**Supplementary materials and methods**

1. **5-aza-2′-deoxycytidine treatment, RNA isolation and semi-quantitative RT-PCR**

CRC cell lines were split to a low density (30% confluence) 12 h before treatment with 2 μM 5-Aza (Sigma-Aldrich, #A3656, Missouri, USA). Growth medium conditioned with 5-Aza at 2 μM was exchanged every 24 h for a total of 96 h. Total RNA was prepared by TRIzol reagent (ThermoFisher Scientific, #350302). Agarose gel electrophoresis and spectrophotometric analysis were used to check RNA quality and quantity. Total RNA (5 μg) was used to synthesize first-strand cDNA according to the manufacturer’s instructions (ThermoFisher Scientific, #K1691). The mixed cDNA solution was diluted to100 μl with water. The cDNA quality was evaluated by PCR amplification of GAPDH, thermal cycling parameters were as follows: 95°C 5 min, 1cycle; 95°C 30 s, 63°C 30 s, 72 °C 40s, 25 cycles; and 72°C 5min, 1 cycle. Three qualified cDNA reactions were put together for RT-PCR detection. Semi-quantitative RT-PCR was amplified for 32 cycles and 2.5 μl of diluted cDNA template was used for 25 μl PCR reaction. Thermal cycling parameters were as follows: 95°C 5 min, 1cycle; 95°C 30s, 64°C 30s, 72°C 40s, 3 cycles; 95°C 30s, 61°C 30s, 72°C 40s, 3 cycles; 95°C 30s, 58°C 30s, 72 °C 40s, 3 cycles; 95°C 30s, 55°C 30s, 72°C 40s, 26 cycles; and 72°C 5min, 1 cycle. 72°C 5min, 1 cycle. The amplified PCR products were examined by 1.5% agarose gels. Primer sequences are listed in Supplementary Table1.

1. **DNA extraction, bisulfite modification, methylation specific PCR, and bisulfite sequencing**

Genomic DNA was extracted by the proteinase K method. DNA (2 μg) in a volume of 50 μl was denatured by NAOH (final concentration, 0.2 M) for 15min at 37°C. Fresh prepared 30 μl of 10mM hydroquinone (Sigma-Aldrich, #H9003) and 520 μl of 3M sodium bisulfite (Macklin, Shanghai, China, #S874829) at pH 5, were added and mixed, and samples were incubated under mineral oil at 50°C for 16h. Modified DNA was purified using the Wizard DNA purification resin according to the manufacturer (Promega, Madison, USA, #A7280) and eluted into 50 μl of water. Modification was completed by NaOH (final concentration, 0.3 M) treatment for 5 min at room temperature, followed by ethanol precipitation. DNA was resuspended in water and used immediately or stored at -20°C. Methylation-specific PCR (MSP) primers were designed according to genomic sequences around transcriptional start sites (TSS) and synthesized (BGI, Shenzhen, China) to detect unmethylated (U) and methylated (M) alleles. MSP amplification conditions were as follows: 95°C 5min, 1 cycle; 95°C 30s, 60°C 30s, 72°C 40s, 35 cycles; and 72°C 5min, 1 cycle. BSSQ products were amplified by primers flanking the region of MSP products. The cycling conditions were as follows: 95°C 5 min, 1cycle; 95 °C 30 s, 58°C 30 s, 72°C 40 s, 35 cycles; and 72°C 5 min, 1 cycle. Primer sequences are listed in Supplementary Table1.

**3. Immunohistochemistry**

Immunohistochemistry (IHC) staining was performed following previous study1. Antibodies against NRIP3 (Proteintech, #15664-1-AP, Chicago, USA), PI3K110β (Proteintech, #67121-1-Ig), Ser473-p-AKT (Proteintech, #28731-1-AP), and Ser2448-p-mTOR (ZENBIO, #385033, Chengdu, China) were diluted 1:100, 1:400, 1:200, and 1:200, respectively. A German semi-quantitative scoring system was used to evaluate the intensity and extent of the staining area.

**4. MTT and colony formation**

*NRIP3* unexpressed and re-expressed DLD1, RKO and HCT116 cells were seeded for 2 × 103 cells/well, and DKO cells were seeded for 4 × 103 cells/well in 96-well plates. The cell viability was measured by the MTT assay at 0, 24, 48, 72 and 96 h (KeyGEN Biotech, #KGT5251, Jiangsu, China). Absorbance was measured on a microplate reader (ThermoFisher Scientific, Multiskan MK3) at a wavelength of 490 nm. The results were plotted as means ± SD.

