ABBREVIATIONS

Casp1	Caspase 1
CCL2	C-C Motif Chemokine Ligand 2
CCl3	C-C Motif Chemokine Ligand 3
CCR2	C-C Chemokine Receptor Type 2
CD3	Cluster Of Differentiation 3
CD4	Cluster Of Differentiation 4
CD8	Cluster of differentiation 8
CD68	Cluster Of Differentiation 68
CM	Conditioned Medium
COX2	Cyclooxygenase 2
CxCl10	C-X-C Motif Chemokine Ligand 10
CxCl11	C-X-C Motif Chemokine Ligand 11
Dapi	4',6-Diamidin-2-Fenilindolo
DAT	Dopamine Active Transporter
DRD2	Dopamine Receptor D2
Foxa2	Forkhead Box Protein A2
Gal3	Galectin 3
GFAP	Glial Fibrillary Acidic Protein
gp91PHOX	Phagocyte Oxidase
GSK-3β	Glycogen Synthase Kinase-3β
IF	Immunofluorescent
IFN- γ	Interferon-λ
IL-10	Interleukin 10
IL-17	Interleukin 17
IL-17°	Interleukin 17°
IL-18	Interleukin 18
Il-1β	Interleukin 1β
Il-2	Interleukin 2
IL-4	Interleukin 4
IL-6	Interleukin 6
iNOS	Inducible Nitric Oxide Synthase
LPS	Lipopolysaccharide
LRRK2	Leucine-Rich Repeat Kinase 2
MAC-1	Macrophage Antigen Complex-1
MAPT	Microtuble Associated Protein Tau
mDAns	Midbrain Dopaminergic Neurons
MFI	Mean Fluorescence Intensity
NF-kb	Nuclear Factor Kappa-Light-Chain-Enhancer of Activated B Cell
NOS2	Inducible Nitric Oxide Synthase
Nurr1	Nuclear Receptor Related 1
PD	Parkinson's Disease
SNpc	Substantia Nigra Pars Compacta
SPMs	Spleen Macrophages
Str	Striatum
TG	Transgenic
TH	Tyrosine Hydroxylase
TNF-R	Tumor Necrosis Factor Receptor
TNF-α	Tumor Necrosis Factor α
VM	Ventral Midbrain
WT	Wild Type
α-syn	α-Synuclein

Supplementary Figure legends.

Supplementary Figure 1. G2019S combined with aging and chronic low-grade inflammation affects mice motor performance (A) Illustration of the experimental design. To mimic chronic low-grade inflammation, WT and G2019S mice were exposed to intraperitoneal ip injections of a low dose of lipopolysaccharide (LPS) (0.1 mg/kg, twice weekly, i.p.) administrated for 12 consecutive weeks administered twice a week for 12 weeks starting at 3 M or 7 M (green arrows); sterile NaCl was used as a control. Each group was sacrificed at different time points (red arrows), central/peripheral tissues were processed as described, and serum was collected for peripheral cytokine detection. **(B)** Weekly clinical evaluations (body weight, coat condition, lethargy, reluctance to move, grooming behavior) showed no effect of treatment except for a significant increase in body weight of G2019S at the indicated time point. Unpaired t-test, * $p< 0.05$, n=5. (**C, D**) The accelerated rotarod test was performed to assess motor coordination from 3 M to 16 M in saline- and LPS-treated mice. Two-way ANOVA followed by post hoc Bonferroni for multiple comparisons was used: § $p < 0.05$, §§ $p < 0.01$ §§§ $p < 0.001$ vs 3 M within genotype, two tailed t test was also performed for difference within each time point between WT and G2019S. * $p < 0.05$, ** $p <0.01$ ns= not significant. n=5

