APPENDIX

	APPENDIX FIGURES AND TABLES	Pg.	
Figure S1.	Characterization of α -syn assemblies used throughout this work	2	
Figure S2.	Immunodetection of α -syn clusters on cell surface.	3	
Figure S3.	Concentration-dependent increase in a-syn clustering	4	
Figure S4.	Example of unambiguous mass spectrometric identification of α 3-NKA obtained from neurons exposed for 10 min to fibrillar α -syn	5	
Figure S5.	Membrane expression of α 3-NKA and chimeric α 3/ α 1-NKA constructs.	6	
Figure S6.	Protocol for the measurement of Na ⁺ dynamics in neurons	7	
Table S1.	(Data used in Figure 1B) Age-dependent clustering of α -syn assemblies	8	
Table S2.	(Data supporting Figure S3) Concentration-dependent clustering of α -syn assemblies	9	
Table S3.	(Data used in Figure 2A and 2B) Time-dependent clustering of α -syn assemblies	10	
Table S4.	Association of α-syn and homer/gephyrin	11	
Table S5.	(Data supporting Figure 3) Neuronal proteins interacting with extracellularly applied oligomeric α-syn assemblies	12	
Table S6.	(Data supporting Figure 3) Neuronal proteins interacting with extracellularly applied fibrillar α -syn assemblies	13-15	
Table S7.Astrocyte proteins interacting with extracellularly applied oligomeric (A) and fibrillar (B) α-syn assemblies		16-18	
Table S8.(Data supporting Fig 4B) Single particle tracking of pHluorin- α 3-NKA in presence of ATTO-550-tagged α -syn		19	
Table S9.(Data supporting Fig 4D and 4E) Single particle tracking of pHluorin- α 3-NKA in presence of unlabeled α -syn		20	
Table S10.	(Data supporting Fig 6B) Enrichment of α 3-NKA over α -syn at synapses	21	
	APPENDIX MATERIAL AND METHODS	Pg.	
Preparation, lab	eling, characterization and assembly of α-syn		
Primary neurona	al cultures		
Pull-down of α-spectrometry (N	syn-S-tag bound protein complexes and sample preparation for mass IS)		
Mass spectrome	Mass spectrometric identification and quantification of the pulled-down proteins		
Co-immunoprec			
In vivo injection	22-30		
Immunohistoche			
Plasmids and Tr			
Single particle the			
Super-resolution			
Sodium Dye Lo	ading, Imaging and Analysis		
Calcium Imagin	g and Analysis		
Software and St	atistics	21	
	APPENDIX REFERENCES	31	

Appendix Figure S1



Appendix Figure S1. Characterization of α-syn assemblies used throughout this work

Electron micrographs of oligomeric (left column) and fibrillar (middle column) untagged, S-tagged, ATTO550- and biotin-labeled wild-type α -syn used throughout this study. The scale bar represents 200 nm. The assembly kinetics assessed by Thioflavin T binding of wild-type untagged (closed circle and solid line) and S-tagged- α -syn (open circles and dashed line), 100 μ M, in 50 mM Tris pH 7.5, 150 mM KCl, are compared in the top panel of the column on the right. The values are mean ± S.E.M. obtained from three independent assembly reactions. No statistically significant difference is observed. The MALDI-TOF mass spectra, from top to bottom of S-tagged, ATTO-550- and biotin-labeled wild-type α -syn are shown. The spectra show that α -syn is labeled on average by one ATTO-550 or one biotin molecule.

Appendix Figure S2



Appendix Figure S2. Immunodetection of α-syn clusters on cell surface

Cultured striatal neurons unexposed or exposed (60 min) to alexa647-labeled oligomeric (25 nM) or fibrillar (0.03 nM) α -syn (red) followed by immunolabeling using α -syn antibody (green) without permeabilization. Almost all alexa647- α -syn clusters were also immunoreactive indicating cell surface localization.





(A-D) 21 DIV neurons exposed for 1 h to ATTO-550-labeled oligomeric (A, C, blue) or fibrillar (B, D, orange) α -syn at indicated concentrations. Note the concentration-dependent increase in the average fluorescence intensity and number per μ m² of α -syn clusters. (See Appendix Table S2 for actual values).





Appendix Figure S4. Example of unambiguous mass spectrometric identification of α3-NKA obtained from neurons exposed for 10 min to fibrillar α-syn

(A) Primary structure coverage of α 3-NKA (Swiss Prot accession number P06687) obtained following onbeads tryptic digestion of the proteins pulled down as described in the material and methods section. The eight amino acid stretches spreads throughout the primary structure, from the N- to the C-terminus, labeled in yellow correspond to peptides identified by nanoLC-MS/MS analysis. α 3-NKA is identified with confidence. The eight peptides cover 12% (117 out of 1030 amino acid residues) of α 3-NKA primary structure.

(B) The MS/MS fragmentation spectra of the eight identified peptides are shown as is their primary structure, their mascot ion score and the m/z and the mass accuracy of the fragmented precursor peptide. The primary structure is determined from the y (blue) and b (red) fragment ions.

Appendix Figure S5



Appendix Figure S5. Membrane expression of α3-NKA and chimeric α3/α1-NKA constructs.

Neurons transfected with non-chimeric α 3-NKA-pHluorin or chimeric α 3/ α 1-NKA-pHluorin (used in **Figure 5A**). Representative images show membrane expression of chimeric constructs similar to that of the α 3-NKA-pHluorin. Scale: 10 μ m.

Appendix Figure S6



Appendix Figure S6. Protocol for the measurement of Na⁺ dynamics in neurons

(A) A representative trace showing changes in the fluorescence intensity (Y-axis) of Na⁺ dye ANG-2 following exchange of solutions as indicated in blue (See Material and Methods for all buffer compositions). First ~10 ml of 0 mM K⁺ recording solution is added to increase Na⁺ level in neurons. Then 0 mM K⁺ recording solution is replaced with normal recording solution to visualize and measure the extrusion/efflux of Na⁺ ions. This step is followed by step-wise Na⁺ calibration as illustrated.

(B) Figure showing different parameters computed and plotted in Figure 9B-D and Figure EV4. Increases in fluorescence measure the relative rise in ANG-2 fluorescence following 0 mM K⁺ recording solution application. The initial slope of decay curve measures the "Max Initial Pumping Rate". Recovery to basal level measures the difference between recovered Na⁺ level and basal level.

(Data used in Figure 1B)

Age-Dependent Clustering of a-Syn Assemblies

Oligomeric α-syn			
	Intensity of Cluste	rs	
	(Normalized to DIV	7)	
	$(Mean \pm SEM)$		
DIV 7	$1.00 \pm 0.03 \text{ (n=20)}$		
DIV 14	$1.23 \pm 0.07 (n=24) (**)$	} (***)	
DIV 21	2.35 ± 0.13 (n=35) (***))()	
	Fibrillar α-syn		
DIV 7	1.00 ± 0.03 (n=41)		
DIV 14	$1.29 \pm 0.03 (n=30) (***)$	} (ns)	
DIV 21	$1.26 \pm 0.04 (n=28) (***)$) (115)	

t-test to compare difference between DIV 7 and DIV 14/21 or DIV 14 and DIV 21 ns = not significant, * p<0.05; ** p<0.01; *** p<0.001

n = field of view (3-experiments on three independent cultures)

(Data supporting Figure S3)

Oligomeric α-syn					
	Intensity of Clusters	No. of Clusters/µm ²			
	(Normalized to lowest conc.)	(Normalized to lowest conc.)			
	$(Mean \pm SEM)$	$(Mean \pm SEM)$			
1.25 nM	$1.00 \pm 0.08 (n=20)$	1.00 ± 0.12 (n=20)			
2.50 nM	$1.51 \pm 0.10 \text{ (n=20) (ns)}$	5.37 ± 0.79 (n=20) (ns)			
6.25 nM	$2.63 \pm 0.29 \text{ (n=20) (ns)}$	$9.20 \pm 1.25 \text{ (n=20) (ns)}$			
12.50 nM	$3.16 \pm 0.30 (n=20) (**)$	$12.64 \pm 2.12 (n=20) (*)$			
25.00 nM	5.56 ± 0.81 (n=20) (***)	22.65 ± 3.84 (n=20) (***)			
50.00 nM	7.83 ± 0.71 (n=20) (***)	31.39 ± 5.96 (n=20) (***)			
	Fibrillar α-syr	1			
0.006 nM	$1.00 \pm 0.04 (n=20)$	$1.00 \pm 0.04 \text{ (n=20)}$			
0.012 nM	$1.31 \pm 0.05 \text{ (n=20) (ns)}$	$1.57 \pm 0.16 (n=20) (**)$			
0.018 nM	1.87 ± 0.30 (n=20) (***)	1.40 ± 0.10 (n=20) (ns)			
0.024 nM	$1.76 \pm 0.06 (n=20) (***)$	$1.60 \pm 0.10 (n=20) (**)$			
0.030 nM	2.04 ± 0.07 (n=20) (***)	$1.74 \pm 0.15 (n=20) (***)$			
0.060 nM	2.57 ± 0.13 (n=10) (***)	2.41 ± 0.16 (n=10) (***)			

Concentration-Dependent Clustering of a-Syn Assemblies

One-way ANOVA with Dunnett's test to compare the difference from lowest concentration used (Oligomer:

1.25 nM; Fibril: 0.006 nM) (2 experiments)

ns = not significant, *p<0.05; **p<0.01; ***p<0.001

(Data used in Figure 2A-B)

Time-Dependent Clustering of a-Syn Assemblies

Oligomeric α-syn					
	Intensity of Clusters	No of Clusters/µm ²			
	(Normalized to 5min)	(Normalized to 5min)			
	$(Mean \pm SEM)$	$(Mean \pm SEM)$			
5 min	$1.00 \pm 0.05 \text{ (n=35)}$	$1.00 \pm 0.06 \text{ (n=35)}$			
60 min	2.04 ± 0.14 (n=35) (***)	$1.39 \pm 0.06 \text{ (n=35) (***)}$			
	Fibrillar α-syn				
5 min	$1.00 \pm 0.02 \text{ (n=35)}$	$1.00 \pm 0.04 \text{ (n=35)}$			
60 min	2.01 ± 0.15 (n=35) (***)	$1.98 \pm 0.09 (n=35) (***)$			

t-test to compare difference from 5min

ns = not significant, *p<0.05; **p<0.01; ***p<0.001

n = field of view; (3-experiments on three independent cultures)

Oligomeric α-syn				
	% Association-Homer	% Association-Gephyrin		
	$(Mean \pm SEM)$	$(Mean \pm SEM)$		
1.25 nM	4.8 ± 1.1 (n=20)	3.7 ± 0.8 (n=20)		
2.50 nM	$12.9 \pm 2.1 \text{ (n=20) (ns)}$	$11.4 \pm 1.8 (n=20) (*)$		
6.25 nM	$19.3 \pm 3.3 (n=20) (***)$	$17.8 \pm 2.8 (n=20) (***)$		
12.50 nM	25.5 ± 3.2 (n=20) (***)	24.8 ± 2.7 (n=20) (***)		
25.00 nM	38.2 ± 2.6 (n=20) (***)	34.3 ± 1.6 (n=20) (***)		
50.00 nM	45.8 ± 2.2 (n=20) (***)	45.7 ± 1.9 (n=20) (***)		
	Fibrillar α-sy	n		
0.006 nM	26.6 ± 1.4 (n=20)	23.4 ± 1.1 (n=20)		
0.012 nM	36.2 ± 1.2 (n=20) (***)	33.1 ± 1.4 (n=20) (***)		
0.018 nM	43.3 ± 1.9 (n=20) (***)	41.1 ± 2.3 (n=20) (***)		
0.024 nM	47.5 ± 1.4 (n=20) (***)	42.3 ± 1.4 (n=20) (***)		
0.030 nM	47.7 ± 0.8 (n=20) (***)	43.9 ± 1.4 (n=20) (***)		
0.060 nM	60.1 ± 1.4 (n=10) (***)	59.9 ± 1.5 (n=10) (***)		

Appendix Table S4 Association of α-Syn and Homer/Gephyrin

One-way ANOVA with Dunnett's test to compare the difference from lowest concentration used (Oligomer:

1.25 nM; Fibril: 0.006 nM) (2 experiments)

ns = not significant, *p<0.05; **p<0.01; ***p<0.001

List of proteins from whole neurons lysates interacting with extracellularly applied oligomeric α-syn

 ∞ : the spectral count ratio is infinite as the protein is pulled-down only with oligometric α -synuclein

Fold change corresponds to the average spectral count ratio of three independent replicates

In bold, the unique plasma membrane protein pulled-down both with oligomeric and fibrillar α-synuclein and presenting both transmembrane and extracellularly exposed regions.

Protein Name	Gene Name	Accession Number	Fold Change (Syn/Ctrl)
40S ribosomal protein S28	Rps28	P62858	7.0
40S ribosomal protein S5	Rps5	P24050	2.2
78 kDa glucose-regulated protein	Hspa5	P06761	5.4
AP-3 complex subunit delta-1	Ap3d1	054774	1.8
ATP synthase subunit beta, mitochondrial	Atp5b	P10719	2.0
Calcium/calmodulin-dependent protein kinase type II subunit gamma	Camk2g	P11730	2.1
Calmodulin	Calm1	P62161	2.5
Creatine kinase B-type	Ckb	P07335	5.0
Cytoskeleton-associated protein 5	Ckap5	A2AGT5	1.7
Dihydropyrimidinase-related protein 3	Dpysl3	Q62188	2.1
Double-stranded RNA-binding protein Staufen homolog 2	Stau2	Q68SB1	1.6
Elongation factor 2	Eef2	P05197	2.0
Elongation factor Tu, mitochondrial	Tufm	P85834	5.0
ERC protein 2	Erc2	Q8K3M6	3.5
Fragile X mental retardation syndrome-related protein 1	Fxr1	Q61584	2.0
Friend of PRMT1 protein	Fop	Q9CY57	2.0
GMP synthase [glutamine-hydrolyzing]	Gmps	Q3THK7	2.0
Heterogeneous nuclear ribonucleoprotein K	Hnrnpk	P61979	1.8
Kinesin-like protein KIF2A	Kif2a	P28740	1.6
Myb-binding protein 1A	Mybbp1a	035821	1.9
Neuron navigator 1	Nav1	Q8CH77	1.6
Non-POU domain-containing octamer-binding protein	Nono	Q5FVM4	1.7
Protein unc-13 homolog A	Unc13a	Q62768	3.5
Protein-L-isoaspartate(D-aspartate) O-methyltransferase	Pcmt1	P22062	2.5
Serine/arginine-rich splicing factor 2	Srsf2	Q62093	2.4
Serine/arginine-rich splicing factor 3	Srsf3	P84104	1.6
SLIT-ROBO Rho GTPase-activating protein 2	Srgap2	Q91Z67	2.5
Sodium/potassium-transporting ATPase subunit alpha-3	Atp1a3	P06687	1.6
Tubulin beta-4B chain	Tubb4b	P68372	1.6
Ubiquitin carboxyl-terminal hydrolase isozyme L1	Uchl1	Q00981	×
Vesicle-associated membrane protein-associated protein A	Vapa	Q9WV55	4.0
WD repeat-containing protein 47	Wdr47	Q8CGF6	2.8

List of proteins from whole neurons lysates interacting with extracellularly applied fibrillar a-syn

 ∞ : the spectral count ratio is infinite as the protein is pulled-down only with fibrillar α -synuclein

Fold change corresponds to the average spectral count ratio of three independent replicates

In bold, the unique plasma membrane pulled-down both with oligomeric and fibrillar α-synuclein, and presenting both transmembrane and extracellularly exposed regions.

