SUPPLEMENTARY MATERIAL

Distinct alpha-Synuclein species induced by seeding are selectively cleared by the Lysosome or the Proteasome in Neuronal Cells

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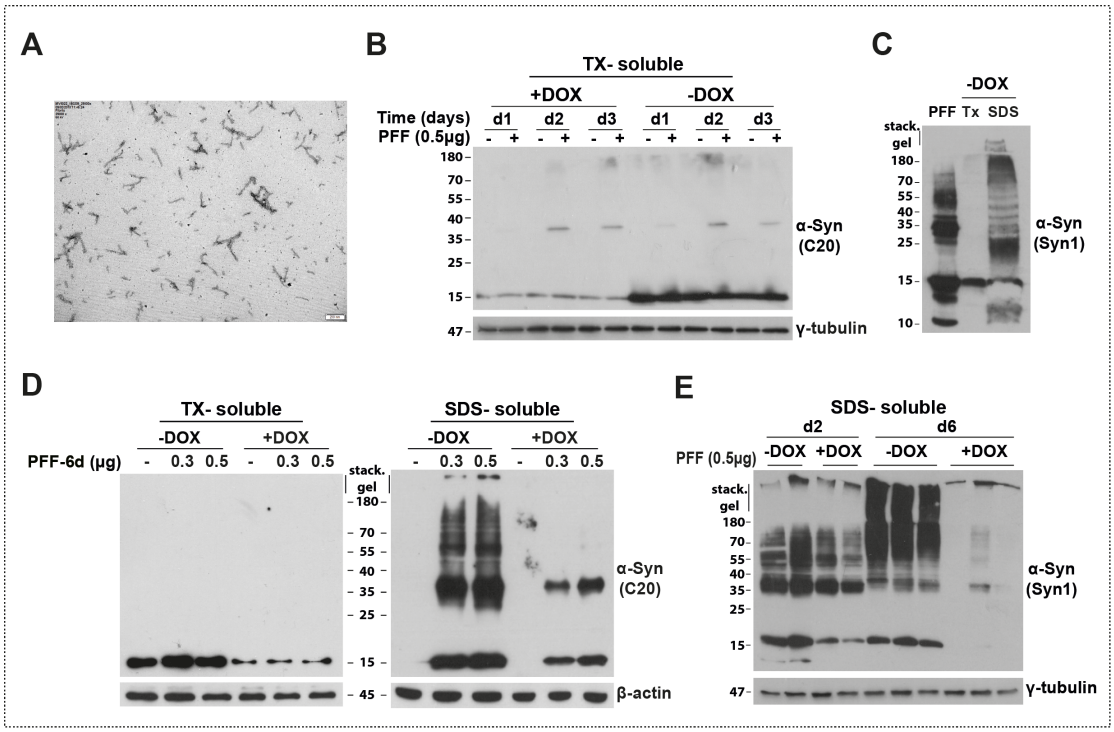
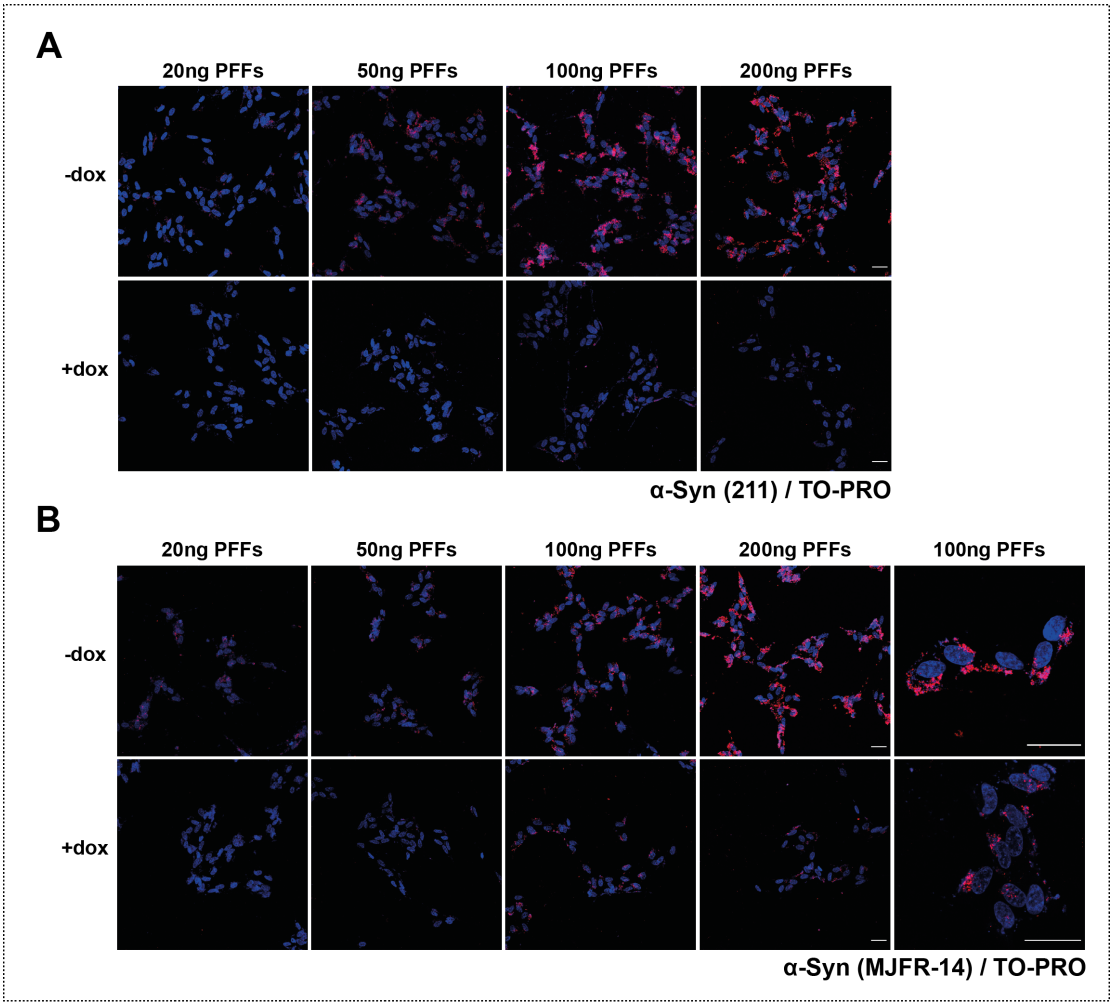
Running title: Clearance of alpha-synuclein aggregates

