

1 **Supporting information**

2 **Supplementary File 1**

3 **Table S1.** Summary of RNA-seq data for each sample

4 **Table S2.** Primers used in this study

5 **Table S3.** Differentially expressed genes related to cellular and textural changes in
6 apple fruit during long-term cold storage

7 **Table S4.** Differentially expressed genes related to sugar and malic acid pathways in
8 apple fruit during long-term cold storage

9 **Table S5.** Differentially expressed genes related to FA and lipid pathways in apple fruit
10 during long-term cold storage

11 **Table S6.** Differentially expressed genes related to hormone pathways in apple fruit
12 during long-term cold storage

13 **Table S7.** Differentially expressed genes related to stress responses in apple fruit
14 during long-term cold storage

15 **Fig. S1.** Correlation of the sequencing data for each sample among biological replicates

16 **Fig. S2.** Volcano plots for differentially expressed genes in the following comparison
17 groups: CS5, 4, 3, and 2 vs CS1; CS5, 4, and 3 vs CS2; CS5 and 4 vs CS3; and CS5 vs CS4

18 **Fig. S3.** Analysis of differentially expressed genes by qRT-PCR and the linear
19 relationship between qRT-PCR and RNA-seq data for related genes

20 **Fig. S4.** Results of the K-means clustering of the expression patterns of differentially
21 expressed genes in apple fruit during long-term cold storage. Apple fruits were stored
22 at 4 °C for 1, 30, 60, 90, and 150 days, and the time-points were designated as CS1,

23 CS2, CS3, CS4, and CS5, respectively. On the basis of RNA-seq data, 6,672 individual
24 transcripts with significant differences (fold-change > 2, $p < 0.05$) in comparisons
25 between each pair of time-points were identified

26 **Fig. S5.** Gene ontology functional analysis of differentially expressed genes (DEGs)
27 related to biological process (A), cellular component (B), and molecular function in the
28 following comparison groups: CS5, 4, 3, and 2 vs CS1; CS5, 4, and 3 vs CS2; CS5 and 4
29 vs CS3; and CS5 vs CS4

30 **Fig. S6.** KEGG pathway enrichment analysis of differentially expressed genes (DEGs) in
31 the following comparison groups: CS5, 4, 3, and 2 vs CS1; CS5, 4, and 3 vs CS2; CS5 and
32 4 vs CS3; and CS5 vs CS4

33 **Fig. S7.** Cluster analysis of differentially abundant metabolites associated with amino
34 acids in apple fruit during long-term cold storage

35 **Fig. S8.** Cluster analysis of differentially abundant metabolites associated with organic
36 acids in apple fruit during long-term cold storage

37 **Fig. S9.** Quality analysis of models based on R^2X or R^2Y and Q^2 values. The comparison
38 groups were as follows: CS5, 4, 3, and 2 vs CS1; CS5, 4, and 3 vs CS2; CS5 and 4 vs CS3;
39 and CS5 vs CS4

40 **Supplementary File 2**

41 **Additional file 1.** Detailed information regarding the annotation of up- and down-
42 regulated DEGs in the following comparison groups: CS5, 4, 3, and 2 vs CS1; CS5, 4, and
43 3 vs CS2; CS5 and 4 vs CS3; and CS5 vs CS4

44 **Additional file 2.** Detailed information regarding the up- and down-regulated

45 metabolites in the following comparison groups: CS5, 4, 3, and 2 vs CS1; CS5, 4, and 3
46 vs CS2; CS5 and 4 vs CS3; and CS5 vs CS4
47 **Additional file 3.** Detailed information regarding the differentially expressed
48 transcription factor genes in apple fruit during long-term cold storage

Table S1. Summary of RNA-seq data for each sample.

Statistical content	CS0 (repeat1)		CS0 (repeat2)		CS0 (repeat3)		CS1 (repeat1)		CS1 (repeat2)		CS1 (repeat3)		CS2 (repeat1)		CS2 (repeat2)		CS2 (repeat3)	
	No.	%																
Total	56078054	100%	48912980	100%	60236584	100%	55687726	100%	47347912	100%	47182372	100%	56572488	100%	46936866	100%	47709640	100%
Mapped	52601036	93.80%	45965792	93.97%	56333742	93.52%	51612544	92.68%	43609768	92.10%	43776706	92.78%	52377258	92.58%	43512718	92.70%	44392826	93.05%
Unique	46492214	88.39%	40979321	89.15%	49705682	88.23%	45081174	87.35%	38261071	87.74%	38243530	87.36%	45402717	86.68%	37984689	87.30%	38708491	87.20%
Multiple	6108822	11.61%	4986471	10.85%	6628060	11.77%	6531370	12.65%	5348697	12.26%	5533176	12.64%	6974541	13.32%	5528029	12.70%	5684335	12.80%
Pair Map	45920592	87.30%	40722254	88.59%	49228738	87.39%	44594200	86.40%	38041574	87.23%	37877782	86.52%	44488194	84.94%	37283622	85.68%	38282752	86.24%
Single	6680444	12.70%	5243538	11.41%	7105004	12.61%	7018344	13.60%	5568194	12.77%	5898924	13.48%	7889064	15.06%	6229096	14.32%	6110074	13.76%

Continue

Statistical content	CS3 (repeat1)		CS3 (repeat2)		CS3 (repeat3)		CS4 (repeat1)		CS4 (repeat2)		CS4 (repeat3)	
	No.	%										
Total	54334176	100%	58608962	100%	49649892	100%	50855728	100%	55353034	100%	56602390	100%
Mapped	50702310	93.32%	54983856	93.81%	46544464	93.75%	47098990	92.61%	51337152	92.74%	52639754	93.00%
Unique	44614615	87.99%	48922522	88.98%	41231633	88.59%	41746440	88.64%	45581029	88.79%	46735348	88.78%
Multiple	6087695	12.01%	6061334	11.02%	5312831	11.41%	5352550	11.36%	5756123	11.21%	5904406	11.22%
Pair Map	43672690	86.14%	48491698	88.19%	40904066	87.88%	41112760	87.29%	45187830	88.02%	46397526	88.14%
Single	7029620	13.86%	6492158	11.81%	5640398	12.12%	5986230	12.71%	6149322	11.98%	6242228	11.86%

Table S2. List of primers used in this study.

Genes	Gene ID	Forward (5' to 3')	Reverse (5' to 3')
FAD2	MD12G1057600	ACAGAGAGGGAGGGAGAAGGTAGAA	GAGGCTAACGGAGGCTTAGAGTA
KCS1	MD01G1087400	CCGTCGTAGTCAGCACCGAAA	CCCGCTGGTAAACGCACTTGTA
SS3	MD05G1347400	TGCTGCTGGTGTGATTATGCTCTT	CCTTCCGTGCTGCGTGGTAAA
ALMT9	MD06G1214800	GGGTAGGTTATGAAGGTGCCAAAGT	CGACTTCCAATCTCCCAGCCTTC
AATP1	MD17G1045300	AGGAAACAAACGGCGAACATCAGACTT	TGCCTGTTGCTCACCAATAACTCTT
EXP1	MD14G1202100	TGTGCGTCTTACTCTGCTCTCTT	CCAAACCAAACCAGACGGACAACTA
WRKY7	MD15G1078200	ATCACAATCACTCCCTCTGCGTTAC	GGCGTTCCCACTAATCCTCTAGG
COR47	MD15G1253900	ACACAGATGAGTATGGAAACCCGAT	TCTTGTTGCTGCTGCCCTCCT
ATG8C	MD05G1347700	GACCTTCAGCGGAGAGAACATGTGTT	GGCCAAAGAGCAGATCTACTTCATG
ACS8	MD15G1302200	TGAGGCACTTACTTAGGTCCAACAC	GGCAAAGCAGACACGGAACCA
SAM1	MD13G1141700	TACGGACACTTCGGGAGGGATG	GTGAAGACTGAAGAGCCGCCATT
HSP9.4	MD03G1036600	CCCGCCCAACCAGCAGTAGTAT	TCCGAATACCAAACAGTAGCAACCA
HistoneH3	MD01G1035300	ATGGCCCGTACCAAGCAAAC	GTACTTACGGATTCACGAAGAGCAAC

Table S3. Differentially expressed genes related to cellular and textural changes in apple fruit during long-term cold storage

