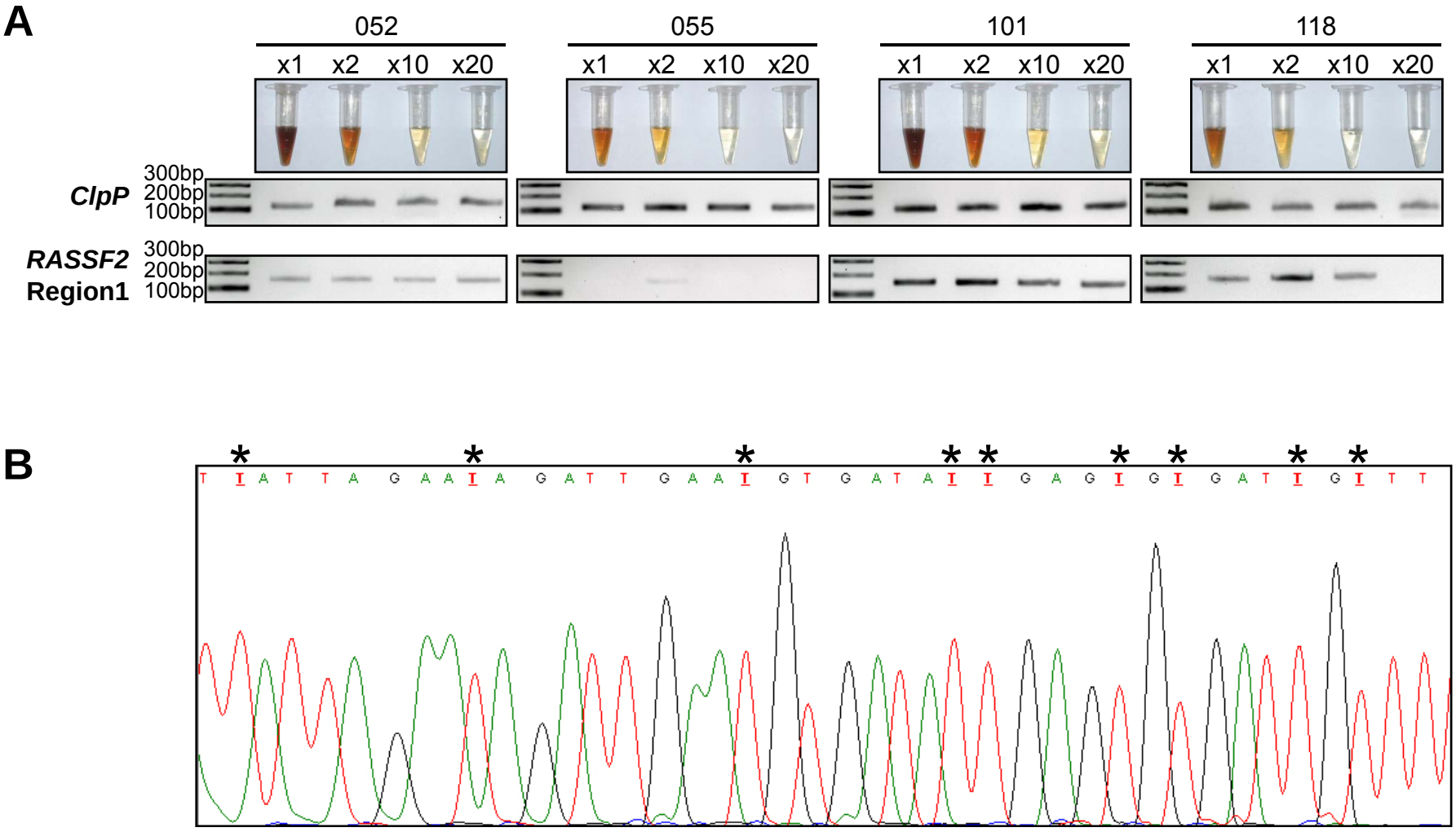
Supplementary Figure 1

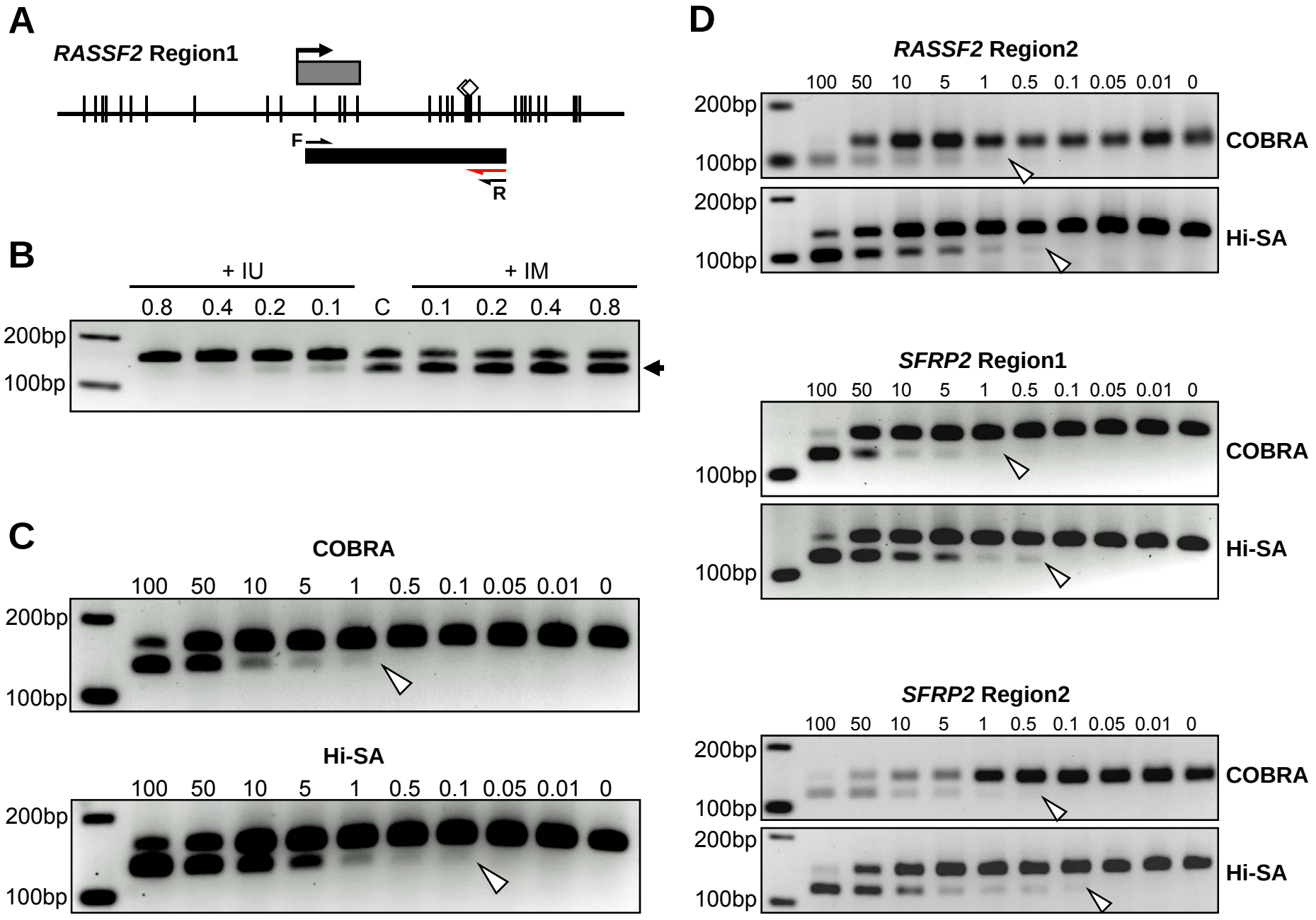


Supplementary Figure 1. One-step bisulfite modification of unpurified fecal DNA

(A) Examples of dissolved feces before bisulfite modification and post-bisulfite PCR products from four representative samples. Solutions of 100 mg feces in 1 ml lysis buffer (x1) were further diluted 2, 10, and 20 times. The top gel shows the amplification of the *E. Coli ClpP* gene used as a control for direct fecal bisulfite modification. The bottom gel shows the amplification of the human *RASSF2* promoter Region 1.

(B) The bisulfite-modified *ClpP* gene sequence obtained from sample 055 (x2). Asterisks indicate unmethylated cytosines converted to uracils by bisulfite modification of unpurified fecal DNA.

Supplementary Figure 2



Supplementary Figure 2. High-Sensitive Assay (Hi-SA)

