Chromium Nuclei Isolation Kit: Data Highlights & Methods Comparison for Single Cell Multiome ATAC + Gene Expression

Introduction

The Chromium Nuclei Isolation Kit offers a highly optimized protocol for isolating nuclei from frozen tissue samples for use in 10x Genomics Single Cell assays. The comprehensive kit streamlines the nuclei isolation process into a single, reproducible workflow. This Technical Note compares gene expression and chromatin accessibility data derived from nuclei isolated using the Chromium Nuclei Isolation Kit versus three alternative nuclei isolation methods in human jejunum, human kidney cancer, adult mouse kidney, and adult mouse brain tissue samples run on the Chromium Next GEM Single Cell Multiome ATAC + Gene Expression assay.

Traditional nuclei isolation protocols often require extensive reagent and protocol optimization based on tissue type, introduce reagent and/or user-mediated variability, and may depend on the use of expensive equipment (e.g. flow sorter). This Technical Note compares nuclei-derived gene expression and chromatin accessibility data between the Chromium Nuclei Isolation Kit and three alternative nuclei isolation protocols: the Nuclei Isolation from Complex Tissues for Single Cell Multiome ATAC + Gene Expression Sequencing Demonstrated Protocol (CG000375) with and without sorting (i.e. 10x DP with and without sorting), and the Salty Ez-10 (i.e. Salty EZ) lysis protocol (Martelotto, 2021).

The 10x DP was optimized with flow sorting to remove debris. While flow sorting is highly effective in removing background and debris, it also presents workflow bottlenecks in terms of instrument accessibility and the inability to run multiple samples in parallel. Even without sorting, the 10x DP requires intricate upstream buffer preparation that adds to experimental complexity. Similarly, the customer-developed Salty EZ protocol requires fresh, homebrew buffer preparation, as well as additional decision points within the protocol (e.g. an additional lysis step and whether or not to use a density gradient to clean up the sample) that increase potential sources of variability and data loss. For both the unsorted 10x DP and the Salty EZ

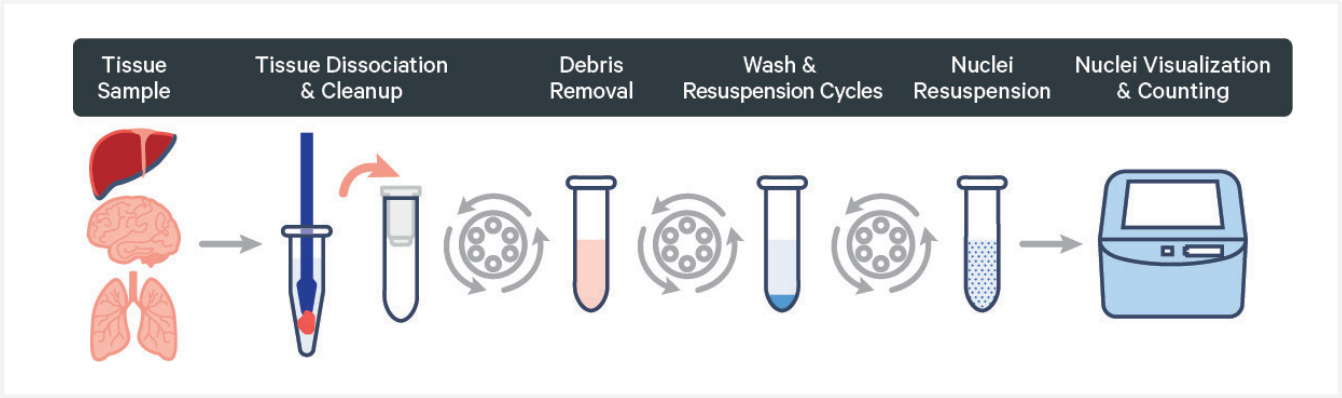

Figure 1. Chromium Nuclei Isolation Kit workflow.

protocols, large debris may not be eliminated from the nuclei suspension and can interfere with downstream microfluidics.

The Chromium Nuclei Isolation Kit not only enables a standardized nuclei isolation process, but its workflow is efficient at removing both large and smaller fragments of debris along with ambient RNA. In the streamlined workflow, frozen tissue samples are homogenized with a pestle in Lysis Buffer and passed through a spin column to isolate nuclei from the disrupted sample. Debris is then removed via centrifugation in Debris Removal Buffer. After a series of wash steps, the isolated nuclei are resuspended in a final resuspension step and loaded directly into the compatible 10x Genomics Single Cell assay. Figure 1 provides a high-level overview of the Chromium Isolation Kit workflow.

Here, a multiomics approach was used to obtain combined gene expression and chromatin accessibility data from the same nucleus. Together, this Technical Note shows that data derived using the Chromium Nuclei Isolation Kit are consistent with other nuclei isolation methods.

Methods

Nuclei were isolated from frozen human jejunum (BioIVT), human kidney cancer (Discovery Life Sciences), adult mouse kidney, and adult mouse brain samples (BioIVT) (each tissue cut into four parts) using the following isolation methods:

- Chromium Nuclei Isolation Reagent Kits Sample Prep User Guide (CG000505)
- Nuclei Isolation from Complex Tissues for Single Cell Multiome ATAC + Gene Expression Sequencing Demonstrated Protocol (CG000375) with sorting
- Nuclei Isolation from Complex Tissues for Single Cell Multiome ATAC + Gene Expression Sequencing Demonstrated Protocol (CG000375) without sorting
- Protocol for nuclei isolation from fresh and frozen tissues using Salty-Ez10 or Salty-Ez50 buffer: compatible with snRNA-Seq and Multiome workflows from 10x Genomics (Martelotto, 2021)

Nuclei samples were processed in parallel according to the Chromium Next GEM Single Cell Multiome ATAC + Gene Expression (CG000338) library construction workflow and run on the Chromium X instrument. 5,000 nuclei were targeted per replicate. The libraries were sequenced and the data were analyzed using Cell Ranger ARC v2.0 with intronic reads included in the analysis. Clusters were annotated using known cell markers and custom scripts. A summary of the overall experimental design is presented in Figure 2.

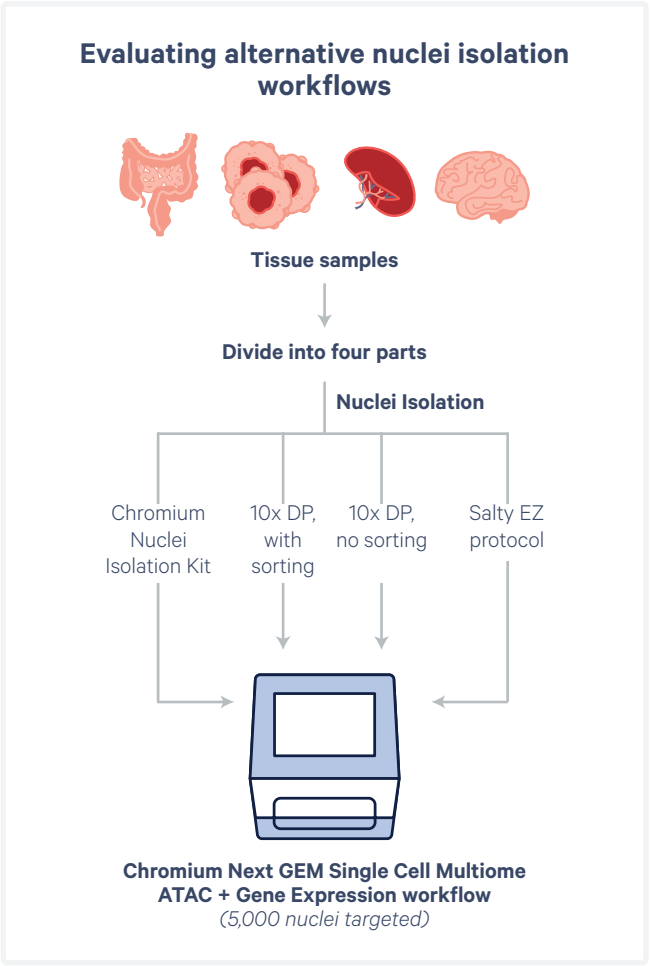

Figure 2. Schematic of experimental design for comparing the Chromium Nuclei Isolation workflow vs. alternative nuclei isolation methods.