Gene ID	name	cluster	Annotation	Cold storage time				
				1/1	2/1	3/1	4/1	5/1
MD11G1084600	TUB7	1	Tubulin beta-7 chain	1	0.42	0.62	0.56	0.74
MD04G1143700	TUB7	1	Tubulin beta-7 chain	1	0.51	0.58	0.44	0.54
MD14G1103900	TUB6	1	Tubulin beta-6 chain	1	0.45	0.16	0.12	0.77
MD12G1158100	TUB3	1	Tubulin beta-3 chain	1	0.11	0.16	0.16	0.18
MD15G1011800	TUB1	1	Tubulin beta-1 chain	1	0.66	0.58	0.32	0.34
MD05G1106800	TUA6	1	Tubulin alpha-6 chain	1	0.21	0.14	0.17	0.27
MD14G1003200	TUA5	1	Tubulin alpha-5 chain	1	0.36	0.27	0.38	0.42
MD12G1004700	TUA5	1	Tubulin alpha-5 chain	1	0.93	0.36	0.3	0.47
MD03G1004400	TUA5	1	Tubulin alpha-5 chain	1	0.6	0.65	0.5	0.75
MD10G1111600	TUA2	1	Tubulin alpha-2 chain	1	0.91	0.67	0.58	0.46
MD10G1179100	PG2	4	Polygalacturonase 2	1	94.54	37.02	24.42	8.88
MD12G1077600	PG1	4	Polygalacturonase 1	1	2.27	0.45	0.39	0.3
MD09G1155100	GAUT15	2	Galacturonosyltransferase 15	1	1.42	1.13	2.39	5.19
MD00G1171900	GAUT14	2	Galacturonosyltransferase 14	1	0.94	0.82	1.43	1.7
MD09G1061900	GAUT14	2	Galacturonosyltransferase 14	1	1.53	1.6	1.96	2.34
MD13G1046300	GATL3	2	Galacturonosyltransferase-like 3	1	0.52	0.07	1.39	7.28
MD16G1048100	GATL3	2	Galacturonosyltransferase-like 3	1	0.57	0.4	1.04	4.08
MD17G1042500	GATL10	2	Galacturonosyltransferase-like 10	1	1.35	1.38	4.11	11.61
MD12G1196400	GAUT7	3	Galacturonosyltransferase 7	1	1.24	1.58	1.46	1.26
MD17G1141200	GAUT15	3	Galacturonosyltransferase 15	1	10.4	26	7.8	15.6
MD05G1204900	GAUT6	7	Galacturonosyltransferase 6	1	0.88	0	4.88	2.65
MD11G1285000	CYCP1;1	4	Cyclin-p3-1	1	1.51	0.99	0.52	0.66
MD15G1288100	CYCD3;2	1	Cyclin-D3-2-like	1	0.5	0.33	0.25	0.23
MD15G1077100	CYCD3	4	Cyclin-D3-1-like	1	1.39	1.31	0.29	0.37
MD15G1381900	CYCD2;1	8	Cyclin-D2-1-like	1	1.24	1.7	0.99	0.64
MD14G1202100	EXP1	2	Expansin-A1-like	1	76.33	60.67	214.33	255
MD06G1195100	EXP1	3	Expansin-A1-like	1	58.56	258.27	266.42	201.75
MD07G1200300	EXP4	4	Expansin-A4-like	1	52.25	32.5	12.5	17
MD03G1090700	EXP4	3	Expansin-A4-like	1	6.43	31	22.14	20.57
MD03G1052400	EXP8	2	Expansin-A8-like	1	2.29	3.14	15.71	19.57
MD07G1233100	EXP8	3	Expansin-A8-like	1	1.75	1.81	1.57	2.04
MD10G1133200	EXLA1	2	Expansin-like A1	1	1.49	0.54	0.87	3.11
MD11G1054500	EXP8	2	Expansin-A4-like	1	0.65	0.27	0.3	1.7
MD03G1028900	CSLE1	2	Cellulose synthase like e1	1	1.06	0.86	3.81	3.11
MD03G1029000	CSLE1	7	Cellulose synthase like e1	1	2.5	2.33	9.42	5.5
MD09G1158600	CSLC05	7	Cellulose-synthase like c5	1	0.3	0.3	8.8	4.4
MD08G1126200	CSLC05	2	Cellulose-synthase like c5	1	1.78	1.09	3.65	5.04
MD09G1037900	CSLC04	2	Cellulose-synthase like c4	1	0.53	0.32	0.38	2
MD17G1038900	CSLC04	2	Cellulose-synthase like c4	1	0.66	0.45	0.65	2.67
MD15G1150300	CESA7	2	Fimbrin-like protein 2	1	0.39	0.27	0.13	2.8
MD09G1119000	FIM2	2	B-hexosaminidase	0.15	1.15	1.85	5.46	0.15
MD15G1150300	CESA7	2	Cellulose synthase catalytic subunit7	1	0.39	0.27	0.13	2.79

Table S4. Differentially expressed genes related to sugar and malic acid pathways in apple fruit during long-term cold storage

Gene ID	name	cluster	Annotation	Cold storage time				
				1/1	2/1	3/1	4/1	5/1
MD13G1148300	A/N-INV	2	Alkaline/neutral invertase	1	1.06	1.4	1.91	2.52
MD05G1363600	PFK2	1	Phosphofructokinase 2	1	0.33	0.26	0.22	0.15
MD17G1180800	PFK3	1	Phosphofructokinase 3	1	0.69	0.33	0.28	0.45
MD01G1037400	PFK5	1	Phosphofructokinase 5	1	0.61	0.31	0.58	0.49
MD17G1180600	PFK3	1	Phosphofructokinase 3	1	0.59	0.38	0.32	0.21
MD10G1024400	FBP	1	Cytosolic fructose-1,6-bisphosphatase	1	0.47	0.75	0.52	0.6
MD00G1040000	FBA2	3	Fructose-bisphosphate aldolase 2	1	0.78	2	1.86	1.35
MD15G1012900	HXK3	1	Hexokinase 3	1	0.52	0.58	0.15	0.35
MD15G1197000	HXK2	1	Arabidopsis thaliana hexokinase 2	1	0.8	0.68	0.49	0.58
MD15G1127900	SPS1F	1	Sucrose phosphate synthase 1F	1	0.84	0.59	0.5	0.29
MD02G1100600	SUS4	5	Arabidopsis thaliana sucrose synthase 4	1	0.11	0.11	0.76	1.11
MD03G1250300	TPS1	4	Trehalose-6-phosphate synthase	1	4.35	1.76	2.06	1.12
MD13G1089600	TPS10	3	Trehalose phosphate synthase	1	1.45	2.44	1.44	1.95
MD00G1171800	TPS10	3	Trehalose phosphate synthase	1	2.85	3.14	3.3	2.93
MD09G1234800	TPS7	1	Trehalose-phosphatase/synthase 7	1	0.59	0.38	0.46	0.53
MD14G1183700	TPS9	13	Trehalose-phosphatase/synthase 9	1	0.59	0.36	0.88	0.52
MD06G1208300	ENO1	3	Enolase 1	1	2.41	5.18	3.51	2.57
MD07G1272600	TPP2	1	Tripeptidyl peptidase ii	1	0.59	0.46	0.36	0.44
MD01G1195500	SDH	10	Sorbitol dehydrogenase-like	1	0.02	0	0	0.01
MD00G1005600	SDH	4	Sorbitol dehydrogenase-like	1	11.43	3.34	0.49	1.89
MD00G1005700	SDH	4	Sorbitol dehydrogenase-like	1	3.33	1.14	0.28	0.5
MD01G1194700	SDH	4	Sorbitol dehydrogenase-like	1	2.87	0.52	0.14	0.17
MD01G1195100	SDH	4	Sorbitol dehydrogenase-like	1	5.97	1.47	0.4	0.4
MD01G1195200	SDH	4	Sorbitol dehydrogenase-like	1	1.64	1.55	0.56	0.65
MD07G1261900	SDH	4	Sorbitol dehydrogenase-like	1	1.35	0.51	0.32	0.35
MD05G1326100	STP1	2	Sugar transporter 1	1	3.8	3.53	2.91	14.67
MD10G1304000	STP1	3	Sugar transporter 1	1	14.75	28.54	18.34	19.23
MD16G1189900	STP13	2	Sugar transport protein 13	1	12.44	9.11	4.22	124.9
MD03G1268800	BE2	1	Isoamylase 2	1	0.53	0.65	0.37	0.36
MD11G1289600	BE2	1	Isoamylase 2	1	0.44	0.43	0.37	0.22
MD14G1017700	SBE2.2	1	Starch branching enzyme 2.2	1	0.62	0.52	0.58	0.46
MD10G1265500	SS4	12	Starch synthase 4	1	0.96	0.33	0.83	0.72
MD05G1347400	SS3	1	Starch synthase 3	1	0.17	0.22	0.45	0.38
MD09G1052000	SS2	1	Starch synthase 2	1	0.62	0.77	0.45	0.45
MD13G1096800	GWD	3	Alpha-glucan water dikinase 1	1	4.85	5.22	3.86	3.37
MD16G1097800	GWD	2	Alpha-glucan water dikinase 1	1	1.45	0.53	1.56	1.94
MD06G1122100	GBSS1	1	Granule bound starch synthase 1	1	0.06	0.47	0.2	0.26
MD07G1159300	GBSS1	4	Granule bound starch synthase 1	1	1.29	0.78	0.51	0.9
MD13G1236000	ISA1	4	Isoamylase 1	1	0.99	0.95	0.41	0.6
MD15G1376800	ISA3	1	Isoamylase 3	1	0.84	0.61	0.18	0.24
MD08G1101700	AMY1	2	Alpha-amylase-like	1	1.31	3.5	7.9	21.5
MD02G1064800	NADP-ME3	8	Arabidopsis thaliana NADP-malic enzyme 3	1	0.91	1.14	0.57	0.46
MD01G1083300	NADP-ME3	8	Arabidopsis thaliana NADP-malic enzyme 3	1	0.84	1.06	0.54	0.44
MD04G1101300	NADP-ME3	8	Arabidopsis thaliana NADP-malic enzyme 3	1	0.76	1.16	0.56	0.45

<i>MD17G1077700</i>	<i>NADP-ME3</i>	8	Arabidopsis thaliana NADP-malic enzyme 3	1	0.94	1.13	0.53	0.53
<i>MD07G1087300</i>	<i>NAD-ME1</i>	1	NAD-dependent malic enzyme 1	1	0.46	0.59	0.62	0.54
<i>MD02G1226700</i>	<i>NAD-ME1</i>	1	NAD-dependent malic enzyme 1	1	0.68	0.61	0.45	0.83
<i>MD16G1219000</i>	<i>NAD-MDH1</i>	1	Cytosolic-NAD-dependent malate dehydrogenase 1	1	0.55	0.5	0.44	0.45
<i>MD06G1214800</i>	<i>ALMT9</i>	2	Aluminum-activated malate transporter 9	1	1.52	2	4.1	9.79
<i>MD13G1247200</i>	<i>AHA2</i>	2	H(+) -atpase 2	1	0.56	0.71	8.16	13.56
<i>MD08G1130200</i>	<i>AHA5</i>	5	H(+) -atpase 5	1	0.63	0.53	1.6	1.42
<i>MD09G1043700</i>	<i>AATP1</i>	2	AAA-atpase 1	1	0.92	0.52	0.8	2.41
<i>MD17G1045300</i>	<i>AATP1</i>	2	AAA-atpase 1	1	3.63	0.68	5.11	9.95

