cancers

MDPI

Supplementary materials

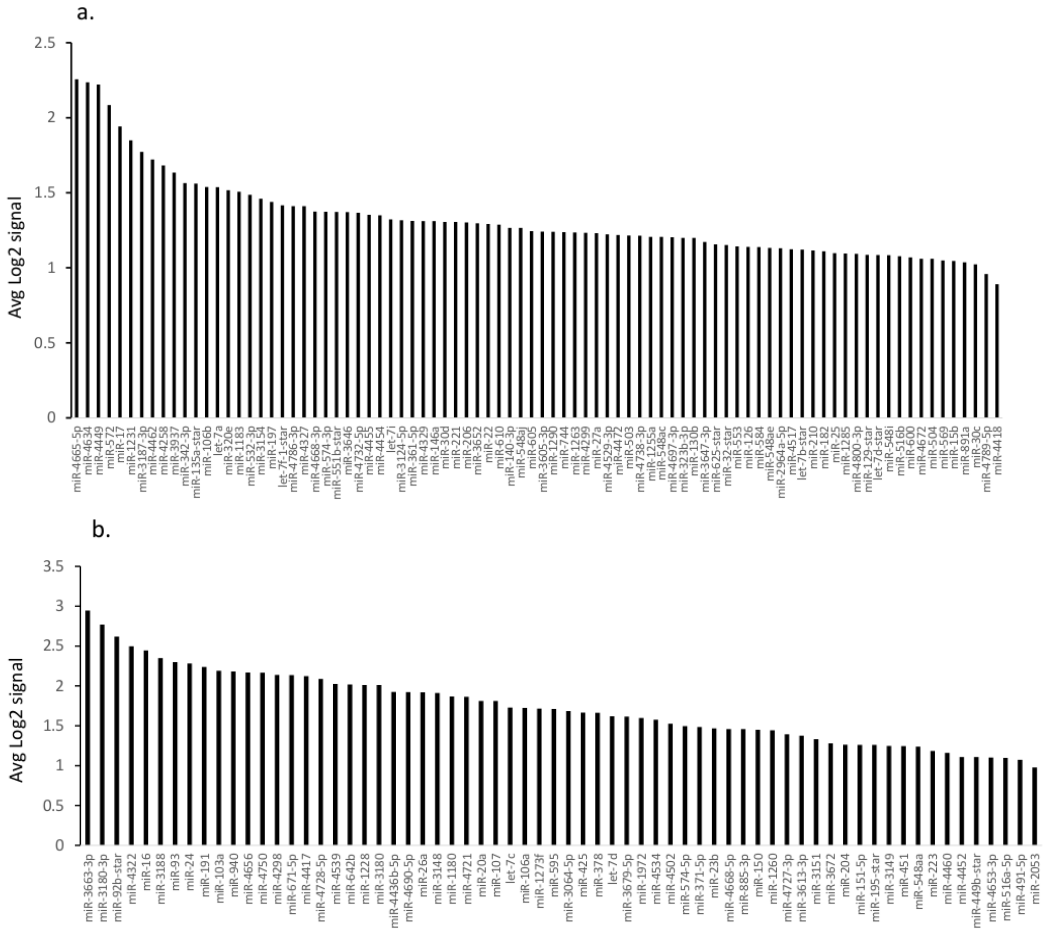

Figure S1. Circulating miRNA expression in the 2nd and 3rd quantile. According to the Absent/Present calling of the Affymetrix algorithm, we divided the circulatory miRNome in five quantiles based on their presence in the analyzed population. We considered the miRNAs in the first quantile as not expressed miRNAs, since they were detectable in less than five subjects. We found 1469 out of the 1734 mature miRNA not expressed in serum, 86 were expressed in the 2nd quantile of the population (considered as rare miRNAs) (**a**), 66 in the 3rd quantile (**b**). MiRNAs are ranked according to their average expression (log2) determined by miRNA microarray.

