## **OPEN PEER REVIEW REPORT 2**

Name of journal: Neural Regeneration Research

Manuscript NO: NRR-D-20-00763

Title: Low-dose lipopolysaccharide as an immune regulator for homeostasis maintenance in the

central nervous system through transformation to neuroprotective microglia

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### **COMMENTS TO AUTHORS**

The manuscript examined relationship between spatin and CRMP3 to determine neural regeneration after spinal cord injury. The authors examined this using several techniques including immunohistochemistry, LC-MS analysis, immunoblotting and neuronal culture. Apparently, the study seems to be well documented, but the manuscript and explanation are actually very poor.

### Major comments

#### Methods:

1. Immunohistochemical staining: How many days after injury were animals terminated for histological samples? The authors described this in the results. It should describe in here.

Moreover, which region of spinal cord did you sectioned for the observation? How many animals did you evaluate this? Show antibodies information in detail. Does the CRMP antibody react with all subtypes?

- 2. Glutathione S-transferase (GST) Pull-down Assay: Which levels of spinal cord did you used for samples? How many sections how much interval sections did you prepare for the assay?
- 3. Plasmids and Constructs: Show information of CRMP3 in detail such as assembly number.
- 4. Western Blotting and Co-Immunoprecipitation (co-IP): Describe the homogenate condition in detail. Show information antibodies in detail.
- 5. Cell Culture and Transfection: Show the neuronal cell preparation in detail.
- 6. Show sample number in the each experiment.

# Results

Descriptions of all results were not enough and became detached from the Figures. Moreover, the authors often did not explain all figures in the text. I commented here only "Rat SCI model establishment" and Figure 1.

The authors described "sham rats appeared normal, whereas sections from SCI model rats exhibited evidence of edema, inflammatory cell infiltration, and consolidation of the spinal cord nucleus (Figure 1A)". However, from figure, they could not recognize because of low magnification images only. The description "sham rats appeared normal" is also quite unclear. What did you mean normal? Moreover, Figure 1 has four figures (A to D). However, the descriptions were only Figure A to C, did not included D.

In the figure 1, they were implausible. The mice were subjected to around Th10 level spinal cord injury in present study. The level of spinal cord is relatively round shape in the coronal plane like sham images of Fig1A. However, the sham images in C and D were different, and evidently a lower thoracic level (Th11-12) or upper lumbar (L1) level.