miR156 switches on vegetative phase change under the regulation of redox signals in apple seedlings

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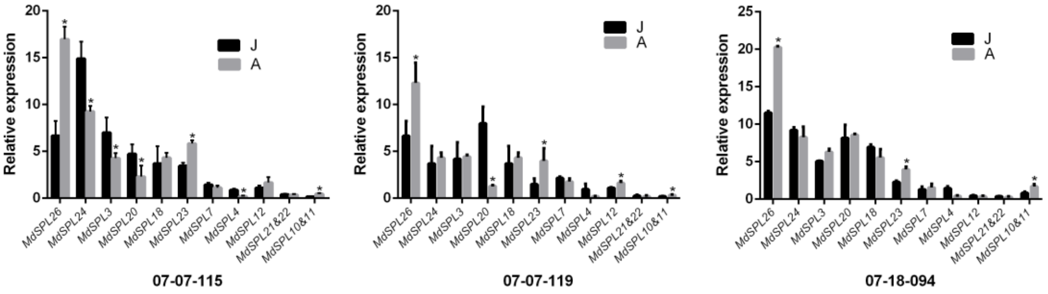
SUPPLEMENTARY DATA



07-07-115

07-07-119

07-18-094

Fig S1. Relative expression of *MdSPLs* in the juvenile (J) and adult (A) phase of three individuals of *Malus asiatica* ‘Zisai Pearl’ × *M. domestica* ‘Red Fuji’. Error bars represent the SD of three biological replicates. Asterisk represents $p < 0.05$ (Duncan’s multiple-range test).

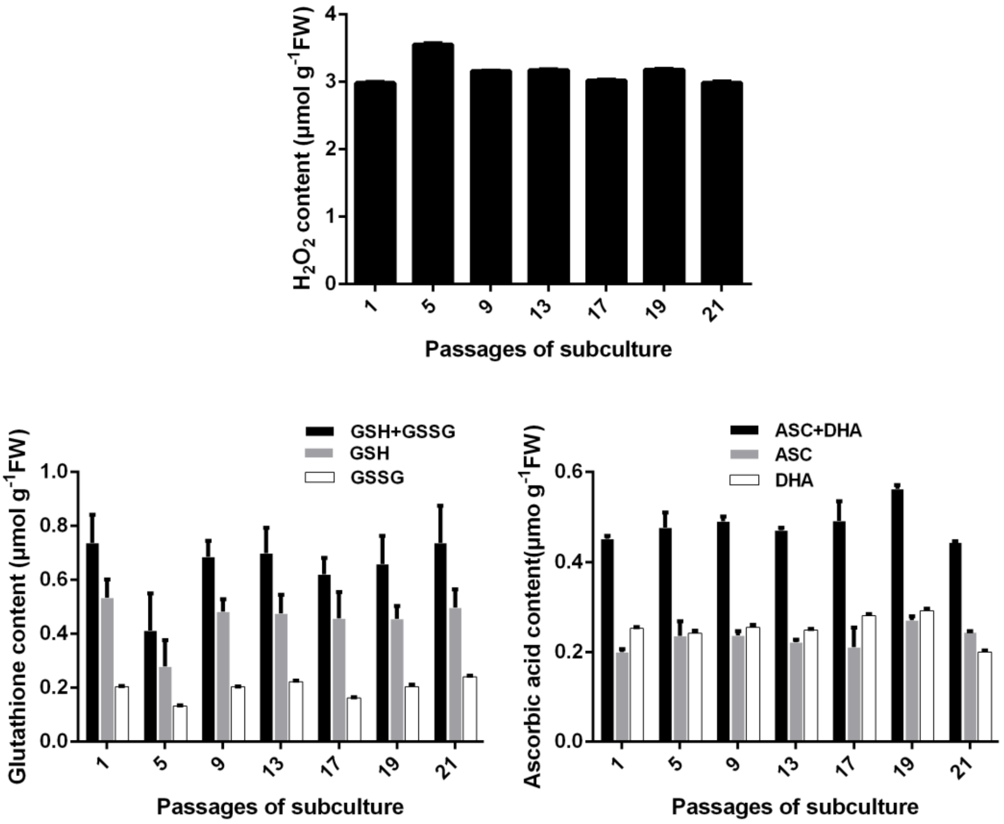


Figure S2. Hydrogen peroxide (H_2O_2), glutathione (GSH), glutathione disulfide (GSSG), ascorbic acid (ASC) and dehydroascorbate (DHA) concentrations in suspension cells of apple '*Orin*' leaf during successive subculture cycles.

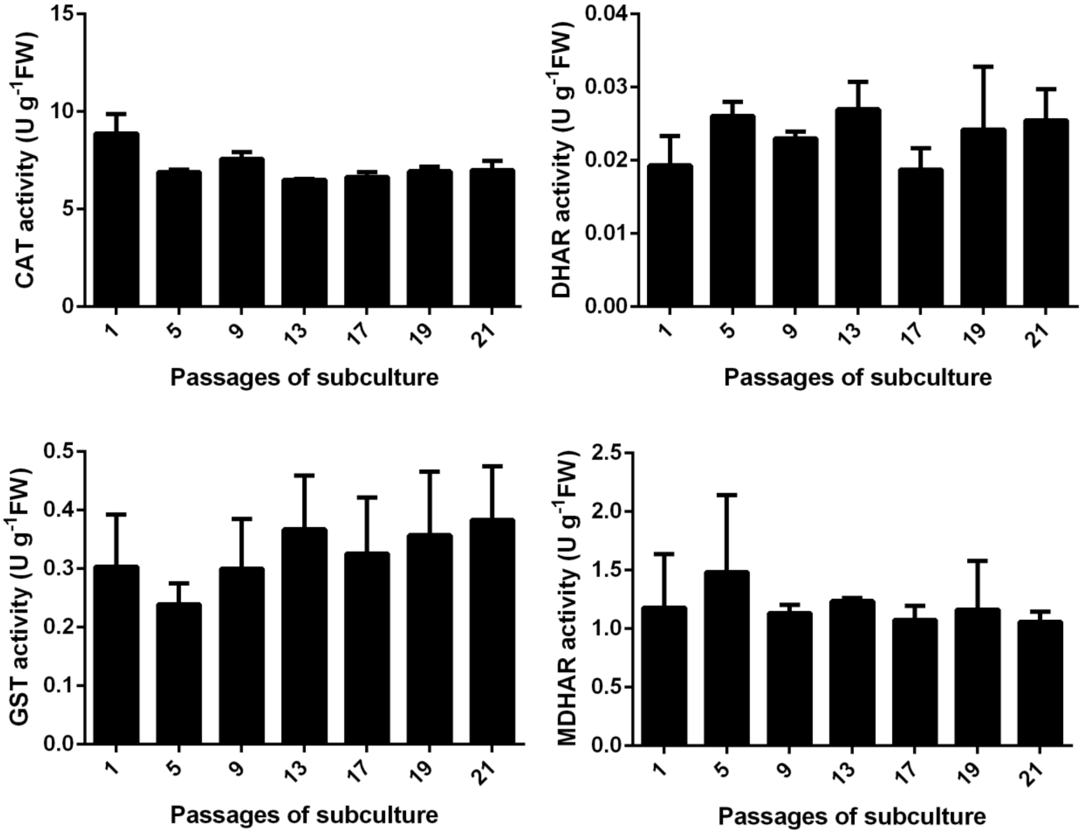


Figure S3. Activity of catalase (CAT), dehydroascorbate reductase (DHAR), glutathione *S*-transferase (GST) and monodehydroascorbate reductase (MDHAR) in suspension cells of apple ‘*Orin*’ leaf during successive subculture cycles.

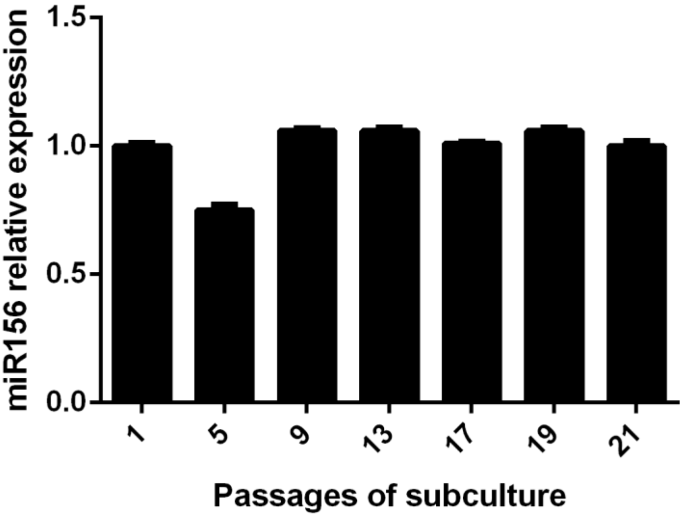


Figure S4. Relative expression of miR156 in suspension cells of apple '*Orin*' leaf during successive subculture cycles.

