

Supporting Information

Kachanovsky et al. 10.1073/pnas.1214808109

M82 M C T L S F M Y P N S L L D G T C K T V 20
1 ATGTGTACCTTGAGTTTATGTATCCTAATTCACCTTCTTGATGGTACCTGCAAGACTGTA
A L G D S K P R Y N K Q R S S C F D P L 40
61 GCTTTGGGTGATAGCAAACCAAGATACAATAAACAGAGAAGTCTTGTGTTGACCCCTTG
R I G N C T D Q Q Q L C G L S W G V D K 60
121 AGAATTGGAATTTGACTGATCAGCAGCAGCTTTGTTGGCTTGAGTTGGGGGGTGGACAAG
A K G R R G G T V S N L K A V V D V D K 80
181 GCTAAGGGAAGAAGAGGGGGTACTGTTTCCAATTTGAAAGCAGTTGTAGATGTAGACAAA
R V E S Y G S S D V E G N E S G S Y D A 100
241 AGAGTGGAGAGCTATGGCAGTAGTGATGTAGAAGAAATGAGAGTGGCAGCTATGATGCC
I V I G S G I G G L V A A T Q L A V K G 120
301 ATTGTTATAGGTTCCAGGAATAGTGGATTGGTGGCAGCAGCAGCTGGCGGTTAAGGGA
A K V L V L E K Y V I P G G S S G F Y E 140
361 GCTAAGGTTTTAGTCTGGAGAAGTATGTTATTCCTGGTGAAGCTCTGGCTTTTACGAG
R D G Y K F D V G S S V M F G F S D K G 160
421 AGGGATGGTTATAAGTTTGTATGTTGTTTATCATCAGTATGTTGGATTGATGATGAGGA
N L N L I T Q A L A A V G R K L E V I P 180
481 AACCTCAATTTAATTAAGCAGTGGCAGCAGTGGACGTAATTAAGATTATACCT
D P T T V H F H L P N D L S V R I H R E 200
541 GACCCAACAACGTACATTTCCACCTGCCAATGACCTTTCTGTTTCGTATACACCGAGAG
Y D D F I E E L V S K F P H E K E G I I 220
601 TATGATGACTTCATTGAAGAGCTTGTGAGTAAATTTCCACATGAAAAGGAAGGATTATC
t³⁰⁰² L L E D L *
AAATTTACAGTGAATGCTGGAAGATCPTTAA
K F Y S E C W K I F N S L N S L E L K S 240
661 AAATTTACAGTGAATGCTGGAAGATCPTTAAATTCCTGAATTCATTGGAACGAAAGTCT
t⁴⁸³⁸ K
AAG
L E E P I Y L F G Q F F K K P L E C L T 260
721 TTGGAGGAACCCATCTACCTTTTGGCCAGTCTTTAAGAAGCCCTTGAATGCTTGACT
L A Y Y L P Q N A G S I A R K Y I R D P 280
781 CTTGCCTACTATTTGCCCAAGATGCTGGTAGCATCGCTCGGAAGTATATAAGAGATCTCT
G L L S F I D A E C F I V S T V N A L Q 300
841 GGGTTGCTGCTTTTATAGATGCAGAGTCTTTATCGTGAGTACAGTTAATGCATTACAA
