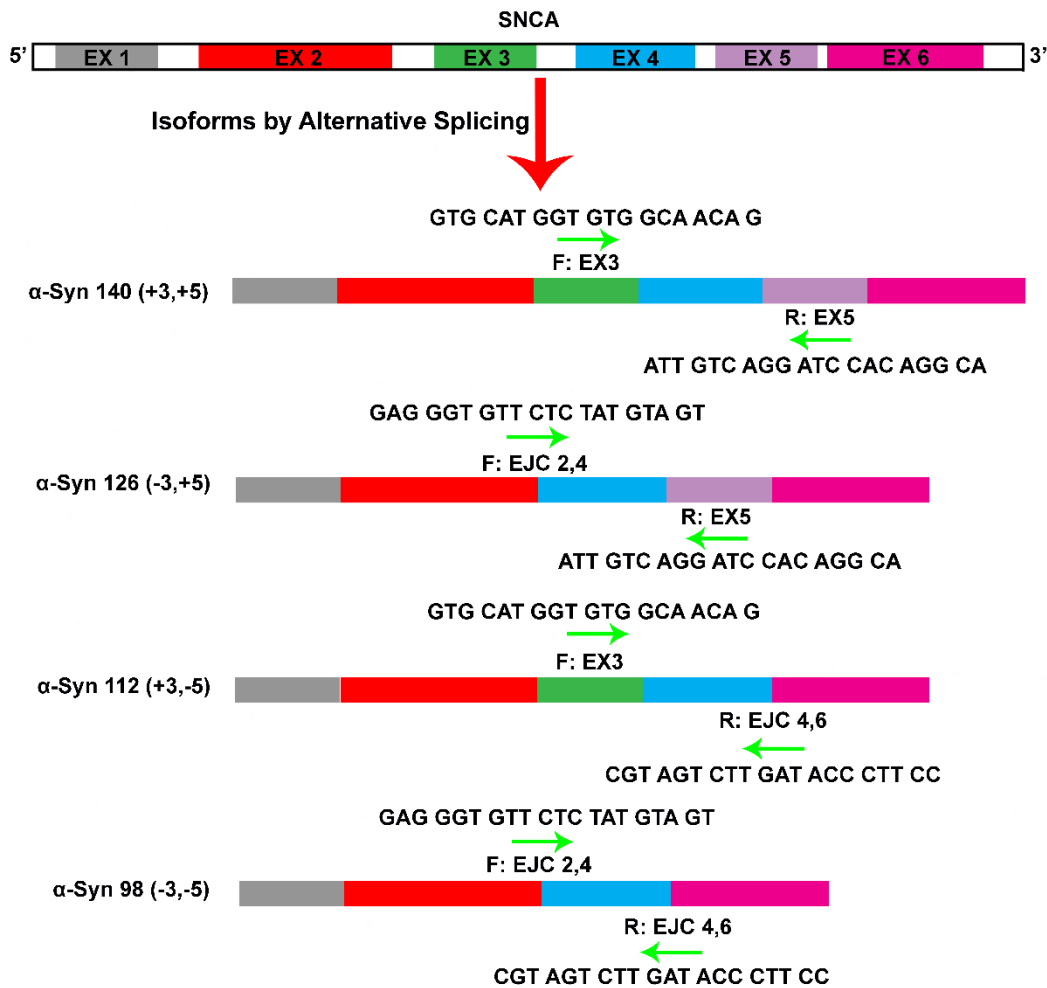
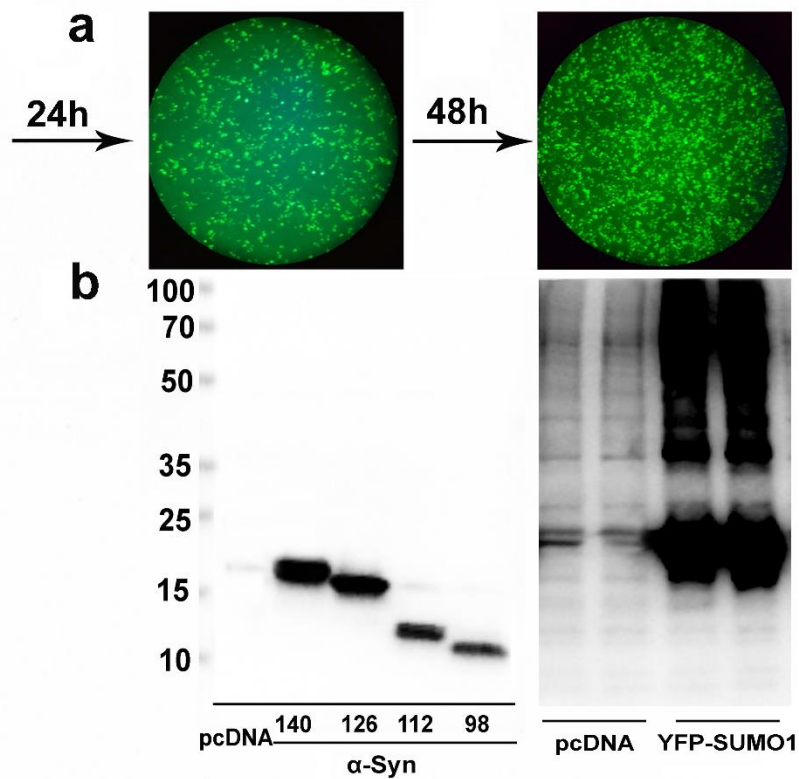


