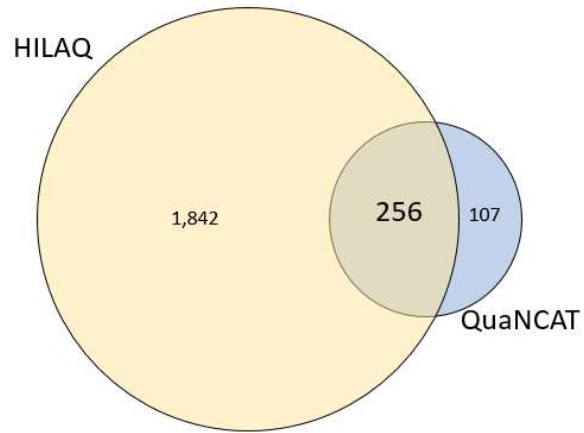


Supplementary Figure 1

Overlap of identification on three replicates of HILAQ or QuaNCAT

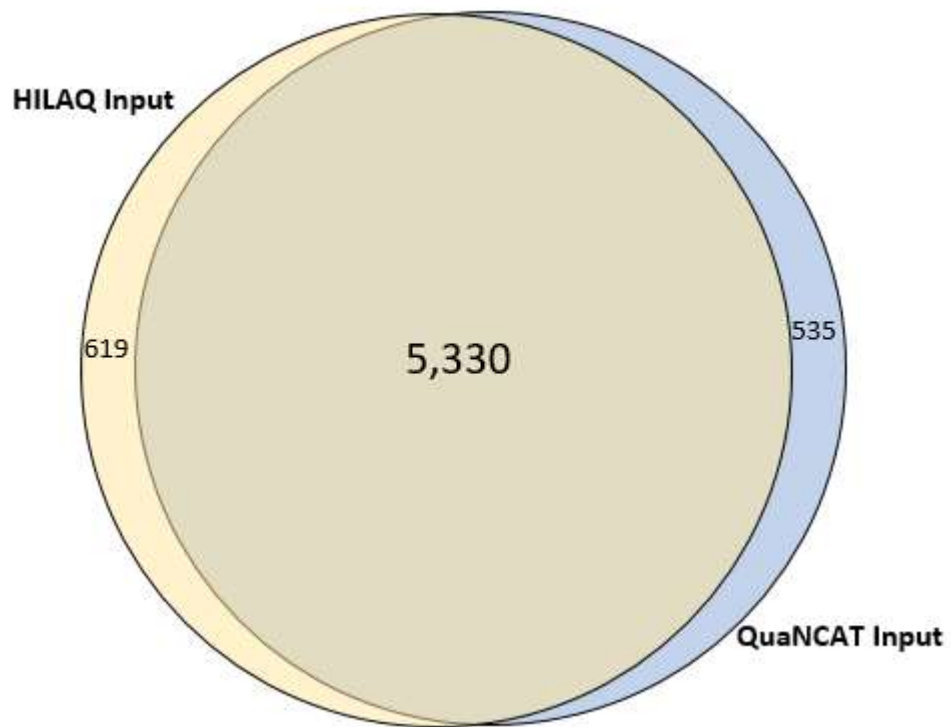
There were 2,098 NSP were confidentially identified by HILAQ and 363 NSP by QuaNCAT from three biological replicates.



Supplementary Figure 2

Venn diagram analysis comparison between HILAQ and QuaNCAT on identification

Number counts came from the overlap of three independent samples of HILAQ or QuaNCAT. There were 256 NSP were confidently identified by both strategies. However, 1,842 NSP were only identified by HILAQ.



Supplementary Figure 3

Over 90% of proteins identified from the baseline of both strategies were in common.

