# ONLINE RESOURCE

# Supplementary Material: Multiple system atrophy prions retain strain specificity after serial propagation in two different Tg(*SNCA**A53T) mouse lines

## Acta Neuropathologica

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## SUPPLEMENTARY MATERIALS AND METHODS

### Human patient neuropathology

Human tissue provided by the Sydney Brain Bank was bisected with one hemisphere randomly designated for fresh dissection and the other for fixation in 15% (vol/vol) buffered formalin [39% (vol/vol) aqueous formaldehyde solution] for 2 weeks before sectioning. Standard neuropathological assessment was done using H&E-stained sections, and a modified Bielschowsky silver stain was used to identify Alzheimer-type pathologies. Immunohistochemical staining for phosphorylated α-synuclein (BD Biosciences USA; 1:7,000), phosphorylated tau (AT8, Thermo Scientific USA; 1:1,000), and β-amyloid (Dako Denmark; 1,500) was performed.

Samples received from the Parkinson's UK Brain Bank were assessed neuropathologically following bisection of the brain with one hemisphere fixed in 10% buffered formalin and the other hemisphere sliced coronally, photographed on a grid, and rapidly frozen. Fixed blocks from 20 key brain regions were stained with H&E and Luxol fast blue (LFB). To diagnose and stage disease, appropriate blocks were stained with antibodies against α-synuclein, β-amyloid, tau, and p62. The MSA diagnosis was based on α-synuclein inclusions in oligodendrocytes [1].

MSA patient samples obtained from the Massachusetts Alzheimer's Disease Research Center (ADRC) Brain Bank were assessed to confirm the diagnosis of multiple system atrophy (MSA). Fresh brains were dissected down the midline with one half fixed in 10% (vol/vol) neutral buffered formalin and coronally sectioned and the other half coronally sectioned before rapid freezing. The fixed tissue was evaluated histologically using a set of blocked regions representative of a variety of neurodegenerative diseases. All blocks were stained with LFB and H&E. Selected blocks were used for immunohistochemical staining for α-synuclein, β-amyloid, and phosphorylated tau. A confirmed MSA diagnosis required the presence of glial cytoplasmic inclusions [2].

Fixed and frozen tissue from two MSA patients was provided by the Neurodegenerative Disease Brain Bank at the University of California, San Francisco. In one case, the brain was dissected own the midline with one half fixed in 10% neutral buffered formalin and subsequently coronally section, and the other half was coronally sectioned before freezing at -80 ºC. In the second case, the fresh brain was cut into ~1 cm coronal sections and alternatively rapidly frozen or fixed in 10% neutral buffered formalin for 72 h. Neuropathological diagnoses were made in accordance with consensus diagnostic criteria using immunohistochemistry.

## SUPPLEMENTARY REFERENCES


1 Alafuzoff I, Ince PG, Arzberger T, Al-Sarraj S, Bell J, Bodi I, Bogdanovic N, Bugiani O, Ferrer I, Gelpi Eet al (2009) Staging/typing of Lewy body related alpha-synuclein pathology: a study of the BrainNet Europe Consortium. Acta Neuropathol 117: 635–652

2 Gilman S, Wenning GK, Low PA, Brooks DJ, Mathias CJ, Trojanowski JQ, Wood NW, Colosimo C, Dürr A, Fowler CJet al (2008) Second consensus statement on the diagnosis of multiple system atrophy. Neurology 71: 670–676

3 Prusiner SB, Woerman AL, Mordes DA, Watts JC, Rampersaud R, Berry DB, Patel S, Oehler A, Lowe JK, Kravitz SNet al (2015) Evidence for α-synuclein prions causing multiple system atrophy in humans with parkinsonism. Proc Natl Acad Sci USA 112: E5308–E5317

4 Woerman AL, Kazmi SA, Patel S, Freyman Y, Oehler A, Aoyagi A, Mordes DA, Halliday GM, Middleton LT, Gentleman SMet al (2018) MSA prions exhibit remarkable stability and resistance to inactivation. Acta Neuropathol 135: 49–63 Doi doi: 10.1007/s00401-017-1762-2