Column 5: "<u>Proteins identified after cross-linking</u>" corresponds to proteins identified after cross-linking. All the other proteins were not identified after cross-link.

Protein name	Gene name	Accession Number	Fold Change (Syn/Ctrl)	Proteins identified after cross-linking
14-3-3 protein gamma	Ywhag	P61982	x	+
14-3-3 protein zeta/delta	Ywhaz	P63102	×	+
40S ribosomal protein S15	RPS15	P62842	×	+
40S ribosomal protein S16	Rps16	P14131	3.0	+
40S ribosomal protein S17	Rps17	P04644	5.0	+
40S ribosomal protein S18	Rps18	P62270	6.7	+
40S ribosomal protein S19	Rps19	P17074	x	
40S ribosomal protein S20	Rps20	P60867	1.6	+
40S ribosomal protein S25	Rps25	P62852	13.0	
40S ribosomal protein S29	Rps29	P62274	5.5	+
40S ribosomal protein S5	Rps5	P24050	9.0	
40S ribosomal protein S7	Rps7	P62082	3.9	
5-azacytidine-induced protein 1	Azi1	Q62036	x	
60S ribosomal protein L22-like 1	Rpl22l1	Q9D7S7	×	
60S ribosomal protein L23	Rpl23	P62830	3.1	+
60S ribosomal protein L27	Rpl27	P61354	2.1	+
60S ribosomal protein L27a	Rpl27a	P18445	2.2	
60S ribosomal protein L38	Rpl38	P63174	x	
Abl interactor 1	Abi1	Q8CBW3	18.0	
Acetyl-CoA acetyltransferase, mitochondrial	Acat1	P17764	x	
Actin filament-associated protein 1	Afap1	Q8VH46	4.3	
Adenomatous polyposis coli protein	Арс	P70478	x	
Adenomatous polyposis coli protein 2	Apc2	Q9Z1K7	x	
ADP/ATP translocase 2	Slc25a5	P51881	3.7	
ADP-ribosylation factor GTPase-activating protein 3	Arfgap3	Q4KLN7	×	
Agrin	Agrn	P25304	x	
Alpha-internexin	Ina	P23565	34.0	
Alpha-tubulin N-acetyltransferase	Atat1	Q6MG11	×	
Amphiphysin	Amph	O08838	×	
AP-3 complex subunit beta-2	Ap3b2	Q9JME5	×	
AP-3 complex subunit delta-1	Ap3d1	054774	4.3	
Apolipoprotein E	Apoe	P02650	×	
Ataxin-2	Atxn2	070305	×	
Ataxin-2-like protein	Atxn2l	Q7TQH0	x	
ATP synthase subunit alpha, mitochondrial	Atp5a1	P15999	1.8	+
ATP synthase subunit d, mitochondrial	Atp5h	P31399	x	
ATP synthase subunit gamma, mitochondrial	Atp5c1	P35435	25.0	+
ATP synthase subunit O, mitochondrial	Atp5o	Q06647	x	
ATPase family AAA domain-containing protein 3	Atad3	Q3KRE0	×	
ATP-dependent RNA helicase DDX3X	Ddx3x	Q62167	2.4	+
Bcl-2-associated transcription factor 1	Bclaf1	Q8K019	10.5	
Calcium-binding mitochondrial carrier protein Aralar1	Slc25a12	Q8BH59	α	
Calmodulin-regulated spectrin-associated protein 2	Camsap2	Q8C1B1	α	
Calmodulin-regulated spectrin-associated protein 3	Camsap3	Q80VC9	x	
CaM kinase-like vesicle-associated protein	Camkv	Q63092	6.0	
CAP-Gly domain-containing linker protein 2	Clip2	055156	x x	
Caskin-1	Caskin1	Q8VHK2	x x	
Catenin delta-2	Ctnnd2	035927	~	ļ
Cell division control protein 42 homolog	Cdc42	P60766		
Centrosomal protein of 170 kDa	Cep170	Q6A065	37.0	
Charged multivesicular body protein 2b	Chmp2b	Q8BJF9	~	
Chromatin target of PRM11 protein	Chtop	Q9CY57	5.3	
CLIP-associating protein 2	Clasp2	Q99JD4	1./	
Coatomer subunit alpha	Сора	Q8CIE6	3.0	

Protein name	Gene name	Accession Number	Fold Change (Syn/Ctrl)	Proteins identified after cross-linking
Cofilin-1	Cfl1	P45592	3.8	+
Cytoplasmic dynein 1 heavy chain 1	Dync1h1	Q9JHU4	62.0	
Dihydropyrimidinase-related protein 1	Crmp1	Q62950	4.5	+
Dihydropyrimidinase-related protein 2	Dpysl2	O08553	1.6	+
Disks large-associated protein 1	Dlgap1	Q9D415	×	
Disks large-associated protein 4	Dlgap4	P97839	x	
DnaJ homolog subfamily A member 1	Dnaja1	P63036	×	
DnaJ homolog subfamily B member 1	Dnajb1	Q9QYJ3	č.	
DnaJ homolog subfamily B member 2	Dnajb2	Q9QYI5	č.	
DnaJ homolog subfamily B member 5	Dnajb5	089114	č.	
DnaJ homolog subfamily B member 6	Dnajb6	054946	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Dynactin subunit 1	Dctn1	P28023	4.0	
Elongation factor 1-alpha 1	Lef1a1	P10126	2.6	+
Elongation factor Tu, mitochondrial	Tufm	P85834	27.5	
Ena/VASP-like protein	EVI	008/19	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Eukaryotic translation initiation factor 3 subunit C	Eif3c	B5DFC8	~	
Glyceraldehyde-3-phosphate dehydrogenase	Gapdh	P04797	2.6	+
Glypican-1	Gpc1	P35053	or.	
Glypican-2	Gpc2	P51653	or.	
Giypican-4	Gpc4	P51655		
beta-1	Gnb1	P54311	×	
Heat shock cognate 71 kDa protein	HSPA8	P63018	5.0	+
Hepatoma-derived growth factor-related protein 2	Hdgfrp2	Q925G1	×	
Heterochromatin protein 1-binding protein 3	Hp1bp3	Q6P747	6.0	
Heterogeneous nuclear ribonucleoprotein D0	Hnrnpd	Q60668	×	+
Heterogeneous nuclear ribonucleoprotein D-like	Hnrpdl	Q9Z130	×	
Heterogeneous nuclear ribonucleoprotein K	Hnrnpk	P61979	4.5	+
Heterogeneous nuclear ribonucleoprotein M	Hnrnpm	Q62826	5.4	
Heterogeneous nuclear ribonucleoprotein U-like protein 2	Hnrnpul2	Q00P19	2.7	+
Histone H2A.J	H2afj	A9UMV8	1.7	
Histone H2A.Z	H2afz	P0C0S6	6.0	
Histone H4	Hist1h4b	P62804	1.6	+
Host cell factor 1	HCTC1	Q61191	~	
IQ motif and SEC7 domain-containing protein 1	Idsec1	Q8RUS2	or.	
Keich-like protein 22	KINIZZ	D322C3		
associated protein 1	Khdrbs1	Q91V33	x	
Kinesin heavy chain isoform 5C	Kif5c	P28738	26.5	
Lamin-B1	Lmnb1	P70615	<u>م</u>	
Lipid phosphate phosphatase-related protein type 4	Lppr4	Q7TME0	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
Liprin-alpha-2	Ppfia2	Q8BSS9	6.0	
MAP/microtubule affinity-regulating kinase 3	Mark3	Q8VHF0	11.0 ~	
MAP/microtubule aminity-regulating kinase 4	Iviark4	Q8CIP4	or.	
MAP7 domain-containing protein 1	Map/d1	AZAJIU	2.7	
Microtubule-associated protein 18	Mapio	P14873	2.7 ∝	+
Microtubule associated protein 15	Map15	PUC3W1	5.0	
Microtubule associated protein 2	Map2	05140	2.5	+
Microtubule-associated protein 4	Map4	063560	3.0	+
Microtubule-associated protein tau	Mapt	D10330	5.0	+
Microtubule-associated protein tau	Mast1	0810W/7	χ	
Mitochondrial glutamate carrier 1	Slc25a22	0906M3	x	
Mitogen-activated protein kinase kinase kinase kinase 4	Map4k4	P97820	x	
Myc box-dependent-interacting protein 1	Bin1	008539	x	
Myosin regulatory light chain 12B	Mvl12h	P18666	×	
Nck-associated protein 1	Nckap1	P28660	4.0	
Neurexin-1-alpha	Nrxn1	Q63372	x	
Neurexin-2-alpha	Nrxn2	Q63374	x	
Neurofilament light polypeptide	Nefl	P19527	x	
Neurofilament medium polypeptide	Nefm	P12839	x	
Neuromodulin	Gap43	P07936	4.0	+
Neuron navigator 1	Nav1	Q8CH77	44.3	

