SUPPLEMENTARY MATERIAL

Supplementary Methods

DISC Study exclusion criteria

The exclusion criteria were being aged <16 or >85 years, being a prisoner at the time of endoscopy, being pregnant or planning to become pregnant, diabetes mellitus, familial adenomatous polyposis syndrome, Lynch Syndrome, known colorectal tumour or prior CRC, prior colorectal resection, active colonic inflammation at endoscopy, iatrogenic perforation at endoscopy, incomplete left-sided examination, colorectal carcinoma discovered at endoscopy, CRC on histology, chemotherapy in the last six months, administering non-steroidal anti-inflammatories e.g. aspirin, anti-coagulants e.g. warfarin or immunosuppressive medication e.g. methotrexate.

Isolation of total RNA

Total RNA for gene expression analyses was extracted from half a rectal mucosal biopsy using the RNeasy Mini Kit (Qiagen, UK) as described by the manufacturers. RNA, for miRNA analyses, was extracted separately from half a rectal mucosal biopsy using the miRNeasy Mini Kit (Qiagen, UK). Tissue disruption was performed by shaking the tissues with five 3mm glass beads (VWR, UK) for 1 minute in Buffer RLT (RNeasy Mini Kit) or QIAzol Lysis Reagent (miRNeasy Mini Kit) using an amalgamator. The lysate and beads were transferred to QiaShredders (Qiagen, UK) for homogenisation. RNA concentration and RNA purity were determined using the NanoDrop 1000 spectrophotometer (Thermo Scientific) and the NanoDrop 1000 Software version 3.7.1. RNA integrity was assessed by agarose gel electrophoresis.

Quantification of gene expression by reverse transcriptase polymerase chain reaction (qPCR)

Two-step reverse transcriptase quantitative polymerase chain reaction (RT-qPCR) was performed. Complementary DNA (cDNA) was synthesised by reverse transcription from 1µg of RNA using the QuantiTect Reverse Transcription Kit (Qiagen, UK) as described by the manufacturers. cDNA was diluted ten times to yield a total volume of 200µl using nuclease-free water.

Quantification of expression of *APC, AXIN2, CTNNB1, FOSL1, GSK3β, c-JUN, SFRP2, WNT5A* and *WNT11* and of the two reference genes *18S* and *β2M* was performed using the QuantiTect® SYBR® Green PCR Kit, QuantiTect® Primer Assays (Qiagen, UK) and the Applied Biosystems® StepOnePlus™ system. Each reaction contained 15µl of master mix (SYBR Green PCR Master Mix, primer assay and RNase-free water) and 5µl of the sample cDNA. PCR cycling was performed with a 15 minute initial activation step at 95°C followed by 40 cycles of 15 seconds denaturation at 94°C, 30 seconds annealing at 55°C and 30 seconds extension at 72°C. Expression of *CCND1, c-MYC, SFRP1* and two reference genes, *18S* and *β2M*, was quantified by qPCR using the Applied Biosystems® StepOnePlus™ system. Each reaction contained 5µl ImmoMix™ (2x) (Bioline, UK), 1µl BSA (Ambion, UK), 0.6µl RNase-free water, 0.2µl ROX (50x) (Invitrogen, UK), 0.1µl $MgCl_2$ (Bioline, UK), 0.06µl SYBR Green (100x) (Invitrogen, UK) and 0.02µl of each forward and reverse primer (100µM) to which 3µl of the sample cDNA were added. PCR cycling was performed with a 10 minute initial activation step at 95°C followed by 40 cycles of 30 seconds denaturation at 95°C, 30 seconds annealing at 60°C and 30 seconds extension at 72°C. All samples were analysed in duplicate.

Quantification of miRNA expression by qPCR

The expression eight miRNAs predicted to be involved in the regulation of the WNT signalling pathway was performed by two-step RT-qPCR. Firstly, 0.8µg of RNA extracted with the miRNeasy Mini Kit was reverse transcribed using the miScript II RT Kit (Qiagen, UK) and miScript HiSpec Buffer as described by the manufacturers. The synthesised cDNA was then diluted ten times with nuclease-free water to yield a total volume of 200µl.

Expression of the eight selected miRNAs and of two reference RNAs, *RNU-6* and *SNORD68*, were quantified by qPCR using the Applied Biosystems® StepOnePlus™ system, miScript SYBR Green PCR Kit and miScript primer assays (Qiagen, UK) as described by the manufacturers.

