Supporting information

Discovery of Conserved and Novel MicroRNAs in *Galdieria sulphuraria*

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The following Supporting information is available for this article:

Figure S1. Biomass of *G. sulphuraria* in the growth phase.

Figure S2. Flowchart of experiment and information analysis in *G. sulphuraria*.

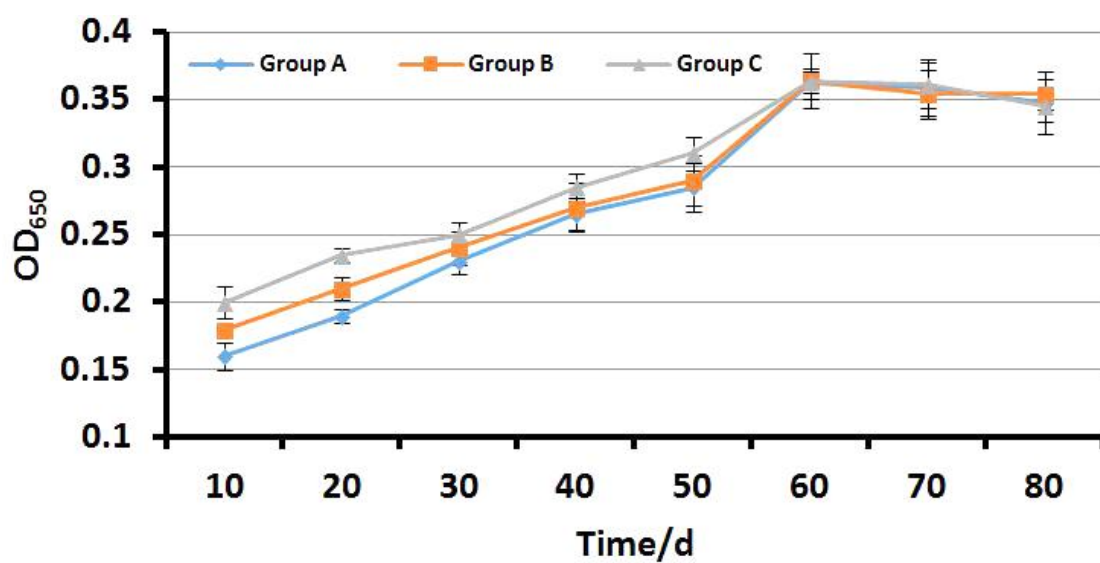**Figure S3.** Distribution, category and number of *G. sulphuraria* sRNAs.

Figure S4. Precursor structures of some representative gsu-miRNAs;

Figure S5. Venn diagrams of predicted targets based on psRobot and TargetFinder;
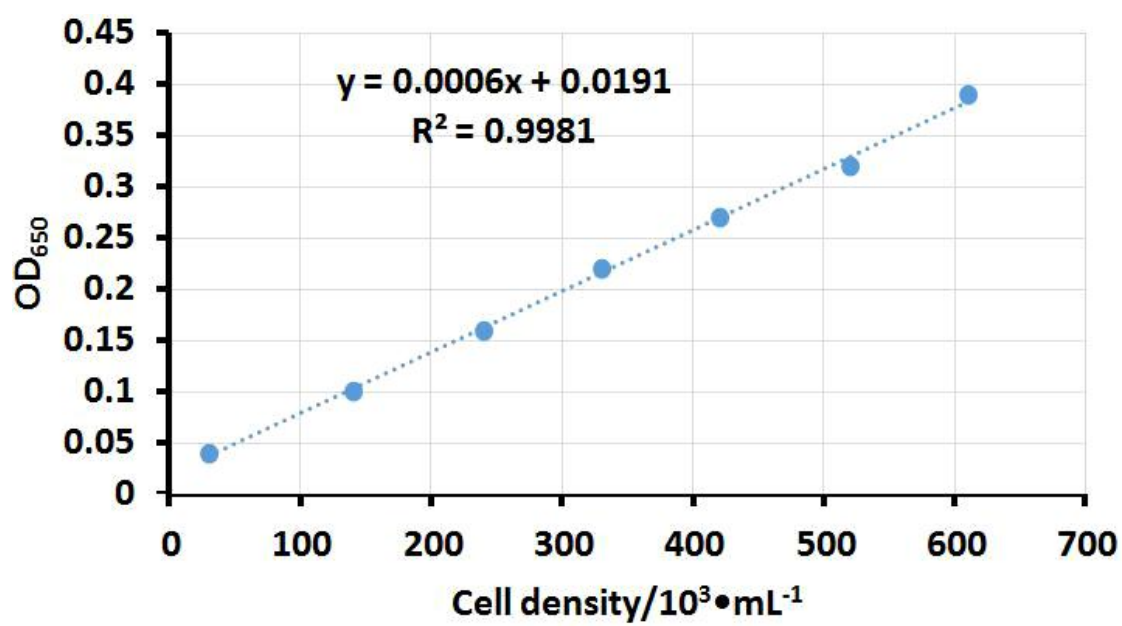
Figure S6. DAGs of enriched GO terms;
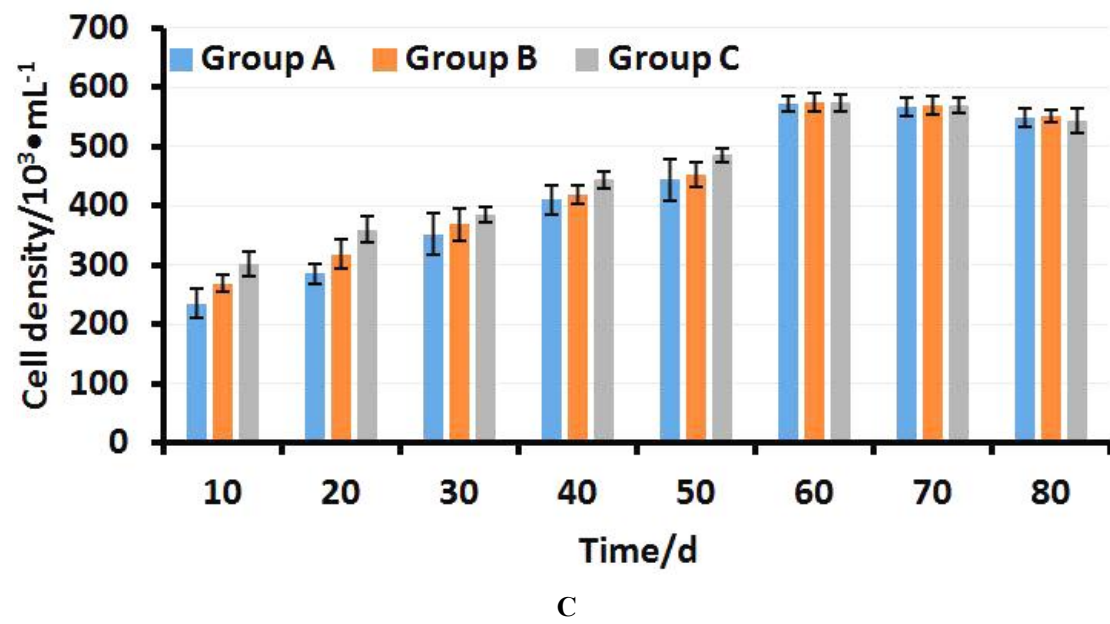
Figure S7. Reference KEGG pathways;