Supplementary Figure 2. **G2019S mice upon LPS treatment show a marked nigrostriatal DAns loss starting at 10M. (A)** Representative confocal images of SNpc from WT and G2019S mice at 10 months of age (M) under NaCl or a low dose of LPS. Tissues were immunostained with TH (green) and nuclear marker DAPI (blue). Scale bar= 300 µm. **(B)** Striatal TH immunofluorescence (IF) response analyzed at 3, 6, 10 and 16 M under NaCl or LPS exposure. G2019S, low-grade inflammation and aging robustly reduced TH-IF in the striatum of G2019S vs. WT mice. A two-tailed t-test performed at 3 months showed no significant difference between genotypes. Two-way ANOVA followed by post hoc Bonferroni was used to analyze the effect of treatment (exposure to NaCl or LPS) and genotype on TH-IF in the striatum starting at 6 months: §§§ $p <0.001$, §§§§ $p <0.0001$ NaCl vs. LPS within genotype; **** $p <0.0001$ WT vs. G2019S within treatment, n=5 **(C)** Representative confocal images of Str from WT and G2019S mice at 10 months of age (M) under NaCl or a low dose of LPS. Tissues were immunostained with TH (green) and nuclear marker DAPI (blue). Scale bar= 300 µm.

Supplementary Figure 3. G2019S synergizes with ageing and low-grade inflammation to induce active pGSK-3β, pα-syn, and pTau. (A, B) Western blot analysis was performed in the ventral midbrain (VM) of 6, 10 and 16 M old WT and G2019S mice exposed to NaCl or LPS treatment as described. Quantification of protein levels is shown relative to the loading control, phosphorylated GSK-3β (pTyr216 GSK-3β); phosphorylated α-syn (pSer129 α-syn) and phosphorylated tau (pSer396 tau) were normalized to the respective control (total GSK-3β, α-syn and tau, respectively). Data represent the mean % ± SEM of n=4. Statistical significance analyzed by two-way ANOVA, followed by post hoc Bonferroni for multiple comparisons: §§ $p <0.01$, §§§ $p <0.001$, §§§§ $p <0.0001$ NaCl vs LPS within genotype; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ *vs* WT within the same treatment group.

Supplementary Figure 4. Age-dependent astrocytosis in the SNpc of WT and G2019S mice. (A-B) Representative confocal images of triple immunofluorescence staining with TH (green), GFAP (red) and DAPI (blue) in SNpc of WT and TG mice at 6M. **(C-D)** Representative confocal images of triple immunofluorescence staining with TH (green), GFAP (red) and DAPI (blue) in SNpc of WT and TG mice at 10M. **(D-E)** Representative confocal images of triple immunofluorescence staining with TH (green), GFAP (red) and DAPI (blue) in SNpc of WT and TG mice at 16M. All sections showed in Figure are matched at the same level of the substantia nigra (Bregma -3.08-3.16 mm). Scale bar = 300 µm.

Supplementary Figure 5. G2019S interacts with aging and chronic low-grade inflammation to drive striatal neurodegeneration. (A) Representative confocal images of dual immunofluorescence staining with GFAP (red) and DAPI (blue) in striatum (Str) under NaCl/LPS at 10 M. Scale bar= 50µm, 20 µm (inserts) **(B)** Quantification of GFAP+/Dapi+ astrocytes in the Str of WT and G2019S mice under NaCl/LPS treatment at the indicated time points. A two-tailed T-test was used for $GFAP^+$ cell counts at 3 M and did not reveal any significant difference between genotypes. A two-way ANOVA followed by Bonferroni post hoc for multiple comparisons was performed to analyze the effect of treatment (exposure to NaCl or LPS) and genotype on $GFAP^+$ cell counts at 6, 10 and 16 M: §§ $p < 0.01$, §§§§ $p < 0.0001$ NaCl vs LPS within genotype; * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ WT vs G2019S within treatment **(C)** Representative confocal images of dual immunofluorescence staining for IBA1 (red) and DAPI (blue) in striatal slices from WT and G2019S mice after LPS or NaCl treatment at 10 M. Scale bar= 50µm, 20 µm (inserts) **(D)** Quantification of IBA1+/Dapi+ cells in Str sections of WT and G2019S mice after LPS or NaCl treatment from 3 to 16 M. All statistical analysis were carried out by two-way ANOVA followed Bonferroni for multiple comparisons: §§§ $p < 0.001$, §§§§ $p < 0.0001$ LPS vs NaCl within each genotype; ** $p < 0.01$, *** $p < 0.001$ WT vs G2019S within the same treatment group.

