Supporting Information

Autophagy-mediated CTR1 turnover orchestrates the reciprocal interaction between autophagy and ethylene signaling

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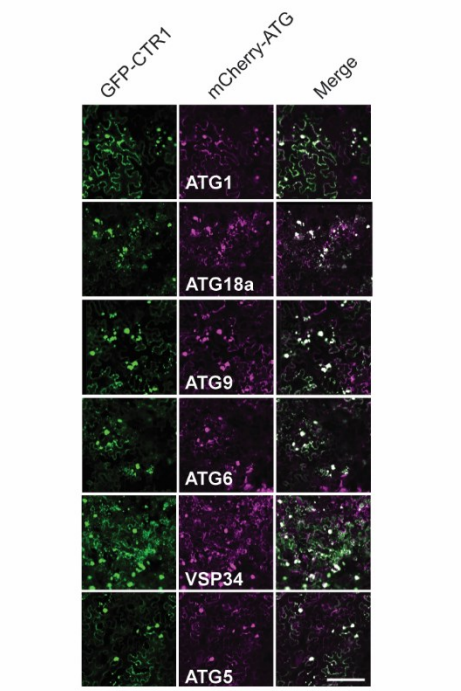
This PDF file includes

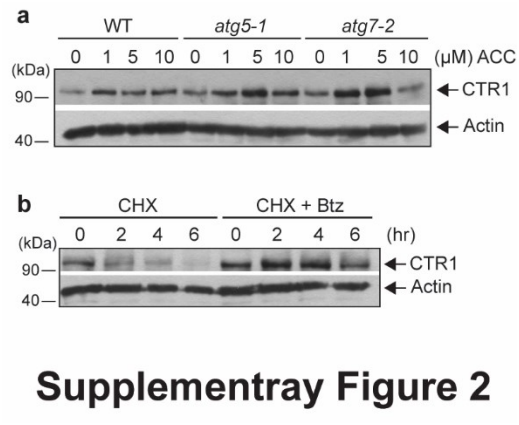
Supplementary Figures 1-7

Supplementary Table 1



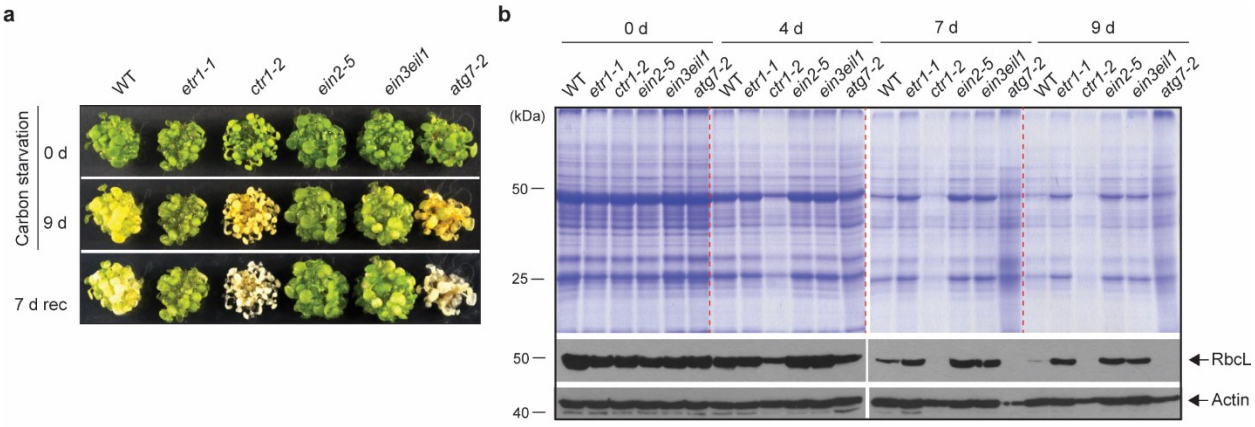
Supplementray Figure 1

Supplementary Figure 1. CTR1 colocalizes with various ATG proteins involved in autophagosome formation. Leaves of *N. benthamiana* were co-infiltrated with Agrobacterium carrying plasmids expressing GFP-CTR1 and the indicated mCherry-ATG proteins. After 3 days, the overlap of GFP and mCherry fluorescence was observed by confocal microscopy. Scale bar, 50 μm.



