Dear editor and reviewers,

Thank you very much for giving us an opportunity to revise our manuscript. We appreciate the editor and reviewers very much for their constructive comments and suggestions on our manuscript entitled "E3 Ubiquitin Ligase TRIM55 promotes metastasis of gastric cancer cells by mediating epithelial-mesenchymal transition" (ID: 79440)

We have studied reviewers' comments carefully. According to the reviewers' detailed suggestions, we have made a careful revision on the original manuscript. All revised portions are marked in red in the revised manuscript which we would like to submit for your kind consideration.

Reviewer #1:
Scientific Quality: Grade C (Good)
Language Quality: Grade B (Minor language polishing)
Conclusion: Major revision
Specific Comments to Authors: In this study, Li et al investigated the role of tripartite motif containing 55 (TRIM55) in gastric cancer progression. They found that TRIM55 was upregulated in GC tissues or cells. By using siRNA or overexpression vector of TRIM55, they showed that TRIM55 promoted GC proliferation. Interestingly, TRIM55 was demonstrated to facilitate GC metastasis by influencing EMT. Here, I have the following concerns.

Response: Thanks for your review.

(1) A major issue is that there are currently not many studies about TRIM55 in tumors. There are two studies showing that TRIM55 is a tumor suppressor in lung cancer and liver cancer (PMID: 34974792 and 30685767). How do the authors explain these inconsistencies? These controversial studies should be included in your discussion.

Response: Thanks for your review. We appreciated it very much for this good suggestion, and we have done it according to your ideas.

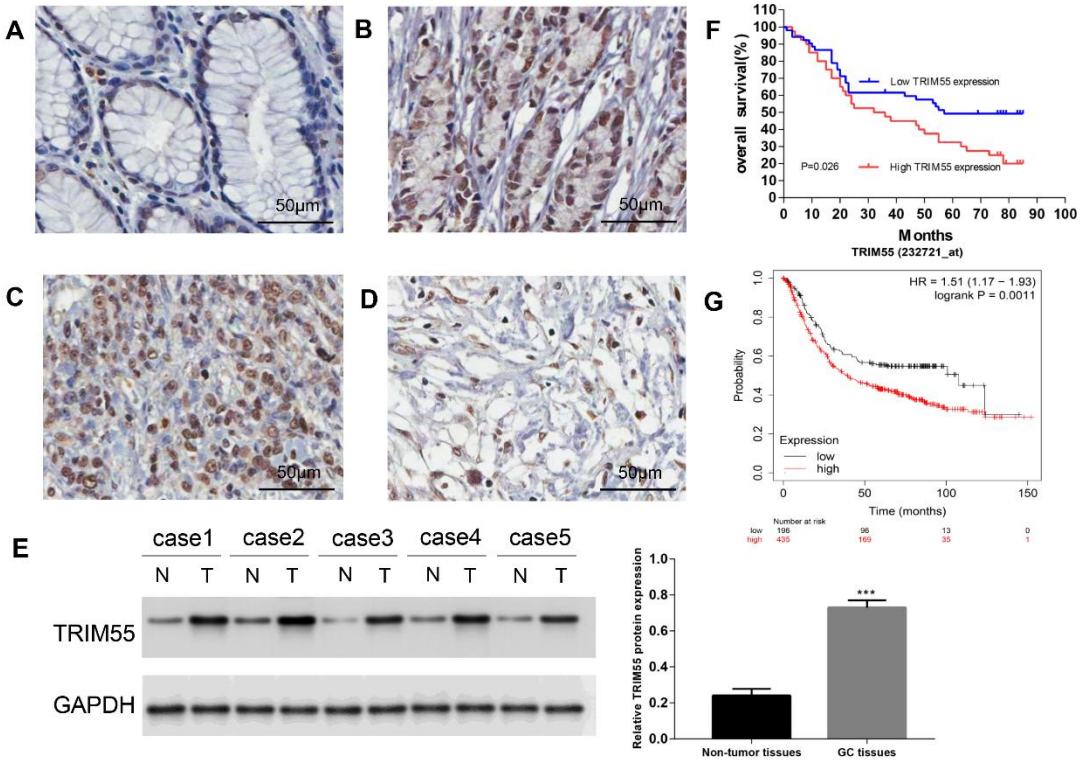
TRIM proteins could serve the ubiquitination function to stabilize or dislocate target proteins in various cellular compartments[1]. Ubiquitination is a post-transcriptional modification that labels the target proteins to be degraded at the proteasome level. Thus, TRIM family members determine both tumor suppressor and oncogenic roles by affecting the signal pathways in cancer development and progression. For example, TRIM29 and TRIM8 exhibited contextual function in different cancers[2-4]. They negatively or positively regulate tumorigenesis and tumor progression by affecting pathways. In our study, we showed that TRIM55 is highly expressed in gastric tumors and cultured tumor cells. TRIM55 has E3 ubiquitin ligase activity and whether it can regulate the EMT-related proteins through ubiquitination requires further investigation.

