An AAV2-A53T Mouse Model Mimics Key Pathologies of Parkinson`s Disease: α-Synuclein Aggregation, Dopaminergic Loss, and Neuroinflammation

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Background

Parkinson's disease (PD) is characterized by α-synuclein (α-syn) phosphorylation and aggregation, dopaminergic loss, and neuroinflammatory responses. However, most preclinical models either lack one or even more of these critical pathological hallmarks or exhibit them only at advanced age. The A53T point mutation in α-syn is a well-documented genetic alteration associated with familial PD, known for its role in promoting oligomerization and aggregation of the protein. Thus, we generated an AAV2-based mouse model recapitulating core features of PD that can be used to investigate A53T-mutated α-syn-related pathologies, downstream effects, and ultimately become a valuable tool for the development of new therapies.

Material and Methods

The substantia nigra (SN) of 13-week-old C57BL/6J mice was injected unilaterally with adeno-associated virus 1/2 (AAV1/2) containing the human A53T mutated α-syn (A53T). The contralateral SN was injected with an AAV-empty control vector. After 9 weeks, coronal tissue sections of the frozen brain were generated and sections displaying the SN and caudate putamen (CPu) were immunofluorescently labeled with antibodies against human α-syn, ionized calcium-binding adaptor molecule 1 (Iba1), tyrosine hydroxylase (TH), dopamine transporter (DAT), α-syn phosphorylated at serine 129 (pSer129 α-syn), cluster of differentiation (CD) 45, CD3 and CD8. Quantification of target proteins was performed macro-based and thus rater-independent. Distribution of AAV-hA53T was analyzed by RNAscope^{TM} *in situ* hybridization technology.

