Electronic Supplementary Material (ESI) for Molecular BioSystems

Dissection the potential characteristic of miRNA-miRNA functional synergistic regulations

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Dissection the potential characteristic underlying miRNA synergism identified by predicted miRNA targets from miRBase

Dissection the potential characteristic underlying miRNA synergism identified by the integrated predicted miRNA target data

1. Supplementary Figures

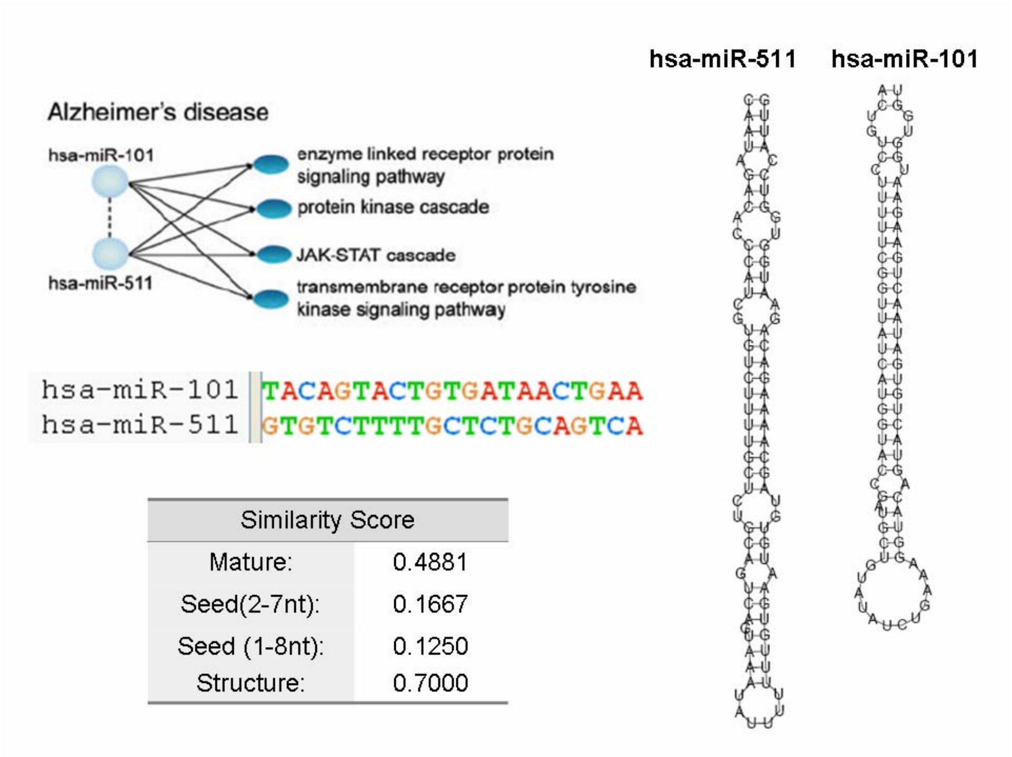

Figure S1. An example of hsa-miR-101 and hsa-miR-511 that synergistically regulate functional modules; these co-regulations are associated with diseases. The two miRNAs show high structural similarity score, however their sequence similarity are relatively low. Non-direct dashed line represents miRNA synergistic action; direct line represents the miRNA regulation to functional modules.