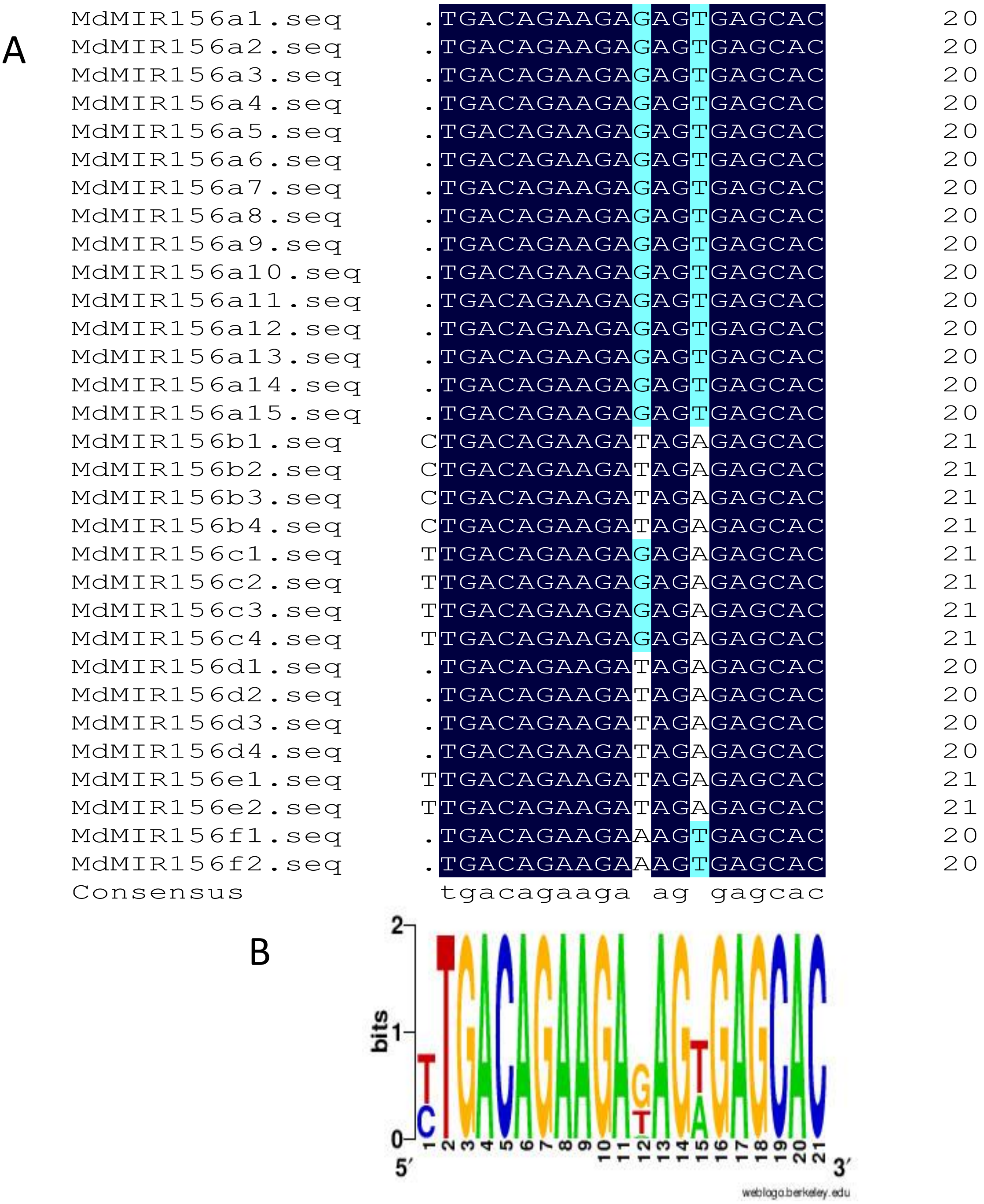


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Figure S5. A. Multiple alignment of the MdMIR156s obtained with DNAMAN software. B. Sequence logo of the MdMIR156s. The height of the letter at each position represents the degree of conservation.

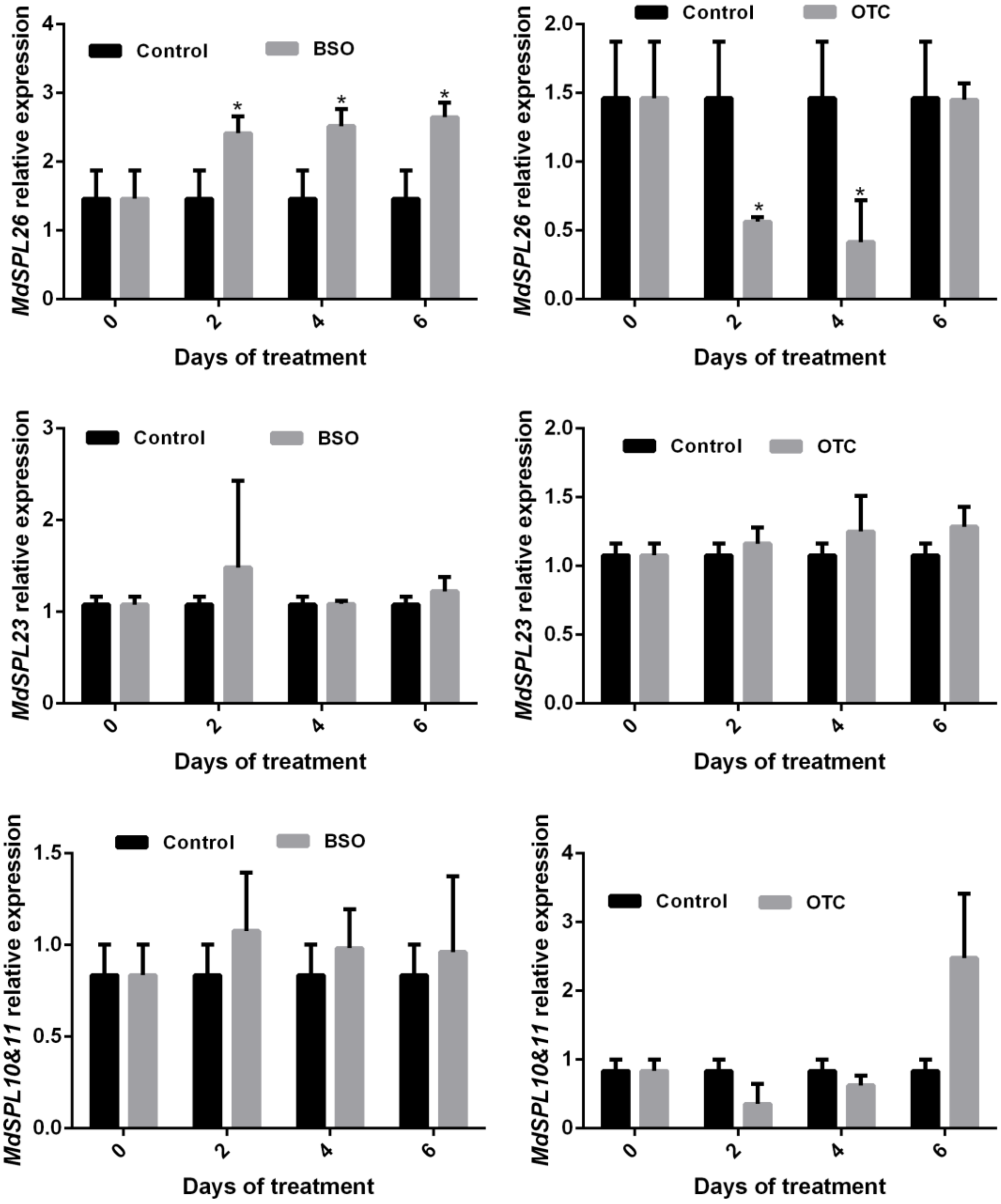

Figure S6. Relative expression of *MdSPL26, MdSPL23* and *MdSPL10&11* of suspension cells of apple '*Orin*' leaf treated with redox-modulating chemicals. The suspension cells were cultured for six days in medium supplemented with either 50 μM L-2-oxothiazolidine-4-carboxylic acid (OTC) or 0.5 mM buthionine sulphoximine (BSO). Error bars represent the SD of three biological replicates.

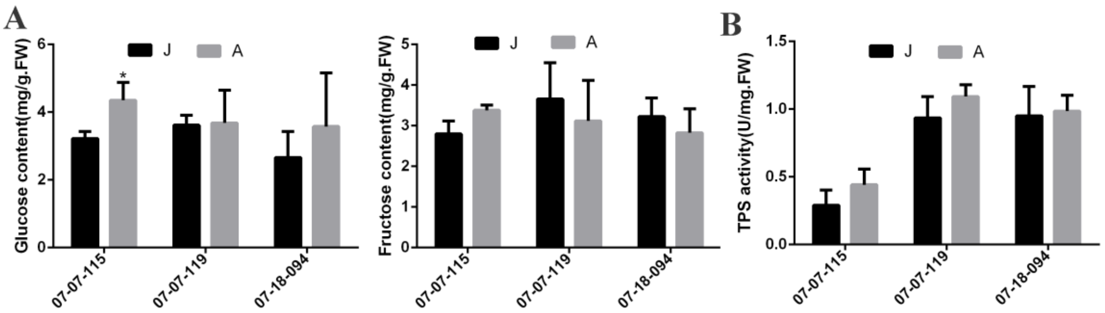

Fig S7. Glucose and Fructose concentrations (A) and activity of Trehalose-6-phosphate synthase (TPS) (B) in the juvenile (J) and adult (A) phase of three individuals of *Malus asiatica* ‘Zisai Pearl’ × *M. domestica* ‘Red Fuji’. Error bars represent the SD of three biological replicates. Asterisk represents $p < 0.05$ (Duncan’s multiple-range test).

