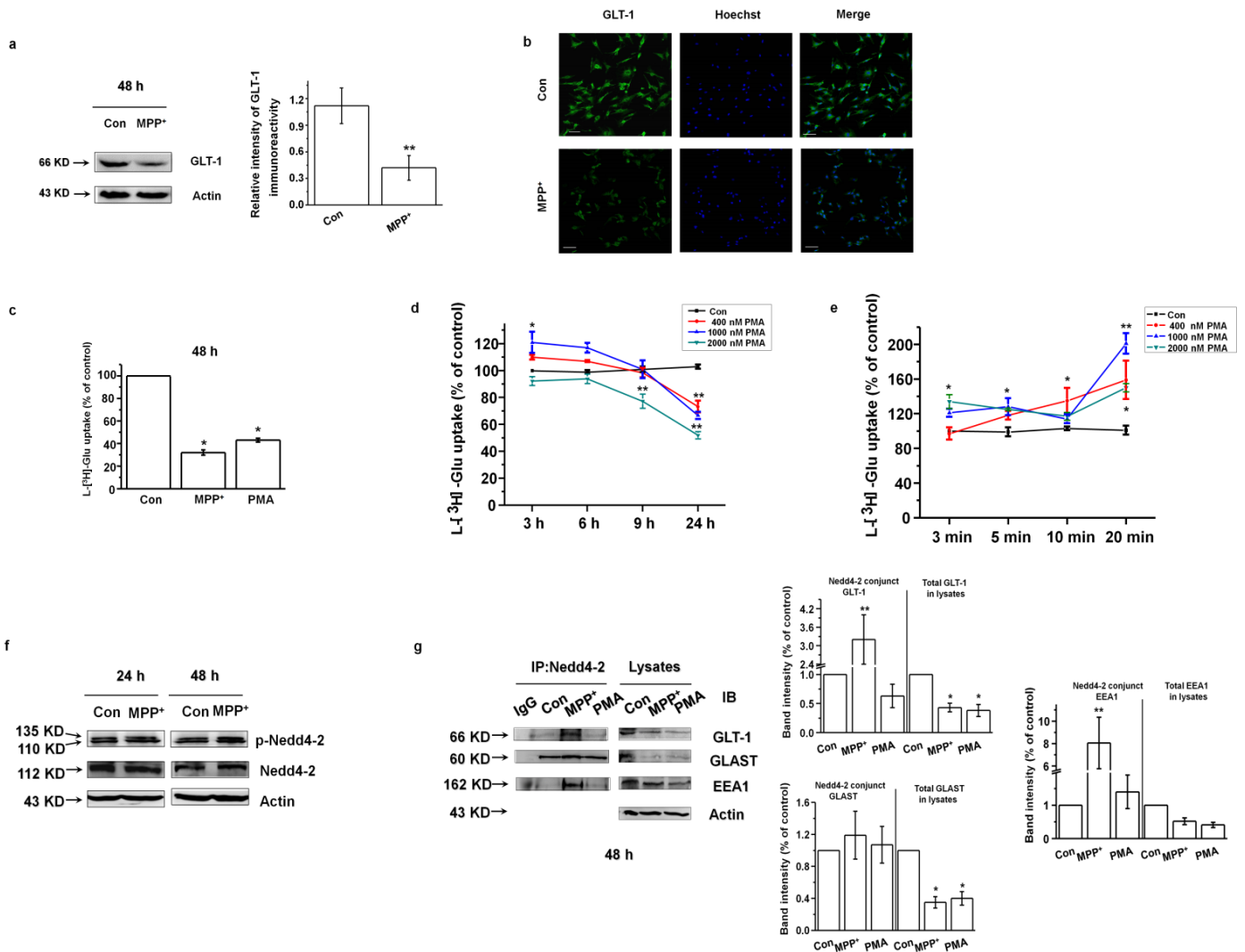
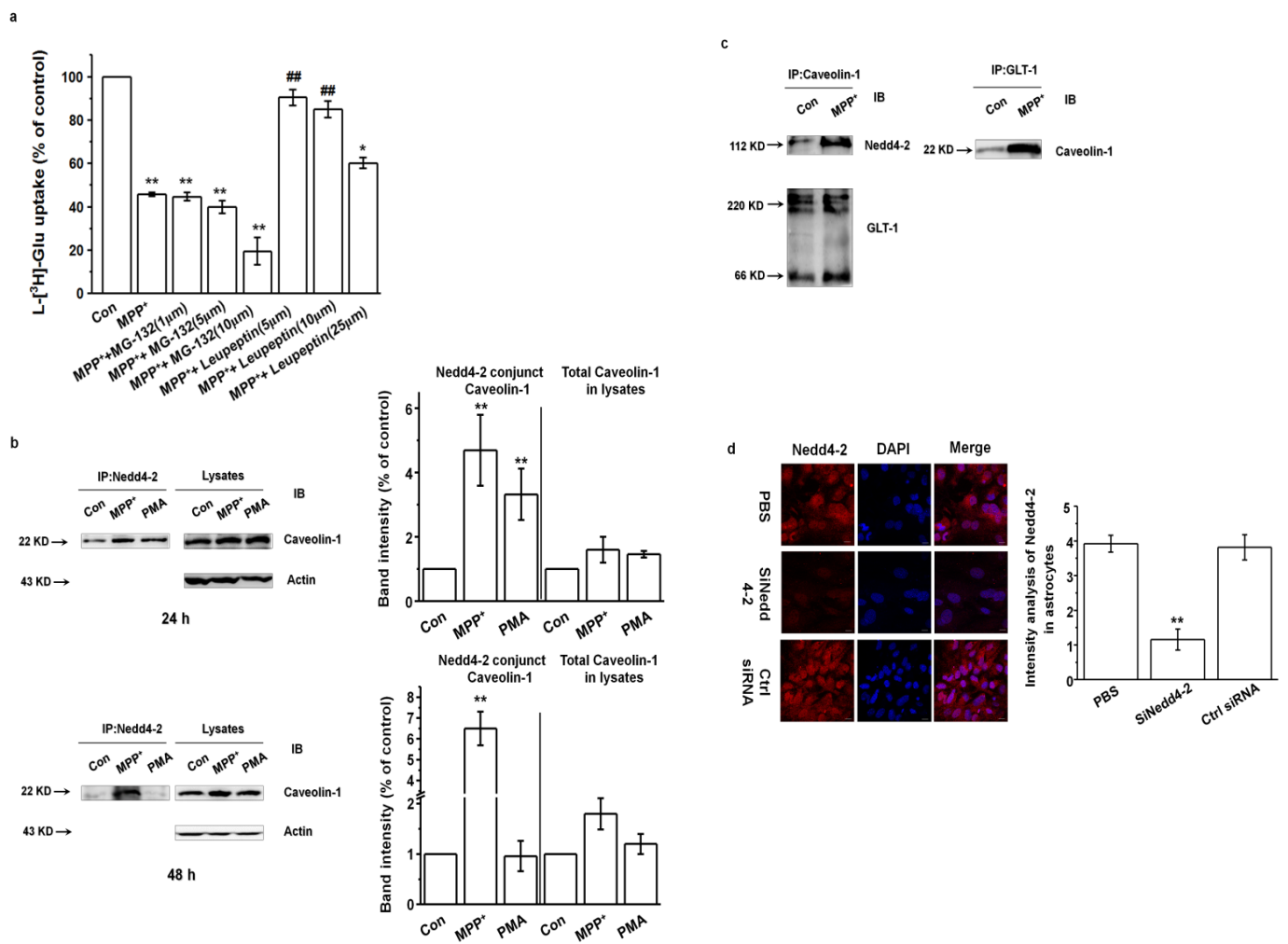


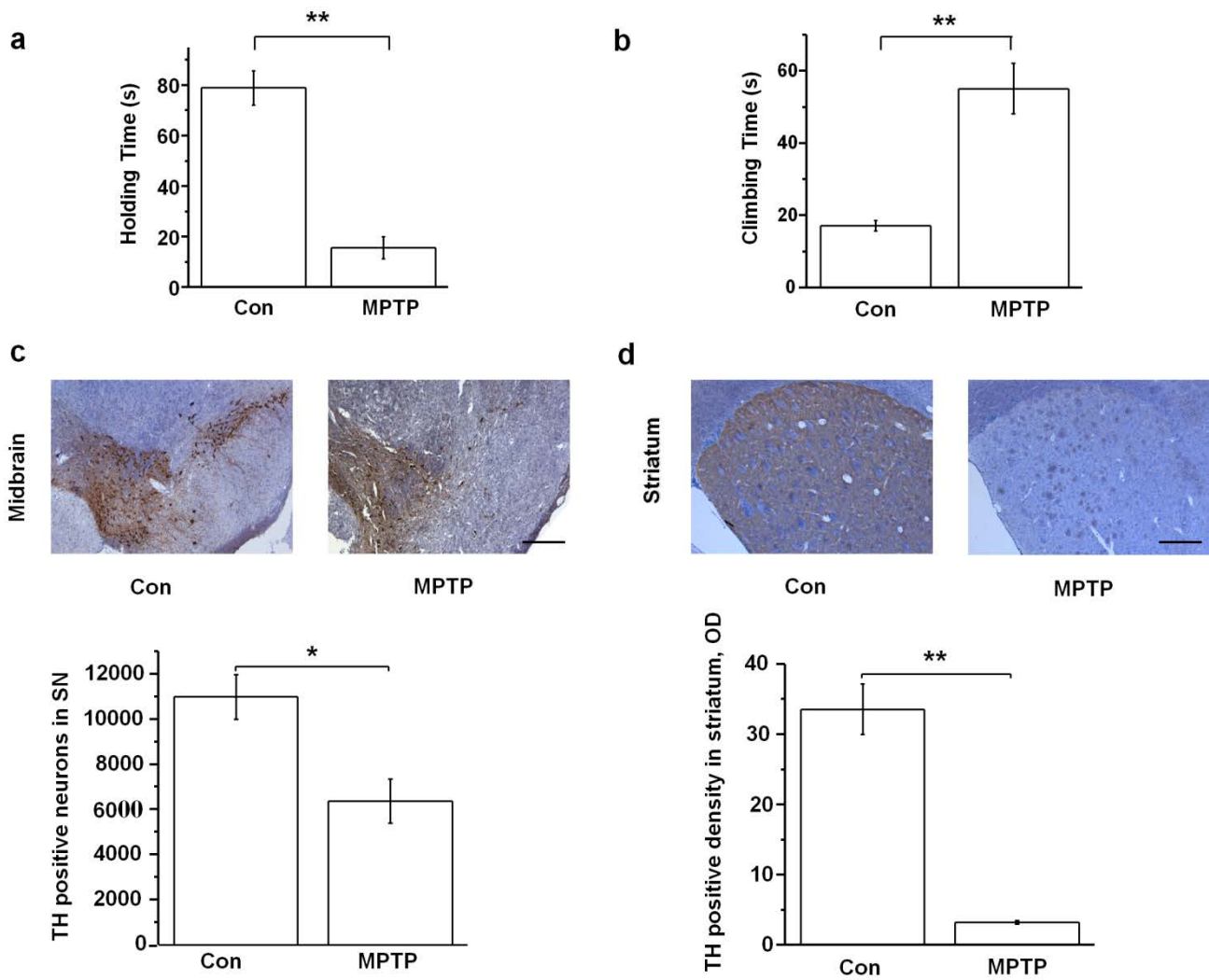
Supplemental Material



Supplementary Figure 1 (a) Western blotting analysis showing that GLT-1 expression is decreased upon MPP⁺ treatment of astrocytes for 48 h. (b) Immunofluorescent staining showing that GLT-1 expression is obviously decreased after 48 h MPP⁺ treatment in astrocytes. Scale bar: 40 μ m. (c) L-[³H]-Glutamic acid uptake assay showing that 1 mM MPP⁺ and 2 μ M PMA treatment for 48 h decreases the glutamate uptake in astrocytes. (d and e) Effects of different concentrations