Supplementary material
for
Evaluating the consistency of differential expression of microRNA detected in human cancers

Xue Gong[1#], Ruihong Wu[1#], Hongwei Wang[1], Xinwu Guo[1], Dong Wang[1,2], Yunyan Gu[1], Yuannv Zhang[1], Wenyuan Zhao[1], Lixin Cheng[1], Chenguang Wang[1§], Zheng Guo[1,2§]

[1] College of Bioinformatics Science and Technology, Harbin Medical University, Harbin 150086, China

[2] Bioinformatics Centre, School of Life Science, University of Electronic Science and Technology of China, Chengdu, 610054, China

[#] contributed equally, [§] Corresponding author

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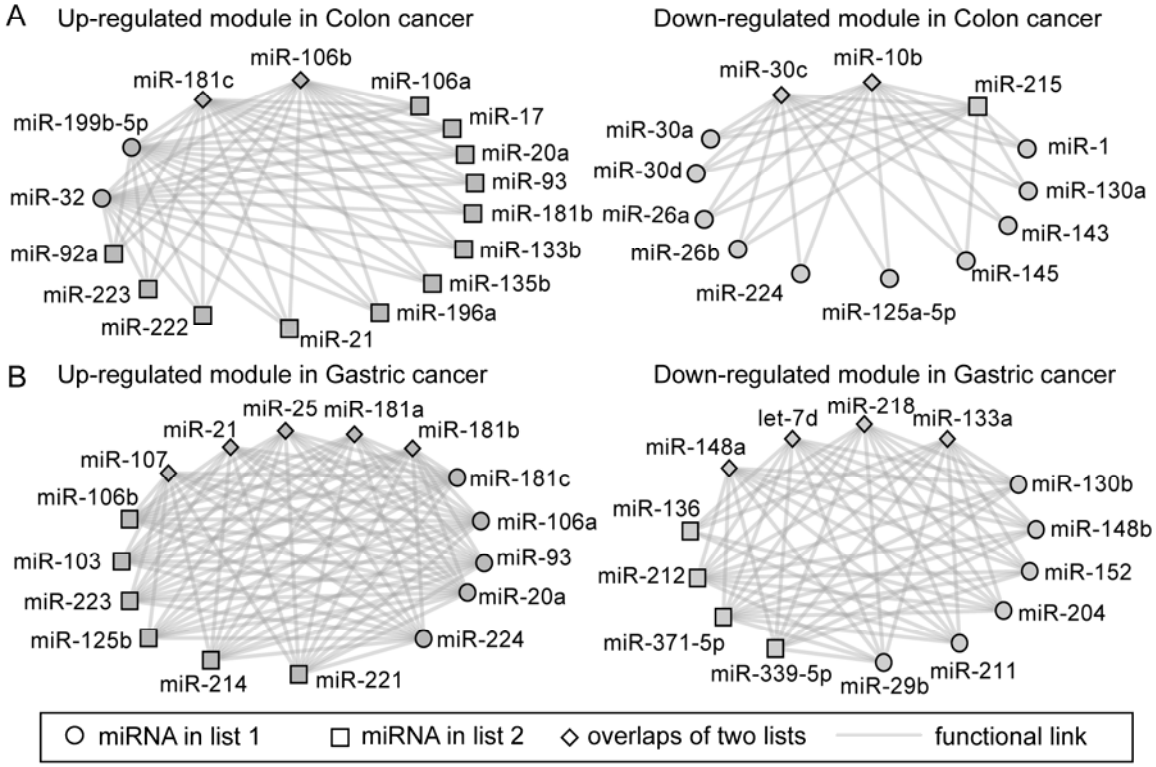
A. Supplementary Data

miRNA-target interaction data

The eight miRNA-target intersection sources are TargetScan (version 5.1, using the conserved targets) (1), miRanda (September 2008 available at http://www.microrna.org/microrna/getDownloads.do) (2), PicTar (based on the conservation in 4 species) (3), miRBase (version 5) (4), DIANA-microT (version 3.0, using the default loose score threshold of 7.3) (5), RNA22 (6), RNAhybrid (7) (extracted from miRNAMap (8)), and PITA(version 6) (9). We mapped all kinds of target IDs to Entrez Gene ID using the SOURCE database (10). The first three widely used sources (TargetScan, miRanda and PicTar) were analyzed separately. For TargetScan, we got 189075 miRNA-gene interactions consisting of 675 miRNAs and 11,758 target genes; for miRanda, we got 732507 miRNA-gene interactions consisting of 677 miRNAs and 12,828 target genes; for PicTar, we got 58301 miRNA-gene interactions consisting of 178 miRNAs and 7034 target genes. Finally, we got 408,931 miRNA-gene interactions consisting of 784 miRNAs and 15,152 target genes documented in at least two of the eight sources.

