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(54) **ANTIBODIES FOR UBIQUITINATED PROTEINS**

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C07K 16/00	(2006.01)
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(57) **ABSTRACT**

The invention relates to particular ubiquitination epitopes, antibodies that specifically recognize and bind to ubiquitinated proteins and peptides (particularly after the ubiquitin is removed by proteolytic cleavage) and to methods of using these epitopes and antibodies.

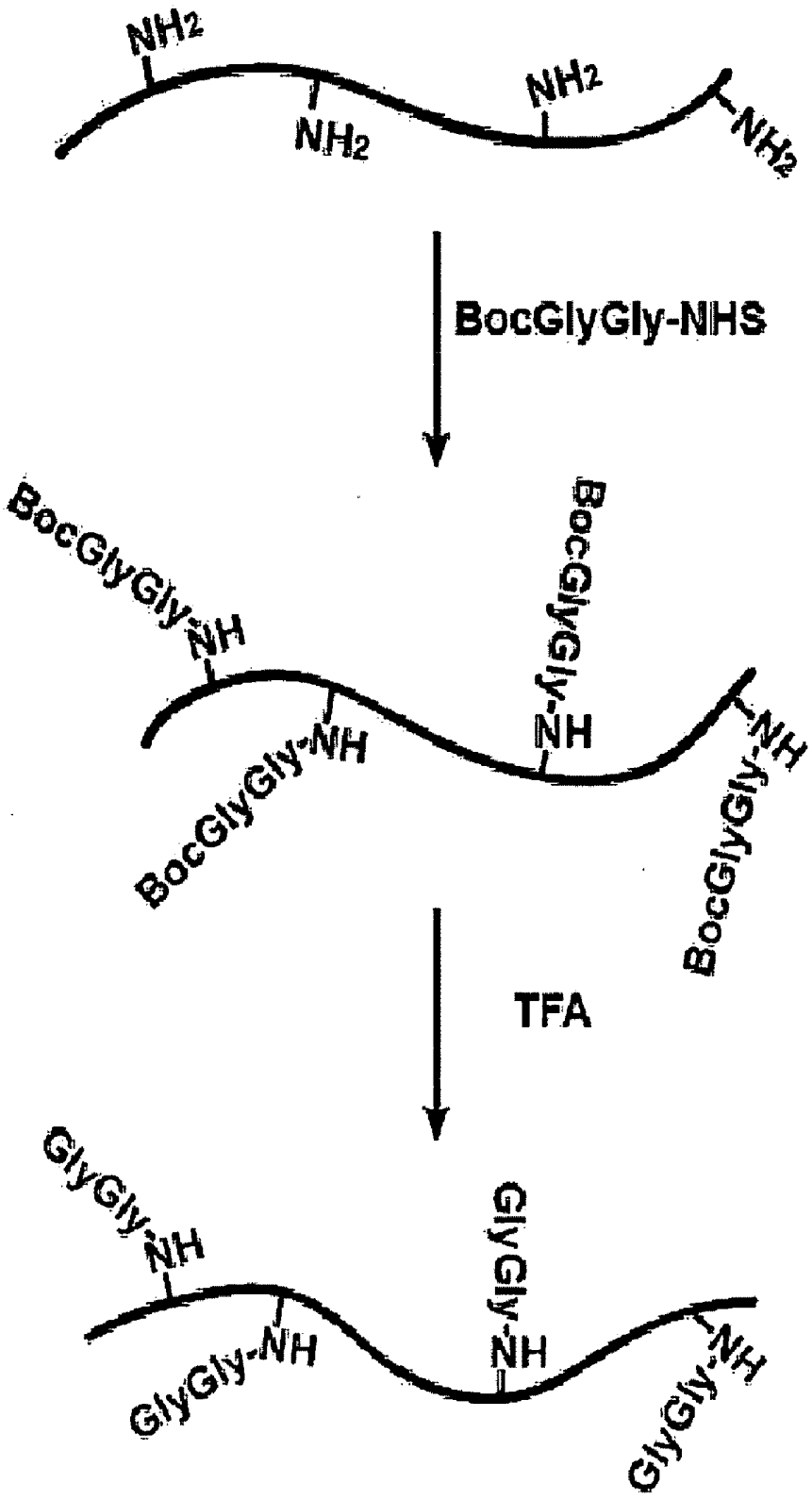

NH2
NH2
NH2
NH2
BocGlyGly-NHS
BocGlyGly-NH
BocGlyGly-NH
BocGlyGly-NH
BocGlyGly-NH
BocGlyGly-NH
TFA
GlyGly-NH
GlyGly-NH
GlyGly-NH
GlyGly-NH

Fig. 1A

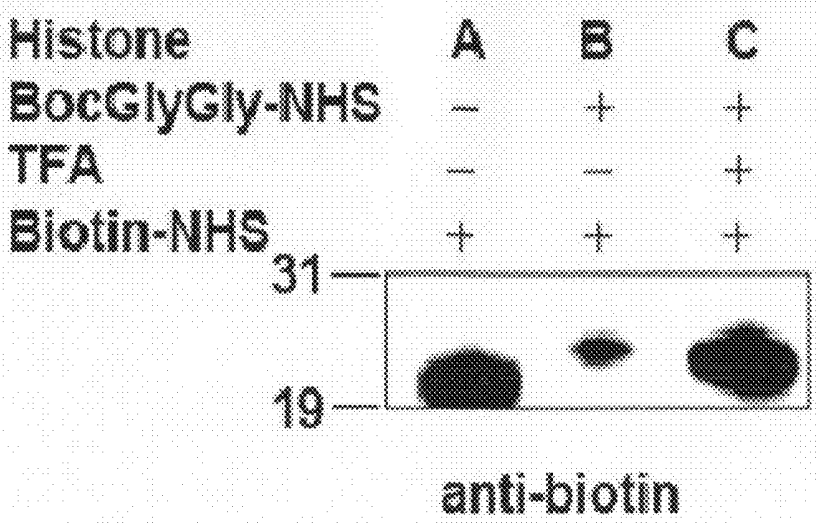

Histone A B C
BocGlyGly-NHS − + +
TFA − − +
Biotin-NHS + + +
31
19
anti-biotin

Fig. 1B

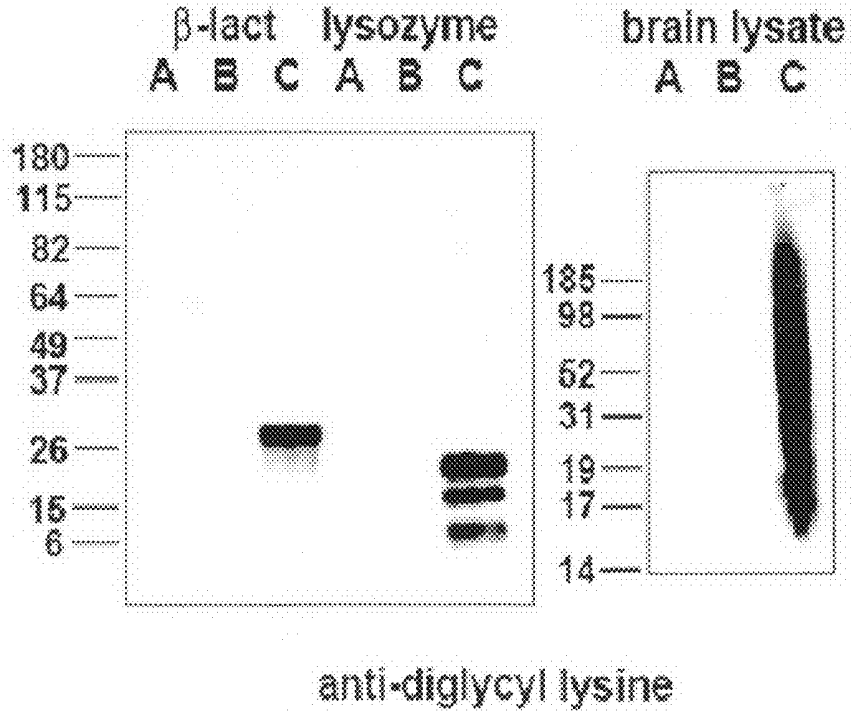

β-lact lysozyme
A B C A B C
brain lysate
A B C
180
115
82
64
49
37
26
15
6
185
98
52
31
19
17
14
anti-diglycyl lysine

Fig. 1C

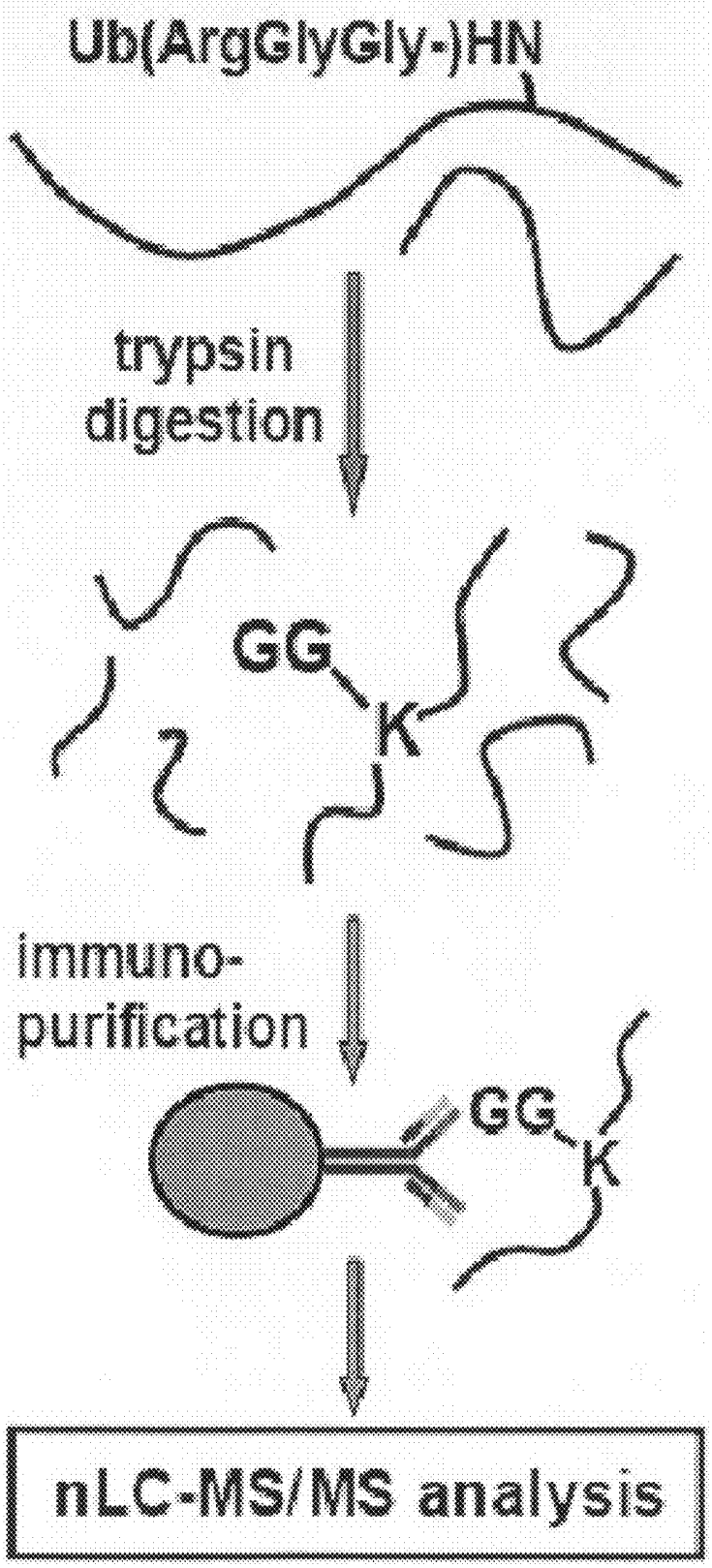

Ub(ArgGlyGly-)HN
trypsin
digestion
GG
K
immuno-
purification
GG
K
nLC-MS/MS analysis

Fig. 2A

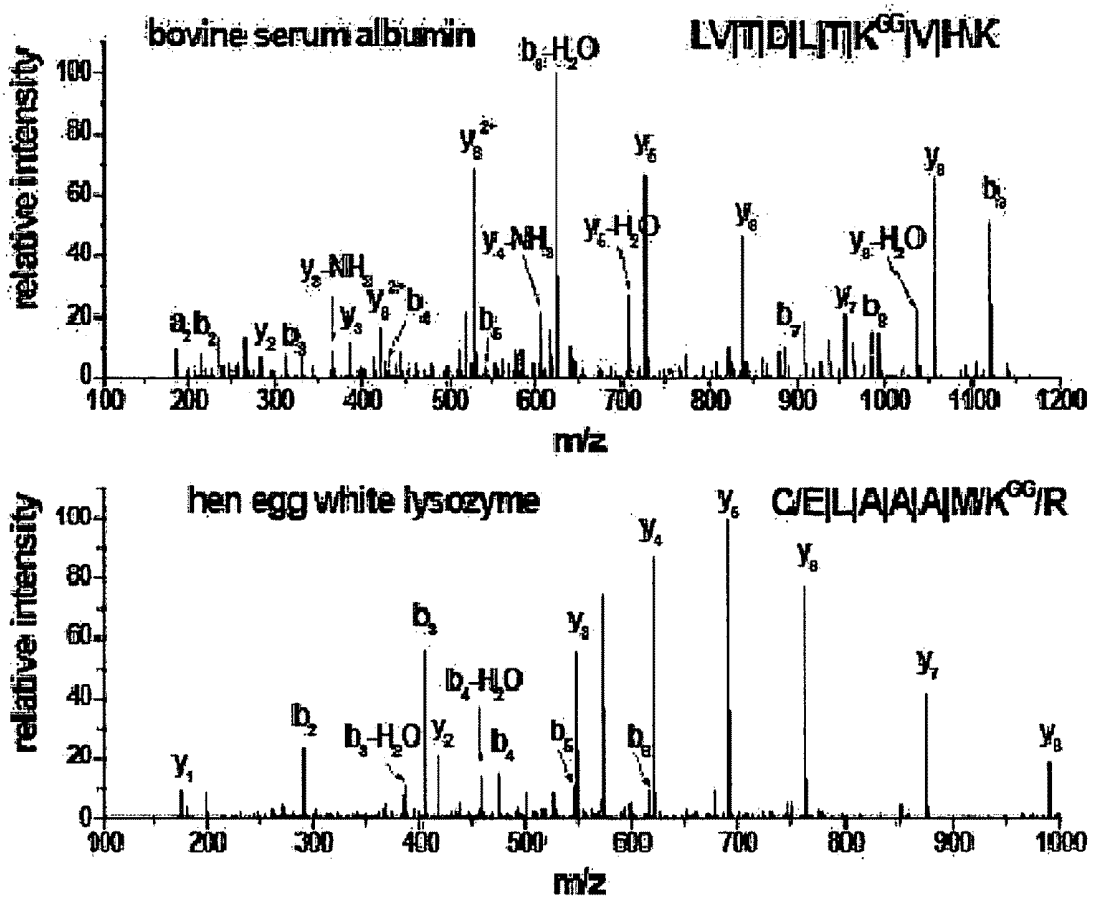

bovine serum albumin
LV|T|D|L|T|K^GG|V|H|K
relative intensity
m/z
hen egg white lysozyme
C/E|L|A|A|A|M/K^GG/R
relative intensity
m/z

Fig.2B

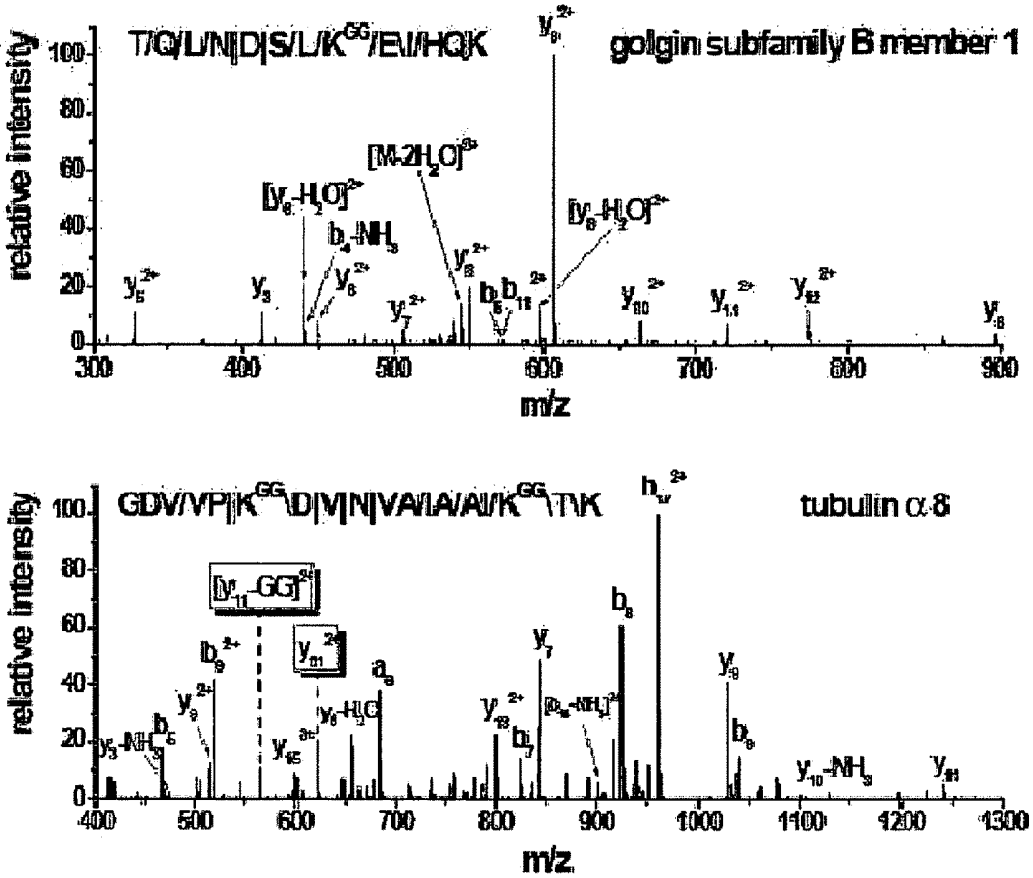


Fig.2C

tissue/culture	HEK293	N2a & sEnd1	rat brain & liver	total
species	Human	Mouse	Rat	
Ub sites	216	94	85	395
Ub proteins	194	72	65	331
reported	49	28	30	107

Fig.2D

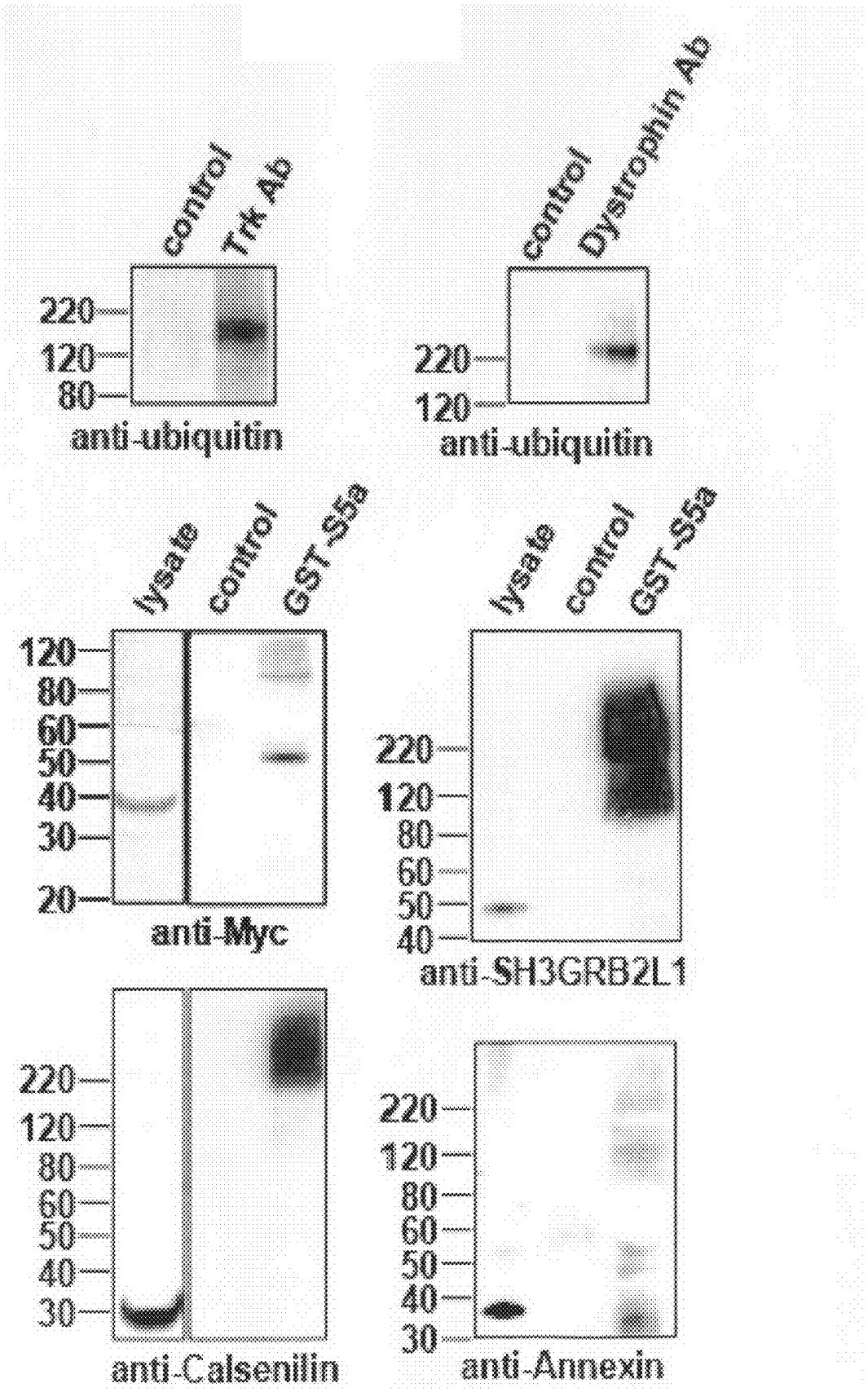

control
Trk Ab
220
120
80
anti-ubiquitin
control
Dystrophin Ab
220
120
anti-ubiquitin
lysate
control
GST-S5a
120
80
60
50
40
30
20
anti-Myc
lysate
control
GST-S5a
220
120
80
60
50
40
anti-SH3GRB2L1
220
120
80
60
50
40
30
anti-Calsenilin
220
120
80
60
50
40
30
anti-Annexin

Fig. 2E

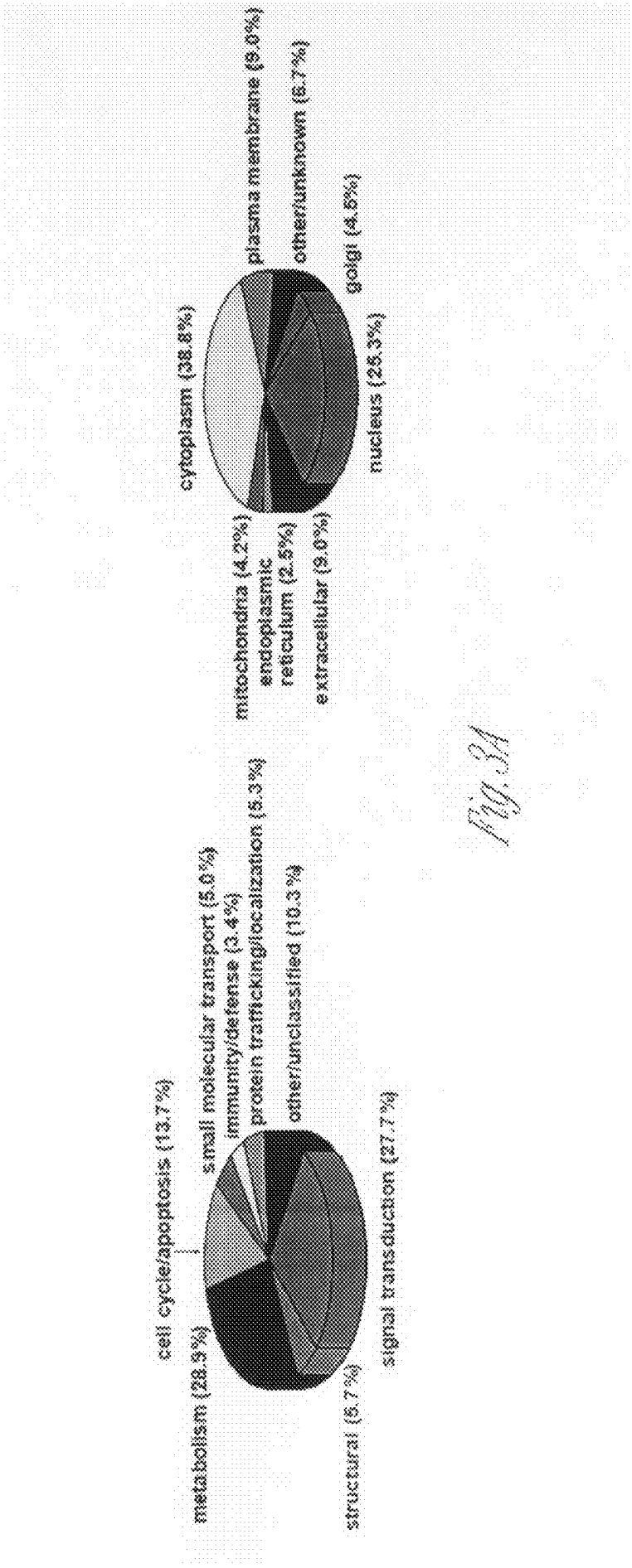

cell cycle/apoptosis (13.7%)
small molecular transport (5.0%)
immunity/defense (3.4%)
protein trafficking/localization (5.3%)
other/unclassified (10.3%)
signal transduction (27.7%)
structural (6.7%)
metabolism (28.9%)
cytoplasm (38.8%)
plasma membrane (9.0%)
other/unknown (6.7%)
golgi (4.5%)
nucleus (28.3%)
extracellular (9.0%)
endoplasmic reticulum (2.5%)
mitochondria (4.2%)

Fig. 3A

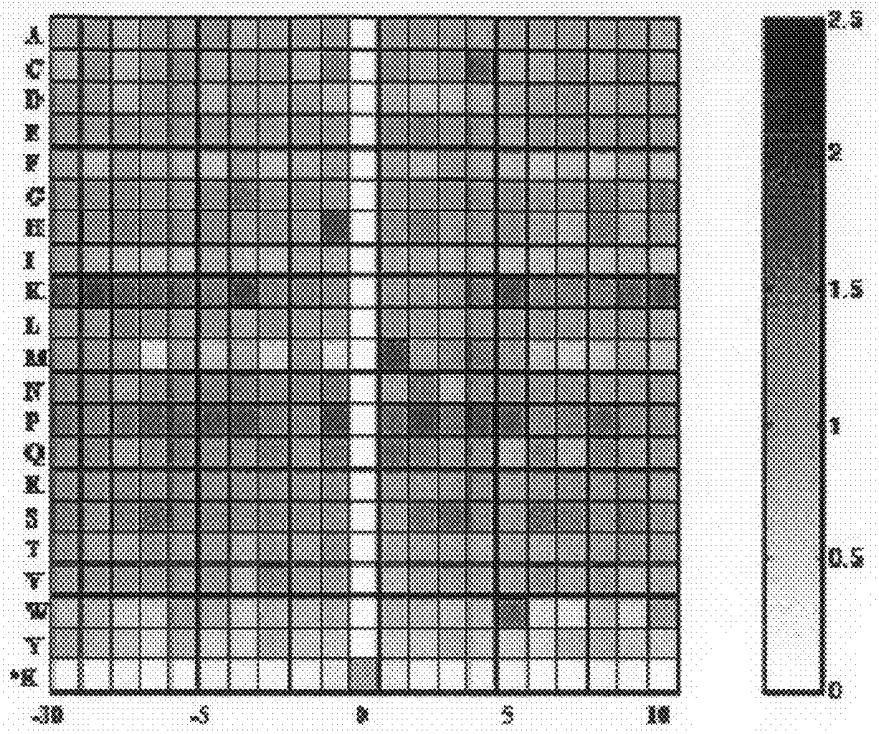

2.5
2
1.5
1
0.5
0

Fig.3B

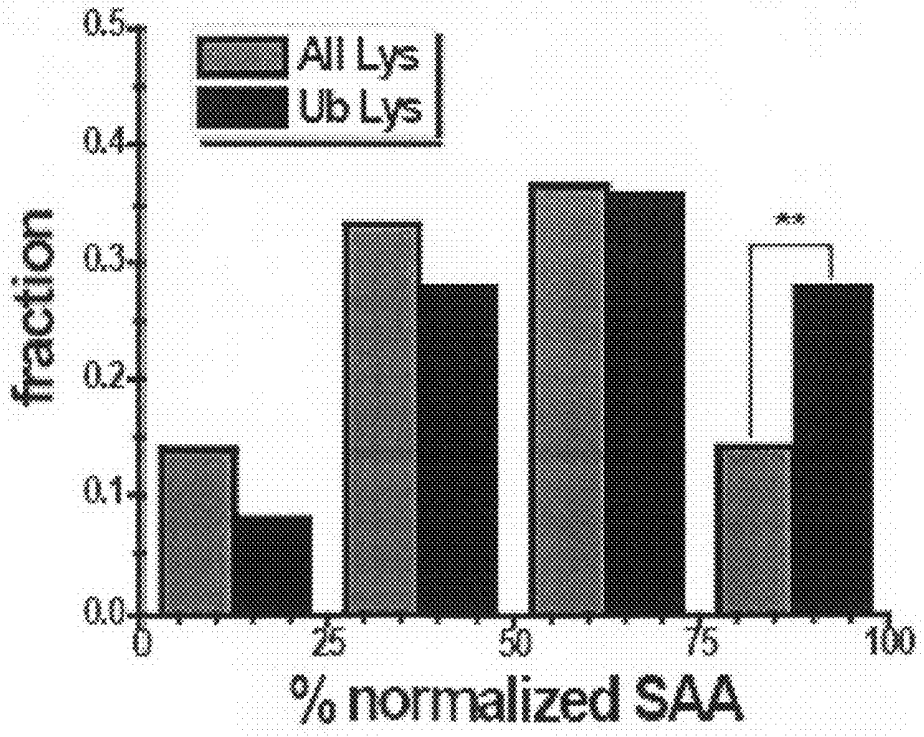

All Lys
Ub Lys
fraction
0.5
0.4
0.3
0.2
0.1
0.0
**
0
25
50
75
100
% normalized SAA

Fig.3C

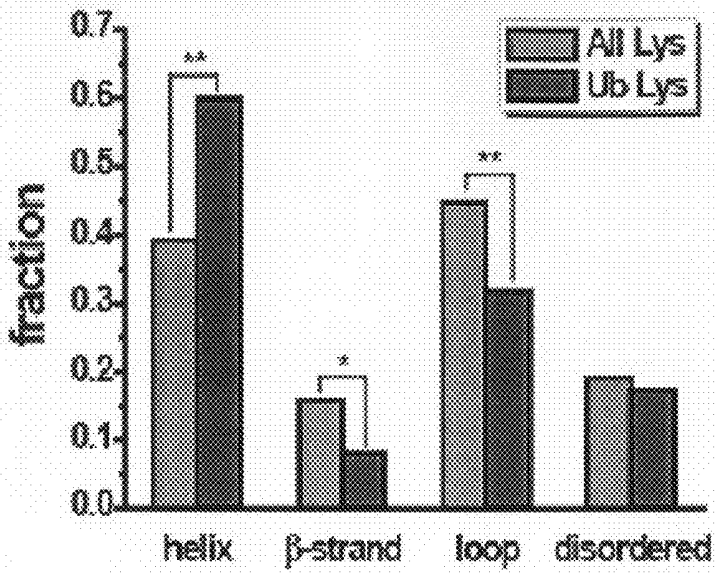

fraction
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
All Lys
Ub Lys
**
*
**
helix
β-strand
loop
disordered

Fig. 3D

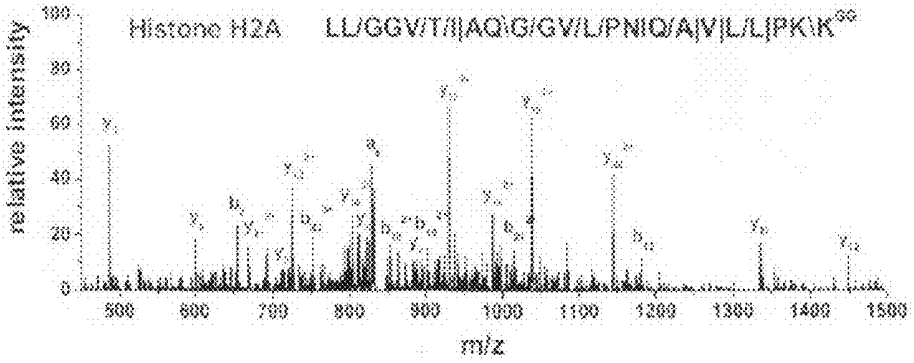

Histone H2A
LL/GGV/T/I|AQ\G/GV/L/PNIQ/A|V|L/L|PK\K^GG
relative intensity
m/z

Fig. 4

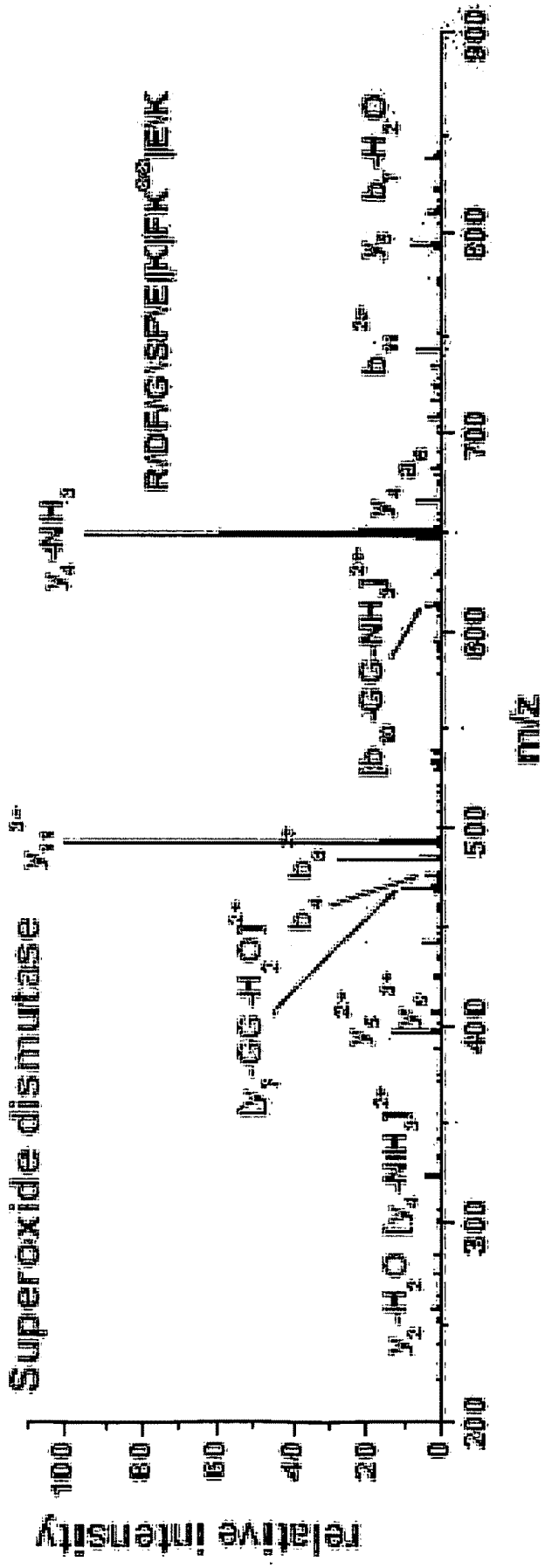

Superoxide dismutase
R/DFNGSPEIQFKEIK
relative intensity
m/z
0
20
40
60
80
100
200
300
400
500
600
700
800
900

Fig. 5A

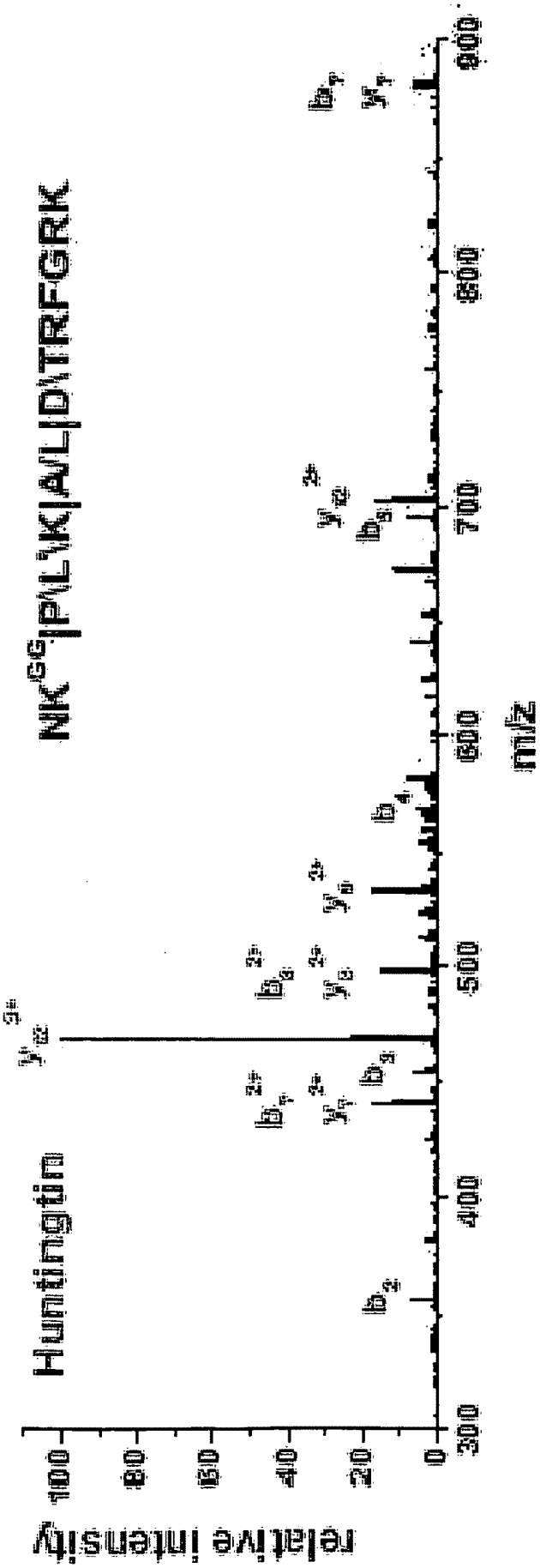

Huntingtin
NK^GG|P\L\K|A/L|D\TRFGRK
relative intensity
m/z
100
80
60
40
20
0
300
400
500
600
700
800
900

Fig. 5B

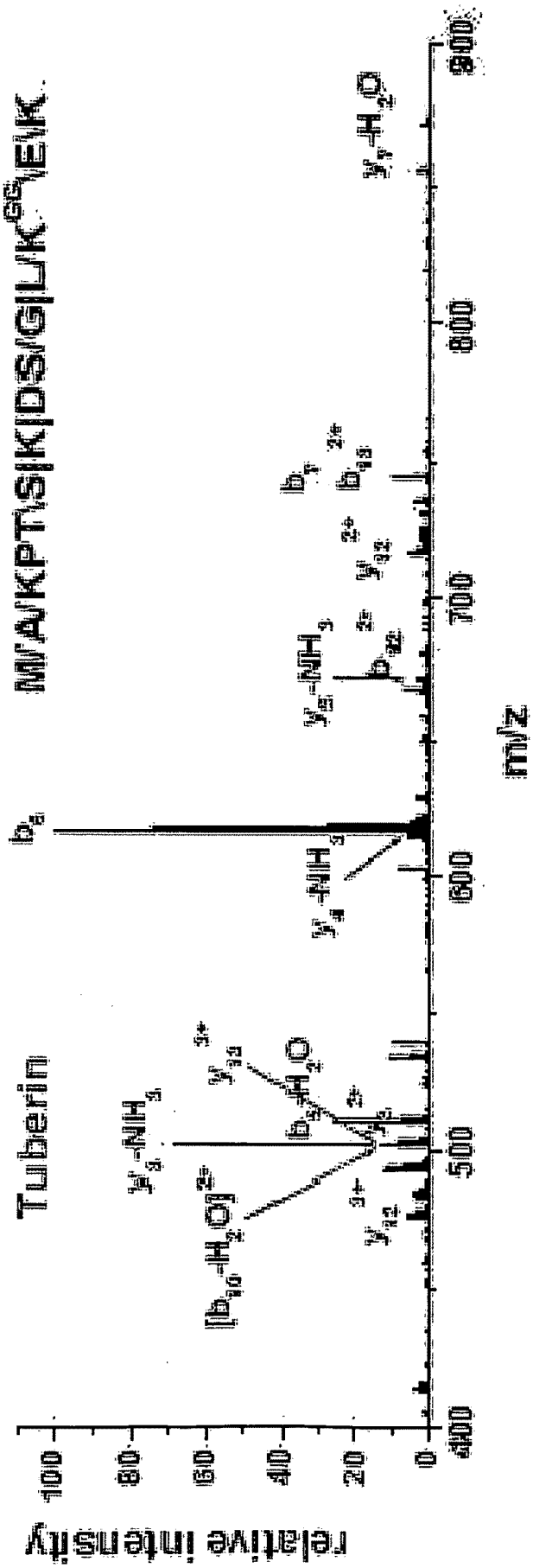

Tuberin
MAKPTSIKDSGLKSVEIK
relative intensity
m/z
100
80
60
40
20
0
400
500
600
700
800
900

Fig. 5C

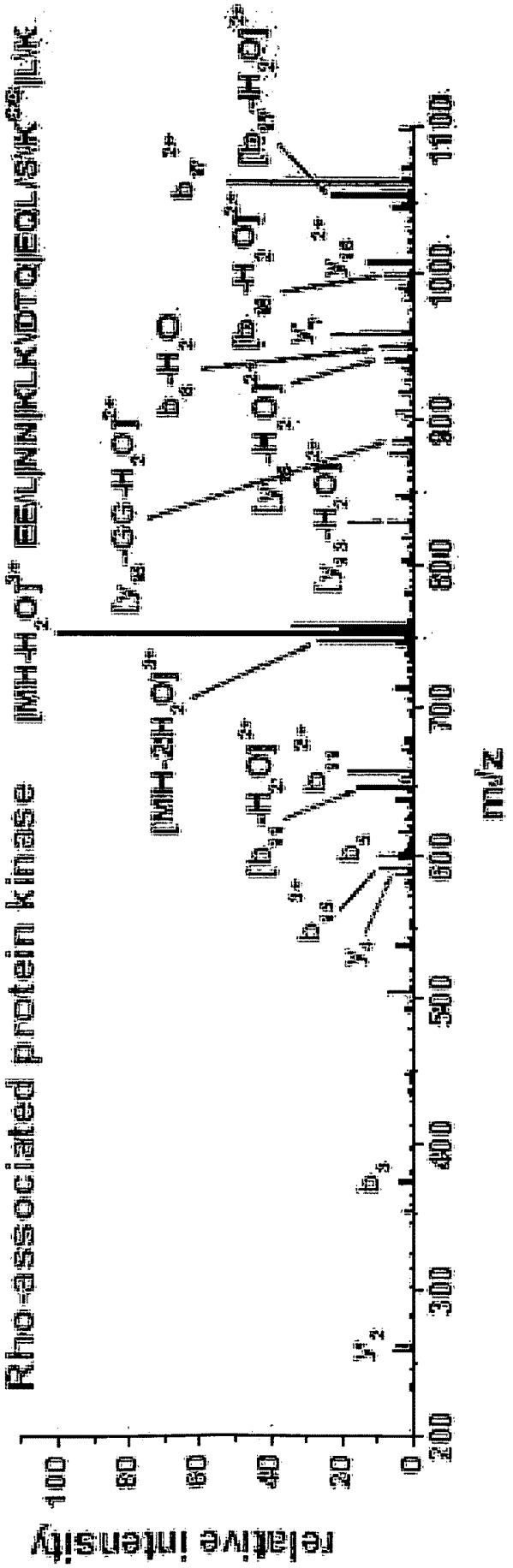

Rho-associated protein kinase
[MH+H2O]+ EELNNKLKVDTQEQLSHK2+LK
relative intensity
m/z
100
80
60
40
20
0
200
300
400
500
600
700
800
900
1000
1100

