Supplementary Information for

Epigenetics override proinflammatory PTGS transcriptomic signature
towards selective hyperactivation of PGE₂ in colorectal cancer

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SUPPLEMENTARY METHODS

TCGA data
Methylation data of 16 cancers types (COAD, STAD, READ, PAAD, THCA, BLCA, BRCA, LGG, PRAD, KIRC, KIRP, LUSC, GBM, LUAD, UCEC, LIHC) were downloaded from The Cancer Genome Atlas (TCGA) (www.cancergenome.nih.gov). For each tumor type, paired tumors and adjacent normal mucosae were analyzed, together with additional unmatched tumors. Sample preparation details can be found in the TCGA documentation section. For our analysis, we used the beta-values obtained from the level_3methylation data. Samples were considered hypermethylated when their mean beta-value was at least the double of the mean of the controls (considering all probes in the area).

Cell lines
Cell lines HCT116, HT-29, DLD-1, SW480, LoVo were obtained from the American Type Culture Collection (ATCC) and grown in Dulbecco’s Modified Eagle Medium (DMEM)/F12 (1:1) supplemented with 100mM Sodium Pyruvate, 200mM L-Glutamine, 10% FBS (Invitrogen), at 37ºC in humidified 95% air and 5% CO₂. HCT116-DKO cells (HCT116 deficient in both DNA methyltransferases [1]) were cultured under the same conditions.

Methylation data
Genomic DNA was extracted from peripheral leukocytes by the salting-out procedure (Miller et al, 1988) QIAmp. The extracted DNA was quantified using a Nano Drop ND 2000c spectrophotometer (NanoDrop Thermo scientific, Wilmington, DE) and stored at 4C. 200-600 ng of DNA were treated with bisulfite using EZ DNA methylation kit (Zymo Research. Cat. No. D5004) according to manufacturer’s recommendations. The incubation profile was 16 cycles at 95C for 30s, 50C for 60 min and a final holding step at 4C [2]. DNA methylation profiles were generated using the Illumina Human Methylation 450K BeadChip assay (technical details of this array are described elsewhere [3]). This array involves whole genome amplification, interrogate the methylation levels of 485,577 CpG sites on bisulfite-treated DNA, covering 99% of RefSeq genes and 96% of CpG island regions such as CpG island, island shore and shelf, 5’ and 3’ UTRs, and promoter and gene body [3]. The methylation level is represented by the β value resulting from the ratio of intensity signal obtained from the methylated allele over the sum of methylated and unmethylated allele intensity bead signals. β values may take any value between 0 (non-methylated) and 1 (completely methylated).

We used raw *.idat files as a starting point for this pipeline, based on the Bioconductor library “minfi”. Here, the outliers were removed by inspecting the overall signal intensity, the distribution of M-values and the control probe profile. We also checked on the sex specification obtained from the results of the Affymetrix Genome-Wide Human SNP 6.0 array (Affymetrix, Santa Clara, USA), using the observed genotypes of SNPs on chromosome X and Y. All individuals who were marked as “male” but with significant amount of heterozygous X genotypes (>=1%), or who were marked as “female” but with high frequency of homozygous X genotypes (>=80%) or Y genotype readings, were individually inspected against original data source. If no satisfactory correction could be obtained these individuals were excluded from further analyses. A clustering between the 19 common SNPs of the HumanMethylation450K array and affy 6.0 to check the correct pairing between N-T was performed. A total of 17 samples were excluded for these reasons. Thus, a final dataset of 240 arrays was used for subsequent analyses.

Probes with a minfi detection p value > 0.01 exceeding 5% of the samples were annotated as missing, we removed probes on chromosomes X and Y and the assays measuring 65 SNPs (from Illumina manifest). Afterwards, subset-quantile within array normalization (SWAN) introduced sub-quantile
normalization for the methylated and unmethylated channels separately, assuming that the distribution of the probes with similar number of CpGs should be similar, irrespective of probe type (n=481,019) [4]. Methylation probes with low variability were removed (SD < 0.04). We discarded 41,082 probes that ambiguously mapped to multiple locations in the human genome with up to two mismatches [5]. We excluded a further 11,765 probes that contained SNPs within 10 bp of the CpG interrogate (from 1,000 genomes project, CEU population). This resulted in a final set of 430,158 probes that were used in all further analyses. We mapped the 485,577 Illumina probes to the human genome sequence (hg18) using UCSC genome browser having a complete annotation of all genes. To set the methylation beta value of each gene promoter we used the median of the beta values of probes located at the 5’UTR, and within 1.5 Kb of the TSS according to the UCSC genome browser annotation. Probes are listed below:

| Gene (HumanMethylation450K array probes) |
|------------------------------------------|
| PTGES2 (cg01311102 cg13880225 cg14092536 cg22637398 cg14324338 cg10219895 cg13826862 cg13833419 cg16020551 cg05930684 cg24730207) |
| PTGES2 (cg18335243 cg08482694 cg17419623 cg09461185 cg24887140 cg26564040 cg10180406 cg25837803 cg23070111 cg25147026 cg16101346) |
| PTGES3 (cg04686114 cg12507325 cg03226218 cg16823406 cg08027110 cg20253639 cg07281688 cg1439834 cg22366350) |
| PTGES5 (cg04831490 cg17683775 cg14390764) |
| TBXAS2 (cg06384607 cg25307468 cg15861540 cg04985611 cg09998229 cg08069088 cg11729169 cg18738857 cg07481491 cg22757101 cg19225308 cg01209635 cg23846712 cg05343404) |
| PTG2R (cg21502048 cg16134491 cg26674715 cg06738602 cg20055841 cg08423052 cg23693907) |
| AKR1B1 (cg18416881 cg16132520 cg13801416 cg06864853 cg10795359 cg14629509 cg21079345 cg02215070 cg09957386 cg08167706 cg23918923 cg04663564) |
| PTGER1 (cg10468702 cg21533997 cg24360051 cg03949996 cg27524460 cg03020379) |
| PTGER2 (cg04311682 cg11822964 cg07844738 cg04706667) |
| PTGER3 (cg18369034 cg23022053 cg10857896 cg24089118 cg18242103 cg24989962 cg17929687 cg02191312 cg05302386 cg09516965) |
| PTGER4 (cg06802812 cg08164315 cg06013215 cg03337243 cg17421097 cg02026948 cg04727116 cg21149775 cg25664622 cg01952088 cg01897756 cg04126707 cg04790357 cg04824304 cg26597539) |
| PTGDS (cg00563932 cg18502630) |
| PTGER5 (cg23647191 cg14963735 cg12739034 cg03726881 cg00915178 cg20751856 cg00781169) |
| PTGIS (cg11746278 cg15543919 cg16537756) |
| PTGFIR (cg04410756 cg09658982 cg24022301 cg03949931 cg27046936 cg03495868 cg24924936 cg00701741 cg02859837 cg19403909) |
| PTG51 (cg13974165 cg00501774 cg10493166 cg14154487 cg12927153 cg13630095) |
| HPGD (cg03772063 cg06369891 cg02822257 cg07372795 cg11073923 cg00906130 cg18164599 cg15474754 cg13181537 cg05527430 cg04555941 cg01084566 cg20455617) |

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Table S1. Samples used in this study

| Series         | Tissue Type                        | no of samples          | Molecular data                  | Data access                        | Reference          |
|----------------|-----------------------------------|------------------------|---------------------------------|------------------------------------|--------------------|
| HGTiP/ICO      | tumor and paired adjacent normal   | 64 cancers 64 adjacent | Gene expression, DNA methylation | this paper                         | this paper         |
| Colonomics     | Healthy mucosa, tumor and adjacent normal | 50 healthy 98 cancers 98 adjacent | Gene expression, DNA methylation | http://www.colonomics.org          | this paper         |
| TCGA           | tumor and adjacent normal          | 254 cancers 38 adjacent | DNA methylation                 | https://tcga-data.nci.nih.gov/tcga/ | Weinstein et al [6]|
| Luo et al.     | Healthy mucosa, tumor and adjacent normal | 19 healthy 42 adenomas 22 adjacent 64 cancers | DNA methylation                 | GEO: GSE48684          | Luo et al [7]      |
### Table S2. Nomenclature of the PTGS pathway genes analyzed in this study.

| Gene     | Protein                                      | Alternative name                          |
|----------|----------------------------------------------|-------------------------------------------|
| AKR1B1   | Aldo-keto reductase family 1, member B1      | PGFS (prostaglandin F2alpha synthase)     |
| HPGD     | Hydroxyprostaglandin dehydrogenase 15-(NAD) |                                           |
| PTGDR    | Prostaglandin D2 receptor                    | DP                                        |
| PTGDS    | Prostaglandin D2 synthase                    |                                           |
| PTGER1   | Prostaglandin E2 receptor 1                  | EP1                                       |
| PTGER2   | Prostaglandin E2 receptor 2                  | EP2                                       |
| PTGER3   | Prostaglandin E2 receptor 3                  | EP3                                       |
| PTGER4   | Prostaglandin E2 receptor 4                  | EP4                                       |
| PTGES    | Prostaglandin E2 synthase                    |                                           |
| PTGES2   | Prostaglandin E2 synthase 2                  |                                           |
| PTGES3   | Prostaglandin E2 synthase 3 (cytosolic)      | cPGES                                     |
| PTGFR    | Prostaglandin F2alpha receptor               | FP                                        |
| PTGIR    | Prostacyclin receptor                        | IP                                        |
| PTGIS    | Prostacyclin synthase                        | PGIS                                      |
| PTGS1    | Prostaglandin-endoperoxide synthase 1        | COX-1 (Cyclooxygenase-1)                  |
| PTGS2    | Prostaglandin-endoperoxide synthase 2        | COX-2 (Cyclooxygenase-1)                  |
| TBXA2R   | Thromboxane A2 receptor                      | TP                                        |
Table S3. Clinicopathological correlates of gene expression in the mucosa of healthy controls (NORMAL) and in CRC patients (ADJACENT MUCOSA and TUMOR)*.

