Supplementary Material

Title: **Investigating the neuroprotective effect of AAV-mediated β-synuclein overexpression in a transgenic model of synucleinopathy**

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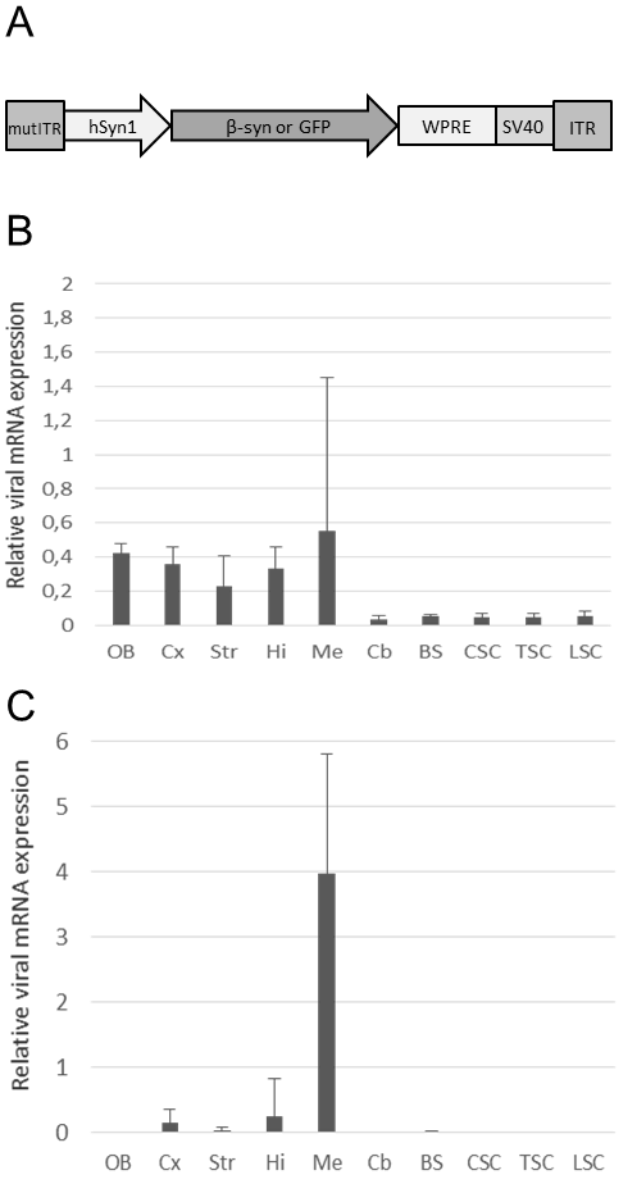
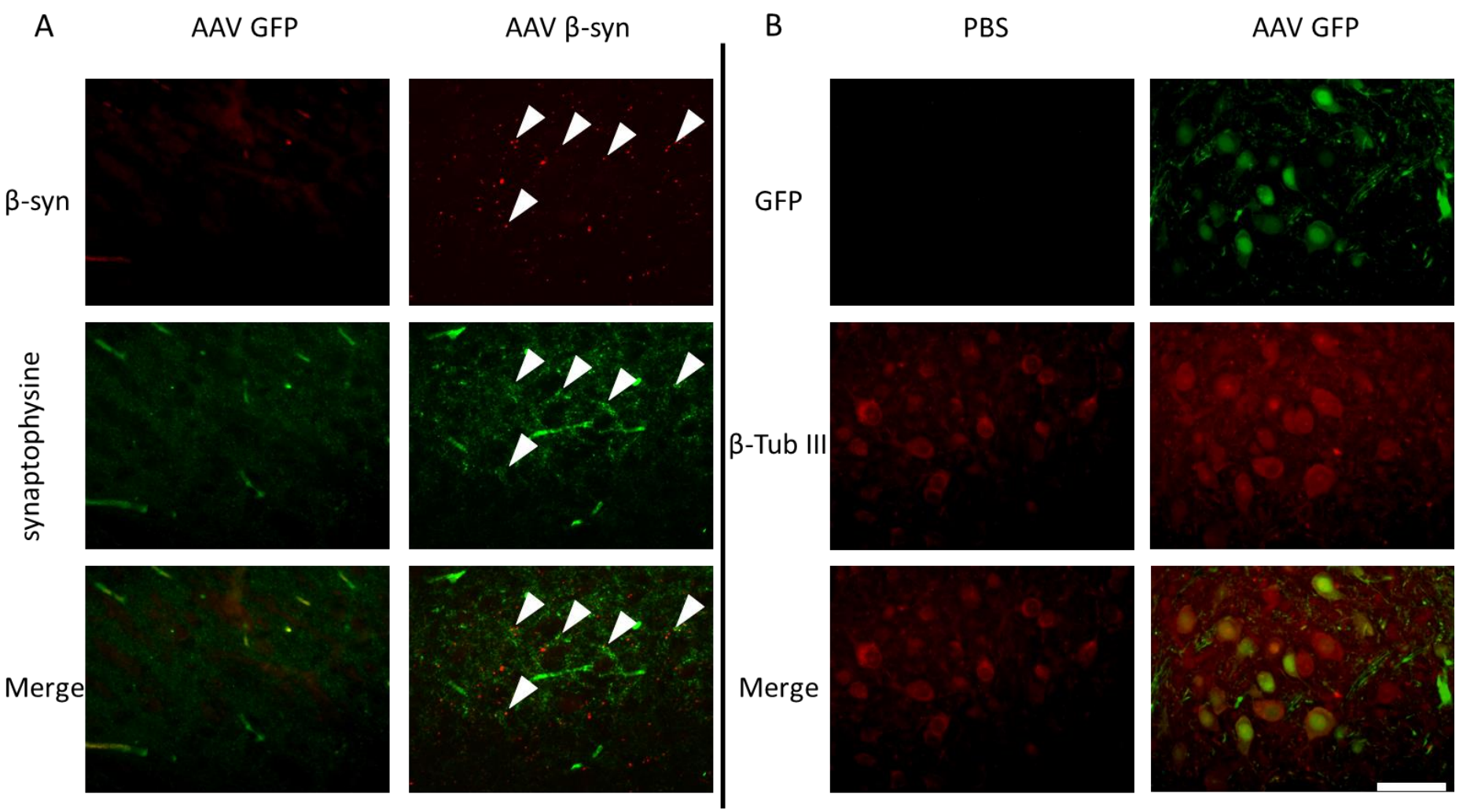
Inventory of Supplementary Material:

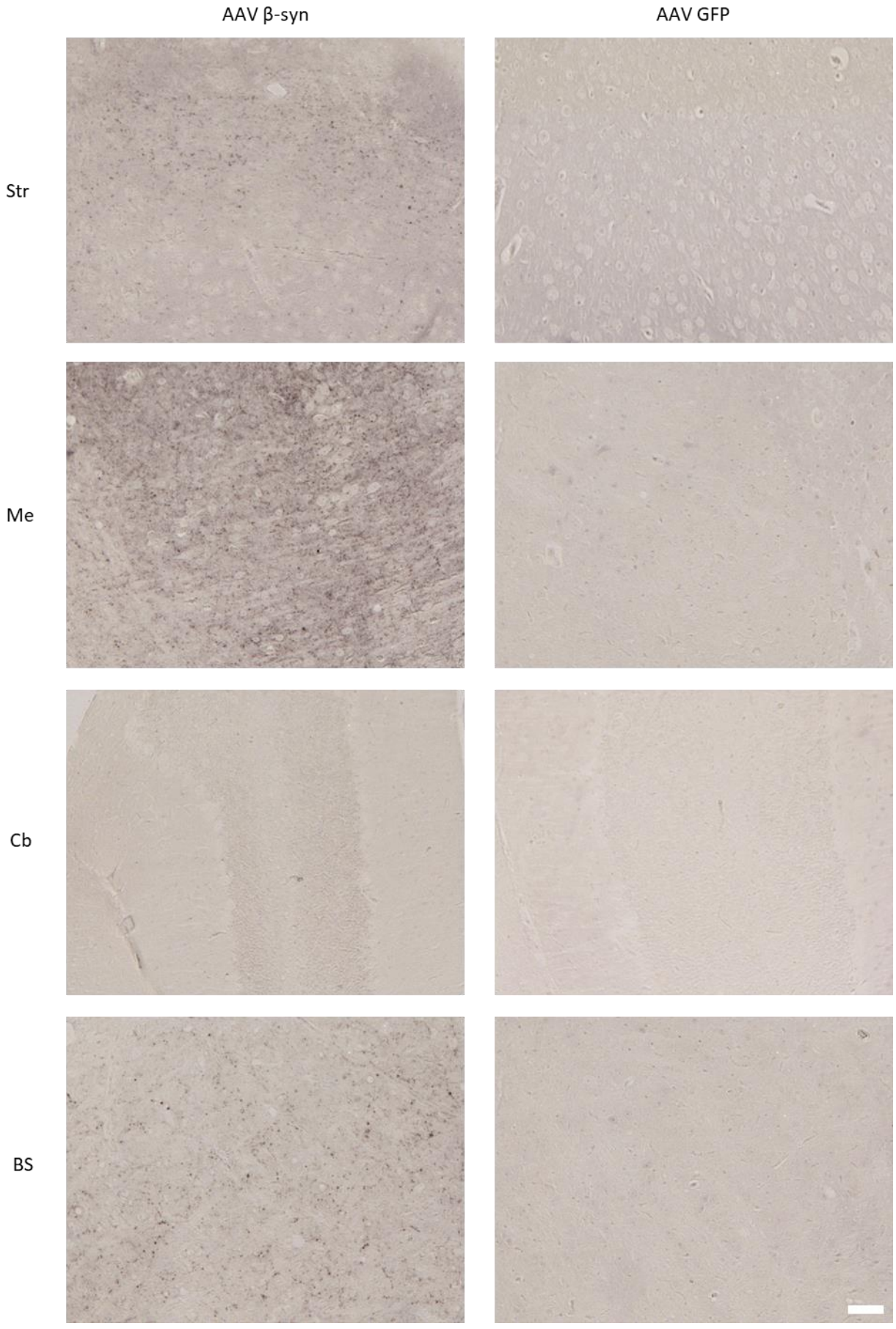


Supplemental figure 1. AAVβ-syn expression in wild-type mice after injection by intracerebroventricular route at birth by qRT-PCR. (A) Schematic representation of the genome of the serotype 9 self-complementary AAV (scAAV) vector used in this study. mutITR, mutated Inverted Terminal Repeat; hSyn1, human Synapsin1 promoter; WPRE, Woodchuck Hepatitis Virus Posttranscriptional Regulatory Element; SV40, Simian Virus 40 polyadenylation signal.

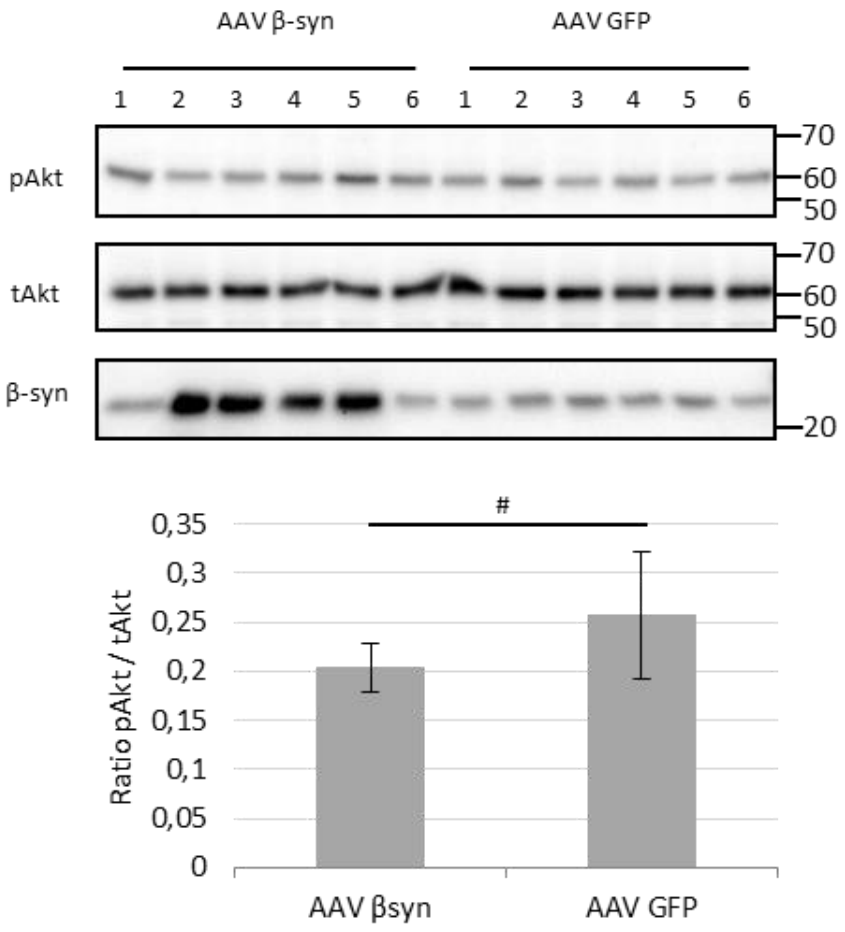
(B) Wild-type B6C3H neonates were inoculated with $9{,}38*10^{8}$ vg of AAVβ-syn per lateral ventricle and euthanized one month later. Levels of human β-syn mRNAs in dissected brains and spinal cord were quantified by qRT-PCR, relative to GAPDH expression (n=6). (C) Two months old wild-type B6C3H mice were inoculated with $3{,}75*10^{8}$ vg of AAVβ-syn in the ventral tegmental area (VTA) and euthanized one month later. Levels of human β-syn mRNAs in dissected brains and spinal cords were quantified by qRT-PCR, relative to GAPDH expression (n=6). OB: olfactory bulbs, Cx: cerebral cortex, Str: striatum, Hi: hippocampus, Mes: mesencephalon, BS: brain stem, CSC: cervical spinal cord, TSC: thoracic spinal cord, LSC: lumbar spinal cord. Data are shown as means ± sd.


