Reviewer's report

Title: Estrogen-mediated inactivation of FOXO3a by the G protein-coupled Estrogen Receptor GPER

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Reviewer: Ratna Vadlamudi

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In this manuscript, authors investigated the mechanism by which E2 signaling modulates FOXO3a localization using breast cancer cell line MCF7. Using GPER and ERalpha specific siRNA as well as GPER specific ligand, authors provided evidence that FOXO3a inactivation occurs via GPER. Further, mechanistic studies revealed that GPER-p110# catalytic subunit of PI3Kinae-EGFR axis play role in this process. Collectively these results suggest that GPER play a critical role in FOXO3A translocation and these findings have implications in understanding the mechanisms of therapy resistance. Overall this is a good study, experiments are well designed and most conclusions are supported by the data. Addressing the few concerns given below will further strengthen the manuscript.

Minor essential revisions needed.

1. All of the studies are done using a single model cell. It is critically needed to confirm these findings using additional ER+ve model cell(s). At the minimum, few of the critical results need to be validated in another cell line.

2. GPER siRNA data is convincing. However, ruling out role of ERs in this process completely is premature at this stage and it is possible that both ER and GPER are capable of facilitating FOXO3a trans-location. As can be seen from the western in Fig 4D, ERalpha knock down is not 100% and some ER is still present and it is possible that remaining small amount of ER may be sufficient for the needed translocation.

3. Further, MCF7 cells express both ERalpha and ERbeta. The study only utilized ERalpha siRNA and excluded the role of other ERbeta in this process of FOX3A translocation by E2. Does GPER agonist facilitate translocation of FOXO3a in ERalpha negative cells?

4. All the studies are done using GFP-tagged FOX03a. It remain unknown if this regulation occurs at the level of endogenous FOXO3a

5. Use of three different names confusing: FOXO3a, FoxO3, FOX3

Level of interest: An article of outstanding merit and interest in its field
Quality of written English: Acceptable

Statistical review: Yes, and I have assessed the statistics in my report.

Declaration of competing interests:

I declare that I have no competing interests