

Monoclonal antibodies targeting cytotoxic α -synuclein oligomers: molecular properties and diagnostic potential

Janni Nielsen, Johanne Lauritsen, Jannik N. Pedersen, Jan S. Nowak, Malthe K. Bendtsen, Giulia Kleijwegt, Matthias M. Schneider, Georg Krainer, Louise Goksøyr, Paul Khalifé, Sanne Simone Kaalund, Susana Aznar, Magnus Kjærgaard, Tuomas P.J. Knowles, Morten Agertou Nielsen, Adam F. Sander, Marina Romero-Ramos, Daniel E. Otzen

Supplementary Information

Supplementary Tables

Table S1. Concentrations of antibodies used for staining of rat striatal sections.

Ab ID	Stock concentration (mg/ml)	ELISA concentration (μ g/ml)	ELISA x 20 (mg/ml)	Volume (μ l/ml) $\left(\frac{ELISA \times 20}{Stock \text{ conc.}} \times 1000\right)$
3-9H9	0.42	1	0.02	47.62
6-3A10-A1	0.28	1	0.02	71.43
8-9H4-E3	0.67	2	0.04	59.70
10-9C8-C1	0.61	1	0.02	32.79
13-3H6-D2	0.55	1	0.02	36.36
14-9E7-A1	0.9	0.5	0.01	11.11
14-IE2-E1	0.52	1.5	0.03	57.69
16-9E2	0.5	1	0.02	40.00
17-5A8-H1	1.12	1.5	0.03	26.79
17-9D12-A1	1.59	0.5	0.01	6.29
18-3A5-H2	2.05	1	0.02	9.76
18-9E10-B1	1.16	2	0.04	34.48
19-2C3-F10	0.9	0.5	0.01	11.11
20-5H12-C10	0.69	2	0.04	57.97
20-9E2-B8	0.77	1.5	0.03	38.96
21-4G12	1.03	2	0.04	38.83

Table S2. Quantification of human pathology with mAb 10-9C8-C1

10-9C8-C1								
Disease	ID	Area	Pigmented neurons	Extracellular inclusions	LBs	LNs	Neuropil “grains”	GCI
CTRL	117	SN	+++	-	-	-	-	-
		WM CTX		-	-	-	-	-
CTRL	384	SN	+++	-	-	-	-	-
		WM CTX		-	-	-	-	-
CTRL	127	SN	++/+++	++	+	++/+++	++/+++	-
		WM CTX		-	-	-	-	-
CTRL	287	SN	+++	-	-	-	-	-
		WM CTX		-	-	-	-	-
PD	684	SN	+	-	-	+	+	-
		WM CTX		-	-	-	-	-
PD	337	SN	- / +	-	- / +	-	- / +	-
		WM CTX		-	-	-	-	- / +
PD	248	SN	++	- / +	+/++	+	+/++	-
		WM CTX		-	-	-	- / +	-
PD	191	SN	+	+++	+++	+++	+++	- / +
		WM CTX		+/++	+	+	+/++	-
DLB	256	SN	+/++	+++	+++	+++	+++	- / +
		WM CTX		+	+	+	++	- / +
DLB	543	SN	+/++	++/+++	++	++	+++	- / +
		WM CTX		-	+	-	- / +	-
MSA	477	SN	+	-	-	-	-	++
		WM CTX		-	-	-	-	++/+++
MSA	578	SN	+	+	-	+	+	+++
		WM CTX		- / +	-	-	+	++
MSA	167	SN	++	+	- / +	-	-	+++
		WM CTX		+	-	+	+	+++
MSA	623	SN	+	++	+/++	+++	++/+++	+++
		WM CTX		+	- / +	+	+/++	++
MSA	312	SN	- / +	+	+	+	+	+++
		WM CTX		-	-	-	+	++

Table S3. Quantification of human pathology with mAb 14-9E7-A1

14-9E7-A1								
Disease	ID	Area	Pigmented neurons	Extracellular inclusions	LBs	LNs	Neuropil “grains”	GCI
CTRL	117	SN	+++	-	-	-	-	-
		WM CTX		-	-	-	-	-
CTRL	384	SN	+++	+	-	- / +	+	-
		WM CTX		-	-	-	+/+++	-
CTRL	127	SN	++/+++	++	++	++/+++	++/+++	-
		WM CTX		-	-	-	-	-
CTRL	287	SN	+++	-	-	-	-	-
		WM CTX		-	-	-	-	-
PD	684	SN	+	- / +	+	+/++	++	-
		WM CTX		-	-	-	-	-
PD	337	SN	NA	NA	NA	NA	NA	NA
		WM CTX		-	-	-	-	- / +
PD	248	SN	++	+	+/++	++	++	-
		WM CTX		-	-	+	- / +	-
PD	191	SN	+	+++	+++	+++	+++	- / +
		WM CTX		+	+	+	++/+++	- / +
DLB	256	SN	+/++	+++	+++	+++	+++	- / +
		WM CTX		+/++	+	+/++	+++	- / +
DLB	543	SN	+	++	++/+++	++	+++	- / +
		WM CTX		-	-	-	- / +	-
MSA	477	SN	+	-	-	-	-	+
		WM CTX		-	-	-	-	++/+++
MSA	578	SN	+	+	-	+	+	+++
		WM CTX		+	-	-	+	++
MSA	167	SN	++	+	- / +	-	+	+++
		WM CTX		+	-	+	+	+++
MSA	623	SN	+	++/+++	++	+++	++/+++	+++
		WM CTX		+	+	++	++/+++	++
MSA	312	SN	- / +	+	+	+	++	+++
		WM CTX		-	-	-	+	++

Table S4. Quantification of human pathology with mAb 17-9D12-A1

17-9D12-A1								
Disease	ID	Area	Pigmented neurons	Extracellular inclusions	LBs	LNs	Neuropil “grains”	GCI
CTRL	117	SN	+++	-	-	-	-	-
		WM CTX		-	-	-	-	-
CTRL	384	SN	+++	-	-	-	-	-
		WM CTX		-	-	-	+	-
CTRL	127	SN	++/+++	+	+	+/++	++/+++	-
		WM CTX		-	-	-	-	-
CTRL	287	SN	+++	-	-	-	-	-
		WM CTX		-	-	-	-	-
PD	684	SN	+	-	-	-	+	-
		WM CTX		-	-	-	-	-
PD	337	SN	- / +	-	-	-	+	-
		WM CTX		-	-	-	-	- / +
PD	248	SN	++	-	+	+	+	-
		WM CTX		-	-	-	-	-
PD	191	SN	+	++/+++	++/+++	++	++/+++	- / +
		WM CTX		+	+	+	++	-
DLB	256	SN	+/++	++/+++	++/+++	++	++	- / +
		WM CTX		+	+	+	++	- / +
DLB	543	SN	+	+	+	+	+/++	-
		WM CTX		-	-	-	-	-
MSA	477	SN	+	-	-	-	-	-
		WM CTX		-	-	-	-	+
MSA	578	SN	+	-	-	+	+	+++
		WM CTX		-	-	-	+	+/++
MSA	167	SN	++	+	- / +	- / +	+	++/+++
		WM CTX		-	-	-	+	++/+++
MSA	623	SN	+	+	+/++	++	++/+++	++/+++
		WM CTX		-	-	+	+	+/++
MSA	312	SN	- / +	+	- / +	-	+/++	+++
		WM CTX		-	-	-	+	- / +

Table S5. Quantification of human pathology with mAb 18-3A5-H2

18-3A5-H2								
Disease	ID	Area	Pigmented neurons	Extracellular inclusions	LBs	LNs	Neuropil “grains”	GCI
CTRL	117	SN	+++	-	-	-	-	-
		WM CTX		-	-	-	-	-
CTRL	384	SN	+++	-	-	-	-	-
		WM CTX		-	-	-	-	-
CTRL	127	SN	++/+++	+	+	++/+++	+/++	-
		WM CTX		-	-	-	-	-
CTRL	287	SN	+++	-	-	-	-	-
		WM CTX		-	-	-	-	-
PD	684	SN	+	-	+	+	+	-
		WM CTX		-	- / +	-	-	-
PD	337	SN	- / +	-	-	-	-	-
		WM CTX		-	-	-	-	-
PD	248	SN	++	+	+	+	+	-
		WM CTX		-	-	-	-	-
PD	191	SN	+	+	+/++	- / +	+	-
		WM CTX		-	-	-	+	-
DLB	256	SN	+/++	+/++	+/++	+	+/++	-
		WM CTX		- / +	- / +	- / +	+	- / +
DLB	543	SN	+	+	+/++	+	+/++	- / +
		WM CTX		-	-	-	-	-
MSA	477	SN	+	-	-	-	-	+/++
		WM CTX		-	-	-	-	+
MSA	578	SN	+	-	-	+	-	++
		WM CTX		-	-	-	-	+
MSA	167	SN	+/++	- / +	-	-	-	++
		WM CTX		-	-	-	+	++/+++
MSA	623	SN	+	+	+/++	+++	+/++	++/+++
		WM CTX		+	- / +	-	+	+/++
MSA	312	SN	- / +	-	-	-	- / +	++
		WM CTX		-	-	-	-	-

