Dear Editor-in-Chief:

Thank you very much for your letter and advice. We have revised the manuscript, and would like to re-submit it for your consideration. We have addressed the comments raised by the reviewers, and the amendments are highlighted in red in the revised manuscript. Point by point responses to the reviewers' comments are listed below this letter.

We hope that the revised version of the manuscript is now acceptable for publication in your journal.

I look forward to hearing from you soon.

Sincerely yours

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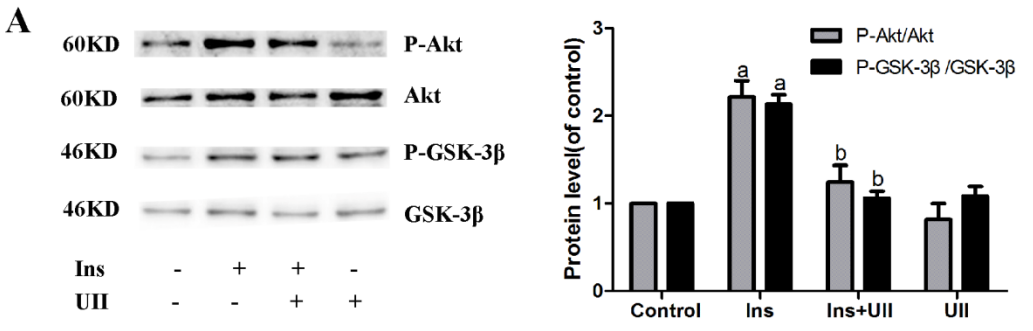
we would like to express our sincere thanks to the reviewers for the constructive and positive comments.

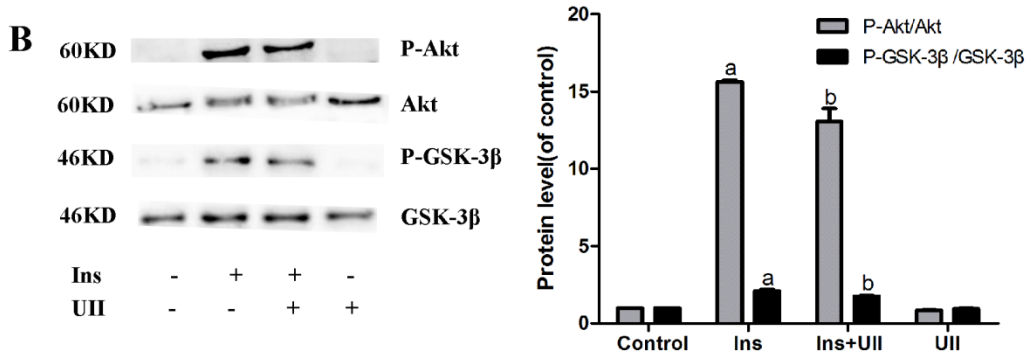
Replies to Reviewer ***00289402***

Specific Comments

1. HepG2 cell line itself is not a very good model for physiological studies, including the glucose consumption and glycogen synthesis. The overall conclusion of this study would be significantly strengthened by examining the regulatory effect of Urotensin II at transcriptional level.

Answers: HepG2 cell line, phenotypically similar to human hepatocytes, was frequently used *in vitro* system for studying insulin resistance on hepatic cells. This reference has been quoted as ref.14, 17, 18, 24 et al. in the revised version. Meanwhile, we validate the consistent key effects of urotensin II on an independent hepatocyte-derived cell line, HuH7, and another hepatic cell line, BEL-7402 as follows supporting figure 1 based on the reviewers' constructive advice. Moreover the regulatory effect of urotensin II at transcriptional level will be further studied in my next research.





Supporting figure 1 UII inhibited insulin receptor-mediated signal transduction in HuH7 cells and BEL-7402 cells. HuH7 cells (A) and BEL-7402 cells (B) were exposed to 100 nM UII for 24 h prior to stimulation with insulin (100 nM, 30 min), and total cell lysates were then subjected to western blotting. UII impaired phosphorylation of Akt and GSK-3β. Data from at least three independent experiments are presented as the mean ± S.D. $^{a}P<0.001$ versus control group; $^{b}P<0.05$ versus insulin treatment alone.

2. Several chemical compounds were used to examine the signaling pathway. The authors need to use more than one inhibitor to eliminate any effects associated with the chemical compound per se.

Answers: Yes, chemical compounds study should be conducted to eliminate the correlation between chemical compounds per se. And each chemical compound had been used alone in revised manuscript Figure 3 and Figure 5 to achieve the goal.

3. Urotensin II was suggested to induce ROS production, which further activates JNK activation to suppress insulin signaling pathway in this article. This hypothesis could be further tested by investigating the effect of JNK inhibitor, in addition to the NADPH-synthase inhibitor in insulin signaling pathway.

Answers: Thanks for the reviewer's suggestion. We had investigated the effect of SP600125, JNK inhibitor, in revised manuscript Figure 3C and D in insulin signaling pathway and glycogen synthesis.

