Supplementary Materials

A Distinctive microRNA (miRNA) Signature in the Blood of Colorectal Cancer (CRC) Patients at Surgery

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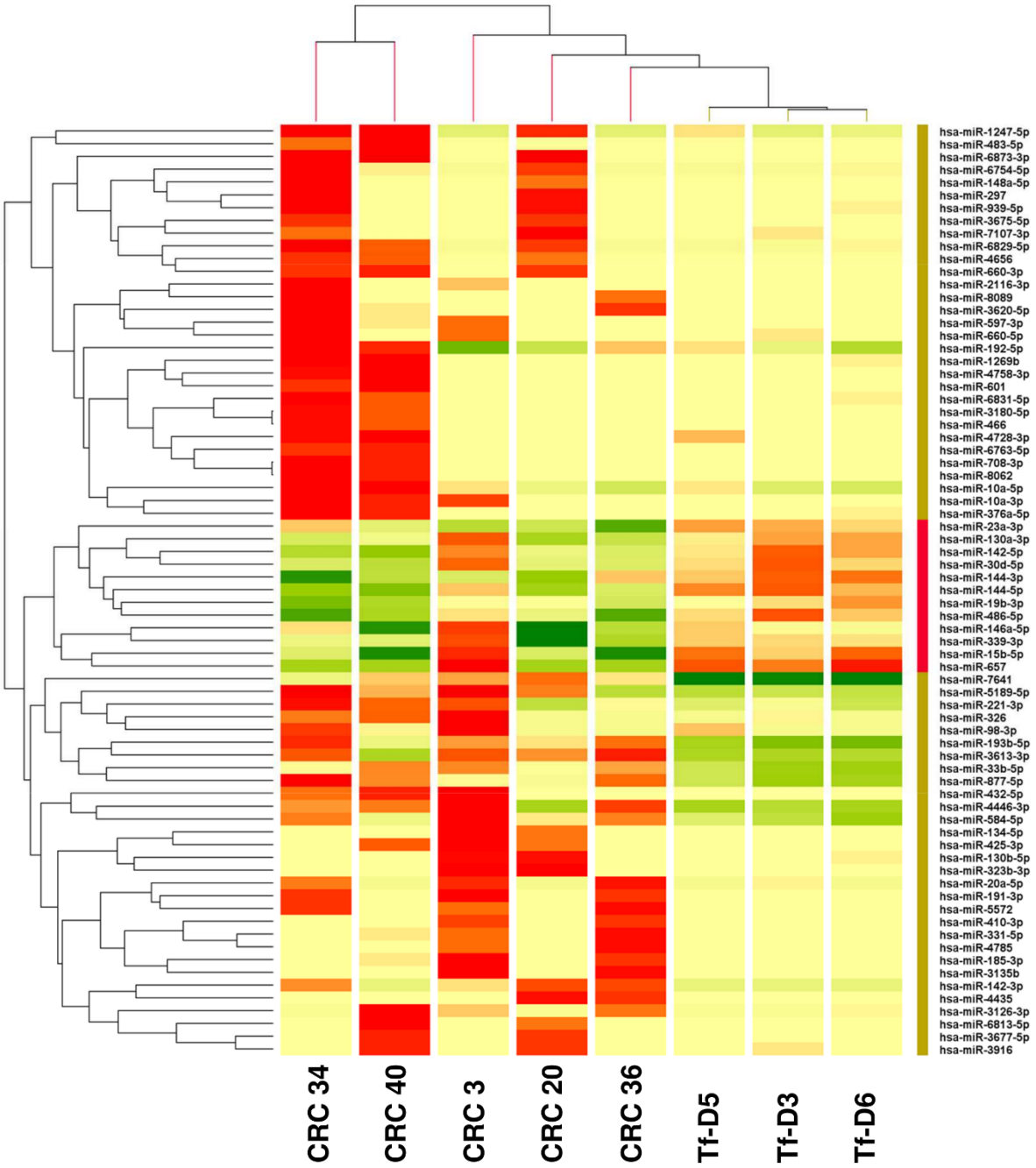

