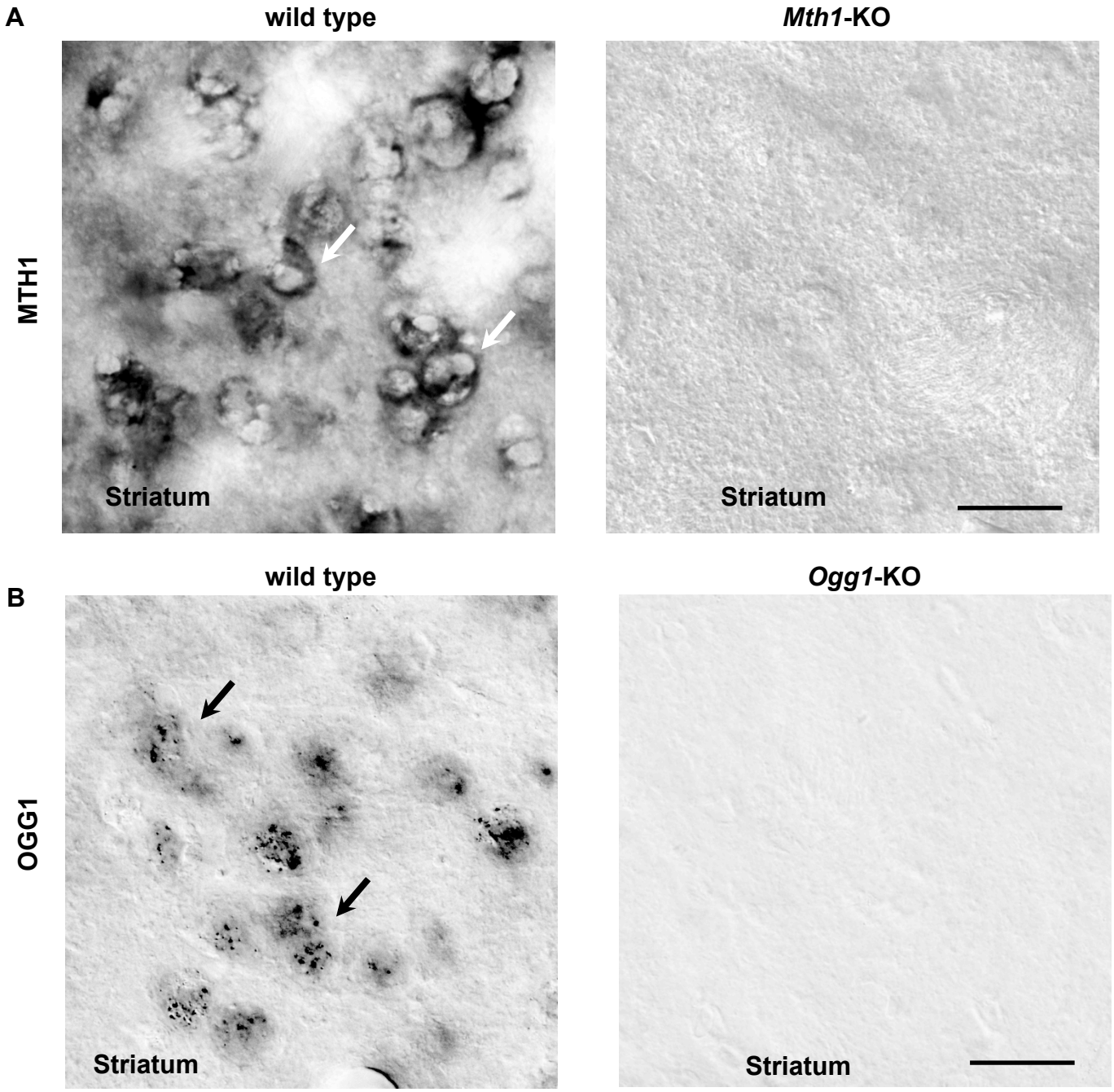
