Supplementary Information

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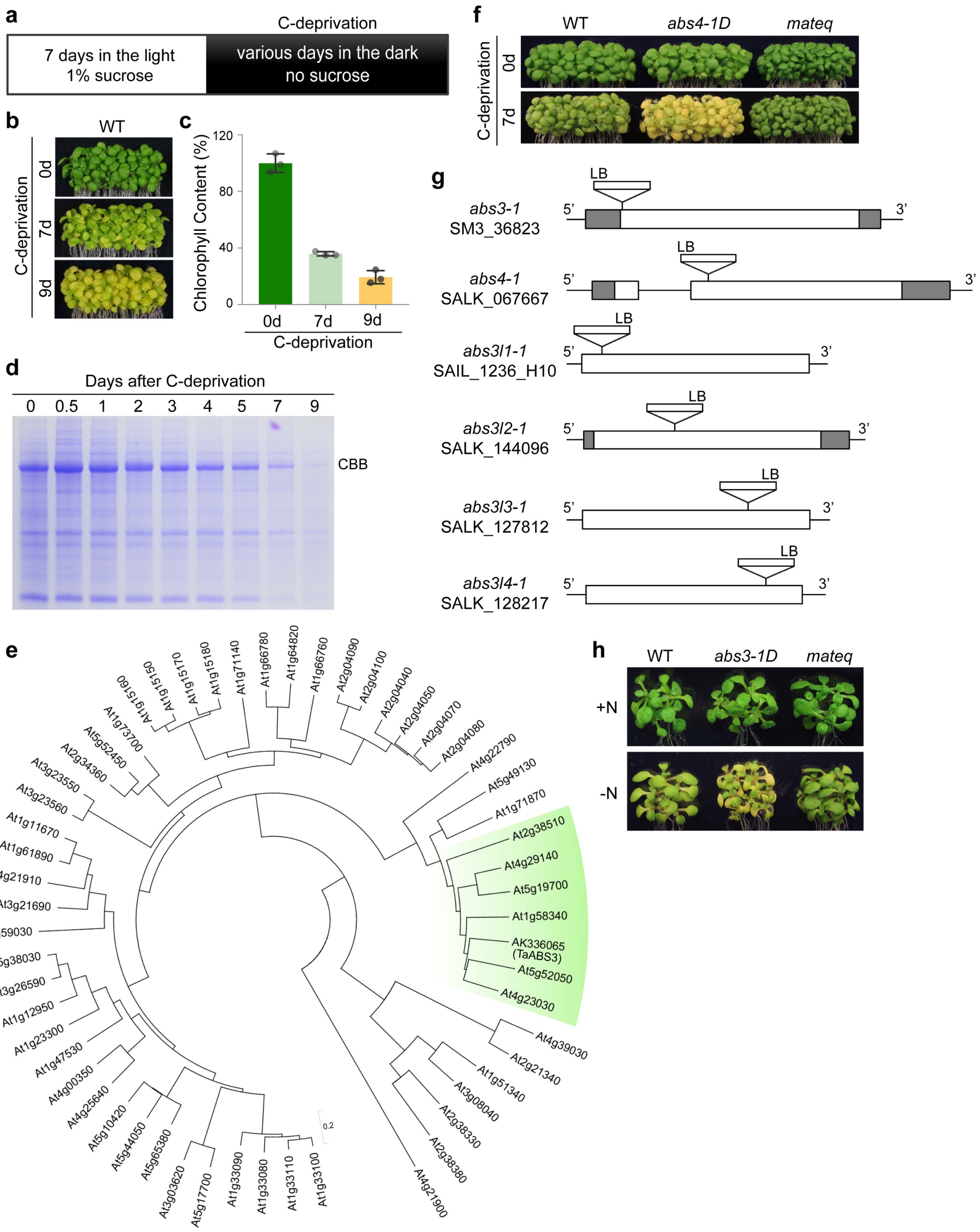
