

Table S1. Statistics of integrated regulatory networks (IRNs).

A. Type of nodes.

Node Type	Protein	TF	miRNA
Number	24174	2278	408

B. TF regulation of genes inferred from PReMod.

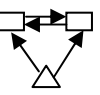

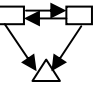

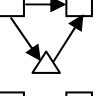

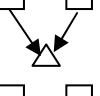

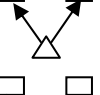

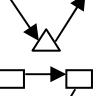

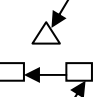

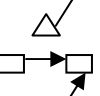

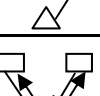

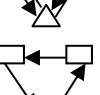

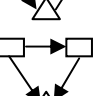

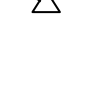

TFs	Edges to miRNA	Edges to TF	Edges to non-TF protein	Toatal edges
405	2190	17548	76633	96371

C. MiRNA regulations of protein genes inferred from miRanda and PicTar.

	miRNAs	Edges to protein	Edges to TF
miRanda	157	39801	7785
PicTar	178	75968	13442
Overlap	129	8629	1876













Table S2. Frequencies of network subgraphs in integrated regulatory networks compared with expected results from random simulations: A. T2m1; B. T1m2; C. T1m1g1. Elements of subgraph: square, TF; triangle, miRNA; ellipse, protein. Besides observed and expected frequencies and Z-score, we also listed P-value (fraction of simulation runs where concerned subgraph appears in the observed frequency or more). We executed 500 runs for T2m1 and T1m2, and 200 runs for T1m1g1), and number of clusters consisted of concerned subgraph members. Subgraphs sharing one or more nodes/edges are classified into the same cluster, and repeat this classification until cluster number reaches minimum. Threshold for significant motifs: Z-score ≥ 3.41 (A), 2.94 (B) and 3.01 (C) and number of clusters ≥ 5 . Different Z-score cutoff values are chosen so that their corrected probabilities of multiple (group size) testing are 0.01. Significant motifs are sorted according to their ratios. Consistent significant motifs are marked with star. Because of space limit and simplicity, only results from PReMode-based networks were listed in A, B and C. The consistent motifs from all 4 data sets were presented in D.

A. T2m1

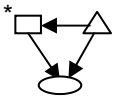
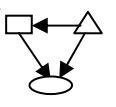
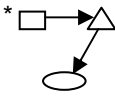
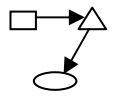
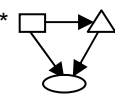
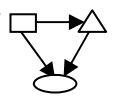
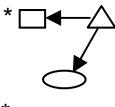
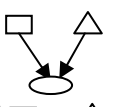
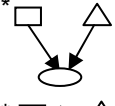
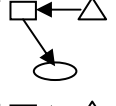
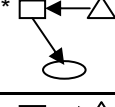
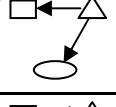
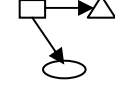
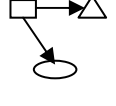
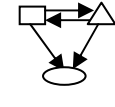
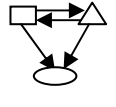
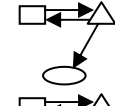
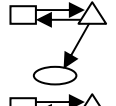
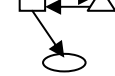
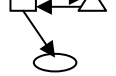
miRanda						PicTar					
Subgraph	Observed	Expected	Ratio	Z-Score	Clusters	Subgraph	Observed	Expected	Ratio	Z-Score	Clusters
* 	350	9.5±5.0	36.84	68.1	9	* 	216	21.1±8.0	10.24	24.4	9
	55	21.7±7.5	2.53	4.5	6		59	24.0±9.1	2.46	3.9	9
* 	1166	855.9±55.1	1.36	5.6	49	* 	1872	1516.0±82.5	1.23	4.3	64
* 	10749	8602.8±258.8	1.25	8.3	76	* 	11253	9394.9±263.2	1.20	7.1	85
* 	12865	10475.2±214.3	1.23	11.2	80	* 	22624	19083.0±471.1	1.19	7.5	99
* 	13936	11530.7±379.6	1.21	6.3	79	* 	11617	10157.8±307.6	1.14	4.7	96
* 	9772	8704.7±277.3	1.12	3.8	81	* 	31755	28182.7±481.2	1.13	7.4	115
* 	18242	16501.0±425.2	1.11	4.1	113	* 	34008	30327.6±316.7	1.12	11.6	131
* 	19119	17374.8±230.3	1.10	7.6	108	* 	25900	23869.9±537.4	1.09	3.8	132
	30	12.7±6.3	2.36	2.8	4		6	0.05±0.2	120.00	27.3	2
	352	291.5±23.9	1.21	2.5	36		1814	1600.0±68.6	1.13	3.1	66
	1205	1093.1±60.4	1.10	1.9	41		47	23.0±8.3	2.04	2.9	6

