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Supplemental Information

Title: Distinct region-specific alpha-synuclein oligomers in A53T transgenic mice: implications for neurodegeneration

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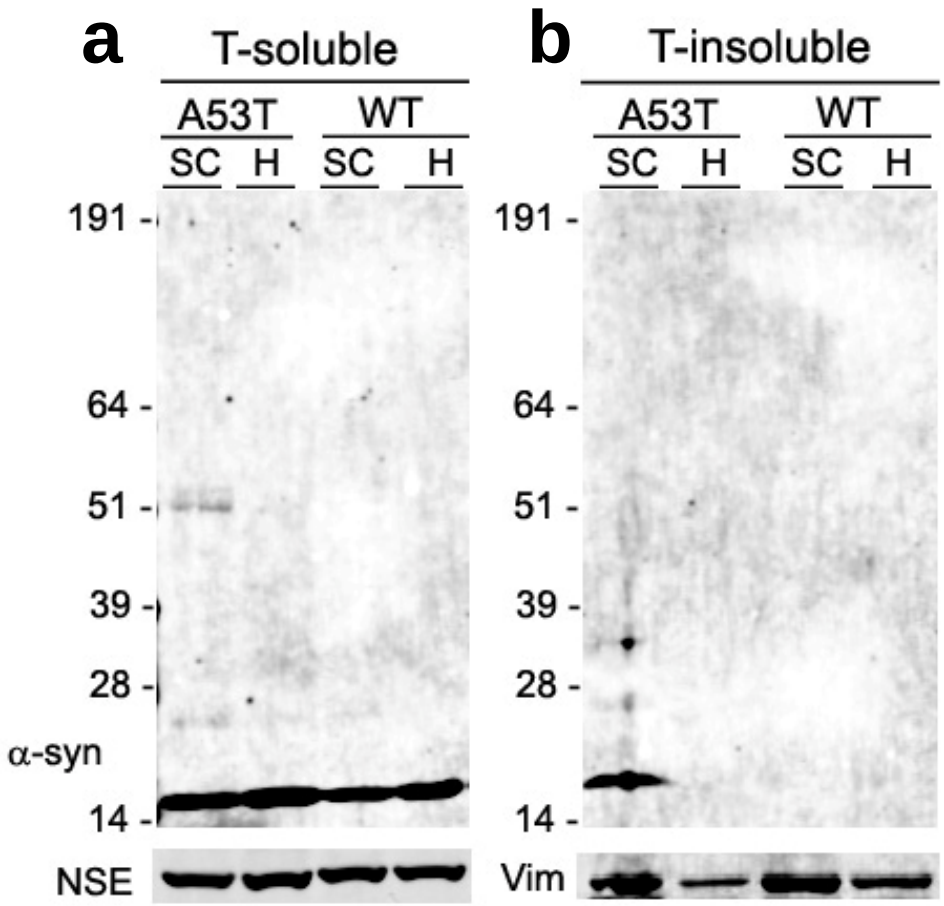
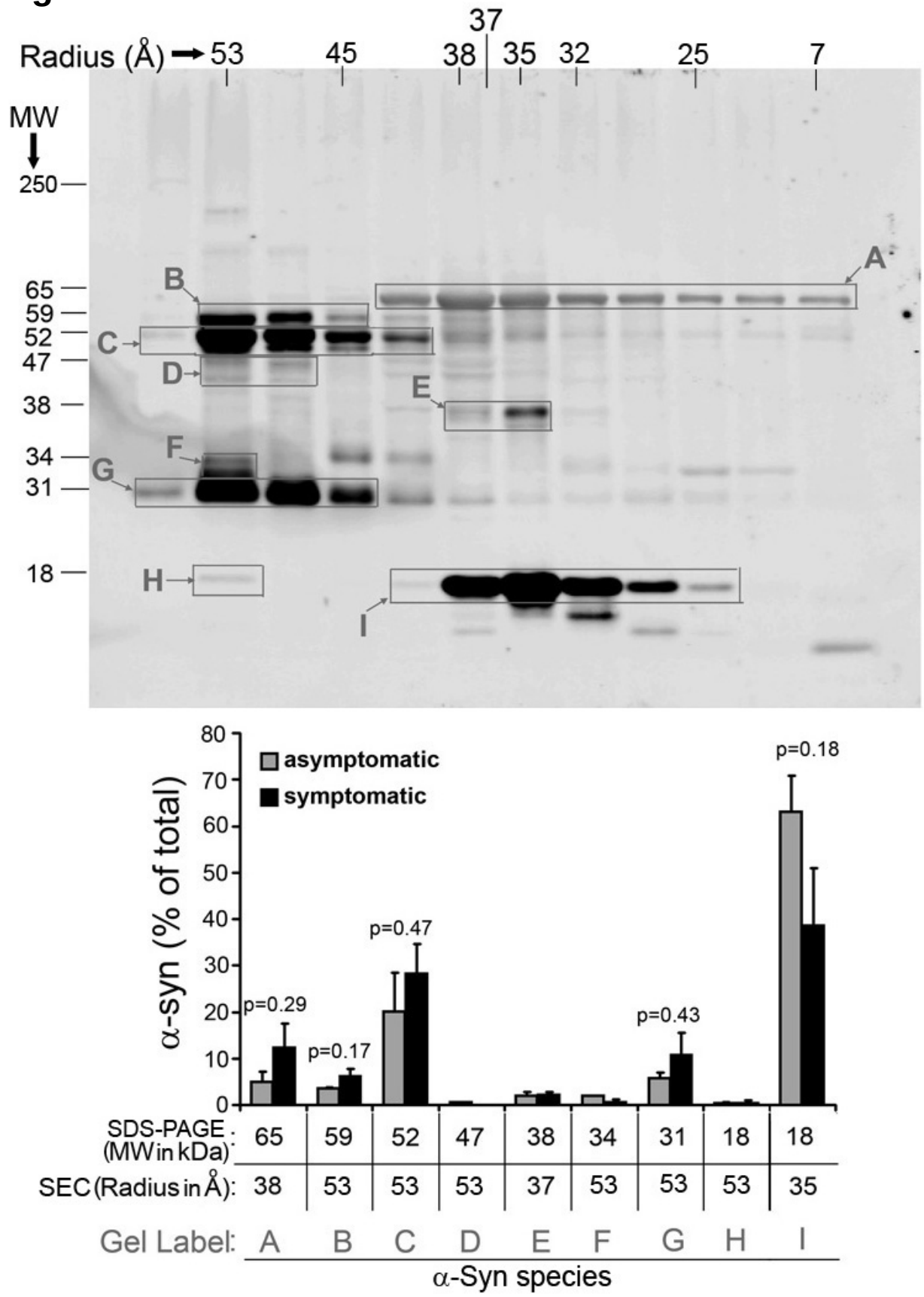
Figure S1



