# α-Synuclein aggregates amplified from patient-derived Lewy bodies recapitulate Lewy body diseases in mice

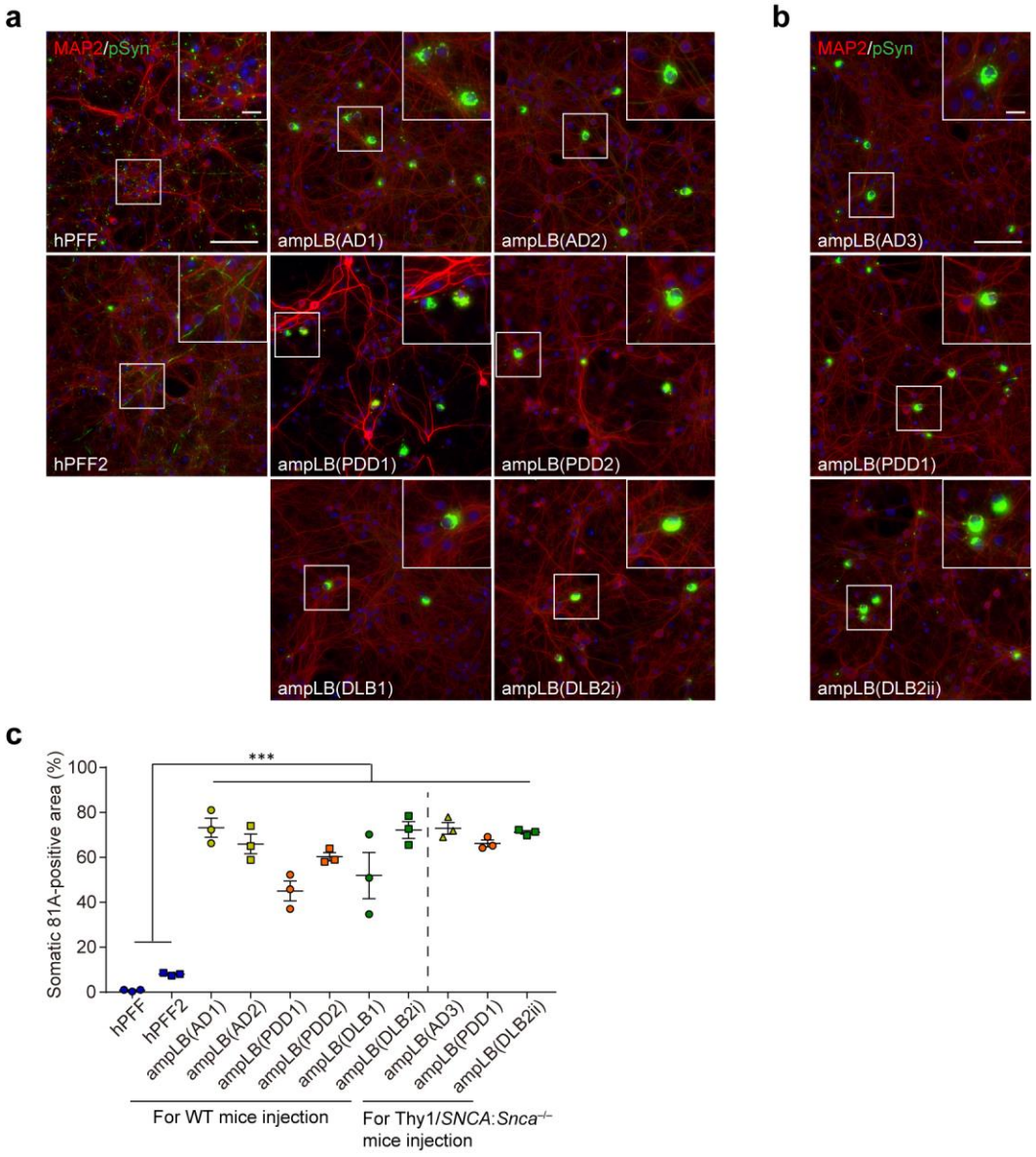
Norihito Uemura[1,*], Nicholas P. Marotta[1], Jahan Ara[1], Emily S. Meymand[1], Bin Zhang[1], Hiroshi Kameda[2], Masato Koike[2], Kelvin C. Luk[1], John Q. Trojanowski[1], Virginia M.-Y. Lee[1,*]

*Correspondence: nuemura@kuhp.kyoto-u.ac.jp, vmylee@upenn.edu

**Affiliations**

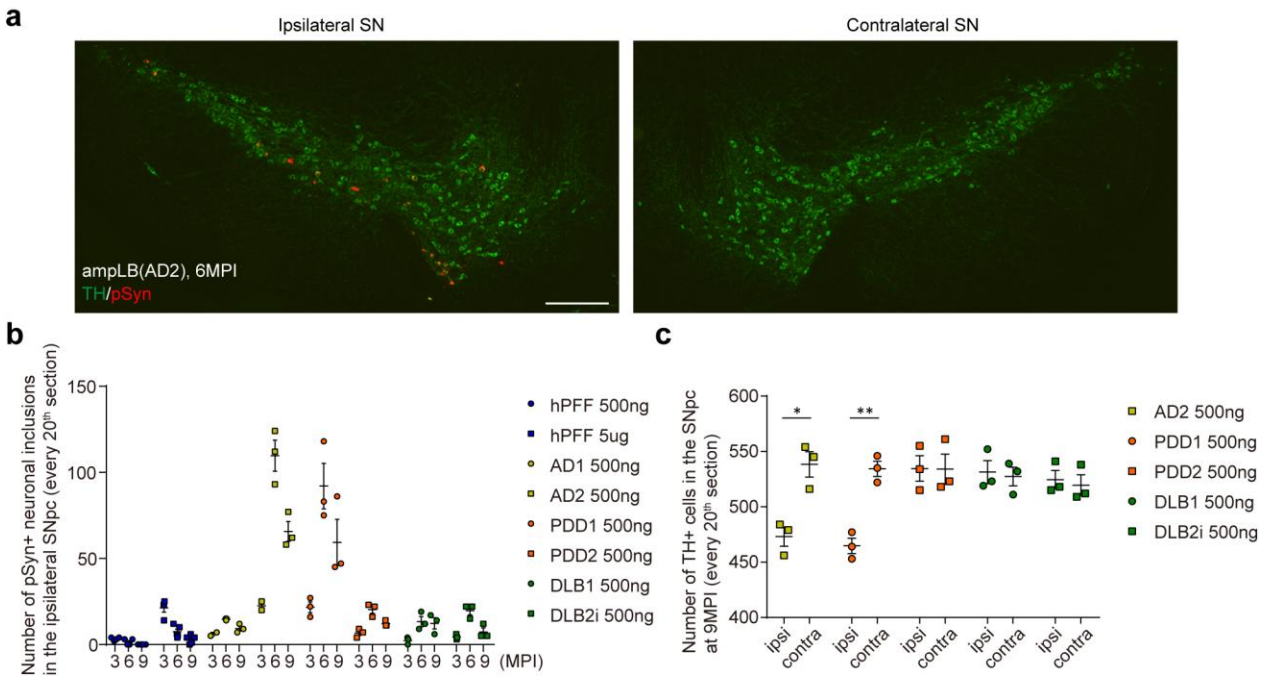
[1]Department of Pathology and Laboratory Medicine, Institute on Aging and Center for Neurodegenerative Disease Research, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, 19104-2676, USA