of PMA (500, 1000, 2000 nM) on astrocytes at different time points. Glutamate uptake was decreased after 6 or more hours of PMA treatment (**panel d**), but was increased at shorter time points (3-20 min; **panel e**). (f) Western blotting analysis showing that Nedd4-2 expression and phosphorylation does not change significantly after 48 h MPP⁺ treatment of astrocytes. (g) Co-IP assay showing the interaction between Nedd4-2 and GLT-1 in MPP⁺-treated astrocytes. The capture antibody in **panel g** was anti-Nedd4-2 and MPP⁺ treatment time was 48 h. Results are expressed as the mean \pm SE of at least 3 independent experiments. One-way ANOVA. ** $p < 0.01$, * $p < 0.05$ versus control.

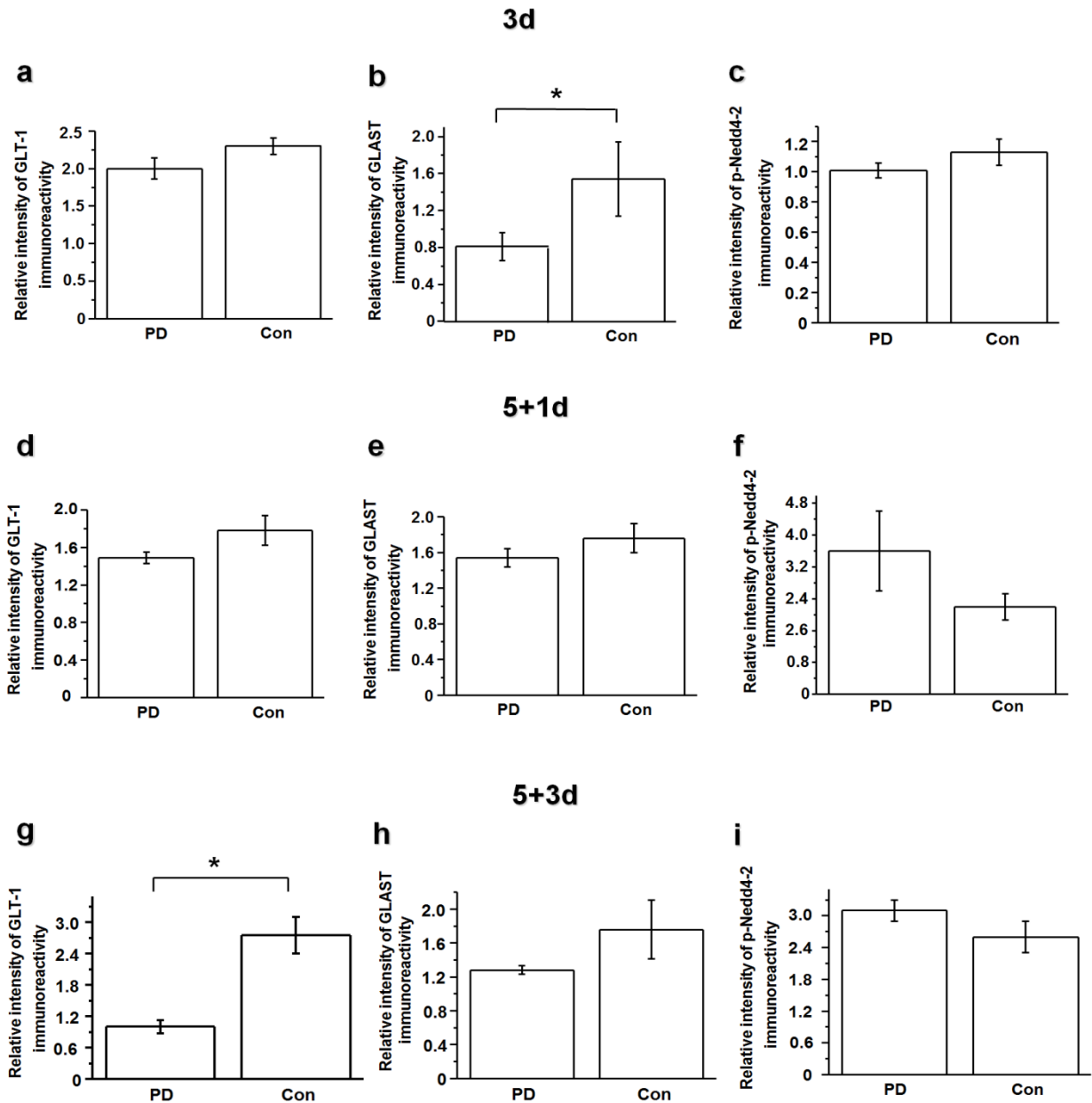


Supplementary Figure 2 (a) Glutamate uptake assay showing that different concentration of Leupeptin increased glutamate uptake in MPP⁺-treated astrocytes, while MG-132 showed no obvious effects. **(b and c)** Co-IP assay showing that Nedd4-2 interacts with caveolin-1 and GLT-1 in MPP⁺-treated astrocytes. In **panel b**, the MPP⁺ treatment was 24 h and 48 h and the capture antibody was Nedd4-2; in **panel c**, the MPP⁺ treatment was 24 h and the capture antibody was caveolin-1 or