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Output tables of the Fisher Exact Testing for enrichment within the substrate populations for experiments M1, M2, M3.

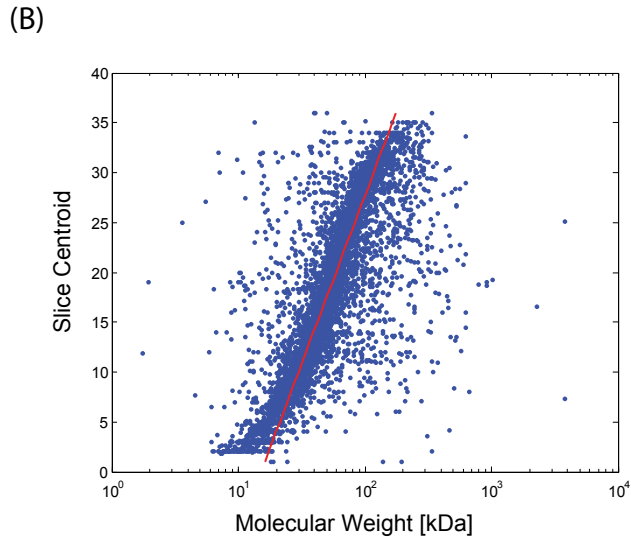
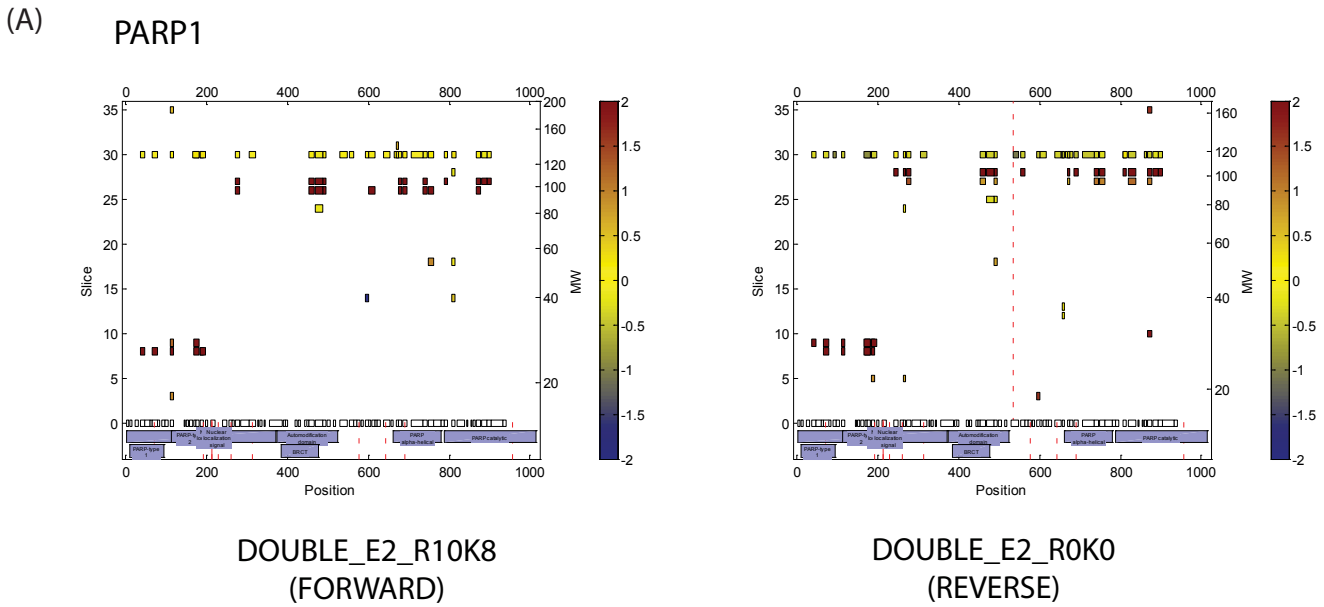


Figure S1: Background information on the experiments and algorithm.

A. Forward and reverse SILAC labeling. Both forward and reverse labeling resulted in the same ratio pattern for the cleaved substrate PARP1. **B.** Correlation of slice numbers to molecular weights. Based on all detected proteins of an experiment we calibrated approximate molecular weight regions along the gel. The red line is the regression line for the calculation of the right y-axis in the 3D cleavage plot.

