Table S1. The basic information of 377 patients with colorectal cancer for ALKAL1 immunohistochemical staining analysis.

|                       | Cases (n) | Percentage (%) |
|-----------------------|-----------|----------------|
| Location              |           |                |
| Rectum                | 168       | 44.6           |
| Colon                 | 196       | 52.0           |
| NA                    | 13        | 3.4            |
| Gender                |           |                |
| Female                | 158       | 41.9           |
| Male                  | 218       | 57.8           |
| NA                    | 1         | 0.3            |
| Age                   |           |                |
| <60                   | 187       | 49.6           |
| ≥60                   | 190       | 50.4           |
| Histologic type       |           |                |
| Non-specific<sup>a</sup> | 316     | 83.8           |
| Other<sup>b</sup>     | 61        | 16.2           |
| Grade                 |           |                |
| G1                    | 28        | 7.4            |
| G2                    | 253       | 67.1           |
| G3                    | 27        | 7.2            |
| NA                    | 69        | 18.3           |
| T classification       |           |                |
| T1                    | 16        | 4.2            |
| T2                    | 68        | 18.0           |
| T3                    | 255       | 67.6           |
| T4                    | 11        | 2.9            |
| NA                    | 27        | 7.3            |
| N classification       |           |                |
| N0                    | 199       | 52.8           |
| N1                    | 91        | 24.1           |
| N2                    | 63        | 16.7           |
| NA                    | 24        | 6.4            |
| M classification       |           |                |
| M0                    | 298       | 79.0           |
| M1                    | 79        | 21.0           |
| Stage                 |           |                |
| Stage I               | 56        | 14.9           |
| Stage   | Count | Percentage |
|---------|-------|------------|
| Stage II | 113   | 30.0       |
| Stage III | 108   | 28.6       |
| Stage IV  | 79    | 21.0       |
| NA       | 21    | 5.5        |

Noted: a) Non-specific type of adenocarcinoma included polypoid adenocarcinoma, tubular adenocarcinoma, and mucious adenocarcinoma. b) Other type included signet ring cell carcinoma, undifferentiated carcinoma, and adenosquamous carcinoma. NA: Not available
| Type of diseases | Cases (n) | Percentage (%) |
|------------------|-----------|----------------|
| Gender           |           |                |
| Male             | 58        | 72.5           |
| Female           | 22        | 27.5           |
| Age              |           |                |
| <60              | 47        | 58.8           |
| ≥60              | 33        | 41.3           |
| Type of diseases |           |                |
| Adenoma          | 40        | 50.0           |
| Inflammation     | 9         | 11.3           |
| Polyp            | 25        | 31.3           |
| Other            | 6         | 7.5            |

* Adenoma includes tubular adenoma and villioustublar adenoma. Inflammation includes ulceration, chronic inflammation and abscess. Polyp includes adenomatous polyp, inflammatory polyp, inflammatory hyperplastic polyp, juvenile polyp and hyperplastic polyp. Other includes multiple diverticulum and congenital megacolon.
Table S3. The basic information of 20 patients with colorectal precancerous lesions for ALKAL1 immunohistochemical staining analysis.

|                          | Cases (n) | Percentage (%) |
|--------------------------|-----------|----------------|
| **Gender**               |           |                |
| Male                     | 12        | 30.0           |
| Female                   | 8         | 20.0           |
| **Age**                  |           |                |
| <60                      | 7         | 17.5           |
| ≥60                      | 12        | 30.0           |
| NA                       | 1         | 2.5            |
| **Histologic**           |           |                |
| Adenomatous polyp with IN| 3         | 7.5            |
| Tubular adenoma with IN  | 8         | 20.0           |
| Villioustublar adenoma with IN | 9    | 22.5           |

* NA: Not available; IN: intraepithelial neoplasia.
Table S4. The basic information of 10 colorectal cancer patients for ALKAL1 mRNA and protein expression analysis.

