

# **Genome-Wide Expression of MicroRNAs is Regulated by DNA Methylation in Hepatocarcinogenesis**

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## Supplementary Materials

### Supplementary Figure Legend

#### **Supplementary FIGURE S1: Comparisons of up- or under-expressed miRNAs between tumor and non-tumor tissues in the discovery set (10 pairs)**

MiR-196b, miR-183, miR-10b<sup>#</sup> and miR-182 were significantly up-regulated, while miR-511, miR-144<sup>#</sup>, miR-486, miR-378, miR-139-5p, and miR-199a-3p were significantly down-regulated in HCC tumor tissues.

#### **Supplementary FIGURE S2: Hierarchical cluster analysis of the top 28 significantly differentially methylated CpG sites between 10 pairs of HCC tumor and adjacent non-tumor tissues**

Blue represents tumor tissue and red adjacent non-tumor tissue. The top 28 significant CpG sites can clearly distinguish HCC tumor from adjacent tissues without misclassification.

#### **Supplementary FIGURE S3: Genome-wide associations between DNA methylation and miRNA expression covered by TLDA and 450K arrays**

A Venn diagram describes the number of miRNAs covered by each assay (TLDA and 450K array), as well as detectable in over 80% samples. There are 628 miRNAs covered by both TLDA and 450K arrays, but only 222 were detectable in over 80% of samples. We focused our analyses on these 222 miRNAs. A total of 1,014 CpG sites were hypomethylated covering 206 miRNAs, and 501 CpG sites were hypermethylated covering 115 miRNAs.

#### **Supplementary FIGURE S4: Comparison of mir-199a-1's host gene (*DNM2*) mRNA expression in HCC tumor, precursor and normal liver tissues**

Significant under-expression of *DNM2* mRNA was observed in HCC tumor compared with precursor and normal liver tissues, which is consistent with the expression pattern of miR-199a in HCC tumor tissue.

#### **Supplementary FIGURE S5: Comparisons of mRNAs in HCC tumor, precursor and normal liver tissues**

A representative target gene for miR-125b (*LIN28B*) is displayed in Figure S5A. *LIN28B* was significantly over-expressed in HCC tumor compared with precursor and normal liver tissues ( $p < 0.001$ ). Figure S5B displays a representative target gene for miR-199a (*mTOR*) that was significantly over-expressed in HCC tumor compared with precursor and normal liver tissues ( $p < 0.001$ ).

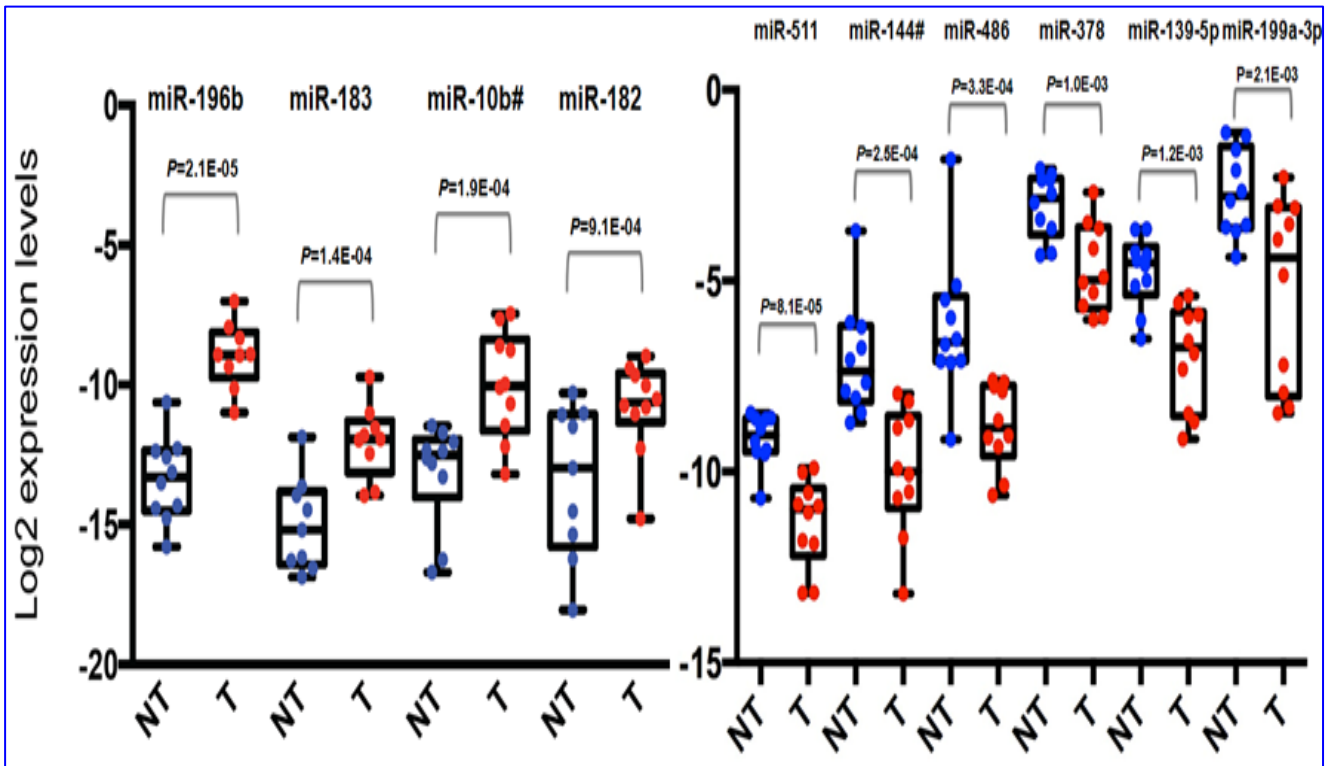
**Supplementary TABLE S1:** Comparison of clinical and pathological characteristics for HCC subjects in the discovery (10 patients) and validation sets (56 patients)