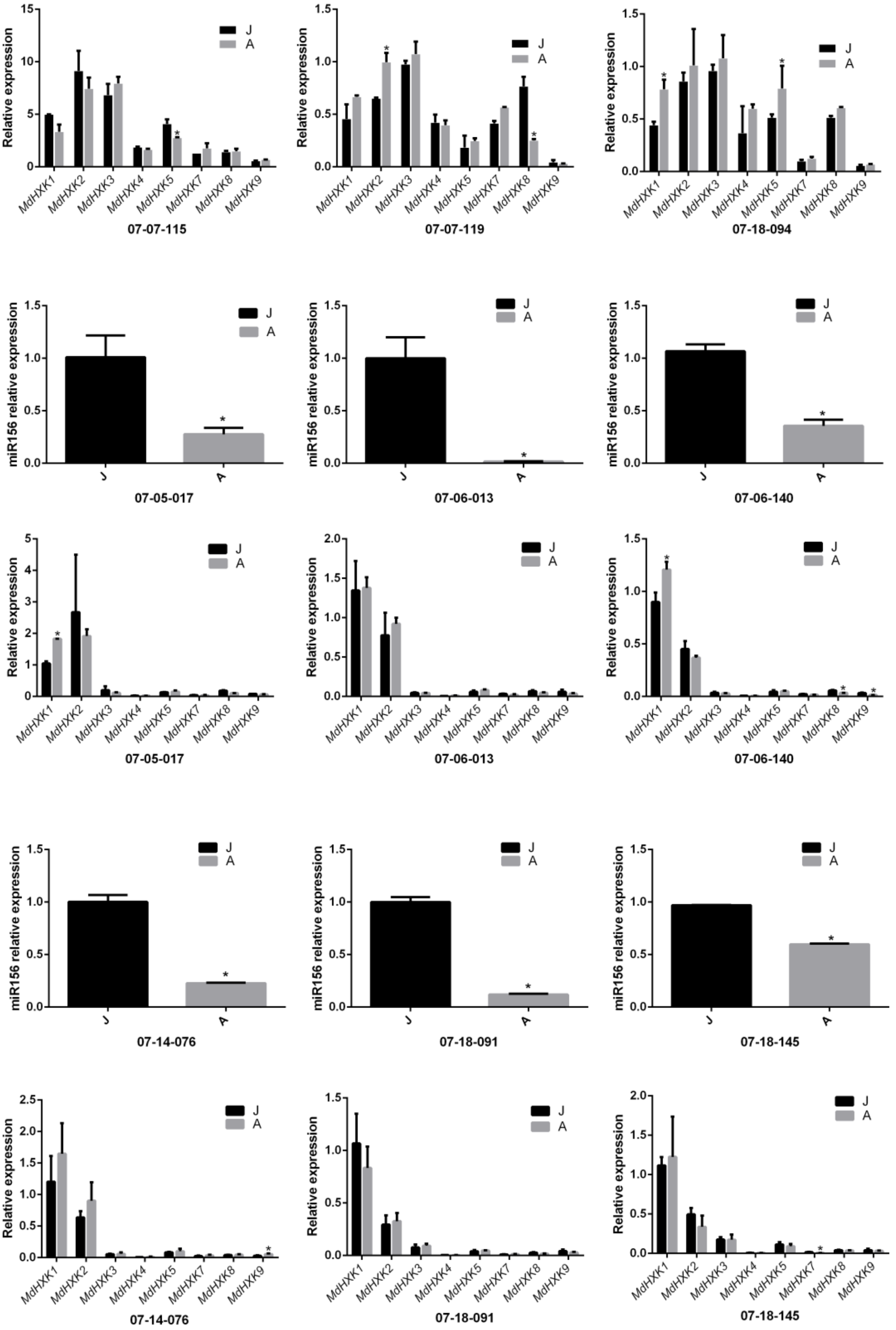

Fig S8. Relative expression of *MdHXKs* in the juvenile (J) and adult (A) phase of three individuals of *Malus asiatica* ‘Zisai Pearl’ × *M. domestica* ‘Red Fuji’. Error bars represent the SD of three biological replicates. Asterisk represents $p < 0.05$ (Duncan’s multiple-range test).

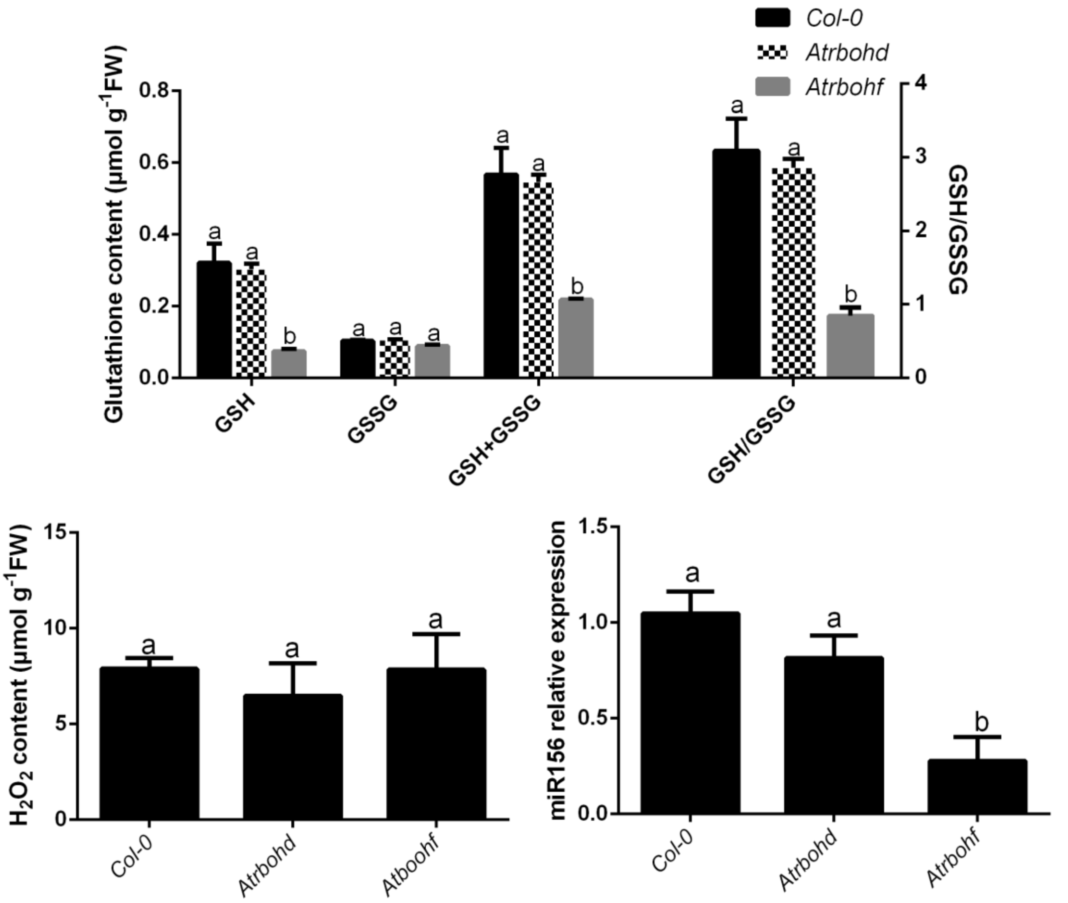

Fig S9. Glutathione (GSH), glutathione disulfide (GSSG) concentrations, GSH/GSSG ratio, Hydrogen peroxide (H_2O_2) and miR156 expression in wild type and *rbohd*, *rbohf* mutants of *Arabidopsis thaliana*.

Error bars represent the SD of three biological replicates. Different letters indicate statistical significance ($p < 0.05$; Duncan's multiple-range test).

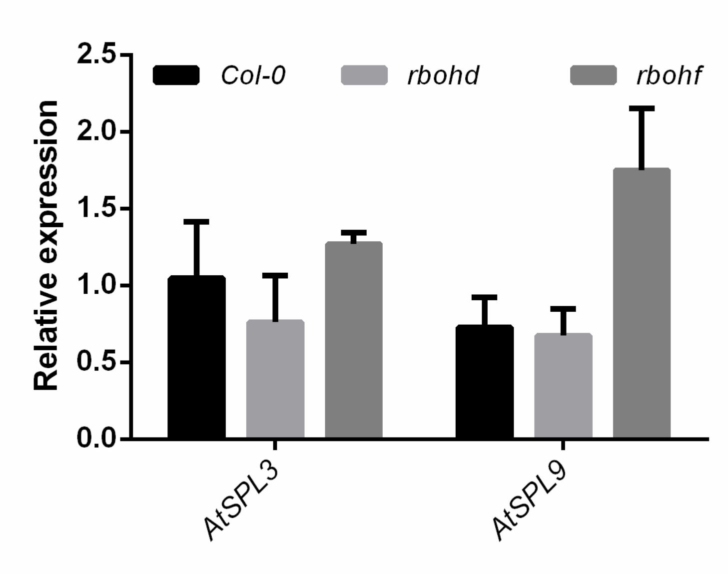

Fig S10. Relative expression of *SPL3* and *SPL9* in wild type and *rbohd*, *rbohf* mutants of *Arabidopsis thaliana*.

