Cuproptosis-induced up-regulation of RBM24 suppresses tumor metastasis via MAPK signaling blockage in colorectal cancer

Keywords
colorectal cancer, metastasis, MAPK signaling pathway, cuproptosis, RBM24

Abstract

Introduction

Cuproptosis is an emerging form of programmed cell death that scientists are linking to tumor progression. Nevertheless, the link between cuproptosis and the metastatic process in colorectal cancer (CRC) is obscure to this day, as are the underlying molecular mechanisms that drive CRC progression in this process.

Material and methods

Bioinformatics, quantitative reverse transcription polymerase chain reaction (qRT-PCR), immunofluorescence (IF), and Western blot (WB) were leveraged to analyze the expression levels of RBM24 in CRC. Cell counting kit-8 (CCK-8) assay, Transwell, and WB assays were conducted to determine the cell proliferation, migration, and invasive potential, alongside the expression analysis of metastasis-related proteins. Intracellular Cu2+ levels were quantified using a Copper Assay Kit. Additionally, the expression of mitogen-activated protein kinase (MAPK) pathway and cuproptosis-related proteins were probed via WB.

Results

RBM24 was under-expressed in CRC, and its forced expression inhibited the metastatic abilities of CRC cells, including migration, invasion, and epithelial-mesenchymal transition (EMT). The use of a MAPK pathway inhibitor could temper the pro-metastatic effects associated with low RBM24 levels. On the molecular level, the combination of copper ionophores with copper ions (Es-Cu) up-regulated RBM24, leading to the inhibition of CRC cell spread. The effects of cuproptosis on CRC cells were nullified by knocking down RBM24.

Conclusions

Elevated levels of cuproptosis-induced cell death disrupt the MAPK signaling cascade, thus suppressing the metastasis of CRC. This discovery sheds new light on the potential application of cuproptosis in oncological treatments.

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1. Introduction

Colorectal cancer (CRC) is characterized by its high malignancy, the difficulty in early detection and treatment, and its poor prognosis[1]. Due to the complex etiology and difficulties in prevention, CRC has become the third most common cancer globally[2, 3]. Despite considerable progress in the field of CRC treatment, the incidence and mortality rates remain on an upward trend, with an incidence rate of 10.2% and a mortality rate of 9.2% in 2021[4]. Due to the unspecific early symptoms of CRC, which can lead to missed diagnosis, or misdiagnosis as alternative gastrointestinal conditions, most CRC patients receive their diagnosis at later stages when cell invasion and distant metastasis have occurred, which greatly hampers the effectiveness of treatment[5-7]. Thus, it is essential to delve into the molecular processes behind metastasis for more effective CRC therapeutic targets.

Emerging research has established a strong link between copper and the occurrence of cancer, with altered copper levels being a new target for cancer therapy[8]. A deeper comprehension of the interplay between copper and CRC paves the way for exploring innovative treatment strategies to improve the prognosis of CRC patients. Copper ionophores or copper-containing compounds elevate the copper levels in cancer cells, inducing oxidative stress and eventually cell death, showing their potential as anti-cancer agents[9]. The class of copper ionophores, which includes Disulfiram (DSF), Elesclomol (ES), and Clioquinol (CQ), is known for its biological effects. Specifically, the DSF-Cu complex has been demonstrated to inhibit NF-κB activity, thus impeding EMT[10]. Experiments applying Es-Cu pulses to CRC cells and cell lines resistant to oxaliplatin showed that Es-Cu cripples the viability of CRC cells[11]. Despite the salient role that cuproptosis plays in cancer progression, the interaction between cuproptosis and metastasis in CRC is not well defined.

RNA-binding proteins (RBPs) are essential in the post-transcriptional regulation of gene expression. Modulating almost every facet of RNA metabolism, they feed into a broad spectrum of physiological and pathological activities[12]. RBM24, part of the RBP family, can bind to numerous target mRNAs, thus affecting mRNA maturation or

translation[13]. Increasing evidence shows that alterations in RBM24 expression and function can disrupt tissue homeostasis and act as a tumor suppressor in a range of cancers[14, 15]. For instance, circsSMARCA5 can up-regulate RBM24 expression by negatively modulating miR-670-5p, which suppresses tumor growth, reduces cell proliferation, and encourages apoptosis[16]. However, the up-regulation of RBM24 in other types of cancer appears to promote tumor growth[13]. Given the versatile role RBM24 plays in cancer progression, further research into its regulatory mechanisms in CRC metastasis is justified.

Our study detected a plunge in RBM24 levels in CRC, and the overexpression of RBM24 was found to mediate the inhibition of tumor metastasis via the mitogen-activated protein kinase (MAPK) signaling pathway. Intriguingly, Es-Cu resulted in an up-regulation of RBM24 in CRC cells. Cuproptosis was found to obstruct the metastasis of CRC cells by modulating the RBM24/MAPK pathway. Overall, these findings contribute to our understanding of the regulatory mechanisms involved in cuproptosis-mediated cancer inhibition and present a promising therapeutic strategy for CRC.

2. Materials and methods

2.1 Bioinformatics analysis

We obtained and filtered the counts file data for CRC patients from The Cancer Genome Atlas (TCGA) database. By leveraging the edgeR package, we conducted an analysis for differentially expressed mRNAs (DEmRNAs) to compare the expression profiles between tumor and normal tissues ($\log2|FC| > 2$, FDR < 0.05). This led to the discovery of 647 tumor tissues and 51 normal tissues, totaling 2185 DEmRNAs, with 1262 up-regulated and 923 down-regulated. To illuminate the potential role of RBM24 in CRC, we categorized the CRC cohort into high and low expression groups based on the median RBM24 expression and performed Gene Set Enrichment Analysis (GSEA) to determine if genes in these groups were enriched in biological functions of significance.

