Ultrasensitive single-cell proteomics workflow identifies >1000 protein groups per mammalian cell

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Method	Protein groups						
	Replicate	Replicate 2	Replicate 3	Average			
	1						
OTOT-HCD 2 FAIMS CVs	1498	1627 1633		1586			
OTIT-CID 2 FAIMS CVs	1729	1844	1933	1835			
OTIT-HCD 2 FAIMS CVs	2171	2009	2003	2061			
OTIT-HCD 3 FAIMS CVs	1847	1937	1803	1862			

Table S1. Protein groups identified from 0.5 ng of HeLa digest

	Sample	Protein groups			Peptide groups				
		R1	R2	R3	Average	R1	R2	R3	Average
FAIMS	SH	1031	1156	980	1056	4064	4395	3276	3912
	SM	972	1192	1126	1097	3063	3595	3232	3297
	SI	989	1265	1322	1192	2978	3842	4195	3672
	Blank	149	200	224	191	320	443	524	429
No FAIMS	SH	532	430	418	460	2460	1814	1545	1940

Table S2. Protein groups identified from single cells and blank sample analyzed by LC/MS

Note: SH: single HeLa cell, SM: single motor neuron, SI: single interneuron; R1,2,3: replicates 1,2,3; Blank: HeLa cell supernatant



Figure S1 The percentrage of detected charged ions from single HeLa cells with/without FAIMS Pro interface incorporated . A) Percentage change of charged ions count (species). B) Percentage change of charged ions abundance.



Figure S2 Venn diagram indicating overlap of identified protein groups among three replicate analyses of single HeLa cells.



Figure S3. Single-cell proteomic interrogation of human spinal motor neurons (MNs) and interneurons (INs). A) Distribution of log2-transformed values for 1118 quantified protein groups. Imputed values shown in dark gray B) Top 15 significantly (Benjamini-Hochberg-corrected FDR<1%) over-represented Gene Ontology Biological Process (GOBP) categories pathways within the subset of proteins significantly enriched in MNs (28 protein groups, red) or INs (11 protein groups, blue) (p<0.05 |Fold Difference| \geq 2). C) Protein-protein interactions among 39 significantly differentially expressed proteins in MNs and INs. Nodes represent individual protein groups, edges represent protein-protein interactions curated by the STRING database (v11). Node color indicates fold difference in protein group abundance, edge width indicates strength of reported interaction (0.4<x<1). Nodes indicated in gray (TARDBP, FUS, SMN) were not detected in single MNs or INs and were manually added to the network as examples of connectivity of enriched-in-MN proteins with protein groups implicated in motor neuron disease.



Figure S4. Representative images showing laser capture microdissection of single motor neurons and interneurons. A1 and A2 show H&E stained tissue before and after microdissection of a motor neuron. A3 shows the same motor neuron transferred to a nanowell. B1–B3 show corresponding images for an interneuron.