

Supplemental information:

Synaptic vesicle endocytosis deficits underlie cognitive dysfunction in mouse models of *GBA*-linked Parkinson's disease and Dementia with Lewy bodies

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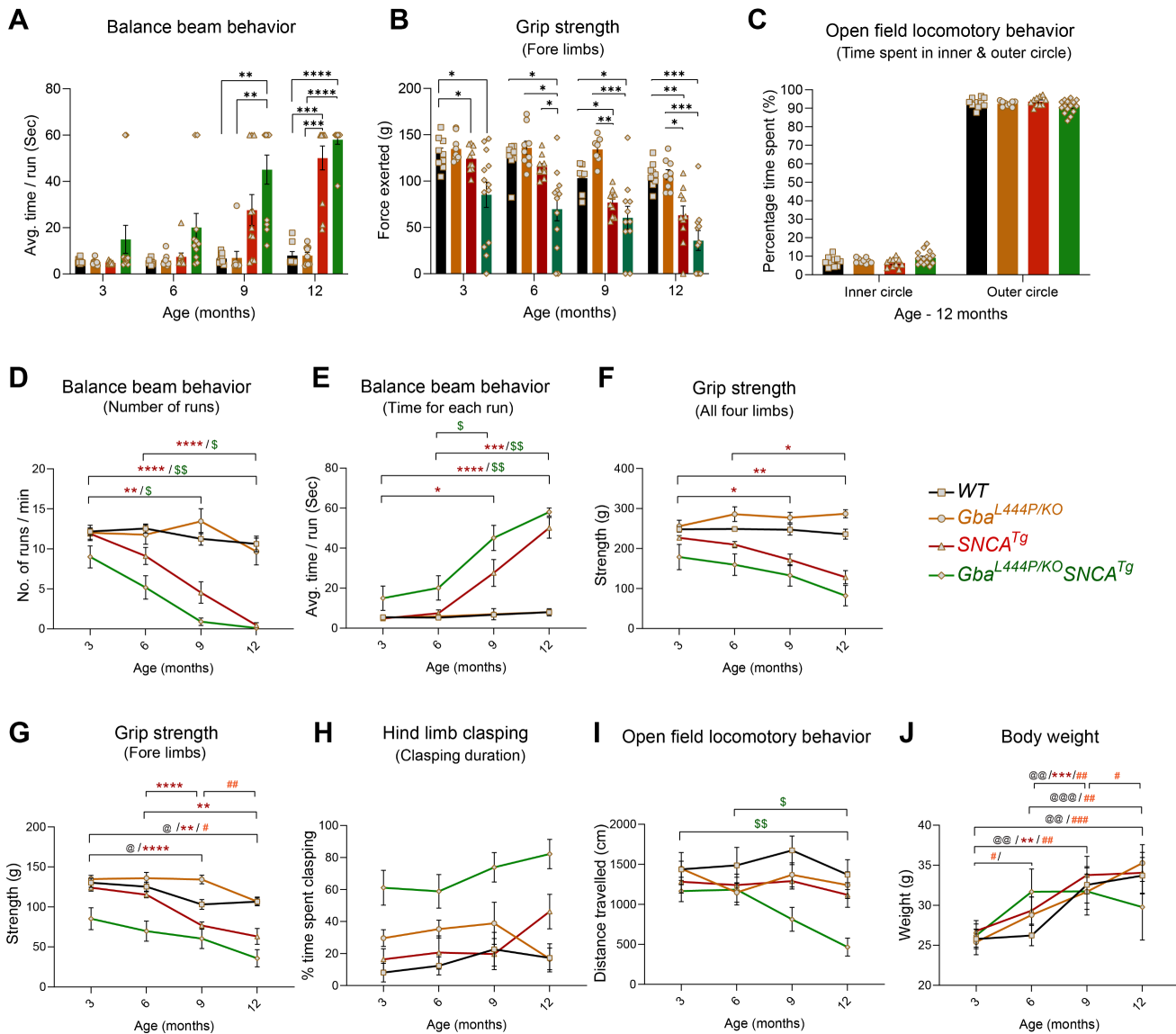
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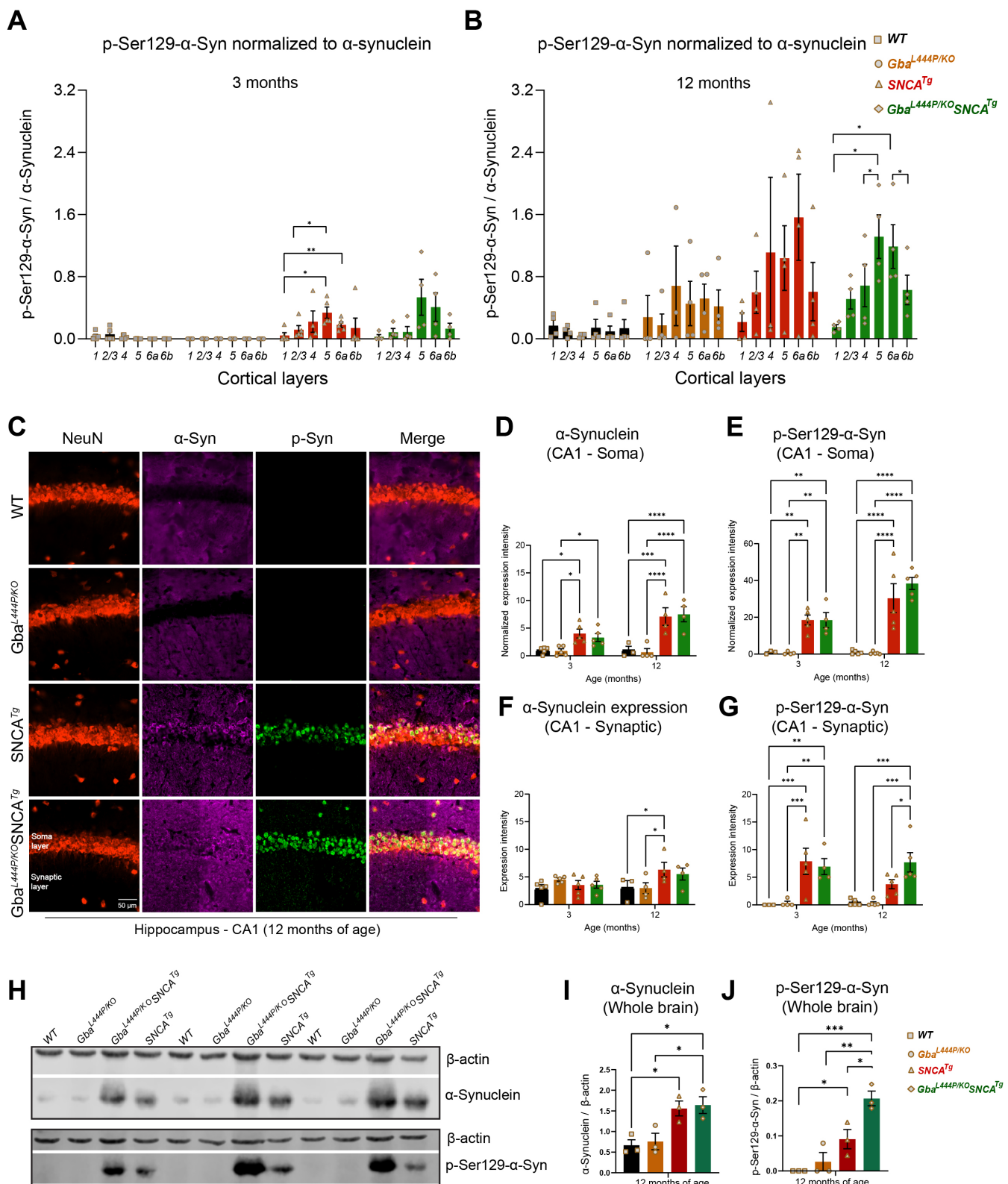
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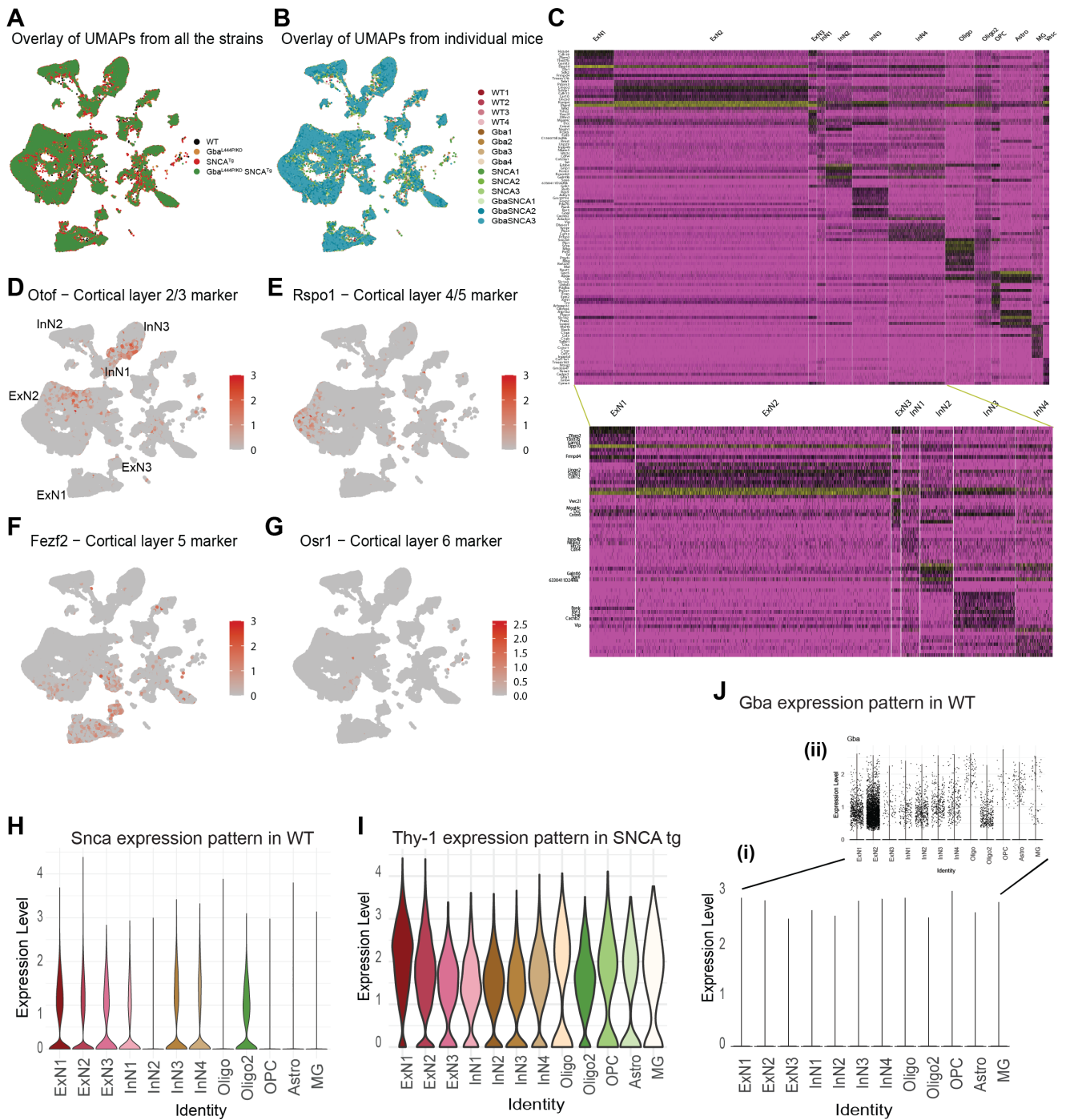


Supplementary Figure 1. Age-related progressive worsening of motor behavior deficits in SNCA tg and Gba-SNCA mice. Longitudinal motor behavior evaluations for WT, Gba, SNCA tg, and Gba-SNCA double mutant mice across 3, 6, 9 and 12 months of age. **A.** Balance beam behavior test showing time taken per each run. **B.** Grip strength of forelimbs. **C.** Time spent in inner and outer circle of an open field as a measure of anxiety-like behavior at 12 months of age. **D.** Balance beam behavior test showing age-related changes in number of runs per minute. **E.** Balance beam behavior test showing age-related changes in time taken per each run. **F.** Age related changes in grip strength (all four limbs). **G.** Age related changes in forelimb grip strength. **H.** Age related changes in hind limb clasp. **I.** Age related changes in open field behavior. **J.** Age related changes in body weight. n = 9-12 mice, sex-balanced cohorts. For **statistics**, two-way repeated measure ANOVA followed by Sidak's multiple comparison test was used for all the comparisons except for **C**. One-way ANOVA followed by Tukey's multiple comparison test was used for **C**. Data are presented as mean ± SEM. @ represent p-value in WT, # represent p-value in Gba mice, * represent p-value in SNCA tg mice, and \$ represent p-value in Gba-SNCA double mutant mice. @p < 0.05, @@p < 0.01, @@@p < 0.001, @@@@p < 0.0001, #p < 0.05, ##p < 0.01, ###p < 0.001, ####p < 0.0001, *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 and, \$p < 0.05, \$\$p < 0.01.