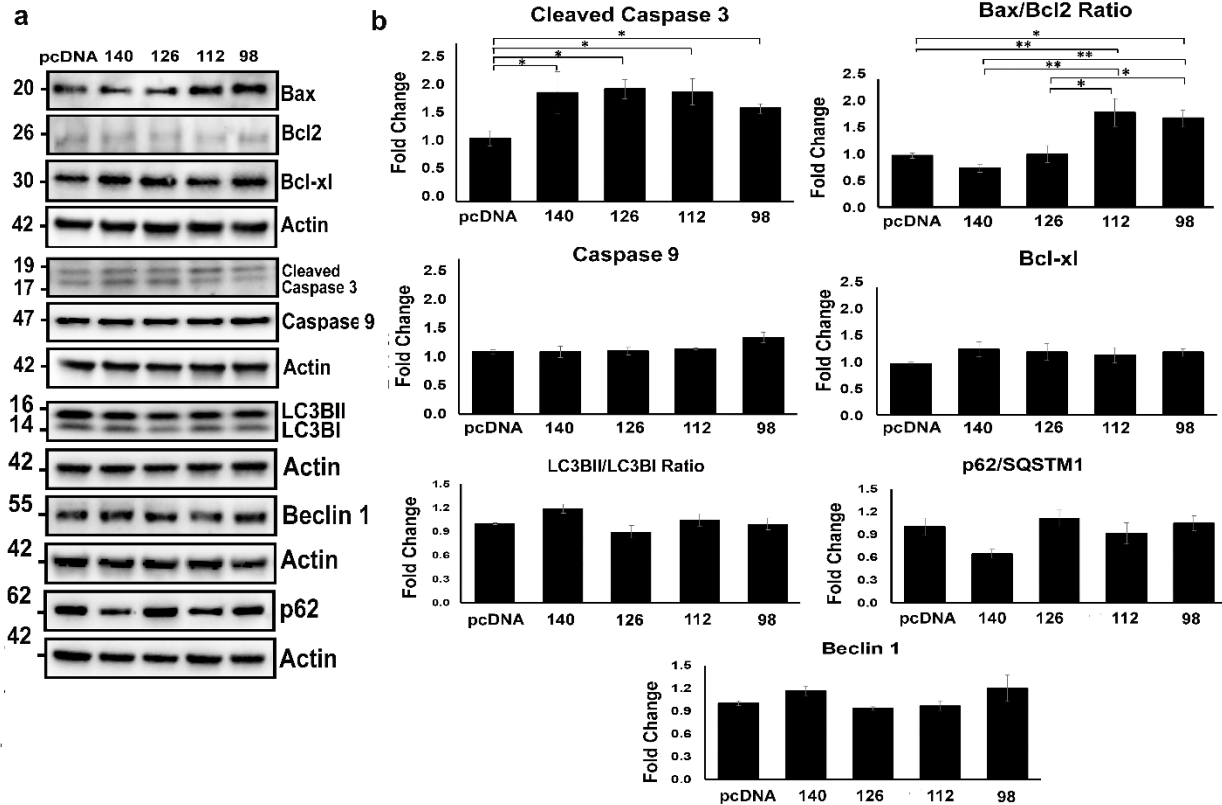
Supplementary Data



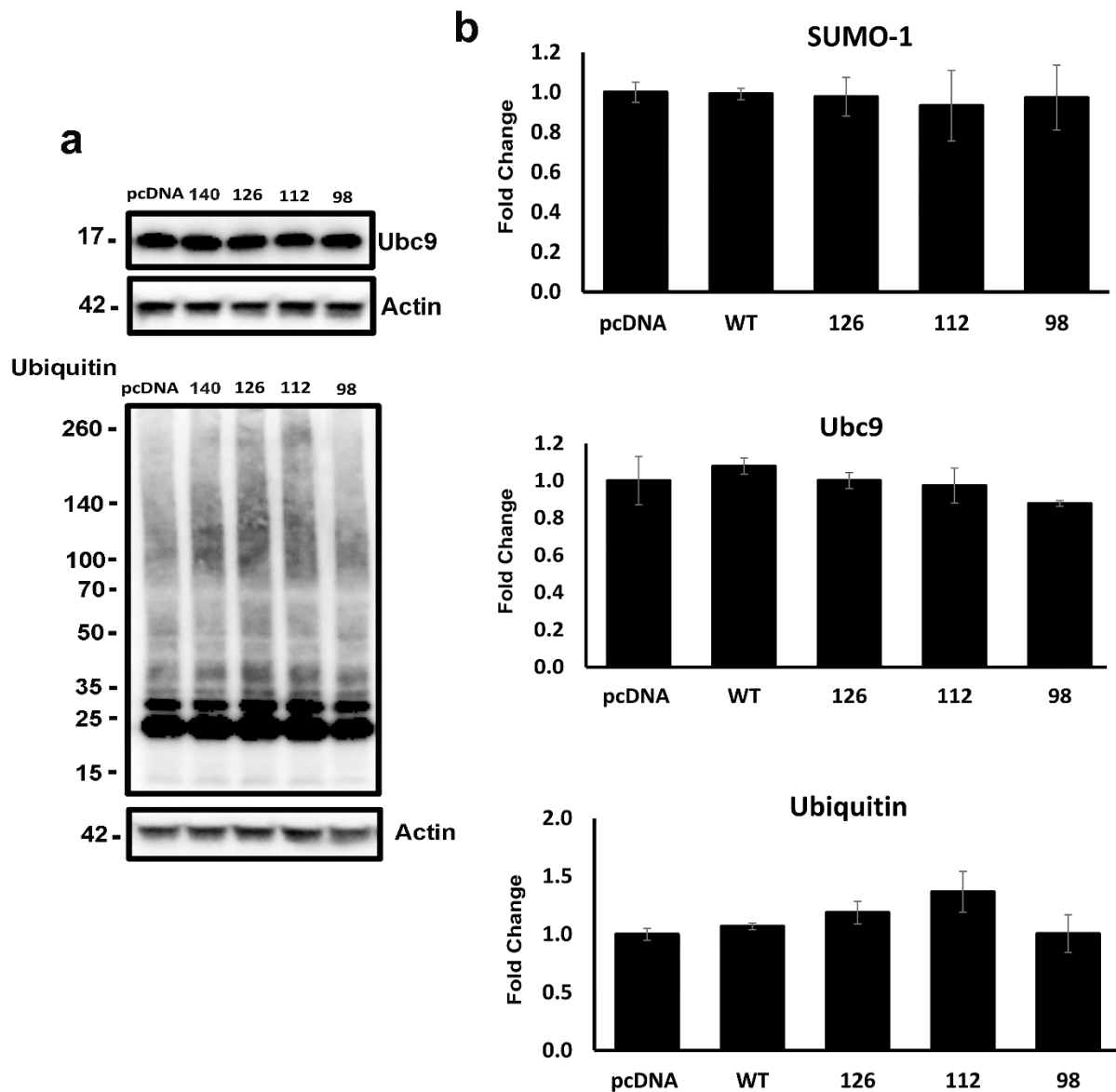
1. Schematic representation of the SNCA genes. Alternative splicing isoforms and exons affected are shown. Six exons encode the full-length α -syn protein, α -syn 140. Three alternatively spliced variants that skip exons 3, 5, or both produce isoforms α -syn 126, α -syn 112, and α -syn 98, respectively. Primers have been designed that amplify cDNA encoding specific α -syn isoforms: two sense primers, one located in exon 3 and one in exon junction 2 and 4 (EJC 2,4), were used in combination with two antisense primers, one located in exon 5 and one in exon junction 4 and 6 (EJC 4,6), to amplify isoforms. The order and sequence of the primers are shown.



2. The efficiency of the overexpression of YFP-SUMO-1 and isoforms. a) Fluorescent image showing the efficiency of overexpression of YFP-SUMO-1 after 24 and 48 h post-transfection. **b)** Representative western blots show the overexpression of pc DNA, the isoforms, and YFP-SUMO-1 in SH-SY5Y cells after 48 h.



3. Effect of overexpression of α -Syn isoforms on apoptosis markers and autophagy. Cells were overexpressed with α -Syn isoforms. After 48 h, they were harvested and checked for apoptosis and autophagy markers. **a)** Representative western blots and **b)** Relative quantifications of apoptosis and autophagy markers in different isoforms overexpression conditions. Data are shown as mean $\pm$ SEM. A *p*-value of less than 0.05 was considered significant in all analyses. * $P < 0.05$ and ** $P < 0.01$.



