

Response to Reviewers' Comments:

Reviewer #1:

Comment 1: Di Wang et al revealed the complex role of MIB1 gene mutation and abnormal expression in the prognosis of GC. The result of the study is of interest that authors found that high expression of MIB1 can serve as a potential prognostic biomarker to identify gastric adenocarcinoma patients with poor clinical prognosis and may play a specific role in immune infiltration. Overall, this study was well conducted with good methodology and intelligible English.

Reply: Thank you for your comment.

Reviewer #2:

Comment 1: Introduction contains the most important data to support the importance of the study. It is recommended to add more introductions about MIB1 to the first part of the text.

Reply: Thank you for your comment. The introductions about MIB1 are indeed not sufficient as the reviewer said, so we enriched this part. The added contents, as follows, also were highlighted in yellow in the last paragraph of the Introduction section.

Mind bomb 1 (MIB1), a large multidomain RING-type E3 ubiquitin-protein ligase[19], which activates Notch signaling by promoting ubiquitination, endocytosis and subsequent activation of Notch ligands, plays a central role in the conduction of Notch signaling pathway. Inhibition of MIB1 led to the decrease of Notch signal activation in mammalian cells, which was fatal to mouse embryos with Notch activation deficiency [20, 21]. Vitro experiments confirmed that MIB1 can induce degradation of suppressor of tumorigenicity 7 protein (ST7) to upregulate the IQ motif containing GTPase activating

protein 1 (IQGAP1) in pancreatic cancer cells to promote tumor growth and progression, and also regulate the resistance of pancreatic cancer cells to gemcitabine[22, 23]. It has been reported that MIB1 was ubiquitous in breast cancer to mediate JAG1 ubiquitination and activate Notch signal[24]. Aside from ubiquitinating the NOTCH ligand, MIB1 also ubiquitinated Ctnnd1 to regulate the migration of cells[25]. However, it remains unclear that the influence of MIB1 gene on gastric cancer because of the limited research on MIB1. Our study aimed to determine whether MIB1 was associated with prognosis in STAD and whether MIB1 could be regarded as a potential therapeutic target.

Comment 2: In Table 1, only row 1 lists the n values for each group, but numbers are incorrect except for 'T stage', 'Sex' and 'OS event', please confirm that using only one n value throughout the table is reasonable? It is proposed to supplement the respective n values in the row for each item.

Reply: Thank you for carefully pointing out what we overlooked. We adjusted the contents of the Table 1 and highlighted the changes.

Reviewer #3:

Comment: I have only one question, why did the authors initially choose Mind Bomb 1 to investigate?

Reply: Thank you for your comment. Our research target is the MIB1 gene for the following reasons: First of all, it is well-known that the occurrence of gastric cancer is a very complicated process regulated by multiple factors and pathways, including the involvement of NOTCH pathway. At present, there were many literatures on the role of ligands and receptors in NOTCH pathway in gastric cancer. Ligand-receptor interactions can affect the NOTCH pathway, so how does this complex affect this pathway? By searching literatures, I found that this process requires E3 ubiquitin protein ligase to promote ubiquitination of Notch ligand, endocytosis and subsequent

activation to activate the Notch signaling pathway. Currently, there are more than 300 E3 ubiquitin ligases. However, studies found that MIB1 played a major role in ubiquitination and transport of Notch ligands in mammals, which might be the only one. Secondly, E3 ubiquitin ligase belongs to ubiquitin proteasome system, which plays an important role in the occurrence and development of tumors. Finally, some studies showed that MIB1 was involved in the proliferation, migration and invasion of some tumors, including prostate cancer, pancreatic cancer and so on. However, there were few studies on its role in gastric cancer. Therefore, the connection between MIB1 and gastric cancer was discussed in our paper.