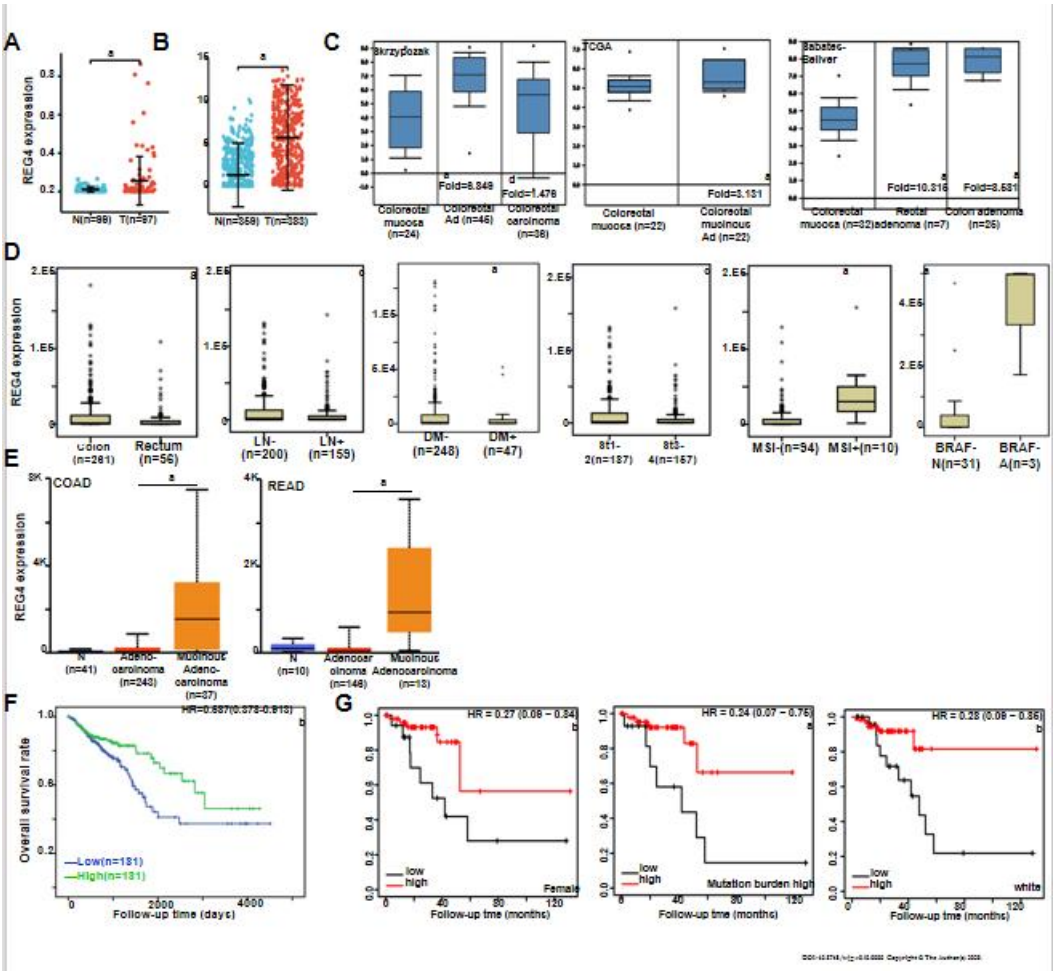
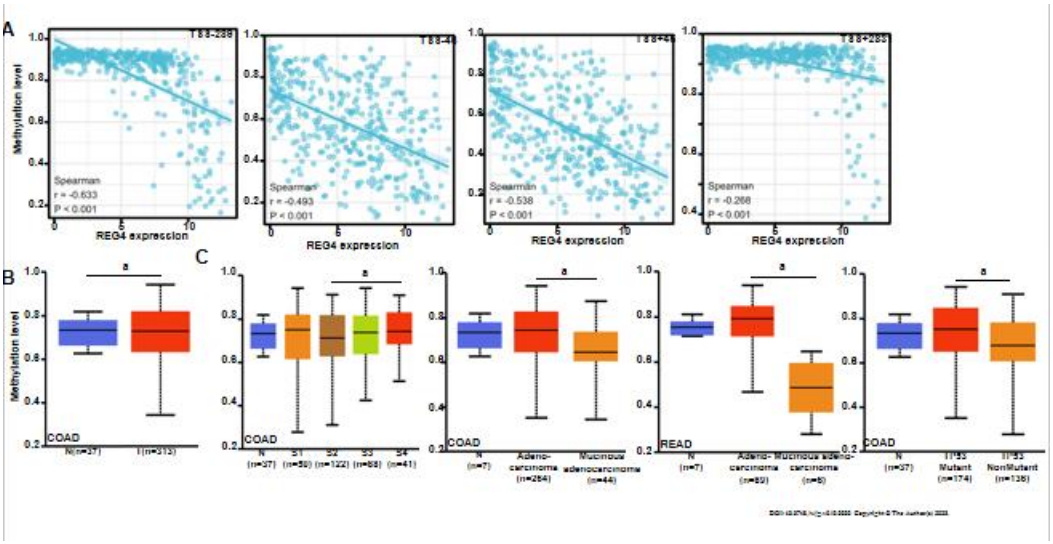
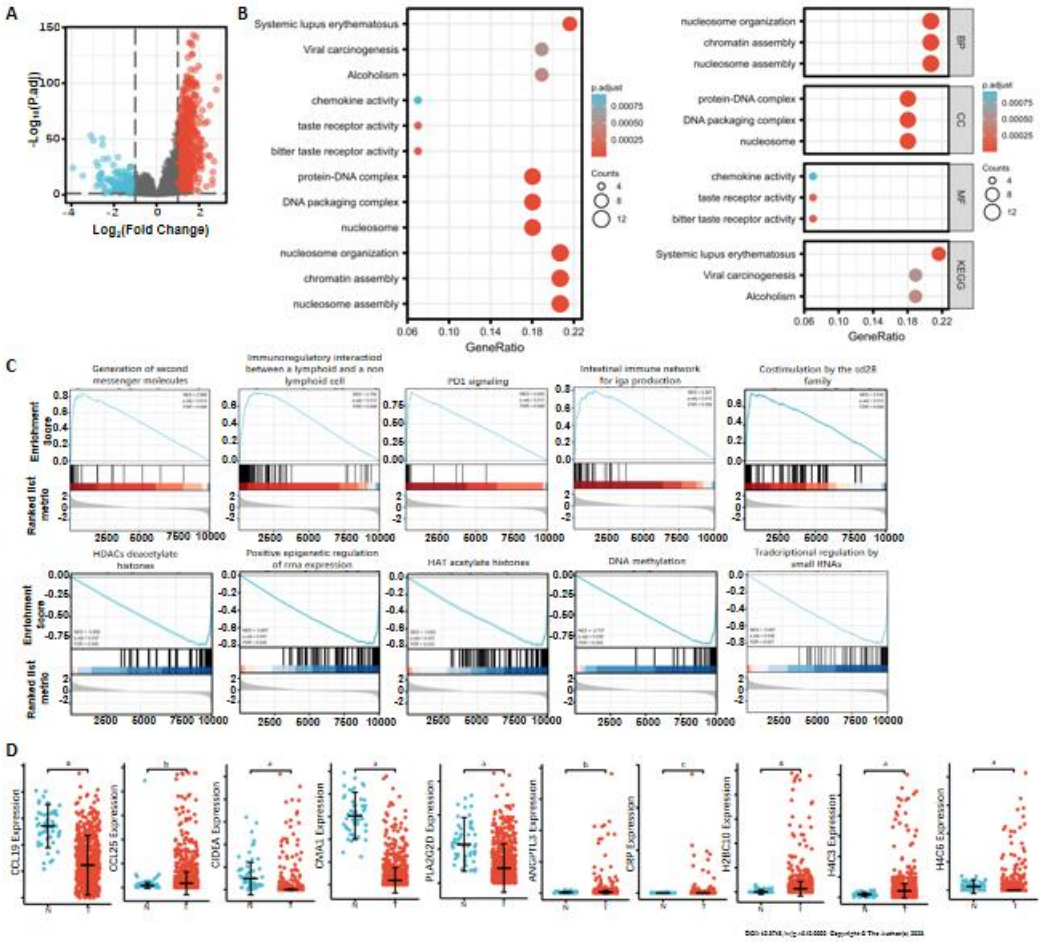