Supplemental Figure 1. Regional specific accumulation of T-soluble oligomers and Triton-insoluble α-syn in symptomatic A53T α-syn transgenic mice.

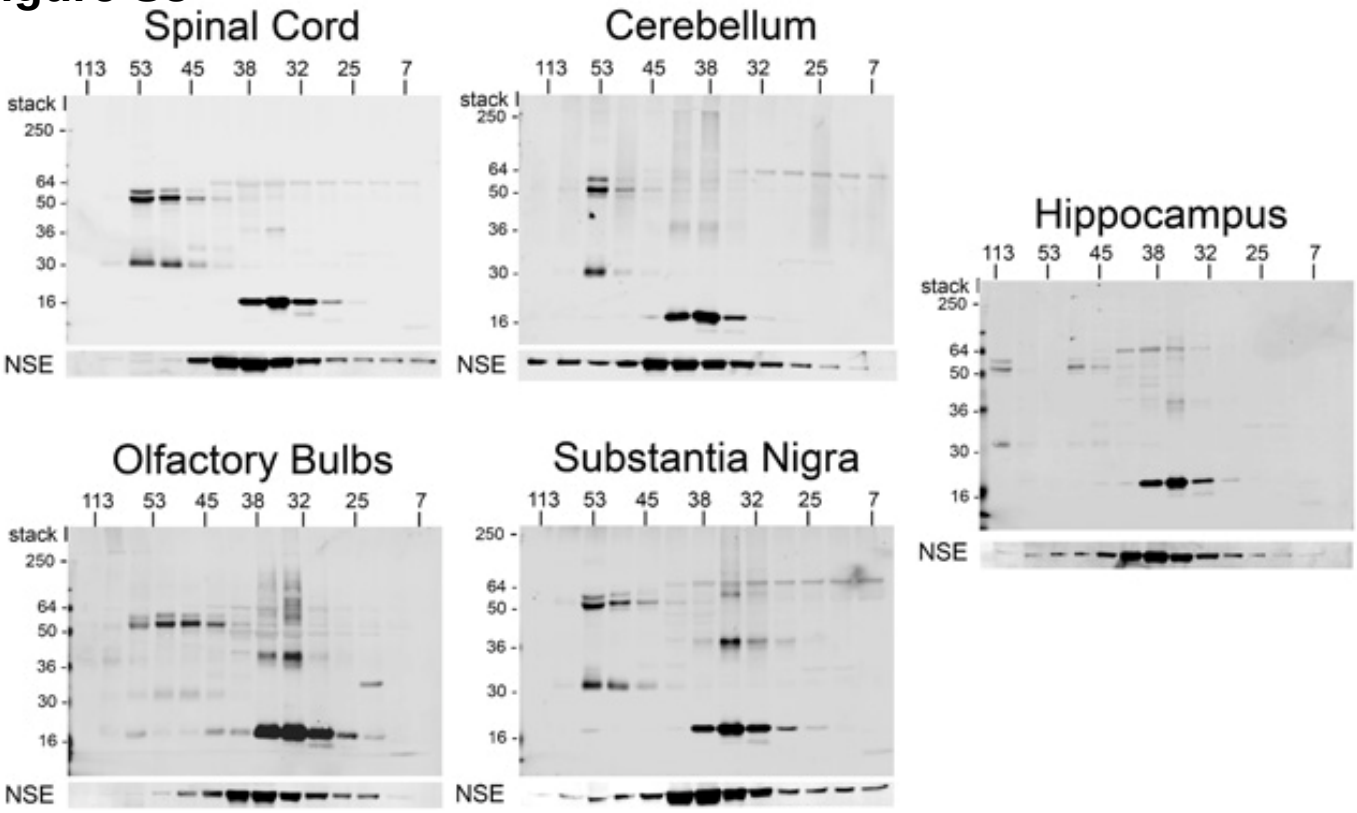
a) SC and Hipp from symptomatic A53T α-syn transgenic mice and age-matched transgenic mice expressing human wild-type α-syn were subjected to extraction with buffer containing 1% Triton X-100 (T-soluble) followed by western blot analysis using the human specific monoclonal anti-α-syn, LB509. A53T and wt α-syn extracts were loaded on the same gel to show a direct comparison of the α-syn levels in each sample. Neural specific enolase (NSE) was used as a loading control. **b)** the Triton-insoluble pellets were extracted with buffer containing 2% SDS (T-insoluble). Vimentin (Vim) was used as a loading control. The markers on the left indicate the mobility of standards with known molecular masses on SDS-PAGE in kDa.

