

55. Liu, Y., Fiskum, G., and Schubert, D. (2002) Generation of reactive oxygen species by the mitochondrial electron transport chain. *J. Neurochem.* 80, 780-787
56. Winkler, B. S., Boulton, M. E., Gottsch, J. D., and Sternberg, P. (1999) Oxidative damage and age-related macular degeneration. *Mol. Vis.* 5, 32
57. Imamura, Y., Noda, S., Hashizume, K., Shinoda, K., Yamaguchi, M., Uchiyama, S., Shimizu, T., Mizushima, Y., Shirasawa, T., and Tsubota, K. (2006) Drusen, choroidal neovascularization, and retinal pigment epithelium dysfunction in SOD1-deficient mice: a model of age-related macular degeneration. *Proc. Natl. Acad. Sci. U S A* 103, 11282-11287
58. Justilien, V., Pang, J. J., Renganathan, K., Zhan, X., Crabb, J. W., Kim, S. R., Sparrow, J. R., Hauswirth, W. W., and Lewin, A. S. (2007) SOD2 knockdown mouse model of early AMD. *Invest. Ophthalmol. Vis. Sci.* 48, 4407-4420
59. Perkins, G. A., Ellisman, M. H., and Fox, D. A. (2003) Three-dimensional analysis of

mouse rod and cone mitochondrial cristae architecture: bioenergetic and functional implications. *Mol. Vis.* 9, 60-73

60. Barron, M. J., Johnson, M. A., Andrews, R. M., Clarke, M. P., Griffiths, P. G., Bristow, E., He, L. P., Durham, S., and Turnbull, D. M. (2001) Mitochondrial abnormalities in ageing macular photoreceptors. *Invest. Ophthalmol. Vis. Sci.* 42, 3016-3022

61. Hopper, R. K., Carroll, S., Aponte, A. M., Johnson, D. T., French, S., Shen, R. F., Witzmann, F. A., Harris, R. A., and Balaban, R. S. (2006) Mitochondrial matrix phosphoproteome: effect of extra mitochondrial calcium. *Biochemistry* 45, 2524-2536

62. Li, Y., Huang, T. T., Carlson, E. J., Melov, S., Ursell, P. C., Olson, J. L., Noble, L. J., Yoshimura, M. P., Berger, C., Chan, P. H., Wallace, D. C., and Epstein, C. J. (1995) Dilated cardiomyopathy and neonatal lethality in mutant mice lacking manganese superoxide dismutase. *Nat. Genet.* 11, 376-381

63. Sandbach, J. M., Coscun, P. E., Grossniklaus, H. E., Kokoszka, J. E., Newman, N. J., and Wallace, D. C. (2001) Ocular pathology in mitochondrial superoxide dismutase

(Sod2)-deficient mice. *Invest. Ophthalmol. Vis. Sci.* 42, 2173-2178

64. Kapphahn, R. J., Giwa, B. M., Berg, K. M., Roehrich, H., Feng, X., Olsen, T. W., and Ferrington, D. A. (2006) Retinal proteins modified by 4-hydroxynonenal: identification of molecular targets. *Exp. Eye. Res.* 83, 165-175

65. Kingma, P. B., Bok, D., and Ong, D. E. (1998) Bovine epidermal fatty acid-binding protein: determination of ligand specificity and cellular localization in retina and testis. *Biochemistry* 37, 3250-3257

66. Krecic, A. M., and Swanson, M. S. (1999) hnRNP complexes: composition, structure, and function. *Curr. Opin. Cell. Biol.* 11, 363-371

67. Dreyfuss, G., Kim, V. N., and Kataoka, N. (2002) Messenger-RNA-binding proteins and the messages they carry. *Nat. Rev. Mol. Cell. Biol.* 3, 195-205

68. Kattapuram, T., Yang, S., Maki, J. L., and Stone, J. R. (2005) Protein kinase CK1alpha regulates mRNA binding by heterogeneous nuclear ribonucleoprotein C in

response to physiologic levels of hydrogen peroxide. *J. Biol. Chem.* 280, 15340-15347

69. Rajagopalan, L. E., Westmark, C. J., Jarzembowski, J. A., and Malter, J. S. (1998)

hnRNP C increases amyloid precursor protein (APP) production by stabilizing APP

mRNA. *Nucleic Acids Res.* 26, 3418-3423

70. White, R., Gonsior, C., Kramer-Albers, E. M., Stohr, N., Huttelmaier, S., and Trotter, J.

(2008) Activation of oligodendroglial Fyn kinase enhances translation of mRNAs

transported in hnRNP A2-dependent RNA granules. *J. Cell. Biol.* 181, 579-586

71. Houle, F., Rousseau, S., Morrice, N., Luc, M., Mongrain, S., Turner, C. E., Tanaka, S.,

Moreau, P., and Huot, J. (2003) Extracellular signal-regulated kinase mediates

phosphorylation of tropomyosin-1 to promote cytoskeleton remodeling in response to

oxidative stress: impact on membrane blebbing. *Mol. Biol. Cell.* 14, 1418-1432

72. Liu, Q., Tan, G., Levenkova, N., Li, T., Pugh, E. N., Jr., Rux, J. J., Speicher, D. W., and

Pierce, E. A. (2007) The proteome of the mouse photoreceptor sensory cilium complex.