|                | Cases (n) | Percentage (%) |
|----------------|-----------|----------------|
| Location       |           |                |
| Colon          | 5         | 50.0           |
| Rectum         | 5         | 50.0           |
| Gender         |           |                |
| Male           | 5         | 50.0           |
| Female         | 5         | 50.0           |
| Age            |           |                |
| ≤60            | 4         | 40.0           |
| >60            | 6         | 60.0           |
| Grade          |           |                |
| G1             | 1         | 10.0           |
| G2             | 8         | 80.0           |
| G3             | 1         | 10.0           |
| Stage          |           |                |
| Stage I        | 1         | 10.0           |
| Stage II       | 5         | 50.0           |
| Stage III      | 4         | 40.0           |
| Stage IV       | 0         | 0.0            |
Table S5. A list of primers used in the reactions for real-time RT-PCR.

| Gene name | forward | reverse |
|-----------|---------|---------|
| GAPDH     | 5'-GCACCGTCAAGGCTGAGAAC -3' | 5'-TGGTGAAGACGCCAGTGGA -3' |
| ACTB      | 5'-TGGCACCCAGCAACAATGAA -3' | 5'-CTAAGTCATAGTCCGCTAGAAGCA -3' |
| ALKAL1    | 5'-AGCAAAACATTTCCACCGACT -3' | 5'-AACTACGATGATGACCGCAAG -3' |
| MMP2      | 5'-GACAGACGGAAGTTCTTGGTG -3' | 5'-GATAGGCTAGCGAAGACACT -3' |
| MMP3      | 5'-TACAAGGAGGCGAGCAAGAC -3' | 5'-GGATAGGCTAGCGAAGACACT -3' |
| MMP7      | 5'-TGTATGGGGAACCTGAGACA-3' | 5'-ATCTCCTCCAGACCTGTCC-3' |
| MMP9      | 5'-CAGTCCACCTTGTGCTCTT -3' | 5'-ATTTCACTCTCCACCGCATC -3' |
| TIMP1     | 5'-TTTCTTCCTCCCTTGCTCTA -3' | 5'-AAAGGGACGCAGCCAGATTT -3' |
| TIMP2     | 5'-GGAAGTGGACTCTGGAAACG -3' | 5'-GGGGGCGGCTGAGATAAACACT -3' |
| TIMP3     | 5'-CTGACAGGTCGCGTCTATGA -3' | 5'-TGCAACGCTACACCCCACGGTGA -3' |
| TIMP4     | 5'-CTTTGCGACAGGCGAAGAGTC -3' | 5'-GTCAGAAGGCACTCGTTAGG -3' |
| CFL1      | 5'-GCAAGAAGGAGATTGGTGG -3' | 5'-GCTTTACCTCGTCAAGCTCTC -3' |
| CFL2      | 5'-ACGTCAAAACCCCTTTAAGAAG -3' | 5'-CTCCAGGACCCCATGTAAGTC-3' |
| HSP90AA1  | 5'-GCCTCTGGTGATGAGATGTG-3' | 5'-ACGTCCACACAAAAGGCTGAGT-3' |
| LIMK1     | 5'-GGAGAGGAAAGGAGCGAGTT-3' | 5'-GCAGTCACAACACCTGAAGC-3' |
| LIMK2     | 5'-ATGCACATCAGTCCCAACAA -3' | 5'-GTCTCCTGCTAATTGCTCCTC -3' |
| TESK1     | 5'-CGTGTGGAGATTTTCACTG-3' | 5'-TGGGGAAGGCTGTTCCCTCCTC -3' |
|  | forward | reverse          |
|---|---------|------------------|
| PDXP | 5'- CGACCCCGAGTGCCCTACT -3' | 5'- CGAGGCTGTCTCCACTGC -3' |
| ROCK1 | 5'- GCACCAGTTGTACCCGATTT-3' | 5'- AGTTGATTGCAAACGAAAGC-3' |
| ROCK2 | 5'- TGGGGTGGAAAGAAATCAGAC -3' | 5'- TCATCGAAATTGCTGCTGTC -3' |
| PTCH | 5'- GCCATGGTTCTGCTCATT TT -3' | 5'- CCTGAATCACTCTGCTGACG -3' |
| HIP1 | 5'- GCAGGAAGTGCTGTAAGG -3' | 5'- GCACTCGCGTTGCTAGACAGA -3' |
| CCND1 | 5'- CGTGGCCTCTAAGATGAAGG -3' | 5'- CCACTTGAGCTTGTCAAGG -3' |
| CCNE2 | 5'- GGAGATCGTCTGCTTGCATTA -3' | 5'- TCAGGCAAAGGTGAGATT -3' |
| HDAC1 | 5'- TGGAATCTATCGCCCTCACA -3' | 5'- CTGCTTGTGTACTCCGACA -3' |
Table S6. The basic information of 377 patients with colorectal cancer for ALKAL1 immunohistochemical staining analysis.