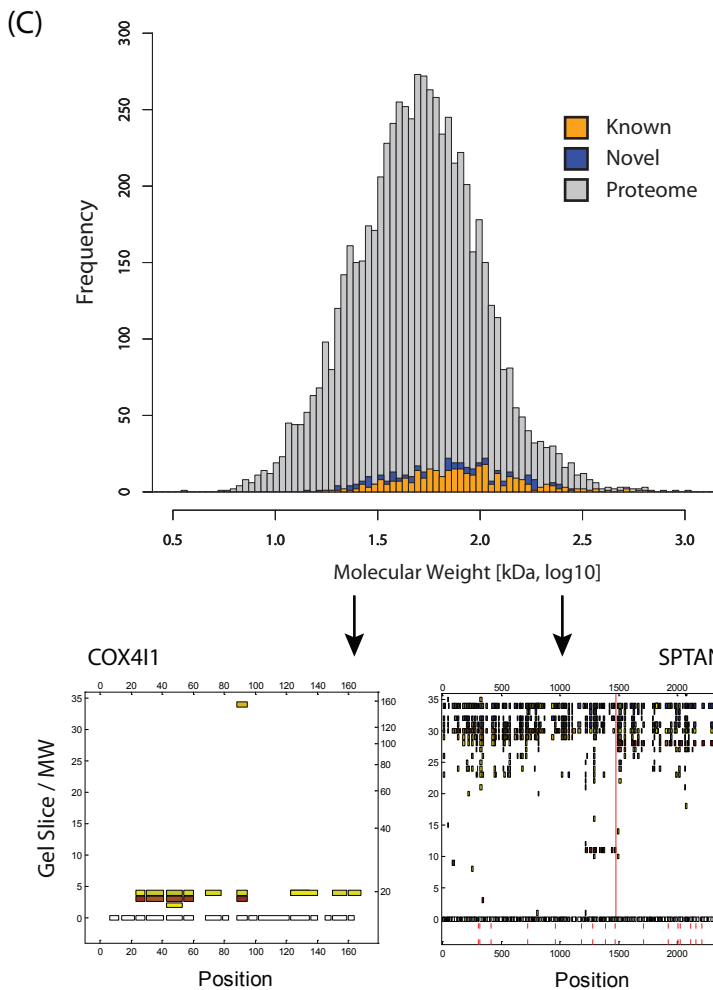
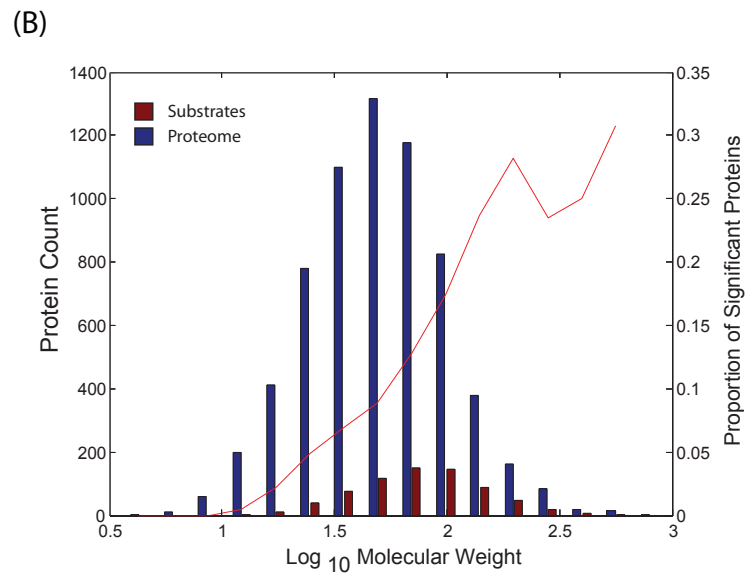
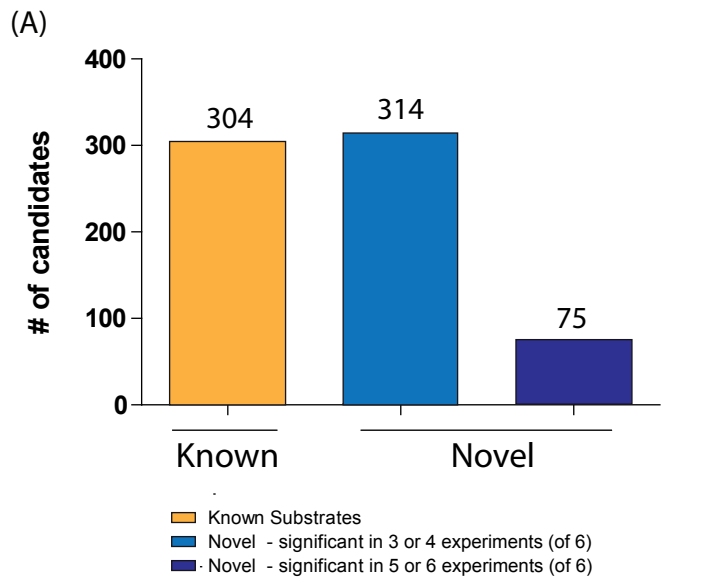


Figure S2: Characterization of the substrate population.

A. Characterization of identified substrates. Numbers of known and novel substrates are depicted. Novel substrates are subdivided depending on their number of significant identifications as cleavage substrate in all six experiments. Only substrates identified significantly at least three times are considered for the analysis.

B. Coverage of the molecular weight region by the cleaved substrates. Whole proteome data as well as substrates are plotted. The red line indicates the proportion of significant substrates within the different molecular weight regions in comparison to the whole proteome.

C. Distribution of molecular weights spanned by the whole proteome experiment and by the cleavage substrates. Proteome data are plotted according to their molecular weight, underlying known and novel substrates are highlighted. Substrates are generally detected between 20 kDa and 250 kDa. Representative examples of the lower and higher molecular weight regions are depicted.