Figure S1. Heatmap of CRC samples versus tumor-free plasmas (FC > 1.5).

	miR-144-3p (copies/μL)	miR-486-5p (copies/μL)	miR-144-5p (copies/μL)	miR-15b-5p (copies/μL)	miR-1247-5p (copies/μL)	miR-584-5p (copies/μL)	miR-483-5p (copies/μL)	miR-10a-5p (copies/μL)	miR-425-3p (copies/μL)	dysregulated miRNA
Tf-D Average ± SD	2.14 ± 0.85	494.70 ± 404.29	1.13 ± 0.42	4.63 ± 2.20	1.97 ± 0.81	0.98 ± 0.27	4.23 ± 1.06	0.54 ± 0.23	0.77 ± 0.58	
CRC 1	0.50	106.40	0.69	2.30	1.40	0.62	3.70	0.30 *	0.28	5 (+1) *
CRC 2	3.10	548.00	3.30	8.50	1.70	2.10	5.90	0.63	1.54	6
CRC 3 #	1.15	430.00	0.55	42.40	1.70	11.00	5.30	1.30	22.30	7
CRC 4	2.70	442.00	3.00	33.40	1.18	5.70	4.60	1.02	8.20	5
CRC 5	0.60	267.00	1.09	7.20	0.40	1.80	4.00	0.23	1.80	6
CRC 6	8.50	538.00	4.70	11.70	2.10	2.00	7.30	0.51	1.90	6
CRC 7	0.60	249.00	1.50	3.30	4.10	2.10	5.00	0.77 *	1.10	4 (+1) *
CRC 8	0.67	239.00	1.20	5.30	2.50	2.00	2.30	0.50	0.54	3
CRC 9	0.61	130.00	1.60	2.40	2.30	1.70	2.90	0.16	0.68	6
CRC 10	0.45	108.70	3.00	2.80	0.98	1.60	2.00	0.08	0.33	6
CRC 11	0.86	143.90	0.95	3.50	1.14	1.32	4.20	0.51	0.72	3
CRC 12	3.8	745.00	6.40	9.70	1.60	2.00	5.90	0.44	0.59	5
CRC 13	0.07	60.00	0.70	5.80	3.30	1.70	4.10	0.22	1.15	6
CRC 14	0.76	90.70	0.46	2.10	0.85	1.41	5.30	0.14	0.19	7
CRC 15	0.54	137.80	0.82	2.10	1.30	1.60	6.30	0.37	0.51	4
CRC 16	0.42	176.00	0.42	3.30	1.80	1.25	3.10	0.52	0.19	3
CRC 17	0.43	146.00	0.72	4.70	1.70	2.00	2.80	0.47	0.87	3
CRC 18	4.40	955.00	2.10	7.00	0.58	2.20	3.20	0.35	0.95	6
CRC 19	0.21	173.00	0.43	4.50	3.80	1.60	2.90	0.66	0.91	5
CRC 20 #	3.40	330.00	3.30	11.10	1.80	1.90	4.80	1.09	2.70	6
CRC 21	1.21	307.00	0.92	7.80	1.70	1.90	4.10	0.57	1.90	4
CRC 22	1.27	177.00	2.90	10.60	2.40	2.90	7.00	0.13	1.50	7
CRC 23	0.82	158.00	1.01	6.50	0.81	0.63	1.70	0.38	1.39	5
CRC 24	1.00	186.00	2.6	7.00	1.50	0.58	5.30	0.47	0.48	5
CRC 26	0.41	152.00	0.63	2.40	0.99	0.48	4.10	0.54	0.38	5
CRC 27	2.40	1978.00	2.70	10.20	1.70	2.30	2.10	0.26	1.90	7
CRC 28	1.90	456.00	2.60	5.60	0.68	0.46	2.00	0.38	0.56	4
CRC 29	1.60	402.00	0.84	4.40	0.62	0.91	3.40	0.13	0.61	2
CRC 30	1.70	271.00	0.96	3.00	1.26	0.94	3.60	0.38	0.47	0
CRC 31	0.52	272.00	0.90	1.50	1.15	0.78	2.30	0.36	0.53	4
CRC 33	0.24	87.50	0.82	5.40	2.80	1.70	4.80	0.70	1.90	5
CRC 34 #	1.00	201.00	0.45	5.80	10.00	6.10	8.80	1.90	2.70	7
CRC 36 #	1.10	71.80	0.24	8.60	1.10	1.20	2.00	0.27 *	1.55	8 (+1) *
CRC 40 #	0.81	76.70	0.50	2.80	7.20	2.50	36.60	0.33	0.79	6
CRC 46	2.10	376.00	0.89	4.70	4.70	1.80	3.40	0.82	1.70	4
CRC Average ± SD	1.48 ± 1.63	319.64 ± 350.31	1.60 ± 1.39	7.41 ± 8.20	2.14 ± 1.93	2.08 ± 1.95	5.05 ± 5.75	0.51 ± 0.37	1.88 ± 3.81	

