Supplementary Information for

Synaptic mechanisms underlying onset and progression of memory deficits caused by hippocampal and midbrain synucleinopathy

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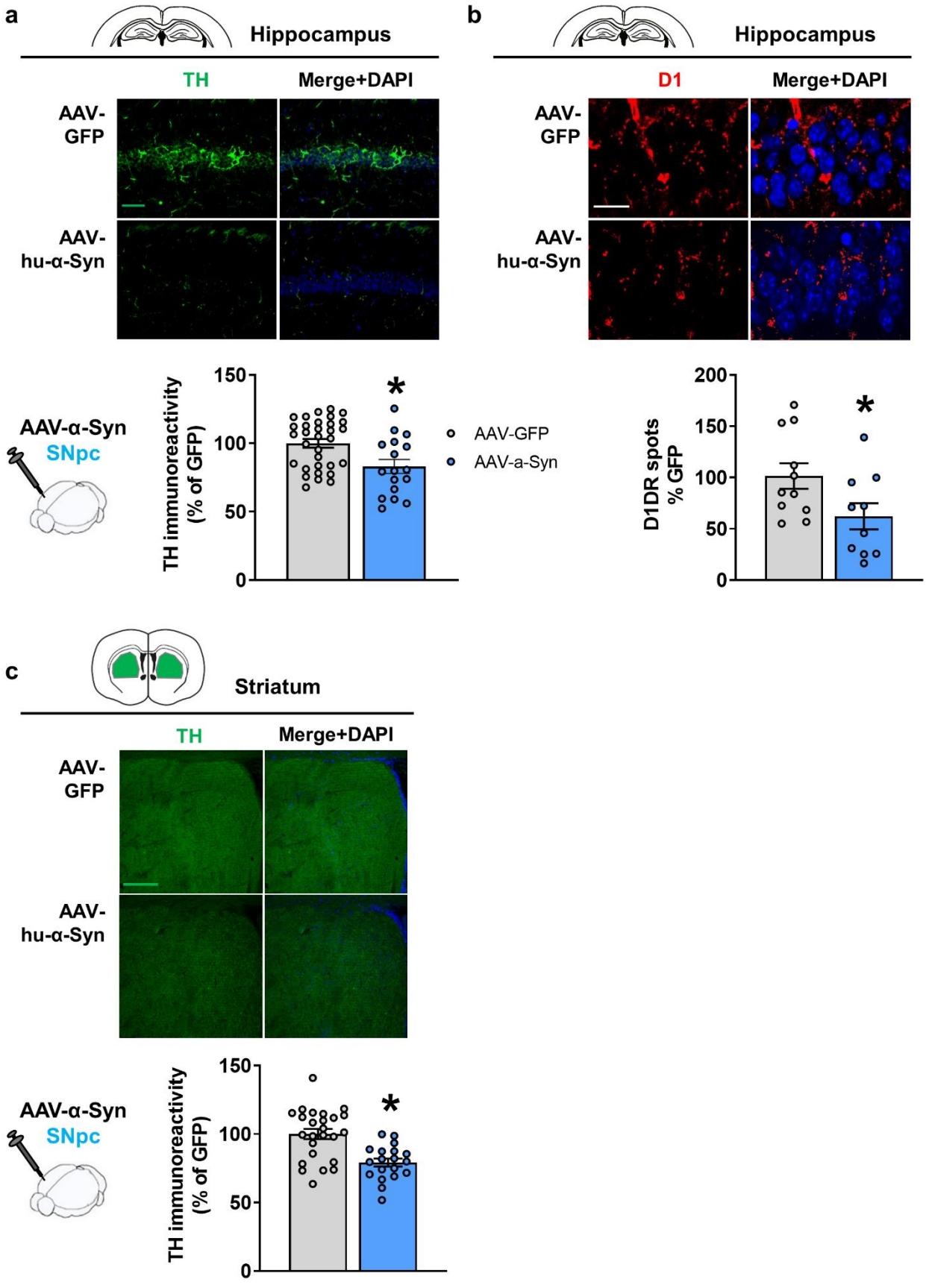
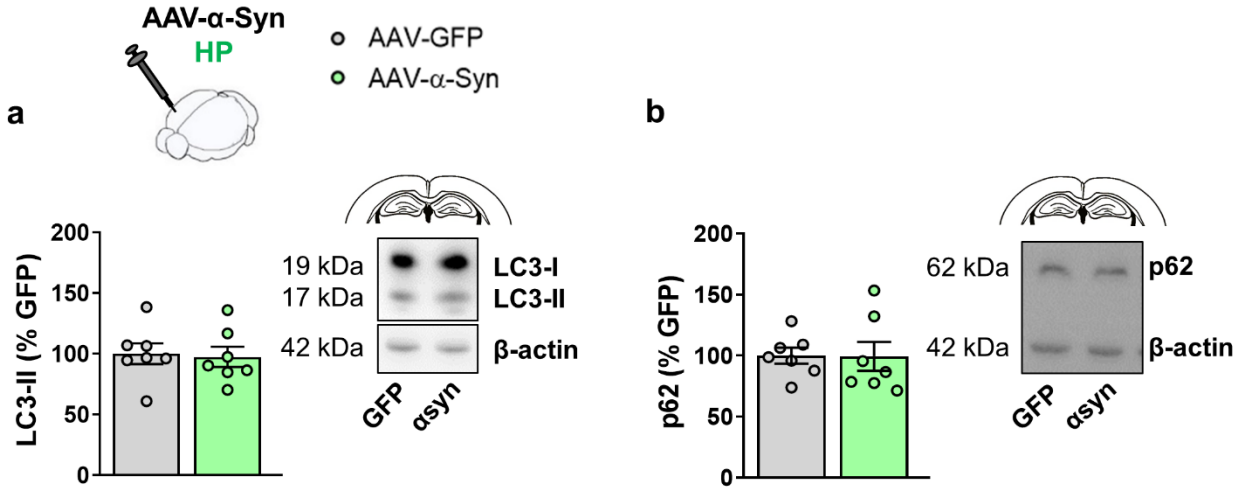
This PDF file includes:

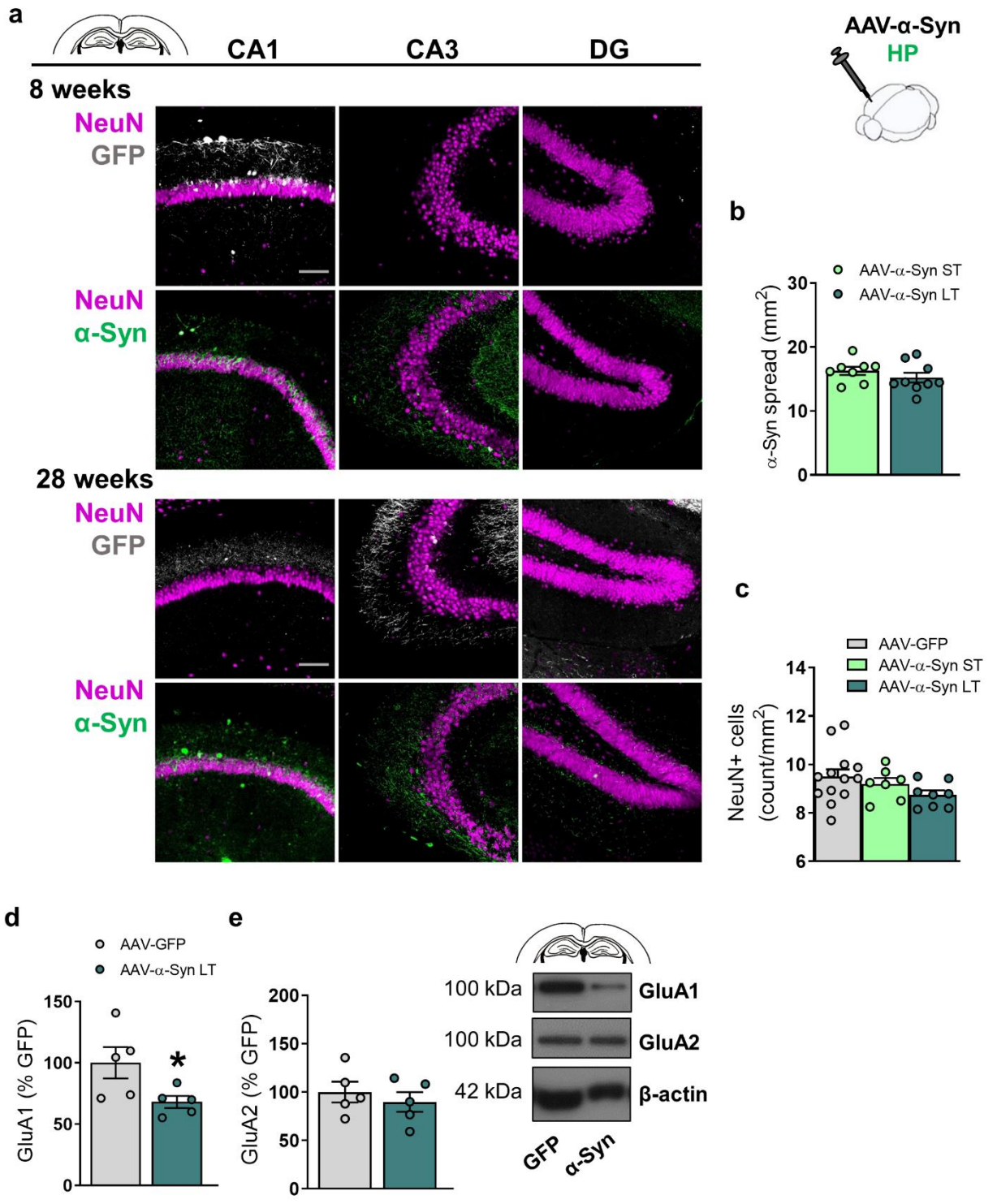
- Supplementary Figures 1 to 6
- Supplementary Table 1
- Uncropped dot/western blots


a
Hippocampus
TH
Merge+DAPI
AAV-GFP
AAV-hu-α-Syn
AAV-α-Syn
SNpc
TH immunoreactivity (% of GFP)
150
100
50
0
*
AAV-GFP
AAV-a-Syn
b
Hippocampus
D1
Merge+DAPI
AAV-GFP
AAV-hu-α-Syn
D1DR spots % GFP
200
150
100
50
0
*
c
Striatum
TH
Merge+DAPI
AAV-GFP
AAV-hu-α-Syn
AAV-α-Syn
SNpc
TH immunoreactivity (% of GFP)
150
100
50
0
*

Supplementary Figure 1. rAAV-mediated overexpression of hu-α-Syn in the midbrain leads to reduced TH immunoreactivity in the striatum and the hippocampus and decreases the number of D1R1 spots in the latter. (a) Overexpression of hu-α-Syn in the SNpc/VTA significantly decreased the percentage of TH immunoreactivity compared to SNpc-GFP mice in the hippocampus (unpaired t test, t = 2.973, P = 0.0046, SNpc-GFP, n = 32; SNpc-hu-α-Syn, n = 17). Representative confocal images of the CA1 are reported for each condition in the upper panel (Scale bar: 50 μm). **(b)** In parallel, SNpc-hu-α-mice show a reduction of D1DR spots compared to rAAV-GFP mice (unpaired t test, t = 2.194, P = 0.0409, SNpc-GFP, n = 11; SNpc-hu-α-Syn, n = 10). Representative confocal images of D1DR spots are reported for each condition in the upper panel (Scale bar: 20 μm). **(c)** SNpc-hu-α-mice show a significantly decreased percentage of TH immunoreactivity in the striatum compared to SNpc-GFP mice (unpaired t test, t = 4.215, P < 0.0001, SNpc-GFP, n = 25; SNpc-hu-α-Syn, n = 19). Representative confocal images of the striatum are reported for each condition in the upper panel (Scale bar: 200 μm). Data are presented as mean ± SEM. * P < 0.05 from SNpc-GFP.

