

LRRK2-G2019S couples metabolic stress to astrocytic senescence through DDB1-mediated p21 stabilization in Parkinson's disease

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The supplementary material comprises Figures S1–S5 and supplementary tables 1-3

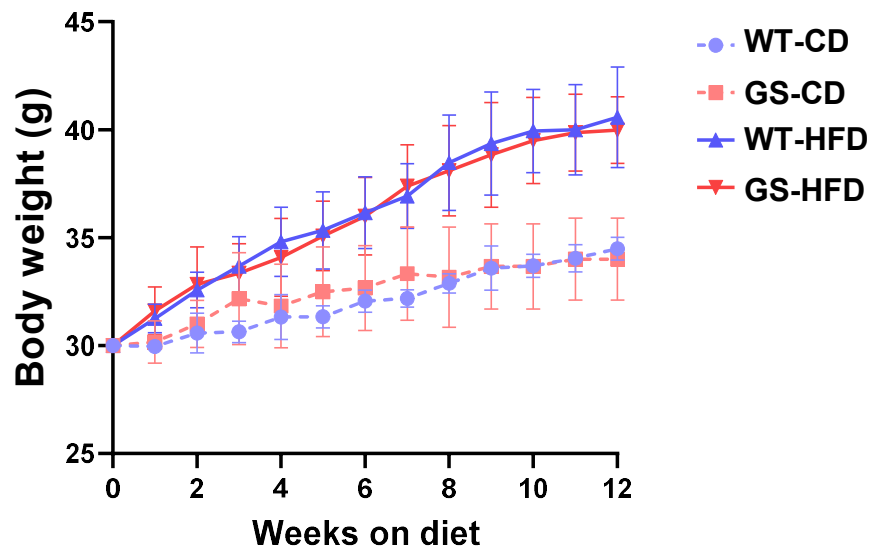
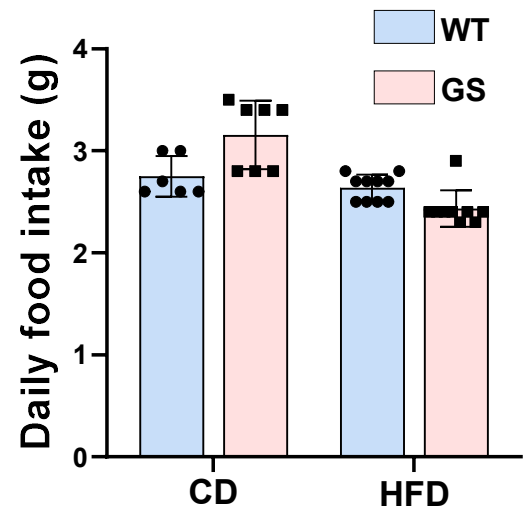
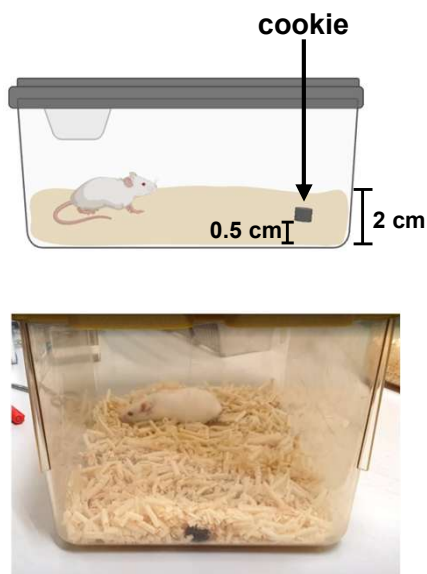
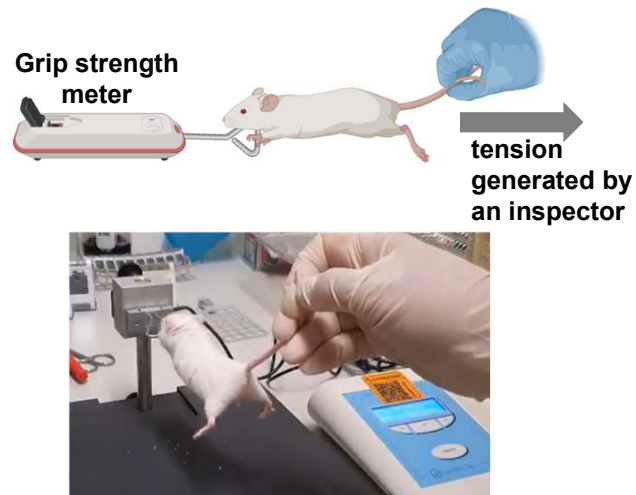
A**B****C****D**

