Reviewer's report

Title: STAMP alters the growth of transformed and ovarian cancer cells

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Reviewer: Kylie Louise Gorringe

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The authors have clarified their paper considerably, and it now makes more sense to me. The additional changes I believe are essential for publication are given below. I don’t think any of these are major – just technical details for the reader’s clarification.

In brief:
- Give full QPCR data for the ovarian panels as a supplementary table (points 3, 4 below)
- Give full % knockdown data on a per cell line basis in Table 1B (points 5, 7 below)
- Some small text changes (points 1, 2, 6 below)

1. Going back to my previous point 3 regarding the QPCR data, I still think the fact that each plate was analysed one time should be explicitly stated in the methods. How about on page 8, line 14 saying “Quantitative real-time PCR (qRT-PCR) was run once for each panel using validated…etc”

2. On page 11 the authors now use the term “endometrial ovarian cancer”. This is incorrect. Endometrial cancer is entirely different from ovarian cancer of the endometrioid subtype. I would just remove the term “endometrial”. I downloaded the Origene data for the 2 ovarian cancer panels and the authors are mistaken – the panels definitely contain a variety of ovarian cancer subtypes, mostly serous (as expected) and with a smaller number of endometrioid and mucinous subtypes. This information is given in the column “diagnosis” and in the pathology reports. E.g. CU000001506 is “Adenocarcinoma of ovary, serous” grade 3, stage III.

3. I still think that the subtype of the tumours is important, however, I don’t feel the manuscript text needs to be changed necessarily. What I would very strongly suggest, to make the paper stronger and more likely to be cited, is to include as a supplementary file the QPCR data from the ovarian panel (and perhaps the others as well?). All this would require is a table with the columns B-J of the Origene “HORT” excel files (i.e. well, position, age, tissue, appearance, diagnosis, grade and stage) plus the QPCR result eg. Ct value. This would allow the reader to further analyse the data themselves to extract the subtype/grade-specific information. This should be very simple for the authors to do as they will have all the data available.
4. Further to this, I can’t quite add up the same numbers as the authors for Figure 2B – In “HORT101” and “HORT201” files that I downloaded from Origene, there are 96 samples listed, but the authors only analysed 83 – did some of these not work? In particular the LMP tumours drop from 9 to 3. Given that each well should have the same amount of cDNA how do you exclude samples without a Ct value when it might mean there is no STAMP present at all, rather than the reaction not working? I’m not actually sure what the correct thing is to do here, because possibly giving these samples a “0” value is inappropriate as well, maybe they really did just not work. I think this illustrates the problems when you can only run each sample once and don’t have a positive expression control in your own hands. The only thing I can suggest is that of giving the full data so readers have access to all the information as stated above in point 3. This is also relevant in terms of subtype – if 2/3 LMP that are included happen to be the mucinous ones, then the interpretation of difference between LMP and malignant (mostly serous) is suspect.

5. For the cell line QPCR data, this is somewhat clearer for me, I now understand that there are two values: a “relative” i.e. % knockdown per cell line, and an “absolute” i.e. amount of RNA present (relative to HeyA8) to enable cross-cell line comparisons. However, I think both of these values should be given in Table 1B. Again this should be very simple for the authors to do as it just means adding in a single line to the table – call one “relative level” and one “absolute level” and then this will correlate with how the data is described in the text. This will enable the reader to interpret the relative knockdown for each cell line itself rather than the average value given in the text.

6. Again for the cell line QPCR data, I think the authors have misunderstood me. In the legend to table 1 they state:

[cell A STAMP mRNA with STAMP siRNA]/[cell A STAMP mRNA with Lamin siRNA]x[cell A STAMP mRNA with Lamin siRNA]/[HeyA8 cell STAMP mRNA with Lamin siRNA]).

If [cell A STAMP mRNA with STAMP siRNA] = a
[cell A STAMP mRNA with Lamin siRNA] = b
[HeyA8 cell STAMP mRNA with Lamin siRNA] = c

Then the authors are calculating a/b x b/c. My school algebra would then tell me that “b” cancels out and the authors are calculating a/c.

I think as it is described in the text makes sense now but the legend should be clarified.

7. Finally, on page 12 the average value of the % knockdown is given as n=21. Presumably this is 7 cell lines x three replicates. However, wouldn’t it be better to average the replicates first and then give the value for n=7? If they all have the same number of replicates it won’t matter for the average but it does make a difference for the standard deviation. This also would be solved by showing the
data for each cell line as suggested above.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**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'