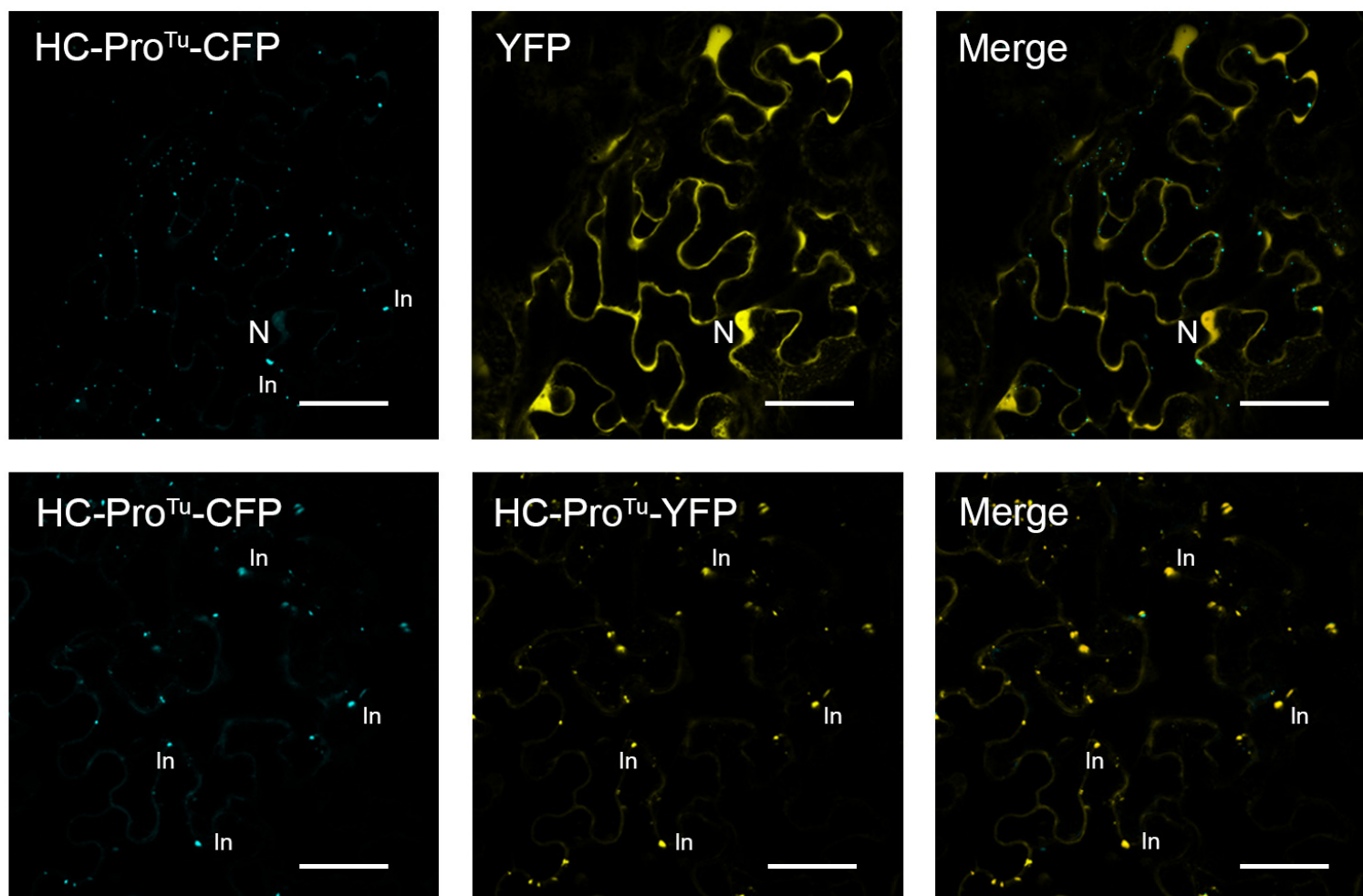
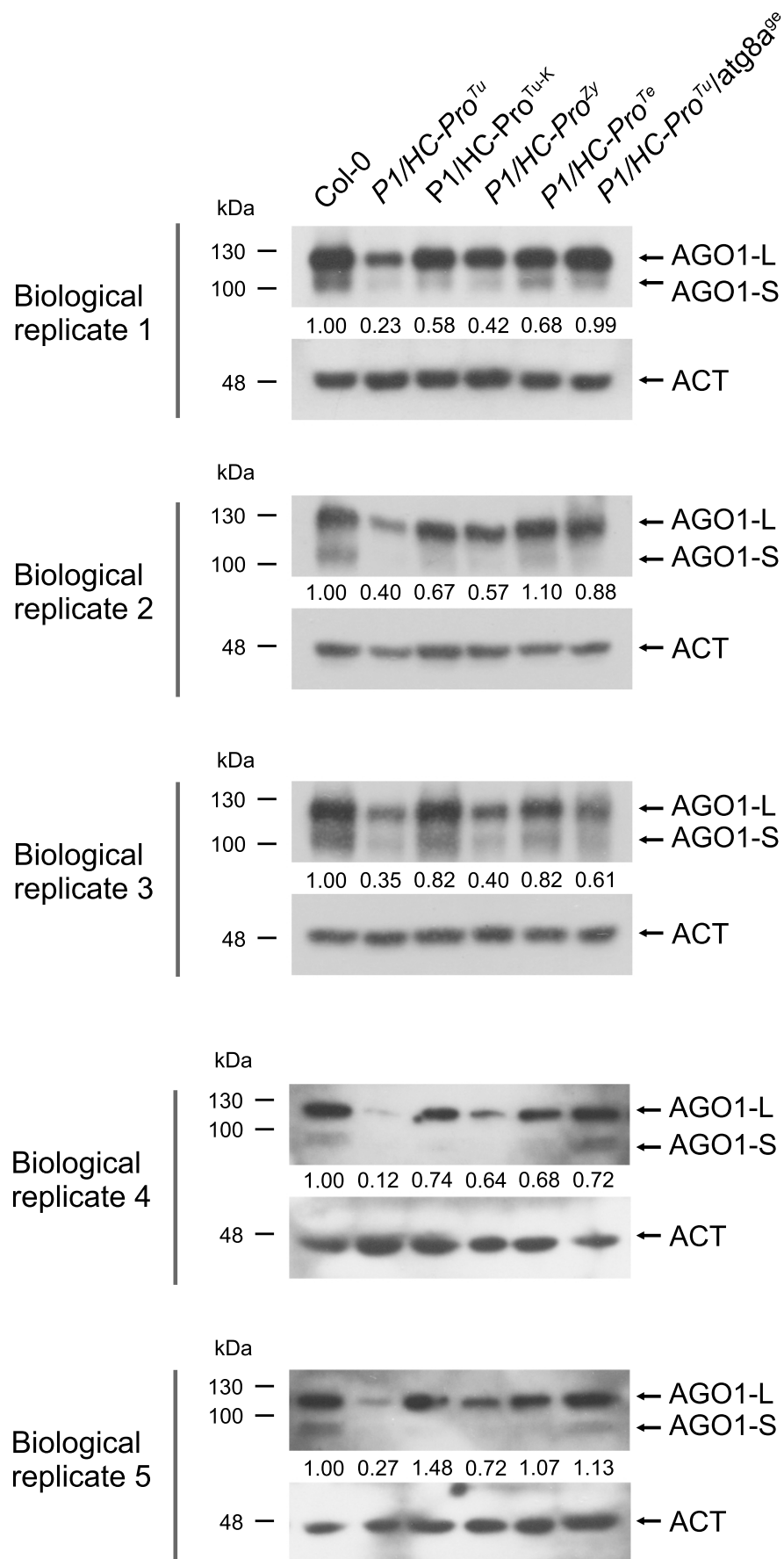
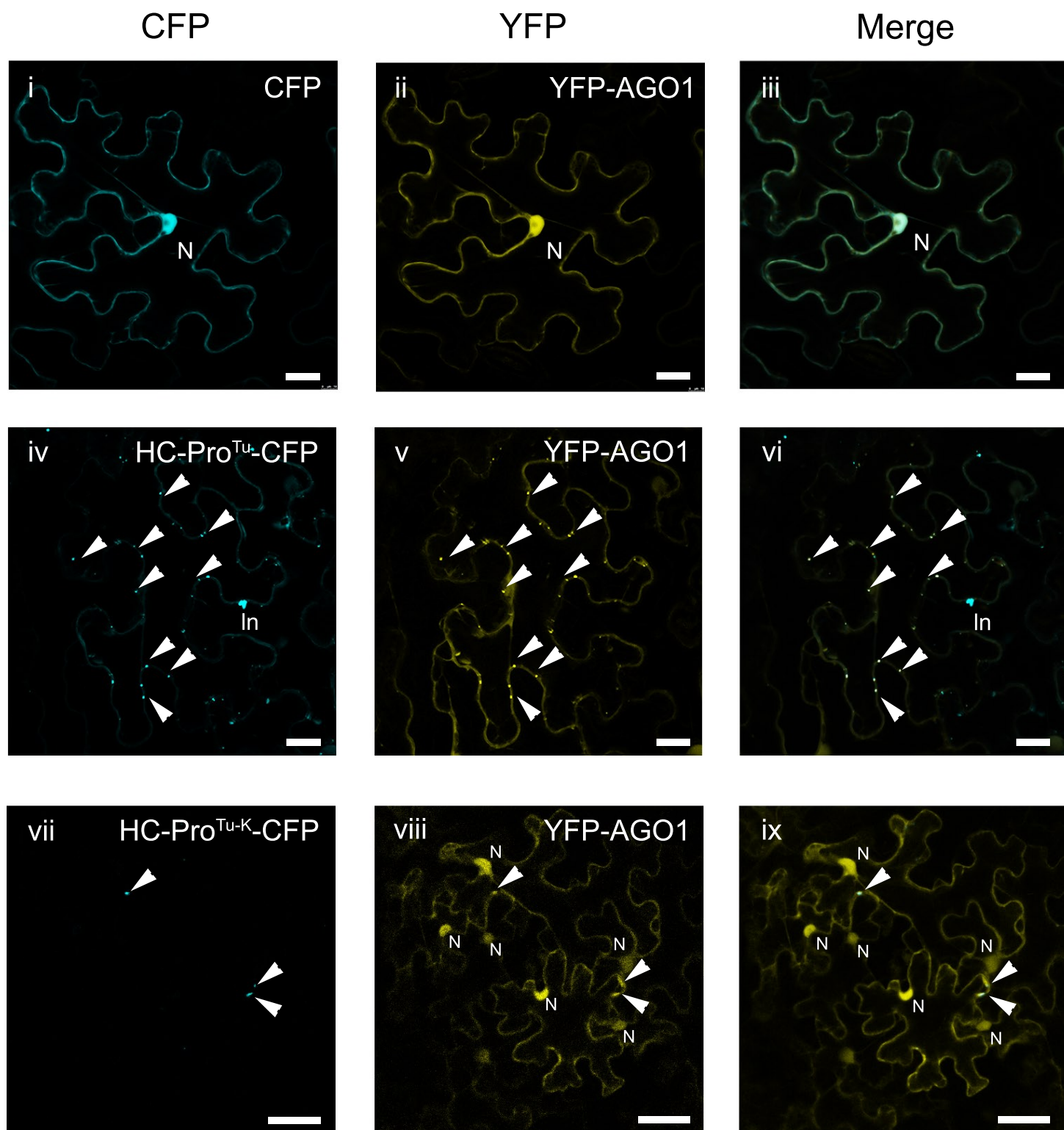




