SUPPLEMENTARY FIGURES AND TABLES

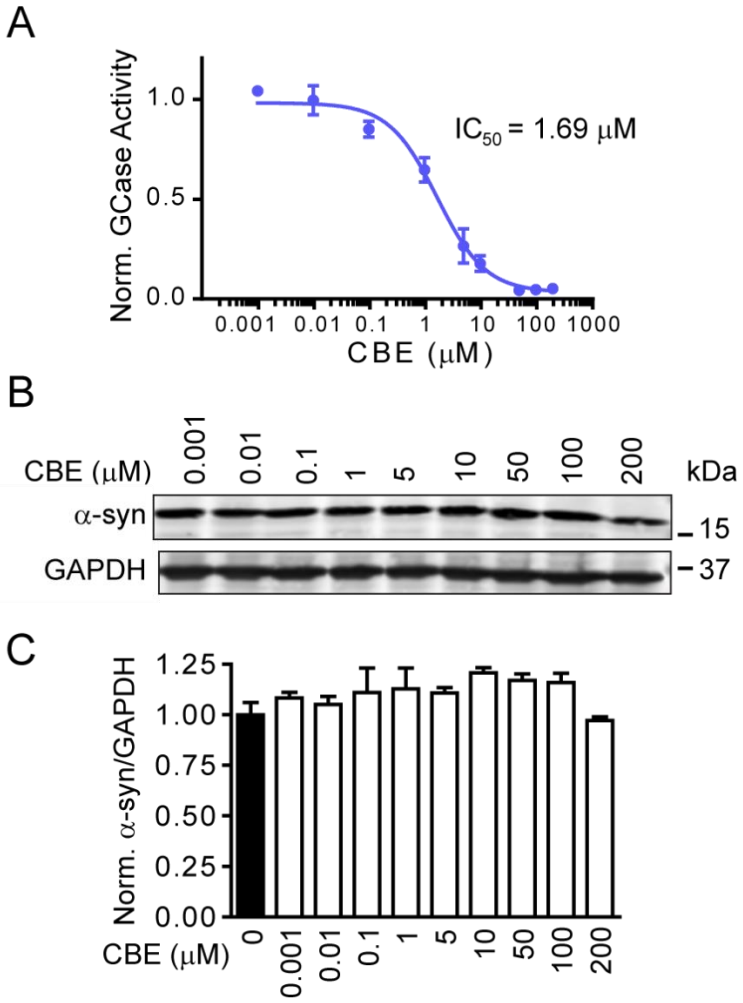

Figure S1. GCase Inhibition Does Not Elevate α-Synuclein Levels in Non-Neuronal Cells, Related to Figure 1 (A) HEK293 cells stably overexpressing α-synuclein (Clone 10) were treated with ascending doses of CBE for 3 days. Cell lysate was used to determine GCase activity. The plotted line is representative of a non-linear fit of values with an estimated IC_{50} of 1.69 μM (n=3-4/group). (B) Parallel samples were assayed for total α-synuclein and GAPDH protein levels. (C) Quantification of α-synuclein levels indicates that there is no significant change with CBE treatment compared to vehicle treatment (one-way ANOVA with Dunnet's multiple comparison test, All $p>0.05$, n=3-4/group). All plots are means, and error bars represent s.e.m.

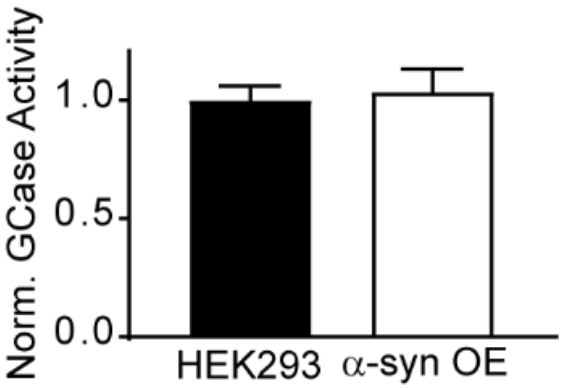

Figure S2. Constitutive α-synuclein expression does not reduce GCase activity, Related to Figure 2 (A) HEK293 cells or HEK293 cells stably overexpressing α-synuclein (Clone 10) were lysed and used to determine GCase activity. No significant difference was found between the two cell types (p=0.7725, unpaired t test, n=17/group). Plot represents means, and error bars represent s.e.m.