B. Supplementary Figure

Supplementary Figure 1



C. Supplementary Figure Legend

Supplementary Figure 1 - The function link model between the two lists of the top ranked 20 DE miRNAs for each cancer. For two lists of DE miRNAs for a cancer, we linked every two functionally similar miRNAs between the lists to construct the model (see *Materials and Methods* in main text). **A,** The model between the top ranked 20 DE miRNAs from Colon99 (cycle and diamond) and the top ranked 20 DE miRNAs from Colon168 (rectangle and diamond); **B,** The model between the top ranked 20 DE miRNAs from Gastric353 (cycle and diamond) and the top ranked 20 DE miRNAs from Gastric41 (rectangle and diamond). For a cancer, the up-regulated module consists of up-regulated miRNAs (left panel) and the down-regulated module consists of down-regulated miRNAs (right panel).

D. Supplementary Results

We have carefully investigated the six datasets for prostate cancer reviewed by Gandellini *et al* (11). One dataset was not publicly available and three datasets included only 8, 4, and 7 normal samples, respectively, which might be too small to get reliable results. Thus, we only analyzed the dataset (denoted as Prostate76) from (12) and the dataset (denoted as Prostate80) from (13). Prostate76 contains 60 tumors (fresh-frozen, macrodissected) and 16 surrounding non-tumor tissues from prostate cancer patients, generated on OSU_CCC version 3.0 chip. Changed miRNA abundance was observed between 35 organ-confined tumors and 17 with extraprostatic extension (12). Prostate80 contains 40 paired tumor (microdissected) and normal tissues from the uninvolved area of these 40 prostatectomy specimens (formalin-fixed paraffin-embedded) from stage T2a/b patients, generated by mirMASA technique, a bead-based hybridization method. Sixteen miRNAs were detected as differentially expressed between 20 patients with early chemical relapse within two years and 20 without clinical relapse within ten years (13).

Prostate76 was preprocessed using the same method for the five datasets analyzed in the main text (see *Materials and Methods*). For Prostate80, only the normalized miRNA expression ratios (Tumor/Norma) were supplied in the original study (13). A total of 83 miRNAs were presented in both datasets. Using the two sample *t*-test followed by BH correction(14), we found 22 DE miRNAs in Prostate76 (FDR< 5%, see Supplementary Table S6). We used the one-sample *t*-test to test the null hypothesis of mean Tumor/Normal ratio of 1 and found eight DE miRNAs in Prostate80 (FDR<

5%, see Supplementary Table S7). Only one miRNA (hsa-miR-221), which was consistently down-regulated across the two datasets, was shared by these two DE miRNA lists. The PO score was only 0.05 from the long list to the short list and 0.13 in the opposite direction. After 10,000 random experiments, we did not observe significance for these two scores (P=0.76). Then, we evaluated whether miRNAs exclusively detected as differentially expressed in one dataset showed potential differential expressions (P less than 0.1) in the other dataset and show the same regulation directions across the two datasets. For the 21 DE miRNAs found exclusively in Prostate76, eight showed P values less than 0.1 in Prostate80 and only four of them showed consistent regulation directions across the two datasets, which was not significantly different from expected by chance (P=0.64, binominal distribution test). For the 7 DE miRNAs found exclusively in Prostate80, only one miRNA (hsa-miR-145) showed a P value less than 0.1 and exhibited consistent down-regulation across the two datasets. The POF score for the lists of the top 10 or 20 miRNAs were only about 0.50 using the four types of miRNA target interaction sources, and no significance was observed (data not shown). The inconsistency may be induced by the difference in the approach of dissection, preservation of specimens (11) and/or the phenotypic heterogeneity between the two studies as mentioned above.

