Supplemental Figure 2

A. Example of small RNAs generated from a single-stranded RNA precursors but without the reported miRNA sequenced (type B loci in Table 2)

1. Pre-miR810

```
                                                AACGGCAUAAUUAGAUCAC,1
                                               AACGGCAUAAUUAGAUCA,1
                                              UAACGGCAUAAUUAGAUCACU,4
                                              UAACGGCAUAAUUAGAUCA,1
                                             CUAACGGCAUAAUUAGAUCACU,4              CGGUGUUCGCUAUAUAUACUAU,4
                                             CUAACGGCAUAAUUAGAUCAC,23              CGGUGUUCGCUAUAUAUACUA,3
                                             CUAACGGCAUAAUUAGAUCA,1               UCGGUGUUCGCUAUAUAUACUA,2
                                            ACUAACGGCAUAAUUAGAUCACU,1            UAUCGGUGUUCGCUAUAUAUACUA,1
                                            ACUAACGGCAUAAUUAGAUCAC,1             UAUCGGUGUUCGCUAUAUAUAC,1
                                            ACUAACGGCAUAAUUAGAUCA,1              UAUCGGUGUUCGCUAUAUAUA,5
                                          AUAACUAACGGCAUAAUUAGAUCA,1            AUAUCGGUGUUCGCUAUAUAU,1
GUUGCCAUCAUAAGCCCACCACAUGUGGCUCGCAUGCUUAAAUAACUAACGGCAUAAUUAGAUCACUUGAUGACGACGUAUAUCGGUGUUCGCUAUAUAUACUAUCUACUGGUAAGUAUAUAUAGCGAACACCGAUAUGCGUCAUCAUCAAGUGAUUUAAUUAUGCCGUUAGUUAAGCAUGCGAGCCACAUGUGGUGAGCUUACGAUGACGAC
((((.((((.(((((.(((((((((((((((((((((((....(((((((((((((((((((((((((((((((((((.((((((((((((((((((((((((((((((((((....))).))))))))))))))))))))))))))))))).))))))))))))))))))))))))))))))))))))))))))))))))).))))).)))).)))))
```

B. Examples of small RNAs generated from double-stranded RNAs or only from the antisense strand of the reported pre-miRNAs (type C loci in Table 2)

1. Pre-miR395c

```
                                                                                                AGGGUUGUUGUGAAGUGUUUGGAG,1
5′ GUAUUAUCAAGAGUUCUCUUUAAGCACUUCAUACGACACCAUUAUUUAUAGGGUUGUUGUGAAGUGUUUGGAGGAACUCUCGGUGUCAU 3′
3′ CAUAAUAGUUCUCAAGAGAAAUUCGUGAAGUAUGCUGUGGUAAUAAAUAUCCCAACAACACUUCACAAACCUCCUUGAGAGCCACAGUA 5′
   ....(((((.(((((((.(((((((((((((((((((((.((..........)))))))).))))))))))))))))))))))))).))))....
                                                                                                UCCUUGAGAGCCACAGUAGUUUGU,1
```

2. Pre-miR395f

```
    GGUAUUAUCGCGGGUUCCCU,1
     5′ UUAUCGCGGGUUCCCUUCAAUCACUUCACAUGGUACUAUUUCAAGGCCUACUAUGUGAAUUGUUUGGGGGAACUCUCGAUGU 3′
     3′ AAUAGCGCCCAAGGGAAGUUAGUGAAGUGUACCAUGAUAAAGUUCCGGAUGAUACACUUAACAAACCCCCUUGAGAGCUACA 5′
        .(((((.(((((((((.(((.((.(((((((((((((.....)).....))))))))))).)).))))))))))))).))))).
                         GGGAAGUUAGUGAAGUGUACCAU,1
CAAACCAUAAUAGCGCCCAAGGGA,1
```

