Supplementary Figures



Fig. S1. Clp protease subunits and their expression during tomato fruit ripening. (A) The cartoon shows the structural organization of the stromal Clp complex. Chaperones unfold and translocate protein substrates into the proteolytic chamber for degradation into small peptides that are emitted through the lateral pores of the chamber. (B) Graphs show the level of transcripts for Clp subunits in the pericarp (flesh) of tomato fruit. The profile of the qPCR reference gene *ACT* (Solyc04g011500) is also shown. Graph in the left represents RNAseq data from the Aharoni lab (www.weizmann.ac.il/plants/aharoni/made-aa-lab) corresponding to immature green (IG), mature green (MG), breaker (B), orange (O), and red ripe (R) fruit. Graph in the right contains data from the tomato eFP browser (http://bar.utoronto.ca/efp_tomato) corresponding to fruit at the following stages: 3 cm (IG), MG, breaker (B), and 10 days after breaker (B+10). In both graphs, results are represented relative to the levels in IG fruit. See Table S2 for accessions.



Fig. S2. HPLC chromatograms of carotenoids in ripe fruit. Pericarp from fruit collected from MT and A94 plants at the R stage (52 DPA) was used for carotenoid extraction and HPLC analysis using the non-plant carotenoid canthaxanthin as an internal standard as described (Rodriguez-Concepcion *et al.*, 2004). Representative HPLC chromatograms are shown. Lutein, β -carotene, and lycopene, together with canthaxanthin, were detected at 470 nm. Phytoene was detected at 290 nm (inset).

