Supplementary Material

Supplementary Method：

Immunoprecipitation

MO3.13 stable cells expressing Vector-FLAG or α-Syn-FLAG and cerebellar tissues from WT and KI mice were lysed in IP buffer supplemented with protease inhibitors. After centrifugation, equal amounts of protein were used for immunoprecipitation, with aliquots retained as input controls. FLAG-tagged proteins in cell lysates were immunoprecipitated using anti-FLAG magnetic beads, whereas α-synuclein in cerebellar lysates was immunoprecipitated using magnetic beads pre-coated with an anti-α-synuclein antibody. After incubation at 25°C for 1 hour, the beads were washed with IP buffer, and the bound proteins were eluted in SDS sample buffer for western blot analysis.

Supplementary Table 1. Primer sequences used for genotyping of mPlp1-hSNCA knock-in mice.

Primer	Primer set	Sequence (5′–3′)
WT-F	①	AGTCTTTCCCTTGCCTCTGCT
WT-R	①	GGGTCTTCCACCTTTCTTCAG
5’arm-F	②	GGGCAGTCTGGTACTTCCAAGCT
5’arm-R	②	TCTCCATTCTGGCTGGGCTTCT

Primer set 1 (WT-F and WT-R) was used to amplify the WT allele, producing an expected 825-bp product. Primer set 2 (5′arm-F and 5′arm-R) was used to amplify the 5′ junction of the mPlp1-hSNCA knock-in allele, producing an expected 390-bp product. All primer sequences are presented in the 5′-to-3′ direction.

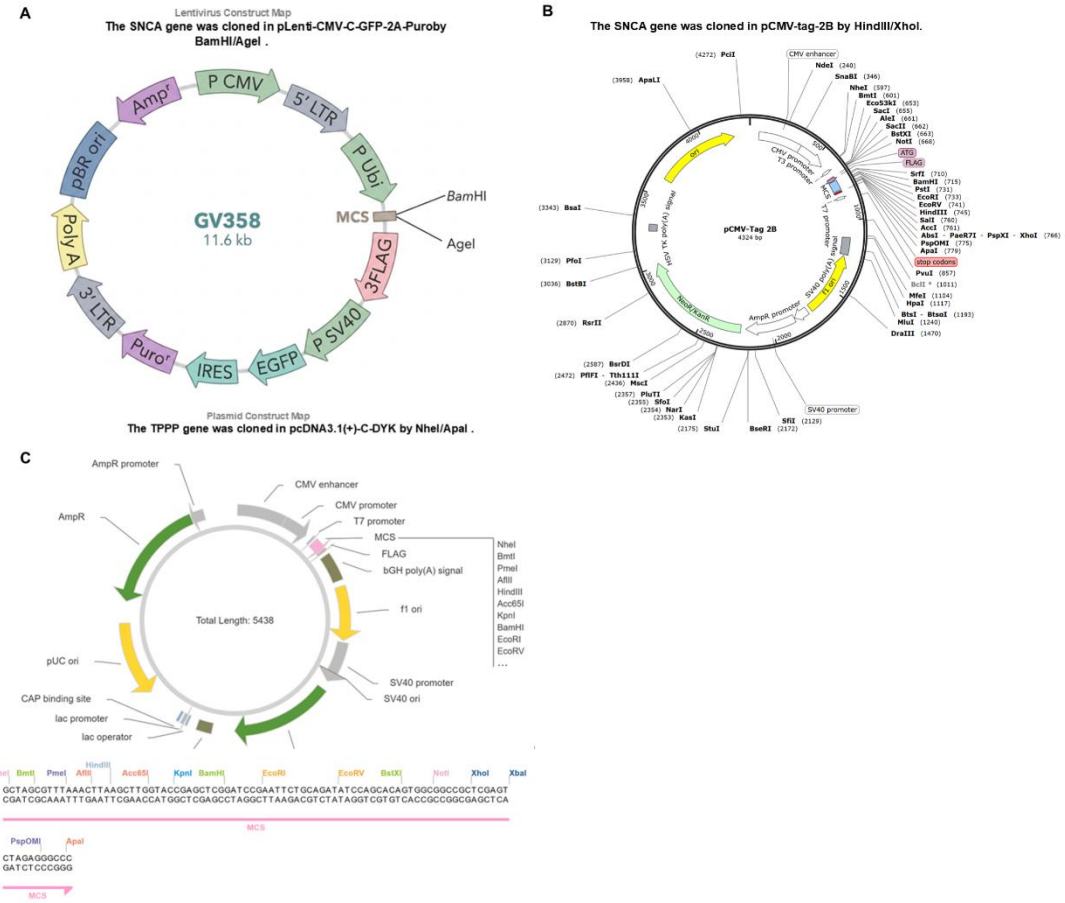
Abbreviations: bp, base pair; F, forward primer; KI, knock-in; R, reverse primer; WT, wild type.

