Supplementary information for: High-throughput single-cell proteomics and transcriptomics from same cells with a nanoliter-scale, spin-transfer approach

AUTHORS:

Pranav Dawar[1], Lye Meng Markillie[1], Sarah M. Williams[1], Hugh D. Mitchell[1], Johannes W. Bagnoli[2], Joshua Cantlon-Bruce[2], Anjali Seth[2], Carter C. Bracken[1], Ljiljana Paša-Tolić[1], Ying Zhu[1,3], James M. Fulcher[1*]

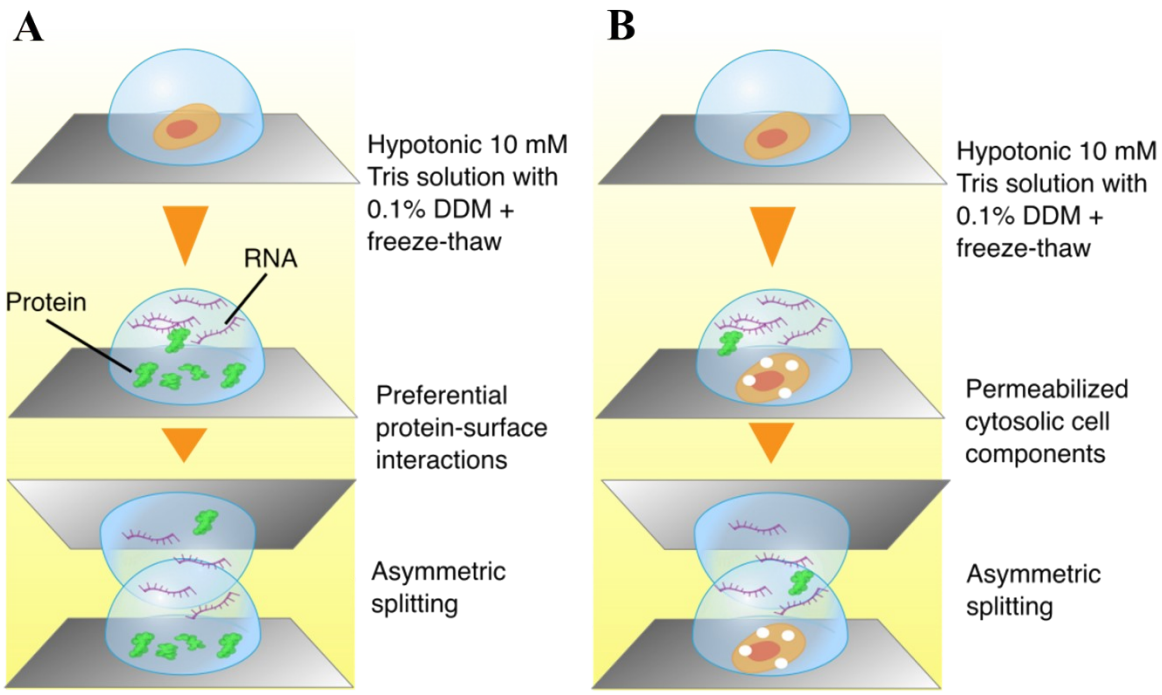
AFFILIATIONS

[1]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington 99354, United States