Supplementary Figure 1 Regenerating gene 4 mRNA expression in colorectal carcinogenesis and subsequent progression. A: Regenerating gene 4 (*REG4*) expression was higher in colorectal cancer (CRC) than normal mucosa by quantitative reverse transcription polymerase chain reaction; B: *REG4* expression was higher in CRC than normal mucosa by xiantao database ($P < 0.05$); C: Skyzypczak's, the cancer genome atlas (TCGA)'s and Sabates-Bellver's datasets were also used for bioinformatics analysis and higher *REG4* expression was detectable in colorectal adenoma and CRC than in normal mucosa ($P < 0.05$); D: TCGA database showed that *REG4* was more highly expressed in colonic than rectal cancers ($P < 0.05$). *REG4* expression was negatively correlated with lymph node metastasis, distant metastasis and tumor, node, and metastasis staging of CRC. *REG4* expression was positively correlated with microsatellite instability and B-raf proto-oncogene mutation ($P < 0.05$); E: *REG4* expression was lower in colorectal adenocarcinomas than mucinous adenocarcinomas by University of Alabama at Birmingham cancer

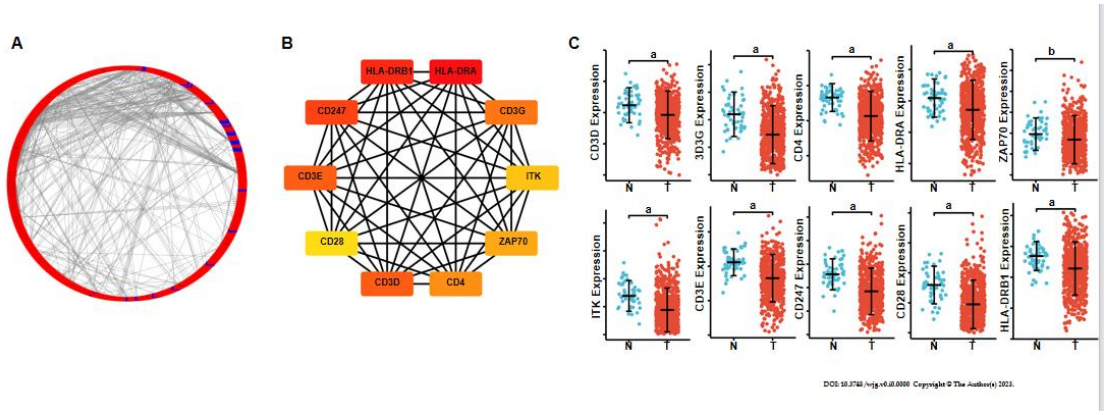
data analysis portal; F: *REG4* mRNA expression was positively related to overall survival rate of the patients with CRC according to TCGA database; G: *REG4* mRNA expression was positively related to overall survival rate of patients with CRC according to Kaplan-Meier plotter. [a]$P < 0.001$. *REG4*: Regenerating gene 4; Ad: Adenocarcinoma; BRAF: B-Raf proto-oncogene; COAD: Colon adenocarcinoma; DM: Distal metastasis; HR: Hazard ratio; LN: Lymph node metastasis; MSI: Microsatellite instability; N: Normal; READ: Rectal adenocarcinoma; TCGA: The cancer genome atlas.



