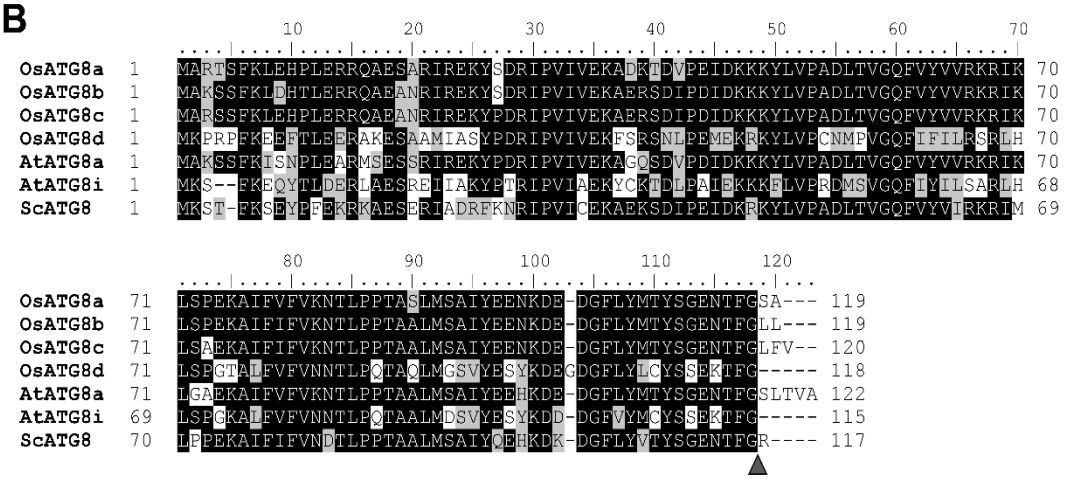
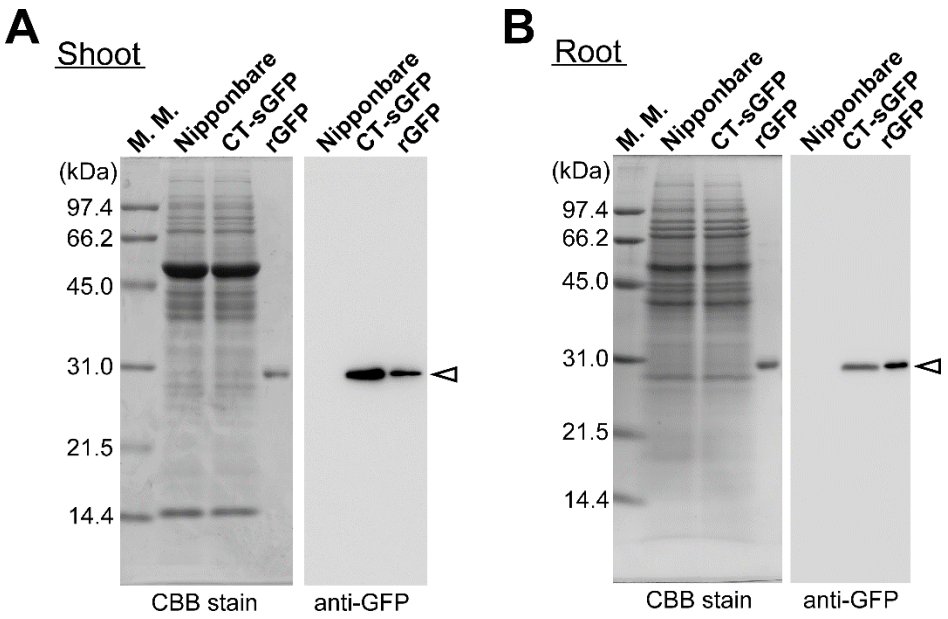
A

Gene name	MSU Locus	RAP-DB Locus	Accession No. or NCBI RefSeq No. for corresponding cDNA	No. of amino acid residues
OsATG8a	LOC_Os07g32800	Os07g0512200	AK059939, NM001066302	119
OsATG8b	LOC_Os04g53240	Os04g0624000	AK121268, NM001060464	119
OsATG8c	LOC_Os08g09240	Os08g0191600	AK121169, NM001067706	120
OsATG8d	LOC_Os11g01010	Os11g0100100	NM001071754	118

