

SUPPLEMENTAL DATA

Fig. S1. Two dimensional acrylamide gel of a mixture of secreted proteins from B35, B50, B103, and B104 nerve-like rat CNS cell lines, 75 µg of each. The majority of the proteins in the gel were identified by LC/MS and are listed in Table S1 along with the other secreted proteins identified in this manuscript.

Fig. S2. Two dimensional acrylamide gel of proteins secreted by AHP cells.

Fig. S3. Western blots of four secreted proteins from four different cell types confirm LC/MS protein identification. Ten micrograms of secreted protein from each cell line were run on 12% SDS acrylamide gels and blotted with the indicated antisera.

Table S1. Table of secreted proteins. All of the identified proteins in this study are listed, roughly starting with the low molecular weight proteins determined in Fig. S1, the gels with a mixture of the secreted proteins from the CNS cell lines master mix. The proteins unique to the different cell types from Fig. 2 (cortical nerve and astrocyte) and AHP (Fig. S2) follow in the right hand columns. Each protein is assigned an accession number and theoretical molecular weight. The probability based Mowse score determined by the Mascot search algorithm is listed. Scores >40 are considered as being significant. However, each protein spot in this table has been identified by LC/MS at least three times in the same position on the gels from three independent experiments. A +* indicates the protein was detected and identified in the B35, B50, B103, B104 master mix and identified again by LC/MS when the secreted proteins from the individual cell lines were run. Otherwise in the case of B35, B50, B103 and B104 the proteins were identified by spot location. All of the proteins from AHP, astrocytes (Astro) and cortical nerve (nerve) were identified independently at least three times by LC/MS and, in addition, by matching the spot position to the master mix when possible.

Table S2. Table of secreted proteins grouped according to known biological function.

