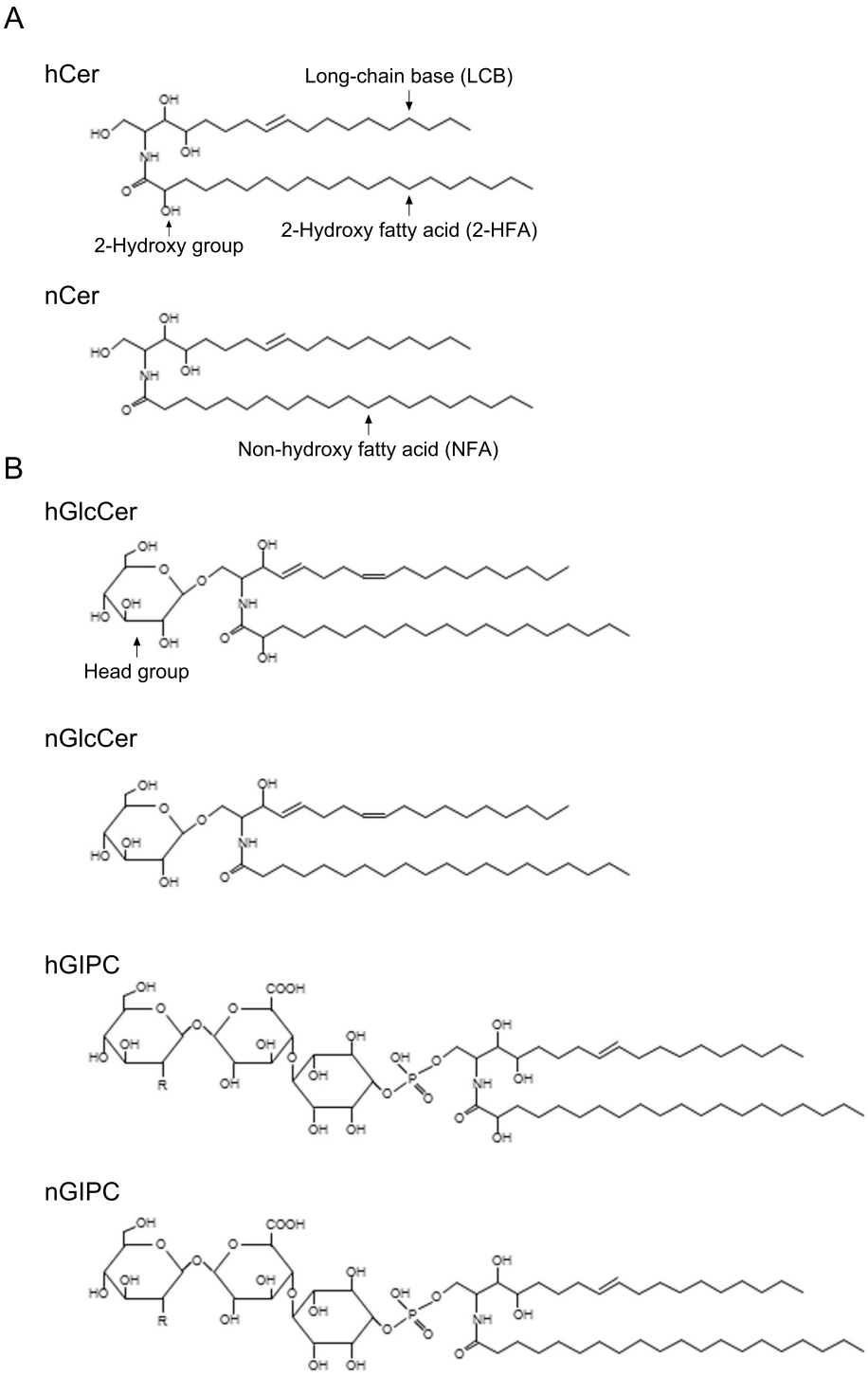
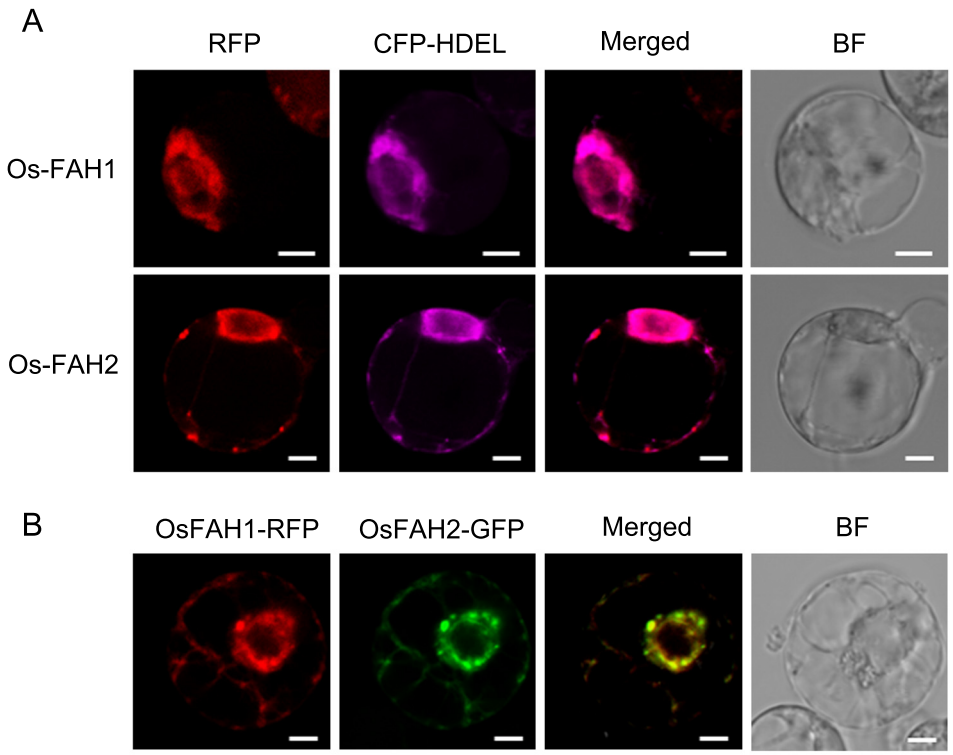