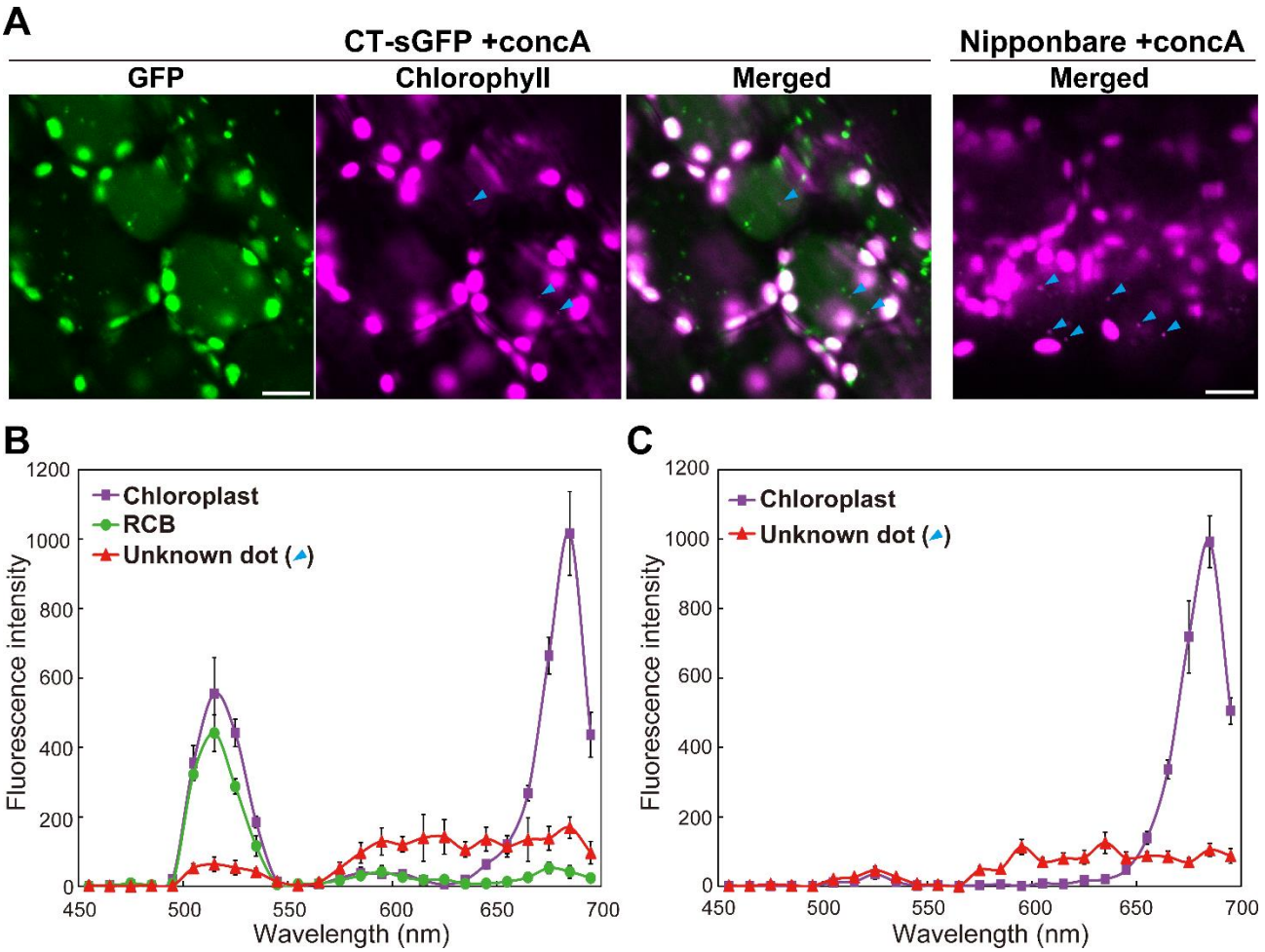
B



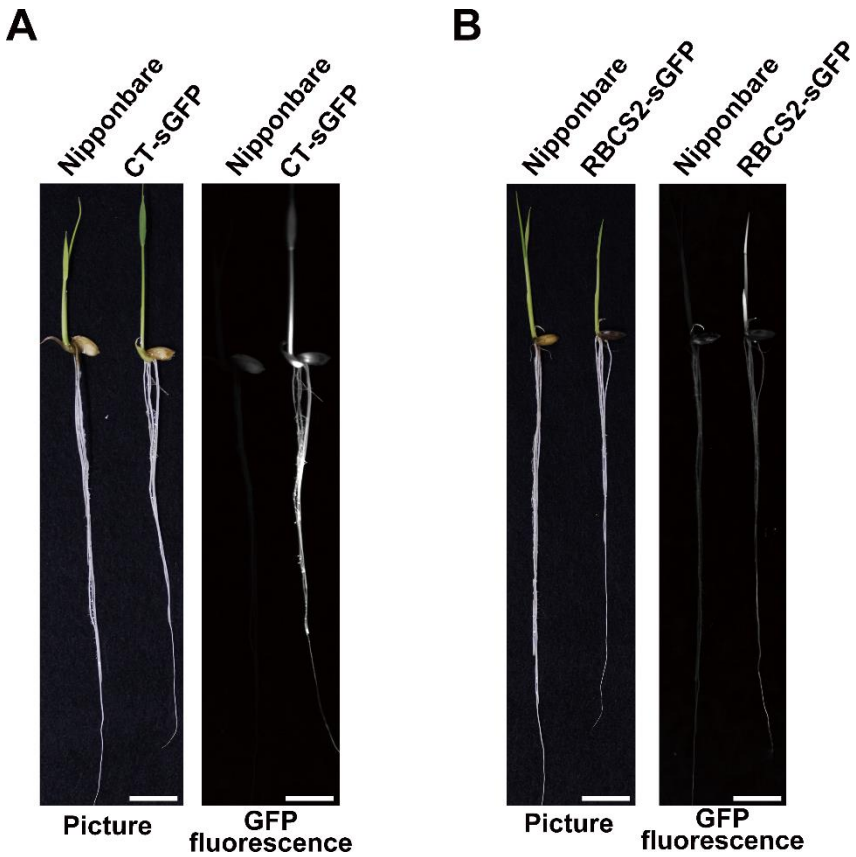
Supplemental Figure S1. *OsATG8* genes characterized in this study. A, List of *OsATG8* genes. For each, the gene name, its locus in the MSU Rice Genome Annotation Project, its locus in the Rice Annotation Project Database (RAP-DB), the accession number or NCBI reference sequence number of the corresponding cDNA data, and the number of encoded amino acid residues are listed. B, Alignment of the deduced amino acid sequences of the *OsATG8* genes, *ScATG8*, *AtATG8a* and *AtATG8i*. The alignment was performed using ClustalW. The arrowhead indicates the processing site by the ATG4 protease.



