Dear Editor:

Thanks for your letter and the reviewers' constructive and professional comments concerning our manuscript (NO. 83661) entitled “Interaction Mechanisms Between Autophagy and Ferroptosis: Potential Role in Colorectal Cancer”. Those comments are very helpful for revising and improving our manuscript and significantly guide our research. We have studied these comments carefully and made modifications which we hope meet with your approval. At the same time, according to the suggestions of the company editor-in-chief, we have supplemented the highlights of the latest cutting-edge research results and refined the content of the manuscript. Since we are non-native English speakers, we have sent the revised manuscript for language polishing to the professional English editing company (AJE) recommended by the BPG publishing group, ensuring the language meets publication requirements.

The following is our point-by-point response to each question raised by the reviewers.

Reviewer #1:

Q1: CRC is not a single disease. It is not clear from the article how autophagy and ferroptosis are distinguished in sporadic or colitis-associated CRC. - It is possible to influence autophagy, and there is experimental evidence that this alters the phenotype of CRC cells.

Reply: We appreciate your time and effort in reviewing this manuscript. Thanks to the reviewer for your professional advice. In the “**INTRODUCTION**” section, we have defined and described autophagy and ferroptosis. Autophagy transports a bulk of cytoplasm or specific cargoes to lysosomes for degradation by forming autophagosomes with a double-membrane structure. Unlike autophagy, the process of ferroptosis

is lipid peroxidation-mediated plasma membrane damage catalyzed by iron accumulation. Morphologically, ferroptosis is represented by obvious shrinkage of the mitochondria with increased membrane density, fewer or no mitochondrial cristae, and outer mitochondrial membrane rupture.

Q2: In any case, I think it is justified to add to the article how it is possible to influence the process of autophagy (e.g., by TLR signaling), how this influences ferropotosis, and whether this has an effect on cancer cell survival, division, and the emergence of the stem cell phenotype.

Reply: Thanks to the reviewer for your professional advice. As the reviewers point out, toll-like receptor (TLR) signaling acts as an immunomodulator that plays a critical role in colitis-associated CRC. We found that TLR4 could mediate autophagy to promote cancer cell migration and invasion. Interestingly, p62 negatively regulates TLR4-mediated autophagy. Therefore, we have supplemented this content in the last paragraph of the "***The role of autophagy in CRC***" section, as follows: Toll-like receptor (TLR) signaling acts as an immunomodulator that regulates inflammatory responses and plays a critical role in colitis-associated CRC[49]. TLR4 mediates the formation of the TRAF6-BECN1 complex, which activates autophagy, facilitating the migration and invasion of cancer cells[50]. P62 negatively regulates TLR4-induced autophagy activation and inhibits cancer cell progression[50].

Q3: It would also be useful to develop a figure highlighting the interactions of autophagy and ferroptosis in cancer cells.

Reply: Thank you for your suggestion. This article focuses on the interaction mechanism between autophagy and ferroptosis in CRC, and draws Figure 2 (below). The crosstalk of autophagy and ferroptosis in other

cancer cells is also mentioned in the "**THE INTERACTION BETWEEN AUTOPHAGY AND FERROPTOSIS**" section. Interested readers can learn more based on the cited references.

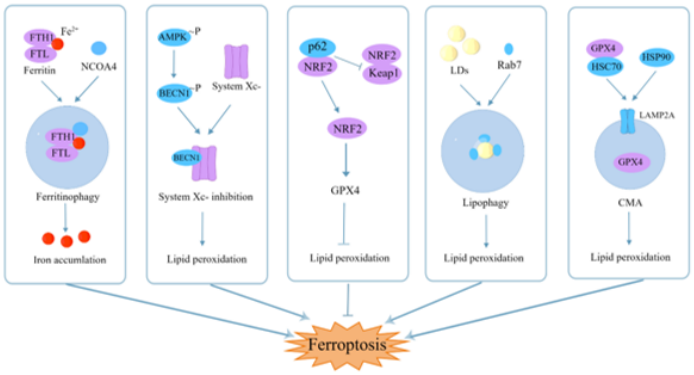

Figure 2 The interaction mechanisms between autophagy and ferroptosis in colorectal cancer (by Figdraw). Fe^{2+}: Ferrous ions; FTH1: Ferritin heavy chain 1; FTL: Ferritin light chain; NCOA4: Nuclear receptor coactivator 4; AMPK: Adenosine monophosphate-activated protein kinase; BECN1: Beclin1; p62: sequestosome 1; NRF2: Nuclear factor erythroid 2-related factor 2; KEAP1: Kelch-like ECH-associated protein 1; GPX4: Glutathione Peroxidase 4; LDs: Lipid droplets; Rab7: The small GTPase; HSC70: Heat shock cognate 71 kDa protein; HSP90: Heat shock protein 90; LAMP2A: Lysosome-associated membrane protein type 2A; CMA: Chaperone-mediated autophagy.