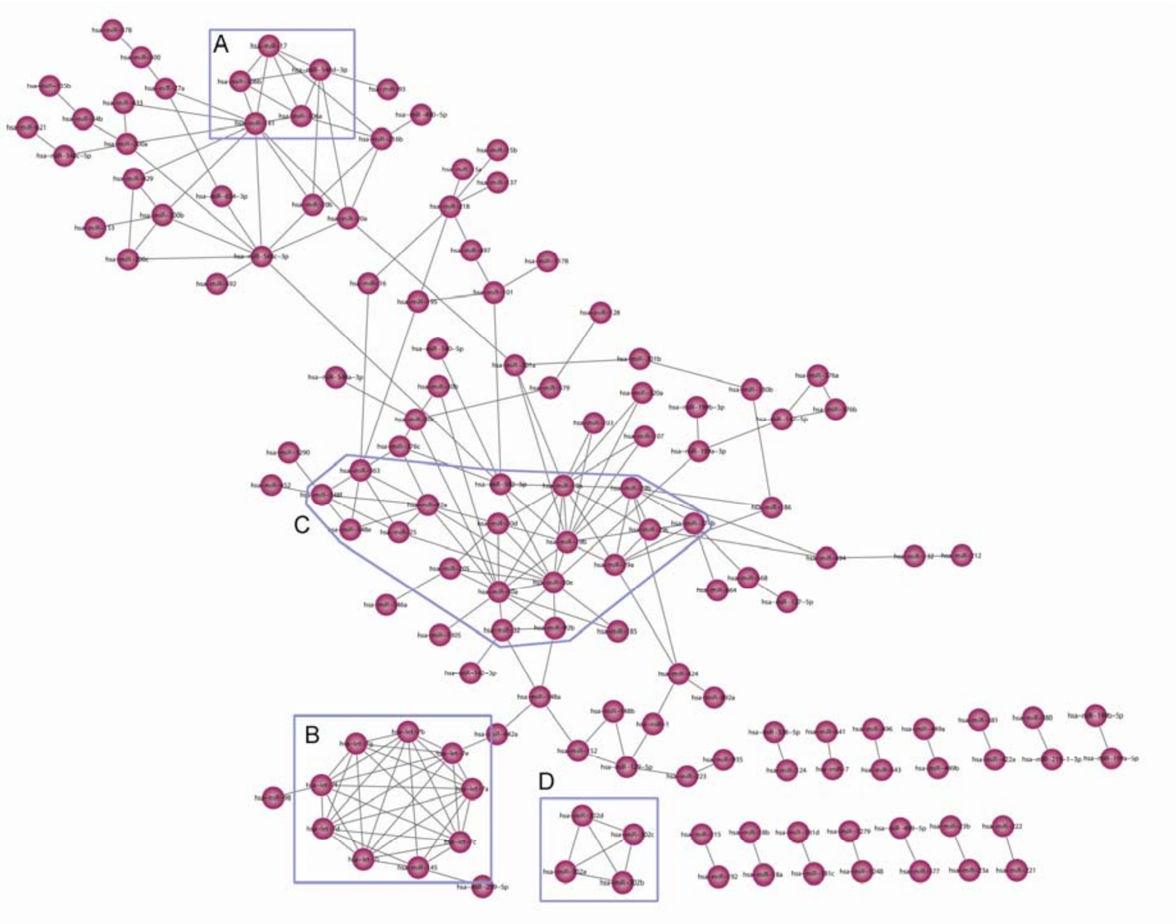

Figure S2. The layout of the sub-network composed of miRNA synergistic regulations which also share at least one same TF. Four miRNA communities are identified which are marked by symbols A to D. The marked orders of communities are the same as in Figure 3.

2. Supplementary Tables

Table S1. All synergistic miRNA pairs in the MFSN constructed using different miRNA target datasets.

Table S2. The statistical information of transcriptional regulations for miRNAs in the MFSN.

Databases	TransmiR	miReg	UCSC Threshold			Total		
			1KB	2 KB	5 KB	1KB	2 KB	5 KB
Number of TF	118	84	140	149	151	264	271	272
Number of miRNAs	116	50	346	426	521	381	451	536
Number of regulations	397	161	1447	2399	5088	1767	2609	4875

Table S3. The detailed TF-miRNA regulatory relationship for miRNAs in the MFSN.