Figure S1. Experimental setup for behavioral testing and metabolic profiling of control diet (CD)- or HFD-fed LRRK2-WT and -GS mice

(A and B) Body weight changes (A) and daily food intake (B) of mice fed a CD or HFD over 12 weeks (n=6-10 per group). Data are expressed as mean $\pm$ SD. **(C)** Schematic illustration of the buried food test (upper) and representative photographs of the test (lower). **(D)** Experimental scheme (upper) and representative photographs (lower) of the forelimb grip strength test.

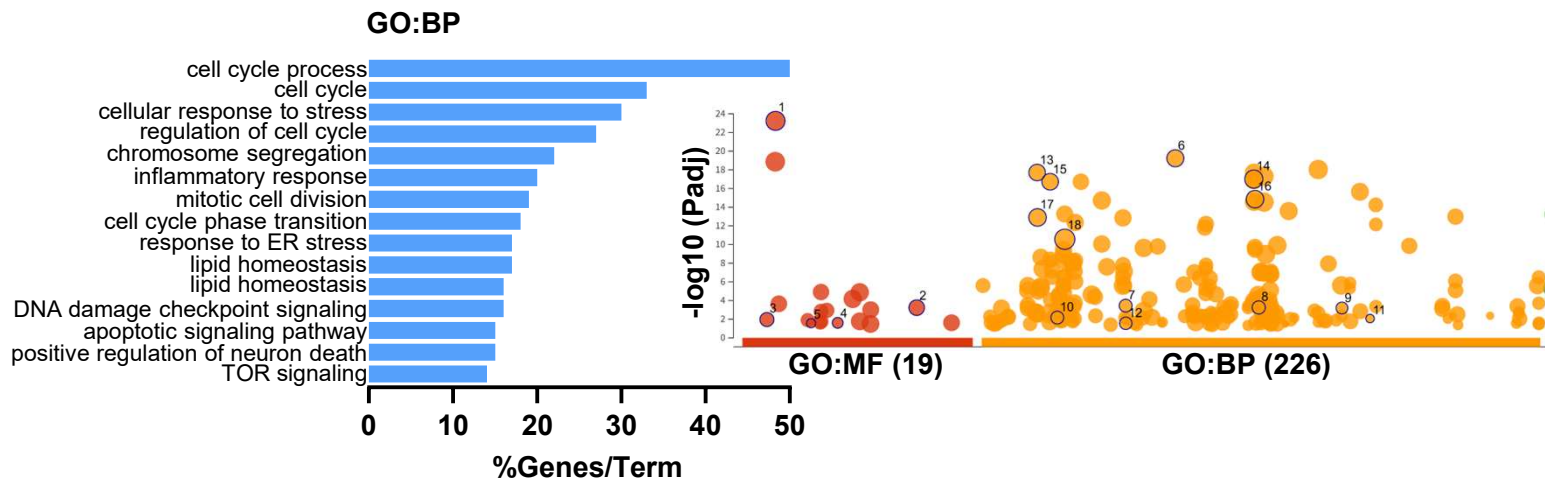
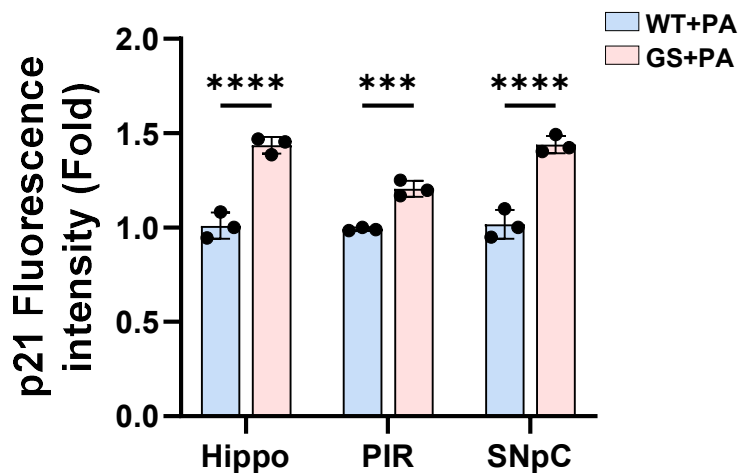
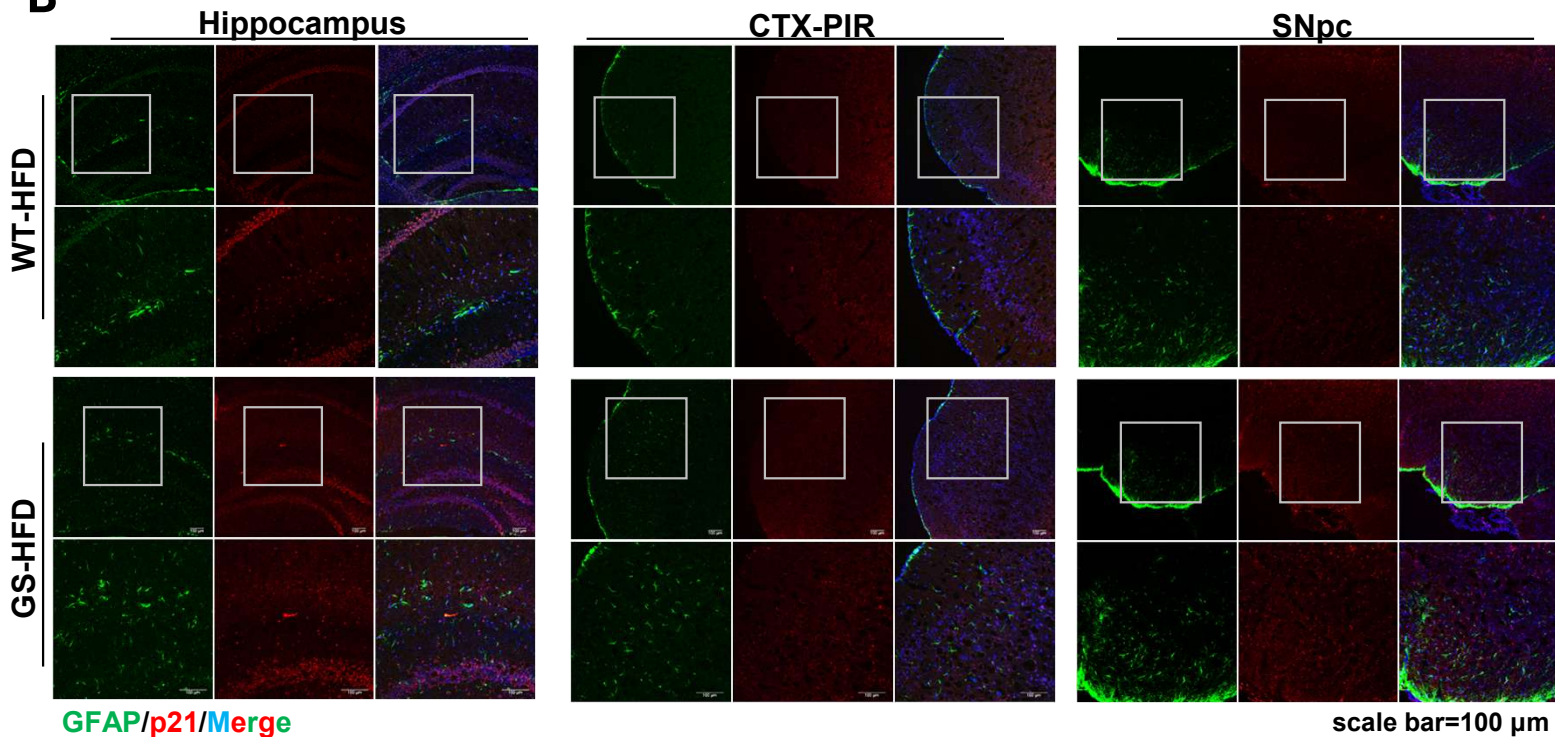
A**B**

Figure S2. Transcriptomic characterization and senescence markers in LRRK2-WT and -GS brains under CD or HFD

(A) GO enrichment analysis generated via g:Profiler. The bar plot (left) depicts the top enriched Biological Process terms, and the bubble plot (right) displays significantly enriched Molecular Function and Cellular Component categories. Circle size corresponds to the number of genes associated with each terms. **(B)** Representative immunofluorescence images (upper) and quantitative summary data (lower) of p21 staining are shown. Scale bar, 100 μm . Quantitative data represent the average of three random fields of view per group from three independent experiments. Data are presented as mean $\pm$ SD (*** $p < 0.001$, **** $p < 0.0001$). Hippo, Hippocampus; SNpc, Substantia nigra pars compacta; PIR, Piriform area.

