

Supplementary information

Supplementary Figure 1. Colonization of *S. mutans* in germ-free (GF) mice.

Supplementary Figure 2. Colonization with *E. coli* expressing *urdA* of the *S. mutans* in GF mice.

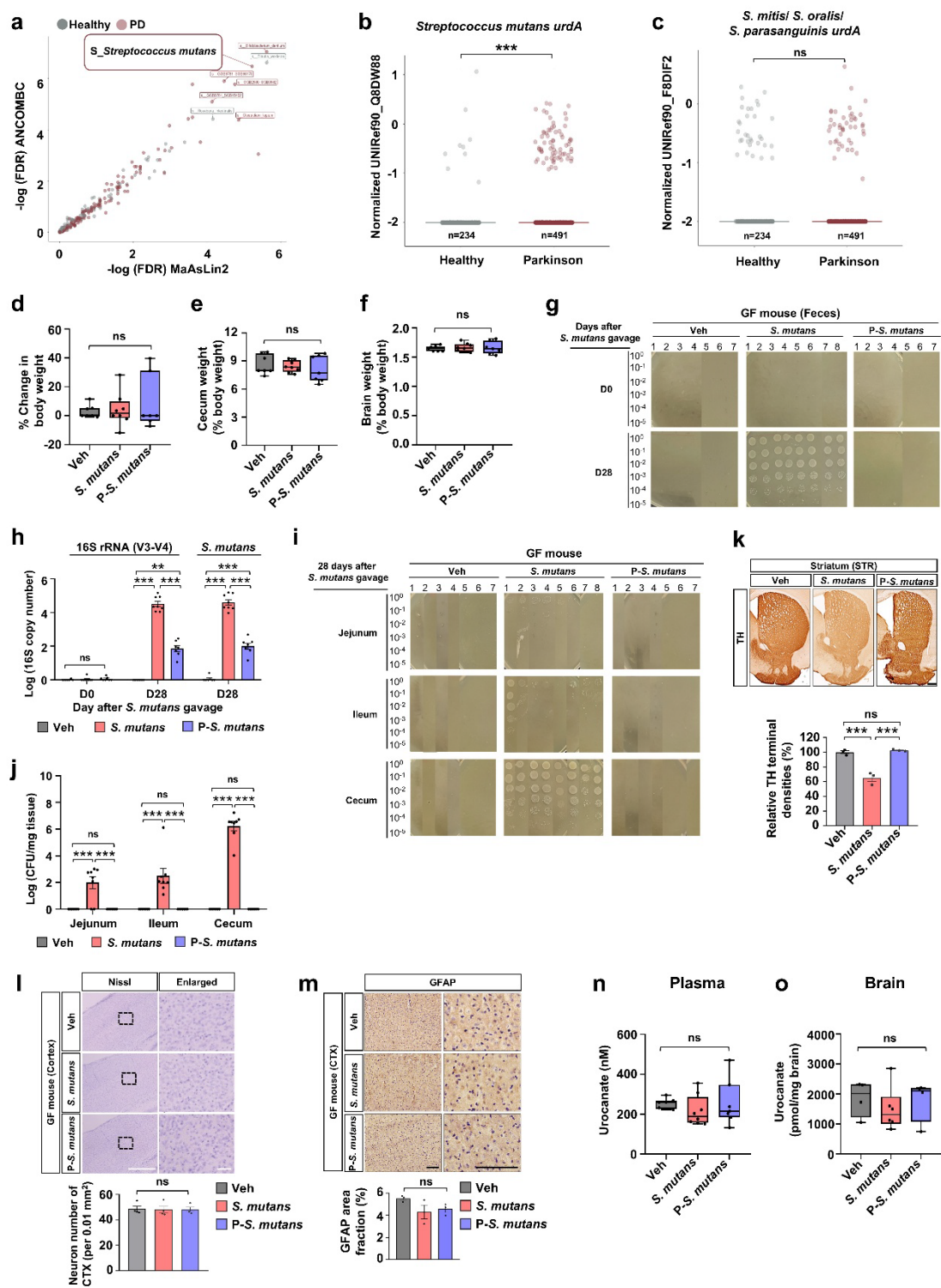
Supplementary Figure 3. Dopaminergic p4E-BP expression, *S. mutans* colonization, physiological changes, and urocanate levels in mice.

Supplementary Figure 4. Contribution of ImP and *S. mutans* to α -synuclein aggregation, mTORC1 signaling, astrogliosis, and microgliosis in in vitro and in vivo models of α -synucleinopathy.

Supplementary Figure 5. Selective dopaminergic toxicity and behavior deficits induced by systemic imidazole propionate, and mTORC1-dependent dopaminergic toxicity and astrogliosis by nigral imidazole propionate injection.

Supplementary Table 1. Demographic summary of PD and healthy cohort in this study.

