Supplementary Information

Cellular Milieu Imparts Distinct Pathological α-Synuclein Stains in α-Synucleinopathies

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Table of contents:

Supplementary Table 1 Clinical Information.

Clinical Information of the Cases Used for the Extraction of Pathological α-Syn

Case No.	Clinical Diagnosis	Pathological Diagnosis	Race	Sex	Age of Disease onset	Age at Death	PMI (h)	Brain Region used
1	MSA-P	MSA	Multi-racial	Female	63	67	11	Cerebellum / Cingulate Gyrus
2	MSA-P	MSA	White	Male	73	77	5	Cerebellum
3	MSA-P	MSA	Asian	Female	47	56	6	Cerebellum / Middle Frontal Gyrus
4	MSA-P	MSA	White	Male	61	72	4.5	Middle Frontal Gyrus
5	MSA-C	MSA	White	Female	55	64	16	Middle Frontal Gyrus
6	MSA-C	MSA	Multi-racial	Male	55	62	24	Middle Frontal Gyrus
7	AD Probable	AD	White	Female	52	62	11	Middle Frontal Gyrus
8	CBS	AD	White	Female	45	55	15	Middle Frontal Gyrus
9	DLB	DLB	White	Male	76	83	6	Cingulate Gyrus
10	PDD	PDD	White	Male	54	75	6.5	Cingulate Gyrus
11	PDD	PDD	White	Female	51	66	12	Middle Frontal Gyrus
12	PDD	PDD	White	Male	49	70	12.5	Cingulate Gyrus
13	PDD	PDD	White	Male	71	74	12	Cingulate Gyrus
14	DLB	PDD	White	Male	66	72	9	Cingulate Gyrus
15	PDD	PDD	White	Male	60	72	19	Cingulate Gyrus
16	Normal	Normal	White	Male	-	59	18	Middle Frontal Gyrus
30	Normal	Normal	White	Male	-	70	10.5	Middle Frontal Gyrus
31	Normal	Normal	Black	Male	-	66	20	Cingulate Gyrus
32	Normal	Normal	White	Male	-	61	15	Cingulate Gyrus
33	Normal	Normal	Black	Female	-	72	6	Middle Frontal Gyrus

Supplementary Table 1 (Continue)

Clinical Information of the Cases Used for IHC Shown in Fig. 1e&f and extended data Fig. 2

Case No.	Clinical Diagnosis	Pathological Diagnosis	Race	Sex	Age of Disease onset	Age at Death	PMI (h)	Brain Region used
3	MSA-P	MSA	Asian	Female	47	56	6	Middle Frontal Gyrus
6	MSA-C	MSA	Multi-racial	Male	55	62	24	Middle Frontal Gyrus
7	AD Probable	AD	White	Female	52	62	11	Hippocampus
12	PDD	PDD	White	Male	49	70	12.5	Middle Frontal Gyrus
17	AD Probable	AD	White	Male	66	71	7	Substantia Nigra
18	AD Probable	AD	White	Male	62	79	8	Middle Frontal Gyrus
19	DLB	DLB	White	Male	67	76	8	Substantia Nigra
20	CBS	DLB	White	Male	78	83	20	Middle Frontal Gyrus
21	DLB	DLB	White	Male	60	68	12	Hippocampus
22	PD (not demented)	PD	White	Male	70	81	7	Substantia Nigra
23	PDD	PDD	White	Male	52	68	7	Hippocampus
24	MSA-C	MSA	Black	Male	61	65	43	Cerebellum
25	MSA-C	MSA	White	Female	53	67	19	Substantia Nigra
26	MSA-C	MSA	White	Male	54	63	24	Hippocampus
27	PD (not demented)	MSA	White	Male	45	54	15	Hippocampus
28	MSA-P	MSA	White	Female	67	76	2	Substantia Nigra
29	MSA-P	MSA	White	Female	50	57	8	Substantia Nigra

Supplementary Table 1 (Continued)

Clinical Information of the Cases Used for IHC and Quantification Shown in Fig. 3d&e

Case No.	Clinical Diagnosis	Pathological Diagnosis	Race	Sex	Age of Disease onset	Age at Death	PMI (h)	Brain Region used
26	MSA-C	MSA	White	Male	54	63	24	Medulla
27	PD (not demented)	MSA	White	Male	45	54	15	Medulla
29	MSA-P	MSA	White	Female	50	57	8	Medulla
34	MSA-P	MSA	White	Male	74	79	16	Medulla
35	PD (not demented)	MSA	White	Female	57	72	14	Hippocampus
36	MSA-P	MSA	White	Male	59	73	8	Hippocampus

Clinical Information of the Cases Used for IHC Shown in Extended Data Fig. 9d

Case No.	Clinical Diagnosis	Pathological Diagnosis	Race	Sex	Age of Disease onset	Age at Death	PMI (h)	Brain Region used
6	MSA-C	MSA	Multi-racial	Male	55	62	24	Middle Frontal Gyrus
25	MSA-C	MSA	White	Female	53	67	19	Substantia Nigra

PMI: postmortem interval

Supplementary Table 2 Biochemical Information for Sarkosyl Insoluble Fractions of Brain Extractions.

Case No.	Brain Region	Diagnosis	α-Syn (ug/ml)	Tau (ug/ml)	Abeta 40 (ng/ml)	Abeta 42 (ng/ml)	Total protein (mg/ml)
1	Cerebellum	MSA-P	22.90	0.09	< LOD	6.70	24.13
1	Cingulate Gyrus	MSA-P	2.26	0.27	< LOD	10.6	5.17
2	Cerebellum	MSA-P	7.52	0.49	< LOD	3.89	12.73
3	Cerebellum	MSA-P	19.01	0.10	2.98	13.98	5.88
3	Middle Frontal Gyrus	MSA-P	1.79	0.43	< LOD	5.50	13.74
4	Middle Frontal Gyrus	MSA-P	6.60	0.47	< LOD	< LOD	8.52
5	Middle Frontal Gyrus	MSA-C	5.54	0.53	< LOD	24.75	13.73
6	Middle Frontal Gyrus	MSA-C	40.00	0.09	< LOD	< LOD	20.33
7	Middle Frontal Gyrus	AD	8.01	17.64	14.64	53.55	11.09
8	Middle Frontal Gyrus	AD	13.55	26.00	10.00	82.30	14.40
9	Cingulate Gyrus	DLB	7.55	2.12	< LOD	73.72	12.30
10	Cingulate Gyrus	PDD	20.66	0.77	< LOD	6.00	41.11
11	Middle Frontal Gyrus	PDD	7.43	0.19	< LOD	22.75	12.76
12	Cingulate Gyrus	PDD	8.49	0.77	1.98	58.47	8.85
13	Cingulate Gyrus	PDD	15.87	2.49	0.75	89.12	7.74
14	Cingulate Gyrus	PDD	9.52	4.35	11.00	33.00	14.13
15	Cingulate Gyrus	PDD	8.28	1.50	< LOD	77.00	12.56
16	Middle Frontal Gyrus	Normal	0.2	0.12	3.85	15.67	7.43
30	Middle Frontal Gyrus	Normal	0.45	0.46	< LOD	< LOD	11.68
31	Cingulate Gyrus	Normal	0.40	0.36	< LOD	< LOD	16.28
32	Cingulate Gyrus	Normal	0.39	0.50	< LOD	18.79	14.56
33	Middle Frontal Gyrus	Normal	0.52	0.62	< LOD	< LOD	16.94

LOD: limit of detection

Supplementary Table 3 Quantification of α-Syn in the Sarkosyl Insoluble Fraction of Neuronal Culture Extractions.

