Author's response to reviews

**Title:** NCOA3 is a selective co-activator of estrogen receptor alpha-mediated transactivation of PLAC1 in MCF-7 breast cancer cells

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

Thank you for considering the manuscript MS: 9112983431062817 entitled “NCOA3 is a selective co-activator of estrogen receptor α - mediated transactivation of PLAC1 in MCF-7 breast cancer cells” for publication in BMC Cancer. Please find enclosed the revised version of our manuscript.

We greatly appreciate and thank the reviewers for their valuable comments and helpful suggestions which will improve the importance of our manuscript. We have revised the manuscript according to the reviewer’s recommendations and addressed each of the concerns raised by the reviewers in the point-to-point reply below.

The rewritten passages are highlighted in red in the revised manuscript.

Reviewer 1 (Ramaiah Nagaraja)
The manuscript by Wagner et al., describes the involvement of cofactors that interact with ER alpha mediated activation of P2 promoter of PLAC1. The study shows that SRC-3 (NCOA3) involvement in the activation of the promoter and associated changes in chromatin modification to a transcriptionally active state and provides evidence for recruitment of transcription activation complex at P2 promoter in MCF-7 breast cancer cells. The study provides additional clues to the activation of PLAC1 by yet another route and defines how the two promoters (P1 and P2) differ in their activation.

PLAC1 is repressed in all normal cells other than placenta. The study uses standard experimental controls and generally accepted techniques.

Minor essential revisions:
1) Transcription from P1 promoter in primary cells was shown recently to be repressed by p53 (Oncogenesis, 2013 Sep 2;2:e67) and the promoter exists in closed chromatin configuration and was demonstrated to be activated by loss of inhibitory chromatin modifications and in the presence of nuclear receptor agonists for RXR# and LXR further activation could be achieved by RB interaction with NCOA2 (SRC-2). Thus both NCOA2 and NCOA3 here seem to play an important role in activation...
under different circumstances in activating P1 or P2 promoters. In light of these observations it would be relevant for the authors to rework their discussion section.

Thank you for this helpful comment, of course the results of this report have to be considered in our manuscript. We have included the recent results of this report in our discussion section.

2) I suggest that the authors should preferably use the current HGNC approved nomenclature for the genes. For example SRC-1, 2, 3 are renamed as Nuclear Receptor Co-activator 1, 2 and 3 respectively (NCOA1, 2 and 3).

We have included the HGNC approved nomenclature NCOA1, 2 and 3 for SRC-1,-2 and -3 in our manuscript accordingly.

3) Do the authors think it is possible to derepress PLAC1 P2 promoter in primary cells by coexpression of ER alpha and NCOA3? It would give us clues regarding how the gene is repressed and if it could be overcome in non-placental tissues (cells).

Co-expression of ER alpha and NCOA3 seems to not play a role in the derepression of PLAC1 P2 promoter. First, PLAC1 expression can be found also in breast cancer cell lines, like SKBR3, which are ER negative. Second, introduction of C/EBPβ-2-ivt RNA alone was sufficient to induce PLAC1 expression in HMEC (human mammary epithelial cells) which are considered to be ER “poor” (Dietze et al., 2004, DOI:10.1038/sj.onc.1207480 and Koslowski et al, 2009, DOI: 10.1074/jbc.M109.031120). We found enhanced recruitment of C/EBPβ-2 to PLAC1 promoter P2 after E2-treatment (Koslowski et al, 2009, DOI: 10.1074/jbc.M109.031120). E2-activated ER alpha presumably associates with C/EBPβ-2, bridging this factor to SP1 and favoring interaction of C/EBPβ-2 with its cognate element. Thus, in cells expressing C/EBPβ-2, co-expression of ER alpha and NCOA3 might enhance the expression of PLAC1 but these two factors are not required to derepress PLAC1 P2 promoter. If regulation of the P2 promoter is attributable only to tissues specific transcription factors or if additional derepression factor are needed, remains to be clarified.

Reviewer 2 (Michael Fant)

Critique: The manuscript by Wagner et al provides convincing evidence that SRC-3 is a transcriptional co-activator of ER alpha mediated trans-activation of Plac1 in breast cancer. These data provide important new insights into the regulatory mechanisms involved in some breast cancers and thus could have significant prognostic and/or therapeutic implications. The approach, techniques, and data are sound. The results are convincing and clearly articulated.

Minor Essential Revisions:
1. Figure 1: The figure should indicate that the differences are statistically significant and the test used. This is stated in the text but not indicated in the figure.

We have included a statistical analysis in Figure 1 A and stated in the figure legend that we used a Student’s t-Test. The differences between NCOA3 expression compared to NCOA1 and 2 is statistically significant in MCF-7 but not in SK-BR-3. This was changed in the text accordingly.

2. In the Abstract and Background the authors state that PLAC1 is “strictly confined” to the differentiated trophoblast. This text should be revised to reflect a recent report by Kong et al (Birth Defects Research -Part A: Clinical and Molecular Teratology, 97(9):571-7. doi:10.1002/bdra.23171.)
demonstrating that Plac1 is also expressed in embryonic tissues where it has a significant effect on brain development. This does not impact the significance of findings in this manuscript but more accurately represents PLAC1’s important role in embryonic development as well as cancer biology.

Thank you for this valuable comment, at the time of submission we were not aware of this report and of course it has to be cited in our manuscript. We have included the very important findings that Plac1 is widely expressed in fetal tissues especially the brain and is suggested to play a major role in fetal brain development in the abstract as well as the background sections of our manuscript.

3. The word "placental" should be removed from the title. It is an inaccurate descriptor in the context of this study of breast cancer.

That is right, thank you for this comment. We have removed “placental” from the title accordingly.

Thank you for considering our resubmission. We hope that our manuscript will now be deemed acceptable for publication in *BMC Cancer*.

Yours sincerely,

Prof. Dr. Ugur Sahin