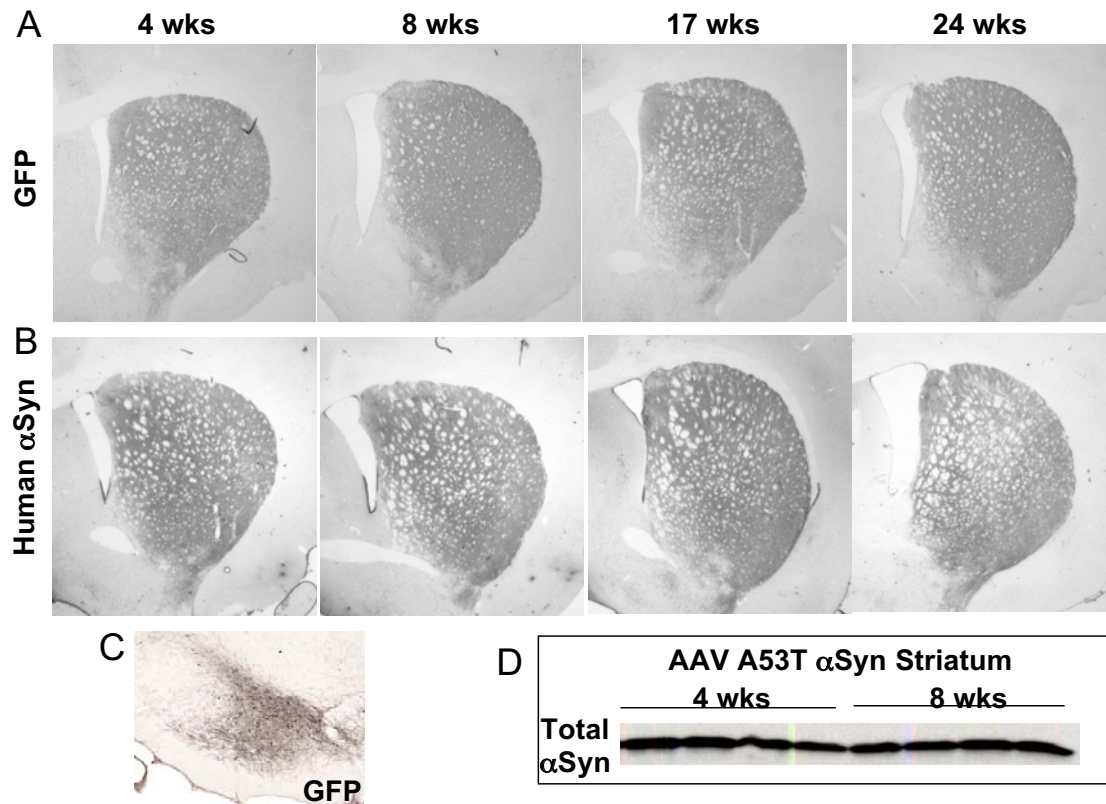


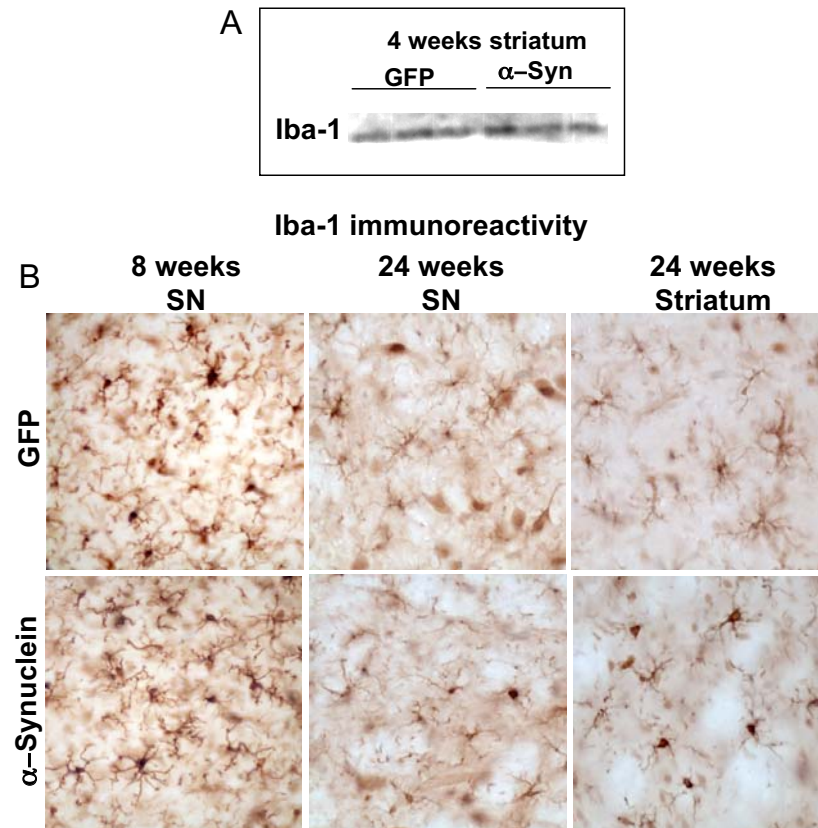
Supp. Figure 1. A: A53T α -synuclein overexpression did not cause loss of TH, DAT and VMAT2 in the striatum at 8 weeks demonstrated by Western blot (D; AAV GFP, n=4; AAV A53T α -synuclein, n=4). Nigral overexpression of AAV A53T α -synuclein under synapsin promoter causes ~32% loss of TH positive neurons at 17 weeks (B) with human α -synuclein positive aggregates in the SN (arrows; C) and signs of axonopathy (D). NeuN was stained in the GFP or A53T α -synuclein overexpressing SN (E). Stereological counting revealed that A53T α -synuclein overexpression significantly reduced number of NeuN-positive cells in the SN at 24 weeks (F), which corroborates the reduction of TH-positive cells at this stage. AAV under synapsin promoter also transduced other neurons in the SN, such as GABAergic neurons in the substantia nigra pars reticulata (G, *; α -synuclein /GAD67 colocalized neurons), which project their terminals to the thalamus. GFP or human α -synuclein-positive fibers were present in the thalamus. Unlike DA terminals in the striatum, α -synuclein-positive terminals in the thalamus did not show obvious pathology (H). Similar findings were also reported in other AAV mediated α -synucleinopathy model (Kirik et al, 2002).