[2]Department of Cell Biology and Neuroscience, Juntendo University Graduate School of Medicine, Tokyo, Japan

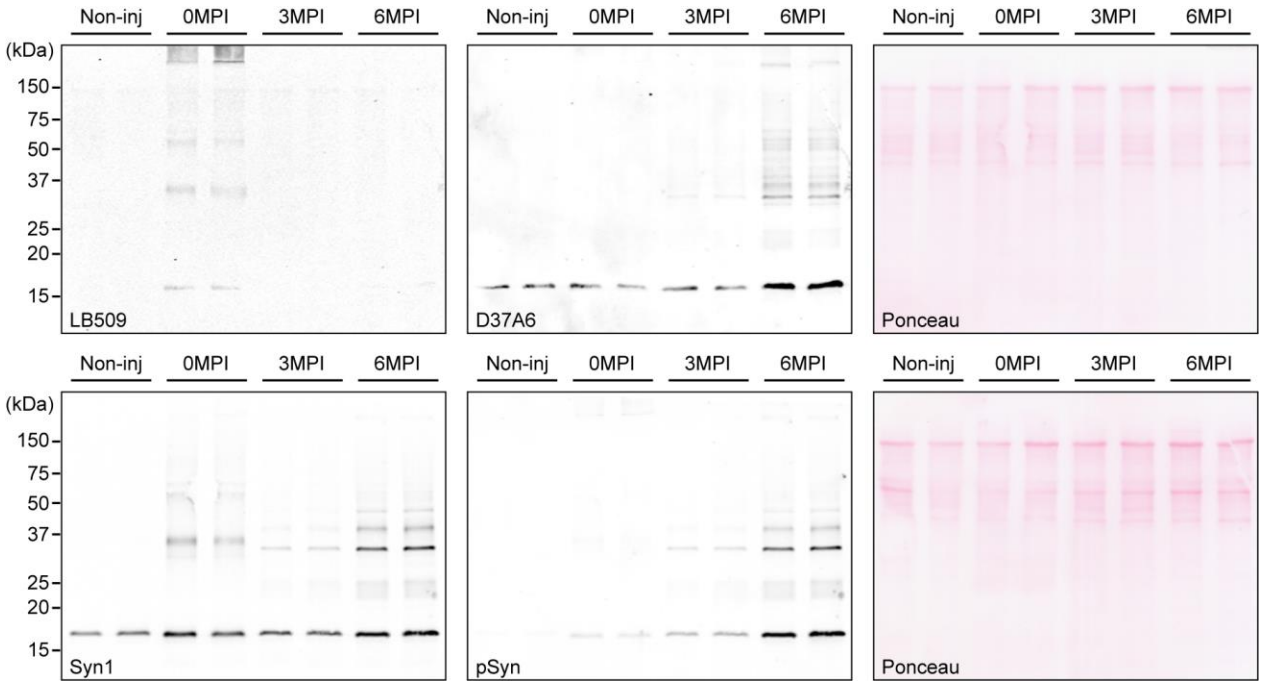



**Supplementary Fig. 1 Validation of LB-αSyn amplification in mouse primary neurons. a, b** Mouse primary hippocampal neurons treated with hPFF and ampLB preparations. Immunocytochemistry with anti-MAP2 and pSyn (81A) antibodies. Multiple hPFF batches, hPFF used for mouse brain injection and another hPFF batch (hPFF2) were tested. AmpLB preparations used for WT mice injection (**a**) and Thy1:*SNCA*/*Snca*$^{-/-}$ mice injection (**b**) were tested. Scale bars 100 µm, 20 µm (inset). **c** Percent of total pSyn-positive pathology in somatic