Table S6. Quantification of human pathology with mAb 20-9E2-B8

20-9E2-B8								
Disease	ID	Area	Pigmented neurons	Extracellular inclusions	LBs	LNs	Neuropil “grains”	GCI
CTRL	117	SN	+++	-	-	-	-	-
		WM CTX		-	-	-	-	-
CTRL	384	SN	+++	+	+	+	+	-
		WM CTX		-	-	-	-	-
CTRL	127	SN	++/+++	+/++	+	++/+++	++/+++	-
		WM CTX		-	-	-	-	-
CTRL	287	SN	+++	-	-	-	-	-
		WM CTX		-	-	-	-	-
PD	684	SN	+	- / +	+	+	+/++	-
		WM CTX		-	-	- / +	+	-
PD	337	SN	- / +	-	-	-	- / +	-
		WM CTX		-	-	-	-	-
PD	248	SN	++/+++	+	+	++	++	-
		WM CTX		-	-	-	-	-
PD	191	SN	+	+++	+++	+++	+++	- / +
		WM CTX		+	+	+	+/++	-
DLB	256	SN	+/++	+++	+++	+++	+++	- / +
		WM CTX		+	+	+/++	++	- / +
DLB	543	SN	+	+++	++/+++	+++	+++	- / +
		WM CTX		-	-	+/++	++	-
MSA	477	SN	NA	NA	NA	NA	NA	NA
		WM CTX		-	-	-	-	+/++
MSA	578	SN	+	- / +	-	+/++	+	+++
		WM CTX		+	-	-	-	++
MSA	167	SN	++	+	- / +	+	+	+++
		WM CTX		+	-	+	+	+++
MSA	623	SN	+	++	+	+++	++	+++
		WM CTX		+	+	++	++	++
MSA	312	SN	- / +	+	+	- / +	- / +	+++
		WM CTX		-	-	-	-	+

Table S7. Demographics of human patients

Clinical diagnosis	N	Gender (M/F)	Age of death	Age of disease onset	PMI (hrs)
CTRL	4	2/1?	82.3 ± 14.7	-	54 ± 36
PD	4	3/1	83.5 ± 11.5	74.8 ± 12.3	62 ± 28
DLB	2	2/0	70.5 ± 6.5	62.5 ± 11.5	86.5 ± 30.5
MSA	5	3/2	67.0 ± 11.0	60.0 ± 12.0	48.5 ± 23.5

PMI: post-mortem interval. CTRL: control. PD: Parkinsons Disease. MSA: Multiple system atrophy. DLB: Dementia with Lewy bodies.

Figure S1

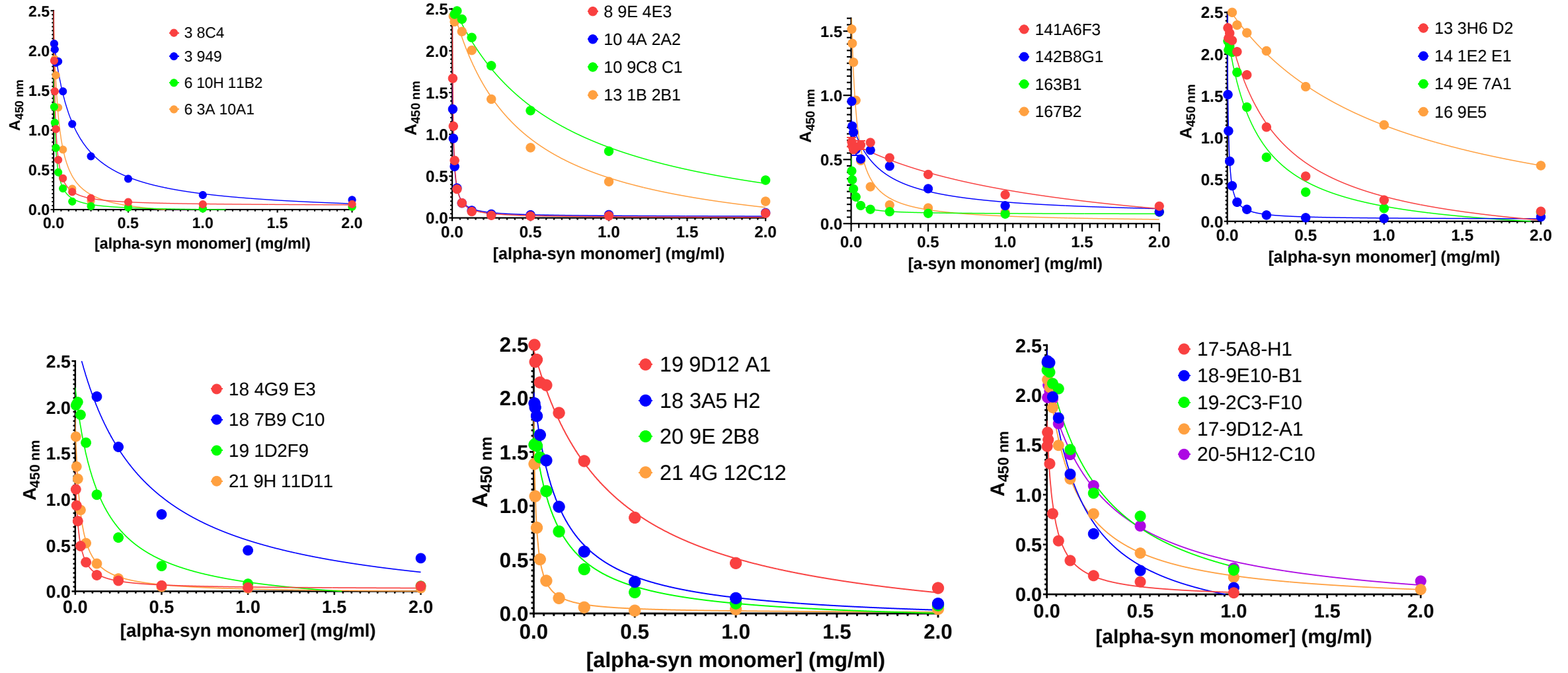
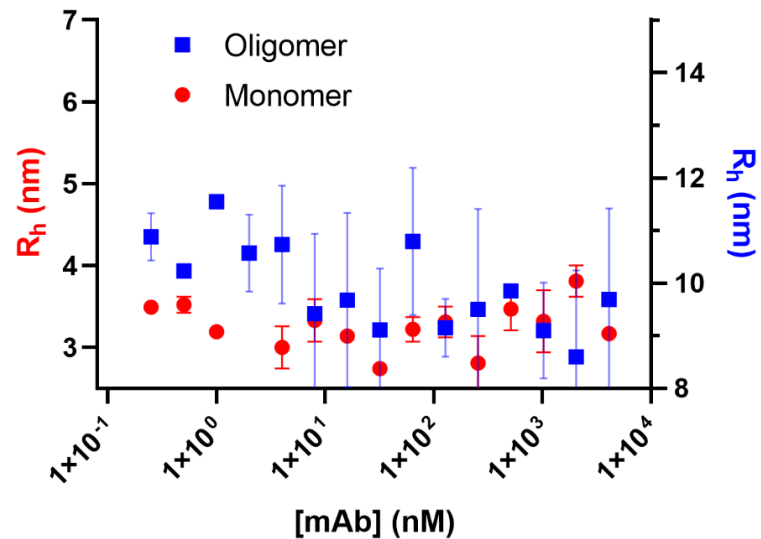


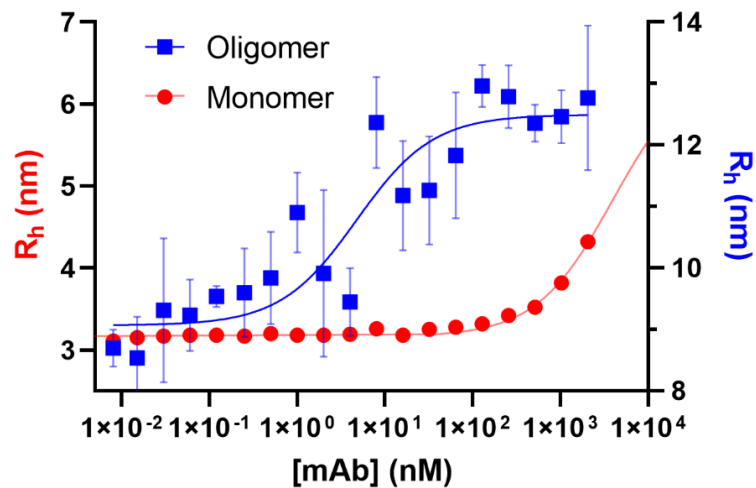
Figure S1. Competition ELISA assays in which immobilized α SO is exposed to a fixed amount of mAb in the presence of 0-2 mg/ml monomeric α -syn in the soluble phase. The steeper the decline in mAb binding, the higher the relative affinity of the mAb for monomeric rather than oligomeric α -syn. Data summarized in **Table 2**.

