

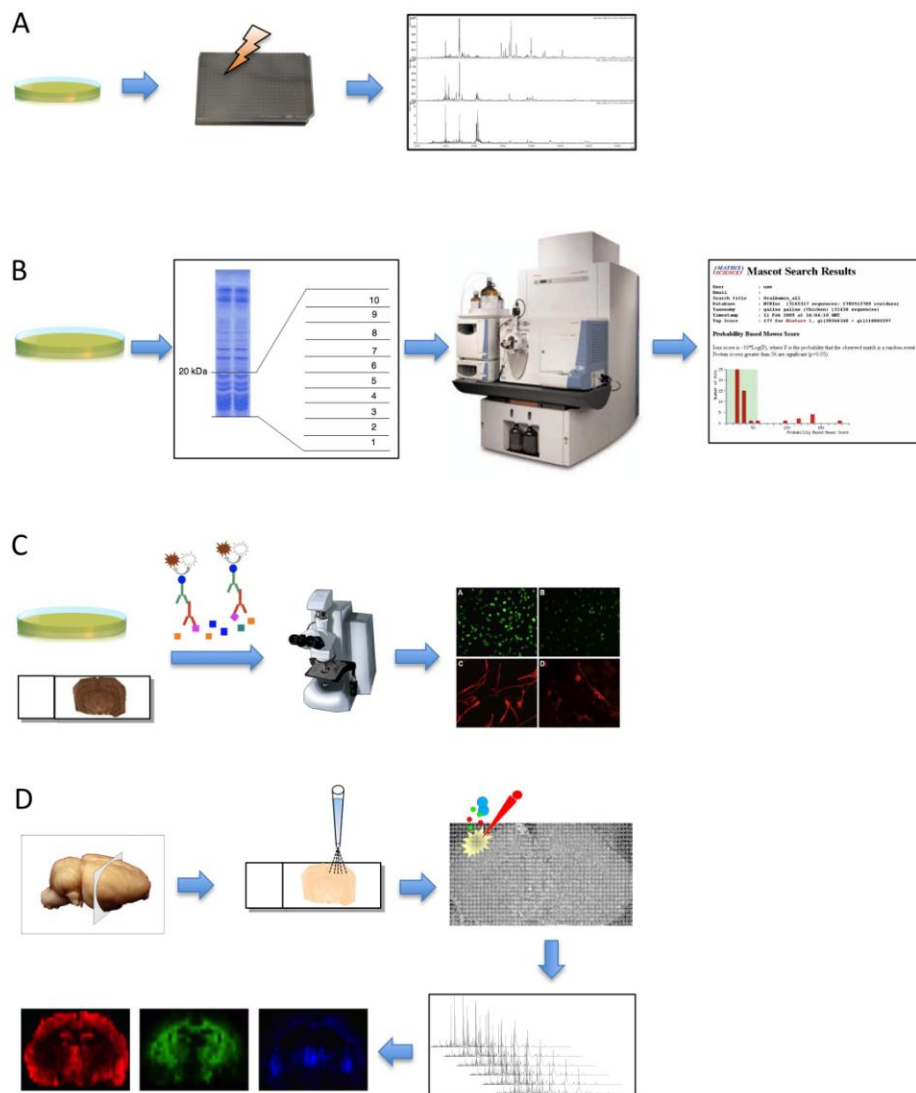
## **Analytical and Bioanalytical Chemistry**

