Appendix for

Limited proteolysis-coupled mass spectrometry captures proteome-wide protein structural alterations and biomolecular condensation in living cells

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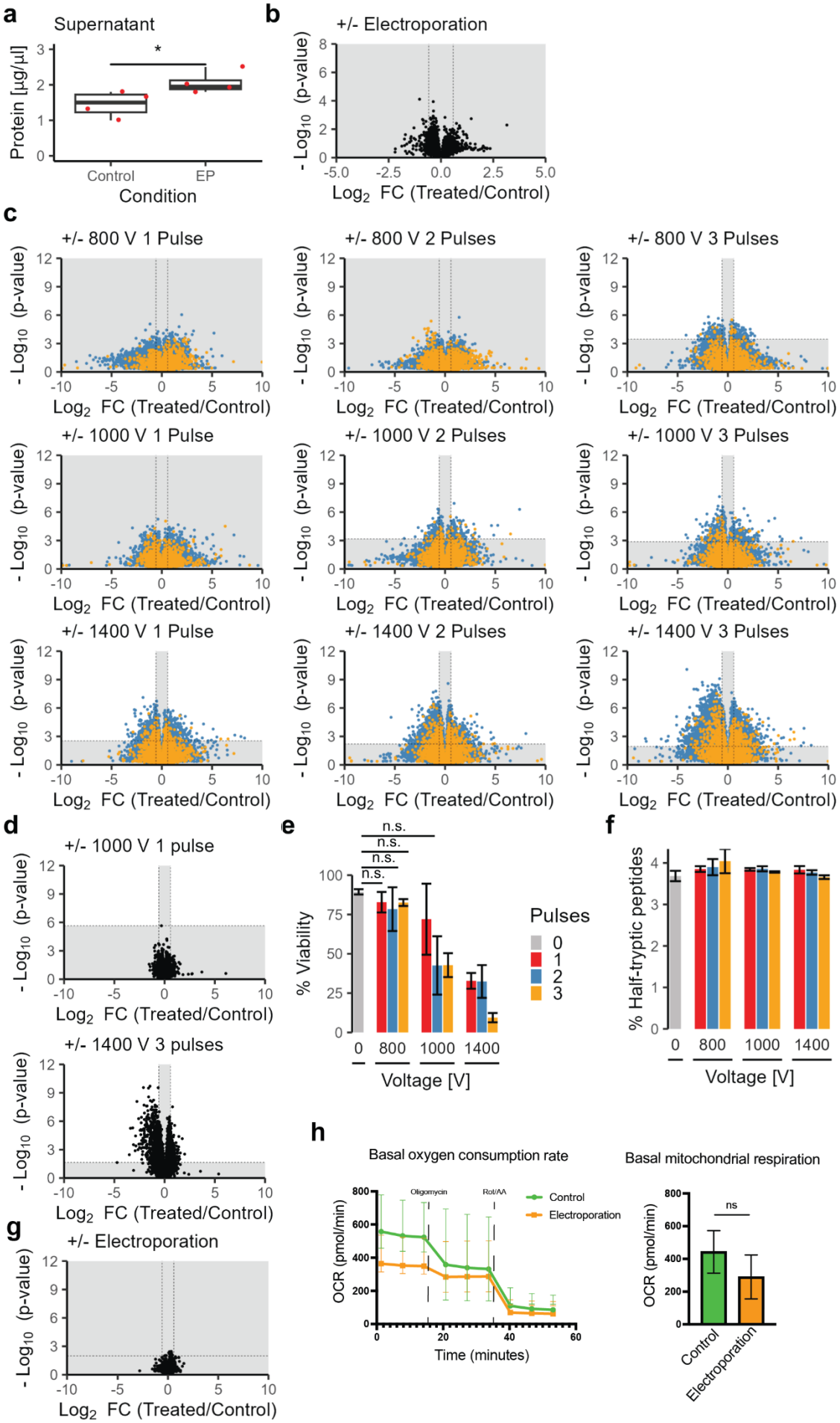
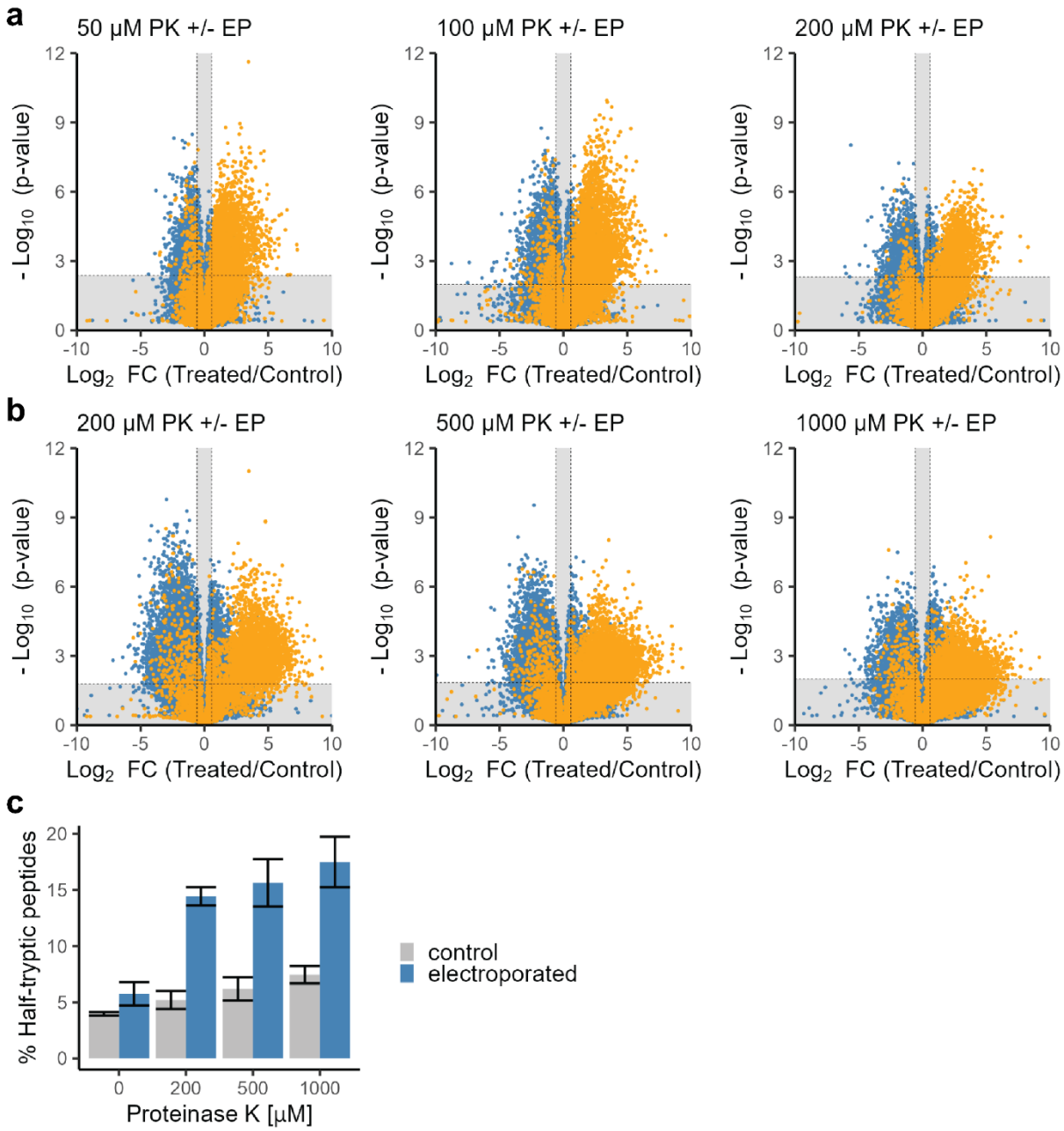
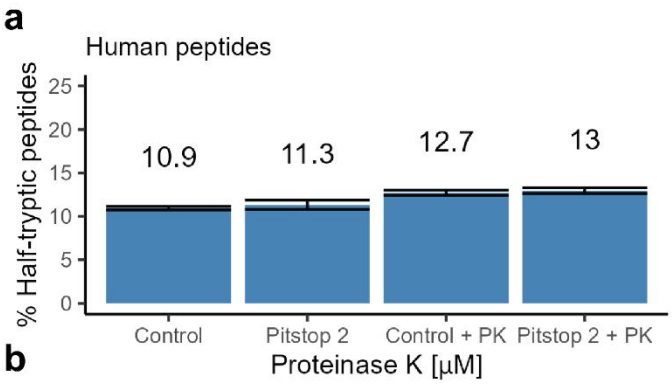
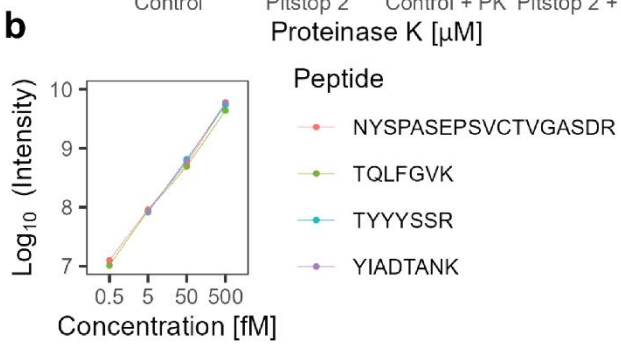