Supplementary Figures



Supplementary Figure S1. A. Negatively stained TEM of *α*-syn pre-formed fibrils (PFFs). Scale bar 200 nm. **B.** HMW *α*-Syn species of Tx-soluble fraction from experiment at Fig. 1B. **C.** Cells (-DOX), incubated with PFFs (0.75 µg) for 6 days, were fractionated (Tx- and SDS-soluble fraction- lane 2 and 3 respectively) and subjected to western immunoblotting against *α*-Syn (Syn1). Lane 1 represent 0.1 µg of recombinant *α*-syn PFFs, used as a control. **D.** 6 days post-PFF, using different amount of fibrils (0, 0.3, 0.5 µg) cells were harvested and subjected to fractionated western immunoblotting (Tx- and SDS- soluble fraction) with an antibody against total *α*-Syn (C20). β-actin was used as a loading control. **E.** After 2 (in duplicate) and 6 days (in triplicate), PFF (0.5µg)- treated cells (- and +DOX) were harvested and subjected to fractionated western immunoblotting with an antibody against *α*-Syn (Syn1) and γ-tubulin.



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963 **Supplementary Figure S2.** Differentiated SH-SY5Y cells, - and +Dox, were incubated
964 with different amounts of PFFs (20, 50, 100, 200 ng) for 6 days, fixed and
965 immunostained with 211, detecting total *α*-Syn (**A**), and MJFR-14, detecting
966 aggregated *α*-Syn species (**B**). Representative confocal images depict *α*-Syn
967 inclusions in -Dox cells. TO-PRO was used to stain the nucleus. Scale bar 30 µm.
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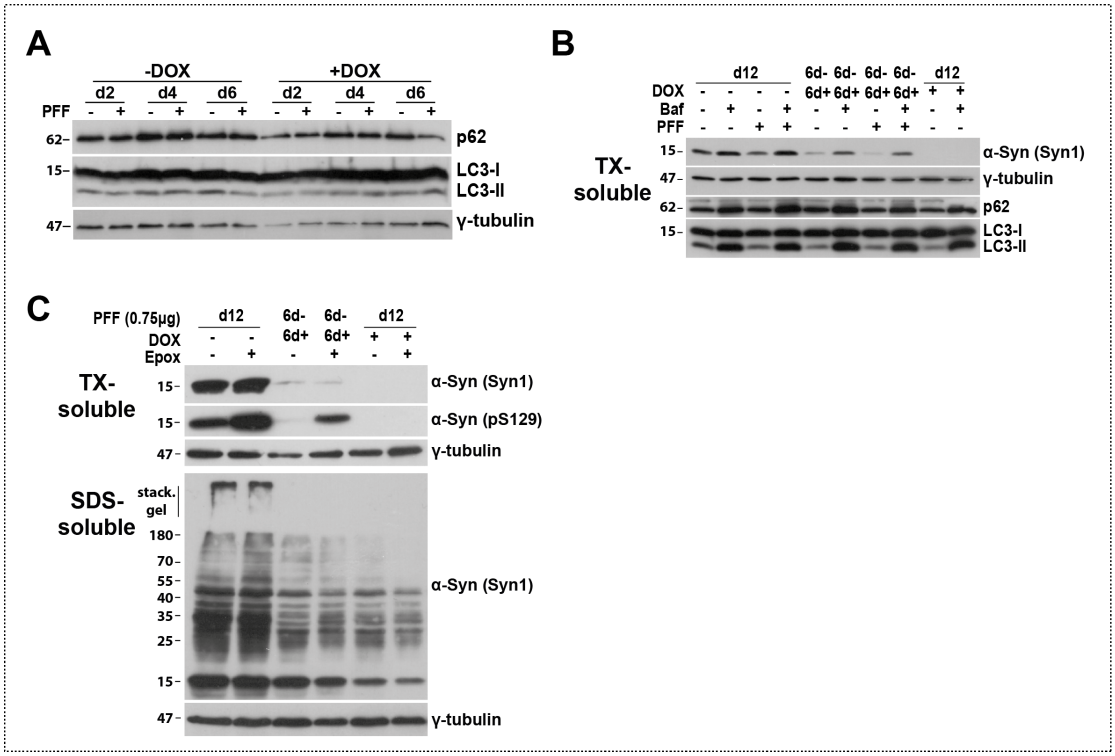