Figure S2


Radius (Å) → 53 45 38 37 35 32 25 7
MW
250
65
59
52
47
38
34
31
18
A
B
C
D
E
F
G
H
I
α-syn (% of total)
asymptomatic
symptomatic
p=0.29
p=0.17
p=0.47
p=0.43
p=0.18
SDS-PAGE (MW in kDa): 65 59 52 47 38 34 31 18 18
SEC (Radius in Å): 38 53 53 53 37 53 53 53 35
Gel Label: A B C D E F G H I
α-Syn species

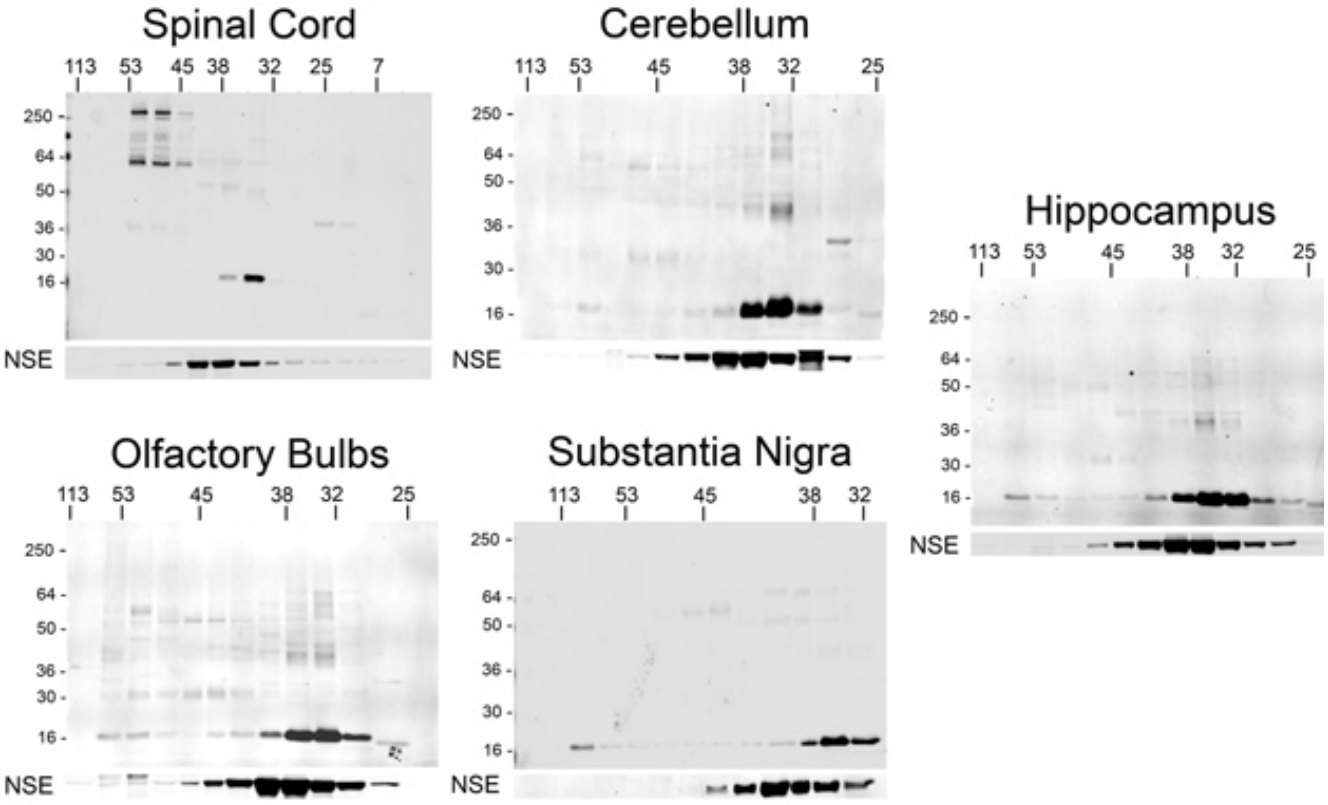
Supplemental Figure 2. Quantification of distinct soluble oligomeric forms of α-syn by native SEC followed by SDS-PAGE / Syn211 western blot analysis. Triton-soluble extract from the SC of a symptomatic A53T α-syn transgenic mouse is shown in the western blot as a representative example. Band labels B, C, D, F, G, H make up the 53Å-size α-syn oligomer, A and E comprise the minor oligomeric species and label I represents the monomer of α-syn. The boxed areas on the blot comprise the regions used for oligomer quantification. Values are presented on the graph (A-I). Any bands that are visible on this particular membrane that were not observed on a consistent basis in replicates or other tissues were omitted from analysis. Values of 'total soluble oligomers' were calculated from species A-H. The horizontal marker indicates the apparent molecular radius in angstroms (Å), while the vertical marker indicates the molecular weight in kDa as determined by SDS-PAGE. Values in the graph are the mean ± SEM (n=3-4). P values were determined by a student's t-test.