Supplementary Table2. Full antibody information

Antibody	Supplier	Catalog no.	IF/IHC dilution	WB dilution
Alexa Fluor 555	Invitrogen	A-21428	1:500	-
Alexa Fluor 647	Invitrogen	A-21245	1:500	-
Calbindin-D28K	Proteintech	14479-1-AP	1:200	-
CNPase	Abcam	ab6319	1:200	-
GAPDH	Proteintech	60004-1-Ig	-	1:2000
GFAP	Proteintech	60190-1-Ig	1:200	-
Goat anti mouse IgG HRP	Invitrogen	31430	-	1:10000
Goat anti Rabbit IgG HRP	Invitrogen	31460	-	1:10000
IBA1	Proteintech	10904-1-AP	1:200	-
OGA	ABclonal	A24124	-	1:1000
OGT	ABclonal	A3501	-	1:1000
pSer129-α-Synuclein	CellSignaling Technology	23706	1:100	1:1000
RL2	Abcam	ab2739	1:200	1:1000
TH	SantaCruz	Sc-25269	1:200	-
α-Synuclein	PTM BIO	PTM-6345	1:100	1:1000
β-actin	Proteintech	66009-1-Ig	-	1:2000

The primary and secondary antibodies used for immunofluorescence (IF), immunohistochemistry (IHC), and western blotting (WB), together with their suppliers, catalog numbers, and working dilutions, are listed below. "- indicates that the antibody was not used for the corresponding application.

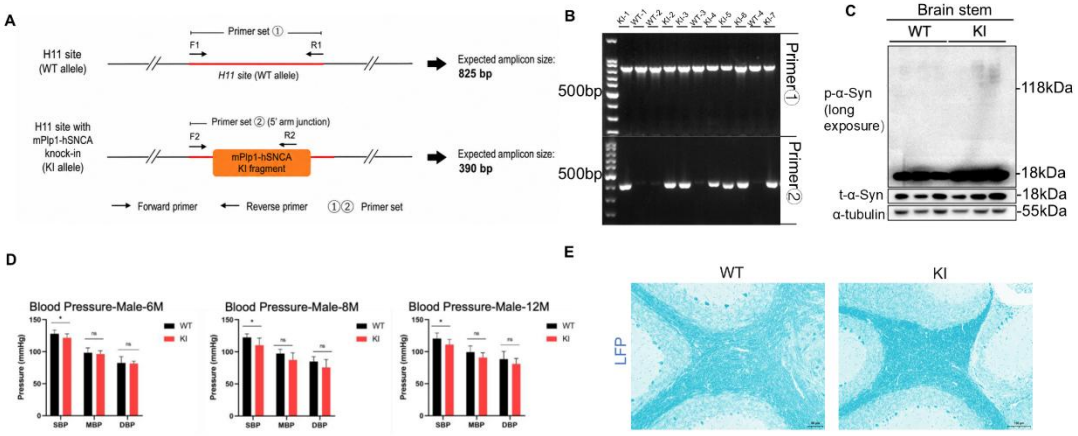
Abbreviations: CD28K, calbindin-D28K; CNPase, 2′,3′-cyclic nucleotide 3′-phosphodiesterase; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; GFAP, glial fibrillary acidic protein; HRP, horseradish peroxidase; IBA1, ionized calcium-binding adaptor molecule 1; IF, immunofluorescence; IHC, immunohistochemistry; OGA, O-GlcNAcase; OGT, O-GlcNAc transferase; pSer129-α-synuclein, α-synuclein phosphorylated at serine 129; RL2, monoclonal antibody recognizing O-linked β-N-acetylglucosamine (O-GlcNAc); TH, tyrosine hydroxylase; WB, western blotting.



