Figure S1: Heat map based on the relative expression of genes and miRNAs in human prostate tissue. Columns represent genes and miRNAs; rows represent prostate tissue samples (red squares: Tu samples, green squares: Tf samples, yellow squares: BPH samples). Transcript levels of genes and miRNAs were normalized to TBP and RNU48, respectively.
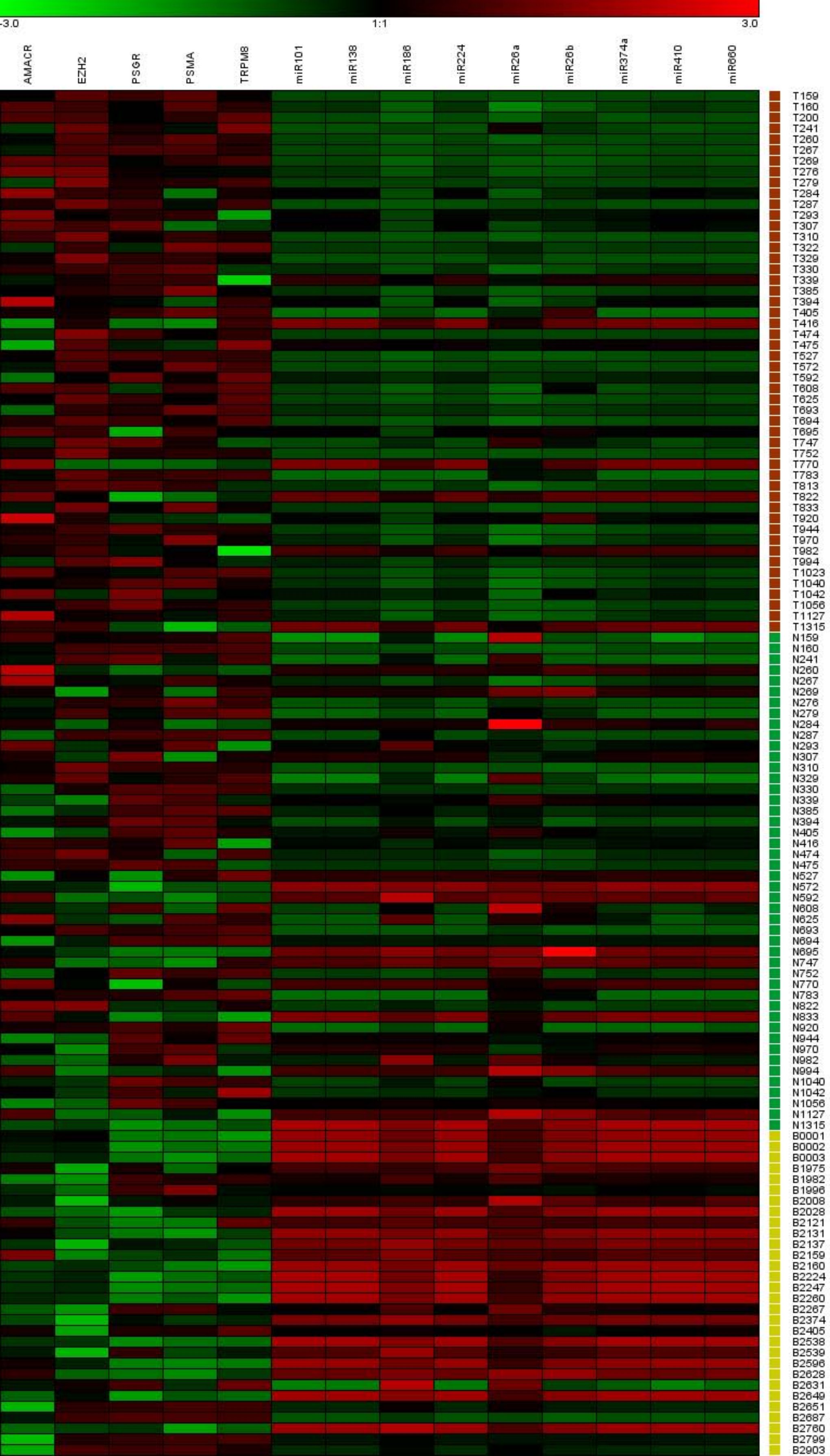
Table S1: Web addresses of the bioinformatics resources used for miRNA prediction.