Figure S1

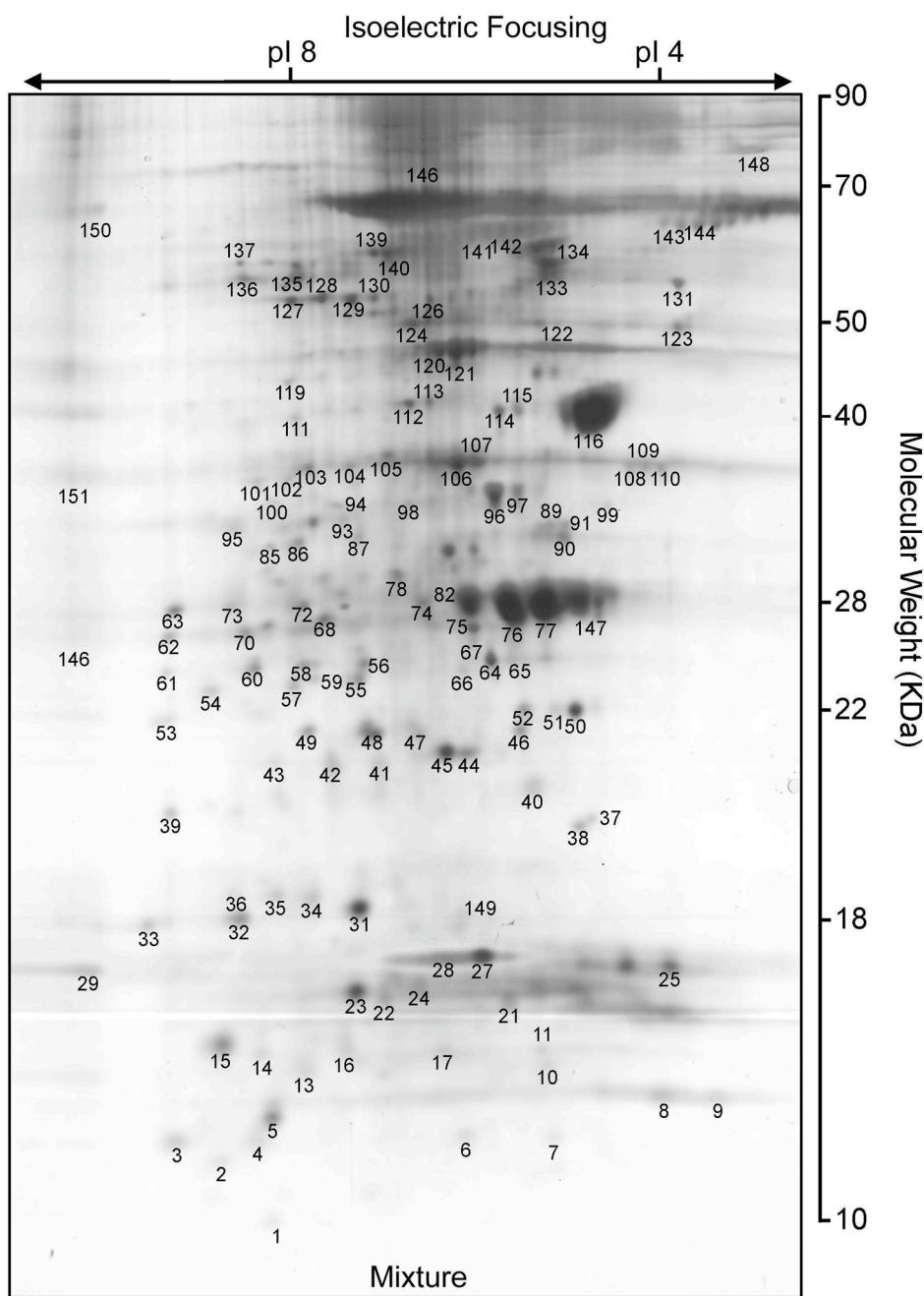


Figure S2

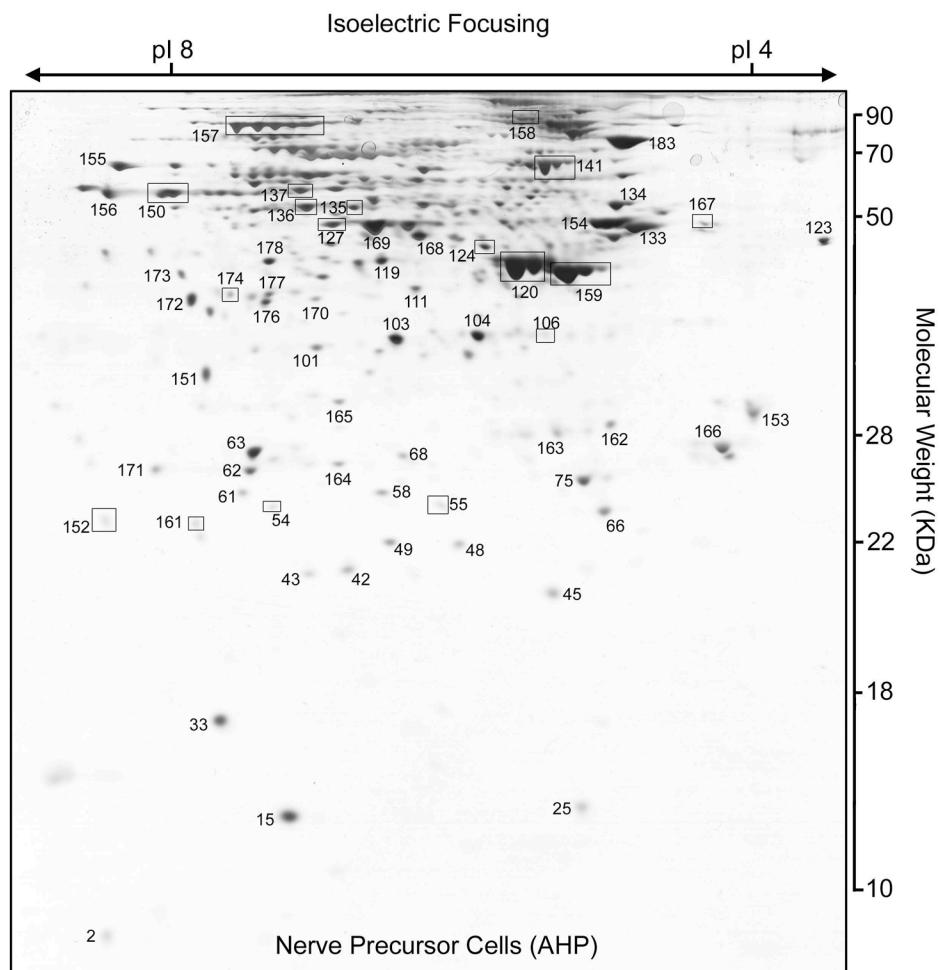


Figure S3

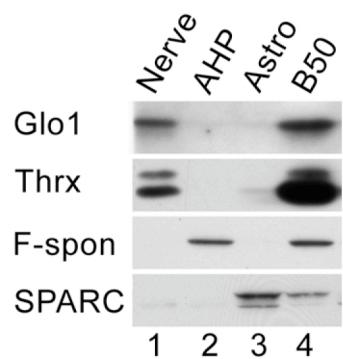


TABLE S1: COMPLETE LIST OF SECRETED PROTEINS

Spot No.	Protein Name	Accession Number	Theor. MW (kDa)	Score	Peptides	Master Mix				AHP	Astro	Nerve
						B35	B50	B103	B104			
1	Nedd 8	13435504	8,967	208	45	+	+	+	+	o	o	o
2	Macrophage migration inhibitory factor beta chain (a)	5542287	12,365	210	2	+	+	o	o	+	o	+
3	Macrophage migration inhibitory factor beta chain (b)	5542287	12,365	74	2	+	+	+	+	o	o	o
4	Macrophage migration inhibitory factor beta chain (c)	5542287	12,365	150	3	+	+	o	+	o	o	o
5	D-dopachrome decarboxylase	6753618	13,069	96	4	+	+	+	+	o	o	o
6	GAPDH (12 kd frag)	63556808	41,160	44	2	+	+	+	+	o	o	o
7	Parathymosin	18204145	11,762	42	1	+	+	+	+	o	o	o
8	Reticulon 4	56207523	12,480	104	3	+	+	+	+	o	o	o
9	Thioredoxin 1 (a)	6755911	11,388	130	3	+	+	+	+	o	o	o
10	Thioredoxin 1 (b)	6755911	11,668	59	2	+	+	+	+	o	o	+
11	Coactosin-like protein (a)	16307409	11,189	69	7	+	+	+	+	o	o	o
13	Profilin 1	1628436	14,978	105	4	+	+	+	+	+	o	o
14	Histidine triad nucleotide binding protein 1	33468857	13,768	51	1	+	+	+	+	o	o	o
15	Fatty acid binding protein 5	6754450	15,127	101	3	+	+	+	+	+	+	+
16	Histone H2A.1	51304	14,153	153	5	+	+	+	o	o	o	o
17	Profilin 2	9506971	15,022	66	1	+	+	+	+	o	o	o
21	Coactosin-like protein 1 (b)	1628436	11,189	80	3	+	+	o	o	o	o	o