Figure S2. MicroRNA content in CRC plasma samples compared with the average values found in tumor-free controls. Plasma miRNAs levels (copies/μL) in CRC (from CRC1 to CRC46) plasma samples, detected by dd-RT-PCR. Dys-regulation of the miRNAs was boxed when an increase/decrease equal or higher of the Tf-D average ± SD was detected. In green boxes the down-regulated miRNA values (values < average of Tf-D ± SD); in red boxes the up-regulated miRNA values (values > average of Tf-D ± SD). Asterisk indicates newly detected samples with the respect to Table 1 proposed in main text. The number of dys-regulated miRNAs is indicated for each CRC sample in the last right column. CRC and Tf-D plasma samples analyzed both by NGS and dd-RT-PCR were marked with hashtag (#).

Table S1. Key examples of up-regulated miRNAs extracted from the 9-miR signature and compared with up-regulation of miR-141-3p, miR-221-3p and miR-222-3p.

	miR-15b-5p (copies/µL)	miR-584-5p (copies/µL)	miR-425-3p (copies/µL)	dysregulated miRNA	miR-141-3p (copies/µL)	miR-221-3p (copies/µL)	miR-222-3p (copies/µL)	dysregulated miRNA	total dysregulated miRNA
Tf-D Average ± SD	**4.63 ± 2.20**	**0.98 ± 0.27**	**0.77 ± 0.58**		**9.3 ± 0.71**	**8.1 ± 1.85**	**60.2 ± 4.86**		
CRC 1	2.30	0.62	0.28	**0**	5.90	**10.30**	**66.10**	**2**	**2**
CRC 2	**8.50**	**2.10**	**1.54**	**3**	6.60	8.90	63.10	**0**	**3**
CRC 3 #	**42.40**	**11.00**	**22.30**	**3**	8.80	**122.00**	**106.00**	**2**	**5**
CRC 4	**33.40**	**5.70**	**8.20**	**3**	8.20	**52.00**	**95.00**	**2**	**5**
CRC 5	**7.20**	**1.80**	**1.80**	**3**	7.70	**12.20**	63.10	**1**	**4**
CRC 6	**11.70**	**2.00**	**1.90**	**3**	7.10	9.20	64.30	**0**	**3**
CRC 7	3.30	**2.10**	1.10	**1**	7.40	6.50	64.70	**0**	**1**
CRC 8	5.30	**2.00**	0.54	**1**	5.80	**10.70**	**65.10**	**2**	**3**
CRC 9	2.40	**1.70**	0.68	**1**	7.10	5.20	61.50	**0**	**1**
CRC 10	2.80	**1.60**	0.33	**1**	**18.40**	6.90	60.20	**1**	**2**
CRC 11	3.50	**1.32**	0.72	**1**	7.90	**13.20**	56.50	**1**	**2**
CRC 12	**9.70**	**2.00**	0.59	**2**	7.30	9.30	59.00	**0**	**2**
