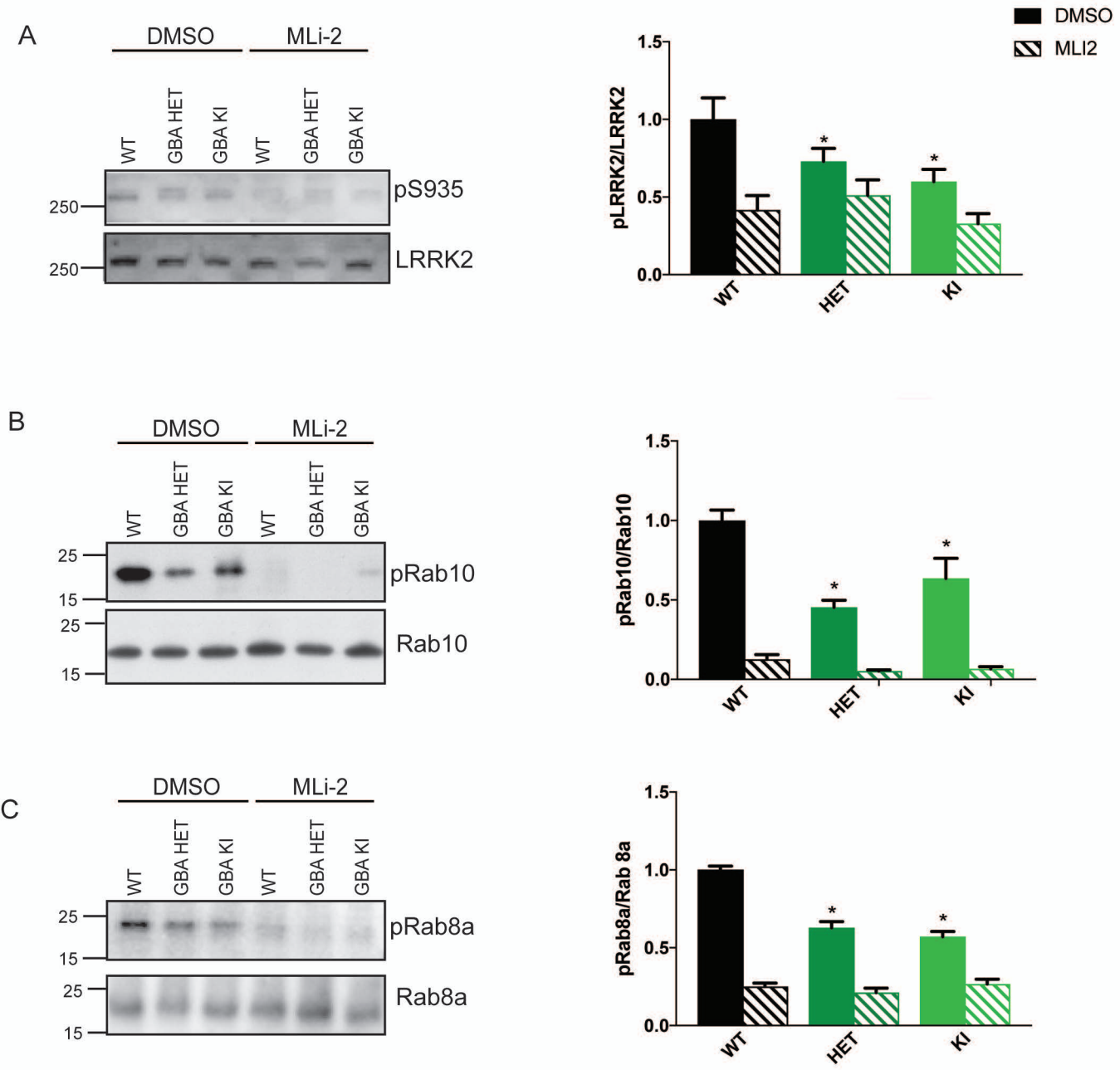
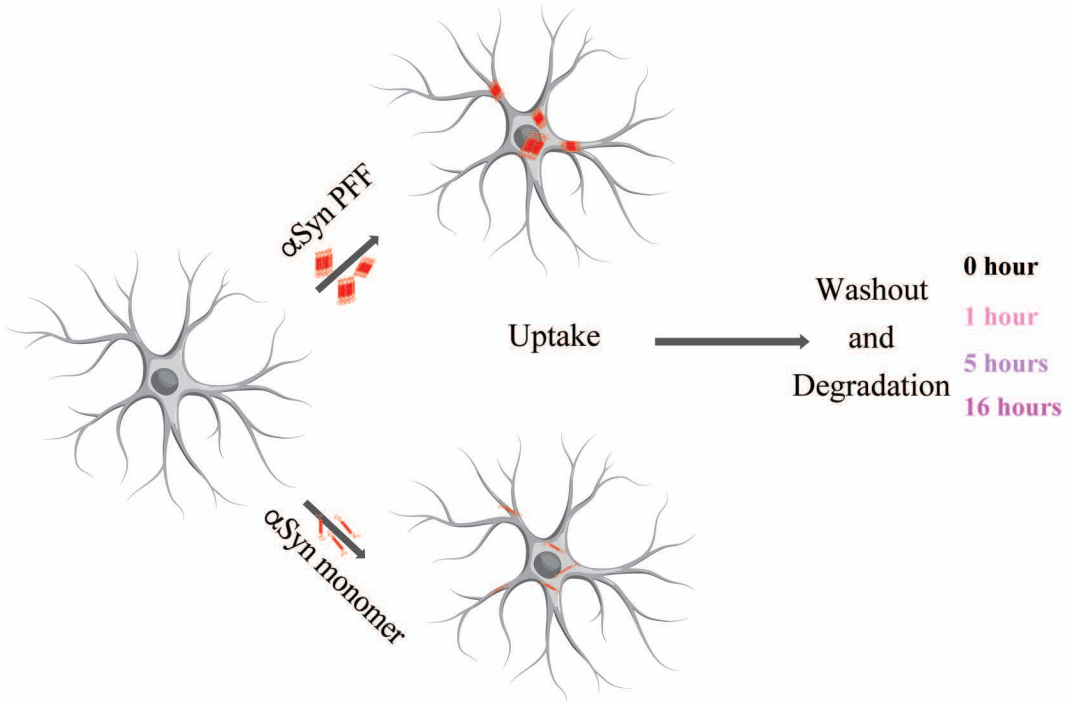