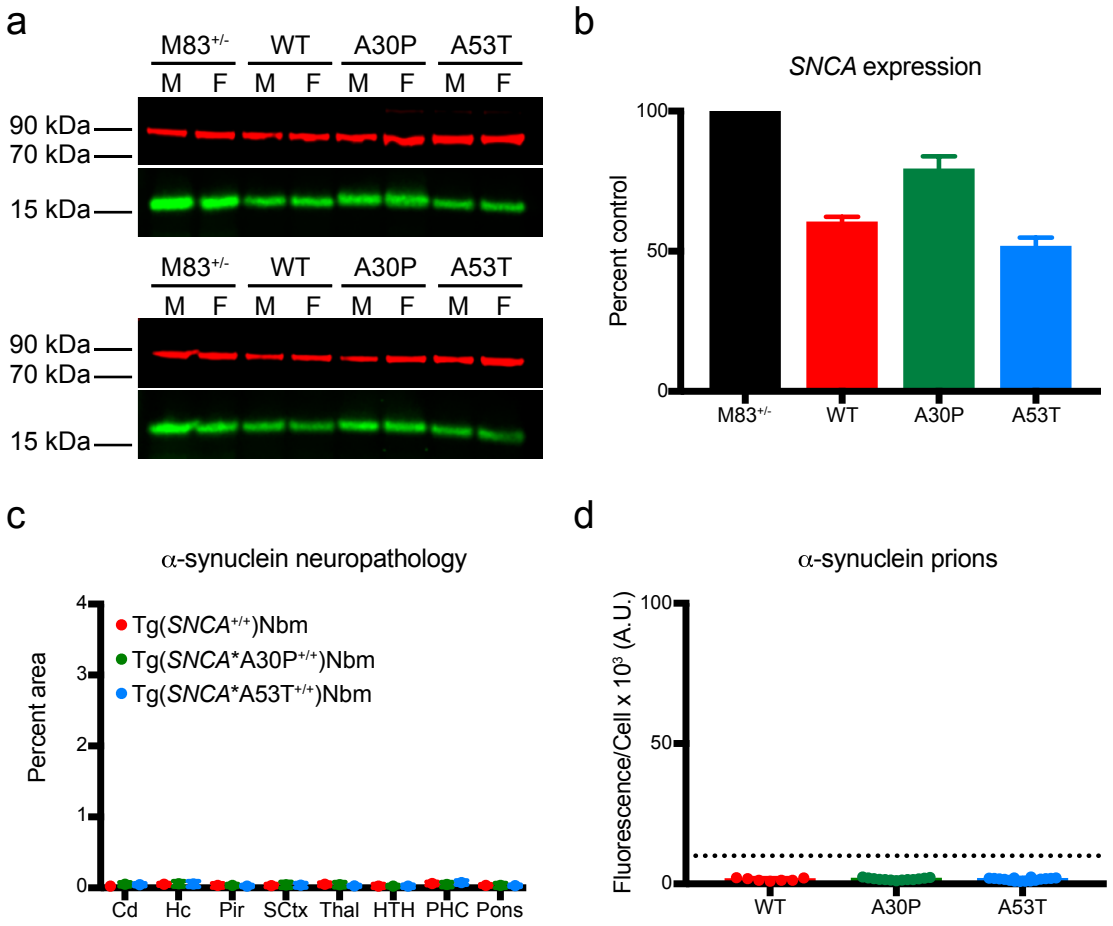


**Fig. S1. Alpha-synuclein expression in Tg(*SNCA*) mice.** (**a**) Total α-synuclein (green) was measured by Western blot in two male and two female two-month-old TgM83$^{+/-}$, Tg(*SNCA*$^{+/+}$)Nbm, Tg(*SNCA**A30P$^{+/+}$)Nbm, and Tg(*SNCA**A53T$^{+/+}$)Nbm mice. Vinculin (red) was used as the protein-loading control. (**b**) Quantification of total α-synuclein from blots shown in (**a**) standardized to expression in TgM83$^{+/-}$ mice. (**c**) Formalin-fixed half-brains from aged Tg(*SNCA*)Nbm mice (WT: 500 days old, n=7; A30P: 450–500 days old, n=8; A53T: 450–625 days old, n=8) were immunostained for phosphorylated α-synuclein in the caudate (Cd), hippocampus and fimbria (Hc), piriform cortex and amygdala (Pir), sensory cortex (SCtx), thalamus (Thal), hypothalamus (HTH), parahippocampal cortex (PHC), and pons. None of the aged mice developed spontaneous α-synuclein neuropathology. One slide containing all brain regions was analyzed per animal. (**d**) Frozen half-brains from the same animals analyzed in (**c**) were homogenized and α-synuclein aggregates were isolated by phosphotungstic acid precipitation. Protein pellets were incubated with α-syn140*A53T–YFP cells to measure α-synuclein prion infectivity. None of the mice tested developed spontaneous α-synuclein prions, as indicated by data points falling below the dotted line ($10 \times 10^3$ arbitrary units, A.U.).

