PROTEOMICS

Supporting Information for Proteomics

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Sarah Dunn Keene, Todd M. Greco, Ioannis Parastatidis, Seon-Hwa Lee, Ethan G. Hughes, Rita J. Balice-Gordon, David W. Speicher and Harry Ischiropoulos

Mass spectrometric and computational analysis of cytokine-induced alterations in the astrocyte secretome

Supporting Information

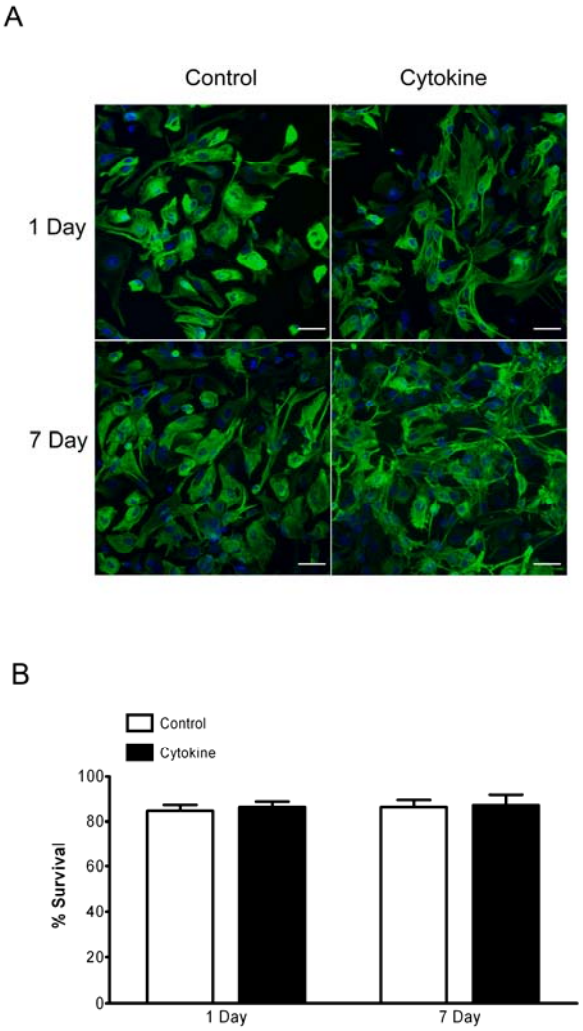

Figure 1. Characterization of primary murine astrocyte cell cultures. (A) Immunohistochemical (IHC) staining for glial fibrillary acidic protein (GFAP) and DAPI. (B) Cell viability measured via flow cytometry (see Experimental Procedures). Minimum event count 20,000 cells per condition (N = 4).

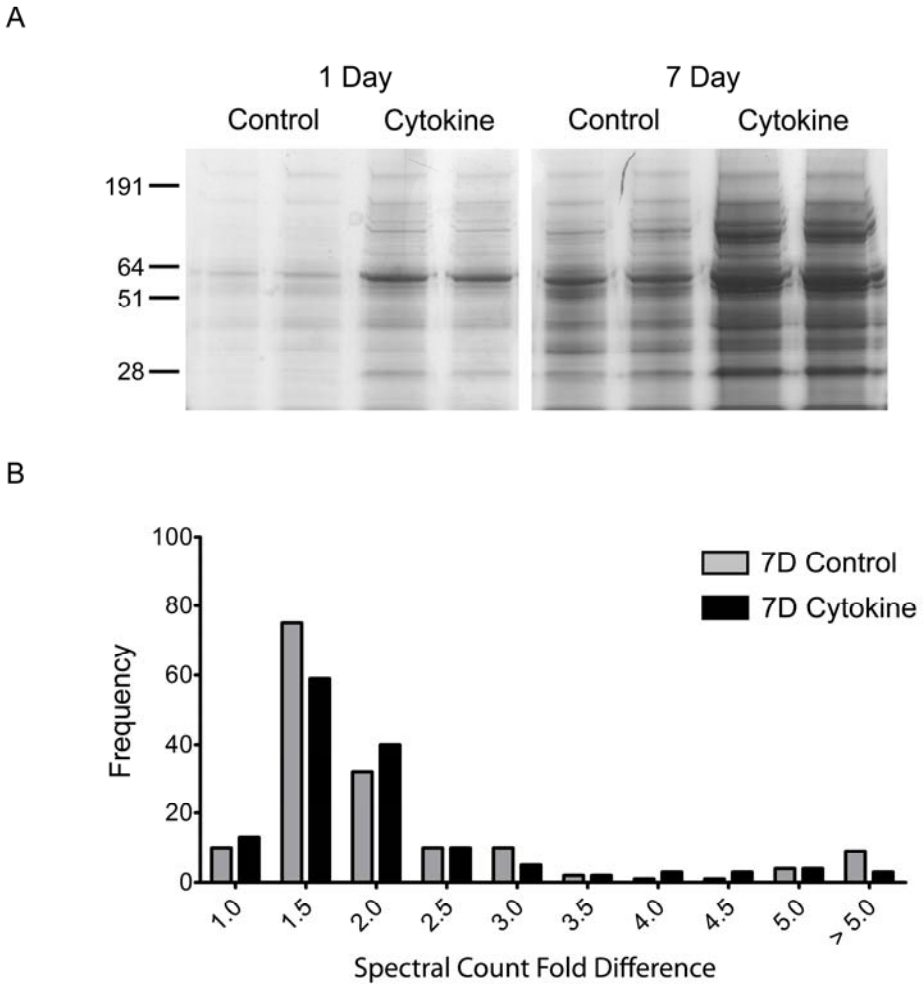

Figure 2. Reproducibility of Gel/LC-MS/MS. (A) ACM protein fraction from biological duplicates were resolved for 2 cm by 1D-SDS-PAGE and visualized with Colloidal Blue. Biological duplicates show similar protein staining patterns. Increased total protein in ACM is observed after cytokine stimulation and after 7 days *in vitro*. (B) Frequency versus spectral count fold difference calculated for secreted proteins identified in biological duplicates from 7D control (gray bars) and 7D cytokine (black bars). Most of the proteins' spectral counts differed by less than 2.5-fold between biological duplicates. This fold difference was selected as the minimum criteria when evaluating cytokine-induced alterations in relative protein abundance between control and cytokine conditions.

Table 1. Unique peptides from unclassified proteins after computational analysis

Protein Name (Synonym)	Accession[A]	MW (kDa)	1D Control	1D Cytokine	7D Control	7D Cytokine
			Unique Peptides[B]			
14-3-3 protein beta	Q9CQV8	28,086	4	4	6	5
14-3-3 protein epsilon	P62259	29,174	11	8	12	8
14-3-3 protein eta	P68510	28,212	3	2	-	2
14-3-3 protein gamma	P61982	28,303	4	4	6	3
14-3-3 protein theta	P68254	27,778	3	4	8	4
14-3-3 protein zeta	P63101	27,771	7	8	8	6
Actin related protein 2/3 complex, subunit 2	Q9CVB6	34,357	2	4	2	3
Actinin, alpha 1	Q7TPR4	103,068	24	24	39	8
Actinin, alpha 4	P57780	104,977	6	32	17	17
Aldehyde dehydrogenase 1 family, member L1	Q8R0Y6	98,709	-	3	4	-
Aldo-keto reductase family 1, member A4	Q9JII6	36,587	4	2	2	3
Aldolase 1, isoform A	Q5FWB7	39,356	4	8	11	6
Aldolase 3, isoform C	Q5SYM1	39,395	2	10	2	4
Annexin A3	O35639	36,371	2	-	6	-
Annexin A5	P48036	35,752	5	3	6	2
Asparaginase like 1	Q8C0M9	33,950	3	4	4	-
Astrocytic phosphoprotein PEA-15	Q62048	15,054	-	4	3	3
Brain glycogen phosphorylase	Q8CI94	96,730	-	2	5	-
Calponin 3, acidic	Q9DAW9	36,429	-	-	2	2
Clathrin, heavy polypeptide (Hc)	Q68FD5	191,557	3	14	6	4
Cofilin 1, non-muscle	P18760	18,560	-	5	5	4
Creatine kinase, brain	Q04447	42,713	9	10	11	9
Cytochrome c	P62897	11,605	3	2	2	2
Dihydropyrimidinase-like 2	O08553	62,278	13	12	12	5
Dimethylarginine dimethylaminohydrolase 1	Q9CWS0	31,381	3	5	3	2
DJ-1 protein	Q99LX0	20,021	2	3	3	3
Enolase 1, alpha non-neuron	P17182	47,141	7	9	8	7
Eukaryotic initiation factor 4AII	P10630	46,402	-	2	5	3
Eukaryotic translation elongation factor 2	P58252	95,314	4	4	5	3
Eukaryotic translation initiation factor 5A	P63242	16,303	-	2	2	3
Fatty acid binding protein 7, brain	P51880	14,893	3	4	4	2
Fatty acid synthase	P19096	272,428	-	5	3	2
Ferritin heavy chain 1	P09528	21,067	-	2	4	4
Ferritin light chain 1	P29391	20,802	4	2	5	6
Filamin-A	Q8BTM8	281,194	9	23	26	15
Filamin-B	Q80X90	277,753	22	29	27	25
Filamin-C	Q8VHX6	291,119	-	-	8	2
Gelsolin-like capping protein	Q99LB4	38,769	-	-	2	4
Glial fibrillary acidic protein (GFAP)	P03995	46,492	11	9	12	12
Glutathione S-transferase, alpha 4	P24472	25,564	3	2	3	2
Glutathione S-transferase, mu 1	A2AE90	25,970	11	10	12	10
Glutathione S-transferase, mu 5	P48774	26,635	-	3	3	-
Glutathione S-transferase, pi 2	P46425	23,537	-	-	5	4
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	P16858	35,810	-	-	2	4

