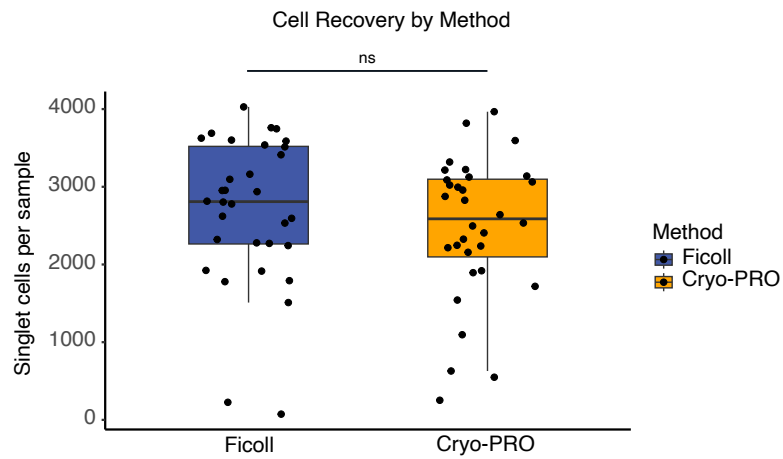
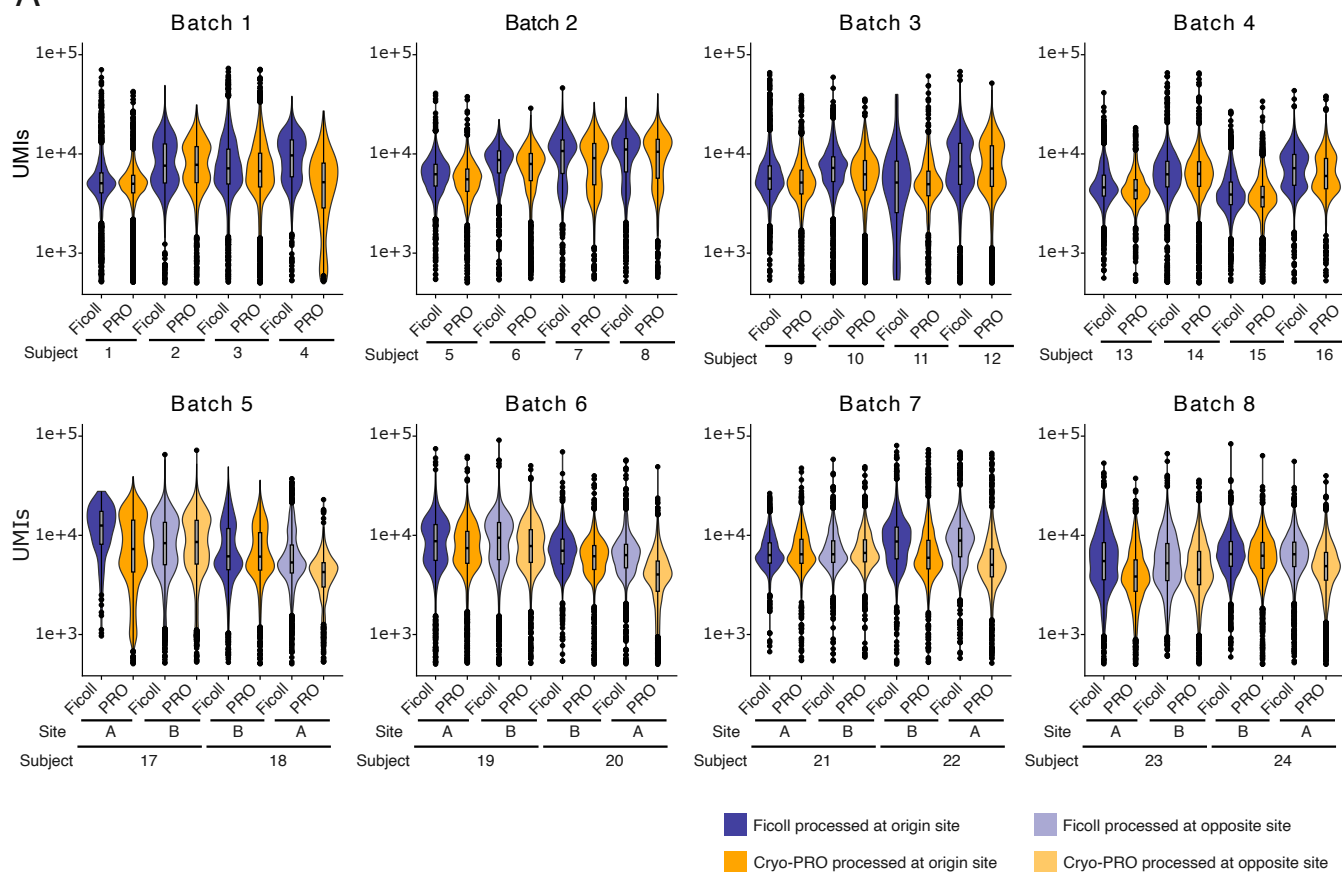




Supplemental Figure 1. Number of singlet cells sequenced per method. Starting blood sample volume was variable in Ficoll samples and was 1 mL in Cryo-PRO samples. Because Ficoll input volumes varied and were not recorded precisely, these values are not a direct comparison of recovery efficiency between methods. Statistical significance was generated using a Welch's two-sample t test assuming unequal variances (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, ns = not significant).