Sample	Total α-Syn (ng/uL)	Human α-Syn (ng/uL)	Percentage of Human α-Syn to Total α-Syn
GCI1-N-P1	127.7	<0.15	<0.12%
GCI2-N-P1	65.1	<0.15	<0.23%
GCI3-N-P1	65.5	<0.15	<0.23%

Supplementary Table 4 Antibodies Used in This Study.

Antibody	Specificity	Host species	Dilution	Source or Reference
81A	p-α-syn (phosphorylated at Ser 129)	mouse monoclonal	1:5000 (ICC), 1:1000 (WB), 1:10000 (IHC)	[1]
SNL-4	α-syn (amino acids 2-11)	rabbit polyclonal	1:500 (WB)	[2]
Syn506	misfolded α-syn	mouse monoclonal	1:5000 (IHC), 1:2500 (IF)	[1]
NAC1	α-syn (amino acids 75-91)	rabbit polyclonal	1:500 (WB)	[3]
LB509	α-syn (amino acids 115-122)	mouse monoclonal	1:500 (WB); 1:2500 (IF)	[4]
Syn211	α-syn (amino acids 121-125)	mouse monoclonal	1:500 (WB)	[5]
Syn102	α-syn (amino acids 131-140)	mouse monoclonal	1:500 (WB)	[6]
HuA	Raised against recombinant human α-syn	rabbit polyclonal	3.3ng/µl (ELISA); 1:500 (WB)	[7]
Syn9027	α-syn (amino acids 130-140)	mouse monoclonal	3.3ng/µl (ELISA); 1:20000 (WB); IP	[8]
MJF-R1	human α-syn	rabbit monoclonal	1:1000 (ELISA)	Abcam (ab138501)
SNL-1	α-syn (C-terminal)	rabbit polyclonal	1:500 (WB)	[2]
Olig-2	Oligodendrocyte transcription factor 2 (Olig-2)	rabbit polyclonal	1:500 (IF and ICC)	Millipore (AB9610)
Iba1	Macrophage/Microglia-Specific Calcium-Binding Protein (Iba1)	rabbit polyclonal	1:500 (IF) and 1:250 (ICC)	Wako (019-19741)
GFAP	Glial Fibrillary Acidic Protein	rabbit polyclonal	1:1000 (IF) and 1:500 (ICC)	Dako (Z0334)
NeuN	Neuronal Nuclei (NeuN)	rabbit polyclonal	1:1000 (IF) and 1:500 (ICC)	Millipore (ABN78)
MAP2	Microtubule-Associated Protein 2 (MAP2)	rabbit polyclonal	1:2000 (ICC)	[9]
PLP	proteolipid protein	Rat monoclonal	1:1 (ICC)	Gift from Dr. Judith B. Grinspan, CHOP, Phiadelphia, PA[10]. The antibody was originally from Dr. Alex Gow, Wayne State University, Detroit, MI
CNP	2',3'-Cyclic-nucleotide 3'-phosphodiesterase	rabbit polyclonal	1:500 (ICC)	[11]
Syn204	α-syn (amino acids 87-110)	mouse monoclonal	1:500 (WB)	[5]
Syn303	α-syn	mouse monoclonal	1:60000 (IHC)	[12]
Syn7015	Raised against strain A α-syn fibrils	mouse monoclonal	1ng/ml (IHC)	[8]

Mouse α-syn	Mouse α-syn	Rabbit monoclonal	1:1000 (WB)	Cell Signaling Technology (4179)
β-tubulin	β-tubulin	mouse monoclonal	1:3000 (WB)	Invitrogen (32-2600)
GAPDH	GAPDH	mouse monoclonal	1:18000 (WB)	Advanced Immunochemical (2-RGM2)

Supplementary Table 5 Statistics.

Figure #	**Compare (Group size n=)**	**Statistical methods**	**P Value**	**t Value**	**F value**	**Degrees of freedom**
Figure 1b	LB vs GCI (LB, n=7; GCI,n=5 different cases)	two tailed, unpaired t-test	0.0009	4.69		10
Figure 1e	LB-7015 vs GCI-7015 (LB, n=9, GCI, n=7 different cases)	Mann Whitney U test	0.0157			
Figure 1f	LB vs GCI (LB, n=9, GCI, n=7 different cases)	two tailed, unpaired t-test	0.001	4.149		14
Figure 1i	All LB vs All GCI (LB,n=9; GCI,n=8 different preparations)	two tailed, unpaired t-test with Welch's correction, using the mean value of each case	0.0015	5.023		7.001
Figure 1j	30ng LB vs 30pg GCI (LB, n=6; GCI, n=5 biologically independent replicates)	two tailed, unpaired t-test with Bonferroni correction	0.9693	0.03952		9
Figure 1j	30ng GCI vs 30µg PFF (GCI, n=5; PFF, n=5 biologically independent replicates)	two tailed, unpaired t-test with Bonferroni correction	0.296	1.118		8
Figure 2b	All LB vs All GCI (GCI, n=8, LB, n=9 different preparations)	two tailed, unpaired t-test with Welch's correction, using the mean value of each case	<0.0001	13.51		7.067
Figure 2c	100pg GCI vs 100ng PFF (n=3 biologically independent replicates)	two tailed, unpaired t-test with Bonferroni correction	0.8687	0.1762		4
Figure 2d	10pg GCI vs 10ng LB (n=3 biologically independent replicates)	two tailed, unpaired t-test with Bonferroni correction	0.5405	0.6683		4

Figure 2f	2ng LB vs 2pg GCI (LB, n=7; GCI, n=4 biologically independent replicates)	two tailed, unpaired t-test	0.2019	1.377		9
Figure 2f	200pg GCI vs 200ng PFF (GCI, n=4; PFF, n=3 biologically independent replicates)	two tailed, unpaired t-test	0.0989	2.024		5
Figure 2g	GCI-WT vs LB-WT at 3 mpi (n=3 mice for both GCI-WT and LB-WT)	two tailed, unpaired t-test with Welch's correction	0.0417	4.744		2.001
Figure 2h	All groups	Two-way ANOVA, with Tukey's HSD	Brain region: p<0.0001; Strains: p=0.2074; Interaction p<0.0001		Brain region: F=60.98; Strain: F=1.613; Interaction F=18.5	Brain region: Df=8; Strain: Df=2; Interaction Df=16
Figure 2h	Cotex: GCI-WT vs PFF-WT (GCI-WT, n=3; PFF-WT, n=4 mice)	Two-way ANOVA, with Tukey's HSD	<0.0001			
Figure 2h	Cotex: GCI-WT vs LB-WT (n=3 mice for both GCI-WT and LB-WT)	Two-way ANOVA, with Tukey's HSD	<0.0001			
Figure 2h	PIR2: GCI-WT vs PFF-WT (GCI-WT, n=3; PFF-WT, n=4 mice)	Two-way ANOVA, with Tukey's HSD	<0.0001			
Figure 2h	PIR2: GCI-WT vs PFF-WT (GCI-WT, n=3; PFF-WT, n=4 mice)	Two-way ANOVA, with Tukey's HSD	<0.0001			
Figure 2h	Str: GCI-WT vs PFF-WT (GCI-WT, n=3; PFF-WT, n=4 mice)	Two-way ANOVA, with Tukey's HSD	p=0.0003			
Figure 2h	Str: LB-WT vs PFF-WT (LB-WT, n=3; PFF-WT, n=4 mice)	Two-way ANOVA, with Tukey's HSD	p=0.0055			
Figure 2h	Hippo: GCI-WT vs PFF-WT (GCI-WT, n=3; PFF-WT, n=4 mice)	Two-way ANOVA, with Tukey's HSD	p=0.0199			
Figure 2h	Hippo: GCI-WT vs LB-WT (n=3 mice for both GCI-WT and LB-WT)	Two-way ANOVA, with Tukey's HSD	p=0.0599			
Figure 2i	all groups	Two-way ANOVA, with Tukey's HSD	Brain region: p<0.0001; Strains: p=0.0027;		Brain region: F=29.89; Strain: F=6.496;	Brain region: Df=8; Strain: Df=2; Interaction Df=16

