

Supporting Information for submission

High-frequency and strong *FAD2* co-suppression: A bottleneck to increase

polyunsaturated fatty acids in seeds

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The following Supporting Information is available for this article:

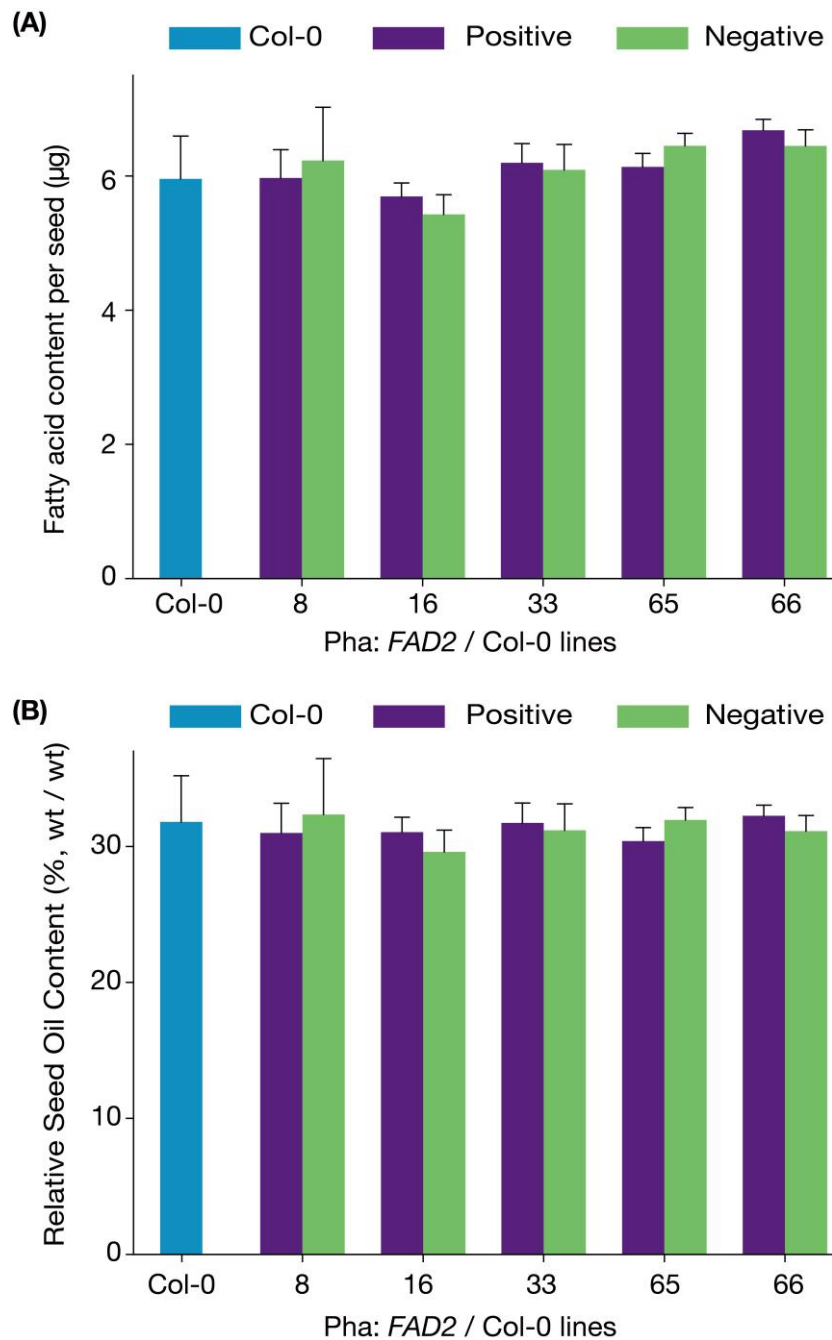


Fig. S1 Fatty acid content per seed **(A)** and relative seed oil content **(B)** of wild type and T₂ Pha: *FAD2* / Col-0 lines.

Positive and negative transgenic seeds of representative T₂ Pha: *FAD2* / Col-0 lines were identified with red fluorescence. Seed weights were determined by using five hundred seeds. Twenty seeds of Col-0, transgenic seeds and non-transgenic seeds were analyzed for oil contents. Pha: *FAD2*/Col-0 refers to *FAD2*, driven by the *Phaseolin* promoter, transferred to wild type Col-0. Error bars indicate SD ($n = 3$).

Table S1 Primers used for mutant genotyping, vector construction and q-RT-PCR

Usage	Primer and sequence (5'-3')
<i>rdr6-11</i>	F: TACTGTCCCTGGCGATCTCT (for sequencing)
Identification	R: CCACCTCACACGTTCTCTT
<i>AtFAD2</i>	AtFAD2-F: ATGGGTGCAGGTGGAAGA
clone	AtFAD2-R: GGTCATAACTTATTGTTGTACCAG
<i>TmFAD2</i>	Sall-TmFAD2-F: <u>GGGGTACCCC</u> ATGGGTGCAGGTGGACGAATGTCTAT
clone	Sall-TmFAD2-R: <u>GGGGTACCCC</u> GGTTCAGTGGTCAAATGAATTATTTCG
<i>BnFAD3</i>	Sall-BnFAD3-F: <u>GGGGTACCCC</u> ATGGTTGTTGCTATGGAC
clone	Sall-BnFAD3-R: <u>GGGGTACCCC</u> TCAATAGAGCTAGGAAGAAAAG
Phaseolin	HindIII-pPha-F: <u>GGGAAGCTT</u> GCCATTGTACTCCCA
promoter	SpeI-pPhaR: <u>CGGACTAGT</u> TAGAGTAGTATTGAATATGAG
SAD2 promoter	pSAD2-F: AGGAGCTCTCAATGTAGTAACACAA
	pSAD2-R: GGACTAGTCCTCTAGATAAAATT
Tubulin	Tub-F: TTTGTGCTCATCTTGCCACGGAAC
For q-RT-PCR	Tub-R: CTCAAGAGGTTCTCAGCAGTACC
Endo FAD2	Endo FAD2-F:CTTCTTCTTCGTAGGGTG
For q-RT-PCR	Endo FAD2-R: TGTTTCTGGAGATGGAGC
Total FAD2	Total FAD2-F:GCTGGATGACACAGTTGGTCTTATCTT
For q-RT-PCR	Total FAD2-R:GGAATGGTGACGGCGATGACTATAC