For colony formation assay, unexpressed and re-expressed DLD1, RKO and HCT116 cells were seeded at a density of 300 cells/well, and DKO cells were seeded for 400 cells/well before and after knockdown of *NRIP3* for growing 14 days in 6-well plates. Cells were fixed with 75% ethanol for 30min and stained with 0.2% crystal violet (Beyotime, C0121, Shanghai, China) and the clone numbers were counted. Each experiment was repeated for three times.

**5. Cell cycle analysis**

All CRC cells were starved for 12 h for synchronization, and then re-stimulated with 10% FBS for 48 h. Cells were fixed with 70% ethanol and stained with propidium following the protocol of the Cell Cycle Detection Kit (KeyGEN Biotech, #KGA512). The cells were then sorted by a FACS Caliber (BD Biosciences, CA) and cell phase distribution was analyzed by the Modifit software (Verity Software House, USA). Each experiment was repeated for three times.

**6. Transwell assay**

For migration study, *NRIP3* unexpressed and re-expressed DLD1 (8×104) , RKO (3×105), HCT116 (2×105) cells, and DKO (1×105) cells before and after knockdown of *NRIP3*, were suspended in 200 μl serum-free medium and added into the upper chamber of an 8µm pore size transwell apparatus (Corning, #3422, New York, USA) for 30 h. Cells, which migrated to the surface of lower chamber membranes, were stained with crystal violet and counted in three independent high-power fields (×200). Each experiment was repeated for three times.

For invasion analysis, *NRIP3* unexpressed and re-expressed DLD1 (8×104), RKO (3×105), HCT116 (2×105) cells, and DKO cells (2×105 ) before and after knockdown of *NRIP3*, were seeded into the upper chamber of a transwell apparatus coated with Matrigel (BD Biosciences, #354234, California, USA) and incubated for 48 h. Then cells invaded to the surface of lower chamber membranes were stained with crystal violet and counted in three independent high-power fields (×200). Each experiment was repeated for three times.

**7. Gene expression array analysis**

RNA from *NRIP3* unexpressed and re-expressed RKO cells was isolated by Trizol reagent (ThermoFisher Scientific, #15596018) and the expression was analyzed by expression array (Agilent, Capitalbio Corp, Beijing, China).

**8. Western blot**

Cell lysis was prepared by RIPA lysis buffer (Beyotime, #P0013B) and western blot was performed following previous study2. Protein was quantified by the BCA protein assay kit (CWBIO, #CW0014S, Jiangsu, China). Antibody dilution was performed following related instructions, including NRIP3 (Proteintech, #15664-1-AP), cyclinE1 (Proteintech, #11554-1-AP), cyclinA2 (Proteintech, #18202-1-AP), cyclinD1 (Proteintech, #60186-1-Ig), AKT (Proteintech, #60203-2-Ig), Ser473-p-AKT (Proteintech, #28731-1-AP), PI3K110β (Proteintech, #67121-1-Ig), β-actin (Proteintech, #66009-1-Ig), mTOR (Cell Signaling Technology, #2983S, Massachusetts, USA), ATR (Cell Signaling Technology, #2790S), Ser428-p-ATR (Cell Signaling Technology, #2853S), Ser2448-p-mTOR (ZENBIO, #385033), Ser1981-p-ATM (ZENBIO, #380751), CHK2 (ZENBIO, #R23921), Thr68-p-CHK2 (ZENBIO, #340766), CHK1 (ZENBIO, #380200), ATM (HuaXingBio, #HX12561, Beijing, China), Ser345-p-CHK1 (Genetex, #GTX100065, Texas, USA).

**References**

1. Du W, Gao A, Herman JG, et al. Methylation of NRN1 is a novel synthetic lethal marker of PI3K-Akt-mTOR and ATR inhibitors in esophageal cancer. *Cancer science*. Jul 2021;112(7):2870-2883. doi:10.1111/cas.14917

2. Yu Y, Yan W, Liu X, et al. DACT2 is frequently methylated in human gastric cancer and methylation of DACT2 activated Wnt signaling. *American journal of cancer research*. 2014;4(6):710-24.