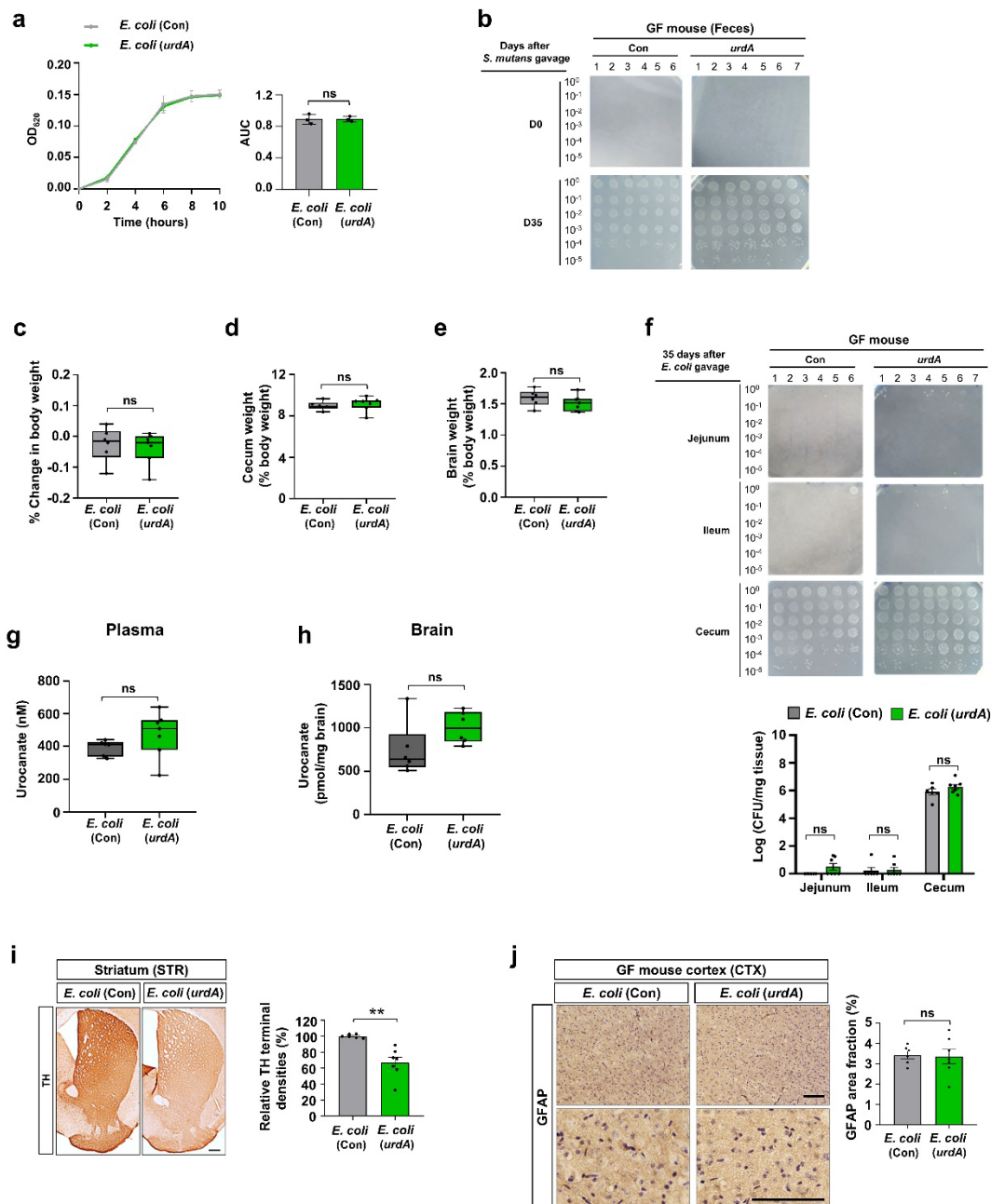
Source Data File for Supplementary information. Unprocessed western blot images of Supplementary Figure 4.



Supplementary Figure 1. Colonization of *S. mutans* in germ-free (GF) mice. **a**, False discovery rate (FDR) of differential abundance analysis using MaAsLin2 (X-axis) and ANCOM-BC (Y-axis), based on species present in > 5% of samples with a relative abundance > 0.05. FDR values were log₁₀-transformed. **b,c**, Normalized gene copy numbers of urocanate

