# Peer Review File

## Reviewer comments

1. Lines 47-62
Please, add proper citations to this paragraph.

Response: We thank the reviewer for the insightful comments. We added proper citations to this paragraph. (see Page 4, line 57-70)

2. Please, provide proper citations for the WGCNA method and package, as described here:
https://horvath.genetics.ucla.edu/html/CoexpressionNetwork/Rpackages/WGCNA/#citation

Response: We thank the reviewer's comments and suggestions. We added proper citations for WGCNA method and package to the paragraph (see Page 4, 72-73; Page 6, line 127-129).

3. Line 72
The Authors write about two networks here, but further in the text they write about one network. Could the Authors explain that?
Please, correct the language:
"differential expressed genes" -> "differentially expressed genes"
Testing and validation data should be independent. In the reviewed paper, testing and validation datasets include the same normal samples. Therefore, the validation performed on validation dataset is not properly done. The decision about using the same normal samples is probably caused by a small number of normal samples. The Authors should use independent dataset for validation or, at least, discuss that issue in the discussion section (lines 305-309).

Response: We apologize for these errors. All language issues have been fixed as advised. We agree with the reviewer that the testing and validation data should be independent. We have discussed that issue in the discussion section as advised (see Page xx, line xx). We have also added the independent validation by the GEO database (see Page 13, line 246-250), which is consistent with our result.

4. Please, could you better describe in the text, what "targeted modules" and "module-traits correlation" are?

Response: We apologize for not clearly expressing the meaning of "targeted modules" and "module-traits correlation". The "targeted modules" more appropriate to say as "modules of interest," which means blue and yellow modules (see Page 2, line 24). The "module-traits correlation" means correlations between modules and traits (see Page 7, line 132).

5.  Line 119.
The Authors write about the threshold. For which variable the threshold was set?

Response: We thank the reviewer for the comment and apologize for not being precise in the original manuscript. We set the thresholds for both blue and yellow modules to screen genes that were highly centered (hub genes) in these modules, and we have made modifications in the text (see Page 8, line 145-147).

6.  Line 127
The Authors write that they used PTC cell lines. In fact, the Nthy Ori3-1 is not PTC cell line. It is a normal human primary thyroid follicular epithelial cell line. Please, make a correction.
Line 138

Response: We thank the reviewer for pointing this out and apologize for our misrepresentation, and we have modified it in the text (see Page 8, line 156-157).

7.  Line 136. The Authors write that Student's t-test was used to compare gene expression between two cell lines. However, two measurements are not enough to calculate the statistical test. Did the Authors perform qRT-PCR in duplicates / triplicates?

Response: We thank the reviewers for their questions. We carried out three repeated experiments and had three sets of data, so we used the Student's t-test.

8.  Line 164
The Authors write: "the cutoff criteria were top 25% row sum of counts of all genes". This sentence is unclear. Could the Authors rephrase it? At the Figure 1 I see that they excluded lowly expressed genes. Did the Authors included 25% genes with highest summary expression? But 4387 (number of analyzed genes) is not 25% of 13693 (total number of genes).

Response: We apologize for not clearly expressing the cutoff criteria. "The cutoff criteria" is better expressed as "the filtering criteria," and the filtering criteria were that row sums of gene counts should be bigger than the upper quantile of the row sums. We have modified it in our text (see Page 10, line 197-198).

9.  Line 181.
The Authors write about "corresponding correlation". To make the paper more easy to

read, could the Authors explain here, between which two variables the correlation was calculated?

Response: We thank the reviewer for the insightful comments and apologize for not clearly expressing the "corresponding correlation." "Corresponding correlation" means the correlation between modules and traits, and we have modified it in the text (see Page 7, line 132).

10. Lines 192-193
The Authors wrote that "31 DEGs from 64 genes matched in the yellow module and 84 DEGs from 111 genes matched in the blue module". However, the yellow module includes 94 genes and blue module includes 896 genes (as described in lines 173-174). So, what the 64 genes from yellow module and 111 genes from blue module are?

Response: We thank the reviewer for the comment and apologize for not being precise in the original manuscript. We used the threshold to screen the highly centered genes for both blue and yellow modules. After the screening with the threshold 0.17 for the yellow module, 64 genes were visualized in which 31 genes were matched to DEGs. After the screening with the threshold 0.27 for the blue module, 111 genes were visualized in which 84 genes were matched to DEGs. We have made modifications in our text (see Page 12, line 224-227).

11. Line 221
There is a misspelling in the gene symbol. It should be KCNN4, not KGNN4.

Response: We apologize for these errors. All language issues have been fixed as advised.

12. Lines 223-224
The Authors write that "four genes (GDF15, LCN2, KGNN4, and SH3BGRL3)
showed much higher expression in T3-T4 than T1-T2 (q value<0.05)". Please, could you clarify in the text, which dataset was used to show that? The test or validation dataset?

Response: We thank the reviewers for this question. We use the clinical-stage matrix to show that and have marked in the text (see Page 9, line 173).

13. Lines 255-260
Please, correct the language in that sentence:
Blue modules -> blue module
Were chose -> were chosen
The expression three genes -> the expression of three genes

Response: We apologize for these errors. All language issues have been fixed as advised.

14. Lines 305-309

The Authors wrote that the study has several limitations. For example, they used only one dataset, and multiple datasets should be used for validation. Why didn't the Authors use another dataset for validation? There are many expression datasets publicly available nowadays. The independent validation would increase the value of the paper.

Response: We thank the reviewer for the insightful comments and agree with the suggestion of reviewer. We have added the independent validation by the GEO database (see Page 13, line 246-250), which is consistent with our result.