22	Histone H2B	280961	15,099	137	6	+	+	+	+	o	o	o
23	Ubiquitin - conjugating enzyme E2N	12838544	17,171	97	3	+	+	+	+	o	o	o
24	Histone H2B (b)	280961	15,009	189	6	+	+	o	+	o	o	o
25	Guanine nucleotide BP B2	56800239	16,322	128	2	+	+	+	**	+	o	o
27	N-cadherin-2 precursor (16 kd frag)	6680902	99,699	216	8	+	+	+	+	o	o	o
28	N-cadherin-2 precursor (16 kd frag)	6680902	99,699	59	3	+	+	o	o	o	o	o
29	Peptidylprolylisomerase C	1000036	17,960	103	4	o	**	**	+	o	o	o
31	Tumor metastatic protein NM 23 (a)	387496	18,072	128	8	+	+	**	+	o	o	+
32	NM 23 (b)	387496	18,672	155	10	o	o	**	o	o	o	o
33	Expressed in non-metastatic cell protein 1	56800494	17,197	197	20	+	+	+	+	+	o	+
34	Cofilin 1, nonmuscle (a)	6880924	18,548	168	4	o	**	**	+	o	o	o
35	Cofilin 1, nonmuscle (b)	6880924	18,548	144	3	o	**	**	+	o	o	o
36	NM 23 (c)	387496	18,072	177	9	o	+	+	+	o	+	o
37	Vimentin (15 kd frag)	55291	53,641	280	11	o	+	o	+	o	o	o
38	Vimentin (15 kd frag)	55291	53,641	75	2	+	+	+	+	o	o	o
39	Cyclophilin C	1000036	19,904	51	2	+	+	+	+	o	o	o
40	Peroxiredoxin 2 (a)	1568575	21,765	274	86	o	+	o	+	+	o	+
41	NM 23 (d)	387496	18,672	78	3	o	o	+	+	o	o	o
42	NM 23 (e)	387496	18,672	121	4	o	+	+	+	+	+	o
43	NM 23 (f)	387496	18,672	144	3	o	+	+	+	+	+	o
44	Peroxiredoxin 2 (b)	1568575	21,765	194	15	+	**	**	+	o	o	o
45	Phosphatidyl ethanolamine BP	92565972	20,787	144	5	+	+	**	+	+	+	o
46	Methylglyoxylase 1	31981282	20,826	59	2	+	+	**	+	o	o	+