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971 **Supplementary Figure S3. A.** After 2, 4 and 6 days, non-treated and PFF (0,75µg)
972 treated cells (- and +DOX) were harvested and subjected to fractionated Western
973 Immunoblotting with an antibody against *α*-Syn (Syn1), p62 and LC3. **B.** Non-treated
974 and PFF (0,75µg) treated cells (- and +DOX, 6d-/6d+), with or without bafilomycin
975 (100nM), were harvested on day 12 and subjected to fractionated western
976 immunoblotting (Tx- and SDS- soluble fraction) with an antibody against *α*-Syn
977 (Syn1), p62 and LC3. **C.** In -DOX and +DOX, PFFs were added and the cells were
978 incubated for 5 days. 12 days after PFF-addition (0,75µg), non-treated and
979 epoxomicin-treated cells (20nM) were harvested and subjected to fractionated
980 western immunoblotting with an antibody against *α*-Syn (Syn1) and pS129 *α*-Syn. γ-
981 tubulin was used as a loading control.
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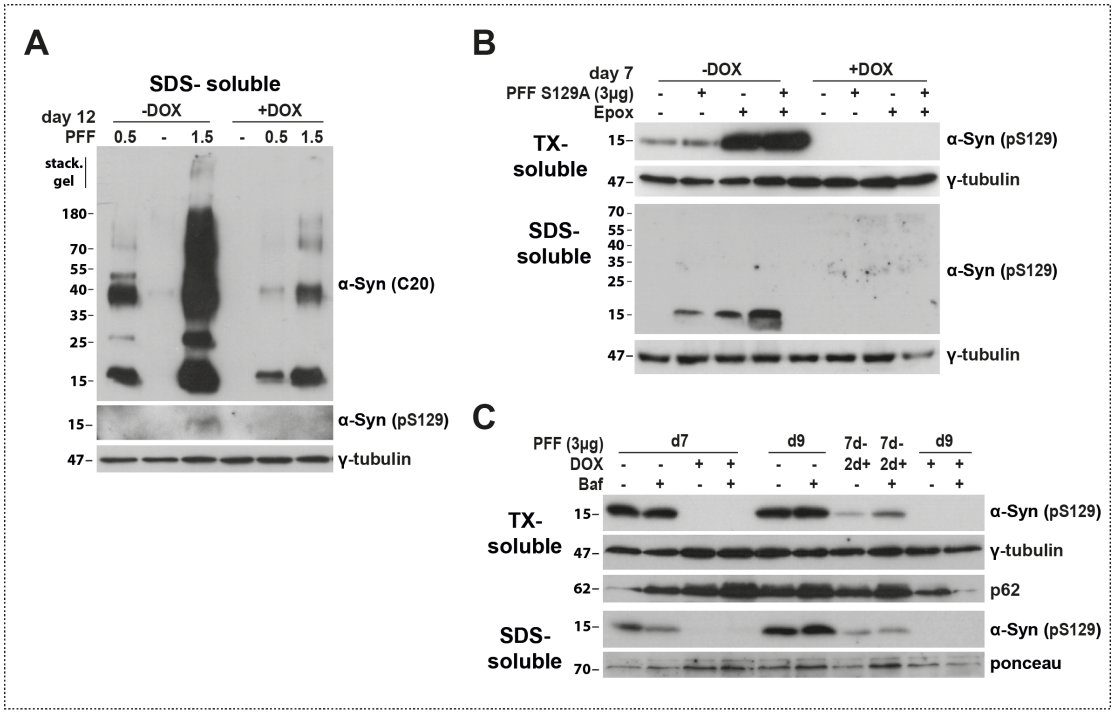

Supplementary Figure S4. A. 12 days post-PFF addition (0, 0.5, 1.5 µg), cells (- or +DOX) were harvested and subjected to fractionated western immunoblotting (SDS fraction) with antibodies against *α*-Syn (C20), pS129 *α*-Syn and γ-tubulin. **B.** After 7 days of high-dose S129A PFF (3 µg) incubation, - and +DOX cells, non-treated and epoxomicin-treated, were subjected to fractionated western immunoblotting (TX-100 and SDS fraction) with anti-pS129 and anti-γ-tubulin antibodies. **C.** 7 and 9 days post-PFF (3 µg), differentiated SH-SY5Y cells (- or +DOX and 7d-/2d+), untreated or treated with bafilomycin for 24 hours, were harvested and subjected to fractionated western immunoblotting (TX-100 and SDS fraction) with anti-pS129, anti-p62, and anti-γ-tubulin antibodies.