1. Mohammadi A, Pour Abbasi MS, Khorrami S, et al. The TRIM proteins in cancer: from expression to emerging regulatory mechanisms. *Clin Transl Oncol* 2022;24(3):460-70. doi: 10.1007/s12094-021-02715-5 [published Online First: 20211013]

2. Esposito JE, De Iuliis V, Avolio F, et al. Dissecting the Functional Role of the TRIM8 Protein on Cancer Pathogenesis. *Cancers (Basel)* 2022;14(9) doi: 10.3390/cancers14092309 [published Online First: 20220506]
3. Deng X, Fu X, Teng H, et al. E3 ubiquitin ligase TRIM29 promotes pancreatic cancer growth and progression via stabilizing Yes-associated protein 1. *J Transl Med* 2021;19(1):332. doi: 10.1186/s12967-021-03007-w [published Online First: 20210805]
4. Xu M, Hu J, Zhou B, et al. TRIM29 prevents hepatocellular carcinoma progression by inhibiting Wnt/β-catenin signaling pathway. *Acta Biochim Biophys Sin (Shanghai)* 2019;51(1):68-77. doi: 10.1093/abbs/gmy151

(2) GC patient samples are needed to check the expression level of TRIM55 by WB.

Response: Thank you for your advice. We have detected the expression level of TRIM55 of 5 GC patient samples by WB.

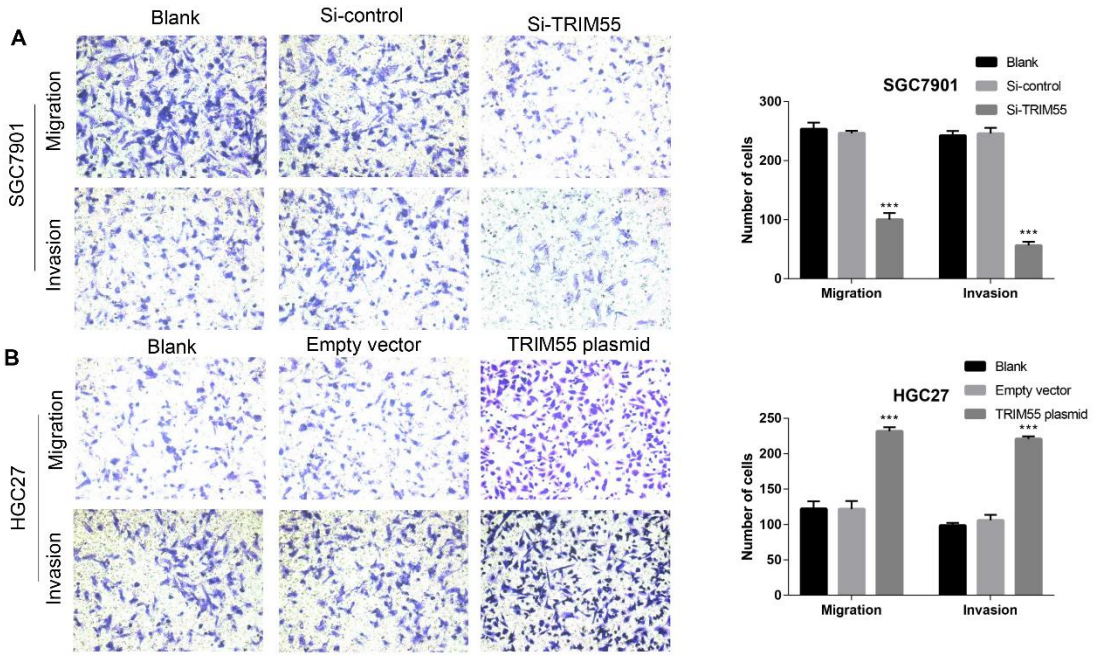


(3) Animal experiment is needed to show the growth promoting effect of TRIM55.

Response: Thanks for your suggestion. In this study, we focused on the effect of TRIM55 on the proliferation and migration of GC in vivo assay. For future research, we will utilize animal models to confirm the role of TRIM55 in gastric cancer cell growth.

(4) In figure 4B, why are the colors in TRIM55 plasmid group not the same as in other groups? They should be united.

Response: Thanks for your kind suggestions, which is valuable for improving the accuracy of the manuscript. We have reunited figure 4B.



