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Supplemental Information

Non-toxic HSC Transplantation-Based Macrophage/Microglia-Mediated GDNF Delivery for Parkinson's Disease

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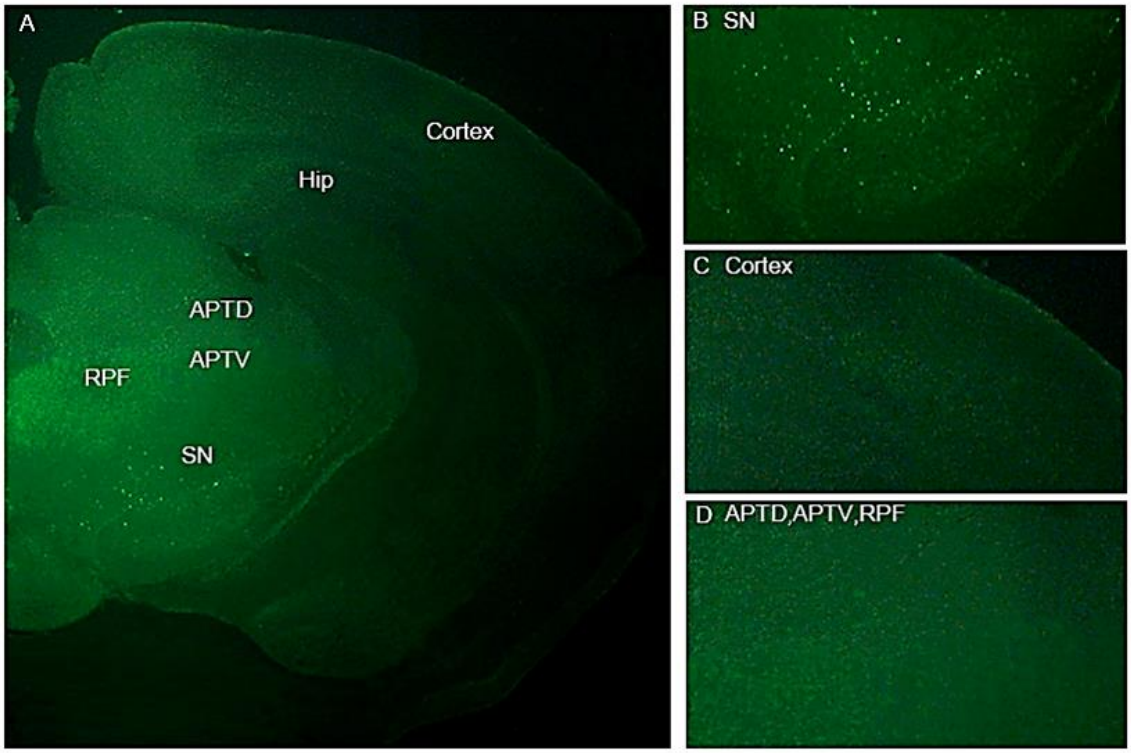

Figure S1. Macrophage infiltration into the midbrain of MitoPark mice. Midbrain sections were from LT-MSP-hGDNF-2A-GFP- and LT-MSP-GFP-transplanted MitoPark mice after 7 cycles of non-toxic HSCT. (A) Representative half brain image synthesized from a midbrain section shows infiltrated gene-modified GFP-expressing macrophages/microglia (in green) in substantia nigra, as well as VTA (original magnification, 40x). (B-D) Enlarged images to focus on substantia nigra (B), Cortex (C), APTD, APTV, RPF (D). Hip: Hippocampus; SN: Substantia Nigra; APTD: anterior pretectal nu, dorsal; APTV: anterior pretectal nu, ventral; RPF: retroparafascicular nu.