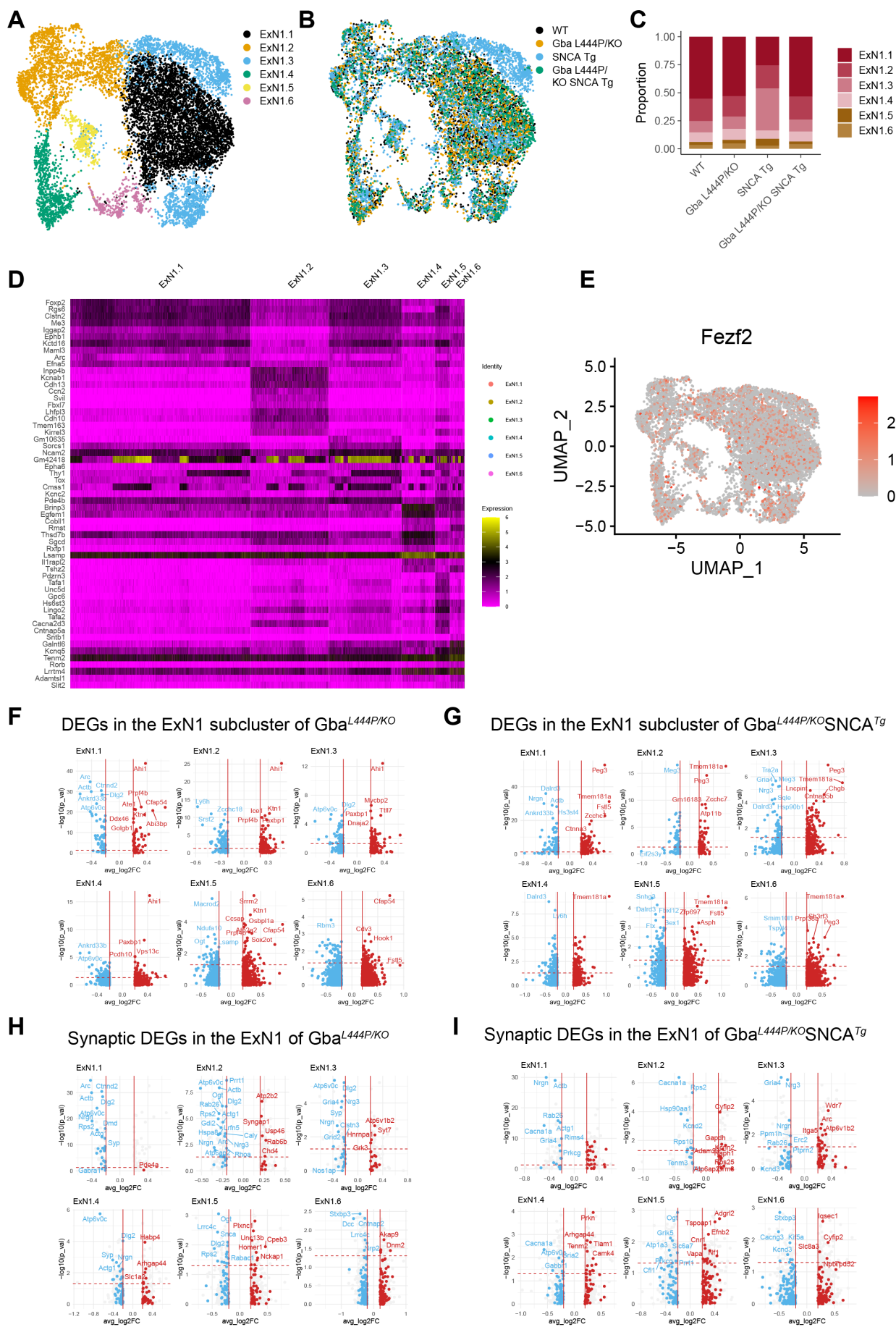


Supplementary Figure 2. α -Synuclein and pSer129- α -syn expression in cortex, hippocampus, and whole brain. **A.** Cortical layer specific pSer129- α -syn expression normalized to α -synuclein expression at 3 months of age. **B.** Cortical layer specific pSer129- α -syn expression normalized to α -synuclein expression at 12 months of age. **C.** Representative images showing α -synuclein and pSer129- α -syn expression in the CA1 subregion of the

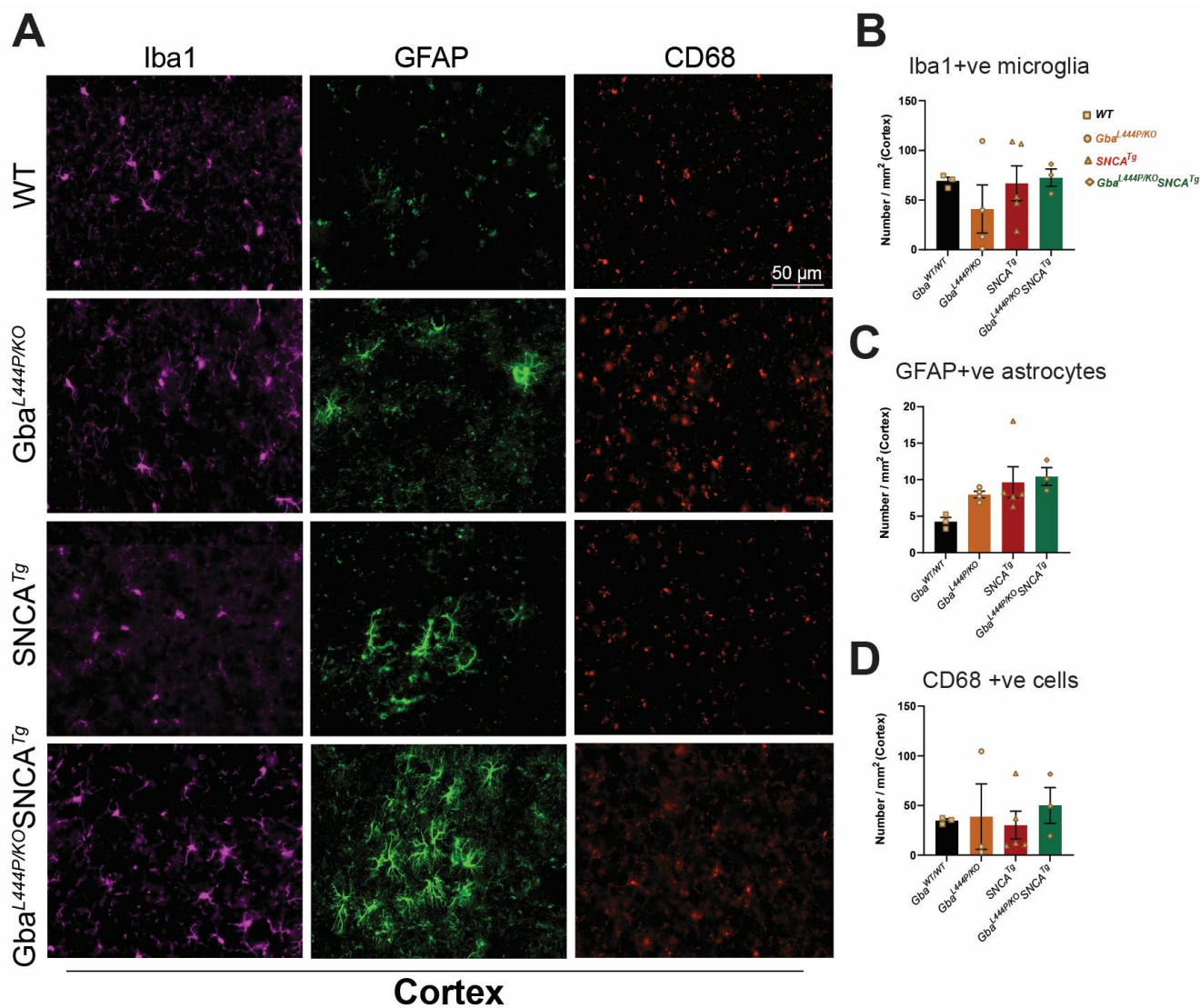
hippocampus in 12 month old mice. Scale = 50 μ m. **D.** Quantification of α -synuclein expression in the soma layer of CA1 hippocampus. **E.** Quantification of pSer129- α -syn expression in the soma layer of CA1 hippocampus. **F.** Quantification of α -synuclein expression in the synaptic layers of CA1 hippocampus. **G.** Quantification of pSer129- α -syn expression in the synaptic layers of CA1 hippocampus. **H.