Results

AAV-A53T Increases α-syn Levels

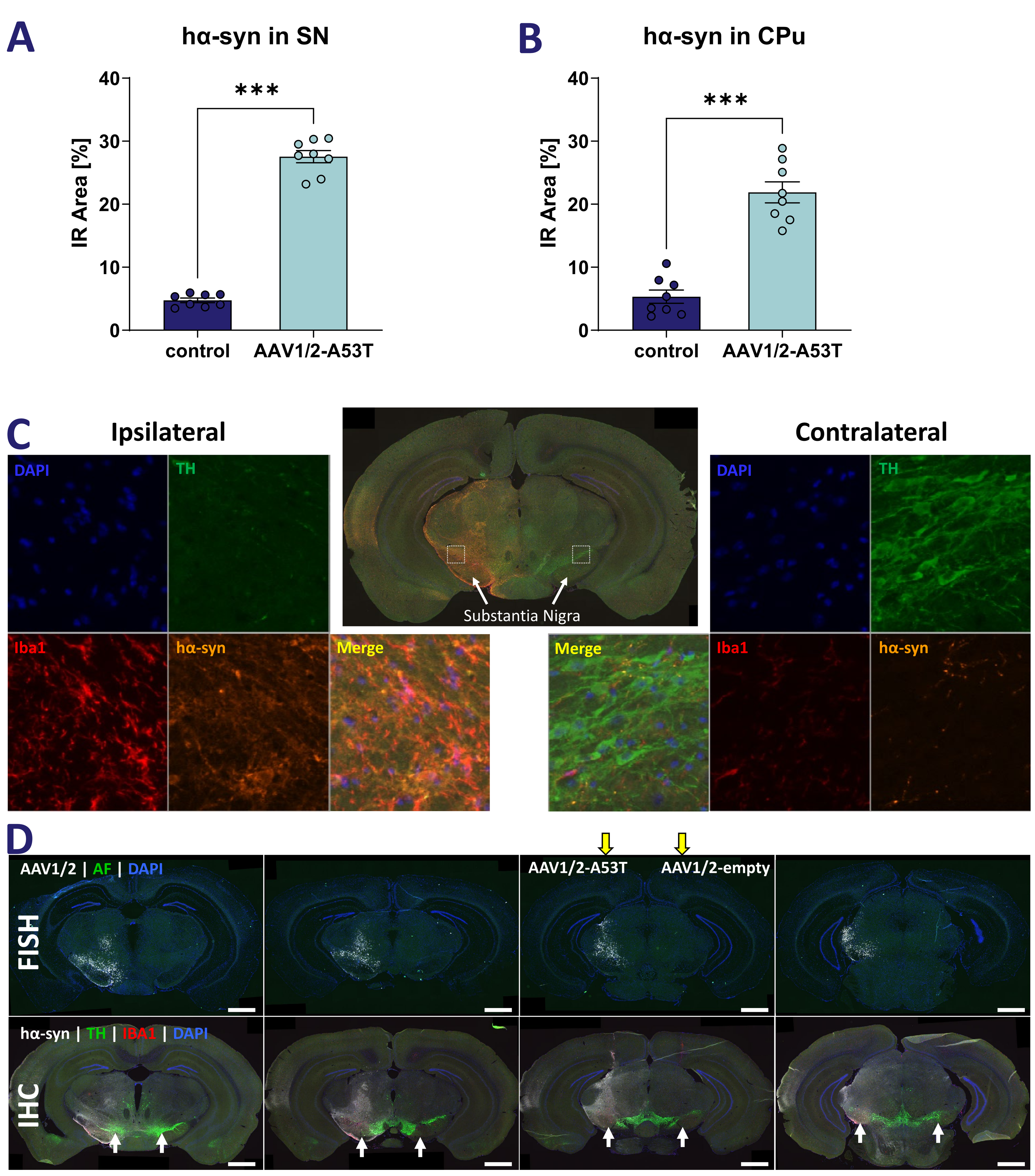

Figure 1: Levels of hα-syn in SN and CPu after injection of AAV1/2-A53T or AAV1/2-empty control vector. Immunoreactive (IR) area of hα-syn in the SN (**A**) and CPu (**B**) evaluated by quantitative immunofluorescence. Mean ± SEM; n = 8 / group; paired t-test; ***p <0.001. Representative images of hα-syn, TH, and Iba1 immunofluorescent labeling in the ipsi- and contralateral SN (**C**). Detection of the WRPE sequence of AAV1/2 construct by fluorescent *in situ* hybridization (FISH, **D**). Representative images of the injection site show FISH-labeling in the SN (AAV2-WPRE, white channel, top row) compared to immunofluorescence labeling (IHC) of hα-syn (white channel, bottom row).

AAV-A53T Increases pSer129 α-syn Levels

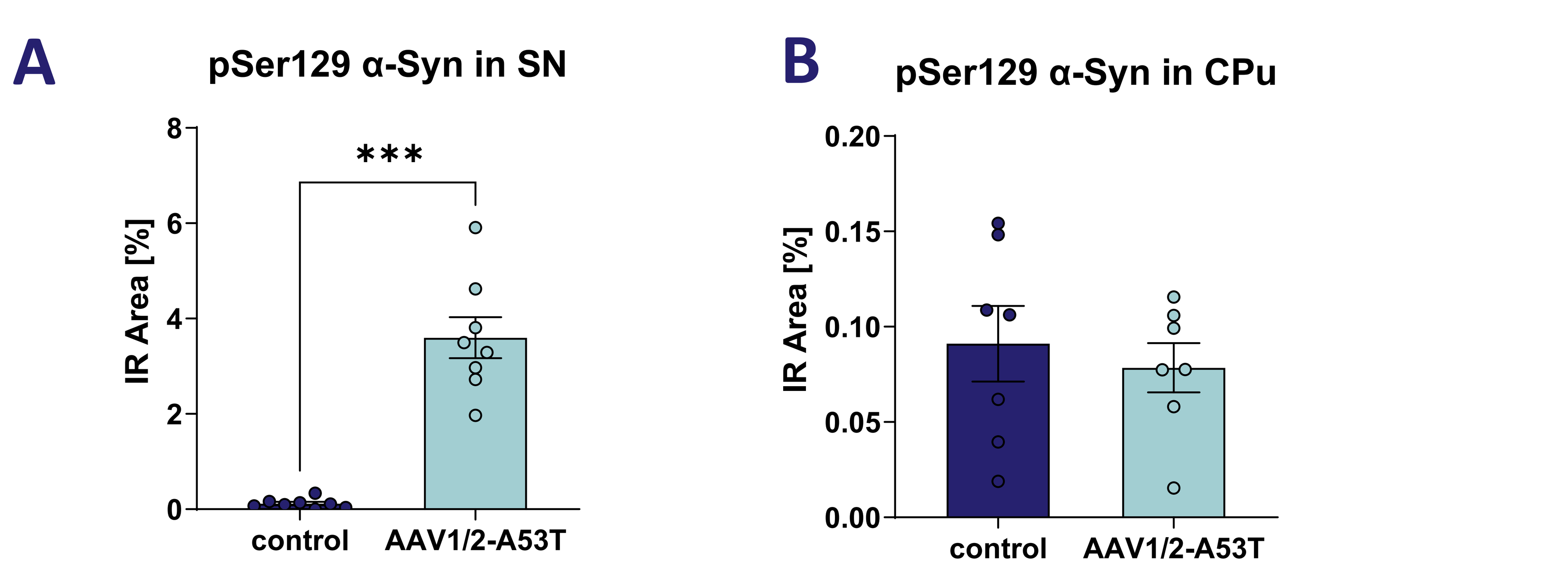

Figure 2: Levels of pSer129 α-syn in SN and CPu after injection of AAV1/2-A53T or AAV1/2-empty control vector. pSer129 α-syn IR area in SN (**A**) and CPu (**B**) evaluated by quantitative immunofluorescence. Mean ± SEM; n = 8 / group; paired t-test; ***p <0.001.