			Interaction p<0.0001		Interaction F=4.673	
Figure 2i	Cotex: GCI-WT vs PFF-WT (GCI-WT, n=3; PFF-WT, n=4 mice)	Two-way ANOVA, with Tukey's HSD	<0.0001			
Figure 2i	Cotex: GCI-WT vs LB-WT (n=3 mice for both GCI-WT and LB-WT)	Two-way ANOVA, with Tukey's HSD	<0.0001			
Figure 3b	GCI-KOM2 vs LB-KOM2 at 1 mpi (n=3 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Bonferroni correction	<0.0001	18.61		4
Figure 3b	GCI-KOM2 vs LB-KOM2 at 3 mpi (n=5 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Welch's correction and Bonferroni correction	0.3391	1.067		4.583
Figure 3b	GCI-KOM2 vs LB-KOM2 at 6 mpi (n=5 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Welch's correction and Bonferroni correction	0.1308	1.835		4.616
Figure 3c	All groups	One-way Anova with Dunnett's Multiple Comparison Test comparing each group with LB-KOM2	0.0041		6.566	between columns: Df=4; within colums: Df=13; Total: Df=17
Figure 3c	LB-KOM2 vs GCI (LB-KOM2, n=4 mice; GCI, n=3 cases)	One-way Anova with Dunnett's Multiple Comparison Test	0.2193			
Figure 3c	LB-KOM2 vs LB (n=4 mice; for LB-KOM2; n=3 cases for LB)	One-way Anova with Dunnett's Multiple Comparison Test	0.0043			
Figure 3c	LB-KOM2 vs GCI-KOM2 (n=4 mice for both LB-KOM2 and GCI-KOM2)	One-way Anova with Dunnett's Multiple Comparison Test	0.2			
Figure 3c	LB-KOM2 vs M83 (n=4 mice for both LB-KOM2 and M83)	One-way Anova with Dunnett's Multiple Comparison Test	0.0024			
Figure 3e	NI vs GCI (n=6 cases for both NI and GCI)	two tailed, unpaired t-test	0.0002	5.734		10
Figure 4c	All groups	One-way Anova with Dunnett's Multiple Comparison Test comparing each group with PFF-KOM2	<0.0001		65.39	between columns: Df=4; within colums: Df=15; Total: Df=19

Figure 4c	PFF-KOM2 200pg vs PFF 200pg (PFF-KOM2, n=6; PFF n=3 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.0045			
Figure 4c	PFF-KOM2 200pg vs PFF 2ng (PFF-KOM2, n=6; PFF n=3 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.0205			
Figure 4c	PFF-KOM2 200pg vs PFF 20ng (PFF-KOM2, n=6; PFF n=3 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.0001			
Figure 4c	PFF-KOM2 200pg vs KOM2 + PFF (PFF-KOM2, n=6; KOM2 + PFF n=5 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.002			
Figure 4d	LB-KOM2 vs LB (LB-KOM2, n=7; LB, n=3 biologically independent replicates)	two tailed, unpaired t-test	0.0439	2.389		8
Figure 4f	All Groups	One-way Anova with Dunnett's Multiple Comparison Test comparing each group with PFF-Oligo-Syn	<0.0001		130.4	between columns: Df=7; within colums: Df=23; Total: Df=30
Figure 4f	PFF-Oligo-Syn 200pg vs PFF-RatH-Syn 200pg (PFF-Oigo-Syn, n=6; PFF-RatH-Syn, n=4 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.0001			
Figure 4f	PFF-Oligo-Syn 200pg vs PFF-RatC-Syn 200pg (PFF-Oigo-Syn, n=6; PFF-RatC-Syn, n=4 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.0001			
Figure 4f	PFF-Oligo-Syn 200pg vs PFF-QBI-Syn 200pg (PFF-Oigo-Syn, n=6; PFF-QBI-Syn, n=4 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.0001			
Figure 4f	PFF-Oligo-Syn 200pg vs PFF 200pg (PFF-Oigo-Syn, n=6; PFF, n=4 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.0001			

Figure 4f	PFF-Oligo-Syn 200pg vs PFF 2ng (PFF-Oigo-Syn, n=6; PFF, n=3 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.0001			
Figure 4f	PFF-Oligo-Syn 200pg vs PFF 20ng (PFF-Oigo-Syn, n=6; PFF, n=3 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.9999			
Figure 4f	PFF-Oligo-Syn 200pg vs PFF 200ng (PFF-Oigo-Syn, n=6; PFF, n=3 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.0001			
Figure 4h	All Groups	One-way Anova with Dunnett's Multiple Comparison Test	0.0025		7.913	between columns: Df=3; within colums: Df=14; Total: Df=17
Figure 4h	Oligo-PFF vs RatC-PFF (Oligo-PFF, n=6; RatC-PFF, n=4 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.0107			
Figure 4h	Oligo-PFF vs RatH-PFF (Oligo-PFF, n=6; RatH-PFF, n=4 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.0045			
Figure 4h	Oligo-PFF vs PFF (Oligo-PFF, n=6; PFF, n=4 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.004			
Figure 4j	All Groups	One-way Anova with Dunnett's Multiple Comparison Test comparing each group with GCI 200pg group.	<0.0001		16.88	between columns: Df=9; within colums: Df=28; Total: Df=37
Figure 4j	GCI 200pg vs GCI-N-P1 200pg (GCI, n=3; GCI-N-P1, n=5 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.3584			
Figure 4j	GCI 200pg vs GCI-N-P2 200pg (GCI, n=3; GCI-N-P2, n=4 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.3156			
Figure 4j	GCI 200pg vs GCI-N-P3 200pg (GCI, n=3; GCI-N-P1, n=4 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.9049			

