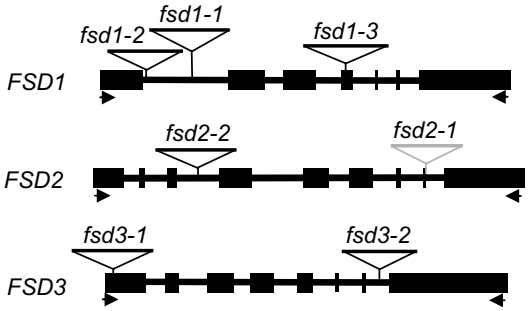
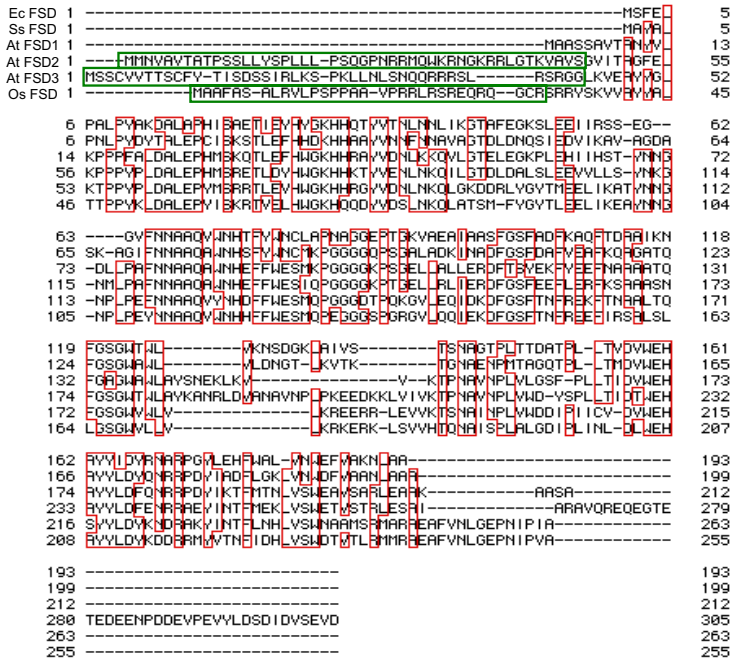
Supplemental Data. Myouga et al. (2008). A heterocomplex of iron superoxide dismutases defends chloroplast nucleoids against oxidative stress and is essential for chloroplast development in *Arabidopsis.*



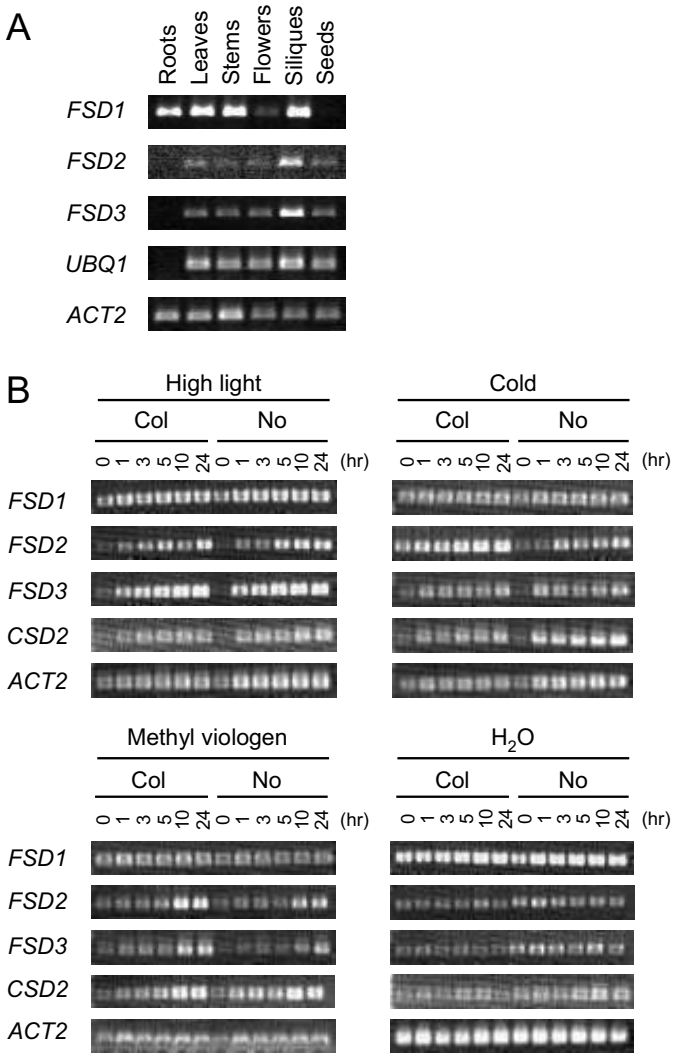
Supplemental Figure 1. Structure of *FSD1*, *FSD2*, and *FSD3* genes and their *Ds*/T-DNA insertion sites.
Three T-DNA insertion sites in the *FSD1* gene (At4g25100), one T-DNA insertion site and one *Ds* insertion site in the *FSD2* gene (At5g51100), and two T-DNA insertion sites in the *FSD3* gene (At5g23310) were mapped. Insertion positions for lines GABI_740E11 (*fsd1-2*), SALK_029455 (*fsd1-1*), GABI_341D04 (*fsd1-3*), SALK_080457 (*fsd2-2*), 11-6562-1 (*fsd2-1*), SALK_103228 (*fsd3-1*), and SAIL_224_E05 (*fsd3-2*) are indicated. Exons are represented by filled black boxes, introns by lines, T-DNA insertion sites by black triangles, and *Ds* insertion site by gray triangle. Short arrows show the positions of primers used for RT-PCR analysis.