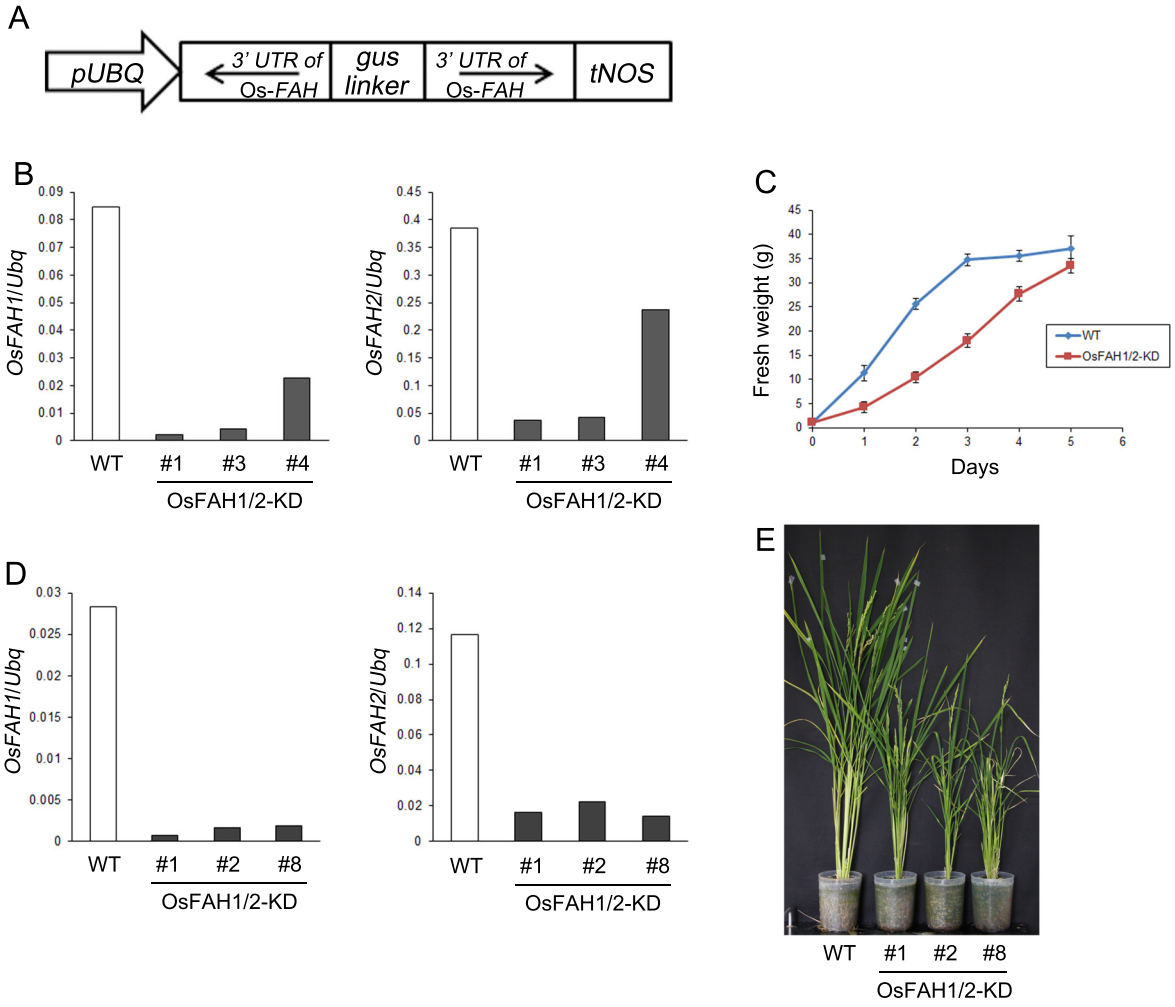