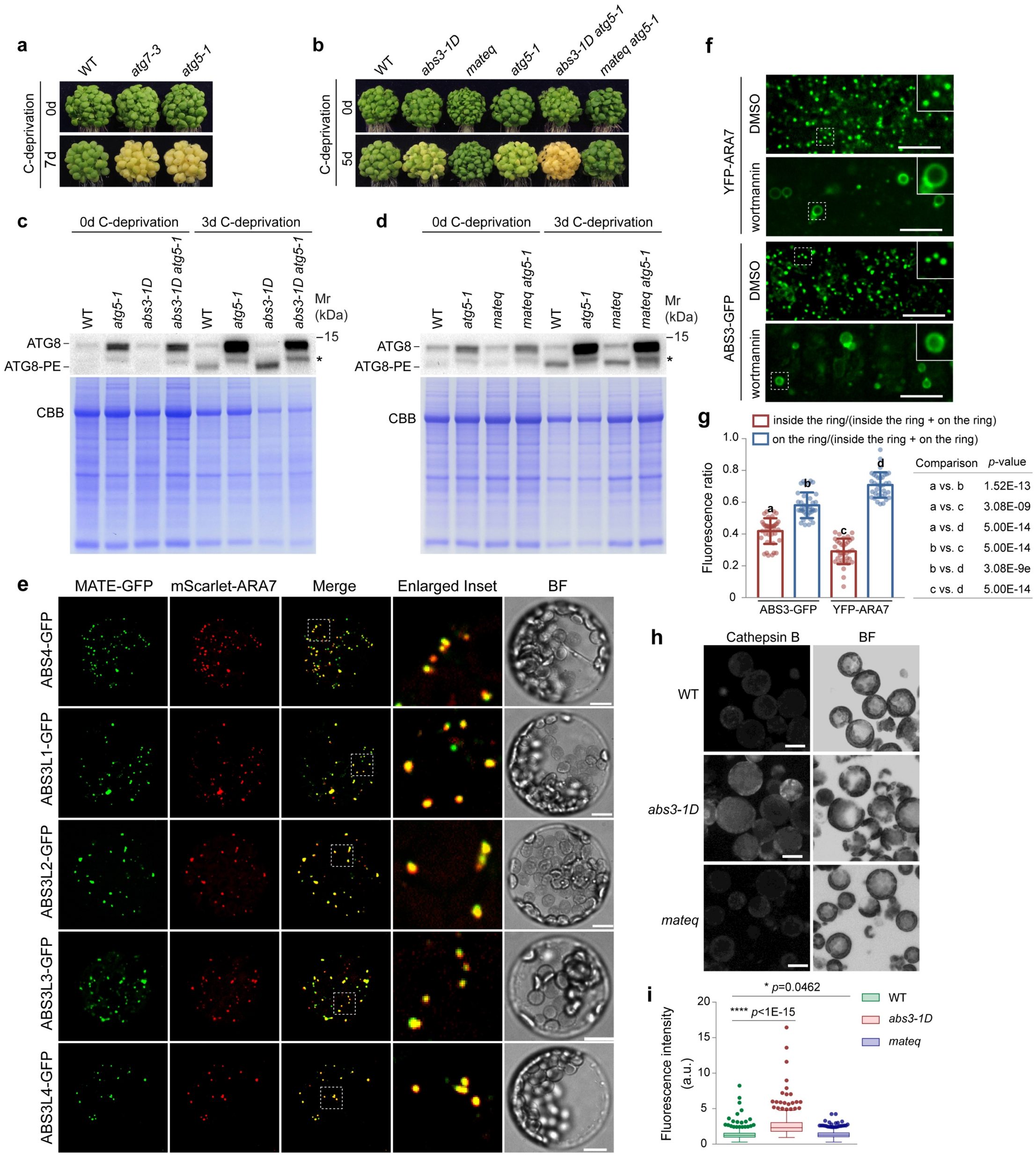
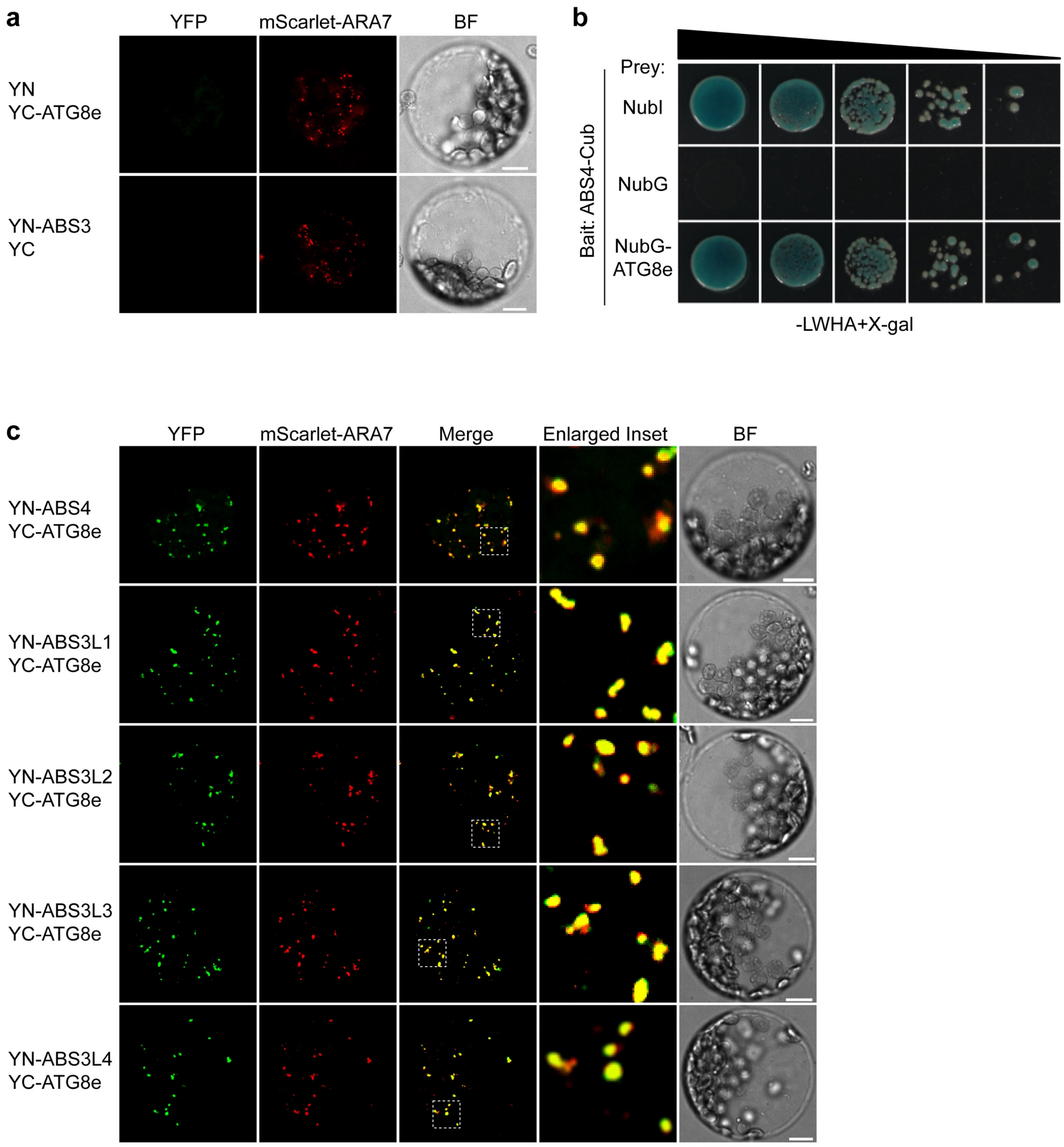