a
Supernatant
Protein [µg/µl]
Control
EP
Condition
b
+/- Electroporation
- Log10 (p-value)
Log2 FC (Treated/Control)
c
+/- 800 V 1 Pulse
+/- 800 V 2 Pulses
+/- 800 V 3 Pulses
+/- 1000 V 1 Pulse
+/- 1000 V 2 Pulses
+/- 1000 V 3 Pulses
+/- 1400 V 1 Pulse
+/- 1400 V 2 Pulses
+/- 1400 V 3 Pulses
d
+/- 1000 V 1 pulse
+/- 1400 V 3 pulses
e
% Viability
n.s.
Pulses
0
1
2
3
Voltage [V]
f
% Half-tryptic peptides
Voltage [V]
g
+/- Electroporation
h
Basal oxygen consumption rate
OCR (pmol/min)
Oligomycin
Rot/AA
Control
Electroporation
Time (minutes)
Basal mitochondrial respiration
ns

Appendix Figure S1: Effects of electroporation. ***(a)*** *The plot shows the concentration of protein in the supernatant of 3 million HEK293 cells electroporated at 1 pulse of 1000 V, and non electroporated cells (electroporated n = 4, *: p < 0.05, t-test.* ***(b)*** *The plot shows protein intensities in the supernatant, comparing samples with and without electroporation of the condition described in (a). Each data points represents a single protein. The shaded gray regions marks significance levels (FC > 1.5, q-value < 0.05, n = 4 replicates). (**c**) The plots show peptide intensities, comparing samples with and without electroporation. Each data point represents a single peptide; half-tryptic peptides are shown in orange and fully tryptic peptides are shown in blue. The shaded gray region marks significance levels (FC > 1.5, q-value < 0.05, n = 5 replicates). (**d**) The plot shows protein intensities comparing electroporated to non electroporated cells. Each data point represents a single protein. The shaded gray region marks significance levels (FC > 1.5, q-value < 0.01, n = 5 replicates). (**e**) The plot shows cell viability at tested electroporation settings. Conditions that do not differ significantly from cells without electroporation are indicated with n.s. (p. adj. > 0.05). (**f**) The plot shows the fraction of half-tryptic peptides under the indicated conditions after in-cell LiP-MS. Colors indicate pulse number as in (e). (**g**) The plot shows protein intensities 4 h after electroporation (1000 V 1 pulse) comparing electroporated to non electroporated cells. Each data point represents a single protein. The shaded gray region marks significance levels (FC > 1.5, q-value < 0.01, n = 5 technical replicates). To generate technical replicates, cells were split into the indicated number of aliquots before treatment. (**h**) Oxygen consumption rate (OCR) of control cells (green) and cells 24h after electroporation (orange) (left) and basal mitochondrial respiration (right) calculated as basal OCR minus non-mitochondrial OCR (post Rot/AA). Data are shown as mean ± SD (n=10 wells per condition). ns, not significant (Mann–Whitney U test).*