Bioinformatic resource	Web address
TargetScanHuman v5.1	http://www.targetscan.org/
TargetScanS	http://genes.mit.edu/tscan/targetscanS2005.html
PicTar (based on conservation in mammals)	http://pictar.mdc-berlin.de/
MicroCosm Targets (formerly miRBase Targets)	http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/
microRNA.org	http://www.microrna.org/microrna/home.do
Human miRNA Targets (optimized intersection: PicTar, TargetScanS)	http://www.diana.pcbi.upenn.edu/cgi-bin/TargetCombo.cgi
DIANA microT v3.0	http://diana.cslab.ece.ntua.gr/microT/
DIANA TarBase v5.0	http://diana.cslab.ece.ntua.gr/tarbase/

Table S2: Sequences of primers and probes used for qPCR.

Gene name (synonym)	Abbreviation (synonym)	Gene ID[a]	Primer / Probe	Sequence 5'-3'	Length of qPCR fragment	Ref.
alpha-methylacyl-CoA racemase	**AMACR**	23600	AMACR FOR AMACR REV AMACR FL AMACR LC	gTATgCCCCgCTgAATCTC TCCTTTgATCAgCAgCTCgTAg ATgCTgTTCCTTCCACCATATTTgCATC-FL LC640-ATgACCTgACCCTTgCCAgTgCgT-PH	312 bp	
enhancer of zeste homolog 2	**EZH2**	2146	EZH2 FOR EZH2 REV EZH2 FL EZH2 LC	gCCAgACTgggAAgAAATCTg TgTgTTggAAAATCCAAgTCA AACCTCTTgAgCTgTCTCAgTCgCA-FL LC640-TACTCTgATTTTACACgCTTCCgCC-PH	277 bp	[1]
prostate-specific G-protein coupled receptor (olfactory receptor, family 51, subfamily E, member 2)	**PSGR** (OR51E2)	81285	PSGR FOR PSGR REV PSGR FL PSGR LC	AACTgCTgTATgggCTCTACTg TgCCACTACATACATggAAAggAgg TCCAgCTATgAgTTCCTgCAACTTCACAC-FL LC640-TgCCACCTTTgTgCTTATTggTATCCCA-PH	179 bp	
prostate-specific membrane antigen (folate hydrolase 1)	**PSMA** (FOLH1)	2346	PSMA FOR PSMA REV PSMA FL PSMA LC	CTTTCTCCTCggCTTCCTCTTC gATTTTCATgTCCCgTTCCA TTCCACTgggATTgAATTTgCTTTgC-FL LC640-AgCTgAAAgTTTTgTTCTgTTCCTgCTAAATg-PH	485 bp	
TATA box binding protein	**TBP**	6908	TBP FOR TBP REV TBP FL TBP LC	gAATATAATCCCAAgCggTTTg ACTTCACATCACAgCTCCCC TTTCCCAgAACTgAAAATCAgTgCC-FL LC640-TggTTCgTggCTCTCTTATCCTCATg-PH	226 bp	[1]
transient receptor potential cation channel, subfamily M, member 8	**TRPM8** (Trp-p8)	79054	TRPM8 FOR TRPM8 REV TRPM8 FL TRPM8 LC	ACgCTTgTgTACCggAATCT CgAgTAATAggAgACACgTCg TTTCCAgACAAACgTgAggAgggC-FL LC640-CATTATAggAATTCTTggCgATCTgCA-PH	167 bp	[1-3]

[a] http://www.ncbi.nlm.nih.gov/gene

FL, fluorescence dye fluorescein; FOR, forward primer; LC640, fluorescence dye LC Red640; PH, phosphorylated 3'-end; REV, reverse primer

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2. Fuessel S, Sickert D, Meye A, Klenk U, Schmidt U, Schmitz M, Rost AK, Weigle B, Kiessling A, Wirth MP: **Multiple tumor marker analyses (PSA, hK2, PSCA, trp-p8) in primary prostate cancers using quantitative RT-PCR.** *Int J Oncol* 2003, **23**(1):221-228.
3. Kiessling A, Fussel S, Schmitz M, Stevanovic S, Meye A, Weigle B, Klenk U, Wirth MP, Rieber EP: **Identification of an HLA-A*0201-restricted T-cell epitope derived from the prostate cancer-associated protein trp-p8.** *Prostate* 2003, **56**(4):270-279.