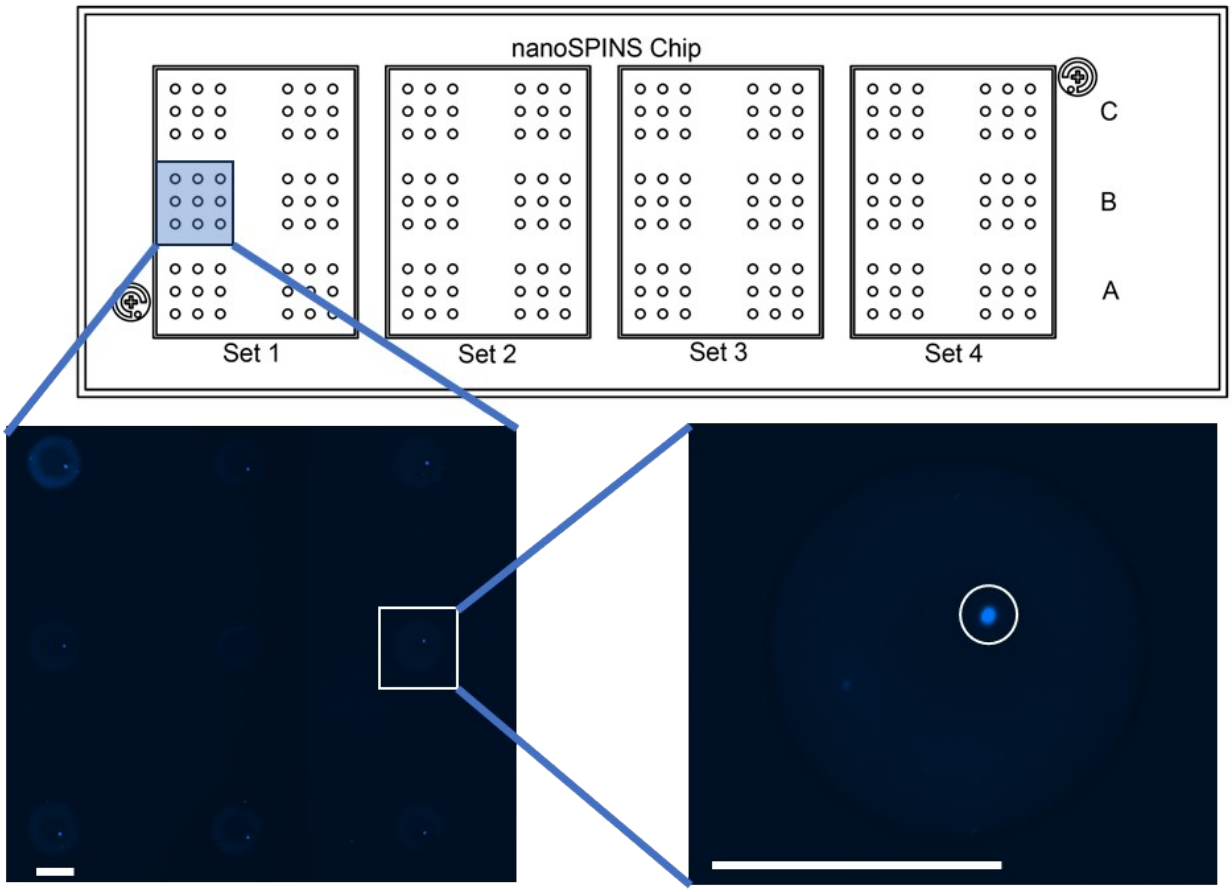
[2]Cellenion SASU, 60 Avenue Rockefeller, Bâtiment BioSerra2, 69008 Lyon, France

[3]Present address: Department of Proteomic and Genomic Technologies, Genentech, 1 DNA Way, South San Francisco, 94080, United States

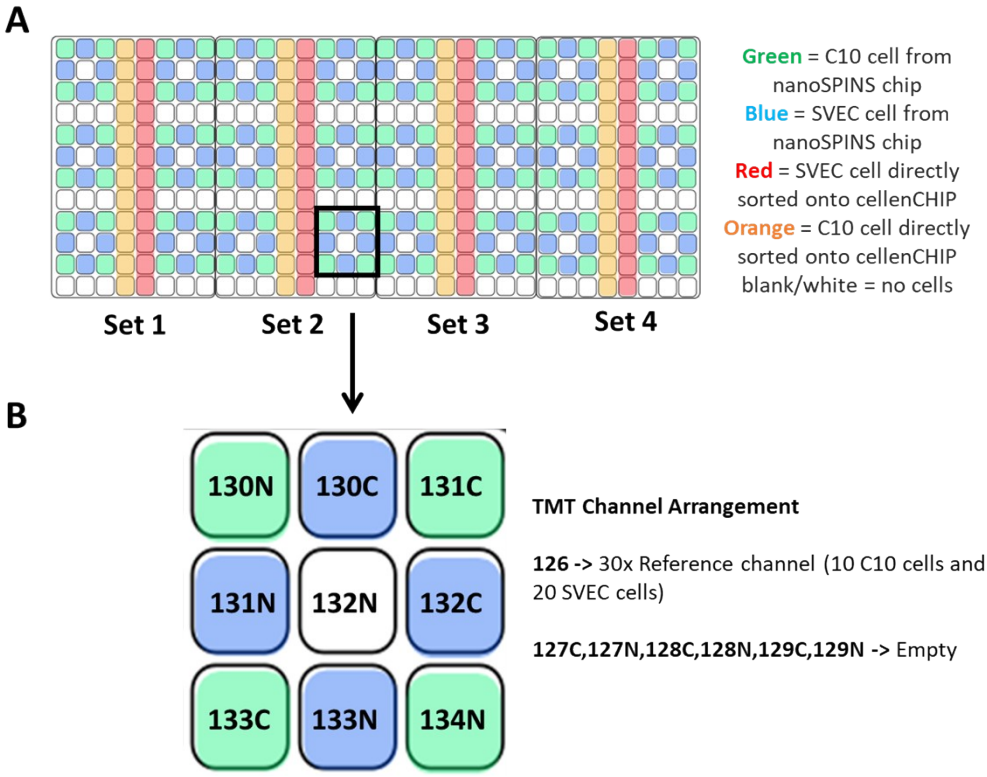
[*]Correspondence: james.fulcher@pnnl.gov