	26436	24780.5±895.5	1.07	1.8	82		1126	839.3±120.5	1.34	2.4	20
	667	607.7±39.7	1.10	1.5	37		951	680.7±123.1	1.40	2.2	19
	16	13.2±4.5	1.21	0.6	3		482	426.0±29.9	1.13	1.9	48
	24	21.4±6.2	1.12	0.4	5		1238	1046.9±142.6	1.18	1.3	20
	2	1.6±1.5	1.25	0.3	1		29721	28521.5±972.4	1.04	1.2	100
	46	44.2±11.3	1.04	0.2	6		612	513.4±85.1	1.19	1.2	19
	342	335.9±72.3	1.02	0.1	10		1258	1189.8±64.0	1.06	1.1	45
	0	0.04±0.19	0.00	-0.2	0		77	63.6±12.6	1.21	1.1	12
	0	0.8±0.9	0.00	-0.8	0		63	49.4±13.2	1.28	1.0	13
	0	1.3±1.3	0.00	-1.0	0		86	78.5±18.6	1.10	0.4	13
	347	453.6±78.7	0.76	-1.4	11		30	27.9±7.2	1.08	0.3	10
	14	25.8±7.1	0.54	-1.7	4		2	2.1±1.6	0.95	0.0	1
	419	604.0±110.3	0.69	-1.7	11		3	3.4±2.3	0.88	-0.2	2
	313	445.3±72.9	0.70	-1.8	15		1063	1129.0±221.2	0.94	-0.3	18
	3	14.9±6.5	0.20	-1.8	2		20	26.4±8.0	0.76	-0.8	5
	308	532.8±104.1	0.58	-2.2	9		0	1.4±1.2	0.00	-1.1	0
	3	17.8±6.3	0.17	-2.3	2		11	21.4±7.4	0.51	-1.4	7
	234	466.4±90.5	0.50	-2.6	12		373	518.9±83.1	0.72	-1.8	16

B. T1m2

miRanda						PicTar					
Subgraph	Observed	Expected	Ratio	Z-Score	Clusters	Subgraph	Observed	Expected	Ratio	Z-Score	Clusters
	5564	4247.7±238.0	1.31	5.5	56		29127	24032.6±334.4	1.21	15.2	80
	16136	13257.5±244.2	1.22	11.8	70		8906	7606.6±294.5	1.17	4.4	68
	682	520.6±102.3	1.31	1.6	12		1153	820.1±121.5	1.41	2.7	21
	3808	3551.0±214.1	1.07	1.2	40		4861	4907.4±298.9	0.99	-0.2	52
	9	9.0±5.3	1.00	0.0	3		20	17.0±6.9	1.18	0.4	6
	68	144.8±29.1	0.47	-2.6	9		333	382.1±79.1	0.87	-0.6	19

C. TImigl

miRanda						PicTar					
Subgraph	Observed	Expected	Ratio	Z-Score	Clusters	Subgraph	Observed	Expected	Ratio	Z-Score	Clusters
	11839	9068.4±179.1	1.31	15.5	121		27528	22685.1±396.2	1.21	12.2	155
	318900	271895.0±3633.5	1.17	12.9	86		601439	522115.0±6411.6	1.15	12.4	106
	15388	13187.3±422.3	1.17	5.2	84		25675	22448.3±690.2	1.14	4.7	101
	548496	477667.0±4043.9	1.15	17.5	131		484968	428255.0±1130.4	1.13	50.2	178
	290533	255762.0±913.1	1.14	38.1	157		618072	552856.0±8024.2	1.12	8.1	164
	414894	376670.0±3209.0	1.10	11.9	131		1633680	1492560.0±11775.5	1.09	12.0	164
	631125	607252.0±17779.0	1.04	1.3	86		710340	702647.0±20759.5	1.01	0.4	106
	274	332.7±67.5	0.82	-0.9	12		1325	1223.8±266.9	1.08	0.4	22
	8513	10758.4±1738.6	0.79	-1.3	12		30882	29754.2±4285.1	1.04	0.3	22
	7451	12350.3±2136.1	0.60	-2.3	12		28311	29418.2±6416.7	0.96	-0.2	22