** Western blot showing α -synuclein and pSer129- α -syn expression in the whole-brain homogenates of WT, Gba mutant, SNCA tg, and Gba-SNCA tg mice at 12 months of age. Original blots are provided in the Supplementary Figure 7. **I.** Quantification of western blots for α -synuclein levels in whole brain. **J.** Quantifications of western blots for pSer129- α -syn levels in the whole brain. n = 3 -5 mice, equal number of males and females were used. One-way ANOVA followed by Tukey's's multiple comparison test was used for statistics. "*" indicates p-value. Data are presented as mean $\pm$ SEM. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.



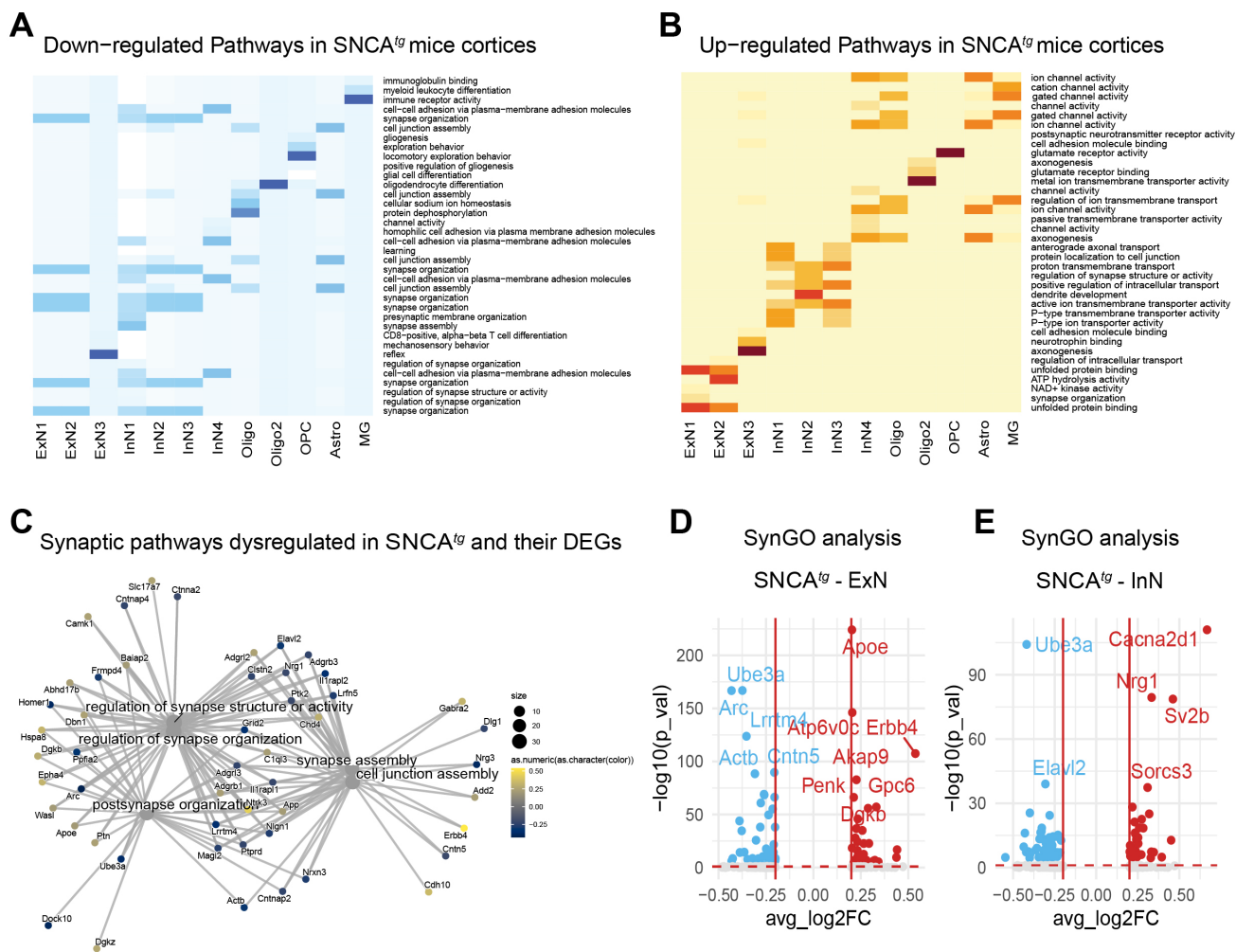
Supplementary Figure 3. UMAPs and expression of marker genes. **A.** Overlay of UMAPs for WT, Gba, SNCA tg, and Gba-SNCA showing lack of large-scale differences due to genotype. **B.** UMAPs for individual WT, Gba, SNCA tg, Gba-SNCA cortical samples. **C.** Cell type markers for the major clusters. **D-G.