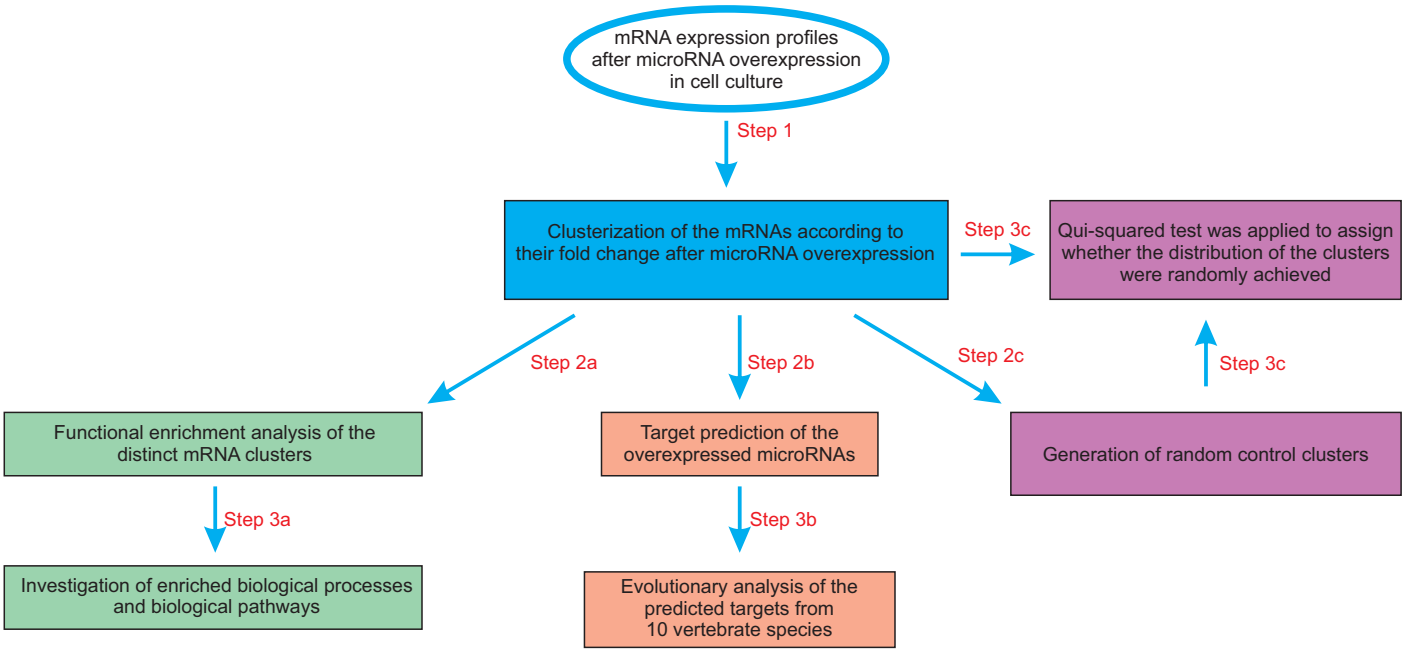
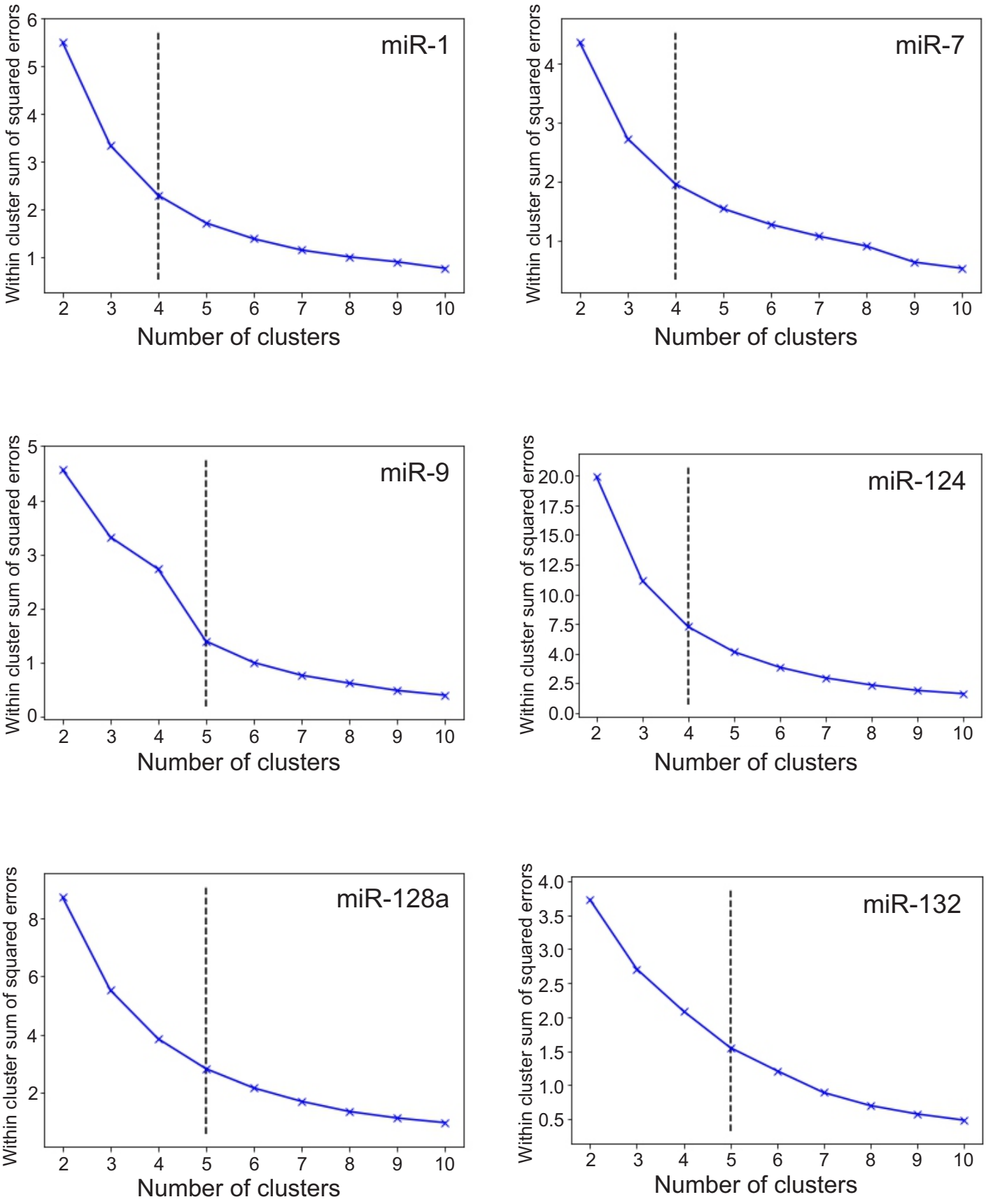
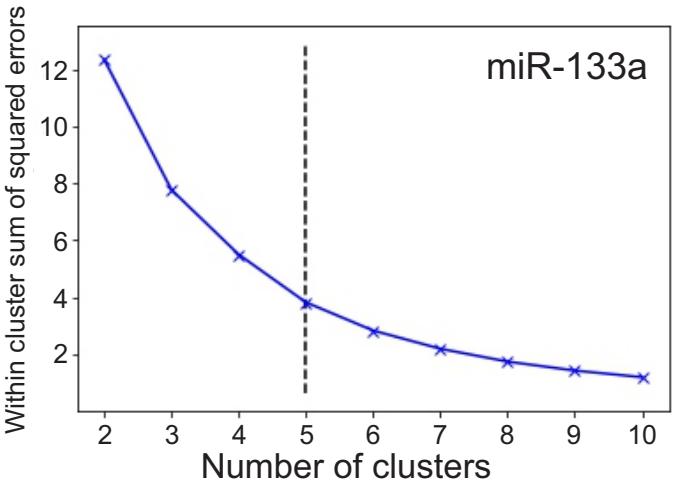
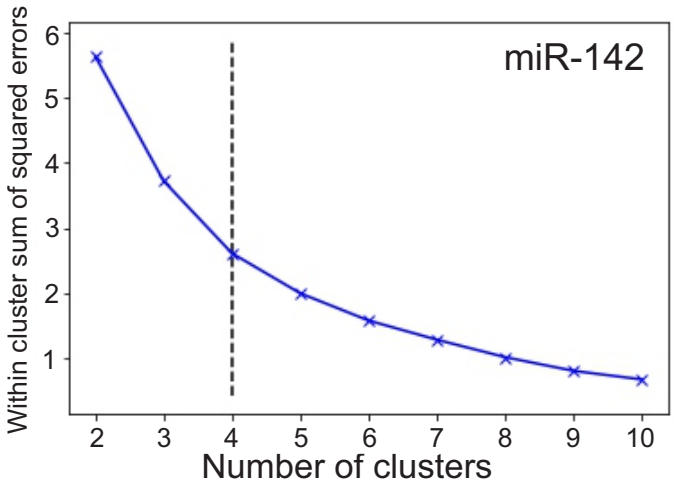
Supplementary Figure S1. Summary of the workflow.

