

Supplementary Information

Effects of local reduction of endogenous α -synuclein using antisense oligonucleotides on the fibril-induced propagation of pathology through the neural network in wild-type mice

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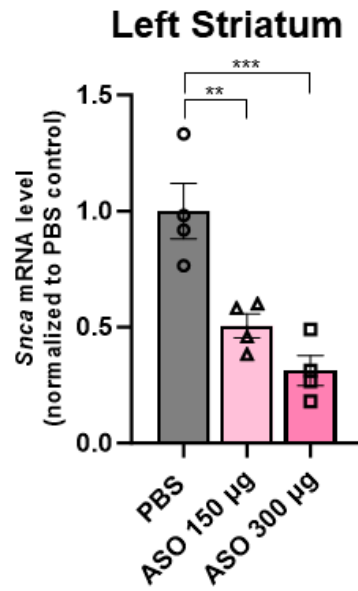
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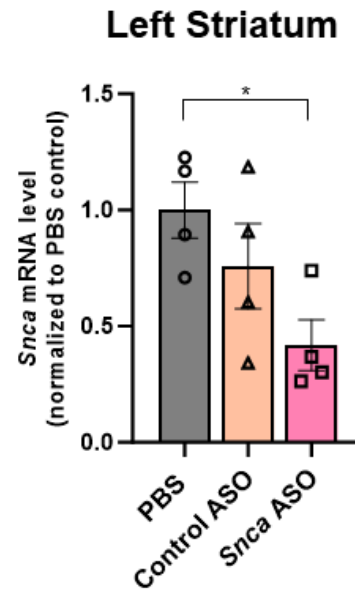
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Supplementary Fig.1

a



b



Supplementary Fig.1 Intrastratial injection of *Snca* ASOs efficiently downregulated the levels of

endogenous *Snca* mRNA. a *Snca* mRNA expression levels from the ipsilateral striatum at 7 days after

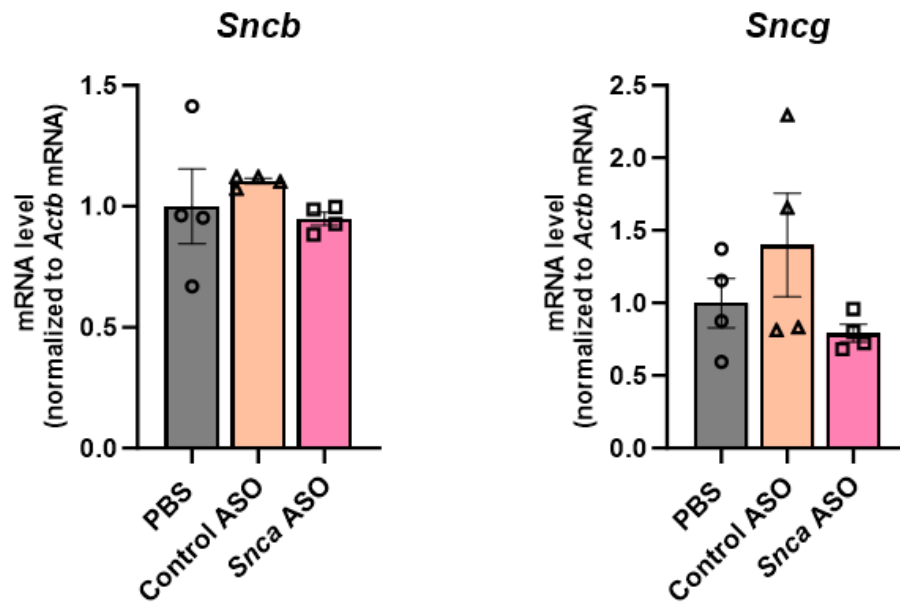
intrastratial injection of PBS, 150 µg *Snca* ASO or 300 µg *Snca* ASO by qRT-PCR (n=4 per each group;

P < 0.01, *P < 0.001). **b** *Snca* mRNA expression levels from the ipsilateral striatum at 14 days after

intrastratial injection of PBS, 300 µg Control ASO or 300 µg *Snca* ASO by qRT-PCR (n=4 per each

group; *P < 0.05). All data are expressed mean ± SEM.

