

Supporting Information

**HyperSCP: Combining Isotopic and Isobaric Labeling for Higher Throughput Single-Cell
Proteomics**

Yiran Liang,¹ Thy Truong,¹ Aubrianna J. Saxton,¹ Hannah Boekweg,² Samuel H. Payne,² Pam
M. Van Ry¹ and Ryan T. Kelly^{1*}

1. Department of Chemistry and Biochemistry, Brigham Young University, Provo, UT, 84602

2. Department of Biology, Brigham Young University, Provo, UT, 84602

*Email: ryan.kelly@byu.edu

Table of Contents

Figure S1: The dimensions of each nanowell in a nested-well and between the nested-wells on a hyperSCP chip. Unit: mm.	S3
Figure S2: Cover slide used for incubation. A PMMA frame was glued to a microscope glass slide. (a) Overview of the cover slide. (b) The top, front and side view of the cover slide with dimensions labeled. Unit: mm.	S4
Figure S3: Flowchart of hyperSCP data processing.	S5
Figure S4: (a) Proteome coverage comparison of TMTpro isobaric labeling only and hyperSCP labeling methods without carrier channels. (b) The percentage of quantifiable protein groups (≥ 2 unique peptides) among the identified protein groups shown in (a). (c) Missing values between heavy and light TMTpro sets using hyperSCP with different gradient times.....	S6
Figure S5: Correlation of the heavy-labeled and light single cells. Condition: 60 min gradients and 10-ng carrier were applied.	S7
Figure S6: The PCA plot of lung cells A549 and HFL1 with 10 ng carrier proteome and 60-min gradient.	S8

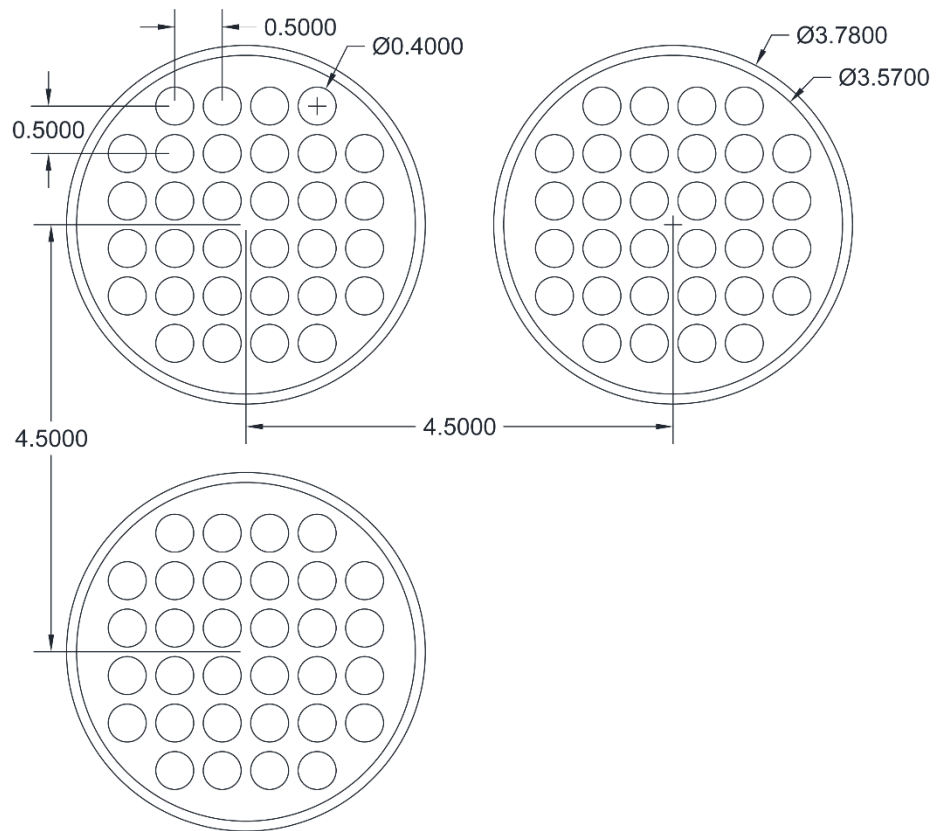


Figure S1: The dimensions of each nanowell in a nested-well and between the nested-wells on a hyperSCP chip. Unit: mm.