3. Supplementary Text

Dissection the potential characteristic underlying miRNA synergism identified by predicted miRNA targets from miRBase

Synergetic miRNA pairs show high seed sequence similarity

In the MFSN based on predicted miRNA targets from miRBase by miRanda algorithm, there are 3872 non-redundant synergistic pairs among 692 miRNAs. Among these miRNAs, 676 miRNAs have sequence information in miRBase database, and they form 3712 miRNA synergistic regulations. For these synergistic miRNA pairs, we respectively calculated their seed sequence and secondary structure similarities and then compared these similarity distributions to the background distributions consisted of all miRNA combinations except for synergistic ones. So, 224,438 (676*675/2-3712) miRNA pairs among 676 miRNAs are assembled the background set. As a result, the average 2-7nt sequence similarity for synergetic miRNA pairs is significantly higher than that for the background set. The average similarity score for synergetic pairs is 0.2932, and that for non-synergetic ones is 0.2532 (p=4.91e-14, computed by the Wilcoxon rank-sum test). In addition, we randomly chose the same number of miRNA pairs from the background set as pseudo-MFSNs, and recalculated their average similarity scores. The average similarity score of the MFSN is also significantly higher than that of random conditions (p<1.0e-4). Moreover, when analyzing different thresholds of seed sequence similarity, we found that the proportions for cooperative miRNA pairs with high similarity are about two times more than those for non-cooperative ones (Table S4). We also got the similar results when analyzing the similarity of 1-8nt seed sequences of miRNAs. These results indicate that miRNA pairs with strong seed sequence similarity are more likely to be functional synergism with each other in the MFSN than random pairs.

Next, we specially focused on the similarity of miRNA synergism between different miRNA families. 96.47% cooperation occurs between miRNA members from different families. As a result, although the average similarities of synergistic miRNA pairs decrease which for 2-7nt and 1-8nt seeds are 0.2760 and 0.2749 respectively, the values are still significant higher than other pairs between different miRNA families (p-values for 1-8nt and 2-7nt seeds respectively are 6.44e-7 and 2.17e-6, computed by the rank-sum test). When analyzing different thresholds of seed sequence similarity, we found that the proportions for cooperative miRNA pairs with high similarity are about two times more than those for non-cooperative ones (Table S4). Therefore, we concluded that synergistic miRNA pairs may exercise their function with the aid of their similar seed sequences.

Table S4. The proportions of synergistic miRNA pairs with strong similarity in seed sequence and secondary structure are higher than those of pairs in the background set under different thresholds of similarity scores.

Similarity	Synergetic /non-synergetic	Thresholds of similarity scores			
		0.9	0.8	0.7	0.6
Seed sequence (2-7nt)	Yes	0.034 (0.013)	0.050 (0.027)	0.050 (0.027)	0.096 (0.073)
	No	0.001 (0.0006)	0.007 (0.006)	0.007 (0.006)	0.043 (0.042)
Seed sequence (1-8nt)	Yes	0.019 (0.001)	0.036 (0.013)	0.045 (0.22)	0.079 (0.056)
	No	0.0005 (6.8e-5)	0.002 (0.0008)	0.007 (0.006)	0.035 (0.033)
Secondary structure	Yes	0.004 (0.0003)	0.040 (0.030)	0.308 (0.2934)	0.703 (0.696)
	No	0.001 (0.0001)	0.030 (0.028)	0.261 (0.257)	0.653 (0.651)

Note: Yes represents all synergistic miRNA pairs in the MFSN, and No represent miRNA pairs with no cooperation in the background set. The values in the brackets represent the proportions of miRNA pairs which originate from different miRNA family.

