Supporting Information for

Scanometric microRNA (Scano-miR) Array Profiling of Prostate Cancer Markers Using Spherical Nucleic Acid (SNA)-Gold Nanoparticle Conjugates

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EXPERIMENTAL SECTION

RNA isolation from the prostate cancer cell line (PC-3). Cells were washed with PBS and lysed with 300 μL of cell disruption and 300 μL of 2X denaturing buffers using a mirVana miRNA isolation kit (Ambion). Following the manufacturer's protocol, total RNA was isolated by adding 600 μL of acid/phenol/chloroform mixture, vortexed, and spun at room temperature for 8 min at 12000 × *g*. The aqueous phase was mixed with 100% ethanol at a 1:1.25 volume ratio and then column filtered. The column was washed, and the RNA was eluted with 100 μL of pre-warmed elution buffer.

RNA isolation from human serum. After informed consent and according to an Investigational Review Board (IRB) approved protocol, whole blood from a healthy donor was collected in standard red top tubes (BD Biosciences) (see Table S3 for clinical information). Five mL of serum was separated by centrifugation of the whole blood sample at 5000 × *g* for 5 min. The serum was mixed with an equal volume of 2X denaturing solution from mirVana miRNA isolation kit (Ambion). The total RNA was isolated as described above with a scaled-up version of the manufacturer's protocol. 100 μL of the filtrate was then mixed with 0.3 M NaCl, 20 μg glycogen, and 1 volume of isopropanol followed by incubation at -80°C for 12 hrs. The mixture was spun for 30 min at 4°C, 16000 × *g*, followed by one wash with 1 mL of 70% ethanol. The pellet was then air-dried for 3 min and resuspended in 10 μL of RNase-free water and the RNA concentration was quantified using the NanoDrop Technologies ND-100 spectrophotometer.

Cell culture Preparation. The PC-3 cell line was purchased from American Type Cell Culture (ATCC, CRL-1435). The cells were grown in RPMI media supplemented with 10 % fetal bovine serum in 5 % CO_2 containing atmosphere and incubated at 37°C. The cells were initially grown in flasks and subcultured in 6 well plates (~25K cells/well). The culture was allowed to incubate for 1 day for suitable attachment prior to $CoCl_2$ treatment.

Hypoxia mimetic treatment. $CoCl_2$ was used to stabilize hypoxia inducible factor 1-alpha (HIF-1α) and induce upregulation of miR-210. The PC-3 cells in the 6 well plates were allowed to reach a

confluence of 70% prior to treatment with $CoCl_2$. For treatment, 300 µM (final concentration) of $CoCl_2$ was added to the PC-3 cells and allowed to incubate for 72 hrs. Total RNA was isolated from $CoCl_2$ treated (6 wells) and untreated (6 wells) PC-3 cells and the miR-210 concentrations were analyzed using both the Scano-miR system and qRT-PCR.

qRT-PCR for miR-210. PC-3 cells cultured with the above-mentioned protocol were analyzed for miR-210 expression levels using qRT-PCR. The $CoCl_2$ treated and untreated PC-3 cells were lysed and total RNA isolated using the previously described mirVana miRNA isolation kit (Ambion). The concentration of total RNA ranged from 70-90 ng/μL with A_{260}/A_{280} = ~ 2 for all samples. All untreated and treated replicates were diluted to 2 ng/μL, and using TaqMan RT kit (part #4366596), TaqMan U6-snRNA, and hsa-miR-210 RT primers, 10 ng (5 μL) of total RNA from each sample were reverse transcribed in 15 μL reaction volumes following manufacturer's protocol (Applied Biosystems, TaqMan MicroRNA Assays PN 4364031E). qRT-PCR reactions were conducted in 384 well plates with 1.33 μL of RT product with TaqMan PCR master mix, TaqMan U6-snRNA, hsa-miR-210 probes in a total volume of 20 μL. An ABI Prism Model 7900 HT instrument was used to perform the qRT-PCR reactions with data analyzed using the comparative Ct method with U6 snRNA utilized as an endogenous control. Known concentrations of purified miRNA-210 (IDT, Coralville, Iowa) were used to generate qRT-PCR reactions to compare miRNA-210 production in PC-3 cells.

Clinical Samples. Two samples of total RNA from human prostate tissues with low grade Gleason score 6 and high grade Gleason score 9 were purchased from Bioreclamation, LLC, Hicksville, NY (Table S3). Samples were stored at -80°C upon arrival and thawed on ice before use.

