

My Personal Reflections and Conclusions from Steps 3-6

Exciting Steps:

- **Step 5: Quality Control (QC):** I really enjoyed this part because it felt like cleaning up and making sure the data was in top shape. Seeing the violin plots and identifying thresholds for filtering cells gave me confidence in the quality of my analysis moving forward.
- **Step 8: Clustering and UMAP:** This step was truly rewarding. Watching the UMAP plot come to life and seeing distinct clusters was exciting—it felt like unlocking hidden patterns in the data. It was even more fascinating to think about what each cluster could represent biologically.

Curious Conclusions:

1. **Cluster Distribution by Mouse Sex:** One interesting outcome was the uniformity of clusters across mouse sex. This suggests that sex might not play a major role in driving the differences in these cell populations, at least in this dataset. However, the abundance of "NA" entries in the metadata caught my attention—it made me wonder how much more I could uncover if this information was complete.
2. **UMAP Insights:** The UMAP plot provided a clear separation of clusters, which made me curious about the biological characteristics of each group. The fact that sex didn't seem to influence clustering was intriguing and hinted at the possibility that other variables, like cell type or tissue-specific markers, are more significant here.

What I Learned About the Biology:

- I gained a deeper appreciation for how critical QC is in single-cell RNA-seq analysis. Filtering out low-quality cells ensures the insights are biologically meaningful.
- The differences between neurons and microglia became more apparent through clustering, reinforcing their distinct roles in brain function and immune response.
- I also learned that biological metadata can be a powerful tool in uncovering hidden patterns, but its utility heavily depends on its completeness and accuracy.

Overall, this part of the project was both challenging and rewarding. It felt like peeling back layers to reveal the biological story behind the data, which was both a fun and insightful experience.