Supplemental Figure 1: Examples of sphingolipids in plants

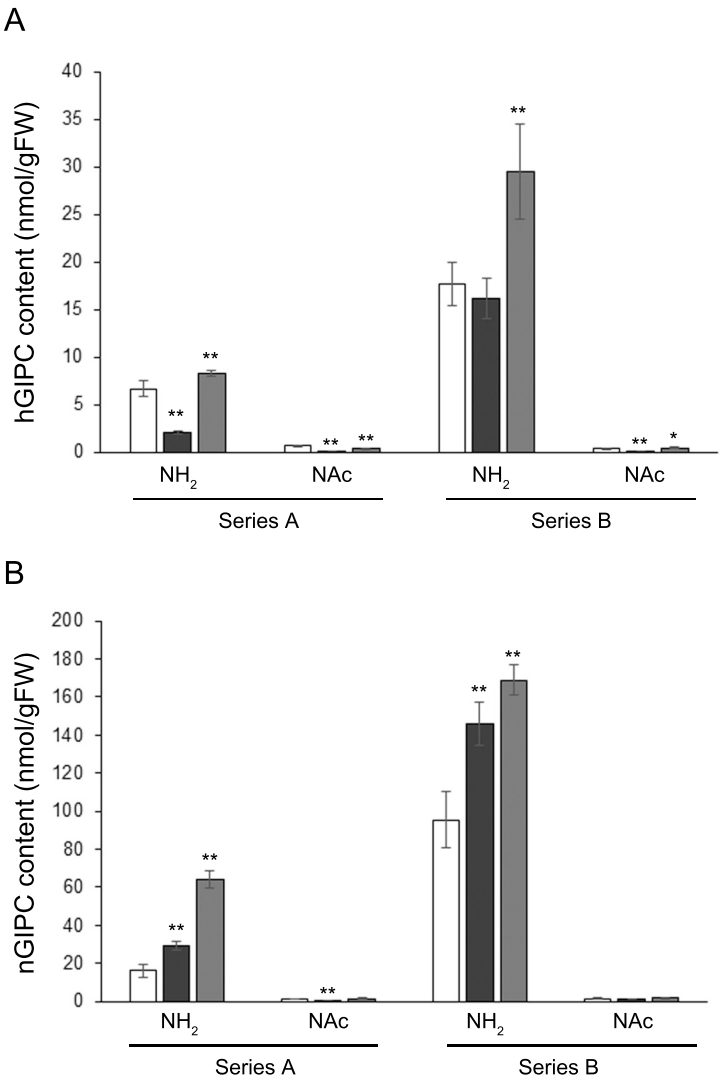
(A) Structures of representative ceramides (Cers). Cers comprise a long-chain base (LCB) amide-linked to a fatty acid. hCer, Cer containing 2-hydroxy fatty acid (2-HFA); nCer, Cer containing non-hydroxy fatty acid (NFA). **(B)** Structures of representative complex sphingolipids. Complex sphingolipids are formed by the addition of head groups to Cers. In plants, glucosylceramide (GlcCer) and glycosylinositolphosphoceramide (GIPC) are exclusive complex sphingolipids. hGlcCer, GlcCer containing 2-HFA; nGlcCer, GlcCer containing NFA; hGIPC, GIPC containing 2-HFA; nGIPC, GIPC containing NFA.



Supplemental Figure 2: Subcellular localization of Os-FAH1 and Os-FAH2 in rice protoplasts
(A) Protoplasts produced from rice suspension culture cells were co-transformed with the plastids encoding CFP-HDEL and either OsFAH1-RFP or OsFAH2-RFP and observed by CLSM. Scale bars = 5 μm. BF, bright field. **(B)** Rice protoplasts were co-transformed with plasmids encoding OsFAH1-RFP and OsFAH2-GFP, and observed by CLSM. Scale bars = 5 μm.