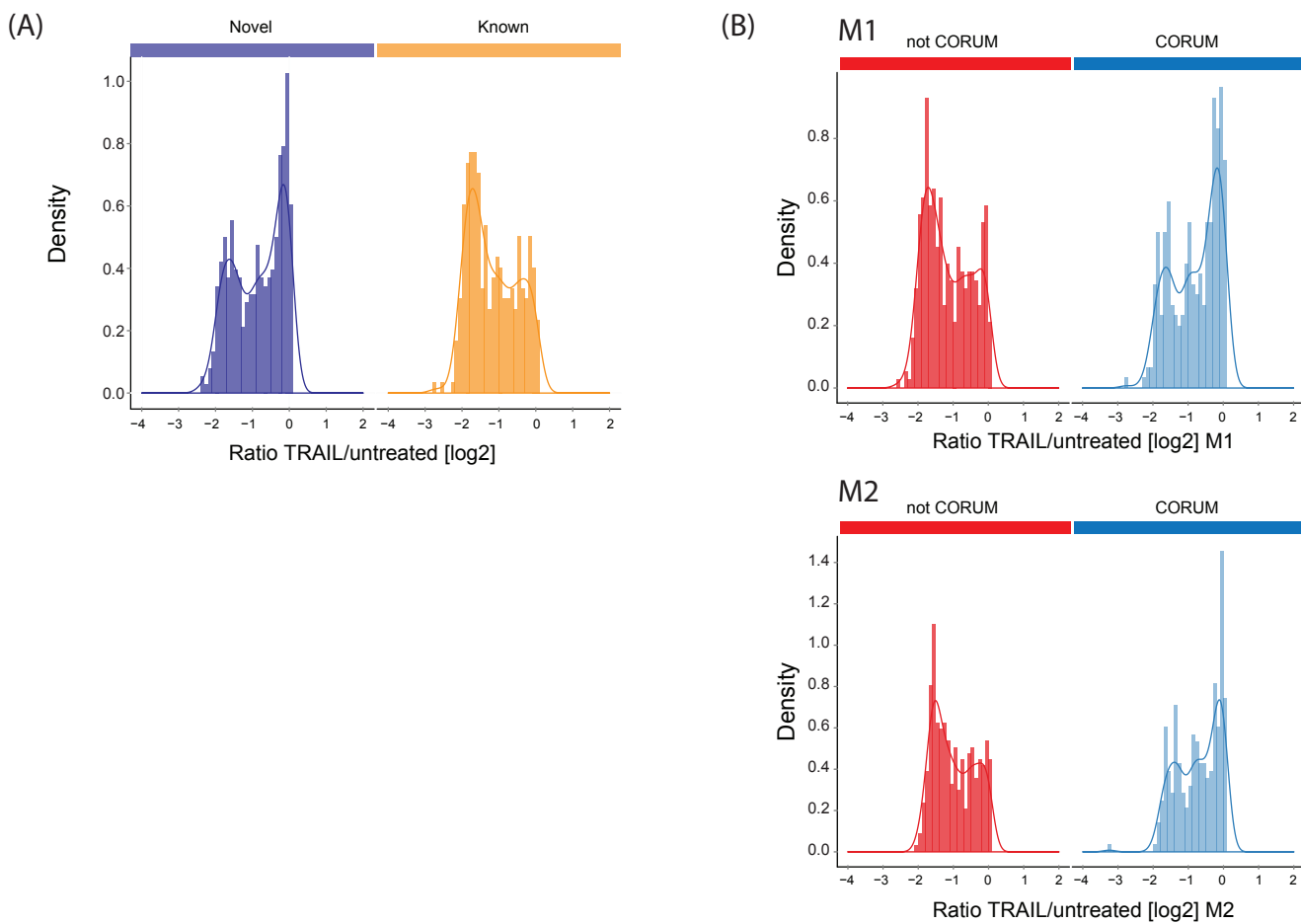


Figure S3: Investigation of the uncleaved ratio distribution of cleaved substrates.

A. Uncleaved ratio distribution of all cleaved substrates split for novel and known substrates. The uncleaved ratio distribution indicates a tendency of novel substrates to be located in the right hand peak in comparison to the population of known substrates. **B.** Separation of the uncleaved ratio population for CORUM and non-CORUM substrates within the experiments M1 and M2. Substrates annotated to CORUM show tendencies to be located in the right hand peak.

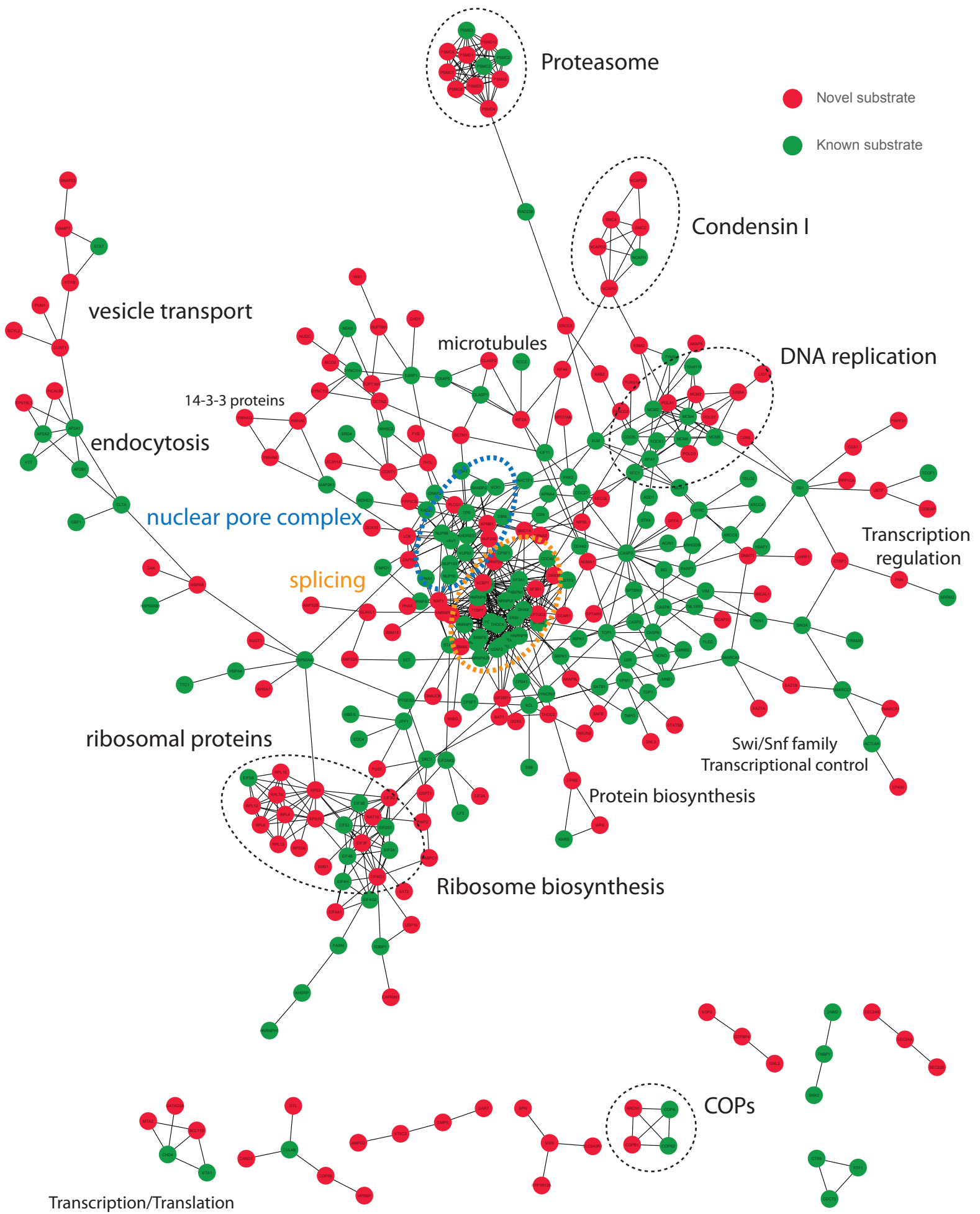
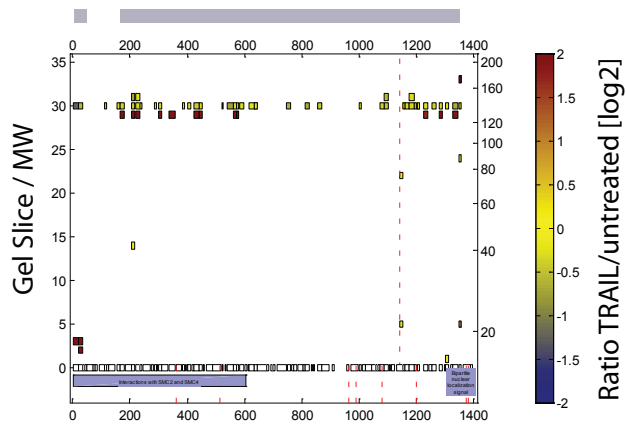