GLT-1. **(d)** Immunofluorescent staining showing that Nedd4-2 expression was decreased in astrocytes at 72 h after transfection with Nedd4-2 siRNA compared with transfection with PBS or negative control siRNA. Scale bar: 30 μm. Results are expressed as the mean ± SE of at least 3 independent experiments. One-way ANOVA. ** $p < 0.01$, * $p < 0.05$ versus control. ## $p < 0.01$ versus MPP⁺ group.



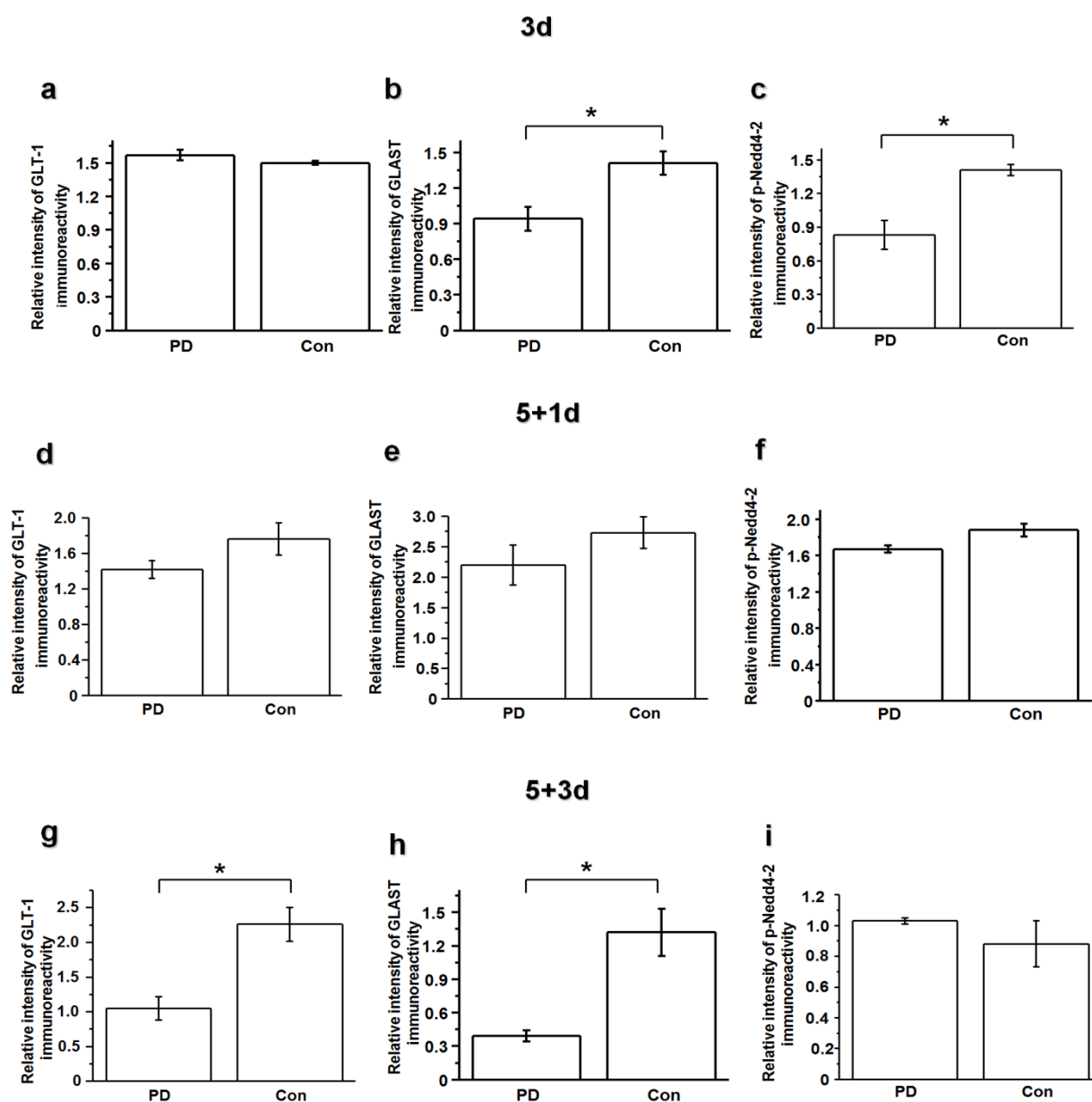
Supplementary Figure 3 Development of a PD mouse model by intraperitoneal injection of MPTP. **(a and b)** Behavior tests. The holding time was shortened (**panel a**) and the climbing