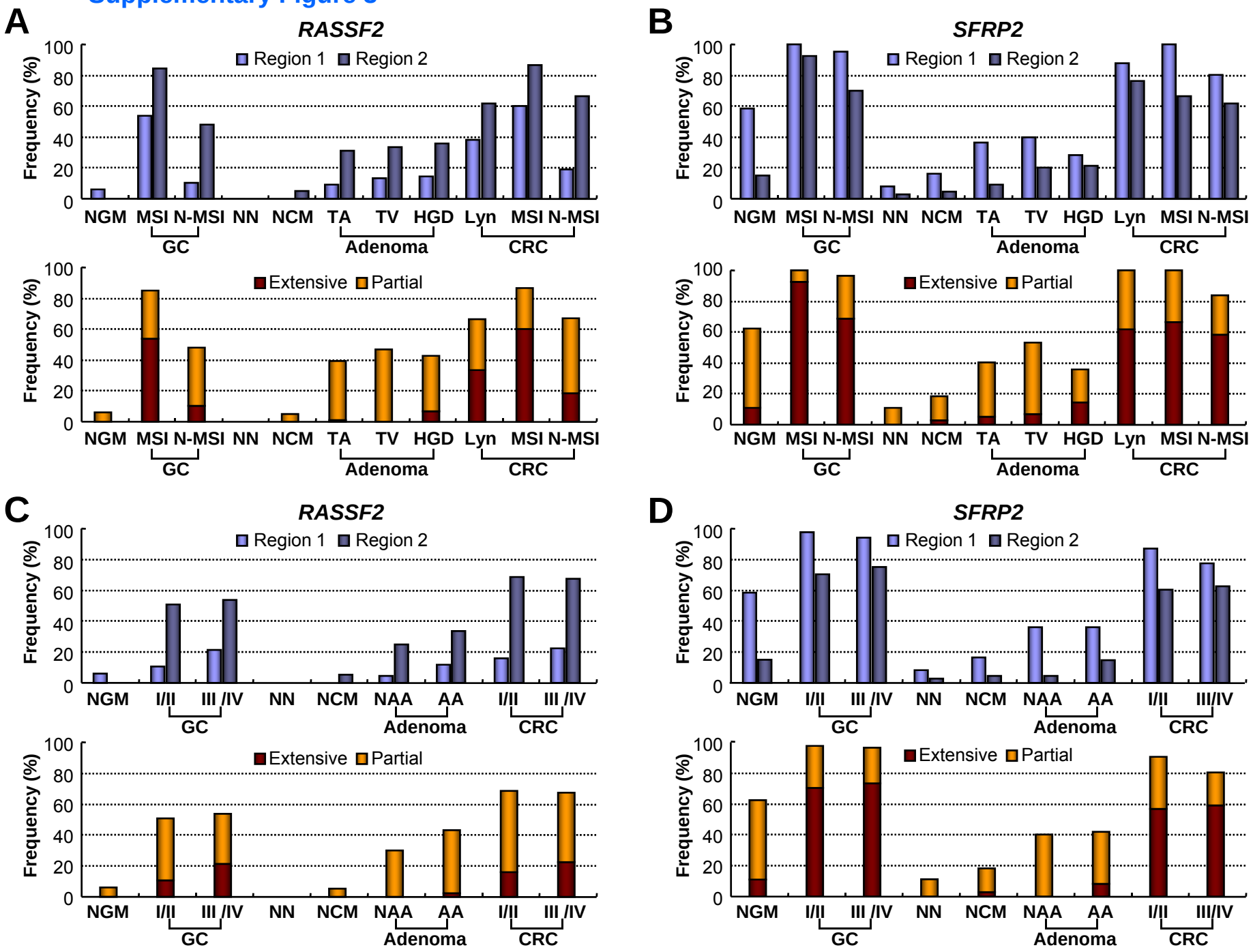
(A) Schematic representation of COBRA and Hi-SA analysis for methylation of *RASSF2* Region 1. Grey square, non-coding exon 1 with arrow showing transcriptional start site; vertical lines, CpG sites; white diamonds, *Hha*I restriction enzyme recognition sequence for COBRA and Hi-SA; thick horizontal line, location of COBRA and Hi-SA products. **Black arrows** indicate 'F' and 'R' primers that are non-methylation specific primers and **a red arrow** indicates the internal primers specific for unmethylated (+IU) or methylated (+IM) used for Hi-SA.

(B) Efficiency of Hi-SA using varying concentrations of +IU or +IM primer added at the final concentrations (μM) shown above the lanes. Bisulfite-modified DNA in which *Sss*I (methyltransferase) -treated DNA and unmethylated, normal colonic epithelial DNA were mixed 1:1, was used as a template. C, COBRA results without internal primers; arrows indicate methylated alleles.

(C) Comparison of the sensitivity of COBRA and Hi-SA for detecting methylation in *RASSF2* Region 1. Percentages of *Sss*I-treated DNA mixed with unmethylated normal colonic epithelial DNA as the template are shown above the lanes. White arrows indicate minimum detectable levels of methylation.

(D) Comparisons of the sensitivity of COBRA and Hi-SA for detecting methylation in *RASSF2* Region 2, *SFRP2* Region 1, and *SFRP2* Region 2.