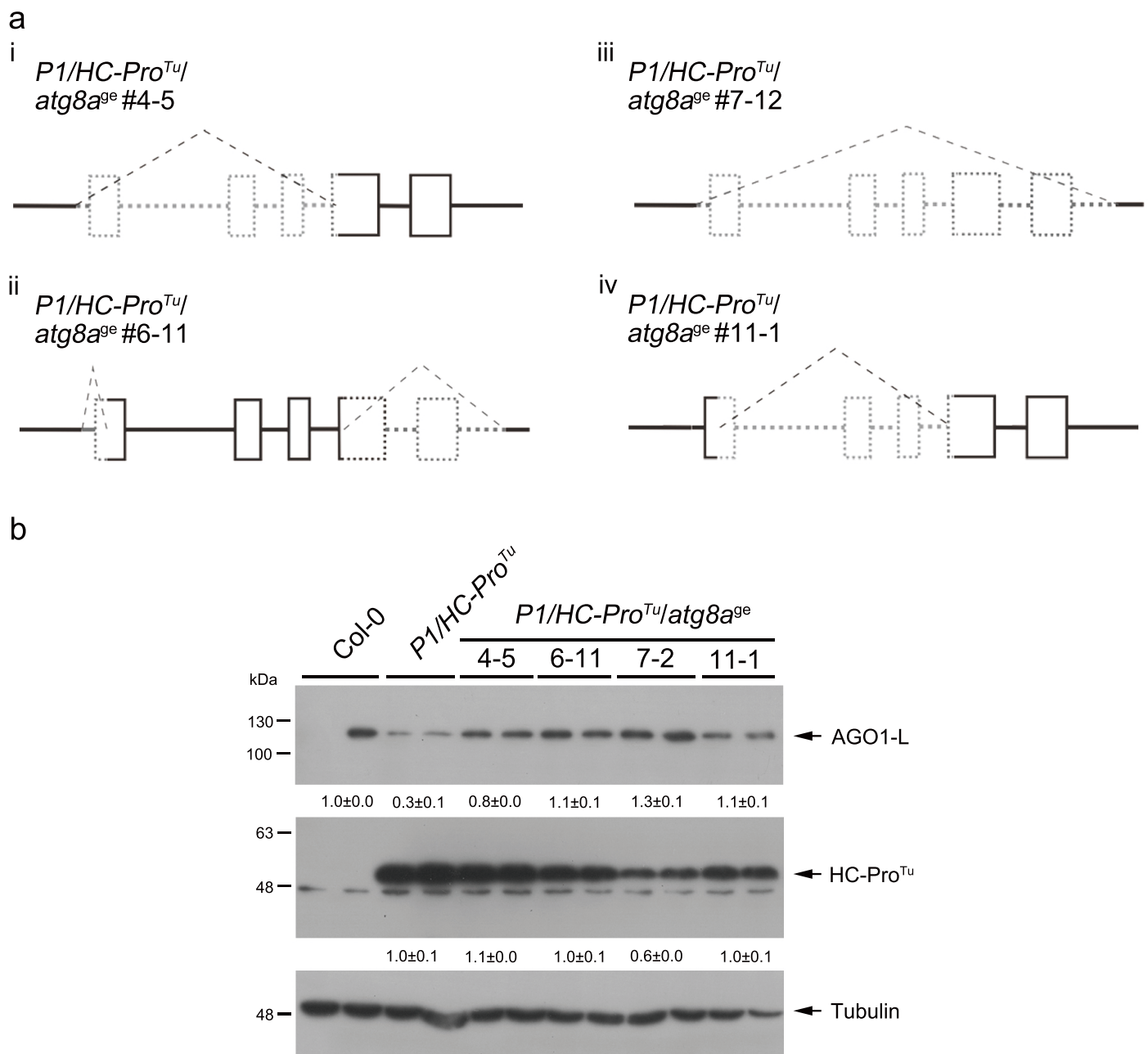
Supplementary Figure 1. Subcellular colocalization and interaction of HC-Pro. Subcellular co-localization of HC-Pro^{Tu}-YFP or YFP with HC-Pro^{Tu}-CFP. Bar, 50 μ m. N, nucleus. In, inclusion body.



