Supplementary Information

Supplementary Fig. 1 Volcanic map of DEGs between DMSO and Chiaurinib groups.

DEGs between DMSO and Chiaurinib groups were displayed by volcanic map. The X-axis represented the difference multiples after log2 conversion, and the Y-axis represented the significant value after log10 conversion. The blue one represented downregulated DEGs, the red one represented up-regulated DEGs, and the gray one represented non-DEG. $\log_2|FC| \geq 1$ and $P < 0.05$.

Supplementary Fig. 2 Gene set enrichment analysis (GSEA) of DEGs DMSO and Chiaurinib groups.

The significantly enriched hallmark pathways in GSEA were listed.

Supplementary Fig. 3 Statistical analysis of ROS levels in CRC cells after treatment with Chiaurinib.

(A-B) Statistical analysis of (A) mitochondrial $O_2^{\bullet-}$ and (B) H_2O_2 in *KRAS*^{WT} SW48, *KRAS*^{G13D} SW48, LoVo-shNC and LoVo-shKRAS cells. Bars represented the mean ± SD of three independent experiments; * P <0.05, ** P <0.01 and *** P <0.001.

Supplementary Fig. 4 The antitumor effects of Chiaurinib in LoVo Xenograft Models.

(A-C) (A)The representative morphology, (B)tumor growth rate, and (C)tumor weight were shown as a result of treatment with Chiaurinib (40 mg/kg) once every three days in LoVo Xenograft Models. Data are shown as mean ± SD; * P <0.05.

Supplementary Fig. 5 The body weight of SW48 Xenograft mice between DMSO and Chiaurinib groups.

The body weight of SW48 Xenograft mice was measured at 27 days after inoculation.

Supplementary Table 1. Primer sequence

Primer	Sequence (5' to 3')
BCL2-F	GGTGGGGTCATGTGTGTGG
BCL2-R	CGGTTCAGGTACTCAGTCATCC
BAX-F	CCCGAGAGGTCTTTTTCCGAG
BAX-R	CCAGCCCATGATGGTTCTGAT
ZMAT3-F	AGAAGCCTTTTGGGCAGGAG
ZMAT3-R	TGCTGCATAGTAATTTCGGAGTT
FAS-F	TCTGGTTCTTACGTCTGTTGC
FAS-R	CTGTGCAGTCCCTAGCTTTCC
TP53AIP1-F	GGTGCCCAAGTTCACGGAG
TP53AIP1-R	CTGGAGAGACCTAGACCAAGG
CDKN1A-F	TGTCCGTCAGAACCCATGC
CDKN1A-R	AAAGTCGAAGTTCCATCGCTC
CDK1-F	AAACTACAGGTCAAGTGGTAGCC
CDK1-R	TCCTGCATAAGCACATCCTGA
EI24-F	TGCCAGAGGAATCAAAGACTCC
EI24-R	TCTCTTGCTTCCGCTCTATACT

MDM2-F	GAATCATCGGACTCAGGTACATC
MDM2-R	TCTGTCTCACTAATTGCTCTCCT
TP53-F	CAGCACATGACGGAGGTTGT
TP53-R	TCATCCAAATACTCCACACGC
β-actin-F	ACTCTTCCAGCCTTCCTTC
β-actin-R	ATCTCCTTCTGCATCCTGTC
Nrf2-F	TCAGCGACGGAAAGAGTATGA
Nrf2-R	CCACTGGTTTCTGACTGGATGT

Materials and methods

Cell lines and culture

The human CRC cell lines (SW48, CaCO2, LoVo, HCT116) were obtained from the American Type Culture Collection (Manassas, USA). Cell lines were authenticated by Cellcook Biotech Co., Ltd, (Guangzhou, China). SW48 *KRAS*^{WT}, SW48 *KRAS*^{G13D}, LoVo-shNC and LoVo-shKRAS cells were previous constructed[1]. All these cells were cultured in RPMI1640 (Gibco, New York, USA, Cat. No. 61870044) and supplemented with 10% FBS (Gibco, Uruguay, 10270-106) at 37°C in a humidified incubator containing 5% CO2.