Table S5. Differentially expressed genes related to FA and lipid pathways in apple fruit during long-term cold storage

Gene ID	name	cluster	Annotation	Cold storage time				
				1/1	2/1	3/1	4/1	5/1
MD12G1057600	FAD2	3	Fatty acid desaturase 2	1	2.09	1.67	1.73	1.72
MD05G1050200	FAAH	1	Fatty acid amide hydrolase	1	0.47	0.57	0.52	0.49
MD09G1022600	KAS III	2	3-ketoacyl-acyl carrier protein synthase III	1	3.59	8.11	10.65	11.82
MD08G1046600	KCS10	1	3-ketoacyl-coa synthase 10	1	0.5	0.33	0.68	0.57
MD01G1087400	KCS1	2	3-ketoacyl-coa synthase 1	1	3.45	2.55	8.77	10.83
MD15G1118900	KCS11	3	3-ketoacyl-coa synthase 11	1	1.7	2.36	1.65	1.5
MD13G1042400	CER6	11	Eceriferum 6	1	2.38	1.11	1.86	2.34
MD13G1166700	MFP2	1	Multifunctional protein 2	1	0.73	0.65	0.61	0.46
MD14G1058600	ENR1	4	Enoyl-acp reductase 1	1	1.23	1.08	0.45	0.81
MD17G1234100	ACX3	1	Acyl-coa oxidase 3	1	0.44	0.63	0.64	0.39
MD15G1364500	KAT2	2	3-ketoacyl-coa thiolase 2	1	2	0.85	4.25	4
MD15G1363500	ACC1	7	Acetyl-coa carboxylase 1	1	0.8	1.43	4.93	2.54
MD17G1117800	AACT1	2	Acetoacetyl-coa thiolase 1	1	0.81	0.89	1.36	2.2
MD09G1128400	AACT1	2	Acetoacetyl-coa thiolase 1	1	0.43	0.37	0.76	1.36
MD08G1063400	SLD2	1	Acyl-lipid (9-3)-desaturase-like	1	0.2	0.12	0.05	0.11
MD08G1087700	ACBP3	1	Acyl-coa-binding domain 3	1	0.74	0.46	0.55	0.8
MD06G1132700	ACP4	4	Acyl carrier protein 4	1	1.51	0.95	0.51	0.74
MD13G1253800	GPP2	1	Glycerol-3-phosphatase 2	1	0.68	0.6	0.47	0.71
MD08G1174800	GPAT3	4	Glycerol-3-phosphate sn-2-acyltransferase 3	1	1.53	0.65	0.88	0.61
MD15G1164300	PAP26	1	Purple acid phosphatase 26	1	0.48	0.52	0.64	0.73
MD15G1020000	PAP1	1	Purple acid phosphatase 1	1	0.16	0.12	0.04	0.02
MD14G1020300	PAP22	1	Purple acid phosphatase 22	1	1.11	0.11	0.06	0.12
MD05G1059300	PAP10	2	Purple acid phosphatase 10	1	0.47	0.15	0.31	2.43
MD13G1016000	PAP3	2	Purple acid phosphatase 3	1	4.37	5.29	4.35	12.4
MD05G1174000	PAP15	3	Purple acid phosphatase 15	1	2.04	2.13	1.71	1.46
MD04G1009700	PAP15	6	Purple acid phosphatase 15	1	2.76	0.24	0.12	3.29
MD16G1134300	DGAT3	1	Diacylglycerol acyltransferase 3	1	0.8	0.88	0.51	0.47
MD02G1020400	MGD2	3	Monogalactosyldiacylglycerol synthase 2	1	1.42	2.49	1.38	1.15
MD12G1161400	SQD2	1	Sulfoquinovosyldiacylglycerol 2	1	0.47	0.51	0.47	0.45
MD07G1125900	DGD2	1	Digalactosyl diacylglycerol deficient 2	1	0.32	0.5	0.56	0.65
MD12G1040400	DGD1	4	Digalactosyl diacylglycerol deficient 1	1	1.08	0.85	0.61	0.54
MD02G1265300	WRI1	1	Activator of spo(min)::luc1	1	0.61	0.78	0.44	0.39
MD06G1106100	LACS4	3	Long-chain acyl-coa synthetase 4	1	1.77	2.84	3.33	2.82
MD12G1052800	SPP1	1	Lipid phosphate phosphatase delta-like	1	0.67	0.25	0.38	0.74
MD04G1173600	LTP3	7	Lipid transfer protein 3	1	1.63	0.69	2.36	1.9

Table S6. Differentially expressed genes related to hormone pathways in apple fruit during long-term cold storage

Gene ID	name	cluster	Annotation	Cold storage time				
				1/1	2/1	3/1	4/1	5/1
ET								
<i>MD13G1141700</i>	<i>SAM1</i>	3	S-adenosylmethionine synthase 2-like	1	2.13	2.59	2.54	2
<i>MD01G1186700</i>	<i>ACS12</i>	3	1-amino-cyclopropane-1-carboxylate synthase 12	1	1.48	2.37	1.46	1.44
<i>MD15G1302200</i>	<i>ACS8</i>	3	1-amino-cyclopropane-1-carboxylate synthase 8	1	298.68	536.53	548.05	511.29
<i>MD06G1090600</i>	<i>ACS6</i>	2	1-aminocyclopropane-1-carboxylic acid (acc) synthase 6	1	63	43.33	28.67	202.67
<i>MD10G1328100</i>	<i>ACO4</i>	3	ethylene forming enzyme	1	10.48	12.47	5.3	3.77
<i>MD05G1354000</i>	<i>ACO4</i>	4	ethylene forming enzyme	1	10.67	9.41	3.73	3.02
<i>MD17G1106300</i>	<i>ACO4</i>	4	ethylene forming enzyme	1	6.02	1.35	0.68	1.53
<i>MD15G1205100</i>	<i>ACO1</i>	7	ACC oxidase 1	1	0.12	0.14	7.46	0.35
<i>MD03G1292200</i>	<i>ERS</i>	4	Ethylene response sensor	1	2.11	1.95	1.19	1.13
<i>MD06G1001100</i>	<i>ERS</i>	4	Ethylene response sensor	1	3.06	2.98	1.27	1.87
<i>MD04G1096400</i>	<i>EIN2</i>	2	ethylene-insensitive protein 2	1	1.25	1.94	2.29	2.53
<i>MD15G1171800</i>	<i>EBF1</i>	2	EIN3-binding F box protein 1	1	0.86	1.79	3.75	5.17
<i>MD13G1130700</i>	<i>Rap2.6L</i>	3	related to AP2 6l	1	1.82	2.1	1.94	1.91
<i>MD13G1163300</i>	<i>AEBP</i>	3	ethylene-responsive element binding protein	1	2.05	2.32	2.47	1.98
<i>MD09G1174400</i>	<i>RAP2.12</i>	4	related to AP2 12	1	2.08	1.68	1.18	1.01
<i>MD17G1152400</i>	<i>RAP2.12</i>	4	related to AP2 12	1	1.57	1.21	0.96	0.67
<i>MD15G1078900</i>	<i>DEAR3</i>	4	DREB and EAR motif protein 3	1	1.33	0.76	0.4	0.28
JA								
<i>MD11G1221200</i>	<i>FAD4</i>	3	Fatty acid desaturase 4	1	89	114	176	112.5
<i>MD12G1057600</i>	<i>FAD2</i>	3	fatty acid desaturase 2	1	2.09	1.67	1.73	1.72
<i>MD02G1073600</i>	<i>PLA2</i>	2	phospholipase A2 gamma	1	3.28	1.77	4.08	5.26
<i>MD02G1317800</i>	<i>LOX2</i>	2	lipoxygenase 2	1	1.36	4.43	2.57	7.29
<i>MD17G1167100</i>	<i>LOX3</i>	2	lipoxygenase 3	1	3.57	1.86	19.36	27.64
<i>MD04G1204000</i>	<i>LOX1</i>	7	lipoxygenase 1	1	2.88	5.6	12.57	6.86
<i>MD13G1045900</i>	<i>AOC4</i>	1	Allene oxide cyclase 4	1	0.59	0.32	0.18	0.31
<i>MD00G1142600</i>	<i>OPR3</i>	2	12-oxophytodienoate reductase 3	1	1.14	1.14	0.89	2.76
<i>MD08G1216100</i>	<i>OPR2</i>	6	12-oxophytodienoate reductase 2	1	0.67	0.32	0.39	1.46
<i>MD15G1003500</i>	<i>OPCL1</i>	3	OPC-8:0 CoA ligase1	1	2.56	2.25	2.45	2.46
<i>MD08G1004000</i>	<i>OPCL1</i>	4	OPC-8:0 CoA ligase1	1	252.5	101	12.5	36
<i>MD04G1155700</i>	<i>ACX4</i>	2	acyl-CoA oxidase 4	1	0.89	1.05	1.16	3.32
<i>MD01G1026400</i>	<i>ACX2</i>	7	acyl-CoA oxidase 2	1	0.89	1.1	1.6	1.03
<i>MD15G1364500</i>	<i>KAT2</i>	2	3-ketoacyl-coa thiolase 2	1	2	0.85	4.25	4
<i>MD16G1127400</i>	<i>JAS1</i>	2	Jasmonate-associated 1	1	0.67	2.8	1.47	32.53
<i>MD13G1127100</i>	<i>JAS1</i>	2	Jasmonate-associated 1	1	1.36	1.38	0.68	3.84
<i>MD17G1164400</i>	<i>JAZ2</i>	2	Jasmonate-zim-domain protein 2	1	0.4	1.1	0.4	23.3
<i>MD15G1220400</i>	<i>JAZ1</i>	2	Jasmonate-zim-domain protein 1	1	8.85	10.5	18.55	17.2
ABA								
<i>MD05G1081400</i>	<i>CYP707A4</i>	4	cytochrome P450, family 707, subfamily A, polypeptide 4	1	2.37	1.81	0.78	0.63
<i>MD03G1088100</i>	<i>CYP707A2</i>	8	cytochrome P450, family 707, subfamily A, polypeptide 2	1	1.08	1.52	0.71	0.56
<i>MD08G1027400</i>	<i>CYP722A1</i>	8	cytochrome P450, family 722,	1	0.66	1.26	0.19	0.52