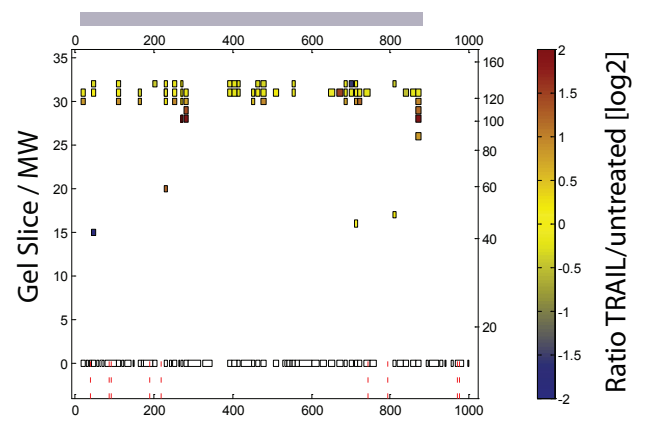
Figure S4: STRING analysis of known and novel substrates.

Substrates were uploaded to STRING and interactions were represented in the Cytoscape environment. Novel substrates are indicated in red, known substrates in green. Categories spanned by several interacting proteins are named within the graphic and encircled for clarity if necessary.

NCAPD2



NCAPG



SMC4

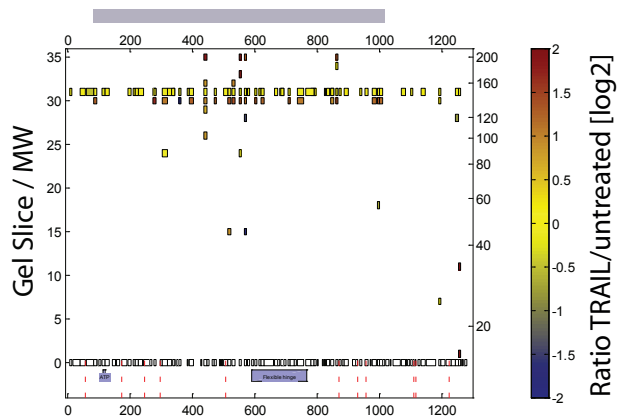
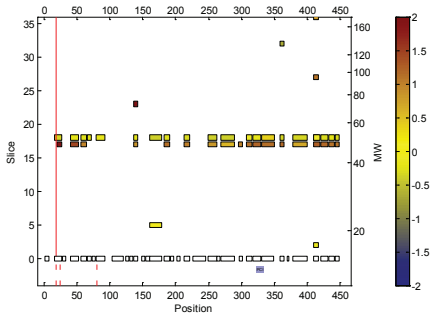


Figure S5: 3D cleavage plots for components of the condensin I complex.

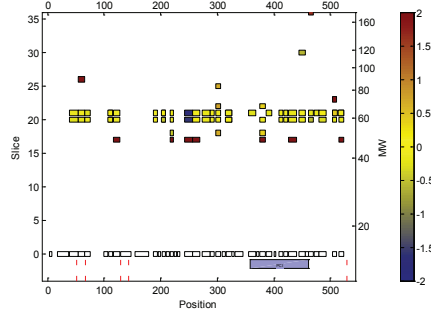
Components from the condensin I complex not displayed in Fig. 7A are plotted. All proteins are cleavage substrates, cleavage fragments are indicated on top of the graph with grey bars.

19S regulatory particle (RP)