Supplemental Figure 2. *SOD* (superoxide dismutase) gene family from *Arabidopsis* and other species.

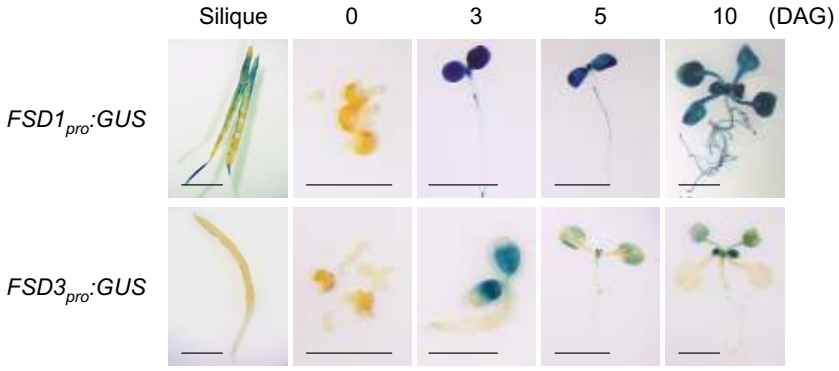
Multiple alignments of deduced amino acid sequences of full-length cDNAs of *FSD1*, *FSD2*, and *FSD3* from *Arabidopsis* (At FSD1, At FSD2, At FSD3) and *FSD* from other species. The sticky ends of the N-termini of At FSD2, At FSD3, and Os FSD, which were deduced by using the TargetP program to be chloroplast transit peptides, are indicated by green squares. The amino acid sequences were aligned by using the ClustalW program (Thompson et al., 1994). Gaps introduced to maximize the alignment are indicated by dashes. Red squares indicate conserved amino acids. At FSD1, At FSD2, and At FSD3 are from *Arabidopsis*, Os FSD is from rice, Ec FSD is from bacteria, and Ss FSD is from cyanobacteria.

Reference: Thompson, J.D., Higgins, D.G., and Gibson, T.J. (1994). CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. 22: 4673-4680.