Bioinformatics Analysis. The signal intensity values, calculated using GenePix Pro 6 software, were generated from two Scano-miR arrays after profiling two samples of 0.8 μg total RNA from human prostate tissues with low grade Gleason score 6 and high grade Gleason score 9, which correspond to less aggressive versus more aggressive prostate cancer forms, respectively, as described in manuscript text. Signal intensity values were used to calculate the fold change in miRNA expression level by

obtaining the ratio of Gleason 9 to Gleason 6. A total of 163 miRNAs were considered deregulated in aggressive CaP as defined by 1.5 fold difference in signal (Table S5). Predicted and validated target genes of the given 163 miRNAs were retrieved from public databases (mirBase, targetScan, tarBase from Diana Labs, mirTarget2 from mirDB, picTar) by the Bioconductor package, RmiR.Hs.miRNA.[3] The identified target genes were then subjected to gene enrichment analysis based on Disease Ontology to include only the CaP-related genes using GeneAnswers, a Bioconductor package of concept-gene enrichment analysis.[4] A total 94 CaP-related genes were identified and remapped back to 161 miRNAs (Figure S1). Comparing with the original 163 miRNAs, "hsa-miR-139-3p" and "hsa-miR-526b" were not found to target the identified CaP-related genes (Table S5). Because a complete prostate-cancer function-gene-miRNA network consists of hundreds of edges and nodes, the complex network was simplified by constructing a function-gene-miRNA network (Figure 5 and Table S6). 1) We excluded deregulated miRNAs that have not been previously identified as differentially expressed miRNAs in CaP and/or multiple human cancer types based on the human miRNA-disease association database (HMDD, 2011).[5,6] 2) We limited our analysis to 13 CaP related genes, which are involved in select Gene Ontology terms (Regulation of apoptosis, cell motility, cell differentiation, response to hormone stimulus, and regulation of protein phosphorylation and metabolic process).[4] Each function is represented by a large yellow node in the network, small blue nodes stand for genes, while green and red nodes are miRNA nodes where red nodes have positive logR ratios and green nodes are associated with negative values (Figure 5). The saturation is proportional to the absolute value of logR ratio.

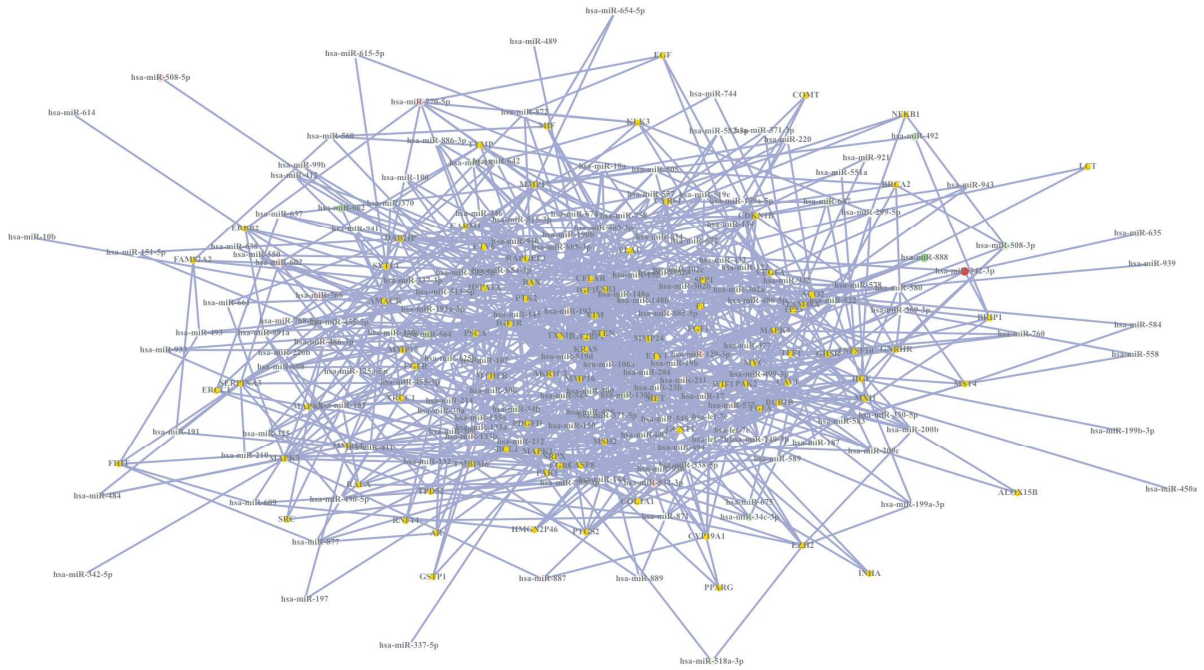

Figure S1. A complete functional prostate cancer-related gene-miRNA network was analyzed; total 94 prostate-cancer related genes (small yellow nodes) were identified and remapped back to 161 miRNAs. Green and red nodes stand for miRNAs with negative and positive logR ratios, respectively. The saturation is proportional to the absolute value of logR ratio.

Table S1. Synthetic oligonucleotide and miRNA sequences used in this study.