Supplementary Fig.1. C-deprivation and N-starvation induced plant senescence.

a, Schematic diagram of the C-deprivation assay in Arabidopsis. **b,** C-deprivation induced senescence in WT Arabidopsis seedlings. **c,** Chlorophyll content in WT during C-deprivation. Data were presented as mean ± s.d., n=3 biological replicates. **d,** Total cellular proteins (normalized to equal amount of fresh weight) from WT during C-deprivation were resolved on SDS-PAGE and stained by CBB. **e,** Phylogenetic tree of Arabidopsis MATE family proteins and TaABS3 (GenBank Accession No. AK336065). **f,** Senescence phenotype of WT, *abs4-1D* and *mateq* before and after 7d C-deprivation. **g,** Diagrams of the T-DNA insertion single *mate* mutants used in this study. **h,** N-starvation phenotypes of WT, *abs3-1D*, and *mateq*. 7-d-old light-grown seedlings were transferred to +N (10 mM KNO_3) or -N (10 mM KCl) plates for 4 days. Experiments in **d**, **f**, and **h** were independently repeated three times with similar results.

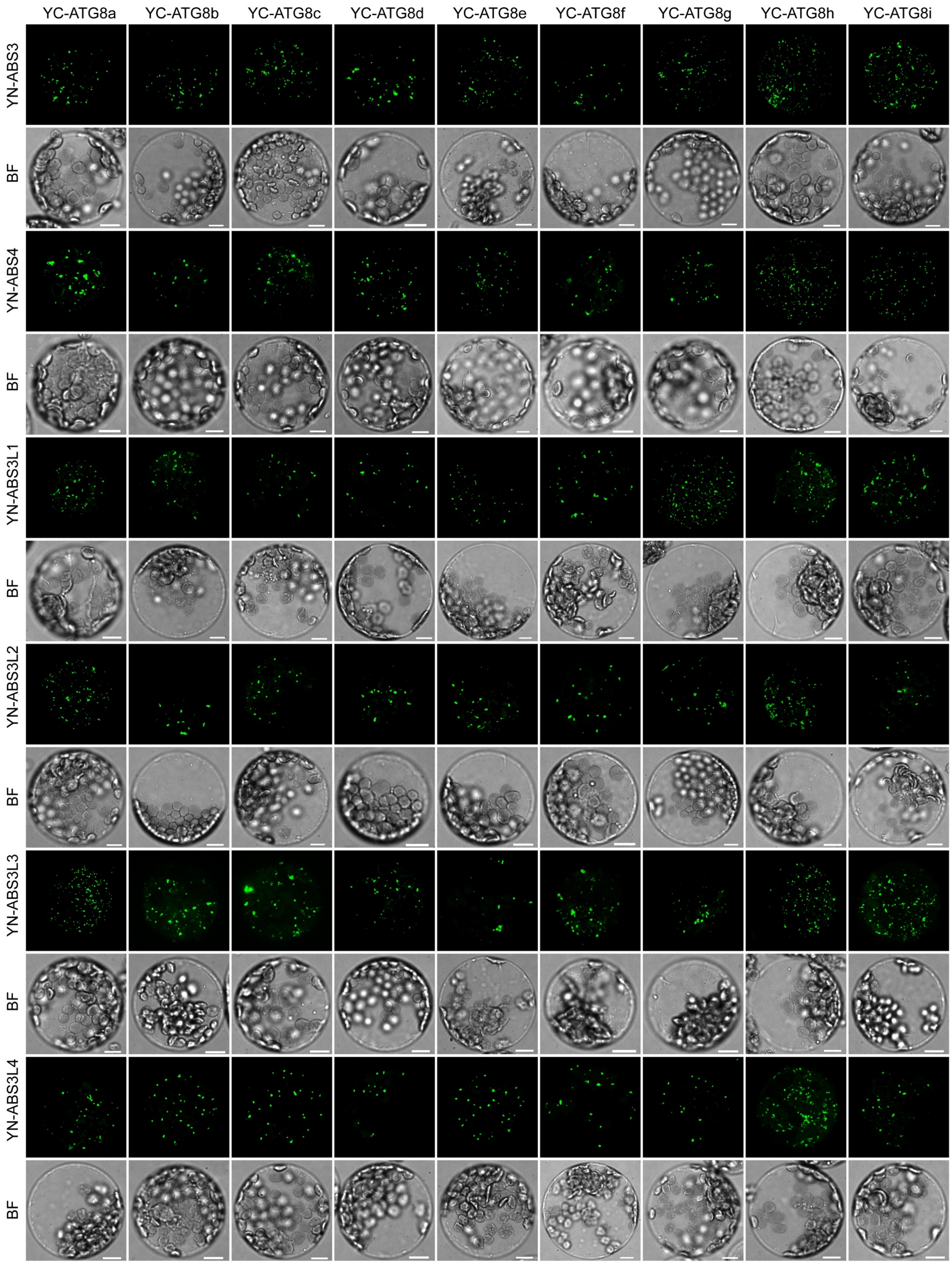


Supplementary Fig. 2. Genetic interaction between *abs3-1D*, *mateq* and *atg5-1*. **a,** Senescence phenotype of WT, *atg7-3*, and *atg5-1* before and after 7d C-deprivation. **b,** Senescence phenotype of WT, *abs3-1D*, *mateq*, *atg5-1*, *abs3-1D atg5-1* double mutant, and *mateq atg5-1* quintuple mutant before and after 5d C-deprivation. **c** and **d,** Total cellular proteins from plants of indicated genotypes before and after 3d C-deprivation were resolved on SDS-PAGE, probed with anti-ATG8 or stained by CBB. **e,** Co-localizations of ABS3 subfamily MATE-GFPs and mScarlet-ARA7 in WT leaf protoplasts. BF, bright field. Bars, 10 μm. **f,** Root epidermal cells of Arabidopsis transgenic lines expressing YFP-ARA7 or ABS3-GFP treated with DMSO or wortmannin. Bars, 10 μm. **g,** Quantification of YFP-ARA7 or ABS3-GFP fluorescence intensities after wortmannin treatment. Mean fluorescence intensity on the MVB rings and inside MVB rings were measured with Fiji-ImageJ. Fluorescence ratio was calculated as indicated. Data were presented as mean ± s.d. (ABS3-GFP, n=37; YFP-ARA7, n=34). *p*-values were determined by one-way ANOVA followed by Tukey's multiple comparisons test. **h,** Representative images of WT, *abs3-1D* and *mateq* cells stained with Magic Red cathepsin B reagent. Bars, 20 μm. **i,** Fluorescence intensity quantifications of cells stained by Magic Red cathepsin B reagent (WT, n=459; *abs3-1D*, n=482; *mateq*, n=469; two-tailed Mann-Whitney test). In boxplots, box represented the interquartile range (IQR), middle line in the box represented the median, whiskers were drawn as defined by the Tukey method (75th percentile + 1.5 IQR and 25th percentile -1.5 IQR). Experiments in **a**-**e** were independently repeated three times with similar results.