Glyoxalase domain containing 4	Q9CPV4	33,317	-	-	4	-
Guanosine diphosphate (GDP) dissociation inhibitor 1	P50396	50,522	2	3	5	-
H4 histone family, member A	P62806	11,367	3	5	2	3
Heat shock 70kDa protein 8 isoform 1	P63017	70,871	7	16	13	5
Heat shock protein 4	Q5NCS5	94,209	5	6	7	
Heat shock protein 90kDa alpha (cytosolic), class A member 1	P07901	84,788	6	6	13	9
Heat shock protein 90kDa alpha (cytosolic), class B member 1	Q71LX8	83,281	6	16	4	5
Heterogeneous nuclear ribonucleoprotein A2/B1	O88569	37,403	-	3	2	3
Hypoxanthine guanine phosphoribosyl transferase 1	P00493	24,570	-	-	3	-
Inositol monophosphatase	O55023	30,436	3	2	3	-
Isocitrate dehydrogenase [NADP] cytoplasmic	O88844	46,660	2	2	4	-
Lactate dehydrogenase 1, A chain	P06151	36,499	5	4	5	5
Lactate dehydrogenase 2, B chain	P16125	36,572	12	10	11	7
Lactoylglutathione lyase	Q9CPU0	20,810	-	3	2	2
Lamin A isoform A	P48678	74,238	-	-	3	2
Leukotriene A4 hydrolase	Q3UY71	69,051	2	-	6	-
Lysosomal alpha-mannosidase precursor (Mannosidase, alpha B)	O09159	114,604	3	5	11	6
Malate dehydrogenase 2, NAD (mitochondrial)	P08249	35,611	11	9	8	5
Malate dehydrogenase, cytoplasmic	P14152	36,511	7	3	6	5
Malic enzyme 1, supernatant	P06801	63,999	-	-	2	2
Mannosidase alpha, class 1A, member 1	Q544T7	73,276	2	2	10	5
Myosin, heavy polypeptide 9, non-muscle isoform 1	Q8VDD5	226,357	-	-	3	-
Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	Q60605	16,930	-	3	5	2
Nestin	Q6P5H2	202,011	-	7	4	-
Nit protein 2	Q9JHW2	30,502	-	-	2	-
Nucleoside-diphosphate kinase 1	P15532	17,208	3	-	4	4
Nucleoside-diphosphate kinase 2	Q01768	17,363	2	4	2	2
Ornithine aminotransferase	P29758	48,355	-	-	3	3
Peroxiredoxin 1	A2AP16	22,176	4	6	7	6
Peroxiredoxin 2 (Thioredoxin peroxidase 1)	Q61171	21,779	3	5	5	4
Peroxiredoxin 5 precursor	P99029	21,897	9	12	8	8
Peroxiredoxin 6 (Acidic calcium-independent phospholipase A2)	O08709	24,871	-	2	4	7
Phosphogluconate dehydrogenase	Q91V28	53,261	4	4	5	2
Phosphoglycerate kinase 1	P09411	44,536	9	11	8	8
Phosphoglycerate mutase 1 (brain)	Q9DBJ1	28,832	3	3	7	5
Phosphoserine aminotransferase 1	Q99K85	40,473	7	-	2	-
Plectin 1	Q9QXS1	534,216	-	-	6	-
Proteasome activator PA28 alpha subunit	P97371	28,673	-	-	3	4
Proteasome subunit, alpha type 1	Q9R1P4	29,547	3	2	3	-
Proteasome subunit, alpha type 2	P49722	25,926	-	2	3	-
Proteasome subunit, alpha type 3	Q9DCD8	28,490	4	2	2	-
Proteasome subunit, alpha type 5	Q3UPK6	26,411	3	2	4	-
Proteasome subunit, alpha type 6	Q9QUM9	27,372	2	3	3	3
Proteasome subunit, alpha type 7	Q9Z2U0	27,855	6	4	5	4
Proteasome subunit, beta type 1	O09061	26,372	4	-	4	-
Proteasome subunit, beta type 3	Q545G0	22,965	2	3	4	-
Proteasome subunit, beta type 5	Q3UZI1	28,532	3	7	4	-
Proteasome subunit, beta type 8	P28063	30,260	2	3	3	-
Purine-nucleoside phosphorylase	P23492	32,277	4	-	5	4

Pyruvate kinase isozyme M2	P52480	57,887	3	5	12	7
Rho GDP dissociation inhibitor (GDI) alpha	Q99PT1	23,407	3	2	5	3
Ribonuclease/angiogenesis inhibitor	Q91VI7	49,816	5	-	3	3
S-adenosylhomocysteine hydrolase	Q5M9P0	47,674	2	-	2	-
SH3-binding domain glutamic acid-rich protein like	Q9JJU8	12,811	2	3	4	4
Soluble calcium-activated nucleotidase 1	Q8VCF1	45,653	2	2	4	3
Spectrin alpha 2	P16546	284,597	-	3	14	-
Spectrin beta 2 isoform 1 or 2	Q62261	274,223	-	2	5	-
S-phase kinase-associated protein 1A	Q9WTX5	18,672	-	3	3	2
Superoxide dismutase 1, soluble	P08228	15,943	4	4	4	5
Thioredoxin reductase 1	Q8CI31	54,337	3	3	2	-
Transaldolase 1	Q93092	37,387	5	-	3	-
Transgelin	P37804	22,576	3	5	7	5
Transgelin 2	Q91VU2	22,395	2	4	6	6
Transitional endoplasmic reticulum ATPase (valosin-containing protein)	Q01853	89,364	14	23	14	4
Transketolase	P40142	67,630	-	-	3	-
Translin	Q62348	26,201	2	-	4	2
Triosephosphate isomerase 1	P17751	26,713	6	4	4	2
Tropomyosin 1, alpha	P58771	32,681	4	6	4	3
Tropomyosin 4, alpha	Q6IRU2	28,468	5	3	6	2
Tubulin, alpha	P05213	50,152	-	4	5	2
Tubulin, beta	Q9ERD7	50,419	-	5	5	-
Ubiquitin carboxy-terminal hydrolase L1	Q9R0P9	24,838	2	3	3	2
Ubiquitin-activating enzyme E1, Chr X	Q02053	117,809	-	4	3	2
UDP-glucose pyrophosphorylase 2	Q91ZJ5	56,979	3	3	3	-
Villin 2	P26040	69,407	-	-	5	4
Vinculin	Q64727	116,717	11	17	19	6
WD repeat domain 1	Q3TJY2	66,407	4	4	3	-

Proteins are reported with their corresponding accession number, molecular weight (MW), and unique peptides identified for each treatment condition.

[A]Accession numbers are reported from the Uniprot database (www.uniprot.org) and, when available, refer to the unprocessed precursor protein.

[B]The average number of unique peptides identified after applying the selection criteria detailed in Experimental Procedures. A null value indicates the protein did not meet the minimum criteria for identification.

Table 2. Protein Prowler N-terminal signal peptide prediction

Protein Name (Synonym)	Accession[a]	SP[b]	MTP[c]	Other[d]
Acid ceramidase precursor (Acylsphingosine deacylase)	Q9WV54	0.99	0	0.01
Aminopeptidase (Plasma glutamate carboxypeptidase)	Q9WVJ3	0.99	0	0.01
Carboxypeptidase E precursor	Q00493	0.99	0	0.01
Chemokine (C-X-C motif) ligand 1	P12850	0.99	0	0.01
Collagen alpha-1(XII) chain precursor	Q60847	0.99	0	0.01
Extracellular matrix protein	Q9QX30	0.99	0	0.01
Growth-arrest-specific protein 6 precursor (GAS-6)	Q61362	0.99	0	0.01
Lysosomal-associated membrane glycoprotein 1 (LAMP1)	P11438	0.99	0	0.01
Metalloproteinase inhibitor 2 precursor (TIMP-2)	P25785	0.99	0	0.01
Basement membrane-specific heparan sulfate proteoglycan core protein	Q05793	0.99	0	0.01
SPARC precursor	Q5NBV5	0.99	0	0.01
Plasma protease C1 inhibitor	P97290	0.99	0	0.01
4632419I22Rik protein	Q6GU68	0.98	0	0.02
Alpha-N-acetylglucosaminidase	O88325	0.98	0	0.02
Amyloid beta A4 protein precursor (APP)	P12023	0.98	0	0.02
Astrocytic phosphoprotein PEA-15	Q62000	0.98	0	0.02
Beta-2-microglobulin precursor	P01887	0.98	0	0.02
Beta-glucuronidase precursor	P12265	0.98	0	0.02
Bone morphogenetic protein 1	P09581	0.98	0	0.02
Cadherin-2 precursor (Neural-cadherin)	P15116	0.98	0	0.01
Calreticulin precursor	P14211	0.98	0	0.02
Cathepsin B precursor	P10605	0.98	0	0.02
Cathepsin L precursor	P06797	0.98	0	0.02
CD109 antigen homolog precursor	Q8R422	0.98	0.01	0.02
Chemokine (C-C motif) ligand 7	Q03366	0.98	0	0.02
Chemokine (C-C motif) ligand 8	Q9Z121	0.98	0	0.02
Chemokine (C-X3-C motif) ligand 1	O35188	0.98	0	0.02
Clusterin precursor (Apolipoprotein J)	Q06890	0.98	0	0.02
Collagen alpha-1(I) chain precursor	P11087	0.98	0	0.02
Collagen alpha-1(IV) chain precursor	P02463	0.98	0	0.02
Collagen alpha-1(VI) chain precursor	Q04857	0.98	0	0.02
Collagen alpha-2(I) chain precursor	Q01149	0.98	0	0.02
Macrophage colony-stimulating factor 1 receptor	Q6NXM5	0.98	0	0.02
Complement C1q tumor necrosis factor-related protein 5 precursor	Q8K479	0.98	0	0.02
Complement C1s-A subcomponent	Q8CG14	0.98	0	0.02
Complement factor B precursor	P04186	0.98	0	0.02
Cyclophilin C-associated protein	O35649	0.98	0	0.02
Cystatin-C precursor (Cystatin-3)	P21460	0.98	0	0.02
Dipeptidyl-peptidase 1 precursor (Cathepsin C)	P97821	0.98	0	0.02
Epididymal secretory protein E1 precursor	Q9Z0J0	0.98	0	0.02
Epididymis-specific alpha-mannosidase precursor	O54782	0.98	0	0.02
Fibromodulin precursor (FM)	P50608	0.98	0	0.02
Fibulin-5 precursor (FIBL-5)	Q9WVH9	0.98	0	0.02
Ganglioside GM2 activator precursor	Q60648	0.98	0	0.02
Glypican-4 precursor (K-glypican)	P51655	0.98	0	0.02
H-2 class I histocompatibility antigen, D-B alpha chain precursor	P01899	0.98	0	0.02
H-2 class I histocompatibility antigen, Q8 alpha chain	P14430	0.98	0	0.02