Figures 3–8 demonstrate that the Chromium Nuclei Isolation Kit yields comparable Multiome-based data (Gene Expression and ATAC data combined) in terms of data capture and complexity, cell clustering, and cell type identification compared to alternative nuclei isolation methods. Immune cell clustering from human jejunum obtained using the Chromium Nuclei Isolation Kit is highlighted in Figure 9. Single Cell gene expression and Multiome ATAC-based data (captured individually) can be found in the Appendix.

Data Highlights & Methods Comparison

Data Yield

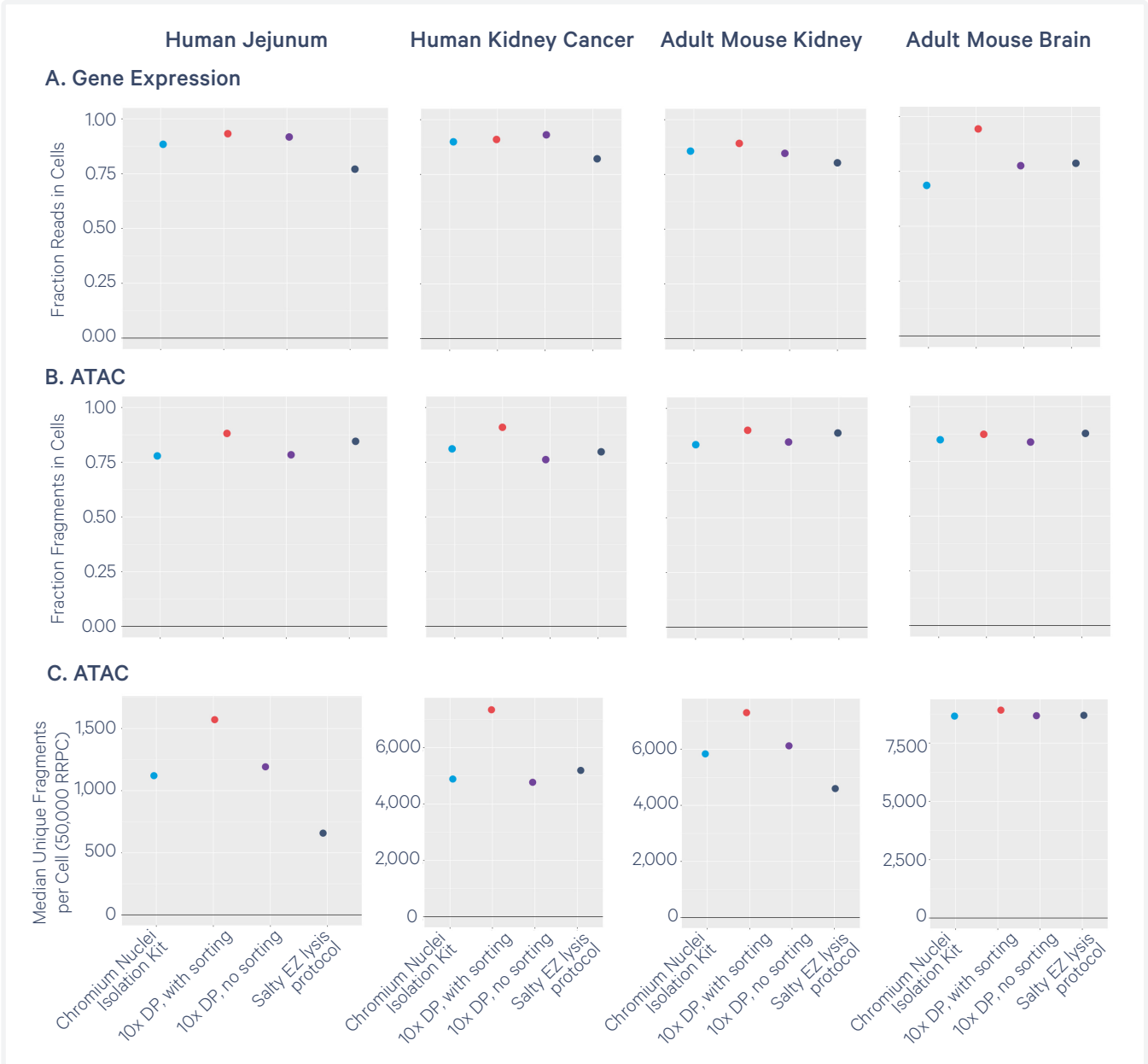

Figure 3. Dot plots show comparable (A) fraction reads in cells, (B) fraction fragments in cells, and (C) median unique fragments per cell from nuclei isolated from human jejunum, human kidney cancer, adult mouse kidney, and adult mouse frozen brain tissue samples run on the Single Cell Multiome ATAC + Gene Expression assay across the different nuclei isolation methods.