time was prolonged (**panel b**) in MPTP-treated mice. The results are expressed as the mean \pm SE. Student's *t*-test. $n = 12$ in each group. $**p < 0.01$. **(c and d)** IHC staining showed that TH expression is decreased in the SN (**panel c**) and striatum (**panel d**) of MPTP-treated mice. Results are expressed as the mean \pm SE. Student's *t*-test. $n = 6$ per group. $*p < 0.05$, $**p < 0.01$ versus control. Scale bar: 100 μ m.

Intensity quantification of protein in Midbrain

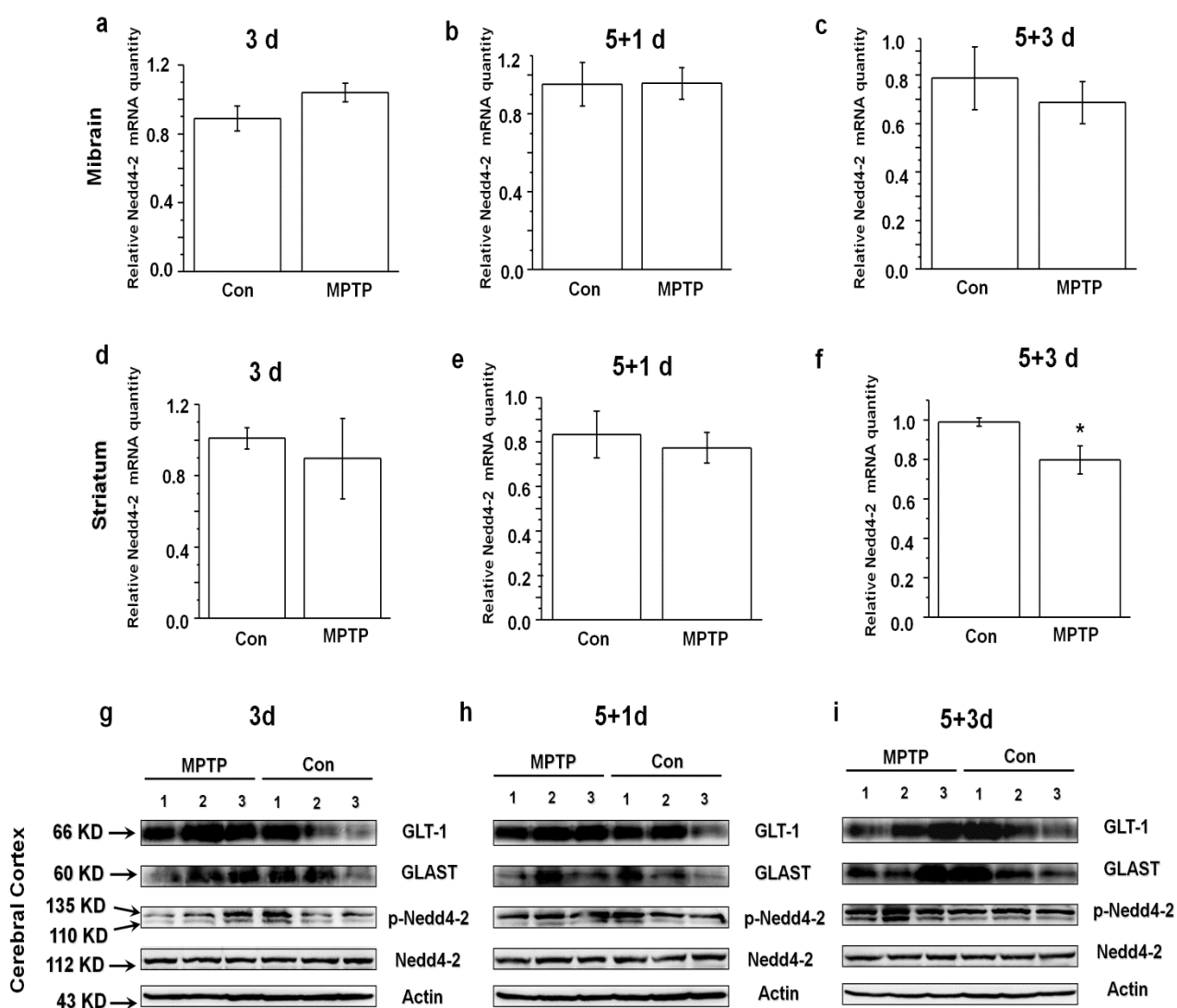