Fig. 5D

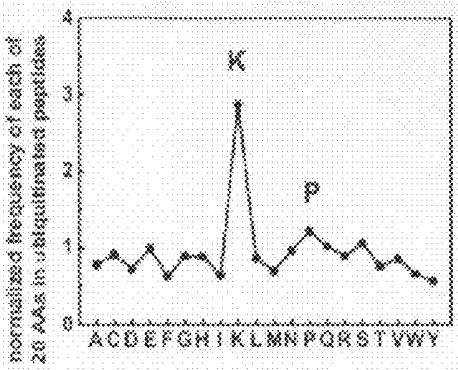


Fig. 6A

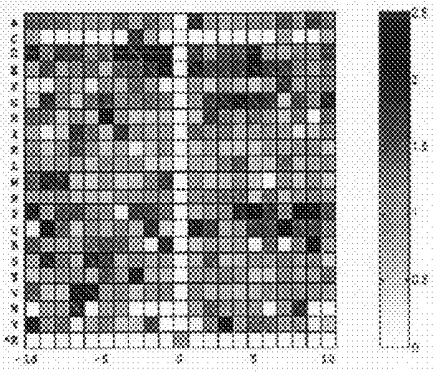


Fig. 6C

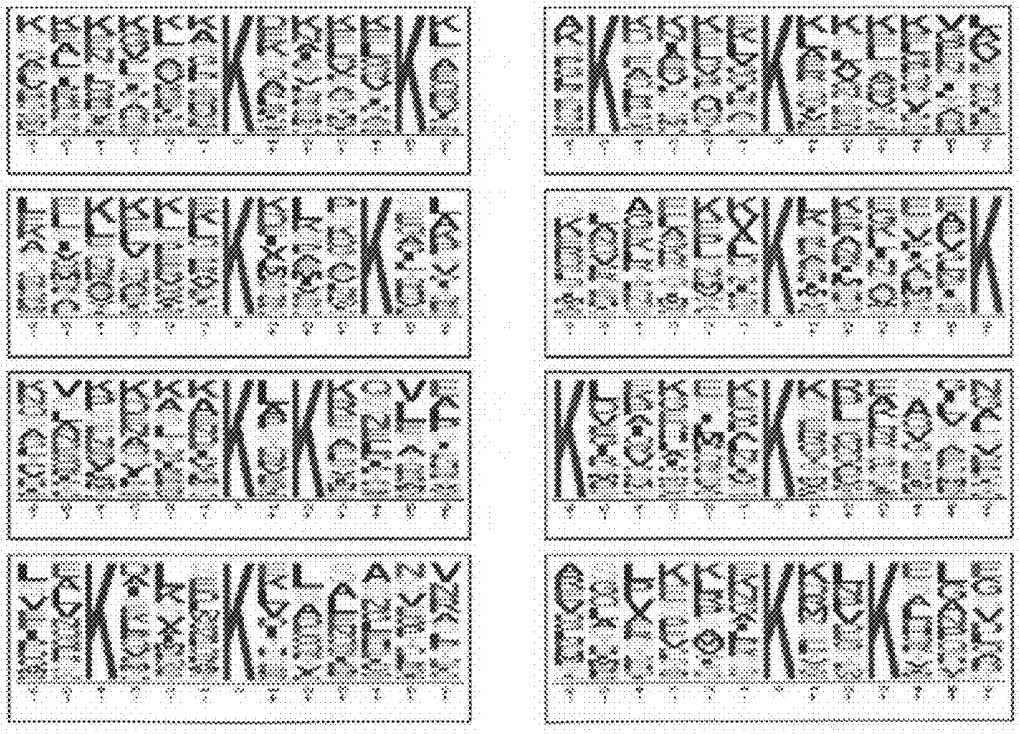


Fig. 6B

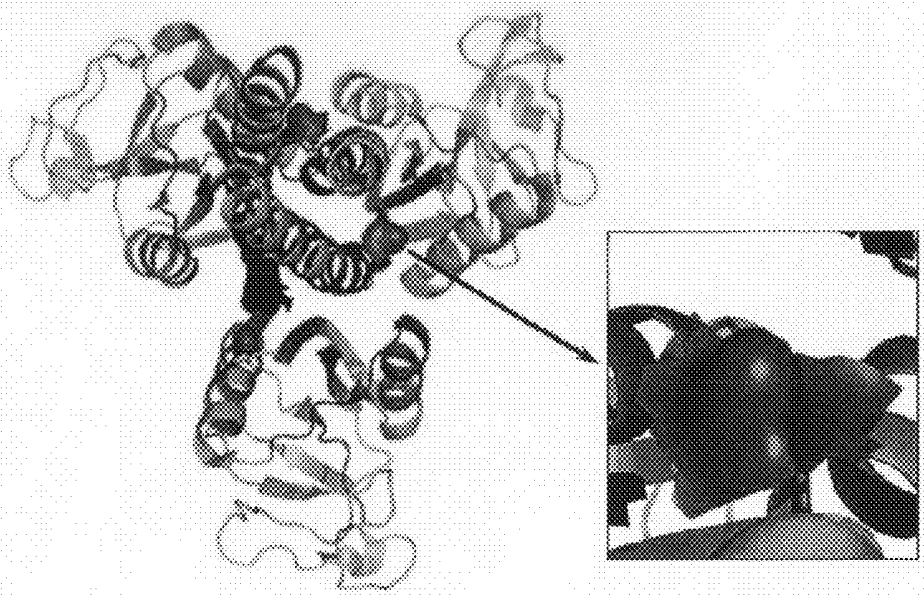


Fig. 7A

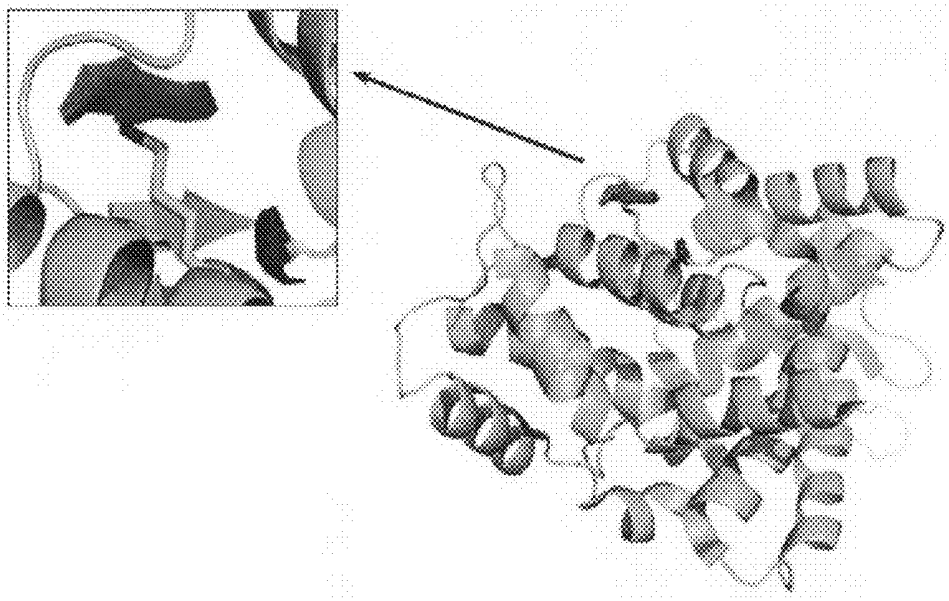


Fig. 7B

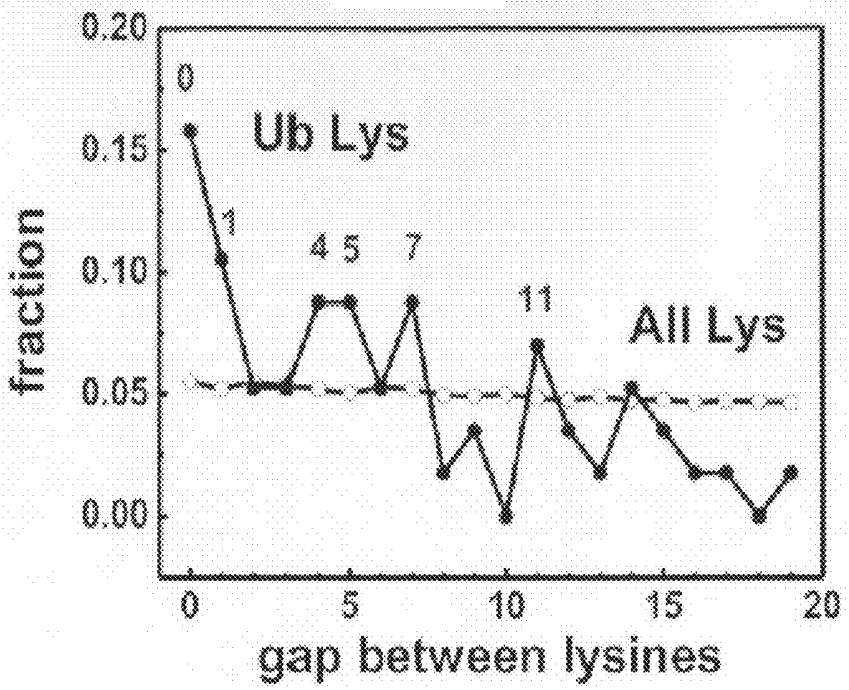

0.20
0.15
0.10
0.05
0.00
fraction
Ub Lys
All Lys
0
1
4 5
7
11
0
5
10
15
20
gap between lysines

Fig. 8A

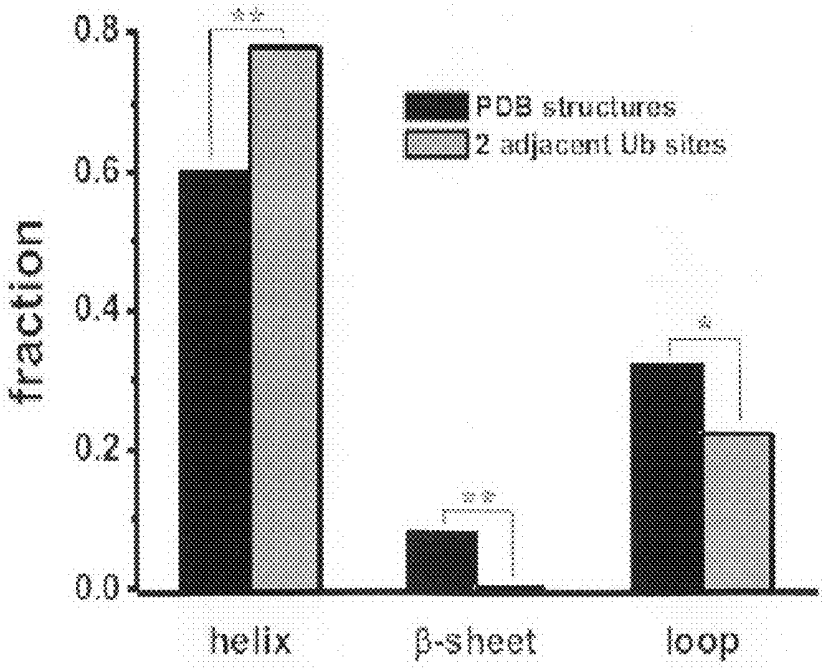

0.8
0.6
0.4
0.2
0.0
fraction
PDB structures
2 adjacent Ub sites
**
**
*
helix
β-sheet
loop

Fig. 8B

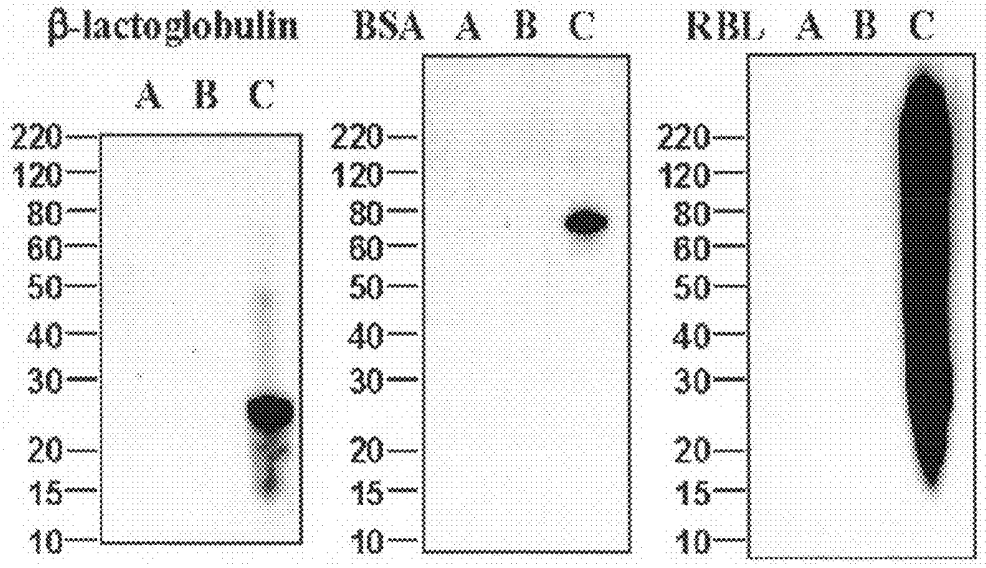


Fig. 9A Fig. 9B Fig. 9C

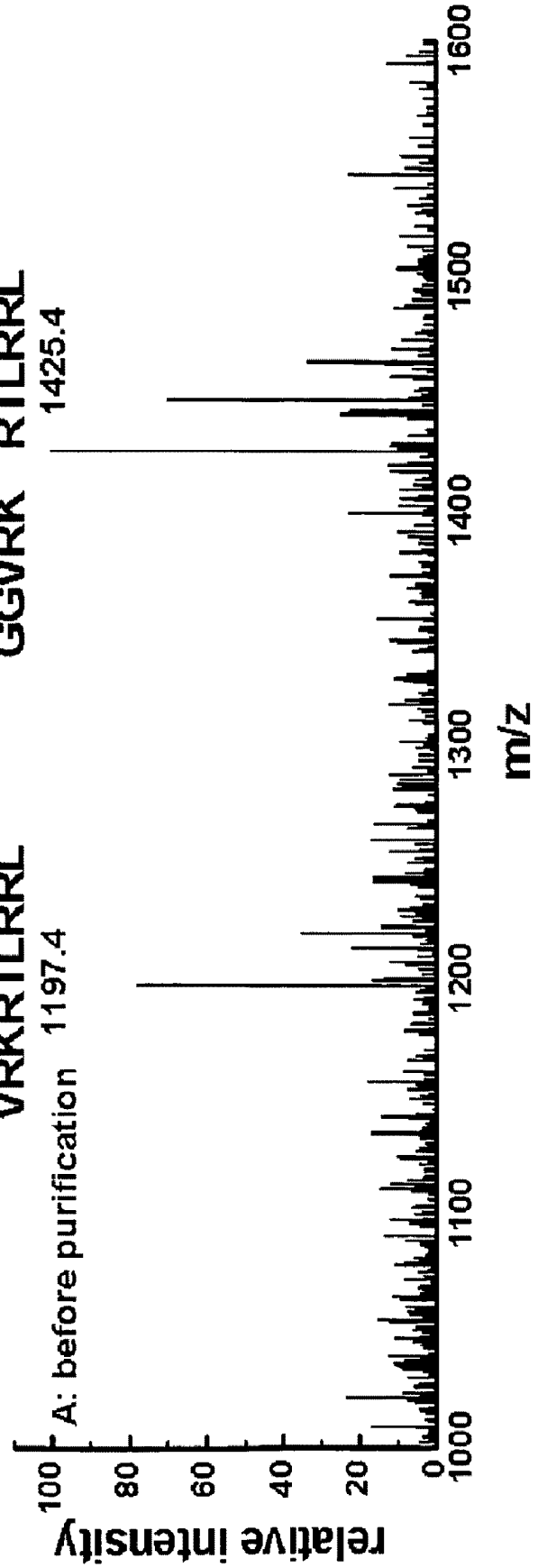

A: before purification
VRKRTLRRL
1197.4
GGVRK^GG RTLRRL
1425.4
relative intensity
100
80
60
40
20
0
1000
1100
1200
1300
1400
1500
1600
m/z

Fig. 10A

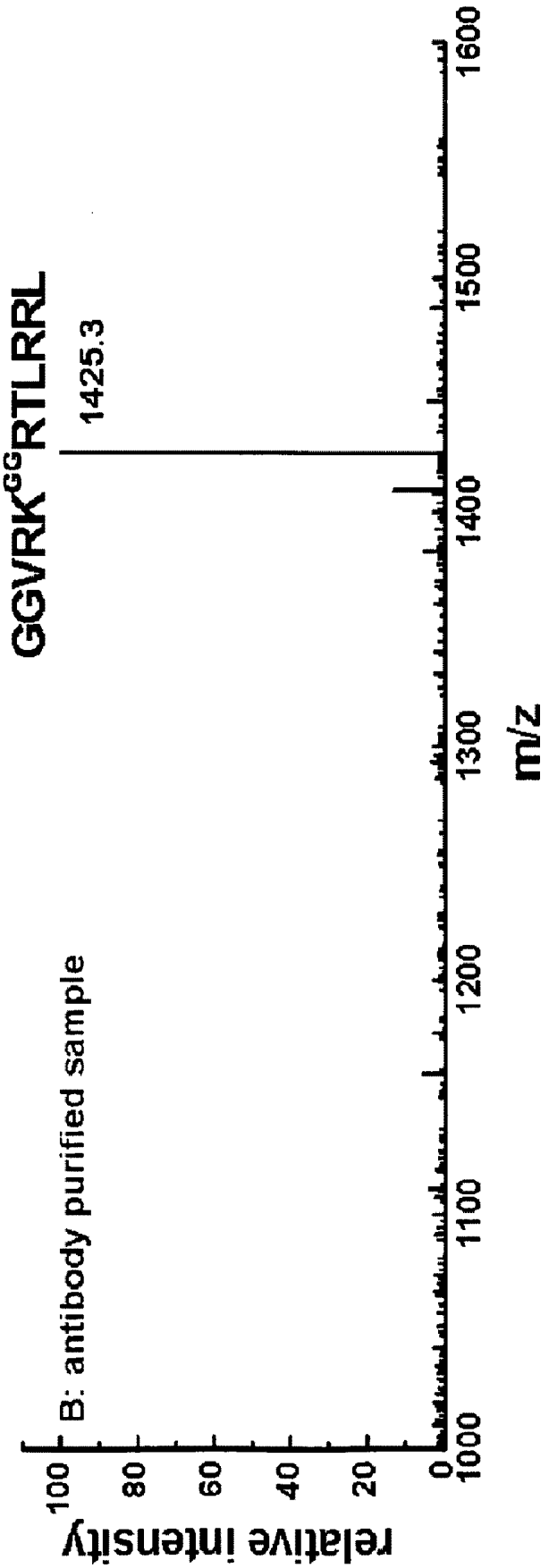

B: antibody purified sample
GGVRK^{GG}RTLRRL
1425.3
relative intensity
100
80
60
40
20
0
1000
1100
1200
1300
1400
1500
1600
m/z

Fig. 10B

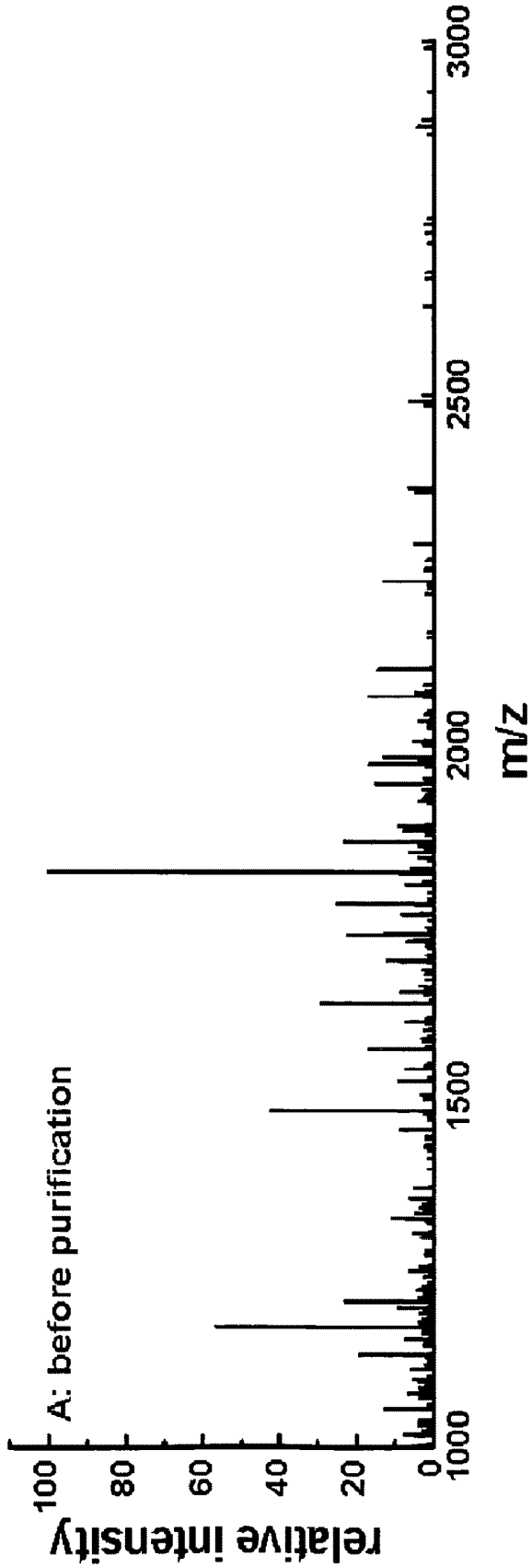

A: before purification
relative intensity
100
80
60
40
20
0
1000
1500
2000
2500
3000
m/z

Fig. 11A

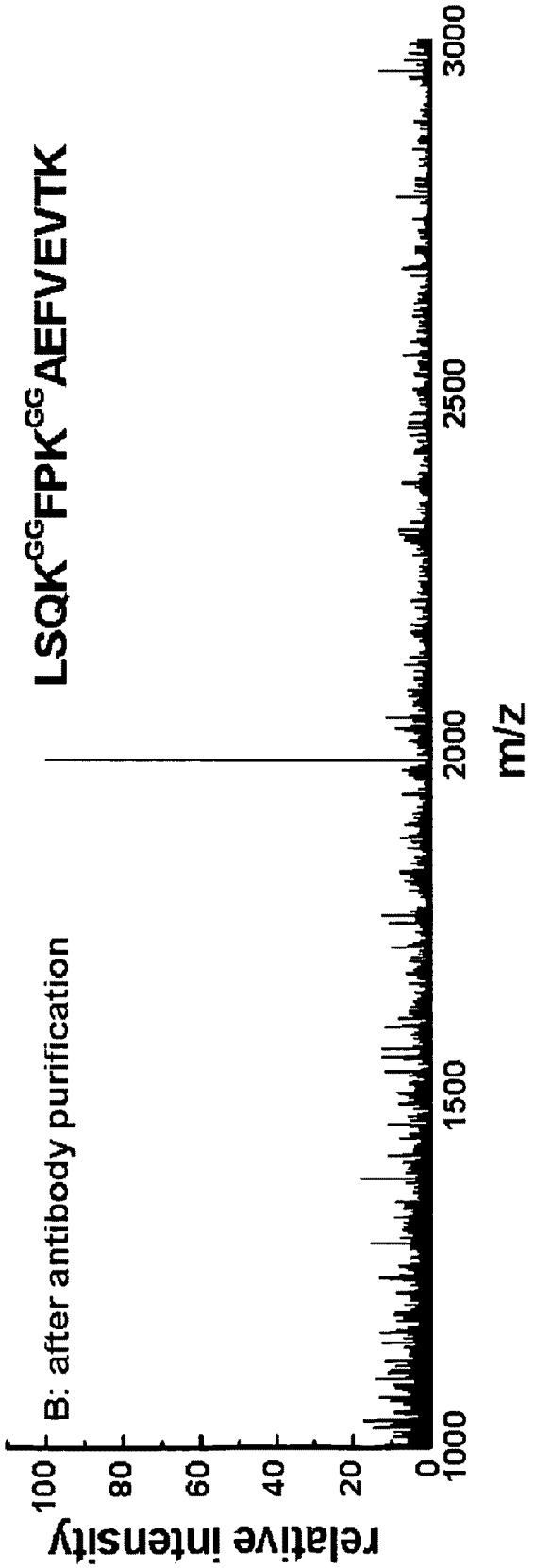

B: after antibody purification
LSQK^{GG}FPK^{GG}AEFVEVTK
relative intensity
100
80
60
40
20
0
1000
1500
2000
2500
3000
m/z

Fig. 11B

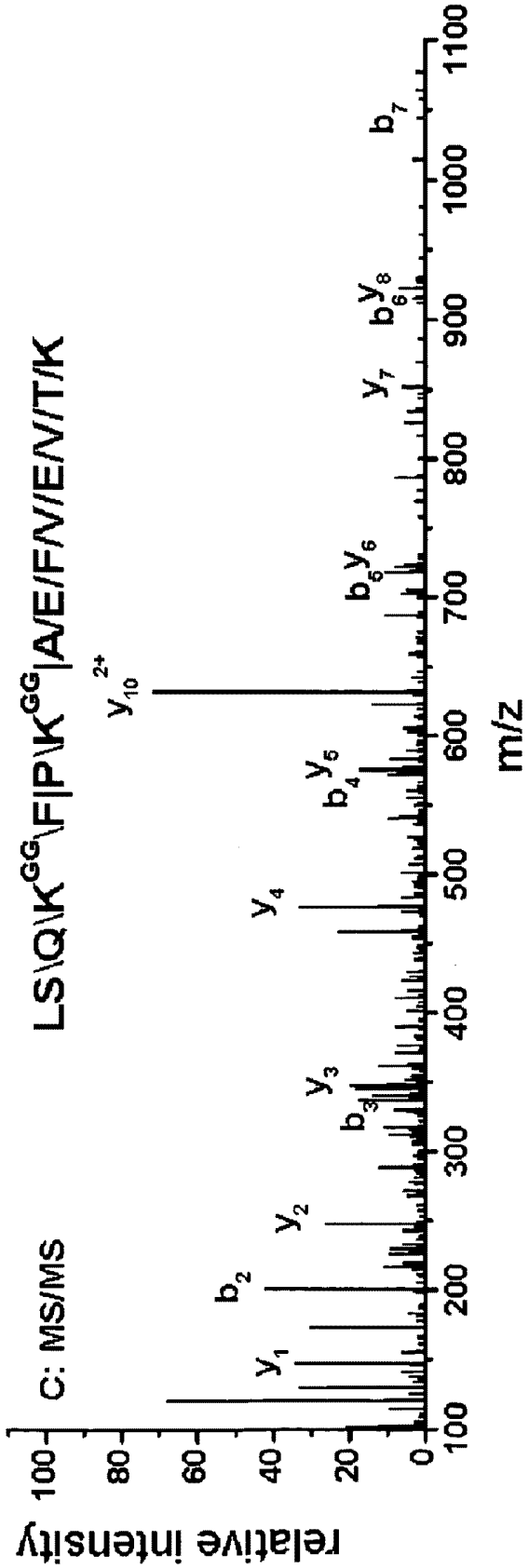

C: MS/MS
LS\Q\K^GG\F|P\K^GG|A/E/F/V/E/V/T/K
relative intensity
m/z
y1
b2
y2
b3
y3
y4
b4 y5
y10 2+
b5 y6
y7
b6 y8
b7
0
20
40
60
80
100
100
200
300
400
500
600
700
800
900
1000
1100

Fig. 11C

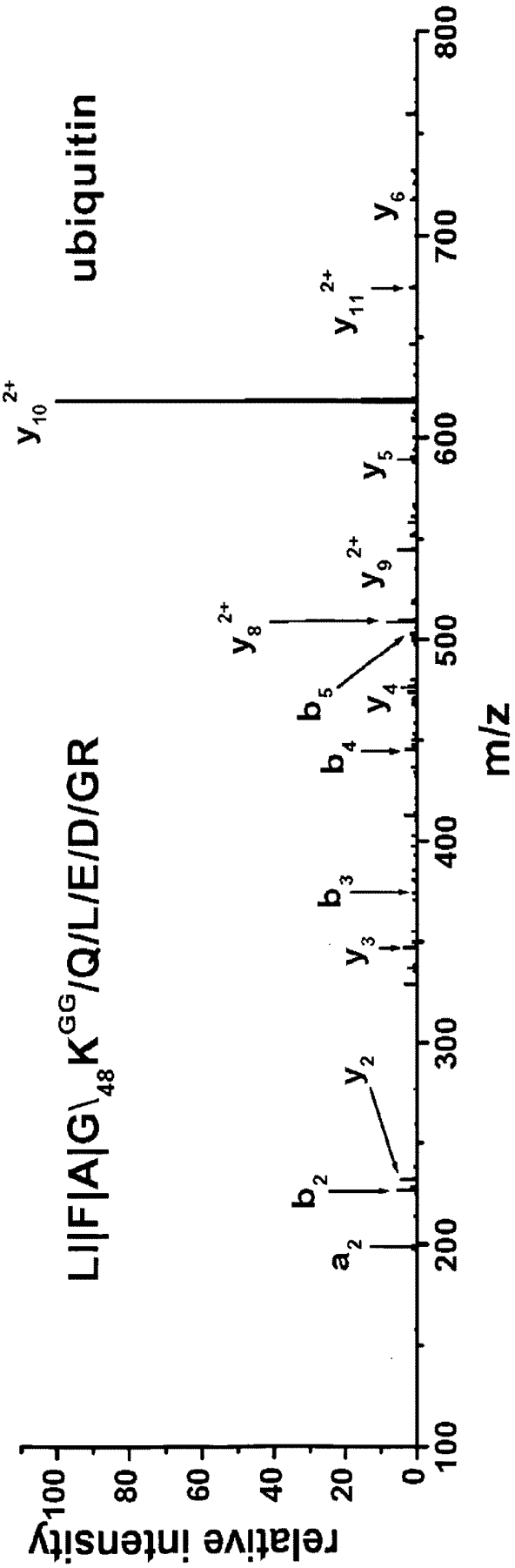

ubiquitin
LI|F|A|G\48KGG/Q/L/E/D/GR
relative intensity
m/z
100
80
60
40
20
0
100
200
300
400
500
600
700
800
a2
b2
y2
y3
b3
b4
b5
y4
y8 2+
y9 2+
y5
y10 2+
y11 2+
y6

Fig. 12

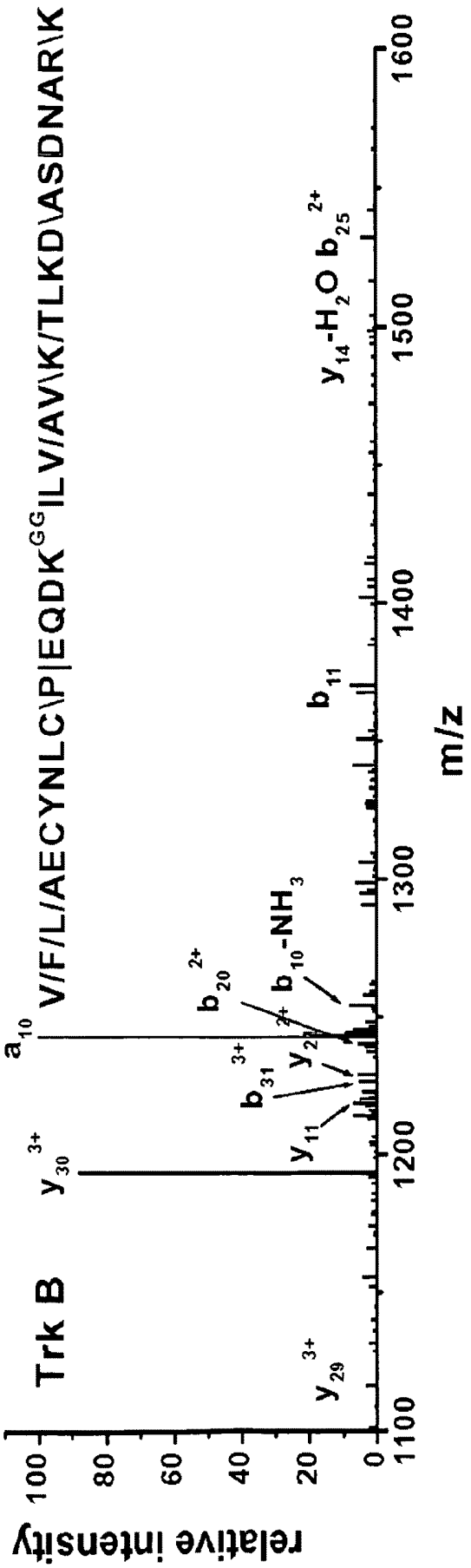
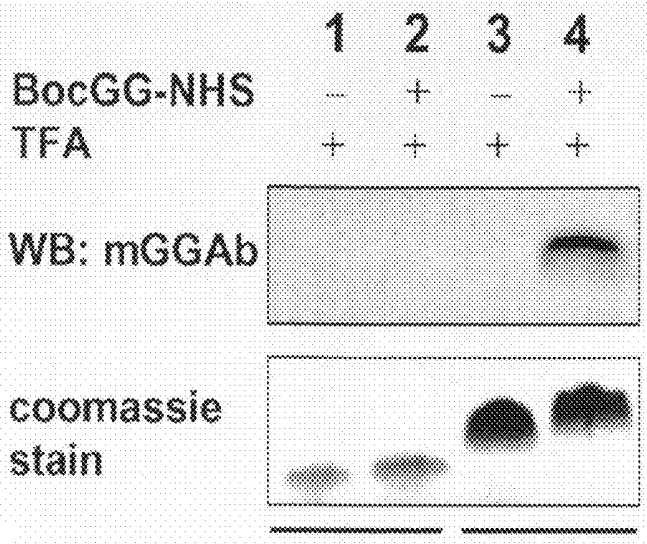
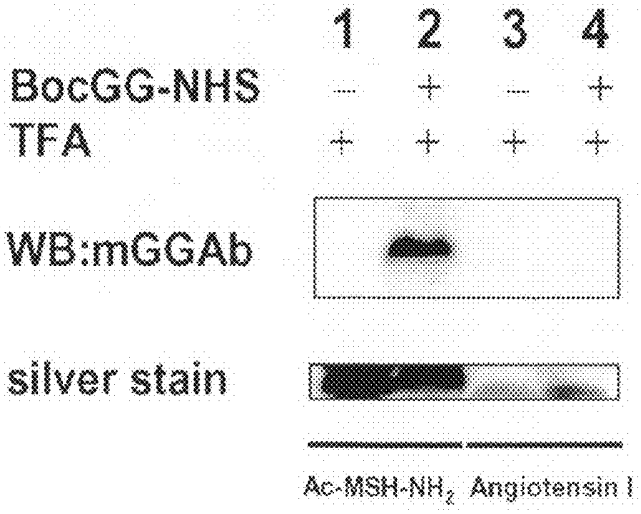

Trk B
V/F/L/AECYNLC\P|EQDK^GG ILV/AV\K/TLKD\ASDNAR\K
relative intensity
m/z
100
80
60
40
20
0
1100
1200
1300
1400
1500
1600
y29 3+
y30 3+
y31 3+
a10
y11
b31 3+
y28 2+
b20 2+
b10-NH3
b11
y14-H2O b25 2+

Fig. 13



K	0	0	5	5
GG-ε-K	0	0	0	5
N-term GG	0	1	0	1

Fig. 14A



K	1	1	0	0
GG-ε-K	0	1	0	0
N-term GG	0	0	0	1

Fig. 14B

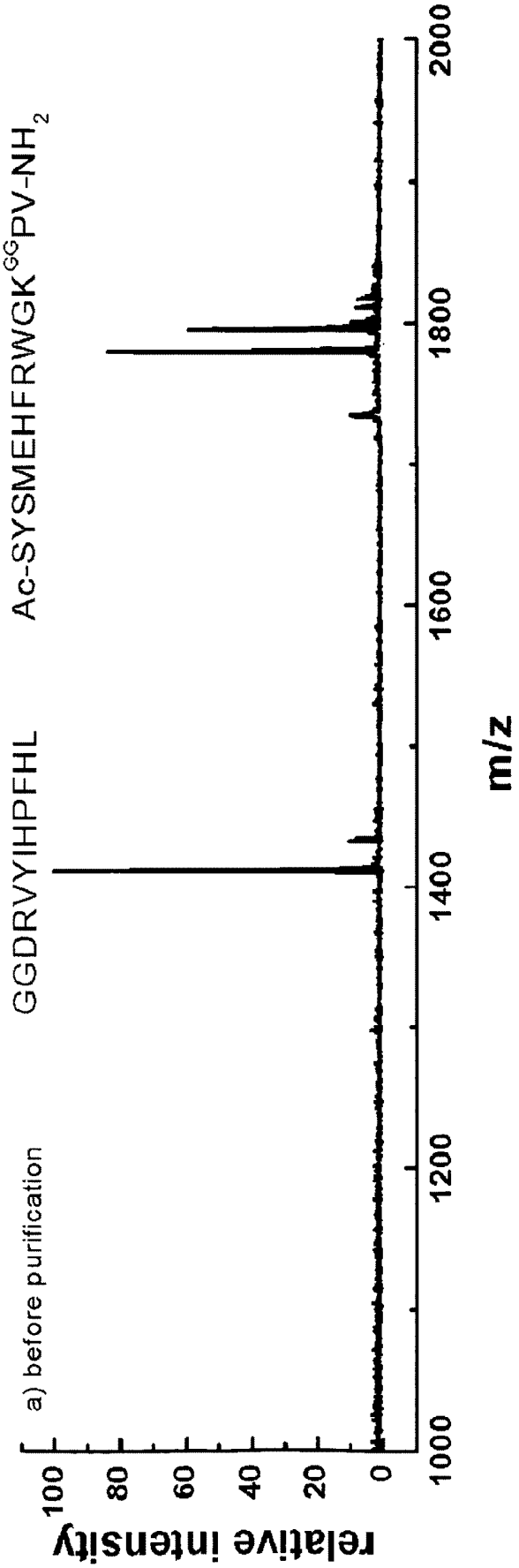

a) before purification
GGDRVYIHPFHL
Ac-SYSMEHFRWGK^{GG}PV-NH$_2$
relative intensity
100
80
60
40
20
0
1000
1200
1400
1600
1800
2000
m/z

Fig. 15A

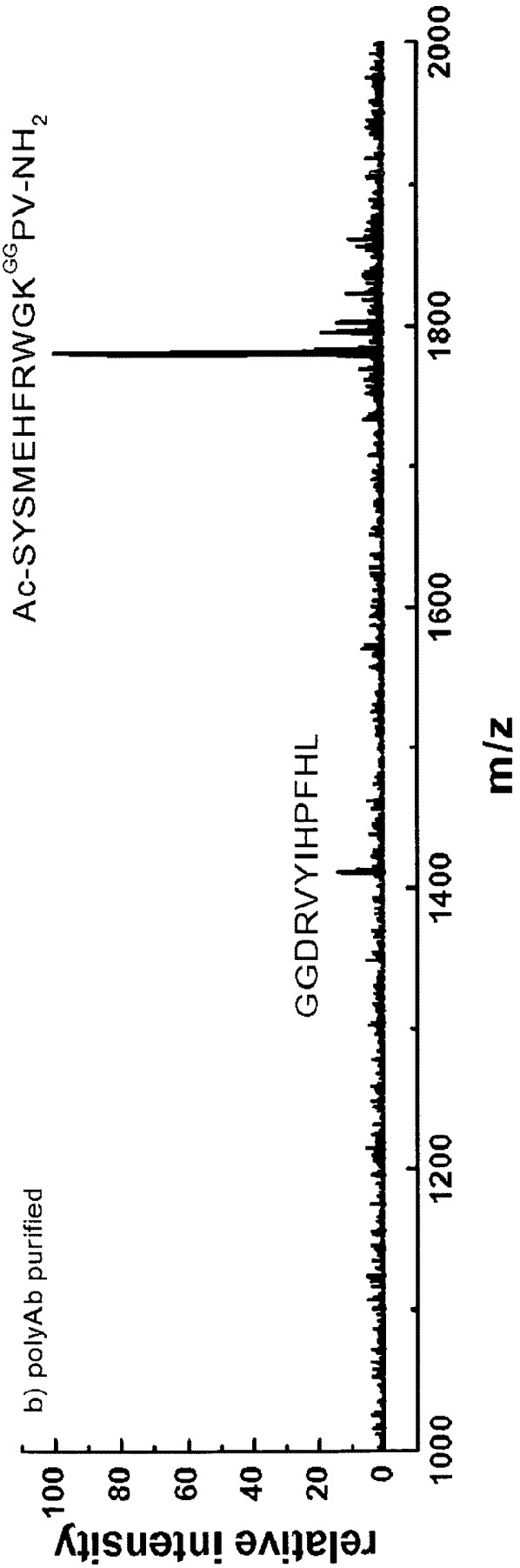

b) polyAb purified
relative intensity
100
80
60
40
20
0
1000
1200
1400
1600
1800
2000
m/z
GGDRVYIHPFHL
Ac-SYSMEHFRWGK^{GG}PV-NH$_2$

Fig. 15B

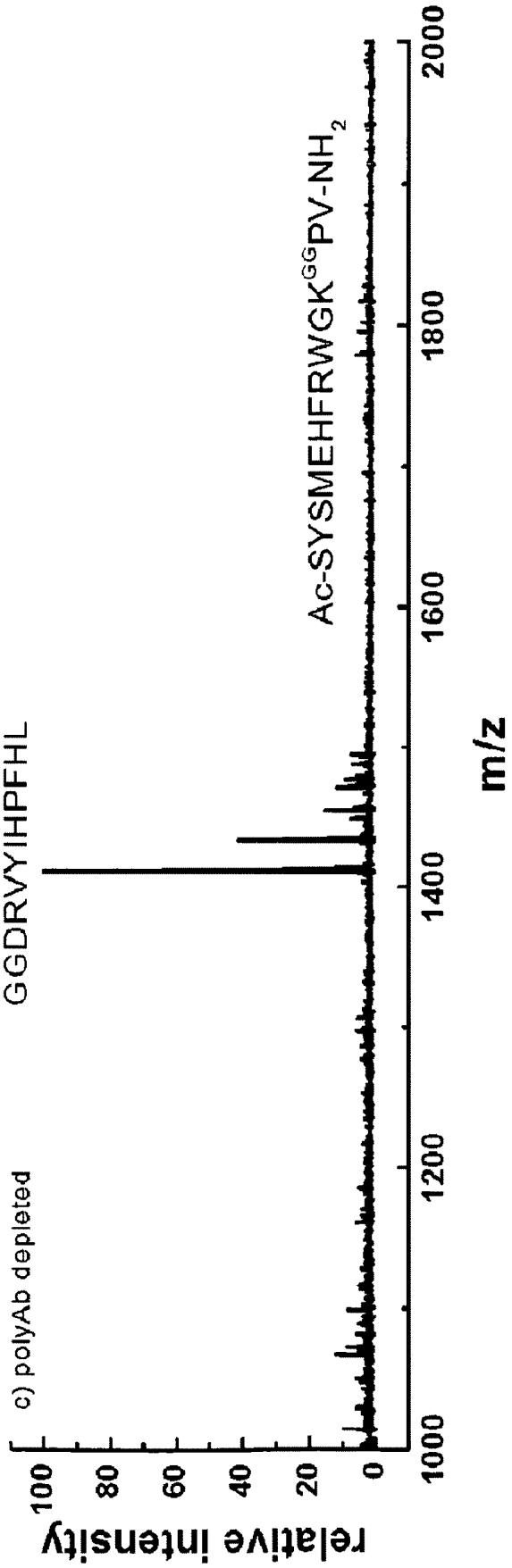
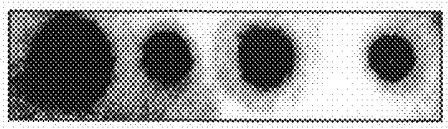

c) polyAb depleted
GGDRVYIHPFHL
Ac-SYSMEHFRWGK^{GG}PV-NH$_2$
relative intensity
100
80
60
40
20
0
1000
1200
1400
1600
1800
2000
m/z

Fig. 15C

GG-insulin 100 10 4 2 ng

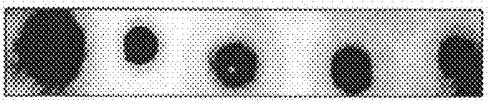
mGG Ab



GG-insulin 17 1.7 0.68 0.34 pmol

Fig. 16A

GG-insulin 100 10 4 2 1 ng



17 1.7 0.68 0.34 0.17 pmol

Fig. 16B

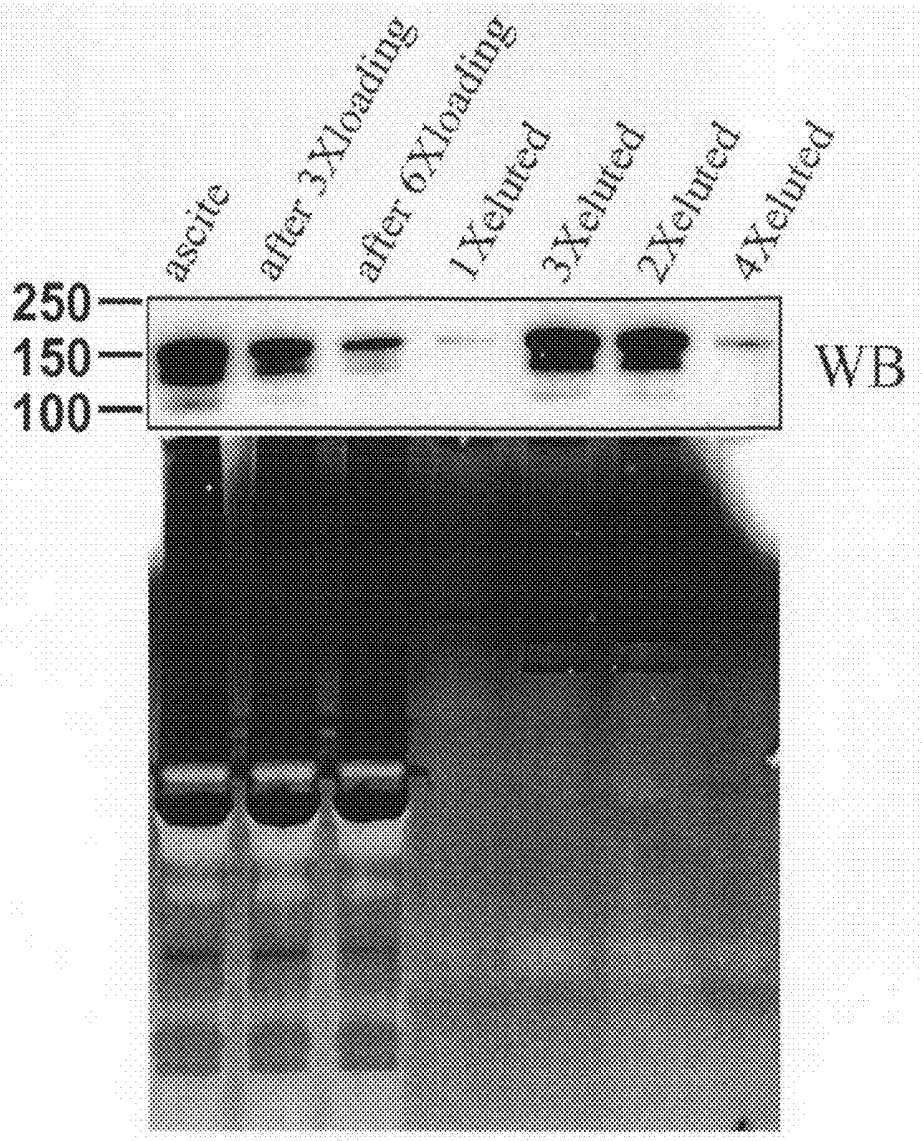


Fig. 17A

GG-beta lactoglubolin

0.1 0.01 μg

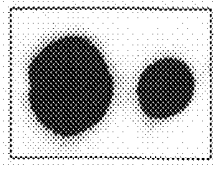


Fig. 17B

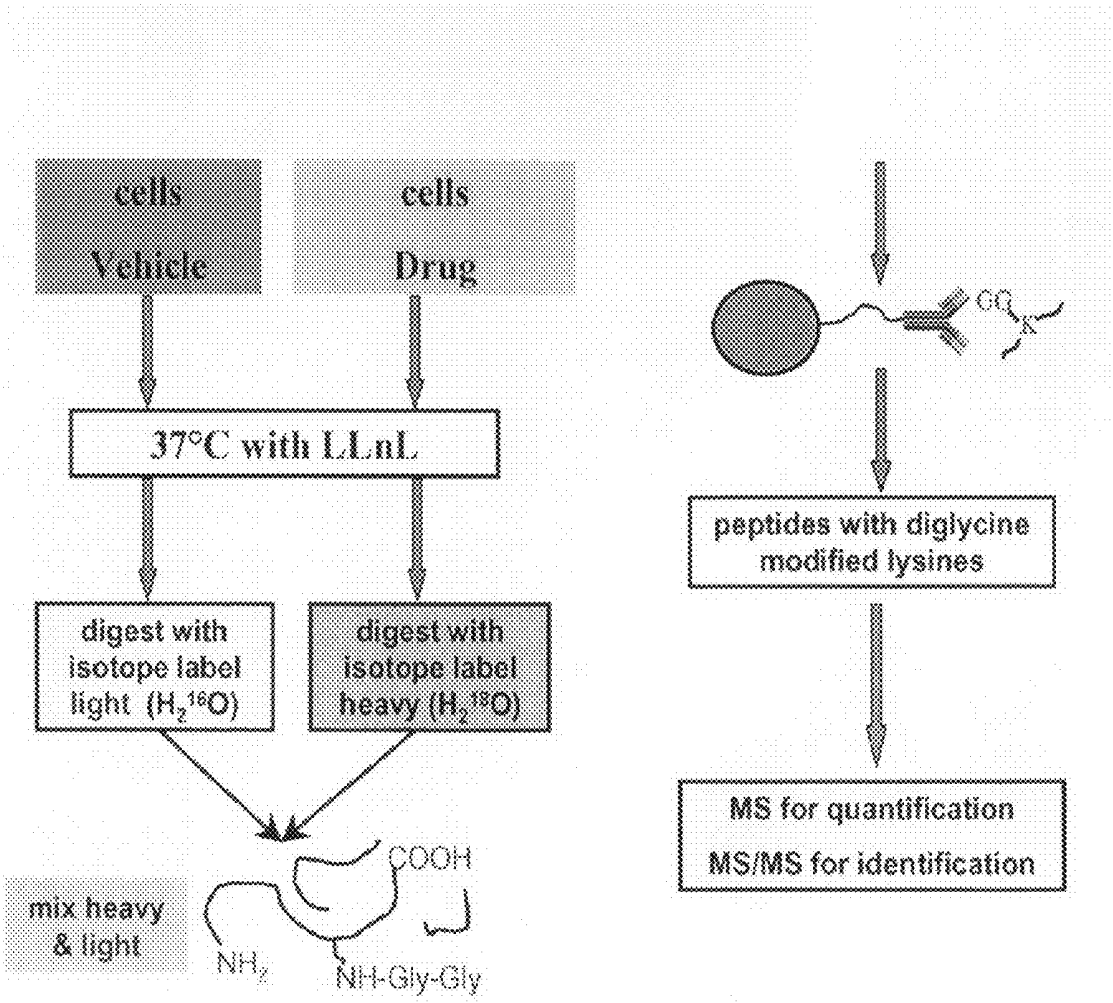

cells
Vehicle
cells
Drug
37°C with LLnL
digest with isotope label light ($H_2^{16}O$)
digest with isotope label heavy ($H_2^{18}O$)
mix heavy & light
COOH
NH_2
NH-Gly-Gly
peptides with diglycine modified lysines
MS for quantification
MS/MS for identification

Fig. 18

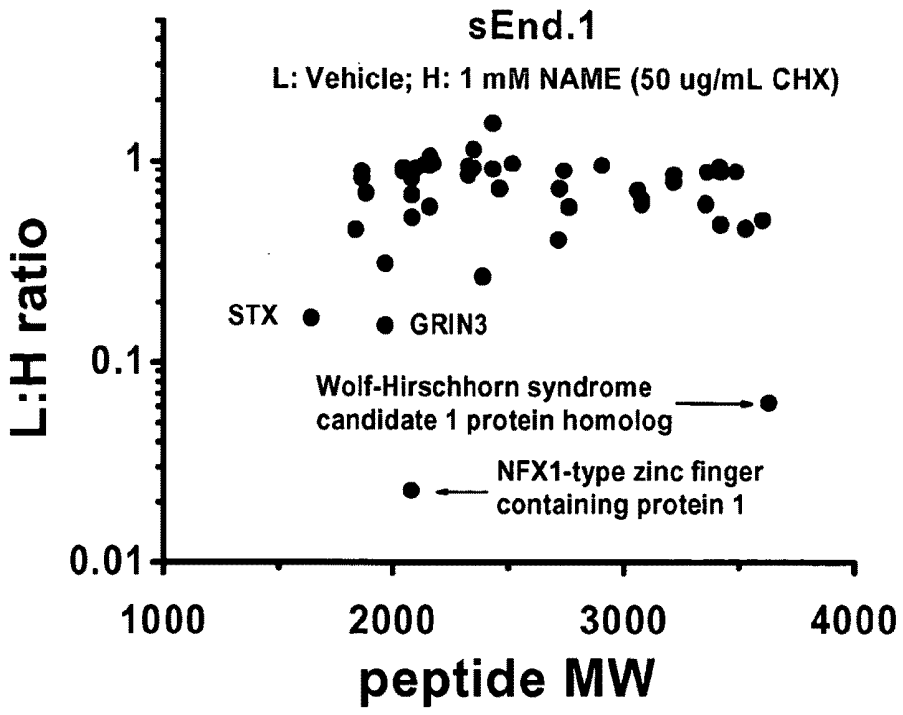

sEnd.1
L: Vehicle; H: 1 mM NAME (50 ug/mL CHX)
L:H ratio
1
0.1
0.01
STX
GRIN3
Wolf-Hirschhorn syndrome candidate 1 protein homolog
NFX1-type zinc finger containing protein 1
1000
2000
3000
4000
peptide MW

