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## Appendix Figure legends

**Appendix Figure S1.** RNA-seq analyses for the genes commonly regulated by SGK1 inhibition and Nurr1+Foxa2 (N+F) overexpression in cultured glia. A and B, Venn-diagram summarizing the overlap of the genes downregulated (A) and upregulated (B) from the glial culture treated with the SGK1 inhibitor GSK-650394 vs. the untreated control (left circle) and the culture transduced with Nurr1+Foxa2 vs. the mock-transduced control (right circle). The numbers of genes are indicated in the Venn-diagram. C–F, GO and KEGG analyses for the genes commonly down- (726 genes, C and E) or upregulated (777 genes, D and F). Red bars indicate p values, and negative logs of the p values are plotted on the X-axis.

**Appendix Figure S2.** SGK1 inhibition upregulates expression and activity of glutamate transporters in cultured glial cells. A and B, Expression of glutamate transporters in RNA-seq and q-PCR analyses. C, Glutamate uptake activity. n=3. ANOVA. Significantly different at p=0.00001#, 0.0018###, 0.0393\* (GLT1), 0.002#, 0.0007### (GLAST) in graph B and p=0.0026#, 0.0001###, 0.012\* in graph C.

**Appendix Figure S3.** The combined  $\alpha$ -syn overexpression + PFF treatment to mDA neuronal cultures induces the formation of  $\alpha$ -syn aggregates detected by immunoblot for SNCA( $\alpha$ -syn) (A) and immunocytochemical analyses against phosphorylated  $\alpha$ -syn at serine 129 (p129- $\alpha$ -syn) (B). n=3, student's t-test. Significantly different at p=0.016\*, 0.0006\*\*, 0.0005\*\*\*, 0.0319\*\*\*\* in graph A and p=0.0054\* in graph B. Scale bar, 20 $\mu$ m.

**Appendix Figure S4.** Effect of the SGK1 inhibitor GSK-650394 treatment on  $\alpha$ -syn monomer and aggregate levels in primary mDA neuron cultures. Seven days after the initial  $\alpha$ -syn PFF treatment, the neuronal cultures were exposed to GSK-650394 for 4 days. The levels of  $\alpha$ -syn monomers and aggregates were detected by immunoblot for SNCA( $\alpha$ -syn) (A) and immunocytochemical analyses against p129- $\alpha$ -syn (B). n=3. ANOVA. Significantly different at p=0.0003#, 0.0003### (monomer), 0.0002#, 0.0007### (dimer), 9.03E-06#, 7.77E-06### (trimer), 1.04E-06#, 1.16E-06### (oligomer) in graph A and p=0.0016\*, 0.0022\*\* in graph B. ns, not significant, Scale bar, 20 $\mu$ m.

**Appendix Figure S5.** SGK1 inhibition in VM-glia potentiates their ability to rescue neuronal cells from mitochondrial and oxidative stress. VM-derived mDA neuron cultures were treated with glial CM (GCM) prepared in VM-glial cultures transduced with sh-Sgk1 (or sh-control as a control) in the presence of H<sub>2</sub>O<sub>2</sub> (150  $\mu$ M, 2hr). Intra-neuronal mitochondrial and oxidative stress was estimated by Mito-timer (A), Mito-Sox (B), DCF-staining (C), and reduced glutathione (GSH) levels (D). ANOVA. n=3. Significantly different at p=1.18E-15#, 9.72E-08##, 0.011\*, 0.048\*\*, 1.98E-10+ in graph A and p=1.98E-24#, 4.99E-15##, 1.43E-08\*, 0.000003\*\*, 1.80E-17+ in graph B and 1.37E-24#, 1.09E-12##, 0.000037###, 1.58E-10\*, 0.0002\*\*, 5.36E-18+ in graph C. Significantly different at p=0.0329#, 0.0054##, 0.0383\* in graph D. Scale bar, 20 $\mu$ m.

**Appendix Figure S6.** Effect of the SGK1 inhibitor GSK-650394 on neuronal viability in primary mDA neuron cultures. A, Endogenous SGK1 expression in the primary cultures of mDA neurons, astrocytes, and microglia. The cultures were derived from mouse VM at E15 (mDA neuron) and post-natal day 5 (astrocyte, microglia). Significantly different at p=0.00002\*, 0.009\*\* in graph A. B, Immunocytochemical analysis to determine mDA neuron degeneration. The pure mDA neuron cultures were treated with GSK-650394 (or DMSO as control). Two days after the inhibitor treatment, mDA neuronal viability and degeneration (induced by H<sub>2</sub>O<sub>2</sub>, 250 $\mu$ M, 3hr) were assessed using TuJ1+ (TH+) neuron counts and neurite outgrowths. n=3. Scale bar, 20 $\mu$ m.