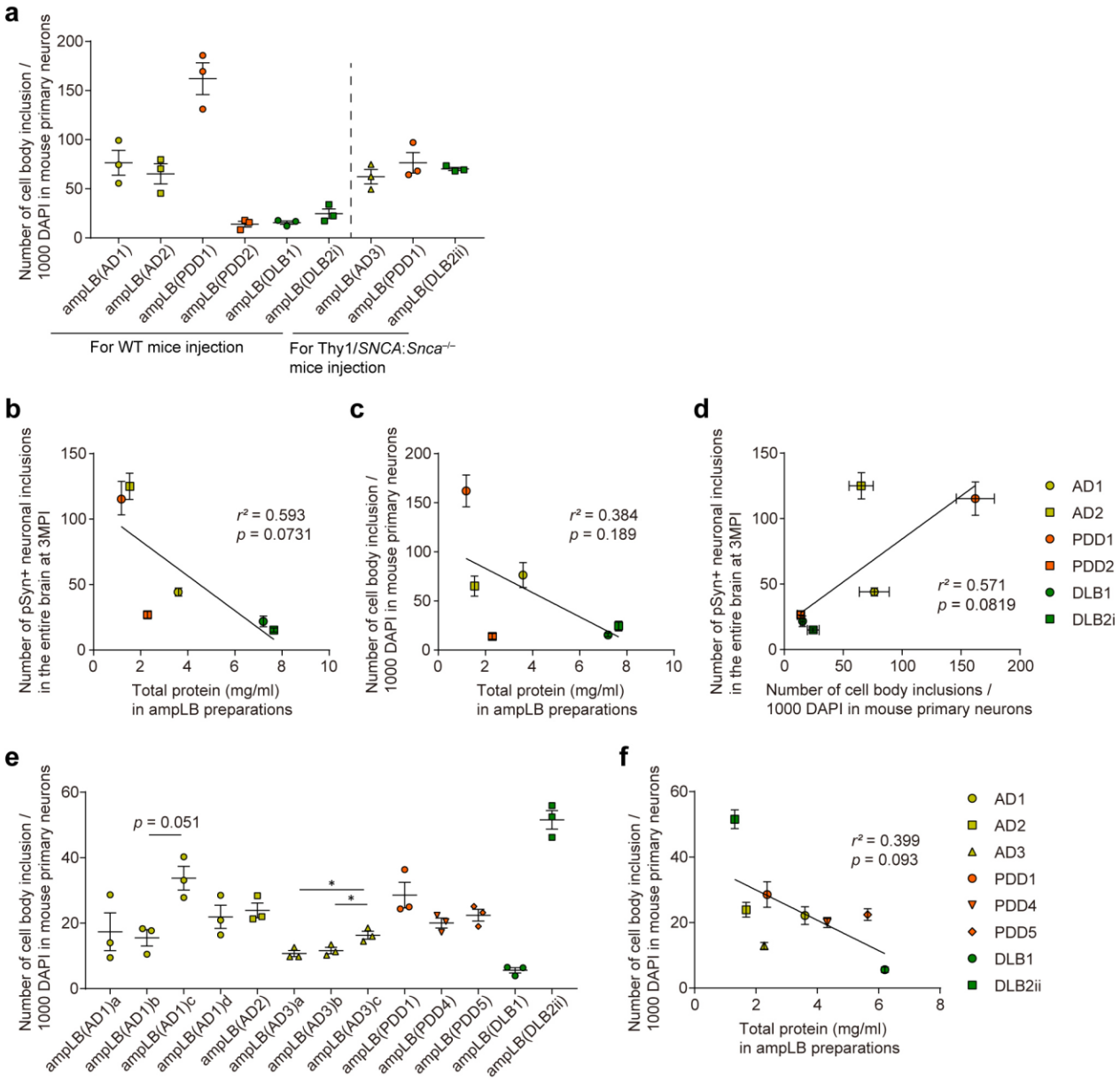
inclusions (n = 3 independent wells per group). One-way ANOVA with a Tukey's post-hoc test was performed; hPFF vs. ampLB(AD1), ampLB(AD2), ampLB(PDD1), ampLB(PDD2), ampLB(DLB1), ampLB(DLB2i), ampLB(AD3), ampLB(PDD1), ampLB(DLB2ii) $p < 0.0001$; hPFF2 vs. ampLB(AD1), ampLB(AD2), ampLB(PDD2), ampLB(DLB1), ampLB(DLB2i), ampLB(AD3), ampLB(PDD1), ampLB(DLB2ii) $p < 0.0001$; hPFF2 vs. ampLB(PDD1) $p = 0.001$). Data are represented as mean ± SEM. Source data are provided as a Source Data file.




**Supplementary Fig. 2 αSyn pathology in the SNpc of ampLB-injected WT mice. a** Double immunofluorescence for tyrosine hydroxylase (TH, green) and pSyn (EP1536Y, red) in the SN of ampLB-injected mice at 6MPI. Scale bar 200 µm. **b** Number of pSyn-positive neuronal inclusions in the SNpc (hPFF 500 ng, n = 4; hPFF 5µg, n = 4, 5, 5; AD1, AD2 500 ng, n = 2, 3, 3; PDD1, PDD2, DLB1, DLB2i 500 ng, n = 3 biologically independent samples). **c** Number of TH-positive cells in the ipsilateral (ipsi) and contralateral (contra) SNpc at 9MPI (n = 3 biologically independent samples per group). A two-tailed unpaired Student's *t*-test was performed between the ipsilateral and contralateral sides for each group (AD2 500 ng $p = 0.0104$; PDD1 500 ng $p = 0.0021$). Data are represented as mean ± SEM. Source data are provided as a Source Data file.