Insulin-like growth factor-binding protein 5	Q07079	0.98	0	0.02
Inter-alpha-trypsin inhibitor heavy chain H3 precursor	Q61704	0.98	0	0.02
Legumain	A2RTI3	0.98	0	0.02
Lipopolysaccharide binding protein	A2AC66	0.98	0	0.02
Low-density lipoprotein receptor-related protein 1 precursor (A2MR)	Q91ZX7	0.98	0	0.02
Lysosomal protective protein precursor	P16675	0.98	0	0.02
Lysozyme C type M precursor	P08905	0.98	0	0.02
Cell adhesion molecule 4 precursor	Q8R464	0.98	0	0.02
mouse fat 1 cadherin	Q9QXA3	0.98	0	0.02
Neutrophil gelatinase-associated lipocalin precursor (Lipocalin 2)	P11672	0.98	0	0.02
Phospholipid transfer protein precursor (Lipid transfer protein II)	P55065	0.98	0	0.02
Platelet-derived growth factor receptor-like protein precursor	Q61147	0.98	0	0.02
Procollagen, type XI, alpha 1	P22777	0.98	0	0.02
Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (Lysyl hydroxylase 1)	Q9R0E2	0.98	0	0.02
protein disulfide isomerase associated 6	Q922R8	0.98	0	0.02
Retinoic acid receptor responder protein 2 precursor	Q9DD06	0.98	0	0.02
Serotransferrin precursor (Transferrin)	Q921I1	0.98	0	0.02
Sulfated glycoprotein 1 precursor (Prosaposin)	Q61207	0.98	0	0.02
Transcobalamin-2 precursor (Transcobalamin II)	O88968	0.98	0	0.02
Acid sphingomyelinase-like phosphodiesterase 3a precursor	P70158	0.97	0	0.03
Apolipoprotein E precursor (Apo-E)	P08226	0.97	0	0.03
Cathepsin D precursor	P18242	0.97	0	0.03
Collagen alpha-2(V) chain precursor	Q3U962	0.97	0	0.03
Complement C1q subcomponent subunit C precursor	Q640N1	0.97	0	0.03
Complement C1r-A subcomponent	Q8CG16	0.97	0	0.03
Fibronectin 1	Q3UGY5	0.97	0.01	0.03
Glia-derived nexin precursor (Serpine 2)	Q07235	0.97	0	0.03
Igfbp3 protein (Insulin-like growth factor binding protein 3)	Q6PE55	0.97	0	0.03
Insulin-like growth factor binding protein 7	Q61581	0.97	0	0.03
Lysyl oxidase-like 3	Q91VN8	0.97	0	0.03
Mini-agrin	Q5EBX5	0.97	0	0.03
Monocyte differentiation antigen CD14 precursor	P10810	0.97	0	0.03
Nidogen 2 protein	Q8R5G0	0.97	0	0.03
Nucleobindin-1 precursor (CALNUC)	Q02819	0.97	0	0.03
Pentraxin-related protein PTX3 precursor	P48759	0.97	0	0.03
Platelet-activating factor acetylhydrolase	Q60963	0.97	0	0.02
protein disulfide isomerase associated 4	P08003	0.97	0	0.03
Renin receptor precursor	Q9CYN9	0.97	0	0.03
Ribonuclease T2 isoform 1	Q9CQ01	0.97	0	0.03
Secretogranin 3	Q8R1D7	0.97	0	0.03
Tenascin precursor (TN-C)	Q80YX1	0.97	0	0.03
Complement C1q subcomponent subunit B precursor	P14106	0.96	0	0.04
Complement C3 precursor	P01027	0.96	0	0.03
Immunoglobulin superfamily containing leucine-rich repeat	Q62356	0.96	0	0.04
Laminin subunit alpha-4 precursor	P97927	0.96	0	0.04
Laminin subunit gamma-1 precursor (Laminin B2 chain)	P02468	0.96	0.01	0.04
Mammalian ependymin related protein-2	Q99M71	0.96	0	0.04
Peptidyl-prolyl cis-trans isomerase B	P24369	0.96	0	0.04
Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3 (Lysyl hydroxylase 3)	Q9R0E1	0.96	0	0.04

Serine protease inhibitor A3N precursor (Serpin A3N)	Q91WP6	0.96	0	0.04
Superoxide dismutase, extracellular	O09164	0.96	0	0.04
Tenascin-N precursor (TN-N)	Q80Z71	0.96	0	0.04
Alpha-2-macroglobulin-P precursor (Alpha-2-macroglobulin)	Q02105	0.95	0	0.04
Beta-1,3-N-acetylglucosaminyltransferase lunatic fringe	O09010	0.95	0	0.05
Biotinidase precursor	Q8CIF4	0.95	0	0.05
Cathepsin S precursor	O70370	0.95	0.01	0.05
EGF-containing fibulin-like extracellular matrix protein 2	Q9JM06	0.95	0	0.05
Insulin-like growth factor binding protein 2	P47877	0.95	0	0.04
Calsyntenin 1	A2A800	0.94	0.02	0.04
Inter-alpha-trypsin inhibitor heavy chain H5	Q8BJD1	0.94	0	0.06
Ceruloplasmin	Q6NZM2	0.93	0	0.07
Complement C4-B precursor	P01029	0.93	0.01	0.06
Sulfhydryl oxidase 1 precursor (Quiescin Q6)	Q8BND5	0.93	0.01	0.06
Neurocan core protein precursor (Chondroitin sulfate proteoglycan 3)	P55066	0.92	0	0.08
Palmitoyl-protein thioesterase 1 precursor (PPT-1)	O88531	0.92	0.02	0.06
N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase	Q64191	0.91	0.01	0.08
Lysosomal alpha-glucosidase precursor (Acid maltase)	P70699	0.91	0	0.09
Chitinase-3-like protein 1 precursor (Cartilage glycoprotein 39)	Q61292	0.9	0	0.1
Cathepsin Z	Q9ES94	0.89	0.01	0.1
Macrophage colony-stimulating factor 1 precursor (CSF-1)	P07141	0.89	0.02	0.09
Thrombospondin 1	Q8CGB2	0.87	0	0.13
Vacuolar ATP synthase subunit S1 precursor	Q9R1Q9	0.87	0.02	0.11
Laminin subunit beta-2 precursor (S-laminin)	Q61245	0.86	0	0.13
Pigment epithelium-derived factor (PEDF)	P97298	0.84	0.02	0.14
Galactocerebrosidase precursor (GALCERase)	P54818	0.83	0.03	0.14
Vitamin K-dependent protein S precursor	Q08761	0.83	0.02	0.15
Endoplasmin precursor (GRP94)	P08113	0.82	0.03	0.15
Mimecan precursor (Osteoglycin)	Q61592	0.81	0.03	0.16
Follistatin-related protein 1 precursor	Q6GQT1	0.8	0.02	0.17
Putative phospholipase B-like 2 precursor	Q3TCN2	0.8	0.02	0.18
Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (Lysyl hydroxylase 2)	Q9R0B9	0.8	0.01	0.2
Beta-hexosaminidase beta chain precursor (Hexosaminidase B)	P20060	0.77	0.04	0.19
N-acetyllactosaminide beta-1,3-N-acetylglucosaminyltransferase	Q8BWP8	0.77	0.02	0.22
Tripeptidyl-peptidase 1 precursor	O89023	0.77	0.01	0.22
Protein disulfide-isomerase A3 precursor	P27773	0.73	0.03	0.25
Plasminogen activator inhibitor 1	P47878	0.72	0.11	0.17
Gamma-glutamyl hydrolase	Q9Z0L8	0.69	0.06	0.25
Endoplasmic reticulum protein ERp29 precursor	P57759	0.67	0.06	0.28
45 kDa calcium-binding protein precursor (SDF-4)	Q61112	0.66	0.06	0.28
N-acetylglucosamine-6-sulfatase precursor (G6S)	Q8BFR4	0.66	0.06	0.28
Protein disulfide-isomerase precursor (PDI)	P09103	0.66	0.05	0.29
Ptprz1 protein (DSD-1 Proteoglycan)	B2RXS8	0.65	0.06	0.29
Intercellular adhesion molecule	Q922B3	0.65	0.06	0.29
Stromelysin-1 precursor (MMP-3)	P28862	0.63	0.05	0.32
Agrin	A2ASQ0	0.61	0.04	0.35
Exostosin-2	P70428	0.54	0.05	0.41
SPARC-like protein 1 precursor (Matrix glycoprotein Sc1)	P70663	0.54	0.1	0.36
78 kDa glucose-regulated protein precursor	P20029	0.53	0.08	0.39