Figure 4j	GCI 200pg vs PFF 200ng (GCI, n=3; GCI-N-P1, n=3 biologically independent replicates)	One-way Anova with Dunnett's Multiple Comparison Test	0.1082			
Figure 4l	All Groups	One-way Anova with Tukey's Multiple Comparison Test	<0.0001		44.43	between columns: Df=3; within colums: Df=24; Total: Df=27
Figure 4l	GCI vs GCI-M-P1 (GCI, n=8; GCI-M-P1, n=6 biologically independent replicates)	One-way Anova with Tukey's Multiple Comparison Test	0.2562			
Figure 4l	GCI-M-P1 vs LB-M-P1 (n=6 biologically independent replicates for both GCI-M-P1 and LB-M-P1)	One-way Anova with Tukey's Multiple Comparison Test	<0.0001			
Figure 4l	LB vs LB-M-P1 (LB, n=8; LB-M-P1, n=6 biologically independent replicates)	One-way Anova with Tukey's Multiple Comparison Test	0.9944			
Extented data Fig. 2b	LB vs GCI at Syn7015 15ng/ml (LB, n=5; GCI, n=4 cases)	two tailed, unpaired t-test	0.0423	2.479		7
Extented data Fig. 2b	LB vs GCI at Syn7015 5ng/ml (LB, n=5; GCI, n=4 cases)	two tailed, unpaired t-test	0.0153	3.187		7
Extented data Fig. 2b	LB vs GCI at Syn7015 1.67ng/ml (LB, n=5; GCI, n=4 cases)	two tailed, unpaired t-test	0.0435	2.459		7
Extented data Fig. 3b	All GCI vs All LB (n=3 biologically independent replicates for MSA-P, MSA-C, AD, PDD, DLB, PFF and PBS)	two tailed, unpaired t-test with Welch's correction	0.0012	6.621		5.003
Extented data Fig. 4b	All GCI vs All LB (n=3 biologically independent replicates for MSA-P, MSA-C, AD, PDD, DLB, PFF and PBS)	two tailed, unpaired t-test with Welch's correction	0.0071	4.387		5.008
Extented data Fig. 4c	All LB vs All GCI (LB, n=9; GCI, n=8 different preparations]	two tailed, unpaired t-test with Welch's correction, using the mean value of each case	<0.0001	12.71		7.102
Extented data Fig. 4e	All GCI vs All LB (n=3 biologically independent replicates for MSA-P, MSA-C,	two tailed, unpaired t-test with Welch's correction	0.0012	6.621		5.003

	AD, PDD, DLB, PFF and PBS)					
Extented data Fig. 4f	All Group	One-way Anova with Tukey's Multiple Comparison Test	<0.0001		33.44	between columns: Df=11; within colums: Df=24; Total: Df=35
Extented data Fig. 4f	10ng IP purified GCI vs 10ng GCI (n=3 biologically independent replicates for both IP purified GCI and GCI)	One-way Anova with Tukey's Multiple Comparison Test	0.9799			
Extented data Fig. 4f	10ng IP purified GCI vs 10ng IP purified LB (n=3 biologically independent replicates for both GCI and LB)	One-way Anova with Tukey's Multiple Comparison Test	<0.0001			
Extented data Fig. 4g	GCI Depleted vs GCI Depleted + PFF (n=3 biologically independent replicates for both group)	two tailed, unpaired t-test with Bonferroni correction	0.7754	0.3052		4
Extented data Fig. 4g	LB Depleted vs LB Depleted + PFF (n=3 biologically independent replicates for both group)	two tailed, unpaired t-test with Bonferroni correction	0.1986	1.539		4
Extented data Fig. 4h	Depleted GCI + 100ng PFF vs 100ng PFF (n=3 biologically independent replicates for both group)	two tailed, unpaired t-test with Bonferroni correction	0.6536	0.4841		4
Extented data Fig. 4i	All group	Two-way ANOVA, with Sidak's multiple comparisons test	Strrains p<0.0001; Ch trestment p<0.0001; interaction p<0.0001		Strrains F=24.66; Ch trestment F=71.89; interaction F=25.65	Strrains Df=2; Ch trestment Df=1; interaction Df=2
Extented data Fig. 4i	GCI without Ch vs GCI with Ch (n=3 biologically independent replicates for both group)	Two-way ANOVA, with Sidak's multiple comparisons test	<0.0001			
Extented data Fig. 4i	PFF without Ch vs PFF with Ch (n=3 biologically independent replicates for both group)	Two-way ANOVA, with Sidak's multiple comparisons test	0.0065			

Extented data Fig. 4i	GCI with Ch vs PFF with Ch (n=3 biologically independent replicates for both group)	Two-way ANOVA, with Sidak's multiple comparisons test	0.0036			
Extented data Fig. 4j	All group	Two-way ANOVA, with Sidak's multiple comparisons test	Strrains p<0.0001; Ch trestment p<0.0001; interaction p=0.0003		Strrains F=17.93; Ch trestment F=92.1; interaction F=13.52	Strrains Df=2; Ch trestment Df=1; interaction Df=2
Extented data Fig. 4j	GCI without Ch vs GCI with Ch (n=3 biologically independent replicates for both group)	Two-way ANOVA, with Sidak's multiple comparisons test	<0.0001			
Extented data Fig. 4j	PFF without Ch vs PFF with Ch (n=3 biologically independent replicates for both group)	Two-way ANOVA, with Sidak's multiple comparisons test	<0.0001			
Extented data Fig. 4j	GCI with Ch vs PFF with Ch (n=3 biologically independent replicates for both group)	Two-way ANOVA, with Sidak's multiple comparisons test	0.2822			
Extented data Fig. 4k	GCI-WT vs LB-WT at 6 mpi (n=3 mice for both GCI-WT and LB-WT)	two tailed, unpaired t-test with Welch's correction	0.2852	1.445		2.002
Extented data Fig. 7b	GCI-KOM2 vs LB-KOM2 at 1 mpi (n=3 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Welch's correction and Bonferroni correction	0.0515	4.212		2.012
Extented data Fig. 7b	GCI-KOM2 vs LB-KOM2 at 3 mpi (n=5 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Welch's correction and Bonferroni correction	0.267	1.259		4.694
Extented data Fig. 7b	GCI-KOM2 vs LB-KOM2 at 6 mpi (n=5 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Welch's correction and Bonferroni correction	0.1069	2.054		4.133
Extented data Fig. 7c	GCI-KOM2 vs LB-KOM2 at 1 mpi (n=3 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Welch's correction and Bonferroni correction	0.0177	7.174		2.046
Extented data Fig. 7c	GCI-KOM2 vs LB-KOM2 at 3 mpi (n=5 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Bonferroni correction	0.5336	0.6505		8
Extented data Fig. 7c	GCI-KOM2 vs LB-KOM2 at 6 mpi (n=5 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Bonferroni correction	0.0673	2.116		8

