Mitochondrial alternative oxidase dependent autophagy involved in ethylene-mediated drought tolerance in *Solanum lycopersicum*

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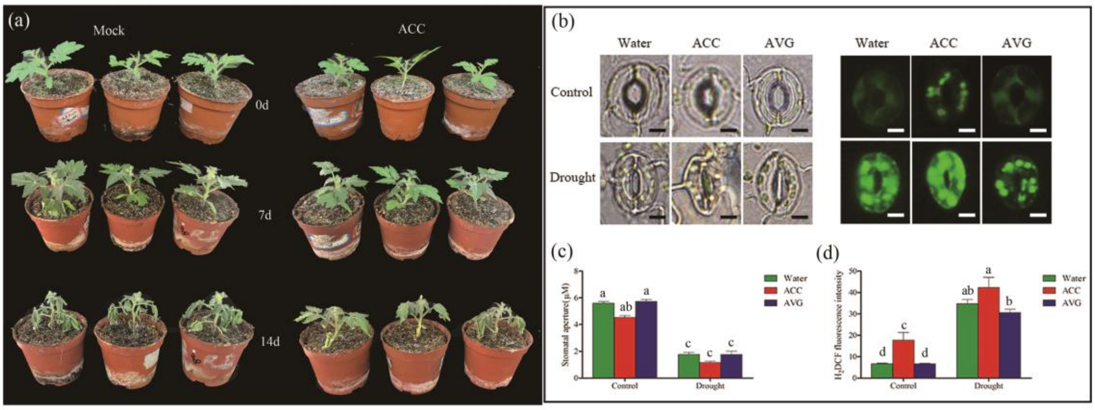

Figure S1 Phynotype and stomatal closure analysis of tomato seedlings in different pretreatments under drought stress. (a) Phenotype of tomato plants during drought stress after 0.1 μM ACC pretreatment. The left side was the control group and the right side was the treatment group. Each group has three parallel samples. (b) Stomatal closure in different treatment groups under microscope. (c) Statistics of horizontal distance of stomatal aperture in different treatment groups. (d) Statistics of green fluorescence intensity after H_2DCF-DA staining. Bars represent mean and standard deviation of values obtained from three biological repeats. Means with the same letter did not significantly differ at P<0.05 according to Duncan multiple range tests.

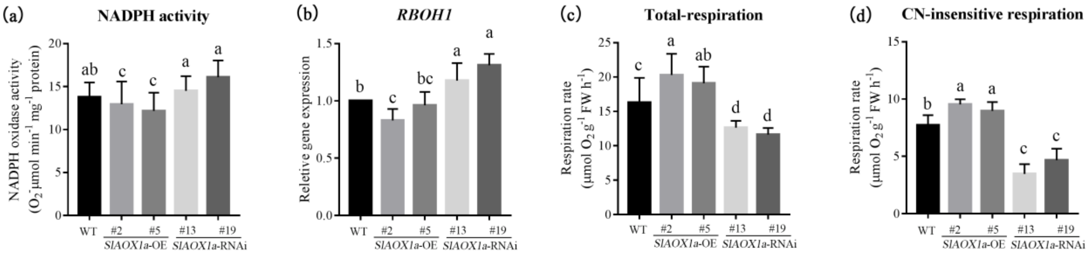

Figure S2 ROS accumulation and respiration rate in transgenic lines. NADPH activity (a), *RBOH1*expression (b), total-respiration rate (c) and CN-insensitive respiration rate (d) in transgenic tomatoes. Bars represent mean and standard deviation of values obtained from three biological repeats. Signifcant differences ($P<0.05$) are denoted by different lowercase letters.