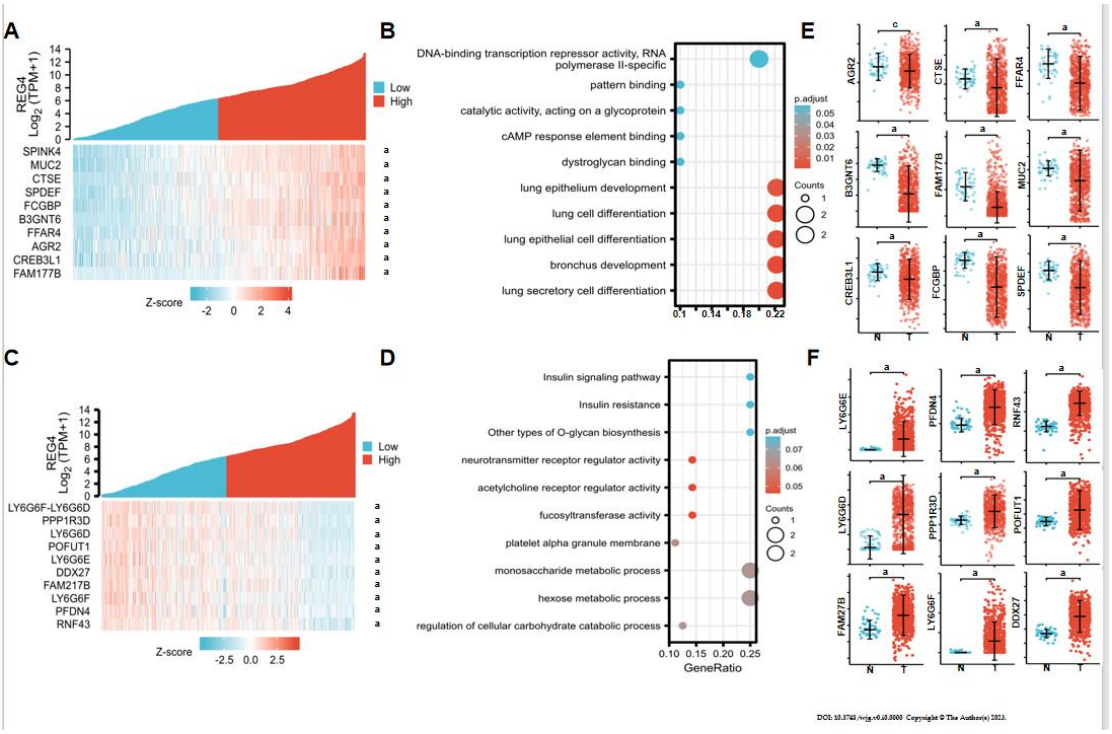
Supplementary Figure 2 Clinicopathological significances of regenerating gene 4 methylation in colorectal cancer. A: We established the linear regression between methylation and mRNA expression of regenerating gene 4 (*REG4*) in colorectal cancer (CRC) using xiantao database; B: Comparison of the methylation level of *REG4* between colon cancer and normal tissues; C: Comparison of *REG4* methylation level with different clinical characteristics of CRC patients based on University of Alabama at Birmingham cancer data analysis portal. $^{a}P < 0.001$. *REG4*: Regenerating gene 4; COAD: Colon adenocarcinoma; READ: Rectal adenocarcinoma; N: Normal; T: Tumor; S: stage; TP53: Tumor protein p53; TSS: Transcription start site.



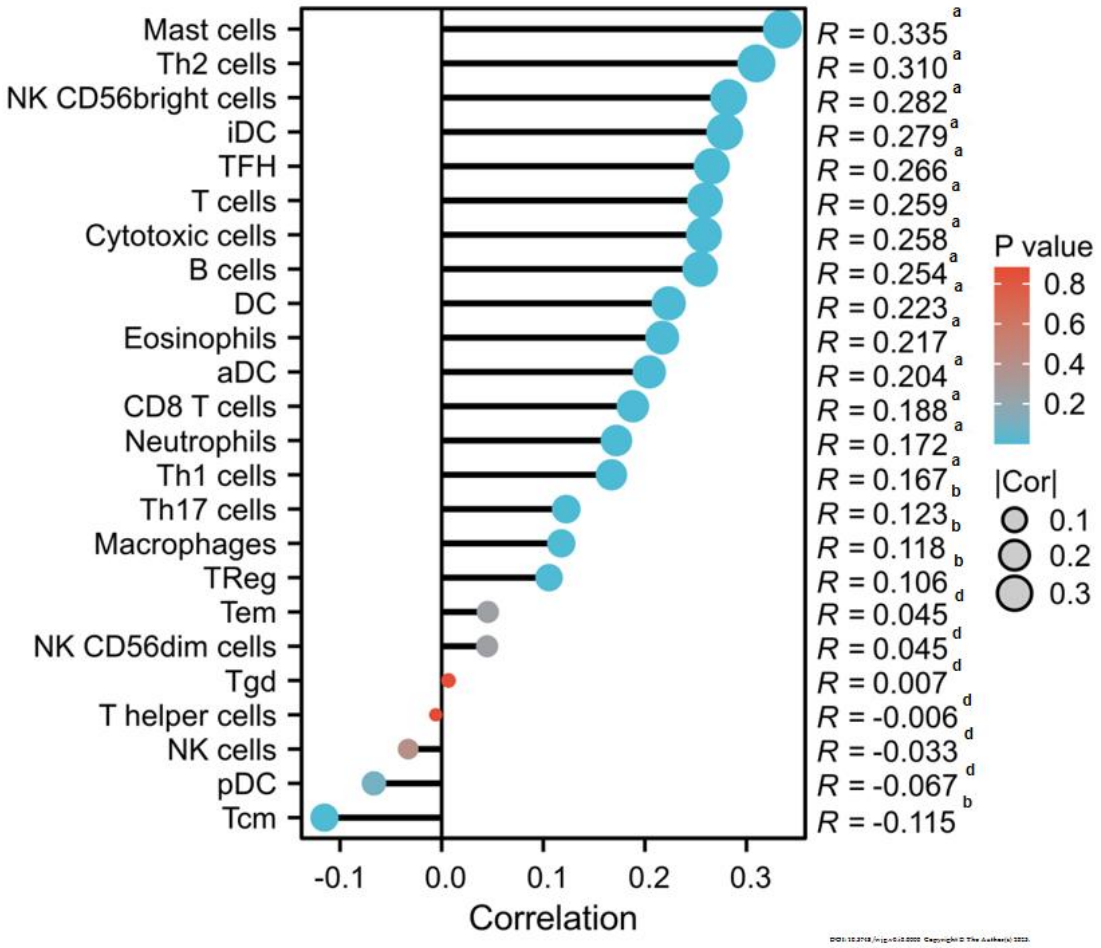
Supplementary Figure 3 Differential genes and related signal pathways about regenerating gene 4 expression in colorectal cancer. A: The volcano map of the differential genes about regenerating gene 4 was shown in colorectal cancer (CRC); B: Differential genes were subjected to signal pathway analysis using kyoto encyclopedia of genes and genomes; C: Differential genes were subjected to signal pathway analysis using gene set enrichment analysis; D: The expression of differential genes was compared between CRC and normal tissues. $^{a}P < 0.001$; $^{b}P < 0.01$. KEGG: Kyoto encyclopedia of genes and genomes; N: Normal; T: Tumor.