2.2 Cell culture

Human colon epithelial cell line FHC and CRC cell lines HT-29, SW480, and DLD-1 were sourced from the BFB - Shanghai Cell Bank (China). They were cultured in DMEM-H medium (HyClone, USA) with 10% fetal bovine serum (FBS, Invitrogen, USA), 100 U/mL penicillin, and 100 μg/mL streptomycin (Invitrogen, USA) under conditions of 5% CO_2 at 37 °C.

2.3 Cell transfection

The RBM24 overexpression (oe-RBM24) and knockdown (si-RBM24) constructs, along with their negative controls (oe-NC, si-NC), were synthesized by Shanghai GeneChem Co., Ltd. The transfection of these vectors into CRC cells was facilitated using Lipofectamine 2000 (Invitrogen, USA) following the provided instructions. After 48 h post-transfection, the cells were collected for further experimental procedures.

The CRC cells were incubated with DMSO or PD0325901 (10 μM), an inhibitor of the MAPK pathway, for 48 h, followed by Cell counting kit-8 (CCK-8), Western blot (WB), and Transwell[17].

2.4 Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

Total RNA was extracted from cells using TRIzol reagent, and the RNA concentration and purity were assessed with a NanoDrop One UV-Vis spectrophotometer. The reverse transcription of RNA to cDNA was facilitated by SuperScript™ III Reverse Transcriptase (Invitrogen, USA). The qRT-PCR was performed on the ABI 7500 PCR system (Applied Biosystems, USA) with the Platinum SYBR Green qPCR SuperMix UDG kit (Invitrogen, USA). The relative expression levels of RBM24 were determined using the $2^{-\Delta\Delta CT}$ method, with GAPDH serving as the internal control. The primer sequences are detailed in the table provided.

2.5 WB

Cells were collected and then lysed in RIPA lysis buffer (Beyotime, China) containing 1% protease and phosphatase inhibitors (Beyotime, China) at 4 °C to extract total proteins. The protein concentration was quantified using the bicinchoninic acid (BCA) assay kit (Beyotime, China). The BCA protein assay kit (Beyotime, China) was employed to measure protein concentrations. Denatured proteins were separated by 10% SDS-PAGE and transferred onto PVDF membranes. The membranes were blocked with

5% BSA for 2 h at 37 °C and incubated overnight at 4 °C with primary antibodies for MMP2 (abcam, UK), MMP9 (abcam, UK), E-Cadherin (CST, USA), Fibronectin (CST, USA), p-ERK/ERK (abcam, UK), p-JNK/JNK (abcam, UK), FDX1 (Abclonal, China), DLAT (Abclonal, China), LIAS (abcam, UK), RBM24 (Proteintech Group, USA), and GAPDH (abcam, UK). The membranes were further incubated with HRP-conjugated secondary goat anti-rabbit IgG (abcam, UK) for 2 h at room temperature. Chemiluminescent detection was performed using the ChemiScope 6000 system (Clinx, China) with NcmECL Ultra (NCM Biotech, China) for imaging.

2.6 CCK-8 assay

Cell proliferation was determined by the CCK-8 kit (Dojindo, Japan). CRC cells were dispensed into a 96-well plate at a concentration of 2×10^3 cells per well. After cell adherence, 10 μL of CCK-8 reagent was introduced at 0 h, 24 h, 48 h, and 72 h. The plate was incubated in a cell culture incubator away from light for 2 h, and the absorbance at 450 nm in each well was measured with a microplate reader.

2.7 Transwell assay

CRC cells, 48 h post-transfection, were resuspended in serum-free medium and placed into the upper compartment of the Transwell at a density of 2×10^4 cells per well with 200 μL of the medium. The lower compartment received 600 μL of medium supplemented with 10% FBS. After a 24-h incubation, cells that had migrated to the exterior of the Transwell membrane were fixed using 4% paraformaldehyde and stained with 0.1% crystal violet. For the invasion assay, cells were seeded in the upper chamber coated with Matrigel, following the same protocol as the migration assay. The number of cells that migrated or invaded was counted under a microscope by selecting three random fields and calculating the mean cell count.

2.8 Immunofluorescence (IF)

The cells were fixed in 75% alcohol for 30 min. Subsequently, they were treated with 0.1% Triton X-100 for permeabilization for 10 min. Next, they were blocked with 5% BSA for 1 h. RBM24 antibody (Proteintech Group, USA) followed by Cy3-conjugated secondary antibody (Abclonal, China) were successively added for incubation. After being washed with PBS, they were observed using an inverted fluorescence microscope.

2.9 Measurement of intracellular copper content

HT-29 cells were cultured in a 6-well plate overnight. On the following day, 200 nM elesclomol-Cu (Es-Cu) was added to the cells, obtained from MCE (USA), at a 1:1 ratio. After incubation for 24 h, the cells were gathered and resuspended in 100 μL of PBS. The Copper (Cu) Content Assay Kit (Solarbio, China) was used to quantify the copper ion concentration inside the cells, according to the supplier's guidelines[18].

2.10 Data analysis

Experiments were conducted in triplicate, and data were analyzed using GraphPad Prism 8 software. The results are reported as mean ± SD. A Student's t-test was chosen for contrasting pairs of groups, whereas the one-way ANOVA was the protocol for assessing differences across multiple groups. $P < 0.05$ is deemed statistically significant.