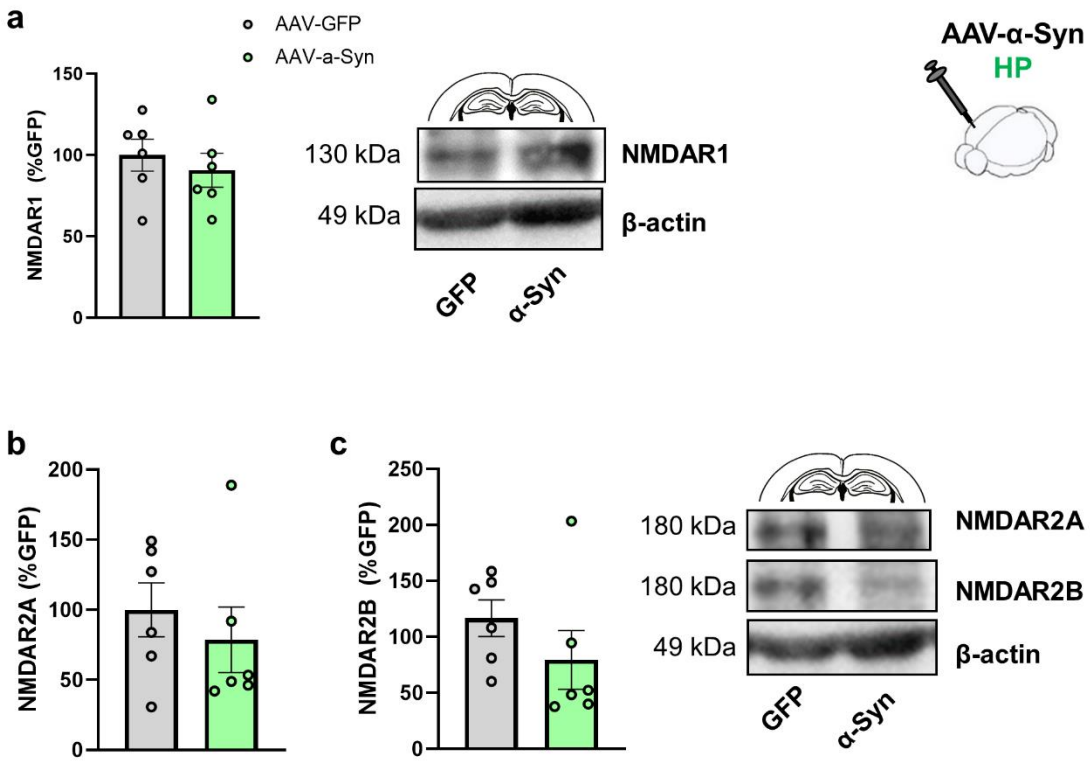


Supplementary Figure 2. rAAV-mediated overexpression of hu-α-Syn does not lead to autophagy impairment. (**a**) Western blot analysis of the autophagosomal membrane marker LC3-II and (**b**) the autophagy substrate p62 in the hippocampus of HP-hu-α-Syn and HP-GFP mice. Representative bands of S2 belong to the same blot of GluA2 reported in Fig. 5d. Data are presented as mean ± SEM.

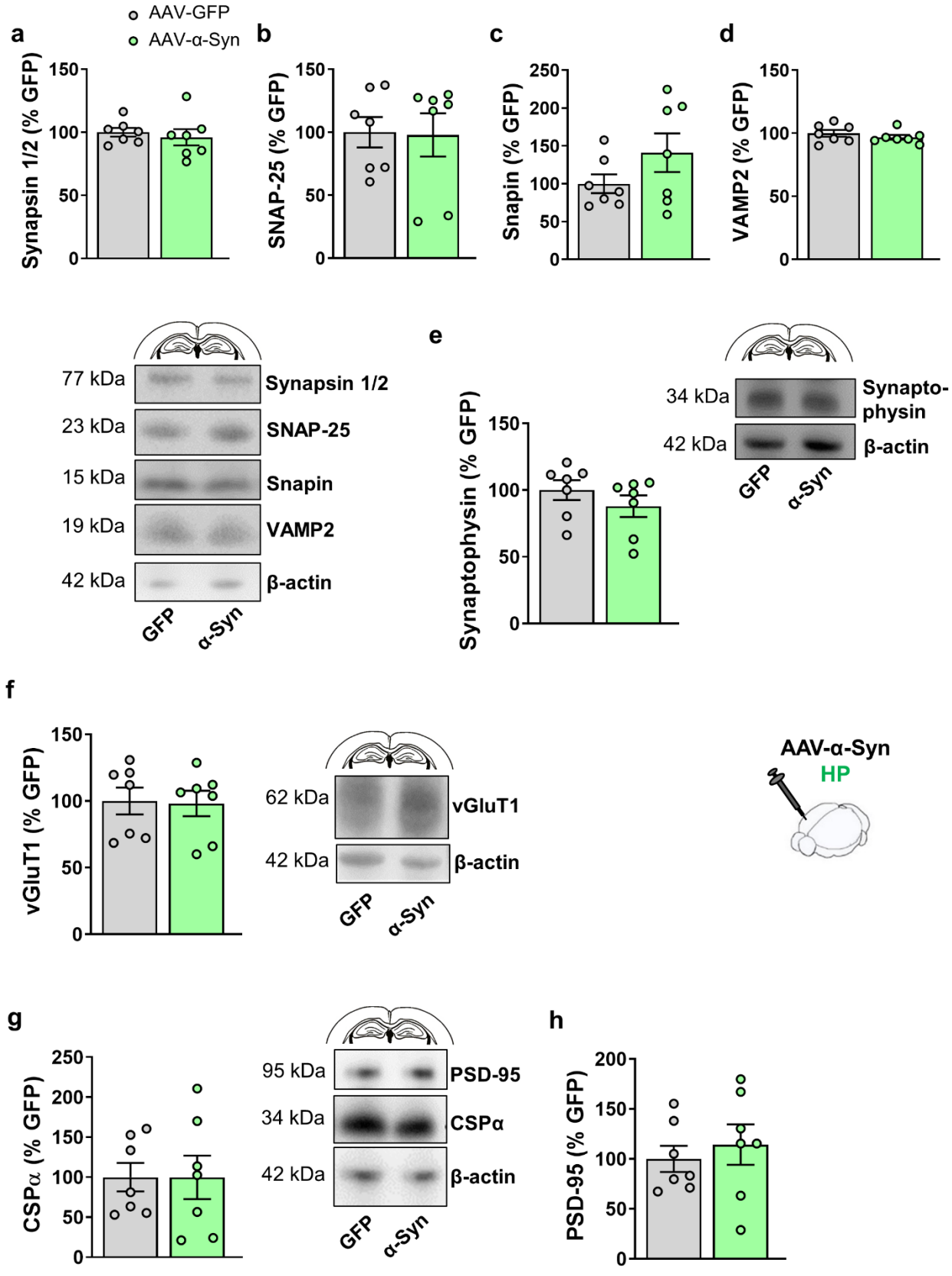


Supplementary Figure 3. Short and long-term rAAV-mediated overexpression of GFP and hu-α-Syn in the hippocampus does not lead to neuronal loss. (**a**) Representative confocal images from CA1, CA3 and DG of control GFP (grey) and hu-α-Syn (green) mice, stained for NeuN (magenta) in the dorsal hippocampus (Scale bar: 100 µm). (**b**) Quantification of hu-α-Syn spread in the dorsal hippocampus after short- and long-term overexpression showed no significant difference between

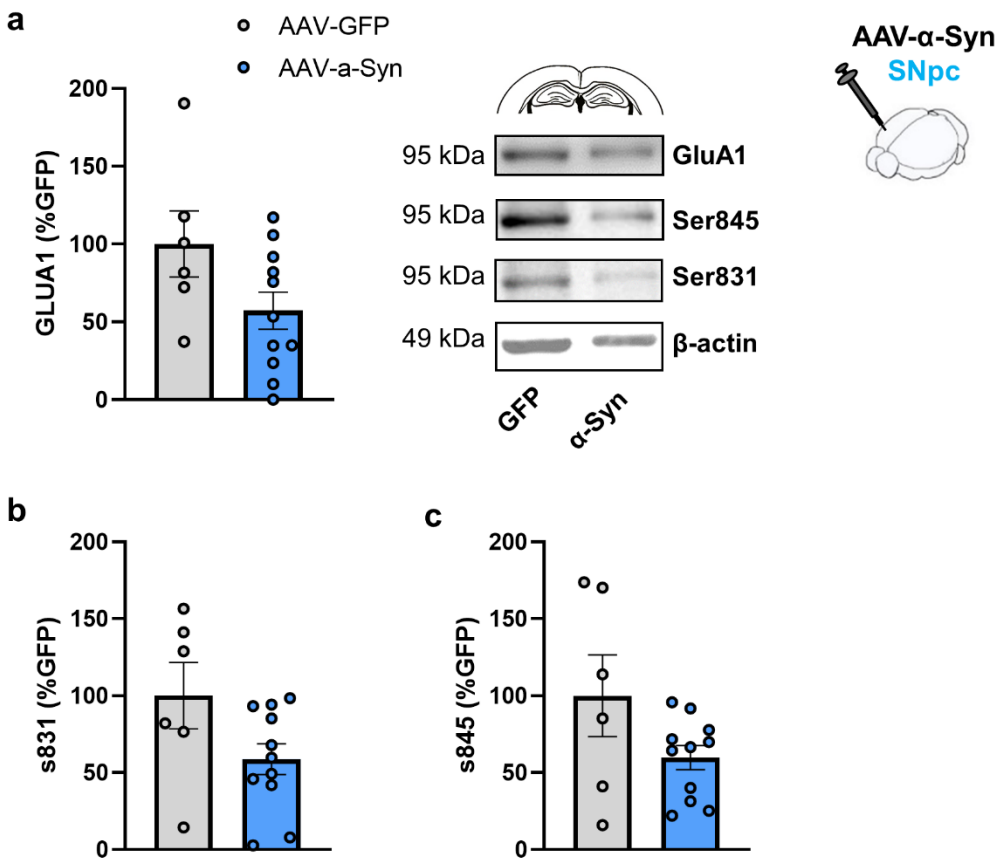
groups. (**c**) Accumulation of α-Syn in the dorsal hippocampus after short- and long-term overexpression of hu-α-Syn does not induce a significant reduction in NeuN-immunoreactivity, when compared to rAAV-GFP control animals. (**d**) Long-term overexpression of hu-α-Syn in the hippocampus decreased GluA1 (unpaired t test, $t = 2.329$, $P = 0.04$, HP-GFP, $n = 5$; α-Syn, $n = 5$) but not GluA2 (**e**), expression. Representative bands for each condition are reported. Data are presented as mean ± SEM. * $P < 0.05$ different from rAAV-GFP.



