

# proteoCHIP EVO 96 – Evotip Pure™ – timsTOF SCP:

## A powerful Trio for Single Cell Proteomics

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### Highlights

- Near loss-less sample preparation using the cellenONE in combination with the proteoCHIP EVO 96
- Easy and fast sample transfer to Evotips via centrifugation
- End-to-end workflow from the cellenONE to the timsTOF SCP
- High quality spectra from single cell samples

### Introduction

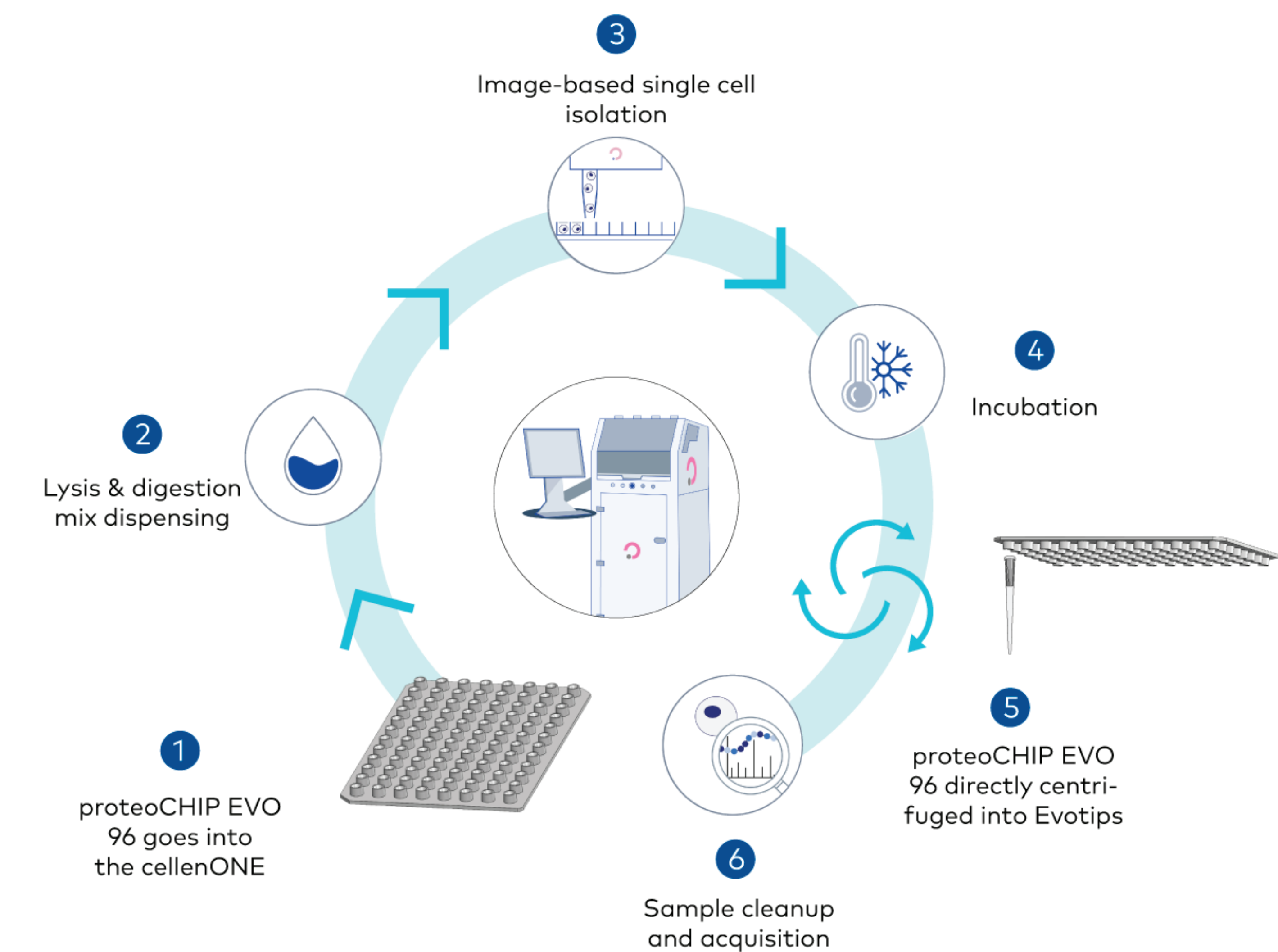