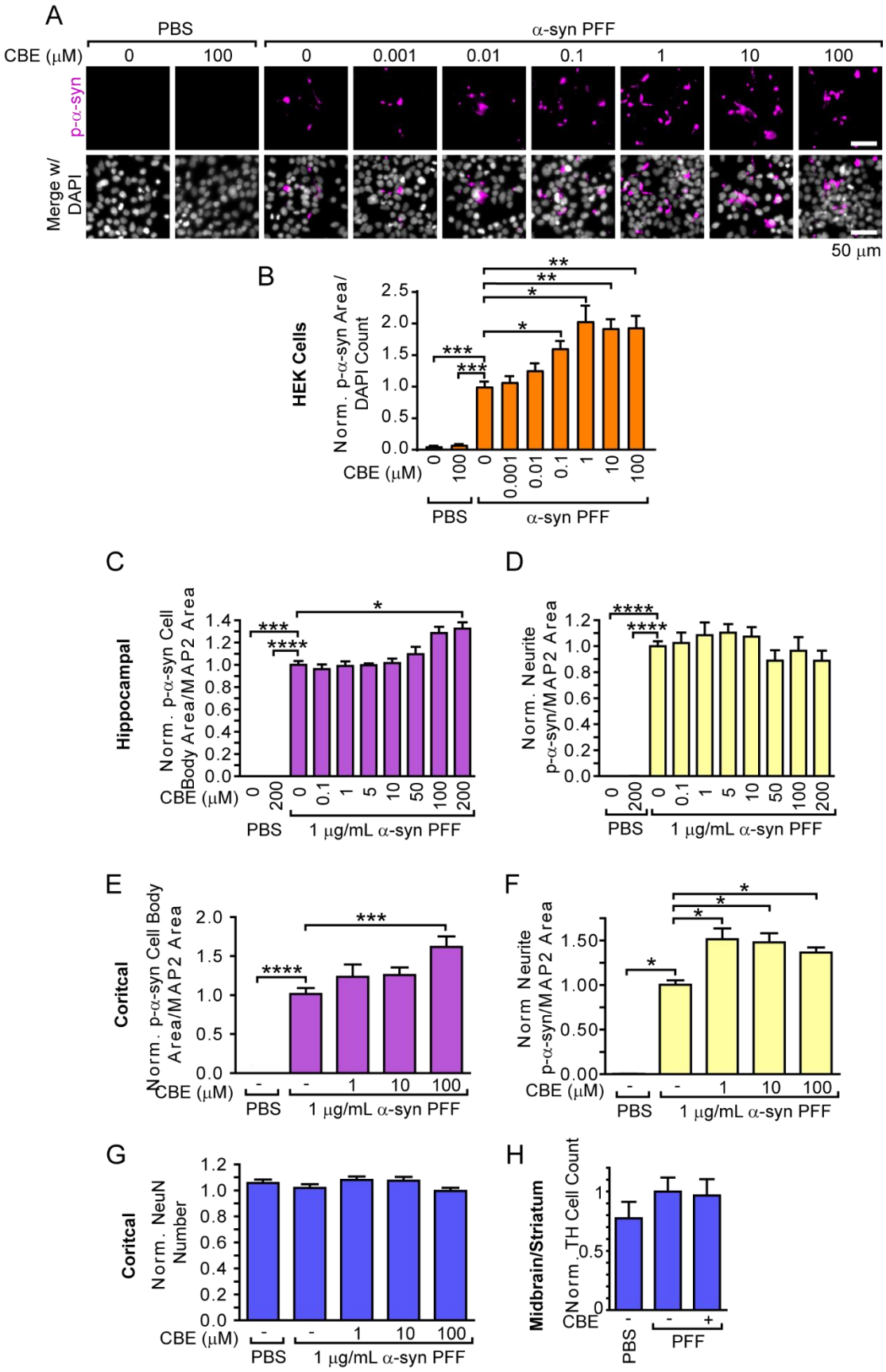
A
PBS
α-syn PFF
CBE (μM)
0
100
0
0.001
0.01
0.1
1
10
100
p-α-syn
Merge w/ DAPI
50 μm
B
HEK Cells
Norm. p-α-syn Area/ DAPI Count
CBE (μM)
PBS
α-syn PFF
C
Hippocampal
Norm. p-α-syn Cell Body Area/MAP2 Area
1 μg/mL α-syn PFF
D
Norm. Neurite p-α-syn/MAP2 Area
E
Coritcal
Norm. p-α-syn Cell Body Area/MAP2 Area
F
Norm Neurite p-α-syn/MAP2 Area
G
Coritcal
Norm. NeuN Number
H
Midbrain/Striatum
Norm. TH Cell Count
CBE
PFF