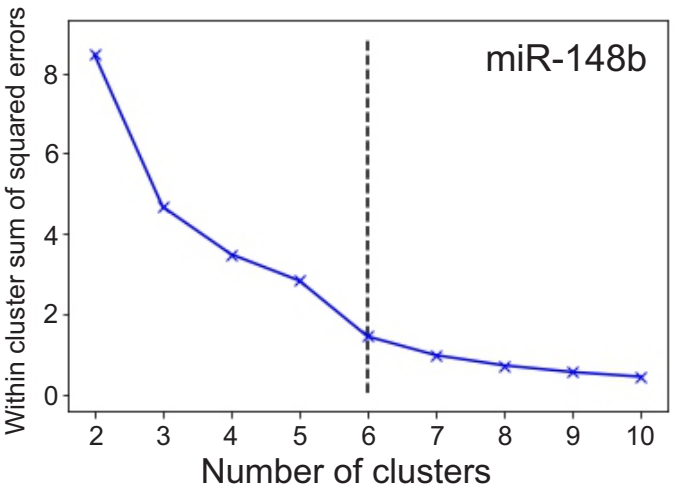


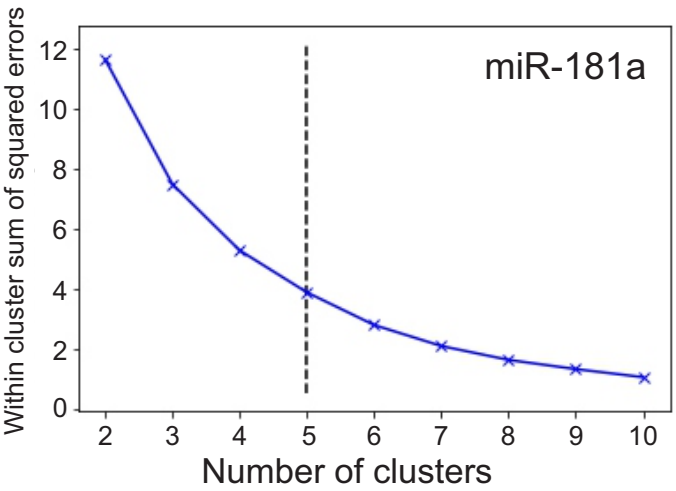
Supplementary Figure S2. Number of clusters identified for the 10 miRNAs analyzed. For each miRNA, mRNAs with similar fold changes (log2) were clustered together using the software Weka (v3.8; Frank et al., 2016) by the k-means method. Nine clusters were tested (from two to ten) and the best cluster number for each array were identified with the elbow curve method, using a python algorithm developed by Sapota et al. (2011).



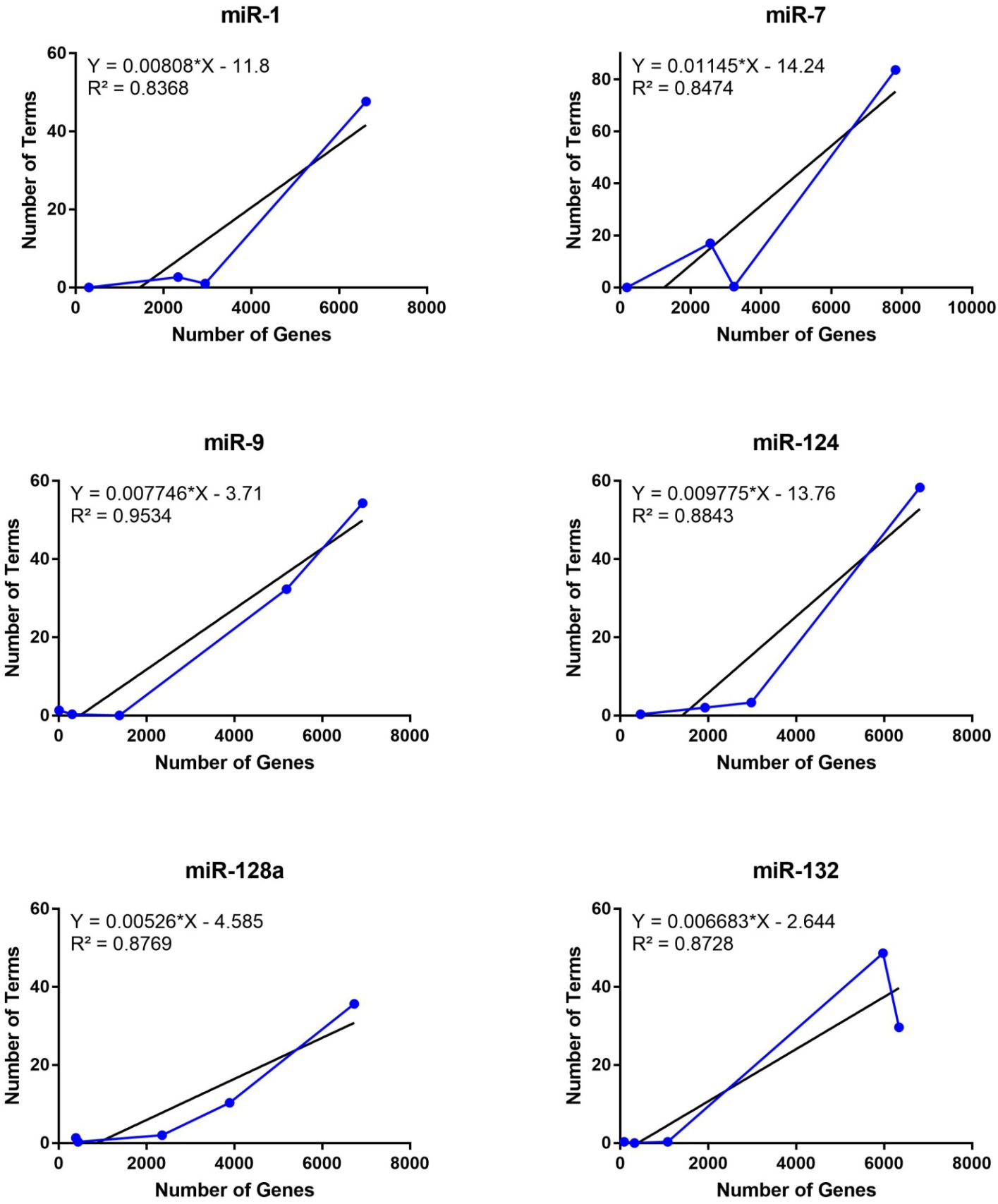

miR-133a
Within cluster sum of squared errors
Number of clusters

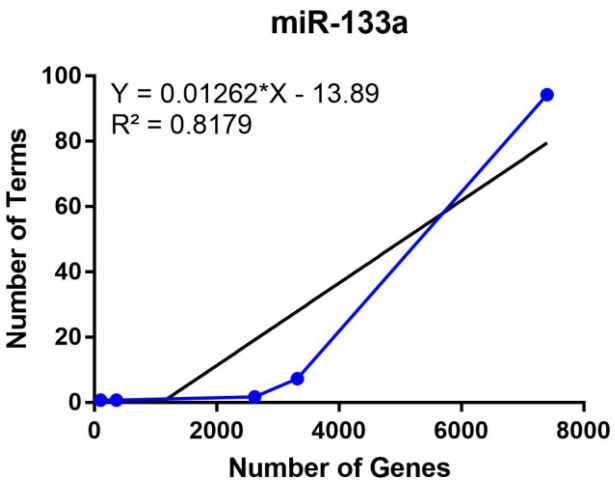

miR-142
Within cluster sum of squared errors
Number of clusters

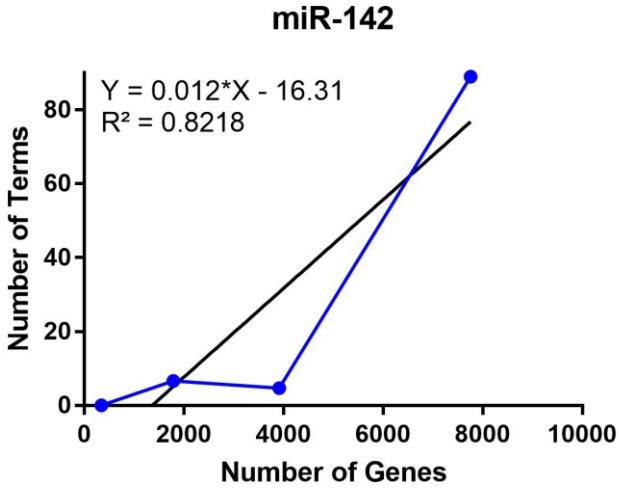

miR-148b
Within cluster sum of squared errors
Number of clusters

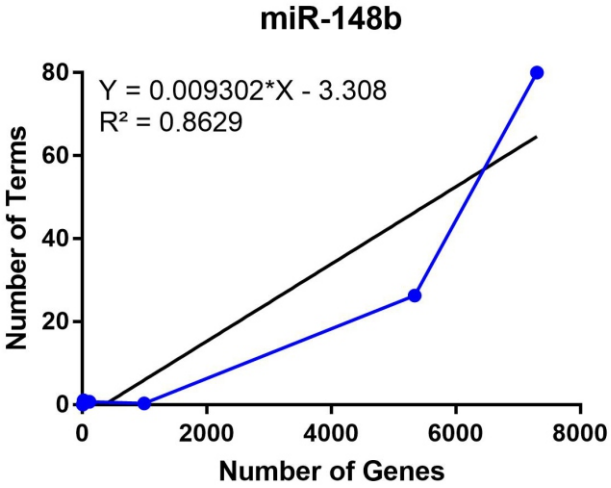

miR-181a
Within cluster sum of squared errors
Number of clusters

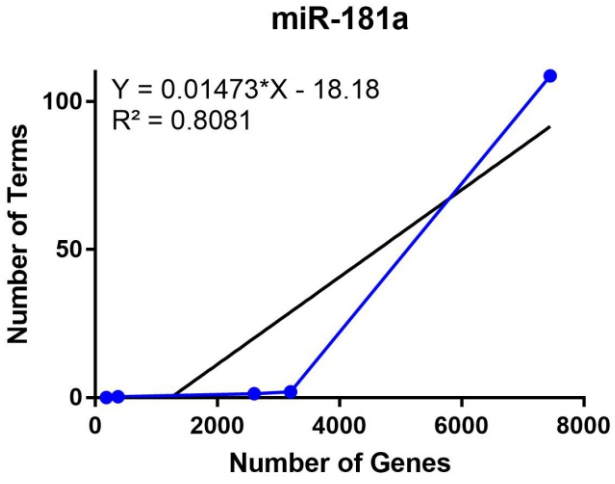
Supplementary Figure S3. Correlation between the number of enriched terms and perturbed targets of the random sets of the 10 miRNAs analyzed. The average number of terms of the three random sets were used. Clusters are ordered by the number of gene members.




