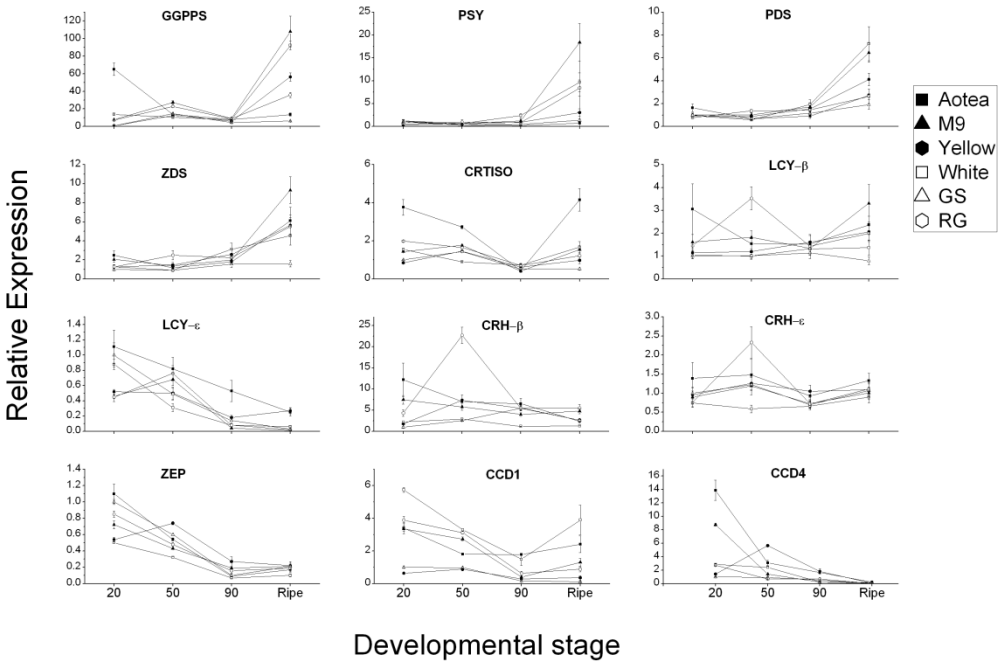

Fig. S1. Expression of apple carotenoid biosynthetic genes in fruit skin of different apple genotypes measured at 20, 50, 90 DAFB and ripe fruit stages. Gene expression was measured relative to *actin*. Error bars are standard errors of the mean from 4 technical replicates.