Beta-hexosaminidase alpha chain precursor (Hexosaminidase A)	P29416	0.53	0.08	0.39
Dipeptidyl-peptidase 2 precursor	Q9ET22	0.52	0.1	0.38
Sortilin-related receptor, LDLR class A repeats-containing	O88307	0.49	0.27	0.24
Bifunctional heparan sulfate N-deacetylase/N-sulfotransferase 1; DAST-1	Q3UHN9	0.48	0.09	0.42
Lysosomal alpha-mannosidase precursor (Mannosidase, alpha B)	O09159	0.44	0.05	0.51
Laminin subunit alpha-5 precursor	Q61001	0.42	0.03	0.55
Vesicular integral-membrane protein VIP36 precursor	Q9DBH5	0.42	0.07	0.51
Adipocyte enhancer-binding protein	Q62165	0.41	0.11	0.48
Meteorin, glial cell differentiation regulator-like	Q8VE43	0.38	0.12	0.5
Plexin domain-containing protein 2 precursor	Q9DC11	0.31	0.07	0.62
Phosphogluconate dehydrogenase	Q91V28	0.29	0.01	0.7
Vascular cell adhesion protein 1 precursor (V-CAM 1)	P29533	0.28	0.13	0.58
Gelsolin precursor (Actin-depolymerizing factor)	P13020	0.27	0.14	0.59
Mannosidase alpha, class 1A, member 1	Q544T7	0.25	0.13	0.62
Glutathione S-transferase, alpha 4	P24472	0.21	0.11	0.68
Galectin-3 (Galactose-specific lectin 3)	P16110	0.19	0.09	0.72
Ferritin light chain 1	P29391	0.17	0.08	0.76
Intermediate filament protein nestin	Q6P5H2	0.16	0.08	0.76
Ca2+-dependent endoplasmic reticulum nucleoside diphosphatase	Q8VCF1	0.15	0.08	0.76
Heat shock protein 90kDa alpha (cytosolic), class B member 1	Q71LX8	0.14	0.09	0.76
Ubiquitin carboxy-terminal hydrolase L1	Q9R0P9	0.14	0.05	0.81
Isocitrate dehydrogenase [NADP] cytoplasmic	O88844	0.11	0.08	0.8
Heat shock protein 4	Q5NCS5	0.11	0.02	0.86
Aminopeptidase puromycin sensitive	Q5PR74	0.1	0.58	0.32
Inositol monophosphatase	O55023	0.1	0.07	0.83
Nit protein 2	Q9JHW2	0.1	0.1	0.8
Vimentin	P20152	0.09	0.38	0.53
Malate dehydrogenase, cytoplasmic	P14152	0.08	0.01	0.91
S-adenosylhomocysteine hydrolase	Q5M9P0	0.07	0.05	0.88
Hypoxanthine guanine phosphoribosyl transferase 1	P00493	0.06	0.05	0.89
Heat shock protein 90kDa alpha (cytosolic), class A member 1	P07901	0.06	0.03	0.91
Superoxide dismutase 1, soluble	P08228	0.06	0.05	0.88
Glutathione S-transferase, mu 5	P48774	0.06	0.06	0.88
Lactate dehydrogenase 1, A chain	P06151	0.04	0.01	0.95
Translin	Q62348	0.04	0.01	0.96
Asparaginase like 1	Q8C0M9	0.03	0.08	0.89
Peroxiredoxin 6 (Acidic calcium-independent phospholipase A2)	O08709	0.02	0.02	0.96
Peptidyl-prolyl cis-trans isomerase A	P17742	0.02	0.02	0.97
Acyl-CoA-binding protein (diazepam-binding inhibitor)	P31786	0.02	0.02	0.96
Phosphatidylethanolamine-binding protein 1 (PEBP-1)	P70296	0.02	0.01	0.96
Profilin 1	P62962	0.02	0.02	0.97
Dystroglycan precursor (Dystrophin-associated glycoprotein 1)	Q62048	0.02	0.02	0.96
Heterogeneous nuclear ribonucleoprotein A2/B1	O88569	0.02	0.02	0.97
Tubulin, alpha	P05213	0.02	0.02	0.97
Annexin A2	P07356	0.02	0.02	0.97
Eukaryotic initiation factor 4AII	P10630	0.02	0.03	0.96
Lactate dehydrogenase 2, B chain	P16125	0.02	0.01	0.96
Spectrin alpha 2	P16546	0.02	0.02	0.96
Fatty acid synthase	P19096	0.02	0.02	0.96

Purine-nucleoside phosphorylase	P23492	0.02	0.02	0.96
Proteasome subunit, beta type 8	P28063	0.02	0.01	0.97
Guanosine diphosphate (GDP) dissociation inhibitor 1	P50396	0.02	0.01	0.97
Actinin, alpha 4	P57780	0.02	0.03	0.96
14-3-3 protein epsilon	P62259	0.02	0.02	0.96
Heat shock 70kDa protein 8 isoform 1	P63017	0.02	0.02	0.96
14-3-3 protein zeta	P63101	0.02	0.02	0.96
14-3-3 protein theta	P68254	0.02	0.02	0.96
Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	Q60605	0.02	0.02	0.96
Rab GDP dissociation inhibitor beta	Q61598	0.02	0.01	0.97
Vinculin	Q64727	0.02	0.02	0.97
Actinin, alpha 1	Q7TPR4	0.02	0.02	0.96
Filamin-B	Q80X90	0.02	0.02	0.96
Thioredoxin reductase 1	Q8CI31	0.02	0.02	0.96
Aldehyde dehydrogenase 1 family, member L1	Q8R0Y6	0.02	0.01	0.97
Ribonuclease/angiogenesis inhibitor	Q91VI7	0.02	0.02	0.97
Rho GDP dissociation inhibitor (GDI) alpha	Q99PT1	0.02	0.02	0.96
Lactoylglutathione lyase	Q9CPU0	0.02	0.02	0.96
Actin related protein 2/3 complex, subunit 2	Q9CVB6	0.02	0.01	0.97
Tubulin, beta	Q9ERD7	0.02	0.02	0.96
S-phase kinase-associated protein 1A	Q9WTX5	0.02	0.02	0.96
Macrophage migration inhibitory factor (MIF)	P34884	0.01	0.02	0.97
Peroxiredoxin 1	A2AP16	0.01	0.03	0.96
Dihydropyrimidinase-like 2	O08553	0.01	0.03	0.96
Proteasome subunit, beta type 1	O09061	0.01	0.03	0.96
Annexin A3	O35639	0.01	0.03	0.96
Glial fibrillary acidic protein	P03995	0.01	0.39	0.6
Phosphoglycerate kinase 1	P09411	0.01	0.03	0.97
Ferritin heavy chain 1	P09528	0.01	0.03	0.96
Nucleoside-diphosphate kinase 1	P15532	0.01	0.03	0.96
Triosephosphate isomerase 1	P17751	0.01	0.03	0.96
Cofilin 1, non-muscle	P18760	0.01	0.02	0.97
Peroxiredoxin 3	P20108	0.01	0.94	0.05
Villin 2	P26040	0.01	0.02	0.97
Transgelin	P37804	0.01	0.02	0.96
Transketolase	P40142	0.01	0.03	0.96
Glutathione S-transferase, pi 2	P46425	0.01	0.02	0.97
Annexin A5	P48036	0.01	0.03	0.96
Fatty acid binding protein 7, brain	P51880	0.01	0.02	0.97
Pyruvate kinase isozyme M2	P52480	0.01	0.02	0.96
Eukaryotic translation elongation factor 2	P58252	0.01	0.02	0.97
Tropomyosin 1, alpha	P58771	0.01	0.02	0.96
14-3-3 protein gamma	P61982	0.01	0.02	0.96
Cytochrome c	P62897	0.01	0.03	0.96
Eukaryotic translation initiation factor 5A	P63242	0.01	0.03	0.96
14-3-3 protein eta	P68510	0.01	0.02	0.97
Proteasome activator PA28 alpha subunit	P97371	0.01	0.03	0.96
Nucleoside-diphosphate kinase 2	Q01768	0.01	0.03	0.97
Creatine kinase, brain	Q04447	0.01	0.03	0.96

WD repeat domain 1	Q3TJY2	0.01	0.02	0.96
Proteasome subunit, alpha type 5	Q3UPK6	0.01	0.03	0.96
Proteasome subunit, beta type 5	Q3UZI1	0.01	0.03	0.96
Aldolase 1, isoform A	Q5FWB7	0.01	0.02	0.96
Aldolase 3, isoform C	Q5SYM1	0.01	0.02	0.96
Peroxiredoxin 2 (Thioredoxin peroxidase 1)	Q61171	0.01	0.02	0.96
Spectrin beta 2 isoform 1 or 2	Q62261	0.01	0.02	0.96
Clathrin, heavy polypeptide (Hc)	Q68FD5	0.01	0.02	0.97
Tropomyosin 4, alpha	Q6IRU2	0.01	0.02	0.97
Brain glycogen phosphorylase	Q8CI94	0.01	0.03	0.97
Myosin, heavy polypeptide 9, non-muscle isoform 1	Q8VDD5	0.01	0.02	0.96
Filamin-C	Q8VHX6	0.01	0.02	0.97
Transgelin 2	Q91VU2	0.01	0.03	0.96
UDP-glucose pyrophosphorylase 2	Q91ZJ5	0.01	0.03	0.96
Transaldolase 1	Q93092	0.01	0.03	0.97
Phosphoserine aminotransferase 1	Q99K85	0.01	0.02	0.97
Gelsolin-like capping protein	Q99LB4	0.01	0.02	0.96
DJ-1 protein	Q99LX0	0.01	0.02	0.97
14-3-3 protein beta	Q9CQV8	0.01	0.02	0.96
Calponin 3, acidic	Q9DAW9	0.01	0.03	0.96
Phosphoglycerate mutase 1 (brain)	Q9DBJ1	0.01	0.02	0.97
Proteasome subunit, alpha type 3	Q9DCD8	0.01	0.02	0.96
Aldo-keto reductase family 1, member A4	Q9JII6	0.01	0.03	0.96
Plectin 1	Q9QXS1	0.01	0.03	0.96
Proteasome subunit, alpha type 1	Q9R1P4	0.01	0.03	0.96
Proteasome subunit, alpha type 7	Q9Z2U0	0.01	0.02	0.96
Valosin containing protein	Q01853	0.01	0.02	0.96
Glucose-6-phosphate isomerase (GPI)	P06745	0	0.14	0.86
Glutathione S-transferase, mu 1	P10649	0	0.02	0.97
Aspartate aminotransferase	P05202	0	0.95	0.04
Malic enzyme 1, supernatant	P06801	0	0.03	0.97
Malate dehydrogenase 2, NAD (mitochondrial)	P08249	0	0.99	0.01
Glyceraldehyde-3-phosphate dehydrogenase	P16858	0	0.05	0.94
Enolase 1, alpha non-neuron	P17182	0	0.03	0.96
Ornithine aminotransferase	P29758	0	0.98	0.02
Lamin A isoform A	P48678	0	0.04	0.96
Proteasome subunit, alpha type 2	P49722	0	0.04	0.95
H4 histone family, member A	P62806	0	0.03	0.97
Peroxiredoxin 5 precursor	P99029	0	0.96	0.03
Ubiquitin-activating enzyme E1, Chr X	Q02053	0	0.03	0.97
Leukotriene A4 hydrolase	Q3UY71	0	0.08	0.92
Proteasome subunit, beta type 3	Q545G0	0	0.02	0.97
Filamin-A	Q8BTM8	0	0.05	0.95
Glyoxalase domain containing 4	Q9CPV4	0	0.98	0.01
Dimethylarginine dimethylaminohydrolase 1	Q9CWS0	0	0.75	0.24
SH3-binding domain glutamic acid-rich protein like	Q9JJU8	0	0.07	0.93
Proteasome subunit, alpha type 6	Q9QUM9	0	0.04	0.96

Proteins are reported in order of decreasing SP (secretory pathway) score calculated by Protein Prowler version 1.2 (pprowler.imb.uq.edu.au/index.jsp)

a) Accession numbers are reported from the Uniprot database (www.uniprot.org) and, when available, refer to the unprocessed precursor protein.
b) Secretory pathway targeting score (signal peptide)
c) Mitochondrion targeting score (mitochondrial targeting peptide)
d) Other targeting score (nucleus, cytoplasm, other)

Table 3. Redundant peptides from proteins in the astrocyte secretome.

