FAS rs2234767 and rs1800682 polymorphisms jointly contributed to risk of colorectal cancer by affecting SP1/STAT1 complex recruitment to chromatin

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FAS rs2234767 (−1377 G>A), rs1800682 (−670 A>G) and FASLG rs763110 (−844 C>T) promoter polymorphisms can influence transcriptional activities of the genes and thus multiple tumors susceptibility. To investigate their association with risk of colorectal cancer (CRC), the three SNPs were genotyped in 878 cases and 884 controls and the results showed that the FAS rs2234767 and rs1800682 were in a high linkage disequilibrium (LD) with each other (D’ = 0.994) and jointly contributed to an increased risk of CRC (without vs. with rs2234767 GG/rs1800682 AA genotypes, adjusted OR = 1.30, 95% CI = 1.05 – 1.61). In vivo ChIP assays evaluated the effect of rs2234767 and rs1800682 on recruitment of SP1 and STAT1, respectively, to chromatin. The results showed SP1 interacting specifically with STAT1 recruited to their respective motifs for transcriptional activation. The mutant alleles rs2234767 A and rs1800682 G jointly affected coupled SP1 and STAT1 recruitment to chromatin. The interplay between SP1 and STAT1 was critical for the functional outcome of rs2234767 and rs1800682 in view of their high LD. In conclusion, the FAS rs2234767 and rs1800682 polymorphisms were in high LD with each other, and they jointly contributed to an increased risk of CRC by altering recruitment of SP1/STAT1 complex to the FAS promoter for transcriptional activation.

Colorectal cancer (CRC) is the third most commonly diagnosed cancers worldwide, accounting for roughly 1.2 million new cases and 600,000 deaths per year¹. CRC is a complex disease resulting from both genetic and epigenetic alterations, including genetic variants² and abnormal DNA methylation patterns³–⁵, among others. Two decades of research on genetic architecture of CRC has revealed that inherited susceptibility is a major component of CRC predisposition, with genetic factors accounting for 12–35% risk of CRC².

A large number of studies have implicated the involvement of deregulated apoptosis pathway in CRC carcinogenesis⁶–⁷. Defect, dysfunction or altered expression of genes encoding key apoptotic proteins modify risk of CRC⁸. FAS, also known as CD95, encoded by FAS gene, is a cell surface factor and important inducer of the extrinsic apoptosis signaling pathway. FAS ligand, FASLG, also known as CD95L, encoded by FASLG gene, is a member of the tumor necrosis factor superfamily. FASLG binding to FAS triggers apoptosis through activation of CASP8⁹. Accumulating evidence suggests that altered expression of FAS and/or FASLG contributes to development of CRC⁹.

Functional SNPs within the promoter region of gene are capable of affecting transcription and subsequently modulating risk of disease¹⁰,¹¹. It has been reported that there are two functional SNPs in the promoter of FAS...
gene (FAS − 1377 G > A, rs2234767; − 670 A > G, rs1800682), which located within the consensus sequences of the SP1 and STAT1 transcription factors (TF) binding sites, respectively. Sibley et al. reported that the rs2234767 A had a greatly reduced ability to bind SP1 compared with rs2234767 G and people with A allele had a significantly increased risk of acute myeloid leukemia (AML); however, both the rs1800682 A and rs1800682 G alleles could bind STAT1 and have no detectable difference in binding affinity. Wu et al. first identified a T to C substitution at position − 844 in the promoter of FASLG gene (FASLG − 844 C > T, rs763110), which located in a putative binding motif for CAAT/enhancer-binding protein β (C/EBPβ). Functional study revealed that − 844 C allele could increase basal FASLG expression, suggesting the − 844 C > T polymorphism may affect the FASLG-mediated apoptotic signaling. A number of studies have been conducted to investigate the association between the three SNPs and a variety of tumors, including esophageal squamous-cell carcinoma (ESCC), squamous cell carcinoma of the head and neck (HNSCC), bladder cancer, and gastric cancer.

In this study, we aimed to determine the association of the FAS rs2234767, rs1800682, and FASLG rs763110 polymorphisms with risk of CRC in a Chinese population and the molecular mechanism underlying the association.

**Methods and Materials**

**Ethics statement.** The study was approved by the institutional review board of Southeast University. Each subject signed an informed consent. The research protocol was carried out in accordance with the approved guidelines.

