Pushing the boundaries for robust and high-throughput single cell analysis with Whisper Flow technology powered by dia-PASEF

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Highlights

- More than 99.5% efficiency from the Evotip Pure, enables high sensitivity for single cell analysis.
- Scalable single cell analysis enabled by utilizing unique analytical properties for storage of samples on the Evotip Pure.
- Whisper methods combined with dia-PASEF maps more than 5,000 proteins from single HeLa cells isolated and digested in a proteoCHIP Evo96 on the cellenONE.

Extremely high sample capture and recovery

Nearly loss-less sample transfer

While most autosamplers utilize a sample loop for injecting limited sample volumes, the Evotip can be loaded with large volumes (>20μL) and used for concentrating dilute samples. Peptides are efficiently concentrated on the Evotip, which enables sample preparation in large volumes that allow for reproducible handling and eliminates the need for prior concentration steps that would severely impair high sensitivity workflows.

We evaluated the efficiency of the Evotip by measuring the capture and recovery of peptides in relation to the amount of sample loaded on the tip. Capture is the amount of sample retained on the tip in relation to the amount of sample lost in the flow-through. Recovery is the amount of sample retained and subtracted the the residual amount of peptide left on the tip after analysis. This is calculated using summed precursor intensity relative to both 1, 5 and 50 ng loaded sample on Evotips. Our results indiciate that the total summed losses are below 0.45%, which for the low loads will be below limit of detection.
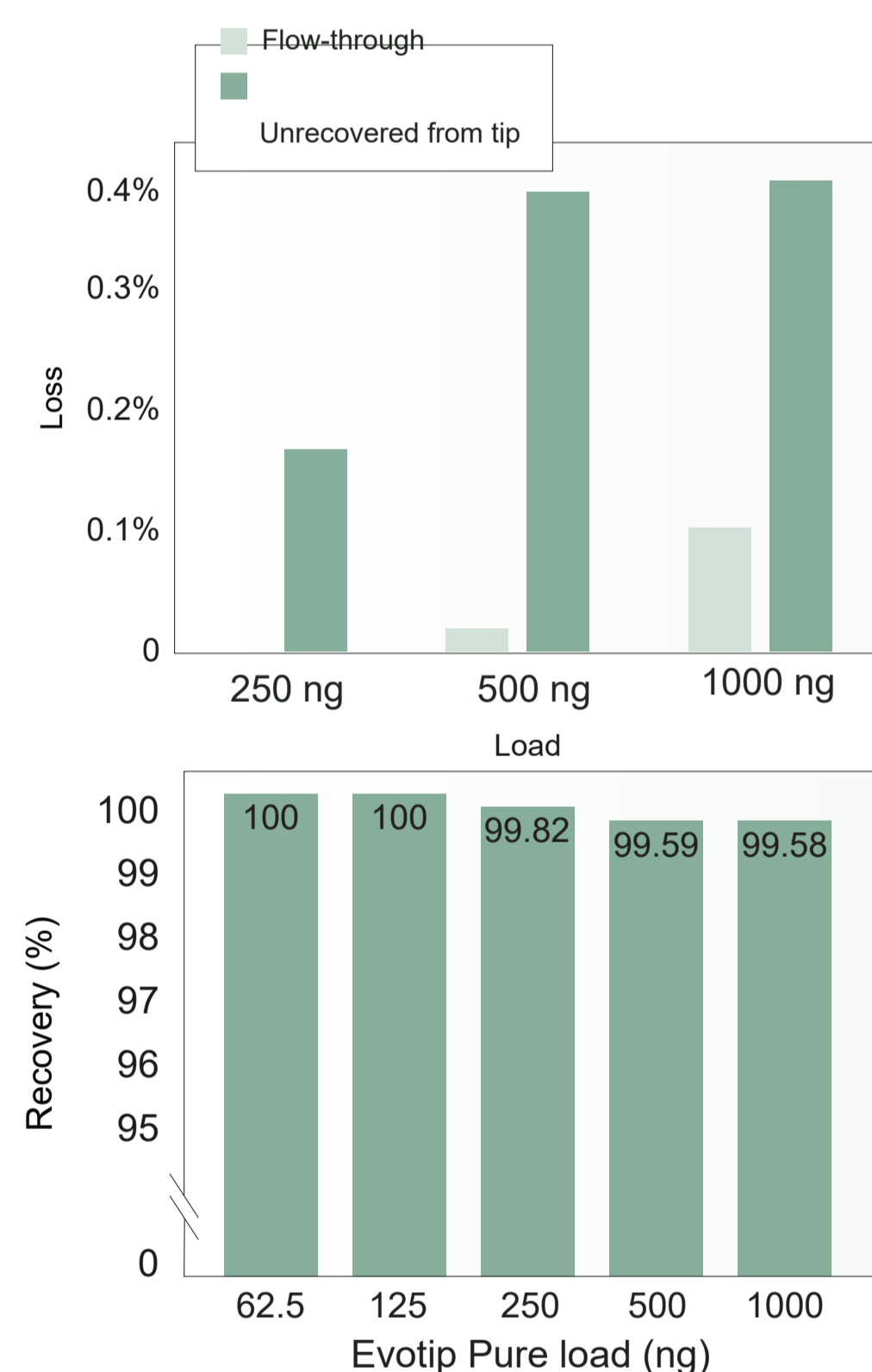
Percentage recovery measured fromEvotip Pure tips loaded with 62.5 - 1000 ng, calculated as the sum of all identified precursors compared to the 50 ng load.