Supplementary Figure 4 The protein expression of glutamate transporters is decreased in the midbrain in MPTP-treated mice. Protein levels in midbrain were quantified using Image J software and actin immunoreactivity was set as the loading control. GLAST expression was decreased in the 3d group (**panel b**), and GLT-1 expression was decreased in the 5+3d group (**panel g**) in the midbrain of MPTP-treated mice. Results are expressed as the mean \pm SE. Student's *t*-test. $n = 3$ per group. * $p < 0.05$ versus control.

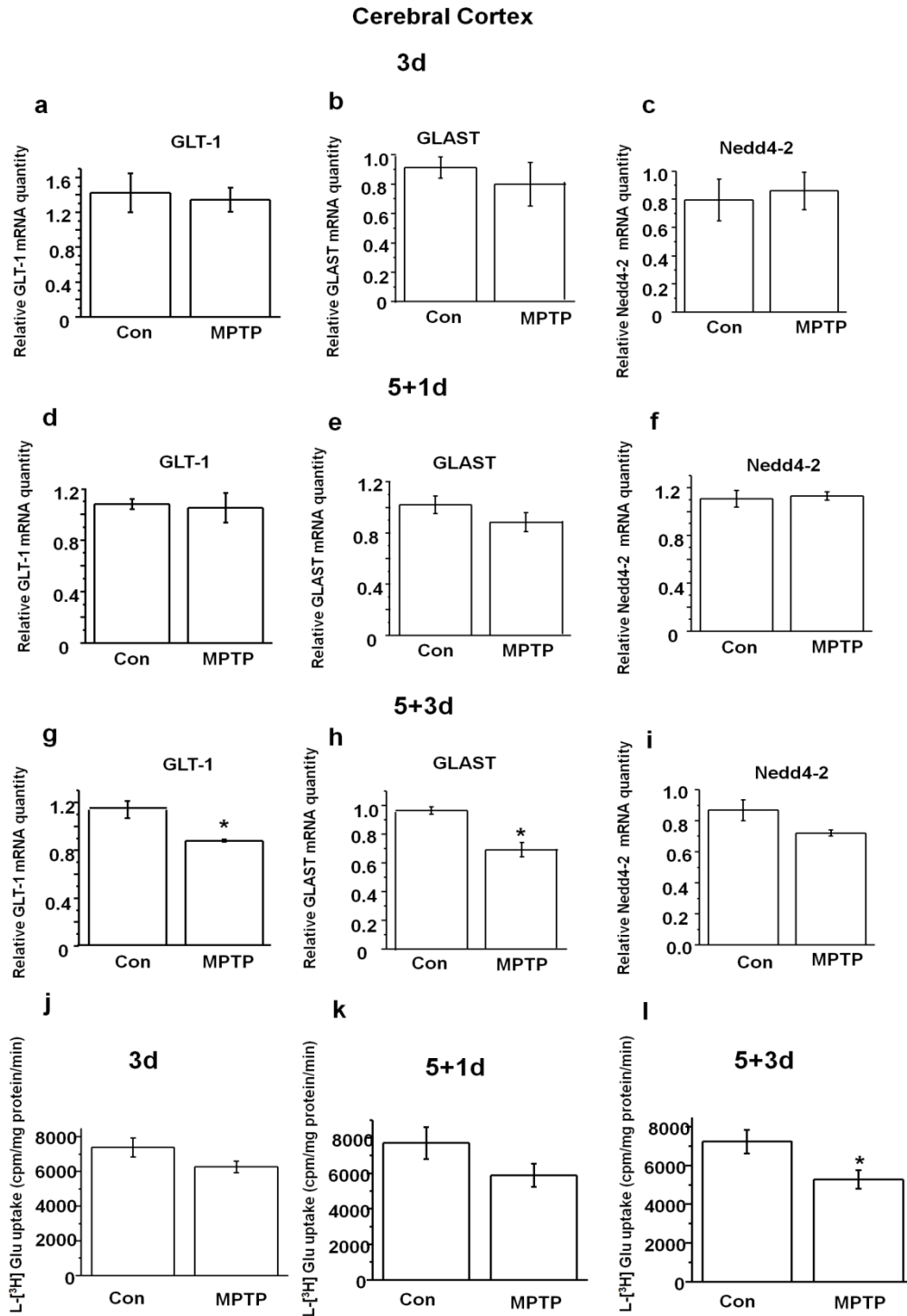
Intensity quantification of protein in Striatum



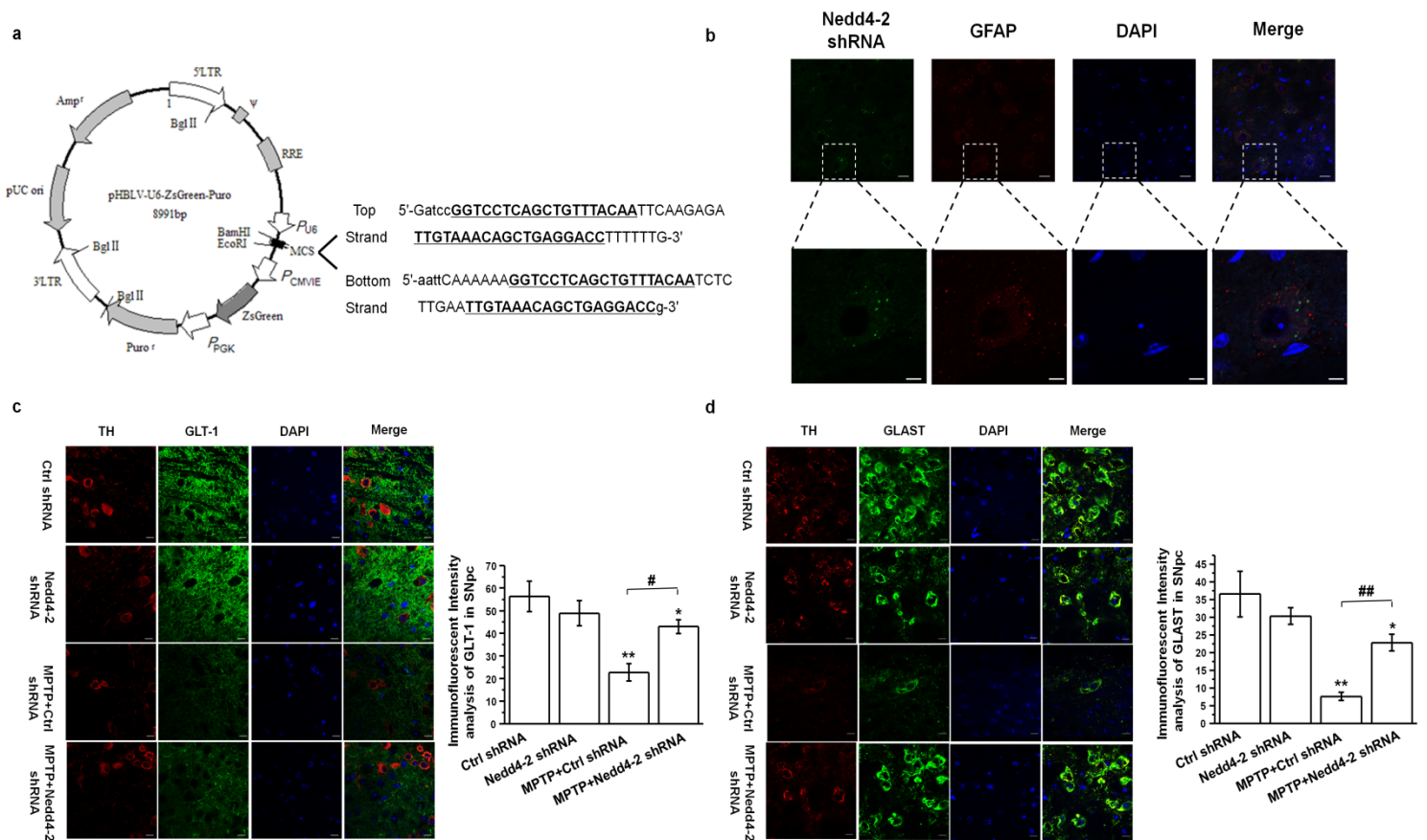
Supplementary Figure 5 The protein expression of glutamate transporters is decreased in the striatum in MPTP-treated mice. Protein levels in striatum were quantified using Image J software and actin immunoreactivity was set as the loading control. GLT-1 expression was decreased in the 5+3d group (**panel g**), and GLAST expression was decreased in the 3d and 5+3d groups (**panel b** and **h**) in the striatum of MPTP-treated mice. p-Nedd4-2/Nedd4-2 expression was decreased in the 3d group (**panel c**) in the striatum of MPTP-treated mice. Results are expressed as the mean \pm SE. Student's *t*-test. $n = 3$ per group. * $p < 0.05$ versus control.