47	Heparan sulfate proteoglycan 2 (frag)	55715901	117,786	106	3	+	+	**	+	o	o	o
48	Peroxiredoxin 2 (c)	1568575	21,765	100	3	+	+	+	+	o	o	o
49	DJ1	55741460	20,008	48	2	+	+	+	+	+	o	+
50	Huntingtin interacting protein	2897818	22,389	91	3	+	+	+	+	o	o	o
51	p22 phox	26354020	20,030	85	6	o	+	+	+	o	o	o
52	Proteasome beta-6	984938	21,668	236	9	+	+	+	+	o	o	o
53	Peroxiredoxin 2 (d)	1568575	22,162	133	5	+	o	o	+	+	o	+
54	GST Yfyf chain B (a)	2624496	23,350	50	1	+	+	**	+	o	o	o
55	GST - P2 (a)	32401425	23,552	106	2	+	+	**	+	+	o	o
56	Peroxiredoxin 6	2072655	24,855	124	4	+	+	+	+	o	o	o
57	GST Yfyf chain B (b)	2624496	23,350	130	2	+	+	+	+	o	o	o
58	Proteasome beta 3	83944082	22,949	116	2	+	+	+	+	+	o	+
60	Proteasome alpha type 5	6679497	25,909	68	2	+	+	+	+	o	o	o
61	Proteasome alpha type 2	6679497	25,909	71	3	+	+	+	+	o	+	+
62	Triosephosphate isomerase (a)	54855	26,679	162	5	+	+	+	+	+	o	+
63	Pyrrolidine-5-carboxylate reductase	13384800	26,153	56	1	+	+	+	+	+	+	+
64	Rho GDP dissociation inhibitor	55742827	23,393	186	7	+	+	+	+	o	o	o
65	GST Yfyf chain B	2624496	23,350	49	1	+	+	**	+	o	o	o
66	HS 27	547679	22,879	204	10	o	o	o	+	+	o	o
67	LI	6755929	24,822	62	4	+	+	+	+	o	o	o
68	ER p29 (a)	19526463	28,805	171	7	+	+	o	+	+	o	o
70	Triosephosphate isomerase 1 (b)	54855	26,679	162	5	+	+	**	+	o	o	o
72	ER p29 (b)	19526463	28,805	78	2	+	+	+	+	o	o	o
73	Heat Shock 8	63664182	33,101	74	2	+	+	+	+	+	+	+
