

Supplementary Material

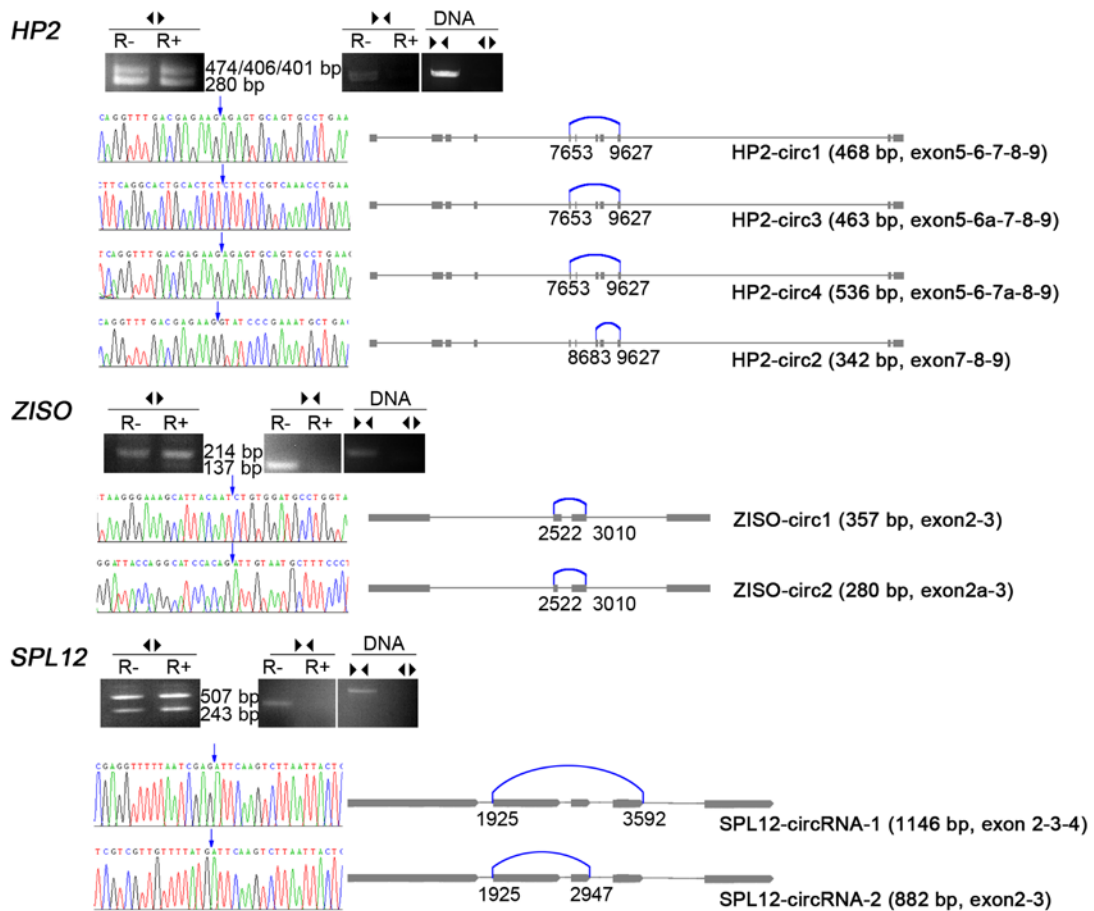
Identification and Functional Characterization of Tomato CircRNAs Derived from Genes Involved in Fruit Pigment Accumulation

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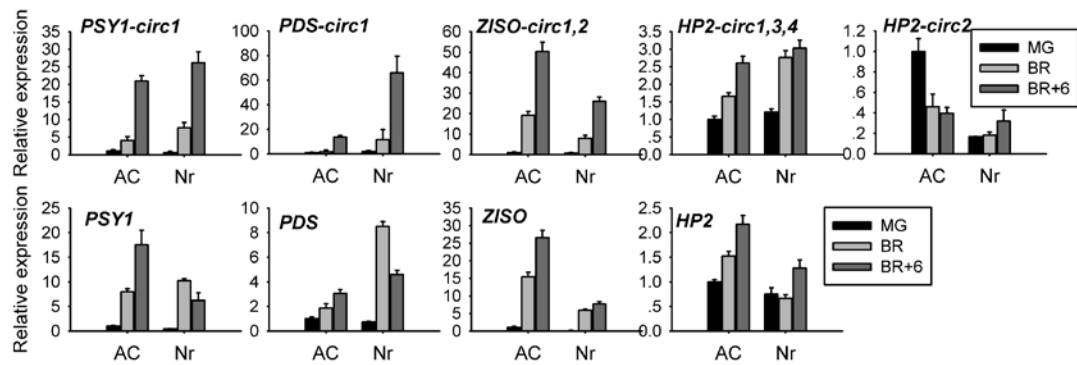
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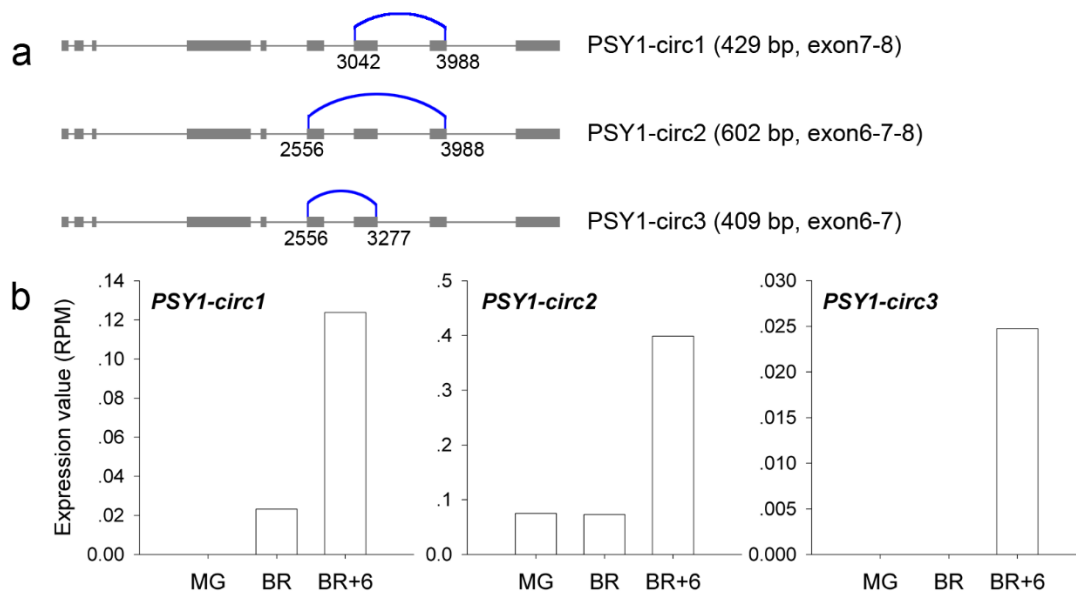


Supplementary Figure 1 Alternative circularization of circRNAs in tomato fruits.