Data Complexity

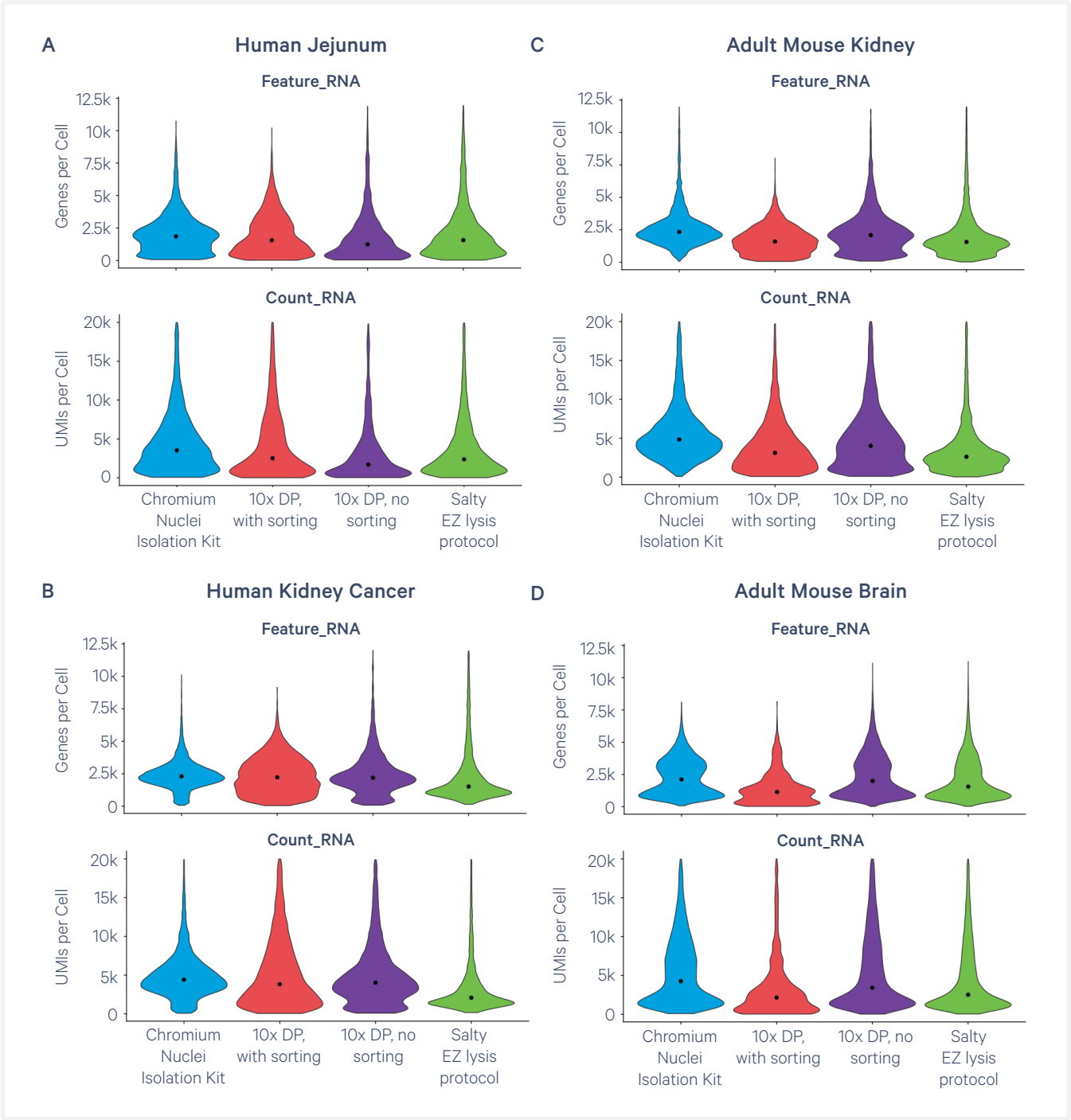

Figure 4. Comparable Gene Expression library complexity was observed in Single Cell Multiome across tissue types and nuclei isolation methods. Violin plots of genes per cell (top panels) and UMIs per cell (bottom panels) after aggregation with *cellranger aggr* in nuclei are shown isolated from (A) human jejunum, (B) human kidney cancer, (C) adult mouse kidney, and (D) adult mouse brain tissue samples using the Chromium Nuclei Isolation Kit compared to the alternative nuclei isolation methods in the Single Cell Multiome ATAC + Gene Expression assay.

Multiome-based (Gene Expression and ATAC combined) Cell Clustering and Cell Type Identification for Human Jejunum

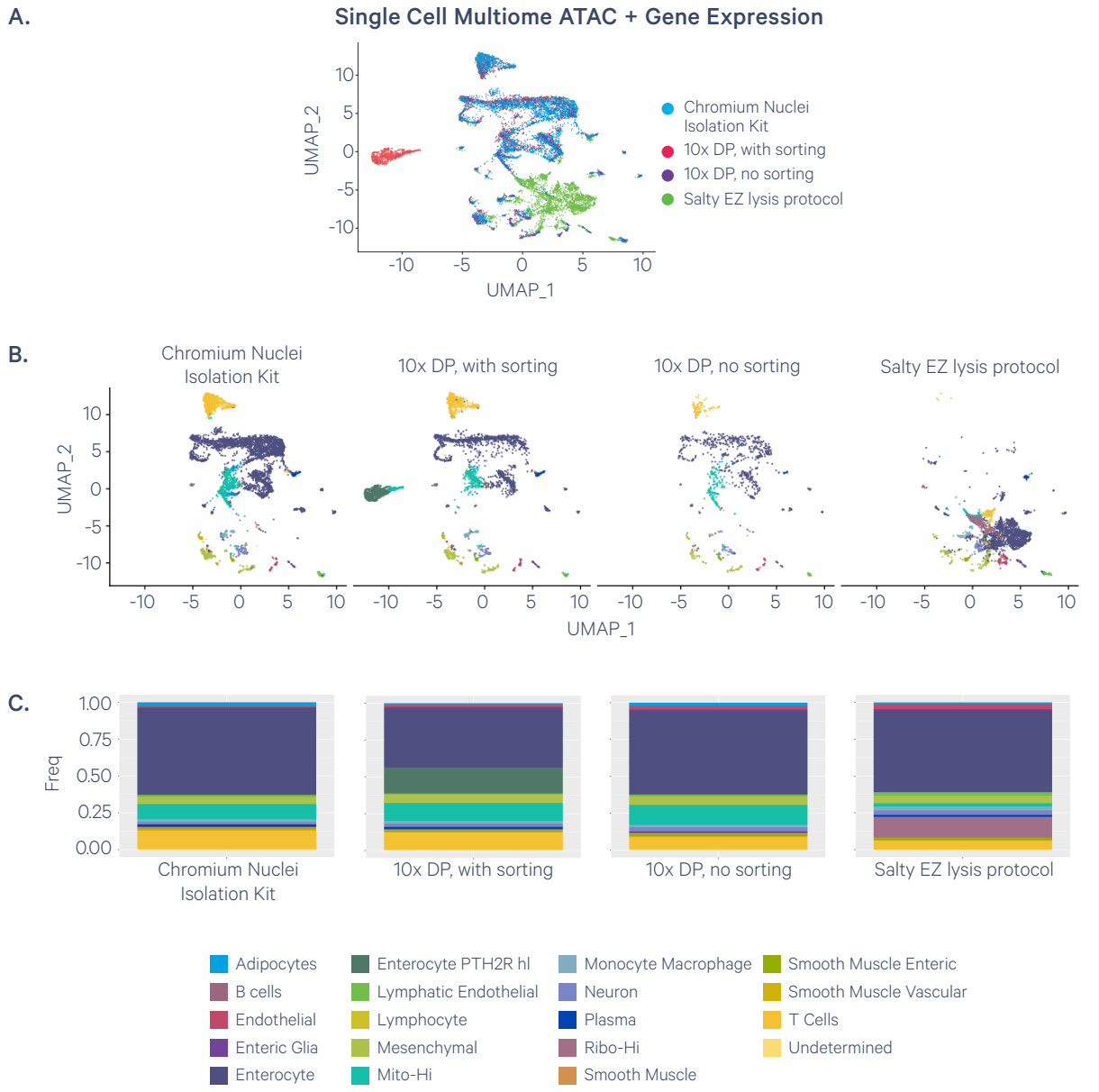

Figure 5. Multiome-based UMAPs (A), Multiome-based cell clustering split by condition (B), and cell type proportions (C) for human jejunum tissue samples profiled using the Single Cell Multiome ATAC + Gene Expression assay across the different nuclei isolation methods. Refer to Appendix A1 for Single Cell Gene Expression and ATAC-based data (captured separately) in the Human Jejunum tissue sample.

