## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

**Figure S1.** Multiple neuronal brain stem-type Lewy bodies and an extracellular brain stem-type Lewy body. The highest number of brain stem-type Lewy bodies identified in a neuron was eight (**A–D**). Because of their spiky halo nature, multiple brain stem-type Lewy bodies were usually interconnected. Multiple brain stem-type Lewy bodies in a neuron each had a similar neurochemical pattern as described previously, but varied in size. In this neuron, the core of the brain stem-type Lewy bodies contained TH (**A**) and tαSYN (**B**), which is surrounded by a shell of tαSYN (**B)** which is not clearly recognized due to plane of focus, which is surrounded by a shell of pαSYN (**C**). Several features defined an extracellular brain stem-type Lewy body (**E–H**) including: (i) central portion containing tαSYN (F**)** and a peripheral shell stained for flαSYN (**G**) or pαSYN (not shown); (ii) the peripheral shell becoming thinned and irregular; (iii) the absence of TH or other evidence of an intracellular location; and (iv) direct contact with a glial or macrophage nucleus (**H**).

**Figure S2.** TH+ neurons in a CTRL substantia nigra. Both flαSYN (**B**) and ubiquitinated proteins (**E**) and can be found in the neuronal soma, nucleus and neurites of TH+ neurons. To identify location, it is necessary to look at all three planes for a particular feature. Normal flαSYN has a documented preference for the neuropil, but has been identified in the soma, nucleus and neurites at a very low level. Normal ubiquitinated proteins are more easily recognized and abundant in those three compartments. To maximize visualization of non-aggregated flαSYN PK-AR should be avoided. Free-floating sections did not require AR to visualize proteins. TH can be seen to be normally highly expressed. All proteins appeared cylindrical to ovoid in shape.

**Figure S3.** Axon bundles innervating the putamen in an age-matched CTRL several axons containing TH can be seen scattered within these bundles. The number of axons transporting TH at any given time was a fraction of the total axons present in this pathway. An axon usually contained a variable number of TH cargo that ranged in axon length from 10 μm to 70 μm. The variable length was not a function of tissue thickness or image acquisition. TH cargo are transported in axons as rod-shaped structures varying in cross-sectional diameter from ~0.5 μm to ~1.2 μm with a length of ~2.5 μm and move along a normal axon in single file (**A, D**). TH+ axons contain a small number of cargo that are positive for ubiquitinated proteins (UB, **B, C**) or flαSYN (**E, F**). To identify location within an axon, it is necessary to look at all three planes for a particular cargo. The orthogonal planes were selected to optimize visualization of one ubiquitinated protein (C) and one flαSYN (F) cargo. A series of adjacent cargo for ubiquitinated proteins or flαSYN was not seen, unlike those for TH.

**Figure S4.** Nerve terminals in mouse striatum and human putamen. Antigen retrieval (AR) was not required with mouse tissue (**A–C**) as it was lightly fixed. TH (**A**, red, Fischer Scientific) was found to frequently colocalize with flαSYN (**B**, green, Sigma) in terminals and distal axon branches in the mouse. TH labeled both the distal axon branch leading to a terminal and the terminal, whereas flαSYN was more often restricted to a terminal. Terminals most often appeared disc shaped. Nerve terminals in human putamen (**D–F** and **G–I**) were labeled for flαSYN (**D, F**, red, Biomol) and TH (**D–F**, dark blue) and were found to frequently colocalize with the terminal specific protein synaptophysin (**E, F**, green) using WB-AR. The need for PK-AR to visualize the majority of TH in human tissue reduced its colocalization with soluble flαSYN and synaptophysin which were more sensitive to PK-AR. PK-AR did not affect a pre-synaptic marker for the glutamatergic system, vGLUT1 (Lower Panel, **H, I**) which did not colocalize with TH.

**Figure S5.** Possible lesions before incidental Lewy body disease. Unique abnormalities were found in the nucleus and soma of rare TH+ neurons in the SN of a CTRL case. Abnormalities included an aggregate in the axon hillock region that contained both flαSYN (**B**) and UB (**E**). UB was also found in neurites in a manner not seen in other CTRL cases (**E**). UB+ Marinesco bodies were seen in many TH+ neurons (as reported by others), some of which also contained the axon hillock aggregate (**E**). Rarely, Marinesco bodies contained TH (**G**) and pαSYN (**H**).

**Table S1.** Antibodies and nuclear stain used in this report. Abbreviations: Rbt = rabbit; Chk = chicken; Gt = goat; Ms = mouse; Gp = guinea pig; FFPE = free-floating paraffin embedded; conc. = concentration; flαSYN = full length human αSynuclein; PK = proteinase K; Synapto = synaptophysi; TH, tyrosine hydroxylase = tαSYN, truncated αSYN; UB = ubiquitin; vGLUT1 = vesicular glutamate transporter 1; WB = water bath.

