Manuscript ID ijms-2189846 – Authors' response to reviewer 1

The authors would like to thank the reviewer for their time in assessing the manuscript and for the useful and insightful comments. We have addressed each of the comments below with responses highlighted in red.

In the manuscript, Winstanley-Zarach et al tried to explore the distribution of polyA sites on articular chondrocyte transcripts in the health and OA cartilage. They found that differential use of polyadenylation sites was modest. Their findings could provide new insight into OA study. Several issues should be addressed in the study.

(1) For the OA samples used in the study, which stage of OA are they from? Early stage? Moderate stage? Or Late stage? The gene expression is different by stages. These patients had late stage OA and were undergoing arthroplasty because of this. Late stage description has been added to the manuscript (line 487).

(2)For the QuantSeq Reverse analysis, total RNA was isolated from tissue cryosections. Why not try to directly isolate RNA from fresh tissue? Cutting cryosections enabled us to ensure we had reproducible full depth cartilage tissue across all samples. This has been mentioned in the paper.

(3) For the culture of human chondrocytes, is it normal culture with oxygen? Because cartilage is in the hypoxia environment, chondrocytes typically have an adapted response to low oxygen environment. Why not use the hypoxia culture for chondrocytes? Whilst the oxygen tension is an influential parameter in the chondrocyte response, it is not standard within the filed to exclusively culture the cell in this environment. It is a distinct technical challenge to exclusively maintain the cells in low oxygen conditions due to the necessity to change media. Exposure to higher levels of oxygen can then rapidly stimulate signalling processes, which would be repeated along the culture period, hindering interpretation of cells' use as a model. We had decided in this study to culture under 20% oxygen in keeping with most studies in the field. Nevertheless, the reviewer raises a very good point about this and a further study of the influence of hypoxia on post transcriptional processes would be very interesting.

(4) Statistical analysis is critical for a study. Could the author indicate the statistical analysis as a sub-section in the method? A section on statistical analysis has been added (4.8).

(5) Could the author clarify the application in OA? Which one could be therapeutic target for OA in the future? We have already described in the discussion the limited changes in alternative polyadenylation that occur as a result of osteoarthritis, whilst also identifying the potential disease associated mechanisms that might result for some of the genes where regulation has been shown to occur (e.g. KMT2A and OSMR). We have added some additional text to the final paragraph of the discussion to re-enforce this.