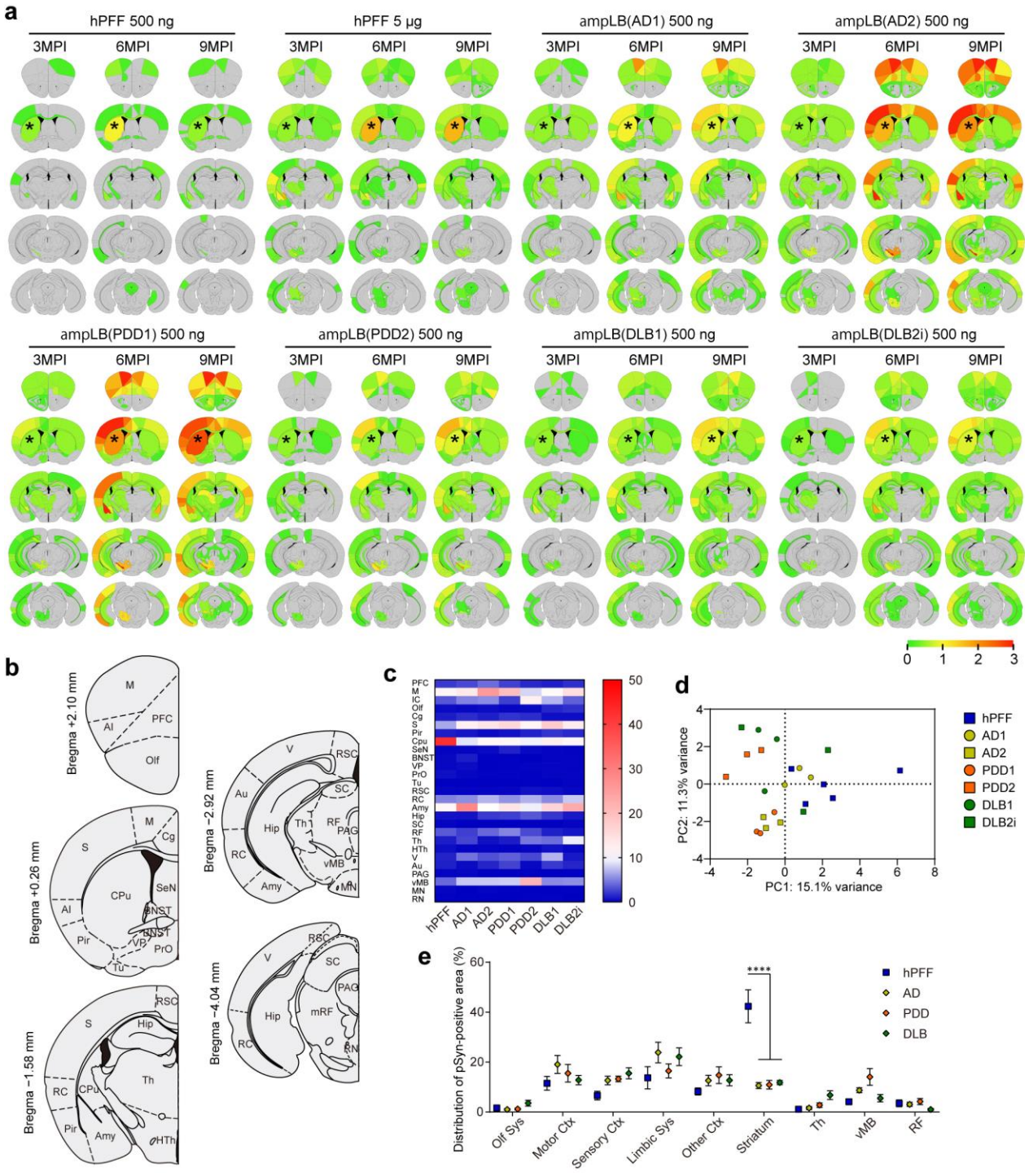



**Supplementary Fig. 3 Biochemical analysis of ampLB-injected WT mouse brains.** Western blot analysis of sarkosyl-insoluble brain lysates of non-injected mice (Non-inj), ampLB (DLB2i)-injected mice at 0MPI (just after injection), 3MPI, and 6MPI. Immunoblots with anti-αSyn antibodies LB509 (human αSyn), D37A6 (mouse αSyn), and Syn1 (human + mouse αSyn), and an anti-pSyn antibody EP1536Y. Images are representative of two independent experiments. Source data are provided as a Source Data file.



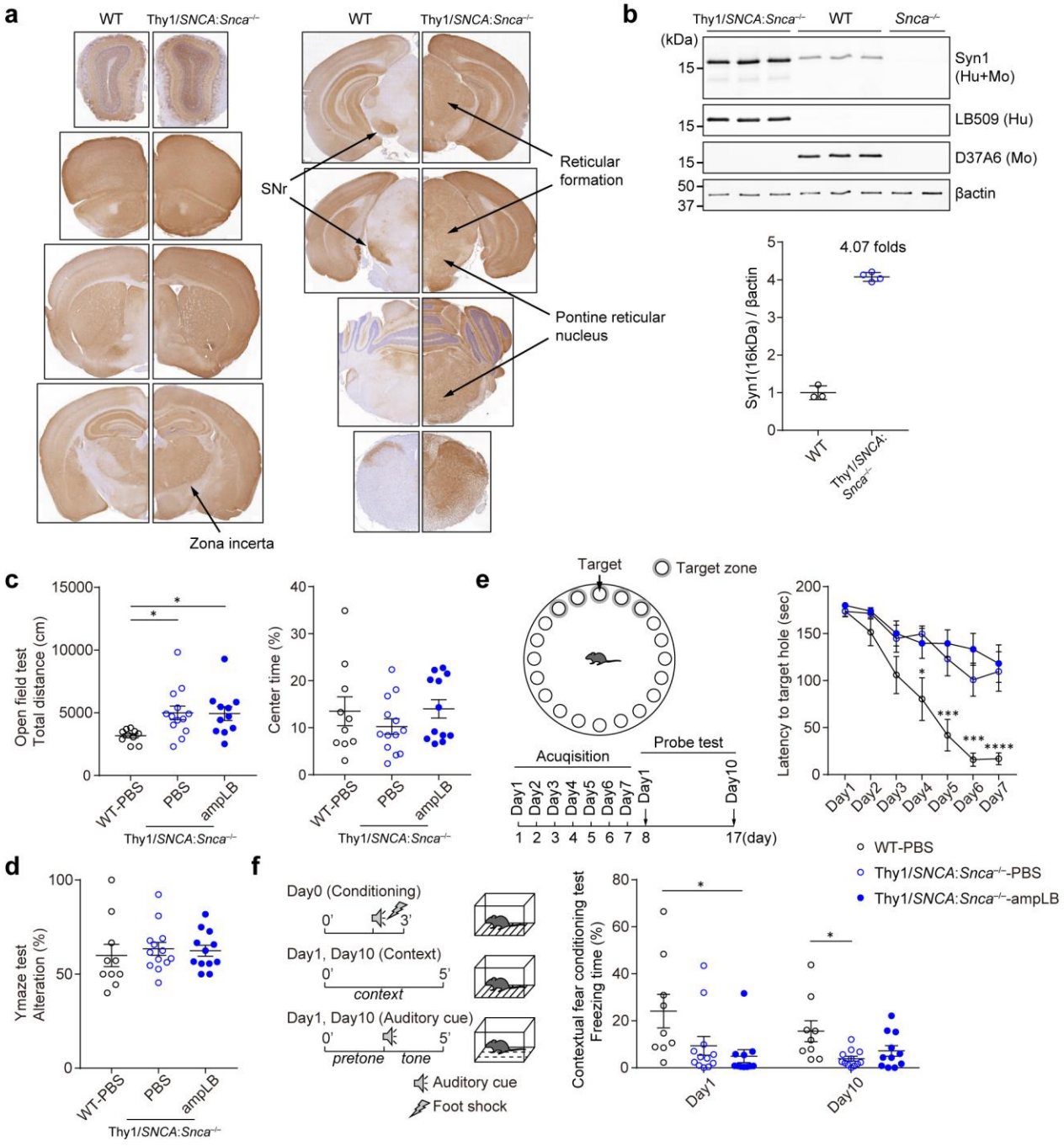

**Supplementary Fig. 4 Correlation among *in vivo* and *in vitro* seeding activity and total protein of ampLB preparations. a** Number of pSyn-positive cell body inclusions per 1,000 DAPI-positive nuclei in mouse primary hippocampal neurons treated with ampLB in Fig. S1 (n = 3 independent wells per group). **b** Correlation between number of pSyn-positive neuronal inclusions in the entire brains of ampLB-injected mice at 3MPI (AD1, AD2, n = 2; PDD1, PDD2, DLB1, DLB2i, n = 3 biologically independent samples) and total protein in ampLB preparations

