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Increased levels of IAA are required for system 2 ethylene synthesis causing fruit softening in peach (*Prunus persica* (L). Batsch)

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Table S1 Oligonucleotide primers used in qRT-PCR

Name	Oligonucleotide sequence
PpACS1 forward	5'- GCCTTCCGGGTTTTTCGA-3'
PpACS1 reverse	5'- GGCGGCCACAACCATGT-3'
PpACS2 forward	5'-GGAAAAGCACTTGAAGAAAATTC-3'
PpACS2 reverse	5'-CGCTCCTTTTGATCCCTTTG-3'
PpACO1 forward	5'- GAATGTCGATAGCCTCGTTCTACA-3'
PpACO1 reverse	5'- GGTGCTGGGCTGATGAATG-3'
PpPG2 forward	5'- AAGGTTTCGTGTGCCGTTGTA-3'
PpPG2 reverse	5'- CGGGATAATGTGAACGAGCAT-3'
<i>Prunus_v4_Contig5085</i> forward	5'- TGCGAAACCAGAATCTTCAAGA-3'
<i>Prunus_v4_Contig5085</i> reverse	5'- CCTCAGTTGACGCTGGATAAAA-3'
actin forward	5'- GATTCCGGTGCCCAGAAGT -3'
actin reverse	5'- CCAGCAGCTTCCATTCCAA -3'

Table S2. Pearson's correlation coefficient (PCC) and signal intensity of 29 filtered genes with similar expression patterns for *PpACS1*

Probe ID	Annotation	PCC	Akatsuki				Manami		Odoroki	
			days after full bloom				Air	Propylene	Air	Propylene
			92	98	104	106				
1 Prunus_AJ533769	Homeobox-leucine zipper protein 17 (HB-17)	0.88	1.4E+02	8.6E+02	2.0E+03	1.5E+03	1.9E+01	1.1E+01	5.8E+01	5.8E+01
2 Prunus_DW348600	ATNUDT2 (Arabidopsis thaliana Nudix hydrolase homolog 2)	0.97	1.3E+02	4.9E+02	1.2E+03	1.5E+03	3.1E+02	5.5E+02	8.1E+02	1.6E+03
3 Prunus_DY652512		0.94	5.8E+03	1.4E+04	4.0E+04	5.1E+04	1.2E+04	2.3E+04	4.8E+04	5.0E+04
4 Prunus_DY654073		0.89	3.3E+03	7.5E+03	1.0E+04	9.6E+03	4.8E+02	7.3E+02	2.9E+03	4.3E+03
5 Prunus_EE488466		0.89	1.1E+04	3.3E+04	5.1E+04	4.1E+04	4.1E+03	7.9E+03	2.5E+04	2.9E+04
6 Prunus_v4_Contig1550	Calcium ion binding protein	0.95	1.2E+03	1.8E+03	4.4E+03	9.9E+03	1.5E+03	2.3E+03	1.3E+04	2.6E+04
7 Prunus_v4_Contig1633		0.94	1.1E+03	2.0E+03	2.3E+03	5.9E+03	6.8E+02	9.1E+02	8.7E+03	5.3E+03
8 Prunus_v4_Contig1891		0.85	1.2E+03	1.6E+03	2.4E+03	2.4E+03	2.1E+02	1.6E+02	3.3E+02	5.0E+02
9 Prunus_v4_Contig1945	REDUCTASE OXIDOREDUCTASE IRON-SULFUR	0.94	1.2E+03	2.1E+03	3.6E+03	8.8E+03	5.6E+02	3.1E+02	1.9E+03	2.8E+03
10 Prunus_v4_Contig2173	IAA9 (indoleacetic acid-induced protein 9)	0.87	7.4E+03	1.1E+04	1.5E+04	1.3E+04	1.3E+03	2.4E+03	7.1E+03	4.1E+03
11 Prunus_v4_Contig2986		0.98	1.9E+03	2.3E+03	3.3E+03	4.5E+03	2.8E+03	2.6E+03	7.2E+03	5.1E+03
12 Prunus_v4_Contig3538	Gibberellin 2-beta-dioxygenase	0.88	1.4E+03	9.2E+03	2.2E+04	4.0E+04	1.4E+04	1.0E+04	2.8E+04	1.5E+04
13 Prunus_v4_Contig3715		0.98	3.7E+02	1.0E+03	1.7E+03	2.9E+03	9.1E+02	1.2E+03	3.0E+03	2.2E+03
14 Prunus_v4_Contig5320		0.92	5.9E+03	7.2E+03	8.1E+03	5.1E+03	6.6E+03	1.1E+04	1.7E+04	1.2E+04
15 Prunus_v4_Contig5832	AUXIN-INDUCED PROTEIN-LIKE MULTIGENE	0.99	7.5E+02	2.0E+03	5.4E+03	9.6E+03	1.6E+04	2.9E+04	4.7E+04	2.8E+04
16 Prunus_v4_Contig5890		0.99	3.4E+02	1.1E+03	2.4E+03	5.2E+03	4.1E+03	6.1E+03	7.0E+03	1.4E+04
17 Prunus_v4_Contig6623	ATSIP2 (ARABIDOPSIS THALIANA SEED IMBIBITION 2)	0.92	5.0E+03	1.1E+04	1.3E+04	1.4E+04	1.7E+03	2.5E+03	1.1E+04	7.3E+03
18 Prunus_v4_Contig7034		0.85	1.7E+03	4.0E+03	5.9E+03	6.2E+03	3.2E+03	2.5E+03	7.3E+03	3.6E+03
19 Prunus_v4_Contig8994	IAA16 (indoleacetic acid-induced protein 16)	0.88	1.8E+02	9.5E+02	1.3E+03	5.5E+03	2.9E+01	2.1E+01	1.7E+02	8.6E+01
20 R_Prunus_CB821238		0.92	1.5E+03	1.6E+03	1.7E+03	2.1E+03	1.4E+03	2.5E+03	6.2E+03	4.6E+03
21 R_Prunus_DW343225		0.95	2.0E+03	2.5E+03	2.5E+03	2.9E+03	6.1E+02	6.8E+02	1.8E+03	1.3E+03
22 R_Prunus_DY636010		0.92	2.1E+03	6.6E+03	1.1E+04	1.0E+04	4.8E+03	7.3E+03	1.7E+04	9.0E+03
23 R_Prunus_v4_Contig2011	GASA5 (GAST1 PROTEIN HOMOLOG 5)	0.94	5.6E+04	1.1E+05	1.8E+05	1.7E+05	1.5E+05	1.4E+05	3.2E+05	2.6E+05
24 R_Prunus_v4_Contig4064		0.93	1.2E+03	2.1E+03	3.0E+03	2.8E+03	4.1E+02	7.5E+02	3.1E+03	1.7E+03
25 ULR_Prunus_v4_Contig1569		0.92	6.1E+02	1.3E+03	3.2E+03	2.3E+03	7.3E+00	8.1E+00	2.3E+01	2.4E+01
26 URF_Prunus_v4_Contig3599		0.87	3.1E+03	3.6E+03	4.3E+03	4.7E+03	3.1E+03	4.6E+03	9.3E+03	7.4E+03
27 URF_Prunus_v4_Contig6298		0.93	9.0E+03	1.5E+04	1.5E+04	2.0E+04	1.2E+04	2.2E+04	5.2E+04	2.7E+04
28 URF_Prunus_v4_Contig772		0.93	7.8E+02	1.1E+03	2.1E+03	1.7E+03	1.1E+02	1.9E+02	1.7E+03	9.3E+02
29 URF_Prunus_v4_Contig9013		0.97	3.3E+04	4.7E+04	6.1E+04	6.2E+04	1.7E+04	2.8E+04	9.0E+04	5.3E+04

