Figure S1 Comparison of the *GFRA1* mRNA level in colon cancer tissues containing high and low levels of dmGFRA1 by Illumina 450K Methylation array using the TCGA datasets.
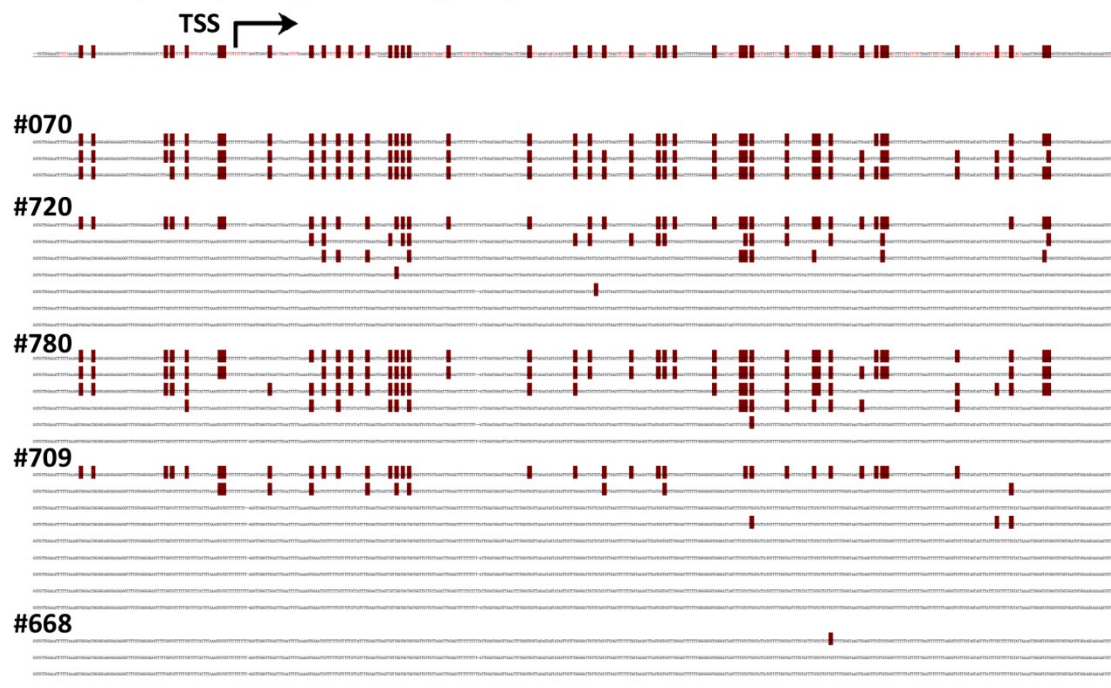
Figure S2 Bisulfite-sequencing results for the methylation status of *GFRA1* **CpG island around transcription start site (TSS-CGI) in 5 representative colon carcinoma samples.**
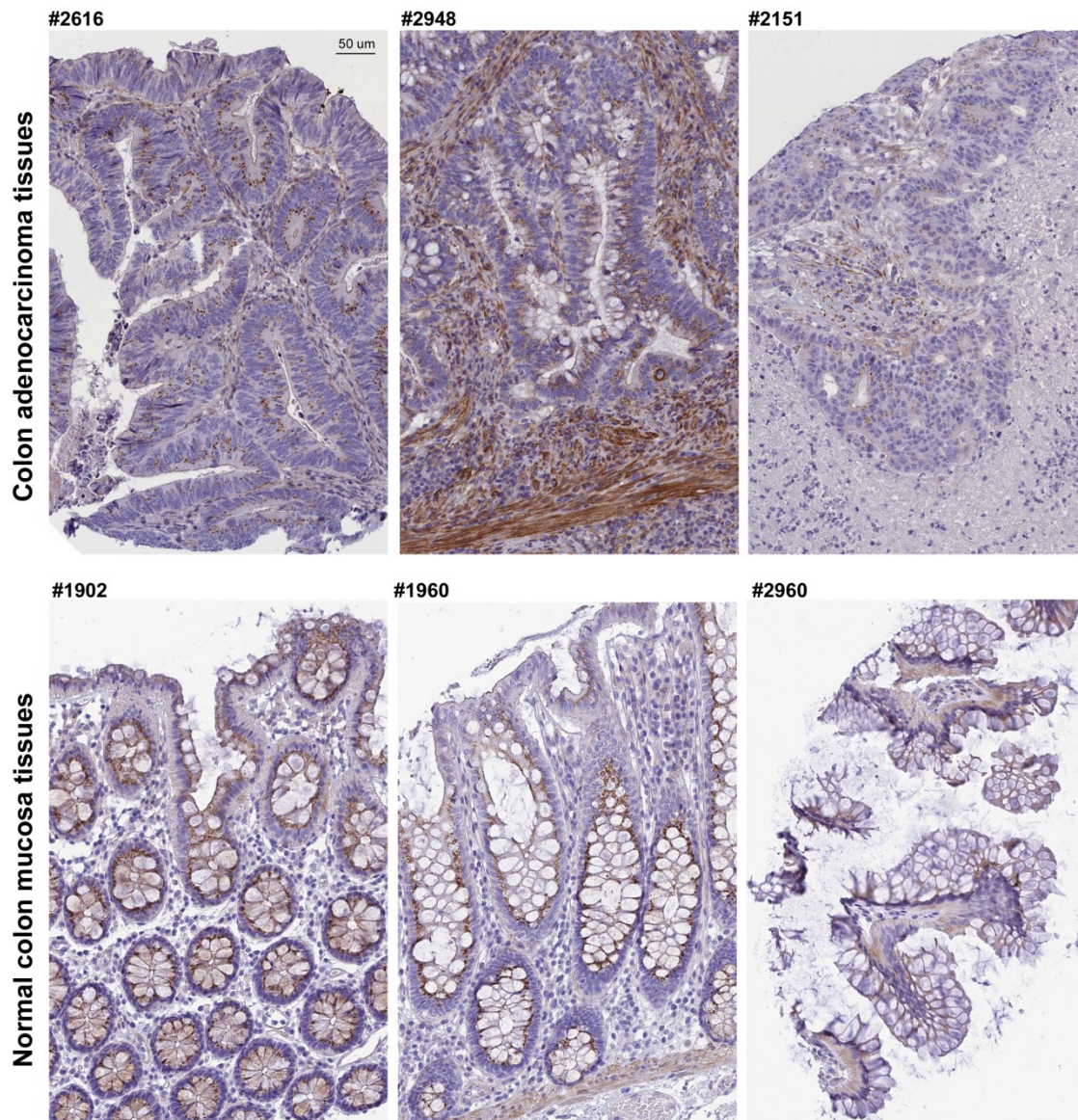
Figure S3 Images of immunohistochemical (IHC) staining of GFRA1 protein in colon adenocarcinoma and normal colon mucosa tissues. The rabbit anti-human GFRA1 antibody HPA043829 (1:200) was in the IHC staining. These images were downloaded and adapted from