D. Common significant network motifs from 4 data sets.

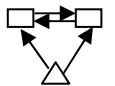
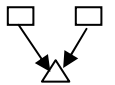
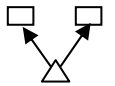
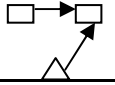
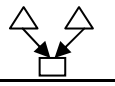
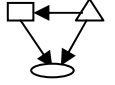
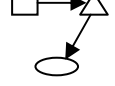
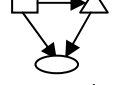
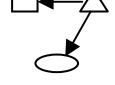
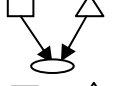


Category	Motifs	PReMod:miRanda		PReMod:PicTar		cTFBS:miRanda		cTFBS:PicTar	
		Ratio	Z-Score	Ratio	Z-Score	Ratio	Z-Score	Ratio	Z-Score
T2m1		36.70	68.1	10.25	24.4	13.60	20.9	12.06	31.1
		1.25	8.3	1.20	7.1	1.23	6.7	1.17	5.1
		1.23	11.2	1.13	7.4	1.28	9.9	1.15	6.3
		1.10	7.6	1.12	11.6	1.09	5.4	1.07	5.4
T1m2		1.22	11.8	1.21	15.2	1.23	8.8	1.19	9.7
T1mig1		1.31	15.5	1.21	12.2	1.28	13.2	1.22	14.3
		1.17	12.9	1.15	12.4	1.14	11.8	1.11	9.1
		1.17	5.2	1.14	4.7	1.12	4.8	1.09	4.1
		1.15	17.5	1.09	12.0	1.17	13.4	1.10	9.0
		1.14	38.1	1.13	50.2	1.12	42.2	1.12	52.7
		1.10	11.9	1.12	8.1	1.13	10.3	1.10	10.7
									

Table S3. Effects of false regulatory relations on detection of the most significant network motif (the miRNA-regulated TF feedback loop), studied by partially shuffling two IRNs based on PreMod. We repeated 100 times for each fraction of randomization, and presented average values in the table.

Shuffle Fraction		0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8
miRanda	Observed	350	233.6	160.4	109.2	76.1	53.6	38.4	29.2	21.1
	Expected	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5	9.5
	SD	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
	Enrichment Ratio	36.7	24.5	16.8	11.4	8.0	5.6	4.0	3.1	2.2
	Z-Score	68.1	44.8	30.2	19.9	13.3	8.8	5.8	3.9	2.3
PicTar	Obs.	216	151.9	109.7	74.5	57.9	49.3	38.6	33.3	28.7
	Expected	21.1	21.1	21.1	21.1	21.1	21.1	21.1	21.1	21.1
	SD	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0	8.0
	Enrichment Ratio	10.2	7.2	5.2	3.5	2.7	2.3	1.8	1.6	1.4
	Z-Score	24.4	16.4	11.1	6.7	4.6	3.5	2.2	1.5	1.0

Table S4. Detection of the most significant network motif (the miRNA-regulated TF feedback loop), using cTFBS set from the "TFBS Conserved" track of UCSC genome browser (see Methods). This set of TFBSs have conservation Z-scores, and we chose 4 values as cutoff to get 4 sets of TF regulations.

TFBS conservation threshold: Z-score (P-Value)		1.64 (0.051)	2.00 (0.023)	2.33 (0.01)	2.58 (0.005)
miRanda	Observed	264	125	109	107
	Expected (SD)	94.2 (16.5)	31.5 (9.6)	8.0 (4.8)	2.7 (2.9)
	Enrichment Ratio	2.8	4.0	13.6	39.6
	Z-Score	10.3	9.8	20.9	36.1
PicTar	Observed	895	446	301	273
	Expected (SD)	275.4 (28.2)	95.8 (18.0)	25.0 (8.9)	8.0 (5.1)
	Enrichment Ratio	3.3	4.7	12.1	34.0
	Z-Score	22.0	19.4	31.1	51.6

