

**Supplemental Table S3.** Fatty acid compositions in seeds of *Brassica napus* lines with mutations in *FAD2* genes grown in a greenhouse.

Line	16:00	16:01	18:00	18:01	18:02	18:03	20:00	20:01
HIOL112/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.55	0.42	1.73	87.26	1.25	2.19	0.62	1.21
HIOL112/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.52	0.42	1.71	87.05	1.26	2.31	0.64	1.24
HIOL112/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.6	0.43	1.72	87.05	1.26	2.34	0.62	1.2
HIOL112/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.52	0.4	1.73	87.11	1.24	2.39	0.63	1.24
HIOL112/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.5	0.41	1.69	87.23	1.25	2.36	0.62	1.23
HIOL112/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.76	0.38	1.66	85.6	1.75	3.48	0.6	1.21
HIOL112/HIOL103/HIOL109 (a5a5/c5c5/--)	3.81	0.38	1.67	85.33	1.75	3.5	0.63	1.25
HIOL112/HIOL103/HIOL109 (a5a5/c5c5/--)	3.82	0.4	1.69	85.27	1.81	3.62	0.61	1.2
HIOL112/HIOL103/HIOL109 (a5a5/c5c5/--)	3.75	0.4	1.6	85.81	1.7	3.38	0.6	1.24
HIOL112/HIOL103/HIOL109 (a5a5/c5c5/--)	3.84	0.39	1.65	85.36	1.79	3.54	0.61	1.22
HIOL112/HIOL103/HIOL109 (a5a5/--/c1c1)	4.43	0.47	1.86	74.2	8.33	7.73	0.66	1.06
HIOL112/HIOL103/HIOL109 (a5a5/--/c1c1)	4.5	0.49	1.83	73.8	8.57	8.04	0.61	1.01
HIOL112/HIOL103/HIOL109 (a5a5/--/c1c1)	4.41	0.47	1.82	74.03	8.54	7.85	0.63	1.05
HIOL112/HIOL103/HIOL109 (a5a5/--/c1c1)	4.53	0.48	1.78	73.78	8.58	7.86	0.64	1.07
HIOL112/HIOL103/HIOL109 (a5a5/--/c1c1)	4.48	0.48	1.85	74.03	8.55	7.78	0.62	1.02
HIOL112/HIOL103/HIOL109 (a5a5/--/--)	4.04	0.34	1.67	72.63	10.04	8.27	0.58	1.17
HIOL112/HIOL103/HIOL109 (a5a5/--/--)	4.07	0.34	1.62	72.32	10.3	8.31	0.58	1.18
HIOL112/HIOL103/HIOL109 (a5a5/--/--)	4.06	0.34	1.7	72.79	10.02	8.02	0.61	1.19
HIOL112/HIOL103/HIOL109 (a5a5/--/--)	4.07	0.34	1.7	72.91	10.03	7.91	0.6	1.16
HIOL112/HIOL103/HIOL109 (a5a5/--/--)	4.13	0.35	1.65	72.23	10.3	8.23	0.6	1.19
HIOL112/HIOL103/HIOL109 (--/c5c5/--)	4.81	0.44	1.74	69.1	12.6	8.37	0.62	1.04
HIOL112/HIOL103/HIOL109 (--/c5c5/--)	4.79	0.44	1.79	68.63	12.87	8.49	0.63	1.04
HIOL112/HIOL103/HIOL109 (--/c5c5/--)	4.65	0.42	1.73	69.22	12.57	8.44	0.63	1.06
HIOL112/HIOL103/HIOL109 (--/c5c5/--)	4.71	0.43	1.73	69.11	12.55	8.48	0.63	1.07
HIOL112/HIOL103/HIOL109 (--/c5c5/--)	4.71	0.43	1.69	68.93	12.79	8.49	0.61	1.06
HIOL112/HIOL103/HIOL109 (--/--/c1c1)	4.28	0.33	1.71	63.15	18.48	9.03	0.61	1.06
HIOL112/HIOL103/HIOL109 (--/--/c1c1)	4.28	0.34	1.75	63.07	18.54	9.14	0.58	1.02
HIOL112/HIOL103/HIOL109 (--/--/c1c1)	4.25	0.34	1.77	63.71	18.13	8.95	0.58	1.02
HIOL112/HIOL103/HIOL109 (--/--/c1c1)	4.25	0.34	1.78	63.3	18.3	9.16	0.59	1.01
HIOL112/HIOL103/HIOL109 (--/--/c1c1)	4.37	0.34	1.75	63.21	18.44	9.03	0.59	1
HIOL112/HIOL103/HIOL109 (--/--/--) Wild type	4.96	0.43	1.74	62.24	18.53	9.17	0.62	1