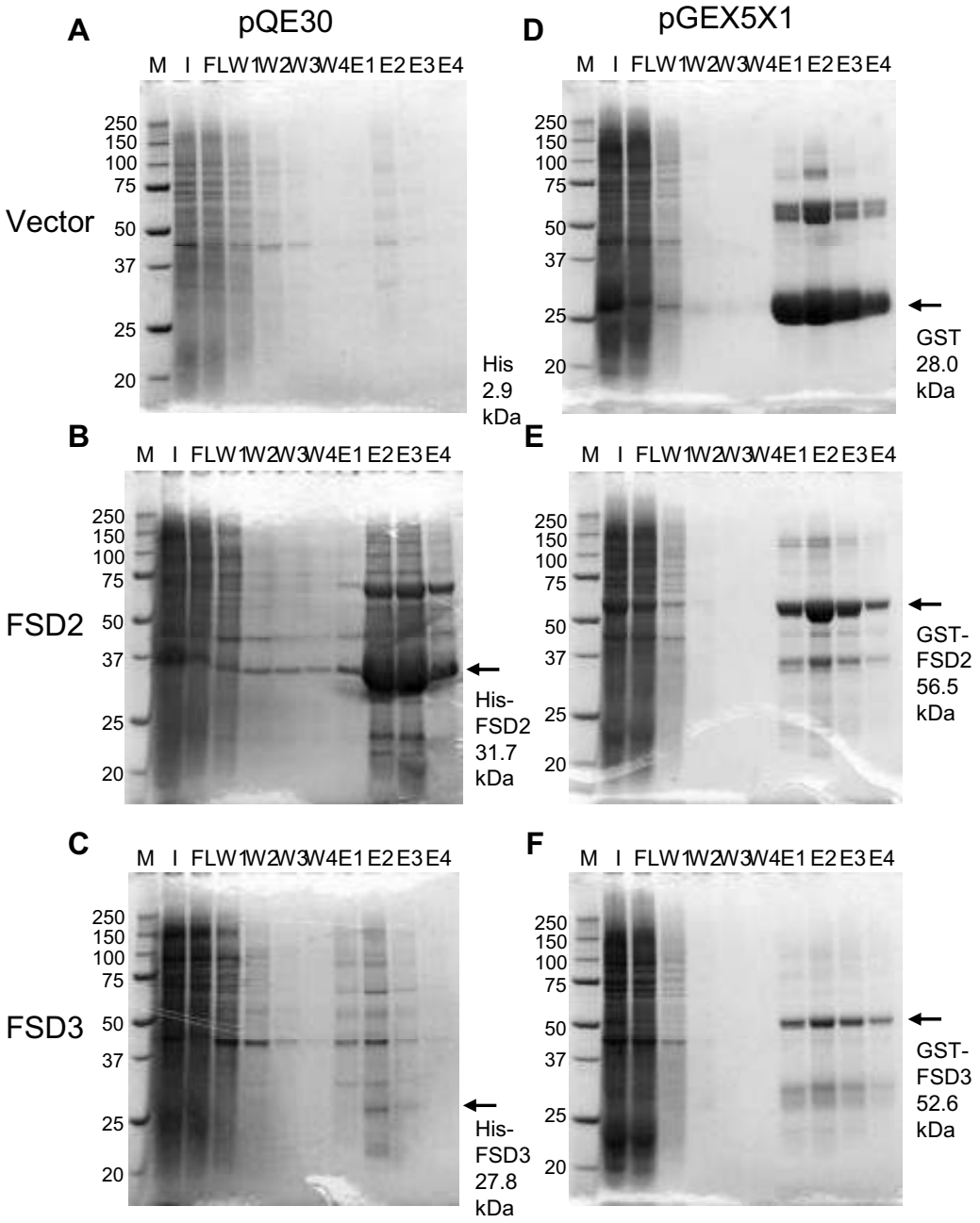


Supplemental Figure 3. RT-PCR analysis of transcripts of *FSD1*, *-2*, *-3*, and *CSD2* in wild-type plants.

(A) RT-PCR analyses showing transcript levels in the organs indicated. *Ubiqutin1* (UBQ1) and *Actin2* (ACT2) were used as controls. Two replicates were performed. **(B)** *FSD1, FSD2, FSD3*, and *CSD2* expression was induced in response to oxidative stress. Wild-type plants (Columbia [Col] and Nossen [No] ecotypes) were treated with high light (photon flux density 240 μ mol m^{-2} s^{-1}), cold (4 ℃), methyl viologen (10 μM), or water (as a negative control) for the numbers of hours indicated. The level of actin transcript was used as a control. Two replicates were performed.