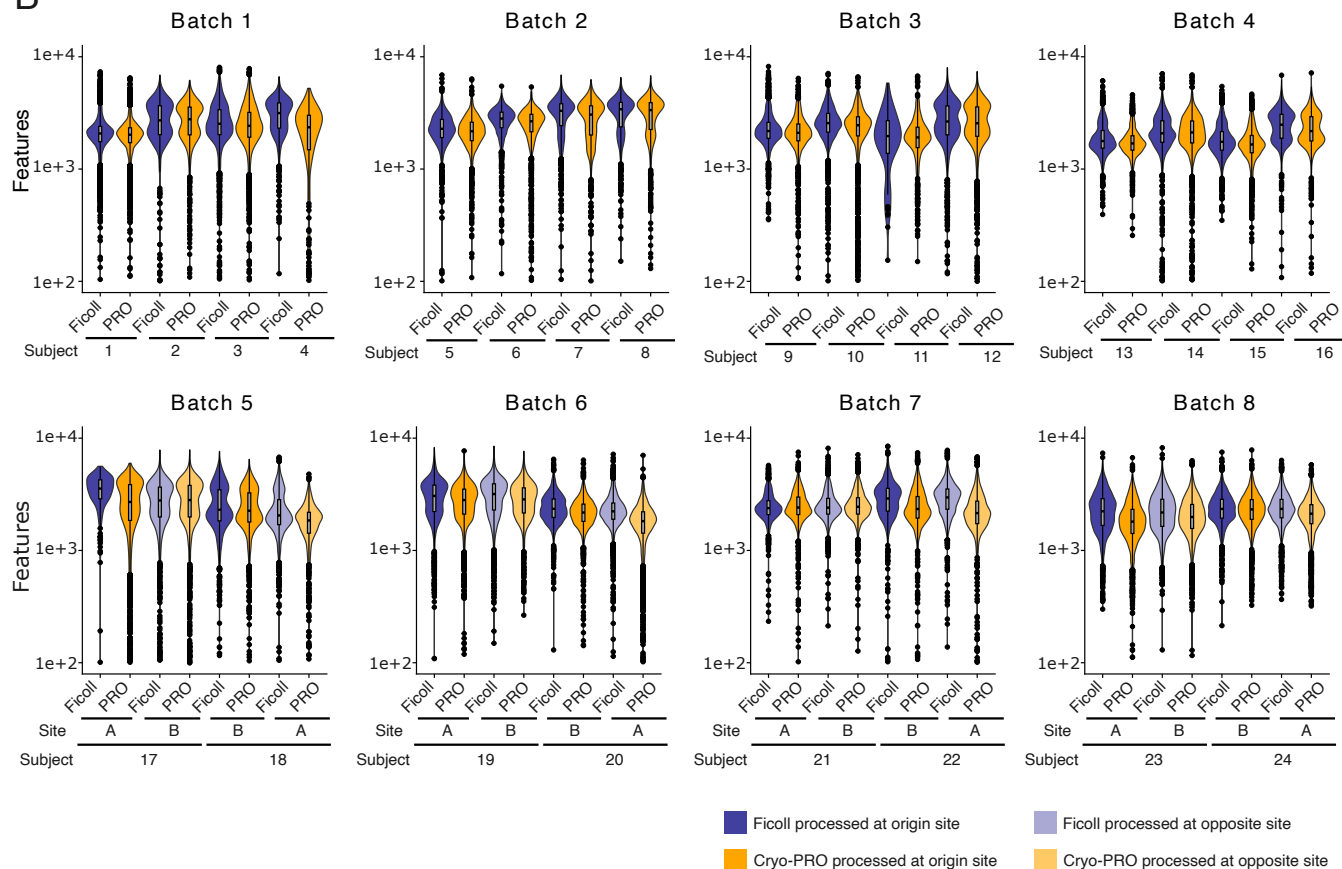
A

Unique Molecular Identifiers Per Sample



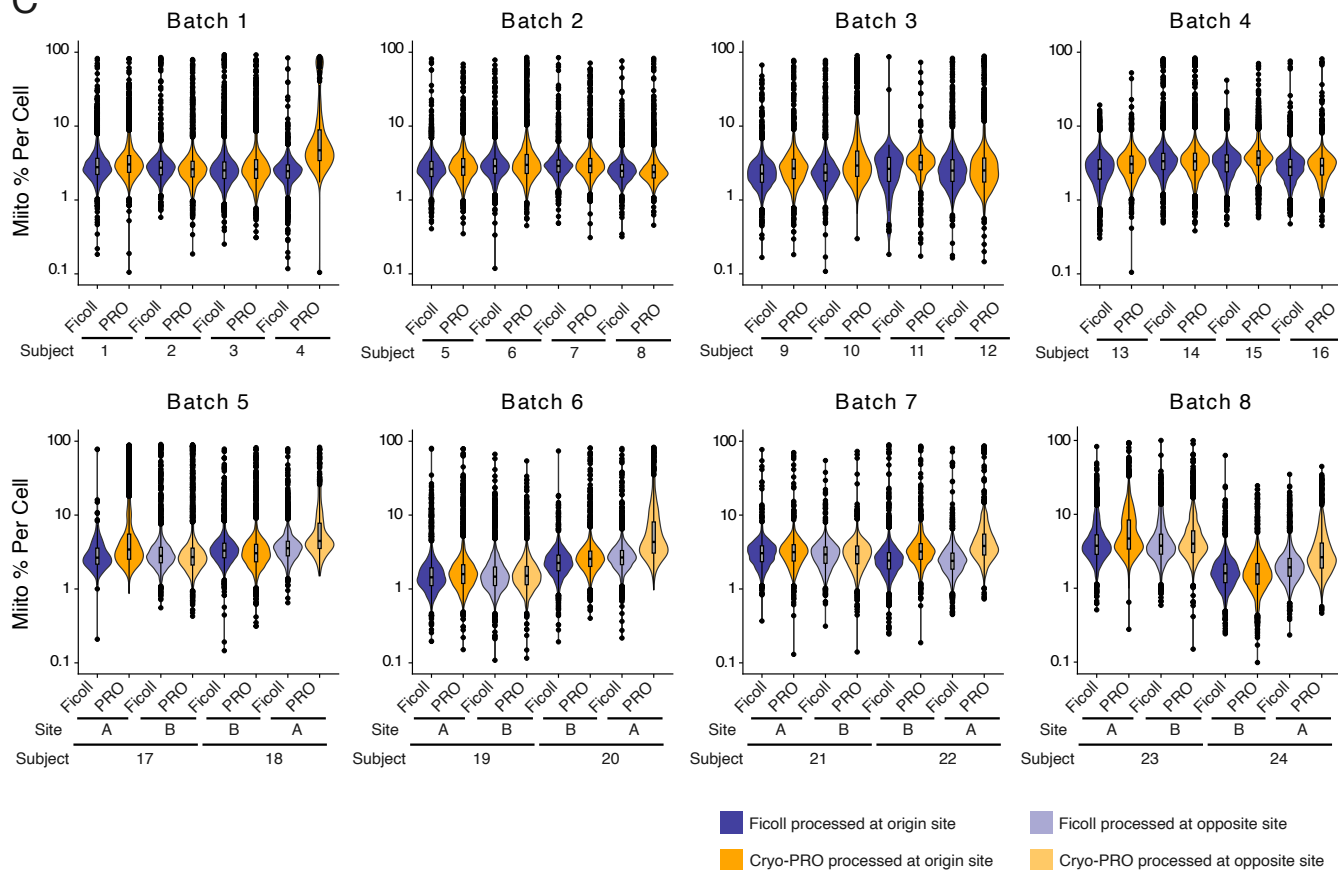
B

Unique Genes Per Sample



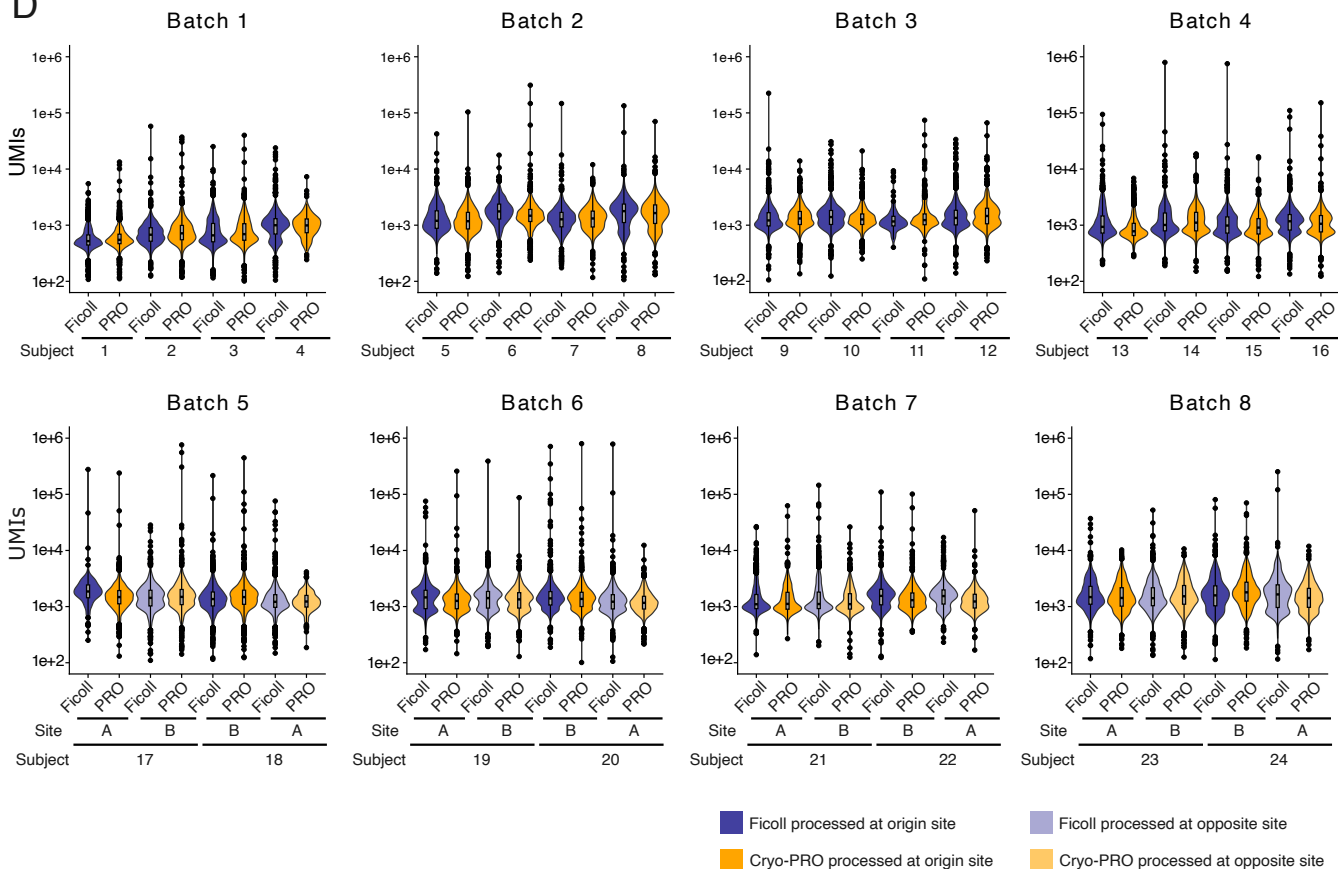
