

**Quantitative Secretome Analysis of Activated Jurkat Cells using Click Chemistry-Based
Enrichment of Secreted Glycoproteins**

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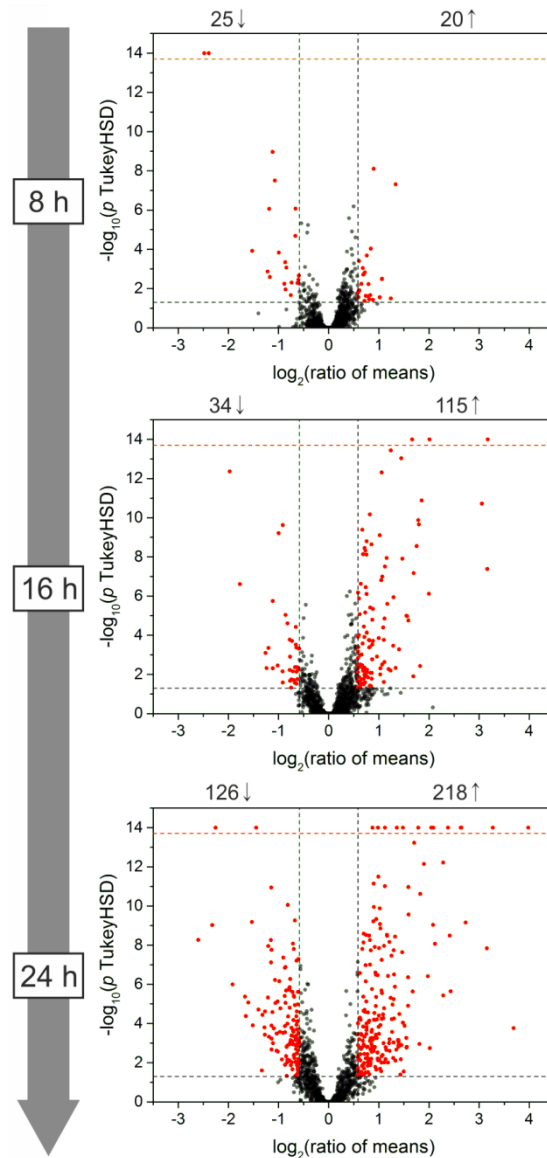
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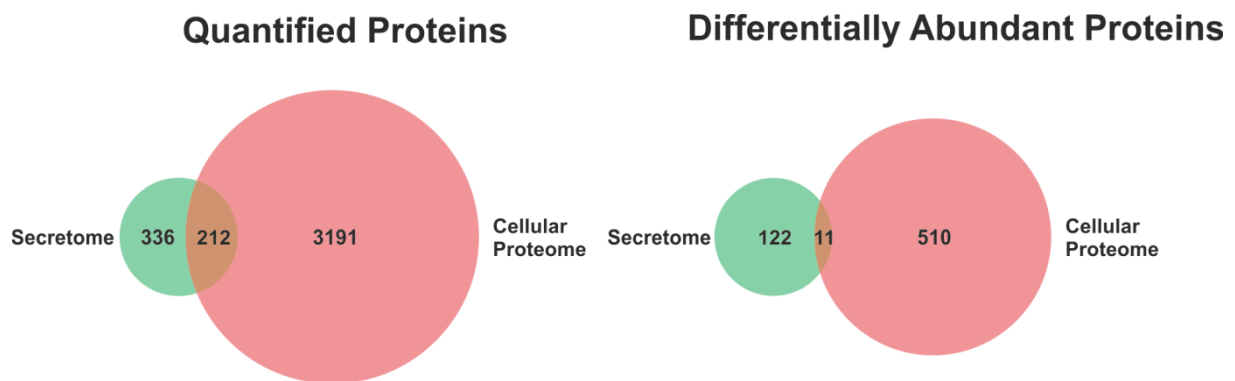
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Supplementary Results: Quantitative analysis of the cellular proteome

In the cellular proteome analysis 4575 proteins (4034 protein groups) were identified and 3403 proteins were quantified by the Progenesis QI software. The activation of Jurkat cells was analyzed in a time-dependent manner and three different time points were investigated (Supplementary Table 3, Supplementary Figure 1). After 8 h, 78 proteins were found to be significantly differentially abundant between activated cells and the inactive group (ANOVA p_{FDR} value ≤ 0.05 , p TukeyHSD ≤ 0.05 , absolute fold change ≥ 1.5). 36 proteins were higher abundant in the inactive group whereas 42 proteins were higher abundant in the activated group. After 16 h, 252 proteins were observed to be differentially abundant of which 171 were higher abundant in activated samples and 81 proteins were higher abundant in inactive samples. The highest number of differentially abundant proteins was observed after 24 h with 521 significantly differentially abundant proteins of which 323 were higher abundant in activated cells and 198 showed a higher abundance in the inactive group, respectively.



Supplementary Figure S1: Volcano plots illustrating the time-dependent analysis of the cellular proteome of activated Jurkat cells. Three time points (8 h, 16 h and 24 h) were analyzed. Positive ratios of means indicate higher protein abundance in the activated groups while negative values indicate higher abundance in the corresponding inactive groups. Only proteins quantified with minimum two unique peptides are displayed. The significance thresholds are indicated by dashed lines (p TukeyHSD ≤ 0.05 , absolute ratio of means ≥ 1.5) and significantly differentially abundant proteins are highlighted in red. Proteins with a p TukeyHSD = 0 are displayed above the red dashed line.



Supplementary Figure S2: Comparison of sets of quantified and differentially abundant proteins between the secretome and cellular proteome analyses. Proteins with a p TukeyHSD lower or equal to 0.05 and an absolute fold change greater or equal to 1.5 between activated and inactive samples were considered to be differentially abundant. With the overlap of quantified proteins mounting up to 212 proteins, there are 11 proteins that are differentially abundant in both, the secretome and the cellular proteome (Supplementary Table 4).

Supplementary Table S3: List of mutual differentially abundant proteins in the secretome complement and the cellular proteome.

UniProt Accession	Gene name	Protein name	Ratio of Means Biotin^{a)}	Ratio of Means Desthiobiotin^{a)}	Ratio of Means Proteome^{a)}
P07686	HEXB	Beta-hexosaminidase subunit beta		1.88	2.96
O43852	CALU	Calumenin		3.16	2.32
P22413	ENPP1	Ectonucleotide pyrophosphatase/phosphodiesterase family member 1	24.69	26.60	9.67
P14625	ENPL	Endoplasmin	1.65	1.97	1.99
P42892	ECE1	Endothelin-converting enzyme 1	1.63	1.67	-1.83
Q99808	S29A1	Equilibrative nucleoside transporter 1		-3.07	-4.77
P01130	LDLR	Low-density lipoprotein receptor		1.50	2.25
P43007	SATT	Neutral amino acid transporter A	-1.57		-2.21
P07602	SAP	Proactivator polypeptide		2.07	-1.56
P02786	TFR1	Transferrin receptor protein 1	-1.62		-1.71
O60701	UGDH	UDP-glucose 6-dehydrogenase		1.79	1.72

^{a)}Statistical significance of differential abundance between activated and inactive cells was tested by ANOVA followed by Tukey's HSD method. All displayed proteins passed the significance threshold of p TukeyHSD ≤ 0.05 .