

Figure S1

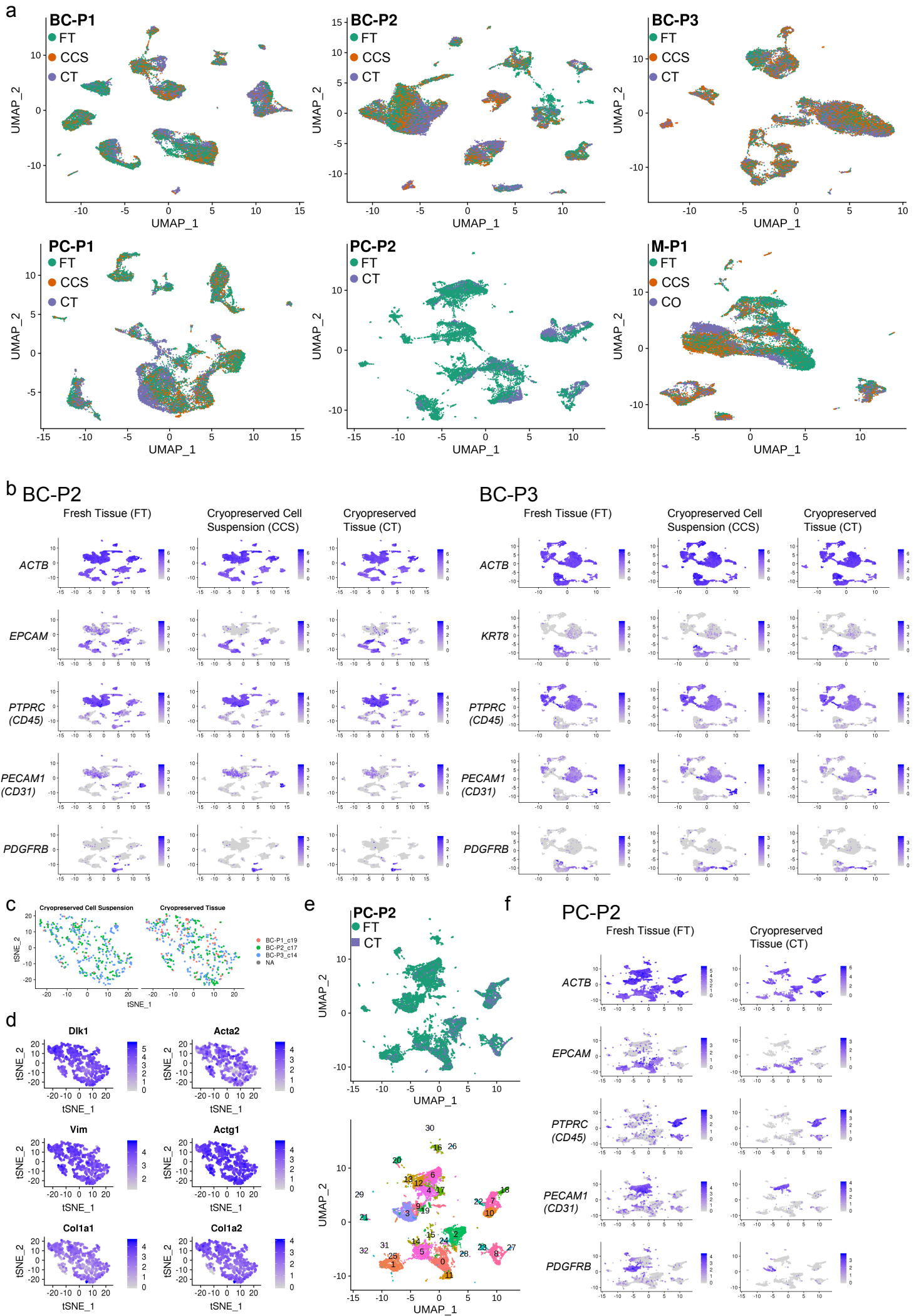
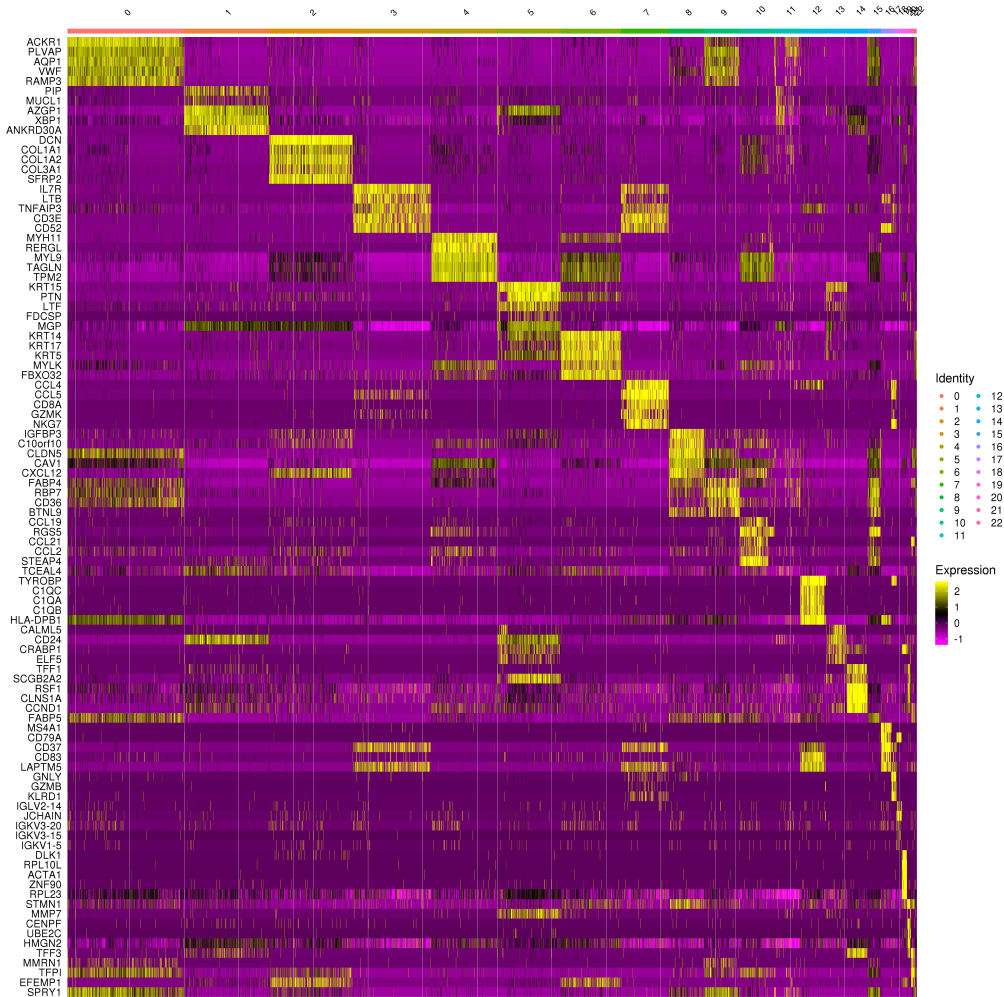


