TUBE-based Mass Spec Proteomics

For Ubiquitome Analysis

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LifeSensors

- Leading Biotech in UPS Drug Discovery and Diagnostic R&D
- ~500 Products, Proteins, Ubiquitin Affinity Reagents (TUBEs), Inhibitors, Assays, Kits and Proprietary Protein Expression Systems (SUMO)
- Drug Discovery, UPS and PROTAC Screening Services
- Profiling Compounds Against Ubiquitin Ligases and DUBs
- Custom Assay Development and Collaborative Research

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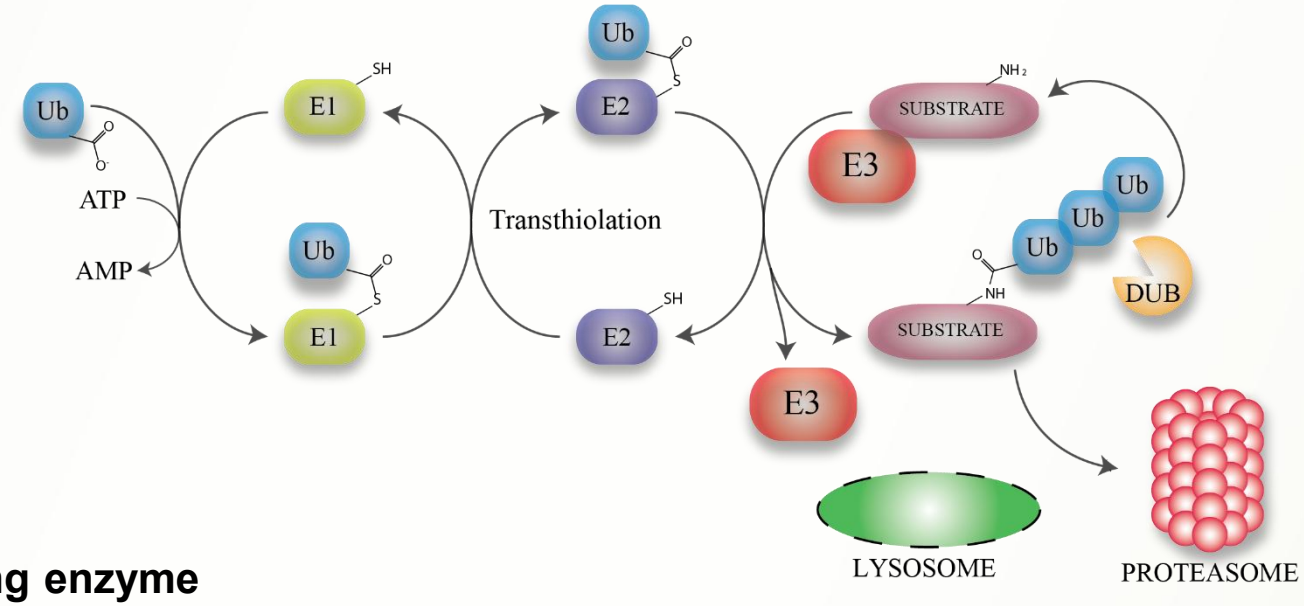
Ubiquitin Proteasome System