|                | Cases (n) | Percentage (%) |
|----------------|-----------|-----------------|
| **Location**   |           |                 |
| Rectum         | 187       | 49.6            |
| Colon          | 178       | 47.2            |
| NA             | 12        | 3.2             |
| **Gender**     |           |                 |
| Female         | 158       | 41.9            |
| Male           | 218       | 57.8            |
| NA             | 1         | 0.3             |
| **Age**        |           |                 |
| <60            | 187       | 49.6            |
| ≥60            | 190       | 50.4            |
| **Histologic type** |     |                 |
| Non-specific   | 316       | 83.8            |
| Other          | 61        | 16.2            |
| **Grade**      |           |                 |
| G1             | 28        | 7.4             |
| G2             | 253       | 67.1            |
| G3             | 27        | 7.2             |
| NA             | 69        | 18.3            |
| **T classification** | |                 |
| T1             | 16        | 4.2             |
| T2             | 68        | 18.0            |
| T3             | 255       | 67.6            |
| T4             | 11        | 2.9             |
| NA             | 27        | 7.3             |
| **N classification** | |                 |
| N0             | 199       | 52.8            |
| N1             | 91        | 24.1            |
| N2             | 63        | 16.7            |
| NA             | 24        | 6.4             |
| **M classification** | |                 |
| M0             | 298       | 79.0            |
| M1             | 79        | 21.0            |
| **Stage**      |           |                 |
| Stage I        | 56        | 14.9            |
| Stage  | Count | Percentage |
|--------|-------|------------|
| Stage II | 113  | 30.0       |
| Stage III | 108 | 28.6       |
| Stage IV   | 79   | 21.0       |
| NA        | 21   | 5.5        |

Noted: a) Non-specific type of adenocarcinoma included polypoid adenocarcinoma, tubular adenocarcinoma, and mucious adenocarcinoma. b) Other type included signet ring cell carcinoma, undifferentiated carcinoma, and adenosquamous carcinoma. NA: Not available
Table S7. The relationship between ALKAL1 IHC expression level and clinical pathological characteristics in 377 patients with colorectal cancer.

| Parameters         | Number of cases | ALKAL1 IHC expression | P values |
|--------------------|-----------------|-----------------------|----------|
|                    |                 | Low | High |     |
| Location           |                 |     |      |     |
| Rectum             | 187             | 92  | 95   | 0.1162 |
| Colon              | 178             | 73  | 105  |     |
| Gender             |                 |     |      |     |
| Female             | 158             | 77  | 81   | 0.2425 |
| Male               | 218             | 93  | 125  |     |
| Age                |                 |     |      |     |
| <60                | 187             | 92  | 95   | 0.1120 |
| ≥60                | 190             | 78  | 112  |     |
| Histologic type    |                 |     |      |     |
| Non-specific<sup>a</sup> | 316           | 146 | 170  | 0.3243 |
| Other<sup>b</sup>  | 61              | 24  | 37   |     |
| Grade              |                 |     |      |     |
| G1-G2              | 281             | 134 | 147  | 0.1532 |
| G3                 | 27              | 9   | 18   |     |
| T classification   |                 |     |      |     |
| T1-2               | 84              | 41  | 43   | 0.4753 |
| T3-4               | 266             | 118 | 148  |     |
| N classification   |                 |     |      |     |
| N0                 | 199             | 100 | 99   | 0.0465 |
| N1-2               | 154             | 61  | 93   |     |
| M classification   |                 |     |      |     |
| M0                 | 298             | 147 | 151  | 0.0013 |
| M1                 | 79              | 23  | 56   |     |
| Stage | Count | No. 1 | No. 2 | No. 3 | P-value |
|-------|-------|-------|-------|-------|---------|
| I-II  | 169   | 92    | 77    |       | 0.0006  |
| III-IV| 187   | 68    | 119   |       |         |

Noted: a) Non-specific type of adenocarcinoma included polypoid adenocarcinoma, tubular adenocarcinoma, and mucous adenocarcinoma. b) Other type included signet ring cell carcinoma, undifferentiated carcinoma, and adenosquamous carcinoma.