Supplementary Information

Hypoxia induces extensive protein and proteolytic remodeling of the cell surface in pancreatic adenocarcinoma (PDAC) cell lines

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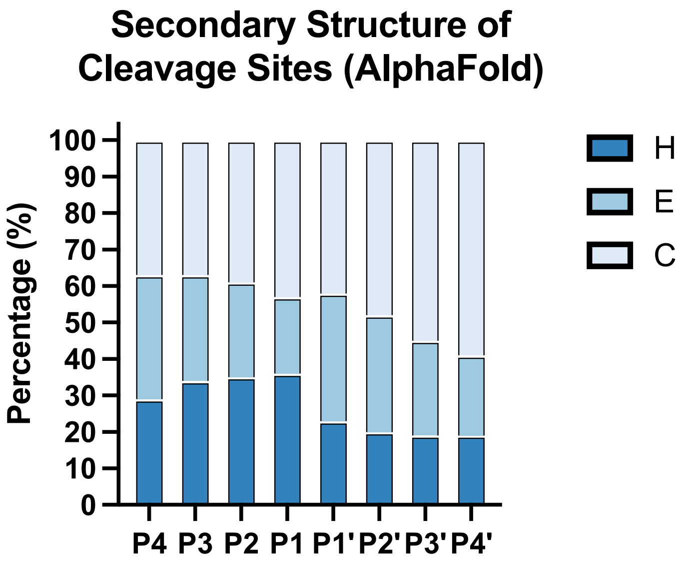
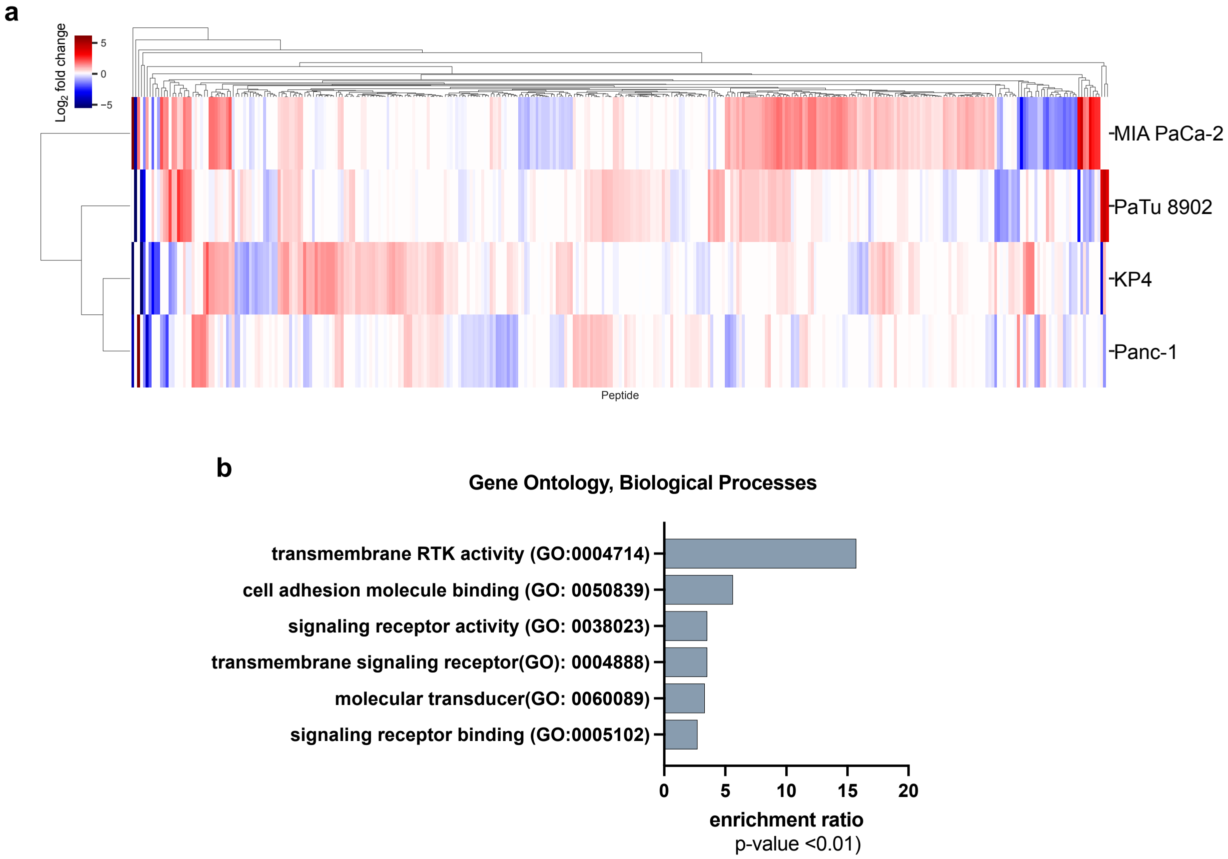
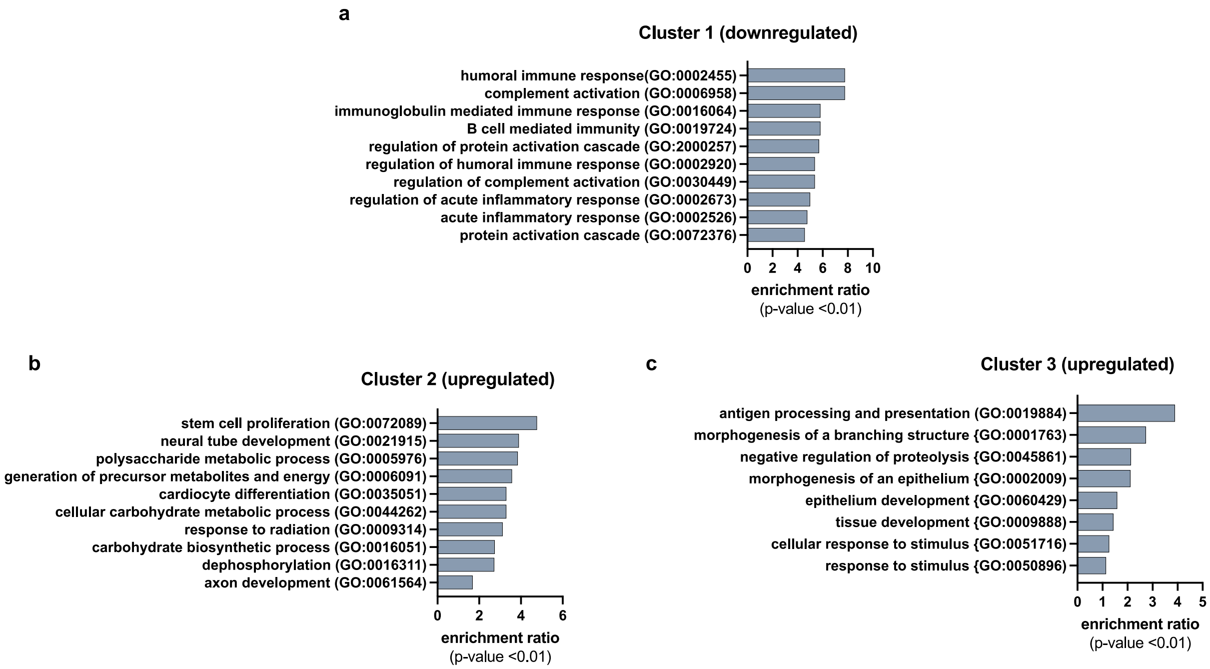


