

SUPPLEMENTARY MATERIAL

Scarce evidence of the causal role of germline mutations in *UNC5C* in hereditary colorectal cancer and polyposis

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Supplementary Table 1. LOH and methylation results for *UNC5C*.

Germline mutation ^a	Family ID (Fig. 1)	Index patient	LOH (informative markers)	Promoter methylation
c.932C>T (p.T311M)	A	III.1	n.a.	n.a.
c.1057G>A (p.D353N)	B	III.1	No LOH (D4S2380, D4S470, D4S1559)	Yes
c.1235A>C (p.D353N)	C	II.8	No LOH (D4S2380, D4S470, D4S1559)	Yes
c.1807C>T (p.R603C)	D	III.1	n.a.	Yes
c.1882_1883delinsAA (p.A628K)	E	III.1	No LOH (D4S2380, D4S470, D4S1559)	n.a.
c.2002G>A (p.A668T)	F	III.4	n.a.	n.a.
c.2210G>A (p.S737N)	G	II.3 and III.1	No LOH (D4S2380, D4S470, D4S1559)	Yes
c.2240A>G (p.D747G)	H	II.2	No LOH (D4S2380)	Yes

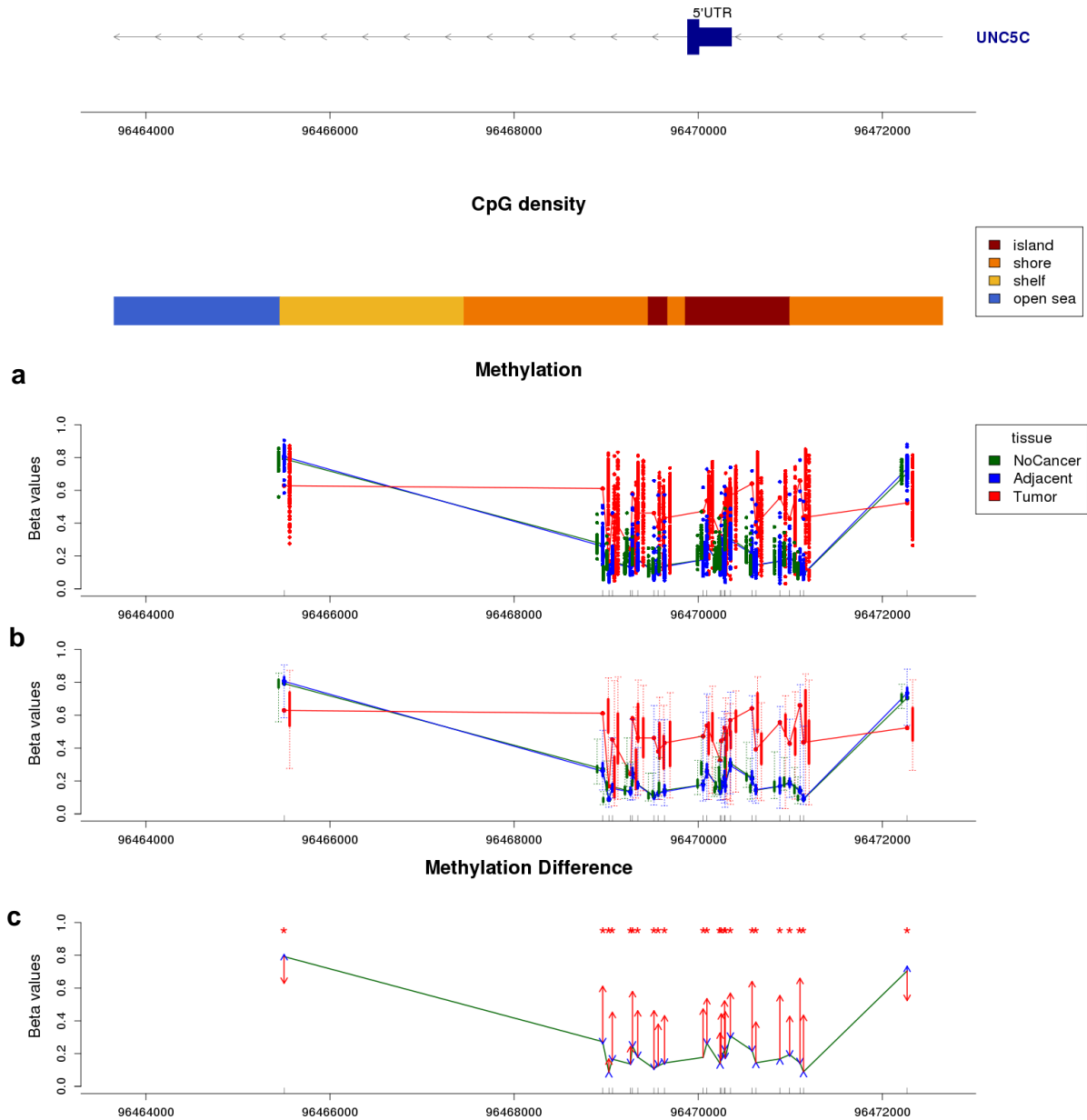
^aRefSeq GRCh37: *UNC5C*, NM_003728

Abbreviations: LOH, loss of heterozygosity; n.a., not available information; n.i., not informative