3. Pre-miR414

```
5′-GUUGCCGCUGCCGUCAUCCUCAUCAUCAUCGUCCUUGCAUGAAACCGCCAGCAAAUCCUUGCGCGGGGGGAGGGGGAAGGGGAGGGGGAGGGGUCGGAGGGGCGGC-3′
3′-CAACGGCGACGGCAGUAGGAGUAGUAGUAGCAGGAACGUACUUUGGCGGUCGUUUAGGAACGCGCCCCCCUCCCCCUUCCCCUCCCCCUCCCCAGCCUCCCCGCCG-5′
   ((((((.((.(((((.(((((.((.((.((.(((((.........((.((.((((....))))).)).)))))))).)).)).)).)))))..)).)))))).)))))))
                  GUAGUAGCAGGAACGUACUUUGGC, 1
                  GUAGGAGUAGUAGUAGCAGGAA, 1
                 AGUAGGAGUAGUAGUAGCAGGAAC,1
                 AGUAGGAGUAGUAGUAGCAGGA,1
               GCAGUAGGAGUAGUAGUAGCAGGA,1
              GGCAGUAGGAGUAGUAGUAGCAGG,1
```

4. Pre-miR416

```
5′-CAAGAUGAGGAGUAUGAAUAGAAAGAAUUAGAAAAGCUACGGUGGUUGUUGAGCAGCGCCAAAAAAAACUCUGCUUUGCUCUGUUUCUGUGUUCGUCCGUACACUGUUCAUCA-3′
3′-GUUCUACUCCUCAUACUUAUCUUUCUUAAUCUUUUCGAUGCCACCAACAACUCGUCGCGGUUUUUUUUGAGACGAAACGAGACAAAGACACAAGCAGGCAUGUGACAAGUAGU-5′
   ...(((((((.((((((((((...((((.((((((((...((((..((.(((.((...)))))).)).)..)))).)))))..))))).))))).))))))).....))).)))))))
                                                                                                CGAAACGAGACAAAGACACAAGCA,1
```

5. Pre-miR420

```
                                                  AUACUUCCAAUGCUCAAAUAAU,1
                                                 AAUACUUCCAAUGCUCAAAUAA,1
                                                 AAUACUUCCAAUGCUCAAAUAAUU,1
                                              UAAAAAUACUUCCAAUGCUCA,1
                                             UUAAAAAUACUUCCAAUGCUC,1
5'-UUCCAACUAAAUUAAUCACGGAAAUGAUCUUUGAAUUGUUAAAAAUACUUCCAAUGCUCAAAUAAUUCCAAGAAAAAUCUUGAAAAUACUUGGACACUCAAAGUACUUAACAAAAUUAUAAUUAGACCAUUUAAUGAUUAAUUUAAUAUGUGGG-3'
3'-AAGGUUGAUUUAAUUAGUGCCUUUACUAGAAACUUAACAAUUUUUAUGAAGGUUACGAGUUUAUUAAGGUUCUUUUUAGAACUUUUAUGAACCUGUGAGUUUCAUGAAUUGUUUUAAUAUUAAUCUGGUAAAUUACUAAUUAAAUUAUACACCC-5'
   .(((((..(((((((((((...(((((.(((.(((.(((((((...(((((....((..(((.((.((.(((((....)))))).)).)).)))..)).....)))))).)))))))..)))).....)).)))))..)))))))))))).....))))
                                        AACAAUUUUUAUGAAGGUUAC, 1
                                             AAUUUUUAUGAAGGUUACGAGU,1
                                               UUUUUAUGAAGGUUACGAGUUUAU,1
                                               UUUUUAUGAAGGUUACGAGUUU,1
                                                UUUUAUGAAGGUUACGAGUUU,6
                                                 UUAUGAAGGUUACGAGUUUAU,1
                                                 UUAUGAAGGUUACGAGUUUAUU,8
                                                  UAUGAAGGUUACGAGUUUAUUA,1
                                                   AUGAAGGUUACGAGUUUAUUAA,2
```

6. Pre-miR439a (antisense strand)

```
UUAAUGGAAAAUCGCAAAACUAUAGAUCAGUUAGGCGAAAUCGCAAGUUUAGCACCCUGUCGAACAACCGCGGUUCGACAGGGAUACCUAUCGAACAACCACAGUUCGACAGGUACAUGUCGAACUGCGUCAGUUCGACAGGGUAGGUGGUCCAAAAA
                              UCAGUUAGGCGAAAUCGCAAGU,1                                                                                  CAGGUACAUGUCGAACUGCGUCAG,1
                                                                                                                                         AGGUACAUGUCGAACUGCGUCA,1
                                                                                                                                       ACAGGUACAUGUCGAACUGCGUCA,1
                                                                                                                                     CGACAGGUACAUGUCGAACUGCGU,1
                                                                                                                                     CGAACAACCACAGUUCGACAGGU,1
                                                                                                                 ACCUAUCGAACAACCACAGU,1
                                                                                                     CGCGGUUCGACAGGGAUACCUAUC,2
                                                                                                 CAACCGCGGUUCGACAGGGAUA,1
                                                                                            ACCCUGUCGAACAACCGCGGUUCG,1
                                                                                           GCACCCUGUCGAACAACCGCGGUU,2
                                                                                             ACCCUGUCGAACAACCGCGGU,2
                                                                                          AGCACCCUGUCGAACAACCGCGGU,4
                                                                                          AGCACCCUGUCGAACAACCGCGG,1
                                                                                         UAGCACCCUGUCGAACAACCGCGG,2
                                                                                        UUAGCACCCUGUCGAACAACCGCG,1
                                                                                     AGUUUAGCACCCUGUCGAACAACC,1
```

