# Author's Point-by-point Response to Reviewers' Comments

**[cancers-1746495]**
**NR2F1, a tumor dormancy marker, is expressed predominantly in cancer-associated fibroblasts and is associated with suppressed breast cancer cell proliferation**

**Reviewer #2 (General comments):**
*The authors present interesting findings that a tumor dormancy marker, NR2F1, is predominantly expressed in the inflammatory CAFs, and high expression of NR2F1 is associated with suppressed immune response and increased density of stromal cells. However, this reviewer has a few concerns that need to be addressed before accepting this article for publication.*

**Response:**
We are grateful to the reviewer for her/his time and effort in reviewing our paper, as well as for pointing out issues to improve the paper.

**Comment 1:**
*This reviewer noticed that all the analysis was performed on the public data sets of bulk RNA-seq or single cell sequencing of primary breast tumors. Can the authors do some analyses using data generated from both primary and metastatic tumors to check if there is any difference of NR2F1 expression, and how NR2F1 expression is correlated with metastasis?*

**Response 1:**
We completely agree with the reviewer that it will be informative to demonstrate the NR2F1 expression in both primary and metastatic breast cancer and the possible correlation of NR2F1 expression with metastasis. In Figure 3C, we present NR2F1 expression in primary breast cancer with and without distant metastases. NR2F1 expression was not increased in the group with later recurrence in four cohorts in this analysis. We also present NR2F1 expression between primary and metastatic breast cancer in Figure 3E with no significant difference. We did not find a clear association of NR2F1 with distant metastasis in this study. On the other hand, NR2F1 expression was higher in primary breast tumors with lymph node metastasis in all four cohorts, as shown in Figure 3B, suggesting an association between NR2F1 and lymph node metastasis. Given these results, we revised the results section as follows.

**Results section (Page 6, line 221):**
**We investigated the association of *NR2F1* expression with metastasis. No significant difference was found in the *NR2F1* expression with and without distant metastasis in 5 independent primary breast cancer cohorts (Figure 3B). On the other hand, *NR2F1* expression was higher in the group with lymph node metastasis in three out of four cohorts (Figure 3C). In addition, there was no difference in NR2F1 expression in primary breast cancer based on based on the known site of relapse (Figure 3D). It was also of interest to compare the expression of *NR2F1* between primary and**

**metastatic breast cancer, but there was no difference observed in the *NR2F1* expression between both groups (Figure 3E).**

**Comment 2:**
*As mentioned by the authors in Figure 3, there is no correlation between NR2F1 expression in the primary breast tumor and late recurrence. The authors need to analyze the single cell sequencing data and show whether the late recurrence in other organs, such as lung, bone, and brain, other than lymph node, is correlated with NR2F1 expression in the CAFs. Otherwise, the authors cannot make a conclusion that CAF-expressed NR2F1 regulates breast tumor dormancy. Moreover, the authors need to provide some, at least minimum, evidence or clues that CAF-expressed NR2F1 is responsible for tumor dormancy regulation.*

**Response 2:**
We completely agree with the reviewer that NR2F1 expression in cancer cells and CAFs should be investigated not only in primary but also in metastatic breast cancer using single-cell sequence in order to prove that NR2F1 expression in CAFs affects late recurrence. The main finding of this study is that NR2F1 is predominantly expressed in CAFs rather than in all other cell types in the TME, and we do not intend to claim that CAF-expressed NR2F1 regulates dormancy. Further, we do not have access to single-cell sequence cohorts of metastatic breast cancer tumors, but it is of our interest, and this will be our future direction. In response to the reviewer, we added the following sentences in the discussion section.

**Discussion section (Page 13, line 427):**
**The current study demonstrated that *NR2F1* expression in the bulk tumor of primary breast cancer is associated with decreased cell proliferation and cancer stem cell-like characteristics. Previous studies mentioned that *NR2F1* was highly expressed in DTCs [67]. We found that NR2F1 is most predominantly expressed in CAFs in the TME of primary breast cancer. However, the findings from our study are not ample to substantiate that CAF-expressed *NR2F1* regulates breast tumor dormancy. Aguirre-Ghiso group recently demonstrated that *NR2F1* agonist treatment induced cancer cell dormancy [21], which raised an expectation that *NR2F1* expression in primary breast cancer may have the possibility to be a biomarker. The novelty of our study is that it is CAFs, not cancer cells, that are the dominant source of *NR2F1* expression in the bulk tumor. To this end, we believe that *NR2F1* expression in the bulk tumor does not reflect the expression in the cancer cells, thus its value as a biomarker is in doubt. *NR2F1* expression in cancer cells of primary breast cancer was not associated with cancer stem cell-like characteristics at all. In order to prove that CAF-expressed *NR2F1* regulates breast tumor dormancy, one needs to analyze the single-cell sequencing data and show whether the late recurrence in other organs, such as lungs, bones, and brain, other than lymph nodes, is correlated with *NR2F1* expression in the CAFs.**

**Comment 3:**
*Line 170, "Figure 1E" should be "Figure 1D".*

**Response 3:**
We thank the reviewer for closely reading our manuscript and pointing out our oversight. We have corrected the results section as answered in Responce1.

We would like to thank the reviewer for his/her thoughtful and thorough review of our manuscript, which significantly strengthened our manuscript.