Tuning *LeSPL-CNR* expression by SlymiR157 affects tomato fruit ripening

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Running title: Roles of miR157/6 in tomato ripening and softening

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Supplementary information

Supplementary Table 1 Primers for construction of *Mr* and *Sir* VIGS and miRNA:GFP reporter vectors

Supplementary Table 2 Primers for quantitative and semi-quantitative RT-PCR detections and genome walking

Supplementary Figure Legends

Supplementary Figure 1 | Localization of the potential SlymiR157/6 target site in *LeSPL-CNR*. (**a**) *LeSPL-CNR* expression from DNA to mRNA to protein. The potential miRNA target site is located in the 3'-UTR of the *LeSPL-CNR* mRNA. The cytosine hypermethylated (^{m}C) region of 286-nt, exons 1 and 2 and relative positions and the mRNA and protein sizes are indicated. (**b**) Comparisons of miR157/6 among different plant species. Non-conserved ribonucleotides are underlined and highlighted red.

Supplementary Figure 2 | Viral delivery of mature SlymiR157 delays tomato fruit ripening. (**a**) PVX-based *Mr* VIGS vectors PVX/SlymiR156 and PVX/SlymiR157. Genome organization (166K, 25K 12K, 8K and coat protein (CP)) of PVX are indicated. (**b**) PVX delivery of mature SlymiR157 postponed ripening of AC fruits. Ripening delayed sectors and pericarp in dissected fruits are green or yellow. (**c**) Expression of SlymiR156 from PVX/SlymiR156 did not affect normal ripening, but enhanced the process of softening after breaker and the red ripe stages. Mock- or empty PVX-treated

AC fruits ripened normally. Photographs were taken at 8, 11, or 14 days after breaker (Br +8, Br + 11, or Br + 14).

Supplementary Table 1 Primers for construction of Mr and Sir VIGS and miRNA:GFP reporter vectors

