

Peer Review File

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Reviewer A

Fetal growth restriction (FGR) is one of the most common fetal complications during pregnancy in the obstetrics department, with poor therapeutic efficacy. The local inflammatory response of the placenta has gradually become known as the main mechanism for the occurrence and development of FGR. In the manuscript “NCOA2-induced secretion of leptin leads to fetal growth restriction via the NF- κ B signaling pathway”, authors investigated the potential role and regulatory functions and mechanisms of NCOA2 in HTR-8/SVneo trophoblast cells. Couple questions are required to be answered before it will be accepted.

(1) What were the correlations between leptin and inflammation? Please state in the introduction.

Reply: Thank you for your advice. We have modified our manuscript as advised to add introduction. (see line 94-110).

(2) What were the main causes leading to fetal growth restriction? Please state in the introduction.

Reply: Thank you for your advice. We have modified our manuscript as advised to add introduction. (see line 73-79).

(3) In the introduction, please supplement related references about the biological roles of leptin, such as (Ann Transl Med. 2021 Apr;9(7):568).

Reply: Thank you for your advice. We have modified our manuscript as advised to add introduction. (see line 94-110).

(4) Please check the HTR-8/SVneo trophoblast cells were cultured in medium with 5% FBS.

Reply: We have examined and confirmed the culture conditions of Human chorionic trophoblast cell line (HTR-8/SVneo cell) was maintained in Roswell Park Memorial Institute (RPMI) 1640 with 5% fetal bovine serum (FBS), 1% penicillin and streptomycin at 37 °C and 5% CO₂.

(5) What was the meaning of “Janpan” in the methods? Please state clearly.

Reply: We have checked the use of this term and this is a supplementary description of where the kit was made

(6) In the figure legends, please supplement the full-name of abbreviations in the figures, such as “OE”.

Reply: Thank you for your advice. We have modified our manuscript as advised to add

introduction. (see line 606-607, and 625-626).

(7) What were the roles of migration or invasion of trophoblast cell in the FGR? Please state in the discussion.

Reply: Thank you for your advice. We have modified our manuscript as advised to add introduction. (see line 402-406).

(8) In the MDAR-checklist, if the item was not applicable, please fill in the n/a.

Reply: Thank you for your advice. We have modified the MDAR-checklist and submitted it again.

Reviewer B

1. Ethical Statement

For research involving human, the article should include

1) a statement that ethical approval was obtained (or a statement that it was not required and why), including the name of the ethics committee(s) or institutional review board(s), the number/ID of the approval(s),

Reply: It is provided in the manuscript. (see in manuscript methods section)

2) and a statement that the participants gave informed consent before taking part (or a statement that it was not required and why).

Reply: It is provided in the manuscript. (see in manuscript methods section)

3) Authors should also state that the study conformed to the provisions of the Declaration of Helsinki (as revised in 2013).

Reply: Thank you for your advice. We have modified the manuscript. (line 179-180)

2. Figure 1F, Figure 1G and Figure 3F were not cited in the main text. Please check.

Reply: Thank you for your advice. We have modified the manuscript.

3. Please indicate the full name of "PE". All abbreviated terms should be full when they first appear.

103 [Leptin is considered to be a molecular marker for the occurrence and development of](#)
104 [PE and FGR, and the increase of its blood level is positively correlated with the](#)
105 [occurrence and severity of FGR and PE \(11-12\). In Mirthe H Schoots et al., Leptin can](#)

Reply: Thank you for your advice. We have modified the manuscript.

4. Please check the below sentence. What is 2013? The publication year of reference 2 is 2009. Why 2013 appears here.

63 intrauterine growth restriction (IUGR) (1). FGR is the second leading cause of perinatal
64 death worldwide with an incidence rate in China of 6.39% (2013) (2). FGR is an

Reply: Thank you for your advice. We have modified the manuscript.

5. Please check if any more references need to be added in the below 3 sentences since you mentioned “Studies”, but only one reference was cited. If not, “studies” should be changed to “a study/a previous study”.

351 Previous studies have shown that LPS induces the activation of NF- κ B signaling
352 pathways which lead to an inflammatory response (25). Recent studies have uncovered
353 the important role of the NF- κ B signaling pathways in regulating inflammation during
354 the dysfunction of trophoblast cells (26). In addition, leptin has been shown to induce
82 Studies have found that the levels of pro-inflammatory cytokines, such as interleukin-
83 8 (IL-8), interferon- γ (IFN- γ), and tumor necrosis factor- α (TNF- α), in the peripheral
84 blood of patients with FGR caused by placental dysfunction are increased (8). These

Reply: Thank you for your advice. We have modified the manuscript.

6. Please check “Janpan” below. Should it be “Japan”?

204 ##Cell Counting Kit-8 (CCK-8) assay
205 CCK-8 analyses was performed according to the manufacturer’s instructions. Briefly,
206 cells were harvested in different time points and incubated for 1 hour at 37 C° with 10
207 μ L CCK-8 reagent (Dojindo, Kumamoto, Janpan). The optical density (OD) value was

Reply: Thank you for your advice. Yes, it should be JAPAN and we have modified the manuscript.