3. Results

3.1 Overexpression of RBM24 inhibits CRC metastasis

As part of our investigation to identify mRNAs involved in the development of CRC, bioinformatics analysis showed that RBM24 expression was down-regulated in CRC tissues (Figure 1A). qPCR and IF experiments demonstrated a significant down-regulation of RBM24 expression in CRC cell lines HT-29, SW480, and DLD-1 when compared to the FHC human colon epithelial cell line (Figure 1B-C). Furthermore, the establishment of oe-NC/oe-RBM24 groups within HT-29 and SW480 cells was executed. qRT-PCR analyses confirmed a significant augmentation in RBM24 expression within the oe-RBM24 group in contrast to the oe-NC group (Figure 1D). The CCK-8 and Transwell assay data showed that the overexpression of RBM24 hindered the proliferative, migratory, and invasive capacities of CRC cells (Figure 1E-F). Following WB analysis of genes related to migration and invasion (MMP2, MMP9) as well as EMT markers (E-cadherin, fibronectin), the data revealed a notable reduction in the expression levels of MMP2, MMP9, and fibronectin, alongside an elevation in E-cadherin expression in HT-29 and SW480 cells overexpressing RBM24 (Figure 1G). In conclusion, the outcomes of this investigation strongly suggest a down-regulation of

RBM24 expression in CRC. Moreover, the overexpression of RBM24 was found to impede the metastatic capabilities of CRC cells.

3.2 RBM24 impedes the metastatic potential of CRC via the MAPK signaling cascade

To investigate the mechanisms of RBM24 in controlling the migration of CRC cells, we conducted GSEA analysis and discovered that RBM24 was associated with the MAPK signaling pathway (Figure 2A). The MAPK pathway is recognized in cellular function, and its aberrant activation can facilitate the EMT process[19]. Subsequently, we silenced RBM24 and further treated HT-29 cells with the MAPK pathway inhibitor PD0325901. The qRT-PCR results manifested that the si-RBM24+DMSO group and si-RBM24+PD0325901 suppressed the expression of RBM24 compared to the si-NC+DMSO group (Figure 2B). WB results revealed that in HT-29 cells with low RBM24 expression, levels of p-ERK and p-JNK were elevated, but no significant change was seen in the levels of ERK and JNK. The application of PD0325901 impaired the stimulatory effect of low RBM24 expression on the MAPK signaling pathway (Figure 2C-D). We also evaluated the proliferative, migratory, and invasive capabilities of HT-29 cells using CCK-8 and Transwell assays. It was shown that low RBM24 expression could facilitate the proliferation, migration, and invasion of HT-29 cells, and the addition of PD0325901 impaired the influence of low RBM24 expression on the biological behavior of CRC cells (Figure 2E-F). Another WB analysis revealed that the knockdown of RBM24 led to an up-regulation of MMP2, MMP9, and fibronectin expression, and a down-regulation of E-cadherin expression, and the addition of PD0325901 returned the protein expression levels to the control levels (Figure 2G). Overall, the inhibition of the MAPK signaling pathway counteracted the metastatic-promoting effect of low RBM24 expression in CRC.

3.3 Cuproptosis up-regulates RBM24 expression in CRC cells

To probe into the molecular mechanisms that govern RBM24 expression, we performed bioinformatics analysis, where a connection between RBM24 and copper ion homeostasis pathways was identified (Figure 3A). Subsequently, we detected the expression of cuproptosis-related proteins in the oe-RBM24/oe-NC group HT-29 cells.

The WB experimental results showed that after overexpression of RBM24, the expressions of FDX1, DLAT, and LIAS were significantly down-regulated, and cuproptosis was activated (Figure 3B). Research has highlighted the pivotal role of cuproptosis in tumor metastasis[20]. Therefore, we hypothesized that the expression of RBM24 might be related to the occurrence of cuproptosis. To test this, we treated HT-29 cells with Es-Cu and measured the intracellular copper ion concentrations much higher than in the NC group (Figure 3C). Additionally, CCK-8 assay results indicated a significant inhibition of CRC cell proliferation with Es-Cu treatment (Figure 3D). qRT-PCR results supported that Es-Cu treatment significantly increased RBM24 expression (Figure 3E). Finally, we detected the protein expression levels of cuproptosis-related genes and RBM24 through WB. The results showed that after treatment with Es-Cu, the expressions of FDX1, DLAT, and LIAS were significantly down-regulated, while the expression of RBM24 was up-regulated significantly (Figure 3F). These results collectively implied that the treatment of CRC cells with copper ionophores and copper ions could up-regulate the expression of RBM24.

3.4 Cuproptosis inhibits the metastasis of CRC cells through the RBM24/MAPK axis

To determine if cuproptosis influences CRC metastasis through the up-regulation of RBM24, Es-Cu was introduced to CRC cells with suppressed RBM24 expression, followed by the assessment of RBM24 expression via qRT-PCR. It was observed that the Es-Cu+si-NC group markedly increased RBM24 expression compared to the DMSO+si-NC group, whereas the Es-Cu+si-RBM24 group alleviated the enhancing effect of Es-Cu on RBM24 expression (Figure 4A). Thereafter, we examined the expression of p-ERK/ERK and p-JNK/JNK by WB and discovered that Es-Cu treatment inhibited the expression of the phosphorylated forms, with the reduction in MAPK signaling pathway gene expression being relieved after RBM24 knockdown (Figure 4B). In addition, CCK-8 and Transwell assay results showed that after Es-Cu treatment, the proliferation, migration, and invasion abilities of HT-29 cells significantly decreased, while knockdown of RBM24 concurrently led to a recovery increase (Figure 4C-E). WB also demonstrated that Es-Cu treatment down-regulated

the expression of MMP2, MMP9, and fibronectin, and up-regulated E-cadherin expression. Knockdown of RBM24 alleviated the changes in migration, invasion, and EMT-related protein expression levels induced by Es-Cu treatment (Figure 4F-G). We are led to the conclusion that elevated levels of cuproptosis disrupted the MAPK signaling cascade, thwarting CRC cell spread.

4 Discussion

Tumor progression and the ensuing metastasis are always the primary causes of mortality in CRC patients, with no effective treatment strategies currently available[20]. However, the regulation of key targets has shown great potential in preventing and treating CRC[21, 22]. Within our research, we identified RBM24, an RNA-binding protein, which was notably under-expressed in CRC and found to inhibit tumor metastasis upon overexpression. Moreover, RBM24 operates by inhibiting the MAPK signaling pathway. We also observed that cuproptosis in CRC can lead to an increase in RBM24 expression. These discoveries help elucidate the role of RBM24 in the processes of migration, invasion, and EMT, all of which are instrumental in the metastasis of CRC.

