# SUPPLEMENT

## Hsp110 Disaggregase Mitigates α-Synuclein Pathology *In Vivo*

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This section has 6 Supplemental Figures and a detailed Material and Methods section

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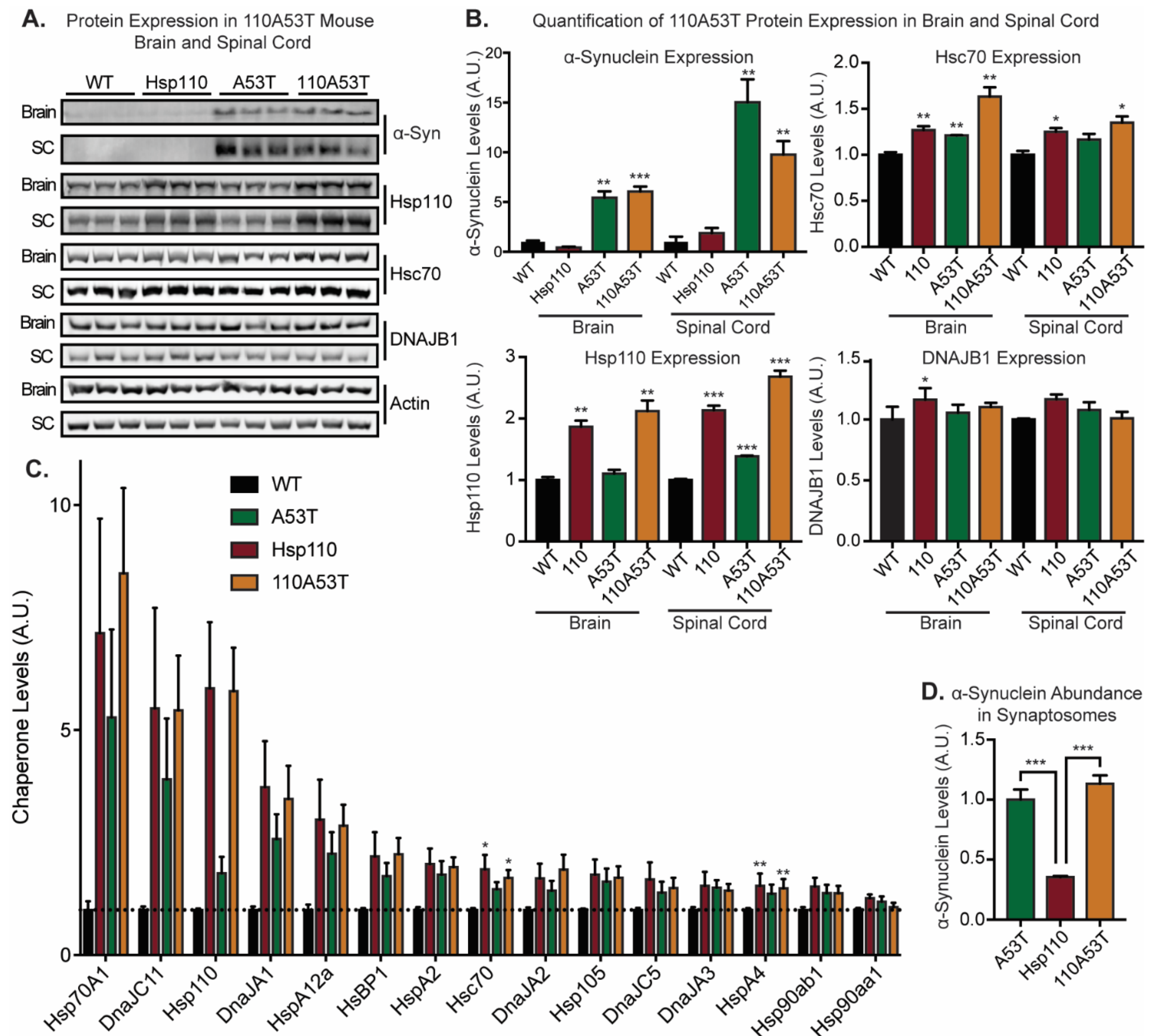


**Figure S1. Characterization of α-synuclein, Hsp110 and 110A53T transgenic mice.** (**A**) Western blot of α-synuclein and chaperone components of the disaggregase levels in whole brain and spinal cord (SC) from littermate wildtype (WT), Hsp110, A53T and 110A53T transgenic mice (n=3 mice/genotype). (**B**) Quantitation of western blots for α-synuclein, Hsc70, Hsp110 and DnaJB1 expression levels in whole mouse brain and spinal cord. (**C**) Relative chaperone abundance in synaptosomes derived from mice of the 4 genotypes, as quantified by LFQ mass spectrometry (N=9 technical replicates and N=2-3 biological replicates). (**D**) Synaptic α-synuclein levels as determined by proteomics. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Data analyzed by Student t-test except for panel C which was analyzed by ANOVA and Dunnett's post-hoc test for multiple comparisons to a control group.

