

**Figure S1.** Assessing selectivity of SR and CB staining. (A) Mouse liver membrane samples were resolved by 1DE (10  $\mu\text{g}$  of total protein per lane). (B) A commercially available mixture of 14 recombinant protein standards was resolved by 1DE (total protein load was 2.5  $\mu\text{g}$  per lane, and the quantity of individual protein species ranged from 80 to 480 ng). Gels were stained with SR (i) or with one of 14 different CB stain formulations and imaged using IRF (ii through xv). Quantitative image analysis was used to determine the  $S^2/N$  of detected proteins for each stain (Figure 1).

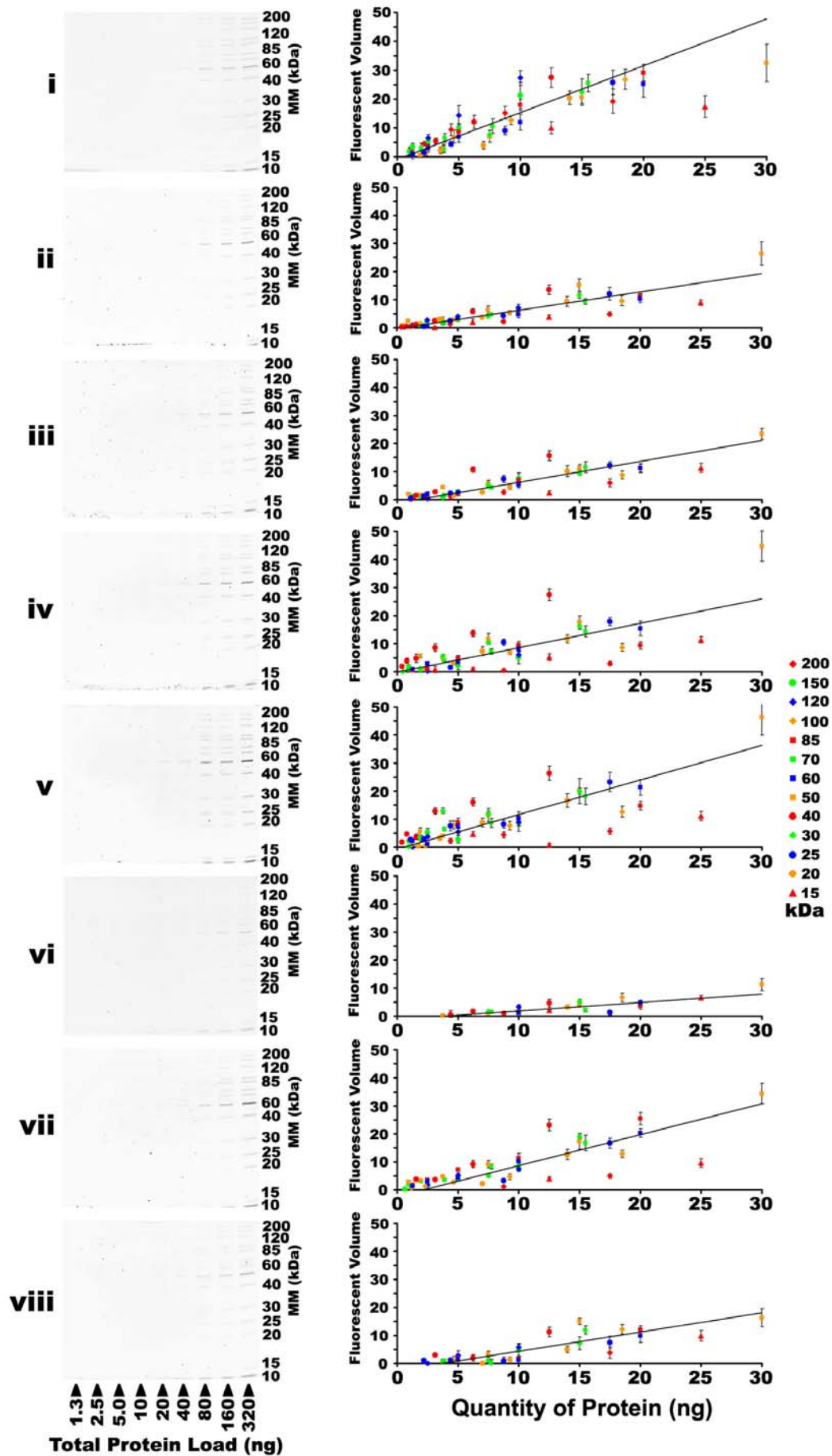


Figure S2 part I

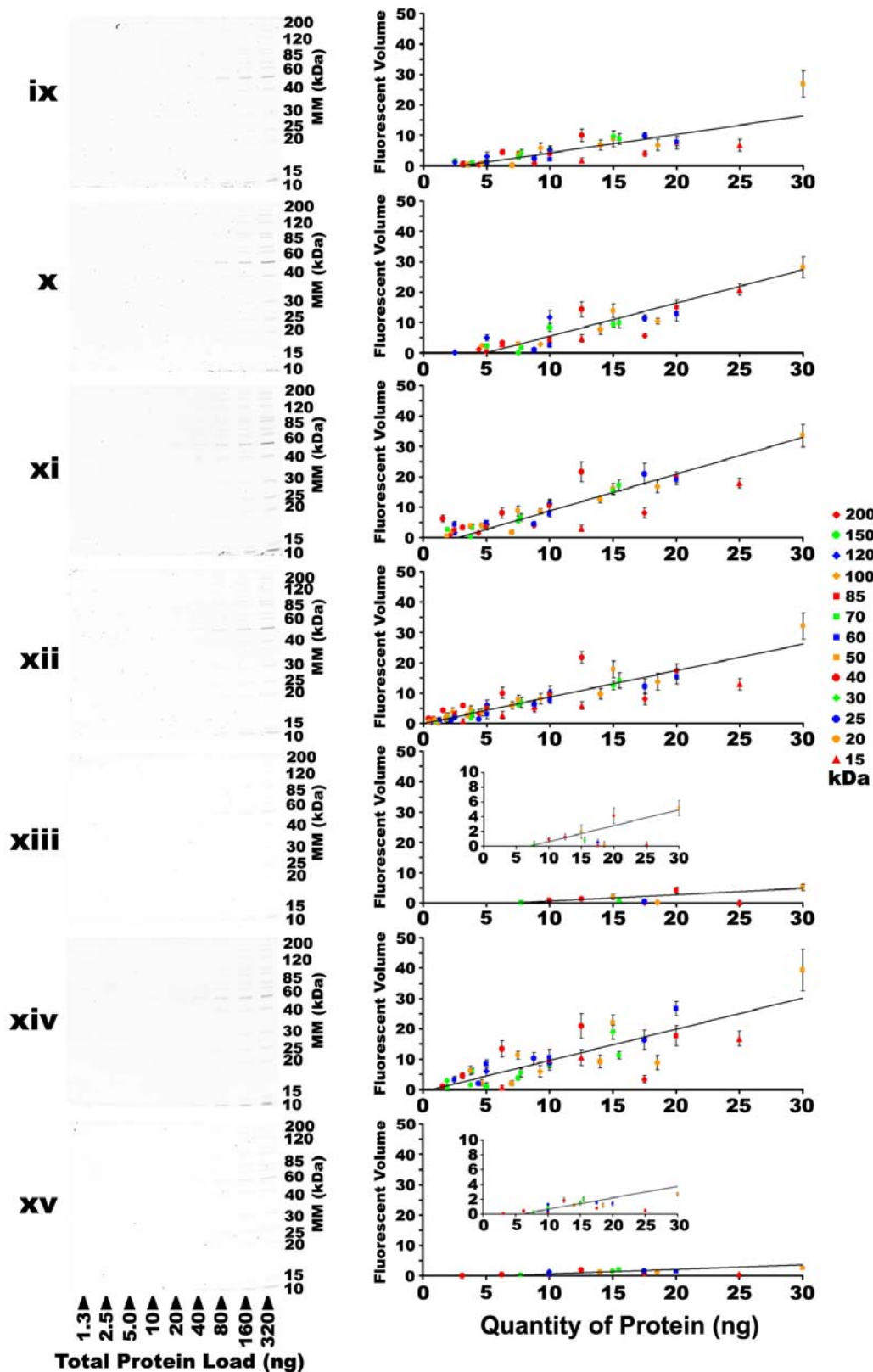
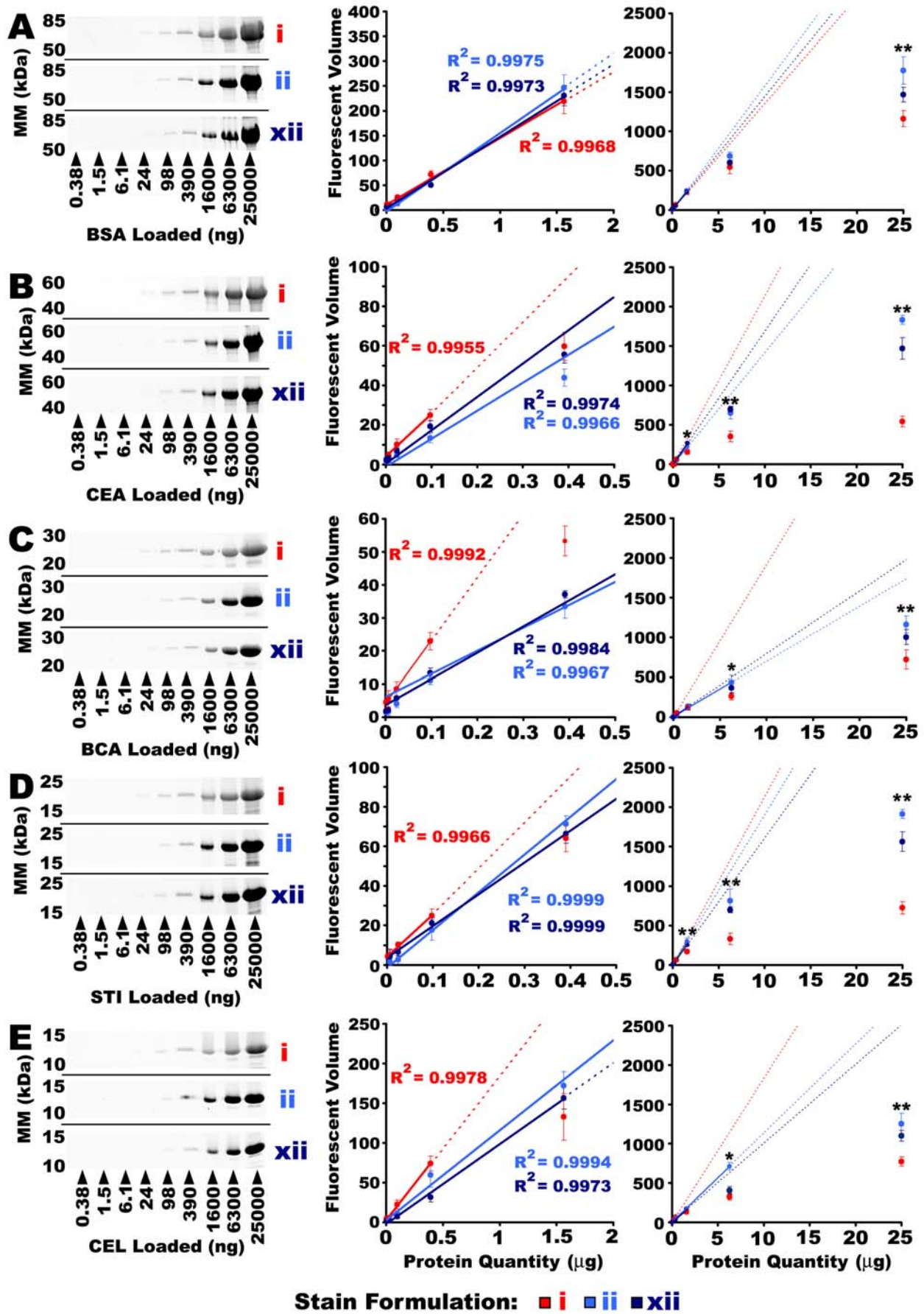


