Supplementary Figure 1. Sequence chromatogram from a patient with serrated polyposis (patient A:II-2) (A) A germline RNF43 splice site mutation c.953-1 G>A in the blood DNA. (B) cDNA sequencing in blood shows a heterozygous 2bp deletion c.953_954 delAG. (C) cDNA sequencing in an adenoma (II-2 AD2) shows homozygous c.953_954 delAG with loss of wild-type allele.
Supplementary Figure 2. (A-I) Histology of serrated polyps and adenomas from 5 germline RNF43 gene carriers in family A. The presence or absence of RNF43 second somatic hit and BRAF or KRAS mutation are shown at the top of each panel. Inset in panel (G), high power view of paneth cells present in the SSA.
Supplementary Figure 3. Increased number of Paneth cells in adjacent colonic mucosa (A) or in the sessile serrated adenoma of patient A:II-2 (B), with corresponding immunohistochemical staining for lysozymes (C). Inset in panel (A) also shows immunostaining for lysozymes.
Supplementary Figure 4. (A) Assessment of CIMP using 5 markers with representative methylation-specific PCR results shown. N.C. normal colon as un-methylated control; UMD, universal methylated DNA as methylation positive control; U, primers specific for unmethylated DNA; M, primers specific for methylated DNA. CIMP positive cases show methylated bands in at least 3 markers.
Supplementary Figure 5. (A) Schematic summary of MLH1 promoter and location of methylation specific PCR primers, pyrosequencing and Illumina methylation array probes. Red arrow denotes region assessed by bisulphite-pyrosequencing, blue arrows denote regions assessed by MS-PCR, with positions indicated relative to the translation start site. (B) Methylation beta value of MLH1 promoter region in 288 TCGA CRCs assessed by Illumina 450K methylation array. Each column is a probe along chromosome 3p and each row is a sample. The color of each box denotes the beta value: Pink, >0.2; Orange 0.1-0.2; white; <0.1. The green box on top denotes a region of 28 illumina probes with methylation associated with MSI-H status and clear-cut negative signals in a subset of MSI-H CRCs and all but 3 cases of MSS CRC. The MLH1me+ MSI CRCs showed low levels of MLH1 expression by RNAseq. Dark and light green color denote MLH1 expression <2 standard deviations (S.D.) or 1.5 to 2 S.D. below the mean, respectively. (C) Representative MS-PCR in 3 MLH1 promoter regions showing concordant methylation in 2 SSAs (SA38 & SA26) with focal MLH1 protein loss and a MSI CRC. SA20 shows discontinuous methylation and no evidence of protein loss. Right panel shows a SSA (SA26) with loss of MLH1 protein in several glands (arrows). Please refer to Figure 4 for SA38.
Supplementary Figure 6. RT-PCR for PTPRK(Exon 1) to RSPO3(Exon2) fusion transcript in sporadic serrated adenomas. The fusion bands were confirmed by cDNA sequencing.