Isolation of DNA and bisulphite modification

DNA was extracted from half a rectal mucosal biopsy using a phenol-chloroform method. DNA purity and concentration were analysed using the NanoDrop 1000 spectrophotometer (Thermo Scientific) and NanoDrop 1000 Software version 3.7.1. Bisulphite modification of DNA was performed using the EZ DNA Methylation Gold™ Kit (Zymo Research, UK) as described by the manufacturers.

Quantification of *SFRP1* methylation by pyrosequencing

Bisulphite-modified DNA was amplified in duplicate by PCR using the PyroMark CpG assay PCR primer (Hs_SFRP1_01_PM) and HotStarTaq Master Mix (Qiagen, UK) in a 24µl total reaction volume. Pyrosequencing was performed in duplicate using a 5µl volume of PCR product, 3µl PyroMark CpG assay sequencing primer (Hs_SFRP1_01_PM) and Pyromark Q96 reagents and run on the Pyromark Q96 ID pyrosequencer (Qiagen) as described by the manufacturers. Appropriate negative and positive controls including unmethylated and 100% methylated DNA were included.

Whole crypt microdissection

The colorectal crypt cell proliferative state (CCPS) was assessed by counting the number of mitotic figures following whole crypt microdissection and histochemical staining of Carnoy's-fixed rectal mucosal biopsies as described by Mills and colleagues [(19)]. Samples were hydrated in 50% ethanol for 10 minutes at room temperature followed by an identical hydration step in 25% ethanol. Samples were then hydrolysed for 10 minutes in 1ml of 1M HCl at 60°C. Schiff reagent (Surgipath™) was used to stain the samples for one hour at room temperature. The Schiff reagent was removed and replaced with 1ml of 45% acetic acid. Stained samples were microdissected immediately using the Olympus SZ40 dissecting microscope and Leica CLS 150X light source. The stained biopsies were placed on a clean microscope slide (Surgipath™, Leica Microsystems, UK) with a drop of 45% acetic acid and arranged so that the bases of the crypts were facing upwards. Using 25G x 5/8" fine gauge hypodermic needles, (Terumo®, Belgium), rows of crypts were carefully teased apart by separating the serosae and muscularis mucosa. Any connective tissue or Peyer's patches were removed. The crypts were covered with a cover slip (Surgipath®, Leica, UK) and slight pressure was applied to spread the crypts. Prepared samples were counted immediately.

Assessment of colorectal crypt cell proliferative state

A light microscope was used to examine the microdissected crypts under 40x magnification to allow visualisation of individual cells in single crypts. Ten intact crypts were randomly selected per sample. Crypt lengths were measured by placing the graticule at the middle of the base of the crypt in the middle plane of focus and widths were measured by placing the graticule at the widest part of the crypt in the middle plane of focus. Crypt volumes were calculated using length and width measurements and assuming a cylindrical shape. Using the length measurement, the crypt was divided into ten compartments equal in size. The number

of mitotic cells in each compartment was counted by focusing up and down through the crypt, commencing with the first compartment at the base of the crypt. Cells in prophase, metaphase, anaphase or telophase were characterised as mitotic.

Supplementary Table 1 WCRF/AICR recommendations for cancer prevention

WCRF/AICR Recommendation	Public Health Goals	Personal Recommendation
1. Body fatness	Median adult BMI to be between 21 and 23, depending on the normal range for different populations. The proportion of the population that is overweight or obese to be no more than the current level, or preferable lower, in 10 years.	Ensure that body weight through childhood and adolescent growth projects towards the lower end of the normal BMI range at age 21. Maintain body weight within the normal range from age 21. Avoid weight gain and increases in waist circumference throughout adulthood.
2. Physical activity	The proportion of the population that is sedentary to be halved every 10 years. Average physical activity levels to be above 1.6.	Be moderately physically active, equivalent to brisk walking, for at least 30 minutes every day. As fitness improves, aim for 60 minutes or more of moderate, or for 30 minutes or more of vigorous, physical activity every day. Limit sedentary habits such as watching television.
3. Foods and drinks that promote weight gain	Average energy density of diets to be lowered towards 125kcal per 100g. Population average consumption of sugary drinks to be halved every 10 years.	Consume energy-dense foods sparingly. Avoid sugary drinks. Consume 'fast foods' sparingly, if at all.
4. Plant foods	Population average consumption of non-starchy vegetables and of fruits to be at least 600g daily. Relatively unprocessed cereals (grains) and/or pulses (legumes), and other foods that are a natural source of dietary fibre, to contribute to a population average of at least 25g non-starch polysaccharide daily.	Eat at least five portions/servings (at least 400g) of a variety of non-starchy vegetables and of fruits every day. Eat relatively unprocessed cereals (grains) and/or pulses (legumes) with every meal. Limit refined starchy foods. People who consume starchy roots or tubers as staples

