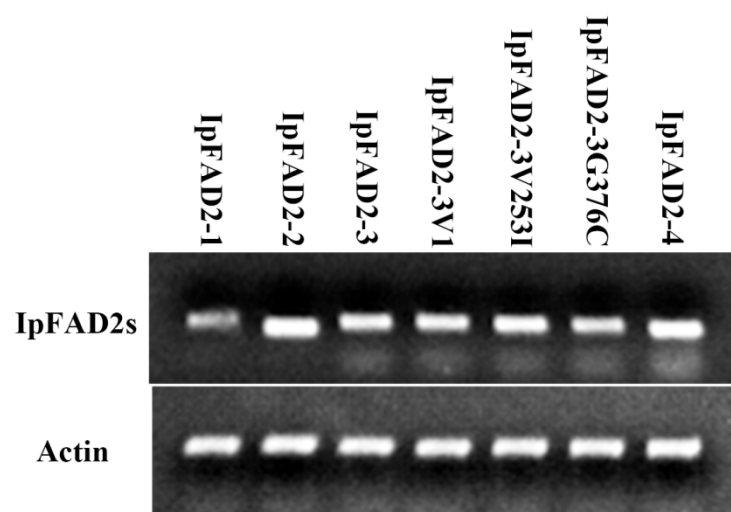
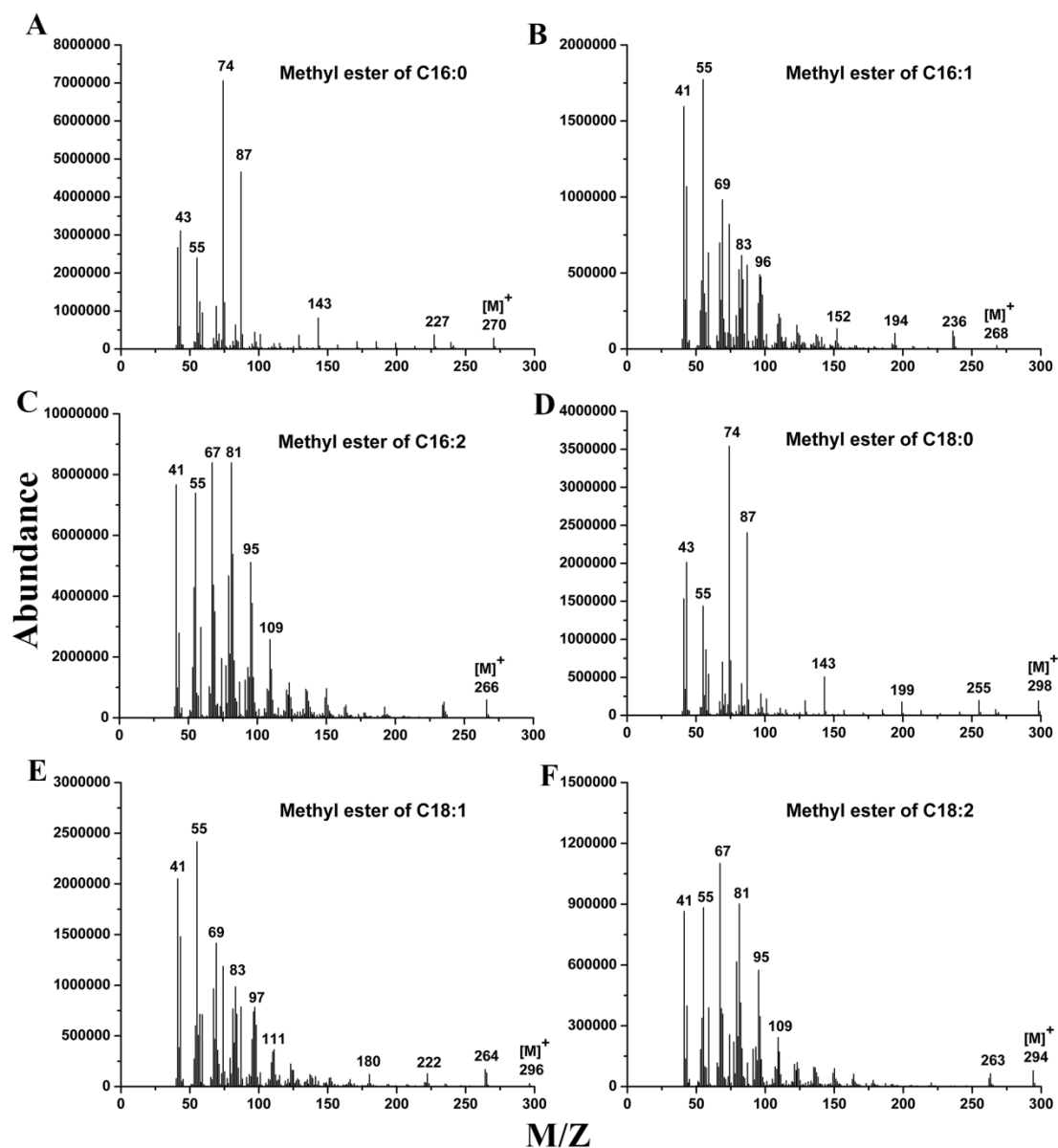
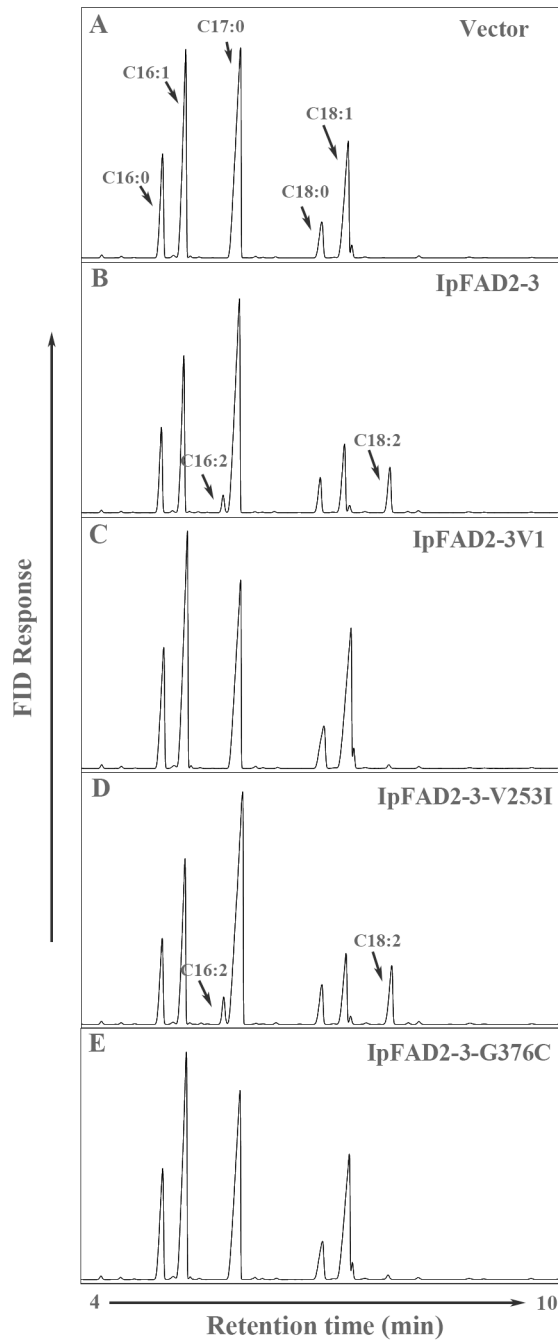




**Supplementary Figure S1.** *IpFAD2s* transcripts in transgenic yeast lines.



**Supplementary Figure S2.** Mass spectrum of fatty acid methyl esters from yeast cells transformed with vector harboring *IpFAD2* genes. Diagnostic fragment ions are labeled in boldface. [M]<sup>+</sup>, Molecular ion.



