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Supplemental information

A structured evaluation of cryopreservation in generating single-cell transcriptomes from cerebrospinal fluid

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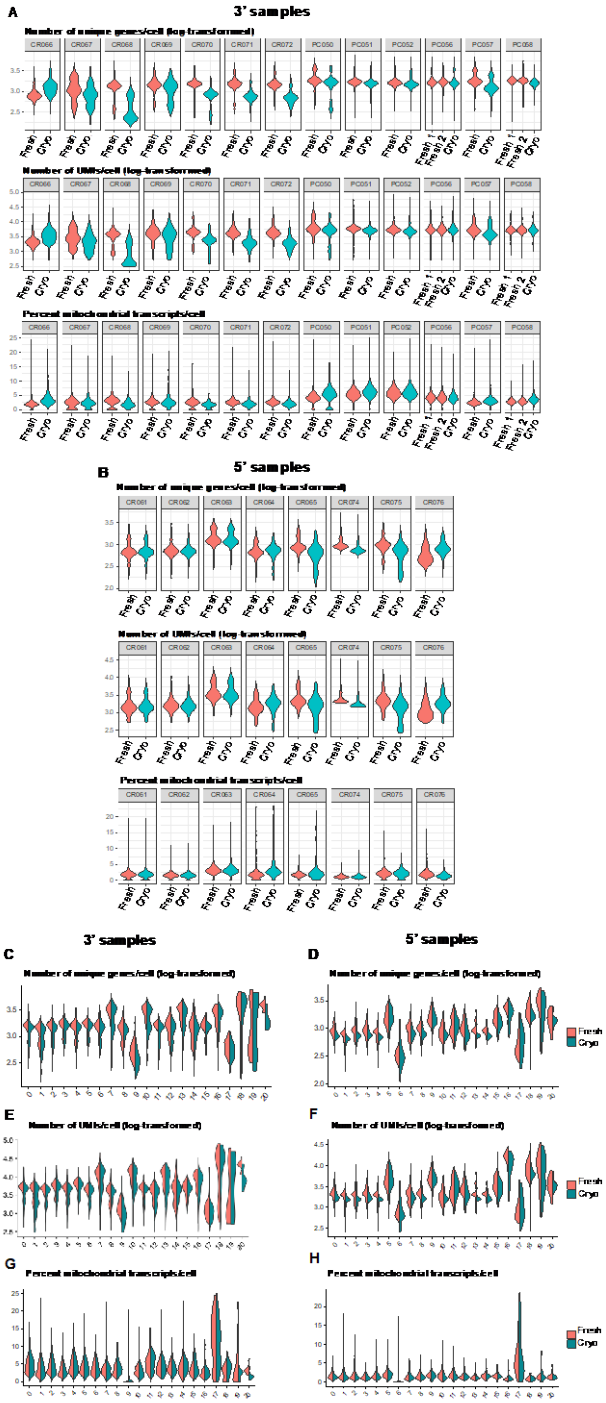

Figure S1: Sample and cell cluster level RNA sequencing quality control (QC), related to Figure 2 and 3. (A) Sequencing QC metrics in 28 samples sequenced using 3' chemistry across Columbia university and University Hospital Basel, and **(B)** 16 samples using 5' sequencing chemistry from University hospital Basel samples. **(A-B)** Violin plot of the number of unique genes, **(A-B)** number of unique transcripts, and **(A-B)** percentage of mitochondrial reads in each fresh-cryopreserved sample-pair. The values of number of detected genes and transcripts is on the log scale. **(C-E-G)** Sequencing QC metrics in 21 clusters from 3' samples, and **(D-F-H)** in 21 clusters from 5' samples. **(C-D)** Violin plot showing the similar distribution of the number of genes, **(E-F)** number of unique transcripts, and **(G-H)** percentage of mitochondrial genes in each cluster. The number of detected genes and transcripts in depicted on a log scale. Pink colored density plots represent fresh cells and blue colored plots represent cryopreserved cells in each cluster.

