

## *Supplemental Material*

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

**Brice A. Jarvis<sup>1</sup>, Trevor B. Romsdahl<sup>2</sup>, Michaela McGinn<sup>1</sup>, Tara J. Nazarenus<sup>3</sup>, Edgar B. Cahoon<sup>3</sup>, Kent D. Chapman<sup>2</sup>, and John C. Sedbrook<sup>1\*</sup>**

<sup>1</sup>School of Biological Sciences, Illinois State University, Normal, IL 61790, USA

<sup>2</sup>BioDiscovery Institute and Department of Biological Sciences, University of North Texas, Denton, TX 76203, USA

<sup>3</sup>Department of Biochemistry and Center for Plant Science Innovation, University of Nebraska-Lincoln, Lincoln, NE 68588 USA

**\*Correspondence:** John Sedbrook, Email: jcseedbr@ilstu.edu

**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.

<b>Genotype</b>	<b>16:0</b>	<b>16:1</b>	<b>18:0</b>	<b>18:1</b>	<b>18:2</b>	<b>18:3</b>	<b>20:1</b>	<b>22:1</b>	<b>24:1</b>
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
<i>fad2-4</i> (-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**
<i>fad2-5</i> (+A)	2.4	0.1	0.4	35.0	0.5	2.5	14.9	39.8	3.8
<i>fad2-6</i> (-29bp)	2.5	0.1	0.4	34.0	0.5	2.7	14.2	41.4	3.6
<i>rod1-3</i> (-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**
<i>rod1-4</i> (+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
<i>rod1-5</i> (+T)	4.3	0.0	0.5	23.1	10.5	12.5	14.3	31.7	2.8
<i>fael-3</i> (-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**
<i>fad2-4 fael-3</i>	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**
<i>rod1-4 fael-3</i>	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.

	<b>Day 1</b>	<b>Day 2</b>	<b>Day 3</b>	<b>Day 4</b>	<b>Day 5</b>	<b>Day 6</b>	<b>Day 7</b>	<b>Day 8</b>	<b>Day 9</b>	<b>Day 10</b>
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
<i>fael-3</i> (-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
<i>rod1-4</i> (+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
<i>fad2-5</i> (+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
<i>fad2-4</i> (-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
<i>rod1-3 fael-3</i>	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
<i>fad2-4 fael-3</i>	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
<i>fad2-5 fael-3</i>	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,  $p < 0.05^*$ ,  $p < 0.01^{**}$ .

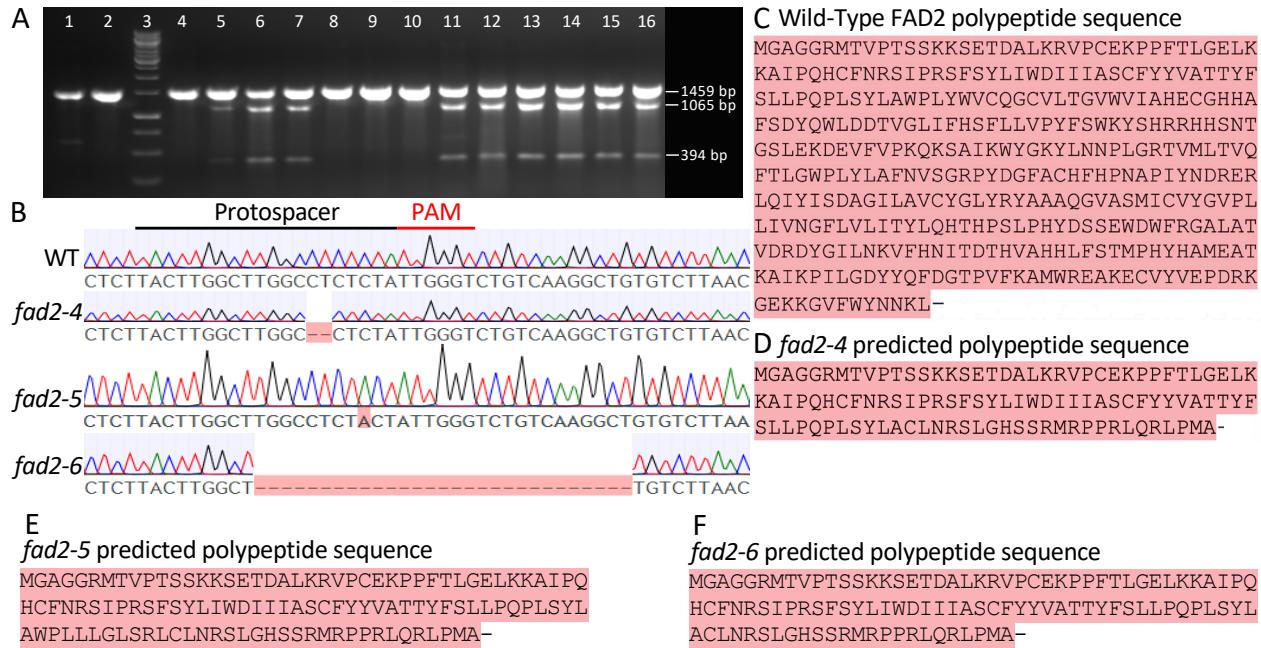
**Table S4.** Amounts of seed germination in constant darkness at different temperatures over a 16-day period. Data can be found in graph form in Figure S5. Values are the means of the number of seeds that germinated on the given day plus/minus standard deviations. 3 biological reps of 25 seeds each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