Pre-miR439a (pre-miRNA)

```
UUUUUGGACCACCUACCCUGUCGAACUGACGCAGUUCGACAUGUACCUGUCGAACUGUGGUUGUUCGAUAGGUAUCCCUGUCGAACCGCGGUUGUUCGACAGGGUGCUAAACUUGCGAUUUCGCCUAACUGAUCUAUAGUUUUGCGAUUUUCCAUUAA
....((((.....(((((((((((...(((.(((((((.((((((((((((.......)))))))))))))...)))))))).)))...)))))))))))))).............((((..(((((.....)))))..))))...)))).....
          ACCUACCCUGUCGAACUGACGCAG,1                                                
```

7. Pre-miR808

```
5'-GAAUGCUGAUUACUACUUCCUCCGUUUCACAAUGUAAGUCAUUCUAGCAUUUUCCACAUUCAUAUUGAUGUUAAUGAAUCUAGAUAGAUAUAUAUGUCUAGAUUCAUUAACAUCAAUAUGAAUGUGGGAAAUGUAAGAAUGACUUACAUUGUGAAAUGGAGGGAGUACAAAGUAUUC-3'
3'-CUUACGACUAAUGAUGAAGGAGGCAAAGUGUUACAUUCAGUAAGAUCGUAAAAGGUGUAAGUAUAACUACAAUUACUUAGAUCUAUCUAUAUAUACAGAUCUAAGUAAUUGUAGUUAUACUUACACCCUUUACAUUCUUACUGAAUGUAACACUUUACCUCCCUCAUGUUUCAUAAG-5'
   (((((((.....(((((((
```

2. Pre-miR529 (note that sequence abundance suggests that both miR529 and miR529* should be 1nt longer than previously reported, which are shown in green and pink, respectively)

```
                    AGAGAGAGAGUACAGCCU,2
                   GAAGAGAGAGAGUACAGCCU,7
                  AGAAGAGAGAGAGUACAGCCUU,1
                  AGAAGAGAGAGAGUACAGCC,8
                  AGAAGAGAGAGAGUACAGC,1
                 AAGAAGAGAGAGAGUACAGCC,1
                 AAGGAAGAAGAGAGAGAGUA,1
                AAAGGAAGAAGAGAGAGAGUA,7                                                    GCUGUACCCUCUCUCUUCUU,1
               UAAAGGAAGAAGAGAGAGAGU,16                                                    GCUGUACCCUCUCUCUUCU,2
                     AGAAGAGAGAGAGUACAGCCU,594                                             GCUGUACCCUCUCUCUUCUUC,22
CGAGAUGUACAUCGAGGCUAAAGGAAGAAGAGAGAGAGUACAGCCUUAGUUCAGAUCACAUAUGACUAUCUUUUGCGAGUCUGAUCGAUACUCUGAUGAAGGCUGUACCCUCUCUCUUCUUCUCUUAGCUUCUUGAUGCUUUCUCU
.((((....((((((((((((..((((((((((((.((((((((((...(((((........((((..(....)..)))).........)))))..)))))))))).))))))))))))..))))))))..)))))...)))))).
```