### **Electronic Supplementary Material**

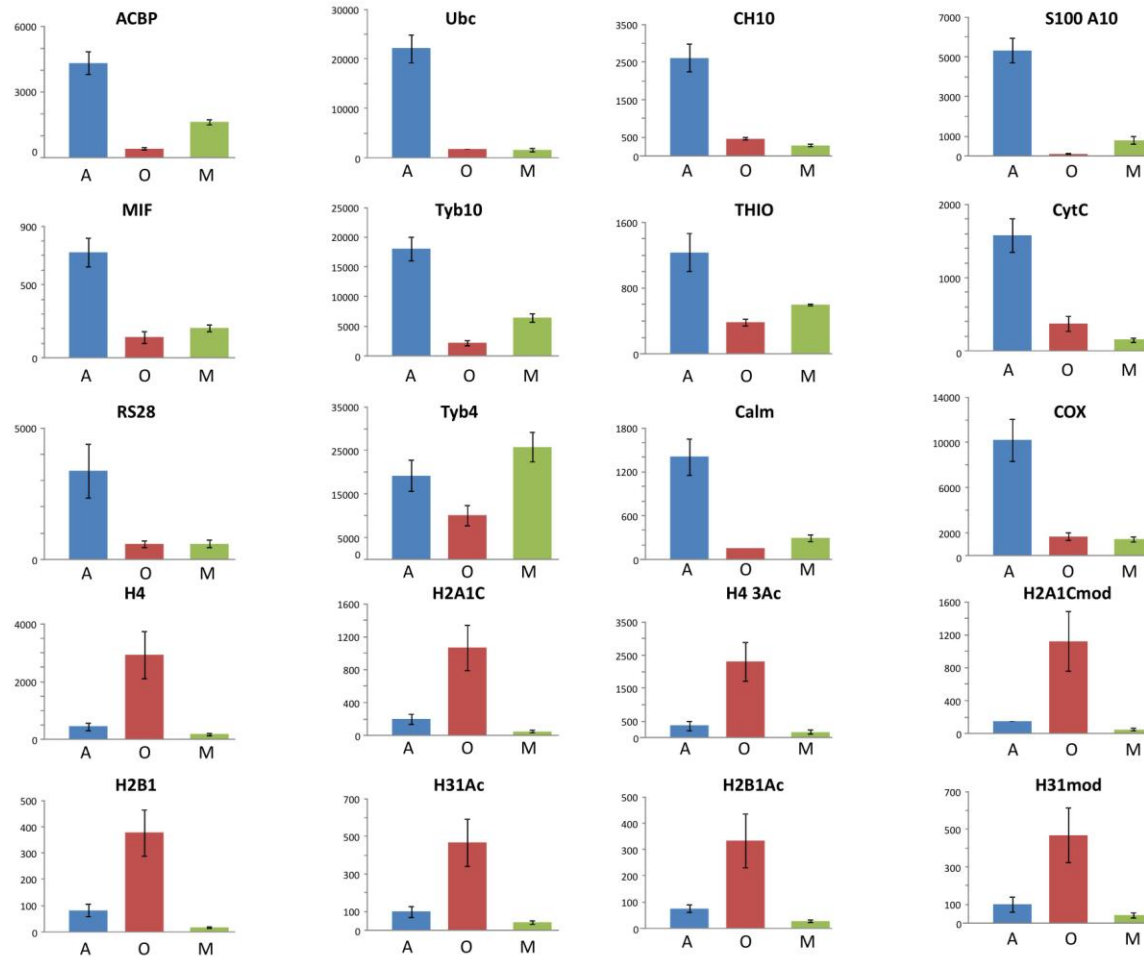
# **MALDI mass spectrometry based molecular phenotyping of CNS glial cells for prediction in mammalian brain tissue**

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**Figure S1.** Experimental Design (A) Intact cell MS workflow. Intact glial cells are suspended in ammonium acetate and placed directly on a MALDI target plate. MS analysis reveals characteristic template mass spectra for each cell type. (B) Proteomic analysis on mixed cells was performed by means of 1DGE and nanoLC-MSMS. A number of nine equidistant consecutive bands in the mass range below 20kDa were excised, followed by in-gel digestion and LC-MSMS analysis of the tryptic peptides. (C) Immunohistochemistry. Double antibody labeling was performed in situ and in vitro. Antibodies against cell specific marker proteins were used for cellular characterization. (D) MALDI IMS. Rodent brain tissue sections were thaw-mounted on conductive glass slides, followed by matrix application. MALDI analysis reveals the ion distribution of single protein masses, which is in line with histological features.



**Figure S2.** Quantitative Intact cell MS data of identified protein species in different cell types. The graphs show the mean normalized mass peak intensity (arbitrary units  $\pm$  SEM) of the respective protein for the different cell types (n=10, 10, and 10). A: astrocytes, O: oligodendrocytes and M: microglia.