Martinez/Needleman-Wunsch DNA Alignment

Minimum Match: 9; Gap Penalty: 1.10; Gap Length Penalty: 0.33

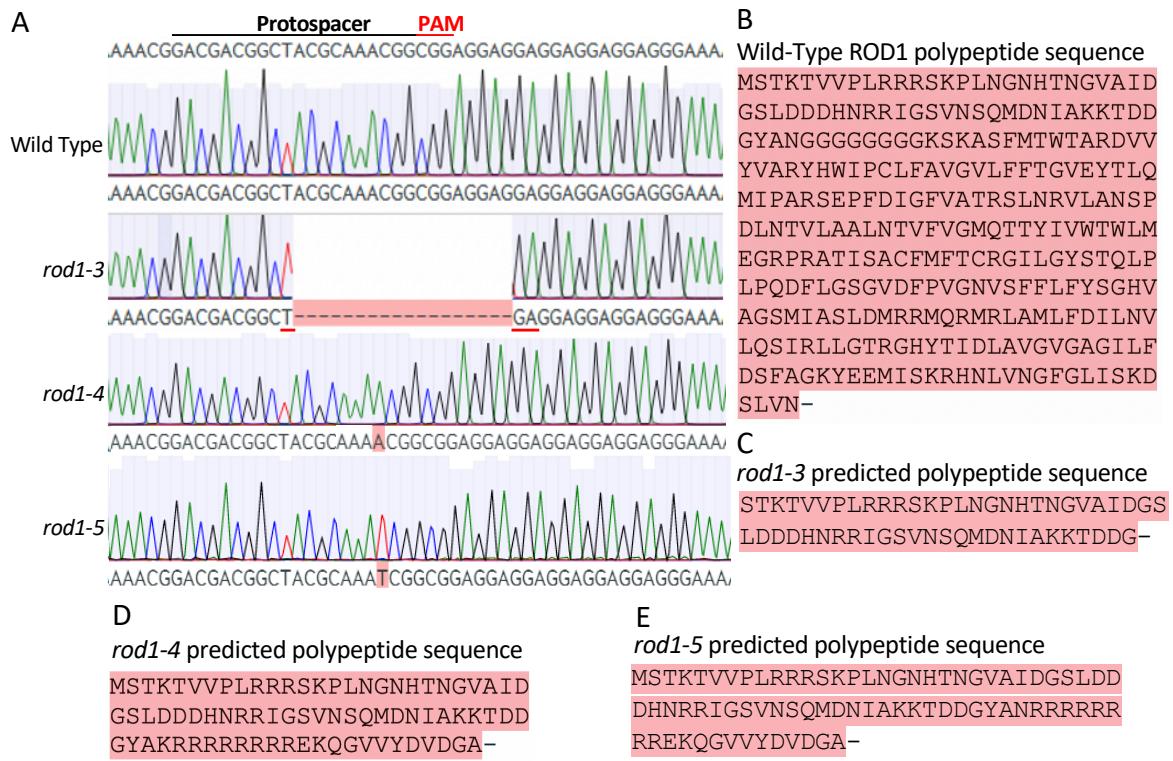
Seq1(1>1152) TaFAD2 ORF GAKE01001774.seq	Seq2(1>1152) AtFAD2 ORF AT3G12120.seq	Similarity	Gap Number	Gap Length	Consensus Length			
(1>1152)	(1>1152)	88.8	0	0	1152			
v10	v20	v30	v40	v50	v60	v70	v80	v90
ATGGGTGCAGGTGGAAGAATGACGGTCTCACTTCTCCAAGAACGATGCCCTAAAGCGTGTGCGAGAACCGCCG	ATGGGTGCAGGTGGAAGAATGCGTTCTACTTCTCCAAGAACGATGCCCTAAAGCGTGTGCGAGAACCGCCG							
ATGGGTGCAGGTGGAAGAATGCGTTCTACTTCTCCAAGAACGATGCCCTAAAGCGAACCACAAAGCGTGTGCGAGAACCGCCG	ATGGGTGCAGGTGGAAGAATGCGTTCTACTTCTCCAAGAACGATGCCCTAAAGCGAACCACAAAGCGTGTGCGAGAACCGCCG							
^10 ^20 ^30 ^40 ^50 ^60 ^70 ^80 ^90	^10 ^20 ^30 ^40 ^50 ^60 ^70 ^80 ^90							
v100	v110	v120	v130	v140	v150	v160	v170	v180
TTCACGCTCGGAGAACTGAAGAAAGCAATCCCACAGCATTGTTCAATCGCTCAATCCCTCGCTTTCTCTACCTTACGGACATC	TTCACGCTCGGAGAACTGAAGAAAGCAATCCCACAGCATTGTTCAATCGCTCAATCCCTCGCTTTCTCTACCTTACGGACATC							
TTC CG T CGGAGA CTGAAGAAAGCAATCCC C GCATTGTTCAA CGCTCAATCCCTCGCTTTCTCTACCTTATC G GACATC	TTC CG T CGGAGA CTGAAGAAAGCAATCCC C GCATTGTTCAA CGCTCAATCCCTCGCTTTCTCTACCTTATC G GACATC							
TTCTCGGTGGGAGACTCTGAAGAAAGCAATCCCACGGCATTGTTCAAACGCTCAATCCCTCGCTTTCTCTACCTTATCAGTGACATC	TTCTCGGTGGGAGACTCTGAAGAAAGCAATCCCACGGCATTGTTCAAACGCTCAATCCCTCGCTTTCTCTACCTTATCAGTGACATC							
^100 ^110 ^120 ^130 ^140 ^150 ^160 ^170 ^180	^100 ^110 ^120 ^130 ^140 ^150 ^160 ^170 ^180							
v190	v200	v210	v220	v230	v240	v250	v260	v270
ATCATAGCCCTTGTCTACTACGTTGCCACCAACTACTTCTCTCTCCAGGCTCTCTACTTGGCTTGGCTCTCTATTTGG	ATCATAGCCCTTGTCTACTACGTTGCCACCAACTACTTCTCTCTCCAGGCTCTCTACTTGGCTTGGCTCTCTATTTGG							
AT ATAGCCCT TGCTTCACTACGTCGCCACCAATTACTTCTCTCTCCAGGCTCTCTACTTGGCTTGGCT CTCTATTGG	AT ATAGCCCT TGCTTCACTACGTCGCCACCAATTACTTCTCTCTCCAGGCTCTCTACTTGGCTTGGCT CTCTATTGG							
ATTATAGCCTCATGCTTCACTACGTCGCCACCAATTACTTCTCTCTCCAGGCTCTCTACTTGGCTTGGCCACTCTATTGG	ATTATAGCCTCATGCTTCACTACGTCGCCACCAATTACTTCTCTCTCCAGGCTCTCTACTTGGCTTGGCCACTCTATTGG							
^190 ^200 ^210 ^220 ^230 ^240 ^250 ^260 ^270	^190 ^200 ^210 ^220 ^230 ^240 ^250 ^260 ^270							
v280	v290	v300	v310	v320	v330	v340	v350	v360