Robust storage of samples until analysis

Further, storage of samples prior to mass spectrometry analysis is often necessary and typically performed in tubes, plates, or HPLC vials. This will lead to various degrees of sample losses, through the additional transfer steps and peptide adsorption to plastics during storage, which is particularly significant when working with low amounts of input material. Here, we show that once captured on the C18 material, single-cell levels of peptides (250 pg HeLa digest) can be stored in a stable manner for 2 weeks without compromising data quality. This feature of the Evotip is crucial in the pursuit of robust, high-throughput single-cell proteomics as it enables experiments to be readied and run for extended periods.
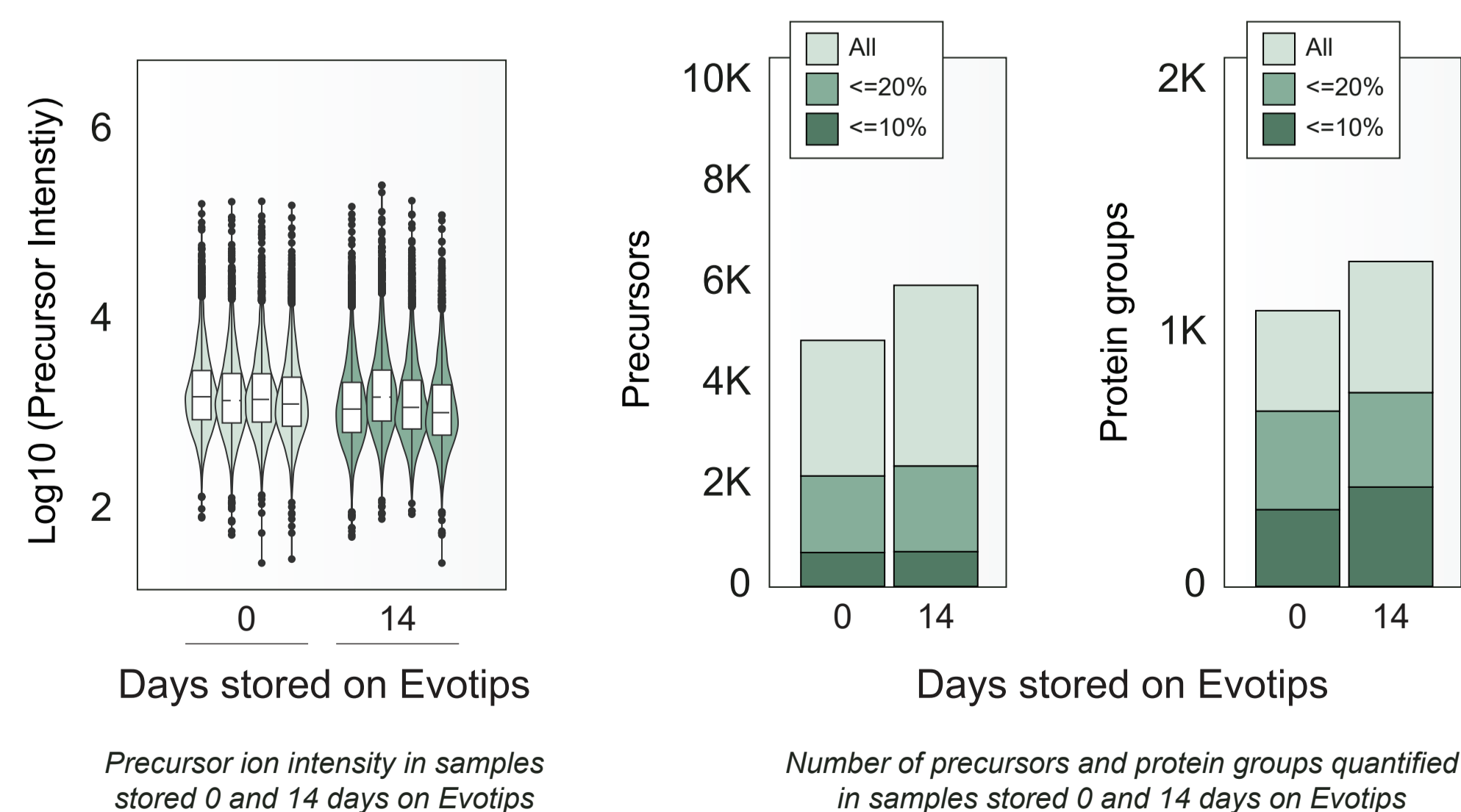