Reviewer #2:

Q1: "relevant studies has indicated significant crosstalk between autophagy and ferroptosis". The "has" should be "have".

Reply: Thank you for your reminder. we have corrected the mistake.

Q2: In the section of "Other potential pathways", I suggest add brief description about the specific autophagy receptor HPCAL1 in ferroptosis.

Reply: Thanks to the reviewer for your professional advice. Following your suggestion, we have added a description about the specific autophagy receptor HPCAL1 in the last paragraph of the "***Other Potential Pathways***" section, as follows:

Furthermore, hippocalcin-like 1 (HPCAL1), a neuronal calcium sensor, was identified as an important negative regulator of lipid synthesis and mTOR signaling activation, thereby blunting lipid metabolism to suppress tumorigenesis in the liver[135]. A recent study showed that HPCAL1

selectively degrades cadherin 2 and promotes lipid peroxidation to induce ferroptosis[136]. This phenomenon has been confirmed in a variety of cancer cells, including pancreatic cancer, non-small cell lung cancer, and bladder cancer cells, but this trend needs to be explored further in CRC.

Q3: In the section of "CONCLUSION AND PERSPECTIVE", I suggest add brief description about clinical translation of ferroptosis in cancer treatment.

Reply: Thank you for your suggestion. In the last paragraph of the "***The role of ferroptosis in CRC***" section, we introduce the role of ferroptosis in CRC treatment, including ferroptosis inducers combined with chemotherapy, emerging nanotechnology, and so on. To make this section more prominent, we have named this paragraph "***Antitumor therapy based on ferroptosis in CRC***". In addition, according to your suggestion, we have added a summary of CRC treatment based on ferroptosis in the "**CONCLUSION** " section, as follows:

Ferroptosis can be used as a new treatment to clear cancer cells. For example, sorafenib itself is a ferroptosis inducer and ferroptosis inducers combined with chemotherapy drugs can overcome drug resistance; in addition, nanoparticulate anticancer drug delivery systems based on ferroptosis have emerged[137, 138]. Although many studies are still in the experimental stage, these results have revealed the great potential of ferroptosis in cancer treatment.

Reviewer #3:

We appreciate your time and effort in reviewing this manuscript. Thank

you for your encouraging comment!

Reviewer #4:

Q1: I suggested the authors should register this review to PROSPERO.

Reply: Thanks very much for taking your time to review this manuscript. Our submitted manuscript is a literature review. We tried to register in PROSPERO, but this database does not receive this type of review.

Q2: The quality of prisma flow chart is lacked. Besides, I suggested the authors should add some published article for this meta-analysis if necessary.

Reply: Thank you for your suggestion. Our manuscript is a literature review that includes two figures: (1) “Figure 1 Mechanisms of ferroptosis”; (2) “Figure 2 The interaction mechanisms between autophagy and ferroptosis in colorectal cancer”. We further refined these two figures.

Q3: Where is the mechanism plot described in manuscript based on its assumed pathogenesis? This concern needs to be addressed.

Reply: Thanks to the reviewer for this question. Our manuscript includes two figures: (1) “Figure 1 Mechanisms of ferroptosis”; (2) “Figure 2 The interaction mechanisms between autophagy and ferroptosis in colorectal cancer”, which are placed at the end of the original text according to the magazine's submission requirements.