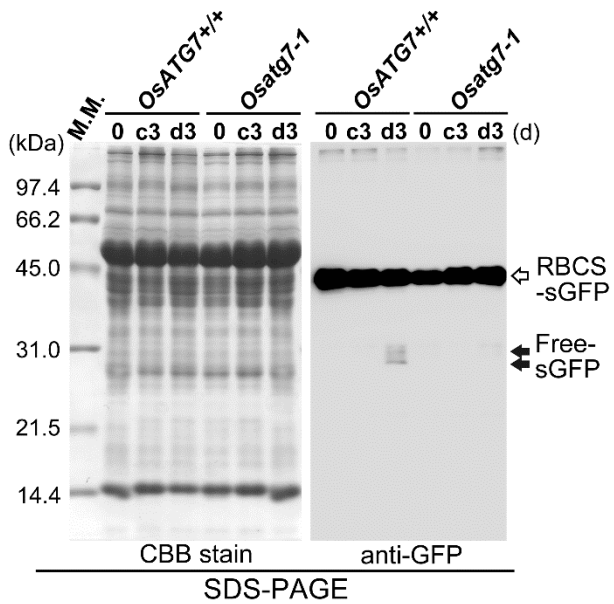
Supplemental Figure S2. Protein analysis in transgenic rice expressing CT-sGFP. Total soluble proteins (10 μg for CBB stain, 4 μg for immunoblotting) from shoots (A) or roots (B) of CT-sGFP-expressing plants were separated by SDS-PAGE, and either stained with Coomassie Brilliant Blue R250 (CBB stain) or subjected to immunoblotting with anti-GFP antibodies. White arrowheads indicate mature form of CT-sGFP after removal of its OsRBCS2 transit peptide. The sizes of molecular mass markers (M. M.; kilodaltons) are indicated at the left of the stained gels.