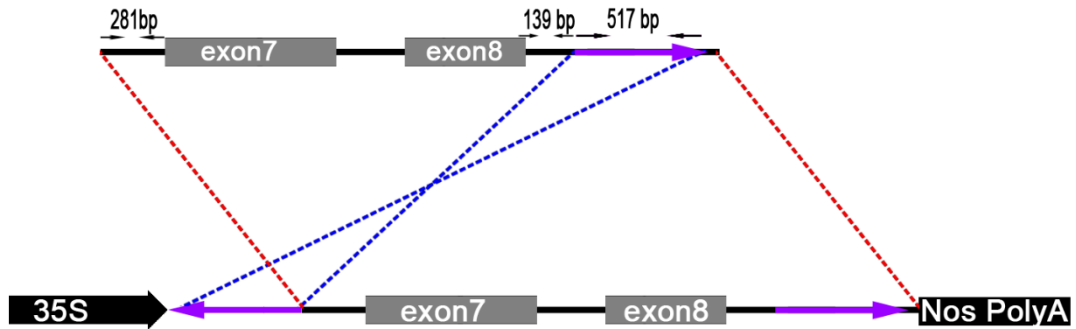
Alternative back-splicing circularization referring to different back-splice sites generated from the same gene was molecularly validated and showed in circRNAs derived from *HP2* and *SPL12* genes. Alternative splicing circularization referring to different transcripts with the same back-splice site was molecularly validated and showed in circRNAs derived from *HP2* and *ZISO* genes.



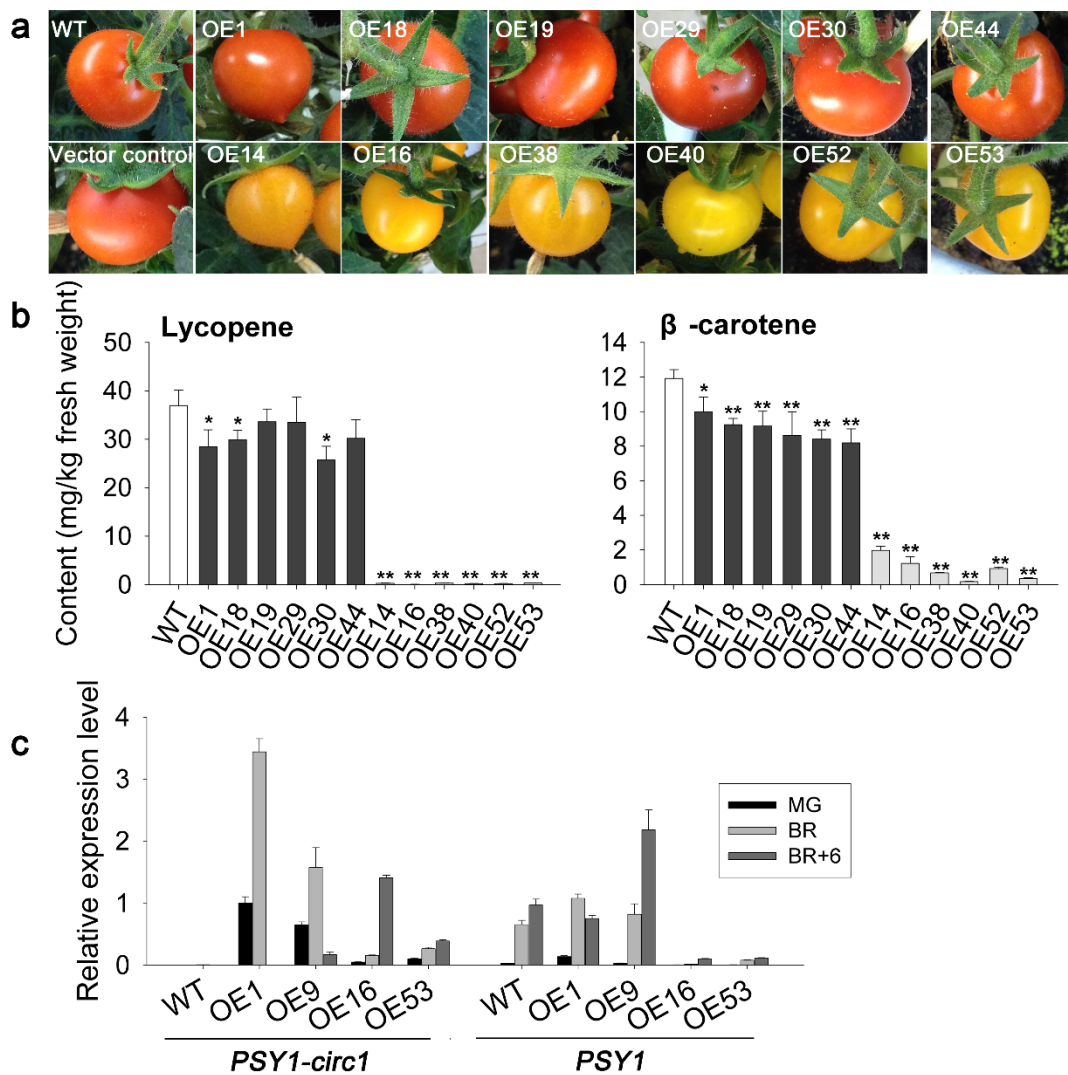
Supplementary Figure 2 Expression profile of a few circRNAs and their parent genes in Ailsa Craig and *Nr* mutant fruits at different ripening stages. The data were normalized to *Actin* and presented as the means \pm SD (n=3).



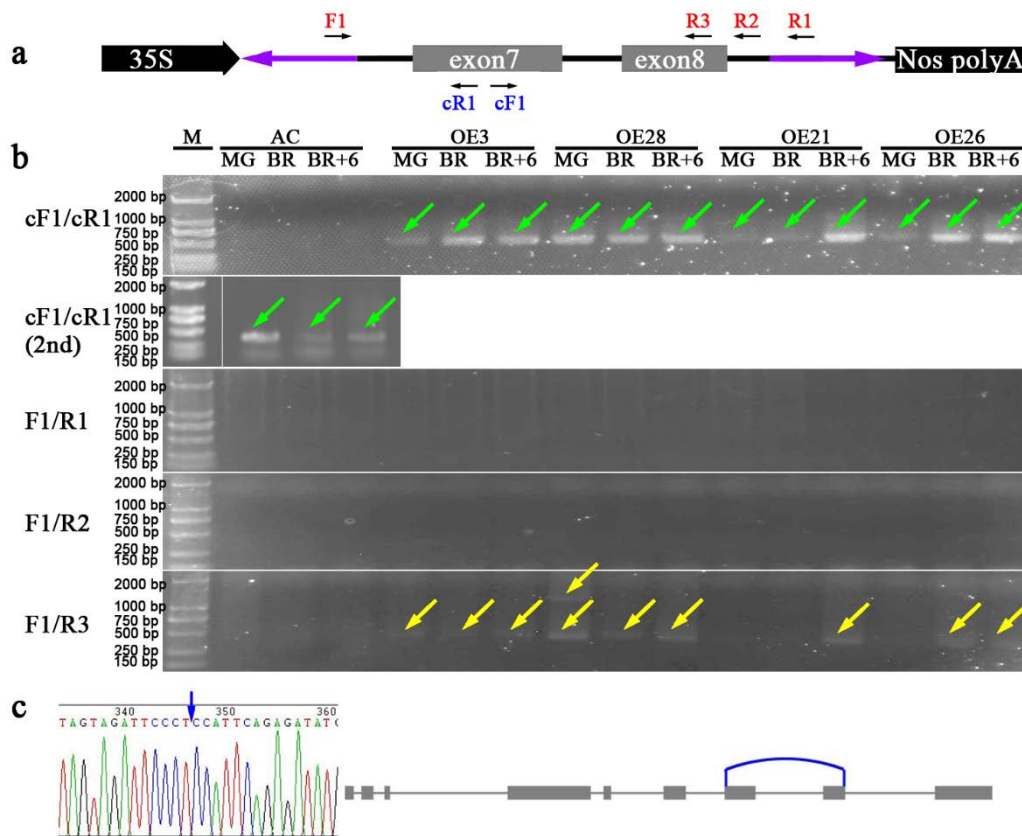
Supplementary Figure 3 Structure and expression profile of canonical circRNAs derived from *PSY1* calculated from RNA-seq data. Blue curves indicate canonical back-splice sites. RPM, reads per million mapped reads.



