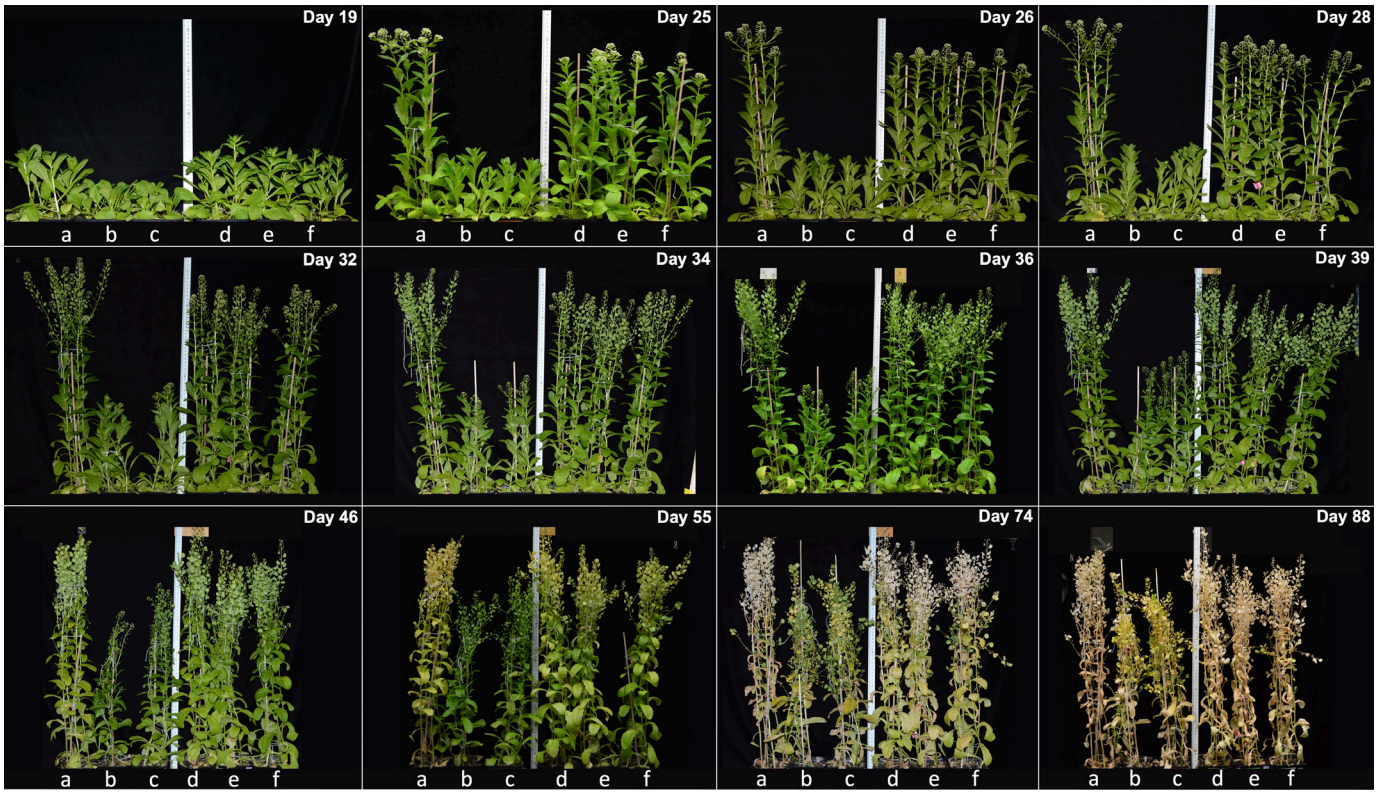

Figure S5. Growth time course of different lipid mutants and wild-type plants grown at the same time and conditions from Day 19 until Day 88 of their plant life cycles. Shown are four plants for each genotype growing in four-inch pots. (a) *rod1 fae1*, (b) *fad2 fae1*, (c) *fad2*, (d) Wild-type Spring 32-10, (e) *fae1*, and (f) *rod1*.