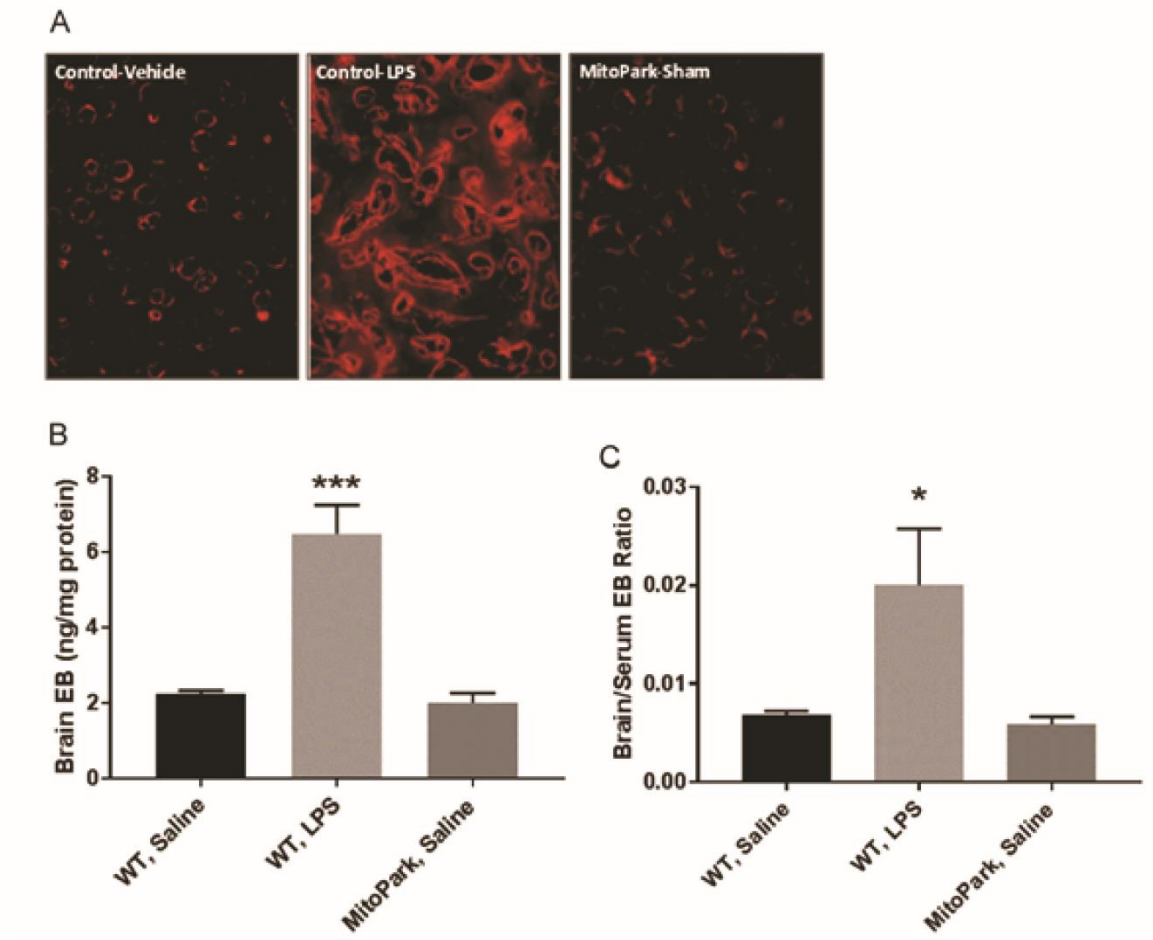

Figure S2. Blood-brain barrier (BBB) permeability to Evan's Blue dye was not increased in MitoPark mice. Approximately 20 week-old MitoPark or WT littermate were mice injected i.p. with LPS (5mg/ml) or saline. After 6h, mice received an i.p. injection of 2% Evan's blue (EB) dye. Two hours later, blood was collected, mice were perfused and brains were harvested for histologic and spectrophotometric analysis of EB dye content. Results are shown as (A) Representative 20X cortical images illustrating diffusion of EB dye signal (600nm) in brain parenchyma only in LPS challenged mice, (B) total EB concentration in cerebral tissue lysates, and (C) the ratio of brain/serum EB concentration. Data represent group means ± SEM, n=3-4 mice per group with both sexes represented in each group. ***$P < 0.001$ vs. all other groups; *$P < 0.05$ vs. all other groups.