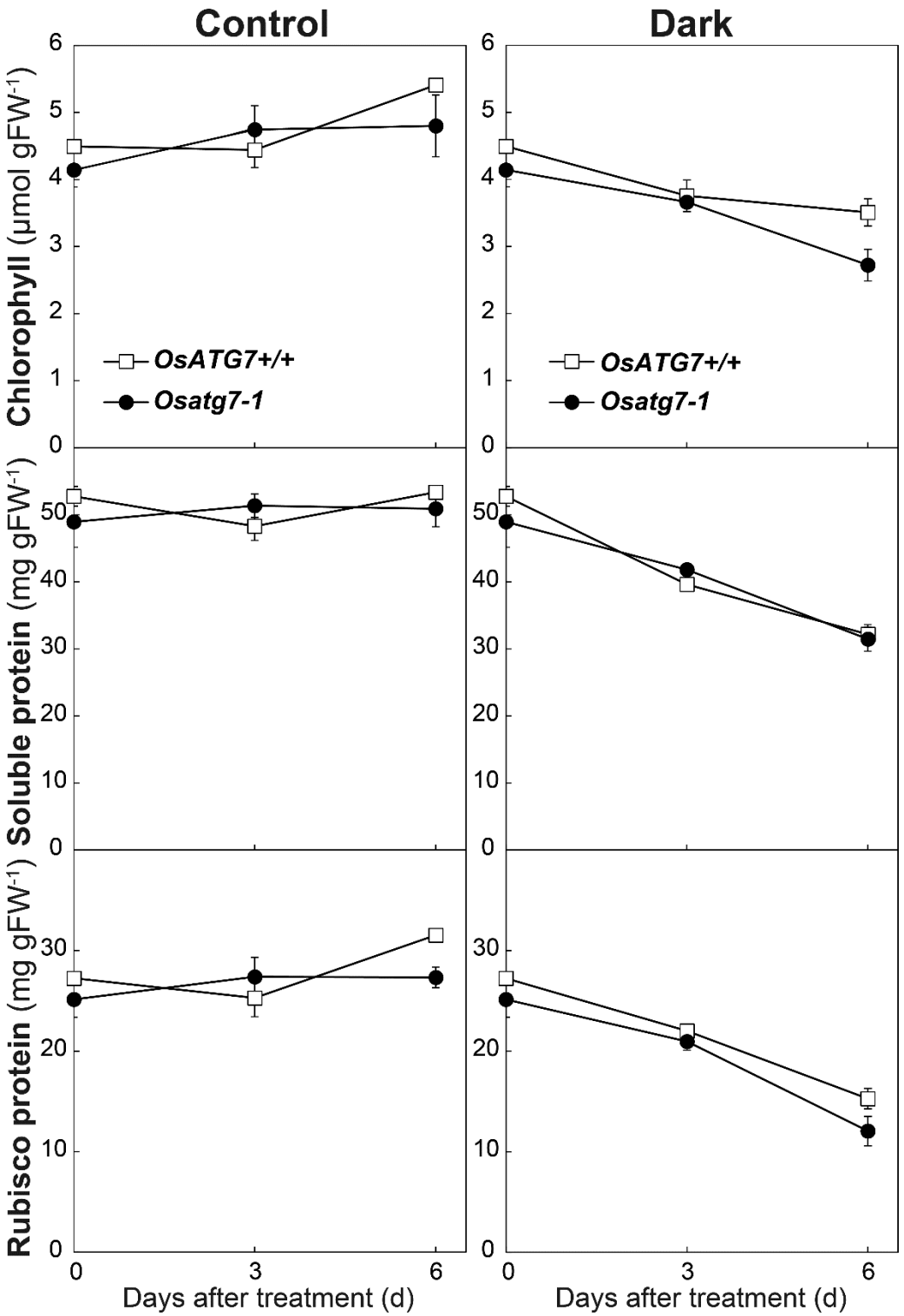
Supplemental Figure S3. Fluorescence spectra of chloroplasts, RCBs and unknown dots observed in rice by LSCM. A, Visualization of RCBs and unknown dots in rice leaves. Excised leaves of CT-sGFP-expressing rice or wild-type Nipponbare plants were incubated in darkness for 20 h in 10 mM Mes-NaOH (pH 5.5) containing 1 μM concanamycin A. Arrowheads indicate observed unknown dots similar to RCBs. GFP fluorescence appears green and chlorophyll autofluorescence appears magenta. In merged images, overlapping signals appear white. Scale bars = 10 μm. In wild-type Nipponbare, a merged image is shown. B, Difference in fluorescence spectra of chloroplasts, RCBs and unknown dots in leaves of CT-sGFP-expressing rice. Spectra between 450 and 700 nm of chloroplasts in the cytoplasm (squares), RCBs in the vacuole (circles) and unknown dots (triangles) excited with 488 nm-laser were obtained at 10-nm resolution. C, Difference in fluorescence spectra of chloroplasts and unknown dots in leaves of wild-type Nipponbare plants. Spectra between 450 and 700 nm of chloroplasts in the cytoplasm (squares) and unknown dots (triangles) excited with 488 nm-laser were obtained at 10-nm resolution. The data represent means ±SE (n = 3).