Figure S2 Part II

**Figure S2.** Assessing sensitivity of SR and CB. A dilution series of a commercially available mixture of recombinant protein standards was resolved by 1DE and detected with either SR (i) or stained with one of 14 different CB formulations (ii through xv) and detected with IRF. Quantification of fluorescent volumes in each band detected in gel images are plotted in the

corresponding right hand panels. Trend lines indicate overall linear fits of the entire dataset. Insets: Y-axes are expanded for stains that produced very low fluorescent signal intensity. Error bars represent SD,  $n = 3$ .



**Figure S3.** Assessing the linear dynamic range of SR and CB. A dilution series of isolated protein standards was resolved by 1DE and stained with either SR, or one of the two most

sensitive CB formulations, ii and xii. In the left hand panels, dilution series of five protein standards are presented in order of decreasing molecular weight: A. BSA; B. CEL, C. BCA; D. STI; E. CEL, imaged with either SR (formulation i) or CB (formulations ii or xii) as indicated. Quantitative image analysis was used to determine the lowest limit of detection and the LDR for each stain / protein. Data near threshold are expanded in the centre panels, and the full stain response is plotted in the right hand panels. The LDR was determined by regression analysis. Solid lines indicate the LDR for each stain. Dotted lines indicate projection of the linear fit beyond the LDR. \* Indicate CB IRF volumes that were significantly greater than SR. (One-way ANOVA.  $p < 0.001$ , Post-hoc Tukey analysis,  $p < 0.05$ ,  $n = 3$ ). Error bars indicate SD.

**Table S1.** Quantitative comparison of CB formulation ii and SR staining of mouse brain proteomes. Mouse brains were pre-fractionated into total soluble and membrane protein fractions, resolved by 2DE, and stained with either SR or CB formulation ii (Figure 7). Quantitative image analysis indicated proteins that were detected by both staining methods, but differed significantly in fluorescent signal volume (FV) between the two (T-test,  $p < 0.05$ ,  $n = 4$ ). All significant quantitative differences are summarized here, 1.5-fold or greater differences are indicated on gel images, Figure 7.

Stain Formulation:			SR (i)		CB ii		Fold Difference			
Spot No. / Fraction			FV	SD	FV	SD	T-test (p)	pl	MW	
1	/	Soluble	11.8	0.15	12.92	0.22	1.1	0.02675	6.3	52
2	/	Soluble	21.14	0.16	22.39	0.33	1.1	0.04048	6.9	96
3	/	Soluble	23.12	0.5	19.95	0.87	1.2	0.04691	4.8	101
4	/	Soluble	14.85	0.49	12.11	0.61	1.2	0.03841	4.5	39
5	/	Soluble	2.88	0	3.48	0.03	1.2	0.00135	5.7	166
6	/	Soluble	54.59	2.21	63.02	0.36	1.2	0.03362	7.5	67
7	/	Soluble	25.48	0.17	19.31	1.33	1.3	0.02288	6.5	63
8	/	Soluble	22.41	0.34	16.93	0.56	1.3	0.00702	8.6	28
9	/	Soluble	3.49	0.06	2.62	0.09	1.3	0.00731	8.8	56
10	/	Soluble	10.28	0.31	7.18	0.19	1.4	0.00671	7	24
11	/	Soluble	7.72	0.33	5.51	0.06	1.4	0.01165	6.8	126
12	/	Soluble	5.19	0.34	3.76	0.08	1.4	0.02794	7	23
13	/	Soluble	68.45	6.27	97.29	4.84	1.4	0.03565	5.6	40
14	/	Soluble	8.77	0.29	5.94	0.48	1.5	0.01914	6.1	14
15	/	Soluble	62.35	2.02	93.01	5.43	1.5	0.0174	6.2	36
16	/	Soluble	24.18	1.29	14.76	0.51	1.6	0.01063	4.3	26
17	/	Soluble	28.17	0.88	17.32	0.19	1.6	0.00345	5.3	24
18	/	Soluble	17.9	0.76	11.01	0.27	1.6	0.00671	7.7	15
19	/	Soluble	7.29	0.56	4.49	0.68	1.6	0.04583	6.3	23
20	/	Soluble	9.59	0.27	5.95	0.21	1.6	0.00439	5.4	26
21	/	Soluble	1.29	0.02	0.82	0.09	1.6	0.01841	6.3	17
22	/	Soluble	51.3	3.47	32.9	4.23	1.6	0.04144	8.8	26
23	/	Soluble	11.99	0.3	19.37	0.34	1.6	0.00189	6.2	79
24	/	Soluble	9.01	0.84	5.22	0.34	1.7	0.02732	3.9	19
25	/	Soluble	114.32	4.46	67.66	11.19	1.7	0.03176	8.6	14
26	/	Soluble	20.58	2.36	11.62	0.24	1.8	0.03323	7.1	22
27	/	Soluble	2.07	0.23	3.75	0.05	1.8	0.00936	6.7	28
28	/	Soluble	9.08	0.5	4.67	0.86	1.9	0.0245	5.9	12
29	/	Soluble	1.31	0.16	0.68	0.02	1.9	0.0303	6.6	22
30	/	Soluble	64.47	3.49	33.88	9.05	1.9	0.04675	9.5	16
31	/	Soluble	9.23	0.67	4.85	0.21	1.9	0.01269	5.5	16
32	/	Soluble	3.46	0.34	1.87	0.26	1.9	0.03447	7.5	21
33	/	Soluble	328.13	4.74	623.56	24.25	1.9	0.00348	5.1	51
34	/	Soluble	10.45	0.27	5.12	0.82	2	0.01269	4.8	22
35	/	Soluble	4.48	0.24	2.27	0.49	2	0.02877	7.6	23
36	/	Soluble	8.51	0.36	4.33	1.19	2	0.04154	8.8	31
37	/	Soluble	1.49	0.13	0.72	0.18	2.1	0.04016	6.2	13
38	/	Soluble	11.16	0.24	5.09	1.64	2.2	0.03534	5.8	13
39	/	Soluble	13.17	0.36	5.67	0.21	2.3	0.00152	8	16
40	/	Soluble	6.33	0.28	2.79	0.41	2.3	0.00981	5.5	22
41	/	Soluble	8.31	0.47	3.47	0.6	2.4	0.01233	5.1	12
42	/	Soluble	3.26	0.33	1.15	0.19	2.8	0.01579	4.1	15
43	/	Soluble	7.41	0.22	2.52	1.13	2.9	0.02654	4	39
44	/	Soluble	2.21	0.13	0.73	0.42	3	0.04157	6.4	15
45	/	Soluble	10.7	0.74	3.56	2.1	3	0.04548	5.3	16