	160	170	180	190	200	210	220
ClpP1a	DLYLFVNSP	G	GWVIPGIAI	YDTMQFVRPD	IHTICLGLAAS	MGSFILAGGO	LTKRIAF
ClpP1b	DLYLFVNSP	G	GWVIPGIAI	YDTMQFVRPD	IHTMCLRLAAS	MGSFTLAGGO	LTKRMIM
ClpP3	DIRLIINSP	G	GSVTAGMGI	YDAMKMCKAD	VSTICMGLAAS	MGAFLLASGS	KGKRYCM
ClpP4	DIRLFINCP	G	GSLSATMAI	FDVVQLVRAD	VSTVALGISAS	TASIILAGGI	KGKRYAM
ClpP5	DIVMYVNSP	G	GSVTAGMAV	FDTMRHIRPD	VSTVCVGLAAS	MGAFLLSAGI	KGKRYSL
ClpP6	DILIYLNCP	G	GSTYSVLAI	YDCMSWIKPK	VGTVCFGAAAS	QGALLLAGGE	KGMRYAM
ClpR1	PVYLYINSS	GTQNDEMETV	GSETEAYAI	ADMIRYCKSD	VYTVNCGMAFG	QAAMLLSQGP	KGFRAVQ
ClpR2	KLYMFINGP	G	GDLTPTLAI	YDIMQSLKSA	VGTHCVGFAYN	LAAFLLAAGE	KGNRCAM
ClpR3	PIYLYINST	GTTRDDGETV	GMEAEGFAI	YDSMMQLQNE	IHTVAVGAAIG	QACLLLAAGI	KGKRFMM
ClpR4	PIYFYINST	GTTKG-GEKL	GYETEAFAV	YDVMSYVKPP	IFTLCVGNAWG	EAALLLAAGS	KGNRAAL
	230	240	250	260	270	280	
CloP1a	230 HAWVMIHEP	240 YSG - FYMAQ V	250 GEFVLEAIE	260 	270 	280	IFMSATE
ClpP1a ClpP1b	230 HAWVMIHEP TENENIPHE	240 YSG - F YMAQ V YP HN VEL	250 GEFVLEAIE SICIPTKGA	260 MAKLRETLTF TVPPDTHILF	270 RVYAEKTGQPVV RVYS	280 VVIHEDMERD	IFMSATE
ClpP1a ClpP1b ClpP3	230 HAWVMIHEP TPNFNIPHF NAKVMIHDP	240 YSG - FYMAQV YP HNVEL LGTSGG KA	250 GEFVLEAIE SICIPTKGA TEMGIRIRE	260 MAKLRETLTF TVPPDTHILF MGYHKMKLNF	270 RVYAEKTGQPVV RVYSI(KILSRVTGQPLE	280 VVIHEDMERD CLIYI	IFMSATE
ClpP1a ClpP1b ClpP3 ClpP4	230 HAWVMIHEP TPNFNIPHF NAKVMIH2P NTRIMIHDP	240 YSG • FYMAQV YP • • • HNVEL LG TSGG • • KA	250 GEFVLEAIE SICIPTKGA TEMGIRIRE	260 MAKLRETLTF TVPPDTHILF MGYHKMKLNF IMHNKDNVIF	270 RVYAEKTGQPVN RVYS	280 VVIHEDMERD CLIYI KIEVDTDRD	IFMSATE
ClpP1a ClpP1b ClpP3 ClpP4 ClpP5	230 HAWVMIHEP TPNFNIPHF NAKVMIH2P NTRIMIH2P NSRIMIH2P	240 YSG - FYMAQV YP HNVEL LG TSGG KA LGGASG QA	250 GEFVLEAIE SICIPTKGA TEMGIRIRE IDVEIQARE	260 MAKLRETLTF TVPPDTHILF MGYHKMKLNF IMHNKDNVIF MIHHKANING	270 RVYAEKTGQPVV RVYSI((ILSRVTGQPLE (IFSNSTGRSYE	280 VVIHEDMERD CLIYI EKIEVDTDRD EQVQKDIDRD	IFMSATE NFMNAWE RYMSPIE
ClpP1a ClpP1b ClpP3 ClpP4 ClpP5 ClpP6	230 HAWVMIHEP TPNFNIPHF NAKVMIH2P NTRIMIH2P NSRIMIH2P	240 YSG - F YMAQ V YP HNVEL LG TSGG KA LGG ASG QA LGG AQG GQ	GEFVLEAIE SICIPTKGA TEMGIRIRE IDVEIQARE SDIEIQANE	260 MAKLRETLTF TVPPDTHILF MGYHKMKLNF IMHNKDNVIF MLHHKANLNG AVOSBOKVDF	270 RVYAEKTGQPVV RVYSIC (ILSRVTGQPLE (IFSNSTGRSYE BYLAYQTGQSLE (MYAAFTGOSLE	280 VVIHEDMERD CLIYI KIEVDTDRD QVQKDIDRD NRINQDTDRD	IFMSATE NFMNAWE RYMSPIE FFMSAKE RFMSSAE
ClpP1a ClpP1b ClpP3 ClpP4 ClpP5 ClpP6 ClpR1	230 HAWVMIHEP TPNFNIPHF NAKVMIH2P NTRIMIH2P NSRIMIH2P NARIMIH2P	240 YSG-FYMAQV YPHNVEL LGTSGGKA LGGASGQA LGGAQGGQ QSGCGGHV	GEFVLEAIE SICIPTKGA TEMGIRIRE IDVEIQARE SDIEIQANE EDVRRQVNE	260 MAKLRETLTF TVPPDTHILF MGYHKMKLNF IMHNKDNVIF MLHHKANLNG AVQSRQKVDF	270 RVYAEKTGQPVV RVYS	220 VVIHEDMERD CLIYI KIEVDTDRD QVQKDIDRD NRINQDTDRD MIQTYTERD	IFMSATE NFMNAWE RYMSPIE FFMSAKE RFMSSAE
ClpP1a ClpP1b ClpP3 ClpP4 ClpP5 ClpP6 ClpR1 ClpR2	230 HAWVMIHEP TPNFNIPHF NAKVMIH2P NTRIMIH2P NSRIMIH2P NARIMIH2P NSSTKLYLP	240 YSG-FYMAQV YPHNVEL LGTSGGKA LGGASGQA LGGAQGGQ QSGCGGHV KVSKSS-GSP	250 GEFVLEAIE SICIPTKGA TEMGIRIRE IDVEIQARE SDIEIQANE EDVRRQVNE TELWIKAKE	260 MAKLRETLTR TVPPDTHILR MGYHKMKLNP IMHNKDNVIP MLHHKANLNG AVQSRQKVDP LESNSEYYLE	270 RVYAEKTGQPVI RVYS	220 VVIHEDMERD CLIVIVI EKIEVDTDRD QVQKDIDRD RINQDTDRD EKKDILRP	IFMSATE NFMNAWE RYMSPIE FFMSAKE RFMSSAE KYFRAQE
ClpP1a ClpP1b ClpP3 ClpP4 ClpP5 ClpP6 ClpR1 ClpR2 ClpR3	230 HAWVMIHEP TPNFNIPHF NAKVMIH2P NTRIMIH2P NSRIMIH2P NARIMIH2P NSSTKLYLP LARIALESP	240 YSG - F YMAQ YP H N V E L LG T SGG KA LG G A SG QA LG G A QG GQ QSG C G G H V KV SK SS - G SP AG A A R GQ A	250 GEFVLEAIE SICIPTKGA TEMGIRIRE IDVEIQARE SDIEIQANE EDVRRQVNE TELWIKAKE DDIRNEAEE	260 MAKLRETLTR TVPPDTHILR MGYHKMKLNP IMHNKDNVIP MLHHKANLNG AVQSRQKVDP LESNSEYYLE LLRIRNYLFP	270 RVYAEKTGQPV RVYS	220 VVIHEDMERD CLIVI EKIEVDTDRD QVQKDIDRD RINQDTDRD EMIQTYTERD EKKKDILRP	IFMSATE NFMNAWE RYMSPIE FFMSAKE RFMSSAE KYFRAQE KRFTAKE
ClpP1a ClpP1b ClpP3 ClpP4 ClpP5 ClpP6 ClpR1 ClpR2 ClpR3 ClpR4	230 HAWVMIHEP TPNFNIPHF NAKVMIH2P NSRIMIH2P NSRIMIH2P NARIMIH2P NSSTKLYLP LARIALESP HAKAMIQQP	240 YSG - FYMAU YP HNVEL LG TSGG KA LGG ASG QA LGG AQG GQ QSG CGG HV KVSKSS - GSP AG AARGQA RAPSSG LMQA	250 GEFVLEAIE SICIPTKGA TEMGIRIRE SDIEIQARE SDIEIQANE EDVRQVNE TELWIKAKE DDIRNEAEE SDVYIRAKE	260 MAKLRETLTR TVPPDTHILR MGYHKMKLNF IMHNKDNVIF MLHHKANLNG AVQSRQKVDF LESNSEYYLE LLRIRNYLFF VLVNRDNLVF	270 RVYAEKTGQPV RVYS	220 VVIHEDMERD CLIVI EKIEVDTDRD QVQKDIDRD RINQDTDRD ENIQTTERD EKKDILRP EKVHKDLSRV	IFMSATE NFMNAWE RYMSPIE FFMSAKE RFMSSAE KYFRAQE KRFTAKE YYMDSIK