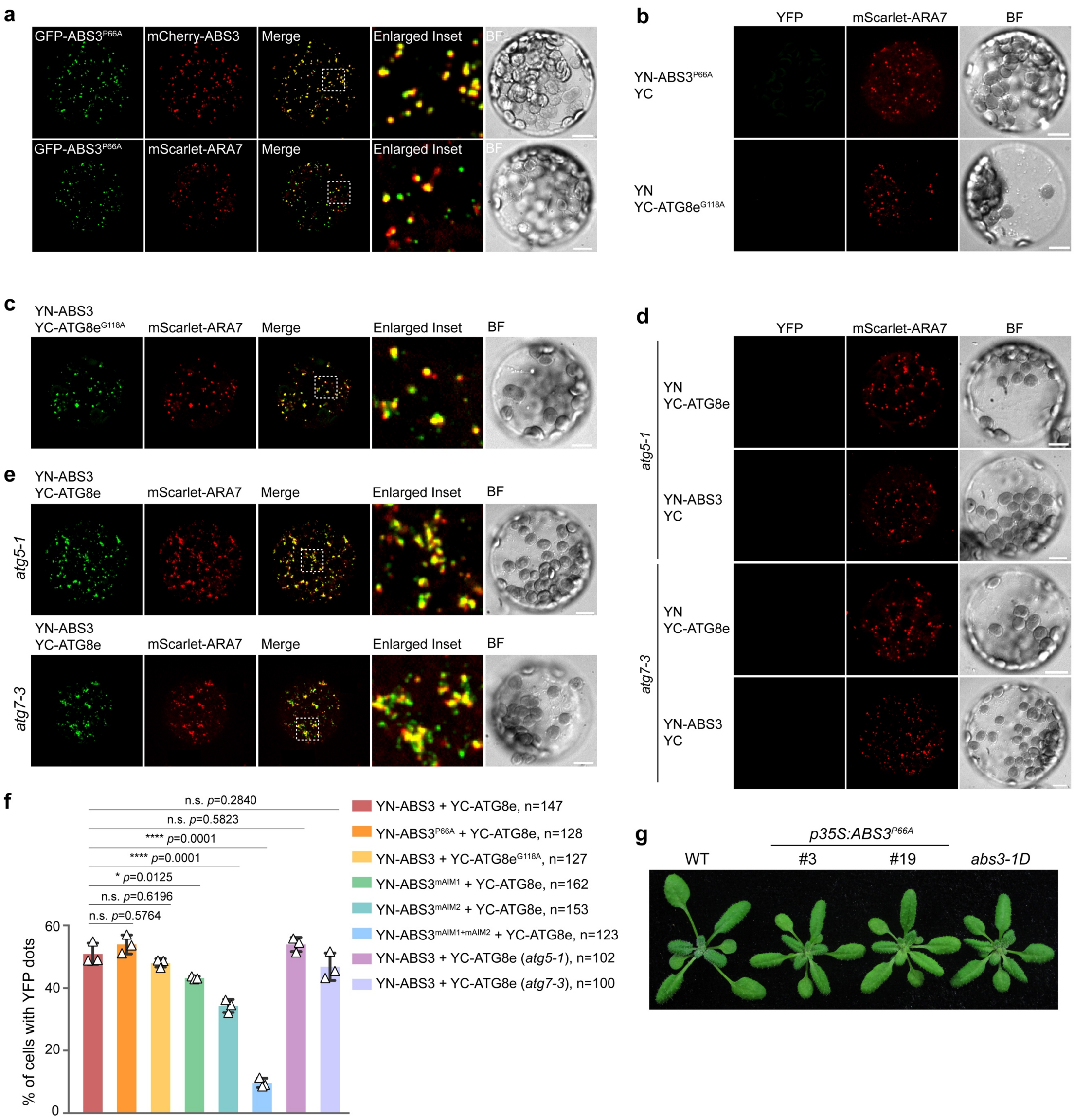
Supplementary Fig. 3. Physical interactions between ABS3-subfamily MATEs and ATG8e.

a, Negative controls for BiFC assay shown in Fig. 3b. Protoplasts were co-transfected with indicated BiFC vectors and *p35S:mScarlet-ARA7*. **b,** Interaction between ABS4 and ATG8e in split-ubiquitin assay. **c,** Protoplasts co-expressing YN-ABS4/ABS3L1/ABS3L2/ABS3L3/ABS3L4, YC-ATG8e, and mScarlet-ARA7 to determine the subcellular localization of their interactions. Bars in **a** and **c**, 10 μm. Experiments in **a-c** were independently repeated three times with similar results.



Supplementary Fig. 4. BiFC analyses of interactions between six ABS3 subfamily MATEs and nine AtATG8s.

Bars, 10 μm. Experiments were independently repeated three times with similar results.