Table S5. Two classes of miRNAs

Class I	Class II	
hsa-mir-10b	hsa-let-7g	hsa-mir-136
hsa-mir-16	hsa-mir-28	hsa-mir-142-3p
hsa-mir-22	hsa-mir-200a	hsa-mir-152
hsa-mir-199b	hsa-mir-103	hsa-mir-15a
hsa-mir-204	hsa-mir-122a	hsa-mir-15b
hsa-mir-132	hsa-mir-208	hsa-mir-211
hsa-mir-212	hsa-mir-31	hsa-mir-185
hsa-mir-191	hsa-mir-223	hsa-mir-34a
hsa-mir-107	hsa-mir-145	hsa-mir-27b
hsa-mir-10a	hsa-mir-198	hsa-mir-33
hsa-mir-218	hsa-mir-126*	hsa-mir-130b
hsa-mir-187	hsa-mir-134	hsa-mir-320
hsa-mir-129	hsa-mir-150	hsa-mir-142-5p
hsa-mir-125b	hsa-mir-153	hsa-mir-182
hsa-mir-219	hsa-mir-29b	hsa-mir-203
hsa-mir-214	hsa-mir-29c	hsa-mir-106b
hsa-mir-199a	hsa-mir-29a	hsa-mir-93
hsa-mir-9*	hsa-mir-217	hsa-mir-101
hsa-mir-137	hsa-mir-143	hsa-mir-139
hsa-mir-100	hsa-mir-182*	hsa-mir-138
hsa-mir-213	hsa-mir-190	hsa-mir-181c
hsa-mir-199a*	hsa-mir-99a	hsa-mir-23b
hsa-mir-124a	hsa-mir-149	hsa-mir-30b
hsa-mir-92	hsa-let-7b	hsa-mir-30d
hsa-mir-9	hsa-let-7c	hsa-mir-32
hsa-mir-195	hsa-mir-98	hsa-mir-206
	hsa-mir-25	hsa-mir-221
	hsa-mir-186	hsa-mir-26a
		hsa-mir-194

Table S6. Expression ranks of two classes of miRNAs in different development stages. Benjamini and Hochberg False Discovery Rate multiple testing correction was applied. Class I miRNAs prefer to express in embryo stage; while class II miRNAs in the adult stage. There are 5 violations among 54 cases (p-Value 1.9E-10).

Name	Class I	Class II	Rand Diff.	Orig. p-Value	Multiple Testing
					Corrected
Uterus	43.08	42.68	0.40	0.47	0.48
Cervix	42.16	42.74	-0.58	0.47	0.48
E7	48.52	47.76	0.76	0.45	0.48
E15	47.16	48.08	-0.92	0.42	0.46
Bladder	45.13	43.42	1.71	0.40	0.45
Adrenal Gland	41.83	39.23	2.60	0.35	0.39
E17	50.64	47.69	2.95	0.33	0.38
Testicle	43.56	46.57	-3.01	0.32	0.38
Breast	46.68	43.30	3.38	0.31	0.37
Frontal Cortex	45.00	41.57	3.43	0.31	0.37
Ovary	45.80	42.06	3.74	0.30	0.38
brain	49.20	45.18	4.02	0.28	0.36
Thymus	48.46	43.38	5.08	0.24	0.32
Prostate	46.42	41.06	5.36	0.22	0.30
Spleen	47.52	41.89	5.63	0.21	0.29
Lung	48.96	43.36	5.60	0.21	0.29
Brain	45.16	39.68	5.48	0.19	0.28
0 hrs	55.27	62.86	-7.59	0.19	0.29
Skeletal Muscle	41.79	35.89	5.90	0.19	0.29
8 hrs	56.65	64.88	-8.22	0.18	0.29
Heart	40.44	34.19	6.25	0.18	0.29
Small Intestine	46.52	40.47	6.05	0.18	0.30
Kidney	48.79	42.09	6.70	0.17	0.30
Liver	41.54	34.81	6.73	0.16	0.29
Lymph	46.32	39.11	7.21	0.15	0.29
E11	42.64	49.45	-6.81	0.14	0.26
Placenta	48.72	39.13	9.59	0.07	0.14
Fallopian	53.48	42.58	10.90	0.05	0.11
Bone Marrow	50.16	39.36	10.80	0.05	0.11
ovary	54.52	42.39	12.13	0.04	0.09
Colon	51.76	39.23	12.53	0.04	0.09
E11.5	36.65	49.38	-12.73	0.04	0.09
12 hrs	53.42	69.46	-16.03	0.04	0.09
Hela S3	51.43	38.92	12.51	0.03	0.09
kidney	55.20	41.61	13.59	0.02	0.06
16 hrs	51.00	68.19	-17.19	0.02	0.07
heart	54.96	42.31	12.65	0.02	0.07
4 hrs	47.04	65.18	-18.14	0.02	0.05
Pancreas	54.88	39.55	15.33	0.01	0.04
thymus	58.32	43.80	14.52	0.01	0.04
E9.5	36.20	53.10	-16.90	0.01	0.04
lung	57.12	42.06	15.06	0.01	0.02
E10.5	33.15	52.56	-19.41	0.01	0.02