($r^2$ = 0.593, $p$ = 0.0731). **c** Correlation between number of pSyn-positive cell body inclusions per 1,000 DAPI-positive nuclei in mouse primary hippocampal neurons treated with ampLB (n = 3 independent wells per group) and total protein in ampLB preparations ($r^2$ = 0.384, $p$ = 0.189). **d** Correlation between number of pSyn-positive neuronal inclusions in the entire brains of ampLB-injected mice at 3MPI and number of pSyn-positive cell body inclusions per 1,000 DAPI-positive nuclei in mouse primary hippocampal neurons treated with ampLB ($r^2$ = 0.572, $p$ = 0.0819). **e** Number of pSyn-positive cell body inclusions per 1,000 DAPI-positive nuclei in mouse primary hippocampal neurons treated with an independent batch of ampLB preparations (n = 3 independent wells per group). The ampLB(AD1)a–d and ampLB(AD3)a–c preparations were generated from the same brain lysates. One-way ANOVA with a Tukey's post-hoc test was performed among the ampLB(AD1) and ampLB(AD3) preparations (ampLB(AD1)b vs. ampLB(AD1)c $p$ = 0.0508; ampLB(AD3)a, ampLB(AD3)b vs. ampLB(AD3)c $p$ = 0.0206, 0.0428). **f** Correlation between number of pSyn-positive cell body inclusions per 1,000 DAPI-positive nuclei in mouse primary hippocampal neurons treated with ampLB (AD1, n = 12; AD3, n = 9; AD2, PDD1, PDD4, PDD5, DLB1, DLB2ii, n = 3 independent wells) and total protein in ampLB preparations ($r^2$ = 0.399, $p$ = 0.093). Data are represented as mean ± SEM. Source data are provided as a Source Data file.



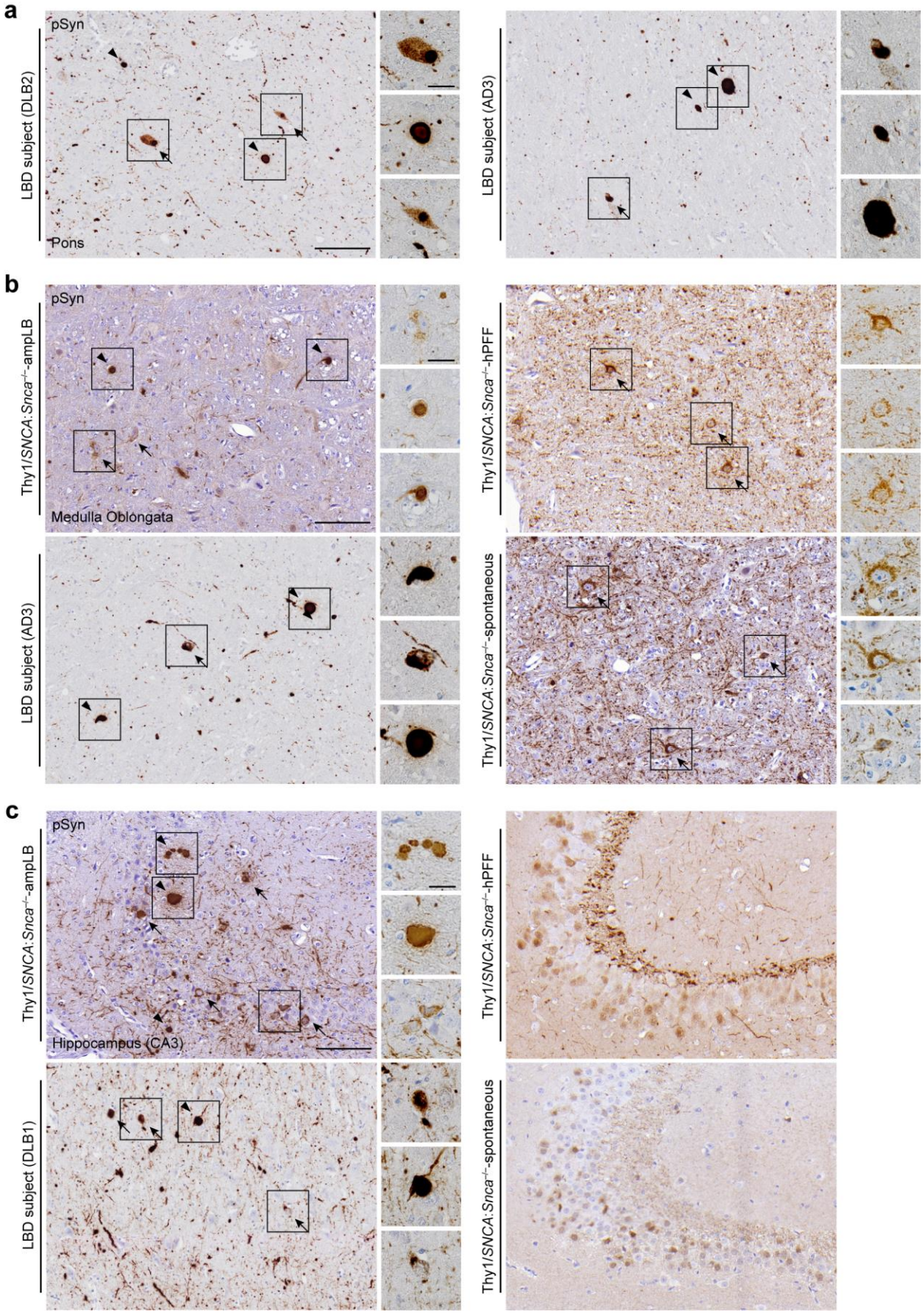

**Supplementary Fig. 5 Differences in distribution of αSyn pathology between hPFF and ampLB in WT mice. a** Heat map colors represent extent of pSyn-positive pathology. **b** Classification of brain regions for quantitative analyses of pSyn-positive area. **c** Heat map colors