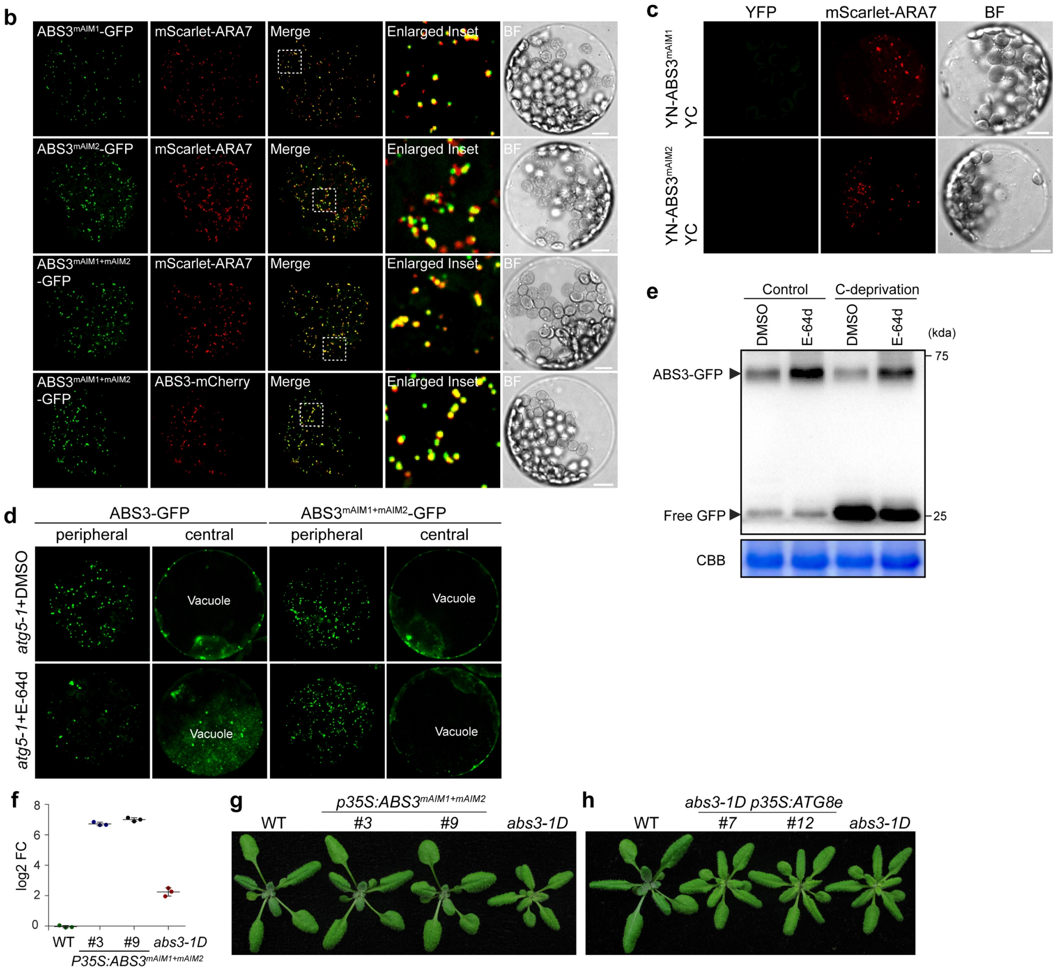
Supplementary Fig. 5. Localization of GFP-ABS3^{P66A} and phenotypes of *p35S:ABS3^{P66A}* overexpression lines.

a, Co-localization of GFP-ABS3^{P66A} with mCherry-ABS3 or mScarlet-ARA7 in Arabidopsis leaf protoplasts. **b** and **d,** Negative controls for BiFC assays shown in Fig. 4c, g-i. Protoplasts were co-transfected with indicated BiFC vectors and *p35S:mScarlet-ARA7*. **c,** Protoplasts co-expressing YN-ABS3, YC-ATG8e^{G118A}, and mScarlet-ARA7. **e,** Co-expressing YN-ABS3, YC-ATG8e, and mScarlet-ARA7 in protoplasts prepared from *atg5-1* or *atg7-3* mutant. Bars, 10 μm in **a-e**. **f,** Quantifications of BiFC assays. Protoplasts were co-transfected with indicated BiFC vectors and *p35S:mScarlet-ARA7*. Percentages of cells showing YFP signals over the total number of cells expressing mScarlet-ARA7 were calculated. Data were shown as mean ± s.d. of three independent sets of experiments and *p*-values were determined by one-way ANOVA followed by Dunnett's multiple comparisons test. **g,**

17-day-old soil-grown WT, two independent lines expressing *p35S:ABS3*^{P66A} and *abs3-1D*. Note that *p35S:ABS3*^{P66A} transgenic plants showed shorter petioles, smaller plant statures, resembling *abs3-1D*. Experiments in **a**-**e**, and **g** were independently repeated three times with similar results.

