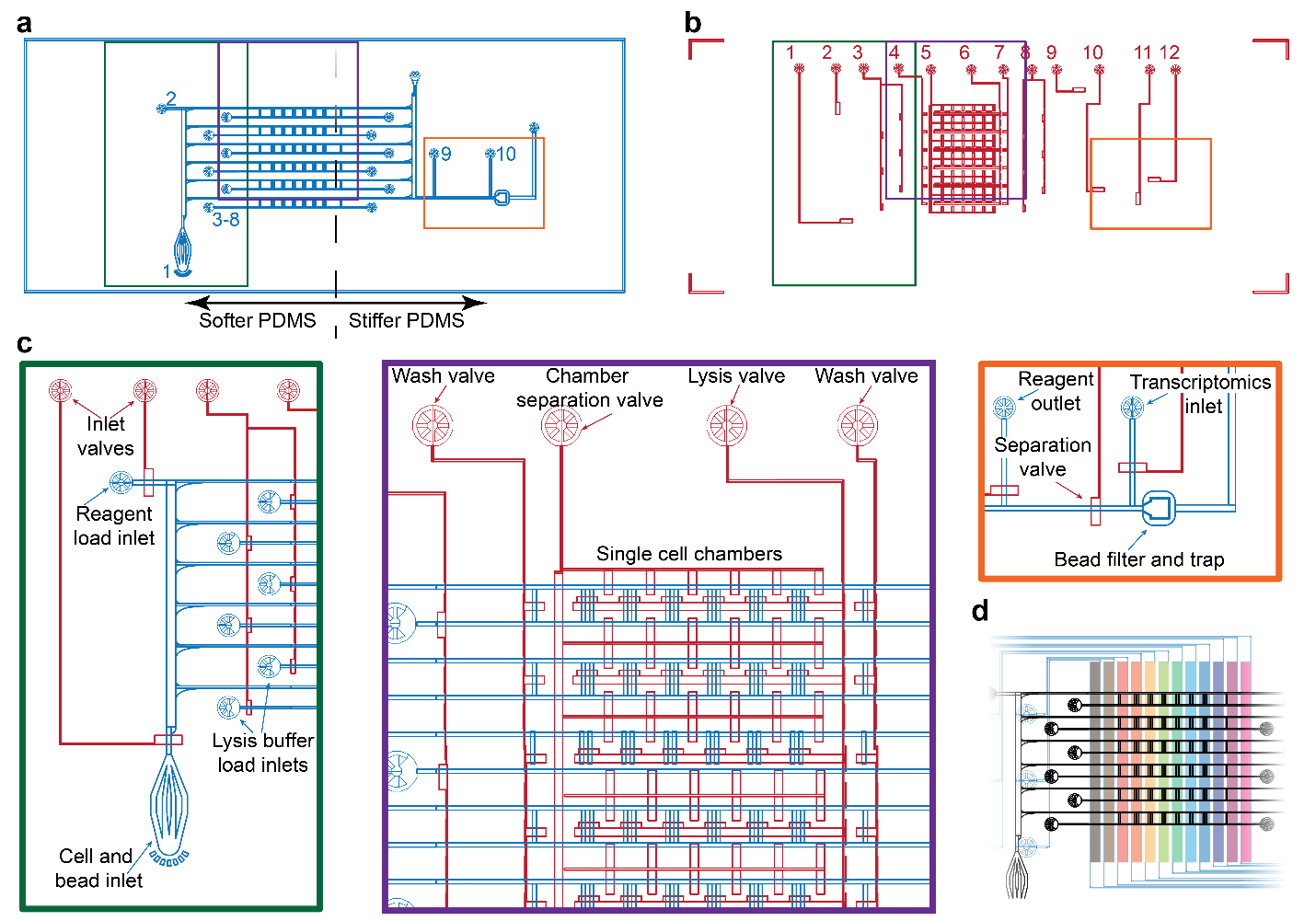
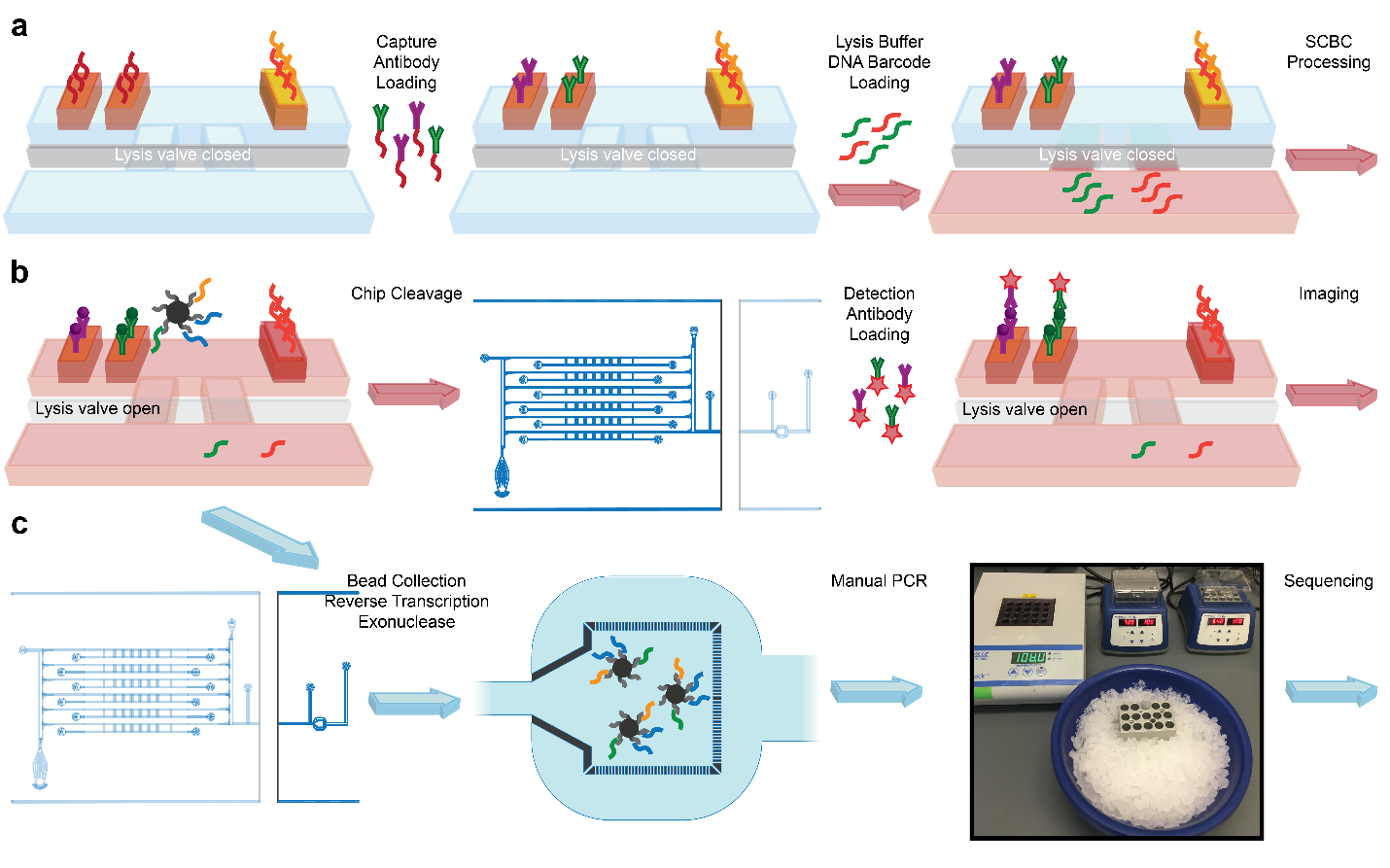
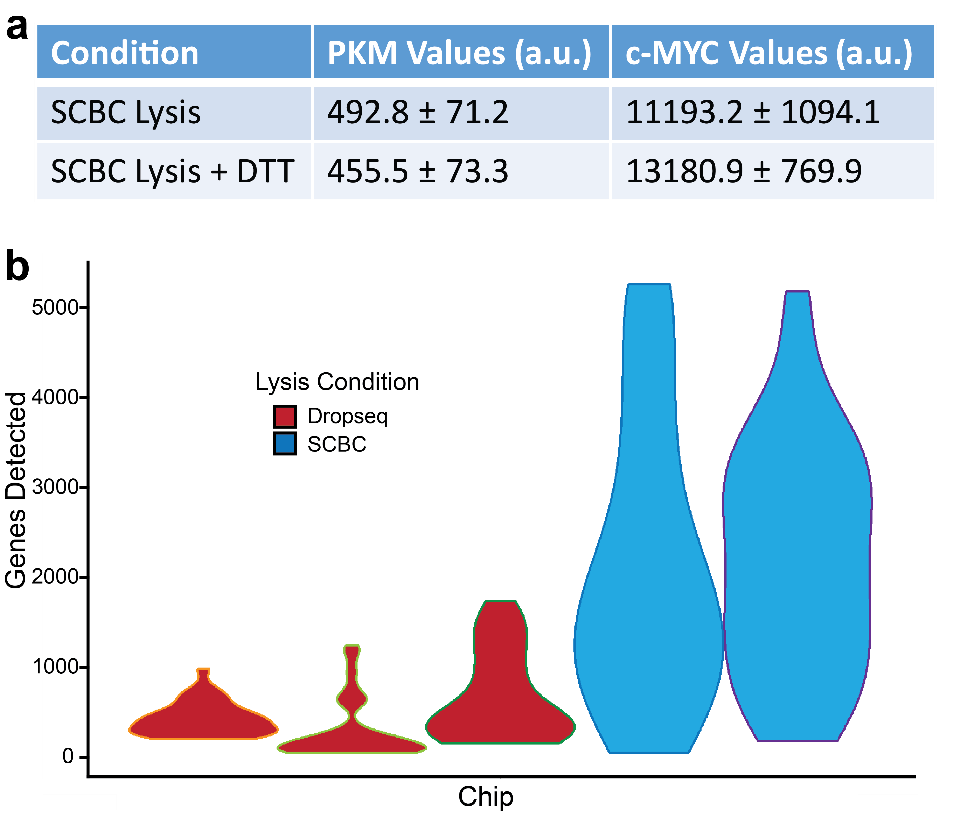
**Electronic Supplementary Information**

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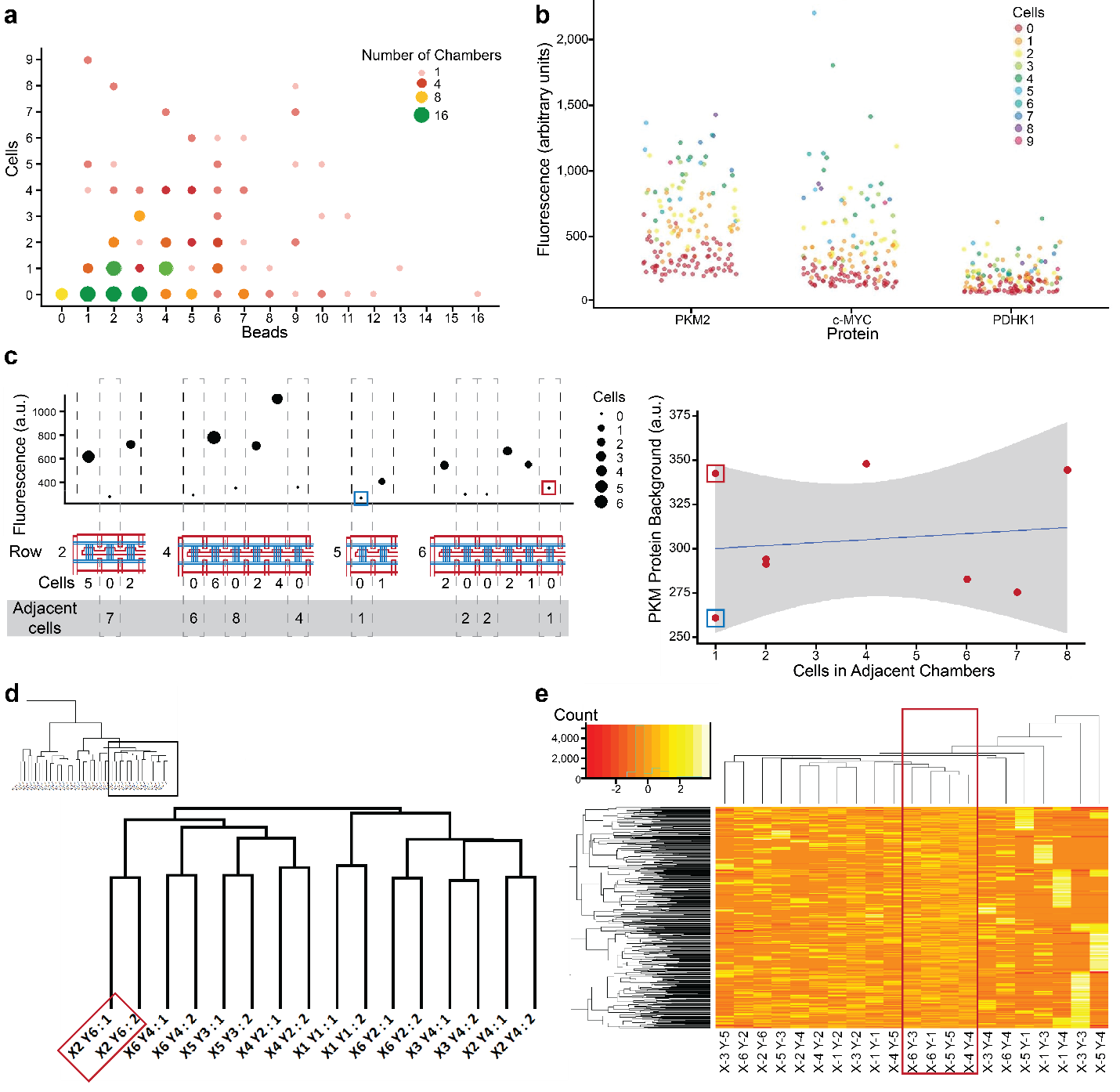
**Supplementary Figure 1.** Multi-omic SCBC schematics. a. The flow layer of the device contained the cell chambers, microfluidic filter, and 10 fluid inlet ports. Softer PDMS was added to the half of the chip with cell chambers and stiffer PDMS was added to the half