Extented data Fig. 7d	GCI-KOM2 vs LB-KOM2 at 1 mpi (n=3 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Welch's correction and Bonferroni correction	0.3468	1.22		2
Extented data Fig. 7d	GCI-KOM2 vs LB-KOM2 at 3 mpi (n=5 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Welch's correction and Bonferroni correction	0.3906	0.9617		4.004
Extented data Fig. 7d	GCI-KOM2 vs LB-KOM2 at 6 mpi (n=5 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Welch's correction and Bonferroni correction	0.3183	1.139		4.004
Extented data Fig. 7e	GCI-KOM2 vs LB-KOM2 at 1 mpi (n=3 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Welch's correction and Bonferroni correction	0.1661	2.136		2.001
Extented data Fig. 7e	GCI-KOM2 vs LB-KOM2 at 3 mpi (n=5 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Welch's correction and Bonferroni correction	0.5626	0.6263		4.334
Extented data Fig. 7e	GCI-KOM2 vs LB-KOM2 at 6 mpi (n=5 mice for both GCI-KOM2 and LB-KOM2)	two tailed, unpaired t-test with Welch's correction and Bonferroni correction	0.0831	2.223		4.477
Extented data Fig. 10d	All Groups	One-way Anova with Dunnett's Multiple Comparison Test comparing each group with PFF-Oligo-Syn	<0.0001		23.56	between columns: Df=5; within colums: Df=12; Total: Df=17
Extented data Fig. 10d	PFF-Oligo-Syn vs PFF-HipN-Syn (n=3 biologically independent replicates for both group)	One-way Anova with Dunnett's Multiple Comparison Test	0.0001			
Extented data Fig. 10d	PFF-Oligo-Syn vs PFF-CtxN-Syn (n=3 biologically independent replicates for both group)	One-way Anova with Dunnett's Multiple Comparison Test	0.0001			
Extented data Fig. 10d	PFF-Oligo-Syn vs PFF-QBI-Syn (n=3 biologically independent replicates for both group)	One-way Anova with Dunnett's Multiple Comparison Test	0.0001			
Extented data Fig. 10d	PFF-Oligo-Syn vs PFF (n=3 biologically independent replicates for both group)	One-way Anova with Dunnett's Multiple Comparison Test	0.0001			
Extented data Fig. 10d	PFF-Oligo-Syn vs PPBS (n=3 biologically independent replicates for both group)	One-way Anova with Dunnett's Multiple Comparison Test	0.0001			

Extented data Fig. 10g	All Groups	One-way Anova with Dunnett's Multiple Comparison Test comparing each group with GCI	0.0003		11.63	between columns: Df=5; within colums: Df=12; Total: Df=17
Extented data Fig. 10g	GCI vs GCI-N-P1 (n=3 biologically independent replicates for both group)	One-way Anova with Dunnett's Multiple Comparison Test	0.9998			
Extented data Fig. 10g	GCI vs GCI-N-P2 (n=3 biologically independent replicates for both group)	One-way Anova with Dunnett's Multiple Comparison Test	0.6404			
Extented data Fig. 10g	GCI vs GCI-N-P3 (n=3 biologically independent replicates for both group)	One-way Anova with Dunnett's Multiple Comparison Test	0.6721			
Extented data Fig. 10g	GCI vs PFF (n=3 biologically independent replicates for both group)	One-way Anova with Dunnett's Multiple Comparison Test	0.0061			
Extented data Fig. 10g	GCI vs PBS (n=3 biologically independent replicates for both group)	One-way Anova with Dunnett's Multiple Comparison Test	0.009			

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Supplementary Fig. 1. Full blots of western blot data

