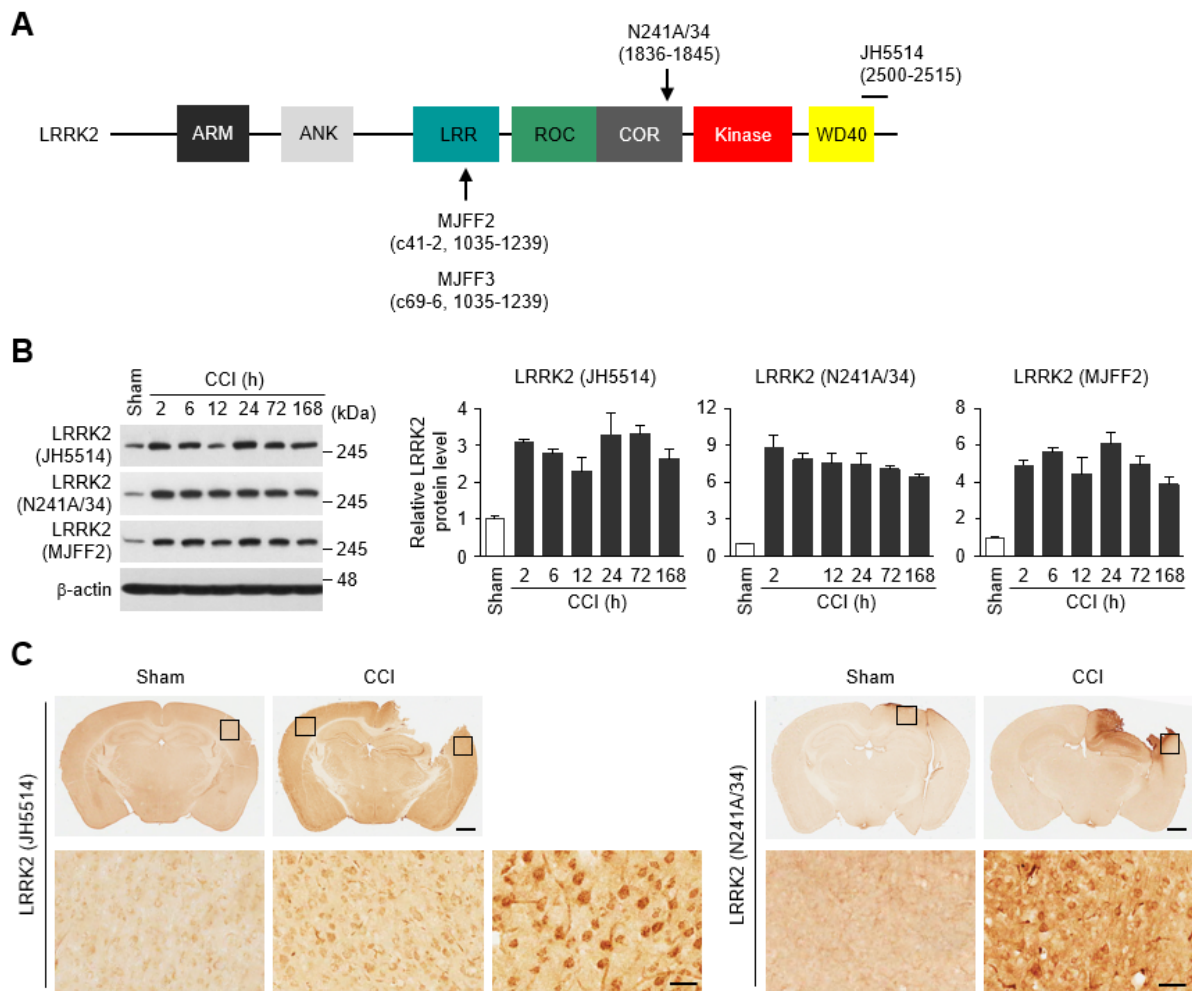
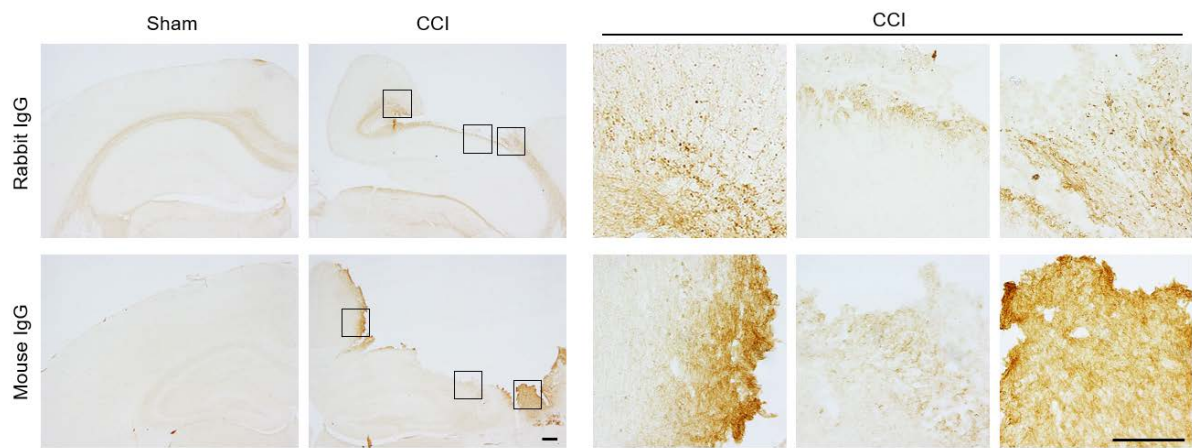


**Title: Brain injury induces HIF-1 $\alpha$ -dependent transcriptional activation of LRRK2 that exacerbates brain damage**