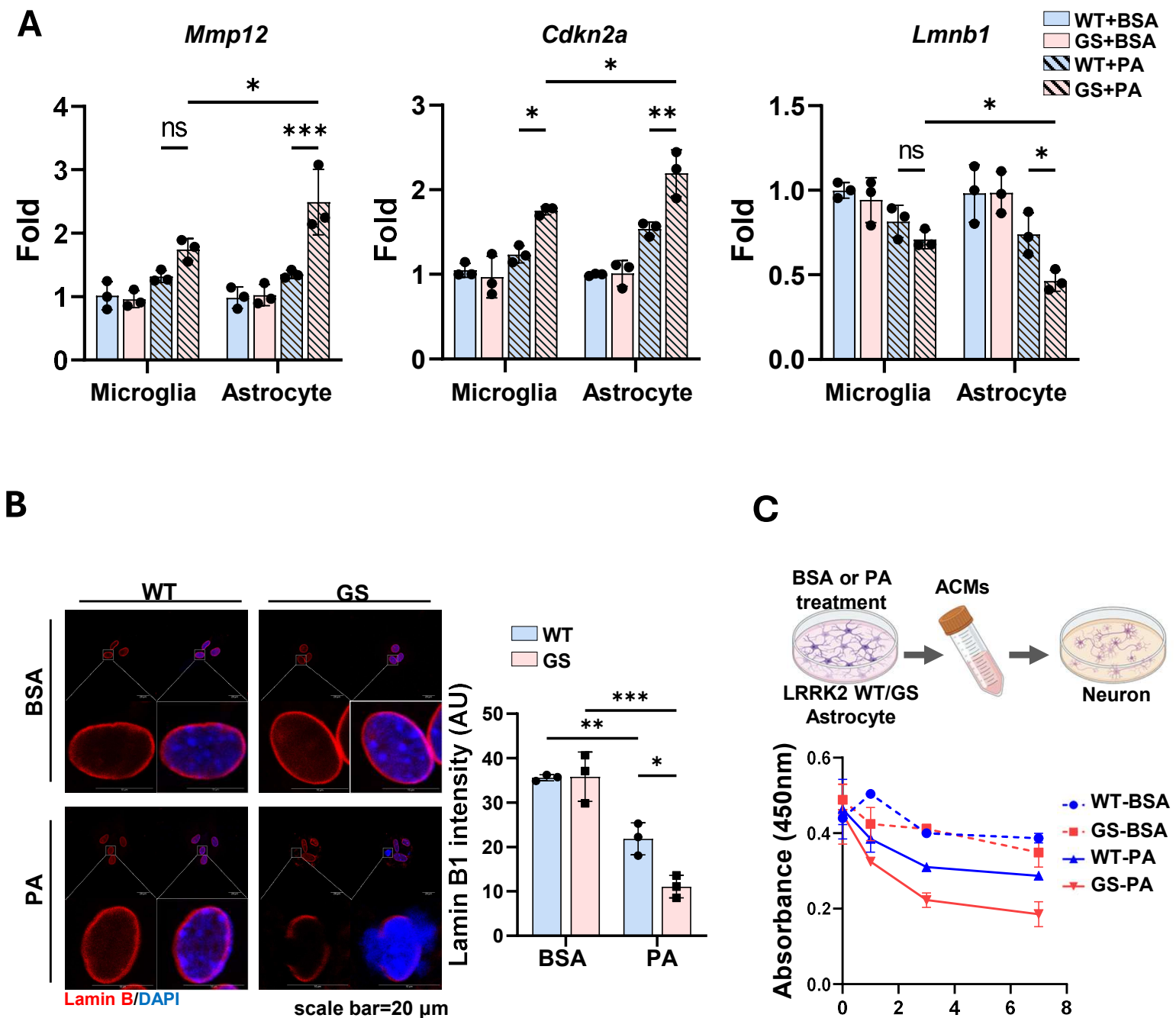


Figure S3. Exacerbation of PA-induced astrocytic senescence and neuronal injury by the LRRK2-GS mutation