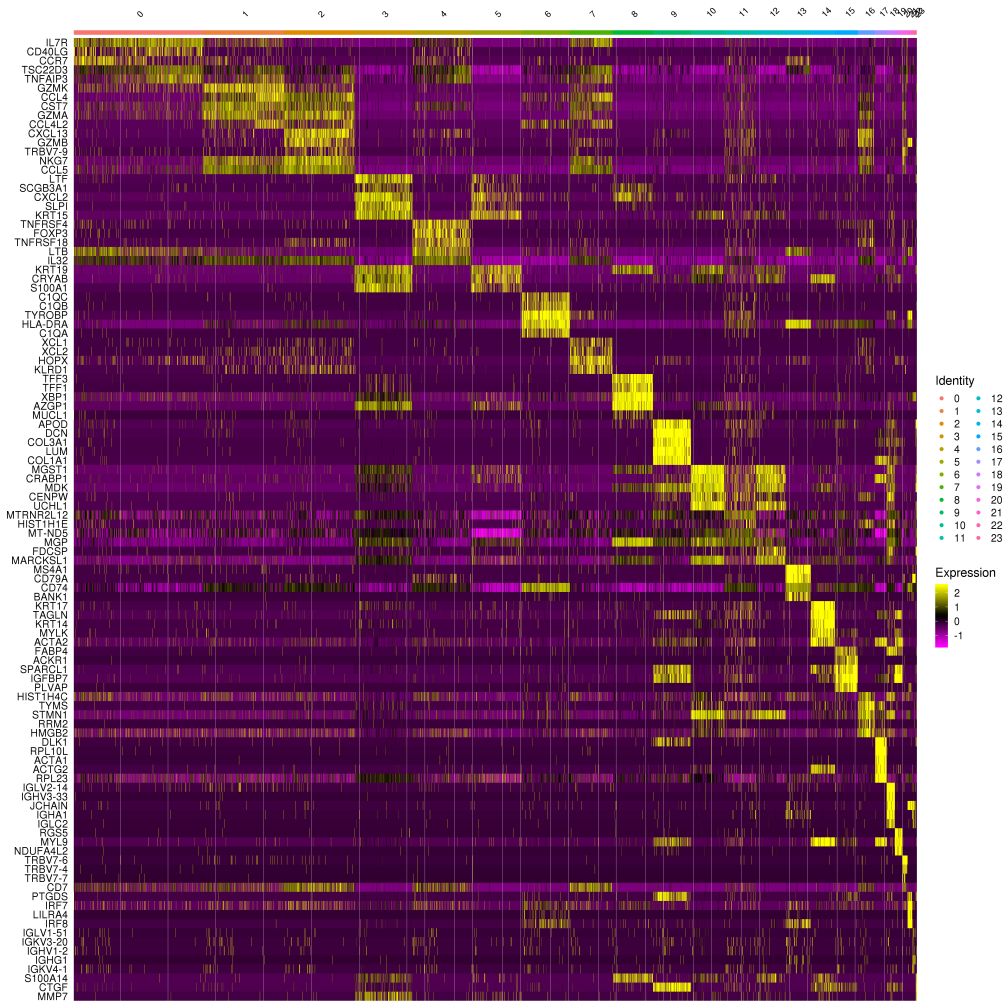
Figure S1. Cryopreservation allows for robust cell-type detection in clinical cancer samples. **a**, UMAP visualisations for the non-batch corrected data for each of the three breast cancer (BC-P1, BC-P2 and BC-P3), two prostate cancer (PC-P1 and PC-P2) and metastatic melanoma case (M-P1). **b**, Featureplot visualisations for additional breast cancer cases BC-P2 and BC-P3. Gene expression shows the conservation of the housekeeping gene *ACTB*, and markers for cancer/epithelial (*EPCAM*), immune (*PTPRC/CD45*), endothelial (*PECAM1/CD31*) and fibroblast/perivascular (*PDGFRB*) clusters following cryopreservation as CCS and CT. **c**, tSNE visualisation showing the high mixability of mouse NIH3T3 fibroblast cell line spike ins (~2%) from the cryopreserved replicates from all three breast cancer cases. Embeddings are split by cells captured from CCS and CT, respectively. Original cluster IDs from Figure 1b are c19 from BC-P1, c17 from BC-P2 and c14 from BC-P3. **d**, Featureplot visualisations of the NIH3T3 cell line fibroblast markers *Dlk1*, *Acta2*, *Vim*, *Actg1*, *Col1a1* and *Col1a2*. **e**, UMAP visualisations for the batch corrected data for PC-P2, which only contains comparisons between FT and CT replicates due to low cell numbers in the CCS replicate. UMAPs are coloured by cryopreserved conditions and cluster IDs. **f**, Featureplot visualisations of gene expression highlighting the conservation of the major cell lineages, as represented in **(b)**.

Figure S2

a BC-P1



b BC-P2



C

Heatmap visualization showing gene expression profiles across 15 samples (S1 to S15). The y-axis lists 100 genes, and the x-axis lists 15 samples. The color scale ranges from 0 (blue) to 14 (red).