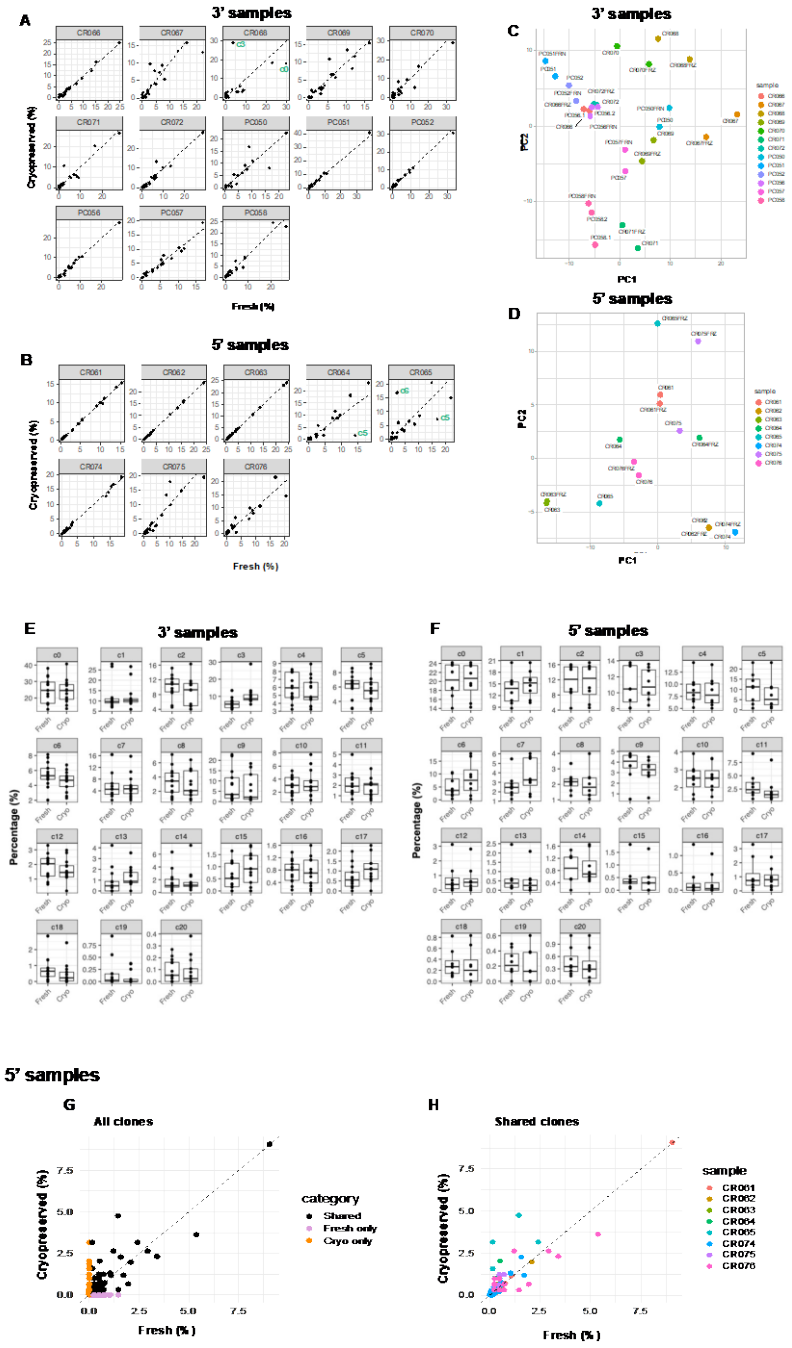

Figure S2. Efficient recovery of CSF cell types upon cryopreservation and TCR and BCR clonotypes, related to Figure 2 and 3. (A) Correlation of cluster frequencies (%) between fresh and cryopreserved samples pairs, analyzed using 3' and **(B)** 5' sequencing chemistry, respectively. **(B)** Clusters with >10% difference in frequency between fresh and cryopreserved pairs are labeled c0: $CD4^+$ TEM, c3: $CD4^+$ TCM **(A)**; c5 and c6: CSF myeloid cells. **(C)** PCA plot of samples based on cluster frequencies for 3' sequencing chemistry samples, **(D)** 5' sequencing chemistry samples. **(E)** Frequencies of individual clusters in fresh and cryopreserved samples sequenced using 3', and **(F)** 5' sequencing chemistry, respectively. Clonotype sharing between fresh and cryopreserved CSF samples and across each cluster processed using 5' sequencing chemistry. **(G)** Frequencies (%) percentage of all identified clonotype. **(H)** Correlation between clonotype frequencies shared between fresh and cryopreserved sample pairs ($p<0.001$).