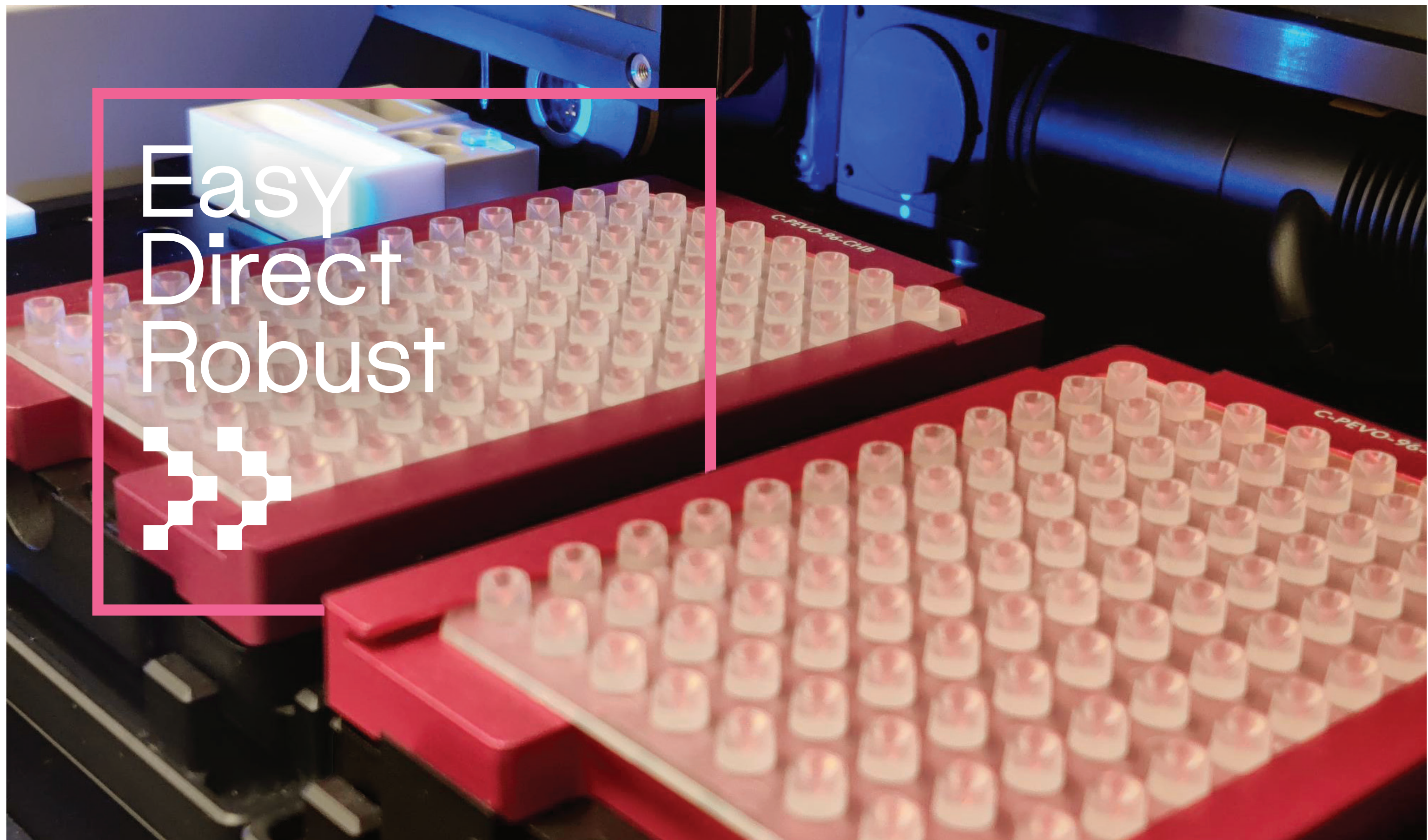
With the recent advancements in LC-MS technology and the introduction of near loss-less sample preparation, Single Cell Proteomics (SCP) has become a meaningful option to investigate cell heterogeneity. The cellenONE cell isolation and nL-dispensing platform in combination with the proteoCHIP EVO 96 allows for sub- $\mu$ L cell processing and a seamless and easy transfer of samples via centrifugation directly to Evotips for robust and fast LC-MS data acquisition of single cell digests.