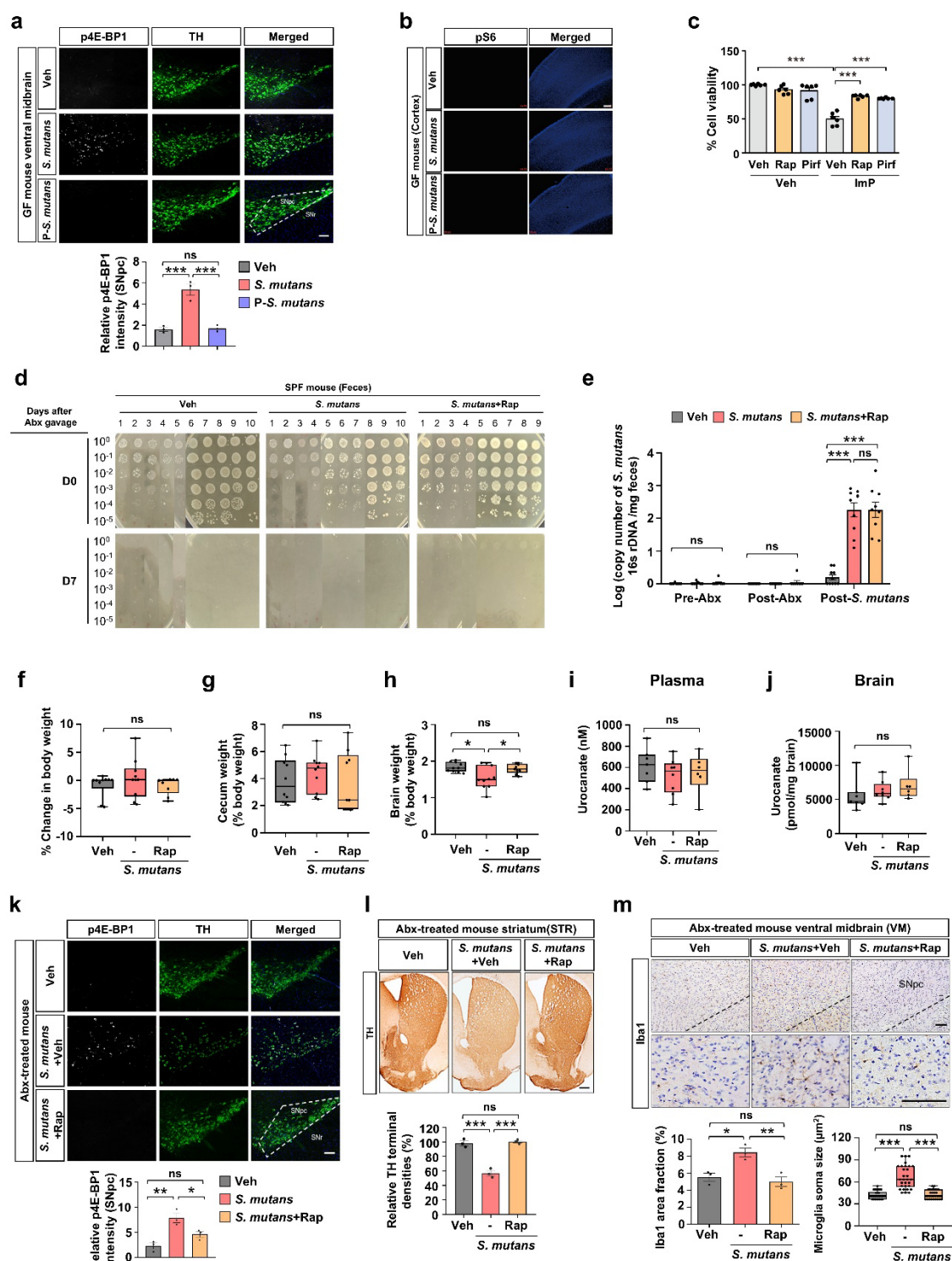
reductase (*urdA*) of *Streptococcus mutans* (Q8DW88, **b**), *Streptococcus mitis*, *S. oralis*, and *S. parasanguinis* (F8DIF2, **c**). **d**, Percent changes in body weight in GF mice gavaged with vehicle (Veh), *S. mutans*, or pasteurized *S. mutans* (P-*S. mutans*) for four weeks ($n = 7$ mice per Veh and P-*S. mutans* group, $n = 8$ mice per *S. mutans* group). **e,f**, Effect of gut-colonized *S. mutans* on cecum weight (**e**) and brain weight (**f**) in the indicated experimental mouse groups (organ weight normalized with body weight (%), $n = 7$ per Veh and P-*S. mutans* group, $n = 8$ per *S. mutans* group). **g**, Viability of *S. mutans* assessed by colony-forming ability by plating serially diluted fecal samples from GF mice before and 28 days after *S. mutans* colonization ($n = 7$ mice per Veh and P-*S. mutans* group, $n = 8$ mice per *S. mutans* group). **h**, Quantification of universal bacterial and *S. mutans*-specific 16S rDNA copy numbers in fecal samples from GF mice before and 28 days after *S. mutans* colonization ($n = 7$ mice per Veh and P-*S. mutans* group, $n = 8$ mice per *S. mutans* group). **i**, Viability of *S. mutans* assessed in terms of colony forming ability by plating serially diluted small intestinal (jejunum, ileum) and cecal samples from GF mice 28 days after *S. mutans* colonization ($n = 7$ mice per Veh and P-*S. mutans* group, $n = 8$ mice per *S. mutans* group). **j**, Quantification of viable colonies from (**i**), expressed as colony-forming units (CFU) per mg tissue ($n = 7$ mice per Veh and P-*S. mutans* group, $n = 8$ mice per *S. mutans* group). **k**, (Upper panel) Representative anti-TH immunohistochemistry images in the striatum from the indicated experimental mouse groups. (Bottom panel) Quantification of TH-positive dopaminergic axon terminal densities in the striatum ($n = 3$ mice per group). Scale bar = 500 μm . **l**, Representative anti-Nissl immunohistochemistry images of cortical neurons from GF mice colonized with vehicle, live *S. mutans*, or P-*S. mutans*. Quantification of Nissl-stained neuron densities in the cortex (CTX) of the indicated experimental mouse groups was shown below ($n = 4$ mice per group). Scale bar = 500, and 50 μm for enlarged images, respectively. **m**, (Upper panel) Anti-GFAP immunohistochemistry images of the CTX of the indicated experimental mouse groups. Magnified images are also presented on the right. (Bottom panel) Quantified % area fraction of GFAP signals in the CTX is shown in the right panel ($n = 3$ mice group). Scale bar = 100 μm . **n**, Quantification of urocanate concentrations in the plasma from GF mice colonized with vehicle, live *S. mutans*, or P-*S. mutans* ($n = 7$ mice per Veh and P-*S. mutans* group, $n = 8$ mice per *S. mutans* group). **o**, Quantification of urocanate concentration in the brains from GF mice colonized with vehicle, live *S. mutans*, or P-*S. mutans* ($n = 4$ mice per Veh and P-*S. mutans* group, $n = 6$ mice per *S. mutans* group). Quantitative data are expressed as the mean $\pm$ S.E.M. $**P < 0.01$, $***P < 0.001$; ns, not significant. *P* values were determined using the Wilcoxon rank-sum test (**b**, **c**), one-way

ANOVA with Tukey's *post hoc* test (**d-f, j-o**) and two-way ANOVA with Tukey's *post hoc* test (**h**).