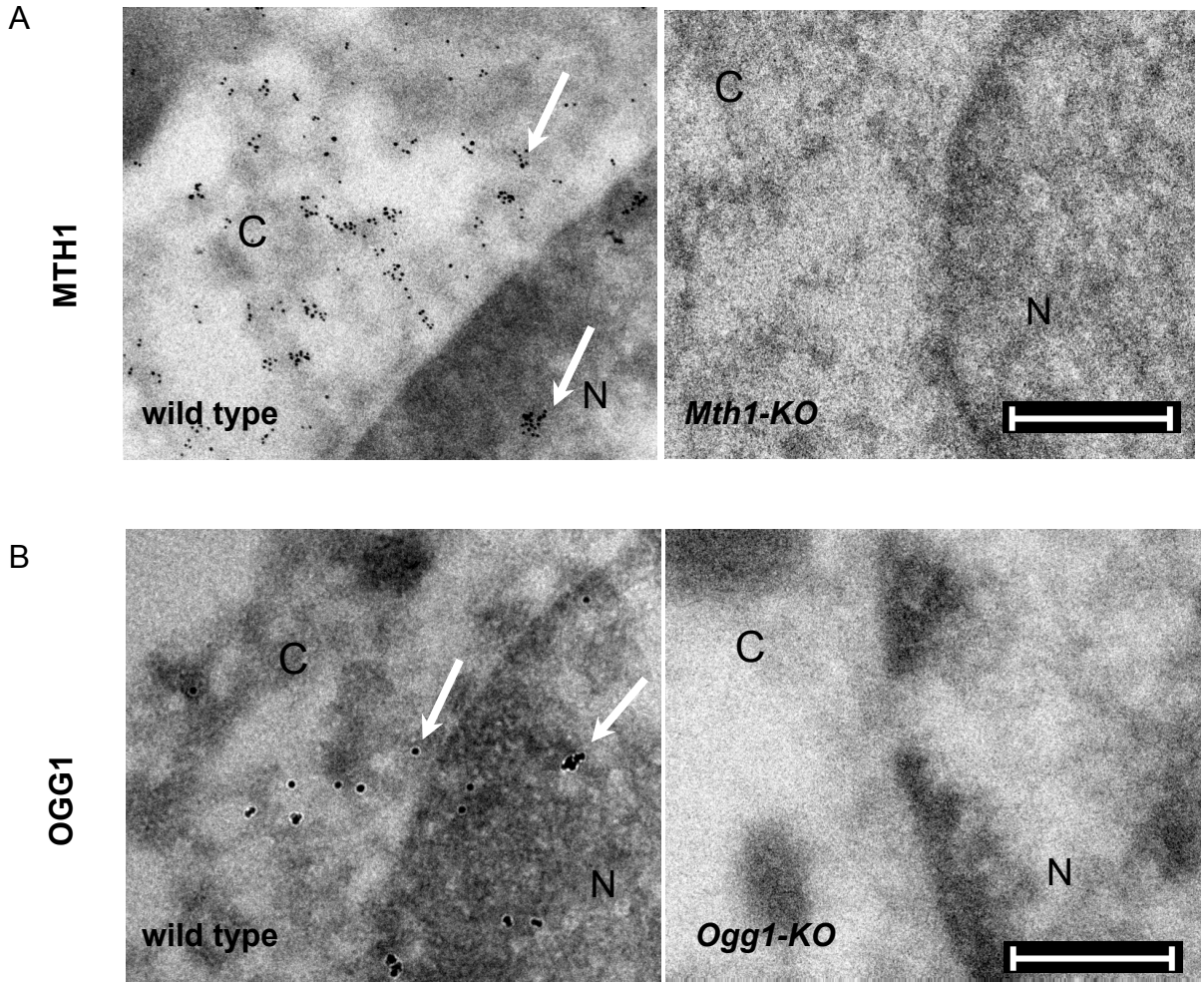
Supplementary Data