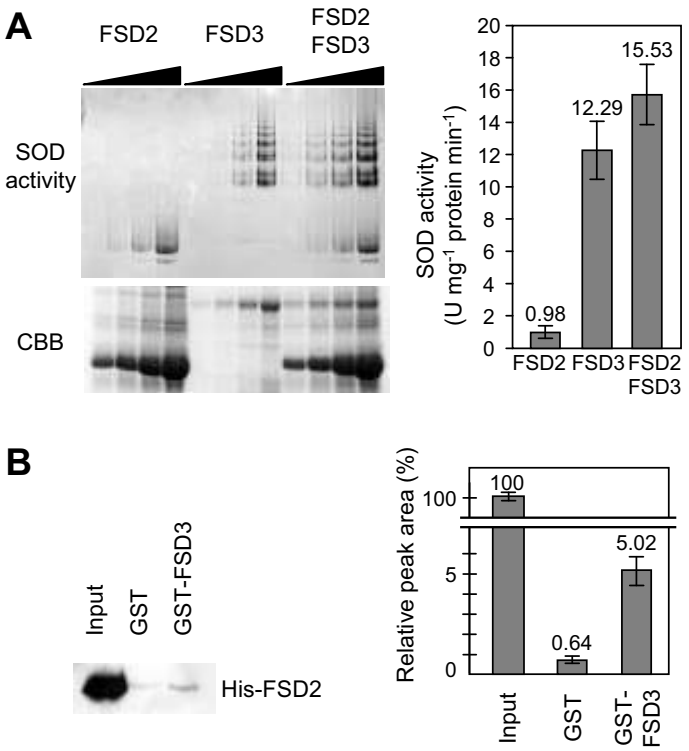


Supplemental Figure 4. Activity of the *FSD1* and *FSD3* promoters is tissue-specific.
GUS expression patterns of *FSD1*$_{pro}$*:GUS* and *FSD3*$_{pro}$*:GUS*-transformed *Arabidopsis* plants (Columbia ecotype) are shown at different developmental stages. Whole seedlings were grown for the number of days after germination (DAG) indicated and siliques were processed for GUS histochemical staining, as described before (Myouga et al., 2006). Each construct was transformed into six *Arabidopsis* plants and data for line 5 are shown; five other lines gave comparable results. Scale bar, 1cm.



Supplemental Figure 5. Total soluble protein profiles of IPTG-induced *E. coli* cells containing the tagged constructs indicated.

Fifteen microliters of IPTG-induced crude extract (I), flow-through (FL), wash (W1–W4), or eluate (E1–E4) was subjected to 12.5% SDS-PAGE and stained with Coomassie brilliant blue. **(A)** pQE30-vector; **(B)** pQE30-FSD2; **(C)** pQ30-FSD3; **(D)** pGEX5X1-vector; **(E)** pGEX5X1-FSD2; **(F)** pGEX5X1-FSD3. Arrows denote induced FSD proteins and show predicted molecular weights of the fusion proteins. Molecular weights (M) of protein markers are shown on the left.



Supplemental Figure 6. SOD (superoxide dismutase) activity of recombinant FSD2 and FSD3 proteins.

(A) Recombinant FSD2 proteins purified by Ni-column (His-FSD2) and FSD3 proteins purified by glutathione Sepharose 4B column (GST-FSD3) were subjected to 10% native-PAGE and their SOD activities were visualized (upper panel). The same amounts of proteins were subjected to 10% SDS-PAGE and stained with Coomassie brilliant blue as a loading control (lower panel). To form heterocomplexes, 1 volume of His-FSD2 and 1/10 volume of GST-FSD3 were mixed together and incubated at 4 ℃ for 2 h. The amount of protein applied to each lane was about 2.5 μg (lane 1), 5 μg (lane 2), 10 μg (lane 3), 20 μg (lane 4), 0.25 μg (lane 5), 0.5 μg (lane 6), 1 μg (lane 7), 2 μg (lane 8), 2.75 μg (lane 9), 5.5 μg (lane 10), 11 μg (lane 11), and 22 μg (lane 12). The enzyme activities of both His-FSD2 and GST-FSD3 homodimers and their heterodimer were assayed (right panel). Data represent the means ± S.D. of three separate experiments (*N*=15). **(B)** His-FSD2 interacted weakly with GST-FSD3 *in vitro*. Proteins were separated by 12.5% SDS-PAGE gel and His-FSD2 was detected by anti-His antibody (left panel) and histograms of relative areas of peak intensities were shown (right panel). Lane 1 represents 10% of the amount of input protein added to the bait beads mixture. Lanes 2 and 3 were reactions using GST alone and GST-tagged FSD3 as the bait protein, respectively. Values were calculated using ImageJ program that can measure areas of peak intensities. Peak areas of Lane 2 and 3 were normalized against that of the peak area measured in Lane 1.

