

SUPPLEMENTARY MATERIAL

Supplementary figures

Figure S1. A) Fluorescence emitted by Venus complementation 24 h post-transfection in HEK293 cells. The single transfection with VN-aSyn or aSyn-VC did not produce any fluorescence. Co-expression of VN-aSyn or VN-Tau WT with the empty -VC half led to background fluorescence, while the expression of the empty VN- half together with aSyn-VC or Tau WT-VC gave rise to some fluorescence-positive cells. **B)** Total levels of Tau and aSyn measured by Western blot. The original membrane was cut at 38 KDa to assess separately the protein levels of aSyn and Tau in the cell lysate of HEK293 cells co-transfected with different BiFC constructs. Stripping of the upper part of the membrane (38-98 KDa) was performed to evaluate the levels of β -Actin (loading control).

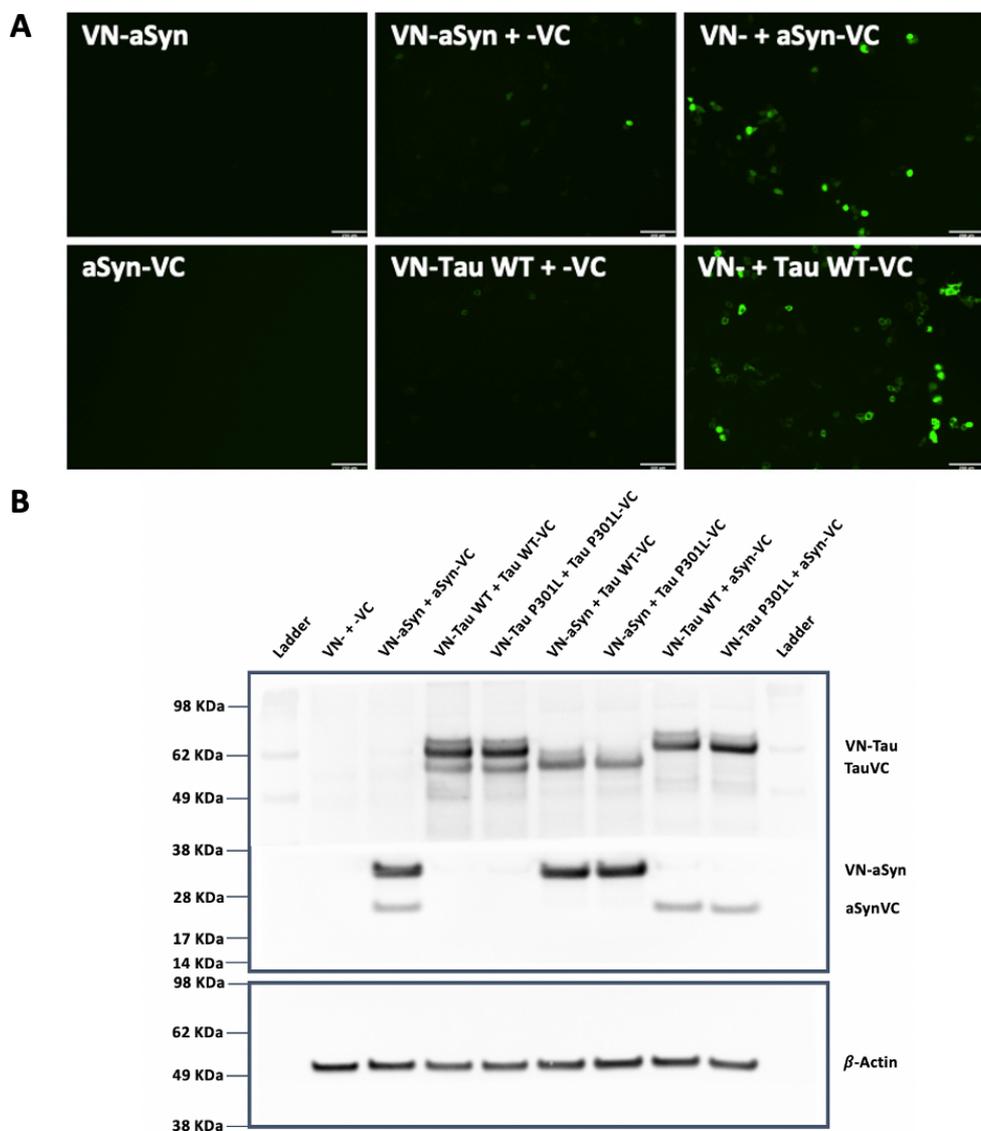


Figure S2. A) Fluorescence emitted by Venus complementation 24 h post-transfection in SH-SY5Y cells. The single transfection with VN-aSyn or aSyn-VC did not produce any fluorescence. Co-expression of VN-aSyn or VN-Tau WT with the empty -VC half led to background fluorescence, while the expression of the empty VN- half together with aSyn-VC or Tau WT-VC gave rise to fluorescence-positive cells. **B)** Time-dependent progressive interaction of BiFC constructs. Protein complementation in SH-SY5Y cells was recorded by live-cell imaging every 30 min after transfection for 24 h. For each condition, a FOV was chosen and tracked, and changes in fluorescence intensity over time in the cells within the FOV were measured. Data obtained every hour is represented. Data are expressed as mean \pm SD. n = 2-3.