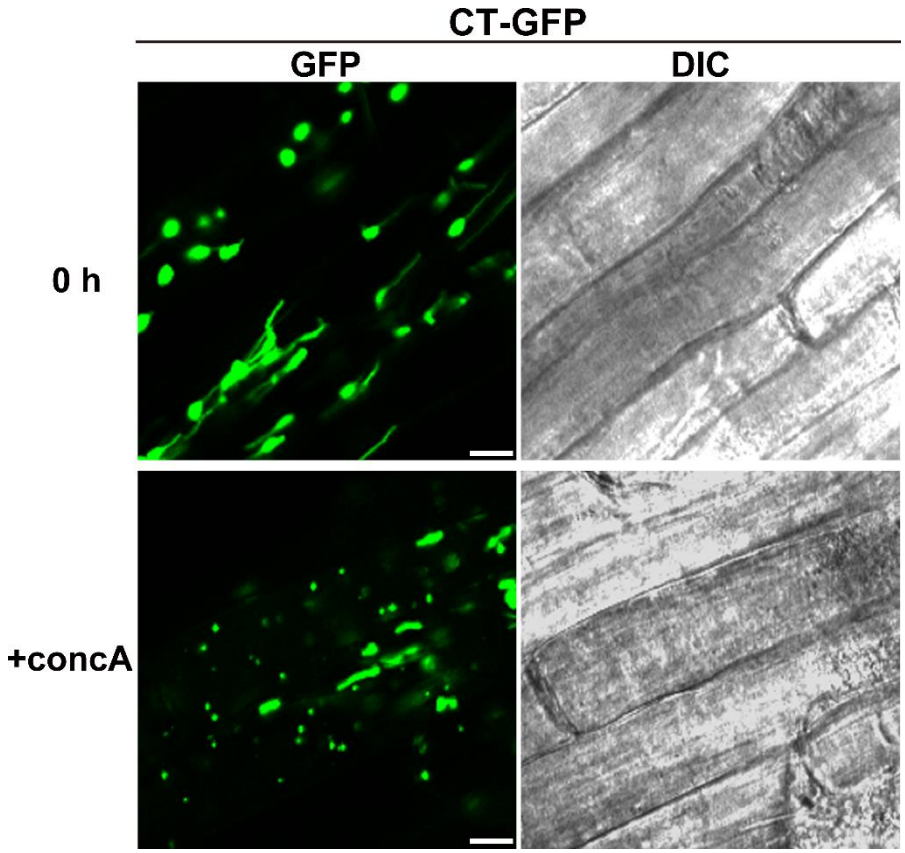
Supplemental Figure S4. Detection of GFP fluorescence in whole rice plants expressing CT-sGFP or OsRBCS2-sGFP. 7-d-old seedlings of CT-sGFP expressing plant and wild-type Nipponbare plant (A) or of OsRBCS2-sGFP expressing plant and wild-type Nipponbare plant (B) were observed using a digital camera (picture) or a fluorescent image analyzer to detect the GFP fluorescence (GFP fluorescence). Scale bars = 10 mm.