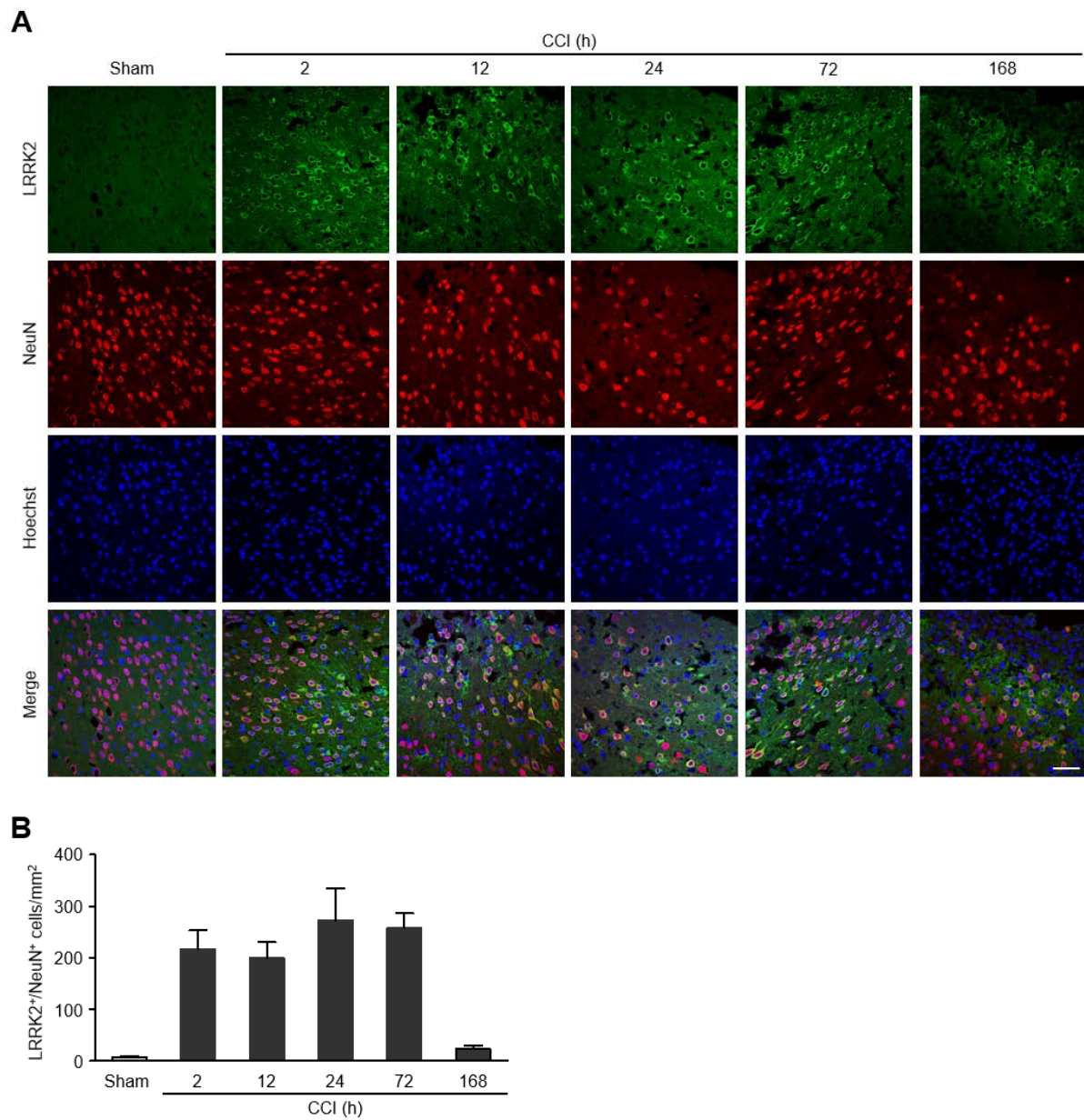
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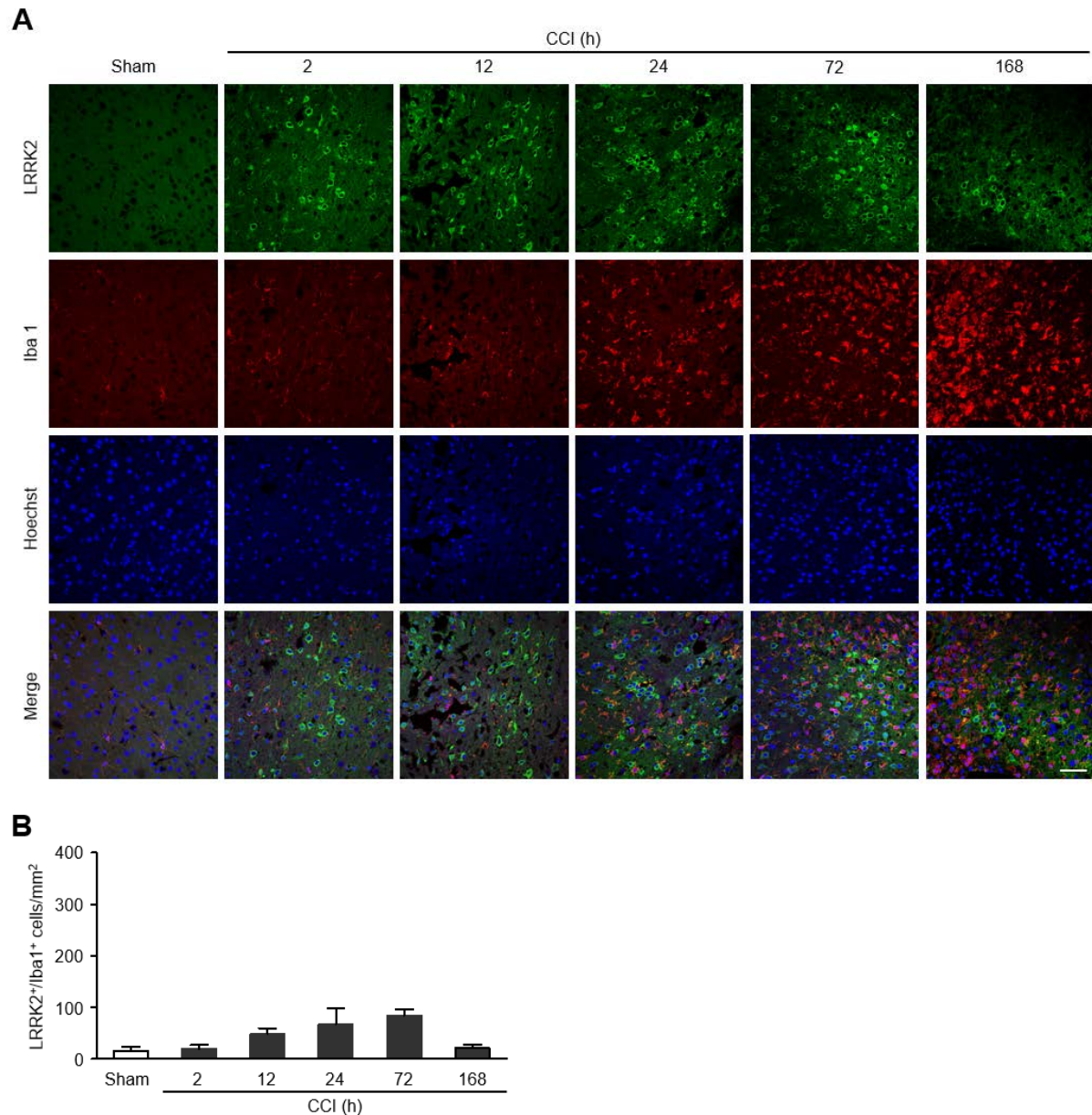
**Supplementary figure 1. LRRK2 induction in *in vivo* TBI model.** (A) Schematic diagram indicating antigen information of LRRK2 antibodies used in this paper. (B) Level of LRRK2 protein in brain lysates from the ipsilateral side of sham and CCI group. Shown are representative blots (B, *left*) and quantification of LRRK2 protein levels (B, *right*) relative to sham. Bar graph shows means  $\pm$  s.d. (n=3). (C) LRRK2 immunostaining from coronal brain sections of sham and CCI group at 24 hr post-injury. Magnified images of indicated area in *upper* rows are presented in *lower* row. Scale bar = 1 mm (*upper* row) and 50  $\mu$ m (*lower* row).