Figure S3. GCase Inhibition Alters the Amount and Morphology of α-Synuclein Pathology in HEK Cells and Primary Neurons, Related to Figure 3 (A) HEK293 cells stably overexpressing α-synuclein (Clone 10) were treated with ascending doses of CBE 1 day prior to transduction with PBS in BioPorter reagent or α-synuclein PFFs in BioPorter reagent and incubated for 3 days post-transduction. Cells were then fixed and stained for pathological pS129 α-synuclein and DAPI. (B) Quantification of pS129 α-synuclein area/DAPI count in HEK293 cells shows appearance of α-synuclein pathology with α-synuclein PFF addition, but not with CBE and a dose-dependent increase in α-synuclein pathology with CBE addition (Kruskal-Wallis test with Dunn's multiple comparison tests comparing all conditions to α-synuclein PFF without CBE condition, PBS-0 μM CBE ***p=0.0004, PBS-100 μM CBE ***p=0.0009, PFF-0.1 μM CBE *p=0.0437, PFF-1 μM CBE *p=0.0261, PFF-10 μM CBE **p=0.0019, PFF-100 μM CBE **p=0.0087, all others p>0.05, n=23-24/group). (C) Primary hippocampal neurons were treated with ascending doses of CBE for 16 days with or without the addition of α-synuclein PFFs for 14 days and stained for pathological pS129 α-synuclein, MAP2 and NeuN. An analysis algorithm was used to separate cell body pathology by size and shape from neuritic pathology. When assessed in this manner, there is a dose-dependent effect of CBE on α-synuclein cell body pathology, but not neuritic pathology (Kruskal-Wallis test with Dunn's multiple comparison tests comparing all conditions to α-synuclein PFF without CBE condition (PBS-0 μM CBE ***p=0.0002, PBS-200 μM CBE ****p<0.0001, PFF-200 μM CBE p=0.0208, all others p>0.05, n=15-18/group). (D) Quantification of neuritic pathology in the same neurons as (C). One-way ANOVA with Dunnett's multiple comparison tests comparing all conditions to α-synuclein PFF without CBE condition (PBS-0 μM CBE ****p<0.0001, PBS-200 μM CBE ****p<0.0001, all others p>0.05, n=15-18/group). (E) Primary cortical neuron α-synuclein cell body pathology assayed in the same manner as described above for hippocampal neurons (One-way ANOVA with Dunnett's multiple comparison tests comparing all conditions to α-synuclein PFF without CBE condition: PBS-0 μM CBE ****p<0.0001, PBS-100 μM CBE ***p=0.0009, all others p>0.05, n=17-18/group). (F) Quantification of neuritic pathology in the same neurons as (E). Kruskal-Wallis test with Dunn's multiple comparison tests comparing all conditions to α-synuclein PFF without CBE condition (PBS-0 μM CBE *p=0.0161, PFF-1 μM CBE *p=0.0110, PFF-10 μM CBE *p=0.0110, PFF-100 μM CBE *p=0.0233, n=17-18/group). (G) Primary cortical neuron number (One-way ANOVA with Dunnett's multiple comparison tests comparing all conditions to α-synuclein PFF without CBE condition: All p>0.05, n=17-18/group). (H) Total TH cell number reveals no effect of α-synuclein PFF addition or of CBE addition (one-way ANOVA with Dunnett's multiple comparison tests comparing all conditions to α-synuclein PFF without CBE condition, all p>0.05, n=6/group Data are represented as mean ± SEM.