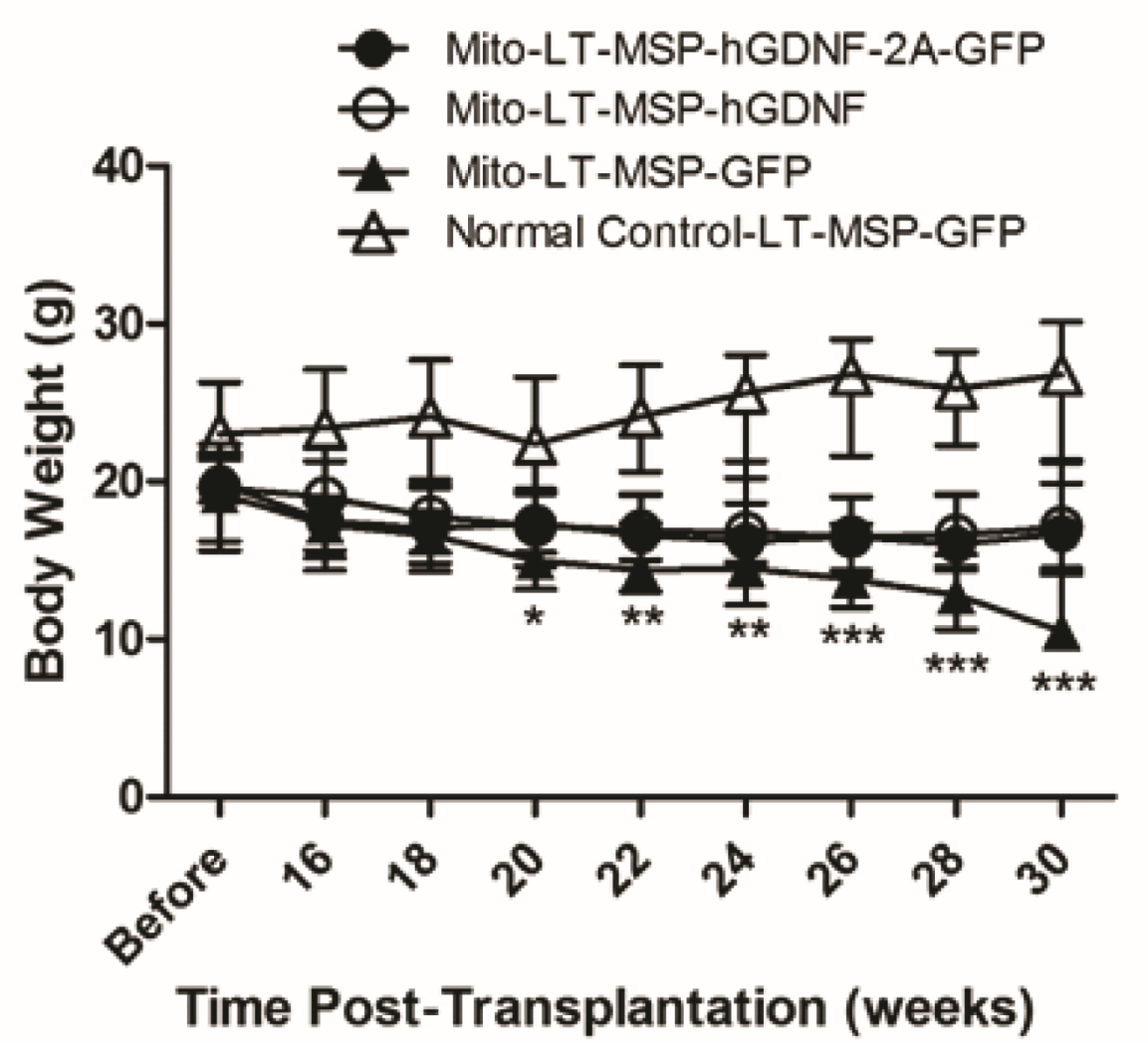

Figure S3. GDNF delivery prevented loss of body weight in MitoPark mice. Body weight was measured every two weeks before and after hGDNF-2A-GFP-, hGDNF- or GFP-expressing HSC transplantation. Each point represents mean ± SEM from 15 animals per treatment group (n=15). Two-way ANOVA was performed, followed by Bonferroni post-test. *P < 0.05, **P < 0.01, ***P < 0.001, Mito-LT-MSP-hGDNF group versus Mito-LT-MSP-hGDNF-2A-GFP and LT-MSP-GFP transplanted MitoPark mice.