RBM24, an RNA-binding motif protein, is a versatile regulator that influences cell proliferation, apoptosis, and differentiation through its effects on pre-mRNA splicing and mRNA stability[13]. Furthermore, it acts as a tumor suppressor across various cancer types. In hepatocellular carcinoma cells, RBM24 is believed to inhibit the formation of multicellular three-dimensional tumor spheres. According to Rong and colleagues, RBM24 directly interacts with and stabilizes phosphatase and tensin homolog (PTEN) mRNA, which in turn inhibits the proliferation, migration, and invasion of CRC cells, blocking CRC tumorigenesis[14]. Our research, echoing the results of past studies, unveiled that the overexpression of RBM24 in CRC cells crippled their migration and invasion abilities, with a concurrent decrease in the expression of fibronectin and MMP2/MMP9, and a surge in E-cadherin expression. This illustrated that RBM24 expression negatively modulated the metastatic behavior of CRC cells.

The MAPK signaling pathway is a central regulatory nexus for a host of cellular functions, including cell proliferation, differentiation, and stress responses[23]. The

human MAPK family predominantly includes three pathways, known as Extracellular Signal-Regulated Kinase (ERK), p38, and c-Jun N-terminal Kinase (JNK)[24, 25]. It has been established that the activation of the MAPK pathway can enhance the proliferation and metastatic capabilities of cancer cells. Peng *et al.* have described that the GXYLT1^{S212*} mutation mainly facilitates CRC metastasis through the activation of the MAPK pathway[26]. It was recently discovered that mutations and increased expression of MAPK signaling pathway-associated molecules, along with excessive activation of this crucial pathway, are common occurrences in CRC. Targeting the MAPK pathway with specific pharmacological inhibitors has been demonstrated to elicit notable anti-cancer activity[27]. For example, PD-0325901, a specific MAPK inhibitor with oral efficacy, is being tested in early-phase clinical trials for a range of cancers, including CRC. In approximately 16% of late-stage CRC patients, the treatment resulted in a response rate and clinical benefit of approximately 5%, with a progression-free survival of 2.8 months[28]. Bioinformatics analysis in our research has revealed the possible involvement of RBM24 in the modulation of the MAPK signaling pathway. Notably, by down-regulating RBM24 and introducing PD0325901, we explored the effects of RBM24 on MAPK signaling in the context of CRC metastasis. We discovered that the inhibition of the MAPK signaling pathway with a specific inhibitor could reduce the increased metastasis observed with low RBM24 expression. Thus, the targeting of RBM24 to suppress MAPK signaling could be a new approach for CRC treatment.

Continuing our investigation into the regulation of RBM24 expression in CRC, bioinformatics analysis suggested a connection between RBM24 and copper ion homeostasis. After adding Es-Cu, the expression of FDX1, DLAT, and LIAS significantly decreased, while the expression level of RBM24 increased. Cuproptosis, a novel copper-mediated form of regulated cell death, is believed to contribute to cancer progression[29]. For example, 4-octyl itaconate (4-OI) inhibits aerobic glycolysis by targeting GAPDH to facilitate cuproptosis in CRC cells, thereby inhibiting tumor growth[11]. The findings of Li *et al*. have highlighted the role of FDX1 in combating tumor growth via cuproptosis in hepatocellular carcinoma and non-small cell lung cancer cell lines[30]. However, the existing studies are just beginning to explore the field

of cuproptosis. More investigation is needed, especially in relation to CRC. Our results revealed that suppressing RBM24 could mitigate the inhibitory effects of Es-Cu on cell migration and invasion. Therefore, a therapeutic approach that combines copper ionophores with copper ions and MAPK inhibitors may present a viable option for CRC therapy.

The findings above established that cuproptosis resulted in an increase in RBM24 expression, which suppressed the MAPK signaling pathway activation and consequently diminished the ability of CRC to metastasize. This research offers a novel perspective for understanding the mechanisms behind CRC development and positions the RBM24 gene as a potential marker for evaluating copper ionophore-based therapies. However, we could not overlook its limitations: the molecular regulatory network involving RBM24 remains to be fully elucidated, and the research was confined to a cellular level and without animal experimental validation. Going forward, we will explore the precise regulatory mechanisms of RBM24 in CRC across various levels and examine its role in the immune response to CRC tumors, aiming to provide new insights and a strong theoretical foundation for the advancement of CRC clinical practice.

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Figure legend

Figure 1: Down-regulation of RBM24 in CRC suppresses tumor metastasis

A: Bioinformatics analysis of RBM24 expression levels; B-C: qRT-PCR and IF detection of RBM24 expression in CRC cell lines and colonic epithelial cell lines; D: qRT-PCR confirmation of RBM24 overexpression in HT-29 and SW480 cells; E: CCK-8 assay for cell proliferation assessment; F: Transwell migration and invasion assays for HT-29 cells; G WB detection of migration and invasion-related (MMP2, MMP9) and EMT-related (E-cadherin, fibronectin) gene expression. All experiments were independently repeated three times, and the mean ± standard deviation was calculated. * represents $P < 0.05$.