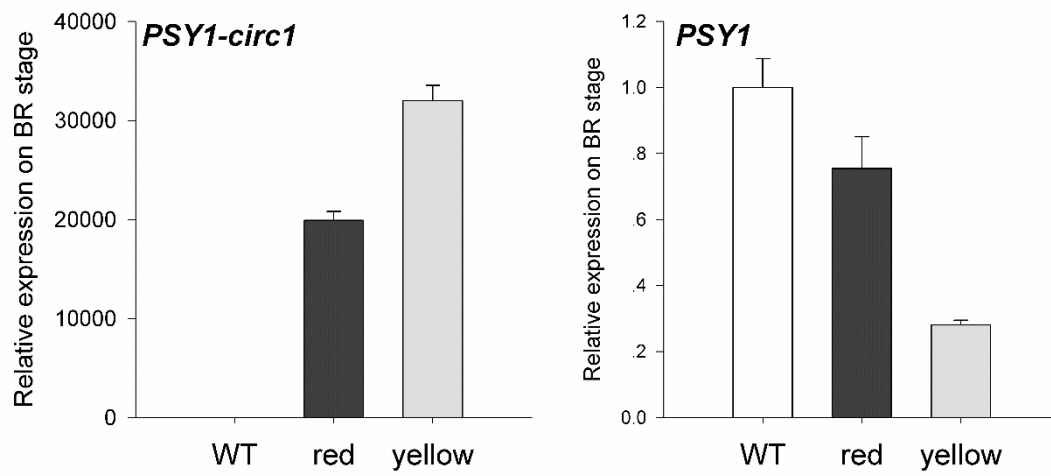
Supplementary Figure 4 Schematic diagram of vector construction for PSY1-circ1 overexpression. An intronic fragment (purple arrow) from the downstream flanking intron of the circularized PSY1-circ1 exons were introduced to the upstream flanking sequence in an opposite orientation, then the tandem fragments were introduced to pCAMBIA1301 vector to replace the *β -Glucuronidase (GUS)* gene, which is under drive of the CaMV 35S promoter.



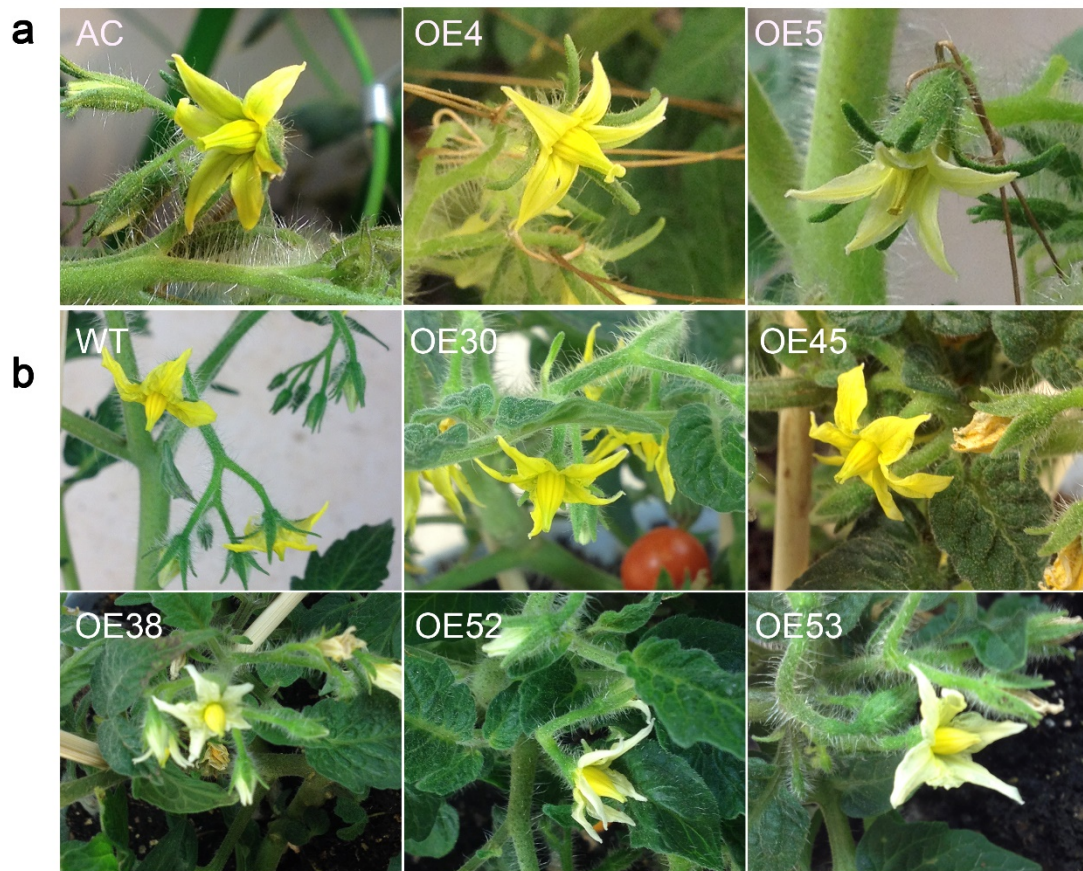
Supplementary Figure 5 Overexpression of PSY1-circ1 in microTom. (a) Fruits of different PSY1-circ1 overexpression tomato lines. WT, wild type. OE1-OE53 stand for transgenic lines. Vector control stands for microTom plants transformed with the empty vector pCAMBIA1301. (b) Lycopene and β -carotene contents in transgenic tomato fruits at breaker+10 stage. White, black and gray bars stand for control, transgenic lines with red fruits and yellow fruits respectively. The data with error bars were expressed as mean \pm SEM (n=4), asterisks indicate statistically significant difference (with respect to control; * p value < 0.05; ** p value < 0.01; two-way ANOVA followed by LSD test). (c) Expression profile of PSY1-circ1 and *PSY1* gene in transgenic tomato fruits at different ripening stages. The data were normalized to *Actin* and presented as means \pm SD (n=5).