the publicly available database in the Human Protein Atlas project (www.proteinatlas.org) [29]. According to the data reliability description, the amounts of GFRA1 protein in human tissues by the antibody staining were not consistent with mRNA expression data, therefore, external verification is pending to confirm the IHC staining results.
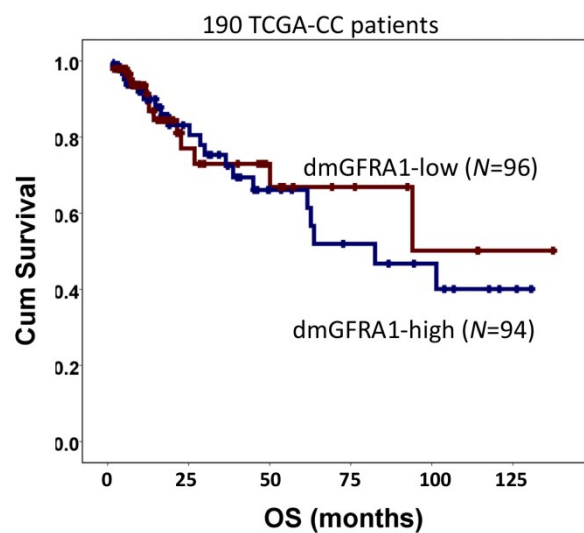
Figure S4 Kaplan-Meier survival curves for 190 CC patients in TCGA with different *GFRA1* demethylation (dmGFRA1) levels by Illumina 450K array.
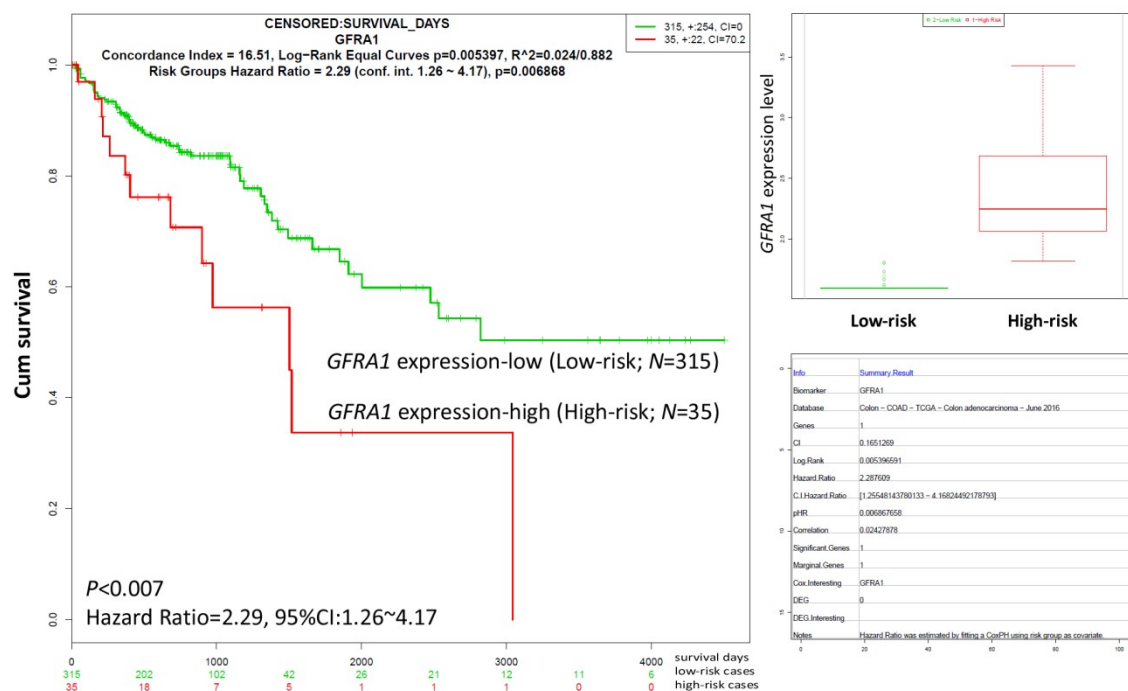
Figure S5 Kaplan-Meier overall survival curves for 350 patients in TCGA with different *GFRA1* mRNA levels in CC samples.