Supplementary Table 1

Identification results for HILAQ and QuaNCAT. Enrichment efficiency (EE) calculated from this formula:

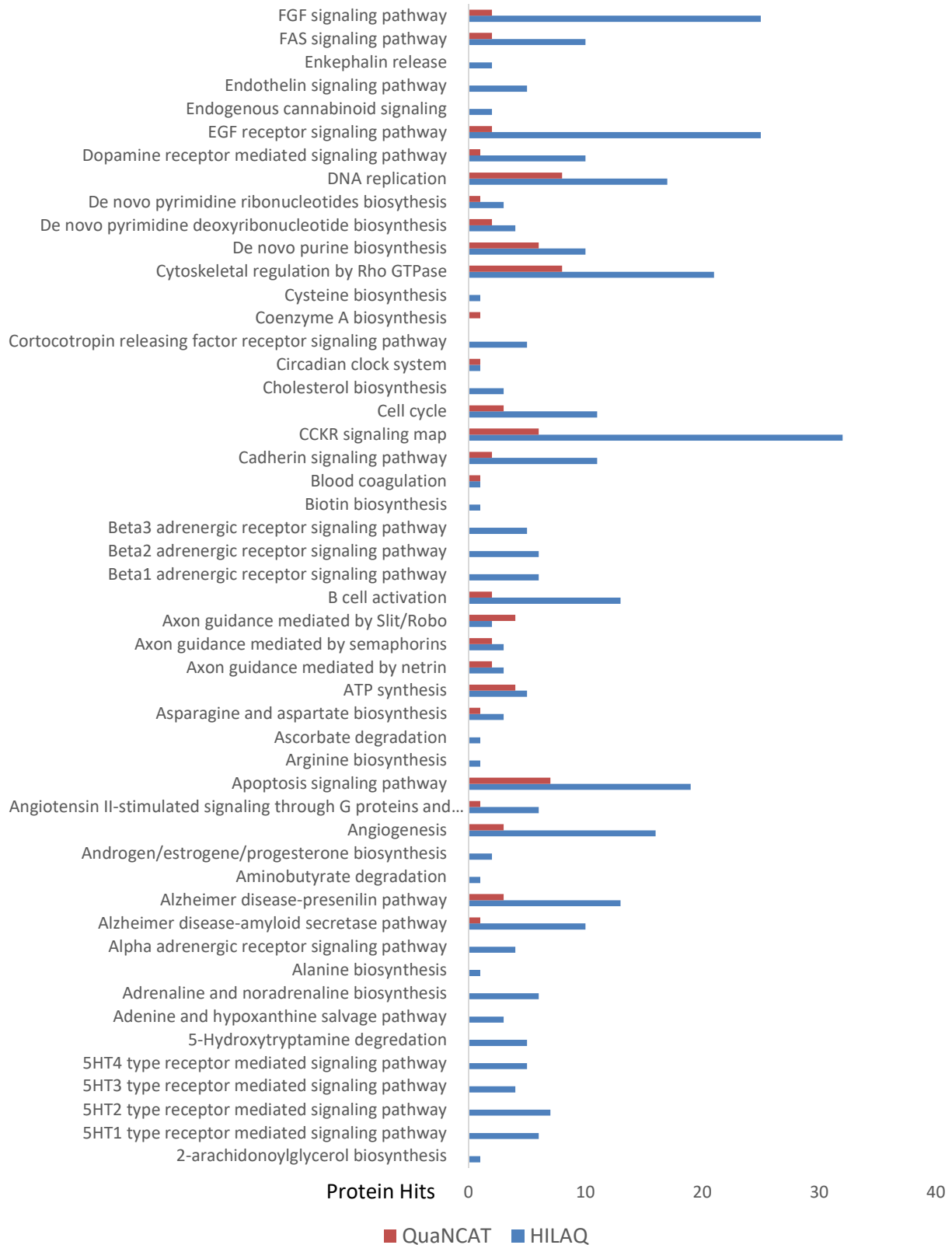
$$EE=100*\text{Modified protein number}/(\text{modified protein number} + \text{unmodified protein number})$$