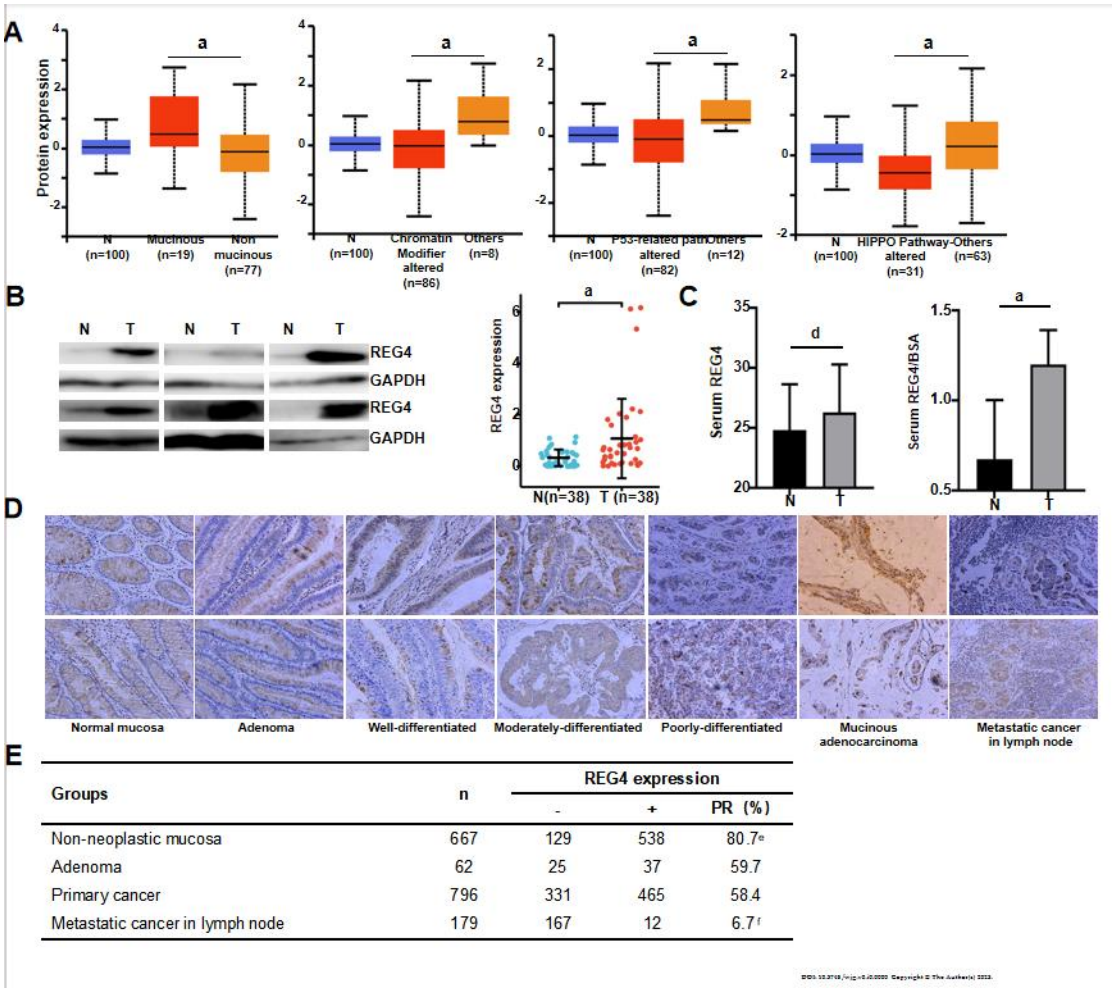
Supplementary Figure 4 Protein-protein interaction network and module analysis about differential genes of regenerating gene 4 in colorectal cancer. A: String was used to identify the protein-protein interaction network of differential genes about regenerating gene 4 in colorectal cancer (CRC); B: Cytoscape was used to find out the top 10 hub nodes ranked by degree; C: The hub genes were compared between CRC and normal tissues. $^{a}P < 0.001$; $^{b}P < 0.01$.



Supplementary Figure 5 regenerating gene 4-related genes and signal pathways in colorectal cancer. A: The top positively related genes of regenerating gene 4 (*REG4*) were screened according to the hot map; B: Positively related genes were subjected to the signal pathway analysis using kyoto encyclopedia of genes and genomes (KEGG); C: The top negatively related genes of *REG4* were screened according to the hot map; D: Negatively related genes were subjected to the signal pathway analysis using KEGG; E: The positively related genes were compared between colorectal cancer (CRC) and normal tissues using Xiantao platform; F: The negatively related genes were compared between CRC and normal tissues using Xiantao platform. *REG4*: Regenerating gene 4.