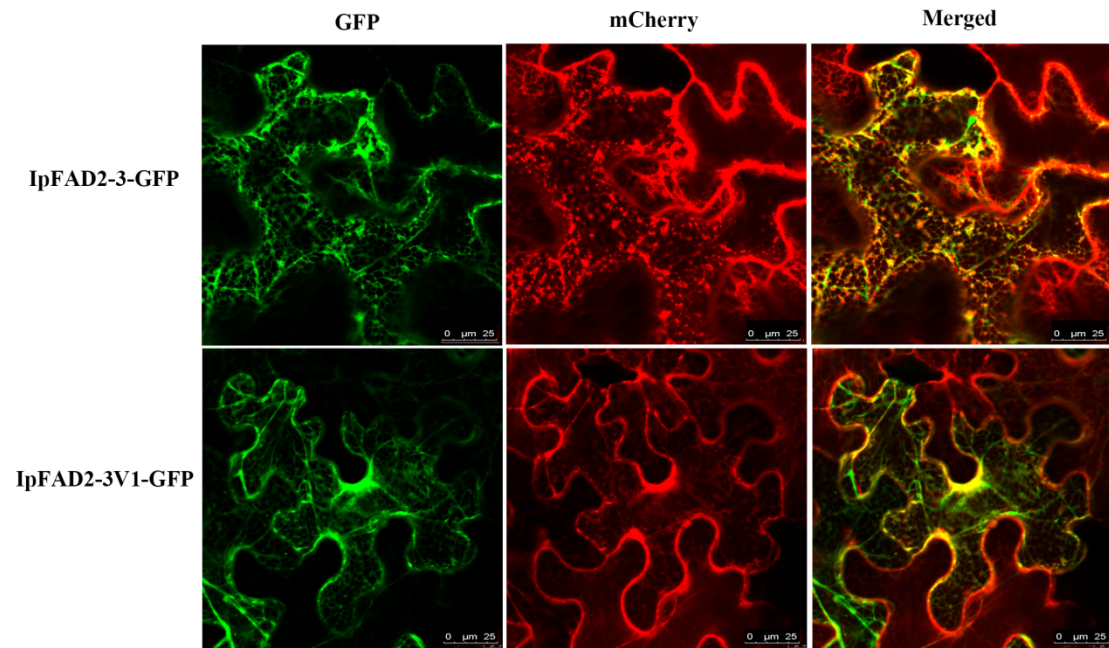
**Supplementary Figure S3.** GC analysis of FAMES isolated from yeast cells expressing IpFAD2-3 and its mutant forms. The FAMES of total lipid were extracted from yeast transformed with empty vector control pESC-his (A), IpFAD2-3 (B), IpFAD2-3V1 (C), IpFAD2-3-V253I (D) and IpFAD2-3-G376C under induction conditions and then analyzed by GC/FID. The peaks of major fatty acid are indicated by arrows. The heptadecanoic acid methyl ester (C17:0) is used as the internal standard.

|            |                                                               |     |
|------------|---------------------------------------------------------------|-----|
| IpFAD2-2   | MGANGVFASSAKHEGKESRIKRMPIYAKPPFTLGKIKKAIPPHCFERSLLRSFSYVGYDLC | 60  |
| IpFAD2-2V1 | MGANGVFASSAKHEGKESRIKRMPIYAKPPFTLGKIKKAIPPHCFERSLLRSFSYVGYDLC | 60  |
| IpFAD2-2V2 | MGANGVFASSAKHEGKESRIKRMPIYAKPPFTLGKIKKAIPPHCFERSLLRSFSYVGYDLC | 60  |
| IpFAD2-2V3 | MGANGVFASSAKHEGKESRIKRMPIYAKPPFTLGKIKKAIPPHCFERSLLRSFSYVGYDLC | 60  |
| IpFAD2-2V4 | MGANGVFASSAKHEGKESRIKRMPIYAKPPFTLGKIKKAIPPHCFERSLLRSFSYVGYDLC | 60  |
| IpFAD2-2V5 | MGANGVFASSAKHEGKESRIKRMPIYAKPPFTLGKIKKAIPPHCFERSLLRSFSYVGYDLC | 60  |
|            | *                                                             |     |
| IpFAD2-2   | ISFLLCYIAITYFHLLPSLAYIAWPIYWILQGCILTGWVWIAHECGHHAFSDYQVDDT    | 120 |
| IpFAD2-2V1 | ISFLLCYIAITYFHLLPSLAYIAWPIYWILQGCILTGWVWIAHECGHHAFSDYQVDDT    | 120 |
| IpFAD2-2V2 | ISFLLCYIAITYFHLLPSLAYIAWPIYWILQGCILTGWVWIAHECGHHAFSDYQVDDT    | 120 |
| IpFAD2-2V3 | ISFLLCYIAITYFHLLPSLAYIAWPIYWILQGCILTGWVWIAHECGHHAFSDYQVDDT    | 120 |
| IpFAD2-2V4 | ISFLLCYIAITYFHLLPSLAYIAWPIYWILQGCILTGWVWIAHECGHHAFSDYQVDDT    | 120 |
| IpFAD2-2V5 | ISFLLCYIAITYFHLLPSLAYIAWPIYWILQGCILTGWVWIAHECGHHAFSDYQVDDT    | 120 |
|            | *                                                             | *   |
| IpFAD2-2   | VGLILHSALFVPYFSWKYSHRRHSNTGSLERDEVFVPKPSRIAUYSKYLNPPGRALS     | 180 |
| IpFAD2-2V1 | VGLILHSALFVPYFSWKYSHRRHSNTGSLERDEVFVPKPSRIAUYSKYLNPPGRALS     | 180 |
| IpFAD2-2V2 | VGLILHSALFVPYFSWKYSHRRHSNTGSLERDEVFVPKPSRIAUYSKYLNPPGRALS     | 180 |
