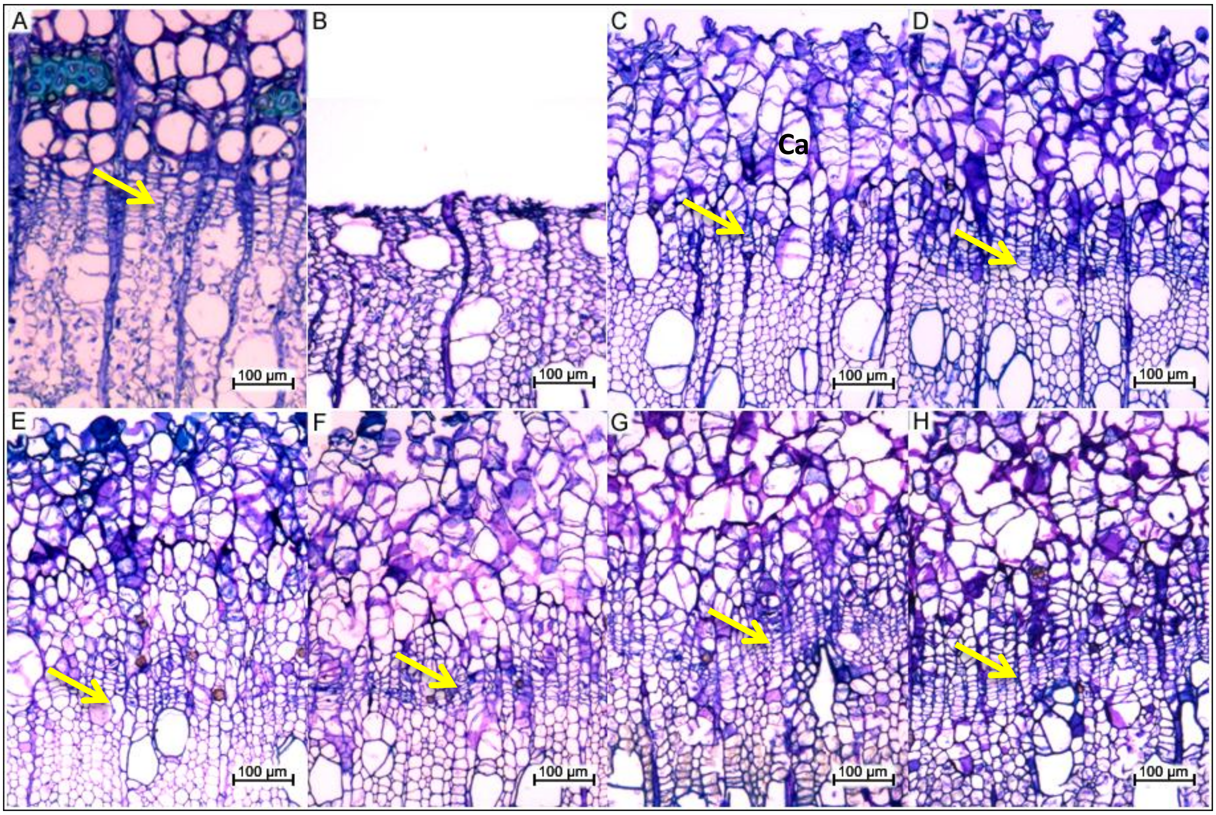

Figure S1. Cross sections of SVS regenerated tissues upon debarking. (A) the phloem, cambium zone and majority part of immature xylem were peeled off with the bark. (B) A few layers of immature xylem remained on the surface of the girdled trunk. (C) The callus covered the whole surface of the girdled trunk on the 7 DAG and discontinuous meristem cells were observed. (D) Continuous, flat meristem cells arranged irregularly between the callus and immature xylem on the 10 DAG. (E) The flat meristem cell layers had increased in size on the 12 DAG. (F) The flat meristem cell layers formed continuous and regular layers that are similar to a vascular cambium on the 16 DAG. (G) The newly cambium-like zone began to differentiate on the 18 DAG. (H) A large number of phloem cells outside the cambium and xylem cells inside the cambium were observed on the 21 DAG. The yellow arrows indicate the meristem cells and cambial region.