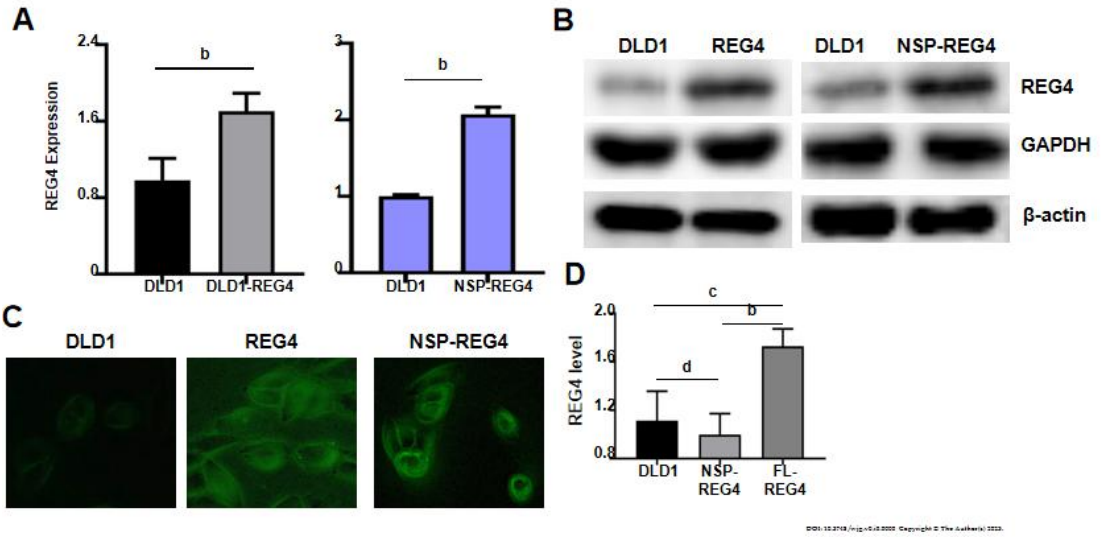
Supplementary Figure 6 Relationship between regenerating gene 4 mRNA expression and immune infiltration in colorectal cancer. In line with Xiantao, mast, T helper (Th) 2, Natural killer CD56 bright, T, cytotoxic, B, CD8 T, Th1 and Th17 cells, interstitial dendritic cell (DC), T follicular helper, activated DC and T regulatory cell in colorectal cancer were positively correlated with regenerating gene 4 (*REG4*) mRNA expression ($P < 0.05$). Central memory T cells were negatively correlated with *REG4* mRNA expression ($P < 0.05$). [a]$P <$ 0.001; [b]$P < 0.01$; [d]No significance. NK: Natural killer; TReg: Regulatory T cell; Tcm: Central memory T cells; Tem: Effector memory T cell; TFH: Follicular helper T cell; Tgd: γδ T cells; DC: interstitial dendritic cell; Th: T helper.



E

Groups	n	REG4 expression		
		-	+	PR (%)
Non-neoplastic mucosa	667	129	538	80.7[e]
Adenoma	62	25	37	59.7
Primary cancer	796	331	465	58.4
Metastatic cancer in lymph node	179	167	12	6.7[f]

Supplementary Figure 7 Expression of regenerating gene 4 protein in colorectal carcinogenesis and subsequent progression. A: University of Alabama at Birmingham cancer data analysisportal database was used to compare regenerating gene 4 (*REG4*) protein with the clinicopathological features of colorectal cancer (CRC); B: We used western blotting to compare *REG4* protein expression between CRC and normal tissues; C: ELISA detection of serum *REG4* expression; D: Immunohistochemically, *REG4* protein was positively expressed in normal epithelial cells, adenoma, well-, moderately and poorly differentiated and mucinous adenocarcinoma, and lymph node metastatic cancer cells; E: *REG4* expression was analyzed during colorectal carcinogenesis and subsequent progression. [a]$P < 0.001$; [d]No significance; [e]$P < 0.001$ *vs* adenoma and primary cancer; [f]$P < 0.001$ *vs* primary cancer. N: Normal; PR: Ppositive rate; *REG4*: Regenerating gene 4; T: Tumor.



Supplementary Figure 8 Expression of regenerating gene 4 protein in colorectal carcinogenesis and subsequent progression. A: Expression of regenerating gene 4 (*REG4*) mRNA was detected by quantitative polymerase chain reaction to verify whether full-length (FL)-*REG4* and nonsignal peptide (NSP)-*REG4* were successfully transfected in DLD-1 cells; B: Expression of *REG4* protein was detected by western blotting to verify whether FL-*REG4* and NSP-*REG4* were successfully transfected in DLD-1 cells; C: Expression of *REG4* protein was detected by immunofluorescence to verify whether FL-*REG4* and NSP-*REG4* were successfully transfected in DLD-1 cells; D: Expression of *REG4* protein was detected by ELISA to verify whether FL-*REG4* and NSP-*REG4* were successfully transfected in DLD-1 cells. $^{b}P < 0.01$; $^{c}P < 0.05$; ^{d}No significance. FL: Full length; NSP: Nonsignal peptide; *REG4:* Regenerating gene 4.