46	/	Soluble	0.49	0.05	0.17	0.02	3	0.01331	9.1	29
47	/	Soluble	10.05	2.02	3.38	0.39	3	0.04428	8.8	12
48	/	Soluble	2.45	0.08	0.8	0.13	3.1	0.00441	8.6	19
49	/	Soluble	10.74	1.92	3.21	0.46	3.3	0.03268	7	14
50	/	Soluble	3.08	0.16	0.92	0.4	3.3	0.01948	4.5	15
51	/	Soluble	4.12	0.82	1.18	0.43	3.5	0.04647	6.7	12
52	/	Soluble	5.11	0.51	1.47	0.7	3.5	0.02728	5.3	14
53	/	Soluble	1.52	0.07	0.41	0.29	3.7	0.03449	3.6	29
54	/	Soluble	2.03	0.16	0.55	0.06	3.7	0.00683	5.9	16
55	/	Soluble	10.19	0.62	2.79	1.14	3.7	0.01496	8	14
56	/	Soluble	2.53	0.39	0.44	0.28	5.7	0.02565	5.5	13
57	/	Soluble	8.14	0.86	1.14	0.29	7.1	0.00832	8.7	13
58	/	Membrane	28.24	0.34	25.3	0.28	1.1	0.01127	5.5	42
59	/	Membrane	29.74	0.31	27.12	0.34	1.1	0.01499	7.3	56
60	/	Membrane	21.95	1.09	18.36	0.39	1.2	0.04806	5.8	30
61	/	Membrane	22.99	0.71	17.78	1.01	1.3	0.02674	5.9	63
62	/	Membrane	5.18	0.15	3.97	0.15	1.3	0.01486	6.2	45
63	/	Membrane	8.3	0.42	6.1	0.34	1.4	0.02855	6.2	52
64	/	Membrane	7.29	0.21	5.17	0.09	1.4	0.00588	6.5	55
65	/	Membrane	6.17	0.07	4.3	0.03	1.4	0.00076	3.9	34
66	/	Membrane	8.82	0.13	5.99	0.8	1.5	0.03852	7.4	60
67	/	Membrane	26.05	2.7	17.41	0.59	1.5	0.04761	7.2	30
68	/	Membrane	3.68	0.04	2.38	0.09	1.5	0.00264	7.9	56
69	/	Membrane	141.86	11.66	205.41	0.88	1.5	0.01651	5.7	45
70	/	Membrane	18.65	1.06	11.56	0.45	1.6	0.01286	4.9	19
71	/	Membrane	15.37	1.99	8.69	0.69	1.8	0.04621	5.9	244
72	/	Membrane	5.64	0.77	3.18	0.24	1.8	0.04989	5.7	39
73	/	Membrane	11.9	0.81	6.67	1.02	1.8	0.02974	4.7	19
74	/	Membrane	9.81	1.12	5.19	0.62	1.9	0.03645	4.4	64
75	/	Membrane	3.35	0.43	1.77	0.14	1.9	0.03894	7	25
76	/	Membrane	7.27	0.33	3.79	1	1.9	0.04259	6.6	41
77	/	Membrane	84.51	15.59	159.1	15.82	1.9	0.04159	5.5	92
78	/	Membrane	0.65	0.08	0.33	0.04	2	0.03652	6.9	23
79	/	Membrane	6.3	0.88	3.13	0.54	2	0.04918	7	28
80	/	Membrane	8.19	0.88	4.05	0.53	2	0.02932	4.8	39
81	/	Membrane	20.65	1	10.17	1.98	2	0.0216	7.2	68
82	/	Membrane	3.06	0.37	1.48	0.18	2.1	0.03197	6.2	42
83	/	Membrane	2.56	0.04	1.22	0.03	2.1	0.00066	7.4	38
84	/	Membrane	5.76	0.13	2.69	0.24	2.1	0.0039	8	55
85	/	Membrane	3.76	0.07	1.72	0.63	2.2	0.04478	6.4	26
86	/	Membrane	6.07	0.33	2.77	0.46	2.2	0.01427	4	20
87	/	Membrane	8.08	0.36	3.59	0.14	2.2	0.00374	4.2	26
88	/	Membrane	22.13	1.2	9.7	0.07	2.3	0.00462	5.1	24
89	/	Membrane	12.7	1.85	5.34	0.65	2.4	0.03378	5.2	29
90	/	Membrane	4.3	0.33	1.62	0.8	2.7	0.04836	6.2	40
91	/	Membrane	5.2	0.16	1.95	0.61	2.7	0.01829	6.3	34
92	/	Membrane	3.22	0.37	1.2	0.35	2.7	0.02975	4.1	87
93	/	Membrane	8.3	1.38	2.97	0.28	2.8	0.03327	4.9	24
94	/	Membrane	3.63	0.12	1.29	0.06	2.8	0.00169	7.6	49
95	/	Membrane	11.06	0.12	3.81	0.22	2.9	0.0006	5.9	27
96	/	Membrane	4.17	0.32	1.43	0.39	2.9	0.01647	3.8	39
97	/	Membrane	11.9	0.07	4.08	0.01	2.9	0.00004	6.2	27
98	/	Membrane	2.01	0.23	0.67	0.26	3	0.03216	4	17
99	/	Membrane	4.02	0.51	1.33	0.04	3	0.01749	4.1	63
100	/	Membrane	2.83	0.22	0.92	0.11	3.1	0.008	4.4	21
101	/	Membrane	4.04	0.09	1.31	0.44	3.1	0.01305	6.1	32