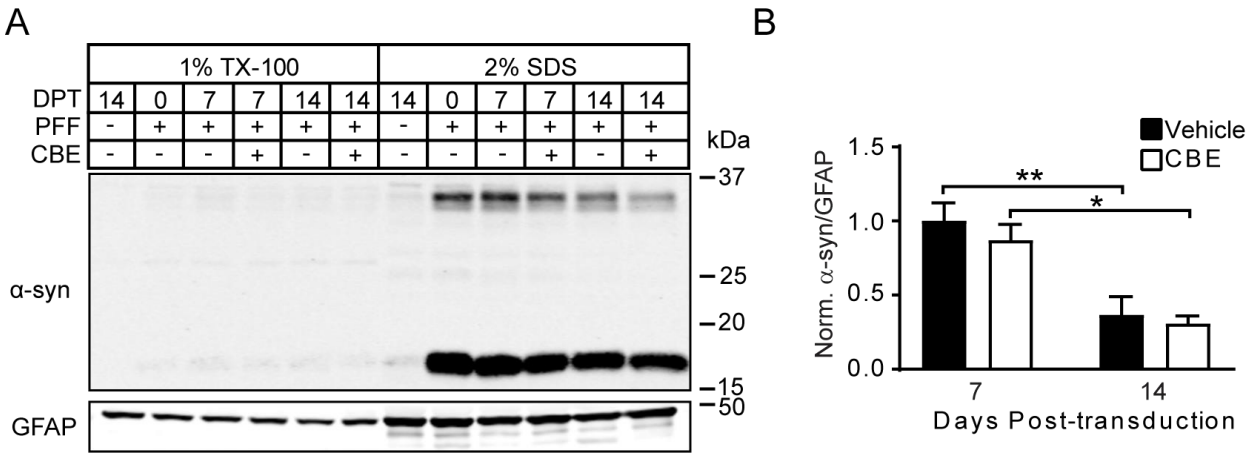

Figure S4. GCase Inhibition Does Not Change the Degradation of Pathological α-Synuclein by Astrocytes, Related to Figure 4 (A) Primary astrocytes were treated with 2.5 µg/mL α-synuclein PFFs at multiple timepoints prior to collecting cell lysate as noted. The astrocytes were then extracted in sequentially stronger detergents to separate aggregated from non-aggregated α-synuclein, which was the majority of α-synuclein present. (B) Quantification of aggregated α-synuclein in primary astrocyte cultures 7-14 days after addition of α-synuclein PFFs. Between days 7-14, over half of α-synuclein is degraded by astrocytes but there is no change in the rate of degradation with CBE treatment (two-way ANOVA (Vehicle vs. CBE p=0.3848) with Sidak's multiple comparisons test comparing within treatment to 7 days post-transduction: Vehicle **p=0.0052, CBE *p=0.0102, n=3/group). Data are represented as mean ± SEM.