Fig. S3. Alignment of the region harboring the catalytic triad of Clp proteolytic subunits. Multiple alignment was performed with the MEGA6 software using the protein sequences listed in Table S2. Triad residues are boxed.



Fig. S4. Position and validation of amiRNA sequences. The upper cartoon shows the regions in the tomato *ClpR1* mRNA targeted by *amiR1_1* (red box) and *amiR1_2* (blue box). The mutations in the *amiR1_1* sequence that render the construct inactive (*amiC*, black box) are highlighted in gray. For validation, amiRNAs were cloned under the control of the 2x35S promoter and the generated constructs were agroinfiltrated in independent tomato leaves. The lower graph shows a qPCR analysis of *ClpR1* transcript levels in leaves transiently expressing the indicated amiRNAs. Data correspond to mean \pm SEM of n=3 leaves and they are represented relative to *ClpR1* transcript levels in leaves agroinfiltrated with a similar vector harboring an unrelated amiRNA (C).



Fig. S5. Transcript levels of ripening marker genes in wild-type and *ClpR1*-silenced **fruits.** (A) Level of transcripts of ripening-related genes *E8* (Solyc09g089580), *PG2A* (Solyc10g080210) and *ACS2* (Solyc01g095080) in the pericarp (flesh) of tomato fruit at the indicated stages of ripening (RNAseq data from the Aharoni lab represented in a log10 scale relative to the levels in IG fruit; see Fig. S1). (B) Quantitative RT-PCR (qPCR) analysis of *E8* transcripts in MT fruits. Mean \pm SEM of n \geq 3 fruits are shown relative to IG samples. (C) Transcript levels of *ClpR1* in fruits from MT and *E8:amiR1* (line A94) plants at the indicated stages. Mean \pm SEM of n \geq 3 fruits are shown relative to MT fruit at the MG stage. No statistically significant differences relative to reference MT samples were found. (D) Levels of *E8*, *PG2A* and *ACS2* transcripts detected by qPCR in ripe fruits from MT and A94 plants. Mean \pm SEM of n \geq 3 fruits are shown relative to MT samples. No statistically significant differences relative to reference MT samples. No statistically significant differences relative to reference MT samples. No statistically significant differences relative to reference MT samples. No statistically significant differences relative to reference MT samples were found.



