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Supplementary Materials for

Intronic enhancers of the human *SNCA* gene predominantly regulate its expression in brain in vivo

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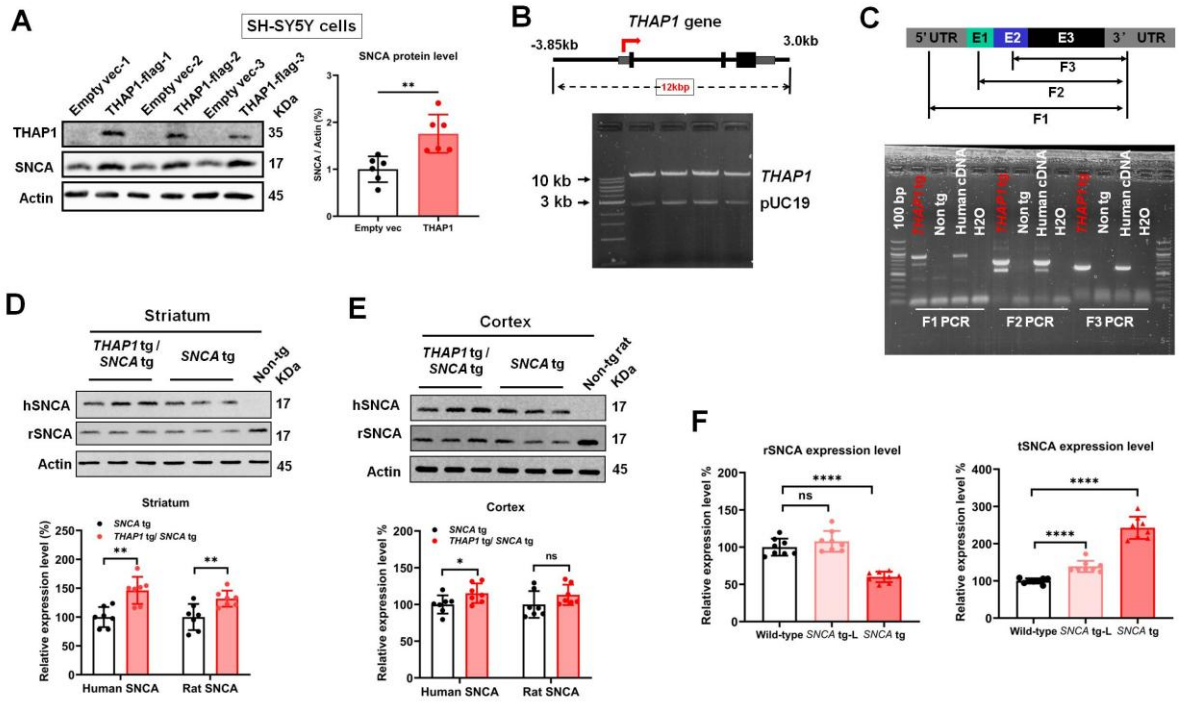

Fig. S1. THAP1 regulates the expression of SNCA *in vitro* and *in vivo*. (A) Western blotting and statistical analysis show the SNCA protein level in THAP1 overexpressed SH-SY5Y cells (THAP1-flag). (B) Strategy to clone the whole human *THAP1* gene (12 kb), including upstream (-3.85 kb) and downstream (3.0 kb) regions of this gene, into pUC19 vector (upper). Restriction enzyme digestion and electrophoresis analysis validate the positive clones (bottom). (C) Reverse-transcript PCR analyzes the expression of *THAP1* mRNA in *THAP1* tg rats. Fragment 1 (F1) and fragment 2 (F2) RT-PCR products both present two bands, because *THAP1* gene has an exon 2 skipping transcripts. (D and E) Western blotting (top) and statistical analysis (bottom) determine the protein level of human SNCA (hSNCA) and rat SNCA (rSNCA) in striatum (D) and cortex (E) of human *THAP1* and human *SNCA* double transgenic rats (*THAP1* tg/ *SNCA* tg), *SNCA* transgenic rats (*SNCA* tg), and non-transgenic wild-type rats (Non-tg). (F) Statistical analysis shows the expression changes of rSNCA and total SNCA (tSNCA) in cortex of wild-type, *SNCA* tg-L, and *SNCA* tg rats. (*: $p<0.05$; **: $p<0.01$; ****: $p<0.0001$. Comparison by unpaired two-tailed *t* test)

