

## **Additional Data**

### **A selective inhibitor of the NLRP3 inflammasome as a potential therapeutic approach for neuroprotection in a transgenic mouse model of Huntington's disease**

Kai-Po Chen<sup>1#</sup>, Kuo-Feng Hua<sup>2,3#</sup>, Fu-Ting Tsai<sup>1</sup>, Ting-Yu Lin<sup>1</sup>, Chih-Yuan Cheng<sup>1</sup>, Ding-I Yang<sup>4</sup>, Hsien-Ta Hsu<sup>5,6</sup>, and Tz-Chuen Ju<sup>1,7\*</sup>

#These authors contributed equally.

\* **Corresponding author: Tz-Chuen Ju**, E-mail: [tzchuen@thu.edu.tw](mailto:tzchuen@thu.edu.tw).

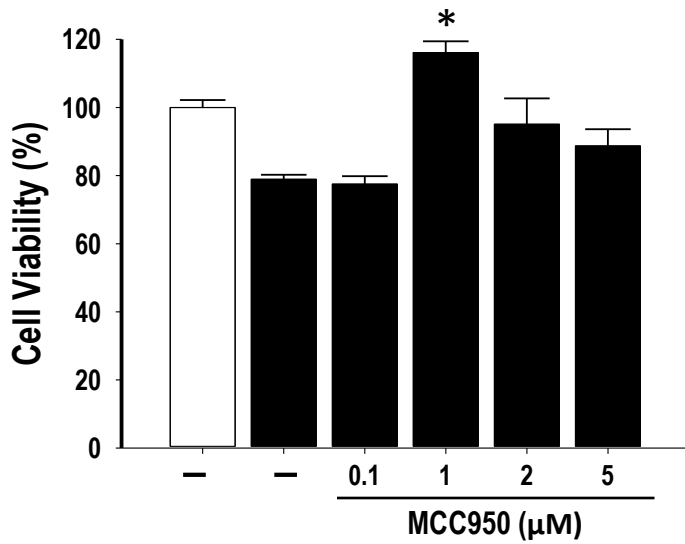
#### **Inventory of Additional Data**

##### **Additional Figures and Legends**

- Additional Figure 1. MCC950 markedly reduces cytotoxicity in striatal progenitor cells.
- Additional Figure 2. MCC950 markedly reduced IL-18 secretion in BV2 microglial cells and in a transgenic mouse model (R6/2) of HD.
- Additional Figure 3. MCC950 downregulated the phosphorylation level of I $\kappa$ -B and P65.

# Additional Figures and Legends

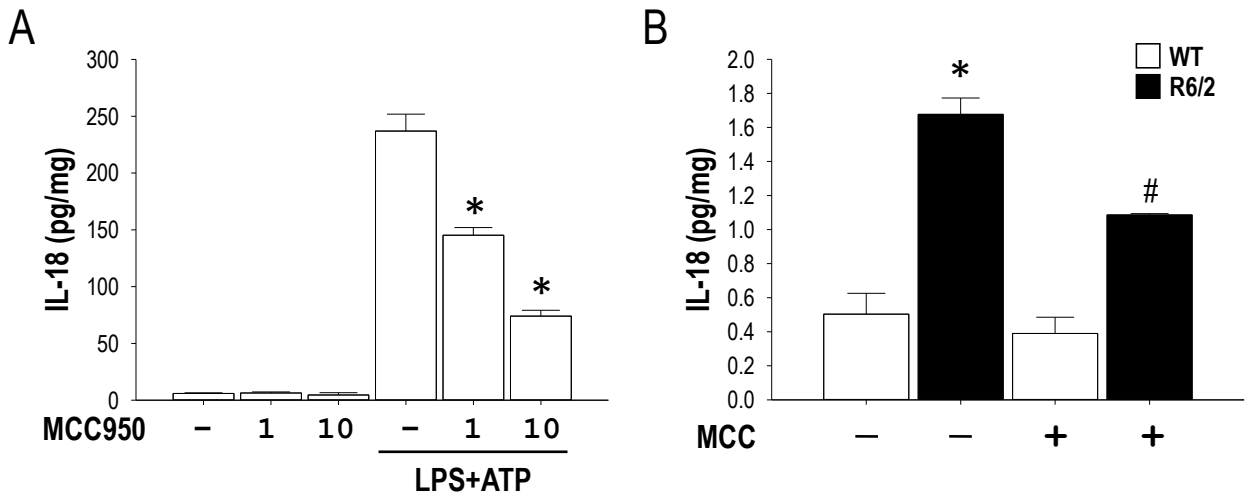
## Figure 1



**Fig. 1. MCC950 markedly reduces cytotoxicity in striatal progenitor cells.** *STHdh*<sup>Q109</sup> cells were incubated for 24 h with MCC950, cell death was quantified using the CCK-8 assay. The data are presented as the mean  $\pm$  SEM from three independent experiments. \* $P < 0.05$  vs. untreated *STHdh*<sup>Q109</sup> cells.