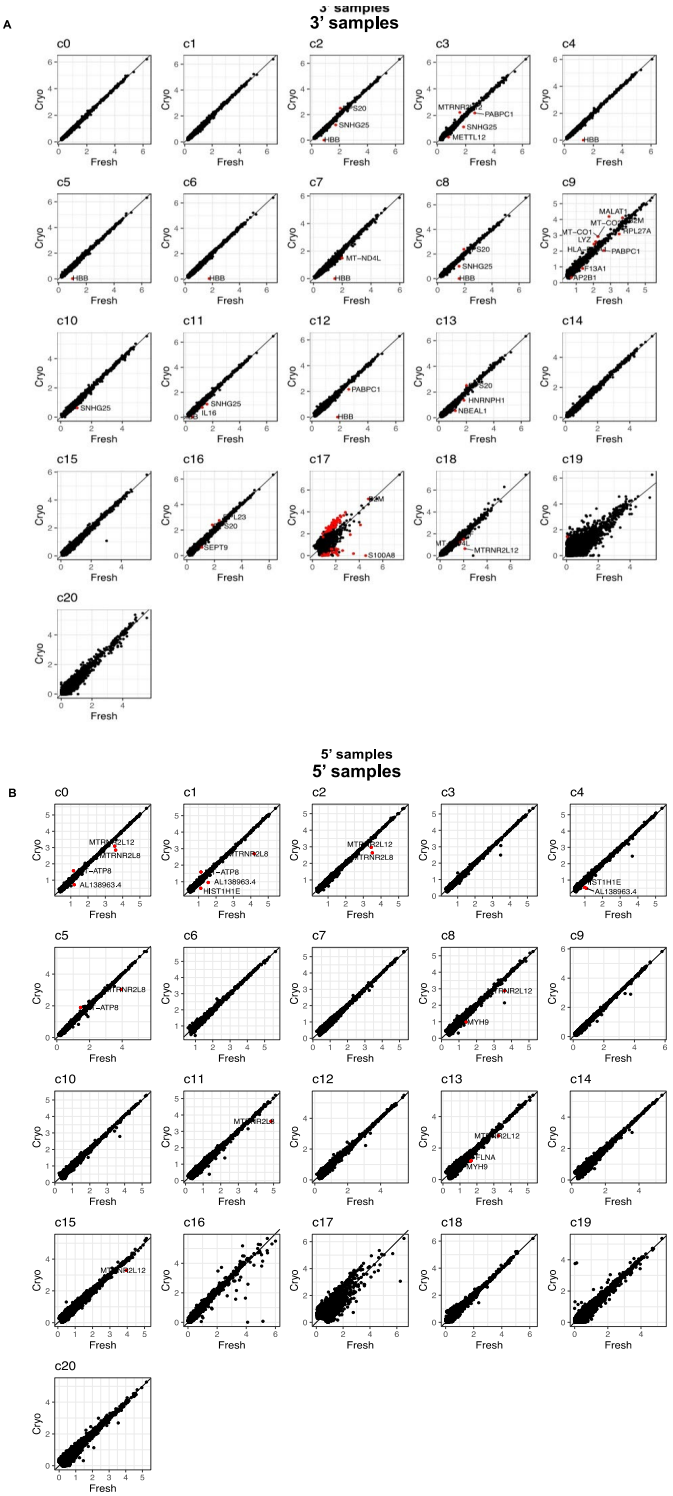

Figure S3. CSF cell type specific gene expression profiles are preserved upon cryopreservation, related to Figure 2 and 3. (A) Gene expression correlations between fresh and cryopreserved samples in individual clusters from 3' samples and **(B)** 5' samples. Differentially expressed genes are labeled in red.