Supplementary Figure 3

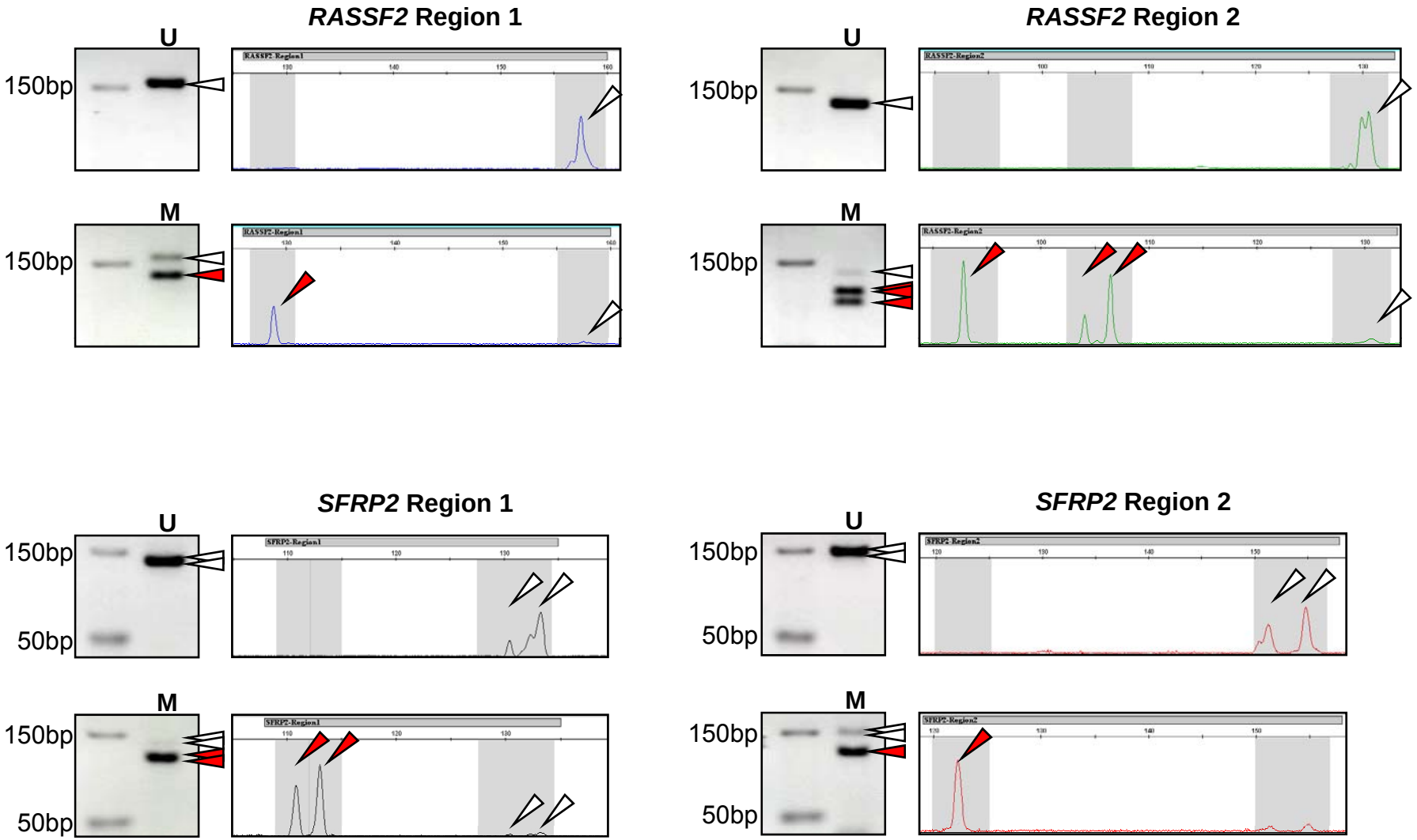


Supplementary Figure 3. Frequency of RASSF2 and SFRP2 methylation in colorectal and gastric tissue

(A-B) Methylation frequency of *RASSF2* **(A)** and *SFRP2* **(B)** categorized by histopathological status in colorectal adenomas and by sporadic MSI and hereditary status in colorectal and gastric cancers. Extensive denotes samples methylated in both Region 1 and 2. Partial denotes samples methylated in either Region 1 or 2.