miR-133a
Y = 0.01262*X - 13.89
R² = 0.8179
Number of Terms
Number of Genes
0
20
40
60
80
100
0
2000
4000
6000
8000

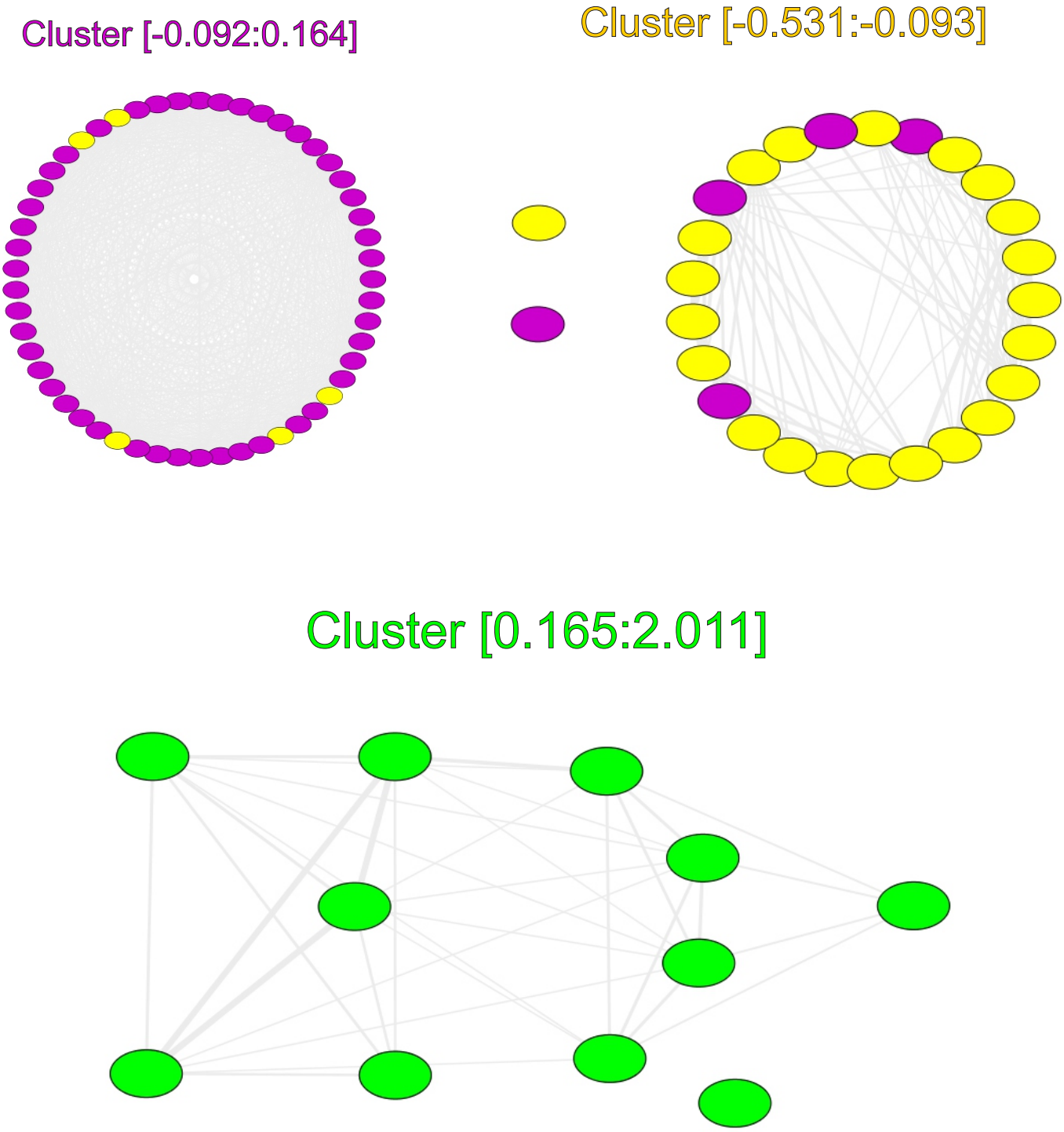

miR-142
Y = 0.012*X - 16.31
R² = 0.8218
Number of Terms
Number of Genes
0
20
40
60
80
0
2000
4000
6000
8000
10000


miR-148b
Y = 0.009302*X - 3.308
R² = 0.8629
Number of Terms
Number of Genes
0
20
40
60
80
0
2000
4000
6000
8000

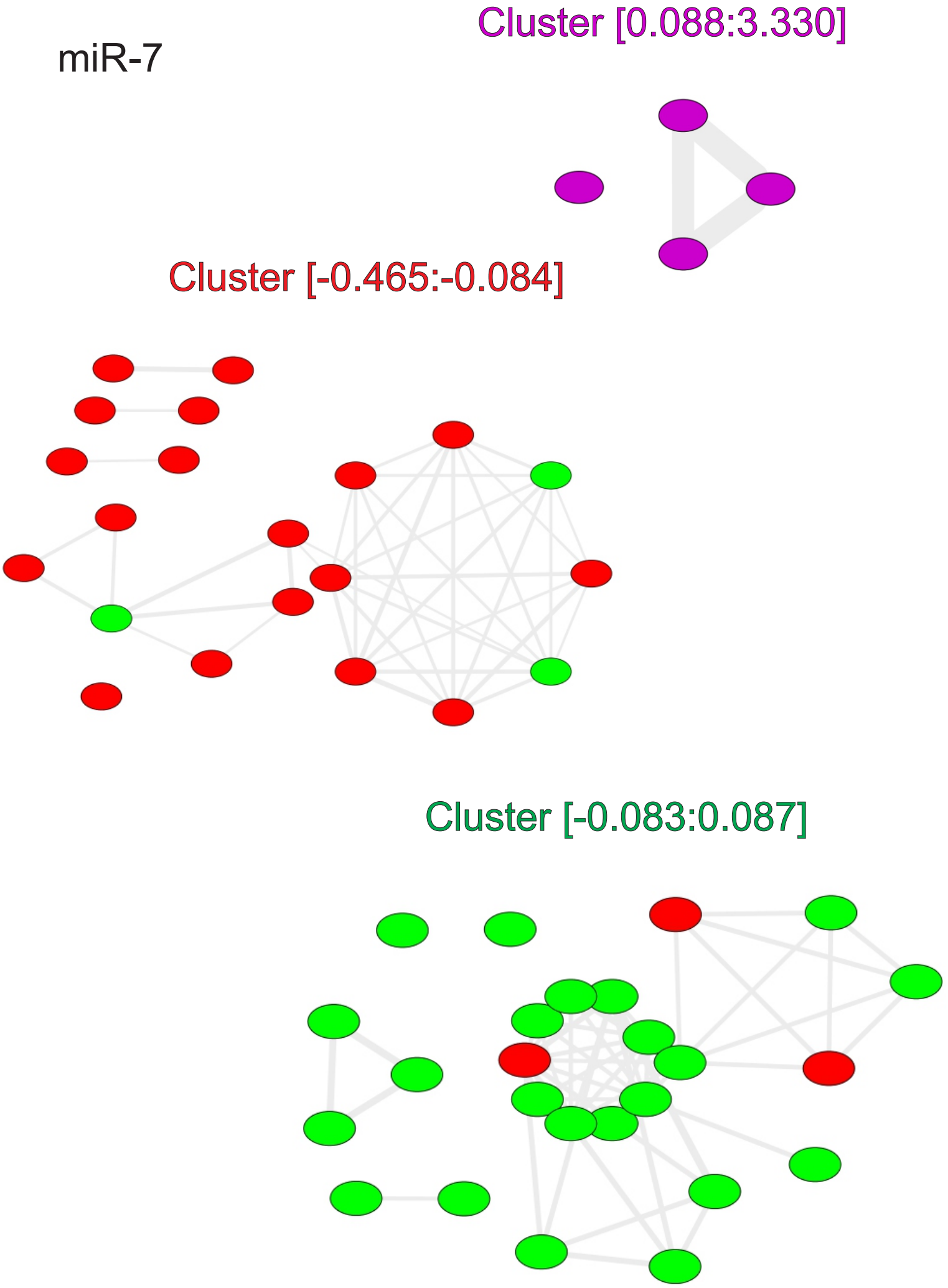

miR-181a
Y = 0.01473*X - 18.18
R² = 0.8081
Number of Terms
Number of Genes
0
50
100
0
2000
4000
6000
8000

Supplementary Figure S4. Semantic similarity of the enriched GO terms of mRNA clusters after transfection of the 10 miRNAs. Each term enriched in the same miRNA cluster is identified by the same color. Enriched GO terms were identified by ToppCluster and summarized by REVIGO to eliminate redundant terms. The numbers within the brackets represent the minimum and maximum fold change values of each cluster. Terms with different colors in a cluster indicate that the same term is also present in the cluster of that color. Grey edges connect highly similar terms, with edge width indicating similarity degree. The name of the enriched terms of each cluster identified by ToppCluster can be found at Supplementary Table S3.

