Supplementary information

Simple and highly efficient detection of PSD95 using a nanobody and its recombinant minibody derivative

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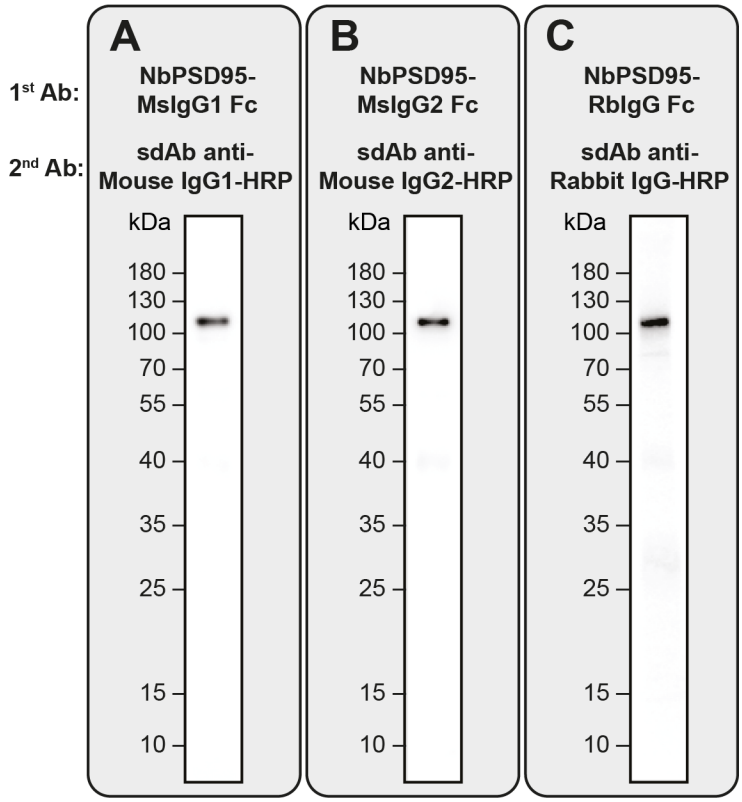

Figure S1: anti-PSD95 minibodies harboring different Fc domains specifically recognize PSD95 in Western blot applications.

Total mouse brain lysates were analyzed by Western blot using minibodies consisting of NbPSD95 fused to a Fc domain from either mouse IgG1 (MsIgG1 Fc; **A**), mouse IgG2 (MsIgG2 Fc; **B**) or rabbit IgG (RbIgG Fc; **C**). Detection was performed using HRP-coupled secondary nanobodies recognizing the respective Fc domains. All three minibodies detected PSD95 with similar specificity.