Synthetic miRNA	Sequence (5' --> 3')
miR-200c	UAAUACUGCCGGGUAAUGAUGGA
miR-21	UAGCUUAUCAGACUGAUGUUGA
miR-20a	UAAAGUGCUUAUAGUGCAGGUAG
miR-143	UGAGAUGAAGCACUGUAGCUC
miR-143*	GGUGCAGUGCUGCAUCUCUGGU
miR-205	UCCUUCAUUCCACCGGAGUCUG
miR-210	CUGUGCGUGUGACAGCGGCUGA
miR-16	UAGCAGCACGUAAAUAUUGGCG
Universal SNA-Au NPs	propylthiol-$(A)_{10}$-TCCTTGGTGCCCGAGTG
miRNA Cloning Linker 2	rApp-CACTCGGGCACCAAGGA-ddC

Table S2. Synthetic miRNA species used for the Latin Square experimental design (concentrations in fM).

Synthetic miRNA	Array 1	Array 2	Array 3	Array 4	Array 5
miR-20a	0	10^2	10^3	10^4	10^5
miR-143	10^2	10^3	10^4	10^5	0
miR-143*	10^3	10^4	10^5	0	10^2
miR-205	10^4	10^5	0	10^2	10^3
miR-210	10^5	0	10^2	10^3	10^4

Table S3. Clinical samples used in this study.

Sample ID	Sample Type	Cancer History	Gender
277[a]	Human serum	No	M
CR562458[b]	Human prostate tissue	Prostate cancer Gleason score 6	M
CR562503[b]	Human prostate tissue	Prostate cancer Gleason score 9	M

[a]A whole blood sample from a healthy human donor was used for Figure 1, 3 and Table S4 to detect miRNAs recovered from human serum.

[b]Two samples of total RNA isolated from human prostate tissue were purchased from Bioreclamation, LLC, Hicksville, NY, and used for Figure 5, Figure S1, and Table S5-6 to detect deregulated miRNAs involved in prostate cancer progression.

Table S4. Serum miRNAs detected from healthy donor.

Serum miRNA	log2-Signal Intensity	Serum miRNA	log2-Signal Intensity	Serum miRNA	log2-Signal Intensity
hsa-miR-148a	15.98	hsa-miR-325	15.26	hsa-miR-302b	13.81
hsa-miR-519d	15.97	hsa-miR-877	15.25	hsa-miR-200c	13.80
hsa-miR-675	15.97	hsa-miR-433	15.22	hsa-miR-522	13.76
hsa-miR-515-3p	15.97	hsa-miR-200b	15.18	hsa-miR-219-2-3p	13.74
hsa-miR-605	15.94	hsa-miR-505	15.13	hsa-miR-617	13.59
hsa-miR-495	15.91	hsa-miR-302d	15.00	hsa-miR-541	13.45
hsa-miR-493	15.84	hsa-miR-943	14.80	hsa-miR-331-3p	13.40
hsa-miR-595	15.84	hsa-miR-150	14.78	hsa-miR-577	13.31
hsa-miR-584	15.80	hsa-miR-18b	14.76	hsa-miR-136	13.30
hsa-miR-337-3p	15.78	hsa-miR-17	14.75	hsa-miR-371-3p	13.28
hsa-miR-144	15.71	hsa-miR-502-5p	14.73	hsa-miR-33b	13.21
hsa-miR-106a	15.69	hsa-miR-18a	14.66	hsa-miR-132	13.20
hsa-miR-558	15.68	hsa-miR-193a-3p	14.62	hsa-miR-637	13.20
hsa-miR-550	15.68	hsa-miR-486-5p	14.60	hsa-miR-324-3p	13.17
hsa-miR-886-3p	15.61	hsa-miR-455-5p	14.57	hsa-miR-652	13.12
hsa-miR-871	15.59	hsa-miR-654-5p	14.53	hsa-miR-383	13.07
hsa-miR-450a	15.57	hsa-miR-342-5p	14.48	hsa-miR-302c	13.02
hsa-miR-154	15.54	hsa-miR-302a	14.48	hsa-miR-363	12.88
hsa-miR-519e	15.50	hsa-miR-578	14.26	hsa-miR-155	12.86
hsa-miR-122	15.41	hsa-miR-875-3p	14.26	hsa-let-7b	12.83
hsa-miR-106b	15.36	hsa-miR-142-3p	14.11	hsa-miR-409-3p	12.68
hsa-miR-338-5p	15.36	hsa-miR-672	14.09	hsa-miR-92a	12.64
hsa-miR-508-3p	15.36	hsa-miR-330-5p	14.02	hsa-miR-592	12.56
hsa-miR-371-5p	15.33	hsa-miR-337-5p	14.02	hsa-miR-151-5p	12.50
hsa-miR-16	15.31	hsa-miR-876-3p	13.99	hsa-miR-610	12.39
hsa-miR-93	15.30	hsa-miR-138	13.83		