Figure S2a

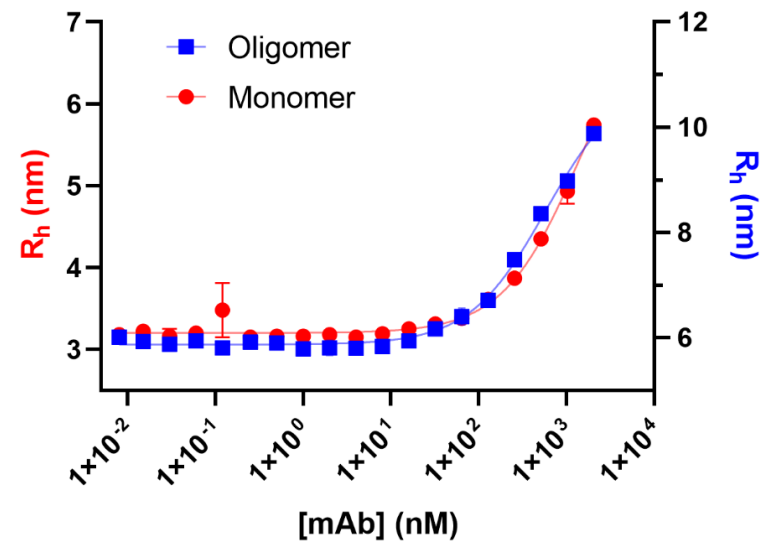
03-8C4



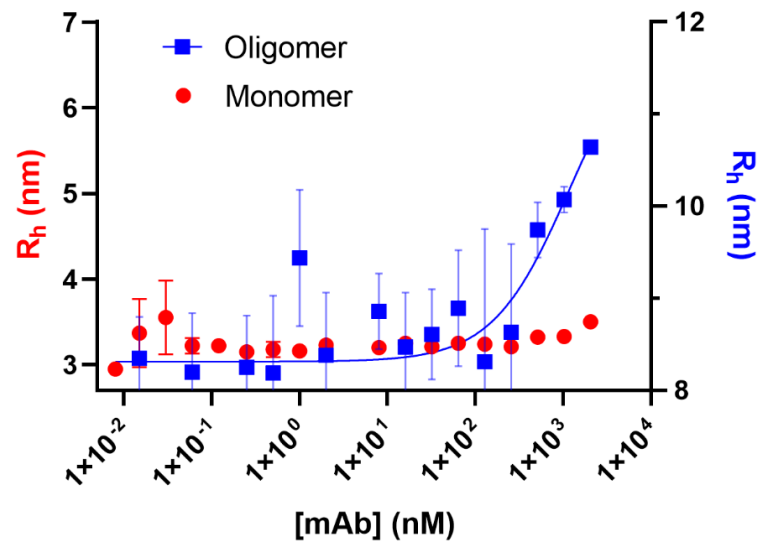
03-9H9



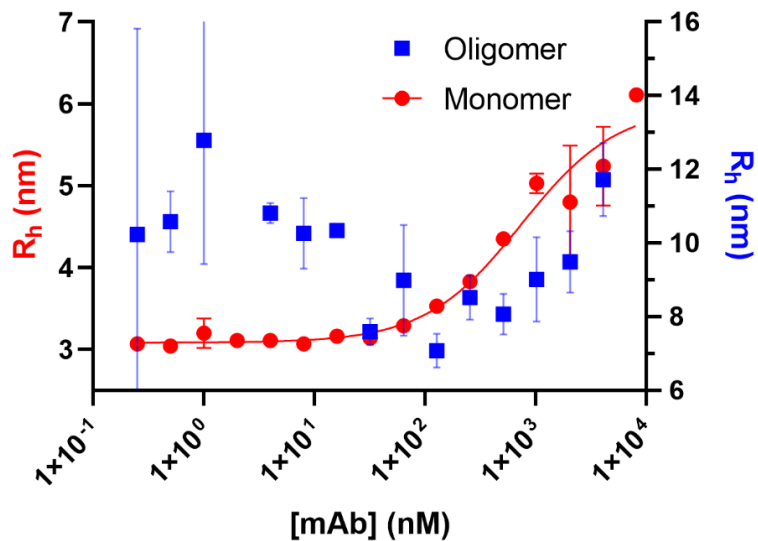
06-3A10-A1



06-10H11-B2



08-9E4-E3



10-4A2-A2

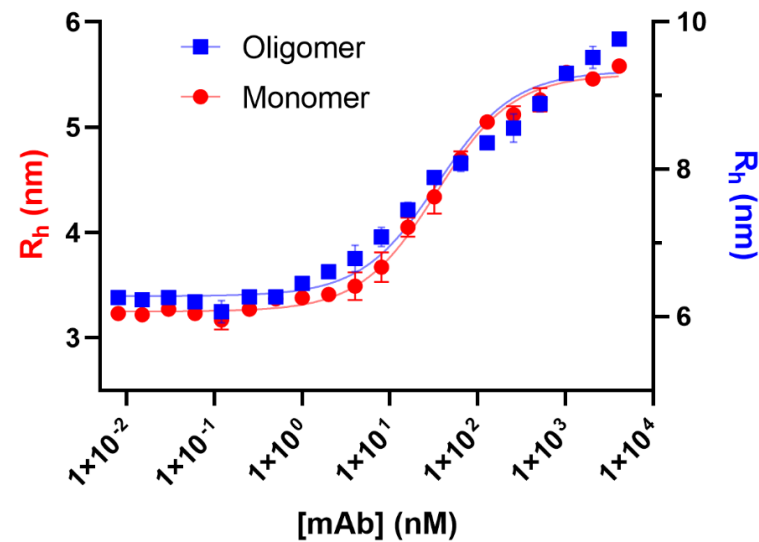


Figure S2a. FIDA plots for the affinity of mAbs for oligomeric and monomeric α -syn. Data summarized in Table 2, columns E-G.

Figure S2a

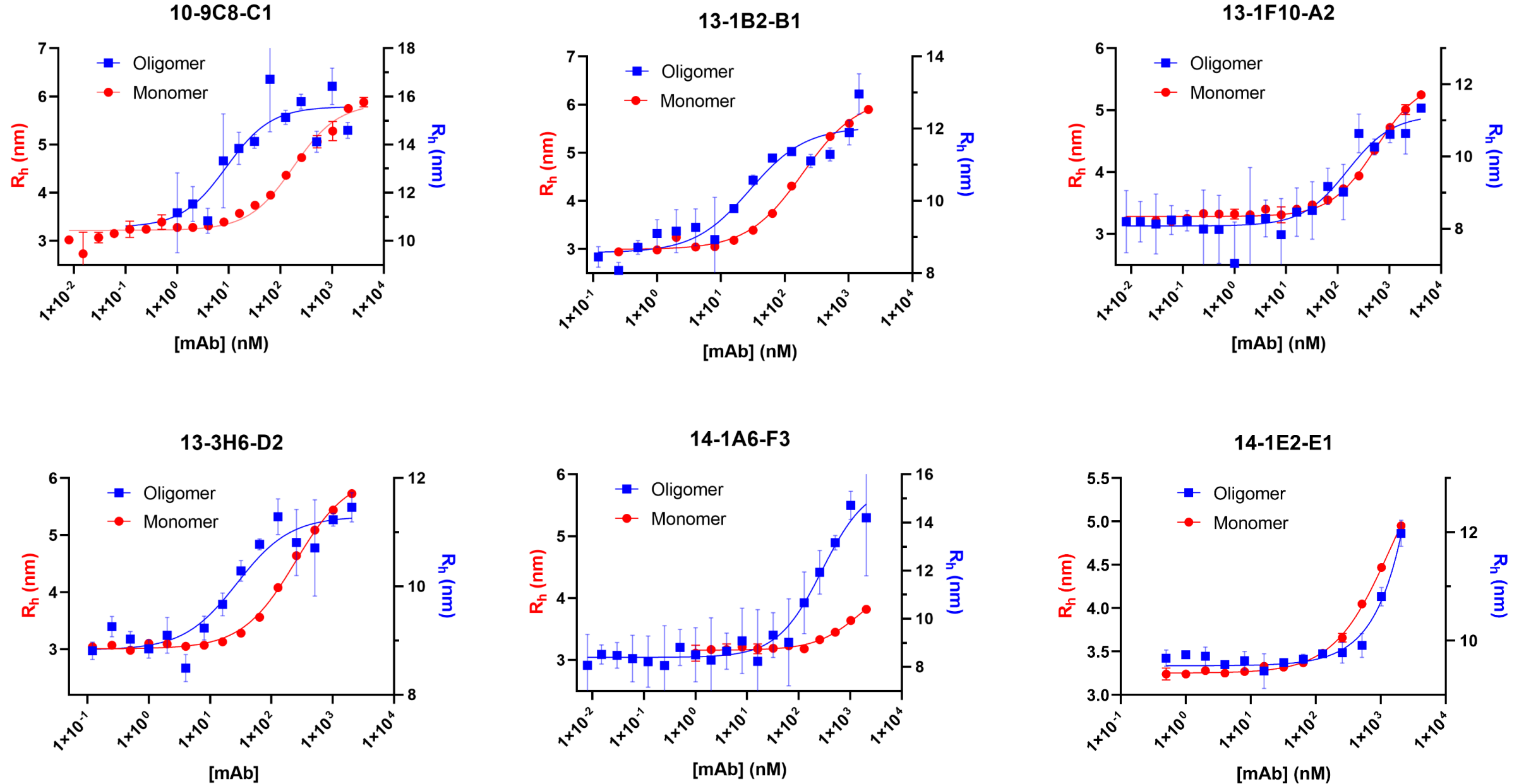


Figure S2a. FIDA plots for the affinity of mAbs for oligomeric and monomeric α -syn. Data summarized in Table 2, columns E-G.