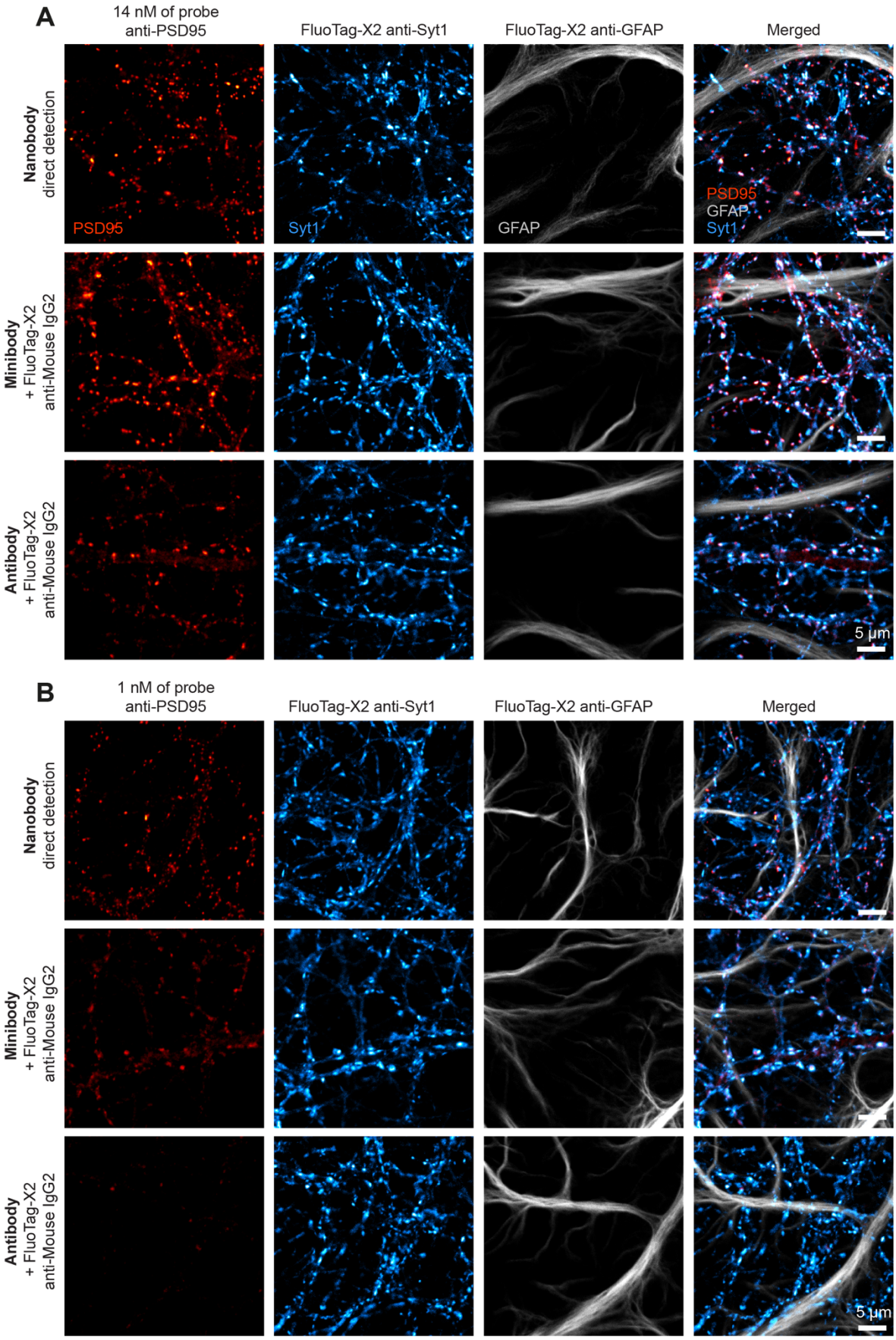

Figure S2: Comparison of NbPSD95 with an anti-PSD95 minibody and a conventional antibody – related to Fig. 3. Figure shows separate channels used for creating overlays displayed in Fig. 3.

