Supplementary Figure Legends

Figure S1. (A) The indicated DKO-1 and SW620 cells or DKs-8 and Caco-2 cells were transfected with a miR-139-5p agomir or antagonir, respectively, using Lipofectamine 2000 according to the manufacturer's instructions. The transfection efficiencies of the miR-139-5p agomir and antagonir were validated by qRT-PCR. (B) To generate stable cell lines, DKO-1 and SW620 cells were infected with lentiviruses at a multiplicity of infection of 100:1. Infection efficiency was confirmed by qRT-PCR 72 h after infection. (C) The indicated cells were injected subcutaneously into nude mice (n = 10). After the tumor size reached approximately 100 mm³, the mice received 5-FU treatment (8 mg/kg/d, i.p. injection). Representative fluorescent images of GFP signals captured from subcutaneous tumors are shown. (D) Left, growth curves of tumors in nude mice injected with the indicated cells. Right, calculated weights and volumes of tumors isolated on day 30 after treatment. The results were analyzed by one-way ANOVA followed by Dunnett’s test compared with the miR ctrl group. (E) Left, representative images of tumor samples that were subjected to IHC for Ki-67 and TUNEL staining. Right, percentages of Ki-67- and TUNEL-positive cells. Bars: 200 µm. The data are presented as the means ± SDs. *, P < 0.05; **, P < 0.01, n.s., not significant.

Figure S2. (A) Transfection with β-catenin siRNAs significantly decreased β-catenin protein levels in DKO-1 and SW620 cells. siβ-catenin#1 was used in Fig. 5B. (B) Transfection with TCF3 and TCF4 siRNAs significantly decreased TCF3 and TCF4 protein levels in DKO-1 cells. siTCF3#1 and siTCF4#2 were used in Fig. 5F. (C) Schematic of the predicted TCF4 binding sites in the promoter region of the MIR139 sequence. The indicated TCF4 binding sites are mapped to their respective locations in MIR139 and are numbered relative to the MIR139 sequence (+1). The sequence of each site and the bases mutated in the mutant promoters are shown.
| No  | Age | Gender | TNM stage     | KRAS status  |
|-----|-----|--------|---------------|-------------|
| 1   | 60  | Female | T3N1aM0       | WT          |
| 2   | 65  | Female | T2N0M0        | WT          |
| 3   | 59  | Female | T3N0M0        | WT          |
| 4   | 72  | Male   | T3N0M0        | WT          |
| 5   | 72  | Male   | T3N0M0        | WT          |
| 6   | 59  | Female | T4bN0M0       | WT          |
| 7   | 72  | Male   | T2N1bM0       | WT          |
| 8   | 69  | Male   | T3N0M0        | WT          |
| 9   | 54  | Male   | T3N1aM0       | WT          |
| 10  | 53  | Male   | T3N1aM0       | WT          |
| 11  | 59  | Female | T2N0M0        | WT          |
| 12  | 47  | Male   | T2N0M0        | WT          |
| 13  | 78  | Male   | T3N0M0        | WT          |
| 14  | 48  | Male   | T3N0M0        | WT          |
| 15  | 54  | Male   | T3N1aM0       | WT          |
| 16  | 54  | Male   | T3N0M0        | WT          |
| 17  | 63  | Male   | T2N0M0        | WT          |
| 18  | 47  | Female | T2N0M0        | WT          |
| 19  | 46  | Male   | T3N0M0        | WT          |
| 20  | 64  | Female | T3N0M0        | WT          |
| 21  | 70  | Male   | T4aN2bM0      | WT          |
| 22  | 74  | Male   | T3N0M0        | WT          |
| 23  | 72  | Male   | T2N0M0        | WT          |
| 24  | 67  | Male   | T2N0M0        | WT          |
| 25  | 59  | Male   | T3N0M0        | WT          |
| 26  | 53  | Female | T3N0M0        | WT          |
| 27  | 53  | Male   | T3N0M0        | WT          |
| 28  | 84  | Female | T3N0M0        | WT          |
| 29  | 82  | Female | T3N0M0        | WT          |
| 30  | 67  | Male   | T3N0M0        | WT          |
| 31  | 62  | Female | T3N1aM0       | Exon2 G13D  |
| 32  | 60  | Female | T3N1bM0       | Exon2 G13C  |
| 33  | 84  | Male   | T2N0M0        | Exon2 G12/G13C |
| 34  | 69  | Male   | T3N0M0        | Exon2 G13D  |
| 35  | 46  | Female | T4aN2bM0      | Exon2 G13D  |
| 36  | 73  | Female | T4aN2aM0      | Exon2 G13D  |
| Case No | Age | Gender | TNM stage | KRAS status |
|---------|-----|--------|-----------|-------------|
| 37      | 50  | Female | T2N0M0    | Exon2 G13D  |
| 38      | 57  | Male   | T3N0M0    | Exon2 G12X/G13C |
| 39      | 75  | Male   | T3N2aM0   | Exon2 G12S/G13D |
| 40      | 73  | Male   | T3N1cM0   | Exon2 G12S/G12D |
| 41      | 70  | Male   | T3N0M0    | Exon2 G12S/G12D |
| 42      | 80  | Male   | T3N0M0    | Exon2 G12X/G13C |
| 43      | 70  | Male   | T4bN1aM1  | Exon2 G12S/G12D |
| 44      | 52  | Female | T3N0M0    | Exon2 G12S/G12D |
| 45      | 62  | Male   | T2N0M0    | Exon2 G12X/G13C |
| 46      | 69  | Male   | T3N1cM0   | Exon2 G12X/G13C |
| 47      | 58  | Female | TisN0M0   | Exon2 G12X/G13C |
| 48      | 59  | Male   | T2N0M0    | Exon2 G12X/G13C |
| 49      | 55  | Female | T3N1bM0   | Exon2 G12X/G13C |
| 50      | 76  | Female | T4bN0M0   | Exon2 G12S/G12D |
| 51      | 71  | Male   | T3N1cM0   | Exon2 G12S/G12D |
| 52      | 71  | Female | T4aN0M0   | Exon2 G12S/G12D |
| 53      | 73  | Female | T3N0M0    | Exon2 G12X/G13C |
| 54      | 61  | Male   | T4aN1bM0  | Exon2 G13D  |
| 55      | 59  | Male   | T3N1cM0   | Exon2 G13D  |
| 56      | 59  | Female | T3N1aM0   | Exon2 G13D  |
| 57      | 75  | Male   | T3N1bM0   | Exon2 G13D  |
| 58      | 64  | Male   | T3N1cM0   | Exon2 G12X/G13C |
| 59      | 28  | Male   | T2N1M0    | Exon2 G12X/G13C |
| 60      | 72  | Female | T3N0M0    | Exon2 G12X/G13C |