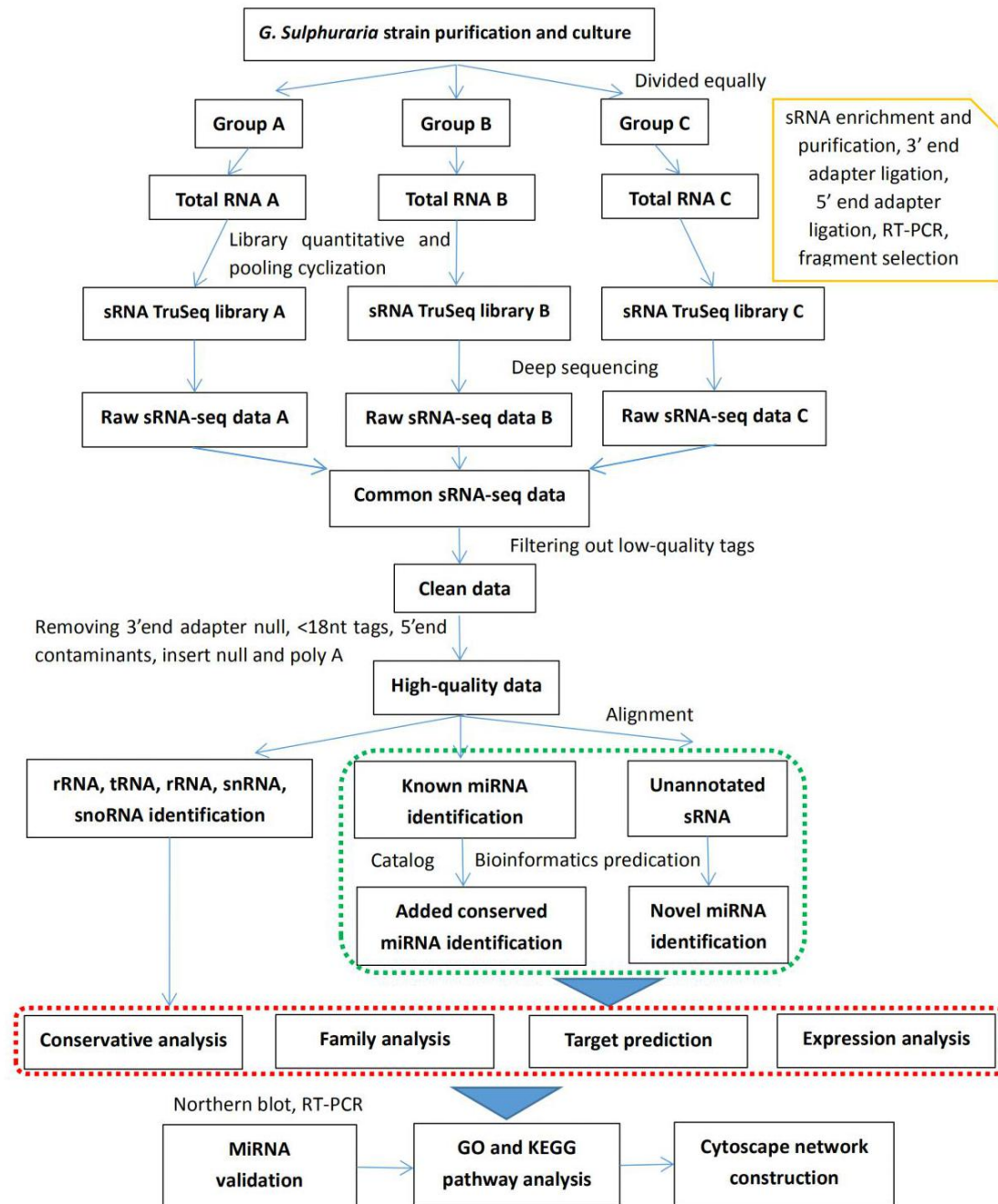**Figure S8.** Cytoscape networks of gsu-miRNAs and their target genes.
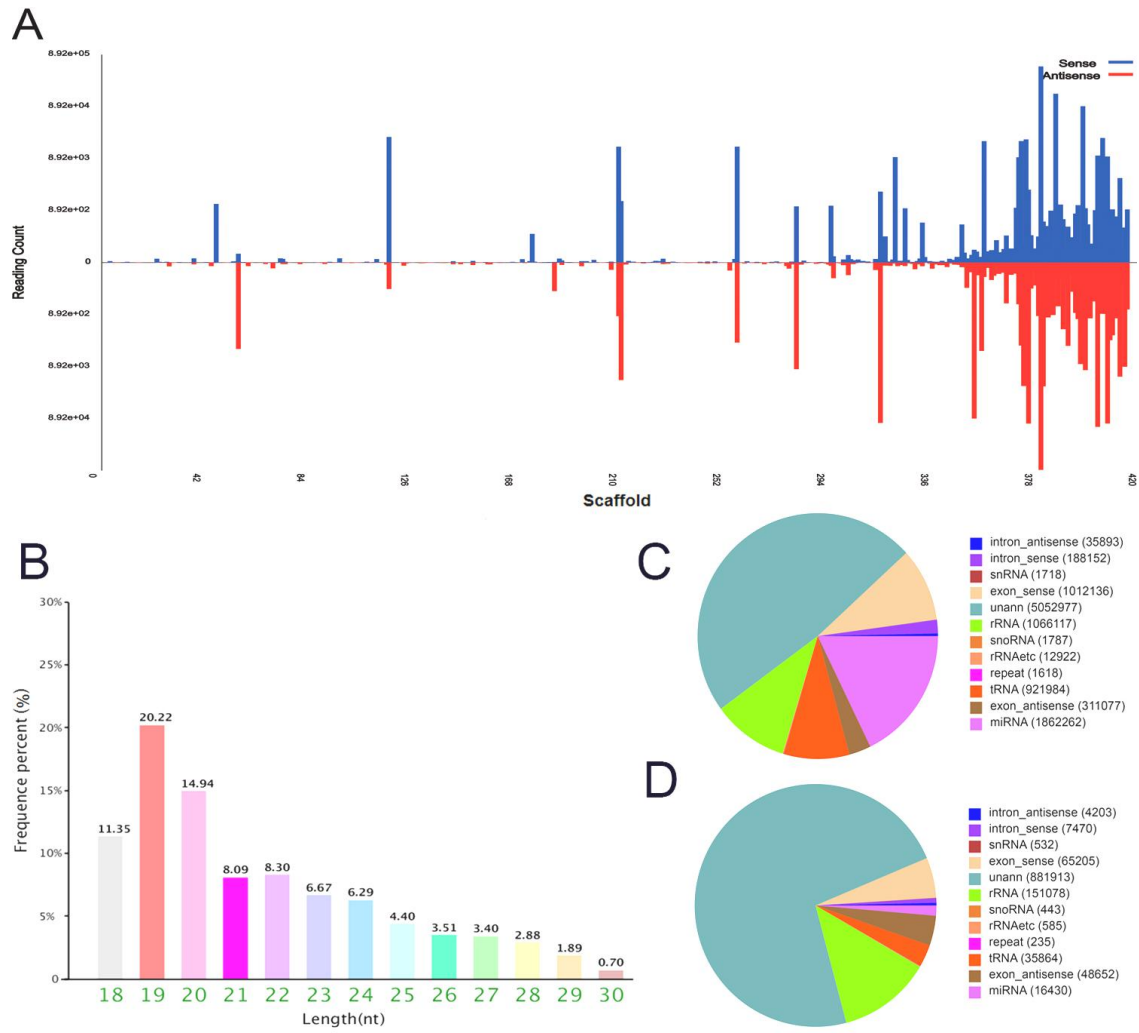
A