74	ER p29 (c)	19526463	28,805	66	3	+	+	+	+	o	o	o
75	Tyrosine hydroxylase (a)	31981925	29,170	314	17	+	**	**	+	+	o	+
76	14-3-3 gamma (a)	3065929	27,000	193	7	+	**	+	+	o	o	o
77	14-3-3 gamma (b)	3065929	27,000	104	5	+	+	+	+	o	o	o
78	Dimethyl arginine hydroxylase 2	7949035	25,352	164	7	+	+	+	+	o	+	+
82	14-3-3 gamma (c)	3065929	27,000	120	5	+	+	+	+	o	o	o
85	Proteasome alpha 1	38328483	29,499	500	69	+	o	o	o	o	o	+
86	Glucose regulated protein (frag) (a)	2506545	72,412	320	+	+	+	+	o	o	o	o
87	Fibrillin 1 precursor (frag)	3913647	312,025	163	+	+	o	+	+	o	o	o
89	Tyrosine hydroxylase (b)	31981925	29,170	58	2	o	o	**	o	o	o	o
90	Tyrosine hydroxylase (c)	31981925	29,170	219	7	o	**	**	+	o	o	o
91	HS 70 (frag)	1661134	70,793	59	1	o	o	+	+	o	o	o
95	Pirin	57164095	32,158	325	20	o	+	o	+	o	o	o
96	Pro-collagen XII alpha 1 (a)	111074529	35,209	89	+	o	+	+	o	o	o	+
97	Pro-collagen XII alpha 1 (b)	111074529	35,209	250	+	o	+	+	o	o	o	o
98	Alpha dystroglycan 1 (frag) (a)	1587060	70,537	67	3	o	+	**	+	+	o	o
99	Proliferating cell nuclear antigen	7242171	28,766	213	8	o	o	+	+	o	o	o
100	Collagen type 1, alpha 2 (frag)	16758080	41,160	58	2	+	o	o	o	o	o	o
101	GAPDH	120702	35,804	80	4	+	o	o	o	+	+	+
102	Aldose reductase	55759	35,774	429	50	+	o	+	o	o	+	o
103	Malic dehydrogenase	14152	63,999	78	5	o	+	+	+	+	+	+
104	LDH chain M (a)	65923	36,474	60	1	o	+	o	+	+	o	+
105	LDH chain B	6678674	36,549	143	4	+	+	+	+	o	o	o

