Supplementary Material

# Supplementary Data

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# Supplementary Figures and Tables

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## Supplementary Figures



**Supplementary Figure S1. The expression level of AC010789.1 in CRC cell lines and tissues. (A)** The qRT-PCR analysis of AC010789.1 expression levels in CRC cell lines and FHC. Data are presented as mean ±SEM from triplicate independent experiments. *P*-values were determined by one-way ANOVA (\* *p* < 0.05). **(B)** Overview of the tissue microarray.



**Supplementary Figure S2. Silencing AC010789.1 inhibits EMT of CRC cells in vitro.** **(A)** The qRT-PCR was performed to verify the relative expression of AC010789.1 in HCT116 and SW1116 cells transfected with two independent siRNAs targeting AC010789.1. **(B, C)** The relative expression levels of E-cadherin and vimentin were determined by qRT-PCR in HCT116 and SW1116 cells with AC010789.1 knockdown. **(D)** Knockdown efficiency verification of sh-NC and sh-AC010789.1 stable cell lines. **(E, F)** The expression of GFP (green) was observed under a fluorescence microscope to determine the transfection efficiency in HCT116 and SW1116 cells. Scale bar: 100μm. Data are presented as mean ±SEM from triplicate independent experiments. *P*-values were determined by one-way ANOVAs or two-tailed Student’s t-test ( \**p* < 0.05,\*\**p* < 0.01, \*\*\* *p* < 0.001).



**Supplementary Figure S3. MiR-432-3p directly binds to AC010789.1 producing anti-EMT effect in CRC cells. (A)** The qRT-PCR was performed to verify the relative expression of miR-432-3p, miR-7854-3p and miR-4796-3p in HCT116 cells transfected with shRNA-NC and sh-AC010789.1. **(B)** The correlation between AC010789.1 expression and miR-432-3p expression levels were measured in CRC tissues by Spearman correlation analysis (n=24). **(C)** The correlation analysis of AC010789.1 expression and miR-4796-3p expression levels were measured in CRC tissues by Spearman correlation analysis (n=24). **(D)** The correlation analysis of AC010789.1 expression and miR-7854-3p expression levels were measured in CRC tissues by Spearman correlation analysis (n=24). **(E, F)** Expression levels of E-cadherin and vimentin were determined by qRT-PCR after transfecting with mimic NC, miR-432-3p mimic, inhibitor NC and miR-432-3p inhibitor in HCT116 and SW1116 cells. Data are presented as mean ±SEM from triplicate independent experiments. *P*-values were determined by two-tailed Student’s t-test (\* *p* < 0.05).



**Supplementary Figure S4.** **ZEB1 is a direct target of miR-432-3p in CRC migration and invasion. (A)** Knockdown efficiency verification of RNA-seq samples. **(B)** ZEB1 expression level in lymph node metastasis and non-lymph node metastasis of CRC tissues in the TCGA database. **(C)** ZEB1 expression level in T1/2 stage and T3/4 stage of CRC tissues in the TCGA database. **(D)** The mRNA level of ZEB1 in HCT116 and SW1116 cells transfected with mimic NC or miR-432-3p mimic. **(E)** The mRNA level of ZEB1 in HCT116 and SW1116 cells transfected with inhibitor NC or miR-432-3p inhibitor. **(F)** The mRNA level of ZEB1 in miR-432-3p overexpressed-HCT116 cells with or without ZEB1 upregulation. **(G)** The mRNA level of ZEB1 in miR-432-3p overexpressed-SW1116 cells with or without ZEB1 upregulation. Data are presented as mean ±SEM from triplicate independent experiments. *P*-values were determined by two-tailed Student’s t-test or one-way ANOVAs (\* *p* < 0.05).



**Supplementary Figure S5. AC010789.1 positively regulates the expression of ZEB1. (A)**The mRNA level of ZEB1 in CRC cells with AC010789.1 overexpression or knockdown **(B)** Correlation analysis of AC010789.1 expression and ZEB1 expression levels in CRC tissues (n=24). **(C)** The mRNA level of ZEB1 in AC010789.1 knockdown-HCT116 cells with or without miR-432-3p inhibitor. **(D)** The mRNA level of ZEB1 in AC010789.1 knockdown-SW1116 cells with or without miR-432-3p inhibitor. Data are presented as mean ±SEM from triplicate independent experiments. *P*-values were determined by two-tailed Student’s t-test or one-way ANOVAs (\* *p* < 0.05).



**Supplementary Figure S6. AC010789.1 expression level correlates with** **Wnt/β-catenin pathway activation in CRC cells. (A, B)** The mRNA expression levels of Wnt-responsive genes in HCT116 and SW1116 cells with AC010789.1 knockdown. **(C)** The expression levels of β-actin, β-catenin and Lamin B1 in the cytoplasm of HCT116 cells. **(D)** The expression levels of Lamin B1, β-catenin and E-cadherin in the nucleus. Data are presented as mean ±SEM from triplicate independent experiments. *P*-values were determined by two-tailed Student’s t-test (\* *p* < 0.05).