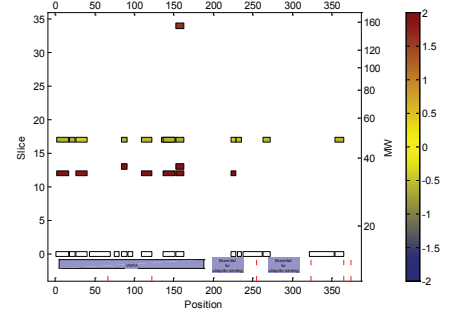
PSMD12,RPN5



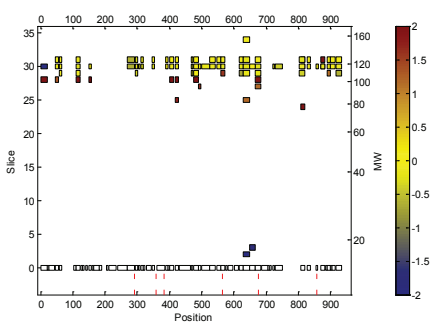
PSMD3,RPN3



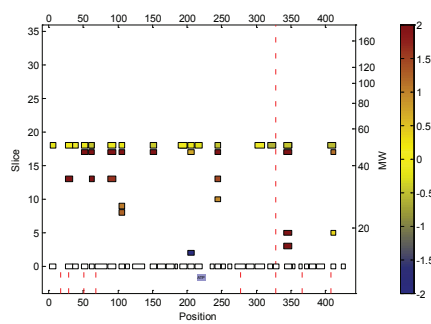
PSMD4,RPN10



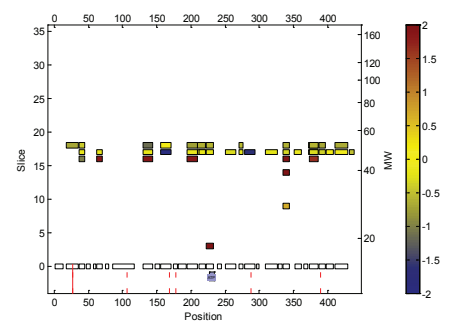
PSMD1,RPN2



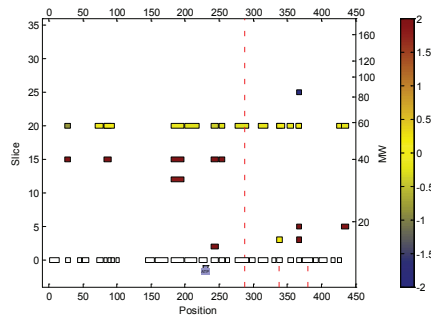
PSMC2,RPT1



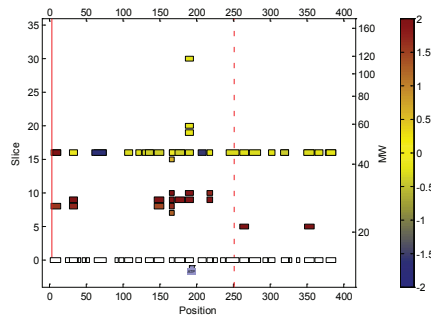
PSMC3,RPT5



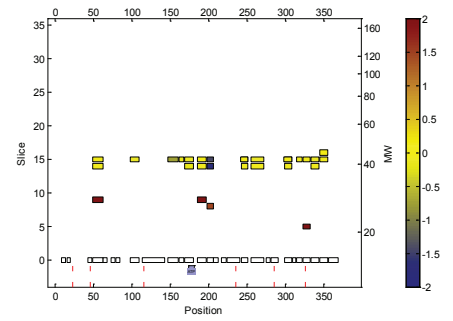
PSMC1,RPT2



PSMC5,RPT6

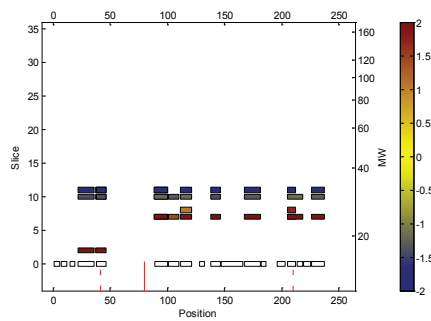


PSMC6,RPT4



PA-28-gamma

PSME3



Core particle (20S proteasome)

PSMA5

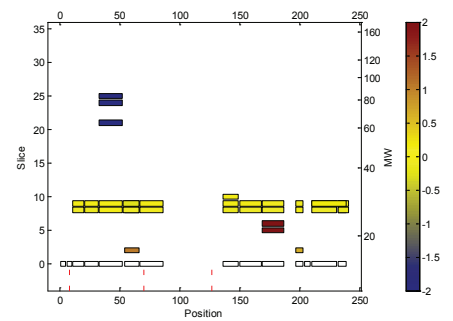


Figure S6: Cleavage of proteasomal proteins.

Cleaved proteins from the proteasome complex are depicted via their 3D cleavage plots. Proteins from both the 19S regulatory particle as well as the 20S core particle were identified as cleaved substrates. Most substrates show low stoichiometries in their uncleaved region.

Supplemental Information

Instructions on how to use MaxQB for visualizing and extracting cleavage information.

Starting from the project page, each of the experiments can be selected (1). Moreover, a list of proteins with an explicit cleavage information is provided (2).

TRAIL induced Apoptosis

standard SILAC experiment

Identification of cleaved substrates

The extrinsic, extracellular ligand induced pathway of apoptosis is executed by caspase protease cascades activating downstream effectors by site-directed proteolysis. Here we identify proteome changes upon induction of apoptosis by the cytokine Tumor Necrosis Factor Related Apoptosis Inducing Ligand (TRAIL) in a Jurkat T cell line. We detect caspase-dependent cleavage substrates by quantifying protein intensities before and after TRAIL induction in SDS gel slices. Apoptotic protein cleavage events are identified by a characteristic SILAC ratio pattern across gel slices that result from differential migration of the cleaved versus the uncleaved protein. We apply a statistical test to define apoptotic substrates in the proteome. Our approach identified more than 650 of these cleaved proteins in response to TRAIL-induced apoptosis, including many previously unknown substrates and cleavage sites. Inhibitor-treatment combined with triple-SILAC demonstrated that the detected cleavage events are caspase-dependent. Proteins located in human organelles such as mitochondria and endoplasmic reticulum were significantly under-represented in the substrate population. Interestingly, caspase cleavage is generally found not only in one but several members of stable complexes, but often with lower stoichiometry. For instance, all five proteins of the condensin I complex were cleaved upon TRAIL treatment. The apoptotic substrate proteome data can be accessed and visualized in the MaxQB database and may prove useful for basic and clinical research into TRAIL-induced apoptosis. The technology described here is extensible to a wide range of other proteolytic cleavage events.

Stoehr Gabi, Schaab Christoph, Gramann Johannes, Mann Matthias: "A SILAC-based approach identifies substrates of caspase-dependent cleavage upon TRAIL-induced apoptosis", MCP submitted

Show proteins with cleavage events ← 2

Experiment Series	Name	Description	Create
1	TRIPLE Inh M2	TRIPLE SILAC exp 10/09/20	
2	TRIPLE Inh M1	TRIPLE SILAC exp 10/09/20	
3	TRIPLE Inh M3	TRIPLE SILAC exp 10/09/20	

For each experiment general information on the experiment is provided.

Experiment Series TRIPLE Inh M3

Details	QC	Cleaved Proteins	Protein Groups	Peptides	Evidences
Type	LC-MS/MS Experiment				
Name	TRIPLE Inh M3				
Description	TRIPLE SILAC experiment in Jurkat T cells; L = untreated; M = 5h TRAIL + z-VAD-FMK; H = 5h TRAIL				
Project	Gabi Apoptosis				
Experimentalist	Gabi Stoehr				
Labeling Method	SILAC				
Enrichment	no Enrichment				
Protease	Trypsin/P				
Separation	SDS-PAGE				
LC Gradient	min				
Column Material	3.0 µm				
Column Length	15.0 cm				
Fragmentation Method					
LC-MS/MS Machine	LTQ Orbitrap Velos				
Created	12/09/11 by schaab				
Raw Files	Apoptosis_Gabi/TRIPLE_Inh_M3_txt				

Experiments

	Name	Cellline	Description
0	E1		
1	E10		
2	E11		
3	E12		
4	E13		

A list of the cleaved proteins can be accessed via the “Cleaved Proteins” tab including cleavage information on the single entries (p-value, FDR, ratio of cleaved peptides, ...) (1).