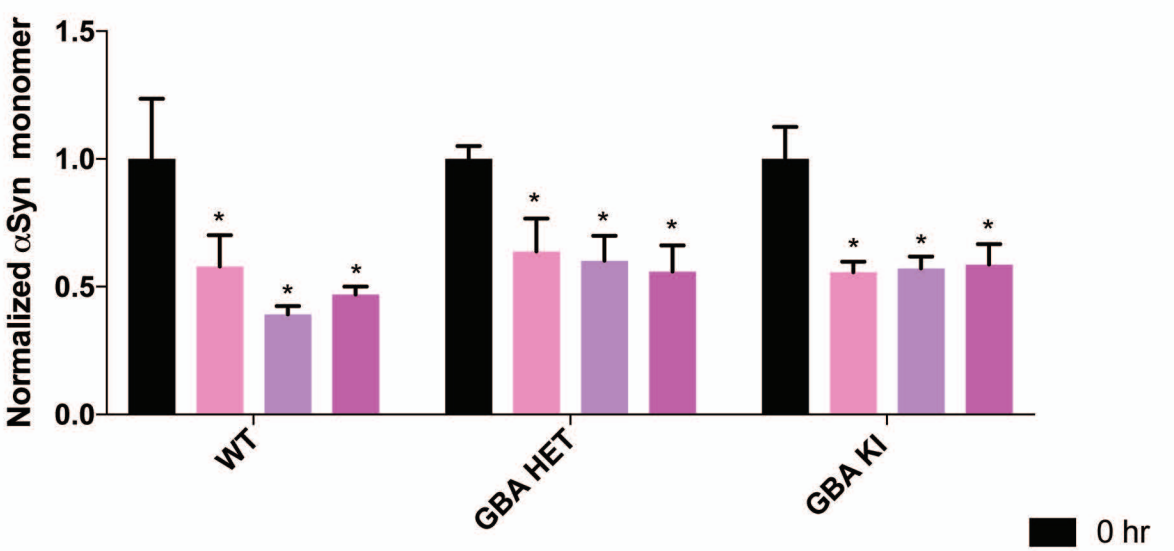

