Supplementary Material

Supplementary Methods

1.1 Measurements of fruit total soluble sugars

Total soluble sugars of tomato fruit were extracted as described previously (Ozaki *et al.*, 2009). Fruit samples (200 mg, each) were crushed in liquid nitrogen. Soluble sugars were extracted with 5 mL of boiling 80% (v/v) ethanol for 1 h, followed by centrifugation at 10,000 *g* at 4 ℃ for 10 min. The process was repeated for complete extraction. Total soluble sugar amount was determined using anthrone reagent and glucose as a standard.

1.2 Determination of fruit firmness and water loss

Fruit firmness of each individual tomato was measured at three points of the equatorial region by using the FT327 fruit pressure tester (Breuzzi Company, Milano, Italy). The probe descended toward the sample with a uniform force and stopped at 10 mm depth. The three measurements were averaged for each fruit and expressed in kg cm^{-2}.

Water loss analysis was performed using 10 fruits from the wild-type and each transgenic line harvested at the mature green stage (MG). The fresh weight of MG fruit was recorded as a starting point. Fruits were placed on the shelf at 25±1 ℃ for 30 days, and a fresh weight was recorded every 5 days. Water loss was calculated as a percentage in fresh weight difference between the starting weight and each individual measurement.

1.3 Determination of fruit color and lycopene content

For surface color assessment, fruits were marked along their equatorial axes and three readings were taken using a Minolta Chromo Meter, model CR 200 colorimeter (Minolta camera Co, Ltd., Osaka, Japan) in terms of lightness (*L*), a green (negative values) to red (positive values) scale (*a*), and saturation, a blue (negative values) to yellow (positive values) scale (*b*). The surface color was reported as hue angle (*H*°) as described previously (Choi *et al.*, 2008).

Lycopene content was analyzed as described previously (Markovic *et al.*, 2006). Fruit samples (5 g, each) were carefully weighed into a 200 mL flask wrapped with aluminum foil to keep out light.

28 The samples of fresh tomatoes were homogenized in a blender (JYL-C012; Joyoung industry Co, Ltd.,
29 Beijing, China) for 30 s. A 100 mL mixture of hexane-acetone-ethanol, 2:1:1 (v/v) was added to the
30 flask and agitated continuously for 10 min on a magnetic stirrer plate. After that, 15 mL of water was
31 added followed by another 5 min of agitation. The solution was separated into distinct polar and
32 nonpolar layers. The hexane solution containing lycopene was filtered into a 0.2 μm filter paper trough;
33 the filtrate was then diluted with a mixture of hexane-acetone-ethanol (2:1:1, v/v). The residue on the
34 filter paper was colorless, indicating rapid and complete extraction of lycopene. Lycopene
35 concentration was estimated by measuring the absorbance of the hexane solution containing lycopene
36 at 472 nm on a spectrophotometer.

37 *1.4 Isolation and purification of tomato mitochondria and Western blot analysis*

38 Mitochondria was isolated and purified as described previously (Bartoli *et al.*, 2006; Lei *et al.*,
39 2010). Tomato pulp was homogenized in 75 mM MOPS (pH 7.5) containing 600 mM sucrose, 4 mM
40 EDTA, 0.2% (w/v) polyvinyl-pyrrolidone (PVP)-40, 8 mM cysteine, and 0.2% (w/v) BSA.
41 Homogenate was centrifuged at 3,000 *g* for 10 min, and then the supernatant was centrifuged at
42 16,000 *g* for 10 min. The resulting pellet was resuspended in 10 mM MOPS (pH 7.2) containing 300
43 mM sucrose, and then layered onto Percoll gradients consisting of 20% Percoll (7.5 mL)/45% Percoll
44 (2.5 mL), centrifuged at 26,000 *g* for 15 min. Mitochondria, recovered at the interface between the 20
45 and 45% Percoll layers, were washed twice with MOPS-sucrose (pH 7.2). All steps were performed
46 under 4 ℃. Protein concentration was determined by the Bradford method using bovine serum albumin
47 as a standard (Bradford, 1976).

48 For SDS–PAGE and western blot analysis, 50 μg of protein from each sample was pretreated
49 with sample buffer (10% [v/v] *β*-mercaptoethanol, 20% [v/v] glycerol, 4% [w/v] SDS, 0.005% [w/v]
50 bromophenol blue and 50 mM Tris, pH 6.8) and boiled for 3 min, electrophoresed in 15%
51 polyacrylamide and then transferred to a nitrocellulose membrane. After transfer, the nitrocellulose
52 membrane was immune blotted as described previously (Lei *et al.*, 2010). The primary antibody was
53 AOX monoclonal antibody (Elthon *et al.*, 1989), provided by Dr. Jian-Ping Yu (Michigan State
54 University). And the secondary antibody was horse anti-mouse IgG alkaline phosphatase conjugate
55 (dilution 1:500). The digital data of band intensity was analyzed densitometrically by scanning the
56 blots with a thin-layer scanner.