**Patients and samples.** A total of 878 CRC patients and 884 healthy controls were enrolled in this study. The detailed information on the subjects has been described elsewhere. Briefly, all patients were recruited from the First Affiliated Hospital of Nanjing Medical University between September 2010 and October 2013. The pathological stage of CRC at the time of diagnosis was classified into Dukes A, B, C and D. All controls were genetically unrelated to the cases and recruited from those who were seeking for health care in the same hospital. After signed the informed consent, all subjects donated 5 ml of venous blood sample for genomic DNA extraction.

**Genotyping.** The genotyping of FAS rs2234767, rs1800682 and FASLG rs763110 was performed by TaqMan allelic discrimination method equipped with ABI 7900 HT Real Time PCR System (Applied Biosystems, CA, USA). The reaction conditions were set as follows: 95 °C for 10 min followed by 40 cycles of 95 °C for 15 sec, and 60 °C for 1 min. The antisera for ChIP reaction was normal mouse IgG (Cat. No. 2027, Santa Cruz Biotechnology, Inc.), normal rabbit IgG (Cat. No. NI01, EMD Chemicals, Inc., Gibbstown, NJ), anti-human SP1 (Cat. No. 9389, Cell Signaling Technology), and anti-human STAT1 (Cat. No. 9172, Cell Signaling Technology). Precipitated genomic DNA was analyzed by quantitative PCR in triplicate measurements for each sample using the following human FAS promoter primers: 5′ − ACCATCCCTCTTATCCACT-3′ (forward) and 5′ − GTAGGTGTAGATGCGTCTGTA-3′ (reverse) for rs2234767; 5′ − CTAAGGGGCTCCCTCTATT-3′ (forward) and 5′ − ACTTGCCGCGATTTGA-3′ (reverse) for rs1800682. Captured genomic DNA was normalized to input material and the samples with different genotypes compared.

**Chromatin immunoprecipitation assay (ChIP).** Human peripheral white blood cells (5 × 10^7 per sample) were fixed for 10 min at 37 °C with 4% formaldehyde. After incubation, fresh glycine was added to a final concentration of 125 mM to stop cross-linking. After 5 min at room temperature, the samples were pelleted in an centrifuge at 3600 rpm (2000 g) for 2 min at 4 °C, washed once with cold PBS plus protease inhibitors, and then repelleted. The pellet was resuspended in 1 ml of PBS and ground the cells using a micro−tissue grinder on ice. Cells were pelleted again as above at 4 °C. ChIP was performed using the ChIP-IT™ Express Magnetic assay kit (Cat. No. 53009, Active Motif). The antisera for ChIP reaction was normal mouse IgG (Cat. No. 2027, Santa Cruz Biotechnology, Inc.), normal rabbit IgG (Cat. No. NI01, EMD Chemicals, Inc., Gibbstown, NJ), anti-human SP1 (Cat. No. 9389, Cell Signaling Technology), and anti-human STAT1 (Cat. No. 9172, Cell Signaling Technology). Precipitated genomic DNA was analyzed by quantitative PCR in triplicate measurements for each sample using the following human FAS promoter primers: 5′ − ACCATCCCTCTTATCCACT-3′ (forward) and 5′ − GTAGGTGTAGATGCGTCTGTA-3′ (reverse) for rs2234767; 5′ − CTAAGGGGCTCCCTCTATT-3′ (forward) and 5′ − ACTTGCCGCGATTTGA-3′ (reverse) for rs1800682. Captured genomic DNA was normalized to input material and the samples with different genotypes compared.

**Statistical analysis.** The Hardy-Weinberg equilibrium (HWE) of the controls’ genotype frequencies was evaluated by a goodness-of-fit chi-square test (χ^2 test). Bonferroni correction for multiple testing was also applied. Crude and adjusted odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to analyze the magnitude of the association between the genotypes and risk of CRC by univariate and multivariate unconditional logistic regression models, respectively. A P-value < 0.05 was considered statistically significant.

**Results**

**Association between the FAS and FASLG polymorphisms and risk of CRC.** The genotype frequencies of FAS rs2234767, rs1800682 and FASLG rs763110 among the controls were all in agreement with Hardy-Weinberg equilibrium (P = 0.11 for rs2234767, 0.060 for rs1800682 and 0.53 for rs763110). As shown in Table 1, the frequencies of rs2234767 mutant A allele was higher in the cases than the controls (39% and 34%, P = 0.013 after Bonferroni correction). Moreover, the frequencies distribution of rs2234767 genotypes were significantly different between the cases and controls (P = 0.0051), which remained significant after Bonferroni correction (P = 0.015). When the rs2234767 GG genotype used as the reference, the heterozygous GA and AA genotypes were both associated with significantly increased risk of CRC (adjusted OR = 1.39, 95% CI = 1.13 – 1.71 for the GA genotype; 1.37, 1.02 – 1.84 for the AA genotype), and the risk did not change substantially under the assumption of a dominant genetic model (adjusted OR = 1.38, 95% CI = 1.14 – 1.68).