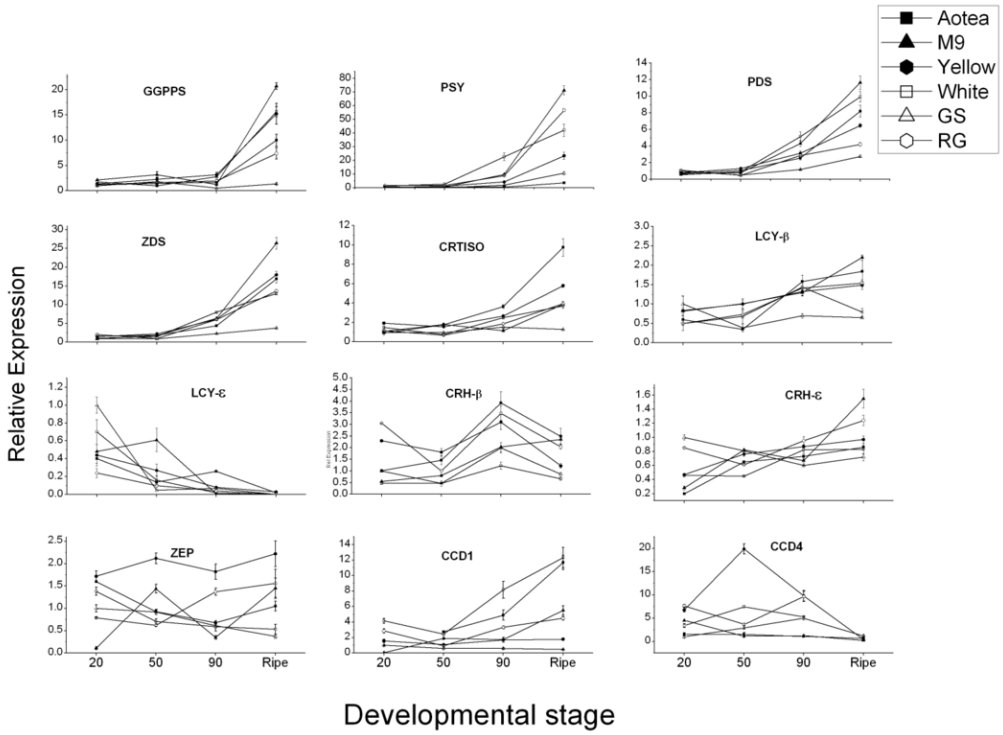

Fig. S2. Expression of apple carotenoid biosynthetic genes in fruit flesh of different apple genotypes measured at 20, 50, 90 DAFB and ripe fruit stages. Gene expression was measured relative to *actin*. Error bars are standard errors of the mean from 4 technical replicates.