Multiome-based (Gene Expression and ATAC combined) Cell Clustering and Cell Type Identification for Human Kidney Cancer

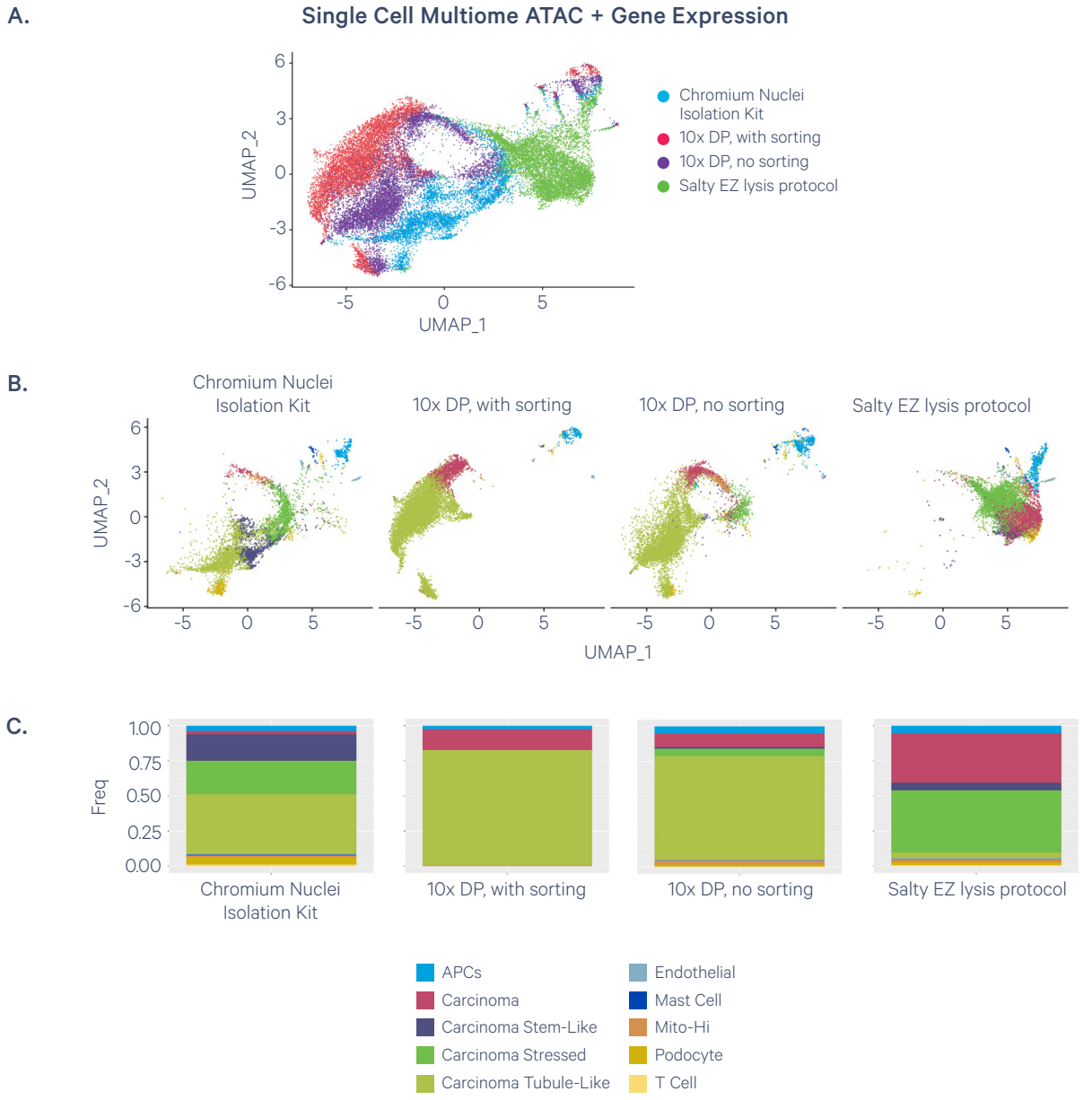

Figure 6. Multiome-based UMAPs (A), Multiome-based cell clustering split by condition (B), and cell type proportions (C) for human kidney cancer tissue samples profiled using the Single Cell Multiome ATAC + Gene Expression assay across the different nuclei isolation methods. Refer to Appendix A2 for Single Cell Gene Expression and ATAC-based data (captured separately) in the Human Kidney Cancer tissue sample.

Multiome-based (Gene Expression and ATAC combined) Cell Clustering and Cell Type Identification for Mouse Adult Kidney

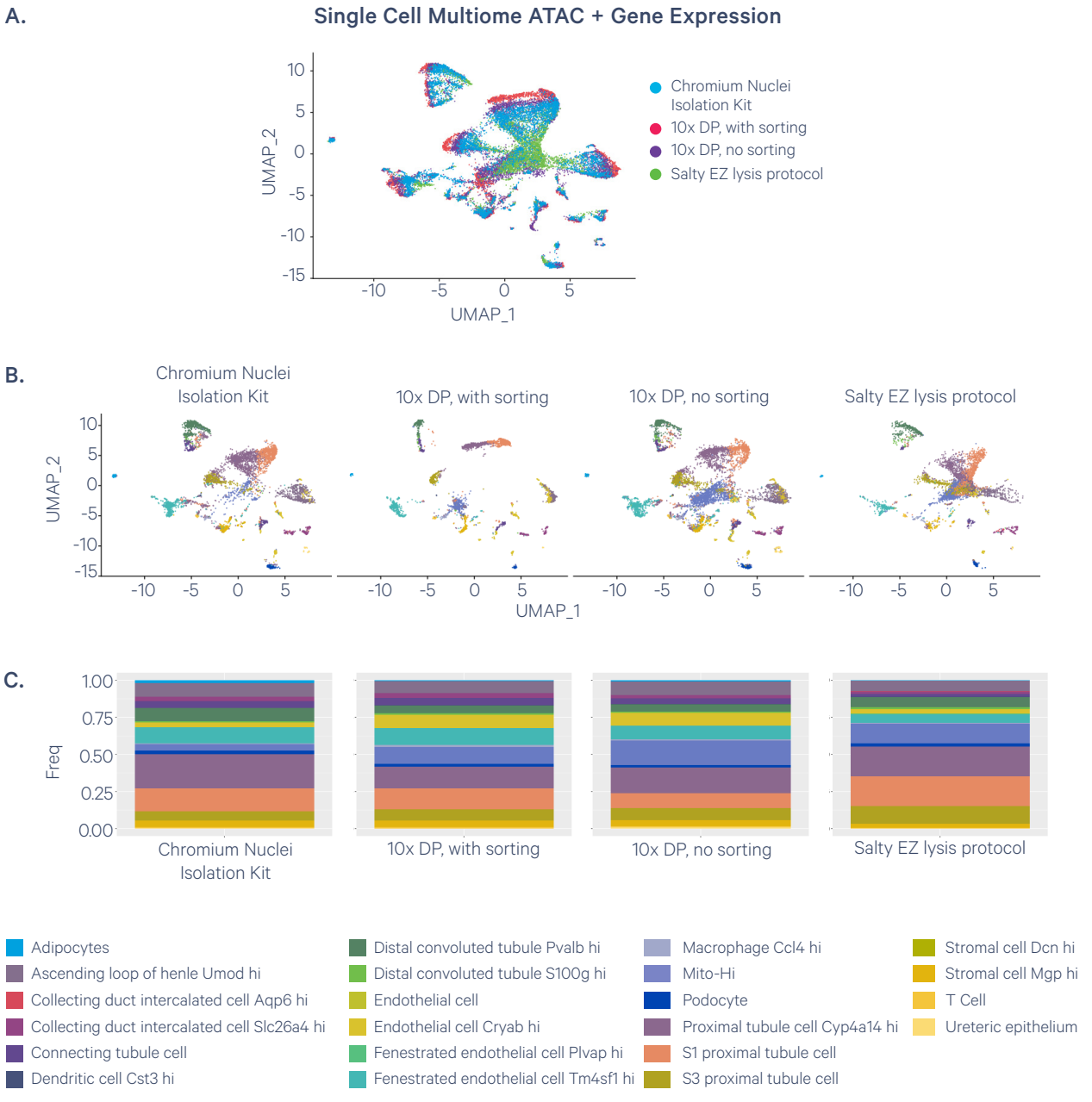

Figure 7. Multiome-based UMAPs (A), Multiome-based cell clustering split by condition (B), and cell type proportions (C) for mouse adult kidney tissue samples profiled using the Single Cell Multiome ATAC + Gene Expression assay across the different nuclei isolation methods. Refer to Appendix A3 for Single Cell Gene Expression and ATAC-based data (captured separately) in the Mouse Adult Kidney tissue sample.