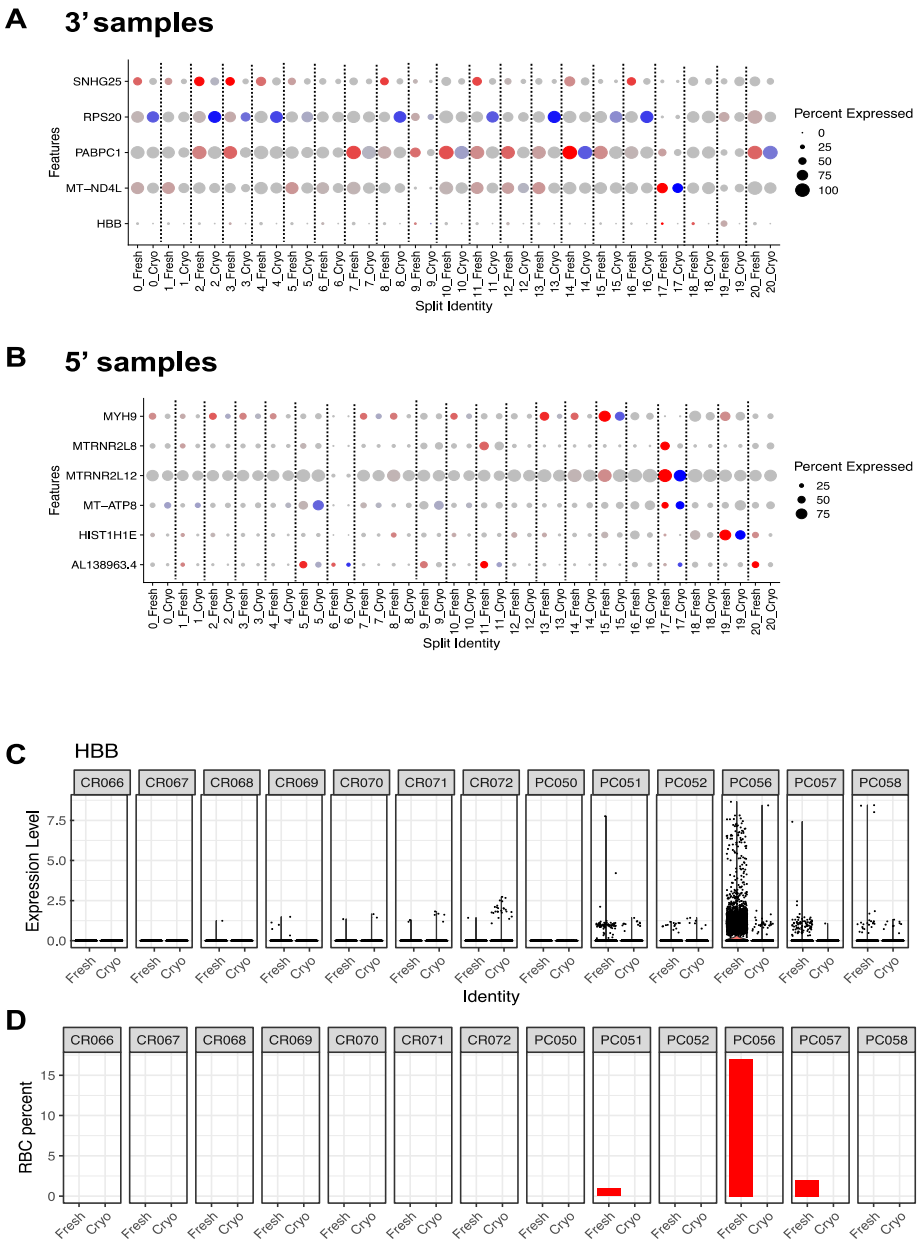

Figure S4. Impact of cryopreservation on gene expression, related to Figure 2 and 3.
(A) Genes susceptible to change in response to the cryopreservation process in 3' sequencing chemistry samples and **(B)** 5' sequencing chemistry samples. Gene expression in each cluster within fresh samples (red) *vs.* cryopreserved samples (blue). Darker colors represent a higher level of expression. Dot size represents the percentage of cells in each cluster, where the gene is expressed. **(C)** The normalized expression levels of *HBB* in cells in five sample-pairs. The percentage of red blood cells identified in each sample before exclusion. **(D)** Genes with higher expression in clusters from fresh (red dots) and cryopreserved samples (blue dots). Darker color represents higher expression. Dot size represents the percentage of cells in each cluster in which the gene is expressed.