C

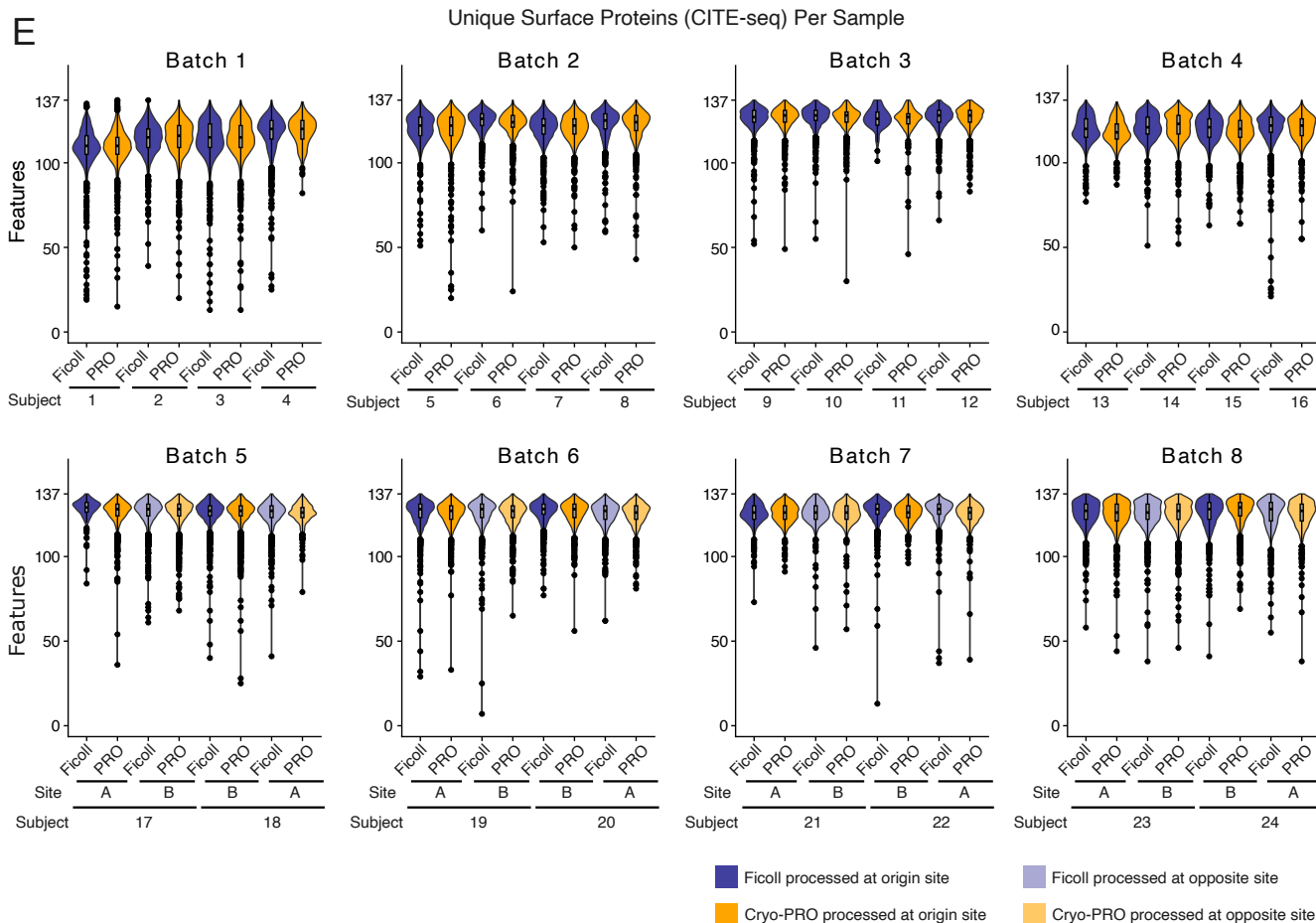
Percent Mitochondrial Genes Per Sample



D

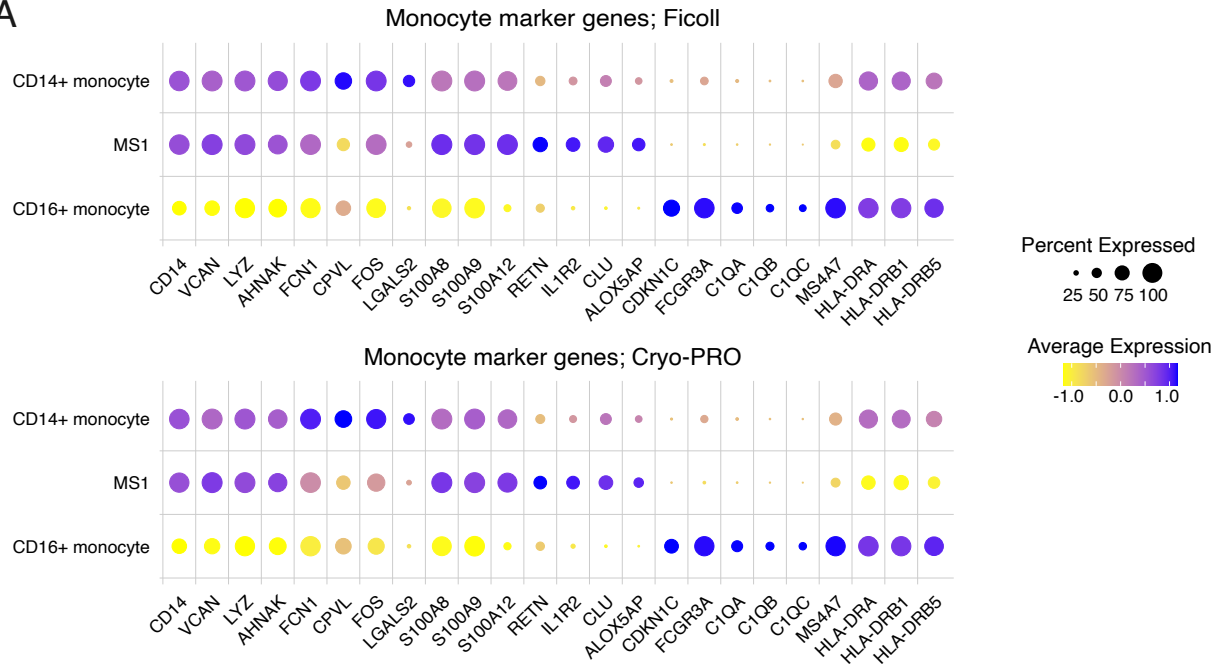
Unique Molecular Identifiers (CITE-Seq) Per Sample