miR-1

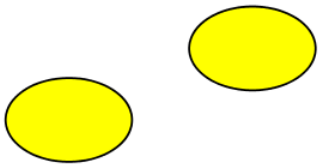


miR-7

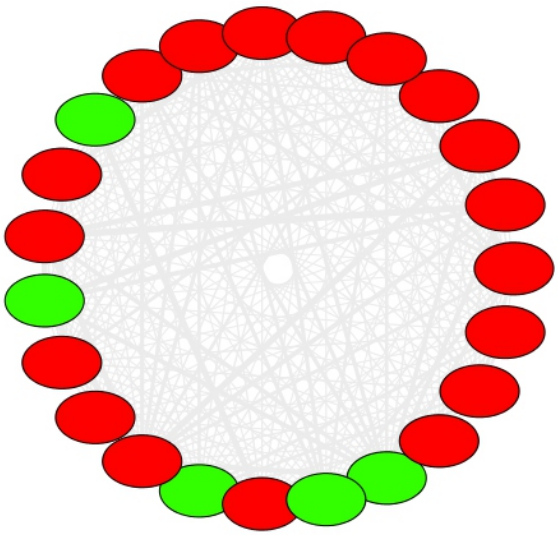


miR-9

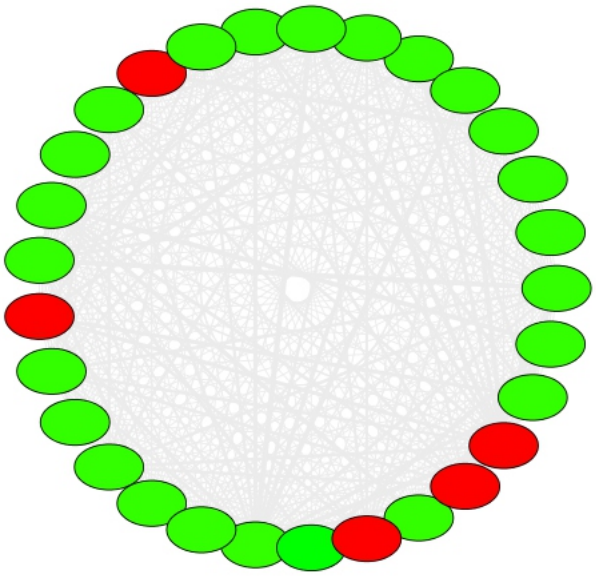
Cluster [0.405:3.413]



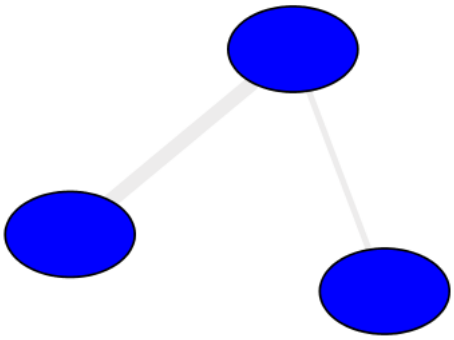
Cluster [-2.232:-0.233]



Cluster [-0.232:0.046]

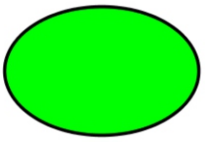


Cluster [0.047:0.402]

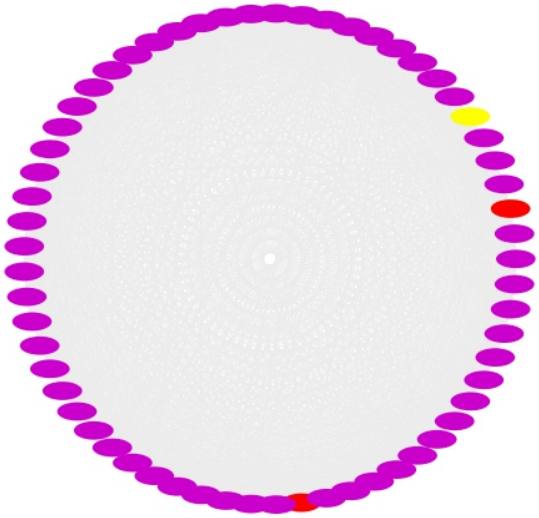


miR-124

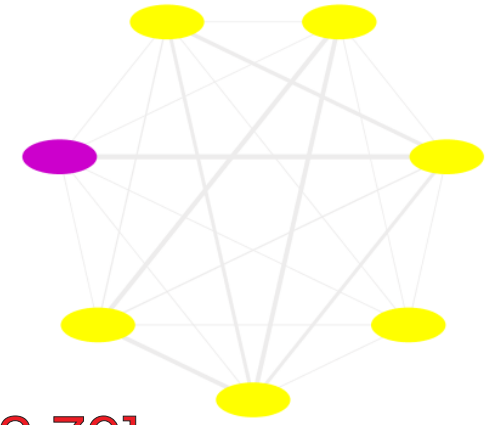
Cluster [-2.608:-0.58]



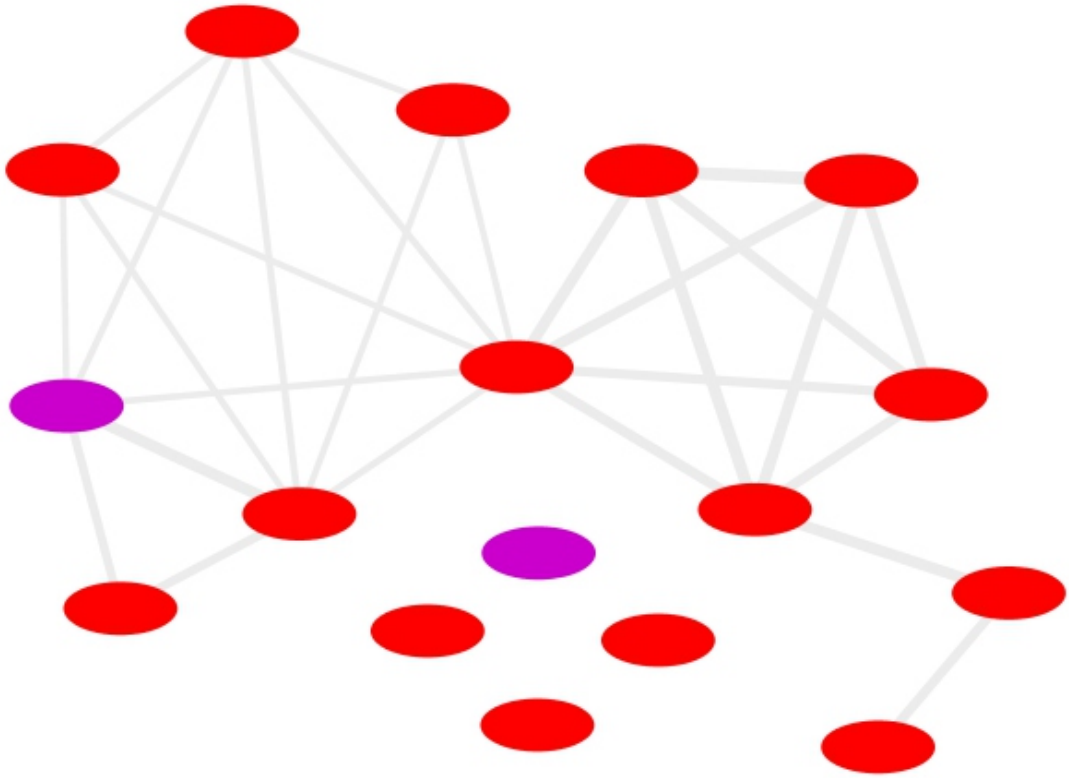
Cluster [-0.09:0.224]



Cluster [-0.576:-0.091]

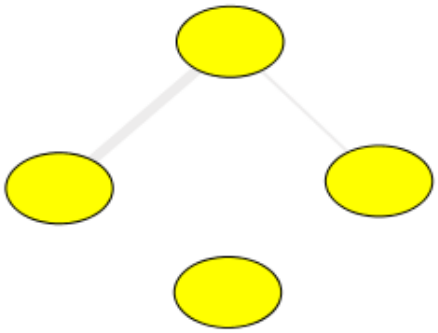


Cluster [0.225:2.79]

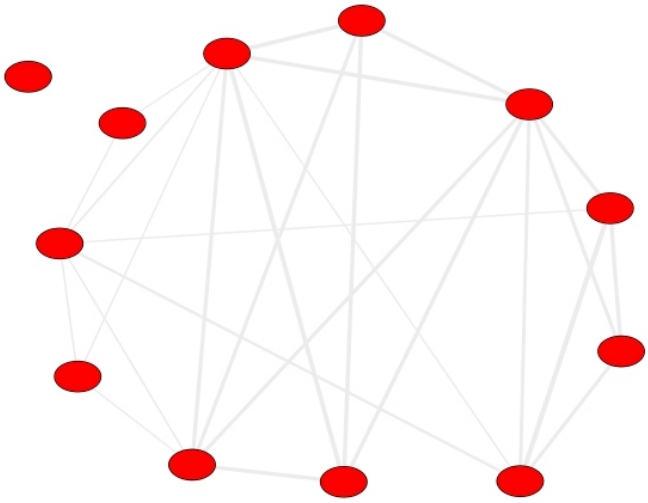


miR-128a

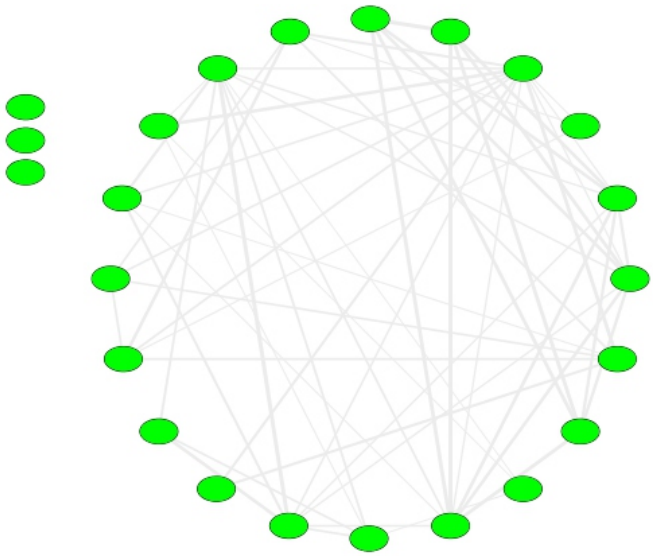
Cluster [0.34:3.676]