Nauroan angigator 3 Nav3 QB0TN7 × Nucloaling and colled-body phosphoprotein 1 NoIc1 P41777 × Prini Pon O35601 × Polymersse detta-interacting protein 3 Poleligi 3 QB80611 × Probatine AC Luma P48579 × Probatine Social Procession P487 × Probatine Social Procession P482 O35129 × Probatine Social Phosial O380740 × × Protein Nuksia Fam54b CSN10 × × Protein Nuksia Napragi CSS12 × × Protein Nuksia Navessia CSS12 × × Protein Nuksia Protein Suksia Protein Suksia × * Protein	Protein name	Gene name	Accession Number	Fold Change (Syn/Ctrl)	Proteins identified after cross-linking
Neurolan ingration protein doublecortin Dex 08809 4.2 Pinin Point 035691 × Pinin Point 035691 × Print Point 035691 × Pridel-like protein 3 Poidigita 0880681 × Problebilito-2 Print 2 035129 × Protein Passon Bin 088718 11.0 Protein Passon Bin 088718 11.0 Protein Nuonoga Hook3 0880149 × Protein Nuonoga Hook3 0880146 × Protein Nuonoga Hook3 Name × Rotein Nurs Haok3 Name × Protein Nuona Haok3 Name × Rotein Nurs Haok3 Name × Rotein Nurs	Neuron navigator 3	Nav3	Q80TN7	×	
Nuclear and colled-body phosphoprotein 1 PNIC P41777 4 Point Problemase deta-interacting protein 3 Poldigla Q88681 4 Prelamin-A/C Imma P44879 4 Problemase deta-interacting protein 3 Prickle 2 Q80724 4 Problem FAMS4B Fam54b Q50719 4 Protein FAMS4B Fam54b Q50710 4 Protein FAMS4B Fam54b Q50710 4 Protein FAMS4B Fam54b Q50710 4 Protein FAMS2 Fam63 PA7709 4 Protein FAMS4 Raba PA7570 4 4 Ras-related protein FAMS4 Raba PA7520 4 4 Ras-related protein FAMS4 Raba PA75220 4 4 Ras-related protein FABS4 RABAB PA75320 <	Neuronal migration protein doublecortin	Dcx	O88809	4.2	
Pinin Pinin O33691 Q Polymerase deta-interacting protein 3 Poldeling O286301 Q Prickie-like protein 2 Prickie-like protein 2 Prickie-like protein 2 Prickie-like protein 2 Protein bason Bin 068779 11.0 Protein fox homolog 3 Fam54b 058119 % Protein foxAD284 Kaa0284 Q80U49 % Rescription foxAD28 Kaa0284 Q80U49 % Rescription foxAD2 Tara Kaa0284 Q80U49 % Rescription foxAD2 Tara Asta00899 % * Rescription foxAD3 Angap13 P80232 %	Nucleolar and coiled-body phosphoprotein 1	Nolc1	P41777	8	
Polymerse deta-interacting protein 3 Poldp3 Q88G81 α Preizmin-X-C Imma P48379 α Protoin like protein 2 Probl Q80724 α Protoin hassion Bin Q88778 11.0 Protein hok homolog 3 Hook3 Q80406 α Protein NGA homolog 3 Hook3 Q80406 α Protein NGA homolog 3 Hook3 Q80406 α Protein NGAD284 Klad2244 Q8049 α Protein NGAD244 Nappall Q55125 α Protein NGAD2 Fully 3 Q51700 α Protein NGAD3 Pully 3 Q51700 α Raschaded Southinum toxin substrat Raphilhin-3A Rapha P47709 α Ras-related protein Rab-8A Rata P6322 α Ras-related protein Rab-1A Rata P6322 α Ras-related protein Rab-1A Rata P6323 α Ras-related protein Rab-1A Rata P63232 α Ras-related protein Rab-1A Rata	Pinin	Pnn	035691	8	
Predsenik protein 2 Lmna P448679 4 Protsich-like protein 2 Pricks-like protein 2 Pricks-like protein 2 4 Protein basson Bsn 088778 11.0 Protein KMA0284 Fam54b 05819 * Protein KMA0284 Kiaa0284 028109 * Protein KMA0284 Kiaa0284 028109 * Protein KMA0284 Nipsnap1 055125 * Protein KMA0284 Kiaa0284 0281040 * Protein NuPS3 Ruly3 055125 * Protein NuPS3 Ruly3 055125 * Protein NuPS3 Ruly3 05500 * Rachaller Status Ruly3 055125 * Rachaller Status Ruly3 055125 * Rachaller Status Ruly3 058322 * * Ras-related protein Rab-A Rab P63322 * * Ras-related protein Rab-A Rab P63322 * * Ras-relate	Polymerase delta-interacting protein 3	Poldip3	Q8BG81	8	
Prickle Q80724 a Probiblin2 Pholbin2 Pholbin2 Pholbin2 Protein bassoon Ban 088778 11.0 Protein FMASB Famble Dock 088778 11.0 Protein FMASB Famble Dock 080149 % Protein MSA0284 C80149 % Protein MSA0284 C80149 % Protein MSA0284 C80149 % Protein MSA0284 Robota % Protein MSA0284 Robota % Protein MAC2 Tanc2 A2A690 % Ras Grasse-activating protein SynGAP Syngap1 Q9QUH6 % Ras-related protein Rab-1A Raba P633220 % <	Prelamin-A/C	Lmna	P48679	×	
Proble O35129 * Protein FAMS4B Fam54b QSN19 * Protein FAMS4B Fam54b QSN19 * Protein KIAA0284 Kiaa0284 Q80U49 * Protein KIAA0284 Nipsnap1 O55125 * Protein KICA0204 Rufy3 QSV19 * Protein KIAA0284 Q80U49 * * Protein NINSnap homolog 1 Nipsnap1 QSS125 * Protein NINC2 Tanc2 AZA690 * Rascrelated protein SymGAP Sympap1 QSUH46 * Ras-related protein Rab-BA Raba PS3220 * Ras-related protein Rab-A Raba PS3220 * Ras-related protein Rab-A Raba PS322 * Ras-related protein Rab-A Raba PS322 * Ras-related protein Rab-A Raba PS322 * Ras-related protein Rab-A Raba PS3220 * Ras-related protein Rab-A Raba PS3220 <td< td=""><td>Prickle-like protein 2</td><td>Prickle2</td><td>Q80Y24</td><td>×</td><td></td></td<>	Prickle-like protein 2	Prickle2	Q80Y24	×	
Protein FAMS40 Ban 088778 11.0 Protein Hook homolog 3 Fmasba QSUI9 ~ Protein KMAS40 Kaa02284 QSBUK6 ~ Protein KMAS2244 Kaa02284 QSBUK6 ~ Protein KNPSnap homolog 1 Nipsnap1 OS5125 ~ Protein KNPSnap homolog 1 PAdo QSBUK6 ~ Protein TANC2 Tanc2 A2A690 ~ Rass Fielded 25 Outliumu toxin substrate 1 Rac1 P63001 ~ + Ras-related 2 Dottilumu toxin substrate 1 Rac1 P63001 ~ + Ras-related protein Rab-A RalaB P63322 ~ - Ras-related protein Rab-A RalaB P63322 ~ - Ras-related protein Rab-A RalaB P62332 ~	Prohibitin-2	Phb2	O35129	×	
Protein Hoxh homolog 3 Fam5ab QSXIII9 CSXIII9 Protein Hoxh homolog 1 Nisan224 QS0U49 CS Protein NisAn0224 Nisan21 QSU49 CS Protein NisAn021 Nisan21 QSVU56 CS Protein NisAn022 Tanc2 A2A690 CS Protein NisAn2 RayPhila PAT709 CS Ras-related protein NisAn2 Raph3a PAT709 CS Ras-related protein Mas Mras OM9898 CS Ras-related protein Rah-A Rala P62301 CS CS Ras-related protein Rah-A Rala P62352 C CS Ras-related protein Rah-A Rala P62353 C CS Ras-related protein Rah-A Rala P6235 C CS	Protein bassoon	Bsn	088778	11.0	
Protein KiAo284 Kiao284 Q88UK6 ~ Protein KiAo284 Kiao284 Q80U49 ~ Protein piCcolo Pclo Q98VK6 ~ Protein piCcolo Pclo Q98VK6 ~ Protein NUPY3 Rufy3 Q5FV/0 ~ Protein TANC2 Tanc2 A2A690 ~ Ras Frances Rather Rather Statuting protein SynGAP Syngapi ~ Ras-related 2 brotein Rath SA Rather Rather Statuting protein SynGAP Rescale Rather Statuting protein Statuting protein Substrate 1 Rec1 PF63001 ~ + Ras-related protein Rath SA Rabs PF3322 ~ - Ras-related protein Rath SA Rabs PF3323 ~ + Ras-related protein Rath SA Rabs PF3322 ~ - - - - Rastrated protein Rath SA Rabs PF3322 ~ - - - - - - - - - - - - - - - - - -	Protein FAM54B	Fam54b	Q5XII9	x	
Protein NiAo2234 Kiaa0224 Q80/49 * Protein NiAona homolog 1 Nipsnap1 OS5125 * Protein NiAona homolog 1 Rufyia Q5FV/0 * Protein RAUC2 Tanc2 A2A690 * Rabphilm-3A Raph3a PA7709 * Ras-related protein SynGAP Syngap1 Q9QUH6 * Ras-related protein Mass Mras O08989 * Ras-related protein Rab-3A Raba P63322 * Ras-related protein Rab-4A Rala P63322 * Ras-related protein Rab-4A Rala P63322 * Ras-related protein Rab-4A Rala P63322 * Ras-related protein Rab-1A Rap1a PC62835 * Ras-related protein Rab-1A Rap1a PC63223 * Regulating synaptic membrane exocytosis protein 1 Ring1a PC63131 * Regulating synaptic membrane exocytosis protein 2 \$rrm2 X * Serine/Arpinne-repotein kinase DCLK1 Dolk1 Q98H9	Protein Hook homolog 3	Hook3	Q8BUK6	×	
Protein piccolo Nipsnap1 OS5125 # Protein piccolo Pclo OgNK56 # Protein RUFY3 Rufy3 OSFV/0 # Rabphlin-SA Rp13a Par709 # Ras GTPase-activating protein SynGAP Syngap1 Q9QUH6 # Ras-related Z Sotuliumur toxin substrate 1 Rac1 P63001 # Ras-related Z Sotuliumur toxin substrate 1 Rac1 P63001 # Ras-related protein Rab-BA RabBa P32280 # Ras-related protein Rab-AA Rab1 P63031 # Ras-related protein Rab-AA Rab1 P63822 # Ras-related protein Rab-AA Rab1 P63835 # Regulating synaptic membrane exocytosis protein 1 Rins1 Q80H9 # Ribosome-binding protein 3 Arftgap33 Q80Y9 # Sertine/Areginine -rich splicing factor 7 Srs77 Q88187 1.6 Sertine/Areginine -rich splicing factor 7 Srs77 Q88197 1.8 Sertine/Arreginine-rortein kinase DCLK	Protein KIAA0284	Kiaa0284	Q80U49	×	
Protein RVF3 Pclo Q9/K50 ~ Protein RVF3 Rufy3 QSFV/0 ~ Rabphilin-3A Rph3a P47709 ~ Ras CIPase-activating protein SynGAP Syngap1 QOUH6 ~ Ras-related forber M-Ras Mras OO8989 ~ + Ras-related protein Rab-BA Raba P53220 ~ + Ras-related protein Rab-AA Raba P63322 ~ + Regulating synaptic membrane exocytosis protein 1 Rints QB/F19 10.0 - Khof Grass-actiption modulator Stm QSKH25 ~ - Serine/Arbreonine-protein Kinase DCLX1 Dclk1 QB/F19 1.8 - Serine/Arbreonine-protein	Protein NipSnap homolog 1	Nipsnap1	055125	x	
Protein RJPT3 Rufy3 QSFV/00 ≪ Protein TANC2 Tanc2 AZA690 ≪ Rab Millin-3A Rph3a P47709 ≪ Ras GTPase-activating protein SynGAP Syngap1 Q9QUH6 ≪ Ras-related C3 botulinum toxin substrate 1 Rac1 P63001 ≪ + Ras-related protein Rab-8A Rab8a P33280 ≪ + Ras-related protein Rab-8A Rab1a P632322 ≪ Ras-related protein Rab-AA Rab1a P62835 ≪ Ras-related protein Rab-AA Rab1a P63833 Q80YP9 ≪ Ribosome-binding protein 3 Arhgp33 Q80YP9 ≪ Ribosome-binding protein 1 Rirhy1 Q61/15 ≪ Setrin-/raginine repetitive matrix protein 2 Srrm2 Q88118 ≪ Setrine/rigrinine-ricits splicing factor 7 Srsf7 Q88197 1.8 Setrine/threonine-protein kinase MARK1<	Protein piccolo	Pclo	Q9JKS6	×	
Protein TANC2 Tanc2 AZA690 ~ Ras Orbanin-3A RpB3a PA7709 ~ Ras Grase-activating protein SynGAP Syngap1 Q9QUH6 ~ Ras-related protein M-Ras Marss Q9QUH6 ~ Ras-related protein Ra-Ras Marss Q90SB99 ~ Ras-related protein Ra-Ra Raba PS3220 ~ Ras-related protein Ra-A Raba PS3220 ~ Ras-related protein Ra-A Raba PS3220 ~ Ras-related protein Ra-A Raba PS3220 ~ Rabor-Carpine repetitive matrix protein 1 Rim1 Q9BI84 1.6 Ribosome-Sinding protein 1 Stm QSCH25 ~ Serine/Arginine -repetitive matrix protein 2 Srm2 QBB137 1.8 Serine/Arginine -rotein kinase DCLX1 Dok12 QSMPA9 <td>Protein RUFY3</td> <td>Rufy3</td> <td>Q5FVJ0</td> <td>×</td> <td></td>	Protein RUFY3	Rufy3	Q5FVJ0	×	
Rabphilin:3A Rph3a P47709 © Ras OTPase-activating protein SynGAP Syngap1. 09QUH6 © Ras-related protein Minkas Mras 09QUH6 © Ras-related protein Ra-SA Rabka P5280 © Ras-related protein Ral-A Raba P63832 © Ras-related protein Ral-A Rapla P63835 © Regulating synaptic membrane exocytosis protein 1 Rims1 Q9JIN4 1.6 Ribosome-binding protein 3 Arhgap33 Q8DVF9 © Ribosome-binding protein 1 Rimb1 Q9JIN4 1.6 SAFB-like transcription modulator Setin/ Arginine-rich splicing factor 7 Srsf7 Q8BU71 1.8 Serine/Arginine-rich splicing factor 7 Srsf7 Q8BU74 1.8 Serine/threonine-protein kinase DCLK2 Dclk1 Q9ILN8 2.9 Serine/threonine-protein kinase MARK1 Mark1 O08678 8.0 Serine/threonine-protein kinase MARK2 Mark2 O08679 11.0 Signal recognition particle 54 Aba protein Sryrip4 Q6KN85 2 <td>Protein TANC2</td> <td>Tanc2</td> <td>A2A690</td> <td>×</td> <td></td>	Protein TANC2	Tanc2	A2A690	×	
Ras Grass-activating protein SynGAP Syngap1 Q9QUH6 Control Ras-related Solutilum toxis substrate 1 Rat P63001 C + Ras-related protein M-Ras Miras O08989 C - Ras-related protein Ra-BA Rababa P63322 C - Ras-related protein Ra-A Rababa P63322 C - Ras-related protein Ra-A Rababa P63322 C - Ras-related protein Ra-A Rababa P63333 C - Regulating synaptic membrane exocytosis protein 1 Ritin O 291144 1.6 - - Ribosome-binding protein 1 Rrbp1 C9915 10.0 - - Serine/regriginine repetititive matrix protein 2 Srm2 Q88118 - - Serine/ribreonine-protein kinase DCLX1 Dclk1 Q911M8 2.9 - Serine/ribreonine-protein kinase MARK1 Mark2 O08679 1.0 - Signal recognition particle 34 Da protein Srp54 Q6A679 1.0 -	Rabphilin-3A	Rph3a	P47709	×	