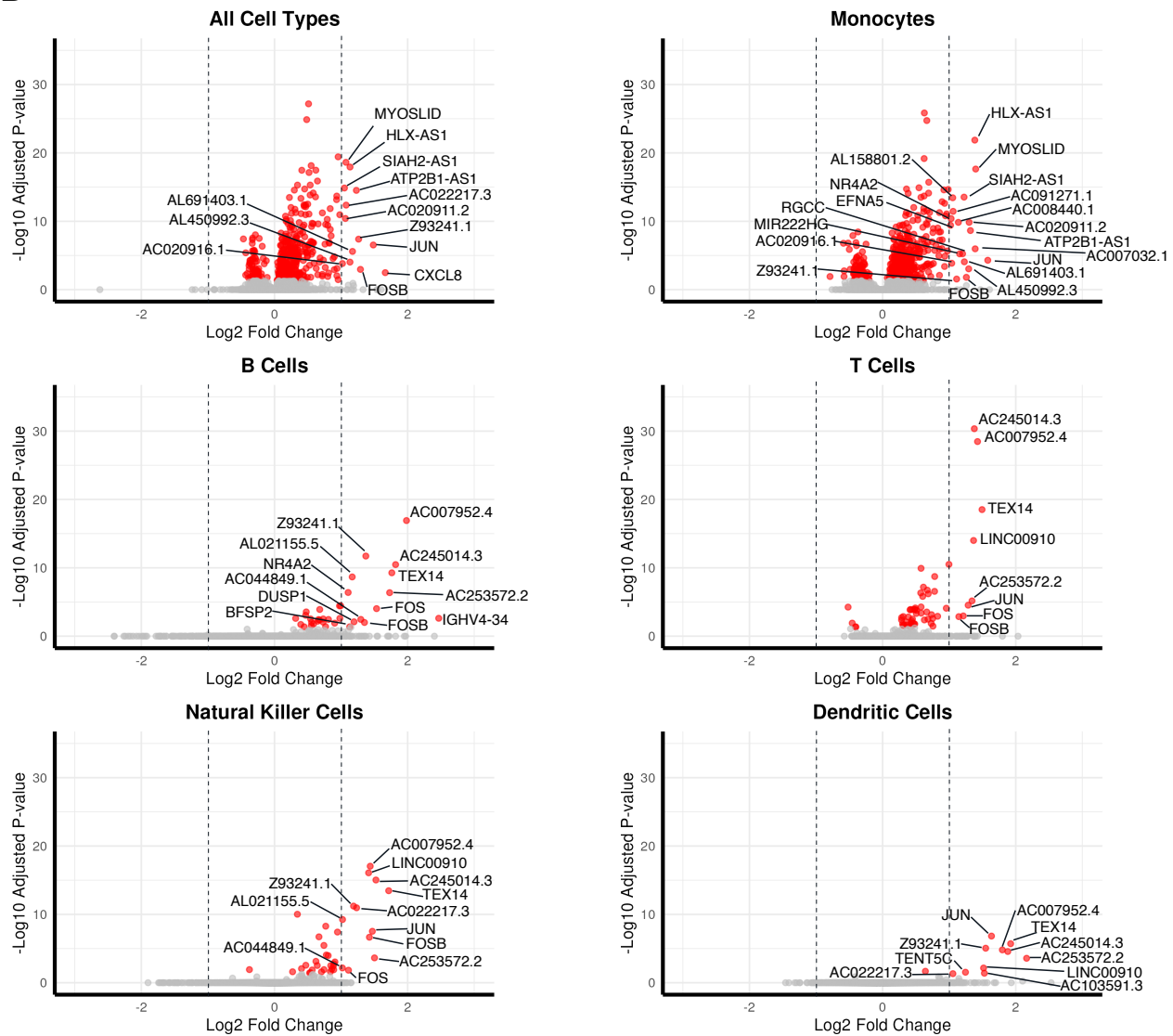


Supplemental Figure 2. Per-sample distributions of **(A)** UMIs of RNA transcripts, **(B)** unique genes, **(C)** percentage of mitochondrial transcripts, **(D)** unique surface protein features detected via CITE-seq, and **(E)** UMIs of surface protein features detected via CITE-seq per cell. Batches represent samples that were thawed, processed and sequenced together. Ficoll and Cryo-PRO samples from the same patient are plotted next to each other. For patients where parallel processing occurred at both clinical sites (bottom rows), the samples processed at the opposite site of enrollment are shown in lighter shades. A total of 137 different surface proteins were queried in the CITE-seq analysis. Violin plots show the distribution of each metric detected per cell; embedded boxplots indicate the median and IQR (whiskers = 1.5x IQR). PRO denotes Cryo-PRO.