Supplementary Fig.2



Supplementary Fig.2 Intrastratial injection of *Snca* ASOs did not affect the levels of *Snca* and *Sncg*

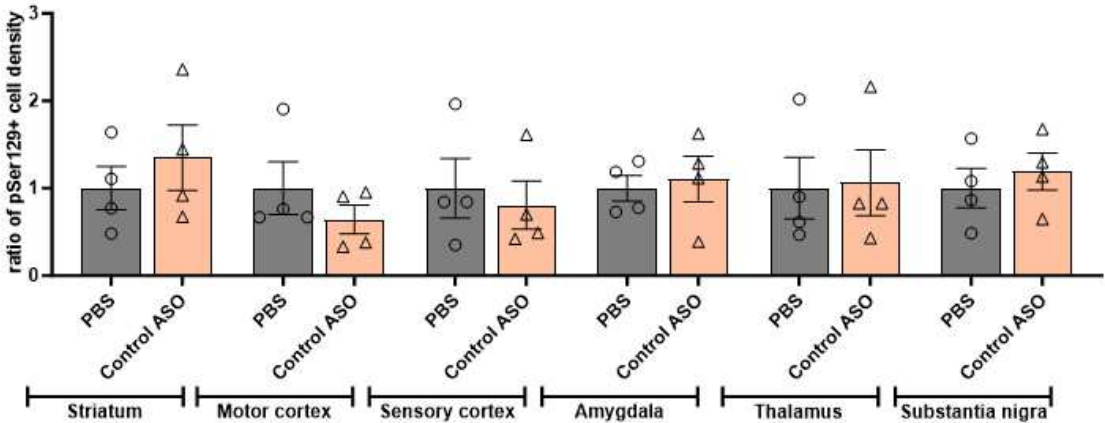
mRNA. *Snca* and *Sncg* mRNA expression levels from the ipsilateral striatum at 14 days after intrastratial injection of PBS, 300 μ g Control ASO, or 300 μ g *Snca* ASO by qRT-PCR (n=4 per each group). All data are expressed mean $\pm$ SEM.