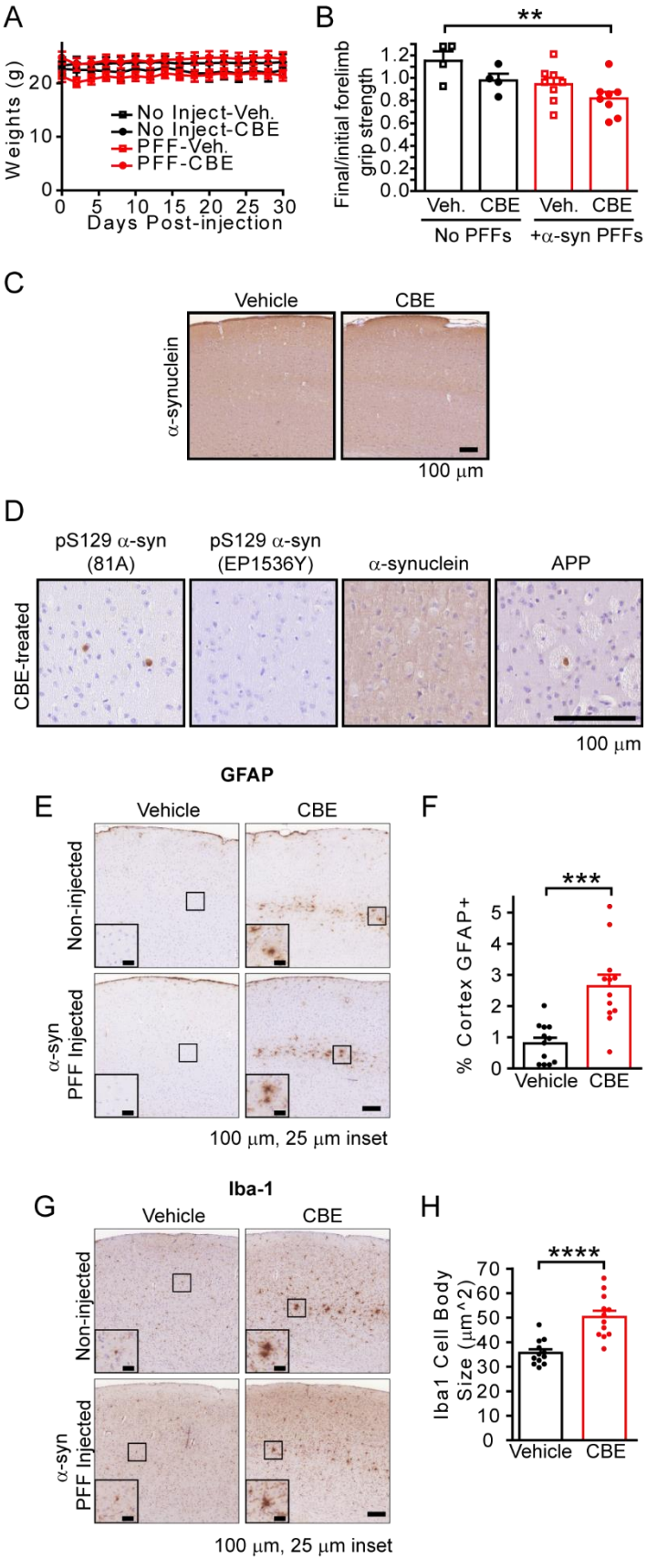
A
Weights (g)
20
10
0
0 5 10 15 20 25 30
Days Post-injection
No Inject-Veh.
No Inject-CBE
PFF-Veh.
PFF-CBE
B
**
Final/initial forelimb grip strength
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Veh. CBE Veh. CBE
No PFFs
+α-syn PFFs
C
Vehicle
CBE
α-synuclein
100 μm
D
pS129 α-syn (81A)
pS129 α-syn (EP1536Y)
α-synuclein
APP
CBE-treated
100 μm
GFAP
E
Vehicle
CBE
Non-injected
α-syn PFF Injected
100 μm, 25 μm inset
F
***
% Cortex GFAP+
5
4
3
2
1
0
Vehicle CBE
Iba-1
G
Vehicle
CBE
Non-injected
α-syn PFF Injected
100 μm, 25 μm inset
H
****
Iba1 Cell Body Size (μm^2)
70
60
50
40
30
20
10
0
Vehicle CBE

Figure S5. GCase Inhibition Induces Gliosis But Does Not Induce α-synuclein Aggregation in Mice, Related to Figure 5 (A) Mice were weighed every other day and mean weight did not change over the course of the study in any of the groups (n=4-8/group). (B) An alternate way to assess grip strength measures is to determine the relative difference from the beginning of the study as final/initial grip strength. By this measure, the combination of α-synuclein PFFs and CBE treatment is the only group significantly different from the non-injected vehicle group (one-way ANOVA with Dunnett's multiple comparisons test PFF-CBE **p=0.0064, all others p>0.05, n=4-8/group). (C) α-synuclein staining of somatosensory cortex from mice that had not been injected with α-synuclein PFFs but were treated with vehicle or CBE. (D) Immunohistochemical staining with various antibodies recognizing pS129 α-synuclein, α-synuclein or APP. While spheroids can be seen in CBE-treated mouse brains and not in controls with 81A, no similar structure can be seen with a second antibody recognizing pS129 α-synuclein (EP1536Y) or an antibody which recognizes total α-synuclein. Some spheroids are recognized with an antibody recognizing APP. Scale bars = 100 μm. All plots are means with individual values shown, and error bars represent s.e.m. (E) CBE treatment of mice, with or without concurrent α-synuclein PFF injection, induces robust astrogliosis as visualized by GFAP staining. Images show matched somatosensory cortex from mice with an enlarged image of the region with high GFAP staining in the insets. (F) The percentage of area occupied by GFAP staining in all cortical regions at 0.98 mm and -0.34 mm relative to Bregma was quantified (unpaired t test with Welch's correction ***p=0.0004, n=12/group). (G) Iba-1 staining reveals microglial profiles. While microglia normally have small cell bodies and extensive processes, CBE treatment induces an activated microglial phenotype with enlarged cell bodies. (H) An object-based algorithm was used to identify Iba-1 cell bodies. The average size of microglial cell bodies was quantified in all cortical regions at 0.98 mm and -0.34 mm relative to Bregma was quantified (unpaired t test with Welch's correction ****p<0.0001, n=12/group). Scale bars = 100 μm (25 μm, inset). All plots are means with individual values shown, and error bars represent SEM.