| IpFAD2-2V3 | VGLILHSALFVPYFSWKYSHRRHSNTGSLERDEVFVPKPSRIAUYSKYLNPPGRALS     | 180 |
| IpFAD2-2V4 | VGLILHSALFVPYFSWKYSHRRHSNTGSLERDEVFVPKPSRIAUYSKYLNPPGRALS     | 180 |
| IpFAD2-2V5 | VGLILHSALFVPYFSWKYSHRRHSNTGSLERDEVFVPKPSRIAUYSKYLNPPGRALS     | 180 |
|            | *                                                             |     |
| IpFAD2-2   | LAVTLLLGWPLYLAFNVSGRPYDRFACHYDPHGP IYSDRERLQIYISDLGIFAATFVLVN | 240 |
| IpFAD2-2V1 | LAVTLLLGWPLYLAFNVSGRPYDRFACHYDPHGP IYSDRERLQIYISDLGIFAATFVLVN | 240 |
| IpFAD2-2V2 | LAVTLLLGWPLYLAFNVSGRPYDRFACHYDPHGP IYSDRERLQIYISDLGIFAATFVLVN | 240 |
| IpFAD2-2V3 | LAVTLLLGWPLYLAFNVSGRPYDRFACHYDPHGP IYSDRERLQIYISDLGIFAATFVLVN | 240 |
| IpFAD2-2V4 | LAVTLLLGWPLYLAFNVSGRPYDRFACHYDPHGP IYSDRERLQIYISDLGIFAATFVLVN | 240 |
| IpFAD2-2V5 | LAVTLLLGWPLYLAFNVSGRPYDRFACHYDPHGP IYSDRERLQIYISDLGIFAATFVLVN | 240 |
| IpFAD2-2   | IAMSQGLAFLIC IYGVPLLIVNGFLVTITYLQHTHPSLPHYDSSEWEWLRGALVTMDRDY | 300 |
| IpFAD2-2V1 | IAMSQGLAFLIC IYGVPLLIVNGFLVTITYLQHTHPSLPHYDSSEWEWLRGALVTMDRDY | 300 |
| IpFAD2-2V2 | IAMSQGLAFLIC IYGVPLLIVNGFLVTITYLQHTHPSLPHYDSSEWEWLRGALVTMDRDY | 300 |
| IpFAD2-2V3 | IAMSQGLAFLIC IYGVPLLIVNGFLVTITYLQHTHPSLPHYDSSEWEWLRGALVTMDRDY | 300 |
| IpFAD2-2V4 | IAMSQGLAFLIC IYGVPLLIVNGFLVTITYLQHTHPSLPHYDSSEWEWLRGALVTMDRDY | 300 |
| IpFAD2-2V5 | IAMSQGLAFLIC IYGVPLLIVNGFLVTITYLQHTHPSLPHYDSSEWEWLRGALVTMDRDY | 300 |
|            | *                                                             |     |
| IpFAD2-2   | GILNKVFHNITDTHVTHHLFSTMPHYHATEATKAIKPILGEFYQFDDTP IYKALWRETKE | 360 |
| IpFAD2-2V1 | GILNKVFHNITDTHVTHHLFSTMPHYHATEATKAIKPILGEFYQFDDTP IYKALWRETKE | 360 |
| IpFAD2-2V2 | GILNKVFHNITDTHVTHHLFSTMPHYHATEATKAIKPILGEFYQFDDTP IYKALWRETKE | 360 |
| IpFAD2-2V3 | GILNKVFHNITDTHVTHHLFSTMPHYHATEATKAIKPILGEFYQFDDTP IYKALWRETKE | 360 |
| IpFAD2-2V4 | GILNKVFHNITDTHVTHHLFSTMPHYHATEATKAIKPILGEFYQFDDTP IYKALWRETKE | 360 |
| IpFAD2-2V5 | GILNKVFHNITDTHVTHHLFSTMPHYHATEATKAIKPILGEFYQFDDTP IYKALWRETKE | 360 |
| IpFAD2-2   | CLYVDPDDGAPEKGVFWYRNQF                                        | 382 |