Fig. S6. Ripening rate of transgenic tomato lines. MT and transgenic plants of the indicated lines were grown in the greenhouse. At least five flowers from each of two plants of every transgenic line (#1 and #2) were tagged at anthesis and left to self-pollinate. The graph represents the mean and SEM of the number of days from the day of anthesis (day 0) to the day when the first signs of chlorophyll loss and carotenoid overaccumulation were visually detected (breaker stage). No statistical differences were found.

CmOR(H) IbOR SlOR DcOR1 DcOR2 BoOR AtOR SlOR-like BoOR-like BoOR-like CrOR	MCRVLVASYPINHLIRPHSFRIDYCWSTC-FTSRL-NSGKERQKLSSRWRWRSMASDST-DSSSSSFAP-SVES-DPSDKTSASFCIIEGPETVQDFAKMELQEIQENIRSHRW MVSGRILSLSSSTTPFHLSTSPF-BSS-RYHLH-GRLKSRVRLR-FMADD-AD-SSSFSS-SVDT-EAPDKNAAGFCIIEGPETVQDFAWELQEIQNIRSRRW MVGCRGLILSCSTTPFSFSTAF-FSS-TYF-H-ANRROITLR-SMASD-AD-ASSYAT-SIDS-ESSENAAGFCIIEGPETVQDFAWELQEIQNIRSRRW MVFSTRILAVSYPLSPLLISR
CmOR(H) Ibor Slor DcOR1 DcOR2 BoOR Ator SloR-like BoOR-like BoOR-like Ator-like Cror	KIPLIMEEVRRLRIQQRIKNAELGISKEERENELPNPPSPIPFLPPLSSENIKLYYVTCYSLIAGIILPGGLIAPTLELKLGLGGTSYEDFIRSVHLPMQLSQVDP KIPLIMEEVRRLRIQQRIKNAELGIITERQENKLPNPPSPIPFLPPLPSSNLKQYYATCISLIAGVHLFGGLIAPTLELKLGLGGTSYADFIRSWHLPMQL
CmOR(H) IbOR SlOR DcOR1 DcOR2 BoOR AtOR SlOR-like BoOR-like AtOR-like CrOR	IVASFSGGAVGVISALMVVEVNNVKQQEHKRCKYCLGTGYLACARCSNTGALVLIE PVSTLNGEHQPLSLPKTERCQNCSGSGKVMCPTCLCTCAMAASEHDPRIDPD* IVASFSGGAVGVISALMVVEINNVKQQEHKRCKYCLGTGYLACARCSSTGSLVLIE PVSTVNRGQPLSPKTERCTNCSGSGKVMCPTCLCTCAMAASEHDPRIDPD* IVASFSGGAVGVISALMVVEINNVKQQEKKRCKYCLGTGYLACARCSSTGSLVLIE PVSTVNRGQPLSPKTERCSNCSGAGKVMCPTCLCTCAMAASEHDPRIDPTD* IVASFSGGAVGVISALMVVEINNVKQQEKKRCKYCLGTGYLACARCSSTGLVLIE PVANNGGDELSPKTERCSNCSGAGKVMCPTCLCTCAMAASEHDPRIDPTD* IVASFSGGAVGVISALMVVEINNVKQQEKKRCKYCLGTGYLACARCSSTGLVLIE PVANNGGDELSPKTERCSNCSGAGKVMCPTCLCTCAMAASEHDPRIDPTD* IVASFSGGAVGVISALMVVEVNNVKQQEKKRCKYCLGTGYLACARCSSTGLVLIE PVANNGGDELSPKTERCSNCSGAGKVMCPTCLCTCAMAASEHDPRIDPTD* IVASFSGGAVGVISALMVVEVNNVKQQEKKRCKYCLGTGYLACARCSSTGLVIE PVSALAGGNHSVSTSKTERCSNCSGAGKVMCPTCLCTCAMAASEHDPRIDPTD* IVASFSGGAVGVISALMVVEVNNVKQQEKKRCKYCGSGYLACARCSSTGLVIE PISVASIAGGNHSVSTSKTERCSNCSGAGKVMCPTCLCTCAMAASEHDPRIDPTD* IVASFSGGAVGVISTALLEANNVQQEKKRCKYCGTGYLACARCSSTGLUTE PISVASIAGGNHSVSTSKTERCSNCSGAGKVMCPTCLCTCAMAASEHDPRIDPTD* IVASFSGGAVGVISTALLEANNVQQEKKRCKYCGTGYLCGTGYLACARCSSTGCUTTPIKRTANCSTGKVMCPTCLCTCAMAASEHDPRIDPTD* IVASFSGGAVGVISTALLEANNVQQEKKRCKYCGTGYLCACASASGCCITEPISVSAIAGCNHSVSTSKTERCSNCSGAGKVMCPTCLCTCAMAASEHDPRIDPTD* IVASFSGGAVGVISTILLENSNVQQEKKRCKYCGTGYLCACASASGCCIS ID PITPPRATNQIMQVATKRCLNCSGAGKVMCPTCLCTCAMASEHDPFDPTD* IVASFSGGAVGVISTILLENSNVQQEKKRCKYCGTGYLCACASASGCCIS ID PITPPRATNQIMQVATKRCLNCSGAGKVMCPTCLCTCAMASEHDPFDPTD* IVASFSGGAVGVISTILEVNNVKQQEKKRCKYCLGTGYLCACASASGCCIS ID PITPPRATNQIMQVATKRCLNCSGAGKVMCPTCLCTCAMASEHDPFDPTD* IVASFSGGAVGVISTILEVNNVKQQEKKRCKYCLGTGYLCACASASGCCSIS ID PITPPRATNQIMQVATKRCLNCSGAGKVMCPTCLCTGAVTASEHDPFDPFD* IVASFSGGAVGVISTILEVNNVKQQEKKRCKYCLGTGYLCACASASGCCSIS IS ID PITPPRATNQIMQVATKRCLNCSGAGKVMCPTCLCTGAVTASEHDPFDPFD*
	CmOR(H) 98 B9 IbOR 51 SIOR 51 DcOB1
	$\begin{array}{c c} & & & & \\ & & & & \\ & & & \\ & & & \\ & & & \\ & & &$

