**This supplementary file contains eight supplementary figures, four supplementary tables and one supplementary appendix.**

Manuscript entitled "**Quantitation of Fecal *Fusobacterium* Improves Performance of Fecal Immunochemical Test in Detecting Advanced Colorectal Neoplasia**"
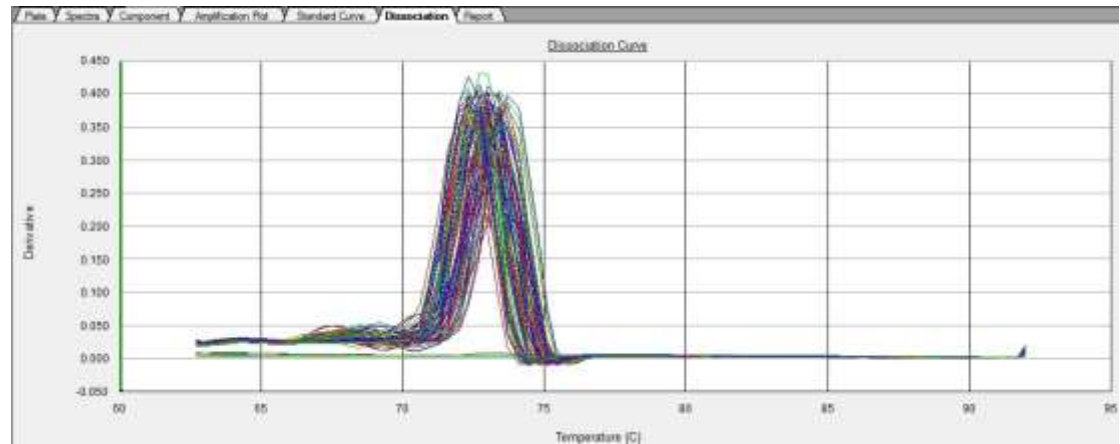
Wong and Kwong et al.

Supplementary Figure 1. Correlation between first and second detection Ct values

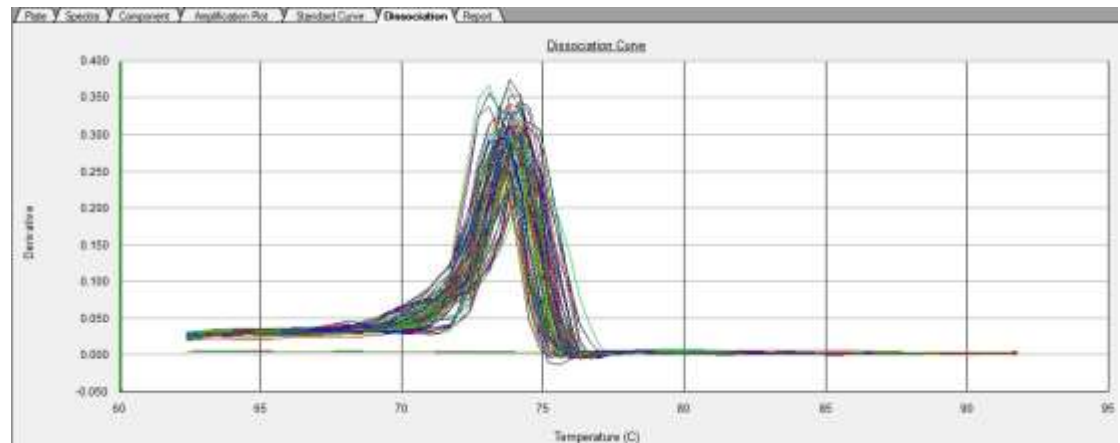of total bacteria for the same stool samples.