**Supplementary TABLE S2:** Significantly aberrant DNA methylation of CpG sites associated with miRNAs in comparison of 10 pairs of HCC tumor and adjacent non-tumor tissues (adjusted  $p < 0.05$ )

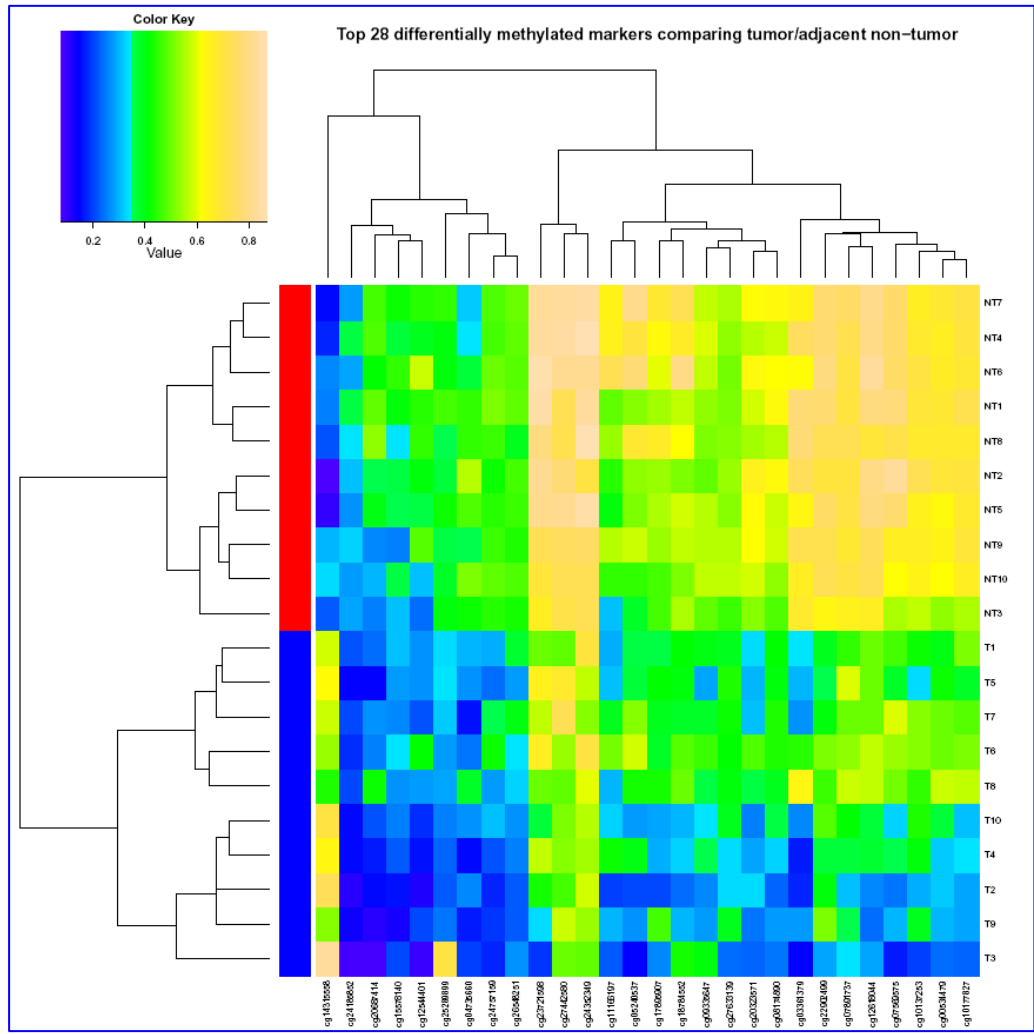
**Supplementary TABLE S3:** Validation of the 28 significant CpG sites in additional 56 pairs of HCC tumor and adjacent non-tumor tissues (adjusted  $p < 0.05$ )