102	/	Membrane	6.69	0.74	2.14	0.49	3.1	0.01844	6.5	29
103	/	Membrane	11.27	0.1	3.53	0.38	3.2	0.00129	3.7	45
104	/	Membrane	14.78	0.52	4.18	3.29	3.5	0.04595	4.2	28
105	/	Membrane	7.59	1.32	2.12	0.42	3.6	0.03043	4.6	25
106	/	Membrane	5.2	0.31	1.44	0.3	3.6	0.00646	6.2	28
107	/	Membrane	7.03	0.14	1.86	0.23	3.8	0.0014	4.4	20
108	/	Membrane	10.59	1.27	2.76	0.21	3.8	0.01324	4.9	21
109	/	Membrane	4.47	0.6	1.03	0.46	4.4	0.0232	5.6	18
110	/	Membrane	5.77	0.19	1.3	0.66	4.5	0.01145	5.2	23
111	/	Membrane	3.15	0.02	0.66	0.01	4.8	0.00003	5.8	22
112	/	Membrane	6.25	1.62	1.15	0.28	5.4	0.04808	7.1	16
113	/	Membrane	9.59	0.08	1.73	1.29	5.6	0.0132	7.2	24
114	/	Membrane	6.93	0.71	1.14	0.44	6.1	0.01033	4.3	59
115	/	Membrane	4.55	0.7	0.7	0.12	6.5	0.01674	5.9	23
116	/	Membrane	4.39	0.35	0.59	0.52	7.5	0.01318	6.2	37
117	/	Membrane	4.71	0.47	0.48	0.27	9.8	0.00816	6.2	23

**Table S2.** Quantitative comparison of CB formulation xii and SR staining of mouse brain proteomes. Mouse brains were pre-fractionated into soluble and membrane protein fractions, resolved by 2DE, and stained with either SR or CB formulation xii (Figure 7). Quantitative image analysis indicated proteins that were detected by both staining methods, but differed significantly in fluorescent signal volume (FV) between the two (T-test,  $p < 0.05$ ,  $n = 4$ ). All significant quantitative differences are summarized here, 1.5-fold or greater differences are indicated on gel images, Figure 7.