represent proportion of pSyn-positive area in each brain region shown in Fig. 5b at 6MPI. **d** Primary component analysis of the distribution of pSyn-positive pathology at 6MPI (hPFF, n = 5; AD1, AD2, PDD1, PDD2, DLB1, DLB2, n = 3 biologically independent samples). **e** Distribution of pSyn-positive pathology in the brain systems (hPFF, n = 5; AD, PDD, DLB, n = 6 biologically independent samples). Olf Sys, olfactory system; Motor Ctx, motor cortex; Sensory Ctx, sensory cortex; Limbic Sys, limbic system; Th, thalamus; vMB, ventral midbrain; RF, reticular formation. Two-way ANOVA with a Sidak's post-hoc test was performed (interaction $p < 0.0001$, hPFF vs. AD, PDD, DLB $p < 0.0001$). Data are represented as mean ± SEM. Source data are provided as a Source Data file.

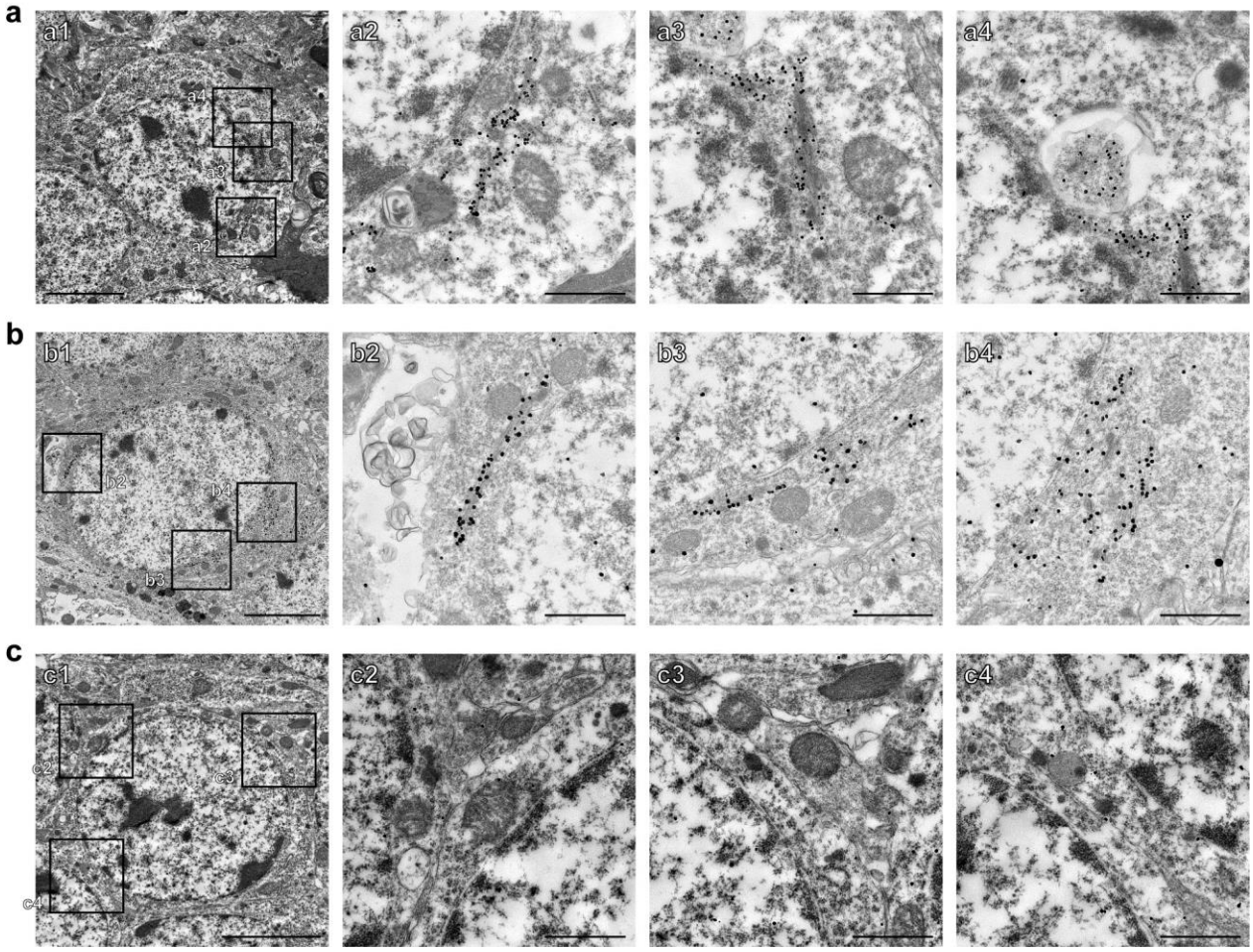



**Supplementary Fig. 6 Modeling LBDs in Thy1:*SNCA*/*Snca*$^{-/-}$ mice. a** Immunohistochemistry with an anti-αSyn antibody (Syn1). SNr, substantia nigra reticulata. **b** Upper panel: Western blot analysis of Triton X-soluble brain lysates of Thy1:*SNCA*/*Snca*$^{-/-}$ mice, WT mice, and *Snca*$^{-/-}$ mice with anti-αSyn (Syn1 [human and mouse αSyn], LB509 [human αSyn], and D37A6 [mouse αSyn]) and βactin antibodies. Lower panel: quantification of Syn1 (16kDa) bands normalized by