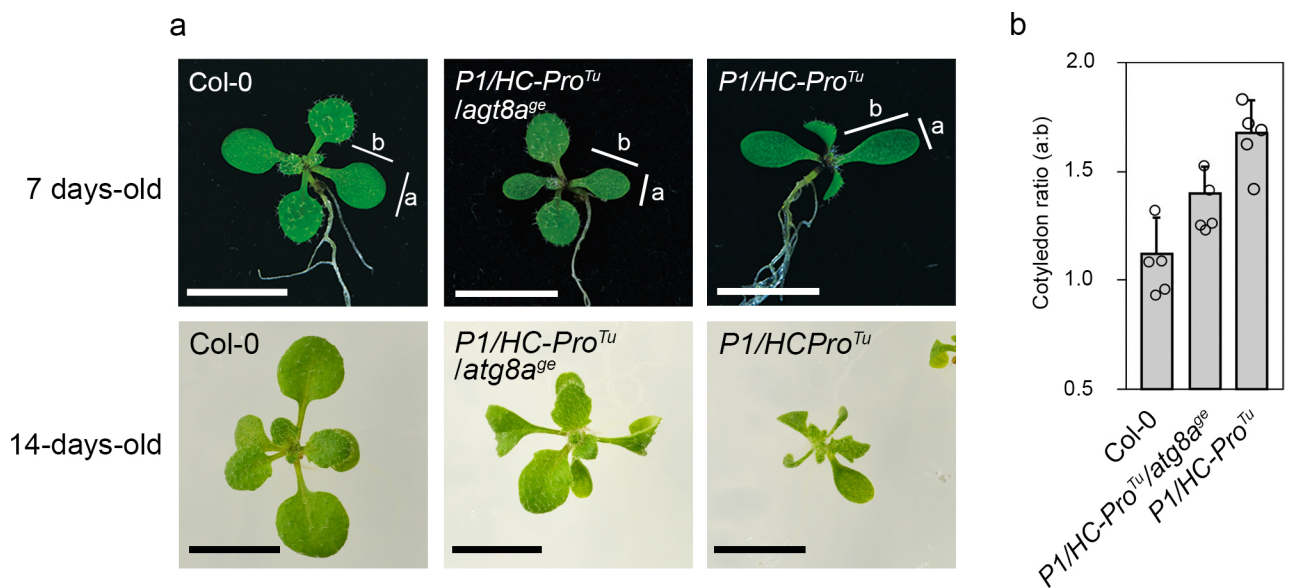
Supplementary Figure 2. Evaluation of the inhibition of P1/HC-Pro^{Tu}-mediated AGO1 degradation. Endogenous AGO1 protein levels (AGO1-L and AGO1-S) in Col-0 and various P1/HC-Pro plants. Actin (ACT) is a loading control. The fold change values are indicated below the gel image. Levels in Col-0 plants were set at 1.0. The average signal strengths of AGO1 relative to those in the non-transformed wild-type plants (Col-0) after normalization against the ACT loading control.



