Supplementary Information in pdf format of the paper titled 'Identification of miRNAs and their targets in wild tomato at moderately and acutely elevated temperatures by high-throughput sequencing and degradome analysis' by Rong Zhou, Qian Wang, Fangling Jiang, Xue Cao, Mintao Sun, Min Liu and Zhen Wu. Supplementary Information of the paper titled 'Identification of miRNAs and their targets in wild tomato at moderately and acutely elevated temperatures by high-throughput sequencing and degradome analysis' by Rong Zhou, Qian Wang, Fangling Jiang, Xue Cao, Mintao Sun, Min Liu and Zhen Wu.

Supplementary Figure S1. Folding structures of novel miRNAs in tomato LA2093 at a control temperature (26/18°C, NT), moderately elevated temperature (33/33°C, MET) and acutely elevated temperature (40/40°C, AET)

Supplementary Figure S2. GO functional annotation of target genes cleaved by conserved miRNAs in tomato LA2093

Supplementary Figure S3. GO enrichment analysis of the target genes that predicted by Targetfinder and detected by degrdome sequencing with different expression levels cleaved by miRNAs with different expression levels in tomato LA2093. **(A)** MET vs NT, **(B)** AET vs NT. The x-axis indicated rich factor; the y-axis indicated GO term. The size of the circle indicated the number of genes with the functions; the different colours of the circle indicated different significance levels.

Supplementary Figure S4. Target plots of some mRNAs from category 0 in tomato LA2093 at **(A)** a control temperature (26/18°C, NT), **(B)** moderately elevated temperature (33/33°C, MET) and **(C)** acutely elevated temperature (40/40°C, AET)