Protein Name (Synonym)	Accession[a]	MW (kDa)	1D Control	1D Cytokine	7D Control	7D Cytokine
			Redundant Peptides[b]			
Extracellular Matrix and Adhesion						
Agrin	A2ASQ0	205.0	8	46	10	60
Cadherin-2 precursor (Neural-cadherin)	P15116	99.8	19	18	43	35
Calsyntenin 1	A2A800	108.9	18	9	29	15
Collagen alpha-1(I) chain precursor	P11087	138.0	3	7	26	11
Collagen alpha-1(IV) chain precursor	P02463	160.7	-	-	2	4
*Collagen alpha-1(VI) chain precursor	Q04857	108.5	-	2	-	5
^Collagen alpha-1(XI) chain precursor	Q61245	181.0	-	-	5	-
Collagen alpha-1(XII) chain precursor	Q60847	340.2	4	21	85	52
Collagen alpha-2(I) chain precursor	Q01149	129.6	-	2	10	8
Collagen alpha-2(V) chain precursor	Q3U962	145.0	4	15	24	11
EGF-containing fibulin-like extracellular matrix protein 2	Q9JM06	49.5	4	8	29	8
Extracellular matrix protein	Q9QX30	62.8	-	-	15	8
Fat 1 cadherin	Q9QXA3	506.0	-	-	13	7
Fibromodulin precursor (FM)	P50608	43.1	6	9	21	10
Fibronectin 1	Q3UGY5	262.8	146	238	375	355
Fibulin-5 precursor (FIBL-5)	Q9WVH9	50.2	7	2	25	3
Galectin-3 (Galactose-specific lectin 3)	P16110	27.5	-	2	6	3
Glypican-4 precursor (K-glypican)	P51655	62.6	-	3	11	10
*Intercellular adhesion molecule (Icam1)	Q922B3	58.9	-	-	-	5
*Laminin subunit alpha-4 precursor	P97927	201.8	-	-	-	11
Laminin subunit alpha-5 precursor	Q61001	404.0	-	12	2	46
*Laminin subunit beta-2 precursor (S-laminin)	Q61292	196.4	-	10	-	34
Laminin subunit gamma-1 precursor (Laminin B2 chain)	P02468	177.3	-	20	5	46
Legumain	A2RTI3	49.4	6	18	25	15
Lysyl oxidase-like 3	Q91VN8	83.7	-	-	4	2
Mammalian ependymin related protein-2	Q99M71	25.5	14	21	20	24
Mimecan precursor (Osteoglycin)	Q62000	34.0	-	4	26	6
Mini-agrin	Q5EBX5	103.4	2	4	2	5
Neurocan core protein precursor (Chondroitin sulfate proteoglycan 3)	P55066	137.2	24	27	31	14
Nidogen 2 protein	Q8R5G0	153.9	4	-	13	3
Basement membrane-specific heparan sulfate proteoglycan core protein	Q05793	469.0	3	34	32	117
SPARC-like protein 1 precursor (Matrix glycoprotein Sc1)	P70663	72.3	-	5	13	11
Tenascin precursor (TN-C)	Q80YX1	231.8	8	28	11	73
Tenascin-N precursor (TN-N)	Q80Z71	173.1	-	-	5	29
Thrombospondin 1	Q8CGB2	129.7	-	2	9	21
Vascular cell adhesion protein 1 precursor (CD106 antigen)	P29533	81.3	9	5	34	66
Vitamin K-dependent protein S precursor	Q08761	74.9	-	-	14	10
Protein Processing and Proteolysis						
78 kDa glucose-regulated protein precursor	P20029	72.4	12	14	15	8
Alpha-2-macroglobulin-P precursor (Alpha-2-macroglobulin)	Q6GQT1	164.3	116	204	301	189
Aminopeptidase (Plasma glutamate carboxypeptidase)	Q9WVJ3	51.8	9	11	19	9
Aminopeptidase puromycin sensitive	Q5PR74	103.3	2	9	4	-
Bone morphogenetic protein 1	Q6NZM2	111.7	2	4	5	4
Calreticulin precursor	P14211	48.0	5	3	4	-

Carboxypeptidase E precursor	Q00493	53.3	27	41	90	71
Cathepsin B precursor	P10605	37.3	47	71	131	136
Cathepsin D precursor	P18242	45.0	21	28	79	69
Cathepsin L precursor	P06797	37.5	17	9	28	39
Cathepsin S precursor	O70370	38.4	-	-	26	15
Cathepsin Z precursor	Q9WUU7	34.0	5	6	13	15
Cystatin-C precursor (Cystatin-3)	P21460	15.5	77	109	232	287
Dipeptidyl-peptidase 1 precursor (Cathepsin C)	P97821	52.4	-	4	2	5
Dipeptidyl-peptidase 2 precursor	Q9ET22	56.3	3	6	-	3
^Endoplasmic reticulum protein ERp29 precursor	P57759	28.8	-	-	3	-
Endoplasmin precursor (GRP94)	P08113	92.5	4	3	9	2
Glia-derived nexin precursor (Serpine 2)	Q07235	44.2	-	15	14	29
^Inter-alpha-trypsin inhibitor heavy chain H3	Q61704	99.0	-	-	11	-
Inter-alpha-trypsin inhibitor heavy chain H5	Q8BJD1	106.7	-	-	3	11
Lysosomal protective protein precursor	P16675	53.8	-	8	14	16
Metalloproteinase inhibitor 2 precursor (TIMP-2)	P25785	24.3	9	23	22	19
Peptidyl-prolyl cis-trans isomerase A	P17742	18.0	9	6	15	11
Peptidyl-prolyl cis-trans isomerase B	P24369	22.7	9	14	11	10
Protein disulfide-isomerase A3 precursor	P27773	56.7	14	24	22	9
Protein disulfide-isomerase A4 precursor	P08003	72.0	-	3	10	2
^Protein disulfide-isomerase A6 precursor	Q922R8	48.1	-	-	4	-
Protein disulfide-isomerase precursor (PDI)	P09103	57.1	4	6	7	-
Pigment epithelium-derived factor (PEDF)	P97298	46.2	57	59	61	57
Plasma protease C1 inhibitor	P97290	55.6	7	3	40	73
Plasminogen activator inhibitor 1	P22777	45.0	-	12	5	17
Serine protease inhibitor A3N precursor (Serpin A3N)	Q91WP6	46.7	12	30	43	51
*Stromelysin-1 precursor (MMP-3)	P28862	53.8	-	3	-	14
Sulfhydryl oxidase 1 precursor (Quiescin Q6)	Q8BND5	82.8	-	-	23	7
Tripeptidyl-peptidase 1 precursor	O89023	61.3	-	-	4	7
Metabolism						
Acid ceramidase precursor (Acylsphingosine deacylase)	Q9WV54	44.7	-	-	8	15
Alpha-N-acetylglucosaminidase	O88325	82.6	-	-	4	5
Aspartate aminotransferase	P05202	47.4	6	5	5	2
^Beta-1,3-N-acetylglucosaminyltransferase lunatic fringe	O09010	42.0	-	-	7	-
^Beta-glucuronidase precursor	P12265	74.2	-	-	6	-
Beta-hexosaminidase alpha chain precursor (Hexosaminidase A)	P29416	60.6	-	-	6	3
Beta-hexosaminidase beta chain precursor (Hexosaminidase B)	P20060	61.1	8	3	27	6
^Bifunctional heparan sulfate N-deacetylase/N-sulfotransferase 1	Q3UHN9	100.7	-	-	3	-
Chitinase-3-like protein 1 precursor (Cartilage glycoprotein 39)	Q61362	43.0	9	75	42	130
Epididymis-specific alpha-mannosidase precursor	O54782	115.6	2	2	16	9
Exostosin-2	P70428	82.1	-	-	5	2
Galactocerebrosidase precursor (GALCERase)	P54818	75.5	-	-	6	3
Gamma-glutamyl hydrolase	Q9Z0L8	35.4	2	2	8	5
Ganglioside GM2 activator precursor	Q60648	20.8	3	9	12	10
Glucose-6-phosphate isomerase (GPI)	P06745	62.8	18	15	15	6
*Lysosomal alpha-glucosidase precursor (Acid maltase)	P70699	106.2	-	-	-	11
N-acetylglucosamine-6-sulfatase precursor (G6S)	Q8BFR4	61.2	-	7	7	6
^N-acetyllactosaminide beta-1,3-N-acetylglucosaminyltransferase	Q8BWP8	47.4	-	-	7	-
N(4)-(beta-N-acetylglucosaminyl)-L-asparaginase	Q64191	37.0	-	8	5	10

