Supplementary data

Supplementary Figure 1. Expression of ATP13A2WT and KRS pathogenic mutants in HEK293 cell stable clones.

Shown are expression levels of exogenous ATP13A2 variants in stable clones and control transfectants (upper panel). Two independent clones for each variant are shown. Exogenous ATP13A2 proteins were by immunoblotting using an anti-V5 antibody. Tubulin is shown as loading control (lower panel).

Supplementary Figure 2. Degradation of ATP13A2WT and the KRS pathogenic mutant proteins.

A.B. Half-life of ATP13A2 variants: HEK293 cells stable expressing ATP13A2WT(A) and three KRS pathogenic mutants (B) were treated with cycloheximide (CHX) treatment for various times (indicated at top of panels) followed by detecting protein level of ATP13A2 variants. Tubulin was detected as loading controls. Note that ATP13A2 pathogenic mutants show shorter half-life than ATP13A2WT.

C. Potential mechanism of ATP13A2 degradation: HEK293 cells stable expressing ATP13A2WT and three KRS pathogenic mutants were either untreated (Control) or treated with solvents (H_2O, DMSO), lysosomal inhibitor NH_4Cl, autophagy inhibitor 3-MA, or proteasomal inhibitor MG132, followed by detecting protein level of ATP13A2 variants. Tubulin was detected as loading controls. Note that ATP13A2 variants may be degraded via multiple mechanisms.

Supplementary Figure 3. Overexpression ATP13A2 does not inhibit cell death induced by high-concentration of Fe^{2+}.

HEK293 cells stably expressing ATP13A2 WT or ATP13A2 DUP22 were treated with $FeCl_2$ at indicated concentration (bottom of the figure) for 72h. Apoptotic cells were counted as described in experimental procedures.

Supplementary Figure 4. ATP13A2 and $MnCl_2$ treatment had little effect on expression of α-synuclein.

N2a cells co-transfected with ATP13A2,α-synuclein and GFP. 36 hr post-transfection, cells were treated with $MnCl_2$ at various concentrations (indicated at bottom of the figure) for 24h, followed by detection of α-synuclein protein. Control: vector tranfection; α-synuclein: vector and α-synuclein transfection; α-synuclein+ATP13A2WT: cotransfection of ATP13A2WT and α-synuclein; α-synuclein+ATP13A2DUP22: cotransfection of ATP13A2DUP22 and α-synuclein. GFP is included as control of transfection efficiency. Actin is detected as loading controls.

Supplementary Figure 5. ATP13A2WT has little effect on Triton X-100 solubility of α-synuclein.

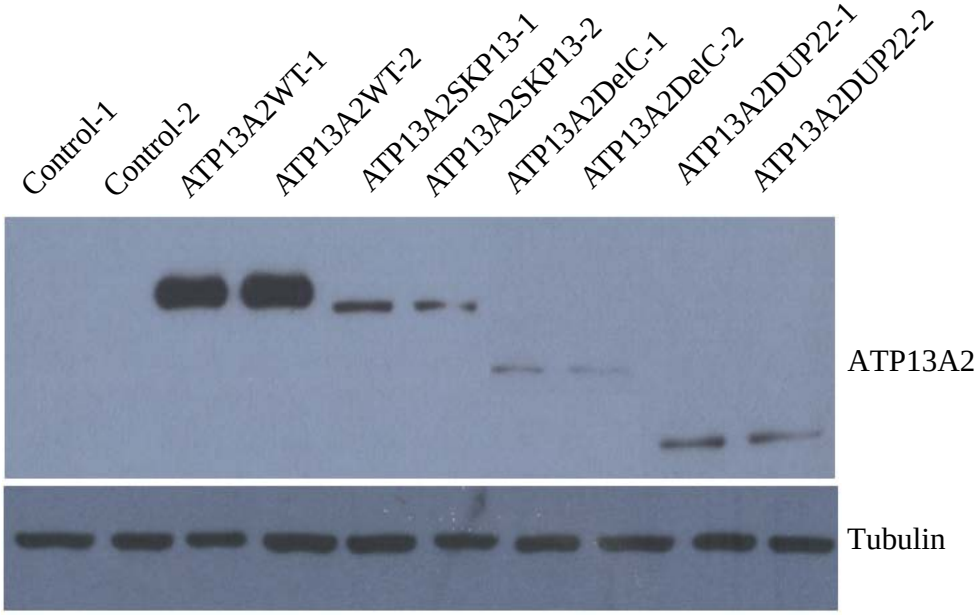
N2a cells transfected with vector alone (NC, Control) and two indepdently clones stably expressing ATP13A2WT (ATP13A2WT1, ATP13A2WT2) were transfected with α-synuclein and GFP. 36 hr post-transfection, cells were subject to analyse α-synuclein solubility in Triton