Figure S1: Predicted secondary structure at cleavage sites
For proteins that did not have a structure deposited on PDB, AlphaFold 2.0 models were used to predict the secondary structure surrounding the identified cleavage sites. H represents helices, E represents residues in sheets, and C represents residues in loop and unstructured locations.



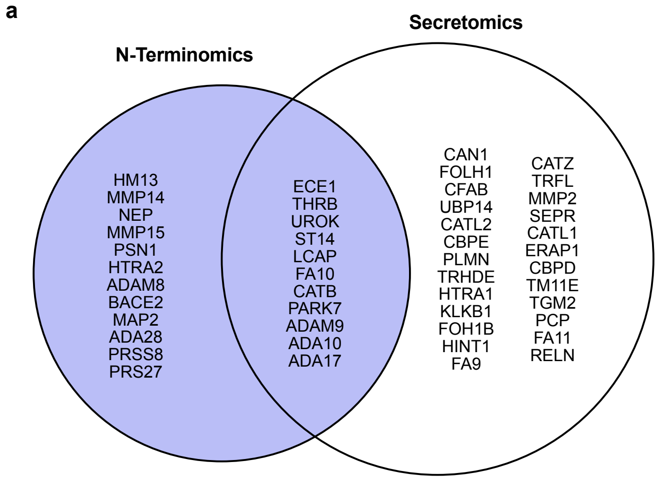
Supplementary Figure S2: Quantification of N-termini abundance changes observed under normoxic conditions.

a. Heatmap of identified N-termini depicting the change in peptide abundance under hypoxic conditions versus normoxic conditions. Red indicates an increased presence in hypoxia while blue indicates an increased presence in normoxia. **b.** GO analysis of all N-termini reveals an over-representation of proteins involved in signaling receptor activity.