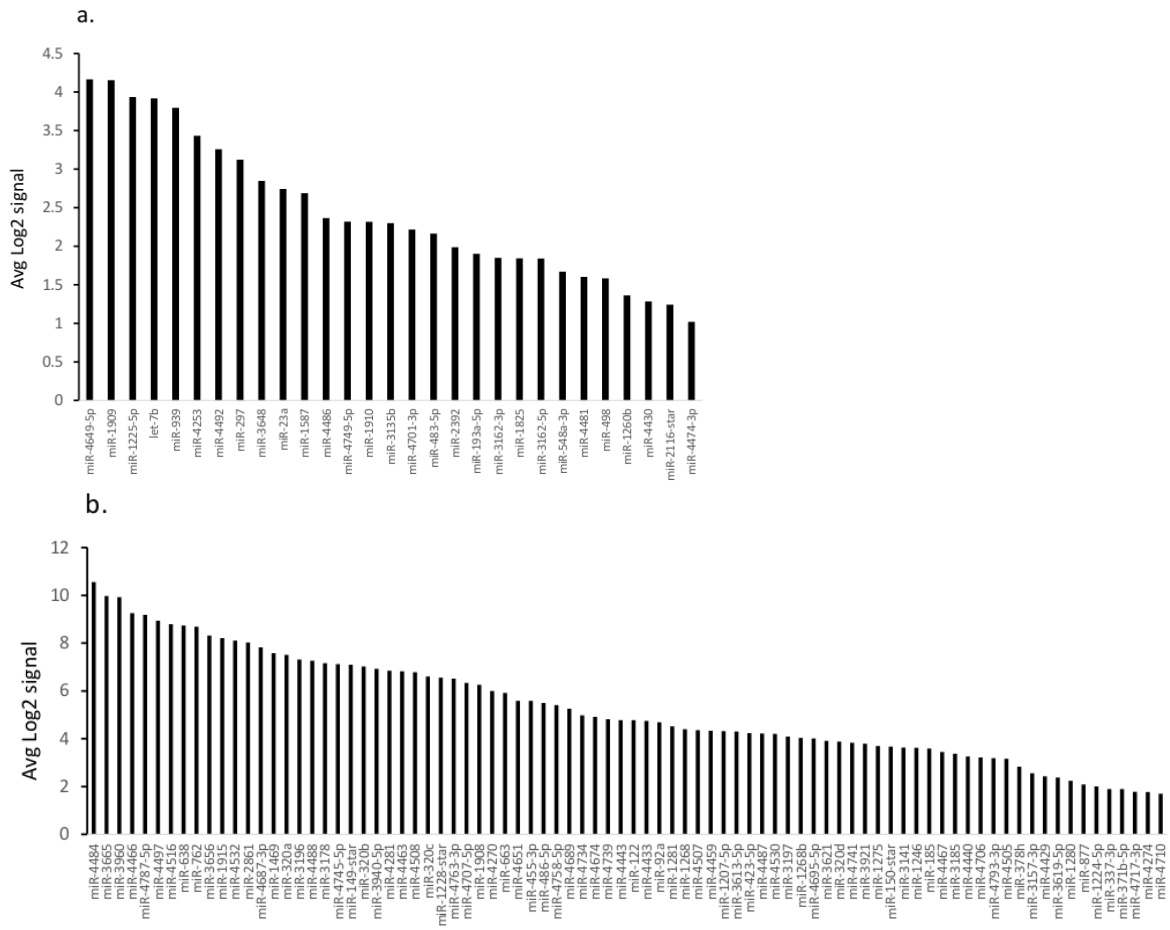

Figure S2. Circulating miRNA expression in the 4th and 5th quantile. According to the Absent/Present calling of the Affymetrix algorithm, we divided the circulatory miRNome in five quantiles based on their presence in the analyzed population. We considered the miRNAs in the first quantile as not expressed miRNAs, since they were detectable in less than five subjects. We found 1469 out of the 1734 mature miRNA not expressed in serum, 86 were expressed in the 4th quantile of the population (**a**), 66 in the 5th quantile. The miRNAs in the 5Th quantile have the highest expression in the serum samples (**b**). MiRNAs are ranked according to their average expression (log2) determined by miRNA microarray.