(A) Real-time qPCR analyses of the indicated mRNA expression levels in primary microglia and astrocytes following treatment with BSA or PA. Data are presented as mean $\pm$ SD of three independent experiments (* p < 0.05, ** p < 0.01, *** p < 0.001. ns, not significant). **(B)** Representative immunofluorescence images (left) and quantitative summary (right) of Lamin B1 staining (Scale bar, 20 μ m). Quantitative data represent the average of five random fields of view per group from three independent experiments. Data are presented as mean $\pm$ SD (* p < 0.05, ** p < 0.01, *** p < 0.001). **(C)** Cell viability of primary neurons assessed via WST-8 assay. Neurons were incubated for 3 days with astrocyte-conditioned medium (ACM) harvested from LRRK2-WT and -GS astrocytes treated with BSA or PA. Data are presented as mean $\pm$ SD of three independent experiments.

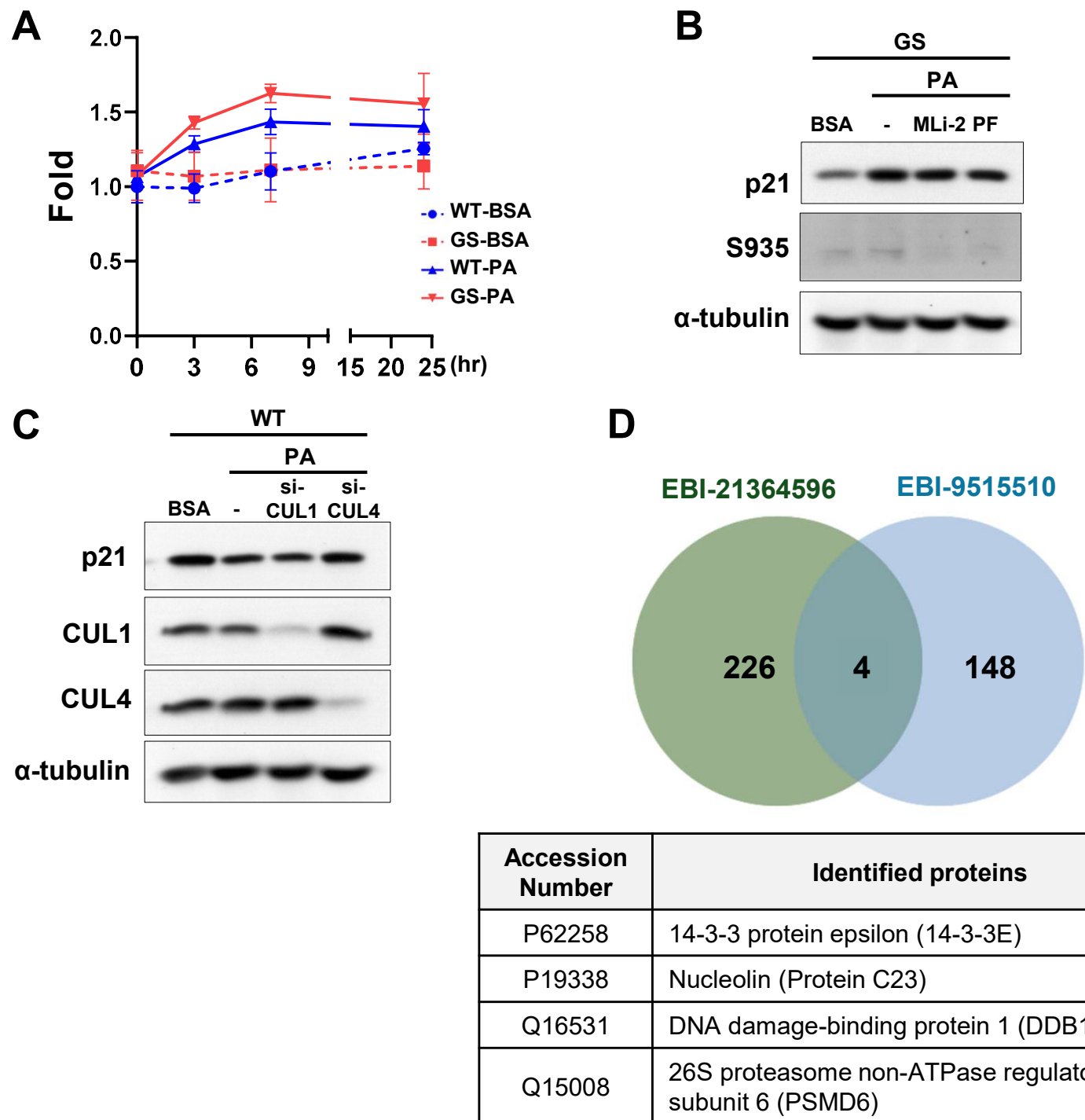
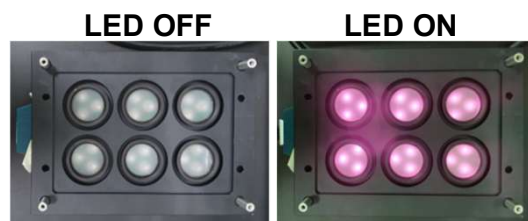
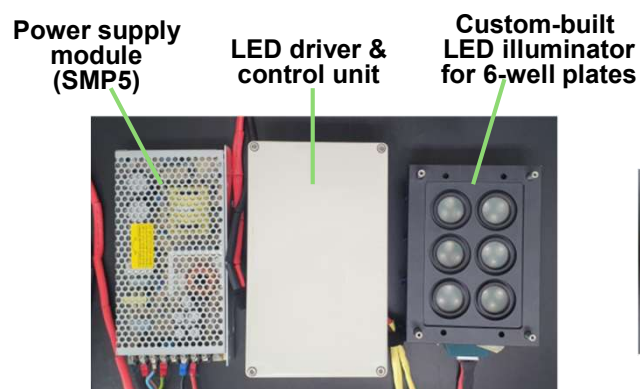