Supplementary Figure 4. Short-term rAAV-mediated overexpression of hu-α-Syn in the hippocampus does not affect the expression of NMDA receptor subunits. (**a-c**). Western blot analysis of the hippocampal tissue in the HP-hu-α-Syn and HP-GFP groups for the NMDAR1 (**a**), NMDAR2A (**b**) and NMDAR2B (**c**) subunits (n = 6 for each experimental group). Representative bands for each condition are reported. Data are presented as mean ± SEM.


a
AAV-GFP
AAV-α-Syn
Synapsin 1/2 (% GFP)
b
SNAP-25 (% GFP)
c
Snapin (% GFP)
d
VAMP2 (% GFP)
77 kDa
Synapsin 1/2
23 kDa
SNAP-25
15 kDa
Snapin
19 kDa
VAMP2
42 kDa
β-actin
GFP
α-Syn
e
Synaptophysin (% GFP)
34 kDa
Synapto-physin
42 kDa
β-actin
GFP
α-Syn
f
vGluT1 (% GFP)
62 kDa
vGluT1
42 kDa
β-actin
GFP
α-Syn
AAV-α-Syn
HP
g
CSPα (% GFP)
95 kDa
PSD-95
34 kDa
CSPα
42 kDa
β-actin
GFP
α-Syn
h
PSD-95 (% GFP)

Supplementary Figure 5. Short-term rAAV mediated overexpression of hu-α-Syn in the hippocampus does not affect the expression of pre- and post-synaptic density proteins. (**a-h**) Short-term accumulation of hu-α-Syn in the mouse hippocampus does not change the expression of several essential proteins involved in synapse function and morphology (GFP, n = 7; hu-α-Syn, n = 7). Representative bands for each condition are reported. Data are presented as mean ± SEM.



Supplementary Figure 6. Long-term overexpression of hu-α-Syn in the midbrain does not affect the hippocampal expression of GluA1 AMPA receptors and phosphorylation. (**a-c**) Western blot analysis of the hippocampal tissue from SNpc-hu-α-Syn mice showing no significant change in the expression of GluA1 and its phosphorylation at serine 845-831 sites compared to SNpc-GFP control (GFP, n = 6; hu-α-Syn, n = 11). Representative bands for each condition are reported. Data are presented as mean ± SEM.

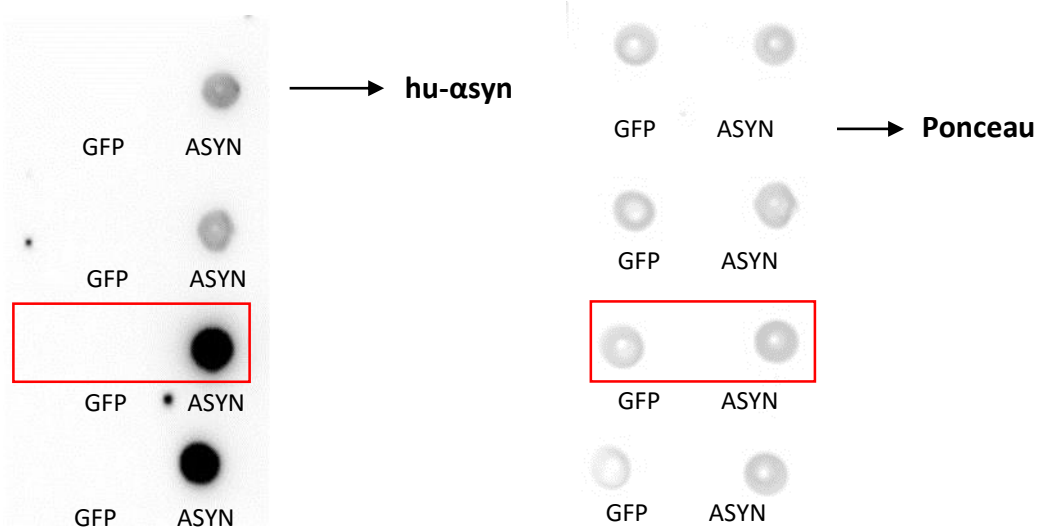
Supplementary Table 1. Number of mice used for behavioral analysis.

	HIPPOCAMPUS				**SUBSTANTIA NIGRA**			
Behavior	4 weeks		24 weeks		4 weeks		24 weeks	
	HP-GFP	HP-hu-α-Syn	HP-GFP	HP-hu-α-Syn	SNpc-GFP	SNpc-hu-α-Syn	SNpc-GFP	SNpc-hu-α-Syn
6-DOT	8	11 (2)	9	15	15 (1)	19 (1)	8 (1)	15
6-IOT	8	13	9	15	15 (1)	20	8 (1)	15
Locomotor activity	7[1]	13	9	15	16	20	9	15
Open field (time in center)	7 [1]	13	9	15	16	20	9	15
Elevated plus maze	8	13	9	15	16	20	9	15
Pre-pulse inhibition	7 [1]	11 [2]	9	15	16	20	9	15
Fear conditioning	8	13	9	15	**ND**	**ND**	**ND**	**ND**

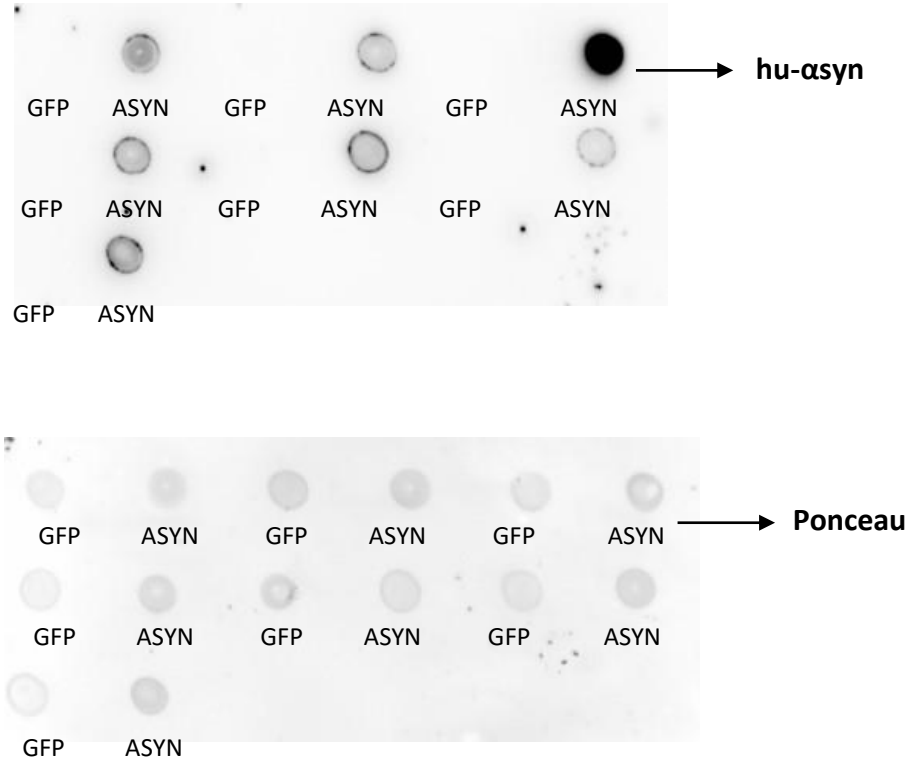
The number of excluded animals because of procedural testing problems or because they did not explore the objects for more than 5 sec is reported in square and round brackets, respectively.