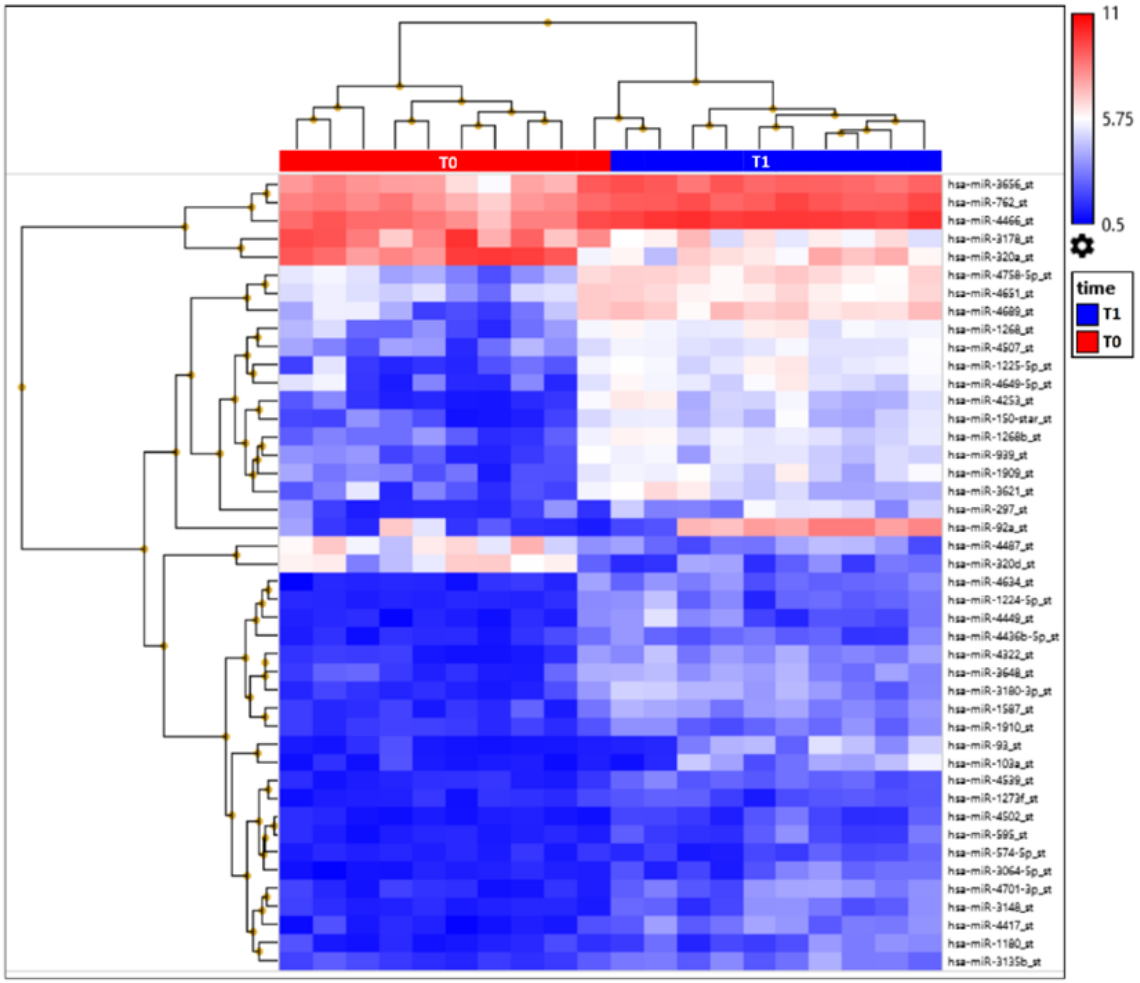

Figure S3. Heatmap with the pseudocolor scale underneath, of the differentially expressed miRNAs between T0 (red) and T1 (blue) in all samples. Unsupervised hierarchical clustering is used to order samples and miRNAs, the log2-transformed microarray signal was considered. The sample tree with optimized leaf-ordering is drawn using Euclidean distances and average linkages for cluster-to-cluster distance.