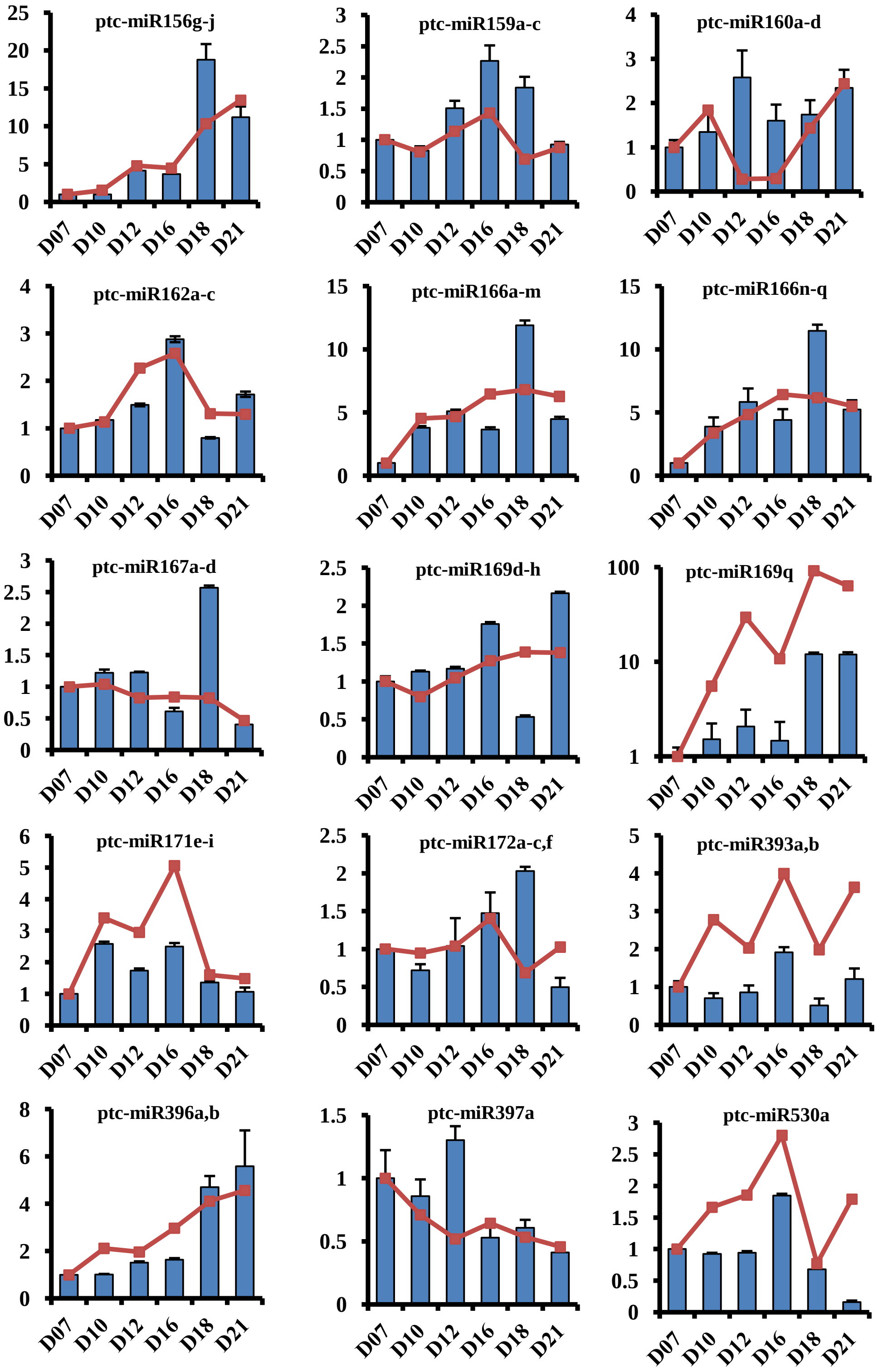

Figure S2. The expression profiles of known miRNAs during SVS regeneration based on sequencing and qRT-PCR analyses. The line chart represents the relative expression fold changes of known miRNAs from solexa sequencing reads and the histogram indicates the relative expression fold changes of known miRNAs analyzed by real-time quantity PCR. Transcript levels on the 7 DAG are set to 1. The X-axis shows the regeneration stages on the 7, 10, 12, 16, 18 and 21 DAG. The Y-axis shows the relative expression fold changes of known miRNAs.