The genotype and allele frequencies distribution of the FASLG rs763110 were not significantly different between the cases and controls (P = 0.71 and P = 0.92; P = 1.0 and P = 1.0 after Bonferroni correction). The allele frequency distribution of the FAS rs1800682 was not significantly different between the cases and controls (P = 0.13 and P = 0.39 after Bonferroni correction). The difference of the rs1800682
Genotype distribution was quasi significant between the cases and controls ($P = 0.060$); however, the difference did not remain significant after Bonferroni correction ($P = 0.18$). Further analysis showed that the rs1800682 polymorphism was associated with an increased risk of CRC under the dominant genetic model ($AG/GG$ vs. $AA$, adjusted OR = $1.26, 95\% CI = 1.04$–$1.53$).

**Association between the combined genotypes of the FAS polymorphisms and risk of CRC.** Linkage disequilibrium analysis (LD) revealed a strong LD between the two FAS polymorphisms among the controls ($D = 0.994$ and $r^2 = 0.848$, $P < 0.001$), suggesting a joint effect between the two FAS polymorphisms. To evaluate the genotype-genotype interaction, we dichotomized the FAS genotypes as either rs2234767 $GG$ or rs2234767 $GA/AA$ and rs1800682 $AA$ or rs1800682 $AG/GG$. When the rs2234767 $GG$/rs1800682 $AA$ genotypes were used as the reference, the (rs2234767 $GA$)/rs1800682 $AA$ genotypes and the (rs2234767 $GA$)/rs1800682 $AG/GG$ genotypes were associated with a significantly higher risk of CRC (adjusted OR = $15.49, 95\% CI = 2.01$–$119.25$ for the (rs2234767 $GA$)/rs1800682 $AA$ genotypes; and $1.30, 1.06$–$1.59$ for the (rs2234767 $GA$)/rs1800682 $AG/GG$ genotypes; Table 2).

**Table 1. Association between FAS rs2234767, rs1800682 and FASLG rs763110 genotypes and risk of CRC.** Bold indicated statistically significant. *$\chi^2$ test for either genotype distributions or allele frequencies between the cases and controls. **Adjusted for multiple comparisons by Bonferroni correction. ***Adjusted for age, sex, smoking and drinking status in logistic regression model.

| SNPs      | Genotype | Cases (n = 878) | Controls (n = 884) | $P^a$ | $P^b$ | Adjusted OR (95% CI)$^c$ |
|-----------|----------|----------------|--------------------|-------|-------|--------------------------|
| FASLG     | CC       | 462 53 470 53  | 0.71 1.0           | 1.00 (Ref) |
| rs763110  | CT       | 354 40 344 39  | 1.06 (0.87–1.28)  |       |
|           | TT       | 62 7 70 8    | 0.90 (0.62–1.30)  |       |
| $P_{total}$ |          |               | 0.92               |       |
| FAS       | GG       | 305 37 385 44 | 0.0051 0.015       | 1.00 (Ref) |
| rs2234767 | GA       | 407 49 372 43 | 1.39 (1.13–1.71)  |       |
|           | AA       | 124 15 114 13 | 1.37 (1.02–1.84)  |       |
| $P_{total}$ |          |               | 0.0042 0.013       |       |
| FAS       | GG       | 305 36 385 44 | 1.00 (Ref)         |       |
| rs1800682 | AG       | 435 50 392 44 | 1.30 (1.06–1.60)  |       |
|           | GG       | 142 16 144 16 | 1.16 (0.88–1.53)  |       |
| $P_{total}$ |          |               | 0.0051 0.14        |       |
| FAS       | AA       | 301 34 348 40 | 0.060 0.18         | 1.00 (Ref) |
| rs1800682 | AG       | 435 50 392 44 | 1.30 (1.06–1.60)  |       |
|           | GG       | 142 16 144 16 | 1.16 (0.88–1.53)  |       |
| $P_{total}$ |          |               | 0.0051 0.14        |       |
| FAS       | AA       | 301 34 348 40 | 0.060 0.18         | 1.00 (Ref) |
| rs1800682 | AG       | 435 50 392 44 | 1.30 (1.06–1.60)  |       |
|           | GG       | 142 16 144 16 | 1.16 (0.88–1.53)  |       |
| $P_{total}$ |          |               | 0.0051 0.14        |       |