Mol. Cell. Proteomics. 6, 1299-1317

73. Folkman, J. (1971) Tumor angiogenesis: therapeutic implications. *N. Engl. J. Med.* 285, 1182-1186

74. MacDonald, N. J., Shivers, W. Y., Narum, D. L., Plum, S. M., Wingard, J. N., Fuhrmann, S. R., Liang, H., Holland-Linn, J., Chen, D. H., and Sim, B. K. (2001) Endostatin binds tropomyosin. A potential modulator of the antitumor activity of endostatin. *J. Biol. Chem.* 276, 25190-25196

75. Stamm, S., Casper, D., Lees-Miller, J. P., and Helfman, D. M. (1993) Brain-specific tropomyosins TMBr-1 and TMBr-3 have distinct patterns of expression during development and in adult brain. *Proc. Natl. Acad. Sci. U S A* 90, 9857-9861

76. Vrhovski, B., Schevzov, G., Dingle, S., Lessard, J. L., Gunning, P., and Weinberger, R. P. (2003) Tropomyosin isoforms from the gamma gene differing at the C-terminus are spatially and developmentally regulated in the brain. *J. Neurosci. Res.* 72, 373-383

77. Cooper, J. A. (2002) Actin dynamics: tropomyosin provides stability. *Curr. Biol.* 12,

R523-525

78. Williams, D. S. (1991) Actin filaments and photoreceptor membrane turnover.

Bioessays 13, 171-178

79. Kennedy, C. J., Rakoczy, P. E., and Constable, I. J. (1995) Lipofuscin of the retinal pigment epithelium: a review. *Eye* 9 (Pt 6), 763-771

Figure legends

Figure 1

Resolution of monkey retina proteins on 2D gels stained with SYPRO Ruby (pH3-10)

Peripheral retina and macula extracted proteins (300 μ g each) were isoelectric focused on pH3-10 IPG strip and then separated on 12% SDS-page gels. Spots identified by LC-MS/MS are marked by spot numbers.

Figure 2

Resolution of monkey retina proteins on 2D gels stained with SYPRO Ruby (pH 4-7).

Peripheral retina and macula extract proteins (300 μ g each) were isoelectric focused on pH4-7 IPG strip. Spots identified by LC-MS/MS are marked by the spot number.

Figure 3

Resolution of monkey retina proteins on 2D gels stained with SYPRO Ruby (pH5-8).

Peripheral retina and macula extract proteins (300 μ g each) were isoelectric focused on pH5-8 IPG strip. Spots identified by LC-MS/MS are marked by the spot number.

Figure 4

Resolution of monkey retina proteins on 2D gels stained with SYPRO Ruby (pH7-10).

Peripheral retina and macula extract proteins (300 μ g each) were isoelectric focused on pH 7-10. Spots identified by LC-MS/MS are marked by the spot number.

Figure 5

Western blot of 8 proteins.

Five micrograms of each sample from the peripheral retina and macula were loaded onto SDS-page gel (for γ -synuclein 15 μ g loading). After transferring to PVDF membrane, the

proteins were detected with antibodies specific to Arrestin-C (1), Mn-SOD (2), γ -synuclein (3), E-FABP (4), Tropomyosin1a Br-1,Br-3 (5), Tropomyosin1a TM311 (6), hnRNPs A2/B1 (7), and hnRNPs C1/C2 (8). Lane P; peripheral retina, Lane M; macula.

Figure 6

2D western blotting of Mn-SOD.

Thirty microgram of each sample from the peripheral retina and macula were separated by 2D gel electrophoresis. After transferring to PVDF membrane, the proteins were detected with antibodies specific to Mn-SOD.

Figure 7

Tissue localization of macula enriched proteins.

Four micrometer paraffin section of monkey eye was stained with hematoxylin and eosin (A), other sections were labeled with antibodies specific to Arrestin-C (B), Mn-SOD (C), γ -synuclein (D), E-FABP (E), Tropomyosin Br-1, Br-3 (F), TM311 to Tropomyosin (G), hnRNPs A2/B1 (H), and hnRNPs C1/C2 (I). GCL, ganglion cell layer; INL, inner nuclear layer; OPL, outer plexiform layer; ONL, outer nuclear layer; PIS, photoreceptor inner segment; POS, photoreceptor outer segment; RPE, retinal pigment epithelial; Ch,

choroid. (Bar; 50 μ)