Fig. 1a

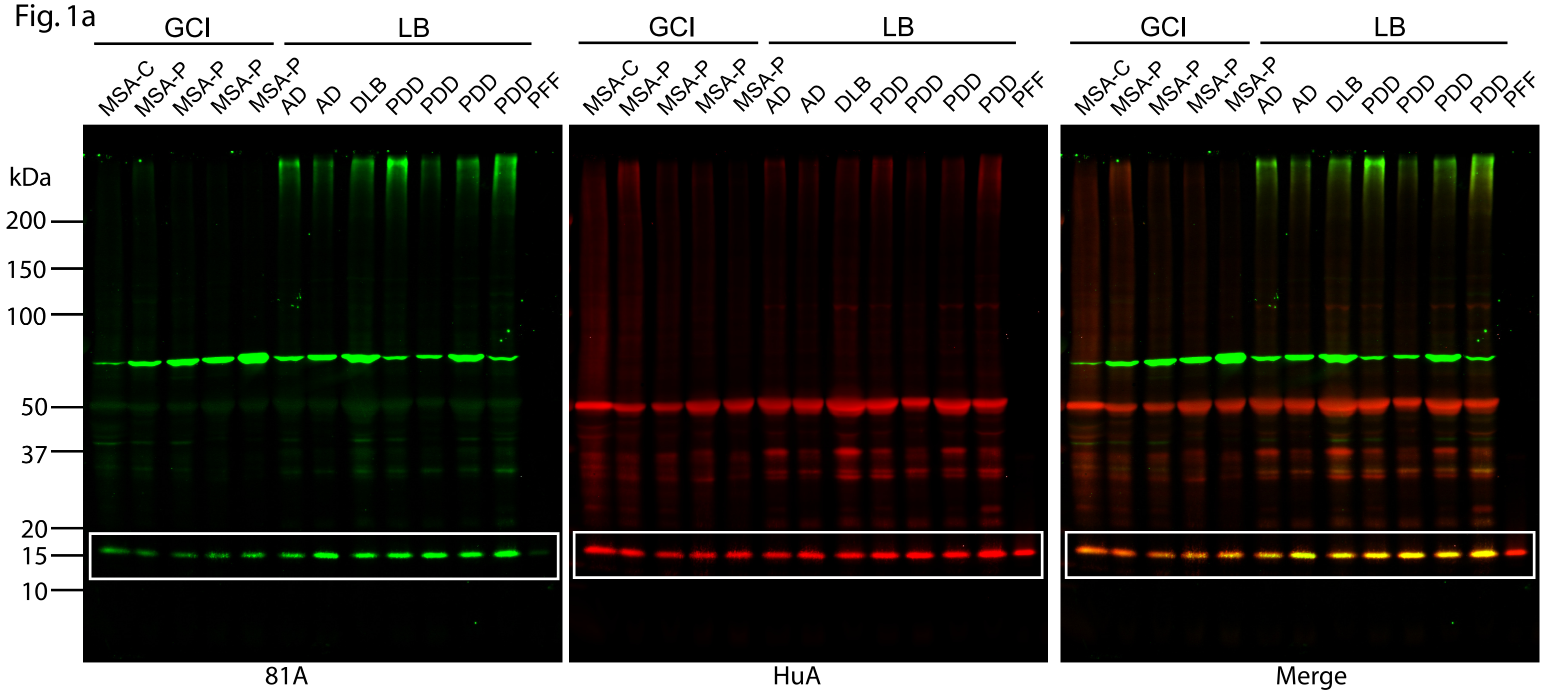


Fig. 1c

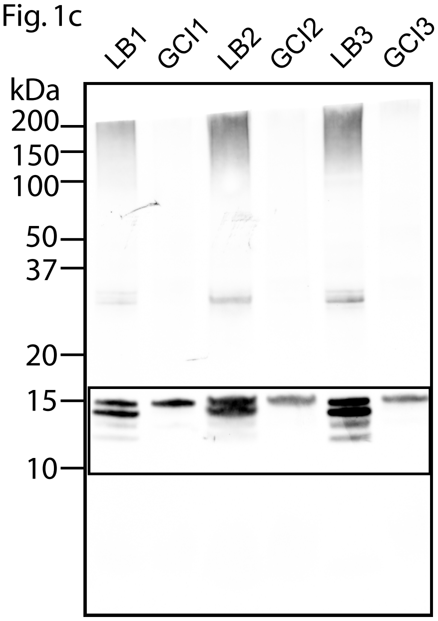


Fig. 1d

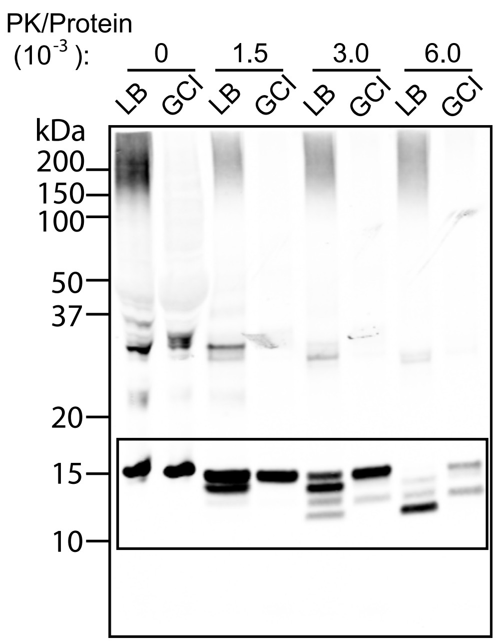
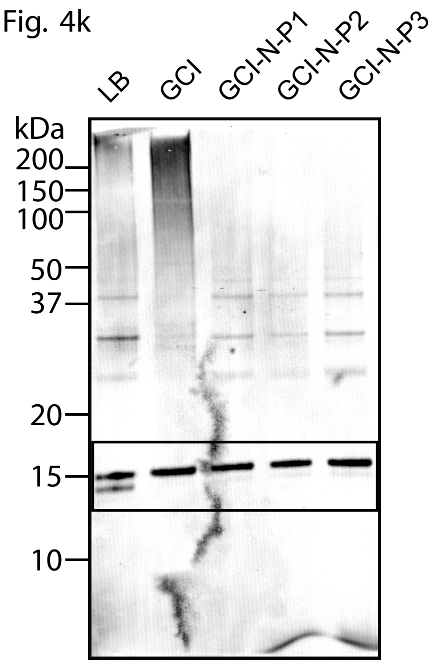
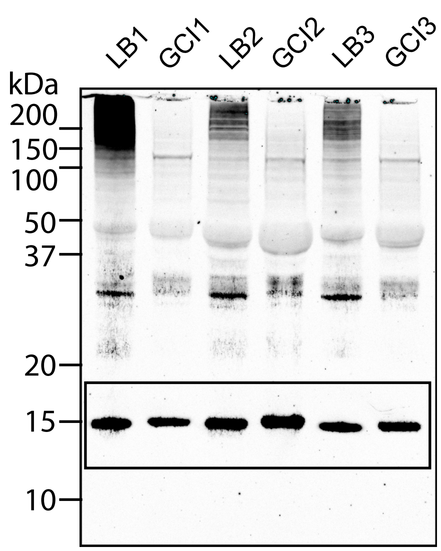


Fig. 4k

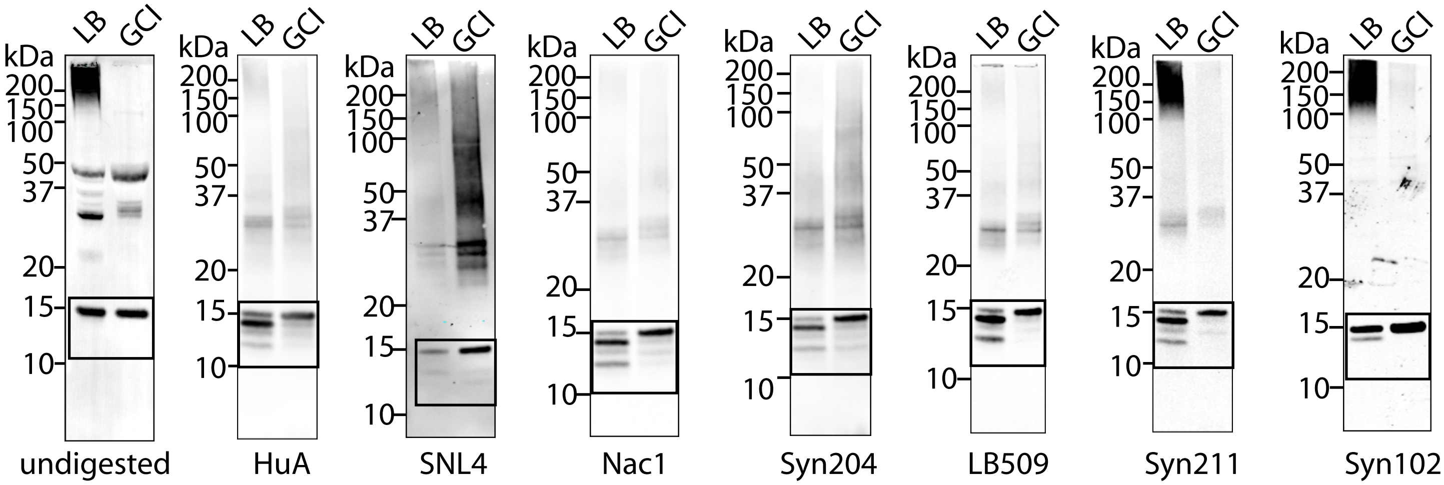




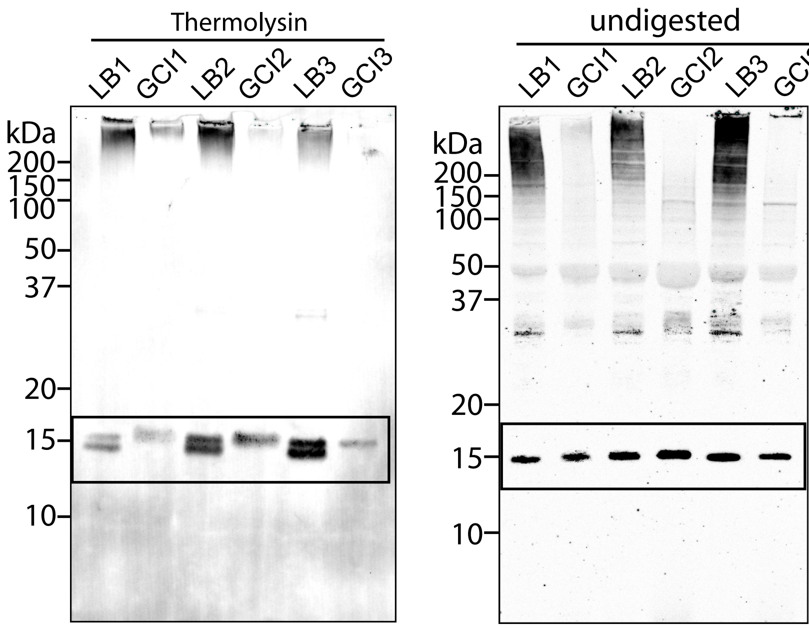
Undigested

Supplementary Fig. 1. Full blots of western blot data (Continue)