		also to ensure intake of sufficient non-starchy vegetables, fruits, and pulses (legumes).
5. Animal foods	Population average consumption of red meat to be no more than 300g a week, very little if any of which to be processed.	People who eat red meat to consume less than 500g a week, very little if any to be processed.
6. Alcoholic drinks	Proportion of the population drinking more than the recommended limits to be reduced by one third every 10 years.	If alcoholic drinks are consumed, limit consumption to no more than two drinks a day for men and one drink a day for women.
7. Preservation, processing, preparation	Population average consumption of salt from all sources to be less than 5g (2g of sodium) a day. Proportion of the population consuming more than 6g of salt a day to be halved every 10 years. Minimise exposure to aflatoxins from mouldy cereals (grains) or pulses (legumes).	Avoid salt-preserved, salted, or salty foods; preserve foods without using salt. Limit consumption of processed foods with added salt to ensure an intake of less than 6g (2.4g sodium) a day. Do not eat mouldy cereals (grains) or pulses (legumes).
8. Dietary supplements	Maximise the proportion of the population achieving nutritional adequacy without dietary supplements.	Dietary supplements are not recommended for cancer prevention.

Supplementary Table 2

Distribution of DISC Study participants' total scores for adherence to the WCRF/AICR recommendations

Total adherence score	n	%	Mean age (SD)
1	5	7	50.0 (4.8)
1.5	1	1	37.0
2	12	16	52.9 (13.1)
2.5	8	11	54.4 (14.6)
3	15	20	47.5 (11.6)
3.5	7	9	52.4 (9.0)
4	13	17	55.5 (16.1)
4.5	3	4	57.0 (5.0)
5	9	12	57.4 (7.2)
5.5	0	0	-
6	2	3	44.0 (19.8)
Mean total adherence score (SD)	3.2 (1.2)		

Supplementary Table 3

Spearman correlation indices and p-values for correlations between total adherence score and measured outcomes.

Outcome	Spearman correlation index	P value
APC	-0.066	0.661
AXIN2	-0.134	0.282
CCND1	-0.293	0.067
CTNNB1	-0.114	0.364
FOSL1	-0.013	0.940
GSK3β	-0.083	0.514
c-JUN	-0.216	0.086
c-MYC	-0.328	**0.039**
SFRP1	-0.117	0.480
SFRP2	0.179	0.168
WNT5A	-0.119	0.353
WNT11	-0.407	**0.003**
miR-17	-0.041	0.765
miR-19a	0.054	0.690
miR-19b	0.131	0.336
miR-20a	0.036	0.793
miR-25	0.021	0.878
miR-93	-0.002	0.986
miR-106b	0.085	0.535
miR-424	0.205	0.129
SFRP1 methylation (mean)	-0.070	0.565
Total mitoses	-0.081	0.505
Proportion of mitotic cells in upper half of crypt	-0.094	0.440
Crypt length	-0.002	0.988
Crypt width	-0.216	0.073
Crypt volume	-0.179	0.139

Supplementary Table 4 Spearman correlation indices and p-values for correlations between measurements for individual recommendations and measured outcomes.