| IpFAD2-2V1 | CLYVDPDDGAPEKGVFWYRNQF                                        | 382 |
| IpFAD2-2V2 | CLYVDPDDGAPEKGVFWYRNQF                                        | 382 |
| IpFAD2-2V3 | CLYVDPDDGAPEKGVFWYRNQF                                        | 382 |
| IpFAD2-2V4 | CLYVDPDDGAPEKGVFWYRNQF                                        | 382 |
| IpFAD2-2V5 | CLYVDPDDGAPEKGVFWYRNQF                                        | 382 |

Supplementary Figure S4. Multiple alignments of predicted amino acid sequences of IpFAD2-2 variants.

|          |            |                                                                |     |
|----------|------------|----------------------------------------------------------------|-----|
| <b>A</b> | IpFAD2-3   | MGAGGRMSVPPSGKVESDVLKRAPDSKPPFTLGQIKKAIPPHCFQRSVLRFSFVYVDLI    | 60  |
|          | IpFAD2-3V1 | MGAGGRMSVPPSGKVESDVLKRAPDSKPPFTLGQIKKAIPPHCFQRSVLRFSFVYVDLI    | 60  |
|          | IpFAD2-3   | IASLFYYVATNYFHLLPHPLPYVTWPIYWAVQGCVLTVGVWVIAHECGHHAFSDYQLDDI   | 120 |
|          | IpFAD2-3V1 | IASLFYYVATNYFHLLPHPLPYVTWPIYWAVQGCVLTVGVWVIAHECGHHAFSDYQLDDI   | 120 |
|          | IpFAD2-3   | VGLTLHSCLLVPYFSWKHSHRRHHSNTGSLDRDEVFVPKQKSGIRWYSKYINNPPGRFLT   | 180 |
|          | IpFAD2-3V1 | VGLTLHSCLLVPYFSWKHSHRRHHSNTGSLDRDEVFVPKQKSGIRWYSKYINNPPGRFLT   | 180 |
|          | IpFAD2-3   | LTITLTGWLPLYLAFNISGRPYDRFACHYDPYGP IYNDREVE IF ISDAGILAVTYGLYR | 240 |
|          | IpFAD2-3V1 | LTITLTGWLPLYLAFNISGRPYDRFACHYDPYGP IYNDREVE IF ISDAGILAVTYGLYR | 240 |
|          | IpFAD2-3   | LAVAKGLAWVLGVGGPLLNVNAFLVLITYLQHTHPSLPHYDSSEWDWLKALATIDRDY     | 300 |
|          | IpFAD2-3V1 | LAVAKGLAWVLGVGGPLLNVNAFLVLITYLQHTHPSLPHYDSSEWDWLKALATIDRDY     | 300 |
| <b>B</b> | IpFAD2-3   | GILNKVFHNITDTHVAHHLFSMMPHYHAMEATKAIKPILGDYYQDGTFFYKAMWREAKE    | 360 |
|          | IpFAD2-3V1 | GILNKVFHNITDTHVAHHLFSMMPHYHAMEATKAIKPILGDYYQDGTFFYKAMWREAKE    | 360 |
|          | IpFAD2-3   | CIYVHPDDDDNQKSKGVFWYTNKLD                                      | 385 |
|          | IpFAD2-3V1 | CIYVHPDDDDNQKSKGVFWYTNKLD                                      | 385 |
|          | IpFAD2-4   | MGANGVFNPNGKDEEKESSESHIKRVTRTKPPFTLSQIKKAIPPHCFERSLLRSFSYVVYD  | 60  |
