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Supplementary Materials

GM1 Oligosaccharide Efficacy in Parkinson's Disease: Protection against MPTP

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S1. Methods

S1.1. MPP⁺ Dose Effect on CGN

S1.1.1. MTT Assay

Viability of CGN after MPP⁺ treatments (25, 50, and 100 μM) was determined by MTT assay, as previously reported [38]. Briefly, after 1 h from GM1-OS incubation (100 μM), cells plated in a 96-well were washed and incubated with 100 μL of 2.4 mM MTT (4 mg/mL in RH) for 1 h at 37 °C in a humidified atmosphere of 95 % air/5 % CO_2. Subsequently MTT was carefully removed and replaced with 2-propanol/formic acid, 95/5 (v/v). Plates were gently shacked prior to read the absorbance at 570 nm with a microplate spectrophotometer (Wallac 1420 VICTOR2TM, Perkin Elmer).

S1.1.2 Morphological Analysis for Neurite Outgrowth Evaluation

After MPP⁺ challenge in the presence or absence of GM1-OS, CGN, were observed by phase contrast microscopy (20X objective, Olympus BX50 microscope; Olympus, Tokyo, Japan). At least 10 fields from each well were photographed for each experiment.

S2. Supplementary Figures

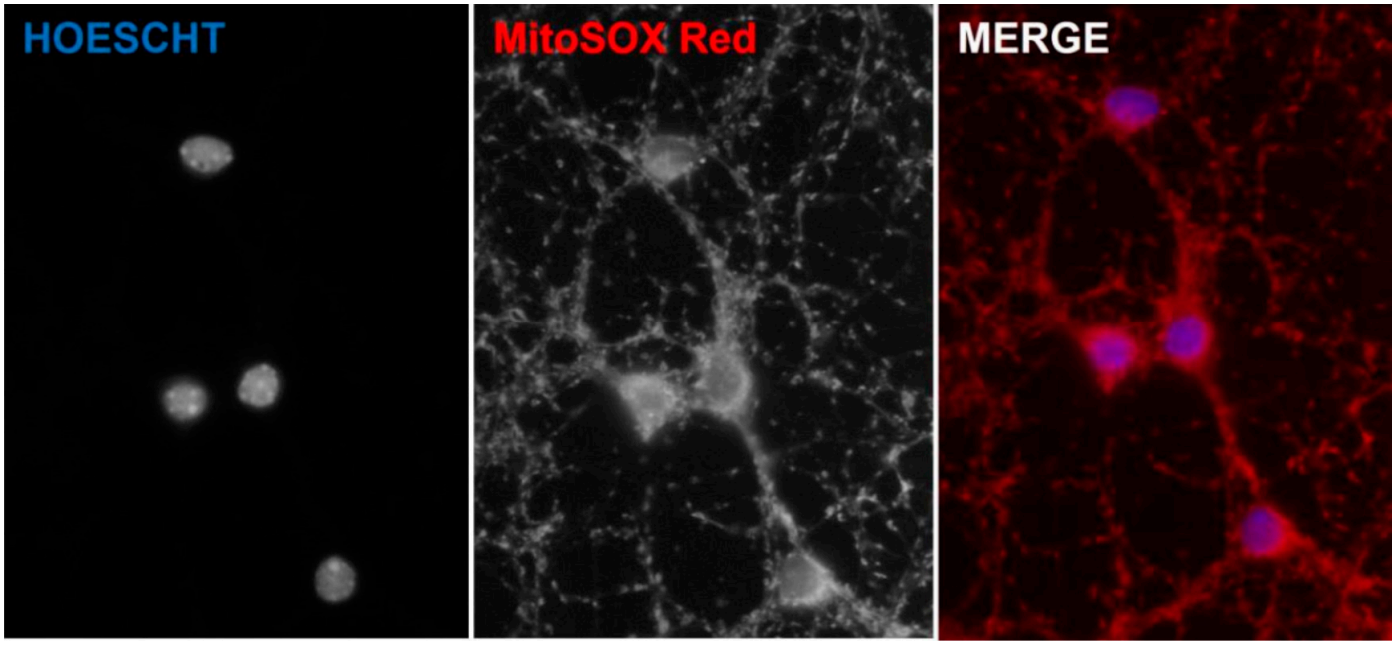

Figure S1. Identification of $O_2^{\bullet-}$ content in mitochondria by MitoSOX™ red dye. Representative image of day 14 primary CGNs 47 stained with 1 μM MitoSOX red dye in HBSS with Ca^{2+} and Mg^{2+} for 10 min at 37 °C. After MitoSOX incubation, PBS-washed CGNs were fixed and nuclei were stained by Hoechst dye. Images were acquired using a NikonEclipse Ni upright microscope with 100X objective (Red: Mitosox; Blue: Nuclei).