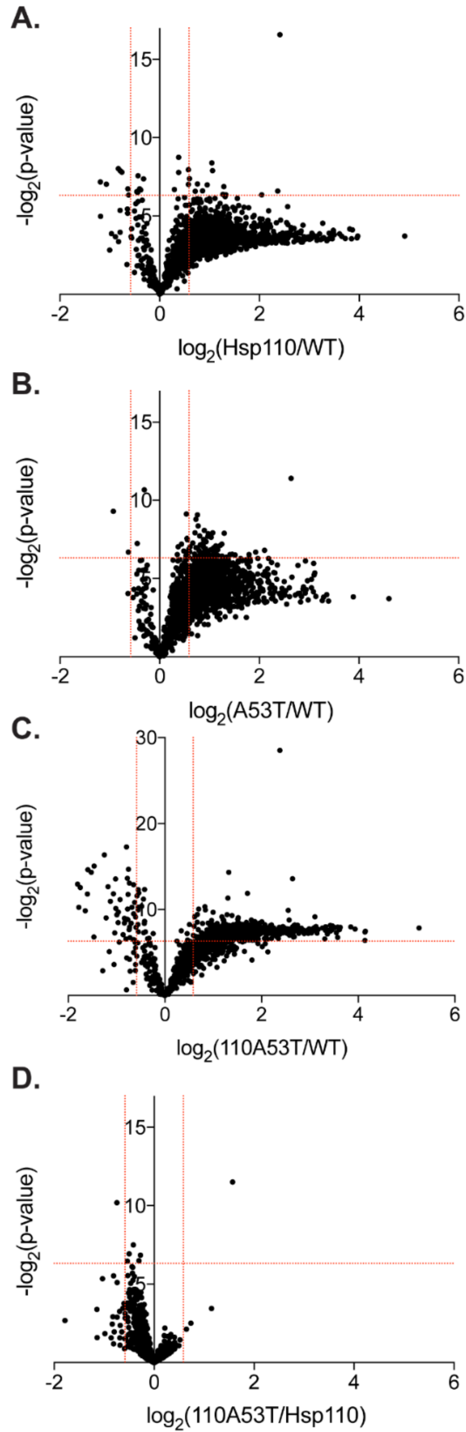


**Figure S2. Volcano plots comparing synaptic proteomes.** Comparisons of (**A**) Hsp110 to WT (**B**), A53T to WT (**C**), 110A53T to WT and (**D**) 110A53T to Hsp110 are shown. Vertical dotted line represents a ± 1.5-fold change, while horizontal dotted line represents a Bonferroni-corrected p=0.05 (0.0125).