Palmitoyl-protein thioesterase 1 precursor (PPT-1)	O88531	34.5	-	-	11	14
Platelet-activating factor acetylhydrolase	Q60963	49.2	-	2	29	3
Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (Lysyl hydroxylase 1)	Q9R0E2	83.6	4	6	24	10
Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 2 (Lysyl hydroxylase 2)	Q9R0B9	84.5	-	5	8	4
Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 3 (Lysyl hydroxylase 3)	Q9R0E1	84.9	-	-	15	11
Putative phospholipase B-like 2 precursor	Q3TCN2	66.7	-	3	10	2
Ribonuclease T2 isoform 1	Q9CQ01	29.6	2	-	6	9
Immune						
Beta-2-microglobulin precursor	P01887	13.8	5	24	24	56
^Cell adhesion molecule 4 precursor	Q8R464	42.7	-	-	6	-
*Chemokine (C-C motif) ligand 7	Q03366	11.0	-	-	-	11
*Chemokine (C-C motif) ligand 8	Q9Z121	11.0	-	-	-	3
*Chemokine (C-X3-C motif) ligand 1 (Fractalkine)	O35188	42.1	-	-	-	9
*Chemokine (C-X-C motif) ligand 1 (Growth-regulated alpha protein)	P12850	10.3	-	6	-	15
Complement C1q subcomponent subunit B precursor	P14106	26.7	-	-	4	5
Complement C1q tumor necrosis factor-related protein 5 precursor	Q8K479	25.4	4	8	7	11
Complement C3 precursor	P01027	186.5	95	575	484	2036
Complement C4-B precursor	P01029	192.9	18	14	156	112
Complement C1q subcomponent subunit C precursor	Q02105	26.0	-	-	7	5
Complement C1r-A subcomponent	Q8CG16	80.1	-	10	23	67
Complement C1s-A subcomponent	Q8CG14	77.4	-	-	18	132
Complement factor B precursor	P04186	85.0	-	-	5	41
Cyclophilin C-associated protein	O35649	64.1	5	-	20	43
H-2 class I histocompatibility antigen, D-B alpha chain precursor	P01899	40.8	-	5	6	13
H-2 class I histocompatibility antigen, Q8 alpha chain	P14430	37.5	-	13	6	22
Immunoglobulin superfamily containing leucine-rich repeat	Q6GU68	45.6	2	7	7	2
Lysozyme C type M precursor	P08905	16.7	-	-	45	66
Macrophage colony-stimulating factor 1 precursor (CSF-1)	P07141	60.6	-	41	39	90
^Macrophage colony-stimulating factor 1 receptor	P09581	109.3	-	-	4	-
Macrophage migration inhibitory factor (MIF)	P34884	12.5	10	8	8	7
Monocyte differentiation antigen CD14 precursor	P10810	39.2	-	2	21	10
Pentraxin-related protein PTX3 precursor	P48759	41.8	6	38	3	34
Platelet-derived growth factor receptor-like protein precursor	Q6PE55	41.9	-	4	4	7
Binding and Transport						
45 kDa calcium-binding protein precursor (SDF-4)	Q61112	42.1	2	2	7	6
^Acyl-CoA-binding protein (DBI)	P31786	10.0	-	-	4	-
*Adipocyte enhancer-binding protein	Q640N1	128.4	-	-	-	12
Annexin A2	P07356	38.7	9	2	13	-
Apolipoprotein E precursor (Apo-E)	P08226	35.9	64	7	177	128
^Biotinidase precursor	Q8CIF4	58.6	-	-	4	-
Ceruloplasmin	Q61147	121.2	19	97	64	300
Dystroglycan precursor (Dystrophin-associated glycoprotein 1)	Q62165	96.9	10	7	26	21
Follistatin-related protein 1 precursor	Q62356	34.6	11	29	16	31
Gelsolin precursor (Actin-depolymerizing factor)	P13020	85.9	6	-	18	3
Rab GDP dissociation inhibitor beta	Q61598	50.5	9	11	20	3
Lipopolysaccharide binding protein	A2AC66	53.1	-	10	10	15
*Lysosomal-associated membrane glycoprotein 1 (LAMP1)	P11438	43.9	-	-	-	7
Neutrophil gelatinase-associated lipocalin precursor (Lipocalin 2)	P11672	22.9	-	33	25	283
Nucleobindin-1 precursor (CALNUC)	Q02819	53.4	2	18	8	8

Phosphatidylethanolamine-binding protein 1 (PEBP-1)	P70296	20.8	11	20	17	14
Phospholipid transfer protein precursor (Lipid transfer protein II)	P55065	54.5	4	-	78	31
Renin receptor precursor	Q9CYN9	39.1	-	-	2	6
SPARC precursor	P07214	34.3	126	73	82	61
Serotransferrin precursor (Transferrin)	Q921I1	76.7	-	-	15	3
^Sortilin-related receptor, LDLR class A repeats-containing	O88307	247.1	-	-	3	-
Sulfated glycoprotein 1 precursor (Prosaposin)	Q61207	61.4	2	7	35	48
Superoxide dismutase, extracellular	O09164	27.4	-	-	8	20
Transcobalamin-2 precursor	O88968	47.6	11	11	33	24
Vacuolar ATP synthase subunit S1 precursor	Q9R1Q9	51.0	-	9	9	16
Vesicular integral-membrane protein VIP36 precursor	Q9DBH5	40.4	-	-	4	5
Cell Growth & Maintenance						
Epididymal secretory protein E1 precursor	Q9Z0J0	16.4	5	13	14	21
Growth-arrest-specific protein 6 precursor (GAS-6)	Q61592	74.6	-	-	11	7
Insulin-like growth factor binding protein 2	P47877	32.8	32	17	86	98
Insulin-like growth factor binding protein 3	P47878	31.7	-	5	6	5
Insulin-like growth factor binding protein 7	Q61581	28.9	-	7	11	71
Insulin-like growth factor-binding protein 5	Q07079	30.4	8	8	20	21
Prolow-density lipoprotein receptor-related protein 1 precursor (A2MR)	Q91ZX7	504.7	-	-	4	8
^Plexin domain-containing protein 2 precursor	Q9DC11	59.6	-	-	4	-
Ptprz1 protein (DSD-1 Proteoglycan)	B2RXS8	175.2	7	5	33	9
Other						
4632419I22Rik protein	Q6NXM5	31.9	-	-	6	3
Acid sphingomyelinase-like phosphodiesterase 3a precursor	P70158	49.9	-	-	4	2
Amyloid beta A4 protein precursor (APP)	P12023	86.7	4	11	25	28
CD109 antigen homolog precursor	Q8R422	161.7	-	5	19	3
Clusterin precursor (Apolipoprotein J)	Q06890	51.7	20	14	119	154
^Meteorin, glial cell differentiation regulator-like	Q8VE43	34.5	-	-	4	-
Peroxiredoxin 3	P20108	28.1	-	-	6	9
Profilin 1	P62962	11.8	16	19	19	14
Retinoic acid receptor responder protein 2 precursor	Q9DD06	18.4	-	-	4	12
Secretogranin 3	P47867	53.3	2	2	32	6
Vimentin	P20152	53.7	77	132	172	108

Proteins are organized by functional category and reported with their corresponding accession number, molecular weight (MW), and redundant peptides identified for each treatment condition.
a) Accession numbers are reported from the Uniprot database (www.uniprot.org) and, when available, refer to the unprocessed precursor protein.
b) The average numbers of redundant peptides identified are reported for protein identifications that passed the selection criteria as detailed in the Experimental Procedures. A null value indicates the protein did not meet the minimum criteria for identification.
^Protein was exclusively detected under control conditions.
*Protein was exclusively detected under cytokine-treated conditions.

Table 4. Redundant peptides from unclassified proteins after computational analysis

Protein Name (Synonym)	Accession[a]	MW (kDa)	1D Control	1D Cytokine	7D Control	7D Cytokine
			Redundant Peptides[b]			
14-3-3 protein beta	Q9CQV8	28,086	8	8	13	8
14-3-3 protein epsilon	P62259	29,174	29	39	41	24
14-3-3 protein eta	P68510	28,212	6	5	-	3
14-3-3 protein gamma	P61982	28,303	10	12	14	9
14-3-3 protein theta	P68254	27,778	4	10	14	5
14-3-3 protein zeta	P63101	27,771	16	35	25	19
Actin related protein 2/3 complex, subunit 2	Q9CVB6	34,357	2	4	4	4
Actinin, alpha 1	Q7TPR4	103,068	50	53	104	14
Actinin, alpha 4	P57780	104,977	8	88	29	23
Aldehyde dehydrogenase 1 family, member L1	Q8R0Y6	98,709	-	4	4	-
Aldo-keto reductase family 1, member A4	Q9JII6	36,587	6	6	3	5
Aldolase 1, isoform A	Q5FWB7	39,356	12	17	27	10
Aldolase 3, isoform C	Q5SYM1	39,395	4	24	4	8
Annexin A3	O35639	36,371	3	-	10	-
Annexin A5	P48036	35,752	12	5	12	2
Asparaginase like 1	Q8C0M9	33,950	6	7	5	-
Astrocytic phosphoprotein PEA-15	Q62048	15,054	-	11	5	6
Brain glycogen phosphorylase	Q8CI94	96,730	-	3	5	-
Calponin 3, acidic	Q9DAW9	36,429	-	-	2	2
Clathrin, heavy polypeptide (Hc)	Q68FD5	191,557	4	16	8	4
Cofilin 1, non-muscle	P18760	18,560	-	7	8	7
Creatine kinase, brain	Q04447	42,713	25	44	74	38
Cytochrome c	P62897	11,605	7	4	4	2
Dihydropyrimidinase-like 2	O08553	62,278	23	21	25	8
Dimethylarginine dimethylaminohydrolase 1	Q9CWS0	31,381	6	9	4	2
DJ-1 protein	Q99LX0	20,021	2	5	7	3
Enolase 1, alpha non-neuron	P17182	47,141	11	20	17	11
Eukaryotic initiation factor 4AII	P10630	46,402	-	3	6	4
Eukaryotic translation elongation factor 2	P58252	95,314	4	8	11	4
Eukaryotic translation initiation factor 5A	P63242	16,303	-	2	3	3
Fatty acid binding protein 7, brain	P51880	14,893	6	8	10	5
Fatty acid synthase	P19096	272,428	-	6	4	2
Ferritin heavy chain 1	P09528	21,067	-	6	9	17
Ferritin light chain 1	P29391	20,802	5	2	11	19
Filamin-A	Q8BTM8	281,194	16	67	66	28
Filamin-B	Q80X90	277,753	28	66	86	55
Filamin-C	Q8VHX6	291,119	-	-	11	2
Gelsolin-like capping protein	Q99LB4	38,769	-	-	5	4
Glial fibrillary acidic protein (GFAP)	P03995	46,492	38	40	73	42
Glutathione S-transferase, alpha 4	P24472	25,564	9	4	9	5
Glutathione S-transferase, mu 1	A2AE90	25,970	48	44	61	38
Glutathione S-transferase, mu 5	P48774	26,635	-	3	6	-
Glutathione S-transferase, pi 2	P46425	23,537	-	-	8	7
Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	P16858	35,810	-	-	2	7
Glyoxalase domain containing 4	Q9CPV4	33,317	-	-	9	-