A
AAV GFP
AAV β-syn
β-syn
synaptophysine
Merge
B
PBS
AAV GFP
GFP
β-Tub III
Merge

Supplemental figure 2. Characterization of the AAVβ-syn or AAVGFP expression after their inoculation in the ventral tegmental area. (A) Co-staining of total β-syn with presynaptic protein synaptophysin in the mesencephalon of sick M83 mice inoculated with AAVβ-syn or AAVGFP in the ventral tegmental area and challenged with MSA/M83 inoculum (mice from the experiment Figure 4E-G). (B) Co-staining of GFP with specific neuronal marker β-tubulin type 3 in the mesencephalon of sick M83 mice inoculated with AAVGFP or PBS and challenged with MSA/M83 inoculum. Scale bar 50µm.


AAV β-syn
AAV GFP
Str
Me
Cb
BS

Supplemental figure 3. Proteinase K-resistant β-syn staining in brain regions of sick M83 mice inoculated with AAV β-syn. Total β-syn detection by immunohistochemistry after PK digestion in the striatum (Str), mesencephalon (Me), cerebellum (Cb) and brain stem (BS) of sick M83 mice inoculated with $3,75*10^8$ vg of AAVβ-syn or $1,09*10^8$ vg of AAVGFP and challenged with MSA/M83 inoculum (mice from the experiment Figure 4E-G). A Proteinase K-resistant punctate pattern was detected in the Str, Me and BS, but not in the Cb, in which only traces of viral mRNA were detected by qRT-PCR. Scale bar 100µm.



Supplemental figure 4. β-syn overexpression in the mesencephalon of M83 mice did not activate Akt signaling. Two months old M83 mice were injected with $3{,}75*10^8$ vg of AAVβ-syn or $1{,}09*10^8$ vg of AAVGFP vectors in the ventral tegmental area (VTA) and challenged one month later by the injection of M83/M83 inoculum in the striatum to accelerate the disease. In this experiment, all mice were euthanized 3 months after the challenge for biochemical analysis (same mice as Figure 5A, B). (A) Detection by Western blot of the Akt protein phosphorylated at the serine 473 (pAkt) and total Akt protein (tAkt) in the mesencephalon. (B) Semi-quantification of the Akt signaling activation, by calculating the ratios pAkt/tAkt. Mice 1 and 6 from the AAVβ-syn group were excluded from the analysis since no β-syn overexpression was detected in these mice. $p>0{,}05$ according to Wilcoxon test.