**Supplementary TABLE S4:** Validation of the inverse correlations between DNA methylation differences and miRNAs fold changes (N=56 pairs)

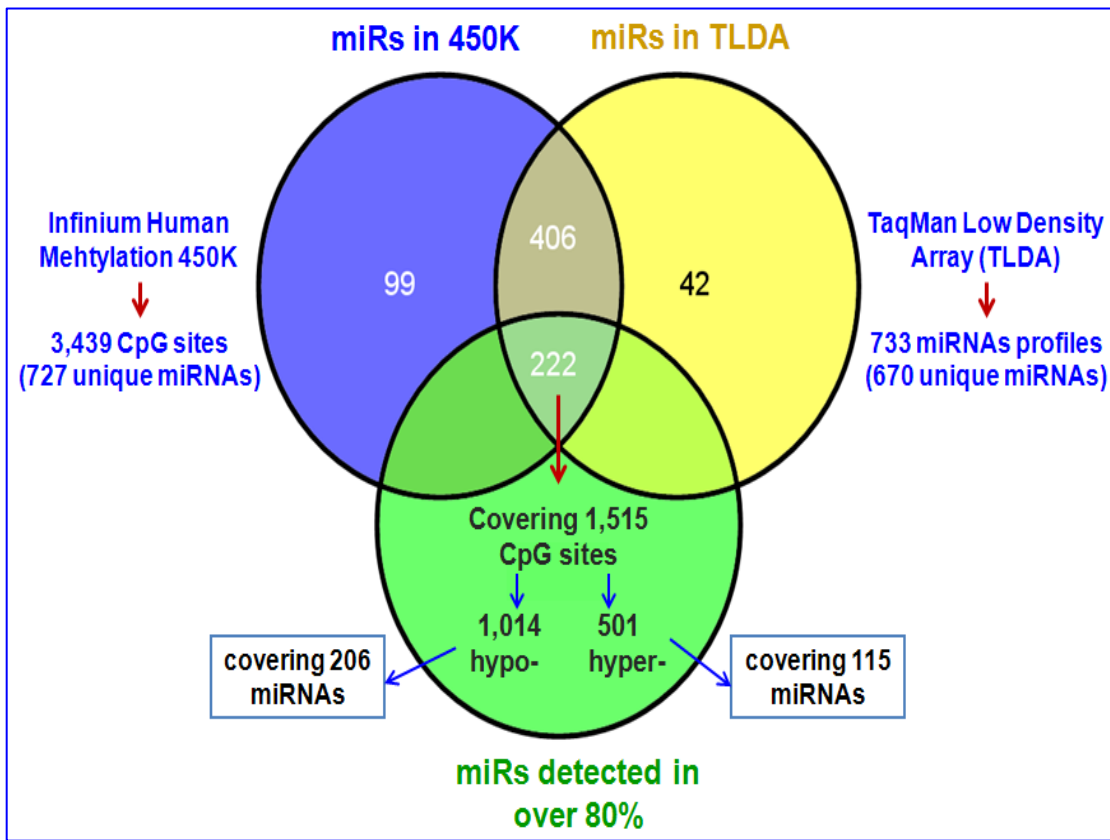
Supplementary FIGURE S1



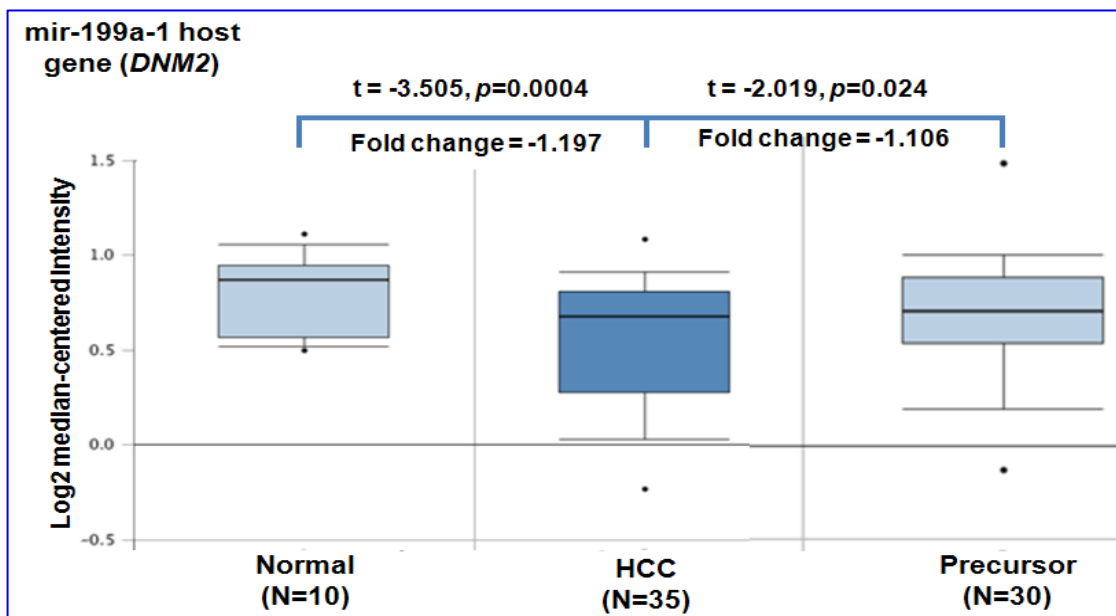
# Supplementary FIGURE S2



Supplementary FIGURE S3

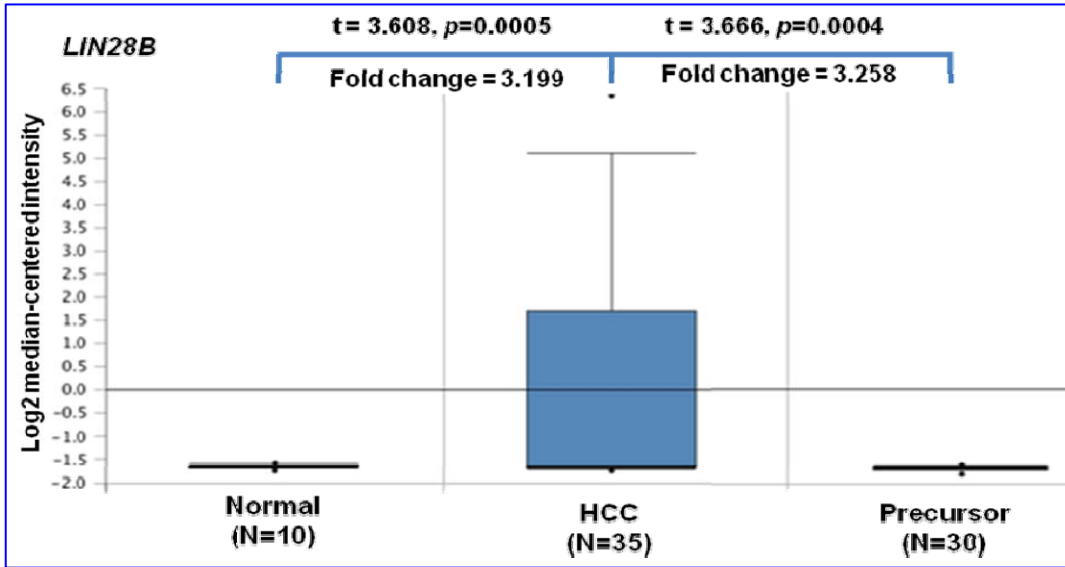


Supplementary FIGURE S4

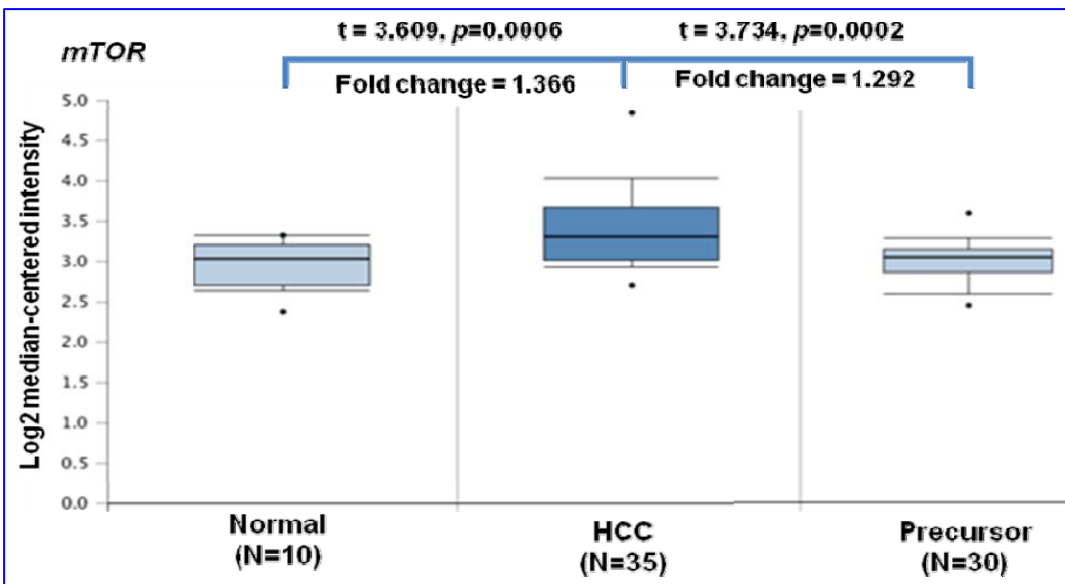


Supplementary FIGURE S5

Supplementary FIGURE S5A



Supplementary FIGURE S5B





**Supplementary TABLE S1:** Comparison of clinical and pathological characteristics for HCC subjects in discovery set (10 patients) and validation set (56 patients)

Variables	Discovery set	Validation set	<i>p</i> values