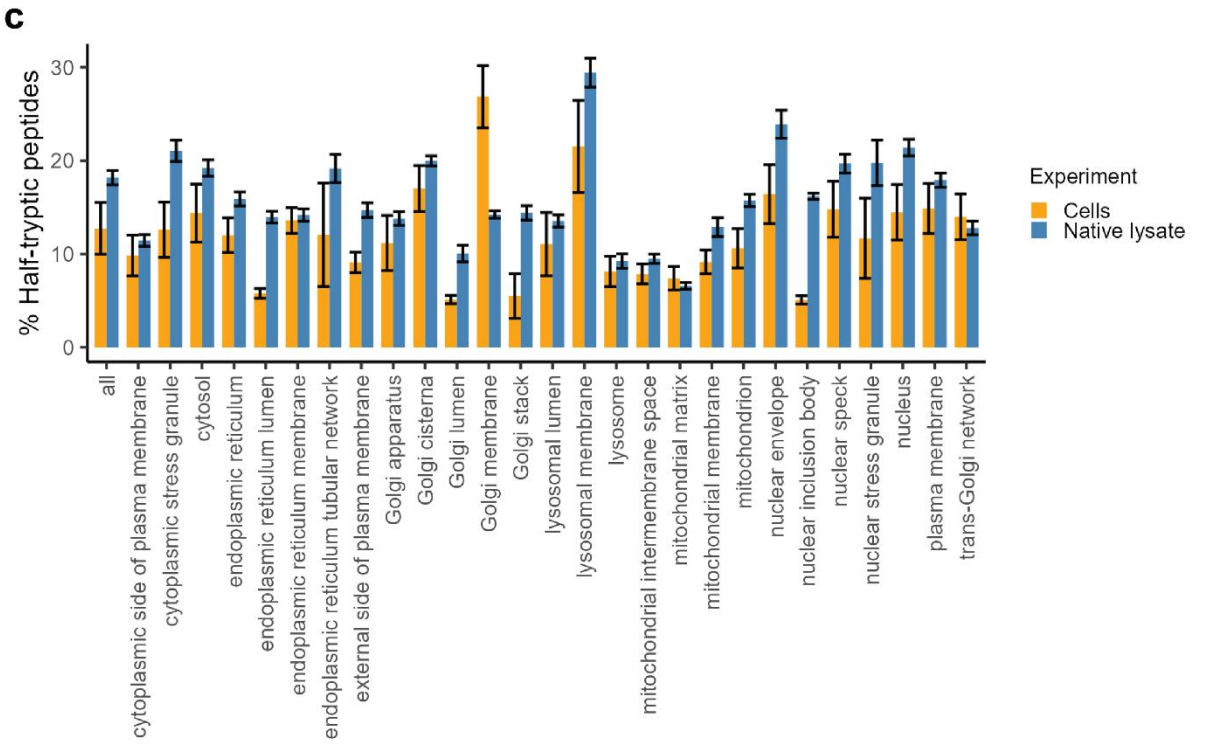

a
50 µM PK +/- EP
100 µM PK +/- EP
200 µM PK +/- EP
b
200 µM PK +/- EP
500 µM PK +/- EP
1000 µM PK +/- EP
- Log10 (p-value)
Log2 FC (Treated/Control)
c
% Half-tryptic peptides
Proteinase K [µM]
control
electroporated
d
Log2 (Intensity)
Condition
Control + 0µM PK
Control + 50µM PK
Control + 100µM PK
Control + 200µM PK
EP + 0µM PK
EP + 50µM PK
EP + 100µM PK
EP + 200µM PK