A
DMSO
MLi-2
WT
GBA HET
GBA KI
WT
GBA HET
GBA KI
250
pS935
250
LRRK2
DMSO
MLI2
pLRRK2/LRRK2
1.5
1.0
0.5
0.0
WT
HET
KI
B
DMSO
MLi-2
WT
GBA HET
GBA KI
WT
GBA HET
GBA KI
25
15
pRab10
25
15
Rab10
pRab10/Rab10
1.5
1.0
0.5
0.0
WT
HET
KI
C
DMSO
MLi-2
WT
GBA HET
GBA KI
WT
GBA HET
GBA KI
25
15
pRab8a
25
15
Rab8a
pRab8a/Rab 8a
1.5
1.0
0.5
0.0
WT
HET
KI

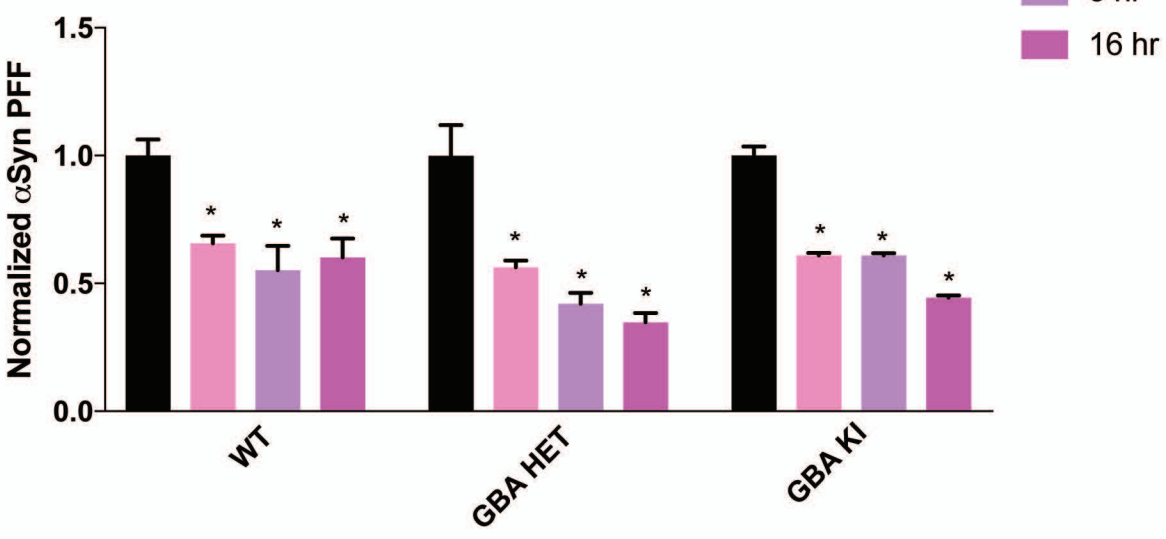
A


αSyn PFF
Uptake
Washout
and
Degradation
0 hour
1 hour
5 hours
16 hours
αSyn monomer