Figure 1

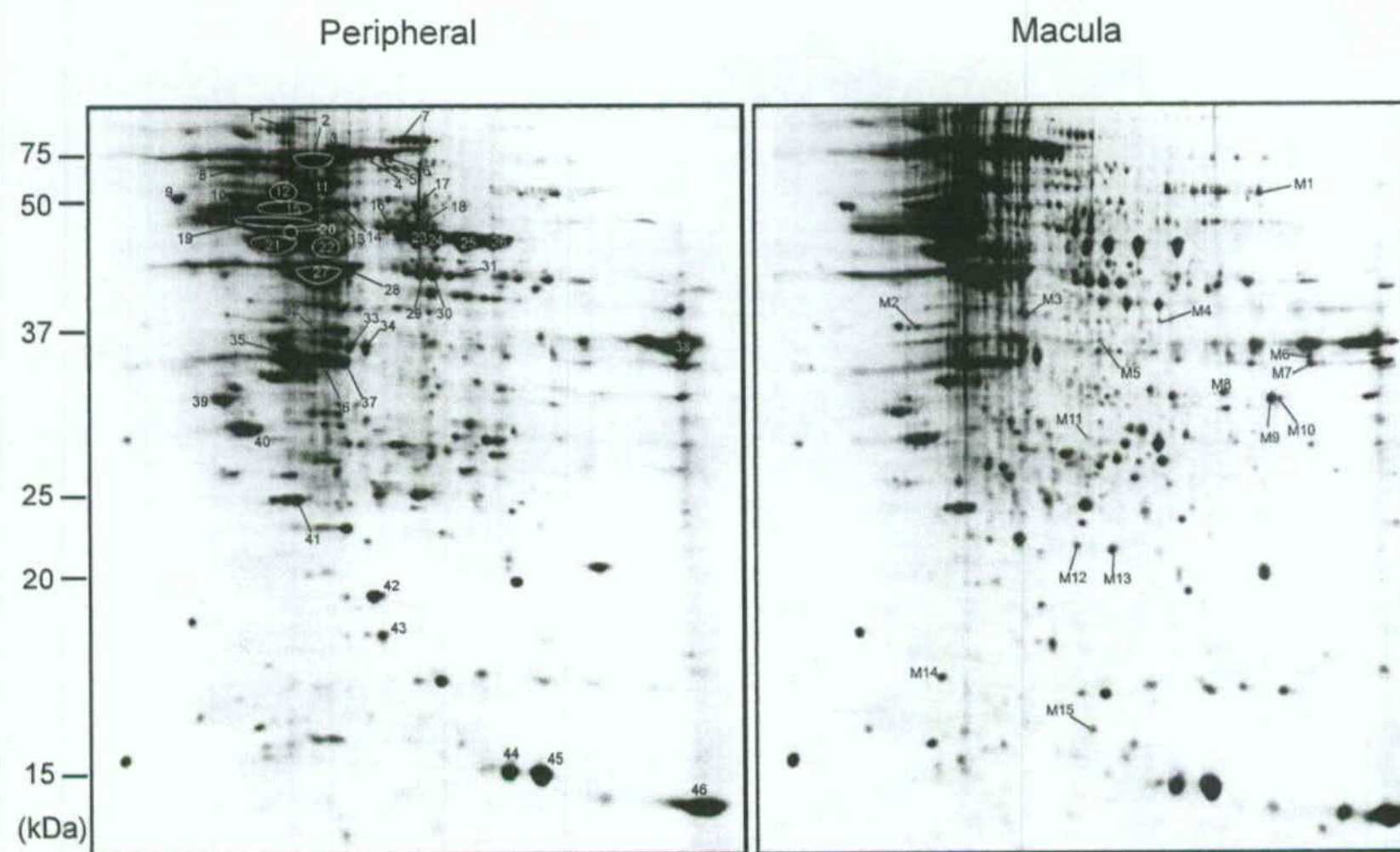


Figure 2

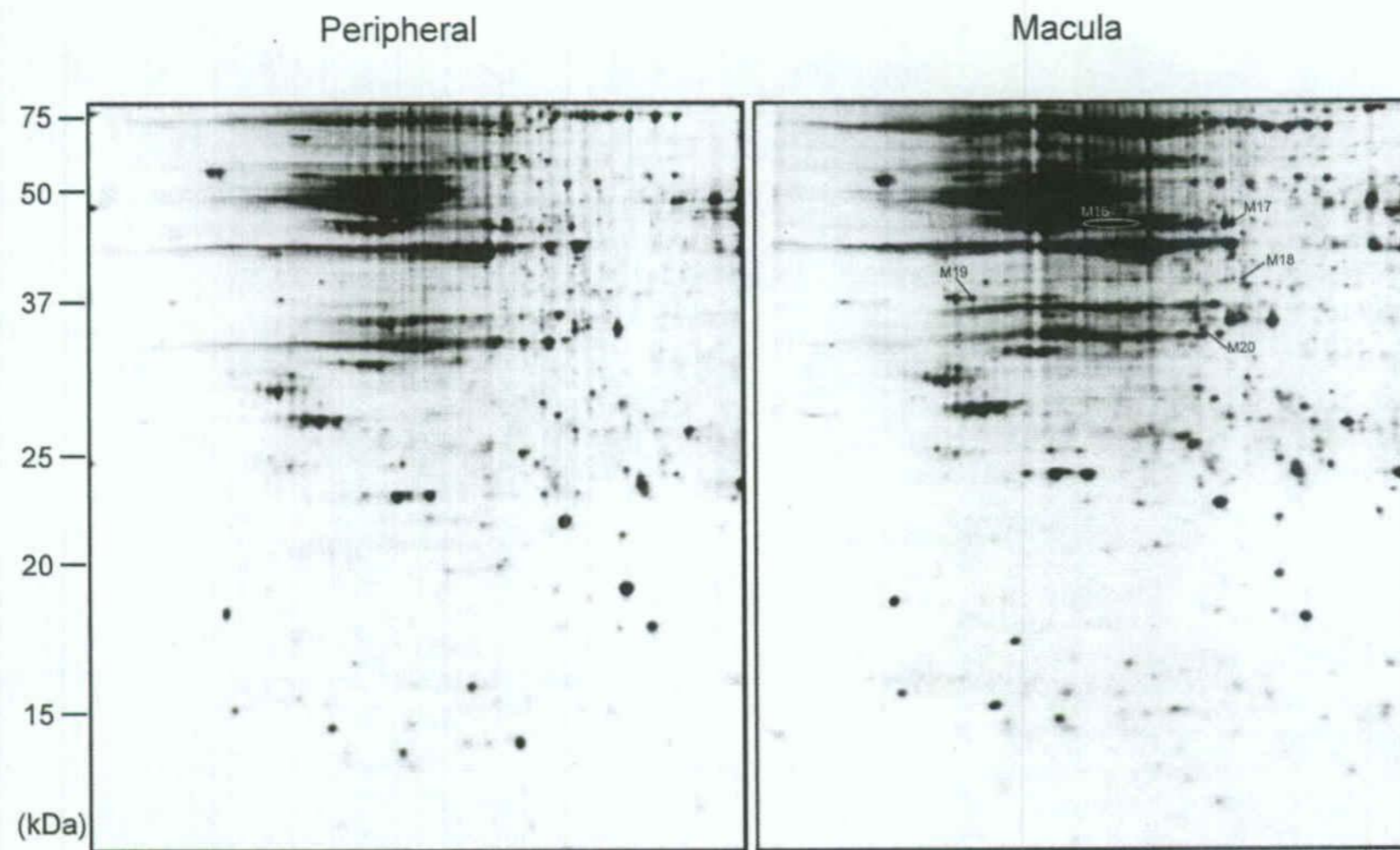


Figure 3

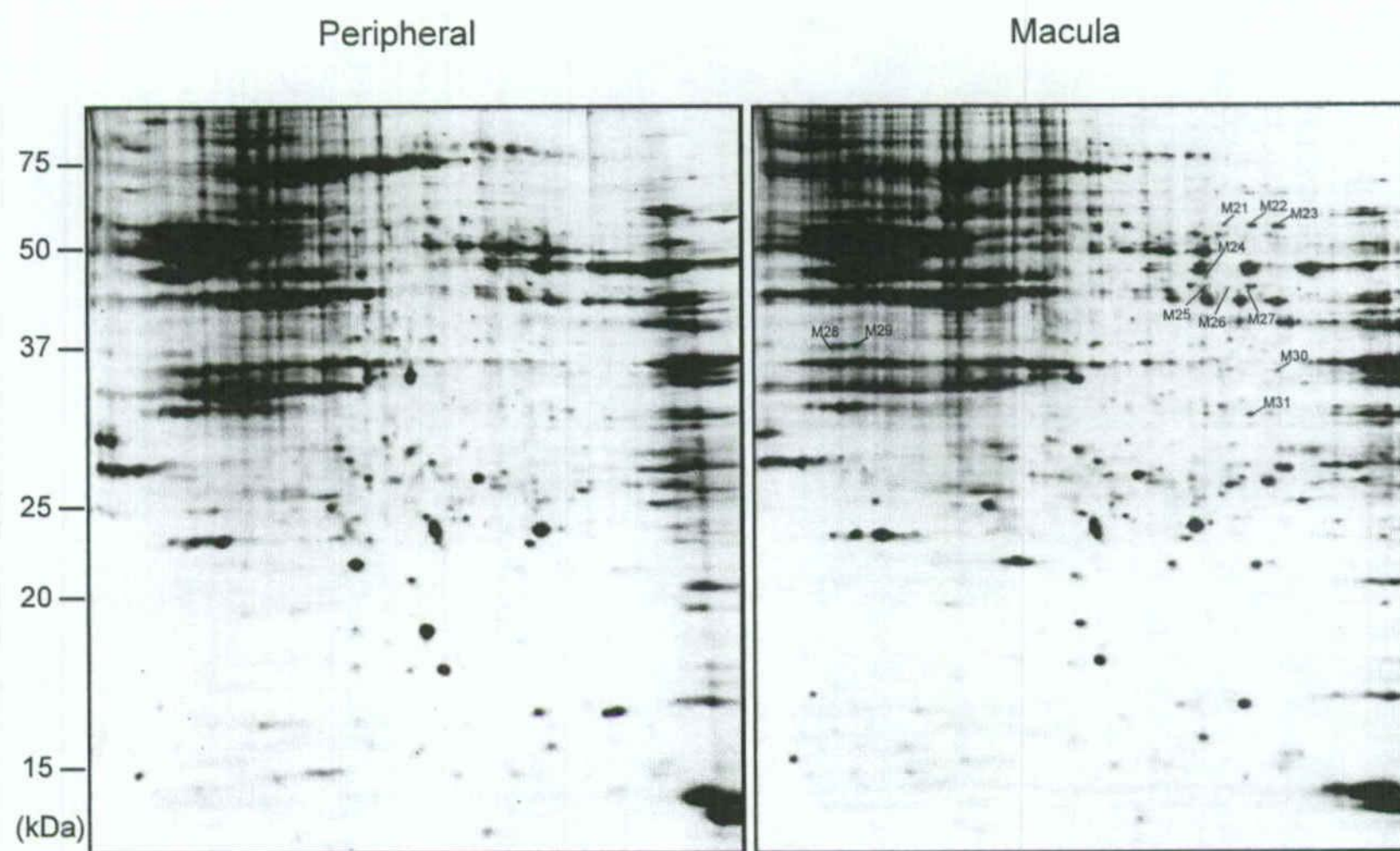


Figure 4

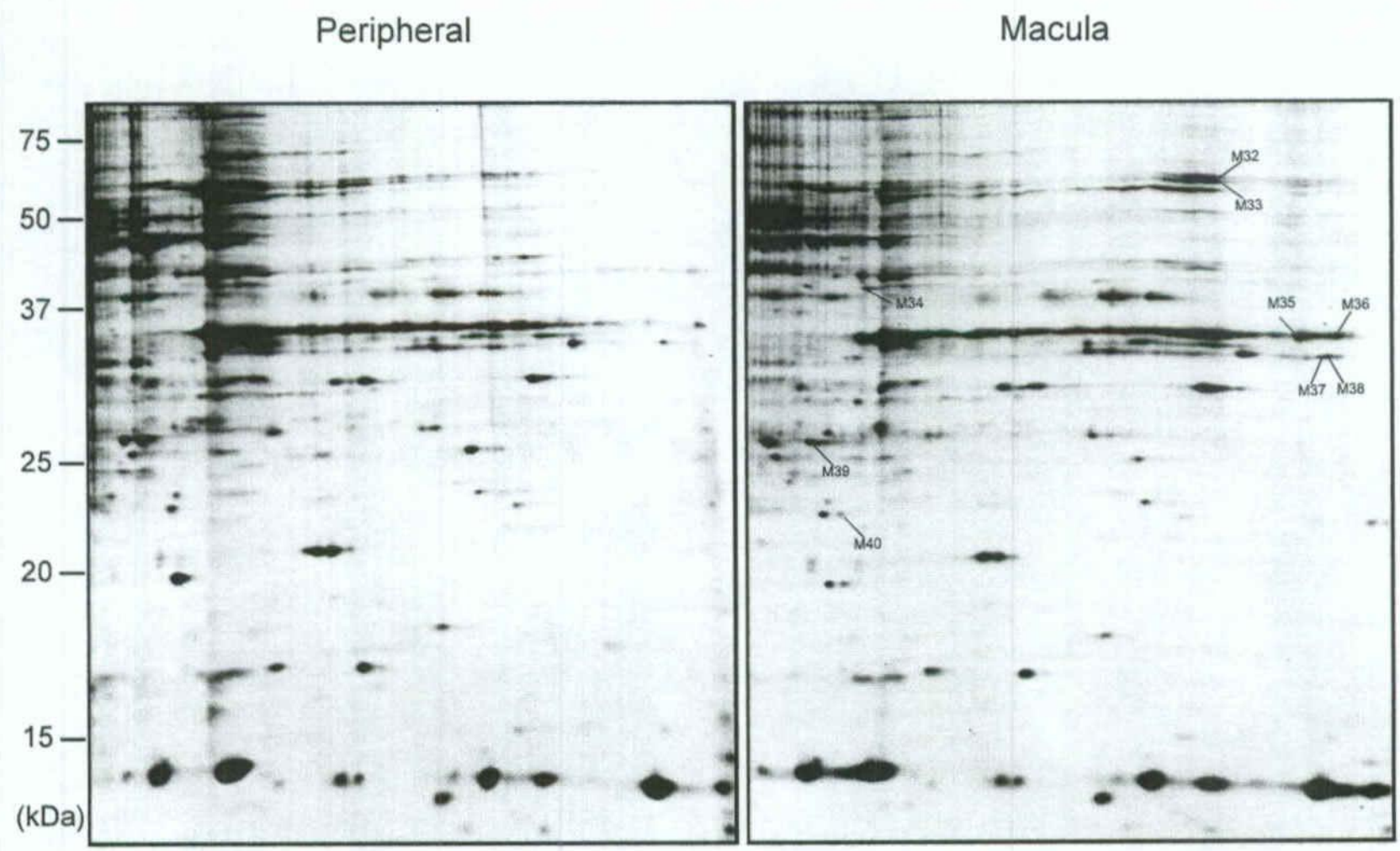


Figure 5

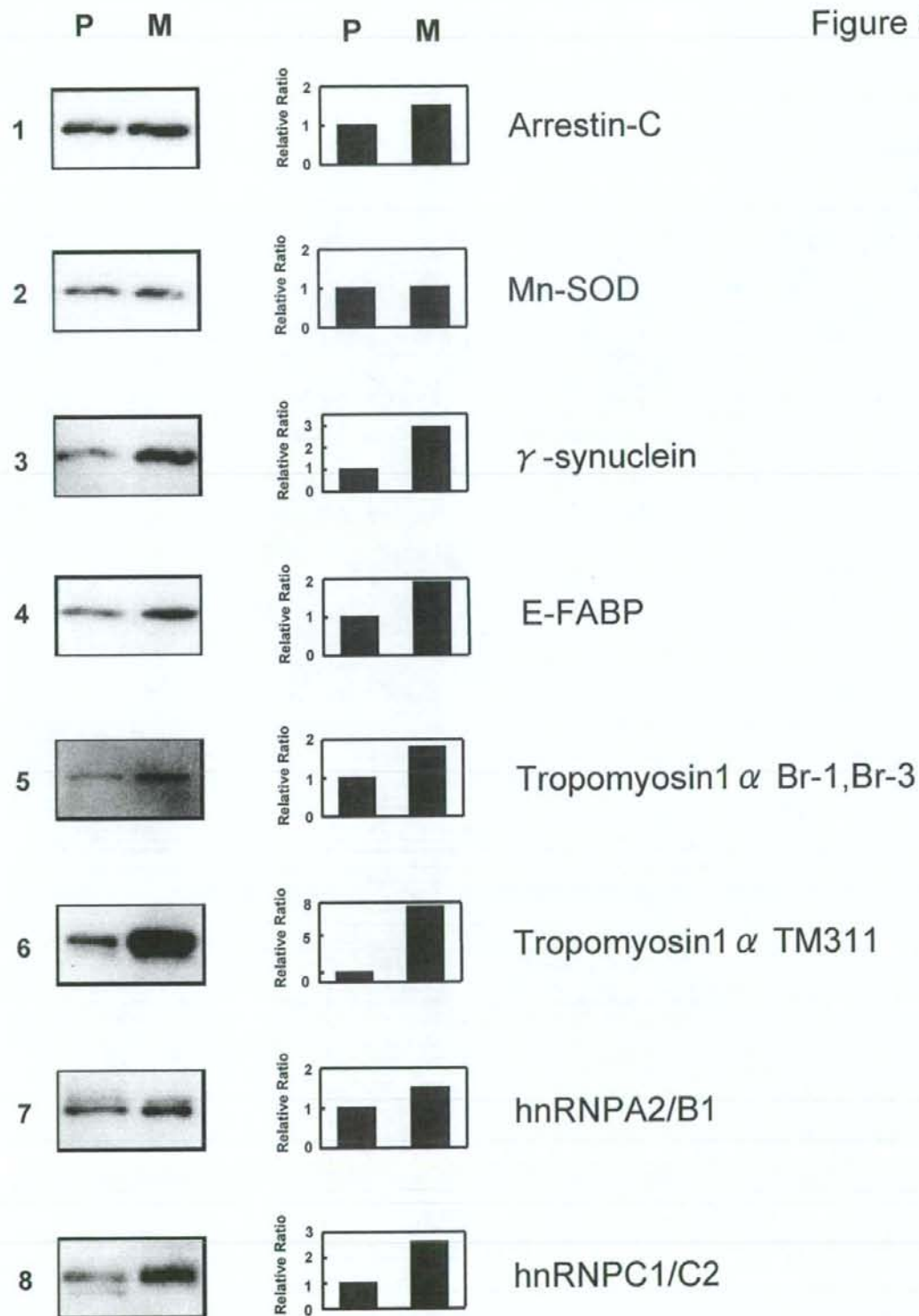


Figure 6

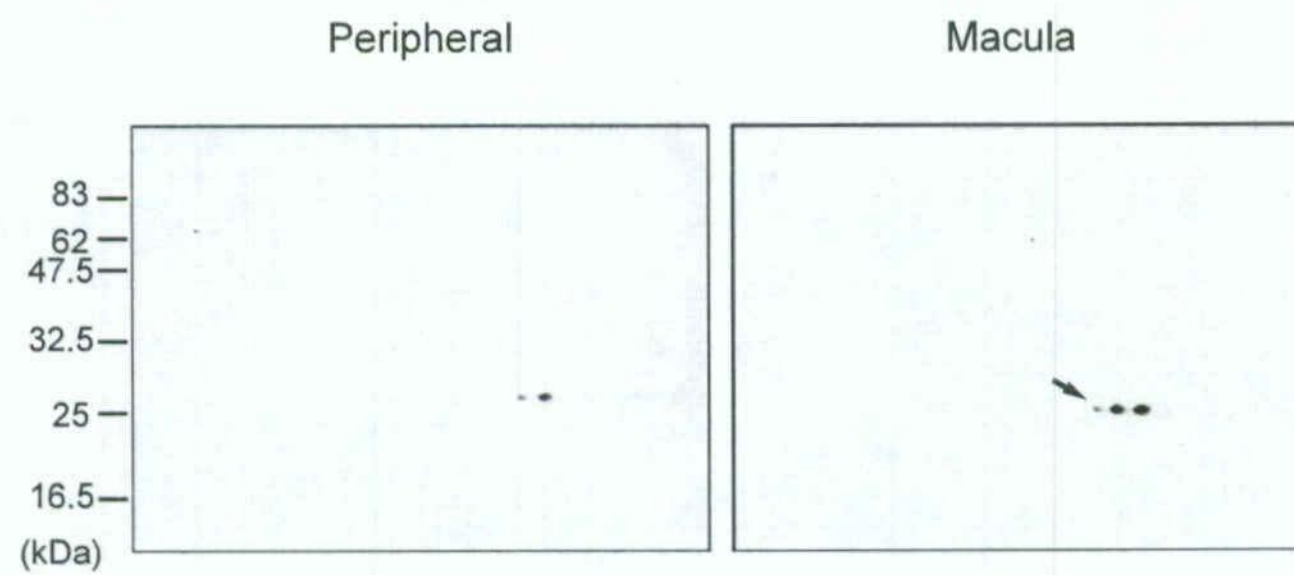
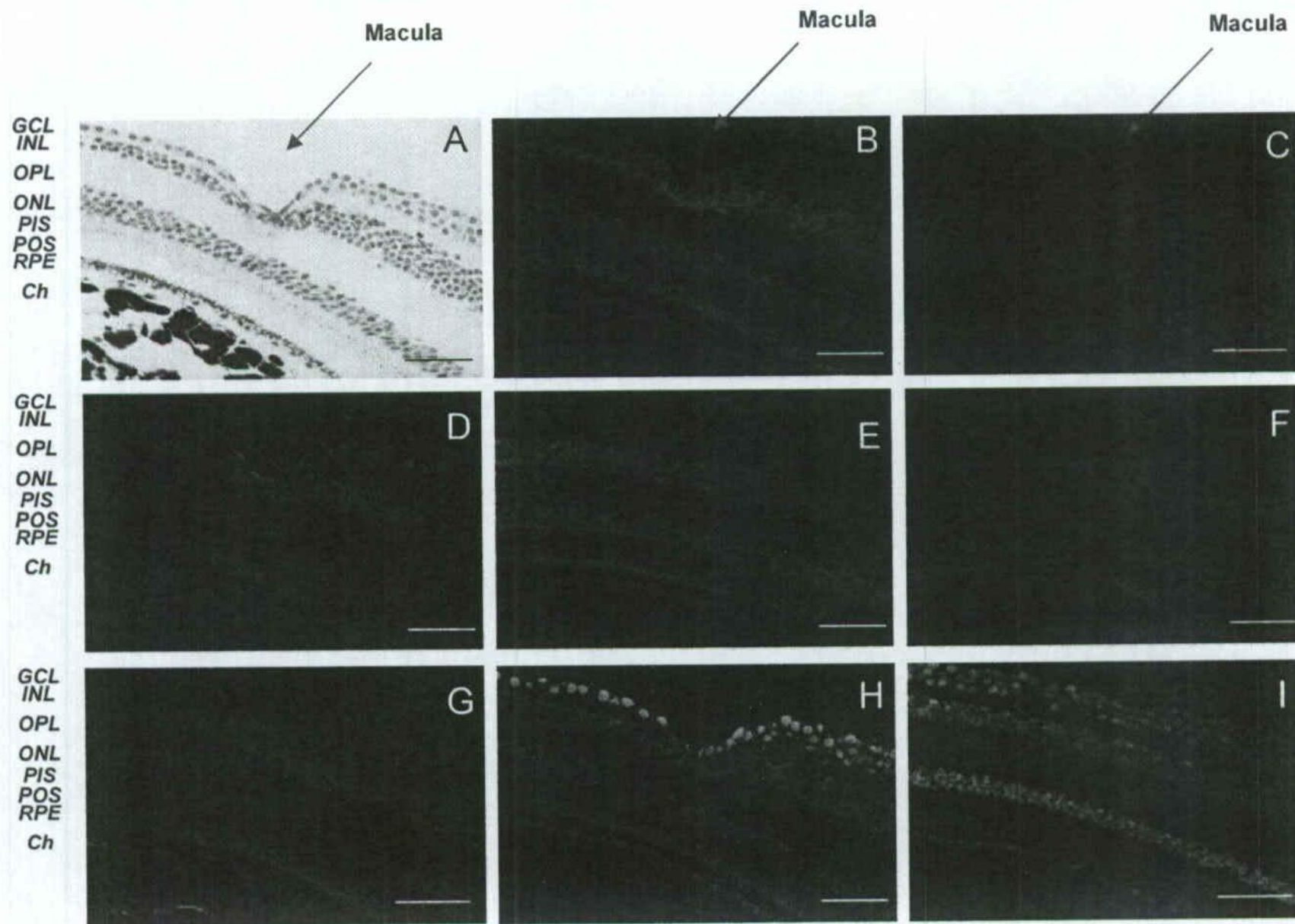


Figure 7



Tables

Table 1

Proteins identified macular and peripheral retina.