**Supplementary figure 2. Immunostaining with control IgG antibodies.** Immunostaining of sham and CCI brain sections collected at 24 hr post-injury with rabbit IgG or mouse IgG control antibodies. Magnified images of indicated area in *left* are presented in *right*. Scale bar = 200 μm.

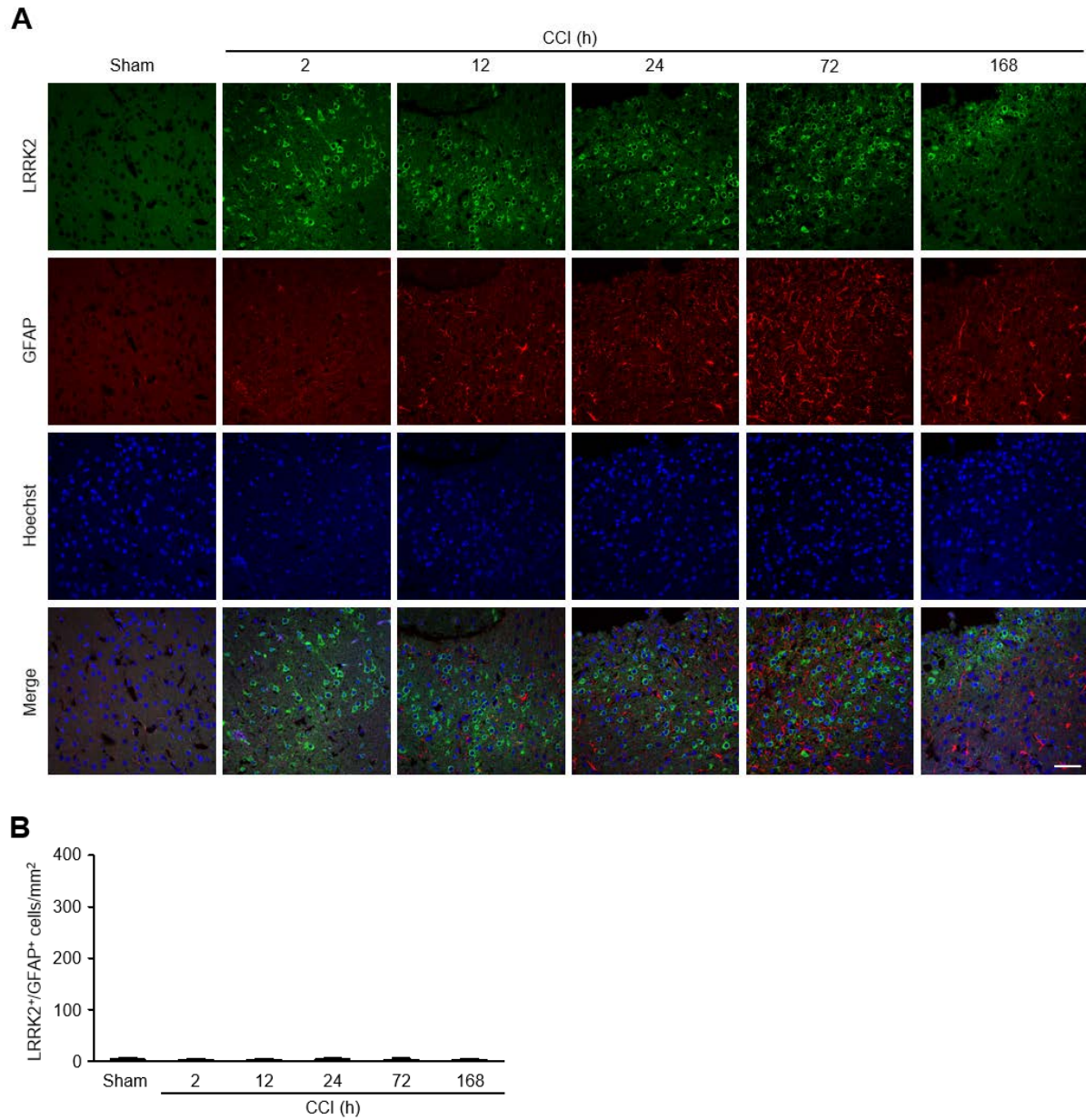


**Supplementary figure 3. LRRK2 induction in neuron after CCI injury.** Co-immunostaining with LRRK2 and NeuN antibodies from coronal brain sections of sham and CCI group. (A) Representative image in pericontusion region of CCI group and corresponding region of sham group immunostained with LRRK2 and NeuN antibodies. Scale bar = 50  $\mu$ m. (B) Numbers of LRRK2 and NeuN double immuno-positive cells (per mm<sup>2</sup>). Bar graph shows means  $\pm$  s.d. (n=3).