T P M I N A S M V L C D R H F G G I N Y 320
901 ACACCAATGATCAATGCAAGCATGGTCTATGTGACAGACATTTGGCGGAATCAACTAC
P V G G V G E I A K S L A K G L D D H G 340
961 CCCGTTGGTGGAGTTGGCGAGATCGCCAAATCCTTAGCAAAGGCTTGGATGATCACGGA
S Q I L Y R A N V T S I I L D N G K A V 360
1021 AGTCAGATACTTTATAGGGCAAATGTTACAAGTATCATTTTGACAAATGGCAAAGCTGTG
G A K L S D G R K F Y A K T I V S N A T 380
1081 GGAGCGAAGCTTCTGACGGGAGGAAGTTTATGCTAAAACCATAGTATCGAATGCTACC
R W D T F F G K L L K A E N L P K E E E N 400
1141 AGATGGGATACCTTTGGAAGCTTTTAAAAGCTGAGAATCTGCCAAAAGAAAGAAAAT
F Q K A Y V K A P S F L S I H M G V K A 420
1201 TTCCAGAAAGCTTATGTAAGACACCTTCTTTTCTTCTATTTCATATGGGAGTTAAAGCA
D V L P P D T D C H H F V L E D D W T N 440
1261 GATGTACTCCACCAGACACAGATTGTCACCATTTGTCCTCGAGGATGATTGGACAAAT
L E K P Y G S I F L S I P T V L D S S L 460
1321 TTGGGAAACCATATGGAAGTATATCTTGAGTATCCCAACAGTCTTGTATTCTCATTG
A P E G H H I L H I F T T S S I E D W E 480
1381 GCCCCAGAAGGACACCATATTTCCACATTTTACAACATCGAGCATGGAAGATTGGGAG
G L S P K D Y E A K K E V V A E R I I S 500
1441 GGACTCTCTCCGAAAGACTATGAAGCGAAGAAAGGTTGTTGCTGAAAGGATTATAAGC
t³⁴⁰⁶ R
AGA
R L E K T L F P G L K S S I L F K E V G 520
1501 AGACTTGA AAAAACACTCTCCAGGGCTTAAGTCATCTATTCTTTAAGGAGGTGGGA
T P K T H R R Y L A R D S G T Y G P M P 540
1561 ACTCCAAAGACCACAGACGATACCTTGTCTGATAGTGGTACCTATGGACCAATGCCA
t⁹⁷⁷⁶ E
GAA
R G T P K G L L G M P F N T T A I D G L 560
1621 CCGGGAACACCTAAGGACTCTGGGAATGCCTTTCAATACCAGTCTATAGATGGTCTFA
Y C V G D S C F P G Q G V I A V A F S G 580
1681 TATTGTGTTGGCGATAGTTGCTTCCAGGACAAGGTGTTATAGCTGTAGCCTTTTCAGGA
V M C A H R V A A D L G F E K K S D V L 600
1741 GTAATGTGCGCTCATCGTGTGACGCTGACTTAGGGTTGAAAAAATCAGATGTGCTG
D S A L L R L L G W L R T L A * 615
1801 GACAGTCTCTTCTTAGACTACTTGGTTGGTTAAGGACACTAGCATGA