with the microfluidic filter to optimize valve function and maintain filter integrity, respectively. Spin coating ensured that both halves remained distinct. b. The control layer contained 12 total fluidic valves. c. The assembled multi-omic SCBC consisted of 3 sections: the inlet section (green), the cell chambers (purple), and the transcriptomics section (orange). Detailed chip operation procedures can be found in Supplementary Table 3. d. Since the X axis of the single cell chambers was not directly addressable by fluid flow, the X-coordinate ssDNA oligos were added to the chip before SCBC assembly. Using a flow pattern mold, each column of single cell chambers was labeled with a distinct X-coordinate ssDNA.



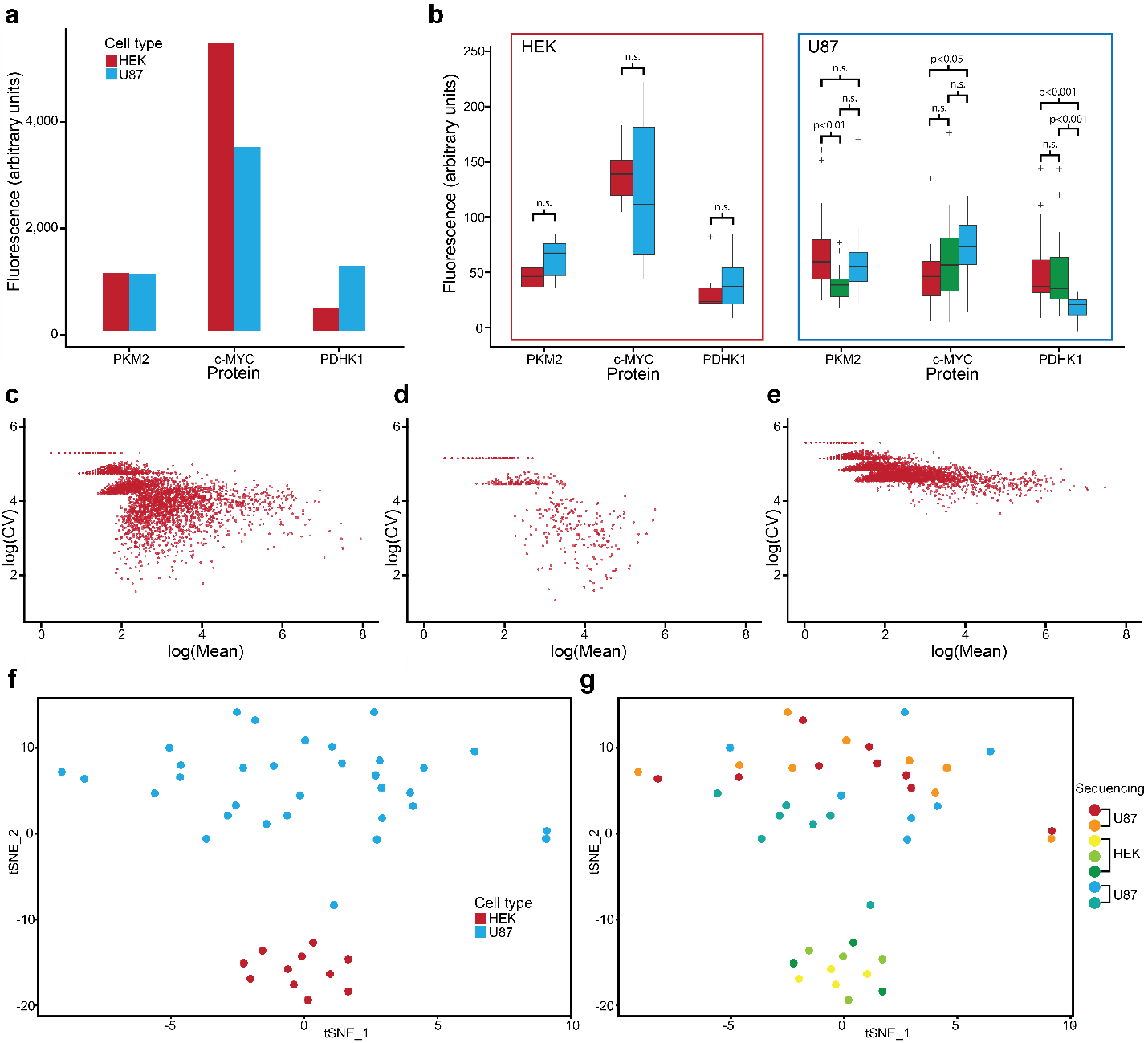
**Supplementary Figure 2.** Multi-omic SCBC operation. a. Before lysis, the chip was prepared by functionalizing the DEAL array into an antibody array and loading the SCBC with lysis buffer, Y-coordinate ssDNA, and DNA displacement oligos. b. After lysis and analyte capture, the chip