Supplementary Figure 6 Identification of RNA isoforms generated by PSY1-circ1 overexpressing vector. (a) Convergent primers F1/R1, F1/R2, and F1/R3 were used for amplification of linear RNAs, and divergent primers cF1/cR1 were used for circular RNA PSY1-circ1 identification. (b) PSY1-circ1 RNAs were successfully amplified in all fruit samples (green arrows), although in Ailsa Craig fruits the PCR products were only visible in the second-round PCR (used ten times diluted 1st PCR products as templates), since the first-round products were too low. Linear RNAs were also detected in some transgenic fruits (yellow arrows), but with lower abundance compared to PSY1-circ1. All PCRs were amplified with 35 cycles, and an equal aliquot of cDNA templates was used among PCRs with different primers. (c) Sanger sequencing of PCR products from (b, cF1/cR1) confirmed the back-splice site and structure of PSY1-circ1. The blue arrow and blue curve indicate the back-splice site.



Supplementary Figure 7 Expression of *PSY1-circ1* and its parent gene *PSY1* in transgenic microTom fruits (T1 generation). White, black and gray bars represent microTom control (WT), transgenic plants (T1 generation from OE1) with red fruits and yellow fruits. The data were normalized to *Actin* and are presented as the means \pm SD (n=3).



Supplementary Figure 8 Flowers of some PSY1-circ1 transgenic plants (Ailsa Craig and microTom). (a) OE4 is a transgenic Ailsa Craig line with yellow petals and red fruits, while OE5 is a transgenic line with white petals and yellow fruits. (b) OE30 and OE45 are transgenic microTom lines with yellow petals and red fruits, while OE38, OE52, and OE53 are transgenic lines with white petals and yellow fruits.

Supplementary Table 1 Putative circular RNAs identified in this study. The putative circRNAs identified by both Segemehl and CIRI were listed.

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SL2.50ch11	50811334	50812180
SL2.50ch12	217442	217915
SL2.50ch12	2065962	2066920
SL2.50ch12	3566900	3567533
SL2.50ch12	10409242	10410399
SL2.50ch12	64401220	64403790
SL2.50ch12	64996487	65000697
SL2.50ch12	65188991	65189273
SL2.50ch12	64171469	64172017
SL2.50ch09	1500972	1503561
SL2.50ch12	65475978	65476430
SL2.50ch08	1276168	1277561
SL2.50ch01	820898	822978
SL2.50ch01	86106056	86106704
SL2.50ch08	25694879	25696508
SL2.50ch08	54212037	54212838
SL2.50ch08	60997789	60999534
SL2.50ch11	5353386	5353797
SL2.50ch10	1307213	1307526

SL2.50ch01	53602896	53604870
SL2.50ch03	66167869	66168990
SL2.50ch06	84413	88510
SL2.50ch06	5405158	5406468
SL2.50ch09	69300335	69300656
SL2.50ch10	57048647	57051100
SL2.50ch11	119413	119977
SL2.50ch11	723254	728197
SL2.50ch11	43321339	43321664
SL2.50ch12	5159090	5159623
SL2.50ch12	40752081	40753680
SL2.50ch01	43301186	43301878
SL2.50ch01	82330208	82334863
SL2.50ch01	90630990	90635761
SL2.50ch02	38332994	38335216
SL2.50ch02	46148680	46149442
SL2.50ch02	54574060	54574355
SL2.50ch03	48957675	48958466
SL2.50ch03	52908503	52909510
SL2.50ch03	69998505	70000400
SL2.50ch05	472149	472586
SL2.50ch05	1098231	1100806
SL2.50ch05	60506583	60509059
SL2.50ch05	63150654	63150977
SL2.50ch06	38408116	38409846
SL2.50ch07	51886032	51886592
SL2.50ch07	51978020	51978765
SL2.50ch07	64404265	64405554
SL2.50ch08	23794785	23795898
SL2.50ch08	51704250	51705225
SL2.50ch08	61380487	61381154
SL2.50ch10	1064902	1065599
SL2.50ch11	6414685	6415189
SL2.50ch11	33341469	33342055
SL2.50ch11	55455170	55455539
SL2.50ch12	3819467	3820138
SL2.50ch12	15390991	15391501
SL2.50ch12	42547078	42548460
SL2.50ch12	66775482	66778458
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SL2.50ch01	1224426	1225750
SL2.50ch01	1291118	1291401
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SL2.50ch01	56475386	56478152
SL2.50ch01	81359434	81360413
SL2.50ch01	93676693	93678517
SL2.50ch01	95189874	95191854
SL2.50ch01	98292433	98294960
SL2.50ch01	98358813	98359417
SL2.50ch02	27234035	27234583
SL2.50ch02	29565234	29573580
SL2.50ch02	41368054	41369164
SL2.50ch02	43284367	43285814
SL2.50ch02	51188457	51190256
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SL2.50ch04	59779836	59780749
SL2.50ch04	60233710	60234096

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SL2.50ch05	63874443	63874933
SL2.50ch06	1760904	1761388
SL2.50ch06	37606914	37607937
SL2.50ch07	976732	977512
SL2.50ch07	7732585	7735844
SL2.50ch07	63869552	63872708
SL2.50ch08	2603215	2606808
SL2.50ch08	61692684	61693435
SL2.50ch08	65682545	65687123
SL2.50ch09	1273900	1274403
SL2.50ch09	1654611	1654980
SL2.50ch09	3824966	3825169
SL2.50ch10	792863	795612
SL2.50ch10	60588123	60588581
SL2.50ch11	5545234	5546917
SL2.50ch11	5964037	5965575
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SL2.50ch11	50612451	50613955
SL2.50ch11	52222851	52223670
SL2.50ch11	55113648	55115281
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SL2.50ch12	37883939	37888582
SL2.50ch12	62109878	62110173
SL2.50ch12	66507278	66512105
SL2.50ch07	59218379	59220089
SL2.50ch05	63418316	63419432
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SL2.50ch02	47800873	47805003
SL2.50ch08	60425246	60425698
SL2.50ch11	30393446	30396689
SL2.50ch01	90619882	90622529
SL2.50ch02	38096019	38097479
SL2.50ch04	16923096	16925145
SL2.50ch06	1805580	1806074
SL2.50ch06	43176125	43177400
SL2.50ch01	82777176	82780246
SL2.50ch01	89729655	89733533
SL2.50ch02	22716424	22718663
SL2.50ch03	64059405	64060002
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SL2.50ch05	10063412	10066958
SL2.50ch05	16810911	16813250
SL2.50ch07	56496311	56496886
SL2.50ch08	55522642	55528313
SL2.50ch10	408375	409635
SL2.50ch10	10862536	10863685
SL2.50ch11	53734462	53744307
SL2.50ch12	37589810	37590042
SL2.50ch12	53519635	53520442
SL2.50ch12	66470736	66471292
SL2.50ch01	35350510	35354615

