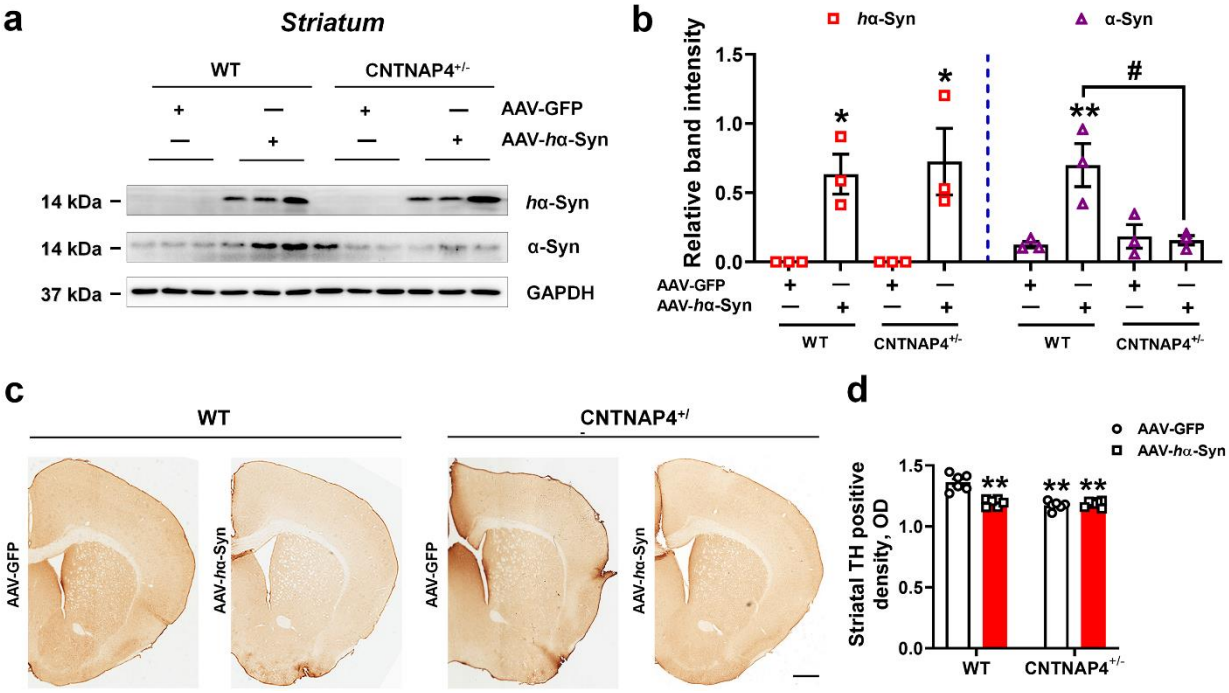
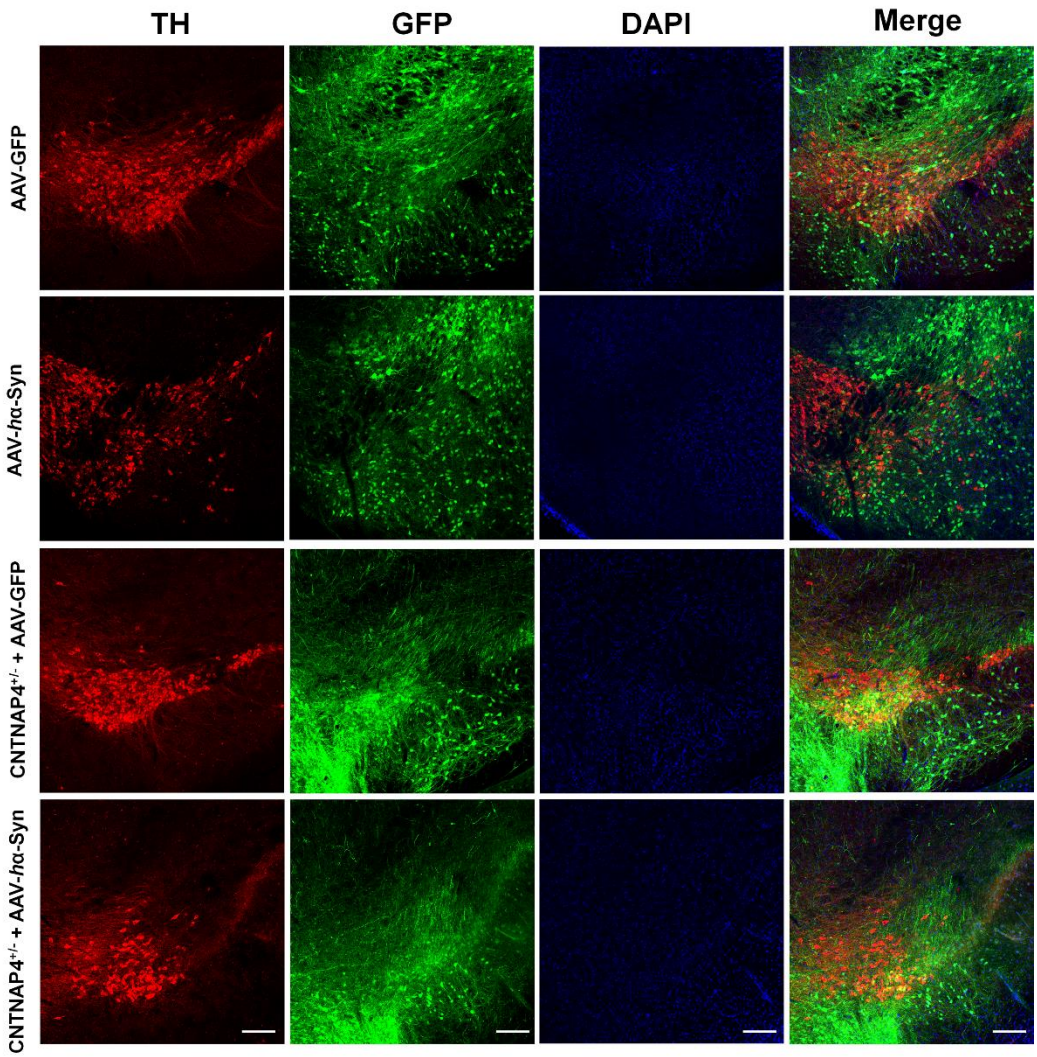
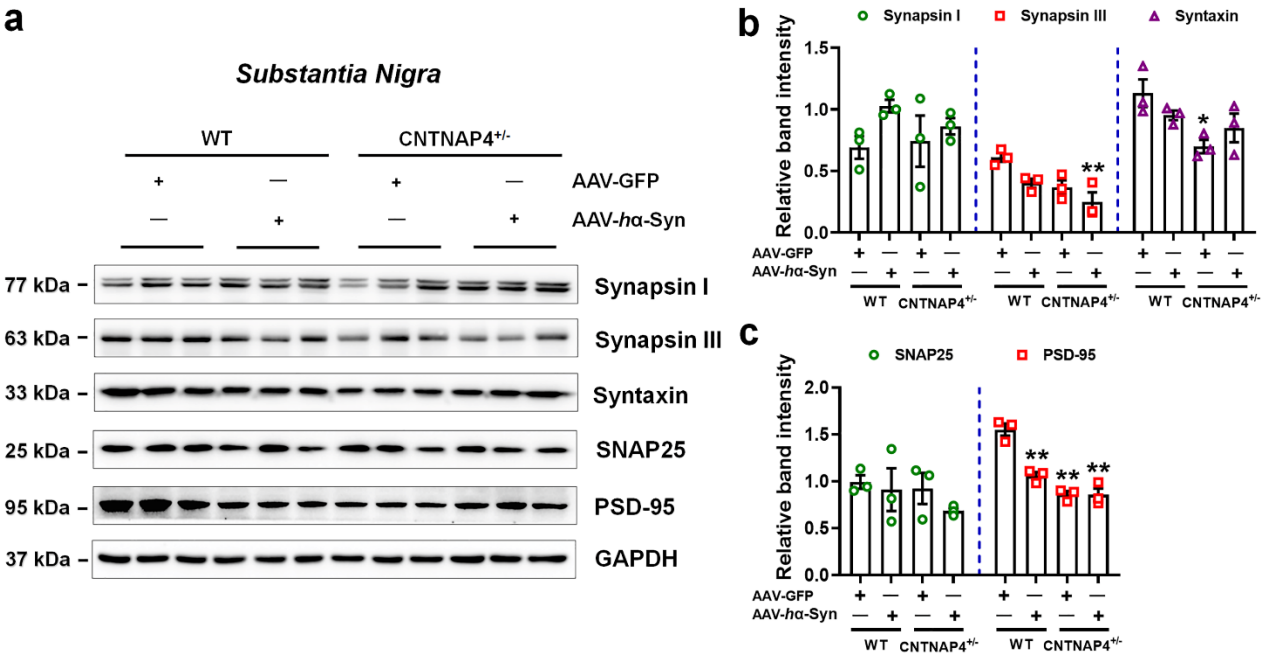
Supplementary Materials



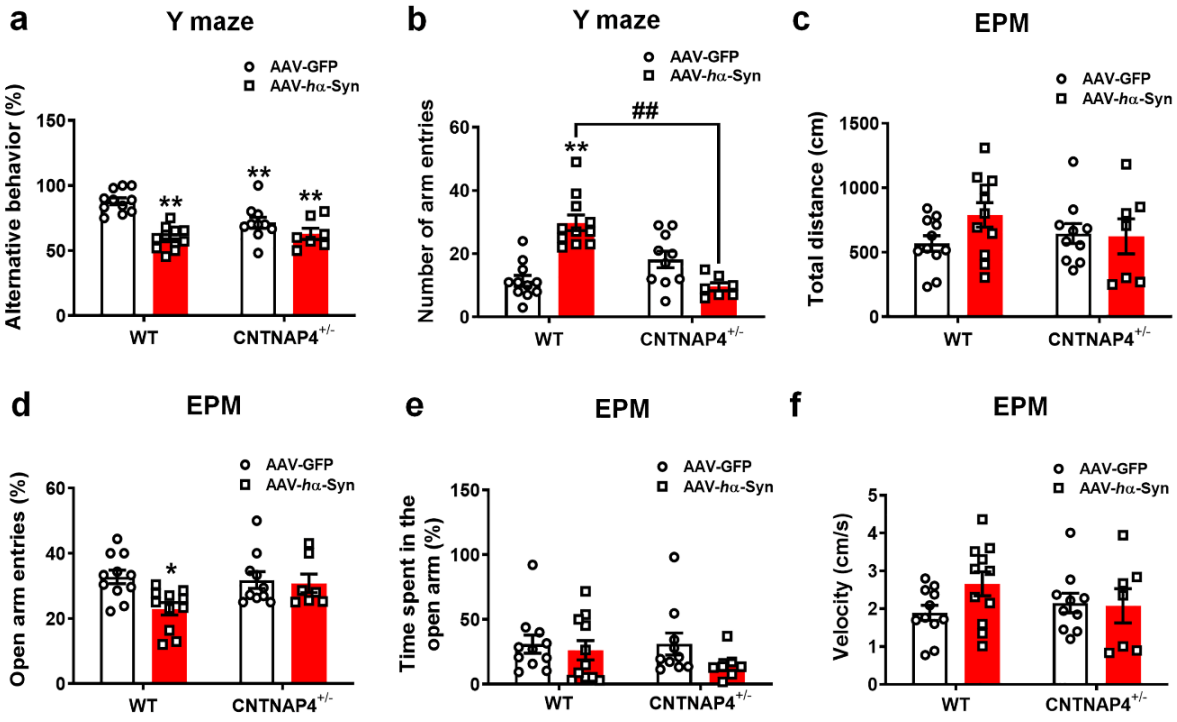
Supplementary Figure 1. Effect of *CNTNAP4* partial deficiency on the striatal α-synuclein pathology and nigral DA neuron expression in AAV-*h*α-Syn injected mice. (**a** and **b**) The protein expression levels of TH, *h*α-Syn, and mouse α-synuclein were determined using Western blotting. N = 3 per group. (**c**) Immunohistochemical staining of striatal TH-positive cells in WT and CNTNAP4$^{+/-}$ mice injected with either AAV-GFP or AAV-*h*α-Syn. Scale bars, 1 mm. (**d**) Quantification of striatal TH-positive density. N = 6 per group. Results are expressed as mean ± SEM. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. WT; $^{\#}p < 0.05$ vs. AAV-*h*α-Syn. Statistical significance was determined using two-way ANOVA + Bonferroni's multiple comparisons test.