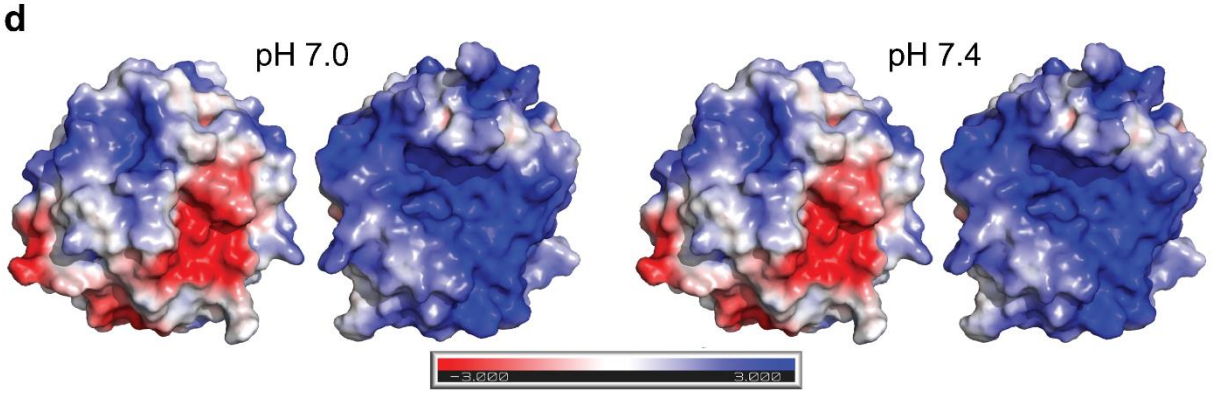
Appendix Figure S2: Effects of PK concentration on proteolytic cleavage. *(**a, b**) The plots show peptide intensities after proteolytic cleavage for cells electroporated with 50 µM to 1000 µM PK, comparing samples with and without electroporation. Each data point represents a single peptide; half-tryptic peptides are shown in orange and fully tryptic peptides are shown in blue. The shaded gray region marks significance levels (FC > 1.5, q-value < 0.05, n = 5 technical replicates except for the condition 200 µM PK -EP in (a) where 2 replicates were excluded because of retention time shifts during mass spectrometry acquisition).(a) lower PK concentration range (50 µM to 200 µM). (b) Higher PK concentration range (200 µM to 1000 µM). (**c**) The plot shows the fraction of half-tryptic peptides under the indicated conditions after in-cell LiP-MS. (**d**) The heatmap shows peptide intensities (stripped sequence level) from the in-cell LiP in figure S2a. Control indicates no electroporation, EP indicates electroporation. Colors in the heatmap indicate log_2-transformed peptide intensities (blue: not quantified). To generate technical replicates, cells were split into the indicated number of aliquots before treatment.*



