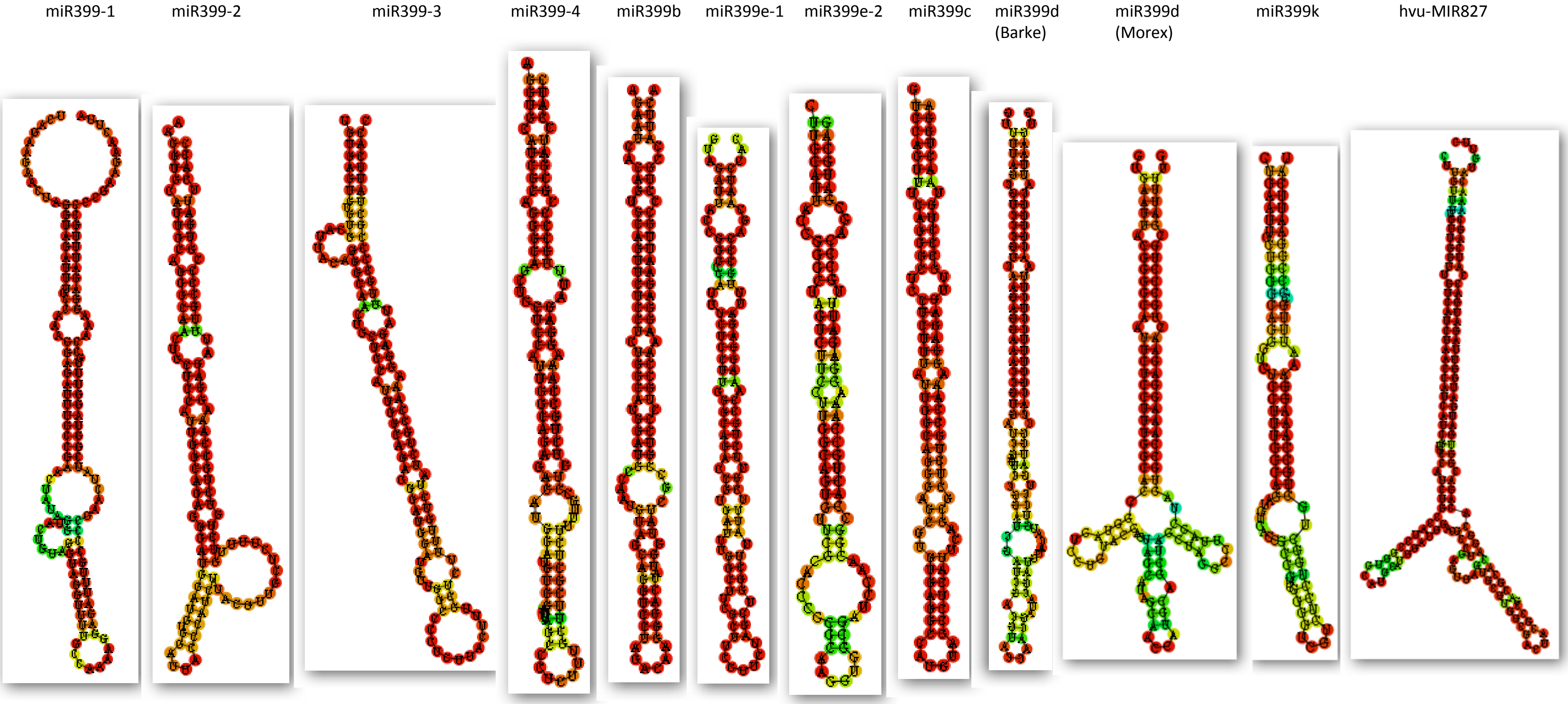

Supplemental Figure 1

hvu-MIR399-1

```
P sufficient                                                                                                                           read count
TCAGAAGAACTAGGTAGATTTGCCAAAGGAGATTTGCCGAACTATAGGTACTGTAGGTAGGTTTTGCCAAAAGGAGATTTGCCCCGAACTATCGGTAGGTTTGCCAAAGGAGATTTGCCCCGAAGAACTTA    6
............(((((((((.((...(((((((((((((......((.......((((((((((........))))))))))))......))))))))))).))...))))))))))).............
                                                                                                     TGCCAAAGGAGATTTGCCCCG             4
                                                                                                     TGCCAAAGGAGAGTTGCCCC              1
                           GGAGATTTGCCGAACTATAGGT                                                                                      1

P deficient
TCAGAAGAACTAGGTAGATTTGCCAAAGGAGATTTGCCGAACTATAGGTACTGTAGGTAGGTTTTGCCAAAAGGAGATTTGCCCCGAACTATCGGTAGGTTTGCCAAAGGAGATTTGCCCCGAAGAACTTA    168 (total count)
............(((((((((.((...(((((((((((((......((.......((((((((((........))))))))))))......))))))))))).))...))))))))))).............
                                                                                                     TGCCAAAGGAGATTTGCCCCG             123
                                                                                                          AAGGAGATTTGCCCCG             4
                                                                                                     TGCCAAAGGAGATTTGC                 4
                                                                      AAGGAGATTTGCCCCG                                                 4
                    TGCCAAAGGAGATTTGC                                                                                                  4
                                          TATAGGTACTGTAGGTAGGTTT                                                                       3
                                        ACTATAGGTACTGTAGGTAGGTT                                                                        3
                                                                                                     TGCCAAAGGAGATTTGCCC               2
                                                                                                     TGCCAAAGGAGATTTGCC                2
                    TGCCAAAGGAGATTTGCC                                                                                                 2
  AGAAGAACTAGGTAGATTTGC                                                                                                                2
TCAGAAGAACTAGGTAGATTTG                                                                                                                 2