Supplementary Fig.3

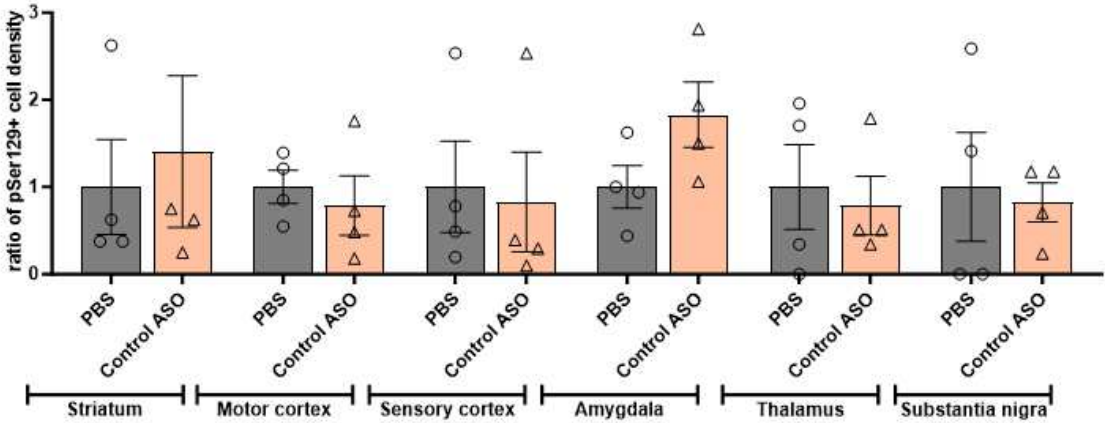
a

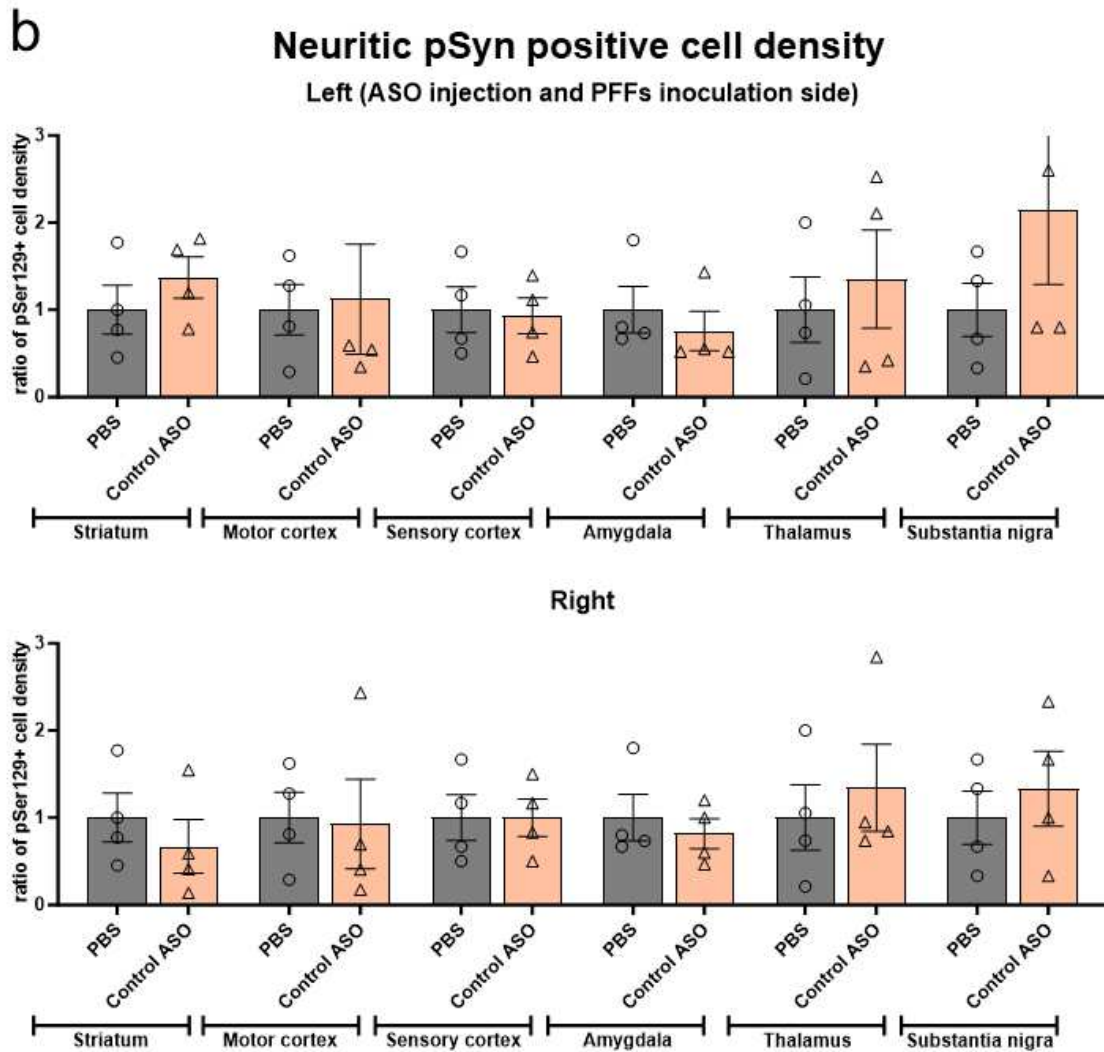
Somatic pSyn positive cell density

Left (ASO injection and PFFs inoculation side)



Right





Supplementary Fig.3 Comparison of pSyn pathologies between the PBS ipsilateral pre-treatment

and the Control ASO ipsilateral pre-treatment groups in WT mice with aSyn PFFs intrastriatal

inoculation. The ratio of cells with pSyn (pSer129) positive somatic (a) and neuritic (b) inclusions

density in the striatum, motor cortex, sensory cortex, amygdala, thalamus, and substantia nigra on each