$y = 0.0006x + 0.0191$

$R^2 = 0.9981$

B

C

Figure S1. Biomass of *G. sulphuraria* in the growth phase. (A) Growth phase analysis of *G. sulphuraria*. (B) Standard curve of *G. sulphuraria* cell density. (C) Cell density of *G. sulphuraria* at OD650nm.

**Figure S2.** Flowchart of experiment and information analysis in *G. sulphuraria*.

Figure S3. Distribution, category and number of *G. sulphuraria* sRNAs. (A) Positional distribution of sRNA tags on *G. sulphuraria* scaffolds. (B) Percentage of different sizes of gsu-sRNAs. (C) Category and number of total gsu-sRNAs. (D) Category and number of unique gsu-sRNAs.

5
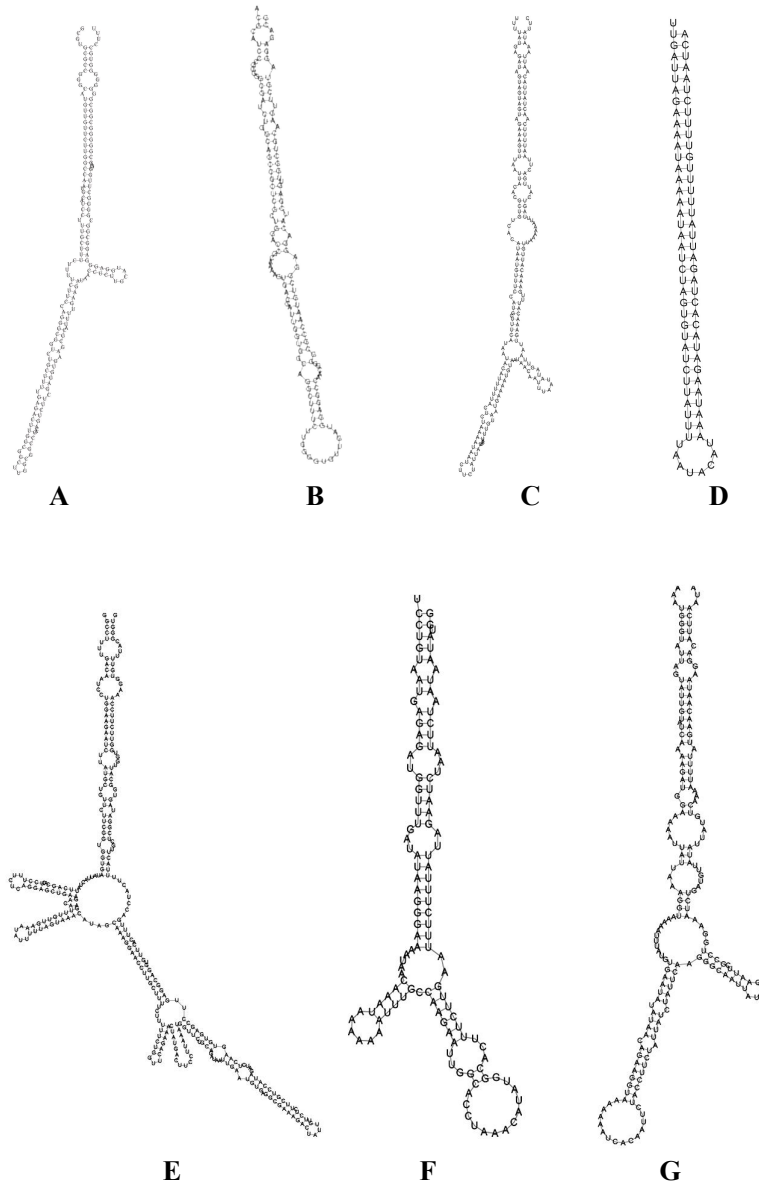
Figure S4. Precursor structures of some representative gsu-miRNAs. (A) Structure of conserved gsu-miR1025-3p. (B) Structure