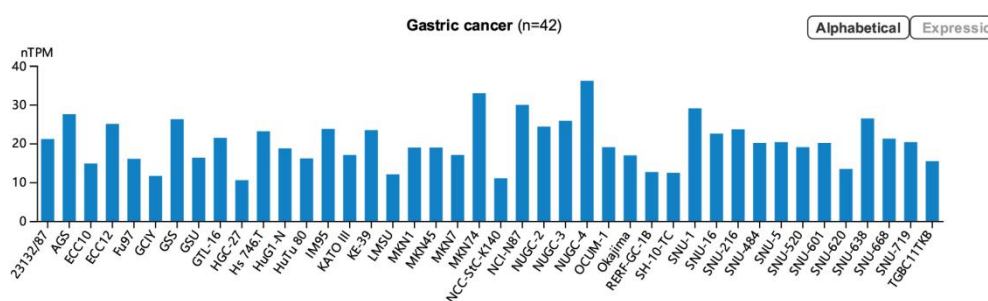# Summary letter

Reviewer #1: The authors investigate the effects of Tousled-like kinase 1 (TLK1) in gastric cancer in vitro and in vivo. While the role of TLK1 was described in other cancers, little is known for gastric cancer yet. Overall, the study is of interest but several points should be addressed and require a major revision:

1. The study uses only a small number of cell lines. It would be interesting to see results with cell lines expressing different levels of TLK1.

Response 1: Dear reviewer, thank you for your valuable advice. We acknowledge the reviewer's concern about the limited number of cell lines used. To address this, we referred to the Human Protein Atlas (HPA) to analyze the expression of TLK1 across various gastric cancer cell lines. This comprehensive evaluation is depicted in Summary letter Figure 1.
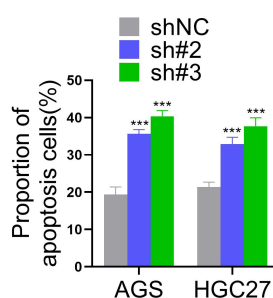
Summary letter Figure 1:

2. The labeling of the FACS data (Fig 4) needs to be improved. Please add % values

Response 2: We are grateful for the suggestion regarding the labeling of our FACS data. We have enhanced the clarity of Figure 4F by incorporating the "%" notation, as illustrated in Summary letter Figure 2.
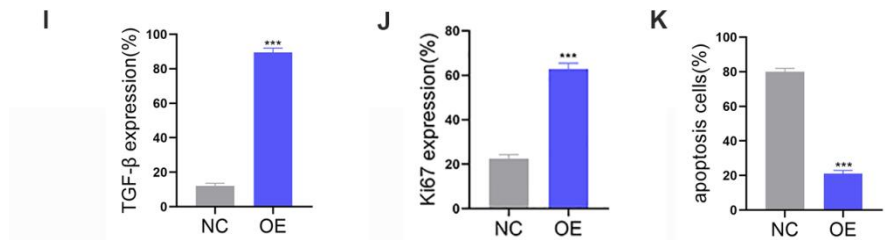
Summary letter Figure 2:

3. What happens when cells are treated with TGFb? this control is missing (Fig 5). What happens when TGFb is blocked (by antibodies) (Fig 6)?

Response 3: Dear reviewer, thank you for your valuable advice. We understand the importance of TGFb control in our experiments. While the current data might not directly influence our primary conclusions, we believe further research in this direction could be pivotal. In our future studies, we intend to utilize TGF-beta inhibitors and agonists to underscore the mechanism through which TLK1 modulates gastric cancer progression via the TGF-beta signaling pathway.

4. IHC data should be quantified

Response 4: In response to the recommendation to quantify our IHC data, we have conducted a quantification, as presented in Summary letter Figure 3. The respective modifications have been incorporated into the manuscript (Fig 6I-K).

Summary letter Figure 3:

5. Figure 1: explain abbreviations on axis of 1A.

Response 5: We are thankful for the insight regarding the abbreviations in Figure 1A. We have elucidated these abbreviations in the accompanying legend for clarity.
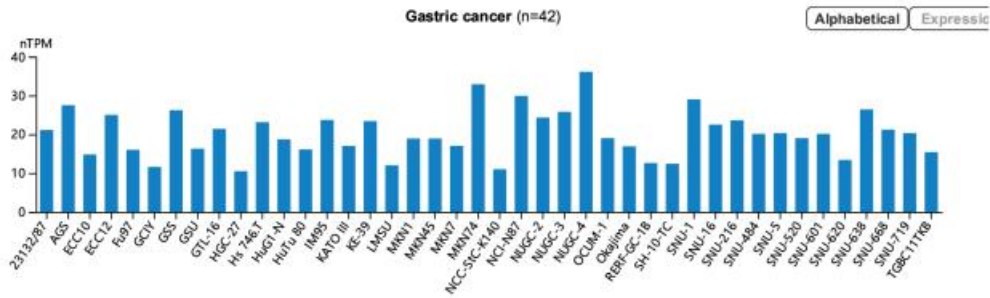
6. The human samples shown in Fig 1 are not described! IRB approval?