|          | IpFAD2-4V1 | MGANGVFNPNGKDEEKESSESHIKRVTRTKPPFTLSQIKKAIPPHCFERSLLRSFSYVVYD  | 60  |
|          | IpFAD2-4V2 | MGANGVFNPNGKDEEKESSESHIKRVTRTKELFTLSQIKKAIPPHCFERSLLRSFSYVVYD  | 60  |
|          | IpFAD2-4   | LLITSLLGYIATTFYHLLPSHLAYSANPIYWILQGCILTGLWVIGHECGHHAFSDYQWVD   | 120 |
|          | IpFAD2-4V1 | LLITSLLGYIATTFYHLLPSHLAYSANPIYWILQGCILTGLWVIGHECGHHAFSDYQWVD   | 120 |
|          | IpFAD2-4V2 | LLITSLLGYIVTTFYHLLPSHLAYSANPIYWILQGCILTGLWVIGHECGHHAFSDYQWVD   | 120 |
|          | IpFAD2-4   | DTVGLILHSALFVPYFSWKYSHRRHHSNICLDRDEVFVPKPKFQIPWYSKLLNNPPGRA    | 180 |
|          | IpFAD2-4V1 | DTVGLILHSALFVPYFSWKYSHRRHHSNICLDRDEVFVPKPKFQIPWYSKLLNNPPGRA    | 180 |
|          | IpFAD2-4V2 | DTVGLILHSALFVPYFSWKYSHRRHHSNICLDRDEVFVPKPKFQIPWYSKLLNNPPGRA    | 180 |
|          | IpFAD2-4   | LGLAITLLLGWPLYLTFNASGRPYDRFACHYDPYSP IYSDRERLHIYISDLGIFAATFVL  | 240 |
|          | IpFAD2-4V1 | LGLAITLLLGWPLYLTFNASGRPYDRFACHYDPYSP IYSDRERLHIYISDLGIFAATFVL  | 240 |
|          | IpFAD2-4V2 | LGLAITLLLGWPLYLTFNASGRPYDRFACHYDPYSP IYSDRERLHIYISDLGIFAATFVL  | 240 |
|          | IpFAD2-4   | YSIAVSQQAFLIC IYGVPLLFVNGFLVTITYLQHTHPSLPHYDSTEUDWLRGALVTVD    | 300 |
|          | IpFAD2-4V1 | YSIAVSQQAFLIC IYGVPLLFVNGFLVTITYLQHTHPSLPHYDSTEUDWLRGALVTVD    | 300 |
|          | IpFAD2-4V2 | YSIAVSQQAFLIC IYGVPLLFVNGFLVTITYLQHTHPSLPHYDSTEUDWLRGALVTVD    | 300 |
|          | IpFAD2-4   | DYGILNKVFHNITDTHVAHHLFSTIPHYHAMEATIAIKPVLGEYYQFDATPFYKALUREA   | 360 |
|          | IpFAD2-4V1 | DYGILNKVFHNITDTHVAHHLFSTIPHYHAMEATIAIKPVLGEYYQFDATPFYKALUREA   | 360 |
|          | IpFAD2-4V2 | DYGILNKVFHNITDTHVAHHLFSTIPHYHAMEATIAIKPVLGEYYQFDATPFYKALUREA   | 360 |
|          | IpFAD2-4   | RECLQVEPDEKGVFWYSNKF                                           | 380 |
|          | IpFAD2-4V1 | RECLQVEPDEKGVFWYSNKF                                           | 380 |
|          | IpFAD2-4V2 | RECLQVEPDEKGVFWYSNKF                                           | 380 |

Supplementary Figure S5. Alignment of predicted amino acid sequences of IpFAD2-3 variants (A) and IpFAD2-4 variants (B).