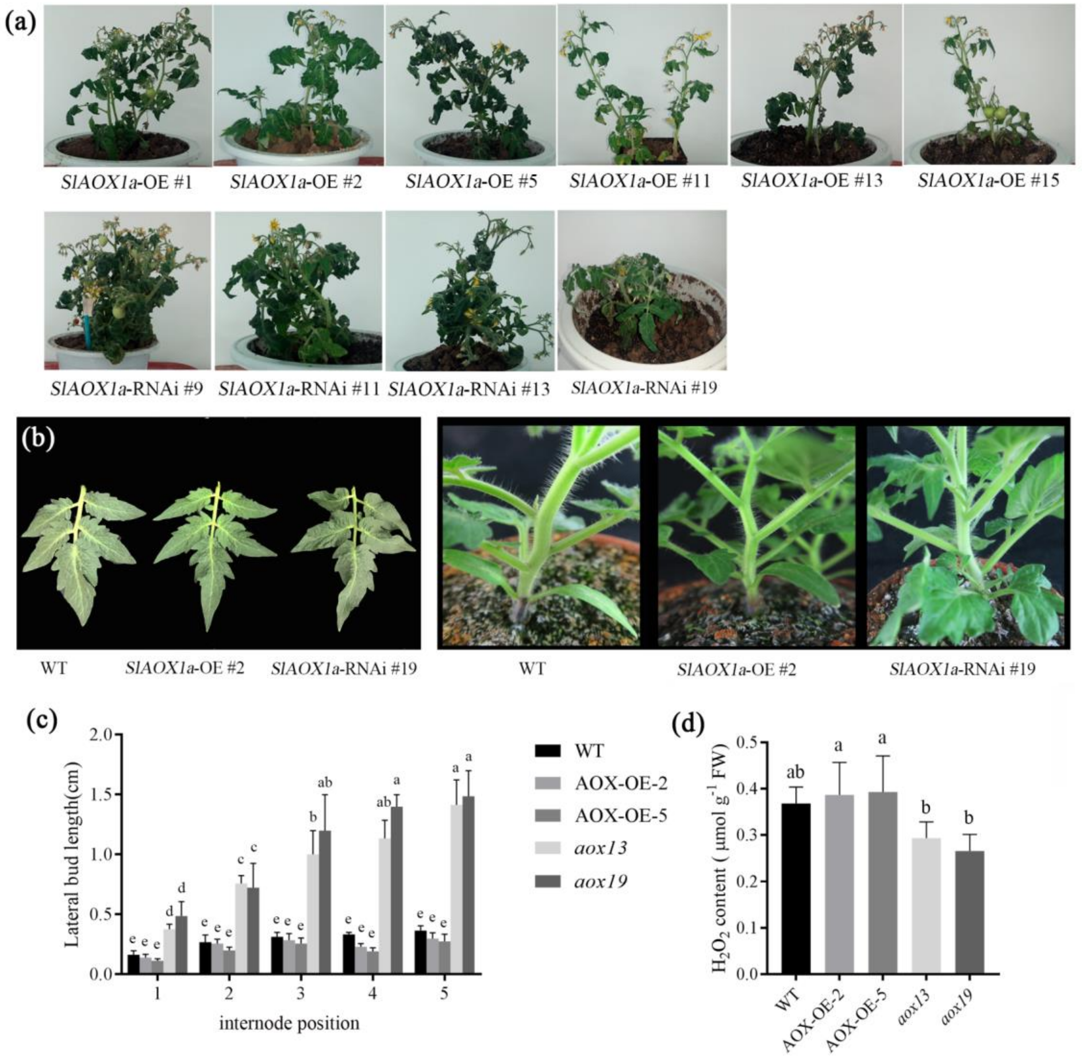

Figure S3 Developmental changes and lateral buds length in transgenic lines. (a) Phenotypes of T0 transgenic tomato lines. (b) Leaf color and later bud growth in transgenic tomato plants. Lateral bud length (c) and H_2O_2 content (d) were detected in transgenic tomato plants. All data are presented as the means of at least 3 biological replicates (± SE). Means with the same letter did not significantly differ at $P<0.05$ according to Duncan multiple range tests.