### Basic SCP workflow with the cellenONE

- The proteoCHIP EVO 96 is placed into the cellenONE
- 300 nL of Master Mix is dispensed into proteoCHIP wells
  - 10 ng/ $\mu$ L Enzyme, 0.2% DDM, 100 mM TEAB
- Cell isolation according to their size, morphology & fluorescence
- Lysis and digestion of cells for 2h at 50 °C with evaporation controls
- Dilution of the samples with 3.2  $\mu$ L of Solvent A

### Sample transfer to Evotips

- The proteoCHIP EVO 96 is placed with the cell digests on the Evotip box with already equilibrated Evotip Pure™
- Centrifuge the samples into the equilibrated Evotip Pure™
- Wash the Evotip Pure™ with 20  $\mu$ L Solvent A
- The samples are ready for measurement

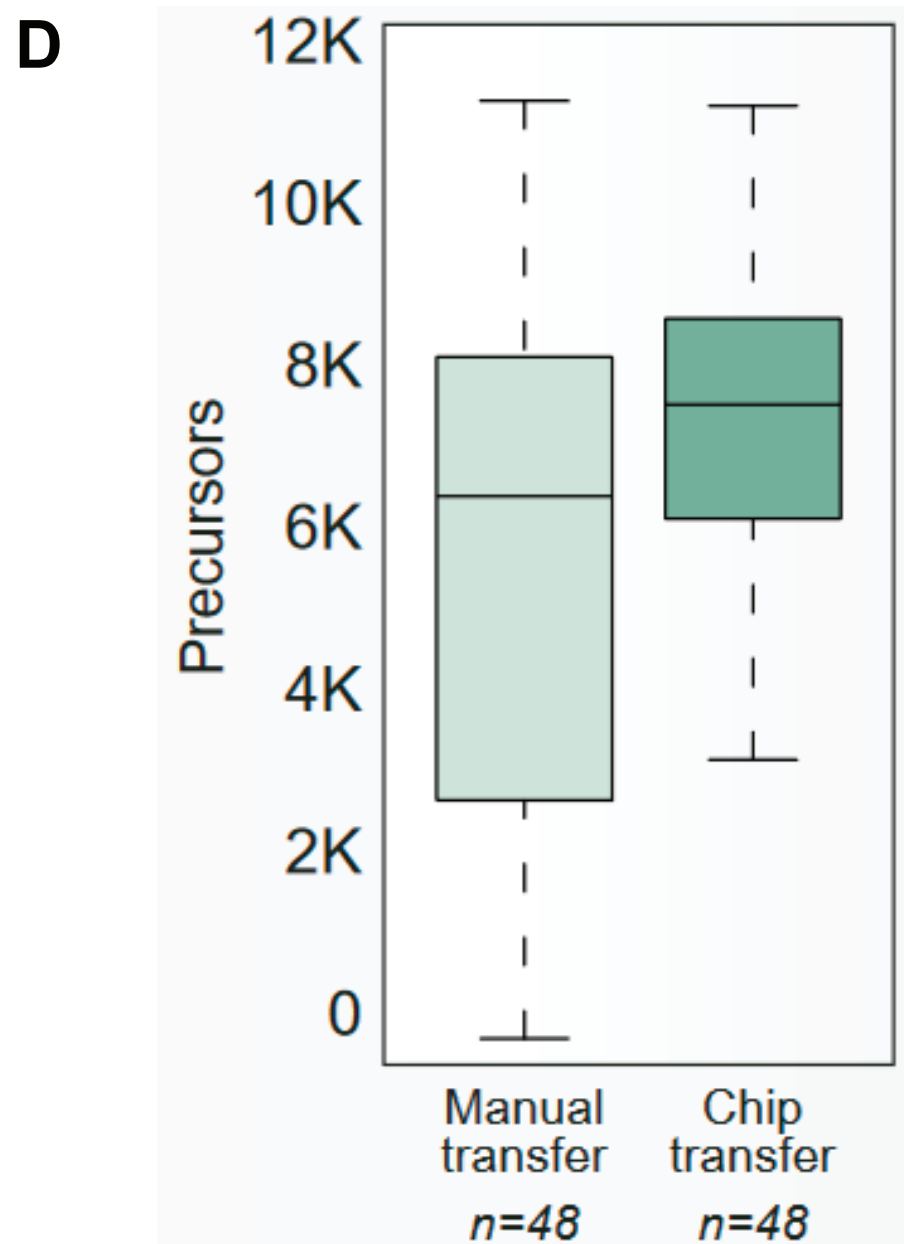
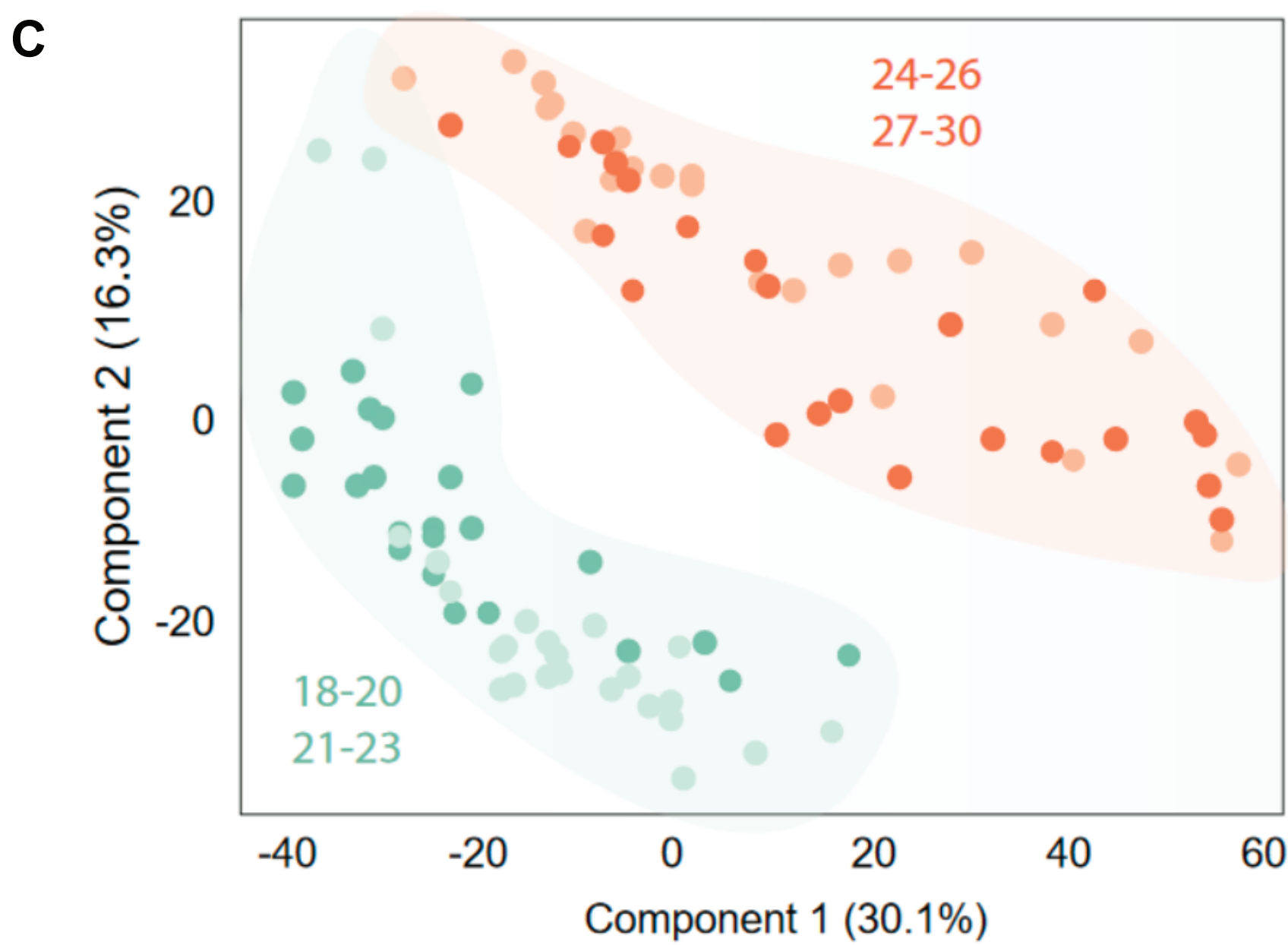
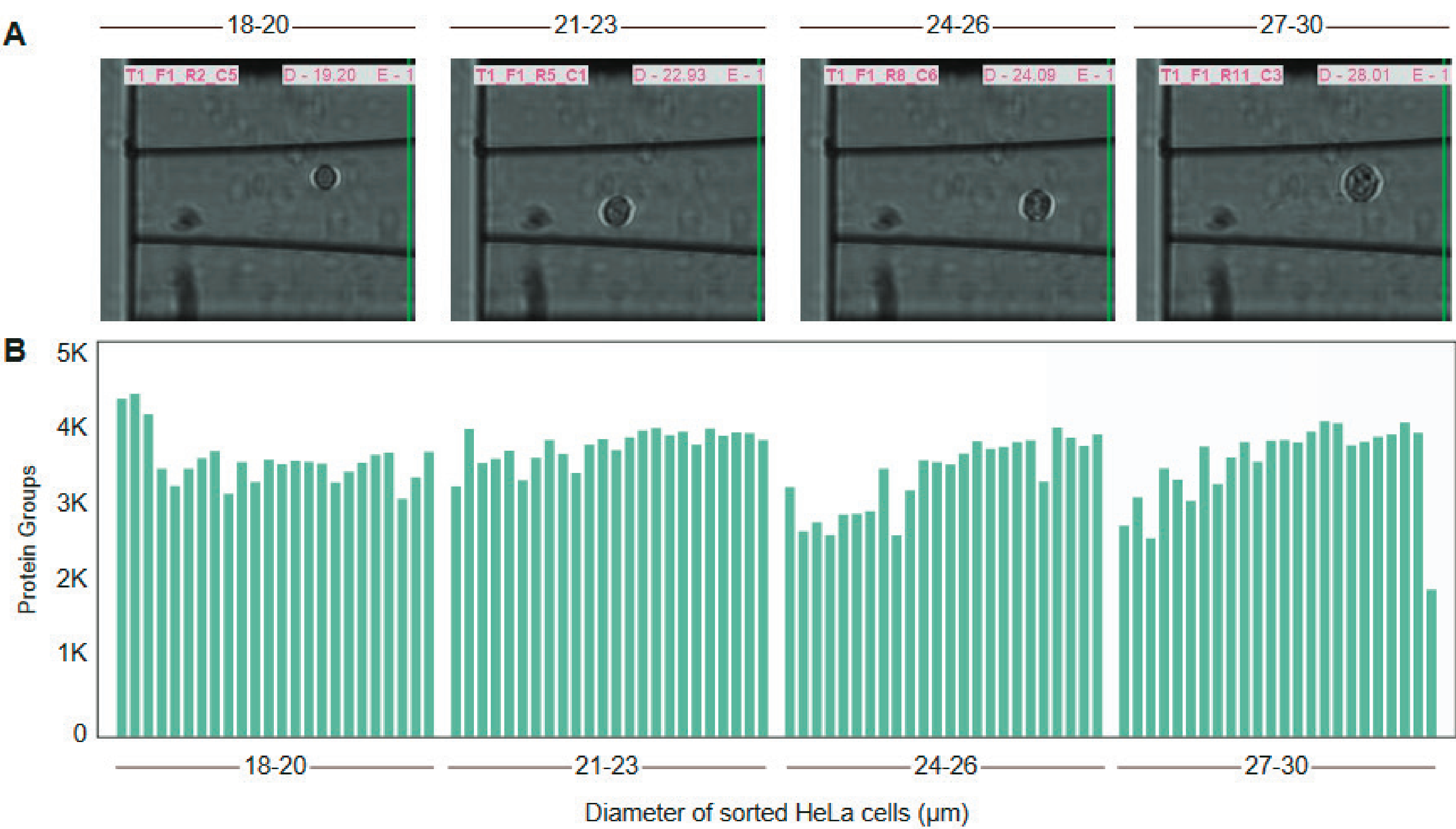


The proteoCHIP EVO 96 workflow



### LC-MS evaluation of single HeLa cells

Single HeLa cells were sorted by the cellenONE based on four defined size bins and processed in the proteoCHIP EVO 96. Afterwards peptides were separated on the Evosep ONE using Whisper 40 SPD and analysed on the timsTOF SCP mass spectrometer. On average 3500 proteins were identified with some injections reaching an analysis depth of 4400 protein groups. Principal component analysis shows separation of cell size groups 18-23  $\mu$ m and 24-30  $\mu$ m cell diameter. An ANOVA analysis reveals more than 2500 proteins are significantly regulated between those groups.



### Summary

The cellenONE provides a streamlined and automated Single Cell Proteomics workflow with the proteoCHIP EVO 96 tailored for near loss-less sample transfer to Evotips for MS analysis.