Results

AAV-A53T Reduces TH and DAT Levels

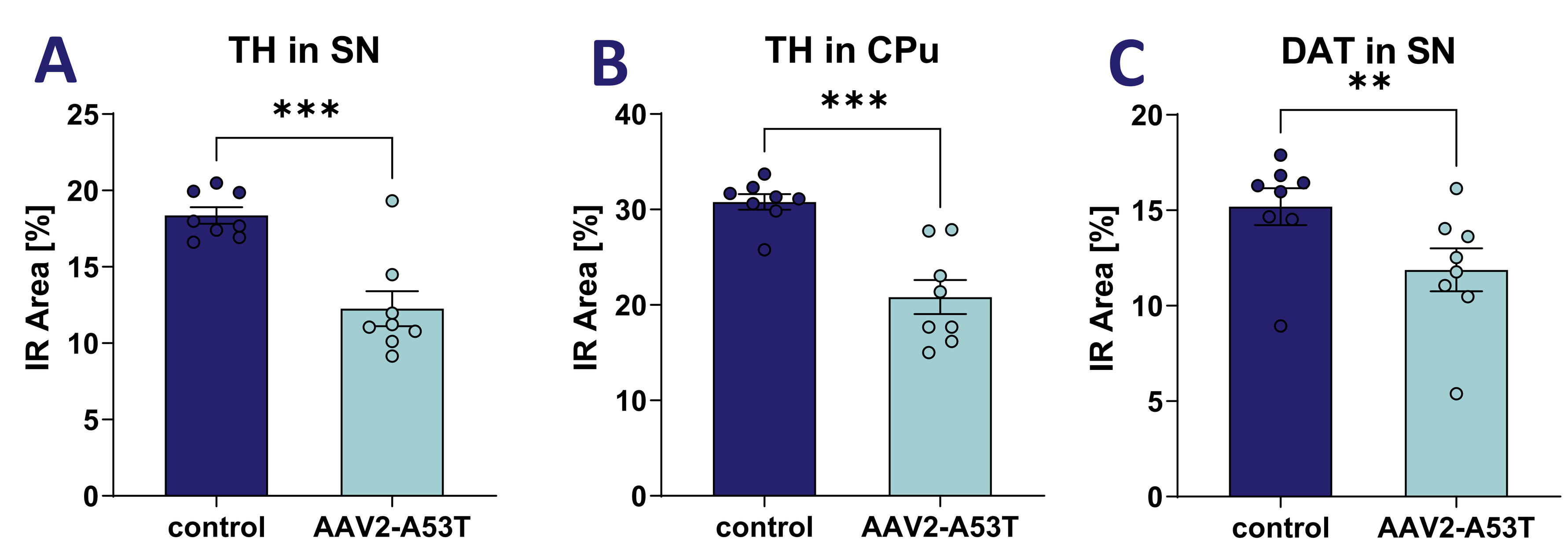

Figure 3: Levels of tyrosine hydroxylase (TH) and dopamine transporter (DAT) in SN and CPu after injection of AAV2-A53T or AAV-empty control vector. Immunoreactive (IR) area of TH in SN (**A**) and CPu (**B**) as well as DAT in SN (**C**) evaluated by quantitative immunofluorescence. Mean ± SEM; n = 8 / group; paired t-test; **p <0.01, ***p <0.001.