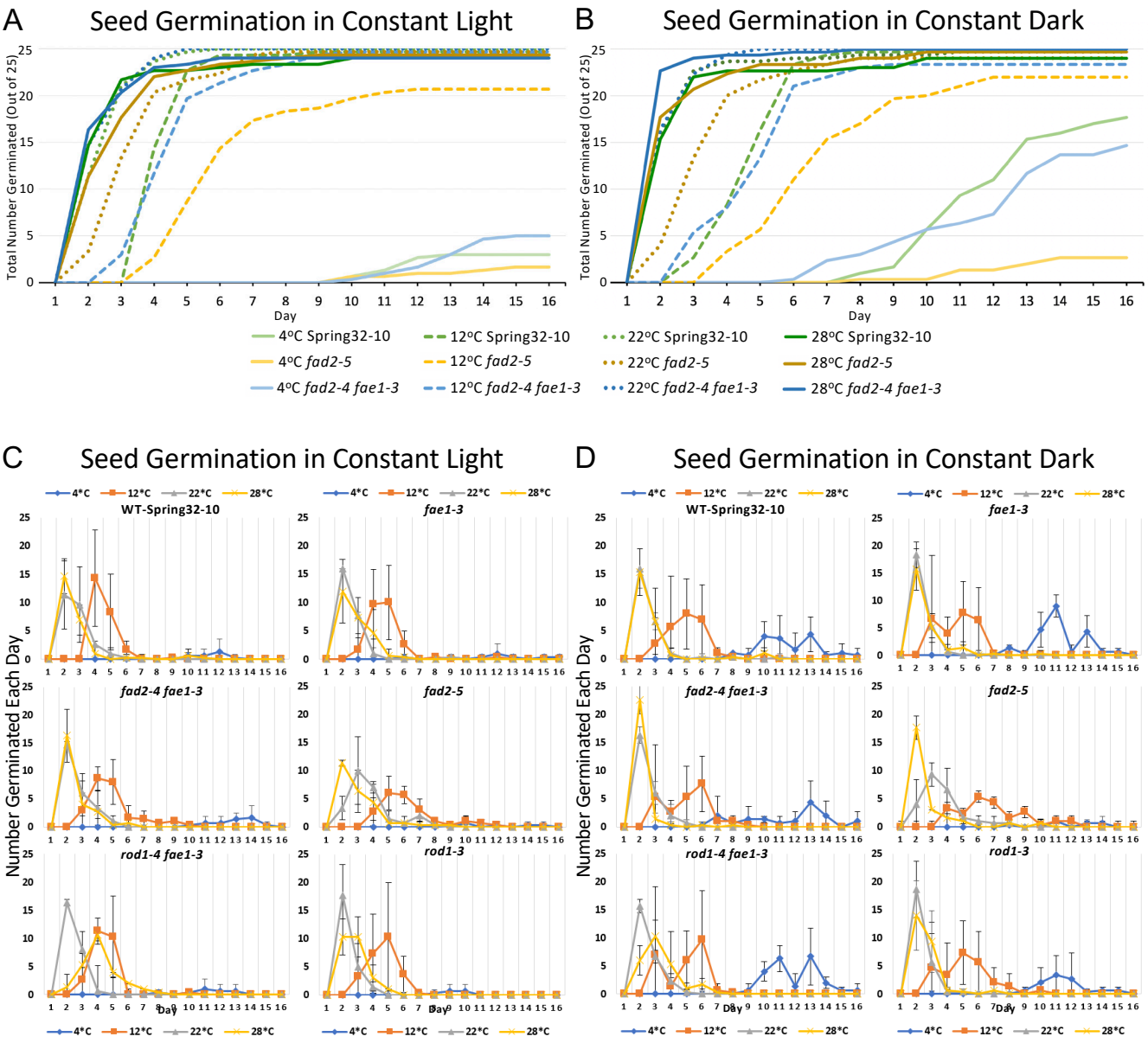

Figure S6A-D. Average amounts of seed germination over a 16-day period under different temperatures and light regimes. Sets of 75 seeds for each genotype were plated onto three agar growth media plates (25 seeds per plate; n = 3) and incubated at 4 ºC, 12 ºC, 22 ºC, or 28 ºC either in constant florescent light (**A, C**) or constant darkness (**B, D**). Seed germination in (**A**) and (**B**) are graphed as cumulative, whereas as (**C**) and (**D**) are graphed as the number that germinated each day. Values and significant differences can be found in Tables S2 and S3. Error bars in (**C**) and (**D**) are standard deviations.