E. Supplementary Tables

Supplementary Table 1 - Differentially expressed miRNAs for Colon99

Supplementary Table 2 - Differentially expressed miRNAs for Colon168

Supplementary Table 3 - Differentially expressed miRNAs for Colon108

Supplementary Table 4 - Differentially expressed miRNAs for Gastric41

Supplementary Table 5 - Differentially expressed miRNAs for Gastric353

Supplementary Table 6 - Differentially expressed miRNAs for Prostate76

Supplementary Table 7 - Differentially expressed miRNAs for Prostate80

Supplementary Table 1 - Differentially expressed miRNAs for Colon99

miRNA	*P* value	adjusted *P* value	deregulation direction
hsa-miR-1	2.78E-12	2.43E-10	down
hsa-miR-145	1.87E-11	1.09E-09	down
hsa-miR-143	6.51E-11	2.85E-09	down
hsa-miR-224	3.00E-09	7.50E-08	down
hsa-miR-30a	2.31E-08	5.05E-07	down
hsa-miR-10b	1.82E-07	3.53E-06	down
hsa-miR-130a	4.12E-07	7.21E-06	down
hsa-miR-199b-5p	5.02E-07	7.99E-06	up
hsa-miR-106b	2.37E-06	3.20E-05	up
hsa-let-7c	7.99E-06	9.98E-05	down
hsa-miR-30d	9.41E-06	1.10E-04	down
hsa-miR-125a-5p	1.21E-05	1.33E-04	down
hsa-miR-125b	1.66E-05	1.71E-04	down
hsa-miR-26b	2.04E-05	1.99E-04	down
hsa-let-7d	2.75E-05	2.41E-04	down
hsa-miR-31	2.75E-05	2.41E-04	up
hsa-miR-32	3.86E-05	3.22E-04	up
hsa-miR-26a	4.22E-05	3.35E-04	down
hsa-miR-30c	5.56E-05	4.10E-04	down
hsa-miR-181c	5.63E-05	4.10E-04	up
hsa-let-7a	9.19E-05	6.43E-04	down
hsa-miR-140-5p	1.12E-04	7.57E-04	down
hsa-miR-27a	1.64E-04	1.07E-03	down
hsa-miR-27b	1.89E-04	1.14E-03	down
hsa-miR-99a	2.30E-04	1.34E-03	down
hsa-miR-184	2.45E-04	1.38E-03	up
hsa-miR-219-5p	5.46E-04	2.99E-03	up
hsa-miR-92a	7.06E-04	3.74E-03	up
hsa-miR-7	7.81E-04	4.02E-03	up
hsa-miR-133b	8.76E-04	4.38E-03	up
hsa-miR-93	1.16E-03	5.62E-03	up
hsa-miR-211	1.77E-03	8.15E-03	up
hsa-miR-185	2.06E-03	9.22E-03	up
hsa-miR-99b	2.17E-03	9.51E-03	down
hsa-miR-122	2.38E-03	1.02E-02	up
hsa-miR-196a	2.76E-03	1.15E-02	up
hsa-miR-181a	3.16E-03	1.29E-02	up
hsa-miR-95	3.99E-03	1.59E-02	up
hsa-miR-25	5.34E-03	1.95E-02	up
hsa-miR-29b	5.35E-03	1.95E-02	down
hsa-miR-195	5.72E-03	2.04E-02	down

hsa-miR-106a	6.37E-03	2.22E-02	up
hsa-miR-21	6.47E-03	2.22E-02	up
hsa-miR-181b	6.62E-03	2.23E-02	up
hsa-miR-192	7.80E-03	2.53E-02	up
hsa-miR-20a	8.44E-03	2.68E-02	up
hsa-miR-127-5p	8.97E-03	2.74E-02	up
hsa-miR-29c	1.09E-02	3.24E-02	down
hsa-miR-128	1.52E-02	4.43E-02	up

Supplementary Table 2 - Differentially expressed miRNAs for Colon168

miRNA	*P* value	adjusted *P* value	deregulation direction
hsa-miR-21	5.66E-10	1.08E-07	up
hsa-miR-192	1.09E-05	6.96E-04	down
hsa-miR-135b	6.42E-05	3.06E-03	up
hsa-miR-10b	1.44E-04	5.17E-03	down
hsa-miR-20a	1.87E-04	5.17E-03	up
hsa-miR-106a	2.07E-04	5.17E-03	up
hsa-miR-92a	2.43E-04	5.17E-03	up
hsa-miR-222	2.79E-04	5.34E-03	up
hsa-miR-30c	3.54E-04	6.14E-03	down
hsa-miR-106b	4.77E-04	6.99E-03	up
hsa-miR-17	4.93E-04	6.99E-03	up
hsa-miR-196a	5.12E-04	6.99E-03	up
hsa-miR-93	5.49E-04	6.99E-03	up
hsa-miR-210	6.08E-04	7.26E-03	up
hsa-miR-215	1.24E-03	1.27E-02	down
hsa-miR-181b	1.77E-03	1.69E-02	up
hsa-miR-223	2.76E-03	2.29E-02	up
hsa-miR-181c	3.21E-03	2.54E-02	up
hsa-miR-133b	3.33E-03	2.54E-02	up
hsa-miR-135a	4.43E-03	3.25E-02	up
hsa-miR-203	5.95E-03	4.06E-02	up
hsa-miR-211	6.92E-03	4.56E-02	up