Specific proteins can be searched by first clicking on the “Search” button at the bottom of the table (2). Each column can then be search for a specific term.

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Experiment Series TRIPLE Inh M3

Details QC Cleaved Proteins Protein Groups Peptides Evidences

Cleaved Proteins ID	Protein IDs	Gene Names	Leading Protein ID	Leading Gene Name	Leading Protein Name	Known Substrate	Detected Cleavage Site	Cleavage p-Value	Cleavage fdr	Ratio Cleaved Peptides	Ratio Uncleaved Peptide
4328	Q4LE64, Q14980, Q9BTE	NUMA1 variant protein, NUQ4LE64		NUMA1 variant protein		no	0	0	1.58932129363276	-0.37502334897691	
5608	P40855, E9PBB5, P40855	PEX19, PEX19, PEX-P40855		PEX19	Peroxisomal biogenesis factor 19	no	0.035	0.3991292134031461	2.139281765762556	-1.91537661053458	
2264	P20936, P20936-2, B4DT	RASA1, RASA1	P20936	RASA1	Ras GTPase-activating protein 1	yes	0	0	2.35444408209437	-1.7584641106292	
4657	O60524, O60524-2	NEMF, NEMF	O60524	NEMF	Nuclear export mediator factor 1	no	0	0	2.59130845029964	-1.93698660043571	
131	Q08170, B4CEMB	SRSF4	Q08170	SRSF4	Serine/arginine-rich splicing factor 4	no	0.002	0.00423404255319149	1.79136740170787	-0.156192215055628	
979	IF10009982, Q9Y2W6	TDRKH, TDRKH, TDRKH, TDRKH	IF10009982	TDRKH	TSOFORM 1 OF TUDOR ANK	no	0	0	2.58070866409789	-1.3905169979634	
6369	Q12739, B4D9W9	TYRPF3	Q12739	TYRPF3	TYR domain family protein	yes	168	0	2.2766910031114	-1.17906079429701	
4757	E9H677, P52594, P52594	AGF1, AGF1, ALESHK7		AGF1		no	0.003	0.0056056338028169	1.42572950241939	-1.97342540216991	
2548	IF10003099, Q12982-2, BNP2, BNP2, BNP2, BNP2	BNP2, BNP2, BNP2, BNP2	IF10003099	BNP2	BCL2/ADENOVIRUS E1B 1	no	0.006	0.0092468538281413	2.9314816345582	-1.7645713455094	
9238	B723U7, Q9ULV3, Q5SYVC12, C1Z1, C1Z1, C1Z1, (B723U7					no	0.008	0.0114121863799203	2.3502538802562	-1.82908647816948	
2549	O40563, A9XU13, IF1007	CCNT1, CCNT1, CCNT1, CCNT1	O40563	CCNT1	Cyclin-T1	no	47	0.005	0.0080484295845998	2.4695511177522	-1.50289367588682
2424	P14921, Q6N087, P14921	ETS1, ETS1, ETS1, ETS1	P14921	ETS1	Protein C-ets-1	no	0.001	0.0027590976682042	1.56532283568238	-1.58327810788659	
1834	P18189, Q55207, Q55207	HDGF, HDGF, HDGF, HDGF	P18189	HDGF	Hepatooma-derived growth factor	no	0.011	0.014605504587156	2.04291311549538	-0.0105112292669604	
2699	Q5T126, Q5T126, E9PCH	FUM1, FUM1, FUM1, FUM1	Q5T126	FUM1		no	0.003	0.0056056338028169	1.36429954129575	-1.35288302347412	
2698	Q14CA3, Q14649, Q5EKTR12, TRIP12, TRIP12, TQ14CA3			TRIP12		no	0.001	0.0027590976682042	1.44245789654969	-1.0901860231177	
8696	E9P9H1, Q102W5, Q102W5	E1F4G1, E1F4G1, E1F4G1, E1F4G1		E1F4G1		no	0	0	1.92367935654827	-1.29441999966676	
4784	Q12619, B4CT54, IF1006	HHR23B, HHR23B, HHR23B, HHR23B	Q12619	HHR23B	Minor histocompatibility protein	no	0	0	1.89976064239532	-1.9564095987211	
6488	A9N051, Q13813-2, Q13813-2, SPTAN1, SPTAN1, A9N051			SPTAN1		no	1475	0	0	1.58035647262302	-1.75630676455551
262	B4DT57, Q9Y860, E9PCB	STK24, STK24, STK24, STK24	B4DT57	STK24		yes	0.002	0.00423404255319149	2.42067599671289	-1.1390642275353	
4404	Q92734, Q5E9V1, Q5E9V1	TFG, TFG/ALK fusion, Q92734		TFG	Protein TFG	no	0.008	0.0114121863799203	1.90406283563285	-1.82060795482012	
5835	Q2M218, Q2M218-2, E9PAAK1, AAK1, AAK1		Q2M218	AAK1	AP2-associated protein kinases	no	0	0	1.40073100863005	-1.86484942413679	

Search Clear Columns Excel View 1 - 60 of 691

When selecting one ID in the “Cleaved proteins” table, further information about the protein within the experiment is provided.