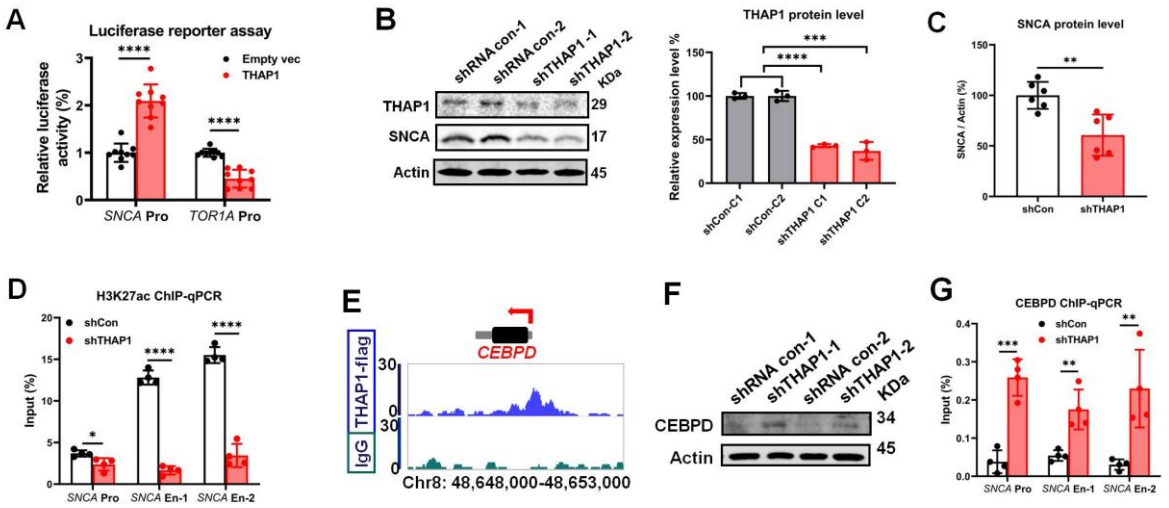

Fig. S2. THAP1 regulates SNCA expression through both directly and indirectly pathways. (A) Luciferase reporter assays analyze the role of THAP1 in regulating *SNCA* promoter and *TOR1A* promoter activities. The *SNCA* promoter (*SNCA* pro, 10.7 kb) or *TOR1A* promoter (*TOR1A* pro) were co-transfected with vector with wild-type *THAP1* cDNA into SH-SY5Y cells, respectively, to determine their regulative interaction. *TOR1A* pro was used as control, as THAP1 can repress its activity. (B) Western blotting and statistical analysis determine the protein level of THAP1 and SNCA in THAP1 knock-down cell lines (shTHAP1) and control cell lines (shCon). (C) Statistical analysis of the SNCA protein level in shTHAP cell lines compared to shCon cell lines. (D) H3K27ac ChIP-qPCR analyze the H3K27ac modification on *SNCA* promoter (SNCA pro) and enhancers (*SNCA* En-1: *SNCA* enhancer 1 region; *SNCA* En-2: *SNCA* enhancer 2 region) in shTHAP1 cell lines and shCon cell lines. (E) Anti-flag ChIP-seq data (using anti-flag antibody and THAP1-flag overexpression cell lines) detect the THAP1 binding site on *CEBPD* gene. (F) Western blotting analyzes the CEBPD protein level in shTHAP1 cells and shCon cells (left). (G) CEPBD ChIP-qPCR shows its binding activities on *SNCA* promoter (*SNCA* pro) and enhancers (*SNCA* En-1 and *SNCA* En-2) in shTHAP1 cell lines and shCon cell lines. (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$; ****: $p<0.0001$. Comparison by unpaired two-tailed *t* test)

