ONLINE RESOURCE

Supplementary Material:
Multiple system atrophy prions transmit neurological disease to mice expressing wild-type human α-synuclein

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MATERIALS AND METHODS

Human patient neuropathology

Neuropathology assessment by the Parkinson's UK Brain Bank was performed using human tissue samples bisected down the midline. One hemisphere was fixed in 10% buffered formalin, and the other hemisphere was sliced coronally, photographed on a grid, and rapidly frozen. The fixed tissue blocks from 20 key brain regions were stained with hematoxylin and eosin (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. An MSA diagnosis was based on α-synuclein inclusions in oligodendrocytes [1].

Neuropathology assessment by the Massachusetts Alzheimer's Disease Research Center (ADRC) Brain Bank was performed using human tissue samples bisected down the midline. One hemisphere was fixed in 10% (vol/vol) neutral buffered formalin and coronally sectioned, and the other hemisphere was 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 H&E and LFB. 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 [3].

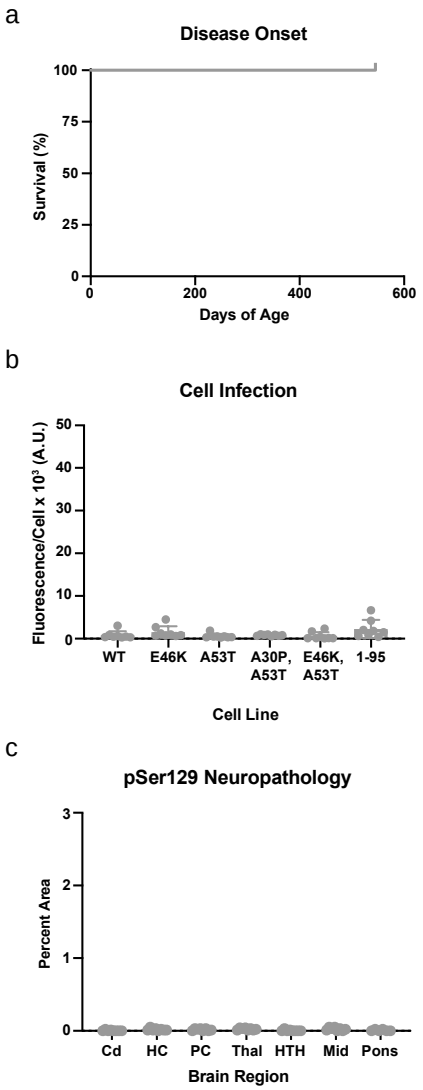

Fig. S1. Hemizygous TgM20$^{+/-}$ mice do not develop spontaneous disease. (**a**) TgM20$^{+/-}$ mice were aged to 546 days (~18 months) and were assessed for neurological signs twice each week. Consistent with previously reported studies [2], hemizygous TgM20$^{+/-}$ mice did not develop spontaneous disease. (**b**) Frozen half-brains from TgM20$^{+/-}$ mice collected at 546 days of age were homogenized and α-synuclein aggregates were isolated by phosphotungstic acid precipitation. Protein pellets were incubated with α-syn140–YFP (WT), α-syn140*E46K–YFP (E46K), α-syn140*A53T–YFP (A53T), α-syn140*A30P,A53T–YFP (A30P,A53T), α-syn140*E46K,A53T–YFP (E46K,A53T), and α-syn1-95*A53T–YFP (1-95) cells to measure α-synuclein prion infectivity. None of the mice tested developed detectable spontaneous α-synuclein prions. (**c**) Formalin-fixed half-brains from TgM20$^{+/-}$ mice collected at 546 days of age were immunostained for phosphorylated α-synuclein. Neuropathology was quantified (percent area) in the caudate (Cd), hippocampus (HC), piriform cortex and amygdala (PC), thalamus (Thal), hypothalamus (HTH), midbrain (Mid), and pons. None of the aged mice developed spontaneous α-synuclein pathology.