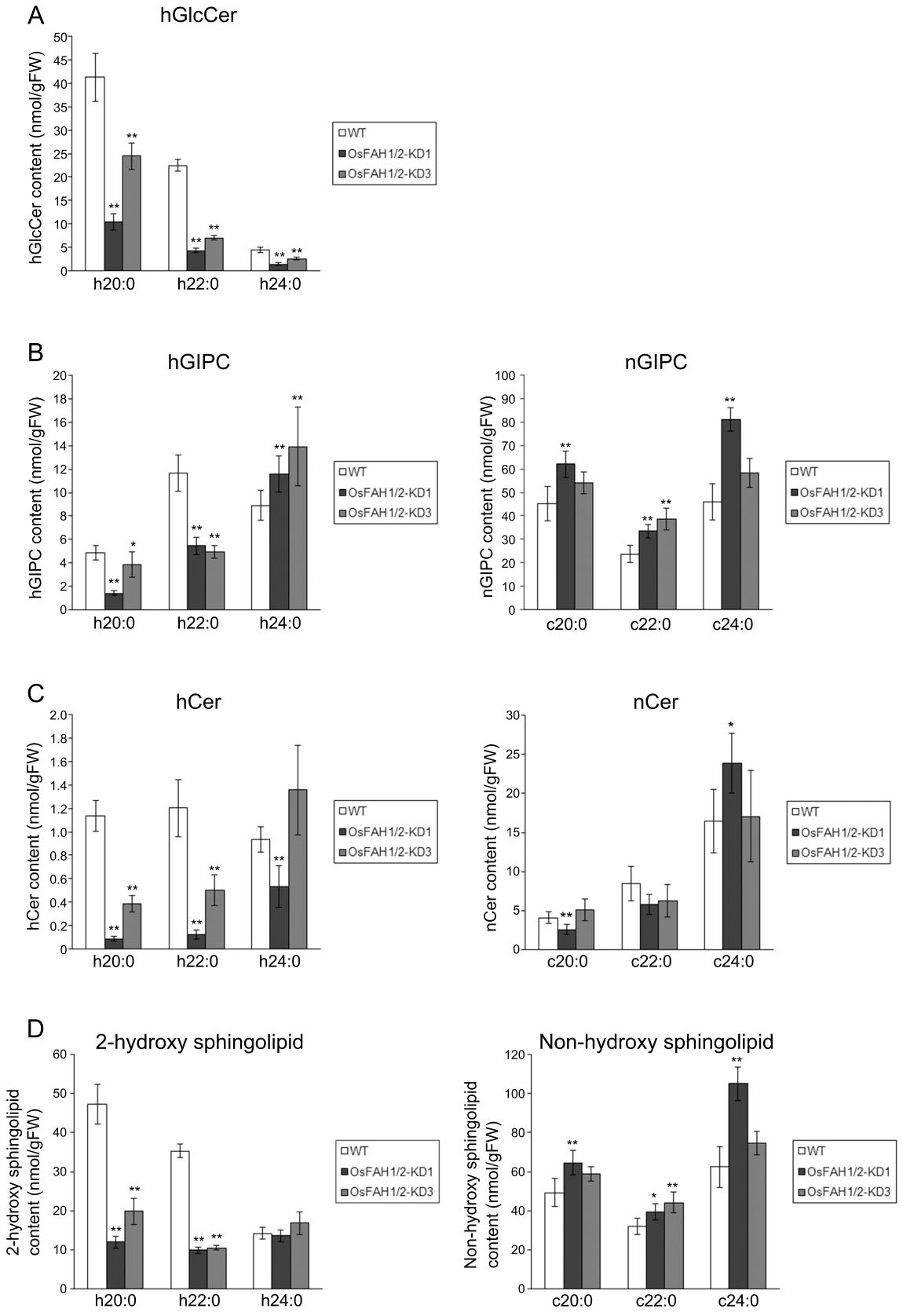


Supplemental Figure 3: Establishment of OsFAH1/2-KD lines using an RNAi system
(A) Schematic image of RNAi constructs. *pUBQ*, ubiquitin promoter, *tNOS*, terminator of the *nopaline synthase* (*NOS*) gene; *gus*, *β-glucuronidase*. **(B)** qRT-PCR of Os-*FAH1* and Os-*FAH2* in OsFAH1/2-KD suspension cells. **(C)** Growth of OsFAH1/2-KD1 suspension cells. **(D)** qRT-PCR of Os-*FAH1* and Os-*FAH2* in OsFAH1/2-KD plants. **(E)** Phenotype of 3-month-old OsFAH1/2-KD plants.

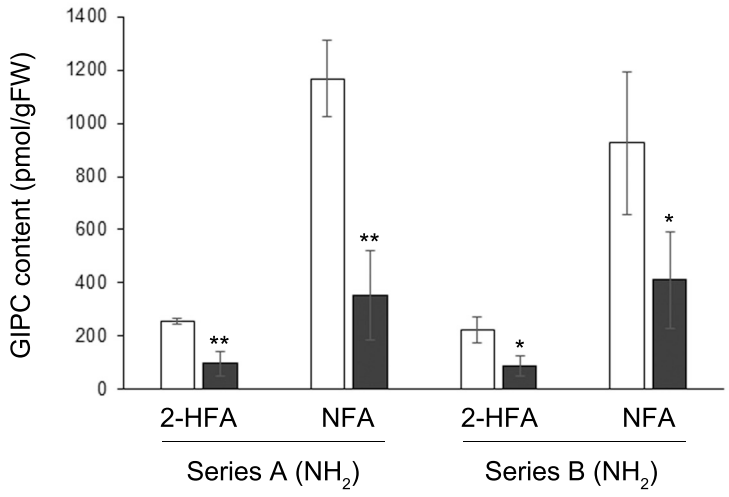


Supplemental Figure 4: GIPC content in whole cells of OsFAH1/2-KD lines

Four types of GIPCs in rice suspension cells were determined by LC-MS/MS. Series A and series B indicate Hex(NH_2 or NAc)-HexA-Ins-P-Cer and Hex-Hex(NH_2 or NAc)-HexA-Ins-P-Cer, respectively, according to Cacas et al. (2013). White, dark gray, or light gray bars indicate WT, OsFAH1/2-KD1 or OsFAH1/2-KD3, respectively.
(A) hGIPCs **(B)** nGIPCs. Data are means ± SD (N = 4). Asterisks indicate significant differences compared to WT (Student's *t*-test; *$p < 0.05$, **$p < 0.01$).