Stain Formulation:		SR (i)		CB xii		Fold Difference			
Spot No. / Fraction		FV	SD	NV	SD		T-test (p)	pl	MW
118 / Soluble		20.69	0.5	18.18	0.54	1.1	0.04016	5.7	73
119 / Soluble		21.14	0.16	19.07	0.46	1.1	0.02592	6.9	96
120 / Soluble		13.17	0.36	11.03	0.25	1.2	0.0201	7.9	17
121 / Soluble		10.28	0.31	8.43	0.45	1.2	0.04125	7	25
122 / Soluble		4.27	0.19	5.3	0.22	1.2	0.03827	6.7	71
123 / Soluble		65.51	2.5	50.97	2.78	1.3	0.03148	8.5	57
124 / Soluble		34.11	1.8	25.74	0.89	1.3	0.02757	7.5	65
125 / Soluble		29.23	1.79	21.78	1.17	1.3	0.03869	5.2	114
126 / Soluble		28.17	0.88	21.22	0.58	1.3	0.01137	5.4	24
127 / Soluble		13.79	0.78	10.94	0.32	1.3	0.04138	8.2	58
128 / Soluble		10.45	0.27	8.06	0.68	1.3	0.04329	5	22
129 / Soluble		2.47	0.18	1.85	0.03	1.3	0.03834	6.6	37
130 / Soluble		6.02	0.05	7.89	0.4	1.3	0.02246	6.8	70
131 / Soluble		7.08	0.57	9.05	0.21	1.3	0.04375	7.4	52
132 / Soluble		193.29	12.73	254.66	13.03	1.3	0.04134	4.7	67
133 / Soluble		34.98	1.27	25.4	2.62	1.4	0.0432	5.9	11
134 / Soluble		22.68	0.72	16.66	1.45	1.4	0.03436	8.4	63
135 / Soluble		9.59	0.27	6.9	0.72	1.4	0.03876	5.6	26
136 / Soluble		5.99	0.18	4.19	0.06	1.4	0.00562	6.6	52
137 / Soluble		2.76	0.13	1.99	0.05	1.4	0.01654	5.3	22
138 / Soluble		11.66	0.29	16.69	0.75	1.4	0.01259	4.8	34
139 / Soluble		11.93	0.85	17.34	0.64	1.5	0.01889	5.4	157
140 / Soluble		28.88	0.31	18.15	1.98	1.6	0.017	6.9	76
141 / Soluble		4.48	0.24	2.82	0.29	1.6	0.02494	7.5	24
142 / Soluble		2.74	0.21	1.58	0.17	1.7	0.02605	7.8	44
143 / Soluble		25.48	0.17	14.65	0.23	1.7	0.00035	6.5	68
144 / Soluble		10.06	0.35	5.93	0.53	1.7	0.01173	8.5	23
145 / Soluble		6.33	0.28	3.8	0.05	1.7	0.00626	5.6	23
146 / Soluble		3.08	0.16	1.86	0.06	1.7	0.00981	4.6	15
147 / Soluble		1.9	0.07	3.45	0.42	1.8	0.03542	7.8	65
148 / Soluble		5.38	0.54	9.58	0.02	1.8	0.00815	7.2	68
149 / Soluble		74.41	9.57	132.06	12.38	1.8	0.03492	3.2	16
150 / Soluble		8.89	0.87	4.66	0.57	1.9	0.02876	5.9	27
151 / Soluble		2.45	0.08	1.32	0.09	1.9	0.0054	8.5	20
152 / Soluble		3.1	0.45	5.75	0.36	1.9	0.02281	7	53
153 / Soluble		8.74	0.08	16.5	1.5	1.9	0.01816	5.7	54
154 / Soluble		7.72	0.33	3.88	0.34	2	0.00757	6.8	129
155 / Soluble		2.34	0.34	1.08	0.15	2.2	0.04005	6.4	25
156 / Soluble		2.07	0.23	4.63	0.13	2.2	0.00509	6.7	31
157 / Soluble		2.53	0.39	0.9	0.06	2.8	0.02807	5.6	13
158 / Soluble		2.03	0.56	6.62	0.49	3.3	0.01296	7.2	117
159 / Soluble		2.21	0.13	0.62	0.33	3.6	0.02356	6.3	15
160 / Soluble		2.03	0.16	0.49	0.33	4.1	0.02667	5.9	17
161 / Soluble		0.89	0.5	7.34	0.03	8.2	0.00302	4.1	64