	BMI		Levels of moderate physical activity		Fruit and vegetable intake		NSP intake		Alcohol intake		Red meat intake		Sodium intake	
Outcome	**ρ**	**P value**	**ρ**	**P value**	**ρ**	**P value**	**ρ**	**P value**	**ρ**	**P value**	**ρ**	**P value**	**ρ**	**P value**
APC	0.192	0.200	-0.145	0.336	-0.004	0.978	-0.591	0.695	-0.198	0.187	0.187	0.212	0.016	0.917
AXIN2	0.217	0.080	-0.131	0.293	-0.137	0.270	-0.265	**0.032**	-0.107	0.391	0.008	0.950	-0.118	0.343
CCND1	0.034	0.831	0.038	0.816	0.088	0.587	0.088	0.587	0.015	0.925	0.206	0.201	0.185	0.251
CTNNB1	0.175	0.164	-0.167	0.184	-0.203	0.105	-0.380	**0.002**	-0.150	0.232	0.007	0.956	-0.264	**0.034**
FOSL1	-0.088	0.603	-0.154	0.362	-0.243	0.146	-0.180	0.284	-0.052	0.757	0.016	0.925	0.008	0.961
GSK3β	0.199	0.114	-0.133	0.295	0.033	0.798	-0.275	**0.028**	-0.058	0.649	-0.053	0.680	-0.215	0.088
c-JUN	0.128	0.312	-0.182	0.150	-0.133	0.293	-0.081	0.524	-0.179	0.157	0.048	0.704	-0.050	0.696
c-MYC	0.053	0.742	-0.190	0.239	-0.081	0.616	-0.055	0.735	-0.085	0.601	0.200	0.214	0.055	0.735
SFRP1	0.145	0.378	-0.089	0.588	0.185	0.258	0.097	0.557	-0.199	0.222	0.027	0.869	0.139	0.396
SFRP2	0.119	0.361	0.034	0.796	0.156	0.229	0.019	0.885	-0.187	0.148	0.008	0.951	-0.009	0.948
WNT5A	0.171	0.180	-0.235	0.064	-0.073	0.569	-0.218	0.086	-0.154	0.228	0.016	0.902	-0.133	0.297
WNT11	0.020	0.891	-0.334	**0.018**	-0.384	**0.006**	-0.261	0.068	0.160	0.266	0.156	0.276	-0.246	0.085
miR-17	-0.151	0.265	-0.022	0.871	-0.177	0.191	-0.271	**0.044**	-0.177	0.191	0.052	0.705	-0.233	0.084
miR-19a	-0.185	0.171	-0.088	0.518	0.025	0.853	-0.031	0.817	0.034	0.803	-0.006	0.964	0.067	0.625
miR-19b	-0.285	**0.034**	-0.000	0.999	0.077	0.573	0.046	0.738	0.178	0.188	-0.060	0.659	0.105	0.439
miR-20a	-0.147	0.278	0.069	0.613	0.036	0.794	-0.083	0.541	0.072	0.595	-0.009	0.946	-0.114	0.403
miR-25	0.037	0.785	0.037	0.784	0.040	0.775	0.105	0.438	-0.092	0.497	0.074	0.589	-0.088	0.519
miR-93	0.023	0.868	-0.101	0.456	-0.123	0.367	-0.041	0.762	-0.076	0.578	0.050	0.716	-0.101	0.456

	BMI		Levels of moderate physical activity		Fruit and vegetable intake		NSP intake		Alcohol intake		Red meat intake		Sodium intake	
Outcome	**ρ**	**P value**	**ρ**	**P value**	**ρ**	**P value**	**ρ**	**P value**	**ρ**	**P value**	**ρ**	**P value**	**ρ**	**P value**
miR-106b	-0.193	0.153	0.041	0.765	-0.073	0.594	-0.087	0.524	0.047	0.732	0.066	0.628	-0.094	0.491
miR-424	-0.123	0.363	0.030	0.828	0.036	0.789	-0.054	0.694	-0.036	0.790	-0.034	0.802	-0.087	0.520
***SFRP1* methylation (mean)**	-0.034	0.781	-0.075	0.538	-0.037	0.761	0.032	0.795	0.035	0.771	0.107	0.375	-0.009	0.941
Total mitoses	-0.079	0.516	0.008	0.946	0.006	0.963	0.005	0.970	0.023	0.853	0.129	0.285	0.012	0.921
Mitotic cells in upper half of crypt (%)	0.068	0.578	0.133	0.271	-0.062	0.608	-0.068	0.576	0.073	0.547	0.168	0.163	-0.072	0.553
Crypt length	0.525	**0.035**	0.034	0.780	-0.018	0.882	-0.010	0.932	-0.101	0.405	0.022	0.858	-0.052	0.669
Crypt width	-0.022	0.860	0.070	0.566	0.072	0.552	0.153	0.208	-0.051	0.677	0.141	0.244	0.094	0.440
Crypt volume	0.086	0.480	0.057	0.637	0.036	0.769	0.121	0.317	-0.077	0.525	0.142	0.241	0.071	0.558