Figure S2a

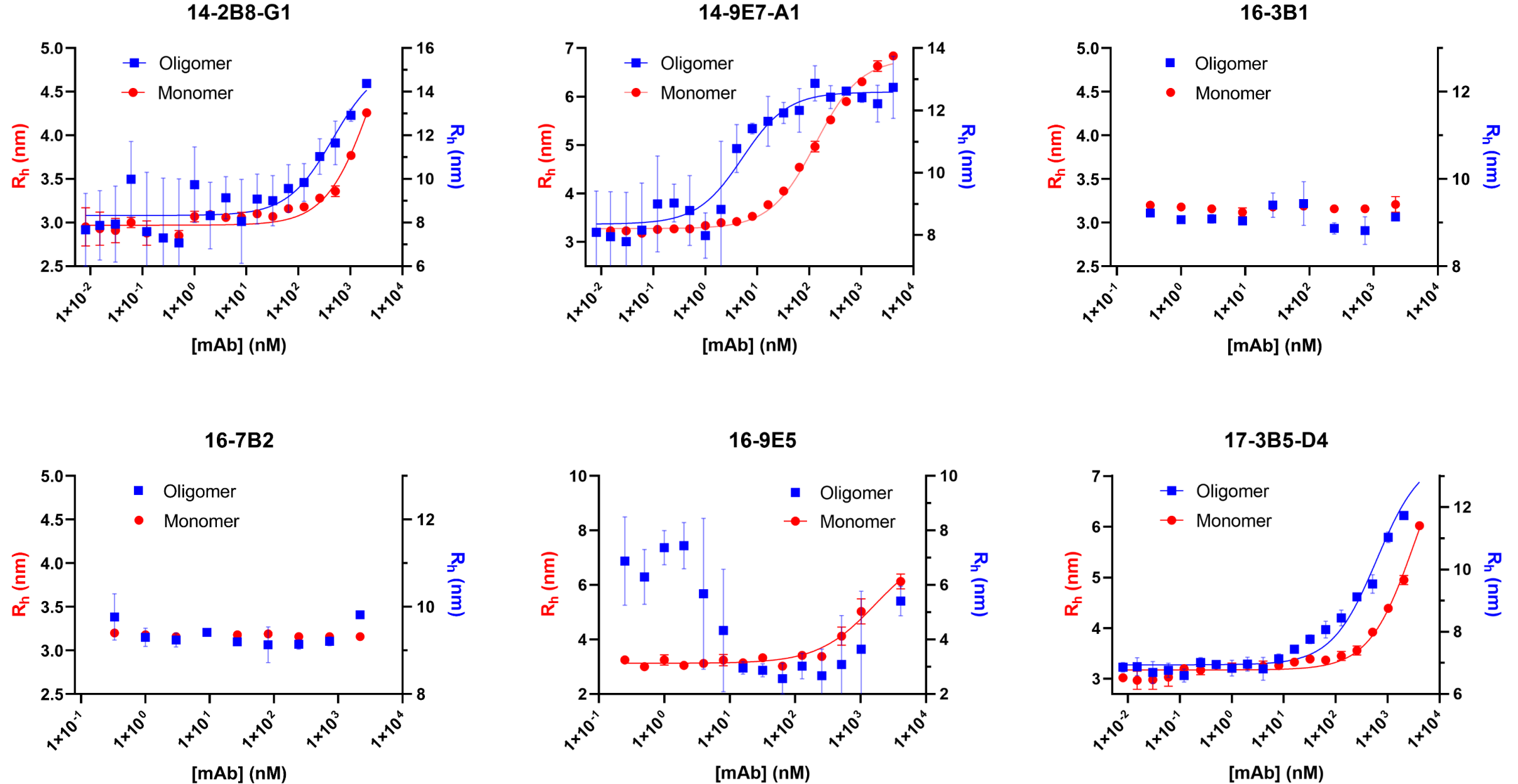


Figure S2a. FIDA plots for the affinity of mAbs for oligomeric and monomeric α -syn. Data summarized in Table 2, columns E-G.

Figure S2a

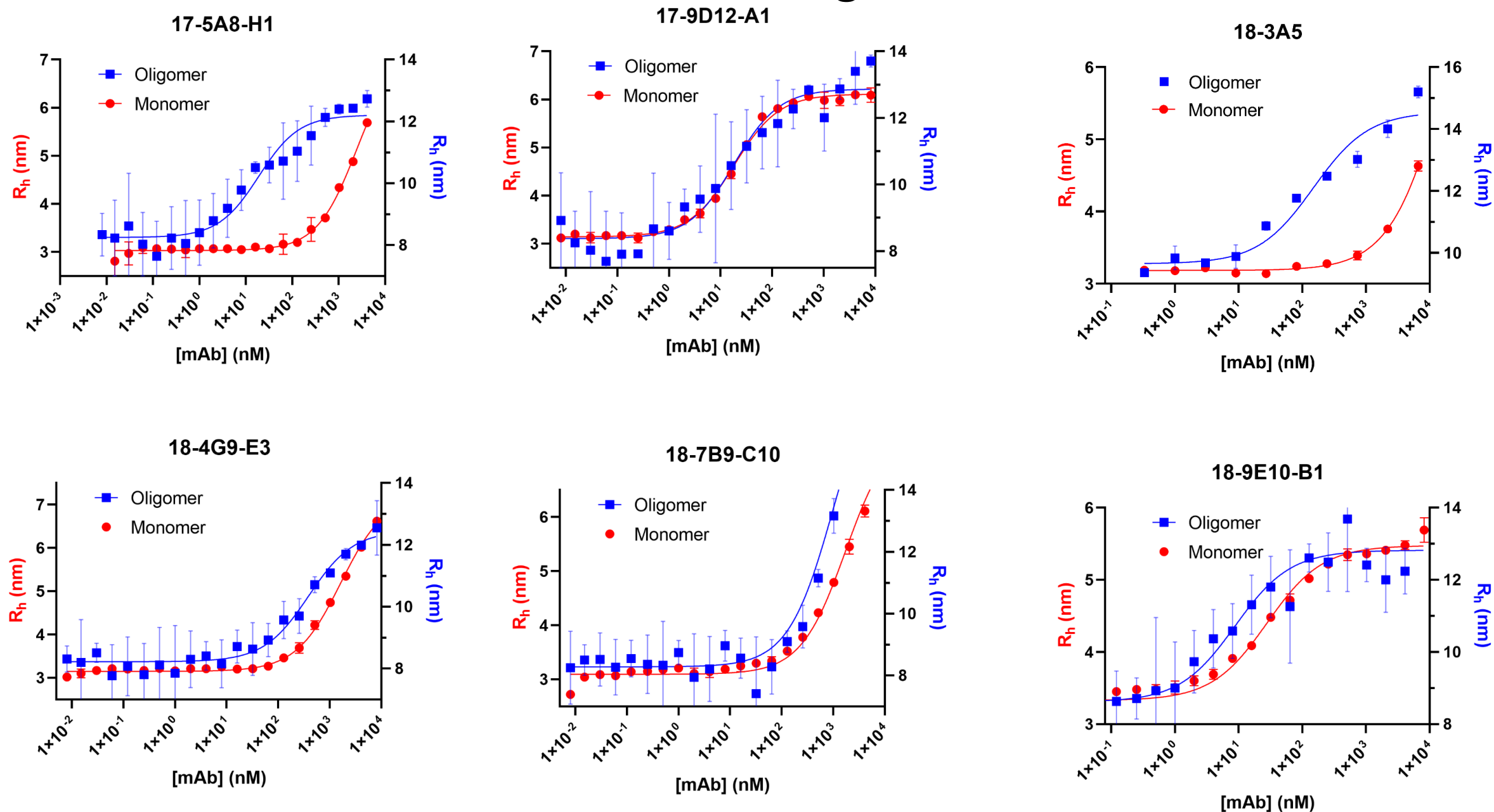


Figure S2a. FIDA plots for the affinity of mAbs for oligomeric and monomeric α -syn. Data summarized in Table 2, columns E-G.