Fig. S1. Mutations in carotenoid *cis-trans* isomerase (*CRTISO*) in four alleles of *tangerine*. The sequence of the wild type, M82 (GenBank accession no. AF416727), served as reference. The mutations in *t^{mic}* (deletion of 348 bp, nucleotides 2214–2496 in GenBank accession no. AF416727) and *t³¹⁸³* (deletion of 282 bp in nucleotides 1092–1440 in GenBank accession no. AF416727) have been previously described (1).

1. Isaacson T, Ronen G, Zamir D, Hirschberg J (2002) Cloning of *tangerine* from tomato reveals a carotenoid isomerase essential for the production of β -carotene and xanthophylls in plants. *Plant Cell* 14(2):333–342.

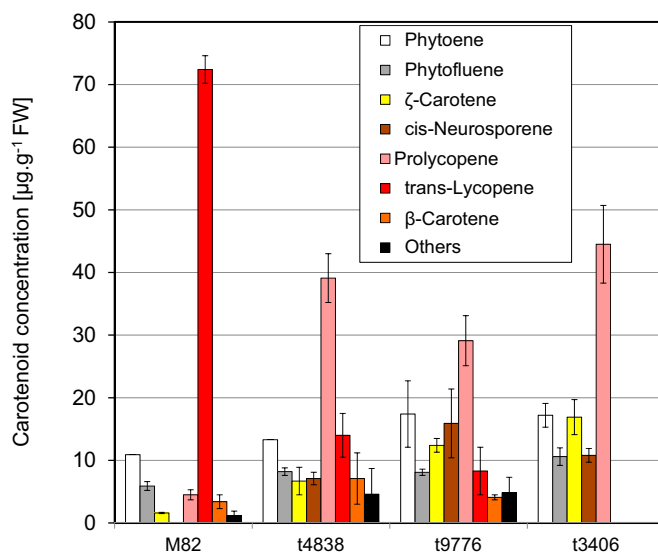


Fig. S2. Carotenoids in fruit of *tangerine* mutants t^{4838} , t^{9776} , and t^{3406} . Total carotenoids were extracted from ripe fruit and analyzed by HPLC ($n = 3$, \pm SEM). FW, fresh weight.

```

1 MSVALLWVVS PCDVSNQTSF MESVREGNRF FDSSRHRNLV SNERINRGGG
51 KQTNNGRKFS VRSAILATPS GERTMTSEQM VYDVVLRQAA LVKRQLRSTN
101 ELEVKPDPII PGNLGLLSEA YDRCGEVCAE YAKTFNLGTM LMPERRRAI
151 WAIIYVWCRRT DELVDGPNAS YITPAALDRW ENRLEDVFNG RPFDMLDGAL
201 SDTVSNFPVD IQPFRDMIEG MRMDLRKSRY KNFDELYLYC YYVAGTVGLM
251 SVPIMGIAPE SKATTESVYN AALALGIANQ LTNILRDVGE DARRGRVYLP
301 QDELAQAGLS DEDIFAGRVT DKWRIFMKKQ IHRARKFFDE AEKGVTELSL
351 ASRFPVWASL VLYRKILDEI EANDYNNFTK RAYVSKSKKL IALPIAYAKS
401 LVPPTKTASL QRA*

```

Fig. S3. Amino acid sequence of phytoene synthase 1 (PSY1) in the mutant *yellow-flesh*³⁷⁵⁶. A transition mutation in codon 151, UGG → UAG of r^{3756} , creates an early stop codon, W151Stop. The sequence of PSY1 in the wild type is according to Unigene SGN-U580527 (http://solgenomics.net/search/unigene.pl?unigene_id=SGN-U580527), which is identical in varieties M82, Rutgers, and Heinz 1706.


```

Psyl -----
Contig ttcgattcaatccatacaccaatatccaacaagagagttaagagacacattggtctagcc
Psyl -----
Contig atctatacgcagattattgagtagtatttttaacagtgcaacataaattcggttttttt
Psyl -----
Contig gtcgattcggttttagaagggaataacacaatattttatagttgaattgtaattca
Psyl -----
Contig attcaggttgatgatacaagttgctcgaatcgatcttgatacatattttacatctct
Psyl -----
Contig taatagtcctttccaccttttaaaactttatatcaacaaaattagacataaaattgtaa
Psyl -----
Contig cgcaacatactttataaagtattggtggtttgggcaaaaacaaacaagatcattaaagg
Psyl -----
Contig atgagcaactctatcttgatatttttaacgattatgatttttttaagaaaactta
Psyl -----
Contig aataaaatattattcaattacttttttatatagtatacacacacctgggcatcaagg
Psyl -----
Contig aggaacgtaacatacaatatttcaatgaaaactctggcgaatattgtttcattatca
Psyl -----
Contig tattgtataaaatcaatagaagatataatatttccagatggttcaagataaaaga
Psyl -----
Contig agtagtgattcaatgaatagatcaaaaggacaacagtaaaacaaatagttgggctag
Psyl -----
Contig gctcaatcaaaaacaaataaattaaagtttaagttcaattgaataaactttcgttaata
Psyl -----
Contig atcattattatataagttttaaaaataatctcaaaaatattcgtgattattattctt
Psyl -----
Contig atccacgaaccccaaaaaaacctaaccaatggatgagtcgatggggccaagtagtgtt
Psyl -----
Psyl cta
Psyl ---

```

Fig. S4. The genomic DNA sequence of *PSY1*. Contig, genomic DNA; Psyl, transcribed sequence. The putative transcription start site (TSS), TATA box, initiation ATG, stop codon, and mutation in *yellow-flesh r³⁷⁵⁶* are marked.