Serum miRNA	log2-Signal Intensity
hsa-miR-638	12.36
hsa-miR-20a	12.30
hsa-miR-367	12.09
hsa-miR-28-5p	12.07
hsa-miR-608	11.87
hsa-let-7c	11.82
hsa-miR-708	11.78
hsa-miR-19a	11.75
hsa-miR-766	11.70
hsa-miR-193a-5p	11.68
hsa-miR-22	11.67
hsa-miR-331-5p	11.66
hsa-miR-769-3p	11.64
hsa-miR-574-5p	11.63
hsa-miR-543	11.62
hsa-miR-380	11.60
hsa-miR-520a-5p	11.59
hsa-miR-148b	11.59
hsa-let-7e	11.56
hsa-miR-615-3p	11.53
hsa-miR-195	11.52
hsa-miR-221	11.50
hsa-miR-218	11.46
hsa-miR-518e	11.44
hsa-miR-188-5p	11.42
hsa-let-7f	11.42
hsa-miR-601	11.39

Serum miRNA	log2-Signal Intensity
hsa-miR-19b	11.38
hsa-miR-141	11.36
hsa-miR-942	11.32
hsa-miR-647	11.26
hsa-miR-517c	11.26
hsa-miR-557	11.24
hsa-miR-190b	11.23
hsa-miR-875-5p	11.20
hsa-miR-432	11.16
hsa-miR-345	11.12
hsa-miR-674	11.12
hsa-miR-651	11.10
hsa-miR-653	11.10
hsa-miR-654-3p	11.09
hsa-miR-517a	11.09
hsa-miR-98	11.08
hsa-miR-770-5p	11.07
hsa-miR-520d-3p	11.06
hsa-miR-373	11.04
hsa-miR-548c-5p	11.03
hsa-miR-129-5p	11.02
hsa-miR-548d-5p	11.01
hsa-miR-519b-3p	10.99
hsa-miR-549	10.98
hsa-miR-296-5p	10.97
hsa-miR-211	10.96
hsa-miR-451	10.96

Serum miRNA	log2-Signal Intensity
hsa-let-7i	10.96
hsa-miR-181d	10.95
hsa-miR-27a	10.94
hsa-miR-449a	10.93
hsa-miR-483-3p	10.92
hsa-miR-628-5p	10.91
hsa-miR-609	10.90
hsa-miR-583	10.90
hsa-miR-668	10.90
hsa-miR-210	10.89
hsa-miR-431	10.88
hsa-miR-152	10.88
hsa-miR-323-5p	10.88
hsa-miR-21	10.87
hsa-miR-30e	10.86
hsa-miR-659	10.85
hsa-miR-335	10.85
hsa-miR-520f	10.83
hsa-miR-542-3p	10.83
hsa-miR-449b	10.83
hsa-miR-508-5p	10.82
hsa-let-7g	10.77
hsa-miR-923	10.77
hsa-miR-365	10.76
hsa-miR-885-5p	10.75
hsa-miR-301b	10.74

Serum miRNA	log2-Signal Intensity
hsa-miR-671-3p	10.73
hsa-miR-516a-5p	10.73
hsa-miR-526b	10.73
hsa-miR-222	10.72
hsa-miR-196b	10.71
hsa-miR-139-5p	10.69
hsa-miR-181b	10.69
hsa-miR-15b	10.68
hsa-miR-941	10.68
hsa-miR-486-3p	10.64
hsa-miR-297	10.64
hsa-miR-143	10.62
hsa-miR-760	10.62
hsa-miR-200a	10.61
hsa-miR-448	10.60
hsa-miR-576-3p	10.59
hsa-miR-615-5p	10.59
hsa-miR-618	10.59
hsa-miR-338-3p	10.58
hsa-miR-589	10.58
hsa-miR-765	10.58
hsa-miR-192	10.58
hsa-miR-886-5p	10.58
hsa-miR-802	10.58
hsa-miR-582-5p	10.58
hsa-miR-133b	10.57
hsa-miR-622	10.57

Serum miRNA	log2-Signal Intensity
hsa-miR-29b	10.57
hsa-miR-340	10.57
hsa-miR-30b	10.56
hsa-miR-507	10.56
hsa-miR-588	10.55
hsa-miR-509-3p	10.55
hsa-miR-551a	10.54
hsa-miR-520g	10.54
hsa-let-7d	10.53
hsa-miR-375	10.51
hsa-miR-939	10.51
hsa-miR-134	10.50
hsa-miR-204	10.50
hsa-miR-516b	10.49
hsa-miR-565	10.49
hsa-miR-593	10.49
hsa-miR-499-5p	10.48
hsa-miR-520c-3p	10.48
hsa-miR-616	10.47
hsa-miR-569	10.47
hsa-miR-370	10.47
hsa-miR-579	10.47
hsa-miR-361-5p	10.46
hsa-miR-532-5p	10.46
hsa-miR-517b	10.43
hsa-miR-887	10.43
hsa-miR-571	10.42

Serum miRNA	log2-Signal Intensity
hsa-miR-190	10.41
hsa-miR-133a	10.40
hsa-miR-525-5p	10.39
hsa-miR-937	10.39
hsa-miR-630	10.38
hsa-miR-15a	10.38
hsa-miR-412	10.37
hsa-miR-519a	10.37
hsa-miR-497	10.36
hsa-miR-126	10.35
hsa-miR-934	10.34
hsa-miR-933	10.34
hsa-miR-25	10.33
hsa-miR-545	10.32
hsa-miR-96	10.32
hsa-miR-635	10.32
hsa-miR-205	10.31
hsa-miR-548b-3p	10.30
hsa-miR-768-5p	10.30
hsa-miR-487a	10.30
hsa-miR-194	10.30
hsa-miR-581	10.29
hsa-miR-101	10.28
hsa-miR-494	10.28
hsa-miR-9	10.27
hsa-miR-767-3p	10.27