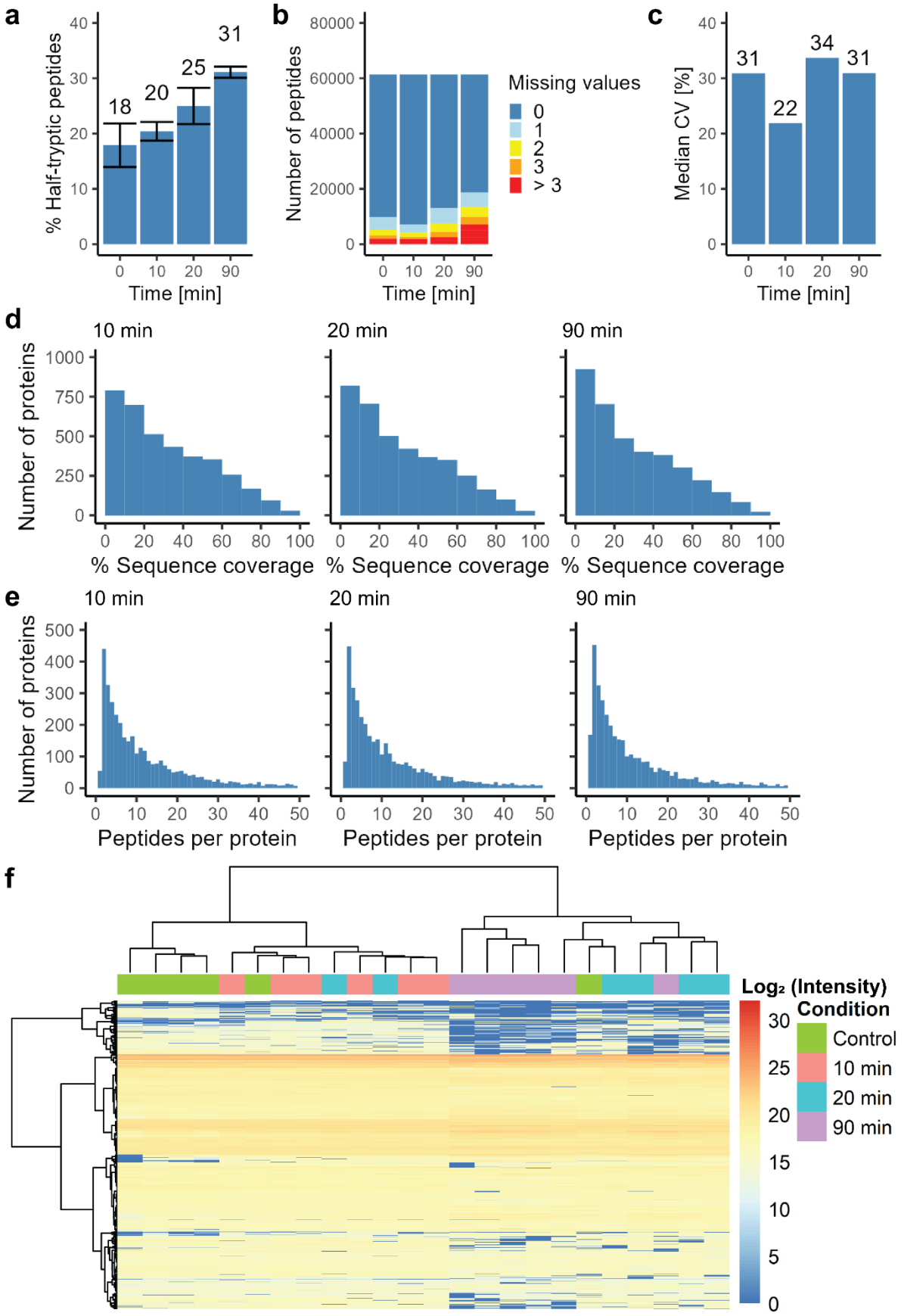


Peptide	Electroporation [fM]	No electroporation [fM]
NYSPASEPSVCTVGASDR	219	48
YIADTANK	384	117
TQLFGVK	356	105
TYYYSSR	231	110
Average	298	95