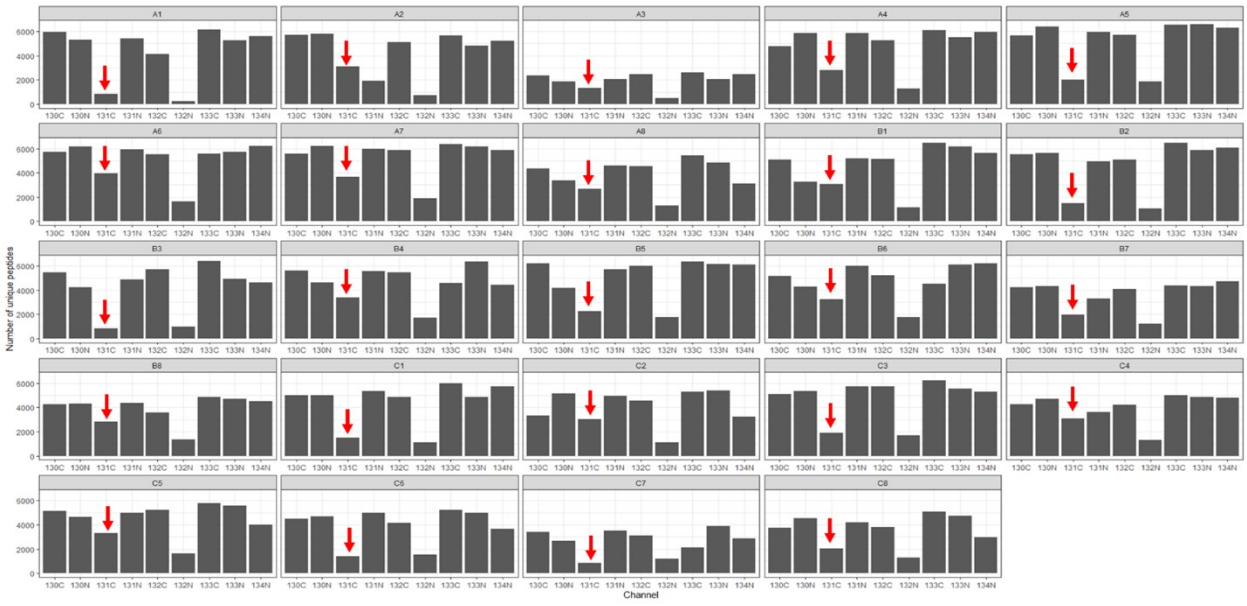
Supplementary Figure 1 – **(A)** Previous model of cell lysis - 0.1% DDM-mediated increased overall cell lysis, with enhanced protein retention due to hydrophobic interactions between protein molecules and the trimethylsilyl-coated surface of the nanoSPLITS chip wells. **(B)** Revised model suggesting that 0.1% DDM primarily induces membrane permeabilization rather than complete lysis, allowing diffusible cytosolic components to enter the droplet while the bulk of the cell remains intact and adhered to the glass chip surface.