AAV-A53T Increases Immune Cell Infiltration

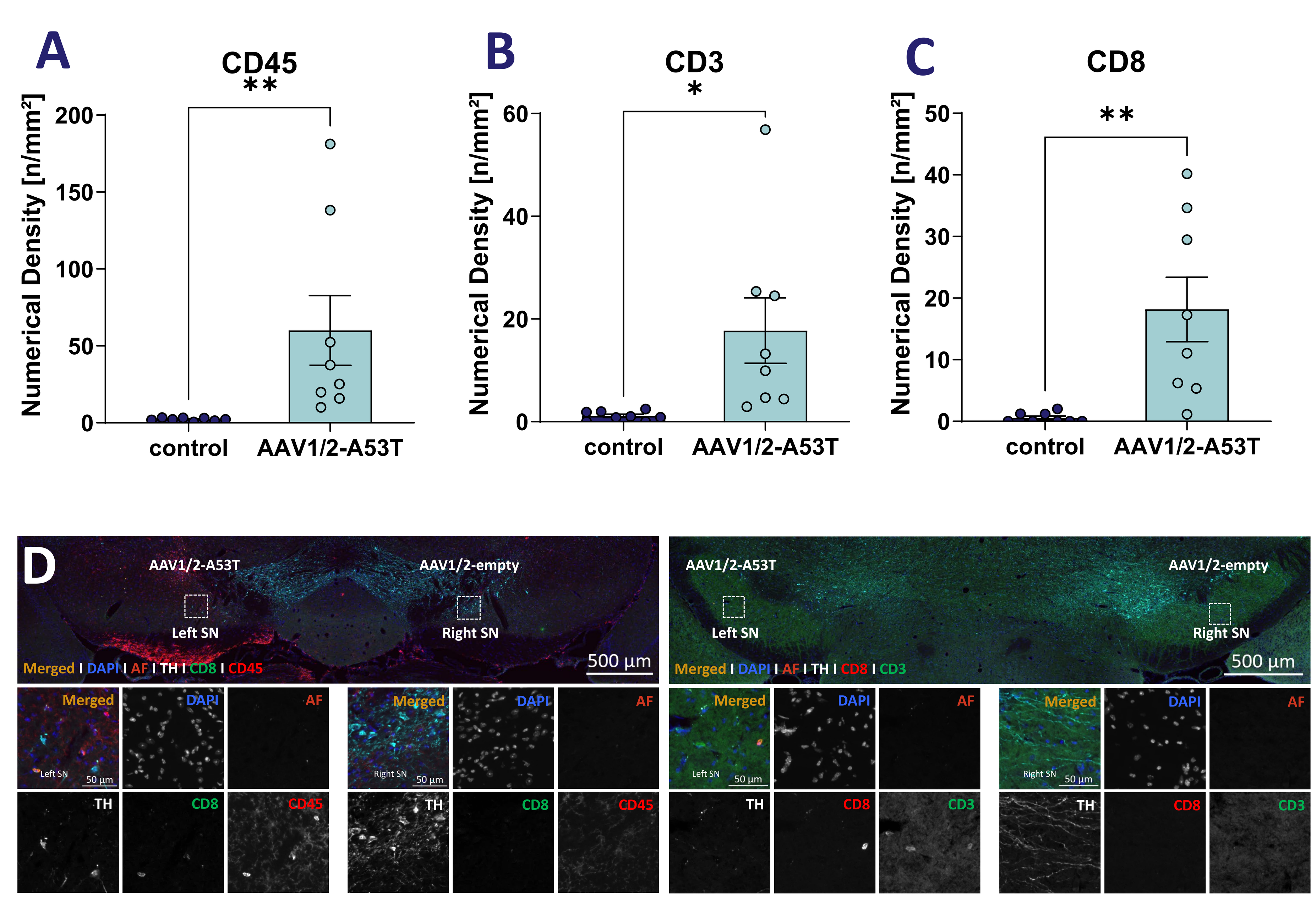

Figure 4: Quantification of CD45, CD3, and CD8 in the SN after injection of AAV1/2-A53T or AAV-empty control vector. Density of CD45-positive cells, indicating leukocytes (**A**), CD3-positive cells, indicating infiltrating T-cells (**B**), and CD8, indicating specifically cytotoxic T-cells (**C**). **D:** Representative images of the SN injected with control vector or AAV1/2-A53T-α-syn and labeled with antibodies against TH, CD8, and CD45 (left) or TH, CD8, and CD3. Both labeling were counterstained for nuclei with DAPI. SN: substantia nigra. Mean ± SEM; n = 8 / group; Wilcoxon matched-pairs signed rank test (**A, C**); paired t-test (**B**); *p <0.05, **p <0.01.

Summary and Conclusion

In conclusion, the AAV2-A53T mouse model replicates key features of Parkinson's disease, such as α-syn aggregation, dopaminergic loss, and immune cell infiltration in the CPu and SN and thus facilitates the analysis of A53T α-syn-linked pathologies and associated signaling pathways. The AAV2-A53T mouse model is therefore a powerful tool to evaluate the molecular basis of synucleinopathies, potentially leading to the development of new therapeutic strategies.

For more information about the model please visit: www.scantox.com
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