Supplementary Methods

RNA isolation and qRT-PCR from serum and cell culture medium

RNA extraction and miRNA enrichment from all sera and culture media samples were performed using the Qiagen miRNeasy Kit (Qiagen, Valencia, CA). Briefly, 250 uL of serum or medium was thawed on ice and centrifuged at 10,000 rpm for 5 minutes to remove cell debris. Next, same amount of the starting material (200 uL) of supernatant was lysed in 5 volumes of Qiazol solution. To normalize any inadvertent sample-to-sample variations during the RNA isolation procedure, Reverse Transcription (RT) and PCR reaction, 25 fmol of synthetic *C. elegans* miRNA (cel-*miR-39*) was added to each denatured sample. Small RNAs were then enriched and purified following the manufacturer's protocol, with the exception that the enriched small RNAs were eluted in 40 uL of nuclease-free water. For miRNA-based RT-PCR assays, 1.67 uL of enriched small RNAs from serum or cell-culture medium were reverse-transcribed using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, San Diego, CA) in a total volume of 5.0 uL. A 1:15 dilution of RT products was used as template for the PCR. PCR reactions for quantifying *miR-21*, *miR-31* and cel-*miR-39* were performed in duplicate, with TaqMan 2× Universal PCR Master Mix, using conditions previously described (1).

RNA isolation and qRT-PCR from formalin-fixed, paraffin-embedded (FFPE) tissues

Total RNA was isolated from FFPE samples using the RecoverAll Total Nucleic Acid Isolation Kit (Ambion Inc., Austin, TX). Briefly, tissue sections were microdissected to enrich for neoplastic cells, followed by deparaffinization and RNA

extraction using the manufacturer's protocol. Total RNA was eluted in the appropriate buffer, and quantified using a NanoDrop Spectrophotometer (NanoDrop Technologies, Wilmington, DE). Reverse transcription reactions were carried out using the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) in a total reaction volume of 15 uL. *MiR-21, miR-31* and *miR-16* were quantified in duplicate by qRT-PCR, using MicroRNA Assay Kits (Applied Biosystems, Foster City, CA). qRT-PCR was performed on an Applied Biosystems 7000 Sequence Detection System with the following cycling conditions: 95°C for 10 min, followed by 45 cycles of 95°C for 15s and 60°C for 1 min. Cycle threshold (Ct) values were calculated by using same threshold cut-off values for each assay to prevent plate-to-plate variations while analyzing data with the SDS 1.4 software (Applied Biosystems, Foster City, CA).

Calculation of miRNA expression

Expression levels of serum or tissue miRNAs were normalized using cel-*miR-39* (for serum or cell media culture samples) and *miR-16* (for tissue samples) using the $2^{-\Delta Ct}$ method. Differences between the groups are presented as ΔCt, indicating differences between Ct values of miRNAs of interest and Ct values of normalizer miRNAs.

Reference

1. Kroh EM, Parkin RK, Mitchell PS, Tewari M. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). *Methods*.2010;50(4):298-301.

Supplementary Table 1: Patient characteristics for serum and tissue *miR-21* expression analysis in the validation set*

	All CRC patients	Patients analyzed for serum analysis	Patients analyzed for tissue analysis	Patients with adenomas	Healthy controls	*P*†
Characteristics	n=200	n=186	n=166	n=43	n=53	
Age(years)						
Mean±SD	67.5±7.5	-	-	66±9.8	64±12.9	0.15
Gender						
Male	117	106	100	30	27	0.62
0.Female	83	80	66	13	26	
TNM stage						
I	46	45	37	-	-	-
II	62	57	51	-	-	-
III	48	43	43	-	-	-
IV	44	41	35	-	-	-

*CRC: colorectal cancer; TNM: tumor-node-metastasis staging system; SD: Standard Deviation ; ns: not significance
†*P* values are two-sided and estimating using the Kruskal–Wallis tests or Chi-square test, as appropriate.

Supplementary Table 2: Actual Numbers divided by optimal cutoff value and the associated values for sensitivity, specificity, PPV and NPV*

Colorectal Cancer (CRC) vs. Controls

	CRC	Controls	Total
serum *miR-21* >0.0019(cutoff)	154	5	160
serum *miR-21* ≤0.0019(cutoff)	32	48	79
Total	186	53	239

PPV (%)	96.3	**Sensitivity (%)**	82.8
NPV (%)	60.8	**Specificity (%)**	90.6

Adenoma vs. Controls

	Adenoma	Controls	Total
serum *miR-21* >0.0013(cutoff)	33	10	43
serum *miR-21* ≤0.0013(cutoff)	10	43	53
Total	43	53	96

PPV (%)	76.7	**Sensitivity (%)**	76.7
NPV (%)	81.1	**Specificity (%)**	81.1

*PPV: Positive Predictive Value; NPV: Negative Predictive Value

Supplementary Table 3: Sensitivity and Specificity after ROC analysis using Bootstrap Methods*

CRC vs. Controls				*Adenoma vs. Controls*			
Sensitivity	**Specificity**	**optimal cutoff**	**95% CI**	**Sensitivity**	**Specificity**	**optimal cutoff**	**95% CI**
91.94	81.13	>0.0013	0.0009-0.00134	81.13	76.74	>0.0013	0.0010-0.00134
Estimated specificity at fixed sensitivity				***Estimated specificity at fixed sensitivity***			
Sensitivity	**Specificity**	**95% CI**	**cutoff**	**Sensitivity**	**Specificity**	**95% CI**	**cutoff**
80	92.45	79.25 - 98.11	>0.002	80	58.49	15.03 - 84.91	>0.0012
90	83.02	50.94 - 92.45	>0.0015	90	35.85	5.66 - 64.15	>0.0009
95	45.28	19.27 - 73.58	>0.001	95	15.09	0.53 - 52.83	>0.0006
97.5	24.53	3.53 - 45.28	>0.0007	97.5	9.43	0.87 - 35.85	>0.0004
Estimated sensitivity at fixed specificity				***Estimated specificity at fixed sensitivity***			
Specificity	**Sensitivity**	**95% CI**	**cutoff**	**Specificity**	**Sensitivity**	**95% CI**	**cutoff**
80	91.94	84.41 - 96.39	>0.0013	80	76.74	58.14 - 90.70	>0.0013
90	82.8	68.82 - 92.47	>0.0019	90	60.47	25.58 - 76.74	>0.0019
95	73.66	57.23 - 84.41	>0.0026	95	34.88	13.54 - 65.12	>0.0026
97.5	64.15	53.83 - 79.57	>0.0033	97.5	25.58	7.24 - 51.16	>0.0032

* Bootstrap bias-correction and accelerated bootstrap methods used. Repeating times:1000. CI: Confidence Interval

Supplementary Table 4: Comparison between ROCCH and adjusted ROCCH by Bootstrap Methods*

		AUC	SE	95% CI	*P*†
CRC vs. Controls	**ROCCH**	0.935	0.06	0.812-0.982	0.80
	adjusted* ROCCH	0.919	0.02	0.867-0.958	
Adenoma vs. Controls	**ROCCH**	0.838	0.11	0.619-0.964	0.84
	adjusted* ROCCH	0.813	0.06	0.691-0.910	

* Bootstrap bias-correction and accelerated bootstrap methods used for adjusting. Repeating times:1000. CRC: Colorectal Cancer; ROCCH:
Receiver Operating Characteristic Convex Hull; AUC: Area under the ROC curve; SE: Standard Error
CI: Confidence Intervals
†A two-sided z-test was used to compare the AUCs of two ROC curves