Supplemental Data

The *Arabidopsis* RNA-binding protein FCA regulates microRNA172 processing in thermosensory flowering

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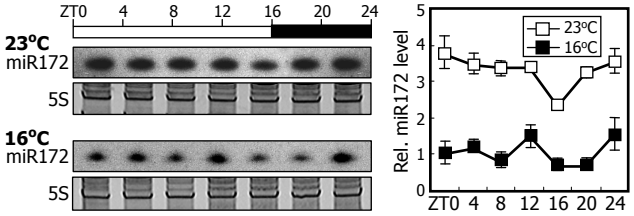

Figure S1. miR172 levels in Col-0 plants grown at either 23 ºC or 16 ºC.
Ten-day-old whole plants grown on MS-agar plates under LDs were harvested at different zeitgeber time (ZT) points. miR172 levels were examined by northern blot analysis. The 5S rRNA (5S) was used as loading control. The intensities of bands on the blots (*left panel*) were quantitated by densitometry of images (*right panel*). Values of 3 representative blots were averaged.

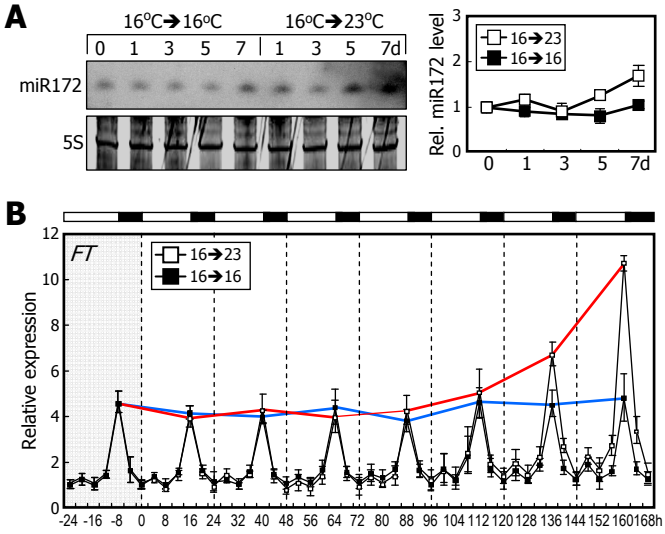

Figure S2. Kinetic measurements of miR172 abundance and *FT* mRNA levels after transfer from 16 ºC to 23 ºC.
Two-week-old plants grown at 16 ºC were either maintained at 16 ºC or transferred to 23 ºC. Whole plants were harvested at different zeitgeber time (ZT) points for up to 7 days.
A, Kinetic measurements of miR172 levels. miR172 levels were examined by northern blot analysis. The 5S rRNA (5S) was used as loading control. The intensities of bands on the blots (*left panel*) were quantitated by densitometry of images (*right panel*), as described in supplemental Fig. S1. d, days after transfer.
B, Kinetic measurements of *FT* mRNA levels. *FT* mRNA levels were determined by quantitative real-time RT-PCR (qRT-PCR). Biological triplicates using different plant samples were averaged. Bars indicate standard error of the mean. The red and blue lines indicate peaks of *FT* mRNA levels in plants grown at 23 ºC and 16 ºC, respectively. h, hours after transfer.