Synergetic miRNA pairs show high structural similarity

To investigate the structure feature of synergetic miRNAs, we computed their structural similarity and found that the mean similarity of synergetic miRNA pairs is significantly higher than that of non-synergetic ones (*p*=1.7038e-16, Wilcoxon rank-sum test). The average similarity score of the MFSN is also significantly higher than that of random conditions (*p*<1.0e-4). Additionally, after discarding synergism occurred in the same miRNA families, synergistic miRNA pairs also show significant high similarity in structure (*p*=4.16e-12, rank-sum test), which is also significant higher than random (*p*<1.0e-4). The fraction of synergetic miRNA pairs with high similarity scores is also more than that of the background set (Table S4). For example, about 3.42% synergetic miRNA pairs' structural similarity scores are above 0.8. We also found 70.34% miRNA pairs in the MFSN are with structural similarity scores above 0.6. These results indicate that synergetic miRNA pairs tend to have significantly high structural similarity which might partially explain their functional cooperative regulations.

Synergistic miRNA pairs are likely to share a common transcriptional regulatory mechanism

Firstly, we investigate the distance distribution of these synergistic miRNA pairs. As a result, there are 300 miRNAs pairs located on the same chromosomes, accounting for 8.08% of the MFSN, and only 81 out of these pairs are located in the same miRNA cluster. We concluded there is no dominant orientation for miRNAs located on the same chromosomes or the same miRNA clusters tend to cooperate with each other.

We further attempted to dissect the potential characteristic of miRNA synergistic regulations from the view of transcriptional regulation. TF-miRNA regulatory relationships are obtained from three databases: TransmiR, miReg and UCSC (Table S5). Totally, 421 miRNAs in the MFSN are regulated by more than one TF when considering 1KB promoters and those two validated datasets, involving 1553 synergetic miRNA pairs. Of these synergetic miRNA pairs, 17.77% (276/1553) share at least one TFs, which is significantly higher than that of non-synergetic miRNA pairs (1.234 fold to the ratio of non-synergistic miRNA pairs, p=1.16e-4, Fisher exact test). Moreover, we found that the average regulation consistence score is significantly higher than that of non-synergetic miRNA pairs (p=1.6494e-5, Wilcoxon rank-sum test); the mean score of synergistic miRNAs is 0.3284, and that of non-synergistic miRNAs is 0.2562. In order to further evaluate the regulation concordance of synergetic miRNAs, we randomly selected the same number of miRNA pairs from the general background and recalculated the consistence score. Consequently, the regulation concordance for synergetic miRNA pairs is also significantly higher than that of random conditions (p<1.0e-4). Additionally, the proportions of synergistic miRNA pairs with strong concordance scores are higher under different thresholds compared with non-synergistic miRNA pairs (Figure S3). We also got the similar results when analyzing the 2KB and 5KB putative promoter regions of miRNAs (data not shown).

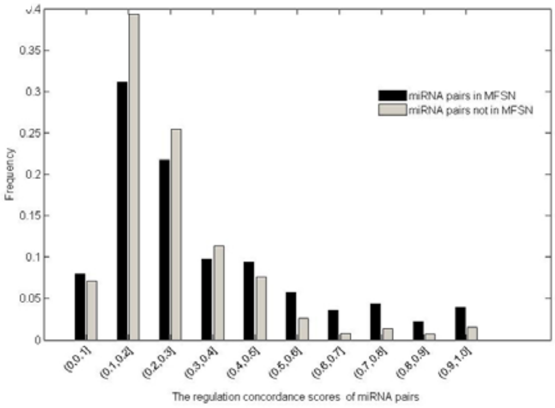

Figure S3. The distributions of regulation concordance scores for synergetic miRNA pairs in the MFSN and other miRNA combinations.

Table S5. The transcriptional regulation information of miRNAs in the MFSN constructed by miRBase.

Databases	TransmiR	miReg	UCSC			Total		
			1KB	2 KB	5 KB	1KB	2 KB	5 KB
Number of TFs	118	84	138	149	150	262	271	271
Number of miRNAs	115	78	406	474	563	445	500	578
Number of regulations	396	267	1732	2880	6056	2144	3149	5821

Note: In the UCSC, three kinds of putative promoter regions of each miRNA are respectively analyzed, defined as 1kb, 2kb and 5kb upstream of miRNA precursor.