Supplementary Figures and Figure Legends



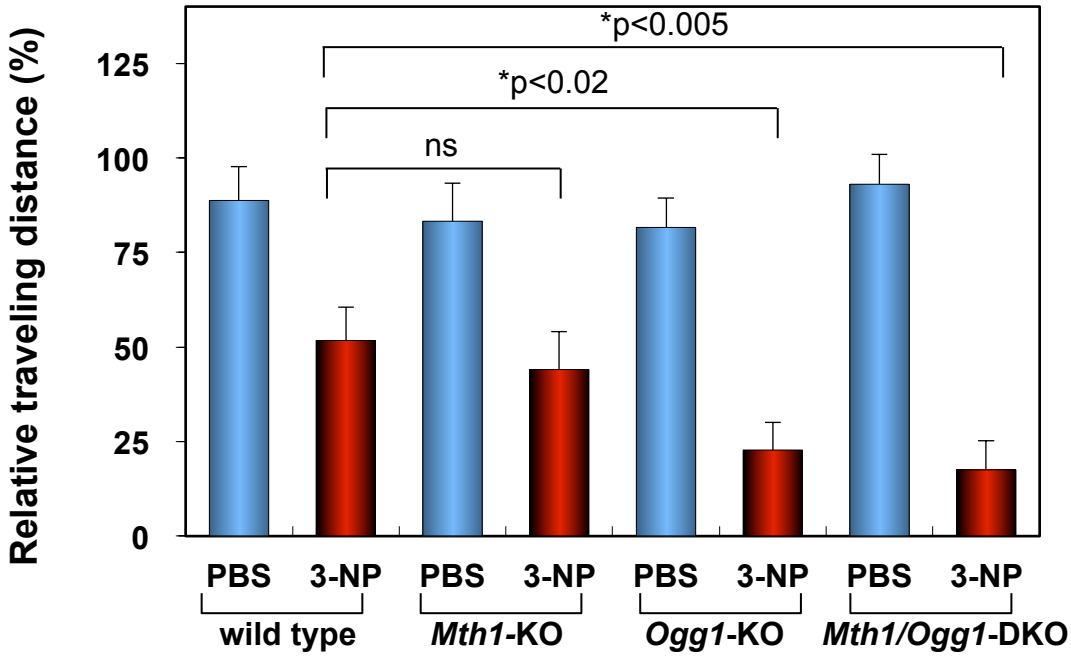
Supplemental Figure 1. Immunohistochemical detection of MTH1 and OGG1 in the mouse striatum.

(A) MTH1 expression was examined using the NB-109 antibody, which reacts with human MTH1; the human protein has 91% sequence identity with mouse and rat proteins. MTH1 was preferentially localized cytoplasm, but no signal was detected in *Mth1*-KO mice. Arrows, MTH1 immunoreactive signals. (B) OGG1 expression was examined using anti-OGG1 antibody. In wild-type striatum, OGG1 was detected in nucleus and cytoplasm, but no signal was detected in *Ogg1*-KO mice. Arrows, OGG1 immunoreactive signals. Bar, 20 μm. Dark blue signals represent immunoreactivity. (A) (B) The images are visualized by differential interference contrast microscopy.



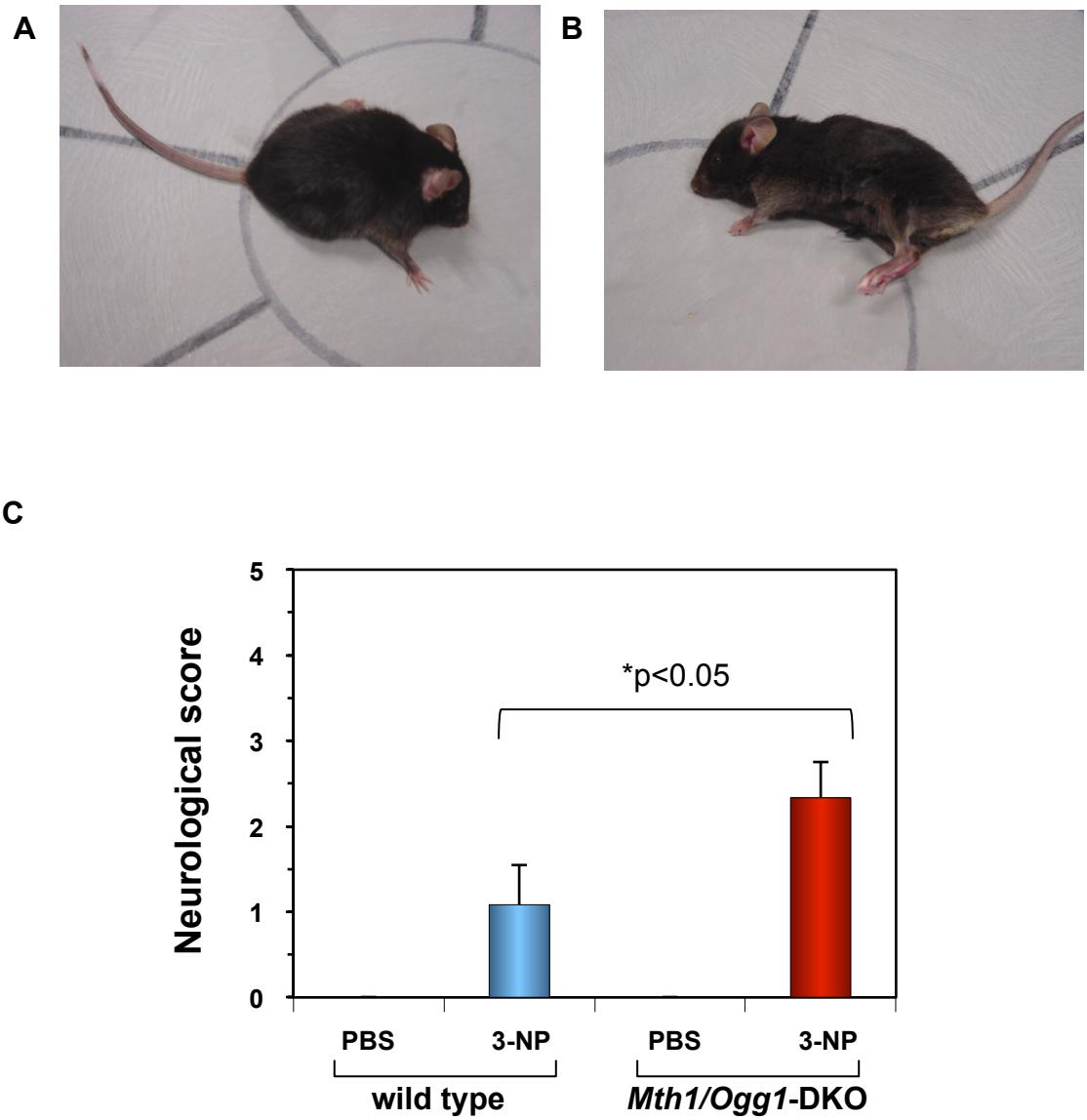
Supplemental Figure 2. Detection of MTH1 and OGG1 in the mouse striatum by immunoelectron microscopy.