Table S3. Pearson's correlation coefficient (PCC) and signal intensity of IAA related genes with similar expression patterns for *PpACS1*

Probe ID	Annotation	PCC	Akatsuki				Manami		Odoroki	
			days after full bloom				Air	Propylene	Air	Propylene
			92	98	104	106				
1 URR_Prunus_v4_Contig5085	indole-3-acetic acid inducible 29	0.95	3.2E+00	2.5E+02	4.3E+03	3.8E+03	1.8E+01	2.7E+00	2.9E+00	4.1E+00
2 URR_Prunus_v4_Contig1538	ATAUX2-11 (indoleacetic acid-induced protein 4)	0.80	3.2E+03	1.1E+04	3.2E+04	2.4E+04	2.0E+01	3.2E+02	4.0E+02	1.6E+02
3 R_Prunus_v4_Contig8766	IAA13 (indoleacetic acid-induced protein 13)	0.79	3.7E+03	7.5E+03	1.5E+04	7.7E+03	1.4E+02	8.1E+02	1.4E+03	1.3E+03
4 R_Prunus_BU046438	SAUR-like auxin-responsive protein family	0.33	1.3E+04	1.8E+04	3.2E+04	1.4E+04	1.6E+01	4.6E+01	2.3E+02	2.3E+01
5 R_Prunus_AJ875835	IAA12 (AUXIN-INDUCED PROTEIN 12)	0.76	2.1E+02	3.0E+02	9.8E+02	7.0E+02	5.5E+01	2.0E+00	1.8E+01	1.9E+01
6 R_Prunus_AJ823619	IAA6 (indoleacetic acid-induced protein 6)	0.69	1.9E+04	5.0E+04	7.1E+04	6.6E+04	8.6E+02	1.2E+03	1.0E+04	1.2E+04