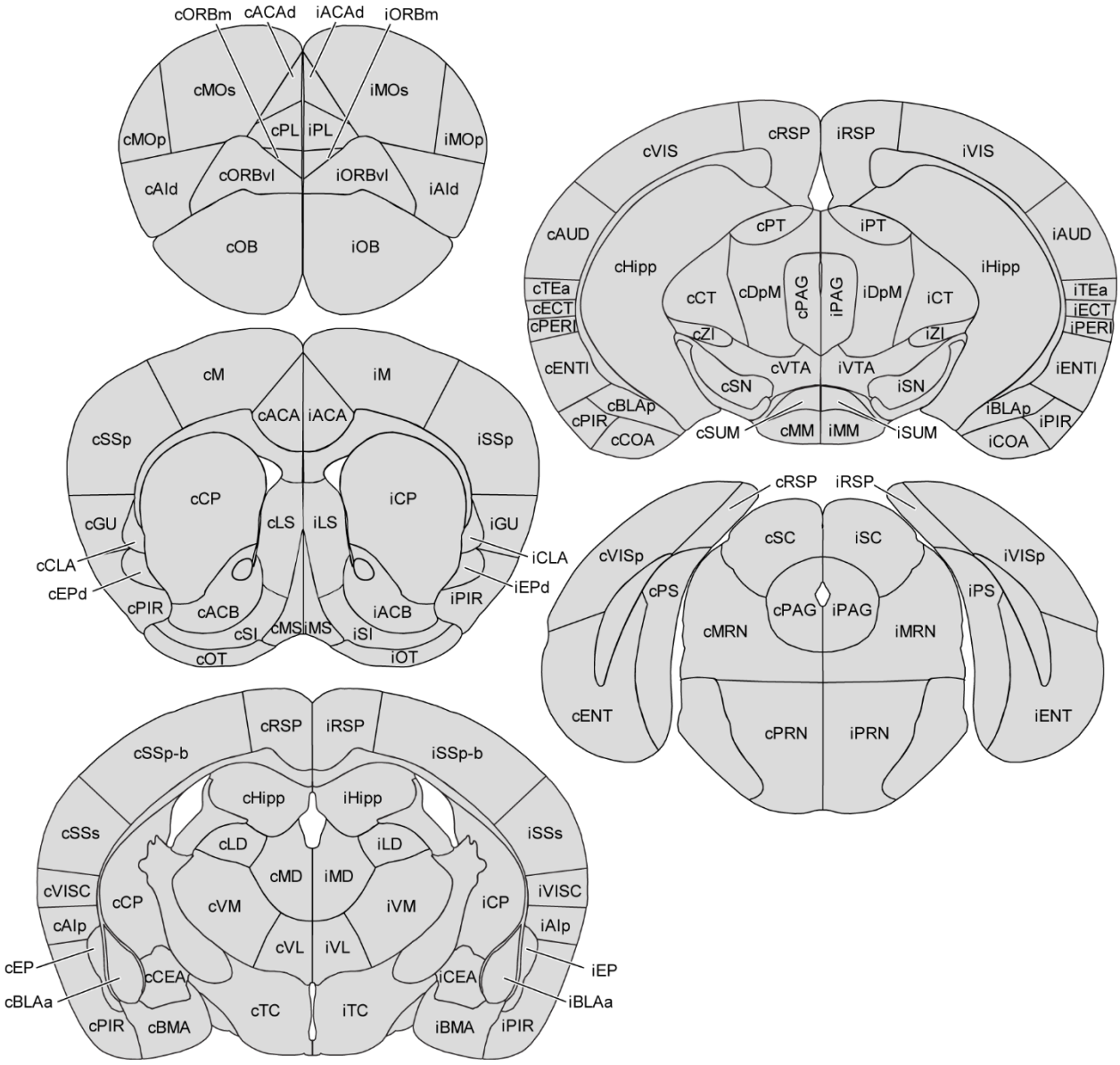

Figure S6. Brain Region Annotation Key, Related to Figure 6 Each of 132 gray matter brain regions used in analysis of pathological burden are labelled here.