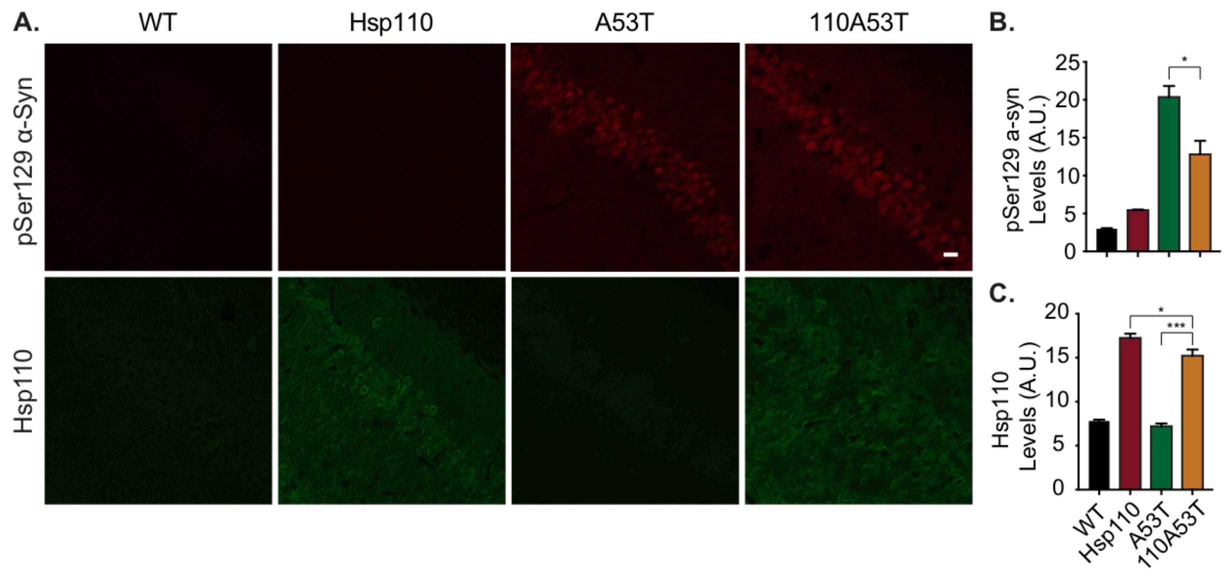


**Figure S3. Characterization of α-synuclein pathology in 110A53T brains.** (**A**) Representative fluorescence images of 9-month WT, Hsp110, A53T, and 110A53T mouse CA1 of hippocampus. First row of images, pSer129 α-synuclein in red, second row Hsp110 in green. Scale bar applies to all images and equal 20 μm. (**B**) Quantification of pSer129 α-synuclein levels, **C**) Quantification of total Hsp110 levels normalized to WT. N = 3-4 mice for the A53T and 11053T genotypes. * p-value <0.05, ** p-value < 0.01, *** p-value < 0.001 two-tailed student t-test. One 110A53T mouse was noted as an outlier and excluded.

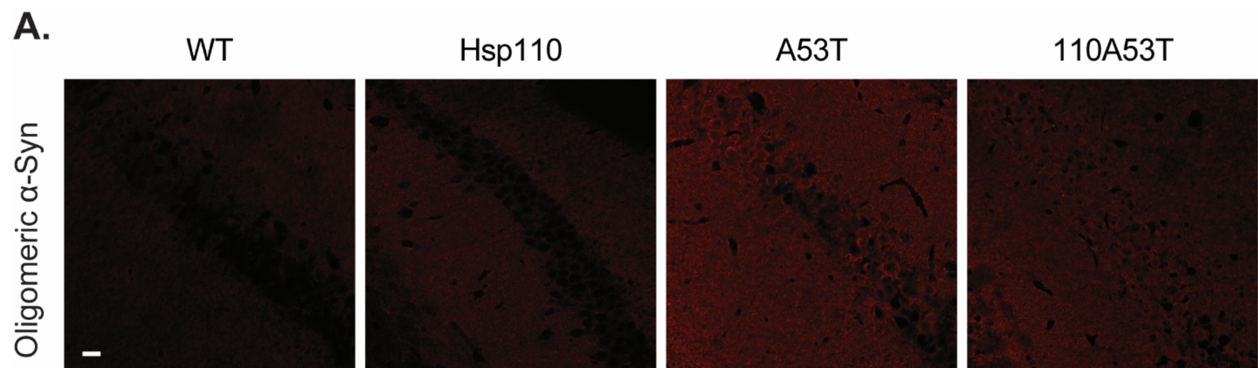


**Figure S4. Characterization of α-synuclein oligomers in 110A53T brain.** (**A**) Representative fluorescence images of 6-month WT, Hsp110, A53T, and 110A53T mouse hippocampus stained with Syn O2 antibody. Scale bar applies to all images and equal 20 μm.