PC-1



PC-2



PC-3

Output of sir_graph () by D. Stewart and M. Zuker



PC-4



dG = -52.3mPC-5

PC-5

Output of sir_graph () by D. Stewart and M. Zuker



PC-6

Output of sir_graph () by D. Stewart and M. Zuker



PC-7

dG = -39.6պPC-8 **PC-8**



dG = -41.3ղPC-9 **PC-9**





Output of sir_graph () by D. Stewart and M. Zuker



PC-11

dG = -33ղPC-12 PC-12







dG = -98.12PC-14

PC-14



dG = -44.2%PC-15

PC-15

Output of sir_graph () by D. Stewart and M. Zuker



PC-16

Output of sir_graph () by D. Stewart and M. Zuker



PC-17

> > dG = -64.3%PC-18

PC-18

Output of sir_graph () by D. Stewart and M. Zuker



PC-19



dG = -68.6%PC-20

PC-20

Output of sir_graph () by D. Stewart and M. Zuker



dG = -60.8%PC-21

PC-21

dG = -79.1%PC-22



Output of sir_graph () by D. Stewart and M. Zuker



PC-23

> > dG = -72.3%PC-24

PC-24



dG = -77.1½PC-25

PC-25



dG = -65.5%PC-26

PC-26



PC-27



dG = -31.8%PC-28

PC-28

Output of sir_graph () by D. Stewart and M. Zuker



dG = -57.22PC-29

PC-29



PC-30



dG = -56.6%PC-31

PC-31



dG = -115.32PC-32





dG = -45.92PC-33

PC-33

Output of sir_graph () by D. Stewart and M. Zuker



dG = -51.6^hPC-34

PC-34


dG = -53.22PC-35

PC-35



dG = -33.92PC-36

PC-36



dG = -38.3½PC-37

PC-37



dG = -79.5½PC-38

PC-38



dG = -55.7₧PC-39

PC-39

Output of sir_graph () by D. Stewart and M. Zuker



PC-40



PC-41

Output of sir_graph () by D. Stewart and M. Zuker



dG = -39.92PC-42

PC-42



dG = -41.1%PC-43

PC-43



PC-44

Output of sir_graph () by D. Stewart and M. Zuker



PC-45



dG = -51.5½PC-46

PC-46



PC-47

> > dG = -52.7%PC-48

PC-48

> > dG = -81.1%PC-49

PC-49

Output of sir_graph () by D. Stewart and M. Zuker



dG = -43.8%PC-50

PC-50



dG = -85.5½PC-51

PC-51



PC-52

3, ٦ 160

dG = -45.1ኪPC-53

PC-53

Output of sir_graph () by D. Stewart and M. Zuker



PC-54

Output of sir_graph () by D. Stewart and M. Zuker



PC-55



dG = -37.2½PC-56

PC-56



PC-57



PC-58

Output of sir_graph () by D. Stewart and M. Zuker



dG = -52.7½PC-59

PC-59



PC-60

Output of sir_graph () by D. Stewart and M. Zuker



dG = -46.4%PC-61

PC-61

Output of sir_graph () by D. Stewart and M. Zuker



PC-62



dG = -68.5½PC-63

PC-63

Output of sir_graph () by D. Stewart and M. Zuker



dG = -29.6%PC-64

PC-64



PC-65



dG = -48.2%PC-66

PC-66

Output of sir_graph () by D. Stewart and M. Zuker



dG = -64.1%PC-67

PC-67



PC-68

20

dG = -54.6½PC-69

PC-69



PC-70
s_0 s_0

dG = -61.8%PC-71

PC-71

Output of sir_graph () by D. Stewart and M. Zuker



PC-72



PC-73

Output of sir_graph () by D. Stewart and M. Zuker



PC-74



PC-75

Output of sir_graph () by D. Stewart and M. Zuker



PC-76



PC-77



dG = -25.2ኪPC-78

PC-78

Output of sir_graph () by D. Stewart and M. Zuker



PC-79

dG = -95.42PC-80

PC-80



dG = -64.1%PC-81

PC-81



PC-82

Output of sir_graph () by D. Stewart and M. Zuker



PC-83



dG = -25.8%PC-84

PC-84



dG = -108.12PC-85





PC-86

5 3 160

dG = -53.7ኪPC-87

PC-87





PC-88



PC-89



PC-90



PC-91



dG = -81.6%PC-92

PC-92

Supplementary Figure S1. Folding structures of novel miRNAs in tomato LA2093 at a control temperature (26/18°C, NT), moderately elevated temperature (33/33°C, MET) and acutely elevated temperature (40/40°C, AET)



Supplementary Figure S2. GO functional annotation of target genes cleaved by conserved miRNAs in tomato LA2093

'regulation of transcription, D...' = 'regulation of transcription, DNA-dependent'; 'intracellular protein transport...' = 'intracellular protein transport'; 'outer membrane-bounded peripla...' = 'outer membrane-bounded periplasmic space'; 'intrinsic to endoplasmic retic...' = 'intrinsic to endoplasmic reticulum membrane'; 'sequence-specific DNA binding...' = 'sequence-specific DNA binding transcription factor activity'.





Supplementary Figure S3. GO enrichment analysis of the target genes that predicted by Targetfinder and detected by degrdome sequencing with different expression levels cleaved by miRNAs with different expression levels in tomato LA2093. (A) MET vs NT, (B) AET vs NT. The x-axis indicated rich factor; the y-axis indicated GO term. The size of the circle indicated the number of genes with the functions; the different colours of the circle indicated different significance levels.



spi-miR160_tae slicing Solyc11g013470.1.1 at nt 554

Solyc11g013470.1.1 position (rts)

spi-miR166a slicing Solyc02g069830.2.1 at nt 629



Solyc02g069830.2.1 position (rts)



spi-miR166g_osa slicing Solyc02g069830.2.1 at nt 629



spi-miR160h_ptc slicing Solyc11g013470.1.1 at nt 554

Solyc11g013470.1.1 position (rts)

spi-miR166a slicing Solyc02g069830.2.1 at nt 629



Solyc02g069830.2.1 position (rts)

spi-miR396a-5p slicing Solyc12g096070.1.1 at nt 332



Solyc12g096070.1.1 position (rts)



spi-miR160_tae slicing Solyc11g013470.1.1 at nt 554

Solyc11g013470.1.1 position (rts)

spi-miR166a slicing Solyc02g069830.2.1 at nt 629



Solyc02g069830.2.1 position (rts)

spi-miR396a-5p slicing Solyc12g096070.1.1 at nt 332



Supplementary Figure S4. Target plots of some mRNAs from category 0 in tomato LA2093 at (**A**) a control temperature (26/18°C, NT), (**B**) moderately elevated temperature (33/33°C, MET) and (**C**) acutely elevated temperature (40/40°C, AET)