57 **References**

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74 peroxide treatment. *Journal of Plant Physiology* **166**, 569-578.

Supplementary Figures

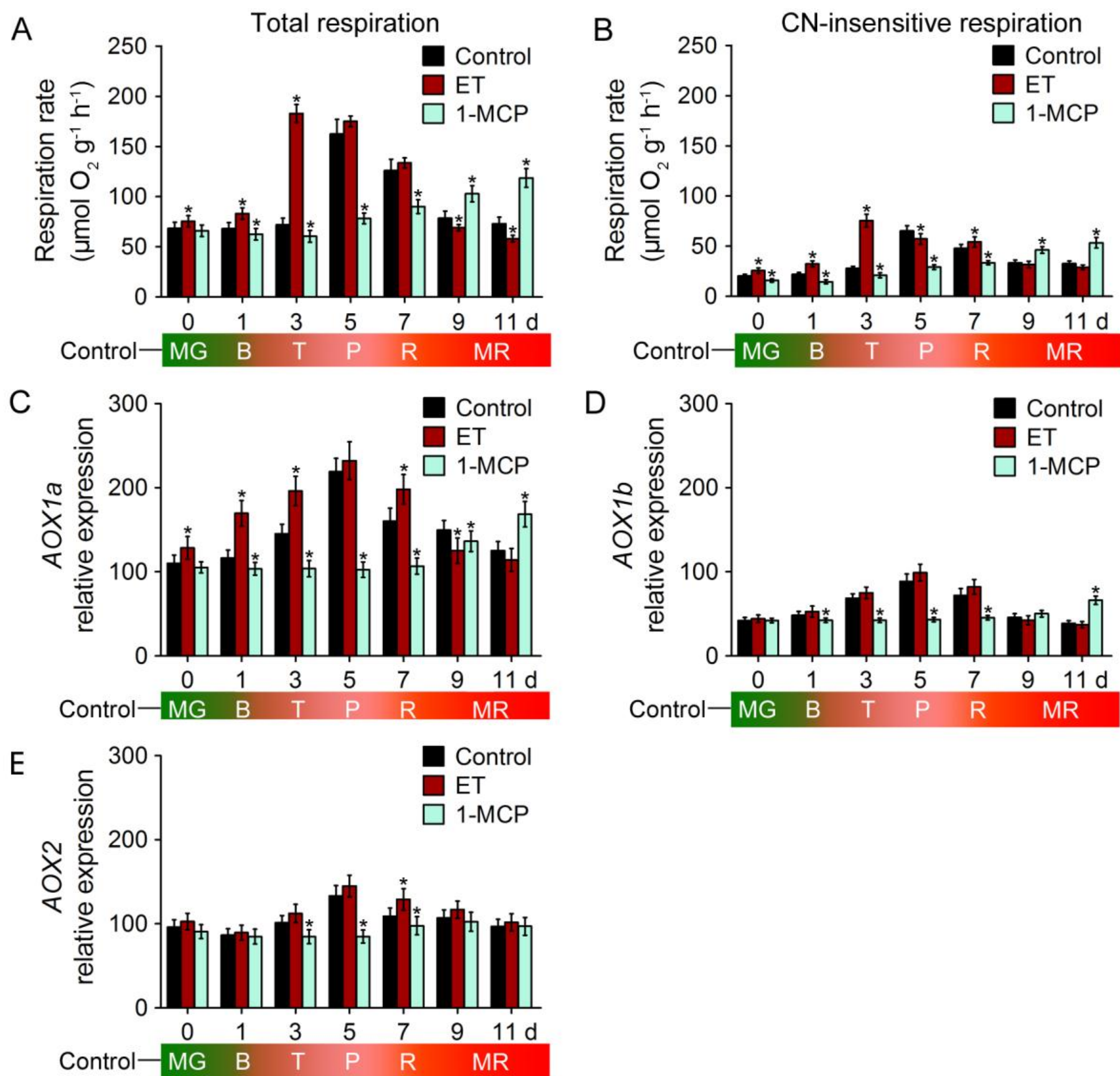

Fig. S1. Response of respiration (A and B) and *LeAOX* genes (C-E) to ET or 1-MCP treatment during postharvest ripening in tomato. For ET and 1-MCP treatments, mature green fruit was selected, washed with water and air-dried; the fruit was then exposed to 500 μL L^{-1} ET or 0.5 μL L^{-1} 1-MCP for 12 h. All of the fruit were stored at 25±1 ℃ until the end of the experiments. The bottom picture represents the postharvest ripening of the control (water treatment). MG, mature green; B, breaker; T, turning; P, pink; R, red; and MR, mature red. Data are the means ± SD of three independent experiments. The asterisks indicate statistically significant differences from the control ($P < 0.05$).