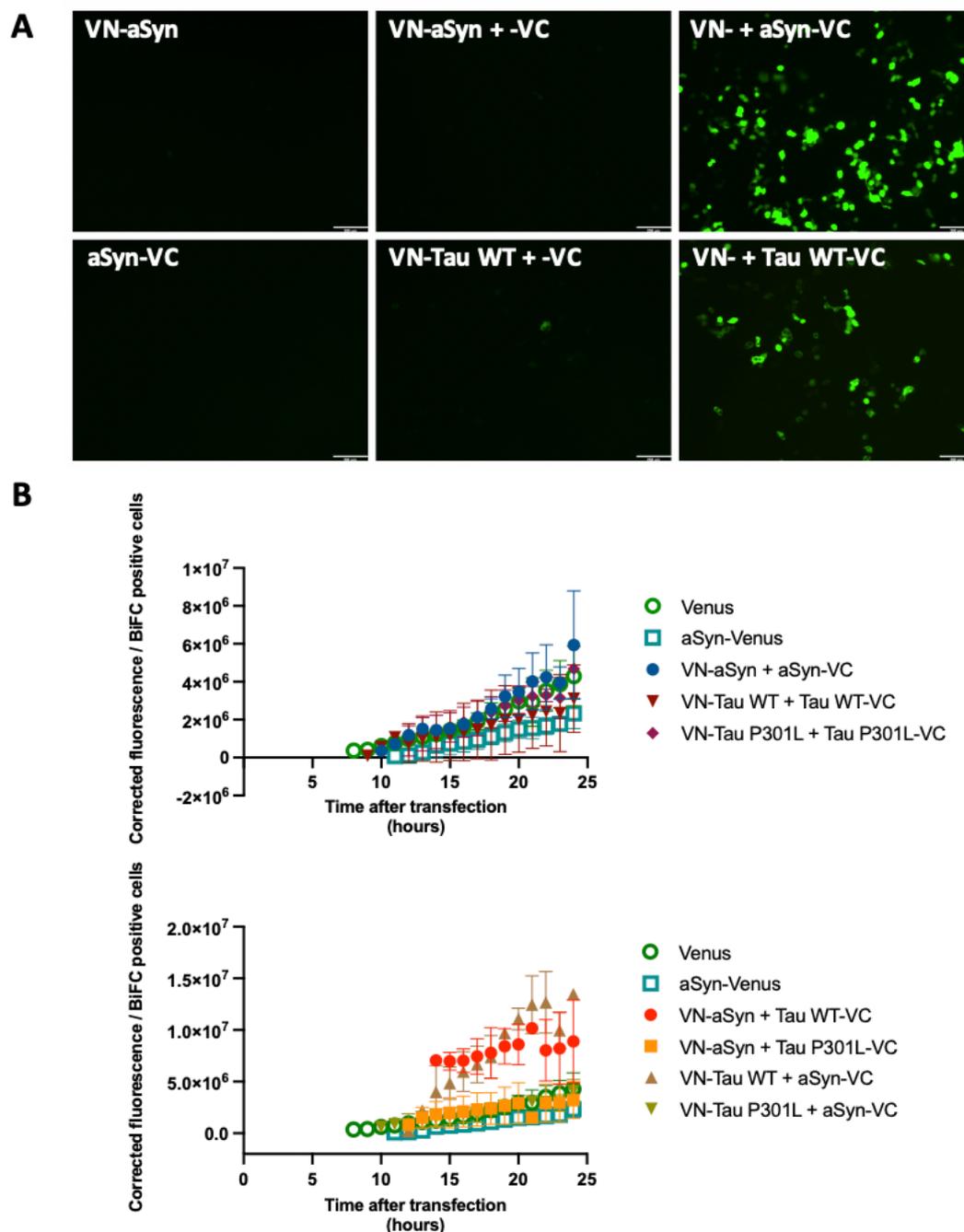
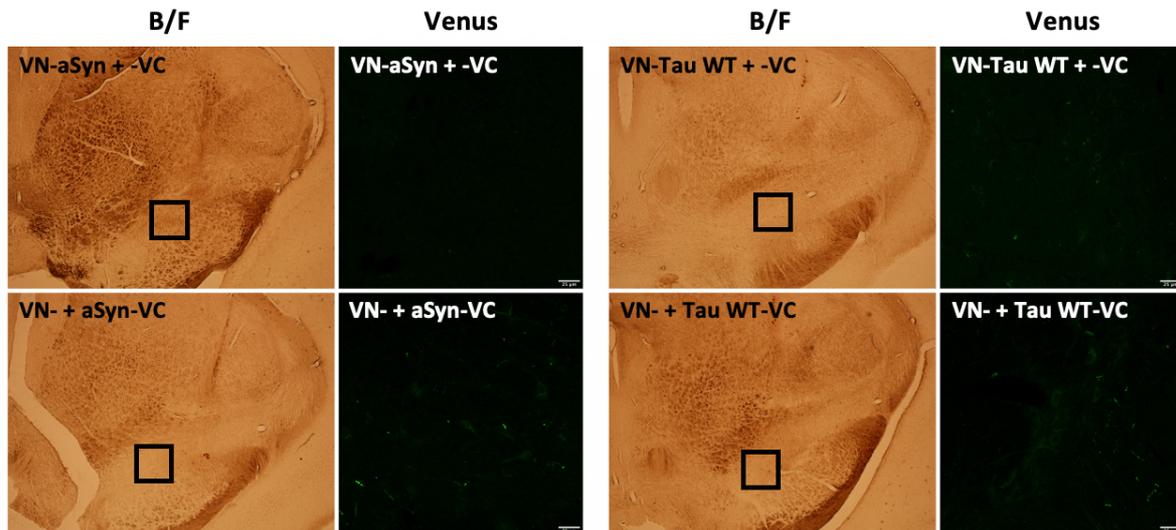