Ras-related C3 botulinum toxin substrate 1 Rac1 P63001 * Ras-related protein M-Ras Mras 008898 * Ras-related protein Rab-8A Rabba P35280 * Ras-related protein Rab-A Rala P63322 * Ras-related protein Rab-A Rapia P62835 * Regulating synaptic membrane exocytosis protein 1 Rims1 Q91844 1.6 Reborne-Inding protein 1 Rrbp1 Q99P15 10.0 SAFB-like transcription modulator Sptin / Q80YF9 * Sertine/Arginine-rich splicing factor 7 Spti / Q8BVF9 * Serine/Arginine-rich splicing factor 7 Srsf / Q8BU9 1.8 Serine/Arginine-rich shase DCLK1 Dclk1 Q91L48 2.9 Serine/threonine-protein kinase MARK2 Mark1 O08678 8.0 Serine/threonine-protein kinase MARK2 Mark1 O08678 6.0 Sodium/potasium-transporting ATPase subunit alpha-3 Atpla19 P06687 6.0 Sodium/potasium-transporting ATPase subunit alpha Tcp1	Ras GTPase-activating protein SynGAP	Syngap1	Q9QUH6	×	
Ras-related protein M-Ras Mras O08989 Coll Ras-related protein Ral-A Rabka P63322 C Ras-related protein Ral-A Rala P63322 C Ras-related protein Ral-A Rala P63322 C Ras-related protein Ral-A Rapla P63322 C Ras-related protein Rase cocytosis protein 1 Rintp Q99PL5 10.0 Saffield transcription modulator Sitm Q88T8 C Serine/Arronine-protein Kinase DCLK1 Dock1 Q91MR8 C Serine/Arronine-protein Kinase MARK2 Mark1 O08678 8.0 Serine/Arronine-protein Kinase MARK2 Mark2 Q68478 C + Sodium/potassium-transporting ATPase subunit beta-1 Strpin Q909K7 C.0 + Sodiam/potassium-transporting ATPase subunit beta-	Ras-related C3 botulinum toxin substrate 1	Rac1	P63001	×	+
Ras-related protein Rab-BA RabBa P53280 * Ras-related protein Rap-1A Rap1a P62835 * Regulating synaptic membrane exocytosis protein 1 Rims1 Q9JIR4 1.6 Rho GTPase-activating protein 13 Arhgap33 Q8079 * Ribosome-binding protein 1 Rirbp1 Q99PL5 10.0 SAFB-like transcription modulator Sitm Q8CH25 * Serine/Arginine-rich splicing factor 7 Serif7 Q8B18 * Serine/Arginine-rich splicing factor 7 Srsf7 Q8B17 1.8 Serine/Artenoine-protein kinase DCLK1 Dclk1 Q9LM8 2.9 Serine/Artenoine-protein kinase DCLK2 Dclk2 Q5MPA9 65.0 Serine/Artenoine-protein kinase MARK1 Mark1 O08679 11.0 Signal recognition particle 54 kDa protein Srp54 Q6AV85 & Sodium/potassium-transporting ATPase subunit beta-1 Atp111 P07340 2.5 Sress-70 protein, mitochondrial MSyn1 P09951 29.7 Synaptional-associated protein 47 Snap47 Q6P630 \$ T-complex protein 1 subunit dplh	Ras-related protein M-Ras	Mras	O08989	×	
Ras-related protein Ra)-A Rala P63322 * Regulating synaptic membrane exocytosis protein 1 Rims1 Q9IR4 1.6 Rho GTPase-activating protein 33 Arhgap33 Q80YF9 * Ribosome-binding protein 1 Rims1 Q9IR4 1.6 Rho GTPase-activating protein 33 Arhgap33 Q80YF9 * Sibosome-binding protein 1 Rrbp1 Q9PL5 10.0 SAFB-like transcription modulator Sitm Q8CH25 * Septin-7 Septi7 O55131 * Serine/arginine repetitive matrix protein 2 SrrT2 Q8BL97 1.8 Serine/Arginine-rice binkinase DCLK2 Dclk2 QSMP49 65.0 Serine/Ithreonine-protein kinase DCLK2 Dclk2 QSMP49 65.0 Serine/Ithreonine-protein kinase MARK1 Mark1 O08678 8.0 Serine/Ithreonine-protein kinase MARK2 Mark2 O8687 6.0 + Sodium/potassium-transporting ATPase subunit alpha-3 Atp1a3 P06687 6.0 + Sodium/potassium-transporting ATPase subunit beta-1 Atp110 P07340 2.5 S Strain 0290VY2 3.2 S S S S Synapsin-1 Syn1 P09051 29.7	Ras-related protein Rab-8A	Rab8a	P35280	×	
Ras-related protein Rap-1A Rap1a P62835 ~ Regulating synaptic membrane exocytosis protein 1 Rims Q0JIR4 1.6 Rho GTPase activating protein 33 Arhgap33 Q80VF9 ~ Ribosome-binding protein 1 Rrbp1 Q99PL5 10.0 SAPE-like transcription modulator Sitm Q8CH25 ~ Septin-7 Sept7 Q8BTI8 ~ Serine/arginine-rich splicing factor 7 Srs77 Q8BTI8 ~ Serine/Arginine-rich splicing factor 7 Srs77 Q8BTI8 ~ Serine/threonine-protein kinase DCLK2 Dclk1 Q9LM8 2.9 Serine/threonine-protein kinase MARK1 Mark1 O08678 8.0 Serine/threonine-protein kinase MARK2 Mark2 O08679 11.0 Signal recognition particle 54 kDa protein Srp54 O6687 6.0 + Sodium/potassium-transporting ATPase subunit beta-1 Atp113 P06687 6.0 + Sodium/potassium-transporting ATPase subunit beta-1 Atp111 P07340 2.5 Sr Synapsin-1 Syn1 P09951 29.7 Synapsin-1 Syn1 P09951 29.7 Synapsin-2 Syn2 Cd337 ~ + Co	Ras-related protein Ral-A	Rala	P63322	×	
Regulating synaptic membrane exocytosis protein 1 Rims1 Q9JIR4 1.6 Rho GTPase-activating protein 33 Arhgap33 Q80YP9 ~ Ribosome-binding protein 1 Rrbp1 Q9PL5 10.0 SAFB-like transcription modulator Stm Q8CH25 ~ Septin-7 Septin-7 Septin-7 Septin-7 Serine/Arginine-rich splicing factor 7 Srsf7 Q8B118 ~ Serine/Arginine-rich splicing factor 7 Srsf7 Q8B17 1.8 Serine/threonine-protein kinase DCLK1 Dclk1 Q9JIM8 2.9 Serine/threonine-protein kinase DCLK2 Dclk2 Q5MPA9 65.0 Serine/threonine-protein kinase MARK1 Mark1 O08679 11.0 Signal recognition particle 54 kDa protein Srp54 Q6AYB5 ~ Sodium/potassium-transporting ATPase subunit alpha-3 Atp133 P06687 6.0 + Sodium/potassium-transporting ATPase subunit beta-1 Strcin1 Q9QXY2 3.2 Stress-7 Srcelas signaling inhibitor 1 Srcin1 Q9QXY2 3.2 Stress-7 Synapsin-2 Syn2 Q63537 ~ - Synapsin-3 Synapt Q63501 ~ + Synapsin-4 Synaptio-1 <td< td=""><td>Ras-related protein Rap-1A</td><td>Rap1a</td><td>P62835</td><td>×</td><td></td></td<>	Ras-related protein Rap-1A	Rap1a	P62835	×	
Rho GTPase-activating protein 1 Arhgap33 Q80YF9 ∞ Ribosome-binding protein 1 Rrbp1 Q9PL5 10.0 SAPE-like transcription modulator Sitm Q8CH25 ∞ Serine/arginine-rich splicing factor 7 Srsf7 Q8BU7 1.8 Serine/arginine-rich splicing factor 7 Srsf7 Q8BU7 1.8 Serine/threonine-protein kinase DCLK2 Dclk1 Q9JLM8 2.9 Serine/threonine-protein kinase DCLK2 Dclk2 QSMPA9 65.0 Serine/threonine-protein kinase MARN1 Mark1 O08678 8.0 Serine/threonine-protein kinase MARN1 Mark1 O08678 8.0 Serine/threonine-protein kinase MARN1 Mark1 O08678 8.0 Sodium/potassium-transporting ATPase subunit alpha-3 Atp1a3 P06687 6.0 + Sodium/potassium-transporting ATPase subunit beta-1 Atp1a3 P06501 ∞ Synaption Syn1 P09551 29.7 Synaptosomal-associated protein 47 Snap47 Q67650 ∞ + T-complex protein 1 subunit alpha Tcp1	Regulating synaptic membrane exocytosis protein 1	Rims1	Q9JIR4	1.6	
Ribosome-binding protein 1 Rrbp1 Q99P15 10.0 SAFB-like transcription modulator Sitm Q8CH25 \propto Septin-7 Sept7 OS5131 \propto Serine/arginine repetitive matrix protein 2 Srm2 Q8BTI8 \propto Serine/arginine-rich splicing factor 7 Srsf7 Q8BU97 1.8 Serine/threonine-protein kinase DCLK1 Dclk1 Q9JLM8 2.9 Serine/threonine-protein kinase MARK1 Mark1 O08678 8.0 Serine/threonine-protein kinase MARK2 Mark2 O08679 11.0 Signal recognition particle 54 kDa protein Srp54 Q6AV85 \propto Sodium/potassium-transporting ATPase subunit beta-1 Atp101 P07340 2.5 Stress-70 protein, mitochondrial HSPA9 O35501 \propto Synapsin-1 Syn1 P09951 29.7 Synapsin-2 Syn2 Q63537 \propto Synapsin-3 Syn2 Q63537 \propto Synaptosomal-associated protein 47 Snap47 Q6F650 \propto T-complex protein 1 subunit alpha Tcp1 P11983 \propto T-complex protein 1 subunit gamma Cct14 P80315 \propto T-complex protein 1 subunit gamma Cct3 P80317	Rho GTPase-activating protein 33	Arhgap33	Q80YF9	×	
SAFE-like transcription modulator SItm Q8CH25 % Septin-7 Sept7 Q55131 % Serine/arginine-rich splicing factor 7 Srs77 Q8BU78 % Serine/arginine-rich splicing factor 7 Srs77 Q8BU78 % Serine/threonine-protein kinase DCLK1 Dclk1 Q9JLM8 2.9 Serine/threonine-protein kinase DCLK2 Dclk2 Q5MPA9 65.0 Serine/threonine-protein kinase MARK1 Mark1 008679 11.0 Signal recognition particle 54 kDa protein Srp54 Q6AVB5 % Sodium/potassium-transporting ATPase subunit beta-1 Atp1a3 P06687 6.0 + Sodium/potassium-transporting ATPase subunit beta-1 Srp11 P07340 2.5 SK SK kinase ignaling inihibitor 1 Src11 Q9QXV2 3.2 Stress-70 protein, mitochondrial HSPA9 O35501 % Synapsin-1 Syn1 P09951 29.7 Synapsin-2 Syn2 Q665337 % Synaptosomal-associated protein 47 Snap47 Q6P650 % T-complex protein 1 subunit deta	Ribosome-binding protein 1	Rrbp1	Q99PL5	10.0	
Septin 7Sept7O55131 \propto Serine/arginine repetitive matrix protein 2Srrm2Q8BT8 \propto Serine/arginine -rich splicing factor 7Srsf7Q8BL971.8Serine/threonine-protein kinase DCLK1Dclk1Q9LM82.9Serine/threonine-protein kinase MARK1Mark1O086788.0Serine/threonine-protein kinase MARK1Mark1O086788.0Serine/threonine-protein kinase MARK2Mark2O0867911.0Signal recognition particle 54 KDa proteinSrp54Q6AYB5 \propto Sodium/potassium-transporting ATPase subunit alpha-3Atp133P066876.0+Sodium/potassium-transporting ATPase subunit alpha-3Kp133P06837 \propto Synapsin-1Srp11Srcin1Q9QXY23.2Stress-70 protein, mitochondrialHSPA9O35501 \propto Synapsin-1Syn1P0995129.7Synapsin-2Syn2Q63837 \propto Synapsin-3Syn2Q63813 \propto +T-complex protein 1 subunit alphaTcp1P11983 \propto +T-complex protein 1 subunit betaCct2P80315 \propto +T-complex protein 1 subunit gammaCct3P80315 \propto +T-complex protein 1 subunit gammaCtd3P80315 \propto +T-complex protein 1 subunit gammaCtd3P80315 \propto +T-complex protein 1 subunit gammaCtd3P80315 \propto +T-comp	SAFB-like transcription modulator	Sltm	Q8CH25	×	
Serine/arginine repetitive matrix protein 2 Srm2 Q8BTB ~ Serine/Arginine-rich splicing factor 7 Srs7 Q8BL97 1.8 Serine/threonine-protein kinase DCLK1 Dclk1 Q9LNM8 2.9 Serine/threonine-protein kinase DCLK2 Dclk2 Q5MPA9 65.0 Serine/threonine-protein kinase MARK1 Mark1 O08678 8.0 Serine/threonine-protein kinase MARK2 Mark2 O08679 11.0 Signal recognition particle 54 kDa protein Srp54 Q6AVB5 ~ Sodium/potassium-transporting ATPase subunit beta-1 Atp1a3 P06687 6.0 + Sodium/potassium-transporting ATPase subunit beta-1 Srcin1 Q9QXY2 3.2 SRC kinase signaling inhibitor 1 Srcin1 Q9QXY2 3.2 Synapsin-1 Syn1 P09951 29.7 Synapsin-2 Syn2 Q65337 ~ Synapsin-3 Syn2 Q65337 ~ Synapsin-4 Cct2 P80314 ~ + T-complex protein 1 subunit alpha Tcp1 P11983 ~<	Septin-7	Sept7	055131	×	
Serine/arginine-rich splicing factor 7 Srsf7 Q8L197 1.8 Serine/threonine-protein kinase DCLK1 Dclk1 Q9JLM8 2.9 Serine/threonine-protein kinase DCLK2 Dclk2 QSMPA9 65.0 Serine/threonine-protein kinase MARK1 Mark1 O08678 8.0 Serine/threonine-protein kinase MARK2 Mark2 O08679 11.0 Signal recognition particle 54 kDa protein Srp54 QGAVB5 ~ Sodium/potassium-transporting ATPase subunit alpha-3 Atp1a3 P06687 6.0 + Sodium/potassium-transporting ATPase subunit beta-1 Atp1b1 P07340 2.5 Stress-70 protein, mitochondrial HSPA9 035501 ~ Synapsin-1 Syn1 P09951 29.7 Synaptosomal-associated protein 47 Snap47 Q648337 ~ T-complex protein 1 subunit alpha Tcp1 P11983 ~ + T-complex protein 1 subunit detta Cct2 P80314 ~ + T-complex protein 1 subunit detta Cct3 P80318 ~ + T-complex protein 1 subunit gamma Cct3 P80318 ~	Serine/arginine repetitive matrix protein 2	Srrm2	Q8BTI8	×	
