Reviewer's report

Title: Proliferation, invasion and activation of cAMP response element binding protein (CREB) through the A2B adenosine receptor in trophoblast cells

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Reviewer: Tanja Groten

Reviewer's report:

Darashchonak and colleagues describe the role of A2B adenosine receptor in a trophoblast cell line. They investigated changes in proliferation, invasion and activation of CREB as functions of the A2B adenosine receptor. Hypoxia, A2B agonist and blocking of activation by an A2B antagonist showed effects on the A2B signaling and trophoblast cell function. The conclusion drawn by the authors reveal a functional role of the A2B in trophoblast reaction to different oxygen concentrations. The study is innovative and of big interest for the research field of impaired trophoblast invasion in pregnancy. However, the study could benefit from small changes.

Specific comments:

1 The title should be changed to clarify the content of the study.

2 Abstract: The result part of the abstract has to be reworded to clarify what is normoxia and what hypoxia (2%, 8%, 21%??)

3 In the abstract and the introduction: either omit to draw the interrelation to preeclampsia or connect the study to intrauterine growth retardation as well. The subject of the study is to explain an important mechanism involved in impaired trophoblast invasion in response to hypoxic conditions. Hypoxia and shallow trophoblast invasion is imperatively related to intrauterine growth restriction and also (but not imperatively) to preeclampsia. The introduction should be reworded to be precise on this issue. The reviewer is aware that data on adenosine and iugr do not exist so far, but probably such data would be of big interest and the paper could give big impact on this. The paper should comment on this.

4 The Method section is straightforward.

5 Results:

1 In general: why sometimes showing the 8% results and sometimes not. May be the clarity of all figures would benefit from omitting the 8% results.

2 It would be nice to see the results for cAMP levels in response to different O2 concentrations in figure 2 to compare the effect of the agonist to the effect of hypoxia.

3 Figure 3 is somehow confusing. Each bar graph pair is normalized to a different
untreated control (by 2, 8 or 21%). This is confusing. What are the differences in proliferation between the three oxygen concentrations? Does activation of the receptor with the agonist bring proliferation back to the level seen in untreated conditions under normoxia? What exactly is the control for C and D? ...untreated with NECA and H89? What is the effect of NECA and H 89 alone compared to normoxic conditions...

4 In figure 4 change the order of B and C.

5 Figure 5, for B and C see comment to figure 3, one graph and different controls?

6 Discussion, again do not over-interpret the relation of the findings to preeclampsia.
Trophoblast invasion is a feature important for multiple pregnancy disorders, preeclampsia might be one of them.

**Level of interest:** An article of outstanding merit and interest in its field

**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'