of conserved gsu-miR6183-3p. (C) Structure of novel gsu-miR2-5p. (D) Structure of novel gsu-miR9-3p. (E) Structure of novel gsu-miR4-3p. (F) Structure of novel gsu-miR5-5p. (G) Structure of novel gsu-miR6-5p.
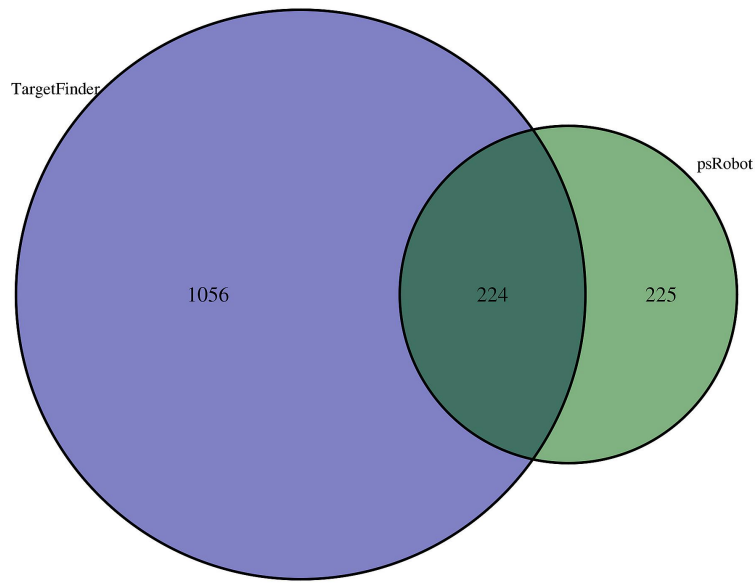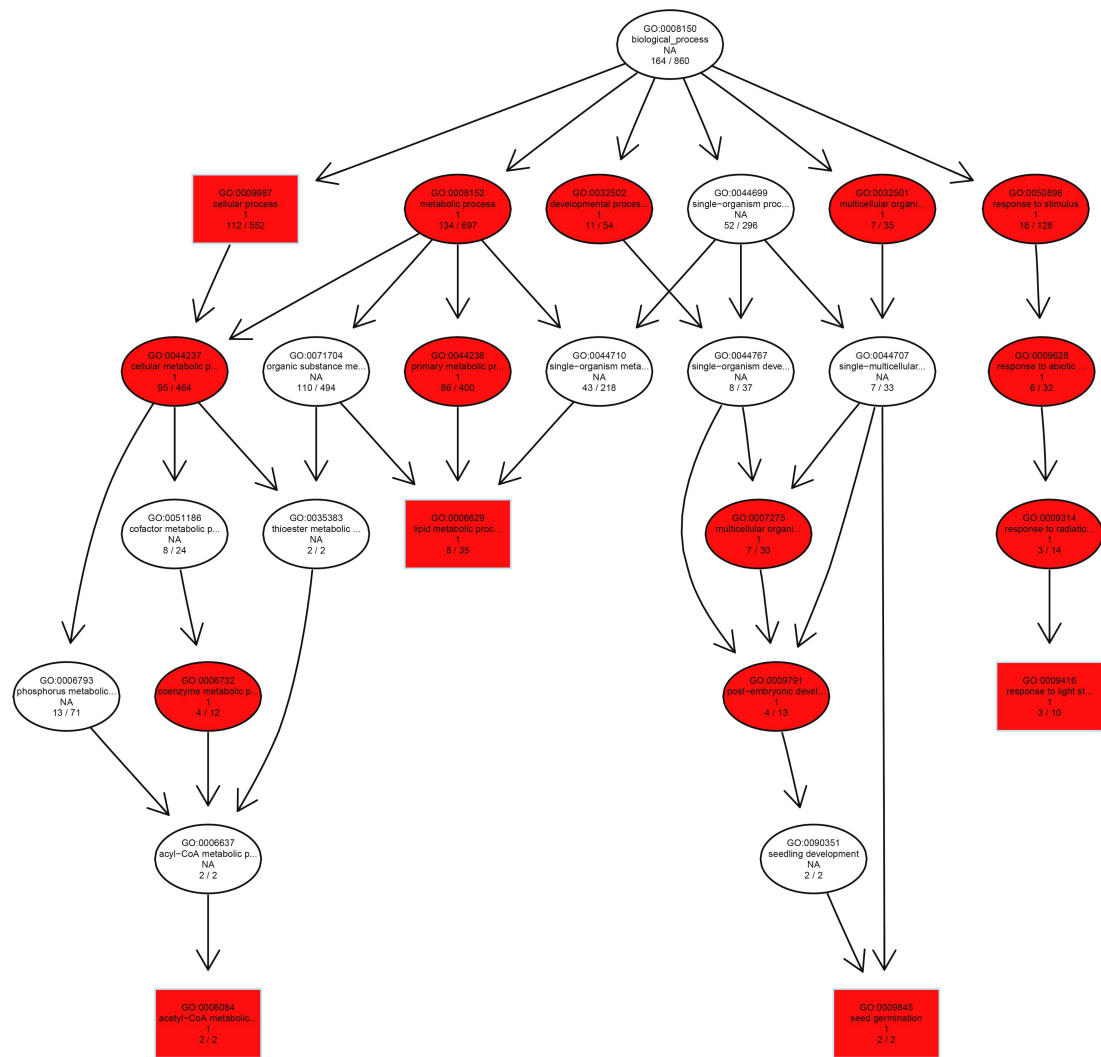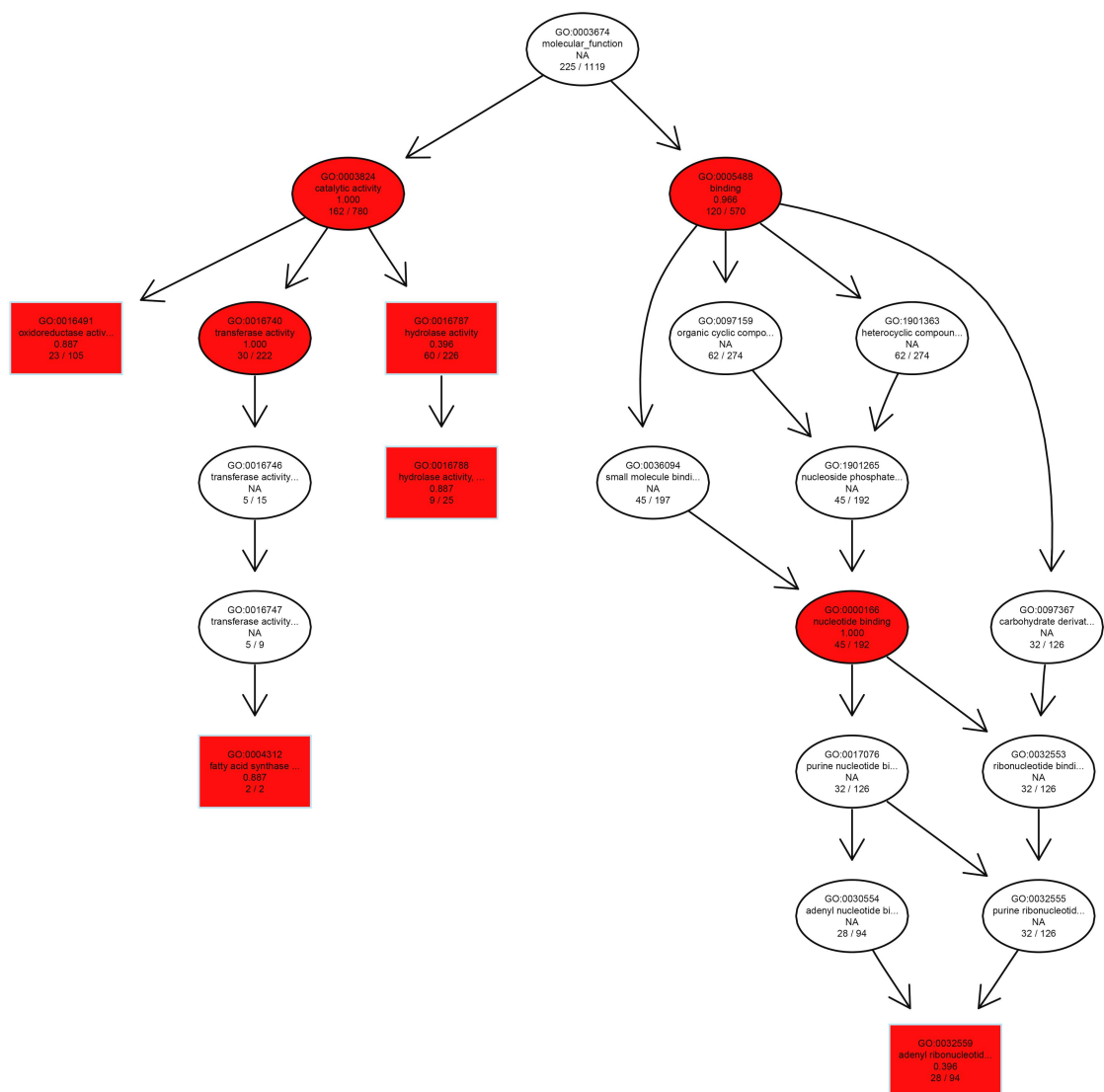
A
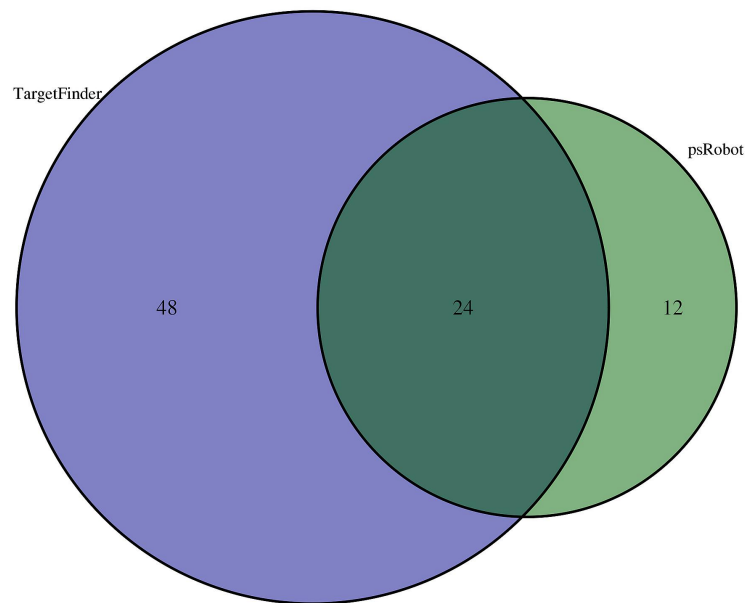
B