                    TGCCAAAGGAGATTTGCCCAA                                                                                              2
                                                                                                     TGCCAAAGGAGATTTGCCCCGAA           1
                                                                                                     TGCCAAAGGAGATTTGCCCCGA            1
                                                                                                     TGCCCAAGGAGATTTGCCCCG             1
                                                                                                      GCCAAAGGAGATTTGCCCCG             1
                                                                                                     TGCCAAAGGAGATTTTCCCCG             1
                                                                                                     TGCCAAAGGAGACTTGCCCCG             1
                                                                                                     TGCCAAAGGAGAGTTGCCCC              1
                                                                                                     TGCCAAAGGAGATTTGCCCC              1
                                                                       AGGAGATTTGCCCCGAACTAT                                           1
                                               GTACTGTAGGTAGGTTTTGCCAAA                                                                1
                                               GTACTGTAGGTAGGTTTTGCCAA                                                                 1
                                               GTACTGTAGGTAGGTTTTGCC                                                                   1
                                       AACTATAGGTACTGTAGGTAGG                                                                          1
```

Supplemental Figure 2

miR827

```
                                               Read count

       TTTTGTTGGTTGTCATCTAACC                    (169)
5′
--- T     T      T                  TT  A    -
   C TGTTT GTTGGT GTCATCTAACCATCATCG  CC TCTG
   | ||||| |||||| ||||||||||||||||||  || ||||
   G ACAAA CGACTA CAGTAGATTGGTAGTAGT  GG AGAC
CTT T     -      C                  --  C    A
3′
     ACAAA-CGACTACCAGTAGATT                      (8935)
```

miR827 (alt)

```
                TTGTCATCTAACCATCATCG             (2)
5′
--- T     T      T                  TT  A    -
   C TGTTT GTTGGT GTCATCTAACCATCATCG  CC TCTG
   | ||||| |||||| ||||||||||||||||||  || ||||
   G ACAAA CGACTA CAGTAGATTGGTAGTAGT  GG AGAC
CTT T     -      C                  --  C    A
3′
              CTACCAGTAGATTGGTAGTA               (10)
```