Figure S3. BiFC interaction in mouse SNpc. 10-12-weeks-old mice were injected in SNpc with AAV6 carrying different BiFC constructs. After 15 weeks, Venus fluorescence was evaluated. Co-expression of VN-aSyn or VN-Tau WT with the empty -VC half did not produce any fluorescence in the injected area, while the expression of the empty VN- half together with aSyn-VC or Tau WT-VC gave rise to some background fluorescence. Boxed area on Bright Field (B/F) images is magnified to the right. Scale bar: 25 μ m.



Supplementary tables

Table S1: List of BiFC constructs used in the study.

Construct	Abbreviation
Venus Full-Length (FL)	Venus
α -synuclein – Venus FL	aSyn-Venus
Empty VN-	VN-
Empty -VC	-VC
VN – α -synuclein	VN-aSyn
α -synuclein – VC	aSyn-VC
VN – Tau WT	VN-Tau WT
Tau WT – VC	Tau WT-VC
VN – Tau P301L	VN-Tau P301L
Tau P301L – VC	Tau P301L-VC

Table S2: List of BiFC constructs injected in mice SNpc by AAV6.

Viral vector	Abbreviation
pAAV2-Syn-haSyn-Venus Full	aSyn-Venus
pA2-Syn-empty Venus N	VN-
pA2-Syn-empty Venus C	-VC
pAAV2-Syn-VN-haSyn	VN-aSyn
pAAV2-Syn-haSyn-VC	aSyn-VC
pA2-Syn-VN-TauWT	VN-Tau WT
pA2-Syn-TauWT-VC	Tau WT-VC

Table S3: List of antibodies used in this study

Abbreviation	Antibody	Dilution	Manufacturer	Method
aSyn	Purified Mouse Anti- α -Synuclein	1:1000	610787 (BD Biosciences)	WB
Total Tau	Anti-Tau antibody	1:1000	ab64193 (ABCAM)	WB
β -Actin	Monoclonal Anti- β -Actin antibody	1:4000	A5441 (Merck)	WB
Anti-mouse HRP	Mouse IgG HRP-conjugated Antibody	1:5000	HAF007 (R&D Systems)	WB
Anti-rabbit HRP	Rabbit IgG HRP-conjugated Antibody	1:5000	HAF008 (R&D Systems)	WB
211	Mouse monoclonal antibody against total aSyn	1:1000	sc-12767 (Santa Cruz Biotechnology)	IHC
Tau-5	Mouse monoclonal antibody against total Tau	1:1000	AHB0042 (Thermo Fisher Scientific)	IHC
pS129	Rabbit monoclonal antibody against aSyn phosphorylated at the S129 epitope	1:1000	AB51253 (ABCAM)	IHC
AT8	Mouse monoclonal antibody against Tau phosphorylated at S202 and T205 epitopes	1:500	MN1020 (Thermo Fisher Scientific)	IHC
Anti-mouse HRP	Goat Anti-Mouse Ig/HRP Antibody	1:400	P044701 (Dako)	IHC
Anti-rabbit	Alexa Fluor 647-Donkey Anti-Rabbit IgG	1:400	711-605-152 (Jackson ImmunoResearch)	IHC
Anti-mouse	Cy3-Donkey Anti-Mouse IgG	1:500	715-165-150 (Jackson ImmunoResearch)	IHC