E1 – Ubiquitin activating enzyme

Requires ATP to attach Ub to E1

E2 – Ubiquitin conjugating enzyme

Transfers Ub from E1 to E3

E3 – Ubiquitin ligases

Transfers Ub to self or substrate

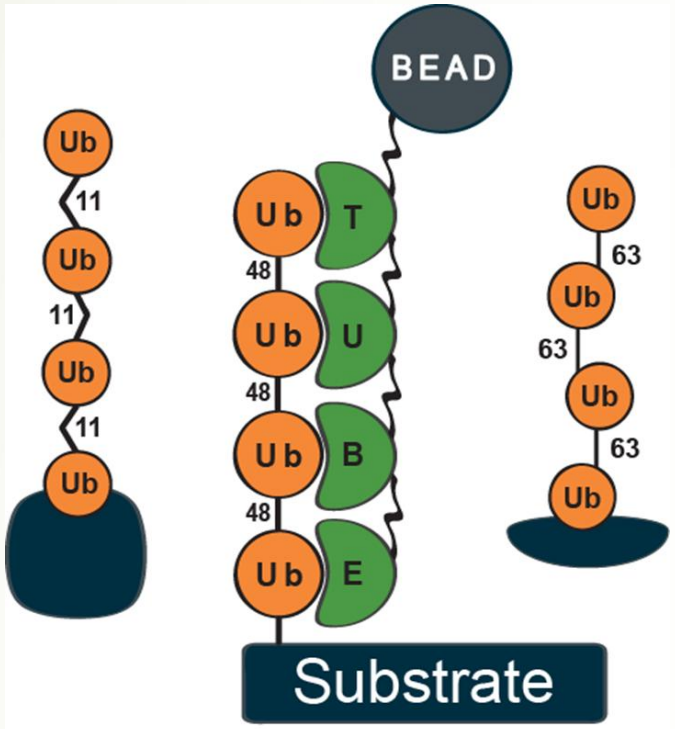
Forms mono-Ub or poly-Ub chains

DUB – Deubiquitinase

Removes mono-Ub or poly-Ub chains

Proteasome – Degrades ubiquitylated proteins

TUBE Properties

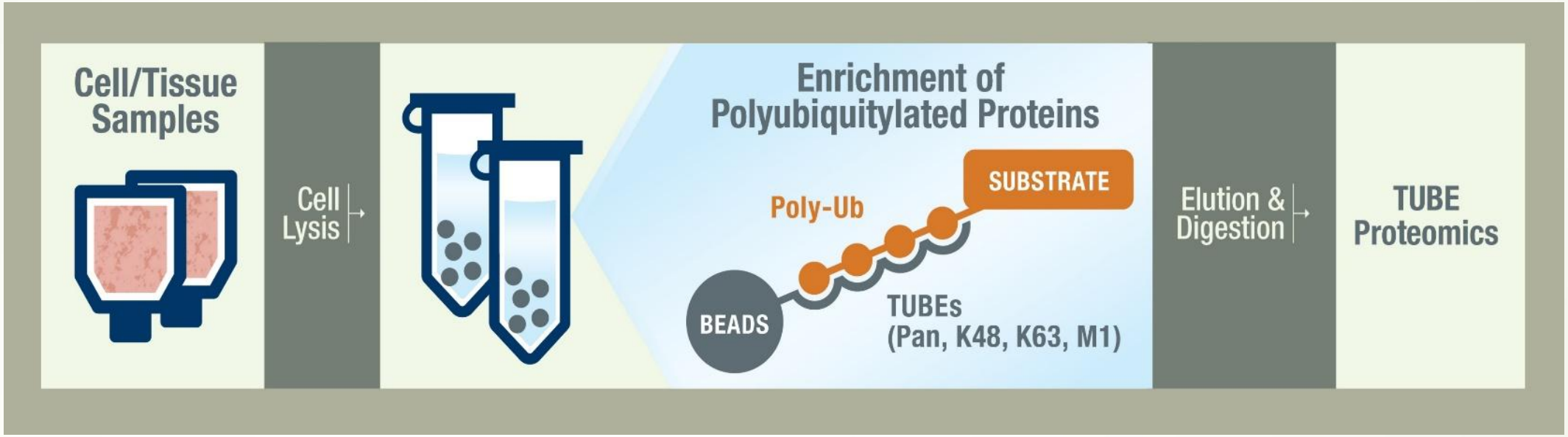


- Natural ubiquitin-binding domains (UBDs)
- Designed and engineered for high affinity and selectivity for polyubiquitin chains
- Superior to antibodies with regards to selectivity and versatile applications
- Labeling with tags (i.e., 6xHis, GST, Biotin, Magnetic, fluorophores, etc.)
- Pan-selective and polyubiquitin chain linkage-selective TUBEs
- Variety of applications, mass spec proteomics, imaging, HTS and biomarkers