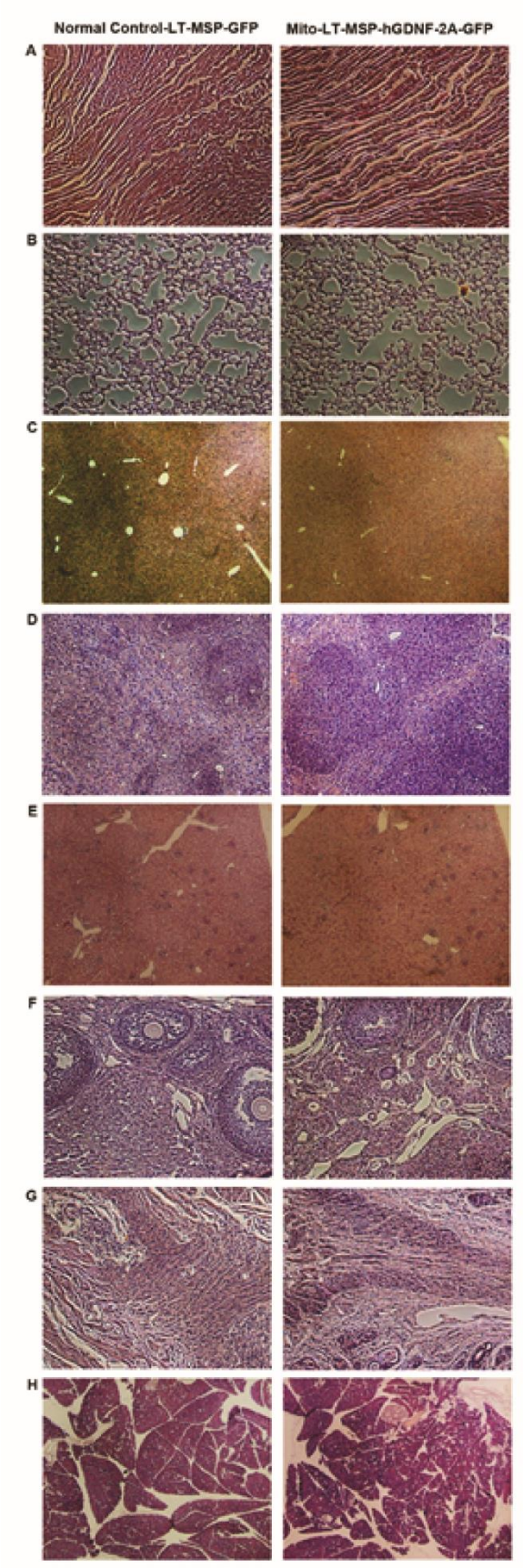

Figure S4. Pathological examination of LT-MSP-GFP-treated normal control mice and LT-MSP-hGDNF-2A-GFP-treated MitoPark mice. Tissues were collected at 4 weeks after the last HSCT, embedded in paraffin, cut to 8 µm thick sections and stained with hematoxylin and eosin (H&E). The histology of the tissues was reviewed by a board-certified veterinary pathologist. Microphotographs are: (A) heart, (B) lung, (C) liver, (D) spleen, (E) kidney, (F) ovary, (G) uterus, and (H) pancreas. There were no significant abnormal findings in the LT-MSP-GFP-treated normal control mice and LT-MSP-hGDNF-2A-GFP-treated MitoPark mice, except insignificant findings including various stages of physiologic estrous, extramedullary hematopoiesis in the liver and spleen, increased binucleate hepatocyte numbers and one case of uterine amyloidosis.