Supplemental Table 1. Restoration of greening in pale green mutants grown under low light conditions.

A

	WT (No)	WT (Col)	*fsd1-1*	*fsd2-1*	*fsd3-1*
Fv/Fm	0.83	0.83	0.83	0.45	0.49
NPQ	0.78	0.79	0.68	0.24	0.34
qL	0.58	0.59	0.55	0.72	0.68

B

	WT (No)	WT (Col)	*fsd1-1*	*fsd2-1*	*fsd3-1*	*fsd2-1* /No	*fsd3-1* /Col
Normal light 1% Suc	0.79	0.75	0.85	0.066	0.024	8.30%	3.20%
Low light 1% Suc	0.48	0.47	0.47	0.17	0.075	35%	15%

C

	Sucrose	WT (No)	WT (Col)	*fsd1-1*	*fsd2-1*	*fsd3-1*	*fsd2-1* /No	*fsd3-1* /Col
Normal light	1%	6.83	4.82	5.08	2.63	2.00	38%	41%
	3%	8.00	5.81	6.09	4.90	3.33	61%	57%
Low light	1%	2.41	2.33	1.90	1.70	1.72	70%	73%
	3%	3.66	3.25	3.41	3.09	2.87	84%	88%

(A) Measurement of chlorophyll fluorescence in one-week-old plants grown under normal light conditions. *Fv/Fm*, *NPQ* and *qL* indicate the maximal efficiency of PSII photochemistry, the non-photochemical quenching coefficient and a parameter estimating the fraction of PSII centers in open sates, respectively. All values are means of five leaf mesurements. **(B)** Chlorophyll content in ten-day-old wild type and *fsd* mutants grown under normal or low light conditions. In addition to the chlorophyll contents (mg/g) of each plant, the ratio of the chlorophyll content of each *fsd* mutant to that of the wild-type plants is shown in the far-right columns. Three replicates were performed. **(C)** Leaf size in ten-day-old wild type and *fsd* mutants grown under normal light or low light on media supplemented with 1% or 3% sucrose. In addition to the leaf size (mm) of each plant, the ratio of average leaf size of each *fsd* mutant to that of the wild type plants is shown in the far-right columns. Each mean value is obtained at least twenty leaf experiments. Col: Columbia; No: Nossen

Supplemental Table 2. Prediction of targeting sequences and subcellular localization of SOD (superoxide dismutase) in *Arabidopsis* by different software tools.

Gene name (AGI code)	TargetP		WOLF PSORT		Predator	PCLR
	Prediction score	(Peptide length)	Prediction score	(Peptide length)	Prediction score	Prediction score
FSD1 (At4g25100)	– 0.128	–	Chlo 6	–	– 0	– 0.078
FSD2 (At5g51100)	cTP 0.771	46	Chlo 11	24	Chlo 0.12	Chlo 0.674
FSD3 (At5g23310)	cTP 0.945	41	Chlo 12	15	Chlo 0.46	Chlo 0.945
CSD1 (At1g08830)	– 0.118	–	Cyto 14	–	0.02	– 0.014
CSD2 (At2g28190)	cTP 0.96	61	Chlo 13	24	Chlo 0.95	Chlo 0.988
CSD3 (At5g18100)	– 0.069	–	Pero 9	32	0.02	– 0.034
MSD1 (At3g10920)	mTP 0.796	6	Mito 10	13	Mito 0.52	– 0.019
MSD-like (At3g56350)	SP 0.957	23	Extr 7	23	ER 0.99	– 0.003