Spot No.	Protein Name	Accession Number	Theor. MW (kDa)	Score	Peptides	Master Mix				AHP	Astro	Nerve
						B35	B50	B103	B104			
106	LDH chain M (b)	65923	36,474	54	4	+	+	+	+	+	+	0
107	Alpha dystroglycan (frag) (b)	1587060	70,537	74	2	+	o	+	+	o	0	0
108	LDH-A (a)	8393706	36,427	100	5	o	+	o	+	o	0	0
109	LDH-A (b)	8393706	36,427	129	6	o	+	o	+	o	0	0
110	LDH-X	2117461	35,664	61	2	o	+	o	+	o	0	0
111	Transaldolase	33859640	37,363	158	8	o	+	o	+	+	o	+
112	Enolase alpha (a)	50926833	47,098	54	2	+	+	+	+	o	0	0
113	Enolase alpha (b)	50926833	47,098	440	13	o	o	+	+	o	0	0
114	Cathepsin L (a)	44870022	37,505	125	3	+	+	+	+	o	0	0
115	Cathepsin L (b)	44870022	37,505	115	2	+	+	+	+	o	0	0
116	Secreted acidic cysteine rich protein	6678077	34,428	255	8	+	+	+	+	o	+	0
119	Phosphoglycerate kinase 1	6679291	44,508	79	3	+	+	+	+	+	o	0
120	gamma-actin (a)	4885049	40,992	152	7	+	+	+	+	+	0	0
121	gamma-actin (b)	4885049	40,992	42	4	+	+	+	+	o	0	0
122	gamma-actin (c)	4885049	40,992	83	4	o	+	+	+	o	0	0
123	Creatine kinase (a)	10946574	42,686	540	25	o	+	+	o	+	0	0
124	Creatine kinase (b)	10946574	42,686	409	4	+	+	+	+	+	+	+
126	Pigment epithelium-derived factor	2961482	46,175	107	2	+	+	+	+	o	0	0
127	Enolase 1alpha (a)	12963491	47,095	171	6	+	+	+	+	+	+	0
128	Enolase 1alpha (b)	12963491	47,095	210	5	+	+	+	+	o	0	0
129	Enolase 1alpha (c)	12963491	47,095	248	7	+	+	+	+	o	0	0
130	Enolase 1alpha (d)	12963491	47,095	138	4	+	+	+	+	o	0	0
131	Calreticulin precursor	436046	47,065	118	3	+	+	+	+	o	0	0
133	Prolyl-4-hydroxylase beta	54777	57,108	201	9	+	+	+	+	o	0	+
134	Heat shock 70 (HS70) (a)	1661134	70,934	410	25	+	+	+	+	o	0	+
135	Procollagen C-proteinase enhancer protein (a)	9506953	50,153	496	31	+	+	+	+	+	+	0
136	Procollagen C-proteinase enhancer protein (b)	9506953	50,153	513	48	+	+	+	+	o	0	0
137	Dihydropyrimidinase 3 (frag)	21666559	738,530	162	5	+	+	+	+	+	+	+
139	Nucleobindin 1 (a)	6679158	53,362	54	2	+	+	+	+	o	0	0
140	Nucleobindin 1 (b)	6679158	53,362	98	3	+	+	+	+	o	0	0
141	HS 70 (b)	1661134	70,934	655	33	+	+	+	+	+	+	+
142	HS 70 (c)	1661134	70,934	433	27	+	+	+	+	o	0	0
143	Nucleobindin 1 (c)	6679158	53,362	196	6	+	+	+	+	o	0	0
144	Nucleobindin 1 (d)	6679158	53,362	69	2	+	+	+	+	o	0	0
146	Triosephosphate isomerase (c)	19526463	26,679	162	6	+	+	+	+	+	+	0
147	14-3-3 gamma (d)	3065929	27,000	104	5	+	+	o	+	+	0	0
148	N-Cadherin (frag)	6680902	99,734	157	6	o	+	+	+	o	0	0
149	Glial maturation factor beta	108869743	16,723	129	7	o	o	o	+	o	0	0
150	M2 pyruvate kinase	1675994	57,744	509	16	+	o	o	+	o	0	+
151	Lamin receptor 1	31560560	32,698	120	3	+	o	o	+	+	o	+
152	GST-p12 (b)	25453412	23,424	434	27	o	+	o	o	+	o	0
154	Tubulin alpha 3	90217	50,064	597	77	o	o	o	o	+	o	0
155	Thioredoxin reductase 1	5410338	54,250	324	47	o	o	o	o	+	o	+