Supplementary Table 1 Primary antibodies used in this study

Name	Dilution	Source	Company	Catalog no.
GAPDH	1: 2000 for WB	Mouse	Proteintech	60004-1-Ig
REG4	1: 1000 for WB, 1: 100 for IHC	Goat	R and D	AF1379
EGFR	1: 10000 for WB	Mouse	Proteintech	66455-1-Ig
EGFR Tyr992	1: 1000 for WB	Rabbit	CST	2235S
EGFR Tyr1068	1: 1000 for WB	Rabbit	CST	2234S
EGFR Tyr1148	1: 1000 for WB	Rabbit	CST	4404S
EGFR Tyr1173	1:1000 for WB	Rabbit	CST	4407S
P-PI3K	1: 1000 for WB	Rabbit	CST	4228S
P-AKT	1: 1000 for WB	Rabbit	Proteintech	4058S
NF-κB	1: 2000 for WB	Rabbit	Proteintech	10745-1-AP
P-NF-κB	1: 1000 for WB	Rabbit	CST	3033S
Bcl-2	1: 2000 for WB	Rabbit	Proteintech	12789-1-AP
BCL-X/L	1: 1000 for WB	Rabbit	Santa Cruz	sc-56021
ACLY	1: 2000 for WB	Rabbit	Proteintech	15421-1-AP
ACC1	1: 2000 for WB	Rabbit	Proteintech	21923-1-AP
P-ACC1	1: 1000 for WB	Rabbit	CST	11818S
AC-H3	1: 2000 for WB, 10 μg for ChIP, 5 μg for Co-IP	Rabbit	Proteintech	61637
AC-H4	1: 2000 for WB, 10 μg for ChIP, 5 μg for Co-IP	Rabbit	Proteintech	39925
ING5	1: 2000 for WB, 10 μg for ChIP	Rabbit	Proteintech	10665-1-AP

HDAC	1: 20000 for WB, 10 μg for ChIP, 5 μg for Co-IP	Mouse	Proteintech	66085-1-Ig
SREBP-1	1: 200 for WB, 10 μ g for ChIP, 10 μg per Co-IP	Mouse	Santa Cruz	66875-1-Ig
ACAT	1: 2000 for WB	Rabbit	Proteintech	16215-1-AP
ADRP	1: 2000 for WB	Rabbit	Proteintech	15294-1-AP
CIDE A	1: 1000 for WB	Rabbit	Proteintech	13170-1-AP
CIDE B	1: 2000 for WB	Rabbit	Proteintech	27600-1-AP
CIDE C	1: 2000 for WB	Rabbit	Proteintech	12287-1-AP
Perilipin 5	1: 2000 for WB	Rabbit	Proteintech	26951-1-AP
TIP47	1: 2000 for WB	Rabbit	Proteintech	10694-1-AP
NEDD4	1: 200 for WB	Mouse	Santa Cruz	sc-518160
NEDD4L	1: 200 for WB	Mouse	Santa Cruz	sc-514954
CBL	1: 200 for WB	Mouse	Santa Cruz	sc-1651
PSMC1	1: 200 for WB	Mouse	Santa Cruz	sc-293484
COP1	1: 200 for WB	Mouse	Santa Cruz	sc-166799
PSMC1	1: 500 For WB	Rabbit	Proteintech	11196-1-AP
Ubiquitin	1: 1000 for WB	Rabbit	Proteintech	10201-2-AP

REG4: Regenerating gene 4; EGFR: Epidermal growth factor receptor; p-PI3K: Phosphorylated phosphoinositide 3-kinase; CST: Cell signaling technology; WB: Western blotting; IHC: Immunohistochemistry; Co-IP: Co-immunoprecipitation; ChIP: Chromosomal immunoprecipitation; ACLY: ATP-citrate lyase; ACC1: Acetyl-CoA carboxylase 1; HDAC: Histone deacetylase; SREBP-1: Sterol-regulatory element binding protein 1; CBL: Cbl proto-oncogene; ACAT: A-cholesterol acyltransferase; ADRP: Adipocyte differentiation-related protein; COP1: COP1 E3 ubiquitin ligase; NF-κB: Nuclear factor-κB; AC-H3: Acetyl-acetyl-histone 3; H4: Histone 4; ING5: Inhibitor of growth protein 5; CIDE: Cell-death-inducing DFF45-like effector; TIP: Tail-interacting protein; NEDD4L: NEDD4 like E3 ubiquitin protein

ligase.

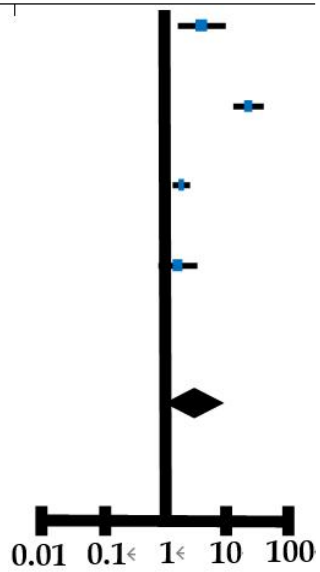
Supplementary Table 2 Main characteristics of eligible studies

Ref.	Yr	Country	Ethnicity	AS	Cases	Control	Risk to cancer	Outcome
Oue *et al*[34]	2005	Japan	Asian	Self-making	36			
Oue *et al*[15]	2007	Japan	Asian	R and D	80			Negative
Li *et al*[30]	2010	Japan	Asian	R and D	320	169	Down	No
Zheng *et al*[28]	2010	Japan	Asian	Self-making	318	330	Down	No
Li *et al*[31]	2010	China	Asian	Self-making	65	24	Down	
Sawada T *et al*[35]	2013	Japan	Asian	R and D	220			Negative
He *et al*[17]	2014	China	Asian	R and D	172			Negative
Kaprio T *et al*[37]	2014	Finland	European	R and D	793			Positive
Zhu *et al*[36]	2015	China	Asian	R and D	186	186	Up	Negative
Zheng *et al*[29]	2010	China	Asian	R and D	60	60	Down	
Wang *et al*[32]	2015	China	Asian	R and D	92	92	Up	
Wang *et al*[33]	2016	China	Asian	R and D	20	60	Up	

AS: Antibody source; Self-making: Self-making antibody.