7 Prunus_v4_Contig9018	indole-3-acetic acid inducible 19	0.82	1.1E+04	3.1E+04	4.8E+04	4.7E+04	1.5E+01	7.2E+02	5.4E+03	6.6E+03
8 Prunus_v4_Contig9000	SAUR-like auxin-responsive protein family	-0.36	4.0E+03	8.2E+03	4.7E+03	1.1E+03	4.9E+01	1.8E+01	1.5E+01	2.9E+00
9 Prunus_v4_Contig565	SAUR-like auxin-responsive protein family	0.48	3.4E+03	6.3E+03	9.7E+03	5.0E+03	3.6E+01	1.6E+01	9.4E+01	4.6E+01
10 Prunus_v4_Contig5592	ATAUX2-11 (indoleacetic acid-induced protein 4)	0.78	5.9E+03	1.8E+04	4.9E+04	3.6E+04	1.8E+02	6.1E+02	9.9E+02	3.8E+02
11 Prunus_v4_Contig1545	IAA16 (indoleacetic acid-induced protein 16)	0.87	5.2E+04	1.8E+05	2.5E+05	2.1E+05	3.2E+03	7.0E+03	6.1E+04	6.4E+03
12 Prunus_BU044903	IAA16 (indoleacetic acid-induced protein 16)	0.92	7.3E+03	4.0E+04	7.5E+04	6.5E+04	1.1E+03	9.3E+02	9.3E+03	1.0E+03
13 Prunus_BU039946	SAUR-like auxin-responsive protein family	0.88	1.2E+02	1.9E+03	5.6E+03	2.9E+03	3.0E+01	3.7E+00	7.8E+00	2.2E+01

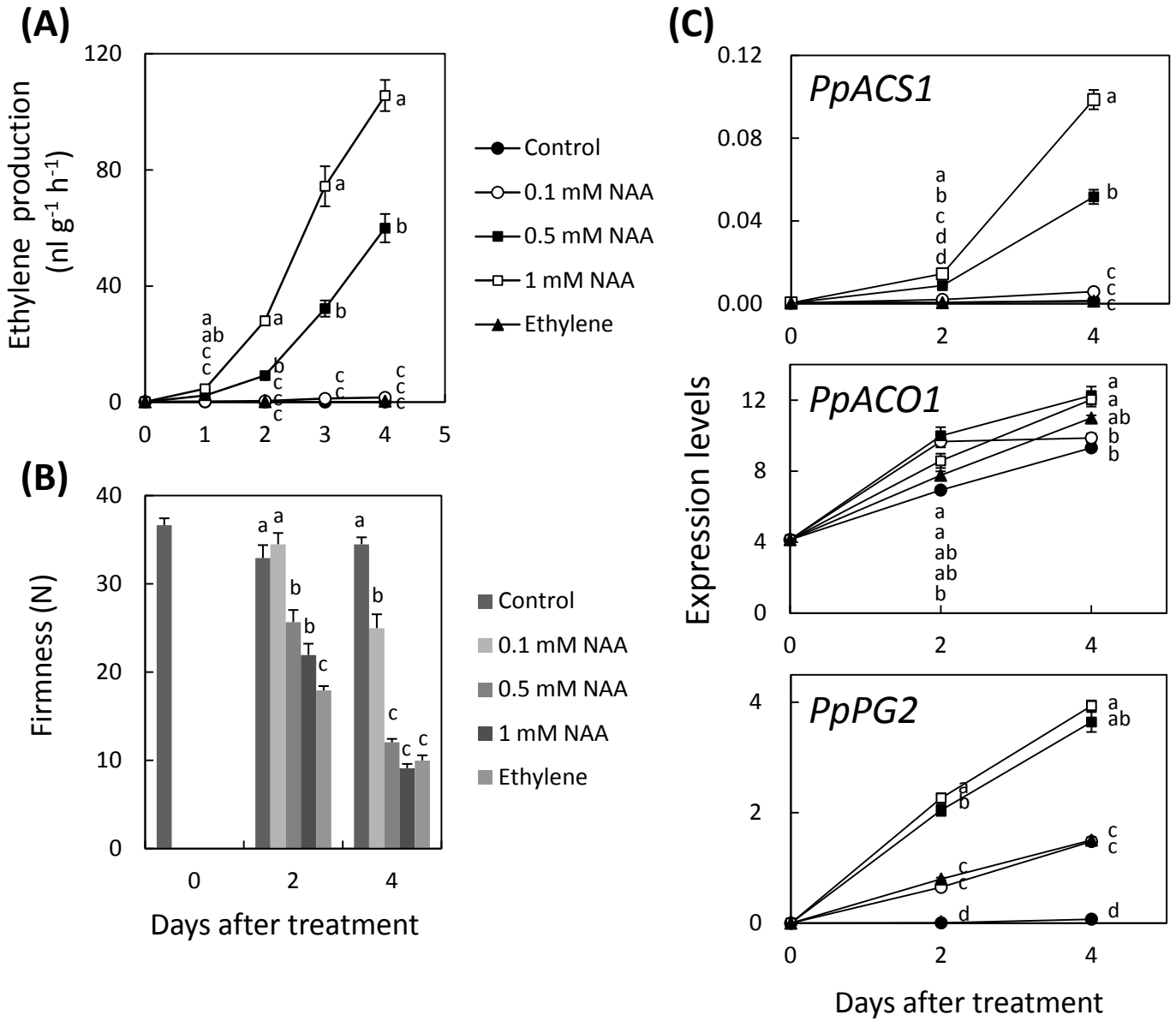
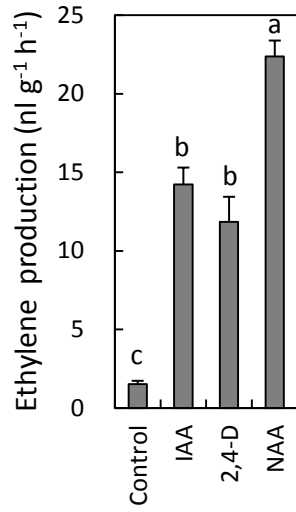