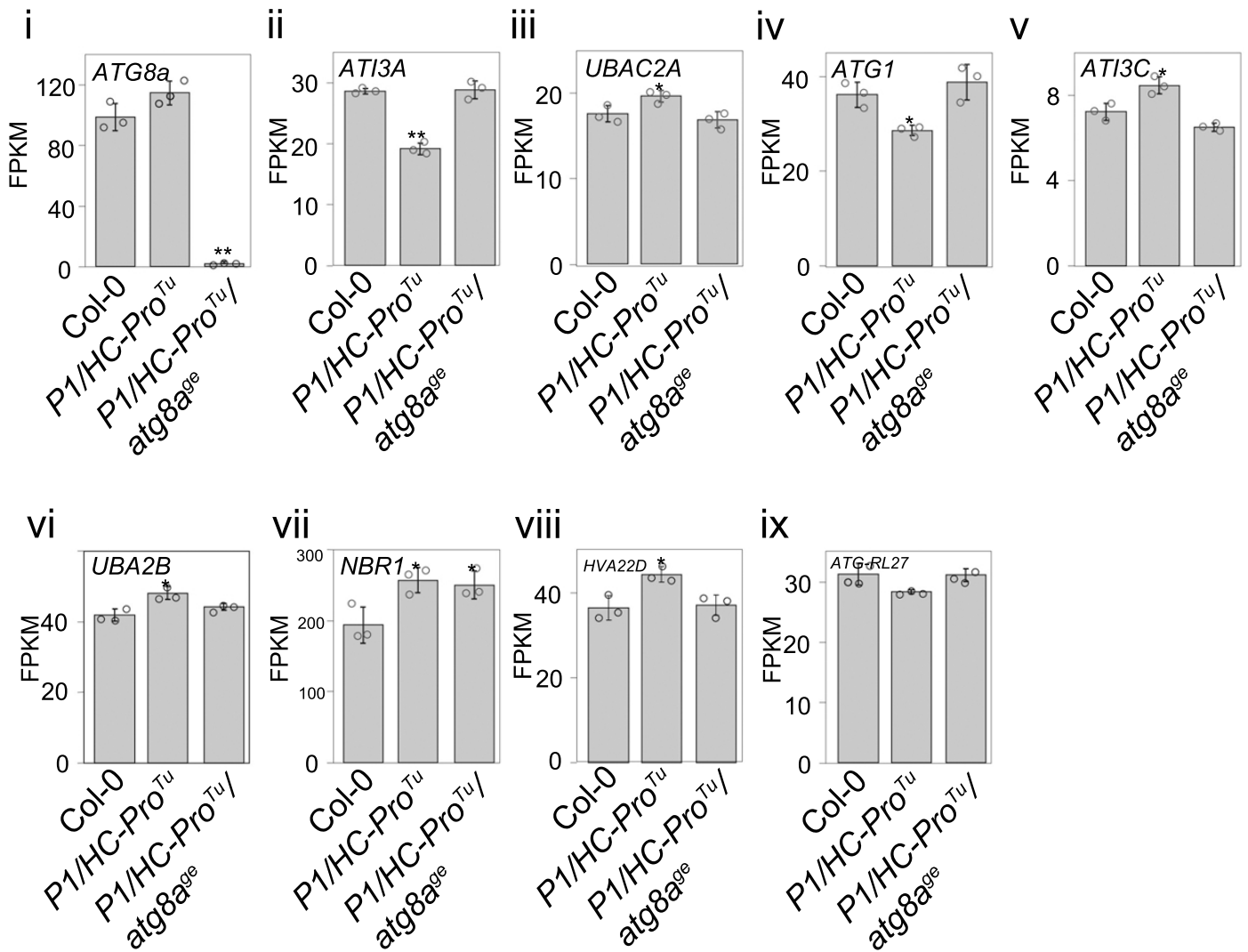
Supplementary Figure 3. Subcellular colocalization and interaction of HC-Pro with AGO1. Subcellular co-localization of YFP-AGO1 or YFP with HC-Pro^{Tu}-CFP or HC-Pro^{Tu-K}-CFP. Bar, 50 μ m. N, nucleus. In, inclusion body. Arrowheads indicate the H-bodies.



