Highly multiplexed single-cell transcriptome and epigenome profiling of cryopreserved tissue enables multi-omic tissue characterization in clinical settings

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Abstract

Tissues are complex structures comprised of many specialized cells, each with distinct functions and underlying regulatory processes. Traditional/bulk characterization techniques are unable to capture such heterogeneity and the complexity of the tissue is lost. High throughput droplet-based methodologies such as single-cell RNA-seq (scRNA-seq) and single-cell ATAC-seq (scATAC-seq) have been recently developed and has allowed for identification of novel cell types and processes involved in normal and pathogenic processes. However, these techniques rely on obtaining fresh tissues and processing immediately after collection which limits their utility in translational applications and clinical settings.

To circumvent these challenges, we established a simple and robust cryopreservation workflow to preserve tissues harvested from a variety of tissues until downstream processing can be performed. Following sample collection and cryopreservation, tissues are thawed and subjected to semi-automated processing with optional cell surface marker enrichment resulting in a cell or nuclei suspension suitable for scRNA-seq and scATAC-seq. The validity of the approach was established by analysis of a diverse range of cryopreserved and fresh tissues. Cell recovery and cell viability were comparable across all tested tissues demonstrating the broad applicability of the approach. Furthermore, profiling by scRNA-seq and scATAC-seq demonstrates that cryopreservation has limited effects on gene expression and chromatin accessibility.

In sum, we have established a workflow to enable analysis of the transcriptome and epigenome at single-cell resolution in translational and clinical samples with equivalent results to fresh tissue. Combining scRNA-seq and scATAC-seq sheds light on the regulatory processes and enables more complete characterization of tissues.
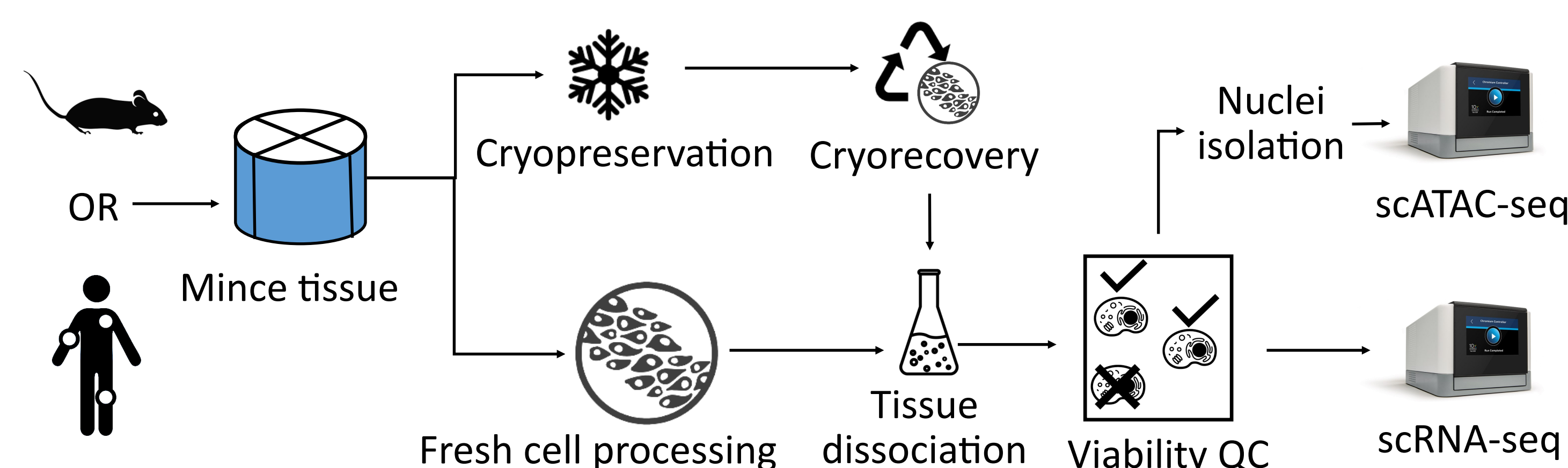
Methods