Supplemental Figure 3

hvu-MIR399b

```
                                                    loop
                                                      ↓
AGAATCACAGTGCAGTTCTCCTCTGGCATGGATGCCAATGTAGCAGGTCCTAGACAAGGGACTATGGTATCGCCGTCCCTGCCAAAGGAGAATTGCCCTGCCATTCA  324(53.4/30,325.7) adj:324+
.((((..(((.(((((((((((.(((((.(((((.(...(((.((((((((......)))))).)).))).).))))).))))).))))))))))).)))..)))).
                                                                               TGCCAAAGGAGAATTGCCCTG         140(23.1/13,103.7) adj:140+ hvu-miR399b-3p  exact-
         GTGCAGTTCTCCTCTGGCATG                                                                               133(21.9/12,448.5) adj:133+ hvu-miR399b-5p  exact-
                                                                               TGCCAAAGGAGAATTGCCC           7(1.2/655.2) adj:7+.lv3p|lv3pT|lv3p#-2-
         GTGCAGTTCTCCTCTGGCA                                                                                 4(0.7/374.4) adj:4+.lv3p|lv3pT|lv3p#-2-
         GTGCAGTTCTCCTCTGGCATGA                                                                              4(0.7/374.4) adj:4+.nta#A|nta#A#1-
                                                                                 CCAAAGGAGAATTGCCCTGCG       4(0.7/374.4) adj:4+.nta#G|nta#G#1-
                                                                               TGCCAAAGGAGAATTGCC            3(0.5/280.8) adj:3+.lv3p|lv3pT|lv3p#-3-
                                                                               TGCCAAAGGAGAATTGCCCTGA         3(0.5/280.8)adj:3+.nta#A|nta#A#1-
                                                                                  CAAAGGAGAATTGCCCTG         2(0.3/187.2) adj:2+.lv5p|lv5pT|lv5p#-3-
                                                                               TGCCAAAGGAGAATTGCCCT          2(0.3/187.2) adj:2+.lv3p|lv3pT|lv3p#-1-
                                                                               TGCCAAAGGAGAATTGCCCTGAA       2(0.3/187.2) adj:2+.nta#A|nta#A#2-
          TGCAGTTCTCCTCTGGCATG                                                                               2(0.3/187.2) adj:2+.lv5p|lv5pT|lv5p#-1-
                                                                                GCCAAAGGAGAATTGCCCTG         2(0.3/187.2) adj:2+.lv5p|lv5pT|lv5p#-1-
                                                  TAGACAAGGGACTATGGTA                                        2(0.3/187.2) adj:2+..
         GTGCAGTTCTCCTCTGGCAT                                                                                1(0.2/93.6) adj:1+.lv3p|lv3pT|lv3p#-1-
                                                  TAGACAAGGGACTATGGTATCG                                     1(0.2/93.6) adj:1+...
         GTGCAGTTCTCCTCTGGCATC                                                                               1(0.2/93.6) adj:1+.nta#C|nta#C#1-
                                                                                 CCAAAGGAGAATTGCCCT          1(0.2/93.6) adj:1+.mv-
                                                                                 CCAAAGGAGAATTGCCCTG         1(0.2/93.6) adj:1+.lv5p|lv5pT|lv5p#-2-
                                                                                 CCAAAGGAGAATTGC             1(0.2/93.6) adj:1+.mv-
                                                                                    AAGGAGAATTGCCCTG         1(0.2/93.6) adj:1+.lv5p|lv5pT|lv5p#-5-
                                                                               TGCCAAAGGAGAATTGCCCTGT        1(0.2/93.6) adj:1+.nta#T|nta#T#1-
                                                  TAGACAAGGGACTATGGTAT                                       1(0.2/93.6) adj:1+...
                                                                              CTGCCAAAGGAGAATTGCCCTG         1(0.2/93.6) adj:1+.lv5p|lv5pE|lv5p#1-
                                                  TAGACAAGGGACTATGGTATC                                      1(0.2/93.6) adj:1+...
             AGTTCTCCTCTGGCATG                                                                               1(0.2/93.6) adj:1+. lv5p|lv5pT|lv5p#-4-
                                                                                GCCAAAGGAGAATTGCCCTGA        1(0.2/93.6) adj:1+.nta#A|nta#A#1-
                                                                               TGCCAAAGGAGAATTGC             1(0.2/93.6) adj:1+.lv3p|lv3pT|lv3p#-4-
```