1. Cases 1, 2, 3, and 4 in Fig. 6A denote Subjects No. 2, 24, 32, and 54 in this table.
2. WT, wild-type.
Supplementary Table 2. Schema of miR-139-5p binding sites in predicted target 3’ UTR sequences of human genes Gene.

| Gene    | Target Site                                                                 | 3’ UTR Position |
|---------|-----------------------------------------------------------------------------|-----------------|
| PRKCA   | 5’ ...AUGACAGGCGCUCGACUGAGAAGA...                                         | 1151-1157       |
|         | 3’ UGACCUCUGUGCAGUCGACGACU                                                  |                 |
| PRKCA   | 5’ ...GUGUCCAGUUAAUUCCUGUAGAA...                                           | 234-240         |
|         | 3’ UGACCUCUGUGCAGCGAGACCU                                                   |                 |
| NFAT5   | 5’ ...AGAAUAUAACCCGAGACACUUAGA...                                          | 3004-3010       |
|         | 3’ UGACCUCUGUGCAGCGAGACCU                                                   |                 |
| ZEB1    | 5’ ...AGUGUAGUAAAGUAGAAGAAGAAGA...                                         | 1424-1430       |
|         | 3’ UGACCUCUGUGCAGCGAGACCU                                                   |                 |
| ZEB1    | 5’ ...UUAAACUUGCCUUGCCUUGAGAAGA...                                         | 1565-1572       |
|         | 3’ UGACCUCUGUGCAGCGAGACCU                                                   |                 |
| RASGRF1 | 5’ ...GAUAAAUAGUAGUAGAAGAAGAAGA...                                         | 887-894         |
|         | 3’ UGACCUCUGUGCAGCGAGACCU                                                   |                 |
| RASGRF1 | 5’ ...UUUUUAUUGCAUAAACCCGAGAAGA...                                         | 932-938         |
|         | 3’ UGACCUCUGUGCAGCGAGACCU                                                   |                 |
| RASGRF1 | 5’ ...CUUGUACUUGAUAUUGCCUUGACAGAA...                                       | 138-144         |
|         | 3’ UGACCUCUGUGCAGCGAGACCU                                                   |                 |
| JUN     | 5’ ...CAAACUGCAAUGAGAAGAGAAGAAGA...                                         | 377-384         |
|         | 3’ UGACCUCUGUGCAGCGAGACCU                                                   |                 |
| JUN     | 5’ ...UCUUUCUGCAUCUUGAGAAGAAGAAGA...                                       | 782-788         |
|         | 3’ UGACCUCUGUGCAGCGAGACCU                                                   |                 |
| DVL1    | 5’ ...CCCCACGUGGUCGUGGUGGUGAGAAGA...                                       | 759-765         |
|         | 3’ UGACCUCUGUGCAGCGAGACCU                                                   |                 |
| FOS     | 5’ ...UAGCUAAUCCCAUAGUAGAAGAAGAAGA...                                       | 526-532         |
|         | 3’ UGACCUCUGUGCAGCGAGACCU                                                   |                 |
| FOS     | 5’ ...UACCCUAGAGGGUCCUGUGAGAAGAAGAAGA...                                   | 96-102          |
|         | 3’ UGACCUCUGUGCAGCGAGACCU                                                   |                 |
| CACNA2D1| 5’ ...GUUGUUUUUACCUUGAAGAAGAAGAAGA...                                     | 942-949         |
|         | 3’ UGACCUCUGUGCAGCGAGACCU                                                   |                 |
| CCND2   | 5’ ...UAAAGUGCGGCUACUGAGAAGAAGAAGA...                                     | 4739-4745       |
|         | 3’ UGACCUCUGUGCAGCGAGACCU                                                   |                 |
| Gene | 5' UG... | 3' UG... |
|------|----------|----------|
| MAPK8 | GUUUACAUUUUCUACUGUAG... | UGACCUCUGUCGCUGCUGCAUCU |
| MAPK8 | GAACUGUUUUUAAACUUGUAG... | UGACCUCUGUCGCUGCUGCAUCU |
| FZD3  | UCCCAGUAGCUAAGACUGUAG... | UGACCUCUGUCGCUGCUGCAUCU |
| FZD3  | UUGGGAUUGCUCCCUCUGUAGA... | UGACCUCUGUCGCUGCUGCAUCU |
| RAP1B | UUGCACCUUGCAGCUGAAGA... | UGACCUCUGUCGCUGCUGCAUCU |
| CTNNB1| UGCAUUGUGAUGGGCCUGAAG... | UGACCUCUGUCGCUGCUGCAUCU |
| CAMK2D| UGGUGUGAAGUAUACUGUAGA... | UGACCUCUGUCGCUGCUGCAUCU |
| PPP2CA| GCUUUAACAGCAUGACUGUAGA... | UGACCUCUGUCGCUGCUGCAUCU |
| ROCK1 | AACUUCAGUGCCCUCACUGA... | UGACCUCUGUCGCUGCUGCAUCU |
| TCF4  | UUGCUAUAAAAUAGACUGUAGU... | UGACCUCUGUCGCUGCUGCAUCU |
| ROCK2 | AAAAAACUAAGACAG--ACUGUAGA... | UGACCUCUGUCGCUGCUGCAUCU |