FIG 2C Dot blot anti-hu-αsyn; ponceau staining

DOT-BLOT#1



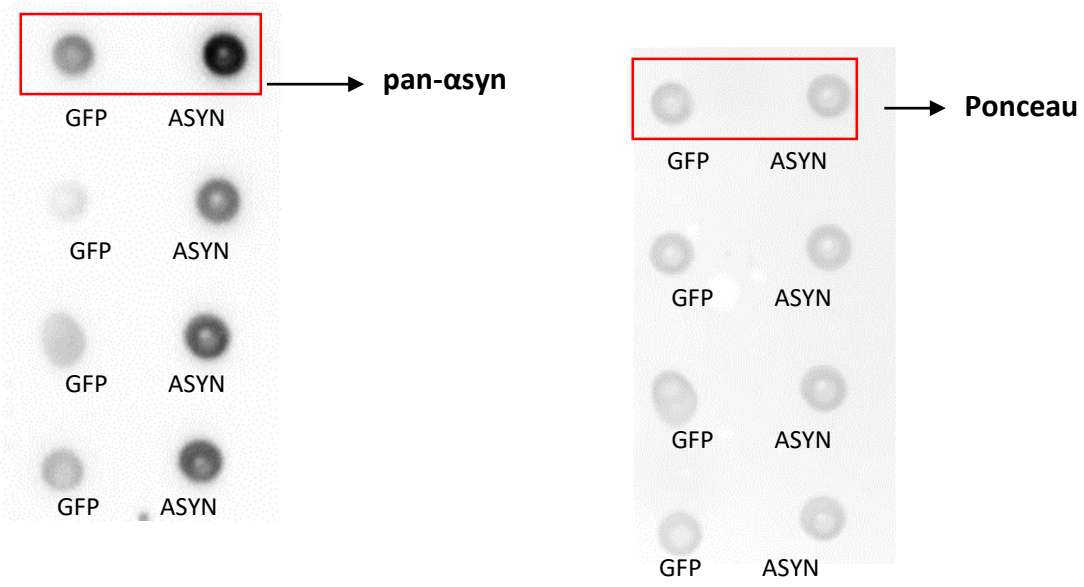
DOT-BLOT#2



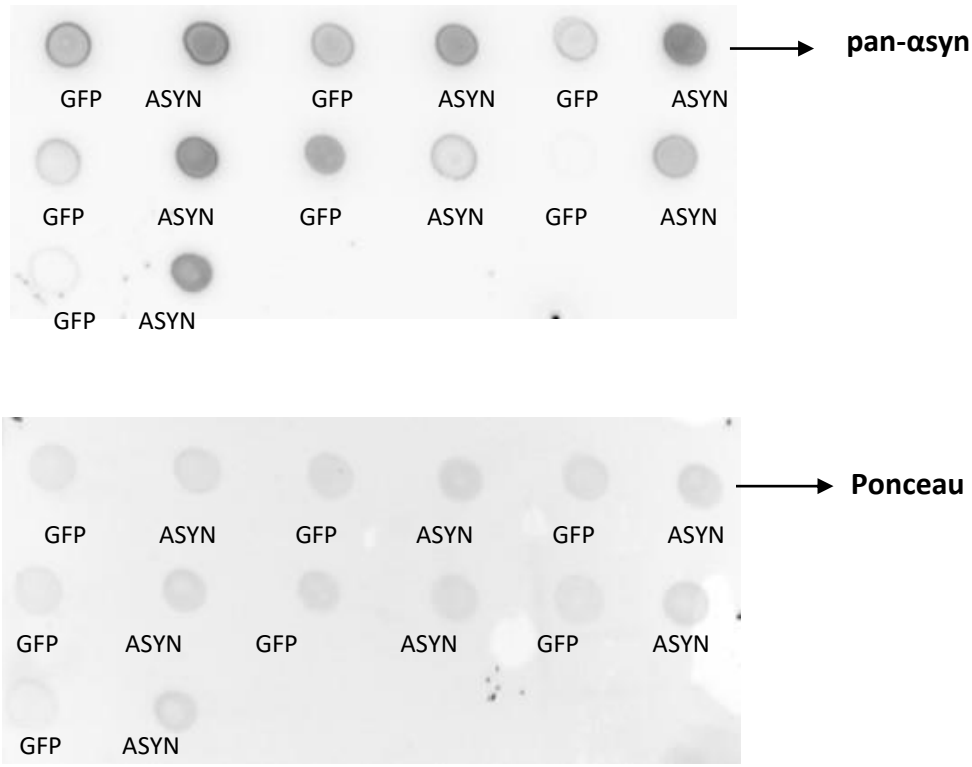
The square box represents bands reported in the figure

FIG 2D Dot blot anti-pan-αsyn; ponceau staining

DOT-BLOT#1

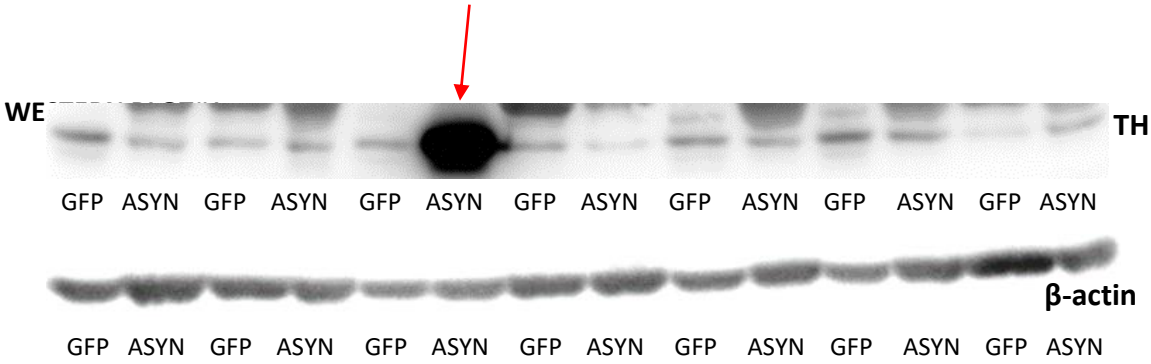


DOT-BLOT#2

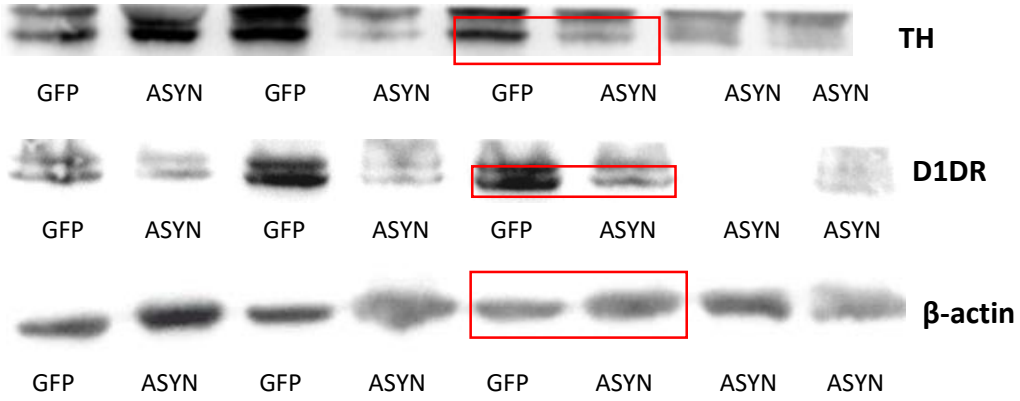


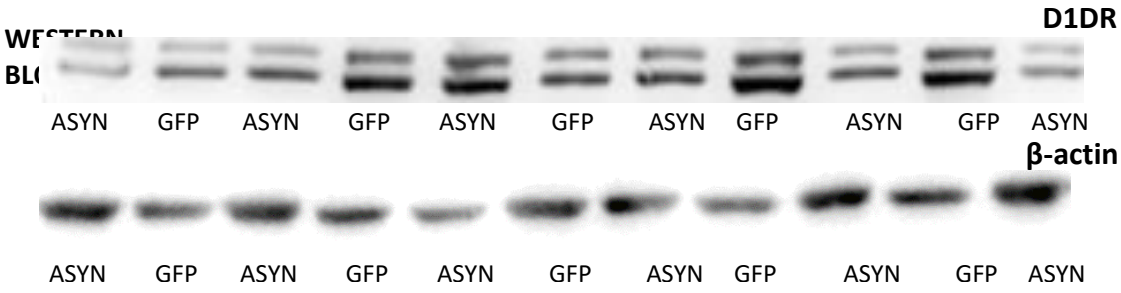
The square box represents bands reported in the figure

FIG 2E,F Anti TH, anti-D1R, anti-β-actin



WESTERN BLOT#2

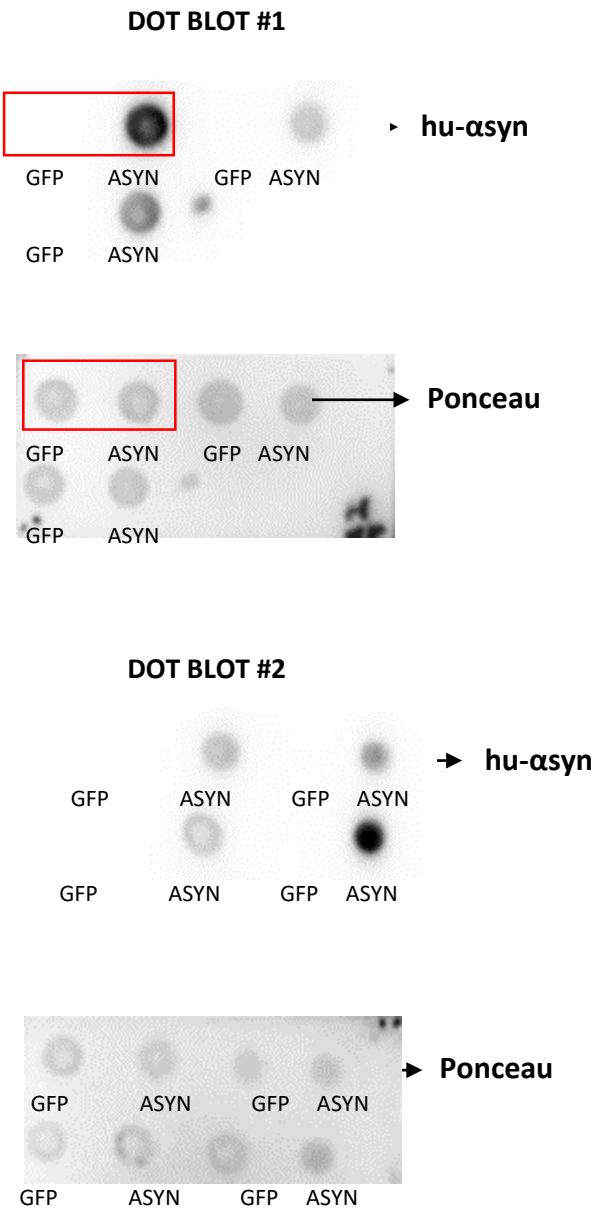




The arrow shows an outlier sample excluded from the analysis

The square box represents bands reported in the figure

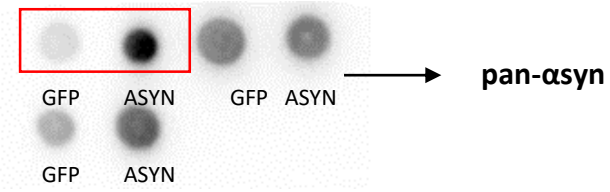
Fig. 4d Dot blot anti-hu-αsyn; ponceau staining



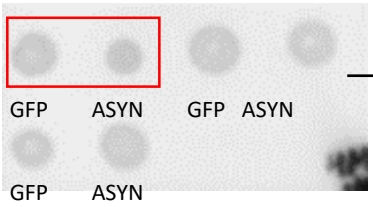
The square box represents bands reported in the figure