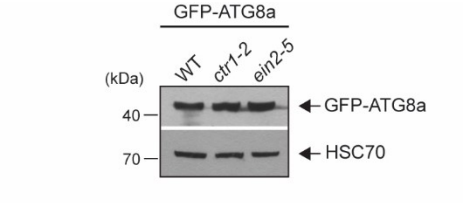
Supplementray Figure 2

Supplementary Figure 2. ACC-induced stabilization of CTR1 is not regulated by autophagy. a. ACC stabilizes CTR1 in both WT and *atg* mutants. Seedlings were grown in MS medium for 3 days in the dark, and treated with different concentrations of ACC in liquid MS medium. Total protein extracts were immunoblotted using anti-CTR1 and anti-Actin antibodies. Actin was detected to show even loading between samples. **b**. The stability of CTR1 is also regulated by the 26S proteasome-ubiquitin pathway. Dark-grown WT seedlings were treated with or without 50 μM Bortezomib (Btz) for 2 hrs, followed by cycloheximide (CHX) treatment to measure its degradation kinetics.



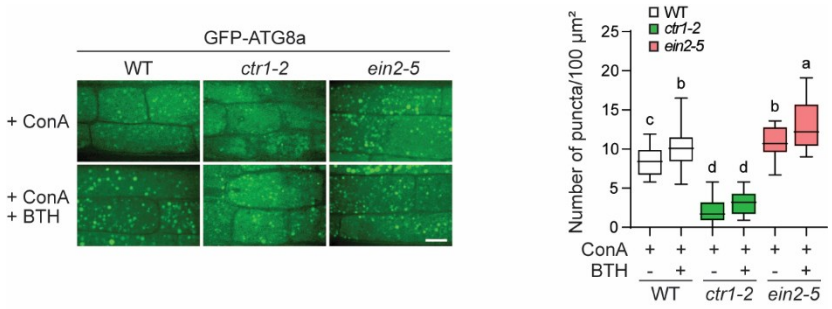
Supplementray Figure 3

Supplementary Figure 3. Ethylene-insensitive mutants are hyposensitive to carbon starvation stress. a. Carbon starvation-induced senescence phenotypes of WT, *ctr1-2*, *atg7-2*, and ethylene-insensitive mutants (*etr1-1*, *ein2-5*, *ein3eil1*). Seven-day-old light-grown seedlings were grown on MS medium without sucrose and transferred to darkness for 9 days, followed by recovery in the light for 7 days (7-rec). **b**. Coomassie brilliant blue-stained SDS-PAGE of total protein extracts of WT, *ctr1-2*, ethylene insensitive mutants, and *atg7-2* mutant. Seven-day-old light-grown seedlings were transferred to total darkness for the indicated times. Seedlings were harvested at the indicated time after carbon starvation, and total cellular proteins were resolved in SDS-PAGE, stained by CBB, or immunoblotted using antibodies against RbcL and actin (loading control).



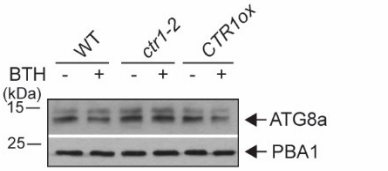
Supplementray Figure 4

Supplementary Figure 4. Expression of GFP-ATG8a in WT, *ctr1-2*, and *ein2-5* mutants. Similar levels of GFP-ATG8a were expressed in WT, *ctr1-2*, and *ein2-5* mutants. Total protein extracts from seedlings were immunoblotted using antibodies against GFP and HSC70 (loading control).