Figure S2a

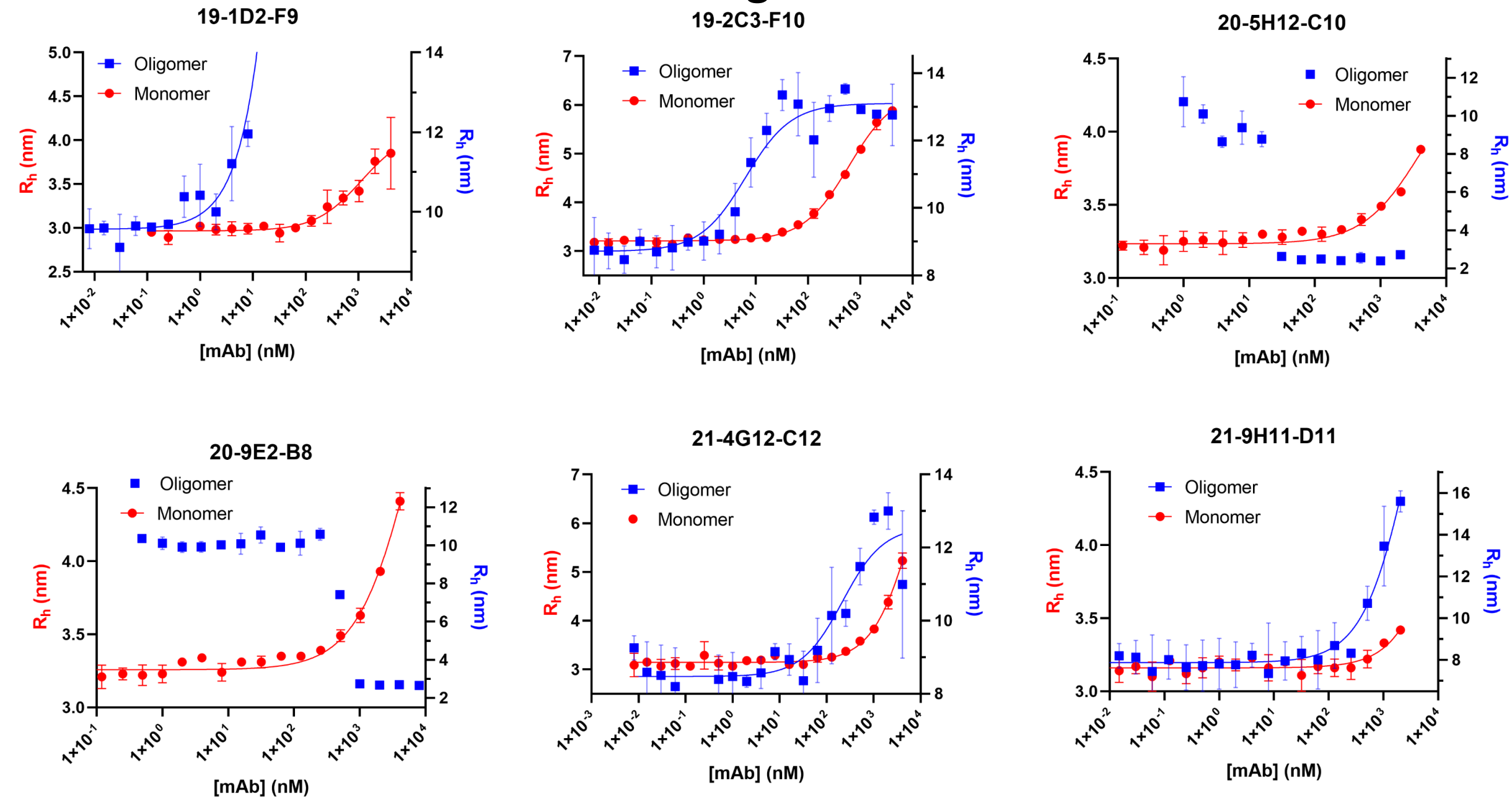


Figure S2a. FIDA plots for the affinity of mAbs for oligomeric and monomeric α -syn. Data summarized in Table 2, columns E-G.

Figure S2b

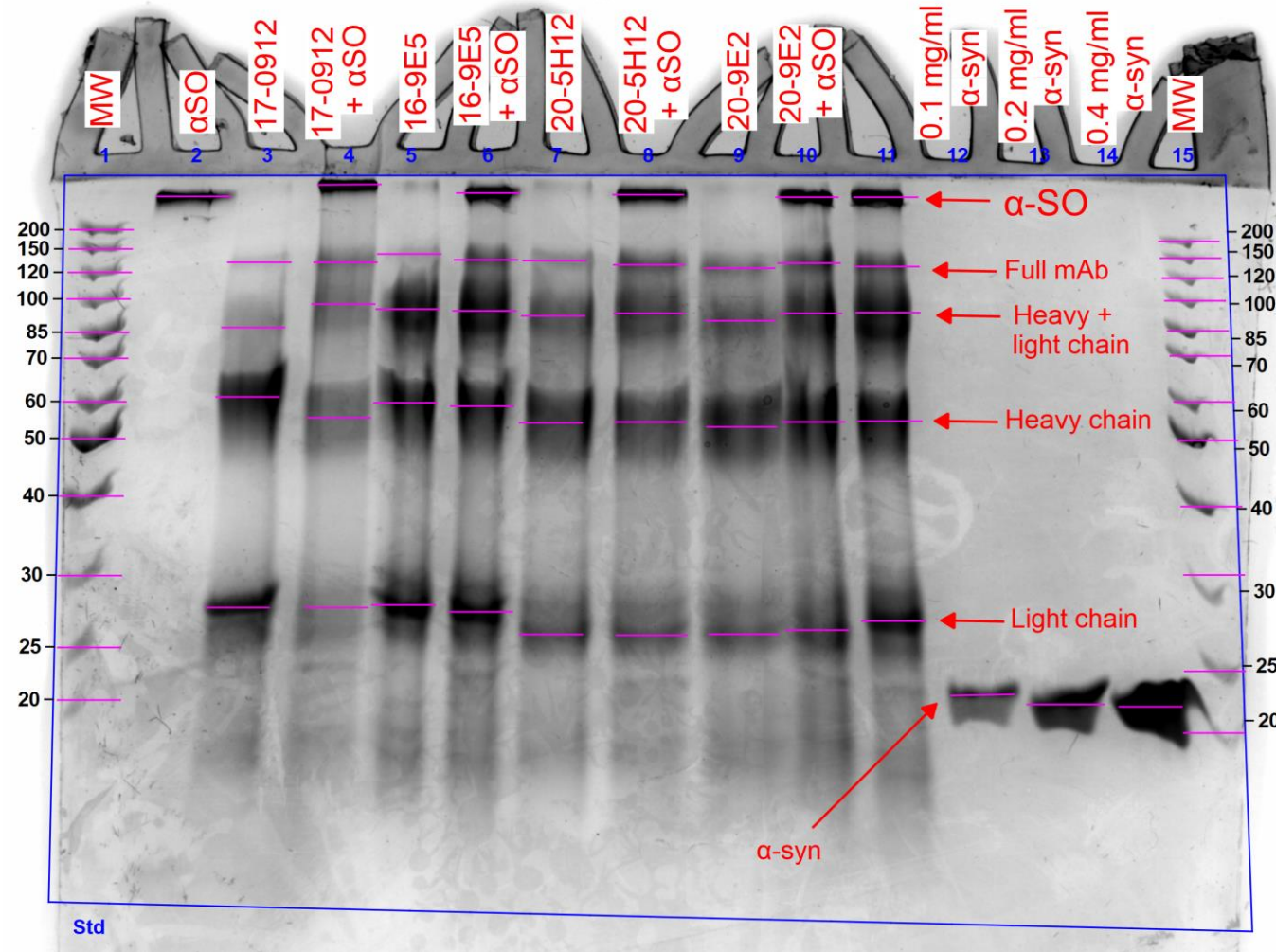


Figure S2b. SDS-PAGE of mAbs 17-9D12-A1 (control), 16-9E5, 20-5H12-C10, and 20-9E2-B8 incubated with and without α SO in lanes 3-10. Pure α SO is shown in lane 2 and monomeric α -syn in lanes 12-14. Lines drawn are meant to guide the eye.

Figure S3a

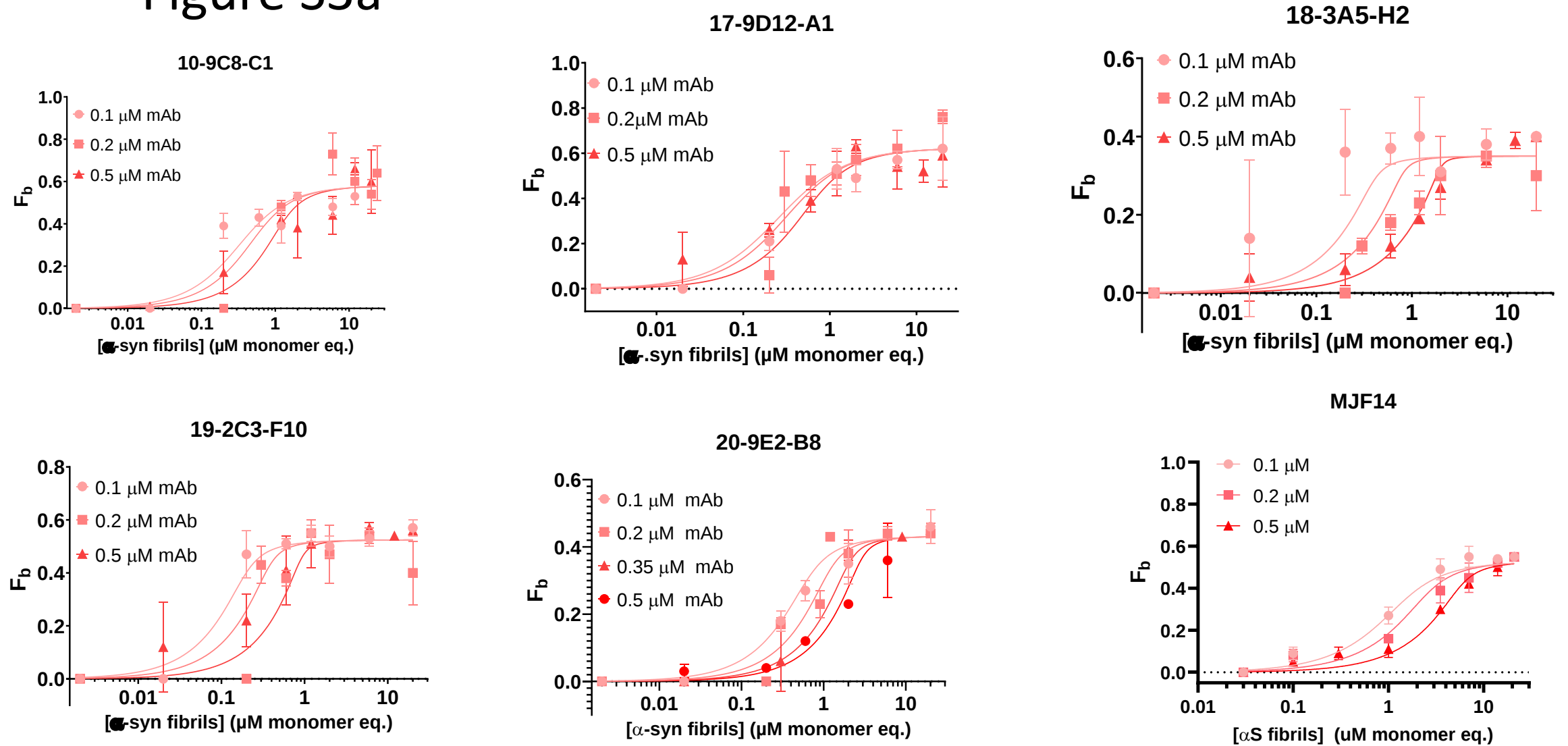


Figure S3a. Fibril-antibody binding curves obtained by microfluidic diffusional sizing (MDS). Data analysed using equation 2.