Multiome-based (Gene Expression and ATAC combined) Cell Clustering and Cell Type Identification for Mouse Adult Brain

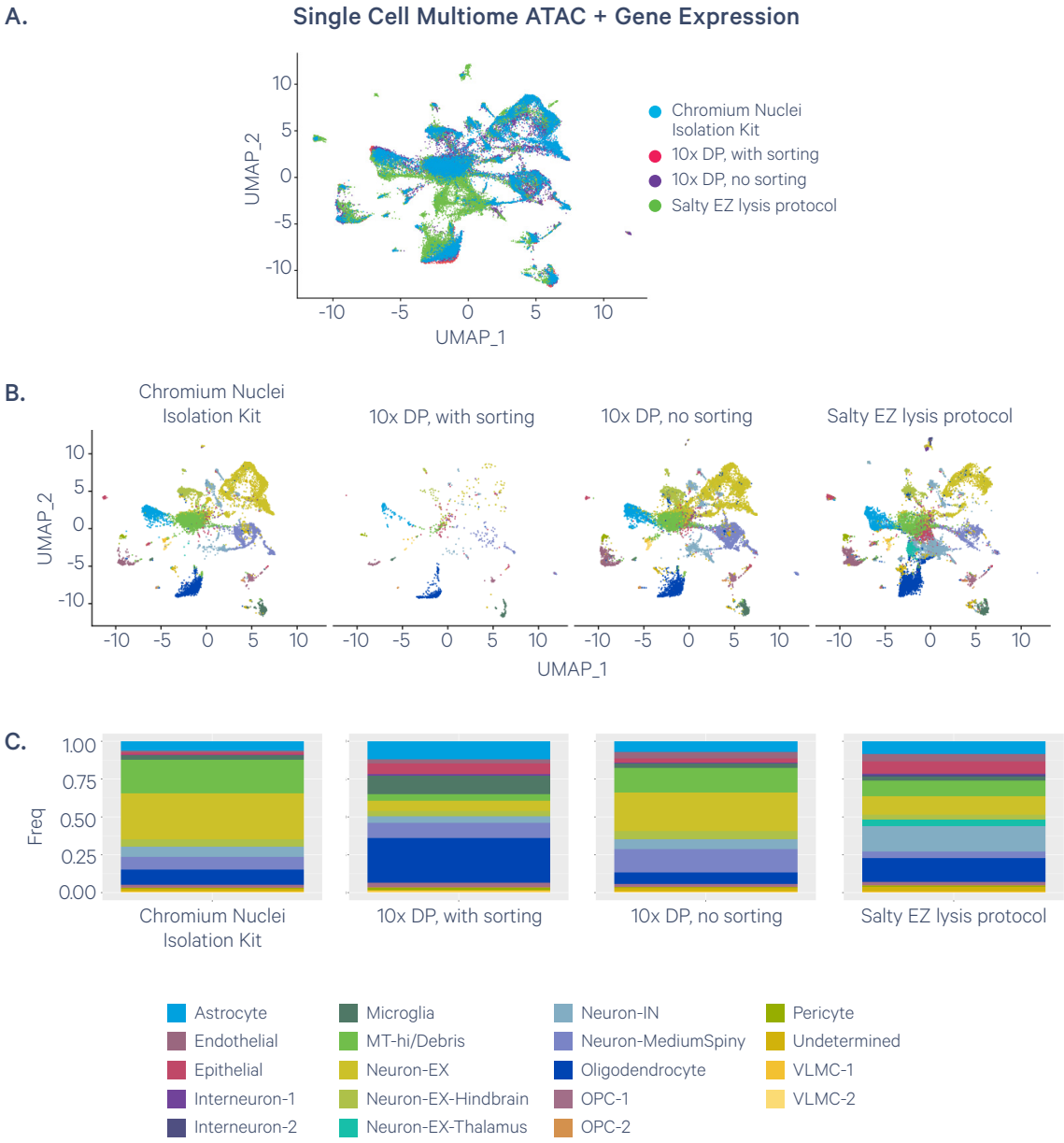

Figure 8. Multiome-based UMAPs (A), Multiome-based cell clustering split by condition (B), and cell type proportions (C) for mouse adult brain tissue samples profiled using the Single Cell Multiome ATAC + Gene Expression assay across the different nuclei isolation methods. Refer to Appendix A4 for Single Cell Gene Expression and ATAC-based data (captured separately) in the Mouse Adult Brain tissue sample.