Age at diagnosis, Mean ± SD	62.7 ± 13.1	58.9 ± 14.9	4.53.E-01
Age at diagnosis, No (%)			
< 60 yrs	3 (30.0)	26 (46.4)	
≥ 60 yrs	7 (70.0)	30 (53.6)	3.35.E-01
Gender, No (%)			
Male	5 (50.0)	45 (80.4)	
Female	5 (50.0)	11 (19.6)	<b>3.91.E-02</b>
Ethnicity, No (%)			
Caucasian	3 (30.0)	31 (55.5)	
African-American	2 (20.0)	4 (7.1)	
Hispanic	2 (20.0)	4 (7.1)	
Asian	3 (30.0)	11 (19.6)	
Unknown/Other	0 (0.0)	6 (10.7)	3.98.E-01
Viral infection, No (%)			
HBV (-), HCV (-)	1 (10.0)	18 (32.2)	
HBV (-), HCV (+)	5 (50.0)	14 (25.0)	
HBV (+), HCV (-)	4 (40.0)	9 (16.1)	
HBV (+), HCV (+)	0 (0.0)	4 (7.1)	
Missing	0 (0.0)	11 (19.6)	7.70.E-02
Cigarette smoking, No (%)			
No	6 (60.0)	21 (37.5)	
Yes	4 (40.0)	32 (57.1)	
Missing	0 (0.0)	3 (5.4)	3.60.E-01
Alcohol drinking, No (%)			
No	5 (50.0)	22 (39.3)	
Yes	5 (50.0)	31 (55.4)	
Missing	0 (0.0)	3 (5.4)	6.64.E-01
AFP (ng/mL), Mean ± SD	6086.8 ± 17237	10275 ± 52910	6.68.E-01
Tumor size (cm), Mean ± SD	7.8 ± 3.9	5.8 ± 4.8	2.12.E-01
Cirrhosis, No (%)			
No	2 (20.0)	15 (26.8)	
Yes	7 (70.0)	41 (73.2)	
Missing	1 (10.0)	0 (0.0)	5.60.E-02
Tumor grade*, No (%)			
I-II	3 (30.0)	20 (35.7)	
III	4 (40.0)	18 (32.1)	
IV	3 (30.0)	15 (26.8)	
Missing	0 (0.0)	3 (5.4)	8.48.E-01
Surgery, No (%)			
Hepatic resection alone	8 (80.0)	20 (35.7)	
Transplant alone	0 (0.0)	32 (57.1)	
Both hepatic resection and transplant	2 (20.0)	4 (7.2)	8.41.E-04
Survival, No (%)			