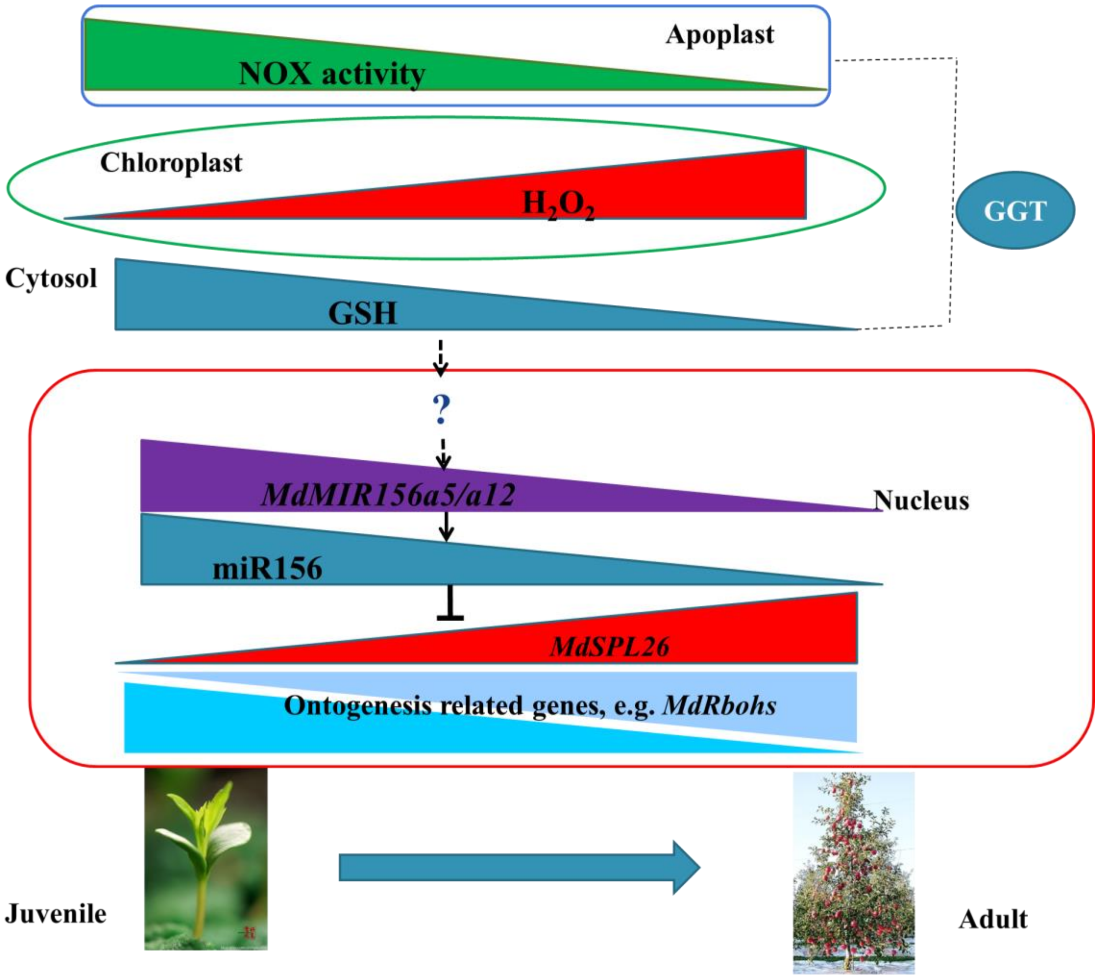

Fig S11. A model for the regulation of miR156 by redox signals to control vegetative phase change in apple seedlings

1. The primary transcript sequence of MdMIR156s

The mature miR156 sequences were obtained from miRBase database (http://www.mirbase.org /, Release 21) and the plant microRNA database (PMRB, http:// bioinformatics.cau.edu.cn/PMRD/), then compared with the apple genome (http://genomics.research.iasma.it/) by BLAST. We selected the ~500bp upstream and ~500bp downstream of mature miR156 as the pri-miR156 sequence.

The positions of the mature miR156 embedded on each pri-MdmiR156 member were marked with red colored fonts.

The pri-MdmiR156 transcript, which has been experimentally confirmed to be pri-MdmiR156, was marked with an asterisk following it's name and the corresponding reference (if there is any) is listed just behind the DNA base sequence.

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Sun C., Zhao Q., Liu D. D., You C. X. & Hao Y. J. Ectopic expression of the apple Md-miRNA 156h gene regulates flower and fruit development in Arabidopsis. Plant Cell Tiss Organ Cult. 112, 343-351 (2013).

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MdMIR156e2

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MdMIR156f1
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MdMIR156f2
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TTAGTTTAAT

2. Predicted secondary structures of the apple precursor miR156s.

We analyzed RNA secondary structure of the candidate sequences (http://mfold.rna.albany.edu/). The positions of the mature miR156 embedded were marked with highlighted yellow color fonts.

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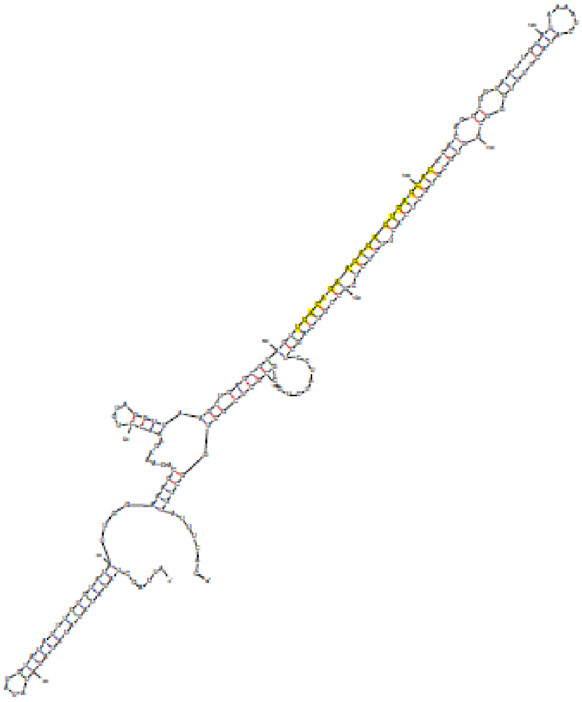

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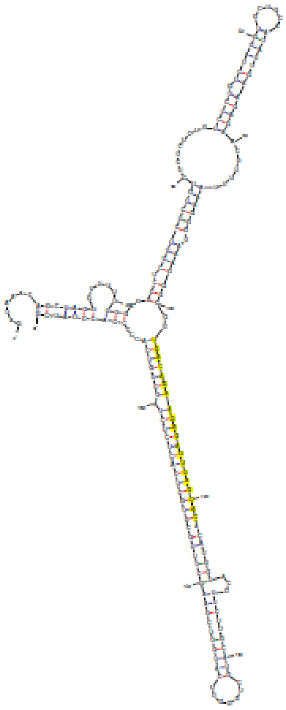

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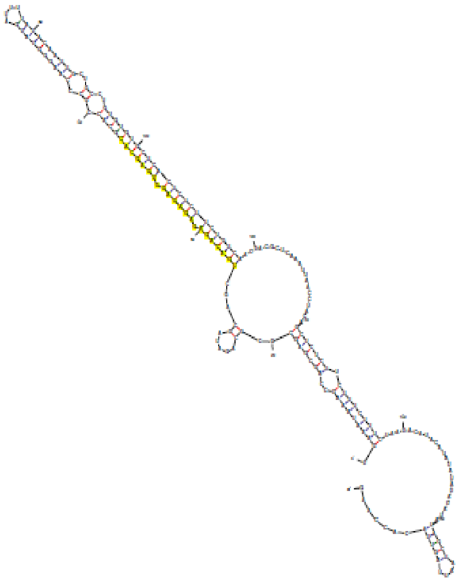

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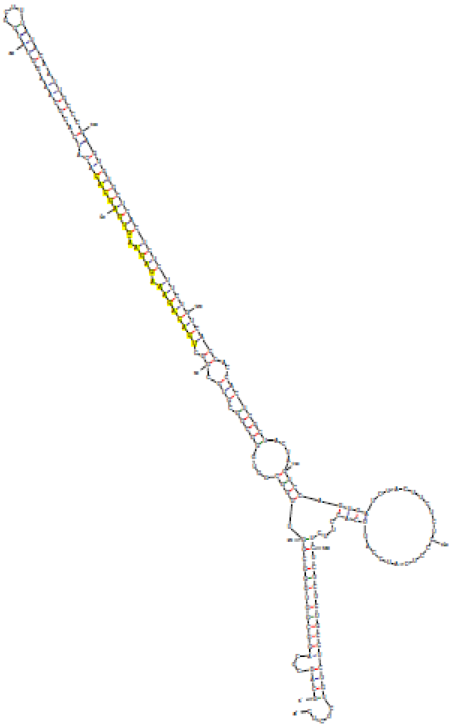

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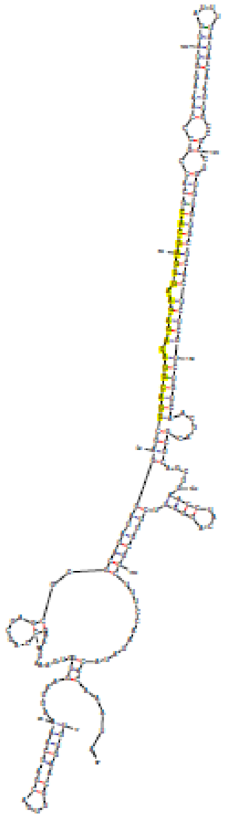