Figure S5. Venn diagrams of predicted targets based on psRobot and TargetFinder. (A) Venn diagram of conserved gsu-miRNAs targets predicted by psRobot and TargetFinder. (B) Venn diagram of novel gsu-miRNAs targets predicted by psRobot and TargetFinder.

A

B

C

D

E

GO:0003674
molecular_function
NA
20 / 1119

GO:0003824
catalytic activity
1.00000
12 / 780

GO:0009055
electron carrier act...
0.00126
3 / 4

GO:0016491
oxidoreductase activ...
1.00000
2 / 105

GO:0016787
hydrolase activity
0.79548
5 / 226

GO:0016874
ligase activity
1.00000
1 / 65

GO:0016614
oxidoreductase activ...
1.00000
1 / 24

GO:0016638
oxidoreductase activ...
NA
1 / 1

GO:0016877
ligase activity, for...
NA
1 / 4

GO:0016901
oxidoreductase activ...
0.60008
1 / 1

GO:0015930
glutamate synthase a...
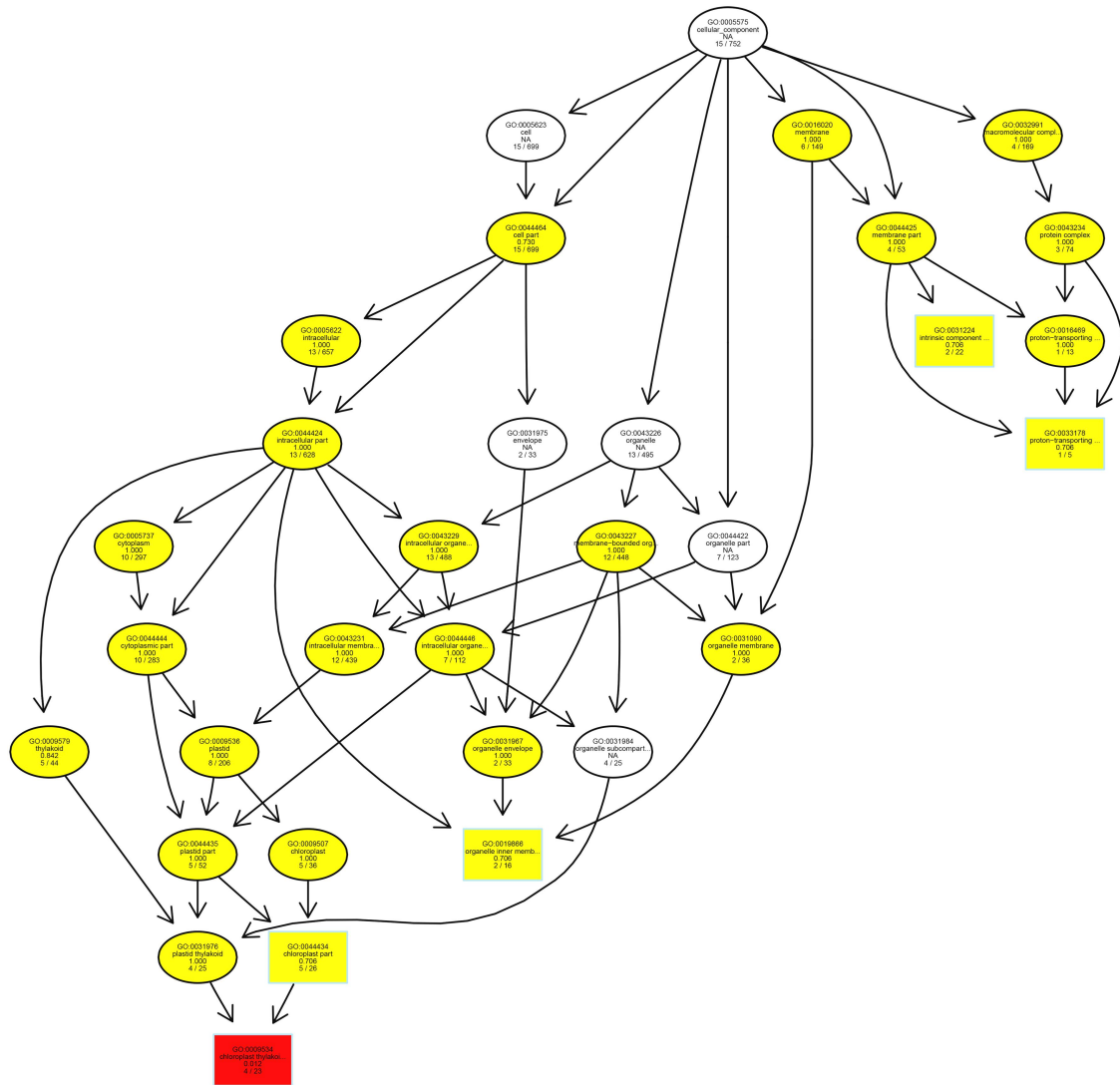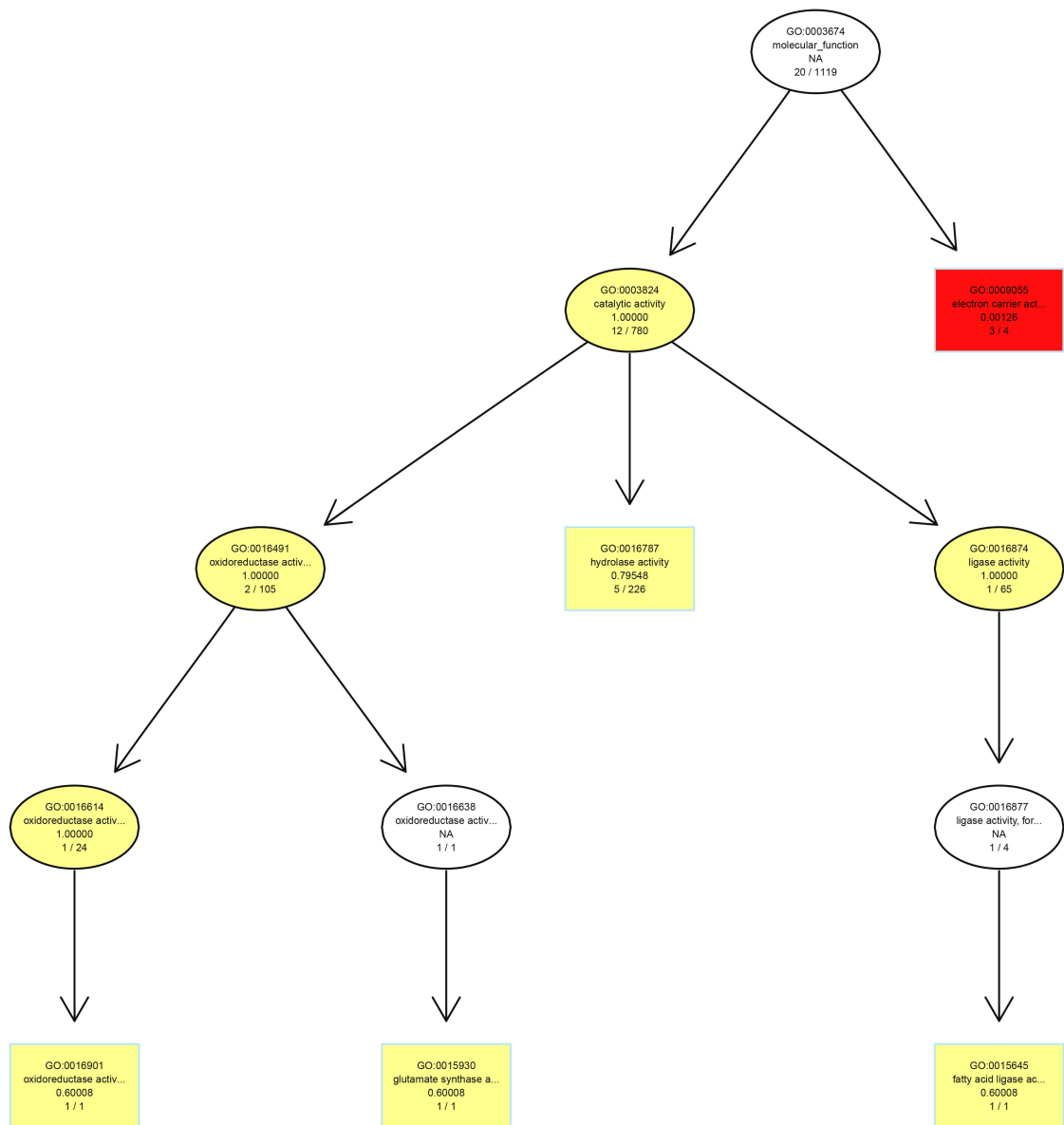0.60008
1 / 1