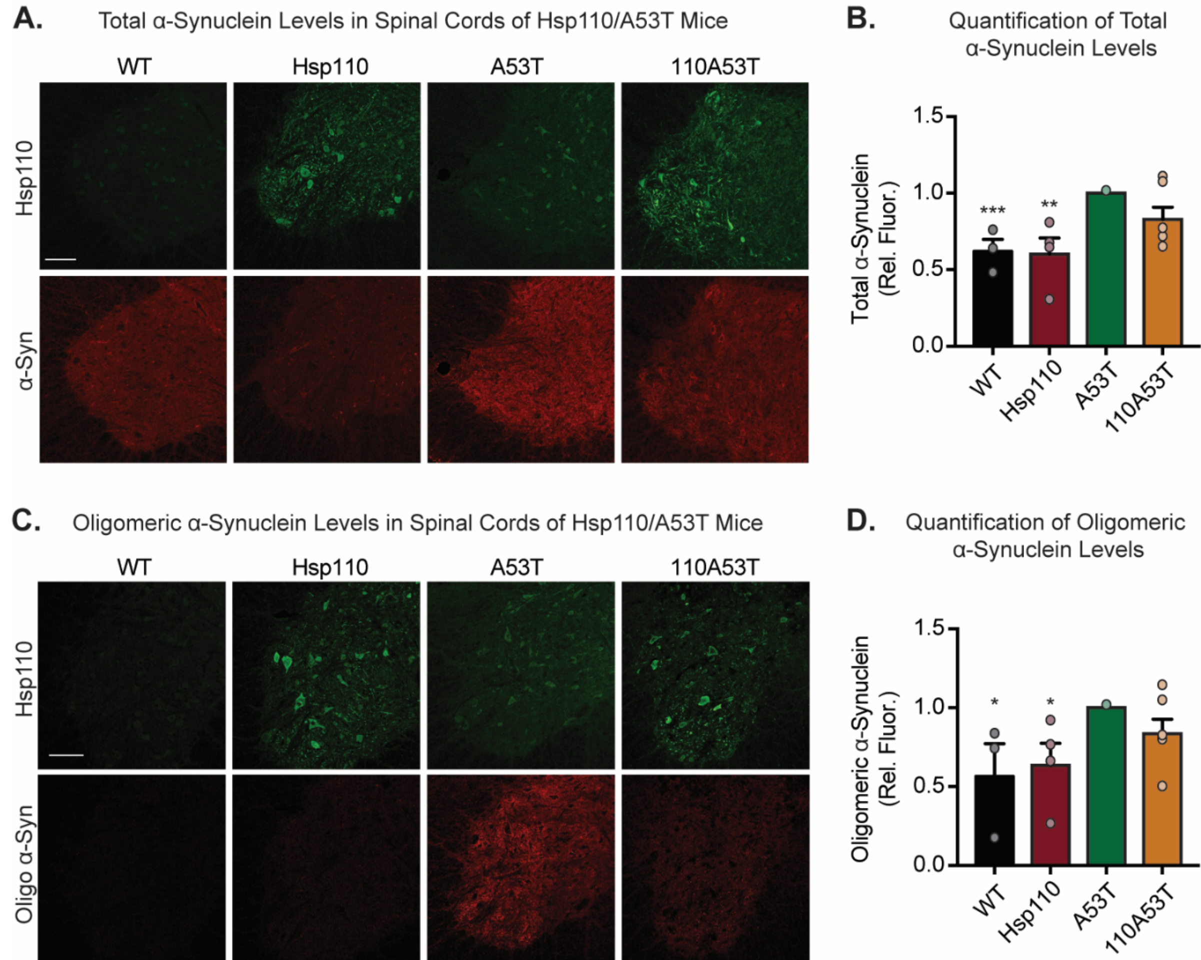


**Figure S5. Characterization of 110A53T mouse spinal cords.** (**A**) Representative fluorescence images of 6-month WT, Hsp110, A53T, and 110A53T mouse ventral horn of L2 spinal cord. Total α-synuclein is shown in red, and Hsp110 in green. (**B**) Quantification of total α-synuclein levels, normalized to A53T. N = 3-6 mice per genotype. ** p-value < 0.01, *** p-value < 0.001 two-tailed student t-test relative to A53T levels. 110A53T rel. A53T p-value = 0.083. (**C**) Representative fluorescence images of 6-month WT, Hsp110, A53T, and 110A53T mouse ventral horn of L2 spinal cord. Oligomeric α-synuclein is shown in red, and Hsp110 in green. (**D**) Quantification of oligomeric α-synuclein levels, normalized to A53T. N = 3-6 mice per genotype. * p-value < 0.05, two-tailed student t-test relative to A53T levels. Hsp110/A53T rel. A53T p-value = 0.139. Scale bar applies to all panels and equals 100 μm.