Figure S3



Supplemental Figure 3. SEC analysis of asymptomatic A53T α-syn transgenic mice. Triton-soluble extracts of the indicated regions of the nervous system from 4-5 month-old human A53T α-syn transgenic mice were analyzed as described in legend of Figure 2, using human specific anti-α-syn antibody, Syn211, and anti-NSE as a loading control. The horizontal marker indicates the molecular size obtained by native SEC in Å, while the vertical marker indicates the mobility of known protein standards with known molecular masses on SDS-PAGE in kDa.

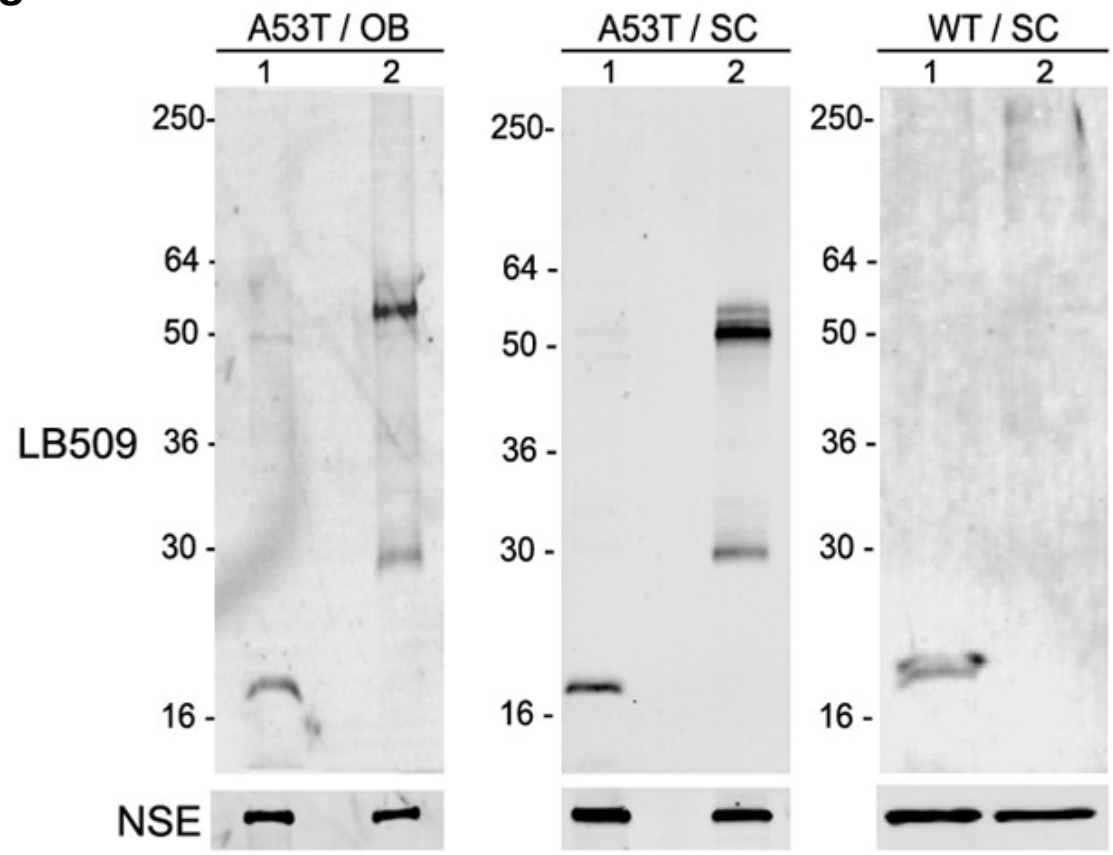