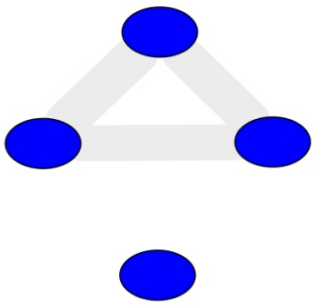
Cluster [-0.413:-0.106]



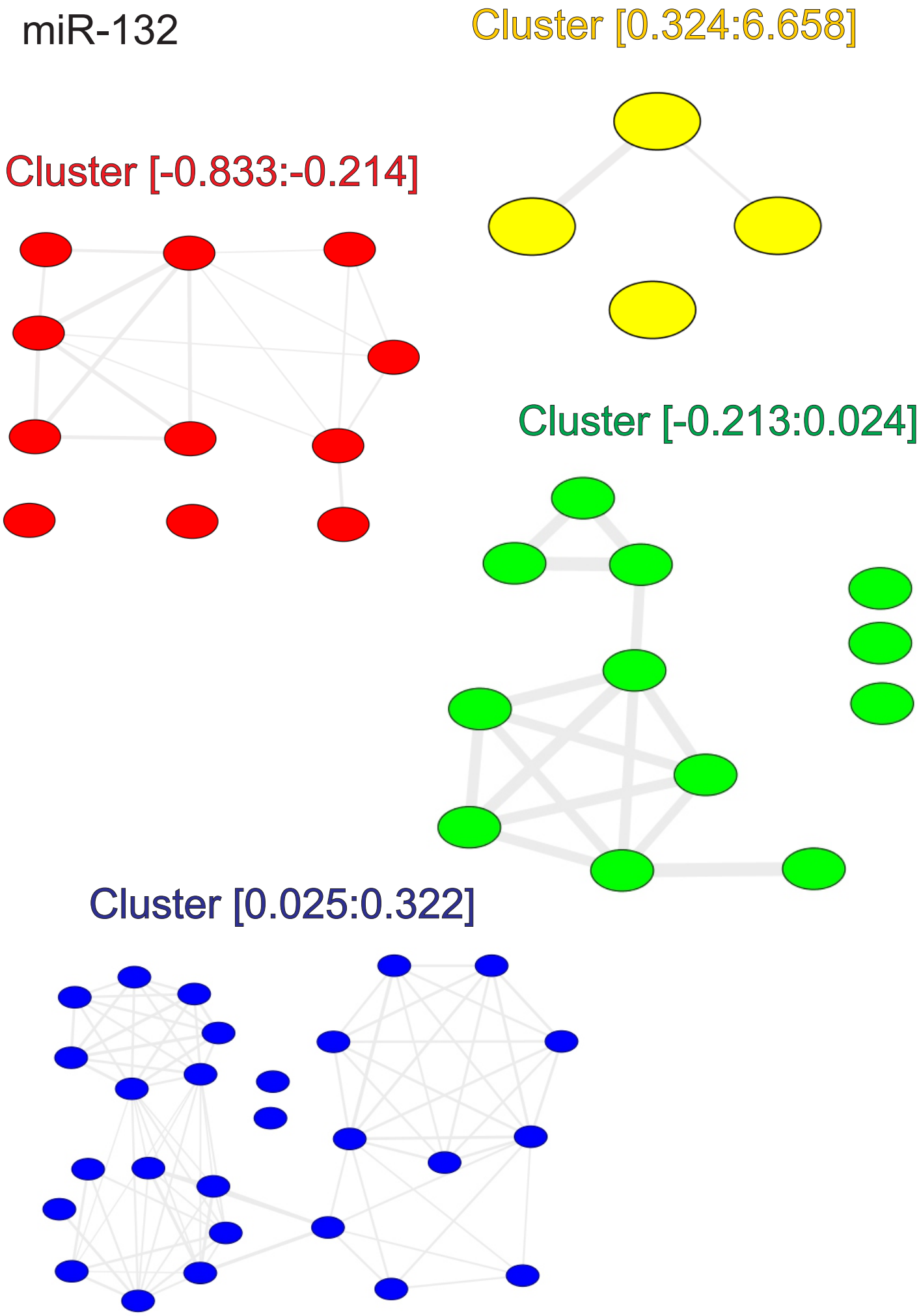
Cluster [-0.105:0.072]



Cluster [0.073:0.339]

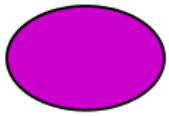


miR-132

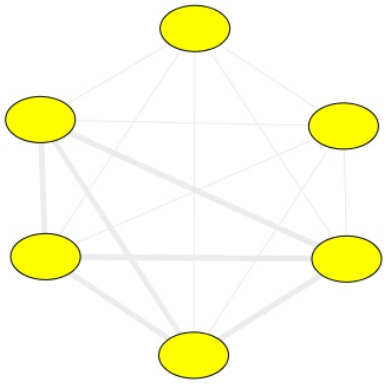

Cluster [0.324:6.658]
Cluster [-0.833:-0.214]
Cluster [-0.213:0.024]
Cluster [0.025:0.322]

miR-133a

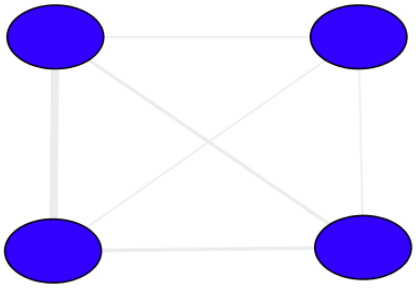
Cluster [-2.156:-0.542]



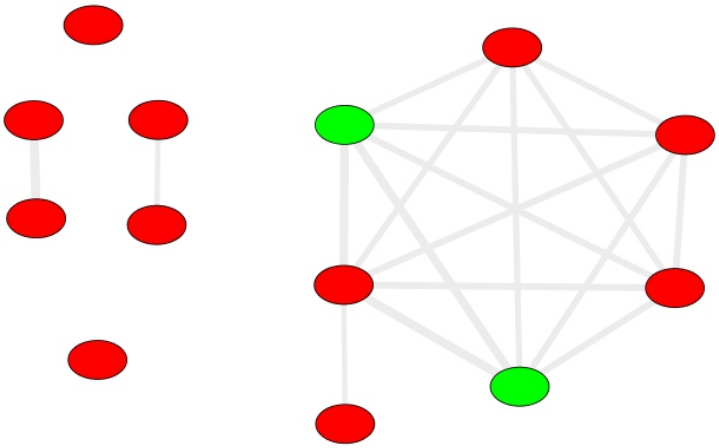
Cluster [0.674:4.128]



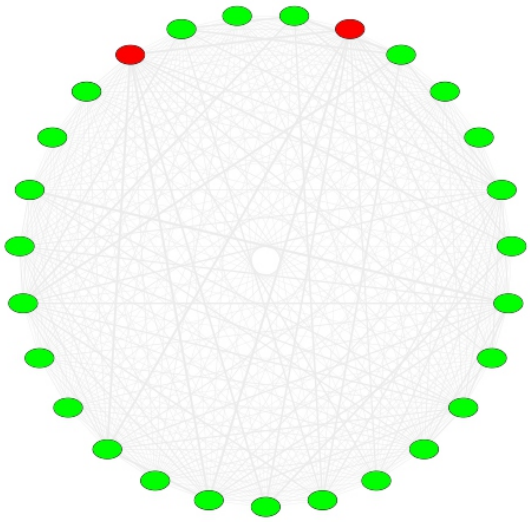
Cluster [0.12:0.666]



Cluster [-0.539:-0.119]

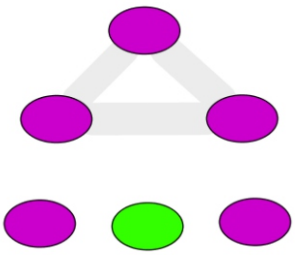


Cluster [-0.118:0.119]

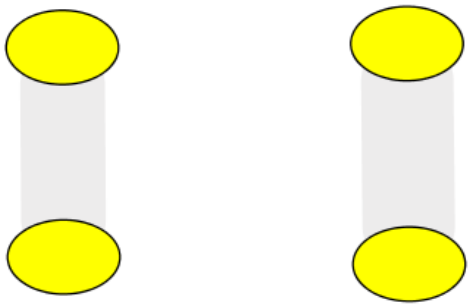


miR-142

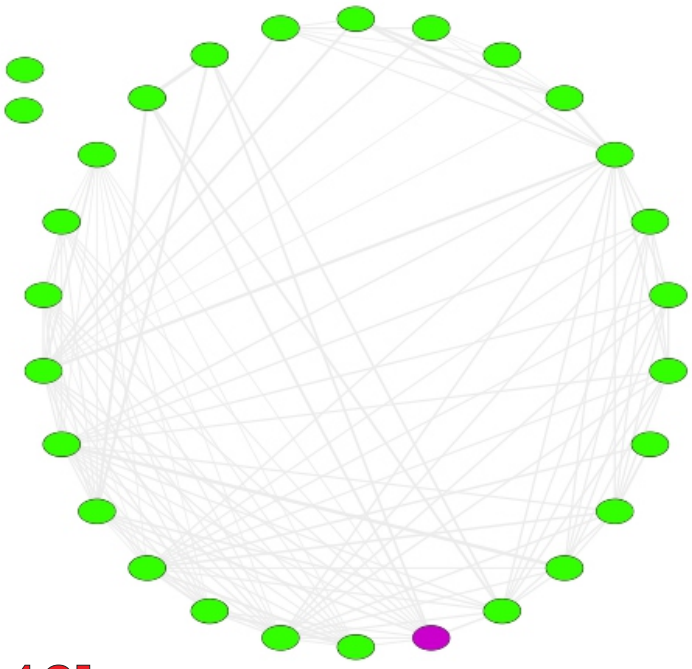
Cluster [-3.89:-0.346]