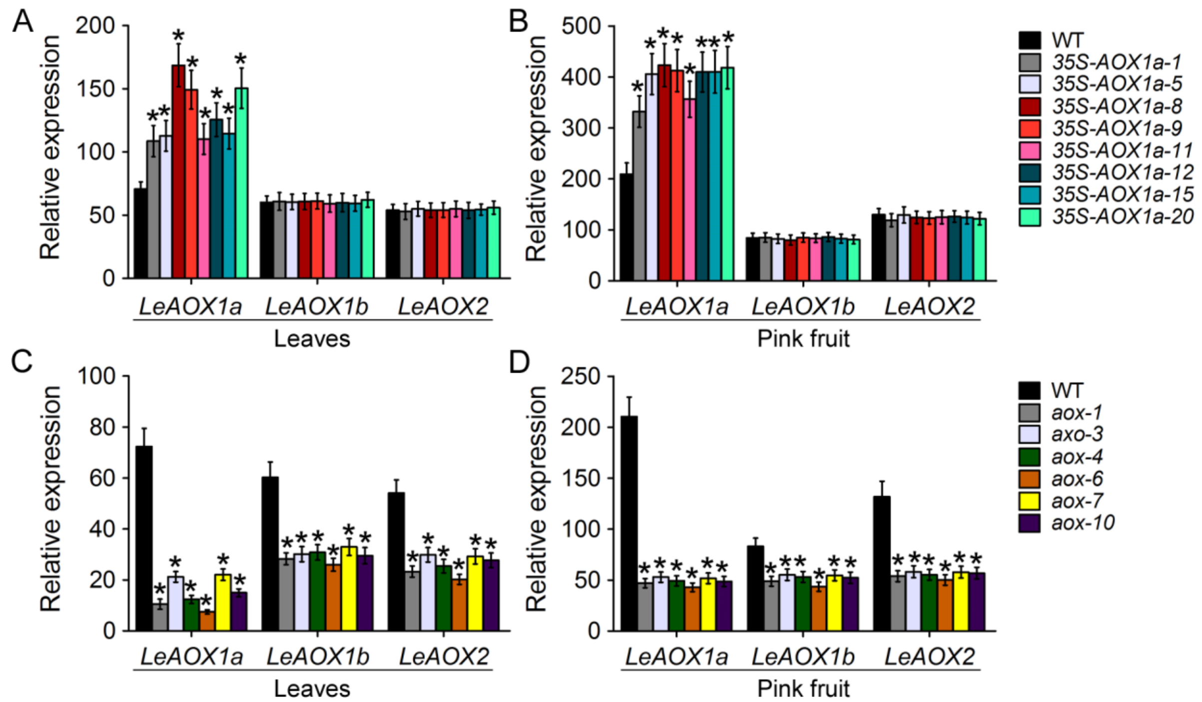

Fig. S2. Transcript levels of *LeAOX* in the transgenic plants. *LeAOX1a*, *LeAOX1b* and *LeAOX2* transcripts were quantified by qRT-PCR analysis using cDNA obtained from total RNA from leaves and pink fruit. Young leaves of 20-day-old plants grown in the artificial climate incubator were collected for extracting total RNA. Data are the means ± SD of three independent experiments. The asterisks indicate statistically significant differences between the wild-type and transgenic plants ($P < 0.05$).