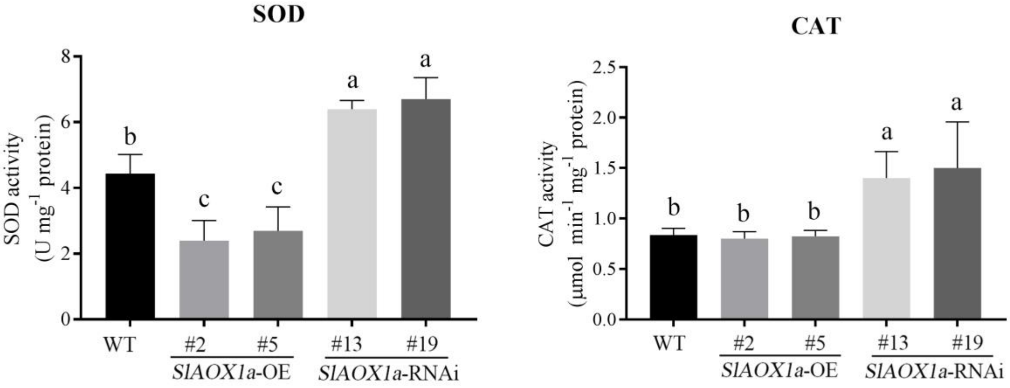

Figure S4 SOD and CAT activity in transgenic tomatoes. Bars represent mean and standard deviation of values obtained from three biological repeats. Signifcant differences ($P<0.05$) are denoted by different lowercase letters.

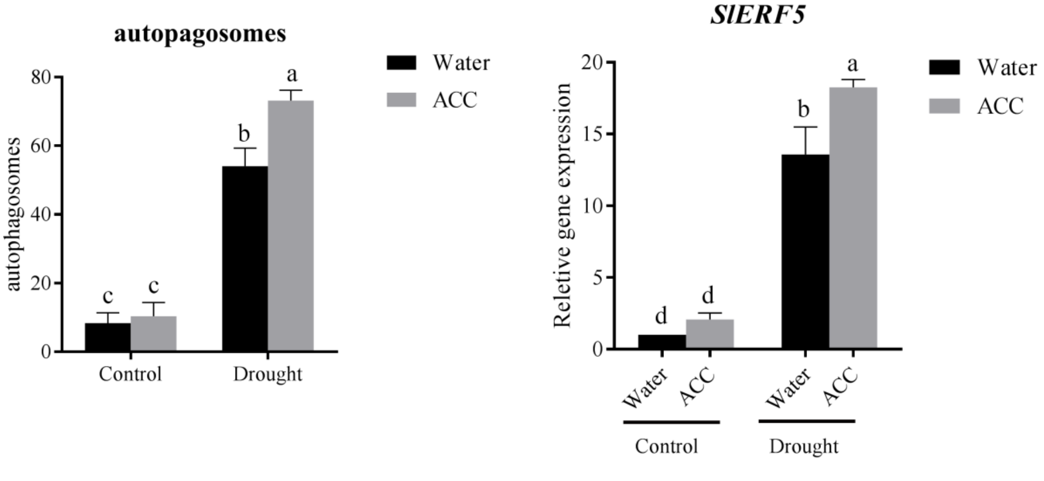

Figure S5 ACC-induced autophagic activity and *SlERF5* transcription under drought stress. Bars represent mean and standard deviation of values obtained from three biological repeats. Means with the same letter did not significantly differ at $P<0.05$ according to Duncan multiple range tests.