Fig. 4e Dot blot anti-pan-αsyn; ponceau staining

DOT BLOT #1

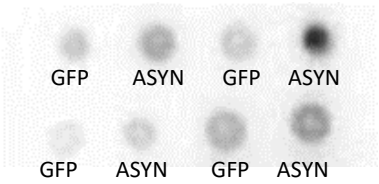


pan-αsyn

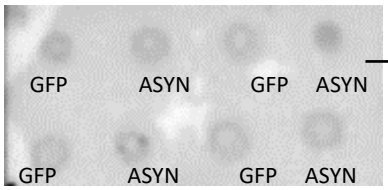


Ponceau

DOT BLOT #2



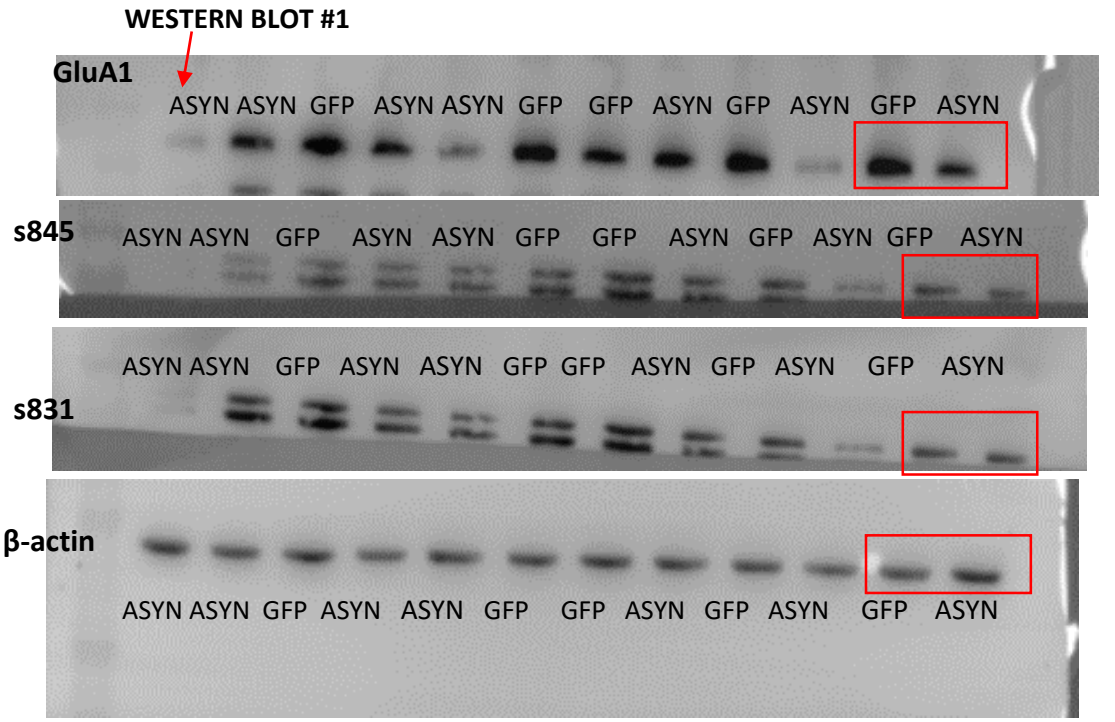
pan-αsyn

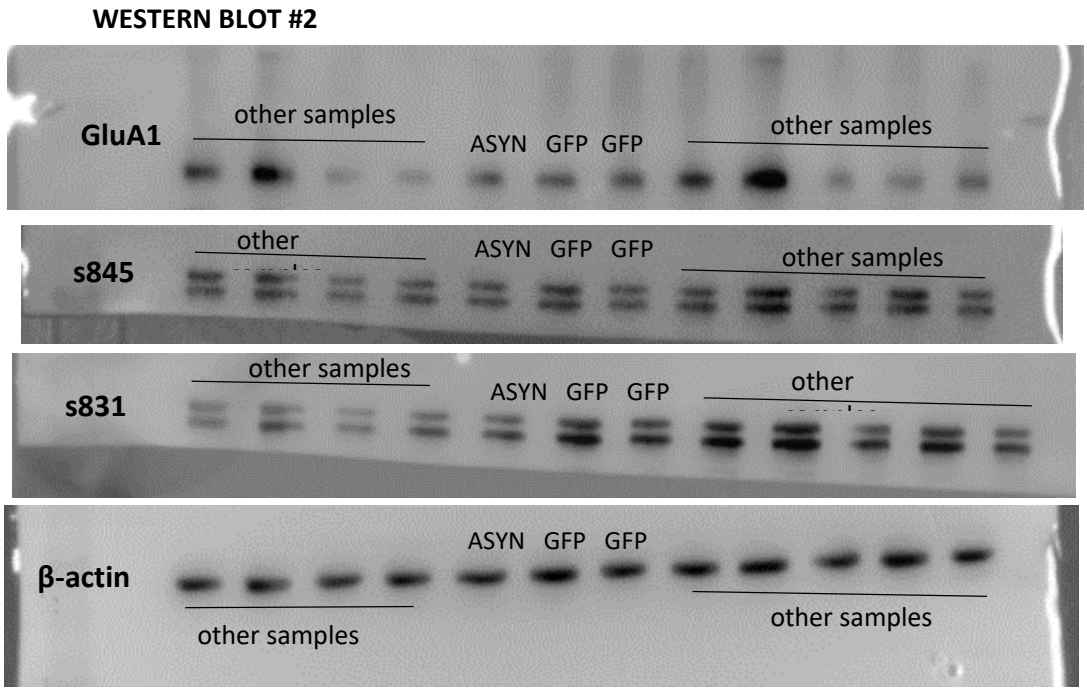


Ponceau

The square box represents bands reported in the figure

Fig. 5A, B, C anti- GluA1; anti-s845; anti-s831; anti-β-actin





The square box represents bands reported in the figure
The first sample blot #1 was excluded and replicated in the second blot (arrow)

Fig. 5D anti- GluA2; anti-β-actin

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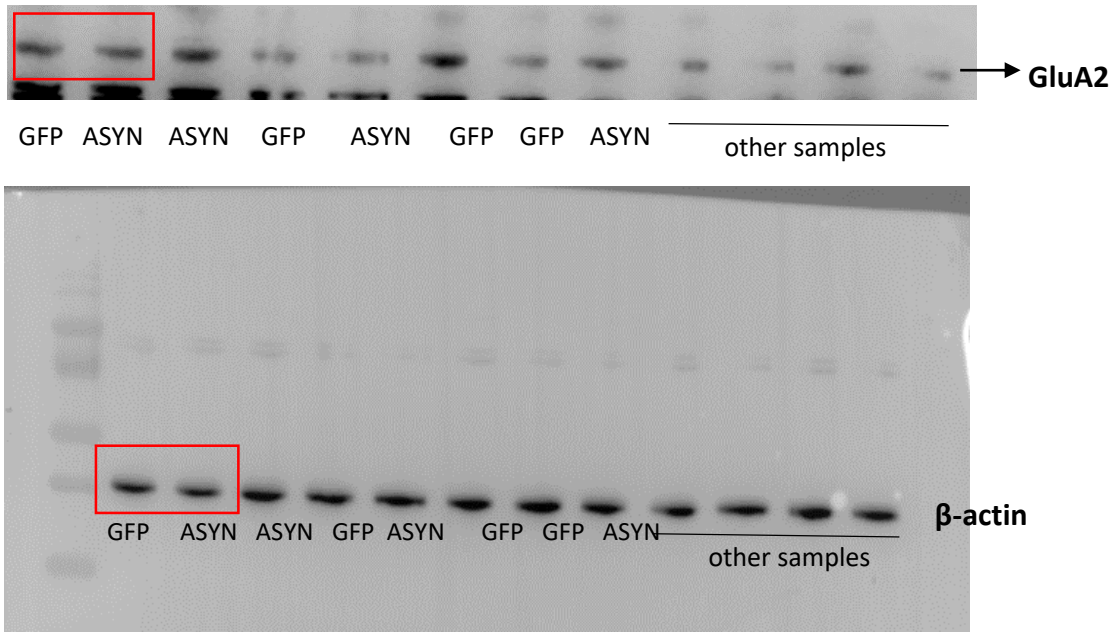

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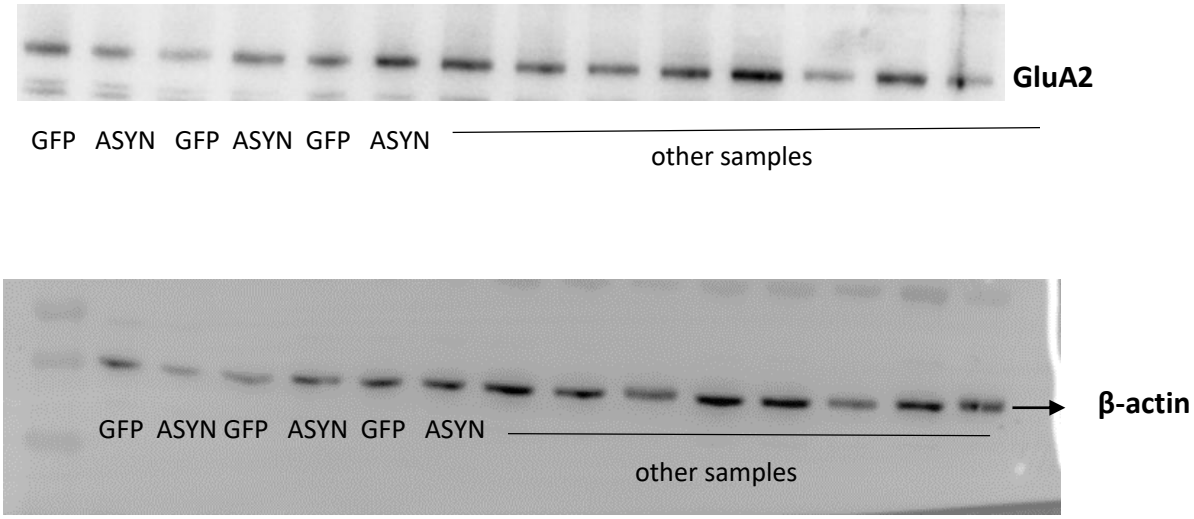

The square box represents bands reported in the figure
These are the same gel of figure S2a. GluA2 was blotted on the same membrane

Fig. S2a anti- LC3; anti-β-actin

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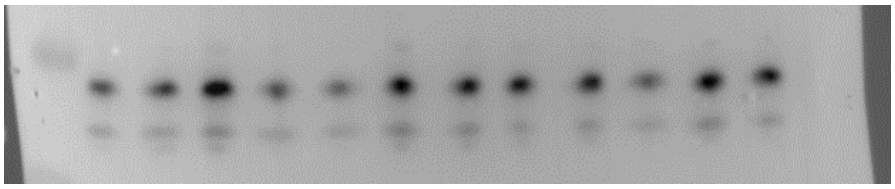
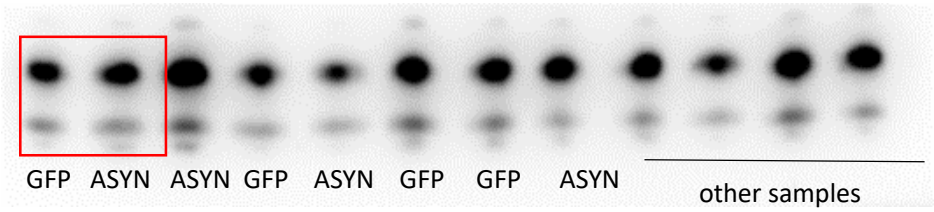
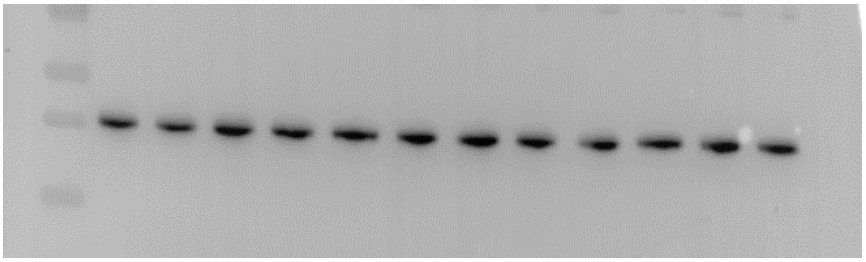