pto-MIR003, ΔG = -61.00

```
   10        20       30         40        50
     U  U     UU    C         UG           AC-|
GAGGA CA AAAGA  GAUG AGCCAAGGA  ACUUGCCGACG   U
CUCCU GU UUUCU  UUAC UCGGUUCUU  UGGACGGUUGC   A
     C  C     UU    A         GU           CUA^
      130       120       110       100
```

pto-MIR037a, ΔG = -57.00

```
  10        20        30        40        50
      A               C            A    GAU
GUUAUA CAUCUUGAUCAAUGG CAUUGUAAGAGU GAAG   C
UAAUAU GUAGAAUUAGUUACC GUGACAUUCUCA CUUU   G
      G               C            -    AU-
  140       130       120       110
```

pto-MIR045b, ΔG = -80.40

```
   10         20        30          40        50
   AAC-   A         GUA   A     .-U|      UUCAU
GUA    AAC CAACUGGAU   GAU GGAGU   GAGACUA     A
CAU    UUG GUUGAUCUG   UUG CCUCA   CUCUGGU     U
   AGGA   -         AUG   -     \ -^      UCUUU
       330        320        310     60
```

pto-MIR048b, ΔG = -72.60

```
 10        20        30        40        50
 GUCUAUUUUUUG                               CA-
A            AUCAGCAAUGUAGUCCUUCCGUCAACUUUGA   G
U            UGGUCGUUACAUCAGGAAGGCAGUUGAAACU   U
 ------------                               CUA
                   120        110      100
```

pto-MIR064b, ΔG = -43.90

```
        10        20            30          40
A         AUUA      A  --  --       .-UAAUUUAA|
 AUUUAUGUA    GUCGAG UA  CA  UGCAAGC          A
 UGAGUACGU    CAGCUU AU  GU  ACGUUUG          U
C         C---      A  UA  AA       \ --------^
    250           240      230
```

pto-MIR072, ΔG = -45.40

```
     10        20        30          40
U      ACAU      AGAU     GG     U     .-C
 UUGCC     UAGAUA    UUUGA  GUGGA GUUUU   G
 AACGG     GUUUGU    AAAUU  CGCCU CAAGA   C
-      ----      GC--     GA     -     \ -
            150         140        130
```

pto-MIR074, ΔG = -71.50

```
    10         20        30        40
AU|         UA    UU       G   CU          -   G
  GGAGAGGGAG  GGGU  UCGGAAG UUA  GGAUGGGUG AGU G
  CCUCUCCCUC  CUUA  AGCCUUU GAU  CCUACCCAC UCA C
CU^         --    CU       G   AC          C   A
   120         110       100        90        80
```

pto-MIR077, ΔG = -24.20

```
      10          20         30         40
    UUCAU    --          AG      .-ACAUAC       C
UCAA     GGAA  GAAAGAAUU  AUGUC        AAGGAGC A
AGUU     CCUU  UUUUUUUGA  UACAG        UUUUUCG U
    UUC--    UA          A-      \ ------       A
    150         140        130              100
```

pto-MIR079, ΔG = -48.00

```
       20        30        40          50
      C          C           A     .-AAA
UGCACG UCUCGUAAUG UUCAUUCUCAC ACGGC     G
AUGUGU AGAGUAUUAC AAGUAAGGGUG UGCCG     U
      C          -           C     \ ---
   110       100         90              60
```

pto-MIR093, ΔG = -38.90

```
     10       20         30               40
A        UG G         U  -    C      AU-------|  UC
 CUGGAAGU  U GGCAUGAGG GU UUGG AAGAAA         GGA  \
 GACCUUUA  A CCGUACUCU CA AACC UUCUUU         CCU  U
-        GU A         C  U    A      AGUAGUAUU^  UU
    90        80        70        60        50
```