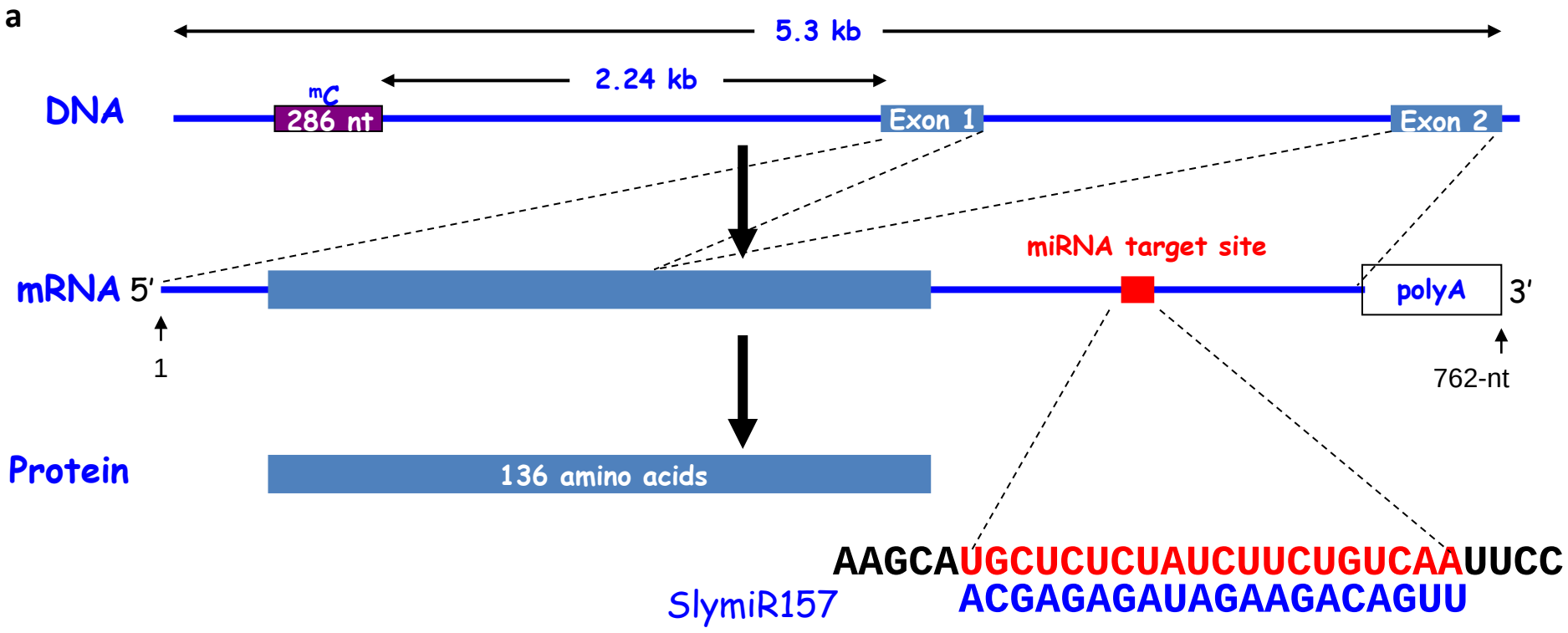
Construct	Primer sequence (5'-3')	Gene or fragment amplified	Use
PVX/pre-SlymiR157	F: CGGGGGATCGATTTTGACAGAAGATAGA (*Cla*I) R: TACATACGGCCGATGACGGAAGCATAGA (*Eag*I)	pre-SlymiR157	PCR cloning pre-SlymiR157 into the *Cla*I and *Eag*I sites of PVX
PVX/SlymiR157	F: CGATTGACAGAAGATAGAGAGCAC (*Cla*I/*Eag*I) R: GGCCGTGCTCTCTATCTTCTGTCAAT (*Eag*I/*Cla*I)	SlymiR157	Cloning mature SlymiR157 into the *Cla*I and *Eag*I sites of PVX
PVX/SlymiR156	F: CGATGACAGAAGAGAGTGAGCAC (*Cla*I/*Eag*I) R: GGCCGTGCTCACTCTCTTCTGTCAT (*Eag*I/*Cla*I)	SlymiR156	Cloning mature SlymiR156 into the *Cla*I and *Eag*I sites of PVX
TRV/pre-SlymiR157	F: GCGGGGTACCTTTGACAGAAGATAGA (*Kpn*I) R: TTGGGGTCTAGAATGACGGAAGCATAGA (*Xba*I)	pre-SlymiR157	PCR cloning pre-SlymiR157 into the *Kpn*I and *Xba*I sites of TRV
PVX/mLeSPL-CNR	F: CCTCACATCGATTAGGAAACTAACAAATGGGAAGGGA (*Cla*I) R: GATGCTCGGCCGTCAGCCCAAATTTTCTCCATGAGAG (*Eag*I)	Nonsense-mutated *LeSPL-CNR*	PCR cloning mLeSPL-CNR into the *Cla*I and *Eag*I sites of PVX
PVX/SlymiR157Tar::GFP	F: CGATATGGTGCTCTCTATCTTCTGTCAAC (*Cla*I/*Eag*I) R: GGCCGTTGACAGAAGATAGAGAGCACCATAT (*Eag*I/*Cla*I)	SlymiR157 target site	Cloning SlymiR157 target sequence into the *Cla*I and *Eag*I site of PVX/GFP
PVX/SlymiR157::GFP	F: CGATATGTTGACAGAAGATAGAGAGCACC (*Cla*I/*Eag*I) R: GGCCGGTGCTCTCTATCTTCTGTCAACATAT (*Eag*I/*Cla*I)	SlymiR157	Cloning SlymiR157 into the *Cla*I and *Eag*I site of PVX/GFP
PVX/mSlymiR157::GFP	F: CGATATGT-GACAGAA-ATAGAGAGCACC (*Cla*I/*Eag*I) R: GGCCGGTGCTCTCTAT-TTCTGTC-ACATAT (*Eag*I/*Cla*I)	SlymiR157 target site	Cloning mutated SlymiR157 into the *Cla*I and *Eag*I site of PVX/GFP

Supplementary Table 2 Primers for quantitative and semi-quantitative RT-PCR detection and genome walking