Table S3: Conditions for qPCR measurements.

	AMACR	EZH2	PSGR	PSMA	TBP	TRPM8	miRNAs
MgCl$_2$	4 mM	4 mM	4 mM	4 mM	4 mM	4 mM	TaqMan Gene Expression Master Mix
Primer (for / rev)	0.5 µM each	0.5 µM each	0.5 µM / 0.25 µM	0.25 µM each	0.3 µM / 0.6 µM	0.5 µM each	Specific TaqMan
Probes (FL / LC)	0.2 µM each	0.2 µM / 0.4 µM	0.2 µM each	0.2 µM / 0.4 µM	0.2 µM / 0.4 µM	0.2 µM each	MicroRNA Assays
Activation (1 cycle)	95°C, 10 min	95°C, 10 min	95°C, 10 min	95°C, 10 min	95°C, 10 min	95°C, 10 min	95°C, 10 min
Amplification (45 cycles)							
Denaturation step	95°C, 10 s	95°C, 1 s	95°C, 1 s	95°C, 10 s	95°C, 10 s	95°C, 10 s	95°C, 15 s
Annealing step	59°C, 10 s	60°C, 15 s	59°C, 10 s	59°C, 15 s	61°C, 15 s	66°C, 15 s	60°C, 60 s
Elongation step	72°C, 10 s	72°C, 20 s	72°C, 10 s	72°C, 20 s	72°C, 10 s	72°C, 10 s	-

FL, probe with fluorescence dye fluorescein; for, forward primer; LC, probe with fluorescence dye LC Red640; rev, reverse primer

Table S4: Distributions of fold expressions of PCa-associated genes and miRNAs in prostate cancer. Depicted are the proportions of fold expression in Tu samples compared to either Tf or BPH samples. A fold expression of ≥2.0 was considered as up-regulation and of ≤-2.0 as down-regulation, whereas the remaining proportion was regarded as an unaltered expression.

Gene / miRNA	Fold expressions: Tu *vs* Tf[median]			Fold expressions: Tu *vs* BPH[median]		
	≤ -2.0-fold down n (%)	unaltered n (%)	≥ 2.0-fold up n (%)	≤ -2.0-fold down n (%)	unaltered n (%)	≥ 2.0-fold up n (%)
AMACR	5 (10%)	5 (10%)	40 (80%)	2 (4%)	4 (8%)	44 (88%)
EZH2	1 (2%)	29 (58%)	20 (40%)	0 (0%)	10 (20%)	40 (80%)
PSGR	9 (18%)	12 (24%)	29 (58%)	3 (6%)	2 (4%)	45 (90%)
PSMA	8 (16%)	14 (28%)	28 (56%)	2 (4%)	5 (10%)	43 (86%)
TRPM8	5 (10%)	15 (30%)	30 (60%)	2 (4%)	5 (10%)	43 (86%)
miR-101	22 (44%)	25 (50%)	3 (6%)	13 (26%)	32 (64%)	5 (10%)
miR-138	36 (72%)	12 (24%)	2 (4%)	36 (72%)	11 (22%)	3 (6%)
miR-186	43 (86%)	7 (14%)	0 (0%)	45 (90%)	5 (10%)	0 (0%)
miR-224	36 (72%)	14 (28%)	0 (0%)	34 (68%)	14 (28%)	2 (4%)
miR-26a	27 (54%)	23 (46%)	0 (0%)	22 (44%)	26 (52%)	2 (4%)
miR-26b	16 (32%)	29 (58%)	5 (10%)	12 (24%)	30 (60%)	8 (16%)
miR-374a	28 (56%)	21 (42%)	1 (2%)	23 (46%)	26 (52%)	1 (2%)
miR-410	35 (70%)	14 (28%)	1 (2%)	38 (76%)	11 (22%)	1 (2%)
miR-660	19 (38%)	30 (60%)	1 (2%)	20 (40%)	29 (58%)	1 (2%)

Table S5: Expression of selected genes in prostate cancer dependent on clinicopathological parameters. Depicted are the median relative transcript levels of the evaluated genes (normalized to TBP) in Tu tissue samples. A Mann–Whitney *U* test with a two-sided 95% confidence interval was used for two group comparisons. Three-group comparisons were carried out by the Kruskal–Wallis test followed by a Mann–Whitney *U* test with a two-sided 95% confidence interval for *post hoc* analyses. Significant differences are highlighted in bold.