Precursor ion intensity in samples stored 0 and 14 days on Evotips

*Number of precursors and protein groups quantified in samples stored 0 and 14 days on Evotips*

Robust and reproducible single cell analysis

More than 5,000 proteins identified in total from single cells

We have developed a prototype chip for seamless integration between sample preparation on the cellenONE and transfer of peptides directly into Evotips. Across 48 replicate injections, we observe a more tight distribution of precursors using direct centrifugation compared to transferring peptides with a pipette confirming that every step in a highly sensitive protocol affects performance and reproducibility.


Workflow for robust and reproducible single cell analysis
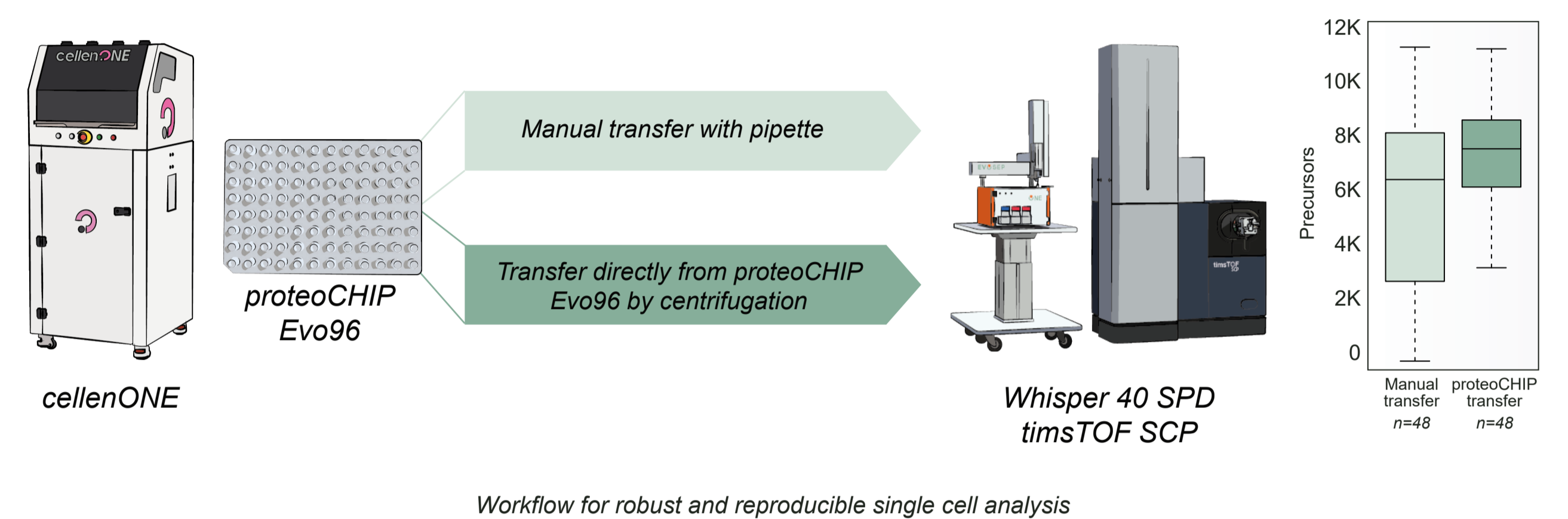
We sorted cells in different groups dependent on size and digested these in the prototype chip and transferred peptides into Evotips via centrifugation. The single-cell proteomes revealed excellent coverage with an average of 3,500 protein groups per cell and close to 5,500 proteins identified in the entire dataset. During the 2.5 days of measurment, the loaded Evotips were stored on the instrument at room temperature with no observed loss in proteome depth.
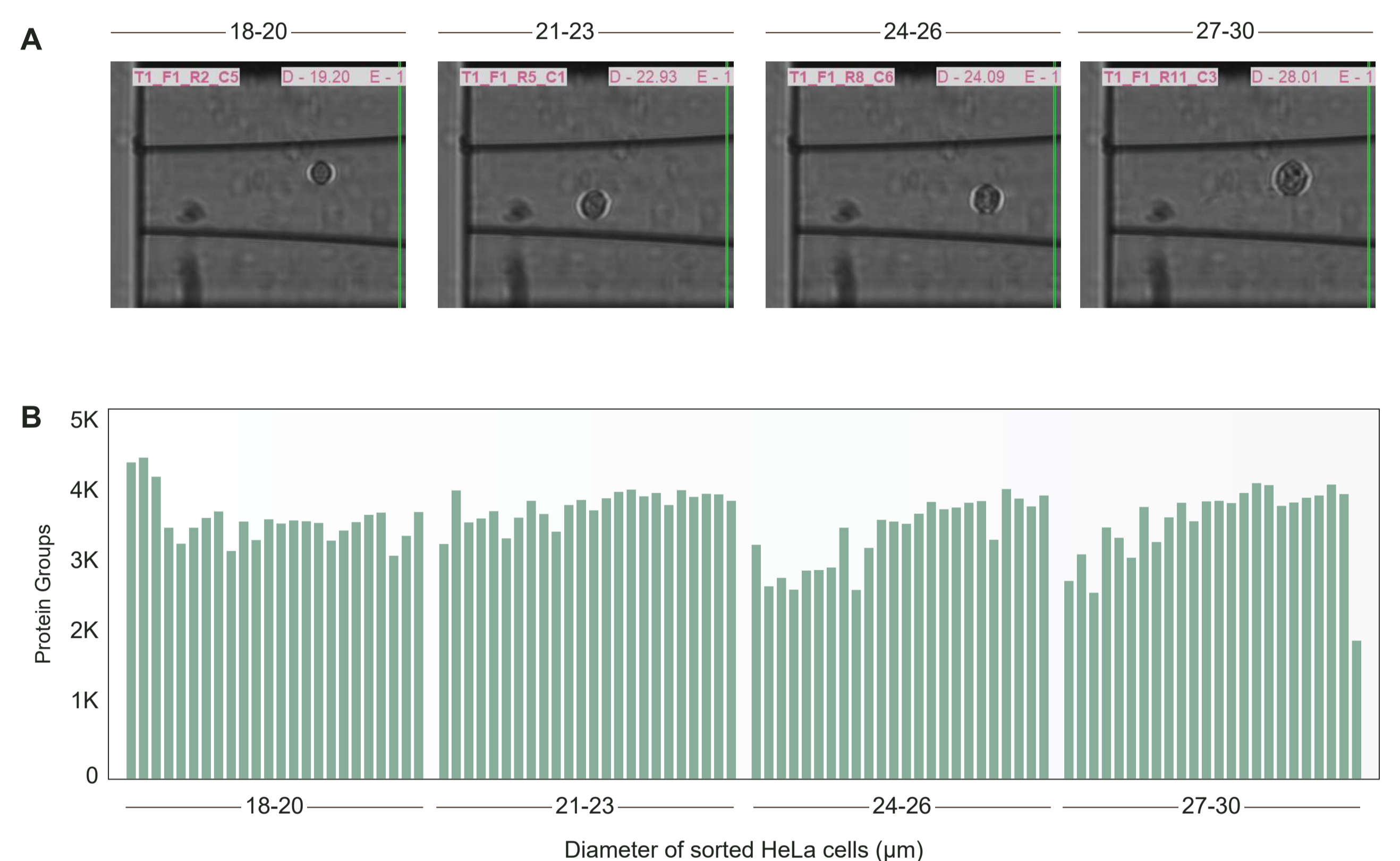
(A) Images of sorted HeLa cells representing the four groups of cells sorted. (B) Protein group identifications from 96 cells analyzed with Whisper 40 SPD with dia-PASEF on a timsTOF SCP and analyzed with DIA-NN.