Supplementary Figure 2. Colonization with *E. coli* expressing *urdA* of the *S. mutans* in GF mice. **a**, Growth curve of *E. coli* MG1655 strains expressing either the empty pUC19 vector (*E. coli* (Con)) or the recombinant plasmid pUC19-*urdA* (*E. coli* (*urdA*)) was monitored by measuring the optical density of the culture medium at 620 nm (OD₆₂₀) every 2 hours. The overall growth of each strain was quantified by calculating the area under the curve (AUC) in the right panel ($n = 3$ per group). **b**, Viability of *E. coli* assessed by colony-forming ability through plating serially diluted fecal samples from GF mice before and 35 days after *E. coli*

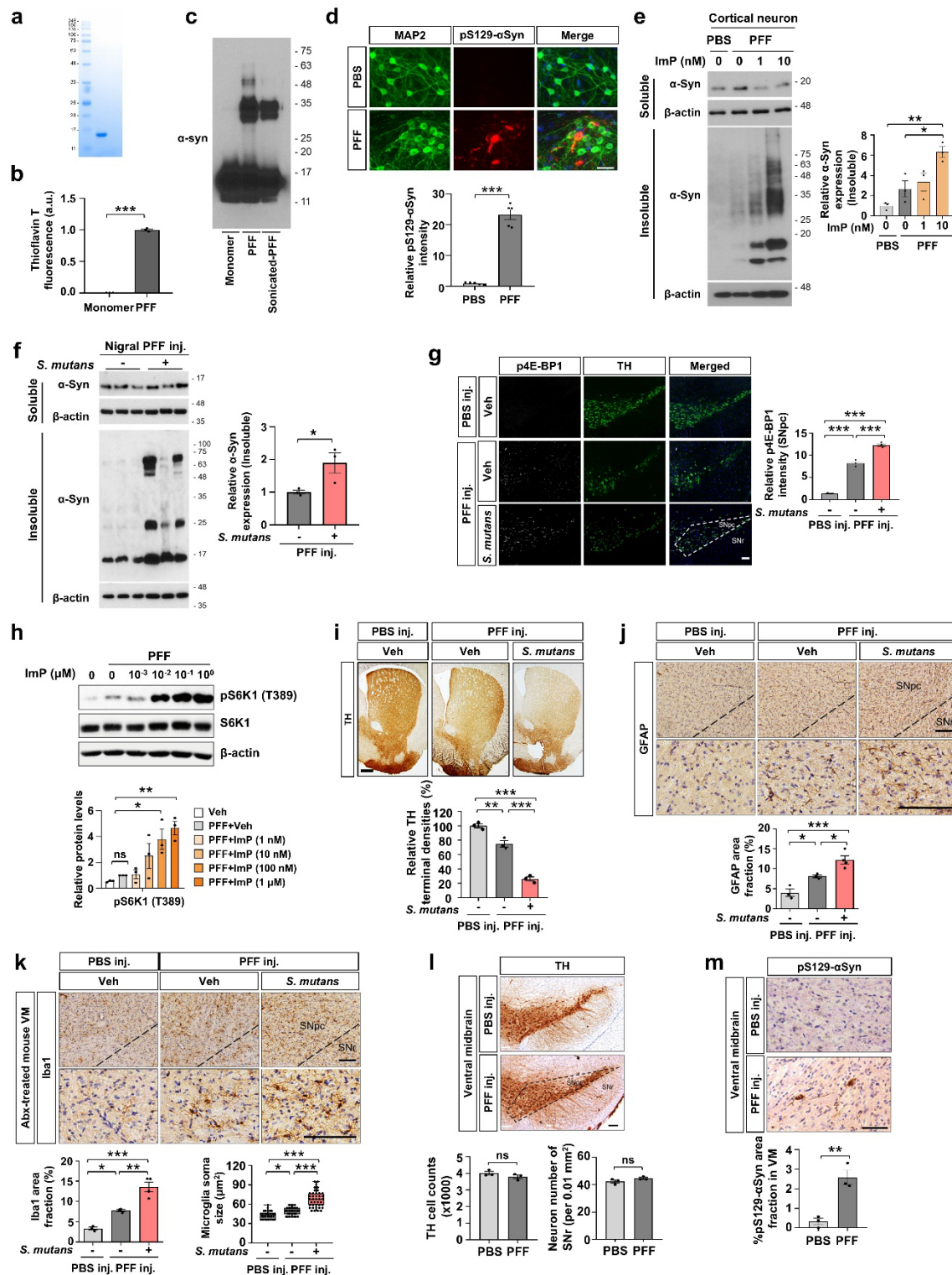
colonization ($n = 6$ mice per *E. coli* (Con) group, $n = 7$ mice per *E. coli* (*urda*) group). CFU, colony-forming unit. **c**, Percent changes in body weight of GF mice gavaged with *E. coli* (Con) or *E. coli* (*urda*) for 35 days ($n = 6$ mice per *E. coli* (Con) group, $n = 7$ mice per *E. coli* (*urda*) group). **d,e**, Effect of gut-colonized *E. coli* (Con) or *E. coli* (*urda*) on cecum weight (**d**) and brain weight (**e**) in the indicated experimental mouse groups (organ weight normalized with body weight (%), $n = 6$ per *E. coli* (Con) group, $n = 7$ per *E. coli* (*urda*) group). **f**, Viability of *E. coli* assessed by colony-forming ability by plating serially diluted samples from small intestinal (jejunum, ileum) and cecum of GF mice 35 days after *E. coli* colonization. Quantification of live *E. coli* in the small intestinal and cecal samples from GF mice are shown below ($n = 6$ mice per *E. coli* (Con), $n = 7$ mice per *E. coli* (*urda*) group). **g,h**, Quantification of urocanate concentration in the plasma (**g**) and brains (**h**) from GF mice gavaged with *E. coli* (Con) or *E. coli* (*urda*) (plasma: $n = 6$ mice per *E. coli* (Con) and $n = 7$ mice per *E. coli* (*urda*) group; brain: $n = 6$ mice per group). **i**, (Left panel) Representative anti-TH immunohistochemistry images of the striatum from the indicated experimental mouse groups. (Right panel) Quantification of TH-stained dopaminergic axon terminal densities in the striatum ($n = 6$ mice per *E. coli* (Con) group, $n = 7$ mice per *E. coli* (*urda*) group). Scale bar = 500 μm . **j**, (Left panel) Representative anti-GFAP immunohistochemistry of the CTX of GF mice colonized with *E. coli* (Con) or *E. coli* (*urda*). Magnified images of the CTX are shown below. (Right panel) Quantified % area fraction of GFAP signals ($n = 6$ mice per *E. coli* (Con) group, $n = 7$ mice per *E. coli* (*urda*) group). Scale bar = 100 μm . Quantitative data are expressed as the mean $\pm$ S.E.M. ns, not significant. *P* values were determined using the unpaired two-tailed Student's *t*-tests.