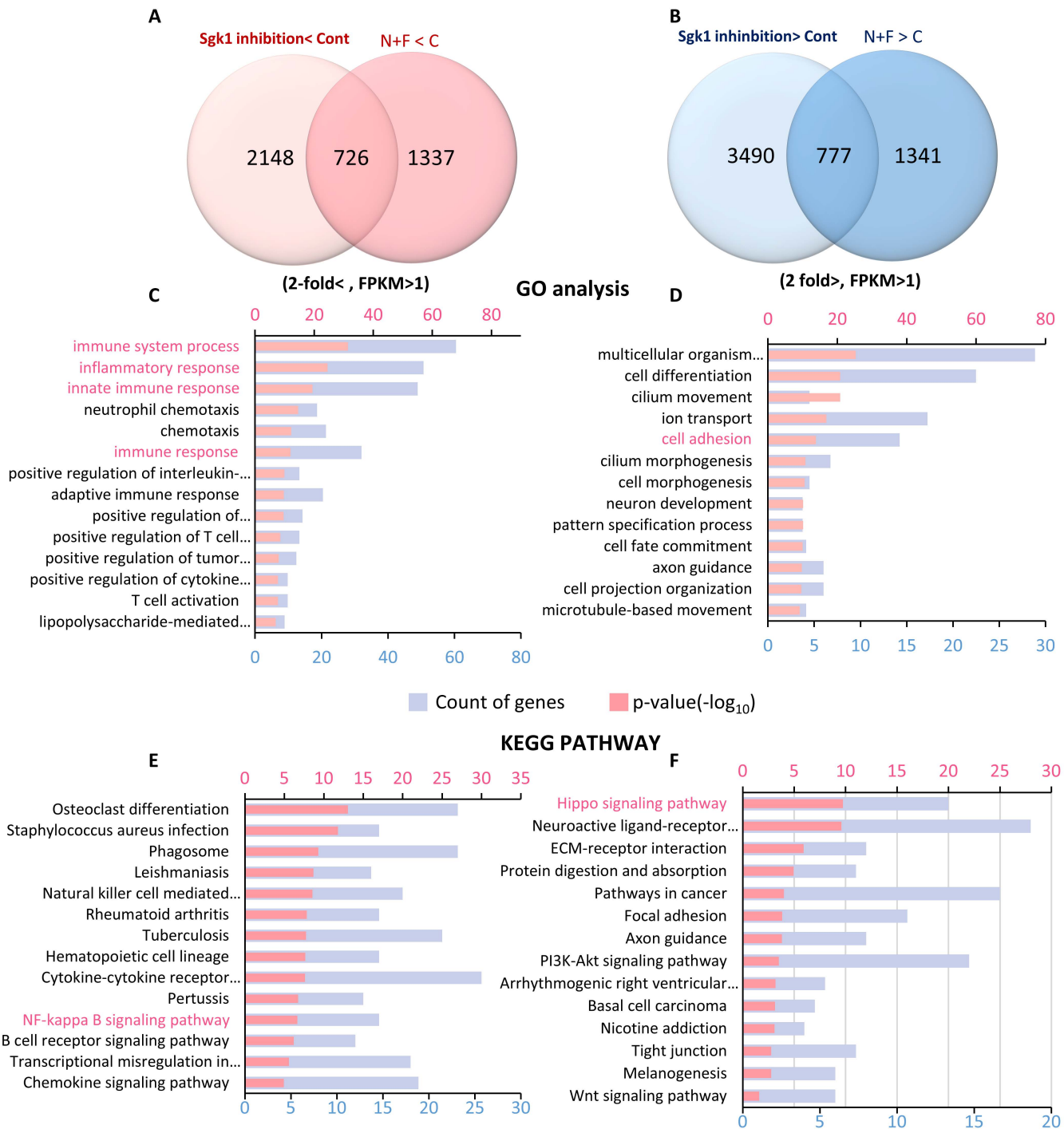
**Appendix Figure S7.** SGK1 inhibition in cultured astrocytes and microglia potentiates their individual and interactive neuroprotective actions in a paracrine manner. A, Schematic presentation of the experimental procedures and groups. On the right, the experimental groups for CM treatments are summarized with numbers and abbreviations. Astrocytes and microglia derived from the VMs of mouse pups were cultured and transduced with the lentivirus expressing sh-Sgk1 (or sh-cont as the control). Medium was conditioned in the cultures of astrocytes (ACM) and microglia (MCM) and directly administered to mDA neuron cultures. To assess the interactive neurotrophic actions between astrocytes and microglia, ACM was administered to cultured microglia for 2 days, and then the microglial CM challenged by ACM

(A-MCM) was collected and administered to cultured mDA neurons. Similarly, astrocyte CM challenged by MCM (M-ACM) was prepared. Cell viability and mDA neuronal resistance to H<sub>2</sub>O<sub>2</sub>-mediated toxic insult (250  $\mu$ M, 4hr) were determined using the CCK8 assay (B,C) and MAP2<sup>+</sup> neuronal (D,E) and TH<sup>+</sup> mDA neuronal (F,G) count. ANOVA. n=3 cultures. Significantly different at p=3.036E-12#, 1.053E-09##, 1.003E-10###, 3.073E-10####, 9.382E-09####, 0.0058\*, 0.0018\*\*, 0.0472&, 0.0347@ in graph B and p=1.088E-12#, 0.029\*, 0.036\*\*, 0.0178\*\*\*, 0.0154\*\*\*\* in graph C. Significantly different at p=6.951E-11#, 4.146E-09##, 3.08E-10###, 4.112E-08####, 2.273E-10#####, 0.0081\*\*, 0.0143+, 0.0393@ in graph D and p=8.145E-13#, 0.0345\*, 0.0042\*\*\*, 0.0309\*\*\*\* in graph E. Significantly different at p=1.414E-12#, 0.00001##, 3.297E-08###, 0.00001####, 8.266E-07#####, 0.0482+ in graph F and 1.058E-15#, 0.0489\*, 0.041\*\*\* in graph G.