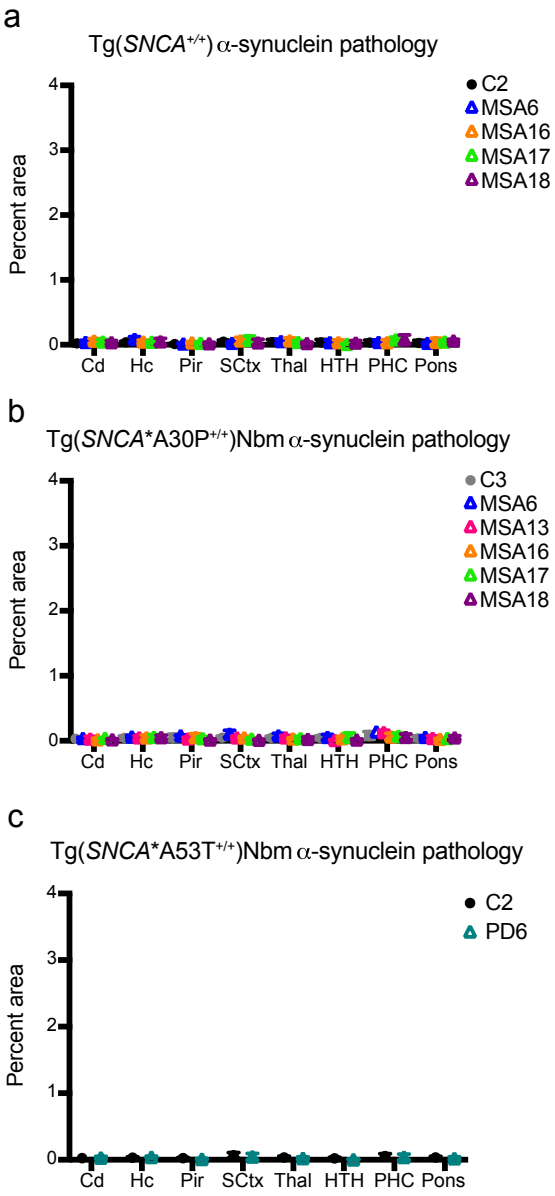


**Fig. S2. Tg(*SNCA*)Nbm mice do not develop neuropathology after inoculation with synucleinopathy patient samples.** (**a**) Tg(*SNCA* $^{+/+}$)Nbm mice were inoculated with brain homogenate from one control (C2; n=7) and five multiple system atrophy (MSA) patient samples (MSA6: n=4; MSA16: n=4; MSA17: n=4; MSA18: n=4), and mice were euthanized 400 days post-inoculation (dpi). (**b**) Tg(*SNCA**A30P$^{+/+}$)Nbm mice were inoculated with brain homogenate from one control (C3; n=8) and five MSA patient samples (MSA6: n=4; MSA13: n=4; MSA16: n=4; MSA17: n=4; MSA18: n=4), and were euthanized 330 dpi. (**c**) Tg(*SNCA**A53T$^{+/+}$)Nbm mice were inoculated with brain homogenate from one control (C2; n=7) and one Parkinson's disease (PD6; n=4) patient sample, and mice were euthanized 330 dpi. Formalin-fixed half-brains from all of the mice were assessed for α-synuclein pathology in the caudoputamen (Cd), hippocampus and fimbria (Hc), piriform cortex and amygdala (Pir), sensory cortex (SCtx), thalamus (Thal), hypothalamus (HTH), parahippocampal cortex (PHC), and pons using one slide per animal. None of the animals showed α-synuclein inclusions.