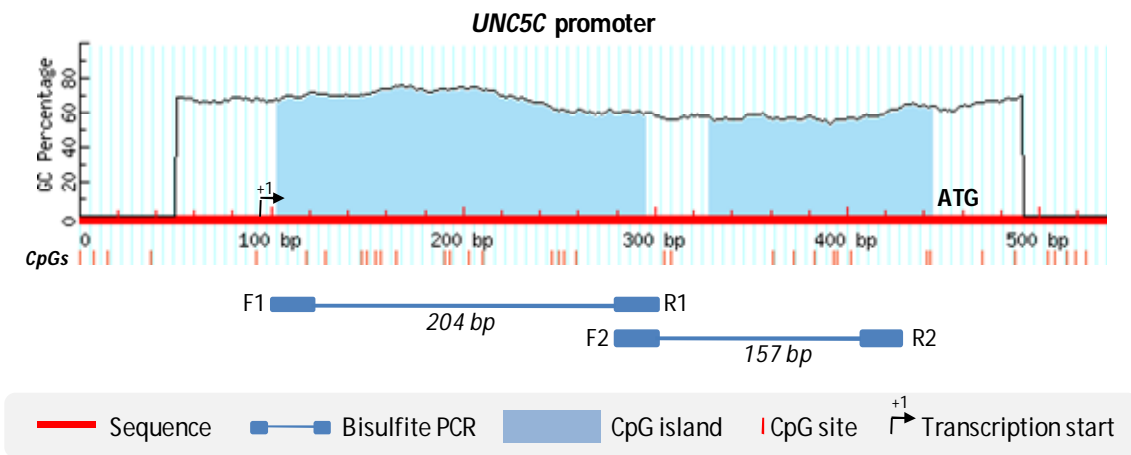
Supplementary Table 2. Primers used in the study and experimental conditions.

	Forward (5'-3')	Reverse (5'-3')	Amplicon size (bp)	Annealing Temp.
Mutation screening				
Exon 1	TCAAACCTCCTCCTCGGCGTC	GGCCGCGGAGCTTGGCGGAC	349	58°C
Exon 2	ATGTGTTAACCATCTCCAC	GTGAATCTTGAAGTGCAATG	301	58°C
Exon 3	AGTATGAATCTTGTGTTGAC	TGGGATTACAGGCGTGAGTC	334	58°C
Exon 4	GCACAGATAAAATACTAAGCCAGAAA	CATACCCTAATTCACCTGCACCA	298	56°C
Exon 5	ATCAGGGGATCCATGCTGAG	TGAACTAGATTGAGACCCTG	264	58°C
Exon 6	GTGTTGTGCATGCACACATG	GGAGATACTTGAATGAGAG	257	58°C
Exon 7	CTCCATCCCTTAAGCAGCTG	CCCTCAATGTCTGCAGTTTG	260	58°C
Exon 8	TATAAGCAGCTGGTTCAATG	AAGTAACAGGGTGTGAAGTG	264	58°C
Exon 9a	CTGAATGGAGAATGCCAATG	GACAGCTTGACGTAAACTC	250	58°C
Exon 9b	CAAAGTGTACAACACCTCAG	AATATGTTTCAAGCTGCCTC	256	58°C
Exon 10	TCATTCCCTTTTACGTGCTG	CATTGGTAAAGTCAGTGCAC	186	58°C
Exon 11	ACCCAAGTGCATAGTTCATG	TGCTCCTGCTTATCTGCATG	296	58°C
Exon 12	TCCTGGGTGACTTAGCCATG	TTCCAGTGAGGTTGAAGAG	400	58°C
Exon 13	GAGAGCTGACAGAAGTGATC	TCTTAGAGATGTCTCCAGAC	255	58°C
Exon 14	GGCAGCAGACCATCTGAGAG	TTCCTGAGTGTCCGCACTCTC	288	58°C
Exon 15	TGTCCTCAGAAATAATGCTG	ACAGAAAGAAGCTAGTCTTG	262	64°C
Exon 16	TAAGTCACACTGTTGAGCAC	TCCTTCATTTCCCCTTCCAG	236	58°C
Splicing analysis				
c.932C>T (exons 4-8)	CAGGAAGTCTTACTCCAGTGTCG	AACATCATCTGAATCAGGAGCA	588	60°C
LOH				
D4S1559	FAM_CTTCTCCAGTTTTTTTCTGAAATTGC	AGAAATAAATGCGTTGAAACCATCC	165	55°C
D4S2380	FAM_ACCTTATTGTGCTATGGAAACA	TCAGCCAAGATTACCTACCG	195	55°C
D4S470	FAM_ATTCTTGGGAAGTCACAAACACTACA	GGTTTAGGCAGCAGTCATTTTCAA	156	55°C
Methylation				
UNC5C_1	GGTGTGTTGTGTGTTTTTATAGG	TTTAAAAATCCCTCTTTCCCAATAC	204	56°C
UNC5C_2	GTATTGGGAAAGAGGGATTTTAAA	AACCCCACTAAACAAAACTAAATC	157	56°C

RefSeq Genes - chr4: 96463655 - 96472655



Supplementary Figure 1. CpG methylation levels in the promoter region of UNC5C in 50 normal colon tissues from cancer-free individuals (green), and in 92 colon tumors (red) and 92 paired normal mucosae (adjacent to the tumor, blue) from sporadic colon cancer patients (with no microsatellite instability). Data obtained from the Illumina Human Methylation Beadchip 450K array. a) Methylation level results represented as stripchart where each dot corresponds to one sample. b) Methylation level results represented as boxplots. c) Methylation differences between tumor and adjacent normal tissue, taking the normal mucosae as reference. Asterisks indicate statistically significant differences (t-test beta with Bonferroni correction). Complete dataset unpublished (in-house data). Almost identical results are obtained when analyzing the publicly available TCGA (The Cancer Genome Atlas) data.



Supplementary Figure 2. Map of the CpG islands (in blue) and CpG sites (red bars) encompassing the *UNC5C* promoter (adapted from MethPrimer program), location of the primers used in the study (blue rectangles) and size of the PCR products. The translation start site of *UNC5C* is indicated by +1.