GCTGTCAAGGCTGTGCTTAACGGAGCTGGTCTAGCTCACGAATGCCGCCACCGCCCTCAGCGACTACCAATGGCTGACGAC	GCTGTCAAGGCTGTGCTTAACGGAGCTGGTCTAGCTCACGAATGCCGCCACCGCCCTCAGCGACTACCAATGGCTGACGAC							
G CTGTCAAGGCTGTGTC TAAC GG TCTGGTCTAGAC CACGAATCGGG ACCACCGCATTAGCGACTACCAATGGCTGGATGAC	G CTGTCAAGGCTGTGTC TAAC GG TCTGGTCTAGAC CACGAATCGGG ACCACCGCATTAGCGACTACCAATGGCTGGATGAC							
GCCTGTCAAGGCTGTGCTTAACGGTATCTGGTCTAGCCCACGAATGCCGCCACCGCATTAGCGACTACCAATGGCTGGATGAC	GCCTGTCAAGGCTGTGCTTAACGGTATCTGGTCTAGCCCACGAATGCCGCCACCGCATTAGCGACTACCAATGGCTGGATGAC							
^280 ^290 ^300 ^310 ^320 ^330 ^340 ^350 ^360	^280 ^290 ^300 ^310 ^320 ^330 ^340 ^350 ^360							
v370	v380	v390	v400	v410	v420	v430	v440	v450
ACAGTCGGCTGATCTTCATTCTCTCTCGTCCCTACTTCTCTGGAAATACGCCACGCCGTCACCATTCACCGGATCA	ACAGTCGGCTGATCTTCATTCTCTCTCGTCCCTACTTCTCTGGAAATACGCCACGCCGTCACCATTCACCGGATCA							
ACAGT GGTCT ATCTTCATTC TTCTCTCTCGTCCCTACTTCTCTGGAA TA AG CA CGCCGTCAACATTCCAACAC CGATC	ACAGT GGTCT ATCTTCATTC TTCTCTCTCGTCCCTACTTCTCTGGAA TA AG CA CGCCGTCAACATTCCAACAC CGATC							
ACAGTTGGCTTATCTCCATTCTCCCTCTCGTCCCTACTTCTCTGGAAATAGTCTAGCATGCCGTCAACATTCCAACACTGGATCC	ACAGTTGGCTTATCTCCATTCTCCCTCTCGTCCCTACTTCTCTGGAAATAGTCTAGCATGCCGTCAACATTCCAACACTGGATCC							
^370 ^380 ^390 ^400 ^410 ^420 ^430 ^440 ^450	^370 ^380 ^390 ^400 ^410 ^420 ^430 ^440 ^450							
v460	v470	v480	v490	v500	v510	v520	v530	v540
CTTAAAAGGACGAAGTGTGTCCTAAACAGAAATCCGCCATCAAATGGTACGGCAAGTACCTCAACAACCCCTCTGGACGACCGTG	CTTAAAAGGACGAAGTGTGTCCTAAACAGAAATCCGCCATCAAATGGTACGGCAAGTACCTCAACAACCCCTCTGGACGACCGTG							
CT GAAA GA GAAGT TTTGTCCC AA CAGAAATC GC ATCAA TGGTACGG AA TACCTCAACAAACCCCTCT GGACGCA C TG	CT GAAA GA GAAGT TTTGTCCC AA CAGAAATC GC ATCAA TGGTACGG AA TACCTCAACAAACCCCTCT GGACGCA C TG							
CTCGAAAGAGATGAAGTATTGTCCCAAAGCAGAAATCAGCAATCAAGTGGTACGGGAAATACCTCAACAAACCCCTCTGGACGACATCATG	CTCGAAAGAGATGAAGTATTGTCCCAAAGCAGAAATCAGCAATCAAGTGGTACGGGAAATACCTCAACAAACCCCTCTGGACGACATCATG							
^460 ^470 ^480 ^490 ^500 ^510 ^520 ^530 ^540	^460 ^470 ^480 ^490 ^500 ^510 ^520 ^530 ^540							
v550	v560	v570	v580	v590	v600	v610	v620	v630