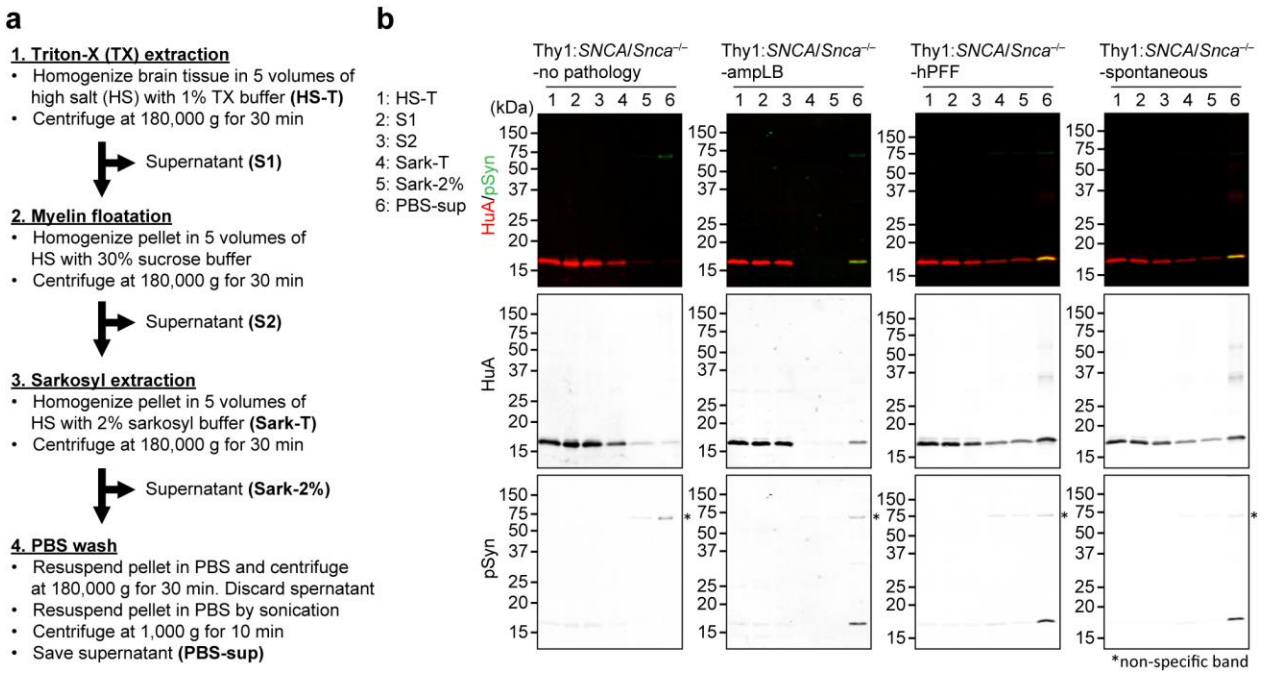
βactin bands (WT, n = 3; Thy1:*SNCA*/*Snca*$^{-/-}$, n = 4 biologically independent samples). **c** Open field test at 7MPI (WT-PBS, n = 10; Thy1:*SNCA*/*Snca*$^{-/-}$-PBS, n = 13; Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB, n = 11 biologically independent samples). Left panel: Total distance traveled. One-way ANOVA with a Tukey's post-hoc test was performed (WT-PBS vs. Thy1:*SNCA*/*Snca*$^{-/-}$-PBS, Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB $p$ = 0.0291, 0.0434). Right panel: Time spent in center area. **d** Y maze test at 7MPI (WT-PBS, n = 10; Thy1:*SNCA*/*Snca*$^{-/-}$-PBS, n = 13; Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB, n = 11 biologically independent samples). **e** Barnes maze test at 7MPI (WT-PBS, n = 10; Thy1:*SNCA*/*Snca*$^{-/-}$-PBS, n = 12; Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB, n = 11 biologically independent samples). Left panel: Schematic representation of experimental design. Right panel: Latency to target hole in acquisition trials. Two-way repeated measures ANOVA with a Tukey's post-hoc test was performed for day (interaction $p < 0.0001$, Day4: WT-PBS vs. Thy1:*SNCA*/*Snca*$^{-/-}$-PBS, Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB $p$ = 0.0021, 0.0123; Day5: WT-PBS vs. Thy1:*SNCA*/*Snca*$^{-/-}$-PBS, Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB $p$ = 0.0002, < 0.0001; Day6: WT-PBS vs. Thy1:*SNCA*/*Snca*$^{-/-}$-PBS, Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB $p$ = 0.0001, < 0.0001; Day7: WT-PBS vs. Thy1:*SNCA*/*Snca*$^{-/-}$-PBS, Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB $p < 0.0001$). **f** Fear conditioning test at 8MPI (WT-PBS, n = 9; Thy1:*SNCA*/*Snca*$^{-/-}$-PBS, n = 12; Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB, n = 11 biologically independent samples). Left panel: Schematic representation of experimental design. Right panel: Freezing time in a contextual fear conditioning test. One-way ANOVA with a Tukey's post-hoc test was performed (Day1: WT-PBS vs. Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB $p$ = 0.0220; Day10: WT-PBS vs. Thy1:*SNCA*/*Snca*$^{-/-}$-PBS $p$ = 0.0118). Source data are provided as a Source Data file.


a
LBD subject (DLB2)
pSyn
Pons
LBD subject (AD3)
b
Thy1/SNCA:Snca−/−-ampLB
pSyn
Medulla Oblongata
LBD subject (AD3)
Thy1/SNCA:Snca−/−-hPFF
Thy1/SNCA:Snca−/−-spontaneous
c
Thy1/SNCA:Snca−/−-ampLB
pSyn
Hippocampus (CA3)
LBD subject (DLB1)
Thy1/SNCA:Snca−/−-hPFF
Thy1/SNCA:Snca−/−-spontaneous

**Supplementary Fig. 7 Similarities and differences in pathological characteristics among Thy1:*SNCA*/*Snca*$^{-/-}$ mouse models and LBD subjects.** Immunohistochemistry with an anti-pSyn antibody (EP1536Y). **a** Pons pathology of the LBD subjects used for brain extraction. **b, c** Medulla oblongata and hippocampus (CA3) pathology of Thy1:*SNCA*/*Snca*$^{-/-}$ mouse models and LBD subjects. Arrows and arrowheads indicate pSyn-positive neuronal inclusions and axonal swelling-like structures, respectively. Scale bars 100 μm, 20 μm (inset).