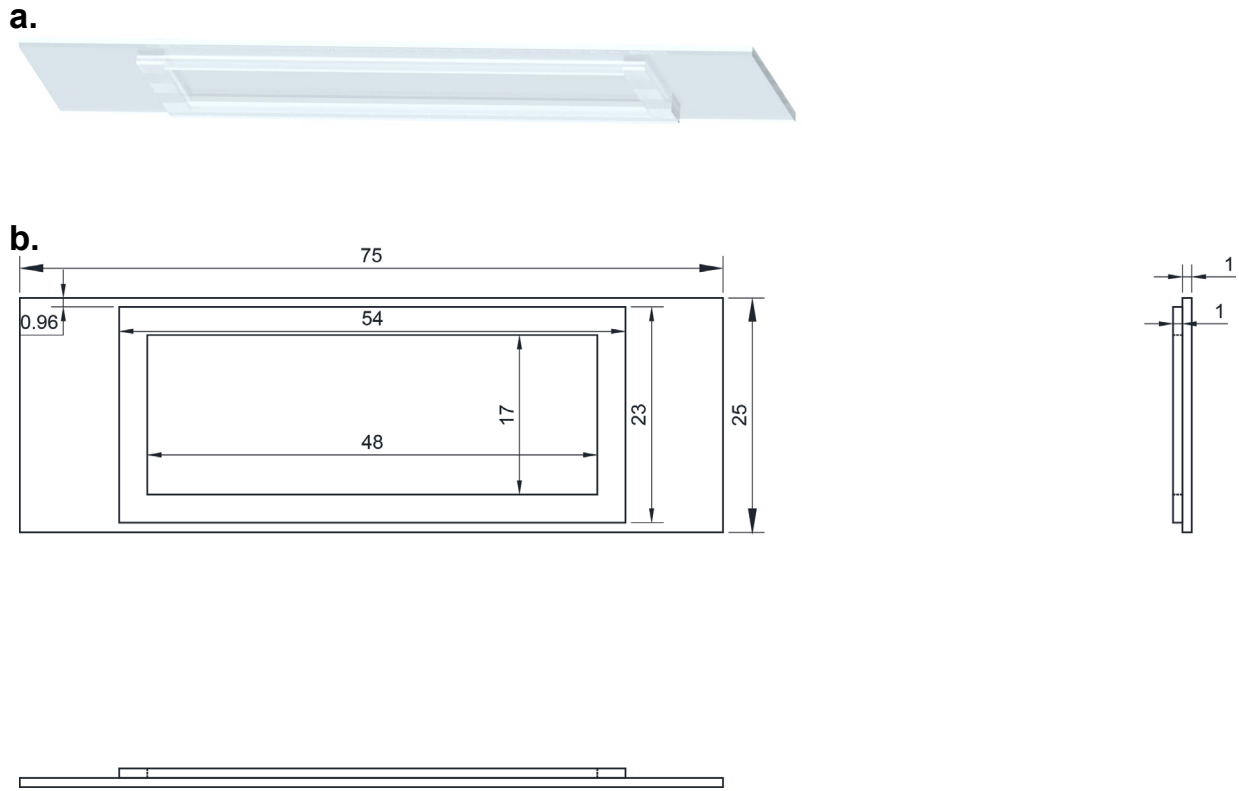


Figure S2: The cover slide for incubation. A PMMA frame was glued to a microscope glass slide. (a) Overview of the cover slide. (b) The top, front and side view of the cover slide with dimensions labeled. Unit: mm.