Fig. 19

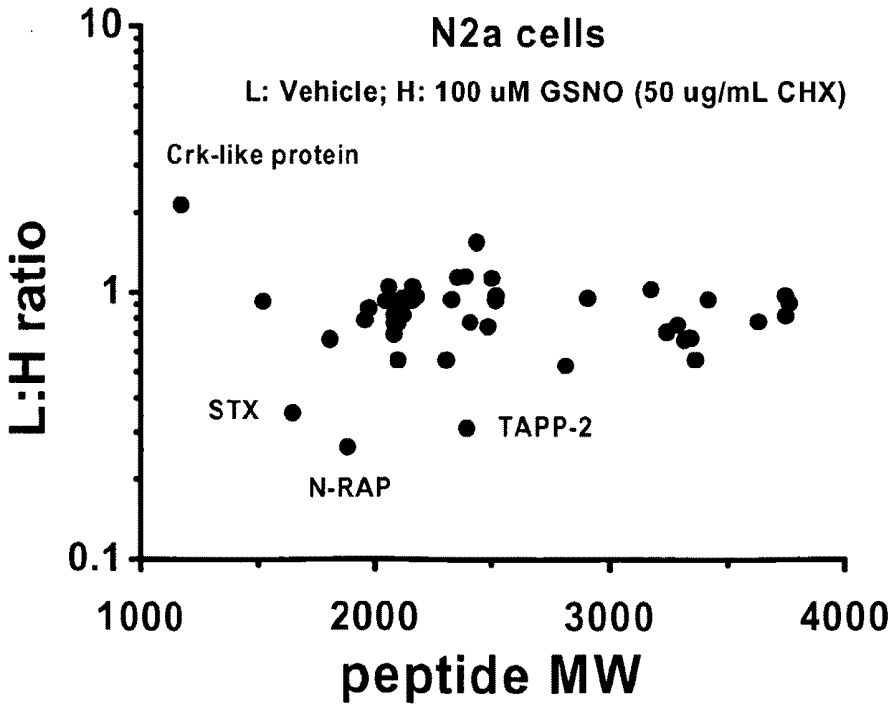

N2a cells
L: Vehicle; H: 100 uM GSNO (50 ug/mL CHX)
L:H ratio
10
1
0.1
Crk-like protein
STX
N-RAP
TAPP-2
1000
2000
3000
4000
peptide MW

Fig. 20

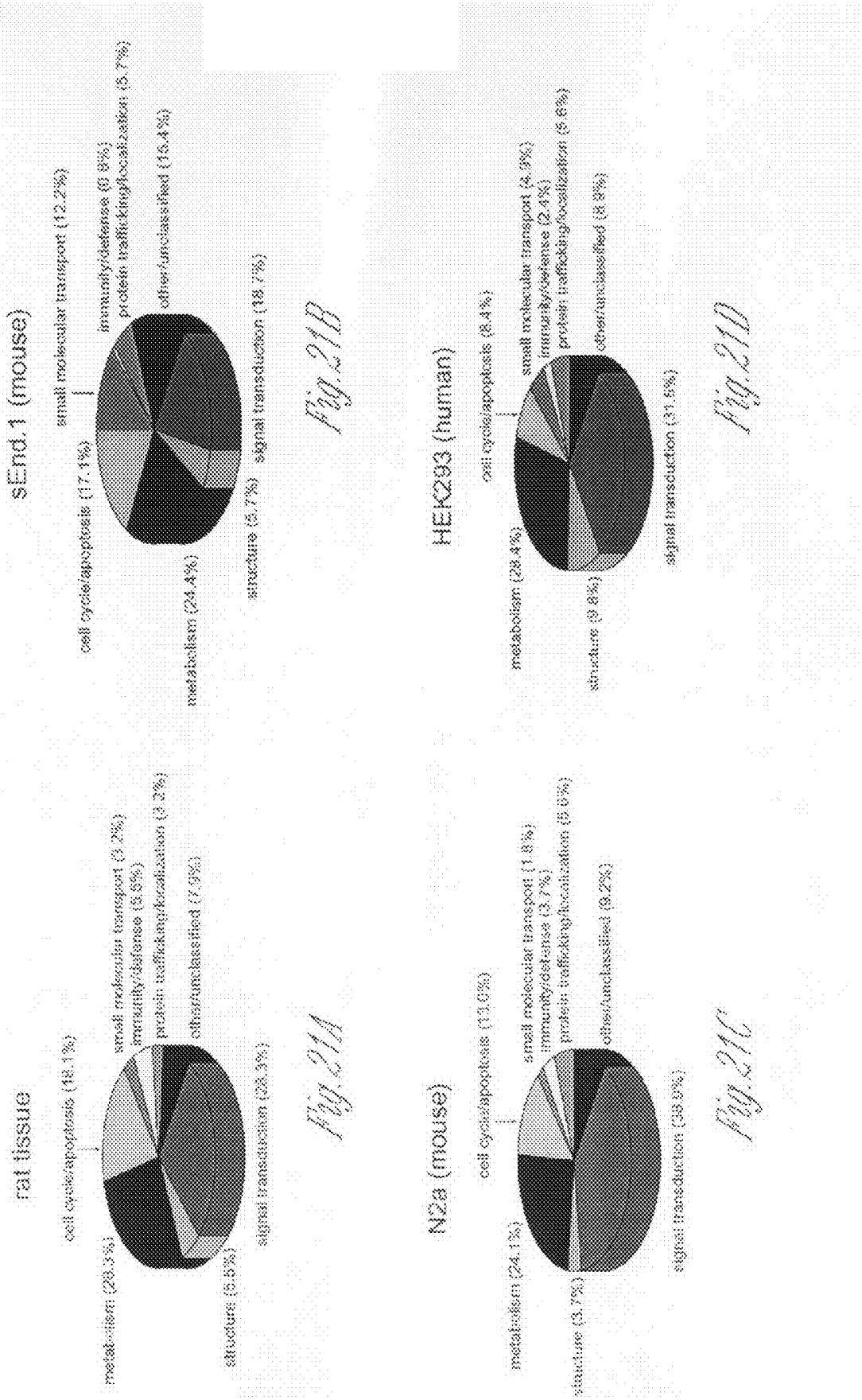


Fig.21A

Fig.21B

Fig.21C

Fig.21D

SEQ ID NO	Peptide Sequence Map	Ubiquitinated Lysines	z	Score	Accession	PROTEIN NAME	Fraction
1	(K)QNE/QA|K/E/MQQMV/k|L|EAE|MDR(R)	K1162k	3	13	Q15147	1-PHOSPHATIDYLINOSITOL-4,5-BISPHOSPHATE PHOSPHODIESTERASE BETA 4	HVW
2	(K)YHNGTkMV\SA|D\AY/K/I\I\PG\SRADFSEEYK(R)	K79k	3	12	P13233	2',3'-CYCLIC-NUCLEOTIDE 3'-PHOSPHODIESTERASE	BVM
3	(K)TP/V/GF/IGLGNM\GN/PMAkNL\M\K(H)	K56k	3	12	P31937	3-HYDROXYISOBUTYRATE DEHYDROGENASE, MITOCHONDRIAL [PRECURSOR]	HLW
4	(K)QYKKA\H\LGT\A/LkAN\P/FG\GASHA\K(G)	K37k	3	12	P62266	40S RIBOSOMAL PROTEIN S23	HLW
5	(K)YAKY\L/PH\S\AGR|Y\A/A/k(R)	K59k	3	13	P46782	40S RIBOSOMAL PROTEIN S5	HLW
6	(K)MDEVY/KVI/L\PNFLA/N\G\G\D\GFQMIk(D)	K512k	3	12	P21589	5'-NUCLEOTIDASE [PRECURSOR]	HLW
7	(R)L/K|VGL|Q\VVAVkA|PGFGDNR(K)	K301k	3	11	*P63039	60 KDA HEAT SHOCK PROTEIN, MITOCHONDRIAL [PRECURSOR]	BVS
8	(K)NK/TG\A\A\PIIDVVRS/GYYK/V\L\Gk\GK(L)	K114k	3	11	*P46776	60S RIBOSOMAL PROTEIN L27A	HLW
9	(R)DVYR/k\G|R|V\F/ANAP\D\S\A/CVIGLRK(K)	K696k	3	11	*P12382	6-PHOSPHOFRUCTOKINASE, LIVER TYPE	SLM
10	(K)ESQ/PEM/S/P/AL\HLMQNLD/Tk\SKL\RPK(R)	K776k	3	11	*Q9Y4K1	ABSENT IN MELANOMA 1 PROTEIN	HLW
11	(R)FGG/NKV/I\EkV|L\I\ANNGIA\AVkCM\R(S)	K119k K131k	3	10	*P11497	ACETYL-COA CARBOXYLASE 1	BVS
12	(R)K/L|G|L/EQQ/HkQEQ/IQEH\k(L)	K202k K210k	3	11	Q6AY33	ACROSIN-BINDING PROTEIN [PRECURSOR]	BVM
13	(R)KIK/ENEFSP\T|NSTSQ/T/VS\SG\A\TNGAE\S\k(T)	K2641k	3	10	*P25054	ADENOMATOUS POLYPOSIS COLI PROTEIN	HLW
14	(R)LQG/PKAG|QM/E\NTNN/FH/NL/YV\kR(H)	K284k	3	12	Q08462	ADENYLATE CYCLASE, TYPE II	HLW
15	(K)K/L|G|L/V/F/DDVVG\IVE/IINSRDVk(V)	K397k	3	12	Q08163	ADENYLYL CYCLASE-ASSOCIATED PROTEIN 1	BVC
16	(R)VVkLE/N\G/E\JETI/A|RFG\S\G\PCK(T)	K123k	3	12	Q9HDC9	ADIPOCYTE PLASMA MEMBRANE-ASSOCIATED PROTEIN	HLW
17	(R)LPIL/N/QP/STQ\IV/ANA\KGAVTG\Ak(D)	K116k	3	10	*Q99541	ADIPOPHILIN	HLW
18	(R)EAQKQA|S\Ek\V\SN\KG\K(R)	K759k	3	12	Q9UHB7	AF4/FMR2 FAMILY MEMBER 4	HLW
19	(K)LPYL/VEL\S/P/DGSDS\RD/K\P\kLYR(L)	K401k	3	11	*P55196	AFADIN	HLW
20	(R)Y/AEDKF/N\ET|T\E/kS\L\K(M)	K407k	3	13	P43652	AFAMIN [PRECURSOR]	HLW
21	(K)CV/YQSLYMGNEPTPTKS\L\S|k(I)	K173k	3	12	O75969	A-KINASE ANCHOR PROTEIN 3	HVW
22	(K)A/LNEEL/HL|QRIN|P|TTVK\Mk(S)	K925k	3	11	Q99996	A-KINASE ANCHOR PROTEIN 9	HLW
23	(K)IL|GTEKG|K|k\Nk(S)	K233k K235k	3	13	Q5XI97	ALANYL-TRNA SYNTHETASE DOMAIN-CONTAINING PROTEIN 1	LVN
24	(R)SN/E/S/L/kHN\I\QPAS\SK(W)	K76k	3	12	O35696	ALPHA-2,8-SIALYLTRANSFERASE 8B	SLC
25	(K)QHLPSSGNGKSF/KA/GGEPSPAQ\P|VC\k|ALDP\R(G)	K228k	3	11	Q9D4H4	ANGIOMOTIN-LIKE PROTEIN 1	SLW
26	(R)SSDQDS/T/NkEA/EAAG\VK\P|A\GVRPR(E)	K389k	3	11	Q92625	ANKYRIN REPEAT AND SAM DOMAIN PROTEIN 1	HLW
27	(K)A/EVV/NG/Y/E/A|KVY\T/VNNV\N\V\ITk(I)	K279k	3	14	Q8IZ07	ANKYRIN REPEAT DOMAIN PROTEIN 13	HLW
28	(K)A/G/DLA/SLK\K\A\FES/G\IP\VD\M/k(D)	K521k	3	13	Q9NU02	ANKYRIN REPEAT DOMAIN PROTEIN 5	HLW
29	(K)K/GLDIPAKKPPG|L\DPPF|K\D\D\K(K)	K1651k	3	11	Q6UB99	ANKYRIN REPEAT DOMAIN-CONTAINING PROTEIN 11	HLW
30	(R)G/LAGY/SG/YGSD|T\HAVFC/VG/R\PK\A\RESk(L)	K267k	3	11	Q8BXP5	ANKYRIN REPEAT DOMAIN-CONTAINING PROTEIN 33	SLW
31	(R)SGRS/A/L/HHAVHSGHL\ETVNLLL/NKGAS/LNVCDk\k(E)	K171k K172k	3	11	Q8BTI7	ANKYRIN REPEAT DOMAIN-CONTAINING PROTEIN 52	NLW
32	(R)IM/V/SR/S\E\V/D\M/LKI\RSEFkR(K)	K308k	3	13	*P07355	ANNEXIN A2	HLW
33	(R)VSSQNL/V/AI/PVYVkHNISF\k\ENS\SCGR(F)	K277k K283k	3	11	P53676	AP-3 COMPLEX SUBUNIT MU-1	BVM
34	(K)LIDVI\SMYR/ELLKD\L\S\k(E)	K4278k	2	11	*P04114	APOLIPOPROTEIN B-100 [PRECURSOR]	HLW
35	(K)VQ/TDKG/TE|V\AT\N\LVILCTG/I\KI\NSSAYRk(A)	K254k	3	11	Q9BRQ8	APOPTOSIS-INDUCING FACTOR 2	HVW
36	(K)QVGVN/PTSIDS|V|VIGk\DQEVK(L)	K89k	3	11	Q7Z2E3	APRATAXIN	HLW
37	(R)LGPMA|L|AFKLRG|L/VD\Rk\R(S)	K230k	3	11	Q2KMM4	ARACHIDONATE 12-LIPOXYGENASE, 12R TYPE	BVC
38	(K)SNN/I/F/L/HEGL\TVKIGDF\GLA\TV\k\TR(W)	K452k	3	11	P04627	A-RAF PROTO-ONCOGENE SERINE/THREONINE-PROTEIN KINASE	NLW
39	(R)GG/V/EkGPAA\L\Rk/AGL|VEK|L\K(E)	K26k K33k	3	12	P07824	ARGINASE-1	BVS
40	(K)G/K/D/KEVH/SI\KR/G\DSGNIk(A)	K171k	3	13	Q9DBU6	ARGININE/SERINE-RICH COILED COIL PROTEIN 1	NLW
41	(K)G/K/D/KE/VHSIkRG\DSG\NIK(A)	K163k	3	11	Q9DBU6	ARGININE/SERINE-RICH COILED COIL PROTEIN 1	NLW
42	(R)VVP/L/D/EIK\EA\ANAANI\H\SFIE\G\L\P\E\K(Y)	K681k	3	13	Q2M3G0	ATP-BINDING CASSETTE SUB-FAMILY B MEMBER 5	HLW
43	(R)YD/SSLk/PVL/K\HVNAL\I\S\PGQk(I)	K1359k K1374k	3	13	*Q09429	ATP-BINDING CASSETTE TRANSPORTER SUB-FAMILY C MEMBER 8	LVC

Fig. 22A

44	(K)GKTT/IKT/G\AS/V/LNk/W/QMNP\YDR(G)	K90k	3	10	*Q91VR5	ATP-DEPENDENT RNA HELICASE DDX1	SLW
45	(K)HGA/ITN/T/K/VQ\YRVSATDL\P\PE\LP\k(A)	K704k	3	12	*P41182	B-CELL LYMPHOMA 6 PROTEIN	HLW
46	(K)V/F/L/AECYNLCP|EQDkILVAV\K/TLKD\ASDNAR\K(D)	K565k	3	9.9	*P15209	BDNF/NT-3 GROWTH FACTORS RECEPTOR [PRECURSOR]	NLW
47	(R)SVMQ/kYLE\DRG|EV\T\F\E\K(I)	K45k	3	11	*P25098	BETA-ADRENERGIC RECEPTOR KINASE 1	HLW
48	(K)Nk|P/RS/P\VV/E/LSKVPLV\QRGSANGL(-)	K667k	3	10	*P25098	BETA-ADRENERGIC RECEPTOR KINASE 1	HLW
49	(R)k/SNPG|W/E/NL\EK(L)	K142k	3	12	Q96T60	BIFUNCTIONAL POLYNUCLEOTIDE PHOSPHATASE/KINASE	HLW
50	(K)AMESAEQK/EQ|G|LS/RDVTTVW\KLR\IV\SYS\k(K)	K2979k	3	11	*P51587	BREAST CANCER TYPE 2 SUSCEPTIBILITY PROTEIN	HLW
51	(R)k/AD/TT/T/PTT/IDPIHEPP\SLPPEPK\T\T\K(L)	K291k	3	11	O60885	BROMODOMAIN-CONTAINING PROTEIN 4	HLW
52	(K)MVE/L/KA\kVEQFEk|L\S|DK(L)	K4193k K4199k	3	11	Q91ZU6	BULLOUS PEMPHIGOID ANTIGEN 1, ISOFORMS 1/2/3/4	SLM
53	(K)G/TQKP/YALKV|L|KKTV|Dk\K(I)	K71k K79k	3	11	P13234	CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE TYPE IV	BVS
54	(K)S/QEG/KPKE\H\TEPK|SL|P\k(Q)	K68k	3	12	P20810	CALPAIN INHIBITOR, CALPASTATIN	HLW
55	(K)k|R/DK\E\LG\L/GRHE\N\A\I\K(Y)	K21k	3	11	O35350	CALPAIN-1 CATALYTIC SUBUNIT	SLW
56	(R)KLGA/S/L/FTI/GF\AIY/E/VP\K\EM\HGN\k(Q)	K517k	3	12	P20807	CALPAIN-3	HLW
57	(K)WAFNLY/DI/N|KDGYIT/K|E/E\M\LAIMk(S)	K192k	3	12	Q9Y2W7	CALSENILIN	HLW
58	(R)SLHAA/AVLL/L\V\IL/kE/QP/S\S\P\A\P\VNGSK(W)	K18k	3	12	O43570	CARBONIC ANHYDRASE XII [PRECURSOR]	HLW
59	(R)VVQk/N/LVFV\VGL/S\Q/R/LA/D\PE\VL\K(R)	K108k	3	12	*O95628	CCR4-NOT TRANSCRIPTION COMPLEX SUBUNIT 4, E3 UBIQUITIN-PROTEIN LIGASE CNOT4	HLW
60	(K)VFk/QP/SK|PEI/VN/KA|PFLE\T\DQLKK(L)	K132k	3	11	*Q61490	CD166 ANTIGEN [PRECURSOR]	NLW
61	(R)SVH|L|K/EG|E\Q\HVDD\VSSA\LkR(F)	K792k	3	13	Q96P48	CENTAURIN DELTA 2	HLW
62	(K)KA/AECKVD\S/IGSGRAI\P|I/kQGILL\K(R)	K370k	3	12	Q96P47	CENTAURIN-GAMMA 3	HLW
63	(K)ISEk/TIQISDIQK\D\LDK|S|k(D)	K1950k K1965k	3	12	Q02224	CENTROMERIC PROTEIN E	HVW
64	(K)QFSkD/T/YSK/P\S\ISGIESDDH|CQ\R\EQELQK(E)	K2038k	3	11	O15078	CENTROSOMAL PROTEIN CEP290	HLW
65	(K)AG/V/S/AE/PTTRTY\D\LNKPPE/FSF\Ek\AR(V), (R)TY/DLN/K\P/PEF/SF/EkA\RVR(K)	K146k	3	12	Q13237	CGMP-DEPENDENT PROTEIN KINASE 2	HVW
66	(K)NTk/QQN/NAA/L/E|RGL\T\k\A\LK(K)	K289k K302k	3	12	Q9NZA1	CHLORIDE INTRACELLULAR CHANNEL PROTEIN 5	HLW
67	(R)ESN\T\A|G\DIFS/K/FSAYI\KNT\k(Q)	K130k	3	11	*Q8BXK9	CHLORIDE INTRACELLULAR CHANNEL PROTEIN 5	SLM
68	(K)V/I/D/KGAGGG\G\AGQ\G\AGAL\A\R/PkVPS\R(N)	K201k	3	14	O95503	CHROMOBOX PROTEIN HOMOLOG 6	HLW
69	(K)E/L/QV/AND/KA\DMV|LkE\VT/M\K(A)	K3243k	3	11	Q8TE73	CILIARY DYNEIN HEAVY CHAIN 5	HLW
70	(R)YSI/PSH\RT\TQ/k/SY\HT\GK(K)	K207k	3	11	P56748	CLAUDIN-8	HLW
71	(R)Rk/DKET\N\S|E\GL\V/NkTR(E)	K1291k K1303k	3	12	Q06194	COAGULATION FACTOR VIII [PRECURSOR]	SLM
72	(K)E/I/L/TkM/RYF|SNI\E\EK\I\K(L)	K217k	3	12	Q8IWL3	CO-CHAPERONE PROTEIN HSCB, MITOCHONDRIAL [PRECURSOR]	HLW
73	(K)DMERRQQQK|L|k\M/QAE\I\K(R)	K172k	3	11	Q9UL16	COILED-COIL DOMAIN-CONTAINING PROTEIN 19	HLW
74	(K)DW/A/N/QR/CSS\P\KG/SARNSLV\KA\k(R)	K231k	3	11	P38432	COILIN	HLW
75	(R)G/P/P/GPAGK|P\GD\DGEA/GK|PGkAGER(G)	K194k	3	12	*P05539	COLLAGEN ALPHA-1 (II) CHAIN [PRECURSOR]	LVN
76	(R)GHPGPPGP/P/GEQG\LP\GAAGKEGTKGD\P\G\PA\GLPGk(D)	K1051k	3	12	*Q9JI03	COLLAGEN ALPHA-1 (V) CHAIN [PRECURSOR]	BVN
77	(R)IE/G/N/Q/G/ARVGLVAVDkGVF\VLNKK\N\K(L)	K599k	3	11	P01026	COMPLEMENT C3 [PRECURSOR]	BVS
78	(K)FW|I\G\L|QR/E|kG\K(C)	K100k	2	12	Q9ET61	COMPLEMENT COMPONENT C1Q RECEPTOR [PRECURSOR]	BVS
79	(R)TSSQNNV/Q/V/L/NTNkT/SAEL\LLPI\K(E)	K957k	3	11	Q62682	CONTACTIN-3 [PRECURSOR]	LVM
80	(R)ENKRIAIL\PD/G/S/L/RILNASKSD\EGk(Y)	K548k	3	11	O94779	CONTACTIN-5 [PRECURSOR]	HLW
81	(K)F/PVDGKVLEG/N/TMA\DESLITGEA/M/P\V/T\K\kPGSTVIAR(S)	K867k	3	12	P35670	COPPER-TRANSPORTING ATPASE 2	HLW
82	(R)DAD/PILIS/L|R/EA\YV/P|SK|QRDLk(I)	K405k	3	13	Q9BR76	CORONIN-1B	HLW
83	(R)k\G|L|F\PF/THVK(I)	K283k	2	10	*P47941	CRK-LIKE PROTEIN	NLW
84	(R)QGAFLVN/T\AR/GGLV|DE|K/ALA\QALk(E)	K280k	3	10	*Q13363	C-TERMINAL BINDING PROTEIN 1	HLW
85	(R)KRP/A\T\D\DS\S/T\Q/N/k/R(A)	K165k	3	11	*P46527	CYCLIN-DEPENDENT KINASE INHIBITOR 1B	HLW
86	(K)LPkT/L/QE/L\RLH/D\NEITkL\K(K)	K145k K159k	3	10	*Q01129	DECORIN [PRECURSOR]	BVS
87	(K)TTKLHFE/A/LM\N\IPVLVLD/V|N/D|D\F\S\EEVTk(Q)	K262k	3	11	Q16854	DEOXYGUANOSINE KINASE, MITOCHONDRIAL [PRECURSOR]	HLW

Fig.22B

88	(K)GS/SQ|QP|NKV/TDK|N\k(M)	K411k	2	11	P49621	DIACYLGLYCEROL KINASE BETA	BVS
89	(K)M/S/Q/DSM/M\KL|KG/M\AAAGRSQGQHk(Q)	K90k	3	10	*O88797	DISABLED HOMOLOG 2	BVW
90	(K)DkDF/AL/DVSANQP|VLVAV\K\M|LRADAN\K(N)	K591k	3	12	Q16832	DISCOIDIN DOMAIN-CONTAINING RECEPTOR 2 [PRECURSOR]	HVW
91	(R)KL|D|SV|kRQ\KY\Nk(E)	K175k K181k	3	11	*Q03468	DNA EXCISION REPAIR PROTEIN ERCC-6	HLW
92	(K)TDk/L/I/GFSK|PI\VRKKLSLSSQLG\SL\Ek(F)	K732k K756k	3	11	Q9UHC1	DNA MISMATCH REPAIR PROTEIN MLH3	HLW
93	(R)GKLQG|HDVDF|L\ITH/PkEGQEAGL\LP\R(V)	K339k	3	12	Q9NP87	DNA POLYMERASE MU	HLW
94	(K)LLKSVE/NL/G\V/SYVk|G\T\EQY\QSK(L)	K65k	3	11	*P49643	DNA PRIMASE LARGE SUBUNIT	HLW
95	(R)RF/A/IGSQVS|E/H/SII/KD\FT\k(Q)	K700k	3	12	*P33992	DNA REPLICATION LICENSING FACTOR MCM5	HLW
96	(K)D/KKVAE|P|D\N\K\k(K)	K194k	3	13	*Q9WUL0	DNA TOPOISOMERASE 1	BVC
97	(R)REDPE/A/G/WLLYLKTGQM/YPV\P\A\N\HLDk(R)	K352k	3	11	Q6ZQJ5	DNA2-LIKE HELICASE	SLW
98	(K)GSRSQkEAF/PLAK|G\E/V\D|TAP|Q\G\N\kDL\K(E)	K575k K593k	3	13	P48382	DNA-BINDING PROTEIN RFX5	HVW
99	(K)DLL/LNTM/SQ/E\EK\AAYLSDP/RA\PPCEYk(D)	K3840k	3	11	*P78527	DNA-DEPENDENT PROTEIN KINASE CATALYTIC SUBUNIT	HLW
100	(K)SPS/A/CLVVGkVV/KGGTG|L\FE/LKQPL\R(-)	K1700k	3	13	O54889	DNA-DIRECTED RNA POLYMERASE I LARGEST SUBUNIT	BVC
101	(K)Y/KEAARLE|I|NVLEk\I\N\E\K(D)	K214k	3	12	P49760	DUAL SPECIFICITY PROTEIN KINASE CLK2	HLW
102	(R)VLSFkTG/I|I|SLCKAH|L\E\D\K(Y)	K3200k	3	15	P11532	DYSTROPHIN	HVW
103	(K)W\SkE|A/TAG/KKS|KS|G\K(L)	K665k	3	12	Q9NV58	E3 UBIQUITIN-PROTEIN LIGASE RNF19	HVW
104	(K)k/GVL/P\T\K|DIR/RMC\k(S)	K650k K663k	2	11	Q9D485	EF-HAND DOMAIN-CONTAINING FAMILY MEMBER C2	SLN
105	(R)DCNSI/P/LVL\GTCKE\TFNLYYM\E\S\DDHGVk(F)	K133k	3	11	P29319	EPHRIN TYPE-A RECEPTOR 3 [PRECURSOR]	NLW
106	(K)S/kQDT/PA/L\P|P/KKPAPPR\P\KP\PSGK(S)	K773k	3	12	*Q9UBC2	EPIDERMAL GROWTH FACTOR RECEPTOR SUBSTRATE 15-LIKE 1	HLW
107	(K)N/D/P/FTSD/P/FTk\NPSLPS\K(L)	K694k	3	11	*Q9UBC2	EPIDERMAL GROWTH FACTOR RECEPTOR SUBSTRATE 15-LIKE 1	HLW
108	(R)TGLSQ/L/HNAL/NDVKD\I/Q\QSLAD/VSKDWRQ/SINTIES\Lk(D)	K107k	3	11	Q6KAR6	EXOCYST COMPLEX COMPONENT 3	SLW
109	(K)NCF|L\CATVT\TERP/V\QVk\VV\KVK(K)	K51k	3	12	Q9NV70	EXOCYST COMPLEX COMPONENT SEC3	HLW, HVW
110	(R)LQAEK|ALVEFTNSPDC|L\S|k(C)	K46k	3	13	Q9UIA9	EXPORTIN 7	HVW
111	(K)SR/KKS/SE|Gk\K(G)	K539k	3	10	*Q04931	FACT COMPLEX SUBUNIT SSRP1	LVN
112	(K)Vk/S/I/V\T\LDGGK/LV\HLQ\K(W)	K82k	3	13	P05413	FATTY ACID-BINDING PROTEIN, HEART	HLW
113	(R)LVWR\N/S\I/R\GSLIK\S\L/SFFLkk(M)	K158k K159k	3	11	Q66H10	F-BOX ONLY PROTEIN 39	BVS
114	(R)HLDLS/G/C\E\kITD/V\ALE\KISR(A)	K395k	3	12	Q9UKA1	F-BOX/LRR-REPEAT PROTEIN 5	HLW
115	(K)Ck/VK/S\L/LH\SPGD\Y/I\L\L\S\ADK(Y)	K280k	3	13	Q9UKT8	F-BOX/WD-REPEAT PROTEIN 2	HLW
116	(R)TPCT/V/SCN\I|P\V/VSGKE/CE\E\I\IRk(G)	K247k	3	10	*P02675	FIBRINOGEN BETA CHAIN [PRECURSOR]	HLW
117	(R)LRS|PPk/kG|L|GS/PTVH\K(V)	K397k K398k	3	11	*Q61851	FIBROBLAST GROWTH FACTOR RECEPTOR 3 [PRECURSOR]	SVW
118	(R)VTEAEIVPM/G/k|N\SHCVRFVP\QEM\GVHTVSVK(Y)	K2150k	3	11	*O75369	FILAMIN B	HLW
119	(R)ML/E/Q|L|L|LAEkCH/RRTVYE\L\ENE\K(H)	K173k	3	12	Q8K4T4	FILAMIN-A-INTERACTING PROTEIN 1	BVS
120	(R)AIV/A/RANS/Lk\N\S\GV/P\DDIFK(L)	K92k	3	12	Q13045	FLIGHTLESS-I PROTEIN HOMOLOG	HLW
121	(R)TV/S/DN|SLS|N/SRGEGk\P\D\L\K(F), (K)RTVSDNSLS|N/SRGE\G/kP\D\L\K(F)	K308k	3	12	Q96RU3	FORMIN-BINDING PROTEIN 1	HLW
122	(K)CN/kLVGG|G/EANPM\NY\N/SYMDEK(N)	K90k	3	11	P26323	FRIEND LEUKEMIA INTEGRATION 1 TRANSCRIPTION FACTOR	SLC
123	(R)YE/L/L/LK|D/YLLK\LP/HG\S\PDS\k(D)	K542k	3	12	*P98174	FYVE, RHOGEF AND PH DOMAIN-CONTAINING PROTEIN 1	HLW
124	(K)E/VK\P|E|TKP\P|E/P/k(K)	K409k	3	11	*P62813	GAMMA-AMINOBUTYRIC-ACID RECEPTOR SUBUNIT ALPHA-1 [PRECURSOR]	LVC
125	(K)KGTDV|M/V\RC|L/k\L|L\K(E)	K334k	3	12	Q8K284	GENERAL TRANSCRIPTION FACTOR 3C POLYPEPTIDE 1	SVW
126	(R)kk/SLAM/LQLYINK|L|D|S\Q\GK(Y)	K382k K383k	3	12	Q92990	GLOMULIN	HLW
127	(R)L/E|I/VSDGK\YG/A/R\DPDT\k(A)	K472k	3	10	*P23818	GLUTAMATE RECEPTOR 1 [PRECURSOR]	SLW
128	(R)N/A/V/NLAVLkLNE\Q\G/L\L\D\KL\K(N)	K773k	3	11	*P42262	GLUTAMATE RECEPTOR 2 [PRECURSOR]	HLW
129	(K)LFHVS|ED/PSD|LNEK/H\P\NLVHk(R)	K1358k	3	12	P35573	GLYCOGEN DEBRANCHING ENZYME	HLW
130	(R)T/Q/L/N|D|S/L/k/E\I/HQK(E)	K3039k	3	15	Q14789	GOLGIN SUBFAMILY B MEMBER 1	HLW

Fig.22C

131	(K)SV/FAKH|G\L/Ek/L/T\P\IGD\K(Y)	K165k	3	11	Q8TAA5	GRPE PROTEIN HOMOLOG 2, MITOCHONDRIAL [PRECURSOR]	HLW
132	(K)GEkE|EED/K|EDEE/K|P\k(I)	K237k K249k	3	11	*P34058	HEAT SHOCK PROTEIN HSP 90-BETA	BVC
133	(R)V/V/S/E/GGkP|K\VQVEYKGET\K(T)	K101k	3	12	*P54652	HEAT SHOCK-RELATED 70 KDA PROTEIN 2	HLW
134	(R)AHG/SKVVAA\V/GDAVk\S|I\D/D\IGGALS\K(L)	K72k	3	12	P02008	HEMOGLOBIN ZETA CHAIN	HLW
135	(R)SPTTNINE/T|I/GA\FQLHIT\DK\P\SI\NTD\k(L)	K219k	3	12	Q9N2K0	HERV-H_2Q24.3 PROVIRUS ANCESTRAL ENV POLYPROTEIN [PRECURSOR]	HLW
136	(K)LFVGGLKGD/V/AEG\DL\IEHFSQFGT/VE\kA\EIIADK(Q)	K126k	3	12	Q13151	HETEROGENEOUS NUCLEAR RIBONUCLEOPROTEIN A0	HLW
137	(K)QTA|K/A\EGLG\D\GYR\L\V/IN\DG\k(L)	K139k	3	11	Q9BX68	HISTIDINE TRIAD NUCLEOTIDE-BINDING PROTEIN 2	HLW
138	(K)AP/k/SP\AK/AKT/VK\P|KAAKPK(T)	K186k	3	11	*P43274	HISTONE H1.4	SLW
139	(K)AL/VQND\T\L|LQV\KG\T\G/ANG\SFk(L)	K115k	3	11	*Q92522	HISTONE H1X	HLW
140	(K)LL/GGV/T/I|AQG/GV/L/PNI\Q/A/V\L/L/PK\k(T)	K120k	3	11	*Q64522	HISTONE H2A TYPE 2-B	NLW
141	(K)QT/AR\k|S|TG\G/K/A\P\R(K)	K10k	3	13	*Q6LED0	HISTONE H3.1	LVC, LVN
142	(K)A/D/I|P/A/k/YA/ETMDRE/FRK\WMK(W)	K193k	3	11	*Q8TEK3	HISTONE-LYSINE N-METHYLTRANSFERASE, H3 LYSINE-79 SPECIFIC	HLW
143	(K)LRFH/P/k|Q/LYF\SA\RQG/EL\QK(V)	K709k	3	12	Q9H9B1	HISTONE-LYSINE N-METHYLTRANSFERASE, H3 LYSINE-9 SPECIFIC 5	HLW
144	(R)MG/R/PE\G\EGTPG/LT\A\K\k(L)	K237k	3	12	Q96ED9	HOOK HOMOLOG 2	HLW
145	(R)KLG/E/G/S|F\A/kVR(E)	K75k	2	10	*P57058	HORMONALLY UP-REGULATED NEU TUMOR-ASSOCIATED KINASE	HLW
146	(R)Nk|P\L\K|A/L|D\TRFGRK(L)	K2511k	3	11	*P42859	HUNTINGTIN (HUNTINGTON DISEASE PROTEIN HOMOLOG)	SLC
147	(R)NG|Q\S\R/L\S\Lk\HG\EK(A)	K337k	3	12	Q92628	HYPOTHETICAL PROTEIN KIAA0232	HLW
148	(K)FG/DRG/N/QLRKMLLQ\N\YLQ|N\Rk(S)	K1699k	3	11	*P70227	INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR TYPE 3	NLW
149	(K)KF/AADFk|TLA/DVLV|Q\E\V\I\K(Q)	K50k	3	11	P49442	INOSITOL POLYPHOSPHATE 1-PHOSPHATASE	SLW
150	(K)T/L|Tk|ED/I|I\K(F)	K933k	2	12	P14735	INSULIN-DEGRADING ENZYME	HLW
151	(K)GWPKT/HPG/G\E/QkE/GTE\ASLQ\I\RG\K(K)	K162k	3	12	P05019	INSULIN-LIKE GROWTH FACTOR IB [PRECURSOR]	HLW
152	(R)G/IDS/S/YRPS\QkDV/EPPKIS/STK\N\I\S\I\k(Q)	K52k K68k	3	12	Q96CB8	INTEGRATOR COMPLEX SUBUNIT 12	HLW
153	(R)kSENT/AIHHY\LE\A\\|L|KVKDRS|PL\R(T)	K391k	3	11	Q13325	INTERFERON-INDUCED PROTEIN WITH TETRATRICOPEPTIDE REPEATS 5	HVW
154	(R)RC/L/I/S/T/DM\HHI\EESF/QE/Ik\RAI\QA\K(D)	K45k	3	14	Q9UHD0	INTERLEUKIN-19 [PRECURSOR]	HLW
155	(K)EQLDA/L/EK|E|T\A\SkLSEM/D\SFNNQLK(C)	K608k	3	11	Q9NZM3	INTERSECTIN 2	HLW
156	(K)Q/KRLQEEKS\Q\D|k(T)	K700k	3	11	Q9Z0R6	INTERSECTIN-2	NLW
157	(K)IPVGSEEG\Y/R\S\LFGQV/L/KD\I\V\E\k(I)	K209k	3	13	P27352	INTRINSIC FACTOR [PRECURSOR]	HLW
158	(K)M/Wk/S/P/NGTIRNI/L/GGT|VFREPIICK(N)	K205k	3	10	*P54071	ISOCITRATE DEHYDROGENASE [NADP], MITOCHONDRIAL [PRECURSOR]	SLW
159	(R)E/V/RPSPSk\T/VKYT/ATVTKGAVTY\T\K\A\K(R)	K334k	3	12	Q92833	JUMONJI PROTEIN	HLW
160	(K)IS/E/LS/L/C/D/LAGSERCk\HQK(S)	K415k	3	12	P97329	KINESIN FAMILY MEMBER 20A	SLW
161	(K)k|L|HAMN\K\E|L\Q\RLQT\AQk(E)	K726k K742k	3	13	Q9QXL2	KINESIN FAMILY MEMBER 21A	SLN
162	(K)Q/LDDK\DDE/I|NQ\QSQ\L\I\E\k(L)	K444k	3	12	Q12840	KINESIN HEAVY CHAIN ISOFORM 5A	HVW
163	(R)NET/PSGDSQT\L/MI\PSN/P/CL\MkR(K)	K143k	3	11	Q8C0N1	KINESIN-LIKE PROTEIN KIF2B	SLC
164	(R)MGk/L/HL/V\D/LA\GSE/RQA|KTG\ATGQ\R(L)	K245k	3	11	Q9Y496	KINESIN-LIKE PROTEIN KIF3A	HLW
165	(R)kR/TFS/LQG\GG/GGGA\NGG\SGG\Q\GK(G)	K277k	3	12	P84550	LADYBIRD HOMEOBOX COREPRESSOR 1	HLW
166	(K)IM/GG/SGTE/VVL/E/KQKS|T\Pk(S)	K787k	3	11	Q9ESV1	LEUCINE ZIPPER PROTEIN 1	BVC
167	(K)IPAk|IS|QMTNLQEL/HLC|HCPA\K(V)	K508k	3	12	Q7L1W4	LEUCINE-RICH REPEAT-CONTAINING PROTEIN 5 [PRECURSOR]	HLW
168	(K)NN/MNR/SNTIQSGPE|GS\L|VkSQ\SLkSI\PE\K(F)	K209k K214k	3	12	Q498T9	LEUCINE-RICH REPEAT-CONTAINING PROTEIN 8C	BVC
169	(R)KISE/N|SYSLDD/L/EIGPGQLSS\STFDSEkNE\S\R(R)	K250k	3	11	Q9UHB6	LIM DOMAIN AND ACTIN-BINDING PROTEIN 1, EPITHELIAL PROTEIN LOST IN NEOPLASM	HLW
170	(R)C/LEHP\N\V\L\KF/I\G/V\LYK/Dk(R)	K407k	3	13	*P53667	LIM DOMAIN KINASE 1	HLW
171	(R)SGKY\L\A\TE\W\NTVSk(L)	K50k	3	12	P61794	LYSOPHOSPHATIDIC ACID RECEPTOR EDG-2	LVC
172	(K)EAEA/FLEK\Y\G/Y/LNE\Q\V\Pk\APTST\R(F)	K52k	3	14	Q9H239	MATRIX METALLOPROTEINASE-28 [PRECURSOR]	HLW

Fig.22D

173	(R)GRQ/T/V/D|k/V/M\GIPK(E)	K812k	3	10	*Q14676	MEDIATOR OF DNA DAMAGE CHECKPOINT PROTEIN 1	HLW
174	(K)KVSFSSP/L|I|LGA|TI/Qk\K(S)	K52k	3	11	O15480	MELANOMA-ASSOCIATED ANTIGEN B3	HLW
175	(K)k|I|PI/KR/TD|I|LK\H/VVGDYR(D)	K77k	3	14	Q9CPR8	MELANOMA-ASSOCIATED ANTIGEN G1	SLN
176	(K)KI/PIK\R\TDI\L\k\H/VVGDYR(D)	K87k	3	11	Q9CPR8	MELANOMA-ASSOCIATED ANTIGEN G1	SLN
177	(K)LQ/N/LQT/VR|L/VFk/I|Q/TQTPRKK(T)	K310k	3	11	Q8N9U0	MEMBRANE TARGETING TANDEM C2 DOMAIN-CONTAINING PROTEIN 1	HLW
178	(K)KGTELLL/GV/DA/LGLHIYD/PE\N\R\L\TPk(I)	K253k	3	11	*P35240	MERLIN	HLW
179	(K)I/AQDLEMY\G\VNYF/T\I\R\Nk(K)	K227k	3	10	*P46662	MERLIN	SLC
180	(R)GSEG/k/P\C\G\E/L/K/KEK(G)	K65k	3	11	*Q14833	METABOTROPIC GLUTAMATE RECEPTOR 4 [PRECURSOR]	HLW
181	(R)VD/GDIILGGL\F\P\VHAk\GERGV\P\CG\ELKKEK(G)	K57k	3	12	P70579	METABOTROPIC GLUTAMATE RECEPTOR 8 [PRECURSOR]	BVM
182	(K)SS/CkENP/FN/RKPS\PAAS\P\A\T\K\K(A)	K631k	3	12	Q8N3F8	MICAL-LIKE PROTEIN 1	HLW
183	(K)VIVKKDK\P\G\kV\E\S\KPSVTEK(E)	K594k	3	12	*P15205	MICROTUBULE-ASSOCIATED PROTEIN 1B	BVW
184	(K)Tk\P\E\EK\K(E)	K644k	2	12	*P15205	MICROTUBULE-ASSOCIATED PROTEIN 1B	LVN
185	(K)SKPSA/ASPK/PGA\LK\ESSDKV/S/R\VA\SPkk(K)	K2282k K2283k	3	10	*P15205	MICROTUBULE-ASSOCIATED PROTEIN 1B	BVC
186	(R)QW/I/GkH\RR\PR\TVSF\QAk(E)	K20k K32k	3	12	Q9CQF8	MITOCHONDRIAL RIBOSOMAL PROTEIN 63	NLW
187	(K)AD/PFSFK/A\RAk/SCG/Ek\DGK(G)	K1010k K1015k	3	10	*O35099	MITOGEN-ACTIVATED PROTEIN KINASE KINASE KINASE 5	NLW, SLW
188	(K)KI\K/T/K/k\S\TW\E\K(V)	K227k	3	11	O60669	MONOCARBOXYLATE TRANSPORTER 2	HVW
189	(K)RARA/D/PT/VESEEAF/k\S/RMEVK(V)	K116k	2	11	Q9R0Q4	MORTALITY FACTOR 4-LIKE PROTEIN 2	SLW
190	(R)A/k/LDSG\R\VLK\Q\ISN\NRk(C)	K326k K341k	3	11	*P09416	MYC PROTO-ONCOGENE PROTEIN	LVM
191	(R)GS/GKDS/HHP\A/RTAH/Y\G\SLP\Qk(S)	K209k	3	10	*P02686	MYELIN BASIC PROTEIN	HLW
192	(K)INkSE\S\V/VYADIR\K(D)	K258k	3	12	Q6AYT8	MYELIN PROTEIN ZERO-LIKE PROTEIN 1 [PRECURSOR]	BVC, LVN
193	(K)EI/FGGNAD\K/kNL/VD/PF/V\EVS\FA\GK(K)	K392k	3	11	Q9NZM1	MYOFERLIN	HLW
194	(R)IEAQNKP/FD\A/K/T/SVFVV/D\P\k\ESYVK(A)	K44k	3	10	*Q9Y623	MYOSIN HEAVY CHAIN, SKELETAL MUSCLE, FETAL	HLW
195	(R)V/FDKE/G/NG/TVMGA\EIR/HV/L\V\TLGEk(M)	K119k	3	12	P60660	MYOSIN LIGHT POLYPEPTIDE 6	HLW
196	(R)G/D/E/V/MV/ELAENGk\K(A)	K67k	3	11	*Q9JLT0	MYOSIN-10	LVN
197	(K)LK/K/L/EE\EQ\II/LE\DQ\N\Ck(L)	K989k	3	10	*P35579	MYOSIN-9	HLW
198	(R)ETNKQI\SKLTI/YD\A\R/PSV\NAV\ANk(A)	K289k	3	11	Q13496	MYOTUBULARIN	HLW
199	(K)Q/k/G/HY\L\A/GKV/I/GEFPGV\V/HCLD\FQK(M)	K4503k	3	11	*P20929	NEBULIN	HLW
200	(K)Q/kGHYVGTLT\AR\D\D\N\K(I)	K4016k	3	10	*P20929	NEBULIN	HLW
201	(K)GF\E/LRLDSLA/FLT\Ak\AK(R)	K808k	3	11	*Q80XB4	NEBULIN-RELATED-ANCHORING PROTEIN	NLW
202	(K)GF/E/LRLDSLA/FLT\AK\Ak(R)	K810k	3	10	*Q80XB4	NEBULIN-RELATED-ANCHORING PROTEIN	NLW
203	(K)kY/L/LL/G\N\AEDS\PD\Q\SGIVADK(S)	K554k	3	12	O95631	NETRIN-1 [PRECURSOR]	HLW
204	(R)LLQ/SAFSkN\ALSkQ\SPK\K\S\P\SA\K(L)	K677k K682k	3	11	P63239	NEUROENDOCRINE CONVERTASE 1 [PRECURSOR]	SLW
205	(R)N/GVG/LEFNH/L\FGYGV\L\D\AG/A\M\VkMA\K(D)	K454k	3	14	P16519	NEUROENDOCRINE CONVERTASE 2 [PRECURSOR]	HLW
206	(K)E/EAk/SPGEAkS/PGEAK\SP/A\EA\KSPG\EAK(S)	K522k K528k	3	11	P19246	NEUROFILAMENT TRIPLET H PROTEIN	SLW
207	(K)AST/DNSPSS\K/A\EDAP\Ak\EEP\K(Q)	K162k	3	13	*P17677	NEUROMODULIN	HLW
208	(K)DQTQkAAT\G\PFD/R/E\HLL\M\Y\LEK(E)	K58k	3	12	Q9NZR1	NEURONAL TROPOMODULIN	HLW
209	(R)SN/SE/I/L/k/QFT/LRE\LRNK(R)	K683k	3	12	Q8R151	NFX1-TYPE ZINC FINGER-CONTAINING PROTEIN 1	SLN
210	(R)FG\S\KAHMERL/EEV\Nk(E)	K391k	3	10	*P29475	NITRIC-OXIDE SYNTHASE	HLW
211	(R)GGDVSD/SKQF\T\YYPL\VE/D\KEEV\Q\Rk(R)	K338k	3	11	*Q00653	NUCLEAR FACTOR NF-KAPPA-B P100 SUBUNIT	HLW
212	(K)Q/LLTGNT/DK/P/IG\MIDR\L\NS\PL\LS\Nk(T)	K677k	3	13	P48552	NUCLEAR FACTOR RIP140	HLW
213	(R)SGTSS/GAG/G\S\I\TRGAPVVVPEL\GkPR(Q)	K1505k	3	10	*Q9WU42	NUCLEAR RECEPTOR COREPRESSOR 2	SLW
214	(R)C/kQLkR/A/A\L\GR\M/C\T\I\I\K(R)	K127k K130k	3	14	Q99P77	NUCLEOLAR GTP-BINDING PROTEIN 1	BVM, LVC, LVN
215	(R)VGDLILAH/L\HkkCPYS/VP\FYP\TF\K(E)	K526k K527k	3	12	Q53GS7	NUCLEOPORIN GLE1	HVW
216	(K)QVKGSA/D\Y/kS\K(K)	K495k	3	12	*Q6P6T5	OCCLUDIN	LVM
217	(K)QV\KGSAD\Y\KS\k(K)	K497k	3	11	*Q6P6T5	OCCLUDIN	LVN
218	(R)I/V\SSIFKVPSS\Q\SI\Hk(A)	K236k	3	12	P23272	OLFACTORY RECEPTOR-LIKE PROTEIN I9	LVC
219	(K)kPY/VAk/L\IELFQN/GA/EV\P\PK\KCAR(V)	K61k K66k	3	10	*Q9Z1N2	ORIGIN RECOGNITION COMPLEX SUBUNIT 1	SLW
220	(K)LFNYN/kVL\P\MNTGVE/A/GETACK\L\ARR(W)	K135k	3	11	P04182	ORNITHINE AMINOTRANSFERASE, MITOCHONDRIAL [PRECURSOR]	BVC
221	(K)EMN/I\L\EL\SH/kkMM\K(K)	K1023k K1024k	3	12	Q9UPQ7	PDZ DOMAIN-CONTAINING RING FINGER PROTEIN 3	HLW
222	(R)GTD/SEHTH/K/A\H\L\VP\EGT\S\K\k(R)	K672k	3	16	Q96RV3	PECANEX-LIKE PROTEIN 1	HLW