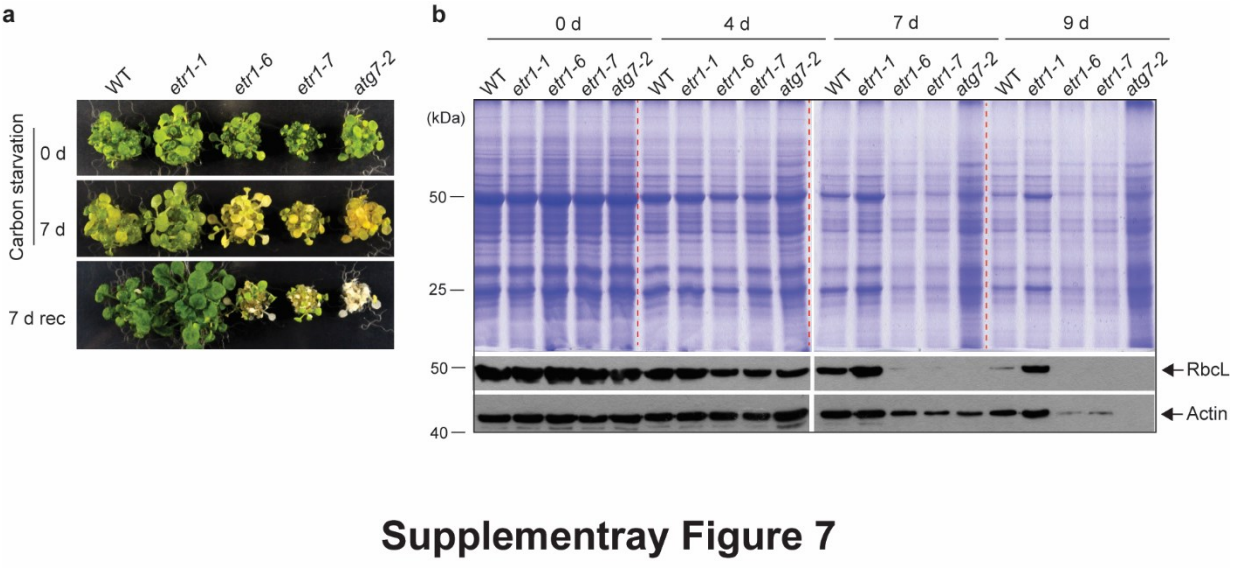
Supplementray Figure 5

Supplementary Figure 5. Autophagosome formation is altered in *ctr1-2* and *ein2-5* in response to BTH treatment. Six-day-old light-grown seedlings were transferred to sucrose-free MS medium with or without benzo-(1,2,3)-thiadiazole-7-carbothioic acid (BTH), a salicylic acid agonist. ConA was also included in both conditions to prevent vacuolar degradation of autophagosomes. Quantification of autophagosome formation in WT, *ctr1-2*, and *ein2-5*. Scale bars, 10 µm. One-way ANOVA with Tukey's post-hoc test was performed, and different letters denote statistically significant differences between groups, $p \leq 0.0001$. ($n \geq 20$, different cells from five to six seedlings for each treatment were used for measurement).



Supplementray Figure 6

Supplementary Figure 6. The steady state levels of endogenous ATG8a in WT, *ctr1-2*, and CTR overexpression lines. Four-day-old dark grown seedlings were treated with or without BTH to induce autophagy. Total protein extracts were analyzed by immunoblotting using anti-ATG8a and PBA1 (loading control) antibodies.



Supplementray Figure 7

Supplementary Figure 7. The *etr1-6* and *etr1-7* loss-of-function mutants are hypersensitive to carbon starvation stress. a. Carbon starvation-induced senescence phenotypes of WT, *etr1-1*, *etr1-6*, and *etr1-7*. Seven-day-old light-grown seedlings were grown on MS medium without sucrose and transferred to darkness for 7 days, followed by recovery in the light for 7 days (7-rec). **b**. Coomassie brilliant blue-stained SDS-PAGE of total protein extracts of WT and *etr1* mutant seedlings. Seven-day-old light-grown seedlings were transferred to total darkness for the indicated times. Seedlings were harvested at the indicated time after carbon starvation, and total cellular proteins were resolved in SDS-PAGE, stained with Coomassie brilliant blue (CBB), or immunoblotted using antibodies against RbCL and actin (loading control).