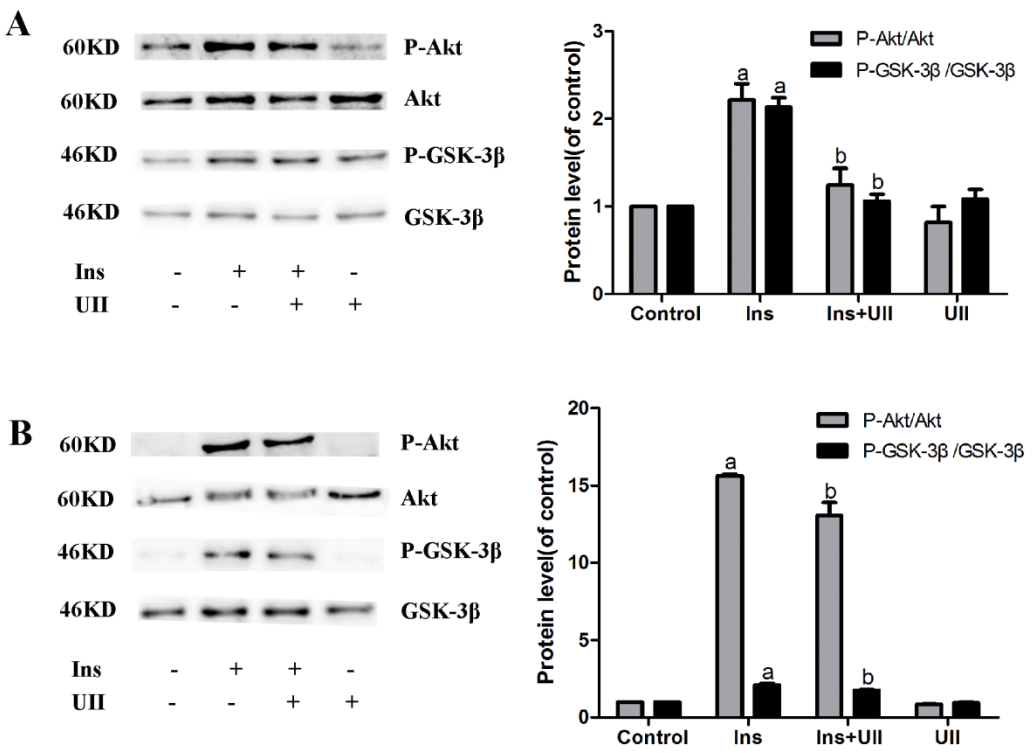
Replies to Reviewer ***02810791***

Minor considerations:

1. HepG2 cells are a tumor-derived cell line, and the use of this cell line might

in part diminish the physiological meaning of the finding. Would the authors be able to reproduce at least some of the key data (PKB phosphorylation, glycogen synthesis) in primary hepatocytes, or an independent hepatocyte-derived cell line (e.g. HuH7) to show that the effects of UII are reproducible to multiple hepatic cell lines?

Answers: Thanks for the reviewer's suggestion. HepG2 cells are indeed a tumor-derived cell line, while the cells, phenotypically similar to human hepatocytes, are frequently used for studying hepatic insulin resistance *in vitro* system. This reference has been quoted as ref.14, 17, 18, 24 et al. in the revised version. And we have validated the key effects of urotensin II on HuH7 cells and BEL-7402 cells as follows supporting figure 1 based on the reviewers' constructive advice, which indicate that the effects of UII are reproducible to multiple hepatic cell lines.

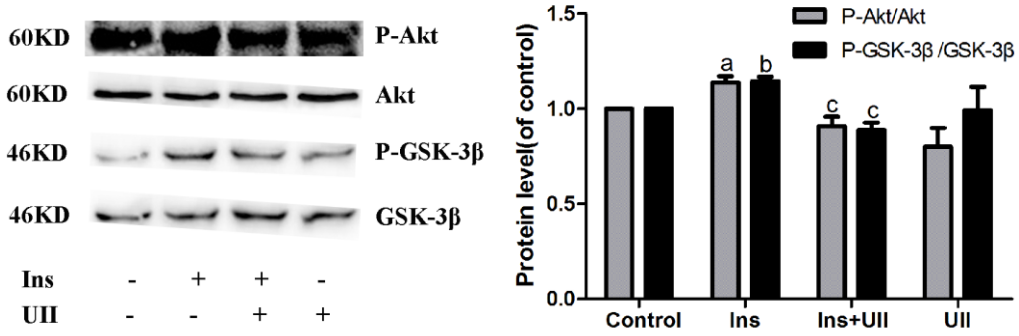


Supporting figure 1 UII inhibited insulin receptor-mediated signal transduction in HuH7 cells and BEL-7402 cells. HuH7 cells (A) and BEL-7402

cells (B) were exposed to 100 nM UII for 24 h prior to stimulation with insulin (100 nM, 30 min), and total cell lysates were then subjected to western blotting. UII impaired phosphorylation of Akt and GSK-3β. Data from at least three independent experiments are presented as the mean ± S.D. $^{a}P<0.001$ versus control group; $^{b}P<0.05$ versus insulin treatment alone.

2. I think an interesting question to address is related to the persistency of the insulin-resistant phenotype. It would be interest in to investigate whether upon UII removal the hepatocytes retain their insulin-resistant state, and in the affirmative case the duration of the insulin-resistant state.

Answers: We performed the experiments with removal of UII, and we found the hepatocytes could retain their insulin-resistant state in 12 hours as follows supporting figure 2



Supporting figure 2 HepG2 cells could retain insulin-resistant state in 12 hours after removal of UII. HepG2 cells were exposed to 100 nM UII for 24 h prior to stimulation with insulin (100 nM, 30 min), then cultured without stimulant for 12 h. Total cell lysates were subjected to western blotting. UII still impaired phosphorylation of Akt and GSK-3β. Data from at least three independent experiments are presented as the mean ± S.D. $^{a}P<0.01$, $^{b}P<0.001$

versus control group; $^{c}P<0.01$ versus insulin treatment alone.

Additional Minor Comments: the paragraph? Comments? (Page 14), is unclear to me. I believe that it should be merged within the discussion.

Answers: Correction has been made in the revised version.