Serum miRNA	log2-Signal Intensity	Serum miRNA	log2-Signal Intensity	Serum miRNA	log2-Signal Intensity
hsa-miR-524-5p	10.27	hsa-miR-556-3p	10.17	hsa-miR-320	10.04
hsa-miR-512-5p	10.27	hsa-miR-326	10.17	hsa-miR-590-3p	10.04
hsa-miR-892a	10.26	hsa-miR-602	10.17	hsa-miR-182	10.03
hsa-miR-490-3p	10.26	hsa-miR-606	10.17	hsa-miR-100	10.03
hsa-miR-149	10.24	hsa-miR-153	10.16	hsa-miR-489	10.02
hsa-miR-198	10.22	hsa-miR-26a	10.15	hsa-miR-186	10.01
hsa-miR-576-5p	10.22	hsa-miR-548d-3p	10.15	hsa-miR-409-5p	10.00
hsa-miR-299-3p	10.22	hsa-miR-573	10.15	hsa-miR-657	10.00
hsa-miR-520e	10.22	hsa-miR-515-5p	10.15	hsa-miR-223	9.98
hsa-miR-625	10.22	hsa-miR-560	10.13	hsa-miR-92b	9.97
hsa-let-7a	10.22	hsa-miR-500	10.13	hsa-miR-921	9.97
hsa-miR-99a	10.21	hsa-miR-498	10.11	hsa-miR-196a	9.97
hsa-miR-642	10.21	hsa-miR-614	10.11	hsa-miR-767-5p	9.97
hsa-miR-376b	10.19	hsa-miR-382	10.09	hsa-miR-665	9.96
hsa-miR-379	10.19	hsa-miR-423-5p	10.08	hsa-miR-644	9.96
hsa-miR-299-5p	10.19	hsa-miR-181c	10.07	hsa-miR-485-5p	9.95
hsa-miR-613	10.18	hsa-miR-1	10.07	hsa-miR-744	9.95
hsa-miR-564	10.17	hsa-miR-521	10.05	hsa-miR-509-3-5p	9.94
hsa-miR-631	10.17	hsa-miR-10a	10.04	hsa-miR-612	9.94

Table S5. List of differentially expressed miRNAs detected from human prostate cancer (CaP) tissue samples with high Gleason grade 9 versus low Gleason grade 6.[a]

miRNA[a]	Signal intensity in Gleason 9	Signal intensity in Gleason 6	Student's t-test *p* value	Ratio Gleason 9 to Gleason 6
hsa-miR-342-3p	14619	70	3.0E-04	209.3049
hsa-miR-532-3p	14429	320	1.0E-03	45.0595
hsa-miR-129-3p	7943	202	7.3E-04	39.2735
hsa-miR-770-5p	11393	291	1.9E-04	39.1878
hsa-miR-766	12615	371	3.3E-04	34.0244
hsa-miR-602	14000	438	7.0E-03	31.9499
hsa-miR-508-5p	9401	295	5.8E-03	31.8750
hsa-miR-192	8060	282	3.7E-04	28.5530
hsa-miR-211	8831	371	3.7E-03	23.8178
hsa-miR-486-3p	12180	514	1.0E-03	23.7063
hsa-miR-921	10363	463	5.8E-04	22.3590
hsa-miR-577	2079	98	7.2E-03	21.2171
hsa-miR-940	10835	547	3.7E-03	19.7983
hsa-miR-543	11402	589	6.8E-03	19.3623
hsa-miR-412	8086	480	1.7E-03	16.8346
hsa-miR-885-5p	13408	824	1.2E-03	16.2780
hsa-miR-133b	8447	520	1.0E-03	16.2340
hsa-miR-190b	1051	66	4.1E-02	15.8476
hsa-miR-150	11162	714	1.3E-03	15.6345
hsa-miR-153	8152	529	5.9E-03	15.4168
hsa-miR-634	14773	966	2.1E-03	15.2950
hsa-miR-887	10133	702	5.0E-03	14.4443
hsa-miR-17	25996	1817	3.7E-04	14.3046