**Table 2. Combined genotype frequencies of the FAS polymorphisms among the cases and controls and their association with risk of CRC.** Bold indicated statistically significant. *$\chi^2$ test for the combined genotype distributions between the cases and controls. **Adjusted for age, sex, smoking and drinking status in logistic regression model.

| Combined genotypes | Cases (n = 836) | Controls (n = 871) | $P$ | Adjusted OR (95% CI)$^b$ |
|--------------------|----------------|--------------------|-----|--------------------------|
|                    | n (%)          | n (%)              |     |                          |
| rs2234767          | FAS rs1800682  | <0.001             |     |                          |
| FAS                |                |                    |     |                          |
| rs2234767          |                |                    |     |                          |
| GG                 | AA             | 287 (34)           | 347 (40) | 1.00 (Reference) |
| GG                 | AG/GG          | 18 (2)             | 38 (3.9) | 0.60 (0.33–1.07) |
| GA/AA              | AA             | 13 (2)             | 1 (0.1) | 15.49 (2.01–119.25) |
| GA/AA              | AG/GG          | 518 (62)           | 485 (56) | 1.30 (1.06–1.59) |
| Trend test         |                |                    | 0.0051 |                          |

**Stratification analysis of the association of FAS combined genotypes with CRC susceptibility by demographic variables.** To control the impact of confounders on the genetic association, we performed stratification analysis. The combined genotypes were dichotomized into two groups, i.e., with rs2234767 $GG$/
rs1800682 AA and without rs2234767 GG/rs1800682 AA, to facilitate further analysis. As shown in Table 3, compared with the rs2234767 GG/rs1800682 AA genotypes, the individuals carrying the combined genotypes without rs2234767 GG/rs1800682 AA had a higher risk of CRC (adjusted OR = 1.28, 95% CI = 1.05 – 1.56), and the risk was more pronounced among the subgroups of age > 60 years, female, never smokers or drinkers, having no family history of cancer (adjusted OR = 1.50, 95% CI = 1.13 – 1.99). However, the combined genotypes were not significantly associated with CRC with low or high grade, which was likely due to the reduced number of subjects. In the stratification of stage, a significantly increased risk was only found between the combined genotypes without rs2234767 GG/rs1800682 AA and CRC with Dukes C and D stage (adjusted OR = 1.33, 95% CI = 1.04 – 1.71; Table 4).

### Table 3. Stratified analysis of the FAS combined genotypes associated with CRC risk by demographic variables. Bold indicated statistically significant. a $\chi^2$ test for the combined genotype distributions between the cases and controls. b Adjusted for age, sex, smoking and drinking status in logistic regression model.

| Variables | Case/control (n) | Combined genotypes (case/control) | With rs2234767 GG/rs1800682 AA | Without rs2234767 GG/rs1800682 AA | $p^a$ | Adjusted OR (95% CI)$^b$ |
|-----------|-----------------|----------------------------------|---------------------------------|----------------------------------|------|--------------------------|
|           | Total           | 836/871                          | n | % | n | % |          |                      |
| Age (years) | ≤ 60            | 436/401                          | 287/347 | 34/40 | 549/524 | 66/60 | 0.019 | 1.28 (1.05–1.56)       |
|           | > 60            | 400/470                          | 131/191 | 33/41 | 269/279 | 67/59 | 0.016 | 1.50 (1.13–1.99)       |
| Sex       | Male            | 510/507                          | 198/207 | 39/41 | 312/300 | 61/59 | 0.51  | 1.08 (0.84–1.39)       |
|           | Female          | 326/364                          | 89/140 | 27/38 | 237/224 | 73/62 | 0.0019| 1.77 (1.26–2.47)       |
| Smoking status | Never        | 558/602                          | 185/244 | 33/40 | 373/358 | 67/60 | 0.0093| 1.39 (1.09–1.77)       |
|           | Ever            | 278/269                          | 102/103 | 37/38 | 176/166 | 63/62 | 0.70  | 1.08 (0.78–1.37)       |
| Drinking status | Never        | 610/655                          | 207/264 | 34/40 | 403/391 | 66/60 | 0.019 | 1.33 (1.05–1.67)       |
|           | Ever            | 226/216                          | 80/83  | 35/38 | 146/133 | 65/62 | 0.51  | 1.16 (0.78–1.72)       |
| Family history of cancer | No          | 642/786                          | 222/323 | 35/41 | 420/463 | 65/59 | 0.012 | 1.34 (1.08–1.67)       |
|           | Yes             | 194/85                           | 65/24  | 34/28 | 129/61  | 66/72 | 0.39  | 0.78 (0.44–1.37)       |