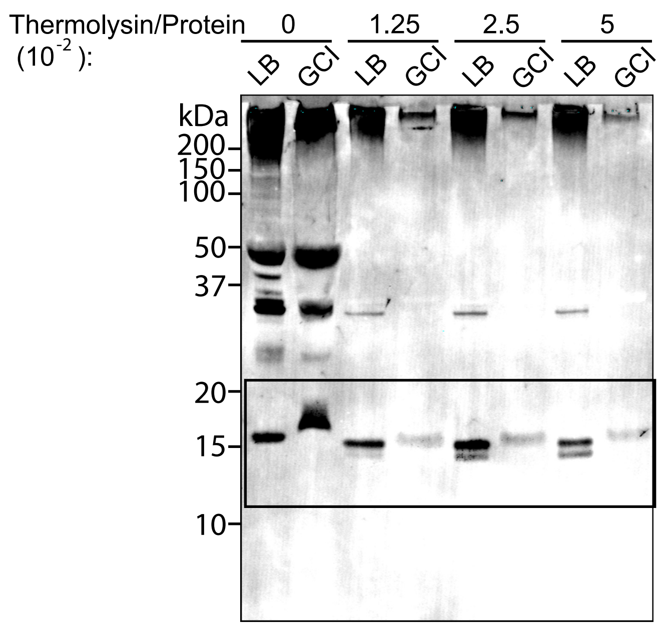
Extended Data Fig. 1b



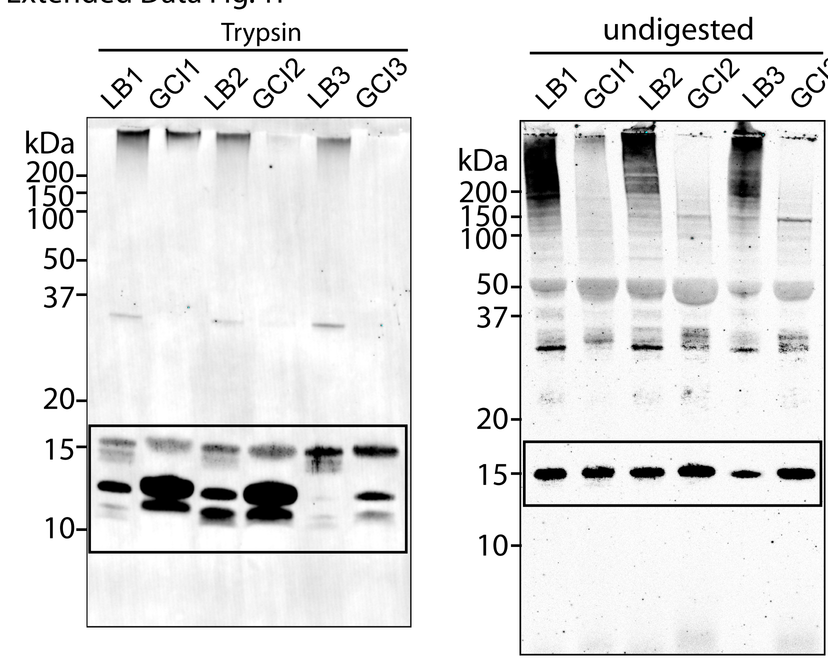
Extended Data Fig. 1d



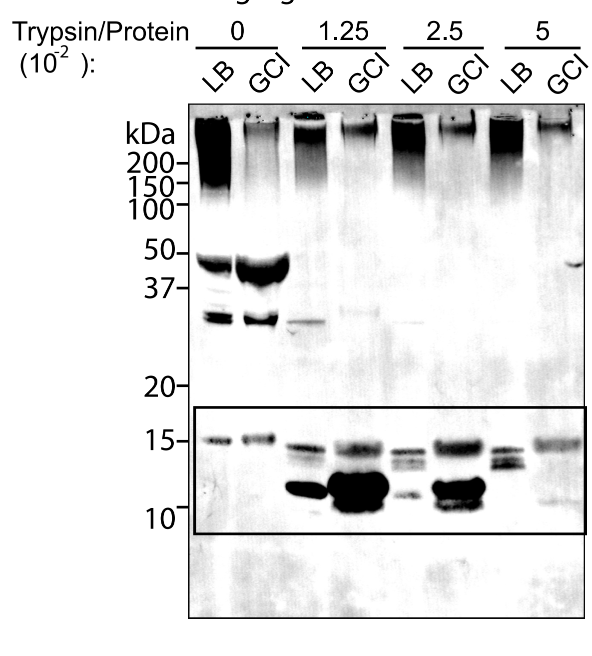
Extended Data Fig. 1e



Extended Data Fig. 1f

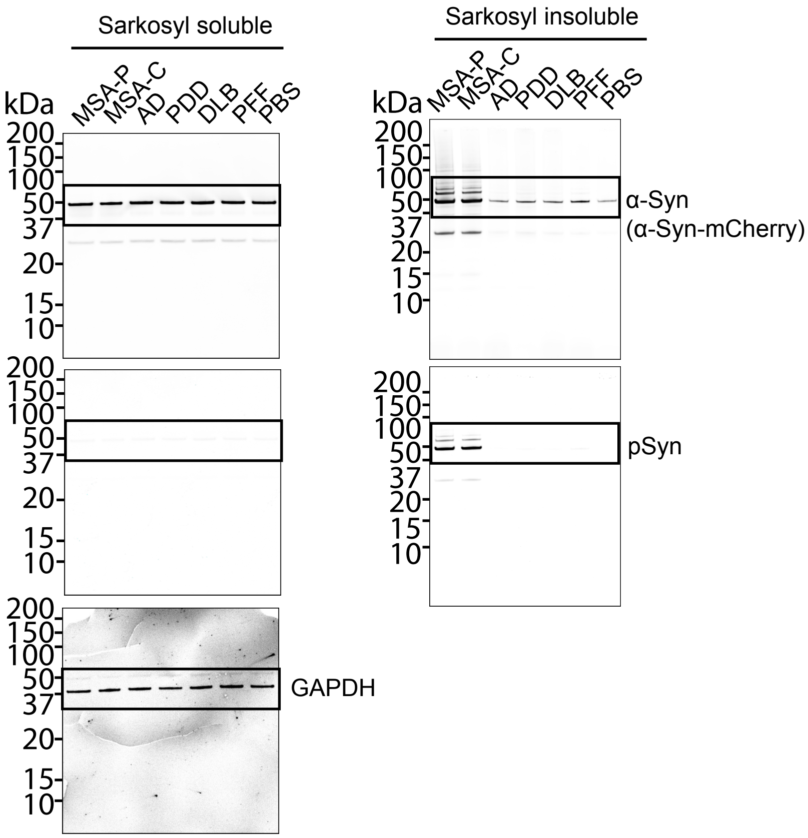


Extended Data Fig. 1g

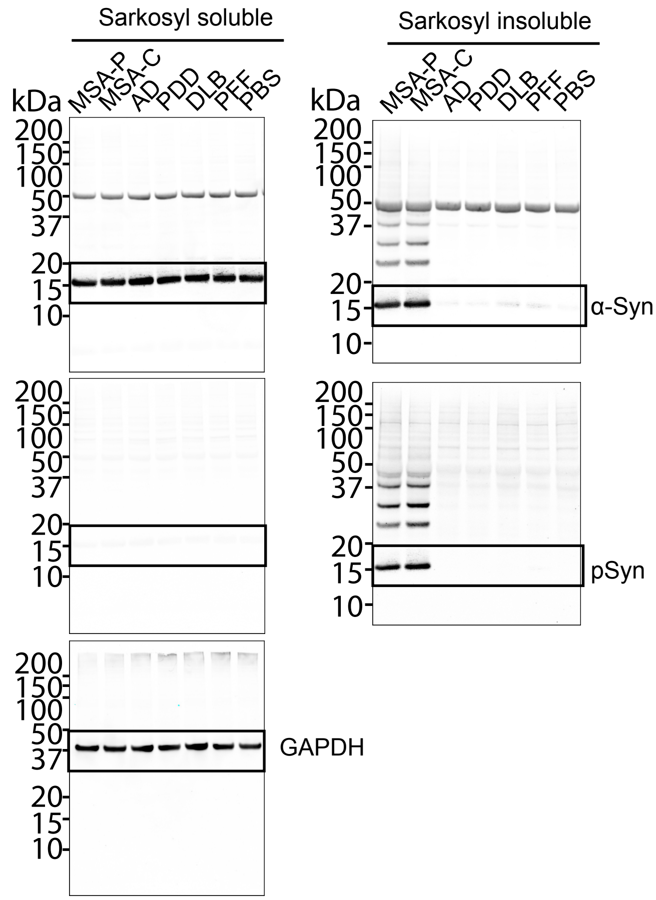


Supplementary Fig. 1. Full blots of western blot data (Continue)