Supplementary Fig. 3. Dopaminergic p4E-BP expression, *S. mutans* colonization, physiological changes, and urocanate levels in mice. **a**, Representative immunofluorescence images of p4E-BP1 and TH in the ventral midbrain coronal sections from the GF mice colonized with vehicle, *S. mutans*, or pasteurized *S. mutans* (P-*S. mutans*). Original red p4E-

BP1 immunofluorescence signals are pseudocolored in white. Quantification of relative p4E-BP1 signal intensities in TH-positive dopamine neurons of the substantia nigra pars compacta is shown below ($n = 3$ mice per group). Scale bar = 100 μm . **b**, Representative immunofluorescence images of S235/S236 phosphorylated ribosomal protein (pS6) in the cortex sections from GF mice colonized with Veh, *S. mutans*, or P-*S. mutans*. Scale bar = 100 μm . **c**, Viability assessment of primary cortical neurons treated with ImP (100 μM , 24 h) in the presence or absence of rapamycin (Rap, 20 nM, 24 h) or Pirfenidone (Pirf, 1 mM, 24 h), determined by the Cell Counting Kit-8 assay ($n = 6$ per group). **d**, Assessment of successful gut sterilization in specific pathogen-free (SPF) mice after treatment with antibiotics cocktail via viable colony formation. Colony forming ability was assessed by plating serially diluted fecal samples from GF mice before and seven days after antibiotics treatment ($n = 10$ mice per Veh and *S. mutans* group, $n = 9$ mice per *S. mutans*+Rap group). **e**, Quantification of the copy number of *S. mutans*-specific 16S rDNA in fecal samples at three time points—pre-Abx (before antibiotics cocktail treatment), post-Abx (after antibiotics cocktail treatment), and post-*S. mutans* (after *S. mutans* administration)—in the indicated experimental groups ($n = 10$ mice per Veh and *S. mutans* group, $n = 9$ mice per *S. mutans*+Rap group). **f**, Percent changes in body weight two weeks after *S. mutans* colonization with or without rapamycin (Rap) in antibiotic-treated mice ($n = 10$ mice per Veh and *S. mutans* group, $n = 9$ mice per *S. mutans*+Rap group). **g,h**, Effect of gut-colonized *S. mutans* on cecum weight (**g**) and brain weight (**h**) in antibiotic-treated mice colonized with *S. mutans* with or without rapamycin (Rap) ($n = 10$ mice per Veh and *S. mutans* group, $n = 9$ mice per *S. mutans*+Rap group). **i,j**, Quantification of urocanate concentration in the plasma (**i**) and brains (**j**) from the indicated experimental groups ($n = 7$ mice per Veh group, $n = 8$ mice per *S. mutans* group, $n = 6$ mice per *S. mutans*+Rap group). **k**, Representative immunofluorescence images of p4E-BP1 and TH in the ventral midbrain coronal sections from the indicated experimental groups. Original red p4E-BP1 immunofluorescence signals are pseudocolored in white. Quantification of relative p4E-BP1 signal intensities in TH-positive dopamine neurons of the substantia nigra pars compacta is shown below ($n = 3$ mice per group). Scale bar = 100 μm . **l**, (Upper panel) Representative anti-TH immunohistochemistry images of the striatum from the indicated experimental mouse groups. (Bottom panel) The quantification of TH-stained dopaminergic axon terminal densities in the striatum ($n = 3$ mice per group). Scale bar = 500 μm . **m**, (Left panel) Inflammatory microgliosis in the ventral midbrains of indicated experimental antibiotic-treated mouse groups determined by anti-Iba1 immunohistochemistry. Magnified images of the SNpc are shown below. (Right panel) Quantified % area fraction of Iba1 signals ($n = 3$ mice per group) and