(A) MTH1 expression was examined using the NB-109 antibody, MTH1 was preferentially localized in cytoplasm and some in nucleus, but no signal was detected in *Mth1*-KO striatum. Arrows, MTH1 immunoreactive signals. Bar, 500 nm. C, cytoplasm; N, nucleus. (B) OGG1 expression was examined using anti-Ogg1 antibody. In wild-type striatum, OGG1 was detected in nucleus, cytoplasm, but no signal was detected in *Ogg1*-KO striatum. Arrows, OGG1 immunoreactive signals. C, cytoplasm; N, nucleus. Bar, 500 nm.



Supplemental Figure 3. Behavioral assessments of mice by open field after exposure to 3-nitropropionic acid in various mouse strains

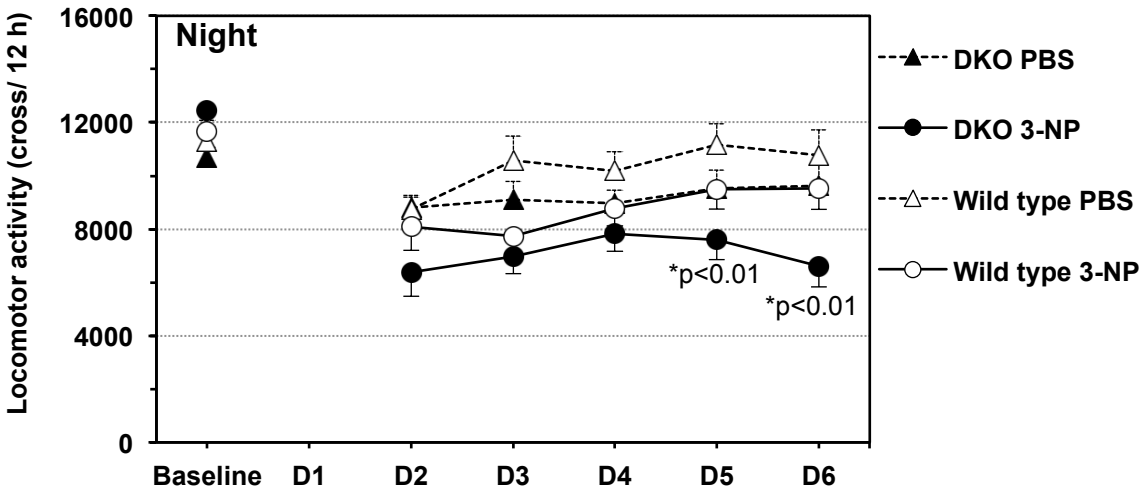
Motor impairments in various mouse strains assessed by open field test after exposure to 3-nitropropionic acid (3-NP, 120 mg/kg) for one week. Wild-type (N=5 for PBS, N=5 for 3-NP), *Mth1*-KO (N=4 for PBS, N=4 for 3-NP), *Ogg1*-KO (N=5 for PBS, N=7 for 3-NP), and *Mth1/Ogg1*-DKO (N=5 for PBS, N=5 for 3-NP) were tested. Compared to wild-type mice exposed to 3-NP, *Ogg1*-KO 3-NP ($p<0.02$), *Mth1/Ogg1*-DKO 3-NP($p<0.005$) (Student's *t*-test), ns, not significant. Relative traveling distance (%) indicates the traveling distance obtained on the 7th day after implantation of an osmotic mini-pump as a percentage of that obtained on the day before the implantation. Data are least squares means (LSMeans) ± SE.