HIOL112/HIOL103/HIOL109 (--/--/--) Wild type	5.08	0.44	1.71	61.72	18.77	9.17	0.64	1.03
HIOL112/HIOL103/HIOL109 (--/--/--) Wild type	4.97	0.42	1.65	62.07	18.71	9.1	0.62	1.05
HIOL112/HIOL103/HIOL109 (--/--/--) Wild type	5.01	0.45	1.7	61.62	19	9.27	0.62	1
HIOL112/HIOL103/HIOL109 (--/--/--) Wild type	5.08	0.44	1.63	61.51	19.26	9.09	0.61	1.01
HIOL112/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.62	0.37	1.42	86.45	1.31	2.69	0.62	1.39
HIOL113/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.66	0.39	1.37	86.54	1.33	2.68	0.59	1.38
HIOL113/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.65	0.38	1.4	86.41	1.31	2.72	0.61	1.42
HIOL113/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.66	0.39	1.38	86.5	1.31	2.7	0.58	1.39
HIOL113/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.7	0.39	1.37	86.4	1.31	2.72	0.59	1.41
HIOL113/HIOL103/HIOL109 (a5a5/c5c5/--)	3.46	0.33	1.68	85.83	1.64	3.67	0.61	1.22
HIOL113/HIOL103/HIOL109 (a5a5/c5c5/--)	3.49	0.35	1.69	85.66	1.71	3.69	0.61	1.22
HIOL113/HIOL103/HIOL109 (a5a5/c5c5/--)	3.5	0.32	1.69	85.42	1.71	3.8	0.64	1.26
HIOL113/HIOL103/HIOL109 (a5a5/c5c5/--)	3.53	0.33	1.66	85.6	1.69	3.67	0.63	1.26
HIOL113/HIOL103/HIOL109 (a5a5/c5c5/--)	3.52	0.34	1.69	85.4	1.76	3.71	0.63	1.28
HIOL113/HIOL103/HIOL109 (a5a5/--/c1c1)	4.02	0.36	1.7	74.71	8.01	8.28	0.6	1.11
HIOL113/HIOL103/HIOL109 (a5a5/--/c1c1)	4.01	0.36	1.71	74.88	7.76	8.19	0.64	1.18
HIOL113/HIOL103/HIOL109 (a5a5/--/c1c1)	4	0.36	1.74	75.1	7.77	8.16	0.6	1.12
HIOL113/HIOL103/HIOL109 (a5a5/--/c1c1)	3.98	0.36	1.67	75.04	7.9	8.11	0.59	1.12
HIOL113/HIOL103/HIOL109 (a5a5/--/c1c1)	4.04	0.37	1.68	75.07	8.04	7.86	0.61	1.12
HIOL113/HIOL103/HIOL109 (--/c5c5/c1c1)	4.15	0.36	1.86	70.97	10.52	9.06	0.65	1.18
HIOL113/HIOL103/HIOL109 (a5a5/--/--)	4.18	0.37	1.76	72.94	9.07	8.57	0.64	1.18
HIOL113/HIOL103/HIOL109 (a5a5/--/--)	4.14	0.35	1.77	73.35	9.04	8.54	0.59	1.08
HIOL113/HIOL103/HIOL109 (a5a5/--/--)	4.16	0.36	1.74	73.35	9.01	8.53	0.59	1.1
HIOL113/HIOL103/HIOL109 (a5a5/--/--)	4.21	0.37	1.75	73.03	9.31	8.47	0.6	1.1
HIOL113/HIOL103/HIOL109 (a5a5/--/--)	4.15	0.35	1.75	72.94	9.21	8.73	0.59	1.1

Line	16:00	16:01	18:00	18:01	18:02	18:03	20:00	20:01
HIOL114/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.93	0.34	1.88	70.22	11.52	9.17	0.64	1.08
HIOL114/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	4	0.33	1.84	70.73	11.36	8.98	0.59	1.01
HIOL114/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	4.04	0.35	1.81	70.62	11.48	8.94	0.58	1.04
HIOL114/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	4.04	0.34	1.8	70.49	11.64	8.92	0.58	1.02
HIOL114/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.99	0.35	1.81	70.83	11.4	8.87	0.58	1.02
HIOL114/HIOL103/HIOL109 (a5a5/c5c5/--)	4.21	0.34	1.83	68.61	12.7	9.63	0.59	0.93