Genes listed on the y-axis (from top to bottom):

- SEPP1
- RLTP
- APOF
- RINASE
- C10C
- S100A8
- FCN1
- LYZ
- S100A9
- GOS2
- COL6A1
- COL6A2
- SERPINE1
- ASPH
- TNFRSF12A
- CD84
- CCL5
- CD88
- GZMK
- NKG2
- TAGLN
- PFN2
- MYL9
- S100A16
- CXCL10
- CXCL1
- CCL8
- ISG15
- ITIH4
- CD52
- IL7R
- CD39
- CD40LG
- ZFP36L2
- ILF3A5
- MSA44A
- SPI1
- MT-ND6
- XCIL
- GNLY
- CD7
- TRDC
- HIST1H1A
- HMG2
- MKI67
- UBE2C
- TOP2A
- IGFBP3
- RAMP2
- PLVAP
- ADP1
- VWF
- STMN1
- HMG2
- TYMS
- RRM2
- ITB
- TNFRSF4
- TNFRSF18
- FOXO3
- IL32
- RGSS
- IGFBP3
- SPARCL1
- NDUFA42
- TIMP1
- DLK1
- RPL10L
- CRABP1
- RPL23
- HIST1H1A
- COL3A1
- PTGDS
- COL1A1
- COL1A2
- POSTN
- MT1X
- MT1H
- MT1M
- MT2A
- GNGL
- CHIR2
- RBP7
- SH3BP2
- HLA-DQA1
- GST3
- LGALS3
- IRF8
- IGKV3-20
- IGLV2-14
- IGKV4-1
- IGKV3-11
- IGLV5-7

Samples listed on the x-axis (from left to right):

- S1
- S2
- S3
- S4
- S5
- S6
- S7
- S8
- S9
- S10
- S11
- S12
- S13
- S14
- S15

d

Heatmap visualization showing gene expression profiles across 15 samples (0-14). The y-axis lists 100 genes, and the x-axis lists 15 samples. A color scale at the top indicates expression levels from 0 (blue) to 15 (red). The heatmap shows various patterns of gene expression, with some genes showing high expression in specific samples, such as KRT17 in sample 0, and others showing more widespread expression.

e PC-P2

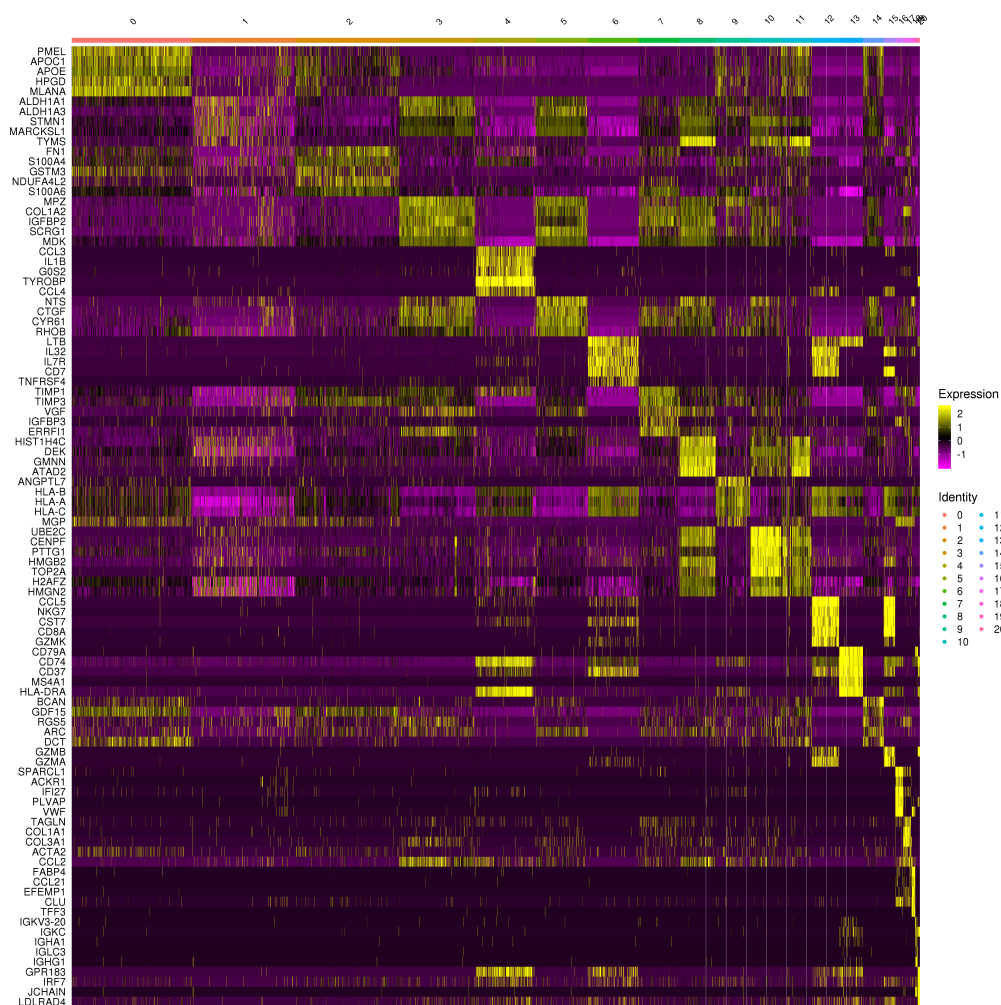
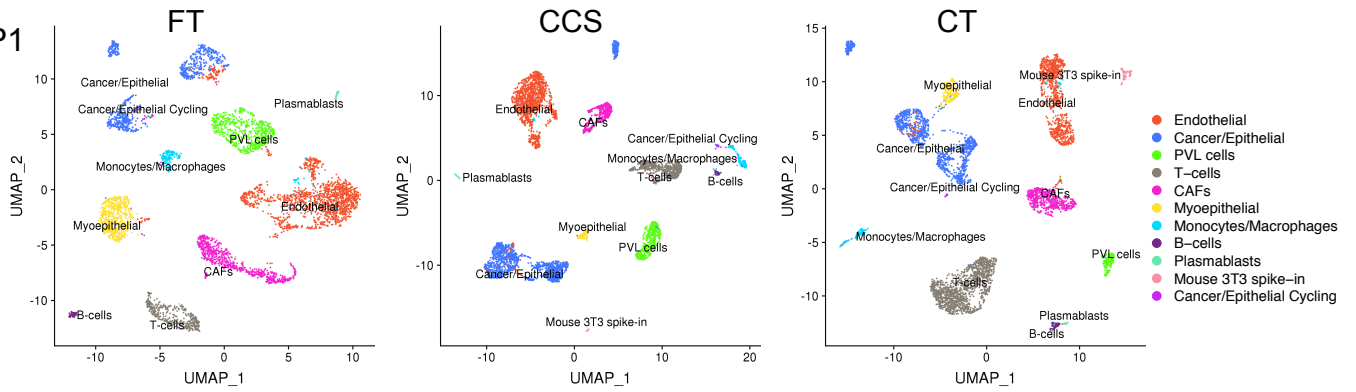


Figure S2. Heatmaps of integrated clusters for breast, prostate and melanoma cancer case.
a-f, Heatmap visualisation of the top 5 differentially expressed genes per cluster for three breast cancer cases BC-P1 (**a**), BC-P2 (**b**) and BC-P3 (**c**), two prostate cancer cases PC-P1 (**d**) and PC-P2 (**e**) and a metastatic melanoma M-P1 (**f**). All cases represent the integrated clustering of all cryopreserved conditions. Differentially gene expression was performed using the MAST method within Seurat v3 with the RNA assay and default parameters. Heatmaps were generated using the DoHeatMap function using Seurat v3. Complete gene lists used are detailed in Supplementary Table 2.