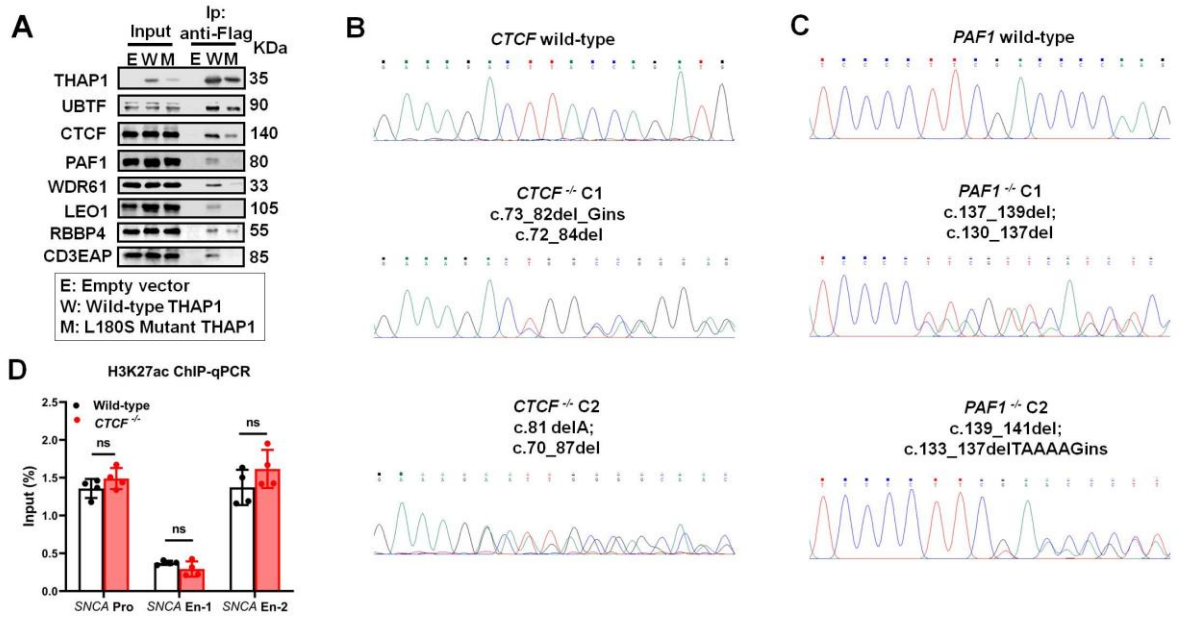

Fig. S3. Identify the interaction partners of THAP1 and their role in regulating SNCA expression. (A) Western blot analyses determine the interactions between THAP1 and some of its potential interaction partners isolated by Tandem Affinity Purification and Mass Spectrometry analysis (TAP-MS). (B) Sanger sequencing confirms two *CTCF* knock-out SH-SY5Y clones (*CTCF*$^{-/-}$ C1 and *CTCF*$^{-/-}$ C2). (C) Sanger sequencing confirms two *PAF1* knock-out SH-SY5Y clones (*PAF1*$^{-/-}$ C1 and *PAF1*$^{-/-}$ C2). (D) ChIP-qPCR shows the activities of *SNCA* promoter (*SNCA* Pro) and enhancers (*SNCA* En-1 and *SNCA* En-2) in wild-type and *CTCF* knock-out (*CTCF*$^{-/-}$) SH-SY5Y cells.