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ProteinGroup TRIPLE Inh M3 - 4328

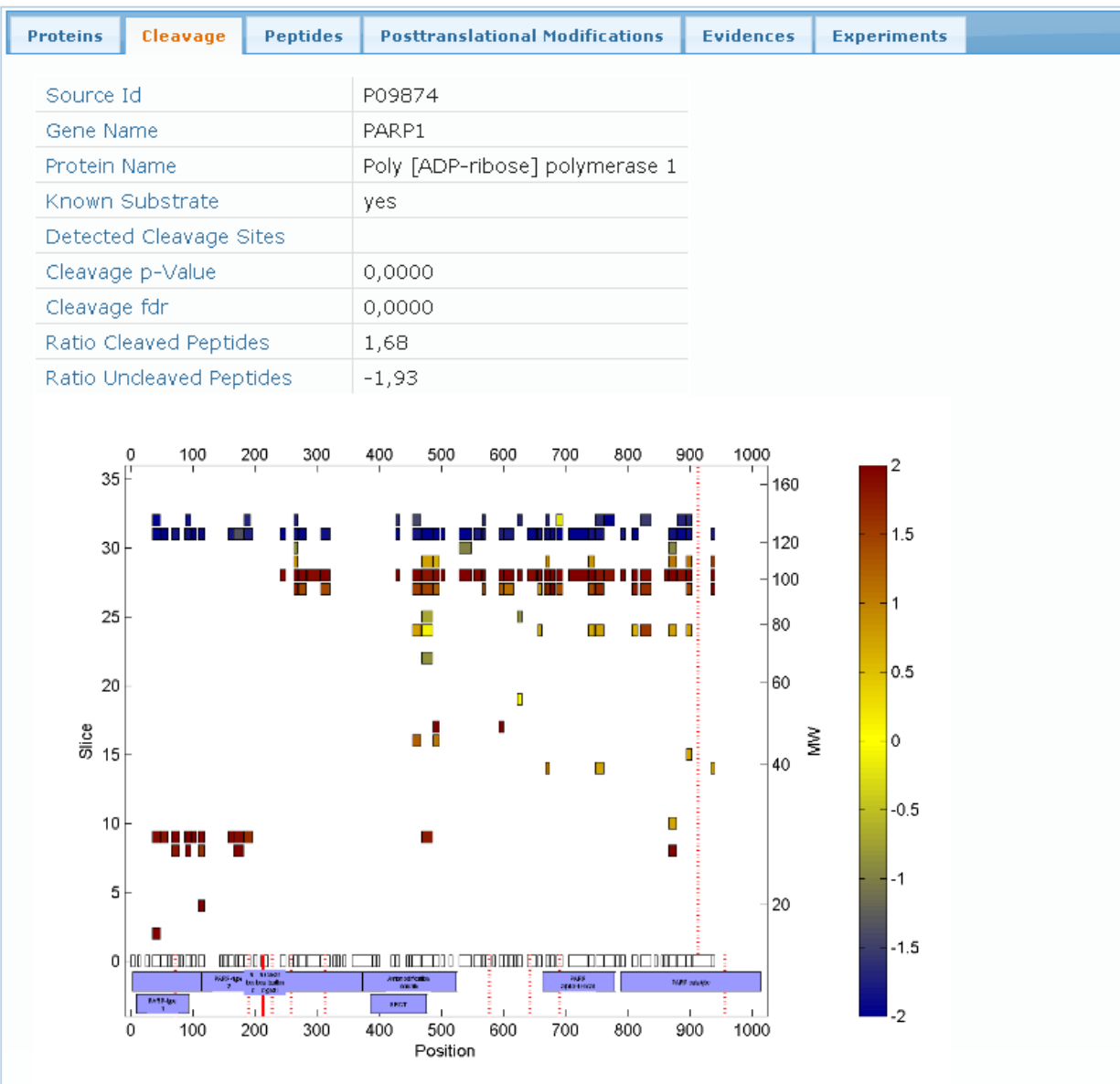
Proteins Cleavage Peptides Posttranslational Modifications Evidences Experiments

Source ID	Source Database	Source Version	Uniprot ID	Protein Name	Gene Name	Synonyms	Description	Molecular Weight	Unique Peptides	Razor Peptides	Total Peptides
Q4LE64	UNIPROT HUMAN	2012_07	Q4LE64	NUMA1 variant protein	NUMA1	NUMA	May be a structural compo	238.859	1	92	92
Q14980	UNIPROT HUMAN	2012_07	Q14980	Nuclear mitotic apparatus	NUMA1	NUMA		238.26	1	92	92
Q9BTE9	UNIPROT HUMAN	2012_07	Q9BTE9		NUMA1			109.279	0	41	41
Q15417	UNIPROT HUMAN	2012_07	Q15417	Trinucleotide repeat-contain	TNRC18	CAGL79_KIAA1056		314.519	0	1	1
IF10093934	IFI HUMAN	3.38		UNCHARACTERIZED PROT	TNRC18			302.845	0	1	1
IF100479962	IFI HUMAN	3.38		MYOSIN-VB	MYO5B			213.756	0	1	1
A9MSW5	UNIPROT HUMAN	2011_10	A9MSW5		TNRC18			140.463	0	1	1
Q9H6Y6	UNIPROT HUMAN	2012_07	Q9H6Y6		MYO5B			111.759	0	1	1
REV_IF100930364	REVERSE							0	1	1	1
REV_IF100806473	REVERSE							0	1	1	1
IF100847324	IFI HUMAN	3.38		40 KDA PROTEIN				39.565	0	1	1
CKPFB	UNIPROT HUMAN	15.14	CKPFB		TNRC18			17.465	0	1	1

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The “Cleavage” table contains all information about the cleavage of this protein within the specific experiment including statistics of the cleavage and the 3D cleavage plot.

ProteinGroup TRIPLE Inh M3 - 5633



Selecting the Source Id in the “Proteins” tab or by selecting the Protein Id at the very beginning leads to the general protein information.