(C-D) Methylation frequency of *RASSF2* **(C)** and *SFRP2* **(D)** in colorectal and gastric tissues according to stage: NN, normal colonic mucosa from subjects with no evidence of colorectal neoplasia; NCM, adjacent normal colorectal mucosa; TA, colorectal tubular adenomas; TV, colorectal tubulovillous adenomas; HGD, colorectal adenomas with high grade dysplasia; Lyn, Lynch syndrome cancer; MSI, sporadic MSI-high cancer; N-MSI, MSI-low and microsatellite-stable cancer; NAA, non-advanced colorectal adenomas; AA, advanced colorectal adenomas; I/II, TNM stage I and II; III/IV, TNM stage III and IV; GC, gastric cancer; CRC, colorectal cancer.

Supplementary Figure 4

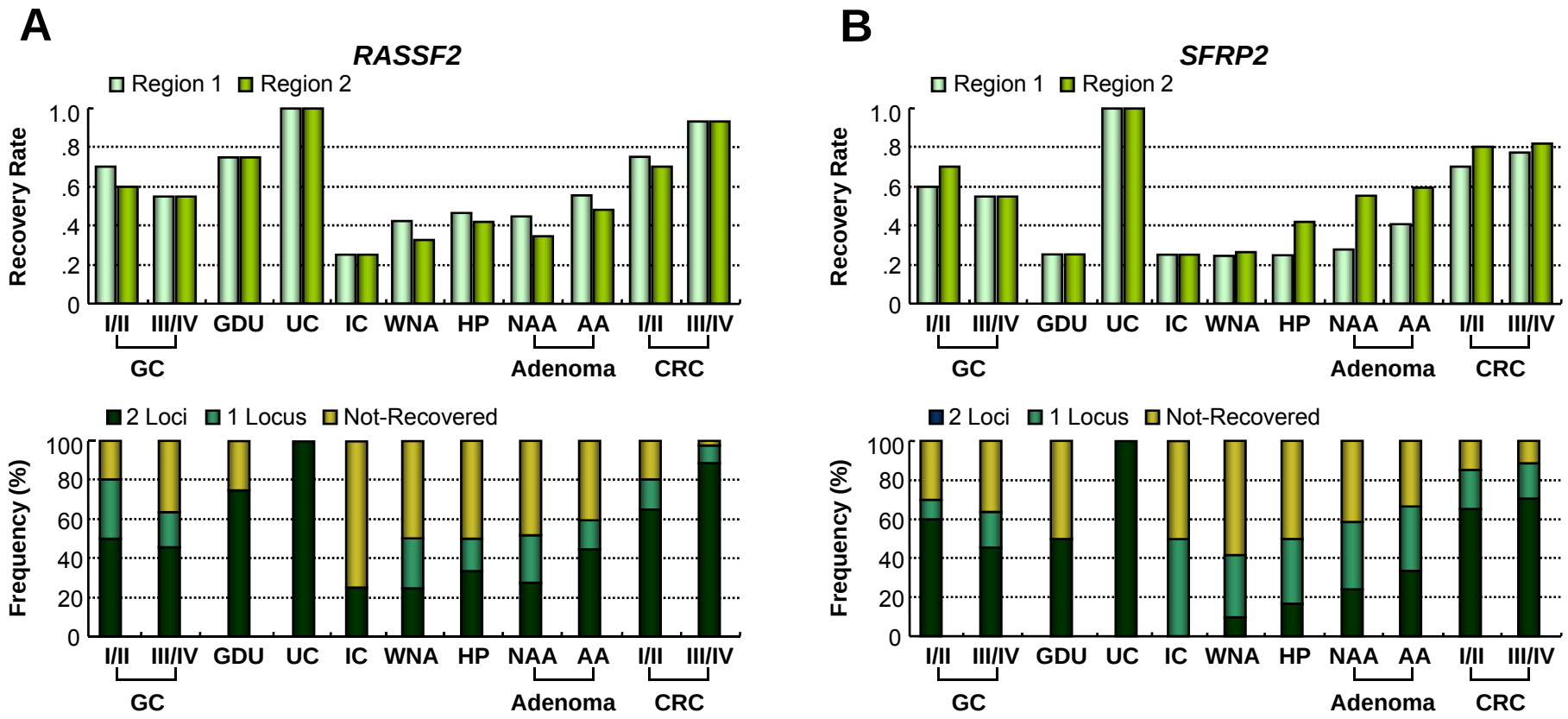


Supplementary Figure 4. Fluorescent High-Sensitive Assay (Hi-SA)

Examples of fluorescent Hi-SA using unmethylated and methylated controls for each marker.

Left panel displays electrophoresis by an agarose gel. Right panel displays electrophoresis by a capillary sequencer. U, unmethylated control; M, methylated control. White arrows, unmethylated alleles; Red arrows, methylated alleles cleaved by *Hha*I restriction enzyme.