Supplementary Table 3 Forest plot for relationship between regenerating gene 4 expression and colorectal cancer

Ref.	Normal Events	Total	Cancer Events	Total	Weight	OR M-H, random	95% CI	OR M-H, random, 95% CI
Li *et al*[31]	15	24	20	65	23.0%	3.75	1.41-9.99	
Li *et al*[30]	147	169	99	320	26.0%	14.92	8.98-24.76	
Zheng *et al*[28]	234	330	184	318	26.7%	1.78	1.28-2.46	
Zheng *et al*[29]	45	60	38	60	24.3%	1.74	0.79-3.81	
Total (95% CI)		583		763	100%	3.64	1.11-11.95	
Total events	441		341					
Heterogeneity: $\tau^2 = 1.35$; $\chi^2 = 50.83$, df = 3 ($P < 0.00001$); $I^2 = 94\%$								
Test for overall effect: Z = 2.13($P = 0.03$)								



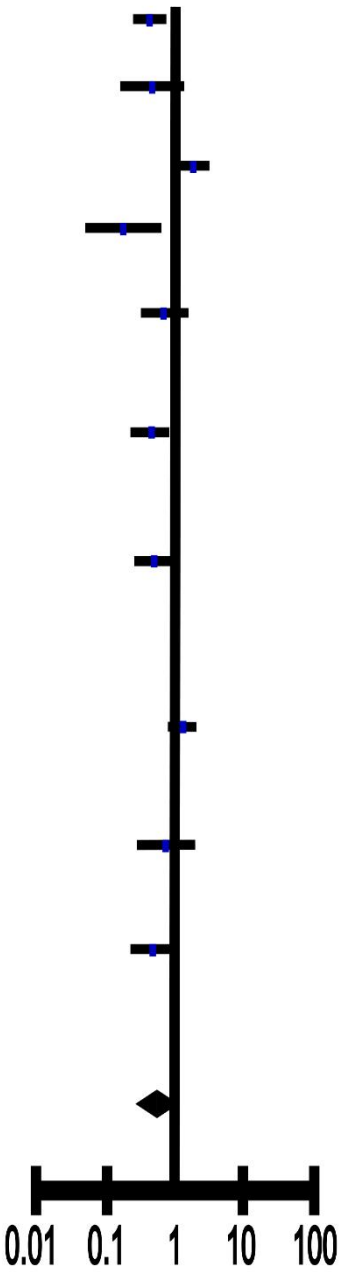
CI: Confidence interval; df: Free degree; M-H: Metropolis-Hastings; OR: Odds ratio.

Supplementary Table 4 Forest plot for the relationship between regenerating gene 4 expression and lymph node metastasis of colorectal cancer

Ref.	LN- Events	LN- Total	LN+ Events	LN+ Total	Weight	OR M-H, random	95% CI	OR M-H, random, 95% CI
He *et al*[17]	56	125	30	47	11.6%	0.46	0.23-0.92	
Li *et al*[31]	7	32	13	32	7.9%	0.41	0.14-1.22	
Li *et al*[30]	67	190	32	130	13.7%	1.67	1.01-2.74	
Oue *et al*[34]	4	20	9	16	5.5%	0.19	0.04-0.85	
Oue *et al*[15]	12	44	11	36	8.9%	0.85	0.32-2.25	
Wang *et al*[33]	7	18	28	42	7.5%	0.32	0.10-1.00	
Wang *et al*[32]	17	43	32	49	10.0%	0.35	0.15-0.81	
Zheng *et al*[38]	110	188	72	126	14.2%	1.06	0.67-1.67	
Zheng *et al*[29]	21	34	17	26	8.1%	0.86	0.30-2.48	
Zhu *et al*[36]	30	96	43	90	12.6%	0.50	0.27-0.90	
Total (95%CI)		790		594	100%	0.62	0.41-0.94	
Total events	331		287					

Heterogeneity: $\tau^2 = 0.27$; $\chi^2 = 25.66$, df = 9 ($P = 0.002$); $I^2 = 65\%$

Test for overall effect: Z = 2.24 ($P = 0.03$)



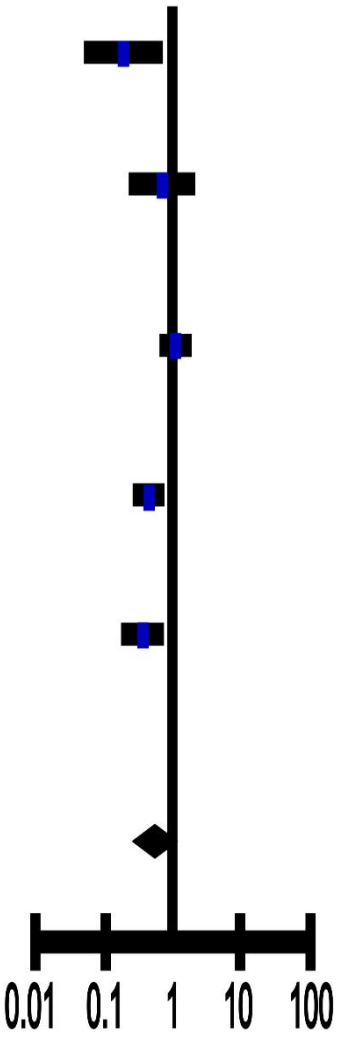
CI: Confidence interval; df: Free degree; M-H: Metropolis-Hastings; OR: odds ratio.

