Supporting Information

VPS35 regulates parkin substrate AIMP2 toxicity by facilitating lysosomal clearance of AIMP2

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Figure S1. VPS35 enhances AIMP2 degradation via lysosome

(a) SH-SY5Y cells were transiently transfected with mock or V5-WT VPS35 and treated with NH₄Cl or distilled water as a vehicle (D/W). At 36 hrs post transfection, NH₄Cl (10 mM, 12 hrs) were used to treat cells. Total protein was extracted and immunoblotted with V5 and AIMP2 antibody to monitor their levels. β -actin was used as an internal loading control. (b) Normalized levels of AIMP2 expression in SH-SY5Y cells transfected with and treated with the indicated combination (n = 3). Error bars represent the mean \pm S.E.M. Two-way ANOVA followed by Bonferroni post hoc analysis was used for multiple group comparison. **P < 0.01, ***P < 0.001



Figure S2. AIMP2 accumulation has no effect on VPS35 levels

(a) SH-SY5Y cells were transiently transfected with control or FLAG-AIMP2. At 48 hrs post transfection, total protein was extracted and immunoblotted with FLAG and VPS35 antibody to monitor their levels. β -actin was used as an internal loading control. (b) Normalized levels of VPS35 in SH-SY5Y cells transfected with mock or FLAG-AIMP2 constructs (n = 3). Error bars represent the mean \pm S.E.M. Unpaired two-tailed Student's *t* test was used for statistical analysis.