hvu-MIR399e-1

```
                                                       loop
                                                     ↓
GTAGATTACCGGGCCATTTCTCCTTGGGCAGACCGTGATTTGGCTTCGCTTCGTTCTAGCTGGCTTATTTCGTTCTGCCAAAGGAGATTTGCCCAGCAATCCAC  296(48.8/27,705) adj:169.4+
...((((.(.((((.(..(((((((.((((((.((.(((..((((..(((.......))).)))).))).)).)))))).)))))))..))))).).))))...
                                                                           TGCCAAAGGAGATTTGCCCAG          226(37.2/21,153.1)adj:113+hvu-miR399e-3pexact-
          GGGCCATTTCTCCTTGGGCAGA                                                                          34(5.6/3,182.3) adj:34+hvu-miR399e-5p exact-
                                                                              CAAAGGAGATTTGCCCAGCAA       4(0.7/374.4)adj:4+.mv-
                                                                           TGCCAAAGGAGATTTGC              4(0.7/374.4)adj:0.6+.lv3p|lv3pT|lv3p#-4-
                                                                            GCCAAAGGAGATTTGCCCAG          3(0.5/280.8)adj:1.5+.lv5p|lv5pT|lv5p#-1-
                                                                         TCTGCCAAAGGAGATTTGCCC            3(0.5/280.8)adj:0.8+.mv-
                                                                                AAGGAGATTTGCCCAGCAATC     3(0.5/280.8)adj:3+.mv-
                                                                           TGCCAAAGGAGATTTGCCCAGT         2(0.3/187.2)adj:1+.nta#T|nta#T#1-
                                                                           TGCCAAAGGAGATTTGCC             2(0.3/187.2)adj:0.3+.lv3p|lv3pT|lv3p#-3-
                                           CTTCGTTCTAGCTGGC                                               2(0.3/187.2)adj:2+...
                                                                           TGCCAAAGGAGATTTGCCC            2(0.3/187.2)adj:0.3+.lv3p|lv3pT|lv3p#-2-
                                                                           TGCCAAAGGAGATTTGCCCAA          2(0.3/187.2)adj:1+.nta#A|nta#A#1-
                                                                           TGCCAAAGGAGATTTGCCCAGA         1(0.2/93.6) adj:0.5+.nta#A|nta#A#1-
                                                                              CAAAGGAGATTTGCCCAGCAAT      1(0.2/93.6) adj:1+.mv-
                                                                                AAGGAGATTTGCCCAGCAAT      1(0.2/93.6) adj:1+.mv-
                                                                               AAAGGAGATTTGCCCAGCAATC     1(0.2/93.6) adj:1+.mv-
          GGGCCATTTCTCCTTGGGCAGAA                                                                         1(0.2/93.6) adj:1+.nta#A|nta#A#1-
                                                                           TGCCAAAGGAGATTTGCCCAT          1(0.2/93.6) adj:0.5+.nta#T|nta#T#1-
    ATTACCGGGCCATTTCTCCTTGGGC                                                                             1(0.2/93.6) adj:1+...
    ATTACCGGGCCATTTCTCCTTGCGC                                                                             1(0.2/93.6) adj:1+...
                                                                                AAGGAGATTTGCCCAGCAATT     1(0.2/93.6) adj:1+.nta#T|nta#T#1-
```

Supplemental Figure 4

A

```
  ↑
  +P                                                                                                                                                                                                                                                                           read count
HvPHO2    5’---TTCTGTAAGTTCTTCGGGGCAAATCTCCTTTGGCA---ATAGTTCGGGGCAAATCTCCTTTTGGCAAAACCTACCTACAGTACCTATAGTTCGGCAAATCTCCTTTGGCAAATCTACCTAGTTCTTCTGATCTCCTTCCTTACATATG---TTGCTCCCTGGACAAATCTCCT---TAGGTTTAGCGTCAGTCCTGTCTGGGCGAACCTCCTTTGACACTAAATCT---CTGGGCAAATCTCCTTTGGCG---3’
  -P           TTCTGTAAGTTCTTCGGGGCA                                                                                                                                                                                                                                           2
                                                                                              GTACCTATAGTTCGGCAAATCTCC                                                                                                                                                         1
  ↓                                                                                                                                                                                            TAGGTTTAGCGTCAGTCCTG                                                            1
                           AAGCCCCGTTTAGAGGAAACCGT                                                                                                                                                                                                                             1
                            AGCCCCGTTTAGAGGAAACCGT                                                                                                                                                                                                                             1
                             GCCCCGTTTAGAGGAAACCGT                                                                                                                                                                                                                             1
                             GCCCCGTTTAGAGGAAACCG                                                                                                                                                                                                                              1
                              CCCCGTTTAGAGGAAACCGT                                                                                                                                                                                                                             112
                               CCCGTTTAGAGGAAACCGT                                                                                                                                                                                                                             6
                                CCGTTTAGAGGAAACCGT                                                                                                                                                                                                                             5
                                 CGTTTAGAGGAAACCGT                                                                                                                                                                                                                             3
                                                     TATCAAGCCCCGTTTAGAGGA                                                                                                                                                                                                     1
                                                                          AAACCGTTTTGGATGGATGTCATG                                                                                                                                                                             1
                                                                           AACCGTTTTGGATGGATGTCATG                                                                                                                                                                             1
                                                                             CCGTTTTGGATGGATGTCATG                                                                                                                                                                             1
                                                                                 TTTGGATGGATGTCATGGATAT                                                                                                                                                                        1
                                                                                  TTGGATGGATGTCATGGATATCA                                                                                                                                                                      2
                                                                                    GGATGGATGTCATGGATATCAA                                                                                                                                                                     1
                                                                                                           CCGTTTAGAGGAAACCGT                                                                                                                                                  203
                                                                                                            CGTTTAGAGGAAACCGT                                                                                                                                                  112
                                                                                                                          CGTTTAGATGGATCAAGAAGA                                                                                                                                2
                                                                                                                           GTTTAGATGGATCAAGAAGACT                                                                                                                              1
                                                                                                                              TAGATGGATCAAGAAGACTAGA                                                                                                                           2
                                                                                                                               AGATGGATCAAGAAGACTAGA                                                                                                                           2
                                                                                                                                 ATGGATCAAGAAGACTAGAGGA                                                                                                                        1
                                                                                                                                  TGGATCAAGAAGACTAGAGGAA                                                                                                                       1
                                                                                                                                   GGATCAAGAAGACTAGAGGAA                                                                                                                       2
                                                                                                                                            AGACTAGAGGAAGGAATGTATA                                                                                                             2
                                                                                                                                            AGACTAGAGGAAGGAATGTATAC                                                                                                            1
                                                                                                                                                                      AACGAGGGACCTGTTTAGAGGA                                                                                   1
                                                                                                                                                                                                    AATCGCAGTCAGGACAGACCCG                                                     1
                                                                                                                                                                                                      TCGCAGTCAGGACAGACCCGCT                                                   1
                                                                                                                                                                                                                    GACCCGCTTGGAGGAAACTGTG                                     1
                                                                                                                                                                                                                            TGGAGGAAACTGTGATTTAGA                              1
                                                                                                                                                                                                                                                    GACCCGTTTAGAGGAAACCG       203
```