Yes	5 (50.0)	33 (58.9)	
No	5 (50.0)	23 (41.1)	5.99.E-01

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\* Edmondson and Steiner grade

**Supplementary TABLE S2:** Significantly aberrant DNA methylation of CpG sites associated with miRNAs in comparison of 10 pairs of HCC tumor and adjacent non-tumor tissues (adjusted  $p < 0.05$ )

TargetID	Mean in HCC tumor tissue	Mean in HCC non-tumor tissue	Mean difference (tumor vs. non-tumor)	Adjusted P value	CHR	MAPINFO	Name of miRNAs	Host genes
cg22902499	0.4172	0.7379	-0.3207	5.44E-04	6	97754012	miR-548H3	MIR548H3
cg20323571	0.3141	0.5941	-0.2800	1.08E-03	1	176999034	miR-488	MIR488;ASTN1;ASTN1
cg05240537	0.3564	0.5961	-0.2397	2.66E-03	X	103346747	miR-1256	MIR1256;MCART6
cg24757159	0.2661	0.4685	-0.2024	8.94E-03	3	99345675	miR-548G	MIR548G
cg27633139	0.3750	0.5307	-0.1556	9.47E-03	4	9558893	miR-548i2	MIR548i2
cg25289899	0.3222	0.4020	-0.0798	9.49E-03	11	2019167	miR-675	H19;MIR675
cg24185852	0.1610	0.3099	-0.1489	9.61E-03	5	168690818	miR-585	MIR585;SLIT3
cg23721598	0.4791	0.7869	-0.3078	1.00E-02	15	79501052	miR-184	MIR184
cg27442580	0.5532	0.7720	-0.2189	1.17E-02	6	97797349	miR-548H3	MIR548H3
cg08174890	0.3533	0.5829	-0.2296	1.26E-02	4	9558077	miR-548i2	MIR548i2
cg10137253	0.3971	0.6663	-0.2692	1.82E-02	14	101507590	miR-300	MIR300
cg15578140	0.2442	0.3627	-0.1185	1.91E-02	7	147718109	miR-548F3	MIR548F3;CNTNAP2
cg11166197	0.3269	0.5248	-0.1979	1.96E-02	X	151561584	miR-105-1	GABRA3;MIR105-1
cg12618044	0.4175	0.7645	-0.3470	2.62E-02	X	103296301	miR-1256	H2BFM;MIR1256
cg04735660	0.2420	0.4271	-0.1851	2.72E-02	1	176999916	miR-488	MIR488;ASTN1;ASTN1
cg00534479	0.3945	0.6490	-0.2545	2.76E-02	5	168196464	miR-218-2	SLIT3;MIR218-2
cg12544401	0.2224	0.4123	-0.1899	2.89E-02	X	103291721	miR-1256	MIR1256
cg07891737	0.4451	0.7052	-0.2601	2.96E-02	19	54215577	miR-519D/miR-517A	MIR519D;MIR517A
cg26548251	0.2969	0.4637	-0.1667	3.36E-02	7	147591462	miR-548F3	MIR548F3;CNTNAP2
cg20687414	0.2064	0.3946	-0.1881	3.73E-02	3	44155764	miR-138-1	MIR138-1
cg09335647	0.3530	0.5481	-0.1950	3.76E-02	7	147097440	miR-548i4	MIR548i4;CNTNAP2
cg24352349	0.5830	0.7996	-0.2166	3.96E-02	22	45597767	miR-1249	C22orf9;C22orf9;MIR1249