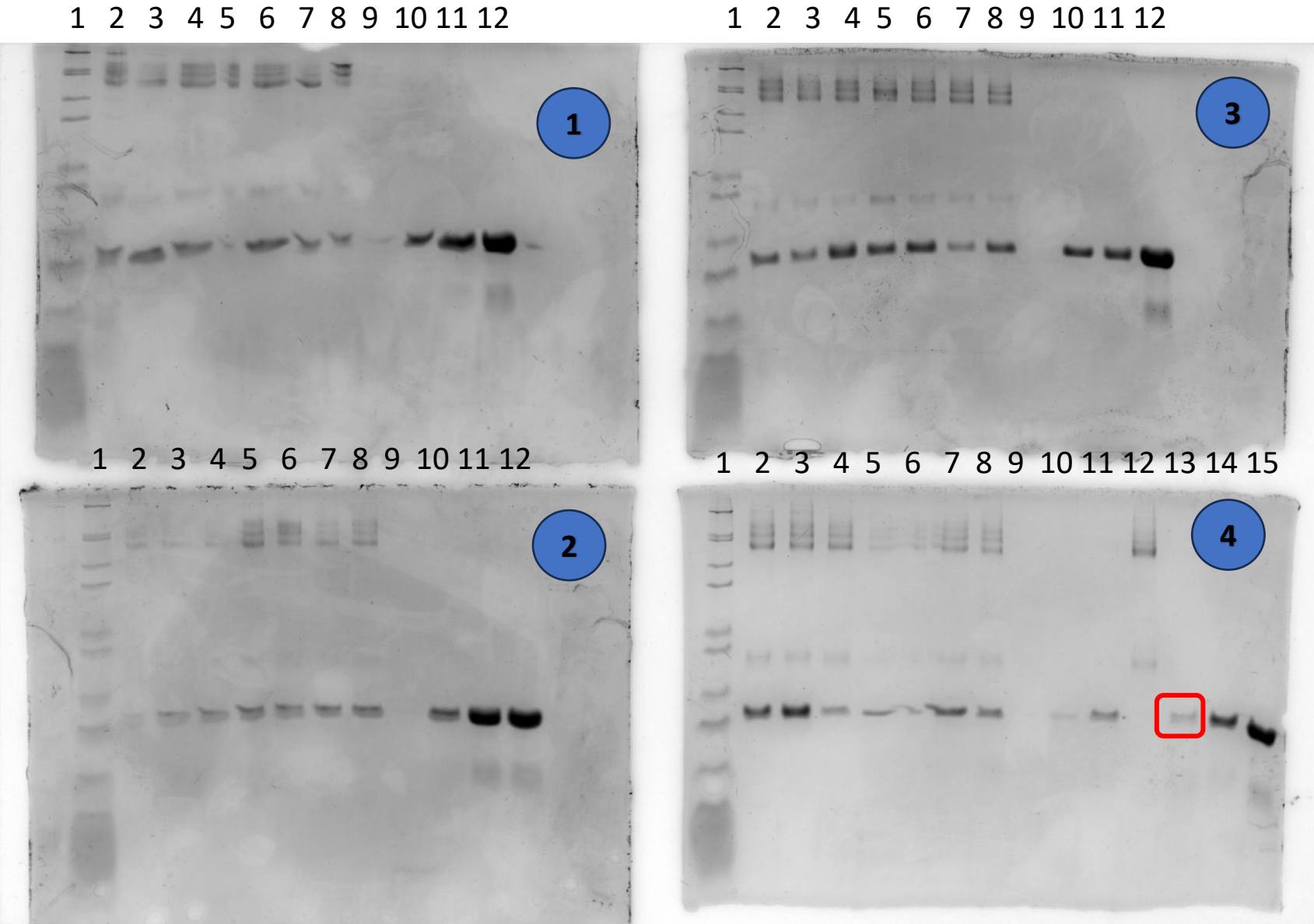


Figure S3b

Lane	Gel1	Gel2	Gel3	Gel4
1	Mwt	Mwt	Mwt	Mwt
2	38C4	131B2	167B2	183A5
3	39H9	133H6	169E5	209E2
4	610H	141A6	184G9	214G1
5	63A1	142B8	187B9	A5A8
6	89E4	141E2	191D2	189E1
7	104A	1419E1	219H11	205H12
8	109C	163B1	179D12	192C3
9	-	-	-	-
10	0.1g/L	0.1g/L	0.1g/L	Mono
11	0.3g/L	0.3g/L	0.3g/L	M+S
12	0.5g/L	0.5 g/L	0.5 g/L	mAb
13	-	-	-	M + beads
14	-	-	-	0.3g/L
15	-	-	-	0.5g/L

Fig. S3b. SDS-PAGE analysis of the soluble fractions left from seeding experiments (Fig. 3b). Note the internal standards (0.1-0.3 mg/ml monomeric α -syn) and the very small amount of soluble α -syn left after seeded fibrillation in the absence of mAbs. 4 of the mAbs lead to retention of 30-40% of monomeric α -syn (see text).

Figure S3c

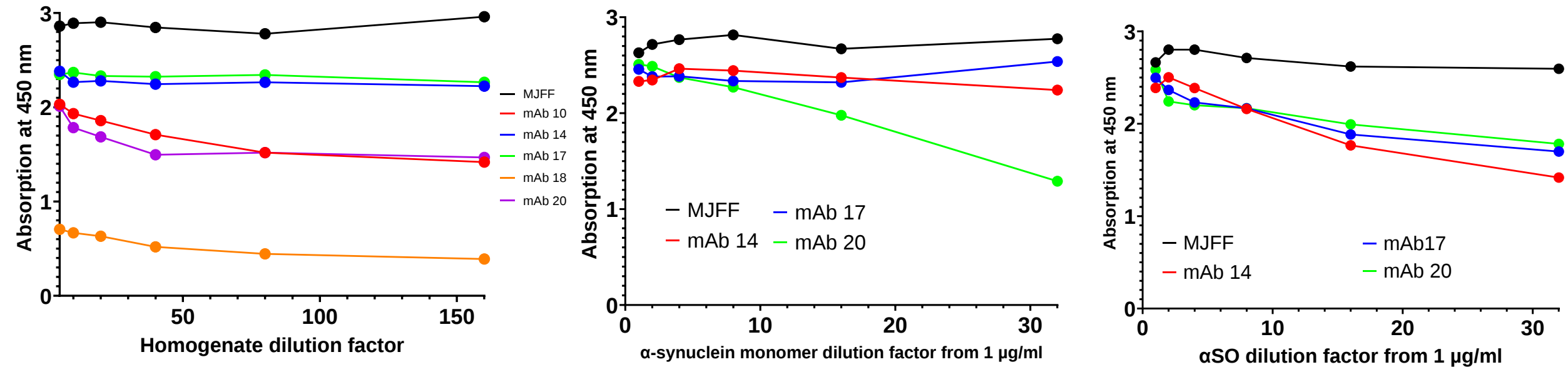


Fig. S3c. Indirect ELISA assays using immobilized brain lysates from a male PD rat. Left panel: Comparison of the binding of the MJF14 mAb with the 5 mAbs selected for further analysis (10-9C8-C1, 14-9E7-A1, 17-9D12-A1, 18-3A5-H2, and 20-9E2-B8). Antibodies indicated by their first two digits only. Middle and right panels: Binding of the indicated antibodies to immobilized monomeric α -syn (middle) and α SO (right).