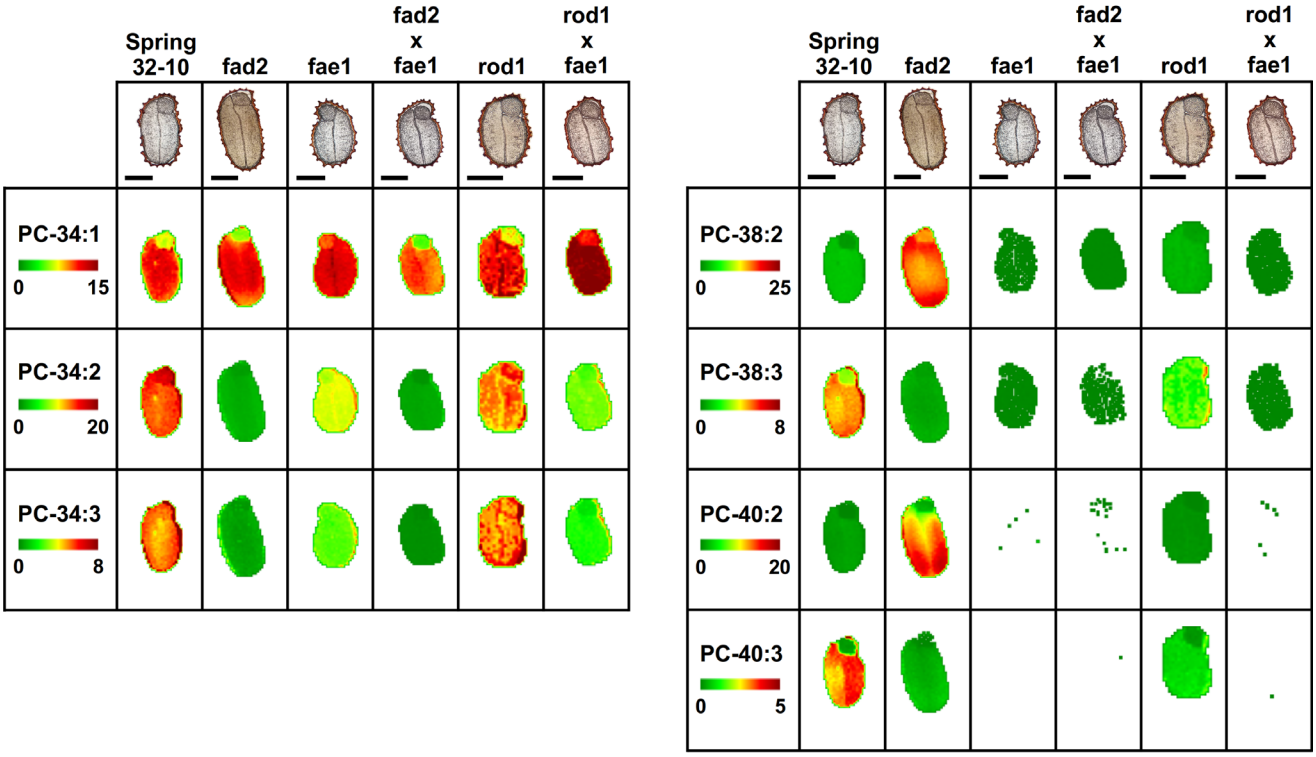

Figure S7. MS imaging of other PC molecular species detected in wild-type Spring 32-10, the various mutants and mutant combinations.