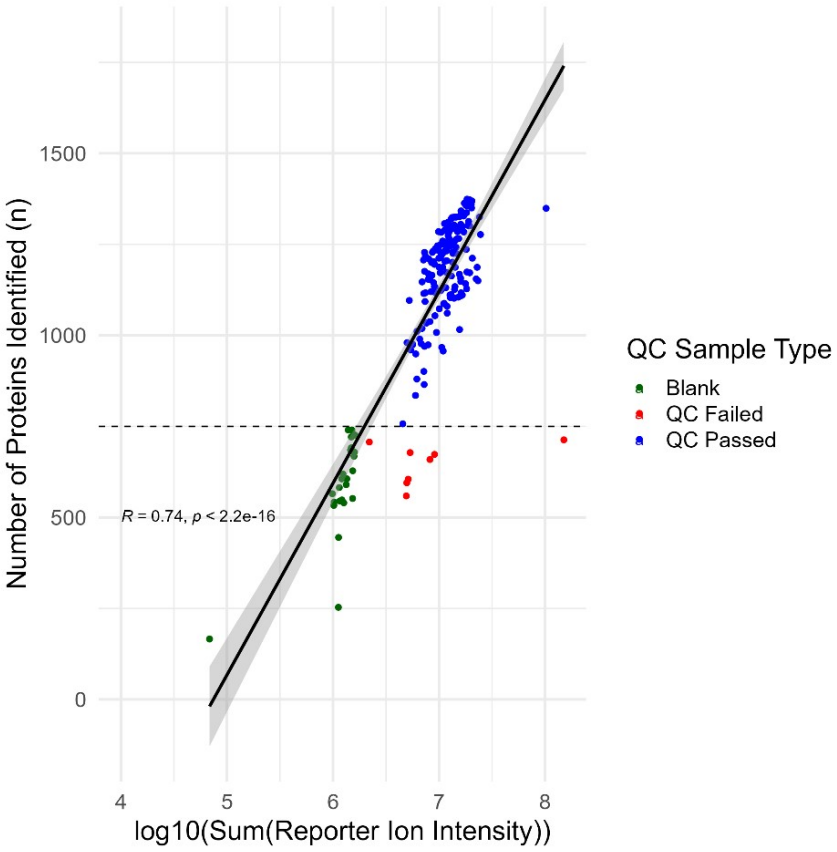
Supplementary Figure 2 – Fluorescent image of a 3×3 array of wells on the nanoSPINS chip, showing eight peripheral wells containing Hoechst-stained single cells sorted into 40 nL of 0.1% DDM, 10 mM HEPES (pH 8.5), and a central control/empty well, demonstrating high cell retention on the nanoSPINS wells following a single freeze-thaw cycle, evaporation, immediate reconstitution with 40 nL nuclease-free water, and centrifugation at 1000 × g.



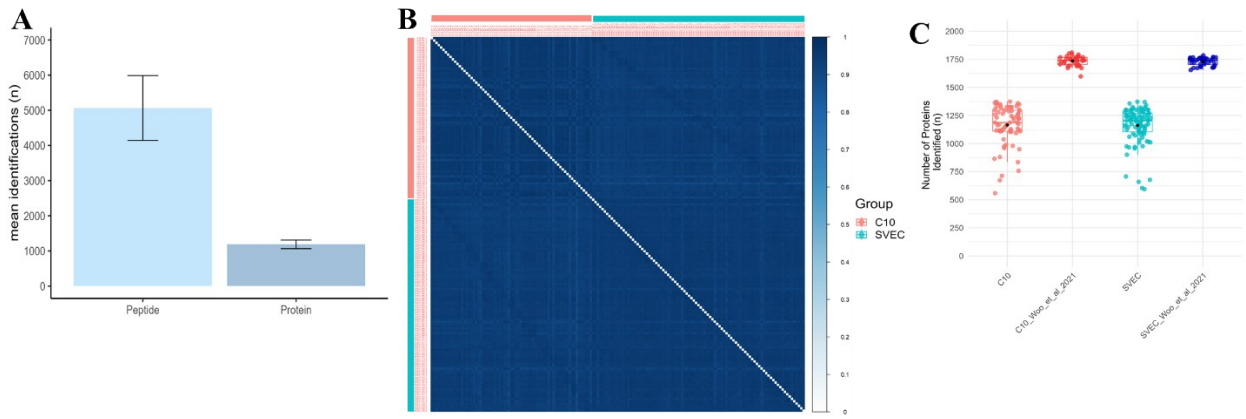
Supplementary Figure 3 – (**A**) Arrangement of samples on cellenCHIP 384 and nanoSPINS array. Green, Blue and Grey colored wells represent C10, SVEC cells and Blanks (negative control) from nanoSPINS chip, respectively. Orange and Red wells represent C10 and SVEC cells directly sorted onto cellenCHIP, respectively. White wells represent empty wells (negative control) for directly sorted cells. (**B**) Zoomed in image representing the arrangement of cells and TMTpro channels on the 3x3 nanoSPINS array. Channels are indicated within each position and additional channels used are noted in text.