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Protein P09874 - Poly [ADP-ribose] polymerase 1

Details	Sequence	Sequence Coverage	Peptides	Modifications	Experiment Series	PrEST	Cleavage	Literature
Organism	Human							
Protein Name	Poly [ADP-ribose] polymerase 1							
Gene Names	PARP1 (ADPRT,PPOL)							
Description	Involved in the base excision repair (BER) pathway, by catalyzing the poly(ADP-ribosylation) of a limited number of acceptor proteins involved in chromatin architecture and in DNA metabolism. This modification follows DNA damages and appears as an obligatory step in a detection/signaling pathway leading to the repair of DNA strand breaks. Mediates the poly(ADP-ribosylation) of APLF and CTRF. Positively regulates the transcription of MTS1 and negatively regulates the transcription of MTS2/TIP150. With EEP1A1 and TRK, forms a complex that acts as a T-helper 1 (Th1) cell-specific transcription factor and binds the promoter of IFN-gamma to directly regulate its transcription, and is thus involved importantly in Th1 cytokine production.							
Location	Chromosome 1 : 226548392-226595774							
Source	UNIPROT HUMAN Version: 2012_07 Id: P09874 ★							
Global FDR	0							
Other Sources	<p>IPi HUMAN Version: 3.26 Id: IPi00953994 Gene Name: PARP1 Protein Name: POLY [ADP-RIBOSE] POLYMERASE 1</p> <p>IPi HUMAN Version: 3.26 Id: IPi00292802 Gene Name: PARP1 Protein Name: POLY [ADP-RIBOSE] POLYMERASE 1</p> <p>UNIPROT HUMAN Version: 15.6 Id: B1JANJ4 Gene Name: PARP1</p> <p>ENSEMBL HUMAN Version: 68 Id: ENSP00000355759</p> <p>IPi HUMAN Version: 3.26 Id: IPi00449049 Gene Name: PARP1 Protein Name: POLY [ADP-RIBOSE] POLYMERASE 1</p>							
External Links	Uniprot Kinexus PhosphoNet							

Information as e.g. general sequence coverages over all stored data can be accessed (for more information on general MaxQB features refer to the publication on MaxQB: Schaab et al., MCP, 2012).

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Protein P09874 - Poly [ADP-ribose] polymerase 1

Details	Sequence	Sequence Coverage	Peptides	Modifications	Experiment Series	PrEST	Cleavage	Literature
<p>Export image as SVG, PNG, or PDF</p>								
<p>Display Options</p> <p>Protease: <input type="text" value="Trypsin/P"/></p> <p>Show peptides per experiment series: <input type="checkbox"/></p> <p>Color peptides by intensity: <input type="checkbox"/></p> <p>Show non-proteotypic peptides: <input type="checkbox"/></p> <p>Proteolytic peptides with masses between 600 and 4000 Da are shown as grey boxes.</p> <p>Refresh</p>								

Again cleavage information can be accessed, this time, not based on a single experiment, but on all experiments. On top, information from literature about known cleavage sites is displayed. The hyperlink guides to the reference. Below, information derived from our experiments is displayed for all experiments – the 3D cleavage plot, cleavage site information as well as statistics on the cleavage per experiment.

Protein P09874 - Poly [ADP-ribose] polymerase 1

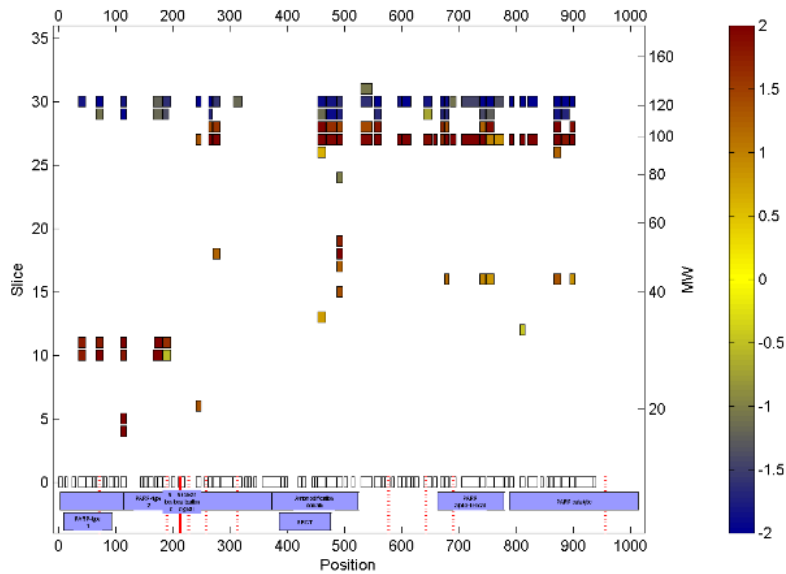
Details	Sequence	Sequence Coverage	Peptides	Modifications	Experiment Series	PrEST	Cleavage	Literature
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Cleavage Sites from Literature

Pos	Source
214	CASBAH

Cleavage Sites determined by Stoehr et al.

Experiment Series	TRIPLE Inh M1
Protein Group	5518
Detected Cleavage Sites	
Cleavage p-Value	0,0000
Cleavage fdr	0,0000
Ratio Cleaved Peptides	1,65
Ratio Uncleaved Peptides	-1,95



Proteins can also be searched directly via the “Search -> Proteins” function. By selecting the Source Id, general protein information will be obtained. In case the protein is known as cleavage substrate but was not identified by our approach, the information about the reference will still be provided in the “Cleavage” tab. In case no “Cleavage” tab is provided, the protein is not known to be a cleavage substrate.

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Protein Query

Search protein by gene or protein name:

Query results

6 protein entries found.

Source Id *	Source Database	Source Version	Protein Name	Gene Name	Synonyms	Description	Organism	Reviewed	Molecular Weight	Chromosome	Start Position
P09874	UNIPROT HUMAN	2012_07	Poly [ADP-ribose] polymer PARP1	PARP1	ADPRT,PPOL	Involved in the base exci	Human	true	113.084	1	226548392
KW11_Step3_001603	MPI-XENO	03.2012	parp1	parp1			Xenopus	true	112.76976		
KW11_Step0_010162	MPI-XENO	03.2012	parp1*	MGC115350			Xenopus	true	66.09867		
KW11_Step0_048397	MPI-XENO	03.2012	parp1*	MGC115350			Xenopus	true	17.30115		
P11103	UNIPROT MOUSE	2012_07	Poly [ADP-ribose] polymer Parp1	Parp1	Adprp,Adprt,Adprt1	Positively regulates the tra	Mouse	true	113.1		
P11103-2	UNIPROT MOUSE	2012_07	Poly [ADP-ribose] polymer Parp1	Parp1	Adprp,Adprt,Adprt1	Positively regulates the tra	Mouse	true	55.237		

Columns | Excel | View 1 - 6 of 6

Advanced search

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