Fig.22E

223	(R)K/S/V/R/P\G\AS/Y/k\R(Q)	K180k	3	11	Q8NEY8	PERIPHILIN 1	HLW
224	(K)GATA/G/ASH|KGD/EVPP|IKK|N\T\kA\PGK(Q)	K198k	3	12	O75167	PHOSPHATASE AND ACTIN REGULATOR 2	HLW
225	(R)EIDK/R/MNS\Ik/P/DLI\QLR\K(T)	K567k	3	11	*Q63787	PHOSPHATIDYLINOSITOL 3-KINASE REGULATORY SUBUNIT ALPHA	LVC, LVM
226	(K)k/QAAEYREI\DKRMN\S\IKPDL\I\QLR(K)	K551k	3	10	*P27986	PHOSPHATIDYLINOSITOL 3-KINASE REGULATORY SUBUNIT ALPHA	HLW
227	(R)L/E/Q\D/LKKQALDN\R/EIDK\k(M)	K294k	3	10	*Q63789	PHOSPHATIDYLINOSITOL 3-KINASE REGULATORY SUBUNIT GAMMA	LVN
228	(R)EP/LRT/IP\LKDV\L\KTHE\CLVk(S)	K256k	3	11	Q9ER55	PLECKSTRIN HOMOLOGY DOMAIN-CONTAINING FAMILY A MEMBER 2	NLW, SLW
229	(K)I/K\PTSA/W/NL\A\Q\KH\k(L)	K667k	3	11	*P54277	PMS1 PROTEIN HOMOLOG 1	HLW
230	(R)SFP/SEKR\G/VL\S/SYPSDVIS/Y\R\GL\RG\SQDk(L)	K474k	3	11	P54279	PMS1 PROTEIN HOMOLOG 2	NLW
231	(R)VDRMEH/SI/GS\IVS/KID/AVIV\K\LEIMERAk(L)	K874k	3	11	*Q13563	POLYCYSTIN-2	HLW
232	(K)ID/kN\E/D/R\K\K(D)	K555k	3	10	*Q96RN5	POSITIVE COFACTOR 2 GLUTAMINE/Q-RICH-ASSOCIATED PROTEIN	HLW
233	(K)GDPF/LAS/PTS\DREIIA\P\K\IkE\R(T)	K364k	3	11	*Q12809	POTASSIUM VOLTAGE-GATED CHANNEL SUBFAMILY H MEMBER 2	HLW
234	(K)LKINN/N/V/F/VDKPAFP/EY\K\V\S\DAk(K)	K208k	3	11	*Q96L42	POTASSIUM VOLTAGE-GATED CHANNEL SUBFAMILY H MEMBER 8	HLW
235	(R)DGALLIAG/S/EAP\KR\GSVLSk/PRT\GG\AG\A\GKPPK(R)	K56k	3	10	*Q9Z351	POTASSIUM VOLTAGE-GATED CHANNEL SUBFAMILY KQT MEMBER 2	SLW
236	(K)CP/K/PS/AQ/EITNLADSLQLEkEVVRV\WF\CNRR(Q)	K442k	3	10	*P31361	POU DOMAIN, CLASS 3, TRANSCRIPTION FACTOR 3	NLW
237	(R)EYSVK\E/ILkLDSN/P\S\K\G\K(D)	K219k	3	11	O75626	PR DOMAIN ZINC FINGER PROTEIN 1	HLW
238	(R)GVSSQ/E/T/A/GI/GASA/HLVNFkG\TDTVAGL\AL\IK\K(Y)	K216k	3	12	P43490	PRE-B CELL ENHANCING FACTOR [PRECURSOR]	HLW
239	(K)YVEAkDCLN/V\L/NK\SN\E\GK(E)	K47k	3	13	Q99471	PREFOLDIN SUBUNIT 5	HLW
240	(K)YVEAKDC/L\NVL\N/k\SN\E\GK(E)	K55k	3	13	Q99471	PREFOLDIN SUBUNIT 5	HLW
241	(K)QYYk/VV\NSYP\LAH/K\V\F\EEK(T)	K281k	3	11	Q9UHI6	PROBABLE ATP-DEPENDENT RNA HELICASE DDX20	HLW
242	(R)WK\V\T/Sk\EA\L\Q\R(I)	K953k	3	11	Q62780	PROBABLE ATP-DEPENDENT RNA HELICASE DDX46	LVN
243	(R)ANQS\V\PEDLVVM/AEQY/KL\N\QQk(R)	K610k	3	13	Q86TM3	PROBABLE ATP-DEPENDENT RNA HELICASE DDX53	HLW
244	(R)G/G/D/GINS\I/GG\G\Q\k\VRLM\K(E)	K89k	3	12	O00469	PROCOLLAGEN-LYSINE,2-OXOGLUTARATE 5-DIOXYGENASE 2 [PRECURSOR]	HLW
245	(R)SVP/AT/KGLLSPLMSRP\EIKV/GDQSGTGRGQk\VLP\k(G)	K184k K188k	3	11	*Q00175	PROGESTERONE RECEPTOR	SLM
246	(K)VT/VV/N\S/I\kP/S\S\PT\E\GK(R)	K657k	3	14	O95447	PROTEIN C21ORF13	HLW
247	(R)EMV/V\Q\Y/ISA/TSK\SIVGSK/VL\GGL\K\NSk(H)	K155k	3	13	O70566	PROTEIN DIAPHANOUS HOMOLOG 2	SLW
248	(R)AVEKYSVK/P/EH\PN/LGSCN\P/S\LYNTFASk(Q)	K304k	3	12	Q9BVV6	PROTEIN KIAA0586	HLW
249	(R)D/NM/SIVLV\C\FS\NAPkVS\DEAV\KK(D)	K300k	3	12	O75688	PROTEIN PHOSPHATASE 2C BETA ISOFORM, PROTEIN PHOSPHATASE 1B	HLW
250	(K)NEAA/F\L\SMTDSk\T\E\GK(F)	K319k	3	15	P35247	PULMONARY SURFACTANT-ASSOCIATED PROTEIN D [PRECURSOR]	HLW
251	(K)LkILM/D/KPE/MNVV\L/K/NVK\P\D\QWVK(L)	K558k	3	12	P55786	PUROMYCIN-SENSITIVE AMINOPEPTIDASE	HLW
252	(R)k/ELN/k\ILE\GRS\K(I)	K429k K433k	3	11	P10515	PYRUVATE DEHYDROGENASE COMPLEX E2 SUBUNIT	HLW
253	(R)DL/KLEN/LM\L\DK\DGH/I/k(I)	K289k	3	10	*P47196	RAC-ALPHA SERINE/THREONINE-PROTEIN KINASE	BVS
254	(R)FYG/AEIVS\A\LDYLH/SG\kIV\YRDLK(L)	K266k	3	10	*Q63484	RAC-GAMMA SERINE/THREONINE-PROTEIN KINASE	BVN
255	(R)DMK/S/N/N/IFLHEGLTVk/IGDFGLA\TVK\S\R(W)	K483k	3	10	*P11345	RAF PROTO-ONCOGENE SERINE/THREONINE-PROTEIN KINASE	LVN
256	(R)LEGNTVG/V/E\AARVI\A\k\ALEK(K)	K71k	3	11	P46060	RAN GTPASE-ACTIVATING PROTEIN 1	HLW
257	(R)Ek/H/C\L\G/M/KLSED\G\TYT\GF\I\K(V)	K218k	3	11	O35141	RAS ASSOCIATION DOMAIN-CONTAINING FAMILY PROTEIN 5	LVC
258	(R)T/L\T/L/IAk/VIQN\L/ANF\SK(F)	K613k	3	12	Q96PV0	RAS GTPASE-ACTIVATING PROTEIN SYNGAP	HLW
259	(-)MA/K/S/AEVk\L\AIFGRAGVGK\S\AIVVR(F)	K8k	3	11	Q8R367	RAS-RELATED AND ESTROGEN-REGULATED GROWTH INHIBITOR	NLW, SLW

Fig. 22F

260	(R)L/L|K/K\E/Q\NK/M\G/VkL\S(-)	K211k	3	12	Q5PQP2	RECEPTOR-BINDING CANCER ANTIGEN EXPRESSED ON SISO CELLS	LVM, LVN
261	(K)ATH|PPPAS\PS/S/LVK/VPSSA\T\GkR(Q)	K492k	3	11	O43566	REGULATOR OF G-PROTEIN SIGNALING 14	HLW
262	(K)EE/L|NN|KLK\DTQ|EQL/S\k|L\K(D)	K996k	3	11	Q62868	RHO-ASSOCIATED PROTEIN KINASE 2	BVN
263	(R)EA/L/G/D/K\APP\K\P\VPk(T)	K103k	3	11	Q5RJS9	RIBOSOME PRODUCTION FACTOR 1	LVN
264	(K)L/AkQNT\Nk\A|K\E\N|L\R(K), (K)L/AK/QNT/N\k\Ak\E\N|L\R(K)	K198k K203k; K	3	11	Q9D6S7	RIBOSOMERE CYCLING FACTOR, MITOCHONDRIAL [PRECURSOR]	SVW
265	(K)LDTP/ATS\D|P/L/SDRGG/RKR\k(R)	K786k	3	12	Q5XI59	RING FINGER PROTEIN 10	LVN
266	(R)RNH/C/F|Q|DFkEE|KP\Q\ENK(T)	K594k	3	12	Q8IYF1	RNA POLYMERASE II TRANSCRIPTION FACTOR SIII SUBUNIT A2	HLW
267	(R)QkCHS/P/PP/K\P\EPFPFGQS/G\Q\K\PALNGGKK(V)	K76k	3	11	Q63068	RNA U SMALL NUCLEAR RNA EXPORT ADAPTER PROTEIN	BVM
268	(K)F/EAKLMPE\E|CFS|PL|DL\F\N\k(I)	K78k	3	13	Q6NXK5	RNA/RNP COMPLEX-1-INTERACTING PHOSPHATASE	SLM
269	(K)VAS\ISFDA/S/KAK\k\P/S/Q/FSGK(I)	K322k	3	12	Q92545	RW1 PROTEIN	HLW
270	(K)RLAV/F/S/Q|PIINk\V|KP\QLLK(T)	K3309k	3	11	Q92736	RYANODINE RECEPTOR 2	HLW
271	(R)k/AQ/A\AE\MKAA\NE|A\E|GK(V)	K4324k	3	13	Q15413	RYANODINE RECEPTOR 3	HLW
272	(K)Y/L/KI/E|E/T|NP\SLAQ\DTVI\I\k(K)	K1715k	3	13	Q9NZJ4	SACSIN	HLW
273	(R)k/GE|I|A|A\S/IAT\HMR/P/YRKKS(-)	K283k	3	14	O75880	SCO1 PROTEIN HOMOLOG, MITOCHONDRIAL [PRECURSOR]	HLW
274	(K)S/D/G\Y/Hk\RP/D/RK\SR(I)	K188k	3	12	Q9QX72	SECIS-BINDING PROTEIN 2	LVN
275	(K)RkD|T/KR|L|VLHMK(N)	K238k	2	11	Q5T4F7	SECRETED FRIZZLED-RELATED PROTEIN 5 [PRECURSOR]	HLW
276	(K)DS/TKD/D/NS\NLGG/k/T\D\E|A\K\G\K(T)	K400k	3	12	P47868	SECRETOGRANIN-3 [PRECURSOR]	BVN, LVC
277	(K)DS/TkDD/NS\NL/G/G|K\TD\EA\K\GK(T)	K391k	3	11	P47868	SECRETOGRANIN-3 [PRECURSOR]	LVN
278	(R)HGYTSS|L\ELPD|N\I|LNFV\k\K(H)	K395k	3	11	Q9C0C4	SEMAPHORIN 4C [PRECURSOR]	HLW
279	(R)D/IkPDN\VLLDV/N\G/H\IR\LAD\F\GS\CLK(M)	K202k	3	11	Q7TT50	SERINE/THREONINE-PROTEIN KINASE MRCK BETA	NLW
280	(R)L/L\S/A\A/KA|PD/R\k(A)	K251k	3	10	*Q63433	SERINE/THREONINE-PROTEIN KINASE N1	LVN
281	(K)LDNT/V/VGQTIWkP/ISNQSWD|Q\K(F)	K411k	3	10	*O08874	SERINE/THREONINE-PROTEIN KINASE N2	BVS
282	(K)E/KKP\IKS|PSKD|AS/S|G/kENR(S)	K337k	3	11	Q61136	SERINE/THREONINE-PROTEIN KINASE PRP4 HOMOLOG	SLW
283	(R)D/LKPE/N/I/L/L/D\C\QGHVV/LT\DF\GLCk(E)	K242k	3	11	*Q9HBY8	SERINE/THREONINE-PROTEIN KINASE SGK2	HLW
284	(K)QNN|TA/K\LVK/Q/LSkS\S\EDE\EL\RK(L)	K122k	3	12	Q96QC0	SERINE/THREONINE-PROTEIN PHOSPHATASE 1 REGULATORY SUBUNIT 10	HLW
285	(K)EE/Gk/SR|TTLP\E\R|PL|T\EVK(A)	K165k	3	12	O55000	SERINE/THREONINE-PROTEIN PHOSPHATASE 1 REGULATORY SUBUNIT 10	LVN
286	(K)A/QGk\KGS/AG/NTW\S\Q\L/SNN\NK(D)	K212k	3	11	Q9Y6X0	SET-BINDING PROTEIN	HLW
287	(K)S/FH/LS|PR|G|PPTS\EPPP\V\PANKP\k(F)	K358k	3	12	P78314	SH3 DOMAIN-BINDING PROTEIN 2	HLW
288	(R)CKSE/G/TLIDLSE\GFSET\SF\N\D\V\k(V)	K39k	3	12	Q9JJS5	SH3 DOMAIN-BINDING PROTEIN 4	LVW
289	(R)A/K/L/TML\N/T\VS/KI\RGQV\k(N)	K82k	3	12	O35964	SH3-CONTAINING GRB2-LIKE PROTEIN 1, ENDOPHILIN-A2	BVM
290	(R)AI/D/E/PNN/CK/G/I\R\CE\k(Q)	K560k	3	12	Q9UL62	SHORT TRANSIENT RECEPTOR POTENTIAL CHANNEL 5	HLW
291	(K)EVCTALL\EA/DVNIkL|VK|Q|L\R(E)	K47k	3	11	Q6AYB5	SIGNAL RECOGNITION PARTICLE 54 KDA PROTEIN	BVS
292	(R)FNQAQEG/NIQN\TV|M\L|D/KQK\ELD\Sk(V)	K145k	3	10	*P42225	SIGNAL TRANSDUCER AND ACTIVATOR OF TRANSCRIPTION 1	SLW
293	(-)ME/NLQSkF\SLV/QGS/N|KKL\NG\M/ED\DGSPPV\k(K)	K7k K30k	3	11	*Q60665	SKI-LIKE PROTEIN	SLW
294	(K)LS/S/GN/SKL/SS|SKFSN/PI\SSS\k(R)	K137k	3	13	O15444	SMALL INDUCIBLE CYTOKINE A25 [PRECURSOR]	HLW
295	(K)D/L/TT/AEK\L\kR\ET\PWK(I)	K1014k	3	14	Q13621	SOLUTE CARRIER FAMILY 12 MEMBER 1	HVW
296	(R)KLL/SQL\Y\AR/k|VIQD\IM\N\K(Q)	K51k	3	11	P16043	SOMATOLIBERIN [PRECURSOR]	SLW
297	(K)SAK/W\RR|P|R/TAk(M)	K160k	3	11	P30680	SOMATOSTATIN RECEPTOR TYPE 2	LVC, LVM, LVN
298	(R)L/K|GLA|LQR\QGkLF\GA\AEVQR(F)	K250k	3	10	*P16086	SPECTRIN ALPHA CHAIN, BRAIN	BVC
299	(R)EVT/MK/K/G/D\V|LTLLS\SI\N\kDWWk(V)	K1012k K1016k	3	10	*P02549	SPECTRIN ALPHA CHAIN, ERYTHROCYTE	HLW
300	(K)YSHLIGKG/A/A/kDAA\HMLQA/NK/T/Y\GC\VP\VANKR(D)	K177k	3	12	Q7TNE3	SPERM-ASSOCIATED ANTIGEN 7	SLW
301	(K)G/P/A/VP/PE/L\D\kH/FLL/CEA\C\GK(C)	K154k	3	12	Q9C004	SPROUTY HOMOLOG 4	HLW
302	(K)KE/QA/L/QLAQ\kMGFP/PNI\VE/SAAE\NMVk(L)	K225k K242k	3	11	Q9P2R7	SUCCINYL-COA LIGASE [ADP-FORMING] BETA-CHAIN, MITOCHONDRIAL [PRECURSOR]	HLW

Fig. 22C

303	(K)R/DF/G\SFE/K|F/kE\K(L)	K132k	3	11	*P09671	SUPEROXIDE DISMUTASE [MN], MITOCHONDRIAL [PRECURSOR]	NLW, SLW
304	(K)R/DF/G\S/FE/K|Fk|E\K(L)	K132k	3	10	*P07895	SUPEROXIDE DISMUTASE [MN], MITOCHONDRIAL [PRECURSOR]	BVW, LVN
305	(R)KK/N/S/L|KDCVAVAGP\LGV\THFLILTkTDNSVYL\k(L)	K90k K98k	3	11	Q91YU8	SUPPRESSOR OF SWI4 1 HOMOLOG	NLW
306	(K)LPV/kI\V\Q\K|NDP\F\VV\D\CSDK(L)	K1815k	3	11	O14497	SWI-SNF COMPLEX PROTEIN P270	HLW
307	(K)TkL/QSE|N|L|K\QSIEK(Q)	K304k	3	11	Q15431	SYNAPTONEMAL COMPLEX PROTEIN 1	HLW
308	(K)M/G|k\KKT/L|V|Vk(K)	K643k K650k	2	12	Q9HCH5	SYNAPTOTAGMIN-LIKE PROTEIN 2	HLW
309	(K)ID/SI/ADHV/NS/A/AVNVEEGTK/NLG\kAAkY\K(L)	K223k K226k	3	11	P56962	SYNTAXIN-17	HLW
310	(R)k/G/P/EDTAQL\AH/AV/LA\KL|N\A\Fk(A)	K193k K213k	3	13	Q64324	SYNTAXIN-BINDING PROTEIN 2	SLW
311	(K)S/AYS/ERL/K/F\NVA/I/k\I\I\DRK(K)	K41k	3	11	Q9BXA7	TESTIS-SPECIFIC SERINE/THREONINE-PROTEIN KINASE 1	HLW
312	(K)KKPNPG/K\Dk\RT/YEP\SSA\TP\V\T\R(S)	K1909k	3	11	P53804	TETRATRICOPEPTIDE REPEAT PROTEIN 3	HLW
313	(K)EPHVEQI\TRkF\R\L|N\S\E\GK(L)	K542k	3	12	Q8WY91	THAP DOMAIN-CONTAINING PROTEIN 4	HLW
314	(R)FKQ/E/GV\L/NSk\V/G|M\DYR(S)	K636k	3	11	P52888	THIMET OLIGOPEPTIDASE	HLW
315	(K) N/ALALF/VLPKE\G\QM/E\SVE\AA\MSS\k(T)	K287k	3	12	P05543	THYROXINE-BINDING GLOBULIN [PRECURSOR]	HLW
316	(K)QLEGA/CYSG\K\LIWkV/T\D\Y|RV|KK(R)	K409k	3	12	P70191	TNF RECEPTOR-ASSOCIATED FACTOR 5	SLW
317	(K)E/D/VSE/SVGA/SGQRP\VFCPVHk\Q\EQL\K(L)	K280k	3	11	Q9UPN9	TRANSCRIPTION INTERMEDIARY FACTOR 1-GAMMA	HLW
318	(R)MITN/E/Q/DLKMFLLSKG/A/SkEVIA\SI\I\S\R(Y)	K118k	3	11	Q99551	TRANSCRIPTION TERMINATION FACTOR, MITOCHONDRIAL [PRECURSOR]	HLW
319	(K)kQPGQDC/P/H\SCNLLCHPGP\CP\P\CPAFMTK(T)	K431k	3	10	*Q12986	TRANSCRIPTIONAL REPRESSOR NF-X1	HLW
320	(R)S/K/W/HIPM|PSGk/G|YFN\F\GK(I)	K50k	3	12	Q8BXN9	TRANSMEMBRANE PROTEIN 87A [PRECURSOR]	NLW, SLW
321	(R)kQ|QA|MWRVP|S|DL\K(M)	K661k	2	10	*O94972	TRIPARTITE MOTIF PROTEIN 37	HVW
322	(R)EL\Nk\LY\P|THA/C/RE\YLK(N)	K183k	3	11	*P17752	TRYPTOPHAN 5-HYDROXYLASE 1	HLW
323	(-)M/A/KPT\S|K|DS/G|L/k\E\K(F)	K12k	3	11	*Q61037	TUBERIN	NLW
324	(R)GDV/VP|k\D|V|N|VA/IA/AI/k\T\K(R)	K326k K336k	3	12	Q6AY56	TUBULIN ALPHA-8 CHAIN	BVS
325	(R)LHEE\WLL/R|EE\k|A\QEE\FR\IK(K)	K77k	3	11	Q64707	U2 SMALL NUCLEAR RIBONUCLEOPROTEIN AUXILIARY FACTOR 35 KDA SUBUNIT-RELATED PROTEIN 1	SLC
326	(R)Gk/M/VSYI/Q/CKE|V\D\YRSDR\R(E)	K327k	3	12	*Q93009	UBIQUITIN CARBOXYL-TERMINAL HYDROLASE 7	HLW
327	(R)SI|P/VLAkWQ/N/SYSIK(V)	K108k	3	13	Q15819	UBIQUITIN-CONJUGATING ENZYME E2 VARIANT 2	HLW
328	(K)FSL/QDPP|N|K|kPk(V)	K490k K492k	3	11	*O70199	UDP-GLUCOSE 6-DEHYDROGENASE	BVC
329	(K)K/GKAES\CGHATV/S|SE/kk|L\K(T)	K368k K369k	3	12	Q9NTX9	UNCHARACTERIZED PROTEIN C20ORF177	HLW
330	(K)FQ/EIAEK|N|MEkL/N\H|I\Ek(S)	K695k K701k	3	13	Q6TFL3	UNCHARACTERIZED PROTEIN C9ORF93	HVW
331	(R)LLk|N|T\E/NM|KGFFGG\LL\E\TKL\K(G)	K232k	3	12	Q8R0A7	UNCHARACTERIZED PROTEIN KIAA0513	SVW
332	(K)F/ALPY\IRD|VAkRV/K/AG\L\Q\K(A)	K242k	3	12	P70697	UROPORPHYRINOGEN DECARBOXYLASE	SLM
333	(K)KMM|SNQYVP|Vk\T\H|EEV\K(M)	K863k	3	11	Q9BZF9	UVEAL AUTOANTIGEN WITH COILED-COIL DOMAINS AND ANKYRIN REPEATS PROTEIN	HVW
334	(K)HEKELM/GL/K|S/N/I/A\ELkk(Q)	K826k K827k	3	12	Q8CGB3	UVEAL AUTOANTIGEN WITH COILED-COIL DOMAINS AND ANKYRIN REPEATS PROTEIN	SLM, SLN
335	(K)EIHVNIEAT/F|K/P\SS/EEYLH|I\T\E\PPSLS\PDTk(L)	K2897k	3	11	P13611	VERSICAN CORE PROTEIN [PRECURSOR]	HLW
336	(K)A/G/EA/ST\ET/K/KLII/k|FK|E\K(N)	K250k	3	13	Q61048	WW DOMAIN-BINDING PROTEIN 4	SLC
337	(K)Y/GLLPSTS\NDFKYGL|L|P\GA\PNDF\k(Y)	K663k	3	14	Q9WTY8	ZINC FINGER AND BTB DOMAIN-CONTAINING PROTEIN 10	LVC
338	(K)HQRI|H\S\G/EK\PYV\CD\YCGk(A)	K737k	3	12	Q8WTR7	ZINC FINGER PROTEIN 473	HLW
339	(K)ED/QPG/HT\K\DL\S/GPTKES/SK/GSPkM\Pk(S)	K324k K327k	3	11	Q92610	ZINC FINGER PROTEIN 592	HLW
340	(K)VE/S/ELLCTRL/L\L\GG\GS/YkCI\K(C)	K257k	3	11	*P70338	ZINC FINGER PROTEIN GFI-1	SLM
341	(R)SPGPDYSLR/L/E\TVPAPGRAEGG/AV/S\A\GES\kMEPR(E)	K49k	3	10	*P70338	ZINC FINGER PROTEIN GFI-1	NLW
342	(K)KAE/NSIGKC|PTRTD/V\S|E\K(A)	K22k	3	12	*Q61602	ZINC FINGER PROTEIN GLI3	SLC
343	(K)MD/L/NN/NS/LkT/kA/QV\PMVLT\AGP\K(W)	K141k K143k	3	12	Q8WW38	ZINC FINGER PROTEIN ZFPM2	HLW

Fig. 22H

ANTIBODIES FOR UBIQUITINATED PROTEINS

[0001] This application claims benefit of the filing date of U.S. Provisional Ser. No. 61/058,084, filed Jun. 2, 2008, the contents of which are specifically incorporated herein by reference.

[0002] This invention was made with government support from the National Institute of Allergy and Infectious Diseases under grant number 5R21-AI068639, and from the National Cancer Institute under grant number 5T32CA062948-13. The U.S. government has certain rights in this invention.

BACKGROUND OF THE INVENTION

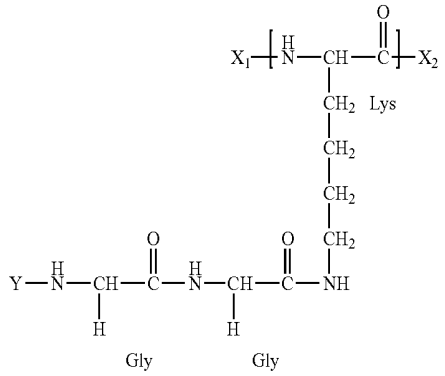
[0003] Ubiquitination has been shown to be a critical step in various cellular processes, including cell division, signal transduction, neurotransmission, and development (Bonifacino and Weissman 1998; Kirkpatrick, Denison et al. 2005), yet elucidation of the proteins targeted by ubiquitination has been difficult because of the technical challenge in recovering modified proteins. The alteration of ubiquitination pathways and patterns of ubiquitination induces many neurodegenerative diseases, such as Alzheimer's disease, Parkinson disease, Huntington disease, and cancers, such as breast cancer, ovarian cancer, as well as numerous other conditions (Jiang and Beaudet 2004). Similarly patterns of ubiquitination can serve as a marker for these diseases or provide insight into the mechanism of diseases or the effects of experimental treatments on cells. Although the mechanisms of these diseases are not the same, all of them significantly alter ubiquitination pathways in one way or another, for example, by mutation, overexpression, or impairment in the function of proteins that directly or indirectly regulate ubiquitination. Ubiquitination can have significant effects on cellular function by affecting protein localization, protein-protein interactions, and protein turnover.

[0004] Unfortunately, not many ubiquitination sites have currently been identified in mammalian cells. Therefore, information on how to manipulate ubiquitination and modulate some of the processes involving ubiquitination is lacking. Furthermore, methods to profile ubiquitination in cells and tissues are lacking, and would require tools that allow for the simple, sensitive, specific, and rapid detection of ubiquitination sites in biological samples.

SUMMARY OF THE INVENTION

[0005] This application describes polyclonal and monoclonal antibodies that have been generated that facilitate identification of ubiquitinated proteins in cells and tissues. These antibodies recognize a fragment of ubiquitin that is created after samples (comprising proteins that are ubiquitinated, either as pure proteins or in a mixture of proteins, such as a cell lysate) are treated with trypsin. The epitope that is recognized by the antibodies includes a diglycine moiety on the epsilon amines of lysines. After cleavage of an ubiquitinated protein, a peptide containing diglycine residues is present on lysine residues within the released peptides. Cleavage can be achieved using a variety of proteases (for example, trypsin or Arg-C can be used to generate a giglycyl moiety on formerly ubiquitinated proteins). The antibodies have been successfully used for immunopurification of ubiquitinated peptides obtained from mammalian cells and many ubiquitination sites have been identified by mass spectrometry of the immunoprecipitated peptides. Use of these antibodies to identify and isolate peptides containing the telltale residues of ubiquitinated lysines from a complex peptide mixture, has many applications. The peptides with these telltale residues can be separated from a complex mixture of peptides, where some peptides contain the these telltale residues and some do not, and analyzed to ascertain the sequences and origins of the ubiquitinated peptides. In addition, the specific sites of ubiquitination within the peptides and the proteins from which the peptides were derived, can be determined. For example, the antibodies described herein can be used to identify specific targets of ubiquitin ligases (there are over 500 in the human genome); to identify ubiquitination events that occur after a drug or other treatment; and to determine the proteins that are affected after a specific ubiquitin ligase inhibitor (e.g. nutlin) is applied to cells. Identification of ubiquitination sites will expand our understanding of the biological role of ubiquitination, will permit the discovery of substrates for specific protein ubiquitination ligases, and will enable the detection of substrates in ubiquitination-related diseases.

[0006] Therefore, one aspect of the invention is an isolated epitope comprising a diglycine (Gly-Gly) present on an epsilon amino group of a lysine in a protein. For example, the diglycine (Gly-Gly) present on the epsilon amino group of the lysine can include the following structure:



[0007] wherein: X_1 is hydrogen or a first peptide sequence;

[0008] X_2 is hydroxy or a second peptide sequence; and

[0009] Y is hydrogen or a ubiquitin peptide, wherein the Y ubiquitin peptide is not a full-length ubiquitin protein.

In some embodiments, the Y group can be Leu-Arg or STLHLVLRLR (SEQ ID NO:344). In other embodiments, the Y group can be hydrogen.

[0010] Another aspect of the invention is an antibody that specifically binds to a diglycine (Gly-Gly) epitope present on an epsilon amino group of a lysine in a protein. For example, the epitope can have the structure provided above, wherein: X_1 is hydrogen or a first peptide sequence; X_2 is hydroxy or a second peptide sequence; and Y is hydrogen or a ubiquitin peptide, wherein the Y ubiquitin peptide is not a full-length ubiquitin protein. Again, the Y group can be Leu-Arg or STLHLVLRLR (SEQ ID NO:344) in some embodiments and in other embodiments, the Y group can be hydrogen. The antibody can be a monoclonal or polyclonal antibody.

[0011] Another aspect of the invention is a method of detecting a ubiquitinated site in a test protein or a mixture of test proteins that comprises

[0012] (i) cleaving a test protein or a mixture of test proteins with a protease that cleaves ubiquitin to form a mixture of cleavage peptides;

[0013] (ii) contacting the cleavage peptides with an antibody that specifically binds to a diglycine (Gly-Gly) epitope present on an epsilon amino group of a lysine; and

[0014] (iii) observing whether the antibody binds to a cleavage peptide, to thereby detect a ubiquitinated site in a test protein or a mixture of test proteins.

The diglycine (Gly-Gly) epitope present on an epsilon amino group of a lysine can have the structure shown above wherein: X_1 is hydrogen or a first peptide sequence; X_2 is hydroxy or a second peptide sequence; and Y is hydrogen or a ubiquitin peptide, wherein the Y ubiquitin peptide is not a full-length ubiquitin protein. In some embodiments, Y is Leu-Arg or STLHLVLRLR (SEQ ID NO:344). In other embodiments, Y is hydrogen. The protease that cleaves ubiquitin can also, in some instances, cleave the test protein or the mixture of test proteins.

[0015] In some embodiments, the protease that cleaves ubiquitin is a protease that cleaves on the C-terminal side of arginine or glutamic acid residues. For example, the protease that cleaves ubiquitin can be trypsin, Arg-C or Glu-C.

[0016] In some embodiments, step (i) is performed in the presence of $H_2{}^{18}O$. Thus, upon hydrolysis of peptide bonds by a protease, the C-terminal hydroxy group will be labeled with heavy oxygen (^{18}O), this allows determination of the amount or proportion of cleavage peptides labeled with ^{18}O. Hence, different samples can be labeled in different ways, even during the proteolytical cleavage step.

[0017] In some embodiments, the method can also include sequencing the cleavage peptide to which the antibody binds to thereby determine the amino acid sequence of the cleavage peptide. For example, the sequencing can be performed by a method comprising mass spectrometry (e.g., LC-MS/MS).

[0018] In some embodiments, the method can also include identifying the ubiquitinated site in the cleavage peptide. The method can further include identifying the test protein from which the cleavage peptide was derived. In addition, the method can also include isolating the test protein.

[0019] The methods and antibodies described herein can be used to identify ubiquitination sites, patterns and profiles in a variety of samples. For example, the sample to be evaluated can be a body fluid, tissue sample, cell lysate, fractionated cellular material, cellular extract, cell culture supernatant, or cultured cells. Similarly, a variety of body fluids can be evaluated using the methods described herein. For example, the body fluid can be saliva, mucous, sweat, whole blood, serum, urine, amniotic fluid, genital fluid, fecal material, marrow, plasma, spinal fluid, pericardial fluid, gastric fluid, abdominal fluid, peritoneal fluid, pleural fluid, synovial fluid, cyst fluid, cerebrospinal fluid, lung lavage fluid, lymphatic fluid, tears, prostatitc fluid, tissue extract, or glandular secretion. In some embodiments, the sample is obtained from a mammal fed a diet containing an isotopically-labeled amino acid. In other embodiments, the sample is obtained from culture media containing an isotopically-labeled amino acid.

[0020] For example, when the sample is a selected cell or tissue type, the method can be adapted to include identifying substantially all prominently ubiquitinated proteins in the cell or tissue type to yield a ubiquitination profile of proteins from the cell or tissue type. In addition, the method can further include comparing the ubiquitination profile of proteins from the cell or tissue type with a ubiquitination profile of proteins from the cell or tissue type after treatment or exposure of the selected cells or tissues to a drug or test agent. The method can further include comparing the ubiquitination profile of proteins from the cell or tissue type with a ubiquitination profile of proteins from cells with a mutation (e.g., a deletion or insertion) or amplification of a gene encoding a ubiquitin ligase, a ubiquitin conjugating enzyme or a ubiquitin activating enzyme. For example, the mutation can substantially eliminate expression or function of an E3 ubiquitin ligase, an E2 ubiquitin conjugating enzyme or an E1 ubiquitin activating enzymes. The amplification of a gene encoding a ubiquitin ligase, a ubiquitin conjugating enzyme or a ubiquitin activating enzyme can lead to overexpression of the ubiquitin ligase, the ubiquitin conjugating enzyme or the ubiquitin activating enzyme.

[0021] Another aspect of the invention is a method of generating an antibody that comprises administering to a mammal an effective amount of an antigen comprising a diglycine (Gly-Gly) epitope linked to an epsilon amino group of a lysine, to generate and immune response against the epitope, to thereby generate an antibody that specifically binds to a diglycine (Gly-Gly) epitope present on an epsilon amino group of a lysine. The lysine to which the diglycine (Gly-Gly) epitope is linked can be part of a larger protein (e.g., a carrier protein, adjuvant molecule or other polymer).

[0022] Thus, the methods described herein can be used to identify the proteins that are ubiquitinated in specific types of cells. For example, cells derived from cancer, or cells and tissues obtained from patients with various diseases that may have altered ubiquitination as a cause or consequence of disease. In addition, the methods can be used to identify proteins that are ubiquitinated in cells that overexpress or have reduced levels of proteins that affect ubiquitination. Moreover, the methods can be used to measure ubiquitination in cells after treatment with a small molecule or a small interfering RNA that is predicted to affect ubiquitination. Assessment of the proteins that are ubiquitinated in these cells constitutes a "profile" of protein ubiquitination. Changes in this profile identify protein ubiquitination events that may be regulated by a specific disease, a protein of interest, or an experimental treatment of interest.

DESCRIPTION OF THE FIGURES

[0023] FIG. **1**A-C illustrates how to make an antibody that selectively recognizes diglycine-modified lysines. FIG. **1**A schematically illustrates antigen synthesis. The ϵ-amine of lysines in histone was modified by Boc-Gly-Gly-NHS and then the Boc group was removed by TFA. The lysines in the final protein contain diglycine adducts on all lysine residues. FIG. **1**B demonstrates the synthesis of diglycine-modified histone. To monitor the reaction of histone with Boc-Gly-Gly-NHS, the presence of amines, such as those in unmodified lysine, was detected by reacting proteins with the amine-modifying agent biotin-NHS, and subsequent western blotting with an anti-biotin antibody. Amines in histone were nearly completely lost after treatment with Boc-Gly-Gly-NHS, indicating near complete modification of all the lysines in histone. Removal of the Boc protecting group with TFA results in the formation of an amine at the N-terminus of the diglycine adduct. This step was essentially complete, as TFA-treated protein exhibited nearly complete recovery of amine reactivity. The position of three samples is slightly shifted due to the alteration of molecular weight and number of positive

charges in the modified and unmodified samples. FIG. **1**C shows the specificity of the purified antibody as evaluated by western blot of protein samples with proteins or lysates in which the lysines were either unmodified (A), or modified with Boc-Gly-Gly (B) or Gly-Gly-(C) adducts, respectively, from β-lactoglobulin, lysozyme, and rat brain lysate.

[0024] FIG. **2**A-E illustrates that ubiquitinated proteins are readily identified by using the procedures and antibodies described herein. FIG. **2**A shows a schematic diagram illustrating one strategy for identifying ubiquitinated proteins by immunoprecipitation of peptides with diglycine-modified lysines and tandem MS analysis. Note that "Ub(Arg-Gly-Gly)" is used to indicate that ubiquitin (with an Arg-Gly-Gly C-terminus) is attached to a ubiquitinated protein via an epsilon amine group of a lysine within the ubiquitinated protein. In this illustration, trypsin is used to cleave the ubiquitinated protein and sever the linkage between the arginine and the glycine near the C-terminus of the ubiquitin protein, thereby liberating a peptide with the diglycyl-lysine epitope, which can be used for immunopurification and detection of the ubiquitination site. FIG. **2**B shows that the methods and antibodies described herein specifically identify ubiquitinated peptides from partially diglycine-modified BSA and lysozyme proteins. The diglycine-modified proteins were trypsinized and immunoprecipitated with control IgG from preimmune serum or with anti-diglycl-lysime antibody. A total of six diglycine-modified peptides (i.d., Gly-Gly-modified peptides) were obtained from BSA and two from diglycine-modified lysozyme using the anti-diglycyl-lysine antibody, while no diglycine-modified peptides were recovered using IgG purified from preimmune serum. An MS/MS spectrum of one diglycine-modified peptide from each protein is presented. FIG. **2**C shows representative spectra of trypsin-digested ubiquitinated peptides obtained from cultured cells and animal tissue. A peptide ion exhibiting loss of Gly-Gly from a lysine is indicated by a shadowed box in the lower panel. Peptide ions exhibiting a loss of Gly-Gly or Gly fragments were observed in 32.3% of the identified proteins. For this analysis, peaks reflecting a Gly-Gly or Gly loss of the appropriate m/z were counted only if they had an intensity of 10% of the most abundant fragment. FIG. **2**D shows the number of ubiquitinated proteins and ubiquitination sites that were identified by LC-MS/MS. The number of proteins identified in experiments conducted by the inventors and reported in the literature is also shown. FIG. **2**E verifies that the antibodies described herein specifically detect ubiquitinated proteins. Proteins from cell cultures and rat brain lysate were purified by GST-S5a pulldown and visualized by western blotting using an antibody directed against the indicated protein. For Trk neurotrophin receptor and dystrophin, proteins were immunoprecipitated and the immunoprecipitate was blotted with an anti-ubiquitin antibody. Agarose beads and protein A or protein G beads were used as a control for nonspecific pulldown and immunoprecipitation.

[0025] FIG. **3**A-D illustrates bioinformatic analysis of ubiquitin remnant-modified lysines. FIG. **3**A shows pie charts of biological processes and subcellular localization of ubiquitinated proteins analyzed by PANTHER and PENCE Proteome Analyst database, respectively. FIG. **3**B illustrates a backbone amino acid sequence analysis of ubiquitinated peptides, showing that the antibodies described herein have no strong backbone sequence preference. A density plot of ratios of ubiquitinated lysines to all lysines was plotted by MATLAB. FIG. **3**C shows the distribution of solvent accessible area (SAA) of all lysines and ubiquitinated lysines as a function of percentage of normalized SAA. The distribution of SAA of ubiquitinated lysines is slightly shifted to a large value. The two distributions are significantly different (χ^2 analysis, $p<0.01$), which mainly resulted from the fraction above 75% SAA. The results were obtained from analysis of 24 PDB structures (25 ubiquitinated lysine and 1066 all lysine residues). FIG. **3**D shows the distribution of secondary structures of all lysines and ubiquitinated lysines obtained from analysis of 24 PDB structures. **p-value<0.01; *p-value<0.05.

[0026] FIG. **4** shows a MS/MS spectrum of a ubiquitinated peptide from Histone H2A, which has a diglycine-modified lysine at C-terminus of the peptide (Lys 120). The results illustrate that Lys 120 in Histone H2A is ubiquitinated. See also, Wang et al., *J Mol Biol* 348, 727-739 (2005); Nickel & Davie, *Biochemistry* 28, 964-968 (1989). Note that in the Swiss-prot database, the histone H2A sequence has a preceding methionine but this methionine was not included in the sequence used in Wang et al. (2005) and therefore the ubiquitinated lysine in that paper is numbered as 119. The presence of C-terminal lysines containing the diglycine modification in some peptides such as the one from Histone H2A, indicates that trypsin can cleave peptides after this modification, albeit less efficiently than after lysines or arginines, presumably because the presence of a positive charge on the amino group of Gly-Gly allows this modified residue to interact with trypsin in a manner similar to the epsilon-amine of lysine.

[0027] FIG. **5**A-D shows representative MS/MS spectra of ubiquitinated peptides from disease-related proteins identified using the methods and antibodies described herein. FIG. **5**A shows a MS/MS spectrum of ubiquitinated peptides from the protein superoxide dismutase, which may play a role in amyotrophic lateral sclerosis (ALS) and other diseases. FIG. **5**B shows a MS/MS spectrum of ubiquitinated peptides from the huntingtin protein involved in Huntington's disease. FIG. **5**C shows a MS/MS spectrum of ubiquitinated peptides from the protein tuberin, which may play a role in Alzheimer's disease. FIG. **5**D shows a MS/MS spectrum of ubiquitinated peptides from the protein Rho-associated protein kinase, which has a variety of effects upon cell adhesion, cell motility, vascular and smooth muscle contraction and cytokinesis.

[0028] FIG. **6**A-C illustrates the frequency of each of the 20 amino acids in the sequences near ubiquitinated lysines in protein sequences obtained from the Swiss-Prot database. FIG. **6**A illustrated the normalized frequency of each of the 20 amino acids within a six amino acid span on either side of ubiquitinated lysines. Normalization was carried out against the frequency calculated for each amino acid using all lysines in all the proteins in the Swiss-Prot database. The ubiquitinated lysines are not counted and every amino acid in the protein in the Swiss-Prot database is counted no more than once. FIG. **6**B shows sequence logo representations of extracted motifs for ubiquitinated peptides, obtained using the Motif-x (Schwartz & Gygi *Nat Biotechnol* 23, 1391-1398 (2005)) search algorithm with a significance of 0.000001. Sequence logos were automatically generated by Motif-x using Weblogo (Schneider et al. *Nucleic Acids Research* 18, 6097-6100 (1990); Crooks et al. Genome Research 14, 1188-1190 (2004)). In sequence logos, the height of each residue is proportional to the frequency in the subset of peptides (Schwartz & Gygi *Nat Biotechnol* 23, 1391-1398 (2005)). FIG. **6**C illustrates a backbone amino acid sequence analysis

of ubiquitinated peptides from yeast. A density plot was prepared as described in FIG. **3**B except that proteins were used that were identified to be ubiquitinated in the yeast from a recent study. Peng et al., *Nat. Biotechnol.* 21, 921-926 (2003). Unlike in mammalian proteins, Asp, Glu, His, Pro are highly enriched (>2.3 times the expected value) at some positions.

[0029] FIG. **7**A-B provides examples of solvent exposed and buried ubiquitinated lysines. FIG. **7**A shows a solvent exposed ubiquitinated lysine for pulmonary surfactant-associated protein (PDB:1PW9). FIG. **7**B shows a solvent buried ubiquitinated site for dystrophin (PDB:1EG3) The snapshots were prepared using PyMOL.

[0030] FIG. **8**A shows the fraction of peptides with two ubiquitination sites as a function of the number of amino acids between the ubiquitination sites. This result indicates that when two lysines are ubiquitinated, they tend to be separated by 0, 1, 4, 5, 7 or 11 amino acids. FIG. **8**B shows the distribution of secondary structures, predicted by PSI-Pred (Jones, *J Mol Biol* 292, 195-202 (1999)) for peptides with two ubiquitination sites. **p-value<0.01:*p-value<0.05.

[0031] FIG. **9**A-C illustrates the specificity of the anti-Gly-Gly-Lysine antibodies described herein. This figure shows a Western blot analysis of the specificity of anti-GG monoclonal antibody (clone 42) for Gly-Gly modified lysines from beta-lactoglobulin (FIG. **9**A), bovine serum albumin (BSA, FIG. **9**B) and rat brain lysate (RBL, FIG. **9**C). The antibody specifically recognizes proteins with Gly-Gly modified lysines and does not interact with unmodified and Boc-Gly-Gly modified proteins. Lane 1 for each of FIG. **9**A-C: intact proteins; lane 2 for each of FIG. **9**A-C: Boc-Gly-Gly-modified proteins; lane 3 for each of FIG. **9**A-C: Gly-Gly-modified proteins.

[0032] FIGS. **10** A and **10**B shows enrichment of a diglycine modified peptide (GlyGly modified PKC substrate 2) with the anti-GG monoclonal antibody (clone 49) from a 1:1 mixture of modified and unmodified peptides. FIG. **10**A and FIG. **10**B show MALDI-TOF-MS spectra of the sample before and after monoclonal antibody purification, respectively.

[0033] FIGS. **11**A, **11**B and **11**C show isolation of a double diglycine modified peptide with monoclonal anti-GG antibody (clone 49) from a bovine serum albumin (BSA) digest. FIGS. **11**A and **11**B are the MALDI-TOF-MS of a peptide containing two diglycine-modified lysines mixed with a BSA digest before and after anti-GG antibody purification, respectively. FIG. **11**C is the MS/MS spectrum of the GG-modified peptide.

[0034] FIG. **12** shows a MS/MS spectrum of a peptide fragment from ubiquitin, which contains a ubiquitin remnant (the diglycine epitope) on K48. This peptide can be detected with either the polyclonal or monoclonal anti-diglycyl-lysine antibodies. In this case, the antibody was immobilized to protein A or protein G beads to facilitate isolation of the ubiquitin peptide containing the diglycine epitope. These results illustrate that ubiquitin can conjugate to other ubiquitin molecules.

[0035] FIG. **13** shows a MS/MS spectrum of a peptide fragment from ubiquitinated TrkB that was digested with trypsin. These results illustrate that membrane-bound ubiquitinated proteins can be detected using the methods and antibodies described herein.