|                  | NORMAL MUCOSA | ADJACENT MUCOSA (AM) | TUMOR (T) | FOLD CHANGE (LOG2(T/AM)) |
|------------------|--------------|----------------------|-----------|-------------------------|
|                  | Sex  | Age | Site | Sex  | Age |Location| Metastasis | Stage | Sex | Age | Site | Metastasis | Stage | Sex | Age | Site | Metastasis |
| **PTGES2**       | 0.4178 | 0.5689| 0.9161| 0.8712| 0.5026| 0.9031| 0.9853| 0.9289| 0.7230| 0.3529| 0.4890| 0.3072| 0.8820| 0.8061| 0.7458| 0.7956| 0.4485| 0.7412 |
| **PTGS2**        | 0.8169 | 0.7871| 0.0660| 0.5679| 0.3495| 0.3174| 0.9853| 0.9289| 0.7985| 0.3529| 0.4105| 0.8277| 0.8820| 0.8061| 0.9810| 0.1546| 0.9458| 0.8752 |
| **PTGES3**       | 0.5617 | 0.5689| 0.3823| 0.8712| 0.8988| 0.1724| 0.8277| 1.0000| 0.7230| 0.7501| 0.7930| 0.7585| 0.8820| 0.8061| 0.9810| 0.5410| 0.5458| 0.8752 |
| **PTGES**        | 0.5617| 0.7871| 0.3790| 0.6451| 0.5761| 0.2311| 0.2800| 0.9289| 0.7333| 0.3529| 0.7959| 0.9119| 0.8820| 0.8061| 0.9097| 0.7956| 0.9458| 0.8752 |
| **TBX2R**        | 0.4882| 0.7295| 0.9385| 0.6451| 0.5761| 0.9031| 0.9855| 0.9289| 0.7230| 0.4632| 0.8490| 0.8277| 0.8820| 0.8061| 0.6911| 0.7956| 0.9485| 0.7412 |
| **PTGER2**       | 0.4178| 0.5689| 0.0660| 0.5679| 0.8988| 0.3284| 0.1304| 0.9289| 0.8956| 0.7808| 0.7959| 0.0192| 0.8820| 0.8508| 0.9779| 0.8311| 0.2080| 0.8752 |
| **AKR1B1**       | 0.6053| 0.5689| 0.3990| 0.5679| 0.8571| 0.9031| 0.8277| 0.9289| 0.9431| 0.3529| 0.7959| 0.6058| 0.8820| 0.8061| 0.7458| 0.7956| 0.9458| 0.8752 |
| **PTGER1**       | 0.4882| 0.7871| 0.2673| 0.6793| 0.5761| 0.9583| 0.8277| 1.0000| 0.8523| 0.6061| 0.3805| 0.9119| 0.8820| 0.8061| 0.6911| 0.6756| 0.9485| 0.8752 |
| **PTGIR**        | 0.7894| 0.7871| 0.4982| 0.8712| 0.8653| 0.9031| 0.4130| 0.9289| 0.7230| 0.3529| 0.8753| 0.6037| 0.8820| 0.8061| 0.6911| 0.8311| 0.3156| 1.0000 |
| **PTGDR**        | 0.6053| 0.5689| 0.3790| 0.8712| 0.3495| 0.9031| 0.4130| 0.6834| 0.7230| 0.5106| 0.0408| 0.3072| 0.8820| 0.8061| 0.9810| 0.2891| 0.2080| 0.8752 |
| **PTGER4**       | 0.4882| 0.7871| 0.4539| 0.6238| 0.5761| 0.9031| 0.5524| 1.0000| 0.9256| 0.7808| 0.7959| 0.6037| 0.8820| 0.8061| 0.9440| 0.7956| 0.3156| 0.8752 |
| **PTGDS**        | 0.4178| 0.7295| 0.2673| 0.6451| 0.8988| 0.3174| 0.0288| 1.0000| 0.7230| 0.5106| 0.8753| 0.6037| 0.8820| 0.8061| 0.7458| 0.7956| 0.9458| 0.8752 |
| **PTGER3**       | 0.7150| 0.7670| 0.1790| 0.6451| 0.8653| 0.7221| 0.8277| 0.9289| 0.7985| 0.3529| 0.7930| 0.9855| 0.8820| 0.8506| 0.7458| 0.8311| 0.9458| 0.8752 |
| **PTGIS**        | 0.5617| 0.7871| 0.3790| 0.5679| 0.5026| 0.9031| 0.9663| 0.9289| 0.7230| 0.3529| 0.6108| 0.8523| 0.8820| 0.8061| 0.9779| 0.6756| 1.0000| 0.8752 |
| **PTGFR**        | 0.4882| 0.7871| 0.1110| 0.6451| 0.8988| 0.9031| 0.5524| 0.6834| 0.8523| 0.3529| 0.7673| 0.6037| 0.8820| 0.8061| 0.6911| 0.7956| 0.9458| 0.7412 |
| **PTGS1**        | 0.1321| 0.7670| 0.1268| 0.5679| 0.3495| 0.9031| 0.8277| 0.9289| 0.7230| 0.5106| 0.0408| 0.6037| 0.8820| 0.8508| 0.9779| 0.1546| 0.9458| 0.8752 |
| **HPGD**         | 0.7894| 0.5689| 0.3790| 0.8712| 0.8988| 0.9031| 0.4130| 0.8610| 0.7985| 0.3529| 0.7959| 0.3011| 0.8820| 0.8061| 0.6911| 0.7956| 0.2080| 0.8752 |