was cleaved in two and separated so that the elevated temperatures used in reverse transcription did not affect the protein measurements. The side of the chip containing the single cell chambers was incubated with detection antibodies and imaged. c. The transcriptomics side of the chip was isolated at elevated temperatures for cDNA generation. After cDNA libraries were created, the filter containing beads was excised, manual PCR was performed on the entire filter, and the transcriptomes were sequenced.



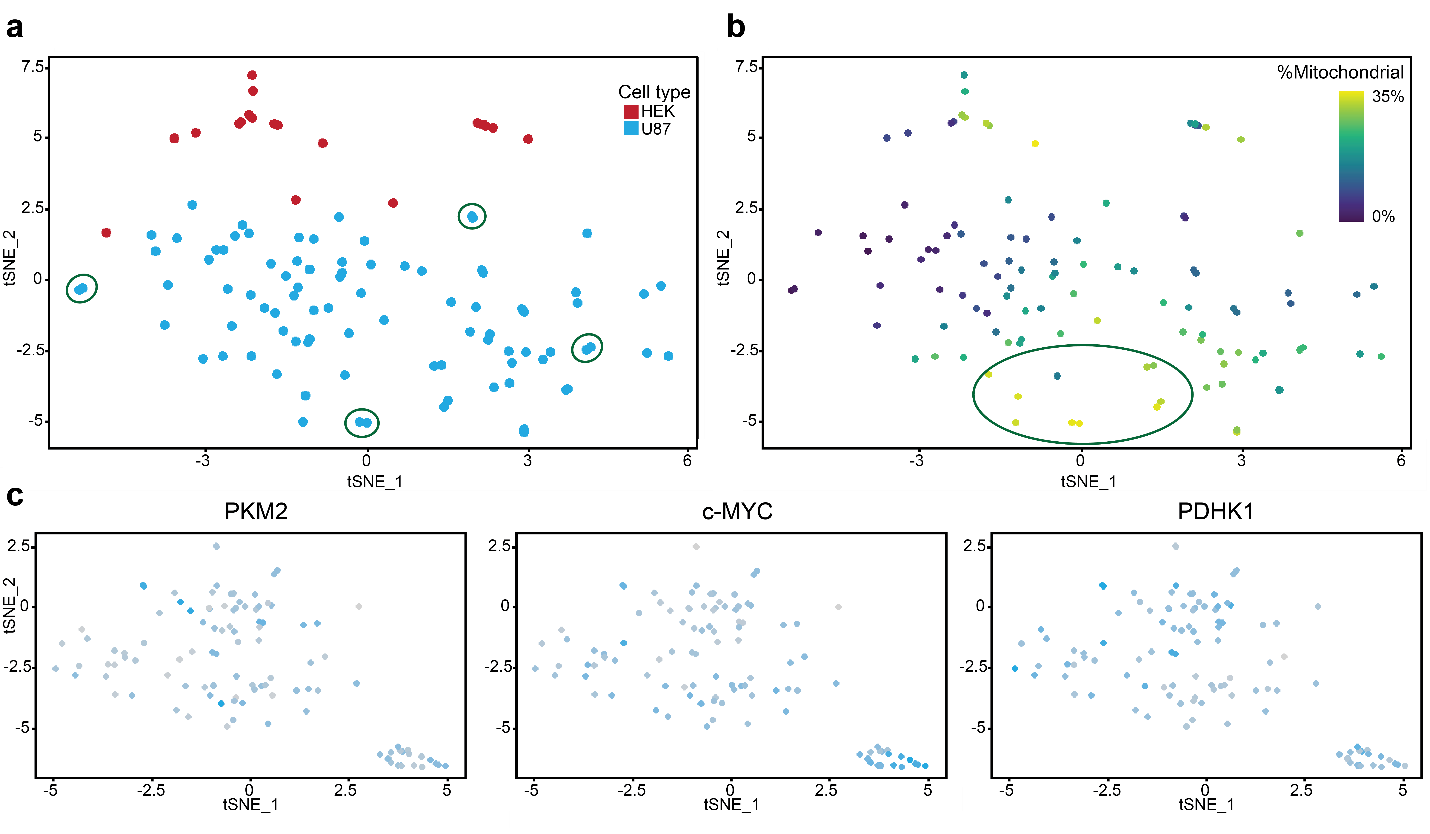
**Supplementary Figure 3.** Lysis buffer considerations. a. Protein measurements with bulk samples were not diminished by the conversion of standard SCBC buffer into multi-omic SCBC buffer (+ DTT). b. Multi-omic SCBC buffer was required for transcriptomics. Transcriptomic metrics were plotted for SCBC chips using standard Dropseq lysis buffer (3 chips, red) and multi-omic SCBC lysis buffer (2 chips, blue). The number of genes detected was sharply increased with the multi-omic SCBC lysis buffer.