[0036] FIGS. **14**A and **14**B illustrate that monoclonal anti-diglycyl antibodies (mGGAb) can distinguish between diglycyl adducts on the epsilon amine of lysine vs. the alpha-amine of proteins. FIG. **14**A shows a western blot (top) and the corresponding Coomassie stained gel (bottom) of electrophoretically separated angiotensin I and MARCKS polypeptides after reaction with Boc-diglycine-NHS, which will conjugate to primary amines on lysines and on the N-terminus of these proteins. Angiotensin I, which contains zero lysines (indicated as "K") while the MARCKS peptide has five lysines. Because angiotensin I does not contain any epsilon amines, there is only a single diglycine modification on the alpha-amine. This is readily detected by Coomassie staining (lower panel of FIG. **14**A), where the modified protein exhibits a small upward mobility shift. However, this peptide exhibits no reactivity with the anti-diglycyl-lysine monoclonal antibodies ("mGGAb") used to probe the Western blot. In contrast, the MARCKS peptide, which contains five lysines, is reactive with the mGGAb after the MARCKS peptide was treated with Boc-diglycine-NHS and the diglycine epitope was introduced onto each of the five epsilon amines and one alpha-amine of the MARCKS peptide. FIG. **14**A further illustrates the specificity of the anti-diglycyl antibodies (mGGAb) towards diglycyl-modified epsilon amines (but not N-terminal alpha-amines) using the peptide, Ac-MSH-NH_2, which contains no N-terminal alpha-amine (the alpha-amine is acetylated), and just one lysine. As shown for the MARCKS peptide in FIG. **14**A, the conjugation of the diglycine to the Ac-MSH-NH_2 peptide is readily detected when (FIG. **14**B). This is in contrast to Angiotensin I, where no diglycine epitopes are detected. The immunoreactivity of mGGAb for the Ac-MSH-NH_2 peptide therefore reflects binding of mGGAb to internal diglycyl-lysine, because the alpha-amine of the Ac-MSH-NH_2 peptide is acetylated.

[0037] FIG. **15**A-C illustrates that the polyclonal anti-diglycyl-lysine antibodies described herein immunoprecipitate peptides with GlyGly-modified lysines but not peptides with N-terminal GlyGly. FIG. **15**A shows the MALDI-TOF-MS spectrum of a mixture of angiotensin I (one N-terminal GlyGly modification) and GlyGly-modified acetylated MSH peptide (one GlyGly-modified lysine). Both peptides are present in approximately equal amounts. FIG. **15**B shows the MALDI-TOF-MS spectrum of angiotensin I/acetylated MSH peptides that bound to the polyclonal anti-diglycyl-lysine antibodies. The GlyGly-modified acetylated MSH peptide, with one internal lysine is present in substantially greater amounts, indicating that this peptide bound to the antibodies whereas the angiotensin I peptide with one N-terminal Gly-Gly did not. FIG. **15**C shows the MALDI-TOF-MS spectrum of angiotensin I/acetylated MSH peptides that did not bind to the polyclonal anti-diglycyl-lysine antibodies. The angiotensin I peptide with one N-terminal Gly-Gly is present in greater amounts than the peptide with the internal Gly-Gly-lysine. These data illustrate that the anti-diglycyl-lysine antibodies can facilitate specific purification of peptides that have GlyGly-modified lysines.

[0038] FIG. **16**A-B illustrate that one of the monoclonal anti-diglycyl-lysine antibody preparations described herein can detect GlyGly-Lysine-modified peptides in amounts at least as low as 170 femtomoles. FIG. **16**A shows a dot blot spotted with a serial dilution of insulin (molecular weight ~5800 Da), which contains only one lysine in its heavy chain that was conjugated to the Gly-Gly moiety using procedures and reagents as described herein. The amount of insulin (in ng) and the corresponding molar quantity (in pmol) was as listed above and below, respectively, the dot blot. As shown, monoclonal antibody 49 binds effectively to amounts of the

GlyGly-lysine-conjugated insulin as low as 2 nanograms (340 fmoles). FIG. **16**B also shows a dot blot spotted with a serial dilution of insulin conjugated to the Gly-Gly moiety. The amount of insulin (in ng) and the corresponding molar quantity (in pmol) was as listed above and below, respectively, the dot blot. As shown, monoclonal antibody 49 binds effectively to amounts of the GlyGly-lysine-conjugated insulin as low as 1 nanogram (170 fmoles).

[0039] FIG. **17**A-B show that affinity purified monoclonal anti-diglycyl-lysine antibodies can detect GlyGly-modified proteins. The tissue culture media in which the hybridoma cell line expressing anti-diglycyl-antibody #49 was growing (ascites) was used as a source of monoclonal antibody and the antibody was purified using diglycine-modified BSA immobilized on Affi-gel. FIG. **17**A shows a silver-stained gel of electrophoretically separated BSA from the flow-through from the Affi-gel (first three lanes) in the gel after the media was incubated with the immobilized diglycine-modified BSA. A blot to detect antibody was also performed and shown above the silver-stained gel. This blot shows that the antibody is depleted from the media by incubation with the immobilized diglycine-modified BSA. Acid elution (last four lanes of FIG. **17**A), results in the elution of specific antibody bands, which can be seen in both the silver stain and Western blot at the top of FIG. **17**A. FIG. **177**B shows a Western dot blot of diglycine-modified lactoglobulin which was separated by SDS-PAGE and transferred to PVDF. The purified antibody readily recognizes 0.01 μg of this protein. Thus, the purified antibody has a high purity and can recognize GlyGly-modified proteins at least at nanogram level.

[0040] FIG. **18** is a schematic diagram illustrating one procedure to quantify ubiquitinated proteins in cells or tissues treated with a drug or other agent, that involves quantitative mass spectrometry. Cells are treated either by vehicle or drug for a period of time in the presence of proteasome inhibitor, such as LLnL. The cells are lysed and then digested with trypsin. The lysate from vehicle-treated cells (vehicle) is digested with protease in the presence of light water ($H_2{}^{16}O$), while the lysate from drug-treated cells is digested with protease in the presence of heavy water ($H_2{}^{18}O$). Therefore the carboxyl termini of cleaved (e.g., tryptic) peptides are labeled differently in the samples from drug treated (^{18}O) and non-treated (^{16}O) cells. The digested samples are combined with and immunopurified by the anti-diglycyl-lysine antibody, then the enriched ubiquitinated peptides are analyzed by tandem mass spectrometry (MS/MS). The sequences determined by MS/MS (e.g., by mass spectrometry software) are used to search protein databases to determine from which protein(s) the peptide(s) originated. The relative quantity of diglycyl-lysine tagged peptides can be determined by extracted ion chromatography (EIC) for the peptides of interest, by observation of the intensity ratios of the light form (^{16}O) and heavy form (^{18}O) of the peptides from, so that the effect of drug on alternation of ubiquitination levels can be investigated. This approach can also be used for quantification of protein ubiquitination between normal tissue and patient tissue for disease diagnostics when the proteasome inhibitor step is skipped. Here protein labeling can also been achieved by SILAC (stable isotope labeling by amino acid in cell culture) for cells.

[0041] FIG. **19** is a graph of the ratio of ubiquitination of a peptide from control (L) cells relative to the ubiquitination of the same peptide from cells exposed to inhibitor of nitric oxide synthase, NAME (N-nitro-L-arginine methyl ester) (H), illustrating how protein ubiquitination changes in murine endothelioma (sEnd.1) cells exposed to NAME (N-nitro-L-arginine methyl ester).

[0042] FIG. **20** is a graph of the ratio of ubiquitination of a peptide from control (L) cells relative to the ubiquitination of the same peptide from cells exposed to a nitric oxide producer, GSNO(S-nitrosoglutathione)(H), illustrating how protein ubiquitination changes in mouse N2a neuroblastoma cells exposed to GSNO(S-nitrosoglutathione).

[0043] FIG. **21**A-D shows that the types of biological processes in which various ubiquitinated proteins are involved varies depending upon the cell type. FIG. **21**A shows the types of biological processes in which rat tissue ubiquitinated cells are involved. FIG. **21**B shows the types of biological processes in which murine endothelioma (sEnd.1) ubiquitinated cells are involved. FIG. **21**C shows the types of biological processes in which mouse neuroblastoma (N2a) ubiquitinated cells are involved. FIG. **21**D shows the types of biological processes in which human embryo kidney (HEK293) ubiquitinated cells are involved.

[0044] FIG. **22**A-H*is* a table listing LC-MS/MS identified diglycine-modified peptides with determined ubiquitination sites. The sequences of ubiquitinated peptides with SEQ ID NO:1-343, 345 and 349-350 are provided. The symbols, \, / and |, represent b-ions, y-ions, and both b-ions and y-ions, respectively. The location of the ubiquitinated lysine in the protein from which each peptide is derived is identified under the column "Ubiquitinated Lysines," using the notation "K" followed by the numerical position of the ubiquitinated lysine in the protein and a lower case "k" to indicate modification by diglycine on the indicated lysine. Proteins with isoforms previously reported to be ubiquitinated are indicated by a "*" in the accession ID column. The parentheses around N-terminal and C-terminal amino acids indicate that these amino acids are not actually present in the sequenced peptide but are present in the larger protein from which the peptide was derived. When a dash surrounded by parentheses is present (-), there is no preceding amino acid because the identified peptide is present at the end of the protein from it is derived. The charge of the peptide is listed under the column labeled "z." The three-letter code in "Fraction" column provides the following information: (1) the first letter indicates the origin of the sample, where H means HEK293, N means N2a, S means sEnd.1, B means rat brain, and L means rat liver; (2) the second letter indicates the treatment, L for LLnL and V for vehicle; (3) the third letter indicates the subcellular fraction, where W means whole lysate, C means cytosolic fraction, M means mitochondrial fraction, N means nuclear fraction, and S means synaptosomal fraction. Ubiquitin remnant-containing peptides that are derived from ubiquitin itself (i.e., polyubiquitin) have been excluded from this table.

DETAILED DESCRIPTION OF THE INVENTION

[0045] The invention relates to an antigen that includes a diglycine moiety on the epsilon (ϵ) amino group of lysine. This antigen can be used to generate antibodies that recognize ubiquitinated proteins. Antibodies directed against such diglycine-lysine-containing epitopes surprisingly recognize ubiquitination in a large variety of proteins, and are highly effective in binding to proteins and peptides that contain a diglycine adduct on lysines, thereby providing a reagent to efficiently isolate, enrich, and/or purify these peptides. These

antibodies exhibit considerable selectivity, insofar as they exhibit negligible binding to peptides or proteins that lack diglycine adducts.

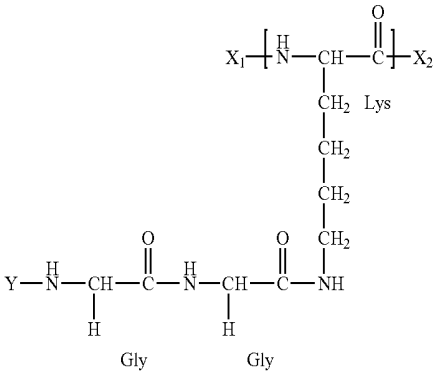
[0046] Antibodies are typically generated against large epitopes, to help insure that the antibody has the specificity and affinity that are needed for antibody binding. Also, antibodies are typically difficult to generate against epitopes that contain repeated amino acids (See Prediction of protein antigenic determinants from amino acid sequences, T P Hopp and K R Woods, Proc. Natl. Acad. Sci. U.S.A., 78: 3824-3828, 1981). However, as described herein the diglycyl-lysine antigen serves as an epitope that can result in the generation of antibodies with sufficient affinity to specifically bind diglycine-modified proteins in numerous applications, such as immunopurification, Western blotting, and dot blotting.

[0047] Ubiquitin is a small (76-amino acid) protein that is ubiquitously expressed in eukaryotes. Ubiquitination refers to the post-translational modification of a protein by the covalent attachment of one or more ubiquitin molecules to epsilon (ϵ) amine groups present on lysine residues in the protein to be ubiquitinated. The most prominent function of ubiquitin is labeling proteins for proteasomal degradation. Besides this function, ubiquitination also controls the stability, function, and intracellular localization of a wide variety of proteins, which complicates the isolation and identification of peptides containing this modification.

[0048] This application describes a method to identify ubiquitinated proteins and ubiquitin-modified lysine residues. In this approach, ubiquitinated proteins are proteolyzed to peptides containing a ubiquitin-derived diglycine adduct which are then affinity isolated and analyzed. Using this approach, the inventors have prepared ubiquitination profiles of specific cell types, and have identified more than 300 mammalian ubiquitinated proteins comprising nearly 400 diglycine-modified lysines. Analysis of these proteins provides insight into ubiquitination consensus sites, potential biological roles for ubiquitination and establishes that the antibodies described herein can be used to profile ubiquitination in biological samples.

Epitopes

[0049] An epitope that is useful for generating antibodies that specifically bind to protein targets of ubiquitination, includes the following structure, where the brackets indicate that the diglycine-lysine epitope can be present in a selected protein:



[0050] wherein X_1 is hydrogen or a first peptide sequence; X_2 is hydroxy or a second peptide sequence; and Y is hydrogen or a ubiquitin peptide, wherein the Y ubiquitin peptide is not a full-length ubiquitin protein. For example, Y can be Leu-Arg or STLHLVRLR (SEQ ID NO:344). The first and second peptide sequences can be peptide sequences from a selected protein of interest (e.g., a carrier). For example, the first peptide sequence can be a first carrier peptide sequence and/or the second peptide sequence can be a second carrier peptide sequence.

[0051] Such an epitope is present on ubiquitinated proteins because ubiquitination occurs by linkage of the C-terminal glycine of ubiquitin to ϵ-amine groups of lysines present in the protein target of ubiquitination. Thus, sequences for human ubiquitin typically contain Gly-Gly at the C-terminus, for example, as illustrated by the following sequence for human ubiquitin (SEQ ID NO:346):

```
1  MQIFVKTLTG KTITLEVEPS DTIENVKAKI QDKEGIPPDQ

41 QRLIFAGKQL EDGRTLSDYN IQKESTLHLV LRLRGG
```

Thus the C-terminus of human ubiquitin typically includes Gly-Gly, and the sequence STLHLVLRLRGG (SEQ ID NO:347) is a longer C-terminal ubiquitin sequence. As described herein, an epitope that includes the Gly-Gly-lysine sequence, where the carboxylate group the Gly-Gly is linked to the ϵ-amine of a lysine, can be used to generate antibodies that specifically recognize ubiquitinated proteins and peptides.

[0052] However, antigenic epitopes useful for making such antibodies can have ubiquitin sequences in addition to the C-terminal Gly-Gly ubiquitin sequences. The length of the epitope amino acid Y sequence extending from the N-terminal glycine shown above can therefore vary. For example, in some embodiments, the epitope can include a Leu-Arg-Gly-Gly (SEQ ID NO:348) moiety that is linked through the C-terminal glycine to an ϵ-amine on a lysine in the same fashion as shown above. The Leu-Arg-Gly-Gly (SEQ ID NO:348) sequence is a longer portion of the C-terminal sequence of ubiquitin. Thus an epitope that includes SEQ ID NO:348 has additional ubiquitin sequences and these additional sequences will be present on ubiquitinated proteins naturally present in various samples that may be analyzed. While the methods described herein for identifying ubiquitination sites typically involve cleavage and removal of most of the ubiquitin sequences, the amount of ubiquitin sequences left on a ubiquitination site varies depending upon the protease selected for cleavage and removal of the ubiquitin. For example, it has been observed that after treatment with trypsin, which cleaves proteins on the C-terminal side of arginine and lysine, most ubiquitinated proteins are cleaved so that peptides with the ubiquitinated site have a diglycine linked to the ϵ-amine of lysine. However, about 20% of these ubiquitinated sites are partially cleaved, which leaves four C-terminal residues, Leu-Arg-Gly-Gly (SEQ ID NO:348), from ubiquitin linked to the ϵ-amine of a lysine residue. Therefore, another epitope of the invention includes Leu-Arg-Gly-Gly (SEQ ID NO:348) linked to the ϵ-amine group of lysine.

[0053] In other embodiments, the epitope is a STLHLVLRLRGG (SEQ ID NO:347) moiety, where the C-terminal glycine of this SEQ ID NO:347 peptide is linked to the

ϵ-amine on a lysine. Such an epitope can be exposed and is readily detectable when ubiquitinated proteins are cleaved with Glu-C.

[0054] One reason to contemplate use of epitopes that include the SEQ ID NO:347 and/or SEQ ID NO:348 sequences is that the Gly-Gly-lysine epitope is very small, with just two glycines on the epsilon amine of lysine residues. Thus, in some cases the affinity and/or specificity of an anti-ubiquitinated site antibody can be improved by use of a longer antigen. The STLHLVLRLRGG (SEQ ID NO:347) moiety linked to the ϵ-amine on a lysine is formed as an epitope after Glu-C cleavage of ubiquitinated proteins, because the Glu-C protease is an enzyme that cleaves on the C-terminal side of glutamic acid. Thus, when a ubiquitin conjugate is cleaved with Glu-C, the remnant from ubiquitin on the ubiquitination sites, is STLHLVLRLRGG (SEQ ID NO:347).

[0055] The diglycine-lysine containing epitope(s) can be placed on one or more lysine residues in a selected protein. Such a protein can be carrier that facilitates generation of antibodies against the epitopes described herein. However, the epitopes described herein can also be placed on any selected protein of interest. In addition, the diglycine-lysine containing epitope(s) can be placed on the amino terminus of a selected protein and/or on any of the lysines present within the selected protein.

[0056] In some embodiments, the diglycine-lysine containing epitope is placed on one lysine, or two lysines, or three lysines, or four lysines, or five lysines, or six lysines, or seven lysines or eight lysines, or nine lysines, or ten lysines, or eleven lysines, or twelve lysines, thirteen lysines, or fourteen lysines, or fifteen lysines, or more than fifteen lysines.

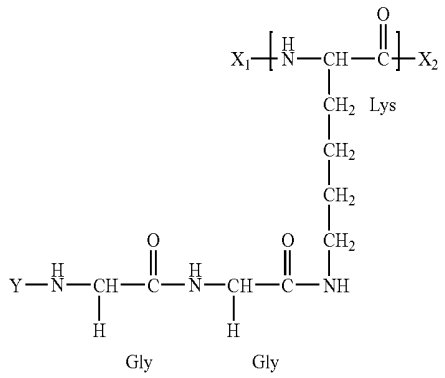
[0057] Multiple copies of the diglycine-lysine containing epitopes can be placed on a selected protein by a method that involves reacting t-butyloxycarbonyl-peptide epitope-N-hydroxysuccinimide with the selected protein. For example, t-butyloxycarbonyl-Gly-Gly-N-hydroxysuccinimide can be reacted with the selected protein. Alternatively, the selected protein can be reacted with t-butyloxycarbonyl-Leu-Arg-Gly-Gly-N-hydroxysuccinimide or with t-butyloxycarbonyl-STLHLVLRLRGG-N-hydroxysuccinimide. Such a reaction can be carried out at room temperature using dimethyl sulfoxide (DMSO) as solvent. The reaction between the selected protein and t-butyloxycarbonyl-peptide epitope-N-hydroxysuccinimide can be repeated to facilitate complete reaction between the amino groups on the selected protein and the t-butyloxycarbonyl-peptide epitope-N-hydroxysuccinimide. The t-butyloxycarbonyl (Boc) group can be removed using trifluoroacetic acid (TFA) to yield a protein with one or more epitopes on the selected protein.

[0058] The protein selected for linkage to an epitope peptide can be any protein of interest to one of skill in the art. The selected protein can be from any species. For example, the selected protein can be a protein from any human or domesticated animal. Examples of species from which the selected protein can be obtained or derive include human, mouse, rat, rabbit, sheep, goat, horse, dog, cat, bird, plant, yeast, and other species.

[0059] The selected protein can have a function of interest or be obtained from a location of interest. For example, the protein can be an enzyme, a contractile protein, a structural protein, a hormonal protein, a storage protein, a transport protein, a secreted protein, a cell membrane protein, a cytoplasmic membrane, a nuclear protein, a protein involved in metabolism, a protein involved in cellular replication, a protein involved in chromosomal replication, a protein involved in transcription of DNA into RNA, as well as other types of proteins. In some embodiments, the protein selected for linkage to a diglycine-lysine epitope is a protein involved in a disease, disorder or condition. Further examples of proteins that can be selected for linkage to a diglycine-lysine epitope are available in various databases, for example, the Swiss-Prot database. FIG. **22** illustrates sequences of a variety of proteins that have, or can have, one or more diglycine-lysine epitopes.

Antibodies Directed Against the DiGlycine-Lysine Epitopes

[0060] Another aspect of the invention is an antibody that specifically binds to a diglycine-lysine epitope, or to a Leu-Arg-Gly-Gly-lysine (where the Leu-Arg-Gly-Gly (SEQ ID NO:348) peptide is linked to the ϵ-amine group of lysine), or to STLHLVLRLRGG-lysine (where the STLHLVLRLRGG (SEQ ID NO:347) peptide is linked to the ϵ-amine group of lysine). Thus, the antibody can specifically bind to an epitope that includes the following structure:



[0061] wherein X_1 is hydrogen or a first peptide sequence; X_2 is hydroxy or a second peptide sequence; and Y is hydrogen or a ubiquitin peptide, wherein the Y ubiquitin peptide is not a full-length ubiquitin protein. For example, Y can be Leu-Arg or STLHLVLRLR (SEQ ID NO:344). The first and second peptide sequences can be peptide sequences from a selected protein of interest (e.g., a carrier). For example, the first peptide sequence can be a first carrier peptide sequence and/or the second peptide sequence can be a second carrier peptide sequence.

[0062] The term "antibody," as used herein, refers to a full-length immunoglobulin molecule or an immunologically-active fragment of an immunoglobulin molecule such as the Fab or $F(ab')_2$ fragment generated by, for example, cleavage of the antibody with an enzyme such as pepsin or co-expression of an antibody light chain and an antibody heavy chain in bacteria, yeast, insect cell or mammalian cell. The antibody can also be an IgG, IgD, IgA, IgE or IgM antibody. In addition, the term "antibody" can include antibody fragment s (e.g., CDR) linked or fused to other polypeptides. Moreover, the "antibody" as used herein means a single antibody or antibody type as well as a plurality of antibodies or types of antibodies.

[0063] As used herein, the term "binds specifically" or "specifically binds," in reference to an antibody/antigen interaction, means that the antibody binds with a particular antigen (e.g., the diglycine-lysine or other epitopes described herein)

without substantially binding to other unrelated antigens. For example, the antibody has at least 50% or greater affinity, preferably about 75% or greater affinity, and more preferably, about 90% or greater affinity, to a particular polypeptide than to other unrelated polypeptides.

[0064] An antibody directed against the epitope(s) can be a polyclonal or monoclonal antibody. Polyclonal antibodies can be obtained by immunizing an animal (e.g., a mammal or bird) with a peptide or protein having one or more of the epitopes of the invention, and then isolating antibodies from the blood of the mammal using standard techniques including, for example, enzyme linked immunosorbent assay (ELISA) to determine antibody titer and protein A chromatography to obtain the antibody-containing IgG fraction.

[0065] A monoclonal antibody is a population of molecules having a common antigen binding site that binds specifically with a particular antigenic epitope. A monoclonal antibody can be obtained by selecting an antibody-producing cell from a mammal that has been immunized with one or more of the epitopes of the invention and fusing the antibody-producing cell, e.g. a B cell, with a myeloma to generate an antibody-producing hybridoma. A monoclonal antibody of the invention can also be obtained by screening a recombinant combinatorial library such as an antibody phage display library using, for example, a peptide or protein with one of the epitopes of the invention. See, for example, PHAGE DISPLAY—A LABORATORY MANUAL, Barbas, et al., eds. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001; and Kontermann & Dübel, ANTIBODY ENGINEERING, Heidelberg: Springer-Verlag. Berlin, 2001. A monoclonal antibody of the invention can also be obtained by screening a recombinant combinatorial library such as a ribosome display library. See for example, Expert Review of Proteomics, 2: 421-430, 2005. Ribosome display: next-generation display technologies for production of antibodies in vitro. Mingyue He and Farid Khan.

[0066] An antibody of the invention can also be a murine, goat, sheep, rabbit, chimeric, humanized or fully human antibody. A murine, goat, rabbit, primate or sheep antibody is an antibody derived entirely from a murine, goat, rabbit, primate or sheep source, for example, an antibody derived from a murine, goat, rabbit, primate or sheep hybridoma generated from the fusion of a murine, goat, rabbit, primate or sheep myeloma cell and a murine, goat or sheep B-lymphocyte cell. A chimeric antibody is an antibody that has variable regions derived from a non-human source, e.g. murine, rabbit, goat or sheep or primate, and constant regions derived from a human source. A humanized antibody has antigen-binding regions, e.g. complementarity-determining regions, derived from a mouse source, and the remaining variable regions and constant regions derived from a human source. A fully human antibody is antibody from human cells or derived from transgenic mice carrying human antibody genes.

[0067] Methods to generate antibodies are well known in the art. For example, a polyclonal antibody of the invention can be prepared by immunizing a suitable animal with peptide or protein having one or more of the epitopes of the invention. The animal can be, for example, a rabbit, goat, sheep, rabbit, hamster, cow, chicken, monkey or mouse. At the appropriate time after immunization, antibody molecules can be isolated from the animal, e.g. from the blood or other fluid of the animal, and further purified using standard techniques that include, without limitation, precipitation using ammonium sulfate, gel filtration chromatography, ion exchange chromatography or affinity chromatography using protein A. In addition, an antibody-producing cell of the animal can be isolated and used to prepare a hybridoma cell that secretes a monoclonal antibody of the invention. Techniques for preparing monoclonal antibody-secreting hybridoma cells are known in the art. See, for example, Kohler and Milstein, *Nature* 256:495-97 (1975) and Kozbor et al. *Immunol Today* 4: 72 (1983). A monoclonal antibody of the invention can also be prepared using other methods known in the art, such as, for example, expression from a recombinant DNA molecule, or screening of a recombinant combinatorial immunoglobulin library using a mutant polypeptide of the invention.

[0068] Methods to generate chimeric and humanized monoclonal antibodies are also well known in the art and include, for example, methods involving recombinant DNA technology. A chimeric antibody can be produced by expression from a nucleic acid that encodes a non-human variable region and a human constant region of an antibody molecule. See, for example, Morrison et al., *Proc. Nat. Acad. Sci. U.S.A.* 86: 6851 (1984). A humanized antibody can be produced by expression from a nucleic acid that encodes non-human antigen-binding regions (complementarity-determining regions) and a human variable region (without antigen-binding regions) and human constant regions. See, for example, Jones et al., *Nature* 321:522-24 (1986); and Verhoeven et al., *Science* 239:1534-36 (1988). Completely human antibodies can be produced by immunizing engineered transgenic mice that express only human heavy and light chain genes. In this case, therapeutically useful monoclonal antibodies can then be obtained using conventional hybridoma technology. See, for example, Lonberg & Huszar, *Int. Rev. Immunol.* 13:65-93 (1995). Nucleic acids and techniques involved in design and production of antibodies are well known in the art. See, for example, Batra et al., *Hybridoma* 13:87-97 (1994); Berdoz et al., *PCR Methods Appl.* 4: 256-64 (1995); Boulianne et al. *Nature* 312:643-46 (1984); Carson et al., *Adv. Immunol.* 38 :274-311 (1986); Chiang et al., *Biotechniques* 7 :360-66 (1989); Cole et al., *Mol. Cell. Biochem.* 62 :109-20 (1984); Jones et al., *Nature* 321: 522-25 (1986); Larrick et al., *Biochem Biophys. Res. Commun.* 160 :1250-56 (1989); Morrison, *Annu. Rev. Immunol.* 10 :239-65 (1992); Morrison et al., *Proc. Nat'l Acad. Sci. USA* 81: 6851-55 (1984); Orlandi et al., *Pro. Nat'l Acad. Sci. U.S.A.* 86:3833-37 (1989); Sandhu, *Crit. Rev. Biotechnol.* 12:437-62 (1992); Gavilondo & Larrick, *Biotechniques* 29: 128-32 (2000); Huston & George, *Hum. Antibodies.* 10:127-42 (2001); Kipriyanov & Le Gall, *Mol. Biotechnol.* 26: 39-60 (2004).

[0069] Antibodies that react and bind to the epitopes described herein may also be isolated from phage antibody libraries using the techniques described in Clackson et al. *Nature* 352: 624-628 (1991), as well as in Marks et al., *J. Mol. Biol.* 222: 581-597 (1991) and U.S. patent application Ser. No. 10/939,309.

[0070] Another method for generating antibodies involves a Selected Lymphocyte Antibody Method (SLAM). The SLAM technology permits the generation, isolation and manipulation of monoclonal antibodies without the process of hybridoma generation. The methodology principally involves the growth of antibody forming cells, the physical selection of specifically selected antibody forming cells, the isolation of the genes encoding the antibody and the subsequent cloning and expression of those genes.

[0071] The antibodies can be mutated to optimize their affinity, selectivity, binding strength or other desirable property. A mutant antibody refers to an amino acid sequence variant of an antibody. In general, one or more of the amino acid residues in the mutant antibody is different from what is present in the reference antibody. Such mutant antibodies necessarily have less than 100% sequence identity or similarity with the reference amino acid sequence. In general, mutant antibodies have at least 75% amino acid sequence identity or similarity with the amino acid sequence of either the heavy or light chain variable domain of the reference antibody. Preferably, mutant antibodies have at least 80%, more preferably at least 85%, even more preferably at least 90%, and most preferably at least 95% amino acid sequence identity or similarity with the amino acid sequence of either the heavy or light chain variable domain of the reference antibody.

[0072] The antibodies of the invention are isolated antibodies. An isolated antibody is one that has been identified and separated and/or recovered from a component of the environment in which it was produced. Contaminant components of its production environment are materials that would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. The term "isolated antibody" also includes antibodies within recombinant cells because at least one component of the antibody's natural environment will not be present. Ordinarily, however, isolated antibody will be prepared by at least one purification step.

[0073] If desired, the antibodies of the invention can be purified by any available procedure. For example, the antibodies can be affinity purified by binding an antibody preparation to a solid support to which the antigen used to raise the antibodies is bound. After washing off contaminants, the antibody can be eluted by known procedures. Those of skill in the art will know of various techniques common in the immunology arts for purification and/or concentration of polyclonal antibodies, as well as monoclonal antibodies (see for example, Coligan, et al., Unit 9, *Current Protocols in Immunology*, Wiley Interscience, 1991, incorporated by reference).

[0074] Monoclonal antibodies can be isolated and purified from hybridoma cultures by a variety of well-established techniques. Such isolation techniques include affinity chromatography with Protein-A Sepharose, size-exclusion chromatography, and ion-exchange chromatography. See, e.g., Coligan, et al., sections 2.7.1-2.7.12 and sections 2.9.1-2.9.3; Barnes, et al., Purification of Immunoglobulin G (IgG), in: METHODS IN MOLECULAR BIOLOGY, Vol. 10, pages 79-104 (Humana Press (1992).

[0075] In some embodiments, the antibody will be purified as measurable by at least three different methods: 1) to greater than 95% by weight of antibody as determined by the Lowry method, and most preferably more than 99% by weight; 2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator; or 3) to homogeneity by SDS-PAGE under reducing or non-reducing conditions using Coomassie blue or, preferably, silver stain.

[0076] The antibodies described herein include immunologically-active fragments of antibodies. Methods of making antibody fragments are also known in the art (see for example, Harlow and Lane, ANTIBODIES: A LABORATORY MANUAL, Cold Spring Harbor Laboratory, New York, (1988), incorporated herein by reference). Antibody fragments of the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* of DNA encoding the fragment. Antibody fragments can be obtained by pepsin or papain digestion of whole antibodies conventional methods. For example, antibody fragments can be produced by enzymatic cleavage of antibodies with pepsin to provide a 5S fragment denoted $F(ab')_2$. This fragment can be further cleaved using a thiol reducing agent, and optionally a blocking group for the sulfhydryl groups resulting from cleavage of disulfide linkages, to produce 3.5S Fab monovalent fragments. Alternatively, an enzymatic cleavage using pepsin produces two monovalent Fab' fragments and an Fc fragment directly. These methods are described, for example, in U.S. Pat. No. 4,036,945 and No. 4,331,647, and references contained therein. These patents are hereby incorporated in their entireties by reference.

[0077] Other methods of cleaving antibodies, such as separation of heavy chains to form monovalent light-heavy chain fragments, further cleavage of fragments, or other enzymatic, chemical, or genetic techniques may also be used, so long as the fragments bind to the antigen that is recognized by the intact antibody. For example, Fv fragments comprise an association of V_H and V_L chains. This association may be noncovalent or the variable chains can be linked by an intermolecular disulfide bond or cross-linked by chemicals such as glutaraldehyde. Preferably, the Fv fragments comprise V_H and V_L chains connected by a peptide linker. These single-chain antigen binding proteins (sFv) are prepared by constructing a structural gene comprising DNA sequences encoding the V_H and V_L domains connected by an oligonucleotide. The structural gene is inserted into an expression vector, which is subsequently introduced into a host cell such as *E. coli*. The recombinant host cells synthesize a single polypeptide chain with a linker peptide bridging the two V domains. Methods for producing sFvs are described, for example, by Whitlow, et al., *Methods: a Companion to Methods in Enzymology*, Vol. 2, page 97 (1991); Bird, et al., Science 242:423-426 (1988); Ladner, et al, U.S. Pat. No. 4,946,778; and Pack, et al., *Biotechnology* 11:1271-77 (1993).

[0078] Another form of an antibody fragment is a peptide coding for a single complementarity-determining region (CDR). CDR peptides ("minimal recognition units") can be obtained by constructing genes encoding the CDR of an antibody of interest. Such genes are prepared, for example, by using the polymerase chain reaction to synthesize the variable region from RNA of antibody-producing cells. See, for example, Larrick, et al., *Methods: a Companion to Methods in Enzymology*, Vol. 2, page 106 (1991). The antibodies described herein can be any CDR-containing polypeptides.

[0079] The antibodies that specifically bind to the diglycine-lysine epitope, and/or to the Leu-Arg-Gly-Gly-lysine epitope, and/or to the STLHLVLRLRGG-lysine epitope can be immobilized onto a solid support. Such immobilized antibodies can facilitate purification and analysis of peptides and proteins that have any of these epitopes. Examples of solid supports to which the antibodies can be immobilized include water miscible particles, such as agarose beads, sepharose beads, column chromatographic matrix materials, microtiter dish wells, and the like. Attachment can be through peptide/protein amine, sulfhydryl, carboxy, carboxylate and other reactive groups in the peptide/protein. For example, the peptides/proteins can be attached to an amine on the surface of the solid support under native condition. Such immobilized antibodies can, for example, be used for immune-purification

of peptides generated from ubiquitinated proteins after cleavage with a protease (e.g., trypsin, Glu-C or Arg-C).

[0080] The antibodies that bind to the diglycine-lysine epitope, and/or to the Leu-Arg-Gly-Gly-lysine epitope, and/or to the STLHLVLRLRGG-lysine epitope can be linked to detectable labels. Alternatively, labeled secondary antibodies can be used to detect the antibodies that bind specifically to the diglycine-lysine epitope, and/or to the Leu-Arg-Gly-Gly-lysine epitope, and/or to the STLHLVLRLRGG-lysine epitope.

[0081] Any detectable label known in the art can be linked to the antibodies, including, for example, a radioactive label (e.g., ^{3}H, ^{125}I, ^{35}S, ^{14}C, ^{32}P, ^{33}P), an enzymatic label, such as horseradish peroxidase, alkaline peroxidase, glucose 6-phosphate dehydrogenase, etc., a chemiluminescent label, such as, acridinium derivatives, luminol, isoluminol, thioesters, sulfonamides, phenanthridinium esters, etc. a fluorescence label, such as, fluorescein (5-fluorescein, 6-carboxyfluorescein, 3'6-carboxyfluorescein, 5(6)-carboxyfluorescein, 6-hexachloro-fluorescein, 6-tetrachlorofluorescein, fluorescein isothiocyanate, etc.), rhodamine, phycobiliproteins, R-phycoerythrin, quantum dots (zinc sulfide-capped cadmium selenide), a thermometric label or an immuno-polymerase chain reaction label. An introduction to labels, labeling procedures and detection of labels is found in Polak and Van Noorden, INTRODUCTION TO IMMUNOCYTOCHEMISTRY, 2nd ed., Springer Verlag, N.Y. (1997) and in Haugland, HANDBOOK OF FLUORESCENT PROBES AND RESEARCH CHEMI (1996), which is a combined handbook and catalogue published by Molecular Probes, Inc., Eugene, Oreg., each of which is incorporated herein by reference. Additional detail can be found in Mattingly, P. G., and Adamczyk, M. (2002) *Chemiluminescent N-sulfonylacridinium-9-carboxamides and their application in clinical assays*, in LUMINESCENCE BIOTECHNOLOGY: INSTRUMENTS AND APPLICATIONS (Dyke, K. V., Ed.) pp 77-105, CRC Press, Boca Raton.

[0082] The detectable label can be bound to the antibody or secondary antibody either directly or through a coupling agent. An example of a coupling agent that can be used is EDAC (1-ethyl-3-(3-dimethylaminopropyl) carbodiimide, hydrochloride), which is commercially available from Sigma-Aldrich (St. Louis, Mo.). Other coupling agents that can be used are also available in the art as are methods for binding a detectable label to an antibody or nucleic acid. Additionally, many detectable labels can be purchased or synthesized that already contain end groups that facilitate the coupling of the detectable label to other proteins (e.g., antibodies) or nucleic acids, such as, N10-(3-sulfopropyl)-N-(3-carboxypropyl)-acridinium-9-carboxamide, otherwise known as CPSP-Acridinium Ester or N10-(3-sulfopropyl)-N-(3-sulfopropyl)-acridinium-9-carboxamide, otherwise known as SPSP-Acridinium Ester.

Detecting Ubiquitinated Proteins

[0083] Another aspect of the invention includes methods of detecting and/or identifying ubiquitinated proteins, including proteins that are the substrates of ubiquitin ligases. Previous strategies developed to identify targets of ubiquitination have largely relied upon educated guesses to identify proteins that are subject to ubiquitination. In most cases, proteins suspected to be ubiquitinated have been identified based on their instability or other properties typically associated with ubiquitination. These proteins are purified by immunoprecipitation and the presence of ubiquitin adducts is determined by anti-ubiquitin western blotting (Banerjee et al. *Drug Metab Dispos* 28, 118-124 (2000)). For example, the susceptibility of proteins to ubiquitination is usually measured by anti-ubiquitin western blot (Banerjee, Kocarek et al. 2000). Lysine mutation experiments have also been used to determine the location of lysine sites or local regions in a specific protein required for ubiquitination (Gregori, Poosch et al. 1990; Treier, Staszewski et al. 1994; Baboshina and Haas 1996). However, such mutation procedures are time consuming and can also stimulate the ubiquitination of nearby lysines after the mutation of the target lysines (Hou, Cenciarelli et al. 1994; Baboshina and Haas 1996). Thus, the lysines identified may be not the ubiquitination sites under physiological condition. These problems are solved by the methods of the invention. Moreover, despite the availability of these approaches, only 119 mammalian proteins have been identified as being ubiquitinated according to UbiProt database (Chemorudskiy et al. *BMC Bioinformatics* 8, 126 (2007)).

[0084] However, using the antibodies and methods described herein, the inventors have identified more than 300 mammalian ubiquitinated proteins comprising nearly 400 diglycine-modified lysines. Analysis of these proteins provides insight into ubiquitination consensus sites and identifies new potential biological roles for ubiquitination.

[0085] The methods for detecting ubiquitination utilize the fact that the C-terminus of ubiquitin is ligated to the ϵ-amine of lysines in ubiquitinated proteins. The three C-terminal residues of ubiquitin are Arg-Gly-Gly. Thus, the C-terminal glycine of ubiquitin is conjugated to a lysine in the target protein to form a ubiquitin conjugate. The methods of this invention employ a protease to cleave this conjugate, for example, after the Arg moiety to yield a Gly-Gly dipeptide remnant that is still conjugated to the lysine present in the target protein. Any protease that can cleave a peptidyl sequence on the C-terminal side of arginine or glutamic acid can be employed in the methods of the invention. Examples of proteases that cleave protein sequences on the C-terminal side of arginine include trypsin and Arg-C. An example of a protease that cleaves proteins on the C-terminal side of glutamic acid is the Glu-C protease.

[0086] Cleavage of a protein or mixture of proteins with such proteases yields a number of peptides, some of which contain the diglycine-lysine epitope, the Leu-Arg-Gly-Gly-lysine epitope, and/or to the STLHLVLRLRGG-lysine epitope, indicating that these peptides were conjugated to ubiquitin. Therefore, detection of any of these epitopes on a peptide after cleavage permits detection of the type and site of ubiquitination in a protein or mixture of proteins.

[0087] Accordingly, one aspect of the invention is a method of detecting ubiquitination in a protein, or mixture of proteins, that involves cleaving the protein, or mixture of proteins, with a protease that cleaves on the C-terminal side of arginine or glutamic acid to yield cleaved peptides, reacting the cleaved peptides with an antibody that specifically binds to a diglycine-lysine epitope, a Leu-Arg-Gly-Gly-lysine epitope, and/or to the STLHLVLRLRGG-lysine epitope, and observing which peptide(s) bind to the antibody. The method can further include identifying the peptide sequence and/or the protein from which the peptide was cleaved.

[0088] The peptide sequence, and/or the protein from which the peptide was cleaved, can be identified by available techniques. Such methods include mass spectrometry, Edman degradation, N-terminal amino acid analysis, C-terminal amino acid analysis and combinations thereof.

[0089] In some instances it may be easier to separate and/or purify the peptides prior to sequencing. Peptide/protein mixtures from ubiquitination studies can be complicated mixtures because each protein can have ten or more peptides after protease cleavage. Typically, only one or a few of these peptides are ubiquitinated. Moreover, when a mixture of tissues and/or cells are used as a sample, only a small portion of the proteins in such a sample is ubiquitinated while the rest is not ubiquitinated. Therefore, only a small percentage of peptides derived from complex mixture proteins in the sample are typically ubiquitinated. This can be a problem when using approaches that analyze and sequence peptides, such as mass spectrometry, because considerable instrument time may need to be devoted to sequencing peptides that do not have a diglycine modification. However, peptides and proteins with the diglycine-lysine epitope, the Leu-Arg-Gly-Gly-lysine epitope, and/or the STLHLVLRLRGG-lysine epitope can be separated from impurities (including any peptides or proteins that do not contain this epitope) using the antibodies that specifically bind to the diglycine-lysine epitope. Peptides that have the diglycine-lysine epitope, the Leu-Arg-Gly-Gly-lysine epitope, and/or the STLHLVLRLRGG-lysine epitope can also be separated by available peptide purification procedures including, for example, by using ion-exchange chromatography, hydrophobic interaction chromatography, gel filtration chromatography, high pressure liquid chromatography, gel electrophoresis and any other procedure available to one of ordinary skill in the art.

[0090] One method for determining the sequence of peptides involves mass spectrometry (MS), for example, MALDI, MALDI-TOF-MS, LC-MS, LC-MS/MS, as well as MS that uses other separation techniques, such as capillary electrophoresis. In some embodiments, liquid chromatography/mass spectrometry can be used, which involves passing the peptides that contain the diglycine-lysine epitope, the Leu-Arg-Gly-Gly-lysine epitope, and/or the STLHLVLRLRGG-lysine epitope through a high pressure liquid chromatography column. At the end of this column, the solution is sprayed into the mass spectrometer out of a narrow nozzle charged to a high positive potential. The collision energy applied to the droplets causes the peptides to fragment at different locations in the peptide so that different "peaks" of the ions can be detected, where the distance between peaks is proportional to the ratio of mass to charge on the fragment. The mass spectrum is analyzed by computer and often compared against a database of previously sequenced proteins in order to determine the sequences of the fragments. This process is then repeated as necessary. For example, if a complex mixture of peptides is present, the peptides can be separated, or if a peptide of interest is very long, the peptide can be partially cleaved (e.g. with a protease) to generate overlapping peptide fragments. These purified and/or partially cleaved peptides can then be analyzed by liquid chromatography/mass spectrometry as described above. Use of a computer can facilitate analysis of the mass spectrometry results (e.g., mass information, or tandem mass spectrometry fragmentation data) so that the sequences can readily be determined of the peptides and/or protein that contain the diglycine-lysine epitope, the Leu-Arg-Gly-Gly-lysine epitope, and/or the STLHLVLRLRGG-lysine epitope.

[0091] Peptides fragment in a reasonably well-documented manner when using mass spectrometry (P. Roepstorrf, J. Fohlmann, *Biomed. Mass Spectrom.,* 1984, 11, 601; R. S. Johnson, K. Biemann, *Biomed. Environ. Mass Spectrom.,* 1989, 18, 945). The protonated molecules fragment along the peptide backbone and also show some side-chain fragmentation with certain instruments (Four-Sector Tandem Mass Spectrometry of Peptides, A. E. Ashcroft, P. J. Derrick in "Mass Spectrometry of Peptides" ed. D. M. Desiderio, CRC Press, Florida, 1990). In general, there are three different types of bonds that can fragment along the amino acid backbone: the NH—CH, CH—CO, and CO—NH bonds. Each bond breakage gives rise to two species, one neutral and the other one charged. Only the charged species is monitored by the mass spectrometer. The charge can stay on either of the two fragments depending on the chemistry and relative proton affinity of the two species. Hence there are six possible fragment ions for each amino acid residue, where three ions can form having the charge retained on the N-terminal fragment, and three ions can form having the charge retained on the C-terminal fragment. The most common cleavage sites are at the CO—NH bonds, which give rise to the b ions and/or the y ions. The mass difference between two adjacent b ions, or y ions, is indicative of a particular amino acid residue. Thus, mass spectrometers have been found to be extremely useful peptide sequencing.

[0092] Another procedure for determining the sequence of peptides involves partial hydrolysis of epitope-containing peptides to generate a series of overlapping peptide fragments. These fragments can be separated and analyzed by N-terminal amino acid analysis, C-terminal amino acid analysis, Edman degradation and analysis procedures and any other available procedures.

[0093] Another aspect of the invention involves obtaining quantitative information on the abundance of diglycine-modified peptides, which will allow those of skill in the art to make inferences about the abundance of the ubiquitinated protein in a sample. One method for obtaining quantitative information involves the use of a SILAC (Stable isotope labeling with amino acids in cell culture) technique in which differences in peptide abundance between two or more samples can be determined. In this approach, at least one sample is prepared by growing the sample in the presence of specific amino acids that contain a label (e.g., a stable heavy isotope). These labeled amino acids can, for example, be one or more of the following amino acids: lysine, leucine, arginine, histidine, etc. This procedure can be performed by adding the labeled amino acids to the culture media for tissue culture cells or cultured tissue, or providing the amino acids to an animal in the diet. The cells incorporate the modified amino acid. When the sample is harvested and digested, the peptides that are derived from the sample that was prepared in the presence of the labeled amino acid will have a higher mobility than control samples, which are cultured in media without these added amino acids. Typically each cell population is different, for example, they may express a different gene or small interfering RNA, or may the different cell populations have significant genomic differences, such as cancer cells versus normal cells. The two cell populations may differ by an experimental treatment, such as treatment with a hormone, drug, small interfering RNA, or a protein. By comparing these samples simultaneously, the relative differences in abundance of diglycine-modified peptides in the sample can be determined. Further information on these procedures is available in Ong et al., Molecular & Cell Proteomics 1: 376-86 (2002); Ong et al., Journal of Proteome Research 2: 173-81 (2003); Ong & Mann, Nature Protocols 1: 2650-60 (2006); Ong & Mann, Methods in Molecular Biology 359: 37-52 (2007).

[0094] Another aspect of this invention is to measure the abundance of diglycine-containing peptides by labeling peptides with heavy water ($H_2{}^{18}O$). In this technique, peptides are obtained from a sample, and then treated with trypsin, but potentially other proteases as well, and $H_2{}^{18}$ to label the carboxyl terminus with ^{18}O. This can impart a 2 or 4 Da mass difference on these peptides, compared to a control sample with is either untreated or labeled with "light" water $H_2{}^{16}O$. The labeling with $H_2{}^{18}O$ often is performed after peptides are obtained by protease digestion, however, peptides can be labeled during the protease digestion step by including $H_2{}^{18}O$ during this step. Further information on this procedure is described in Heller et al. Journal of the American Society for Mass Spectrometry. 14:704-718 (2003); Yao, et al. Anal Chem. 73:2836-2842 (2001).

[0095] Another aspect of this invention involves determining the abundance of diglycine-containing peptides using techniques that are commonly used in quantitative proteomics (see for example: Mass spectrometry based targeted protein quantification: methods and applications. Pan S, Aebersold R, Chen R, Rush J, Goodlett D R, McIntosh M W, Zhang J, Brentnall T A. J Proteome Res. 2009 February; 8(2):787-97.)

[0096] Another aspect of this invention involves use of the antibodies described herein to purify proteins that are modified on lysines with ubiquitin or proteins other than ubiquitin, but that result in a diglycine epitope after protease treatment. For example, such diglycine epitope-tagged proteins can result when ubiquitin or ubiquitin-like protein is expressed in cells, where the ubiquitin and/or the ubiquitin-like protein will become covalently linked to other proteins. Thus, a ubiquitin-like protein could be expressed in cells, e.g. one which already has a carboxyl terminus sequence that would result in a diglycine-adduct on target lysines after digestion. This ubiquitin-like protein could be engineered to contain the desired diglycine adduct, or it may occur naturally in the amino acid sequence. Proteins that are modified with these ubiquitin-like proteins may be isolated or enriched via the epitope tag, for example, after digestion with a protease as described herein. The digested proteins would then be recognized and bound by the anti-diglycyl-lysine antibody.