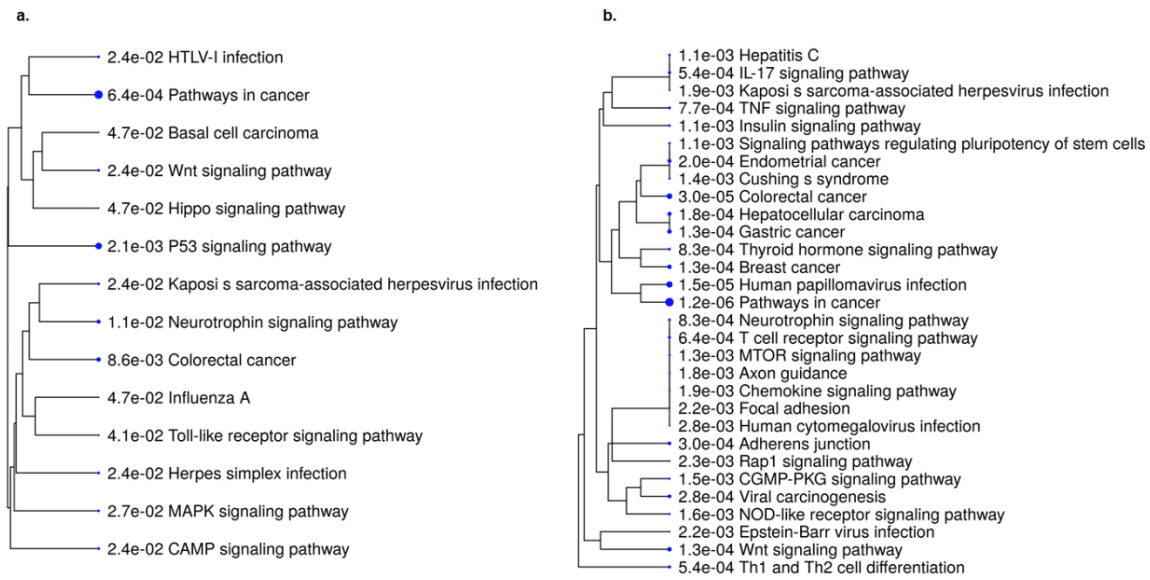

Figure 4. Gene enrichment analysis. (**a**) Dendrogram of the miRNA targets enriched KEGG pathways. (**b**) KEGG enriched pathways for the strongly validated miRNA targets. The area of the blue circles is proportional to the statistical significance of the enrichment.

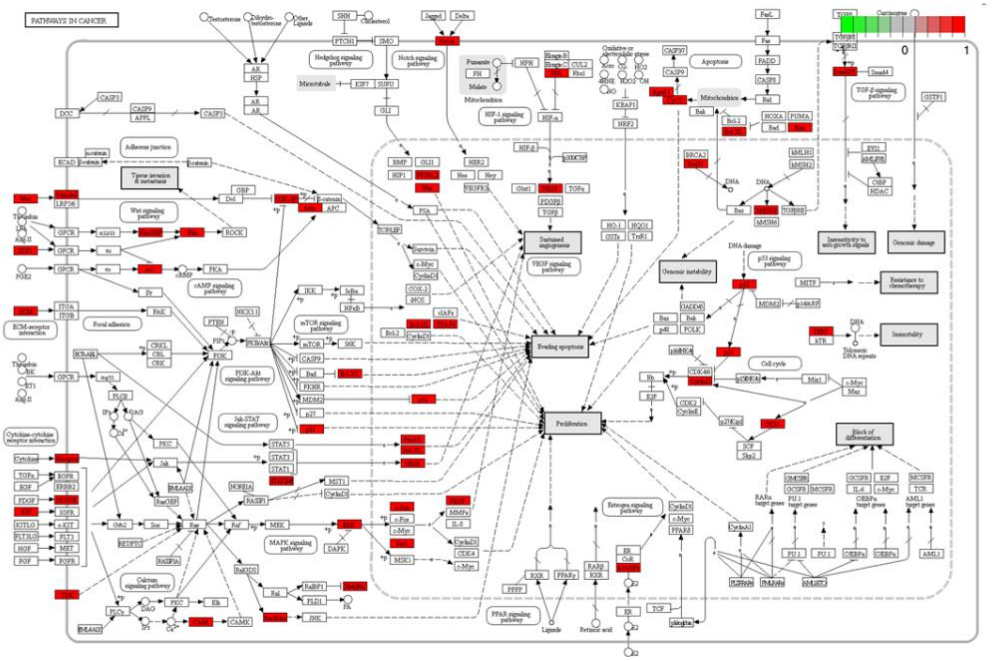

Figure S5. Genes enriched in the "pathways in Cancer" term from KEGG database. Enrichment was based on the miRNA targets downloaded in MirTarBase 7.0 considering both miRNA targets with both "strong" and "less strong" experimental evidences. Enrichment analysis was performed by using the web platform ShinyGo (http://bioinformatics.sdstate.edu/go/).