miRNA[a]	Signal intensity in Gleason 9	Signal intensity in Gleason 6	Student's t-test *p* value	Ratio Gleason 9 to Gleason 6
hsa-miR-199a-5p	8156	581	5.6E-04	14.0272
hsa-miR-220b	9230	674	4.0E-04	13.6897
hsa-miR-99b	7949	592	5.4E-05	13.4280
hsa-miR-891a	9791	737	2.0E-02	13.2794
hsa-miR-455-3p	11971	971	3.0E-04	12.3263
hsa-miR-489	11620	1043	8.5E-04	11.1431
hsa-miR-220	7795	758	5.7E-03	10.2782
hsa-miR-502-5p	20279	1989	1.5E-03	10.1938
hsa-miR-212	9790	1066	6.0E-03	9.1867
hsa-miR-214	10867	1192	2.7E-03	9.1135
hsa-miR-589	9983	1157	9.4E-03	8.6313
hsa-miR-106a	24971	2927	6.6E-03	8.5302
hsa-miR-490-5p	9236	1199	2.9E-03	7.7053
hsa-miR-760	9809	1310	2.5E-05	7.4856
hsa-miR-369-3p	1005	144	1.5E-02	6.9945
hsa-miR-615-5p	6848	1091	7.0E-03	6.2750
hsa-miR-637	9421	1561	3.2E-04	6.0351
hsa-miR-125b	10013	1702	1.3E-02	5.8822
hsa-miR-299-5p	13266	2836	7.1E-03	4.6770
hsa-miR-133a	9417	2065	4.1E-03	4.5615
hsa-miR-872	2227	514	3.9E-02	4.3350
hsa-miR-889	7733	1930	3.0E-04	4.0072
hsa-miR-580	1015	256	4.8E-02	3.9716
hsa-miR-148b	2471	686	5.6E-03	3.6005
hsa-miR-494	10411	3029	1.5E-04	3.4371
hsa-miR-135a	961	302	1.5E-03	3.1844
hsa-miR-609	1045	370	1.4E-02	2.8215

miRNA[a]	Signal intensity in Gleason 9	Signal intensity in Gleason 6	Student's t-test *p* value	Ratio Gleason 9 to Gleason 6
hsa-miR-486-5p	13553	4971	7.7E-03	2.7267
hsa-miR-23b	1256	553	1.4E-02	2.2711
hsa-miR-672	4466	2799	4.7E-02	1.5953
hsa-miR-100	1525	1248	2.0E-02	1.5220
hsa-miR-499-3p	3254	4965	9.0E-03	0.6555
hsa-miR-519e	27000	41430	5.9E-03	0.6517
hsa-miR-337-5p	3295	5184	9.4E-03	0.6355
hsa-miR-132	2496	3969	7.6E-04	0.6290
hsa-miR-768-5p	8240	13864	2.8E-02	0.5943
hsa-miR-560	809	1392	4.4E-02	0.5811
hsa-miR-942	1913	3418	3.6E-02	0.5596
hsa-let-7e	2530	4707	4.5E-02	0.5375
hsa-miR-936	1245	2321	3.9E-02	0.5364
hsa-miR-519d	20525	39835	4.0E-03	0.5152
hsa-miR-200c	4334	8503	3.9E-03	0.5097
hsa-miR-30c	2071	4201	2.2E-02	0.4929
hsa-miR-342-5p	9074	18751	2.1E-03	0.4839
hsa-miR-513-5p	1624	3527	1.3E-02	0.4606
hsa-miR-151-5p	2269	4953	1.3E-03	0.4581
hsa-miR-654-5p	5296	11713	8.4E-04	0.4521
hsa-miR-302c	2075	4680	5.9E-03	0.4434
hsa-miR-204	1357	3231	3.9E-02	0.4199
hsa-miR-134	2463	6555	7.4E-03	0.3757
hsa-miR-515-3p	14838	41630	2.4E-03	0.3564
hsa-miR-210	1477	4167	4.6E-02	0.3544
hsa-miR-107	1140	3225	4.8E-02	0.3534
hsa-miR-346	1011	2869	1.7E-02	0.3525

miRNA[a]	Signal intensity in Gleason 9	Signal intensity in Gleason 6	Student's t-test *p* value	Ratio Gleason 9 to Gleason 6
hsa-miR-943	3756	10769	5.3E-04	0.3488
hsa-miR-557	1601	4771	8.7E-03	0.3356
hsa-miR-608	1428	4444	3.7E-02	0.3214
hsa-miR-583	3938	12391	1.0E-02	0.3178
hsa-miR-674	1622	5130	3.8E-02	0.3162
hsa-miR-377	807	2618	6.8E-03	0.3082
hsa-miR-758	1150	3872	4.0E-02	0.2971
hsa-miR-939	1382	4704	1.9E-02	0.2938
hsa-miR-199a-3p, has-miR-199b-3p	1015	3515	4.8E-02	0.2889
hsa-miR-433	3730	13061	2.5E-02	0.2856
hsa-miR-885-3p	838	3013	4.5E-02	0.2782
hsa-miR-675	14361	52208	2.5E-03	0.2751
hsa-miR-484	1232	4709	2.3E-02	0.2617
hsa-miR-325	3063	11783	3.4E-02	0.2599
hsa-miR-582-3p	849	3408	3.5E-02	0.2491
hsa-miR-371-3p	1292	5591	1.1E-02	0.2311
hsa-miR-330-5p	2031	8787	5.9E-03	0.2311
hsa-miR-187	948	4106	1.5E-02	0.2309
hsa-miR-545	956	4240	2.1E-02	0.2256
hsa-miR-370	1500	6821	4.3E-02	0.2199
hsa-miR-191	1433	6537	4.5E-03	0.2191
hsa-miR-122	3979	18370	5.0E-03	0.2166
hsa-miR-30b	1437	6813	1.2E-02	0.2109
hsa-miR-10b	813	3901	8.1E-03	0.2084
hsa-miR-302b	1306	6404	3.0E-02	0.2039
hsa-miR-614	937	4631	3.8E-02	0.2024