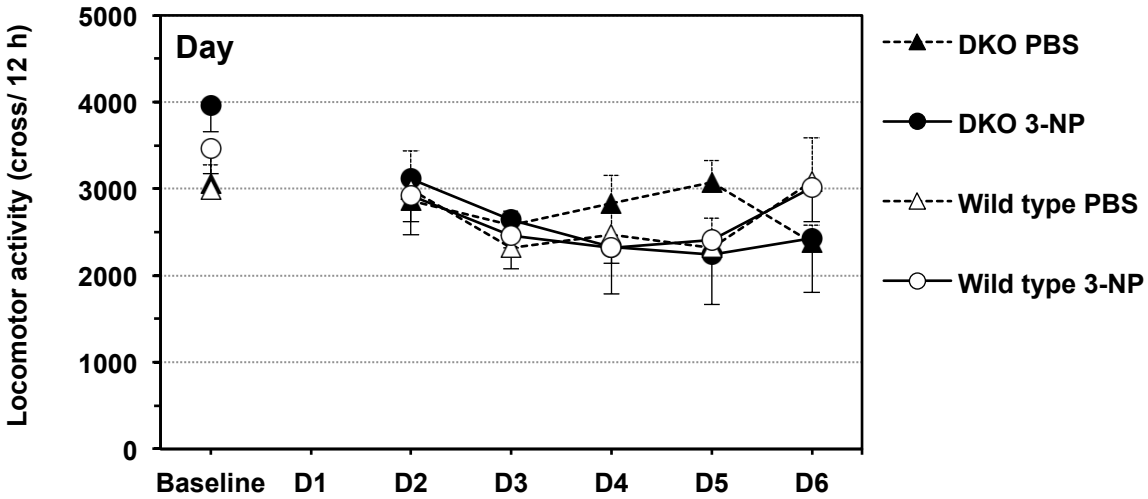
Supplemental Figure 4. Motor impairment observed on the 7th day of chronic exposure to 3-NP

Wild-type mice (N=10 for PBS, N=12 for 3-NP) and *Mth1/Ogg1*-DKO mice (N=11 for PBS, N=12 for 3-NP) were monitored for 10 min on the 7th day of exposure to 3-NP (120 mg/kg). Neurological score was defined as follows: (score=0, normal behavior; 1, general slowness of displacement resulting from mild hindlimb impairment; 2, incoordination and marked gait abnormality; 3, hindlimb paralysis; 4, incapacity to move resulting from forelimb and hindlimb impairment shown in A; 5, recumbency shown in B (Guyot MC et al., 1997). C*, Mth1/Ogg1*-DKO mice exhibited significantly higher neurological scores compared to wild type ($p<0.05$, Mann-Whitney U-test). Data are shown with means ± SE.