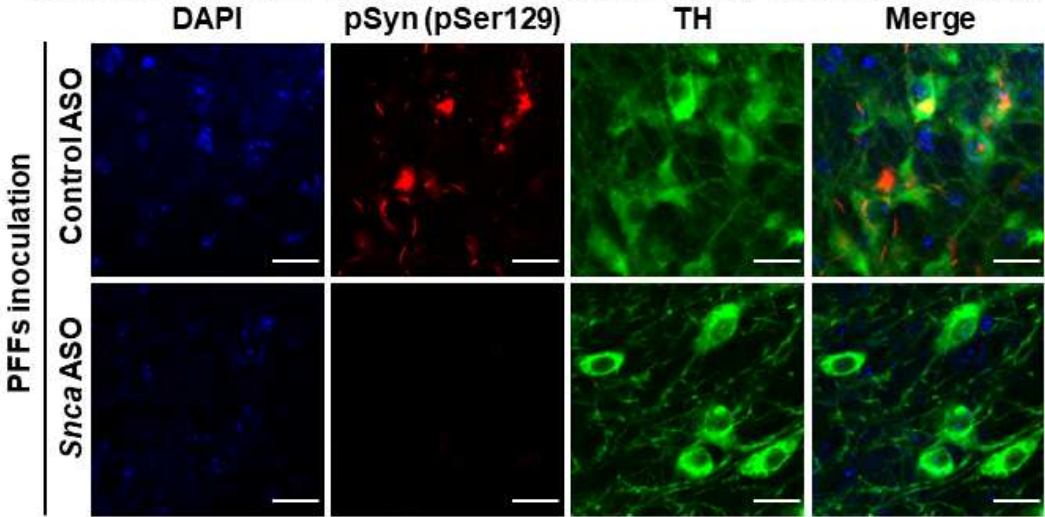
side of the brain (n=4 per each group). The ratio of pSyn (pSer129) positive cell density to the average of

that from the PBS-pre-treated group is shown. The “motor (sensory) cortex” corresponds to the primary

motor (somatosensory) cortex. All data are expressed mean $\pm$ SEM.

Supplementary Fig.4

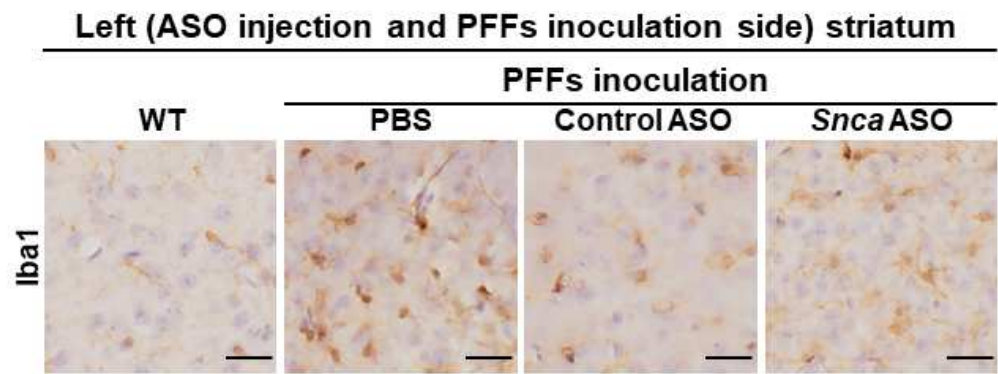
Left (ASO injection and PFFs inoculation side) Substantia nigra