**Table S1.** Protein identification results using 1DGE/LC-MSMS

	Protein <sup>[1]</sup>	Protein Entry <sup>[2]</sup>	Accession <sup>[2]</sup>	theor. Mass [Da] <sup>[3]</sup>	Score <sup>[5]</sup>	Peptides <sup>[6]</sup>
1	Acyl-CoA-binding protein	ACBP_RAT	P11030	10027	139	8
2	Actin, aortic smooth muscle	ACTA_RAT	P62738	42009	71	1
3	Actin, alpha skeletal muscle	ACTS_RAT	P68136	42051	44	3
4	Adaptor protein complex AP-2 subunit sigma	AP2S1_RAT	P62744	17018	31	3
5	Apolipoprotein E	APOE_RAT	P02650	35753	90	2
6	ADP-ribosylation factor 2	ARF2_RAT	P84082	20746	106	2
7	ADP-ribosylation factor 4	ARF4_RAT	P61751	20397	96	5
8	ADP-ribosylation factor 5	ARF5_RAT	P84083	20530	112	5
9	ADP-ribosylation factor-like protein 1	ARL1_RAT	P61212	20412	44	1
10	ADP-ribosylation factor-like protein 3	ARL3_RAT	P37996	20457	50	1
11	Actin-related protein 2/3 complex subunit 5	ARPC5_RAT	Q4KLF8	16320	40	2
12	ATPase inhibitor, mitochondrial	ATIF1_RAT	Q03344	12248	53	1
13	ATP synthase subunit e	ATP5I_RAT	P29419	8255	114	3

14	ATP synthase subunit beta	ATPB_RAT	P10719	56354	29	3
15	ATP synthase subunit delta	ATPD_RAT	P35434	17595	125	3
16	Beta-2-microglobulin	B2MG_RAT	P07151	13720	69	3
17	Calmodulin	CALM_RAT	P62161	16838	43	1
18	Calreticulin	CALR_RAT	P18418	47995	64	1
19	chaperonin 10, 10 kDa heat shock protein	CH10_RAT	P26772	10902	247	10
20	RhoA activator C11orf59 homolog	CK059_RAT	Q6P791	17721	88	1
21	Contactin-4	CNTN4_RAT	Q62845	113393	29	5
22	Cytochrome c oxidase subunit 41	COX41_RAT	P10888	19515	68	5
23	Cytochrome c oxidase subunit 5A	COX5A_RAT	P11240	16130	132	5
24	Cytochrome c oxidase subunit 5B	COX5B_RAT	P12075	13915	193	11
25	Protein CutA	CUTA_RAT	Q6MGD0	18659	41	4
26	Cytochrome c oxidase subunit 7A2	CX7A2_RAT	P35171	9353	62	4
27	Cytochrome b5	CYB5_RAT	P00173	15355	44	1
28	Cytochrome C	CYC_RAT	P62898	11605	196	7

29	Cystatine b	CYTB_RAT	P01041	11196	56	3
30	Defender against cell death 1	DAD1_RAT	P61805	12497	48	1
31	Dynein light chain roadblock-type 1	DLRB1_RAT	P62628	10990	68	2
32	D-dopachrome decarboxylase	DOPD_RAT	P80254	13133	110	4
33	Dynein light chain 1, cytoplasmic	DYL1_RAT	P63170	10366	61	4
34	Dynein light chain 2, cytoplasmic	DYL2_RAT	Q78P75	10350	75	5
35	Dynein light chain Tctex-type 1	DYLT1_RAT	Q9Z336	12452	37	3
36	Elongin-C	ELOC_RAT	P83941	12473	64	2
37	Ephrin type-b receptor 6	EPHB6_RAT		111669	32	9
38	Electron transfer flavoprotein subunit alpha, mitochondrial	ETFA_RAT	P13803	34951	30	1
39	Fatty acid-binding protein, epidermal	FABPE_RAT	P55053	15059	184	3
40	Fatty acid-binding protein, brain	FABPB_RAT	P55051	14864	132	3
41	Mitochondrial fission 1 protein	FIS1_RAT	P84817	16995	80	2
42	FK506 binding protein 1A	FKB1A_RAT	Q62658	11923	192	7
43	Ferritin light chain 1	FRIL1_RAT	P02793	20749	119	3