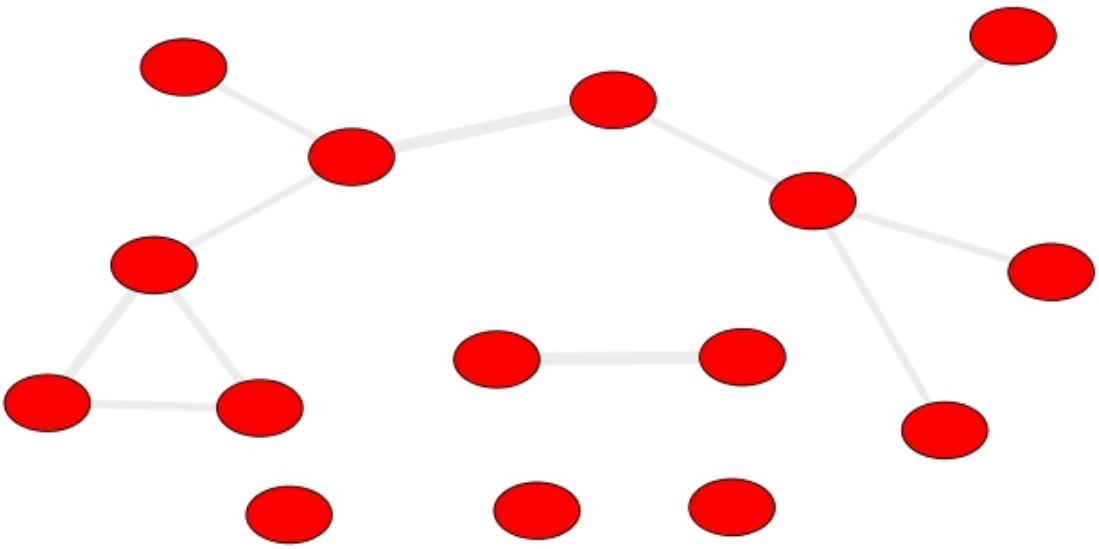
Cluster [0.134:2.367]



Cluster [-0.045:0.133]

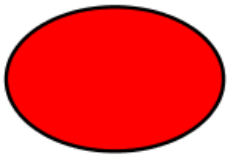


Cluster [-0.344:-0.046]

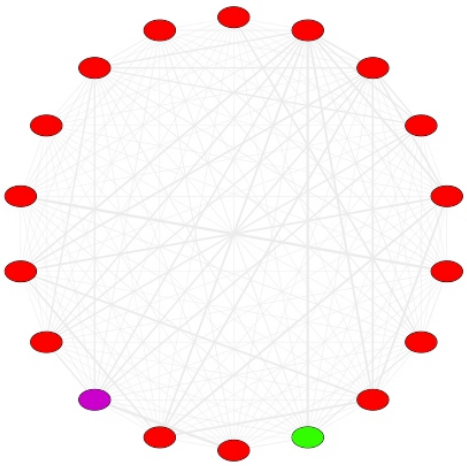


miR-148b

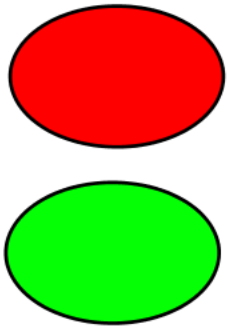
Cluster [-2.544:-0.336]



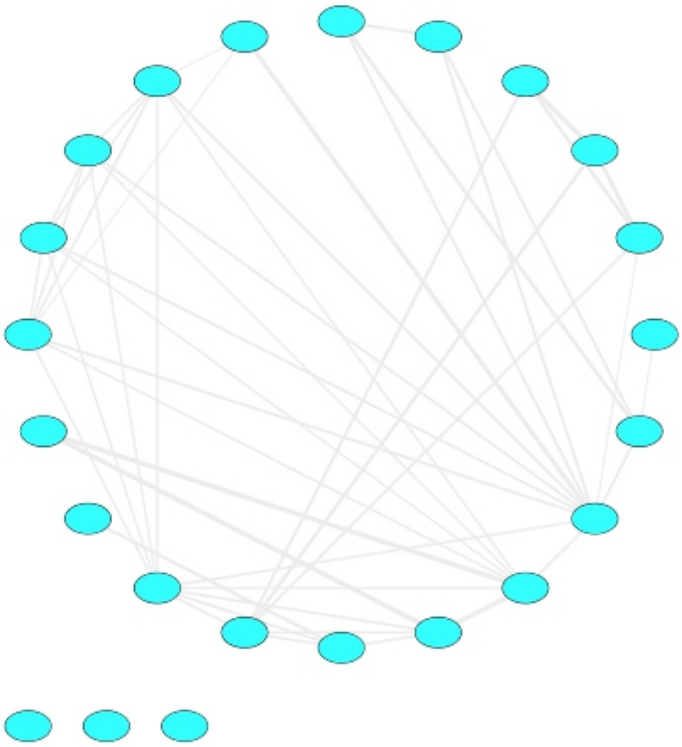
Cluster [0.05:0.749]



Cluster [0.76:3.427]

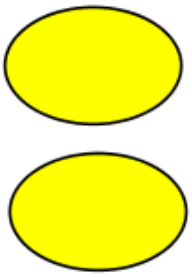


Cluster [-0.335:0.049]

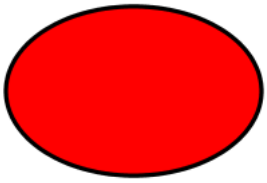


miR-181a

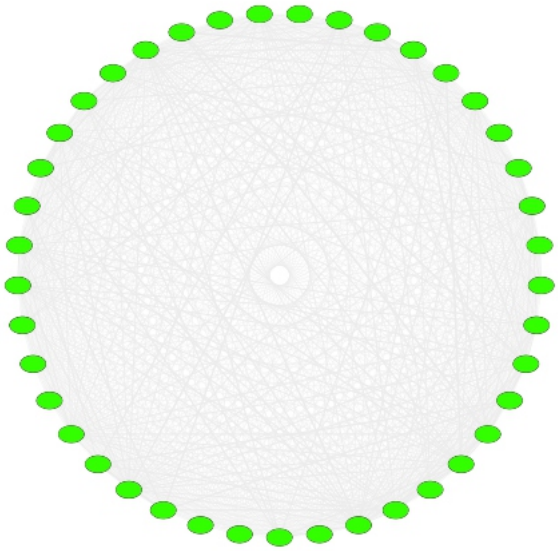
Cluster [0.415:2.751]



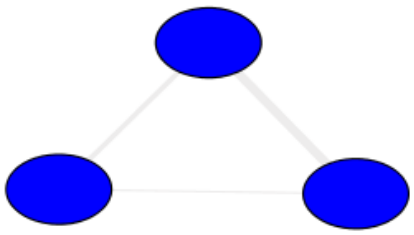
Cluster [-0.331:-0.065]



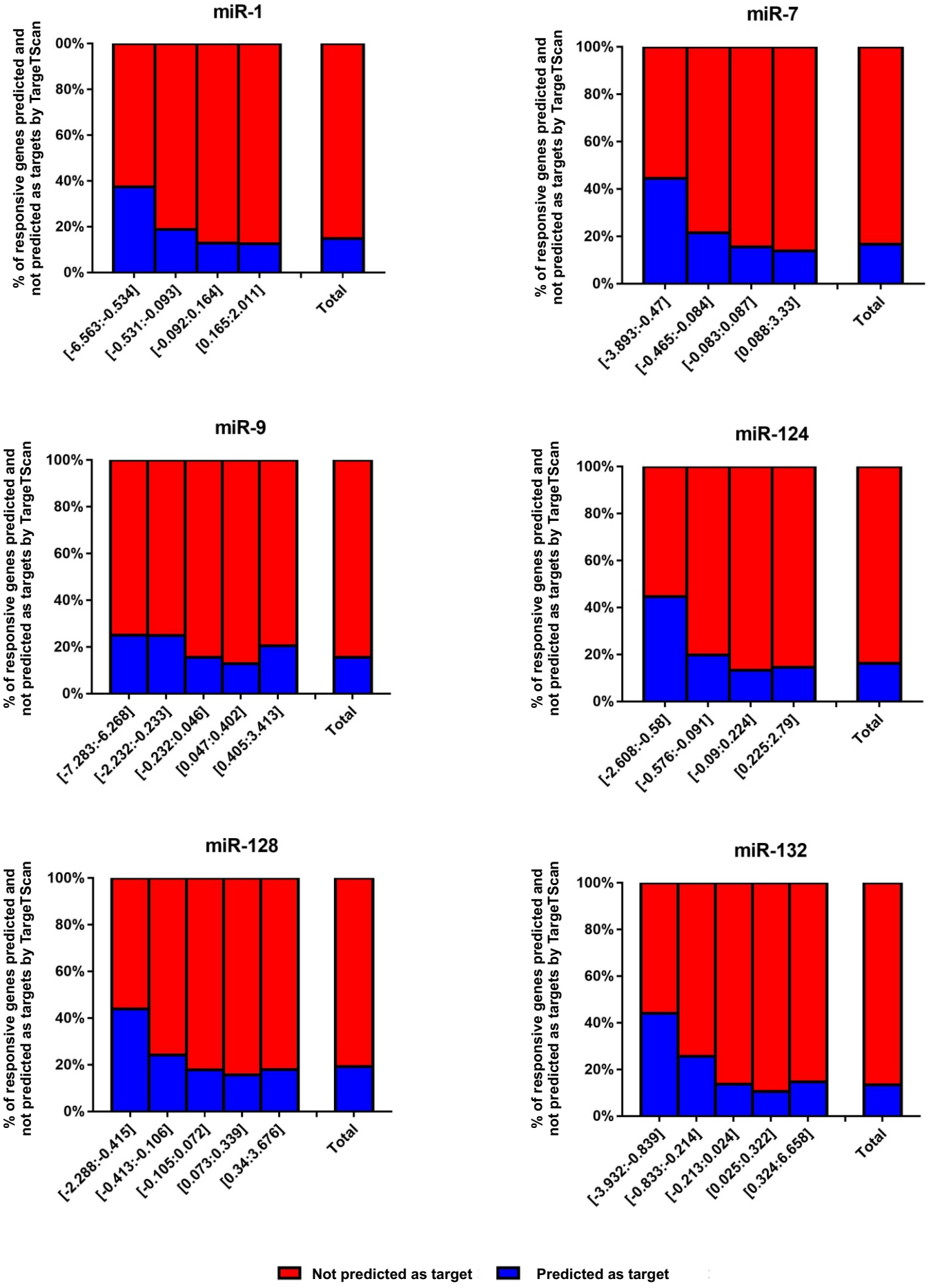
Cluster [-0.064:0.09]