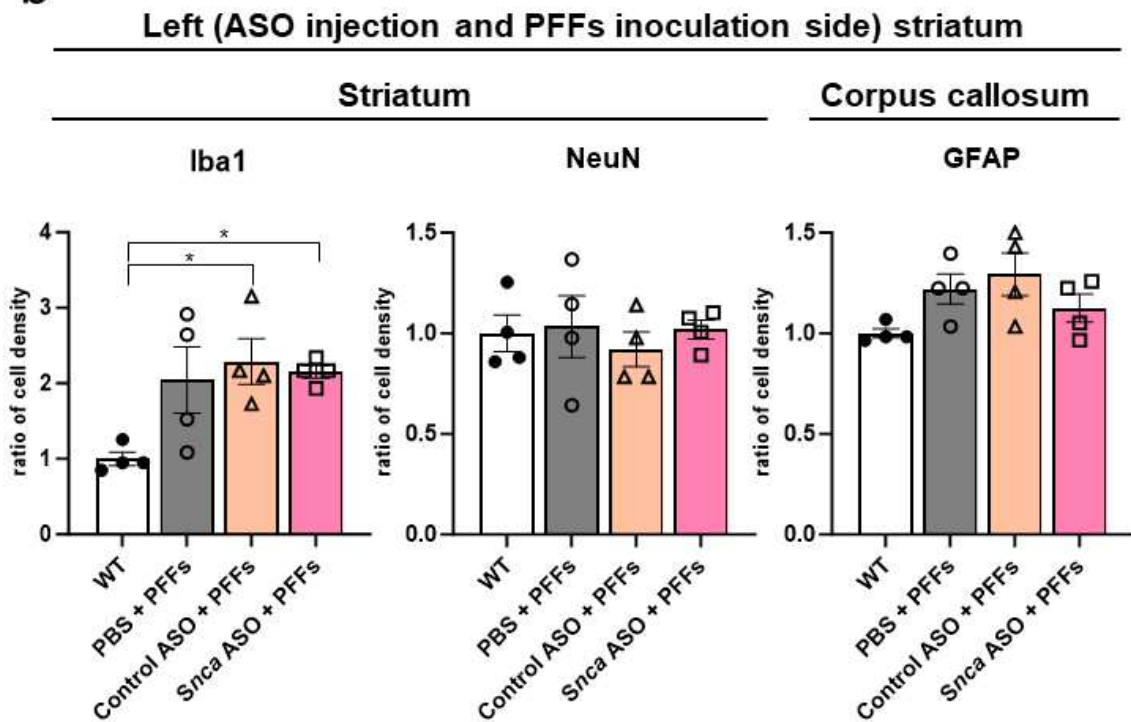
Supplementary Fig.4 Intrastriatal ipsilateral pre-treatment of *Snca* ASOs prevents the appearance of pSyn pathologies in the tyrosine hydroxylase (TH) positive neurons of ipsilateral substantia nigra. Representative images of double-labeled immunofluorescence with pSyn (pSer129) and TH in the left substantia nigra. DNA is stained with DAPI. The scale bar represents 20 μ m.

Supplementary Fig.5

a



b



Supplementary Fig.5 ASO ipsilateral pre-treatment did not affect the cell viability and host

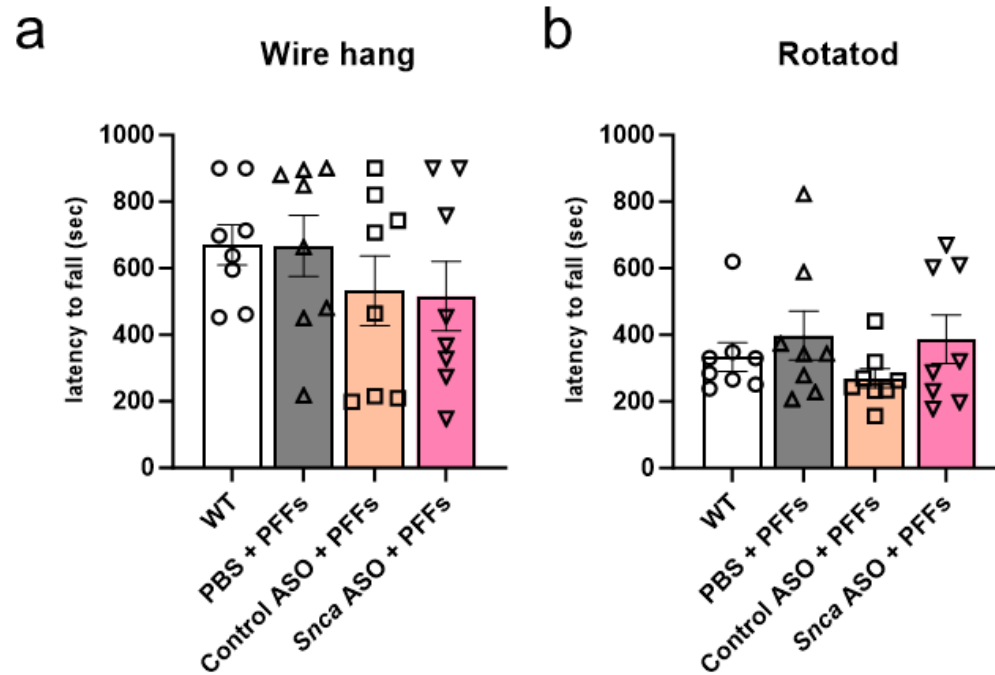
immune response. **a** Representative images of Iba1-positive cells in the left striatum wild-type (WT)