LC3-I

LC3-II



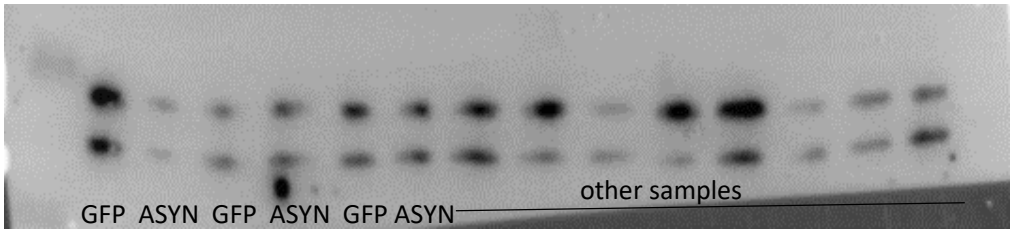
LC3 (higher exposure)



β-actin

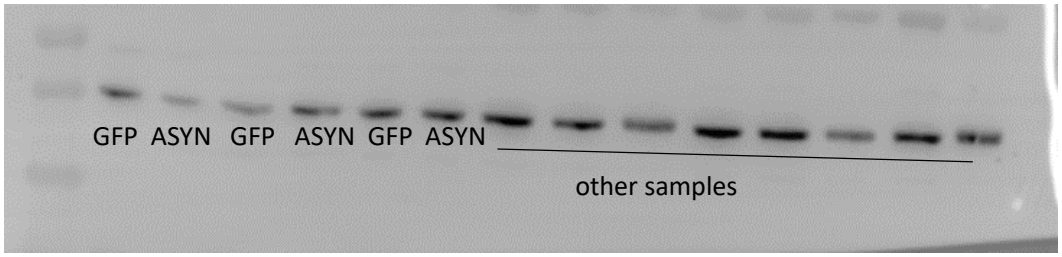
β-actin (lower exposure)

WESTERN BLOT #2



LC3-I

LC3-II

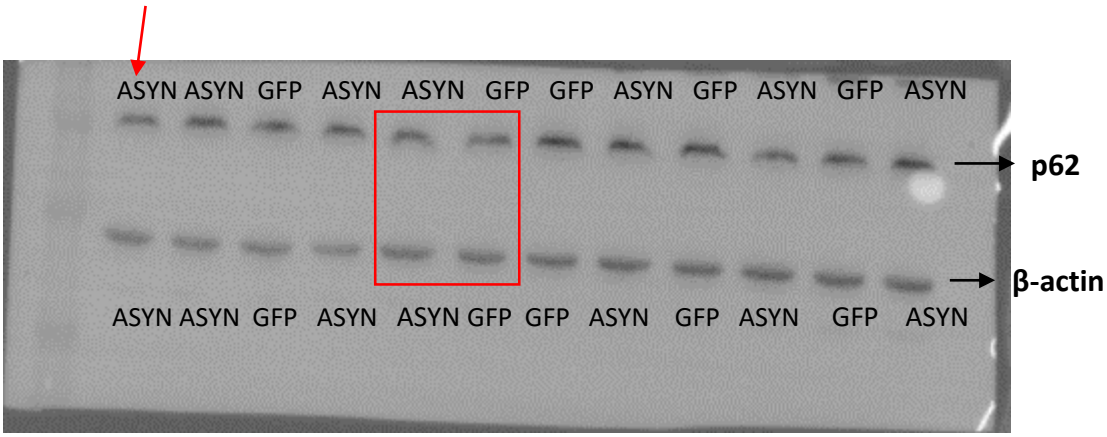


β-actin

The square box represents bands reported in the figure
These are the same gel of figure 5D. LC3 was blotted on the same membrane

Fig. S2b anti- p62 anti-β-actin

WESTERN BLOT #1



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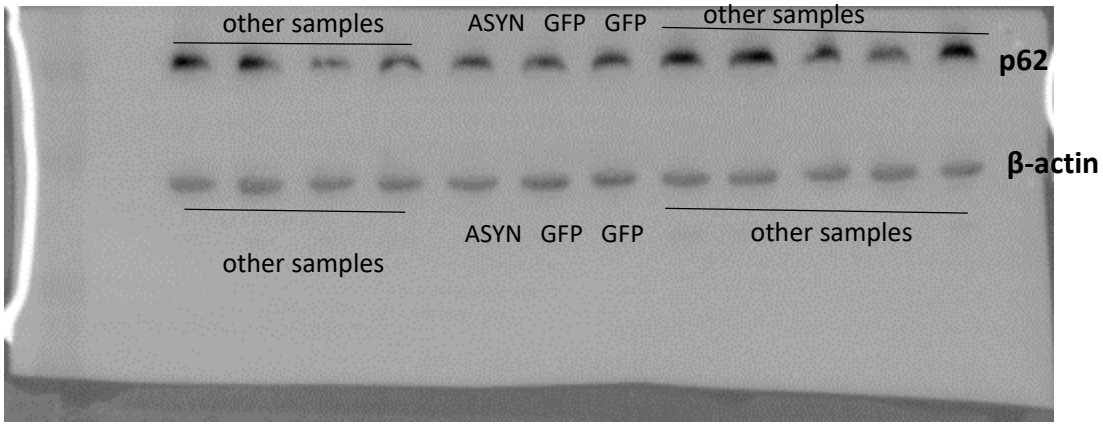

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These are the same gel of figure 5A-C. P62 was blotted after the stripping
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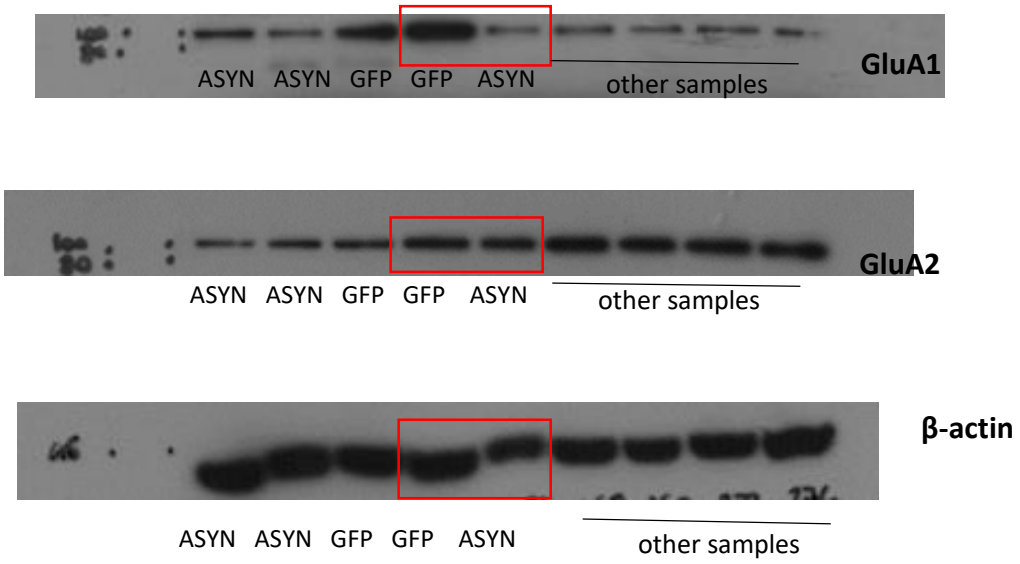
Fig. S3D-E anti- GluA1; anti- GluA2; anti-β-actin

WESTERN BLOT #1



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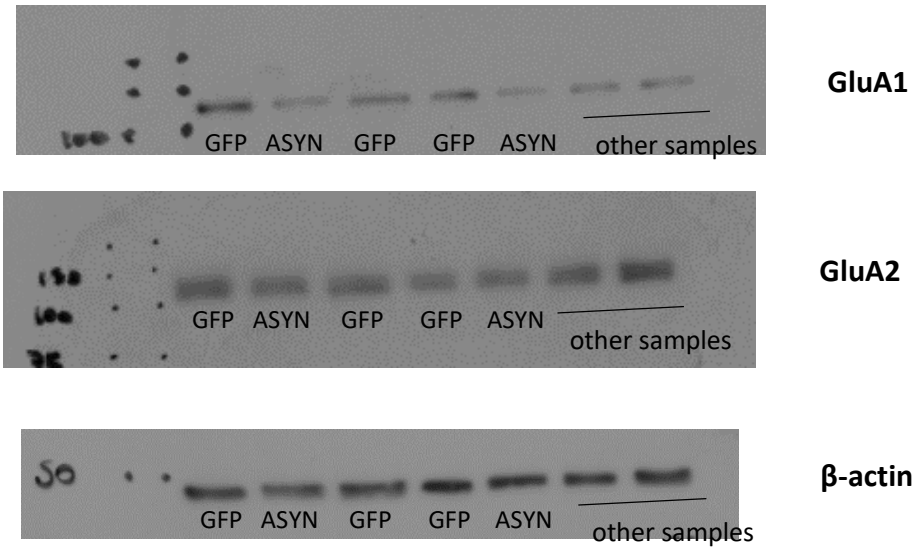

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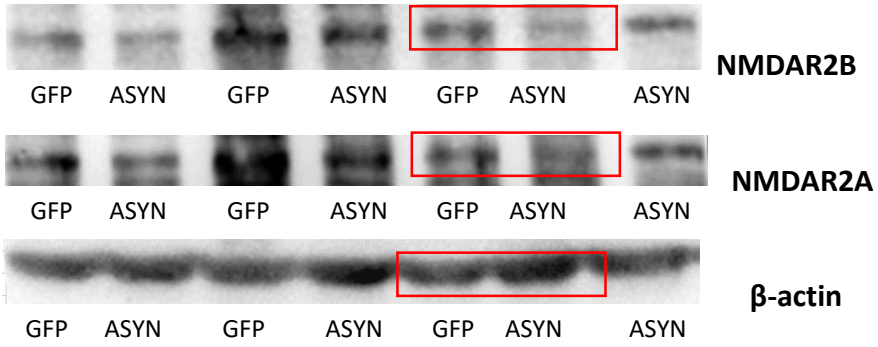
Fig S4A,B,C Anti NMDAR1, anti-NMDAR2A, anti-NMDAR2B, anti-β-actin

WESTERN BLOT#1



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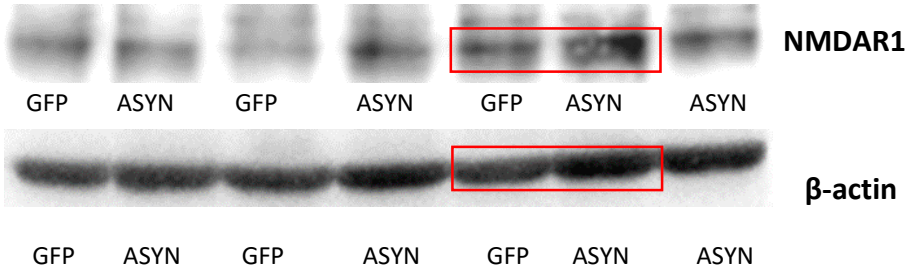