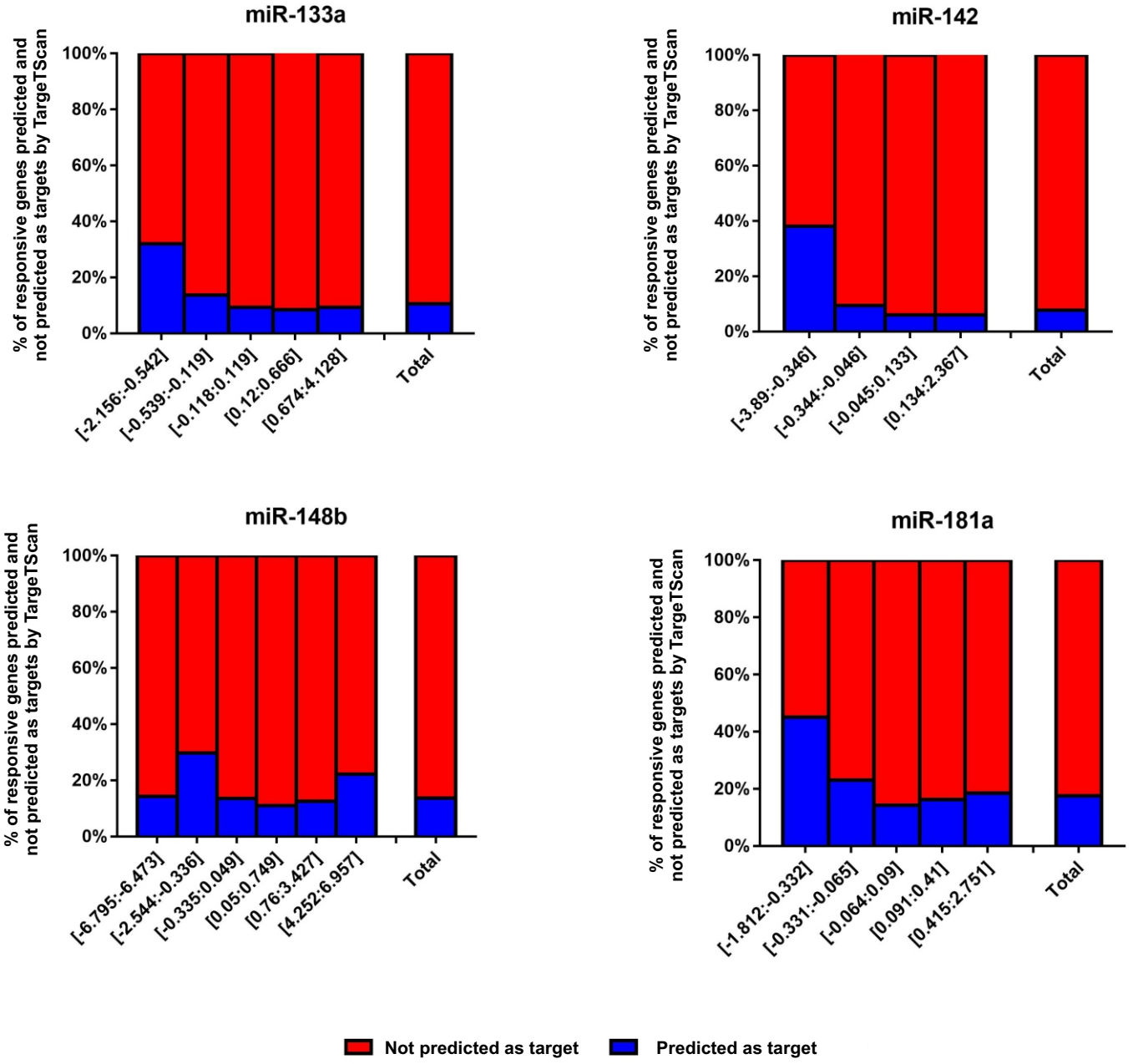


Cluster [0.091:0.41]

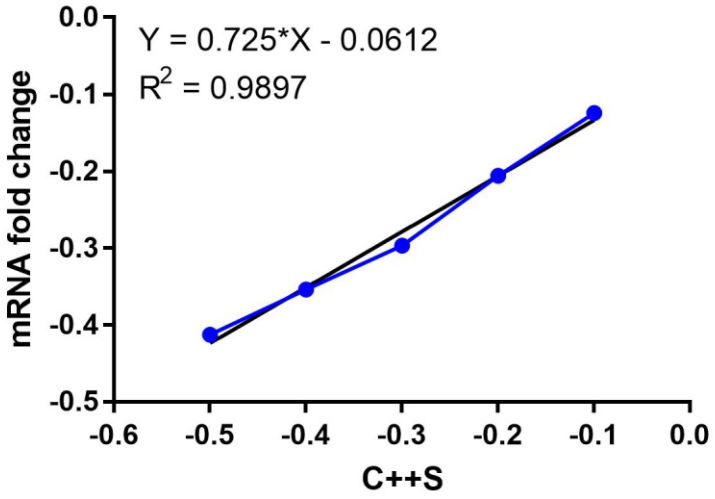


Supplementary Figure S5. Correlation between mRNA fold change and the number predicted targets of the 10 miRNAs.