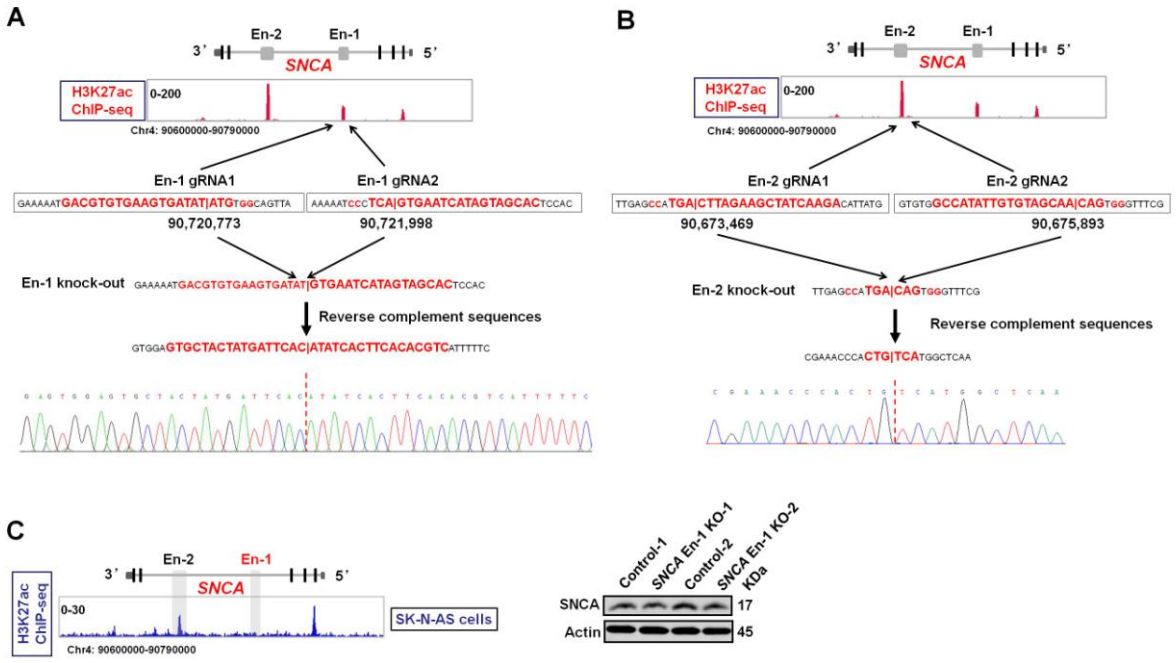

Fig. S4. Characterize the function of *SNCA* enhancers using CRISRP/Cas9 system. (A and B) The strategies and the gRNA sequences used to knock-out of *SNCA* En-1 (A) and En-2 (B) in SH-SY5Y cells (top). Sanger-sequencing determines the positive knock-out clones (bottom). (C) H3K27ac ChIP-seq detected the *SNCA* enhancer and promoter in SK-N-AS neuroblastoma cells. The *SNCA* En-1 region is inactivated in this cell line (Left). Western blotting shows the SNCA protein level in control and *SNCA* En-1 KO SK-N-AS cells (Right).