Western Blotting

Western blotting was performed according to a standard protocol, as described previously[2]. The total proteins were collected using SDS lysis buffer (Beyotime, P0013G), and protein concentration were determined by Bicinchoninic Acid (KeyGen, KGP902). Nuclear extracts were obtained using the NE-PER Nuclear and cytoplasmic extraction reagents (Thermo Scientific, Massachusetts, USA, 78833) according to the manufacturer's instructions. The following primary antibodies were used: CDK1(AF1516), P21(AP021) from Beyotime(Shanghai, China); Bcl-2(sc-7382), Bax(sc-6236)from Santa Cruz (California, USA); P53(CBL404) from Merck Millipore (Boston, USA); Aurora B(ab2254), p-Aurora B(ab115793), KRAS(ab180772), Nrf2(ab31163) from Abcam(Cambridge, UK); β-actin (A5441) from Sigma-Aldrich (St. Louis, USA). HRP-conjugated anti-rabbit IgG (Cell Signaling Tech, #7074) and anti-mouse IgG (Sigma-Aldrich, AP308P) were used as secondary antibodies. Proteins were determined using ECL Plus Reagent (Millipore, WBKLS0100).

RNA isolation and RT-qPCR

Total RNA was extracted using TRIzol (Thermo Scientific, 15596026) according to the manufacturer's protocol[2]. First-strand cDNA synthesis was performed using 500 nanograms of total RNA, and the RT-qPCR analysis system was performed using iQ SYBR Green Supermix and the iCycler Real-time PCR Detection System (Bio-Rad, USA). β-actin was used for normalization. The PCR primer sequences are listed in Supplementary Table 1.

Immunofluorescence staining

After fixed in 4% paraformaldehyde, cells were blocked with goat serum at 37℃ for 1h. They were incubated with rabbit Ki67(Millipore, AB9260) antibodies at 4°C overnight, then were incubated with FITC conjugated goat anti-rabbit IgG (Dako, Glostrup, Denmark, K500711) at 37℃ for 1 h after three times washing. Finally, the

cell nucleus was stained with DAPI (Sigma-Aldrich, D9542).

Cell Counting Kit-8 (CCK8)

Cell proliferation was measured via cell viability with a Cell Counting Kit-8 (Dojindo, Japan). CRC cells were seeded into 96-well plates and cultured for24, 48 and 72 h. Then, 100 μl CCK8 reagent was added to 96-well plates and incubated for 2 h. The absorbance (OD450 nm) was measured using a microplate reader (TECAN, Switzerland) and calculated.

Colony formation assay

CRC cells were plated in 6-well dishes (500 cell/dish) and then incubated for 2 weeks for colony formation with or without Chiauranib. After 14 days, cell colonies were then fixed in 4% polyformaldehyde and stained with 0.1% crystal violet. All colonies were counted separately for each sample, and the relative colony numbers were calculated.

Dihydroethidium (DHE) staining

Reactive oxygen species (ROS) were measured with DHE staining following incubation at 37 °C for 30 min (Beyotime, Shanghai, China). PBS was used instead of DHE in the negative control group. The fluorescence intensity was quantified with the ImageJ software program (National Institutes of Health, Bethesda, MD, USA).

Terminal deoxynucleotidyl transferase mediated dUTP nick end labeling (TUNEL) staining

Tissue sections were deparaffinized and hydrated in xylene and gradient concentrations of ethanol, then incubated in proteinase K at room temperature for 30 min and stained with TUNEL kit (Sigma-Aldrich, St. Louis, MO, USA). Label solution was used instead of TUNEL reagent in the negative control group. All the images were captured by a fluorescence microscope (DFC700T, Leica, Germany). Cells that were positive for TUNEL staining and aligned with DAPI staining were considered apoptotic cells and counted.