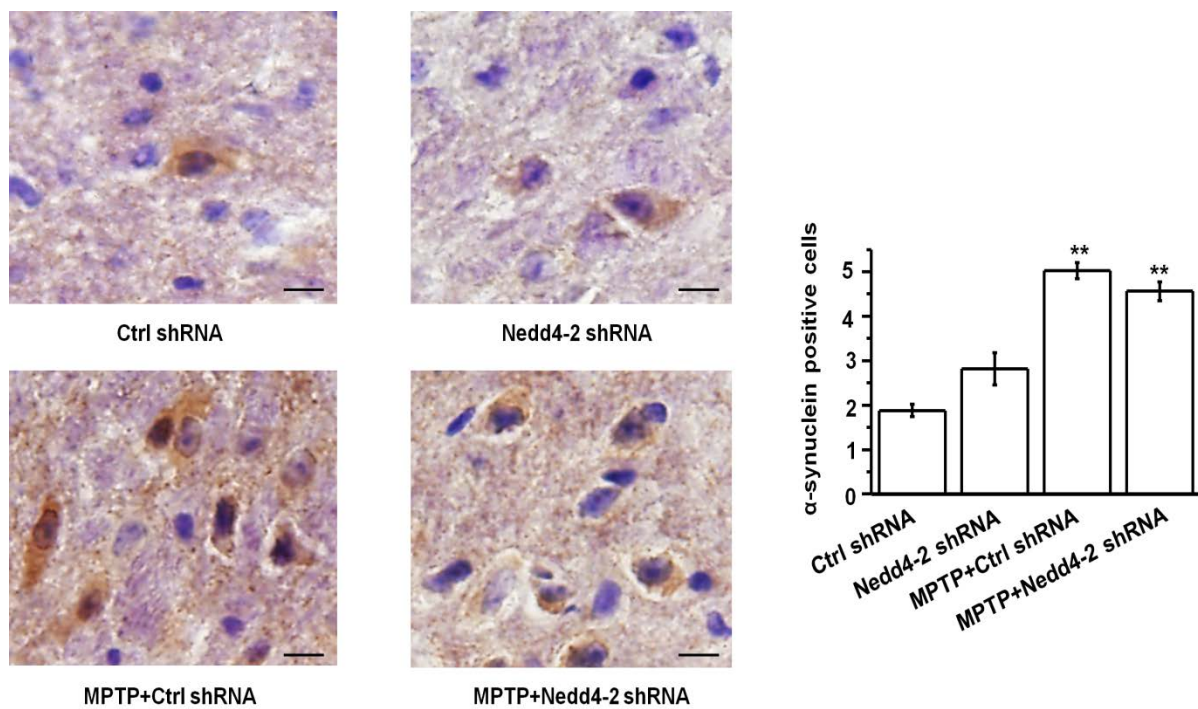
Supplementary Figure 6 Nedd4-2 mRNA expression in the midbrain and striatum of MPTP-treated mice. (a–f) qPCR assay showing Nedd4-2 mRNA expression in the midbrain and striatum. Nedd4-2 mRNA expression was decreased in the 5+3d group in the striatum (**panel f**). (g–i) The protein expressions of glutamate transporters, p-Nedd4-2 and Nedd4-2 in the cerebral cortex were unchanged in MPTP-treated mice. Results are expressed as the mean \pm SE. Student's *t*-test. *n* = 3 per group. * *p* < 0.05 versus control.



Supplementary Figure 7 Glutamate transporters and Nedd4-2 mRNA expression in cerebral cortex of MPTP-treated mice. (a–i) qPCR analysis showing glutamate transporters and Nedd4-2 mRNA expression in cerebral cortex from MPTP-treated mice at different time points. GLT-1 and GLAST mRNA expression was decreased in the 5+3d group in the cerebral cortex (panel g and h). (j–l) The glutamate uptake in the synaptosomes was decreased in 5+3d group in the cerebral cortex in MPTP-treated mice. Student's *t*-test. *n* = 6 per group. * *p* < 0.05 versus control.



Supplementary Figure 8 (a) Design of an Nedd4-2 lentivirus vector (LV) (LV-sh[NEDD4-2]) with PGK-driven puromycin resistance and CMV IE-driven ZsGreen expression. LV-sh[NEDD4-2] was constructed to express shRNA targeting *Nedd4-2* from the U6 (RNA polymerase III) promoter and were generated by ligating annealed oligonucleotides encoding shNedd4-2 (in the figure) or a control sequence into the *Bam*H I/*Eco*R I sites. (b) Images of Nedd4-2 shRNA (GFP) co-labeling with GFAP confirms that the lentivirus vector targets astrocytes in the SN region. Scale bar: 40 μ m at the top and 20 μ m at the bottom. (c and d) Immunofluorescent images of TH co-labeling with GLT-1 or GLAST. Scale bar: 30 μ m.



Supplementary Figure 9 Effect of Nedd4-2 knockdown on the α -synuclein expression in the SN. IHC staining showing that Nedd4-2 knockdown has no obvious effects on α -synuclein expression in the SN of MPTP-treated mice. Results are expressed as the mean \pm SE. One-way ANOVA. $n = 6$ per group. ** $p < 0.01$. Scale bar: 40 μ m.