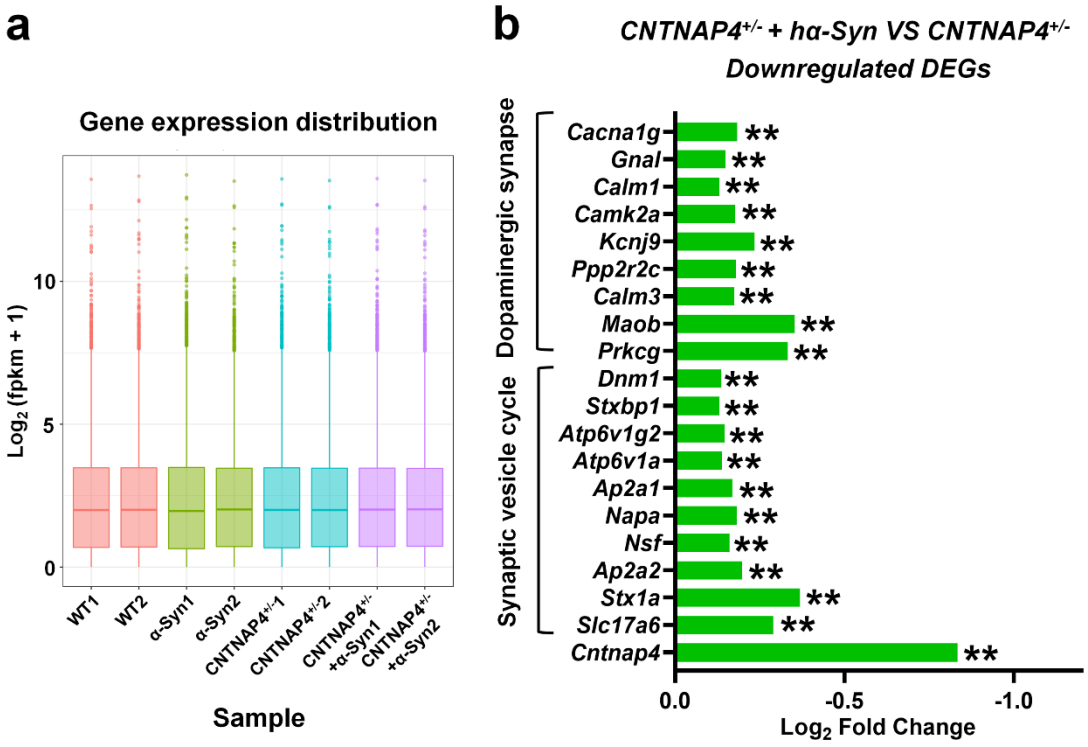
Supplementary Figure 2. Effect of α-synuclein overexpression on the nigral DA neuron expression in *CNTNAP4* partial deficiency mice. Immunofluorescence staining of TH-positive cells in the SNpc of WT and CNTNAP4$^{+/-}$ mice injected with either AAV-GFP or AAV-*h*α-Syn. Scale bars, 120 μm.



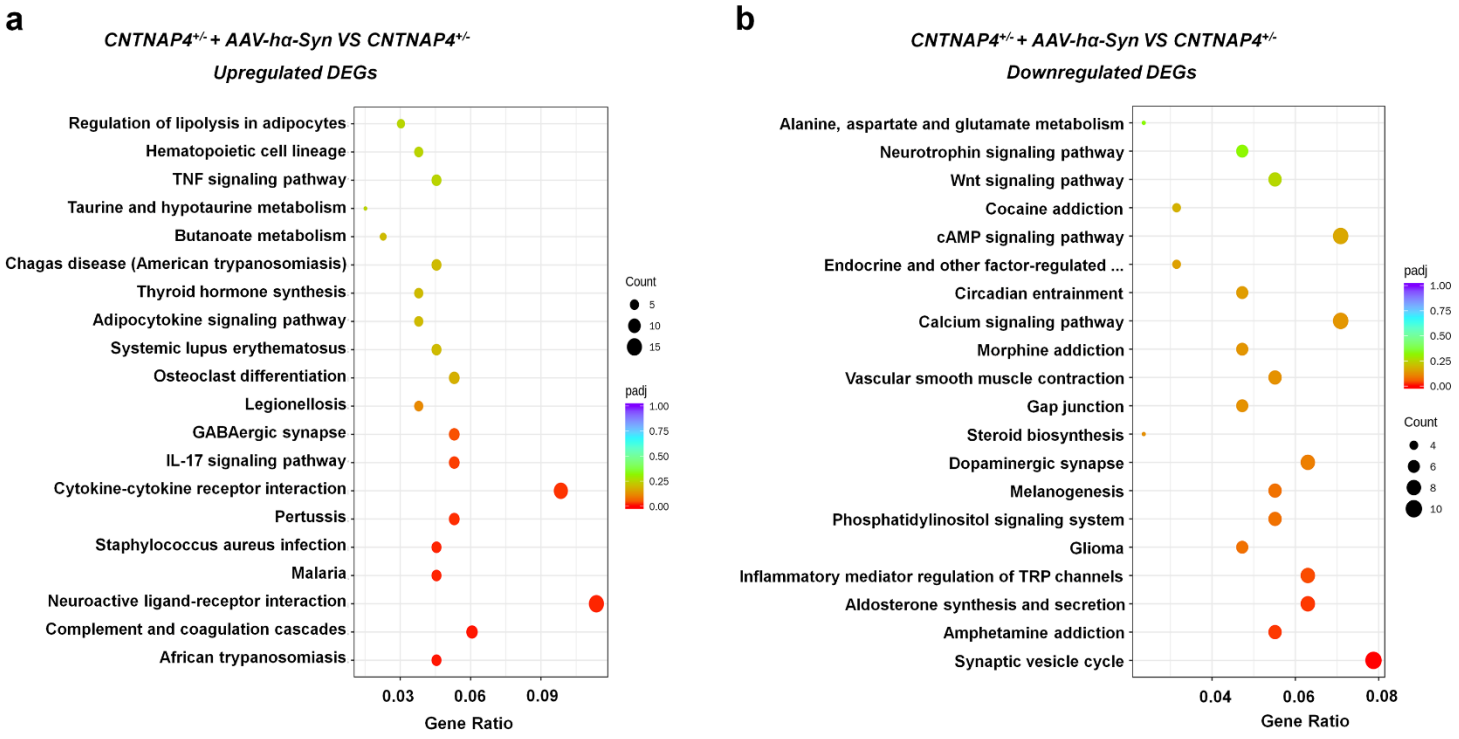
Supplementary Figure 3. Effect of α-synuclein burden on the nigral synaptic protein expression in *CNTNAP4* partial deficiency mice. (**a–c**) The nigral protein expression levels of synapsin I, synapsin III, syntaxin, SNAP25, and PSD-95 in WT and CNTNAP4$^{+/-}$ mice injected with either AAV-GFP or AAV-*h*α-Syn were determined using western blotting. N = 3 per group. Results are expressed as mean ± SEM. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. WT. Statistical significance was determined using two-way ANOVA + Bonferroni's multiple comparisons test.



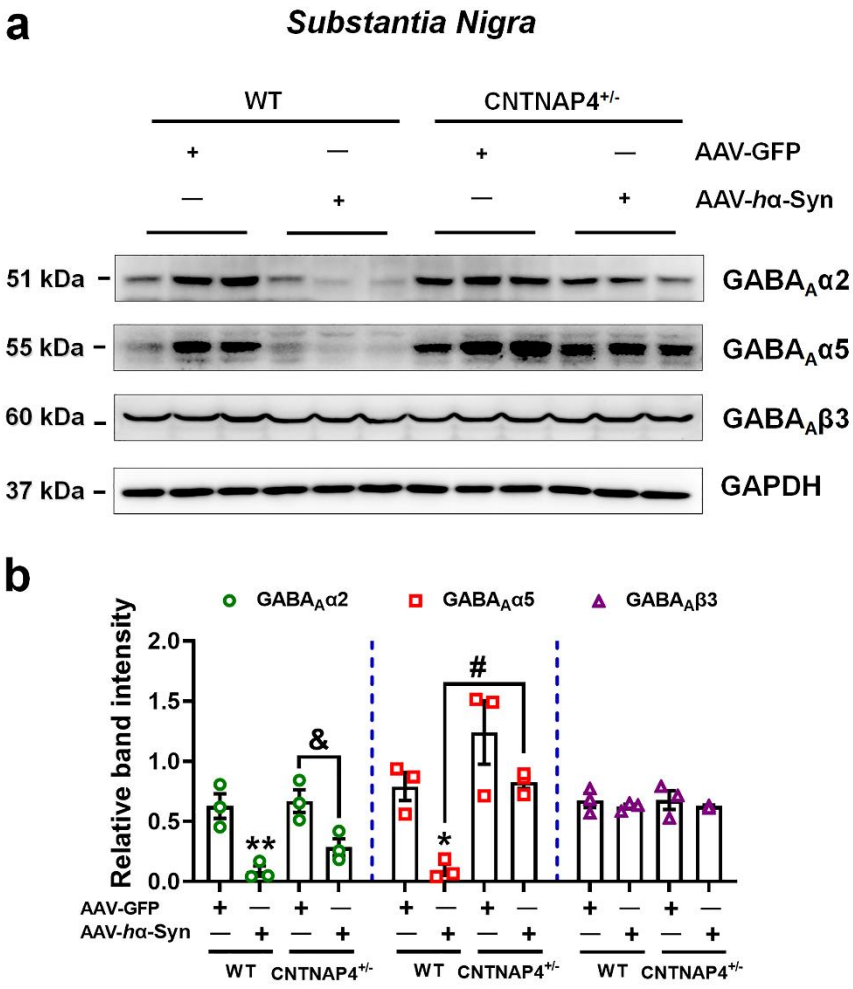
Supplementary Figure 4. Behavioral tests of *CNTNAP4* partial deficient mice by α-synuclein burden. (**a** and **b**) Y maze test of alternative behavior and number of arm entries. (**c–f**) EPM test of total distance travelled, open arm entries, time spent in open arm, and movement speed. Results are expressed as mean ± SEM. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. WT; $^{\#\#}p < 0.01$ vs. AAV-*h*α-Syn. Statistical significance was determined using two-way ANOVA + Bonferroni's multiple comparisons test.