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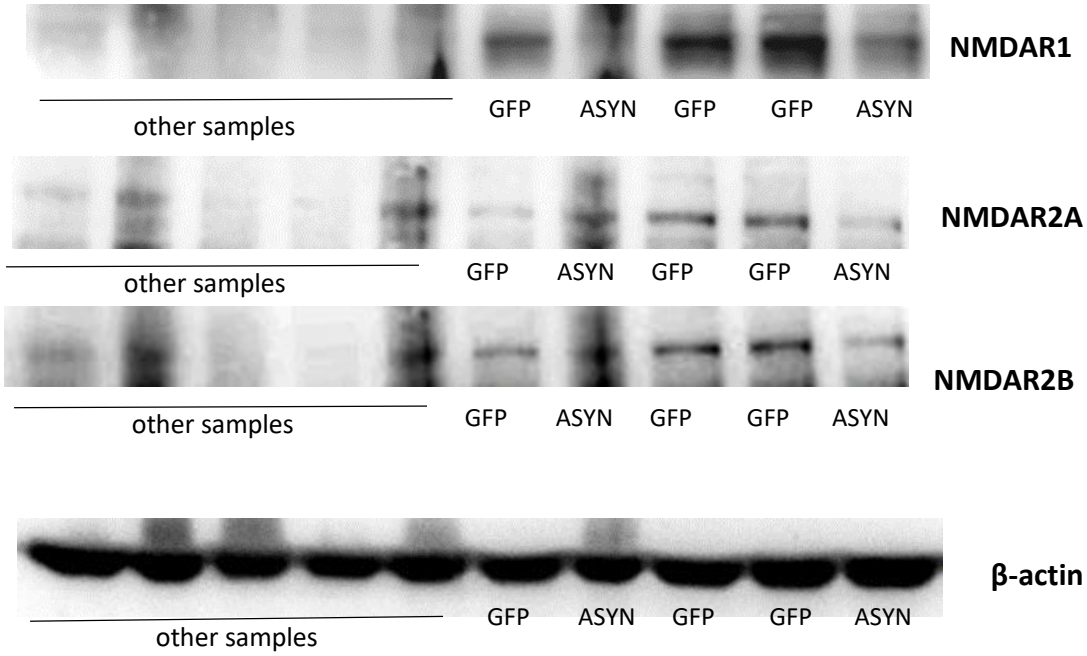
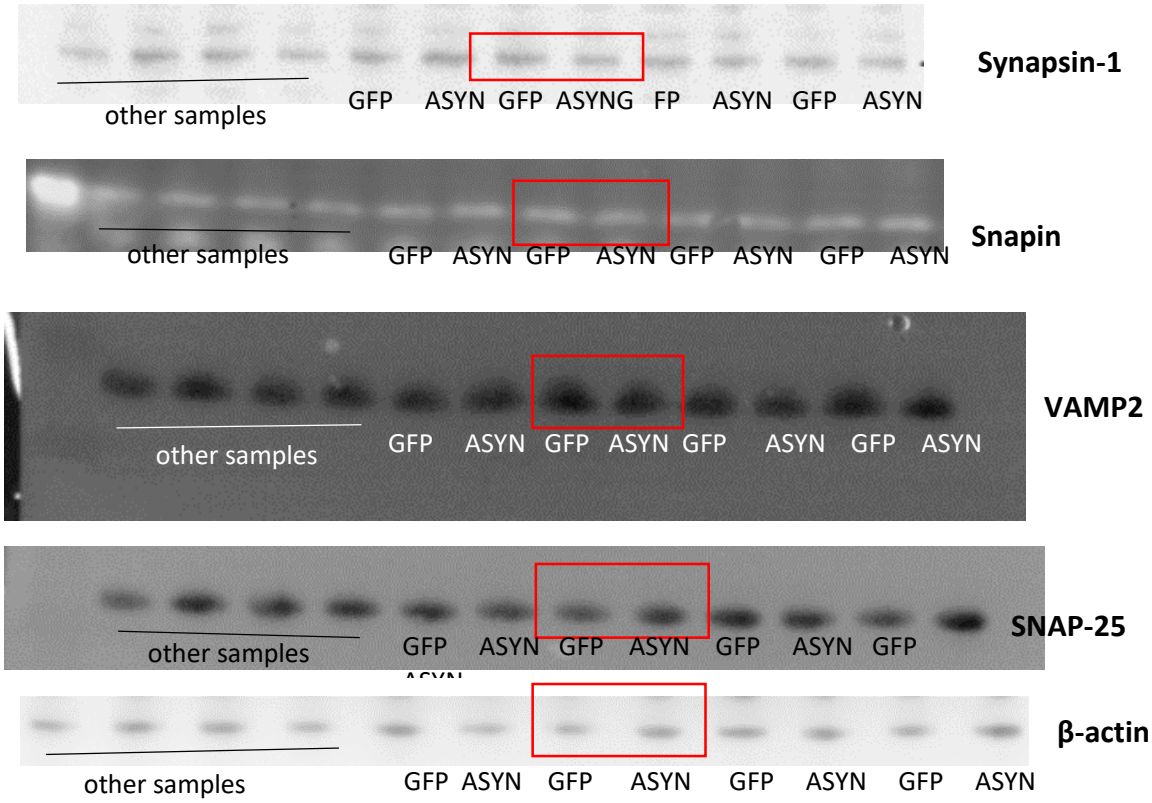


The square box rapresents bands reported in the figure

Fig. S5A, B, C, D Anti-Synapsin-1; anti-SNAP-25; anti-Snapin; anti-VAMP2; anti-β-actin

WESTERN BLOT #1



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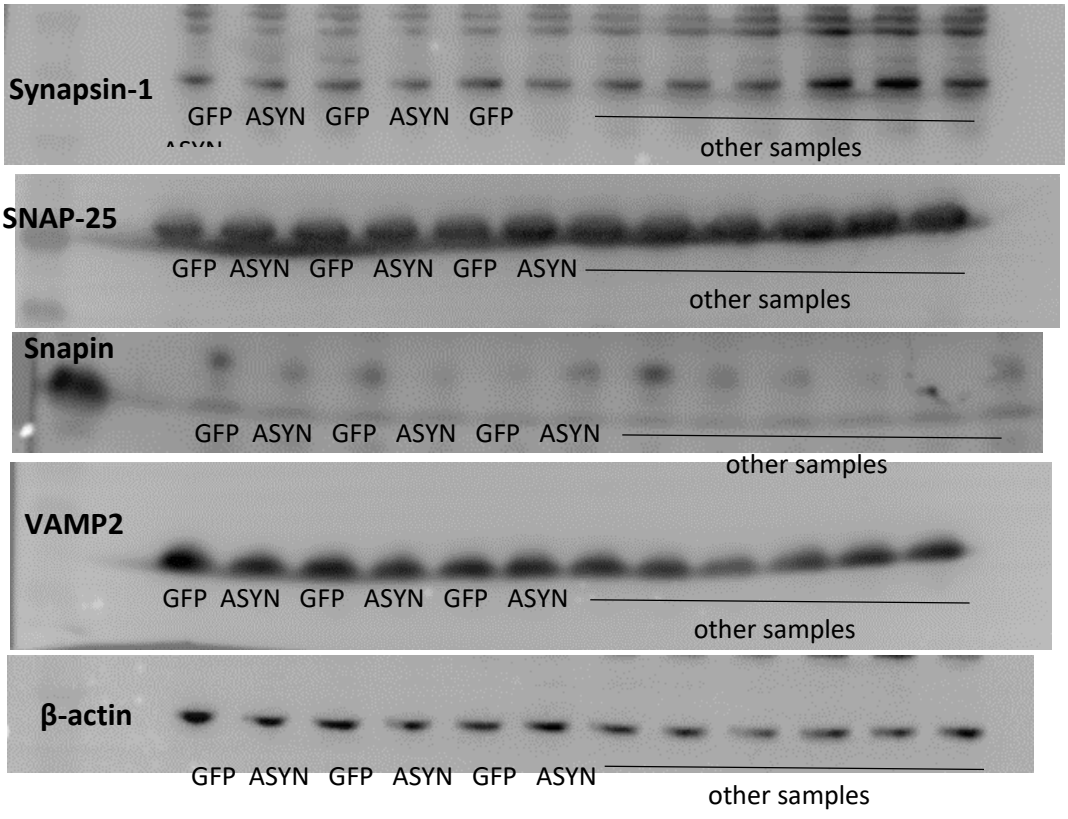

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Fig. S5E Anti-Synaptophysin; anti-β-actin

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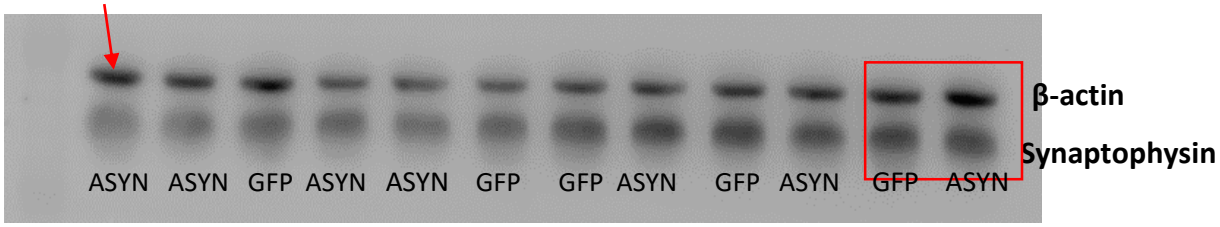

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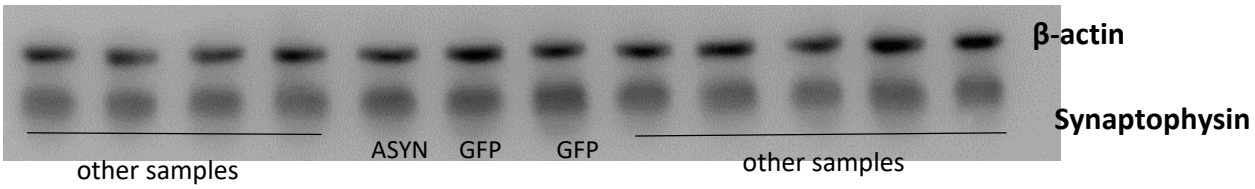
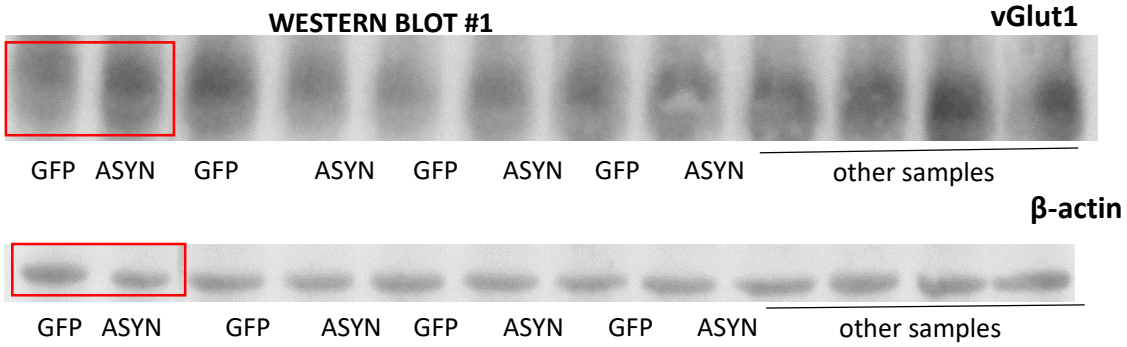