162	/	Membrane	24.09	0.03	25.11	0.11	1	0.00545	6.5	62
163	/	Membrane	30.64	0.07	32.67	0.33	1.1	0.01326	7.2	90
164	/	Membrane	29.74	0.31	32.17	0.42	1.1	0.02189	7.3	56
165	/	Membrane	71.81	1.72	81.09	1.19	1.1	0.02456	5.6	61
166	/	Membrane	31.49	0.33	21.8	2.97	1.4	0.04422	5.9	69
167	/	Membrane	22.13	1.2	15.28	1.48	1.4	0.03631	5	24
168	/	Membrane	13.09	0.99	9.03	0.54	1.4	0.03643	5.5	27
169	/	Membrane	11.9	0.81	8.42	0.38	1.4	0.03143	4.6	18
170	/	Membrane	7.97	0.51	11.03	0.12	1.4	0.01445	7	90
171	/	Membrane	6.07	0.33	4.17	0.21	1.5	0.02022	4	19
172	/	Membrane	5.2	0.16	3.35	0.49	1.6	0.03692	6.3	31
173	/	Membrane	3.76	0.07	2.29	0.47	1.6	0.0485	6.4	27
174	/	Membrane	25.55	1.68	15.03	0.68	1.7	0.01449	5.5	142
175	/	Membrane	5.69	0.56	3.24	0.18	1.8	0.02718	5.9	29
176	/	Membrane	4.3	0.33	2.4	0.29	1.8	0.02605	6.1	35
177	/	Membrane	8.67	0.47	4.76	0.43	1.8	0.01314	8	59
178	/	Membrane	9.59	0.08	5.18	0.6	1.9	0.0094	7.2	24
179	/	Membrane	3.68	0.04	1.98	0.21	1.9	0.00807	7.9	53
180	/	Membrane	7.6	0.96	4.06	0.14	1.9	0.03565	8	64
181	/	Membrane	12.38	0.41	6.45	1.39	1.9	0.02855	8	27
182	/	Membrane	20.65	1	10.53	1.59	2	0.01676	7.2	68
183	/	Membrane	2.83	0.22	1.4	0.36	2	0.04051	4.3	21
184	/	Membrane	7.46	0.22	3.6	0.86	2.1	0.02566	4.2	142
185	/	Membrane	22.99	0.71	11.08	3.67	2.1	0.04585	5.9	63
186	/	Membrane	8.29	0.31	4	0.63	2.1	0.01301	5.9	75
187	/	Membrane	5.2	0.31	2.5	0.23	2.1	0.00998	6.1	28
188	/	Membrane	2.4	0.04	1.15	0.07	2.1	0.00173	6.4	14
189	/	Membrane	11.27	0.1	5.27	0.76	2.1	0.00803	3.8	48
190	/	Membrane	11.06	0.12	5.15	0.29	2.1	0.00137	5.8	27
191	/	Membrane	7.03	0.14	3.26	0.54	2.2	0.0109	4.4	20
192	/	Membrane	4.04	0.09	1.85	0.62	2.2	0.03864	6.1	30
193	/	Membrane	2.93	0.34	1.31	0.07	2.2	0.02167	4.1	120
194	/	Membrane	3.87	0.48	1.67	0.39	2.3	0.03701	6.3	148
195	/	Membrane	4.17	0.32	1.74	0.35	2.4	0.01847	3.8	39
196	/	Membrane	4.39	0.35	1.71	0.35	2.6	0.01673	6.1	33
197	/	Membrane	9.03	0.71	3.23	0.71	2.8	0.0147	8.1	80
198	/	Membrane	2.36	0.08	0.77	0.14	3.1	0.00508	5.1	15
199	/	Membrane	4.71	0.47	1.53	0.66	3.1	0.0312	6.1	24
200	/	Membrane	2.03	0.04	0.66	0.01	3.1	0.0005	6.1	22