**Supplementary Figure S6.** Subcellular localization of IpFAD2-3 and IpFAD2-3V1. Subcellular localization of IpFAD2-3 and IpFAD2-3V1 with GFP fusion protein in *Nicotiana benthamiana* leaves. Images were taken with a confocal laser scanning microscope. Green, GFP fluorescence image; mCherry, red fluorescent image; Merged, the merged signals simultaneously excited by GFP and mCherry fusion proteins. Bars: 25  $\mu$ m.

**Supplementary Table S1. Sequence identity of the deduced amino acids in FAD2 genes.**

|           | IpFAD2-2 | IpFAD2-3 | IpFAD2-4 | AtFAD2 | GmFAD2-2B | HaFAD2-2 | VfFAD2 |
|-----------|----------|----------|----------|--------|-----------|----------|--------|
| IpFAD2-1  | 73.90%   | 93.02%   | 73.13%   | 76.74% | 83.46%    | 73.90%   | 85.53% |
| IpFAD2-2  |          | 73.13%   | 82.95%   | 70.03% | 74.42%    | 69.77%   | 74.16% |
| IpFAD2-3  |          |          | 71.83%   | 76.49% | 82.69%    | 74.16%   | 85.79% |
| IpFAD2-4  |          |          |          | 67.70% | 72.87%    | 66.15%   | 72.09% |
| AtFAD2    |          |          |          |        | 76.49%    | 71.83%   | 77.52% |
| GmFAD2-2B |          |          |          |        |           | 73.39%   | 82.95% |
| HaFAD2-2  |          |          |          |        |           |          | 73.90% |

The GenBank accession numbers of the proteins represented in this figure are shown in Figure 2.

**Supplementary Table S2. Primers used in this study.**

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|                  |                                                         |
|------------------|---------------------------------------------------------|
| IpFAD2-1-F       | CGGAATTCATGGGTGCTGGTGGAAGAAT                            |
| IpFAD2-1-R       | CGGACTAGTTCAATCAAATTTGTTTCGAGTACC                       |
| IpFAD2-2-F       | CGGAATTCATGGGAGCCAATGGAGTGTTT                           |
| IpFAD2-2-R       | CGGACTAGTTCAAACTGGTTCGGTACCAGAAC                        |
| IpFAD2-3-F       | CGGAATTCATGGGTGCTGGCGGTAGAATGT                          |
| IpFAD2-3-R       | CGGACTAGTTTAATCCAATTTATTTGTGTACC                        |
| IpFAD2-4-F1      | RWMRSKKMDYRRAACAATGGG                                   |
| IpFAD2-4-F2      | AATTTTGGAAAATTCGAATTCATGGGAGCCAATGGAGTGT                |
| IpFAD2-4-R       | TTGTAATCCATCGATACTAGTTTAAACTTGTTACTGTACCAG<br>AAAACCCCT |
| AtFAD2-F         | CGGAATTCATGGGTGCAGGTGGAAGAAT                            |
| AtFAD2-R         | CGGACTAGTTCATAACTTATTGTTGTACCAGT                        |
| VfFAD2-F         | CGGAATTCATGGCTTCCACCACTGCTCTT                           |
| VfFAD2-R         | CGGACTAGTTCAAACTTCTTGTTAT                               |