r=0.978
p<0.001
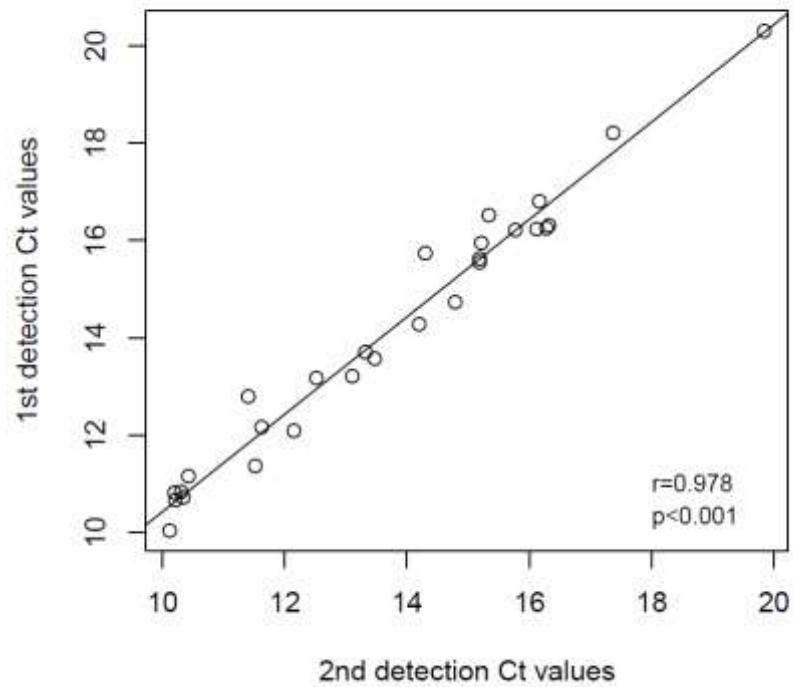
Supplementary Figure 2. Quantitative PCR melt curves of the microbial marker *Fn* (*A*), *Pa* (*B*) and *Pm* (*C*), respectively.
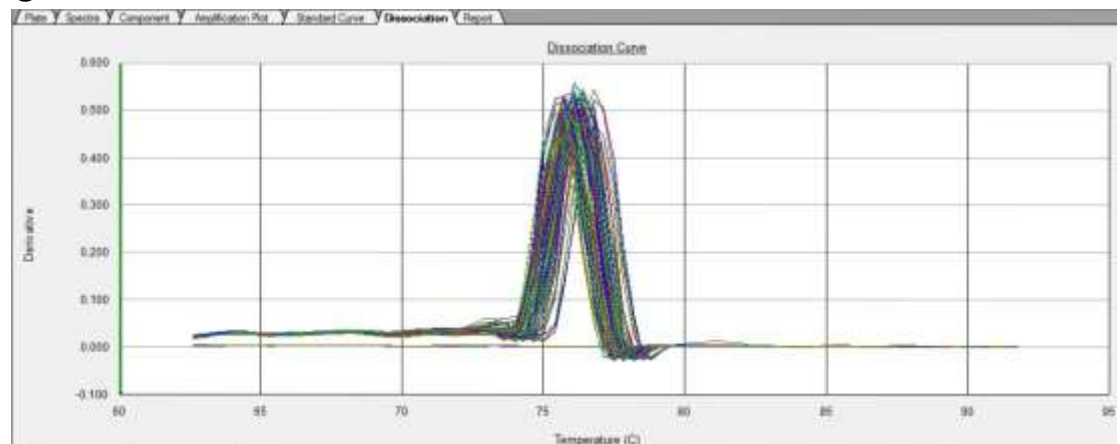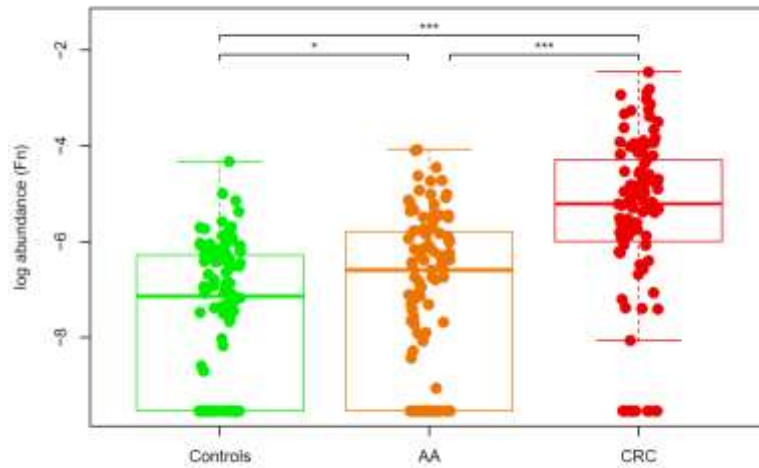
A

B

C

Supplementary Figure 3. Relative abundance of the microbial marker *Fn* (*A*), *Pa* (*B*) and *Pm* (*C*) in CRC, advanced adenoma (AA) and control samples. The Mann–Whitney U two-tailed test was used for comparisons. ***p<0.001, **p<0.01, *p<0.05

