Additional File

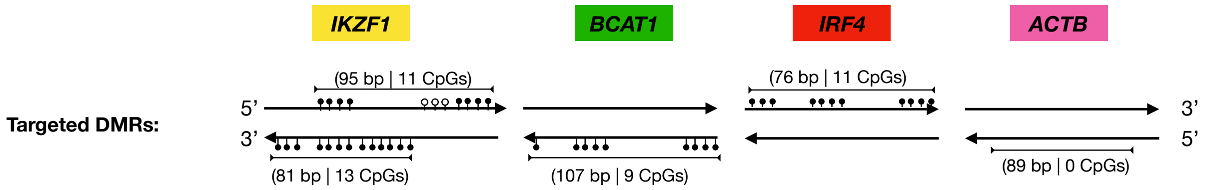

Figure S1. The multi-panel real-time PCR assay. (A) Schematic representation of the investigated multi-panel qPCR assay, which targets differentially-methylated regions (DMRs) in *BCAT1* ((Branched Chain Amino Acid Transaminase 1), *IKZF1* (IKAROS Family Zinc Finger 1) and *IRF4* (Interferon Regulatory Factor 4) and a control region in *ACTB* (beta-actin). The *IKZF1* PCR assay component detects the targeted DMR on both strands (non-complementary subsequent to bisulphite conversion). Partially methylation in the *IKZF1* DMR residing on the sense strand was detectable by utilization of degenerate probes. The *IKZF1* probes were all labelled with the same fluorophore, FAM. Detection of the other amplicon targets were reported using probes labelled with HEX (*BCAT1*), Texas Red (*IRF4*) and Cy5 (*ACTB*).

Table S1. Positivity[1] by progression of cellular atypia (dysplasia in adenomas) and degree of invasion (T stage in cancer) in cases without disseminated disease.

	N	Any gene	BCAT1	IKZF1	IRF4
		Count of Positives, n (%, 95%CI)			
584 adenomas[2]					
HGD	35	9 (25.7, 14-42)	5 (14.3, 6-29)	6 (17.1, 4-30)	7 (20.0, 6-34)
LGD	549	65 (11.8, 9-15)	35 (6.4, 5-9)	32 (5.8, 4-8)	18 (3.3, 2-5)
P-value[3]		0.017	0.072	0.009	<0.0001
94 cancers without disseminated disease[4]					
T1N0M0	24	6 (25.0, 6-44)	1 (4.2, -5-13)	5 (20.8, 55-83)	2 (8.3, -4-20)
T2N0M0	17	10 (58.8, 33-85)	7 (41.2, 15-67)	6 (35.3, 10-61)	5 (29.4, 5-54)
T3N0M0[5]	45	39 (86.7, 76-97)	21 (46.7, 32-62)	31 (68.9, 55-83)	24 (53.3, 38-69)
T4N0M0	8	8 (100, 63-100)	7 (87.5, 58-117)	7 (87.5, 58-117)	7 (87.5, 6-117)
P-value[6]		<0.0001	<0.0001	<0.0001	<0.0001

[1] At least one PCR replicate positive for DNA methylation in any gene.

[2] The severity of dysplasia was available for 584 of 616 adenomas.

[3] Z-score population t-test.[4] T stage in cancer cases without disseminated disease.

[5] 57 Stage II (T3N0M0 and T4N0M0) in study cohort, but only 53 had full TNM information.

[6] Ordinary one-way ANOVA against T1N0M0.

Table S2. Test accuracy[1] for detection of advanced adenomas and adenomas with high grade dysplasia based on all possible combinations of genes.

Gene combination	**TP**	**Sensitivity** % (95%CI)	**AUC** (95%CI)	**TN**	**Specificity** % (95%CI)
Advanced adenoma (N = 337):					
BCAT1 only	29	8.6 (6.0-12.1)	0.516 (0.479-0.553)	776	94.6 (92.9-96.0)
IRF4 only	20	5.9 (3.8-9.0)	0.503 (0.467-0.540)	802	97.8 (96.6-98.6)
IKZF1 only	29	8.6 (6.0-12.1)	0.516 (0.479-0.553)	785	95.7 (94.1-96.9)
BCAT1 and-or *IRF4*	38	11.3 (8.3-15.1)	0.530 (0.492-0.567)	761	92.8 (90.8-94.4)
IKZF1 and-or *IRF4*	37	11.0 (8.0-14.8)	0.528 (0.491-0.565)	773	94.3 (92.5-95.7)
BCAT1 and-or *IKZF1*	47	14.0 (10.6-18.1)	0.543 (0.505-0.580)	750	91.5 (89.4-93.2)
Any of the 3 genes	53	15.7 (12.2-20.0)	0.552 (0.514-0.589)	739	90.1 (87.9-92.0)
Application of BCAT1 Replicate Rule:					
Any of the 3 genes	37	11.0 (8.1-14.8)	0.526 (0.488-0.563)	772	94.1 (92.3-95.6)
Adenoma with high-grade dysplasia (N = 35):					
BCAT1 only	5	14.3 (5.8-29.9)	0.545 (0.441-0.648)	776	94.6 (92.9-96.0)
IRF4 only	7	20.0 (9.7-36.2)	0.573 (0.467-0.679)	802	97.8 (96.6-98.6)
IKZF1 only	6	17.1 (7.7-33.1)	0.559 (0.454-0.664)	785	95.7 (94.1-96.9)
BCAT1 and-or *IRF4*	8	22.9 (11.8-39.3)	0.587 (0.480-0.694)	761	92.8 (90.8-94.4)
IKZF1 and-or *IRF4*	8	22.9 (11.8-39.3)	0.587 (0.480-0.694)	773	94.3 (92.5-95.7)
BCAT1 and-or *IKZF1*	7	20.0 (9.7-36.2)	0.573 (0.467-0.679)	750	91.5 (89.4-93.2)
Any of the 3 genes	9	25.7 (14.0-42.3)	0.602 (0.494-0.709)	739	90.1 (87.9-92.0)
Application of BCAT1 Replicate Rule:					
Any of the 3 genes	8	22.9 (11.8-39.3)	0.587 (0.480-0.694)	772	94.1 (92.3-95.6)

[1] The true- and false-positive rates in cases with advanced adenomas (n=337) or adenomas with high grade dysplasia (n=35) and cases without neoplasia (n = 820) were used for sensitivity (for indicated adenomas) and specificity (for neoplasia) estimates. TP: Counts of true positives. TN: Counts of true negatives. AUC, Area Under Curve.