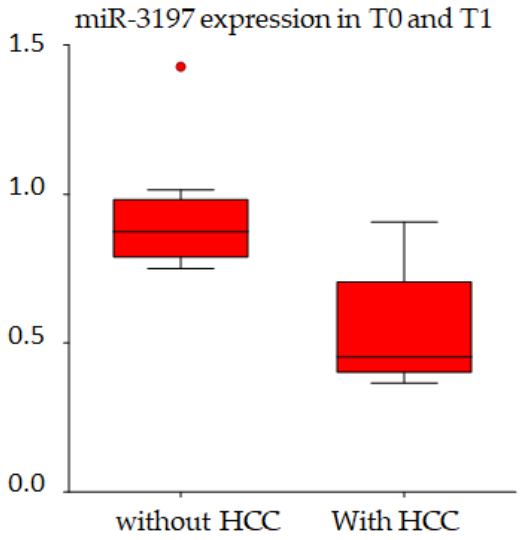

Figure S6. Mir-3197 expression in the discovery cohort at T0 and T1.

Table 1. Circulating miRNA differential expression between T0 and T1 in all samples.

ID	Avg. Signal (log2)		Standard deviation		FC	*p*-value	FDR *p*-value	Accession
	T0	T1	T0	T1				
miR-92a	1.54	7.79	1.98	2.46	-76.08	0.0005	0.0401	MIMAT0000092
miR-1225-5p	1.86	5.50	1.55	0.44	-12.49	1.33E-06	0.0004	MIMAT0005572
miR-4253	1.41	4.87	1.46	0.83	-11.05	1.19E-05	0.0022	MIMAT0016882
miR-297	1.46	4.52	0.93	1.06	-8.32	2.80E-07	0.0001	MIMAT0004450
miR-4689	3.88	6.74	1.74	0.45	-7.25	1.07E-05	0.0021	MIMAT0019778
miR-150-star	1.95	4.75	1.26	0.66	-6.98	1.33E-06	0.0004	MIMAT0004610
miR-4649-5p	2.59	5.39	1.82	0.53	-6.97	4.30E-05	0.0061	MIMAT0019711
miR-93	0.97	3.66	0.41	1.40	-6.46	0.0003	0.0310	MIMAT0000093
miR-1268b	2.61	5.25	1.16	0.46	-6.24	1.99E-06	0.0005	MIMAT0018925
miR-103a	1.01	3.64	0.44	1.52	-6.15	0.0007	0.0486	MIMAT0000101
miR-939	2.49	5.09	1.39	0.58	-6.05	1.68E-05	0.0030	MIMAT0004982
miR-3180-3p	1.33	3.92	0.78	0.80	-6.00	5.59E-07	0.0003	MIMAT0015058
miR-1909	2.84	5.26	1.14	0.67	-5.38	1.22E-06	0.0004	MIMAT0007883
miR-3621	2.41	4.80	1.48	0.96	-5.22	0.0003	0.0316	MIMAT0018002
miR-1268	3.23	5.55	1.34	0.37	-4.99	1.69E-06	0.0005	MIMAT0005922
miR-4758-5p	4.33	6.62	1.30	0.38	-4.91	3.36E-05	0.0050	MIMAT0019903
miR-1587	1.49	3.57	0.62	0.48	-4.22	1.16E-07	8.55E-05	MIMAT0019077
miR-4417	1.02	3.07	0.56	0.61	-4.15	2.15E-07	0.0001	MIMAT0018929
miR-4322	1.37	3.38	0.85	0.53	-4.02	1.21E-07	8.55E-05	MIMAT0016873
miR-3648	1.79	3.74	0.96	0.52	-3.86	1.11E-05	0.0022	MIMAT0018068
miR-4701-3p	1.35	3.22	0.39	0.70	-3.66	2.69E-09	3.79E-06	MIMAT0019799
miR-4507	3.57	5.26	1.05	0.24	-3.22	4.99E-06	0.0011	MIMAT0019044
miR-4634	1.31	2.80	0.89	0.50	-2.80	2.53E-05	0.0040	MIMAT0019691
miR-1224-5p	1.28	2.75	0.67	0.84	-2.78	0.0003	0.0295	MIMAT0005458
miR-4449	1.21	2.64	0.76	1.15	-2.69	0.0007	0.0486	MIMAT0018968
miR-3148	1.24	2.65	0.24	0.72	-2.67	1.81E-07	0.0001	MIMAT0015021
miR-3064-5p	1.07	2.41	0.25	0.83	-2.54	1.34E-06	0.0004	MIMAT0019864
miR-1180	1.23	2.51	0.47	0.85	-2.41	8.93E-06	0.0019	MIMAT0005825
miR-3656	7.76	8.98	0.97	0.28	-2.33	0.0003	0.0301	MIMAT0018076
miR-4539	1.31	2.51	0.26	0.35	-2.30	8.06E-10	1.52E-06	MIMAT0019082
miR-1910	1.74	2.94	0.29	0.55	-2.30	4.23E-09	4.77E-06	MIMAT0007884
miR-4436b-5p	1.39	2.57	0.59	0.67	-2.28	0.0005	0.0394	MIMAT0019940
miR-4466	8.66	9.84	0.68	0.17	-2.26	2.11E-05	0.0034	MIMAT0018993
miR-3135b	1.74	2.90	0.38	0.53	-2.24	1.31E-06	0.0004	MIMAT0018985
miR-4651	5.06	6.17	1.10	0.37	-2.16	0.0005	0.0416	MIMAT0019715
miR-1273f	1.29	2.28	0.38	0.43	-1.98	5.16E-05	0.0071	MIMAT0020601
miR-762	8.19	9.16	0.62	0.23	-1.96	6.35E-05	0.0085	MIMAT0010313
miR-4502	1.01	1.89	0.22	0.56	-1.84	4.21E-06	0.0011	MIMAT0019038
miR-595	1.17	1.93	0.19	0.68	-1.69	2.07E-05	0.0034	MIMAT0003263
miR-574-5p	1.23	1.87	0.23	0.56	-1.55	0.0005	0.0392	MIMAT0004795
miR-4487	5.70	3.29	1.18	0.88	5.31	2.12E-05	0.0034	MIMAT0019021
miR-3178	8.38	5.87	1.07	0.70	5.69	1.37E-06	0.0004	MIMAT0015055
miR-320a	8.97	6.42	1.33	0.95	5.85	0.0002	0.0239	MIMAT0000510
miR-320d	5.86	2.56	1.50	1.01	9.87	7.64E-05	0.0094	MIMAT0006764