A

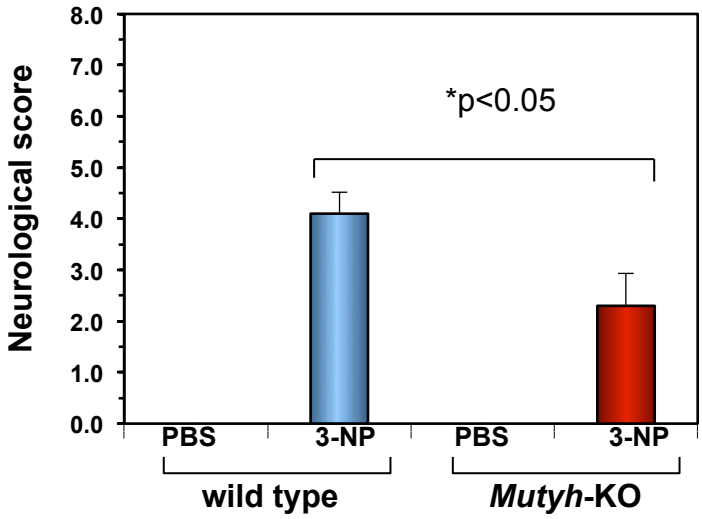


B



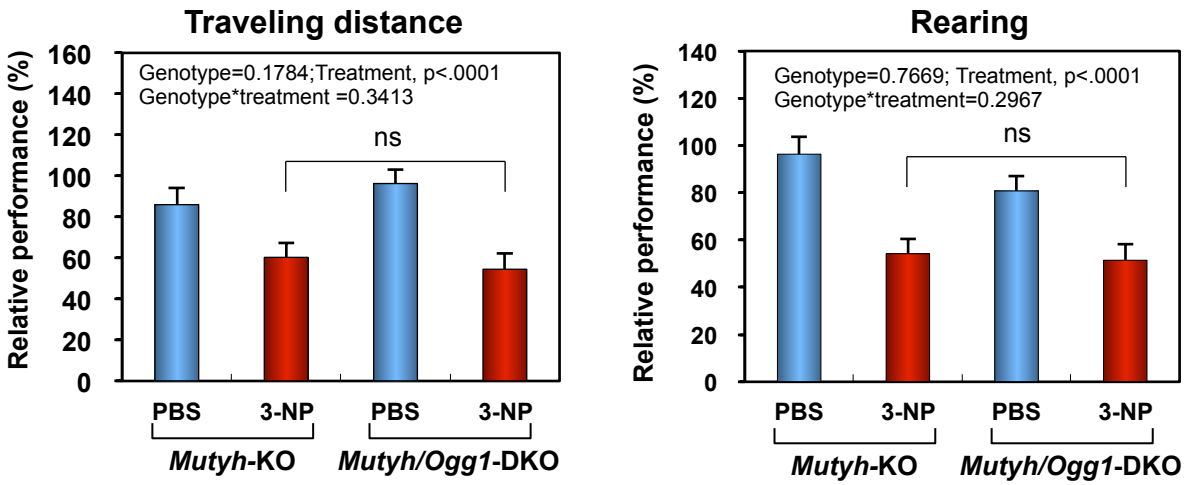
Supplemental Figure 5. Impairment in spontaneous locomotor activity after exposure to 3-NP.

(A) Night-phase locomotor activity observed in the home cage. (B) Day-phase locomotor activity observed in the home cage. Wild-type (N=6 for PBS, N=9 for 3-NP) and *Mth1/Ogg*1-DKO mice (N=6 for PBS, N=8 for 3-NP) were examined. In comparison to wild-type mice, the DKO mice exhibited greater decrease in night-phase locomotor activity on the day 5 (D5) and day 6 (D6) after exposed to 3-NP, however, there was no a significant difference on day-phase locomotor activity. Data are shown with mean ± SE. *p, Student's t test (DKO 3-NP vs Wild type 3-NP).



Supplemental Figure 6. Motor impairment in wild-type and *Mutyh*-KO mice on the 7th day of exposure to higher dose of 3-NP .

Wild-type mice (N=6 for PBS, N=10 for 3-NP) and *Mutyh*-KO mice (N=6 for PBS, N=10 for 3-NP) were monitored for 10 min on the 7th day of exposure to 3-NP (150 mg/kg). Wild-type mice exhibited more severe score compared to *Mutyh*-KO ($p<0.05$, Mann-Whitney U-test). Data are shown with mean ± SE.



Supplemental Figure 7. Behavioral assessments of mice by open field test after exposure to 3-nitropropionic acid between *Mutyh*-KO and *Ogg1/Mutyh*-DKO.

Mutyh-KO (N=7 for PBS, N=5 for 3-NP) and *Ogg1/Mutyh*-DKO (N=6 for PBS, N=7 for 3-NP) were subjected to open field test on the 7th day of exposure to 3-NP (120 mg/kg). There was no a significant effect of genotype, ns, not significant. Relative performnce (%) indicates the traveling distance or number of rearing obtained on the 7th day after implantation of an osmotic mini-pump as a percentage of those obtained on the day before the implantation. Data are least squares means (LSMeans) ± SE.

Supplementary materials:

Supplemental Movie 1. A control *Mth1/Ogg1*-DKO mouse that received PBS infusions for a week exhibited normal spontaneous motor activity in open field.

Supplemental Movie 2. An *Mth1/Ogg1*-DKO mouse that received 3-NP infusions for 1 week (120 mg/kg/day) exhibited a dramatic decrease in the amount of spontaneous motor activity in the open field.