[0097] Once the sequence of an ubiquitin epitope-containing peptide is known, the protein from which that peptide was derived can be identified because the complete sequence of the human genome and the proteins encoded therein is known. Thus, protein databases can be searched to ascertain what proteins have the sequence of the ubiquitin epitope-containing peptide. The identities of the ubiquitin epitope-containing peptides and the proteins from which they are derived can therefore be determined using these and other types of peptide/protein separation and sequencing methods combined with bioinformatics analysis and/or proteomic sequence searches.

[0098] Moreover, the antibodies that bind to the diglycine-lysine epitope, and/or to the Leu-Arg-Gly-Gly-lysine epitope, and/or to the STLHLVLRLRGG-lysine epitope can be used in any immunopurification, immunoassay or other procedure for detecting and evaluating ubiquitinated proteins and/or peptides. For example, the antibodies can be used for detection, purification and/or quantification of ubiquitinated proteins and/or peptides using methods known to one of ordinary skill in the art such as histochemical staining, Western Blot analysis, dot blot analysis, immunoprecipitation, enzyme-linked immunosorbent assays (ELISA) and the like. Measurement of the ubiquitinated proteins and/or peptides can include measurements of fragments of the protein or peptide, wherein the fragments arise from enzymatic or chemical hydrolysis of the protein or peptide.

[0099] For example, a ubiquitinated protein can be detected by forming a complex between the ubiquitinated protein or peptide (or an proteolytic digest of such a ubiquitinated protein or peptide) and a labeled antibody that binds to the diglycine-lysine epitope, and/or to the Leu-Arg-Gly-Gly-lysine epitope, and/or to the STLHLVLRLRGG-lysine epitope. After formation of the labeled complex, the amount of label in the complex can be detected or quantified using techniques known in the art. For example, if an enzymatic label is used, the labeled complex is reacted with a substrate for the label that gives a detectable and/or quantifiable reaction such as the development of color. If the label is a radioactive label, the label is detected and/or quantified using a scintillation counter. If the label is a fluorescent label, the label is detected and/or quantified by stimulating the label with a light of one color (which is known as the "excitation wavelength") and detecting another color (which is known as the "emission wavelength") that is emitted by the label in response to the stimulation. If the label is a chemiluminescent label, the label is detected and/or quantified by detecting the light emitted either visually or by using luminometers, x-ray film, high speed photographic film, a CCD (charged coupled device) camera, etc. For solution phase assays, once the amount of the label in the complex has been detected and/or quantified, the concentration of ubiquitinated protein or peptide in the test sample is determined, for example, by use of a standard curve that has been generated using serial dilutions of the ubiquitinated protein or peptide of known concentration.

[0100] Immunoassays can be used to detect ubiquitinated proteins and/or peptides that employ any of the polyclonal or monoclonal antibodies that bind to any of the epitopes described herein. In some embodiments, the immunoassay involves immunohistochemistry, radioimmunoassays, competitive binding assays, sandwich assays, or immunoprecipitation assays. Antibodies that bind to the diglycine-lysine epitope, and/or to the Leu-Arg-Gly-Gly-lysine epitope, and/or to the STLHLVLRLRGG-lysine epitope can be combined or attached to a detectable label as described herein. The choice of label used will vary depending upon the application and can be made by one skilled in the art.

[0101] Radioimmunoassays typically use radioactivity in the measurement of complexes between antibodies ubiquitinated proteins and/or peptides. Hence, in such a method, the antibody is radio-labeled. The antibody is reacted with unlabeled ubiquitinated proteins or peptides. The radio-labeled complex can be separated from unbound material, for example, by precipitation and/or centrifugation. The amount of complex is then quantified either by measuring the radiation directly or by observing the effect that the radiolabel has on a fluorescent molecule, such as dephenyloxazole (DPO). The latter approach requires less radioactivity and is more sensitive. This approach, termed scintillation, measures the fluorescent transmission of a dye solution that has been excited by a radiolabel, such as ^{3}H or ^{32}P. The extent of binding is determined by measuring the intensity of the fluorescence released from the fluorescent particles. This method, termed scintillation proximity assay (SPA), has the advantage

of being able to measure antibody complexes formed in situ without the need for washing off unbound radioactive antibody.

[0102] Competitive binding assays rely on the ability of a labeled competitor to compete with the test sample analyte for binding with a limited amount of antibody. The labeled competitor may be a ubiquitinated polypeptide, peptide, fragment, variant or derivative thereof. The amount of test sample is inversely proportional to the amount of competitor that becomes bound to the antibodies. To facilitate determination of the amount of competitor that becomes bound, the antibodies employed are generally made insoluble either before or after the competition. This is done so that the competitor and analyte that are bound to the antibodies may be conveniently separated from the competitor and analyte that remain unbound.

[0103] Sandwich assays involve the use of two antibodies, each capable of binding to a different immunogenic portion, or epitope, of the product to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three part complex (David & Greene, U.S. Pat. No. 4,376,110). The second antibody may itself by labeled with a detectable moiety (direct sandwich assays) or may be measured using a third antibody that binds the second bonding entity and is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme.

[0104] Typically, sandwich assays include "forward" assays in which the antibody bound to the solid phase is first contacted with the sample being tested to extract the ubiquitinated proteins or peptides from the sample by formation of a binary solid phase complex between the immobilized antibody and the ubiquitinated proteins or peptides. After a suitable incubation period, the solid support is washed to remove unbound fluid sample, including any unreacted ubiquitinated proteins or peptides, if any. The solid support is then contacted with the solution containing an unknown quantity of labeled antibody (which functions as a label or reporter molecule). After a second incubation period to permit the labeled antibody to react with the complex between the immobilized antibody and the ubiquitinated proteins or peptides, the solid support is washed a second time to remove the unreacted labeled antibody.

[0105] Other types of sandwich assays that may be used include the so-called "simultaneous" and "reverse" assays. A simultaneous assay involves a single incubation step wherein the labeled and unlabeled antibodies are, at the same time, both exposed to the sample being tested. The unlabeled antibody is immobilized onto a solid support, while the labeled antibody is free in solution with the test sample. After the incubation is completed, the solid support is washed to remove unreacted sample and uncomplexed labeled antibody. The presence of labeled antibody associated with the solid support is then determined as it would be in a conventional "forward" sandwich assay.

[0106] In a "reverse" assay, stepwise addition is utilized, first of a solution of labeled antibody to a test sample, followed by incubation, and then later by addition of an unlabeled antibody bound to a solid support. After a second incubation, the solid phase is washed in conventional fashion to free it of the residue of the sample being tested and the solution of unreacted labeled antibody. The determination of labeled antibody associated with a solid support is then determined as in the "simultaneous" and "forward" assays.

[0107] Hence, the antibodies described herein can be used in a variety of immunoassays, immuno-detection and immuno-purification procedures.

Other Uses of the Antibodies and Methods

[0108] Specifically enrich peptides derived from ubiquitinated proteins and significantly increase efficiency for high-throughput identification of ubiquitination sites.

[0109] By using the antibodies described herein in conjunction with immunopurification procedures and subsequent analysis of the peptides isolated with LC-MS/MS, about 400 ubiquitination sites in over 300 proteins from mammalian cells and tissues have successfully been identified under physiological conditions. Most of the ubiquitination sites have not previously been identified.

[0110] Identification of substrates for specific ubiquitin ligase and identification of potential targets for ubiquitination-related diseases. In human ubiquitination pathways, there are about five hundred ubiquitin ligases (E3) interacting with substrates and ubiquitin conjugating enzymes (E2), which carry ubiquitin from ubiquitin activating enzymes (E1). The E3s are highly diverse and have different crystal structures, such as HECT, RING or ubiquitin interacting complex, during the transfer of ubiquitin molecule from E2 to substrates. The diversity of the E3 ligases indicates that these ligases may recognize various substrates. However, up to now, only limited substrates have been identified for specific E1, E2 and E3 ligases due to the difficult technical challenges of substrate identification. However, the antibodies described herein can facilitate these analyses so that the specific substrates for specific E1, E2 or E3 ligases can be identified.

[0111] Diagnostics of ubiquitination-related diseases. Many diseases, such as Alzheimer's disease, Parkinson's disease, breast cancer, etc, are correlated with the alteration of ubiquitination pathways, for example, deficiency of ubiquitin enzymes, accumulation of ubiquitinated proteins, etc. Currently, these diseases are diagnosed through clinical abnormality, usually at a late and incurable stage. Using the methods described herein these diseases may be diagnosed at an early stage, permitting early treatment options for the patient.

[0112] The common feature of these diseases is the alteration of ubiquitinated proteins in human cells. Using the antibodies described herein and optionally other pre-purification techniques, such as GST-S5A or an anti-ubiquitin antibody, one of skill in the art may quantify the relative abundance of the ubiquitinated proteins from patient sample or normal sample and then to identify ubiquitination-related diseases at early stage.

[0113] The experiments described herein have identified many disease-related ubiquitinated proteins, such as Huntingtin, Dystrophin, Apolipoprotein B-100, Superoxide dismutase, Tuberin, Rho-associated protein kinase 2, Neurexin-1-alpha precursor, etc., in normal cells. These studies demonstrated that the antibodies described herein can be used to identify ubiquitination-related diseases.

[0114] Drug screening for ubiquitination-related diseases. In order to treat ubiquitination-related diseases, it is desirable to find drugs that reverse the abnormal ubiquitination pathways toward normal. In the drug screening stage, the use of the antibodies described herein and subsequent mass spectrometry analysis can identify the change of the ubiquitination level in the presence of different drugs in abnormal cells,

thereby identifying possible drug candidates for treatment of ubiquitination-related diseases. Similarly, certain diseases, whether they involve abnormalities in ubiquitination or not, may benefit from increased or decreased ubiquitination of a specific protein that may affect the manifestation of a specific condition, biological state, or disease. In this case, in the drug screening stage, the use of the antibodies described herein and subsequent mass spectrometry analysis can identify the change of the ubiquitination level in the presence of different drugs in normal or abnormal cells, thereby identifying proteins that are affected by a specific drug or other treatment, and thereby suggesting potential uses for a drug or other treatment.

[0115] The following non-limiting Examples illustrate certain aspects of the invention.

EXAMPLE 1

Materials and Methods

[0116] The Example illustrates some of the materials and methods used in developing the subject matter of the claims.

[0117] Antigen design and antibody purification. Lysine-rich histone (10 mg, sample A) from calf thymus (type III-S, Sigma) was dissolved in 100 mM $NaHCO_3$ buffer (10 ml) at pH 10. 50 mM t-butyloxycarbonyl-Gly-Gly-N-hydroxysuccinimide (500 μl, Boc-Gly-Gly-NHS; Derrien D., et al., Glycoconj J. 1989, 6, 241-55) in DMSO was added to histone solution and the reaction was carried out at room temperature for 1 h by shaking on a plate rotator. This step was repeated three additional times and sample B was obtained. For deprotection of the Boc group, neat trifluoroacetic acid (6 ml, TFA, Sigma) was added and the solution was shaken for 2 h at room temperature. The reaction was neutralized and stopped by adding 10 M NaOH dropwise on ice (sample C). All of sample C and part of sample A and sample B were dialyzed four times against 20 mM acetic acid followed by lyophilization. The degree of the reaction was accessed by anti-biotin (Sigma) western blot after samples A, B, and C were reacted with 5 mM biotin-NHS (Sigma) for 10 min. The absence of biotin incorporation as measured by the absence of a signal by Western blotting, indicates that all the amines in the protein were reacted with the Boc-Gly-Gly-NHS. The same protocol was used to prepare Boc-Gly-Gly- and Gly-Gly-modified β-lactoglobulin, hen egg white lysozyme, bovine serum albumin, and rat brain lysate for antibody evaluation, all of which were prepared and tested for their ability to incorporate Gly-Gly and serve as antigens.

[0118] These Gly-Gly-protein antigens were used for antibody production in rabbits (Invitrogen). Bleeds were collected at five, eight, ten weeks. The antibody was purified by Melon gel IgG purification kit (Pierce) according to the manufacturer's protocol and evaluated using β-lactoglobulin, hen egg white lysozyme, and rat brain lysate. The 10-week antibody was used in all experiments due to the high specificity and higher titer.

[0119] Cell culture and subcellular fractionation. Human embryo kidney (HEK293), mouse neuroblastoma (N2a) and murine endothelioma (sEnd.1) cells were cultured in Dulbecco's modified Eagle's medium (DMEM, Invitrogen) with 4.5 g/l glucose for HEK293 and N2a cells and 1 g/l glucose for sEnd.1 cells, respectively, supplemented with 10% fetal bovine serum, 100 units/ml penicillin G, and 100 μg/ml streptomycin at 37° C. When the confluence reached ~60% after three days, cells were treated with the proteasome inhibitor 50 μM N-acetyl-Leu-Leu-norleucinal (LLnL, Calbiochem) in DMSO and incubated for 24 h prior to harvest.

[0120] Cells were cultured in five or twenty 10-cm-diameter Petri dishes for the samples without and with further subcellular fractionation, respectively. The cells were briefly washed with phosphate buffered saline (PBS) buffer and centrifuged at 1000 g for 10 min at 4° C. The cell pellet was dissolved in lysis buffer containing 150 mM NaCl, 50 mM tris-HCl, 250 mM glucose, 50 μM LLnL, 5 mM iodoacetamide, 1 mM phenylmethylsulfonyl fluoride (PMSF) at pH 7.4.

[0121] For animal tissues, rat brain and rat liver (~2 g) were briefly washed with lysis buffer and cut into small pieces. Lysis buffer (five volumes) was added to the tissue. The samples were homogenized in a Dounce homogenizer with 10-15 strokes. Rat tissues were filtered through four layers cheese cloth to remove cell debris. The homogenate was centrifuged at 1000 g at 4° C. to obtain the crude nuclear fraction. The supernatant was further centrifuged at 100,000 g for 90 min. The pellet was regarded as crude mitochondrial fraction and the supernatant as cytosolic fraction. In order to obtain crude synaptosomal fraction from rat brain, the resulting supernatant after removing nuclear fraction was further centrifuged at 10,000 g for 15 min and the pellet was collected. All the pellets were washed twice with 10 ml lysis buffer and centrifuged again at the corresponding speeds and the pellets were dissolved in 2 ml (for rat tissues) or 0.5 ml (for cell cultures) freshly prepared lysis buffer with 6 M urea at pH 8. The cytosolic fraction was concentrated using Amicon Ultra centrifugal filter tubes (5 kDa molecular weight cutoff, Millipore) and the pH was adjusted to 8. For the samples without fractionation, lysis buffer with 6 M urea (pH 8) was directly added.

[0122] Sample preparation and purification. All the samples were sonicated for 10 s on ice and adjusted to 20 mM dithiothreitol for 1 h at 25° C. Iodoacetamide (Sigma) was added to a final concentration of 80 mM and incubated at 25° C. for 30 min in the dark and then desalted (Zeba desalting spin columns, Pierce) with buffer exchange to 20 mM ammonia bicarbonate. The desalted samples were digested with TPCK-treated trypsin (Sigma) at 37° C. for 24 h. The digestion was quenched by addition of a final concentration of 1 mM PMSF (in ethanol) twice, each for 30 min at room temperature.

[0123] Affinity resin was prepared by coupling the antibody to NHS-activated agarose (Sigma) in 0.1 M sodium bicarbonate buffer with 0.5 M NaCl for 24 h. The resin was washed with 0.1 M tris buffer. Peptide-containing samples were diluted to 5 ml (with PBS) followed by incubation with antibody resin for 2 h at 4° C. Then the sepharose beads were washed with 5 ml PBS, 5 ml 0.5 M NaCl in PBS, 5 ml distilled water (twice each) followed by elution by eight times of 400 μl 0.1% formic acid in 50% acetonitrile in distilled water. The eluted samples were filtered through a 0.2 μm spin column (Pall Life Sciences) and concentrated to 50 μl for nano LC-MS/MS analysis.

[0124] LC-MS/MS analysis. The samples were analyzed by nano LC-MS/MS to obtain peptide sequence information using the similar settings as described in the literature (Hao et al., *Proc Natl Acad Sci USA* 103, 1012-1017 (2006). The nano LC-MS/MS was performed with an 1100 series LC coupled to an XCT plus ion trap mass spectrometer. The purified samples were injected onto a 0.3×5 mm Zorbax 300SB-C18 sample-enrichment column at a flow rate of 3 μl/min 100%

solvent A. Peptides were resolved on a 0.075×150 mm Zorbax 300SB-C18 analytical column at a flow rate of 0.35 μl/min with a gradient of 5-45% solvent B for 40 min. Solvent A was 0.1% formic acid (Fluka) and 3% acetonitrile (Fisher) in MS/MS grade water (Fisher) and solvent B consisted of 0.1% formic acid and 90% acetonitrile. The LC column eluate was introduced into the ion trap mass spectrometer via electrospray using a 15 μm diameter silica emitter needle. Mass spectra were acquired in positive-ion mode with automated data-dependent MS/MS on the four most intense ions from precursor MS scans and every selected precursor peak was analyzed no more than twice within 1 min.

[0125] Database search of MS/MS data for peptide and protein identification. Analysis of MS/MS spectra for peptide and protein identification was performed by protein database searching with Spectrum Mill software (Rev A.03.02, Agilent) against Swiss-Prot database released in February 2007. Raw MS/MS spectra were first extracted to MS/MS spectra that could be assigned to at least four y- or b-series ions and searched against the Swiss-Prot database. During the extraction step, scans with the same precursor ±1.4 m/z were merged within a time frame of ±15 s, charges up to a maximum of 5 were assigned to the precursor ion, and the ^{12}C peak was determined by the Data Extractor. Key search parameters were a minimum matched peak intensity of 50%, a precursor mass tolerance of ±2.5 Da, and a product mass tolerance of ±0.7 Da. The fixed modification was iodoacetamide for cysteines for all the samples. A variable modification of diglycine was set for lysine residues and the maximal modification number was set as two. The false positive rate was estimated by searching the MS/MS spectra against a random protein database with same number of amino acids for each entry as the original database for several groups of extracted MS/MS spectra. The threshold used for peptide identification was a score of >10 and an SPI % (the percentage of assigned spectrum intensity of total spectrum intensity) of >60%, which gave a 5% rate of false positive. In the peptide list, only the highest scoring member of each peptide group is shown and only peptides with a charge state of 2 and 3 are reported. Finally, all MS/MS spectra were manually validated and the spectra with low quality fragmentations were removed. The peptides with a score between 10 and 11 are included only when the ubiquitination of the corresponding proteins has been reported previously.

[0126] Search parameters were adjusted so that four trypsin missed cleavages were allowed. This increased tolerance is based on our finding that ubiquitination increased the number of missed cleavages. The ubiquitin-remnant containing peptides had an average length of 20.7 amino acids. This is higher than the average size of tryptic peptides identified by ion trap mass spectrometry and can be explained by a high number of trypsin missed-cleavages. On average, 1.5 missed cleavages per peptide were found without counting the modified lysines. As a control, the number of missed-cleavage sites for a rat brain lysate with only cysteines blocked with iodoacetamide showed that tryptic peptides have an average length of 15.6 amino acids and 0.32 missed-cleavages under similar experimental conditions. Thus, the number of missed cleavages is nearly five times higher for ubiquitinated peptides. Moreover, the inventors observed that 35% of the ubiquitinated peptides have basic amino acids (Arg or Lys) within 2 amino acids from the ubiquitination site, which hampers the efficiency of trypsin digestion (see also, Benore-Parsons et al. *Arch Biochem Biophys* 272, 274-280 (1989); Schlosser et al. *Anal Chem* 73, 170-176 (2001)) while under control conditions, only 12% of peptides exhibit similarly close to Arg or Lys residues. Unlike an analysis of tryptic phosphopeptides (Molina et al. *Proc Natl Acad Sci USA* 104, 2199-2204 (2007)), no significant difference was observed in the frequency of acidic amino acids within two amino acids between diglycine-modified lysines and lysines from control samples. Our results indicate that ubiquitination hinders trypsin digestion, most likely because of the large size of ubiquitin chain or the remaining residues after trypsin digestion of ubiquitin, the presence of basic amino acids in proximity to the ubiquitination sites, and the branching topology of the ubiquitination sites. A similar phenomenon has also been found for phosphopeptides (Molina et al. *Proc Natl Acad Sci USA* 104, 2199-2204 (2007)). For this reason, a higher number of missed-cleavage sites have been allowed during the database search although higher charge states are filtered out.

[0127] Biochemical validation of ubiquitination. Some identified ubiquitinated proteins were verified by western blotting pulldowns obtained with glutathione agarose immobilized GST-S5a. Note that S5a is a subunit of the 26S proteasome and has strong affinity for polyubiquitin chains (Wang et al., *J Mol Biol* 348, 727-739 (2005)). The expression vector, pGEX4T2-S5a, was transformed and grown in BL21 (DE3) *E. coli* and protein expression was induced by addition of 200 μM isopropyl-beta-D-thiogalactopyranoside for 3 h at 37° C. The cells were centrifuged and the pellet was lysed, sonicated and centrifuged again in lysis buffer (100 mM NaCl, 20 mM tris-HCl, 1% triton X-100, 2 mM EDTA) with freshly prepared 1 mM PMSF and 10 mM DTT at pH 7.4. The supernatant was incubated with glutathione-agarose beads (Sigma) for 2 h at 4° C. and the beads were washed three times with lysis buffer adjusted to 0.5 M NaCl. Protein lysates from HEK293 cells and rat brain were incubated with GST-S5a-containing agarose beads overnight at 4° C. and washed with lysis buffer adjusted to 300 mM NaCl four times. Then the agarose beads were incubated with 2×SDS sample loading buffer with β-mercaptoethanol at 100° C. for 10 min and samples were subjected to western blotting. For some proteins, such as the Trk neurotrophin receptor and dystrophin, the protein was immunoprecipitated and the immunoprecipitate was blotted with an anti-ubiquitin antibody. Agarose beads and protein A or protein G beads were used as a control for nonspecific pulldown and immunoprecipitation.

[0128] Ubiquitin antibody (P4D1), c-Myc antibody (9E10) and Trk antibody (C-14) were from Santa Cruz, calsenilin antibody (75-005) and SH3GRB2L antibody (75-049) were from UC Davis/NINDS/NIMH NeuroMab facility, dystrophin antibody (3B7) and annexin antibody (EH7a) were from Developmental Studies Hybridoma Bank.

[0129] Bioinformatic analysis. Protein biological processes were analysed and clustered by PANTHER (Thomas et al. *Genome Res* 13, 2129-2141 (2003)) after converting Swiss-Prot accession numbers of identified ubiquitinated proteins into RefSeq protein accession numbers by the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Dennis et al. *Genome Biol* 4, P3 (2003)) online gene ID conversion function. In total, 582 biological processes were found in the database and the category was further grouped into eight classes. Protein subcellular localization of ubiquitinated proteins was extracted from the database provides by PENCE Proteome Analyst (Lu et al., *Bioinformatics* 20, 547-556 (2004)). Note that some proteins have multiple subcellular localizations.

[0130] The density plot for the diglycine-modified lysines was calculated as follows: A subset of protein sequence, 10 amino acids on either side of modified lysines, was extracted from the whole protein sequence. The frequency of each of the 20 individual amino acids at each position from −10 to +10 was calculated for diglycine-modified lysines and this value was normalized to the frequency of the same amino acid at the same position using all lysines in the Swiss-Prot database to obtain a relative ratio. If the ratio in one position (say −1) for a specific amino acid (say Pro) is larger than 1, there is a commensurately higher likelihood for Pro at the −1 position to be adjacent to a ubiquitinated lysine. The highest relative ratio detected was 2.1 and the range of the colormap was set from 0 to 2.5. The density plot was prepared by MATLAB.

[0131] To access the structural features of ubiquitinated lysine residues for mammalian proteins, we searched crystal structures for all the ubiquitinated proteins in protein database bank (PDB). In total, 24 PDB structures contained lysines that we found are susceptible to ubiquitination (25 modified lysines and 1066 totally lysines). In cases where multiple PDB structures for a single protein are reported, the structure with best quality was used. The secondary structure types for lysines were determined using the program DSSP (Kabsch et al., *Biopolymers* 22, 2577-2637 (1983)). H and G were considered to be helix, E and B to be strand, S, T and others for loop. The fraction of each secondary structure type of modified lysines was compared to that of all the lysine residues in 24 PDB structures. The secondary structures for peptides with two diglycine modified lysines were predicted by PSI-Pred (Jones, *J Mol Biol* 292, 195-202 (1999)) since most of them do not have crystal structures. The disordered region was predicted by DisEMBL (Linding et al. *Structure* 11, 1453-1459 (2003)) for all identified ubiquitinated proteins and the information for modified lysines and all lysines was extracted. The relative solvent accessible area (SAA) for the modified and all lysines in 24 crystal structures was calculated using NACCESS (Hubbard et al. *J Mol Biol* 220, 507-530 (1991)) with a probe of 1.4 Å, which corresponds to the size of a water molecule.

EXAMPLE 2

Identifying Ubiquitinated Proteins and Ubiquitination Sites

[0132] This Example illustrates novel methods for identifying ubiquitinated proteins and ubiquitination sites using an antibody that selectively binds to the diglycine remnant in peptides generated from tryptic digestion of biological samples. Using this immunoaffinity approach coupled to nano LC-MS/MS, more than 300 ubiquitinated proteins and nearly 400 ubiquitination sites were identified. Of these ubiquitinated proteins, 224 have not previously been known to be ubiquitinated. These experiments illustrate that the immunoaffinity profiling methods described herein have broad utility in characterizing the occurrence and extent of ubiquitination in diverse tissues and disease states.

[0133] To generate an antibody that recognizes peptides containing the ubiquitin remnant, a protein antigen was prepared that contained one or more diglycine adducts the terminal amine of the side chain of lysines naturally present in the protein. The scheme of this antigen synthesis is shown in FIG. **1**A. First, the lysine-rich type III-S histone was reacted with Boc-Gly-Gly-NHS, a reagent that specifically reacts with amines to form an amide-linked Boc-Gly-Gly adduct on amines. Reaction with Boc-Gly-Gly-NHS resulted in modification of nearly all amines in histone which, as detected by the marked inability of the modified protein to become biotinylated by the lysine-modifying reagent biotin-NHS, which was assessed using anti-biotin western blot analysis (FIG. **1**B, lane B). The modified protein was treated with trifluoroacetic acid (TFA) to remove the Boc protecting group, which resulted in the adduct being trimmed to the desired Gly-Gly modification on lysine residues (FIG. **1**A). Quantitative conversion of the Boc-Gly-Gly adduct, which does not contain an amine, to Gly-Gly, which contains an amine, was confirmed by the ability of the TFA-treated protein to be readily biotinylated by biotin-NHS (FIG. **1**B, lane C).

[0134] The diglycine-modified histone was injected into rabbits to generate immune serum. The rabbits were bled and antisera were collected at four, eight, and ten weeks after immunization. The titer of the antibody was shown in Table 1.

TABLE 1

Titers of antibody collected at different times (weeks) and dilutions.

	Dilution									
	50	100	200	400	800	1600	3200	6400	12800	25600
0 wks	0.0603	0.3471	0.2211	0.1371	0.1074	0.0957	0.0675	0.0662	0.0618	0.0927
4 wks	0.6067	0.4718	0.3828	0.2218	0.1358	0.0995	0.0863	0.0665	0.0687	0.0594
8 wks	1.6441	1.0971	0.5834	0.2892	0.1986	0.1321	0.0913	0.0708	0.0705	0.0612
10 wks	2.5281	1.9679	1.0901	0.7851	0.4781	0.4533	0.1524	0.1097	0.0794	0.0682

[0135] Antibodies purified from immune serum exhibited pronounced specificity for peptides containing the diglycine modification on ϵ-amine of lysines. The antibodies failed to interact with unmodified lysozyme, which contains two separate internal Gly-Gly sequences, and lactoglobulin (FIG. **1**C, left panel, lane A), or the corresponding proteins modified with Boc-Gly-Gly (FIG. **1**C, left panel, lane B), but showed strong reactivity with proteins that contained the Gly-Gly modification (FIG. **1**C, left panel, lane C). These results indicate that the antibody recognizes diglycine-modified amines. Similarly, the antibody exhibited negligible reactivity with rat brain lysate (FIG. **1**C, right panel, lane A), and with rat brain lysate modified with Boc-Gly-Gly (FIG. **1**C, right panel, lane B). However, the antibody exhibited significant reactivity with Gly-Gly-modified proteins from rat brain lysate (FIG. **1**C, right panel, lane C). The absence of reactivity of the antibody with unmodified rat brain lysate demonstrates the high degree of specificity of the antibody for proteins that contain the ubiquitin remnant.

[0136] The crude antibody was purified by Melon gel IgG purification kit (Pierce) according to the manufacturer's protocol and evaluated using histone and rat brain lysate with or without modified lysines (sample A: with intact lysines; B: with Boc-Gly-Gly-modified lysines; C: with Gly-Gly-modified lysines). A western blot of three samples against the diglycl-lysine antibody, FIG. **1**C, showed that the antibody has high specificity, which can selectively recognize diglycine modified lysines. The rat brain lysate result shown in FIG. **1**C also indicated that the antibody affinity does not depend on backbone amino acid sequences since it can recognize various proteins in the tissue.

[0137] In addition, this antigen was used to immunize mice to develop B-cells producing antibody to the diglycine residue. B cells with this property were then hybridized to mouse myeloma cells to make hybridomas producing monoclonal antibodies in methods well known in the art. Hybridoma clones 41, 42, 45, 49 and 50 were developed which produced effective anti-diglycine monoclonals as shown in FIGS. **9**-**11**. FIG. **9** shows that the antibody from clone 42 specifically recognizes proteins with Gly-Gly modified lysines and does not interact with unmodified and Boc-Gly-Gly modified proteins. FIG. **10** demonstrates that the anti-GG monoclonal antibody from clone 49 simplifies analysis of ubiquitination sites in a simple peptide mixture and FIG. **11** shows that this anti-GG monoclonal antibody simplifies analysis of ubiquitination sites in a complex peptide mixture.

[0138] Next the anti-diglycl-lysine antibody was used to immunoprecipitate diglycine-modified peptides from a peptide mixture. A flow chart for sample preparation, immunoprecipitation, and MS/MS analysis is shown in (FIG. **2**A). To test the ability of the antibody to recover diglycine-modified peptides, diglycine-modified proteins and immunoprecipitated diglycine-modified tryptic peptides were reacted with the anti-diglycyl-lysine antibody. BSA and lysozyme were partially modified with Boc-Gly-Gly-NHS as above, treated with TFA to liberate the Boc moiety, and then trypsinized. While immunoprecipitation with preimmune serum failed to immunoprecipitate peptides, immunoprecipitation with the immune serum recovered peptides from both diglycine-modified BSA and diglycine-modified lysozyme (FIG. **2**B). Thus, the antibody is capable of enriching peptides containing diglycine-modified lysines.

[0139] The diversity of lysine ubiquitination was then assessed in tissue culture cells and animal tissues. Lysates were prepared from human embryonic kidney cells (HEK293), mouse endothelioma cells (sEnd.1), and rat brain and liver. To increase the types of ubiquitinated proteins analysed, crude lysates, as well as subcellular fractions, including nuclear, mitochondrial, and in some cases, synaptosomal fractions were prepared for analysis (see Example 1). Lysates were digested with trypsin and diglycine-modified peptides were immunopurified and subjected to LC-MS/MS followed by database searching and spectral validation (for examples see FIG. **2**C). A full list of ubiquitinated peptides with ubiquitination sites, fragmentation map, score, charge states, corresponding Swiss-Prot accession number, protein name, whether the protein has been previously found to be ubiquitinated, and tissue of origin is provided in FIG. **22**.

[0140] The majority of ubiquitin remnant-containing peptides have three charges, in part because the Gly-Gly adduct has its own N-terminal amine. Diglycine-modified lysines were detected both within the peptide sequence as well as at the N- and C-termini of peptides (FIG. **1**). The presence of C-terminal diglycine-modified lysines indicates that trypsin can cleave at the carboxyl side of this lysine adduct, consistent with a recent report (Denis et al. *Proteomics* 7, 868-874 (2007). Most peptides contained a single diglycine-modified lysine, although 51 peptides did contain two diglycine-modified lysines.

[0141] The presence of diglycine-modified lysines was detected in the MS/MS spectra by the presence of fragmentation ions separated by the m/z corresponding to diglycine-modified lysine. However, in some MS/MS spectra, ions consistent with the loss of Gly-Gly or Gly fragments were detected, and in some cases, both the peptide ions with and without this loss were detected, thus providing further confirmation for the presence of a diglycine adduct in these peptides. For example, the loss of a Gly-Gly fragment in a diglycine-containing tubulin peptide ion is indicated by a pair of MS/MS fragments (FIG. **2**C). Only 32.3% of peptides derived from tissue lysates, and 22% of diglycine-modified peptides from synthetically Gly-Gly-modified lysozyme and BSA exhibited loss of Gly-Gly or Gly. Because this loss is not universal for ubiquitinated peptides, a finding which has been observed previously (Warren et al., *Rapid Commun Mass Spectrom* 19, 429-437 (2005)), this feature cannot be regarded as a fingerprint for diglycine-modified peptides.

[0142] The ubiquitinated proteins and peptides identified in human and mouse tissue culture cells, as well as rat tissues, are summarized in FIG. **2**D. In total, over 300 ubiquitinated proteins and peptides were identified through our experiments, of which 224 (68%) have not previously been known to be ubiquitinated. Among the identified proteins, 268 proteins have one ubiquitination site, 61 have two, and two proteins have three or more ubiquitination sites. Although the other ubiquitin-like proteins that appear to be conjugated to large numbers of proteins, such as SUMO and ISG15, have an amino acid sequence that would not result in a diglycine adduct on lysines after trypsin digestion (Pedrioli et al. *Nat Methods* 3, 533-539 (2006); Denison et al., *Curr Opin Chem Biol* 9, 69-75 (2005)) it is important to validate that proteins found to contain a diglycine modification are indeed ubiquitinated under physiological conditions. Therefore, a subset of the identified proteins was selected and assessed to confirm that the proteins were ubiquitinated as determined by MS/MS spectra by another technique—immunoprecipitation or pull-down procedures.

[0143] In pull-down experiments, the GST-S5a was used to enrich for polyubiquitinated proteins. GST-S5a is a polyubiquitin-binding protein derived from the proteasome (Wang et al. *J Mol Biol* 348, 727-739 (2005)). The anti-Gly-Gly-Lys antibodies were used for immunoprecipitation experiments to precipitate proteins containing these epitopes. The ubiquitin adducts were then detected on a western blot using an anti-ubiquitin antibody.

[0144] In both these experiments, high molecular weight species were readily detected that were specifically associated with GST-S5a pull-down or immunoprecipitates, however the control samples did not exhibit bands indicative of such ubiquitinated proteins (FIG. **2**E).

[0145] The ubiquitination targets include disease-related proteins, such as huntingtin, dystrophin, apolipoprotein B-100, superoxide dismutase, tuberin, and Rho-associated protein kinase (some spectra are shown in FIG. **5**. The different proteins identified by ubiquitin remnant immunoaffinity profiling have roles in a wide range of biological processes, of which the largest number are involved in signal transduction

and metabolism (FIG. **3**A, left panel). Additionally, proteins were found that have roles in cell cycle/apoptosis, the immune system, protein trafficking/localization, and small molecule transport, consistent with previously reported roles for ubiquitination (Kirkpatrick et al., *Nat Cell Biol* 7, 750-757 (2005); Bonifacino & Weissman, *Annu Rev Cell Dev Biol* 14, 19-57 (1998); Rechsteiner, *Annu Rev Cell Biol* 3, 1-30 (1987); Nandi et al., *J Biosci* 31, 137-155 (2006); Mukhopadhyay & Riezman, *Science* 315, 201-205 (2007); Sun & Chen, *Curr Opin Cell Biol* 16, 119-126 (2004); Pickart, *Mol Cell* 8, 499-504 (2001)). Interestingly, some proteins identified are involved in processes that have not previously been linked to ubiquitination. For example several proteins linked to protein translation, including 40S ribosomal subunits S23, S5, and the 60S subunit L27a, were identified. This suggests that the stability or function of the ribosome is regulated by ubiquitination. Several mitochondrially-localized metabolic enzymes, including 3-hydroxyisobutyrate dehydrogenase, pyruvate dehydrogenase complex E2 subunit, succinyl-CoA ligase, isocitrate dehydrogenase, and ornithine aminotransferase were found to be ubiquitinated, suggesting a role for ubiquitination and the cognate E3 ubiquitin ligases in regulating mitochondrial metabolic function. Some of the proteins found to be ubiquitinated extend recent findings regarding the role of ubiquitination in certain cellular processes. For example, while histone H2 ubiquitination has been described (Wang et al. *Nature* 431, 873-878 (2004); Nickel & Davie, *Biochemistry* 28, 964-968 (1989)), the inventors find that histone H3 and H1 isoforms are also ubiquitinated, as well as histone acetyltransferases, supporting the idea that ubiquitin contributes to epigenetic gene regulation through multiple pathways. Several cellular effects mediated by molecular motors are affected by ubiquitination (Gordon & Roof, *Proc Natl Acad Sci USA* 98, 12515-12520 (2001)) indeed, the results identify specific kinesin and myosin isoforms and subunits that may mediate these effects. These studies also identify numerous transcription factors and kinases that are targets for ubiquitination, supporting the well-characterized role for ubiquitination in regulating signal transduction in cells.

[0146] The subcellular distribution of the detected proteins is likely to reflect, in part, the subcellular fractions that were used for MS/MS analysis. Subcellular localization analysis of the identified proteins using PENCE Proteome Analyst (Lu et al., *Bioinformatics* 20, 547-556 (2004)) indicates that most ubiquitinated proteins are cytosolic and nuclear proteins (FIG. **3**A, right panel), which is consistent with the general observation that ubiquitin is primarily in intracellular protein (Bonifacino & Weissman, *Annu Rev Cell Dev Biol* 14, 19-57 (1998)). However, nine of the identified ubiquitinated proteins are located in endoplasmic reticulum, including apolipoprotein B-100. In some cases secreted proteins have been found to be ubiquitinated (Lu et al., *Endocrinology* 147, 5611-5623 (2006); Meerovitch et al., *J Biol Chem* 272, 6706-6713 (1997)) while in other cases, such as with apolipoprotein B-100, proteins can be retrogradely exported from the endoplasmic reticulum to the cytosol, where they are ubiquitinated and degraded (Liao et al., *J Cell Biochem* 89, 1019-1029 (2003)) and may reflect a mechanism to degrade proteins that misfold while in the ER (Galan et al., *FASEB J* 12, 315-323 (1998); Plemper & Wolf, *Mol Biol Rep* 26, 125-130 (1999).

[0147] Next, the large number of ubiquitinated proteins was used to gain insight into how lysine ubiquitination might be regulated at the level of primary and secondary structure. Interestingly, we noticed that ubiquitin remnant-modified lysines tended to be localized in lysine-rich regions of proteins. Examination of the amino acid sequences surrounding the modified lysine revealed that 9.6% of the residues within a six amino acid span on either side are lysines, which is almost three times the expected abundance of lysine residues (3.3%) within a six amino acid span on either side of all lysines based on an analysis of all the proteins in the Swiss-Prot database (FIG. **6**A). Consensus ubiquitination site analysis using Motif-x (Schwartz & Gygi, *Nat Biotechnol* 23, 1391-1398 (2005)) indicated that adjacent lysines were a common feature of possible motifs (FIG. **6**B). The presence of multiple lysines may facilitate ubiquitination by providing multiple alternate adjacent ubiquitin acceptor sites at a specific area in a protein. To compare all 20 amino acids for their propensity to be adjacent to ubiquitinated lysines, a density map was prepared indicating the frequency of each amino acid, at any of the ten proximal positions on either side of the ubiquitinated lysines, compared to the frequency of that amino acid next to lysines in general, as assessed by surveying the Swiss-Prot database. This analysis indicates that other lysines are present near the ubiquitinated lysine residues. However, Cys, His, Met, and Pro, were also found to be slightly enriched at certain positions, although not to the same extent as lysine residues (FIG. **3**B). In comparison, the inventors have found that for the recently identified ubiquitinated proteins in yeast, Asp, Glu, His and Pro are highly enriched (>2.3 times the expected value) at some positions (FIG. **6**C).

[0148] The inventors found that ubiquitinated lysines tend to appear on protein surfaces in preferred structural contexts. For 24 of the proteins identified in this study, structural information was present in Protein Data Bank (PDB), allowing the exposure of lysine residues to solvent to be measured. Measurements of the solvent accessible area of lysine residues in these proteins indicate that ubiquitinated lysines tend to be more solvent exposed than other lysines (FIG. **6**C). If lysines with more than 25% surface exposed are considered solvent exposed residues, 92% (23/25) of the ubiquitinated lysines are exposed, which is higher than that of all lysines (85%). This is in agreement with a ubiquitination site survey for yeast proteins (Catic et al., *Bioinformatics* 20, 3302-3307 (2004)). Interestingly, in some cases, the ubiquitinated lysine is fully buried (for example, see FIG. **7**). In these proteins, ubiquitination may be regulated by stimuli that induce the exposure of the lysine to the surface.

[0149] Secondary structure analysis for all lysines and ubiquitinated lysines indicates that ubiquitinated lysines prefer helical structures compared to all lysines, although ubiquitination sites can also be found in other structural contexts (FIG. **3**D). This result is slightly different from a previous analysis of ubiquitinated yeast proteins, in which ubiquitination was found preferentially in both loops and helices (Catic et al., *Bioinformatics* 20, 3302-3307 (2004)). In some cases, peptides contained two ubiquitination sites. Structural prediction algorithms indicate that these lysines are likely to be in helical structures, with a higher likelihood observed for those pairs of ubiquitinated lysines that are closer together (FIG. **8**). Additional crystal structures of proteins that are susceptible to ubiquitination are needed to more fully assess the structural contexts of ubiquitinated lysines.

[0150] Ubiquitination is a critical effector pathway of numerous signaling pathways and misregulation of ubiquitination has been implicated in cancer, neurodegenerative dis-

eases, and other diseases (Jiang & Beaudet, *Curr Opin Pediatr* 16, 419-426 (2004)). The recent development of small molecule antagonists of specific E3 enzymes suggests that pharmacological manipulation of ubiquitination may be a major therapeutic strategy in various disorders (Nalepa et al. *Nat Rev Drug Discov* 5, 596-613 (2006)). The approaches described here demonstrate a simple and robust strategy to identify endogenously-ubiquitinated proteins in cells and tissues. Although different tissues and subcellular fractions were probed, the approaches described here will have utility in profiling changes in ubiquitination elicited by various signaling molecules, drugs, and in disease states. Additionally, although the above studies do not implicate specific consensus sequences in ubiquitination, specific sequence requirements may be detected when evaluating the proteins regulated by specific E1, E2, and E3 enzymes. Furthermore, identification of the proteins whose ubiquitination is dependent on specific E1, E2, or E3 enzymes may help to identify which of these ubiquitin ligases might serve as useful drug targets.

EXAMPLE 3

Anti Diglycyl-Lysine Antibodies can be Used to Identify Ubiquitinated Ubiquitin, and the Specific Ubiquitin-Ubiquitin Linkages

[0151] Ubiquitin is often present as polyubiquitin chains, with multiple ubiquitins attached in a chain or other branched configuration on proteins. The C-terminus of ubiquitin is conjugated to other ubiquitins via one of its seven lysine residues. The antibodies described here can be used to detect the presence or abundance of specific ubiquitin chains, due to the detection of ubiquitin-specific peptides.

[0152] FIG. **12** shows a MS/MS spectrum of a fragment from ubiquitin, containing a ubiquitin remnant diglycine on K48. This peptide can be detected with either the polyclonal or monoclonal anti-diglycyl-lysine antibodies. In this case, the antibody was immobilized to protein A or protein G beads. Cellular proteins were extracted from HEK293 cells, digested by trypsin, and immunoprecipitated by GlyGly-antibody and detected on ion-trap or Q-TOF LC-MS/MS.

[0153] The detection of different ubiquitinated lysines on ubiquitin itself can provide a way for quantification of different ubiquitin chains, which may change under various physiological conditions, such as ER stress.

EXAMPLE 4

Purification of Peptides from the Membrane Protein TrkB Using Anti-Diglycyl-Lysine Antibodies

[0154] This Example illustrates that the anti-diglycyl-lysine antibodies can be used to study ubiquitination in (1) membrane proteins; and (2) heterologously expressed proteins.

[0155] The TrkB cDNA was expressed in mouse N2a cells, and the TrkB protein was purified by immunoprecipitation with TrkB-specific antibodies. TrkB protein was then digested with trypsin and reimmunoprecipitated with anti-diglycyl-lysine antibodies.

[0156] As shown in FIG. **13**, LC-MS/MS analysis revealed that the TrkB protein had a peptide with a ubiquitination site.

EXAMPLE 5

Anti-Diglycyl-Lysine Antibodies are Highly Specific for the Diglycyl-Lysine Epitope

[0157] This Example illustrates the specificity of the anti-diglycyl-lysine antibodies

Anti-Diglycyl-Lysine Antibodies Recognize GlyGly modifications on the epsilon-, but not alpha-amine.

[0158] Experiments were conducted to determine whether monoclonal anti-diglycyl antibodies (mGGAb) can distinguish between diglycyl adducts on the epsilon amine of lysine vs. the alpha-amine of proteins. Angiotensin I, which contains zero lysines (indicated as "K" in FIG. **14**) was synthesized with the diglycyl modification selectively on the alpha amine by reaction with Boc-diglycine-NHS. As a control, another aliquot of angiotensin I was treated with vehicle. The small tables below the western blots shown in FIGS. **14**A and **14**B, list the number of diglycyl moieties incorporated on the epsilon amine of lysines, and the number of diglycyl moieties incorporated on alpha-amines. Because angiotensin I does not contain any epsilon amines, there is only a single diglycine modification on the alpha-amine. This is readily detected by Coomassie staining (FIG. **14**A), where the modified protein exhibits a small upward mobility shift. As illustrated in FIG. **14**A, this peptide exhibits no reactivity with the anti-diglycyl-lysine monoclonal antibodies ("mGGAb") was used to probe the Western blot.

[0159] In contrast, the MARCKS peptide, which contains five lysines, is reactive with the mGGAb after the MARCKS peptide was treated with Boc-diglycine-NHS (FIG. **14**A). Thus, results indicate that the diglycine epitope was introduced onto the five epsilon amines and the one alpha-amine of the MARCKS peptide (FIG. **14**A).

[0160] The specificity of the anti-diglycyl antibodies (mGGAb) towards diglycyl-modified epsilon amines but not alpha-amines is tested in more detail using a peptide, Ac-MSH-NH_2, which contains no alpha-amine (the alpha-amine is acetylated), and just one lysine. As shown for the MARCKS peptide in FIG. **14**A, the conjugation of the diglycine to the Ac-MSH-NH_2 peptide is readily detected when (FIG. **14**B). This is in contrast to Angiotensin I, where no diglycine epitopes are detected. The immunoreactivity of mGGAb for the Ac-MSH-NH_2 peptide therefore reflects binding of mGGAb to internal diglycyl-lysine, because the alpha-amine of the Ac-MSH-NH_2 peptide is acetylated.

The Polyclonal Anti-Diglycyl-Lysine Antibody Immunoprecipitates Peptides with GlyGly-Modified Lysines but not Peptides with N-Terminal GlyGly

[0161] Peptides with GlyGly modifications on the ϵ-amine of lysines were isolated by the anti-diglycyl-lysine antibody. Anti-diglycyl-lysine antibody bound to beads was incubated with a 1:1 mixture of GlyGly-modified Angiotensin I (one N-terminal GlyGly modification) and GlyGly-modified acetylated MSH peptide (one GlyGly-modified lysine). The beads were washed with 2xPBS, eluted with 0.1% TFA to remove the Boc moiety. MALDI-TOF-MS was used to detect the unpurified peptides in the original mixture (FIG. **15**A), as well as the peptides that bound to immobilized anti-diglycyl-lysine antibodies (FIG. **15**B), and the peptides in the "flow through," i.e., the liquid solution after incubation with immobilized antibody (FIG. **15**C). The sequences of the peptides in

the peaks are indicated in the spectra. These data illustrate that the anti-diglycyl-lysine antibodies can be used to selectively recover a peptide containing a diglycyl-modified lysine (FIG. **15**B), while the flow through contains the peptide with the diglycyl modification on the alpha-amine. Hence, the anti-diglycyl-lysine antibodies can facilitate specific purification of peptides that have GlyGly-modified lysines.

A Monoclonal Anti-Diglycyl-Lysine Antibody Preparation can Detect at Least 170 Femtomoles of GlyGly-Lysine-modified Peptides.