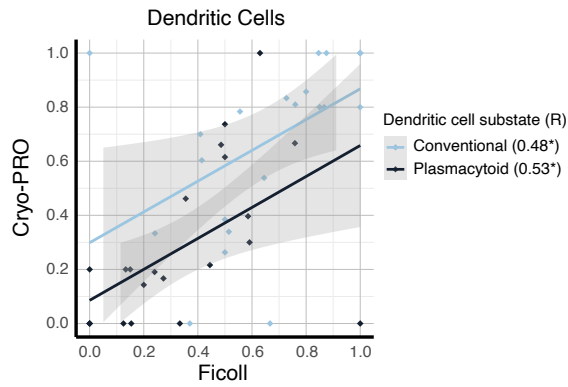
A



B



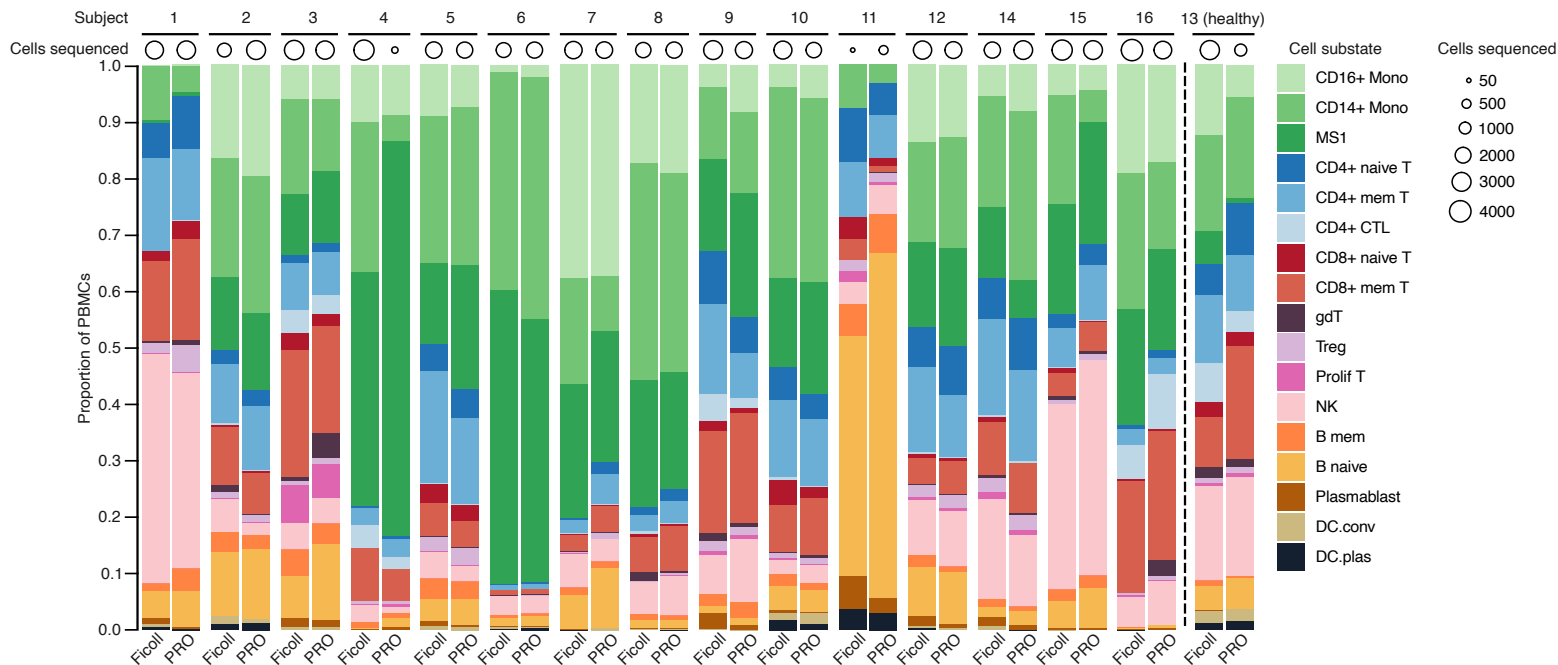
Supplemental Figure 3. Dot plots of marker gene expression by each monocyte substate **(A)**. Color represents scaled relative expression (blue = higher expression). Size represents the proportion of cells in each substate where the feature was detected. Volcano plots showing genes differentially up-regulated (positive Log2FC) or down-regulated (negative Log2FC) in Ficoll compared to Cryo-PRO after pseudobulk analysis **(B)**. Genes with adjusted p-values of less than 0.05 are shown in red; those with $p < 0.05$ and $\text{abs}(\log_2\text{FC}) > 1$ are labeled. Plots are shown for differential gene expression among all cells (top left) and for each major cell type (subsequent plots).



Supplemental Figure 4. Scatter plot of dendritic cell substate proportion from Ficoll and Cryo-PRO. Each point represents the proportion of one cell substate from one patient sample, as measured by each method. Each cell substate is represented by a different color. Proportion is the number of cells of one cell substate divided by the total number of dendritic cells from that patient sample. Patient-paired Ficoll:Cryo-PRO samples are plotted to assess correlation in method for each patient. Trendlines indicate a linear regression fit with shaded 95% confidence intervals. Pearson's correlations (R) are shown for all correlations (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

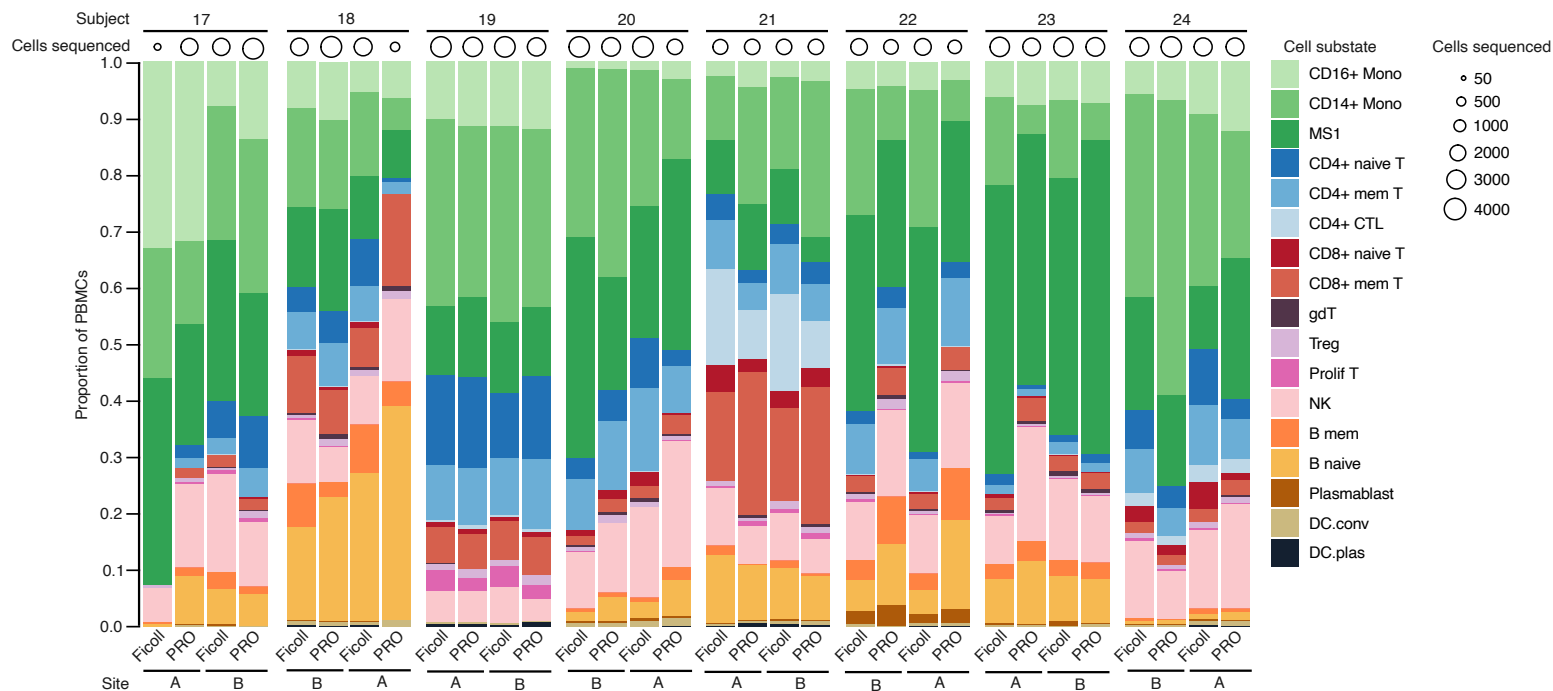
A

Cell Substate Proportion Per Sample (Samples Processed at Origin Site)

