Supplemental Files-Materials and Methods

Plasmids and siRNA transfection

Cells were transfected with plasmid or siRNA for STAT3 or GSTP1, separately by Lipofectamine®3000, as per manufacturer's instructions.

Cell proliferation assay(CCK8)

Medium in 96-well plate was replaced with 100ul mixture (complete medium: CCK8 reagent = 9: 1), incubated at 37 ℃ in 5% CO_2 for 2-4h. The OD value at 450nm of each well was detected by enzyme labeling instrument, and the OD value was adjusted to zero based on the blank control group. The cell proliferation curve was plotted with the mean value of 5 multiple holes in each group.

Cell invasion assays in vitro

Cell in vitro invasion assays were performed to assess cell invasion ability. In brief, 200 μl of serum free media containing 5×10^4 cells were added into the upper chamber with Matrigel（corning，BD Biocoat）and allowed to invade toward the lower chamber with 10% fetal bovine serum (FBS). After incubation for an appropriate period of time at 37°C, the cells were fixed with methanol for 30 min and stained with crystal violet solution for 20 min. Migrated cells were photographed and counted in six random regions with an inverted microscope.

Western blotting

The Immunoblotting of proteins were extracted with a lysis buffer and then quantified by a bicinchoninic acid protein assay. Equivalent amounts of cell lysates were separated using SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Roche Applied Sciences). Membranes were immunoblotted overnight at

4℃ with anti-β-actin antibodies (Proteintech, Bioleaf Science, China, 1:1000), GSTP1 (Abcam, London, England, 1:200), STAT3 (Abcam, London, England, 1:200), GST (Abcam, London, England 1:1000), followed by the appropriate second antibodies. The bands were visualized using Pierce ECL Western Blotting Substrate (Thermo Scientific). Image density of the immunoblotting was determined by Gel densitometry (Bio-Rad).

Reverse transcription-quantitative PCR (qRT-PCR)

Cultured cells and the tissue samples RNA was extracted using Trizol reagent (Invitrogen, USA). The expression level of GSTP1 was analyzed by ABI PRISM 7500 Fast Real-Time PCR System. The relative mRNA levels were calculated using the comparative Ct method (ΔΔCt). Primer sequences for qRT-PCR are as follow:

GSTP1: Fwd: CGG GGT ACC ATG CCG CCC TAC ACC GT; Rev: CCG CTC GAG TCA CTG TTT CCC GTT GCC ATT;

STAT3: Fwd: CAG CAG CTT GAC ACA CGG TA; Rev: AAA CAC CAA AGT GGC ATG TGA.

Human and animals tissue samples and cell lines

Fresh CRC tissues were collected immediately after resection from 20 patients who underwent CRC resection without prior radiotherapy and chemotherapy at the Department of General Surgery in Nanfang Hospital in 2019 year. Paraffin-embedded CRC primary tumors samples from 20 patients at Department of Pathology in Nanfang Hospital. For the use of these clinical materials for research purposes, prior patient's consent and approval from the Institute Research Ethics Committee were

obtained. Paraffin-embedded mice CRC tumors samples from the model constructed by our previous experiments [1].

All cell lines were obtained from American Type Culture Collection. Details are showed in supplementary B. The human Colorectal cancer cell lines (SW480, SW620, HCT116 and LOVO) were cultured in high glucose 1640 (GIBCO, Gaithersburg, USA) supplemented with 10% fetal bovine serum (GIBCO, Gaithersburg, USA). The media was supplemented with 100 μg/ml streptomycin and 100 U/ml penicillin (GibcoTM, Thermo Fisher Scientific, Waltham, MA, USA) and the cells above were incubated at 37°C in a humidified chamber containing 5% CO_2. The stable knocking down GSTP1 cancer cells (SW620/shGSTP1 and HCT116/shGSTP1 cell lines) were from previous study.

References:

1.FeiFei W, HongHai X, YongRong Y, PingXiang W, JianHua W, XiaoHui Z, JiaoYing L, JingBo S, Kun Z, XiaoLi R, Lu Q, XiaoLiang L, ZhiQiang C, Na T, WenTing L, YanQing D, Li L. FBX8 degrades GSTP1 through ubiquitination to suppress colorectal cancer progression. Cell Death Dis. 2019 Apr 25;10(5):351.