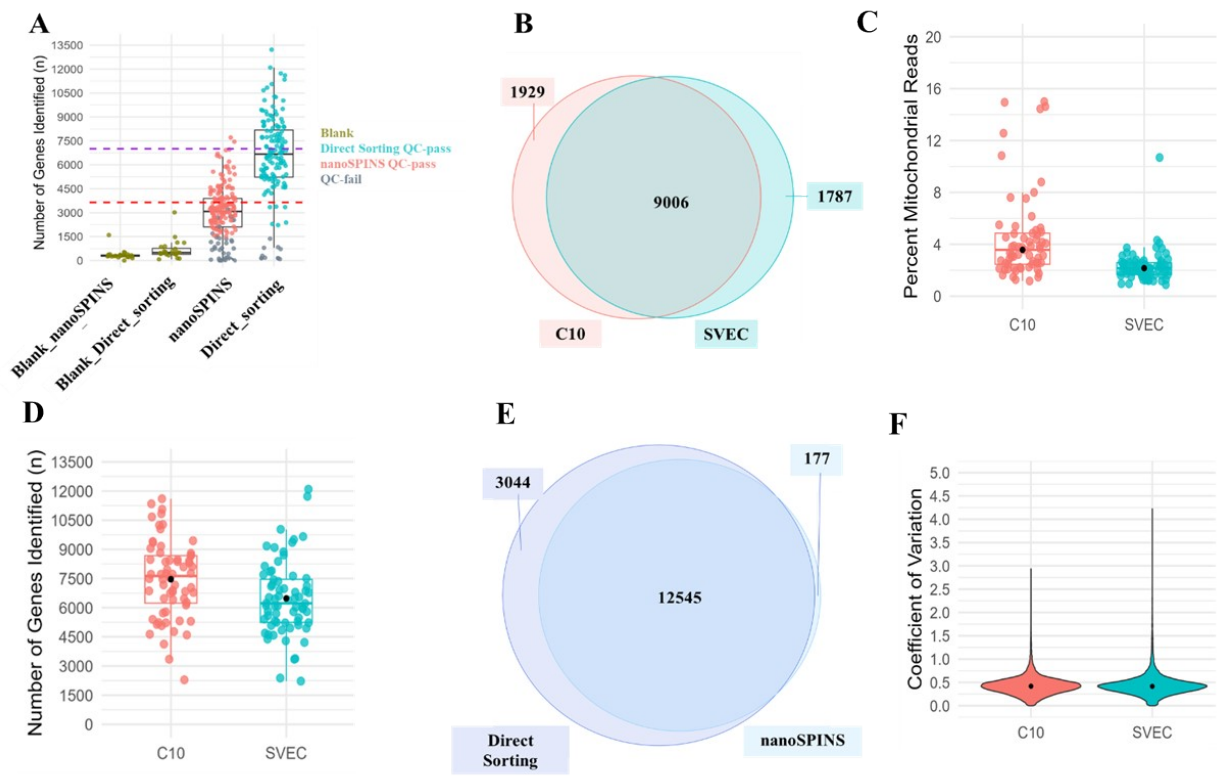
Supplementary Figure 4 – Number of unique peptides identified per TMT channel across all 24 TMT batches. Each barplot corresponds to a single sample and red arrows indicate the 131C channel with consistently reduced coverage.