**Supplementary Figure 4**. Additional proteomic and transcriptomic quality metrics. a. The beads and cells were distributed such that the average chamber contained 1.25 cells and 3.8 beads. b. Raw protein data across all chips confirmed a proportional increase of captured proteins with an increase in cell number. c. The quality of single cell isolation was determined by testing zero-cell chambers for any dependence of fluorescent signal on the number of cells in neighboring chambers. Well-isolated zero-cell chambers should demonstrate some variability in fluorescent intensity, but without any correlations to the number of neighboring cells. Protein measurements for background chambers containing zero cells on a chip were plotted along with their neighboring chambers by fluidic access (left). Only adjacent cells in a row were accessible by microfluidic channel. The size of the points indicates the number of cells in that particular chamber. For each chamber with zero cells, the number of cells in the two neighboring chambers (or one neighboring chamber for zero cell chambers at the end of a row) was plotted against the protein signal measurement (right). There was no statistically significant correlation between fluorescence of zero-cell background chambers and the number of cells in neighboring chambers by Spearman correlation (p=0.65, 0.09, 0.69 for PKM, c-MYC, PDHK1). For example, two such chambers each with 1 neighboring cell have much different fluorescent intensities (red and blue squares). d. Transcriptome sequencing required manual PCR to ensure that no beads were lost. Resampling the manual PCR solutions and sequencing showed that each cell was most similar to its replicate (red box). e. Hierarchical clustering of single chamber transcripts using only highly variable genes showed that there was little similarity between cells and the location of the chambers had little effect on the clustering, even among those cells that were most similar by gene expression (highlighted). The only neighboring chambers that were also neighbors by hierarchical clustering (Row 1, Columns 3 and 4) were the 3rd most distinct neighbors by clustering.



**Supplementary Figure 5**. Cell type specific distinctions and chip-to-chip comparisons. a. The bulk measurements of proteins showed a similar signature of higher c-MYC levels in HEK cells. HEK cells had lower PDHK1 levels than U87 cells in bulk, but this was not observed in SCBC measurements. This was likely due to low signal intensity and resolution in SCBC measurements of PDHK1 for both cell types. b. A comparison of protein levels within cell types and across chips showed that both chips with HEK cells were similar, and some differences existed between U87 chips (student’s t-test, Bonferroni adjusted). No U87 chip was completely distinguishable from any other U87 chip across all three proteins, and the number of cells was relatively small for each chip. c. The coefficient of variation (CV) for highly variable genes expressed by average cells from a sequencing run is plotted. Each point represents a gene plotted by its mean expression and CV across all chambers on a chip in either U87 cell chips, HEK cell chips (d), and all chips combined (e), showing that mixing U87 and HEK cells resulted in a large increase in CV. The average CV values for U87, HEK, and all chips on the log scale is 4.55, 4.33, and 5.08, respectively. Due to gene dropouts, some genes were not detected in each sequencing run, resulting in less expression and higher CV for those genes (top left of plots). f. When only single cell chambers were plotted, t-SNE plots produced well-separated clusters of HEK and U87 cells, requiring a lower perplexity value of 10 due to the small number of cells (default 30). g. When labeling the single cell t-SNE by individual chip, clusters of each cell type were not easily subdivided by their chip of origin, but instead cells measured by different chips were distributed across the t-SNE cluster for each cell type. This suggests that cell type-specific transcriptomic signatures were reproducible by chip, and batch effects from different chips were not easily observed, with the exception of one chip.