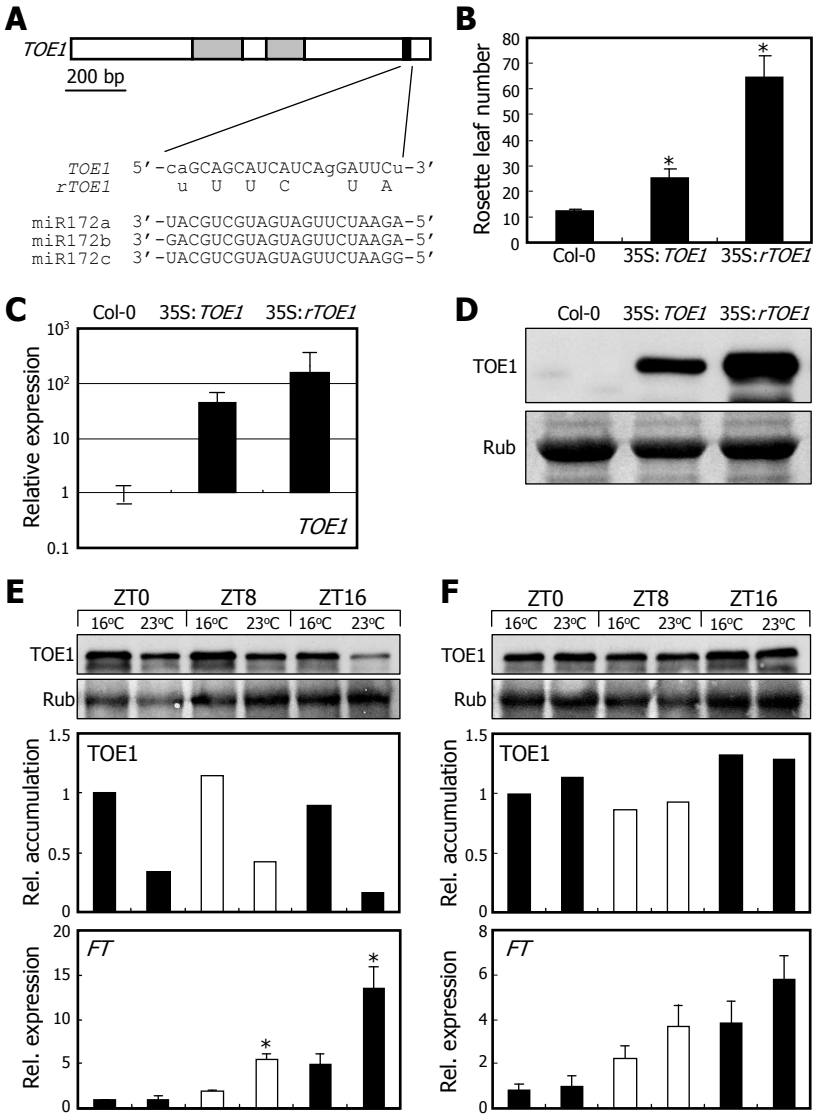

Figure S3. Reduced effects of low temperatures on *FT* expression in transgenic plants overexpressing a miR172-resistant *TOE1* (*rTOE1*) gene.

A, *TOE1* gene constructs examined. bp, base pair.

B, Flowering phenotypes of transgenic plants overexpressing *TOE1-MYC* and *rTOE1-MYC* gene fusions (35S:*TOE1* and 35S:*rTOE1*, respectively). The 35S:*TOE1* and 35S:*rTOE1* transgenic plants were grown in soil under LDs until flowering. Numbers of rosette leaves of 20 plants were counted and averaged for each plant genotype. Statistical significance of the measurements was determined using a student *t*-test (*P<0.01). Bars indicate standard error of the mean.

C and *D*, Levels of *TOE1* mRNA (*C*) and TOE1 protein (*D*) in the 35S:*TOE1* and 35S:*rTOE1* transgenic plants. *TOE1* mRNA levels were determined by qRT-PCR, as described in supplemental Fig. S2*B*. Levels of TOE1 proteins were examined by Western blot analysis using an anti-MYC antibody.

E and *F*, Levels of TOE1 protein (*top and middle panels*) and *FT* mRNA (*bottom panels*) in the 35S:*TOE1* (*E*) and 35S:*rTOE1* (*F*) transgenic plants grown at either 23 ºC and 16 ºC. Whole plants grown on MS-agar plates for 10 days were used for preparation of total protein extract and RNA.

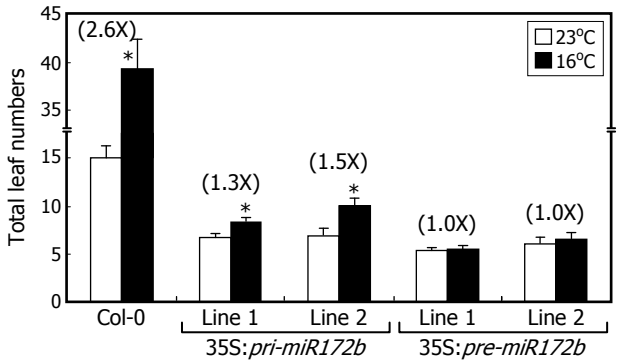

Figure S4. Flowering phenotypes of 35S:*pri-miR172b* and 35S:*pre-miR172b* transgenic plants at 16 °C. Plants were grown in soil at either 23 °C or 16 °C under LDs until flowering. Two independent lines were examined for the 35S:*pri-miR172b* and 35S:*pre-miR172b* transgenic plants. Numbers of total leaves of 20 plants were averaged for each plant genotype and statistically treated using a student *t*-test (*P<0.01). Bars indicate standard error of the mean. The numbers in parentheses refer to the ratios of total leaf numbers at 16 °C and 23 °C (16 °C/23 °C).