Annexin V/propidium iodide flow cytometric analysis

CRC cell staining with Annexin V and PI was carried out using an Annexin V-FITC/PI Apoptosis Detection kit (Merck, Germany). A total of $1x10^6$ cells were incubated at 37°C for 30 minutes before centrifugation to collect the cell pellet, then resuspended in a Ca^{2+}-enriched binding buffer and analysed using a Beckman Coulter flow cytometry. Data were calculated using CellQuest software.

ROS assays

Cells were plated at a density of 200000 cells per well in 12-well plates. After 48 hours, DCF-DA (Sigma) and MitoSOX (Invitrogen) was added to each well at a concentration of 5 μM. After 30 min, the samples were run on the flow cytometer to detect fluorescence. Each sample was collected using 20,000 events.

Tumor xenograft

Male BALB/c nude mice (4-weeks-old, 16-18 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China). Based on a previously described standard protocol, the mice were randomly divided into the indicated groups. 1×10^6 SW48 or LoVo cells were inoculated into the inguinal folds of mice (n = 8 in each cell line per group). Tumor volumes were measured with an external caliper, and calculated according to the following formula: Volume = length×width^2／2. At 27 days

after inoculation, the mice were sacrificed, and the tumors were dissected, weighed, taken photos, and stored at -80°C for further experiments.

siRNA and transfection

P53 siRNA, Nrf2 siRNA and control siRNA were purchased from RiboBio (Guangzhou, China). According to the manufacturer's instructions, transfections were performed at approximately 60% confluency using RNAiMAX (Invitrogen, USA). After 48 hours, confirmation of interference was carried out using real-time quantitative PCR (RT-qPCR) and Western blotting.

RNA-seq

RNA sequence was performed with BGISEQ-500 platform. In brief, the first step in the workflow involved purifying the poly-A containing mRNA molecules using poly-T oligo-attached magnetic beads. Following purification, the mRNA was fragmented into small pieces using divalent cations under elevated temperature. The cleaved RNA fragments were copied into first strand cDNA using reverse transcriptase and random primers. This was followed by second strand cDNA synthesis using DNA Polymerase I and RNase H. These cDNA fragments then had the addition of a single 'A' base and subsequent ligation of the adapter. The products were then purified and enriched with PCR amplification. We then quantified the PCR yield by Qubit and pooled samples together to make a single strand DNA circle (ssDNA circle), which gave the final library. DNA nanoballs (DNBs) were generated with the ssDNA circle by rolling circle replication (RCR) to enlarge the fluorescent signals at the sequencing process. The DNBs were loaded into the patterned nanoarrays and single-end read of 50 bp were read through on the BGISEQ-500 platform for the following data analysis study. For this step, the BGISEQ-500 platform combined the DNA nanoball-based nanoarrays and stepwise sequencing using Combinational Probe-Anchor Synthesis Sequencing Method. Tools such as HISAT2, Bowtie2, Cluster etc. were used for the following bioinformatics analysis. In the analysis of differentially expressed genes (DEGs), the genes that had more than double fold change and the corrected P value is less than or equal to 0.05 were defined as DEGs. With DEGs, we performed KEGG pathway classification and Gene set enrichment analysis (GSEA) using R.

Statistical analysis

The variability of the data is presented as the SD (mean±SD) and was assessed with Student's *t* test between two groups. For multiple groups, significant differences were determined using one-way ANOVA. Statistical significance was defined at $p<0.05$.

Reference:

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2. Jiang P, Huang M, Qi W, Wang F, Yang T, Gao T, Luo C, Deng J, Yang Z, Zhou T, Zou Y, Gao G, Yang X. Fubp1 promotes neuroblastoma proliferation via enhancing glycolysis-a new possible marker of malignancy for neuroblastoma. *Journal of experimental & clinical cancer research : CR*. 2019;38:400