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SL2.50ch01	88759973	88764323
SL2.50ch01	90528374	90529066
SL2.50ch01	97103363	97105089
SL2.50ch02	1288194	1308248
SL2.50ch02	24613596	24616154
SL2.50ch02	50657274	50659209
SL2.50ch03	13176287	13179618
SL2.50ch03	59664353	59667292
SL2.50ch03	60523573	60527185
SL2.50ch03	62186809	62187576
SL2.50ch03	65355537	65357605
SL2.50ch04	627113	627743
SL2.50ch04	5362849	5363376
SL2.50ch04	5962291	5964041
SL2.50ch04	25067738	25068642
SL2.50ch04	33533730	33538568
SL2.50ch04	41770520	41772196
SL2.50ch04	55653007	55654852
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SL2.50ch05	19101465	19105008
SL2.50ch05	63350049	63350677
SL2.50ch06	2044518	2047758
SL2.50ch06	32768836	32770193
SL2.50ch06	43791629	43792446
SL2.50ch07	1268573	1269134
SL2.50ch07	2441518	2441759
SL2.50ch07	10375803	10376859
SL2.50ch07	64249019	64250668
SL2.50ch08	25491703	25492378
SL2.50ch08	55522642	55526071
SL2.50ch08	61288390	61289042
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SL2.50ch09	3556710	3569693
SL2.50ch09	64197764	64198629
SL2.50ch09	70260435	70265364
SL2.50ch10	92386	93935
SL2.50ch10	208225	208693
SL2.50ch10	58624104	58625733
SL2.50ch11	3356043	3356413
SL2.50ch11	7831727	7832592
SL2.50ch11	55193423	55194347
SL2.50ch12	3472364	3473572
SL2.50ch12	37739240	37739821
SL2.50ch01	80715471	80721707
SL2.50ch01	90605614	90607989
SL2.50ch09	67723512	67724089
SL2.50ch11	55524913	55525902
SL2.50ch11	53736751	53741379
SL2.50ch01	90630990	90633168
SL2.50ch09	72086110	72089608
SL2.50ch01	90947891	90950791
SL2.50ch04	55751401	55752727
SL2.50ch07	977078	977512
SL2.50ch07	10523474	10525759
SL2.50ch08	64142078	64143336
SL2.50ch09	1500972	1503099
SL2.50ch09	33689613	33691770

SL2.50ch11	41541444	41543579
SL2.50ch11	49247210	49248279
SL2.50ch01	1695810	1697212
SL2.50ch03	4328007	4328953
SL2.50ch03	61828193	61829642
SL2.50ch03	69945580	69948922
SL2.50ch07	59014613	59015192
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SL2.50ch08	58606436	58607082
SL2.50ch09	62488802	62490152
SL2.50ch10	11326837	11327338
SL2.50ch12	4533376	4533872
SL2.50ch01	13177	13661
SL2.50ch01	56472387	56475756
SL2.50ch01	80404610	80411443
SL2.50ch01	96699865	96700805
SL2.50ch02	25127863	25128299
SL2.50ch02	53580399	53581188
SL2.50ch03	52969572	52972893
SL2.50ch03	68944514	68945013
SL2.50ch04	62793474	62797977
SL2.50ch05	1387522	1388010
SL2.50ch05	6708895	6711557
SL2.50ch05	60344954	60349570
SL2.50ch06	40776441	40779461
SL2.50ch07	56332486	56335535
SL2.50ch08	6990847	6991816
SL2.50ch10	63788551	63789351
SL2.50ch11	36507684	36510824
SL2.50ch12	2352797	2354696
SL2.50ch12	66578393	66578819
SL2.50ch01	81898868	81899402
SL2.50ch01	84289805	84290540
SL2.50ch01	87477286	87482023
SL2.50ch01	92232690	92235858
SL2.50ch02	44770475	44772818
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SL2.50ch04	65620535	65625927
SL2.50ch05	46228872	46234509
SL2.50ch06	71344	73717
SL2.50ch06	31181398	31183175
SL2.50ch06	48983142	48984276
SL2.50ch07	4200218	4201053
SL2.50ch07	21389876	21391076
SL2.50ch08	1472887	1480923
SL2.50ch08	55522642	55525092
SL2.50ch09	2755542	2762584
SL2.50ch09	31893586	31896198
SL2.50ch09	31896266	31896797
SL2.50ch11	10033364	10034334
SL2.50ch12	1611519	1615179
SL2.50ch12	1620849	1624951
SL2.50ch12	29110650	29111090
SL2.50ch12	66108134	66110253
SL2.50ch01	1122379	1122719
SL2.50ch01	13979184	13979678
SL2.50ch01	54560020	54568676
SL2.50ch01	88125455	88126083

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SL2.50ch02	44817076	44817429
SL2.50ch02	46850433	46852137
SL2.50ch03	3009332	3010131
SL2.50ch03	8201277	8203496
SL2.50ch03	55183863	55197548
SL2.50ch03	62197202	62199662
SL2.50ch03	65242618	65243396
SL2.50ch03	66803073	66803827
SL2.50ch03	69770063	69773085
SL2.50ch03	70637392	70641648
SL2.50ch04	2264722	2270861
SL2.50ch04	18195180	18195633
SL2.50ch04	45321116	45322667
SL2.50ch04	60108702	60109162
SL2.50ch05	5225922	5227056
SL2.50ch05	65691748	65693428
SL2.50ch06	34134687	34135147
SL2.50ch06	42528895	42538395
SL2.50ch06	43174977	43177400
SL2.50ch06	43254780	43256242
SL2.50ch06	43638262	43641934
SL2.50ch06	48027396	48028196
SL2.50ch07	3730434	3737549
SL2.50ch07	6949732	6950824
SL2.50ch07	7668400	7669088
SL2.50ch07	21208786	21210064
SL2.50ch07	65842911	65843684
SL2.50ch08	54720018	54720661
SL2.50ch08	58407097	58409623
SL2.50ch09	5793547	5793920
SL2.50ch09	22837104	22837779
SL2.50ch09	31113070	31127995
SL2.50ch09	40063351	40066414
SL2.50ch09	48789677	48790242
SL2.50ch09	67151648	67155803
SL2.50ch10	890903	892387
SL2.50ch10	3051159	3051822
SL2.50ch10	18504927	18515676
SL2.50ch11	6614010	6614625
SL2.50ch11	25025604	25026201
SL2.50ch11	52805479	52805944
SL2.50ch12	61932127	61933038
SL2.50ch12	62055340	62055807
SL2.50ch12	64996487	65000252
SL2.50ch12	66004439	66005898
SL2.50ch12	66212221	66216669
SL2.50ch01	83846390	83847922
SL2.50ch01	90605614	90612102
SL2.50ch03	30990	32428
SL2.50ch08	46339680	46340152
SL2.50ch04	6532541	6534728
SL2.50ch12	66127788	66128276
SL2.50ch01	94484383	94487046
SL2.50ch02	49623047	49623805
SL2.50ch04	60065839	60066532