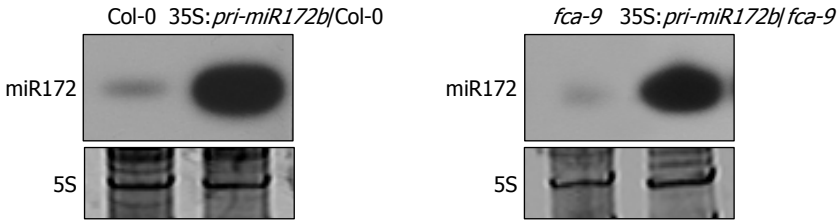

Figure S5. mlR172 levels in 35S:*pri-miR172b* transgenic plants in Col-0 and *fca-9* backgrounds. Ten-day-old whole plants grown on MS-agar plates at 23 °C were used for total RNA extraction. miR172 levels were examined by northern blot analysis. The 5S rRNA (5S) was used as loading control. Note that miR172s are extremely higher in the 35S:*pri-miR172b* transgenic plants than in Col-0 plants and *fca-9* mutant.

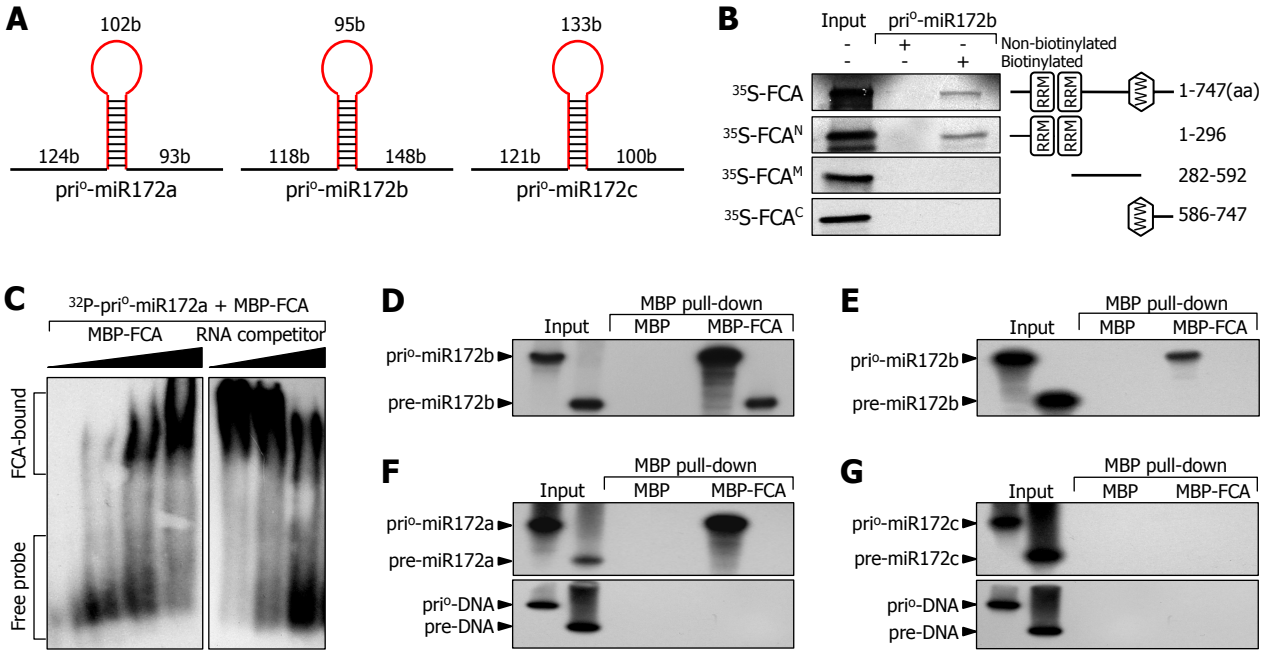

Figure S6. *in vitro* binding of FCA protein to pri°-miR172a, pri°-miR172b, and pri°-miR172c RNAs. Recombinant FCA protein was prepared as maltose binding protein (MBP)-FCA fusion in *Escherichia coli* cells. The *in vitro* transcribed and ^{32}P-labeled pri°-miR172 and pre-miR172 RNAs were used in the assays.
A, Constructs of pri°-miR172a, pri°-miR172b, and pri°-miR172c RNAs examined. The pri°-miR172 RNAs contain 93-148 nucleotides at both sides of the stem-loop structures (designated in red) of the pre-miR172 RNAs.
B, *in vitro* RNA binding assays using truncated FCA proteins. The ^{35}S- labeled FCA deletions prepared by *in vitro* translation were used in the pull-down assays. The *in vitro* transcribed and biotinylated pri°-miR172b RNA was used. RRM, RNA recognition motif. WW, tryptophan-tryptophan motif. aa, amino acid.
C, Electrophoretic mobility shift assays (EMSAs) on FCA binding to pri°-miR172a RNA. Increasing amounts of recombinant MBP-FCA fusion proteins (0, 0.1, 0.5, and 2 μg) were added to the assay mixtures (*left panel*). To verify specific binding, increasing amounts of unlabeled pri°-miR172a RNA (0x, 5x, and 25x) were also included in the assays (*right panel*).