Intestinal Immune Cell Subpopulations

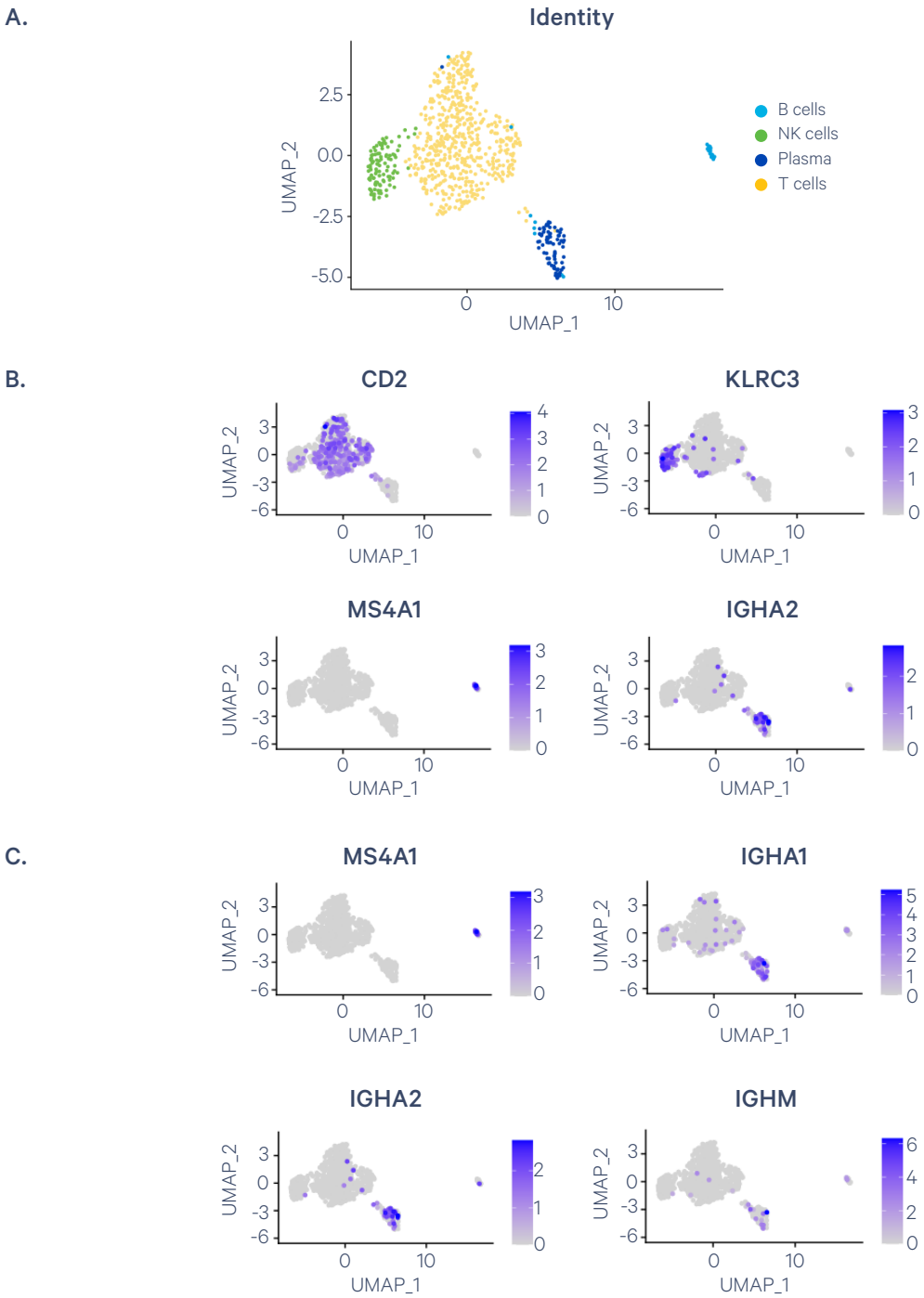

Figure 9. UMAP displaying clusters of immune cell subpopulations derived from human jejunum tissue profiled using the Single Cell Multiome ATAC + Gene Expression assay and the Chromium Nuclei Isolation Kit (A). CD2=T cells, KLRC3=NK cells, MS4A1=B cells, and IGHA2=Plasmsa are markers for lymphocytes (B) and MS4A1, IGHA1, IGHA2, and IGHM are markers for plasma cells (C). MS4A1 distinguishes traditional B cells from plasma cells positive for IGHA1/IGHA2 and less commonly IGHM.

Conclusions

High quality nuclei-derived gene expression and chromatin accessibility data can be generated on multiple tissue types using the Chromium Nuclei Isolation Kit for single cell sequencing. The data presented in this Technical Note clearly demonstrate that data capture and complexity using the Chromium Nuclei Isolation Kit were highly consistent with alternative nuclei isolation methods. Similar cell clusters and cellular populations were detected across the different isolation methods. A loss of rare cell types using the 10x DP with sorting protocol was observed, likely due to the extensive wash steps involved in flow cytometry. The data also demonstrate how a multiomics approach provides a more complete assessment for understanding mammalian tissue biology.

One particular data highlight is found in the nuclei isolated from the human jejunum sample using the Chromium Nuclei Isolation Kit. The mucosa of the intestinal lumen is a key barrier keeping the plethora of bacteria and other antigens in the gut from invading the host. This barrier is kept in balance in large part by the actions of resident T, B, and NK lymphocytes, all of which are clearly separable in the referenced datasets (B cell: Spencer et al., 2016; T cell: Ma et al., 2019; and NK cell: Poggi et al., 2019). The signature expression of immunoglobulin A by plasma cells in the intestinal B cell response is clearly captured in these data, as well as a rarer population of B cells expressing primarily memory B cell markers. While the T cell population is captured in a single cluster, it is polarized by effector T (CD8+) and helper T (CD4+) signatures. Finally, the NK cell population expressed a mixture of resting and regulatory markers, indicative of their complex role in the intestine.

In summary, the standardized protocol and reduced workflow time of the Chromium Nuclei Isolation Kit help to reduce variability across samples and maximize cell type composition, making it a reliable option for isolating nuclei from frozen tissues for use in 10x Genomics Single Cell assays.

References

1. Chromium Next GEM Single Cell Multiome ATAC + Gene Expression User Guide (CG000338)
2. Chromium Nuclei Isolation Reagent Kits Sample Prep User Guide (CG000505)
3. L Martelotto. Protocol for nuclei isolation from fresh and frozen tissues using Salty-Ez10 or Salty-Ez50 buffer: compatible with snRNA-Seq and Multiome workflows from 10x Genomics. *protocols io* (2021)
4. Ma, H., Tao, W. & Zhu, S. T lymphocytes in the intestinal mucosa: defense and tolerance. Cell Mol Immunol 16, 216–224 (2019)
5. Nuclei Isolation from Complex Tissues for Single Cell Multiome ATAC + Gene Expression Sequencing Demonstrated Protocol (CG000375)
6. Poggi et al. Human Gut-Associated Natural Killer Cells in Health and Disease. Frontiers in Immunology (2019)
7. Spencer, J., Sollid, L. The human intestinal B-cell response. Mucosal Immunol 9, 1113–1124 (2016)