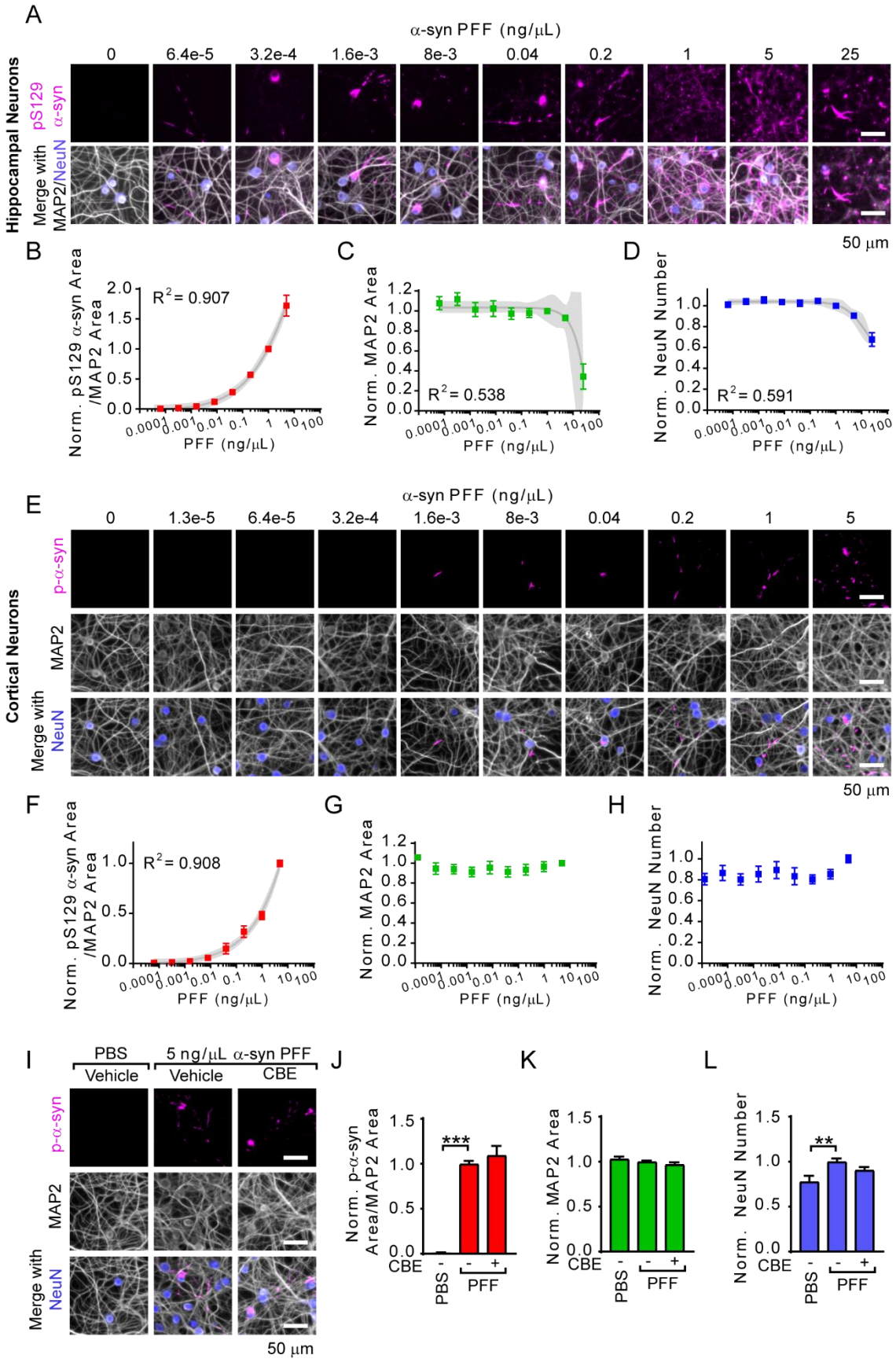
A
α-syn PFF (ng/μL)
0
6.4e-5
3.2e-4
1.6e-3
8e-3
0.04
0.2
1
5
25
Hippocampal Neurons
pS129 α-syn
Merge with MAP2/NeuN
50 μm
B
Norm. pS129 α-syn Area /MAP2 Area
R2= 0.907
PFF (ng/μL)
C
Norm. MAP2 Area
R2= 0.538
PFF (ng/μL)
D
Norm. NeuN Number
R2= 0.591
PFF (ng/μL)
E
α-syn PFF (ng/μL)
0
1.3e-5
6.4e-5
3.2e-4
1.6e-3
8e-3
0.04
0.2
1
5
Cortical Neurons
p-α-syn
MAP2
Merge with NeuN
50 μm
F
Norm. pS129 α-syn Area /MAP2 Area
R2= 0.908
PFF (ng/μL)
G
Norm. MAP2 Area
PFF (ng/μL)
H
Norm. NeuN Number
PFF (ng/μL)
I
PBS
5 ng/μL α-syn PFF
Vehicle
Vehicle
CBE
p-α-syn
MAP2
Merge with NeuN
50 μm
J
Norm. p-α-syn Area/MAP2 Area
***
CBE
PBS
PFF
K
Norm. MAP2 Area
CBE
PBS
PFF
L
Norm. NeuN Number
**
CBE
PBS
PFF