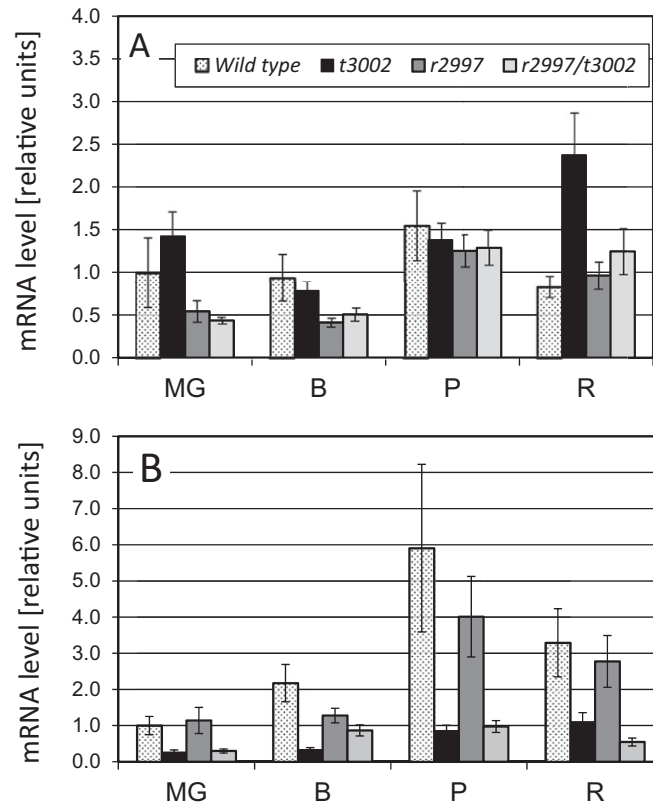


Fig. S5. Expression of *PSY2* (A) and *CRTISO* (B) during fruit development in the wild type (Rutgers), *t³⁰⁰²*, *r²⁹⁹⁷*, and double mutant. RNA was extracted from fruit samples at four developmental stages: mature green (MG), breaker (B), pink (P), and ripe (R). Relative amounts of mRNA were determined by quantitative RT-PCR and were normalized with *HIS3* transcript ($n = 5$, \pm SEM).

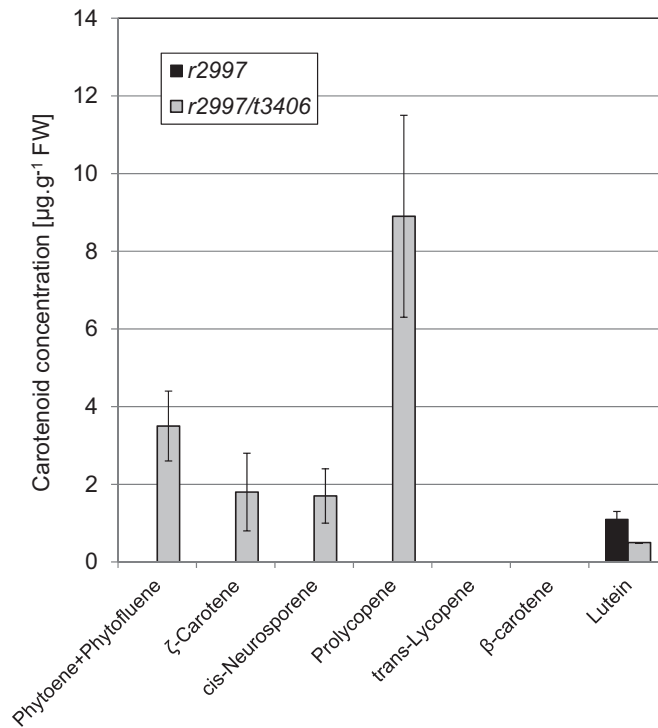


Fig. S6. Carotenoids in fruit of the double mutant r^{2997}/t^{3406} ($n = 3$, \pm SEM).