A

B

C
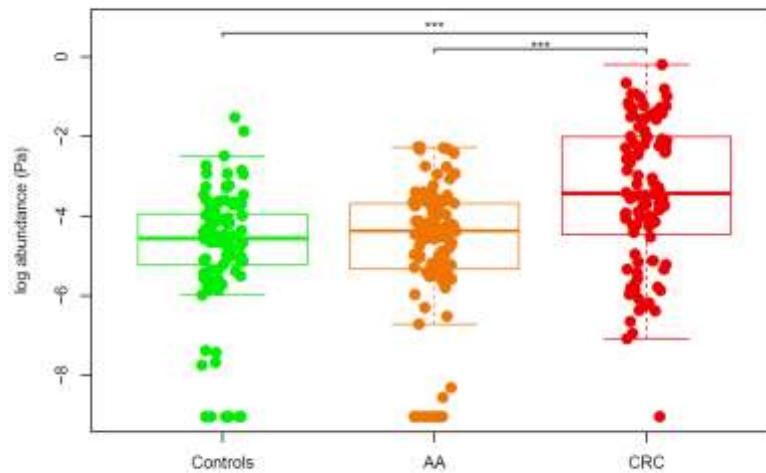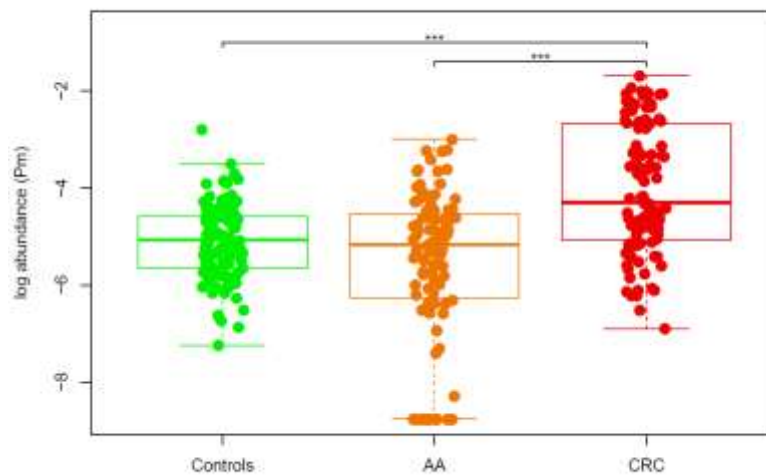
Supplementary Figure 4. Diagnostic performance with the AUC, sensitivities and specificities of FIT, individual microbial markers and their combinations for the diagnosis of CRC (*A*) or advanced adenoma (AA) (*B*).

A

B
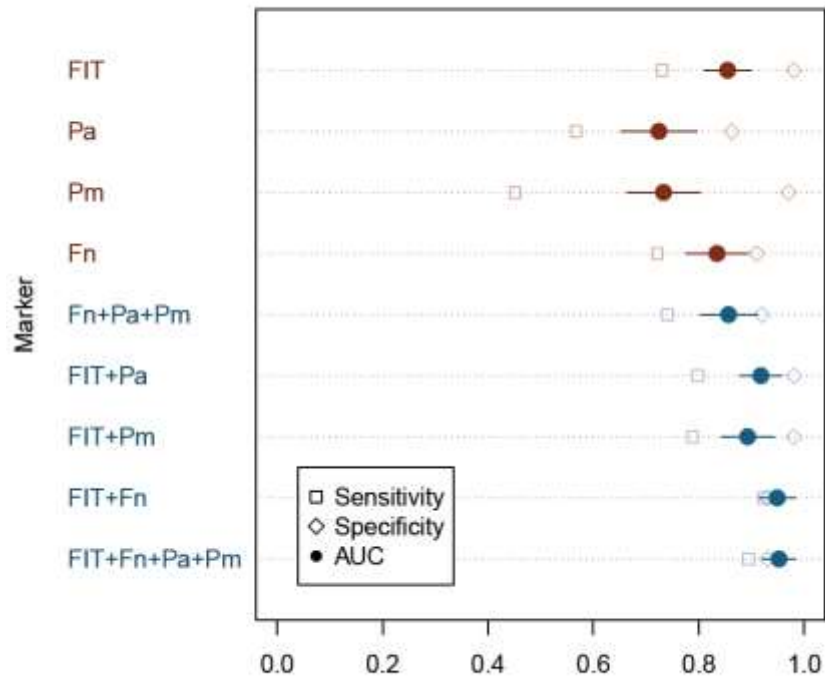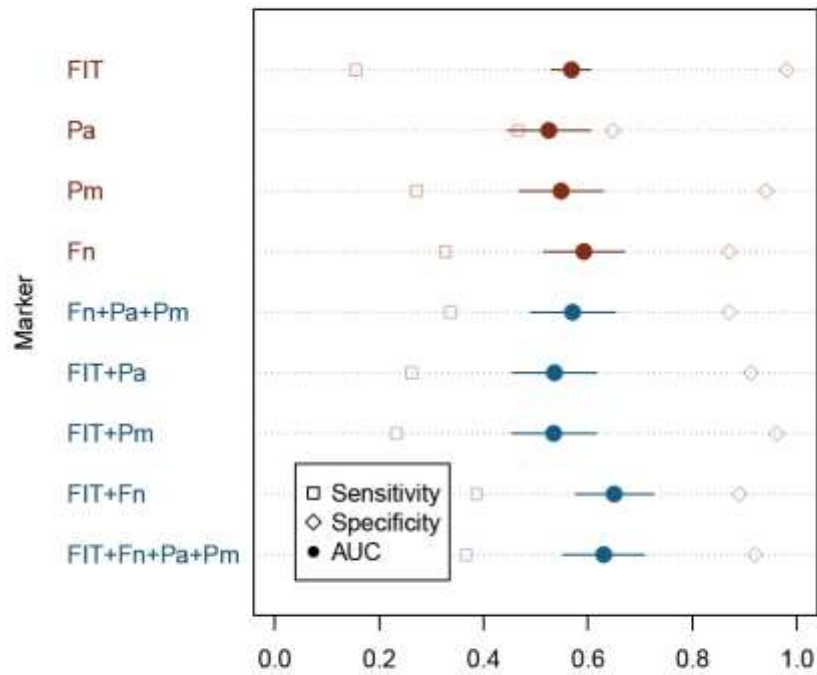
Supplementary Figure 5. ROC analysis of individual microbial markers for the diagnosis of CRC.