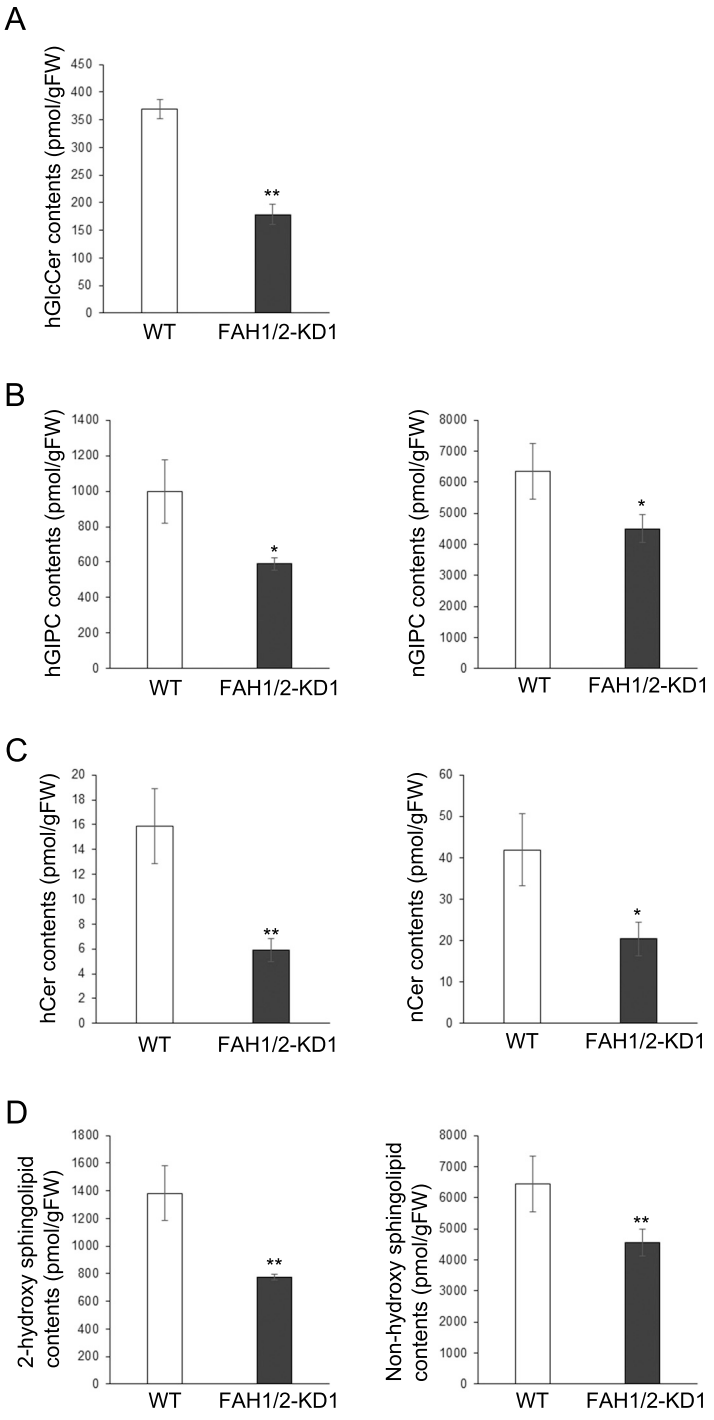


Supplemental Figure 5: Fatty acid content of sphingolipids in OsFAH1/2-KD lines
Sphingolipid fatty acids with 20, 22 or 24 carbon chain length, with or without a 2-hydroxy group, in WT and OsFAH1/2-KD suspension cells were determined by LC-MS/MS and are shown as the amount of each class (**A**, GlcCer; **B**, GIPC; **C**, Cer; **D**, total sphingolipid). Data are means ± SD (N = 4). Asterisks indicate signifiant differences compared to WT (Student's t-test, *$p < 0.05$, **$p < 0.01$).

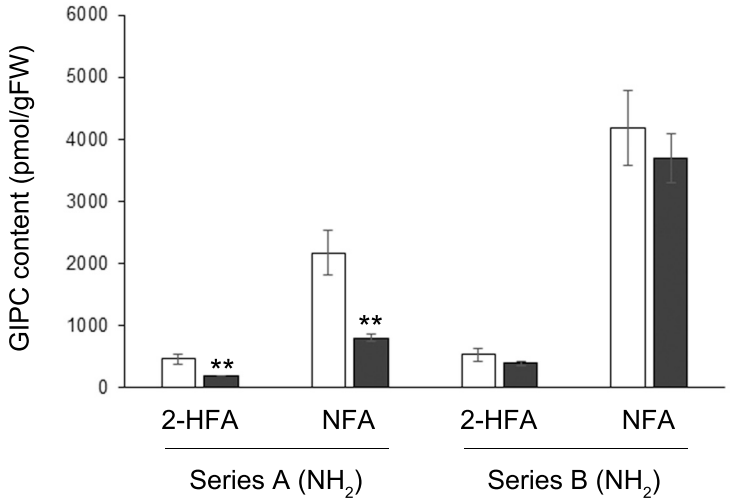


Suppelemental Figure 6: GIPC content in DRM of OsFAH1/2-KD1

Two types of GIPCs in DRM of rice suspension cells (series A (NH_2) and series B (NH_2)) were determined by LC-MS/MS. White or dark gray bars indicate WT or OsFAH1/2-KD1, respectively. Data are means ± SD (N = 3). Asterisks indicate significant differences compared to WT (Student's t-test; *$p < 0.05$, **$p < 0.01$). 2-HFA: 2-hydroxy fatty acid. NFA: non-hydroxy fatty acid. GIPCs containing NAc were too minor to be detected.

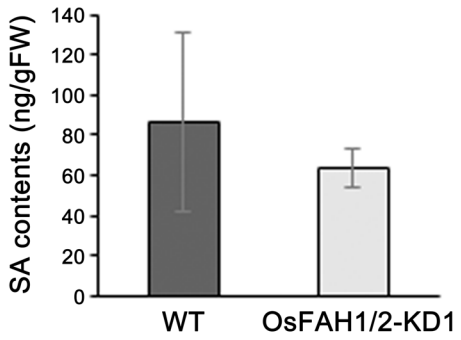


Supplemental Figure 7: Sphingolipid content in PM of OsFAH1/2-KD lines
Amounts of each sphingolipid class (**A**, GlcCer; **B**, GIPC; **C**, Cer; **D**, total sphinglipid) in PM of WT and OsFAH1/2-KD1 suspension cells are shown for 2-HFA- and NFA-containing types. Data are means ± SD ($N = 4$). Asterisks indicate significant differences compared to WT (Student's *t*-test; *$p < 0.05$, **$p < 0.01$).