# Additional Figures and Legends

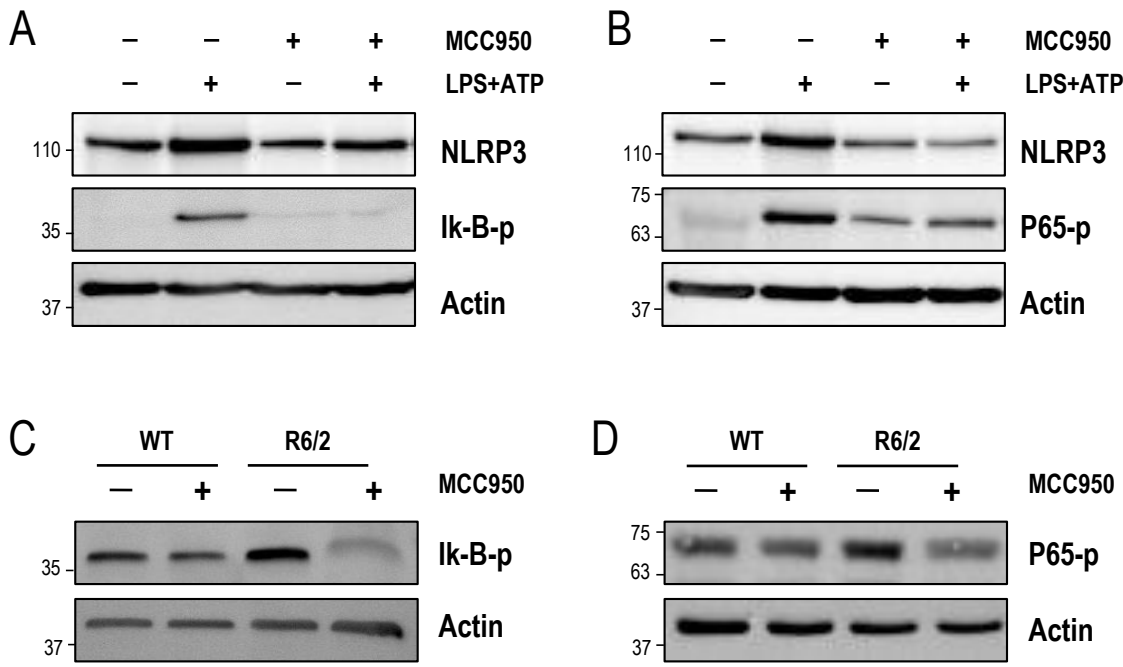
## Figure 2



**Fig 2. MCC950 markedly reduced IL-18 secretion in BV2 microglial cells and in a transgenic mouse model (R6/2) of HD.** (A) BV2 microglia were incubated for 2 h with LPS (1  $\mu\text{g}/\text{mL}$ ) followed by incubation with MCC950 (1 and 10  $\mu\text{M}$ ) for 2 h. The cells were then incubated with ATP (1 mM for 6 h). IL-18 expression levels were measured using the ELISA. \* $P < 0.05$  compared to LPS and ATP treated cells ( $n = 3$  for each condition). (B) Mice were treated daily with MCC950 (10 mg/kg of body weight; oral administration) or water for 5 weeks from the age of 7 weeks. The levels of IL-18 in the striatum were measured by ELISA ( $n = 3$  for each condition). The data are presented as the mean  $\pm$  SEM. \* $P < 0.05$ , between WT and R6/2 mice; # $P < 0.05$  vs. water-treated R6/2 mice.

# Additional Figures and Legends

## Figure 3



**Fig 3. MCC950 downregulated the phosphorylation level of Iκ-B and P65.** (A, B) BV2 microglia were incubated for 4 h with LPS (1 μg/mL) followed by incubation with MCC950 (1 μM) for 2 h. The cells were then incubated with ATP (1 mM for 24 h). Total lysates of BV2 cells were assessed by western blot analysis to determine the phosphorylation levels of Iκ-B and P65. The molecular mass is indicated in kilodaltons. (C, D) Mice were treated daily with MCC950 (10 mg/kg of body weight; oral administration) or water for 5 weeks from the age of 7 weeks. Striatal lysates were analyzed using western blot analysis. The molecular mass is indicated in kilodaltons.