[0162] A "dot blot" was used to test the sensitivity of the monoclonal anti-diglycyl-lysine antibody. Insulin (Molecular Weight ~5800 Da) contains only one lysine in its heavy chain, which is readily conjugated to the Gly-Gly moiety using procedures and reagents as described herein. The fact that GlyGly-modified insulin contains just one GlyGly-modified lysine residue provides a convenient way to spot known amounts of diglycyl-modified protein on PVDF membranes. Dot blots were spotted with serially diluted amounts of GlyGly-modified insulin so that the amount of insulin (in ng) and the corresponding molar quantity (in pmol) was as listed above and below, respectively, the dot blots shown in FIGS. **16**A and **16**B. The affinity of the monoclonal antibody was assessed by probing these dot blot with monoclonal antibody 49 using a 1:2 dilution of ascites and 1:1000 dilution of secondary antibody and visualized by ECL Plus™ Western Blotting Detection System (GE Healthcare).

[0163] As shown in FIG. **16**A-B, this anti-diglycyl-lysine monoclonal antibody can detect at least 1 ng (170 fmol) of GlyGly-lysine-conjugated insulin in the presence of 1 mg/mL BSA.

Affinity Purified Monoclonal Anti-Diglycyl-Lysine Antibody can Detect GlyGly-Modified Proteins.

[0164] This experiment evaluates whether the monoclonal anti-diglycyl-lysine antibody can be purified and still detect diglycine-modified proteins. In this experiment, the tissue culture media in which the hybridoma cell line expressing anti-diglycyl-antibody #49 was growing (ascites) was used as a source of monoclonal antibody. The antibody was purified using diglycine-modified BSA immobilized on Affi-gel. In the first three lanes in the gel shown in FIG. **17**A, the silver stain shows the flow-through from Affi-gel after the media was incubated with the immobilized diglycine-modified BSA. A blot to detect antibody was also performed and shown above. This blot shows that the antibody is depleted from the media by incubation with the immobilized diglycine-modified BSA. Acid elution (last four lanes of FIG. **17**A), results in the elution of specific antibody bands, which can be seen in both the silver stain and Western blot at the top of FIG. **17**A. FIG. **17**B shows a dot blot of diglycine-modified lactoglobulin. The purified antibody readily recognizes 0.01 μg of this protein. Thus, the purified antibody has a high purity and can recognize GlyGly-modified proteins at least at the nanogram level.

EXAMPLE 6

Quantifying Ubiquitination Changes after Drug Treatment

[0165] The Example illustrates how protein ubiquitination changes in cells exposed to an inhibitor of nitric oxide synthase, NAME (N-nitro-L-arginine methyl ester).

[0166] sEnd.1 cells typically express large quantities of nitric oxide, which can be inhibited by applying the drug NAME. To identify proteins that are ubiquitinated by endogenous nitric oxide, sEnd.1 cells were treated either by vehicle (DMSO) or 1 mM NAME (dissolved in DMSO) for a period of 1-6 hours in the presence of proteasome inhibitor, LLnL (50 μM) and protein synthesis inhibitor, cycloheximide (CHX, 50 μg/mL). The proteasome inhibitor allows ubiquitinated proteins to accumulate without being rapidly digested so that there will be sufficient ubiquitinated proteins to readily be immunopurified. The protein synthesis inhibitor was used to prevent new proteins from being synthesized, which would complicate the analysis.

[0167] The cells were lysed followed by trypsin digestion overnight, where the vehicle-treated lysate was digested in the presence of light water ($H_2{}^{16}O$) and the lysate from NAME treated cells was digested in the presence of heavy water ($H_2{}^{18}O$). Therefore, the carboxyl termini of tryptic peptides were labeled differently for sample from NAME-treated (^{18}O) and non-treated cells (^{16}O).

[0168] The digested (^{16}O and ^{18}O labeled) samples were mixed with, and immunopurified by, the anti-diglycyl-lysine antibody. The isolated diglycyl-lysine-containing (ubiquitinated) peptides were analyzed by tandem mass spectrometry. The MS/MS spectra were searched against Swiss-Prot database by Spectrum Mill to identify the type of protein that the various peptides were derived from and the relative quantity of diglycyl-lysine-containing (ubiquitinated) peptides was determined from the extracted ion chromatography (EIC) of the ubiquitinated peptides.

[0169] Each peptide was therefore detected on the mass spectrometer as a pair. One member of the pair is derived from the sample prepared with light water, the other from the sample that was labeled with heavy water. The intensity ratio of the light form (^{16}O) and heavy form (^{18}O) of the peptides from the spectrum or chromatogram can be used for quantification of the ubiquitinated peptides in two samples. Therefore a L:H (light to heavy) ratio can be obtained. If ubiquitination is not affected by the drug treatment, then the ratio is going to be 1:1. If the sample that was treated with NAME causes an increase in the abundance of a ubiquitinated peptide, the L/H ratio will drop because the amount of the "heavy" peptide will be greater than the amount of "light" peptide (H>L).

[0170] In this experiment, the ubiquitination level for most proteins did not change so that the ratio of L:H was close to 1. However, the ubiquitination level of several proteins, STX (Sialyltransferase X), GRIN3 (G protein-regulated inducer of neurite outgrowth 3), Whsc1 (Wolf-Hirschhom syndrome candidate 1 protein homolog), Znfx1 (NFX1-type zinc finger containing protein 1), was significantly increased upon NAME treatment (FIG. **19**). These proteins may have a role in nitric oxide signaling that is modulated by ubiquitination upon inhibition of nitric oxide synthase.

EXAMPLE 7

Quantifying Ubiquitination Changes after Drug Treatment

[0171] The Example illustrates how protein ubiquitination changes in mouse N2a neuroblastoma cells exposed to a nitric oxide producer, GSNO(S-nitrosoglutathione).

[0172] In this experiment, a cell line where nitric oxide is not expressed at readily detectable levels, mouse N2a neuro-

blastoma cells, was used to ascertain whether a nitric oxide generating drug would increase or decrease ubiquitination. This would identify ubiquitination events that are operably linked to this drug. Because certain NO donors have been shown to affect ubiquitination pathways, this is a demonstration of how a drug of interest can be tested to see whether it affects protein ubiquitination.

[0173] N2a cells were treated either with vehicle (DMSO) or with 100 μM GSNO (dissolved in DMSO) for a period of 1-6 hours in the presence of proteasome inhibitor, LLnL (50 μM) and protein synthesis inhibitor, cycloheximide (CHX, 50 μg/mL). The GSNO releases nitric oxide to the cells.

[0174] After this treatment, the cells were lysed and the proteins in the lysate were digested with trypsin overnight. In particular, the lysate from vehicle-treated cells was digested with light water ($H_2{}^{16}O$), while the lysate from GSNO-treated cells was digested with heavy water ($H_2{}^{18}O$). Therefore, the carboxyl termini of tryptic peptides from drug treated (^{18}O) and non-treated cells (^{16}O) were labeled differently. The digested samples (^{16}O and ^{18}O labeled) were mixed with, and immunopurified by, the anti-diglycyl-lysine antibody. The isolated ubiquitinated peptides were analyzed by tandem mass spectrometry and the MS/MS spectra were searched against Swiss-Prot database by Spectrum Mill to identify the proteins from the peptides were derived. To quantify the ubiquitinated peptides, extracted ion chromatography (EIC) was used, where the intensity ratio of the light form (^{16}O) and heavy form (^{18}O) of the peptides was used to ascertain the relative amounts of the ubiquitinated peptides in two samples. The larger the ratio of L:H, the less the protein is ubiquitinated in the GSNO treated samples, and vice visa.

[0175] In this experiment, the ubiquitination level for most proteins did not change very much so that the L:H ratios for these proteins was close to 1. However, the ubiquitination of several proteins, STX (Sialyltransferase X), N-RAP (Nebulin-related-anchoring protein), and TAPP-2 (Tandem PH domain-containing protein 2), was significantly increased upon GSNO treatment (FIG. **20**). While, the ubiquitination level of Crk-like protein is significantly reduced upon the treatment. These proteins are possible nitric oxide effectors upon stimulation of nitric oxide.

EXAMPLE 8

Different Types of Proteins are Ubiquitinated in Different Types of Cells

[0176] This Example illustrates that different types of proteins are ubiquitinated in different types of cells.

[0177] FIG. **21**A-D shows in what types of biological processes various ubiquitinated proteins are involved, in different cell lines (send.1, N2a, HEK293) and rat tissue. The biological processes were analyzed by PANTHER and were categorized into eight groups: signal transduction, structure, metabolism, cell cycle/apoptosis, small molecular transport, immunity and defense, protein rafficking/localization, and others/unclassified. For each cell line and tissue, ubiquitinated proteins are mainly involved in signal transduction, metabolism, and cell cycle/apoptosis.

[0178] However, there are some differences between the different cell lines. For example, in mouse N2a neuroblastoma cell (a cancer cell line), about 40% of ubiquitinated proteins are involved in signal transduction, which is more than twice the number of signal transduction proteins in mouse subcutaneous haemangioma endothelial cell line, sEnd.1 (a noncancerous line). Only 3.7 percent of ubiquitinated proteins are related to cell structure in the N2a cancer cells compared to 5.7 percent in sEnd.1 cells. In sEnd.1 cell, 12% of ubiquitinated proteins are related to small molecular transport, which is much higher (2.5 to 7 times) than that in other cell lines tested.

[0179] Therefore, the ubiquitination profile can distinguish different cell types, indicating that specific proteins or specific ubiquitination events, or combinations thereof, can also be used as markers to distinguish between these different cell types, states, or populations.

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[0240] All patents and publications referenced or mentioned herein are indicative of the levels of skill of those skilled in the art to which the invention pertains, and each such referenced patent or publication is hereby incorporated by reference to the same extent as if it had been incorporated by reference in its entirety individually or set forth herein in its entirety. Applicants reserve the right to physically incorporate into this specification any and all materials and information from any such cited patents or publications.

[0241] The specific methods and compositions described herein are representative of preferred embodiments and are exemplary and not intended as limitations on the scope of the invention. Other objects, aspects, and embodiments will occur to those skilled in the art upon consideration of this specification, and are encompassed within the spirit of the invention as defined by the scope of the claims. It will be readily apparent to one skilled in the art that varying substitutions and modifications may be made to the invention disclosed herein without departing from the scope and spirit of the invention. The invention illustratively described herein suitably may be practiced in the absence of any element or elements, or limitation or limitations, which is not specifically disclosed herein as essential. The methods and processes illustratively described herein suitably may be practiced in differing orders of steps, and that they are not necessarily restricted to the orders of steps indicated herein or in the claims. As used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "an antibody" includes a plurality (for example, a solution of antibodies or a series of antibody preparations) of such antibodies, and so forth. Under no circumstances may the patent be interpreted to be limited to the specific examples or embodiments or methods specifically disclosed herein. Under no circumstances may the patent be interpreted to be limited by any statement made by any Examiner or any other official or employee of the Patent and Trademark Office unless such statement is specifically and without qualification or reservation expressly adopted in a responsive writing by Applicants.

[0242] The terms and expressions that have been employed are used as terms of description and not of limitation, and there is no intent in the use of such terms and expressions to exclude any equivalent of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention as claimed. Thus, it will be understood that although the present invention has been specifically disclosed by preferred embodiments and optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the appended claims.

[0243] The invention has been described broadly and generically herein. Each of the narrower species and subgeneric groupings falling within the generic disclosure also form part of the invention. This includes the generic description of the invention with a proviso or negative limitation removing any subject matter from the genus, regardless of whether or not the excised material is specifically recited herein.

[0244] Other embodiments are within the following claims. In addition, where features or aspects of the invention are described in terms of Markush groups, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group.

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20 25 30

Asp Lys Glu
35

<210> SEQ ID NO 32
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 32

Arg Ile Met Val Ser Arg Ser Glu Val Asp Met Leu Lys Ile Arg Ser
1 5 10 15

Glu Phe Lys Arg Lys
20

<210> SEQ ID NO 33
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 33

Arg Val Ser Ser Gln Asn Leu Val Ala Ile Pro Val Tyr Val Lys His
1 5 10 15

Asn Ile Ser Phe Lys Glu Asn Ser Ser Cys Gly Arg Phe
20 25

<210> SEQ ID NO 34
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 34

Lys Leu Ile Asp Val Ile Ser Met Tyr Arg Glu Leu Leu Lys Asp Leu

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1               5                   10                  15

Ser Lys Glu


<210> SEQ ID NO 35
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 35

Lys Val Gln Thr Asp Lys Gly Thr Glu Val Ala Thr Asn Leu Val Ile
1               5                   10                  15

Leu Cys Thr Gly Ile Lys Ile Asn Ser Ser Ala Tyr Arg Lys Ala
            20                  25                  30


<210> SEQ ID NO 36
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 36

Lys Gln Val Gly Val Asn Pro Thr Ser Ile Asp Ser Val Val Ile Gly
1               5                   10                  15

Lys Asp Gln Glu Val Lys Leu
            20


<210> SEQ ID NO 37
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 37

Arg Leu Gly Pro Met Ala Leu Ala Phe Lys Leu Arg Gly Leu Val Asp
1               5                   10                  15

Arg Lys Arg Ser
            20


<210> SEQ ID NO 38
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 38

Lys Ser Asn Asn Ile Phe Leu His Glu Gly Leu Thr Val Lys Ile Gly
1               5                   10                  15

Asp Phe Gly Leu Ala Thr Val Lys Thr Arg Trp
            20                  25


<210> SEQ ID NO 39
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 39

Arg Gly Gly Val Glu Lys Gly Pro Ala Ala Leu Arg Lys Ala Gly Leu
1               5                   10                  15

Val Glu Lys Leu Lys Glu
            20


<210> SEQ ID NO 40
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<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 40

Lys Gly Lys Asp Lys Glu Val His Ser Ile Lys Arg Gly Asp Ser Gly
1 5 10 15

Asn Ile Lys Ala
20

<210> SEQ ID NO 41
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Mus muculus

<400> SEQUENCE: 41

Lys Gly Lys Asp Lys Glu Val His Ser Ile Lys Arg Gly Asp Ser Gly
1 5 10 15

Asn Ile Lys Ala
20

<210> SEQ ID NO 42
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 42

Arg Val Val Pro Leu Asp Glu Ile Lys Glu Ala Ala Asn Ala Ala Asn
1 5 10 15

Ile His Ser Phe Ile Glu Gly Leu Pro Glu Lys Tyr
20 25

<210> SEQ ID NO 43
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 43

Arg Tyr Asp Ser Ser Leu Lys Pro Val Leu Lys His Val Asn Ala Leu
1 5 10 15

Ile Ser Pro Gly Gln Lys Ile
20

<210> SEQ ID NO 44
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 44

Lys Gly Lys Thr Thr Ile Lys Thr Gly Ala Ser Val Leu Asn Lys Trp
1 5 10 15

Gln Met Asn Pro Tyr Asp Arg Gly
20

<210> SEQ ID NO 45
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 45

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Lys His Gly Ala Ile Thr Asn Thr Lys Val Gln Tyr Arg Val Ser Ala
1               5                   10                  15

Thr Asp Leu Pro Pro Glu Leu Pro Lys Ala
            20                  25


<210> SEQ ID NO 46
<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 46

Lys Val Phe Leu Ala Glu Cys Tyr Asn Leu Cys Pro Glu Gln Asp Lys
1               5                   10                  15

Ile Leu Val Ala Val Lys Thr Leu Lys Asp Ala Ser Asp Asn Ala Arg
            20                  25                  30

Lys Asp


<210> SEQ ID NO 47
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 47

Arg Ser Val Met Gln Lys Tyr Leu Glu Asp Arg Gly Glu Val Thr Phe
1               5                   10                  15

Glu Lys Ile


<210> SEQ ID NO 48
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 48

Lys Asn Lys Pro Arg Ser Pro Val Val Glu Leu Ser Lys Val Pro Leu
1               5                   10                  15

Val Gln Arg Gly Ser Ala Asn Gly Leu
            20                  25


<210> SEQ ID NO 49
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 49

Arg Lys Ser Asn Pro Gly Trp Glu Asn Leu Glu Lys Leu
1               5                   10


<210> SEQ ID NO 50
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 50

Lys Ala Met Glu Ser Ala Glu Gln Lys Glu Gln Gly Leu Ser Arg Asp
1               5                   10                  15

Val Thr Thr Val Trp Lys Leu Arg Ile Val Ser Tyr Ser Lys Lys
            20                  25                  30


<210> SEQ ID NO 51
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-continued

<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 51

Arg Lys Ala Asp Thr Thr Thr Pro Thr Thr Ile Asp Pro Ile His Glu
1 5 10 15

Pro Pro Ser Leu Pro Pro Glu Pro Lys Thr Thr Lys Leu
20 25

<210> SEQ ID NO 52
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 52

Lys Met Val Glu Leu Lys Ala Lys Val Glu Gln Phe Glu Lys Leu Ser
1 5 10 15

Asp Lys Leu

<210> SEQ ID NO 53
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 53

Lys Gly Thr Gln Lys Pro Tyr Ala Leu Lys Val Leu Lys Lys Thr Val
1 5 10 15

Asp Lys Lys Ile
20

<210> SEQ ID NO 54
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 54

Lys Ser Gln Glu Gly Lys Pro Lys Glu His Thr Glu Pro Lys Ser Leu
1 5 10 15

Pro Lys Gln

<210> SEQ ID NO 55
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 55

Lys Lys Arg Asp Lys Glu Leu Gly Leu Gly Arg His Glu Asn Ala Ile
1 5 10 15

Lys Tyr

<210> SEQ ID NO 56
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 56

Arg Lys Leu Gly Ala Ser Leu Phe Thr Ile Gly Phe Ala Ile Tyr Glu
1 5 10 15

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Val Pro Lys Glu Met His Gly Asn Lys Gln
            20                  25


<210> SEQ ID NO 57
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 57

Lys Trp Ala Phe Asn Leu Tyr Asp Ile Asn Lys Asp Gly Tyr Ile Thr
1               5                   10                  15

Lys Glu Glu Met Leu Ala Ile Met Lys Ser
            20                  25


<210> SEQ ID NO 58
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 58

Arg Ser Leu His Ala Ala Ala Val Leu Leu Leu Val Ile Leu Lys Glu
1               5                   10                  15

Gln Pro Ser Ser Pro Ala Pro Val Asn Gly Ser Lys Trp
            20                  25


<210> SEQ ID NO 59
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 59

Arg Val Val Gln Lys Asn Leu Val Phe Val Val Gly Leu Ser Gln Arg
1               5                   10                  15

Leu Ala Asp Pro Glu Val Leu Lys Arg
            20                  25


<210> SEQ ID NO 60
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 60

Lys Val Phe Lys Gln Pro Ser Lys Pro Glu Ile Val Asn Lys Ala Pro
1               5                   10                  15

Phe Leu Glu Thr Asp Gln Leu Lys Lys Leu
            20                  25


<210> SEQ ID NO 61
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 61

Arg Ser Val His Leu Lys Glu Gly Glu Gln His Val Asp Asp Val Ser
1               5                   10                  15

Ser Ala Leu Lys Arg Phe
            20


<210> SEQ ID NO 62
<211> LENGTH: 27
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 62

Lys Lys Ala Ala Glu Cys Lys Val Asp Ser Ile Gly Ser Gly Arg Ala
1 5 10 15

Ile Pro Ile Lys Gln Gly Ile Leu Leu Lys Arg
20 25

<210> SEQ ID NO 63
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 63

Lys Ile Ser Glu Lys Thr Ile Gln Ile Ser Asp Ile Gln Lys Asp Leu
1 5 10 15

Asp Lys Ser Lys Asp
20

<210> SEQ ID NO 64
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 64

Lys Gln Phe Ser Lys Asp Thr Tyr Ser Lys Pro Ser Ile Ser Gly Ile
1 5 10 15

Glu Ser Asp Asp His Cys Gln Arg Glu Gln Glu Leu Gln Lys Glu
20 25 30

<210> SEQ ID NO 65
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 65

Lys Ala Gly Val Ser Ala Glu Pro Thr Thr Arg Thr Tyr Asp Leu Asn
1 5 10 15

Lys Pro Pro Glu Phe Ser Phe Glu Lys Ala Arg Val
20 25

<210> SEQ ID NO 66
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 66

Lys Asn Thr Lys Gln Gln Asn Asn Ala Ala Leu Glu Arg Gly Leu Thr
1 5 10 15

Lys Ala Leu Lys Lys
20

<210> SEQ ID NO 67
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 67

Arg Glu Ser Asn Thr Ala Gly Ile Asp Ile Phe Ser Lys Phe Ser Ala

-continued

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1               5                   10                  15

Tyr Ile Lys Asn Thr Lys Gln
            20


<210> SEQ ID NO 68
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 68

Lys Val Ile Asp Lys Gly Ala Gly Gly Gly Gly Ala Gly Gln Gly Ala
1               5                   10                  15

Gly Ala Leu Ala Arg Pro Lys Val Pro Ser Arg Asn
            20                  25


<210> SEQ ID NO 69
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 69

Lys Glu Leu Gln Val Ala Asn Asp Lys Ala Asp Met Val Leu Lys Glu
1               5                   10                  15

Val Thr Met Lys Ala
            20


<210> SEQ ID NO 70
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 70

Arg Tyr Ser Ile Pro Ser His Arg Thr Thr Gln Lys Ser Tyr His Thr
1               5                   10                  15

Gly Lys Lys


<210> SEQ ID NO 71
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 71

Arg Arg Lys Asp Lys Glu Thr Asn Ser Glu Gly Leu Val Asn Lys Thr
1               5                   10                  15

Arg Glu


<210> SEQ ID NO 72
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 72

Lys Glu Ile Leu Thr Lys Met Arg Tyr Phe Ser Asn Ile Glu Glu Lys
1               5                   10                  15

Ile Lys Leu


<210> SEQ ID NO 73
<211> LENGTH: 19
<212> TYPE: PRT
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-continued

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 73

Lys Asp Met Glu Arg Arg Gln Gln Gln Lys Leu Lys Met Gln Ala Glu
1 5 10 15

Ile Lys Arg

<210> SEQ ID NO 74
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 74

Lys Asp Trp Ala Asn Gln Arg Cys Ser Ser Pro Lys Gly Ser Ala Arg
1 5 10 15

Asn Ser Leu Val Lys Ala Lys Arg
20

<210> SEQ ID NO 75
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 75

Arg Gly Pro Pro Gly Pro Ala Gly Lys Pro Gly Asp Asp Gly Glu Ala
1 5 10 15

Gly Lys Pro Gly Lys Ala Gly Glu Arg Gly
20 25

<210> SEQ ID NO 76
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 76

Arg Gly His Pro Gly Pro Pro Gly Pro Pro Gly Glu Gln Gly Leu Pro
1 5 10 15

Gly Ala Ala Gly Lys Glu Gly Thr Lys Gly Asp Pro Gly Pro Ala Gly
20 25 30

Leu Pro Gly Lys Asp
35

<210> SEQ ID NO 77
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 77

Arg Ile Glu Gly Asn Gln Gly Ala Arg Val Gly Leu Val Ala Val Asp
1 5 10 15

Lys Gly Val Phe Val Leu Asn Lys Lys Asn Lys Leu
20 25

<210> SEQ ID NO 78
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 78

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Lys Phe Trp Ile Gly Leu Gln Arg Glu Lys Gly Lys Cys
1               5                   10


<210> SEQ ID NO 79
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 79

Arg Thr Ser Ser Gln Asn Asn Val Gln Val Leu Asn Thr Asn Lys Thr
1               5                   10                  15

Ser Ala Glu Leu Leu Leu Pro Ile Lys Glu
            20                  25


<210> SEQ ID NO 80
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 80

Arg Glu Asn Lys Arg Ile Ala Ile Leu Pro Asp Gly Ser Leu Arg Ile
1               5                   10                  15

Leu Asn Ala Ser Lys Ser Asp Glu Gly Lys Tyr
            20                  25


<210> SEQ ID NO 81
<211> LENGTH: 39
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 81

Lys Phe Pro Val Asp Gly Lys Val Leu Glu Gly Asn Thr Met Ala Asp
1               5                   10                  15

Glu Ser Leu Ile Thr Gly Glu Ala Met Pro Val Thr Lys Lys Pro Gly
            20                  25                  30

Ser Thr Val Ile Ala Arg Ser
        35


<210> SEQ ID NO 82
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 82

Arg Asp Ala Asp Pro Ile Leu Ile Ser Leu Arg Glu Ala Tyr Val Pro
1               5                   10                  15

Ser Lys Gln Arg Asp Leu Lys Ile
            20


<210> SEQ ID NO 83
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 83

Arg Lys Gly Leu Phe Pro Phe Thr His Val Lys Ile
1               5                   10


<210> SEQ ID NO 84
<211> LENGTH: 26
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-continued

<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 84

Arg Gln Gly Ala Phe Leu Val Asn Thr Ala Arg Gly Gly Leu Val Asp
1 5 10 15

Glu Lys Ala Leu Ala Gln Ala Leu Lys Glu
20 25

<210> SEQ ID NO 85
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 85

Arg Lys Arg Pro Ala Thr Asp Asp Ser Ser Thr Gln Asn Lys Arg Ala
1 5 10 15

<210> SEQ ID NO 86
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 86

Lys Leu Pro Lys Thr Leu Gln Glu Leu Arg Leu His Asp Asn Glu Ile
1 5 10 15

Thr Lys Leu Lys Lys
20

<210> SEQ ID NO 87
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 87

Lys Thr Thr Lys Leu His Phe Glu Ala Leu Met Asn Ile Pro Val Leu
1 5 10 15

Val Leu Asp Val Asn Asp Asp Phe Ser Glu Glu Val Thr Lys Gln
20 25 30

<210> SEQ ID NO 88
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 88

Lys Gly Ser Ser Gln Gln Pro Asn Lys Val Thr Asp Lys Asn Lys Met
1 5 10 15

<210> SEQ ID NO 89
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 89

Lys Met Ser Gln Asp Ser Met Met Lys Leu Lys Gly Met Ala Ala Ala
1 5 10 15

Gly Arg Ser Gln Gly Gln His Lys Gln
20 25

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<210> SEQ ID NO 90
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 90

Lys Asp Lys Asp Phe Ala Leu Asp Val Ser Ala Asn Gln Pro Val Leu
1 5 10 15

Val Ala Val Lys Met Leu Arg Ala Asp Ala Asn Lys Asn
20 25

<210> SEQ ID NO 91
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 91

Arg Lys Leu Asp Ser Val Lys Arg Gln Lys Tyr Asn Lys Glu
1 5 10

<210> SEQ ID NO 92
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 92

Lys Thr Asp Lys Leu Ile Gly Phe Ser Lys Pro Ile Val Arg Lys Lys
1 5 10 15

Leu Ser Leu Ser Ser Gln Leu Gly Ser Leu Glu Lys Phe
20 25

<210> SEQ ID NO 93
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 93

Arg Gly Lys Leu Gln Gly His Asp Val Asp Phe Leu Ile Thr His Pro
1 5 10 15

Lys Glu Gly Gln Glu Ala Gly Leu Leu Pro Arg Val
20 25

<210> SEQ ID NO 94
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 94

Lys Leu Leu Lys Ser Val Glu Asn Leu Gly Val Ser Tyr Val Lys Gly
1 5 10 15

Thr Glu Gln Tyr Gln Ser Lys Leu
20

<210> SEQ ID NO 95
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 95

Arg Arg Phe Ala Ile Gly Ser Gln Val Ser Glu His Ser Ile Ile Lys
1 5 10 15

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Asp Phe Thr Lys Gln
            20


<210> SEQ ID NO 96
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 96

Lys Asp Lys Lys Val Ala Glu Pro Asp Asn Lys Lys Lys
1               5                   10


<210> SEQ ID NO 97
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 97

Arg Arg Glu Asp Pro Glu Ala Gly Trp Leu Leu Tyr Leu Lys Thr Gly
1               5                   10                  15

Gln Met Tyr Pro Val Pro Ala Asn His Leu Asp Lys Arg
            20                  25


<210> SEQ ID NO 98
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 98

Lys Gly Ser Arg Ser Gln Lys Glu Ala Phe Pro Leu Ala Lys Gly Glu
1               5                   10                  15

Val Asp Thr Ala Pro Gln Gly Asn Lys Asp Leu Lys Glu
            20                  25


<210> SEQ ID NO 99
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 99

Lys Asp Leu Leu Leu Asn Thr Met Ser Gln Glu Glu Lys Ala Ala Tyr
1               5                   10                  15

Leu Ser Asp Pro Arg Ala Pro Pro Cys Glu Tyr Lys Asp
            20                  25


<210> SEQ ID NO 100
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 100

Lys Ser Pro Ser Ala Cys Leu Val Val Gly Lys Val Val Lys Gly Gly
1               5                   10                  15

Thr Gly Leu Phe Glu Leu Lys Gln Pro Leu Arg
            20                  25


<210> SEQ ID NO 101
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens
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<400> SEQUENCE: 101

Lys Tyr Lys Glu Ala Ala Arg Leu Glu Ile Asn Val Leu Glu Lys Ile
1 5 10 15

Asn Glu Lys Asp
20

<210> SEQ ID NO 102
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 102

Arg Val Leu Ser Phe Lys Thr Gly Ile Ile Ser Leu Cys Lys Ala His
1 5 10 15

Leu Glu Asp Lys Tyr
20

<210> SEQ ID NO 103
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 103

Lys Trp Ser Lys Glu Ala Thr Ala Gly Lys Lys Ser Lys Ser Gly Lys
1 5 10 15

Leu

<210> SEQ ID NO 104
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 104

Lys Lys Gly Val Leu Pro Thr Lys Asp Ile Arg Arg Met Cys Lys Ser
1 5 10 15

<210> SEQ ID NO 105
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 105

Arg Asp Cys Asn Ser Ile Pro Leu Val Leu Gly Thr Cys Lys Glu Thr
1 5 10 15

Phe Asn Leu Tyr Tyr Met Glu Ser Asp Asp His Gly Val Lys Phe
20 25 30

<210> SEQ ID NO 106
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 106

Lys Ser Lys Gln Asp Thr Pro Ala Leu Pro Pro Lys Lys Pro Ala Pro
1 5 10 15

Pro Arg Pro Lys Pro Pro Ser Gly Lys Ser
20 25

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<210> SEQ ID NO 107
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 107

Lys Asn Asp Pro Phe Thr Ser Asp Pro Phe Thr Lys Asn Pro Ser Leu
1 5 10 15

Pro Ser Lys Leu
20

<210> SEQ ID NO 108
<211> LENGTH: 40
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 108

Arg Thr Gly Leu Ser Gln Leu His Asn Ala Leu Asn Asp Val Lys Asp
1 5 10 15

Ile Gln Gln Ser Leu Ala Asp Val Ser Lys Asp Trp Arg Gln Ser Ile
20 25 30

Asn Thr Ile Glu Ser Leu Lys Asp
35 40

<210> SEQ ID NO 109
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 109

Lys Asn Cys Phe Leu Cys Ala Thr Val Thr Thr Glu Arg Pro Val Gln
1 5 10 15

Val Lys Val Val Lys Val Lys Lys
20

<210> SEQ ID NO 110
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 110

Arg Leu Gln Ala Glu Lys Ala Leu Val Glu Phe Thr Asn Ser Pro Asp
1 5 10 15

Cys Leu Ser Lys Cys
20

<210> SEQ ID NO 111
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 111

Lys Ser Arg Lys Lys Ser Ser Glu Gly Lys Lys Gly
1 5 10

<210> SEQ ID NO 112
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 112

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Lys Val Lys Ser Ile Val Thr Leu Asp Gly Gly Lys Leu Val His Leu
1               5                   10                  15

Gln Lys Trp


<210> SEQ ID NO 113
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 113

Arg Leu Val Trp Arg Asn Ser Ile Arg Gly Ser Leu Ile Lys Ser Leu
1               5                   10                  15

Ser Phe Phe Leu Lys Lys Met
            20


<210> SEQ ID NO 114
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 114

Arg His Leu Asp Leu Ser Gly Cys Glu Lys Ile Thr Asp Val Ala Leu
1               5                   10                  15

Glu Lys Ile Ser Arg Ala
            20


<210> SEQ ID NO 115
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 115

Lys Cys Lys Val Lys Ser Leu Leu His Ser Pro Gly Asp Tyr Ile Leu
1               5                   10                  15

Leu Ser Ala Asp Lys Tyr
            20


<210> SEQ ID NO 116
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 116

Arg Thr Pro Cys Thr Val Ser Cys Asn Ile Pro Val Val Ser Gly Lys
1               5                   10                  15

Glu Cys Glu Glu Ile Ile Arg Lys Gly
            20                  25


<210> SEQ ID NO 117
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 117

Arg Leu Arg Ser Pro Pro Lys Lys Gly Leu Gly Ser Pro Thr Val His
1               5                   10                  15

Lys Val
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<210> SEQ ID NO 118
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 118

Arg Val Thr Glu Ala Glu Ile Val Pro Met Gly Lys Asn Ser His Cys
1 5 10 15

Val Arg Phe Val Pro Gln Glu Met Gly Val His Thr Val Ser Val Lys
20 25 30

Tyr

<210> SEQ ID NO 119
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 119

Arg Met Leu Glu Gln Leu Leu Leu Ala Glu Lys Cys His Arg Arg Thr
1 5 10 15

Val Tyr Glu Leu Glu Asn Glu Lys His
20 25

<210> SEQ ID NO 120
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 120

Arg Ala Ile Val Ala Arg Ala Asn Ser Leu Lys Asn Ser Gly Val Pro
1 5 10 15

Asp Asp Ile Phe Lys Leu
20

<210> SEQ ID NO 121
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 121

Arg Thr Val Ser Asp Asn Ser Leu Ser Asn Ser Arg Gly Glu Gly Lys
1 5 10 15

Pro Asp Leu Lys Phe
20

<210> SEQ ID NO 122
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 122

Lys Cys Asn Lys Leu Val Gly Gly Gly Glu Ala Asn Pro Met Asn Tyr
1 5 10 15

Asn Ser Tyr Met Asp Glu Lys Asn
20

<210> SEQ ID NO 123
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

-continued

<400> SEQUENCE: 123

Arg Tyr Glu Leu Leu Leu Lys Asp Tyr Leu Leu Lys Leu Pro His Gly
1 5 10 15

Ser Pro Asp Ser Lys Asp
20

<210> SEQ ID NO 124
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 124

Lys Glu Val Lys Pro Glu Thr Lys Pro Pro Glu Pro Lys Lys
1 5 10

<210> SEQ ID NO 125
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 125

Lys Lys Gly Thr Asp Val Met Val Arg Cys Leu Lys Leu Leu Lys Glu
1 5 10 15

<210> SEQ ID NO 126
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 126

Arg Lys Lys Ser Leu Ala Met Leu Gln Leu Tyr Ile Asn Lys Leu Asp
1 5 10 15

Ser Gln Gly Lys Tyr
20

<210> SEQ ID NO 127
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 127

Arg Leu Glu Ile Val Ser Asp Gly Lys Tyr Gly Ala Arg Asp Pro Asp
1 5 10 15

Thr Lys Ala

<210> SEQ ID NO 128
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 128

Arg Asn Ala Val Asn Leu Ala Val Leu Lys Leu Asn Glu Gln Gly Leu
1 5 10 15

Leu Asp Lys Leu Lys Asn
20

<210> SEQ ID NO 129
<211> LENGTH: 23
<212> TYPE: PRT

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<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 129

Lys Leu Phe His Val Ser Glu Asp Pro Ser Asp Leu Asn Glu Lys His
1 5 10 15

Pro Asn Leu Val His Lys Arg
20

<210> SEQ ID NO 130
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 130

Arg Thr Gln Leu Asn Asp Ser Leu Lys Glu Ile His Gln Lys Glu
1 5 10 15

<210> SEQ ID NO 131
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 131

Lys Ser Val Phe Ala Lys His Gly Leu Glu Lys Leu Thr Pro Ile Gly
1 5 10 15

Asp Lys Tyr

<210> SEQ ID NO 132
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 132

Lys Gly Glu Lys Glu Glu Glu Asp Lys Glu Asp Glu Glu Lys Pro Lys
1 5 10 15

Ile

<210> SEQ ID NO 133
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 133

Arg Val Val Ser Glu Gly Gly Lys Pro Lys Val Gln Val Glu Tyr Lys
1 5 10 15

Gly Glu Thr Lys Thr
20

<210> SEQ ID NO 134
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 134

Arg Ala His Gly Ser Lys Val Val Ala Ala Val Gly Asp Ala Val Lys
1 5 10 15

Ser Ile Asp Asp Ile Gly Gly Ala Leu Ser Lys Leu
20 25

-continued

<210> SEQ ID NO 135
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 135

Arg Ser Pro Thr Thr Asn Ile Asn Glu Thr Ile Gly Ala Phe Gln Leu
1 5 10 15

His Ile Thr Asp Lys Pro Ser Ile Asn Thr Asp Lys Leu
20 25

<210> SEQ ID NO 136
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 136

Lys Leu Phe Val Gly Gly Leu Lys Gly Asp Val Ala Glu Gly Asp Leu
1 5 10 15

Ile Glu His Phe Ser Gln Phe Gly Thr Val Glu Lys Ala Glu Ile Ile
20 25 30

Ala Asp Lys Gln
35

<210> SEQ ID NO 137
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 137

Lys Gln Thr Ala Lys Ala Glu Gly Leu Gly Asp Gly Tyr Arg Leu Val
1 5 10 15

Ile Asn Asp Gly Lys Leu
20

<210> SEQ ID NO 138
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 138

Lys Ala Pro Lys Ser Pro Ala Lys Ala Lys Thr Val Lys Pro Lys Ala
1 5 10 15

Ala Lys Pro Lys Thr
20

<210> SEQ ID NO 139
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 139

Lys Ala Leu Val Gln Asn Asp Thr Leu Leu Gln Val Lys Gly Thr Gly
1 5 10 15

Ala Asn Gly Ser Phe Lys Leu
20

<210> SEQ ID NO 140
<211> LENGTH: 26
<212> TYPE: PRT

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<213> ORGANISM: Mus musculus

<400> SEQUENCE: 140

Lys Leu Leu Gly Gly Val Thr Ile Ala Gln Gly Gly Val Leu Pro Asn
1 5 10 15

Ile Gln Ala Val Leu Leu Pro Lys Lys Thr
20 25

<210> SEQ ID NO 141
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 141

Lys Gln Thr Ala Arg Lys Ser Thr Gly Gly Lys Ala Pro Arg Lys
1 5 10 15

<210> SEQ ID NO 142
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 142

Lys Ala Asp Ile Pro Ala Lys Tyr Ala Glu Thr Met Asp Arg Glu Phe
1 5 10 15

Arg Lys Trp Met Lys Trp
20

<210> SEQ ID NO 143
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 143

Lys Leu Arg Phe His Pro Lys Gln Leu Tyr Phe Ser Ala Arg Gln Gly
1 5 10 15

Glu Leu Gln Lys Val
20

<210> SEQ ID NO 144
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 144

Arg Met Gly Arg Pro Glu Gly Glu Gly Thr Pro Gly Leu Thr Ala Lys
1 5 10 15

Lys Leu

<210> SEQ ID NO 145
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 145

Arg Lys Leu Gly Glu Gly Ser Phe Ala Lys Val Arg Glu
1 5 10

<210> SEQ ID NO 146
<211> LENGTH: 16

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<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 146

Arg Asn Lys Pro Leu Lys Ala Leu Asp Thr Arg Phe Gly Arg Lys Leu
1 5 10 15

<210> SEQ ID NO 147
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 147

Arg Asn Gly Gln Ser Arg Leu Ser Leu Lys His Gly Glu Lys Ala
1 5 10 15

<210> SEQ ID NO 148
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 148

Lys Phe Gly Asp Arg Gly Asn Gln Leu Arg Lys Met Leu Leu Gln Asn
1 5 10 15

Tyr Leu Gln Asn Arg Lys Ser
20

<210> SEQ ID NO 149
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 149

Lys Lys Phe Ala Ala Asp Phe Lys Thr Leu Ala Asp Val Leu Val Gln
1 5 10 15

Glu Val Ile Lys Gln
20

<210> SEQ ID NO 150
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 150

Lys Thr Leu Thr Lys Glu Asp Ile Ile Lys Phe
1 5 10

<210> SEQ ID NO 151
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 151

Lys Gly Trp Pro Lys Thr His Pro Gly Gly Glu Gln Lys Glu Gly Thr
1 5 10 15

Glu Ala Ser Leu Gln Ile Arg Gly Lys Lys
20 25

<210> SEQ ID NO 152
<211> LENGTH: 29
<212> TYPE: PRT

-continued

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 152

Arg Gly Ile Asp Ser Ser Tyr Arg Pro Ser Gln Lys Asp Val Glu Pro
1 5 10 15

Pro Lys Ile Ser Ser Thr Lys Asn Ile Ser Ile Lys Gln
20 25

<210> SEQ ID NO 153
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 153

Arg Lys Ser Glu Asn Thr Ala Ile His His Tyr Leu Glu Ala Leu Lys
1 5 10 15

Val Lys Asp Arg Ser Pro Leu Arg Thr
20 25

<210> SEQ ID NO 154
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 154

Arg Arg Cys Leu Ile Ser Thr Asp Met His His Ile Glu Glu Ser Phe
1 5 10 15

Gln Glu Ile Lys Arg Ala Ile Gln Ala Lys Asp
20 25

<210> SEQ ID NO 155
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 155

Lys Glu Gln Leu Asp Ala Leu Glu Lys Glu Thr Ala Ser Lys Leu Ser
1 5 10 15

Glu Met Asp Ser Phe Asn Asn Gln Leu Lys Cys
20 25

<210> SEQ ID NO 156
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 156

Lys Gln Lys Arg Leu Gln Glu Glu Lys Ser Gln Asp Lys Thr
1 5 10

<210> SEQ ID NO 157
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 157

Lys Ile Pro Val Gly Ser Glu Glu Gly Tyr Arg Ser Leu Phe Gly Gln
1 5 10 15

Val Leu Lys Asp Ile Val Glu Lys Ile
20 25

-continued

<210> SEQ ID NO 158
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 158

Lys Met Trp Lys Ser Pro Asn Gly Thr Ile Arg Asn Ile Leu Gly Gly
1 5 10 15

Thr Val Phe Arg Glu Pro Ile Ile Cys Lys Asn
20 25

<210> SEQ ID NO 159
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 159

Arg Glu Val Arg Pro Ser Pro Ser Lys Thr Val Lys Tyr Thr Ala Thr
1 5 10 15

Val Thr Lys Gly Ala Val Thr Tyr Thr Lys Ala Lys Arg
20 25

<210> SEQ ID NO 160
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 160

Lys Ile Ser Glu Leu Ser Leu Cys Asp Leu Ala Gly Ser Glu Arg Cys
1 5 10 15

Lys His Gln Lys Ser
20

<210> SEQ ID NO 161
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 161

Lys Lys Leu His Ala Met Asn Lys Glu Leu Gln Arg Leu Gln Thr Ala
1 5 10 15

Gln Lys Glu

<210> SEQ ID NO 162
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 162

Lys Gln Leu Asp Asp Lys Asp Asp Glu Ile Asn Gln Gln Ser Gln Leu
1 5 10 15

Ile Glu Lys Leu
20

<210> SEQ ID NO 163
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

-continued

<400> SEQUENCE: 163

Arg Asn Glu Thr Pro Ser Gly Asp Ser Gln Thr Leu Met Ile Pro Ser
1 5 10 15

Asn Pro Cys Leu Met Lys Arg Lys
20

<210> SEQ ID NO 164
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 164

Arg Met Gly Lys Leu His Leu Val Asp Leu Ala Gly Ser Glu Arg Gln
1 5 10 15

Ala Lys Thr Gly Ala Thr Gly Gln Arg Leu
20 25

<210> SEQ ID NO 165
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 165

Arg Lys Arg Thr Phe Ser Leu Gln Gly Gly Gly Gly Gly Gly Ala Asn
1 5 10 15

Gly Gly Ser Gly Gly Gln Gly Lys Gly
20 25

<210> SEQ ID NO 166
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 166

Lys Ile Met Gly Gly Ser Gly Thr Glu Val Val Leu Glu Lys Gln Lys
1 5 10 15

Ser Thr Pro Lys Ser
20

<210> SEQ ID NO 167
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 167

Lys Ile Pro Ala Lys Ile Ser Gln Met Thr Asn Leu Gln Glu Leu His
1 5 10 15

Leu Cys His Cys Pro Ala Lys Val
20

<210> SEQ ID NO 168
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 168

Lys Asn Asn Met Asn Arg Ser Asn Thr Ile Gln Ser Gly Pro Glu Gly
1 5 10 15

Ser Leu Val Lys Ser Gln Ser Leu Lys Ser Ile Pro Glu Lys Phe

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            20                  25                  30


<210> SEQ ID NO 169
<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 169

Arg Lys Ile Ser Glu Asn Ser Tyr Ser Leu Asp Asp Leu Glu Ile Gly
1               5                   10                  15

Pro Gly Gln Leu Ser Ser Ser Thr Phe Asp Ser Glu Lys Asn Glu Ser
            20                  25                  30

Arg Arg


<210> SEQ ID NO 170
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 170

Arg Cys Leu Glu His Pro Asn Val Leu Lys Phe Ile Gly Val Leu Tyr
1               5                   10                  15

Lys Asp Lys Arg
            20


<210> SEQ ID NO 171
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 171

Arg Ser Gly Lys Tyr Leu Ala Thr Glu Trp Asn Thr Val Ser Lys Leu
1               5                   10                  15


<210> SEQ ID NO 172
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 172

Lys Glu Ala Glu Ala Phe Leu Glu Lys Tyr Gly Tyr Leu Asn Glu Gln
1               5                   10                  15

Val Pro Lys Ala Pro Thr Ser Thr Arg Phe
            20                  25


<210> SEQ ID NO 173
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 173

Arg Gly Arg Gln Thr Val Asp Lys Val Met Gly Ile Pro Lys Glu
1               5                   10                  15


<210> SEQ ID NO 174
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 174
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Lys Lys Val Ser Phe Ser Ser Pro Leu Ile Leu Gly Ala Thr Ile Gln
1               5                   10                  15

Lys Lys Ser


<210> SEQ ID NO 175
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 175

Lys Lys Ile Pro Ile Lys Arg Thr Asp Ile Leu Lys His Val Val Gly
1               5                   10                  15

Asp Tyr Arg Asp
            20


<210> SEQ ID NO 176
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 176

Lys Lys Ile Pro Ile Lys Arg Thr Asp Ile Leu Lys His Val Val Gly
1               5                   10                  15

Asp Tyr Arg Asp
            20


<210> SEQ ID NO 177
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 177

Lys Leu Gln Asn Leu Gln Thr Val Arg Leu Val Phe Lys Ile Gln Thr
1               5                   10                  15

Gln Thr Pro Arg Lys Lys Thr
            20


<210> SEQ ID NO 178
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 178

Lys Lys Gly Thr Glu Leu Leu Leu Gly Val Asp Ala Leu Gly Leu His
1               5                   10                  15

Ile Tyr Asp Pro Glu Asn Arg Leu Thr Pro Lys Ile
            20                  25


<210> SEQ ID NO 179
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 179

Lys Ile Ala Gln Asp Leu Glu Met Tyr Gly Val Asn Tyr Phe Thr Ile
1               5                   10                  15

Arg Asn Lys Lys
            20
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<210> SEQ ID NO 180
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 180

Arg Gly Ser Glu Gly Lys Pro Cys Gly Glu Leu Lys Lys Glu Lys Gly
1 5 10 15

<210> SEQ ID NO 181
<211> LENGTH: 32
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 181

Arg Val Asp Gly Asp Ile Ile Leu Gly Gly Leu Phe Pro Val His Ala
1 5 10 15

Lys Gly Glu Arg Gly Val Pro Cys Gly Glu Leu Lys Lys Glu Lys Gly
20 25 30

<210> SEQ ID NO 182
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 182

Lys Ser Ser Cys Lys Glu Asn Pro Phe Asn Arg Lys Pro Ser Pro Ala
1 5 10 15

Ala Ser Pro Ala Thr Lys Lys Ala
20

<210> SEQ ID NO 183
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 183

Lys Val Ile Val Lys Lys Asp Lys Pro Gly Lys Val Glu Ser Lys Pro
1 5 10 15

Ser Val Thr Glu Lys Glu
20

<210> SEQ ID NO 184
<211> LENGTH: 9
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 184

Lys Thr Lys Pro Glu Glu Lys Lys Glu
1 5

<210> SEQ ID NO 185
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 185

Lys Ser Lys Pro Ser Ala Ala Ser Pro Lys Pro Gly Ala Leu Lys Glu
1 5 10 15

Ser Ser Asp Lys Val Ser Arg Val Ala Ser Pro Lys Lys Lys
20 25 30

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<210> SEQ ID NO 186
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 186

Arg Gln Trp Ile Gly Lys His Arg Arg Pro Arg Thr Val Ser Phe Gln
1 5 10 15

Ala Lys Glu

<210> SEQ ID NO 187
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 187

Lys Ala Asp Pro Phe Ser Phe Lys Ala Arg Ala Lys Ser Cys Gly Glu
1 5 10 15

Asp Gly Lys Gly
20

<210> SEQ ID NO 188
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 188

Lys Lys Ile Lys Thr Lys Lys Ser Thr Trp Glu Lys Val
1 5 10

<210> SEQ ID NO 189
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 189

Lys Arg Ala Arg Ala Asp Pro Thr Val Glu Ser Glu Glu Ala Phe Lys
1 5 10 15

Ser Arg Met Glu Val Lys Val
20

<210> SEQ ID NO 190
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 190

Arg Ala Lys Leu Asp Ser Gly Arg Val Leu Lys Gln Ile Ser Asn Asn
1 5 10 15

Arg Lys Cys

<210> SEQ ID NO 191
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 191

Arg Gly Ser Gly Lys Asp Ser His His Pro Ala Arg Thr Ala His Tyr
1 5 10 15

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Gly Ser Leu Pro Gln Lys Ser
            20