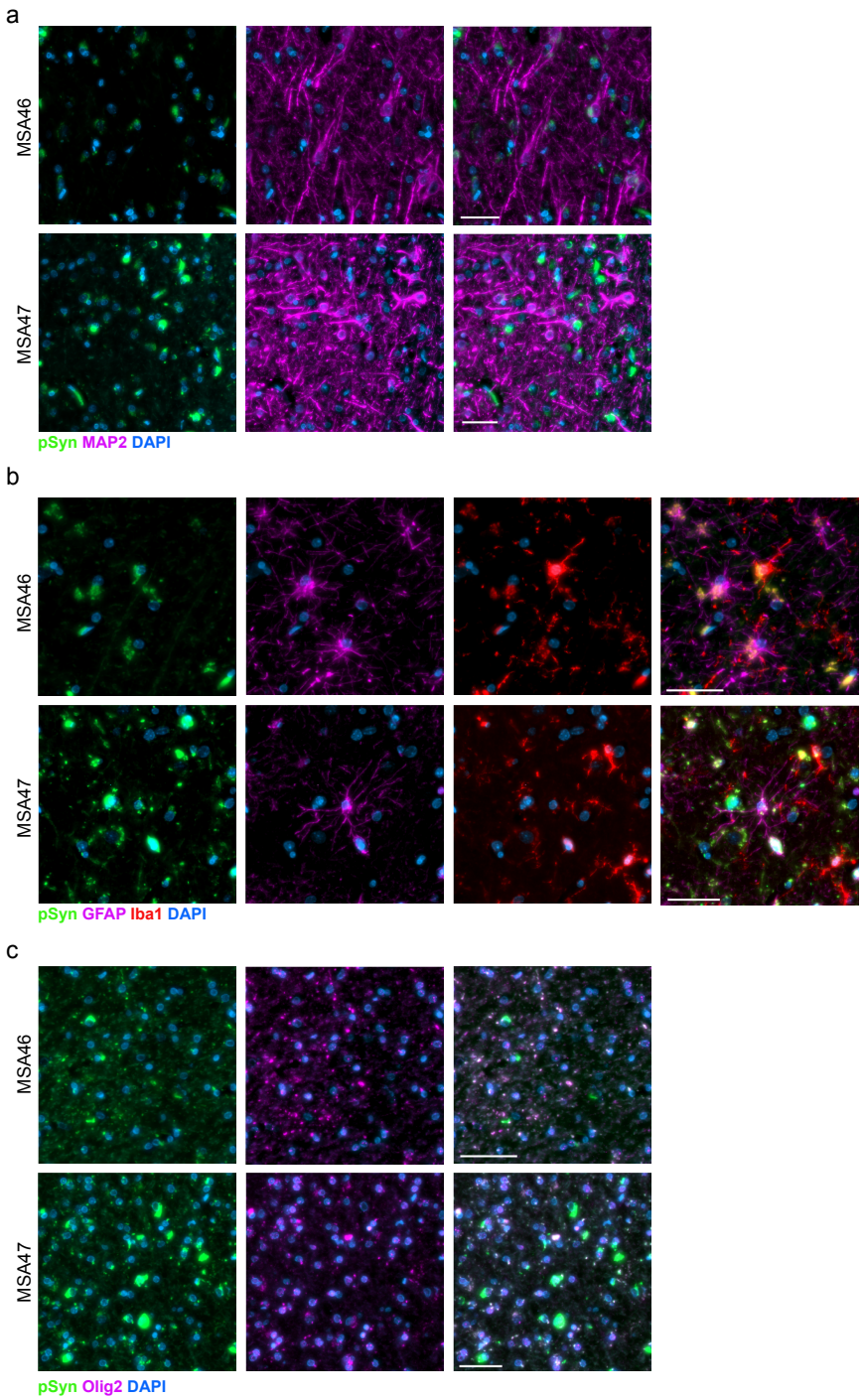


**Fig. S3. Alpha-synuclein inclusions in MSA patient samples co-localize with multiple cell types.** Formalin-fixed sections from the insula/putamen of two MSA patients were analyzed for co-localization of phosphorylated α-synuclein (green) in neurons (MAP2 staining in purple; panel **a**), astrocytes and microglia (GFAP in purple and Iba1 in red, respectively; panel **b**), and oligodendrocytes (Olig2 in purple; panel **c**). While a handful of α-synuclein inclusions co-localized with neurons, astrocytes, and microglia, the majority of phosphorylated α-synuclein was found in oligodendrocytes. DAPI in blue. Scale bars, 50 μm. One section per patient sample was analyzed.