Table S1. Pigment concentration in young leaves of t^{3002} , r^{2997} , the double mutant r^{2997}/t^{3002} , and the corresponding isogenic wild type (Rutgers)

	Concentration (µg/g fresh weight)			
	Rutgers	t^{3002}	r^{2997}	r^{2997}/t^{3002}
Phytoene	<1	8.6 ± 4.3	<1	5.6 ± 2.5
Phytofluene	0	2.6 ± 1.3	0	1.5 ± 0.5
β-Carotene	60.3 ± 13.8	56.1 ± 8.3	54.1 ± 15.9	60.0 ± 8.5
β-Cryptoxanthin	7.9 ± 1.6	5.8 ± 1.0	6.6 ± 2.2	6.5 ± 0.4
Zeaxanthin	1.7 ± 0.3	3.0 ± 0.4	1.8 ± 1.1	3.0 ± 1.1
Antheraxanthin	10.0 ± 4.4	13.3 ± 3.1	8.2 ± 1.7	11.7 ± 1.4
Violaxanthin	70.0 ± 28.0	64.9 ± 18.2	60.3 ± 17.2	64.7 ± 8.5
Neoxanthin	65.9 ± 18.6	47.5 ± 13.0	57.2 ± 15.9	50.1 ± 5.1
α-Cryptoxanthin	5.3 ± 1.4	5.9 ± 1.1	8.2 ± 2.6	8.8 ± 1.8
Lutein	110.4 ± 36.4	60.1 ± 18.9	101.0 ± 30.5	68.1 ± 12.5
Unidentified	22.8 ± 4.4	17.8 ± 4.1	10.3 ± 3.3	19.9 ± 2.1
Total carotenoids	354.5 ± 106.2	285.5 ± 69.9	307.8 ± 78.5	292.6 ± 31.2
Chlorophyll	2292.6 ± 237.2	1165.7 ± 79.3	2226.8 ± 439.3	1679.2 ± 139.9

Total pigments were extracted with acetone and analyzed by HPLC (µg/g fresh weight) ($n = 5$, \pm SEM).

Table S2. Carotenoid content in ripe fruit of *yellow-flesh* alleles r^{2997} and r^{3756} , the F1 progeny of a cross $r^{3756} \times r^{2997}$, and their corresponding isogenic wild type, M82 ($\mu\text{g/g}$ fresh weight) ($n = 3, \pm\text{SEM}$)

	Concentration ($\mu\text{g/g}$ fresh weight)		
	r^{2997}/r^{2997}	r^{3756}/r^{3756}	r^{2997}/r^{3756}
Phytoene	<1.0	0	0
Phytofluene	0	0	0
ζ -Carotene	0	0	0
<i>cis</i> -neurosporene	0	0	0
Prolycopene	0	0	0
<i>trans</i> -lycopene	<1.0	0.9 ± 0.2	0
β -Carotene	0.7 ± 0.3	0	0
Lutein	1.5 ± 0.4	1.0 ± 0.2	4.0 ± 3.3
Total carotenoids	2.5 ± 0.4	1.9 ± 0.4	4.0 ± 3.3

Table S3. Primers used in quantitative RT-PCR amplifications of tomato genes

Gene	Forward	Reverse
<i>PSY1</i>	5'-AACTTGTTGATGGCCCAAAC-3'	5'-CTGTATCGGACAAAGCACCA-3'
<i>Psy2</i>	5'-AGTTCCTGCTAGTAGATGGCC-3'	5'-GGGCACTAGAGATCTTGCA-3'
<i>CRTISO</i>	5'-CCACCAGACACAGATTGTCA-3'	5'-GACTTAAGCCCTGGGAAGAG-3'
<i>His3</i>	5'-GCTTCGATTTTCAGAGGCTTG-3'	5'-TTCAAAGAGCCCAACGAAGT-3'
<i>Actin</i>	5'-TTGCTGACCGTATGAGCAAG-3'	5'-GGACAATGGATGGACCAGAC-3'