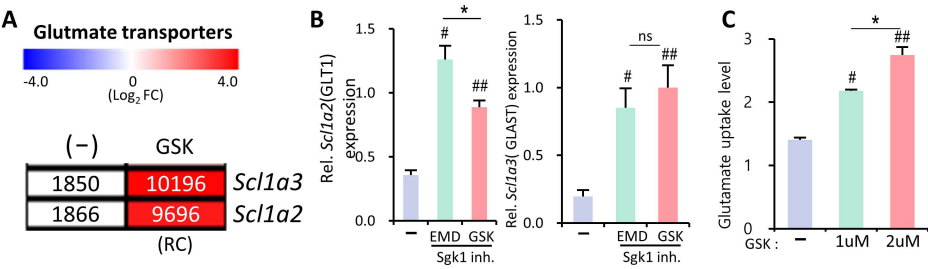
**Appendix Figure S8.** SGK1 expression upregulated in the SN of  $\alpha$ -syn-PD model mice. SGK1 expression levels were estimated in the midbrain SN of normal control (-) and PD (+) mice treated with the combined  $\alpha$ -syn-lentivirus+PFF (1 month after  $\alpha$ -syn injection). A and B, total SGK1 expression levels estimated by real-time PCR (A) and IHC (B). Significantly different at p=0.001\* in graph A and p=1.4E-06\* in graph B. C, Cell type-specific SGK1 expressions assessed in neuron (MAP2a<sup>+</sup>), astrocyte (GFAP<sup>+</sup>), and microglia (IBA1<sup>+</sup>). SGK1 positive cells were indicated by arrow heads. Significantly different at p=0.039\*, 0.013\*\*, 0.038\*\*\* in graph C. Student's t-test. n=3 (A), 3 (control) and 5 (PD) mice (B and C). Scale bar, 20 $\mu$ m.

**Appendix Figure S9.** Schematic summary for the action mechanisms of glial SGK1 inhibition to ameliorate PD pathologies.

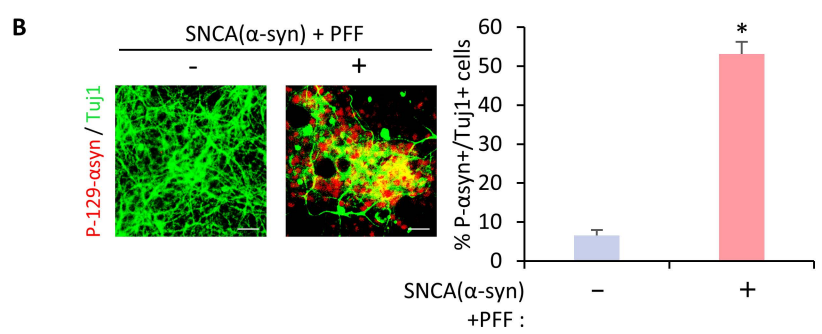
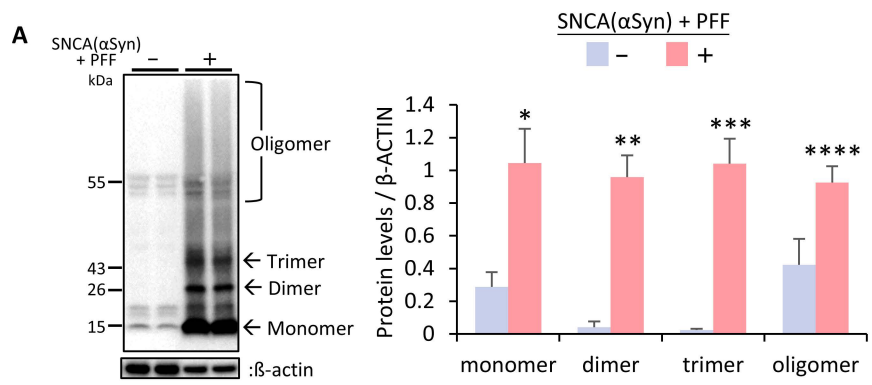
Appendix Figure S1