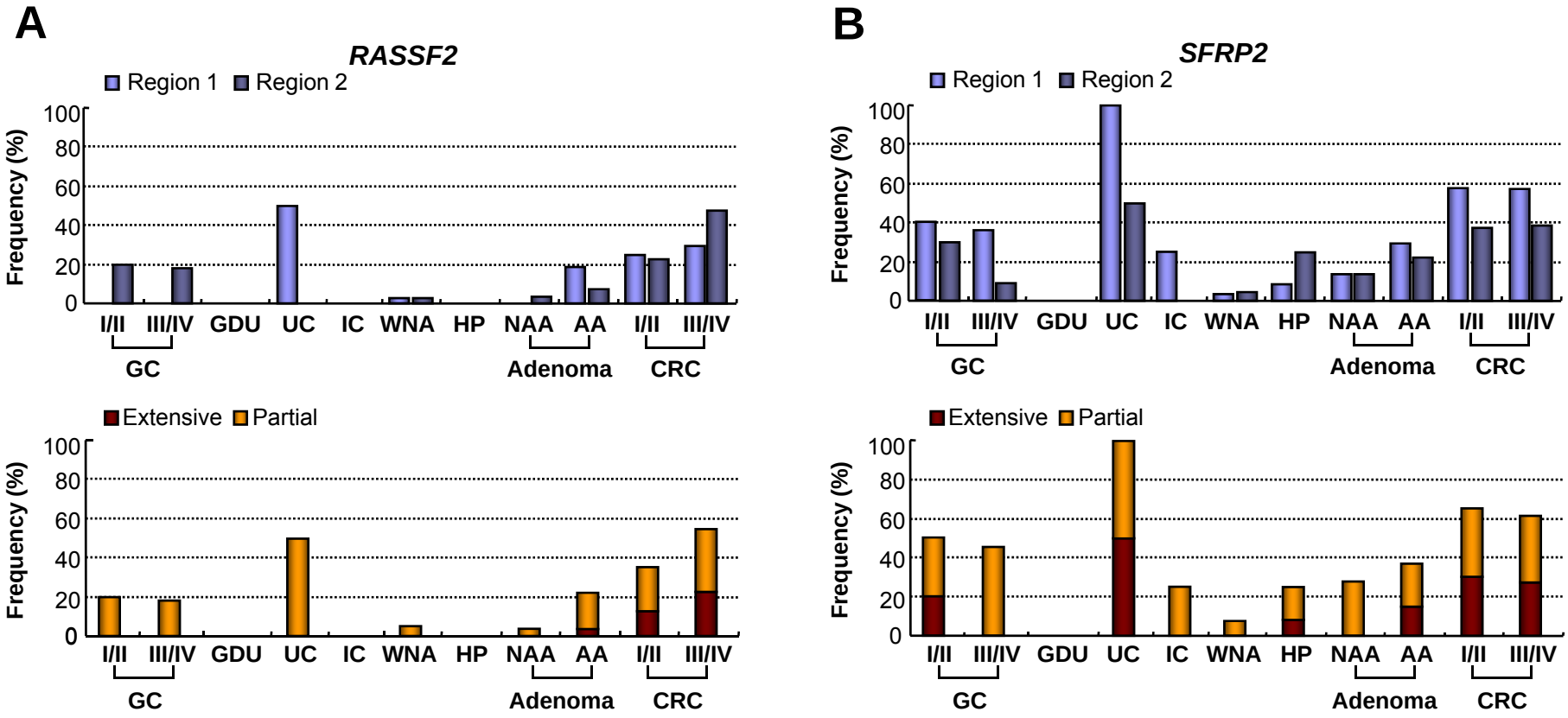
Supplementary Figure 5



Supplementary Figure 5. Recovery status of human fecal DNA

Recovery rates and frequencies of *RASSF2* **(A)** and *SFRP2* **(B)** in fecal samples from patients with colorectal carcinoma (CRC) and gastric carcinoma (GC) categorized by colonoscopy and endoscopy. TNM tumor stage (I/II or III/IV) was determined by histopathological examination of surgically resected lesions. GDU, gastric/duodenal ulcer; UC, ulcerative colitis; IC, ischemic colitis; WNA, subjects without neoplastic or active diseases; HP, hyperplastic colorectal polyps; NAA, non-advanced colorectal adenomas; AA, advanced colorectal adenomas.