X-100. Cells were lysed in 1% Triton X-100 to total cell lysates and incubating on ice for 1h. The lysates were centrifuged at15,000 X g at 4 °C for 20 min. The supernatant was designated Triton X-100 soluble components. The pellet (Triton X-100 insoluble fraction) was further dissolved in 2% SDS-containing lysis buffer and sonicated for 20s. Protein concentration was determined using a Lowry protein assay. 20μg of each cell lysates was separated on 15% SDS PAGE gels followed by immunoblotting analysis of α-synuclein. Note that overexpression of ATP13A2 did not change the level of α-synuclein in both detergent soluble and insoluble fractions. NT, transfected with GFP alone; Control: transfected with α-synuclein+GFP; ATP13A2WT1: Cells stably expressing ATP13A2 contransfected with α-synuclein+GFP; ATP13A2WT2: Cells stably expressing ATP13A2 contransfected with α-synuclein+GFP. GFP is included as control of transfection efficiency.

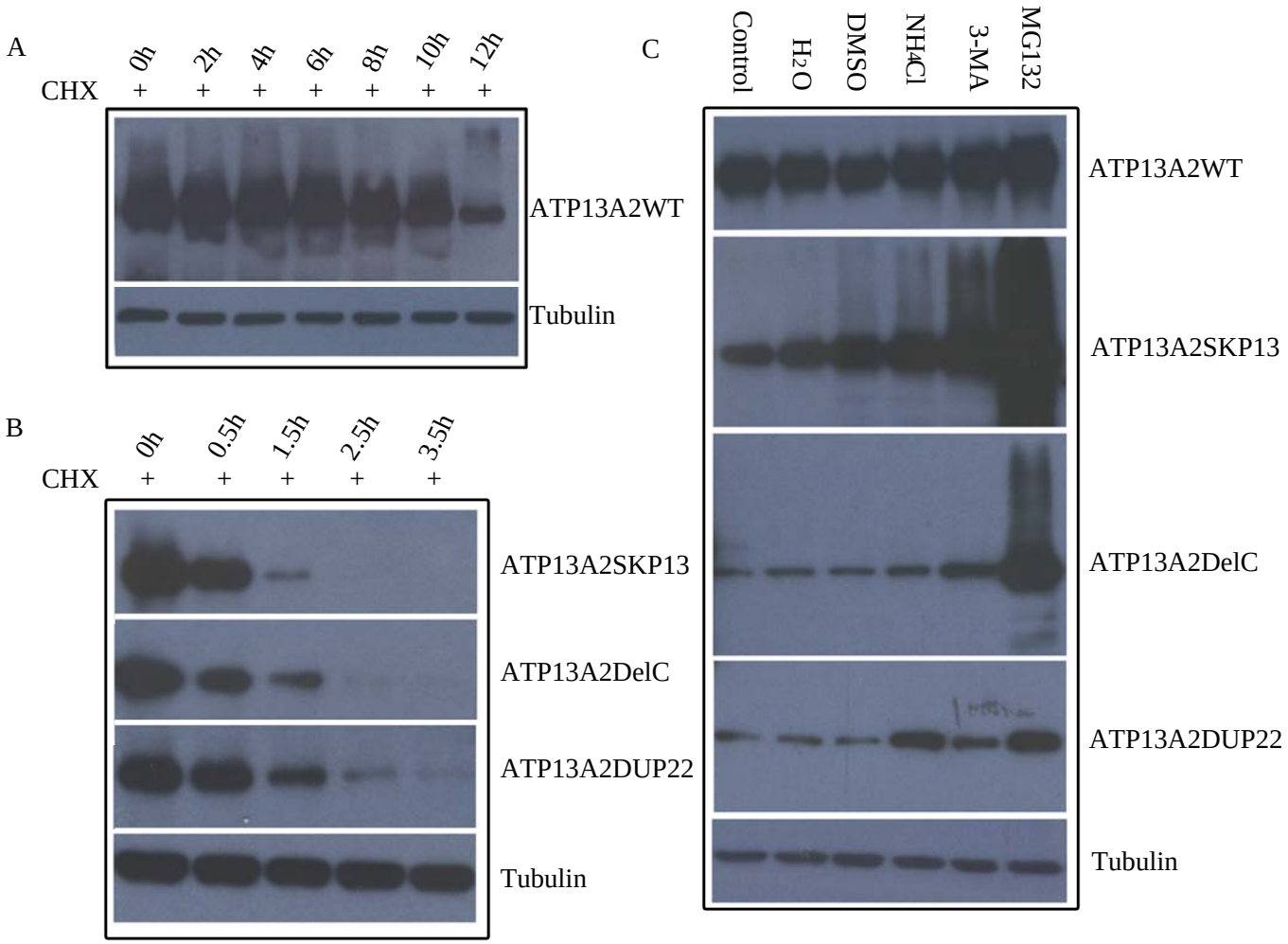
Supplementary Figure 6. ATP13A2WT has little effect on expression of endogenous α-synuclein in the presence of excess $MnCl_2$