Supplementary Figure 1. Construction of SNCA- and TPPP-expressing vectors.
(A) Schematic map of the lentiviral vector used to generate SNCA-expressing stable cells.

(B) Schematic map of the pCMV-tag-2B plasmid used for TPPP expression. The human *SNCA* sequence was inserted into the vector using the *HindIII* and *XhoI* restriction sites.

(C) Schematic map of the pcDNA3.1(+)-C-DYK plasmid used for TPPP expression. The human *TPPP* sequence was cloned into the vector using the *NheI* and *ApaI* restriction sites.



Supplementary Figure 2. Genotyping and preliminary characterization of MSA mice.

(A) Schematic representation of the PCR strategy used to distinguish wild-type (WT) and knock-in (KI) alleles at the H11 locus.

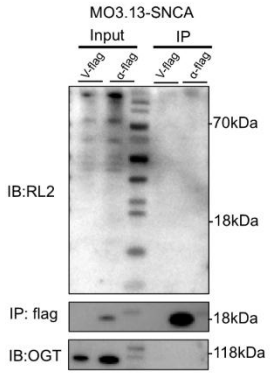
(B) Representative agarose gel electrophoresis images showing PCR genotyping results obtained using primer sets 1 and 2.

(C) Representative immunoblots of brainstem lysates from WT and KI mice showing p-α-Syn (long exposure), t-α-Syn, and α-tubulin.

(D) SBP, MBP, and DBP in male WT and KI mice at 6, 8, and 12 months of age.

(E) LFB-stained sections from WT and KI mice. Scale bars are indicated in the images. Scale bars, 100μm

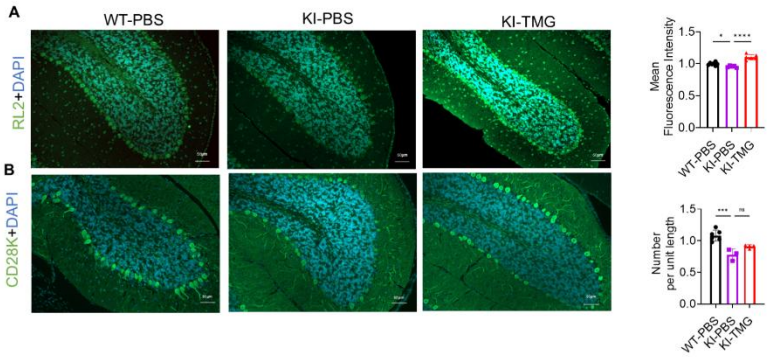
Abbreviations: p-α-Syn: phosphorylated α-synuclein; t-α-Syn: total α-synuclein SBP: Systolic blood pressure; MBP: mean blood pressure; DBP: diastolic blood pressure; LPB: Luxol fast blue.



Supplementary Figure 3. Co-immunoprecipitation analysis of α-synuclein O-GlcNAcylation and its association with OGT.

Cell lysates were incubated with anti-FLAG magnetic beads to immunoprecipitate FLAG-tagged α-syn in MO3.13-SNCA stable lines. Input lysates and immunoprecipitates were analyzed by immunoblotting with the RL2 antibody to assess the O-GlcNAcylation of immunoprecipitated α-synuclein and with an anti-OGT antibody to evaluate the association between α-synuclein and OGT. Immunoblotting with an anti-FLAG antibody confirmed the successful immunoprecipitation of α-Syn-FLAG.