Serine/threonine-protein kinase DCLK1 Dclk1 Q9LIM8 2.9 Serine/threonine-protein kinase MARK1 Mark1 O08678 8.0 Serine/threonine-protein kinase MARK1 Mark2 O08679 11.0 Signal recognition particle 54 kba protein Srp54 Q6AVBS ~ Sodium/potassium-transporting ATPase subunit beta-1 Atp131 P06687 6.0 + Sodium/potassium-transporting ATPase subunit beta-1 Atp110 P07340 2.5 SRC kinase signaling inhibitor 1 Srcin1 Q9UX2 3.2 Synapsin-1 Syn1 P09951 29.7 Synapsin-2 Syn2 Q63337 ~ Synaptosomal-associated protein 47 Snap47 Q6P650 ~ T-complex protein 1 subunit alpha Tcp1 P11983 ~ T-complex protein 1 subunit detta Cct2 P80314 ~ + T-complex protein 1 subunit detta Cct4 P80315 ~ T-complex protein 1 subunit detta Cct4 P80317 ~ T-complex protein 1 subunit gamma Ct53 P62996 + T-complex protein 1 subunit gamma Trasformer-2 P62996 + Trasformer-2 protein homolog beta Tra2b P62996 + <	Serine/arginine-rich splicing factor 7	Srsf7	Q8BL97	1.8	
Serine/threonine-protein kinase DCLK2DCLk2QSMPA965.0Serine/threonine-protein kinase MARK1Mark1O086788.0Serine/threonine-protein kinase MARK2Mark2O867911.0Signal recognition particle 54 kDa proteinSrp54QGAYB5~Sodium/potassium-transporting ATPase subunit alpha-3Atp1a3P06876.0+Sodium/potassium-transporting ATPase subunit beta-1Atp111P073402.5SR kinase signaling inhibitor 1Srcin1Q9QXY23.2Synapsin-1Syn1P0995129.7Synapsin-2Syn2Q63537~Synapsin-1Syn1P0995129.7Synaptosomal-associated protein 47Snap47Q6P650~T-complex protein 1 subunit alphaTcp1P11983~T-complex protein 1 subunit betaCct2P80314~T-complex protein 1 subunit gammaCct3P80318~T-complex protein 1 subunit gammaCct3P80317~T-complex protein 1 subunit gammaCct6aP80317~T-restican-1Spock1Q5M7V8~Traf2 and NCk-interacting protein kinaseTnik1Q92V1~Traf2 and NCk-interacting protein kinaseTnik1Q80428~Trifunctional enzyme subunit alph, mitochondrialHadhaQ64428~Transforming protein RIAA1107Kiaa1107Q807V3~VeinetrianTpm3Q567V8~Vimentin </td <td>Serine/threonine-protein kinase DCLK1</td> <td>Dclk1</td> <td>Q9JLM8</td> <td>2.9</td> <td></td>	Serine/threonine-protein kinase DCLK1	Dclk1	Q9JLM8	2.9	
Serine/threonine-protein kinase MARK1 Mark1 O08678 8.0 Serine/threonine-protein kinase MARK2 Mark2 O08679 11.0 Signal recognition particle 54 kDa protein Srp54 Q6AYB5 \$\pi\$ Sodium/potassium-transporting ATPase subunit beta-1 Atp1a3 P06687 6.0 + Sodium/potassium-transporting ATPase subunit beta-1 Atp1a1 P07340 2.5 SRC kinase signaling inhibitor 1 Srcin1 Q9QXY2 3.2 Stress-70 protein, mitochondrial HSPA9 O35501 \$\pi\$ Synapsin-1 Syn1 P09951 29.7 Synapsin-2 Syn2 Q63537 \$\pi\$ Synapsin-1 Syn2 Q63537 \$\pi\$ Synaptin-2 Syn2 Q63537 \$\pi\$ Synaptin-1 Stro1400 Q8BH13 \$\pi\$ T-complex protein 1 subunit alpha Tcp1 P11983 \$\pi\$ T-complex protein 1 subunit delta Cct4 P80315 \$\pi\$ T-complex protein 1 subunit gamma Cct3 P80318 \$\pi\$ T-complex protein 1 subunit gamma Cct4 P80315 \$\pi\$ T-complex protein 1 subunit gamma Tct410 Q62288 \$\pi\$ Thyroid hormone receptor-associated protein 3 <td>Serine/threonine-protein kinase DCLK2</td> <td>Dclk2</td> <td>Q5MPA9</td> <td>65.0</td> <td></td>	Serine/threonine-protein kinase DCLK2	Dclk2	Q5MPA9	65.0	
Serine/threonine-protein kinase MARK2 Mark2 OB679 11.0 Signal recognition particle 54 kDa protein Srp54 Q6AYB5 X Sodium/potassium-transporting ATPase subunit alpha-3 Atp1a3 P06687 6.0 + Sodium/potassium-transporting ATPase subunit beta-1 Atp1b1 P07340 2.5 S Srcsing of the protein, mitochondrial HSPA9 O35501 X X Synapsin-1 Syn1 P09951 29.7 S Synapsin-1 Syn1 P09951 29.7 S Synapsin-1 Syn2 Q63537 X X Synapsin-1 Synapsin-1 Synap Q87650 X X Synapsin-2 Syn2 Q63537 X X X Synapsin-1 Subunit alpha Tcp1 P11983 X X X Synapsin-2 Synapsin-1 P11983 X	Serine/threonine-protein kinase MARK1	Mark1	008678	8.0	
Signal recognition particle 54 kba protein Srp54 QGAYB5 ** Sodium/potassium-transporting ATPase subunit alpha-3 Atp1a3 P06687 6.0 + Sodium/potassium-transporting ATPase subunit beta-1 Atp1a1 P07340 2.5 SRC kinase signaling inhibitor 1 Srcin1 Q9QXY2 3.2 Synapsin-1 Syn1 P0951 29.7 Synapsin-1 Syn2 Q63537 ~ Synapsin-2 Syn2 Q63537 ~ Synapsin-1 Syn1 P09951 29.7 Synapsin-2 Syn2 Q63537 ~ <	Serine/threonine-protein kinase MARK2	Mark2	008679	11.0	
Sodium/potassium-transporting ATPase subunit alpha-3Atp1a3P066876.0+Sodium/potassium-transporting ATPase subunit beta-1Atp1b1P073402.5	Signal recognition particle 54 kDa protein	Srp54	Q6AYB5	×	
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Synapsin-1Syn1P0995129.7Synapsin-2Syn2Q63537 \propto Synaptosomal-associated protein 47Snap47Q6P6S0 \propto TBC1 domain family member 108Tbc1d10bQ8BHL3 \propto T-complex protein 1 subunit alphaTcp1P11983 \propto T-complex protein 1 subunit deltaCct2P80314 \propto +T-complex protein 1 subunit gammaCct3P80315 \propto T-complex protein 1 subunit gammaCct3P80317 \propto T-complex protein 1 subunit gammaCct4P80317 \propto T-complex protein 1 subunit gammaCct3P80317 \propto T-complex protein 1 subunit gammaCct6aP80317 \propto T-complex protein 1 subunit gammaCtf6aP80317 \propto T-complex protein 1 subunit gammaTreft1Q9QYV1 \propto Tomoregulin-1Tmrap3Q5M7V8 \propto Traf2 and NCK-interacting protein kinaseTnikP83510 \propto Traf2 and NCK-interacting protein kinaseTrikP83510 \propto Trifunctional enzyme subunit alpha, mitochondrialHadhaQ64428 \propto Tropomyosin alpha-3 chainTpm3Q63610 \propto Uncharacterized protein NIAA1107Kiaa1107Q80TK0 \propto VimentinVimP3100032.5Voltage-dependent anion-selective channel protein AVapaQ9WV55 \propto Vinger-dependent anion-selective channel protein 1Vdac1Q609325.0WD 1 \propto Vinger-dependent anio	Stress-70 protein, mitochondrial	HSPA9	035501		
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Synaptosomal-associated protein 47Snap47Q6P650CTBC1 domain family member 10BTbc1d10bQ8BHL3CT-complex protein 1 subunit alphaTcp1P11983CT-complex protein 1 subunit betaCct2P80314CT-complex protein 1 subunit deltaCct4P80315CT-complex protein 1 subunit gammaCct3P80318CT-complex protein 1 subunit gammaCct6aP80317CT-complex protein 1 subunit zetaCct6aP80317CTestican-1Spock1Q62288CThyroid hormone receptor-associated protein 3Thrap3Q5M7V8CTomoregulin-1Tmeff1Q9QYV1CTraf2 and NCK-interacting protein kinaseTrikP82996C+Transformer-2 protein RhoARhoaP61589C-Trifunctional enzyme subunit alpha, mitochondrialTpm3Q63610C-Uncharacterized protein KIAA1107Kiaa1107Q80TK0CCViscel-associated membrane protein-associated protein AVapaQ9WV55C-VointentinVimP3100032.5VVimentinVimP3100032.5Voltage-dependent anion-selective channel protein 18Zc3h18QGTQE1CZZZinc finger CCCH domain-containing protein 18Zc3h18QGTQE1CZ	Synapsin-2	Syn2	Q63537	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
TBC1 domain family member 108 TbC1d10b Q8BHL3 ~ T-complex protein 1 subunit alpha Tcp1 P11983 ~ T-complex protein 1 subunit beta Cct2 P80314 ~ + T-complex protein 1 subunit delta Cct4 P80315 ~ + T-complex protein 1 subunit gamma Cct3 P80318 ~ - T-complex protein 1 subunit zeta Cct6a P80317 ~ - T-complex protein 1 subunit zeta Cct6a P80317 ~ - Testican-1 Spock1 Q62288 ~ - - Thyroid hormone receptor-associated protein 3 Thrap3 QSM7V8 ~ - - Taf2 and NCK-interacting protein kinase Tnik P83510 ~ - - - Transformer-2 protein homolog beta Tra2b P62996 ~ + - - - - Transforming protein RhoA Rhoa P61589 ~ - - - - - - - - - - - - - -	Synaptosomal-associated protein 47	Snap47	Q6P6S0	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
I-complex protein 1 subunit alphaIcp1P11983*T-complex protein 1 subunit betaCct2P80314\$+T-complex protein 1 subunit deltaCct4P80315\$*T-complex protein 1 subunit gammaCct3P80318\$\$T-complex protein 1 subunit zetaCct6aP80317\$\$Testican-1Spock1Q62288\$\$\$Thyroid hormone receptor-associated protein 3Thrap3Q5M7V8\$\$Tomoregulin-1Tmeff1Q9QYV1\$\$\$Traf2 and NCK-interacting protein kinaseTnikP83510\$\$Transformer-2 protein homolog betaTra2bP62996\$\$\$Trifunctional enzyme subunit alpha, mitochondrialHadhaQ64428\$\$Tropomyosin alpha-3 chainTpm3Q63610\$\$Uncharacterized protein KIAA1107Kiaa1107Q80TK0\$\$Vesicle-associated membrane protein-associated protein AVapaQ9WV55\$\$VimentinVimP3100032.5\$\$Voltage-dependent anion-selective channel protein 1Vdac1Q60932\$.0\$WD repeat-containing protein 37Wdr37Q8CBE3\$\$Zinc finger CCCH domain-containing protein 18Zc3h18Q6TQE1\$\$Zw10interactorZwintG8VII3\$\$	IBC1 domain family member 10B	Tbc1d10b	Q8BHL3	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	
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Inrap3 QSM/V8 ~ Tomoregulin-1 Tmeff1 Q9QYV1 ~ Traf2 and NCK-interacting protein kinase Tnik P83510 ~ Traf2 and NCK-interacting protein kinase Tnik P83510 ~ Trafsformer-2 protein homolog beta Tra2b P62996 ~ + Transforming protein RhoA Rhoa P61589 ~ + Trifunctional enzyme subunit alpha, mitochondrial Hadha Q64428 ~ - Tropomyosin alpha-3 chain Tpm3 Q63610 ~ - - Uncharacterized protein KIAA1107 Kiaa1107 Q80TK0 ~ - - Vesicle-associated membrane protein-associated protein A Vapa Q9WV55 ~ - - Vimentin Vim P31000 32.5 - - - - WD repeat-containing protein 37 Wdr37 Q8CBE3 ~ - - - Zinc finger CCCH domain-containing protein 18 Zc3h18 Q6TQE1 ~ - - -	Testican-1	Spock1	Q62288	or.	
Tomoreguin-1Timerr1Q9QYV1~Traf2 and NCK-interacting protein kinaseTnikP83510~Transformer-2 protein homolog betaTra2bP62996~+Transforming protein RhoARhoaP61589~+Trifunctional enzyme subunit alpha, mitochondrialHadhaQ64428~-Tropomyosin alpha-3 chainTpm3Q63610~Uncharacterized protein KIAA1107Kiaa1107Q80TK0~Vesicle-associated membrane protein-associated protein AVapaQ9WV55~VimentinVimP3100032.5WD repeat-containing protein 37Wdr37Q8CBE3~Zinc finger CCCH domain-containing protein 18Zc3h18Q6TQE1~ZW10 interactorZwintO8VII 3~	Invroid normone receptor-associated protein 3	Inrap3	Q5M7V8	or.	
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Interstorting protein KloA Kloa P61589 ~ Trifunctional enzyme subunit alpha, mitochondrial Hadha Q64428 ~ Tropomyosin alpha-3 chain Tpm3 Q63610 ~ Uncharacterized protein KlAA1107 Kiaa1107 Q80TK0 ~ Vesicle-associated membrane protein-associated protein A Vapa Q9WV55 ~ Vimentin Vim P31000 32.5 Voltage-dependent anion-selective channel protein 1 Vdac1 Q60932 5.0 WD repeat-containing protein 37 Wdr37 Q8CBE3 ~ Zinc finger CCCH domain-containing protein 18 Zc3h18 Q6TQE1 ~ ZW10 interactor Zwint O8VII 3 ~	Transforming protoin PhaA	Dhoc	P62996	 02	+
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Visibility Kial107 Q801K0 ~ Vesicle-associated membrane protein-associated protein A Vapa Q9WV55 ~ Vimentin Vim P31000 32.5 Voltage-dependent anion-selective channel protein 1 Vdac1 Q60932 5.0 WD repeat-containing protein 37 Wdr37 Q8CBE3 ~ Zinc finger CCCH domain-containing protein 18 Zc3h18 Q6TQE1 ~ ZW10 interactor Zwint O8VII 3 ~	Hupphryosin alpha-s chain	Ipm3	Q03010	 02	
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Worsz Worsz Worsz Zinc finger CCCH domain-containing protein 18 Zc3h18 Q6TQE1 ZW10 interactor Zwint O8VII 3	WD repeat containing protein 27	VUACI		5.U ∝	
ZW10 interactor ZW10 interacto	Zinc finger CCCH domain-containing protoin 19	7c3h19		x	
	ZW10 interactor	Zwint	08113	×	