CRC 13	5.80	**1.70**	1.15	**1**	9.60	**12.20**	**66.80**	**2**	**3**
CRC 14	2.10	**1.41**	0.19	**1**	7.40	5.80	60.90	**0**	**1**
CRC 15	2.10	**1.60**	0.51	**1**	7.30	8.20	64.30	**0**	**1**
CRC 16	3.30	1.25	0.19	**0**	9.80	6.10	52.10	**0**	**0**
CRC 17	4.70	**2.00**	0.87	**1**	**14.30**	8.60	59.30	**1**	**2**
CRC 18	**7.00**	**2.20**	0.95	**2**	9.70	9.00	62.50	**0**	**2**
CRC 19	4.50	**1.60**	0.91	**1**	9.30	**19.80**	**67.80**	**2**	**3**
CRC 20 #	**11.10**	**1.90**	**2.70**	**3**	9.40	**12.50**	61.50	**1**	**4**
CRC 21	**7.80**	**1.90**	**1.90**	**3**	7.40	**11.00**	47.80	**1**	**4**
CRC 22	**10.60**	**2.90**	**1.50**	**3**	9.90	**13.20**	63.20	**1**	**4**
CRC 23	6.50	0.63	**1.39**	**1**	**10.10**	**12.80**	57.20	**2**	**3**
CRC 24	**7.00**	0.58	0.48	**1**	**12.30**	6.80	22.30	**1**	**2**
CRC 26	2.40	0.48	0.38	**0**	**12.20**	5.00	58.70	**1**	**1**
CRC 27	**10.20**	**2.30**	**1.90**	**3**	**10.30**	**10.10**	**71.60**	**3**	**6**
CRC 28	5.60	0.46	0.56	**0**	9.70	7.20	43.20	**0**	**0**
CRC 29	4.40	0.91	0.61	**0**	**11.70**	**11.80**	31.40	**2**	**2**
CRC 30	3.00	0.94	0.47	**0**	7.80	6.40	**66.20**	**1**	**1**
CRC 31	1.50	0.78	0.53	**0**	9.00	8.70	**65.40**	**1**	**1**
CRC 33	5.40	**1.70**	**1.90**	**2**	**11.70**	**18.60**	55.60	**2**	**4**
CRC 34 #	5.80	**6.10**	**2.70**	**2**	**20.00**	4.30	**321.00**	**2**	**4**
CRC 36 #	**8.60**	1.20	**1.55**	**2**	**12.40**	6.10	**277.00**	**2**	**4**
CRC 40 #	2.80	**2.50**	0.79	**1**	**19.90**	1.90	**270.00**	**2**	**3**
CRC 46	4.70	**1.80**	**1.70**	**2**	**18.10**	3.40	**323.00**	**2**	**4**
CRC Average ± SD	**7.41 ± 8.20**	**2.08 ± 1.95**	**1.88 ± 3.81**		**10.21 ± 3.80**	**13.60 ± 20.56**	**88.38 ± 78.00**		

Table S2. Clinical data of recruited CRC patients. NA = not available; ADC = Adenocarcinoma; # mutation detected in plasma samples; * mutations detected in tumor tissue. Tumor localization, tumor size and TNM classification are reported in the table: pT = size of the primary tumor and grade of nearby tissue invasion; pN = grade of regional lymph nodes involvement; M = presence of distant metastasis.