**Video S1.** A TH+ neuron with two attached brain stem-type Lewy bodies from a PDP case labeled for tαSYN (red), flαSYN (green), TH (violet) and the nucleus (DAPI, light blue). The video starts with all channels visible. Two brain stem-type Lewy bodies of different sizes are joined together, along with an eccentric nucleus to the left of the large brain stem-type Lewy body. The TH+ neuron is surrounded by the nuclei of other cells. The video starts by turning off the flαSYN channel revealing both tαSYN and TH present in the brain stem-type Lewy bodies (purple). Then, the tαSYN channel is then turned off revealing only the TH channel (violet) and nuclei. All channels are turned on and the cell is zoomed and rotated. The volume viewer is turned off and an orthogonal slice viewer (0.5 μm thickness) is turned on and moved through the cell from bottom to top stopping first in the midportion of the large brain stem-type Lewy body where the TH channel is turned off revealing the large brain stem-type Lewy body to have a concentric ring of tαSYN (red) surrounded by flαSYN (green) with a spiky appearance. The flαSYN (green) channel is then turned off. All channels turned on and the orthogonal viewer is moved to the center of the smaller brain stem-type Lewy body. The TH channel is turned off, the two brain stem-type Lewy bodies can be seen joined together by flαSYN. The spiky outer ring of a brain stem-type Lewy body can be seen. The center of the brain stem-type Lewy bodies can be seen and would be filled with a variety of proteins some of which would be ubiquitinated. The flαSYN channel is turned off, then all channels are turned on, the orthogonal slicer finishes its path. The orthogonal viewer is turned off and the volume viewer turned on and the cell is reduced in size and oriented as it began. For all videos using a PDP case and the SN, the FFFF method was used for IHC with no antigen-retrieval and a tissue thickness of 30 μm.

**Video S2.** A TH+ neuron in the SN with both neuronal diffuse cytoplasmic accumulation and a Pale body from a PDP case labeled for tαSYN (red), pαSYN (green), TH (violet) and the nuclei with DAPI (light blue). The video starts with all channels

on, the tαSYN (red), pαSYN (green) channels are then turned off revealing TH and the nucleus, the cell is then enlarged and rotated, the nuclear channel turned off, the TH channel turned off and the tαSYN (red) channel turned on and then off, the pαSYN (green) channel turned on, and the tαSYN (red) channel turned on again. The volume display is turned off the orthogonal slicer turned on and moved through the neuron in both directions revealing a pale body and details of localization of fibrils in the soma and axon. The orthogonal slicer is stopped at the level of the pale body and the tαSYN (red) and pαSYN (green) channels turned on and off. The neuron is then returned to its original orientation and magnification.

**Video S3.** A neuron from a CTRL case labeled for TH (red), flαSYN (green) and nucleus (blue). The video starts with TH and nuclei visible, then flαSYN is turned on then off, the cell is magnified and rotated, then flαSYN is turned back on, rotation continued, flαSYN turned off and the image reduced. TH can be seen as punctate in the soma, axons and dendrites. flαSYN can be seen as punctate in synapses, but also rarely in the cell soma and nucleus.

**Video S4.** A TH+ dystrophic neurite (DN) in the SN from a PDP case was labeled for tαSYN (red), ubiquitin (green), TH (violet) and nuclei counterstained with DAPI (light blue). The video starts with all channels on and the DN in the center of the image surrounded by TH+ neurites (violet). The image is zoomed, tαSYN (red) and ubiquitin (green) are turned off, then on and the DN rotated ~90° to look down the length of the DN. The orthogonal slicer is turned on and the volume view turned off, different channels are turned on and off, then the DN enlarged and individual channels examined. tαSYN, ubiquitin and TH can be seen within the DN and a laminar pattern not seen with these proteins.

**Video S5.** Two TH+ axons are shown in the axon pathway adjacent to the Put in a CTRL case. The section was labeled for flαSYN (red), TH (violet) and nuclei counterstained with DAPI (light blue). A 15 μm FFPE section was used in the absence of PK-AR to demonstrate flαSYN. The video starts showing the two axons and two nuclei. The video begins to zoom in on the lower portion of the axons and the DAPI channel turned off. The image is rotated to demonstrate the size, shape and discrete nature of both TH and flαSYN as axonal cargo being transported along an axon. The oblique slicer is then used in two planes to show the flαSYN cargo (red) are located in the axon between TH cargo. flαSYN can also be seen to be located in puncta, possibly outside of axons. The image is then returned to its initial size and position.

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