SL2.50ch07	68007493	68010577
SL2.50ch12	65128136	65129767
SL2.50ch01	89492725	89493458
SL2.50ch01	89730975	89733533
SL2.50ch06	36729690	36734553
SL2.50ch08	60432213	60433729
SL2.50ch09	69299833	69300656
SL2.50ch10	46300785	46302324
SL2.50ch01	673286	676121
SL2.50ch01	81306099	81308949
SL2.50ch02	43124517	43125172
SL2.50ch02	49502888	49503487
SL2.50ch03	68944597	68945013
SL2.50ch07	10523453	10527102
SL2.50ch08	58926211	58927066
SL2.50ch09	67135125	67136390
SL2.50ch12	10201277	10203029
SL2.50ch01	82651832	82654137
SL2.50ch01	87427188	87427482
SL2.50ch01	88165816	88166171
SL2.50ch02	23574028	23583508
SL2.50ch03	976173	979129
SL2.50ch03	24749210	24749624
SL2.50ch03	64453761	64456536
SL2.50ch03	70459987	70461341
SL2.50ch04	358448	358951
SL2.50ch04	65406403	65406865
SL2.50ch05	2577529	2579535
SL2.50ch05	58563460	58564809
SL2.50ch07	21578418	21593436
SL2.50ch07	67546087	67547159
SL2.50ch08	25829679	25831964
SL2.50ch08	64305831	64306523
SL2.50ch10	1889965	1891454
SL2.50ch10	14126858	14128072
SL2.50ch10	58507010	58507991
SL2.50ch11	49473872	49476376
SL2.50ch12	48120404	48122121
SL2.50ch01	75014690	75015790
SL2.50ch01	75465377	75468823
SL2.50ch01	81659771	81661855
SL2.50ch01	90949911	90950791
SL2.50ch01	93092828	93094156
SL2.50ch02	1284294	1289542
SL2.50ch02	51010954	51012186
SL2.50ch03	2927940	2932099
SL2.50ch03	4967203	4969713
SL2.50ch03	56799637	56801379
SL2.50ch04	2939441	2942441
SL2.50ch04	7376097	7380752
SL2.50ch04	29416785	29420401
SL2.50ch04	41784452	41785026
SL2.50ch06	76764	80638
SL2.50ch06	45900772	45902417
SL2.50ch07	61913265	61919613
SL2.50ch08	64146684	64148175
SL2.50ch10	19704196	19706313
SL2.50ch10	49988106	49993179

SL2.50ch10	58658191	58659219
SL2.50ch11	5534827	5535370
SL2.50ch11	7981451	7983985
SL2.50ch11	33971742	33972203
SL2.50ch11	52742483	52744792
SL2.50ch12	3437518	3437863
SL2.50ch12	26607299	26611856
SL2.50ch12	61691393	61691682
SL2.50ch01	3098317	3098878
SL2.50ch01	55928143	55931501
SL2.50ch01	72325238	72325995
SL2.50ch01	76871263	76871888
SL2.50ch01	89821301	89826056
SL2.50ch01	92249361	92253835
SL2.50ch01	92872760	92881110
SL2.50ch02	22862344	22864338
SL2.50ch02	52183957	52188681
SL2.50ch03	3511408	3516225
SL2.50ch03	53126735	53127804
SL2.50ch03	69952270	69953293
SL2.50ch04	209926	210329
SL2.50ch04	1208879	1214277
SL2.50ch05	534133	534597
SL2.50ch05	10082545	10089432
SL2.50ch06	15696210	15697299
SL2.50ch06	37606914	37607781
SL2.50ch06	40863584	40864081
SL2.50ch06	45008998	45009354
SL2.50ch06	46282005	46282353
SL2.50ch06	49661667	49666403
SL2.50ch07	279900	286050
SL2.50ch07	2482147	2491155
SL2.50ch07	7408758	7411930
SL2.50ch07	64111958	64112701
SL2.50ch08	1583168	1584778
SL2.50ch08	2379362	2381616
SL2.50ch10	468727	469595
SL2.50ch10	1081541	1086146
SL2.50ch10	2411566	2413051
SL2.50ch10	40326425	40327209
SL2.50ch10	58609556	58611315
SL2.50ch10	61947171	61948408
SL2.50ch11	8422662	8422939
SL2.50ch11	53734462	53741997
SL2.50ch12	29122638	29127812
SL2.50ch12	47338038	47338869

Supplementary Table 2 Molecularly identified circRNAs in Ailsa Craig fruits

Gene name	Circle length (bp)	Identical to bioinformatic prediction	Splicing pattern	Cyclized exons*	circRNA name
<i>ETR1</i>	962	Yes	Canonical	exon 3-4-5-6	
	766	No	Non-canonical	exon 3a-4-5-6a	
<i>ETR3</i>	764	Yes	Canonical	exon 5-6-7	
	594	No	Non-canonical	exon 5a-6-7a	
	636	No	Canonical	exon 5-6	
<i>ETR4</i>	544	No	Non-canonical	exon 2a-3a	ETR4-circ1
	199	No	Non-canonical	exon 2b-3b	ETR4-circ2
<i>EIL3</i>	481	No	Non-canonical	exon 2a-2a	
	240	No	Non-canonical	exon 2b-2b	
	433	No	Non-canonical	exon 2c-2c	
<i>JERF1</i>	619	No	Non-canonical	exon 1a-2a	
	449	No	Non-canonical	exon 1b-2b	
	340	No	Non-canonical	exon 1c-2c	
	353	No	Non-canonical	exon 1d-2d	
<i>ACO6</i>	652	No	Non-canonical	exon 2a-3-4a	ACO6-circ1
	531	No	Non-canonical	exon 1a-2-3a	ACO6-circ2
	401	No	Non-canonical	exon 2b-3b	ACO6-circ3
	311	No	Non-canonical	exon 2c-3c	ACO6-circ4
<i>CNR</i>	90	No	Non-canonical	exon 3a-3a	
	69	No	Non-canonical	exon 3b-3b	
	281	No	Non-canonical	exon 2a-3c	
<i>NAC3</i>	314	No	Non-canonical	exon 2a-3a	
<i>SPL1</i>	644	No	Canonical	exon 2a-3	
	736	No	Canonical	exon 2-3	
	858	Yes	Canonical	exon 3-4-5-6	
<i>SPL12</i>	1146	Yes	Canonical	exon 2-3-4	SPL12-circ1
	882	No	Canonical	exon 2-3	SPL12-circ2
<i>PSY1</i>	316	No	Non-canonical	exon 8a-9a	
	227	No	Non-canonical	exon 8b-9b	
	270	No	Non-canonical	exon 7a-8-9c	
	316	No	Non-canonical	exon 8c-9d	
	429	Yes	Canonical	exon 7-8	PSY1-circ1
	405	No	Non-canonical	exon 6a-7-8d	
	510	No	Non-canonical	exon 5a-6-7-8e	
<i>PDS</i>	406	No	Non-canonical	exon 7a-8-9-10a	
	600	No	Non-canonical	exon 6a-7-8-9-10b	
	309	No	Non-canonical	exon 7b-8-9a	
	545	No	Non-canonical	exon 6b-7-8-9-10c	
	996	No	Non-canonical	exon 6-7-8-9-10-11-12-13-14a	
	645	No	Non-canonical	exon 6c-7-8-9-10d	
	571	No	Non-canonical	exon 6-7-8-9-10e	
	515	No	Non-canonical	exon 6d-7-8-9-10f	
	763	No	Non-canonical	exon 4a-5-6-7-8-9-10g	
483	Yes	Canonical	exon 6-7-8-9	PDS-circ1	
<i>HP2</i>	468	Yes	Canonical	exon 5-6-7-8-9	HP2-circ1
	342	No	Canonical	exon 7-8-9	HP2-circ2
	463	Yes	Canonical	exon 5-6a-7-8-9	HP2-circ3
	536	Yes	Canonical	exon 5-6-7a-8-9	HP2-circ4
<i>ZISO</i>	357	Yes	Canonical	exon 2-3	ZISO-circ1
	280	Yes	Canonical	exon 2a-3	ZISO-circ2

<i>HP1</i>	387	No	Non-canonical	exon 18a-18a
<i>CCD1</i>	249	No	Non-canonical	exon 12a-13-14a
	440	No	Non-canonical	exon 12b-13-14b

