Author's response to reviews

**Title:** SOCS3 inhibiting migration of A549 cells correlates with PYK2 signaling in vitro.

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**Author's response to reviews:** see over
Dear Editors,

Thank you for your letter. We are very pleased to learn that our manuscript is suitable for publication in BMC Cancer with some critical revisions. We have worked hard to ensure that our revised manuscript conforms to the journal style and our files are correctly formatted as possible as we can. We are sure that the processing charge of £850.00 will be payable if the article is accepted.

We found the reviewers’ comments quite helpful and revised the manuscript in detail. We have completed the revised manuscript according to the reviewers’ recommendations, and checked the manuscript carefully. We greatly appreciate both your help and that of the reviewers concerning improvement to this manuscript. We wish that the revised manuscript is now acceptable for publication. Moreover, we’ll keep the manuscript being revised until you’re satisfied with it. Thank you very much! If there are any more problems, please contact me without hesitation.

Sincerely yours,
Xueshan Qiu

The point-by-point response to the reviewers is shown as follows:

To dear Prof. Thomas Gudermann: Thank you for your detailed suggestions.

Major compulsory revisions:

1) The western blot data were analyzed statistically and represented as mean±SD of three independent experiments, and the p-values were indicated in the corresponding sections of the revised manuscript, and highlighted in red. The densitometric scanning and statistical analysis have been completed before we wrote the manuscript. The densitometric scanning and statistical analysis of each band were shown in Additional file 1. Therefore the data are credible.

2) We have previously demonstrated that the methylation-associated down-regulation of SOCS3 expression was a general phenomenon in lung cancer tissues and in A549, LH7 and BE1 (pulmonary giant cell lines with low and high metastatic potentials respectively) cells. We will carry on further experiments on methylation status and expression of SOCS3 in some other
lung cancer cell lines. The methylation status of SOCS3 in HBE, A549, LH7 and BE1 cells was shown in Additional file 2. We utilized A549 cells to perform the subsequent experiments, because A549 cells are easy to grow in vitro and get stable results with a high transfection efficiency.

3) We considered that the antibodies used in the experiments were essentially specific, although most of them are polyclonal, and some irrelevant bands were detected in the preliminary experiments. Therefore we took some measures to solve the problem: 1) more than three independent experiments were performed; 2) the molecular weight markers were used; 3) the membranes were blocked with 5% BSA for 4 hours or even longer; 4) the membranes were cropped covering only a narrow area of the gel according to the molecular weight markers, and a small section of proteins were transferred to the membranes. No irrelevant bands were detected in the cropped membranes, as the figures showed.

4) The “p-value of 0.000” is a result provided by the SPSS 13.0 software directly. We mean “p<0.05” actually, which was highlighted in red in the revised manuscript. We are sorry to make your misunderstanding.

5) There is no effect on PYK2 mRNA levels after expression of the SOCS-box construct, as we previously demonstrated by RT-PCR and have shown in the revised Fig.6. The results were omitted because PYK2 mRNA levels were unchanged no matter the SOCS-box construct was transfected or not. There is no impact of β-lactacystin pretreatment upon transfection of the SOCS-box construct, as we previously demonstrated and have shown in the revised Fig.6. The results were also omitted because PYK2 expression, Tyr402 and ERK1/2 phosphorylations were unchanged with β-lactacystin pretreatment.

6) We are sorry to explain that we have no wild-type myc-tagged SOCS3 construct, because of our limited funds and techniques. Therefore the experiment of wild-type SOCS3 transfection was not included. However, we will make great efforts to have this portion supplemented in future researches. Detected SOCS3 mutants with molecular weight markers have been shown in the revised Fig.7, as professor Thomas Gudermann suggested. We feel very sorry about the typographical error in the first lane because of our carelessness, which have already been corrected.

7) We were confused by the results for a long time as well and we have repeated the experiment of immunoprecipitation for five times and got the same results. Therefore we explained that “the
roles of SOCS3-KIR mutant were observed definitely, however the potential interaction as we presumed between the exogenous SOCS3-KIR mutant and PYK2 was not detected. We supposed that SOCS3-KIR mutant interacted with PYK2 principally by the central SH2 domain, generating the inhibition effects, however the binding was a functional interaction, unstable and readily reversible, and the effects would be transient without the help of KIR domain, thus could not be detected by immunoprecipitation in vitro. Therefore we concluded that the KIR domain is possibly required for a more stable binding of SOCS3 with PYK2 based on the SH2 domain. Further investigation is required to demonstrate our point of view”, which was included in the revised manuscript and highlighted in red.

Minor essential revisions:

8) The constructs depicted in Fig.4 have been explained in more detail and supplemented in the text and in the corresponding figure legend, which were highlighted in red.

9) We are so sorry about the typographical and grammatical errors in the text, and we have discovered and revised the errors as possible as we can. We hope that the revised manuscript would be acceptable.

To dear Prof. Donatella Tramontano: Thank you for your constructive recommendations.

Minor essential revisions:

(1) We are so sorry about the typographical and grammatical errors in the text, and we have found and revised the errors as possible as we can. Some paragraphs of the text were simplified or abandoned as professor Donatella Tramontano suggested. We hope that the revised manuscript would be accepted.

(2) We have searched PubMed to update the references in the specific field of “PYK2 and migration” and supplemented necessary references as [6], [9] and [10], which was included in the revised manuscript and highlighted in red.