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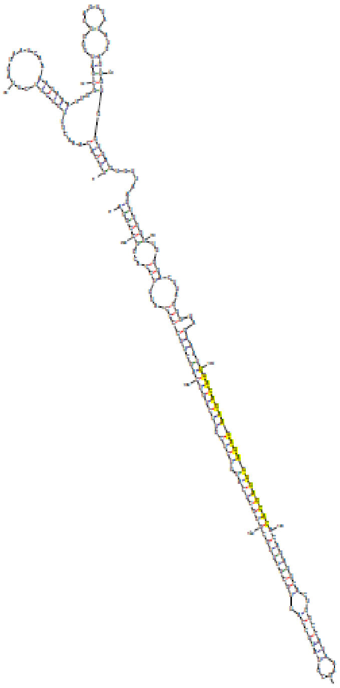

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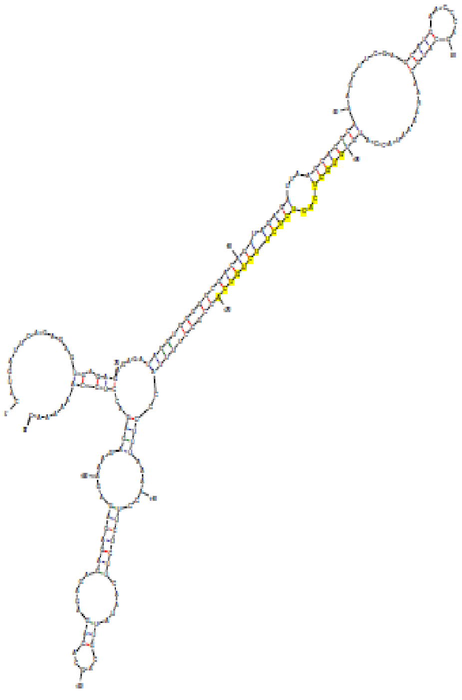

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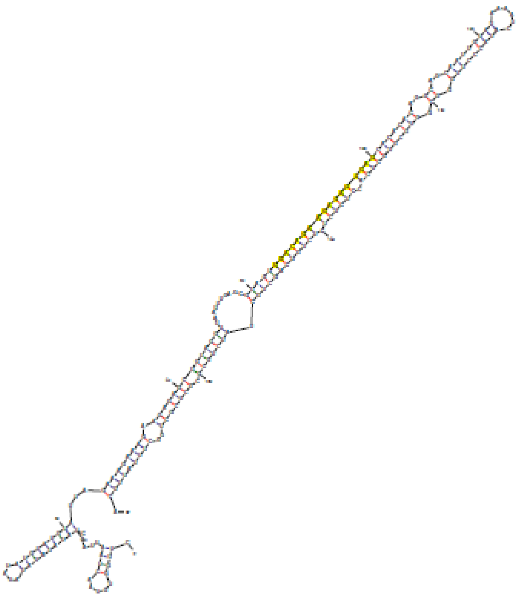

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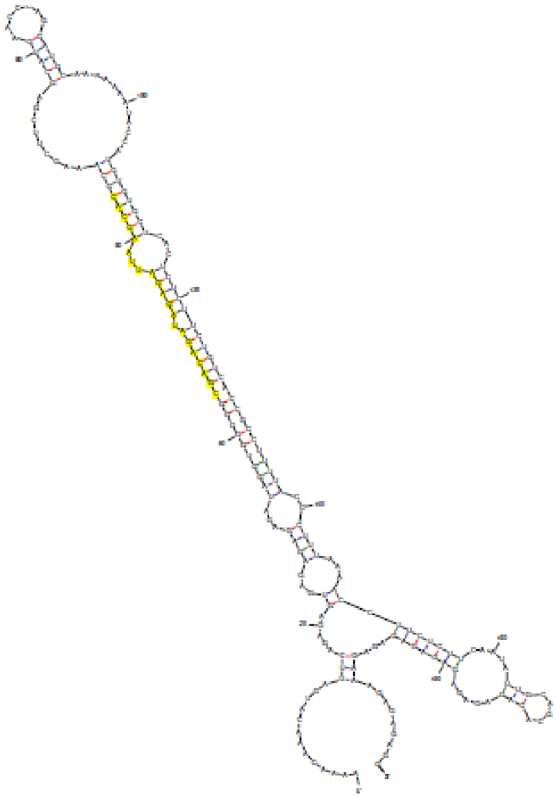

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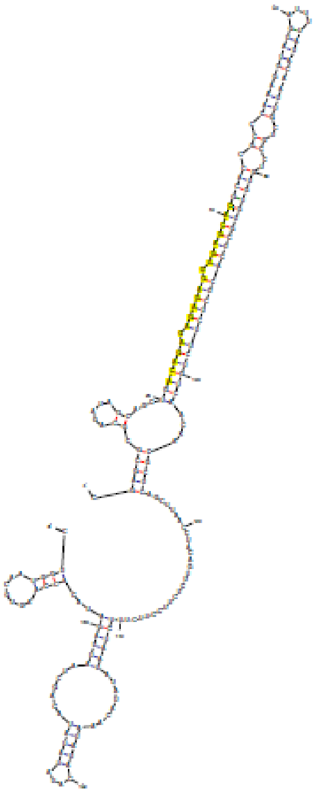

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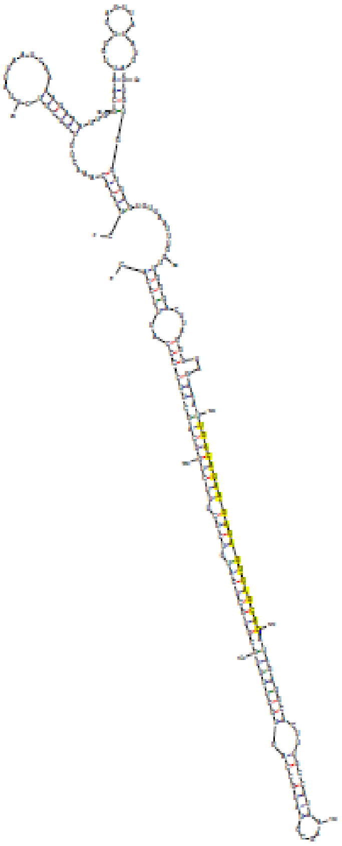

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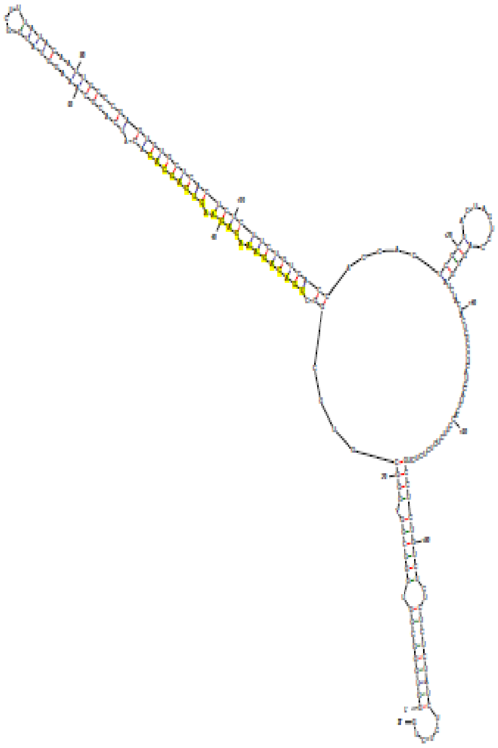

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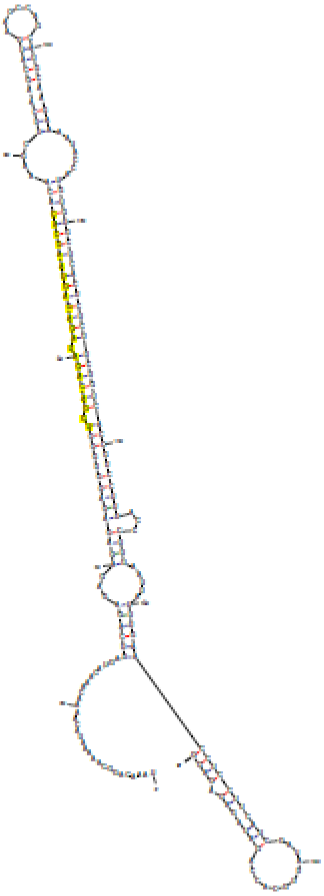