Table S2. Circulating miRNA differential expression between T0 and T1 in samples with HCC.

ID	Avg. Signal (log2)		Standard deviation		FC	*p*-value	FDR *p*-value	Accession
	T0	T1	T0	T1				
miR-297	1.48	4.55	1.00	0.96	-8.36	8.96E-05	0.0297	MIMAT0004450
miR-1268b	2.41	5.43	1.49	0.51	-8.11	0.0002	0.0504	MIMAT0018925
miR-3180-3p	1.24	3.77	1.06	1.05	-5.77	0.0002	0.0585	MIMAT0015058
miR-4417	0.91	3.00	0.63	0.67	-4.26	4.26E-05	0.0185	MIMAT0018929
miR-4701-3p	1.14	3.16	0.43	0.56	-4.04	4.90E-07	0.0009	MIMAT0019799
miR-1180	1.32	3.33	0.53	0.79	-4.02	1.31E-05	0.0067	MIMAT0005825
miR-4322	1.39	3.34	1.15	0.65	-3.86	9.83E-05	0.0308	MIMAT0016873
miR-1910	1.76	3.46	0.22	0.50	-3.25	2.41E-06	0.0023	MIMAT0007884
miR-3148	1.09	2.71	0.34	0.42	-3.06	8.15E-06	0.0051	MIMAT0015021
miR-3064-5p	1.06	2.66	0.22	0.92	-3.05	5.32E-05	0.0214	MIMAT0019864
miR-4767	1.10	2.38	0.40	0.53	-2.44	6.66E-06	0.0047	MIMAT0019919
miR-3135b	1.81	3.02	0.44	0.58	-2.32	3.00E-05	0.0141	MIMAT0018985
miR-1273f	1.19	2.29	0.52	0.12	-2.13	0.0002	0.0467	MIMAT0020601
miR-4539	1.37	2.43	0.24	0.47	-2.07	1.41E-06	0.0020	MIMAT0019082
miR-483-5p	3.48	1.70	1.08	0.29	3.44	4.01E-06	0.0032	MIMAT0004761
miR-3178	9.00	6.02	0.73	0.53	7.91	9.38E-06	0.0053	MIMAT0015055

Table 3. Circulating miRNA differential expression between T0 and T1 in samples without HCC.

