**A point to point answer to the reviewers comments**

Dear Editor,

Please find enclosed the revised manuscript in Word format (file name: 501-review.doc).

**Title:** **Notch2 regulates MMP9 via PI3K/AKT signaling in human gastric carcinoma cell MKN-45**

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**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 501

The manuscript has been considerably improved according to the suggestions of reviewers:

1. Format has been revised to conform the WJG requirements;

2. Revision has been made according to the suggestions of the reviewer

1. The acronym MMP9 has been defined.
2. Fig 1 D and Fig 4 were adjusted. Now p–AKT and MMP9 bars are distinctly visible.
3. Title has been altered to: Notch2 regulates MMP9 via PI3K/AKT signaling in human gastric carcinoma cell MKN-45
4. Histograms and WB were presented side by side for clarity.
5. The two sentences mentioned in the comments were corrected, please refer to the revised manuscript on pages XXX and YYY.
6. The optimal transfection efficiency was achieved with 25µM of Notch2 siRNA for 48h. However, this was not mentioned in the abstract for the sake of simplicity.
7. More details were added to the Material and Methods.
8. The reviewer’s comments “……Why the MMP9 activity is not the same at 24h and 48h for the controls Mock and siRNA Scramble?.....” were answered as follows: it is reasonable that MMP9 expressions at 2 different time points differ.
9. The reviewer’s comments “……The relevance of this cell line (MKN-45) to the current study is unclear,……”.

In our preliminary studies, Notch2 was found to be highly expressed in MKN-45 (but GES) cells, hence the use of knockdown approach.

1. Fig 1A aims to show the efficiency of transfection.
2. The reviewer’s comments: “There is an apparent 10x decrease in Notch2 mRNA but a much lesser decrease in protein. What other Notch are expressed in this cell line.” We examined other Notch receptors but only Notch2 was found to be distinctively silenced.
3. 48 hr after transfection was found to be the optimal time point in our preliminary experiments.
4. The percent transfection and knockdown was mentioned in this paper. Notch2 expression was dramatically decreased after Notch2 siRNA transfection (1.0000±0.0974% vs. 0.1161±0.0385%, p<0.01 by qRT-PCR).
5. “There is re-use of data from Figure 1 (the N2ICD bands and the B-actin are re-used, which is not allowable). Either combine this with the initial data OR repeat the experiment? How many times was this done?“

The same data for NICD and -actin were used in the Fig1C and Fig3B as the same blots were re-probed different proteins. All assays were performed in triplicate, and data analysis was performed using SPSS19.0. Comparison of the differences between the groups was performed using a one-way ANOVA followed by the Bonferroni correction.

3. References were updated and all typos were corrected.

The authors thank the editors and reviewers for reviewing this manuscript.

Sincerely yours,



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