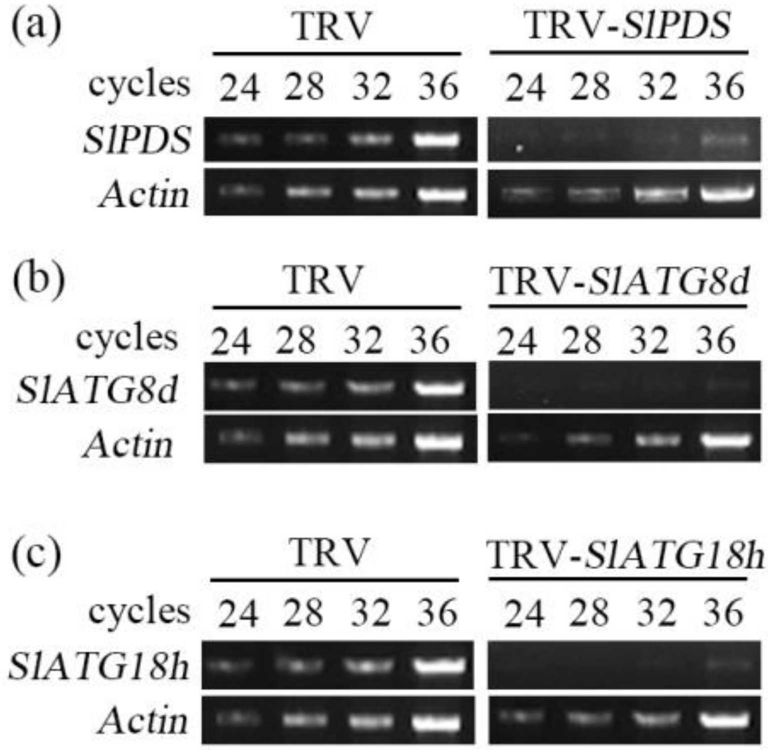

Figure S6 Silence efficiency of *ATG8d/ATG18h* in WT plants. (a) Silence efficiency of *SlPDS* in TRV and TRV-*SlPDS* plants. (b) Silence efficiency of *SlATG8d* in TRV and TRV-*SlATG8d* plants. (c) Silence efficiency of *SlATG18h* in TRV and TRV-*SlATG18h* plants. *Actin* was used as control. *Actin* band was the same in TRV plants. The PCR programme cycles was set as 24, 28, 32 and 36, respectively. Three independent experiments were performed with similar results.

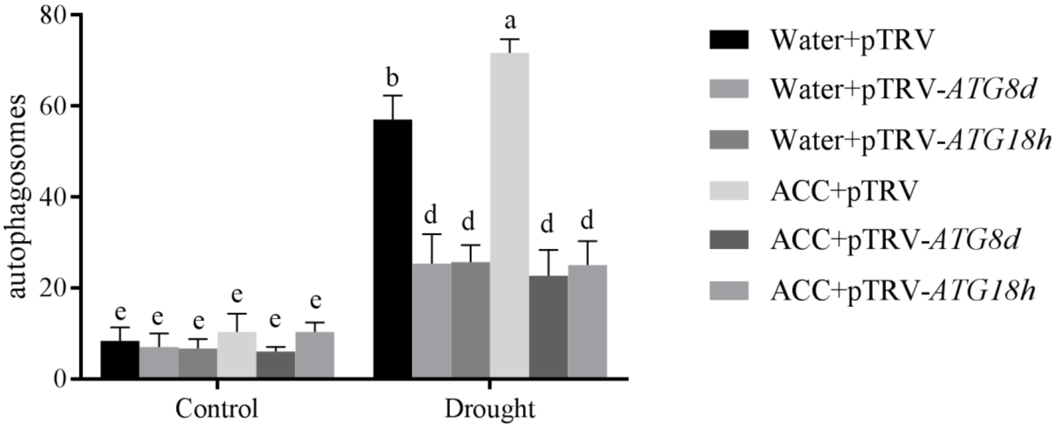

Figure S7 Autophagic activity in *ATG8d/ATG18h*-silenced plants. Bars represent mean and standard deviation of values obtained from three biological repeats. Means with the same letter did not significantly differ at $P<0.05$ according to Duncan multiple range tests.