Score, accuracy, or confidence of a given prediction of each SOD gene by TragetP, WOLF PSORT, Predator, and PCLR programs is shown under the name of the predicted location in each column. The predicted length of targeting sequences (peptide length, given in amino acids) is indicated. "–" denotes negative prediction. cTP, chloroplast transit peptide; mTP, mitochondrial targeting peptide; SP, secretory signal peptides required for translocation across the cytoplasmic membrene through endoplasmic reticulum (ER)/Golgi apparatus; Extr, extracellular; Cyto, cytosolic; Chlo, chloroplast; Mito, mitochondria; Pero, peroxisome; ER, endoplasmic reticulum.

Supplemental Table 3. Bonferroni multiple comparison test.

Comparison	Mean Difference	*t*	P value	
WT vs FSD2-OE	-111.13	3.242	*	P<0.05
WT vs FSD3-OE	-100.05	2.919	*	P<0.05
WT vs CSD2-OE	-179.87	5.248	***	P<0.001
WT vs Double-OE	-245.6	7.165	***	P<0.001
FSD2-OE vs FSD3-OE	11.083	0.3234	ns	P>0.05
FSD2-OE vs CSD2-OE	-68.733	2.005	ns	P>0.05
FSD2-OE vs Double-OE	-134.47	3.923	**	P<0.01
FSD3-OE vs CSD2-OE	-79.817	2.329	ns	P>0.05
FSD3-OE vs Double-OE	-145.55	4.246	***	P<0.001
CSD2-OE vs Double-OE	-65.733	1.918	ns	P>0.05

The significance of differences between control and test values was determined by the two-tailed multiple *t*-test with Bonferroni correction (four comparisons in five groups). If the value of *t* is greater than 2.828 then the P value is less than 0.05 (*P<0.05). Mean difference indicates a measure of statistical dispersion.

Supplemental Table 4. Nucleotide sequences of gene-specific primers.