Table S1. Definition of *GFRA1* demethylation (dmGFRA1)-high and -low by different assays

Assay		Criteria
DHPLC	dmGFRA1-positive/-negative	When a dmGFRA1-peak in PCR products for a sample was detected in DHPLC analysis by fluorescence detector, the sample was defined as the dmGFRA1-positive sample, otherwise, dmGFRA1-negative sample.
450KMethyl array	dmCpG	The methylation level for each CpG site was expressed using the β value, calculated as M/(M+U), where M is the signal from methylated beads, and U is the signal from unmethylated beads at the targeted CpG site. When the β value for a CpG site was <0.2, it was classified as demethylation-positive CpG (dmCpG).
	dmGFRA1-high/-low	The total number of dmCpG sites was used to represent the *GFRA1* demethylation level for each sample. The *median* dmCpG number of 2 for the 268 samples was used as the cutoff value to define dmGFRA1. A sample containing ≥2 dmCpG sites was classified as dmGFRA1-high; otherwise, dmGFRA1-low.
MethyLight	dmGFRA1-high/-low	The *median* value of dmGFRA1 proportion (68.4%) for colon cancer tissue samples was used as the cutoff to define dmGFRA1-high and dmGFRA1-low.

Table S2. Comparison of the *GFRA1* demethylation (dmGFRA1) prevalence by MethyLight in colon cancer (CC) and paired surgical margin (SM) tissue samples from patients with various clinicopathological characteristics