ATGTTAACCGTCCAGTTCACCCCTGGCTGGCCCTGTACTTAGCCTCAACGCTCGGGAGACCCCTACGACGGGTTCGCTTGCACTTC	ATGTTAACCGTCCAGTTCACCCCTGGCTGGCCCTGTACTTAGCCTCAACGCTCGGGAGACCCCTACGACGGGTTCGCTTGCACTTC							
ATGTTAACCGTCCAGTT CCT GG TGGCCCTTGTACTTAGCCTT AACGCTCTC GG AGACC TA GACGGGTTCGCTTGCA CTTC	ATGTTAACCGTCCAGTT CCT GG TGGCCCTTGTACTTAGCCTT AACGCTCTC GG AGACC TA GACGGGTTCGCTTGCA CTTC							
ATGTTAACCGTCCAGTTGTCTCGGGCTGGCCCTGTACTTAGCCTTAAACGCTCTCGTGTGACGGGTTCGCTTGCACTTC	ATGTTAACCGTCCAGTTGTCTCGGGCTGGCCCTGTACTTAGCCTTAAACGCTCTCGTGTGACGGGTTCGCTTGCACTTC							
^550 ^560 ^570 ^580 ^590 ^600 ^610 ^620 ^630	^550 ^560 ^570 ^580 ^590 ^600 ^610 ^620 ^630							
v640	v650	v660	v670	v680	v690	v700	v710	v720
CACCCAAACGCTCCCATCTACAACGACCGTGAACGCCCTCAGATATACTCGGATGCTGGTATCTCGCCGCTGTACGGCTCTAC	CACCCAAACGCTCCCATCTACAACGACCGTGAACGCCCTCAGATATACTCGGATGCTGGTATCTCGCCGCTGTACGGCTCTAC							
CCC AACCTCCATCTACAA GACCG GAACGCCCTCAGATATACTCCTCGTGTGACGGGTTCTGCTGTACGGCTCTAC	CCC AACCTCCATCTACAA GACCG GAACGCCCTCAGATATACTCCTCGTGTGACGGGTTCTGCTGTACGGCTCTAC							
TTCCCCAACGCTCCCATCTACAATGACCGAGAACGCCCTCAGATATACTCTCGTGTGACGGGTTCTGCTGTACGGCTCTAC	TTCCCCAACGCTCCCATCTACAATGACCGAGAACGCCCTCAGATATACTCTCGTGTGACGGGTTCTGCTGTACGGCTCTAC							
^640 ^650 ^660 ^670 ^680 ^690 ^700 ^710 ^720	^640 ^650 ^660 ^670 ^680 ^690 ^700 ^710 ^720							
v730	v740	v750	v760	v770	v780	v790	v800	v810
CGTTACGCTGCTGCACAAGGGATGCCCTCGATGATCTGGCTTACGGACTACCGCTTCTGATGACGGGTTCTGCTGTACGATCACA	CGTTACGCTGCTGCACAAGGGATGCCCTCGATGATCTGGCTTACGGACTACCGCTTCTGATGACGGGTTCTGCTGTACGATCACA							
CGTTACGCTGCTGCACAAGGGATGCCCTCGATGATCTGGCTTACGGACTACCGCTTCTGATGACGGGTTCTGCTGTACGATCACA	CGTTACGCTGCTGCACAAGGGATGCCCTCGATGATCTGGCTTACGGACTACCGCTTCTGATGACGGGTTCTGCTGTACGATCACA							
^730 ^740 ^750 ^760 ^770 ^780 ^790 ^800 ^810	^730 ^740 ^750 ^760 ^770 ^780 ^790 ^800 ^810							
v820	v830	v840	v850	v860	v870	v880	v890	v900
TACTTGCGACGACACCATCACCCCTCGTGGCTCACTACGATTCTACAGTGGACTGGCTCAGGGAGCTTGGCTACCGTAGACAGAGAC	TACTTGCGACGACACCATCACCCCTCGTGGCTCACTACGATTCTACAGTGGACTGGCTCAGGGAGCTTGGCTACCGTAGACAGAGAC							
TACTTGCGACGACACCATCACCCCTCGTGGCTCACTACGATTCTACAGTGGACTGGCTCAGGGAGCTTGGCTACCGTAGACAGAGAC	TACTTGCGACGACACCATCACCCCTCGTGGCTCACTACGATTCTACAGTGGACTGGCTCAGGGAGCTTGGCTACCGTAGACAGAGAC							
