Supplemental Material

α-Synuclein Negatively Regulates PKCδ Expression to Suppress Apoptosis in Dopaminergic Neurons by Reducing p300 HAT Activity

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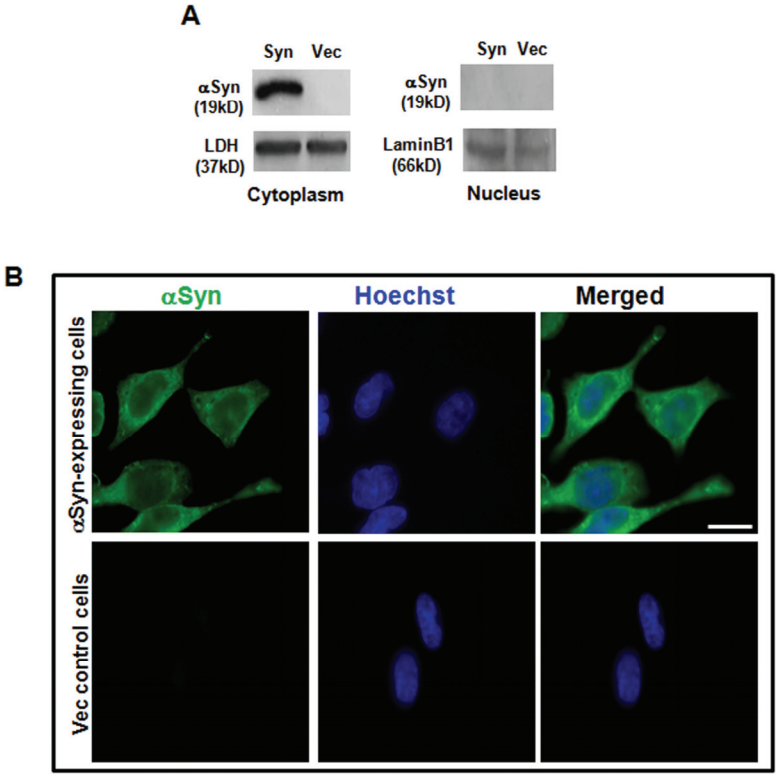
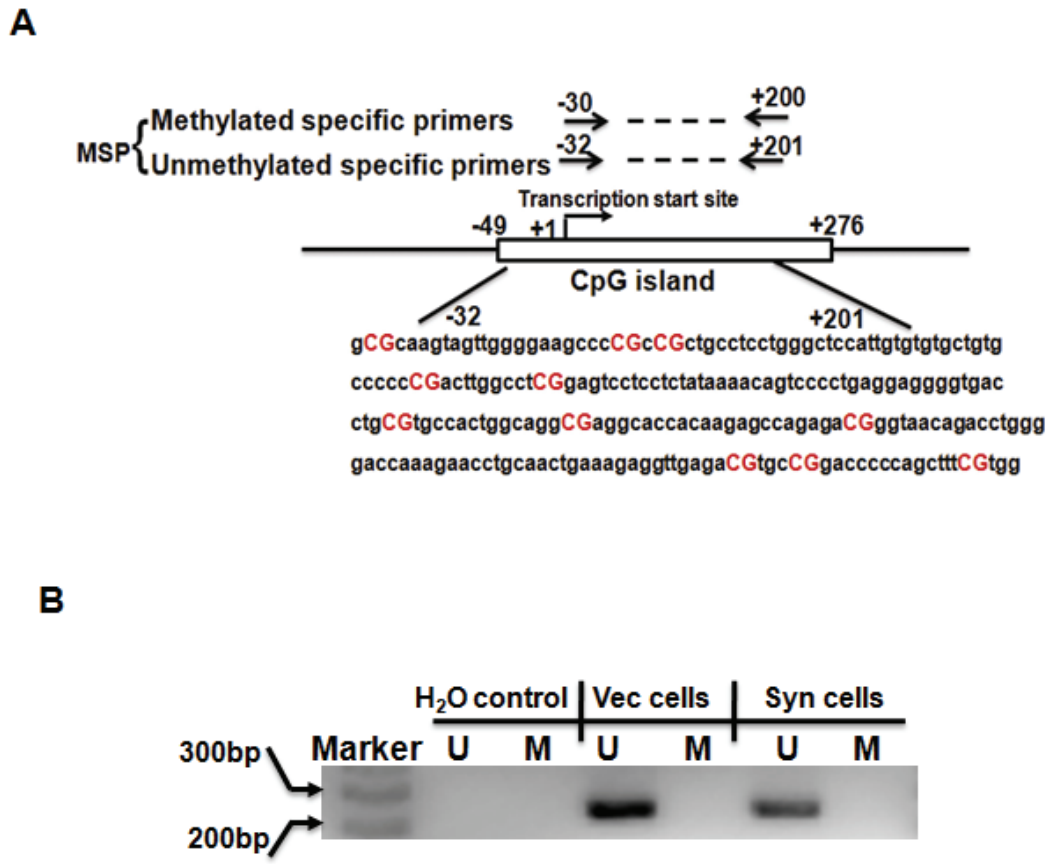
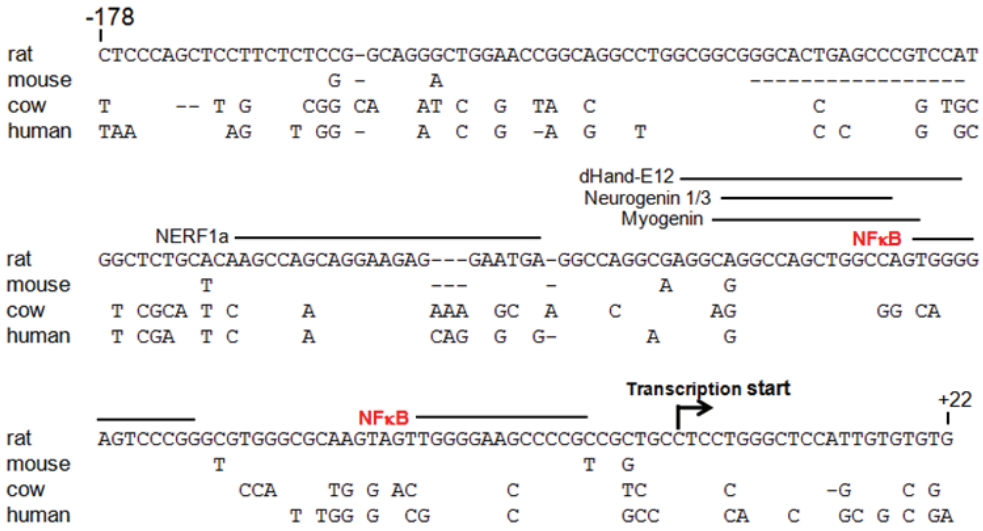