TUBE-based Mass Spec Ubiquitin Proteomics

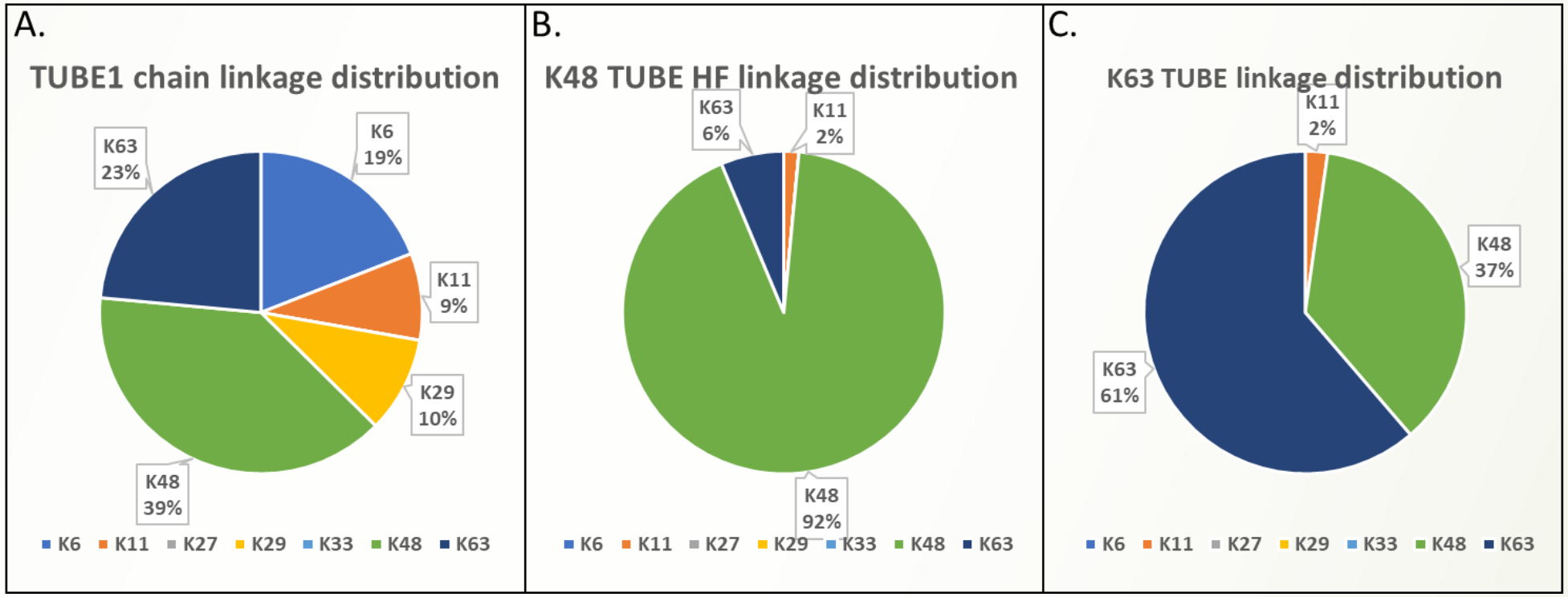
- TUBE applications has simplified ubiquitin proteomics
- Rapid and quantitative analysis of biomarkers from cells and tissues
- Quantitative method for examining drug (DUB, Ligase, PROTAC) effects in cells
- Inexpensive and simple, no need for SILAC or other labeling protocols
- Superior to Di-Gly ubiquitin proteomics