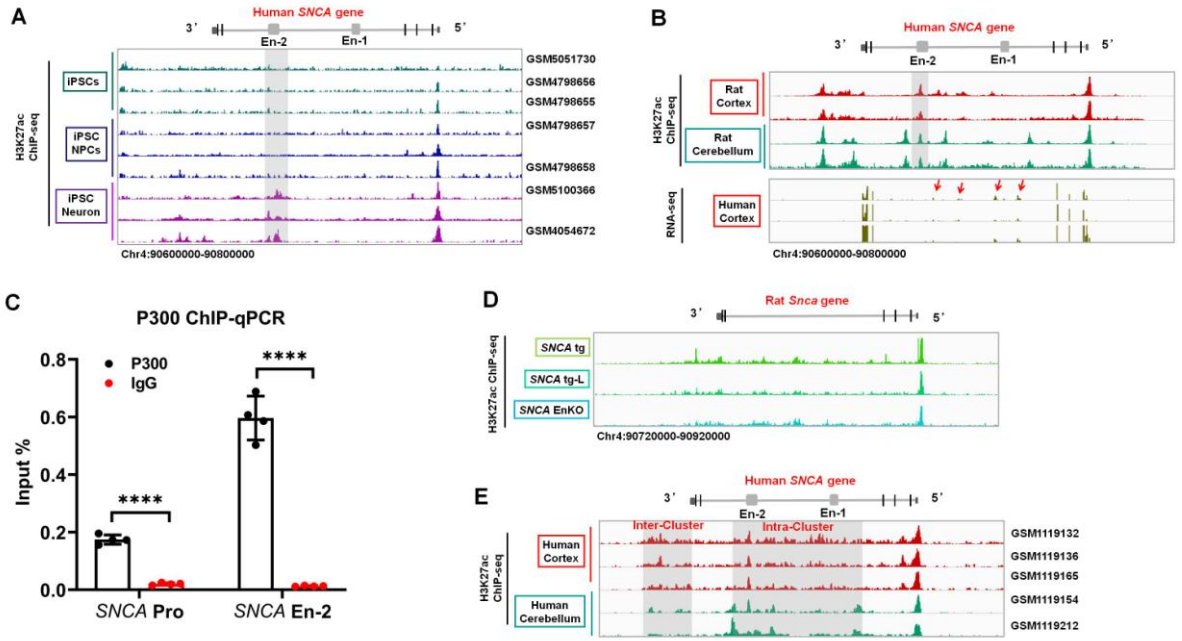

Fig. S5. **Lineage-dependent activation of *SNCA* enhancers in cells and in brain.** (A) The H3K27ac modification status (identified by H3K27ac ChIP-seq) of human *SNCA* gene during neuronal differentiation, including iPSCs, iPSCs derived neural progenitor cells (iPSC-NPCs), and iPSC-derived neurons, like midbrain dopaminergic neurons (iPSC-Neurons). Right side shows the GEO accession number of the re-used raw data. (B) The H3K27ac modification status of human *SNCA* gene in cortex and cerebellum of *SNCA* tg rats and *SNCA* tg-L rats (top). RNA-seq analysis of total RNA isolated from human frontal cortex showed potential enhancer RNA (eRNA) (bottom). (Red arrows indicate the expression of eRNA). Grey squares indicate the enhancer region of *SNCA* En-2. (C) ChIP-qPCR and statistical analyses show the P300 binding activities on *SNCA* promoter and En-2 in *SNCA* tg rat cortex. (D) ChIP-seq shows the H3K27ac modification on rat *Snca* gene in cortex of *SNCA* tg rats, *SNCA* tg-L, and *SNCA* EnKO rats. (E) The status of H3K27ac modification on human *SNCA* gene in human cortex and cerebellum. Grey squares indicate the enhancer clusters of Inter-Cluster (left) and Intra-Cluster (right). (****: $p<0.0001$. Comparison by unpaired two-tailed *t* test)