Figure S3

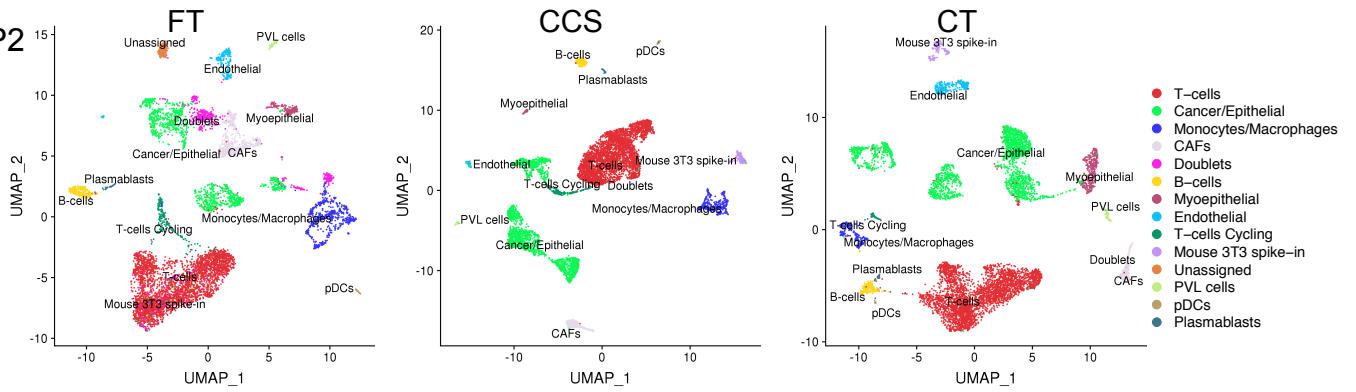
a

BC-P1



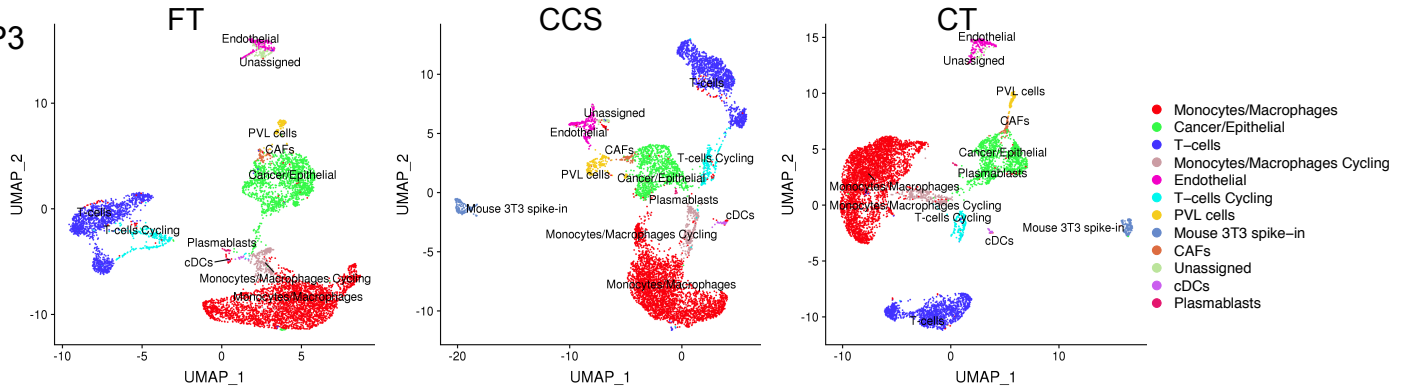
b

BC-P2



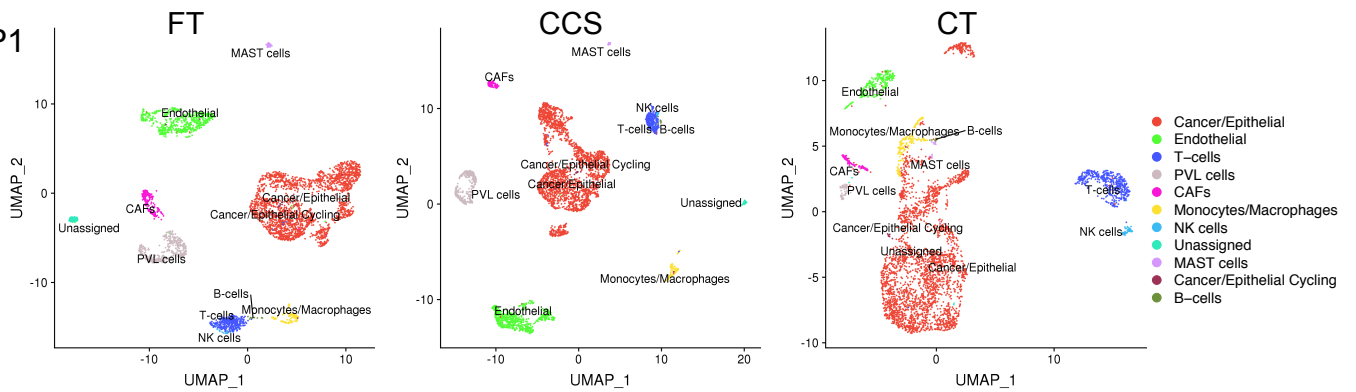
c

BC-P3



d

PC-P1



e

M-P1

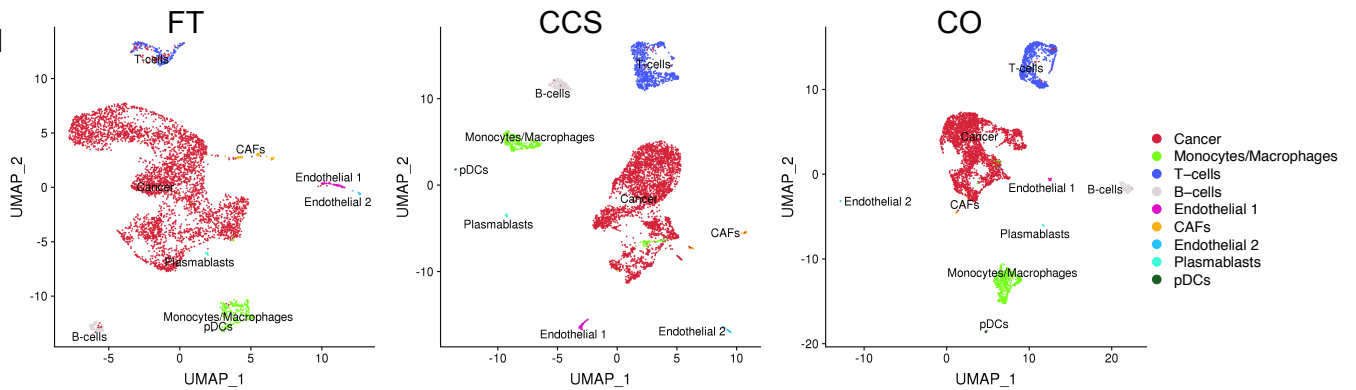


Figure S3. Unbiased clustering and cluster annotation in individual fresh tissue and cryopreserved samples. UMAP visualisations for individual fresh tissue (FT), cryopreserved cell suspension (CCS), cryopreserved tissue (CT) and cryopreserved overnight (CO) samples for the breast cancer cases BC-P1 (**a**), BC-P2 (**b**) and BC-P3 (**c**), prostate cancer case PC-P1 (**d**) and metastatic melanoma case M-P1 (**e**). All matched individual datasets were down sampled to the lowest replicate cell number and analysed independently using unbiased clustering with the default resolution of 0.8 and UMAP dimensionality reduction with the top 20 principal components. Cell annotations plotted are derived from the integrated datasets as in Figure 1b and Figure 2a-b.

Figure S4

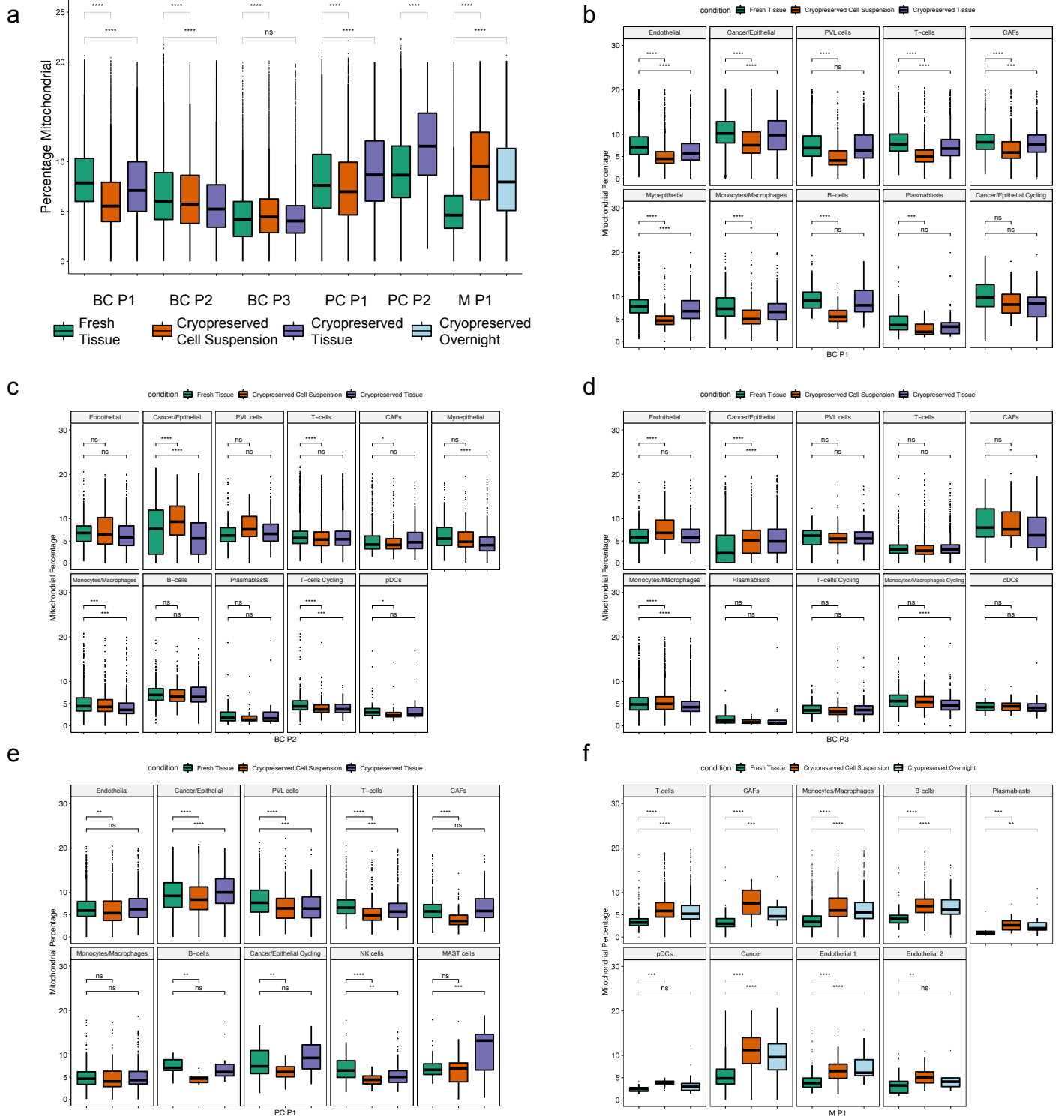
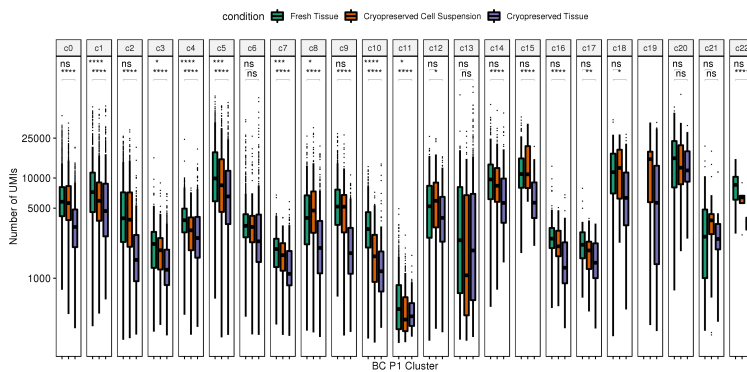