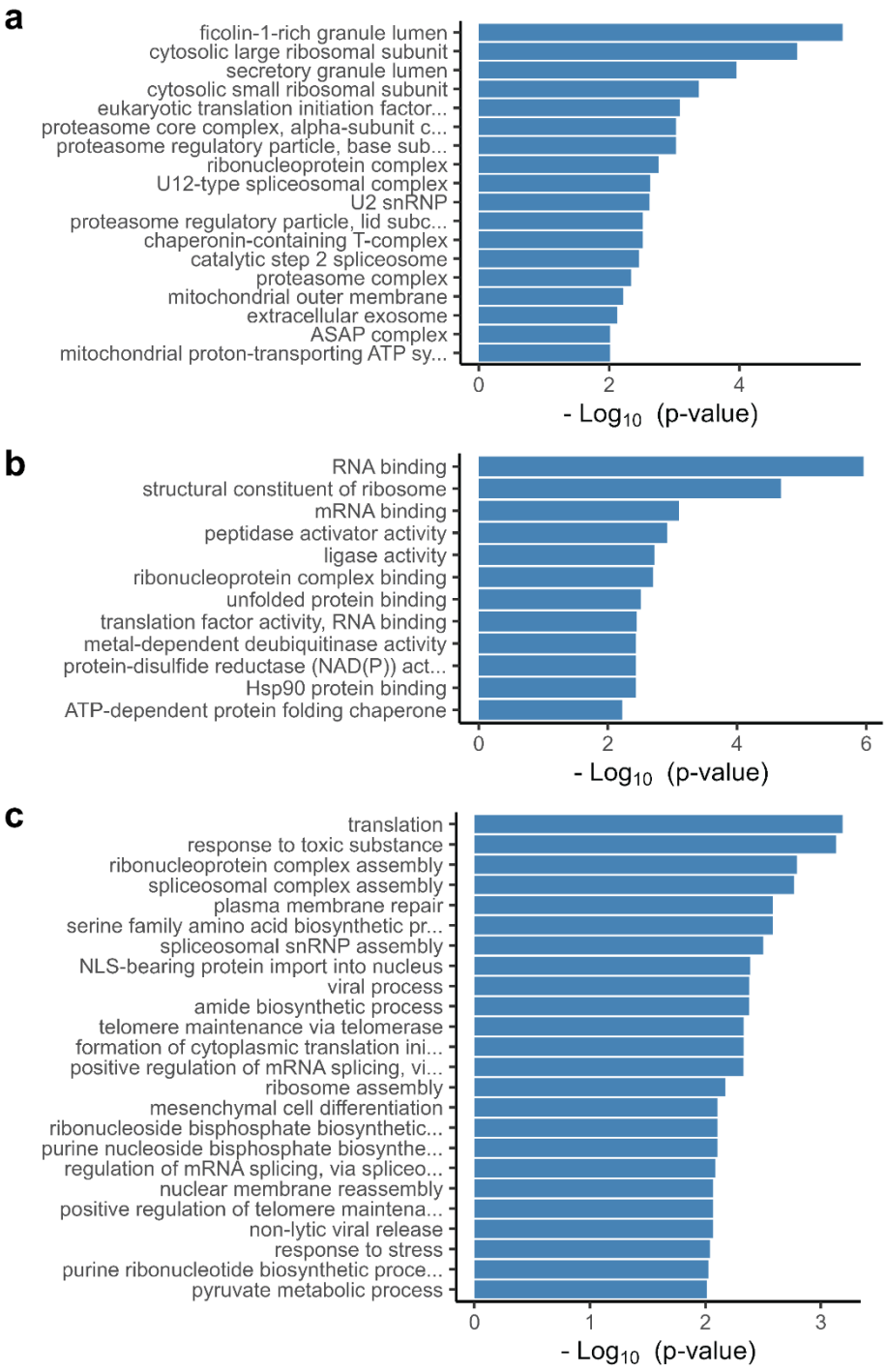


Appendix Figure S3: PK uptake upon electroporation and mass spectrometry coverage. *(**a**) HEK293 cells treated with 30 µM of the endocytosis inhibitor Pitstop 2 dissolved in DMSO for 15 min prior to in-cell LiP-MS, compared to cells treated only with DMSO. The plot shows the fraction of half-tryptic peptides under the indicated conditions in absence of electroporation. (**b**) Intensity of heavy labeled peptides specific for PK spiked into HEK293 lysate at indicated concentrations. The table reports the quantity of PK peptides for in-cell LiP-MS samples. (**c**) Fraction of half-tryptic peptides of organelles quantified in DMSO treated cells (in-cell LiP-MS) or native lysates (standard LiP-MS). Only peptides quantified in at least 3 replicates are considered (in-cell LiP-MS n = 6; standard LiP-MS n = 4 technical replicates). To generate technical replicates, cells were split into the indicated number of aliquots before treatment. (**d**) Electrostatic potential of proteinase K surface (PDB 2prk) at the indicated pH, units ranging from -3 kT/e (red) to 3 kT/e (blue).*



Appendix Figure S4: Quality control of in-cell LiP-MS data of arsenite treatment. *(**a-f**) Comparison of HEK293 cells treated with arsenite for the indicated time compared to untreated cells and analyzed with in-cell LiP-MS. (a) Fraction of half-tryptic peptides relative to total peptide intensity for the experiments. (b) Number of*

peptides with missing values per treatment. The plots report the number replicates per condition, in which a specific peptide was not quantified. A missing value of 0 indicates that the peptide was quantified in all six replicates, 1 indicates that the peptide was not quantified in 1 out of 6 replicates. (c) Median coefficient of variation (CV) of peptide intensities per treatment. (d) Overall sequence coverage of experiments, considering peptides detected in at least 3 replicates per condition. (e) The number of peptides per protein detected in at least three replicates per condition. (f) Heatmap of peptide intensities (stripped sequence level). Colors above the heatmap correspond to the indicated conditions.



Appendix Figure S5: Effects of arsenite on cellular physiology. *(**a**-**c**) Comparison of HEK293 cells treated with sodium arsenite for 90 min to untreated cells, but taking into account only peptides with the strongest changes (FC > 1.5, q-value < 0.0005, n = 6 biological replicates). Gene ontology enrichment analysis of proteins with peptide-level structural changes upon arsenite treatment (q-value < 0.01). (a) Cellular compartment. (b) Molecular function. (c) Biological process. Each replicate was biologically independent, consisting of cells grown separately prior to treatment*