^820 ^830 ^840 ^850 ^860 ^870 ^880 ^890 ^900	^820 ^830 ^840 ^850 ^860 ^870 ^880 ^890 ^900							
v910	v920	v930	v940	v950	v960	v970	v980	v990
TATGGAATCTGAACAAGGTCTTACACATCACGGACACGCCACGTCGGCTTACCGACCTGTTCTGACGATGCCGATTACCATGCGAT	TATGGAATCTGAACAAGGTCTTACACATCACGGACACGCCACGTCGGCTTACCGACCTGTTCTGACGATGCCGATTACCATGCGAT							
TA GGAATC TGAACAAGGT TTCCACACAT AC GACAC CACGTGGCTCA CACCTGTTCTCGAC ATGCCGATT A GC ATG	TA GGAATC TGAACAAGGT TTCCACACAT AC GACAC CACGTGGCTCA CACCTGTTCTCGAC ATGCCGATT A GC ATG							
TACGGAATCTGAACAAGGTGTTCCACACATTACAGACACACACGTGGCTCATCACCTGTTCTGACAAATGCCGATTATAACGCAATG	TACGGAATCTGAACAAGGTGTTCCACACATTACAGACACACACGTGGCTCATCACCTGTTCTGACAAATGCCGATTATAACGCAATG							
^910 ^920 ^930 ^940 ^950 ^960 ^970 ^980 ^990	^910 ^920 ^930 ^940 ^950 ^960 ^970 ^980 ^990							
v1000	v1010	v1020	v1030	v1040	v1050	v1060	v1070	v108
GAGGCCACGAAGGCATAAAGGGATACTCGGGACTATTACCAAGGTTGATGAAACACCGCTTCTCAAGGGATGTTGGAGGGAGGGCAAG	GAGGCCACGAAGGCATAAAGGGATACTCGGGACTATTACCAAGGTTGATGAAACACCGCTTCTCAAGGGATGTTGGAGGGAGGGCAAG							
GA GC AC AAGGGATAAAGGC AT CT GG GACTATTACCAAGGTTGATGAAACACCGCTTCTCAAGGGATGTTGGAGGGAGGGCAAG	GA GC AC AAGGGATAAAGGC AT CT GG GACTATTACCAAGGTTGATGAAACACCGCTTCTCAAGGGATGTTGGAGGGAGGGCAAG							
GAAGCTACAAAGGGATAAAGGCATAAAGGCATTCTGGAGACTATTACCAAGGTTGATGAAACACCGCTTCTGATGAGGATGTTAGGGAGGCAAAG	GAAGCTACAAAGGGATAAAGGCATAAAGGCATTCTGGAGACTATTACCAAGGTTGATGAAACACCGCTTCTGATGAGGATGTTAGGGAGGCAAAG							
^1000 ^1010 ^1020 ^1030 ^1040 ^1050 ^1060 ^1070 ^108	^1000 ^1010 ^1020 ^1030 ^1040 ^1050 ^1060 ^1070 ^108							
v1090	v1100	v1110	v1120	v1130	v1140	v1150		
GAGTGTGTCTATGTAGAACCGGACAGGGAAAGGTGAGAAGAAAGGTGTTCTGGTACAACAAACAAGGTGTA	GAGTGTGTCTATGTAGAACCGGACAGGGAAAGGTGAGAAGAAAGGTGTTCTGGTACAACAAACAAGGTGTA							
GAGTGT TCTATGTAGAACCGGACAGG AAGGTGAGAAGAAAGGTGTTCTGGTACAACAAACAAGGTGTA	GAGTGT TCTATGTAGAACCGGACAGG AAGGTGAGAAGAAAGGTGTTCTGGTACAACAAACAAGGTGTA							
GAGTGTATCTATGTAGAACCGGACAGGGAAAGGTGACAAGGAAAGGTGTTCTGGTACAACAAACAAGGTGTA	GAGTGTATCTATGTAGAACCGGACAGGGAAAGGTGACAAGGAAAGGTGTTCTGGTACAACAAACAAGGTGTA							
^1090 ^1100 ^1110 ^1120 ^1130 ^1140 ^1150	^1090 ^1100 ^1110 ^1120 ^1130 ^1140 ^1150							