Figure S4. Percentage of mitochondrial transcripts across cryopreservation conditions and cell types. a-f, Percentage of mitochondrial genes per cell across cryopreservation conditions: Fresh Tissue (FT; green), Cryopreserved Cell Suspension (CCS; orange), Cryopreserved Tissue (CT; purple) and Cryopreserved Overnight (CO; light blue). Comparisons are plotted across all cells (**a**) and faceted by annotated cell type for each of the tumour cases analysed BC-P1 (**b**), BC-P2, (**c**), BC-P3 (**d**), PC-P1 (**e**) and M-P1 (**f**). Statistical significance was computed using an unpaired wilcoxon test where P-values denoted by asterisks: * $p < 0.05$, $p < 0.01$, * $p < 0.001$ and **** $p < 0.0001$.

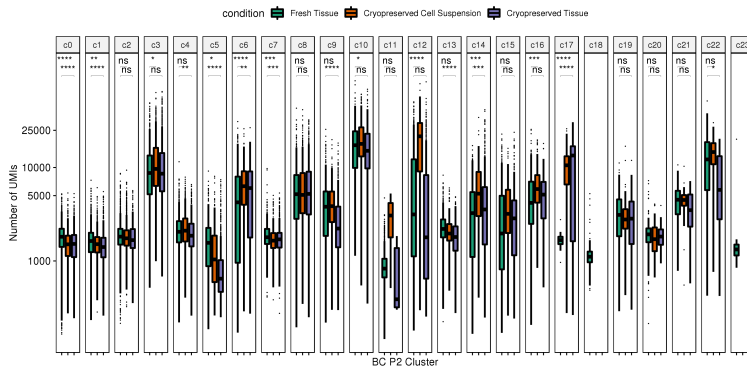
2

[illegible]

b

Figure 3 displays box plots of the number of genes in each BC P2 cluster across three conditions: Fresh Tissue (green), Cryopreserved Cell Suspension (orange), and Cryopreserved Tissue (purple). The y-axis represents the number of genes (0 to 5000). The x-axis shows BC P2 clusters c0 to c23. Statistical significance is indicated by asterisks above the clusters.

Cluster	Condition	Significance
c0	Fresh Tissue	****
c1	Fresh Tissue	****
c2	Fresh Tissue	*
c3	Fresh Tissue	*
c4	Fresh Tissue	**
c5	Fresh Tissue	****
c6	Fresh Tissue	****
c7	Fresh Tissue	ns
c8	Fresh Tissue	ns
c9	Fresh Tissue	****
c10	Fresh Tissue	ns
c11	Fresh Tissue	ns
c12	Fresh Tissue	ns
c13	Fresh Tissue	****
c14	Fresh Tissue	****
c15	Fresh Tissue	ns
c16	Fresh Tissue	ns
c17	Fresh Tissue	****
c18	Fresh Tissue	
c19	Fresh Tissue	ns
c20	Fresh Tissue	ns
c21	Fresh Tissue	ns
c22	Fresh Tissue	*
c23	Fresh Tissue	



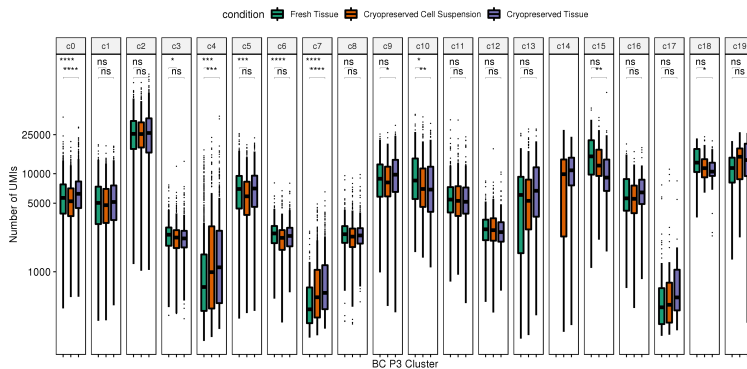
C

condition: Fresh Tissue, Cryopreserved Cell Suspension, Cryopreserved Tissue

Number of Genes

BC P3 Cluster

Significance levels: ns (not significant), * (p < 0.05), ** (p < 0.01), *** (p < 0.001), **** (p < 0.0001).



d

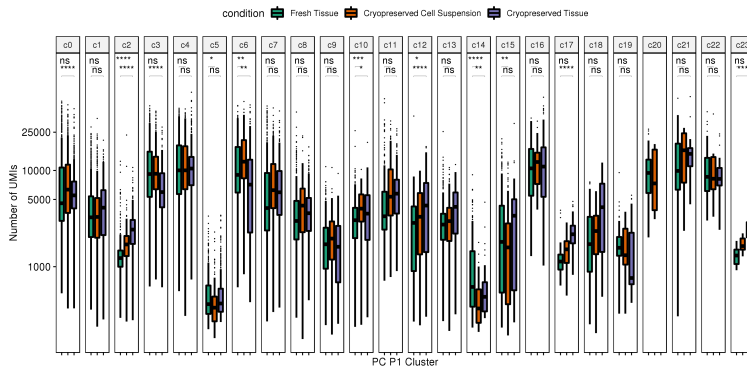
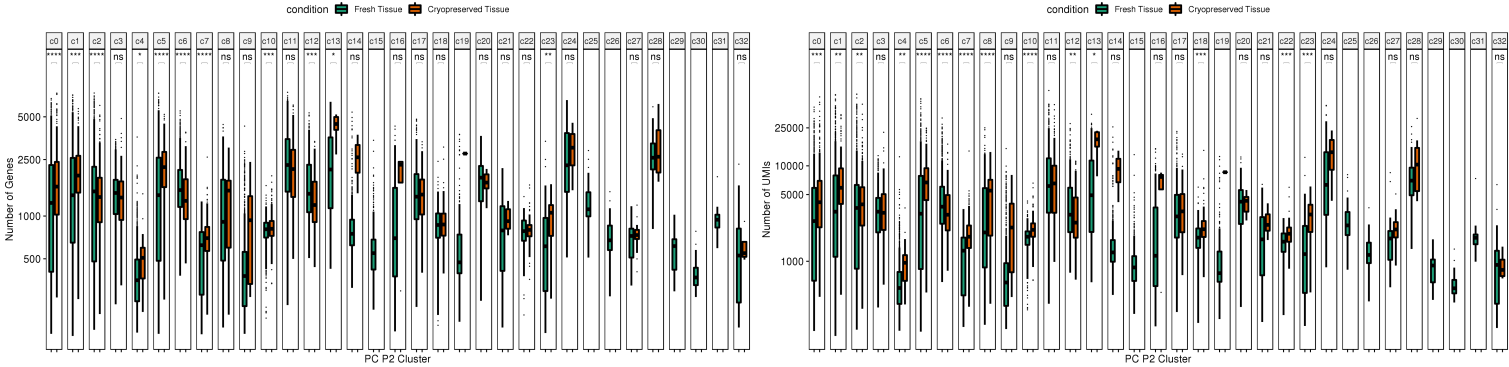
[illegible]