Supplementary Table 3 - Differentially expressed miRNAs for Colon108

miRNA	*P* value	adjusted *P* value	deregulation direction
hsa-miR-135b	2.91E-33	1.37E-30	up
hsa-miR-224	4.28E-25	6.70E-23	up
hsa-miR-147	8.29E-24	7.79E-22	down
hsa-miR-30a	9.99E-24	7.83E-22	down
hsa-miR-9	2.73E-18	9.15E-17	down
hsa-miR-30d	7.19E-17	1.88E-15	down
hsa-miR-195	8.56E-17	2.12E-15	down
hsa-miR-10b	8.38E-15	1.71E-13	down
hsa-miR-96	3.33E-14	5.79E-13	up
hsa-miR-93	1.20E-13	1.88E-12	up
hsa-miR-215	2.51E-11	3.06E-10	down
hsa-miR-106a	3.78E-11	4.34E-10	up
hsa-miR-17	5.48E-11	5.99E-10	up
hsa-miR-150	1.55E-10	1.59E-09	down
hsa-miR-31	2.99E-10	2.87E-09	up
hsa-miR-7	3.31E-10	3.05E-09	up
hsa-miR-95	1.02E-09	8.55E-09	up
hsa-miR-92a	7.09E-09	5.38E-08	up
hsa-miR-197	8.18E-09	6.11E-08	down
hsa-miR-218	9.08E-09	6.66E-08	down
hsa-miR-1	2.37E-08	1.66E-07	down
hsa-miR-138	2.47E-08	1.68E-07	down
hsa-miR-193a-3p	5.11E-08	3.29E-07	up
hsa-miR-27a	5.96E-08	3.79E-07	up
hsa-miR-29c	1.79E-07	9.69E-07	down
hsa-miR-140-5p	2.03E-07	1.08E-06	down
hsa-miR-99b	2.06E-07	1.09E-06	down
hsa-miR-155	2.53E-07	1.31E-06	down
hsa-miR-125a-5p	4.47E-07	2.14E-06	down
hsa-miR-128	9.73E-07	4.23E-06	down
hsa-miR-20a	1.85E-06	7.61E-06	up
hsa-miR-30c	1.86E-06	7.61E-06	down
hsa-miR-191	2.47E-06	9.35E-06	down
hsa-miR-23a	4.98E-06	1.76E-05	up
hsa-miR-203	3.95E-05	1.22E-04	up
hsa-miR-133b	4.41E-05	1.35E-04	up
hsa-miR-185	6.66E-05	1.98E-04	down
hsa-miR-30b	6.95E-05	2.03E-04	up
hsa-miR-100	7.22E-05	2.08E-04	down
hsa-miR-181c	7.78E-05	2.23E-04	up
hsa-miR-25	1.22E-04	3.29E-04	up

hsa-miR-24	6.20E-04	1.50E-03	up
hsa-miR-15a	7.14E-04	1.70E-03	down
hsa-miR-21	8.18E-04	1.92E-03	up
hsa-miR-212	1.21E-03	2.76E-03	down
hsa-miR-210	1.23E-03	2.80E-03	up
hsa-miR-135a	1.60E-03	3.53E-03	down
hsa-miR-34a	1.65E-03	3.63E-03	up
hsa-miR-149	2.03E-03	4.37E-03	down
hsa-miR-32	2.83E-03	5.91E-03	up
hsa-let-7f	3.03E-03	6.25E-03	up
hsa-miR-29b	3.32E-03	6.72E-03	up
hsa-miR-106b	3.35E-03	6.76E-03	up
hsa-miR-136	3.61E-03	7.22E-03	up
hsa-miR-196a	4.32E-03	8.56E-03	down
hsa-miR-33b	4.84E-03	9.47E-03	up
hsa-miR-204	6.59E-03	1.26E-02	down
hsa-let-7g	7.54E-03	1.43E-02	down
hsa-miR-206	1.32E-02	2.35E-02	down
hsa-miR-221	2.12E-02	3.64E-02	down
hsa-miR-27b	2.75E-02	4.57E-02	down
hsa-miR-29a	2.77E-02	4.58E-02	up