Abundant proteins in macular and peripheral retina are identified by LC-MS/MS and listed.

^(a) Spot numbers correspond to the numbers on gel images in figure 1.

^(b) Accession no. corresponds to UniProtKB/Swiss-Prot database (Release 48.8).

^(c) MW and pI is theoretical score by Bioworks ver.3.1.

*Oxidation of methionine.

Spot no. ^(a)	Protein name	Database Accession no. ^(b)	MW (kDa) ^(c)	pI ^(d)	Sequence coverage (%)	Precursor ion MH+	Charge	XC	Residue	Sequence
1	Heat shock protein HSP 90-alpha	P07900	84.5	4.94	10.53	1243.44	2	3.66	100-111	ADLNNLGTIAK
						1152.24	2	3.03	283-291	YIDQEELNK
						1834.87	2	4.55	299-313	NPDDITNEEYGEFYK
						1265.40	2	3.09	345-354	RAPFDLFENR
						1169.23	2	2.23	446-455	LGIHEDSQNR
						1225.38	2	2.85	489-498	HIYYITGETK
						1236.32	2	3.23	499-509	DQVANSFVER
1	Heat shock protein HSP 90-beta	P08238	83.1	4.97	6.09	1545.72	2	2.34	41-54	ELISNASDALDKIR