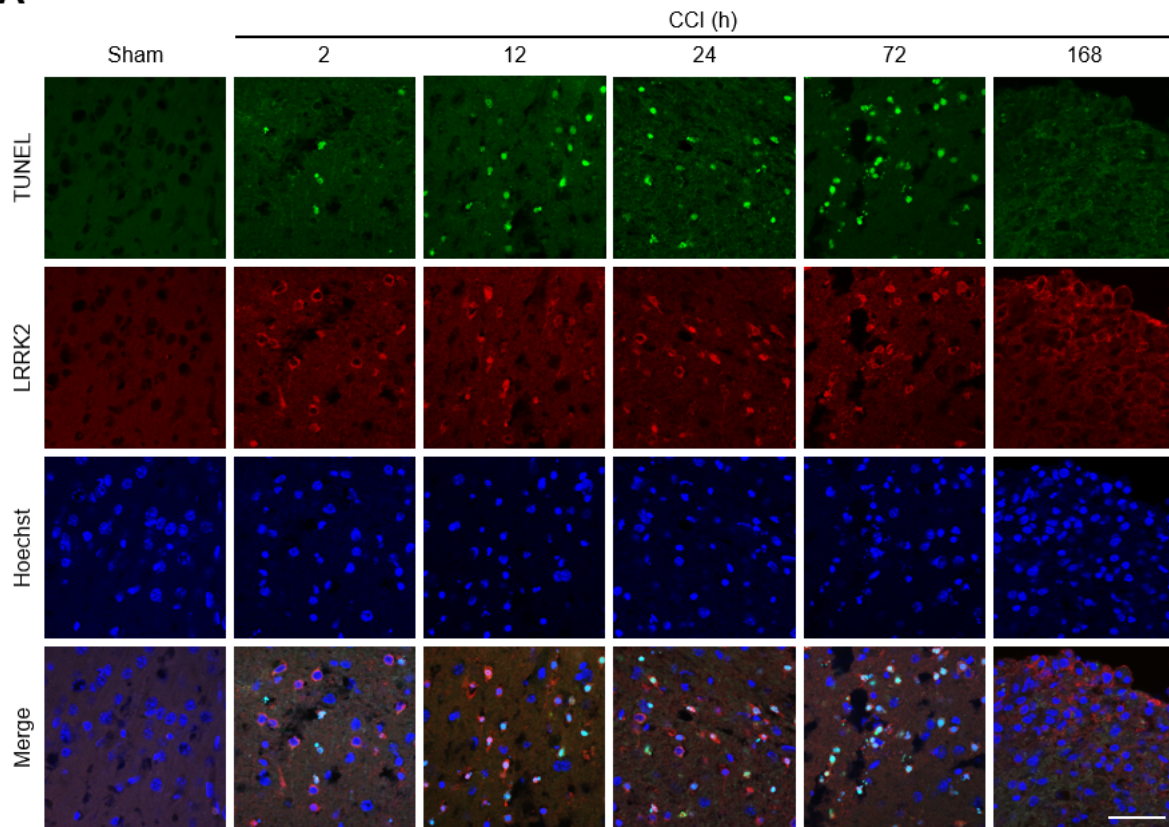
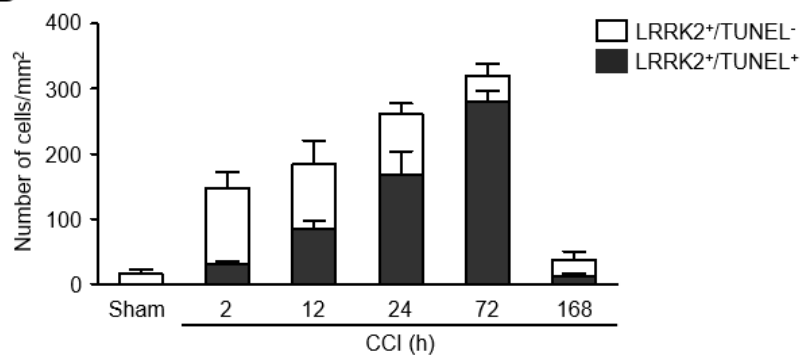


**Supplementary figure 4. LRRK2 induction in microglia after CCI injury.** Co-immunostaining with LRRK2 and Iba1 antibodies from coronal brain sections of sham and CCI group. (A) Representative image in pericontusion region of CCI group and corresponding region of sham group immunostained with LRRK2 and Iba1 antibodies. Scale bar = 50  $\mu$ m. (B) Numbers of LRRK2 and Iba1 double immuno-positive cells (per mm<sup>2</sup>). Bar graph shows means  $\pm$  s.d. (n=3).

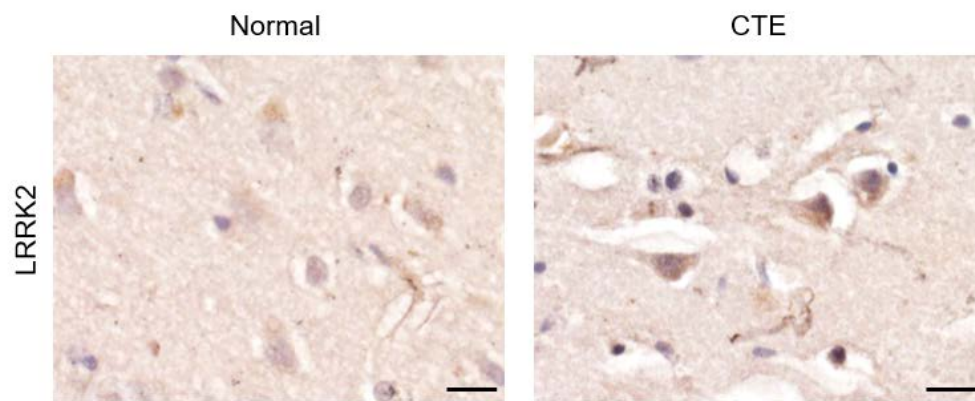




**Supplementary figure 5. LRRK2 induction in astrocyte after CCI injury.** Co-immunostaining with LRRK2 and GFAP antibodies from coronal brain sections of sham and CCI group. (a) Representative image in pericontusion region of CCI group and corresponding region of sham group immunostained with LRRK2 and GFAP antibodies. Scale bar = 50  $\mu$ m. (b) Numbers of LRRK2 and GFAP double immuno-positive cells (per mm<sup>2</sup>). Bar graph shows means  $\pm$  s.d. (n=3).

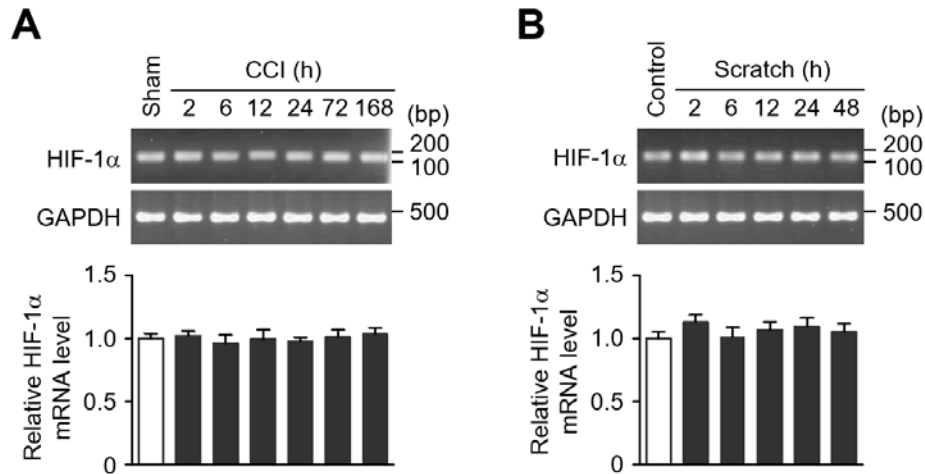
**A****B**

**Supplementary figure 6. Apoptosis in LRRK2 induced cells after CCI injury.** TUNEL staining and LRRK2 immunostaining from coronal brain sections of sham and CCI group. (A) Representative TUNEL image in pericontusion region of CCI group and corresponding region of sham group. Scale bar = 50  $\mu$ m. (B) Numbers of LRRK2<sup>+</sup>/TUNEL<sup>+</sup> and LRRK2<sup>+</sup>/TUNEL<sup>-</sup> cells (per mm<sup>2</sup>). Bar graph shows means  $\pm$  s.d. (n=3).