List of proteins from whole astrocytes lysates interacting with extracellularly applied oligomeric a-syn

 ∞ : the spectral count ratio is infinite as the protein is pulled-down only with fibrillar α -synuclein **Fold change** corresponds to the average spectral count ratio of three independent replicates

Protein name	Gene name	Accession Number	Fold Change (Syn/Ctrl)
14-3-3 protein epsilon	Ywhae	P62260	2.7
40S ribosomal protein S9	Rps9	P29314	1.8
60S ribosomal protein L13a	Rpl13a	P35427	1.7
60S ribosomal protein L26	Rpl26	P61255	2.7
60S ribosomal protein L7a	Rpl7a	P62425	2.2
78 kDa glucose-regulated protein	Hspa5	P06761	4.4
Alpha-enolase	Eno1	P04764	1.7
Arginyl-tRNA synthetase, cytoplasmic	Rars	P40329	x
Aspartyl-tRNA synthetase, cytoplasmic	Dars	P15178	1.9
Bifunctional aminoacyl-tRNA synthetase	Eprs	Q8CGC7	1.6
Destrin	Dstn	Q7M0E3	7.0
Elongation factor 1-delta	Eef1d	Q68FR9	2.0
FH1/FH2 domain-containing protein 1	Fhod1	Q6P9Q4	3.0
Low-density lipoprotein receptor-related protein 2	Lrp2	P98158	6.5
Lysyl-tRNA synthetase	Kars	Q99MN1	2.2
Major vault protein	Mvp	Q62667	7.0
NHP2-like protein 1	Nhp2l1	P55770	3.0
Polyadenylate-binding protein 1	Pabpc1	Q9EPH8	4.5
Protein kinase C delta-binding protein	Prkcdbp	Q9Z1H9	2.0
Protein lin-7 homolog C	Lin7c	Q792I0	1.6
Putative ATP-dependent RNA helicase DHX30	Dhx30	Q5BJS0	2.3
Septin-2	Septin2	Q91Y81	1.7
SPATS2-like protein	Spats2l	Q5U2T3	2.2
Stress-70 protein, mitochondrial	Hspa9	P48721	2.5
Tricarboxylate transport protein, mitochondrial	Slc25a1	P32089	2.0

Table S7B

List of proteins from whole astrocytes lysates interacting with extracellularly applied fibrillar α -syn

change corresponds to the average spectral count ratio of three independent replicates

Protein name	Gene name	Accession Number	Fold Change (Syn/Ctrl)
ADP/ATP translocase 2	Slc25a5	P51881	2.3
Apolipoprotein E	Apoe	P02650	x
ATP synthase subunit gamma, mitochondrial	Atp5c1	P35435	15.0
ATP synthase subunit 0, mitochondrial	Atn50	006647	<u>~</u>
Cell division control protein 42 homolog	Cdc42	P60766	x
Charged multivesicular body protein 2h	Chmn2h	0881F9	x
CLIP-associating protein 2	Clasp2		16.0
Cofilin-1	Clasp2 Cfl1	D/15502	2.0
78 KDa Glucose-regulated protein	Hsna5	P06761	34.0
Drat homolog subfamily A member 1	Dispas	P62026	34.0 X
Churican 4	Chc4		x
Giypicali-4	Gpt4	P31035	ox .
	LINIDI	P70015	ox .
PIIIII Pas related C2 betwlinum tovin substrate 1	PIIII Pac1	035091	oc oc
Ras-related C3 botulinum toxin substrate 1	Raci	P63001	a c
Ras-related protein Rab-8A	карва	P35280	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Ras-related protein Ral-A	Raia	P63321	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Ras-related protein Rap-1A	Rapia	P62835	~
Vesicle-associated membrane protein-associated protein A	Vapa	Q9WV55	4.0 ~
Voltage-dependent anion-selective channel protein 1	Vdac1	Q60932	x L
Long-chain fatty acid transport protein 1	Slc27a1	P97849	1.6
2',3'-cyclic-nucleotide 3'-phosphodiesterase	Спр	P13233	œ
40S ribosomal protein S15	RPS15	P62842	x
40S ribosomal protein S16	Rps16	P14131	2.2
40S ribosomal protein S17	Rps17	P04644	8.0
40S ribosomal protein S18	Rps18	P62270	6.2
40S ribosomal protein S25	Rps25	P62852	4.5
40S ribosomal protein S29	Rps29	P62274	3.0
40S ribosomal protein S7	Rps7	P62082	2.0
60S ribosomal protein L22-like 1	Rpl22l1	Q9D7S7	x
60S ribosomal protein L24	Rpl24	P83732	1.8
60S ribosomal protein L38	Rpl38	P63174	x
Actin, cytoplasmic	Actb	P60711	x
ADP-ribosylation factor GTPase-activating protein 2	Arfgap2	Q3MID3	x
ADP-ribosylation factor GTPase-activating protein 3	Arfgap3	Q4KLN7	x
Alpha-actinin-1	Actn1	Q9Z1P2	x
Alpha-crystallin B chain	CRYAB	P05811	x
Annexin A1	Anxa1	P07150	2.0
AP-2 complex subunit mu	Ap2m1	P84091	2.5
Histone H3.3	H3f3b	P84245	2.2
Ataxin-2	Atxn2	070305	x
ATP-dependent RNA helicase DDX3X	Ddx3x	Q62167	2.9
Band 4.1-like protein 5	Epb41l5	Q5FVG2	x
Calmodulin	Calm1	P62161	2.5
Calmodulin-regulated spectrin-associated protein 2	Camsap2	Q8C1B1	x
Calponin-1	Cnn1	Q08091	\propto
Cdc42 effector protein 1	Cdc42ep1	A1A5P0	x
Chordin-like protein 1	Chrdl1	Q76LD0	9.0
Cytoplasmic dynein 1 heavy chain 1	Dync1h1	Q9JHU4	1.6
DnaJ homolog subfamily A member 2	Dnaja2	Q9QYJ0	x
DnaJ homolog subfamily B member 1	Dnajb1	Q9QYJ3	x
DnaJ homolog subfamily B member 6	Dnaib6	054946	x
Elongation factor 1-alpha 1	Eef1a1	P10126	2.0
Elongation factor Tu, mitochondrial	Tufm	P85834	5.0
Filamin-A	Flna	Q8BTM8	1.8

Protein name	Gene name	Accession Number	Fold Change (Syn/Ctrl)
Friend of PRMT1 protein	Fop	Q9CY57	5.0
G kinase-anchoring protein 1	Gkap1	Q5XIG5	3.0
Glial fibrillary acidic protein	Gfan	P47819	3.2
Glutathione peroxidase 1	Gpx1	P04041	2.0
Golgi-associated plant pathogenesis-related protein 1	Glinr2	09015	7.0
Heat shock cognate 71 kDa protein	НСРДЯ	P63018	3.4
Heterogeneous nuclear ribonucleoprotein A1	Hnrnna1	P0/256	3. - α
Heterogeneous nuclear ribonucleoprotein A1	Hnrnnd	060668	x
Heterogeneous nuclear ribonucleoprotein G retrogene-like	Rhmyrtl	000008	1.6
Heterogeneous nuclear ribonucleoprotein U like protein 2		P 04300	25.0
Heterogeneous nuclear ribonucleoprotein 0-like protein 2	Hirripulz Hirring2h1		23.0 x
Heterogeneous nuclear ribonucleoproteins A2/B1			α α
	HISTINIC	P15864	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
Histone H2A.2	HZafz	PUCUS6	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
IQ motif and SEC7 domain-containing protein 1	Iqsec1	Q8R0S2	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~
KH domain-containing, RNA-binding, signal transduction-	Khdrbs1	Q60749	~
Kinesin-1 heavy chain	Kit5b	Q2PQA9	~ ~
MAP7 domain-containing protein 1	Map7d1	A2AJI0	æ
Microtubule-associated protein 2	Map2	P15146	5.4
Microtubule-associated protein 4	Map4	Q5M7W5	3.9
Microtubule-associated protein 6	Map6	Q63560	x
Mitochondrial import inner membrane translocase subunit	Timm44	O35094	\propto
Multifunctional protein ADE2	Paics	P51583	3.0
Myosin regulatory light chain 12B	Myl12b	P18666	3.8
Myosin-10	Myh10	Q61879	1.6
Nestin	Nes	P21263	x
Non-muscle caldesmon	Cald1	Q62736	4.4
Nuclear pore complex protein Nup214	Nup214	Q80U93	\propto
Nuclear pore complex protein Nup98-Nup96	Nup98	P49793	x
PDZ and LIM domain protein 5	Pdlim5	Q62920	19.0
PDZ and LIM domain protein 7	Pdlim7	Q3TJD7	6.0
Pleckstrin homology-like domain family B member 1	Phldb1	Q6PDH0	34.0
Plectin	Plec	P30427	4.8
Pre-B-cell leukemia transcription factor-interacting protein 1	Pbxip1	A2VD12	1.8
Prelamin-A/C	Lmna	P48679	3.3
Protein FAM164A	Fam164a	O8BJH1	3.0
Protein lin-7 homolog C	Lin7c	088952	1.7
Ras-related protein Rab-13	Rab13	P35286	x
Ras-related protein Rab-8B	Rab20	P61028	x
Rho-related GTP-binding protein RhoG	Rhog	P84096	x
Ribosome biogenesis regulatory protein homolog	Rrs1	A1A5P2	3.0
Sentin-7	Sent7	055131	1.8
Serine/arginine renetitive matrix protein 2	Srrm2	OSBIDI	<u>α</u>
Serine/diginine repetitive matrix protein 2			32.0
Serine/threenine-protein phosphatase PGAM5_mitochondrial	DCIKI Pgam5	056285	7.0
Sernin H1 (Heat shock protein 47)	Sorninh1	D1027/	6.7
Signal recognition particle E4 kDa protein	Serpinit	P19524	0.7 x
Signal recognition particle 34 KDd protein 2	Sorber	F143/0	x
Sorbin and Shi utiliani-containing protein 2		035413	2.2
Stress-70 protein, mitochonarial	HSPA9	035501	3.3
Sumue:quinone oxidoreductase, mitocnondrial	Sqrai	Q9K112	5.5
i rirunctional enzyme subunit alpha, mitochondrial	Hadna	Q64428	4./
I ritunctional enzyme subunit beta, mitochondrial	Hadhb	Q60587	3.0
Vimentin	Vim	P31000	2.4
Vinexin	Sorbs3	Q9R1Z8	x
Voltage-dependent anion-selective channel protein 2	Vdac2	P81155	x

(Data supporting Fig 4B)

Single Particle Tracking of pHluorin-α3-NKA in Presence of ATTO-550-tagged α-Syn

Median Diffusion Coefficient (μm²/s)					
Oligomeric α-syn					
OUT	0.11345 (n=1871)				
IN	0.0671 (n=394) (***)				
Fibrillar α-syn					
OUT	0.0995 (n=1305)				
IN	0.0704 (n=364) (***)				

Kolmogorov-Smirnov statistical analysis to test the difference in distribution; (***p<0.001) n= no of QDs (3-experiments on three independent cultures)

(Data supporting Fig 4D and 4E)

Single Particle Tracking of pHluorin-α3-NKA in Presence of Unlabeled α-syn

(In red: Percentage difference from median value of control)

Oligomeric α-syn								
(SYNAPTIC)								
	No of QDs	Median Diffusion Coefficient (um ² /s)		Median Area Explored (um ²)				
Control	268	0.0551		0.0884				
Oligomer-5 min	308	0.0440 (**)	-20%	0.0702 (***)	-21%			
Oligomer-60 min	306	0.0385 (***)	-30%	0.0602 (***)	-32%			
Oligomeric α-syn								
(EXTRA-SYNAPTIC)								
Control	1273	0.1021		0.2148				
Oligomer-5 min	1277	0.0968 (ns)	-5%	0.2028 (*)	-6%			
Oligomer-60 min	1360	0.0823 (***)	-19%	0.1679 (***)	-22%			
Fibrillar α-syn								
(SYNAPTIC)								
Control	373	0.0603		0.0917	,			
Fibril-5 min	528	0.0546 (*)	-9%	0.0668 (***)	-27%			
Fibril-60 min	466	0.0476 (***)	-21%	0.0641 (***)	-30%			
Fibrillar α-syn								
(EXTRA-SYNAPTIC)								
Control	1234	0.0925		0.1631				
Fibril-5 min	1484	0.0694 (***)	-25%	0.1228 (***)	-24%			
Fibril-60 min	1520	0.0583 (***)	-36%	0.1010 (***)	-38%			

Kolmogorov-Smirnov test to compare difference in distribution relative to "Control"

ns = not significant, *p<0.05; **p<0.01; ***p<0.001

4-experiment each for oligomeric and fibrillar α-syn was performed on independent cultures and on separate

days.

(Data supporting Fig 6B)

Enrichment of α3-NKA over α-Syn at Synapses

	Fluorescence Intensity of α3-NKA clusters			
	Normalized to Control-synaptic			
	$(Mean \pm SEM)$			
	Synaptic	Extra-Synaptic		
Control	$1.00 \pm 0.06 \text{ (n=60)}$	$0.38 \pm 0.02 $ (n=60)		
Oligomer-5 min	$1.22 \pm 0.06 \text{ (n=60) (**)}$	0.42 ± 0.02 (n=60) (ns)		
Oligomer-60 min	$1.25 \pm 0.09 \text{ (n=60) (**)}$	$0.39 \pm 0.01 \text{ (n=60) (ns)}$		
Fibril-5 min	1.18 ± 0.05 (n=60) (*)	0.41 ± 0.01 (n=60) (ns)		
Fibril-60 min	$1.42 \pm 0.07 \text{ (n=60) (***)}$	0.48 ± 0.02 (n=60) (ns)		

One-way ANOVA with Dunnett's test to compare the difference from control

ns = not significant, *p<0.05; **p<0.01; ***p<0.001

n = field of view; (3-experiments on three independent cultures)

MATERIAL AND METHODS

Preparation, labeling, characterization and assembly of α -syn

Recombinant wild type (WT) and C-terminally S-tagged (α -syn-S-tag) human α -syn were expressed and purified as described previously (Ghee et al, 2005). α -syn concentration was determined spectrophotometrically using an extinction coefficient of 5960 M⁻¹cm⁻¹ at 280 nm. Pure monomeric α -syn (0.2–0.5 mM) in 50 mM Tris–HCl, pH 7.5, 150 mM KCl (buffer A) was filtered through sterile 0.22 µm filters and stored at -80 °C. For oligomers and fibrils formation, monomeric α -syn in buffer A were respectively incubated at 4°C for 7 days or 37°C for 4 days under continuous shaking in a thermomixer (Eppendorf, Germany) set at 600 rpm, respectively. Assembly into fibrils was monitored using Thioflavin T binding. Aliquots (10 µl) were withdrawn at different time intervals from the assembly reaction and mixed with 400 µl of Thioflavin T (10 µM) in water and Thioflavin T fluorescence (Excitation wavelengths: 480 nm) was recorded using a Cary Eclipse Spectrofluorometer (Varian Inc., Palo Alto, USA). Oligomeric α -syn was separated from monomeric α -syn by size exclusion chromatography using a Superose[®] 6 HR10/30 column (GE Healthcare) equilibrated in phosphate buffered saline (PBS) buffer. Fibrillar α -syn was separated from monomeric α -syn through 2-cycles of sedimentation at 15000g and re-suspension of the pellet (**Appendix Figure S1**).

