Title

Microglial response promotes neurodegeneration in the *Ndufs4 KO* mouse model of Leigh syndrome

Authors

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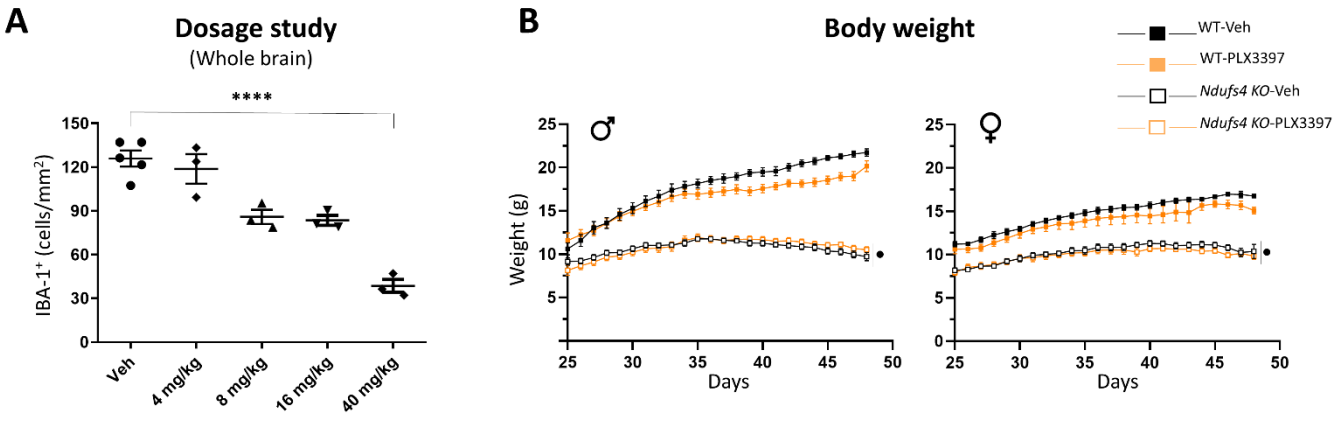
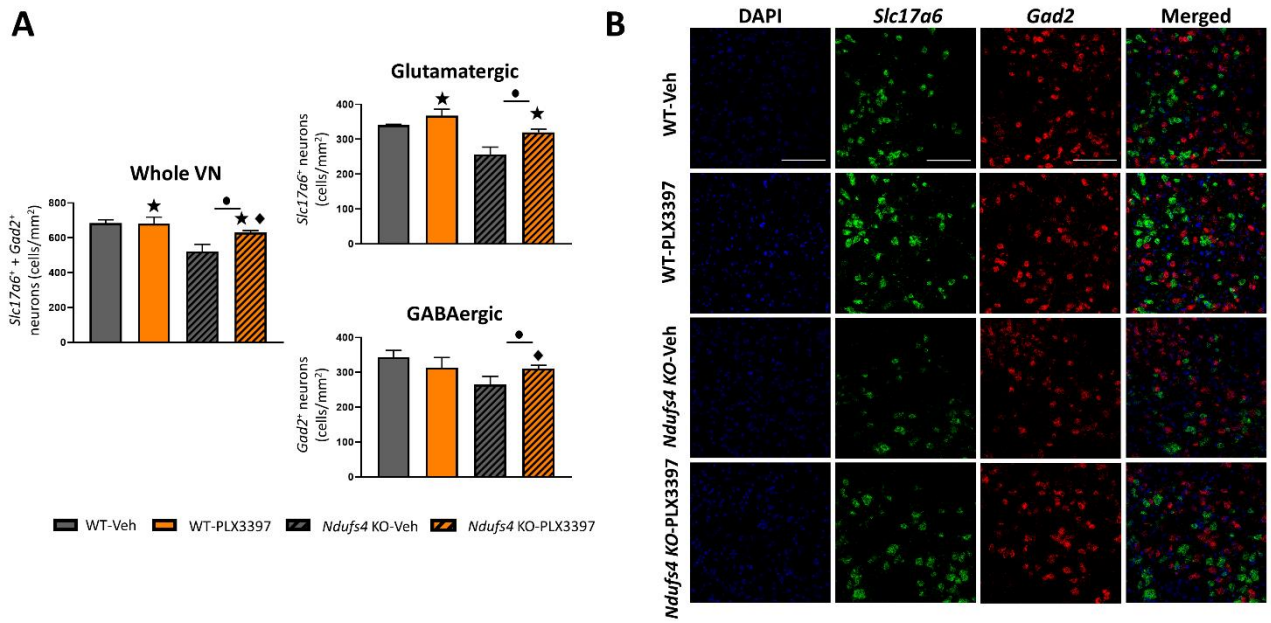
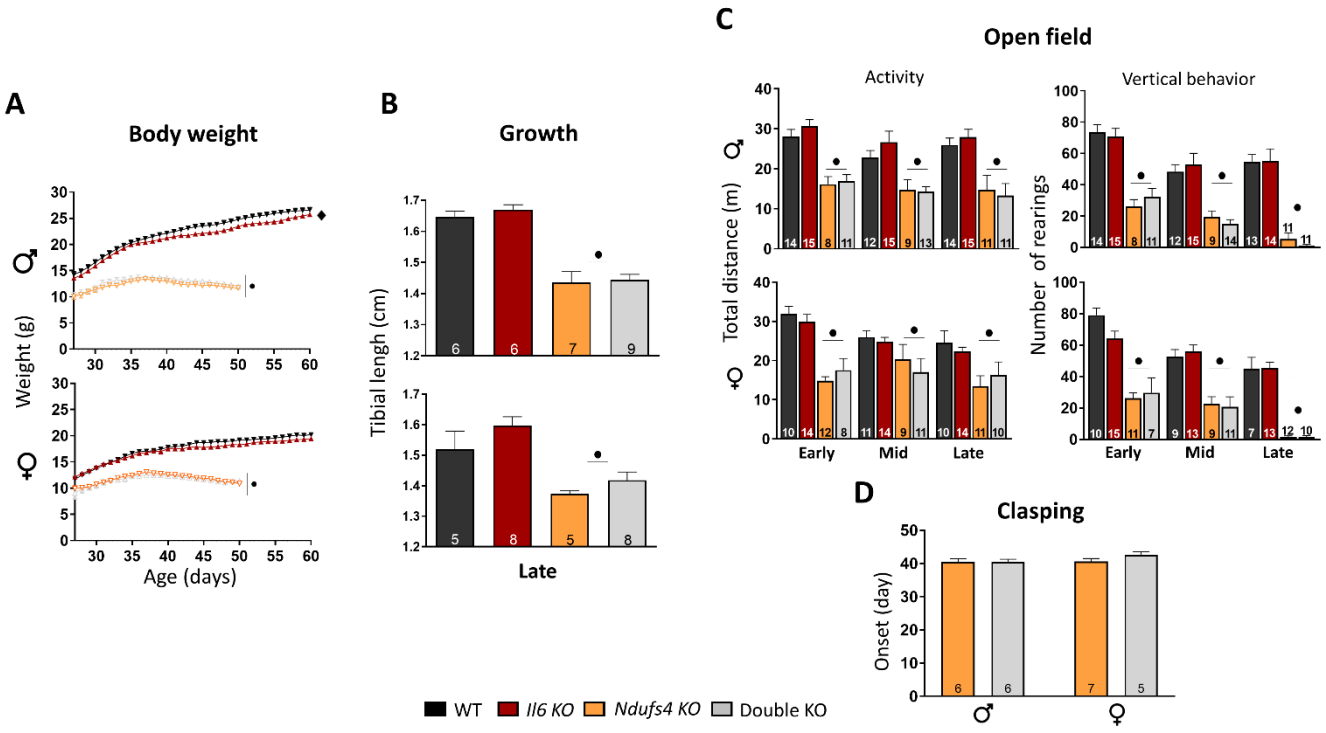