Response 6: We understand the importance of detailing our human sample sourcing. We have delineated the approval details in line 208 of the manuscript. The study was sanctioned by the Ethics Committee of Anhui Medical University. We have uploaded the pertinent approval documentation to the submission system for reference.
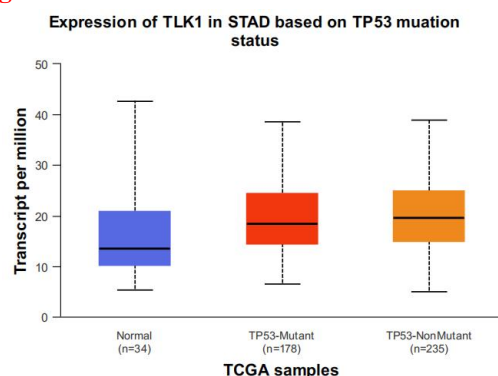
7. Only a limited number of cell lines is used (Fig 1). Why was the analysis not run against public databases like CCLE? Also, cell lines need to be better described, e.g. on p53 status, DNA repair systems etc as this is linked to TLK1.

Response 7: We thank the reviewer for highlighting the significance of utilizing public databases like CCLE. Unfortunately, due to ongoing updates in the CCLE database, we couldn't extract the relevant data. However, as previously mentioned, our reference to the HPA database (Summary letter Figure 1) should provide some comprehensive insights. Regarding the correlation between TLK1, p53, and DNA repair systems, our analysis of the TCGA database indicates no significant association between TLK1 and p53 in gastric cancer (Summary letter Figure 4). Additionally, pathway enrichment analysis did not identify a link between TLK1 and DNA repair (Summary letter Figure 5). Nevertheless, we will consider a deeper exploration of potential molecules associated with TLK1 in subsequent studies.
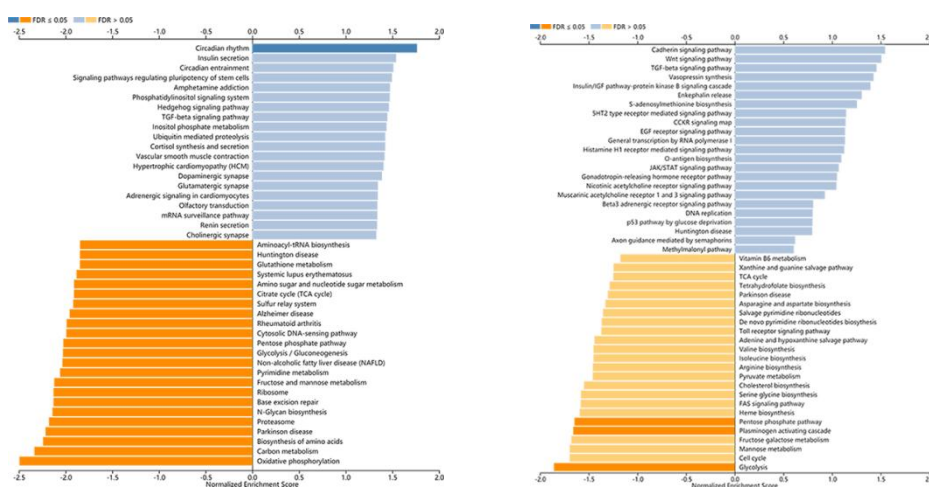
Summary letter Figure 1：

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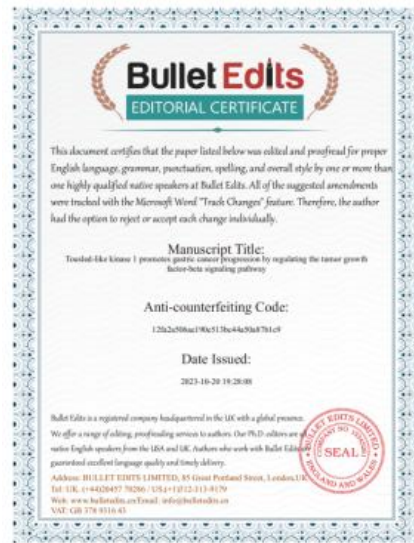**Expression of TLK1 in STAD based on TP53 muation status**

8. The densitometry analysis in Fig 1C does not match the blots in Fig 1B. Bands 1 and 3 should be <1 if data are relative to GAPDH

Response 8: We acknowledge the discrepancy noted by the reviewer. Our quantitative method entails computing the ratio of the TLK1 gray value to the GAPDH gray value, normalizing the ratio of GES-1 to 1.Thank you very much.