D and *E*, *in vitro* binding of MBP-FCA protein to pri°-miR172b and pre-miR172b RNAs. After binding of RNAs to the MBP-FCA fusion protein, the beads were rinsed 10 times with RNA binding buffer containing either 0.1% Triton X-100 (*D*) or 0.3% Triton X-100 (*E*).
F and *G*, *in vitro* binding of MBP-FCA protein to pri°-miR172a and pri°-miR172c RNAs. PCR-amplified and ^{32}P-labeled DNA fragments corresponding to the RNA molecules were also included in the assays (*lower panels*). It was found that the MBP-FCA fusion protein bound to the pri°-miR172a RNA (*F*) but did not bind to the pri°-miR172c RNA (*G*).

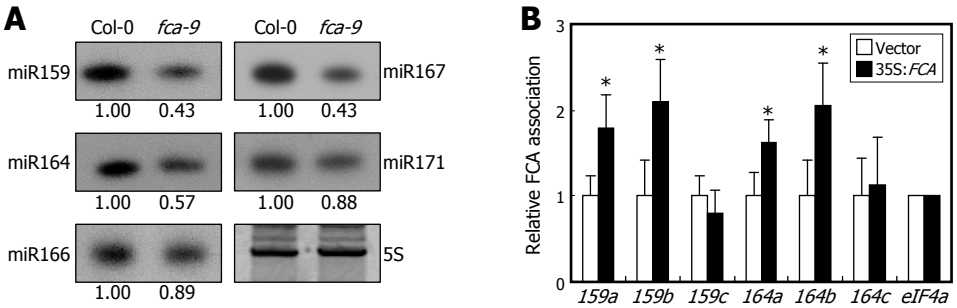

Figure S7. FCA binding to primary transcripts of miRNAs that are temperature-insensitive.
A, Levels of miRNAs in *fca-9* mutant. Two-week-old plants grown at 23 °C on MS-agar plates were harvested at ZT16. miRNA levels were examined by northern blot analysis. Note that the levels of miR159, miR164, and miR167 are reduced detectably in the *fca-9* mutant.
B, RNA immunoprecipitation (RIP) assays on *in vivo* binding of FCA to pri-miR159 and pri-miR164 transcripts. For RIP assays, transgenic plants overexpressing a *FCA-MYC* gene fusion under the control of the Cauliflower Mosaic Virus (CaMV) 35S promoter (35S:*FCA*) were used. Transgenic plants containing the 6xMYC-pBA vector alone were used as control. Total proteins extracted from the 35S:*FCA* transgenic plants and control plants were immunoprecipitated with an anti-MYC antibody. RNA samples eluted from the protein-RNA complexes were analyzed by qRT-PCR (*t*-test, *P<0.05).