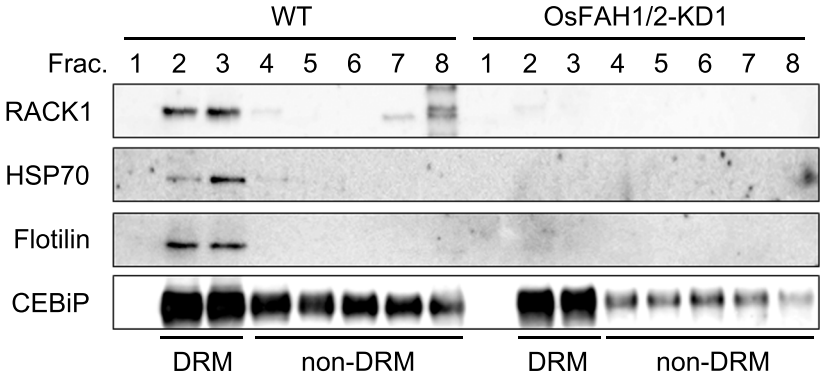


Supplemental Figure 8: GIPC content in PM of OsFAH1/2-KD1

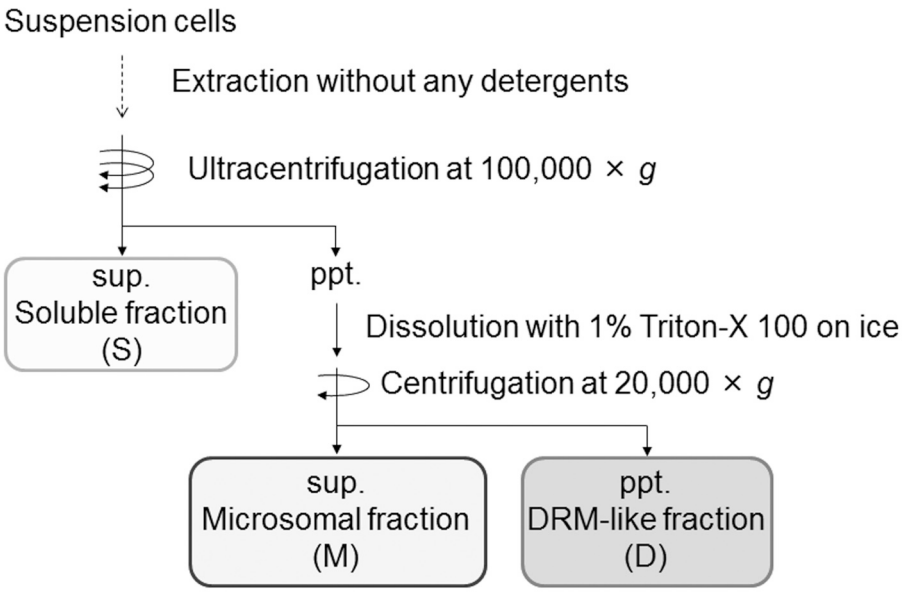
Two types of GIPCs in PM of rice suspension cells (series A (NH_2) and series B (NH_2)) were determined by LC-MS/MS. White or dark gray bars indicate WT or OsFAH1/2-KD1, respectively. Data are means ± SD (N = 3). Asterisks indicate significant differences compared to WT (Student's t-test; $^{**}p < 0.01$). 2-HFA: 2-hydroxy fatty acid. NFA: non-hydroxy fatty acid. GIPCs containing NAc were too minor to be detected.



Supplemental Figure 9: SA content in OsFAH1/2-KD suspension cells
SA was extracted from rice suspension cells and determined by LC-MS/MS. Data are means ± SD (*N* = 4).