pto-MIR014a, ΔG = -48.30

```
        10        20        30        40          50
ACAUAUCCAUU                       A       .-AAAAAUUA|
           AAAUGAUGACAUGGACACCAAAA CUUACAA          G
           UUUACUACUGUACCUGUGGUUUU GAAUGUU          C
AAAAAAAAAAU                       A       \ --------^
 .       180       170       160       150
```

pto-MIR043c, ΔG = -29.80

```
        10        20         30          40
U       CUA  U       -    G       .-GCCCA|   A
 GGAGAGU   CA AUAUCAC AAAA UAGCUGA       CGAG \
 UUUUUCA   GU UAUAGUG UUUU GUUGACU       GUUC A
-       CC-  -       C    G       \ -----^   A
  130         120       110
```

pto-MIR047a, ΔG = -81.10

```
         10        20        30        40
-   G        A     U            UC        AU
 GGG CUUACAUG AGAGG AGAGAGUGUAAU  AGCCAAGG  GA
 UCC GAAUGUAC UCUUC UCUCUCAUGUUA  UCGGUUCC  UU
A   G        -     U            CA        CG
  160        150       140       130       120
```

pto-MIR056a, ΔG = -53.20

```
         20          30        40         50
    -   UU  --      U                   C-
UGCC ACG  GU  UCAUUCC CUUCCUAAAAUGGCUUCUC  U
GUGG UGU  CA  AGUAAGG GAAGGAUUUUAUCGAAGAG  A
    C   GG  UA      -                   UA
    130       120        110        100
```

pto-MIR071, ΔG = -72.30

```
        10        20        30        40
AU|         UA    UU       G    C         -  CG
  GGAGAGGGAG  GGAU  UCGGAAG UUAU GGGUGGGUG AG  G
  CCUCUCCCUC  CUUA  AGCCUUU GAUA CCUACCCAC UC  C
CU^         --    CU       G    C         C  AU
   130         120       110       100        90
```

pto-MIR073, ΔG = -54.80

```
     10       20        30          40
G                      A           |   CUACAAA
 CAACACUUGCAUUACCAAAGUA CUUCUUCAA--GGAU       C
 GUUGUGAACGUAAUGGUUUUGU GAAGAAGUU  CCUG       G
-                      C         \ ^   UCA----
        150       140       130         70
```

pto-MIR075, ΔG = -20.90

```
       20        30        40          50
-------   U     UCU      UA   .-C|    CAUUA
       CUG CCGUU   UUACGA  GCU   AUAUG     U
       GAC GGCAA   GAUGUU  CGG   UAUAC     A
UUAAUAG   U     CCU      UG   \ -^    AAAUU
110       100        90              60
```

pto-MIR078, ΔG = -44.80

```
    10         20          30
CAUCAACA  G     G   C-     U-     .-A   CG
        GU CUGGA UAG  UCAGU  GGUU   GAG  U
        CA GACCU GUC  GGUCA  CCAA   CUC  A
UACUAACC  -     G   UA     CC     \ -   A-
.        210       200       190
```

pto-MIR080, ΔG = -27.30

```
       10        20          30        40
C     UUG-----         AC  U      .-CUUUAAU    .-AUUA|
 GUGUC        AAGUUAAUG  CA AUAAAC         AGCC      U
 CAUAG        UUUAGUUGU  GU UGUUUG         UUGG      A
A     UGUCACUA         GA  -      \ -------    \ ----^
     190       180        170
```

pto-MIR094, ΔG = -28.30

```
    10           20         30          40
G     UCAG--  GU          CC  -   --|    UUCG
 GAGUG      GA  CCAUGCUCUC  UC UGA  AUCGG    \
 UUCAC      CU  GGUGCGGGAG  AG GCU  UGGUC    C
-     CCUAAA  UG          --  U   UC^    UUCU
   80        70         60           50
```

Figure S3. Predicted stem-loop structures of novel pre-miRNAs based on their cloned miRNA sequences.