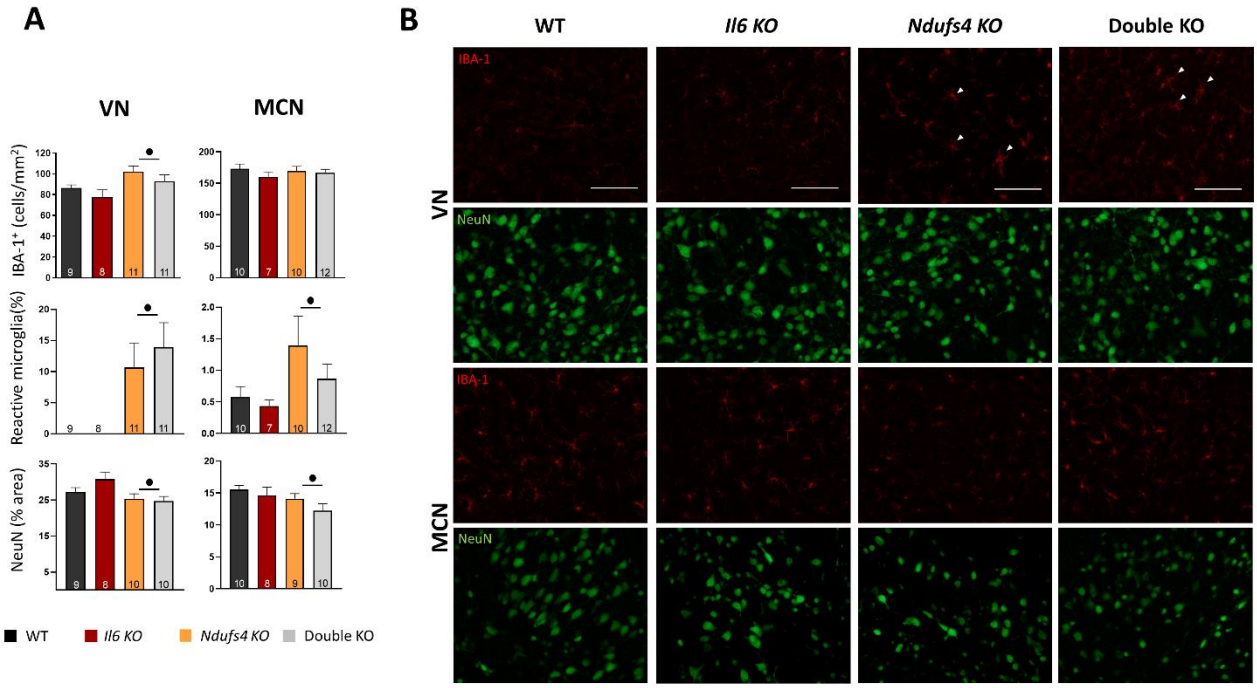
Supplementary Figure 1. (A) Reduction of IBA-1⁺ cells at different PLX3397 doses in brains of WT mice after 14 days of treatment. Dosage at 40 mg/kg was able to reduce around 70% of IBA-1⁺ cells. One-way ANOVA followed by Tukey's multiple comparisons test for statistics. **(B)** Total body weight for WT-Veh, WT-PLX3397, *Ndufs4 KO*-Veh, and *Ndufs4 KO*-PLX3397 of male and female mice (n = 7-15). GEE statistical test. **** $p \leq 0.0001$, ● $p \leq 0.05$ (WT vs. *Ndufs4 KO*).