20 hrs	46.31	68.91	-22.60	0.00	0.02
64 hrs	45.00	66.84	-21.84	0.00	0.02
28 hrs	45.73	68.61	-22.88	0.00	0.02
56 hrs	44.88	67.32	-22.43	0.00	0.02
liver	57.12	38.88	18.24	0.00	0.01
24hrs	47.77	69.23	-21.46	0.00	0.01
32 hrs	43.81	68.02	-24.21	0.00	0.01
96 hrs	40.54	66.65	-26.11	0.00	0.02
40 hrs	41.85	68.67	-26.82	0.00	0.00
48 hrs	40.69	69.21	-28.52	0.00	0.00

Table S7. Top 5 biased functions to each of two TF classes that regulate two miRNA classes respectively: GO items with positive Z-score prefer class I and negative for class II. All GO items associated with transcription factor are excluded. We found 943 GO items associated with class I TFs, and 225 GO items for class II TFs. Percentages of concerned GO among total GO items are denoted as p_I for class I and p_{II} for class II, and p is the expected percentage for both groups.

	GO item	p_I	p_{II}	p	Enrichment	Z_I	Function
Class I	GO:0007165	0.0148	0	0.01	1.49	1.51	signal transduction
	GO:0009887	0.0085	0	0.0057	1.49	1.14	organogenesis
	GO:0007275	0.0233	0.0089	0.0186	1.25	1.08	development
	GO:0007399	0.0064	0	0.0043	1.49	0.98	neurogenesis
	GO:0006915	0.0042	0	0.0028	1.49	0.8	apoptosis
Class II	GO:0006955	0.0042	0.0178	0.0087	2.05	-1.47	immune response
	GO:0006629	0.0021	0.0089	0.0043	2.05	-1.04	lipid metabolism
	GO:0005524	0.0032	0.0089	0.0051	1.76	-0.81	ATP binding
	GO:0007420	0.0032	0.0089	0.0051	1.76	-0.81	brain development
	GO:0016021	0.0032	0.0089	0.0051	1.76	-0.81	integral to membrane

Figure S1. Distribution of TF-miRNA co-regulation Z-scores. Z-score for any pair (assuming TF i and miRNA j) is calculated as followed. For each gene, it has a probability $p_{tfi} = T_{tfi} / N_{gn}$ to be regulated by TF i , where T_{tfi} : number of target protein genes by TF i , and N_{gn} : total protein genes. Similarly, the probability for this gene being regulated by miRNA j is $p_{mij} = T_{mij} / N_{gn}$, where T_{mij} : number of target protein genes by miRNA j . Expected number of genes co-regulated by TF i and miRNA j is $N_{gn} p_{ij}$ (where $p_{ij} = p_{tfi} p_{mij}$) and standard deviation is $\sqrt{N_{gn} p_{ij} (1 - p_{ij})}$. Thus, Z score is $Z_{tfi:mij} = (T_{tfi:mij} - N_{gn} p_{ij}) / \sqrt{N_{gn} p_{ij} (1 - p_{ij})}$, where $T_{tfi:mij}$ is number of observed protein genes co-regulated by this TF-miRNA pair.

Figure S2. Network motif profile of miRNAs: **A.** PicTar data set; **B.** miRanda data set. A network subgraph is represented by a six-bit number. Six bits denote six states respectively: miRNA regulating TF 1, TF 1 regulating miRNA, TF 1 regulating TF 2, miRNA regulating TF2, TF 2 regulating miRNA and TF 2 regulating TF 1. Each state has two values: 1 (regulation exists) and 0 (regulation does not exist). For example, 100100 denotes the subgraph where two TFs regulated by a miRNA.

Figure S3. Expression difference between class I and II miRNAs in tumor (T) and normal (N) tissues: a bar is averaged rank of class I miRNAs subtracted by that of class II miRNAs. Positive sign indicates higher expression of class II miRNAs and negative for class I. Other abbreviations used: BLDR, bladder; BRST, breast; KID, kidney; Lvr, liver; MELA, melanoma; MESO, mesothelioma; PAN, pancreas; PROST, prostate; UT, uterus.

TF-miRNA co-regulation