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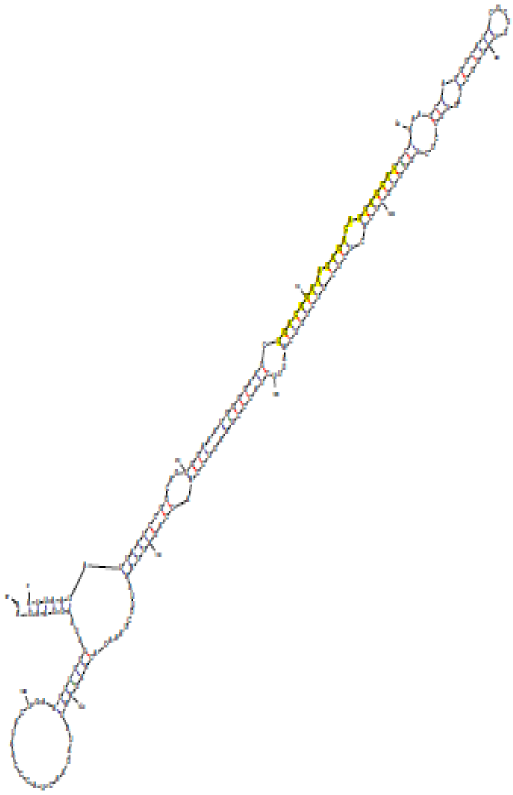

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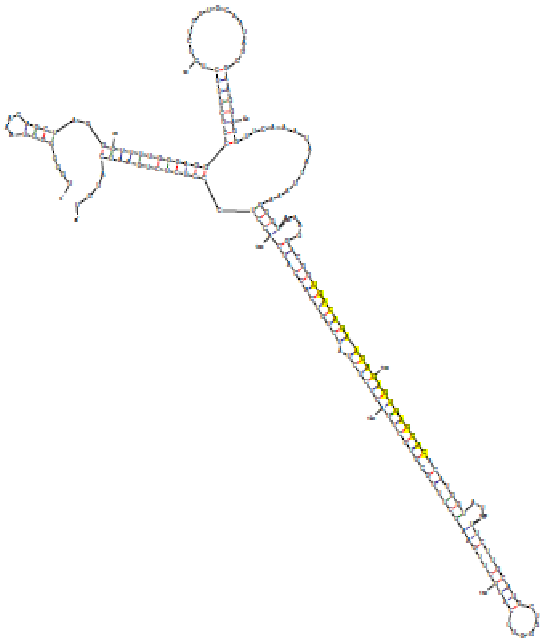

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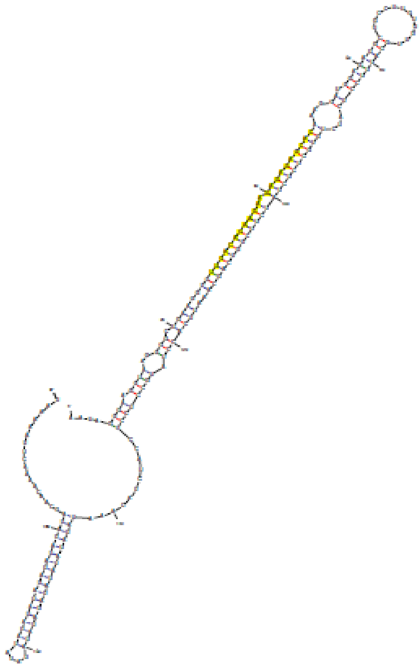

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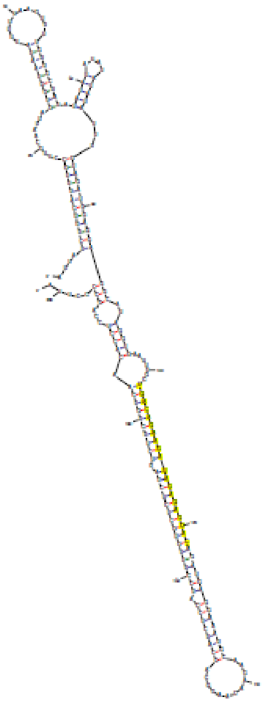

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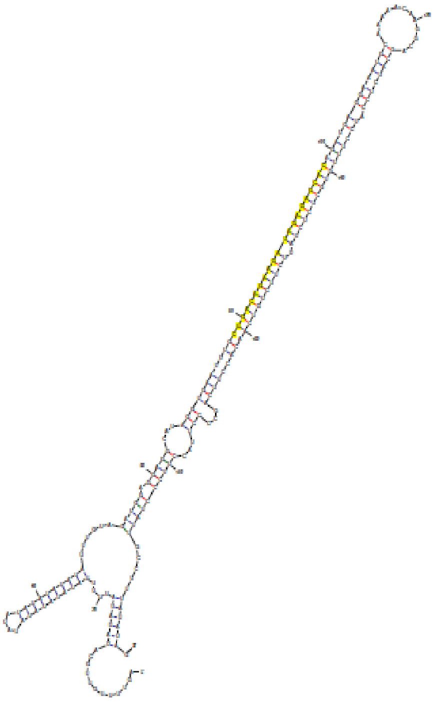

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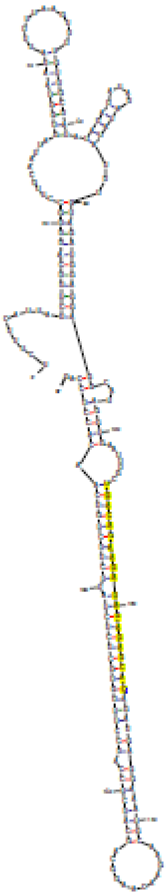

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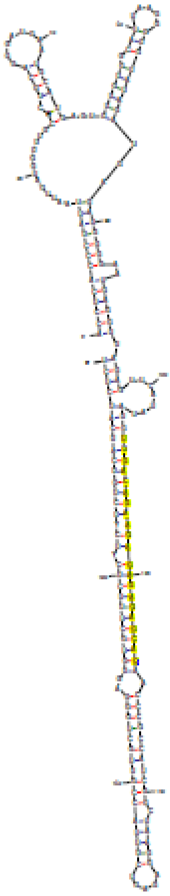

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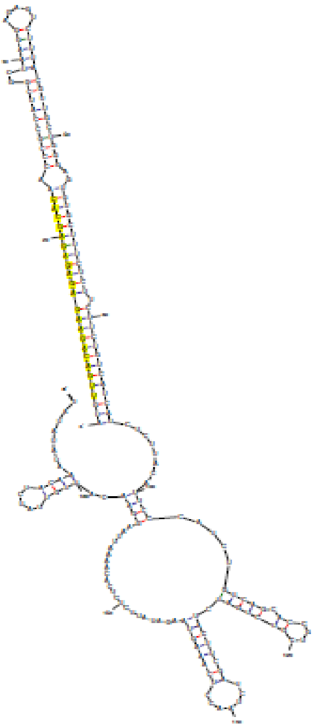

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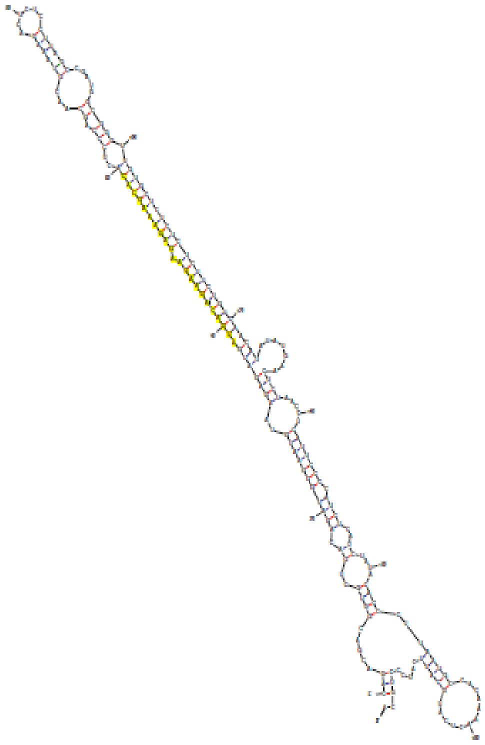

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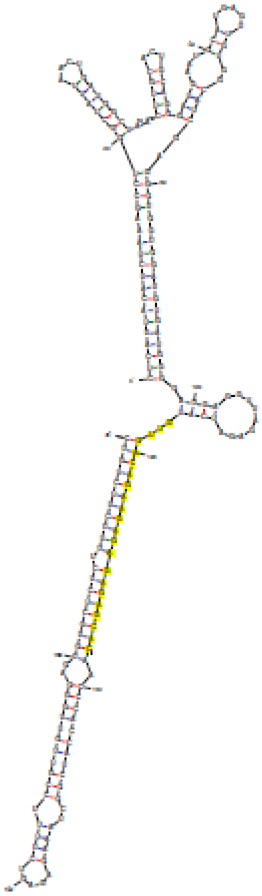

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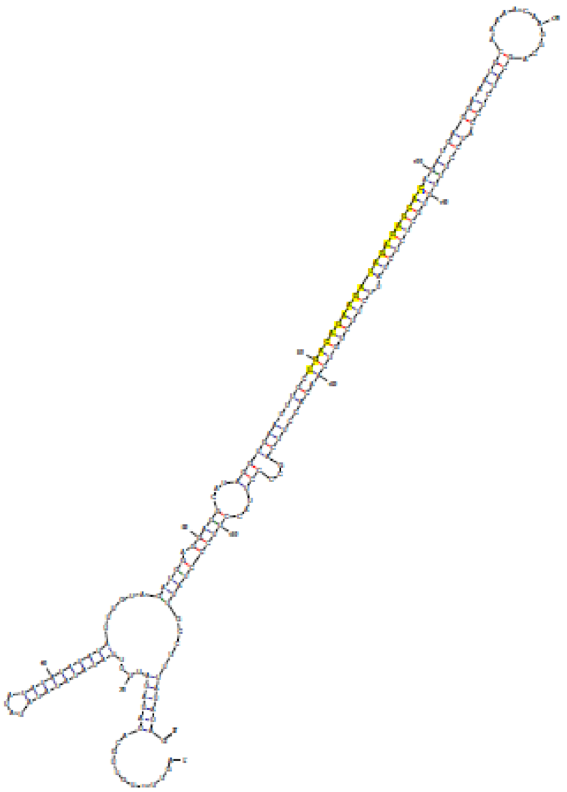