Figure S4. Mechanistic insights into LRRK2-GS-mediated p21 regulation and bioinformatic analysis of LRRK2-interacting proteins

(A) Real-time qPCR analysis showing a time-course of p21 mRNA levels in LRRK2-WT and -GS astrocytes treated with BSA or PA for 2 days. Data are presented as mean $\pm$ SD of three independent experiments. (B) Western blot analysis of LRRK2-GS astrocytes pretreated with 100 nM MLI-2 or 1 μ M PF06447475 (PF) for 10 min prior to PA addition. (C) Western blot analysis of the indicated proteins in LRRK2-GS astrocytes transfected with siRNAs targeting CUL1 or CUL4. (D) Venn diagrams illustrating LRRK2-interacting proteins identified through interactome analysis of two European Bioinformatics Institute (EBI) IntAct datasets (EBI-21364596 and EBI-9515510), with overlapping proteins summarized in the accompanying table.

A

Parameter [unit]	Red LED (650 nm)	NIR LED (850 nm)
Center wavelength [nm]	~650	~850
Average radiant power [mW]	40	40
Area irradiated (35mm dish) [cm ²]	9.62	9.62
Irradiance at target [mW/cm ²]	4.16	4.16
Energy density [J/cm ²]	0-2.5	0-2.5
Exposure duration [sec]	0-600	0-600
Application technique	15mm between LED and cells	15mm between LED and cells
Number of treatments	once daily for 2 days	once daily for 2 days

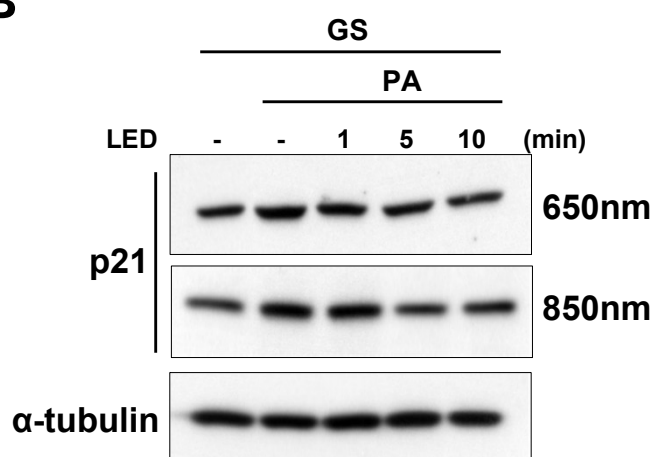
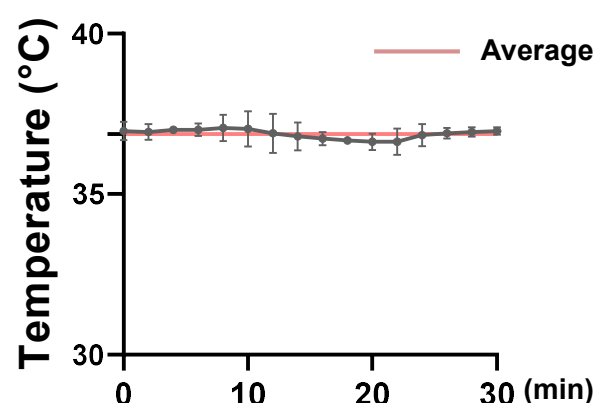
B**C**