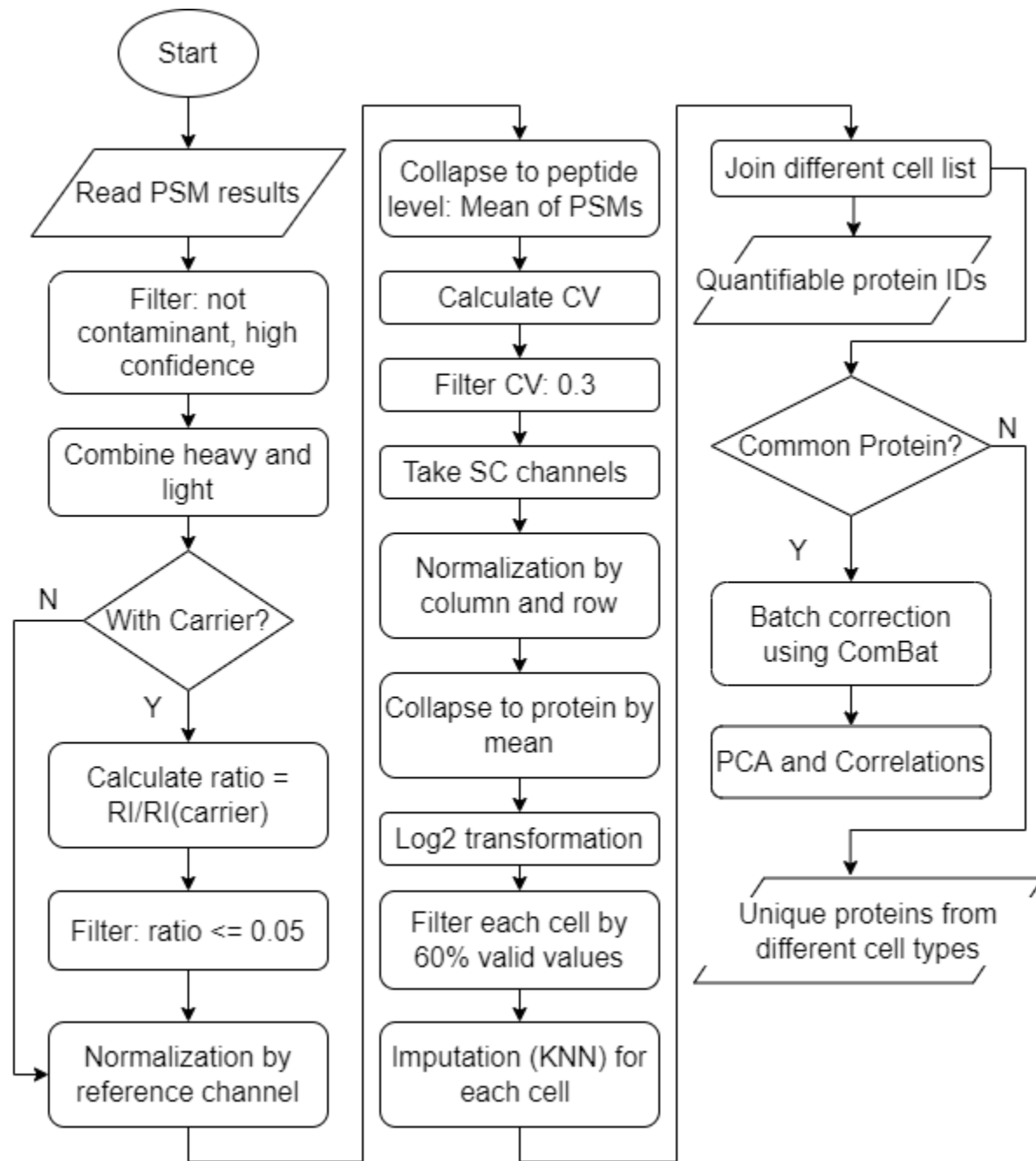


Figure S3: The flowchart of hyperSCP data processing.