miRNA[a]	Signal intensity in Gleason 9	Signal intensity in Gleason 6	Student's t-test *p* value	Ratio Gleason 9 to Gleason 6
hsa-miR-526b	805	3990	3.4E-02	0.2017
hsa-miR-145	1504	7483	1.5E-02	0.2010
hsa-miR-19b	841	4223	2.2E-02	0.1990
hsa-miR-411	1022	5337	4.2E-03	0.1914
hsa-miR-886-3p	4638	24576	2.6E-02	0.1887
hsa-miR-654-3p	937	5325	3.9E-03	0.1761
hsa-miR-302d	2261	13053	4.4E-02	0.1732
hsa-miR-140-3p	973	5894	8.5E-03	0.1651
hsa-miR-493	5441	33349	1.1E-03	0.1631
hsa-miR-200b	2656	16552	5.0E-03	0.1605
hsa-miR-551a	1022	6465	3.5E-05	0.1580
hsa-miR-139-3p	1144	7505	1.4E-02	0.1524
hsa-miR-30a	878	5819	1.8E-03	0.1510
hsa-miR-941	1058	7067	1.2E-02	0.1496
hsa-miR-125a-5p	1007	6776	5.6E-03	0.1486
hsa-miR-578	1338	9090	8.2E-03	0.1472
hsa-miR-550	3354	23297	1.8E-03	0.1440
hsa-miR-144	3775	26510	3.1E-03	0.1424
hsa-miR-744	763	5443	8.3E-03	0.1401
hsa-miR-638	718	5534	6.3E-03	0.1298
hsa-miR-193a-3p	3915	31099	6.4E-04	0.1259
hsa-miR-768-3p	763	6244	1.5E-02	0.1221
hsa-miR-558	3239	26822	1.3E-02	0.1208
hsa-miR-933	666	5594	5.1E-03	0.1190
hsa-miR-371-5p	977	8281	1.7E-02	0.1180
hsa-miR-338-5p	4980	47655	2.0E-03	0.1045
hsa-miR-877	3616	34617	1.8E-03	0.1044

miRNA[a]	Signal intensity in Gleason 9	Signal intensity in Gleason 6	Student's t-test *p* value	Ratio Gleason 9 to Gleason 6
hsa-miR-455-5p	4327	41788	3.7E-04	0.1035
hsa-miR-152	611	6159	3.0E-03	0.0992
hsa-miR-505	2805	29750	3.4E-03	0.0943
hsa-miR-642	562	6108	6.1E-03	0.0921
hsa-miR-220c	535	6121	1.8E-03	0.0874
hsa-miR-302a	1068	12242	4.1E-03	0.0872
hsa-miR-584	2938	34195	1.2E-03	0.0859
hsa-miR-18a	1014	12035	2.7E-02	0.0843
hsa-miR-483-3p	474	6310	5.7E-03	0.0751
hsa-miR-661	445	5935	1.2E-02	0.0749
hsa-miR-495	3022	40805	3.7E-04	0.0741
hsa-miR-871	2764	39241	6.0E-04	0.0704
hsa-miR-197	453	6736	5.0E-04	0.0672
hsa-let-7b	733	11056	2.6E-03	0.0663
hsa-miR-522	442	7152	3.3E-03	0.0618
hsa-miR-18b	720	11699	2.3E-03	0.0615
hsa-miR-337-3p	2543	41586	1.1E-04	0.0611
hsa-miR-450a	1703	28130	7.0E-04	0.0605
hsa-miR-603	398	6923	5.6E-03	0.0575
hsa-miR-518a-3p	526	9396	8.1E-03	0.0560
hsa-miR-148a	2374	45394	4.1E-04	0.0523
hsa-miR-300	360	6970	3.5E-04	0.0517
hsa-miR-635	282	5655	9.6E-04	0.0499
hsa-let-7c	457	10645	6.9E-03	0.0429
hsa-miR-130b	202	6446	1.8E-02	0.0314
hsa-miR-34b	190	6254	4.5E-03	0.0303
hsa-miR-508-3p	528	21551	2.0E-02	0.0245

miRNA[a]	Signal intensity in Gleason 9	Signal intensity in Gleason 6	Student's t-test *p* value	Ratio Gleason 9 to Gleason 6
hsa-miR-647	131	5631	8.1E-03	0.0232
hsa-miR-34c-3p	154	6662	8.5E-03	0.0231
hsa-miR-492	110	5442	8.0E-03	0.0201
hsa-miR-564	91	5692	1.7E-02	0.0159
hsa-miR-662	95	7030	1.5E-02	0.0135
hsa-miR-888	63	6665	5.5E-03	0.0095

[a]All miRNAs were found to target prostate-cancer related genes except "hsa-miR-139-3p" and "hsa-miR-526b".