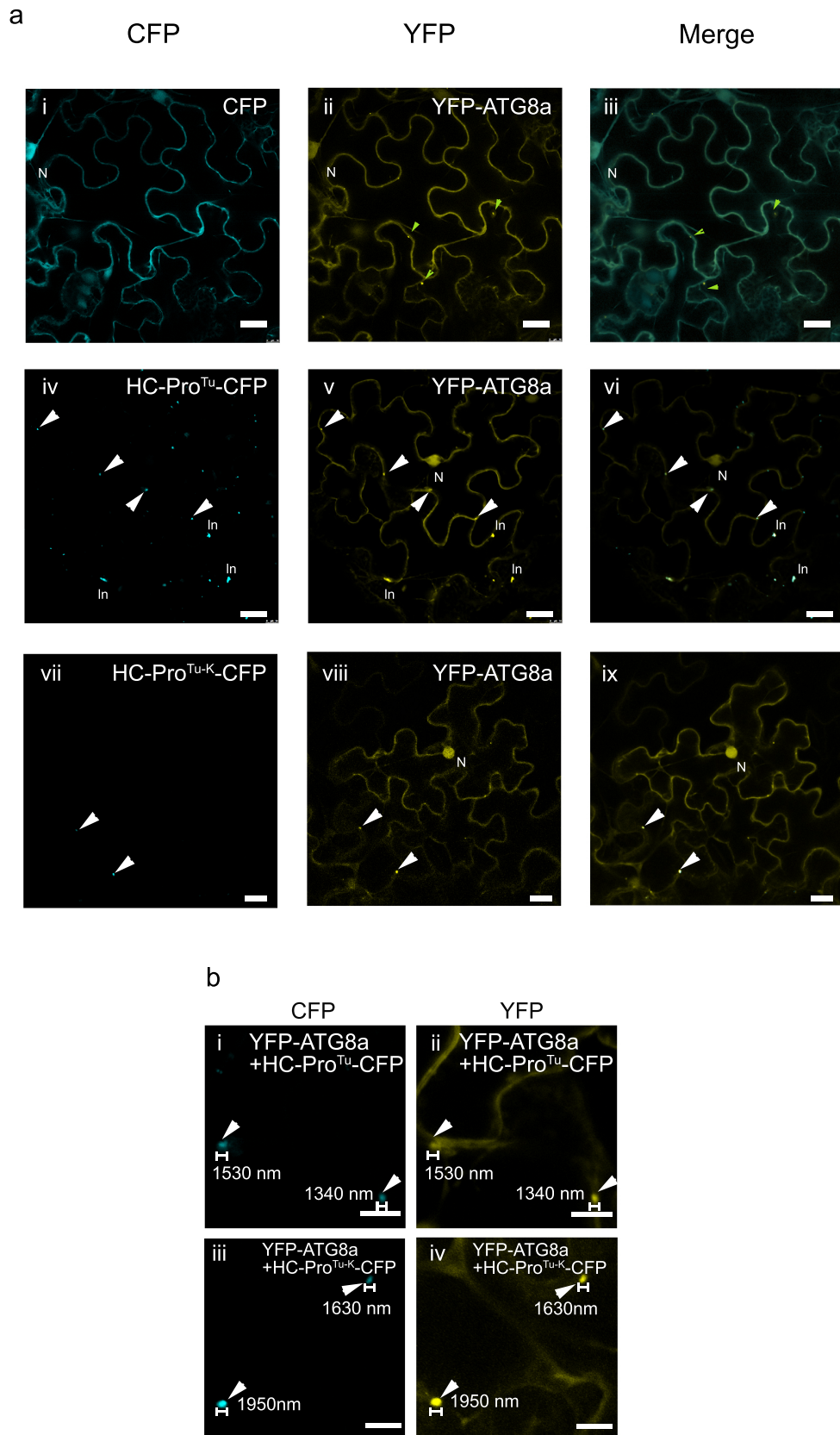
Supplementary Figure 4. *ATG8a* gene knockout in the *P1/HC-Pro^{Tu}* background. (a) Diagram of the gene-editing lines for *P1/HC-Pro^{Tu}/atg8a^{ge}*, including lines #4-5 (i), #6-11 (ii), #7-2 (iii), and #11-1 (iv). (b) AGO1-L (the large form of AGO1) and HC-Pro^{Tu} detection in Col-0, *P1/HC-Pro^{Tu}*, and *P1/HC-Pro^{Tu}/atg8a^{ge}* plants. Tubulin was used as a loading control.



Supplementary Figure 5. Evaluation of the morphogenic phenotype of *P1/HC-Pro^{Tu}/atg8a^{ge}* plants. (a) Phenotypes of wild-type Col-0, *P1/HC-Pro^{Tu}*, and *P1/HC-Pro^{Tu}/atg8a^{ge}* plants. (n=5) Bar, 0.5 cm. The length of the cotyledons is indicated as "a", whereas the width of the leaf is indicated as "b". (b) The ratio of a:b for cotyledons of various plants (n=5). Data are presented as mean values +/- standard deviations. Source data are provided as a Source Data file.

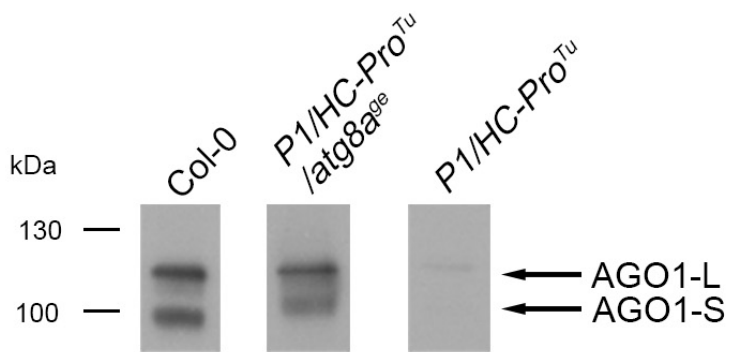


Supplementary Figure 6. Relative expression of autophagy-related genes in *P1/HC-Pro^{Tu}* and *P1/HC-Pro^{Tu}/atg8a^{ge}* plants compared to those expressions in *Col-0* plants. Data are presented as mean values +/- standard deviations. (n=3). Significant differences based on Student's t-test; * indicates *P* values <0.05. ** indicates *P* values <0.01. Source data are provided as a Source Data file.