Chung_Supp. Figure 1



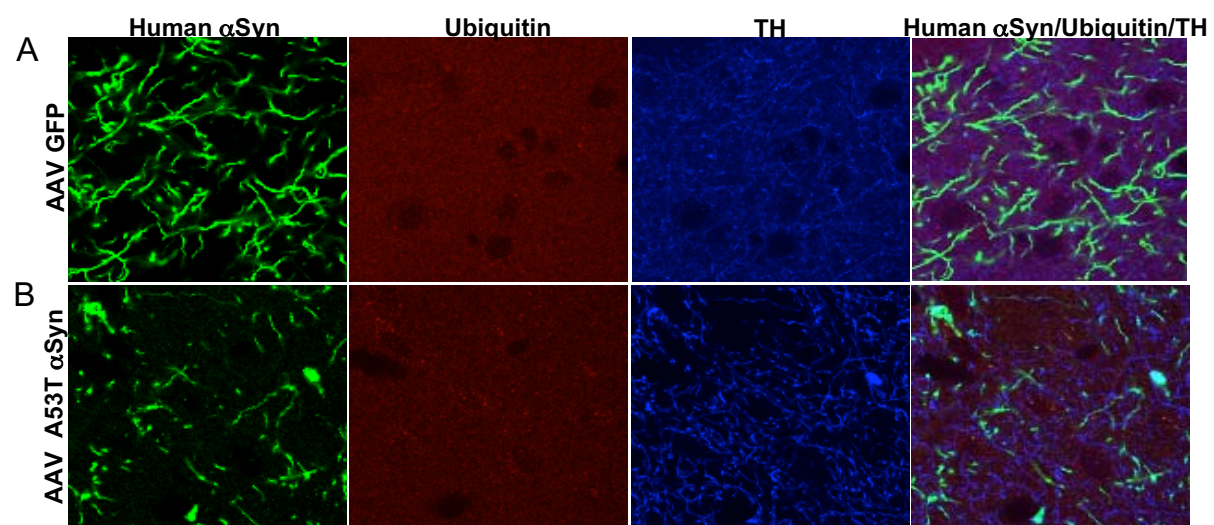
Supp. Figure 2. Striatal GFP immunoreactivity was assessed at 4, 8, 17 and 24 weeks after AAV GFP injection. Intensities of the GFP staining are robust and consistent between times, suggesting that transgene expression by AAV is very efficient and lasting (A). Human α -synuclein immunoreactivity was also determined over time in the A53T α -synuclein overexpressing striatum. Human α -synuclein staining intensities are robust and consistent till 17 weeks. Human α -synuclein intensity was reduced at 24 weeks due to the dramatic loss of DA neurons in the SN and corresponding TH-positive fibers in the striatum at this time (B). AAV GFP injected SN at 4 weeks was stained with anti-GFP antibody (C). Total synuclein (both rat and human) expression levels were compared between 4 and 8 weeks by Western blot analysis. The results demonstrate that total α -synuclein levels are consistent between 4 and 8 weeks (D).

Chung_Supp. Figure 2



Supp. Figure 3. Iba-1 Western blot analysis shows that Iba-1 expression levels were not changed by A53T α -synuclein overexpression in the striatum at 4 weeks (A). In the SN at 8 and 24 weeks post AAV GFP or A53T α -synuclein injection, there was no apparent changes in intensity of the staining and morphology of microglia whereas in the striatum at 24 weeks, mildly activated microglial cells were present (B), suggesting that the inflammation seen in the striatum at 8 weeks persisted until 24 weeks post A53T α -synuclein injection.

Chung_Supp. Figure 3



Supp. Figure 4. Human α -synuclein, ubiquitin and TH were stained in the GFP (A) or α -synuclein overexpressing (B) striatum at 8 weeks. There was no apparent increase in ubiquitin immunoreactivity in dystrophic and bulging axons in the A53T α -synuclein overexpressing striatum (B).