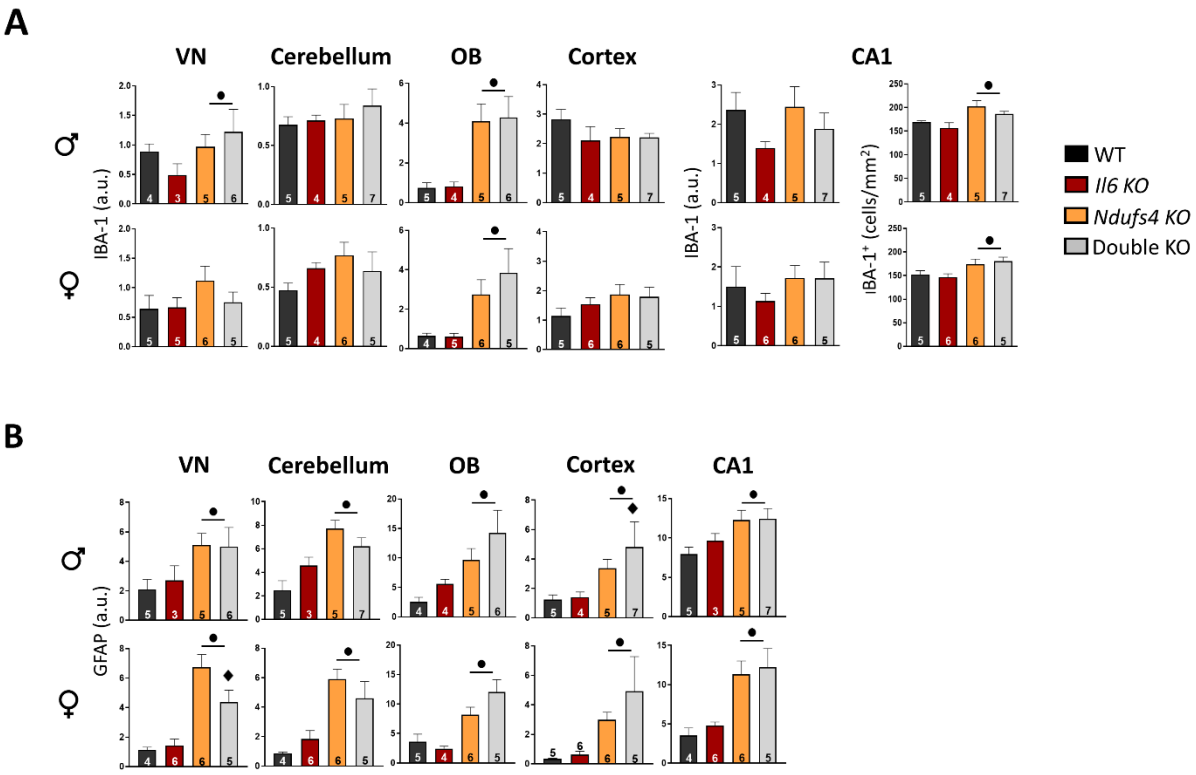
Supplementary Figure 2. Microglial elimination protects from glutamatergic and GABAergic neuronal loss in the VN of *Ndufs4 KO* mice. (A) Number of neurons of both glutamatergic and GABAergic subtypes in the whole VN of *Ndufs4 KO* mice (left) and the number of neurons subdivided by the neuronal subtype (right). GzLM statistical tests. **(B)** Representative ISH images of glutamatergic and GABAergic neurons of the different genotypes in the non-lesioned area of the VN. ● p≤ 0.05 (WT vs. *Ndufs4 KO*), ★ treatment effect p≤ 0.05 (Veh vs. PLX3397),◆ p≤ 0.05 interaction between both factors. Scale bar 100 µm.



