Supplementary Material

An inducible Alpha-Synuclein expressing neuronal cell line model for Parkinson's disease[#]

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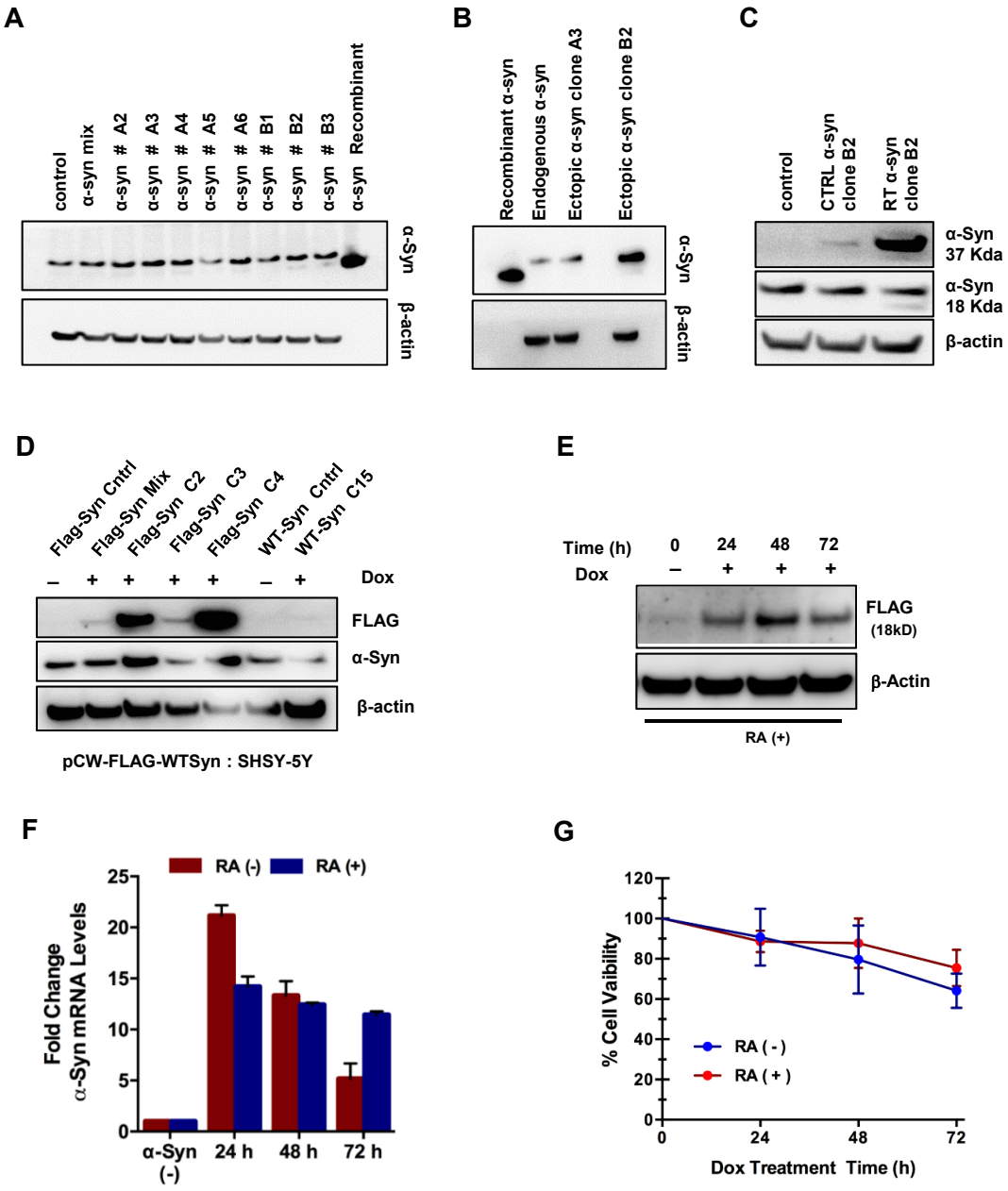
Figure S1.


A
control
α-syn mix
α-syn # A2
α-syn # A3
α-syn # A4
α-syn # A5
α-syn # A6
α-syn # B1
α-syn # B2
α-syn # B3
α-syn Recombinant
α-Syn
β-actin
B
Recombinant α-syn
Endogenous α-syn
Ectopic α-syn clone A3
Ectopic α-syn clone B2
α-Syn
β-actin
C
control
CTRL α-syn clone B2
RT α-syn clone B2
α-Syn 37 Kda
α-Syn 18 Kda
β-actin
D
Flag-Syn Cntrl
Flag-Syn Mix
Flag-Syn C2
Flag-Syn C3
Flag-Syn C4
WT-Syn Cntrl
WT-Syn C15
– + + + + – + Dox
FLAG
α-Syn
β-actin
pCW-FLAG-WTSyn : SHSY-5Y
E
Time (h) 0 24 48 72
Dox – + + +
FLAG (18kD)
β-Actin
RA (+)
F
RA (-) RA (+)
Fold Change α-Syn mRNA Levels
25
20
15
10
5
0
α-Syn (-)
24 h
48 h
72 h
G
% Cell Vaibility
120
100
80
60
40
20
0
RA (-)
RA (+)
0
24
48
72
Dox Treatment Time (h)

Supplementary Fig. S1. Constitutive versus inducible α-synuclein SHSY-5Y Stable line clone selection.

(**A**) SHSY-5Y cells were transfected using lipofectamin 2000 reagent. 24 hours after transfection cells were plated on a 96-well plate containing culture medium supplemented with G418 sulphate for selection of stably transfected cells. Resistant cell colonies were selected for expansion and analyzed for α-synuclein protein expression. α-Syn A3 and B2 clone were selected. (**B**) After several passages clone A3 cell line presented reduced α-Syn expression similar to control protein levels. Clone B2 was selected for further experiments. However, after several passages this line also presented reduced α-Syn. (**C**) Transient re-transfection (RT) of pCDNA- α-Syn vector into clone B2 cell line did not increase the levels of monomeric α-synuclein, but rather of enhance the formation of aggregates at 37kDa. (**D**) SHSY-5Y cells were transfected using lipofectamin 2000 reagent according to the respective manufacturer's instructions. 24 hours after transfection cells were plated on a 96-well plate containing culture medium supplemented with puromycin for selection of stably transfected cells. Resistant cell colonies were selected for expansion and analyzed for FLAG protein expression. FLAG α-Syn C4 clone was selected based on stronger expression of ectopic α-synuclein in comparison to FLAG α-Syn C2. (**E**) Immunoblot characterizing time-dependent induction of FLAG α-synuclein in 7 days differentiated cells. (**F**) α-synuclein mRNA abundance quantified by real-time PCR showed a significant time-dependent decreased of α-synuclein transcript in RA (-) (undifferentiated) cells, whereas RA (+) (differentiated) α-synuclein transcript reduction was less. (**G**) Survival rate comparison between undifferentiated RA (-) and differentiated cells RA (+) overexpressing α-synuclein.