Supplementary Table 1. Primers used in this study

Primers for ENTRY gateway vectors	
CTR1-CACC-F	CACCATGGAAATGCCCGGTAGAAGATCT
CTR1-R-WS	TTACAAATCCGAGCGGTTGGGC
ATG8a-F	GCTTGATATCGAATTCAATGGCTAAGAGTTCCTTCAAGATC
ATG8a-R	CCGCTCTAGAACTAGTTCAAGCAACGGTAAGAGATCCAA
ATG8a-F for mCherry	ACGGATCCATGGCTAAGAGTTCCTTCAAGA
ATG8a-R for mCherry	TGGCGGCCGCTCTAGATCAAGCAACGGTAAGAGATCC
ATG1-F	GGGGCCCCCCCTCGAGATGGAGTCGGCACGACTTGT
ATG1-R	TGCTCACCATGGATCCAAGATGAGACCGACGATGCTGT
ATG5-F	GGGGCCCCCCCTCGAGATGGCGAAGGAAGCGGTC
ATG5-R	TGCTCACCATGGATCCCCTTTGAGGAGCTTTCACAAGGAC
ATG6-F	GGAGGCCAGTGAATTCATGAGGAAAGAGGAGATTCCAG
ATG6-R	CGAGCTCGATGGATCCCTAAGTTTTTTTACATGAAGGCTT
ATG9-F	GGGGCCCCCCCTCGAGATGATGAGCAGTGGGCATAAGGG
ATG9-R	TGCTCACCATGGATCCCCGTAATGTGGTGCTTGATGTTG
ATG18A-F	GGGGCCCCCCCTCGAGATGGCCACCGTATCTTCTTCCTC
ATG18A-R	TGCTCACCATGGATCCGAAAACTGAAGGCGGTTTCAGACAG
VPS34-F	GGGGCCCCCCCTCGAGATGGGTGCGAACGAGTTTC
VPS34-R	TGCTCACCATGGATCCACGCCAGTATTGAGCCCATCTG
CIP8-F	GGGCCCCCCCTCGAGATGTCCGATGCTCCGTCGTCTTC
CIP8-R	CGCTCTAGAACTAGTGTAACGAGAAGTTGAAGAAGAAGAAG
Primers for quantitative RT-PCR	
ERF1-RT-F	ACGTTCTCAACCGCCTACAG
ERF1-RT-R	CGGACTCGCTCTCTGGTG
CTR1-RT-F	CATGGAAGCGTCCATCATTTGCA
CTR1-RT-R	TGGGCAGCAAAAGAATGCTGAG
ATG1a-RT-F	TGAACCCAGATCCAACCACG
ATG1a-RT-R	GAGTGGCAGCACTTGTTTCG
ATG2-RT-F	AATGGATAGCAAGTGGAAGC
ATG2-RT-R	AGATAGACCTACCGTTAGCC
ATG5-RT-F	GAAGGAAGCGGTCAAGTATGT
ATG5-RT-R	TCTTGGTGCTAACACAAGAGC
ATG7-RT-F	GAAGATTGTCTAGGTCGTGG
ATG7-RT-R	CCTGCTTTCTCTTGTATCGG
ATG8a-RT-F	GACAATTTGTATACGTGGTTCGT
ATG8a-RT-R	TCAAGCAACGGTAAGAGATCCA
ATG9-RT-F	GGATGATGTCCGCTTGGAGT
ATG9-RT-R	TCTCTGCTCACGACGAGTTG
ATG11-RT-F	ACCGGGAGGAATTATTGGAG
ATG11-RT-R	GGACTTCAAGACGACCGAAT
ATG13a-RT-F	ATTTCAGCTCGGAGTGGTCG
ATG13a-RT-R	GGCATCTGTAGAAGCTCCCAT
ATG18a-RT-F	CAAAGGAGTGTTACCGAGGTAT

ATG18a-RT-R	ATCCATGCCAAGAATAACAACG
TOR1-RT-F	ATACGCTGCCTGTGGGAAAT
TOR1-RT-R	CGGACAGCCAAAAGTGCTTG
RAPTOR1B-RT-F	GCGGTCCGCAAACTGTAATC
RAPTOR1B-RT-R	TGGAACTCTGTGCTTGGGTC
LST8-1-RT-F	TCCTGCAAACAAATATCTAGCGAC
LST8-1-RT-R	CCATCCACTGAGAAGACGCA
ACTIN2-RT-F	ATTCAGATGCCCAGAAGTCTTGTT
ACTIN2-RT-R	ACGGTCAGCGATACCTGAGAAC

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