Pa, AUC=0.72
Pm, AUC=0.73
Fn, AUC=0.83
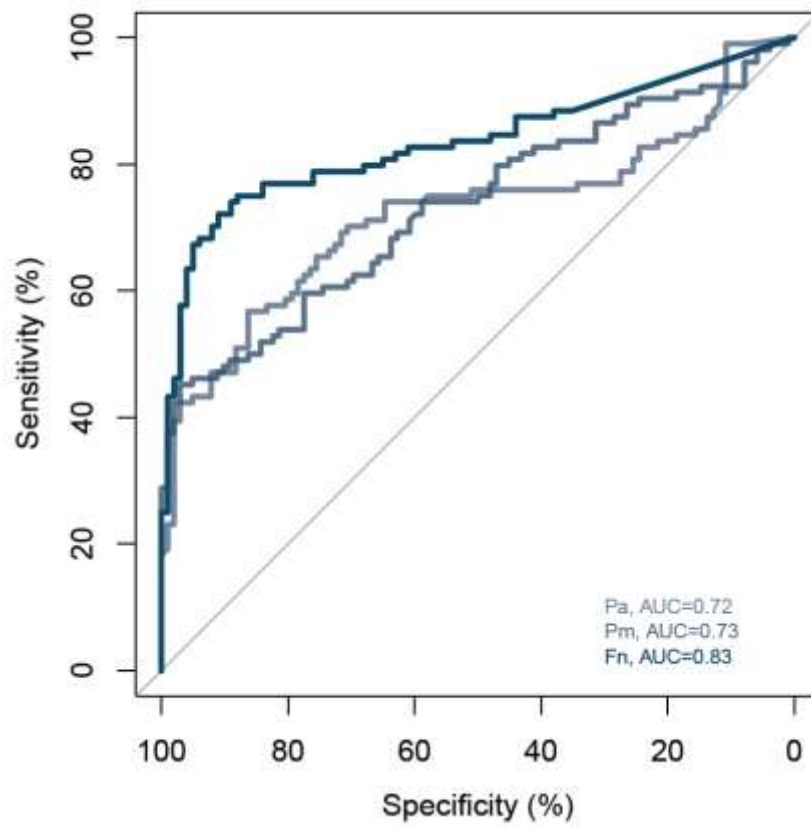
Supplementary Figure 6. The ROC analysis of FIT, marker *Fn* and their combined

test for diagnosing advanced adenoma in the discovery (*A*) and validation (*B*) cohorts.