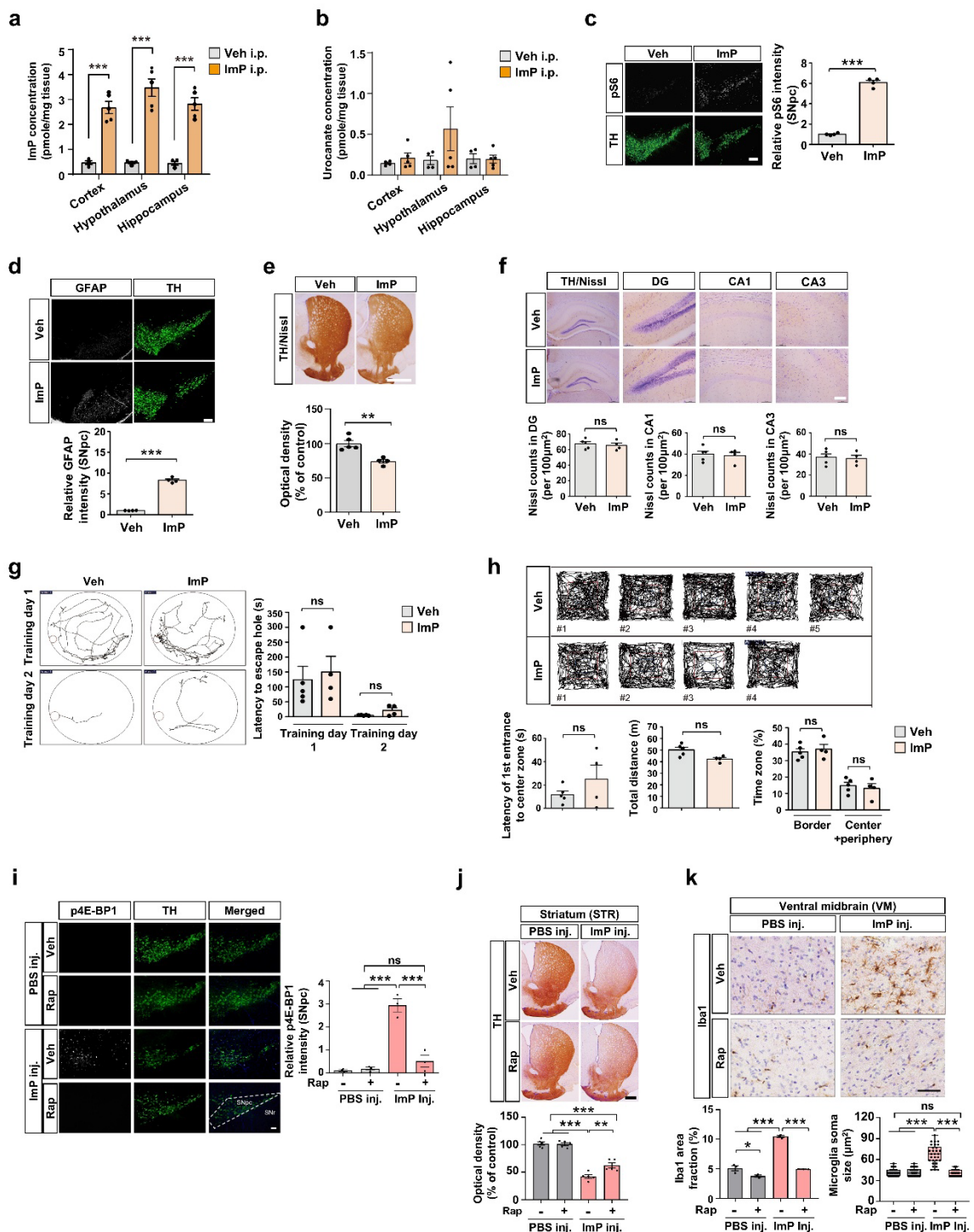
microglial soma size ($n = 30$ microglia from 3 mice per group). Scale bar = 100 μm . Quantitative data are expressed as the mean $\pm$ S.E.M. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$; ns, not significant. P values were determined using one-way ANOVA with Tukey's *post hoc* test (**a**, **c**, **f-m**) and two-way ANOVA with Tukey's *post hoc* test (**e**).



Supplementary Fig. 4. Contribution of ImP and *S. mutans* to α -synuclein aggregation, mTORC1 signaling, astrogliosis, and microgliosis in *in vitro* and *in vivo* models of α -synucleinopathy. **a, SDS-PAGE analysis of purified recombinant α -synuclein by Coomassie Blue staining. **b**, Quantification of thioflavin T fluorescence for both α -synuclein monomers**