**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.

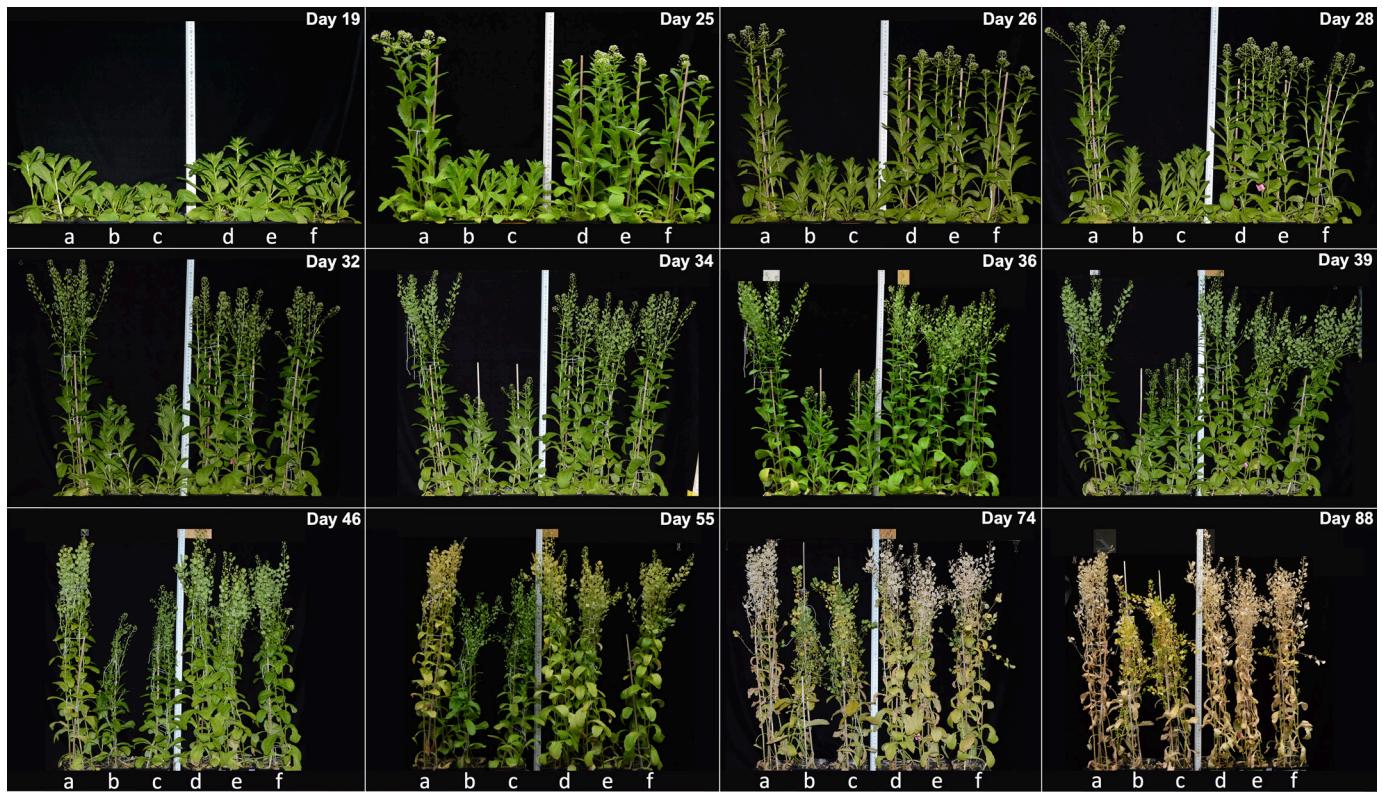


**Figure S2.** Characterization of the CRISPR-induced *Thlaspi arvense* *fad2* mutants' sequences. (A) Electrophoresed T7 endonuclease I-digested PCR products scoring wild type (WT) *FAD2* (1459 bp band) versus *fad2* mutations (partial digestion of 1459 bp band producing 1065 and 394 bp products). Lanes 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/SaCas9 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).