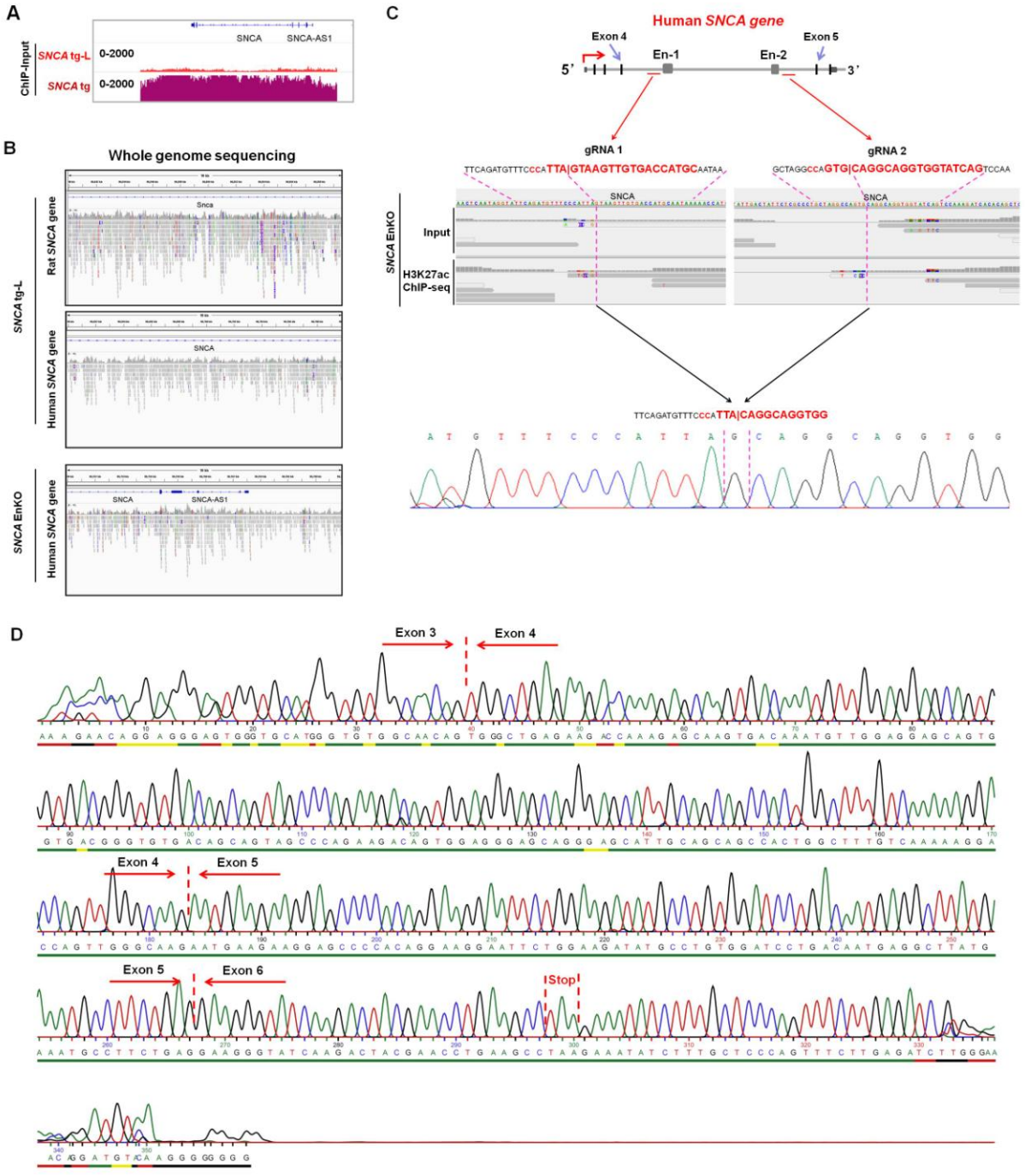

Fig. S6. Generation and validation of the human *SNCA* intronic enhancer clusters knocking-out (*SNCA* EnKO) rat model. (A) IGV browser views the whole genome sequencing raw reads intensity of human *SNCA* gene in *SNCA* tg-L rats and *SNCA* tg rats. (B) IGV browser views the reads intensity of rat *Snca* gene and human *SNCA* gene in *SNCA* tg-L rats (top). IGV gene browser view shows reads intensity of human *SNCA* gene in *SNCA* EnKO rats (bottom). (C) Schematic graph presents the location of gRNA and *SNCA* gene structure (top). IGV browser view the cutting sites generated by CRISPR/Cas9 techniques (middle). Sanger-sequencing confirmed the re-ligation of two residual ends generated by

CRISP/Cas9 system after knocking-out the *SNCA* large intronic enhancer. (D) Sanger-sequencing of hSNCA cDNA PCR product amplified from *SNCA* EnKO cDNA. No aberrant splicing or exon skipping was detected.