*: Lowercase a-g indicates alternative splicing sites

Supplementary Table 3 Circular RNAs derived from PSY1 gene

Samples	Type	Name	Back-splice site	POS1	POS2	INF	RPM
ACMG-1	canonical	PSY1-circ2	GGGGTAATATATGATGCGTTTTG GGCCATCAACAAGTTCATCTGT TCTTCTGCACCATAAGGGAATC TACTAGCTGAGCTCAATTCTGT CACGCCTTTCTCT	4327521	4328953	splits:2:10:2 :C:P	0.051
ACMG-2	canonical	PSY1-circ2	TGGGCCATCAACAAGTTCATCT GTTCTTCTGCACCATAAGGGAA TCTACTAGCTGAGCTCAATTCT GTCACGCCTTTCTCTGCCTCATC AAAGAACTTTCT	4327521	4328953	splits:1:1:1: C:P	0.024
ACBR-1	non-canonical	PSY1-circ4	CGTACCAGCAACATAATAACAA TAAAGGTATAGTTCGTCGAAGT TTTTAAGTTGTTGTAGTCATTGG CTTCAATCTCATCTAGTATTTTG CGGTACAAGAC AGAAAAGGCGTGACAGAATTGA GCTCAGCTAGTAGATTCCCTTA TGGTGCAGAAGAACAGATGAA CTTGTTGATGGCCAAACGCAT CATATATTACCCCGG AGTTCCTTGATGAGGCAGAGGA AGGCGTGACAGAATTGAGCTC AGCTAGTAGATTCCTCCATTC AGAGATATGATTGAAGGAATGC GTATGGACTTGAGA	4328061	4329710	splits:2:2:2: C:P	0.046
	canonical	PSY1-circ2		4327521	4328953	splits:1:31:1 2:C:P	0.023
	canonical	PSY1-circ1		4328007	4328953	splits:1:22:1 2:C:P	0.023
ACBR-2	canonical	PSY1-circ2	CCACCTATCTAAGGCTGCCGGG GTAATATATGATGCGTTTGGGC CATCAACAAGTTCATCTGTTCTT CTGCACCATAAGGGAATCTACT AGCTGAGCTCAA TATGAAGAAACAAATACATAGG GCAAGAAAGTTCTTTGGCCAT TTGACATGCTCGATGGTGCTTT GTCCGATACAGTTTCTAACTTTC CAGTTGATATTC TACTGAGAACGACAAGCTCAAT ACAAGGTTATTTTGCCAGAATA GTAGACAAAGGTGATAAATAA GAGAACTAAAGATACGAGG TATGGGATATTGTAA	4327521	4328953	splits:2:33:1 2:C:P	0.05
	non-canonical	PSY1-circ5		4327629	4328903	splits:1:1:1: C:P	0.025
	ciRNA			4329384	4329616	splits:1:1:1: C:P	0.025
	canonical	PSY1-circ2	GGGCCATCAACAAGTTCATCTG TTCTTCTGCACCATAAGGGAAT CTACTAGCTGAGCTCAATTCTG TCACGCCTTTCTCTGCCTCATCA AAGAACTTTCTT CAGAATTGAGCTCAGCTAGTAG ATTCCCTCCATTGAGAGATATG ATTGAAGGAATGCGTATGGACT TGAGAAAATCGAGATACAAAA ACTTCGACGAACTA ATATTACCCCGGCAGCCTTAGA TAGGTGGGAAAATAGGCTAGA AGATGTTTTCAATGGGCGGCCA TTTGACATGCTCTACCTATTCCG GGGAATTTGGGCT CACTCATCAACCCAACCGTACC AGCAACATAATAACAATAAAGG ACAAGAGATTTTGCATATGCAA TAGGTAATGCAATCAACTTCTTT GATTTGCTCACA	4327521	4328953	splits:10:11 6:33:C:P	0.248
	canonical	PSY1-circ1		4328007	4328953	splits:5:76:3 3:C:P	0.124
	non-canonical	PSY1-circ6		4326837	4327645	splits:2:2:2: C:P	0.05
	non-canonical	PSY1-circ7		4328082	4329784	splits:2:2:3: C:P	0.05

ACRed-1			GTTTCTTCATAAAGATTCTCCAT TTATCGGTCACCCCTTCCAGCAA ATATATCTTCATCGGATAGACCT GCCTCATCAAAGAACTTTCTTG CCCTATGTATT	4328811	4328912	splits:2:2:2: C:P	0.05
non- canonical	PSY1-circ8		GCCATCAACAAGTTCATCTGTT CTTCTGCACCATATCTTCTCCAA CATCTCTGAGTATGTTAGTTAAT TGATTTGCGATCCCCAGAGCCA AAGCAGCATTAA	4327521	4328242	splits:1:116: 83:C:P	0.025
canonical	PSY1-circ3		ACGAACTATACCTTTATTGTTAT TATGTTGCTGGTACGGTTGGGT TGATGAGTGTCCAATTATGGG TATCGCCCCTGAAATAGGCTAG AAGATGTTTTCA	4327600	4328148	splits:1:1:1: C:P	0.025
non- canonical	PSY1-circ9		AGCTCATTTTCTATATTTTGAT ATGTAATATAGCAAAAAAAT TATGTGCGTAAAAACACAGCTTT GTACTTACAAAGAACAACGGA AGCTAAATACGG	4328244	4328740	splits:1:1:1: C:P	0.025
ciRNA							

canonical	PSY1-circ2		GAAAGGCGTGACAGAATTGAG CTCAGCTAGTAGATTCCTTAT GGTGCAGAAGAACAGATGAAC TTGTTGATGGCCCAAACGCATC ATATATTACCCCGGC	4327521	4328953	splits:6:121: 20:C:P	0.151
non- canonical	PSY1-circ10		ATTGAAGCCAATGACTACAACA ACTTCACAAAGAGAGCATATGT GAGCAAATCAAAGAAGTCTACT TGCTCAAGATGAATTAGCACA GGCAGGTCTATCC	4328776	4329742	splits:4:4:4: C:P	0.101
non- canonical	PSY1-circ11		CCACTCTCAAACAAAATTACCC ACTTTCTTTTTGTACCTTCCAA ACTTTACTTAGTTTATAGAAAAT AAATTACTCCTAGTAGATTCCC ACTAGAGAATA	4326363	4326466	splits:2:2:2: C:P	0.05
non- canonical	PSY1-circ12		AAGACGTTTAACTTAGGAACTA TGCTAATGACTCCCGAGAGAAG AAGGGCTATCTGGGCAATATAT GTATGGTGCAGAAGGCAGCCTT GGTGAAGAGGCAA	4326775	4327097	splits:2:2:11 7:C:P	0.05
non- canonical	PSY1-circ13		CCGATAAATGGAGAATCTTTAT GAAAGGGCTATCTGGGCAATAT ATGTATGGTGCAGAAGAACAG ATGAACTTGTTGATGGCCCAA CGCATCATATATTA	4327075	4328870	splits:2:2:2: C:P	0.05
non- canonical	PSY1-circ14		ACCCAACCGTACCAGCAACATA ATAACAATAAAGGTATCTCAAT TCTGTACGCCTTTCTCTGCCTC ATCAAAGAACTTTCTTGCCCTAT GTATTTGTTTC	4328078	4328934	splits:1:1:1: C:P	0.025
ACRed-2							