### Table 4. Association between the FAS combined genotypes and progression of CRC. Bold indicated statistically significant. a $\chi^2$ test for the combined genotype distributions between the cases and controls. b Adjusted for age, sex, smoking and drinking status in logistic regression model.

| Variables | Combined genotypes | With rs2234767 GG/rs1800682 AA | Without rs2234767 GG/rs1800682 AA | $p^a$ | Adjusted OR (95% CI)$^b$ |
|-----------|-------------------|---------------------------------|----------------------------------|------|--------------------------|
|           |                   | n | % | n | % |          |                      |
| Controls (n = 871) |                   | 347 | 40 | 524 | 60 | 1.00 (reference) |
| Cases (n = 836) | Tumor grade       | Low | 24 | 40 | 36 | 60 | 0.98 | 0.99 (0.58–1.70) |
|           |                   | Intermediate | 219 | 34 | 427 | 66 | 0.018 | 1.30 (1.05–1.61) |
|           |                   | High | 44 | 34 | 86 | 66 | 0.19 | 1.30 (0.88–1.91) |
| Dukes stage | A + B             | 150 | 35 | 275 | 65 | 0.11 | 1.22 (0.96–1.55) |
|           | C + D             | 137 | 33 | 274 | 67 | 0.025 | 1.33 (1.04–1.71) |

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**Association between the combined genotypes of the FAS polymorphisms and progression of CRC.** We further evaluated the association between the FAS combined genotypes and grade and stage of CRC. When compared with the rs2234767 GG/rs1800682 AA genotypes, the combined genotypes without rs2234767 GG/rs1800682 AA were associated with a significantly increased risk of CRC with intermediate grade (adjusted OR = 1.30, 95% CI = 1.05 – 1.61; Table 4). However, the combined genotypes were not significantly associated with CRC with low or high grade, which was likely due to the reduced number of subjects. In the stratification of stage, a significantly increased risk was only found between the combined genotypes without rs2234767 GG/rs1800682 AA and CRC with Dukes C and D stage (adjusted OR = 1.33, 95% CI = 1.04 – 1.71; Table 4).
rs2234767 A coordinates with rs1800682 G to attenuate binding affinity of SP1 and STAT1 to FAS promoter in vivo. To determine the molecular mechanisms underlying the association of FAS polymorphisms with an increased risk of CRC, the effect of the rs2234767 and rs1800682 polymorphisms on the ability of SP1 and STAT1 transcription factors to bind the endogenous FAS promoter region was assessed by ChIP assays. Because the rs2234767 G and rs1800682 A alleles were in high LD with each other as mentioned above, there existed only three combined genotypes in our samples for ChIP assays. Finally, a total of 9 people with three different combined genotypes, i.e., rs2234767 GG/rs1800682 AA, rs2234767 GA/rs1800682 AG, and rs2234767 AA/rs1800682 GG, were selected for ChIP assays.

As shown in Fig. 1A, the region with rs2234767 G and rs1800682 A alleles were readily immunoprecipitated by anti-SP1 and anti-STAT1, respectively, but not IgG, demonstrating SP1 and STAT1 interact specifically with the FAS promoter in chromatin (left). Interestingly, the ability of SP1 and STAT1 to bind the FAS promoter was dramatically decreased with the increase of mutant alleles. The amount of chromatin with the rs2234767 GA/rs1800682 AG genotypes captured by anti-SP1 and anti-STAT1 was 6.9% and 12%, respectively, of that with the rs2234767 GG/rs1800682 AA genotypes (middle). The homozygous mutant genotypes rs2234767 AA/rs1800682 GG eliminated both the SP1 and STAT1 recruitment to the FAS promoter (right). Furthermore, as assessed by sequential ChIP (Re-ChIP), the SP1 interacting with STAT1 was recruited to the SP1 (Fig. 1B, left) and STAT1 motifs (Fig. 1C, left) for transcriptional regulation. The mutant alleles could also eliminate the SP1/STAT1 complex recruitment to the SP1 (Fig. 1B, middle and right) and STAT1 motifs (Fig. 1C, middle and right). We also selected 9 other people with three combined genotypes for reproducibility of ChIP assays, and found the results were consistent with those mentioned above (Fig. S1). Our results indicated that SP1 and STAT1 contributed equally to activate the transcription of FAS in CRC and the interplay between these factors was critical for the functional outcome of FAS rs2234767 and rs1800682 in view of their high LD.