Rep	HILAQ					QuaNCAT				
	Modified		Unmodified		EE	Modified		unmodified		EE
	Protein	Peptide	Protein	peptide		Protein	Peptide	Protein	peptide	
1	3,705	31,248	1,053	6,114	77.8%	847	2,979	4,826	40,433	14.9%
2	3,151	31,738	842	4,402	78.9%	715	2,237	4,866	37,732	12.8%
3	2,932	26,849	673	4,257	81.3%	702	2,199	4,325	29,620	14.0%

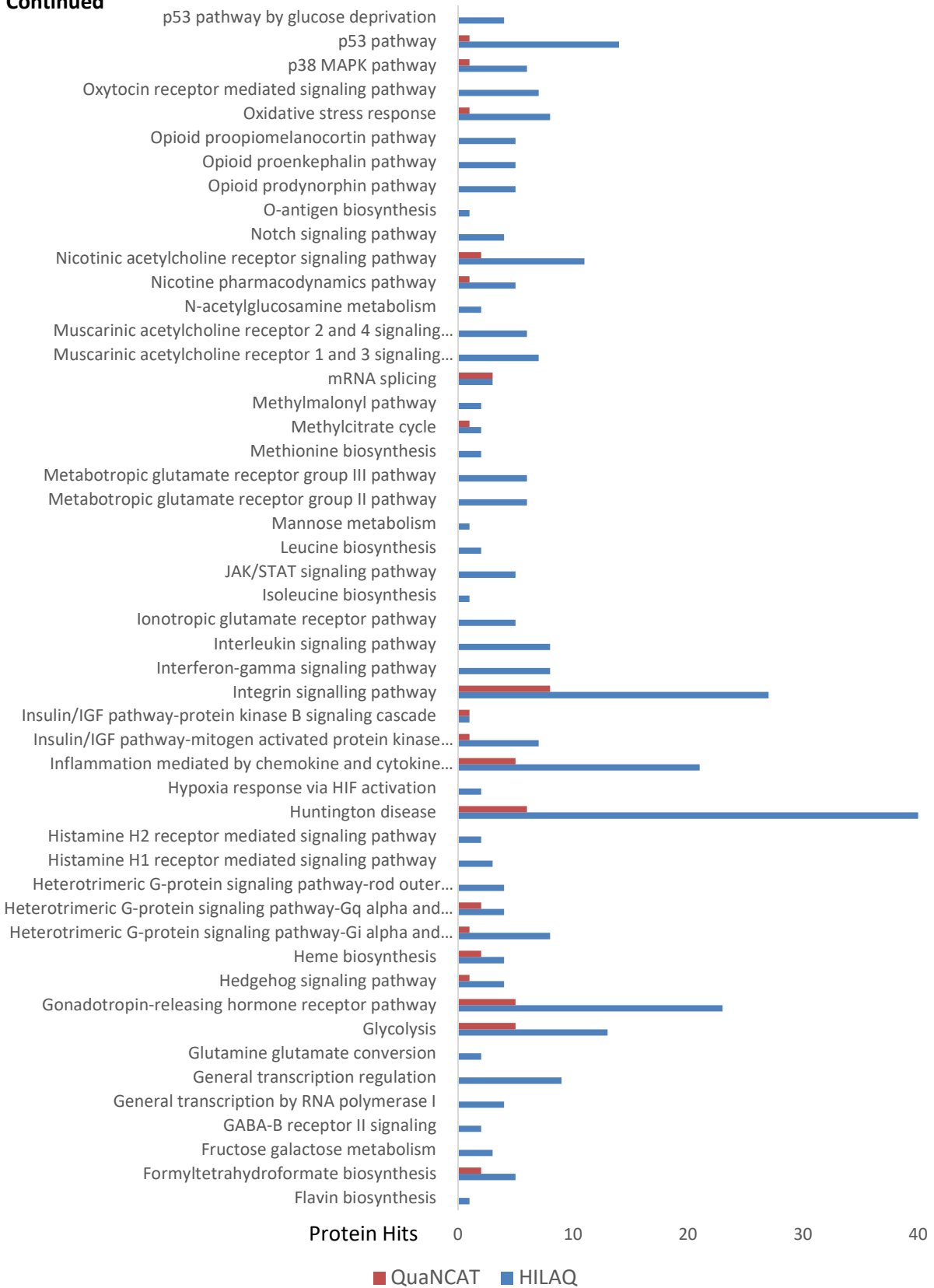
Supplementary Table 2: Quantification efficiency comparison between HILAQ and QuaNCAT. Only protein identified or quantified with sigma less than or equal 0.5 in all three independent biological replicates were counted for all other statistics.