ID	Avg. Signal (log2)		Standard deviation		FC	*p*-value	FDR *p*-value	Accession
	T0	T1	T0	T1				
miR-1225-5p	1.85	5.47	1.57	0.59	-12.29	0.0001	0.0440	MIMAT0005572
miR-297	1.37	4.66	0.97	1.26	-9.75	4.10E-05	0.0180	MIMAT0004450
miR-1909	2.30	5.13	0.83	0.93	-7.08	1.09E-05	0.0073	MIMAT0007883
miR-3180-3p	1.55	4.24	0.47	0.58	-6.46	4.15E-05	0.0180	MIMAT0015058
miR-1268	3.34	5.69	1.38	0.43	-5.08	6.93E-05	0.0261	MIMAT0005922
miR-1587	1.28	3.61	0.36	0.41	-5.05	2.12E-06	0.0030	MIMAT0019077
miR-150-star	1.96	4.20	0.66	0.73	-4.71	0.0001	0.0440	MIMAT0004610
miR-4701-3p	1.49	3.59	0.38	0.89	-4.29	6.86E-06	0.0064	MIMAT0019799
miR-4417	1.13	3.15	0.52	0.61	-4.05	5.85E-05	0.0235	MIMAT0018929
miR-4322	1.41	3.40	0.43	0.46	-3.97	1.09E-05	0.0073	MIMAT0016873
miR-1910	1.37	2.71	0.27	0.60	-2.53	2.79E-06	0.0031	MIMAT0007884
miR-4539	1.26	2.56	0.30	0.25	-2.46	3.53E-07	0.0009	MIMAT0019082
miR-1184	0.92	1.82	0.07	0.39	-1.86	1.72E-05	0.0088	MIMAT0005829

Table S4. Experimentally validated miRNA targets with "strong evidence".

miRNA	Gene targets	Description
hsa-miR-3178	TRAF3	Member of the TNF receptor associated factor (TRAF) protein family. TRAF proteins associate with, and mediate the signal transduction from, members of the TNF receptor (TNFR) superfamily.
hsa-miR-1228-3p	MOAP1	Modulator of Apoptosis 1. The protein encoded by this gene was identified by its interaction with apoptosis regulator BAX protein. This protein contains a Bcl-2 homology 3 (BH3)-like motif, which is required for the association with BAX. When overexpressed, this gene has been shown to mediate caspase-dependent apoptosis.
hsa-miR-1180-3p	CSNK2A2	Casein Kinase 2 Alpha 2. Casein kinase 2 is a serine/threonine protein kinase that phosphorylates acidic proteins such as casein. It is involved in various cellular processes, including cell cycle control, apoptosis.
	TCEAL1	Transcription Elongation Factor A Like 1. Members of this family may function as nuclear phosphoproteins that modulate transcription in a promoter context-dependent manner It may exert its effects via protein-protein interactions with other transcriptional regulators rather than via direct binding of DNA.
	TNIP2	TNFAIP3 Interacting Protein 2. Acts as an inhibitor of NFkappaB activation. The encoded protein is also involved in MAP/ERK signaling pathway in specific cell types
hsa-miR-1246	DYRK1A	Dual Specificity Tyrosine Phosphorylation Regulated Kinase 1A. This gene encodes a member of the Dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) family. It may play a significant role in a signaling pathway regulating cell proliferation.
	GSK3B	Glycogen Synthase Kinase 3 Beta. It is a negative regulator of glucose homeostasis and is involved in energy metabolism, inflammation, ER-stress, mitochondrial dysfunction, and apoptotic pathways.
	AXIN2	Presumably plays an important role in the regulation of the stability of beta-catenin in the Wnt signaling pathway. Mutations in this gene have been associated with colorectal cancer with defective mismatch repair.
	PRKAR1A	Protein Kinase CAMP-Dependent Type I Regulatory Subunit Alpha. This protein was found to be a tissue-specific extinguisher that down-regulates the expression of seven liver genes in hepatoma x fibroblast hybrids. A nonconventional nuclear localization sequence (NLS) has been found for this protein which suggests a role in DNA replication via the protein serving as a nuclear transport protein for the second subunit of the Replication Factor C.
	NFIB	Nuclear Factor I B. Diseases associated with NFIB include Breast Adenoid Cystic Carcinoma and Lacrimal System Cancer. Among its related pathways are RNA Polymerase III Transcription Initiation and FOXA1 transcription factor network.
hsa-miR-483-5p	SRF	Serum Response Factor. This gene encodes a ubiquitous nuclear protein that stimulates both cell proliferation and differentiation. It is a member of the MADS (MCM1, Agamous, Deficiens, and SRF) box superfamily of transcription factors. This protein binds to the serum response element (SRE) in the promoter region of target genes. This protein regulates the activity of many immediate-early genes, for example c-fos, and thereby participates in cell cycle regulation, apoptosis, cell growth, and cell differentiation. This gene is the downstream target of many pathways; for example, the mitogen-activated protein kinase pathway (MAPK) that acts through the ternary complex factors (TCFs).
	ALCAM	Activated Leukocyte Cell Adhesion Molecule. This gene encodes activated leukocyte cell adhesion molecule (ALCAM), also known as CD166 (cluster