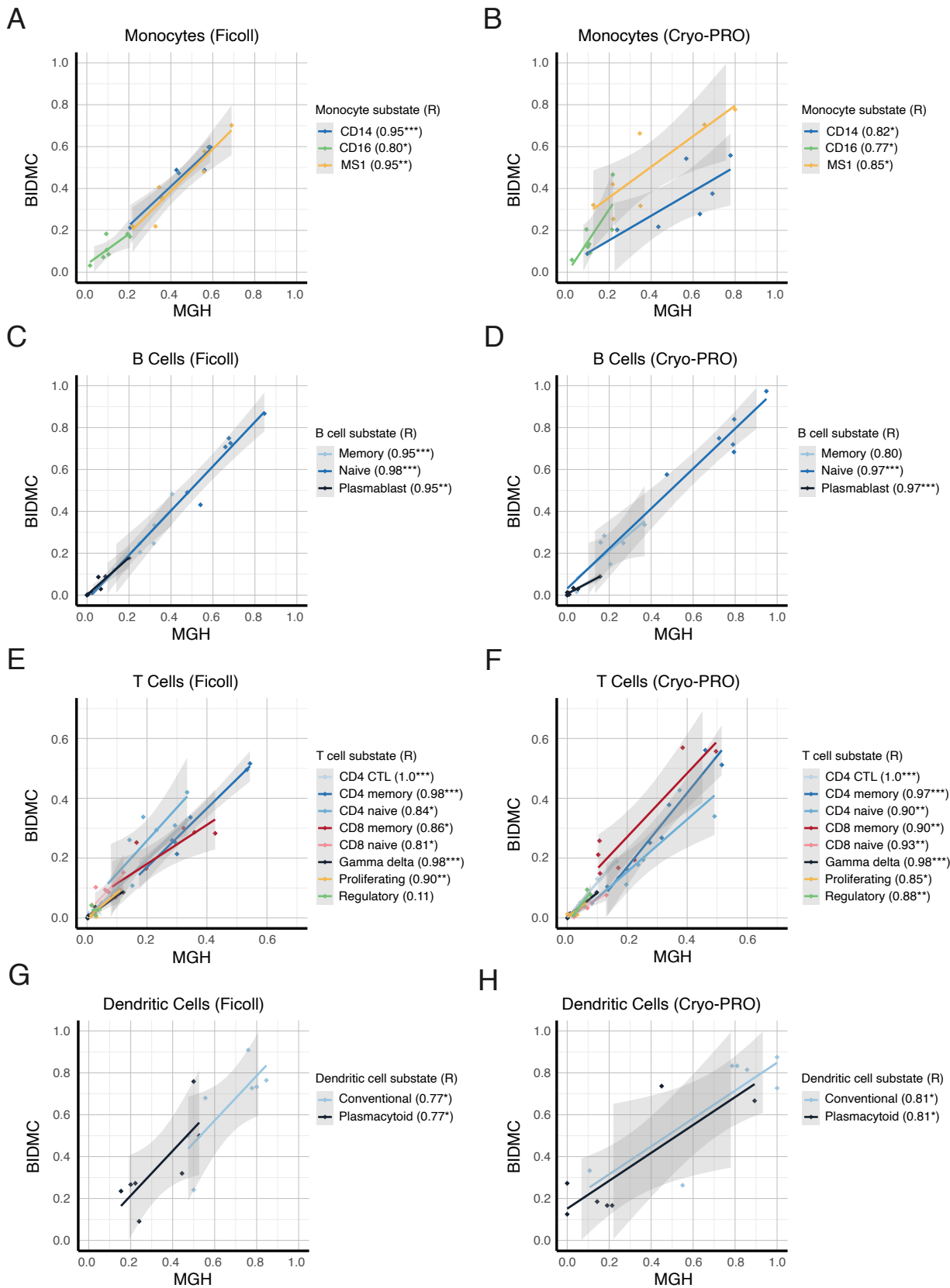


B

Cell Substate Proportion Per Sample (Samples Processed at Both Centers)



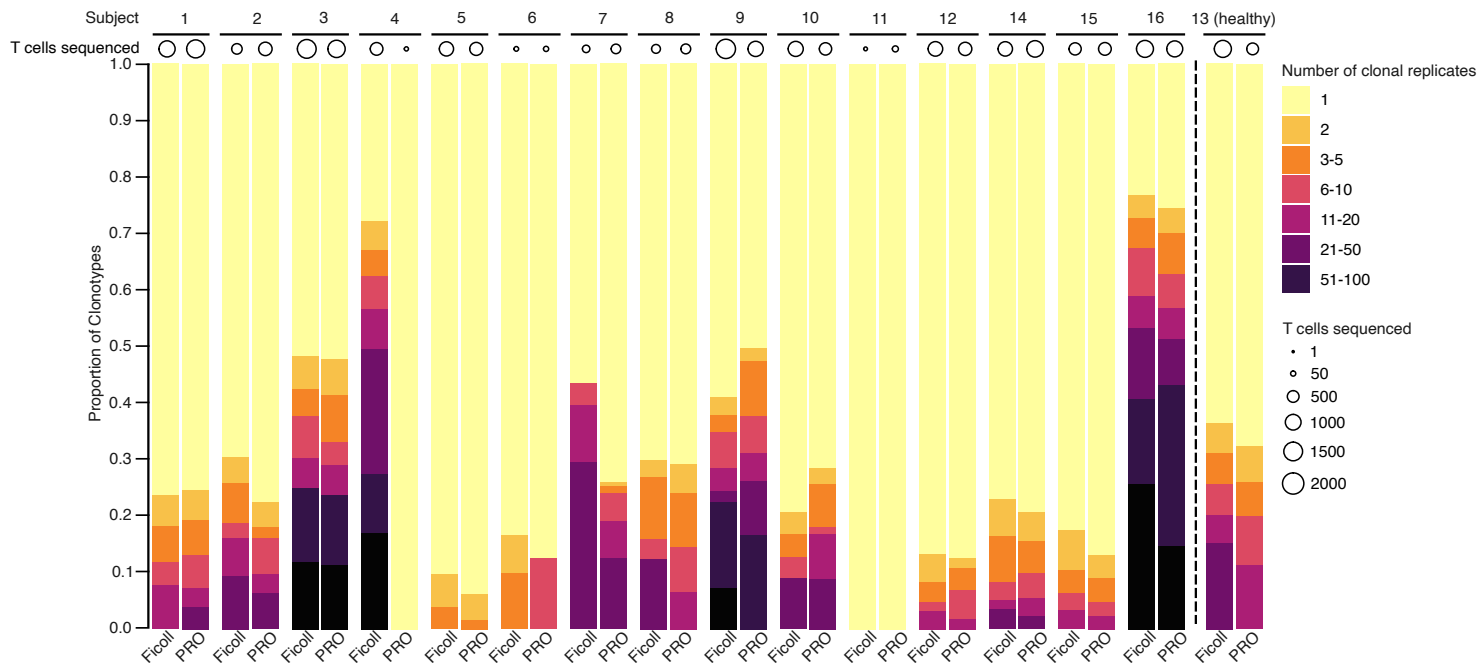
Supplemental Figure 5. Cell substate proportions for samples processed at single centers **(A)** and technical duplicate samples processed at both centers **(B)**. Samples from the same patient processed using different methods are shown next to each other. In **(B)**, the corresponding pair of technical duplicates processed at the non-origin site are shown subsequently, with the labeled site indicating where each sample was processed. PRO denotes Cryo-PRO.



Supplemental Figure 6. Scatter plots of cell substate proportions from different processing sites. Each cell substate is represented by a different color. Proportion is the number of cells of one cell substate divided by the total number of cells from its cell type from that patient sample. The patient-paired Ficoll:Ficoll samples and Cryo-PRO:Cryo-PRO samples from the two different enrollment sites are plotted to assess correlation of technical duplicates for each patient. Trendlines indicate a linear regression fit with shaded 95% confidence intervals. Pearson's correlations (R, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$) are shown for all correlations.