* Gene expression was analysed with SurePrint arrays. Data were obtained from the Colonomics project and show Wilcoxon P values adjusted using Benjamin and Hochberg method.
Gene expression values in regard to the features that are statistically significant (from Table S3)

| LOCATION | PTGDS MEAN | SD   | P   |
|----------|------------|------|-----|
| site_Left (n=61) | 7.4264 | 0.9349 | 0.0288 |
| site_Right (n=39) | 8.0669 | 1.1159 |       |

| AGE | JT | MEAN | SD | P   |
|-----|----|------|----|-----|
| age_<=65 (n=26) | 3.3739 | 1.4867 | 0.0408 |
| age_>65 (n=74) | 2.5285 | 0.6461 |       |

| AGE | PTGDR MEAN | SD  | P   |
|-----|------------|-----|-----|
| age_<=65 (n=26) | 4.9576 | 1.0601 | 0.0408 |
| age_>65 (n=74) | 4.2757 | 0.9525 |       |

| LOCATION | PTGER2 MEAN | SD   | P   |
|----------|-------------|------|-----|
| site_Left (n=61) | 3.8527 | 0.8970 | 0.0192 |
| site_Right (n=39) | 4.8345 | 1.5711 |       |
Table S4. Clinicopathological correlates of DNA methylation in the mucosa of healthy controls (NORMAL) and in CRC patients (ADJACENT MUCOSA and TUMOR)*.

