Supporting Information

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

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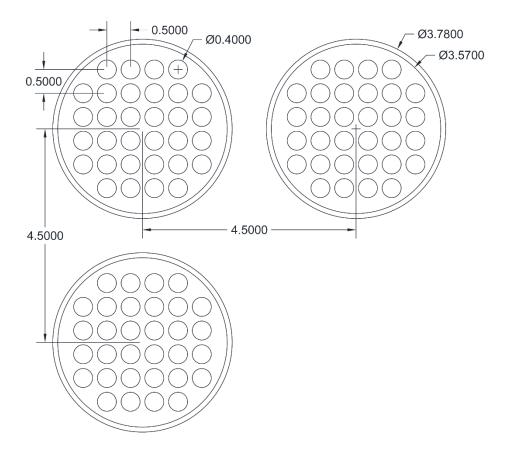
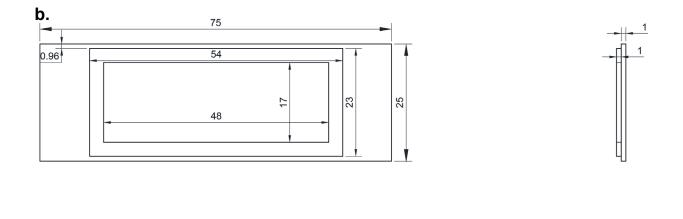
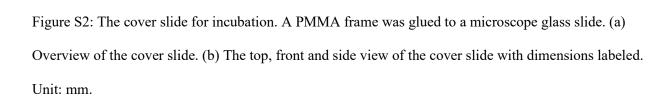


Figure S1: The dimensions of each nanowell in a nested-well and between the nested-wells on a hyperSCP chip. Unit: mm.







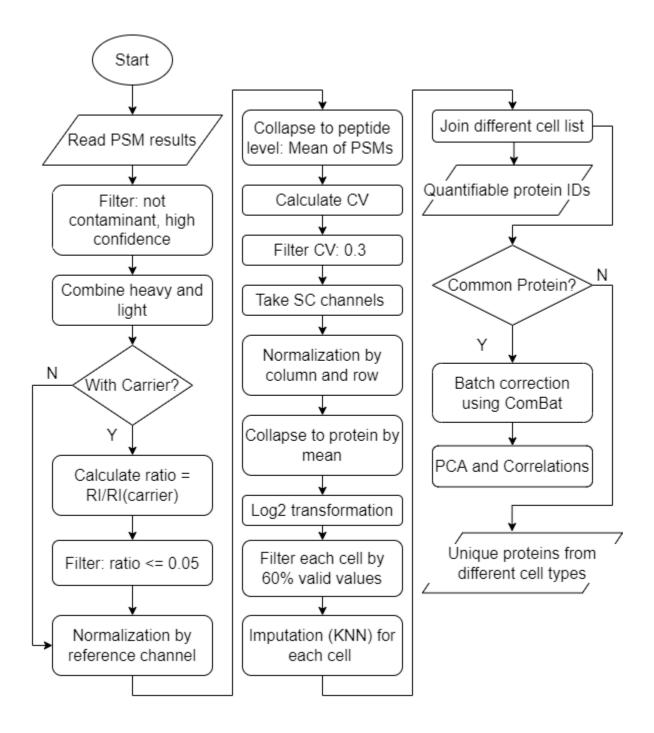


Figure S3: The flowchart of hyperSCP data processing.

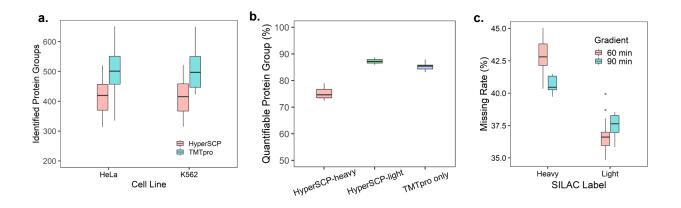


Figure S4: (a) Proteome coverage comparison of TMTpro isobaric labeling only and hyperSCP labeling methods without carrier channels. (b) The percentage of quantifiable protein groups ( $\geq 2$  unique peptides) among the identified protein groups shown in (a). (c) Missing values between heavy and light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

SILAC HeLa	HeLa	SILAC K562	K562	SILAC HFL1	HFL1	SILAC A549	A549	
	Corr: 0.914	Corr: -0.064	Corr: -0.008	Corr: -0.082	Corr: -0.091	Corr: 0.033	Corr: 0.063	SILAC HeLa
0.2 0.0 -0.2 -0.4 -0.6		Corr: -0.054	Corr: -0.072	Corr: -0.045	Corr: -0.045	Corr: 0.053	Corr: 0.014	HeLa
0 -1 -2			Corr: 0.958	Corr: -0.406	Corr: -0.389	Corr: -0.127	Corr: -0.147	SILAC K562
0 -1 -2				Corr: -0.399	Corr: -0.400	Corr: -0.134	Corr: -0.137	K562
1.0 0.5 0.0 -0.5 -1.0				$\int \!$	Corr: 0.949	Corr: -0.099	Corr: -0.048	SILAC HFL1
1.0 0.5 0.0 -0.5 -1.0					$\int_{-\infty}$	Corr: -0.060	Corr: -0.083	HFL1
0.5 0.0 -0.5 -1.0							Corr: 0.891	SILAC A549
0.5								A549
-0.50 -0.25 0.00	0.0.0	0 - 7	0 - 7	-1.0 0.5 0.5	-1.0 0.5 0.5	-1.0 -0.5 -0.0	0.0	2

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

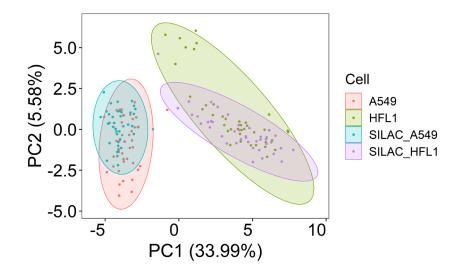


Figure S6: The PCA plot of lung cells A549 and HFL1 with 10 ng carrier proteome and 60-min gradient.