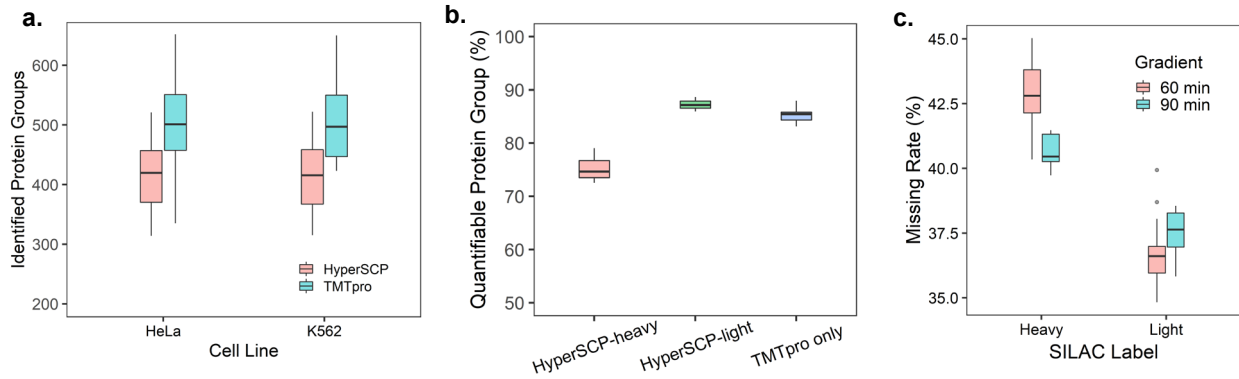


Figure S4: (a) Proteome coverage comparison of TMTpro isobaric labeling only and hyperSCP labeling methods without carrier channels. (b) The percentage of quantifiable protein groups (≥ 2 unique peptides) among the identified protein groups shown in (a). (c) Missing values between heavy and light TMTpro sets using hyperSCP with different gradient time. All quantifiable proteins in one MS run are counted as 100%.

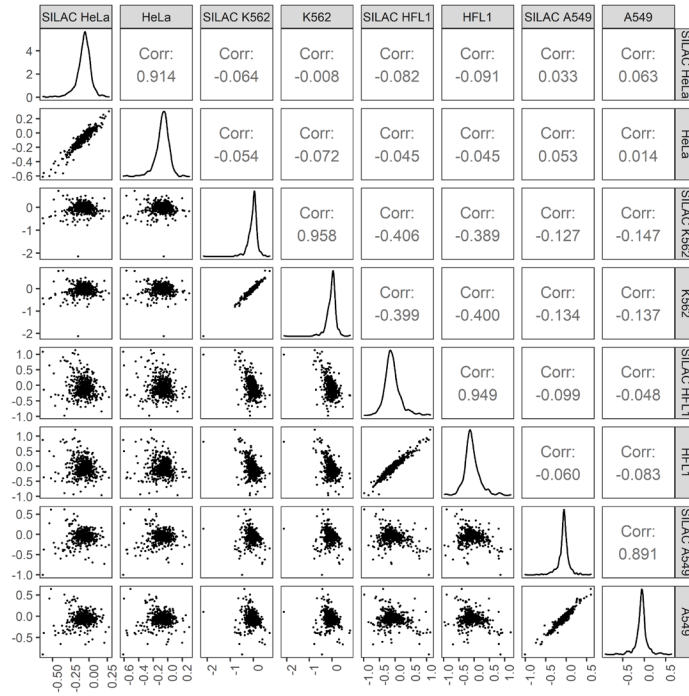


Figure S5: Correlation of the heavy and light labeled single cells. Condition: 60 min gradients and 10-ng carrier were applied.

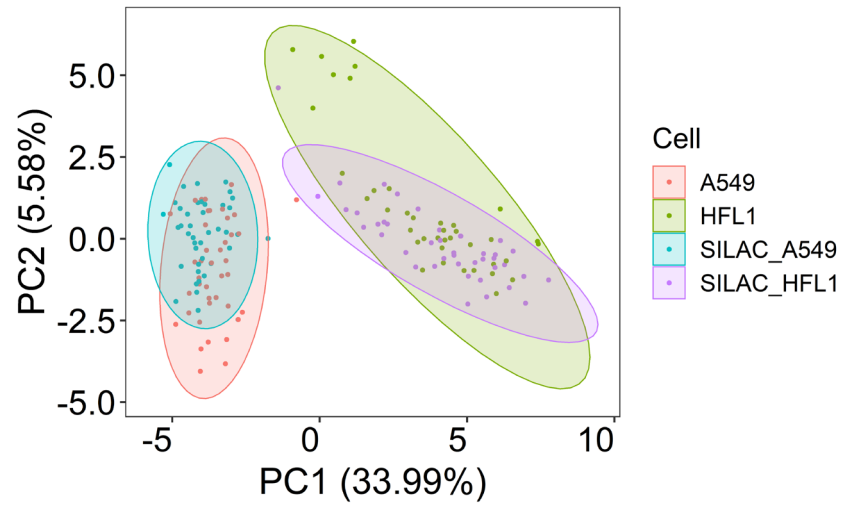


Figure S6: The PCA plot of lung cells A549 and HFL1 with 10 ng carrier proteome and 60-min gradient.