Guanosine diphosphate (GDP) dissociation inhibitor 1	P50396	50,522	2	3	6	-
H4 histone family, member A	P62806	11,367	5	12	5	6
Heat shock 70kDa protein 8 isoform 1	P63017	70,871	10	22	30	8
Heat shock protein 4	Q5NCS5	94,209	6	7	10	-
Heat shock protein 90kDa alpha (cytosolic), class A member 1	P07901	84,788	13	12	30	8
Heat shock protein 90kDa alpha (cytosolic), class B member 1	Q71LX8	83,281	13	34	10	5
Heterogeneous nuclear ribonucleoprotein A2/B1	O88569	37,403	-	7	8	8
Hypoxanthine guanine phosphoribosyl transferase 1	P00493	24,570	-	-	3	-
Inositol monophosphatase	O55023	30,436	4	4	3	-
Isocitrate dehydrogenase [NADP] cytoplasmic	O88844	46,660	3	3	5	-
Lactate dehydrogenase 1, A chain	P06151	36,499	15	11	14	15
Lactate dehydrogenase 2, B chain	P16125	36,572	38	31	34	14
Lactoylglutathione lyase	Q9CPU0	20,810	-	6	2	2
Lamin A isoform A	P48678	74,238	-	-	7	4
Leukotriene A4 hydrolase	Q3UY71	69,051	3	-	7	-
Lysosomal alpha-mannosidase precursor (Mannosidase, alpha B)	O09159	114,604	3	10	26	10
Malate dehydrogenase 2, NAD (mitochondrial)	P08249	35,611	25	24	15	9
Malate dehydrogenase, cytoplasmic	P14152	36,511	14	6	8	5
Malic enzyme 1, supernatant	P06801	63,999	-	-	2	2
Mannosidase alpha, class 1A, member 1	Q544T7	73,276	2	16	16	13
Myosin, heavy polypeptide 9, non-muscle isoform 1	Q8VDD5	226,357	-	-	9	-
Myosin, light polypeptide 6, alkali, smooth muscle and non-muscle	Q60605	16,930	-	5	7	4
Nestin	Q6P5H2	202,011	-	9	5	-
Nit protein 2	Q9JHW2	30,502	-	-	2	-
Nucleoside-diphosphate kinase 1	P15532	17,208	3	-	6	9
Nucleoside-diphosphate kinase 2	Q01768	17,363	3	11	3	4
Ornithine aminotransferase	P29758	48,355	-	-	3	3
Peroxiredoxin 1	A2AP16	22,176	14	20	19	24
Peroxiredoxin 2 (Thioredoxin peroxidase 1)	Q61171	21,779	7	14	14	12
Peroxiredoxin 5 precursor	P99029	21,897	-	9	8	20
Peroxiredoxin 6 (Acidic calcium-independent phospholipase A2)	O08709	24,871	38	48	35	19
Phosphogluconate dehydrogenase	Q91V28	53,261	9	13	10	4
Phosphoglycerate kinase 1	P09411	44,536	22	18	13	12
Phosphoglycerate mutase 1 (brain)	Q9DBJ1	28,832	3	6	12	9
Phosphoserine aminotransferase 1	Q99K85	40,473	8	-	2	-
Plectin 1	Q9QXS1	534,216	-	-	7	-
Proteasome activator PA28 alpha subunit	P97371	28,673	-	-	4	4
Proteasome subunit, alpha type 1	Q9R1P4	29,547	7	4	4	-
Proteasome subunit, alpha type 2	P49722	25,926	-	2	6	-
Proteasome subunit, alpha type 3	Q9DCD8	28,490	9	3	2	-
Proteasome subunit, alpha type 5	Q3UPK6	26,411	4	3	5	-
Proteasome subunit, alpha type 6	Q9QUM9	27,372	4	8	4	4
Proteasome subunit, alpha type 7	Q9Z2U0	27,855	10	8	8	7
Proteasome subunit, beta type 1	O09061	26,372	8	-	7	-
Proteasome subunit, beta type 3	Q545G0	22,965	4	6	7	-
Proteasome subunit, beta type 5	Q3UZI1	28,532	6	11	7	-
Proteasome subunit, beta type 8	P28063	30,260	2	4	5	-
Purine-nucleoside phosphorylase	P23492	32,277	5	-	7	4

Pyruvate kinase isozyme M2	P52480	57,887	4	7	17	12
Rho GDP dissociation inhibitor (GDI) alpha	Q99PT1	23,407	8	8	13	14
Ribonuclease/angiogenesis inhibitor	Q91VI7	49,816	6	-	3	3
S-adenosylhomocysteine hydrolase	Q5M9P0	47,674	3	-	3	-
SH3-binding domain glutamic acid-rich protein like	Q9JJU8	12,811	3	4	5	5
Soluble calcium-activated nucleotidase 1	Q8VCF1	45,653	2	4	5	4
Spectrin alpha 2	P16546	284,597	-	3	18	-
Spectrin beta 2 isoform 1 or 2	Q62261	274,223	-	2	5	-
S-phase kinase-associated protein 1A	Q9WTX5	18,672	-	5	4	3
Superoxide dismutase 1, soluble	P08228	15,943	18	30	15	27
Thioredoxin reductase 1	Q8CI31	54,337	6	5	2	-
Transaldolase 1	Q93092	37,387	6	-	3	-
Transgelin	P37804	22,576	9	15	18	13
Transgelin 2	Q91VU2	22,395	4	11	12	10
Transitional endoplasmic reticulum ATPase (valosin-containing protein)	Q01853	89,364	27	57	31	5
Transketolase	P40142	67,630	-	-	4	-
Translin	Q62348	26,201	3	-	7	3
Triosephosphate isomerase 1	P17751	26,713	9	6	6	3
Tropomyosin 1, alpha	P58771	32,681	7	11	7	4
Tropomyosin 4, alpha	Q6IRU2	28,468	8	4	14	6
Tubulin, alpha	P05213	50,152	-	7	8	2
Tubulin, beta	Q9ERD7	50,419	-	5	7	-
Ubiquitin carboxy-terminal hydrolase L1	Q9R0P9	24,838	2	6	6	2
Ubiquitin-activating enzyme E1, Chr X	Q02053	117,809	-	4	4	2
UDP-glucose pyrophosphorylase 2	Q91ZJ5	56,979	3	4	3	-
Villin 2	P26040	69,407	-	-	8	6
Vinculin	Q64727	116,717	14	30	46	8
WD repeat domain 1	Q3TJY2	66,407	7	6	4	-

Proteins are reported with their corresponding accession number, molecular weight (MW), and redundant peptides identified for each treatment condition.

a) Accession numbers are reported from the Uniprot database (www.uniprot.org) and, when available, refer to the unprocessed precursor protein.

b) The average number of redundant peptides identified after applying the selection criteria detailed in Experimental Procedures. A null value indicates the protein did not meet the minimum criteria for identification.

Materials and Methods

Chemicals and Materials

The following primary antibodies were used to detect the mouse protein for Western blot experiments: anti-ApoE (1:1000, Biodesign, Saco, ME), anti-C3 complement (1:500, Cedarlane, Burlington, NC), anti-ceruloplasmin (1:500, BD Biosciences, San Jose, CA), and anti-CXCL1 (1:2500, Abcam, Cambridge, MA). 9-Oxo-10E,12Z-octadecadienoic acid (9-oxo-ODE), 13-oxo-9Z,11E-octadecadienoic acid (13-oxo-ODE), 15-oxo-5Z,8Z,11Z,13E-eicosatetraenoic acid (15-oxo-ETE), 9(R)-hydroxy-10E,12Z-octadecadienoic acid (9(R)-HODE), 9(S)-hydroxy-10E,12Z-octadecadienoic acid (9(S)-HODE), 13(R)-hydroxy-9Z,11E-octadecadienoic acid (13(R)-HODE), 13(S)-hydroxy-9Z,11E-octadecadienoic acid (13(S)-HODE), 11(R)-hydroxy-5Z,8Z,12E,14Z-eicosatetraenoic acid (11(R)-HETE), 11(S)-hydroxy-5Z,8Z,12E,14Z-eicosatetraenoic acid (11(S)-HETE), 12(R)-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid (12(R)-HETE), 12(S)-hydroxy-5Z,8Z,10E,14Z-eicosatetraenoic acid (12(S)-HETE), 15(R)-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid (15(R)-HETE), 15(S)-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid (15(S)-HETE), 9-oxo-11α,15S-dihydroxy-prosta-5Z,13E-dien-1-oic acid (PGE_2), 9-oxo-11β,15S-dihydroxy-prosta-5Z,13E-dien-1-oic acid (11β-PGE_2), 9-oxo-11α,15S-dihydroxy-(8β)-prosta-5Z,13E-dien-1-oic acid (8-iso-PGE_2), 9α,15S-dihydroxy-11-oxo-prosta-5Z,13E-dien-1-oic acid (PGD_2), 9α,11α,15S-trihydroxy-prosta-5Z,13E-dien-1-oic acid ($PGF_{2\alpha}$), 9α,11β,15S-trihydroxy-prosta-5Z,13E-dien-1-oic acid (11β-PGF_2), 9α,11α,15S-trihydroxy-(8β)-prosta-5Z,13E-dien-1-oic acid (8-iso-$PGF_{2\alpha}$), 9-oxo-11α,15S-dihydroxy-prosta-5Z,13E-dien-1-oic-3,3,4,4-$^2H_4$ acid ([$^2H_4$]-PGE_2), 9α,11α,15S-trihydroxy-prosta-5Z,13E-dien-1-oic-3,3,4,4-$^2H_4$ acid ([$^2H_4$]-$PGF_{2\alpha}$), [$^2H_4$]-13(S)-hydroxy-9Z,11E-octadecadienoic acid ([$^2H_4$]-13(S)-HODE), and [$^2H_8$]-15(S)-hydroxy-5Z,8Z,11Z,13E-eicosatetraenoic acid ([$^2H_8$]-15(S)-HETE), and NS-398, (N-[2-cyclohexyloyl-4-nitrophenyl] methane-sulfonamide) were purchased from Cayman Chemical Co. (Ann Arbor, MI). Diisopropylethylamine (DIPE), 2,3,4,5,6-pentafluorobenzyl bromide (PFB-Br) was purchased from Sigma-Aldrich (St. Louis, MO).