4. Effect of overexpression of α -Syn isoforms on SUMOylation and ubiquitination.

a) Representative western blots and **b)** Relative quantifications of SUMO-1, Ubc9, and ubiquitin in different isoforms overexpression conditions. Data are shown as mean $\pm$ SEM. A *p*-value of less than 0.05 was considered significant in all analyses.

Table 1. List of antibodies used in Western blotting (WB), and immunofluorescence (IF).

Antibody	Company	Concentration	Cat Number
Anti-BAX	Cell Signaling, Danvers, MA, USA	WB: 1:1000	5023
Anti-Bcl2	Cell Signaling, Danvers, MA, USA	WB: 1:1000	15071
Anti-Bcl-xl	Cell Signaling, Danvers, MA, USA	WB: 1:1000	3498
Anti-Cleaved Caspase-3	Cell Signaling, Danvers, MA, USA	WB: 1:1000 IF: 1:100	9654
Anti-Caspase-9	Cell Signaling, Danvers, MA, USA	WB: 1:1000	9508S
Anti-Drp1	Cell Signaling, Danvers, MA, USA	WB: 1:1000	5391T
Anti-LC3B	Cell Signaling, Danvers, MA, USA	WB: 1:1000	43566
Anti-SUMO-1	Cell Signaling, Danvers, MA, USA	WB: 1:1000 IF: 1:100	4930
Anti-SUMO-1 (SM1/495) [Biotin]	Bio-Techne Srl	IF: 1:100	NBP2-34716B
Anti-p62/SQSTM1	Cell Signaling, Danvers, MA, USA	WB: 1:1000 IF: 1:100	88588
Anti- α -Synuclein	Cell Signaling, Danvers, MA, USA	WB: 1:1000	2647
Anti-phosphor-Ser 129 α -Synuclein	Cell Signaling, Danvers, MA, USA	WB: 1:1000	23706
Anti-Ubiquitin	Cell Signaling, Danvers, MA, USA	WB: 1:1000	3933
Anti-Ubc9	Cell Signaling, Danvers, MA, USA	WB: 1:1000	4786
Anti-DJ1	Cell Signaling, Danvers, MA, USA	WB: 1:1000	5933
Anti-Parkin	Cell Signaling, Danvers, MA, USA	WB: 1:1000	4211
Anti β -actin Rabbit	Thermo Fisher Sc, Waltham, MA USA	WB: 1:1000	MA533078
Anti β -actin Mouse	Thermo Fisher Sc, Waltham, MA USA	WB: 1:1000	MA1-140
Anti-Beclin1	Abcam, Cambridge, CB2 0AX, UK	WB: 1:1000	ab217179
Anti-aggregated α -Synuclein 5G4	Merck KGaA, Darmstadt, DE	WB: 1:1000	MABN389
Goat anti-Rabbit IgG (H+L) Alexa Fluor 488	Thermo Fisher Sc, Waltham, MA USA	IF: 1:500	A-11008
Goat anti-Rabbit IgG (H+L) Alexa Fluor 594	Thermo Fisher Sc, Waltham, MA USA	IF: 1:500	A-110012
Goat anti-Mouse IgG (H+L) Alexa Fluor 488	Thermo Fisher Sc, Waltham, MA USA	IF: 1:500	A-11001
Goat anti-Mouse IgG (H+L) Alexa Fluor 594	Thermo Fisher Sc, Waltham, MA USA	IF: 1:500	A-11005
Anti- α -Synuclein 2A7 (61-95)	Bio-technie, Minneapolis, MN USA	WB: 1:1000	NBP1-05194
Anti-aggregated α -Synuclein [MJFR-14-6-4-2]	Abcam, Cambridge, CB2 0AX, UK	IF: 1:500	Ab-209538
DYKDDDDK Tag (D6W5B)	Cell Signaling, Danvers, MA, USA	WB: 1:1000 IP: 1:50	14793

Table.2 Interaction analysis of Ubc9 with different Alpha-syn isoforms.

Ligand	Total Binding energy (kcal/mol)	The ligand nearest interacting residues
Isoform-140	-44.80	TYR-136, TYR-133, GLU-126, GLU-130, GLU-139
Isoform-126	-62.50	GLU-112, ASP-121, GLN-120, GLU-116, MET-113
Isoform-112	-22.51	GLN-79, VAL-82, ASP-107, TYR-108, GLU-111
Isoform-98	-31.74	ILE-74, LEU-8, PHE-4, SER-73, MET-5