Workflow for TUBE-based Ubiquitin Proteomics



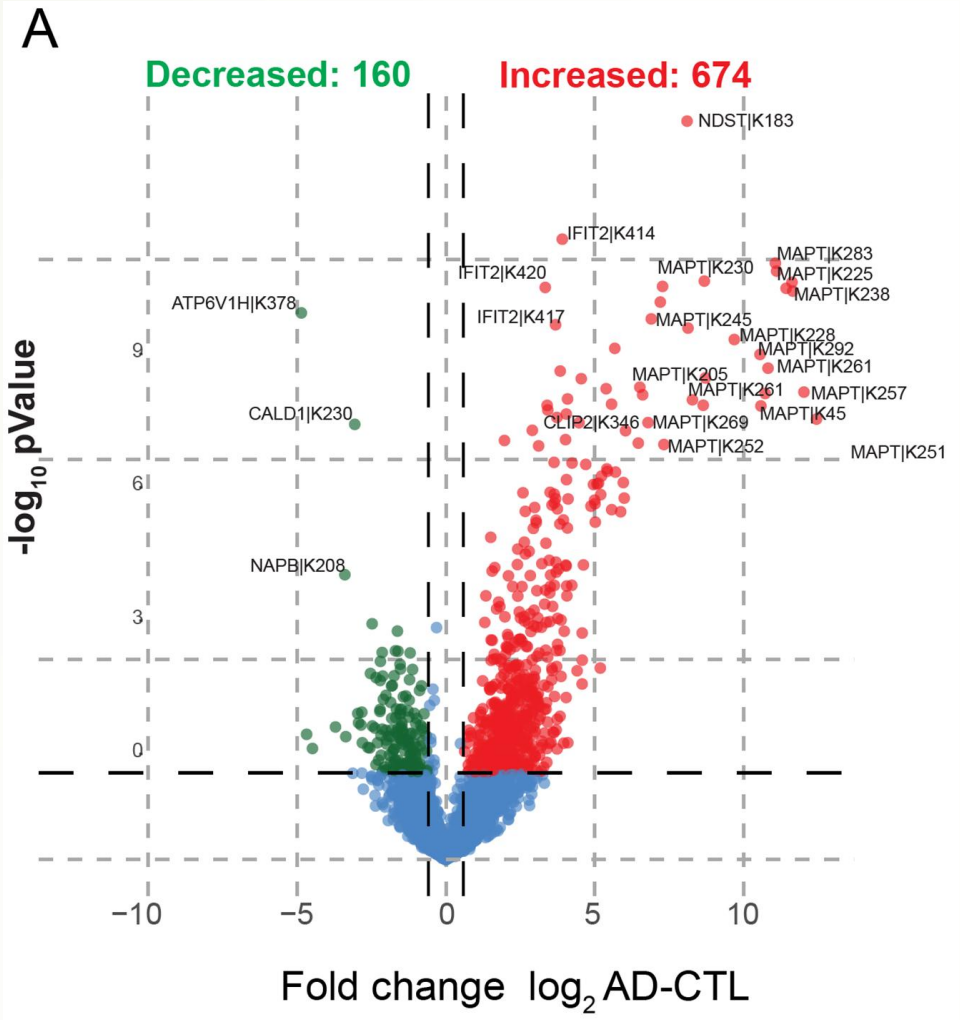
TUBE proteomics is initiated by incubating cell/tissue lysates with TUBEs. After eluting the enriched polyubiquitylated fraction, it is subjected to a tryptic digest followed by MS analysis. LifeSensors provides a comprehensive list in an Excel sheet with all proteins, peptides, and ubiquitylated sites that were detected by MS as well as a quality report summarizing the results of the experiment.

Linkage Distribution of Polyubiquitin Chains Based on LC-MS/MS Identification of Ubiquitin Remnant Peptides



Ubiquitylated proteins were enriched from cell lysates using **A.** Magnetic TUBE1, **B.** K48 TUBE HF, **C.** or K63 TUBE. K63 TUBE enrichment levels in this case may be negatively affected by the presence of K48/K63 heterotypic or branched polyubiquitin chains.

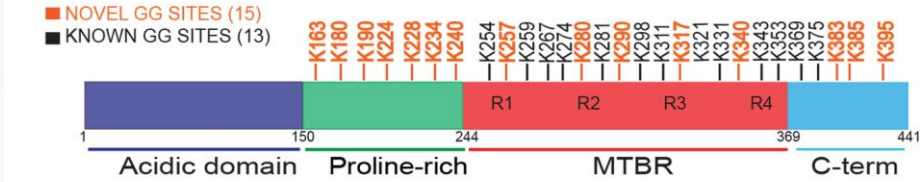
Quantitative TUBE-based Proteomic Analysis of Brains from Alzheimer's Diseases Patients



Red and green dots represent differentially increased or decreased ubiquitylated peptides in the AD vs. control, respectively.