** Cortical layer specific markers overlaid over UMAPs. Note, that Fezf2, the marker for Layer 5 neurons, labels ExN1 neuronal subcluster. **H.** Violin plots for expression of Snca in WT for denoted cell types. **I.** Violin plots for expression of Thy-1 in SNCA tg for denoted cell types. **J.** Violin (i) and dot (ii) plots for expression of Gba in WT for denoted cell types. n = 4 for WT and Gba mice, and n = 3 for Gba-SNCA and SNCA tg mice.



Supplementary Figure 4: Cortical transcriptomic signatures of ExN1. **A.** UMAP showing the six subclusters found in ExN1. **B.** Overlay of the six subclusters of ExN1 in WT, Gba, SNCA tg, and Gba-SNCA samples. **C.** Proportion of cells in each ExN subcluster in the four genotypes. **D.** Heatmap showing characteristic cell type marker expression in the subclusters of ExN1. Note that high Arc expression is a characterizing marker trait of the largest subcluster ExN1.1. **E.** UMAP showing layer 5 marker Fezf2 expression pattern across ExN1 subclusters. **F.** DEGs found in the subclusters of ExN1 in Gba mice. **G.** DEGs found in the subclusters of ExN1 in Gba-SNCA mice. **H.** SynGO analysis showing synapse associated DEGs in ExN1 subclusters of Gba cortex. **I.** SynGO analysis showing synapse associated DEGs in ExN1 subclusters of Gba-SNCA cortex. Note that among all DEGs in ExN1 (**F** and **G**), DEGs are evenly distributed within up and down regulated DEGs, while the synapse associated DEGs (**H** and **I**) are selectively downregulated in all Gba as well as Gba-SNCA ExN1 subclusters. The downregulated DEGs involved in synapse vesicle cycle pathways were largely the same as shown in the main analyses, covering all cortical clusters in Fig. 4A-B and E-F, but in addition shows *Rab26* to be similarly downregulated in both genotypes (in ExN1.2 and ExN1.1, respectively). n = 4 for WT and Gba mice, and n = 3 for Gba-SNCA and SNCA tg mice.