Nucleotide sequences of gene-specific primers used to confirm *Ds* or T-DNA insertion		
fsd1-1	*FSD1*-Fw	5′-GTAGGAACGGGTCAAGTTGG-3′
	FSD1-Rv	5′-CCTTCAAGCTCGGTTCCAAG-3′
fsd1-2	*FSD1*-Fw2	5′-GTGTGCTCTCCTCACTTCA-3′
	FSD1-Rv2	5′-CCTTCAAGCTCGGTTCCAAG-3′
fsd1-3	*FSD1*-Fw3	5′-CAACGCTGCTCAGGTGATAA-3′
	FSD1-Rv3	5′-GGCACTTACAGCTTCCCAAG-3′
fsd2-1	*FSD2*-Fw	5′-GCTCGTATGGGATTATTCTG-3′
	FSD2-Rv	5′-CTCTGGTACTTCATCATCTGG-3′
fsd2-2	*FSD2*-Fw2	5′-GTGGCTGTTTCCGGTGTTAT-3′
	FSD2-Rv2	5′-ACGACCAACACAGAGTAAGC-3′
fsd3-1	*FSD3*-Fw	5′-AGCTCACGGCTTGAATCTTG-3′
	FSD3-Rv	5′-GACCGTAGTAAGCTTCAACC-3′
fsd3-2	*FSD3*-Fw2	5′-CGGGAATCCTTTACCCGAGTT-3′
	FSD3-Rv 2	5′-AGACTGCCCAAGTTTCCA-3′
csd2-1	*CSD2*-Fw	5′-GCCGAATTTGAGAAACCAGT-3′
	CSD2-Rv	5′-TTCACAGAACCATTGAATCAGC-3′
SALK	T-DNA in-tern-al primer	5′-ACCACCATCAAACAGGATTTTC-3′
GABI-Kat	T-DNA in-ternal primer	5′-ATATTGACCATCATACTCATTGC-3′
SAIL	T-DNA in-ternal primer	5′-TTCATAACCAATCTCGATACAC-3′
Nucleotide sequences of gene-specific primers used for RT-PCR analysis		
FSD1	Fw	5′-TCAAGTGCTGTCACCGCAAA-3′
	Rv	5′-TTAAGCAGAAGCAGCCTTGG-3′
FSD2	Fw	5′-TGAATGTTGCAGTGACAGCC-3′
	Rv	5′-TCTTTGCACTGCTCGAGCAA-3′
FSD3	Fw	5′-AGACCAAGAGTGCGAAGTAG-3′
	Rv	5′-TTCACAAACGCTTCTGCACG-3′
CSD1	Fw	5′-TGGCGAAAGGAGTTGCAGTT-3′
	Rv	5′-TGGCAATCAGTGATTGTGAAG-3′
CSD2	Fw	5′-GCATTCTCATCTCCTTCTCG-3′
	Rv	5′-GTTTCCAGTGGTCAGACTAA-3′
CSD3	Fw	5′-AGAGGAAATCTGAGAGCGGT-3′
	Rv	5′-AGATTTCAGCAACACCGTTTG-3′
MSD1	Fw	5′-GAGGATCAGAGGGATTCAGA-3′
	Rv	5′-TGGCACTACCAAGAGATCCT-3′
MSD-like	Fw	5′-TGGAACCGTGTCTCGAATCA-3′
	Rv	5′-CAAAGGATCATGCGGTGGTT-3′
ACT2	Fw	5′-CTAAGCTCTCAAGATCAAAGG-3′
	Rv	5′-ACATTGCAAAGAGTTTCAAGGT-3′
UBQ1	Fw	5′-CGACAATGTCAAGGCCAAGA-3′
	Rv	5′-TGGTTGCTGTGACCACACTT-3′
Nucleotide sequences of gene-specific primers used in complementation tests of mutants and construction of overexpressors		
FSD1 cDNA	Fw	5′-CACCATGGCTGCTTCAAGTGCTGTCACCG-3′
	Rv	5′-AGCAGAAGCAGCCTTGGCG-3′
FSD2 pro-moter + gene	Fw	5′-CACCAAGATGACAGAAGGGAAGCAGT-3′
FSD2 cDNA	Fw	5′-CACCATGATGAATGTTGCAGTGACAGC-3′
	Rv	5′-GTCAACCTCAGATACATCG-3′
FSD3 pro-moter + gene	Fw	5′-CACCAGTCCTGCAGTATTGGGTGATG-3′