			subfamily A, polypeptide 1					
<i>MD05G1207300</i>	<i>NCED3</i>	4	Nine-cis-epoxycarotenoid dioxygenase 3	1	2.42	0.45	0.08	0.14
<i>MD05G1282700</i>	<i>NCED5</i>	4	Nine-cis-epoxycarotenoid dioxygenase 5	1	2	1.45	0.59	0.55
<i>MD07G1033100</i>	<i>ABA2</i>	4	ABA deficient 2	1	1.42	1.07	0.78	0.76
<i>MD09G1171000</i>	<i>PSY1R</i>	2	PSY1 receptor	1	1.76	0.34	3.03	3.55
<i>MD00G1082100</i>	<i>PSY1R</i>	2	PSY1 receptor	1	0.94	0.69	0.81	3.42
<i>MD11G1018500</i>	<i>PSY1R</i>	2	PSY1 receptor	1	0.88	0.67	0.67	2.15
<i>MD04G1165000</i>	<i>PYL4</i>	2	PYR1-like 4	1	1.61	1.19	1.58	9.4
<i>MD01G1158500</i>	<i>PYL4</i>	2	PYR1-like 4	1	1	5	31.9	33
<i>MD15G1060800</i>	<i>PYL2</i>	2	PYR1-like 2	1	4.25	6.25	13.17	72.92
<i>MD12G1057000</i>	<i>PP2C</i>	2	probable protein phosphatase 2C	1	2.84	2.08	1.82	4.8
<i>MD14G1057600</i>	<i>PP2C</i>	2	probable protein phosphatase 2C	1	0.57	0.34	1.33	1.41
<i>MD10G1102000</i>	<i>PP2C</i>	2	probable protein phosphatase 2C	1	0.77	1.05	2.17	2.74
<i>MD05G1124200</i>	<i>PP2C</i>	2	probable protein phosphatase 2C	1	1.08	1.18	1.42	2.12
GA								
<i>MD09G1217400</i>	<i>GA3OX1</i>	4	gibberellin 3-beta-dioxygenase 1-like	1	3.82	3.3	1.75	0.25
<i>MD10G1262000</i>	<i>GA2OX2</i>	2	gibberellin 2-oxidase	1	28	76.5	131	474
<i>MD16G1148400</i>	<i>GA2OX4</i>	2	gibberellin 2-oxidase 4	1	27	23.22	38.89	170.89
<i>MD03G1273300</i>	<i>GID1B</i>	1	gibberellin receptor GID1B-like	1	1.03	0.68	0.84	0.49
<i>MD09G1264800</i>	<i>RGL2</i>	1	spur-type DELLA protein	1	0.7	0.43	0.28	0.16
<i>MD15G1180500</i>	<i>GAI</i>	1	spur-type DELLA protein	1	0.26	0.11	0.03	0.27
<i>MD00G1092500</i>	<i>GASA1</i>	1	gibberellin-regulated protein 1-like	1	0.29	0.07	0.03	0.06
<i>MD17G1039200</i>	<i>GASA14</i>	1	gibberellin-regulated protein 14-like	1	0.01	0	0	0.01
IAA								
<i>MD11G1178400</i>	<i>AAO4</i>	2	aldehyde oxidase 4	1	1.13	3	2.63	10
<i>MD15G1098700</i>	<i>YUC6</i>	1	indole-3-pyruvate monooxygenase YUCCA6-like	1	0.84	0.83	0.56	0.48
<i>MD05G1092300</i>	<i>GH3.1</i>	1	probable indole-3-acetic acid-amido synthetase GH3.1	1	0.02	0.02	0.02	0.03
<i>MD05G1092900</i>	<i>GH3.1</i>	1	probable indole-3-acetic acid-amido synthetase GH3.1	1	0.73	0.78	0.43	0.61
<i>MD15G1290900</i>	<i>GH3.1</i>	4	probable indole-3-acetic acid-amido synthetase GH3.1	1	1.45	0.74	0.38	0.37
<i>MD13G1025300</i>	<i>PIN3</i>	4	auxin efflux carrier component 3	1	1.48	0.74	0.7	1.09
<i>MD10G1176400</i>	<i>IAA11</i>	1	indole-3-acetic acid inducible 11	1	0.32	0.73	0.5	0.45
<i>MD05G1205800</i>	<i>IAA16</i>	1	indoleacetic acid-induced 16	1	0.34	0.46	0.1	0.75
<i>MD04G1225100</i>	<i>IAA16</i>	1	indoleacetic acid-induced 16	1	0.92	0.64	0.23	0.44
<i>MD15G1191800</i>	<i>IAA27</i>	1	indole-3-acetic acid inducible 27	1	0.62	0.27	0.15	0.16
<i>MD02G1057200</i>	<i>IAA27</i>	1	indole-3-acetic acid inducible 27	1	0.16	0.13	0.4	0.6
<i>MD13G1137000</i>	<i>IAA31</i>	1	indole-3-acetic acid inducible 31	1	0.94	0.24	0.11	0.07
<i>MD08G1207300</i>	<i>IAA9</i>	1	indole-3-acetic acid inducible 9	1	0.23	0.18	0.47	0.4
<i>MD06G1132100</i>	<i>ARF1</i>	1	auxin response factor 2	1	0.34	0.15	0.2	0.13
<i>MD05G1309400</i>	<i>ARF3</i>	1	auxin response factor 3-like	1	0.54	0.32	0.5	0.36
<i>MD14G1131900</i>	<i>ARF9</i>	1	auxin response factor 9	1	0.45	0.26	0.39	0.54

Table S7. Differentially expressed genes related to stress responses in apple fruit during long-term cold storage

Gene ID	name	cluster	Annotation	Cold storage time				
				1/1	2/1	3/1	4/1	5/1
MD04G1054400	OST1	1	Open stomata 1	1	0.59	0.38	0.61	0.66
MD06G1046300	OST1	1	Open stomata 1	1	0.3	0.22	0.01	0.01
MD05G1081700	COR27	4	Cold regulated gene 27	1	2.31	1.13	0.25	0.6
MD15G1253900	COR47	4	Cold regulated gene 47	1	2.53	1.97	0.23	0.11
MD05G1291300	COR413	1	Cold regulated 314 thylakoid membrane 1	1	0.59	0.52	0.52	0.42
MD02G1139700	XERO1	7	Dehydrin xero 1	1	0.88	1.63	7.74	3.04
MD14G1147300	BON2	2	Bonzai 2	1	0.35	0.56	4.79	3.81
MD03G1272800	AG18G	7	Autophagy 18	1	24.1	10.8	33.2	11.5
MD05G1347700	ATG8C	3	Autophagy 8c	1	3.67	7.61	6.17	5.37
MD10G1322100	ATG8C	3	Autophagy 8c	1	1.61	2.49	1.67	1.72
MD13G1141800	APG8H	3	Autophagy 8h	1	2.96	4.77	2.3	3.83
MD03G1214400	CRLK1	11	Calcium/calmodulin-regulated Receptor-like kinase 1	1	2.62	2.15	1.36	2.16
MD15G1318400	CAMTA3	2	Calmodulin-binding transcription activator 3	1	1.31	1.41	2.09	2.54
MD12G1044400	CPK2	2	calcium-dependent protein kinase 3	1	0.6	0.73	0.13	3.6
MD12G1072600	CDPK19	2	calcium-dependent protein kinase 19	1	1.32	1.46	2.11	2.02
MD03G1200500	CPK9	2	calmodulin-domain protein kinase 9	1	2.19	1.92	2.45	3.3
MD06G1186000	CDPK1	3	calcium-dependent protein kinase 1	1	1.89	3.07	2.22	2.18
MD14G1192200	CDPK1	3	calcium-dependent protein kinase 1	1	1.91	2.69	2.8	2.54
MD05G1041800	CPK4	3	calcium-dependent protein kinase 4	1	1.62	2.29	2.47	2.14
MD15G1007200	CPK29	4	calcium-dependent protein kinase 29	1	8.05	6	1.63	2.53
MD01G1216700	CNGC1	2	Cyclic nucleotide-gated channel 1	1	1.96	1.38	2.42	4.83
MD15G1106000	CNGC14	2	cyclic nucleotide-gated channel 14	1	0.6	0.27	1.5	3.93
MD14G1243200	CNGC20	2	Cyclic nucleotide-gated channel 20	1	0.5	0.5	0.58	4.5
MD14G1239400	MKK9	3	MAP kinase kinase 9	1	2.11	2.48	1.99	1.5
MD02G1084700	MKK2	4	MAP kinase kinase 2	1	1.66	0.44	0.84	0.58
MD15G1147300	MAPK6	1	MAP kinase 6	1	0.71	0.83	0.68	0.41
MD13G1024800	MPK15	1	MAP kinase 15	1	0.62	0.46	0.26	0.39
MD06G1089500	MPK4	8	MAP kinase 4	1	0.85	1.09	0.39	0.28
MD14G1234900	MPK9	11	MAP kinase 9	1	2.27	1.09	1.52	2.07
MD15G1253700	MPK7	11	MAP kinase 7	1	7.8	6.8	9.2	2
MD03G1108500	MAPK3	4	MAP kinase 3	1	2.84	1.78	1.51	1.67
MD09G1151800	MPK20	4	MAP kinase 20	1	1.59	1.17	0.78	0.96
MD07G1299100	APRR5	4	Pseudo-response regulator 5	1	2.07	0.99	0.93	0.96
MD01G1228200	APRR5	4	Pseudo-response	1	2.48	1.87	1.66	1.7