Supplementary Figure S3: GO Analysis of secretomics

a. Cluster 1 identifies downregulated proteins involved in regulating the immune and inflammatory responses. **b.** Cluster 2 identifies moderately upregulated proteins involved in regulating cellular metabolism and development **c.** Cluster 3 identifies upregulated proteins involved in antigen processing/presentation, as well as those involved in tissue development and response to stimuli.



b

N-Terminomics Median Log_2 Enrichment

Peptide	MIA PaCa-2	KP4	Panc-1	PaTu 8902
UROK_177	n.d.	n.d.	n.d.	-0.53
UROK_179	n.d.	n.d.	-0.09	-0.26
UROK_181	1.47	n.d.	n.d.	0.77

Secretomics Median Log_2 Enrichment

Protein	MIA PaCa-2	KP4	Panc-1	PaTu 8902
UROK	2.25	2.29	1.39	1.91

N-Terminomics Median Log_2 Enrichment

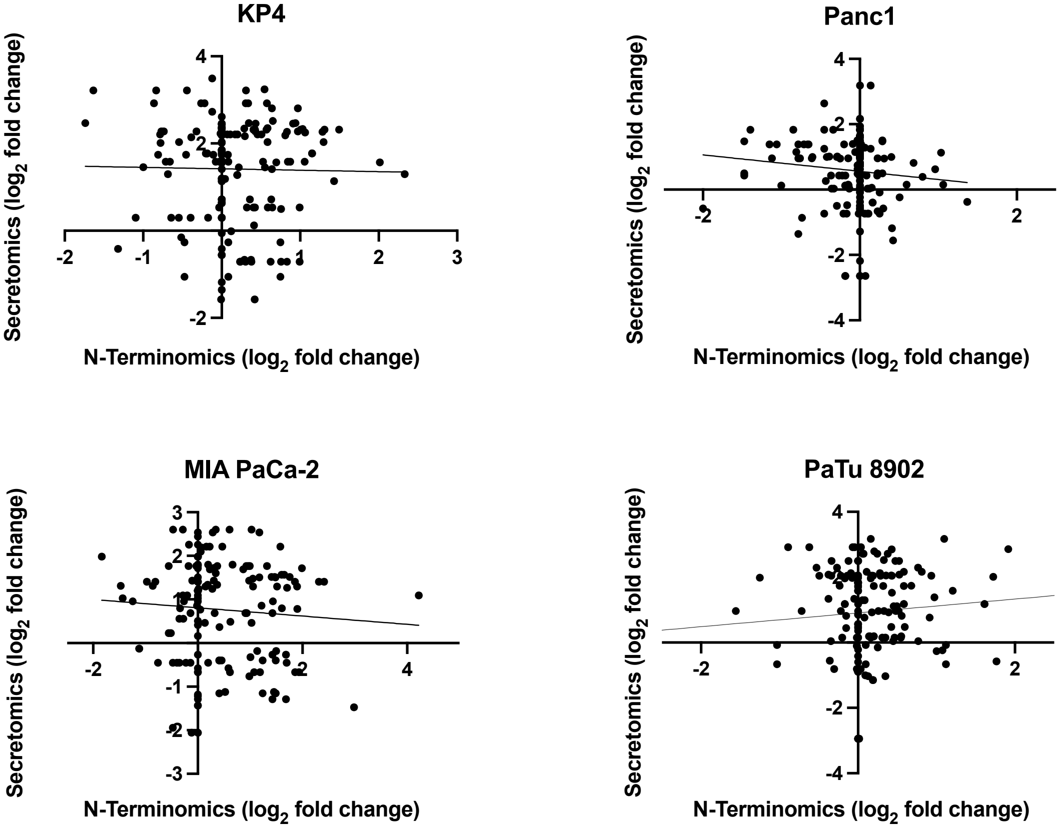
Peptide	MIA PaCa-2	KP4	Panc-1	PaTu 8902
ADA10_214	n.d.	n.d.	n.d.	1.49

Secretomics Median Log_2 Enrichment

Protein	MIA PaCa-2	KP4	Panc-1	PaTu 8902
ADA10	1.92	0.79	0.23	0.96

Supplementary Figure S4: Shared proteases identified in N-terminomics and secretomics

a. Venn diagram displaying functionally-diverse proteases identified in N-terminomics, secretomics, and shared between MS approaches. **b.** Cleavages observed at activating sites of urokinase and ADA10 and their associated secreted protein levels with positive values reflecting more cut in hypoxia vs normoxia and negative values the opposite. n.d. = not detectable.



Supplementary Figure S5: Correlation plots between N-terminomics and secretomics data Identified N-termini (x-axis) and their changes under hypoxic conditions plotted against their respective change in protein levels in secretomics (y-axis) datasets show little correlation between proteolysis and shed/secreted proteins.