						1040.11	2	2.43	55-63	YESLTDPSK
						1243.44	2	3.66	95-106	ADLNNLGTIAK
						1152.24	2	3.03	275-283	YIDQEELNK
2	Heat shock cognate 71 kDa protein	P11142	70.9	5.37	29.41	1488.58	2	2.96	37-49	TTPSYVAFTDTER
						1666.84	2	4.15	57-71	NQVAM*NPTNTVFDAK
						1271.38	2	2.13	78-88	FDDAVVQSDM*K
						1181.32	2	3.19	103-112	VQVEYKGETK
						1633.85	2	3.48	113-126	SFYPEEVSSM*VLTK
						1269.49	2	3.69	127-137	M*KEIAEAYLGK
						1200.37	2	3.82	160-171	DAGTIAGLNVLR
						1789.07	2	4.31	172-188	IINEPTAAAIAYGLDKK
						1252.47	2	3.36	237-246	M*VNHFAIEFK
						1254.37	2	2.60	302-311	FEELNADLFR
						1482.67	2	3.92	329-342	SQIHDIVLVGGSTR
						1082.23	2	2.37	349-357	LLQDFFNGK
						773.86	1	1.80	452-458	DNNLLGK
						1018.15	2	2.43	501-509	ITITNDKGR
						990.09	2	2.54	510-517	LSKEDIER
1142.31	2	2.30	518-526	M*VQEAKEYK						
1320.45	2	3.71	540-550	NSLESYAFNM*K						