Supplemental Figure 1. α-Synuclein was exclusively located in the cytoplasm in αsyn-expressing N27 cells. ***A*****,** Cytoplasmic and nuclear extracts from αsyn-expressing (Syn) and vector control (Vec) N27 cells were prepared and subjected to immunoblotting analysis of αsyn. LDH (cytoplasmic fraction) and Lamin B1 (nuclear fraction) were used as loading controls. ***B*****,** Stained cells were mounted on slides and visualized under a Nikon TE2000 fluorescence microscope. Images were obtained with a SOPT digital camera. A representative image of αsyn immunostaining (green) and Hoechst staining (blue) is shown. Staining of αsyn-expressing (top panels) and vector control (bottom panels) cells with αsyn reveals immnuoreactivity specificity in the cytoplasm but not in the nucleus of αsyn-expressing cells. Scale bar, 10μm.



Supplemental Figure 2. α-Synuclein does not affect the methylation status of PKCδ promoter. ***A***, Schematic map of the putative promoter-associated CpG island region showing the location of MSP primers and the sequence of the region studied by MSP. The CpG dinucleotide is shown in red capital letters. ***B***, MSP analysis of methylation status in PKCδ promoter. Bisulfite-modified DNA was used for MSP with primers specific for methylated (M) and unmethylated (U) DNA. Water blank was used as a negative control.



Supplemental Figure 3. Sequence alignment of the proximal PKCδ promoter. The proximal rat PKCδ promoter sequence (-178 to +22, relative to the transcription start site) was aligned with the homologous sequences from the mouse, human, and cow genome using a DiAlign professional program. Sequence differences are indicated and gaps introduced to maximize homology are marked by dashes. The highly conserved TFBSs are labeled, and the NFκB sites are highlighted in red.

Supplemental Table 1: List of primer sequences used in the study.

Primer	Sequence (5'-3')	Amplicon
PKCδ Fg	GTCTATCTCGAGCACTCTCCTGAAGCCCACCATG	1901
PKCδ Rg	GTCTATAAGCTTCACACACAATGGAGCCCAGGAG	
PKCδ Fs	GGGCTACGTTTTATGCAGCT	700
PKCδ Rs	AGCAGGTCTGGGAGCTCACT	
PKCα Fs	TGAACCCTCAGTGGAATGAGT	325
PKCα Rs	GGCTGCTTCCTGTCTTCTGAA	
PKCε Fs	CCACCAAGCAGAAGACCAAC	466
PKCε Rs	TTTGTGGACGACGCAGGTAC	
PKCη Fs	GAAGGAGAGTCCATCAAGTC	497
PKCη Rs	TCAGCGTAGACCTGGAAATG	
PKCζ Fs	GGGACGAAGTGCTCATCATC	541
PKCζ Rs	GAGGACCTTGGCATAGCTTC	
PKCλ Fs	GCAGTGAGGTTCGAGATATG	380
PKCλ Rs	CCAGCAGTTTGCAGTTGATG	
GAPDH Fs	CAATGCATCCTGCACCACCAAC	320
GAPDH Rs	CATACTTGGCAGGTTTCTCCAG	
PKCδ Fq	TAAGCCCAAAGTGAAATCCC	138
PKCδ Rq	ACAAAGGAGAAGCCCTTGAA	
β-actin Fq	ATCGCTGACAGGATGCAGAAG	76
β-actin Rq	TCAGGAGGAGCAATGATCTTGA	
Methylated F	CGTAAGTAGTTGGGGAAGTTTC	230
Methylated R	CACGAAAACTAAAAAT CCGAC	
Unmethylated F	GGTGTAAGTAGTTGGGGAAGTTTT	233
Unmethylated R	CCACAAAAACTAAAAATCC AAC	
ChIP F	ACAAGCCAGCAGGAAGAGGA	163
ChIP R	TTATAGAGGAGGACTCCGAGGC	

F, Forward; R, Reverse; g, genomic PCR for cloning the rat PKCδ promoter; s, semiquantitative RT-PCR; q, quantitative RT-PCR.

Supplemental Table 2: Sense sequences of the oligonucleotides used in EMSAs.

Probe/Competitor	Sense oligonucleotide (5'-3')
PkcδNFkB1	GTAGTTGGGGAAGCCCCGCC (-20 to -8)
PkcδNFkB1 mutant	GTAGTT**agct**AAGCCCCGCC
PkcδNFkB2	GCCAGTGGGGAGTCCCGGGC (-51 to -39)
PkcδNFkB2 mutant	GCCAGT**agct**AGTCCCGGGC
NFkB consensus	AGTTGAGGGGACTTTCCCAGGC
AP-1	CGCTTGATGACTCAGCCGGAA

Nucleotide sequences of the consensus binding motif are underlined. The localizations of the PKCδ NFκB sites, relative to the transcription start site, are shown. Mutated base pairs in mutant oligos are highlighted in bold and in lowercase.