A

B
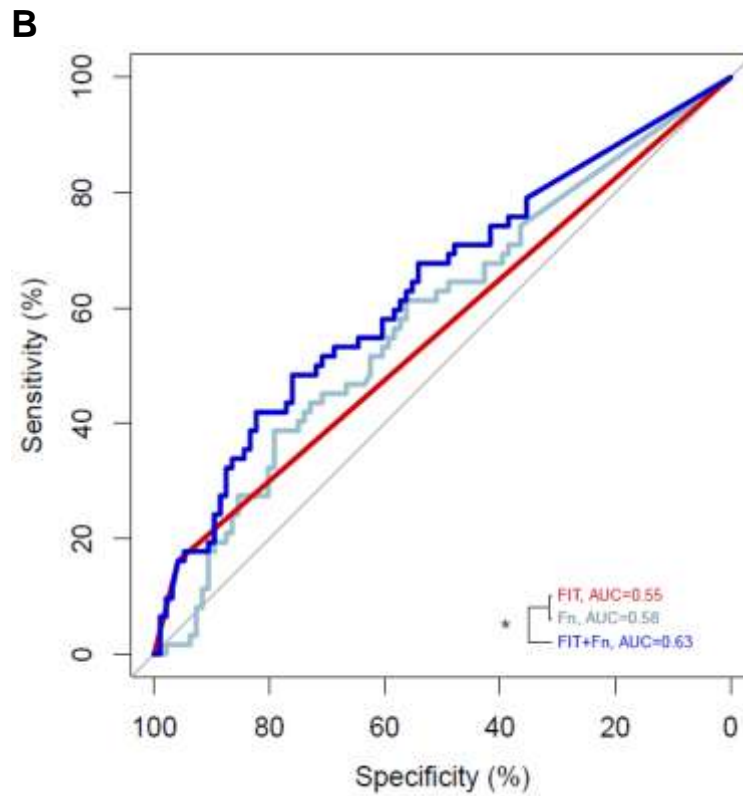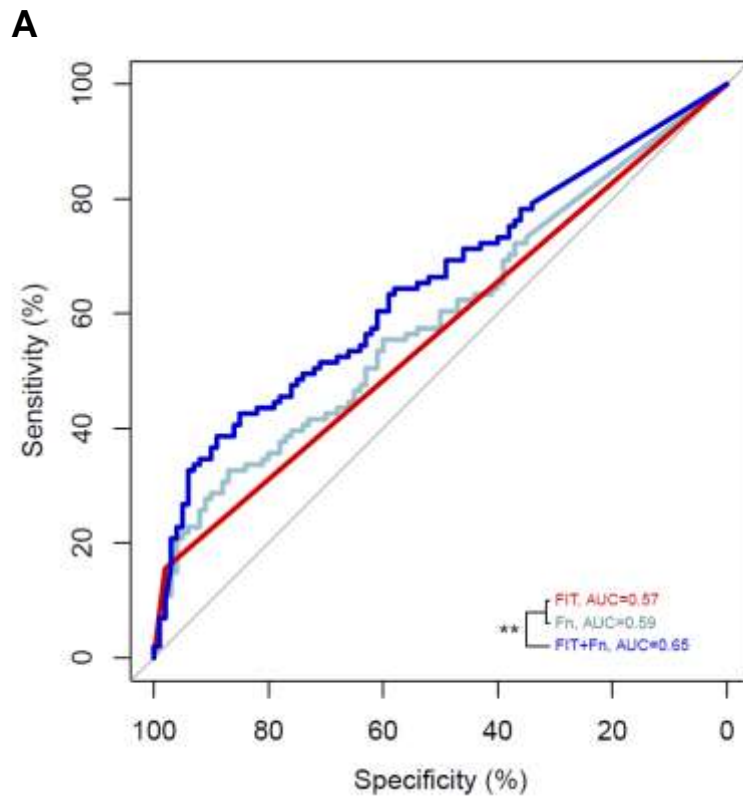
Supplementary Figure 7. The advanced adenoma samples detected by FIT (red), missed by FIT and detected by marker *Fn* (blue), and missed by both test (yellow). The dotted lines indicate the threshold of the individual test above which samples are regarded as positive.
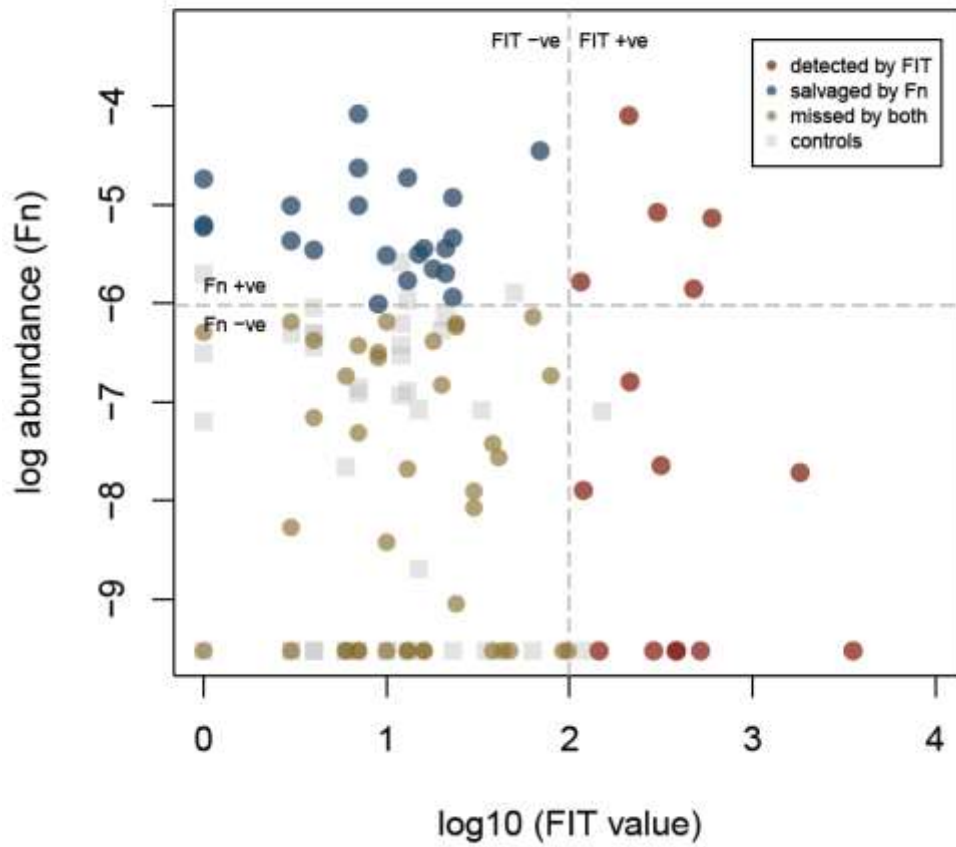
Supplementary Figure 8. Relative abundance of the microbial marker *Fn* in the validation (*A*) and combined (*B*) cohorts. The Mann–Whitney U one-tailed test was used for two-group comparisons for the validation cohort. ***p<0.001, **p<0.01, *p<0.05