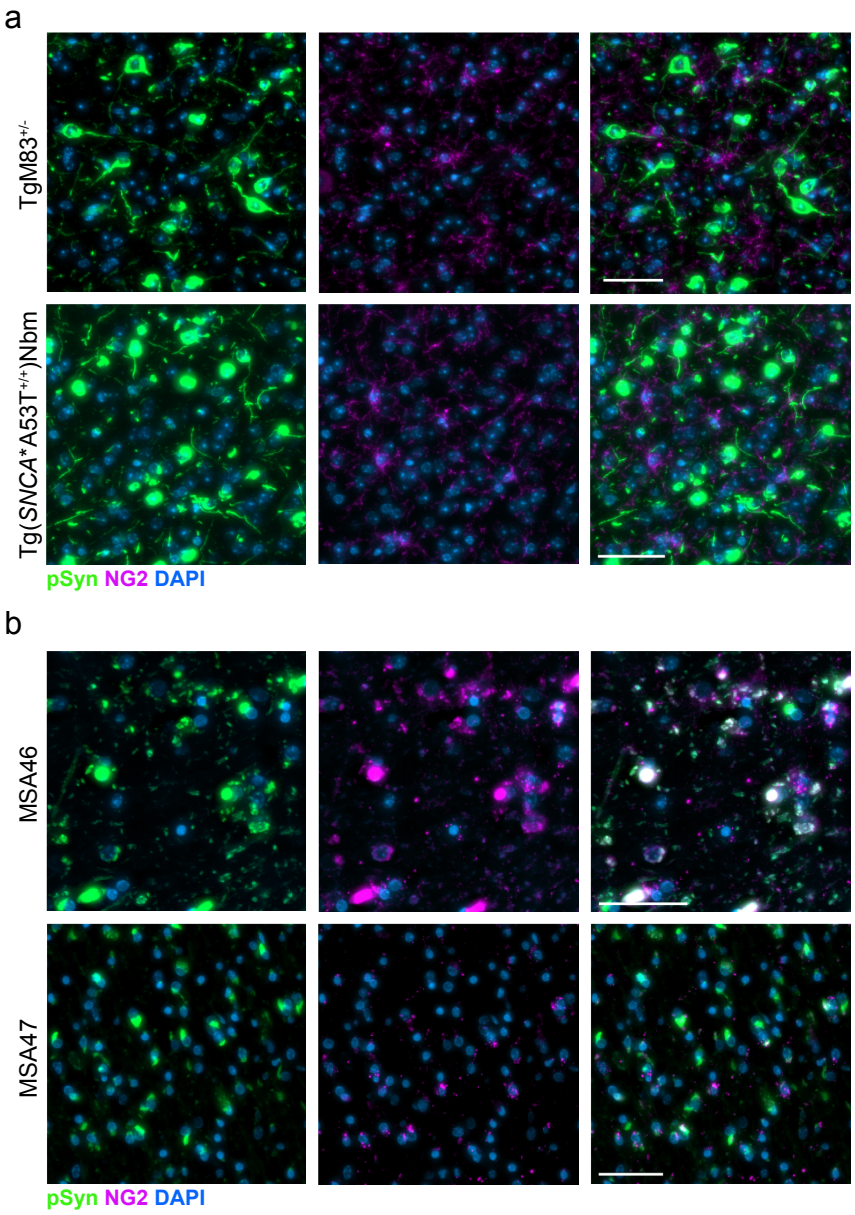


**Fig. S4. Alpha-synuclein inclusions in MSA patient samples, but not in Tg mice, co-localize with NG2 cells.** Formalin-fixed sections from (**a**) the midbrain of TgM83$^{+/-}$ (n=2) and the piriform cortex of Tg(*SNCA**A53T$^{+/+}$)Nbm mice (n=10), both inoculated with MSA patient samples, as well as (**b**) two MSA patient samples, were tested for co-localization of phosphorylated α-synuclein (green) with polydendrocytes (NG2-positive cells, shown in purple). Alpha-synuclein inclusions in the two MSA patient samples co-localized with NG2-positive cells, but co-localization was not observed in either of the two Tg mouse models. DAPI in blue. Scale bars, 50 µm. One slide per mouse or patient sample was analyzed.