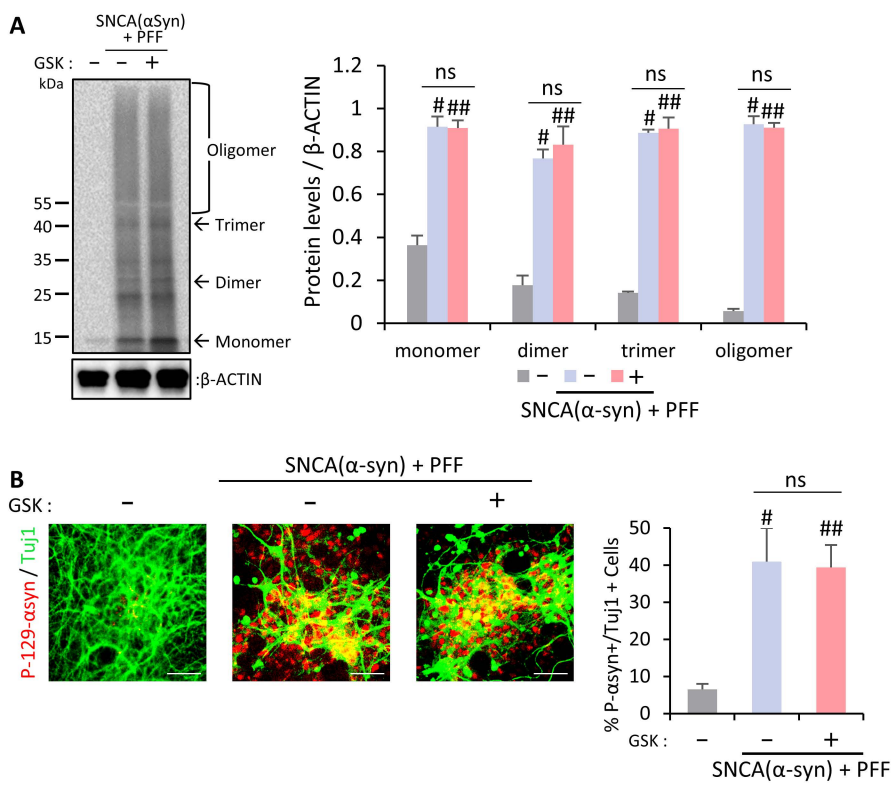
# Appendix Figure S2



Appendix Figure S3

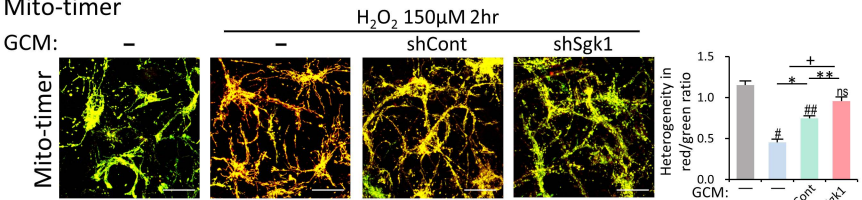


Appendix Figure S4

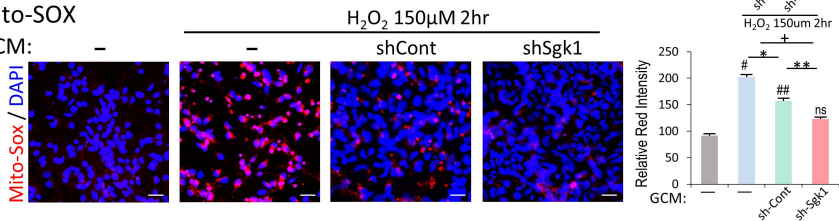


## Appendix Figure S5

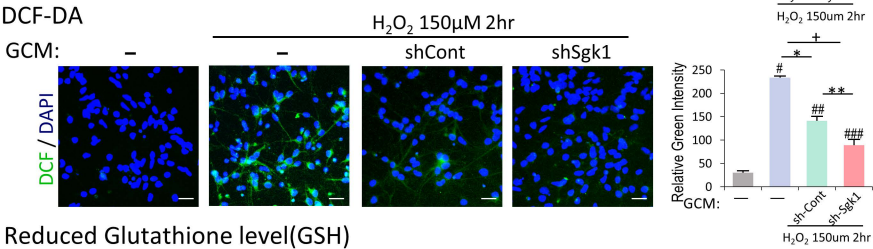