D. Examples where the most abundant small RNA species (in green) is not the reported miRNA

1. Pre-miR167d (the expected miRNA shown in red is not the most abundant small RNA species from pre-miR167d-j)

```
          UGAAGCUGCCAGCAUGAUCUGAUG,1
          UGAAGCUGCCAGCAUGAUCUGAU,9
          UGAAGCUGCCAGCAUGAUCUG,1293
          UGAAGCUGCCAGCAUGAUCU,170
          UGAAGCUGCCAGCAUGAUC,68
          UGAAGCUGCCAGCAUGAU,17
           GAAGCUGCCAGCAUGAUCUGA,28
           GAAGCUGCCAGCAUGAUCUG,2
           GAAGCUGCCAGCAUGAUCU,1
           GAAGCUGCCAGCAUGAUC,1
            AAGCUGCCAGCAUGAUCUGA,3
          UGAAGCUGCCAGCAUGAUCUGA,9104                                   GAUCAUGCUGUGCAGUUUCAUC,1
CAUUAGGAGCUGAAGCUGCCAGCAUGAUCUGAUGAGUGCUUAUUAGGUGAGGGCAGAAUUGACUGCCAAAACAAAGAUCAGAUCAUGCUGUGCAGUUUCAUCUGCUUGUG
(((.((.((.(((((((((((((((((((((((....(((.....)))...(((((.....)))))........)))))))))))))).))))))))).)).)).)))
```

2. Pre-miR444 (the previously reported miRNA shown in red is not the most abundant small RNAs generated and no corresponding miRNA* found; instead, the most abundant small RNA from this precursor is another 21 nt variant which is shown in green and annotated as miR444e by Sunkar et al. 2008, its corresponding miRNA* is shown in pink)

```
AGGTGGCAACTGCATAATTTG,14
AGAGGTGGCAACTGCATAATT,2
AGAGGTGGCAACTGCATAAT,1
TAGAGGTGGCAACTGCATAATT,27
TAGAGGTGGCAACTGCATAAT,9
TAGAGGTGGCAACTGCATAA,8
TAGAGGTGGCAACTGCAT,1
CTAGAGGTGGCAACTGCATAA,2
CTAGAGGTGGCAACTGCATA,2
GCTAGAGGTGGCAACTGCATAATT,2
GCTAGAGGTGGCAACTGCAT,3
GCTAGAGGTGGCAACTGCA,1
AGCTAGAGGTGGCAACTGCAT,55
AGCTAGAGGTGGCAACTGCA,6
AAGCTAGAGGTGGCAACTGCA,1
GCTAGAGGTGGCAACTGCATA,171
```

```
TTGCTGCCTCAAGCTTGCTGC,1
AGTTGCTGCCTCAAGCTT,1
CAGTTGCTGCCTCAAGCTT,1
GCAGTTGCTGCCTCAAGCTTG,8
GCAGTTGCTGCCTCAAGCTT,2
TGCAGTTGCTGCCTCAAGCT,3
TGCAGTTGCTGCCTCAAGC,4
TGCAGTTGCTGCCTCAAG,1
TATGCAGTTGCTGCCTCAAGCTTG,1
TTATGCAGTTGCTGCCTCAAGCTT,1
AGTTATGCAGTTGCTGCCTCA,1
AAGTTATGCAGTTGCTGCCTC,2
CAAGTTATGCAGTTGCTGCCTC,1
CAAGTTATGCAGTTGCTGCCT,8
TGCAGTTGCTGCCTCAAGCTT,325
```

```
ATGCAATTGGGGGCAGCAAGCTAGAGGTGGCAACTGCATAATTTGCAAGAAATTGTTGGCTGAAGATCATACCGATGATATTCTTGCAAGTTATGCAGTTGCTGCCTCAAGCTTGCTGCCTCCTGTTGCCA
..(((((.(((((((((((((((.((((..(((((((((((((((((((((((((..((((.((.....)).)).)))..)).))))))))))))))))))))))))..)))).))))))))))))))).)))))..
```