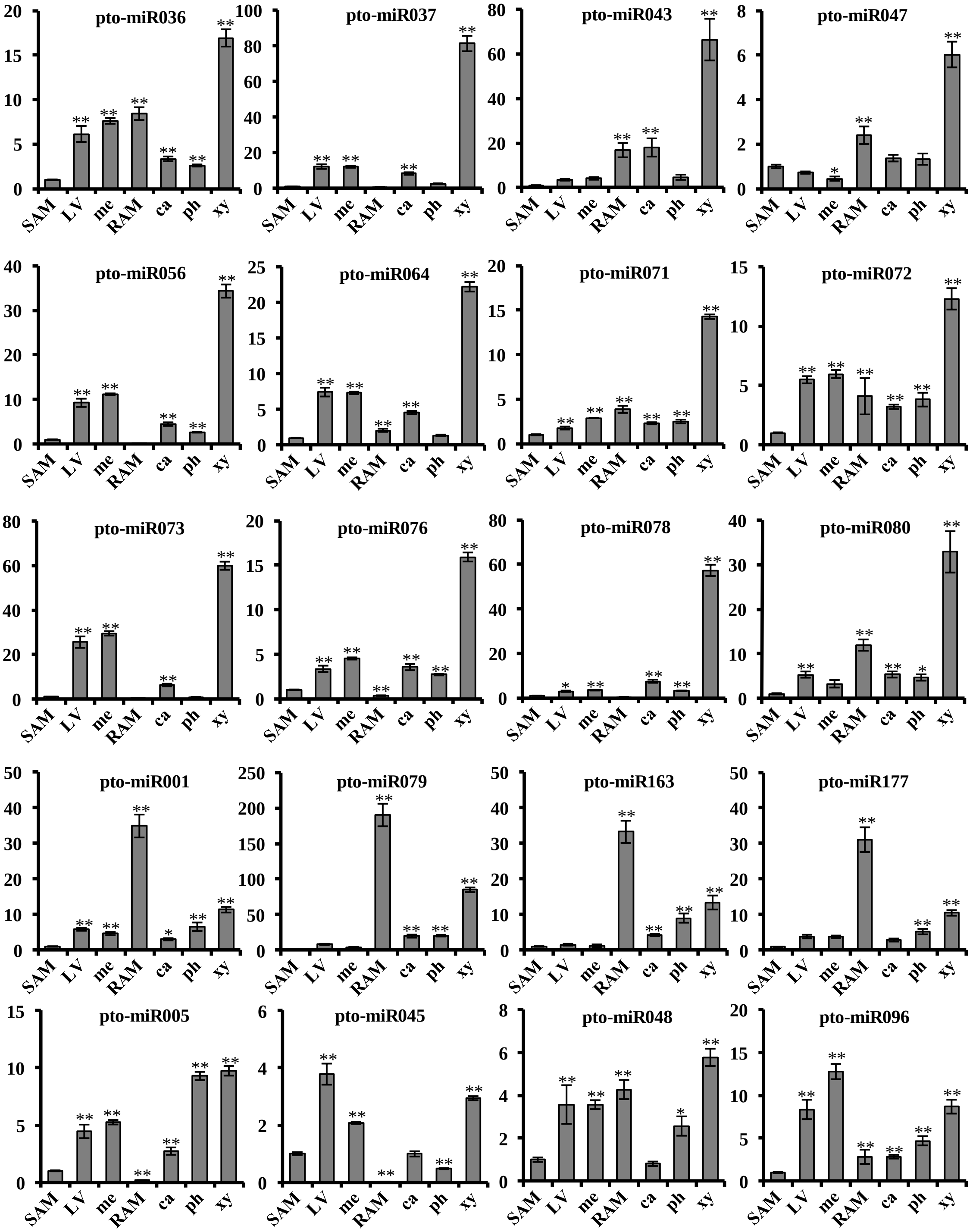

Figure S4. The expression of novel miRNAs in different tissues of *P. tomentosa* by universal qPCR. SAM, shoot apical meristem; LV, leaf vein; me, mesophyll; RAM, root apical meristem; ca, cambium; ph, phloem; xy, xylem. ** *P*-value≤0.01; * 0.01<*P*-value≤0.05.