Table S6. A function-gene-miRNA network analysis of 35 deregulated miRNAs and 5 select Gene Ontology terms (Node 1), and 13 prostate-cancer related genes (Node 2).

Node1: Biological Process/miRNA	Node2: Gene Target
cell differentiation	TP53
cell differentiation	PTEN
cell differentiation	PAK2
cell differentiation	PAK1
cell differentiation	NFKB1
cell differentiation	MMP14
cell differentiation	ERBB2
cell differentiation	COL1A1
cell differentiation	BCL2
cell differentiation	AR
regulation of cell motility	PTEN
regulation of cell motility	COL1A1
regulation of cell motility	BCL2
regulation of protein phosphorylation and metabolic process	TP53
regulation of protein phosphorylation and metabolic process	TGFA
regulation of protein phosphorylation and metabolic process	PTEN
regulation of protein phosphorylation and metabolic process	PAK2
regulation of protein phosphorylation and metabolic process	PAK1
regulation of protein phosphorylation and metabolic process	NFKB1
regulation of protein phosphorylation and metabolic process	MIF
regulation of protein phosphorylation and metabolic process	ERBB2
regulation of protein phosphorylation and metabolic process	BCL2
regulation of apoptosis	TP53
regulation of apoptosis	PTEN
regulation of apoptosis	PAK2

Node1: Biological Process/miRNA	Node2: Gene Target
regulation of apoptosis	NFKB1
regulation of apoptosis	MIF
regulation of apoptosis	ERBB2
regulation of apoptosis	CASP8
regulation of apoptosis	BCL2
response to hormone stimulus	PTEN
response to hormone stimulus	MMP14
response to hormone stimulus	ERBB2
response to hormone stimulus	COL1A1
response to hormone stimulus	BCL2
response to hormone stimulus	AR
hsa-let-7b	TP53
hsa-let-7b	PAK1
hsa-let-7b	COL1A1
hsa-let-7b	CASP8
hsa-let-7c	TP53
hsa-let-7c	PAK1
hsa-let-7c	COL1A1
hsa-let-7e	TP53
hsa-let-7e	PAK1
hsa-let-7e	COL1A1
hsa-miR-106a	PTEN
hsa-miR-106a	CASP8
hsa-miR-106a	BCL2
hsa-miR-125b	ERBB2
hsa-miR-125b	BCL2
hsa-miR-129-3p	TP53
hsa-miR-129-3p	CASP8

Node1: Biological Process/miRNA	Node2: Gene Target
hsa-miR-130b	TGFA
hsa-miR-130b	PTEN
hsa-miR-130b	CASP8
hsa-miR-130b	AR
hsa-miR-144	TGFA
hsa-miR-144	PTEN
hsa-miR-148a	TGFA
hsa-miR-148a	PTEN
hsa-miR-148a	MIF
hsa-miR-150	TP53
hsa-miR-150	PAK1
hsa-miR-150	MMP14
hsa-miR-150	COL1A1
hsa-miR-152	TP53
hsa-miR-152	TGFA
hsa-miR-152	PTEN
hsa-miR-152	MIF
hsa-miR-153	PTEN
hsa-miR-153	BCL2
hsa-miR-17	PTEN
hsa-miR-17	CASP8
hsa-miR-197	MMP14
hsa-miR-200b	PAK2
hsa-miR-200c	PAK2
hsa-miR-212	MMP14
hsa-miR-212	COL1A1
hsa-miR-214	PTEN
hsa-miR-214	PAK2

Node1: Biological Process/miRNA	Node2: Gene Target
hsa-miR-23b	TGFA
hsa-miR-23b	PTEN
hsa-miR-23b	PAK2
hsa-miR-23b	BCL2
hsa-miR-302a	PAK2
hsa-miR-302b	PAK2
hsa-miR-302c	PAK2
hsa-miR-302d	PAK2
hsa-miR-30a	PAK1
hsa-miR-30a	BCL2
hsa-miR-30a	AR
hsa-miR-30b	PAK1
hsa-miR-30b	BCL2
hsa-miR-30b	AR
hsa-miR-30c	PAK1
hsa-miR-30c	BCL2
hsa-miR-30c	AR
hsa-miR-338-5p	BCL2
hsa-miR-34b	PAK1
hsa-miR-34b	BCL2
hsa-miR-371-3p	TP53
hsa-miR-371-5p	COL1A1
hsa-miR-486-5p	PTEN
hsa-miR-494	PTEN
hsa-miR-494	PAK1
hsa-miR-519d	PTEN
hsa-miR-519d	CASP8
hsa-miR-519e	NFKB1

Node1: Biological Process/miRNA	Node2: Gene Target
hsa-miR-582-3p	PTEN

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