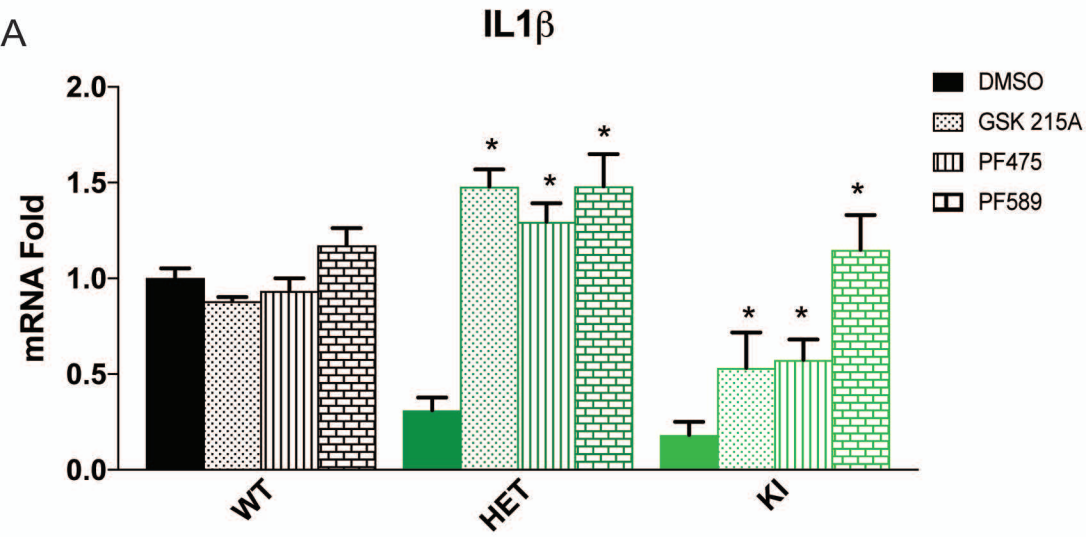
B


Normalized αSyn monomer
1.5
1.0
0.5
0.0
WT
GBA HET
GBA KI
0 hr
1 hr
5 hr
16 hr

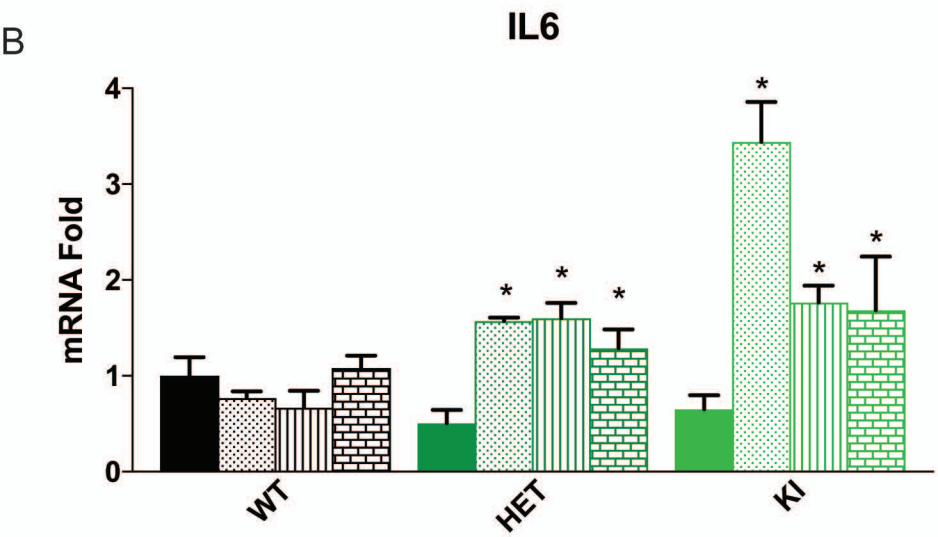
C


Normalized αSyn PFF
1.5
1.0
0.5
0.0
WT
GBA HET
GBA KI

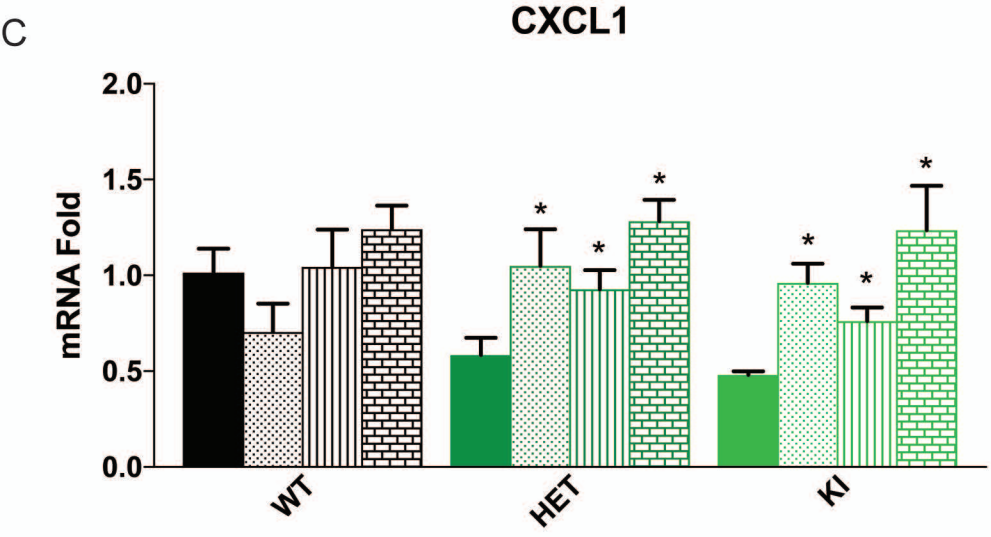
A


IL1β
mRNA Fold
2.0
1.5
1.0
0.5
0.0
WT
HET
KI
DMSO
GSK 215A
PF475
PF589

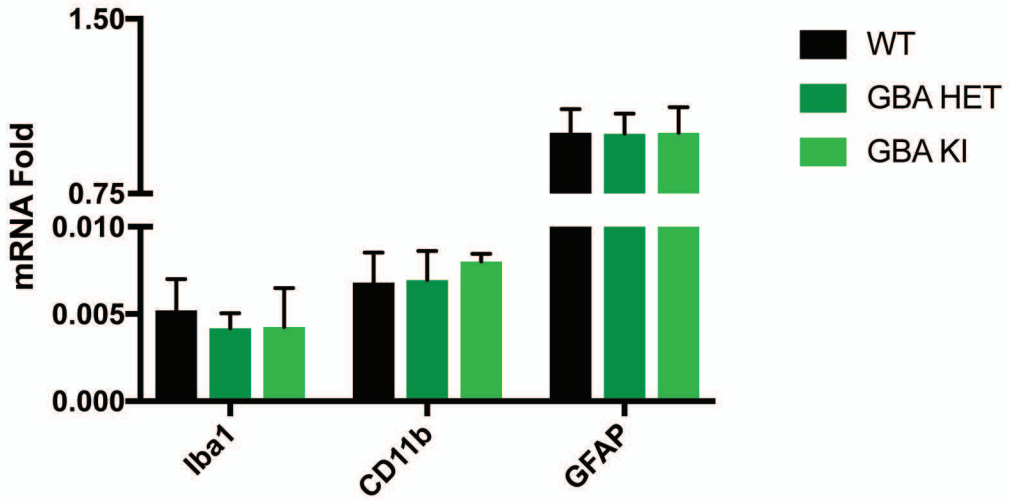
B


IL6
mRNA Fold
4
3
2
1
0
WT
HET
KI

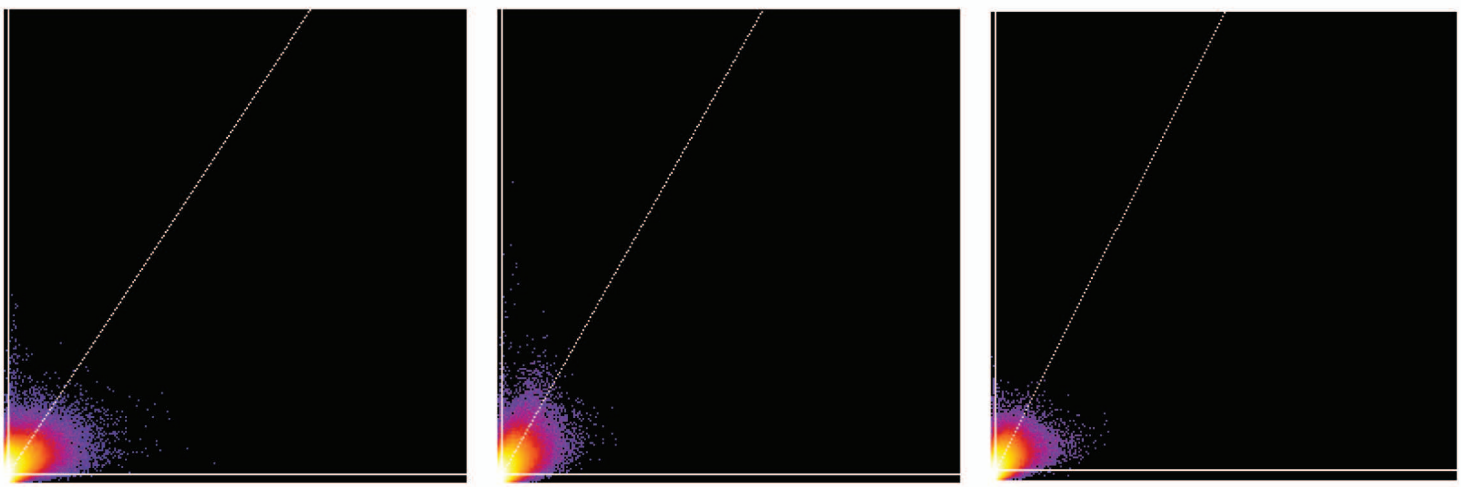
C


CXCL1
mRNA Fold
2.0
1.5
1.0
0.5
0.0
WT
HET
KI

A



B



Genotype	Spearman's Rank Correlation	t-statistics	degrees of freedom
WT	0.56	697.5	1048574
GBA HET	0.49	579.8	1048574
GBA KI	0.45	526.04	1048574

Supplementary Methods

Primary mouse astrocyte culture

All experiments in this manuscript were conducted with wild-type, heterozygous and homozygous knock-in littermates. We acquired C57BL/6NWT and *GBA1 D409V* heterozygous and homozygous knock-in mice on the C57BL/6N background from Jackson Labs. Het-Het crosses were used to obtain WT, WT/KI and KI/KI