156	Transketolase	12018252	71,141	616	76	o	o	o	o	+	+	+
157	Elongation factor 2	600159	95,249	329	18	o	o	o	o	+	+	+
158	F-spondin	50510627	82,002	248	9	o	+	o	o	+	o	0
159	Beta actin	49868	39,161	295	19	+	o	o	o	+	+	+
161	GST YfylfA	576133	23,463	220	18	+	o	o	+	+	o	0
162	Inositol phosphatase	3914098	30,416	139	8	+	o	o	o	+	o	0
163	Proteasome alpha 3	3914438	28,387	264	22	o	o	o	o	+	o	+
164	Presenilin-2	6755188	27,355	165	7	+	+	+	+	o	+	+
165	Phosphatidyl inositol transfer protein alpha	6679337	31,873	155	4	+	o	+	+	o	0	0
166	ER p29 (d)	19526463	28,805	171	7	o	+	o	o	+	o	0
167	Retinoblastoma binding protein	1016275	51,738	416	26	o	+	o	+	o	0	0
168	GDP dissociation inhibitor 1 (a)	40254781	50,505	631	63	+	o	+	+	+	+	+
169	LOC433182 protein	53734652	50,209	800	128	o	o	+	+	+	+	+
170	Transaldolase	33859640	33,363	130	5	o	+	o	+	+	o	0
171	Triosephosphate isomerase 1 (b)	54855	26,696	162	5	+	+	o	+	+	o	0
172	Aldolase 3	14198249	39,307	145	4	o	+	o	+	+	o	+
173	Aspartate aminotransferase (a)	871422	46,203	154	11	o	o	o	+	+	o	+
174	Aspartate aminotransferase (b)	811422	46,203	154	11	o	o	o	o	+	o	+
176	Alpha fetoprotein (a)	191765	47,195	81	2	o	+	o	+	+	o	0
177	Alpha fetoprotein (b)	191765	47,195	84	14	o	o	o	o	+	o	0
178	Dihydropyridinase-2	40254595	62,239	446	25	o	o	o	o	+	o	+
179	Proteasome alpha 4	6755196	29,452	273	11	o	o	o	o	o	o	+
180	Serine protease 27	94382825	59,818	69	8	o	+	o	+	o	0	+
181	Platelet-activating factor acetylhydrolase	7505363	46,640	203	9	o	o	o	o	o	o	+
182	Glucose-regulated protein precursor (b)	2506545	72,377	1178	47	o	o	+	+	o	+	+
183	HS 90	40556608	83,229	1045	33	o	o	+	+	o	+	+
184	ER ATPase p97	94408011	88,796	1336	56	o	o	o	o	o	o	+
185	Follistatin related protein 7	2498391	34,516	90	8	o	o	o	o	o	+	o
186	Nonmuscle alpha tropomyosin	207351	32,711	558	33	o	o	o	o	o	+	o
187	Procollagen type 3 (frag) (a)	57916	62,293	317	33	o	o	o	o	o	+	o
188	Procollagen type I, alpha 2 (frag) (a)	16758080	129,486	349	70	o	o	o	o	o	+	o
189	Procollagen type I, alpha 2 (frag) (b)	16758080	129,486	92	7	o	o	o	o	o	+	o
190	Procollagen type I, alpha 2 (frag) (c)	16758080	129,486	119	3	o	o	o	o	o	+	o
191	Procollagen type I, alpha 2 (frag) (d)	16758080	129,486	210	28	o	o	o	o	o	+	o
192	Lumican (proteoglycan)	13591983	38,255	282	20	o	o	o	o	o	+	o
193	Procollagen type I, alpha 1 (frag)	15214623	56,628	120	3	o	o	o	o	o	+	o
194	Procollagen type I, alpha 2 (frag) (e)	16758080	129,486	135	4	o	o	o	o	o	+	o
195	Procollagen C protease	9506953	50,153	355	17	o	o	o	o	o	+	o
196	Protein disulfide isomerase	129731	56,916	680	45	o	o	o	o	o	+	o
197	Ser/Cys protease inhibitor F1	29293811	46,436	596	23	o	o	o	o	o	+	o
A	Bovine serum albumin	76874	66,430	953	o	o	o	o	o	+	+	+