Supplementary Table S7. GO functional annotation of the target genes cleaved by novel miRNAs in tomato LA2093

Transcript_ID	Annoation	miRNA	GO term	GO function	GO functionos
Solyc12g014350.1.1	Leucine-rich repeat transmembrane protein kinase	PC-52-5p	GO:0006468	biological process	protein phosphorylation
Solyc01g105370.2.1	Aldolase-type TIM barrel family protein	PC-44-3p	GO:0055114	biological process	oxidation-reduction process
Solyc07g051850.2.1	aspartic proteinase A1	PC-46-5p	GO:0006508	biological process	proteolysis
Solyc07g051850.2.1	aspartic proteinase A1	PC-46-5p	GO:0006629	biological process	lipid metabolic process
Solyc02g091190.2.1	idenosyl-L-methionine-dependent methyltransferases superfamily prot	PC-69-3p	GO:0008152	biological process	metabolic process
Solyc12g014350.1.1	Leucine-rich repeat transmembrane protein kinase	PC-52-5p	GO:0004672	molecular function	protein kinase activity
Solyc12g014350.1.1	Leucine-rich repeat transmembrane protein kinase	PC-52-5p	GO:0005515	molecular function	protein binding
Solyc12g014350.1.1	Leucine-rich repeat transmembrane protein kinase	PC-52-5p	GO:0005524	molecular function	ATP binding
Solyc01g105370.2.1	Aldolase-type TIM barrel family protein	PC-44-3p	GO:0016491	molecular function	oxidoreductase activity
Solyc01g105370.2.1	Aldolase-type TIM barrel family protein	PC-44-3p	GO:0018580	molecular function	nitronate monooxygenase activity
Solyc07g051850.2.1	aspartic proteinase A1	PC-46-5p	GO:0004190	molecular function	spartic-type endopeptidase activity
Solyc02g091190.2.1	idenosyl-L-methionine-dependent methyltransferases superfamily prot	PC-69-3p	GO:0008168	molecular function	methyltransferase activity

Supplementary Table S8. KEGG pathway analysis of the target genes cleaved by miRNAs in tomato LA2093