Figure S5. Configuration of the LED array system and its effects on p21 expression

(A) Photographs of the LED array system showing the experimental set up, consisting of a custom-built LED illuminator, power supply module, and LED driver (upper), and table detailing the LED parameter settings (lower). **(B)** Western blot analyses of the p21 expression in LRRK2-GS primary astrocytes treated with BSA or PA with or without 650 or 850 nm LED irradiation for the indicated time points. **(C)** Temperature variation over time as measured by the thermistor.

Table S1. List of primers used for qPCR

Transcripts	Forward	Reverse
<i>Il1b</i>	TGGACCTTCCAGGATGAGGACA	G TTCATCTCGGAGCCTGTAGTG
<i>Cxcl2</i>	CATCCAGAGCTTGAGTGTGACG	GGCTTCAGGGTCAAGGCAAAC
<i>Mmp12</i>	CACACTTCCCAGGAATCAAGCC	TTTGGTGACACGACGGAACAGG
<i>Cdkn2a</i>	TGTTGAGGCTAGAGAGGATCTTG	CGAATCTGCACCGTAGTTGAGC
<i>Lmnb1</i>	AGGAAGAGCTGGAGCAGACCTA	GCAGGTTAGAGAGCTGTGAGGA
<i>Gapdh</i>	ACCCAGAAGACTGTGGATGG	CACATTGGGGGTAGGAACAC

Table S2. List of primary antibodies used in this work

			Working dilution	
Primary antibody	Supplier (Cat.Number)	RRID	IB	IF
Anti-Tyrosine Hydroxylase	Pel-Freez (P40101-150)	AB_2617184	1:1000	
Anti-p21	Santa Cruz Biotechnology (sc-6246)	AB_628073	1:1000	1:100
Anti-p-Histone H2AX	Cell signaling Technology (2577)	AB_2118010	1:1000	1:200
Anti-Lamin B1	Santa Cruz Biotechnology (sc-377000)	AB_2861346	1:1000	
Anti-GFAP	Cell signaling Technology (3670)	AB_561049		1:200
Anti-MAP2	Abcam (ab32454)	AB_776174		1:100
Anti-Ubiquitin	Cell signaling Technology (43124)	AB_2799235	1:1000	
Anti-CUL4A	Abcam (ab72548)	AB_1268363	1:500	
Anti-DDB1	Santa Cruz Biotechnology (sc-376860)	AB_2894825	1:500	
Anti-LRRK2	Abcam (ab133474)	AB_2713963	1:1000	
Anti-alpha-Tubulin	Santa Cruz Biotechnology (sc-8035)	AB_628408	1:1000	

Table S3. Comparison of genes expression in LRRK2-WT and -GS brain**Cell cycle process**

GeneID	Symbol	Gene Name	Fold change (GS/WT)
18858	Pmp22	peripheral myelin protein 22	21.067395
68724	Arl8a	ADP-ribosylation factor-like 8A	3.667550
227800	Rabgap1	RAB GTPase activating protein 1	3.242238
12575	Cdkn1a	cyclin-dependent kinase inhibitor 1A (P21)	3.153955
217653	Mis18bp1	MIS18 binding protein 1	-5.558376
108912	Cdca2	cell division cycle associated 2	-5.824093
70645	Oip5	Opa interacting protein 5	-6.023742
76464	Casc5	cancer susceptibility candidate 5	-6.142262
72415	Sgol1	shugoshin-like 1 (S. pombe)	-6.583509
77744	Bora	bora, aurora kinase A activator	-8.636958
213391	Rassf4	Ras association (RalGDS/AF-6) domain family member 4	-10.619078
109212	Fam64a	family with sequence similarity 64, member A	-11.840625
66977	Nuf2	NUF2, NDC80 kinetochore complex component, homolog	-15.281631
107995	Cdc20	cell division cycle 20	-17.316875
16906	Lmnbl	lamin B1	-2.649408