Co-regulated miRNAs are located in the central of the MFSN

Next, we specially focused on 276 miRNAs synergistic regulations which share at least one TF, involving 248 miRNAs. Here, these miRNAs' position bias in the MFSN is investigated by dividing miRNAs in the network into two groups: co-regulatory miRNAs and others. Three measures are used to characterize miRNA's centrality in the MFSN, degree, betweenness centrality and closeness centrality. As shown in Table S6, all the average centrality values of co-regulation miRNAs are much greater than that of other miRNAs in MFSN, indicating that co-regulation miRNAs indeed play central roles in the MFSN. The median degree of co-regulation miRNAs is 13 and that of other miRNAs is 7; and the median betweenness and closeness of co-regulation miRNAs are also higher. In addition, we got the similar results by analyzing different transcriptional regulation datasets (Table S6). The discovery of the significant difference in the centrality between co-regulatory miRNAs and others suggests a functional importance of miRNAs in the first group. Therefore, we concluded that dysregulations of these co-regulation miRNAs are more likely to cause diseases; indeed, we found 56.45% of these miRNAs are annotated as disease miRNAs in the database of miR2Disease, while the percentage for miRNAs without co-regulations is 47.97%.

Table S6. Three topological centrality features and their corresponding medians between co-regulatory miRNAs and other miRNAs in the MFSN. P values are calculated by Wilcoxon rank sum test.

Upstream threshold	miRNA categories	Topological features		
		Degree	Betweenness	Closeness
1KB	sharing TFs	13	1245.0832	4.9975e-4
	Others	7	489.2087	4.7103e-4
	P value	1.52e-13	4.43e-11	1.82e-12
2KB	sharing TFs	12	1161.7630	4.9751e-4
	Others	7	472.8617	4.6685e-4
	P value	1.44e-14	1.87e-11	6.63e-14
5KB	sharing TFs	10	845.5227	4.8780e-4
	Others	7	584.9804	4.6729e-4
	P value	7.94e-6	1.52e-4	1.99e-6

Dissection the potential characteristic underlying miRNA synergism identified by the integrated predicted miRNA target data

Synergetic miRNA pairs show high seed sequence similarity

In the MFSN based on the integrated predicted miRNA target data, there are 6172 non-redundant synergistic pairs among 592 miRNAs. Among these miRNAs, 538 miRNAs have sequence information in miRBase database, and they form 5562 miRNA synergistic regulations. For these synergistic miRNA pairs, we respectively calculated their seed sequence similarities and secondary structure similarity, and then compared these similarity distributions to the background distributions consisted of all miRNA combinations except for synergistic ones. So, 138,891 (538*537/2-5562) miRNA pairs among 538 miRNAs are assembled the background set. As a result, the average 2-7nt sequence similarity for synergetic miRNA pairs is significantly higher than that for the background set. The average similarity score for synergetic pairs is 0.2835, and that for non-synergetic ones is 0.2537 (p=6.40e-16, computed by the Wilcoxon rank-sum test). In addition, we randomly chose the same number of miRNA pairs from the background set as pseudo-MFSNs, and recalculated their average similarity scores. The average similarity score of the MFSN is also significantly higher than that of random conditions (p<1.0e-4). Moreover, as shown in Table S7, 3.25% synergetic miRNA pairs are with similarity score above 0.8, which is much higher than that of the background set. We also found 7.48% miRNA pairs in the MFSN with similarity scores above 0.6. We also got the similar results when analyzing the similarity of 1-8nt seed sequences of miRNAs. These results indicate that miRNA pairs with strong seed sequence similarity are more likely to be functional synergism with each other in the MFSN than random pairs.