3. Pre-miR820a (the reported miRNA was only sequenced once and its corresponding miRNA* was not sequenced in our study. A second 24 nt long small RNA, which was from the 3′ arm and in phase with the expected miRNA*, is shown in pink)

```
GCCUCGUGGAUGGACCAGGAGCUC,2
GCCUCGUGGAUGGACCAGGA,2
GGCCUCGUGGAUGGACCAGGAG,14
GGCCUCGUGGAUGGACCAGGA,7
GGCCUCGUGGAUGGACCAGG,2
GGCCUCGUGGAUGGACCAG,2
CGGCCUCGUGGAUGGACCAGGAG,7
CGGCCUCGUGGAUGGACCAGGA,1
CGGCCUCGUGGAUGGACCAGG,1
UCGGCCUCGUGGAUGGACCAGGA,1397
UCGGCCUCGUGGAUGGACCAGG,386
UCGGCCUCGUGGAUGGACCAG,94
UCGGCCUCGUGGAUGGACCA,3
UCGGCCUCGUGGAUGGACC,8
UCGGCCUCGUGGAUGGAC,7
UGCGUCGGCCUCGUGGAUGGACCA,1
ACAUGCGUCGGCCUCGUGGAUGGA,1
UCGGCCUCGUGGAUGGACCAGGAG,2208
```

```
GAAGUCGAUGCUCCAGGUCCGUCCA,1
GCCUUAAGGAAGUCGAUGCUC,1
GCCUUAAGGAAGUCGAUGCU,1
UGCCUUAAGGAAGUCGAUGCUC,2
UGCCUUAAGGAAGUCGAUGCU,7
UGCCUUAAGGAAGUCGAUG,1
CUGCCUUAAGGAAGUCGAUGCUCC,1
CUGCCUUAAGGAAGUCGAUGCU,2
ACUGCCUUAAGGAAGUCGAUGCUC,3
ACUGCCUUAAGGAAGUCGAUGCU,2
AACUGCCUUAAGGAAGUCGAUGCUC,6
AACUGCCUUAAGGAAGUCGAUGCU,44
AACUGCCUUAAGGAAGUCGAUGC,1
AACUGCCUUAAGGAAGUCGAUG,2
GAACUGCCUUAAGGAAGUCGAUGCU,11
GAACUGCCUUAAGGAAGUCGAUGC,3
GAACUGCCUUAAGGAAGUCGAUG,3
ACGAACUGCCUUAAGGAAGUCGA,1
AACGAACUGCCUUAAGGAAGUCGAU,1
AACGAACUGCCUUAAGGAAGUCGA,4
```

```
CGCCUGCAUUGUUCCAAGAGUGUC,1
```

```
UCUGUUGAAAGAUAGACCUCUGAUUCGUACAUGCGUCGGCCUCGUGGAUGGACCAGGAGCUCAACAUUCCUUAAGGUUGUUCUUUCAAACCCAUACAACACCGCCUGCAUUGUUCCAAGAGUGUCUUGGAUGAAAGUAGGAAGUGGAACCUUGUUAGGGUUGGAACGAACUGCCUUAAGGAAGUCGAUGCUCCAGGUCCGUCCAGGAGGACGACUCAGCUAGGAGUUAGAGGUUUAUCUUUGAACACA
..((((.(((((((((((((((((((.((..((.((((.((((.((((((((((.(((((((.((.((((((((((.((((.(((.((((.((((((..(((((((..(((((.((..((((((.....)))))).)).)))))..)))).....)))).)))))).))).))))).))))))))))))))).)).)))))).))))))))))).))))).))).)).))))))))))))))))))).))))..
```

4. Pre-miR1318 or Pre-miR1432 (both miR1318 and miR1432 (in orange and red, respectively) are not the most abundant small RNA from this locus)

```
                    AGGAGAGAUGACACCGAC,3
                    AGGAGAGAUGACACCGACA,5
                   CAGGAGAGAUGACACCGAC,1
                   CAGGAGAGAUGACACCGACA,1
                  UCAGGAGAGAUGACACCGAC,73
                  UCAGGAGAGAUGACACCGA,1
                  UCAGGAGAGAUGACACCGACA,197
                  UCAGGAGAGAUGACACCGACAU,1
                 AUCAGGAGAGAUGACACCGA,1
                 AUCAGGAGAGAUGACACCGAC,23
                 AUCAGGAGAGAUGACACCGACAU,2
                 AUCAGGAGAGAUGACACCGACAUC,1
                GAUCAGGAGAGAUGACACCGAC,1
            UGUGAUCAGGAGAGAUGACAC,1
                  AUCAGGAGAGAUGACACCGACA,337
GGGGGGUUUGGGCCCUGUGAUCAGGAGAGAUGACACCGACAUCGCCGGAAUUCGUUCUUGGUCUUGUGCCAUGAUGAAUUGAUGGUCCGUUUGAUGCAGGUGUCAUCUCCCCUGAACAUAGGACUGACUGACCCUGA
..(((((((.((.((((((.(((((.(((((((((((..(((((.(((((((((((..((((.....)))).)))))))......))))..))))))..))))))))))).))))).)))))).)).)..)))))))..
```