B

```
  ↑
  +P
HvIPS1    5’---TCGGAGTGTGCCCTCGGCCA---AGGAAGCTCTGGGCGAAACGGCCA---GGCGGCGGCGACTTCTCA---GGGCAACTTCTATCCTTTGGCA---ATGTGTATTGTATCCTAAA---ATGGGTGTATGTGTGATCCTGTT---3’
  -P           TCGGAGTGTGCCCTCGGCCA                                                                                                                                                                                                                                            1
                                      AGGAAGCTCTGGGCGAAACGGCCA                                                                                                                                                                                                                 1
  ↓                                                              GGCGGCGGCGACTTCTCA                                                                                                                                                                                            1
                                                                                                               ATGTGTATTGTATCCTAAA                                                                                                                                             1
```

C

```
  ↑
                                                                                 AGCTAGCTAGCTAGGCGGGTG                                                                                                                                                                 1
  +P                                    CGTGTACGTAGCTCCTGTCGGC                                                                                                                                                                                                                 1
HvIPS2    5’---ACGCCAGCCACTGCTAGCTAGCTGGCGTGTACGTAGCTCCTGTCGGCGACGACCGGCCGGCGA---AGCTAGCTAGCTAGGCGGGTG---GGGCAACTATACATCTTTGGCAATGTCTTCTTCTTCTTCCTCCTCCTCCGATGGATTATTGGAAGATTGTA---GTAAACTGTGTCCGCATCCTT---3’
  -P                           GCTAGCTGGCGTGTACGTAGCTCCT                                                                                                                                                                                                                       1
                                        CGTGTACGTAGCTCCTGTCGGC                                                                                                                                                                                                                 1
  ↓                                           CGTAGCTCCTGTCGGCGA                                                                                                                                                                                                               1
                                                                                 AGCTAGCTAGCTAGGCGGGTG                                                                                                                                                                 1
                                                                                                         GGGCAACTATACATCTTTGGCA                                                                                                                                                1
                                                                                                                                                            TGGATTATTGGAAGATTGTA                                                                                               1
               TGCGGTCGGTGACGATCGATC                                                                                                                                                                                                                                           2
                                      CCGCACATGCATCGAGGACAGC                                                                                                                                                                                                                   1
                                       CGCACATGCATCGAGGACAGC                                                                                                                                                                                                                   1
                                                        CAGCCGCTGCTGGCCGGCCGCT                                                                                                                                                                                                 1
                                                                                                                                  AGAAGAAGAAGAAGGAGGAGGAG                                                                                                                      1
                                                                                                                                                                                   CATTTGACACAGGCGTAGGAA                                                                       1
```

Note:

1. Sequencing reads in black are identical to the gene sequence.
2. Sequencing reads in blue are reverse complementary to the gene sequence.
3. miR399 target sites and target mimicking sequences are underlined.
4. Sequencing reads above the gene sequence were from P deficient (-P) barley shoots, while sequencing reads below the gene sequence were from P sufficient (+P) barley shoots.

Supplemental Figure 5