### A. Mito-timer



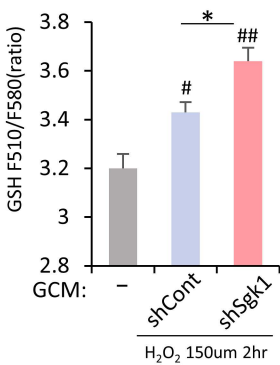
### B. Mito-SOX



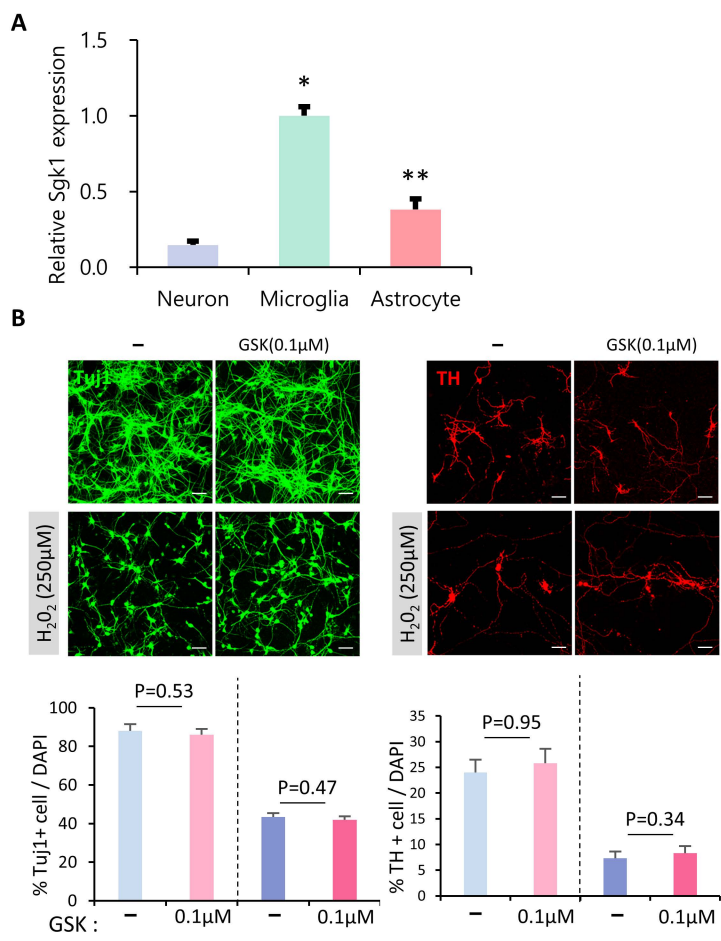
### C. DCF-DA



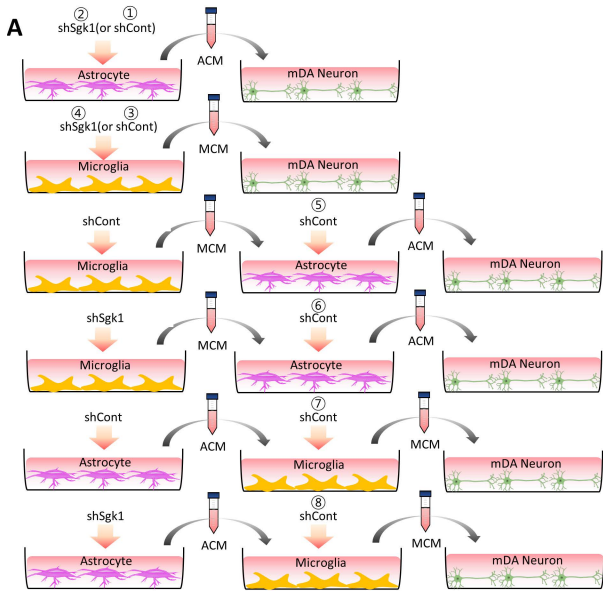
#### D. Reduced Glutathione level(GSH)