Appendix A1

Single Cell Gene Expression & ATAC-based Data for Human Jejunum

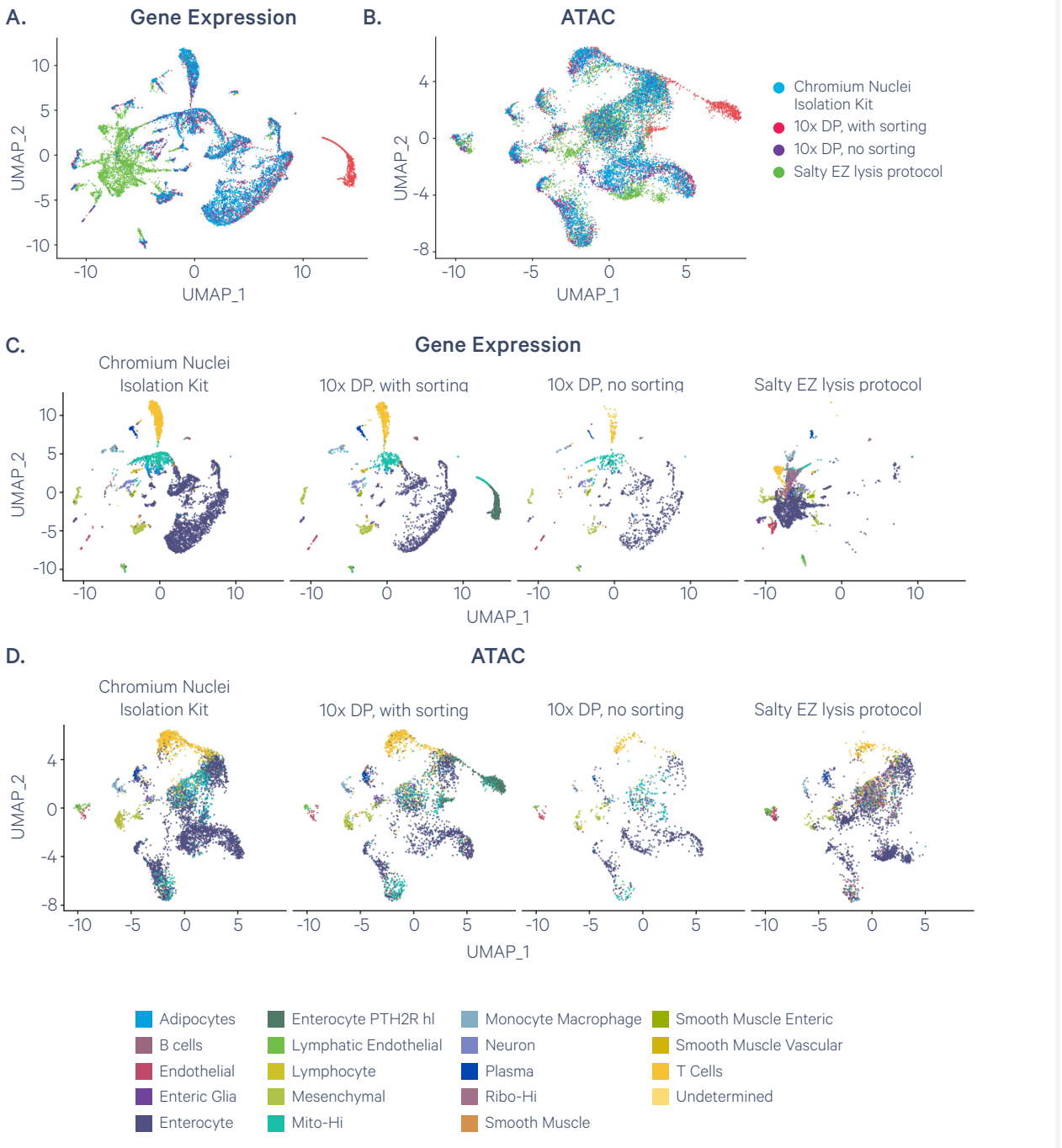

Figure 10. Gene expression (A) and ATAC-based UMAPs (B), gene expression cell clustering split by condition (C), and ATAC-based cell clustering split by condition for human jejunum tissue samples profiled using the Single Cell Multiome ATAC + Gene Expression assay across the different nuclei isolation methods.

Appendix A2

Single Cell Gene Expression & ATAC-based Data for Human Kidney Cancer

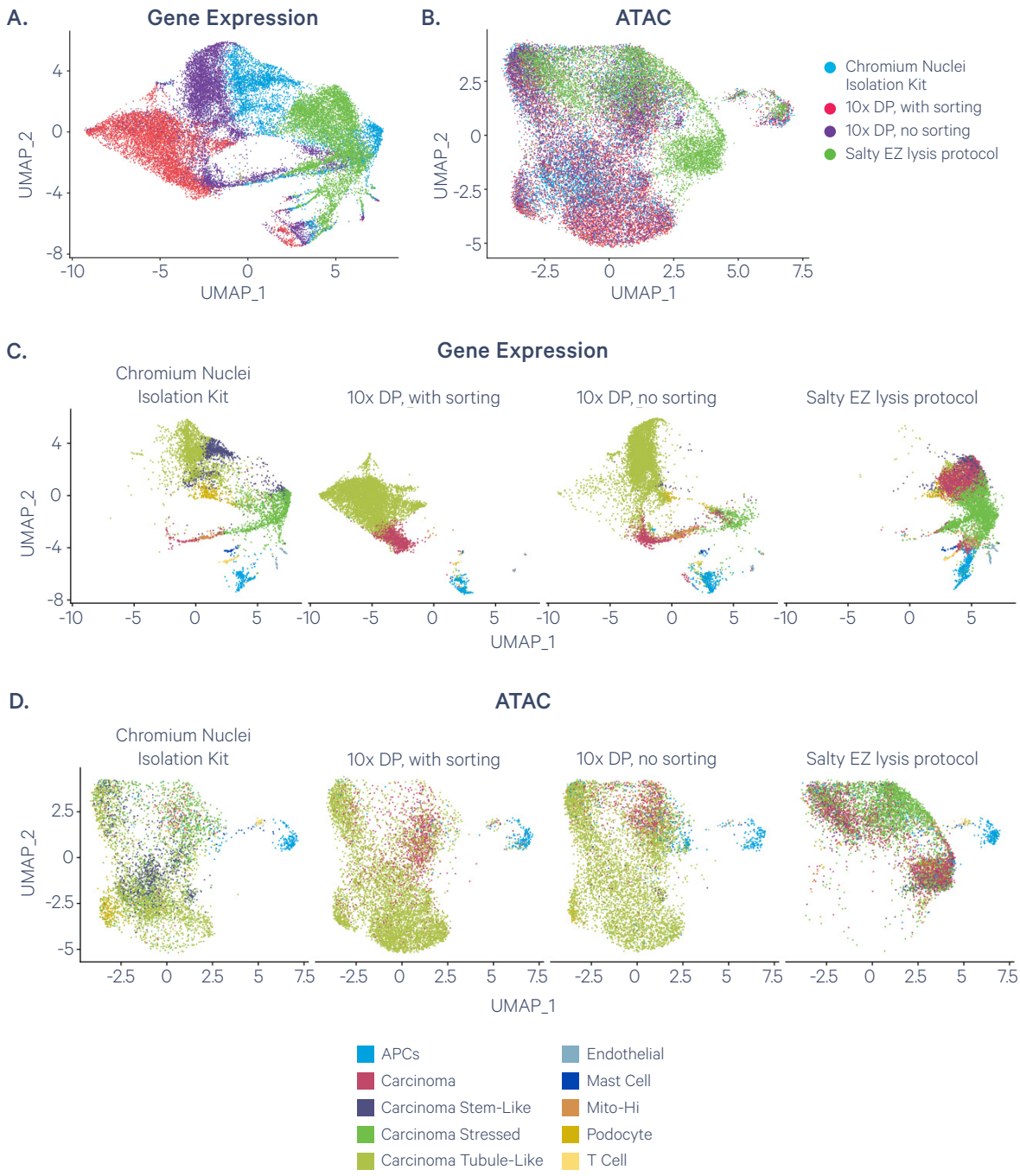

Figure 11. Gene expression (A) and ATAC-based UMAPs (B), gene expression cell clustering split by condition (C), and ATAC-based cell clustering split by condition for human kidney cancer tissue samples profiled using the Single Cell Multiome ATAC + Gene Expression assay across the different nuclei isolation methods.