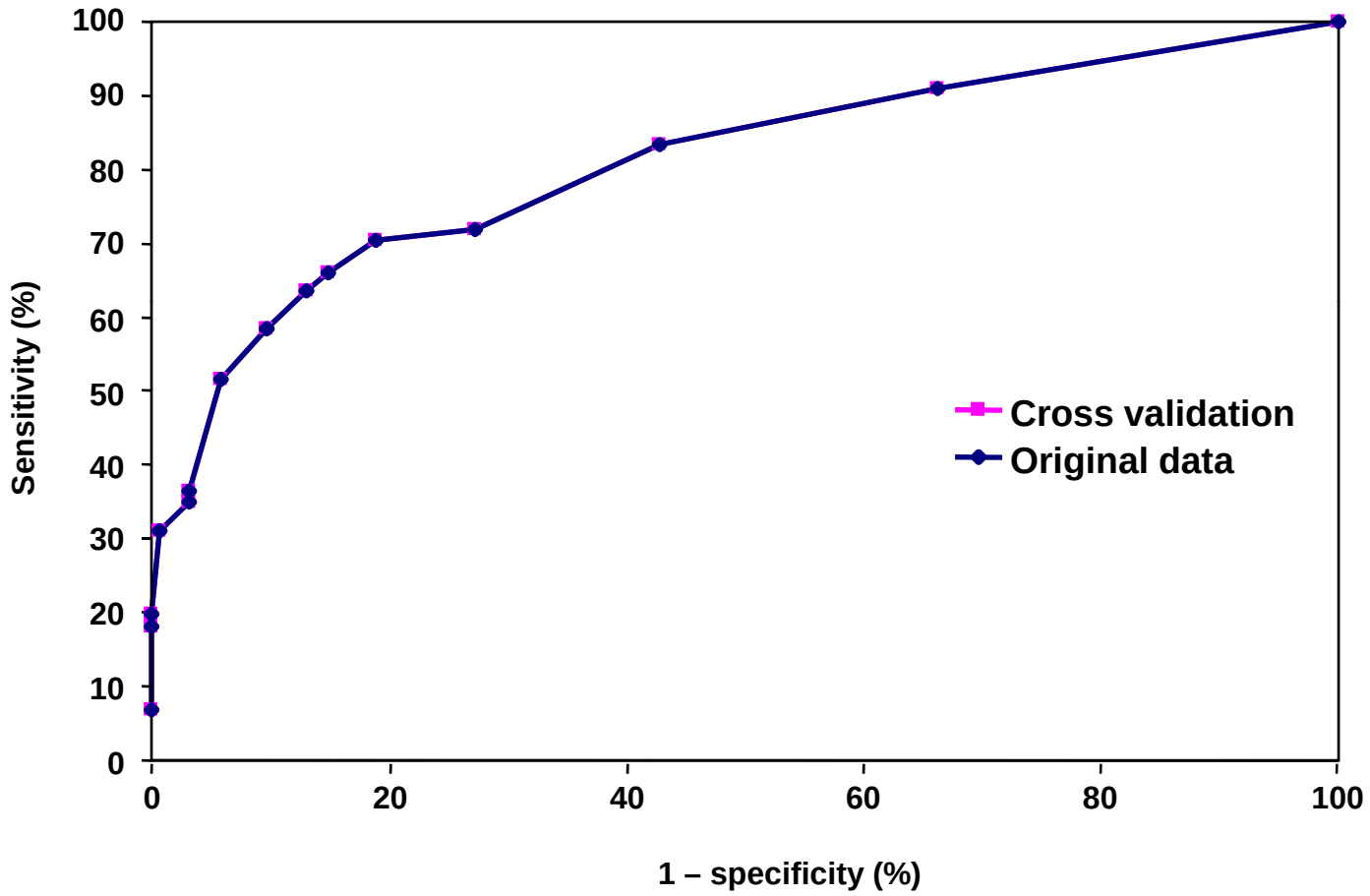
Supplementary Figure 6



Supplementary Figure 6. Methylation status of human fecal DNA

Frequencies of *RASSF2* **(A)** and *SFRP2* **(B)** methylation in fecal samples expressed as the number of loci methylated. TNM tumor stage (I/II or III/IV) was determined by histopathological examination of surgically resected lesions. GDU, gastric/duodenal ulcer; UC, ulcerative colitis; IC, ischemic colitis; WNA, subjects without neoplastic or active diseases; HP, hyperplastic colorectal polyps; NAA, non-advanced colorectal adenomas; AA, advanced colorectal adenomas.