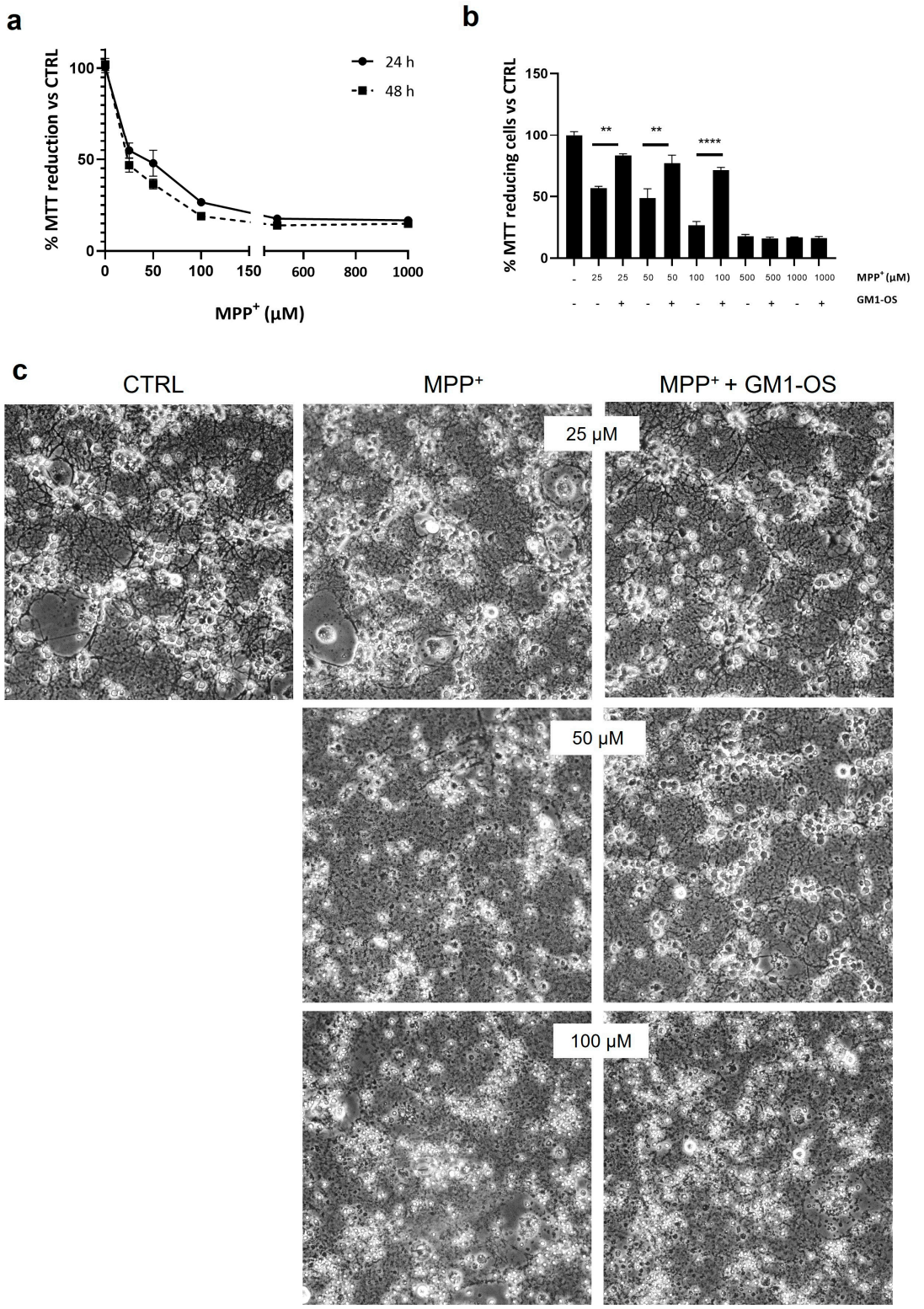

Figure S2. GM1-OS protected CGN injured with MPP+. (**a**) Dose-response effect of MPP+ exposure on CGN after 24 h (straight line) or 48 h (dashed line). MTT assay was performed to measure cell viability; (**b**) MTT assay of MPP+ exposed CGN at the indicated concentrations in the presence or absence of 100 µM GM1-OS administered 1 h prior to MPP+; (**c**) Representative phase contrast images of CGN exposed to MPP+ at the indicated concentrations in the presence or absence of 100 µM GM1-OS (administered 1 h prior to MPP+). Magnification 20X. All values are expressed as mean ± SEM (n = 4. ** $p < 0.01$, **** $p < 0.0001$ One-way ANOVA followed by Tukey's multiple comparisons test).