regulator 5								
<i>MD08G1086500</i>	<i>STZ</i>	2	Salt tolerance zinc finger	1	1.94	0.77	1.89	4.17
<i>MD15G1071400</i>	<i>STZ</i>	2	Salt tolerance zinc finger	1	0.73	0.29	0.34	1.69
<i>MD07G1192900</i>	<i>ZAT11</i>	2	Zinc finger of <i>Arabidopsis thaliana</i> 11	1	0	0.43	0.71	32.21
<i>MD10G1184100</i>	<i>MYB15</i>	4	MYB domain protein 15	1	124	34.5	12	12
<i>MD04G1225400</i>	<i>PR4</i>	2	PR4	1	4.48	10.32	33.76	106.05

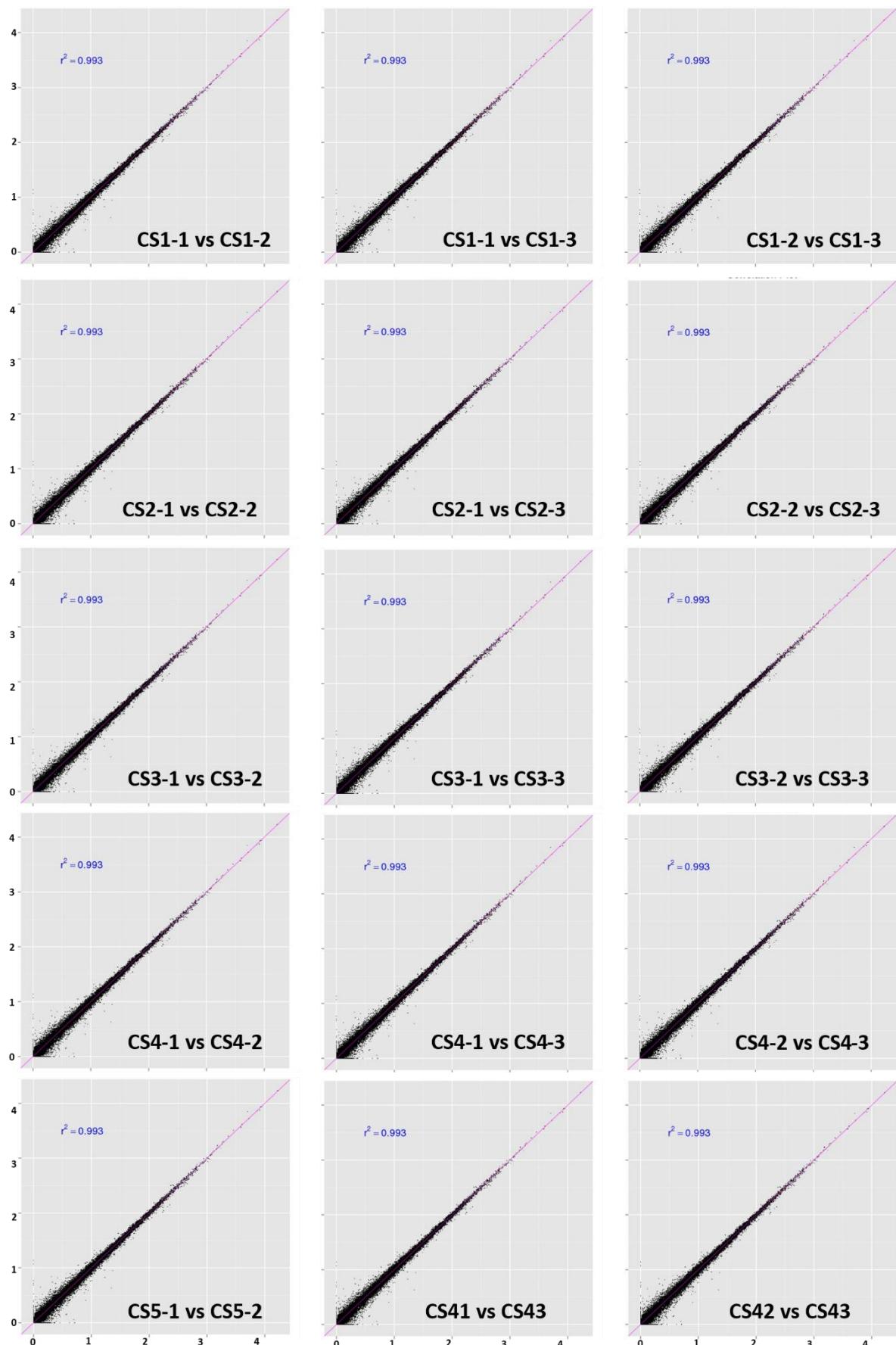


Fig. S1. Correlation of the sequencing data for each sample among biological replicates.

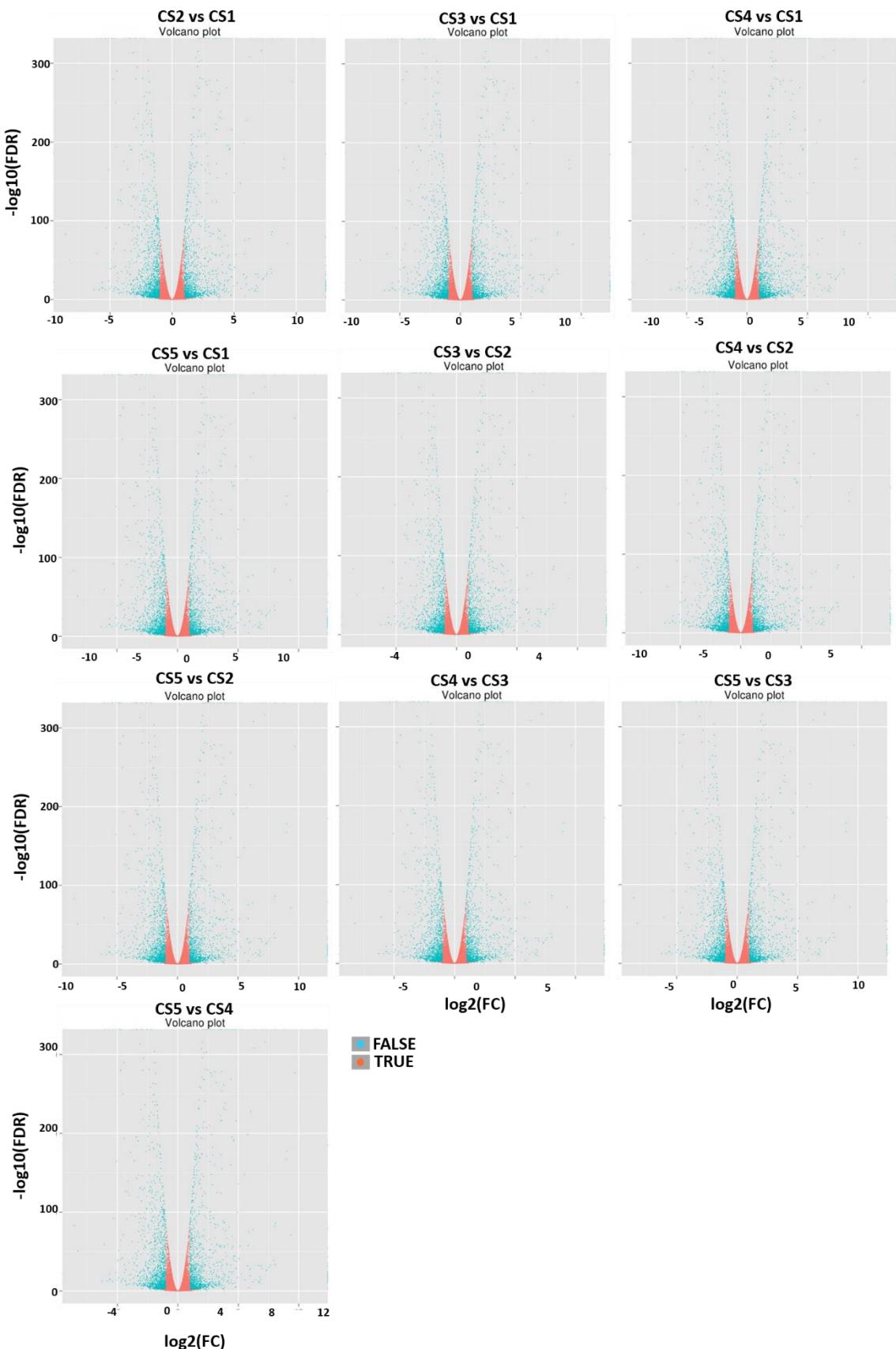


Fig. S2. Volcano plots for differentially expressed genes in the following comparison groups: CS5, 4, 3, and 2 vs CS1; CS5, 4, and 3 vs CS2; CS5 and 4 vs CS3; and CS5 vs CS4.