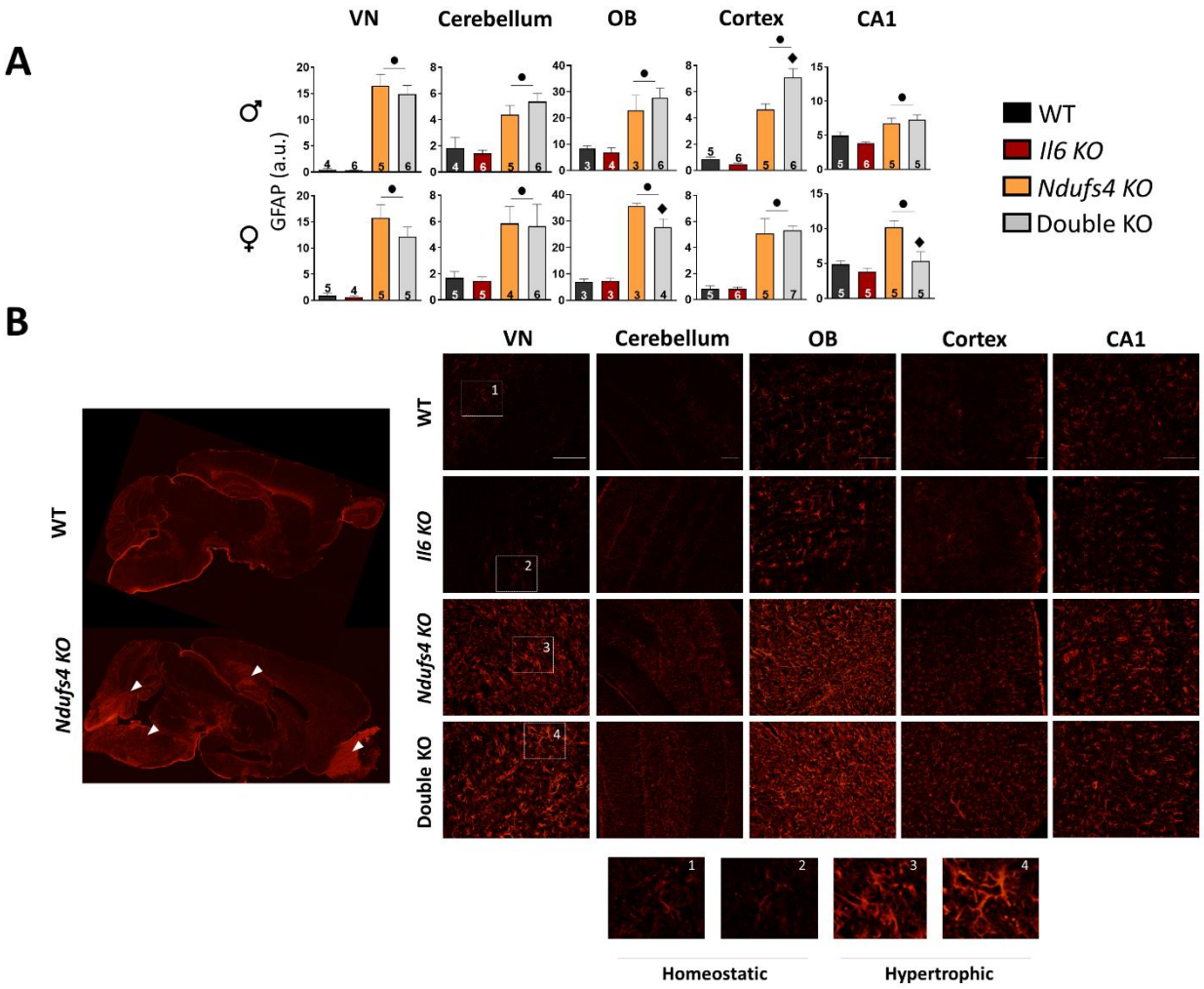
Supplementary Figure 3. IL-6 deficiency in Ndufs4 KO mice does not modify any other motor phenotype. (A) Body weight curve for WT, *Il6 KO*, *Ndufs4 KO,* and Double KO mice (n = 11-17). **(B)** Growth measured by tibial length. **(C)** Total distance travelled and vertical behaviour in the open-field test. **(D)** Day of onset of clasping behaviour in *Ndufs4 KO* and Double KO mice. GEE, GzLM, and Student's t-test statistical test for body weight, growth and open field, and clasping, respectively. ● p≤ 0.05 (WT vs. *Ndufs4 KO*),◆ p≤ 0.05 interaction between both factors.



