Supplementary Information

Interaction of Myelin Basic Protein with Cytoskeletal and Signaling Proteins in Cultured Primary Oligodendrocytes and N19 Oligodendroglial Cells

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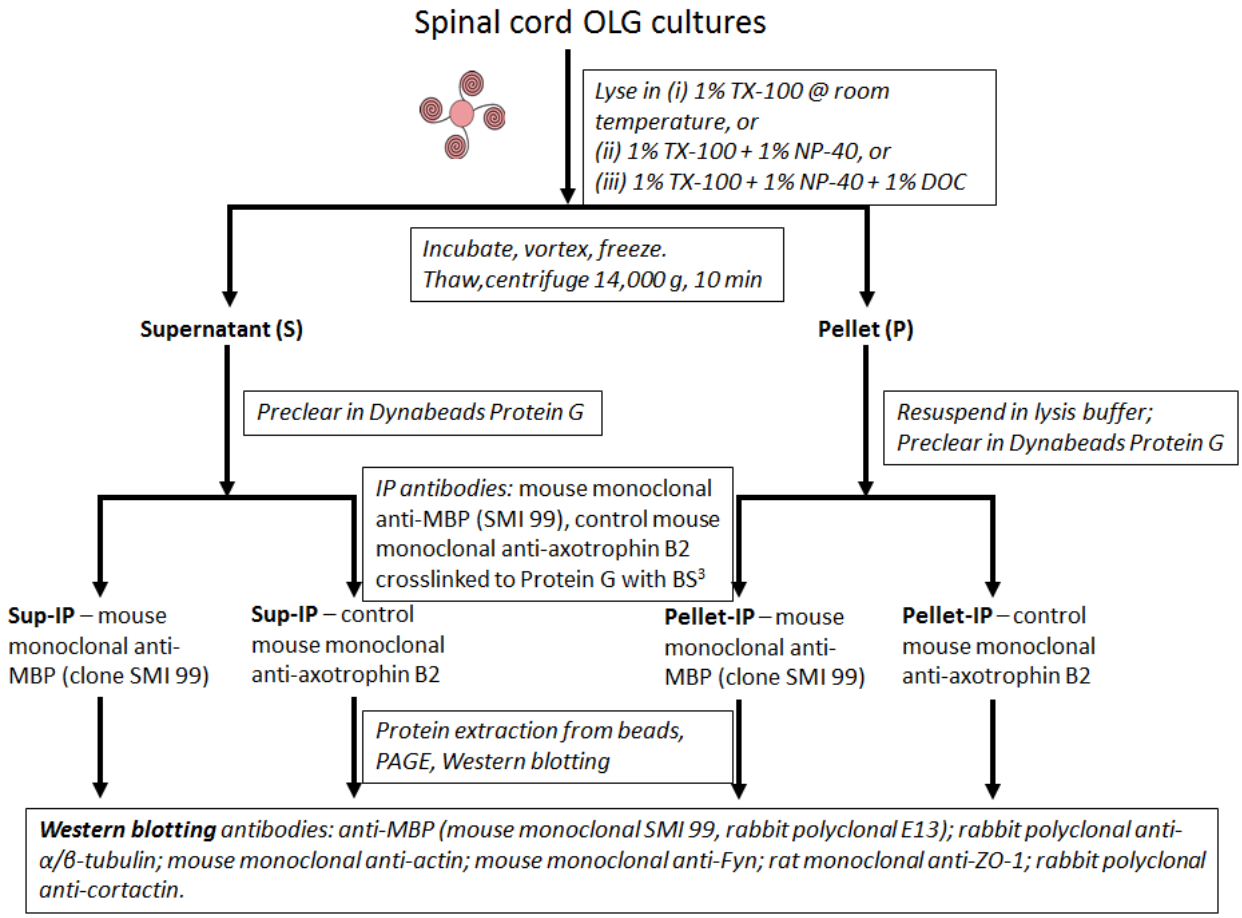
Supplementary Figure S1. Flowchart of procedures used for cell extraction, immunoprecipitation, and western blotting.