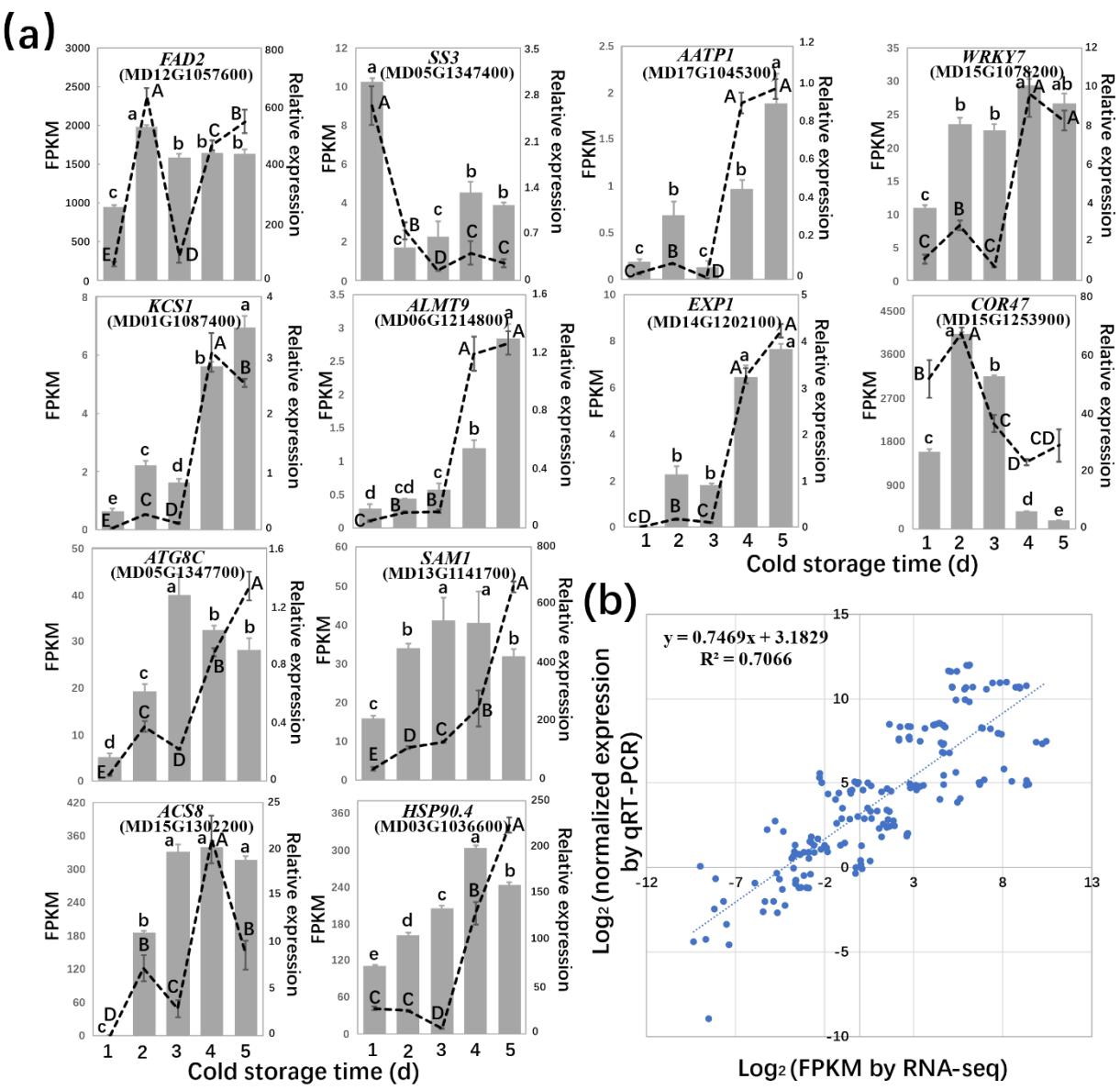


Fig. S3. Analysis of differentially expressed genes by qRT-PCR and the linear relationship between qRT-PCR and RNA-seq data for related genes.

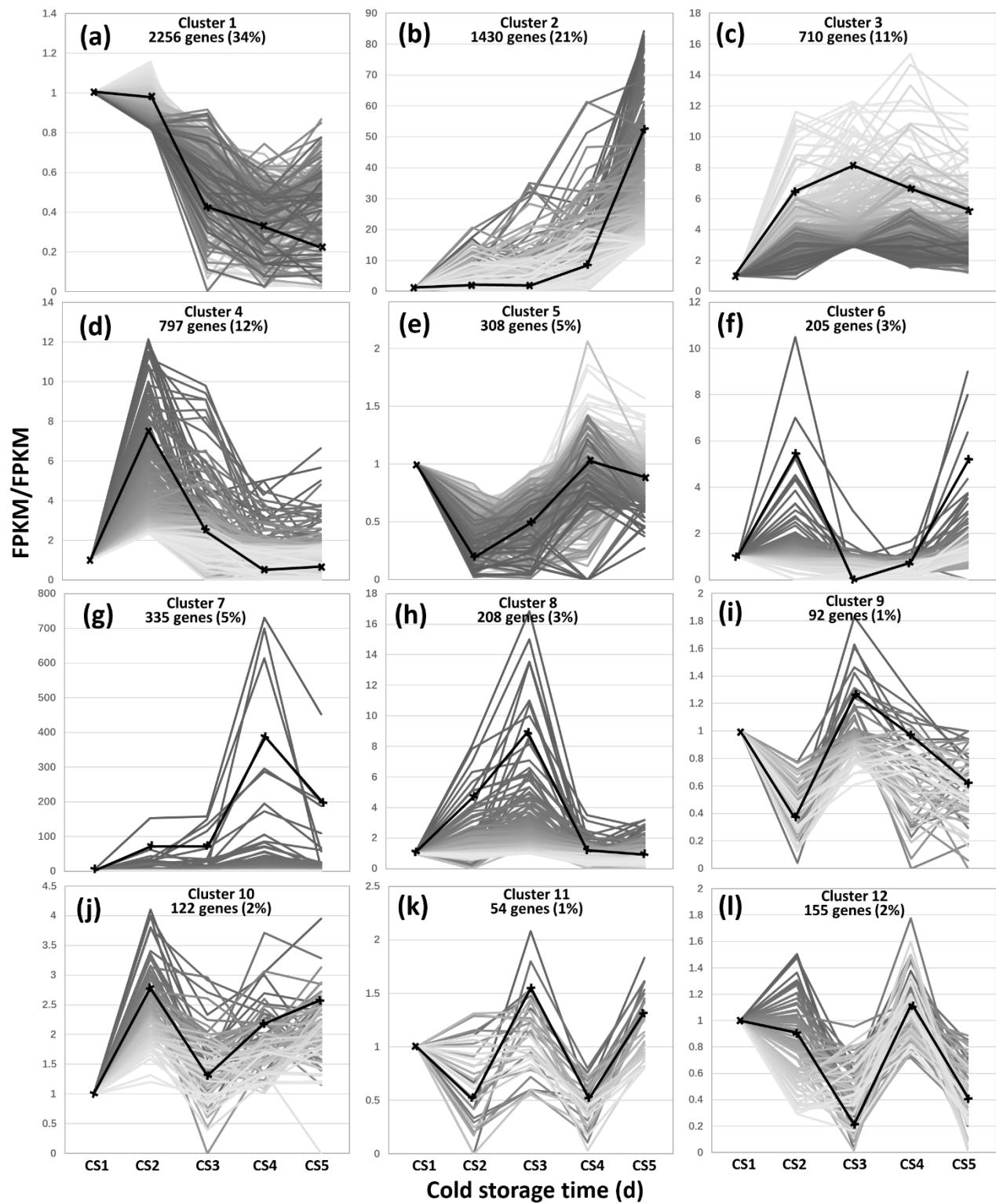


Fig. S4. Results of the K -means clustering of the expression patterns of differentially expressed genes in apple fruit during long-term cold storage. Apple fruits were stored at 4 °C for 1, 30, 60, 90, and 150 days, and the time-points were designated as CS1, CS2, CS3, CS4, and CS5, respectively. On the basis of RNA-seq data, 6,672 individual transcripts with significant differences (fold-change > 2, $p < 0.05$) in comparisons between each pair of time-points were identified.