		of differentiation 166), which is a member of a subfamily of immunoglobulin receptors with five immunoglobulin-like domains (VVC2C2C2) in the extracellular domain. This protein binds to T-cell differentiation antigene CD6, and is implicated in the processes of cell adhesion and migration
	FAM160B2	Family with Sequence Similarity 160 Member B2.
	MAPK3	Mitogen-Activated Protein Kinase 3. MAP kinases, also known as extracellular signal-regulated kinases (ERKs), act in a signaling cascade that regulates various cellular processes such as proliferation, differentiation, and cell cycle progression in response to a variety of extracellular signals. This kinase is activated by upstream kinases, resulting in its translocation to the nucleus where it phosphorylates nuclear targets.
	CKB	Creatine Kinase B. The protein encoded by this gene is a cytoplasmic enzyme involved in energy homeostasis. The encoded protein reversibly catalyzes the transfer of phosphate between ATP and various phosphogens such as creatine phosphate. It acts as a homodimer in brain as well as in other tissues, and as a heterodimer with a similar muscle isozyme in heart.
	RHOA	Ras Homolog Family Member A. Rho proteins promote reorganization of the actin cytoskeleton and regulate cell shape, attachment, and motility. Overexpression of this gene is associated with tumor cell proliferation and metastasis
	NOTCH3	Neurogenic Locus Notch Homolog Protein 3. In *Drosophila*, notch interaction with its cell-bound ligands (delta, serrate) establishes an intercellular signalling pathway that plays a key role in neural development. Homologues of the notch-ligands have also been identified in human, but precise interactions between these ligands and the human notch homologues remains to be determined.
	TERT	Telomerase Reverse Transcriptase. Telomerase expression plays a role in cellular senescence, as it is normally repressed in postnatal somatic cells resulting in progressive shortening of telomeres. Deregulation of telomerase expression in somatic cells may be involved in oncogenesis.
	CSF1	Colony Stimulating Factor 1. the protein encoded by this gene is a cytokine that controls the production, differentiation, and function of macrophages.
	STOML2	Stomatin Like 2. The function remains largely unknown. Previous studies have suggested that SLP-2 may serve a role in stabilizing the mitochondrial inner membrane, regulating ion channel conductance and the organization of sphingolipid and cholesterol-rich lipid rafts. Studies revealed that SLP-2 is overexpressed in numerous types of cancer tissues and is involved in the progression and development of cancer.
hsa-miR-1207-5p	STAT6	Signal Transducer and Activator of Transcription 6. In response to cytokines and growth factors, STAT family members are phosphorylated by the receptor associated kinases, and then form homo- or heterodimers that translocate to the cell nucleus where they act as transcription activators. This protein plays a central role in exerting IL4 mediated biological responses. It is found to induce the expression of BCL2L1/BCL-X(L), which is responsible for the anti-apoptotic activity of IL4.
	CD151	CD151 Molecule (Raph Blood Group). The protein encoded by this gene is a member of the transmembrane 4 superfamily, also known as the tetraspanin family. The proteins mediate signal transduction events that play a role in the regulation of cell development, activation, growth and motility. This encoded protein is a cell surface glycoprotein that is known to complex with integrins and other transmembrane 4 superfamily proteins. It is involved in cellular processes including cell adhesion and may regulate integrin trafficking and/or function. This protein enhances cell motility, invasion and metastasis of cancer cells.