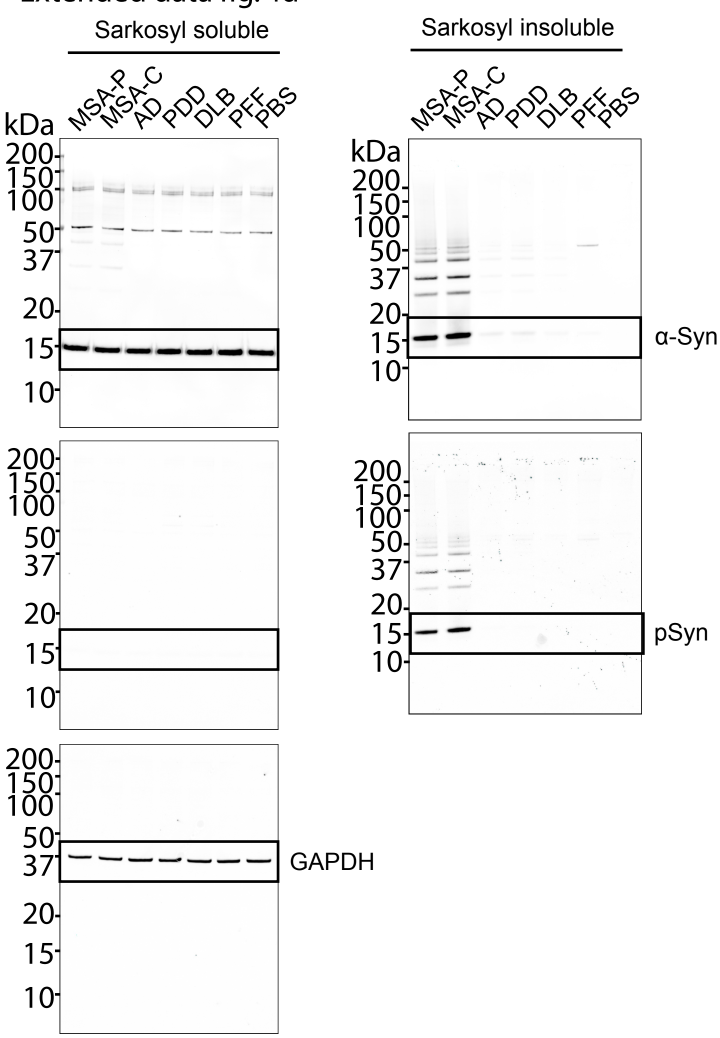
Extended data fig. 3a



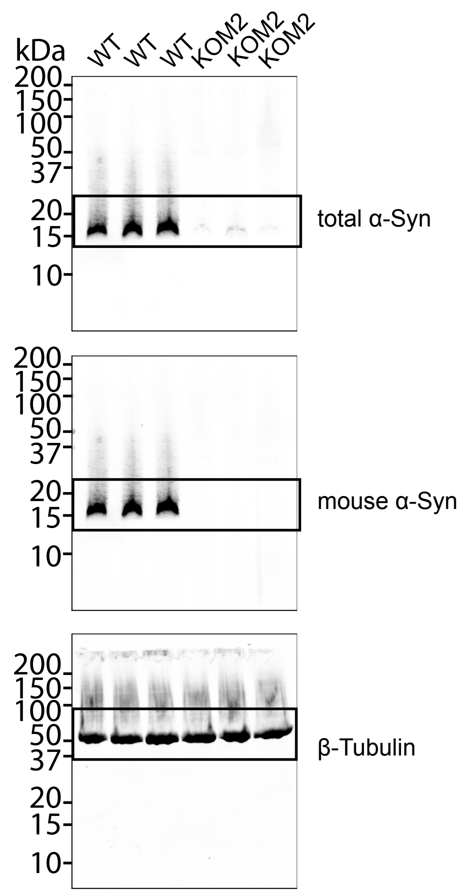
Extended data fig. 4a



Extended data fig. 4d

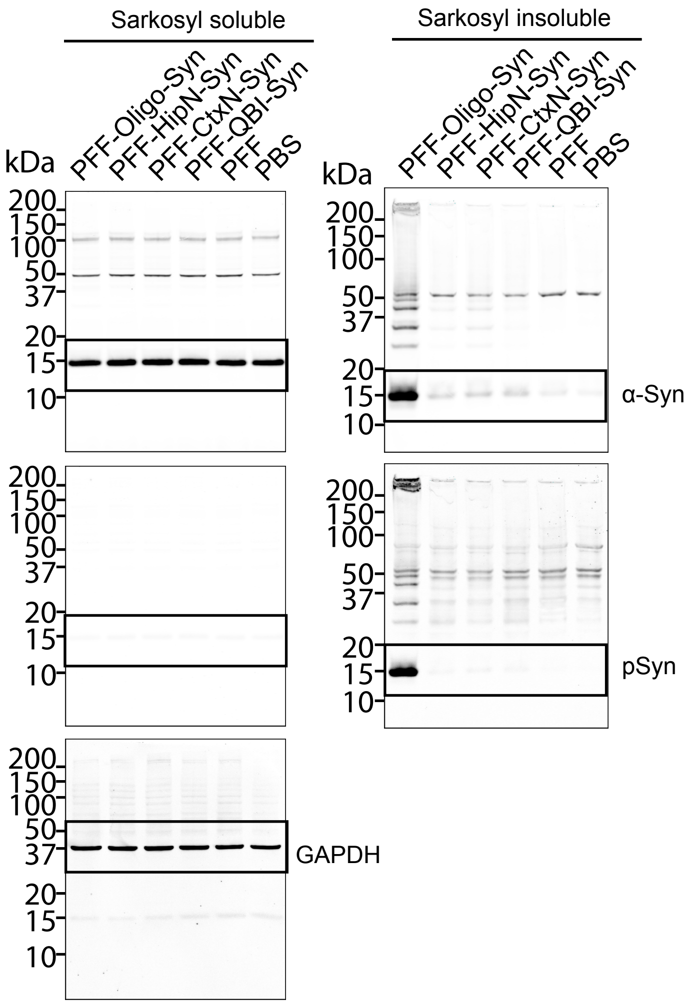


Extended data fig. 6b



Supplementary Fig. 1. Full blots of western blot data (continue)

Extended Data Fig. 10c



Extended Data Fig. 10f