| Gene  | Sex | Age | Site | Stage | Sex | Age | Site | Metastasis | Sex | Age | Site | Metastasis | FOLD CHANGE (LOG2(T/AM)) |
|-------|-----|-----|------|-------|-----|-----|------|------------|-----|-----|------|------------|------------------------|
| PTGES2 | 0.9605 | 0.9740 | 0.5372 | 0.8843 | 0.5233 | 0.7111 | 1.0000 | 0.9229 | 0.6120 | 0.6366 | 0.6029 | 0.9164 | 0.4045 | 0.2976 | 0.9398 | 0.9712 | 0.7733 | 0.6260 |
| PTGS2 | 0.7344 | 0.3789 | 0.4412 | 0.7105 | 0.5233 | 0.9648 | 0.0003 | 0.9229 | 0.8218 | 0.9638 | 0.4661 | 0.0045 | 0.9960 | 0.4973 | 0.6197 | 0.8025 | 0.0776 | 0.7049 |
| PTGES3 | 0.7344 | 0.9745 | 0.4412 | 0.8843 | 0.8580 | 0.9648 | 0.3016 | 0.9229 | 0.3513 | 0.9638 | 0.1518 | 0.8415 | 0.9960 | 0.9668 | 0.9398 | 0.5515 | 0.6336 | 0.6993 |
| PTGES | 0.7344 | 0.9745 | 0.7467 | 0.7105 | 0.3636 | 0.3876 | 0.0953 | 0.9229 | 0.6120 | 0.9638 | 0.2437 | 0.6323 | 0.9960 | 0.9061 | 0.6239 | 0.8676 | 0.7733 | 0.6993 |
| TBA2R | 0.7344 | 0.9745 | 0.7467 | 0.9155 | 0.5233 | 0.7111 | 0.0057 | 0.9996 | 0.3513 | 0.9638 | 0.1518 | 0.3276 | 0.4045 | 0.2812 | 0.9398 | 0.0618 | 0.9840 | 0.6260 |
| PTGER2 | 0.9662 | 0.8878 | 0.4412 | 0.8843 | 0.4070 | 0.9648 | 0.0319 | 0.9229 | 0.3513 | 0.9638 | 0.1518 | 0.9164 | 0.3843 | 0.2106 | 0.9398 | 0.3336 | 0.7733 | 0.6993 |
| AKR1B1 | 0.6770 | 0.9745 | 0.1611 | 0.9155 | 0.8369 | 0.9648 | 1.0000 | 0.9996 | 0.8929 | 0.2988 | 0.6795 | 0.4383 | 0.9996 | 0.9162 | 0.6197 | 0.9712 | 0.4973 | 0.7122 |
| PTGER1 | 0.7344 | 0.9745 | 0.9477 | 0.8843 | 0.5233 | 0.9648 | 0.0497 | 0.9229 | 0.3513 | 0.9638 | 0.7975 | 0.8415 | 0.9996 | 0.2812 | 0.6197 | 0.9330 | 0.4973 | 0.6993 |
| PTGIR | 0.8839 | 0.3087 | 0.1412 | 0.8843 | 0.9114 | 0.9648 | 0.0120 | 0.9229 | 0.9683 | 0.9638 | 0.9748 | 0.6323 | 0.9996 | 0.7744 | 0.9398 | 0.9350 | 0.2995 | 1.0000 |
| PTGDOR | 0.3274 | 0.9917 | 0.4412 | 0.8843 | 0.5839 | 0.9648 | 0.0139 | 0.9229 | 0.3513 | 0.9638 | 0.5581 | 0.9164 | 0.4045 | 0.2812 | 0.9398 | 0.8025 | 0.7733 | 0.6260 |
| PTGER4 | 0.7042 | 0.9745 | 0.4412 | 0.8843 | 0.5233 | 0.7111 | 0.5555 | 0.9229 | 0.8218 | 0.9638 | 0.9748 | 0.9549 | 1.0000 | 0.2812 | 0.6239 | 0.8025 | 0.7733 | 0.9950 |
| PTGDS | 0.4550 | 0.3087 | 0.3832 | 0.9155 | 0.5233 | 0.9648 | 0.0003 | 0.9229 | 0.9683 | 0.9638 | 0.8915 | 0.9164 | 0.9996 | 0.9162 | 0.9398 | 0.9712 | 0.4973 | 0.9981 |
| PTGER3 | 0.7029 | 0.9745 | 0.7467 | 0.8843 | 0.5233 | 0.9671 | 0.8086 | 1.0000 | 0.9683 | 0.9638 | 0.9748 | 0.0578 | 0.9996 | 0.9668 | 0.9398 | 0.9330 | 0.1363 | 0.7049 |
| PTGIS | 0.3274 | 0.3136 | 0.3832 | 0.8843 | 0.5064 | 0.3792 | 0.5498 | 0.9229 | 0.6120 | 0.9638 | 0.2437 | 0.6323 | 0.9996 | 0.4973 | 0.9398 | 0.5515 | 0.7733 | 0.9950 |
| PTGFR | 0.7042 | 0.0129 | 0.0841 | 0.9155 | 0.3636 | 0.0038 | 0.7648 | 0.9229 | 0.6120 | 0.9638 | 0.6169 | 0.9164 | 0.9996 | 0.2976 | 0.6197 | 0.8025 | 0.7733 | 0.6993 |
| PTG51 | 0.7042 | 0.9745 | 0.9477 | 0.8843 | 0.3636 | 0.9648 | 0.0008 | 0.9229 | 0.3513 | 0.9638 | 0.1745 | 0.8415 | 0.5430 | 0.2812 | 0.9398 | 0.3336 | 0.4973 | 0.6993 |
| HPGD | 0.7042 | 0.9745 | 0.9477 | 0.8843 | 0.5233 | 0.9648 | 0.1179 | 0.9229 | 0.3513 | 0.9638 | 0.9748 | 0.8415 | 0.9996 | 0.2106 | 0.6197 | 0.9712 | 0.7733 | 0.9981 |

* Gene expression was analysed with SurePrint arrays. Data were obtained from the Colonomics project and Wilcoxon P values adjusted using Benjamin and Hochberg method.
Gene expression values in regard to the features that are statistically significant (from Table S4)