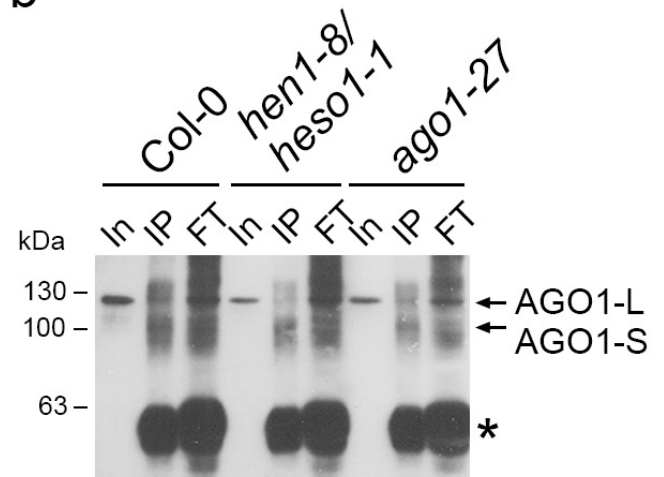


Supplementary Figure 7. Subcellular co-localization and interaction of HC-Pro with ATG8a. (a) Subcellular co-localization of HC-Pro^{Tu}-CFP or HC-Pro^{Tu-K}-CFP with YFP-ATG8a. Bar, 50 μ m. N, nucleus. In, inclusion body. Arrowheads indicate the H-bodies. (b) Image size measurements for speckle-like structures. (i) CFP channel for HC-Pro^{Tu}-CFP. (ii and iv) YFP channel for YFP-ATG8a. (iii) CFP channel for HC-Pro^{Tu-K}-CFP. Bar, 50 μ m.