**Supplementary Fig. 8 Immunoelectron microscopy images of neuronal inclusions in ampLB- and hPFF-injected Thy1:*SNCA*/*Snca*$^{-/-}$ mice. a–c** Immunoelectron micrograph of neurons in the DG with immunogold-labeled pSyn (EP1536Y). **a** pSyn-positive neuron in ampLB-injected Thy1:*SNCA*/*Snca*$^{-/-}$ mice. **b** pSyn-positive neuron in hPFF-injected Thy1:*SNCA*/*Snca*$^{-/-}$ mice. **c** pSyn-negative neuron in ampLB-injected Thy1:*SNCA*/*Snca*$^{-/-}$ mice. Bundles of pSyn-positive filamentous structures in the cytosol (**a2**, **a3**, and **b2–4**) and pSyn-positive amorphous structures in an autophagosome-like vacuole (**a4**). Scale bars 5 μm (**a1**, **b1**, and **c1**) and 1 μm (**a2–4**, **b2–4**, **c2–4**).




**Supplementary Fig. 9 Biochemical extraction of pathological αSyn from Thy1:*SNCA*/*Snca*$^{-/-}$ mouse brains. a** Brief description of the mouse brain extraction protocol. **b** Western blot analysis of brain lysates of Thy1:*SNCA*/*Snca*$^{-/-}$ mice without αSyn pathology (Thy1:*SNCA*/*Snca*$^{-/-}$-no pathology), ampLB-injected Thy1:*SNCA*/*Snca*$^{-/-}$ mice (Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB), hPFF-injected Thy1:*SNCA*/*Snca*$^{-/-}$ mice (Thy1:*SNCA*/*Snca*$^{-/-}$-hPFF), and Thy1:*SNCA*/*Snca*$^{-/-}$ mice with spontaneous αSyn pathology (Thy1:*SNCA*/*Snca*$^{-/-}$-spontaneous). Samples containing 10 µg of total protein was loaded to each lane. Immunoblots with anti-human αSyn (HuA) and pSyn (81A) antibodies. Images are representative of three independent experiments. Source data are provided as a Source Data file.

**Supplementary Table 1. Clinical information.** Clinical information of the cases used for the extraction of pathological αSyn and immunohistochemistry.

| **For extraction of pathological αSyn** | | | |
|---|---|---|---|
| **Case** | **Age of Disease Onset** | **Age at Death** | **PMI (h)** |
| AD1 | 68 | 74 | 19 |
| AD2 | 64 | 72 | 8 |
| AD3 | 59 | 70 | 18 |
| PDD1 | 60 | 72 | 19 |
| PDD2 | 72 | 79 | 6 |
| PDD3 | 42 | 59 | 10 |
| PDD4 | 44 | 73 | 30 |
| PDD5 | 53 | 75 | 6 |
| DLB1 | 78 | 83 | 9 |
| DLB2 | 53 | 63 | 9 |
| Control | - | 62 | 16 |

| **For immunohistochemistry shown in Fig. 6** | | | |
|---|---|---|---|
| **Case** | **Age of Disease Onset** | **Age at Death** | **PMI (h)** |
| LBD | 59 | 76 | 18.5 |

**Supplementary Table 2. Biochemical information for sarkosyl-insoluble fractions of LBD brain extractions.**

| Case | Brain region used | Total protein (µg/µl) | αSyn (ng/µl) | Used for |
|---|---|---|---|---|
| AD1 | Middle Frontal Gyrus | 14.6 | 43 | AmpLB injection into WT mice |
| AD2 | Middle Frontal Gyrus | 8.5 | 58 | AmpLB injection into WT mice |
| AD3 | Middle Frontal Gyrus | 13.5 | 144 | AmpLB injection into Thy1:*SNCA*/*Snca*$^{-/-}$ mice |
| PDD1 | Middle Frontal Gyrus | 9.3 | 95 | AmpLB injection into WT and Thy1:*SNCA*/*Snca*$^{-/-}$ mice |
| PDD2 | Middle Frontal Gyrus | 7.6 | 36 | AmpLB injection into WT mice |
| PDD3 | Middle Frontal Gyrus | 17.5 | 68 | Brain lysate injection into WT mice |
| PDD4 | Middle Frontal Gyrus | 24 | 20 | Transduction into mouse primary hippocampal neurons |
| PDD5 | Middle Frontal Gyrus | 17.5 | 19 | Transduction into mouse primary hippocampal neurons |
| DLB1 | Middle Frontal Gyrus | 7.2 | 12 | AmpLB injection into WT mice |
| DLB2i | Middle Frontal Gyrus | 14.9 | 20 | AmpLB injection into WT mice |
| DLB2ii | Middle Frontal Gyrus | 8.8 | 140 | AmpLB injection into Thy1:*SNCA*/*Snca*$^{-/-}$ mice |
| Control | Middle Frontal Gyrus | 4.8 | N.D. | Brain lysate injection into WT mice |

N.D., not determined.

**Supplementary Table 3. Biochemical information for sarkosyl-insoluble fractions of Thy1/SynKO mouse brain extractions.**

| Mice | Total protein (µg/µl) | αSyn (ng/µl) |
|---|---|---|
| Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB(PDD1)-1 | 5.11 | 15.0 |
| Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB(PDD1)-2 | 3.26 | 6.32 |
| Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB(DLB2ii)-1 | 3.75 | 10.2 |
| Thy1:*SNCA*/*Snca*$^{-/-}$-ampLB(DLB2ii)-2 | 5.55 | 15.85 |
| Thy1:*SNCA*/*Snca*$^{-/-}$-hPFF-1 | 4.21 | 40.0 |
| Thy1:*SNCA*/*Snca*$^{-/-}$-hPFF-2 | 2.49 | 65.0 |
| Thy1:*SNCA*/*Snca*$^{-/-}$-spontaneous-1 | 1.68 | 26.8 |
| Thy1:*SNCA*/*Snca*$^{-/-}$-spontaneous-2 | 4.25 | 53.5 |
| Thy1:*SNCA*/*Snca*$^{-/-}$-no pathology | 4.18 | 1.57 |