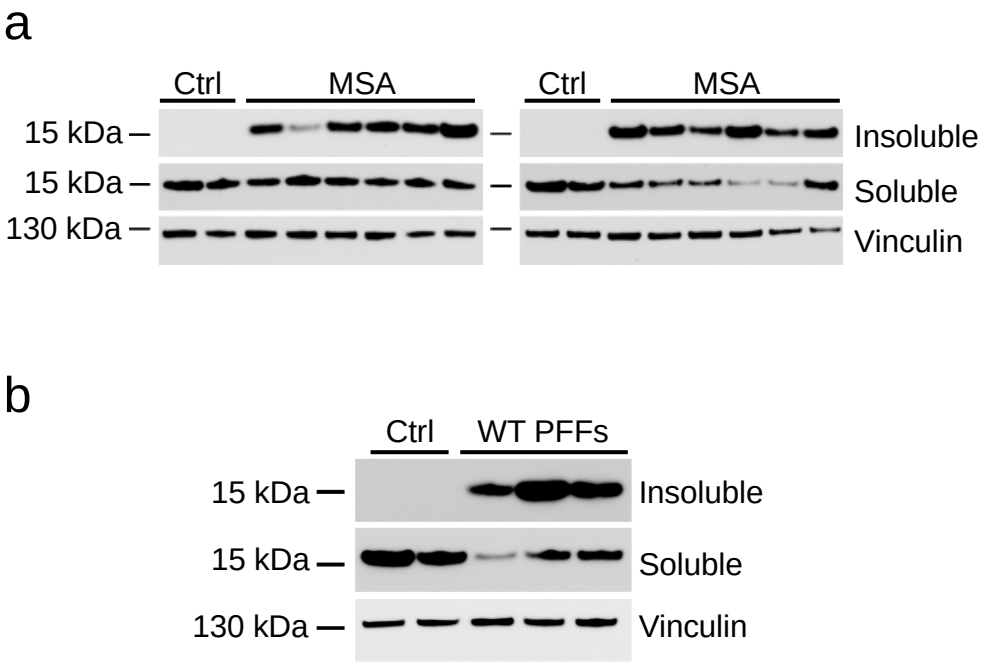

Fig. S2. MSA patient samples and WT PFFs induce the formation of detergent-insoluble α-synuclein aggregates. Eight-week-old TgM20$^{+/-}$ mice were inoculated with control patient sample and either (**a**) MSA patient samples or (**b**) WT PFFs. Brain homogenates from asymptomatic control mice euthanized 475 days postinoculation (dpi) or symptomatic MSA-inoculated or WT PFF–inoculated mice were analyzed for the presence of sarkosyl-insoluble α-synuclein aggregates. (**a**) Two sets of Western blots tested eight mice each. Each blot contains one sample from each inoculation group (two control and six MSA). No phosphorylated insoluble α-synuclein was detected in control-inoculated mice, but it was present in MSA-inoculated animals. (**b**) Two brain homogenates from control-inoculated TgM20$^{+/-}$ mice and three homogenates from WT PFF–inoculated animals were assayed. No insoluble phosphorylated α-synuclein was detected in control-inoculated mice, but it was present in terminal mice inoculated with WT PFFs. For all blots, top and middle panels probed with EP1536Y primary antibody. Top panel is sarkosyl-insoluble protein, middle is soluble protein. Bottom panel shows vinculin as a loading control for the soluble blot.

Table S1. Patient sample information.

Patient	Neurological Disease	Age at Death	Sex	Brain Region	Brain Bank
C9	None	62	M	Putamen	University of Miami
C17	None	65	M	Putamen	University of Miami
MSA5	MSA	60	M	Basal ganglia	Parkinson's UK
MSA6	MSA	61	F	Basal ganglia	Parkinson's UK
MSA13	MSA	75	M	Substantia nigra	MADRC[b]
MSA14	MSA-C[a]	76	M	Basal ganglia	MADRC[b]
MSA16	MSA-C[a]	61	F	Basal ganglia	MADRC[b]
MSA17	MSA	60	M	Substantia nigra	MADRC[b]

[a]Multiple system atrophy—cerebellar subtype
[b]Massachusetts Alzheimer's Disease Research Center

SUPPLEMENTARY REFERENCES

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2 Giasson BI, Duda JE, Quinn SM, Zhang B, Trojanowski JQ, Lee VM (2002) Neuronal α-synucleinopathy with severe movement disorder in mice expressing A53T human α-synuclein. Neuron 34: 521–533

3 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