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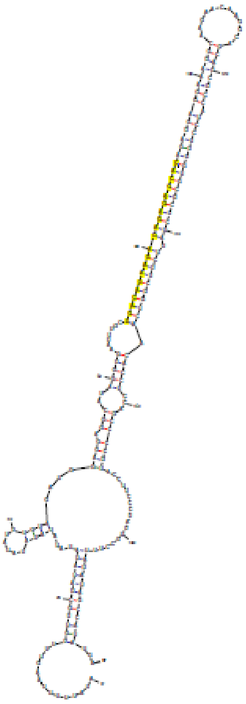

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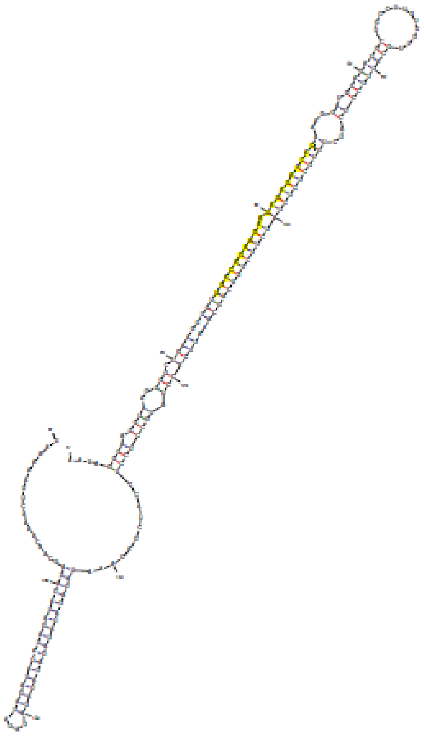

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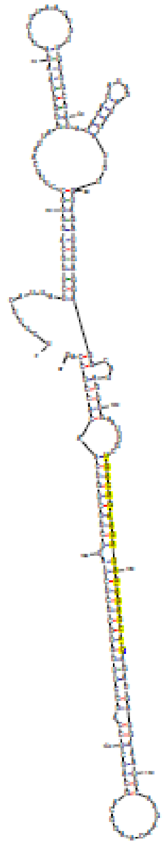

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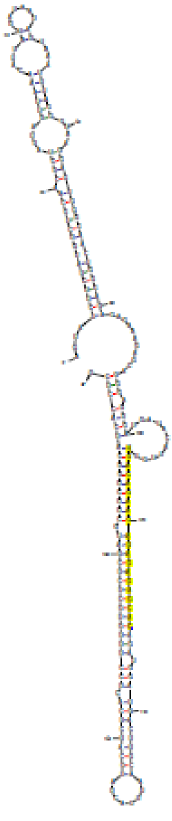

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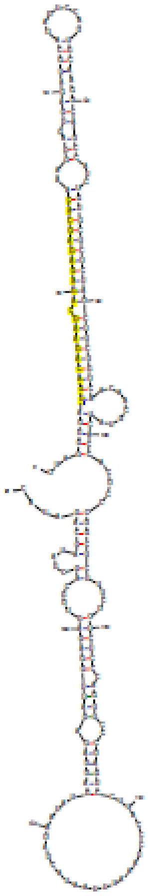

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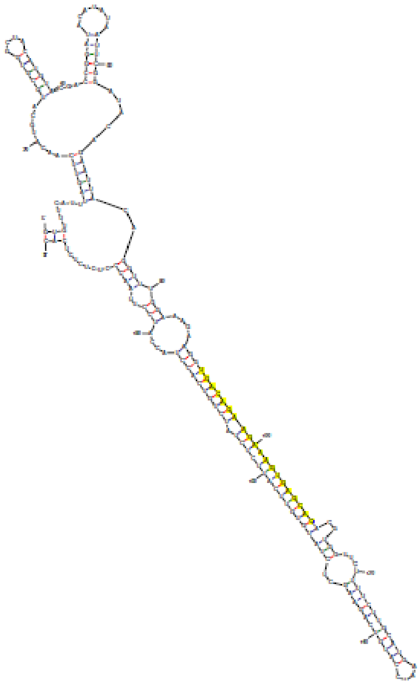

Table S1 Changes in hydrogen peroxide (H_2O_2) concentration in suspension cells of apple 'Orin' leaf cultured in medium supplemented with menadione (MED)

Average ±Standard deviation	Days of treatment			
	0	2	4	6
Control	0.21±0.04	0.15±0.07cd	0.19±0.04b	0.28±0.02c
50 μM MED	0.21±0.04	0.59±0.15a	0.56±0.02a	0.54±0.08b
250 μM MED	0.21±0.04	0.32±0.08bc	0.51±0.16a	0.41±0.02bc
500 μM MED	0.21±0.04	0.37±0.08b	0.64±0.22a	0.80±0.17a

Three replicates for each treatment. Data values in this table represented as 'average ± standard deviation'. Different letters following data values indicate statistical significance ($p < 0.05$; Duncan's multiple-range test).

Table S2 Changes in hydrogen peroxide (H_2O_2) concentration in suspension cells of apple 'Orin' leaf cultured in medium supplemented with diphenyleneiodonium (DPI)

Average ±Standard deviation	Days of treatment			
	0	2	4	6
Control	0.21 ±0.04	0.15 ±0.02bc	0.19 ±0.04b	0.28 ±0.02c
50 μM DPI	0.21 ±0.04	0.12 ±0.04c	0.56 ±0.02a	0.54 ±0.08b
250 μM DPI	0.21 ±0.04	0.33 ±0.12a	0.51 ±0.16a	0.41 ±0.02bc
500 μM DPI	0.21 ±0.04	0.30 ±0.08ab	0.64 ±0.22a	0.80 ±0.17a

Three replicates for each treatment. Data values in this table represented as 'average ± standard deviation'. Different letters following data values indicate statistical significance ($p < 0.05$; Duncan's multiple-range test).

Table S3 Changes in GSH/GSSG ratio in suspension cells of apple 'Orin' leaf cultured in medium supplemented with L-2-oxothiazolidine-4-carboxylic acid (OTC).

Average ±Standard deviation	Days of treatment			
	0	2	4	6
Control	2.03±0.11	1.70±0.03b	1.80±0.05b	2.49±0.04a
25μM OTC	2.03±0.11	1.57±0.29b	1.79±0.33b	2.31±0.10a
50μM OTC	2.03±0.11	2.58±0.26a	2.40±0.27a	2.34±0.23a
100μM OTC	2.03±0.11	2.93±0.57a	2.31±0.02a	2.18±0.30a

Three replicates for each treatment. Data values in this table represented as 'average ± standard deviation'. Different letters following data values indicate statistical significance ($p < 0.05$; Duncan's multiple-range test).

Table S4 Changes in GSH/GSSG ratio in suspension cells of apple 'Orin' leaf cultured in medium supplemented with Buthionine Sulphoximine (BSO).

Average ±Standard deviation	Days of treatment			
	0	2	4	6
Control	2.03 ±0.11	1.70 ±0.03a	1.80 ±0.05a	2.49 ±0.04a
0.1mM BSO	2.03 ±0.11	0.70 ±0.18bc	1.37 ±0.05b	1.55 ±0.25b
0.5mM BSO	2.03 ±0.11	0.93 ±0.33b	1.25 ±0.16b	0.87 ±0.09c
1mM BSO	2.03 ±0.11	0.47 ±0.26c	0.47 ±0.17c	0.36 ±0.07d

Three replicates for each treatment. Data values in this table represented as 'average ± standard deviation'. Different letters following data values indicate statistical significance ($p < 0.05$; Duncan's multiple-range test).