Supplementary Table 4 - Differentially expressed miRNAs for Gastric41

miRNA	*P* value	adjusted *P* value	deregulation direction
hsa-miR-212	1.21E-05	2.81E-03	down
hsa-miR-218	2.76E-05	3.22E-03	down
hsa-miR-223	1.07E-04	8.31E-03	up
hsa-miR-96	1.88E-04	9.85E-03	down
hsa-miR-339-5p	2.33E-04	9.85E-03	down
hsa-miR-181b	2.71E-04	9.85E-03	up
hsa-miR-371-5p	2.96E-04	9.85E-03	down
hsa-miR-25	5.25E-04	1.53E-02	up
hsa-miR-103	6.65E-04	1.64E-02	up
hsa-miR-21	7.03E-04	1.64E-02	up
hsa-miR-181a	8.91E-04	1.73E-02	up
hsa-miR-136	9.83E-04	1.76E-02	down
hsa-miR-125b	1.17E-03	1.94E-02	up
hsa-miR-221	1.99E-03	2.89E-02	up
hsa-miR-214	2.12E-03	2.90E-02	up
hsa-miR-133a	2.59E-03	3.35E-02	down
hsa-miR-107	3.31E-03	4.06E-02	up
hsa-miR-106b	3.89E-03	4.53E-02	up

Supplementary Table 5 - Differentially expressed miRNAs for Gastric353

miRNA	*P* value	adjusted *P* value	deregulation direction
hsa-miR-148a	3.83E-24	1.30E-21	down
hsa-miR-21	1.98E-21	2.24E-19	up
hsa-miR-148b	2.12E-20	1.80E-18	down
hsa-miR-181b	4.76E-20	3.24E-18	up
hsa-miR-93	2.57E-16	1.25E-14	up
hsa-miR-130b	3.53E-16	1.50E-14	down
hsa-let-7d	5.07E-15	1.73E-13	down
hsa-miR-218	5.20E-14	1.47E-12	down
hsa-miR-181c	2.66E-13	6.46E-12	up
hsa-miR-20a	8.92E-13	2.02E-11	up
hsa-miR-25	1.34E-12	2.85E-11	up
hsa-miR-106a	1.93E-12	3.64E-11	up
hsa-miR-204	2.11E-12	3.71E-11	down
hsa-miR-211	2.18E-12	3.71E-11	down
hsa-miR-133a	7.08E-11	1.09E-09	down
hsa-miR-107	1.03E-09	1.46E-08	up
hsa-miR-181a	8.04E-09	1.09E-07	up
hsa-miR-152	1.82E-08	2.14E-07	down
hsa-miR-29b	3.85E-08	4.37E-07	down
hsa-miR-224	1.13E-07	1.11E-06	up
hsa-miR-335	1.14E-07	1.11E-06	up
hsa-let-7e	1.59E-07	1.46E-06	down
hsa-miR-92a	1.59E-07	1.46E-06	up
hsa-miR-103	1.82E-07	1.62E-06	up
hsa-miR-212	2.91E-07	2.48E-06	down
hsa-miR-29c	4.07E-07	3.38E-06	down
hsa-miR-135b	4.98E-07	3.94E-06	up
hsa-miR-183	4.98E-07	3.94E-06	up
hsa-miR-30d	9.20E-07	6.95E-06	down
hsa-miR-199a-5p	1.35E-06	9.76E-06	up
hsa-let-7a	1.78E-06	1.26E-05	down
hsa-miR-1	2.04E-06	1.42E-05	down
hsa-miR-222	3.17E-06	2.03E-05	up
hsa-miR-30c	4.14E-06	2.51E-05	down
hsa-miR-17	5.07E-06	2.93E-05	up
hsa-miR-26a	5.07E-06	2.93E-05	down
hsa-miR-26b	5.08E-06	2.93E-05	down
hsa-miR-371-5p	7.94E-06	4.43E-05	down
hsa-miR-30a	9.18E-06	5.03E-05	down
hsa-miR-223	1.05E-05	5.64E-05	up
hsa-miR-29a	1.07E-05	5.69E-05	down