Figure 2: RBM24 impedes CRC metastatic spread by modulating the MAPK pathway

A: The interaction of RBM24 with the MAPK signaling pathway (NES=1.55, FDR=0.347); B: qRT-PCR measurement of RBM24 expression in groups receiving si-NC+DMSO, si-RBM24+DMSO, and si-RBM24+PD0325901; C-D: WB evaluation of the expression of proteins in the MAPK signaling pathway (p-ERK/ERK, p-JNK/JNK); E: CCK-8 assessment of cell proliferation in different groups; F: Transwell assays evaluated HT-29 cell migration and invasion; G: WB analysis of E-cadherin, fibronectin, MMP2, and MMP9 expression levels. All experiments were independently repeated three times, and the mean ± standard deviation was calculated. * denotes $P < 0.05$ when compared to si-NC+DMSO; # denotes $P < 0.05$ when compared to si-RBM24+DMSO;

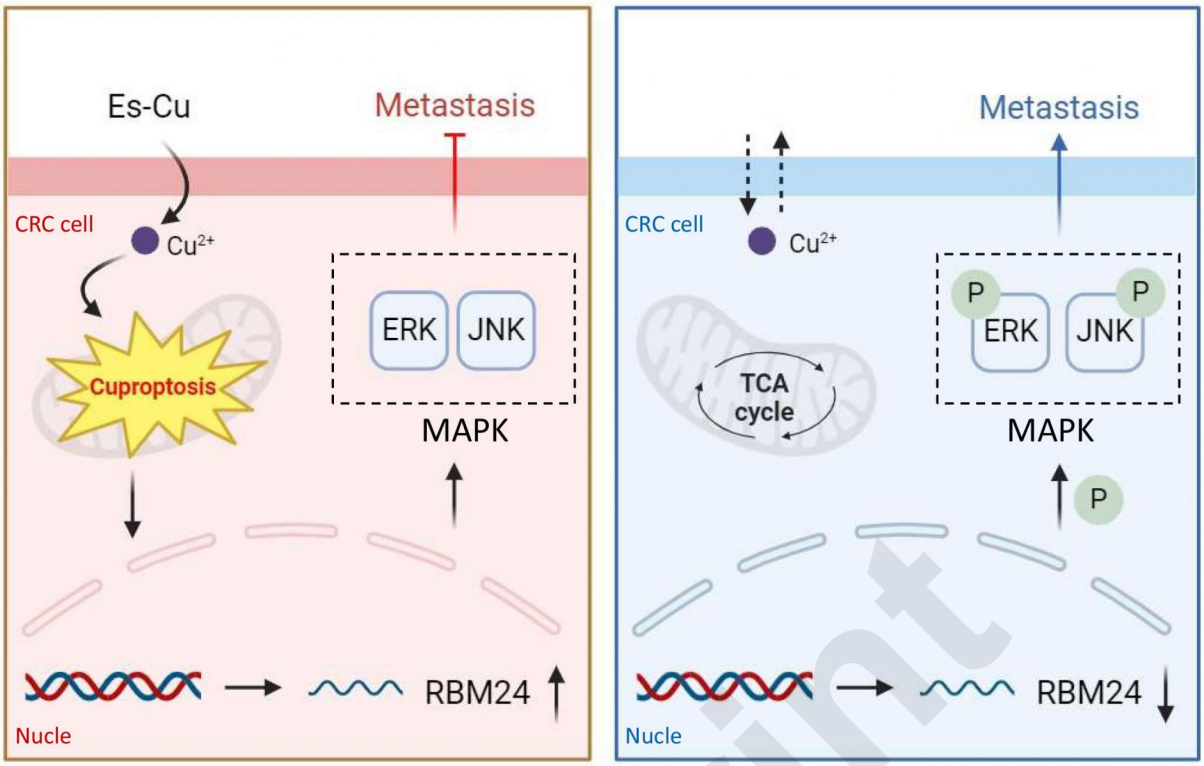
ns denotes $P > 0.05$ when compared to si-NC+DMSO.

Figure 3: Cuproptosis up-regulates RBM24 expression in CRC cells

A: Enrichment of RBM24 in pathways associated with copper ion homeostasis (NES= 0.81, FDR=0.822); B: WB detection of cuproptosis-related proteins (FDX1, DLAT, and LIAS); C: HT-29 cells treated with 200 nM Es-Cu (1:1 ratio), followed by copper ion level determination using a detection kit; D: CCK-8 assay to evaluate cell proliferation in both groups; E: qRT-PCR to measure RBM24 mRNA expression levels in both groups; F: WB detection of FDX1, DLAT, LIAS and RBM24 in both groups. All experiments were independently repeated three times, and the mean ± standard deviation was calculated. * denotes $P < 0.05$.

Figure 4: Cuproptosis inhibits the metastasis of CRC cells through the RBM24/MAPK axis