Primer	Sequences (5' - 3')	Gene or fragment amplified	Annealing temperature	Detection	Orgin
LeSPL-CNR-F	TGGAAACTAACAAATGGGAAGGG	*LeSPL-CNR*	58℃	qRT-PCR for *LeSPL-CNR* RNA	*LeSPL-CNR*
LeSPL-CNR-R	GGCGATGGTATGGCTTGG				
LeMADS-RIN-F	AAGGAACCCAAACTTCATCAG	*LeMADS-RIN*	58℃	qRT-PCR for *LeMADS-RIN* RNA	*LeMADS-RIN*
LeMADS-RIN-R	TTGTCCCAAATCCTCACCTA				
LeHB1-F	CTACGACGAGCAGTCACCG	*LeHB1*	58℃	qRT-PCR for *LeHB1* RNA	*LeHB1*
LeHB1-R	GGAACCATACAGCCACCT				
SlAP2a-F	GGAGTATGAATCCGATGAAGGT	*SlAP2a*	58℃	qRT-PCR for *SlAP2a* RNA	*SlAP2a*
SlAP2a-R	CGATTCCAAATTGTGGTCTT				
SlTAGL1-F	GCAATAACTCCCTGCCTGTA	SlTAGL1	55℃	qRT-PCR for *SlTAGL1*RNA	*SlTAGL1*
SlTAGL1-R	AGATGAAGAGCCTTGACCC				
SlymiR157-F	TATGATGGAATGCTGCTGC	Pre-SlymiR157	58℃	qRT-PCR for pre-miR157 RNA	Pre-SlymiR157
SlymiR157-R	GCATAGAGAGCACAAATGAAGT				
PVX-F	CAGTGTTGGCTTGCAAACTAG	PVX::pre-SlymiR157	58℃	qRT-PCR for recombinant	PVX/pre-SlymiR157
SlymiR157-R	GCATAGAGAGCACAAATGAAGT			PVX::pre-SlymiR157 RNA	
PVX-F	CAGTGTTGGCTTGCAAACTAG	PVX::SlymiR157 RNA	58℃	qRT-PCR for recombinant	PVX/SlymiR157
PVX/SlymiR157-R	GGCCGTGCTCTCTATCTTCTGTCAAT			PVX::SlymiR157 RNA	
SlymiR157	TTGACAGAAGATAGAGAGCACA	pre-SlymiR157/mature SlymiR157	58℃	qRT-PCR and genome walking	SlymiR157
SlymiR156a	TTGACAGAAGATAGAGAGCAAA	SlymiR156a	58℃	qRT-PCR	SlymiR156a
PVX-F	CAGTGTTGGCTTGCAAACTAG	PVX::SlymiR157Tar::GFP	58℃	Semi qRT-PCR for recombinant	PVX/pre-SlymiR157Tsr::GFP
GFP-R	TTTGTGTCCAAGAATGTTTCCATCTTC	PVX::SlymiR157::GFP		PVX::pre-SlymiR157Tar::GFP RNA	
		PVX::mSlymiR157::GFP			
		PVX::GFP			

Supplementary Figure 1 (Hong)

a



b

Tomato SlymiR157	UUGACAGAAGAUAGAGAGCA
Tomato SlymiR157a	UUGACAGAAGAUAGAGAGCAC
Arabidopsis miR157	UUGACAGAAGAUAGAGAGCA
Nicotiana benthamiana miR157	UUGACAGAAGAUAGAGAGCA
Tomato SlymiR156	UGACAGAAGAGAGUGAGCAC
Arabidopsis miR156	UGACAGAAGAGAGUGAGCAC
Glycine max miR156	UUGACAGAAGAUAGAGAGCA
Populus tricharpa miR156	UUGACAGAAGAUAGAGAGCA
Selaginella moellendorffii miR156	UGACAGAAGAUAGAGAGCA
Rice miR156	UGACAGAAGAGAGAGAGCA
Medicago truncatula miR156	UGACAGAAGAGAGAGAGCA
Zea mays miR156	UGACAGAAGAGAGAGAGCA

Supplementary Figure 2 (Hong)