Patient ID	Sex	Age (years)	Tumor localization	Diagnosis	pT	pN	M	Grading	Tumor volume (diameter)	KRAS#	KRAS*	NRAS*	BRAF*
CRC 1	M	63	Colon	Mucinous ADC of colon	3	2	1	NA	3.5 cm	WT	WT	WT	WT
CRC 2	M	54	Rectum	ADC of rectum	0	0	0	NA	NA	NA	WT	WT	WT
CRC 3	F	65	Colon	Mucinous ADC of colon	2	0	0	1	1.8 cm	G12D	G12D	WT	WT
CRC 4	F	72	Colon	ADC of colon	1	0	0	1	4.7 cm	G12D	G12D	WT	WT
CRC 5	M	36	Colon	ADC of colon	4a	1b	1	3	5.0 cm	G60D	WT	WT	WT
CRC 6	M	75	Colon	ADC of right colon	4a	2b	1	3	2.0 cm	WT	WT	WT	V600E
CRC 7	M	51	Sigmoid colon	ADC of sigmoid colon	3	0	0	2	4.5 cm	WT	WT	WT	WT
CRC 8	M	42	Sigmoid colon	ADC of sigmoid colon	2	0	0	2	3.0 cm	WT	WT	WT	WT
CRC 9	M	60	Sigmoid colon	ADC of sigmoid colon	4a	0	0	2	3.0 cm	WT	WT	WT	WT
CRC 10	F	78	Rectum	ADC of rectum	3	0	0	2	3.0 cm	WT	WT	WT	WT
CRC 11	M	76	Sigmoid colon	ADC of sigmoid colon	3	0	0	3	5.0 cm	G12A	G12A	WT	WT
CRC 12	M	50	Colon	ADC of right colon	3	1a	1	2	4.5 cm	WT	WT	WT	WT
CRC 13	F	83	Colon	ADC of right colon	3	0	0	2	4.0 cm	WT	WT	WT	V600E
CRC 14	F	76	Rectum	ADC of rectum	2	1b	0	3	3.0 cm	WT	WT	WT	WT
CRC 15	F	65	Colon	ADC of colon	2	0	0	2	6.0 cm	NA	WT	WT	WT
CRC 16	M	77	Colon	ADC of left colon	3	0	0	2	3.5 cm	NA	WT	WT	WT
CRC 17	F	54	Colon	ADC of colon	4a	1b	0	2	5.0 cm	G12D	G12D	WT	WT
CRC 18	M	75	Colon	ADC of colon	3	1b	0	2	4.0 cm	A146T	A146T	WT	WT
CRC 19	M	82	Colon	ADC of right colon	2	0	0	2	3.5 cm	WT	WT	WT	WT
CRC 20	M	72	Colon	ADC of right colon	3	0	0	2	4.0 cm	NA	WT	WT	WT
CRC 21	M	78	Colon	ADC of right colon	3	0	0	2	4.5 cm	G13D	G13D	WT	WT
CRC 22	M	77	Colon	ADC of right colon	3	0	0	2	4.5 cm	G12V	G12V	WT	WT
CRC 23	M	64	Rectum	ADC of rectum	4a	0	0	2	3.0 cm	G12V	G12V	WT	WT
CRC 24	M	52	Sigmoid colon	Mucinous ADC of sigmoid colon	4b	1a	0	3	4.0 cm	G12D	G12D	WT	WT
CRC 26	M	83	Rectum	ADC of rectum	2	1b	0	2	4.0 cm	NA	WT	WT	WT
CRC 27	F	85	Colon	Villous tubular adenoma	1	0	0	1	2.5 cm	G12R	G12R	WT	WT
CRC 28	M	53	Colon	ADC of right colon	3	0	0	2	5.0 cm	G13D	G13D	WT	WT
CRC 29	M	47	Rectum	ADC of rectum	1	0	0	2	< 1.0 mm	WT	WT	WT	WT
CRC 30	M	71	Rectum	ADC of right colon	3	0	0	2	2.5 cm	NA	WT	WT	WT
CRC 31	M	83	Colon	ADC of right colon	3	1	0	2	4.0 cm	G12D	G12D	WT	WT
CRC 33	M	55	Peritoneal carcinosis	Mixed adeno-neuroendocrine carcinoma (MANEC)	4	0	0	mixed	5.0 cm	G12V	G12V	WT	WT
CRC 34	F	74	Colon	Metastatic ADC of colon	4	1	0	3	NA	G13D	G13D	WT	WT
CRC 36	F	44	Rectum	ADC of rectum	3	1	0	3	NA	NA	G13D	WT	WT
CRC 40	F	79	Colon	Metastatic ADC of colon	4	2	1	3	NA	G12A	G12A	WT	WT
CRC 46	F	59	Colon	Metastatic ADC of colon	4	1	NA	2	NA	NA	WT	WT	WT

Table S3. Examples of molecular targets of the miRNA-signature.