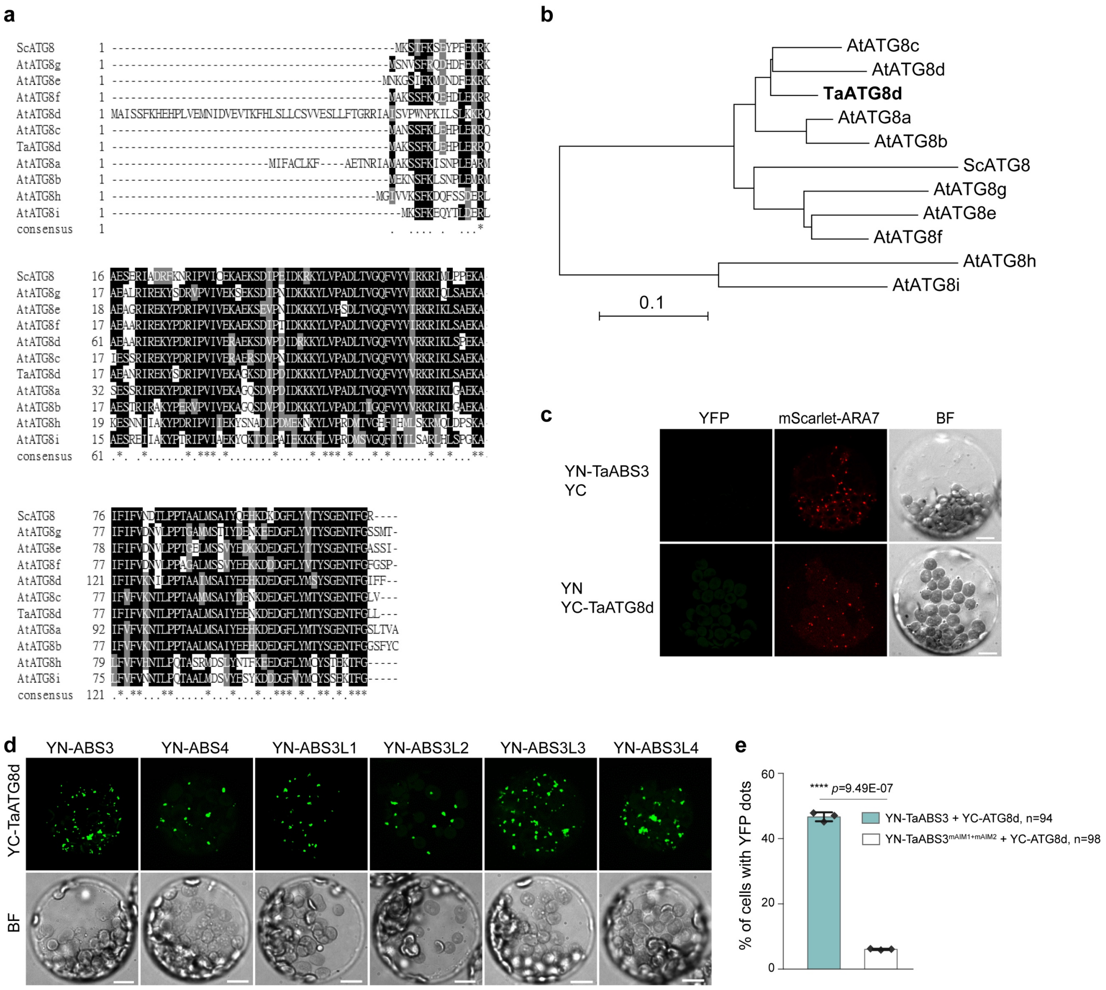
a

	Motif	Start	End	LIR Sequence	PSSM Score
AIM1	WxxL	276	281	RGWAPL	15 (4.2e-02)
AIM2	WxxL	461	466	GLWCGL	12 (1.1e-01)



Supplementary Fig. 6. Two AIMs mediate the ATG8-ABS3 interaction.

a, Two AIMs in ABS3 predicted by the iLIR program. **b,** Co-localizations of ABS3^{mAIM1}-GFP/ABS3^{mAIM2}-GFP/ABS3$^{mAIM1+mAIM2}$-GFP with mScarlet-ARA7 and co-localization of ABS3$^{mAIM1+mAIM2}$-GFP with ABS3-mCherry in protoplasts. **c,** Negative controls for BiFC assays shown in Fig. 5b. Protoplasts were co-transfected with indicated BiFC vectors and *p35S:mScarlet-ARA7*. **d,** Subcellular distribution of transiently expressed ABS3-GFP and ABS3$^{mAIM1+mAIM2}$-GFP in *atg5-1* protoplasts mock-treated with DMSO or treated with E-64d. Illustrated are images of the same protoplasts focused to the cell periphery or to the center of the cell. Bars, 10 μm in **b-d**. **e,** C-deprivation stimulated ABS3-GFP degradation. C-deprivation and E-64d treatment were carried out as in Fig. 5d with 5-d-old seedlings of *p35S:ABS3-GFP* transgenic lines. ABS3-GFP and free GFP were detected by immunoblotting with anti-GFP. **f,** RT-qPCR analysis of *ABS3* transcripts levels in plants shown in **g**. The log2 FC was calculated with respect to the expression levels in the WT. Data were shown as mean ± s.d., n=3 biological replicates. **g,** Phenotype of soil grown 16-d-old WT, two independent lines expressing *p35S:ABS3*$^{mAIM1+mAIM2}$, and *abs3-1D*. Note that *p35S:ABS3*$^{mAIM1+mAIM2}$ lines resemble WT. **h,** Phenotype of soil grown 20-d-old WT, two independent lines expressing *p35S:ATG8e* in *abs3-1D* background, and *abs3-1D*. Note that *abs3-1D p35S:ATG8e* lines resemble *abs3-1D*. Experiments in **a-e**, **g,** and **h** were independently repeated three times with similar results.



Supplementary Fig. 7. Cross species interaction between ABS3 subfamily MATEs and TaATG8d.

a, Multiple sequence alignments of ScATG8, TaATG8d, and AtATG8s. **b,** Phylogenetic tree of Arabidopsis ATG8s and TaATG8d. **c,** Negative controls for BiFC assays shown in Fig. 6b. Protoplasts were co-transfected with indicated BiFC vectors and *p35S:mScarlet-ARA7*. **d,** Protoplasts co-expressing YN-ABS3/ABS4/ABS3L1/ABS3L2/ABS3L3/ABS3L4, and YC-TaATG8d to detect interactions between Arabidopsis MATEs and TaATG8d. Bars, 10 μm in **c** and **d**. Experiments in **c** and **d** were independently repeated three times with similar results. **e,** Quantifications of BiFC assays shown in Fig. 6b and 6e. Data were shown as mean ± s.d. of three independent sets of experiments and *p*-value was determined by two-tailed Student's *t*-test.

Supplementary Table 1. Primers used in this study.

Notes	Primer Name	Primer Sequence
For vector construction		
pUC18-p35S:YN-ABS3; pUC18-p35S:GFP-ABS3^{P66A}	29140 F	CAT GGATCC ATGTGTAACCCATCAACAAC
	SPYN29140 R	CAT CTCGAG TTAATAAAGCACCGTGATGC
MetYC ABS3-CubPLV	29140CubPLV F	ATGTGTAACCCATCAACAACAAC
	29140CubPLV R	ATAAAGCACCGTGATGCGAATCA
pMAL-c4X-ABS3/ABS3^{P66A}	29140SacI F	CAT GAGCTC GATGTGTAACCCATCAACAAC
	29140EcoRI R	CAT GAATTC TTAATAAAGCACCGTGATGC
pUC18-p35S:ABS4-GFP; *MetYC ABS4-CubPLV*	58340BamHI F1	CAT GGATCC ATGTGTAATTCAAAACCATC
	58340BamHI R1	CAT GGATCC AACCAACATGGTTCTCATC
pUC18-p35S:YN-ABS4	58340BamHI R2	CAT GGATCC CTAAACCAACATGGTTCTCATC
pUC18-p35S:YN-ABS3L1	19700BamHI F	CAT GGATCC ATGGAAACCCCAAACATCATC
	19700BamHI R	CAT GGATCC TCAGTCAGTAGCGACAGTGAC
pUC18-p35S:YN-ABS3L2	52050BamHI F	CAT GGATCC ATGAGTCAATCAAATCGTGTC
	52050BamHI R	CAT GGATCC CTACTTATCAACCATCCCAGC
pUC18-p35S:ABS3L3-GFP	23030BamHI F	CAT GGATCC ATGGCAGCTCCTTTGTTGATG
	23030gfp R	CATG CCATGG AACCACCACCACCACCACCGAAGACAAG ATTTTCTTCTAT
pUC18-p35S:YN-ABS3L3	23030XhoI R	CAT CTCGAG CTAGAAGACAAGATTTTCTTC
pUC18-p35S:ABS3L4-GFP	38510BamHI F	CAT GGATCC ATGCAGGTGGGAGAGGAGATGGC
	38510GFP R	CATG GGATCC ACCACCACCACCACCACCATTCGTATTT TGTAGTAAACC
pUC18-p35S:YN-ABS3L4	38510BamHI R	CAT GGATCC TCAATTCGTATTTTGTAGTAAAC
pUC18-p35S:YC-ATG8a	ATG8aBamHI F	CAT GGATCC ATGATCTTTGCTTGCTTG
	ATG8aSacI R	CAT GAGCTC TCAAGCAACGGTAAGAGATC
pUC18-p35S:YC-ATG8b	ATG8bBamHI F	CAT GGATCC ATGGAGAAGAACTCCTTCAAG
	ATG8bXhoI R	CAT CTCGAG TTAGCAGTAGAAAGATCCACC