Figure 1. Schematic of sample processing workflow. Primary tissues are resected from donors, such as clinical or research models. Upon excision, fresh tissue is coarsely minced. One aliquot of tissue is cryopreserved in a proprietary DMSO-based cryopreservation buffer and stored at −80°C until cryorecovery and tissue dissociation. A second aliquot of tissue is immediately dissociated. Fresh and cryorecovered samples are processed identically on different days. Samples are subjected to enzymatic tissue dissociation by either manual or semi-automated processing. The resulting single-cell suspension is counted to determine yield and cell viability. Dead cell removal and/or cell surface marker enrichment may optionally be performed. Aliquots of the single-cell suspension are split for scRNA-seq, whereas a nuclei suspension is prepared for scATAC-seq (10X Genomics).

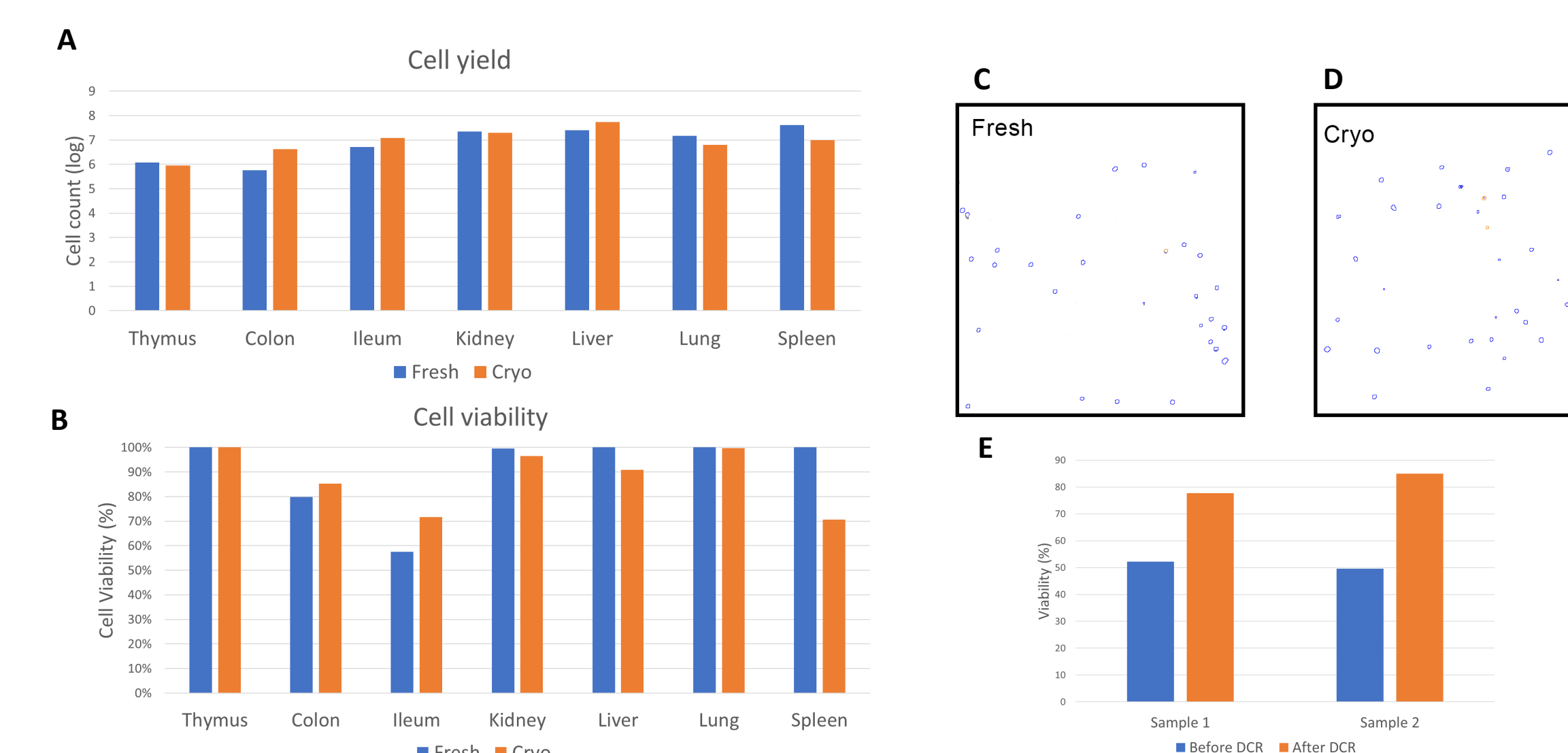## Viability of fresh and cryopreserved tissues