Table S5 Primer sequences for reverse transcription and RT-PCR of mature miR156 and 5s RNA

Primers	Sequence (5′→3′)
RT-miR156	GTCACATCGTATCGTGAAGCTGCGCAGCTGATGTGACGTGCTCAC
miR156	F: TGCACTAGCGTGTGACAGAAGA
	R: ACATCGTATCGTGAAG
RT-5s rRNA	GTCACATCGTATCGTGAAGCTGCGCAGCTGATGTGACTGGATTGG
5s rRNA	F: TGCACTAGCGTGTAGAGGAACC
	R: ACATCGTATCGTGAAG

Table S6 Primer sequences of *MdMIR156s MdHYL1* and *MdSPLs*

Primers	Sequence (5′→3′)
MdMIR156 a1	F:GCTGGGAGTAAGAGGGTATCTA
	R:CTCCTTCTGCTCTCCCAATTC
MdMIR156 a5	F:AGCTGACGGAGAGAGAAGT
	R:CTGAGCGTAGTTGACAGAAGAG
MdMIR156 a6	F: ACAGAGGCACTTGCCATAAA
	R: TAATCCGGCAGTGGAAATGG
MdMIR156 a9	F:AGCTGACGGAGAGAGAAGT
	R:GAGAGTGAGCACACATGGTATT
MdMIR156 a10	F:CAGGAAGGAGAATTGGGAGAAA
	R:TCTCTTCCCGCAAAGGAATG
MdMIR156 a12	F:CACGCAAAGGTATGGCTTTATAC
	R:GCAGAGAGAGAGAAGGTGAATG
MdMIR156 d1	F:GACAGAAGATAGAGAGCACAGATG
	R: AGGTGTTGACAGAAGAAT
MdHYL1	F: TTCCCATGCGCCTTCTTT
	R: GCTGACTGCTCTGCTATCTTAC
MdSPL3	F: CTCATCAGCAGTCGGCGTCA
	R:TCGGGGTTAAGAGAAAGGGCAG
MdSPL4	F:CCCATGTACCTTCATGGAAAGTCAAG
	R:GCAAATGTTTGCCCACCATTTCT
MdSPL7	F:TGGTGGACTTTGATAAGGTGAAGAGA
	R:ACTTCGTTCGTCAGTTGCGGA
MdSPL10&11	F:GGAGCATGAGAGAAACTTTTTTCAAGTA
	R:CACATTGTTGCTTGCACGCC
MdSPL12	F:GTCCTTGCCATGCCCGAT
	R:TCAGGTTTTCCAGTTCCCACA
MdSPL18	F:AGGTTCAGCAGGCGGGACTA
	R:TCGTCGGAAAGTGATTGGAGG
MdSPL20	F:GCCAAGAGATACTCCCTACTGACATTC
	R:CTGGAACTGCTGAAGCTGTGTTG
MdSPL21&22	F:CAAGATATTCGTGCGGATTCGATTAA
	R:CTGTGGAAATGTTGGAATTCTGATGC
MdSPL23	F:TCCCCTGATGTCCGAGTGCT
	R:TTTCAGATGCGTGCCCCGAC
MdSPL24	F:GCTTACCGAGAAAAACAAATCCCAT
	R:AGACTGAACTGGACTGTGCTGTGT
MdSPL26	F:GTCACCACTACTCATCCAGGTCAGA
	R:TCCTCCTCATCATGTGAGCATGAGAATT
β-Actin	F:TGGTGAGGCTCTATTCCAAC
	R:TGGCATATACTCTGGAGGCT

Table S7 Detailed list of MIR156 gene family in apple

MiRNA name	Sequence	Contig	Length	Strand	Match position	Star sequence
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MIR156 a2	TGACAGAAGAGAGTGAGCAC	MDC015254.139	20	+	2152	ACTGTCTATCTCTCACTCGTG
MIR156 a3	TGACAGAAGAGAGTGAGCAC	MDC010428.318	20	-	7697	GTGCTCACTCTCTTCTGTCA
MIR156 a4	TGACAGAAGAGAGTGAGCAC	MDC007399.176	20	-	6603	GTGCTCACTCTCTTCTGTCA
MIR156 a5	TGACAGAAGAGAGTGAGCAC	MDC010428.318	20	-	17326	GTGCTCACTCTCTTCTGTCA
MIR156 a6	TGACAGAAGAGAGTGAGCAC	MDC018927.245	20	+	11206	ACTGTCTTTCTCTGTACTCGTG
MIR156 a7	TGACAGAAGAGAGTGAGCAC	MDC022546.57	20	-	2800	CACGAATTAGAGATAGACAGC
MIR156 a8	TGACAGAAGAGAGTGAGCAC	MDC010428.308	20	+	6933	ACTGTCTCTCTCTCCACTC
MIR156 a9	TGACAGAAGAGAGTGAGCAC	MDC002074.373	20	-	2158	ACTGTCTATCTCTCACTCGTG
MIR156 a10	TGACAGAAGAGAGTGAGCAC	MDC038184.5	20	-	20083	GTGCTCACTCTCTTCTGTCA
MIR156 a11	TGACAGAAGAGAGTGAGCAC	MDC022169.138	20	+	7417	ACTGTCTTTCTCTGTACTCGTG
MIR156 a12	TGACAGAAGAGAGTGAGCAC	MDC015520.219	20	-	7939	GTGCTCACTCTCTTCTGTCA
MIR156 a13	TGACAGAAGAGAGTGAGCAC	MDC013580.195	20	-	17918	GTGCTCACTCTCTTCTGTCA
MIR156 a14	TGACAGAAGAGAGTGAGCAC	MDC022241.434	20	-	30246	GTGCTCACTCTCTTCTGTCA
MIR156 a15	TGACAGAAGAGAGTGAGCAC	MDC011231.822	20	+	32339	ACTGTCTATCTCTCATTCGTG
MIR156 b1	CTGACAGAAGATAGAGAGCAC	MDC007657.477	21	-	1890	GTGCTCACTCTCTTCTGTCA
MIR156 b2	CTGACAGAAGATAGAGAGCAC	MDC018442.71	21	+	5569	ACTGTCTTCTTATCTCTCGTG
MIR156 b3	CTGACAGAAGATAGAGAGCAC	MDC012217.482	21	+	9927	AACTGTCTTCTTATCTCTCGTG
MIR156 b4	CTGACAGAAGATAGAGAGCAC	MDC018690.154	21	+	10957	ACTGTCTTCTTATCTCTCGTG
MIR156 c1	TTGACAGAAGAGAGAGAGCAC	MDC038783.9	21	+	1407	TACTGTCTYCTYCTCTTTCGTG
MIR156 c2	TTGACAGAAGAGAGAGAGCAC	MDC008280.307	21	+	3	ACTGTCTTCTTCTCTTTCGTG
MIR156 c3	TTGACAGAAGAGAGAGAGCAC	MDC019519.287	21	-	801	GTGCTCTCTCTCTTCTGTCAA
MIR156 c4	TTGACAGAAGAGAGAGAGCAC	MDC019519.284	21	+	5666	CTGTCTTCTTTTCTCTCGTG
MIR156 d1	TGACAGAAGATAGAGAGCAC	MDC012217.482	20	+	9928	ACTGTCTTCTTATCTCTCGTG
MIR156 d2	TGACAGAAGATAGAGAGCAC	MDC018442.71	20	+	5570	ACTGTCTTCTTATCTCTCGTG
MIR156 d3	TGACAGAAGATAGAGAGCAC	MDC007657.477	20	-	1890	GTGCTCTCTATCTTCTGTCA
MIR156 d4	TGACAGAAGATAGAGAGCAC	MDC018690.154	20	+	10958	ACTGTCTTCTTATCTCTCGTG
MIR156 e1	TTGACAGAAGATAGAGAGCAC	MDC002621.233	21	+	15660	ACTGTCTTCGTATCTCTCGTG
MIR156 e2	TTGACAGAAGATAGAGAGCAC	MDC002441.399	21	-	5519	GTGCTCACTCTCTTCTGTCA
MIR156 f1	TGACAGAAGAAAGTGAGCAC	MDC013721.403	20	-	6675	CACAAGTAAGAGATAGACAGT
MIR156 f2	TGACAGAAGAAAGTGAGCAC	MDC013721.399	20	+	9390	ACTGTCTATCTCTTACTTGTG

Table S8 Primer sequences of *MdRboh* family members

Primers	Sequence (5′→3′)
Rboh D1	F:GCACCAAGTTACCACACAAATC
MDP0000703059	R:GTTCCCGTGTCACCCAATAA
Rboh D2	F:GCAGAGAGTCTGGGTTATGATG
MDP0000262620	R:CTCGAATGCCTCCTCTCTTTC
Rboh D3	F:CGGCACAGGACTACAAGAAATA
MDP0000421679	R:TCATCTTCCTTGCCCTTCATC
Rboh H1	F:ATGGGCATCAAGAGACATCTG
MDP0000160005	R:TGCTCCCTTGTTACCCAATAAA
Rboh H2	F:GATGCGAGGTCTGCTCTTATT
MDP0000195681	R:TGAGTTCTTATCCGGCTTTCTG
Rboh K1	F:CTAATAAGCACTATACAAGCGTTGC
MDP0000280452	R:TGAGAAGATGTTGAACCAGTCC
Rboh K2	F:TTTGGTGACCCTTCAGTCT
MDP0000261507	R:CGGAGTCCGGGAGAGAA

Table S9 Primer sequences of *MdHXK* family members

Primers	Sequence (5′→3′)
HXK1	F: CTTTCTGCTGCTGGGATCTT
MDP0000181206	R: TGGTGCGGAATTCAGTGTAG
HXK2	F: GCAATGGATGGTGGTTTGTATG
MDP0000309677	R: GTTTGAGTGCTCGACAGAAATG
HXK3	F: GGTAGCTTTGGATGGTGGATTA
MDP0000823956	R: ATCCCAGACCCATCGTTAGA
HXK4	F: GGAACGTGCACATGCAATAC
MDP0000643891	R: CTGTGAAGCAGTGAAGGAGTT
HXK5	F: CGGAGAGGAAGTTGCAGAAA
MDP0000849870	R: CGATTCCTAGGTATTGTGAGTGAG
HXK7	F: GAGCAAGATATTGGGACGATGA
MDP0000251415	R: GGCCCTGTAGTTTGGGAATAG
HXK8	F: CACTAGATCTTGGCGGTACTAAC
MDP0000175245	R: GAGGAATGGCTTGTCGATACA
HXK9	F: CCTGGAGCTAAGCTGTGAAA
MDP0000316781	R: CCATCGGGTAGTTTATCCACAA