Table S1: Antibodies

Figure	Panel(s)	Product	Product number	Provider	Description	Application	Dilution/ Conc.
Fig. 2A		Monoclonal anti-FLAG HRP	A8592	Merck	HRP-conjugated anti-FLAG antibody (clone M2)	ELISA	1:5000
Fig. 2B	upper panels	FluoTag-X2 anti-PSD95 AZDye568	N3702-AF568-L	NanoTag Biotechnologies	Fluorophore-conjugated NbPSD95	IF, direct	1:500
Fig. 2C	upper left panel	Recombinant anti-PSD95 Antibody (Minibody), Mouse IgG1 Fc fusion	N3782	NanoTag Biotechnologies	NbPSD95 fused to mouse IgG1 Fc domain	WB, 1st Ab	1:1000
Fig. 2C	lower panels	Mouse anti-beta-Actin	251 011	Synaptic Systems	Monoclonal antibody	WB, 1st Ab	1:1000
Fig. 2C	upper right panel + lower panels	sdAb anti-MsIgG1 HRP	N2005-HRP	NanoTag Biotechnologies	HRP-conjugated anti-Mouse IgG1 nanobody	WB, 2nd Ab	1:4000
Fig. 2C	upper right panel	Recombinant anti-PSD95 Antibody (Minibody), Rabbit Fc fusion	N3783	NanoTag Biotechnologies	NbPSD95 fused to rabbit IgG Fc domain	WB, 1st Ab	1:1000
Fig. 2C	upper right panel	sdAb anti-RbIgG HRP	N2405-HRP	NanoTag Biotechnologies	HRP-conjugated anti-Rabbit IgG nanobody	WB, 2nd Ab	1:4000
Fig. 2D		FluoTag-X2 anti-PSD95 AZDye568	N3702-AF568-L	NanoTag Biotechnologies	Fluorophore-conjugated NbPSD95	IF, direct	1:500
Fig. 2D		FluoTag-X2 anti-GFAP Atto488	N3802-At488-L	NanoTag Biotechnologies	Fluorophore-conjugated anti-GFAP nanobody	IF, direct	1:500
Fig. 3A, Fig. 3B	upper row	FluoTag-X2 anti-PSD95 AbberiorStar635P	N3702-Ab635P-L	NanoTag Biotechnologies	Fluorophore-conjugated NbPSD95	IF, direct IF, direct	14 nM 1 nM
Fig. 3A, Fig. 3B	middle row	Recombinant anti-PSD95 Antibody (Minibody), Mouse IgG2 Fc fusion	N3785	NanoTag Biotechnologies	NbPSD95 fused to mouse IgG2 Fc domain	IF, 1st Ab IF, 1st Ab	14 nM 1 nM
Fig. 3A, Fig. 3B	lower row	Monoclonal anti-PSD95 antibody	MABN68	Millipore	Monoclonal antibody (Mouse IgG2)	IF, 1st Ab IF, 1st Ab	14 nM 1 nM
Fig. 3A, B	two lower rows	FluoTag-X2 anti-Mouse IgG2 AbberiorStar635P	N2702-Ab635P	NanoTag Biotechnologies	Fluorophore-conjugated anti-Mouse IgG2 nanobody	IF. 2nd Ab	1:500
Fig. 3		FluoTag-X2 anti-Syt1 AZDye568	N2302-AF568-L	NanoTag Biotechnologies	Fluorophore-conjugated anti-Synaptotagmin1 nanobody	IF, direct	1:500
Fig. 3		FluoTag-X2 anti-GFAP Atto488	N3802-At488-L	NanoTag Biotechnologies	Fluorophore-conjugated anti-GFAP nanobody	IF. direct	1:500

Table S1: Antibodies (continued)

Figure	Panel(s)	Product	Product number	Provider	Description	Application	Dilution/ Conc.
Fig. 4		FluoTag-X2 anti-PSD95 AbberiorStar635P	N3702-Ab635P-L	NanoTag Biotechnologies	Fluorophore-conjugated NbPSD95	IF, direct	1:500
Fig. 4		FluoTag-X2 anti-Syt1 AZDye568	N2302-AF568-L	NanoTag Biotechnologies	Fluorophore-conjugated anti-Synaptotagmin1 nanobody	IF, direct	1:500
Fig. 4		FluoTag-X2 anti-GFAP Atto488	N3802-At488-L	NanoTag Biotechnologies	Fluorophore-conjugated anti-GFAP nanobody	IF, direct	1:500
Fig. 5		FluoTag-X2 anti-PSD95 Sulfo-Cy3	N3702-SC3-L	NanoTag Biotechnologies	Fluorophore-conjugated NbPSD95	IHC, direct	1:500
Fig. 6		FluoTag-X2 anti-PSD95 AbberiorStar635P	N3702-Ab635P-L	NanoTag Biotechnologies	Fluorophore-conjugated NbPSD95	IHC, direct	1:500
Fig. 6		Recombinant anti-Synaptotagmin antibody	105 008	Synaptic Systems	Rabbit monoclonal recombinant antibody	IHC, 1st Ab	2 µg/mL
Fig. 6		FluoTag-X2 anti-Rabbit IgG AbberiorStar580	N2402-Ab580-L	NanoTag Biotechnologies	Fluorophore-conjugated anti-Rabbit IgG2 nanobody	IHC, 2nd Ab	1:500
Fig. 7		Recombinant anti-PSD95 Antibody (Minibody), Rabbit Fc fusion	N3783	NanoTag Biotechnologies	NbPSD95 fused to rabbit IgG Fc domain	IHC-P, 1st Ab	1 µg/mL
Fig. 7		Secondary anti-Rabbit Biotin	111-065-144	Jackson Immuno Research	Biotin-SP AffiniPure Goat Anti-Rabbit IgG	IHC-P, 2nd Ab	5 µg/ml
Fig. 7		Avidin-Biotin Complex (ABC)-HRP Kit	PK-4000	Vector Laboratories	ABC-HRP Kit	IHC-P, amplification	prepared according to manufacturer's instructions