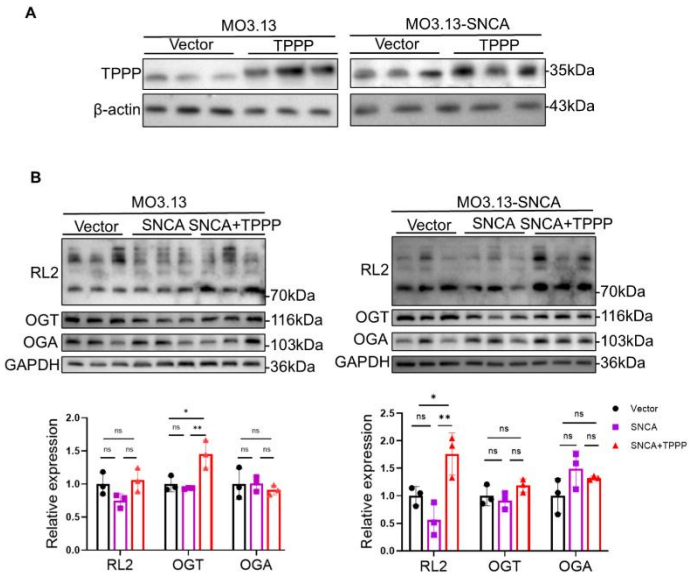
Abbreviations: IB, immunoblotting; IP, immunoprecipitation; OGT, O-GlcNAc transferase; RL2, an antibody recognizing O-linked β-N-acetylglucosamine; α-Syn, α-synuclein；α-Flag, α-Syn-FLAG; v-Flag, vector-Flag.



Supplementary Figure 4. Effects of TMG treatment on O-GlcNAcylation and Purkinje cells in the cerebellum of mPlp1-hSNCA knock-in mice.

(A) Representative immunofluorescence images of cerebellar sections from WT mice treated with PBS (WT-PBS), KI mice treated with PBS (KI-PBS), and KI mice treated with TMG (KI-TMG). Global O-GlcNAcylation was detected using the RL2 antibody (green), and nuclei were counterstained with DAPI (blue). Scale bars, 50 μm.

(B) Representative immunofluorescence images of calbindin-positive Purkinje cells in the cerebellum. Purkinje cells were labeled with an anti-calbindin-D28K antibody (CD28K; green), and nuclei were counterstained with DAPI (blue). Scale bars, 50 μm. Data are presented as the mean ± SEM from independent experiments. Each point represents one independent biological replicate. **P*<0.05, ***P*<0.01, ****P*<0.001, and *****P*<0.0001; ns, not significant.



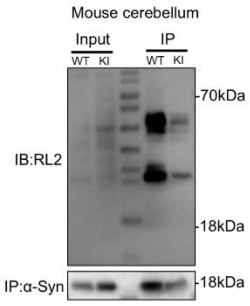
Supplementary Figure 5. Effects of SNCA and TPPP expression on cellular O-GlcNAc homeostasis.

(A) Representative immunoblots confirming TPPP expression in parental MO3.13 and MO3.13-SNCA cells transfected with the TPPP expression plasmid or empty vector.

(B) Representative immunoblots and quantitative analyses of global O-GlcNAcylation, OGT, an OGA expression in parental MO3.13 cells and MO3.13-SNCA cells following transfection with vector, SNCA, or SNCA plus TPPP.

Data are presented as the mean ± SEM from independent experiments. Each point represents one independent biological replicate. **P*<0.05, ***P*<0.01, ****P*<0.001, and *****P*<0.0001; ns, not significant.

Abbreviations: OGT, O-GlcNAc transferase; RL2, an antibody recognizing O-linked β-N-acetylglucosamine.



Supplementary Figure 6. Immunoprecipitation analysis of α-synuclein O-GlcNAcylation in the cerebellum of WT and KI mice.

Cerebellar tissue lysates from WT and KI mice were incubated with anti-α-synuclein magnetic beads to immunoprecipitate α-synuclein. Input lysates and immunoprecipitates were analyzed by immunoblotting with the RL2 antibody to assess the O-GlcNAcylation of immunoprecipitated α-synuclein. Immunoblotting with an anti-α-synuclein antibody confirmed the successful immunoprecipitation of α-synuclein.

Abbreviations: IB, immunoblotting; IP, immunoprecipitation; KI, knock-in; RL2, an antibody recognizing O-linked β-N-acetylglucosamine; WT, wild type; α-Syn, α-synuclein.