Figure 2. Majority of dissociated tissues have comparable yield and viability after cryopreservation. A, B: Seven mouse tissues were excised and either dissociated immediately or cryopreserved. Yield (A) and viability (B) were equivalent for all tissues except spleen. C, D: Representative images of viability count of fresh (C) and cryopreserved (D) thymus tissue. Blue circles = live cells, red = dead cells. E: Viability can be improved when needed by an optional dead cell removal via MACS.

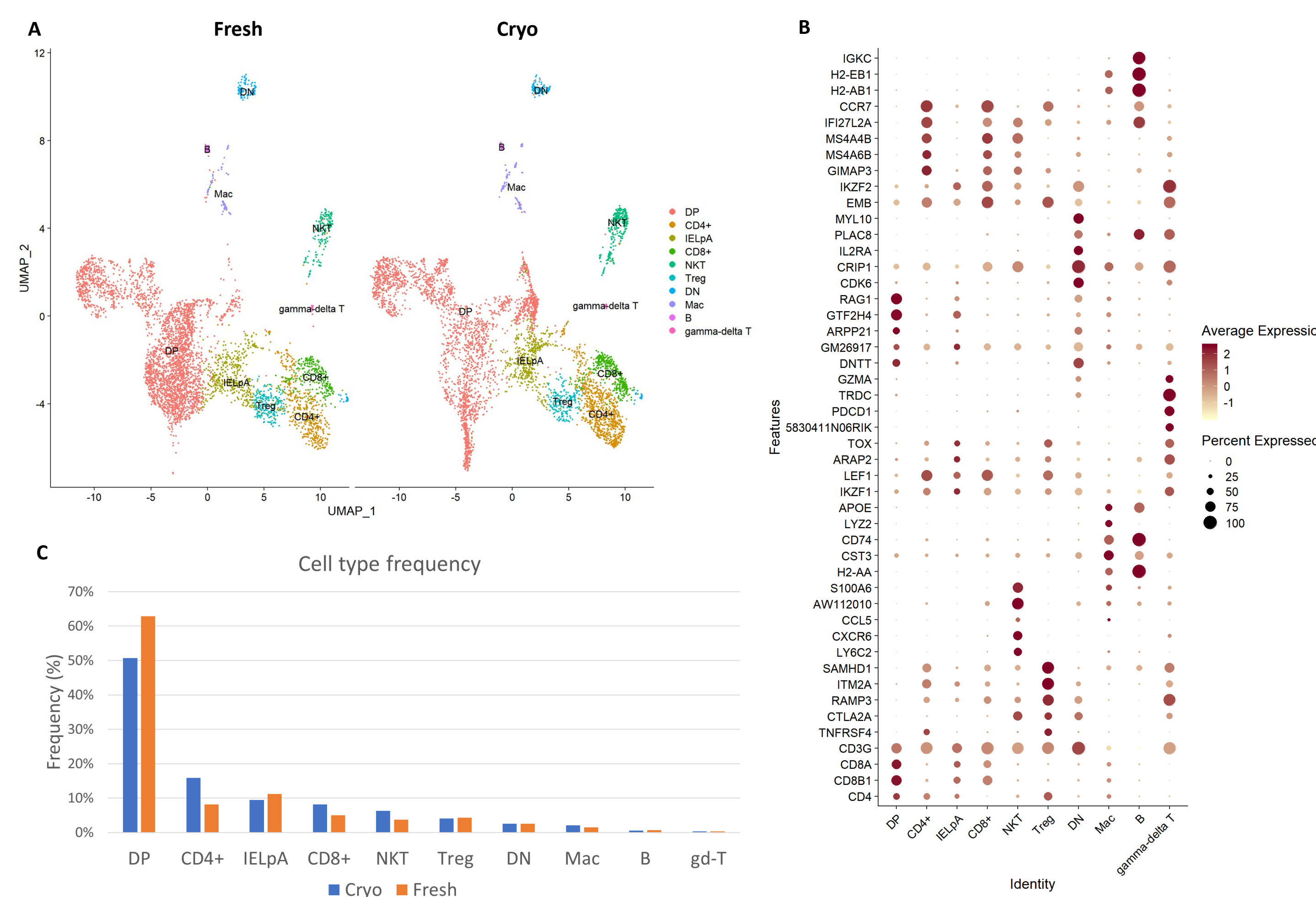## scRNA-seq clustering is comparable

Figure 3. Comparable clustering is obtained in fresh and cryopreserved tissues. A: Fresh (left) and cryopreserved (right) cells were sequenced by 10X scRNA-seq 3' assay and clustered by UMAP projection. Raw data generated by Cell Ranger was processed and integrated using Seurat v3. The putative cell type identities of each cluster were determined by identifying the most differentially expressed genes within each cluster (panel B) and comparing to a previously published thymus single-cell RNA-seq dataset (Park et al., bioRxiv 2020.01.28.911115). This resulted in identification of clusters such as those representing Double Positive (DP) and Double Negative (DN) T cells, as well as single-positive (CD8+ or CD4+) and various other immune cell types. C: The relative proportions of each cell type is plotted.