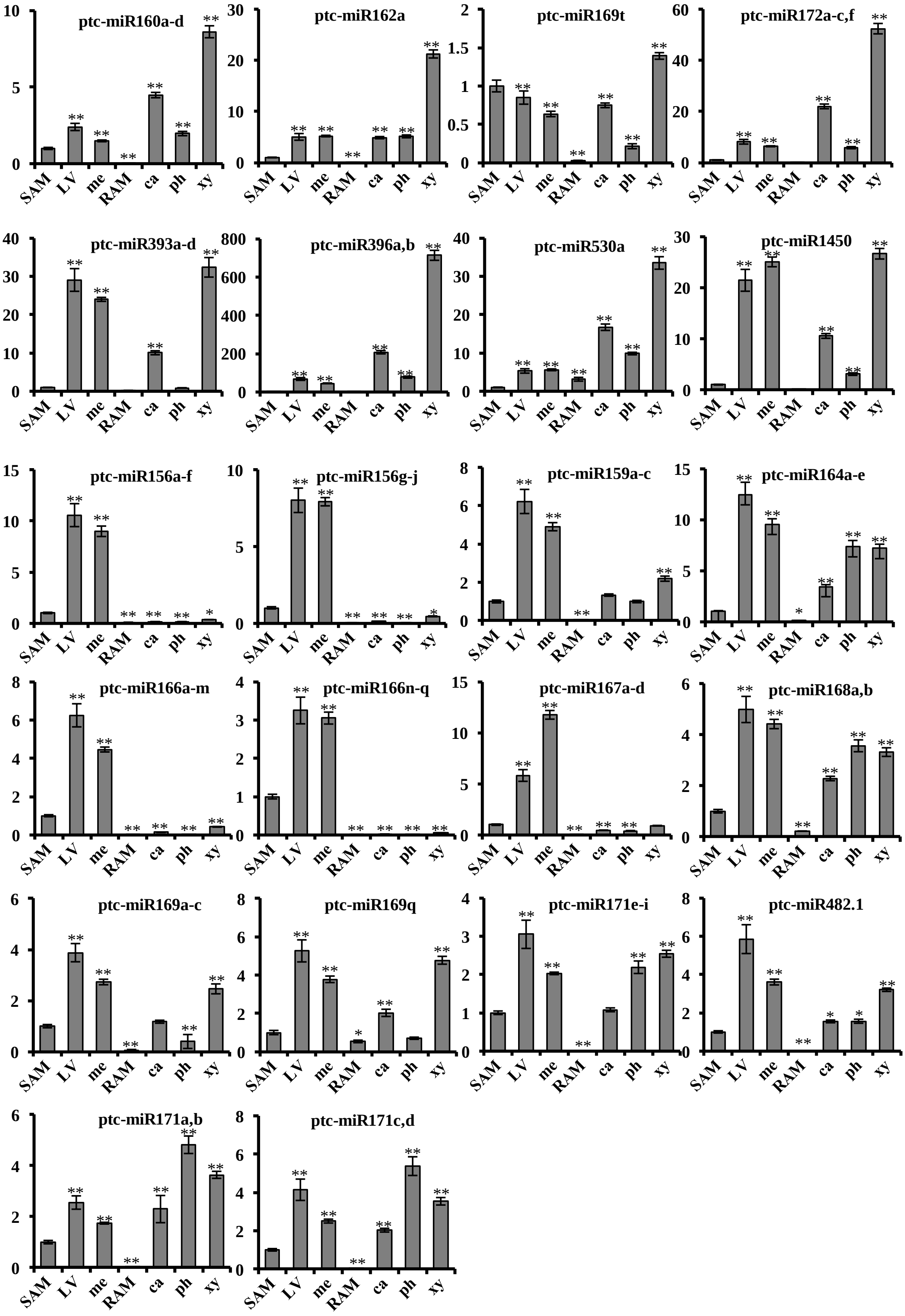

Figure S5. The expression of known miRNAs in different tissues of *P. tomentosa* by universal qPCR. SAM, shoot apical meristem; LV, leaf vein; me, mesophyll; RAM, root apical meristem; ca, cambium; ph, phloem; xy, xylem. ** *P*-value≤0.01; * 0.01<*P*-value≤0.05.