Next, we specially focused on the similarity of miRNA synergism between different miRNA families. 97.41% cooperation occurs between miRNA members from different families. As a result, although the average similarities of synergistic miRNA pairs decrease which for 2-7nt and 1-8nt seeds are 0.2724 and 0.2527 respectively, the values are still significant higher than other pairs between different miRNA families (p-values for 1-8nt and 2-7nt seeds respectively are 1.44e-10 and 2.96e-10, computed by the rank-sum test). When analyzing different thresholds of seed sequence similarity, we found that the proportions for cooperative miRNA pairs with high similarity are more than those for non-cooperative ones (Table S7). We concluded that synergistic miRNA pairs may exercise their function with the aid of their similar seed sequences.

Synergetic miRNA pairs show high structural similarity

To investigate the structure feature of synergetic miRNAs, we computed their structural similarity and found that the mean similarity of synergetic miRNA pairs is significantly higher than that of non-synergetic ones (p=8.6324e-38, Wilcoxon rank-sum test). The average similarity score of the MFSN is also significantly higher than that of random conditions (p<1.0e-4). Additionally, after discarding synergism occurred in the same miRNA families, synergistic miRNA pairs also show significant high similarity in structure (p=1.46e-30, rank-sum test), which is also significant higher than random (*p*<1.0e-4). The fraction of synergetic miRNA pairs with high similarity scores is more than it of the background set (Table S7). These results indicate that synergetic miRNA pairs tend to have significantly high structural similarity which might partially explain their functional cooperative regulations.

Table S7. The proportions of synergistic miRNA pairs with strong similarity in seed sequence and secondary structure are higher than those of pairs in the background set under different thresholds of similarity scores.

Similarity	Synergetic /non-synergetic	Thresholds of similarity scores			
		0.9	0.8	0.7	0.6
Seed sequence (2-7nt)	Yes	0.023 (0.008)	0.032 (0.017)	0.032 (0.017)	0.075 (0.060)
	No	0.001 (0.0008)	0.008 (0.007)	0.008 (0.007)	0.045 (0.044)
Seed sequence (1-8nt)	Yes	0.013 (0.0009)	0.022 (0.007)	0.032 (0.017)	0.070 (0.055)
	No	0.0006 (8.9e-5)	0.002 (0.001)	0.008 (0.007)	0.037 (0.035)
Secondary structure	Yes	0.001 (0)	0.084 (0.073)	0.439 (0.427)	0.830 (0.827)
	No	0.0009 (0.0004)	0.059 (0.056)	0.374 (0.370)	0.774 (0.722)

Note: Yes represents all synergistic miRNA pairs in the MFSN, and No represent miRNA pairs with no cooperation in the background set. The values in the brackets represent the proportions of miRNA pairs which originate from different miRNA family.

Synergistic miRNA pairs are likely to share a common transcriptional regulatory mechanism

Firstly, we investigate the distance distribution of these synergistic miRNA pairs. As a result, there are 726 miRNAs pairs located on the same chromosomes, accounting for 11.76% of the MFSN, and only 116 out of these pairs are located in the same miRNA cluster. We concluded there is no dominant orientation for miRNAs located on the same chromosomes or the same miRNA clusters tend to cooperate with each other.

We further attempted to dissect the potential characteristic of miRNA synergistic regulations from the view of transcriptional regulation. TF-miRNA regulatory relationships are obtained from three databases: TransmiR, miReg and UCSC (Table S8). Totally, 312 miRNAs in the MFSN are regulated by more than one TF when considering 1KB promoters and those two validated datasets, involving 2630 synergetic miRNA pairs. Of these synergetic miRNA pairs, 19.13% (503/2630) share at least one TFs, which is significantly higher than that of non-synergetic miRNA pairs (1.4778 fold to the ratio of non-synergistic miRNA pairs, *p*<1.0e-32, Fisher exact test). As a result, the mean score of synergistic miRNAs is 0.2628, and that of non-synergistic miRNAs is 0.2497. In order to further evaluate the regulation concordance of synergetic miRNAs, we randomly selected the same number of miRNA pairs from the general background and recalculated the consistence score. Consequently, the regulation concordance for synergetic miRNA pairs is also significantly higher than that of random conditions (*p*=0.0137). Additionally, the proportions of synergistic miRNA pairs with strong concordance scores are higher under different thresholds compared with non-synergistic miRNA pairs (Figure S4). We also got the similar results when analyzing the 2KB and 5KB putative promoter regions of miRNAs (data not shown).