littermates. Astrocytes were prepared postnatally from individual pups that were genotyped from tail snips. Each brain was individually plated in 2 sister flasks, according to standard astrocyte culture protocols. Genotyping was conducted using primers from Jackson labs (Forward JAX 15580 5' CAG TTC ACA CAG TGT TGG AGC 3' and Reverse JAX 15581 5' AGG TGA TCG TAT TTC AGT GGC 3'). Microglial contamination was quantified to be ~2% by flow cytometry. Mean Ct values for microglial marker, Iba1 and Cd11b, as quantified by qPCR experiment, are greater than 30 cycles, which is 50-fold less than astrocyte marker, GFAP gene expression (Fig S4).

Western Blot

Cells were lysed in cell lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 0.5 mM EDTA, 0.5% (v/v) sodium deoxycholate, 1% (v/v) NP-40, pH 8) with protease and phosphatase inhibitor for 30 min. The lysates were centrifuged at 14,000 × *g* for 15 min at 4°C, and the supernatant protein was quantified using BCA assay. For isolation of the nuclear fraction, the pellet were dissolved in nuclear lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 0.5 mM EDTA, 0.5% (v/v) sodium deoxycholate, 0.1% (v/v) SDS, 1% (v/v) NP-40, pH 8) with protease and phosphatase inhibitor, centrifuged at 14,000 × *g* for 15 min at 4 °C, and the supernatant nuclear fraction was quantified using BCA assay. Total protein was normalized, mixed with 1X SDS-PAGE loading buffer, denatured at 95°C for 5 min, resolved on an SDS-polyacrylamide gel, transferred to PVDF membrane. For dot blots, total protein was blotted on a nitrocellulose membrane without boiling. Membranes were blocked with 5% bovine serum albumin (Sigma). Blots were probed with primary antibodies to pT73-Rab10 (abcam ab230261), Rab10 (cell signaling 8127S), pT72-Rab8a (abcam ab230260), Rab8a (abcam ab188574), pS935-LRRK2 (abcam 133450), LRRK2 (clone, 8629). Secondary antibodies conjugated to horseradish peroxidase were used for detection using autoradiography. Data was quantified using ImageJ across 3 independent experiments, each with biological triplicates.