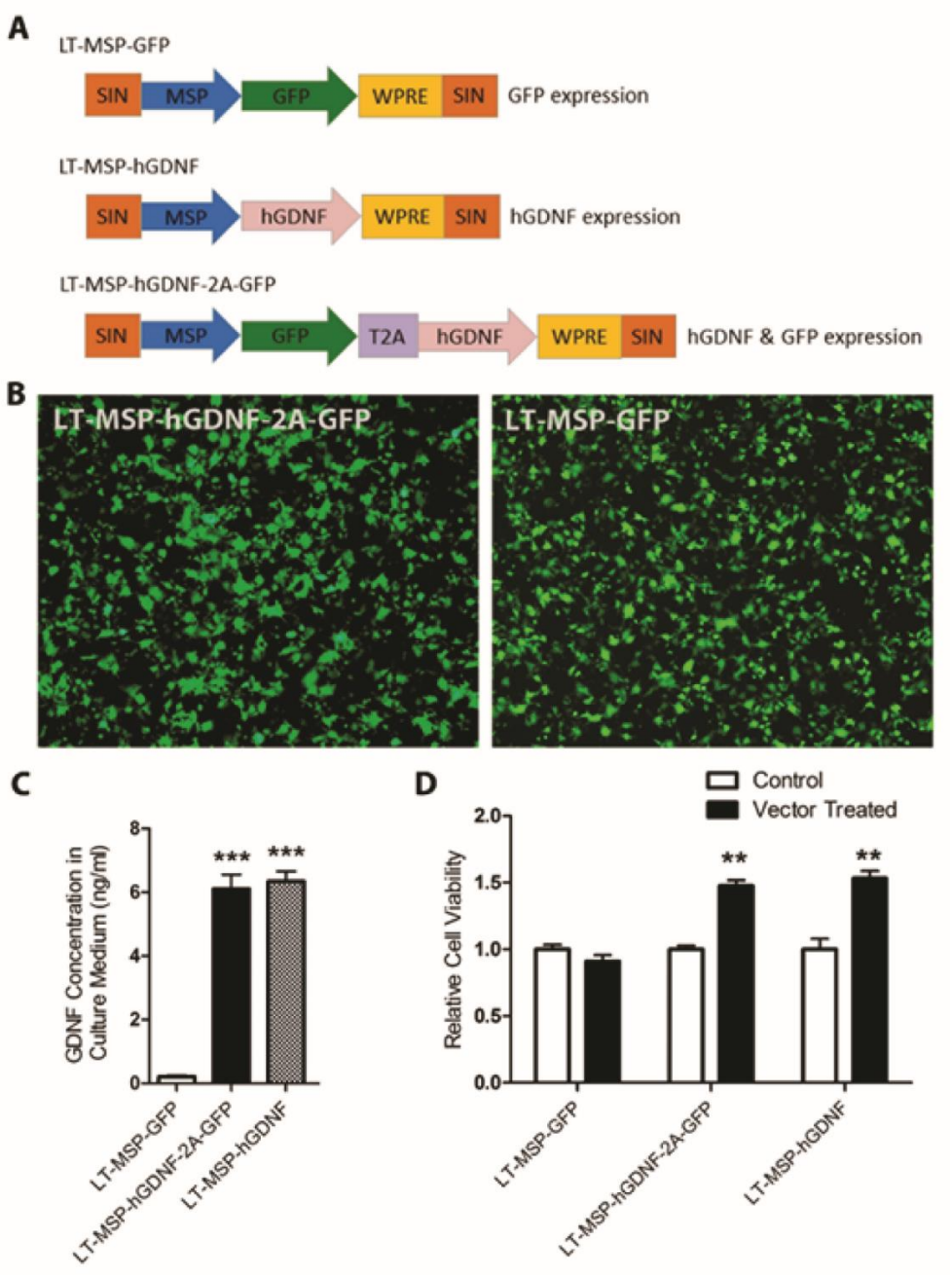

Figure S5. Design and validation of lentiviral vectors. (A) Schematic diagram of LT-MSP-GFP, LT-MSP-hGDNF and LT-MSP-hGDNF-2A-GFP vectors. (B) GFP expression in LT-hGDNF-2A-GFP- and LT-MSP-GFP-transfected 293T cells 24 hours post-transfection. (C) The GDNF production in LT-MSP-GFP-, LT-GDNF-2A-GFP- and LT-MSP-GDNF transfected RAW 264.7 cell cultures. Each bar represents mean ± SEM from six samples per treatment group (n = 6). One-way ANOVA was performed, followed by Tukey's post-test. ***, $P < 0.01$ versus LT-MSP-GFP. (D) The GDNF transfection-induced GDNF secretion protected the viability of SH-SY5Y cells exposed to MPP+. The SH-SY5Y cells were pre-incubated with the medium collected from LT-GDNF-2A-GFP, LT-MSP-GDNF and LT-MSP-GFP transfected RAW 264.7 cell culture separately for 24 h, their corresponding controls were incubated with non-vector treated culture medium, followed by the addition of 300 µM MPP+ for 24 h. The cell viability was determined by MTT assay. Each bar represents mean ± SEM from six wells per treatment group (n = 6). Two-way ANOVA was performed, followed by Bonferroni post-test. **, $P < 0.01$ versus corresponding non-vector treated control.