## Appendix Figure S6

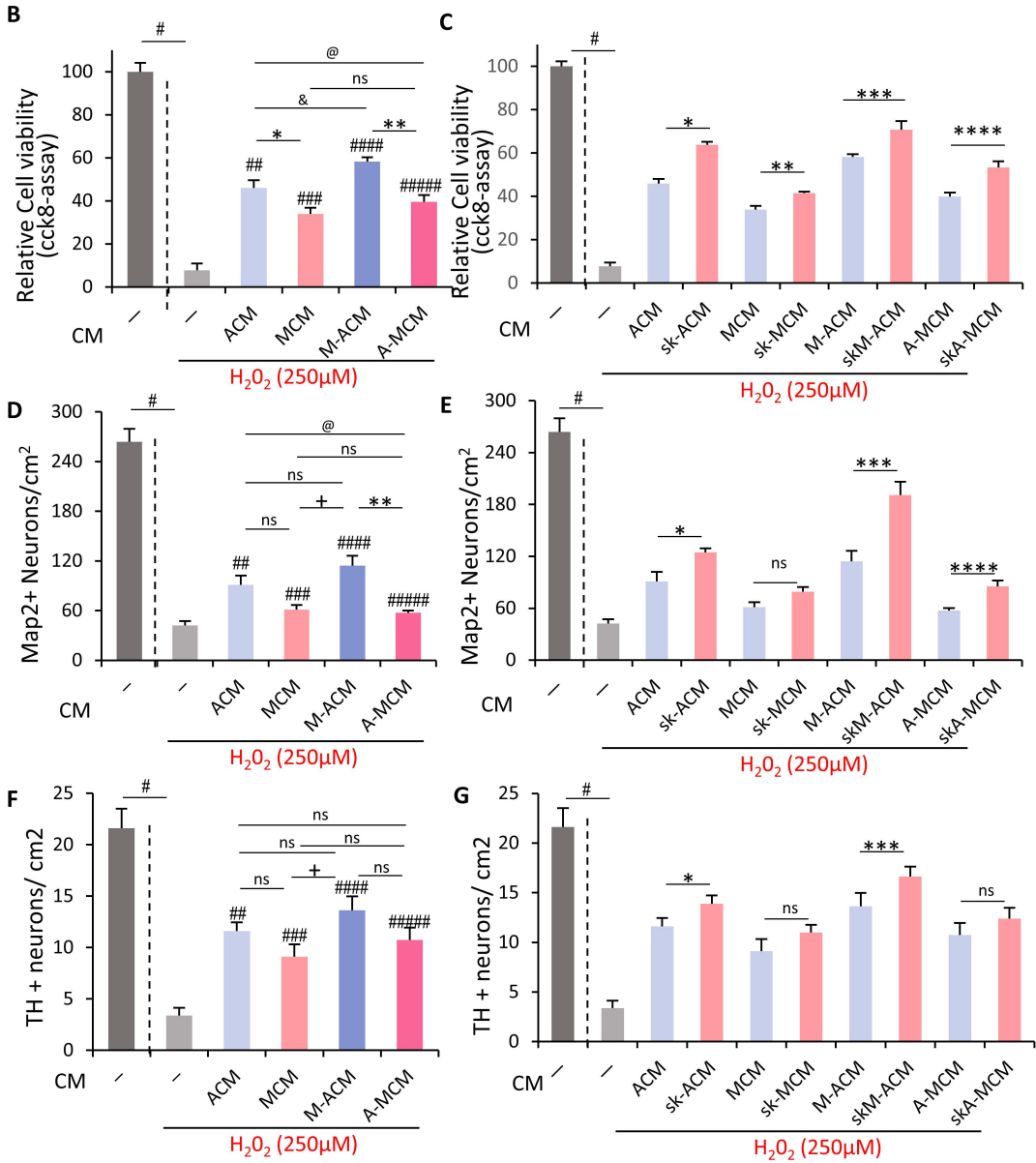


Appendix Figure S7

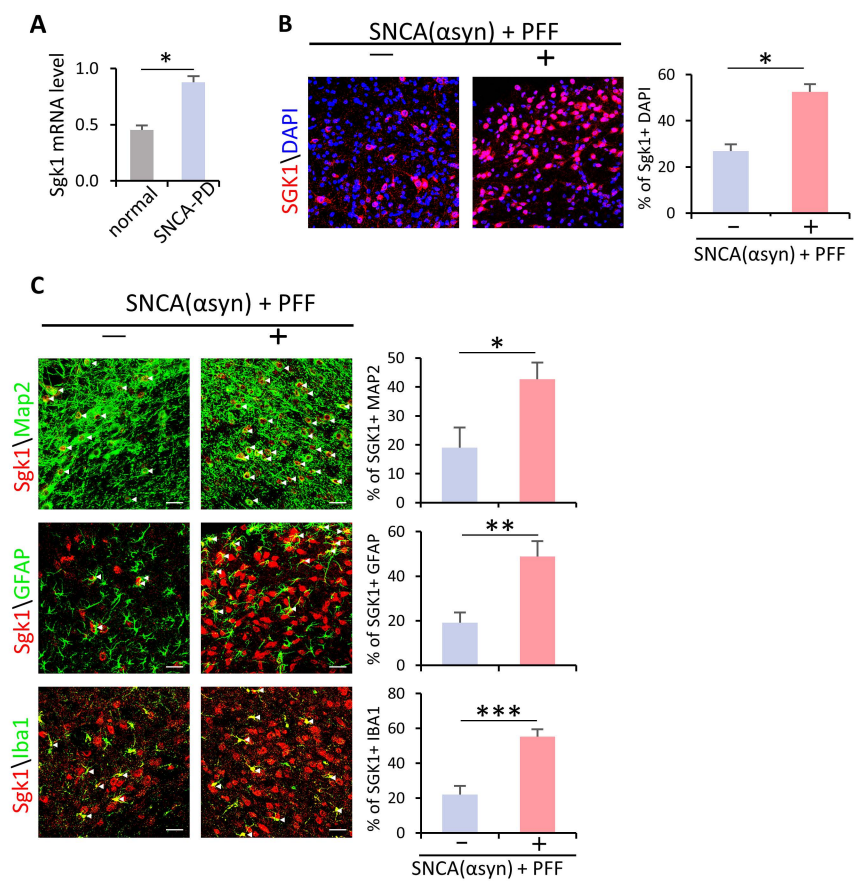


Summary for CM treatment    Abbreviation

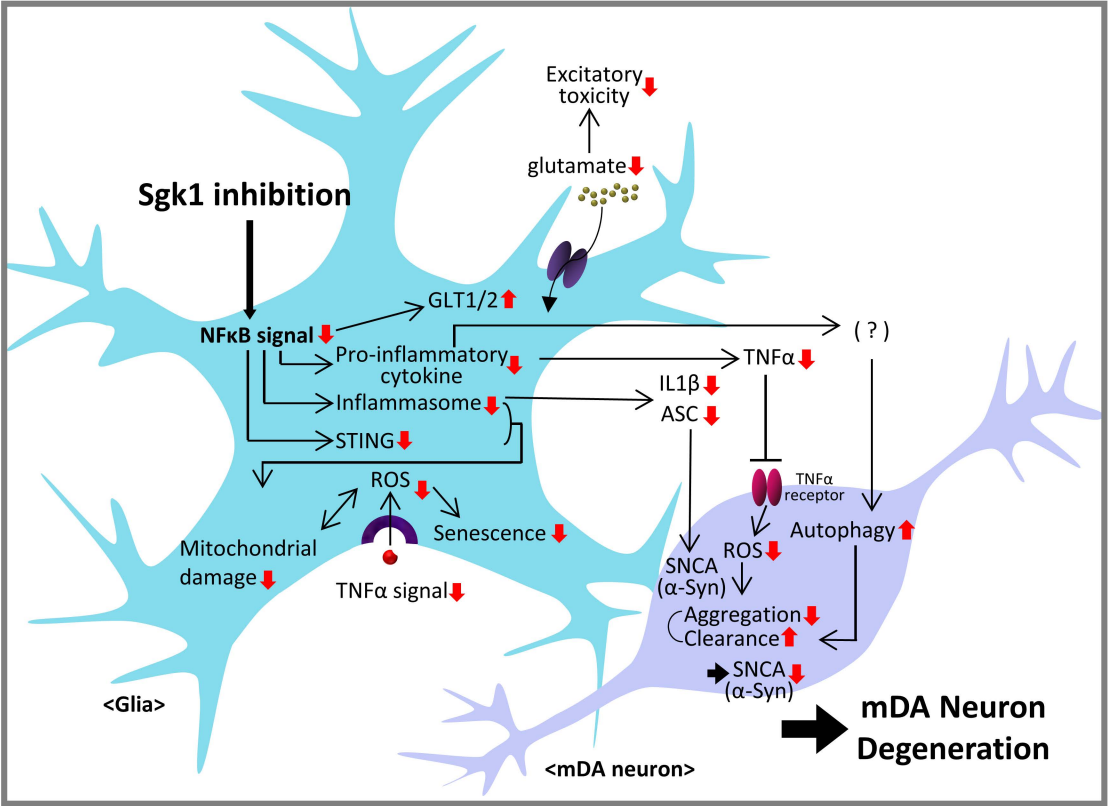
- ① shC-ACM :                      ACM
- ② shSgk1-ACM :                sk-ACM
- ③ shC-MCM :                    MCM
- ④ shSgk1-MCM:                sk-MCM
- ⑤ shC-MCM→shC-ACM:        M-ACM
- ⑥ shSgk1-MCM→shC-ACM:    skM-ACM
- ⑦ shC-ACM→shC-MCM:        A-MCM
- ⑧ shSgk1-ACM →shC-MCM:    skA-MCM



Appendix Figure S8



Appendix Figure S9



**Appendix Table S1. Primer sequences used for qPCR reactions.**

| Gene           | Primer Sequence(Sense/Antisense) |                             |
|----------------|----------------------------------|-----------------------------|
| Casp1          | CAC AGC TCT GGA GAT GGT GA       | TCT TTC AAG CTT GGG CAC TT  |
| DAI            | GCA TTC AAC ATG CAA ACA CC       | GGT CGT GTC CTC TAG CAA GC  |
| GLAST          | AGT AAG TGG CGG TTT CGG AG       | CAGAAGCTCCCCAGGAAAGG        |
| GLT1           | ATC CTG GGA GCA GTA TGT GG       | CTG ACA GCC CTG TGA TGA GA  |
| iNOS           | CAC CTT GGA GTT CAC CCA GT       | ACC ACT CGT ACT TGG GAT GC  |
| IFI16          | AGG CGG TCA AAC CAA ACA AG       | GGG TGA GCC TCC CTT GAA AT  |
| IL-6           | TGA AGG ACT CTG GCT TTG TCT      | ATG GAT GCT ACC AAA CTG GAT |
| IL- 1 $\beta$  | CTG TGA CTC GTG GGA TGA TG       | GGG ATT TTG TCG TTG CTT GT  |
| IFN- $\beta$   | TGC CCT CTC CAT CGA CTA CA       | GCT GAG GTT GAG CCT TCC AT  |
| NLRP3          | ATG CTG CTTCGA CAT CTC CT        | AAC CAA TGC GAG ATC CTG AC  |