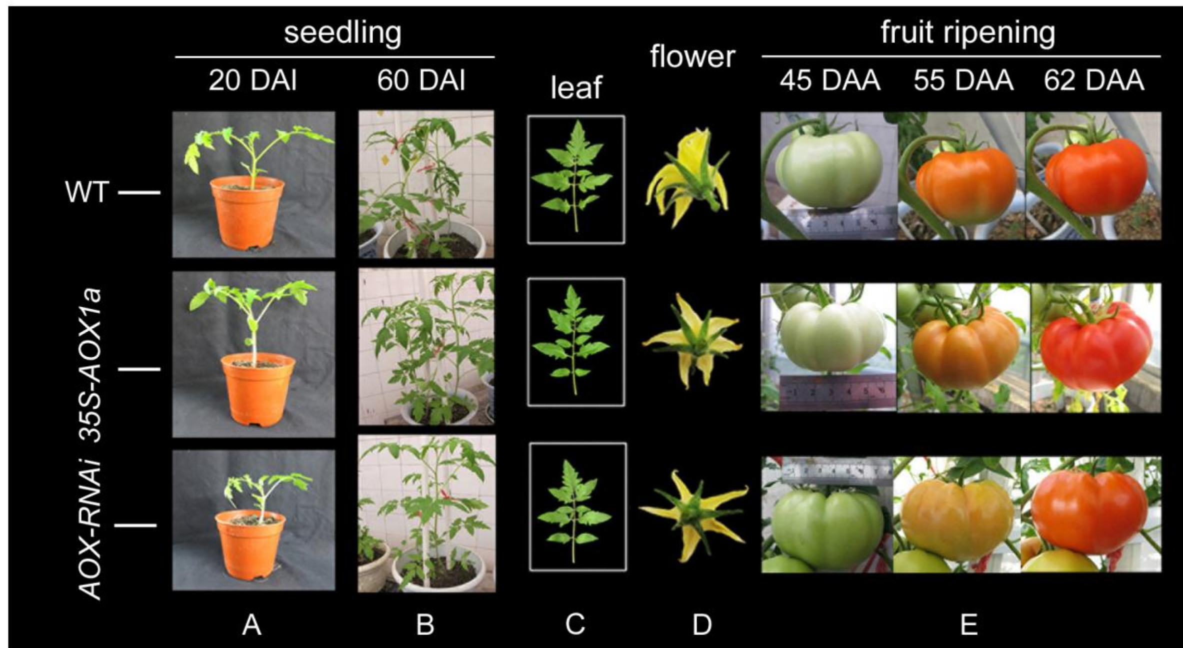

Fig. S3. Representative phenotypes of wild-type (WT) and transgenic plants in the T_1 generation. (A and B) Representative 20- and 60-day-old seedlings of the WT and transgenic plants grown under white-light conditions with a 16 h light/8 h dark photoperiod; DAI, days after incubation. (C) Fully expanded leaves; (D) Comparison of flowers of WT and transgenic plants and the variation in flower sepal and petal numbers; (E) Comparison of on-vine fruit ripening between WT and transgenic plants. DAA, days after anthesis.

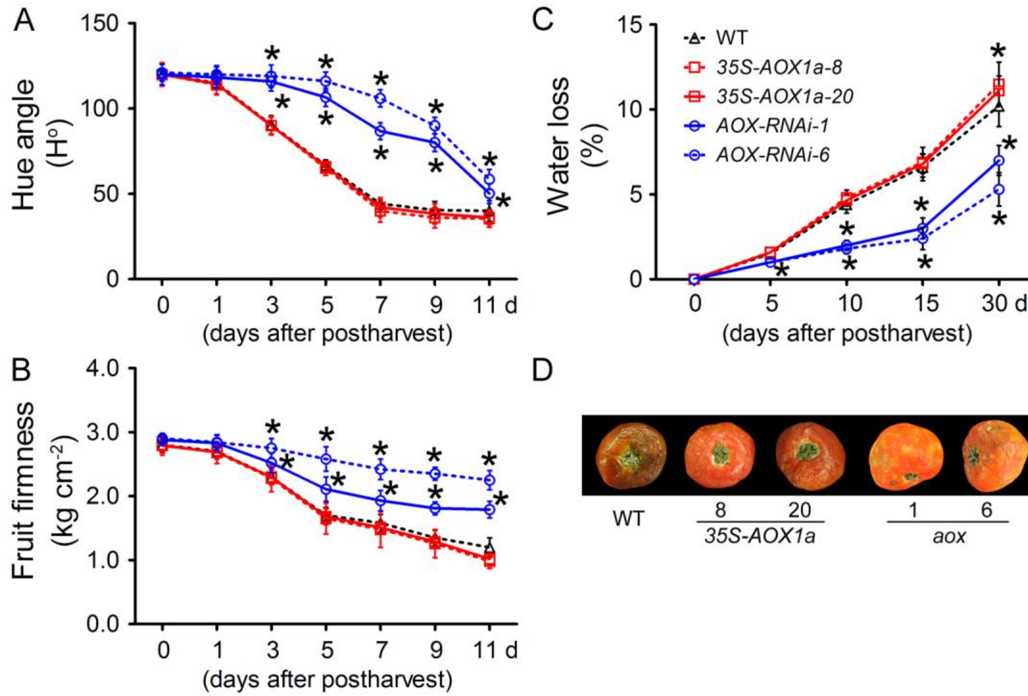

Fig. S4. Changes in the Hue angle (A), fruit firmness (B) and water loss (C) during fruit ripening in wild-type (WT) and transgenic tomatoes. (D) WT and transgenic fruit at 50 d after postharvest. Data are the means ± SD of three independent experiments. The asterisks indicate statistically significant differences between the WT and transgenic fruit ($P < 0.05$).