Overlap of 3 replicates	HILAQ	QuaNCAT
Identification	2,098	363
Quantification	1,926	353
Quantification efficiency	91.80%	97.20%

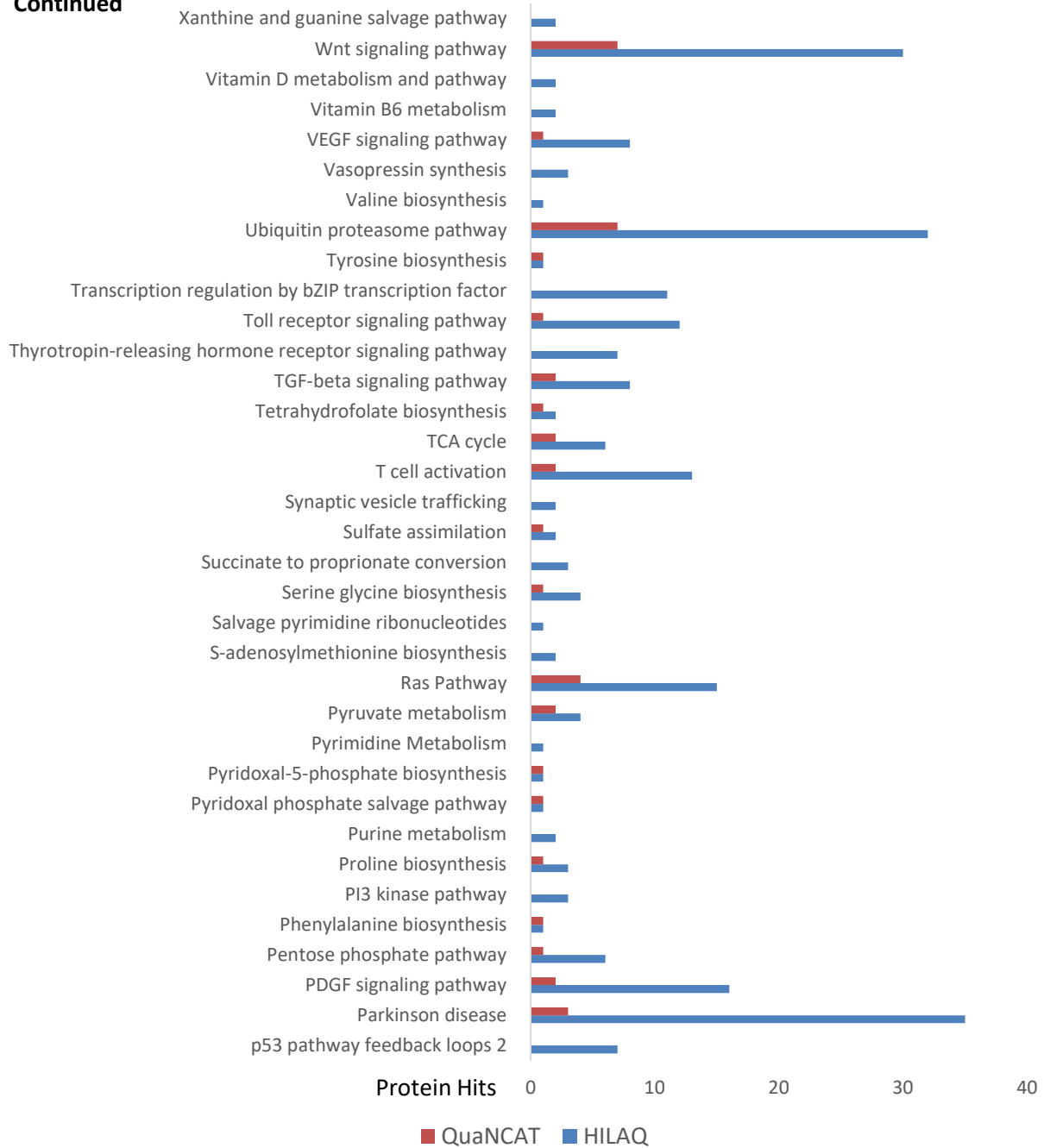
Pathways analysis



Continued

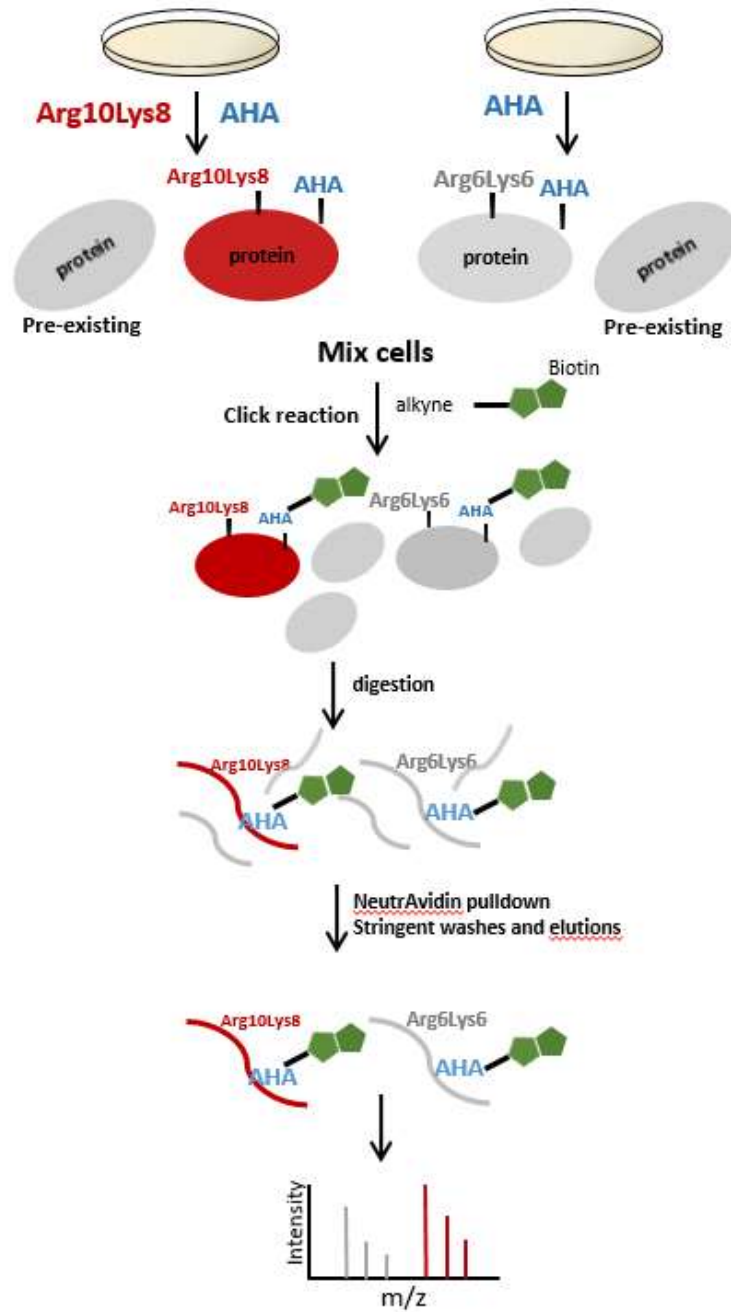


Continued



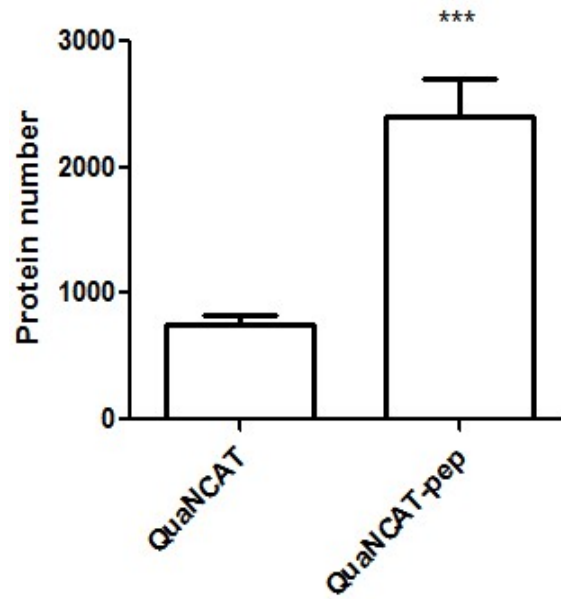
Supplementary Figure 4

Pathway annotation analysis. Proteins quantified by all three replicate of HILAQ or QuaNCAT were annotated using web based tool PANTHER (<http://pantherdb.org/>). There are two times more pathways enriched in HILAQ than QuaNCAT.