Apoptosis/Cell death

GeneID	Symbol	Gene Name	Fold change (GS/WT)
21933	Tnfrsf10b	tumor necrosis factor receptor superfamily, member 10b	28.446975
27280	Phlda3	pleckstrin homology-like domain, family A, member 3	16.420051
74041	Ddias	DNA damage-induced apoptosis suppressor	8.996434
53619	Blcap	bladder cancer associated protein homolog (human)	7.149191
69065	Chac1	ChaC, cation transport regulator 1	4.616928
22029	Traf1	TNF receptor-associated factor 1	3.490080
68048	Aen	apoptosis enhancing nuclease	3.456671
66854	Trim35	tripartite motif-containing 35	3.317677
21981	Ppp1r13b	protein phosphatase 1, regulatory (inhibitor) subunit 13B	2.730793
52440	Tax1bp1	Tax1 (human T cell leukemia virus type I) binding protein 1	2.331837
67171	Dram2	DNA-damage regulated autophagy modulator 2	2.286182
13730	Emp1	epithelial membrane protein 1	2.965496
16904	Gzmm	granzyme M (lymphocyte met-ase 1)	1.642303

Inflammatory response

GeneID	Symbol	Gene Name	Fold change (GS/WT)
244237	Tnfrsf26	tumor necrosis factor receptor superfamily, member 26	7.113469
216850	Kdm6b	KDM1 lysine (K)-specific demethylase 6B	5.323752
21938	Tnfrsf1b	tumor necrosis factor receptor superfamily, member 1b	3.404186
56312	Nupr1	nuclear protein transcription regulator 1	2.047254
19225	Ptgs2	prostaglandin-endoperoxide synthase 2	8.289069
20310	Cxcl2	chemokine (C-X-C motif) ligand 2	5.085842
17381	Mmp12	matrix metalloproteinase 12	2.304712

Continue

Response to endoplasmic reticulum stress

GeneID	Symbol	Gene Name	Fold change (GS/WT)
30934	Tor1b	torsin family 1, member B	1.832415
240832	Tor1aip2	torsin A interacting protein 2	1.586899
13198	Ddit3	DNA-damage inducible transcript 3	2.491884
27368	Tbl2	transducin (beta)-like 2	-8.073159

Lipid metabolism

GeneID	Symbol	Gene Name	Fold change (GS/WT)
105501	Abhd4	abhydrolase domain containing 4	13.877412
68262	Agpat4	1-acylglycerol-3-phosphate O-acyltransferase 4 (lysophosphatidic acid acyltransferase, delta)	11.513756
67630	Samd8	sterile alpha motif domain containing 8	3.690706
17777	Mttp	microsomal triglyceride transfer protein	3.350090
56442	Serinc1	serine incorporator 1	2.925487
66569	Gdpd1	glycerophosphodiester phosphodiesterase domain containing 1	2.566603
16835	Ldlr	low density lipoprotein receptor	-3.001132
210992	Lpcat1	lysophosphatidylcholine acyltransferase 1	-3.318206
68603	Pmvk	phosphomevalonate kinase	-3.349363
235293	Sc5d	sterol-C5-desaturase (fungal ERG3, delta-5-desaturase) homolog (S. cerevisae)	-3.939775
56473	Fads2	fatty acid desaturase 2	-4.010522
231070	Insig1	insulin induced gene 1	-4.028390
20249	Scd1	stearoyl-Coenzyme A desaturase 1	-4.343416
13121	Cyp51	cytochrome P450, family 51	-4.421748
14137	Fdft1	farnesyl diphosphate farnesyl transferase 1	-4.504343
15490	Hsd17b7	hydroxysteroid (17-beta) dehydrogenase 7	-5.130810
208715	Hmgcs1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1	-5.355396
16987	Lss	lanosterol synthase	-6.071151
68671	Pcyt2	phosphate cytidyltransferase 2, ethanolamine	-6.260061
15446	Hpgd	hydroxyprostaglandin dehydrogenase 15 (NAD)	-7.315146
192156	Mvd	mevalonate (diphospho) decarboxylase	-8.901509
74754	Dhcr24	24-dehydrocholesterol reductase	-9.544017
170439	Elovl6	ELOVL family member 6, elongation of long chain fatty acids (yeast)	-10.419205