		Case number	Proportion of dmGFRA1 alleles, % [a]			
			SM	*P*-value [b]	CC	*P*-value
Age	<60	60	99.77 (99.55-99.88)	**0.001**	94.22 (85.70-97.27)	0.150
	≥60	84	99.58 (99.37-99.71)		91.73 (84.77-95.68)	
Sex	Male	88	99.65 (99.44-99.86)	0.290	91.89 (84.37-96.70)	0.387
	Female	56	99.65 (99.41-99.80)		93.36 (86.84-96.63)	
Location	Sigmoid	73	99.58 (99.37-99.78)	**0.016**	91.63 (83.94-95.94)	0.100
	Others	71	99.70 (99.55-99.85)		93.57 (87.24-97.09)	
Differentiation	Poor	13	99.74 (99.61-99.86)	0.203	97.09 (95.31-99.28)	**0.001**
	Mod./Well	131	99.62 (99.42-99.83)		91.79 (84.70-95.88)	
Vascular embolus	Negative	123	99.62 (99.42-99.83)	0.220	91.83 (84.99-96.14)	0.155
	Positive	21	99.70 (99.58-99.84)		95.46 (91.42-97.15)	
pTNM stage	I+II	72	99.64 (99.43-99.80)	0.272	91.54 (84.32-96.44)	0.549
	III+IV	70	99.67 (99.40-99.87)		93.28 (86.39-96.79)	
Local invasion	T1-2	11	99.59 (99.17-99.84)	0.794	91.83 (78.60-99.63)	
	T3	71	99.62 (99.43-99.85)		94.29 (86.95-98.64)	**0.006** [c]
	T4	60	99.67 (99.42-99.82)		91.39 (84.56-95.45)	
Lymphatic metastasis	N0	73	99.65 (99.43-99.80)	0.486	91.45 (84.17-96.25)	0.305
	N1-2	71	99.65 (99.40-99.86)		93.57 (86.80-97.09)	
Distant metastasis	M0	126	99.64 (99.42-99.84)	0.800	91.81 (84.92-96.20)	0.105
	M1	18	99.66 (99.55-99.83)		94.24 (92.25-97.23)	
(All)		144	99.65 (99.42-99.84)		92.73 (85.43-96.70)	**0.000** [d]

NOTE: Numbers underlined: the highlighted values denote that a statistically significant difference was observed. [a] *Median* (25%-75% range) proportion of demethylated *GFRA1* alleles (dmGFRA1); [b] Mann-Whitney *U*-test; [c] T3 vs. T4; [d] SM vs. CC

Table S3. Comparison of *GFRA1* demethylation (dmGFRA1; by Illumina 450KMethyl array) and expression status in colon cancer patients in TCGA datasets with various clinicopathological characteristics

		dmGFRA1-high proportion (%)	Relative *GFRA1* mRNA level [a]	Proportion (%) of *GFRA1* expression-high [c]	*P*-value [d]
Age	<60	74/89 (52.8)	0.53 (0.23-1.11)	64/125 (51.2)	0.753
	≥60	86/179 (48.0)	0.52 (0.22-0.97)	163/329 (49.5)	
Sex	Male	77/144 (53.5)	0.53 (0.22-1.00)	121/240 (50.4)	0.851
	Female	56/124 (45.2)	0.52 (0.20-1.02)	106/214 (49.5)	
Location	Sigmoid	40/75 (53.3)	0.58 (0.24-0.93)	81/150 (54.0)	0.231
	Others	93/193 (48.2)	0.50 (0.22-1.04)	146/304 (48.0)	
Vessel embolus	Negative	72/153 (47.0)	0.47 (0.19-0.95) [b]	107/234 (45.7)	**0.060**
	Positive	43/83 (51.8)	0.57 (0.24-1.12)	97/176 (55.1)	
pTNM stage	I+II	66/144 (45.8)	0.49 (0.20-0.92)	116/251 (46.2)	**0.077**
	III+IV	63/117 (53.8)	0.56 (0.23-1.10)	105/192 (54.7)	
Local invasion	T1-2	20/47 (42.5)	0.57 (0.27-0.92)	46/88 (52.3)	0.545
	T3	97/183 (53.0)	0.50 (0.21-0.97)	149/309 (48.2)	
	T4	15/37 (40.5)	0.66 (0.25-1.21)	31/56 (55.4)	
Lymphatic metastasis	N0	72/154 (46.7)	0.50 (0.21-0.92)	126/267 (47.2)	0.153
	N1-3	61/114 (53.5)	0.56 (0.23-1.12)	101/187 (54.0)	
Distant metastasis	M0	91/183 (49.7)	0.50 (0.22-0.92)	158/333 (47.5)	**0.080**
	M1	20/35 (57.1)	0.61 (0.24-1.05)	38/64 (59.4)	
(All)		133/268 (49.6)	0.53 (0.22-1.00)	227/454 (50.00)	

[a] *Median* (25%-75% range); [b] Mann-Whitney *U* test, *P*=0.039; [c] The *median GFRA1* mRNA level was used as the cutoff value to define expression-high and -low; [d] Chi-square test