Figure S4

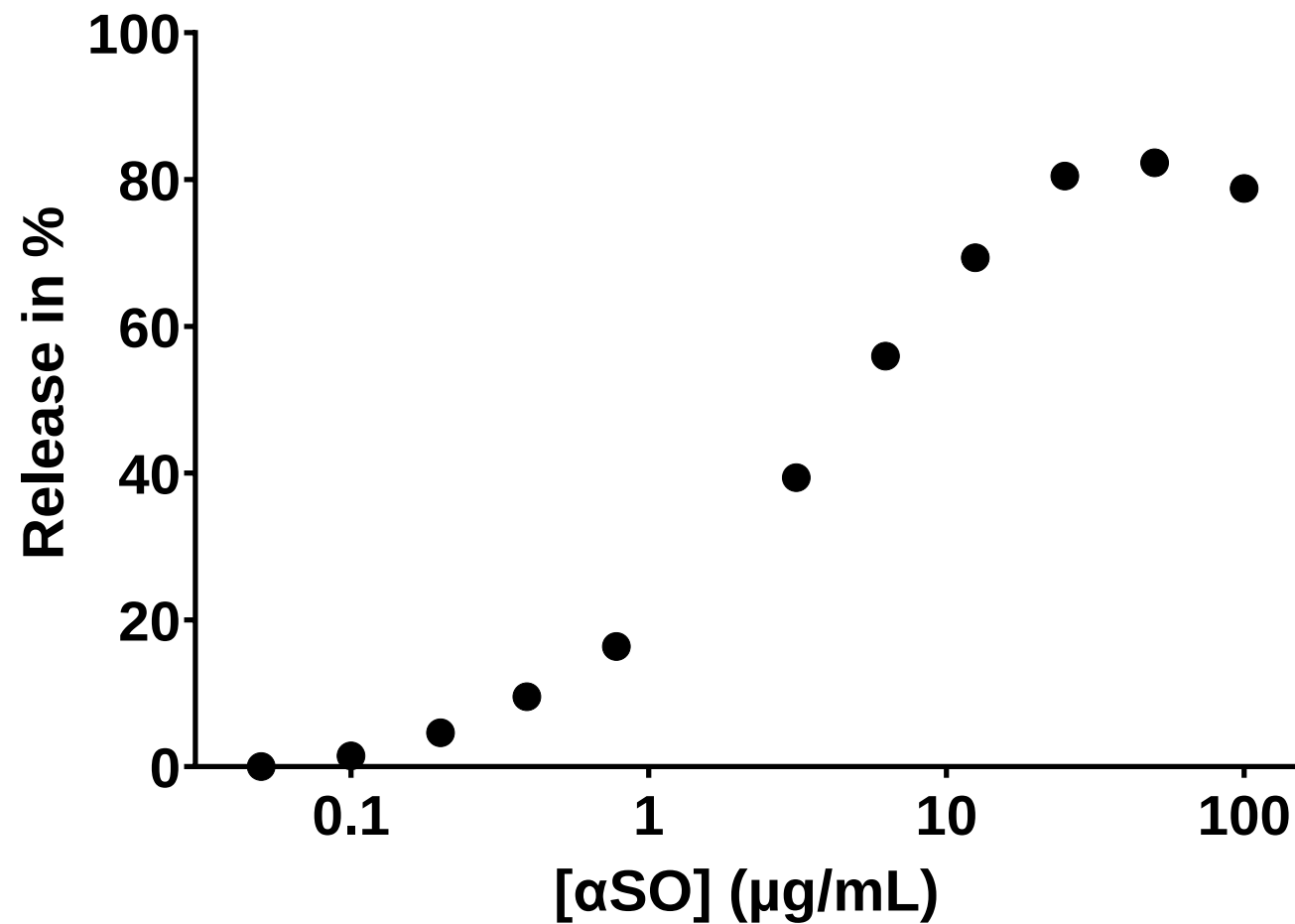


Figure S4. Calibration curve for release of calcein from DOPG vesicles by 0.05-100 $\mu\text{g/ml}$ αSO .

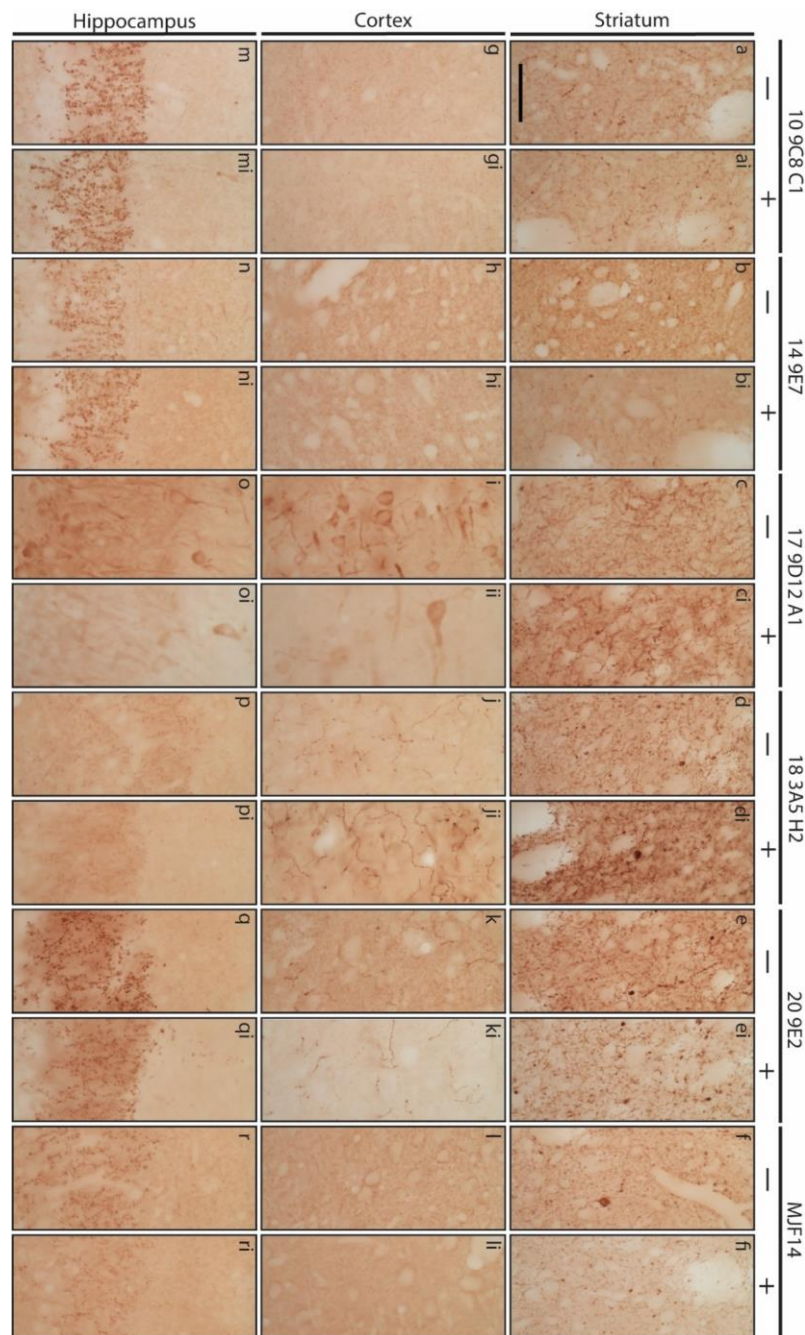


Figure S5

Figure S5. Pre-incubation of the mAbs with monomeric human α -syn. Staining of tissue using 5 mAbs generated in this study along with commercial antibody MJF4. Columns left to right show results for striatum, cortex, and hippocampus, respectively. For each antibody, the first row of figures are obtained without prestaining and the second row with preincubation, with monomeric α -syn. Scalebar=50 μ m.