|                  | NORMAL MUCOSA | ADJACENT MUCOSA | TUMOR       |
|------------------|---------------|-----------------|-------------|
| **AGE**          |               |                 |             |
| **PTGFR**        | MEAN | SD  | P    |
| age_<=65 (n=35)  | 0.3431 | 0.0379 | 0.0129 |
| age_>65 (n=26)   | 0.3940 | 0.0528 |         |
| **AGE**          | MEAN | SD  | P    |
| age_<=65 (n=26)  | 0.3526 | 0.0772 | 0.0038 |
| age_>65 (n=74)   | 0.3830 | 0.0575 |         |
| **LOCATION**     | MEAN | SD  | P    |
| site_Left (n=61) | 0.2091 | 0.1670 | 0.0045 |
| site_Right (n=39)| 0.3735 | 0.2304 |         |
| **PTGFS2**       | MEAN | SD  | P    |
| site_Left (n=27) | 0.1228 | 0.0791 | 0.0003 |
| site_Right (n=34)| 0.1355 | 0.0300 |         |
| **TBX2A2R**      | MEAN | SD  | P    |
| site_Left (n=27) | 0.2244 | 0.0396 | 0.0057 |
| site_Right (n=34)| 0.2476 | 0.0329 |         |
| **PTGER2**       | MEAN | SD  | P    |
| site_Left (n=27) | 0.1770 | 0.0287 | 0.0319 |
| site_Right (n=34)| 0.1953 | 0.0306 |         |
| **PTGER1**       | MEAN | SD  | P    |
| site_Left (n=27) | 0.1904 | 0.0251 | 0.0497 |
| site_Right (n=34)| 0.1969 | 0.0161 |         |

Continued in next page
# ADJACENT MUCOSA

| LOCATION | PTGIR | MEAN  | SD    | P    |
|----------|-------|-------|-------|------|
| site_Left (n=27) | 0.8312 | 0.0264 | 0.0120 |
| site_Right (n=34) | 0.8208 | 0.0169 | NA    |

| LOCATION | PTGDR | MEAN  | SD    | P    |
|----------|-------|-------|-------|------|
| site_Left (n=27) | 0.2255 | 0.0505 | 0.0139 |
| site_Right (n=34) | 0.2432 | 0.0342 |       |

| LOCATION | PTGDS | MEAN  | SD    | P    |
|----------|-------|-------|-------|------|
| site_Left (n=27) | 0.7668 | 0.0216 | 0.0003 |
| site_Right (n=34) | 0.7473 | 0.0214 |       |

| LOCATION | PTGS1 | MEAN  | SD    | P    |
|----------|-------|-------|-------|------|
| site_Left (n=27) | 0.2117 | 0.0269 | 0.0008 |
| site_Right (n=34) | 0.2310 | 0.0213 |       |
### Table S5. Information of CRC patients studied in the expression preliminary analysis and in the DNA methylation dissociation curve analysis.

| Id   | AGE | SEX | STAGE |
|------|-----|-----|-------|
| CR10 | 49  | M   | II    |
| CR12 | 65  | M   | III   |
| CR13 | 86  | F   | III   |
| CR15 | 69  | M   | III   |
| CR16 | 56  | F   | III   |
| CR17 | 76  | F   | III   |
| CR18 | 75  | F   | III   |
| CR19 | 72  | F   | III   |
| CR20 | 63  | M   | III   |
| BH001| 76  | M   | III   |
| BH002| 74  | F   | II    |
| BH003| 84  | M   | III   |
| BH005| 71  | M   | II    |
| BH006| 59  | F   | III   |
| BH007| 48  | M   | III   |
| BH017| 74  | F   | II    |
| BH021| 62  | F   | III   |
| BH022| 84  | F   | III   |
| BH026| 80  | F   | II    |
| BH027| 64  | M   | III   |
| BH028| 68  | M   | III   |
| BH038| 81  | M   | III   |
| BH046| 67  | M   | II    |
| BH050| 73  | M   | II    |
| BH051| 51  | F   | II    |
| ID009| 67  | M   | II    |
| ID014| 64  | F   | II    |
| ID016| 70  | F   | III   |
| ID017| 31  | F   | III   |
| ID025| 67  | M   | II    |
| ID046| 68  | M   | II    |
| ID063| 74  | F   | III   |
| ID072| 73  | M   | III   |
| ID078| 62  | M   | II    |
| ID102| 78  | M   | II    |
| ID114| 76  | F   | II    |
| ID118| 51  | F   | III   |
| ID138| 80  | F   | II    |
| ID143| na  | F   | III   |
| ID145| 73  | F   | II    |
| ID146| 65  | F   | II    |
| ID154| 54  | M   | III   |
| ID186| 56  | M   | II    |
| ID223| 75  | M   | II    |
| ID303| 67  | F   | II    |
| ID346| 64  | M   | II    |
| ID369| 76  | M   | III   |
| ID377| 81  | M   | III   |
| ID385| 64  | F   | III   |
| ID405| na  | M   | na    |
| ID437| 59  | F   | II    |
| ID450| 56  | M   | III   |
| ID495| 71  | F   | II    |
| ID528| 63  | F   | II    |
| ID541| 72  | M   | II    |
| ID544| 61  | F   | III   |
| ID549| 79  | M   | III   |
| ID551| 58  | F   | III   |
| ID555| 49  | F   | III   |
| ID557| 75  | M   | III   |
| ID563| na  | M   | na    |
| ID597| na  | F   | na    |
| ID608| 73  | F   | III   |
| ID628| na  | F   | na    |
Table S6. Primers used in this study for the expression analysis of PTGS pathway genes.