Discovery of Novel Tau Ubiquitination Sites using TUBE-based Mass Spec Proteomics

A



B

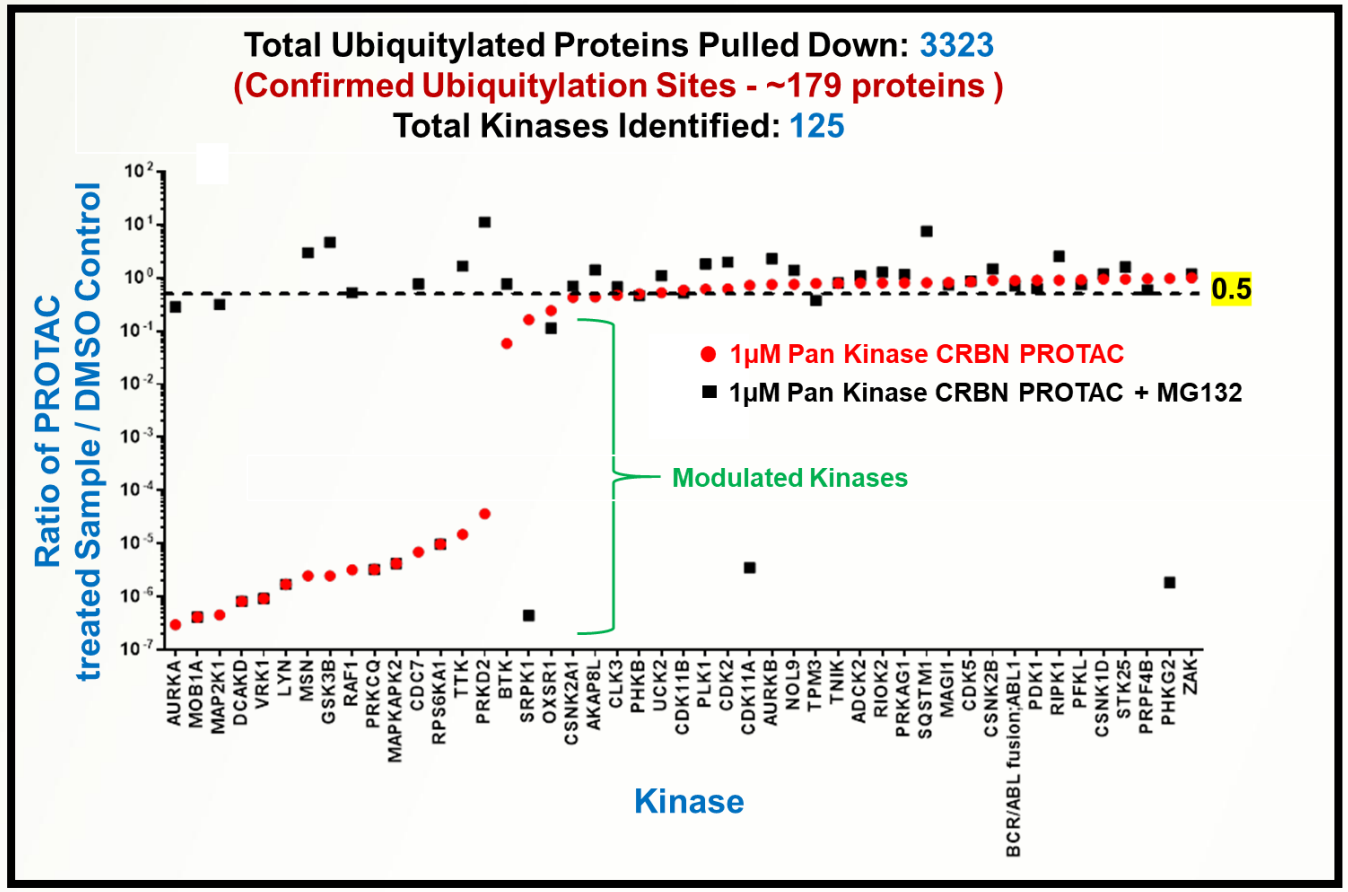
	Ubiquitin Site	Ubiquitin Peptides	Log2 (AD/CTL)	pValue (AD/CTL)	PEP
Proline-rich	K163	GAAPPGQ**K**GQANATR	2.46	1.93E-04	2.91E-03
	K180	TPPAP**K**TPPSSGEPPK	3.73	2.33E-07	1.82E-09
	K190	TPPSSGEPP**K**SGDR	4.02	5.01E-07	7.02E-26
	K224	TPSLPTPPTREP**K**K	7.27	2.52E-09	3.69E-24
	K234	TPP**K**SPSSAK	4.54	6.11E-08	9.38E-04
	K240	TPPKSPSSA**K**SR	3.00	5.53E-03	2.71E-19
MTBR	K254	LQTAPVPMPDL**K**NVK	11.11	1.48E-09	8.44E-201
	K257, K259	NV**K**S**K**IGSTENLK	9.69	1.58E-08	7.27E-246
	K259, K267	S**K**IGSTENL**K**HQPGGGK	8.68	2.10E-09	0.00E+00
	K267	IGSTENL**K**HQPGGGK	11.65	2.96E-09	0.00E+00
	K274	HQPGGG**K**VQIINKK	8.13	1.06E-08	5.03E-163
	K274, K311,317*	HQPGGG**K**VQIVY**K**PVDLS**K**VTSK	10.58	1.57E-07	2.90E-32
	K280, K281	VQIIN**KK**LDLSNVQSK	3.51	5.40E-05	9.81E-44
	K281, K290	**K**LDLSNVQS**K**CGSK	7.32	5.93E-07	9.43E-195
	K290	CGS**K**DNIK	5.57	5.64E-06	8.01E-04
	K298	DNI**K**HVPGGGSVQIVYKPVDLSK	6.79	2.78E-07	1.34E-34
	K311, K317	HVPGGGSVQIVY**K**PVDLS**K**VTSK	10.72	1.02E-07	1.71E-148
	K317	PVDLS**K**VTSK	12.03	9.70E-08	2.22E-129
	K321	VTS**K**CGSLGNIHHKPGGGQVEVK	10.82	4.25E-08	1.59E-106
	K331	CGSLGNIHH**K**PGGGQVEVK	5.02	8.67E-06	2.77E-06
	K340	CGSLGNIHHKPGGGQVEV**K**SEK	5.87	6.05E-06	3.47E-237
	K343	SE**K**LDFK	11.07	1.13E-09	1.81E-03
	K353	VQS**K**IGSLDNITHVPGGGNK	4.24	1.13E-06	2.87E-07
	K369	IGSLDNITHVPGGGN**K**K	5.08	2.33E-06	1.23E-05
C-term	K375	KIETH**K**LTFR	3.79	4.36E-05	4.70E-04
	K383	LTFRENA**K**AK	3.56	4.79E-06	4.85E-04
	K385, K395	A**K**TDHGAEIVY**K**SPVVSGDTSPR	6.03	3.68E-07	3.00E-34
	K395	TDHGAEIVY**K**SPVVSGDTSPR	6.51	8.20E-08	2.27E-81