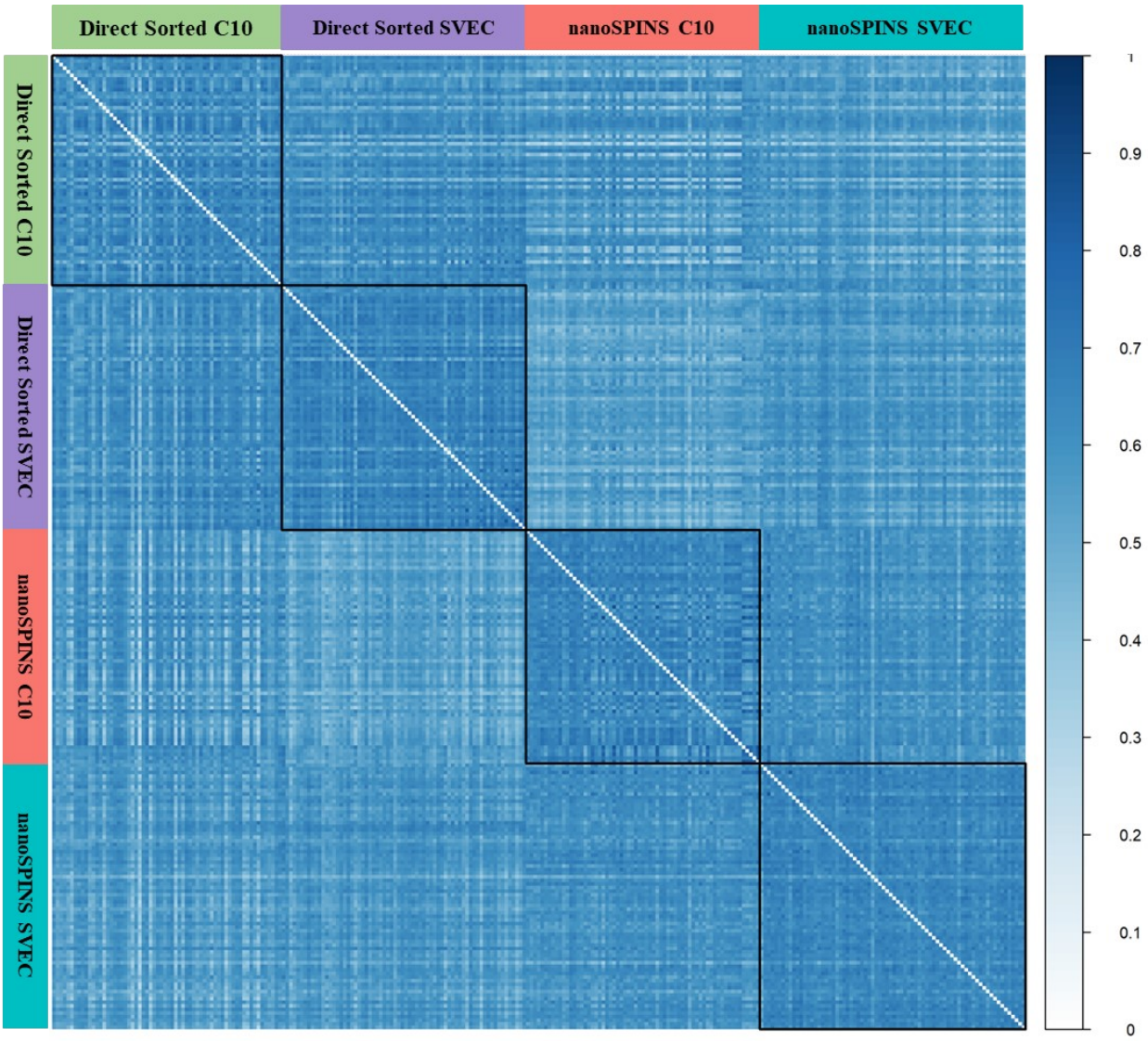
Supplementary Figure 5 – Scatter plot of all C10, SVEC, and blank samples comparing overall TMT reporter ion intensities with the corresponding number of protein identifications. The x-axis represents the sum of all valid reporter ion intensities within 1.5 standard deviations of the IQR (to exclude outliers) while the y-axis represents the number of proteins identified for the corresponding sample. Linear regression is presented with 95% confidence intervals and the corresponding Spearman correlation in text. Dashed horizontal line represents the QC threshold of 750 protein identifications.



Supplementary Figure 6 – **(A)** Mean number of detected peptides and proteins from a total 160 QC-passed single cells (69 C10 and 91 SVEC). Error bars indicate standard deviations (± s.d.). **(B)** Clustering matrix showing Pearson correlations across 160 single cells using log2-transformed protein intensities. The color scale indicates the range of Pearson correlation coefficients. **(C)** Comparison of scProteomic identifications in C10 and SVEC cell in this study with prior TMT-proteomics analysis of C10 and SVEC cells by Woo et al. 2021.