cg10177827	0.3933	0.6765	-0.2833	3.96E-02	14	101511425	miR-487B/miR-381	MIR487B;MIR381
cg18784552	0.3780	0.6123	-0.2343	4.35E-02	X	113885574	miR-1912	MIR1912;HTR2C
cg07569575	0.3885	0.7204	-0.3319	4.44E-02	5	168691938	miR-585	SLIT3;MIR585
cg03361379	0.3041	0.6996	-0.3954	4.68E-02	7	157367159	miR-153-2	PTPRN2;PTPRN2;MIR153-2;PTPRN2
cg17895907	0.3512	0.5711	-0.2199	4.78E-02	X	146366330	miR-514-3/miR-514-2	MIR514-3;MIR514-2
cg14315558	0.6215	0.2117	0.4098	4.99E-02	21	26934682	miR-155HG	MIR155HG

**Supplementary TABLE S3:** Validation of the 28 significant CpG sites in additional 56 pairs of HCC tumor and adjacent non-tumor tissues (adjusted  $p < 0.05$ )

TargetID	Mean in HCC tumor tissue	Mean in HCC non-tumor tissue	Mean difference (tumor vs. non-tumor)	Adjusted P value	CHR	MAPINFO	Name of miRNAs	Host genes
cg22902499	0.4333	0.7277	-0.2945	9.71E-17	6	97754012	miR-548H3	MIR548H3
cg20323571	0.2885	0.5780	-0.2895	9.27E-24	1	176999034	miR-488	MIR488;ASTN1;ASTN1
cg05240537	0.3728	0.6486	-0.2758	7.31E-17	X	103346747	miR-1256	MIR1256;MCART6
cg24757159	0.2874	0.4781	-0.1907	1.36E-16	3	99345675	miR-548G	MIR548G
cg27633139	0.3648	0.5513	-0.1865	2.27E-17	4	9558893	miR-548i2	MIR548I2
cg25289899	0.2865	0.4153	-0.1288	2.16E-10	11	2019167	miR-675	H19;MIR675
cg24185852	0.1734	0.3085	-0.1351	3.46E-22	5	168690818	miR-585	MIR585;SLIT3
cg23721598	0.4487	0.7566	-0.3079	1.04E-16	15	79501052	miR-184	MIR184
cg27442580	0.4493	0.7353	-0.2860	6.68E-15	6	97797349	miR-548H3	MIR548H3
cg08174890	0.3638	0.5888	-0.2250	1.90E-18	4	9558077	miR-548i2	MIR548I2
cg10137253	0.4059	0.6654	-0.2595	4.34E-19	14	101507590	miR-300	MIR300
cg15578140	0.2326	0.3489	-0.1163	2.53E-12	7	147718109	miR-548F3	MIR548F3;CNTNAP2
cg11166197	0.3728	0.5662	-0.1934	4.82E-13	X	151561584	miR-105-1	GABRA3;MIR105-1
cg12618044	0.4226	0.7702	-0.3476	9.10E-19	X	103296301	miR-1256	H2BFM;MIR1256
cg04735660	0.2641	0.4619	-0.1979	5.55E-18	1	176999916	miR-488	MIR488;ASTN1;ASTN1
cg00534479	0.3706	0.6521	-0.2815	1.10E-18	5	168196464	miR-218-2	SLIT3;MIR218-2
cg12544401	0.2452	0.4749	-0.2298	4.49E-19	X	103291721	miR-1256	MIR1256
cg07891737	0.4199	0.6837	-0.2638	1.47E-16	19	54215577	miR-519D/miR-517A	MIR519D;MIR517A
cg26548251	0.2865	0.4431	-0.1566	3.51E-11	7	147591462	miR-548F3	MIR548F3;CNTNAP2
cg20687414	0.2009	0.4088	-0.2079	5.21E-17	3	44155764	miR-138-1	MIR138-1
cg09335647	0.3079	0.5441	-0.2362	6.87E-20	7	147097440	miR-548i4	MIR548I4;CNTNAP2
cg24352349	0.6480	0.7787	-0.1307	2.57E-10	22	45597767	miR-1249	C22orf9;C22orf9;MIR1249
cg10177827	0.4204	0.6903	-0.2699	1.30E-19	14	101511425	miR-487B/miR-381	MIR487B;MIR381
cg18784552	0.3516	0.5914	-0.2398	5.67E-16	X	113885574	miR-1912	MIR1912;HTR2C

cg07569575	0.3930	0.7246	-0.3317	2.48E-19	5	168691938	miR-585	SLIT3;MIR585
cg03361379	0.3158	0.6886	-0.3729	1.61E-20	7	157367159	miR-153-2	PTPRN2;PTPRN2;MIR153-2;PTPRN2
cg17895907	0.3012	0.5452	-0.2440	1.63E-18	X	146366330	miR-514-3/miR-514-2	MIR514-3;MIR514-2
cg14315558	0.5998	0.2146	0.3852	3.11E-17	21	26934682	miR-155HG	MIR155HG