a

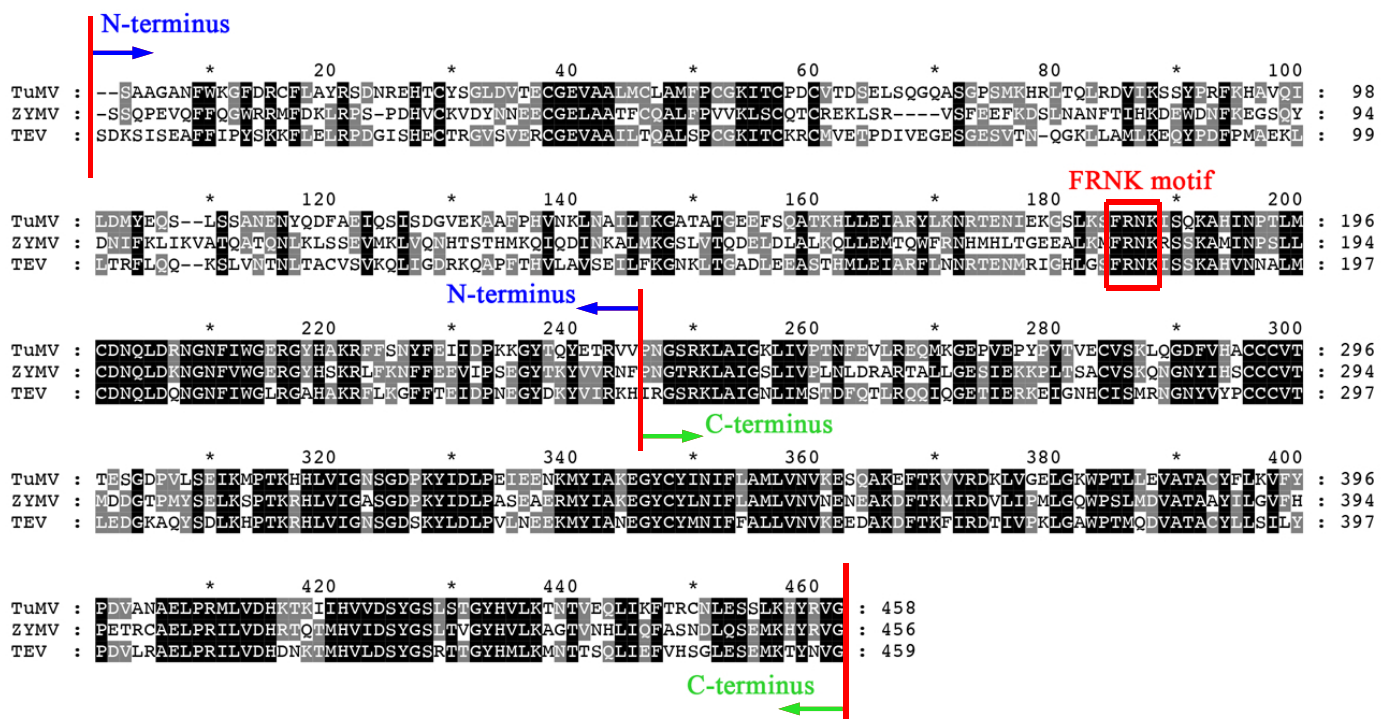


b



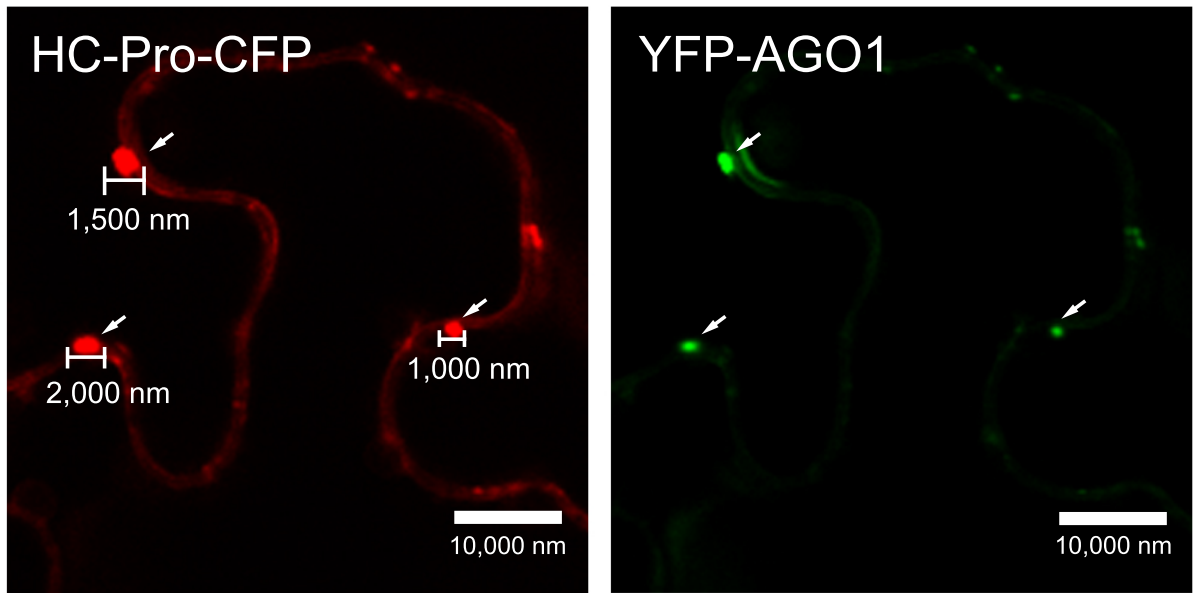
Supplementary Figure 8. Evaluation of the amount of AGO1-IP by western blotting.

(a) AGO1-IP samples from 1-week-old Col-0, *P1/HC-Pro^{Tu}*, and *P1/HC-Pro^{Tu}/atg8a^{ge}* plants. (b) AGO1-IP samples from 1-week-old Col-0, *hen1-8/heso1-1*, and *ago1-27* mutants. AGO1-L, the large form of AGO1. AGO1-S, the small form of AGO1.

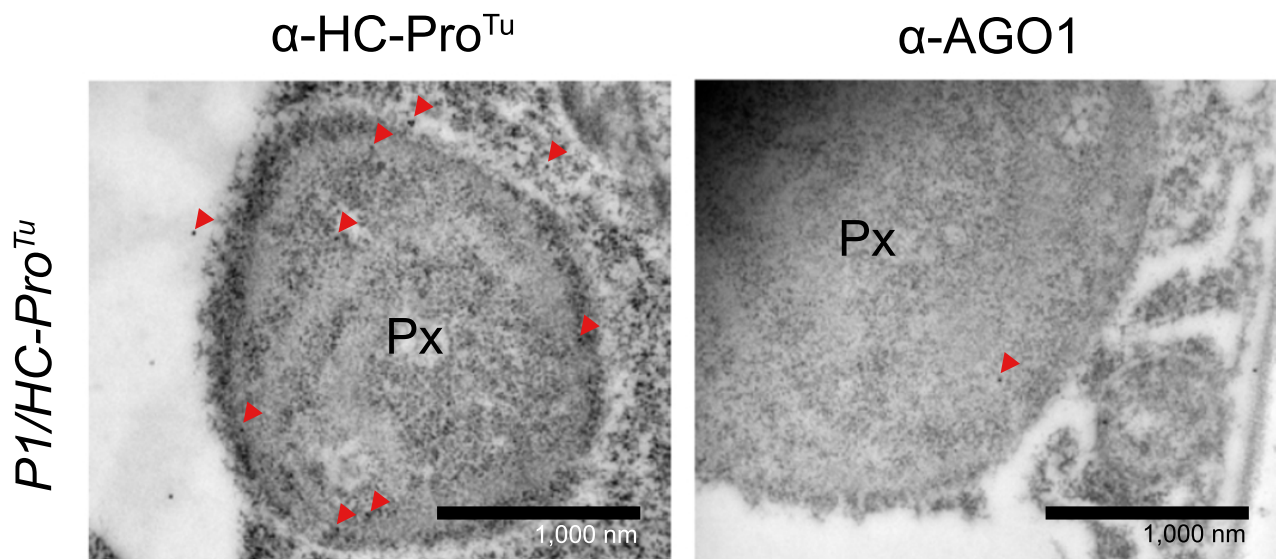


Supplementary Figure 9. Amino acid alignment of HC-Pros. The amino sequences of HC-Pro^{Tu}, HC-Pro^{Zy}, and HC-Pro^{Te} were aligned to compare the sequence variation.

a



b



Supplementary Figure 10. Subcellular localization of HC-Pro^{Tu}, and AGO1 within leaf cells of *P1/HC-Pro^{Tu}* plants. (a) Confocal images depicting the colocalization of HC-Pro-CFP and YFP-AGO1 fluorescence within H-bodies. Arrows indicate the H-bodies. (n=10) Bar, 10,000 nm. (b) Transmission electron microscopy (TEM) images of peroxisomes labeled with immunogold particles. Red arrowheads indicate the presence of immunogold signals of HC-Pro^{Tu} or AGO1 inside of peroxisome (Px). (n=3) Bar, 1,000 nm.

Supplementary Table 1. The primer list for this study.