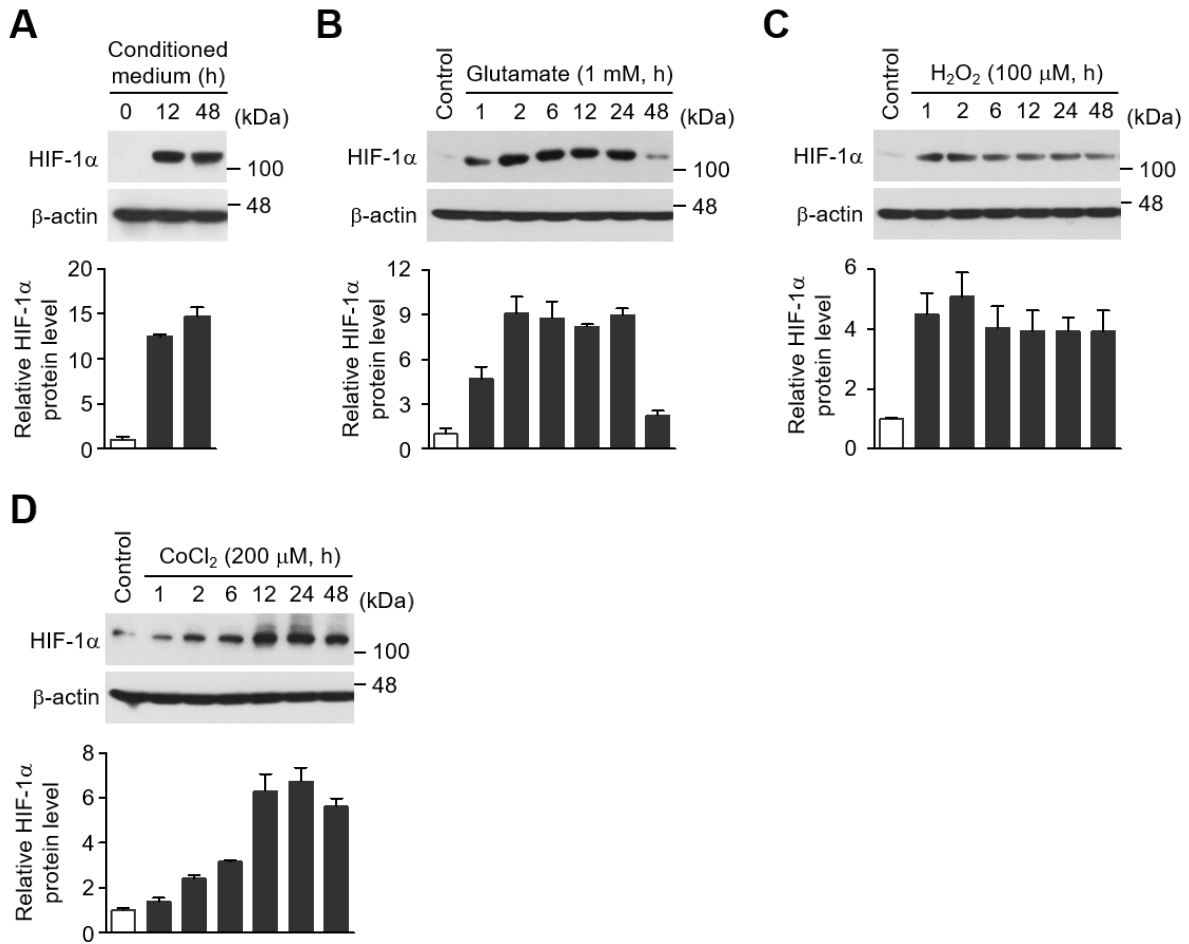
**Supplementary figure 7. LRRK2 induction in the frontal cortex of CTE patients.** Representative images of LRRK2 immunostaining in postmortem brain section from normal subject and CTE patient. Scale bar = 20  $\mu$ m.