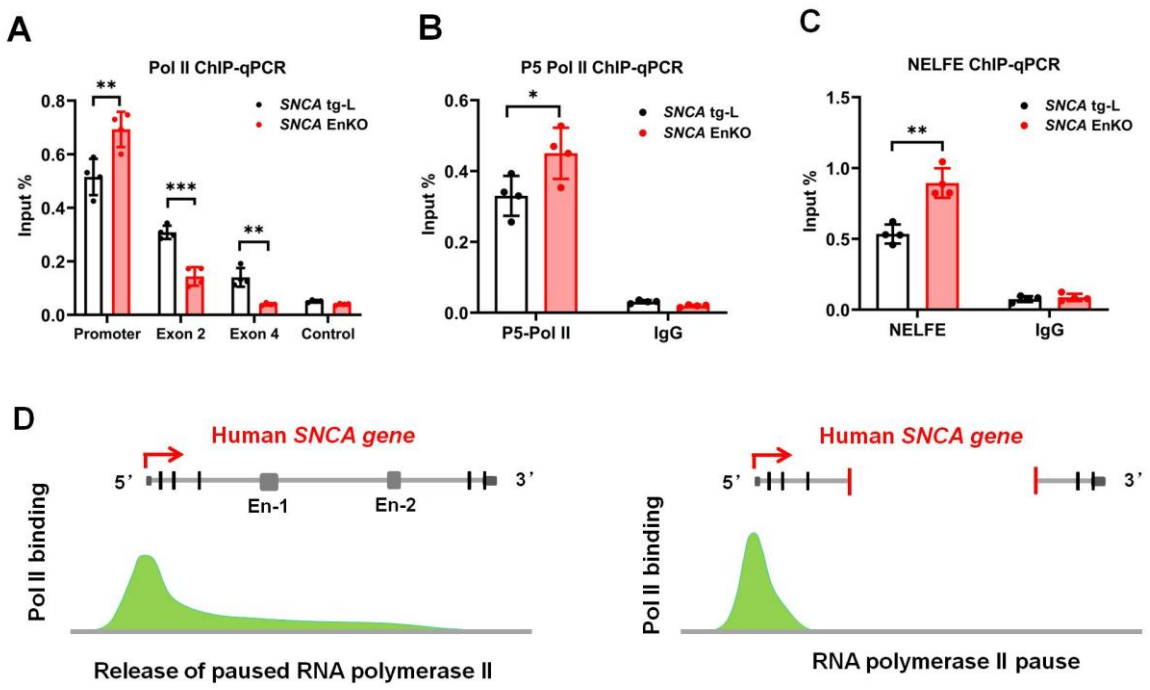

Fig. S7. The human *SNCA* intronic enhancer clusters regulate RNA polymerase II pause. (A) ChIP-qPCR quantitative analyses show the binding activities of polymerase II (Pol II) on the human *SNCA* promoter and different regions of gene body (exon 2 and exon 4) in *SNCA* tg-L and *SNCA* EnKO rat cortex. (Control: unrelated genomic region 10 kb upstream of the *SNCA* gene) (n=4 rats in each group). (B) ChIP-qPCR quantitative analysis shows the binding of phosphorylated (Ser5) polymerase II (P5 Pol II) on the human *SNCA* promoter in *SNCA* tg-L and *SNCA* EnKO rat cortex (n=4 rats in each group). (C) ChIP-qPCR analysis determines the binding activities of negative elongation factor E (NELFE) to the human *SNCA* promoter in *SNCA* tg-L and *SNCA* EnKO rat cortex (n=4 rats in each group). (D) Schematic graphs present the releasing of paused RNA polymerase II on the human *SNCA* gene in *SNCA* tg-L rat cortex (left) and the pausing RNA polymerase II on *SNCA* promoter in *SNCA* EnKO rat cortex (right). (*: $p<0.05$; **: $p<0.01$; ***: $p<0.001$. Comparison by unpaired two-tailed *t* test)

Table S1. List of gRNA sequences and genotype PCR primers.