HPLC grade hexane, methanol and isopropanol were obtained from Fisher Scientific Co. (Fair Lawn, NJ). Gases were supplied by BOC Gases (Lebanon, NJ).

Nitric oxide metabolite analysis

Nitric oxide-derived products (nitrate, nitrite, S-nitrosothiols, N-nitroso-, and iron nitrosyl) were quantified by chemical reduction to nitric oxide followed by ozone-based chemiluminescent detection using Nitric Oxide Analyzer 280 (Sievers, Boulder, CO). Briefly, helium gas was bubbled through an acidified (1 N) vanadium (III) chloride solution (50 mM) maintained at 90 °C in a jacketed glass purge vessel. Aliquots (20 μL) of ACM, serum-free media, or nitrite standards were injected into the glass purge vessel. Concentrations of nitric oxide products were calculated using linear best-fit curves constructed against nitrite standards and were reported after correcting for the content of nitric oxide products quantified in serum-free media.

Immunohistochemistry and cell morphological analysis

Astrocyte cultures were fixed with cold methanol for 20 min at –20 °C, followed by 50:50 methanol:acetone for 5 min at –20 °C. Immunodetection of GFAP or Cd11b were performed using a mouse anti-GFAP antibody (1:250, BD Pharmingen, San Jose, CA) or a rat anti-CD11b antibody (1:100, AbD Serotec, Raleigh, NC), respectively. Antigens were visualized with goat anti-mouse secondary antibodies conjugated to either Alexa Fluor 488 or 546 (Invitrogen, Carlsbad, CA). The nuclei were visualized by DAPI staining (1:10,000). Morphological analyses were performed by counting GFAP-positive cells with and without processes from 3-6 fields from at least 3 independent experiments.

Targeted lipidomics profile of conditioned media

Astrocytes were cultured and treated for 7D as described in Materials and Methods. A portion of the ACM filtrate (3 ml) was transferred into a borosilicate glass tube. Tubes containing cell culture media alone (3 ml) were spiked with the following amounts of authentic lipid standards: 20, 50, 100, 200, 500, 1000, 2000 pg. A mixture of internal standards ($[^2H_8]$-5(*S*)-HETE, $[^2H_8]$-12(*S*)-HETE, $[^2H_8]$-15(*S*)-HETE, $[^2H_4]$-9(*S*)-HODE, $[^2H_4]$-13(*S*)-HODE, $[^2H_4]$-PGE_2, $[^2H_4]$-PGD_2, $[^2H_4]$-11β-PGF_2, $[^2H_4]$-$PGF_{2\alpha}$, $[^2H_4]$-8-*iso*-$PGF_{2\alpha}$-PFB, 1 ng each) was added to each analytical sample and standard solution. The analytical samples and standard solutions were adjusted to pH 3 with 2.5 N hydrochloric acid. Lipids were extracted with diethyl ether (4 ml × 2) and the organic layer was then evaporated to dryness under nitrogen. The residue was dissolved in 100 μL of acetonitrile and treated with 100 μL of PFB-Br in acetonitrile (1:19, v/v) followed by 100 μL of DIPE in acetonitrile (1:9, v/v). The solution was heated at 60 ^oC for 60 min, allowed to cool, evaporated to dryness under nitrogen at room temperature, and re-dissolved in 100 μL of hexane/ethanol (97:3, v/v). Analysis of the PFB derivatives by normal phase chiral LC-electron capture APCI/MRM/MS analysis was conducted on a 20 μL aliquot of this solution along with PFB derivatives of 24 authentic lipids and 10 heavy isotope analog internal standards as described below.

Liquid separation and mass spectrometric analysis of lipids

A Waters Alliance 2690 HPLC system (Waters Corp., Milford, MA) was used for separation of lipids. For the normal phase chiral LC-APCI/MS analysis, a Chiralpak AD-H column (250 × 4.6 mm i.d., 5 μm; Chiral Technologies, Inc., West Chester, PA) was employed with a flow rate of 1.0 mL/min. Separations were performed at 30 ^oC using a linear gradient. Solvent A was hexane and solvent B was methanol/isopropanol (1:1, v/v). The mobile phase gradient was as follows: 2 % B at 0 min, 2 % B at 3 min, 3.6 % B at 11 min, 8 % B at 15 min, 8 % B at 27 min, 50 % B at 30 min, 50 % B at 35 min, and 2 % B at 37 min.

A Finnigan TSQ Quantum Ultra AM mass spectrometer (Thermo Fisher, San Jose, CA) was used for the detection of targeted lipids. The instrument was equipped with an APCI source and operated in the negative ion mode maintaining unit resolution for both parent and product ions during MRM analyses. Operating conditions were as follows: vaporizer temperature was 450 ^{o}C, heated capillary temperature was 250 ^{o}C, with a discharge current of 30 μA applied to the corona needle. Nitrogen was used for the sheath gas, auxiliary gas and ion sweep gas set at 25, 3 and 3 (in arbitrary units), respectively. Collision-induced dissociation (CID) was performed using argon as the collision gas at 1.5 mTorr in the second (rf-only) quadrupole. An additional dc offset voltage was applied to the region of the second multipole ion guide (Q0) at 10 V to impart enough translational kinetic energy to the ions so that solvent adduct ions dissociate to form sample ions.

Targeted chiral LC-electron capture APCI/MRM/MS analysis was conducted using PFB derivatives of 24 lipids and 10 heavy isotope analog internal standards using the following MRM transitions: 9- and 13-oxo-ODE-PFB, *m/z* 293 → 113 (collision energy, 21 eV); 15-oxo-ETE-PFB, *m/z* 317 → 273 (collision energy, 14 eV); 9(*R*)- and 9(*S*)-HODE-PFB, *m/z* 295 → 171 (collision energy, 18 eV); [$^{2}H_{4}$]-9(*S*)-HODE-PFB, *m/z* 299 → 172 (collision energy, 18 eV); 13(*R*)- and 13(*S*)-HODE-PFB, *m/z* 295 → 195 (collision energy, 18 eV); [$^{2}H_{4}$]-13(*S*)-HODE-PFB, *m/z* 299 → 198 (collision energy, 18 eV); 5(*R*)- and 5(*S*)-HETE-PFB, *m/z* 319 → 115 (collision energy, 15 eV); [$^{2}H_{8}$]-5(*S*)-HETE-PFB, *m/z* 327 → 116 (collision energy, 15 eV); 8(*R*)- and 8(*S*)-HETE-PFB, *m/z* 319 → 155 (collision energy, 16 eV); 11(*R*)- and 11(*S*)-HETE-PFB, *m/z* 319 → 167 (collision energy, 16 eV); 12(*R*)- and 12(*S*)-HETE-PFB, *m/z* 319 → 179 (collision energy, 14 eV); [$^{2}H_{8}$]-12(*S*)-HETE-PFB, *m/z* 327 → 184 (collision energy, 14 eV); 15(*R*)- and 15(*S*)-HETE-PFB, *m/z* 319 → 219 (collision energy, 13 eV); [$^{2}H_{8}$]-15(*S*)-HETE-PFB, *m/z* 327 → 226 (collision energy, 13 eV); PGE_2-PFB, PGD_2-PFB, 11β-PGE_2-PFB, 8-*iso*-PGE_2-PFB, *m/z* 351 → 271 (collision energy, 18 eV); [$^{2}H_{4}$]-PGE_2-PFB, [$^{2}H_{4}$]-PGD_2-PFB, *m/z* 355 → 275 (collision energy, 18 eV); 11β-PGF_2-PFB, $PGF_{2\alpha}$-PFB, 8-*iso*-$PGF_{2\alpha}$-PFB, *m/z* 353 → 309 (collision energy, 18 eV);

$[^2H_4]$-11β-PGF_2-PFB, $[^2H_4]$-$PGF_{2\alpha}$-PFB, $[^2H_4]$-8-*iso*-$PGF_{2\alpha}$-PFB, *m/z* 357 → 313 (collision energy, 18 eV).

Enzyme-Linked ImmunoSorbent Assay (ELISA)

The levels of IL-6 were deterimined by a colorimetric ELISA kit (Pierce, Rockford, IL), and the levels of NGF were measured by Chemikine Sandwich ELISA kit (Chemicon, Billerica, MA), following the manufacturer's instructions. Serum-free media was used for dilution of the standards and unknowns.

Western blot analysis

Protein concentration was measured using the Bradford reagent (Bio-rad, Hercules, CA). ACM protein samples were boiled in Laemmli sample buffer and then separated by either 10 % 1-D SDS-PAGE or 10 % NuPAGE gels. For the analysis of proteins that had increased or decreased abundance as assessed by redundant peptides, equal amounts of protein (between 2-6 μg) were loaded per lane. Detection of these proteins from 1D ACM required samples to be concentrated further by Amicon Microcon Ultracel YM-3 filters (Millipore, Billerica, MA). Following electrophoresis, proteins were transferred to PVDF membranes (Millipore, Billerica, MA) and blocked in TBS containing 0.5% tween (TBS-t) and 5 % milk. Membranes were then incubated in TBS-t containing 5% milk and primary antibody (see Chemicals and Materials). Membranes were then washed in TBS-t, incubated with appropriate secondary antibodies conjugated to Alexa Fluor 680 (1:5000, Invitrogen, Carlsbad, CA) for 1 hour in TBS-t containing 1% milk, and visualized using the Odyssey Infrared Imaging system (Licor Biosciences, Lincoln, NE).

Statistical analyses

Graphs were constructed and statistical analyses were performed using GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA). Unless otherwise stated, statistical significance was performed by two-tailed unpaired t-test. For data that did not conform to Gaussian distributions, the non-parametric Mann-Whitney test was performed.