Transcript ID	Annoation	miRNA	KEGG ID	KEGG name
Solyc04g008740.2.1	Pyruvate kinase family protein	spi-miR2275c-5p_ata	ko00010	Glycolysis / Gluconeogenesis
Solyc01g073740.2.1	Citrate synthase family protein	spi-miR396b-p5_osa	ko00020	Citrate cycle (TCA cycle)
Solyc03g115820.2.1	D-ribulose-5-phosphate-3-epimerase	spi-miR3630-3p_vvi	ko00030	Pentose phosphate pathway
Solyc03g115820.2.1	D-ribulose-5-phosphate-3-epimerase	spi-miR3630-3p_vvi	ko00040	Pentose and glucuronate interconversions
Solyc04g008740.2.1	Pyruvate kinase family protein	spi-miR2275c-5p_ata	ko00230	Purine metabolism
Solyc03g005260.2.1	ATP sulfurylase 1	spi-miR395a_stu	ko00230	Purine metabolism
Solyc03g005730.2.1	propyl malate isomerase large subuni	spi-miR384-5p_stu	ko00290	Valine, leucine and isoleucine biosynthesis
Solyc03g005260.2.1	ATP sulfurylase 1	spi-miR395a_stu	ko00450	Selenoamino acid metabolism
Solyc08g063080.2.1	sulfoquinovosyldiacylglycerol 1	spi-miR9470-3p	ko00520	mino sugar and nucleotide sugar metabolism
Solyc09g020190.2.1	non-specific phospholipase C1	spi-miR9470-3p	ko00562	Inositol phosphate metabolism
Solyc09g020190.2.1	non-specific phospholipase C1	spi-miR9470-3p	ko00564	Glycerophospholipid metabolism
Solyc04g008740.2.1	Pyruvate kinase family protein	spi-miR2275c-5p_ata	ko00620	Pyruvate metabolism
Solyc04g008740.2.1	Pyruvate kinase family protein	spi-miR2275c-5p_ata	ko00710	Carbon fixation in photosynthetic organisms
Solyc05g054080.2.1	ubiquitin protein ligase 5	spi-miR9471a-3p	ko04120	Ubiquitin mediated proteolysis
Solyc06g061000.2.1	casein kinase II beta subunit 4	spi-miR319-p5_stu	ko04310	Wnt signaling pathway
Solyc06g061000.2.1	casein kinase II beta subunit 4	spi-miR319-p5_stu	ko04520	Adherens junction
Solyc06g061000.2.1	casein kinase II beta subunit 4	spi-miR319-p5_stu	ko04530	Tight junction
Supplementary	Table S9. Specific	qRT-PCR primers	s for the miRNAs identified in tomato LA2093	
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Number	miRNAs	NAs Stem-loop primer		qRT-PCR primer	Sequence (5'to 3') for qRT-PCR	Length of amplified fragment
1 spi-miR159	GTCGT & TTCTGTGCTGCGTGCA & CTCTCTCCCA CTCA & TACCACCA CCTC	50	F	CGGACGAGTTTGGATTGA	60	
	UICUTATICIUICIUCUUUUAACIUICICUCACIUAATACUACUAUCIC		R	TATTCTGTGCTGGGTGGA		
2 spi-miR168a-5p	GTCGTATTCTGTGCTGGGTGGAACTGTCTCGCACTGAATACGACGTCCCG	50	F	CGGACGAGTCGCTTGGTG	67	
			R	CGTATTCTGTGCTGGGTGGA	02	
3 spi-miR171d	GTCGTATTCTGTGCTGGGTGGAACTGTCTCGCACTGAATACGACCGTGAT	50	F	AAGGACGAGTTGAGCCGC	60	
			R	GTATTCTGTGCTGGGTGG	00	
4 spi-miR319a	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAGGAG	50	F	ACGGCGTTGGACTGAAGG	60	
			R	CGCAGGGTCCGAGGTATT	00	
5 spi-miR408b-3p_stu	GCGTGGTCCACACCACCTGAGCCGCCACGACCACGCAGCCAGGG	44	F	CAACAATGCACTGCCTCTTCC	57	
			R	TCCACACCACCTGAGCCG		
6 PC-49-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACCACGTT	50	F	CCGCCGCTATGAGATAAGTTC	63	
			R	CAGTGCAGGGTCCGAGGTA		
7 PC-65-3p	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGGGGT	50	F	CGGTCAACATGTGTTCTCAGGT	65	
			R	CAGTGCAGGGTCCGAGGTAT		
Reference U6snRNA		1	F	GGAACGATACAGAGAAGATTAGCA	A ,	
	UOSIIKINA	SIIKINA GIGUAGGGIUUGAGGIIIIIGGAUUAIIIUIUGAI	/	R	GTGCAGGGTCCGAGGT	/

Number	Target genes	Primer	Sequence (5'to 3')	Length of amplified fragment	miRNAs	
1	Solyc12g014120.1.1	F	TTACCACCACCATCAACAAT	152	ani miD150	
		R	ACAGACGACCAACTTAACTC	132	spi-mik139	
2	Solyc11g013470.1.1	F	CGCCAATCCCACAGTTTC	171	spi-miR160a	
		R	GGGCTACTGAGTCCAATCGT	171		
3	Solyc06g072300.2.1	F	TCTTGATGATGATGATGGA	160	spi-miR168a-5p	
		R	CTCACGCAGAACAATATC	109		
4	Solyc11g013150.1.1	F	CAGGTGACTCCAATAACAATC	190	spi-miR171d	
		R	AATACGCAGCCAATCTCT	189		
5	Solyc02g070550.2.1	F	TCAAGCGAAGAAGAAGAAGAAGAAGGA	160	spi-miR8029_stu	
		R	GAAGCGGTCTGAGAAGGTATAGG	102		
6	Solyc02g091190.2.1	F	CAGCGTGAGATTGACATT	192	PC-69-3p	
		R	AAGAACTTGACATTGGTGAA	182		
Reference	Actin	F	GAAATAGCATAAGATGGCAGACG	150	/	
		R	ATACCCACCATCACACCAGTAT	139		

Supplementary Table S10. Specific qRT-PCR primers for the target genes cleaved by miRNAs in tomato LA2093