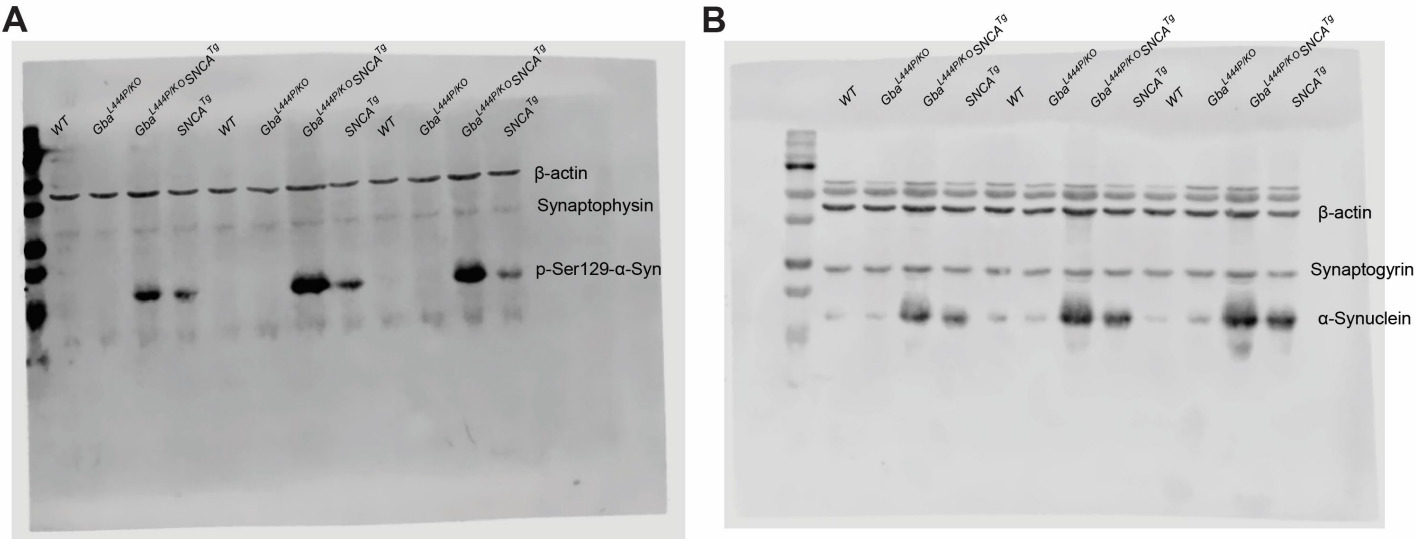


Supplementary Figure 5. Glial signatures in *Gba* and *Gba*-SNCA mice. **A.** Representative images showing cortical expression of Iba1, GFAP, and CD68 in WT, *Gba*, SNCA tg, and *Gba*-SNCA tg mice at 12 months of age. **B.** Number of Iba1 positive microglia in the cortex at 12 months. **C.** Number GFAP positive astrocytes in the cortex at 12 months. **D.** Number of CD68 (activated microglia marker) positive cells in the cortex at 12 months. n = 3 -5 mice, equal number of males and females were used. One-way ANOVA followed by Tukey's's multiple comparison test was used for statistics. "*" indicates p-value. Data are presented as mean ± SEM. Scale = 50 μm.



Supplementary Figure 6. Transcriptional signatures of SNCA tg cortex. A-B, Heatmap showing the most significant cortical gene ontology (GO) biological pathway alterations for each cell type, in 12-month old SNCA tg mice as revealed by unbiased analysis of enrichment of genome-wide corrected DEGs. C, Cnet plot for synapse pathways with associated DEGs in SNCA tg mice. D, SynGO analysis of DEGs in ExN and, E, in InNs of SNCA tg mice. n = 4 for WT and Gba mice, and n = 3 for Gba-SNCA and SNCA tg mice.

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Supplementary Figure 7: Original Western blots of whole brain lysates showing α-synuclein and pSer129-α-synuclein expression. A. pSer129-α-synuclein blotted alongside β-actin and Synaptophysin (Synaptophysin not included in the manuscript). **B.** Total α-synuclein blotted alongside β-actin and Synaptogyrin-3 (Synaptogyrin-3 not included in the manuscript). n = 3 mice/genotype