Targets	gRNA sequences	Positive genotype primers	Negative genotype PCR
CTCF	GAAAGACTTACCAGAGACGC	F: GCCTAATTCATTCACCAAAGGG R: GGTGTGGCATATCATGGGTT	
PAF1	GGTGATGAACTTGGGGTCGA	F: TTCTTCTCCACTAGGCCCAA R: CTCTGGGGAGGAACTCAGAA	
SNCA En-1	1: GTGCTACTATGATTCACTGA 2: GACGTGTGAAGTGATATATG	F: CAAACTCACAGCGTAGACAA R: AATTTCCTGGATGCTCAGTG	F: CAAACTCACAGCGTAGACAA R: AATTTCCTGGATGCTCAGTG
SNCA En-2	1: GCCATATTGTGTAGCAACAG 2: TCTTGATAGCTTCTAAGTCA	F: TTGCATTTGCGTATTTGTGC R: ACTTGGCTGAATCTGAGTCC	F: GGGAATATTGGTGAGCATGG R: GGATCTAAGGACCCTCAACA

Table S2. List of all antibodies used in this study

Target	Sources	Company	Clone / Catalog Nr.	RRID
THAP1	Rabbit	Proteintech	12584-1-AP	AB_2201672
Flag	Mouse	Sigma	F1804	AB_262044
CEBPD	Mouse	Santa Cruz Biotechnology	sc-365546	
CTCF	Rabbit	Cell signaling	D31H2 / 3418	AB_2086791
H3K27ac	Rabbit	Cell signaling	D5E4 / 8173	AB_10949503
H3K4me3	Rabbit	Cell signaling	C42D8 / 9751	AB_2616028
TH	Rabbit	Millipore	657012	AB_696697
Total SNCA	Mouse	BD Biosciences	610786	AB_398107
Human SNCA	Mouse	Cell signaling	Syn204 / 2647	AB_2302251
Rat SNCA	Rabbit	Cell signaling	D37A6 / 4179	AB_1904156
UBTF	Mouse	Santa Cruz Biotechnology	F-9 / sc-13125	AB_671403
PAF1	Rabbit	Cell signaling	D9G9X / 12883	AB_2798052
WDR61	Rabbit	Proteintech	22536-1-AP	AB_11232419
LEO1	Rabbit	Proteintech	12281-1-AP	AB_10640429
CTR9	Rabbit	Cell signaling	D1Z4F / 12619	AB_2797971
Rpb1 CTD	Mouse	Cell signaling	4H8 / 2629	AB_2167468
P5 Rpb1 CTD (Ser5)	Rabbit	Cell signaling	D9N5I / 13523	AB_2798246
P2 Rpb1 CTD (Ser2)	Rabbit	Cell signaling	E1Z3G / 13499	AB_2798238
P300	Rabbit	Cell signaling	E8S2V / 57625	
NELFE	Rabbit	Proteintech	10705-1-AP	AB_513966
Beta-Actin	Mouse	Cell signaling	4967	AB_330288
Normal Rabbit IgG	Rabbit	Cell signaling	2729	AB_1031062
Mouse IgG Isotype	Mouse	Invitrogen	31903	AB_10959891

Table S3. List of primers used for ChIP-qPCR

Regions	Sequences	PCR product (bp)
SNCA promoter	F: GGAAAATGGTGATAGTGGCG; R: TGAGGGTGAAAGGGAGACTA	97
SNCA En-1	F: TTCATCTTCTCCAGGCACAG; R: GGTTACTTTTGTGGGGTGTG	87
SNCA En-2	F: AATAATTGCCGACTGTGACC; R: GTGATGGAGGGAAATCGTGT	96
SNCA Exon 2	F: GCTGAGAAAACCAAACAGGG; R: GAACAAGCACCAAACTGACA	103
SNCA Exon 4	F: GTCAAAAAGGACCAGTTGGG; R: CCAGGCCTCACATGAAAATG	96
SNCA Control	F: GTCTATGCCCTGTTTAGCAATC; R: TCTTACCCTGTAGGACTTTCAT	96