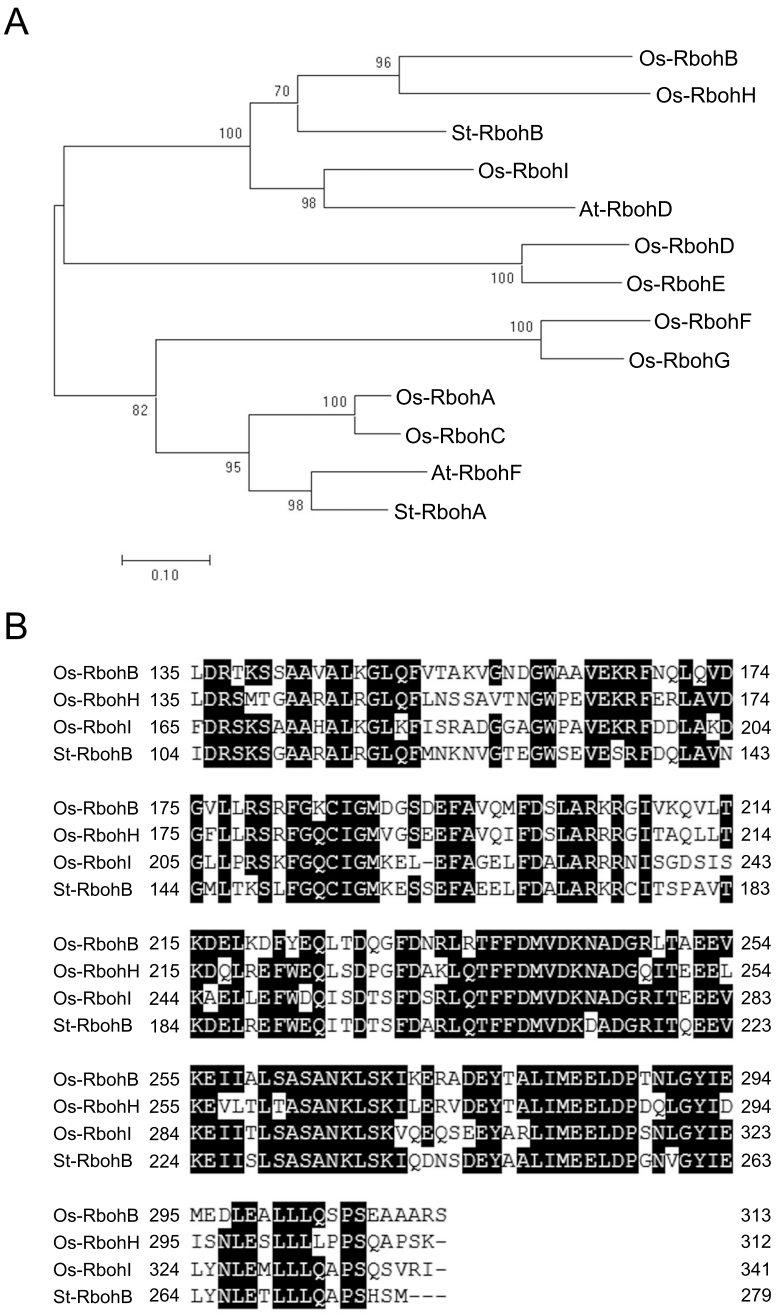


Supplemental Figure 10: Protein analysis of PM fractions
Immunoblot analysis of RACK1, HSP70, Flotilin and CEBiP in PM fractions. Fractions 2 and 3 contain DRMs and fractions 4-8 contain the non-DRM fractions.



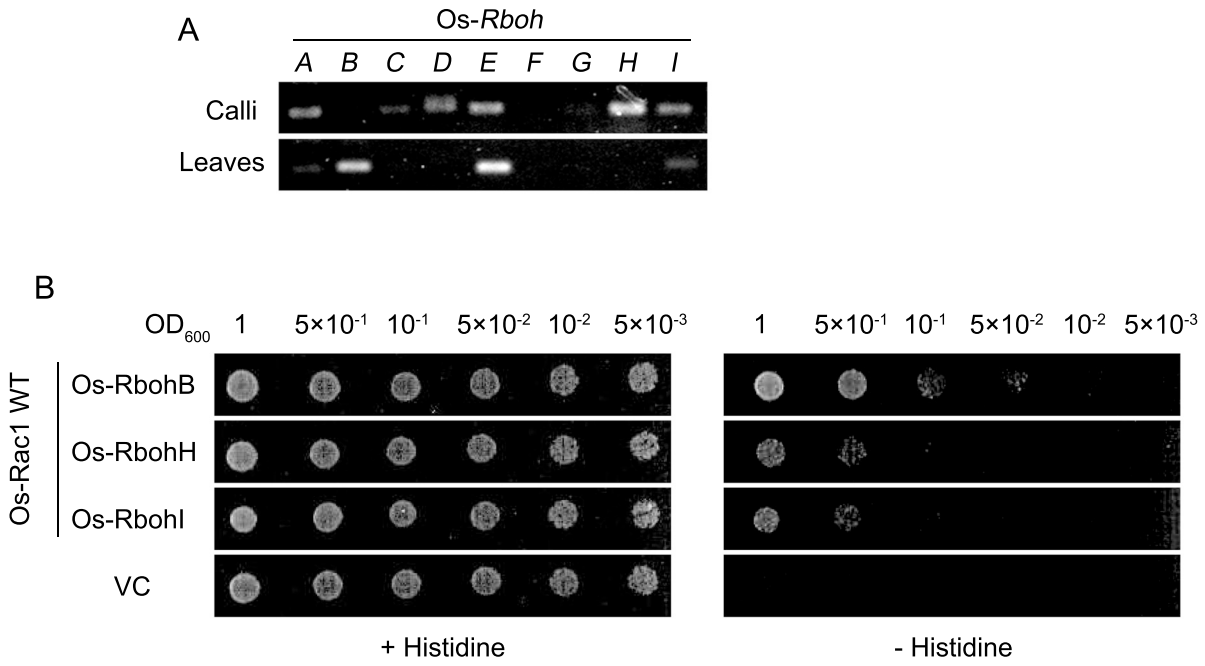
Supplemental Figure 11: Flow chart of fractionation of rice suspension cells

Extracts from WT and OsFAH1/2-KD1 were centrifuged at 100,000 × *g* and divided into supernatant (soluble fraction, S) and pellet. The pellet was dissolved with 1% Triton X-100 on ice at 30 min, centrifuged at 20,000 × *g*, and again divided into supernatant (microsomal fraction, M) and pellet (microsomal DRM-like fraction, D). Mircosomal DRM-like fraction was markedly concentrated to detect a small amout of PM proteins