AtOR SIOR-like

DcOR-like

BoOR-like

AtOR-like CrOR

Fig. S7. Alignment of OR proteins from several plants. Multiple alignment was performed with the MEGA6 software using the following protein sequences: *Chlamydomonas reinhardtii* CrOR (Cre06.g279500.t1.1), *Cucumis melo* CmOR(H) (A0A0D3MU50.1), *Arabidopsis thaliana* AtOR (AT5G61670.1) and AtOR-like (AT5G06130.2), *Brassica oleracea* BoOR (Bol036294) and BoOR-like (Bol024498), *Daucus carota* DcOR1 (DCAR_020166), DcOR2 (DCAR_009463), and DcOR-like (DCAR_009172), *Ipomoea batatas* IbOR (HQ828087), and *Solanum lycopersicum* SIOR (Solyc03g093830.2.1) and SIOR-like (Solyc09g010110). A Maximum Likelihood tree constructed with these data is also shown.

99

56

100



Fig. S8. Expression pattern of genes encoding carotenoid biosynthetic enzymes and chaperones controlling their stability during tomato fruit ripening. Data correspond to publicly available RNAseq results (left graph) and eFP browser data (right graph) from tomato pericarp samples as described in Fig. S1. Values are represented in a log10 scale relative to the levels in IG fruit. Accessions are listed in Table S1.