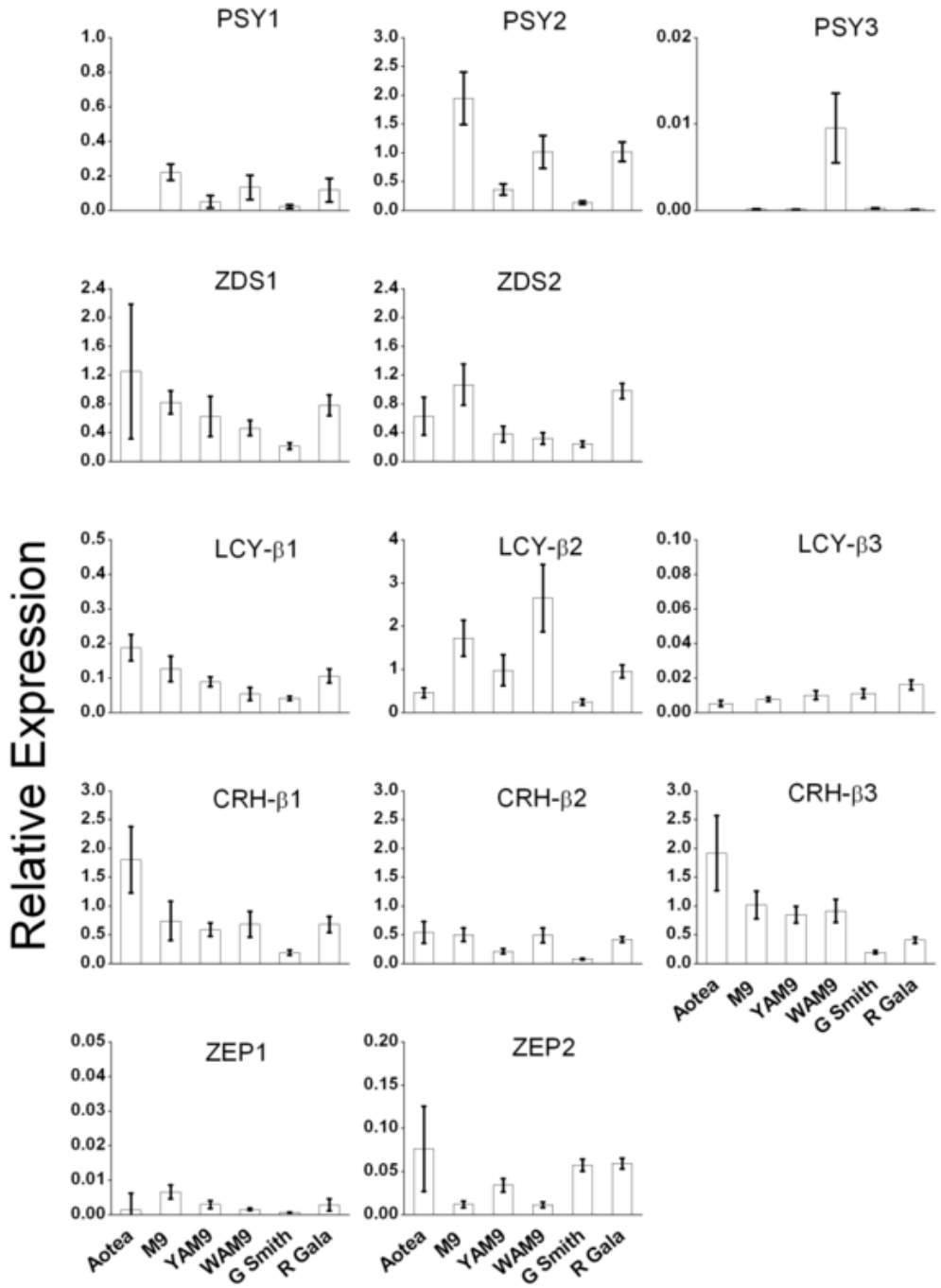

Fig. S3. Expression of apple carotenoid gene homologs, using gene specific primers, in ripe fruit skin of different apple genotypes. Gene expression was measured relative to the apple *actin* gene and the error bars represent standard errors of the mean from 4 technical replicates.