Appendix A3

Single Cell Gene Expression & ATAC-based Data for Adult Mouse Kidney

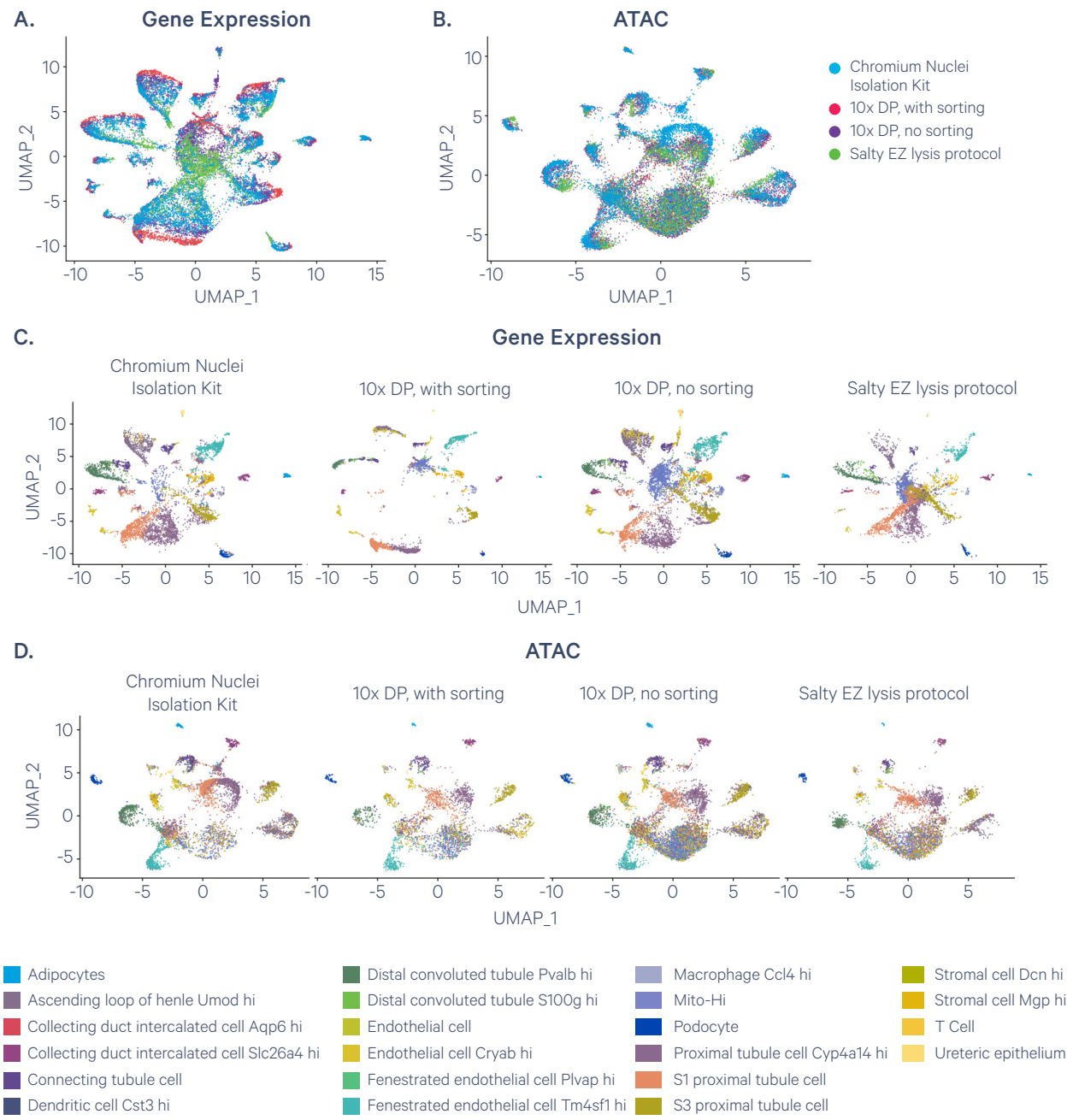

Figure 12. Gene expression (A) and ATAC-based UMAPs (B), gene expression cell clustering split by condition (C), and ATAC-based cell clustering split by condition for adult mouse kidney tissue samples profiled using the Single Cell Multiome ATAC + Gene Expression assay across the different nuclei isolation methods.

Appendix A4

Single Cell Gene Expression & ATAC-based Data for Adult Mouse Brain

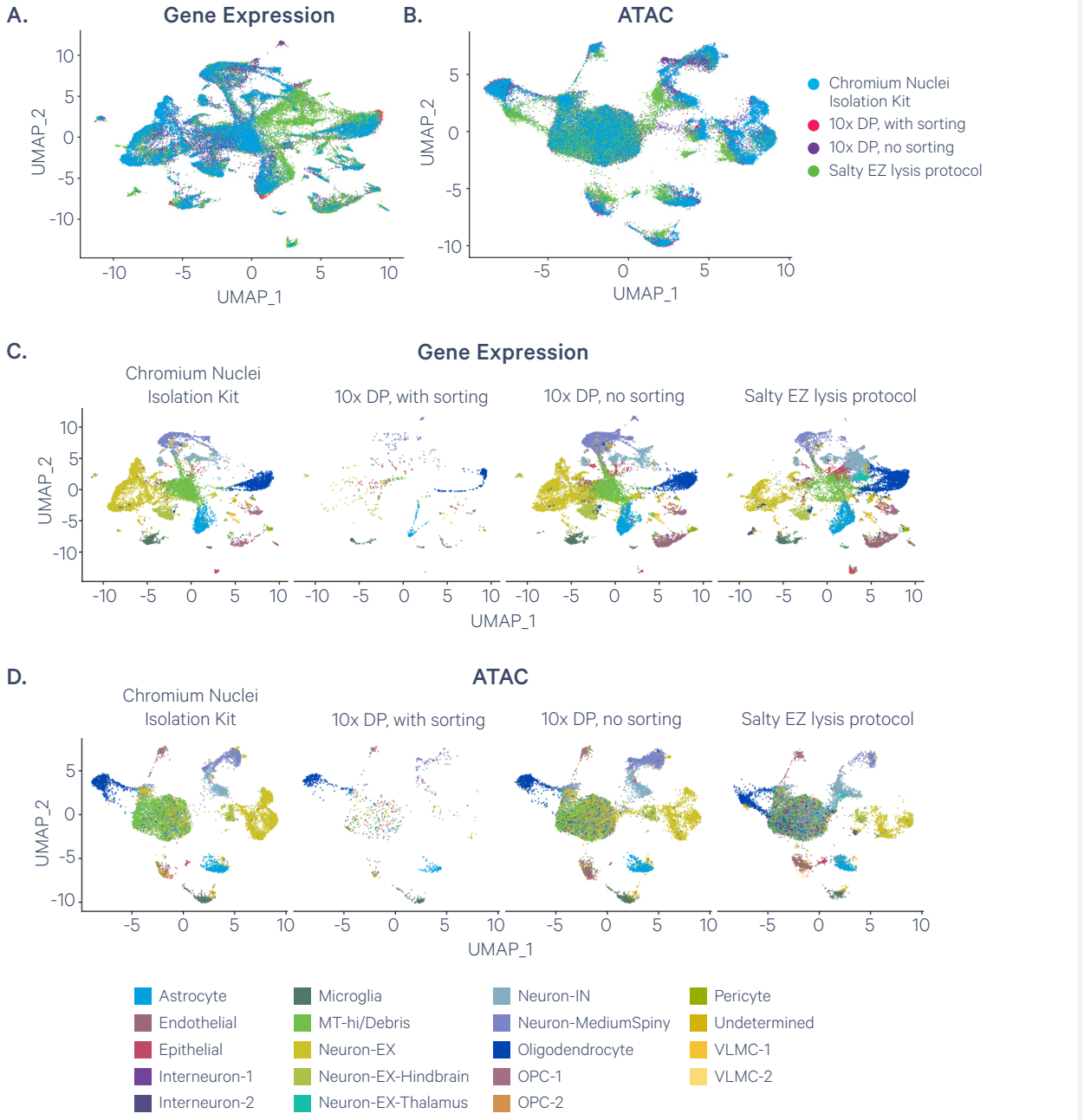

Figure 13. Gene expression (A) and ATAC-based UMAPs (B), gene expression cell clustering split by condition (C), and ATAC-based cell clustering split by condition for adult mouse brain tissue samples profiled using the Single Cell Multiome ATAC + Gene Expression assay across the different nuclei isolation methods.

Document Revision Summary

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