Supplementary Table S1. Interpretation of **Figure 1** – Western blot of primary OLGs extracted with buffer containing 1% TX-100. Samples were immunoprecipitated with anti-MBP (SMI 99) or control Abs, and detected using rabbit polyclonal anti-MBP (E13), mouse monoclonal anti-MBP (SMI 99), rabbit polyclonal anti-tubulin, and mouse monoclonal anti-actin antibodies.

(Abbreviations: "n.d." = "not done"; "-" = "*nil*"; l.c. = IgG light chain; h.c. = IgG heavy chain.)

Figure-Lane	**Description**	**Blotting 1°-Ab**	**Detection**	**Interpretation**
F1-L1	Supernatant	MBP E13	3 bands, 16, 24, 27 kDa	14-, 18.5-, 21.5-kDa MBP isoforms
		MBP SMI 99	2 bands, 24, 27 kDa	18.5-, 21.5-kDa MBP isoforms
		Tubulin	1 band, 55 kDa	Tubulin
		Actin	1 band, 45 kDa (see Fig. 2a,b)	Actin
F1-L2	Sup-IP anti-MBP SMI 99	MBP E13	3 bands, 16, 24, 27 kDa; 1band, 30 kDa	14-, 18.5-, 21.5-kDa MBP isoforms; IgG l.c. (after reaction with BS^3)
		MBP SMI 99	2 bands, 24, 27 kDa; 1 band, 30 kDa	18.5-, 21.5-kDa MBP isoforms; IgG l.c. (after reaction with BS^3)
		Tubulin	-	
		Actin	1 band, 45 kDa	Actin
F1-L3	Sup-IP control	MBP E13	3 bands, 16, 24, 27 kDa; 1 band, 30 kDa	14-, 18.5-, 21.5-kDa MBP isoforms; IgG l.c. (after reaction with BS^3)
		MBP SMI 99	2 bands, 24, 27 kDa; 1 band, 30 kDa	18.5-, 21.5-kDa MBP isoforms; IgG l.c. (after reaction with BS^3)
		Tubulin	-	
		Actin	1 band, 45 kDa	Actin
F1-L4	Pellet	MBP E13	3 weak bands, 16, 24, 27 kDa	14-, 18.5-, 21.5-kDa isoforms of MBP
		MBP SMI 99	2 bands, 24, 27 kDa	18.5-, 21.5-kDa MBP isoforms

		Tubulin	1 band, 55 kDa	Tubulin
		Actin	1 band, 45 kDa	Actin
F1-L5	Pellet-IP anti-MBP SMI 99	MBP E13	3 bands, 16, 24, 27 kDa; 1 band, 30 kDa	14-, 18.5-, 21.5-kDa MBP isoforms; IgG l.c. (after reaction with BS^3)
		MBP SMI 99	2 bands, 24, 27 kDa; 1 band, 30 kDa	18.5-, 21.5-kDa MBP isoforms; IgG l.c. (after reaction with BS^3)
		Tubulin	1 band, 55 kDa	Tubulin
		Actin	1 band, 45 kDa	Actin
F1-L6	Pellet-IP control	MBP E13	4 weak bands, 16, 24, 27, 30 kDa	14-, 18.5-, 21.5-kDa MBP isoforms; IgG l.c. (reacted with BS^3)
		MBP SMI 99	3 bands, 24, 27, 30 kDa	18.5-, 21.5-kDa MBP isoforms; IgG l.c. (reacted with BS^3)
		Tubulin	-	
		Actin	1 band, 45 kDa	Actin
F1-L7	IgG anti-MBP SMI 99	MBP E13	-	
		MBP SMI 99	-	
		Tubulin	-	
		Actin	1 band, 55 kDa	IgG h.c.
F1-L8	IgG control	MBP E13	-	
		MBP SMI 99	1 band, 28 kDa	IgG l.c.
		Tubulin	-	
		Actin	1 band, 55 kDa	IgG h.c.