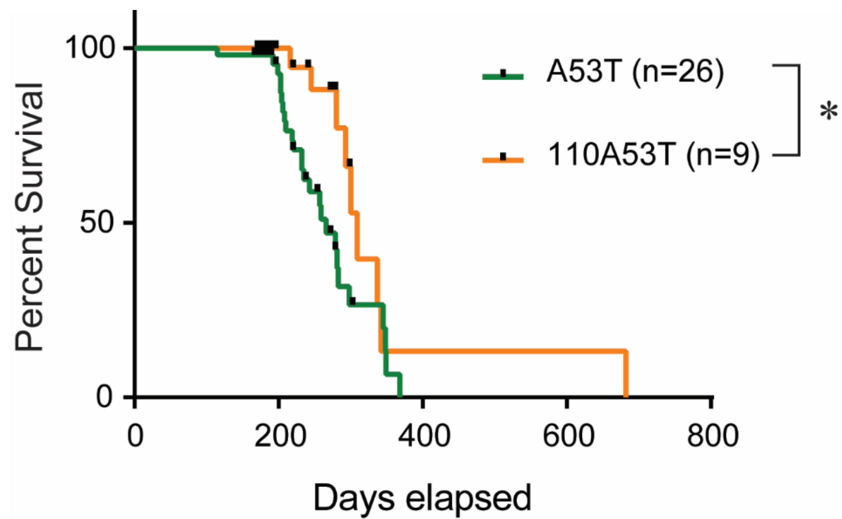


**Figure S6. 110A53T transgenics have significantly improved survival compared to A53T littermates.** Kaplan Meier analysis of littermate A53T and 110A53T mice. Only mice with severe motor phenotypes at the denoted end points are included in the curve. Mice used for experiments are censored. * $p<0.05$.

## MATERIALS AND METHODS

**Antibodies (RRID Information).** α-Synuclein (BD Biosciences 610786, RRID: AB_398107), phosphorylated α-synuclein (BioLegend; 825701, RRID: AB_2564891, Abcam ab51253, RRID: AB_869973, Wako 015-25191, RRID: AB_2537218), Hsp110 (Thermo Fisher Scientific PA5-58671, RRID: AB_2642577), Hsc70 (Enzo Life Sciences ADI-SPA-815-D, RRID: AB_2039279), DNAJB1 (Abcam ab69402, RRID: AB_1209575), Actin (MP Biomedicals 691001, RRID: AB_2336056 at 1:10000), Synuclein Oligomer (BioLegend 847601, RRID:AB_2632700). Tyrosine hydroxylase (TH, Abcam ab76442, RRID: AB_1524535)

**HEK293T overexpression and fibril assay.** HEK293T aggregation assay was completed as described in Taguchi et al., 2017 (35). HEK293T cells were transfected with human α-synuclein-GFP (AddGene 40822), empty pCAG vector, and/or CAG-driven human hsp110 cDNA (HspA4L (Apg1, HSPH3, isoform1)). 0.2mg/mL of oligomeric α-synuclein was used to seed intracellular aggregation. Quantitation of percent of cells with aggregates was completed manually through random visualization of ≥100 cells per replicate.

**Generation of Hsp110/A53T mouse line.** The double transgenic Hsp110/A53T mouse line was generated by crossing the Thy-1 Hsp110 transgenic line and Thy-1 α-synuclein A53T transgenic line, both previously described in detail (31, 38). All lines were kept hemizygous with respect to the expressed transgene. All experiments were done at 6 months of age, unless otherwise noted.

**Mass spectrometric analysis.** Synaptosomes of the denoted genotypes were prepared as described previously (47). Label free quantification on these samples was done on a Thermo Fusion Orbitrap as done previously (48). Proteins with at least 2 independent peptides were analyzed after normalizing both to spiked internal standards and total spectral counts. Pathways altered in the 4 genotypes were identified using STRING analysis.

**Immunohistochemistry of brain and spinal cord sections.** Brains were harvested, and one hemisphere was fixed with 4% PFA, cryoprotected in 30% sucrose and frozen in OCT. While spinal columns were post-fixed in Cal-Rite decalcifying/fixation solution (ThermoFisher Scientific 5501) for 2 days. Spinal cords were dissected, incubated in 30% sucrose for 2 days, and frozen in OCT. Brains and spinal cords were then sectioned into 30 µm and 7 µm sections, respectively onto X-tra slides (Leica Biosystems 3800200) and dried for 10 min. Slides were washed in PBS for 5 min to remove OCT, permeabilized in in 0.5% Triton X-100 in PBS, and blocked in 0.1% Triton X-100 and 2% goat serum in PBS for 30-60 min at room temperature. Slides were then incubated in primary antibody in blocking solution overnight at 4°C, washed in PBS, and incubated in secondary antibody in blocking solution for 1 hour at room temperature. All samples were imaged using the Zeiss Laser Scanning Microscope 710 and 800. Primary antibodies include α-synuclein (1:500), Hsp110 (1:100), phosphorylated Ser 129 α-synuclein (Abcam ab51253 at 1:1000) and oligomeric α-synuclein (1:100). Goat Alexa Flour secondary antibodies were used at 1:500 dilution for all samples. Quantification of immunostainings in brains and spinal cords was performed using ImageJ. Analysis was done blind to genotype.