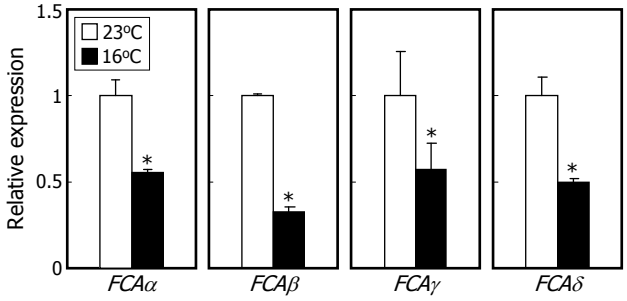

Figure S8. Effects of ambient temperature on *FCA* gene transcription.
Plants were grown on MS-agar plates at either 23 °C or 16 °C for 10 days before harvesting whole plants. mRNA levels were determined by qRT-PCR (*t*-test, *P<0.05), as described in supplemental Fig. S2*B*. Primer pairs used are specific to individual alternatively spliced variants of *FCA* gene and listed in supplemental Table S1.

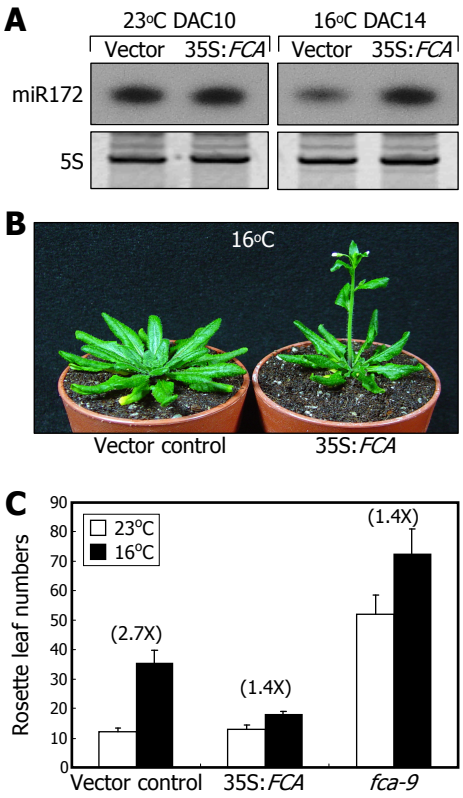

Figure S9. Molecular and morphological characterization of 35S:*FCA* transgenic plants and *fca-9* mutant.

The 35S:*FCA* transgenic plants and vector control plants were used for the assays.

A, miR172 abundance. Plants were grown on MS-agar plates at either 23 °C for 10 days or at 16 °C for 14 days before harvesting whole plants for extraction of total RNA. DAC, days after cold-imbibition.

B, Phenotypes of 35S:*FCA* transgenic plant grown at 16 °C. Plants were grown in soil at 16 °C for 2 months under LDs before taking photographs.

C, Flowering phenotypes of 35S:*FCA* transgenic plants and *fca-9* mutant at 23 °C and 16 °C. Plants were grown in soil at either 23 °C or 16 °C under LDs until flowering. Thirty plants were counted and averaged for each plant genotype. Bars indicate standard error of the mean. The numbers in parentheses refer to the ratios of rosette leaf numbers at 16 °C and 23 °C (16 °C/23 °C).

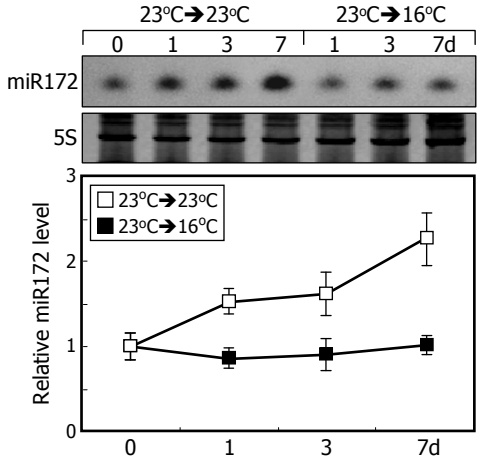

Figure S10. Kinetic measurements of miR172 level in *fy-2* mutant after transfer from 23 °C to 16 °C. Seven-day-old plants grown on MS-agar plates at 23 °C were further grown at either 23 °C or 16 °C for up to 7 days. Whole plants were harvested at ZT14 for total RNA extraction. miRNA levels were examined by northern blot analysis (*upper panel*). The 5S rRNA was used as loading control. The intensities of blots were quantitated by densitometry of images (*lower panel*), as described in supplemental Fig. S1. d, days.