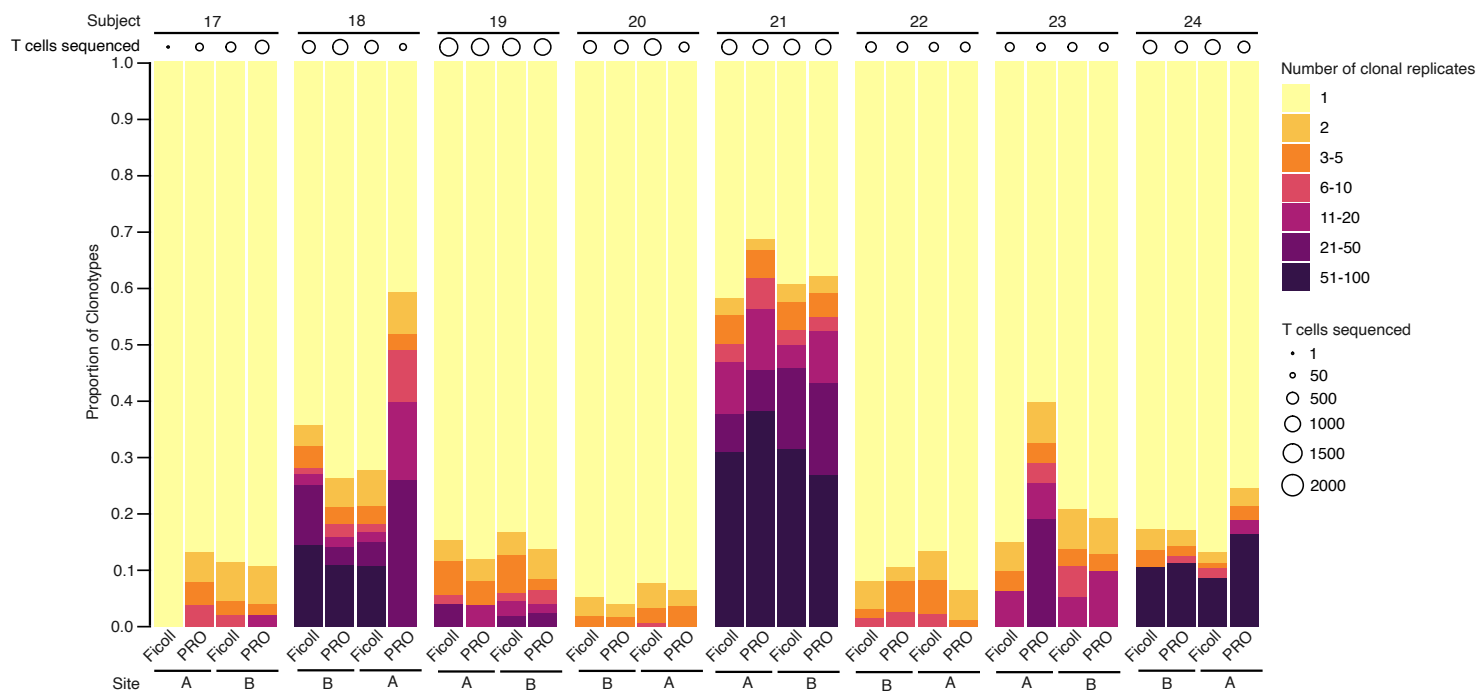
A

Clonal Proportion Per Sample (Samples Processed at Origin Site)

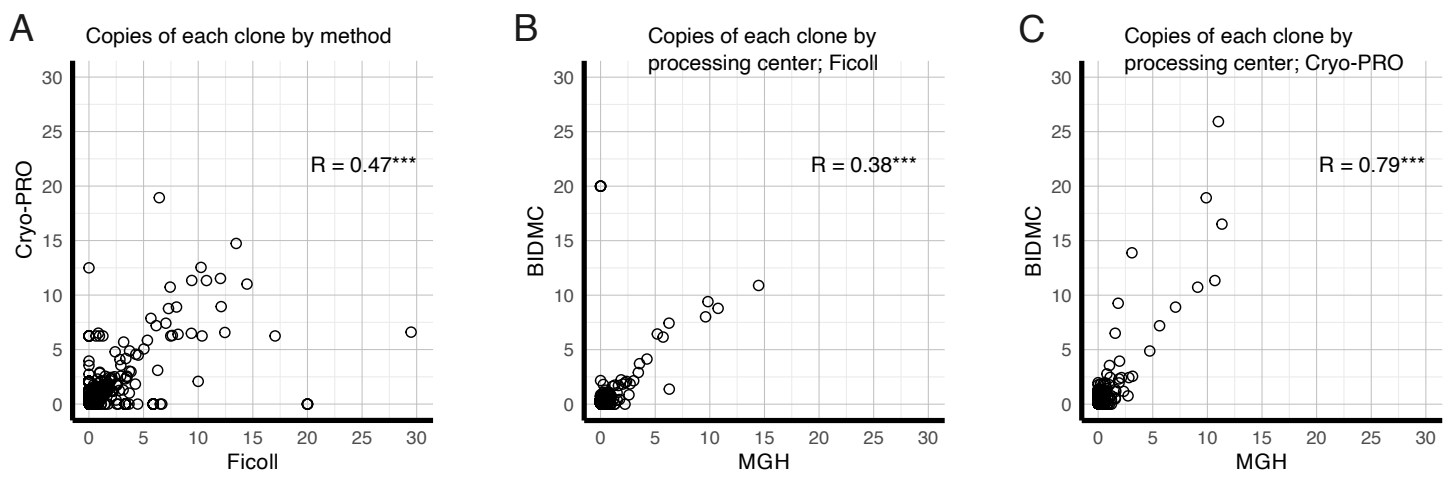


B

Clonal Proportion Per Sample (Samples Processed at Both Centers)