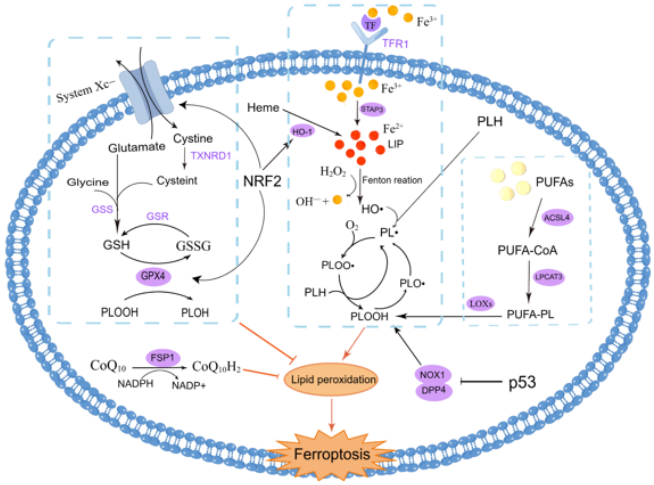

Figure 1 Mechanisms of ferroptosis (by Figdraw). TXNRD1: Thioredoxin reductase 1; GSS: Glutathione synthetase; GSH: Glutathione; GSSG: Oxidized glutathione; GSR: Glutathione-disulfide reductase; GPX4: Glutathione peroxidase 4; PLOOH: Phospholipid hydroperoxides; PLOH: Phosphatidyl alcohol; Fe^{3+}: Ferric ion; Fe^{2+}: Ferrous ion; TF: Transferrin; TfR1: Transferrin receptor 1; STEAP3: Six-transmembrane epithelial antigen of prostate 3; LIP: labile iron pool; H_2O_2: Hydrogen peroxide; HO•: Hydroxyl radicals; PL•: Phospholipid radical; PLH: Phospholipid; PLO•: Phospholipid alkoxyl radical; PLOO•: Phospholipid peroxyl radical; PUFAs: Polyunsaturated fatty acids; PUFA-PL: Polyunsaturated-fatty-acid-containing phospholipid; ACSL4: Acyl-CoA synthetase long-chain family member 4; LPCAT3: Lysophosphatidylcholine acyltransferase 3; LOXs: Lipoxygenases; NRF2: Nuclear factor erythroid 2-related factor 2; HO-1: Haem oxygenase 1; FSP1: Ferroptosis suppressor protein 1; CoQ_{10}: Ubiquinone; $CoQ_{10}H_2$: The reduced form of ubiquinone; p53: Tumor protein p53; DPP4: Dipeptidyl-peptidase-4; NOX1: A member of the NADPH oxidase protein family.

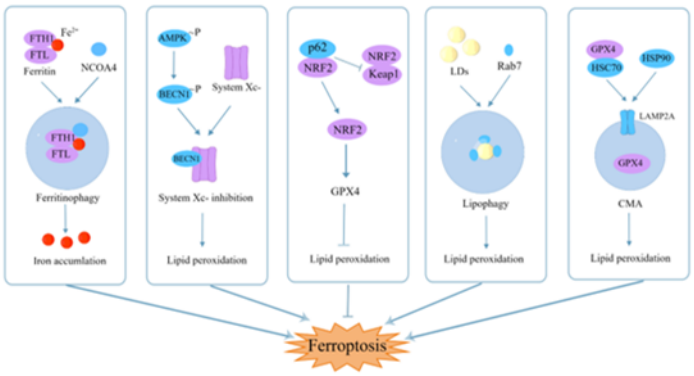

Figure 2 The interaction mechanisms between autophagy and ferroptosis in colorectal cancer (by Figdraw). Fe^{2+}: Ferrous ions; FTH1: Ferritin heavy chain 1; FTL: Ferritin light chain; NCOA4: Nuclear receptor coactivator 4; AMPK: Adenosine monophosphate-activated protein kinase; BECN1: Beclin1; p62: sequestosome 1; NRF2: Nuclear factor erythroid 2-related factor 2; KEAP1: Kelch-like ECH-associated protein 1; GPX4: Glutathione Peroxidase 4; LDs: Lipid droplets; Rab7: The small GTPase; HSC70: Heat shock cognate 71 kDa protein; HSP90: Heat shock protein 90; LAMP2A: Lysosome-associated membrane protein type 2A; CMA: Chaperone-mediated autophagy.

Q4: To my knowledge, discussion should updated solution or treatment for this issue. Could the authors conduct related treatment comparison for traditional therapy associated with Colorectal Cancer?

Reply: Thanks to the reviewer for your professional advice. The treatment

of CRC includes surgical resection, cytotoxic chemotherapy, biological therapy, immunotherapy, and their combinations. However, drug resistance in CRC significantly reduces the effectiveness of systemic treatment. Some studies have shown that certain drugs or compounds such as cisplatin, β-elemene can induce ferroptosis to inhibit the growth and metastasis of CRC. In the "***Antitumor therapy based on ferroptosis in CRC***" section, we describe the role of ferroptosis in CRC treatment. In addition, we have added a summary in the last paragraph of the article.

Q5: There are some grammatical errors in this paper. Generally, this work may be not suitable for publication until major concerns to be addressed in World Journal of Gastrointestinal Oncology.

Reply: We thank the reviewer for pointing out this issue. According to the reviewer's suggestion, we carefully examined, read, and considered the expression and grammar of the sentence. We have sent the revised manuscript for language polishing to the professional English editing company (AJE) recommended by the BPG publishing group, ensuring the language meets publication requirements.

Q6: Literature limitations should be added.

Reply: Thank you for your suggestion. We have reduced the original literature to 139. However, new content has been added based on comments by other reviewers, and references have also been added, which currently stands at 145.