**Western Blotting.** Protein blots were run on 12% acrylamide gels and transferred overnight onto PVDF transfer membrane. Membranes were blocked for 1 hour at room temperature in 5% w/v milk and 5% goat serum in TBST. Membranes were then incubated overnight at 4°C in blocking buffer with primary antibody, washed in TBST, and incubated 1 hour at room temperature in blocking buffer with secondary antibody. Primary antibodies include α-synuclein (1:1000), actin (1:10000), and oligomeric α-synuclein (1:250). LI-COR Goat IRDye secondary antibodies were used at 1:6000 dilution for all samples.

**Purification of alpha-synuclein and generation of preformed fibrils (PFFs).** Expression of α-synuclein in BL21 cells and purification was performed as described (49). Briefly, after induction of protein expression, the bacterial pellet was lysed and boiled to remove unwanted proteins. α-Synuclein was purified by size exclusion chromatography followed by anion exchange. Fractions with one band corresponding to the molecular weight of α-synuclein were pooled and concentrated. The majority of endotoxin was removed using the Pierce high capacity endotoxin removal spin columns. Endotoxin units were 0.017 units per microgram of protein. α-Synuclein protein was stored at -80°C. Fibrils were generated by shaking 350 μM (5 mg/mL) α-synuclein for 7 days in 50 mM Tris-HCl, pH 7.5, 150 mM KCL (50). After fibril formation, a sedimentation assay at 100,00*xg* was performed to confirm that >50% of synuclein was found in the pellet. Transmission electron microscopy was performed to confirm that 10-15 nm fibrils at an average length of >200 μm were generated. Immediately before stereotaxic injections, 100 μL fibrils were sonicated with a 1/8" probe tip sonicator for a total of 30 seconds.

**Stereotaxic injections and imaging of α-synuclein spread.** At 7 months of age, Hsp110 transgenic mice and littermate controls were injected with 0.1 mg/kg buprenorphine and 5 mg/kg carprofen. Mice were anesthetized with isofluorane and stereotactically injected with 2 μL of 5 mg/mL of sonicated fibrils into the right striatum or 2 μL of 5 mg/ mL monomeric α-synuclein (51). Coordinates were 1.0 mm posterior, 2.0 mm, and -3.2 mm ventral.

Six weeks after injections, mice were anesthetized with isoflurane and transcardially perfused with 0.9% saline, 0.005% sodium nitroprusside, 10 units/mL heparin followed by 4% paraformaldehyde in PBS. Brains were dissected, post-fixed in 4% paraformaldehyde for 18 hours at 4°C, cryoprotected with 30% sucrose in PBS, flash frozen in isopentane, and stored at -80°C. Forty μm coronal sections were rinsed with TBS, incubated in 3% $H_2O_2$, rinsed, incubated in 10 mM sodium citrate, pH 6.0, 0.05% Tween-20, for 30 min at 37° C. Sections were blocked and permeabilized with 5% normal goat serum, 0.1% Triton-X 100, TBS, rinsed and incubated in primary antibody in 3% normal serum TBS for 24h at 4° C. Primary antibodies included anti-pS129-α-synuclein (Abcam at 1:5000; (52)), tyrosine hydroxylase (1:1000), and Hsp110 (1:100). After rinsing, sections were incubated in secondary antibody (Alexa-Fluor conjugated secondary antibodies from ThermoFisher) in 3% normal serum and TBS overnight at 4° C. After rinses, sections were using Prolong Gold (ThermoFisher). Immunofluorescent images were captured using a Leica TCS-SP5 laser scanning confocal microscope. The phosphorylated p-S129 α-synuclein in substantia nigra was quantified using Image J. Analysis was done blind to genotype.