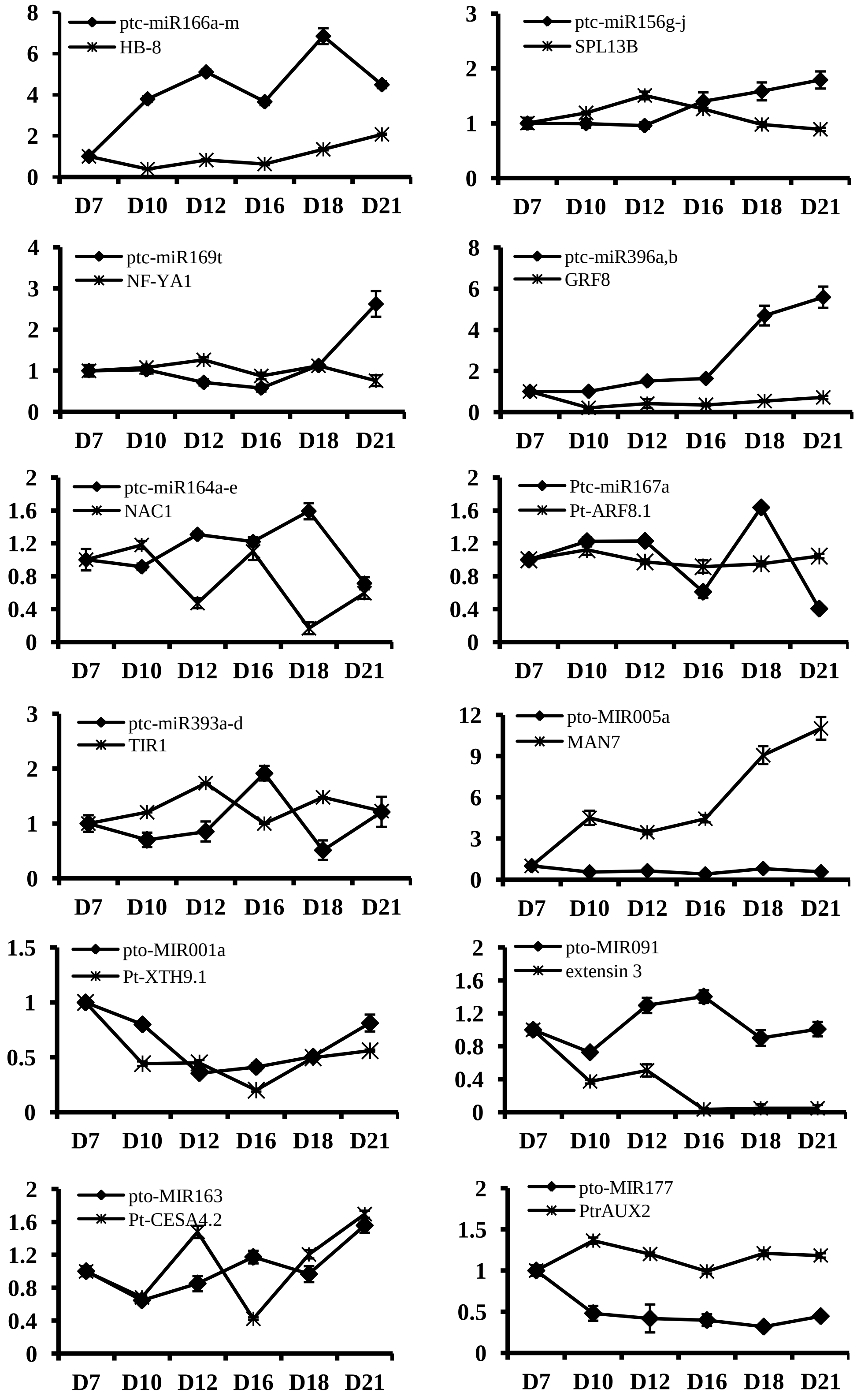

Figure S7. The expression profiles of miRNAs and their target genes during SVS regeneration as validated by universal qRT-PCR.