Figure S4



Supplemental Figure 4. SEC analysis of mice expressing human WT α-syn. Triton-soluble extracts of the indicated regions of the nervous system from 11-13 month-old human WT α-syn transgenic mice were analyzed as described in legend of Figure 2, using human specific anti-α-syn antibody, Syn211, and anti-NSE as a loading control. The horizontal marker indicates the molecular size obtained by native

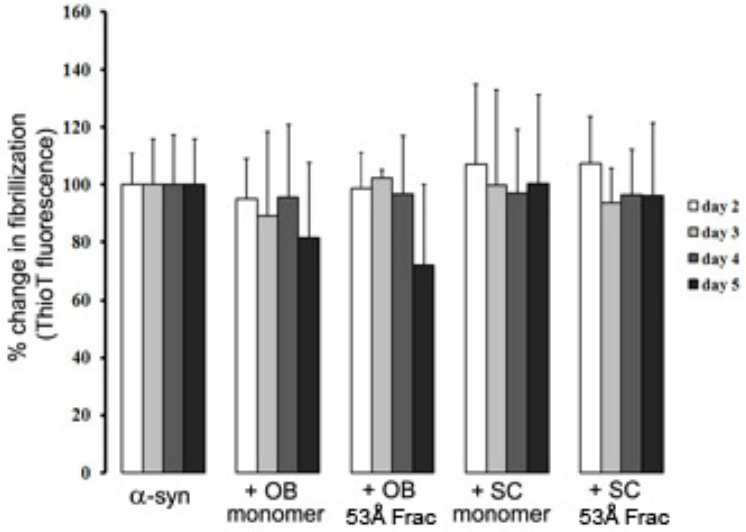
SEC in Å, while the vertical marker indicates the mobility of known protein standards with known molecular masses on SDS-PAGE in kDa.