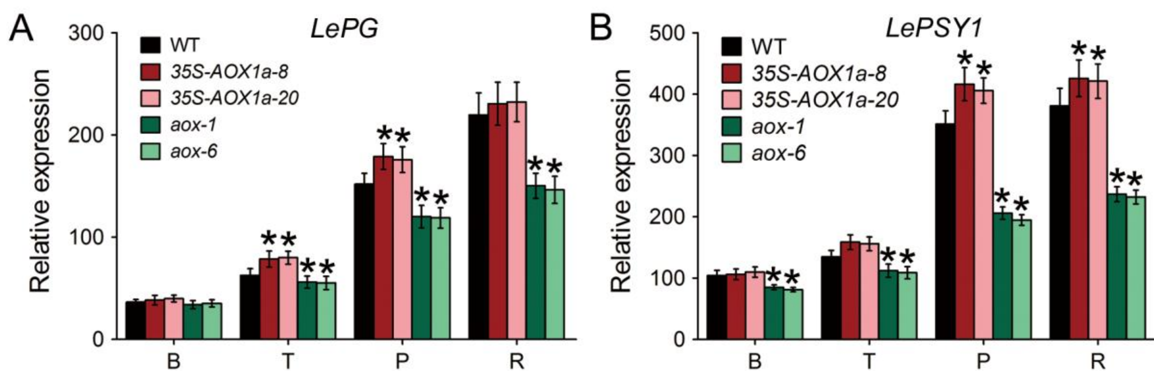

Fig. S5. Changes in the expression of *LePG* (A) and *LePSY1* (B) during ripening in wild-type (WT) and transgenic tomatoes. Data are the means ± SD of three independent experiments. The asterisks indicate statistically significant differences between the WT and transgenic fruit ($P < 0.05$). B: breaker stage; T: turning stage; P: pink stage; R: red stage.

Table S1. Primers used for Real-time PCR analysis

Gene	Accession number	F primer	R primer
LeAOX1a	*AY034148*	GAATGGTGGGAGGTATGT	AGGCAGTCGCCAGTAATC
LeAOX1b	*AY034149*	TCCTCCACTGTAAATCCC	GGTAGCCCTTCAACTCAT
LeAOX2	*AY324396*	CGCAAGTTCGAGCACAGT	TAGGCAGTCTCCAGTAGTCAAT
LePG	*X05656*	ACCAACGGCCTTAACCTTCTG	ATTTTTGCACGTAGCCTCTGATG
LePSY1	*EF534739*	AAAGTTGGTTTGCCTGTC	ATGTCATCGTCCGTTCTC
LeACS2	*X59145*	TATGGAGAGTTATTATAAACGATGTTA	CTAAGTACATAGACCAGTTGTCAATAC
LeACS4	*AF548375*	ATTCACTAGAGGACTTGAAGAAATAG	CAAGCTTTATAACTTTATTTGATTGTA
LeACO1	*X58273*	TATTTATTCAATACACTTAGGAAAACA	ACTTGAGAGATATTAGAAGTAGGAAGA
LeACO4	*AB013101*	ACTATCCTCCTTGTCCCA	TAGTCTCCACAGCCTTCA
LeETR4	*AY600438*	TGGTTGTAATGGCAGTCT	ATCAGCAGCCGATAAGGAA
NR	*AY600437*	CCGAATTCTCTTTGGGACGAAACGAGATA	CCGGTACCATTTGTATTGCTTCAGGGCTA
LeEIL3	*AF328786*	GCCTGGAAGGTTGGTGTT	GCTGTATGGGCAGTGAAG
LeERF1	*AY044236*	AAAGATGTCAAGCCCACT	GTTCCTAACCAAACCCTA
RIN	*AF448522*	GCCTATAAGTTACGGATACGA	TTGCCATACTCTTCTTGACA
NOR	*AY573802*	AGCCACTTGGTTGTGATAA	CATCGTCCTCGTTGTTCT
CNR	*DQ672601*	GCCTGTGTTATTGGTATTGG	ATGATTATCCGTGCCTTCC
ACTIN1	*U60482*	CACCATTGGGTCTGAGCGAT	GGGCGACAACCTTGATCTTC
H4	*X69179*	CAAGAGGCATAGGAAGGTT	ACAGAGTCACGAATCACAT