| PGC1 $\alpha$  | ATGTGTCGCCTTCTTGCTCT             | ATCTACTGCCTGGGGACCTT        |
| PYCARD(ASC)    | ACA GAA GTG GAC GGA GTG CT       | CTC CAG GTC CAT CAC CAA GT  |
| SGK1           | GTA CCC TGC TCT CGC CTG          | ATC TGT ATC CCG ATC CGC CT  |
| TMEM173(STING) | CCC TCT CAA TCT CTC CTG TGC      | CTC AGA TCA TCA GGC ACC CC  |
| TNF- $\alpha$  | AGA TGT GGA ACT GGC AGA GG       | CCC ATT TGG GAA CTT CTC CT  |

**Appendix Table S2. Primary antibodies used in this study.**

| Antibodies                    | Immunostaining | Western Blot | Company                               |
|-------------------------------|----------------|--------------|---------------------------------------|
| Mouse monoclonal Antibody(Ab) |                |              |                                       |
| ASC                           | 1:500          | 1:1000       | <sup>14</sup> Santa Cruz              |
| Caspase-1(p10)                |                | 1:1000       | <sup>14</sup> Santa Cruz              |
| Caspase-1(p20)                |                | 1:500        | <sup>2</sup> AdipoGen                 |
| CD16/32                       | 1:500          |              | <sup>3</sup> BD Biosciences           |
| GFAP                          | 1:200          |              | <sup>9</sup> MP Biomedicals           |
| GFP                           | 1:1000         |              | <sup>8</sup> Thermo Fisher Scientific |
| MAP2                          | 1:1000         |              | <sup>15</sup> Sigma–Aldrich           |