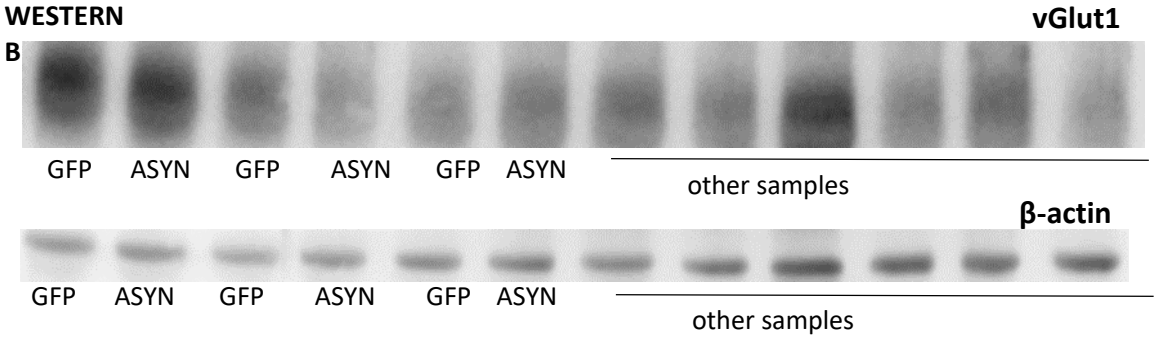
The square box rapresents bands reported in the figure

The first sample blot #1 was excluded and replicated in the second blot (arrow)

These are the same gel of figure 5A-C. Synaptophysin was blotted on the same membrane

Fig. S5F Anti-vGlut1; anti-β-actin

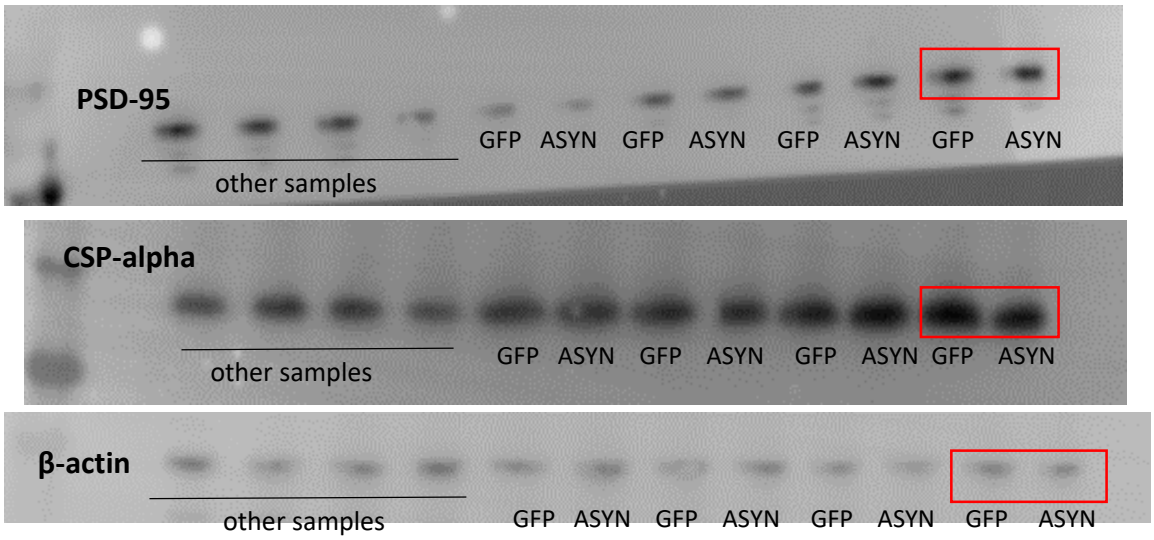




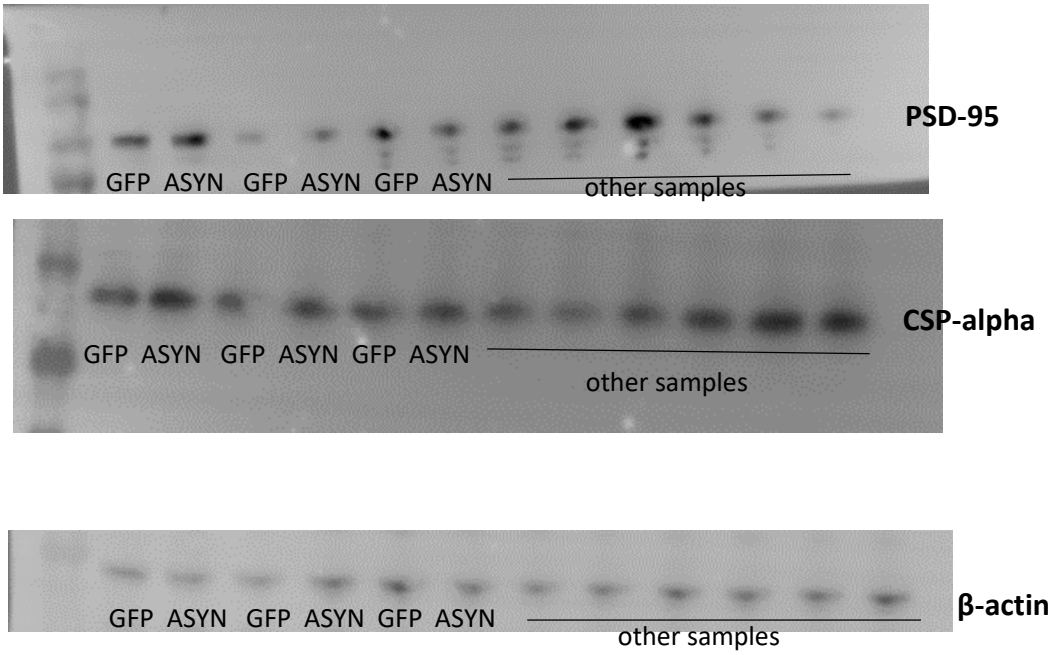
The square box rapresents bands reported in the figure

Fig. S5G-H Anti-PSD-95; anti-CSP-alpha; anti-β-actin

WESTERN BLOT #1



WESTERN BLOT #2

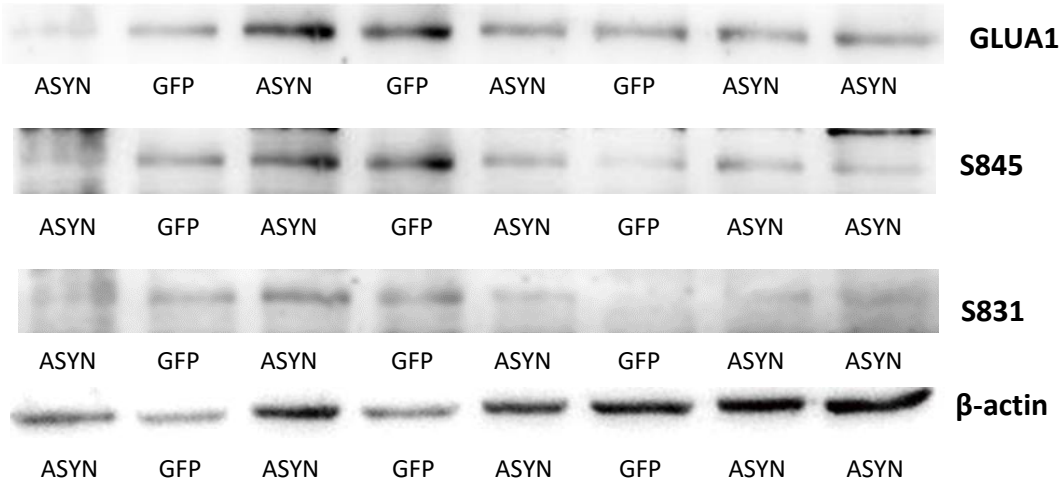


The square box rapresents bands reported in the figure

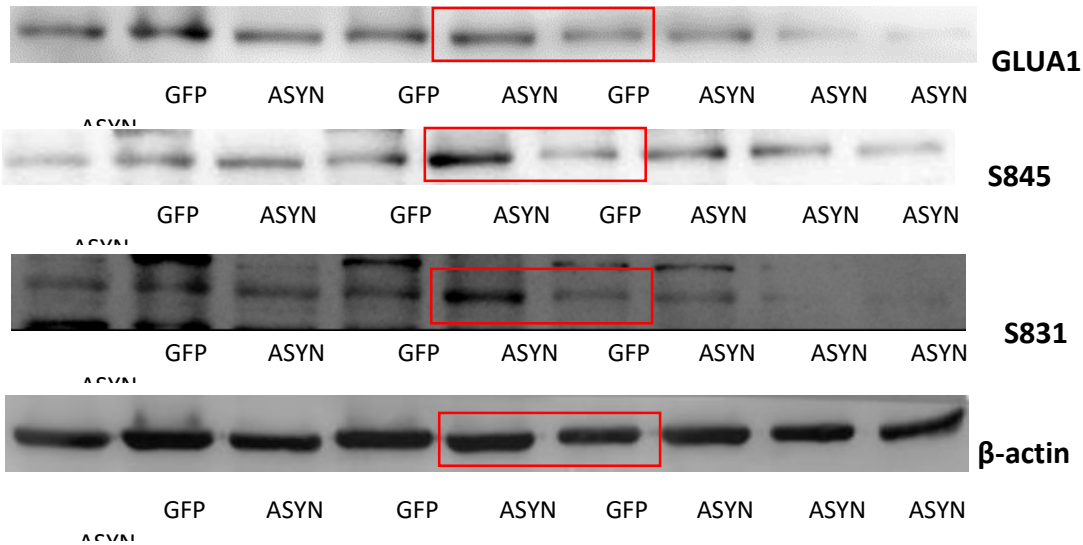
anti- GluA1; anti-s845; anti-s831; anti-β-actin

Fig S6 A,B,C

Western Blot #1



WESTERN BLOT#2



The square box rapresents bands reported in the figure