**Supplementary Figure 6**. Additional multi-omic metrics. a. t-SNE clustering using the full gene set showed some clustering by cell type, but less clearly than the clustering obtained by using highly variable genes obtained from public data sets. Manual PCR resampled replicates were included in the t-SNE analysis, demonstrating the same replicate clustering observed in Supplementary Figure 4a (examples highlighted). b. When choosing highly variable genes in an unsupervised manner, non-cell type-specific transcriptome features could be observed, such as increased mitochondrial gene capture. This can be observed in the high proportion of mitochondrial genes in the circled group of cells, which affected clustering on t-SNE using the full gene set. c. Protein expression was superimposed on the t-SNE plot from Figure 5b, with U87 cells occupying the top left and HEK cells the bottom right. In this study, the major cell-type specific protein signature was the increased c-MYC expression in HEK cells. Protein values are log transformed and scaled to the minimum and maximum values measured, then projected onto the t-SNE plot using the bulk variable gene set.

Supplementary Table 1. DNA sequences used for multi-omic SCBC.

|  |  |
| --- | --- |
| DEAL Barcode and Displacement DNA |  |
| B-DNA | 5'Amine - AAAAAAAAAAGCCTCATTGAATCATGCCTA |
| B'-DNA | 5'Amine - AAAAAAAAAATAGGCATGATTCAATGAGGC |
| C-DNA | 5'Amine - AAAAAAAAAAGCACTCGTCTACTATCGCTA |
| C'-DNA | 5'Amine - AAAAAAAAAATAGCGATAGTAGACGAGTGC |
| D-DNA | 5'Amine - AAAAAAAAAAATGGTCGAGATGTCAGAGTA |
| D'-DNA | 5'Amine - AAAAAAAAAATACTCTGACATCTCGACCAT |
| I-DNA | 5'Amine - AAAAAAAAAATGCCCTATTGTTGCGTCGGA |
| I'-DNA | 5'Cy3 - AAAAAAAAAATCCGACGCAACAATAGGGCA |
| P-DNA | TGGTTGCCGCTACAGGTGGATAAAAAAAAAA - Amine3' |
| P'-Displacement DNA | ATCCACCTGTAGCGGCAACCA - Cy33' |
|  |  |
| Location Barcodes | DEAL-bindingPrimer\_\_SequencingRegion\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Bcode\_PolyA\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |
| X-Location Column 1 | TAGCGGCAACCAATTCTATTGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACCATAAAAAAAAAAAAAAAAAAAAAAAAA |
| X-Location Column 2 | TAGCGGCAACCAATTCTATTGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAATGTAAAAAAAAAAAAAAAAAAAAAAAAA |
| X-Location Column 3 | TAGCGGCAACCAATTCTATTGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAGCAAGAAAAAAAAAAAAAAAAAAAAAAAAA |
| X-Location Column 4 | TAGCGGCAACCAATTCTATTGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTCTCTAAAAAAAAAAAAAAAAAAAAAAAAA |
| X-Location Column 5 | TAGCGGCAACCAATTCTATTGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTGCTTGAAAAAAAAAAAAAAAAAAAAAAAAA |
| X-Location Column 6 | TAGCGGCAACCAATTCTATTGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCTCGCGAAAAAAAAAAAAAAAAAAAAAAAAA |
|  | Primer\_\_SequencingRegion\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_Bcode\_PolyA\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |
| Y-Location Row 1 | ATTCTATTGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGAATTGAAAAAAAAAAAAAAAAAAAAAAAAA |
| Y-Location Row 2 | ATTCTATTGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGTTAGACAAAAAAAAAAAAAAAAAAAAAAAAA |
| Y-Location Row 3 | ATTCTATTGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGACGACTAAAAAAAAAAAAAAAAAAAAAAAAA |
| Y-Location Row 4 | ATTCTATTGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAATACGAAAAAAAAAAAAAAAAAAAAAAAAA |
| Y-Location Row 5 | ATTCTATTGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGAATTTGAAAAAAAAAAAAAAAAAAAAAAAAA |
| Y-Location Row 6 | ATTCTATTGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGTGAACAAAAAAAAAAAAAAAAAAAAAAAAA |
|  |  |
| Sequencing Primers |  |
| Bead Oligo | TTTTTTTAAGCAGTGGTATCAACGCAGAGTACJJJJJJJJJJJJNNNNNNNNTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT |
| SMART Primer | AAGCAGTGGTATCAACGCAGAGT |
| P5-SMART | AATGATACGGCGACCACCGAGATCTACACGCCTGTCCGCGGAAGCAGTGGTATCAACGCAGAGT\*A\*C |
| Location ssDNA Oligomer Primer | ATTCTATTGTCTCGTGGGCTCGG |
| Template Switch Oligo | AAGCAGTGGTATCAACGCAGAGTGAATrGrGrG |
| Custom Read 1 Primer | GCCTGTCCGCGGAAGCAGTGGTATCAACGCAGAGTAC |
|  |  |
| \* - Phophorothioate |  |
| r - RNA |  |

Supplementary Table 2. Antibodies and vendors used for multi-omic SCBC.