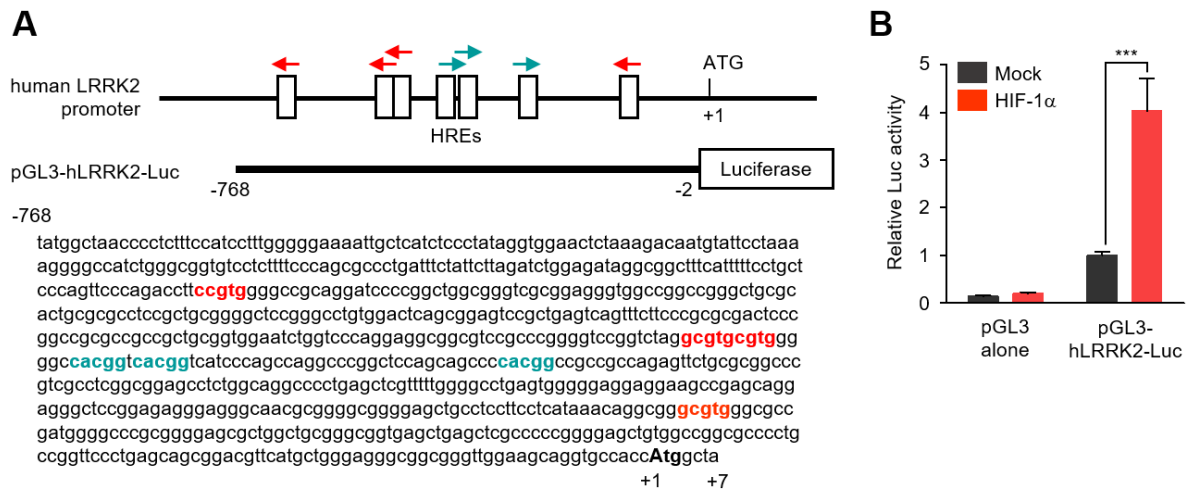


**Supplementary figure 8. The level of *HIF-1 $\alpha$*  mRNA in *in vivo* and *in vitro* TBI models.**

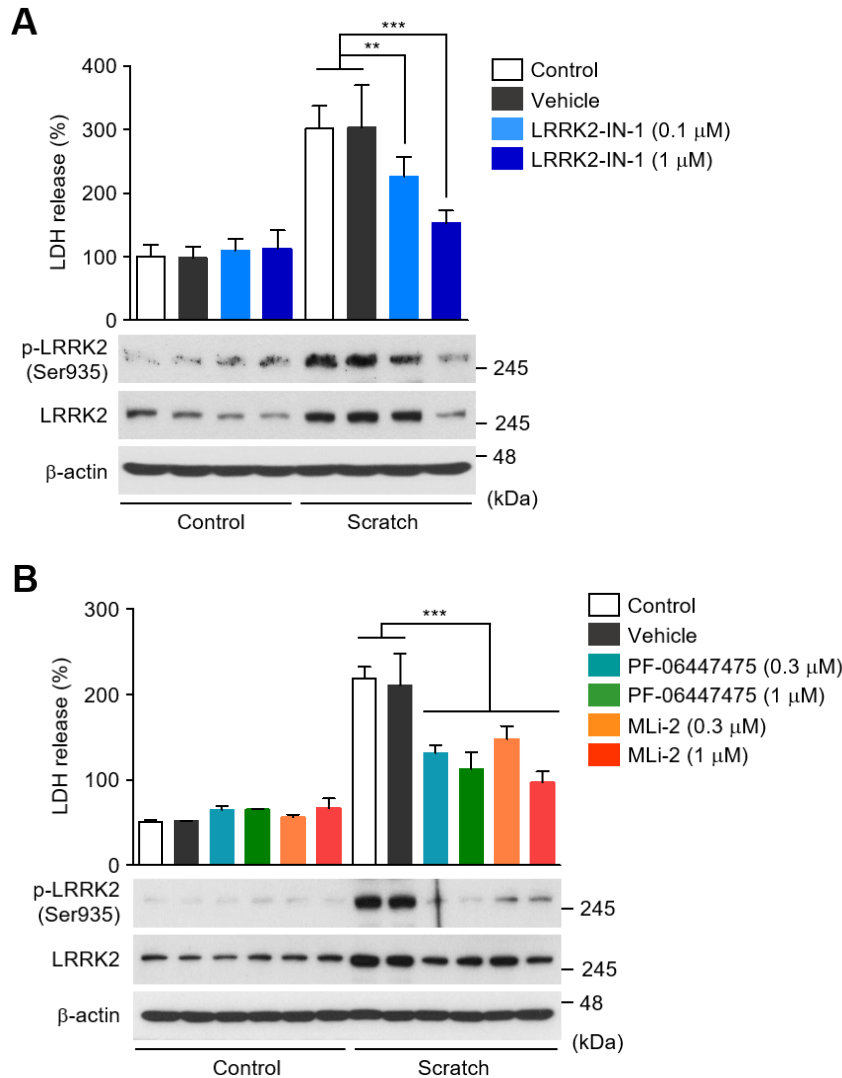
(A) The level of *HIF-1 $\alpha$*  mRNA in Sham and CCI brains. Shown are representative gel images (A, top) and quantification of *HIF-1 $\alpha$*  mRNA (A, bottom) relative to sham. Bar graph shows means  $\pm$  s.d. (n=3). (B) The level of *HIF-1 $\alpha$*  mRNA in control and scratch injured cortical neurons. Shown are representative gel images (B, top) and quantification of *HIF-1 $\alpha$*  mRNA (B, bottom) relative to control. Bar graph shows means  $\pm$  s.d. (n=3).



**Supplementary figure 9. HIF-1 $\alpha$  induction after treatment of neuronal toxic assaults.** Levels of HIF-1 $\alpha$  in cortical neurons treated with conditioned medium from scratch-injured cortical neurons (A), 1 mM glutamate (B), 100  $\mu$ M H<sub>2</sub>O<sub>2</sub> (C), and 200  $\mu$ M CoCl<sub>2</sub> (D). Shown are representative immunoblots (*top*) and quantification of LRRK2 protein level (*bottom*). Bar graph shows mean  $\pm$  s.d. (n=3).

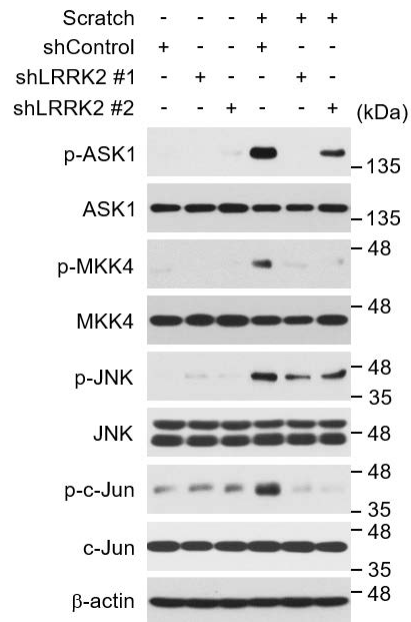
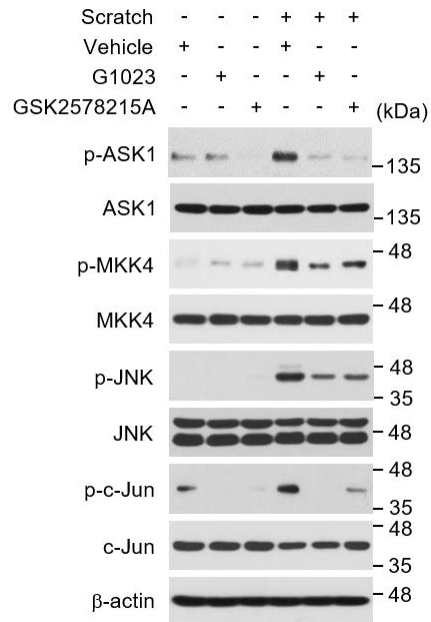


**Supplementary figure 10. Transcriptional regulation of human *LRRK2* by HIF-1α.** (A) Putative HRE sites in human *LRRK2* promoter and promoter sequence. (B) pGL3-hLRRK2-Luc and pcDNA3-HA-HIF-1α-WT were co-transfected into human neuroblastoma SH-SY5Y cells and luciferase activity was measured after 48 hr. Bar graph shows means  $\pm$  s.d. (n=3). One-way ANOVA followed by Newman-Keuls post hoc test was performed. \*\*\* $p < 0.001$ .

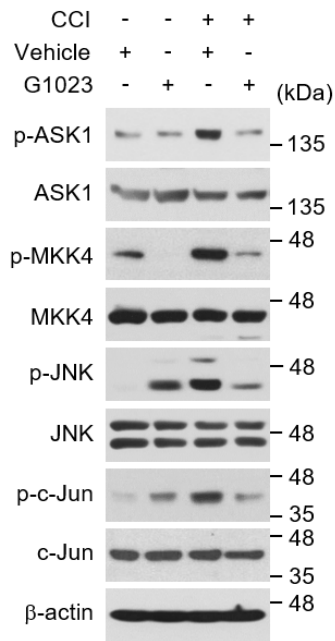


**Supplementary figure 11. The effect of LRRK2 kinase inhibitors on scratch induced neuronal toxicity.** Cortical neurons were pretreated with vehicle (0.05% DMSO) or LRRK2 kinase inhibitors, (A) LRRK2-IN-1 (0.1 and 1  $\mu$ M), (B) PF-06447475 (0.3 and 1  $\mu$ M), or (B) MLI-2 (0.3 and 1  $\mu$ M)) for 1 hr prior to scratch injury at DIV 10. After 48 hrs, the analyses described below were performed. LDH release assay (*top*). LDH release level relative to control neurons treated with vehicle control. Bar graph shows means  $\pm$  s.d. (n=3). Levels of total and phospho-S935 LRRK2 protein in control and scratch-injured cortical neurons treated with LRRK2-IN-1, PF-06447475, MLI-2, or vehicle control were measured. Representative immunoblots (bottom) are shown. One-way ANOVA followed by Newman-Keuls post hoc test was performed for all experiments. \*\* $p < 0.005$ , \*\*\* $p < 0.001$ .



