SJD Pediatric Cancer Center
Barcelona Children's Hospital

April 30th, 2024

Dear Dr. Lu Wang / Editor,

Thank you very much for giving us the opportunity to address the reviewers' comments to our manuscript now entitled "KDM6 demethylases contribute to EWSR1-FLI1-driven oncogenic transformation in Ewing Sarcoma".

We sincerely thank the reviewers for their suggestions and comments, which have been very helpful and insightful. Below we summarize the main changes included in this revision:

-We have included new immunoprecipitation data in support of the direct interaction between KDM6A and of KDM6B with BRG1.

- We have demonstrated the recovery of expression of target genes using an enzymatically inactive KDM6A mutant, thus confirming the demethylase independent role of KDM6A in EwS.

-We have introduced ChIP-seq data on EZH2 to balance the characterization of H3K27me3 distribution.

- We have performed an entirely new battery of H3K27me3 ChIP-seq experiments in KDM6A and KMD6B KO cells with spike-in.

- We have added data on EWSR1-FLI1 overexpression in HeLa cells to understand the KDM6A and KDM6B redistribution.

We are confident that we have successfully answered to all the reviewers' feedback and that our research marks a significant step forward in the understanding of the molecular mechanisms of Ewing sarcoma tumorigenesis. Our point by point responses to the reviewers comments are included for your reference.

Thank you so much for considering our manuscript and for the opportunity that you have given to our work.

With best wishes,

Jaume Mora, M.D., Ph.D.

Scientific Director. Pediatric Cancer Center Barcelona (PCCB).