Peer Review File

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Reviewer A

The paper titled "ERα promotes SUMO1 transcription by binding with the ERE and enhances SUMO1-mediated protein SUMOylation in breast cancer" is interesting. The results showed that E2 promoted the transcription and protein expression of SUMO1 via ERα binding to a 1/2ERE in the SUMO1 promoter, and that E2-ERα also augmented SUMO1-mediated Ras SUMOylation and mediated cellular responses in ER-positive BC. However, there are several minor issues that if addressed would significantly improve the manuscript.

1) Aberrant ERα signaling is recognized as a major contributor to the development of breast cancer. What is the molecular mechanism underlying the regulation of ERα in breast cancer? It is suggested to add relevant contents.

Reply 1: We have added some relevant contents in Page 3, line 3-16.

2) The description of some methods in this study is too simplistic, please describe in detail.

Reply 2: We have added some relevant contents in the methods.

3) It is suggested to increase the research progress of the relationship between estrogen receptor and breast cancer in the discussion.

Reply 3: We have added some relevant contents in Page 10, line 3-10.

4) What is the effect of this study on further breast cancer treatment and prognosis? Please add relevant content to the discussion.

Reply 4: This study reinforces the concept that ERα-induced SUMO1 expression is vital to the regulation of BC proliferation; and that targeting ERα-SUMO1 to attenuate protein SUMOylation may be a novel therapeutic inhibitor of BC development.

5) What are the effects of neoadjuvant chemotherapy on the expression of ER and Her2 in breast cancer patients? It is suggested to add relevant contents.

Reply 5: We have added some relevant contents in Page 10, line 3-10.

6) The introduction part of this paper is not comprehensive enough, and the similar

papers have not been cited, such as “An immunohistochemical panel of three small ubiquitin-like modifier genes predicts outcomes of patients with triple-negative breast cancer, PMID: 33842251”, “Tamoxifen induces stem-like phenotypes and multidrug resistance by altering epigenetic regulators in ERα+ breast cancer cells, PMID: 33294429”. It is recommended to quote the articles.

Reply 6: We have added some relevant contents in Page 3, line 25-27.

7) How does ERα control RNA biology in cytoplasm? What is the next research plan? It is suggested to add relevant contents.

Reply 7: We have added some relevant contents in Page 10, line 5-12. We further study the role of selective estrogen receptor modulators (such as TAM) in the treatment of ERα+ breast tumors.

Reviewer B

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

17 ERα signals will help develop new strategies for treating cancer patients.Previous
18 studies have shown that the ubiquitin proteasome system (UPS) is involved in the
19 regulation of ERα stability(29). E3 ubiquitin ligases induce 26S proteasome mediated

I have revised.

2. Figure 1:

1) Figure 1 is not clear enough. Please resubmit it in higher resolution.

I have completed the modifications

2) Please indicate the meaning of # in Figure 1B legend.

I have completed the modifications on page 15, line 7

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

I have completed the modifications on page 15, line 16

3. Figure 2:

1) Figure 2 is not clear enough. Please resubmit it in higher resolution.

2) Please indicate the full name of “ERα”, “ERE”, “WT”, “TSS” in the legend.

I have completed the modifications on page 16, line 3

4. Figure 3:

Please indicate the magnification for cell maps in Figure 3C legend.

I have completed the modifications on page 16, line 14

5. Figure 4:

Please indicate the full name of "ERα", "BC", "WT" in the legend.

I have completed the modifications on page 16, line 25

6. Figure S1:

1) Figure S1 is not clear enough. Please resubmit it in higher resolution.

2) Please check your Figure S1C legend. There is no * in Figure S1C, but you indicated in the legend.

We have deleted this part of the content.

(C) T47D cells had been treated with vehicle, 1 mM E2 or 0.1 mM ICI alone or in combination for 24 h, and the SUMO1 expression was detected by western blot. *, compared with the untreated group, P<0.05. (D-E) RT-PCR was used to detect the SUMO1 expression

3) Please indicate the meaning of # in Figure S1D legend.

7. Figure S5 (Proposed model showing the crosstalk):

Please confirm your Figure S5 is original and created/made by the authors. Otherwise, the copyright permission is needed.

We confirm the Figure S5 is original and created/made by the authors.

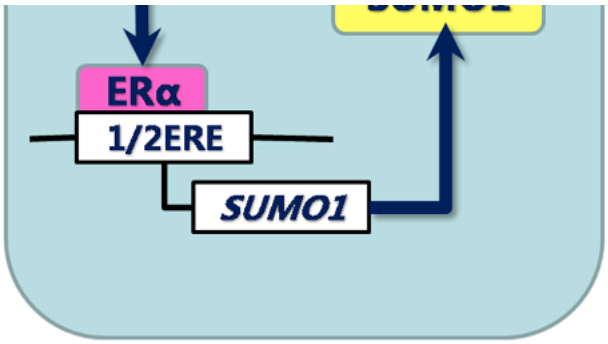

Figure S5. Proposed model showing the crosstalk between E2-ERa signaling and SUMO1 mediated protein SUMOylation.

8. Figure S6:

There is no Figure S6 in your Supplementary material, but you cited it in the main text. Please check.

development. Collectively, our findings indicated that *SUMO1* was the target of ERα, and that protein SUMOylation regulated BC development (Figure S6).

15 **Figure S5** Expression of VEFGR and HIF1α after SUMOylation detected by western
16 blot. ↵
17 ↵
18 **Figure S6** Schematic diagram of crosstalk between E2-ERα signaling and
19 SUMO1-mediated protein SUMOylation. ↵

We have made modifications to the content of S5 and S6, and the modified content is in the Supplementary Information file, page 10

9. Appendix 1 was not cited in the main text. Please add.

The content in Appendix 1 has been cited separately in the article.