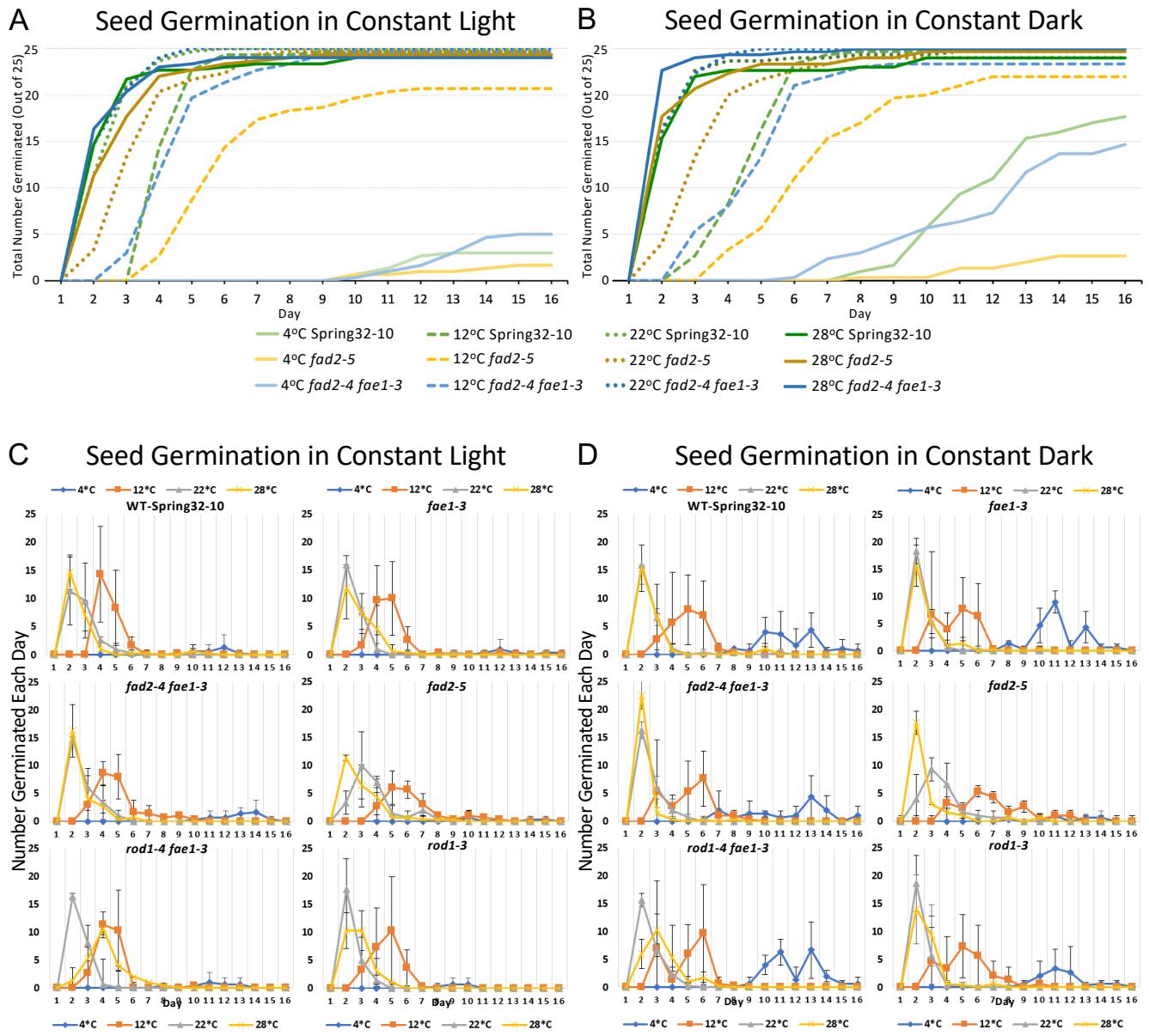
**Figure S3.** Nucleotide sequence alignment of the *Thlaspi arvense* *TaROD1* ORF (top sequence) versus the *Arabidopsis thaliana* *AtROD1* ORF (AT3G15820.1). The *TaROD1* ORF sequence was derived from transcriptome assembly contig GAKE01006801.1 (Dorn et al., 2014), which shares 100% identity with *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.



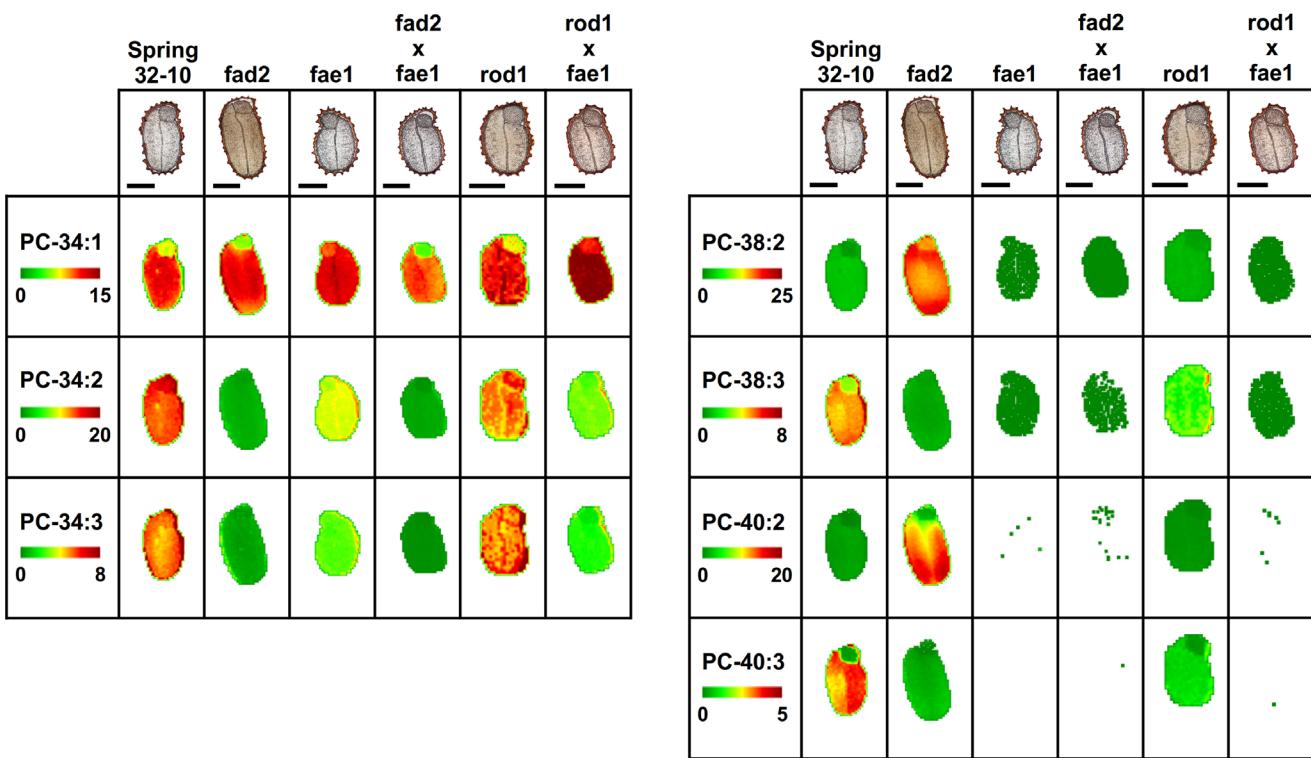
**Figure S4.** Characterization of the CRISPR-induced *Thlaspi arvense* *rod1* mutants' sequences. (A) *Tarod1* DNA sequence chromatograms showing the nature of each mutation. *TaROD1* coding sequences homozygous for wild type (top sequence); 18 bp deletion (*rod1-3*); +A insertion (*rod1-4*); and +T insertion (*rod1-5*). All three mutations are located as expected at the CRISPR/*SpCas9* 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).