| GmFAD2-2B-F      | CGGAATTCATGGGGGCTGGTGGCCGAAGTCT                         |
| GmFAD2-2B-R      | CGGACTAGTTCACAACTTATTGTTGTACCAAAAT                      |
| HaFAD2-2-F       | CGGAATTCATGGGTGCCGGCGGGCGGATGTCTG                       |
| HaFAD2-2-R       | CGGACTAGTTCATATCTTGTTACGGTACCAGTAAAC                    |
| mIpFAD2-1-F      | ATAACCAGAAGACAAAAiGCGTATTTTGGT                          |
| mIpFAD2-1-R      | aTTTTGTCTTCTGGTTATCTTCATCTGGAT                          |
| mIpFAD2-2-F      | ATGACGGAGCTCCCGAAAAAiGCGTGTCTGGT                        |
| mIpFAD2-2-R      | aTTTTTCGGGAGCTCCGTCATCTGGATCCACAT                       |
| mAtFAD2-F        | AGGGAAGGTGACAAGAAATGTGTGTACTGGT                         |
| mAtFAD2-R        | ATTTCTTGTCACCTTCCCTGTCCGGTTCT                           |
| mVfFAD2-F        | ATGGTGATGAAAGCAAATGTGTGTACTG                            |
| mVfFAD2-R        | ATTTGCTTTCATCACCATCATCTGCCT                             |
| mGmFAD2-3-F      | AGTACTCAGAGCAAATGTGTATTTTGGT                            |
| mGmFAD2-3-R      | ATTTGCTCTGAGTACTTTGATCTGGCT                             |
| mHaFAD2-2-F      | ATGAGGAGGTTAAGGATtGTGTTTACTGGT                          |
| mHaFAD2-2-R      | aATCCTTAACCTCCTCATCTTTATCAACAT                          |
| IpFAD2-3-V253I-F | CGCTTGGGTCTTTTGTGTTTATGGAGGACCAT                        |
| IpFAD2-3-V253I-R | ATGGTCCTCCATAAACACAAAGAACCCAAGCG                        |
| IpFAD2-3-G376C-F | AACCAGAAGAGCAAAGGCGTATTTTGGT                            |
| IpFAD2-3-G376C-R | CTTTGCTCTTCTGGTTATCATCATCAT                             |
| IpFAD2-3-GFP-F   | CGGACTAGTATGGGTGCTGGCGGTAGAAT                           |
| IpFAD2-3-GFP-R   | AGGCGCGCCTATCCAATTTATTTGTGTACC                          |
| IpFAD2-1-qPCR-F  | AAAGCCATTCCACCTCATTG                                    |
| IpFAD2-1-qPCR-R  | ATAACCCAAACGCCAGTCAG                                    |



|                 |                                 |
|-----------------|---------------------------------|
| IpFAD2-2-qPCR-F | CGGAGGCCACTAAAGCAATC            |
| IpFAD2-2-qPCR-R | GCATCAAAACTGGTTCCGGT            |
| IpFAD2-3-qPCR-F | CTTGGCCAGATCAAGAAAGC            |
| IpFAD2-3-qPCR-R | AACTCCGGTGAGGACACATC            |
| IpFAD2-4-qPCR-F | GTGGGTCGATGACACAGTTG            |
| IpFAD2-4-qPCR-R | ATGGGATTTGGGACTTAGGC            |
| IpEF1A-F        | GCCTGGTATGGTTGTGACCT            |
| IpEF1A-R        | GGATCATCCTTAGAGTTAGA            |
| G376S-F         | ATAACCAGAAGAGCAAAAGCGTATTTTGGT  |
| G376S-R         | TTTGTCTCTTCTGGTTATCATCATCATCT   |
| G376A-F         | ATAACCAGAAGAGCAAAAGCCGTATTTTGGT |
| G376A-R         | GCTTTGCTCTTCTGGTTATCATCATCATCT  |
| Actin-F         | AGTTGCCCCAGAAGAACACC            |
| Actin-R         | TACCGGCAGATTCCAAACCC            |