125-131, 1475-1481, 493-499, 4107-4113, 9124-9130, 824-830, 349-355, 1938-1945, 365-372, 538-545, 3950-3956, 1156-1163
## Supplementary Table 3. Primer sequences used in the study.

| Primer name | Primer sequences |
|-------------|------------------|
| **Primers used for Sanger sequencing of KRAS** | |
| KRAS-exon2 (G12, G13) sense | 5’-GTTCATAATATAGTCACATTTTCA-3’ |
| KRAS-exon2 (G12, G13) antisense | 5’-TCTATTGTTGGATCATATTCCG-3’ |
| KRAS-exon3 (Q61) sense | 5’-TCTCCCTTCTCAGGATTC-3’ |
| KRAS-exon3 (Q61) antisense | 5’-ATTTATATGGCAAAATACAAAG-3’ |
| KRAS-exon4 (A146) sense | 5’-TCTAGAACAGTGACACAAAAC-3’ |
| KRAS-exon4 (A146) antisense | 5’-GAGAGAAAAACTGATAATTTAATGAC-3’ |
| KRAS-exon4 (K117) sense | 5’-CTTTCCAGAGAACAAATTAAAG-3’ |
| KRAS-exon4 (K117) antisense | 5’-TCAATAAAAGGAATTCCATAACTTCT-3’ |
| **Primers for TCF4 site-directed construct in miR-139-5p promoter** | |
| (-5000/-1bp) sense | 5’-TATAGGTACCAAGGCACAGCTCTCTTAACCG-3’ |
| (-3802/-1bp) sense | 5’-TATAGGTACCAATAACTCTGGATGTCAGCTTG-3’ |
| (-1694/-1bp) sense | 5’-TATAGGTACCGTGCAGCAGCTCCTCTC-3’ |
| (-566/-1bp) sense | 5’-TATAGGTACCTTGGGAGGAAGTTTGTGGTGC-3’ |
| Antisense | 5’-ATATAAGCTTCTGAGCCAGTCCGCTCC-3’ |
| **Primers for TCF4 site-directed mutagenesis in miR-139-5p promoter** | |
| binding site 1 mutation sense | 5’-GGCTTTCCTGGGATagcagcgatcAAATAACTCTG-3’ |
| binding site 1 mutation antisense | 5’-CAGAGTTATTTTgtcgetgtctCCAGGAGCC-3’ |
| binding site 2 mutation sense | 5’-AGGTGTCCTCAAtgacgtcgcagcGGTGATAGTGA-3’ |
| binding site 2 mutation antisense | 5’-TCACTATACCGctcgetcgcagcTGAGGACACCT-3’ |
| **Primers used for ChIP against TCF4 in the miR-139-5p promoter** | |
| distant region sense | 5’-AAAGTCTCCTTAAACCGTGCA-3’ |
| distant region antisense | 5’-TCATCCGAAAAATCTCTC-3’ |
| binding site 1 sense | 5’-CTGACATAATTTACCCAGCC-3’ |
| binding site 1 antisense | 5’-ACTCTGCTCATTACACAGG-3’ |
| binding site 2 sense | 5’-GATTGGAATCATGAGGCTTCT-3’ |
| binding site 2 antisense | 5’-GAGAGGCTTTTGCAACCTCT-3’ |