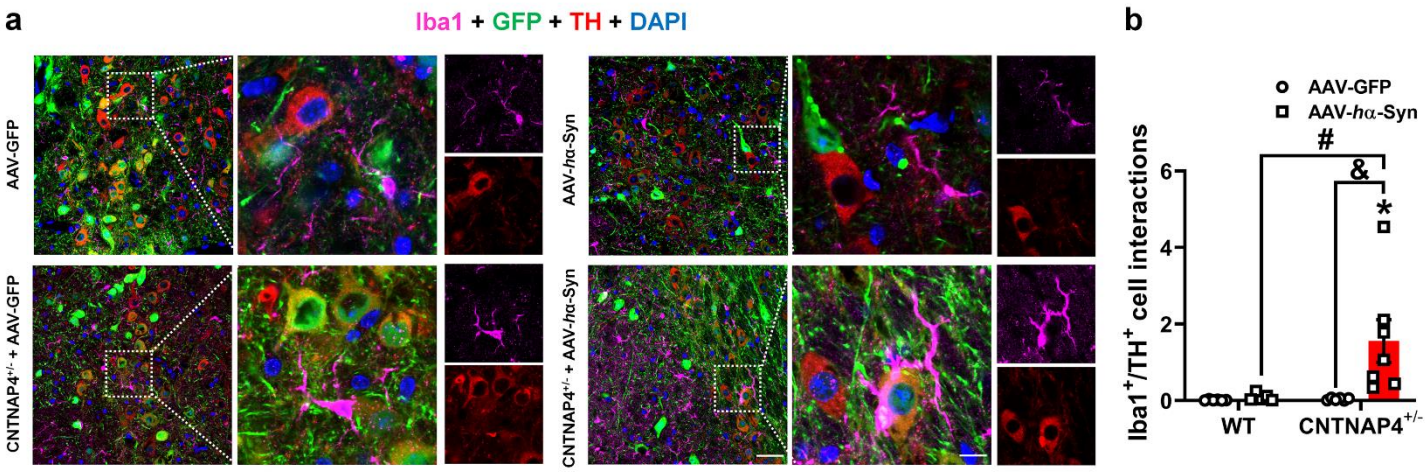
Supplementary Figure 5. DEGs of *CNTNAP4* partial deficiency mice by α-synuclein burden. (**a**) Gene expression distribution of RNA-seq analysis in WT and CNTNAP4$^{+/-}$ mice injected with either AAV-GFP or AAV-*h*α-Syn. (**b**) Downregulated DEGs were enriched in "dopaminergic synapse" and "synaptic vesicle cycle" KEGG pathways between CNTNAP4$^{+/-}$ + AAV-*h*α-Syn and CNTNAP4$^{+/-}$ mice.



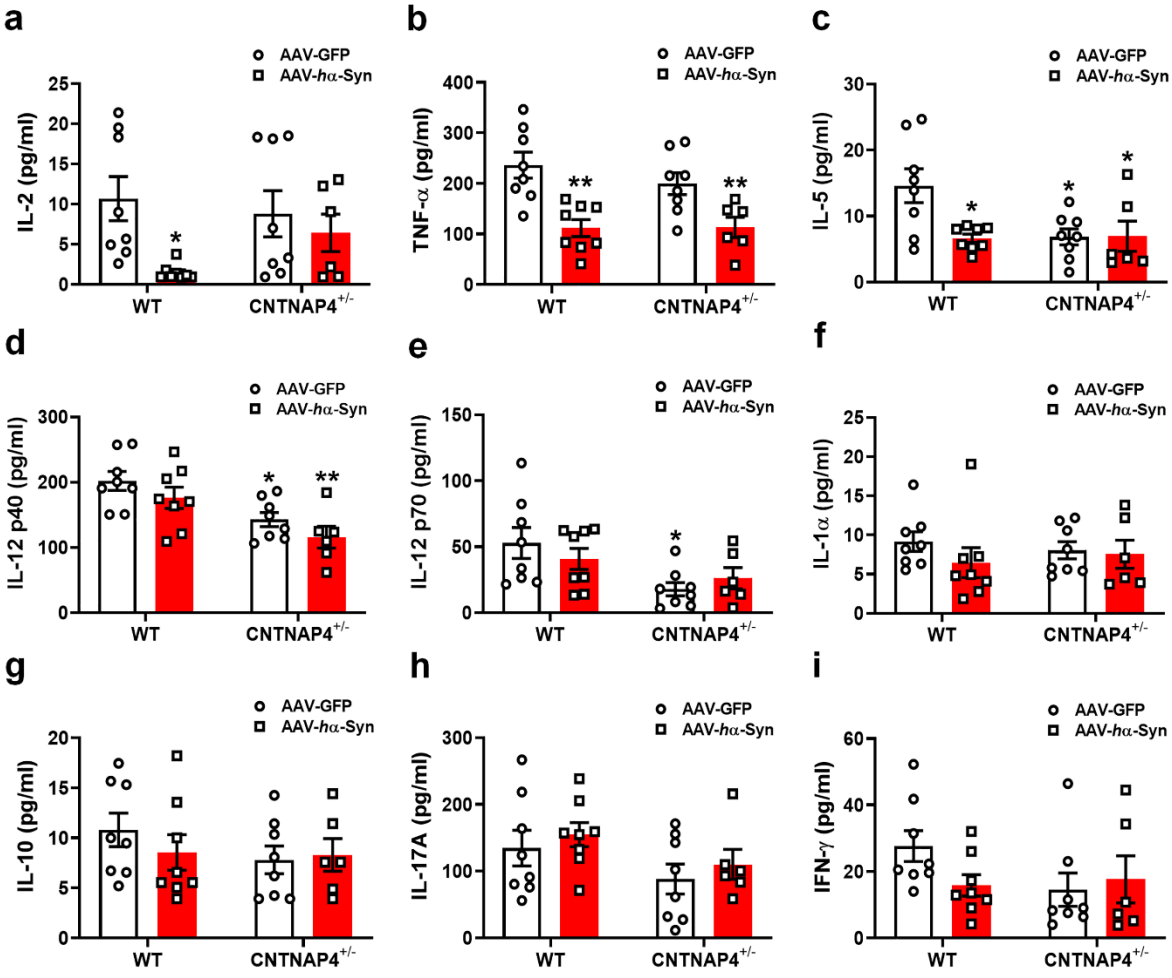
Supplementary Figure 6. KEGG pathways enriched by DEGs in *CNTNAP4* partial deficient mice by α-synuclein burden. (**a** and **b**) KEGG pathways enriched by up- and down-regulated DEGs between CNTNAP4$^{+/-}$ + AAV-*h*α-Syn and CNTNAP4$^{+/-}$ mice.



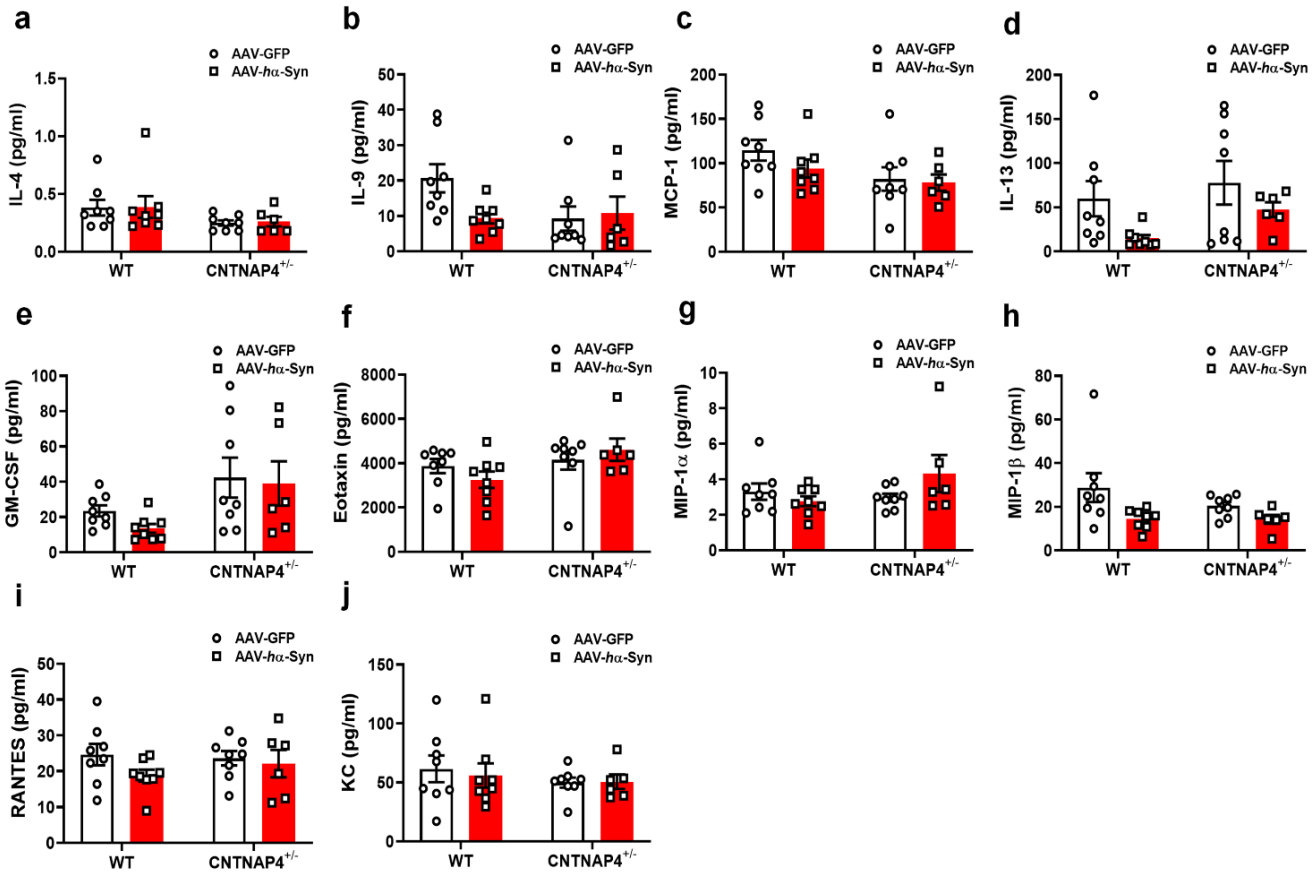
Supplementary Figure 7. Effect of α-synuclein burden on the nigral GABAergic protein expression in *CNTNAP4* partial deficient mice. (**a** and **b**) The nigral protein expression levels of $GABA_A\alpha2$, $GABA_A\alpha2$, and $GABA_A\beta3$ in WT and $CNTNAP4^{+/-}$ mice injected with either AAV-GFP or AAV-*h*α-Syn were determined using Western blotting. N = 3 per group. Results are expressed as mean ± SEM. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. WT; $^{\#}p < 0.05$ vs. AAV-*h*α-Syn; $^{\&}p < 0.05$ vs. $CNTNAP4^{+/-}$. Statistical significance was determined using two-way ANOVA + Bonferroni's multiple comparisons test.



Supplementary Figure 8. Microglial expression upon α-synuclein burden in CNTNAP4$^{+/-}$ mice. (**a**) Immunofluorescence staining of Iba1-merging TH cells in the SNpc of WT and CNTNAP4$^{+/-}$ mice injected with either AAV-GFP or AAV-*h*α-Syn. (**b**) Quantification of the interaction area of Iba1 and TH cells. Scale bars, 40 μm. Magnified images are shown in the right column of panel a. Scale bars, 8 μm. N = 5–7. Results are expressed as mean ± SEM. $^{*}p < 0.05$ vs. WT; $^{\#}p < 0.05$ vs. AAV-*h*α-Syn; $^{\&}p < 0.05$ vs. CNTNAP4$^{+/-}$. Statistical significance was determined using two-way ANOVA + Bonferroni's multiple comparisons test.



Supplementary Figure 9. Effect of α-synuclein burden on the serum cytokine expression in *CNTNAP4* partial deficient mice. (**a**–**i**) Serum expression levels of IL-2, TNF-α, IL-5, IL-21 p40, IL-21 p70, IL-1α, IL-10, IL-17A, and IFN-γ were determined using Mouse Magnetic Luminex Assay. N = 8, 8, 8, and 6 in the WT + AAV-GFP, WT + AAV-*h*α-Syn, CNTNAP4$^{+/-}$ + AAV-GFP, and CNTNAP4$^{+/-}$ + AAV-*h*α-Syn groups, respectively. Results are expressed as mean ± SEM. $^{**}p < 0.01$, $^{*}p < 0.05$ vs. WT. Statistical significance was determined using two-way ANOVA + Bonferroni's multiple comparisons test.



Supplementary Figure 10. Serum cytokine expression levels by α-synuclein burden in CNTNAP4$^{+/-}$ mice. (**a**–**j**) Serum expression levels of IL-4, IL-9, MCP-1, IL-13, GM-CSF, Eotaxin, MIP-1α, MIP-1β, RANTES, and KC were determined using Mouse Magnetic Luminex Assay. N = 8, 8, 8, and 6 in the WT + AAV-GFP, WT + AAV-*h*α-Syn, CNTNAP4$^{+/-}$ + AAV-GFP, and CNTNAP4$^{+/-}$ + AAV-*h*α-Syn groups, respectively. Results are expressed as mean ± SEM. Statistical significance was determined using two-way ANOVA + Bonferroni's multiple comparisons test.