Interestingly, the number of proteins identified does not correlate with cell size in this experiment, but indeed we see a nice separation of the cell size groups, where the bigger cells, 24-26 μm and 27-30 μm are nicely separated from the 18-20 μm and 21-23 μm cells in a principal component analysis. From an ANOVA analysis, we find more than 2,500 proteins to be significantly regulated between the groups.
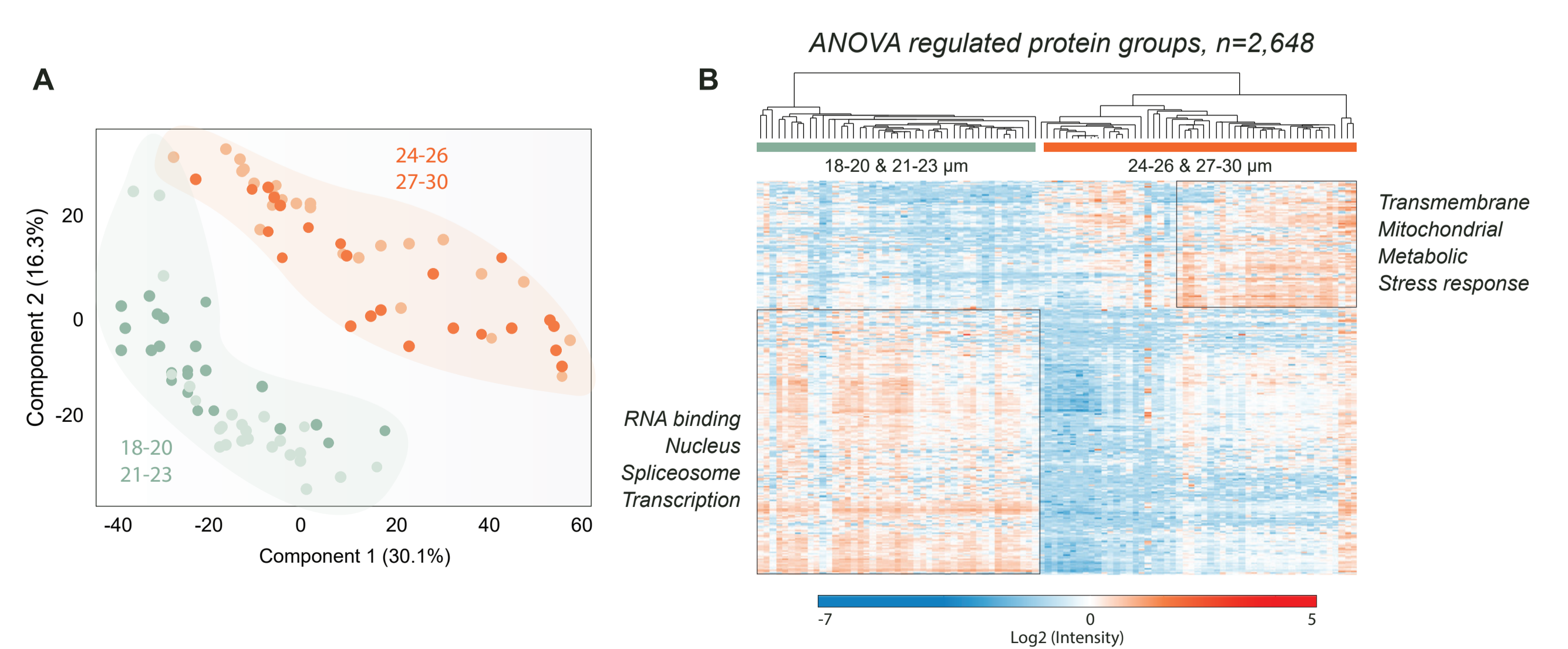
(A) Principal component analysis of the global proteome. (B) Hierarchical clustering analysis of ANOVA regulated protein groups.