mice 30 days after PFFs inoculation and PBS, Control ASO or *Snca* ASO injection 14 days before. The

scale bar represents 20 μ m. **b** The ratio of Iba1- and NeuN- positive cell density in the left striatum and

the ratio of GFAP- positive cell density in the left corpus callosum. The ratio of cell density to the average of that measured in the WT group is shown (n = 4 per each group; *P < 0.05). The density of Iba1-positive cells equally increased in the PFFs inoculation groups compared to the WT group. All data are expressed mean $\pm$ SEM.

Supplementary Fig.6



Supplementary Fig.6 ASO ipsilateral pre-treatment did not affect the motor function. a Wire hang

test (a) and rotarod tests (b) of wild-type (WT) mice and 30 days after PFFs inoculation and PBS, Control

ASO or *Snca* ASO injection 14 days before (n=8 per each group). All data are expressed mean $\pm$ SEM.

Supplementary Table.1 ASOs used in this study.

	Sequence (5'–3')
<i>Snca</i> ASO	CCAACATTGTCACTTGCTC
Control ASO	ATCCTTCTATCGGTCACTCA

Note: Red font indicates 2'-O-methoxyethyl (MOE); black font indicates DNA; all phosphorothioate (PS) backbone; A, adenine; ASO, antisense oligonucleotide; C, cytosine; C in the wing portion of ASO, 5-methylcytosine; G, guanine; U, uracil.

Supplementary Table.2 Primary antibodies used in this study.

Primary Antibodies	Type	Source	Dilution
Anti-aSyn (Synuclein-1)	Mouse monoclonal	BD Transduction Laboratories (Clone 42)	1:1000
Anti-phosphorylated aSyn (pSer129)	Rabbit monoclonal	Abcam (ab51253)	1:2000
Anti-phosphorylated aSyn (pSer #64)	Mouse monoclonal	FUJIFILM Wako Pure Chemical Corporation (#64)	1:1000
Anti-tyrosine hydroxylase	Mouse monoclonal	Millipore (MAB318)	1:2000
Anti-p62	Guinea pig polyclonal	PROGEN (GP62-C)	1:1000
Anti-ubiquitin	Rabbit monoclonal	Abcam (ab140601)	1:15000
Anti-NeuN	Chicken polyclonal	Millipore (ABN91)	1:3000
Anti-GFAP	Goat polyclonal	Abcam (ab53554)	1:1000
Anti-Iba1	Rabbit polyclonal	FUJIFILM Wako Pure Chemical Corporation (019-19741)	1:1000
Anti-phosphorothioate	Rabbit polyclonal	Ionis Pharmaceuticals	1:15000
Anti-β-actin (HRP conjugated)	Mouse monoclonal	Proteintech (HRP-60008)	1:10000

Supplementary Table.3 Primers and probe used in this study.

Gene	TaqMan probe (Applied Biosystems)
Mouse <i>Suca</i> (NM_001042451.2)	Mm00447331_m1
Mouse <i>Sncb</i> (NM_033610.2)	Mm00504325_m1
Mouse <i>Sncg</i> (NM_011430.3)	Mm00488345_m1

Gene	Forward	Rerverse	Probe
Mouse <i>Actb</i> (NM_007393.5)	CGCGAGCACAGCTTCTTTG	CATGCCGGAGCCGTTGTC	CACACCCGCCACCAGTTCGCCATG