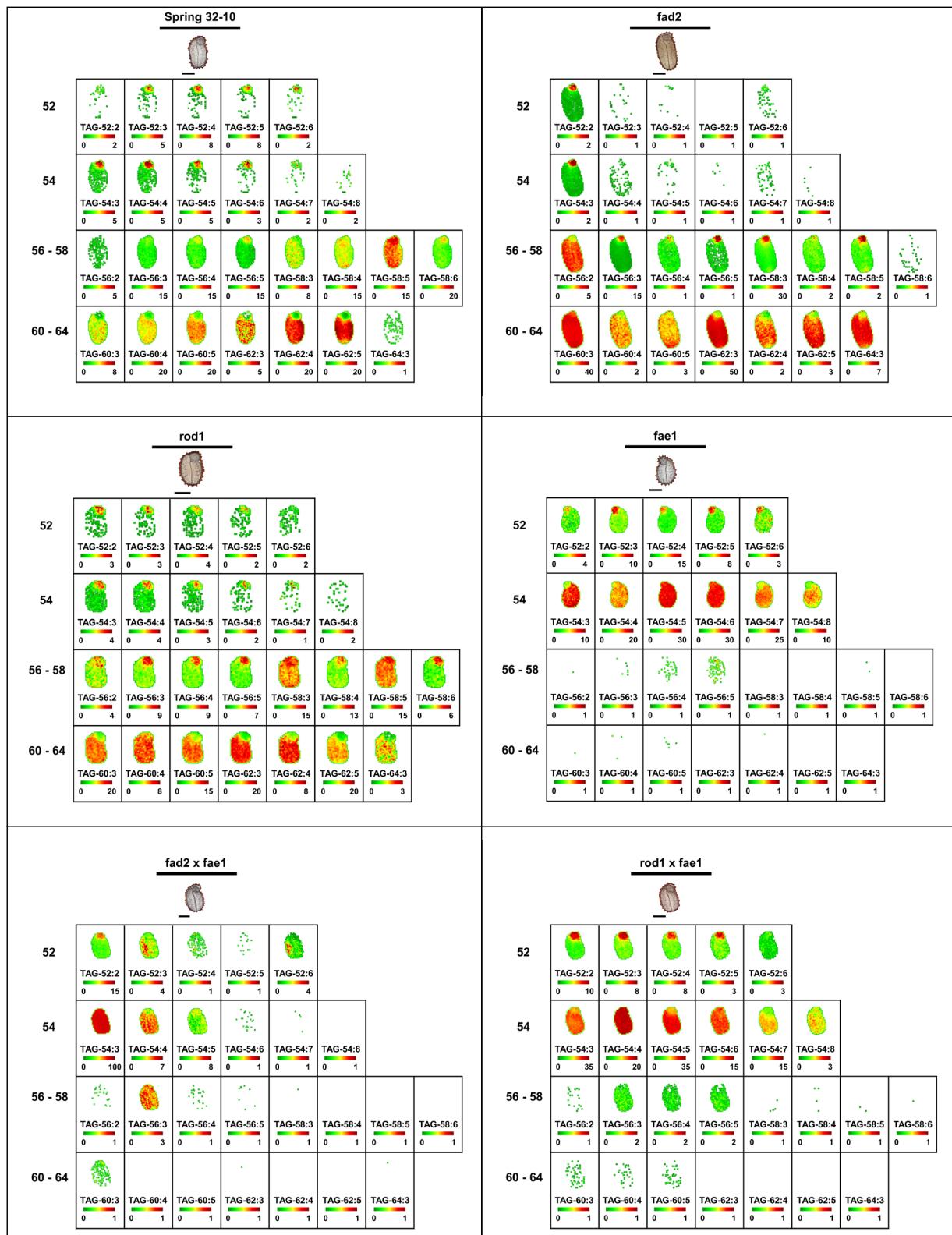
**Figure S5.** Growth time course of different lipid mutants and wild-type plants grown at the same time and conditions from Day 19 until Day 88 of their plant life cycles. Shown are four plants for each genotype growing in four-inch pots. (a) *rod1 fae1*, (b) *fad2 fae1*, (c) *fad2*, (d) Wild-type Spring 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 °C, 12 °C, 22 °C, or 28 °C either in constant fluorescent 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.