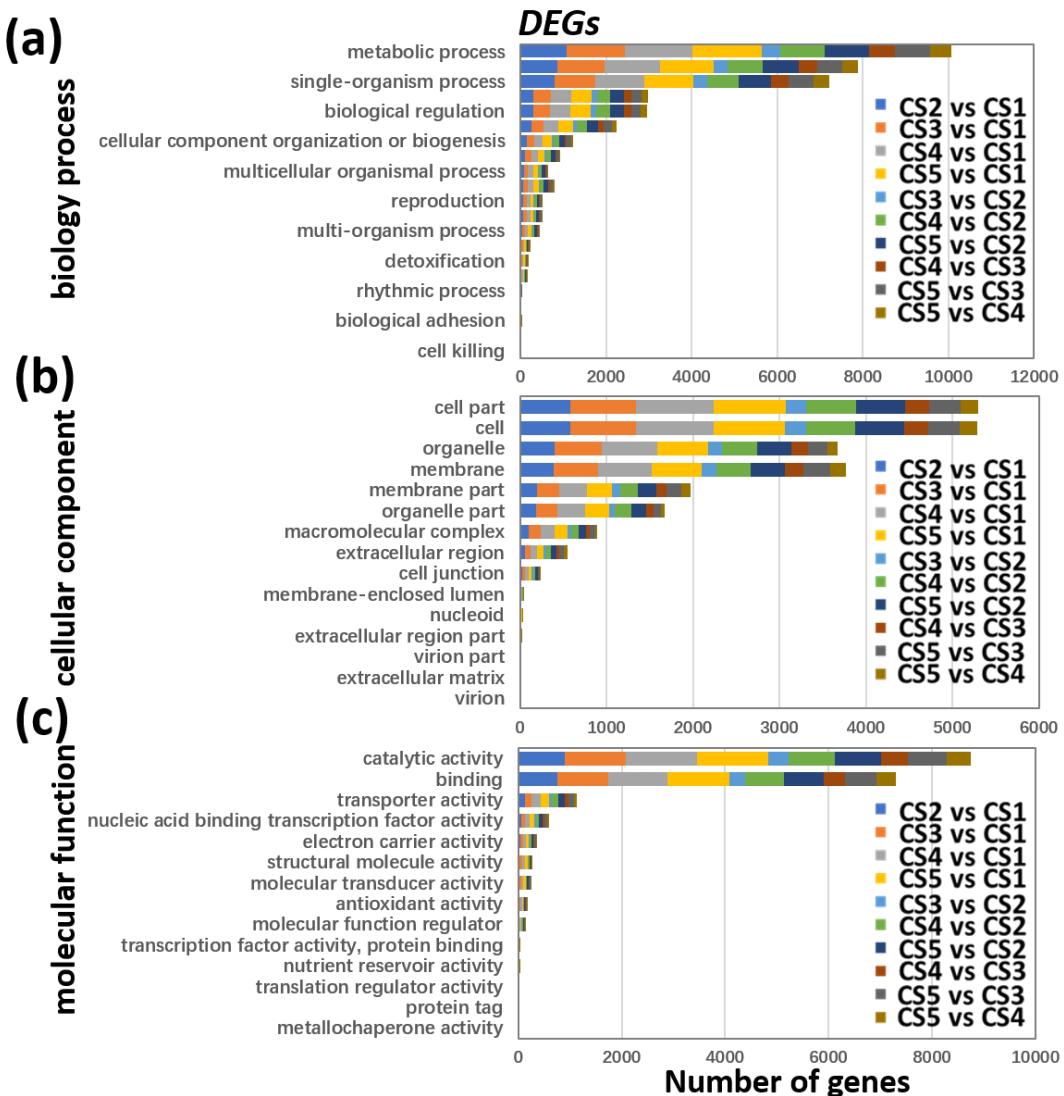


Fig. S5. Gene ontology functional analysis of differentially expressed genes (DEGs) related to biological process (A), cellular component (B), and molecular function in the following comparison groups: CS5, 4, 3, and 2 vs CS1; CS5, 4, and 3 vs CS2; CS5 and 4 vs CS3; and CS5 vs CS4.

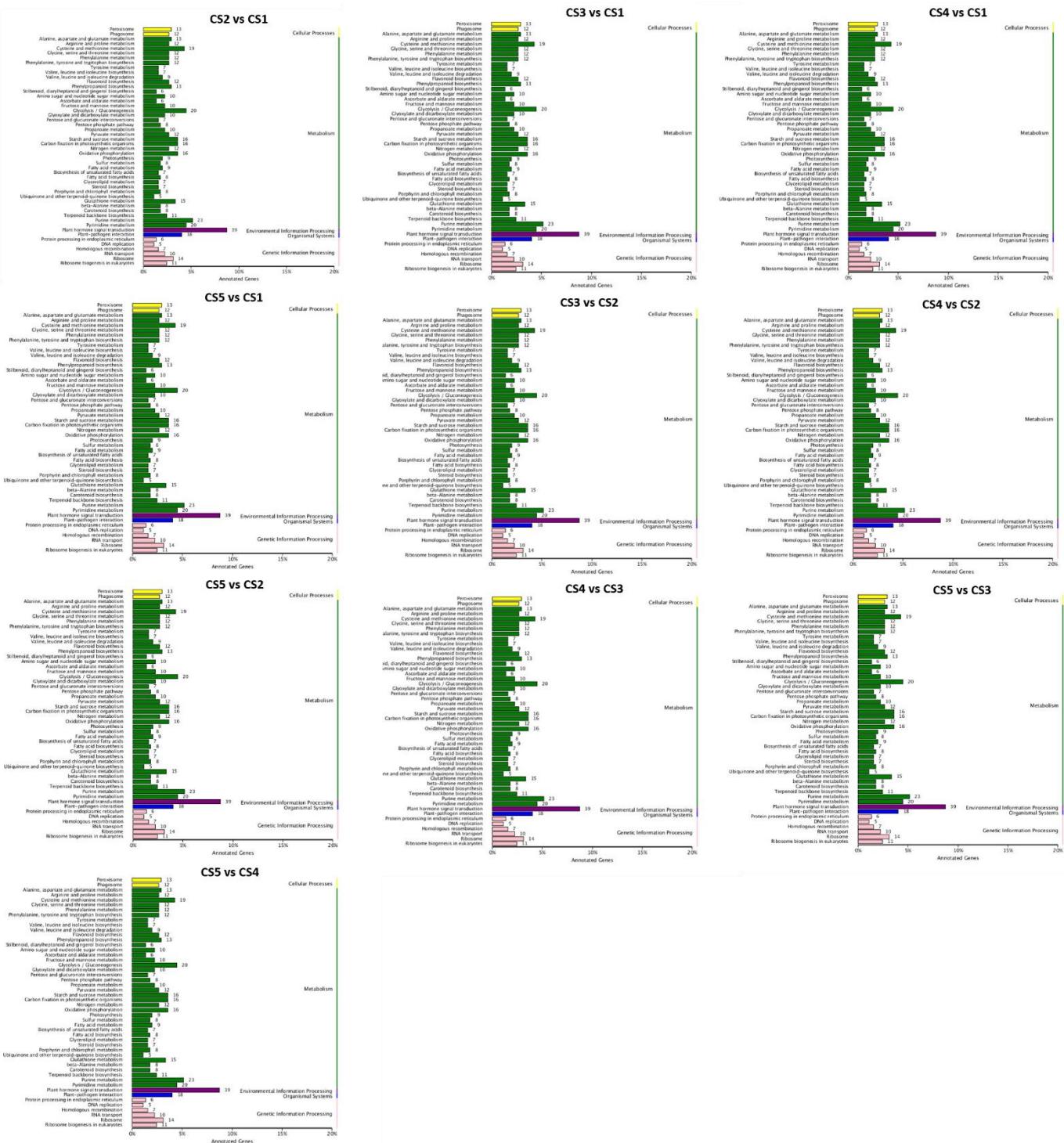


Fig. S6. KEGG pathway enrichment analysis of differentially expressed genes (DEGs) in the following comparison groups: CS5, 4, 3, and 2 vs CS1; CS5, 4, and 3 vs CS2; CS5 and 4 vs CS3; and CS5 vs CS4.