* Exon 13 skip (residues 275-306)

A) Schematic representation of Tau protein domains and ubiquitylation sites. Residues are numbered according to Tau 441 isoform (P10636-8). A total of 15 Novel ubiquitylation sites are indicated in orange.

B) Statistical analysis (Student's t test) indicates a significant fold change increase in Tau ubiquitin site intensities in AD compared to controls ($p<0.05$). These sites need to be further studied to add a better understanding to Tau biology.

TUBE-based Mass Spec Analysis of Cells Treated with Cereblon-pan-kinase PROTAC



- K562 cells were treated with PROTAC (1 μM) for 120 min and cell lysates (3 mg) were pulled down using magnetic TUBE1 (Cat# UM401M) overnight at 4°C and eluted.
- The eluted sample was run on a short gel and an in-gel trypsin digestion was performed prior LC-MS.
- Relative change in quantitative signals from kinases between treated vs DMSO treated sample and PROTAC + MG132 vs DMSO + MG132 treated sample were plotted to identify the modulated proteins.

TUBE-based Mass Spec Service

- Help customer identify ubiquitylation patterns specific to drug treatment
- Optimized for both cell and tissue lysates
- Customer provides cell pellets, we do the rest
- Superior to other ubiquitin proteomic methods such as Di-Gly
- TUBE-based proteomics to assess specificity of PROTAC drug
- Identification of the polyubiquitylation site(s) (number & position) on the protein sequence
- All IP and data belong to the customer
- Work performed under CDA and Master Service Agreement
- Fee for service model, defined milestone-based agreement

Contact Us!

We are your partner for TUBE-based Mass Spec Proteomics

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