| Gene   | Product position | Forward primer          | Reverse primers            | Product size (bp) |
|--------|------------------|-------------------------|---------------------------|------------------|
| AKR1B1 | chr7:134136426-134143806 | CCGTCTCTGCTCAACAAC | TACACATGGGCACAGTCGAT | 138              |
| PTGS1  | chr9:125152594-125154599   | AAACCTACACCTCCTCCA    | CCCCCATCTCTATCATACTCT    | 163              |
| PTGS2  | chr1:186643812-186645076   | GAATTACCCAGTTGTGAATC  | TTCTACAGAGGGAGGAT       | 278              |
| HPGD   | chr4:175413148-175414389   | AAATCTGAAAAAGAAAGAAA  | AAAATGAAATCCTTGGAG      | 186              |
| PTGDR  | chr14:52735343+52741617    | ACCGTGCTCTCCTATAGTG   | AAAATTTGAAAAATATCGG     | 205              |
| PTGDS  | chr9:139875286+13987611    | TAAATGCAATGGGAGGAT    | AGGGCTCGGGGAAGAA        | 110              |
| PTGER1 | chr19:14583514-14584209    | CTGCTGGACCCAAATGC     | TGGCGCATGGATGATC        | 144              |
| PTGER2 | chr14:52782009+52794013    | GCAAGGAGAGGGAAGG     | AATGTGAAAAACCTAAGGC     | 176              |
| PTGER3 | chr1:71478094-71512579     | CTTTCTTGAGCTCTGGCTTG  | TCTTCTGCTTCTCCTGGT      | 290              |
| PTGER4 | chr5:40681909-81373848     | ATCTTACTCATATGGCAAG   | ATTTTACTGACTCTCTG       | 114              |
| PTGES  | chr9:132502095-132515232   | CACGCTGCTGTCATC       | AGGAAAGGAGGGTAG         | 218              |
| PTGES2 | chr9:130883406-130884707   | GTGCGGTGCTGCTGGG     | TGCTTGCCGGGGGACATT      | 195              |
| PTGES3 | chr12:57058139-57060035    | GGTGTTGATAGGGATG      | GGTTAAGTGGCAATAC        | 214              |
| PTGFR  | chr1:78959186+78963629     | CGATTTAGTTGCTCCTGGT   | CTATGTAAGGATCCTATTCG    | 112              |
| PTGIR2 | chr1:712124350-71244424    | GCCGATCGTCTGCTTCTCC   | TCTTCTGCTTCTCCTTCTC     | 75               |
| PTGIS2 | chr1:48140729-48156126     | CCGTGGTCCTCTCTCAGT    | GCAACTTCCACAGGGCAT      | 72               |
| TBX2AR | chr19:3595775-3599890       | TGGGGATCATGGTGGTG     | CGGGCGGGCGGCCAGG        | 201              |

1 Genome Browser Version Feb. 2009 (GRCh37/hg19)
2 from reference [8] (references are listed in page 4)
3 from reference [9]
Table S7. Primers used in this study for dissociation curve analysis and bisulfite sequencing of DNA methylation content.