The gRNA sequences for <i>ATG8a</i> knockout (underlines indicate PAM sequence)	
T1 ^{8a}	5'-TCGATCGTCTGCTAGATCGGG <u>GGG</u> -3'
T2 ^{8a}	5'-AGAGACTAATCGAATCGCAAT <u>TGG</u> -3'
T3 ^{8a}	5'-TGTACCAGCTGATCTAACAGT <u>TGG</u> -3'
T4 ^{8a}	5'-TGACGATTAATAAACCCAAAG <u>GGG</u> -3'
For qPCR	
P_ATG8a-qPCR	5'-CTCTCGAGGCAAGGATGAGT-3'
M_ATG8a-qPCR	5'-TAGCTTTCTCAGCTCCAAGC-3'
P_ATI3C-qPCR	5'-CGGTGACTGCGAAGCAAATA-3'
M+ATI3C-qPCR	5'-GGAACAGAGAGCTGATCCGA-3'
P_AGO1_qPCR	5'-CTCAGGTGGTGGACGTGGTT-3'
M_AGO1_qPCR	5'-TTGCTGTTGTGGTGGTTGCC-3'
P_GRF1_qPCR	5'-CCGGACAGATGGAAAGAAAT-3'
M_GRF1_qPCR	5'-GGCGGCCTCTGTTAATATGT-3'
P_ARF16_qPCR	5'-GCCCCGTTAAGCTCTGTTCTGG-3'
M_ARF16_qPCR	5'-TGGAGGAGGAGGAGGAGGTG-3'
P_UBQ-qPCR	5'-CACTTCACTTGGTCTTGCCT-3'
M_UBQ-qPCR	5'-TATCCTGGATCTTGGCCTTC-3'
For <i>MYB33-230</i> cloning	
For-T7-MYB33	5'-TAATACGACTCACTATAGGGATTTCAAAACTTGCAGCTTCTCAG-3'
Rev-MYB33-230	5'-GGAGATCAGAGTGTGGAGGAG-3'

Supplementary Table 2. Reagents and Chemicals

Experiment	Reagent	Company names	Catalog Numbers
plant treatment	MS media	PhytoTech Labs	M519
	E64-d	Sigma	88321-09-9
	MG132	Sigma	1211877-36-9
SEC&northern	Tris-HCl	BIOMAN	77-86-1
	NaCl	Honeywell	7647-14-5
	DTT	BioShop	3483-12-3
	Protease Inhibitor Cocktail	Roche Applied Science	4693132001
	Proteasome Inhibitor Set II	Calbiochem	539165
	HiPrep 16/60 Sephacryl S-200 HR column	GE Healthcare	17-1166-01
	TRIzol	Invitrogen	15596018
	glycogen	Invitrogen	AM9510
	acrylamide	BIO-RAD	1610156
	urea	Amresco	57-13-6
protein purification	³² P radioisotope	Revvity	BLU502Z250UC
	HiTrap FF column	GE Healthcare	17-5319-01
	imidazole	BioShop	288-32-4
	EDTA	BioBasic	60-00-4
	MgCl ₂	Sigma	7791-18-6
	GSTrap FF column	GE Healthcare	17513001
	glutathione	Sigma	70-18-8
HEN1 methylation assay and β-Elimination	SAM	new england biolabs	B9003S
	borax	Sigma	1330-43-4
	boric acid	Sigma	10043-35-3
	sodium periodate	Sigma	7790-28-5
	glycerol	Sigma	56-81-5
	Sephadex G-25 resin	GE Healthcare	17057202
	NaOH	PanReac AppliChem	1310-73-2
AGO1-IP&RISC assay	NP-40	Sigma	MFCD01779855
	α-HA	Santa Cruz	sc-7392
	protein A-agarose beads	Santa Cruz	sc-20001
	pGEM-T easy vector	Promega	A1360
	MEGAscript T7 Transcription Kit	Invitrogen	AM1334
	[α- ³² P] UTP	PerkinElmer	BLU507H250UC
	G50 resin	GE Healthcare	GE17-0573-02
	TBE	Bioman	TBE051000
	chemiluminescence film	GE Healthcare	28906839
	NaOAc	Invitrogen	AM9740
	centrifuge tube filters	Costar	8160
	KCl	Bio Basic	7447-40-7
EMSA	ATP	Invitrogen	AM1334
	GTP	Invitrogen	AM1334
	RiboLock	Thermo Scientific	EQ0381
	HEPES	Sigma	7365-45-9
	T4 polynucleotide kinase	new england biolabs	M0201S
	bromophenol blue	Sigma	115-39-9
	xylene cyanol	Bio Basic	2650-17-1
Western blotting	acrylamide 37.5:1	BIO-RAD	1610158
	APS	Sigma	7727-54-0
	TEMED	Bio Basic	110-18-9
	SDS	Bioman	151-21-3
	2-mercaptoethanol	Sigma	60-24-2
	Hybond-P PVDF membrane	GE Healthcare	1060023
	α-ACT	Sigma	AS132640
transcriptome	SuperScript III First-Strand Synthesis System	Invitrogen	18080051
	Plant Total RNA Extraction Miniprep System	VIOGENE	GPR1001
TEM	PF-Total RNA Purification Kit	XenoHelix	9366RTR
	paraformaldehyde	Electron Microscopy Sciences, Hatfield, USA	240327-51
	glutaraldehyde	Electron Microscopy Sciences, Hatfield, USA	2220621
	LR White resin	Agar Scientific, UK	832024
	BSA	Sigma-Aldrich, USA	7906
	anti-rabbit IgG	Jackson Immuno Research	148042
	uranyl acetate	Electron Microscopy Sciences, Hatfield, USA	22400
LC-MS/MS	lead citrate	Electron Microscopy Sciences, Hatfield, USA	17800
	iodoacetamide (IAA)	Sigma-aldrich	I1149
	DL-Dithiothreitol(DTT)	Sigma-aldrich	D0632
	TEAB(Triethylammonium bicarbonate)	Sigma-aldrich	T7408
	trypsin	Promega	V5111
	TFA(TFA–Trifluoroacetic acid, HPLC Grade, 99.5+%)	ALFA Aesar	44630
	25-cm-length C18 Acclaim PepMap column	thermo	164941
	formic acid	Honeywell	33015-500ML
	Acetonitrile	Supelco	1.00030.4000