Supplementary Tables

	Arabidopsis		Tomato	
	Protein	Gene	Protein	Gene
Name	abbreviation	identifier	abbreviation	identifier
deoxyxylulose-5-phosphate synthase	DXS	At4g15560	DXS1	Solyc01g067890
plastidial Hsp100 type B chaperone	ClpB3	At5g15450	SICIpB3	Solyc02g088610
phytoene synthase	PSY	At5g17230	PSY1	Solyc03g031860
phytoene desaturase	PDS	At4g14210	PDS	Solyc03g123760
ζ-carotene desaturase	ZDS	At3g04870	ZDS	Solyc01g097810
15- <i>cis</i> -ζ-carotene isomerase	Z-ISO	At1g10830	Z-ISO	Solyc12g098710
carotenoid isomerase	CRTISO	At1g06820	CRTISO	Solyc10g081650
hannana R. avalana		A+2~10220	LCYB	Solyc10g079480
lycopene p-cyclase	LCYB	At3g10230	CYCB	Solyc06g074240
lycopene ε–cyclase	LCYE	At5g57030	LCYE	Solyc12g008980
non home di iran peratangid hudrovulaga	BCH1	At4g25700	BCH1	Solyc06g036260
	BCH2	At5g52570	BCH2	Solyc03g007960
	CYP97A3	At1g31800	CYP97A29	Solyc04g051190
cytochrome P450 carotenoid hydroxylase	CYP97B3	At4g15110	CYP97B3	Solyc05g016330
	CYP97C1	At3g53130	CYP97C11	Solyc10g083790
	0001	A+2~62520	CCD1A	Solyc01g087250
	CCDT	Al3963520	CCD1B	Solyc01g087260
corotopoid closurado dioxurandos	00004	A14-40470	CCD4A	Solyc08g075480
caroteriolu cleavage dioxygeriase	CCD4	Al4g19170	CCD4B	Solyc08g075490
	CCD7	At2g44990	CCD7	Solyc01g090660
	CCD8	At4g32810	CCD8	Solyc08g066650
	AtOR	At5g61670	SIOR	Solyc03g093830
	AtOR-like	At5g06130	SIOR-like	Solyc09g010110

Table S1. Carotenoid-related proteins.

Table S2. Tomato homologues of the Clp protease complex subunits.

Protein abbreviation	Arabidopsis gene identifier	Tomato gene identifier	Identity (%) ²	Subcellular localization ³
ClpP1	AtCg00670	emb AM087200 ¹	75	Р
		Solyc01g007490 ¹	75	other
		Solyc09g065790	71	other
ClpP2	At5g23140	Solyc04g009310	85	М
ClpP3	At1g66670	Solyc02g091280	75	Р
ClpP4	At5g45390	Solyc08g075750	64	Р
ClpP5	At1g02560	Solyc01g100520	78	Р
ClpP6	At1g11750	Solyc10g051310	70	Р
ClpR1	At1g49970	Solyc10g049710	66	Р
ClpR2	At1g12410	Solyc08g079620	68	Р
ClpR3	At1g09130	Solyc01g099690	77	Р
ClpR4	At4g17040	Solyc08g077890	76	Р
ClpC1	At5g50920	Solyc12g042060	90	Р
ClpC2	At3g48870	Solyc03g118340	86	Р
ClpD	At5g51070	Solyc03g117950	67	Р
ClpT1	At4g25370	Solyc03g007110	56	Р
ClpT2	At4g12060	Solyc08g079660	47	Р

¹ Same sequence

² Compared to the corresponding Arabidopsis protein sequence

³ TargetP (P, plastid; M, mitochondria)