Primers	Sequences
FCAα-F	5′-TTTAATGCAGGCTGTTGTTTTG
FCAα-R	5′-CAGTTCCCCCAGGAAGAGTG
FCAβ-F	5′-ACAGAAGAAGAAATCCGTCCCT
FCAβ-R	5′-AAAATGGAGATTGACATACCTTGC
FCAγ-F	5′-GCTCTTGTCGCAGCAAACTC
FCAγ-R	5′-GATCCAGCCCACTGTTGTTT
FCAδ-F	5′-CAGCTGCAGTAAGTCAGAGCG
FCAδ-R	5′-CACCTTGCTTTCACCCGTTA
FT-F	5′-TCCTAGCAACCCTCACCTCC
FT-R	5′-ATTGTAGAAAACTGCGGCCA
MIR159a-F	5′-GCTATGGATCCCATAAGCCC
MIR159a-R	5′-GAGCTCCCTTCAATCCAAAGA
MIR159b-F	5′-ATGGAGGGTTTAGCAGGGTG
MIR159b-R	5′-GAGCTCCCTTCAATCCAAAGA
MIR159c-F	5′-CATAGAGAGTGCGCGGTGTT
MIR159c-R	5′-AAGGAGCTCCCTTCAATCCA
MIR164a-F	5′-ATGCGGGTGAGAATCTCCAT
MIR164a-R	5′-GGGTGAAGAGCTCATGTTGG
MIR164b-F	5′-AAGATGGAGAAGCAGGGCAC
MIR164b-R	5′-TCATGATGGTGAAGATGGGC
MIR164c-F	5′-ACTTGATGGAGAAGCAGGGC
MIR164c-R	5′-ATCCATTGACGATTGCATCC
MIR172a-F	5′-GACTAATTTCCGGAGCCACG
MIR172a-R	5′-TAGTCGTTGATTGCCGATGC
MIR172b-F	5′-GGATCCATGGAAGAAAGCTCA
MIR172b-R	5′-GCAGCATCATCAAGATTCTCAT
MIR172c-F	5′-CATCAACCAGCTACTGTTCGC
MIR172c-R	5′-GTGTAAGCCACTGATTGCAGC
MIR172d-F	5′-TGGCAGTCATTGTTTGCTATTG
MIR172d-R	5′-CGCTGCAGCATCATCAAGA
MIR172e-F	5′-GGCTAGCCTTTGGTGGATGT
MIR172e-R	5′-TCCCACTCGATAAGGAACCC
MIR398a-F	5′-TGAAATTTCAAAGGAGTGGCAT
MIR398a-R	5′-GGGAGATTCAAAGGGGTGAC
MIR398b-F	5′-TGGATCTCGACAGGGTTGAT
MIR398b-R	5′-AAGAGCTCAGCAGGGGTGAC
MIR398c-F	5′-TGGATCTCGACAGGGTTGATAT
MIR398c-R	5′-AAGAGCTCAGCAGGGGTGAC
MIR399a-F	5′-AAATGCATTACAGGGTAAGATCTCTATT
MIR399a-R	5′-AGAAGAATTACAGGGCAAATCTC
MIR399b-F	5′-TCACTAGTTTTAGGGCGCCTC
MIR399b-R	5′-GAACCAGTTTCAGGGCAACTC
MIR399c-F	5′-GGAGCAGTAATAGGGCATCTTTC
MIR399c-R	5′-GAAGCAGTGACAGGGCAACT
MIR399d-F	5′-GGTTGGATTACTGGGCGAAT
MIR399d-R	5′-TTTTGCATGGGATGAATTGC
MIR399e-F	5′-GAAAGCATTACAGGGCGAATC
MIR399e-R	5′-TGAAGCATTGCGAGGCAAAT
MIR399f-F	5′-ATATGCATTACAGGGCAAGATCA
MIR399f-R	5′-AAGAGAATTACCGGGCAAA
TOE1-F	5′-TCGGATCATCGCCTTTACTG
TOE1-R	5′-TGATGCTGCTGCAACTGAGA

Table S1. Primers used in qRT-PCR. F, forward primer; R, reverse primer.