and PFF ($n = 3$ per group). **c**, Immunoblot of recombinant α -synuclein (α -Syn) monomer, non-sonicated and sonicated PFF detected with anti- α -synuclein antibody. **d**, (Upper panel) Representative immunofluorescence images showing the α -Syn aggregation marker S129 phosphorylated α -Syn (pS129- α -Syn) and neuronal marker microtubule-associated protein 2 (MAP2) in mouse primary cultured cortical neurons treated with α -Syn PFF (1 μ g treated once and incubated for fourteen days), counterstained with DAPI. Scale bar = 50 μ m. Bottom panel) Quantification of relative immunofluorescence intensities of pS129- α -Syn in the indicated experimental groups ($n = 5$ per group). **e**, (Left Panel) Representative western blots of α -Syn expression in the Triton X-100-soluble and insoluble fractions prepared from primary cultured cortical neurons treated with PFF (0.1 μ g/mL) followed by treatment with the indicated concentrations of ImP (1, 10 nM). (Right panel) Quantification of relative expression levels of insoluble α -synuclein expression in the indicated experimental groups ($n = 3$ per group). β -actin serves as an internal loading control for both soluble and insoluble fractions. **f**, (Left panel) Immunoblot of α -Syn expression in the Nonidet P-40 (NP-40)-soluble and insoluble fractions from the ventral midbrains of the indicated experimental mouse groups ($n = 3$ mice per group). β -actin serves as an internal loading control. **g**, Representative immunofluorescence images of p4E-BP1 and TH in the ventral midbrain coronal sections from the PBS-injected control or nigral α -Syn PFF-injected mice colonized with *S. mutans* or vehicle. Nuclei were counterstained with DAPI. Original red pS6 immunofluorescence signals are pseudocolored in white. Quantification of relative p4E-BP1 signal intensities in TH-positive dopamine neurons of the SNpc is shown in the right panel ($n = 3$ mice per PBS-injected and PFF-injected group, $n = 4$ PFF + *S. mutans* group). Scale bar = 100 μ m. **h**, (Upper panel) Effects of ImP on pS6K1 (T389), and S6K1 in primary cortical neurons of α -synucleinopathy induced by PFF treatment (0.1 μ g/mL for seven days). ImP was treated at the indicated concentrations (1, 10, 100, 1000 nM) every three days for a total of six days. (Bottom panel) Quantification of relative expression levels of pS6K1 (T389) in the experimental groups. normalized to β -actin internal loading control ($n = 3$ per group). **i**, (Upper panel) Representative anti-TH immunohistochemistry images of the striatum from the indicated experimental mouse groups. (Bottom panel) The quantification of TH-stained dopaminergic axon terminal densities in the striatum of each mouse group ($n = 3$ mice per group). Scale bar = 500 μ m. **j**, (Left panel) Assessment of inflammatory astrogliosis in the ventral midbrains of PBS-injected control or nigral α -Syn PFF-injected mice colonized with *S. mutans* or vehicle determined by anti-GFAP immunohistochemistry. Magnified images are shown below. (Right panel) Quantified % area fraction of GFAP signals ($n = 3$ mice per PBS-injected and PFF-injected group, $n = 4$ PFF + *S.*

mutans group). Scale bar = 100 μ m. **k**, (Upper panel) Assessment of inflammatory microgliosis in the ventral midbrains of PBS-injected control or nigral α -Syn PFF-injected mice colonized with *S. mutans* or vehicle determined by anti-Iba1 immunohistochemistry. Magnified images are shown below. (Bottom panel) Quantified % area fraction of Iba1 signals ($n = 3$ mice per PBS-injected and PFF-injected group, $n = 4$ PFF + *S. mutans* group) and microglial soma size ($n = 30$ microglia from 3 mice per PBS-injected and PFF-injected group, $n = 40$ microglia from 4 mice per PFF + *S. mutans* group). Scale bar = 100 μ m. **l**, (Upper panel) Representative anti-TH immunohistochemistry of ventral midbrains from the PBS-injected control or nigral α -Syn PFF-injected SPF mice (10 μ g α Syn PFF, 1-month incubation). (Bottom panel) Stereological counts of TH-stained dopaminergic neurons in the SNpc and Nissl-stained neurons in the SNr of the indicated mouse group ($n = 3$ mice per group). Scale bar = 100 μ m. **m**, (Upper panel) Representative immunohistochemistry images showing pS129- α -Syn in the ventral midbrain of the PBS-injected control or nigral α -Syn PFF-injected mice (10 μ g α -Syn PFF, 1-month incubation). (Bottom panel) Quantified % area fraction of pS129- α -Syn signals ($n = 3$ mice per group). Scale bar = 100 μ m. Quantitative data are expressed as the mean $\pm$ S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ns, not significant. P values were determined using one-way ANOVA with Tukey's *post hoc* test (**e**, **g-k**) and unpaired two-tailed Student's *t*-tests (**b**, **d**, **f**, **l**, **m**).



Supplementary Fig. 5. Selective dopaminergic toxicity and behavior deficits induced by systemic imidazole propionate, and mTORC1-dependent dopaminergic toxicity and astrogliosis by nigral imidazole propionate injection. a, Measurement of ImP concentration

in brain subregions (cortex, hypothalamus, and hippocampus) from mice following daily intraperitoneal (i.p.) injection of ImP ($n = 5$) or vehicle (Veh, 1% DMSO, $n = 4$) for three days.

b, Measurement of urocanate concentration in brain subregions (cortex, hypothalamus, and hippocampus) from mice following daily intraperitoneal injection ImP ($n = 5$) or vehicle (Veh, 1% DMSO, $n = 4$) for three days.

c, (Left panel) Representative immunofluorescence images of pS6 and TH in the ventral midbrain coronal sections from mouse groups intraperitoneally injected with 20 μ g ImP for three weeks. Original red pS6 immunofluorescence signals are pseudocolored in white. (Right panel) Quantification of relative pS6 signal intensities in TH-positive dopamine neurons of the substantia nigra pars compacta ($n = 4$ mice per group).

d, (Upper panel) Representative immunofluorescence images of GFAP and TH in the ventral midbrain coronal sections from the mouse groups with the indicated treatments. Original red GFAP immunofluorescence signals are pseudocolored in white. (Bottom panel) Quantification of relative GFAP signal intensities in the SNpc with TH-positive dopamine neurons ($n = 4$ mice per group). Scale bar = 100 μ m.

e, (Left panel) Representative anti-TH immunohistochemistry images (Nissl counterstained) of the striatum from each experimental mouse group. Magnified images showing dopaminergic axon terminals were also presented below. (Right panel) Relative optical densities of TH fibers in the striatum of ImP-treated mice compared to vehicle-treated control ($n = 5$ mice per Veh group, $n = 4$ mice per ImP group). Scale bar = 800 and, 100 μ m for enlarged images, respectively.