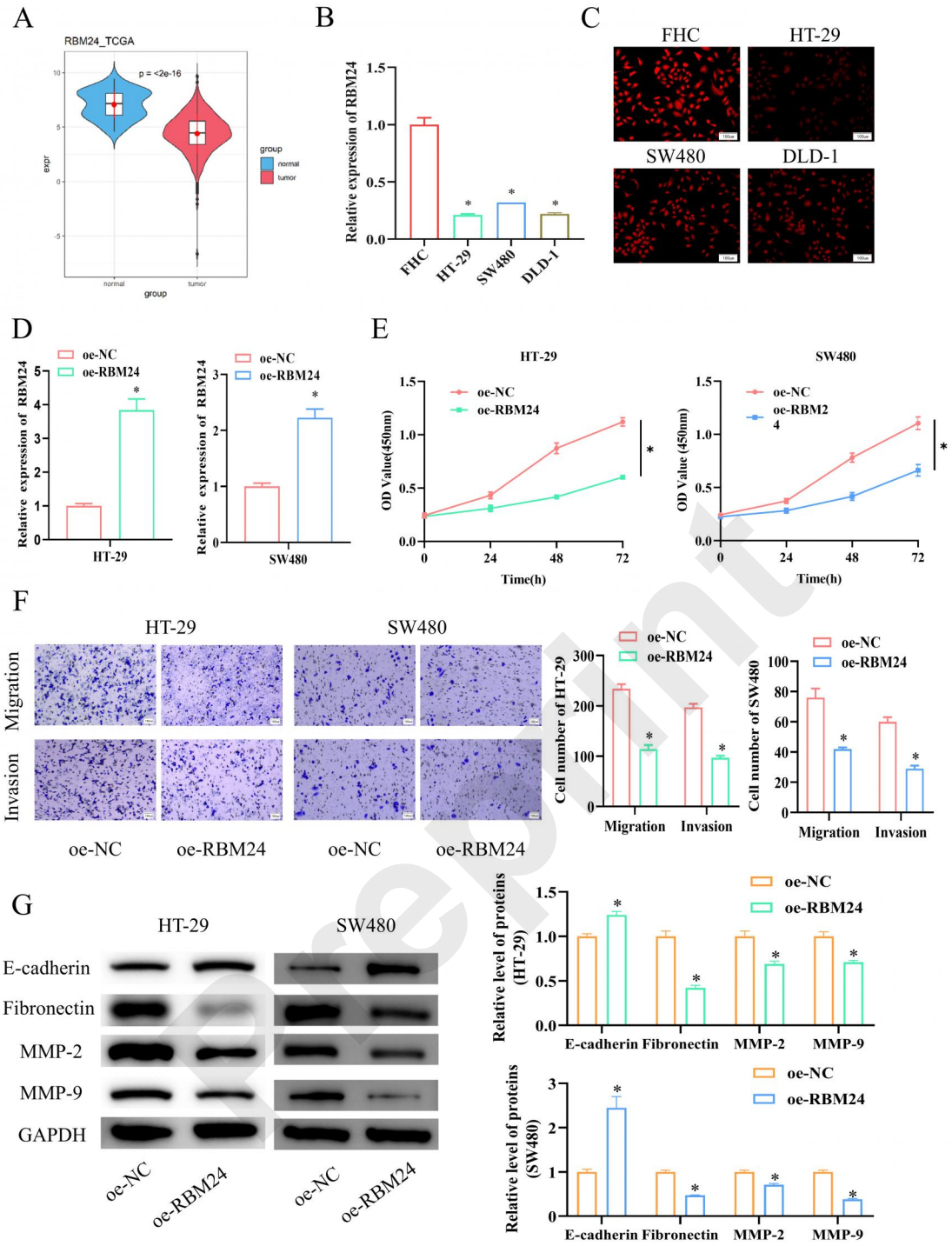
A: qRT-PCR determination of RBM24 levels in the DMSO+si-NC, Es-Cu+si-NC, and Es-Cu+si-RBM24 conditions; B: WB analysis quantified the expression of p-ERK/ERK and p-JNK/JNK; C: CCK-8 assay gauged the proliferation rate of HT-29 cells across the groups; D-E: Transwell migration and invasion assays for HT-29 cells; F-G: WB analysis for the expression of E-cadherin, fibronectin, and MMP2, MMP9. All experiments were independently repeated three times, and the mean ± standard deviation was calculated. * indicates $P < 0.05$ compared to the DMSO+si-NC group; # indicates $P < 0.05$ compared to the Es-Cu+si-NC group; ns denotes $P > 0.05$ when compared to si-NC+DMSO.