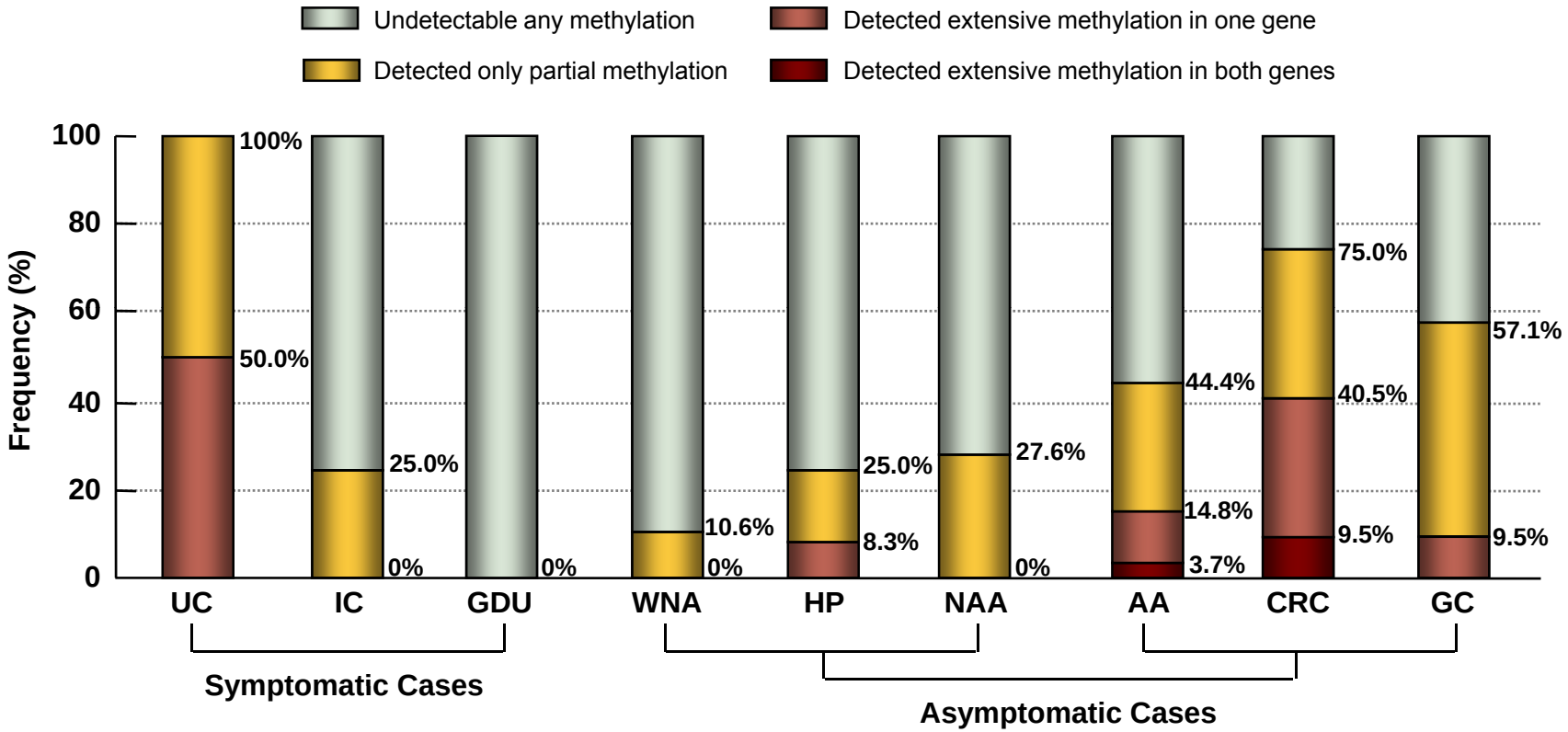
Supplementary Figure 7



Supplementary Figure 7. Comparison of ROC curves for the cross-validation

The effect of dropping one observation is so slight that the ROC curve for the cross-validation was identical to the one for the combination score using the original coefficients from the logistic regression for the full data (original data). ROC curves for various cutoff levels to screen subjects with advanced lesions (colorectal cancer, colorectal advanced adenomas and gastric cancer) from subjects with non-advanced lesions (colorectal non advanced adenomas, colorectal hyperplastic polyps and subjects without neoplastic or active diseases), respectively. Sensitivity and 1 minus specificity (ROC curves) are shown at various threshold values.

Supplementary Figure 8



Supplementary Figure 8. **Cumulative frequency of extensive and partial methylation in the *RASSF2* and/or *SFRP2* genes**

The cumulative frequency of fecal samples with extensive and partial methylation in the *RASSF2* and/or *SFRP2* genes. TNM tumor stage (I/II or III/IV) was determined by histopathological examination of surgically resected lesions. GDU, gastric/duodenal ulcer; UC, ulcerative colitis; IC, ischemic colitis; WNA, subjects without neoplastic or active diseases; HP, hyperplastic colorectal polyps; NAA, non-advanced colorectal adenomas; AA, advanced colorectal adenomas.