| Gene   | Outer forward primer | Outer reverse primer | Inner forward primer | Inner reverse primer | Product size (bp) |
|--------|-----------------------|----------------------|----------------------|----------------------|------------------|
| AKR1B1 | GAGTATTTTTTTTATTTTATT | TTTCCCACCAATAACCAAC | AATTTTAGGGTGATTTGTGT | AAACAAAAAACTCTTTCTAC | 325              |
| HPGD   | GGGTTTTTTTTTTTATTTTTT | CTATCAACTACCTACCAAT | GAAGTTGGTGGTTAGAGAAA | AAACAAAAACTTTTAAAAC | 403              |
| PTGDR  | ATTTTTAGTATTTTTTTTTTTT | AACACCACCCCAACCCC  | AAAACAAAAAAATACCAAA  | GAGTTTGGTTTATTGAGAA | 420              |
| PTGDS  | TTTTTGAGGAGGAGTGGAG  | ATCTATACCTACCTACCTAC | GTTTTAGTGGTGTTAGG     | AAACAAAAAACTTTTAAAAC | 304              |
| PTGER1 | AAGAGGTAGTAGTAGTAGTG  | AAACCTACACATTCTCAA  | GAAGGGTTGTTAGTAGTG    | CTTACACCTCTCTAAAC  | 294              |
| PTGER2 | TAAGTTTTTTTTTGAAGTAGT | TCCAAACTACCTCTCTCACT | GTTTTTATTTTTTTTTTAGTTT | ACTACCTCTCTCTAATT | 469              |
| PTGER3 | GAAAAGGTGTTTATTTTAGTG | TCACCATACACTACCTAAT | TTGGTGTGTGAGTTAGTTAG  | AATCCTCTCTACATAC  | 382              |
| PTGER4 | GTTTAGTATGAGTAGTTAG  | CCTCTATACAAACCTTTCT | GGATAGGGGTTTAAGAAGAA | TTCTCTCCTCATACCTC | 365              |
| PTGES2 | GGTATTTTTTGGTTTTTATT | TACATTAAAATCTTTTAAAC | GAGTTGTTGAGTTAGTTAG  | AACTACCTCTACTACCTC | 401              |
| PTGES3 | GTtagTaagTagtagtg     | cacaAACTACATTATCACC | gTtagTagtagtgTtTaa    | AACTACCTCTACTACCTC | 383              |
| PTGFR  | TATTATTATTATTTTATTAT  | TACATTTAACCTACCTCT  | GAGGAGAGAGAGGAGG      | AAATATACCTCTATCCCA  | 297              |
| PTGIS  | GGTAGAGATGAGTATTTTGA  | ATAATACCTCTACCTAC  | GAGTTGTTGAGTTAGTTAG  | AACTACCTCTACTACCTC | 427              |
| PTGIS_A | GTTTTTTTTTGTTAAGAAGGTGT | ATAAAATACCTAAAACATAATACAA | TTAAGAAGTTGTAAGGTTGG  | AACTCTAACCTTCTATAATAC  | 316             |
| PTGIS_B | TTTTTAATGTGTTTGGGTGGG | CTTCCACCCATTACCTCTTTA | GAAATTTTTTTGGGACTGTTG  | CACTCTCTACCTACCTAC  | 346              |
| PTGS1  | AGATTTAGGTAGTTATTTTA  | TCTATTTTCCAAATACAA  | TATGTATAGGAGGTGGT  | CTTCTCTCTACCTACCTC | 321              |
| PTGS2  | GAGGTTGAGAAGTTTATTAGT | CATAACTTATATTTTCTCATTTAT | GAGGTTGAGAAGTTTTAGGT  | CATAACTTATATTTTCTCATTTAT | 476         |
| TBX2R  | GTTGGTATAGGTAGTAGTG   | ATACCTAAAAATCTCAAAA | TAGTTGGTTGATTAGG      | CCAAAAAACTTAAATACCC | 325              |

1 from reference [8] (references are listed in page 4)
Figure S1. PTGS pathway expression profiling in CRC cell lines. (A) Expression profile of a panel of 5 colorectal cancer cell lines shows the same trends observed in primary colorectal tumor tissues (see Figure 1), being most transcripts downregulated or fully silenced. The only transcripts that keep or increase their expression levels are the PGE2 synthases (PTGES, PTGES2 and PTGES3). As a reference, we included the mean expression values for the tumor tissues analyzed by real time PCR (Figure 1A). (B) Gene expression was analyzed by qRT-PCR in HCT116 and its DNMT double knockout cell line, HCT116-DKO (referred to as DKO). Expression levels in DKO cells were normalized to HCT116. Black bars correspond to genes that undergo hypermethylation a large proportion of colorectal tumors and all of the 5 cell lines analyzed (see Figure 2A-D). PTGIS was only detected in DKO cells, not in HCT116, being the log₂(DKO/HCT116) value arbitrary in this case (marked with a white *). No detectable levels of AKR1B1 and PTGFR, which are fully methylated in HCT116, were found in HCT116, neither in DKO.
Figure S2. DNA methylation beta values quantified with Infinium Methylation450K arrays for a cohort of 19 mucosae from healthy individuals (green), 22 adjacent normal tissues (blue), 42 adenomas (yellow) and 64 colorectal cancers. Normal mucosae from healthy and CRC patients display comparable profiles, while altered patterns affect in a similar way adenomas and carcinomas. Data were obtained from Luo et al. (supplementary reference 7).
Figure S3: Mutations in PTGS pathway genes in CRC. (A) Mutation al rates of PTGS pathway genes in CRC (data obtained from the TCGA database). Deleterious mutation definition based on TCGA criteria. (B) Survival plot for CRC patients with and without deleterious mutations in PTGFR. Survival is greatly affected by the presence of deleterious mutations in this gene, which is frequently hypermethylated in colorectal tumors.
Figure S4: Principal component analysis of DNA methylation profiles in genes of the COX pathway in normal mucosa from healthy individuals, and the adjacent normal mucosa and tumor sample of patients with colorectal cancer. Samples from the left and right colon are depicted with distinct markers.
Figure S5. Survival analysis of CRC patients according to gene expression levels. Data were obtained from four datasets (GEO: GSE14333, GSE12945, GSE17536, GSE17537) with follow-up information available to analyze the survival rate depending on gene expression. The PrognoScan algorithm (Mizuno et al, 2009, supplementary reference 10) was used to classify the patients into either the High or Low expression group (minimum p-value approach). Survival curves were constructed using the Kaplan-Meier method.