Supplementary Figure 5

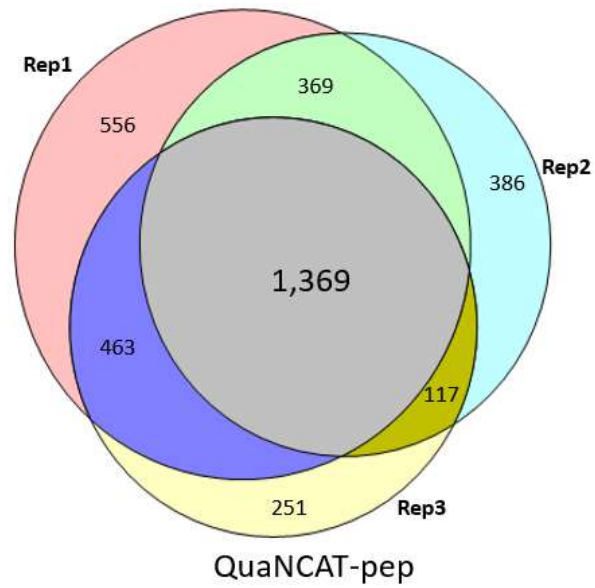
QuANCAT-pep workflow. This worked flow was modified based on QuANCAT. The only difference is protein were digested firstly followed with peptide enrichment.



Supplementary Figure 6

The NSP identified were significantly increased by QuaNCAT-pep. (** $p < 0.001$; one tailed unpaired t-test, comparison with QuaNCAT)

a






b

Strategy	Confident NSP	Input proteins	NSP sensitivity
QuaNCAT-pep	1,369	5,865	23.3%

Supplementary Figure 7

(a) Venn diagram analysis on the three biological replicates of QuaNCAT-pep. (b) Only the proteins quantified in all three replicates were counted for the calculation of NSP sensitivity.

Name		p-value range	# Molecules
Cellular Assembly and Organization		8.98E-03 - 1.82E-09	43
Cellular Function and Maintenance		8.98E-03 - 1.82E-09	39
Cell Morphology		8.98E-03 - 8.00E-09	48
Cellular Development		8.98E-03 - 1.25E-07	47
Cellular Growth and Proliferation		8.98E-03 - 1.25E-07	52



Supplementary Figure 8

Molecular and cellular functions analysis by Ingenuity IPA software package (version 28820210).