Figure S7. α-Synuclein PFFs Induce Dose-Dependent Pathology, and High Pathology Abrogates the Effect of GCase Inhibition, Related to Figure 8 (A) α-Synuclein pathology was titrated in primary **hippocampal** neurons by treating with various concentrations of α-synuclein PFFs and staining for pathological pS129 α-synuclein (magenta), MAP2 (gray) and NeuN (blue). Pathology is reduced at lower concentrations, though a high degree of pathology can be seen in single neurons even at very low concentrations. At 25 ng/µL α-synuclein PFFs, there is substantial neuron loss, and therefore pathology measures at this concentration are more difficult to interpret. (B) α-Synuclein PFF dose is directly related to the amount of pathology developed, and this relationship is well fit by a sigmoidal curve (gray line represents a best-fit line with the 95% confidence interval in light gray, $R^2 = 0.907$, n=72). There is also a non-linear reduction in MAP2 area (C) and NeuN number (D), (gray line represents a best-fit line with the 95% confidence interval in light gray, $R^2 = 0.538$ and 0.591, respectively, n=81). (E) α-Synuclein pathology was titrated in primary **cortical** neurons by treating with various concentrations of α-synuclein PFFs and staining for pathological pS129 α-synuclein (magenta), MAP2 (gray) and NeuN (blue). (F) α-Synuclein PFF dose is directly related to the amount of pathology developed, and this relationship is well fit by a sigmoidal curve (gray line represents a best-fit line with the 95% confidence interval in light gray, $R^2 = 0.908$, n=68). At no dose was α-synuclein pathology toxic in cortical neurons, so MAP2 are (G) and NeuN number (H) were not able to be fit by a sigmoidal curve. (I) Treatment of cortical neurons with 5 ng/µL α-synuclein PFFs induces a higher amount of α-synuclein pathology than in previous experiments, and 100 µM CBE treatment does not further elevate pathology. (J) Quantification of pS129 α-synuclein area/MAP2 area reveals no change in pathology with CBE treatment (Kruskal-Wallis test with Dunn's multiple comparisons test to PFF-Vehicle: PBS-Vehicle ***p=0.0006, PFF-CBE p>0.9999, n=8-9/group). (G) Quantification of MAP2 area reveals no change with CBE treatment (one-way ANOVA with Dunnett's multiple comparisons test to PFF-Vehicle: All p-values are greater than 0.05, n=8-9/group). (H) Quantification of NeuN reduction in neurons with CBE treatment (one-way ANOVA with Dunnett's multiple comparisons test to PFF-Vehicle: PBS-Vehicle **p=0.0068, PFF-CBE p=0.3089, n=8-9/group). Scale bars = 50 µm. Data are represented as mean ± SEM.

Table S1. Clinical information for cases used for extraction of pathological α-synuclein

Case No.	Clinical Diagnosis	Pathological Diagnosis	Race	Sex	Age of Disease onset	Age at Death	PMI (h)	Brain Region Used	α-Synuclein (μg/ml)
1	AD Prob.	AD	White	Female	52	62	11	Midfrontal	21.57
2	PDD	PDD	White	Female	51	66	12	Midfrontal	24.56
3	CBS	AD	White	Female	45	55	15	Midfrontal	22.43
4	PDD	PDD	White	Male	60	72	19	Cingulate	8.28
5	DLB	DLB	White	Male	76	83	6	Cingulate	7.55
6	DLB	PDD	White	Male	66	72	9	Cingulate	9.52
7	PDD	PDD	White	Male	49	70	12.5	Cingulate	8.49

PMI: postmortem interval

Table S1, Related to Figure 8. A list of demographic and pathology information for the four human cases used in this study for extraction of pathological α-synuclein.