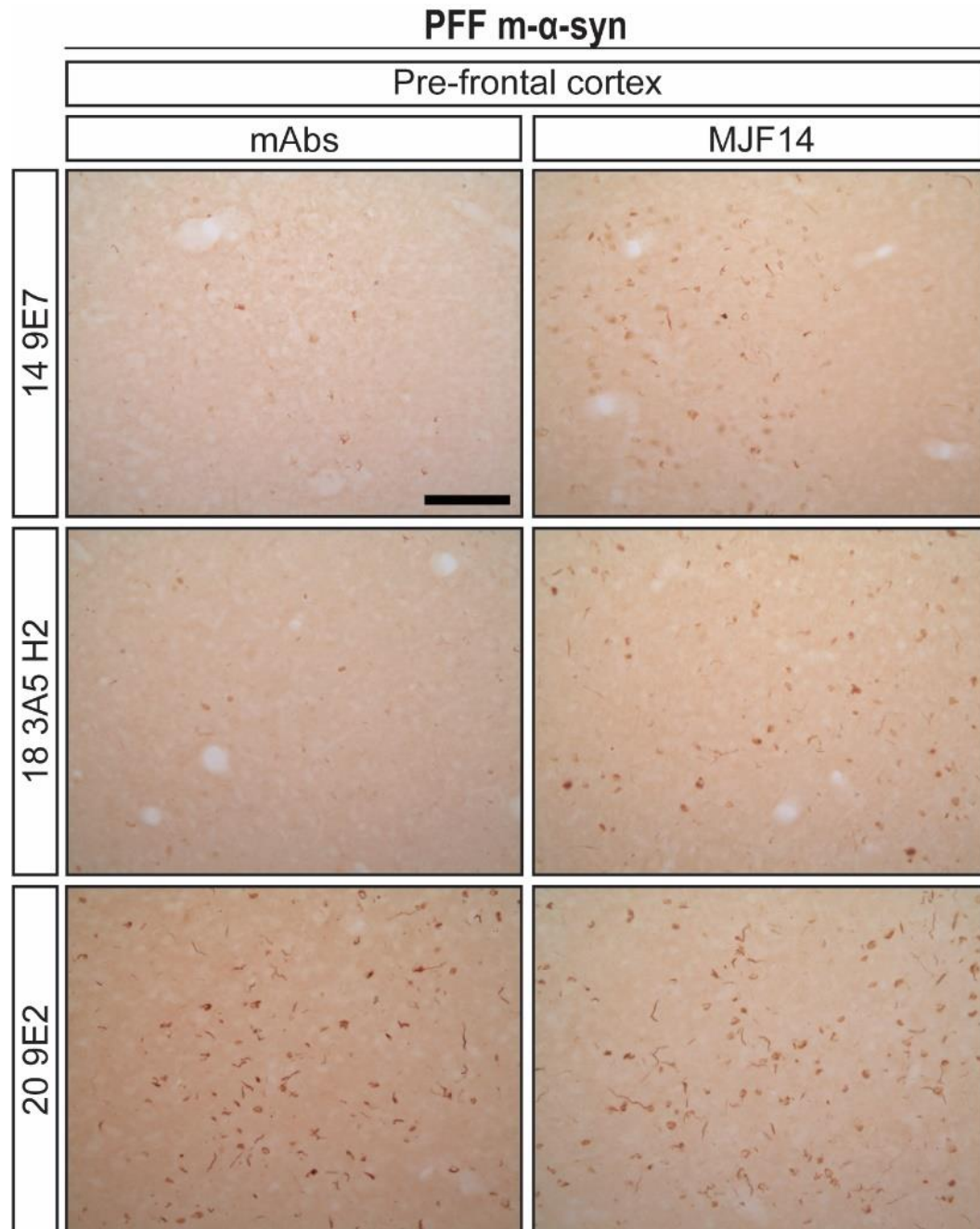


Figure S6

Figure S6. Prefrontal cortex of the PFF m-a-syn model. IHC done with mAbs (left column) or the commercial ab MJF14 (left column). Scalebar = 100 μ m.

Figure S7

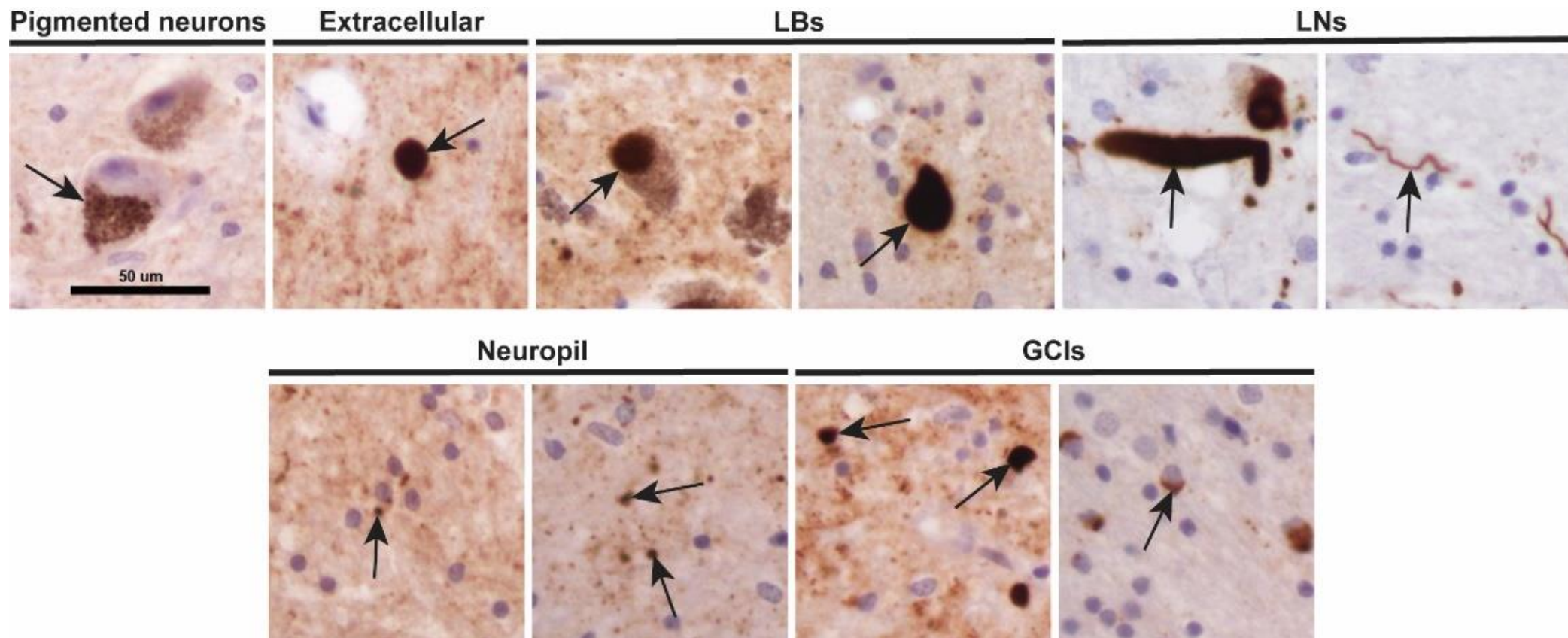


Figure S7. Prefrontal cortex of the PFF m-a-syn model. IHC done with mAbs (left column) or the commercial ab MJF14 (left column). Scalebar = 100μm.

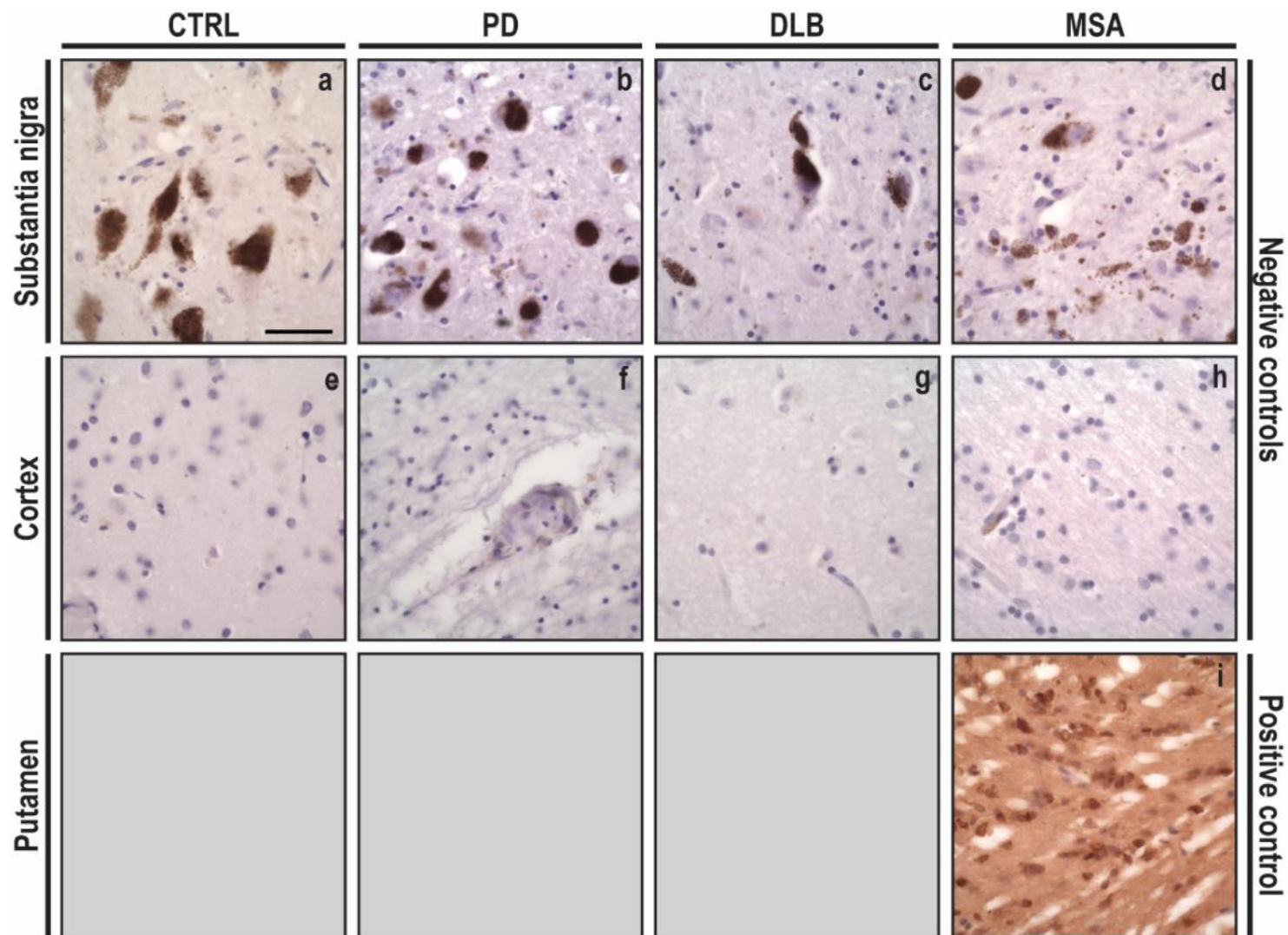


Figure S8

Figure S8. Positive and negative controls. **a-d)** Negative controls of substantia nigra for each group, omitting the primary mAb. **e-h)** Negative controls of the white matter cortex, omitting the primary mAb. **i)** Positive control of putamen from a MSA patient, stained with the commercial anti- α -syn antibody (Genetex). Scalebar = 50 μ m.