pUC18-p35S:YC-ATG8c	ATG8cBamHI F	CAT GGATCC ATGGCTAATAGCTCTTTCAAG
	ATG8cXhoI R	CAT CTCGAG TTAAACCAAACCAAAGGTGTTC
pUC18-p35S:YC-ATG8d	ATG8dBamHI F	CAT GGATCC ATGGCGATTAGCTCCTTCAAGC
	ATG8dXhoI R	CAT CTCGAG TTAGAAGAAGATCCCGAACGTG
pUC18-p35S:GFP-ATG8e; *pUC18-p35S:YC-ATG8e*	ATG8eBamHI F	CAT GGATCC ATGAATAAAGGAAGCATC
	ATG8eBamHI R	CAT GGATCC TTAGATTGAAGAAGCACCGAA
pUC18-p35S:YC-ATG8e^{G118A}	ATG8eG118AR	CAT GGATCC TTAGATTGAAGAAGCAGCGAA
NX33 NubG-ATG8e	NubG-ATG8e F	ATGAATAAAGGAAGCATCTTTAA
	NubG-ATG8e R	TTAGATTGAAGAAGCACCGAATG
XN21 ATG8e-NubG	ATG8eN117 R	GAATGTGTTCTCGCCACTGTAAG
pUC18-p35S:YC-ATG8f	ATG8fBamHI F	CAT GGATCC ATGGCAAAAAGCTCGTTC
	ATG8fXhoI R	CAT CTCGAG TTATGGAGATCCAAATCC
pUC18-p35S:YC-ATG8g	ATG8gBamHI F	CAT GGATCC ATGAGTAACGTCAGCTTCAGG
	ATG8gXhoI R	CAT CTCGAG TTAAGTCATTGACGATCC
pUC18-p35S:YC-ATG8h	ATG8hBamHI F	CAT GGATCC ATGGGGATTGTTGTCAAGTC
	ATG8hXhoI R	CAT CTCGAG TTAGCCGAAAGTTTTCTCGG
pUC18-p35S:YC-ATG8i	ATG8iBamHI F	CAT GGATCC ATGAAATCGTTCAAGGAAC
	ATG8iXhoI R	CAT CTCGAG TCAACCAAAGGTTTTCTCAC
pUC18-p35S:YC-TaATG8d	TaATG8 F	CATG GGATCC ATGGCGAAGAGCTCGTTCAAG
	TaATG8 R	CATG GGATCC GCCAGCGCCATCTAGAGCAAC
pUC18-p35S:GFP-TaABS3; pUC18-p35S:YN-TaABS3	AK336065 F1	CATG GGATCC ATGACGTCCTGCGGAGGCGCCAC
	AK336065 R1	CATG GGATCC GTGTAGCTATCAACACCGATC
pUC18-p35S:mScarlet-ARA7	ARA7BamHI F	CAT GGATCC ATGGCTGCAGCTGGAAACAAG
	ARA7 R	CAT CTCGAG CTAAGCACAACAAGATGAGCT
pUC18-p35S:mScarlet	mScarlet_NT-GF	TGGAGAGAACACGGGGGACTAGGCCTATGGTTTCTAAGGGAGAAGCTG
	mScarlet_CT-GR	CGATCGGGGAAATTCGAGCTTTACTTATACAATTCATCCATTCCTCCA
For site-directed mutagenesis		
ABS3 P66A	29140P66A F	CACGTTAGCTTTTGCGATCGCCGTGAC
	29140P66A R	GTCACGGCGATCGCAAAAGCTAACGTG
	29140W278A F	GATTGTTTCCGCGGGGCTGCTCCGGC