**Supplementary TABLE S4:** Validation of the inverse correlations between DNA methylation differences and miRNAs fold changes (N=56 pairs)

miRNAs (chromosome location)	CpG sites	Distance to miRNA (bp)	Methylation difference (T vs. NT)	Fold change (T vs. NT)	Spearman Correlation Coefficients	p values
<b>mir-10a</b>	cg15649236	195	0.1	-1.45	0.04	6.69.E-01
17q21.32	cg01572694	246	0.03	-1.45	-0.03	7.26.E-01
46,657,200-46,657,309	cg14884929	347	-0.01	-1.45	0.28	<b>3.20.E-03</b>
<b>mir-18a</b>	cg17799287	1241	-0.07	1.27	-0.04	7.09.E-01
13q31.3	cg07641807	1046	-0.09	1.27	-0.06	5.17.E-01
(92,002,997-92,003,088)	cg23665802	667	-0.09	1.27	-0.01	9.26.E-01
	cg02297838	551	-0.11	1.27	-0.08	4.01.E-01
<b>mir-125b-1</b>	cg02101355	142	0.02	-1.51	-0.23	<b>1.35.E-02</b>
11q24.1	cg03891346	173	0.004	-1.51	-0.27	<b>3.90.E-03</b>
121,970,465-121,970,552	cg24150623	540	0.11	-1.51	-0.3	<b>1.40.E-03</b>
	cg06749053	667	0.08	-1.51	-0.29	<b>1.60.E-03</b>
	cg20475322	680	0.17	-1.51	-0.29	<b>1.80.E-03</b>
	cg24603444	782	0.09	-1.51	-0.27	<b>3.90.E-03</b>
	cg16865908	0	0.04	-1.51	-0.18	6.20.E-02
	cg26916936	61	0.06	-1.51	-0.1	3.04.E-01
	cg07685357	1402	0.07	-1.51	-0.29	<b>2.10.E-03</b>
<b>mir-182</b>	cg16576544	0	-0.09	2.31	-0.18	5.39.E-02
7q32.2	cg24423782	85	-0.09	2.31	-0.15	1.19.E-01
129,410,223-129,410,332	cg17677032	95	-0.04	2.31	-0.01	8.82.E-01
	cg04579608	103	-0.07	2.31	-0.11	2.66.E-01
	cg13713066	325	-0.08	2.31	-0.16	9.76.E-02
<b>mir-199a-1</b>	cg27648270	0	0.07	-2.58	-0.61	<b>1.00.E-04</b>

19p13.2	cg18544365	6	0.06	-2.58	-0.61	<b>1.00.E-04</b>
10,928,102-10,928,172	cg23047544	39	0.08	-2.58	-0.61	<b>1.00.E-04</b>
	cg02660440	61	0.08	-2.58	-0.63	<b>1.00.E-04</b>
	cg06754197	150	0.04	-2.58	-0.55	<b>1.00.E-04</b>
	cg02907064	155	0.07	-2.58	-0.59	<b>1.00.E-04</b>
	cg23068797	377	0.09	-2.58	-0.61	<b>1.00.E-04</b>
	cg03216043	467	0.1	-2.58	-0.6	<b>1.00.E-04</b>
	cg13965612	524	0.07	-2.58	-0.54	<b>1.00.E-04</b>
<b>mir-1180</b>	cg04864152	15	-0.01	1.57	-0.15	1.06.E-01
	cg20272287	18	-0.02	1.57	-0.15	1.10.E-01
	cg02796621	678	-0.05	1.57	0.23	<b>1.54.E-02</b>
	cg02206323	1181	-0.05	1.57	0.23	<b>1.64.E-02</b>
	cg26619894	1277	-0.06	1.57	0.28	<b>2.90.E-03</b>