Supplementary Table S2. Interpretation of **Figure 2(a,b)** – Western blot of primary OLGs extracted with buffer containing 1% TX-100 plus 1% NP40. Samples were immunoprecipitated with anti-MBP (SMi 99) or a control Ab, and detected using rabbit polyclonal anti-MBP (E13), rabbit polyclonal anti-tubulin, mouse monoclonal anti-actin, rat monoclonal anti-ZO-1, and mouse monoclonal anti-Fyn antibodies.

(Abbreviations: "n.d." = "not done"; "-" = "*nil*"; l.c. = IgG light chain; h.c. = IgG heavy chain; 1^{o}-Ab = primary antibody.)

Figure-Lane	**Description**	**Blotting 1^{o}-Ab**	**Detection**	**Interpretation**
F2-L1	Standard	MBP E13	1 band, 24 kDa	18.5-kDa MBP
		Tubulin	1 band, 55 kDa	Tubulin
		Actin	1 band, 45 kDa	Actin
		ZO-1	n.d.	
		Fyn	n.d.	
F2-L2	Supernatant	MBP E13	4 bands, 16, 23, 24, 27 kDa	14-, 17-, 18.5-, 21.5-MBP isoforms
		Tubulin	1 band, 55 kDa	Tubulin
		Actin	1 band, 45 kDa	Actin
		ZO-1	several bands, 140-220 kDa	ZO-1 isoforms
		Fyn	1 band, 60 kDa	Fyn
F2-L3	Sup-IP anti-MBP SMI 99	MBP E13	4 bands, 16, 23, 24, 27 kDa	14-, 17-, 18.5-, 21.5-MBP isoforms
		Tubulin	1 band, 55 kDa	Tubulin
		Actin	1 band, 45 kDa	Actin
		ZO-1	several bands, 140-220 kDa	ZO-1 isoforms
		Fyn	1 band, 60 kDa	Fyn
F2-L4	Sup-IP control	MBP	2 bands, 16, 24 kDa	14-, 18.5-kDa MBP isoforms
		Tubulin	1 weak band, 55 kDa	Tubulin
		Actin	1 band, 45 kDa	Actin
		ZO-1	1 band, 220 kDa	ZO-1 isoform
		Fyn	1 weak band, 60 kDa; 1 band, 75 kDa	Fyn; crosslinked IgG l.c. + h.c.
F2-L5	Pellet	MBP	3 weak bands, 23, 24, 27 kDa	17-, 18.5-, 21.5-kDa MBP isoforms
		Tubulin	1 band, 55 kDa	Tubulin
		Actin	1 band, 45 kDa	Actin
		ZO-1	1 band, 220 kDa	ZO-1 isoform

		Fyn	1 band, 60 kDa	Fyn
F2-L6	Pellet-IP anti-MBP SMI 99	MBP	4 bands, 16, 23, 24, 27 kDa	14-, 17-, 18.5-, 21.5-kDa MBP isoforms
		Tubulin	1 band, 55 kDa	Tubulin
		Actin	1 band, 45 kDa	Actin
		ZO-1	several bands, 140-220 kDa	ZO-1 isoforms
		Fyn	1 band, 60 kDa	Fyn
F2-L7	Pellet-IP control	MBP	4 bands, 16, 23, 24, 27 kDa	14-, 17-, 18.5-, 21.5-kDa MBP isoforms
		Tubulin	1 band, 55 kDa	Tubulin
		Actin	1 band, 45 kDa	Actin
		ZO-1	1 band, 220 kDa	ZO-1 isoform
		Fyn	1 band, 60 kDa; 1 band, 75 kDa	Fyn; crosslinked IgG l.c. + h.c.
F2-L8	IgG anti-MBP SMI 99	MBP	-	
		Tubulin	-	
		Actin	1 band, 55 kDa	IgG h.c.
		ZO-1	-	
		Fyn	1 band, 55 kDa	IgG h.c.
F2-L9	IgG control	MBP	-	
		Tubulin	-	
		Actin	1 band, 55 kDa	IgG h.c.
		ZO-1	-	
		Fyn	1 band, 55 kDa	IgG h.c.