Oligomeric or fibrillar α -syn in PBS were labeled by addition of 2 molar excess of the aminoreactive fluorescent dye ATTO-550 (Reference: AD 550-35, ATTO-Tech GmbH) or biotin using EZ-link Sulfo-NHS-Biotin (sulfosuccinimidobiotin, Perbio Science, UK). Labeling was performed following the manufacturer's recommendations. Unreacted dye or biotin were removed by size exclusion chromatography or three cycles of sedimentation and suspension in PBS for oligomeric or fibrillar α -syn, respectively. The amount of incorporated ATTO 550 and biotin was assessed by mass spectrometry (**Appendix Figure S1**). The samples were de-salted (with 5% acetonitrile, 0.1% Trifluoroacetic acid (TFA)) and eluted from a C18 reversed-phase Zip-Tip (Millipore, Billerica, MA, USA) in 50% acetonitrile, 0.1% TFA. Peptide samples were mixed in a ratio of 1:5 to 1:20 (v/v) with sinapinic acid (10 mg/mL) in 50% acetonitrile and 0.1% TFA) and spotted (0.5 μ L) on a stainless steel MALDI target (Opti-TOF; Applied BioSystems). MALDI-TOF-TOF MS spectra were acquired with a MALDI-TOF/TOF 5800 mass spectrometer (Applied Biosystems) using linear mode acquisition. External calibration was performed using unmodified WT α -syn. Acquisition and data analysis were performed using the Data Explorer software from Applied Biosystems.

The nature of all α -syn assemblies used was routinely assessed using a Jeol 1400 (Jeol Ltd., Peabody, MA) Transmission Electron Microscope (TEM) after adsorption of the samples onto carbon-coated 200-mesh grids and negative staining with 1% uranyl acetate. The images were acquired with a Gatan Orius CCD camera (Gatan). The particle concentration of oligomeric and fibrillar α -syn samples was assessed by analytical ultracentrifugation (AUC) and quantitative transmission electron microscopy (TEM) as previously described (Pieri et al, 2012). The particle concentration of α -syn (tagged and untagged) was obtained by dividing α -syn monomeric concentration by the average number of molecules (as measured by AUC and TEM). For our preparation, the average number of molecules measured for oligomeric and fibrillar α -syn was 40 and 8333, respectively. The concentration of oligomeric α -syn was 25 nM and that of fibrillar α -syn was 0.03 nM, unless specified, corresponding to 1 μ M or 0.25 μ M monomeric α -syn, respectively.

Aβ-oligomers preparation, purification and characterization has been recently described in Shrivastava et al, 2013a.

Primary neuronal cultures

All cultures were prepared from 18-day-old Sprague-Dawley rat embryos (Janvier Labs, France). Pull down and proteomics studies were performed on pure cultures of rat cortical neurons plated on 10 cm plates precoated with 80 mg/ml poly-D, L-ornithine. Cortical neurons were used, as they can be prepared in larger quantities (4 X 10^6 cells/dish) as required for these experiments. Pure neuronal cultures were maintained in astrocyte-conditioned neuronal medium supplemented with cytosine-arabinoside (5 μ M) as has been recently described (Shrivastava et al, 2013a). All other experiments were performed on rat striatal neuronal cultures plated on 18 mm coverslips pre-coated with 80 mg/ml poly-D, L-ornithine (Renner et al, 2010). Freshly dissociated (trypsin) striatal cells were plated (10^5 cells/well) in neuronal attachment media consisting of 10% horse serum, 1 mM sodium pyruvate, and 2 mM glutamine in MEM for 3 h. The attachment medium was replaced and cells were maintained in serum-free neurobasal medium supplemented with B27 (1X) and glutamine (2 mM). After 2 days, culture medium was supplemented with cytosinearabinoside to restrict the growth of astrocytes. Cells were maintained by supplementing with fresh medium every week.

Pull-down of α -syn-S-tag bound protein complexes and sample preparation for mass spectrometry (MS)

Oligomeric or fibrillar α -syn-S-tag (40 μ M monomer concentration) were added to the culture medium of 2 weeks old pure cortical neuron cultures of rat (3-4 culture dishes per condition). Unexposed neurons were used as control. After 10 minutes, cells were washed twice with 1X PBS and scraped on ice in 50 mM Hepes-KOH (pH 7.5), 2 mM EDTA, 0.1% Triton X-100, supplemented with complete protease inhibitor cocktail (Roche). The extracts were flash frozen in liquid nitrogen and stored at -80°C. Cell lysis was completed by sonication and the protein concentration in the extracts determined using BCA assay kit (Thermo Scientific). To pull down oligomeric or fibrillar α -syn-S-tag together with their specific protein partners, 0.5 mg of total protein extracts were incubated with S-protein agarose (200 μ l settled resin) (Novagen) equilibrated in 500 μ l binding buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, 0.1% Triton X-100, Complete protease inhibitors) for 1 h at 4 °C under gentle agitation. Extracts from control-unexposed neurons were also incubated with S-protein agarose beads and used as control. After 3 washes with 5 ml

binding buffer and 3 washes with 5 ml Triton-free binding buffer, the resin was re-suspended in 400 µl of 50 mM ammonium bicarbonate pH 8 in the presence of 0.1% RapiGest (Waters corporation, Milford, MA) and heated at 95°C for 10 min. Proteins were then reduced in the presence of 10 mM dithiotreitol (DTT) at 56°C for 30 min and alkylated in 20 mM iodoacetamide (Sigma) at room temperature in the dark for 45 min. Proteins bound to the S-protein agarose beads were digested on the resin by incubating the samples overnight at 37 °C in the presence of 0.8 µg trypsin Promega Gold (Promega, Madison, WI). After digestion, the samples were centrifuged for 10 min at 16000g to discard the resins. Trypsin digestion and RapiGest treatment in the supernatants were stopped by addition of 0.5% TFA and incubation at 37°C for 45 min. The tryptic peptide samples were spun for 10 min at 16000g and the supernatants were stored at -80°C for MS analysis.

In the experiments where the cross-linker DTSSP was used to cross-link fibrillar α -syn-S-tag to its partner membrane proteins, 2 weeks old cortical neurons were treated for 30 minutes with chondroitinase (0.02u/ml) prior to the addition of fibrillar α -syn-S-tag to favor the interactions. Chemical cross-linking with DTSSP (1 mM in PBS, Pierce, Waltham, MA)) was carried out for 30 min at room temperature prior to cell scraping and pull-down of α -syn-S-tag cross-linked proteins. For MS identification of α -syn cross-linked proteins further treatment and protein digestion was performed as described above. For the α 3-NKA peptide targeted identification strategy, the nanoLC-MS/MS data were processed automatically using the Thermo Proteome Discoverer software (version 1.4) and the SEQUEST search engine with α 3-NKA primary structure and the following chemical modifications: cysteine carbamidomethylation as fixed modification and methionine oxidation, N-terminal acetylation, and monolink modification of K, S, Y and T residues with DTSSP as variable modifications.

Mass spectrometric identification and quantification of the pulled-down proteins

For each pull down, 15 µl of tryptic peptide digests were analyzed by nanoLC-MS/MS using an EASY-nLC II high performance liquid chromatography (HPLC) system (Proxeon, Thermo- Scientific, Waltham, MA) coupled to the nanoelectrospray ion source of a Linear Ion Trap-OrbitrapVelos mass spectrometer (Thermo Scientific). Peptide separation was performed on a reversed phase C18 nano HPLC column (100 µm inner diameter, 5 µm C18 particles, 15 cm length, NTCC-360/100-5) from Nikkyo Technos (Nikkyo Technos Co., Ltd., Tokyo, Japan). The peptides were loaded at a pressure-dependent flow rate corresponding to a maximum pressure of 200 bars and eluted at a flow rate of 300 nl/min using a two slope gradient of first 5 to 20% solvent B for 60 min, followed by 20 to 40% solvent B in 40 min and a washing step at 100% solvent B. Solvent A was 0.1% formic acid in water, and solvent B was 0.1% formic acid in 100% acetonitrile. NanoLC-MS/MS experiments were conducted in the data-dependent acquisition mode. The mass of the precursors was measured with a high resolution (60,000 full weight at half maximum) in the Orbitrap. The 20 most intense ions, above an intensity threshold of 5000 counts, were selected for CID fragmentation and

analysis in the LTQ. NanoLC-MS/MS data were processed automatically using the Scaffold software (version 3.6.4) and the SwissProt_18112011 database with both the Mascot (Perkins et al., 1999) (Version: 2.3.02) and the X! Tandem (Craig & Beavis, 2004) (Version CYCLONE 2010.12.01.1) search engine, a specific trypsin digestion with up to 2 missed cleavages, a tolerance of 0.5 Da for fragment monoisotopic masses and 6 ppm for parent monoisotopic mass tolerance, and the following chemical modifications: Carbamidomethylation of Cys as fixed modification, and dehydration, ammonia loss, oxidation of methionine and N-acetylation as variable modifications. For the Scaffold analysis, thresholds were set at 90% minimum for peptides and 99% and 2 unique peptides minimum for protein.

Identified proteins were quantified by a label-free proteomic approach using spectral counting (Liu et al, 2004) with the Scaffold software. Comparison between controls and samples identified some proteins as 60S ribosomal proteins, nucleolin and ribonuclease inhibitor as major contaminants. Among them nucleolin presented the best reproducibility among experiments and was chosen to normalize the spectral counting data. Only proteins identified with at least 2 unique peptides in at least 2 replicates were quantified. Only proteins with a spectral count ratio, between the cells exposed to α -syn (either oligomeric or fibrillar) and the control cells, above 1.6 and a p-value <0.05 were considered as significantly increased in the pull-down and thus considered as α -syn interacting proteins. Spectral count ratios presented in **Appendix Table S5-6** were calculated from averaged spectral counts of three independent replicates. Finally the membrane protein annotation was obtained using the NCBI annotation tool of the Scaffold software. For proteins annotated as membrane proteins, validation of the membrane localization was performed with data reported in the literature (see validated membrane protein list in **Appendix Table S5-6**).

Co-immunoprecipitation of α 3-NKA and α -syn

Total protein extracts (1.5 mg in in RIPA buffer, 50 mM Tris-HCl pH 7.5, 50 mM NaCl, 2 mM EDTA, 0.5 mM sodium deoxycholate, 0.5% NP-40%, Complete protease inhibitors) were pre-incubated with protein A sepharose (GE Healthcare) for 1h at 4°C. The supernatant was incubated with Goat polyclonal anti Na+/K+ -ATPase alpha3 (C-16, Santa Cruz Catalogue # sc-16052) (5 μ g) overnight at 4 °C under gentle agitation. Samples were then incubated with protein A-sepharose beads (100 μ l settled resin) (GE-Healthcare) for 1 h at 4 °C under gentle agitation. As a negative control, protein extracts were incubated with protein A-sepharose beads in the presence of pre-immune goat IgGs. After incubation, the beads were washed with RIPA buffer. The protein A-bound protein complex were denatured with SDS-PAGE buffer for 5 min at 95 °C, resolved on 10% polyacrylamide gels and probed with anti α -syn (1:2000, BD Biosciences Cat # 610787) antibody. Blots were stripped (2h, heating at 50°C in 62.5 mM Tris HCl pH 6.8, 2% SDS, 100 mM beta-mercaptoethanol) and re-probed with an anti α -tubulin (1:4000, Abcam mouse monoclonal, DM1A, Cat # ab7291) antibody.

In vivo injection of α -syn assemblies and tissue preparation

Sprague-Dawley rats were obtained from Janvier Labs, France and maintained at the animal house facility (École Normale Supérieure) until surgery. Oligomeric or fibrillar α -syn injection was performed in 10-week old rats (1 male and 1 female for each α -syn subtype). Following anesthesia (106 mg/kg ketamine and 7.5 mg/kg xylazine), animals were placed on a stereotaxis apparatus. A tiny hole in the skull was opened and 5 μ l of α -syn (oligomer: 100 μ M, fibril: 20 μ M monomer concentration) were pressure-injected at a depth of 4.5 mm in the striatum at a flow rate of 0.5 μ l/min (from the bregma: anteroposterior (AP) 0mm, mediolateral (ML) +3 mm, dorsoventral (DV) +4.5 mm). 8 hr (oligomeric) or 24 hr (fibrillar) following injection, animals were anesthetized using 80 mg/kg pentobarbital intra peritoneal and trans-cardially perfused with 4% paraformaldehyde. Their brains were collected and cryo-protected using sucrose before sectioning. 30 μ m thick coronal sections were prepared using cryostat maintained at -20°C. Brain sections were stored in 1X PBS-sodium azide solution and immunohistochemistry performed within 10 days.

Immunohistochemistry, immunocytochemistry and image acquisition and quantification

Brain sections were extensively washed in 1X PBS to remove azide followed by blocking in 0.1% gelatin and 0.2% Triton-X-100 in 1X PBS for 45 min. Sections were then incubated overnight with the appropriate primary antibodies diluted in PBS: Rabbit-Homer (1:1000, Synaptic System), Mouse Gephyrin (1:1000, Synaptic System), Mouse MAP2 (1:1500, Millipore), Mouse- α 3-NKA (XVIF9-G10) (1:1000, Thermo Scientific), Rabbit-Synapsin (1:1500, Synaptic System). Following 3 washes (20 min each), slices were incubated with appropriate secondary antibodies (FITC or cy5 conjugated; 1:500, 3 hr). After washing for 2 hr, sections were mounted onto glass slides using Vectashield (Vector Labs). Nuclear stain (DAPI) was added in the mounting medium (1:400). Images were acquired using a Leica confocal TCS SP5 microscope and processed using ImageJ and Metamorph (Molecular Devices) software. For each animal, staining was performed in randomly chosen 5-6 sections within ±250 µm from the site of injection. In addition images were acquired from nearly 8-10 randomly chosen regions within the striatum.