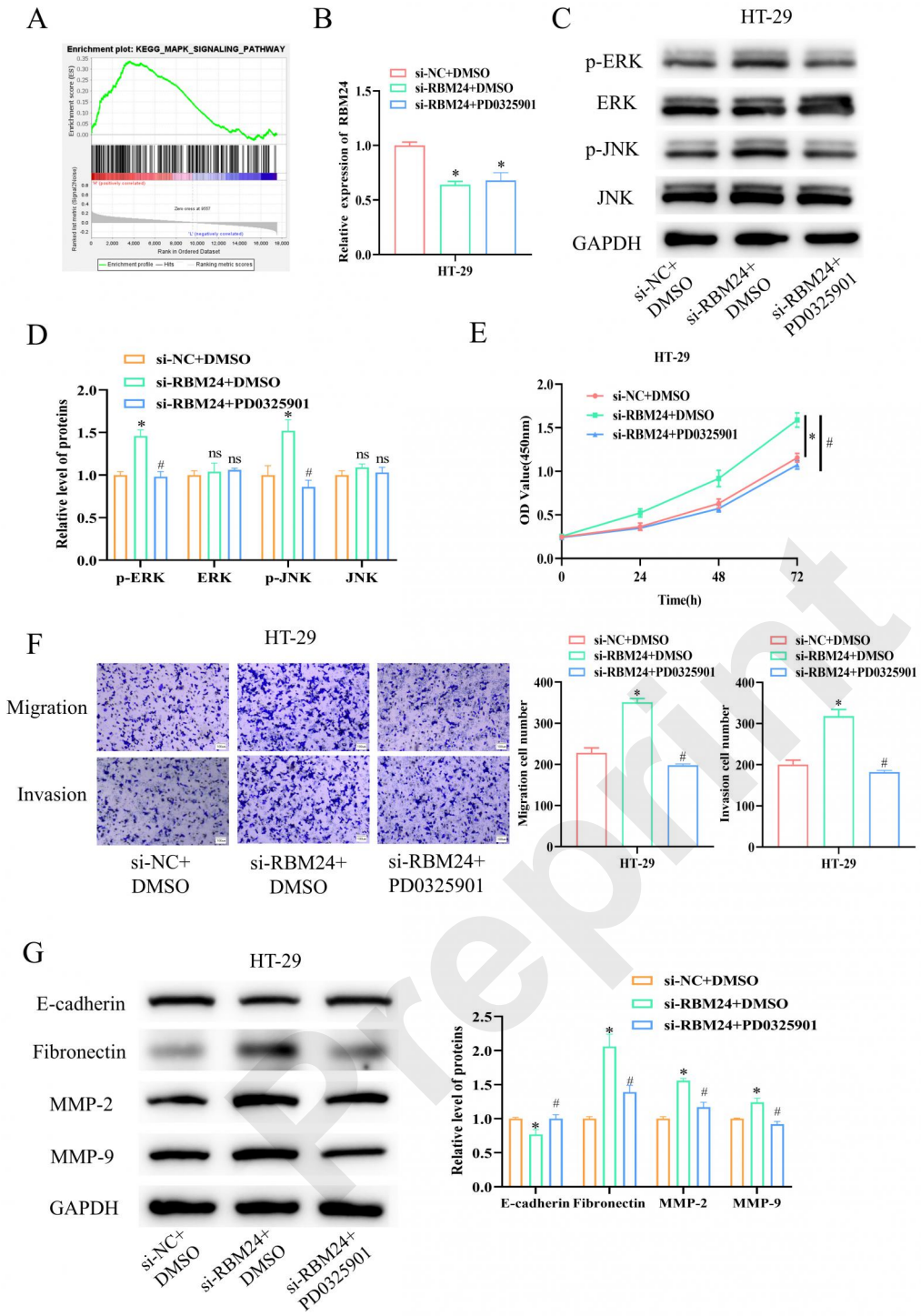


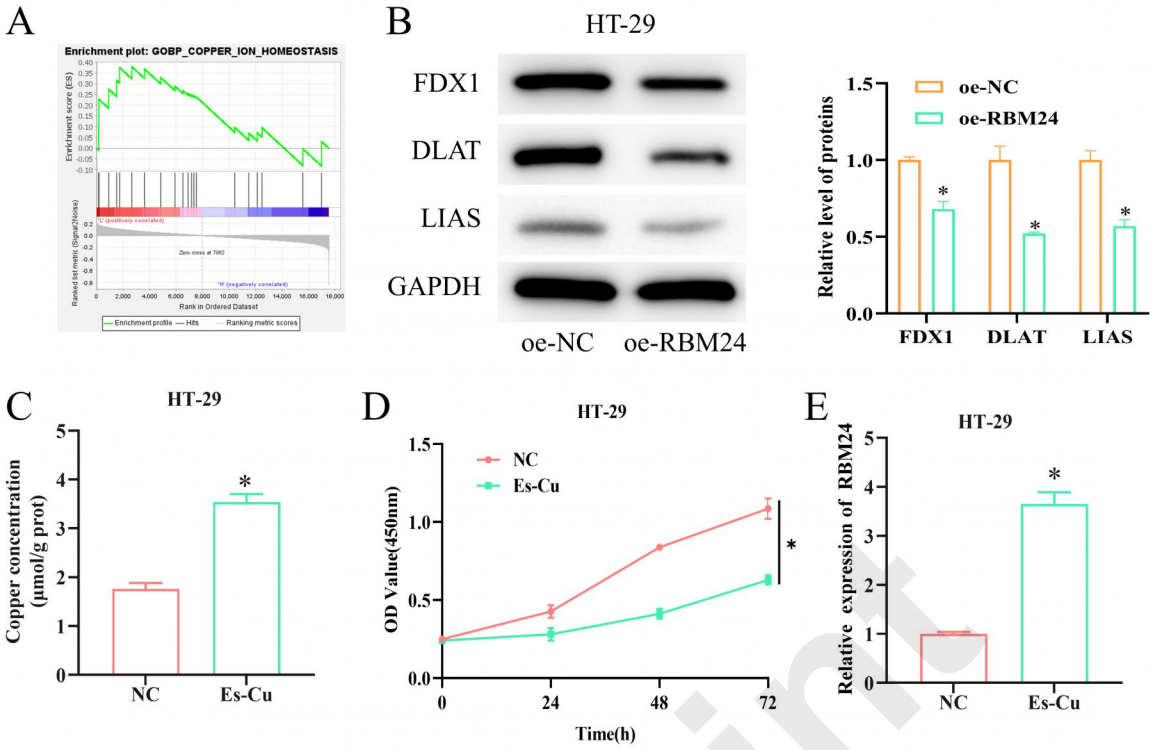
Cuproptosis-induced upregulation of RBM24 suppresses tumor metastasis via MAPK signaling blockage in colorectal cancer