Supplementary Table S3. Interpretation of **Figure 3** – Western blot of primary OLGs extracted with buffer containing 1% TX-100 plus 1% NP40. Samples were immunoprecipitated using mouse monoclonal anti-MBP (SMI 99) or control antibodies, and detected using rabbit polyclonal anti-cortactin antibody.

(Abbreviations: "n.d." = "not done"; "-" = "*nil*".)

Figure-Lane	**Description**	**Blotting 1^{o}-Ab**	**Detection**	**Interpretation**
F3-L1	Supernatant	cortactin	2 bands, 78, 82 kDa	cortactin isoforms
F3-L2	Blank	cortactin	-	
F3-L3	Pellet	cortactin	2 bands, 78, 82 kDa	cortactin isoforms
F3-L4	Sup-IP anti-MBP SMI 99	cortactin	2 bands, 78, 82 kDa	cortactin isoforms
F3-L5	Sup-IP control	cortactin	2 weak bands, 78, 82 kDa	cortactin isoforms
F3-L6	Blank	cortactin	-	
F3-L7	Pellet-IP anti-MBP SMI 99	cortactin	2 bands, 78, 82 kDa	cortactin isoforms
F3-L8	Pellet-IP control	cortactin	2 bands, 78, 82 kDa	cortactin isoforms
F3-L9	IgG anti-MBP SMI 99	cortactin	-	
F3-L10	IgG control		-	

Supplementary Table S4. Interpretation of **Figure 4** – Western blot of primary OLGs extracted with buffer containing 1% TX-100 plus 1% NP40 plus 1% DOC. Samples were immunoprecipitated with monoclonal anti-MBP (SMI 99) or control antibodies, and detected using rabbit polyclonal anti-MBP (E13), rabbit polyclonal anti-tubulin, mouse monoclonal anti-actin, and rat monoclonal anti-ZO-1 antibodies.

(Abbreviations: "n.d." = "not done"; "-" = "*nil*".)

Figure-Lane	**Description**	**Blotting 1°-Ab**	**Detection**	**Interpretation**
F4-L1	Standard	MBP	1 band, 24 kDa	18.5 kDa MBP isoform
		Tubulin	1 band, 55 kDa	Tubulin
		Actin	1 band, 45 kDa (see Fig. 2)	Actin
		ZO-1	n.d.	
F4-L2	Supernatant	MBP	3 bands, 16, 24, 27 kDa	14, 18.5, 21.5 kDa MBP isoforms
		Tubulin	1 heavy band, 55 kDa	Tubulin
		Actin	1 heavy band, 45 kDa	Actin
		ZO-1	3 bands, 140-220 kDa	ZO-1 isoforms
F4-L3	Sup-IP anti-MBP SMI 99	MBP	2 bands, 24, 27 kDa; 1 band 30 kDa	18.5, 21.5 kDa MBP isoforms; IgG l.c. (after reaction with BS^3)
		Tubulin	-	
		Actin	1 weak band, 45 kDa	Actin
		ZO-1	-	
F4-L4	Sup-IP control	MBP	1 band, 30 kDa	IgG l.c. (after reaction with BS^3)
		Tubulin	-	
		Actin	1 weak band, 45 kDa	Actin
		ZO-1	-	
F4-L5	Pellet	MBP	3 weak bands, 16, 24, 27 kDa	14, 18.5, 21.5 kDa MBP isoforms
		Tubulin	1 band, 55 kDa	Tubulin
		Actin	1 band, 45 kDa	Actin
		ZO-1	-	
F4-L6	Pellet-IP anti-MBP SMI 99	MBP	1 band, 30 kDa	IgG l.c. (after reaction with BS^3)

		Tubulin	-	
		Actin	-	
		ZO-1	-	
F4-L7	Pellet-IP control	MBP	1 band, 30 kDa	IgG l.c. (after reaction with BS^3)
		Tubulin	-	
		Actin	-	
		ZO-1	-	
F4-L8	IgG anti-MBP SMI 99	MBP	-	
		Tubulin	-	
		Actin	-	
		ZO-1	-	
F4-L9	IgG control	MBP	-	
		Tubulin	-	
		Actin	-	
		ZO-1	-	