A
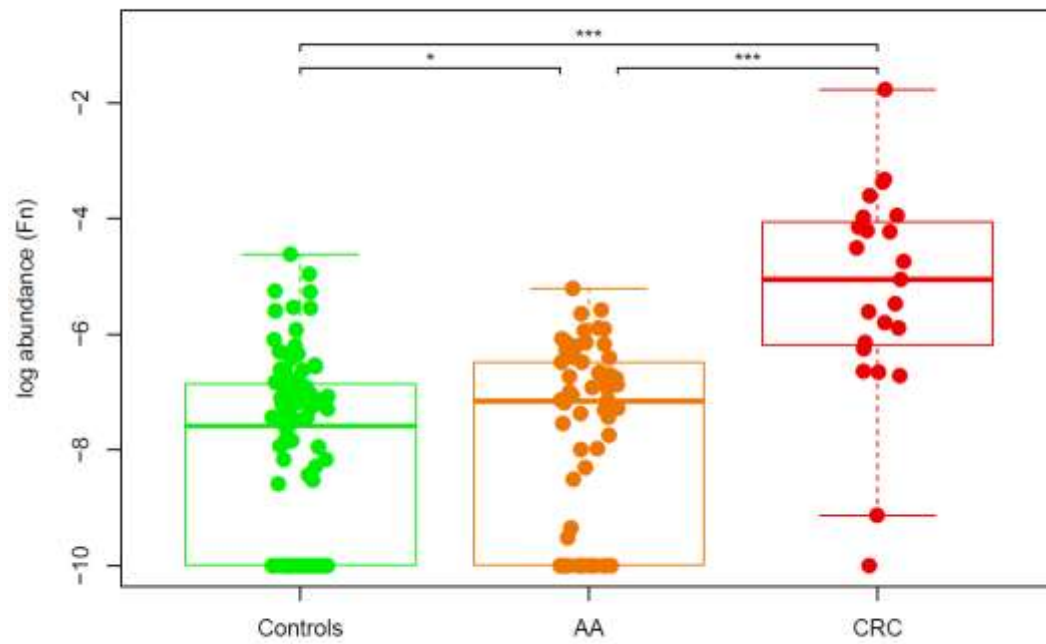
B
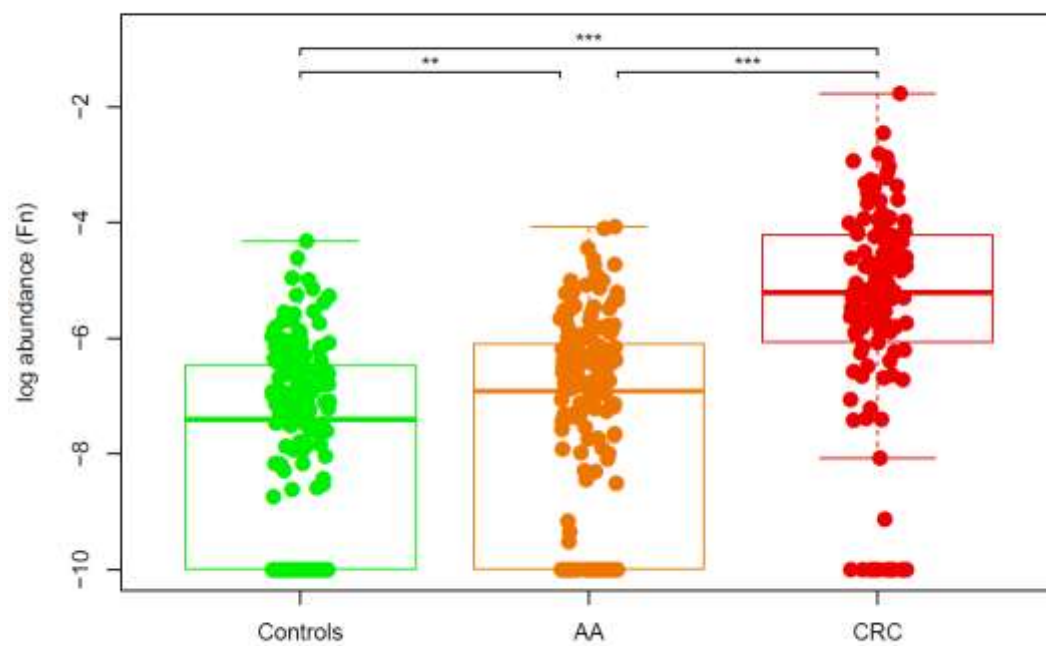
Supplementary Table 1. Background demographic of the study cohorts and location of the most advanced neoplasm.