Table S8. The transcriptional regulation information of miRNAs in the MFSN constructed by the integrated predicted miRNA target data.

Databases	TransmiR	miReg	UCSC			Total		
			1KB	2 KB	5 KB	1KB	2 KB	5 KB
Number of TFs	127	84	140	149	151	273	280	281
Number of miRNAs	124	54	356	438	534	400	472	558
Number of regulations	417	178	1497	2499	5300	1880	2765	5117

Note: In the UCSC, three kinds of putative promoter regions of each miRNA are respectively analyzed, defined as 1kb, 2kb and 5kb upstream of miRNA precursor.

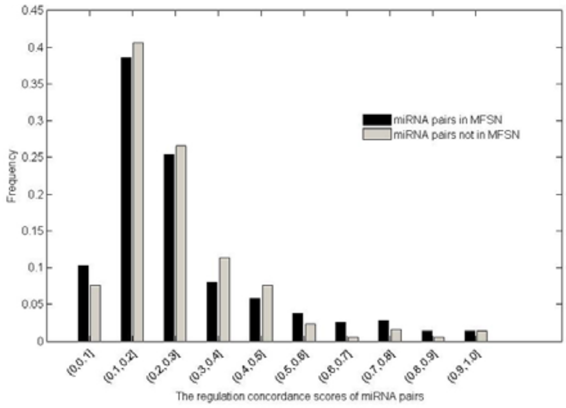

Figure S4. The distributions of regulation concordance scores for synergetic miRNA pairs in the MFSN and other miRNA combinations.

Co-regulated miRNAs are located in the central of the MFSN

Next, we specially focused on 503 miRNAs synergistic regulations which share at least one TF, involving 185 miRNAs. Here, these miRNAs' position bias in the MFSN is investigated by dividing miRNAs in the network into two groups: co-regulatory miRNAs and others. Three measures are used to characterize miRNA's centrality in the MFSN, degree, betweenness centrality and closeness centrality. As shown in Table S9, all the average centrality values of co-regulation miRNAs are much greater than that of other miRNAs in MFSN, indicating that co-regulation miRNAs indeed play central roles in the MFSN. The median degree of co-regulation miRNAs is 31 and that of other miRNAs is 8; and the median betweenness and closeness of co-regulation miRNAs are also higher. In addition, we got the similar results by analyzing different transcriptional regulation datasets (Table S9). The discovery of the significant difference in the centrality between co-regulatory miRNAs and others suggests a functional importance of miRNAs in the first group. Therefore, we concluded that dysregulations of these co-regulation miRNAs are more likely to cause diseases; indeed, we found 73.51% of these miRNAs are annotated as disease miRNAs in the database of miR2Disease, while the percentage for miRNAs without co-regulations is 21.30%.

Table S9. Three topological centrality features and their corresponding medians between co-regulatory miRNAs and other miRNAs in the MFSN. P values are calculated by Wilcoxon rank sum test.

Upstream threshold	miRNA categories	Topological features		
		Degree	Betweenness	Closeness
1KB	sharing TFs	31	1.1905e+3	7.2150e-4
	Others	8	63.0626	6.2035e-4
	P value	3.62e-31	3.02e-28	1.20e-28
2KB	sharing TFs	26.5	976.0792	7.1251e-4
	Others	7	48.7639	6.1538e-4
	P value	9.20e-29	1.5314e-26	1.21e-26
5KB	sharing TFs	21	534.8406	6.8918e-4
	Others	5	27.1598	5.9844e-4
	P value	7.38e-26	3.49e-22	5.80e-25