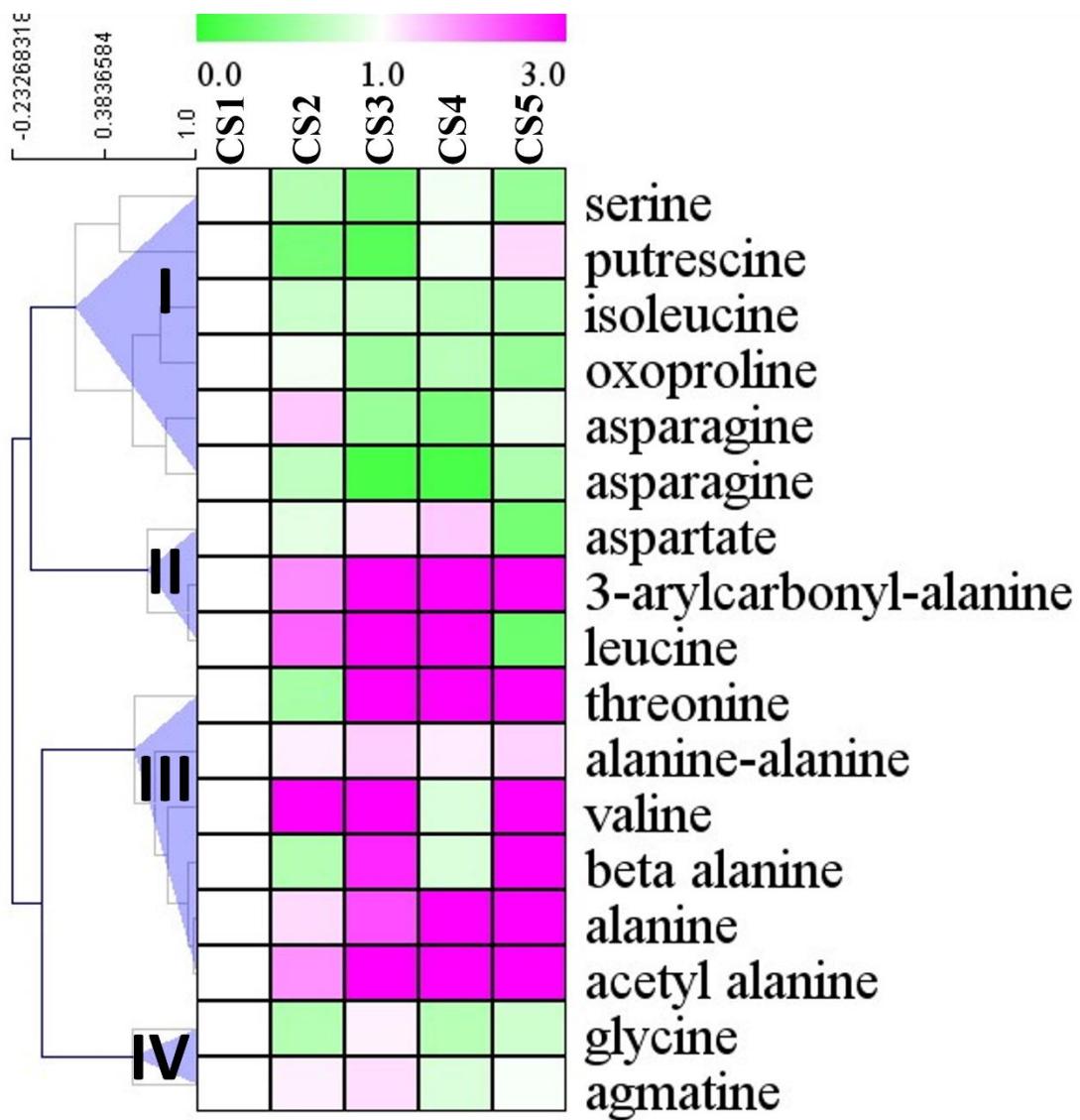


Fig. S7. Cluster analysis of differentially abundant metabolites associated with amino acids in apple fruit during long-term cold storage.

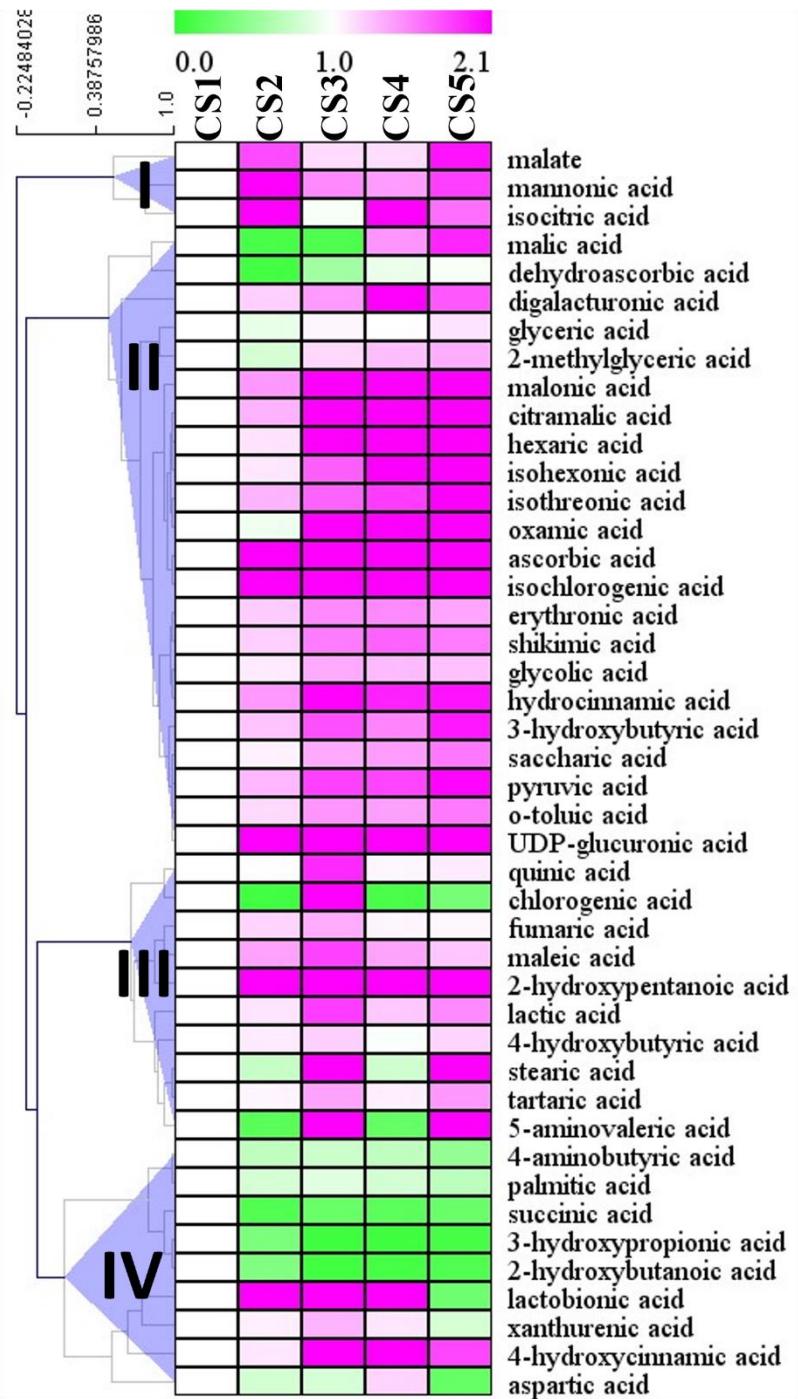


Fig. S8. Cluster analysis of differentially abundant metabolites associated with organic acids in apple fruit during long-term cold storage.

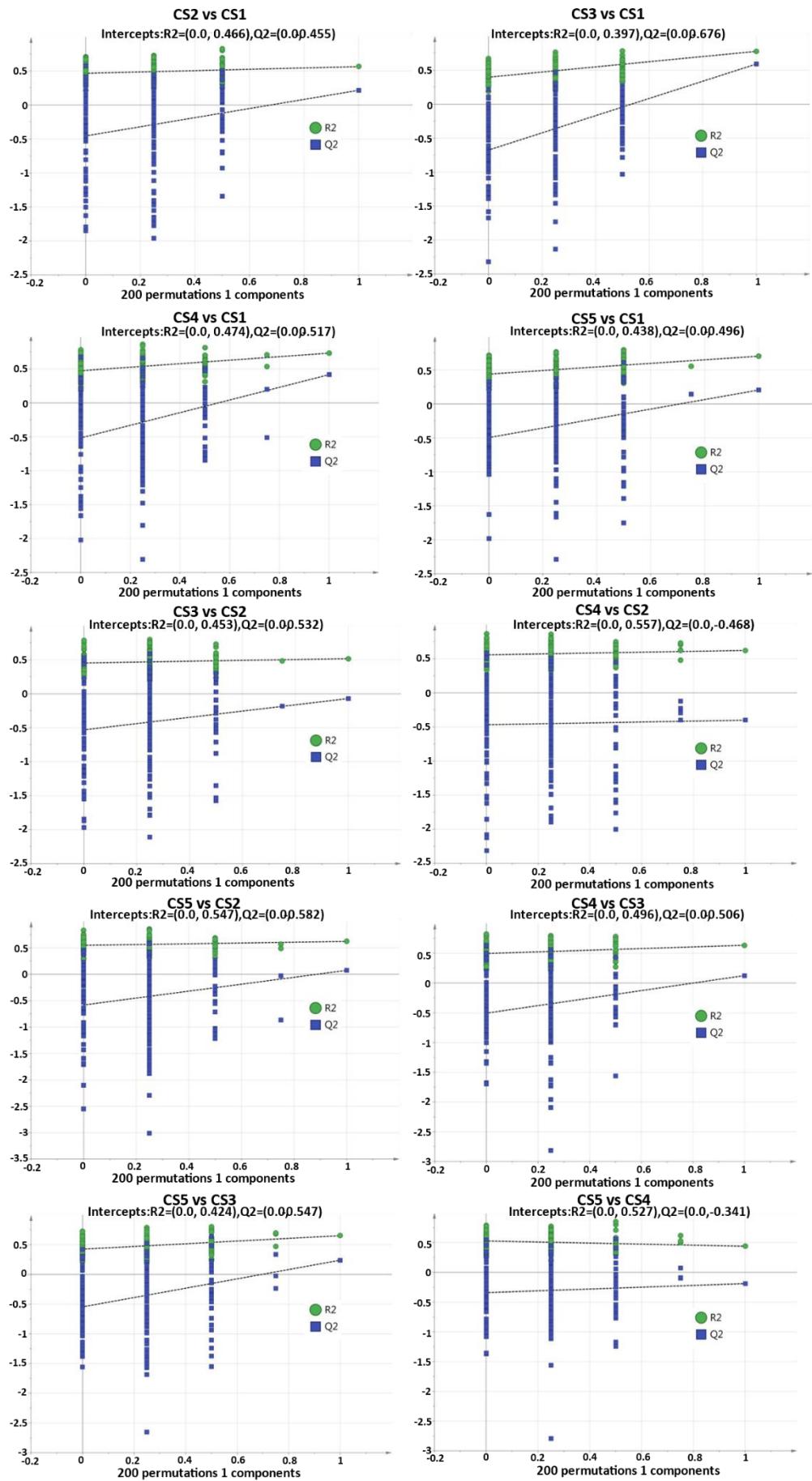


Fig. S9. Quality analysis of models based on R^2X or R^2Y and Q^2 values. The comparison groups were as follows: CS5, 4, 3, and 2 vs CS1; CS5, 4, and 3 vs CS2; CS5 and 4 vs CS3; and CS5 vs CS4.