44	Glutaredoxin-1	GLRX1_RAT	Q9ESH6	11879	76	2
45	Granulin 3	GRN_RAT (GRN3)	P23785	6008	151	3
46	78 kDa glucose-regulated protein	GRP78_RAT	P06761	72347	55	1
47	Histone H2A type1	H2A1_RAT	P02262	14077	285	32
48	Histone H2A type1C	H2A1C_RAT	P0C169	14105	292	24
49	Histone H2A type1E	H2A1E_RAT	P0C170	14119	325	33
50	Histone H2A type3	H2A3_RAT	Q4FZT6	14121	86	5
51	Histone H2A.Z	H2AZ_RAT	P0C0S7	13553	67	4
52	Histone H2B type1	H2B1_RAT	Q00715	13990	90	17
53	Histone H3.1	H31_RAT	Q6LED0	15404	107	8
54	Histone H3.3	H33_RAT	P84245	15328	51	2
55	Histone H4	H4_RAT	P62804	11367	1273	61
56	Heterogeneous nuclear ribonucleoprotein C	HNRPC_RAT	P17132	18020	29	2
57	Interferon-induced transmembrane protein 3	INIB_RAT	P26376	14971	58	2
58	Kinesin-like protein KIF3C	KIF3C_RAT	O55165	89816	47	6

59	Pyruvate kinase isozymes M1/M2	KPYM_RAT	P11980	57818	47	1
60	Galectin-1	LEG1_RAT	P11762	14857	76	2
61	Lysosomal alpha-glucosidase	LYAG_RAT	Q6P7A9	106207	72	1
62	Lysozyme C-1	LYSC1_RAT	P00697	16729	34	1
63	Matrix Gla protein	MGP_RAT	P08494	12037	41	1
64	Macrophage migration inhibitory factor	MIF_RAT	P30904	12477	448	15
65	Myotrophin	MTPN_RAT	P62775	12861	119	2
66	Myosin light chain 6	MYL6_RAT	Q64119	16975	108	5
67	Nucleoside diphosphate kinase A	NDKA_RAT	Q05982	17193	87	6
68	Nucleoside diphosphate kinase B	NDKB_RAT	P19804	17283	58	5
69	NHP2-like protein 1	NH2L1_RAT	P55770	14174	139	5
70	Nuclear transport factor 2	NTF2_RAT	P61972	14478	37	3
71	Protein disulfide-isomerase A6	PDIA6_RAT	Q63081	48173	111	2
72	Astrocytic phosphoprotein PEA-15	PEA15_RAT	Q5U318	15040	38	1
73	Peptidyl-prolyl cis-trans isomerase A	PPIA_RAT	P10111	17874	212	15



74	Peptidyl-prolyl cis-trans isomerase B	PPIB_RAT	P24368	23803	72	1
75	Peroxiredoxin-5, mitochondrial	PRDX5_RAT	Q9R063	22179	148	20
76	Profilin-1	PROF1_RAT	P62963	14957	149	6
77	Profilin-2	PROF2_RAT	Q9EPC6	15002	120	3
78	Parathyrosin	PTMS_RAT	P04550	11559	73	1
79	Ras-related protein 2	RABP2_RAT	P51673	15933	38	3
80	Retinol-binding protein 1	RET1_RAT	P02696	15834	132	3
81	60S ribosomal protein L22	RL22_RAT	P47198	14789	79	2
82	60S ribosomal protein L23	RL23_RAT	P62832	14865	242	6
83	60S ribosomal protein L27	RL27_RAT	P61354	15798	37	1
84	60S ribosomal protein L30	RL30_RAT	P62890	12784	213	4
85	60S ribosomal protein L38	RL38_RAT	P63174	8218	112	6
86	60S ribosomal protein L40	RL40_RAT	P62986	6181	32	1
87	60S acidic ribosomal protein P2	RLA2_RAT	P02401	11692	819	20
88	40S ribosomal protein S12	RS12_RAT	P63324	14525	112	4