Immunocytochemistry was performed as per standard protocol and used previously (Shrivastava et al, 2013a). Permeabilization was performed before antibody incubation as antibody against α 3-NKA has intracellular epitope. Even after permeabilization, α 3-NKA immunoreactivity was pre-dominantly found on the plasma membrane as observed previously (Azarias et al, 2013). Following primary antibody incubation for 1 hr: Rabbit-Homer (1:400, Synaptic System), Mouse-Gephyrin (1:400, Synaptic System), Mouse- α 3-NKA (1:3000, Thermo Scientific), Rabbit-Synapsin (1:800, Synaptic System), Rabbit Tau (1:400, Synaptic System), Mouse MAP2 (1:400, Millipore). For α -syn staining, no permeabilization was performed and mouse monoclonal antibody was used (Clone 42/ α -syn; BD Bioscience). Images were acquired using Leica Inverted Spinning Disk microscope (DM5000B, Coolsnap HQ2 camera, Cobolt lasers).

Confocal and spinning disk images were filtered by wavelet decomposition using an interface implemented in Metamorph (Racine et al, 2007). Wavelet decomposition allows the separation of small and large structures (clusters) based on their fluorescence intensities. This approach was used to generate background free masks showing sites that are enriched (cluster) with a specific protein of interest (homer/gephyrin/synapsin/ α 3-NKA) and has been used in previous publications (Bannai et al, 2009; Renner et al, 2010; Shrivastava et al, 2013a). "Intensity of cluster" means total fluorescence intensity per cluster. Co-localization and/or apposition between the clusters of two images were determined using the masks using Matlab where the total fluorescence intensity of clusters was quantified on the original images after identifying the clusters that were totally or partially co-localizing on the masks (Renner et al, 2010; Shrivastava et al, 2013a). No of fields" in the figure legend refer to the number of microscopic field (1392 x 1042 pixel) that were quantified. Intensity Correlation Quotient (ICQ) was computed on entire field of view using plugin in ImageJ as per the instructions (Li et al, 2004).

Plasmids and Transfection

TMR-Dendra construct was prepared by replacing GFP with Dendra fluorescent protein (Ribrault et al, 2011). Extracellular pHluorin-tagged α 3-NKA and extracellular EGFP-tagged β 1-NKA plasmids were generated and characterized by Thomas Liebmann. pHluorin was inserted in the 2nd extracellular loop between Trp³⁰⁷ and Leu³⁰⁸ (Rat sequence: NP_036638). Chimeric α 3/ α 1-NKA-a, b and c were generated using site-directed mutagenesis kit (Agilent). Transfection was performed using lipofectamine-2000 (Invitrogen). Transfection medium (TM) was composed of 1 mM sodium pyruvate and 2 mM glutamine in nerobasal medium (Invitrogen). 0.5 µg of plasmid and 2 µl of lipofectamine-2000 reagent were added separately in 50 µl of TM. After 10 min, the two solutions were mixed and left for another 15 min at room temperature. During this period, culture medium from cells was replaced with pre-warmed TM. The culture medium was stored at 37°C. 100 µl of Lipofectamine-plasmid mix was then added on top of cells. After 30 min, cells were washed with TM and replaced with original culture medium.

Single particle tracking and analysis

Quantum dot (QD) based single particle tracking (SPT) protocol and analysis methods have been used and described in several previous publications (Renner et al, 2010, Shrivastava et al, 2013a). For all experiments, α -syn exposure was performed on live neurons in the culture medium and in an incubator maintained at 37°C/5%CO₂. Unbound α -syn was washed before experiments. All the washing and imaging was performed in MEM recording medium (Phenol red-free MEM, 33 mM glucose, 20 mM HEPES, 2 mM glutamine, 1 mM sodium pyruvate, and 1X B27). For SPT of α -syn, biotin labeled α -syn assemblies were used. Following exposure to α -syn assemblies, cells were incubated with streptavidin-QD-605nm (1:5000, 2 min). For SPT of α 3-NKA, cells were transfected with pHluorin-tagged α 3-NKA plasmid. Neurons were

labeled using GFP-antibody pre-coupled with QD-605 nm (pre-coupling protocol: 1 µl rabbit-GFP antibody + 1 μ l F_{ab}-QD-605 + 7 μ l 1X PBS, mix and gently shake for 30 min, add 1 μ l 1X casein and shake for additional 15 min) (Renner et al, 2009). Synapses were labeled and identified using FM4-64 labeling. All SPT-experiments were performed on neurons aged DIV 16-17. Transfection was preformed on DIV 14. Tracking and analysis was performed using SPTrack v4, homemade software in Matlab (MathWorks) (Renner et al, 2010). The center of the QD fluorescence spot was determined by Gaussian fit with a spatial resolution of 10-20 nm. The spots in a given frame were associated with the maximum likely trajectories estimated on previous frames of the image sequence. Trajectories with a minimum length of 15 consecutive frames were used. The mean square displacement (MSD) was calculated using MSD(ndt) = $(N - n)^{-1} \Sigma_{i=1}^{N-n}$ $[(x_{i+n} - x_i)^2 + (y^{i+n} - y_i)^2]$, where x_i and y_i are the coordinates of an object on frame I, N is the total number of steps in the trajectory, dt is the time between two successive frames, and ndt is the time interval over which displacement is averaged (Saxton and Jacobson, 1997; Triller and Choquet, 2008). The diffusion coefficient D was calculated by fitting the first two to five points of the MSD plot versus time with the equation MSD(t) $= 4D_{2-5t} + 4\sigma_x^2$, with σ_x is the spot localization accuracy in one direction. Explored area represents the distribution of MSD values at a given interval of time. The explored area of individual trajectories was calculated as the area covered by trajectory in μm^2 during a time-interval of 600 ms to 900 ms. Analysis of the explored area reveals the heterogeneity of the diffusion in the population of trajectories and allows applying statistical tests on MSD data.

Super-resolution STORM imaging and analysis

Stochastic Optical Reconstruction Microscopy (STORM) imaging, buffer composition and analysis was recently described (Specht et al, 2013; Shrivastava et al, 2013b). STORM was performed following immunolabeling of α 3-NKA (Mouse- α 3-NKA, 1:3000, 1 hr). Alexa Fluor 647-conjugated (Invitrogen) secondary antibodies were used. Imaging (Alexa647 alone or Alexa647/Dendra) was performed under reducing condition with buffer composed of PBS (pH 7.4), glucose (10%), β-mercaptoethylamine (50 mM), glucose oxidase (0.5 mg/ml), and catalase (40 mg/ml), and degassed with N₂ (Specht et al, 2013). For 2color STORM involving ATTO488 dye, 10 mM β-mercaptoethylamine was used (Dempsey et al, 2011). STORM imaging was carried out on an inverted Nikon Eclipse Ti microscope equipped with a 100X oilimmersion objective (N.A. 1.49 with a microscope-inbuilt 1.5X lens) using an Andor iXon EMCCD camera (image pixel size, 107 nm). Alexa fluor 647 was imaged using laser 639 nm (1 kW, used at 500 mW) for a 50 ms exposure time. A low-wavelength laser (532 nm (0.5 kW), used at 25-75 mW) was used to convert Alexa fluor 647 from off to on state. TMD-Dendra was imaged using laser 561 nm (0.5kW, used at 300mW) while activating with 405 nm laser (100 mW power, used at 1-10 mW). ATTO488 was imaged with 488 nm laser (0.5kW, used at 75-100 mW) and activated using 405 nm laser. Single molecules detection and rendering was recently described (Shrivastava et al, 2013b). For a3-NKA-Alexa647 quantification, individual detections were rendered using a pixel size of 5 nm and Gaussian of 10 nm to visualize clusters and separate closely spaced clusters. This intensity-based render images were then thresholded using ImageJ to isolate clusters of α 3-NKA. The threshold-images were then masked on top on individual detections to compute number of detections per cluster. Distance between clusters measures the separation between the centroid of two nanoclusters of α 3-NKA. This was measured within a maximum radius of 300 nm from the centroid of a given nanocluster. For α -syn-Alexa647, TMD-Dendra and α 3-NKA visualization, rendering was done with a pixel size of 20 nm and Gaussian radius of 10 nm.

An implementation of the DBSCAN (Density-Based Spatial Clustering of Applications with Noise) algorithm (Ester et al, 1996) was used for the density based clustering of α 3-NKA detections. This approach can identify clusters in large spatial data sets by looking at the local density of points. A lowest 'density threshold' of minimum 5 detections within a radial distance of 500 nm was used. Higher thresholds used decreasing radial distances. Detections below local 'density threshold' or threshold distance were considered non-clustered.

TetraspeckTM microsphere multicolor beads (0.1 μ m, Invitrogen, T7279) were used for all STORM imaging experiments to correct for stage drift and 2-color-alignment (for 2-color STORM). Beads were diluted to 1:200 (in 1X PBS) and applied for 2 min before imaging. Unbound beads were washed extensively (~10-12 washed). Stage drift (x/y) was corrected over sliding window of 1000 frames (Specht et al, 2013; Shrivastava et al, 2013b). For 2-color STORM images, positions of at least 2-3 beads were simultaneously aligned using ImageJ to ensure correct alignment of two channels.

Sodium Dye Loading, Imaging and Analysis

Recordings were performed on primary striatal cultures on day *in vitro* 16–21. Cells exposed or not to α -syn assemblies were loaded with the acetoxymethyl ester derivative forms of the Na⁺ sensitive cytosolic ANG2 (Asante NaTRIUM Green 2; 37°C, 5% C0₂) at 5 μ M for 30 min in the cell culture medium (Neurobasal, 2% B27, 0.25% L-Glutamine). After washing, the coverslips were placed on a hot plate maintained at 37°C for 20 min in "recording solution" (110 mM NaCl, 4 mM KCl, 1.5 mM CaCl₂, 1.2 mM MgSO₄, 25 mM NaHCO₃, 1 mM NaH₂PO₄, 20 mM HEPES, 10 mM glucose, pH 7.4). For imaging, coverslips were mounted on a heated chamber attached with perfusion system for rapid exchange of solutions. ANG2 fluorescence was excited at 490 nm and collected above 500 nm, using a Zeiss Axioscope Observer D1 equipped with a 40X, 1.3NA oil objective and an Andor Ixon camera. The K⁺ free recording solution (~25 ml, 0 mM K⁺) had the same composition, except that the NaCl and KCl concentrations were 114 mM and 0 mM, respectively. The 0 mM K⁺ recording solution was replaced with normal recording solution and recovery to basal level was monitored until a plateau was reached. At the end of each experiment, neurons were super-fused with Na⁺ calibration solutions containing stepwise increasing concentrations of Na⁺ in the presence of 3 μ M gramicidin, 10 μ M monensin, and 1 mM ouabain until a plateau was reached (**Appendix Figure S6**). Na⁺

calibration solutions contained $[Na^+ + K^+] = 165 \text{ mM}$, 136 mM gluconate, 1.2 mM MgSO₄, 0.78 mM KH₂PO₄, 20 mM HEPES, 1.3 mM CaCl₂, pH adjusted to 7.2 with KOH.

In each experiment, 8-10 regions of interest (ROI) were selected around primary or secondary dendrites from 3-5 cells (using ImageJ). The fluorescence levels for each ROI were measured against time. The Na⁺ data were then analyzed using custom-written code in MATLAB (MathWorks). The fluorescence (F) was corrected for bleaching, smoothed using a 7-point moving average, and normalized to the fluorescence levels of the calibration solutions containing 10mM Na⁺ (F_{10mM}). The increase in peak was defined as F/F_{10mM} (peak) – F/F_{10mM} (baseline). The recovery to basal was defined as $[F/F_{10mM}$ (peak) – F/F_{10mM} (plateau after the peak)]/ $[F/F_{10mM}$ (peak) – F/F_{10mM} (baseline)]x100 (See pictorial representation in **Appendix Figure S6**). The Na⁺ extrusion rate was quantified by fitting the recovery slope to a bi-exponential equation. The apparent maximum initial pumping rate was taken as the absolute value of the maximum derivative of the fitted function.

Calcium Imaging and Analysis

 Ca^{2+} imaging was performed following Fluo-4 (1 µM) labeling of neurons for 5 min at 37°C in MEM recording medium as previously (Renner et al, 2010). After final wash, cells were allowed to recover for 5-10 min before imaging. Images were acquired on an inverted spinning disk microscope (Nikon Eclipse Ti with Yokogawa spinning disk) at 10X magnification using a 491 nm wavelength laser (100 mW, Cobolt Calypso). Imaging was performed in a controlled environment at 37°C and 5% CO2. Time-lapse images were acquired at 0.2 Hz at 50% laser power and 50 ms exposure time to minimize photo-toxicity. Glutamate (100 µM final concentration) was manually applied using a pipette. For quantification, regions of interest were selected on cell body and total-fluorescence intensity was determined on background-subtracted images (ImageJ). For statistical analysis, ratio of change in fluorescence following glutamate application was calculated. The ratio represents the average intensity of 5 images recoded after glutamate addition divided by the average of 5-images obtained before glutamate application.

Software and Statistics

All analysis of immunocytochemistry, immunohistochemistry, SPT, Na⁺ imaging and STORM were processed and analyzed on MATLAB (Mathworks). Microsoft Excel, Adobe Illustrator, ImageJ and Graph Pad Prism were used for the preparation of figures. The statistical test used is mentioned in figure legends (and supplementary tables). For SPT, Kolmogorov-Smirnov test was used to test the differences in distribution. Pooled diffusion coefficient values from multiple experiments were used as in previous studies (Bannai et al, 2009; Renner et al, 2009, Renner et al, 2010; Shrivastava et al, 2013a, b).

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