Reviewer's report

Title: SOCS3 inhibiting migration of A549 cells correlates with PYK2 signaling

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Reviewer: Thomas Gudermann

Reviewer's report:

In the present paper the authors address the question whether the calcium-activated tyrosine kinase Pyk2 is regulated by SOCS3. The authors show that SOCS3 expression is reduced in a lung adenocarcinoma cell line because of methylation within exon 2. Up-regulation of SOCS3 inhibits Pyk2 signalling and Pyk2-dependent migration of lung carcinoma cells.

Major compulsory revisions:

(1) The authors have to provide a statistical analysis of the Western blot data shown in Fig. 1a as well as in all the other figures shown. If several independent experiments were performed, densitometric scanning and statistical analysis should be possible. Without any proof of reproducibility, the data are not convincing.

(2) Is methylation-associated down-regulation of SOCS3 expression a specific feature of A549 cells or is this a more general phenomenon? The authors should analyse additional lung cancer cells or cell lines to substantiate their conclusion.

(3) How specific are the antibodies used, for instance in Fig. 2a. The authors should show a larger portion of the Western blots and not only a small section. Were any other bands detected?

(4) On several occasions in the text statistical significance is indicated by a p-value of 0.000. What does that mean?

(5) It is difficult to compare Figures 5 and 6 because necessary control experiments are missing in Fig. 6. Is there any effect on Pyk2 mRNA levels after expression of the SOCS-box construct? What is the impact of lactacystin treatment upon transfection of the SOCS-box construct?

(6) In Fig. 7 the authors show by an immunoprecipitation approach that the SH2 and the KIR region are essential for SOCS3 to interact with Pyk2. How is the situation if wild-type myc-tagged SOCS3 is expressed? Molecular weight markers should be indicated in Fig.7 and the typographical error in the first lane has to be corrected.

(7) Fig. 7 shows that the SOCS3-KIR construct does not interact with Pyk2. However, Fig. 5 is interpreted to show increased proteasome-mediated degradation of Pyk2 in response to SOCS-KIR expression. How can that be explained if Pyk2 and the SOCS3-KIR construct fail to interact physically?

Minor essential revisions:
(8) The constructs depicted in Fig. 4 have to be explained in more detail in the text and in the corresponding figure legend.

(9) There are lots of typographical and grammatical errors in the text. The manuscript desperately needs language editing by a native speaker.

**What next?**: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

**Level of interest**: An article of importance in its field

**Quality of written English**: Needs some language corrections before being published

**Statistical review**: No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests**:

I declare that I have no competing interests.