Supplementary File 1. The detailed procedures and experimental consumables

1. Cell culture

The normal human intestinal epithelial cell line HIEC and colon cancer cell lines(HCT116, HT29, and SW620) were obtained from Cancer Research Institute, Central South University. Cells were cultured in DMEM medium, supplemented with 10% fetal bovine serum(FBS, bio-channel, Jiangsu, China) and 1% antibiotics(penicillin and streptomycin). Cells were incubated at 37℃ in the cell incubator with 5% CO2.

2. RNA isolation and real-time qPCR

Total RNAs were extracted using TRIzol reagent and cDNA was prepared from total RNA according to the manufacturer's protocol (Cowin Biotech). Real-time qPCR was used to measure the relative gene expression with the SYBR RT PCR kit (Cowin Biotech). The amount of target gene was quantified by the comparative CT method using glyceraldehyde 3- phosphate dehydrogenase (GAPDH) as the normalization control. The primer sequences are as follows: GAPDH, forward 5'-GCATTGCCCTCAACGACCAC-3' and reverse 5'-CCACCACCCTGTTGCTGTAG-3'; and FDX1, forward 5'-TTCAACCTGTCACCTCATCTTTG-3' and reverse 5'-TGCCAGATCGAGCATGTCATT-3'.

3. Western blotting.

Cells were lysed in RIPA buffer in the presence of a protease inhibitor cocktail (Biosharp). Protein was quantified using a BCA Protein Assay Kit (Biosharp) and then separated using 10% SDS-PAGE and transferred onto PVDF membranes. The membranes were blocked with 5% non-fat milk and then incubated with primary antibodies at 4°C overnight. The primary antibodies used were as follows: FDX1 (proteintech, Cat.12592-1-AP), GAPDH(proteintech, Cat.60004-1-Ig). Membranes were washed in TBST solution and then incubated with secondary antibodies at room temperature. Signals were detected by an enhanced chemiluminescence detection system (Bio-Rad, CA, USA).

4. Immunohistochemistry of colon cancer tissues.

A total of 32 pairs of colon cancer tissues and adjacent normal tissues were respectively collected from 32 colon cancer patients at the Xiangya Hospital of Central South University (Changsha, China). The study was approved by the Medical Ethics Committee of Xiangya Hospital Central South University and informed consent was obtained from each participant. All specimens were confirmed by histopathological examination. Immunohistochemistry used a rabbit-enhanced polymer assay system (ZSGB-BIO). Tissue sections were blocked with 5% BSA and incubated with rabbit anti-FDX1 (Proteintech, Cat.12592-1-AP) at 4°C overnight. After washing, the sections were incubated with reaction enhancers and then with secondary antibodies for 40 min. Colour reaction was performed with Diaminobenzidine(DAB) and hematoxylin. Sections were dehydrated and then preserved with a neutral resin.

5. Wound healing and transwell assays

The migration ability was examined via wound healing assays and transwell assays. Cells in culture plates were scraped with a 10-μL pipette tip. Images were respectively captured at 0h, 24h, and 48h after wounding. Transwell cell culture inserts were used in a 24-well cell culture

plate. a total of 5×10^4 cells were incubated in the top chamber with 100ul serum-free medium and 600 μL medium containing 20% FBS was added into the lower chamber. Cells on the bottom surface were fixed with 100% methanol and stained with 0.5% crystal violet after 24h incubation.

6. CCK-8 assay and colony formation assay

The CCK-8 assay was used to detect the proliferation ability of tumor cells. Firstly, we constructed the FDX1 overexpression cell models, tumor cells were stably transfected with a vector expressing the FDX1 and their control vectors, respectively. After 48h, cells were trypsinized and seeded in 96-well plates at a density of 4×10^3 cells/well with 100μL complete medium. Then, 10 μL of 5 mg/mL CCK-8 reagent (Beyotime) was added into each well to measure the absorbance at 450nm after incubating for 2h at 37°C.

7. Flow cytometry

The Apoptosis Kit (Biosharp) was applied to detect the dead cells of each sample. Cells were collected 48 h after being transfected with vectors expressing FDX1 and their negative control vectors. Each sample was resuspended in 500 μl binding buffer and then incubated with 5 μl PI fluorescent dyes. The dead cells were detected based on the fluorescence of PI using the WeimiBio-Tech flow cytometer and data was further analyzed with CytoSYS software.