Discussion

In the present study, we analyzed the association of FAS rs2234767, rs1800682 and FASLG rs763110 polymorphisms with risk of CRC in a Chinese population. The FAS rs2234767 and rs1800682 polymorphisms had effect on increasing risk of CRC and a joint effect on risk and progression, but not for the FASLG rs763110 polymorphism. The joint effects of the two FAS polymorphisms on risk of CRC were more pronounced among the subgroups with age >60 years, female, never smokers, never drinkers, having no family history of cancer, and CRC with intermediate grade and with Dukes C and D stage. Functional studies revealed that the SP1 interacting with STAT1 was recruited to the SP1 and STAT1 motifs within the promoter of FAS for transcriptional regulation, and the interplay between these factors was critical for the functional outcome of FAS rs2234767 and rs1800682 in view of their high LD. Given the role of FAS/FASLG pathway in carcinogenesis, it is biologically plausible
that the rs2234767 and rs1800682 polymorphisms may modulate the risk of CRC by attenuating SP1/STAT1 complex-mediated transcriptional activation of FAS, which in turn dampening FAS apoptotic pathway.

Colorectal cancer is a complex disease and develops through a multistage process. During the process, colorectal epithelial cells accumulate a number of molecular changes and eventually become fully malignant cells. These molecular changes involve mutations in the well-defined genes or pathways such as APC, mismatch repair genes like MLH1, and SMAD4, and epigenetic changes such as global DNA hypomethylation in repetitive sequences (satellite and LINE repeats) and promoter hypermethylation of tumor suppressor genes like MLH1, RUNX3 and SEPT9. It is now well accepted that genes that regulate apoptosis are important variables in cancer development. A lot of studies have shown that alteration of FAS and FASLG expression decreases the apoptotic activity and facilitates tumor cells evading or suppressing the immune system. Deregulated FAS and FASLG expression are common features of most human malignancies and associated with progression of a variety of tumors, including CRC.

Therefore, the functional variants of the FAS and FASLG genes which were capable of influencing their expression could be expected to have effect on cell death and thus, carcinogenesis.

**References**

1. Brenner, H., Kloos, M. & Pox, C. P. Colorectal cancer. *Lancet* **383**, 1490–1502 (2014).
2. Peters, U., Bien, S. & Zubair, N. Genetic architecture of colorectal cancer. *Ann NY Acad Sci* **1356**, 1–14 (2015).
3. Bardhan, K. & Liu, K. Epigenetics and colorectal cancer pathogenesis. *Cancers (Basel)* **5**, 676–713 (2013).
4. Ng, J. M. & Yu, J. Promoter hypermethylation of tumour suppressor genes as potential biomarkers in colorectal cancer. *Int J Mol Sci* **16**, 2472–2496 (2015).
5. Colussi, D., Brandi, G., Bazzoli, F. & Ricciardiello, L. Molecular pathways involved in colorectal cancer: implications for disease behavior and prevention. *Int J Mol Sci* **14**, 16365–16385 (2013).
6. Yang, S. Y., Sales, K. M., Fuller, B., Seifalian, A. M. & Winslet, M. C. Apoptosis and colorectal cancer: implications for therapy. *Trends Mol Med* **15**, 225–233 (2009).
7. Mehlen, P. & Tauszig-Delamasure, S. Dependence receptors and colorectal cancer. *Gut* **63**, 1636–1646 (2014).
8. Hoogwater, F. J., Steller, E. J., Westendorp, B. F. & Borel Rinkes, I. H. & Kranenburg, O. CD95 signaling in colorectal cancer. *Biochim Biophys Acta* **1826**, 189–198 (2012).
9. Askshenazi, A. & Salvesen, G. Regulated cell death: signaling and mechanisms. *Annu Rev Cell Dev Biol* **30**, 337–356 (2014).
Author Contributions

R.C. and M.W. conceived and designed the experiments. S.W., S.W. and Q.M. performed the experiments. S.W., and X.L. analyzed the data. R.C. and M.W. contributed reagents/materials/analysis tools. S.W. and R.C. wrote the paper.

Additional Information

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