Supplementary Figure 7 – **(A)** Box plot showing the distributions of numbers of genes identified from 96 C10 cells, 96 SVEC cells and 24 blank/control processed using nanoSPINS platform and 72 SVEC cells and 72 C10 cells sorted directly and respective 24 blank/control. "QC_fail" cells represents cells with less than 1500 gene ids and less than 50% of the mapped reads overall. Red and Purple dash line represents mean number of genes identified in high quality nanoSPINS cells and directly sorted cells. **(B)** Venn diagram showing the overlap between high-confidence genes identified from quality controlled C10 (presented in red) and SVEC (presented in Strong cyan) cells processed using nanoSPINS workflow. **(C)** Box plot showing the distributions of number of reads mapped to mitochondrial genes in QC-passed C10 cells and SVEC cells. Centerlines and "black" points represents the distribution median. **(D)** Box plot showing the distributions of gene identification numbers for QC-passed 68 C10 cells and 64 SVEC cells sorted directly. Centerlines represents the distribution median and "black" points represents the distribution mean. **(E)** Venn diagram showing the overlap between high-confidence genes identified from quality-controlled cells processed using nanoSPINS workflow (n = 139; LightSkyBlue) and cells sorted directly (n = 132; RoyalBlue). **(F)** Violin plots showing the coefficient of variations of gene expression from



Supplementary Figure 8 - Clustering matrix displays pairwise Pearson correlation coefficients among 271 QC-passed single cells, including 74 SVEC and 65 C10 cells processed via the nanoSPINS workflow, and 68 SVEC and 64 C10 cells sorted directly onto the nanoSPINS chip. The color scale indicates the range of Pearson correlation coefficients.

	nanoSPINS	nanoSPLITS	scSTAP	CITE-Seq	REAP-Seq
Measurement Types	Global	Global	Global	mRNA (Global), Protein (targeted)	mRNA (Global), Protein (targeted)
Cell-type used in the manuscript	C10,SVEC(~10-20µm)	C10,SVEC,Islet cells (~10-20µm)	Oocytes (~80-100µm)	HeLa, 4T1, 3T3 and CBMCs	PBMCs
Proteins identified per cell	~1100-1350	~1500-3000	>2600	13	45-82
Transcripts identified per cell	~2500-7000	~1,500-8000	>19000	400-600	>500
Key technical requirements	CellenONE, nanoSPINS Chip, CellenCHIP	CellenONE, nanoSPLITS Chip	scSTAP platform (based on Sequential Operation Droplet Array)	10x Single-cell sorter, DNA-barcoded antibodies	10x Single-cell sorter, DNA-barcoded antibodies
Approach (proteomics)	TMT labelled	Label free	Label free	oligo-tagged antibody staining	oligo-tagged antibody staining
Throughput (per experiment)	384 on a single array	48 on a single array	1 sample	>8000 samples	>8000 samples
Reference	This study	[1]	[2]	[3]	[4]

Supplementary Table 1 – Table comparing characteristics such as throughput, measurement depth, and measurement type(s) in recently developed single-cell proteotranscriptomics approaches.

References

(1) Fulcher, J. M.; Markillie, L. M.; Mitchell, H. D.; Williams, S. M.; Engbrecht, K. M.; Degnan, D. J.; Bramer, L. M.; Moore, R. J.; Chrisler, W. B.; Cantlon-Bruce, J. Parallel measurement of transcriptomes and proteomes from same single cells using nanodroplet splitting. *Nature Communications* **2024**, *15* (1), 1-13.

(2) Jiang, Y.-R.; Zhu, L.; Cao, L.-R.; Wu, Q.; Chen, J.-B.; Wang, Y.; Wu, J.; Zhang, T.-Y.; Wang, Z.-L.; Guan, Z.-Y. Simultaneous deep transcriptome and proteome profiling in a single mouse oocyte. *Cell Reports* **2023**, *42* (11).

(3) Stoeckius, M.; Hafemeister, C.; Stephenson, W.; Houck-Loomis, B.; Chattopadhyay, P. K.; Swerdlow, H.; Satija, R.; Smibert, P. Simultaneous epitope and transcriptome measurement in single cells. *Nature methods* **2017**, *14* (9), 865-868.

(4) Peterson, V. M.; Zhang, K. X.; Kumar, N.; Wong, J.; Li, L.; Wilson, D. C.; Moore, R.; McClanahan, T. K.; Sadekova, S.; Klappenbach, J. A. Multiplexed quantification of proteins and transcripts in single cells. *Nature biotechnology* **2017**, *35* (10), 936-939.