Use	Name	Sequence (5'-3')
	miR1-A	GGGGACAACTTTTCTATACAAAGTTGCTCCCCAAACACACGCTCGGA
	miR1-B	GGGGACAACTTTATTATACAAAGTTGTCCCCATGGCGATGCCTTAA
	R1_1 I-s	GATTTTAGGCCGATGGATACCTTCTCTCTTTTGTATTCCA
	RI_III-a R1_1III*e	
	R1_1 IV*a	GAATTTAGGGCGATGGATACTTTCTACATATATATTCCTA
	R1 2 I-s	GATTCTACAATAGGCATGCGCAGCTCTCTTTTGTATTCCA
	R1_2 II-a	AGCTGCGCATGCCTATTGTAGAATCAAAGAGAATCAATGA
	R1_2 III*s	AGCTACGCATGCCTAATGTAGATTCACAGGTCGTGATATG
	<u>R1_2 IV^a</u>	
	CII-a	AGAAGGTATCCTACGGCCTAAAATCAAAGAGAATCAATGA
	C III*s	AGAAAGTATCCTACGCCCTAAATTCACAGGTCGTGATATG
	C IV*a	GAATTTAGGGCGTAGGATACTTTCTACATATATATTCCTA
	ACT-F	CCTTCCACATGCCATTCTCC
	ClpR1-R	GAAGAGAATCTGAAAAGAAG
	ClpR2-F	CACTGCTAAAGAAGCTCTTG
	ClpR2-R	CAGTGATATCCCTCGGCG
	ClpR3-F	TTCTTTTCAAGCTTCCGTTGA
	CIDR3-R	
	ClpR4-F	TGAGGCACGGCAACTCGC
	ClpP3-F	TTGGTTGATGCTGTTATAGATGAC
	ClpP3-R	TTTTGGTGGAGGTGCATCCT
	ClpP4-F	ATTGACGGTGTAATTGACAGAGA
	CIPP4-R	
	ClpP5-R	CAAGTGGTTGAAGGGCTTTCA
	ClpP6-F	GTTCAATGAGTACGAATCCGG
	ClpP6-R	GGCATGATGGGCGGATTAG
	ClpB3-F	TCGACCGGGTAAATCTTGAG
	CIPB3-R	
	DXS1-P	GCCTCTCTGGTTTGTCCAAG
	PSY1-F	GCCATTGTTGAAAGAGAGAGGGTG
	PSY1-R	AGGCAAACCAACTTTTCCTCAC
	LCYE-F	GCCACAAGAACGAAAACGAC
	LCTE-R	TTGTGGCCCATAGAAAGGAG
	LCYB-R	GGCATCGAAAAACCTTCTTG
	CYCB-F	TGGCAAGGGTTCCTTTCTTC
	CYCB-R	AGTCATGTTTGAGCCATGTCC
RT-qPCR	BCH1-F	TCCTTCTCTTCCTTTCTCCTC
	BCH2-F	CTCGGACAAATTTGATGGTGT
	BCH2-R	CCTTCGGTTGACTTCCTTTTC
	CYP97A29-F	TTGGGAAGAAGCCGATAGATT
	CYP97A29-R	
	CYP97B3-R	CCACGACAATAAGTCGCAAAT
	CYP97C11-F	GCTGTGGTTCCATCTCTTCAC
	CYP97C11-R	GCCAGAAATTGCATCAGGTAA
	CCD1A-F	TTGATTACCTGCCGCCTTGT
	CCD1A-R	
	CCD1B-R	CCTCATCTCATACAACTCATTTG
	CCD4A-F	GTGGGGTAGTGAGTAGACATCC
	CCD4A-R	ACGTCCTACATGCCACTATGC
	CCD4B-F	GGAATGGTGAGCAGACATCC
	CCD4B-R	
	CCD7-R	TGCATATTCAACCACAAGAAGG
	CCD8-F	CCTTCTGAACCATTCTTTGTGG
	CCD8-R	AACCCAACAACCATGTAGCC
	OR-F	TTGGGACTAGGAGGCACATC
	OR-I-F	GGATTATGGTCTTCGGTGGA
	OR-I-R	ACCTGGCTCAATTGCATAGG
	ACS2-F	CGTTTGAATGTCAAGAGCCAGG
	ACS-R	TCGCGAGCGCAATATCAAC
	PG2A-F	
	F8-F	AGCTGCAAGTTGGAGAGACACG
	E8-R	CCGCATGGAGTTGGAAATTC

Table S3. Primers used in this work.

Table S4. Comparison of reference genes for qPCR analysis of carotenoid-related gene expression during tomato fruit ripening.

		Gene	Primer	Expression level at
Gene type	Gene name	identifier	efficiency	MG / B / B+10 ⁽³⁾
	CAC	Solyc08g006960	0.88 ⁽¹⁾ - 0.93 ⁽²⁾	24.28 / 21.75 / 25.65
Reference	EXP	Solyc07g025390	0.88 ⁽¹⁾ - 0.87 ⁽²⁾	13.40 / 8.05 / 14.91
	ACT	Solyc04g011500	0.96	163.12 / 124.90 / 162.10
Corotonoid	ClpR1	Solyc10g049710	0.99	132.75 / 197.65 / 341.93
carotenoid-	DXS1	Solyc01g067890	0.90	59.54 / 176.03 / 295.39
reidleu	PSY1	Solyc03g031860	0.91	188.53 / 2466.46 / 3813.63

¹ Gonzalez-Aguilera *et al.*, 2016

² Exposito-Rodriguez *et al.*, 2008

³ Tomato eFP browser values at the indicated ripening stages (see Fig. S1 and S8).

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