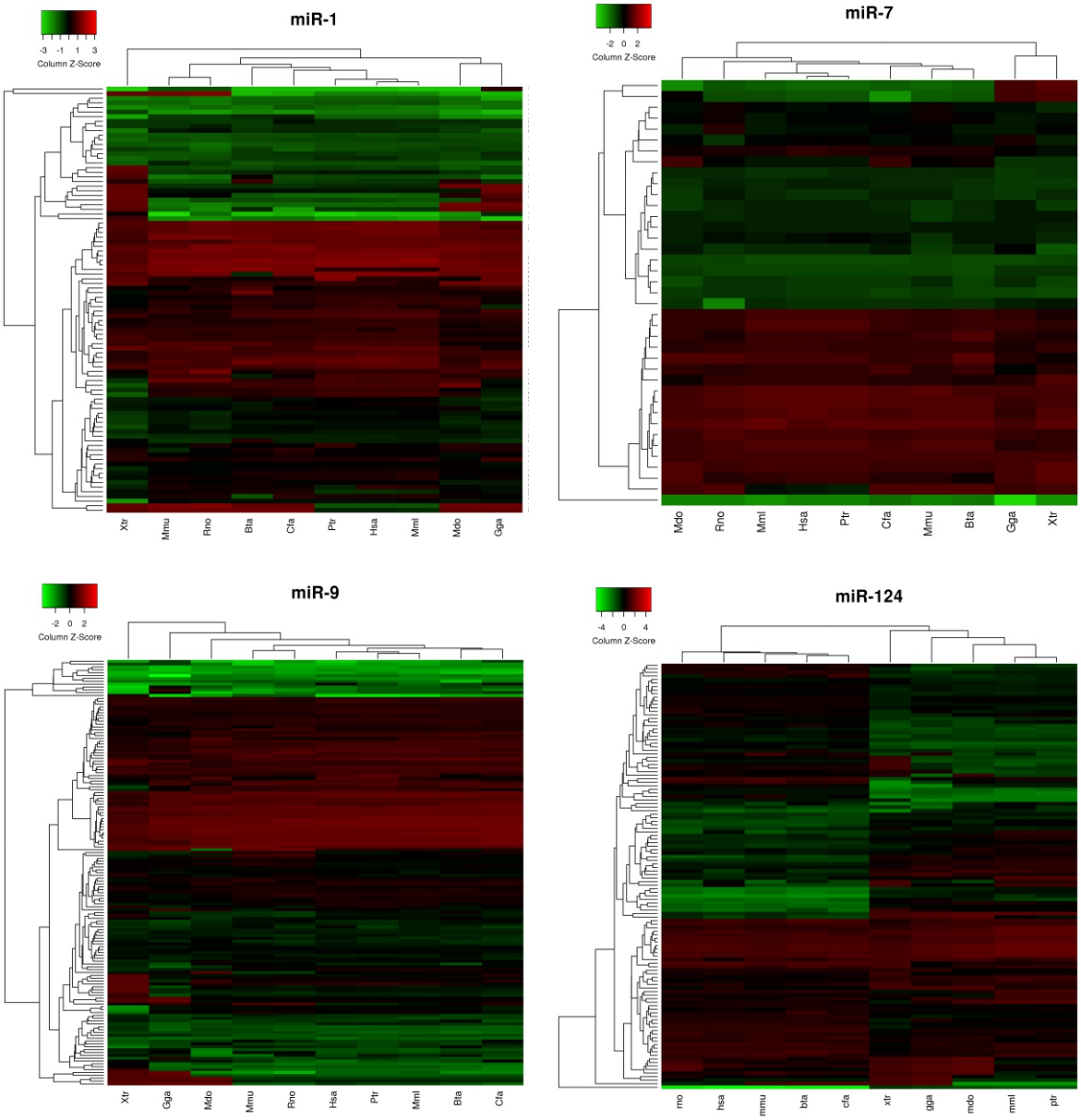


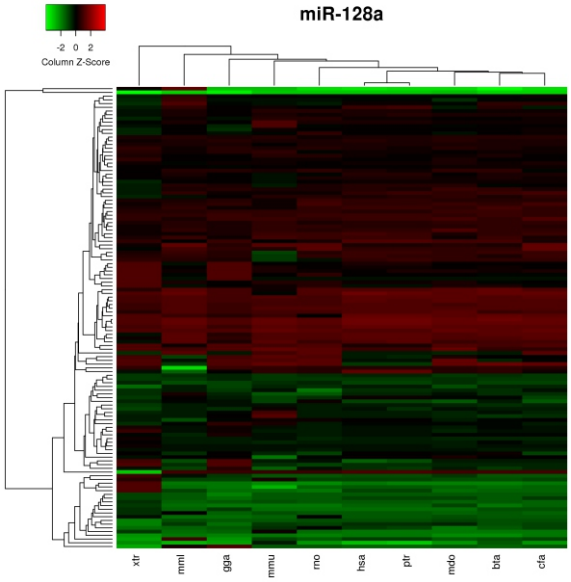

miR-133a
% of responsive genes predicted and not predicted as targets by TargeTScan
100%
80%
60%
40%
20%
0%
[-2.156:-0.542]
[-0.539:-0.119]
[-0.118:0.119]
[0.12:0.666]
[0.674:4.128]
Total
miR-142
% of responsive genes predicted and not predicted as targets by TargeTScan
100%
80%
60%
40%
20%
0%
[-3.89:-0.346]
[-0.344:-0.046]
[-0.045:0.133]
[0.134:2.367]
Total
miR-148b
% of responsive genes predicted and not predicted as targets by TargeTScan
100%
80%
60%
40%
20%
0%
[-6.795:-6.473]
[-2.544:-0.336]
[-0.335:0.049]
[0.05:0.749]
[0.76:3.427]
[4.252:6.957]
Total
miR-181a
% of responsive genes predicted and not predicted as targets by TargeTScan
100%
80%
60%
40%
20%
0%
[-1.812:-0.332]
[-0.331:-0.065]
[-0.064:0.09]
[0.091:0.41]
[0.415:2.751]
Total
Not predicted as target
Predicted as target

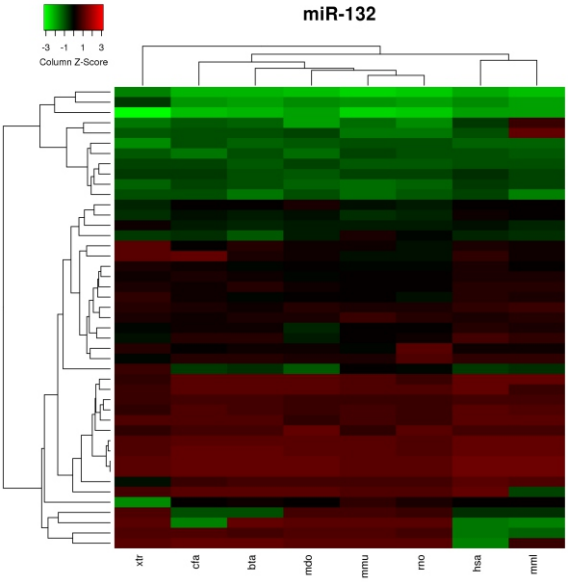
Supplementary Figure S6. Correlation between mRNA fold change and Context++Score. To perform this correlation, for each miRNA we separated the targets predicted by TargetScan in five groups according to its Context++Score ([0.1:0.2[; [0.2:0.3[; [0.3:0.4[; [0.4:0.5[and [0.5:0.6[). Then we calculated the average fold change of the predicted targets of each group. Finally, for each group, we calculated the average fold change of the ten miRNAs.

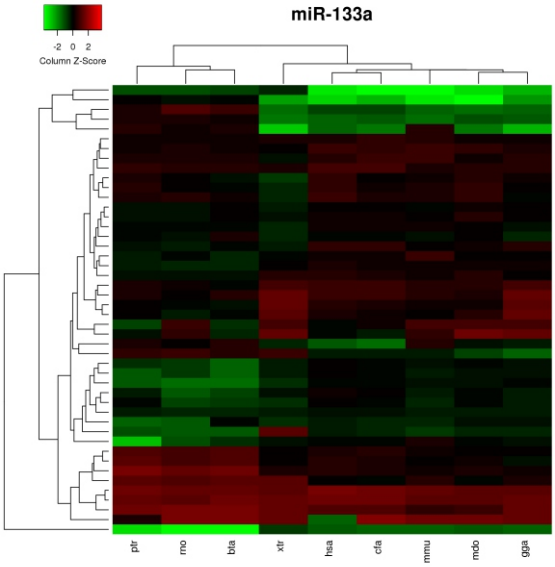


Supplementary Figure S7. Heatmaps of Context++Scores of the predicted targets of the ten miRNAs analyzed among ten vertebrate species. Negative Context++Score values are represented in green, positive values are represented in red, and near-zero values are represented in black. Lower Context++Score values represent stronger miRNA regulation levels.




miR-128a
-2 0 2
Column Z-Score
xtr
mml
gga
mmu
mo
hsa
ptr
mdo
bta
cfa


miR-132
-3 -1 1 3
Column Z-Score
xtr
cfa
bta
mdo
mmu
mo
hsa
mml


miR-133a
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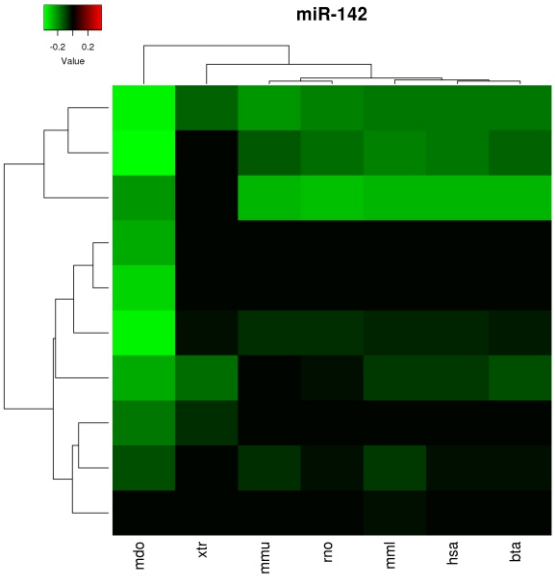
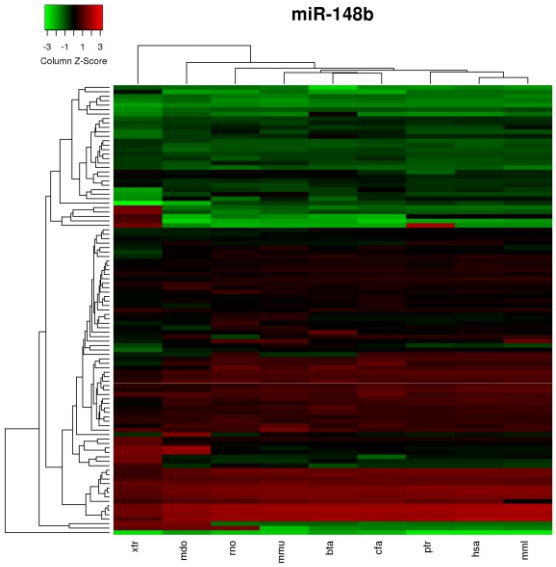
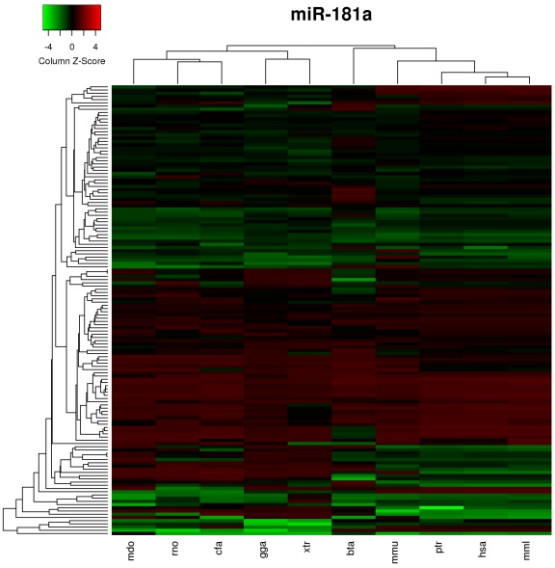
Column Z-Score
ptr
mo
bta
xtr
hsa
cfa
mmu
mdo
gga


miR-142
-0.2 0.2
Value
mdo
xtr
mmu
mo
mml
hsa
bta


miR-148b
-3 -1 1 3
Column Z-Score
xtr
mdo
mo
mmu
bta
cfa
ptr
hsa
mml


miR-181a
-4 0 4
Column Z-Score
mdo
mo
cfa
gga
xtr
bta
mmu
ptr
hsa
mml