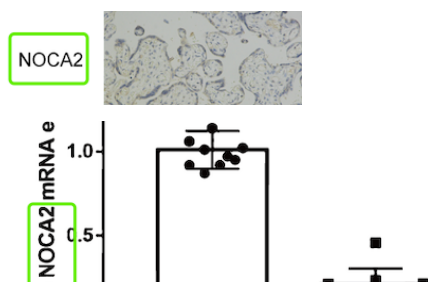
7. Please check below data in your main text. It’s 58 in your Figure 1C.

348 Co-expression analysis was performed to identify the co-expressed DEGs.
349 According to Venn analysis, 57 co-expressed up-regulated DEGs (Figure 1C) and 18

Reply: Thank you for your advice. We have modified the manuscript.

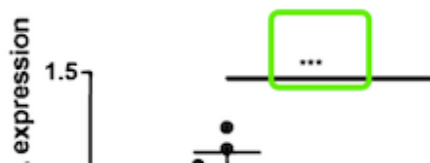
9. Figure 2:

1) Please revise all “NOCA2” to “NCOA2”.



Reply: Thank you for your advice. We have modified the figure.

2) Please indicate the meaning of *** in the legend.



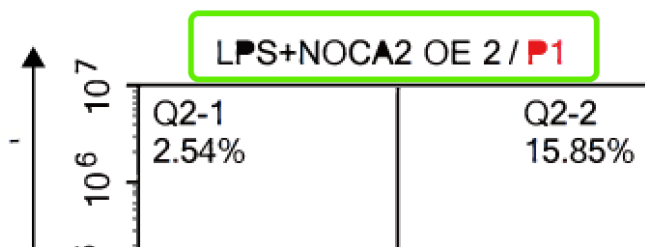
Reply: Thank you for your advice. We have modified the manuscript.

3) Please indicate the full name of “qPCR” in the legend.

Reply: Thank you for your advice. We have modified the manuscript.

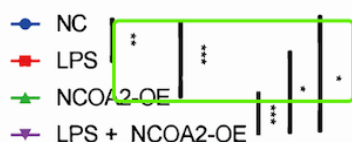
10. Figure 3:

1) The words are not clear in Figure 3C. Please revise.



Reply: Thank you for your advice. We have modified the figure.

2) Please indicate the meaning of *, **, ***, ns in the legend.



Reply: Thank you for your advice. We have modified the figure.

3) Please indicate the staining method and magnification for cell maps in Figure 3B and D legend.

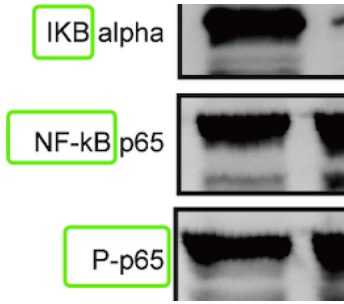
Reply: Thank you for your advice. We have modified the manuscript.

4) Please indicate the full name of “OD”, “NC”, “OE”, “IL” in the legend.

Reply: Thank you for your advice. We have modified the manuscript.

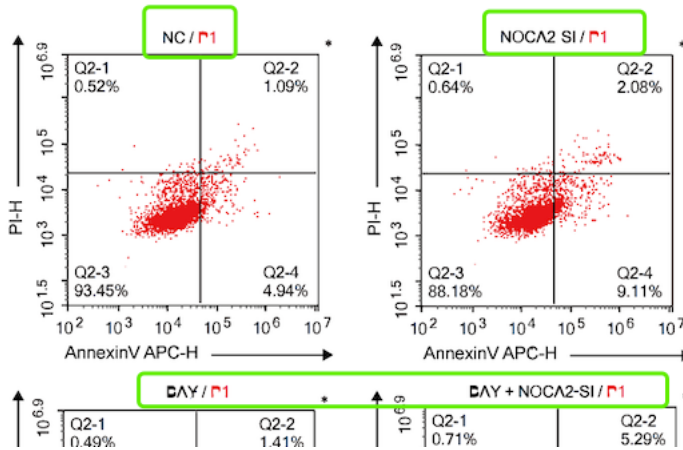
11. Figure 4:

1) Please revise all below 3 words to “IκB, NF-κB, p-NF-κB/p65” in Figure 4A and B.



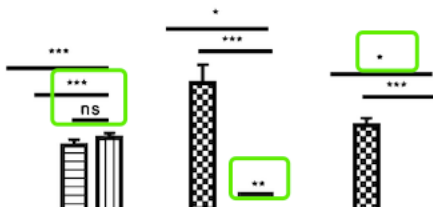
Reply: Thank you for your advice. We have modified the figure.

2) The words are not clear in Figure 4D. Please revise.



Reply: Thank you for your advice. We have modified the figure.

3) Please indicate the meaning of *, **, ***, ns in the legend.



Reply: Thank you for your advice. We have modified the figure.

4) Please indicate the staining method and magnification for cell maps in Figure 4C and E legend.

Reply: Thank you for your advice. We have modified the manuscript.

5) Please indicate the full name of “OD”, “NC”, “OE”, “ELISA” in the legend.

Reply: Thank you for your advice. We have modified the manuscript.

6) Please check the below sentence in your main text. It looks like its meaning is inconsistent with your Figure 4F and G.

376 proliferation and migration, and inhibited apoptosis (Figure 4B-4E). Moreover, the
377 level of IL-1 β and IL-6 was significantly decreased (Figure 4F) whereas the secretion
378 of leptin was increased (Figure 4G) in *NCOA2* knockdown HTR-8/SVneo cells,

Reply: Thank you for your advice. We have modified the manuscript.