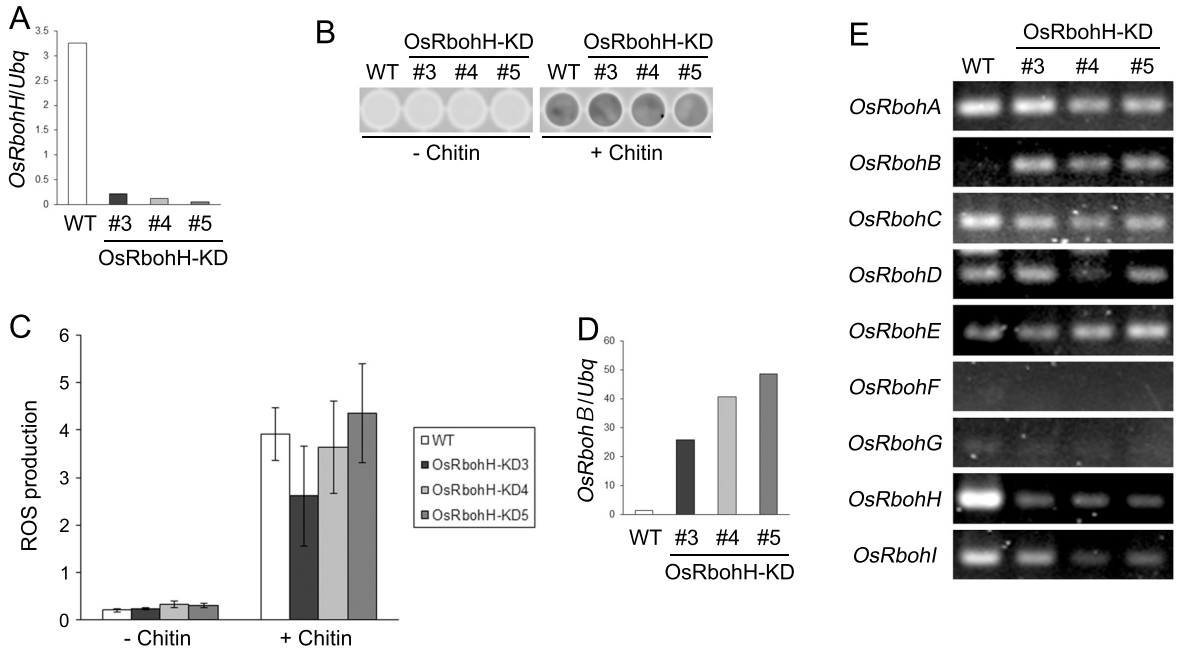
Supplemental Figure 12: Phylogenetic relationships and comparison of Rboh proteins

(A) A phylogenetic tree of Rboh proteins from rice, potato, and Arabidopsis. The bootstrap values are shown in percentage on the internal nodes. **(B)** Comparison of deduced amino acid sequences of the N-terminal EF-hand motif of Os-RbohB, Os-RbohH, Os-RbohI, and St-RbohB.



Supplemental Figure 13: Analysis of Os-Rbohs

(A) Expression levels of Os-*RbohA*-Os-*RbohI* in rice calli and leaves were analyzed by RT-PCR **(B)** Interaction of Os-Rbohs with Os-Rac1 WT in the yeast two hybrid system. Yeast cells containing bait and prey constructs were tested for binding on minimum medium without histidine. The images display the growth of each line (OD_{600} = 1 to 5 × 10^{-3}) after 3 days of incubation at 30°C. Os-Rac1 WT and Os-Rbohs were used as bait and prey constructs, respectively. The combination of empty vectors was used as a vector control (VC).



Supplemental Figure 14: Analysis of OsRbohH-KD lines

(A) **q**RT-PCR analysis of Os-*RbohH* transcription in OsRbohH-KD suspension cells. **(B)** Example of ROS detection in OsRbohH-KD suspension cells by L-012 reagent. **(C)** Quantification of ROS production in OsRbohH-KD suspension cells. Data are means ± SD (*N* = 4). **(D)** qRT-PCR of Os-*RbohB* transcription in OsRbohH-KD suspension cells **(E)** Transcriptional analysis of Os-*RbohA*-Os-*RbohI* in OsRbohH-KD suspension cells by RT-PCR.

Supplemental Table 1. Primers used in this study.

Primer name	Sequence (5'-3')
Primers for RNAi	
OsFAH1 F	GACTTGCGCTGCATTTTGCA
OsFAH1 R	GCAGATTGCCCTCTCTACCA
OsFAH2 F	CTGAGCCACTGACAGAAATG
OsFAH2 R	ATCTGAATTCAGAACATTGGATGTC
OsRbohB F	GGTACAAATTGAGAAATACC
OsRbohB R	GGTCACAACAAAGAGAACTTC
OsRbohH F	GATCATCGGATTCATCGTCG
OsRbohH R	GTGGTAAGCTTTATATCATCG
Primers for construction	
OsFAH1 F	ATGGTTGCAGAGGCCTTTAC
OsFAH1 R	AGAGCTCTTCTTGTCGATGG
OsFAH2 F	ATGGTTGTCCAGGAGTTCAC
OsFAH2 R	GTTGTTCTTTCCGGTGGTCT
OsRac1 F	ATGAGCTCGGCGGCGGC
OsRac1 R	CTACGCGAAACAAGCGCTTC
Primers for qRT-PCR	
OsFAH1 F	GCCTGTTGGGTTATGTGATGT
OsFAH1 R	TGGTATTTCTTGAGGTGTTTTCC
OsFAH2 F	CAAAGAGCCAGCGAAAAATC
OsFAH2 R	AGTGATGAGGTGATGCCAAA
Ubq F	AACCAGCTGAGGCCCAAGA
Ubq R	ACGATTGATTTAACCAGTCCATGA
OsRac1 F	CGCCTGGTGTTCCTGTAGTT
OsRac1 R	CCTCAGTTCTTCTCCCTGCT
OsPIP1;1 F	CACTGATGCCAAGAGGAATG
OsPIP1;1 R	ACCAGGAACACCGCAAAA
OsPIP1;2 F	ACCGACGCCAAGAGGAAC
OsPIP1;2 R	GAACCAAGAACACCGCAAAC
OsPIP1;3 F	GCCACCATCCCCATCACC
OsPIP1;3 R	AACGGACCAACCCAGAAAA
OsRbohB F	CAGCAAGGCGAAGAAGAAAC
OsRbohB R	CCTCTGAACCACTCAAACGA
OsRbohH F	CCACCACCTCTTCGTCATC
OsRbohH R	TGTCGTCTGCTTCCACCAA
OsRbohI F	GCGGAGACGGACAAGAAG
OsRbohI R	TGAGCGACTGGAGCATAG