SH-SY5Y cells were transfected with ATP13A2. 36 hr post-transfection, cells were treated with $MnCl_2$ at various concentration (top of the figure) for 24h, followed by detection of endogenous α-synuclein protein using an anti-α-synuclein antibody (Cell Signal Technology) (A). Exogenous ATP13A2 was detected by immunoblotting using an anti-V5 tag antibody (B). Quantitative results of five independent experiments were shown (C), Note: ATP13A2WT has little effect on expression of endogenous α-synuclein in the presence of excess $MnCl_2$. Control: vector tranfection; ATP13A2WT: transfection of ATP13A2WT; ATP13A2DUP22: transfection of ATP13A2DUP22. GAPDH is detected as a loading control.

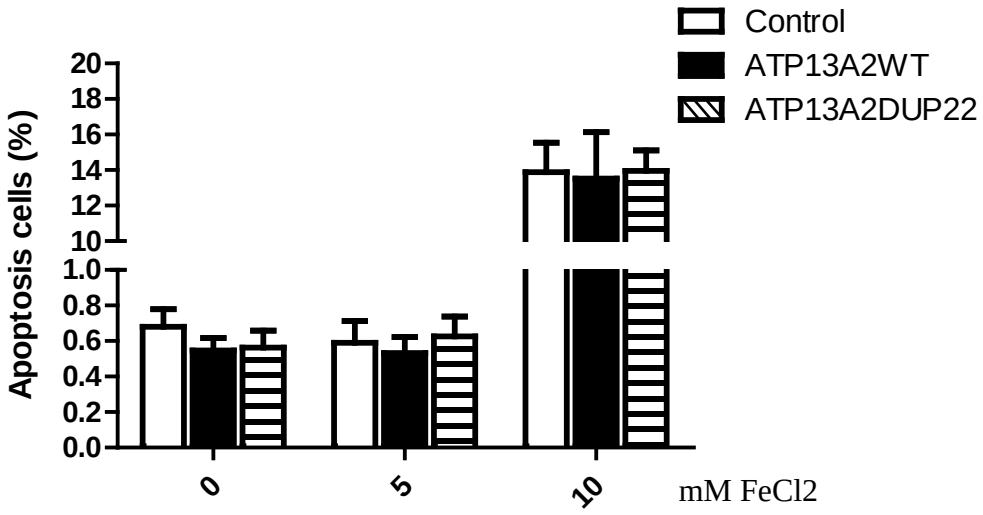
Supplementary Figure 1



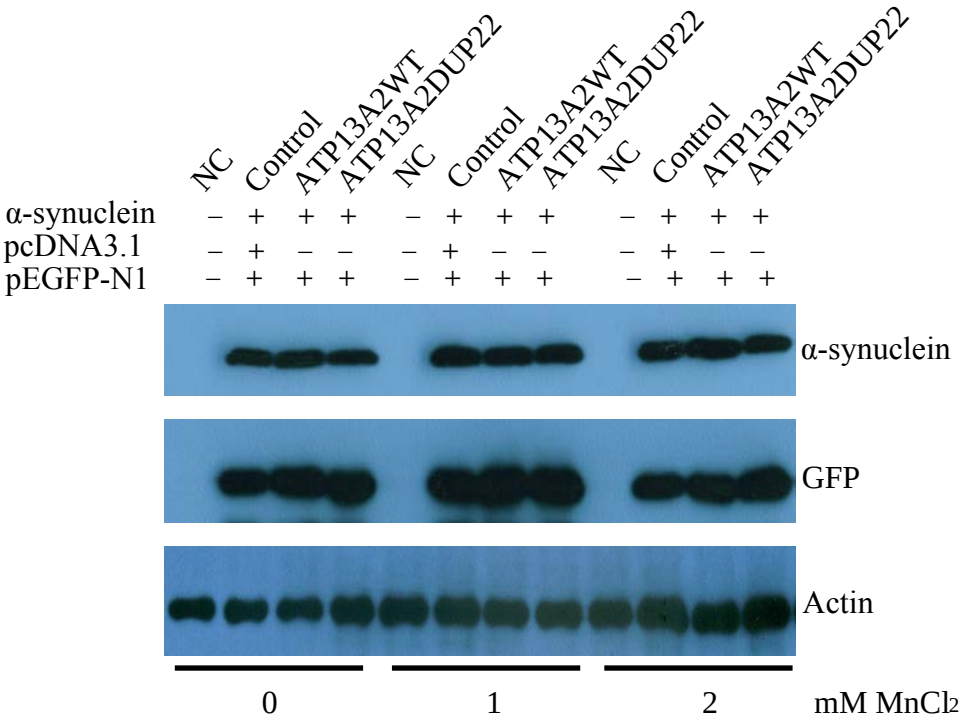
Supplementary Figure 2



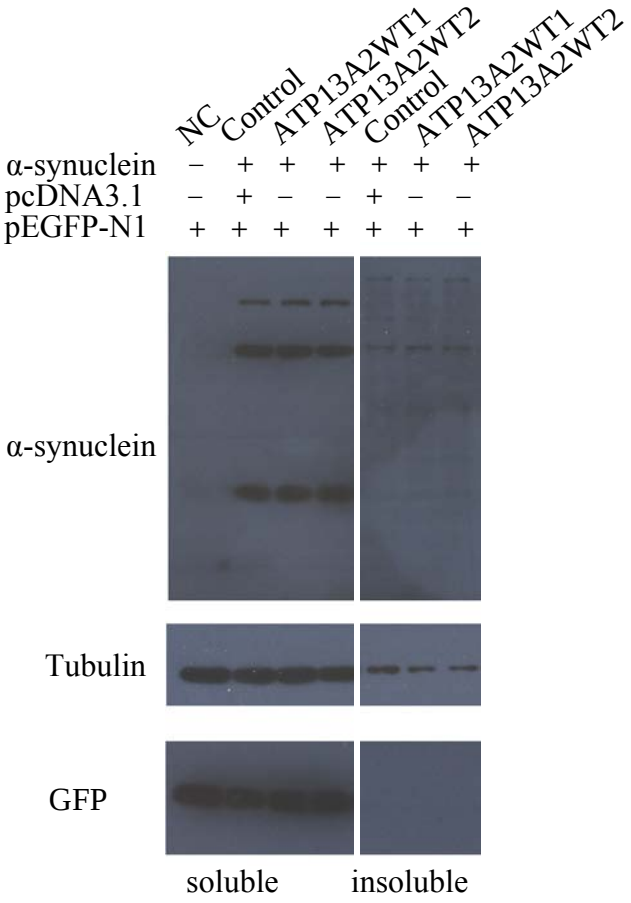
Supplementary Figure 3



Supplementary Figure 4



Supplementary Figure 5



Supplementary Figure 6