<210> SEQ ID NO 192
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 192

Lys Ile Asn Lys Ser Glu Ser Val Val Tyr Ala Asp Ile Arg Lys Asp
1               5                   10                  15


<210> SEQ ID NO 193
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 193

Lys Glu Ile Phe Gly Gly Asn Ala Asp Lys Lys Asn Leu Val Asp Pro
1               5                   10                  15

Phe Val Glu Val Ser Phe Ala Gly Lys Lys
            20                  25


<210> SEQ ID NO 194
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 194

Arg Ile Glu Ala Gln Asn Lys Pro Phe Asp Ala Lys Thr Ser Val Phe
1               5                   10                  15

Val Val Asp Pro Lys Glu Ser Tyr Val Lys Ala
            20                  25


<210> SEQ ID NO 195
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 195

Arg Val Phe Asp Lys Glu Gly Asn Gly Thr Val Met Gly Ala Glu Ile
1               5                   10                  15

Arg His Val Leu Val Thr Leu Gly Glu Lys Met
            20                  25


<210> SEQ ID NO 196
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 196

Arg Gly Asp Glu Val Met Val Glu Leu Ala Glu Asn Gly Lys Lys Ala
1               5                   10                  15


<210> SEQ ID NO 197
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 197
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Lys Leu Lys Lys Leu Glu Glu Glu Gln Ile Ile Leu Glu Asp Gln Asn
1               5                   10                  15

Cys Lys Leu


<210> SEQ ID NO 198
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 198

Arg Glu Thr Asn Lys Gln Ile Ser Lys Leu Thr Ile Tyr Asp Ala Arg
1               5                   10                  15

Pro Ser Val Asn Ala Val Ala Asn Lys Ala
            20                  25


<210> SEQ ID NO 199
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 199

Lys Gln Lys Gly His Tyr Leu Ala Gly Lys Val Ile Gly Glu Phe Pro
1               5                   10                  15

Gly Val Val His Cys Leu Asp Phe Gln Lys Met
            20                  25


<210> SEQ ID NO 200
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 200

Lys Gln Lys Gly His Tyr Val Gly Thr Leu Thr Ala Arg Asp Asp Asn
1               5                   10                  15

Lys Ile


<210> SEQ ID NO 201
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 201

Lys Gly Phe Glu Leu Arg Leu Asp Ser Leu Ala Phe Leu Thr Ala Lys
1               5                   10                  15

Ala Lys Arg


<210> SEQ ID NO 202
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 202

Lys Gly Phe Glu Leu Arg Leu Asp Ser Leu Ala Phe Leu Thr Ala Lys
1               5                   10                  15

Ala Lys Arg


<210> SEQ ID NO 203
<211> LENGTH: 23
<212> TYPE: PRT
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-continued

<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 203

Lys Lys Tyr Leu Leu Leu Gly Asn Ala Glu Asp Ser Pro Asp Gln Ser
1 5 10 15

Gly Ile Val Ala Asp Lys Ser
20

<210> SEQ ID NO 204
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 204

Arg Leu Leu Gln Ser Ala Phe Ser Lys Asn Ala Leu Ser Lys Gln Ser
1 5 10 15

Pro Lys Lys Ser Pro Ser Ala Lys Leu
20 25

<210> SEQ ID NO 205
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 205

Arg Asn Gly Val Gly Leu Glu Phe Asn His Leu Phe Gly Tyr Gly Val
1 5 10 15

Leu Asp Ala Gly Ala Met Val Lys Met Ala Lys Asp
20 25

<210> SEQ ID NO 206
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 206

Lys Glu Glu Ala Lys Ser Pro Gly Glu Ala Lys Ser Pro Gly Glu Ala
1 5 10 15

Lys Ser Pro Ala Glu Ala Lys Ser Pro Gly Glu Ala Lys Ser
20 25 30

<210> SEQ ID NO 207
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 207

Lys Ala Ser Thr Asp Asn Ser Pro Ser Ser Lys Ala Glu Asp Ala Pro
1 5 10 15

Ala Lys Glu Glu Pro Lys Gln
20

<210> SEQ ID NO 208
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 208

Lys Asp Gln Thr Gln Lys Ala Ala Thr Gly Pro Phe Asp Arg Glu His
1 5 10 15

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Leu Leu Met Tyr Leu Glu Lys Glu
            20


<210> SEQ ID NO 209
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 209

Arg Ser Asn Ser Glu Ile Leu Lys Gln Phe Thr Leu Arg Glu Leu Arg
1               5                   10                  15

Asn Lys Arg


<210> SEQ ID NO 210
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 210

Arg Phe Gly Ser Lys Ala His Met Glu Arg Leu Glu Glu Val Asn Lys
1               5                   10                  15

Glu


<210> SEQ ID NO 211
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 211

Arg Gly Gly Asp Val Ser Asp Ser Lys Gln Phe Thr Tyr Tyr Pro Leu
1               5                   10                  15

Val Glu Asp Lys Glu Glu Val Gln Arg Lys Arg
            20                  25


<210> SEQ ID NO 212
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 212

Lys Gln Leu Leu Thr Gly Asn Thr Asp Lys Pro Ile Gly Met Ile Asp
1               5                   10                  15

Arg Leu Asn Ser Pro Leu Leu Ser Asn Lys Thr
            20                  25


<210> SEQ ID NO 213
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 213

Arg Ser Gly Thr Ser Ser Gly Ala Gly Gly Ser Ile Thr Arg Gly Ala
1               5                   10                  15

Pro Val Val Val Pro Glu Leu Gly Lys Pro Arg Gln
            20                  25


<210> SEQ ID NO 214
<211> LENGTH: 19
<212> TYPE: PRT
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-continued

<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 214

Arg Cys Lys Gln Leu Lys Arg Ala Ala Leu Gly Arg Met Cys Thr Ile
1 5 10 15

Ile Lys Arg

<210> SEQ ID NO 215
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 215

Arg Val Gly Asp Leu Ile Leu Ala His Leu His Lys Lys Cys Pro Tyr
1 5 10 15

Ser Val Pro Phe Tyr Pro Thr Phe Lys Glu
20 25

<210> SEQ ID NO 216
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 216

Lys Gln Val Lys Gly Ser Ala Asp Tyr Lys Ser Lys Lys
1 5 10

<210> SEQ ID NO 217
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 217

Lys Gln Val Lys Gly Ser Ala Asp Tyr Lys Ser Lys Lys
1 5 10

<210> SEQ ID NO 218
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 218

Arg Ile Val Ser Ser Ile Phe Lys Val Pro Ser Ser Gln Ser Ile His
1 5 10 15

Lys Ala

<210> SEQ ID NO 219
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 219

Lys Lys Pro Tyr Val Ala Lys Leu Ile Glu Leu Phe Gln Asn Gly Ala
1 5 10 15

Glu Val Pro Pro Lys Lys Cys Ala Arg Val
20 25

<210> SEQ ID NO 220
<211> LENGTH: 28
<212> TYPE: PRT

-continued

<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 220

Lys Leu Phe Asn Tyr Asn Lys Val Leu Pro Met Asn Thr Gly Val Glu
1 5 10 15

Ala Gly Glu Thr Ala Cys Lys Leu Ala Arg Arg Trp
20 25

<210> SEQ ID NO 221
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 221

Lys Glu Met Asn Ile Leu Glu Leu Ser His Lys Lys Met Met Lys Lys
1 5 10 15

<210> SEQ ID NO 222
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 222

Arg Gly Thr Asp Ser Glu His Thr His Lys Ala His Leu Val Pro Glu
1 5 10 15

Gly Thr Ser Lys Lys Arg
20

<210> SEQ ID NO 223
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 223

Arg Lys Ser Val Arg Pro Gly Ala Ser Tyr Lys Arg Gln
1 5 10

<210> SEQ ID NO 224
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 224

Lys Gly Ala Thr Ala Gly Ala Ser His Lys Gly Asp Glu Val Pro Pro
1 5 10 15

Ile Lys Lys Asn Thr Lys Ala Pro Gly Lys Gln
20 25

<210> SEQ ID NO 225
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 225

Arg Glu Ile Asp Lys Arg Met Asn Ser Ile Lys Pro Asp Leu Ile Gln
1 5 10 15

Leu Arg Lys Thr
20

<210> SEQ ID NO 226

-continued

<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 226

Lys Lys Gln Ala Ala Glu Tyr Arg Glu Ile Asp Lys Arg Met Asn Ser
1 5 10 15

Ile Lys Pro Asp Leu Ile Gln Leu Arg Lys
20 25

<210> SEQ ID NO 227
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 227

Arg Leu Glu Gln Asp Leu Lys Lys Gln Ala Leu Asp Asn Arg Glu Ile
1 5 10 15

Asp Lys Lys Met
20

<210> SEQ ID NO 228
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 228

Arg Glu Pro Leu Arg Thr Ile Pro Leu Lys Asp Val Leu Lys Thr His
1 5 10 15

Glu Cys Leu Val Lys Ser
20

<210> SEQ ID NO 229
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 229

Lys Ile Lys Pro Thr Ser Ala Trp Asn Leu Ala Gln Lys His Lys Leu
1 5 10 15

<210> SEQ ID NO 230
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 230

Arg Ser Phe Pro Ser Glu Lys Arg Gly Val Leu Ser Ser Tyr Pro Ser
1 5 10 15

Asp Val Ile Ser Tyr Arg Gly Leu Arg Gly Ser Gln Asp Lys Leu
20 25 30

<210> SEQ ID NO 231
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 231

Arg Val Asp Arg Met Glu His Ser Ile Gly Ser Ile Val Ser Lys Ile
1 5 10 15

-continued

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Asp Ala Val Ile Val Lys Leu Glu Ile Met Glu Arg Ala Lys Leu
            20                  25                  30


<210> SEQ ID NO 232
<211> LENGTH: 11
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 232

Lys Ile Asp Lys Asn Glu Asp Arg Lys Lys Asp
1               5                   10


<210> SEQ ID NO 233
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 233

Lys Gly Asp Pro Phe Leu Ala Ser Pro Thr Ser Asp Arg Glu Ile Ile
1               5                   10                  15

Ala Pro Lys Ile Lys Glu Arg Thr
            20


<210> SEQ ID NO 234
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 234

Lys Leu Lys Ile Asn Asn Asn Val Phe Val Asp Lys Pro Ala Phe Pro
1               5                   10                  15

Glu Tyr Lys Val Ser Asp Ala Lys Lys
            20                  25


<210> SEQ ID NO 235
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 235

Arg Asp Gly Ala Leu Leu Ile Ala Gly Ser Glu Ala Pro Lys Arg Gly
1               5                   10                  15

Ser Val Leu Ser Lys Pro Arg Thr Gly Gly Ala Gly Ala Gly Lys Pro
            20                  25                  30

Pro Lys Arg
        35


<210> SEQ ID NO 236
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 236

Lys Cys Pro Lys Pro Ser Ala Gln Glu Ile Thr Asn Leu Ala Asp Ser
1               5                   10                  15

Leu Gln Leu Glu Lys Glu Val Val Arg Val Trp Phe Cys Asn Arg Arg
            20                  25                  30

Gln
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<210> SEQ ID NO 237
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 237

Arg Glu Tyr Ser Val Lys Glu Ile Leu Lys Leu Asp Ser Asn Pro Ser
1 5 10 15

Lys Gly Lys Asp
20

<210> SEQ ID NO 238
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 238

Arg Gly Val Ser Ser Gln Glu Thr Ala Gly Ile Gly Ala Ser Ala His
1 5 10 15

Leu Val Asn Phe Lys Gly Thr Asp Thr Val Ala Gly Leu Ala Leu Ile
20 25 30

Lys Lys Tyr
35

<210> SEQ ID NO 239
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 239

Lys Tyr Val Glu Ala Lys Asp Cys Leu Asn Val Leu Asn Lys Ser Asn
1 5 10 15

Glu Gly Lys Glu
20

<210> SEQ ID NO 240
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 240

Lys Tyr Val Glu Ala Lys Asp Cys Leu Asn Val Leu Asn Lys Ser Asn
1 5 10 15

Glu Gly Lys Glu
20

<210> SEQ ID NO 241
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 241

Lys Gln Tyr Tyr Lys Val Val Asn Ser Tyr Pro Leu Ala His Lys Val
1 5 10 15

Phe Glu Glu Lys Thr
20

<210> SEQ ID NO 242
<211> LENGTH: 13
<212> TYPE: PRT

-continued

<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 242

Arg Trp Lys Val Thr Ser Lys Glu Ala Leu Gln Arg Ile
1 5 10

<210> SEQ ID NO 243
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 243

Arg Ala Asn Gln Ser Val Pro Glu Asp Leu Val Val Met Ala Glu Gln
1 5 10 15

Tyr Lys Leu Asn Gln Gln Lys Arg
20

<210> SEQ ID NO 244
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 244

Arg Gly Gly Asp Gly Ile Asn Ser Ile Gly Gly Gly Gln Lys Val Arg
1 5 10 15

Leu Met Lys Glu
20

<210> SEQ ID NO 245
<211> LENGTH: 37
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 245

Arg Ser Val Pro Ala Thr Lys Gly Leu Leu Ser Pro Leu Met Ser Arg
1 5 10 15

Pro Glu Ile Lys Val Gly Asp Gln Ser Gly Thr Gly Arg Gly Gln Lys
20 25 30

Val Leu Pro Lys Gly
35

<210> SEQ ID NO 246
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 246

Lys Val Thr Val Val Asn Ser Ile Lys Pro Ser Ser Pro Thr Glu Gly
1 5 10 15

Lys Arg

<210> SEQ ID NO 247
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 247

Arg Glu Met Val Val Gln Tyr Ile Ser Ala Thr Ser Lys Ser Ile Val
1 5 10 15

-continued

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Gly Ser Lys Val Leu Gly Gly Leu Lys Asn Ser Lys His
            20                  25


<210> SEQ ID NO 248
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 248

Arg Ala Val Glu Lys Tyr Ser Val Lys Pro Glu His Pro Asn Leu Gly
1               5                   10                  15

Ser Cys Asn Pro Ser Leu Tyr Asn Thr Phe Ala Ser Lys Gln
            20                  25                  30


<210> SEQ ID NO 249
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 249

Arg Asp Asn Met Ser Ile Val Leu Val Cys Phe Ser Asn Ala Pro Lys
1               5                   10                  15

Val Ser Asp Glu Ala Val Lys Lys Asp
            20                  25


<210> SEQ ID NO 250
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 250

Lys Asn Glu Ala Ala Phe Leu Ser Met Thr Asp Ser Lys Thr Glu Gly
1               5                   10                  15

Lys Phe


<210> SEQ ID NO 251
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 251

Lys Leu Lys Ile Leu Met Asp Lys Pro Glu Met Asn Val Val Leu Lys
1               5                   10                  15

Asn Val Lys Pro Asp Gln Trp Val Lys Leu
            20                  25


<210> SEQ ID NO 252
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 252

Arg Lys Glu Leu Asn Lys Ile Leu Glu Gly Arg Ser Lys Ile
1               5                   10


<210> SEQ ID NO 253
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 253
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Arg Asp Leu Lys Leu Glu Asn Leu Met Leu Asp Lys Asp Gly His Ile
1               5                   10                  15

Lys Ile


<210> SEQ ID NO 254
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 254

Arg Phe Tyr Gly Ala Glu Ile Val Ser Ala Leu Asp Tyr Leu His Ser
1               5                   10                  15

Gly Lys Ile Val Tyr Arg Asp Leu Lys Leu
            20                  25


<210> SEQ ID NO 255
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 255

Arg Asp Met Lys Ser Asn Asn Ile Phe Leu His Glu Gly Leu Thr Val
1               5                   10                  15

Lys Ile Gly Asp Phe Gly Leu Ala Thr Val Lys Ser Arg Trp
            20                  25                  30


<210> SEQ ID NO 256
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 256

Arg Leu Glu Gly Asn Thr Val Gly Val Glu Ala Ala Arg Val Ile Ala
1               5                   10                  15

Lys Ala Leu Glu Lys Lys
            20


<210> SEQ ID NO 257
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 257

Arg Glu Lys His Cys Leu Gly Met Lys Leu Ser Glu Asp Gly Thr Tyr
1               5                   10                  15

Thr Gly Phe Ile Lys Val
            20


<210> SEQ ID NO 258
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 258

Arg Thr Leu Thr Leu Ile Ala Lys Val Ile Gln Asn Leu Ala Asn Phe
1               5                   10                  15

Ser Lys Phe
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-continued

<210> SEQ ID NO 259
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 259

Met Ala Lys Ser Ala Glu Val Lys Leu Ala Ile Phe Gly Arg Ala Gly
1 5 10 15

Val Gly Lys Ser Ala Ile Val Val Arg Phe
20 25

<210> SEQ ID NO 260
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 260

Arg Leu Leu Lys Lys Glu Gln Asn Lys Met Gly Val Lys Leu Ser
1 5 10 15

<210> SEQ ID NO 261
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 261

Lys Ala Thr His Pro Pro Pro Ala Ser Pro Ser Ser Leu Val Lys Val
1 5 10 15

Pro Ser Ser Ala Thr Gly Lys Arg Gln
20 25

<210> SEQ ID NO 262
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 262

Lys Glu Glu Leu Asn Asn Lys Leu Lys Asp Thr Gln Glu Gln Leu Ser
1 5 10 15

Lys Leu Lys Asp
20

<210> SEQ ID NO 263
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 263

Arg Glu Ala Leu Gly Asp Lys Ala Pro Pro Lys Pro Val Pro Lys Thr
1 5 10 15

<210> SEQ ID NO 264
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 264

Lys Leu Ala Lys Gln Asn Thr Asn Lys Ala Lys Glu Asn Leu Arg Lys
1 5 10 15

<210> SEQ ID NO 265

-continued

<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 265

Lys Leu Asp Thr Pro Ala Thr Ser Asp Pro Leu Ser Asp Arg Gly Gly
1 5 10 15

Arg Lys Arg Lys Arg
20

<210> SEQ ID NO 266
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 266

Arg Arg Asn His Cys Phe Gln Asp Phe Lys Glu Glu Lys Pro Gln Glu
1 5 10 15

Asn Lys Thr

<210> SEQ ID NO 267
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 267

Arg Gln Lys Cys His Ser Pro Pro Pro Lys Pro Glu Pro Phe Pro Phe
1 5 10 15

Gly Gln Ser Gly Gln Lys Pro Ala Leu Asn Gly Gly Lys Lys Val
20 25 30

<210> SEQ ID NO 268
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 268

Lys Phe Glu Ala Lys Leu Met Pro Glu Glu Cys Phe Ser Pro Leu Asp
1 5 10 15

Leu Phe Asn Lys Ile
20

<210> SEQ ID NO 269
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 269

Lys Val Ala Ser Ile Ser Phe Asp Ala Ser Lys Ala Lys Lys Pro Ser
1 5 10 15

Gln Phe Ser Gly Lys Ile
20

<210> SEQ ID NO 270
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 270

Lys Arg Leu Ala Val Ph e Ser Gln Pro Ile Ile Asn Lys Val Lys Pro

-continued

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1               5                   10                  15

Gln Leu Leu Lys Thr
            20


<210> SEQ ID NO 271
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Homo  sapiens

<400> SEQUENCE: 271

Arg Lys Ala Gln Ala Ala Glu Met Lys Ala Ala Asn Glu Ala Glu Gly
1               5                   10                  15

Lys Val


<210> SEQ ID NO 272
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo  sapiens

<400> SEQUENCE: 272

Lys Tyr Leu Lys Ile Glu Glu Thr Asn Pro Ser Leu Ala Gln Asp Thr
1               5                   10                  15

Val Ile Ile Lys Lys
            20


<210> SEQ ID NO 273
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 273

Arg Lys Gly Glu Ile Ala Ala Ser Ile Ala Thr His Met Arg Pro Tyr
1               5                   10                  15

Arg Lys Lys Ser
            20


<210> SEQ ID NO 274
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 274

Lys Ser Asp Gly Tyr His Lys Arg Pro Asp Arg Lys Ser Arg Ile
1               5                   10                  15


<210> SEQ ID NO 275
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 275

Lys Arg Lys Asp Thr Lys Arg Leu Val Leu His Met Lys Asn
1               5                   10


<210> SEQ ID NO 276
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 276
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-continued

```
Lys Asp Ser Thr Lys Asp Asp Asn Ser Asn Leu Gly Gly Lys Thr Asp
1               5                   10                  15

Glu Ala Lys Gly Lys Thr
            20


<210> SEQ ID NO 277
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 277

Lys Asp Ser Thr Lys Asp Asp Asn Ser Asn Leu Gly Gly Lys Thr Asp
1               5                   10                  15

Glu Ala Lys Gly Lys Thr
            20


<210> SEQ ID NO 278
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 278

Arg His Gly Tyr Thr Ser Ser Leu Glu Leu Pro Asp Asn Ile Leu Asn
1               5                   10                  15

Phe Val Lys Lys His
            20


<210> SEQ ID NO 279
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 279

Arg Asp Ile Lys Pro Asp Asn Val Leu Leu Asp Val Asn Gly His Ile
1               5                   10                  15

Arg Leu Ala Asp Phe Gly Ser Cys Leu Lys Met
            20                  25


<210> SEQ ID NO 280
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 280

Arg Leu Leu Ser Ala Ala Lys Ala Pro Asp Arg Lys Ala
1               5                   10


<210> SEQ ID NO 281
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 281

Lys Leu Asp Asn Thr Val Val Gly Gln Thr Ile Trp Lys Pro Ile Ser
1               5                   10                  15

Asn Gln Ser Trp Asp Gln Lys Phe
            20


<210> SEQ ID NO 282
<211> LENGTH: 21
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-continued

<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 282

Lys Glu Lys Lys Pro Ile Lys Ser Pro Ser Lys Asp Ala Ser Ser Gly
1 5 10 15

Lys Glu Asn Arg Ser
20

<210> SEQ ID NO 283
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 283

Arg Asp Leu Lys Pro Glu Asn Ile Leu Leu Asp Cys Gln Gly His Val
1 5 10 15

Val Leu Thr Asp Phe Gly Leu Cys Lys Glu
20 25

<210> SEQ ID NO 284
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 284

Lys Gln Asn Asn Thr Ala Lys Leu Val Lys Gln Leu Ser Lys Ser Ser
1 5 10 15

Glu Asp Glu Glu Leu Arg Lys Leu
20

<210> SEQ ID NO 285
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 285

Lys Glu Glu Gly Lys Ser Arg Thr Thr Leu Pro Glu Arg Pro Leu Thr
1 5 10 15

Glu Val Lys Ala
20

<210> SEQ ID NO 286
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 286

Lys Ala Gln Gly Lys Lys Gly Ser Ala Gly Asn Thr Trp Ser Gln Leu
1 5 10 15

Ser Asn Asn Asn Lys Asp
20

<210> SEQ ID NO 287
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 287

Lys Ser Phe His Leu Ser Pro Arg Gly Pro Pro Thr Ser Glu Pro Pro

-continued

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1               5                   10                  15

Pro Val Pro Ala Asn Lys Pro Lys Phe
            20                  25


<210> SEQ ID NO 288
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 288

Arg Cys Lys Ser Glu Gly Thr Leu Ile Asp Leu Ser Glu Gly Phe Ser
1               5                   10                  15

Glu Thr Ser Phe Asn Asp Val Lys Val
            20                  25


<210> SEQ ID NO 289
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 289

Arg Ala Lys Leu Thr Met Leu Asn Thr Val Ser Lys Ile Arg Gly Gln
1               5                   10                  15

Val Lys Asn


<210> SEQ ID NO 290
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 290

Arg Ala Ile Asp Glu Pro Asn Asn Cys Lys Gly Ile Arg Cys Glu Lys
1               5                   10                  15

Gln


<210> SEQ ID NO 291
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 291

Lys Glu Val Cys Thr Ala Leu Leu Glu Ala Asp Val Asn Ile Lys Val
1               5                   10                  15

Lys Gln Leu Arg Glu
            20


<210> SEQ ID NO 292
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 292

Arg Phe Asn Gln Ala Gln Glu Gly Asn Ile Gln Asn Thr Val Met Leu
1               5                   10                  15

Asp Lys Gln Lys Glu Leu Asp Ser Lys Val
            20                  25


<210> SEQ ID NO 293
<211> LENGTH: 31
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<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 293

Met Glu Asn Leu Gln Ser Lys Phe Ser Leu Val Gln Gly Ser Asn Lys
1 5 10 15

Lys Leu Asn Gly Met Glu Asp Asp Gly Ser Pro Pro Val Lys Lys
20 25 30

<210> SEQ ID NO 294
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 294

Lys Leu Ser Ser Gly Asn Ser Lys Leu Ser Ser Ser Lys Phe Ser Asn
1 5 10 15

Pro Ile Ser Ser Ser Lys Arg
20

<210> SEQ ID NO 295
<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 295

Lys Asp Leu Thr Thr Ala Glu Lys Leu Lys Arg Glu Thr Pro Trp Lys
1 5 10 15

Ile

<210> SEQ ID NO 296
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 296

Arg Lys Leu Leu Ser Gln Leu Tyr Ala Arg Lys Val Ile Gln Asp Ile
1 5 10 15

Met Asn Lys Gln
20

<210> SEQ ID NO 297
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 297

Lys Ser Ala Lys Trp Arg Arg Pro Arg Thr Ala Lys Met
1 5 10

<210> SEQ ID NO 298
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 298

Arg Leu Lys Gly Leu Ala Leu Gln Arg Gln Gly Lys Leu Phe Gly Ala
1 5 10 15

Ala Glu Val Gln Arg Phe
20

-continued

<210> SEQ ID NO 299
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 299

Arg Glu Val Thr Met Lys Lys Gly Asp Val Leu Thr Leu Leu Ser Ser
1 5 10 15

Ile Asn Lys Asp Trp Trp Lys Val
20

<210> SEQ ID NO 300
<211> LENGTH: 34
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 300

Lys Tyr Ser His Leu Ile Gly Lys Gly Ala Ala Lys Asp Ala Ala His
1 5 10 15

Met Leu Gln Ala Asn Lys Thr Tyr Gly Cys Val Pro Val Ala Asn Lys
20 25 30

Arg Asp

<210> SEQ ID NO 301
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 301

Lys Gly Pro Ala Val Pro Pro Glu Leu Asp Lys His Phe Leu Leu Cys
1 5 10 15

Glu Ala Cys Gly Lys Cys
20

<210> SEQ ID NO 302
<211> LENGTH: 29
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 302

Lys Lys Glu Gln Ala Leu Gln Leu Ala Gln Lys Met Gly Phe Pro Pro
1 5 10 15

Asn Ile Val Glu Ser Ala Ala Glu Asn Met Val Lys Leu
20 25

<210> SEQ ID NO 303
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 303

Lys Arg Asp Phe Gly Ser Phe Glu Lys Phe Lys Glu Lys Leu
1 5 10

<210> SEQ ID NO 304
<211> LENGTH: 14
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

-continued

<400> SEQUENCE: 304

Lys Arg Asp Phe Gly Ser Phe Glu Lys Phe Lys Glu Lys Leu
1 5 10

<210> SEQ ID NO 305
<211> LENGTH: 35
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 305

Arg Lys Lys Asn Ser Leu Lys Asp Cys Val Ala Val Ala Gly Pro Leu
1 5 10 15

Gly Val Thr His Phe Leu Ile Leu Thr Lys Thr Asp Asn Ser Val Tyr
20 25 30

Leu Lys Leu
35

<210> SEQ ID NO 306
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 306

Lys Leu Pro Val Lys Ile Val Gln Lys Asn Asp Pro Phe Val Val Asp
1 5 10 15

Cys Ser Asp Lys Leu
20

<210> SEQ ID NO 307
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 307

Lys Thr Lys Leu Gln Ser Glu Asn Leu Lys Gln Ser Ile Glu Lys Gln
1 5 10 15

<210> SEQ ID NO 308
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 308

Lys Met Gly Lys Lys Lys Thr Leu Val Val Lys Lys
1 5 10

<210> SEQ ID NO 309
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 309

Lys Ile Asp Ser Ile Ala Asp His Val Asn Ser Ala Ala Val Asn Val
1 5 10 15

Glu Glu Gly Thr Lys Asn Leu Gly Ala Ala Lys Tyr Lys Leu
20 25 30

<210> SEQ ID NO 310
<211> LENGTH: 23
<212> TYPE: PRT

-continued

<213> ORGANISM: Mus musculus

<400> SEQUENCE: 310

Arg Lys Gly Pro Glu Asp Thr Ala Gln Leu Ala His Ala Val Leu Ala
1 5 10 15

Lys Leu Asn Ala Phe Lys Ala
20

<210> SEQ ID NO 311
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 311

Lys Ser Ala Tyr Ser Glu Arg Leu Lys Phe Asn Val Ala Ile Lys Ile
1 5 10 15

Ile Asp Arg Lys Lys
20

<210> SEQ ID NO 312
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 312

Lys Lys Lys Pro Asn Pro Gly Lys Asp Lys Arg Thr Tyr Glu Pro Ser
1 5 10 15

Ser Ala Thr Pro Val Thr Arg Ser
20

<210> SEQ ID NO 313
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 313

Lys Glu Pro His Val Glu Gln Ile Thr Arg Lys Phe Arg Leu Asn Ser
1 5 10 15

Glu Gly Lys Leu
20

<210> SEQ ID NO 314
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 314

Arg Phe Lys Gln Glu Gly Val Leu Asn Ser Lys Val Gly Met Asp Tyr
1 5 10 15

Arg Ser

<210> SEQ ID NO 315
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 315

Lys Asn Ala Leu Ala Leu Phe Val Leu Pro Lys Glu Gly Gln Met Glu
1 5 10 15

-continued

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Ser Val Glu Ala Ala Met Ser Ser Lys Thr
            20                  25


<210> SEQ ID NO 316
<211> LENGTH: 24
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 316

Lys Gln Leu Glu Gly Ala Cys Tyr Ser Gly Lys Leu Ile Trp Lys Val
1               5                   10                  15

Thr Asp Tyr Arg Val Lys Lys Arg
            20


<210> SEQ ID NO 317
<211> LENGTH: 28
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 317

Lys Glu Asp Val Ser Glu Ser Val Gly Ala Ser Gly Gln Arg Pro Val
1               5                   10                  15

Phe Cys Pro Val His Lys Gln Glu Gln Leu Lys Leu
            20                  25


<210> SEQ ID NO 318
<211> LENGTH: 30
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 318

Arg Met Ile Thr Asn Glu Gln Asp Leu Lys Met Phe Leu Leu Ser Lys
1               5                   10                  15

Gly Ala Ser Lys Glu Val Ile Ala Ser Ile Ile Ser Arg Tyr
            20                  25                  30


<210> SEQ ID NO 319
<211> LENGTH: 31
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 319

Lys Lys Gln Pro Gly Gln Asp Cys Pro His Ser Cys Asn Leu Leu Cys
1               5                   10                  15

His Pro Gly Pro Cys Pro Pro Cys Pro Ala Phe Met Thr Lys Thr
            20                  25                  30


<210> SEQ ID NO 320
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 320

Arg Ser Lys Trp His Ile Pro Met Pro Ser Gly Lys Gly Tyr Phe Asn
1               5                   10                  15

Phe Gly Lys Ile
            20


<210> SEQ ID NO 321
<211> LENGTH: 15
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<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 321

Arg Lys Gln Gln Ala Met Trp Arg Val Pro Ser Asp Leu Lys Met
1 5 10 15

<210> SEQ ID NO 322
<211> LENGTH: 18
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 322

Arg Glu Leu Asn Lys Leu Tyr Pro Thr His Ala Cys Arg Glu Tyr Leu
1 5 10 15

Lys Asn

<210> SEQ ID NO 323
<211> LENGTH: 15
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 323

Met Ala Lys Pro Thr Ser Lys Asp Ser Gly Leu Lys Glu Lys Phe
1 5 10 15

<210> SEQ ID NO 324
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 324

Arg Gly Asp Val Val Pro Lys Asp Val Asn Val Ala Ile Ala Ala Ile
1 5 10 15

Lys Thr Lys Arg
20

<210> SEQ ID NO 325
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 325

Arg Leu His Glu Glu Trp Leu Leu Arg Glu Glu Lys Ala Gln Glu Glu
1 5 10 15

Phe Arg Ile Lys Lys
20

<210> SEQ ID NO 326
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 326

Arg Gly Lys Met Val Ser Tyr Ile Gln Cys Lys Glu Val Asp Tyr Arg
1 5 10 15

Ser Asp Arg Arg Glu
20

<210> SEQ ID NO 327

-continued

<211> LENGTH: 17
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 327

Arg Ser Ile Pro Val Leu Ala Lys Trp Gln Asn Ser Tyr Ser Ile Lys
1 5 10 15

Val

<210> SEQ ID NO 328
<211> LENGTH: 13
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 328

Lys Phe Ser Leu Gln Asp Pro Pro Asn Lys Pro Lys Val
1 5 10

<210> SEQ ID NO 329
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 329

Lys Lys Gly Lys Ala Glu Ser Cys Gly His Ala Thr Val Ser Ser Glu
1 5 10 15

Lys Lys Leu Lys Thr
20

<210> SEQ ID NO 330
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 330

Lys Phe Gln Glu Ile Ala Glu Lys Asn Met Glu Lys Leu Asn His Ile
1 5 10 15

Glu Lys Ser

<210> SEQ ID NO 331
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 331

Arg Leu Leu Lys Asn Thr Glu Asn Met Lys Gly Phe Phe Gly Gly Leu
1 5 10 15

Glu Thr Lys Leu Lys Gly
20

<210> SEQ ID NO 332
<211> LENGTH: 21
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 332

Lys Phe Ala Leu Pro Tyr Ile Arg Asp Val Ala Lys Arg Val Lys Ala
1 5 10 15

Gly Leu Gln Lys Ala
20

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<210> SEQ ID NO 333
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 333

Lys Lys Met Met Ser Asn Gln Tyr Val Pro Val Lys Thr His Glu Glu
1 5 10 15

Val Lys Met

<210> SEQ ID NO 334
<211> LENGTH: 19
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 334

Lys His Glu Lys Glu Leu Met Gly Leu Lys Ser Asn Ile Ala Glu Leu
1 5 10 15

Lys Lys Gln

<210> SEQ ID NO 335
<211> LENGTH: 33
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 335

Lys Glu Ile His Val Asn Ile Glu Ala Thr Phe Lys Pro Ser Ser Glu
1 5 10 15

Glu Tyr Leu His Ile Thr Glu Pro Pro Ser Leu Ser Pro Asp Thr Lys
20 25 30

Leu

<210> SEQ ID NO 336
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 336

Lys Ala Gly Glu Ala Ser Thr Glu Thr Lys Lys Leu Ile Ile Lys Phe
1 5 10 15

Lys Glu Lys Asn
20

<210> SEQ ID NO 337
<211> LENGTH: 26
<212> TYPE: PRT
<213> ORGANISM: Rattus norvegicus

<400> SEQUENCE: 337

Lys Tyr Gly Leu Leu Pro Ser Thr Ser Asn Asp Phe Lys Tyr Gly Leu
1 5 10 15

Leu Pro Gly Ala Pro Asn Asp Phe Lys Tyr
20 25

<210> SEQ ID NO 338
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

-continued

<400> SEQUENCE: 338

Lys His Gln Arg Ile His Ser Gly Glu Lys Pro Tyr Val Cys Asp Tyr
1 5 10 15

Cys Gly Lys Ala
20

<210> SEQ ID NO 339
<211> LENGTH: 27
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 339

Lys Glu Asp Gln Pro Gly His Thr Lys Asp Leu Ser Gly Pro Thr Lys
1 5 10 15

Glu Ser Ser Lys Gly Ser Pro Lys Met Pro Ser
20 25

<210> SEQ ID NO 340
<211> LENGTH: 23
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 340

Lys Val Glu Ser Glu Leu Leu Cys Thr Arg Leu Leu Leu Gly Gly Gly
1 5 10 15

Ser Tyr Lys Cys Ile Lys Cys
20

<210> SEQ ID NO 341
<211> LENGTH: 36
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 341

Arg Ser Pro Gly Pro Asp Tyr Ser Leu Arg Leu Glu Thr Val Pro Ala
1 5 10 15

Pro Gly Arg Ala Glu Gly Gly Ala Val Ser Ala Gly Glu Ser Lys Met
20 25 30

Glu Pro Arg Glu
35

<210> SEQ ID NO 342
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 342

Lys Lys Ala Glu Asn Ser Ile Gly Lys Cys Pro Thr Arg Thr Asp Val
1 5 10 15

Ser Glu Lys Ala
20

<210> SEQ ID NO 343
<211> LENGTH: 25
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 343

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Lys Met Asp Leu Asn Asn Asn Ser Leu Lys Thr Lys Ala Gln Val Pro
1               5                   10                  15

Met Val Leu Thr Ala Gly Pro Lys Trp
            20                  25


<210> SEQ ID NO 344
<211> LENGTH: 10
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: A synthetic polypeptide

<400> SEQUENCE: 344

Ser Thr Leu His Leu Val Leu Arg Leu Arg
1               5                   10


<210> SEQ ID NO 345
<211> LENGTH: 20
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 345

Arg Thr Tyr Asp Leu Asn Lys Pro Pro Glu Phe Ser Phe Glu Lys Ala
1               5                   10                  15

Arg Val Arg Lys
            20


<210> SEQ ID NO 346
<211> LENGTH: 76
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 346

Met Gln Ile Phe Val Lys Thr Leu Thr Gly Lys Thr Ile Thr Leu Glu
1               5                   10                  15

Val Glu Pro Ser Asp Thr Ile Glu Asn Val Lys Ala Lys Ile Gln Asp
            20                  25                  30

Lys Glu Gly Ile Pro Pro Asp Gln Gln Arg Leu Ile Phe Ala Gly Lys
        35                  40                  45

Gln Leu Glu Asp Gly Arg Thr Leu Ser Asp Tyr Asn Ile Gln Lys Glu
    50                  55                  60

Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly
65                  70                  75


<210> SEQ ID NO 347
<211> LENGTH: 12
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 347

Ser Thr Leu His Leu Val Leu Arg Leu Arg Gly Gly
1               5                   10


<210> SEQ ID NO 348
<211> LENGTH: 4
<212> TYPE: PRT
<213> ORGANISM: Artificial Sequence
<220> FEATURE:
<223> OTHER INFORMATION: A synthetic polypeptide
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<400> SEQUENCE: 348

Leu Arg Gly Gly
1


<210> SEQ ID NO 349
<211> LENGTH: 22
<212> TYPE: PRT
<213> ORGANISM: Homo sapiens

<400> SEQUENCE: 349

Lys Arg Thr Val Ser Asp Asn Ser Leu Ser Asn Ser Arg Gly Glu Gly
1               5                   10                  15

Lys Pro Asp Leu Lys Phe
            20


<210> SEQ ID NO 350
<211> LENGTH: 16
<212> TYPE: PRT
<213> ORGANISM: Mus musculus

<400> SEQUENCE: 350

Lys Leu Ala Lys Gln Asn Thr Asn Lys Ala Lys Glu Asn Leu Arg Lys
1               5                   10                  15
```
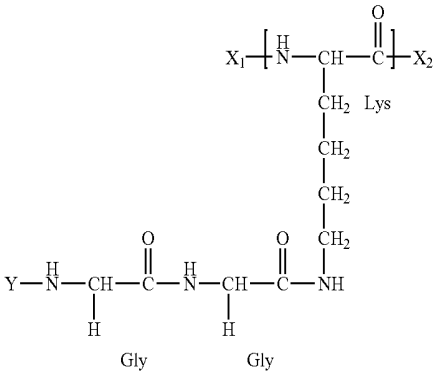

What is claimed:

1. An isolated epitope comprising a diglycine (Gly-Gly) linked to on an epsilon amino group of a lysine, where the lysine can be within a protein.

2. The epitope of claim **1**, wherein the diglycine (Gly-Gly) linked to the epsilon amino group of the lysine comprises the following structure:



wherein: X_1 is hydrogen or a first peptide sequence;

X_2 is hydroxy or a second peptide sequence; and

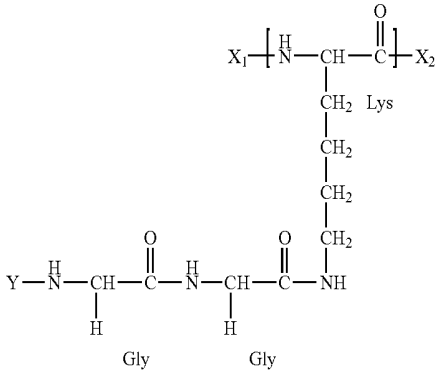
Y is hydrogen or a ubiquitin peptide, wherein the Y ubiquitin peptide is not a full-length ubiquitin protein.

3. The epitope of claim **2** wherein Y is Leu-Arg or STLHLVLRLR (SEQ ID NO:344).

4. The epitope of claim **2**, wherein Y is hydrogen.

5. An antibody that specifically binds to a diglycine (Gly-Gly) epitope linked to an epsilon amino group of a lysine in a protein.

6. The antibody of claim **5**, wherein the epitope comprises the following structure:



wherein: X_1 is hydrogen or a first peptide sequence;

X_2 is hydroxy or a second peptide sequence; and

Y is hydrogen or a ubiquitin peptide, wherein the Y ubiquitin peptide is not a full-length ubiquitin protein.

7. The antibody of claim **6**, wherein Y is Leu-Arg or STLHLVLRLR (SEQ ID NO:344).

8. The antibody of claim **6**, wherein Y is hydrogen.

9. The antibody of claim **5**, wherein the antibody is a polyclonal antibody.

10. The antibody of claim **5**, wherein the antibody is a monoclonal antibody.

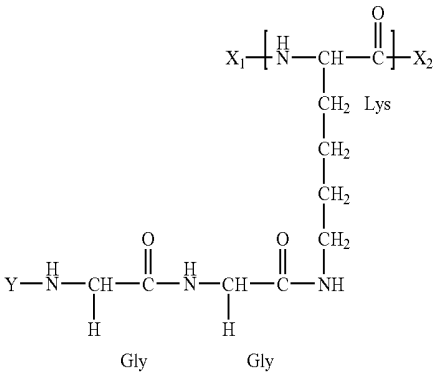
11. A method of detecting a ubiquitinated site in a protein present in a sample, wherein the method comprises

(i) cleaving protein(s) in the sample with a protease that cleaves ubiquitin to form a mixture of cleavage peptides;

(ii) contacting the cleavage peptides with an antibody that specifically binds to a diglycine (Gly-Gly) epitope linked to an epsilon amino group of a lysine; and

(iii) observing whether the antibody binds to a cleavage peptide, to thereby detect a ubiquitinated site in a test protein or a mixture of test proteins.

12. The method of claim **11**, wherein the diglycine (Gly-Gly) epitope linked to an epsilon amino group of a lysine comprises the following structure:



wherein: X_1 is hydrogen or a first peptide sequence;

X_2 is hydroxy or a second peptide sequence; and

Y is hydrogen or a ubiquitin peptide, wherein the Y ubiquitin peptide is not a full-length ubiquitin protein.

13. The method of claim **12**, wherein Y is Leu-Arg or STLHLVLRLR (SEQ ID NO:344).

14. The method of claim **12**, wherein Y is hydrogen.

15. The method of claim **11**, wherein the protease that cleaves ubiquitin also cleaves protein(s) in the sample.

16. The method of claim **11**, wherein the protease that cleaves ubiquitin is a protease that cleaves on the C-terminal side of arginine or glutamic acid residues.

17. The method of claim **11**, wherein the protease that cleaves ubiquitin is trypsin, Arg-C or Glu-C.

18. The method of claim **11**, further comprising sequencing the cleavage peptide to which the antibody binds to thereby determine the amino acids sequence of the cleavage peptide.

19. The method of claim **18**, wherein the sequencing is performed by a method comprising mass spectrometry.

20. The method of claim **18**, further comprising identifying the ubiquitinated site in the cleavage peptide.

21. The method of claim **18**, further comprising identifying the protein from which the cleavage peptide was derived.

22. The method of claim **21**, further comprising isolating the protein from which the cleavage peptide was derived.

23. The method of claim **11**, wherein step (i) is performed in the presence of $H_2{}^{18}O$.

24. The method of claim **23**, wherein the amount or proportion of cleavage peptides labeled with ^{18}O is determined.

25. The method of claim **11**, wherein the sample is a body fluid, tissue sample, cell lysate, fractionated cellular material, cellular extract, cell culture supernatant, or cultured cells.

26. The method of claim **25**, wherein the body fluid is saliva, mucous, sweat, whole blood, serum, urine, amniotic fluid, genital fluid, fecal material, marrow, plasma, spinal fluid, pericardial fluid, gastric fluid, abdominal fluid, peritoneal fluid, pleural fluid, synovial fluid, cyst fluid, cerebrospinal fluid, lung lavage fluid, lymphatic fluid, tears, prostatitc fluid, tissue extract, or glandular secretion.

27. The method of claim **11**, wherein the sample is obtained from a mammal fed a diet containing an isotopically-labeled amino acid.

28. The method of claim **11**, wherein the sample is obtained from culture media containing an isotopically-labeled amino acid.

29. The method of claim **11**, wherein the sample comprises a selected cell or tissue type and the method further comprises identifying substantially all ubiquitinated proteins in the cell or tissue type to yield a ubiquitination profile of proteins from the cell or tissue type.

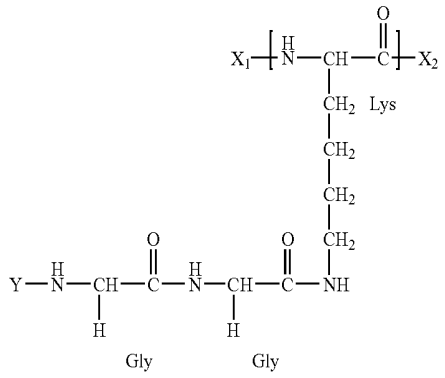
30. The method of claim **29**, further comprising comparing the ubiquitination profile of proteins from the cell or tissue type with a ubiquitination profile of proteins from the cell or tissue type after treatment or exposure of the selected cells or tissues to a drug or test agent.

31. The method of claim **29**, further comprising comparing the ubiquitination profile of proteins from the cell or tissue type with a ubiquitination profile of proteins from cells with a mutation in, or an amplification of, a gene encoding a ubiquitin ligase, a ubiquitin conjugating enzyme or a ubiquitin activating enzyme.

32. The method of claim **31**, wherein the mutation substantially eliminates expression or function of an E3 ubiquitin ligase, an E2 ubiquitin conjugating enzyme or an E1 ubiquitin activating enzymes (E1).

33. A method of generating an antibody that comprises administering to a mammal an effective amount of an antigen comprising a diglycine (Gly-Gly) epitope linked to an epsilon amino group of a lysine, to generate an immune response against the epitope, to thereby generate an antibody that specifically binds to a diglycine (Gly-Gly) epitope present on an epsilon amino group of a lysine.

34. The method of claim **33**, wherein the epitope comprises the following structure:



wherein: X_1 is hydrogen or a first peptide sequence;

X_2 is hydroxy or a second peptide sequence; and

Y is hydrogen or a ubiquitin peptide, wherein the Y ubiquitin peptide is not a full-length ubiquitin protein.

35. The method of claim **34**, wherein Y is Leu-Arg or STLHLVLRLR (SEQ ID NO:344).

36. The method of claim **34**, wherein Y is hydrogen.

37. The method of claim **33**, wherein the lysine is within a protein.

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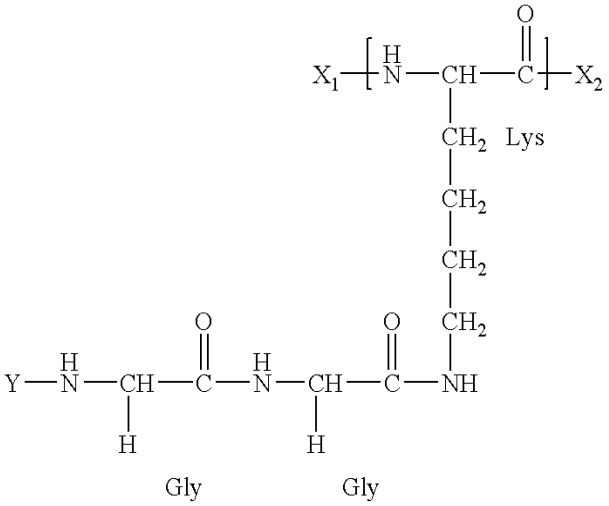
本发明涉及特定的遍在蛋白化表位，特异性识别并结合泛素化蛋白质和肽的抗体（特别是在通过蛋白水解切割除去遍在蛋白后）和使用这些表位和抗体的方法。