89	40S ribosomal protein S13	RS13_RAT	P62278	17222	66	1
90	40S ribosomal protein S14	RS14_RAT	P13471	16259	115	5
91	40S ribosomal protein S15A	RS15A_RAT	P62246	14840	45	1
92	40S ribosomal protein S16	RS16_RAT	P62250	16445	99	3
93	40S ribosomal protein S17	RS17_RAT	P04644	15510	37	2
94	40S ribosomal protein S18	RS18_RAT	P62271	17719	180	7
95	40S ribosomal protein S19	RS19_RAT	P17074	16085	72	7
96	40S ribosomal protein S20	RS20_RAT	P60868	13373	92	6
97	40S ribosomal protein S21	RS21_RAT	P05765	9127	53	3
98	40S ribosomal protein S25	RS25_RAT	P62853	13742	78	2
99	40S ribosomal protein S26	RS26_RAT	P62856	13015	55	2
100	40S ribosomal protein S27	RS27_RAT	Q71TY3	9461	57	3
101	40S ribosomal protein S27L	RS27L_RAT	P24051	9477	74	2
102	40S ribosomal protein S28	RS28_RAT	P62859	7841	104	4
103	40S ribosomal protein S29	RS29_RAT	P62275	6677	39	1

104	40S ribosomal protein S30	RS30_RAT	P62864	6648	40	4
105	Protein S100-A10	S10AA_RAT	P05943	11075	32	4
106	Protein S100-A11	S10AB_RAT	Q6B345	11065	82	2
107	Sulfated glycoprotein 1	SAP_RAT	P10960	61124	170	9
108	Superoxide dismutase [Cu-Zn]	SODC_RAT	P07632	15912	223	5
109	Superoxide dismutase [Mn], mitochondrial	SODM_RAT	P07895	24674	32	2
110	Microsomal signal peptidase 18kDa subunit	SPC18_RAT	P42667	20586	84	4
111	Signal sequence receptor subunit delta	SSRD_RAT	Q07984	18980	65	3
112	Small ubiquitin-related modifier 2	SUMO2_RAT	P61959	10740	74	2
113	Tubulin beta-2A chain	TBB2A_RAT	P85108	49907	32	1
114	Thioredoxin	THIO_RAT	P11232	11673	65	7
115	Mitochondrial import inner membrane translocase subunit Tim13	TIM13_RAT	P62076	10458	114	1
116	Transmembrane protein 109	TM109_RAT	Q6AYQ4	26242	54	2
117	Mitochondrial import receptor subunit Tom20	TOM20_RAT	Q62760	16429	88	1
118	Triosephosphate isomerase	TPIS_RAT	P48500	26849	30	2

119	Thioredoxin domain-containing protein 12	TXD12_RAT	Q498E0	19019	38	2
120	Thymosin beta 4	TYB4_RAT	P62329	5053	32	1
121	Ubiquitin-conjugating enzyme E2 variant 2	UB2V2_RAT	Q7M767	16353	47	1
122	Ubiquitin-conjugating enzyme E2 N	UBE2N_RAT	Q9EQX9	17124	78	1
123	Ubiquitin	UBIQ_RAT	P62989	8565	99	11
124	Ribonuclease UK114	UK114_RAT	P52759	14303	42	3
125	Up-regulated during skeletal muscle growth protein 5	USMG5_RAT	Q9JJW3	6408	49	1
126	V-type proton ATPase subunit F	VATF_RAT	P50408	13370	43	2
127	Voltage-dependent anion-selective channel protein 1	VDAC1_RAT	Q9Z2L0	30756	125	4
128	Voltage-dependent anion-selective channel protein 2	VDAC2_RAT	P81155	31746	72	1
129	Voltage-dependent anion-selective channel protein 3	VDAC3_RAT	Q9R1Z0	30798	72	2

[1] Protein name as listed in the uniprot knowledgebase

[2] Uniprot Database Entry and accession number. ([www.uniprot.org](http://www.uniprot.org))

[3] Theoretical protein mass in Da calculated for the listed sequence without post translational modification.

[4] Mascot score, using *MudPIT* scoring with a significance threshold of  $p < 0.05$ . The false positive rate was  $FPR < 5\%$ .

[5] Number of peptide matches assigned to the corresponding protein identity.