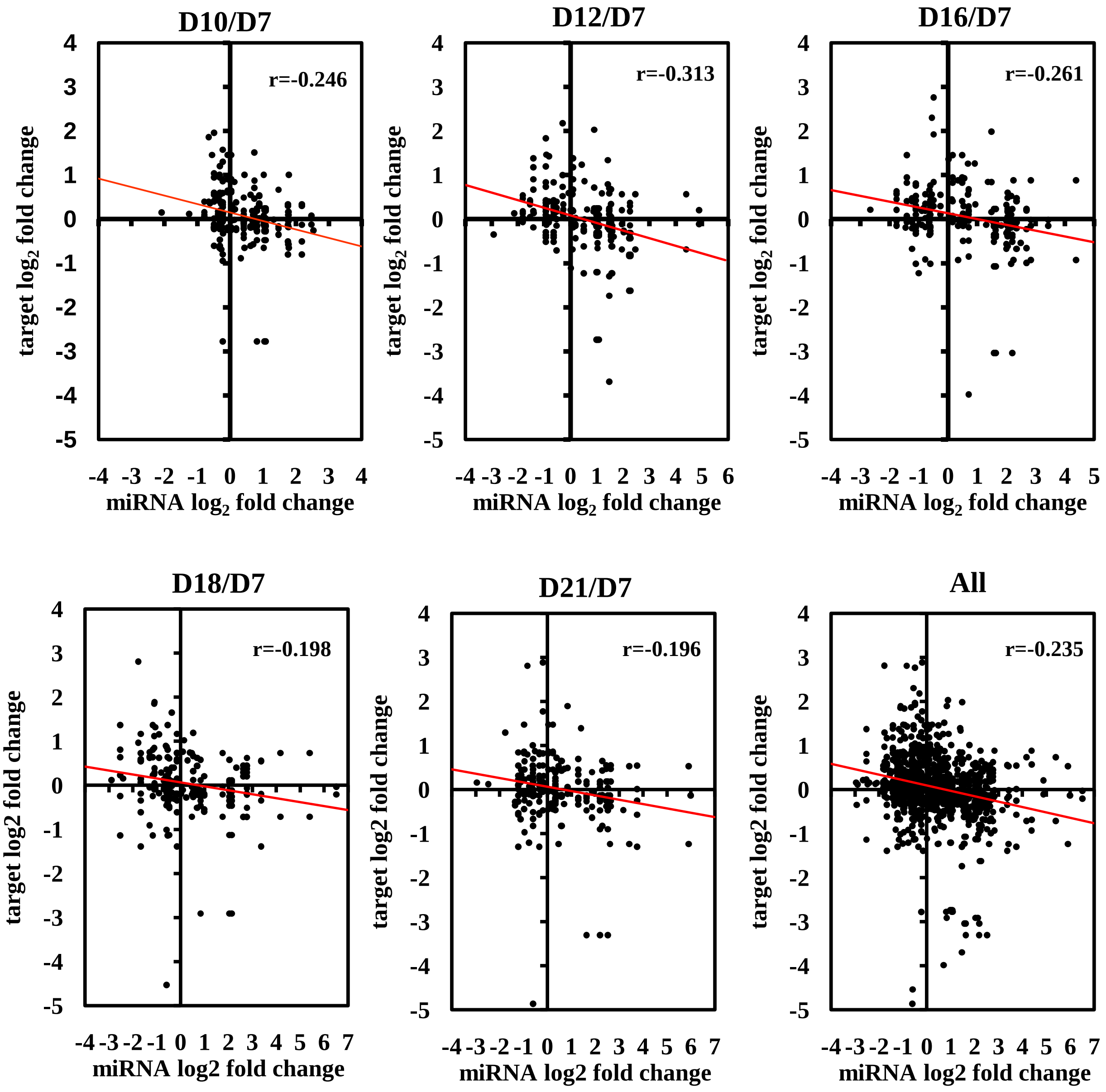

Figure S8. Pearson Correlation Coeffcient between the expression of miRNAs and their target genes during SVS regeneration.