Supplementary Figure 6. G2019S interacts with LPS to enhance COX2, IL-18 and TNF-R expression in VM at 10M. Gene expression showed significantly higher expression levels of cyclooxygenase 2 (COX2) and IL-18, (but not IL-17) in G2019S LPS-treated mice compared to WT counterparts. TNF-R mRNA increased significantly under LPS in both genotypes. No differences were measured in C-X-C motif chemokine, CXCL11 and IL-1β mRNAs, while the anti-inflammatory cytokines IL-4 and IL-10 showed no detectable expression levels (not shown) in both WT and G2019S mice under LPS regimen in the same age group. Data (normalized transcript levels relative to β-actin are shown) represent the mean ± SEM of n= 5-6 mice/age group/treatment/genotype. Two-way ANOVA followed Bonferroni for multiple comparisons, §§ $p < 0.01$, §§§ $p < 0.001$, §§§§ $p < 0.0001$ NaCl vs LPS within genotype; *** $p < 0.001$, **** $p < 0.0001$ WT vs G2019S within treatment.

Supplemental Figure 7. Astrocyte-CCR2 interactions, CCl2 and Gal3 protein expression in the ventral midbrain (VM) of LRRK2 aged G2019S mice under low-grade inflammation at 10 M. (A) Triple immunostaining of GFAP (red), CCR2 (green), and DAPI (blue) at 10 M in WT (A) and G2019S mice under NaCl showing no detectable (WT) or weak (TG) CCR2-IF signal. Scale bar= 100 µm. **(B, C)** Triple immunostaining of GFAP (red), CCR2 (green), and DAPI (blue) at 10 M in WT (B) and G2019S mice under LPS showing GFAP+ cells and CCR2-IF cells in SNpc of both genotypes. Scale bar= 50 µm. **(D)** Quantification of CCR2, CCl2 and Gal3 protein levels relative to the loading control (β-actin) in the VM of 10 M -old WT and G2019S mice. Mean ± SEM; §§ p <0.01, §§§§ p <0.0001 NaCl vs LPS within genotype; **** p <0.0001 WT vs G2019S within treatment, n=5 (CCR2), and n=4 (CCl2, Gal3) mice/age-group/treatment/genotype. **(E)** Representative Western blot of CCR2, CCl2 and Gal3 in the VM of WT and G2019S mice under NaCl/LPS at 10 M showing significantly upregulated CCR2, CCl2 and Gal3 protein levels in G2019S vs. WT counterparts.

Supplementary Figure 8. LRRK2 G2019S and low-grade inflammation exacerbate colon α-syn aggregation. (A) Intestinal cryosections stained for aggregated α-synuclein (MJF-14, green), TH (red) and DAPI (blue) from WT and G2019S mice treated with LPS or NaCl at 16-18 months. **(B)** Intestinal cryosection stained for aggregated α-synuclein (MJF-14, green), TH (red) at 63X magnification from G2019S mice treated with LPS. Scale bar= 100µm.