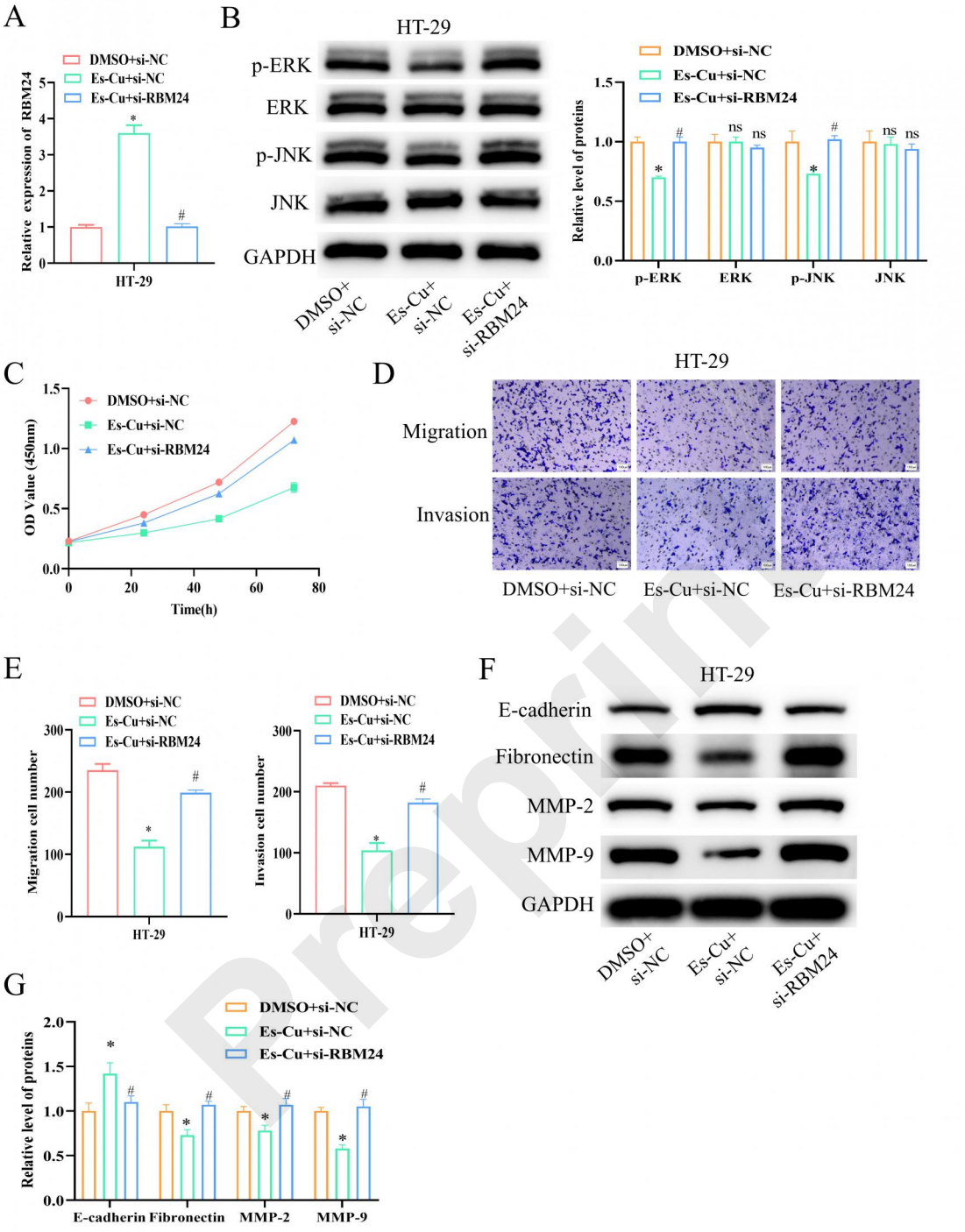
Table1. Primer set for qPCR

Gene	Primer sequence (5'→3')
RBM24	F: GCTGGATGCCGGTTGTTAAG
	R: GCACAAAAGCCTGCGGATAG
GAPDH	F: AAGGTGAAGGTCGGAGTCAAC
	R: GGGGTCATTGATGGCAACAATA


A
RBM24_TCGA
p = <2e-16
expr
group
normal
tumor
B
Relative expression of RBM24
FHC
HT-29
SW480
DLD-1
C
FHC
HT-29
SW480
DLD-1
D
oe-NC
oe-RBM24
Relative expression of RBM24
HT-29
SW480
E
HT-29
SW480
oe-NC
oe-RBM24
OD Value(450nm)
Time(h)
F
HT-29
SW480
Migration
Invasion
oe-NC
oe-RBM24
Cell number of HT-29
Cell number of SW480
G
HT-29
SW480
E-cadherin
Fibronectin
MMP-2
MMP-9
GAPDH
Relative level of proteins (HT-29)
Relative level of proteins (SW480)


A
Enrichment plot: KEGG_MAPK_SIGNALING_PATHWAY
B
si-NC+DMSO
si-RBM24+DMSO
si-RBM24+PD0325901
Relative expression of RBM24
HT-29
C
HT-29
p-ERK
ERK
p-JNK
JNK
GAPDH
si-NC+ DMSO
si-RBM24+ DMSO
si-RBM24+ PD0325901
D
si-NC+DMSO
si-RBM24+DMSO
si-RBM24+PD0325901
Relative level of proteins
p-ERK
ERK
p-JNK
JNK
ns
E
HT-29
si-NC+DMSO
si-RBM24+DMSO
si-RBM24+PD0325901
OD Value(450nm)
Time(h)
F
HT-29
Migration
Invasion
si-NC+ DMSO
si-RBM24+ DMSO
si-RBM24+ PD0325901
si-NC+DMSO
si-RBM24+DMSO
si-RBM24+PD0325901
Migration cell number
Invasion cell number
HT-29
G
HT-29
E-cadherin
Fibronectin
MMP-2
MMP-9
GAPDH
si-NC+ DMSO
si-RBM24+ DMSO
si-RBM24+ PD0325901
si-NC+DMSO
si-RBM24+DMSO
si-RBM24+PD0325901
Relative level of proteins
E-cadherin
Fibronectin
MMP-2
MMP-9


A
Enrichment plot: GOBP_COPPER_ION_HOMEOSTASIS
Enrichment score (ES)
Ranked list metric (Signal2Noise)
Rank in Ordered Dataset
Enrichment profile
Hits
Ranking metric scores
B
HT-29
FDX1
DLAT
LIAS
GAPDH
oe-NC
oe-RBM24
Relative level of proteins
oe-NC
oe-RBM24
FDX1
DLAT
LIAS
C
HT-29
Copper concentration (μmol/g prot)
NC
Es-Cu
D
HT-29
NC
Es-Cu
OD Value(450nm)
Time(h)
E
HT-29
Relative expression of RBM24
NC
Es-Cu
F
HT-29
FDX1
DLAT
LIAS
RBM24
GAPDH
NC
Es-Cu
NC
Es-Cu
Relative level of proteins
FDX1
DLAT
LIAS
RMB24


A
DMSO+si-NC
Es-Cu+si-NC
Es-Cu+si-RBM24
Relative expression of RBM24
HT-29
B
HT-29
p-ERK
ERK
p-JNK
JNK
GAPDH
DMSO+ si-NC
Es-Cu+ si-NC
Es-Cu+ si-RBM24
Relative level of proteins
p-ERK
ERK
p-JNK
JNK
ns
C
OD Value (450nm)
Time(h)
D
HT-29
Migration
Invasion
DMSO+si-NC
Es-Cu+si-NC
Es-Cu+si-RBM24
E
Migration cell number
Invasion cell number
HT-29
F
HT-29
E-cadherin
Fibronectin
MMP-2
MMP-9
GAPDH
G
Relative level of proteins
E-cadherin
Fibronectin
MMP-2
MMP-9