HIOL114/HIOL103/HIOL109 (a5a5/c5c5/--)	4.17	0.34	1.88	68.77	12.33	9.76	0.6	0.94
HIOL114/HIOL103/HIOL109 (a5a5/c5c5/--)	4.24	0.35	1.85	68.95	12.33	9.66	0.59	0.94
HIOL114/HIOL103/HIOL109 (a5a5/c5c5/--)	4.3	0.36	1.83	68.52	12.77	9.53	0.59	0.92
HIOL114/HIOL103/HIOL109 (a5a5/c5c5/--)	4.21	0.34	1.87	69.08	12.18	9.58	0.6	0.92
HIOL114/HIOL103/HIOL109 (a5a5/--/c1c1)	4.22	0.34	2.04	62.33	18.27	10.22	0.6	0.86
HIOL114/HIOL103/HIOL109 (a5a5/--/c1c1)	4.19	0.34	2.08	62.55	18.24	10.06	0.6	0.87
HIOL114/HIOL103/HIOL109 (a5a5/--/c1c1)	4.21	0.33	2.05	62.21	18.62	9.98	0.61	0.87
HIOL114/HIOL103/HIOL109 (a5a5/--/c1c1)	4.22	0.34	2.12	62.45	18.24	10	0.62	0.87
HIOL114/HIOL103/HIOL109 (a5a5/--/c1c1)	4.21	0.35	2.04	62.65	18.16	10	0.61	0.86
HIOL114/HIOL103/HIOL109 (a5a5/--/--)	4.33	0.35	1.69	60.86	19.25	10.86	0.55	0.91
HIOL114/HIOL103/HIOL109 (a5a5/--/--)	4.32	0.36	1.67	61.01	19.4	10.61	0.54	0.9
HIOL114/HIOL103/HIOL109 (a5a5/--/--)	4.35	0.36	1.66	61.16	19.25	10.6	0.54	0.9
HIOL114/HIOL103/HIOL109 (a5a5/--/--)	4.35	0.35	1.63	60.87	19.59	10.6	0.53	0.88
HIOL114/HIOL103/HIOL109 (a5a5/--/--)	4.3	0.36	1.67	61.26	19.16	10.6	0.55	0.91
HIOL116/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	4.03	0.37	1.82	76.93	6.76	7.27	0.6	1.05
HIOL116/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.96	0.36	1.77	77.31	6.6	7.23	0.59	1.03
HIOL116/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.99	0.36	1.76	77.35	6.49	7.29	0.58	1.05
HIOL116/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.96	0.36	1.76	77.33	6.57	7.33	0.57	1.03
HIOL116/HIOL103/HIOL109 (a5a5/c5c5/c1c1)	3.99	0.34	1.8	77.17	6.48	7.4	0.61	1.06
HIOL116/HIOL103/HIOL109 (a5a5/c5c5/--)	4.2	0.35	1.78	74.85	8.04	7.96	0.59	1
HIOL116/HIOL103/HIOL109 (a5a5/c5c5/--)	4.13	0.34	1.73	75.47	7.84	7.74	0.57	1.03
HIOL116/HIOL103/HIOL109 (a5a5/c5c5/--)	4.07	0.34	1.66	75.56	7.79	7.84	0.56	1.04
HIOL116/HIOL103/HIOL109 (a5a5/c5c5/--)	4.13	0.34	1.74	75.52	7.84	7.73	0.57	1.02
HIOL116/HIOL103/HIOL109 (a5a5/c5c5/--)	4.13	0.33	1.73	75.71	7.65	7.82	0.55	1.01
HIOL116/HIOL103/HIOL109 (a5a5/--/c1c1)	4.02	0.33	1.65	74.52	8.15	8.47	0.55	1.07
HIOL116/HIOL103/HIOL109 (a5a5/--/c1c1)	4.12	0.37	1.65	73.57	9.13	8.34	0.53	1.08
HIOL116/HIOL103/HIOL109 (a5a5/--/c1c1)	4.06	0.34	1.67	74.3	8.65	8.3	0.53	1.06
HIOL116/HIOL103/HIOL109 (a5a5/--/c1c1)	4.03	0.33	1.69	74.8	7.85	8.16	0.63	1.17
HIOL116/HIOL103/HIOL109 (a5a5/--/c1c1)	4.06	0.34	1.69	74.41	8.45	8.35	0.55	1.05
HIOL116/HIOL103/HIOL109 (a5a5/--/--)	4.38	0.34	1.72	66.64	14.76	9.28	0.58	1.02
HIOL116/HIOL103/HIOL109 (a5a5/--/--)	4.46	0.36	1.74	67.44	14.29	9.13	0.55	0.95
HIOL116/HIOL103/HIOL109 (a5a5/--/--)	4.44	0.34	1.71	66	15.23	9.44	0.58	1
HIOL116/HIOL103/HIOL109 (a5a5/--/--)	4.44	0.36	1.75	66.64	14.88	9.26	0.56	0.97
HIOL116/HIOL103/HIOL109 (a5a5/--/--)	4.39	0.34	1.76	67.14	14.54	9.13	0.57	0.98