Figure S5 (cont.)

e
BC-P2



f
M-P1

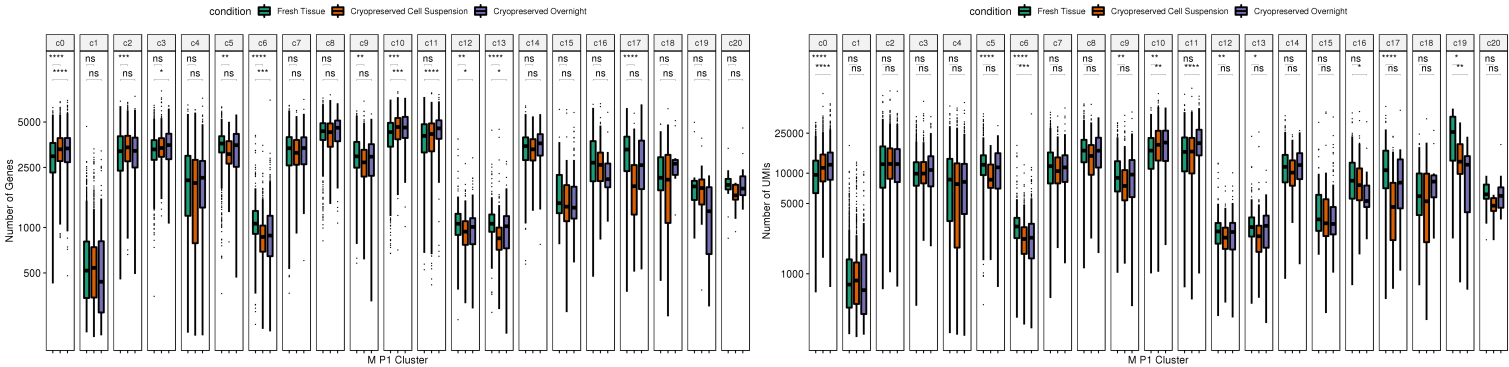


Figure S5. Number of genes and UMIs per cluster. a-f, Number of genes (left) and UMIs (right) detected per cell per cluster across FT (green), CCS (orange), CT (purple) and CO (purple; melanoma case only) replicates of breast cancer cases BC-P1 **(a)**, BC-P2 **(b)** and BC-P3 **(c)**, prostate cancer cases PC-P1 **(d)** and PC-P2 **(e)** and a metastatic melanoma M-P1 **(f)**. Sequencing libraries were down sampled to equal number of mapped reads per cell using cellranger aggregate function to account for differences from sequencing depth. Statistical significance was determined using an unpaired Student's *t*-test.