f, (Upper panel) Coronal hippocampal brain section from each experimental mouse group stained with Nissl. Magnified images for subregions of the hippocampus (dentate gyrus (DG), CA1, and CA3) are presented on the right. (Bottom panel) Quantification of Nissl-stained hippocampal neurons in the indicated subregions ($n = 5$ mice per Veh group, $n = 4$ mice per ImP group).

g, (Left panel) Representative mouse exploratory trace on the circular arena in the Barnes maze test carried on the first and the second trial (1-day interval) examining spatial learning and memory. The cage hidden under the arena is indicated by a red circle. (Right panel) Latency to find the target hole in the Barnes maze in two separate trials for each mouse group treated with ImP or vehicle ($n = 5$ mice per Veh group, $n = 4$ mice per ImP group).

h, (Upper panel) Representative open field exploratory path by mice treated with vehicle (Veh, 1% DMSO) or 20 μ g ImP for three weeks. (bottom panel) The latency to enter the central zone, total distance traveled, and % time spent in the indicated zones (border or center/periphery zones) for the mice of each experimental group in the open field test ($n = 5$ mice per Veh group, $n = 4$ mice per ImP group).

i, Representative immunofluorescence images of p4E-BP1 and TH in the ventral midbrain coronal sections from mice with intranigral brain injections of PBS or ImP with or without mTORC1 inhibitor,

rapamycin (Rap). Original red p4E-BP1 immunofluorescence signals are pseudocolored in white. Quantification of relative p4E-BP1 signal intensities in TH-positive dopamine neurons of the SNpc is shown in the right panel ($n = 3$ mice per group). Scale bar = 100 μm . **j**, (Upper panel) Representative anti-TH immunohistochemistry images of the striatum from the indicated experimental mouse groups. (Bottom panel) The quantification of TH-stained dopaminergic axon terminal densities in the striatum of each mouse group ($n = 5$ mice per group). Scale bar = 500 μm . **k**, (Upper panel) Inflammatory microgliosis in the ventral midbrains of mice with nigral ImP or PBS injection with or without rapamycin treatment determined by anti-Iba1 immunohistochemistry. (Bottom panel) Quantified % area fraction of Iba1 signals ($n = 3$ mice per group) and microglial soma size in the SNpc ($n = 30$ microglia from 3 mice per group). Scale bar = 100 μm . Quantitative data are expressed as the mean $\pm$ S.E.M** $P < 0.01$, *** $P < 0.001$; ns, not significant. P values were determined using unpaired two-tailed Student's t -tests (**a-h**) and one-way ANOVA with Tukey's *post hoc* test (**i-k**).

Supplementary Table 1. Demographic summary of PD and healthy cohort in this study

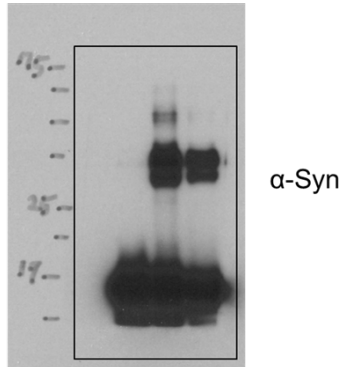
	PD	Healthy	<i>P</i> value
n	65	65	-
Men, n (%)	28 (43)	30 (46)	>0.999
Age at sampling	65.2 ± 0.9	63.9 ± 0.9	0.816
HY stage at sampling	2.2 ± 0.7	NA	-
PD duration at sampling	9.5 ± 2.9	NA	-
Type 2 diabetes, n (%)	6 (9)	NA	-

Abbreviations: HY, Hoehn and Yahr; NA, not applicable; PD, Parkinson's disease. Data are presented as mean ± S.E.M. or as proportions. *P* values were calculated using chi square test for categorical variables.

Source Data File for Supplementary information. Unprocessed western blot images of Supplementary Figure 4.

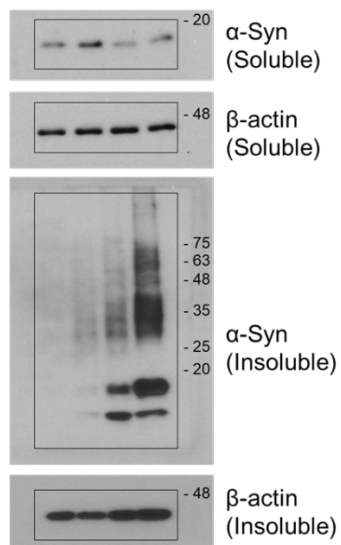
Source data Supplementary Fig. 4c

Unprocessed Western Blots

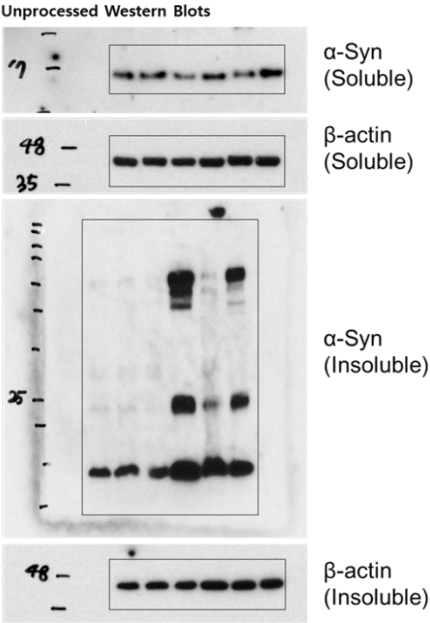


Source data Supplementary Fig. 4e

Unprocessed Western Blots



Source data Supplementary Fig. 4f



Source data Supplementary Fig. 4h

