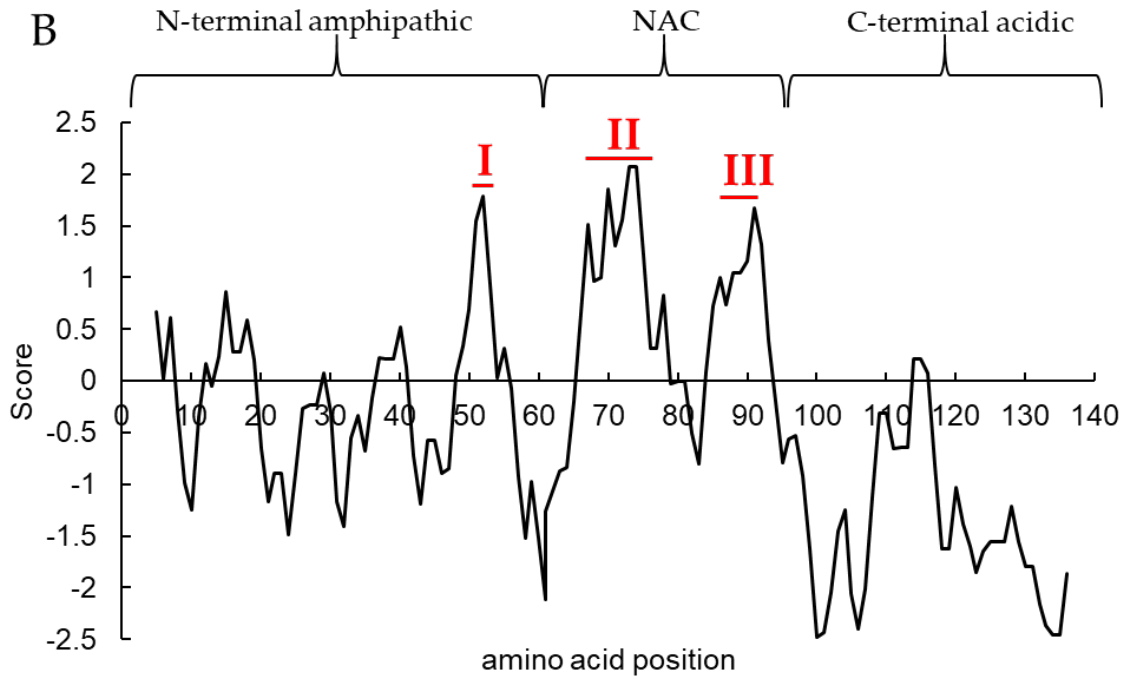


Supplementary Figure S1. Expression of Recombinant αSyn in Tet-On Cells. (A) Western blot of αSyn protein (with or without recombinant αSyn) following knockdown with αSyn specific siRNA. 15 kDa band is the monomer, >250 kDa band is nonspecific. αSyn plasmid is pCS2-SNCA. beta-Actin detected by western blot is a loading control. (B) RT-qPCR analysis of mRNA levels of recombinant αSyn in the cells treated with αSyn specific siRNA and in the control cells. (C) Western blot of αSyn protein following transfection with increasing concentration of the pCS2-SNCA plasmid (0.05-1.5 ng/μl). (D) Quantification of western blots using ImageJ. Normalized to beta-Actin protein levels and then to αSyn protein levels at 1.5 ng/μl concentration. Graph shows mean values ± SE with a total of n= 3 independent experiments. (E) RT-qPCR analysis of αSyn mRNA levels following transfection with increasing concentration of the pCS2-SNCA plasmid (0-1.5 ng/μl). Normalized to beta-Actin mRNA levels and then to αSyn mRNA levels at 1.5 ng/μl concentration. Graph shows mean values ± SE with a total of n= 3 independent experiments.

A

MDVFMKGLSK AKEGVVAAE KTKQGVAAE GKTKEGVLYV GSKTKEGVVH
 GVATVAEKT EQVTNVGGAV VTGVTAVAQK TVEGAGSIAA ATGFVKKDQL
 GKNEEGAPQE GILEDMPVDP DNEAYEMPSE EGYQDYEP



C

Site (as labeled on panel B)	Amino acid Position	Amino acid sequence	GRAVY value
I	51-52	GV	1.9
II	69-75	AVVTGVT	1.8
III	88-92	IAAAT	1.84

Supplementary Figure S2. Hydrophobic Domains of α Syn Protein. (A) Amino acid composition of α Syn. (B) Kyte-Doolittle hydropathy plot of full length α Syn. Hydrophobicity score was calculated using <https://web.expasy.org/protscale/>. Domains with positive hydrophobic score are labeled as I, II and III. α Syn structural domains are amphipathic (1-60), non-amyloid beta component, NAC (61-95) and acidic domain (96-140 amino acids). (C) GRAVY values of selected hydrophobic domains. Average hydropathy value of possible interacting domains were calculated using <http://www.gravy-calculator.de/>.

Supplementary Table S1. Sequences of siRNAs Used for Targeted Knockdown.

siRNA	Target NM	Sequence	Company	Concentration Used (nM)
siSNCA (α Syn)	NM_000345.3	5'-UGGCAACAGUGGCUGAGAA-3'	Dharmacon	11.25
siSRP54	NM_003136.4	5'-GAAAUGAACAGGAGUCAAUdTdT-3'	Dharmacon	13.75
siAGO2-1	NM_012154	5'-GCACGGAAGUCCAUCUGAAUU-3'	Dharmacon	10
siAGO2-2	NM_012154	5'-GCAGGACAAAGAUGUAUUAUU-3'	Dharmacon	10
siAGO2-3	NM_012154	5'-GGGUCUGUGGUGAUAAAUAUU-3''	Dharmacon	10
siAGO2-4	NM_012154	5'-GUAUGAGAACCCAAUGUCAUU-3'	Dharmacon	10

Supplementary Table S2. Sequences of Primers Used for RT-qPCR Analysis.

Target	Target NM	Forward	Reverse
SNCA (α Syn)	NM_000345.3	TGCCTGTGGATCCTGACAAT	TACGACTCACTATAGTTCTAGAGGC
SRP54	NM_003136.4	ACACCCGATCCTCTTGCTAC	CAGTACGGATGGTGCCAAAG
AGO2	NM_012154	AGGACTTCAGATGGACTTCCG	ACGGGCATCGAGATCAAGGT
beta-Actin	NM_001101.3	CACCTTCTACAATGAGCTGCG	TAGCACAGCCTGGATAGCAAC
HPRT	NM_000194.3	CTGAGGATTTGGAAAGGGTGTT	ATCTCCTTCATCACATCTCGAG
GAPDH	NM_002046	GTCTCCTCTGACTTCAACAGCG	ACCACCCTGTTGCTGTAGCCAA
OmpA		AACTACTGGAGCTGCCTCG	GCACACACCATCGGCACTC

Supplementary Table S3. Demographic Characteristics of Substantia Nigra Samples

Diagnosis	Age (Years)	Sex (M/F)	PMI (Hours) *
PD	78	F	21
PD	79	M	19
PD	88	M	19
PD	77	M	11
PD	82	M	21
PD	75	M	6
PD	79	F	21
Healthy	74	M	16
Healthy	38	M	24
Healthy	83	F	18
Healthy	89	F	5
Healthy	64	M	14
Healthy	87	M	21

* PMI is post-mortem interval