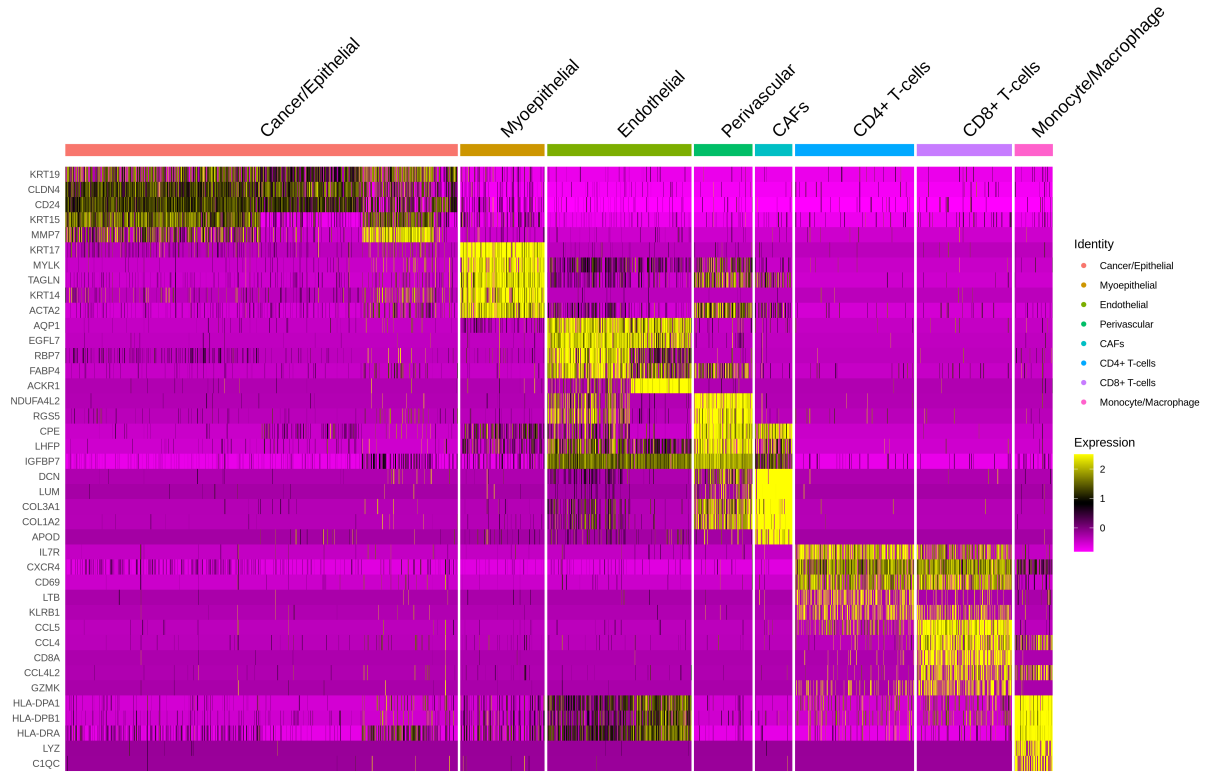
Figure S6



Figure S6. Cryopreservation maintains the detection of biological pathways in additional cases. **a-c**, Sensitivity of pathway enrichment scores detected in clusters across cryopreserved replicates. Additional representative cases of breast cancer BC-P2 (**a**) and BC-P3 (**b**) and prostate cancer PC-P2 (**c**) are shown. The minimum, mean and maximum $-\log_{10}$ q-value are plotted in the error bars of each GO pathway. All DEGs from each cluster were passed on to the ClusterProfiler package for functional enrichment with the CC sub-ontology under the human org.Hs.eg.db database. GO pathway descriptions can be found in Supplementary Table 3. **d-f**, Enrichment scores for gene ontology pathways that are unique to cryopreservation conditions for each annotated cell type: cryopreserved cell suspension (CCS; **d**), cryopreserved tissue (CT; **e**) and cryopreserved cell suspension after overnight cold storage (CO; **f**). Comparisons were performed for each annotated cell type from each matched condition, which were first down sampled by total cell number and total number of sequencing reads per sample. For the CCS (**d**) and CT (**e**) conditions, only pathways that were shared across multiple cases were analysed. Only the top 10 pathways based on enrichment scores are plotted. DEGs from each cell type and condition (Supplementary Table S6) were passed on to the ClusterProfiler package for functional enrichment with the CC sub-ontology under the human org.Hs.eg.db database. All GO pathway descriptions can be found in Supplementary Table S7.

Supplementary Figure 7

a



b

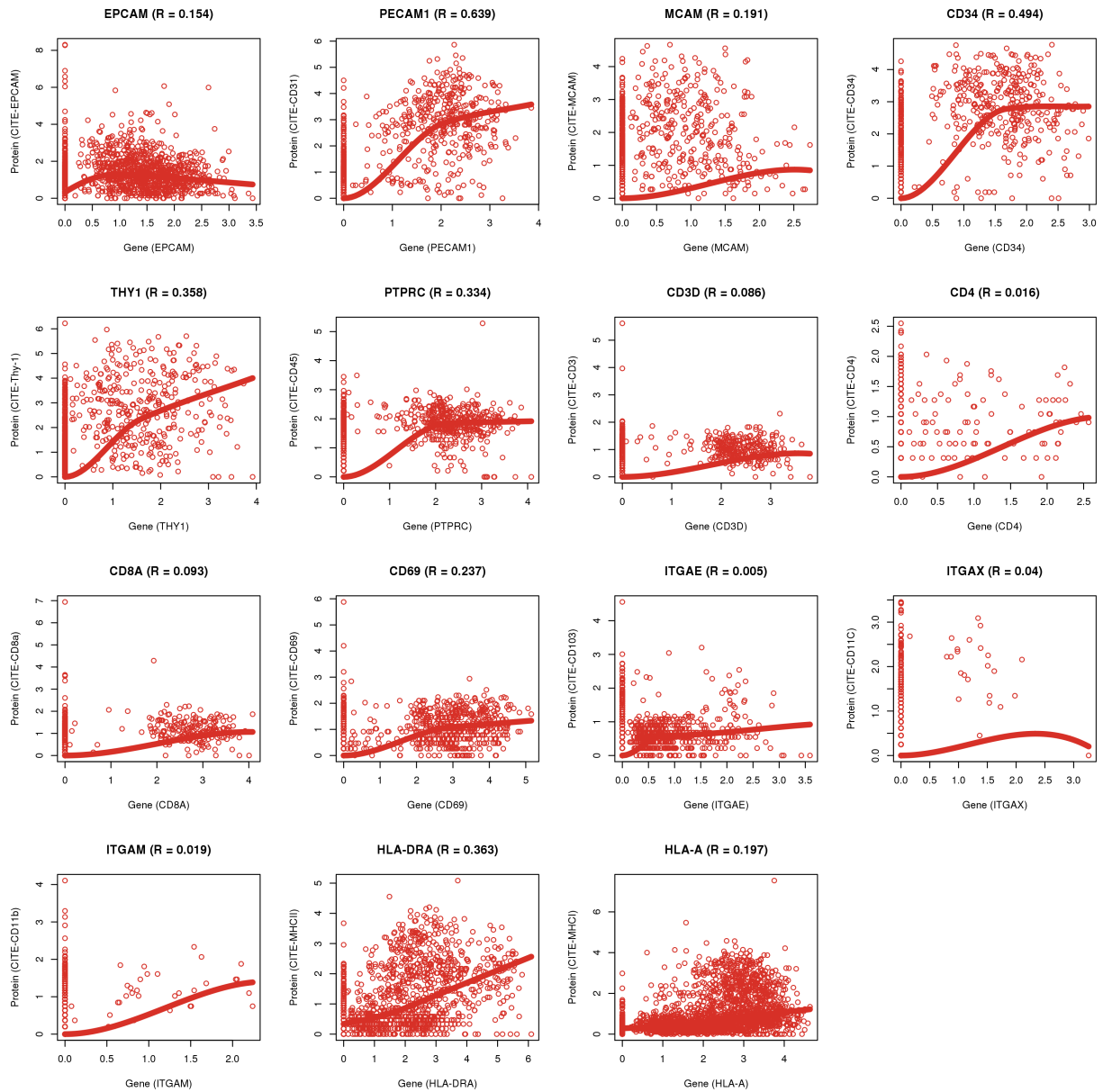


Figure S7. Cryopreservation provides high quality immunophenotyping using CITE-Seq.

a, Heatmap visualisation of the top 5 differentially expressed genes of indicated canonical cell type markers for an independent breast cancer case for the CITE-Seq experiment.

Differentially gene expression was performed using the MAST method within Seurat v3 with the RNA assay and default parameters. Heatmaps were generated using the DoHeatMap function using Seurat v3. **b**, Correlation plots between protein and genes for the panel of 15 markers used for CITE-Seq. Correlation values (adjusted- R^2) were computed using linear regression in *R* to model the log-normalised gene expression value and corresponding ADT levels.