(5) The English of the manuscript should be improved. Check this sentence "Tripartite motif containing 55 (TRIM55), a member of TRIM protein, its role in gastric cancer (GC) is unknow." In addition, the tenses of the captions in Results section should be united.

Response: Thanks for your suggestion! We have made corresponding revisions.

The role of tripartite motif containing 55 (TRIM55), a member of the TRIM protein family, in gastric cancer (GC) is unknown.

We have unified the tense of the captions in the Results section.

(6) The content of this reference "Studies have shown that Mir-30-5-5p can regulate the expression of TRIM55 in SARS-COV infection[7]" is not accurate. Authors need to check and revise it.

Response: Thank you for your suggestion. We have made corresponding revisions as you suggested.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: In the work titled "TRIM55 promotes metastasis of gastric cancer cells by mediating epithelial-mesenchymal transition" by Weiwei Li etc., the authors found that TRIM55 expression levels were significantly increased in GC cell lines and tissues, high expression of TRIM55 was correlated with poor prognosis of GC patients, moreover, TRIM55 can regulate the expression of EMT-related proteins in GC

cells. This is an interesting study that may add some novel information on the effect of TRIM55 on GC. The work is logically designed, the idea is new and interesting. Although, there are several concerns that need to be addressed.

Thanks for your kind review.

1. In my opinion, the most obvious weakness of this work is the lack of in-depth mechanism study. The authors should add more mechanism study in the manuscript, or add your research plan at least.

Response: Thank you for your encouraging and constructive comments. In a subsequent study, more thorough experiments based on knock-out cell line and animal models combined with high-throughput bioinformatic analysis are needed to shed light on the ubiquitination role of TRIM55.

2. The concept of EMT was first reported in embryology area. EMT is a biological process which is of great importance in embryogenesis and organ development. I suggest that the research process and some discoveries of EMT could be added in the discussion or introduction section. Some references could be cited, "EMT Transition States during Tumor Progression and Metastasis", "Exosomes Regulate the Epithelial-Mesenchymal Transition in Cancer" for example, or any other similar references.

Response: Thank you for your suggestion.

The progression of EMT is regulated by translational factors and epigenetic modification[13]. Also, microRNAs and long non-coding RNAs are involved in EMT regulation as post-translational regulators[14]. The tumor cells or other stromal cells can secret exosomes. Exosomes are extracellular vesicles with a lipid bilayer containing proteins, lipids and functional RNAs, which can transfer information between tumor cells or between tumor cells and the tumor microenvironment, thereby regulating the EMT process[15, 16]. As EMT plays essential physiological roles, EMT-targeted therapy combined with conventional chemotherapy can improve the sensitivity of tumor cells to drugs.

13 Lu W, Kang Y. Epithelial-Mesenchymal Plasticity in Cancer Progression and Metastasis. *Dev Cell* 2019; **49**(3): 361-374 [PMID: 31063755 PMCID: PMC6506183 DOI: 10.1016/j.devcel.2019.04.010]

14 Huang Y, Hong W, Wei X. The molecular mechanisms and therapeutic strategies of EMT in tumor progression and metastasis. *J Hematol Oncol* 2022; **15**(1): 129 [PMID: 36076302 PMCID: PMC9461252 DOI: 10.1186/s13045-022-01347-8]

15 Jiang J, Li J, Zhou X, Zhao X, Huang B, Qin Y. Exosomes Regulate the Epithelial-Mesenchymal Transition in Cancer. *Front Oncol* 2022; **12**: 864980 [PMID: 35359397 PMCID: PMC8964004 DOI: 10.3389/fonc.2022.864980]

16 Kim H, Lee S, Shin E, Seong KM, Jin YW, Youn H, Youn B. The Emerging Roles of Exosomes as EMT Regulators in Cancer. *Cells* 2020; **9**(4) [PMID: 32252322 PMCID: PMC7226841 DOI: 10.3390/cells9040861]

3. It seems that the magnification is inconsistent in the Figure1A-D. It is suggested to add a scale in every micrograph.
Response: Thank you for your comments! We have added the scale in Figure1A-D.

4. "CASC20" was mentioned in the legend of Figure 1F, please check.
Response: Thank you for your reminding. We apologize for the mistake in the manuscript. We have made the corresponding corrections in the legend of Figure 1F.

We sincerely hope that this revised manuscript has addressed all your comments and suggestions. We appreciated for reviewers' warm work earnestly, and hope that the correction will meet with approval. Once again, thank you very much for your comments and suggestions.
Furthermore, we have had the manuscript polished with the help of editing service and have marked out in the revised manuscript.

Kind regards,
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