Table 1. List of antibodies used for immunohistochemistry and western blot

Antibody name	Company	Dilution	RRID
CCl2, goat polyclonal	R&D	1:200	AB_354500
CCR2, goat polyclonal	Thermo Fisher Scientific	1:500	AB_557978
CD11b, rabbit	Abcam	1:1000	AB_2650514
CD11b, rat	Biolegend	1:500	AB_312784
CD3, mouse	Santa Cruz Biotechnology	1:500	AB_2228831
CD3, rat	Bio-Rad	1:50	AB_323775
CD4, goat	Santa Cruz Biotechnology	1:100	AB_2073236
CD4, rat	Bio-Rad	1:300	AB_1898234
CD4, mouse	Santa Cruz Biotechnology	1:100	AB_627055
CD68, rat	Bio-Rad	1:300	AB_2074849
CD8, rat	Bio-Rad	1:50	AB_322770
DAT, rabbit	Proteintech	1:200	AB_2879116
Gal3(MAC-2)	Cedarlane	1:500	AB_10060357
GFAP, mouse	Sigma	1:250	AB_2827276
GFAP, rabbit	sigma	1:1000	AB_2905668
GSK-3β, mouse	ECM Biosciences	1:200	AB_2115216
HuD+HuC, rabbit	Abcam	1:400	AB_2864321
Iba-1, goat polyclonal	Abcam	1: 200	AB_870576
Iba-1, rabbit polyclonal	Wako	1:500	AB_2889406
iNOS, mouse	Cell signaling technology	1:200	AB_1078202
LRRK2, rabbit	clone 41-2, Abcam by MJFF	1:1000	AB_2713963
MAP2, rabbit	Cell Signaling	1:500	AB_10693782
NeuN, rabbit	Abcam	1:1000	AB_2716282
Nurr1, goat polyclonal	R&D	1:300	AB_2153894
phospho-alpha-Synuclein (Ser^{129}), rabbit monoclonal (clone LS4-1B1)	Millipore	1:1000	AB_673008
phospho-GSK-3β (p-Tyr^{216}), rabbit	Abcam	1:500	AB_2533691
Phospho-LRRK2-S^{935}, rabbit	Abcam by MJFF	1:1000	AB_2864018
phospho-Tau ($pSer^{396}$), rabbit polyclonal	Sigma aldrich	1:200	AB_261757
phospho-Tau ($pThr^{181}$)	Thermo Fisher scientific	1:200	AB_1087704
Tau, mouse	RayBiotech	1:200	AB_11217610
TH, mouse	STEMCELL Technologies	1:200	AB_215512
TH, rabbit	Pel-Freez	1:200	AB_2313713
TH, sheep	Pel-Freez Biologicals	1:200	AB_2935637

TH, sheep	Pel-Freez Biologicals	1:1000	AB_461070
TUBB3, mouse	Biolegend	1:400	AB_2313773
α-syn (C-20)-R, rabbit	Santa Cruz Biotechnology	1:200	AB_2192953
α-syn aggregate, MJFR-14-6-4-2, rabbit	Abcam	1/5000	AB_2714215
β-actin, rabbit	Cell Signaling	1:1000	AB_10694076

Table 2. List of probes and IDs used for quantitative Real time PCR

PROBES	**IDs**
Casp6	Mm00438053-m1
CCl3	Mm 00441258-m1
CxCl10	Mm 00445235-m1
CxCl11	Mm00444662-m1
Drd1a	Mm01353211-m1
Drd2	Mm00438545-m1
Foxa2	Mm01976556-s1
GAPDH	Mm99999915-g1
Gfap	Mm 00546086-m1
Gp91phox	Mm01287743-m1
GSK3b	Mm00444911-m1
IL-10	Mm12088386-m1
IL-17	Mm00439618-m1
IL-18	Mm00434226-m1
Il1b	Mm00434228-m1
IL-4	Mm00445259-m1
Itgam (Mac1)	Mm 00434455-m1
LRRK2	Mm00481934_m1
Map2	Mm00485231-m1
Nfkb1	Mm00476361-m1
Nos 2	Mm 00440485-m1
Nurr 1	Mm 00443056-m1
Slc6a3 (Dat)	Mm 00438388-m1
Tau (Mapt)	Mm00521988-m1
Th	Mm 00447546-m1
Tnf	Mm00443258-m1
Tnfrsf1b	Mm00441875-m1
α-syn (SNCA)	Mm00458965-m1

Table 3 List of software

Software name	Version	URL
ImageJ	1.53t	*https://imagej.net/*
Luminex xPONENT	4.3	*https://www.luminexcorp.com/xponent/*
Bio-Plex Manager	6.2	*http://www.bio-rad.com/en-us/product/bio-plex-manager-software-standard-edition*
ImageQuantity One	4.6	*http://www.bio-rad.com/en-us/product/quantity-one-1-d-analysis-software*
GraphPad Prism	9	*http://www.graphpad.com/*

Table 4 Mice purchased from Jackson Laboratory

Genotype	Nomenclature	*RRID*
WT	C57BL/6J	*IMSR_JAX:000664*
Transgenic	C57BL/6J LRRK2*G2019S 2AMjff/J	*IMSR_JAX:018785*

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