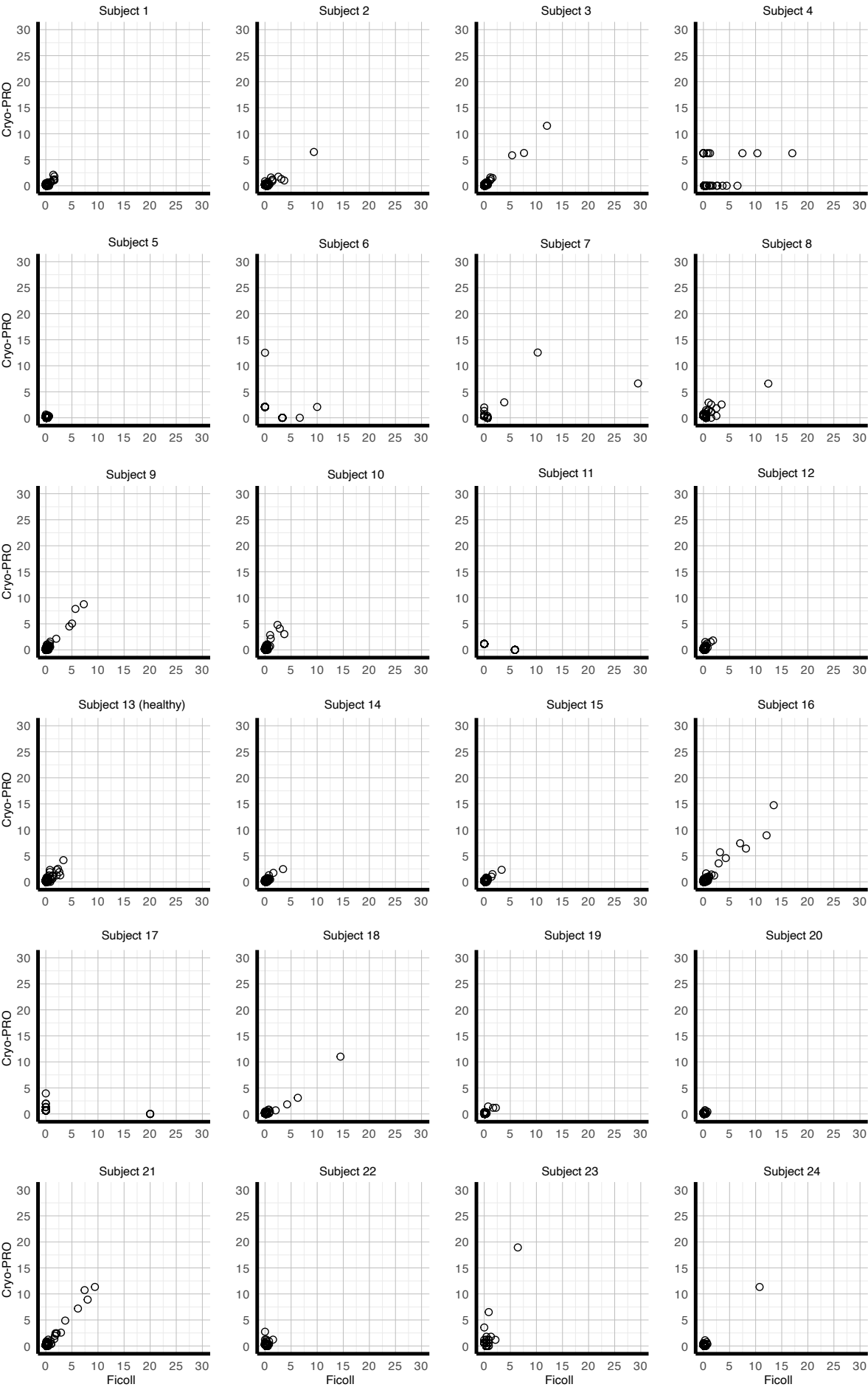


Supplemental Figure 7. Clonal expansion proportions for samples processed at single centers (**A**) and technical duplicate samples processed at both centers (**B**). Samples from the same patient processed using different methods are shown next to each other. In (B), the corresponding pair of technical duplicates processed at the non-origin site are shown subsequently, with the labeled site indicating where each sample was processed. PRO denotes Cryo-PRO.



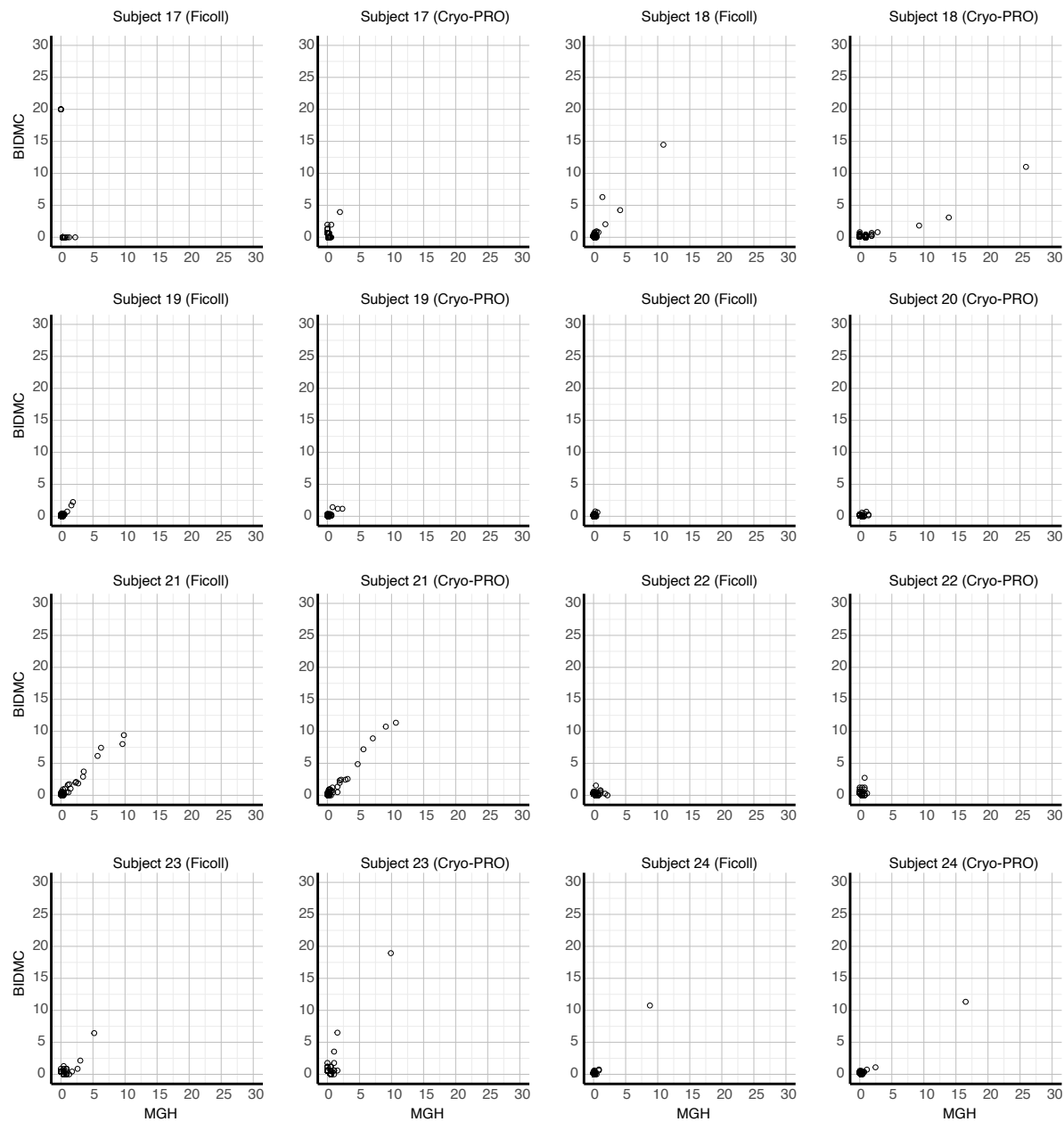
Supplemental Figure 8. Scatter plot of the clonotype sequence frequency for all samples processed by different methods at single centers (**A**) and for technical duplicate samples processed at both centers by Ficoll (**B**) and Cryo-PRO (**C**). The proportion of each unique sequence out of total TCR transcripts in each sample is plotted against its corresponding sample to compare similarities in Ficoll vs Cryo-PRO (A), or in processing center (B,C). Pearson's correlations (R , $*p < 0.05$, $**p < 0.01$, $***p < 0.001$) are shown for all correlations.

Comparison of the proportion of each T cell receptor sequence detected between methods



Supplemental Figure 9. Scatter plot of clonotype sequence frequency for all samples processed by different methods at single centers, individually plotted for each subject. The proportion of each unique sequence out of total TCR transcripts in each Ficoll sample is plotted against its corresponding Cryo-PRO sample to compare similarities in TCR clonal proportion.

Comparison of the proportion of each T cell receptor sequence detected between processing centers



Supplemental Figure 10. Scatter plot of the clonotype sequence frequency for technical duplicate samples processed at both centers by Ficoll (left) and Cryo-PRO (right), individually plotted for each subject. The proportion of each unique sequence out of total TCR transcripts in each MGH sample is plotted against its corresponding BIDMC sample to compare similarities in TCR clonal proportion.