GO:0015645
fatty acid ligase ac...
0.60008
1 / 1

F

Figure S6. DAGs of enriched GO terms. (A) DAG of the top 5 enriched GO terms in BP based on conserved gsu-miRNAs. (B) DAG of the top 5 enriched GO terms in CC based on conserved gsu-miRNAs. (C) DAG of the top 5 enriched GO terms in MF based on conserved gsu-miRNAs. (D) DAG of the top 5 enriched GO terms in BP based on novel gsu-miRNAs. (E) DAG of the top 5 enriched GO terms in CC based on novel gsu-miRNAs. (F) DAG of the top 5 enriched GO terms in MF based on novel gsu-miRNAs. DAGs show the topological relationships of the 5 most highly enriched GO terms. Circles and boxes with different colors represent enriched GO terms.

RNA TRANSPORT

A

PENTOSE PHOSPHATE PATHWAY

B

Figure S7. Reference KEGG pathways. (A) The most significantly enriched pathway map for conserved gsu-miRNA target genes. (B) The most significantly enriched pathway map for novel gsu-miRNA target genes. In the reference pathways map, small boxes represent proteins or enzymes, and red ones indicate the candidate target genes encoding them. The small circles represent metabolites. The arrows represent different metabolic pathways. A detailed introduction to the pathways can be found online at http://www.genome.jp/dbget-bin/www_bget?pathway:map03013 and http://www.genome.jp/kegg-bin/show_pathway?map00030, respectively.

A

B

Figure S8. Cytoscape networks of gsu-miRNAs and their target genes. (A) Cytoscape networks of conserved gsu-miRNAs and their target genes. (B) Cytoscape networks of novel gsu-miRNAs and their target genes.