Figure S5



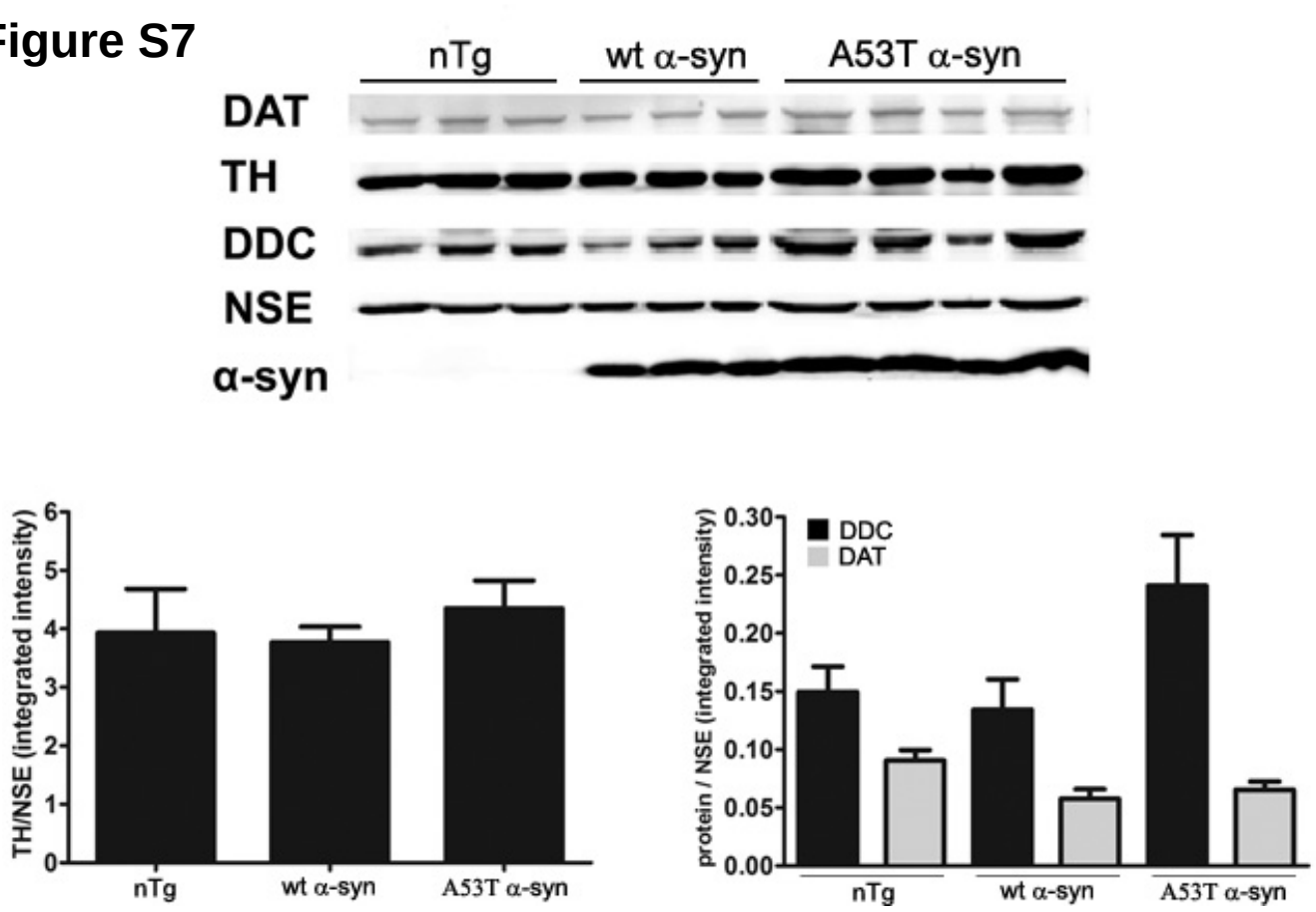
Supplemental Figure 5. Detection of α-syn oligomers from OB and SC of A53T α-syn transgenic mice using monoclonal antibody LB509. Triton-soluble extracts from the OB of A53T α-syn transgenic mice in **a)** SC of A53T α-syn trainsgenic mice in **b)** and SC of WT α-syn transgenic mice in **c)** were separated by SEC and the fractions corresponding to the 53Å oligomers and the monomer of the protein are shown in lanes 2 and 1 of each blot respectively. NSE was used as used as control to assure equal loading across the regions.

Figure S6



Supplemental Figure 6. Properties of Triton-soluble fractions derived from WT α-syn transgenic mice in seeding the fibrillization of recombinant α-syn protein. The % change in RFU as recorded by ThioT fluorescence compared to incubating the protein alone, without addition of material, is reported. None of the SEC fractions derived from WT α-syn transgenic mice have an effect on the kinetics of recombinant α-syn fibril formation. (n=4-5, mean values ± SEM, $p>0.05$ student's t-test)

Figure S7



Supplemental Figure 7. Assessment of dopaminergic markers in the striatum of symptomatic A53T α-syn transgenic mice. Striatal lysates from A53T, wt α-syn, and age-matched nTg mice were analyzed by western blot analysis to determine the protein levels of dopamine transporter (DAT), tyrosine hydroxylase (TH), DOPA decarboxylase (DDC), and α-syn. NSE was used as loading control. Protein levels were analyzed by densitometry and normalized to NSE. Values represent the mean ± SEM (n=3-4).