Parameter	Discovery Cohort			Validation Cohort			Overall
	CRC	AA	Controls	CRC	AA	Controls	
N	104	103	102	23	62	96	490
Age							
Mean ± SD	66.9 ± 10.1	61.3 ± 6.6	57.1 ± 5.8	63.8 ± 12.3	58.1 ± 5.4	58.6 ± 7.7	60.4 ± 8.2
Range	44 – 90	49 – 80	39 – 70	51 – 78	46 – 67	38 – 89	38 – 90
Gender							
Male	65 (62.5%)	66 (64.1%)	69 (67.7%)	14 (60.9%)	43 (75.8%)	53 (55.2%)	310 (63.3%)
Female	39 (37.5%)	37 (35.9%)	33 (32.3%)	9 (39.1%)	19 (24.2%)	43 (44.8%)	180 (36.7%)
Tumour location							
Proximal	28 (26.9%)	43 (41.7%)	NA	7 (30.4%)	24 (38.7%)	NA	NA
Distal	76 (73.1%)	60 (58.3%)		16 (69.6%)	38 (61.3%)		

Supplementary Table 2. Test performance of FIT, the microbial markers and their combination for CRC. The AUC of the markers were compared with FIT. Two-sided Delong's test was used for microbial markers, whereas one-sided Delong's test was used for the combinational markers to test for incremental gain in AUC.

Marker	Threshold	Sensitivity	Specificity	AUC	Compare with FIT
CRC model					
FIT	100 ng/mL	73.1% (64.4-81.8%)	98.0% (95.1-100%)	0.86 (0.81-0.90)	Reference
Fn	1.5×10^{-6}	72.1% (62.5-80.8%)	91.0% (85.0-96.0%)	0.83 (0.78-0.89)	Not significant
Pa	2.7×10^{-4}	56.7% (47.1-66.4%)	86.3% (79.4-93.1%)	0.72 (0.65-0.80)	Not significant
Pm	1.6×10^{-4}	45.2% (35.6-54.8%)	97.1% (93.1-100%)	0.73 (0.66-0.80)	Not significant
FIT+*Fn*	0.166	92.3% (86.5-97.1%)	93.0% (88.0-97.0%)	0.95 (0.92-0.98)	*p*<0.001
FIT+*Pa*	0.762	79.8% (71.1-87.5%)	98.0% (95.1-100%)	0.92 (0.88-0.96)	*p*<0.001
FIT+*Pm*	0.798	78.9% (71.1-86.5%)	98.0% (95.1-100%)	0.89 (0.84-0.94)	*p*=0.026
FIT+*Fn*+*Pa*+*Pm*	0.218	89.4% (83.7-95.2%)	93.0% (87.0-97.0%)	0.95 (0.92-0.98)	*p*<0.001

Supplementary Table 3. Test performance of FIT, the microbial markers and their combination for advanced adenoma (AA). The AUC of the markers were compared with FIT. Two-sided Delong's test was used for microbial markers, whereas one-sided Delong's test was used for the combinational markers to test for incremental gain in AUC.

Marker	Threshold	Sensitivity	Specificity	AUC	Compare with FIT
AA model					
FIT	100 ng/mL	15.5% (8.7-22.3%)	98.0% (95.1-100%)	0.57 (0.53-0.61)	Reference
Fn	9.6×10^{-7}	32.7% (23.8-41.6%)	87.0% (80.0-93.0%)	0.59 (0.51-0.67)	Not significant
Pa	5.5×10^{-5}	46.6% (36.9-55.3%)	64.7% (55.9-73.5%)	0.52 (0.44-0.60)	Not significant
Pm	6.9×10^{-7}	27.2% (19.4-36.0%)	94.1% (89.2-98.0%)	0.55 (0.47-0.63)	Not significant
FIT+*Fn*	0.464	38.6% (28.7-48.5%)	89.0% (83.0-95.0%)	0.65 (0.58-0.73)	*p*=0.007
FIT+*Pa*	0.468	26.2% (18.5-35.0%)	91.2% (85.3-96.1%)	0.54 (0.46-0.62)	Not significant
FIT+*Pm*	0.496	23.3% (15.5-32.0%)	96.1% (92.2-99.0%)	0.54 (0.45-0.62)	Not significant
FIT+*Fn*+*Pa*+*Pm*	0.479	36.6% (27.7-45.5%)	92.0% (86.0-97.0%)	0.63 (0.55-0.71)	*p*=0.034

Supplementary Table 4. Test performance of FIT, marker *Fn* and both markers for CRC and advanced adenoma (AA) in the validation and combined cohorts, fitting the model from the discovery cohort. The AUC of the markers were compared with FIT. Two-sided Delong's test was used for microbial markers, whereas one-sided Delong's test was used for the combinational markers to test for incremental gain in AUC.