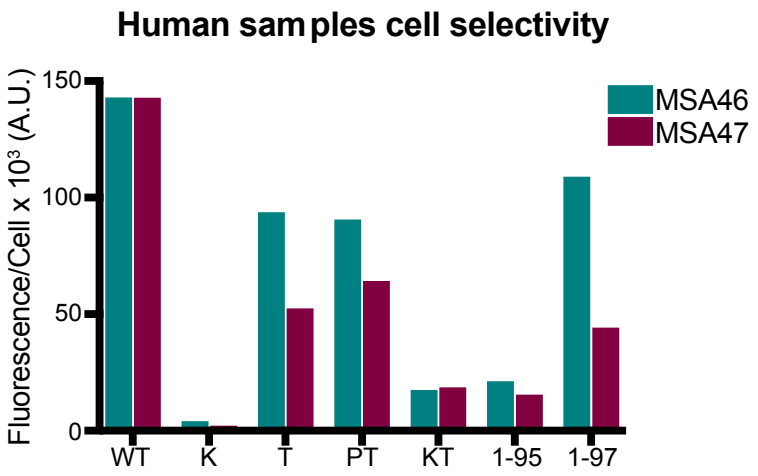


**Fig. S5. MSA patient samples selectively infect α-syn–YFP cells.** Alpha-synuclein prions were isolated from two MSA patient sample brain homogenates via PTA-precipitation. The pelleted prions were incubated with HEK cells expressing WT or mutant α-synuclein. Mutations include E46K (K), A53T (T), A30P,A53T (PT), E46K,A53T (KT), A53T truncated at residue 95 (1-95), and A53T truncated at residue 95 (1-97). MSA prions robustly infected cells expressing WT, T, PT, and 1-97 α-synuclein; however, the E46K mutation and truncation of the protein at residue 95 impaired MSA prion replication. Data reported × $10^3$ arbitrary units (A.U.).

**Table S1. Patient sample information.**

| Patient | Disease | Age at Death | Sex | Brain Region | Country |
|---|---|---|---|---|---|
| C2 | None | 88 | F | Left cortex | Uppsala University |
| C3 | None | 63 | M | Left cortex | Uppsala University |
| C14 | None | 60 | M | Substantia nigra | University of Miami |
| C16 | None | 59 | M | Putamen | University of Miami |
| C17 | None | 65 | M | Putamen | University of Miami |
| MSA6 | MSA | 61 | F | Basal ganglia | Parkinson's UK |
| MSA13 | MSA | 75 | M | Substantia nigra | MADRC[†] |
| MSA16 | MSA | 61 | F | Basal ganglia | MADRC[†] |
| MSA17 | MSA | 60 | M | Substantia nigra | MADRC[†] |
| MSA18 | MSA | 54 | M | Substantia nigra | MADRC[†] |
| MSA46 | MSA | 65 | F | Insula/Putamen | UCSF[#] |
| MSA47 | MSA | 77 | F | Insula/Putamen | UCSF[#] |
| PD2 | PD | 65 | M | Substantia nigra | Parkinson's UK |
| PD3 | PD | 77 | M | Basal ganglia | Parkinson's UK |
| PD6 | PD | 79 | M | Substantia nigra | Sydney Brain Bank |
| PD16 | PD | 76 | M | Substantia nigra | Parkinson's UK |
| PD19 | PD | 87 | M | Substantia nigra | Parkinson's UK |

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[#]*Neurodegenerative Disease Brain Bank at the University of California, San Francisco*

**Table S2. Incubation times in Tg(*SNCA*) mice inoculated with control and MSA patient samples.**

| Patient Sample | Mean incubation time ± SD (d) | | | |
|---|---|---|---|---|
| | TgM83$^{+/-}$ | Tg(*SNCA*$^{+/+}$)Nbm | Tg(*SNCA**A30P$^{+/+}$)Nbm | Tg(*SNCA**A53T$^{+/+}$)Nbm |
| C2 | >400* | >400 | >330 | >330 |
| C3 | n.d. | >400 | >330 | >330 |
| C14 | >400 | >400 | >330 | >330 |
| C16 | n.d. | >400 | >330 | >330 |
| C17 | n.d. | >400 | >330 | >330 |
| MSA6 | 108 ± 29$^{\dagger}$ | >400 | >330 | >330 |
| MSA13 | 113 ± 26$^{\dagger}$ | >400 | >330 | >330 |
| MSA16 | n.d. | >400 | >330 | >330 |
| MSA17 | 121 ± 30 | >400 | >330 | >330 |
| MSA18 | 289 ± 71* | >400 | >330 | >330 |

*n.d., not determined.*

**Data published in [4].*

*$^{\dagger}$Data published in [3].*

**Table S3. MSA prion propagation in Tg(*SNCA*) mice.**

| Patient Sample | Mean cell infection ± SD, fluorescence/cell ($10^3$ A.U.)* |
|---|---|
| PD2 | 2.2 ± 0.8 |
| PD3 | 1.8 ± 0.5 |
| PD6 | 1.8 ± 1.0 |
| PD16 | 1.8 ± 0.6 |
| PD19 | 2.2 ± 0.8 |

**Measurements made from five images per well, n = 6 wells. Phosphotungstic acid (PTA)-precipitated samples were diluted in DPBS 1:10 before testing on α-syn140*A53T–YFP cells.*