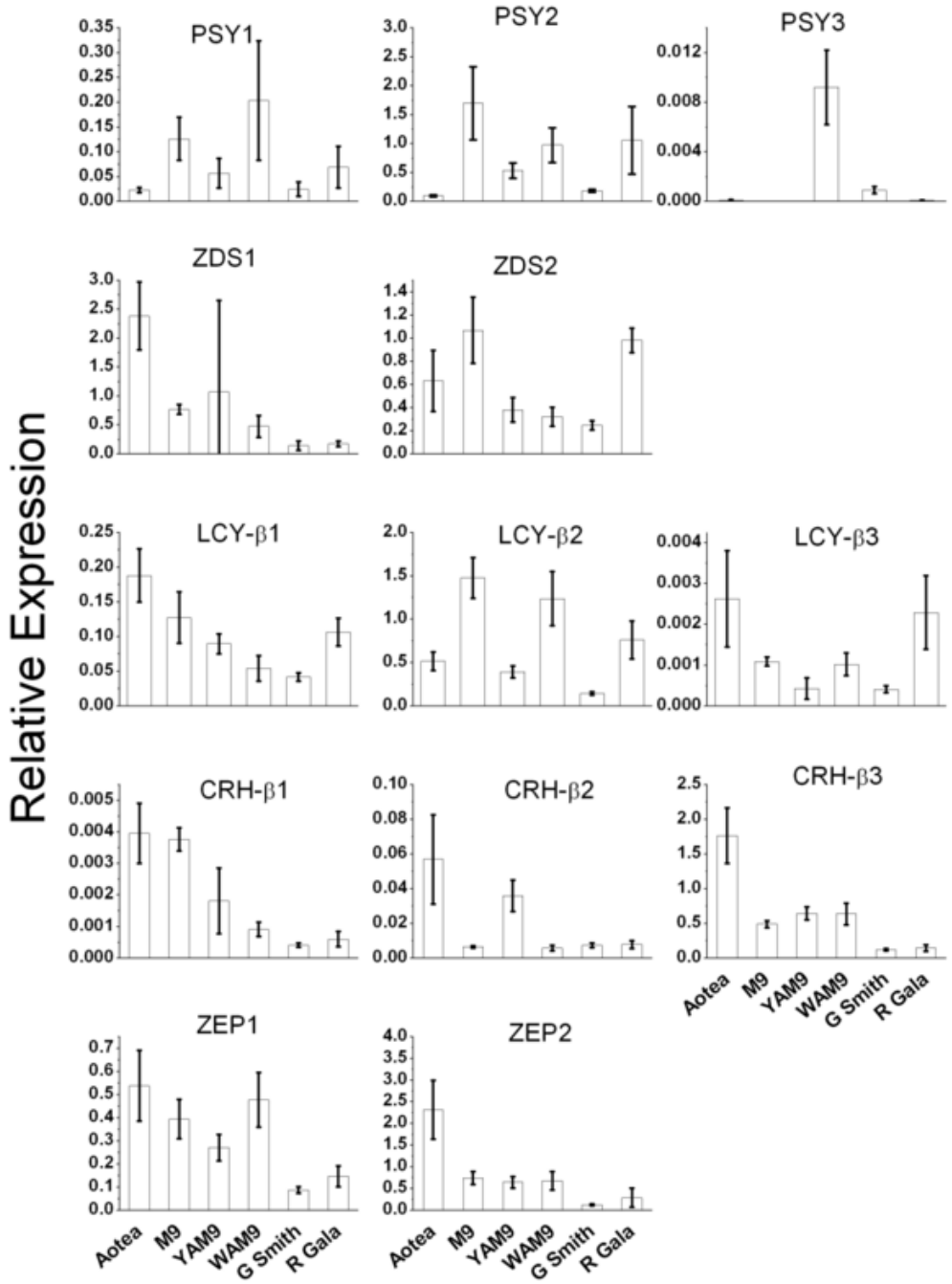

Fig. S4. Expression of apple carotenoid gene homologs, using gene specific primers, in ripe fruit flesh of different apple genotypes. Gene expression was measured relative to the apple *actin* gene and the error bars represent standard errors of the mean from 4 technical replicates.

Table S1. Primers used in quantitative PCR measurements were designed using PRIMER3 software with a stringent set of conditions

Name of primer	**Sequence (5'-3')**
MdACTIN F	TGACCGAATGAGCAAGGAAATTACT
MdACTIN R	TACTCAGCTTTGGCAATCCACATC
MdGGPS F	ACCCCCTCAAAACCACCATAC
MdGGPS R	CCTCTGTGAGGGTCTCTTGCTT
MdPSY F	CAGAGACATGGTAGAAGGAATGAG
MdPSY R	CCAACAGTTCCAGCAACATAGTAG
MdPSY1 F	GAGAAGGTGTATGAAGTGGTGCTG
MdPSY1 R	TTCAAACCTTCAGTAATCCGTTCA
MdPSY2 F	GAGAAGATGCTAGGAGAGGAAGAGT
MdPSY2 R	CTTTGCCACTTGTCAGTCACCTTCC
MdPSY3 F	GAAGCATATGAGAGGTGCAAGAAC
MdPSY3 F	AGTTCATCTGTCCTCCTACACCAA
MdPDS F	ACAAGACTGTACCAGGTTGTGAAC
MdPDS R	GCTCCTTCCATTGAGGCTAAATAC
MdZISO F	GGTAATATGGTGCCTGGCTCACACGAT
MdZISO R	CTCCTGTCACCATTCCAAGCACCAA
MdZDS F	CCAAGGTTTAGAAGTCACTTGGTC
MdZDS R	CAGGTGTCTTCTGATCAGGTCTAA
MdZDS1 F	CAAAGAGGTCCTCCTCCTTCTGG
MdZDS1 R	GGTAGAACTCTGGTTCAGGTGGAA
MdZDS2 F	TCCAGTAACCGGTCGCTGTATAGT
MdZDS2 R	CGTTAACCCCCATGTCAGAAAC
MdCRITSO F	TCACTTGAGGAGCCAATCTACC
MdCRTISO R	GCGAGCTATATCCCCAGCATT
MdLCYB F	AGTTCGGTACCTTGGTTCAGATAG
MdLCYB R	ACAGAAGAACTCCCTTTGTCTCCT
MdLCYB1 F	GCTAGTAGCAGTGCTCTTCTGGAG
MdLCYB1 R	CTAGGTCCACAACAAGACCCTTTG
MdLCYB2 F	CTTGGATCCTTATTACTGGCAAGG
MdLCYB2 R	ATATCAAACCTAGATGGGGTGGAG
MdLCYB3 F	CTCAAGACTCTGTTGTTGGAGAGG
MdLCYB3 R	CGCACAGAATAGAGGACTCGAACT
MdLCYE F	CGGAGCCACTATGATTAGAACCTA
MdLCYE R	AGTGACCTTCAAAAGTAGGCAGTG
MdCRHB F	AGTCCTCCTACGTCATTCCAAAAC
MdCRHB R	CCATCACTACATAGACGGTGAAAC
MdCRHB1 F	ACCTTCTAGGCTAAGCTCCACTCC
MdCRHB1 R	GATGTCGACAGAATCAGGCTCTC
MdCRHB2 F	GAGCTCTCTGCTCGGATTTCTT
MdCRHB2 R	CTAGTAGCCCAGTAGGAACACCAA
MdCRHB3 F	CAGCTGCAGTCTTAACATGGACGT