Figure S1

(A)



(B)

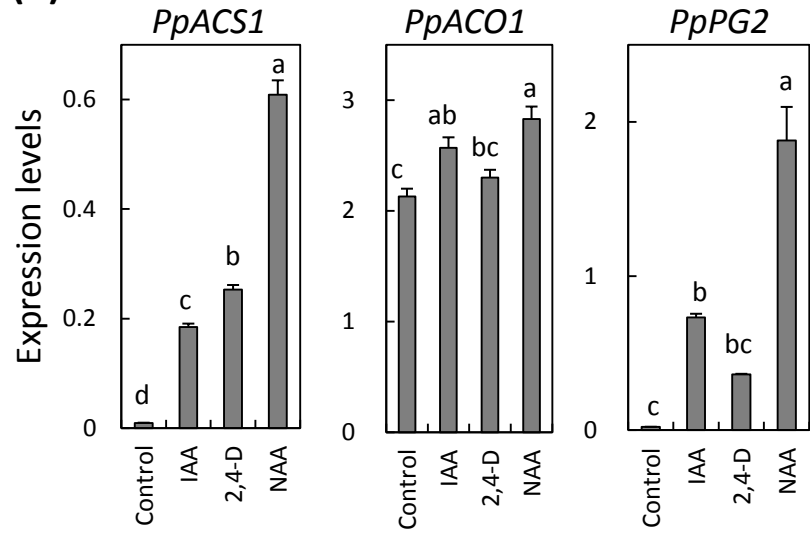


Figure S2

Figure Legends (Supplementary data)

Fig. S1 Effects of NAA on 'Odoroki' fruit ripening and gene expression. (A) Ethylene production, (B) flesh firmness of NAA-treated 'Akatsuki' fruit. Harvested mature fruit of 'Manami' were treated with 0.1 mM, 0.5 mM and 1 mM NAA each day or continuous $20 \mu\text{l L}^{-1}$ ethylene. Vertical bars represent the SE ($n = 10$). (C) Relative transcript abundance of *PpACS1*, *PpACO1*, and *PpPG2*. The steady-state levels were normalized to *actin*. Data are mean values \pm SD of three individual experiments, each performed in triplicate. Different letters indicated significant differences within the same day at $p < 0.05$ using Tukey's *post hoc* test.

Fig. S2 Effect of some auxins on ethylene production and expression patterns of ripening-related genes of 'Manami'. (A) Ethylene production, and (B) relative transcript abundance of *PpACS1*, *PpACO1*, and *PpPG2* in mesocarp disks of 'Manami' treated with 0.5 mM IAA, 0.5 mM 2,4-D, or 0.5 mM NAA. Tissue cylinders (9 mm in diameter) were excised from 'Manami' mesocarp with a cork borer, 5 mm thick discs were cut with a razor blade from the cylinders, and 42 discs were used for each treatment. The prepared discs were placed on paper filters soaked with auxin or anti-auxin solution and stored at 28 °C for 18 h. Vertical bars of ethylene production represent the SE ($n = 10$). (B) The steady-state levels were normalized to *actin*. Data are mean values \pm SD of three individual experiments, each performed in triplicate. Different letters indicated significant differences at $p < 0.05$ using Tukey's *post hoc* test.