|  |  |  |
| --- | --- | --- |
| Capture Antibodies | Vendor | # |
| Human/Mouse/Rat PKM2 Antibody | R&D | AF7244 |
| Human c-Myc Antibody | R&D | AF3696 |
| Anti-Mitochondrial Pyruvate dehydrogenase kinase 1 antibody | Abcam | ab110335 |
|  |  |  |
| Detection Antibodies |  |  |
| PKM2 (D78A4) Rabbit mAb AF647 Conjugate\* | Cell Signaling Technology | 4053 |
| c-Myc (D84C12) Rabbit mAb AF647 Conjugate | Cell Signaling Technology | 13871 |
| PDHK1 (C47H1) Rabbit mAb AF647 Conjugate\* | Cell Signaling Technology | 3820 |
|  |  |  |
| \* - Custom order |  |  |

Supplementary Table 3. Detailed multi-omic SCBC operation. Inlets and valves are those indicated in Supplementary Figure 1a, b.

|  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- |
| Step | Open Valves | Closed Valves | Inlets to use | Solution added | Duration | Condition |  | Purpose |
| 1 | All |  | 2 | 3% BSA in PBS |  |  |  | Fill chambers |
| 2 |  | All |  |  | 1 hr |  |  | Pressurize valves and block |
| 3 | 2,5,10 |  | 2 | Capture antibodies | 1 hr | Incubate 37 °C |  | Convert ssDNA barcode to antibody barcode |
| 4 |  |  | 2 | 1.5% BSA in PBS | 1 hr |  |  | Final blocking |
| 5 | 11 | 10 | 2 | PBST |  |  |  | Wash |
| 6 | 3,8 | 2,11 | 3-8 | Lysis buffer |  |  |  | Lysis buffer, Y-coordinate ssDNA, and displacement ssDNA loading |
| 7 |  | 3,8 |  |  |  |  |  | Holding pattern during cell preparation |
| 8 | 1,9 |  | 1 | Cell and Bead mixture |  |  |  | Load cells and beads into chambers |
| 9 |  | 1,5,9 |  |  |  |  |  | Holding pattern for cell lysis |
| 10 | 1,2,3,4 |  | 2 | PBST |  |  |  | Wash out excess cells and beads on cell/bead loading side of chip |
| 11 | 7,8,9,10 | 1,2,3,4 | 9 | PBST |  |  |  | Wash out excess cells and beads on transcriptomic side of chip |
| 12 | 6 | 7,8,9,10 |  |  | 15 min | On ice |  | Lyse cells |
| 13 |  | 6 |  |  | 15 min | On ice |  | Lyse cells |
| 14 |  |  |  |  | 2 hr | On shaker |  | Lyse cells |
| 15 | 2,3,4 |  | 2 | 6X SSC |  |  |  | Device conditioning |
| 16 | 5,11 | 3,4 | 2 | 6X SSC |  |  |  | Bead collection |
| 17 |  | 11 |  |  |  |  |  | Holding pattern for chip cleavage |
| Protein Chip Processing | | | | | | | | |
| 18 | 10 |  | 2 | Detection antibodies | 1 hr |  |  | Detection antibody development |
| 19 |  |  | 2 | PBST | 30 min |  |  | Wash |
| Transcriptome Chip Processing | | | | | | | | |
| 20 | 12 |  | 10 | Reverse transcriptase | 30 min |  |  | Reverse transcription |
| 21 |  |  | 10 | Reverse transcriptase | 90 min | Incubate 42 °C |  | Reverse transcription |
| 22 |  |  | 10 | TE-SDS/TE-TW/Tris |  |  |  | Wash |
| 23 |  |  | 10 | Exonuclease | 45 min | Incubate 37 °C |  | Exonuclease |
| 24 |  |  | 10 | TE-SDS/TE-TW/H2O |  |  |  | Wash |

Supplementary File 1. AutoCAD drawings of microfluidic molds.

Supplementary File 2. R script to execute analysis pipeline.