**A****B**

**Supplementary figure 12. LRRK2 dependent regulation of AKS-1-MKK-JNK/c-Jun pathways after scratch injury.** Cortical neurons were (A) infected with lentivirus (pLL3.7-shControl, shLRRK2 #1, #2) at DIV8 and (B) treated with LRRK2 kinase inhibitors, 1  $\mu$ M G1023 and 1  $\mu$ M GSK2578215A at DIV10. Scratch injury was applied at DIV10 and cells were harvested at 48 hr post-injury. Immunoblots were performed with indicated antibodies.



**Supplementary figure 13. LRRK2 dependent regulation of AKS-1-MKK-JNK/c-Jun pathways after CCI injury.** LRRK2 kinase inhibitor, G1023 was daily applied of 50 mg/kg through peritoneal injection for 3 days post-injury. Peri-contusional area of CCI group and corresponding area of sham group treated with vehicle or G1023 were collected, and subjected into immunoblots with indicated antibodies.

## Supplementary Table

**Supplementary Table 1. Human tissue information for normal subjects and subjects with CTE.**

| Case     | Age | Sex | CTE stage |
|----------|-----|-----|-----------|
| Normal 1 | 87  | F   | 0         |
| Normal 2 | 88  | M   | 0         |
| Normal 3 | 86  | M   | 0         |
| Normal 4 | 87  | F   | 0         |
| Normal 5 | 67  | M   | 0         |
| Normal 6 | 82  | M   | 0         |
| Normal 7 | 70  | M   | 0         |
| CTE 1    | 84  | M   | 4         |
| CTE 2    | 73  | M   | 4         |
| CTE 3    | 80  | M   | 4         |
| CTE 4    | 73  | M   | 3         |
| CTE 5    | 76  | M   | 4         |
| CTE 6    | 93  | M   | 4         |
| CTE 7    | 69  | M   | 4         |

**Supplementary Table 2. List of antibodies.**

| <b>Primary antibodies</b>  |                           |                 |           |               |
|----------------------------|---------------------------|-----------------|-----------|---------------|
| <b>Antigen</b>             | <b>Supplier</b>           | <b>Cat. No.</b> | <b>WB</b> | <b>IHC/IF</b> |
| LRRK2                      | Abcam                     | ab133475        | 1:2000    |               |
| LRRK2                      | Abcam                     | ab133474        | 1:2000    | 1:300         |
| LRRK2                      | Millipore                 | ABN187          | 1:2000    | 1:500         |
| LRRK2                      | Neuromab                  | 73-253          | 1:200     | 1:100         |
| p-LRRK2 (Ser935)           | Abcam                     | ab133450        | 1:1000    |               |
| $\beta$ -actin             | Abcam                     | ab6276          | 1:4000    |               |
| HIF-1 $\alpha$             | Novus Biologicals         | NB100-105       | 1:2000    | 1:250         |
| HIF-1 $\alpha$             | Novus Biologicals         | NB100-479       | 1:2000    | 1:250         |
| MAP2                       | Thermo Fisher Scientific  | MA512826        |           | 1:500         |
| MAP2                       | Abcam                     | ab32454         |           | 1:1000        |
| Iba1                       | Abcam                     | ab5076          |           | 1:1000        |
| GFAP                       | Abcam                     | ab7260          |           | 1:1000        |
| GFAP                       | Sigma-Aldrich             | G3893           |           | 1:2000        |
| NeuN                       | Cell Signaling Technology | 12943           |           | 1:500         |
| NeuN                       | Millipore                 | MAB377          |           | 1:500         |
| Cleaved Caspase-3          | Cell Signaling Technology | 9661            | 1:1000    |               |
| PARP                       | Cell Signaling Technology | 9532            | 1:2000    |               |
| Bcl-2                      | Cell Signaling Technology | 2870            | 1:2000    |               |
| p53                        | Cell Signaling Technology | 2524            | 1:2000    |               |
| HA.11 Epitope Tag          | Covance                   | MMS-101p-200    | 1:2000    |               |
| Aquaporin 4                | Millipore                 | AB3594          | 1:2000    |               |
| MMP2                       | Santa Cruz Biotechnology  | sc-80201        | 1:4000    |               |
| MMP9                       | Abcam                     | ab38898         | 1:2000    |               |
| p-SAPK/JNK (Thr183/Tyr185) | Cell Signaling Technology | 9251            | 1:2000    |               |
| SAPK/JNK                   | Cell Signaling Technology | 9252            | 1:4000    |               |
| p-c-Jun (Ser63)            | Cell Signaling Technology | 2361            | 1:2000    |               |
| c-Jun                      | Cell Signaling Technology | 9165            | 1:2000    |               |
| p-SEK1/MKK4 (Ser257)       | Cell Signaling Technology | 4514            | 1:2000    |               |
| MKK4                       | Santa Cruz Biotechnology  | sc-376838       | 1:2000    |               |
| p-ASK1 (Thr845)            | Santa Cruz Biotechnology  | sc-109911       | 1:2000    |               |
| ASK1                       | Santa Cruz Biotechnology  | sc-390275       | 1:2000    |               |
| Mouse IgG (70 mg/ml)       | Sigma-Aldrich             | I5381           |           | 1:100         |
| Rabbit IgG (100 mg/ml)     | Sigma-Aldrich             | I5006           |           | 1:300         |

**Supplementary Table 2. List of antibodies (continued).**

| <b>Secondary antibodies</b>           |                           |                 |           |               |
|---------------------------------------|---------------------------|-----------------|-----------|---------------|
| <b>Name</b>                           | <b>Supplier</b>           | <b>Cat. No.</b> | <b>WB</b> | <b>IHC/IF</b> |
| anti-Mouse IgG (H+L)-HRP              | Cell Signaling Technology | 7076            | 1:5000    |               |
| anti-Rabbit IgG (H+L)-HRP             | Cell Signaling Technology | 7074            | 1:5000    |               |
| anti-Goat IgG (whole molecule)-HRP    | Sigma-Aldrich             | A5420           | 1:5000    |               |
| anti-Mouse IgG (H+L)-Biotin           | KPL                       | 16-18-15        |           | 1:500         |
| anti-Rabbit IgG (H+L)-Biotin          | Vector Laboratories       | BA-1000         |           | 1:500         |
| anti-Goat IgG (H+L)-Biotin            | Vector Laboratories       | BA-5000         |           | 1:500         |
| anti-Rabbit IgG (H+L)-Alexa Fluor 488 | Thermo Fisher Scientific  | A11034          |           | 1:500         |
| anti-Rabbit IgG (H+L)-Alexa Fluor 568 | Thermo Fisher Scientific  | A10042          |           | 1:500         |
| anti-Mouse IgG (H+L)-Alexa Fluor 488  | Thermo Fisher Scientific  | A11029          |           | 1:500         |
| anti-Mouse IgG (H+L)-Alexa Fluor 568  | Thermo Fisher Scientific  | A10037          |           | 1:500         |
| anti-Goat IgG (H+L)-Alexa Fluor 594   | Vector Laboratories       | A21468          |           | 1:500         |