Supplementary Table 4 Fruit color of PSY1-circ1 transgenic tomatoes (Ailsa Craig and microTom)

a. Fruit color of *PSY1-circ1* overexpression lines (AC, T0 generation)

Fruit color	OE lines	Total
Yellow	OE5, OE8, OE11, OE19, OE21, OE23, OE26	7
Red	OE3, OE4, OE7, OE12, OE13, OE14, OE15, OE28	8

b. Fruit color of *PSY1-circ1* overexpression lines (microTom, T0 generation)

Fruit color	OE lines	Total
Yellow	OE14, OE16, OE38, OE40, OE52, OE53	6
Red	OE1, OE9, OE11, OE15, OE17, OE18, OE19, OE22, OE23, OE24, OE27, OE28, OE29, OE30, OE35, OE36, OE41, OE43, OE44, OE45, OE50, OE51, OE55, OE56	24

c. Fruit color of *PSY1-circ1* overexpression lines (microTom, hygromycin-resistant T1 generation plants)

	OE lines	Yellow progenies	Red progenies
Yellow lines	OE16	12	0
	OE38	10	3
	OE53	8	0
Red lines	OE1	9	18
	OE9	0	7
	OE11	4	2
	OE18	0	15
	OE44	0	11

Supplementary Table 5 Primers used in this study

Name	Sequence (5'-3')	Annotation
PSY1-cF1	GTTGGAGAAGATGCCAGAAG	Divergent primers for <i>PSY1-circ1</i>
PSY1-cR1	CCATACGCATTCTTCAATC	
PSY1-IF1	TTGGTTTGCCTGTCTGTG	Convergent primers for <i>PSY1</i>
PSY1-IF2	TGTATCGGACAAAGCACC	
PDS-cF1	CTATGTCAAAGGCACTCAACT	Divergent primers for <i>PDS-circ1</i>
PDS-cR1	CCTAATTCTCCAAACAGGTTCT	
PDS-IF1	GACAGGGTGACAGATGAGGT	Convergent primers for <i>PDS</i>
PDS-IF2	CACAAAAGCATCTCCCTCG	
ZISO-cF1	TATGAGGATTACCAGGCATC	Divergent primers for <i>ZISO-circ1~2</i>
ZISO-cR1	CCATAACTGCACTCCATCGT	
ZISO-IF1	AAATGCCACCAAAGAGC	Convergent primers for <i>ZISO</i>
ZISO-IR1	GTCAACAGCCGCTACCTC	
HP2-cF1	GATGGCTTCCTTGCCTTGT	Divergent primers for <i>HP2-circ1~4</i>
HP2-cR1	GGCGTGGATGTTGTTTGAG	
HP2-IF1	TTGGCAGTGTTGATGGTG	Convergent primers for <i>HP2</i>
HP2-IR1	GTTTGGAGGTGCTACGAAG	
PSY1-div-F	TCAGCTAGTAGATCCCTCCATTC	Divergent primers for <i>PSY1-circ1</i> (for Real-time PCR)
PSY1-div-R	CCTTTGATTTCAGGGGCGATAC	
PSY1-con-F	GGACAAGTTTCATGGAATCAGT	Convergent primers for <i>PSY1</i> (for Real-time PCR)
PSY1-con-R	TGGAGTAGCCAAAATAGCAGAC	
PDS-div-F	TGCTATGTCAAAGGCACTCAAC	Divergent primers for <i>PDS-circ1</i> (for Real-time PCR)
PDS-div-R	CATGCAGCTACCTGAAGAAACC	
PDS-con-F	GCCGGTGACTACACGAAACAG	Convergent primers for <i>PDS</i> (for Real-time PCR)
PDS-con-R	ACGCTTGCTTCCGACAACCTC	
HP2/1-div-F	CAGGTTTGACGAGAAGAGAGTGC	Divergent primers for <i>HP2-circ1,3,4</i> (for Real-time PCR)
HP2/1-div-R	TCAACAGGTGATGTCGGTCC	
HP2/2-div-F	TGATGGCTTCCTTGCCTTGT	Divergent primers for <i>HP2-circ2</i> (for Real-time PCR)
HP2/2-div-R	CAGCATTTCCGGATACCTTCTC	
HP2-con-F	AGCTATTGACCGCCATAGACAG	Convergent primers for <i>HP2</i> (for Real-time PCR)
HP2-con-R	GGGTGGAAGAGGAAGGAACA	
ETR4-cF1	CAAGGAGACATCTGGGTAATC	Divergent primers for <i>ETR4-circ1~2</i>
ETR4-cR1	GCACACAGTGAAACTCAAGC	
ETR4-IF1	TGAGCAGCGGCTTCTTGT	Convergent primers for <i>ETR4</i>
ETR4-IR1	GCACACAGTGAAACTCAAGC	
ACO6-cF1	CCAAAGTTAGCAACTACCCACC	Divergent primers for <i>ACO6-circ1~4</i>
ACO6-cR1	CTTGCCACCATTCCTTG	
ACO6-IF1	ATGGTGGCAAGTAAAGGC	Convergent primers for <i>ACO6</i>
ACO6-IR1	AGTAGGACCCTTTGAGCC	
ZISO-div-F	ACTAGAGGTAGCGGCTGTTGAC	Divergent primers for

ZISO-div-R	GGGAAAGCATTACAATCTGTGG	<i>ZISO-circ1~2</i> (for Real-time PCR)
ZISO-con-F	ATGGGGACCGGAGGTTAGC	Convergent primers for <i>ZISO</i> (for Real-time PCR)
ZISO-con-R	GCATAATGGGGTGGAGGAAGTA	
SPL12-cF1	GGAAATGGGAGTGTCAAAG	Divergent primers for <i>SPL12-circ1~2</i>
SPL12-cR1	CAGCCAATGTATGGTAACGAG	
SPL12-IF1	AGCAGGTTCCATAGCGTGTC	Convergent primers for <i>SPL12</i>
SPL12-IR1	CAGCCAATGTATGGTAACGAG	
Actin-con-F	GTCCTCTTCCAGCCATCCAT	Convergent primers for <i>actin</i> (for Real-time PCR)
Actin-con-R	ACCACTGAGCACAATGTTACCG	
F1	ATTTCTTGGTTGGTAGGGTG	Primers used for identification of circRNA and linear RNAs in PSY1-circ1 overexpression plants.
R1	TGCGAGGTTCAATTTCTTGGT	
R2	ATCGACCTAGAAATACTCAACAC	
R3	CTGCCTGTGCTAATTCATCTTG	
cF1	GTTGGGTTGATGAGTGTTC	
cR1	CAAGTCCATACGCATTCC	
PSY1-circ-v-IF	CATTTCTTGGTTGGTAGGGTG	Primers used for identification of linear RNAs generated from PSY1-circ1 overexpression vector. (for Real-time PCR)
PSY1-circ-v-IR	CTACAAACATTTCCGCTAGGCTG	
PDS-circ-v-IF	GAACTTATGGACAACCCAATCAC	Primers used for identification of linear RNAs generated from PDS-circ1 overexpression vector. (for Real-time PCR)
PDS-circ-v-IR	CGTAGAATCTACCAGAAAAGACAGC	