Parameter	AMACR	EZH2	PSGR	PSMA	TRPM8
	P values and median relative transcript levels				
Age	p = 0.635	p = 0.778	p = 0.114	p = 0.869	p = 0.165
≤ median (n=25)	2228.90	0.99	67.79	29.56	52.31
> median (n=25)	1771.34	0.90	25.02	15.76	31.18
PSA	p = 0.705	p = 0.705	p = 0.079	p = 0.869	p = 0.308
≤ median (n=25)	2341.57	0.99	67.79	16.91	31.85
> median (n=25)	1935.56	0.90	38.70	29.49	52.31
Tumor stage	p = 0.915	p = 0.661	p = 0.247	p = 0.676	**p = 0.030**
pT1+2 (n=23)	2341.57	0.99	66.95	15.89	52.44
pT3+4 (n=27)	1935.56	0.90	38.70	28.03	25.14
Gleason score	p = 0.706	p = 0.354	p = 0.128	p = 0.906	p = 0.421
< 7 (n=16)	2369.88	0.86	65.35	25.88	38.16
7 (n=19)	1303.14	0.99	60.30	28.01	41.13
> 7 (n=15)	1771.34	0.90	15.69	28.03	16.49
Initial metastases	p = 0.225	p = 0.410	p = 0.160	p = 0.297	p = 0.182
N0 M0 (n=40)	2339.05	0.98	50.89	25.88	38.55
N+/M+ (n=10)	969.76	0.84	43.31	28.02	27.56

Table S6: Expression of selected miRNAs in prostate cancer dependent on clinicopathological parameters. Depicted are the median relative transcript levels of the evaluated miRNAs (normalized to RNU48) in Tu tissue samples. A Mann–Whitney *U* test with a two-sided 95% confidence interval was used for two group comparisons. Three-group comparisons were carried out by the Kruskal–Wallis test followed by a Mann–Whitney *U* test with a two-sided 95% confidence interval for *post hoc* analyses. Significant differences are highlighted in bold.

Parameter	miR-101	miR-138	miR-186	miR-224	miR-26a	miR-26b	miR-374a	miR-410	miR-660
	P values and median relative transcript levels (x10-3)								
Age	p = 0.388	p = 0.643	p = 0.877	p = 0.915	p = 0.299	p = 0.091	p = 0.509	p = 0.090	p = 0.432
≤ median (n=25)	1.57	0.24	36.60	1.12	194.32	53.32	17.91	0.12	13.60
> median (n=25)	1.47	0.15	40.49	1.27	149.10	35.59	17.24	0.00	11.87
PSA	p = 0.662	p = 0.657	p = 0.907	p = 0.432	p = 0.607	p = 0.786	p = 0.218	p = 0.572	p = 0.720
≤ median (n=25)	1.56	0.24	38.43	1.38	194.32	51.32	19.88	0.00	14.84
> median (n=25)	1.47	0.14	40.49	0.99	165.54	52.95	16.24	0.09	13.32
Tumor stage	p = 0.778	p = 0.262	**p = 0.028**	p = 0.838	p = 0.459	p = 0.969	p = 0.414	p = 0.635	p = 0.711
pT1+2 (n=23)	1.47	0.43	47.70	1.27	149.62	51.50	20.02	0.00	11.42
pT3+4 (n=27)	1.59	0.13	33.89	1.12	194.32	52.04	16.82	0.09	13.98
Gleason score	p = 0.633	p = 0.866	p = 0.742	p = 0.721	p = 0.752	p = 0.495	p = 0.913	p = 0.914	p = 0.786
< 7 (n=16)	1.48	0.39	41.37	1.43	194.66	50.70	18.36	0.11	14.92
7 (n=19)	1.56	0.15	38.03	1.08	175.64	52.95	17.91	0.09	11.95
> 7 (n=15)	1.44	0.15	40.49	0.93	149.10	51.50	17.36	0.00	13.98
Initial metastases	p = 0.370	p = 0.480	**p = 0.005**	p = 0.369	p = 0.528	p = 0.865	p = 0.913	p = 0.625	p = 0.445
N0 M0 (n=40)	1.47	0.34	44.86	1.30	177.19	51.77	17.63	0.05	11.91
N+/M+ (n=10)	1.66	0.07	31.18	0.89	194.32	52.13	17.80	0.09	14.92