Supplemental Figure S5. Processing of OsRBCS-sGFP is not detected in *OsATG7* knockout plants. Total soluble proteins from equal volumes of leaves before treatment or 3 d-control leaves (c3) and 3 d-darkened leaves (d3) of *OsATG7* knockout plants (*Osatg7-1*) or corresponding wild-type plants (*OsATG7*+/+) expressing OsRBCS2-sGFP were separated by SDS-PAGE, and either stained with Coomassie Brilliant Blue R250 (CBB stain) or subjected to immunoblotting with anti-GFP antibody. White arrow indicates the mature form of OsRBCS2-sGFP after cleavage of the OsRBCS2 transit peptide and black arrows indicate free-sGFP released from OsRBCS2-sGFP processing. The sizes of molecular mass markers (M. M.; kilodaltons) are indicated at the left of the stained gels.



Supplemental Figure S6. Changes in chlorophyll, soluble protein and Rubisco protein in individually-darkened leaves of *Osatg7* knockout rice. Contents of chlorophyll, soluble protein and Rubisco protein in control leaves and individually-darkened leaves of *OsATG7* knockout plants (*Osatg7-1*; closed circles) or corresponding wild-type plants (*OsATG7*+/+; open squares) are shown. The data represent means ±SE (*n* = 3-4).



Supplemental Figure S7. Visualization of autophagic degradation of non-green plastids in Arabidopsis roots. Roots of Arabidopsis expressing CT-GFP were excised and observed immediately (0 h), or incubated in darkness for 20 h in the presence of 1 μM concanamycin A. GFP fluorescence images and DIC images obtained simultaneously are shown. Scale bars = 10 μm.

List of primer sequences for vector construction

Name		Primer sequences
OsATG8a (CDS)	F	CACCATGGCCAGGACTTCCTTCAAG
	R	TTACGCAGAGCCGAATGTGTTCTC
OsATG8d (CDS)	F	CACCATGAAGCCCAGGCCCTTC
	R	TCAACCGAATGTCTTCTCGCTGCTG
OsRBCS2	F	CACCATGGCCCCCTCCGTGATGGC
(transit peptide)	R	GCACCTGATCCTGCCGCCATT
OsRBCS2	F	CACCGCATGCCTGCAGCAAAGAA
(promoter, CDS)	R	GTTGCCACCAGACTCCTCGCA

List of primer sequences for genotyping

Name		Primer sequences
OsATG7	F	CGCTTACTACCGTGCGTTGC
	R	ACAATCGCTCCGACGAACC
OsATG7 (F)	F	TTGCTTCAGGCACATAATCAGG
Tos17 (R)	R	ATTGTTAGGTTGCAAGTTAGTTAAGA

List of primer sequences for cloning of OsATG8s

Name		Primer sequences
OsATG8a	F	CCTTCGATCGGTTGGAGATG
	R	ACCTGGCCATCCATGTTTATTTAC
OsATG8b	F	AGTCGCGTGAGTTTCCGCTCC
	R	GCAAAGAATATACACTTGTTTGGCAAGTC
OsATG8c	F	GGTAGTAGTGAAGAAAGCAAGG
	R	AGGGATTTGGAACATGCTGCTAGAC
OsATG8d	F	AGCTACAGAGAGATTCGCATCAAG
	R	TATTATTGACGCCCGAGGTAAAAG

Supplemental Table S1. The list of primers used in this study.