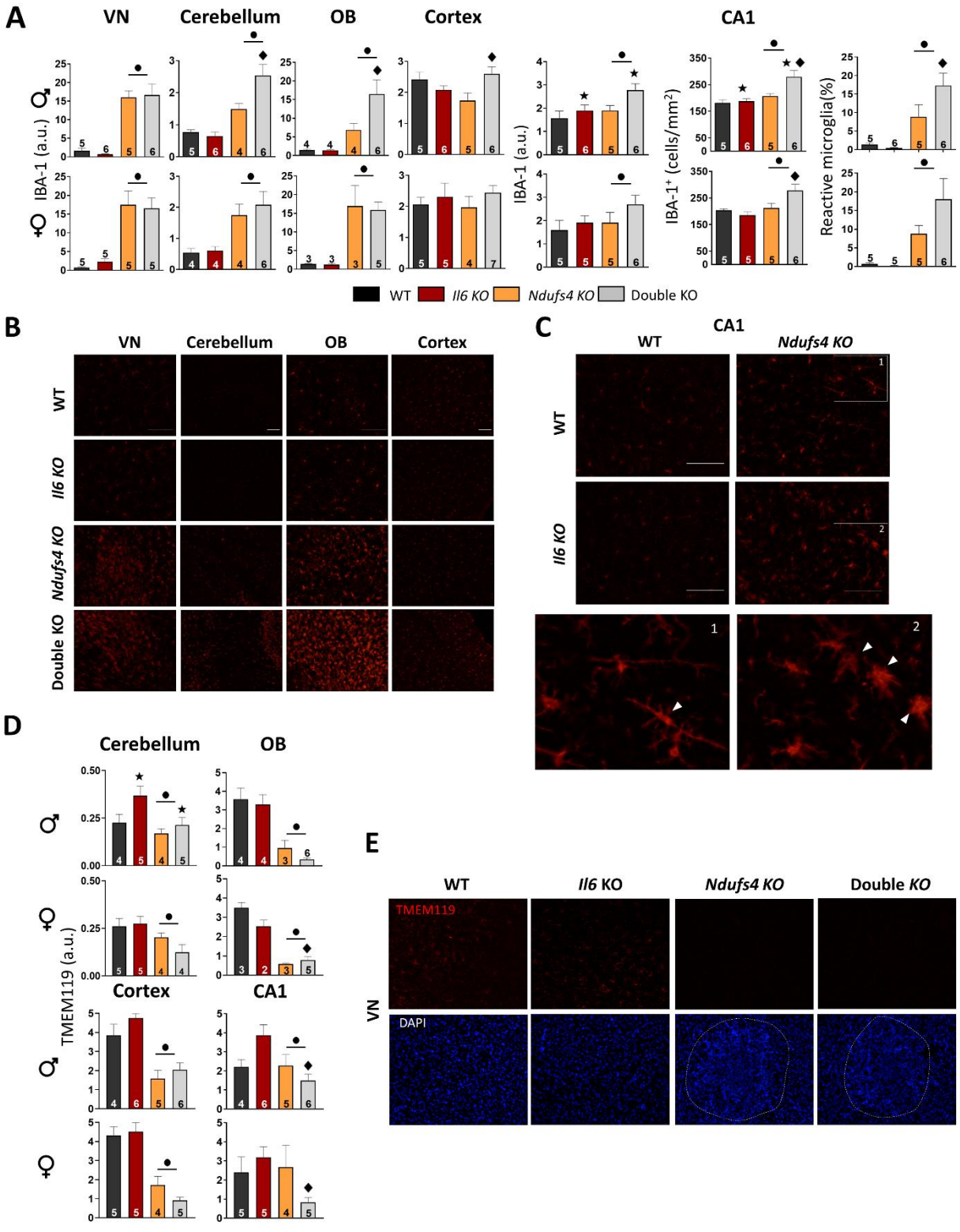
Supplementary Figure 4. Neuronal loss and presence of reactive microglia in the VN and MCN of NDUFS4-deficient mice at the mid stage of the disease. (A) Total count of IBA-1^{+} cells and percentage of those that present reactive morphology together with the percentage of area occupied by NeuN staining in VN and MCN. Both sexes are represented together. GzLM statistical test **(B)** Representative images of IBA-1 and NeuN immunofluorescence. White arrows indicate hypertrophied microglia. ● Ndufs4 effect p≤ 0.05 (WTs vs. *Ndufs4 KO*s). Scale bar 100 µm. MCN (middle cerebellar nuclei), VN (vestibular nuclei).



