**Peer Review File**

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**Reviewer A**

1. Could this work be validated in a second independent cohort in order to conform to REMARK guidelines. Can we be confident in this biomarker if it has only been tested in 146 samples?
2. Would the authors consider using Survminer package in RStudio to determine optimal cut off points for high and low expression groups
3. Have the authors performed extensive antibody specificity testing to ensure the antibody does not bind to off target proteins
4. Did the authors score the sections manually or digitally? If the authors manually scored could they consider rescoring using QuPath (free digital software)
5. Did the authors use tissue microarrays or full tumour sections?

Comment 1: Could this work be validated in a second independent cohort in order to conform to REMARK guidelines. Can we be confident in this biomarker if it has only been tested in 146 samples?
Reply 1: Thank you for your valuable feedback. We highly appreciate your idea. Due to the strict diagnostic screening criteria and the relatively low proportion of triple-negative breast cancer cases, the current study of this queue is already a more comprehensive and larger triple-negative breast cancer queue study in our center. Additionally, we have cancer tissues and adjacent tissues as controls for every case to ensure the accuracy of the tests. Therefore, we have great confidence in this biomarker.
Changes in the text: None

Comment 2: Would the authors consider using Survminer package in RStudio to determine optimal cut off points for high and low expression groups
Reply 2: Thank you for your valuable feedback. RStudio, as an important statistical tool, is increasingly being applied in statistical analysis. We have also come across numerous articles and studies that use SPSS to determine accurate cut-off points. For our current research on queue data, the SPSS method can also provide precise and reliable cut-off points.
Changes in the text: None

Comment 3: Have the authors performed extensive antibody specificity testing to ensure the antibody does not bind to off target proteins

Reply 3: The manufacturer produces the monoclonal antibody following strict international guidelines for monoclonal antibody production. Before leaving the factory, the antibody undergoes rigorous peptide validation to ensure its specificity for binding to the target protein.
Changes in the text: None

Comment 4: Did the authors score the sections manually or digitally? If the authors manually scored could they consider rescoring using QuPath (free digital software)
Reply 4: We appreciate your valuable feedback. Currently, our method for evaluating KLHL22 expression is manual scoring, which involves three independent senior pathologists who have no prior knowledge of clinical pathology data. To ensure accuracy, we only select a score as a result when at least two pathologists agree on the rating. In cases where there are differing opinions, a rediagnosis is conducted, and a discussion is held to reach a consensus.Additionally, we conduct literature research and there have been reports suggesting that manual counting of tumor cells for immunohistochemical expression can be as accurate as software counting, and sometimes even more efficient. DOI: 10.1267/ahc.20-00032
Changes in the text: None

Comment 5: Did the authors use tissue microarrays or full tumour sections?
Reply 5: Our research uses tissue microarrays for experiments
Changes in the text: we have modified our text as advised (see Page 4, line 80-81).

**Reviewer B**

Comment 1: While the focus of the manuscript is on the correlation between KLHL22 and TNBC, it would be valuable to explore its correlation with other types of breast cancer, as this could enhance the value of KLHL22 as a TNBC biomarker. The authors should consider investigating the correlation survival curves between KLHL22 and ER, PGR, HER2, and other breast cancer subtypes.
Reply 1: Thank you for your valuable feedback. It is true that breast cancer has multiple subtypes, such as classification based on immune protein expression or molecular testing. However, our main focus of research is triple-negative breast cancer, which is a less common subtype with poor prognosis and treatment outcomes among breast cancer types. As for your specific question, in our preliminary statistics, KLHL22 protein did not show any clear correlation with ER, HER2, or other markers. Therefore, we did not proceed with further survival analysis. Given that our study is limited to a single center and a small cohort, we hope to collaborate with multiple centers in future

research to gather more data and validate the correlation between KLHL-22 and commonly detected markers in breast cancer, such as ER and HER-2. Once again, we appreciate your valuable input, which has been highly beneficial to us.

Changes in the text: None