MdCRHB3 R	GTTGGAAATCTATCGCCTAGAACG
MdCRHE F	GGTTCCGGATCAGAAAATTAGC
MdCRHE R	ACGAAGACGAGGATGATGTAGC
MdZEP F	CTTGAAGGAAGGTCACCCAGTT
MdZEP R	CACTCTCCGGTCAATAGCACAC
MdZEP1 F	CCACAGCCCAGTCTACTCTTAATC
MdZEP1 R	CTCATTCCCCAAGCAAGAGCTA
MdZEP2 F	GGTTGTGTTACTGGGGATAGGA
MdZEP2 R	TTCCACTGCAGGAGTGAATGTA
MdVDE F	GTCGAAGTTCAACGGAAAGTGG
MdVDE R	CTCCAGGACAAGTTCCCTACAAG
MdCCD1 F	GCACCAGTTGCTGGATATCACT
MdCCD1 R	GTGACGTCCTAACGTAGCGAGA
MdCCD4 F	CTTCCGCTATGGCTTTATACGG
MdCCD4 R	AGTCATGGAGGAACGTTGGAGT

Table S2. Carotenoid concentrations (µg/g FW) in apple fruit flesh measured by HPLC analysis at 20, 50, 90 DAFB and ripe fruit stages. Concentrations were determined as beta-carotene equivalents per gram fresh weight of tissue.

	Apple fruit cultivar and developmental stages																							
	Aotea				M9				YAM9				WAM9				Granny Smith				Royal Gala			
Compound	20	50	90	Ripe	20	50	90	Ripe	20	50	90	Ripe	20	50	90	Ripe	20	50	90	Ripe	20	50	90	Ripe
Neoxanthin isomer	0.79			3.40			0.22	0.35				2.35												0.51
Neoxanthin	3.86	2.11	0.71	2.35	3.29	0.97	0.19	0.31	1.63	0.98	0.26	1.66	1.43	0.37	0.25	0.16	1.80	0.55	0.21	0.17	3.46	0.50	0.15	0.29
Violaxanthin	0.87		0.24	3.13								1.96				0.16								0.54
Luteoxanthin				1.93								0.36				0.18								0.20
Lutein	12.05	6.03	2.18		5.99	1.62	0.20	0.28	6.55	3.59	0.71		6.59	1.88	0.55		5.08	2.35	0.89	0.28	6.30	0.90	0.39	
Zeaxanthin				0.47														0.30					0.14	
β–cryptoxanthin				6.42								0.32												
β–carotene	6.37	3.06	0.98	5.06	3.73	0.96	0.14	0.17	3.59	2.11	0.33	0.60	3.96	1.21	0.37	0.15	2.55	1.16	0.44	0.17	3.40	0.55	0.28	
cis β-carotene	1.27	1.45	0.27	0.46	0.99	0.37			1.16	0.98		0.19	0.96	0.34			0.70	0.37	0.16		1.02			
Other carotenoids	4.02	2.64	1.34	6.44	3.42	0.89	0.24	0.38	1.93	1.32	0.38	1.31	1.79	0.52		0.16	2.46	1.03	0.26	0.19	3.78	0.52	0.19	0.25
Total carotenoid	29.23	15.28	5.72	29.66	17.42	4.80	1.00	1.48	14.86	8.98	1.68	9.06	14.73	4.33	1.17	0.81	12.59	5.76	1.95	0.81	17.96	2.47	1.16	1.79
Chlorophyll a	10.90	20.50	0.30	0.10	8.70	5.20	0.80	0.00	14.30	13.70	4.90	0.10	6.00	3.70	3.90	0.00	5.00	5.10	0.30	0.50	11.00	5.20	0.30	0.20
Chlorophyll b	12.80	26.30	0.20	0.00	11.20	6.50	1.00	0.50	14.30	13.60	5.90	0.00	7.30	4.50	4.70	0.00	5.80	6.40	0.20	0.70	12.60	6.60	0.20	0.20