Supplementary Figure 5. Analysis of microgliosis and astrocyte reactivity at the mid stage in both male and female mice in different brain regions. (A) IBA-1 mean fluorescence intensity quantification in VN, cerebellum, cortex, and CA1. **(B)** GFAP mean fluorescence intensity quantification in VN, cerebellum, cortex, and CA1. GzLM statistical test. ● Ndufs4 effect p≤ 0.05 (WTs vs. *Ndufs4 KO*s), ◆ p≤ 0.05 interaction between both factors. VN (vestibular nuclei), OB (olfactory bulb).



Supplementary Figure 6. IL-6 deficiency barely modifies astrocyte reactivity in *Ndufs4 KO* mice at the late stage. (A) Quantification of relative GFAP fluorescence intensity at the late stage in VN, cerebellum, OB, cortex, and CA1. GzLM statistical tests. Numbers within bars represent the "n" of the group **(B)** Representative images of GFAP immunofluorescence of the different brain regions at the late stage of the disease in male mice. At the left, whole-brain sagittal GFAP staining showing regions of astrogliosis in *Ndufs4 KO* mice (white arrows). At the right, representative images of GFAP staining in VN, Cerebellum, OB, cortex, and CA1 of the different genotypes. Magnification shows homeostatic and hypertrophic astrocyte morphology. Scale bar 100 µm. ● Ndufs4 effect p≤ 0.05 (WTs vs. *Ndufs4 KO*s), ◆ p ≤ 0.05 interaction between both factors. VN (vestibular nuclei), OB (olfactory bulb).



Supplementary Figure 7. Analysis of microgliosis at the late stage of both females and males in different brain regions revealed increased microglial response in male *Ndufs4 KO* mice with IL-6 deficiency. (A) Quantification of relative IBA-1 fluorescence intensity at the late stage in VN, cerebellum, OB, cortex, and CA1. **(B and C)** Representative images of IBA-1 staining in VN, Cerebellum, OB, cortex, and CA1 of the different genotypes. White arrows indicate activated

microglial morphology (1. hypertrophic and 2. ameboid microglial morphology). **(D)** Quantification of relative TMEM119 fluorescence intensity at the late stage in the cerebellum, OB, cortex, and CA1. **(E)** Representative images of TMEM119 signal and DAPI in the VN of the different genotypes. The dashed lines in *Ndufs4 KO* and Double KO images indicate accumulation of cells where massive lesions are classically found, note that no TMEM119 signal is detected compared to the strong IBA-1 staining in the VN. **(A and D)** Numbers within bars represent the "n" of the group. GzLM statistical test for A and D. ● Ndufs4 effect p≤ 0.05 (WTs vs. *Ndufs4 KO*s), ★ IL-6 effect p≤ 0.05 (WT vs. *Il6 KO*s),◆ p≤ 0.05 interaction between both factors. Scale bar 100 µm. VN (vestibular nuclei), OB (olfactory bulb).