RESPONSE TO REVIEWERS COMMENTS

ROUND 2

REVIEWER #3

The authors have properly addressed the reviewer's remarks, and the revised version of the manuscript demonstrates improved quality in the study design (including the addition of READ and gene ontology/enrichment) and the presentation of results. The manuscript could be considered for acceptance if the following (minor) remarks are considered by the authors:

Point 1: The TCGA-COAD and TCGA-READ datasets should have been merged into a single validation dataset; however, the authors have added TCGA-READ only as an independent cohort. However, this issue does not impact the overall quality of the study.

Response: We thank the reviewer for reviewing our work and providing suggestions which have contributed to and increased the overall quality of the study. For **Fig 3**, we did the TCGA-based validation of the top 10 differentially expressed miRNAs identified in our NGS analysis using **Cancer MIRNome (CancerMIRNome (jialab-ucr.org) (COAD – accessed on 12 June, 2022, READ – accessed on 25 December, 2024)**. This information has been given in the text of the manuscript (**"Results" Section 2.4, line no. 263-266; Fig 3 legend line no. 281-287**). Hence this figure represents the expression of the miRNAs between normal and tumour tissues of TCGA-COAD and TCGA-READ cohorts separately.

For Fig 4, we were unable to merge the TCGA-COAD and TCGA-READ datasets into a single validation dataset. However, we **have integrated** the **TCGA-COAD** and **TCGA-READ cohort data** by determining the Average CPM (AveCPM) for each miRNA between Normal-Young, Normal-Old, Tumour-Young and Tumour-Old subsets of the two cohorts. miRNA levels were visualized by plotting the Log_2 AveCPM corresponding to each subset **("Results" Section 2.4, line no. 288-313; Fig 4 A-J and Figure 4 legend line no. 315-325)**. Details regarding the methodology of data acquisition and processing from TCGA-COAD and TCGA-READ

databases have been included in the Materials and Methods section **(Section 4.13, line no. 813-830)**.

Point 2: The manuscript requires thorough proofreading and correction of minor spelling mistakes/typos, inconsistencies in the use of capital and lowercase letters (e.g., for words in brackets), and spaces before/after mathematical operators (e.g., <, >, =).

Response: We appreciate the reviewer's concern and have tried to correct the typographical errors, spelling mistakes, typing inconsistencies that the reviewer has pointed out to the best of our ability.

Point 3: Lines 30, 144, 184, 810 (and wherever else applicable): TCGA-COAD and READ should be written as TCGA-COAD and TCGA-READ.

Response: We have corrected the indicated areas in the manuscript as applicable.

Point 4: Vendor affiliations in the Methods section: Use the full affiliation the first time only; subsequent references should include the vendor's name only.

Response: We have done the necessary correction in the manuscript.