MicroRNA	Validated molecular target	Reference
miR-144-3p	***mTOR***: miR-144-3p suppresses proliferation and induces apoptosis of osteosarcoma cellular models.	*Ren et al., 2018* [1]
	PRR11: miR-144-3p induces cell cycle arrest and apoptosis in pancreatic cancer cells.	*Li et al., 2017* [2]
	BCL6: miR-144-3p inhibits cell proliferation of colorectal cancer cells.	*Sun et al., 2020* [3]
miR-486-5p	***PIK3R1***: miR-486-5p regulates the migration and invasion of colorectal cancer cellular models.	*Zhang et al., 2018* [4]
miR-144-5p	**CCNE1 and CCNE2:** miR-144-5p regulates cell cycle in bladder cancer models.	*Matsushita et al., 2015* [5]
miR-15b-5p	***HPSE2***: miR-15b-5p promotes growth and metastasis in breast cancer models.	*Wu et al., 2020* [6]
	Axin2: miR-15b-5p promote cell proliferation and invasion in liver cancer	*Dong et al., 2019* [7]
	RECK: miR-15b-5p facilitates the tumorigenicity predicts tumour recurrence in prostate cancer	*Chen et al., 2018* [8]
miR-1247-5p	**Wnt3:** miR-1247-5p functions as a tumor suppressor in human hepatocellular carcinoma models.	*Chu et al., 2017* [9]
miR-584-5p	***ROCK-1:*** miR-584-5p regulates invasion and migration thyroid carcinoma models.	*Xiang et al., 2015* [10]
miR-483-5p	***ERK1:*** miR-483–5p suppresses the proliferation and induced a G0/G1 arrest of glioma cells.	Wang et al., 2012 [11]
miR-10a-5p	***MMP14:*** miR-10a-5p suppresses colorectal cancer metastasis regulating the epithelial-to-mesenchymal transition.	Liu et al., 2017 [12]
miR-425-3p	**AKT1*:*** miR-425-3p confers cisplatin resistance in NSCLC.	Ma et al., 2019 [13]

Table S4. NGS analysis data.

miRNAs differentially expressed in CRC Vs Tf-D				
miRNAs expression is normalized and log2 transformed				
# Notes: Created from Advanced Analysis operation: significance Analysis.				
#Experiment: CRC-project_smallRNAseq				
#corrected p-value cut-off:0.45				
#Fold change cut-off:2.0				
#Selected Test:	*Moderated T-Test*			
#Entity List:	*Fold change >= 2.0 CRC Vs Tf-D*			
#Interpretation:	*Type*			
#p-value computation:	*Asymptotic*			
#Multiple Testing Correction:	***Benjamini-Hochberg***			
miRNA ID	**p (Corr) ([CRC] Vs [Tf-D])**	**p ([CRC] Vs [Tf-D])**	**Regulation ([CRC] Vs [Tf-D])**	**FC (abs) ([CRC] Vs [Tf-D])**
hsa-miR-10a-5p	0,4018353	0,24126972	up	2,8678014
hsa-miR-1247-5p	0,4018353	0,19052348	up	7,9928136
hsa-miR-144-3p	0,3119489	0,01949680	down	2,7872958
hsa-miR-144-5p	0,1726657	0,00863329	down	2,6813111
hsa-miR-15b-5p	0,4018353	0,16873904	down	2,4655366
hsa-miR-425-3p	0,4018353	0,21251409	up	2,1048949
hsa-miR-483-5p	0,4355741	0,38219790	up	2,5161827
hsa-miR-486-5p	0,3196733	0,02797141	down	2,5935428
hsa-miR-584-5p	0,4018353	0,06719047	up	2,9576976

Table 5. List of employed miRNA assays.

miRNA name	Assay ID
hsa-miR-10a-5p	000387
hsa-miR-1247-5p	002898
hsa-miR-141-3p	000463
hsa-miR-144-3p	002676
hsa-miR-144-5p	002148
hsa-miR-15b-5p	000390
hsa-miR-221-3p	000524
hsa-miR-222-3p	002676
hsa-miR-425-3p	002302
hsa-miR-483-5p	002328
hsa-miR-486-5p	001278
hsa-miR-584-5p	001624
cel-miR-39-3p	000200

Reference

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