Marker	Cohort	Threshold	Sensitivity	Specificity	AUC	Compare with FIT
CRC model						
FIT	Validation	100 ng/mL	73.9% (56.5-91.3%)	95.8% (91.7-99.0%)	0.85 (0.76-0.94)	Reference
Fn	Validation	1.2×10^{-6}	91.3% (78.3-100%)	80.2% (71.9-87.5%)	0.89 (0.80-0.98)	Not significant
FIT+*Fn*	Validation	0.281	82.6% (65.2-95.7%)	94.8% (90.6-99.0%)	0.96 (0.92-0.99)	*p*=0.0014
FIT	All	100 ng/mL	73.2% (65.4-81.1%)	96.9% (94.4-99.0%)	0.85 (0.81-0.89)	Reference
Fn	All	1.5×10^{-7}	73.2% (65.4-80.3%)	90.8% (86.7-94.4%)	0.85 (0.80-0.90)	Not significant
FIT+*Fn*	All	0.235	88.2% (81.9-93.7%)	94.4% (90.8-97.5%)	0.95 (0.92-0.98)	*p*<0.001
AA model						
FIT	Validation	100 ng/mL	16.1% (8.1-25.8%)	95.8% (91.7-99.0%)	0.56 (0.51-0.61)	Reference
Fn	Validation	9.4×10^{-7}	38.7% (25.8-51.6%)	79.2% (70.8-87.5%)	0.58 (0.49-0.67)	Not significant
FIT+*Fn*	Validation	0.445	48.4% (35.5-61.3%)	76.0% (67.7-84.4%)	0.63 (0.55-0.72)	*p*=0.031
FIT	All	100 ng/mL	15.3% (10.4-20.9%)	96.9% (94.4-99.0%)	0.56 (0.53-0.59)	Reference
Fn	All	9.4×10^{-7}	47.9% (40.5-55.8%)	70.0% (63.3-76.0%)	0.59 (0.53-0.65)	Not significant
FIT+*Fn*	All	0.445	57.7% (49.7-65.0%)	67.4% (60.7-74.0%)	0.65 (0.59-0.70)	*p*<0.001

Supporting Appendix. The FITTER checklist for the reporting of studies using fecal immunochemical tests for hemoglobin

Topic	Item	Priority	Documentation
Specimen collection and handling			
	Name of specimen collection device and supplier (address).	Essential	Page 10
	Description of specimen collection device (vial with probe/stick, card, other).	Essential	Page 10
	Description of specimens used if an *in vivo* study (single or pooled feces, artificial matrix with added blood, etc).	Essential for laboratory evaluations	NA
	Details of fecal collection method (sampling technique and number of samples).	Essential	Page 10
	Who collected the specimens from the samples (patient, technician, etc).	Essential	Page 7
	Number of fecal specimens used in the study (single, pooled, individual patient feces).	Essential for laboratory evaluations	Page 12
	Mean mass of feces collected.*	Essential	NA
	Volume of buffer into which specimen is taken by probe, applicator stick or card.*	Essential	Page 10
	Time and storage conditions of fecal specimen from "passing" to sampling, including time and temperature (median and range).	Essential for laboratory evaluations	Page 7
	Time and storage of collection devices from specimen collection to analysis, including time and temperature (median and range). A concise description of process from collection to analysis is recommended.	Essential	Page 7
Analysis			
	Name of analyser, model, supplier (address), number of systems if more than one used.	Essential	Page 10
	Number of times each sample was analysed.	Essential	Page 10
	Analytical working range* and whether samples outside this range were diluted (factor) and reassayed.	Essential for laboratory evaluations	NA
	Source of calibrator(s) (supplier with address), number of calibrator(s), how concentrations were assigned* and details of calibration process including frequency.	Essential for laboratory evaluations	NA
	Analytical imprecision*, ideally with number of samples analysed, concentrations, and mean, SD and	Essential for all studies	NA

	CV.		
Quality management			
	Source (address) or description of internal quality control materials, number of controls, assigned target concentrations and ranges, how target concentrations were assigned, rules used for acceptance and rejection of analytical runs.	Desirable for laboratory evaluations	NA
	Participation in external quality assessment schemes: (name and address of scheme), frequency of challenges, performance attained.	Desirable for laboratory evaluations	NA
	Accreditation held by the analytical facility (address).	Desirable for laboratory evaluations	NA
	The number, training and expertise of the persons performing the analyses and recording the results.	Essential	Page 10
Result handling			
	Mode of collection of data – manual recording or via automatic download to IT system, single or double reading.	Desirable	NA
	Units used, with conversion to µg Hb/g feces if ng Hb/mL used.	Essential	Page 10
	Cut-off concentration(s) if used and explanation of how assigned locally or by manufacturer.*	Essential	Page 10
	Were the analysts blinded (masked) to the results of the reference investigation and other clinical information?	Essential	Page 10
*information available from manufacturer or supplier			

Note: NA=not applicable