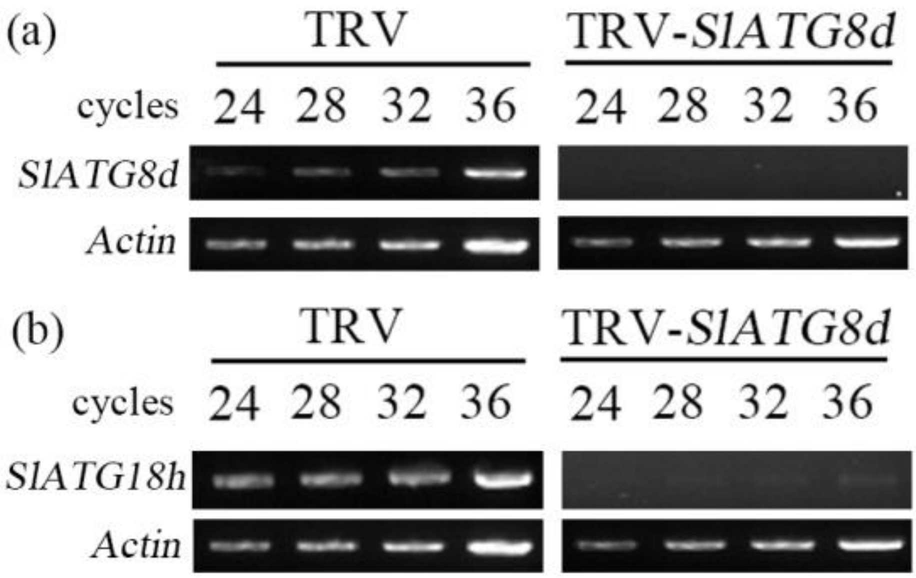

Figure S8 Silence efficiency of *ATG8d/ATG18h* in *aox19* plants. (a) Silence efficiency of *SlATG8d* in TRV and TRV-*SlATG8d* plants. (b) Silence efficiency of *SlATG18h* in TRV and TRV-*SlATG18h* plants. The PCR programme cycles was set as 24, 28, 32 and 36 respectively. *Actin* was used as control. *Actin* band was the same in TRV and TRV-*SlATG8d* plants, respectively. Three independent experiments were performed with similar results.

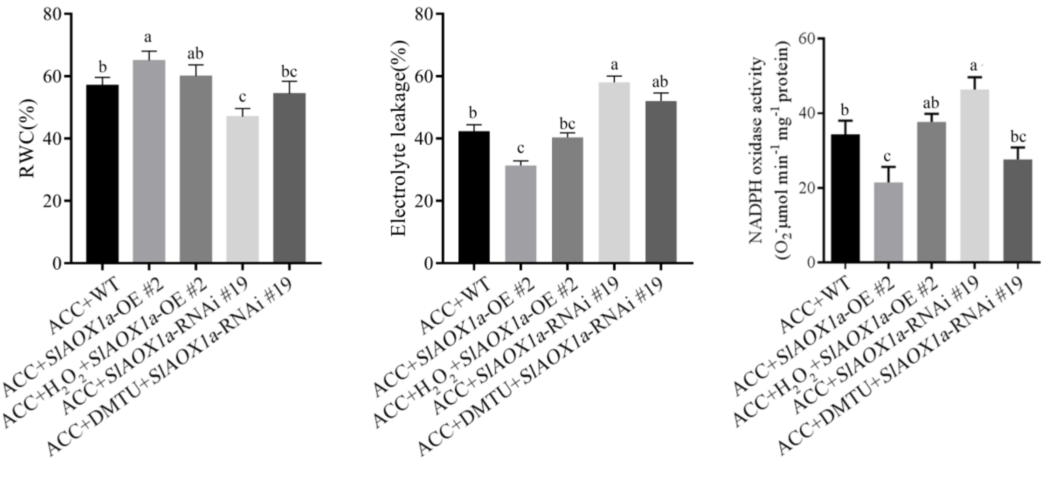

Figure S9 Modified ROS signaling lead to different drought responses in ACC pre-treated transgenic tomatoes. Bars represent mean and standard deviation of values obtained from three biological repeats. Signifcant differences ($P<0.05$) are denoted by different lowercase letters.