TABLE S2. SELECTED PROTEINS BY FUNCTION

<i>Chaperone/Redox</i>		
Spot Number	Protein Name	Function
2, 3, 4	Macrophage inhibition factor β	protein isomerase
9, 10	Thioredoxin 1	oxyreductase
29	Peptidyl proly isomerase	isomerase
40, 44, 48, 53	Peroxiredoxin 2	oxyreductase
46	Methylglyoxylase 1	detoxification
51	P22 phox	regulation
54, 57, 65	GST Yfyf chain B	detoxification
55	GST p2	detoxification
56	Peroxiredoxin 6	oxyreductase
59	Peroxiredoxin 3	oxyreductase
66	Heat shock 27	protein binding
68, 72, 74, 166	ER protein 29	protein binding
73	H58	protein binding
86, 182	Glucose regulated protein	protein binding
134, 91	HS70	protein binding
152	GST(b) p22	detoxification
156	Thioredoxin reductase 1	oxyreductase
162	GST Yfyf chain A	detoxification
176, 177	Alpha fetoprotein	antioxidant
183	Heat shock 90	protein binding
196	Protein disulfide isomerase	oxyreductase

<i>Cell-Cell Interaction</i>		
Spot Number	Protein Name	Function
8	Reticulon 4	adhesion
27, 28	N-Cadherin-2	protein binding
31, 32, 36, 41, 42, 43	Tumor metastatic protein NM 23	nucleotide binding
39	Cyclophilin C	protein binding
47	Heparin sulfate proteoglycan 2	protein binding
63	Pyrroline-5-carboxylate reductase	reductase
87	Fibrillin precursor	regulation
96, 97	Procollagen XII alpha1	protein binding
98, 107	Alpha dystroglycan	protein binding
100, 188, 189, 190, 191, 194	Procollagen 1alpha2	protein binding
114, 115	Cathepsin-L	protease
116	SPARC	ion binding
126	Pigment epithelial factor	signalling
135, 136, 195	Procollagen C-protease	protease
147, 76, 77, 82	14-3-3 protein gamma	protein binding
149	Glial maturation factor	signalling
158	F-Spondin	protein binding
180	Procollagen 3alpha	protein binding
181	Platelet activating factor	acetyl reductase
185	Follistatin-related protein	carbohydrate binding
193	Procollagen 1alpha1	protein binding
197	Serine/Cysteine protease inhibitor f1	inhibitor

<i>Carbohydrate/Amino Acid Metabolism</i>		
Spot Number	Protein Name	Function
62, 70, 146, 171	Triosephosphate isomerase	isomerase
78	Dimethyl arginine hydrolase	hydrolase
101	GAPDH	dehydrogenase
102	Aldose reductase	oxyreductase
103	Malic dehydrogenase	dehydrogenase
104, 105, 106, 108, 109, 110	LDH	oxyreductase
111, 170	Transaldolase	transferase
112, 113, 127, 128, 129, 130	Enolase alpha	lyase

119	Phosphoglycerate kinase 1	kinase
123, 124	Creatine kinase	transferase
150	Pyruvate kinase	kinase
156	Transketolase	transferase
162	Inositol phosphatase	hydroxylase
167	Retinoblastoma binding protein	hydrolase
172	Aldolase 3	lyase
173, 174	Aspartate amino transferase	transferase

Protein Metabolism

Spot Number	Protein Name	Function
1	Nedd 8	protein binding
7	Parathyrosin	co-activator
23	Ubiquitin-conjugating enzyme 2	ligase
52	Proteasome beta 6	peptidase
58	Proteasome beta 3	peptidase
60	Proteasome alpha 5	peptidase
61	Proteasome alpha 2	peptidase
67	Ubiquitin hydrolase L1	hydrolase
85	Proteasome alpha 7	peptidase
131	Calreticulin precursor	protein binding
133	Prolyl-4-hydroxylase beta	hydroxylase
151	Lamin receptor	structural
157	Elongation factor 2	nucleotide binding
163	Proteasome alpha 3	peptidase
164	Presenilin 2	protein binding
179	Proteasome alpha 4	peptidase
180	Serine protease 27	protease
184	ER ATPase 1	hydroxylase

Nucleotide Binding/Metabolism

Spot Number	Protein Name	Function
14	Histidine triad nucleotide BP1	hydroxylase
16	Histone H2A type 1	protein binding
22	Histone 2B	protein binding
25	Guanidine nucleotide binding protein 2	protein binding
33	Expressed non-metastatic cell protein 1	unknown
64	Rho GDP dissociation inhibitor	protein binding
137	Dihydropyrimidinase 3	hydrolase
139, 140, 143, 144	Nucleobindin 1	nucleic acid binding
168, 198	GDP dissociation inhibitor 1	enzyme regulation
178	Dihydropyrimidinase 2	hydrolase

Structural

Spot Number	Protein Name	Function
11, 21	Coactosin-like protein 1	cytoskeleton
13	Profilin 1	cytoskeleton
17	Profilin 2	cytoskeleton
34, 35	Cofilin	cytoskeleton
37, 38	Vementin	cytoskeleton
120, 121, 122	α -Actin	cytoskeleton
154	Tubulin α 3	cytoskeleton
159	β -Actin	cytoskeleton
186	Nonmuscle tropomyosin	cytoskeleton

Lipid Metabolism

Spot Number	Protein Name	Function
15	Fatty acid binding protein	lipid binding
50	Huntingtin-interacting protein	transferase
165	Phosphotidyl inositol transfer protein α	lipid binding

Neurotransmitter Metabolism

Spot Number	Protein Name	Function
5	Dopachrome decarboxylase	lyase
49	DJ1	protein binding
75, 89, 90, 153	Tyrosine hydroxylase	oxygenase