FSD3 cDNA	Fw	5′-CACCATGAGTTCTTGTGTTGTGACGAC-3′
	Rv	5′-AGCGATTGGGATGTTGGGTT-3′
CSD2 cDNA	Fw	5′-CACCATGGCTGCCACCAACACAATC-3′
	Rv	5′-GAGCGGCGTCAAGCCAATCA-3′
M13	Fw	5′-GTAAAACGACGGCCAG-3′
	Rv	5′-CAGGAAACAGCTATGAC-3′
pGWB internal primer	Fw	5′-CAAGTTTGTACAAAAAAGCAGG-3′
	Rv	5′-CCACTTTGTACAAGAAAGCTG-3′
pBE-F internal primer	Fw	5′-CTATCCTTCGCAAGACCCTTCCTC-3′
	Rv	5′-GATGTGCTGCAAGGCGATTAAGTTG-3′
Nucleotide sequences of gene-specific primers used for promoter-GUS constructs		
FSD1 promoter	Fw	5′-CACCGAAGTAAACCAAGCCCCTCA-3′
	Rv	5′-AGCCATTCTTTGTAATTGAAGC-3′
FSD2 promoter	Fw	5′-CACCAAGATGACAGAAGGGAAGCAGT-3′
	Rv	5′-ATTCATCATCTTCACTCAAAGCG-3′
FSD3 promoter	Fw	5′-CACCAGTCCTGCAGTATTGGGTGATG-3′
	Rv	5′-ACTCATAGTTCCTCCCACT-3′
CSD2 promoter	Fw	5′-CACCCGAGAACCCCGTGGATGTATTT-3′
	Rv	5′-AGCCATGGCTGCTGCTATGT-3′
Nucleotide sequences of gene-specific primers used in yeast two-hybrid analysis and immunoprecipitation assay		
FSD1	Fw	5′-CGGAATTCATGGCTGCTTCAAGTGCTGTC-3′
	Rv	5′-CGGGATCCTTAAGCAGAAGCAGCCTTGGC-3′
FSD2 (–cTP)	Fw	5′-CGGAATTCATGGGTGTTATCACAGCTGGATTTG-3′
	Rv	5′-CGGGATCCTTAGTCAACCTCAGATACATCG-3′
FSD2 (+cTP)	Fw	5′-CGGAATTCATGATGAATGTTGCAGTGACAGC-3′
FSD3 (–cTP)	Fw	5′-CGGAATTCATGGGTGGTTTAAAGGTTGAAGCTTA-3′
	Rv	5′-CGGGATCCTTAAGCGATTGGGATGTTGGGTT-3′
FSD3 (+cTP)	Fw	5′-CGGAATTCATGAGTTCTTGTGTTGTGACGAC-3′
CSD2 (–cTP)	Fw	5′-CGGAATTCATGTCCGCGGCGAAGAAGGCTG-3′
	Rv	5′-CGGGATCCTTAGAGCGGCGTCAAGCCAAT-3′
CSD2 (+cTP)	Fw	5′-CGGAATTCATGGCTGCCACCAACACAATC-3′
pGBK internal primer	Fw	5′-TCATCGGAAGAGAGTAGT-3′
	Rv	5′-GTCACTTTAAAATTTGTATAC-3′
pGAD internal primer	Fw	5′-CTATTCGATGATGAAGATACCCCACCAAACCC-3′
	Rv	5′-GTGAACTTGCGGGGTTTTTCAGTATCTACGATT-3′
Nucleotide sequences of gene-specific primers used in qRT-PCR analysis		
FSD1	Fw	5′-AAGTGCTGTCACCGCAAACT-3′
	Rv	5′-CAAGAAGCTCTCCTGATGGT-3′
FSD2	Fw	5′-CACAGCTGGATTTGAGCTGA-3′
	Rv	5′-AATCTGAGGAGCTCTCCAGT-3′
FSD3	Fw	5′-GGTCTCGAGGTGGTTTAAAG-3′
	Rv	5′-AACACCCTTTTGAGGCGTGT-3′
CSD2	Fw	5′-GAAAGCGTTGACAGTTGTTTC-3′
	Rv	5′-CATCGGCATTGGCATTTATG-3′
UBQ1	Fw	5′-GATGGCCGTACTTTGGCTGA-3′
	Rv	5′-CAGCTCTTGGGTGAAGACGA-3′
Nucleotide sequences of gene-specific primers for plastid-encoded genes used in RT-PCR analysis		
psaA	Fw	5′-ATGATTATTCGTTCGCCG-3′
	Rv	5′-CGGTAAAGATACATGTAC-3′
psbA	Fw	5′-TGCAATTTTAGAGAGACGCG-3′
	Rv	5′-AAGTTCCCACTCACGAC-3′
petB	Fw	5′-ATGAGTAAAGTTTATGATTGG-3′
	Rv	5′-TTACACCAAAAGATGCGGT-3′
clpP	Fw	5′-CATGCCTATTGGCGTTCC-3′
	Rv	5′-ATGGATCATTACCCTAGCG-3′
atpB	Fw	5′-ATGAGAACAAATCCTACTAC-3′

atpB	Rv	5′-TATGGATAGGAGATGTTGT-3′
ndhB	Fw	5′-AGAGGAAATCTGAGAGCGGT-3′
	Rv	5′-AGATTTCAGCAACACCGTTTG-3′
accD	Fw	5′-ATGGATGAAGACATGGTCTC-3′
	Rv	5′-AACTAGCTGTCACTCCACC-3′
rpoA	Fw	5′-CGATTCAACGATCTGATCAA-3′
	Rv	5′-TTTCTAGAATGTCTAATATCTT-3′
ycf2	Fw	5′-ATGAAAGGACATCAATTC-3′
	Rv	5′-CACTAAGATTTCGAATAG-3′
Actin	Fw	5′-CTAAGCTCTCAAGATCAAAGG-3′
	Rv	5′-ACATTGCAAAGAGTTTCAAGGT-3′