scATAC-seq and integration with scRNA-seq

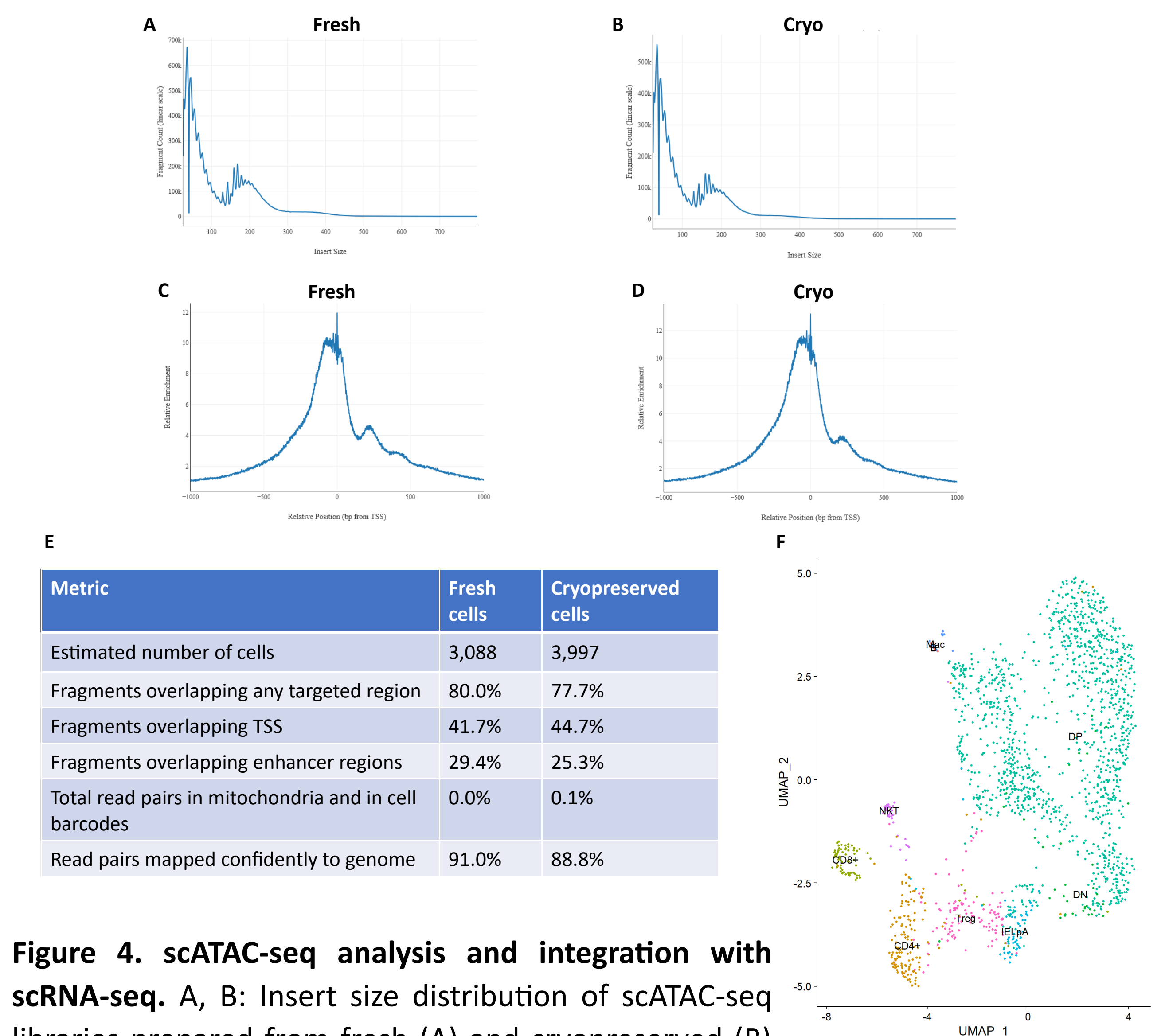| Metric	Fresh cells	Cryopreserved cells
Estimated number of cells	3,088	3,997
Fragments overlapping any targeted region	80.0%	77.7%
Fragments overlapping TSS	41.7%	44.7%
Fragments overlapping enhancer regions	29.4%	25.3%
Total read pairs in mitochondria and in cell barcodes	0.0%	0.1%
Read pairs mapped confidently to genome	91.0%	88.8%

Figure 4. scATAC-seq analysis and integration with scRNA-seq. A, B: Insert size distribution of scATAC-seq libraries prepared from fresh (A) and cryopreserved (B) cells, and corresponding TSS enrichment plots (C: fresh; D: cryopreserved). Library QC summary is also shown (E). F: The putative cell type cluster identities identified by scRNA-seq were integrated with scATAC-seq. Raw data generated by Cell Ranger was processed and normalized using Seurat v3. Gene activity matrix is derived from counts in the gene body and promoter regions (upstream 2kb from TSS), and the matrix is then used to integrate with scRNA data to determine putative cell types.

Conclusions & Future Directions

scRNA-seq and scATAC-seq are powerful techniques for investigating gene expression and chromatin signatures in complex tissues. Since these methods require viable cells as input, cryopreservation of samples is often necessary in lieu of immediate processing. To aid in translating these approaches to the clinic, we have developed a simple workflow to cryopreserve tissues while minimizing transcriptional and epigenomic alterations.

Future directions include:

- Continued development and validation of the approach with additional tissue types
- Deeper characterization of scATAC-seq findings and multi-omic data integration
- Applying the approach to cell surface marker/feature barcoding

Acknowledgements