ELISA

384-well MaxiSorp plates (Nunc, Inc) were coated with capturing antibody syn1 (BD Biosciences 610787) diluted 1:500 in coating buffer (NaHCO3 with 0.2% NaN3, pH 9.6) overnight at 4°C. Following 3 washes with PBS/0.05% Tween-20 (PBST), plates were blocked for 1hr at 37 °C in blocking buffer (1.125% fish skin gelatin; PBS-T). After 3 washes, samples were loaded, in duplicate and at two dilutions, and incubated at room temperature for 2hrs. Biotinylated hSA4 Ab (commercially MJFR1 from abcam) was generated using 200 µg Sulfo-NHS-LC Biotin (Pierce), diluted in blocking buffer and added to the plate for 1 hr at 37 °C. Following 5 washes, ExtrAvidin phosphatase (Sigma) diluted in blocking buffer was applied for 30min at 37 °C. Color development was carried out by using fast-p-nitrophenyl phosphate (Sigma) and monitored at 405 nm every 2.5 min for up to 60 min. Saturation kinetics were examined for identification of time point(s) where standards and sample dilutions were in the log phase.

Supplementary Figure Legends

Figure S1. GBA1 D490V knockin astrocytes show downregulation of endogenous wild-type LRRK2 kinase activity. **(A)** Examination of LRRK2 phosphorylation at S935 using Western blot, shows a modest loss of signal in *GBA1* mutant astrocytes. Phosphorylation of **(B)** Rab10 and **(C)** Rab8a, putative LRRK2 kinase substrates, is decreased in *GBA1* mutant astrocytes, as detected by Western blot using phospho-Rab10/8a specific antibodies and **(D)** quantified using ImageJ. 30 minutes MLi-2 treatment abrogates phosphorylation of LRRK2 S935, Rab10 and Rab8a. Total protein levels remain constant across genotypes and treatments (N=3).

Figure S2. GBA1 D490V mutation does not substantially alter degradation of exogenous alpha-synuclein by astrocytes. (A) Experimental schematic demonstrating α-synuclein (αSyn) monomer or PFF treatment of WT and *GBA1* mutant astrocytes, followed by washout of extraneous protein and degradation of internalized protein for 1-16 hours. Detection of **(B)** monomer by ELISA and **(C)** PFF by dot blot, show no differences in protein degradation capacity between genotypes. Quantifications for each genotype is normalized to their respective 0 hour time points (N=3, * $p<0.05$, ANOVA followed by Tukey's post-hoc test)

Figure S3. LRRK2 kinase inhibitors, GSK, PF475 and PF589, rescues the deficits in inflammatory response caused by *GBA1 D409V* knockin mutation. Quantitative PCR of GSK2578215A treated (1 μM, 3 days) or PF475-treated (100 nM, 3 days) or PF589-treated (120 nM, 3 days) and LPS-induced (100 ng/ml or 50 EU/ml, 6 hours) pro-inflammatory cytokines, **(A)** IL1β, **(B)** IL6 and **(C)** CXCL1 in GBA1 mutant astrocytes, display rescue of mRNA expression when compared to DMSO-treated control. Data is normalized to actin expression and collated from 3 biological replicates. (N=3, *$p<0.05$, ANOVA followed by Tukey's post-hoc test)

Figure S4. (A) Quantification of microglial contamination using qPCR of microglial markers Iba1 and Cd11b show less than 50 fold expression when compared to astrocytic marker, GFAP. **(B)** Coloc2 quantification of LAMP1-LAMP2 colocalization are shown as scatter plot (upper panel) for n=50 cells and represented as Spearman rank correlation values (lower panel).