

Figure S1. *FAD2* cosuppression frequencies and average net change in (18:2+18:3)/18:1 ratios in T_1 seeds in wild type or the designated mutant backgrounds.

(A) Cosuppression frequencies, the lines with a lower average (18:2+18:3)/18:1 ratio than their respective host controls are considered as cosuppression lines.

(B) Average net change of (18:2+18:3)/ 18:1 ratios in T_1 seeds in wild type or the designated mutant backgrounds. Net change refers to the difference in average fatty acid proportions between transgenic seeds and wild type Col-0 or corresponding mutant seeds. The average net change refers to the average net change of all lines detected.

(C) Boxplots showing the distribution of (18:2+18:3)/ 18:1 ratios in T_1 seeds in wild type or the designated mutant backgrounds. The red bars represent values of (18:2 + 18:3)/18:1 ratio in wild type Col-0 seeds and corresponding mutant seeds.

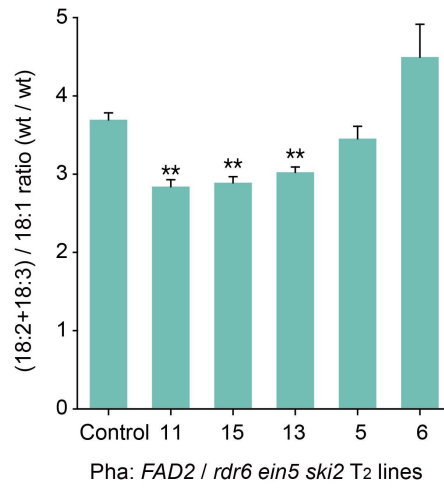


Figure S2. Ratios of (18:2+18:3)/18:1 proportions in seeds of wild type and T₂ Pha::*FAD2/rdr6 ein5 ski2* lines.

Pha::*FAD2/rdr6 ein5 ski2* refers to *FAD2*, driven by the Phaseolin promoter, transferred to the triple *rdr6 ein5 ski2*. Error bars indicate SD (n = 3). Ten T₂ seeds with red fluorescence are used in each replicate. Asterisk (**) indicates a significant difference (t-test, P < 0.01)

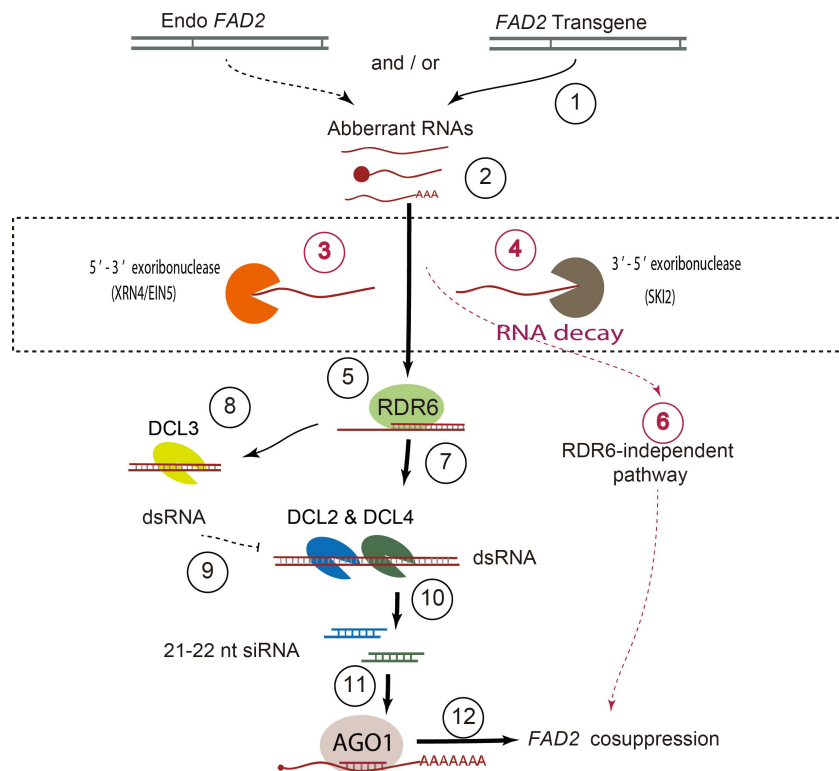


Figure S3. Suggested mechanistic pathway for *FAD2* cosuppression.

- (1) The aberrant RNA triggered by transgene transcription. However, it is uncertain whether active aberrant RNAs are produced from the endogenous gene.
- (2) The active aberrant RNAs are deduced to be incomplete RNA without 5' cap or 3' tail, or both. This hypothesis is supported by the fact that *FAD2* cosuppression is inhibited by EIN5 or SKI2, which degrades aberrant RNA without a 5' cap or 3' tail, respectively.
- (3) and (4) RNA decay system are involved in *FAD2* cosuppression.
- (5) Aberrant RNAs, devoid of degradation by the cytoplasmic RNA decay system, are converted to double-strand RNAs by RDR6. Here, *FAD2* cosuppression is also mediated by RDR6.
- (6) A potential mechanism of *FAD2* cosuppression is independent of RDR6. The weak cosuppression fatty acid phenotype observed in *Pha::FAD2/rdr6 ein5 ski2* lines suggest that the mechanism is inhibited by EIN5 and/or SKI2 and results in a weaker silencing than that created by the RDR6-mediated mechanism.
- (7) Double-strand RNAs, generated by RDR6, are processed into 21-22 nt siRNAs by DCL2 and DCL4. *FAD2* cosuppression depends on the presence of the two dicers, DCL2 and DCL4. Either the DCL2 or DCL4 mutation alone, could not fully release *FAD2* cosuppression.
- (8) and (9) Considering DCL3 could convert double-strand RNA from RDR6 into small RNAs, inhibition of PTGS by DCL3 may occur through completion with substrates of DCL2 and DCL4.
- (10) and (11) 21 and 22 nt siRNAs, generated by DCL2 and DCL4 respectively, result in *FAD2* cosuppression as they account for 80% of the siRNA pool present in cosuppressionors. More importantly, dysfunction of DCL2 and DCL4 release *FAD2* cosuppression
- (12) AGO1 binds siRNAs and slices the target mRNA (*FAD2* in this study).