					903.02	2	3.29	121-129	GVNVSALSR
					1317.52	2	3.10	221-232	LPANHPLLTGQR
					1051.16	2	2.45	281-289	GNEM*SEVLR
					1397.53	2	2.25	290-301	DFPELTM*EVDGK
					1532.75	2	4.18	309-323	TALVANTSNM*PVAAR
					1799.00	2	3.81	365-381	LAEM*PADSGYPAYLGAR
					1869.18	2	3.94	537-552	TVGM*LSNM*IAFYDM*AR
					1248.41	2	2.11	586-596	FKDPLKDGEAK
					1817.96	2	3.68	599-613	SDYAQLLEDM*QNAFR
2	Lamin B2	Q03252	67.7	5.29	17.67				
					1427.67	2	3.18	46-57	ALELENDRLLLK
					1192.30	2	2.94	58-67	ISEKEEVTR
					1238.33	2	3.58	74-84	ALYESELADAR
					1089.18	2	2.77	152-161	GLESDVAELR
					1434.50	2	3.60	271-284	LSSDQNDKAASAAR
					1770.05	2	3.50	292-306	M*RLESLSYQLSGLQK
					1453.69	2	2.67	362-373	LALDM*EINAYRK
					975.04	2	2.10	374-381	LLEGEEER
					1520.69	2	4.09	542-555	TVLVNADGEEVAM*R
2	Heat shock 70 kDa protein 1	P08107	70.1	5.48	16.54				
					1488.58	2	2.96	37-49	TTPSYVAFTDTER
					1659.83	2	4.00	57-71	NQVALNPQNTVFDK
					1688.91	2	4.24	172-187	IINEPTAAAIAYGLDR
					1676.68	2	3.22	221-236	ATAGDTHLGGEDFDNR
					1110.25	2	2.57	349-357	LLQDFFNDR
					1018.15	2	2.43	501-509	ITITNDKGR
					1004.12	2	2.44	510-517	LSKEEIER
					1142.31	2	2.30	518-526	M*VQEAKEYK
					1304.46	2	2.81	540-550	NALESYAFNM*K
2	Stress-70 protein, mitochondrial	P38646	73.7	5.87	9.57				
					1570.88	2	3.07	160-173	LYSPSQIGAFVLM*K
					1243.40	2	3.54	207-218	DAGQISGLNVLR
					1646.87	2	4.48	219-234	VINEPTAAALAYGLDK

3	Stress-70 protein, mitochondrial	P38646	73.7	5.87	8.25	1291.44	2	3.38	395-405	VQQTVDLFR
						1243.40	2	2.60	207-218	DAGQISGLNVL
						1646.87	2	4.44	219-234	VINEPTAAALAYGLDK
						1291.44	2	2.51	395-405	VQQTVDLFR
3	Serum albumin	P02768	69.4	5.92	6.90	1809.96	2	2.32	469-485	SQVFSTAADGQTQVEIK
						1150.31	2	2.76	66-75	LVNEVTEFAK
						961.14	2	2.44	427-434	FQNALLVR
						1512.73	2	2.34	439-452	VPQVSTPTLVEVSR
4	Serum albumin	P02768	69.4	5.92	6.90	1129.38	2	2.83	549-558	KQTALVELVK
						1651.88	2	3.32	250-264	AEFAEVSKLVTDLTK
						1640.88	2	2.71	348-360	DVFLGM*FLYEYAR
						1512.73	2	2.82	439-452	VPQVSTPTLVEVSR
5	Serum albumin	P02768	69.4	5.92	7.39	1150.31	2	2.70	66-75	LVNEVTEFAK
						1658.78	2	3.62	414-426	QNCLEFEQLGEYK
						1512.73	2	3.13	439-452	VPQVSTPTLVEVSR
						1129.38	2	2.98	549-558	KQTALVELVK
6	Serum albumin	P02768	69.4	5.92	3.94	1150.31	2	2.55	66-75	LVNEVTEFAK
						1512.73	2	2.78	439-452	VPQVSTPTLVEVSR
7	Serotransferrin	P02787	77.1	6.81	3.72	1274.41	2	2.88	226-236	HSTIFENLANK
						1883.00	2	3.38	237-251	ADRDQYELLCLDNTR
8	Neurofilament triplet L protein	P07196	61.4	4.64	23.99	1073.14	2	2.30	91-99	AQLQDLNDR
						1125.22	2	2.35	107-115	VHELEQQNK
						1155.41	2	3.43	116-125	VLEAELLVLR
						1062.11	2	2.99	147-156	LAAEDATNEK
						1025.09	2	2.90	177-184	YEEVLSR
						1002.11	2	2.53	196-205	KGADEAALAR