ABS3 W278A L281A	29140W278A R	CCCGCGAGACGCAGAGCCGGAGCAG
ABS3 W463A L466A	29140W463A F	GGGTTTAATGGGCTTGCTGTAGGAGC
	29140W463A R	AATCTGCGCCGCAAGAGCTCCTACAG
TaABS3 W303A L306A	336065W303A F	GAGAGCTTCCGTGGGGCTGGCGAGGC
	336065W303A R	CAGAGCCAGGCTGATAGCCTCGCCAG
TaABS3 W488A L491A	336065W488A F	GACTTCGAGGGCCTGGCTCTGGGGGC
	336065W488A R	GGCCTGCGCCGCCAGAGCCCCCAGAG
ATG8eG118A	ATG8eBamHI F	CAT GGATCC ATGAATAAAGGAAGCATCTTT
	ATG8e G118A R	CAT GGATCC TTAGATTGAAGAAGCAGCGAA
For genotyping		
SALK T-DNA lines	SALK_LB	GAACAACACTCAACCCTATCTC
SAIL T-DNA lines	SAIL_LB3	TAGCATCTGAATTTCATAACCAATCTCGA TACAC
SAIL_129_B07	ATG5 F4	CAAGATCTTTCACCAGTGTC
	ATG5 R2	TTGTATAGGCATCAAGATCAC
SAIL_11_H07	ATG7 qF1	GATTGGCGCGACTCAGATTT
	ATG7 F1	GAGCATTACCGTCTCTTAAC
	ATG7 R2	CATGGCGCATTACCATGTAG
SALK_127812	23030c F	CATGGATCCTAATCATGGCAGCTCCTTT GTTG
	23030gfp R	CATGCCATGGAACCACCACCACCACCAC CGAAGACAAGATTTTCTTCTAT
SALK_128217C	38510 F2	AGTATTGGTGGTATGAGATC
	38510 R1	TTTAGGTATCGATGATGCTC
For RT-qPCR		
ABS3 (AT4G29140)	29140 qF1	GCATCATTCACCCCGTAACC
	29140 qR1	TTGTGATGGAGGAAGCGACT
SGR1 (AT4G22920)	SGR1 qF1	GCGGTGGCCATTTCCTTTTA
	SGR1 qR1	AGTTCCCATCTCCATGCACA
CAB3 (AT1G29910)	CAB3 qF2	TACGGATCCGACCGAGTCAA
	CAB3 qR1	GTGTCCCATCCGTAGTCTCC
PETC (AT4G03280)	PETC qF1	GCCATGGATCCCAATACAAC
	PETC qR1	AGTTTCCACCCATGGAACAA
*PRPL1*3 (AT1G78630)	78630 qF1	GTGAAGTGTGAAGCTGAGCC
	78630 qR1	TTCCAGATGTCAGGGCCATT
TaABS3 (AK336065)	AK336065 seqF	AGTCCATAAACCTGCCACTC
	AK336065L qR1	AGAAGCAAGAGGAGCAGGTT
ATG8e (AT2G45170.1)	ATG8e qF1	TGAAGCTGGAAGGATCAGGG
	ATG8e qR1	GTGTTCTCGCCACTGTAAGT
ACT2 (AT3G18780)	ACT2 qF	CTTGCACCAAGCAGCATGAA
	ACT2 qR	CCGATCCAGACACTGTACTTCCTT
SAG12 (AT5G45890)	SAG12qF3	TGTCGCCGTTTAGGTACCAAAA
	SAG12qR3	CAACAACATCCGCAGCTGC
ORE1 (AT4G03280)	ORE1qF1	CTTACCATGGAAGGCTAAGATGGG
	ORE1qR1	TCGGGTATTTCCGGTCTCTCAC

Supplementary Table 2. Vectors used in this study.

Vector Name	Source
For transient expression in protoplasts	
pUC18-p35S:GFP	Wang et al.,2015
pUC18-p35S:mCherry	Wang et al.,2015
pUC18-p35S:mScarlet	This study
pUC18-p35S:ABS3-GFP	Wang et al.,2015
pUC18-p35S:ABS4-GFP	Wang et al.,2015
pUC18-p35S:ABS3L1-GFP	Wang et al.,2015
pUC18-p35S:ABS3L2-GFP	Wang et al.,2015
pUC18-p35S:ABS3L3-GFP	This study
pUC18-p35S:ABS3L4-GFP	This study
pUC18-p35S: $ABS3^{mAIM1}$ *-GFP*	This study
pUC18-p35S: $ABS3^{mAIM2}$ *-GFP*	This study
pUC18-p35S:$ABS3^{mAIM1+mAIM2}$*-GFP*	This study
pUC18-p35S:GFP-$ABS3^{P66A}$	This study
pUC18-p35S:GFP-ATG8e	This study
pUC18-p35S:GFP-TaABS3	This study
pUC18-p35S:GFP-$TaABS3^{mAIM1+mAIM2}$	This study
pUC18-p35S:mCherry-ABS3	This study
pUC18-p35S:mScarlet-ARA7	This study
pUC18-p35S:YN	Waadt et al.,2008
pUC18-p35S:YC	Waadt et al.,2008
pUC18-p35S:YN-ABS3	This study
pUC18-p35S:YN-ABS4	This study
pUC18-p35S:YN-ABS3L1	This study
pUC18-p35S:YN-ABS3L2	This study
pUC18-p35S:YN-ABS3L3	This study
pUC18-p35S:YN-ABS3L4	This study
pUC18-p35S:YN-TaABS3	This study
pUC18-p35S:YN-$ABS3^{P66A}$	This study
pUC18-p35S:YN-$ABS3^{mAIM1+mAIM2}$	This study
pUC18-p35S:YN-$ABS3^{mAIM1}$	This study
pUC18-p35S:YN-$ABS3^{mAIM2}$	This study
pUC18-p35S:YC-ATG8a	This study
pUC18-p35S:YC-ATG8b	This study
pUC18-p35S:YC-ATG8c	This study
pUC18-p35S:YC-ATG8d	This study
pUC18-p35S:YC-ATG8e	This study

pUC18-p35S:YC-ATG8f	This study
pUC18-p35S:YC-ATG8g	This study
pUC18-p35S:YC-ATG8h	This study
pUC18-p35S:YC-ATG8i	This study
pUC18-p35S:YC-TaATG8d	This study
pUC18-p35S:YC-ATG8e^{G118A}	This study
For Split-Ubiquitin assay	
XN21_GW	ABRC CD3-1734
NX33_GW	ABRC CD3-1736
XNWT_GW	ABRC CD3-1738
NWTX_GW	ABRC CD3-1739
MetYC_GW	ABRC CD3-1740
XN21 ATG8e-NubG	This study
NX33 NubG-ATG8e	This study
MetYC ABS3-CubPLV	This study
MetYC ABS4-CubPLV	This study
For plant transformation	
pBI111L	Yu et al.,2004
pBI111L-p35S:ABS3-GFP	This study
pBI111L-pABS3:ABS3-GFP	This study
pBI111L-p35S: ABS3^{P66A}	This study
pBI111L-p35S: ABS3$^{mAIM1+mAIM2}$	This study
pBI111L-p35S: ABS3$^{mAIM1+mAIM2}$*-GFP*	This study
pBI111L-p35S: TaABS3	This study
pBI111L-p35S: TaABS3$^{mAIM1+mAIM2}$	This study
pBI111L-p35S:ATG8e	This study
For expression in *E. coli*	
pGEX 4T-1	27-4580-01, GE Healthcare
pGEX 4T-1 GST-ATG8e	This study
pMAL-c4X	E8000S, New England BioLabs
pMAL-c4X-ABS3	This study
pMAL-c4X-ABS3^{P66A}	This study