hsa-miR-19a	1.19E-05	6.21E-05	up
hsa-miR-30b	1.35E-05	6.96E-05	down
hsa-miR-331-5p	2.11E-05	1.07E-04	down
hsa-miR-221	2.46E-05	1.21E-04	up
hsa-miR-191	2.57E-05	1.25E-04	up
hsa-miR-216a	2.90E-05	1.39E-04	up
hsa-miR-214	3.72E-05	1.73E-04	up
hsa-miR-199b-5p	1.40E-04	6.25E-04	up
hsa-miR-7	3.48E-04	1.53E-03	up
hsa-miR-128	3.74E-04	1.59E-03	up
hsa-miR-155	4.55E-04	1.91E-03	up
hsa-miR-15a	4.77E-04	1.95E-03	up
hsa-miR-30e	7.01E-04	2.84E-03	down
hsa-miR-370	1.08E-03	4.25E-03	down
hsa-miR-16	1.09E-03	4.25E-03	up
hsa-miR-34a	1.31E-03	4.93E-03	up
hsa-let-7b	1.39E-03	5.09E-03	down
hsa-miR-31	1.48E-03	5.34E-03	down
hsa-miR-339-5p	2.19E-03	7.60E-03	down
hsa-miR-338-5p	2.24E-03	7.63E-03	down
hsa-let-7c	2.35E-03	7.93E-03	down
hsa-miR-345	3.10E-03	9.93E-03	up
hsa-miR-196a	3.13E-03	9.93E-03	up
hsa-miR-194	3.33E-03	1.04E-02	up
hsa-miR-145	3.35E-03	1.04E-02	down
hsa-miR-196b	4.35E-03	1.33E-02	up
hsa-miR-140-5p	4.79E-03	1.44E-02	down
hsa-miR-95	4.99E-03	1.49E-02	up
hsa-miR-219-5p	5.82E-03	1.72E-02	down
hsa-miR-141	7.31E-03	2.14E-02	up
hsa-miR-126	8.09E-03	2.33E-02	down
hsa-miR-328	8.15E-03	2.33E-02	up
hsa-miR-136	1.07E-02	2.97E-02	down
hsa-miR-135a	1.13E-02	3.10E-02	down
hsa-miR-127-5p	1.41E-02	3.77E-02	up
hsa-miR-101	1.58E-02	4.13E-02	up
hsa-miR-323-5p	1.79E-02	4.65E-02	up

Supplementary Table 6 - Differentially expressed miRNAs for Prostate76

miRNA	*P* value	adjusted *P* value	deregulation direction
hsa-miR-218	5.95E-07	3.81E-05	down
hsa-let-7i	8.06E-07	4.12E-05	up
hsa-miR-128	3.68E-06	1.57E-04	down
hsa-miR-26a	5.80E-06	2.12E-04	up
hsa-miR-25	4.53E-05	1.16E-03	up
hsa-miR-194	4.96E-05	1.16E-03	up
hsa-miR-33a	1.81E-04	3.09E-03	up
hsa-miR-7	2.16E-04	3.25E-03	down
hsa-miR-196a	7.02E-04	7.49E-03	up
hsa-miR-129-5p	1.93E-03	1.41E-02	down
hsa-miR-193a-5p	2.31E-03	1.51E-02	down
hsa-miR-133a	2.77E-03	1.73E-02	down
hsa-miR-144	4.23E-03	2.35E-02	down
hsa-miR-16	4.31E-03	2.35E-02	up
hsa-let-7b	4.51E-03	2.35E-02	down
hsa-miR-338-5p	5.32E-03	2.52E-02	down
hsa-miR-10a	8.40E-03	3.51E-02	down
hsa-miR-221	9.55E-03	3.76E-02	down
hsa-miR-150	1.10E-02	4.24E-02	up
hsa-miR-20a	1.15E-02	4.32E-02	up
hsa-miR-342-5p	1.28E-02	4.74E-02	up
hsa-miR-92a	1.34E-02	4.82E-02	up

Supplementary Table 7 - Differentially expressed miRNAs for Prostate80

miRNA	*P* value	adjusted *P* value	deregulation direction
hsa-miR-222	2.62E-09	2.99E-07	down
hsa-miR-221	1.23E-07	6.99E-06	down
hsa-miR-145	9.62E-07	3.66E-05	down
hsa-let-7a	6.10E-06	1.74E-04	up
hsa-miR-23b	2.52E-04	5.75E-03	down
hsa-miR-141	6.77E-04	1.29E-02	up
hsa-miR-100	9.80E-04	1.60E-02	down
hsa-let-7e	2.33E-03	3.33E-02	up

C. Supplementary Reference

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