9. Minor points: The manuscript needs language and style revision. Words like "gamut" are unusual and sometimes sentences or phrases are repeated, e.g. lines 68 and 76. Please also avoid abbreviations in the abstract and explain them upon first use in the main text.

Response 9: We appreciate the feedback on the language and style of our manuscript. We have conducted thorough language editing, and the Language polishing certificate can be found in Summary letter Figure 6. Furthermore, we have rectified repeated phrases and clarified abbreviations as advised.

Reviewer #2: TLK1 promotes gastric cancer progression by regulating TGF-beta signaling pathway " by RuoChuan Sun et al. provides convincing evidence for a role of tousled-like kinase 1 in gastric cancer. The manuscript has to be carefully corrected.

1. Once an abbreviation is defined it should be used. Abbreviations in the title are not recommended. For example: EDU is not explained in the Abstract and TLK1 Is defined two times. "Gastric cancer (GC) ranks as the fifth most prevalent cancer worldwide and stands as the third leading cause of cancer-related mortality[1].Endoscopic interventions serve as the cornerstone for managing early-stage Gastric cancer (GC)," Again GC is defined 2 times. Various spaces are missing. The information regarding e.g. radiation resistance is repetitive. Please correct. Introduction again used abbreviations without explanation.

Response 10: We acknowledge the inconsistencies in the usage of abbreviations. We have now ensured that abbreviations, once defined, are consistently applied throughout the manuscript. Moreover, superfluous definitions have been eliminated for clarity.Thank you very much.

2. EdU or EDU, what is the correct writing?

Response 11: The appropriate notation is "EDU". We've amended our manuscript to reflect this consistency.

3. Mouse model, please indicate gender and age of the mice. How many animals were used. How many had to be killed before the end of the experiment?

Response 12: In response to the query about our mouse model, we utilized four-week old male nude mice. Each group comprised 6 mice, and there were no premature fatalities. We have incorporated this information into the methodology section (Lines 185).

4. "A p-value of ≤ 0.05 " should be "A p-value of <0.05

Response 13: We have corrected the p-value notation in the manuscript (Lines 204) as "<0.05".

5. What was the rationale to select AGS and HGC27 for sh experiments and SGC7901 for overexpression?

Response 14: We selected AGS and HGC27 cell lines for knockdown experiments due to their relatively high TLK1 expression. Conversely, the SGC7901 cell line, with its lower TLK1 expression, was chosen for overexpression studies.Thank you very much.

6. "demonstrated that TLK1 overexpression promoted SGC7901 cell proliferation, invasion, and migration (Fig. 2B-G). Similarly, analogous phenomena were observed in AGS and HGC27 GC cell lines" Please correct this sentence, there was no overexpression in these latter cell lines.

Response 15: We genuinely appreciate the reviewer pointing out the oversight. The sentence has been revised to: "Colony formation, EdU, and Transwell assays disclosed that TLK1 overexpression promoted SGC7901 cell proliferation, invasion, and migration (Figs. 2B – 2G). TLK1 knockdown had the opposite effects on AGS and HGC27 cell lines (Figs. 3 and 4)."Thank you very much.

7. Please explain GES-1 in legend of figure 1. Also explain the abbreviations of 1A.

Response 16: We have elaborated on GES-1 and other abbreviations in Figure 1A in the figure legend for clarity.Thank you very much.

8. In case that P<0.05 is not used in the figure it should be deleted from the figure legend.

Response 17: Notations in the figure legends have been updated to remove redundant or superfluous information.Thank you very much.

Reviewer #3:
Please provide molecular weights in western blot data.

Response 18: We appreciate the feedback and have now added the molecular weight (KD) for each band in Figures 1-6.

Please give a larger crop of the western blot so that the specificity of the antibody is addressed.

Response 19: To validate the antibody's specificity, we have presented extended immunoblot results for TLK1 overexpression, showcasing the full PVDF membrane in Summary letter Figure 7. Thank you very much.

Summary letter Figure 7 :