## Supplementary Tables

Supplementary Table S1. Characteristics of 86 CRC patients

|  |  |
| --- | --- |
| **variables** | **No. of patients (%)** |
| **Age** |  |
| <63 | 41 (47.7) |
| ≥63 | 45 (52.3) |
| **Sex** |  |
| Male | 50 (58.1) |
| Female | 36 (41.9) |
| **Tumor stage** |  |
| T1/2 | 28 (32.6) |
| T3/4 | 58 (67.4) |
| **Lymph node metastasis** |  |
| Negative | 49 (57) |
| Positive | 37 (43) |

Supplementary Table S2. Clinicopathological characteristics of patients with CRC used for ISH assay

|  |  |  |
| --- | --- | --- |
| **variables** |  | **No. of patients (%)** |
|  |
| **Age (year)** |  |  |
|  | ≤65 | 45 (44.6) |
|  | ＞65 | 56 (55.4) |
| **T stage** |  |  |
|  | T1/T2 | 6 (5.9) |
|  | T3/T4 | 94 (93.1) |
|  | Not record | 1 (1) |
| **TNM stage** |  |  |
|  | Ι/II | 61(60.4) |
|  | III/IV | 40 (39.6) |
| **N stage** |  |  |
|  | N0 | 61 (60.4) |
|  | N1/N2 | 40 (39.6) |
| **M stage** |  |  |
|  | M0 | 100 (99) |
|  | M1 | 1 (1) |
| **Sex** |  |  |
|  | Female | 51 (50.5) |
|  | Male | 50 (49.5) |
| **grade** |  |  |
|  | I/ II | 84 (83.2) |
|  | III/Ⅳ | 17 (16.8) |

Supplementary Table S3. Primer sequences for qRT-PCR.

|  |  |  |
| --- | --- | --- |
| **Gene** | **Forward (5’-3’)** | **Reverse (5’-3’))** |
| β-Actin | CATGTACGTTGCTATCCAGGC | CTCCTTAATGTCACGCACGAT |
| AC010789.1 | TGCATCCCTGGCAATACTCAG | GGAGTGCTGTGCATTCATTGG |
| ZEB1 | GCACAACCAAGTGCAGAAGA | GCCTGGTTCAGGAGAAGATG |
| E-cadherin | GGGGTCTGTCATGGAAGGTG | GAAACTCTCTCGGTCCAGCC |
| Vimentin | ACCGCACACAGCAAGGCGAT | CGATTGAGGGCTCCTAGCGGTT |
| β-catenin | GGCTTGGAATGAGACTGCTG | GGTCCATACCCAAGGCATCC |
| Cyclin D1 | GATGCCAACCTCCTCAACGA | GGAAGCGGTCCAGGTAGTTC |
| c-MYC | CCCTCCACTCGGAAGGACTA | GCTGGTGCATTTTCGGTTGT |
| JUN | AGTGCCGAAAAAGGAAGCTG | CTGCTGCGTTAGCATGAGTT |
| TCF1 | AGGTGCGTGTCTACAACTGG | TGGACCTTACTGGGGGAGAG |
| CD44 | CCCAGCAACCCTACTGATGA | TGATCCAGGGACTGTCTTCG |
| U6 | CTCGCTTCGGCAGCACA | AACGCTTCACGAATTTGCGT |

**Supplementary Table S4. Clinical variables for colorectal cancer in TCGA database.**

|  |  |
| --- | --- |
| **variables** | **Total (647)** |
| **Age (years)** |  |
| ≥65 | 388 |
| ＜65 | 256 |
| Not report | 3 |
| **Gender** |  |
| Male | 342 |
| Female | 302 |
| not report | 3 |
| **TNM stage** |  |
| I+II | 353 |
| III+IV | 275 |
| Not report | 19 |
| **Lymph node metastasis** |  |
| Negative | 369 |
| Positive | 271 |
| Not report | 7 |

Supplementary Table S5. Correlation between tissue lncRNA concentrations and clinicopathological characteristics of patients with CRC [median (interquartile range)]

|  |  |  |  |
| --- | --- | --- | --- |
| **Parameters** | **Total cases** | **AC010789.1** | **P** |
| **Age** | | | 0.60 |
| <63 | 41 | 5.35(0.98-16.1) |  |
| ≥63 | 45 | 5.50(0.86-40.1) |  |
| **Sex** | | | 0.91 |
| Male | 50 | 5.42(1.00-21.97) |  |
| Female | 36 | 5.30(0.60-29.21) |  |
| **Tumor stage** | | | 0.97 |
| T1-T2 | 28 | 4.57(0.89-34.32) |  |
| T3-T4 | 58 | 5.68(1.00-19.50) |  |
| **Lymph node metastasis** | | | 0.02\* |
| Negative | 49 | 2.23(0.72-16.38) |  |
| Positive | 37 | 11.95(3.14-31.64) |  |