Table S1: Antibodies (continued)

Figure	Panel(s)	Product	Product number	Provider	Description	Application	Dilution/ Conc.
Fig. S1A		Recombinant anti-PSD95 Antibody (Minibody), Mouse IgG1 Fc fusion	N3782	NanoTag Biotechnologies	NbPSD95 fused to mouse IgG1 Fc domain	WB, 1st Ab	1:1000
Fig. S1A		sdAb anti-MsIgG1 HRP	N2005-HRP	NanoTag Biotechnologies	HRP-coupled anti-Mouse IgG1 nanobody	WB, 2nd Ab	1:4000
Fig. S1B		Recombinant anti-PSD95 Antibody (Minibody), Mouse IgG2 Fc fusion	N3785	NanoTag Biotechnologies	NbPSD95 fused to mouse IgG2 Fc domain	WB, 1st Ab	1:1000
Fig. S1B		sdAb anti-MsIgG2 HRP	N2705-HRP	NanoTag Biotechnologies	HRP-coupled anti-Mouse IgG2 nanobody	WB, 2nd Ab	1:4000
Fig. S1C		Recombinant anti-PSD95 Antibody (Minibody), Rabbit Fc fusion	N3783	NanoTag Biotechnologies	NbPSD95 fused to rabbit IgG Fc domain	WB, 1st Ab	1:1000
Fig. S1C		sdAb anti-RbIgG HRP	N2405-HRP	NanoTag Biotechnologies	HRP-coupled anti-Rabbit IgG nanobody	WB, 2nd Ab	1:4000
Fig. S2A, Fig. S2B	1st rows	FluoTag-X2 anti-PSD95 AbberiorStar635P	N3702-Ab635P-L	NanoTag Biotechnologies	Fluorophore-conjugated NbPSD95	IF, direct IF, direct	14 nM 1 nM
Fig. S2A, Fig. S2B	2nd rows	Recombinant anti-PSD95 Antibody (Minibody), Mouse IgG2 Fc fusion	N3785	NanoTag Biotechnologies	NbPSD95 fused to mouse IgG2 Fc domain	IF, 1st Ab IF, 1st Ab	14 nM 1 nM
Fig. S2A, Fig. S2B	3rd rows	Monoclonal anti-PSD95 antibody	MABN68	Millipore	Monoclonal antibody (Mouse IgG2)	IF, 1st Ab IF, 1st Ab	14 nM 1 nM
Fig. S2A, B	2nd/3rd rows	FluoTag-X2 anti-Mouse IgG2 AbberiorStar635P	N2702-Ab635P	NanoTag Biotechnologies	Fluorophore-conjugated anti-Mouse IgG2 nanobody	IF. 2nd Ab	1:500
Fig. S2		FluoTag-X2 anti-Syt1 AZDye568	N2302-AF568-L	NanoTag Biotechnologies	Fluorophore-conjugated anti-Synaptotagmin1 nanobody	IF, direct	1:500
Fig. S2		FluoTag-X2 anti-GFAP Atto488	N3802-At488-L	NanoTag Biotechnologies	Fluorophore-conjugated anti-GFAP nanobody	IF, direct	1:500

IF: Immunofluorescence; **IHC**: Immunohistochemistry; **IHC-P**: Immunohistochemistry on paraffin-embedded sections, **WB**: Western blot;
1st Ab: Primary antibody; **2nd Ab**: Secondary antibody