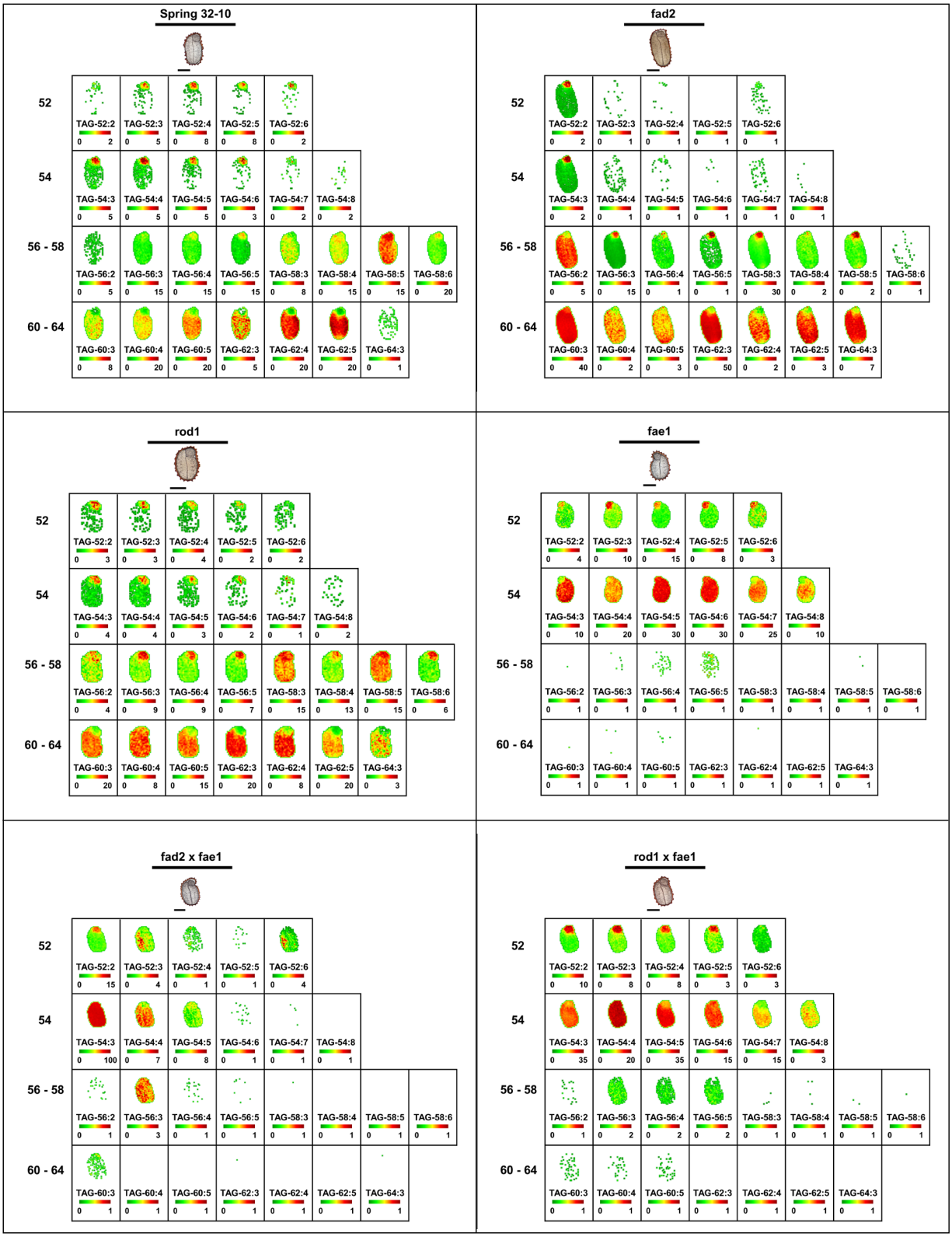

Figure S8. MS imaging of each TAG molecular species detected for wild type and mutant seeds.