Supplementary Table 5 Forest plot for the relationship between regenerating gene 4 expression and tumor, node, and metastasis staging of colorectal cancer

Ref.	Stage I-II Events	Total	Stage III-IV Events	Total	Weight	OR M-H, random	95% CI	OR M-H, random, 95% CI
Oue *et al*[34]	4	20	9	16	10.6%	0.19	0.04-0.85	
Oue *et al*[15]	11	43	12	37	17.6%	0.72	0.27-1.89	
Wang *et al*[32]	39	64	109	192	26.1%	1.19	0.67-2.12	
Zheng *et al*[28]	29	95	44	91	25.6%	0.47	0.26-0.86	
Zhu *et al*[36]	17	43	32	49	20.0%	0.35	0.15-0.81	
Total (95%CI)		265		385	100%	0.55	0.31-0.98	
Total events	100		206					

Heterogeneity: $\tau^2 = 0.24$; $\chi^2 = 9.94$, df = 4 ($P = 0.04$); $I^2 = 60\%$

Test for overall effect: $Z = 2.02$ ($P = 0.04$)



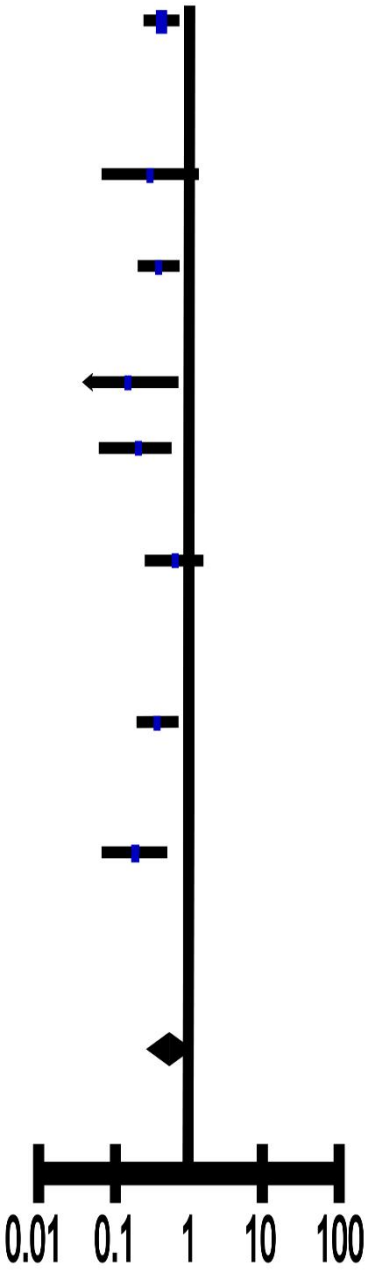
CI: Confidence interval; df: Free degree; M-H: Metropolis-Hastings; OR: Odds ratio.

Supplementary Table 6 Forest plot for the relationship between regenerating gene 4 expression and degree of differentiation of colorectal cancer

Ref.	Well/moderate Events	Total	Poor/mucinous/undiff Events	Total	Weight	OR M-H, Fixed	95% CI	OR M-H, Fixed, 95% CI
Kaprio *et al*[37]	173	712	39	80	37.3%	0.34	0.21-0.54	
Li *et al*[31]	13	53	7	11	6.1%	0.19	0.05-0.74	
Li *et al*[30]	87	297	12	23	11.1%	0.38	0.16-0.89	
Oue *et al*[34]	2	9	5	6	3.3%	0.06	0.00-0.82	
Oue *et al*[15]	17	71	6	9	5.7%	0.16	0.04-0.70	
Wang *et al*[32]	35	71	14	21	7.7%	0.49	0.18-1.35	
Zheng *et al*[28]	160	286	23	29	12.9%	0.33	0.13-0.84	
Zhu *et al*[36]	53	158	20	28	15.9%	0.20	0.08-0.49	
Total (95%CI)		1657		207	100%	0.30	0.22-0.41	
Total events	540		126					

Heterogeneity: $\tau^2 = 4.88$; df = 7 ($P = 0.67$); $I^2 = 0\%$

Test for overall effect: Z = 7.58 ($P < 0.00001$)

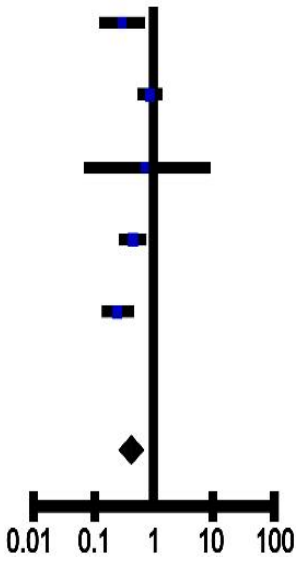


						ous/undiff derate

CI: Confidence interval; df: Free degree; M-H: Metropolis-Hastings; OR: Odds ratio; undiff: Undifferentiated.

Supplementary Table 7 Forest plot for the relationship between regenerating gene 4 expression and overall survival rate of colorectal cancer patients

Ref.	O-E	Variance	Weight	Peto OR Exp [(O-E) /V], Fixed	95% CI	Peto OR Exp [(O-E) /V], Fixed, 95% CI
He *et al*[17]	–6	5.46	11.6%	0.33	0.14-0.77	
Li *et al*[30]	–3.84	19.36	41.1%	0.82	0.53-1.28	
Oue *et al*[15]	–0.03	0.62	1.3%	0.95	0.08-11.48	
Sawada *et al*[35]	–7.23	12.88	27.3%	0.57	0.33-0.98	
Zhu *et al*[36]	– 11.63	8.83	18.7%	0.27	0.14-0.52	
Total (95% CI)			100%	0.54	0.41-0.72	
Heterogeneity: χ^2 = 9.23; df = 4 (P = 0.06); I^2 = 57%						
Test for overall effect: Z = 4.18 (P < 0.0001)						



CI: Confidence interval; df: Free degree; M-H: Metropolis-Hastings; OR: Odds ratio; *REG4*: Regenerating gene 4.

Supplementary Table 8 Relationship between regenerating gene 4 expression and histological subtyping of colorectal cancer

WHO classification	*n*	*REG4* expression		
		-	+	PR (%)
Well-differentiated adenocarcinoma	307	117	190	61.9
Moderately differentiated adenocarcinoma	287	128	159	55.4
Poorly differentiated carcinoma	172	81	91	52.9
Mucinous adenocarcinoma	27	4	23	85.2[a]

[a]$P < 0.05$ *vs* well-, moderately and poorly differentiated adenocarcinoma. WHO: World health organization; *REG4*: Regenerating gene 4.