Author's response to reviews

Title: LATS2 is De-methylated and Overexpressed in Nasopharyngeal Carcinoma and Predicts Poor Prognosis of the Patients

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Author's response to reviews: see over
Dear Editor:

We would like to appreciate the reviewers for their review to our manuscript. We have carefully evaluated the reviewers’ critical comments and thoughtful suggestions, responded to these suggestions point-by-point, and revised the manuscript accordingly. All changes made to the text are in blue color so that they may be easily identified. With regard to the reviewers’ comments and suggestions, we reply as follows:

**Answer to Reviewer (Hiroshi Nojima)**

**Criticism #2**

*In terms of Lats2 function in cancer cells, the authors should not ignore the contribution of the Hippo pathway to the regulation of tumor growth. It is important to examine, for example, the phosphorylation levels of the Lats2 target protein such as YAP, using Phospho-YAP (Ser127) antibody, which is one of the key players of the Hippo pathway. Since this is an easy and essential experiment, the authors should perform it I would suggest to purchase from, for example, Cell Signaling Company the anti phospho-YAP (Ser127) antibody and the anti-YAP antibody to perform immunohistchemistry using the patients’ samples.*

As shown in Additional file 2 in the revised manuscript, expression of p-YAP (Ser127) #4911, Cell Signaling Technology was examined by immunohistochemistry in 122 nasopharyngeal carcinoma (NPC). p-YAP was detected in both cytoplasm and nuclear of NPC tumor cells, as well as of
nasopharyngeal epithelium. Kaplan-Meier survival analysis revealed that p-YAP expression was not correlated to the patients survival (log-rank test, $X^2 = 0.519, P = 0.471$). When the patients were stratified by clinical stage, no differences were found in overall survival between low level and high level p-YAP expression in NPC patients with early stage and advanced stage. Spearman’s correlation analysis revealed that LATS2 expression in NPC tumor was not correlated with expression of p-YAP (Spearman’s correlation coefficient $r = 0.146, p = 0.109$).

Criticism #4

However, no data are presented that confirm the authenticity of the immunostained signals as Lats2 itself. The authors should perform peptide competition in immunohistochemistry and siRNA mediated LATS2 knockdown in the cell lines and confirm the disappearance of these signals in order to show that these antibody signals are not derived from background or nonspecific recognition of other proteins. This query is posed because Lats2 localizes in the nucleus only during M phase, but these cells are mainly at the interphase. It seems to me that these immunostained images are not from Lats2 proteins.

Fig.4C is western blot. I am talking about the immunohistchemistry. If the authors have siRNA samples, it is an easy experiment to perform it using the same set of samples.

As shown in Additional file 1 in the revised manuscript, consecutive sections and 5-8F cells were incubated either in LATS2 primary antibody or with
a non-immunized goat IgG antibody to confirm the specificity of the primary antibodies. LATS2 staining revealed that overexpression of LATS2 was observed in both the nuclear and cytoplasm of the NPC tumor cell and 5-8F cell line. Whereas no immunostaining was detected in NPC tumor tissue and 5-8F cell line which were incubated with a non-immunized goat IgG antibody. Further immunohistochemistry staining revealed that remarkably decreased expression of LATS2 in nuclear of 5-8F cells which were transfected with 25nM and 50nM LATS2 siRNA1, and no expression of LATS2 was observed in 5-8F cells treated with high concentration of 75nM LATS2 siRNA1. These results suggested that the positive signals should be specific for LATS2 protein, and siRNA mediated LATS2 knockdown in the 5-8F cells resulted in reduction or disappearance of the signals.

**Answer to Reviewer (Miho Ohsugi):**

**Minor Essential Revisions**

_in the point-by-point response to my comment #4, although they stated that they referred red Figures 2C-2F in consecutive order, but actually they didn't. Authors should interchange Figure 2C with 2D, and 2E with 2F to referer the figures consecutive order._

We thank the reviewer for the suggestion. Fig.2C, Fig.2D, Fig.2E and Fig.2F were referred to in the text in consecutive order._