**Supplementary Table 3. List of oligomer sequences.**

| Name                                                                                                 | Primer sequences                                                                                                                                           | Product (bp) | Genbank accession number |
|------------------------------------------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------|--------------|--------------------------|
| Mouse gene-specific primers used for RT-PCR analysis (Fig. 1a, 2a, 3a, 7a, and Supplementary Fig. 8) |                                                                                                                                                            |              |                          |
| GAPDH                                                                                                | Forward 5'-GTGATCATCTCCGCCCTTCTGC-3'<br>Reverse 5'-GATGCTGCTCTTACCACTCTCTTG-3'                                                                             | 443          | AK140794                 |
| LRRK2                                                                                                | Forward 5'-AGCTGGTTCTAGTCGCTTG-3'<br>Reverse 5'-GTAGTCTGCACCTCAGCAAC-3'                                                                                    | 310          | AY792512                 |
| HIF-1 $\alpha$                                                                                       | Forward 5'-CTCATCACTGTGGCACTTCC-3'<br>Reverse 5'-TCACTTCACTGCTTAGACCAAC-3'                                                                                 | 126          | AF003695                 |
| IL-1 $\beta$                                                                                         | Forward 5'-TCCAGGA TGAAGACATGAGCAC-3'<br>Reverse 5'-GACGCTCACACACGACGAGTTA-3'                                                                              | 105          | M15131                   |
| IL-6                                                                                                 | Forward 5'-CCACTTCAACAAGTCGAGGCTTA-3'<br>Reverse 5'-CCAGTTGGTAGCATCATCATTTTC-3'                                                                            | 169          | X54542                   |
| TNF- $\alpha$                                                                                        | Forward 5'-CTTCTGCTACTGAACCTCGG-3'<br>Reverse 5'-CAGGCTTGTCACCTCGAATTTT-3'                                                                                 | 134          | X02611                   |
| Human gene-specific primers used for qPCR analysis (Supplementary Fig. 3)                            |                                                                                                                                                            |              |                          |
| LRRK2                                                                                                | Forward 5'-GAGGCGCTTCGAGCTATT-3'<br>Reverse 5'-CTGAA TCCACGGA TTCCAA-3'                                                                                    |              | AY792511                 |
| GAPDH                                                                                                | Forward 5'-GAAA TCCCATCACCA TCTTC-3'<br>Reverse 5'-GAGGCTGTGTCTCA TACTTCTC-3'                                                                              |              | M133197                  |
| shRNA oligonucleotides for mouse LRRK2 (Fig. 5a-5f and Supplementary Fig. 12a)                       |                                                                                                                                                            |              |                          |
| shControl                                                                                            | Forward 5'-TGGCTGAAGTACTTCGAATGCTTTCGAAGA GAGACATTTTCGAGTACTACGCGTTTTTC-3'<br>Reverse 5'-TGGAGAAAAAAGCGCTGAGTACTGGAATGTCTCTTGAAGACATTTCGAA GTACTCAGCGCA-3' |              |                          |
| shLRRK2 #1                                                                                           | Forward 5'-TGTCTTACTACTTCAGATATTTTCAAGAGAAATATGTAAGTGTAAAGCTTTTTTC-3'<br>Reverse 5'-TGGAGAAAAAAGCTTACTTACTTTCACGATATTTCTCTGAAAAATATCGTGAAGTAGTACCA-3'      |              | AY792512                 |
| shLRRK2 #2                                                                                           | Forward 5'-TAACTGTATGATCA GGGCTTAATTCAGAGATTTCCTTGAATTGAGGCTGACTATCAGCTTAA-3'<br>Reverse 5'-TGGAGAAAAAAGTTGATGTCAGGCTGAATTCCTTGAATTGAGGCTGACTATCAGCTTAA-3' |              | AY792512                 |
| Primers used for dominant negative human HIF-1 $\alpha$ construct (Fig. 3g-3i and 4c) <sup>#</sup>   |                                                                                                                                                            |              |                          |
| HA-DN-HIF-1 $\alpha$ -pCDNA3                                                                         | Forward 5'-GGGATTCGCGCGAA GTAAAGAATCT-3'<br>Reverse 5'-GGATATTC TCATTGTCAAGAAGCCTACT-3'                                                                    |              |                          |
| Primers used for mouse and human LRRK2 promoter constructs (Fig. 4 and Supplementary Fig. 10)        |                                                                                                                                                            |              |                          |
| pGL-3-mLRRK2-Luc                                                                                     | Forward 5'-GGGTACCGCTGTGGTGGCATGTGAG-3'<br>Reverse 5'-GCTGAGAGGTGCAGCGCGGGGACT-3'                                                                          |              | AC099704                 |
| HRE1-mut                                                                                             | Forward 5'-GGGGACTTGGAAACAATGTGTCACTTAGAA TTT-3'<br>Reverse 5'-AAATTCTAA GTGACACATGTTCCAAAGTCCC-3'                                                         |              |                          |
| HRE2-mut                                                                                             | Forward 5'-CCACCTTCAATATTGGGTACTTGGAAAG-3'<br>Reverse 5'-CTTCAAGTAA CCAATATTGAAGGGGTGG-3'                                                                  |              |                          |
| HRE3-mut                                                                                             | Forward 5'-GCACCTTACTACCAATCAAAAGGTGA AAAAC-3'<br>Reverse 5'-GTTTCACTTGA TTGGTAGTAAAGTGC-3'                                                                |              |                          |
| HRE4-mut                                                                                             | Forward 5'-GAAAAACCTTACTAATTGAGCA TTTTCTT-3'<br>Reverse 5'-AAGAAAAATGCTCAATTAGTAAGGTTTTTC-3'                                                               |              |                          |
| pGL-3-mLRRK2-Luc                                                                                     | Forward 5'-GGTACCGATGGCTAACCCCTCTTTC-3'<br>Reverse 5'-CTCAGGCTGGCACCCTGCTTCCAAC-3'                                                                         |              | AC079630                 |
| Primers used in ChIP analysis (Fig. 4f)                                                              |                                                                                                                                                            |              |                          |
| mLRRK2 promoter-HRE3                                                                                 | Forward 5'-GGTACTTGGAAAGCAATCC-3'<br>Reverse 5'-GATGGGTAAAGGTGAGG-3'                                                                                       | 232          | AC099704                 |
|                                                                                                      |                                                                                                                                                            |              |                          |

<sup>#</sup> Sequences for the restriction enzymes BamHI and EcoRV, with an extra *g* added to improve digestion efficiency, are underlined and in italics.