|                          |        |        |                                       |
|--------------------------|--------|--------|---------------------------------------|
| NeuN                     | 1:100  |        | <sup>10</sup> Millipore               |
| Nitrated $\alpha$ syn    |        | 1:1000 | <sup>7</sup> Invitrogen               |
| P-129- $\alpha$ syn      | 1:1000 |        | <sup>4</sup> BioLegend                |
| TH                       | 1:200  |        | <sup>6</sup> Immunostar               |
| Tuj1                     | 1:1500 |        | <sup>4</sup> BioLegend                |
| YAP                      |        | 1:2000 | <sup>13</sup> Santa Cruz              |
| $\alpha$ -Synuclein      | 1:1000 | 1:2000 | <sup>2</sup> BD Biosciences           |
| $\beta$ -actin           |        | 1:2000 | <sup>7</sup> Invitrogen               |
| Rabbit polyclonal Ab     |        |        |                                       |
| cGAS                     |        | 1:2000 | <sup>5</sup> Cell signaling           |
| HMGB1                    |        | 1:1000 | <sup>5</sup> Cell signaling           |
| GFP                      | 1:1000 |        | <sup>8</sup> Thermo Fisher Scientific |
| Iba-1                    | 1:200  |        | <sup>14</sup> Wako                    |
| IKK $\alpha$ / $\beta$   |        | 1:1500 | <sup>5</sup> Cell signaling           |
| IL-6                     |        | 1:1000 | <sup>5</sup> Cell signaling           |
| I $\kappa$ B $\alpha$    |        | 1:2000 | <sup>5</sup> Cell signaling           |
| IRF3                     |        | 1:2000 | <sup>5</sup> Cell signaling           |
| Lamin B1                 |        | 1:1000 | <sup>5</sup> Cell signaling           |
| LATS1                    |        | 1:1000 | <sup>5</sup> Cell signaling           |
| LATS2                    |        | 1:1000 | <sup>5</sup> Cell signaling           |
| MMP3                     |        | 1:1000 | <sup>5</sup> Cell signaling           |
| MST1                     |        | 1:1000 | <sup>5</sup> Cell signaling           |
| MST2                     |        | 1:1000 | <sup>5</sup> Cell signaling           |
| PGC1 $\alpha$            |        | 1:1000 | <sup>1</sup> Abcam                    |
| P16 INK4A                |        | 1:1000 | <sup>5</sup> Cell signaling           |
| p65 (NF $\kappa$ B)      |        | 1:1000 | <sup>5</sup> Cell signaling           |
| p-IKK $\alpha$ / $\beta$ |        | 1:1000 | <sup>5</sup> Cell signaling           |
| p-I $\kappa$ B $\alpha$  |        | 1:2000 | <sup>5</sup> Cell signaling           |
| p-p65(p-NF $\kappa$ B)   |        | 1:1500 | <sup>5</sup> Cell signaling           |
| p-TBK1                   |        | 1:1000 | <sup>5</sup> Cell signaling           |

|                    |        |        |                             |
|--------------------|--------|--------|-----------------------------|
| p-IRF3             |        | 1:2000 | <sup>5</sup> Cell signaling |
| p-s127-YAP         |        | 1:2000 | <sup>5</sup> Cell signaling |
| p-s397-YAP         |        | 1:2000 | <sup>5</sup> Cell signaling |
| SGK1               |        | 1:1000 | <sup>10</sup> Millipore     |
| SGK1               |        | 1:500  | <sup>1</sup> Abcam          |
| STING              |        | 1:1000 | <sup>1</sup> Abcam          |
| TH                 | 1:1000 |        | <sup>11</sup> Pel-freez     |
| TBK1               |        | 1:1000 | <sup>5</sup> Cell signaling |
| Tuj1               | 1:1500 |        | <sup>4</sup> BioLegend      |
| TNF $\alpha$       |        | 1:1000 | <sup>5</sup> Cell signaling |
| YAP/TAZ            |        | 1:1000 | <sup>5</sup> Cell signaling |
| Rat polyclonal Ab  |        |        |                             |
| DAT                | 1:200  |        | <sup>1</sup> Abcam          |
| Goat polyclonal Ab |        |        |                             |
| IL-1 $\beta$       |        | 1:2000 | <sup>12</sup> R&D Systems   |
| TNF $\alpha$       |        | 1:2000 | <sup>12</sup> R&D Systems   |

1. Abcam, Cambridge, MA, UK
2. AdipoGen, San Diego, CA, USA
3. BD Biosciences, Franklin Lakes, NJ, USA
4. BioLegend, San Diego, CA, USA
5. Cell signaling, Danvers, MA, USA
6. Immunostar, Hudson, WI, USA
7. Invitrogen, Carlsbad, CA, USA
8. Thermo Fisher Scientific, Waltham, MA, USA
9. MP Biomedicals, Santa Ana, CA, USA
10. Millipore, Pittsburgh, PA, USA
11. Pel-Freez, Rogers, AR, USA
12. R&D Systems, Minneapolis, MN, USA
13. Santa Cruz Biotechnology, Santa Cruz, CA, USA
14. Wako, Osaka, Japan
15. Sigma–Aldrich, Saint Louis, MO, USA