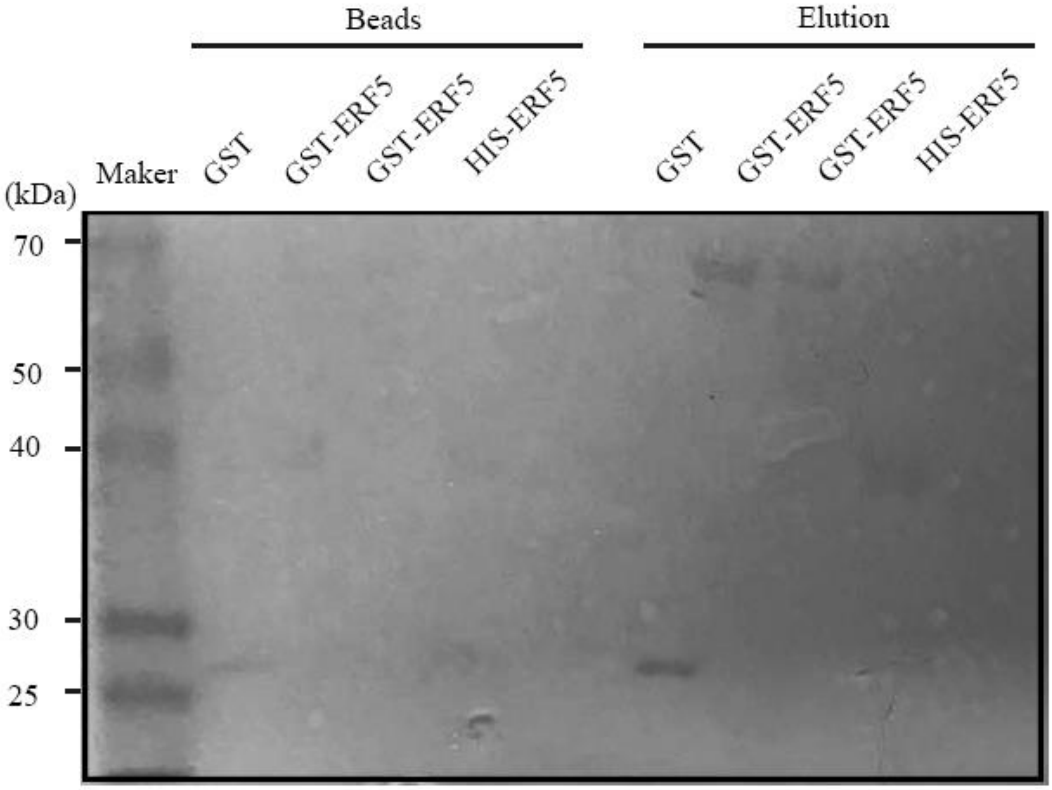

Figure S10 Purified ERF5 protein for EMSA assay.

Primers for Transgenic tomato

Gene	Accession number	Forward primer (5’-3’)	Reverse primer (5’-3’)
SlAOX1a	NM_001247188	ATGATGACCCGTGGAGCA	TGGTGAACCCATAGTGATT
RNAi	NM_001247188	AATGGTGGGAGGTATGTT	AGGCAGTCGCCAGTAATC

Primers for VIGS assay:

Gene	Accession number	Forward primer (5’-3’)	Reverse primer (5’-3’)
SlATG8d	Sl10g006270	AAGGCTGACAGAAGTGA	GTACGAAACAGACATAAGAT
SlATG18h	Sl07g064060	GGAGTGGAGGTAATGGTT	GTTCTTTGCGATTCTGTTT
SlPDS	NM_001247166	GCTCTAGACTGGTCACAAACCGATAC	CGGGATCCCACCTCATCTGTCACCCT

Primers for RT-PCR assay:

Gene	Accession number	Forward primer (5’-3’)	Reverse primer (5’-3’)
NPTII:		GAGAGGCTATTCGGCTATG	CTCAGAAGAACTCGTCAAGA
35S/NOS		CGTAAGGGATGACGCACAA	AAGACCGGCAACAGGATT

Primers for qRT-PCR

Gene	Accession number	Forward primer (5’-3’)	Reverse primer (5’-3’)
SlAOX1a	AY034148	GAATGGTGGGAGGTATGT	GTACGAAACAGACATAAGAT
SlAOX1b	AY034149	TCCTCCACTGTAAATCCC	GGTAGCCCTTCAACTCAT
SlAOX2	AY324396	CGCAAGTTCGAGCACAGT	TAGGCAGTCTCCAGTAGTCAAT
SlATG8d	Sl10g006270	AAGGCTGACAGAAGTGA	ACTAACGAACTTGGGTGA
SlATG8f	Sl08g078820	GGCAATCATGTCTGCAATCT	TCAAAGCTACAGTTCGCTCAG
SlATG13a	Sl03g096790	GATGTCGACACTTCCGATTC	TTGCAGATCCCTGAGAAGAG
SlATG18h	Sl07g064060	GGAGTGGAGGTAATGGTT	GTTCTTTGCGATTCTGTTT
SlATG18b	Sl07g006120	TTGAGGAGACAGCAACACCT	TGTTCTGATGGTTGACGTTG
SlERF5	NM_001330445	ATGGGTT CTCCACAAGA	AGAGTCGAACAATACTAT
SlRBOH1	NM_001247197	AATAACAAAA CTAACGGATG	GGAGACGTTTGTTTAATAGT
SlATG8d-Promoter		CCGCTCGAGAAATAATCTAGTCACCCTA	CCCAAGCTTAATTCAGTCTTTTCTGTCGATCTCG
SlATG18h-Promoter		CCGCTCGAGCTTGTGGCAACTAAATGAAATGAAA	CCCAAGCTTATCACCGTTCTTGATAACCCCTAGG

Table S1 Primers used in this assay.