Chai et al., http://www.jcb.org/cgi/content/full/jcb.201512016/DC1

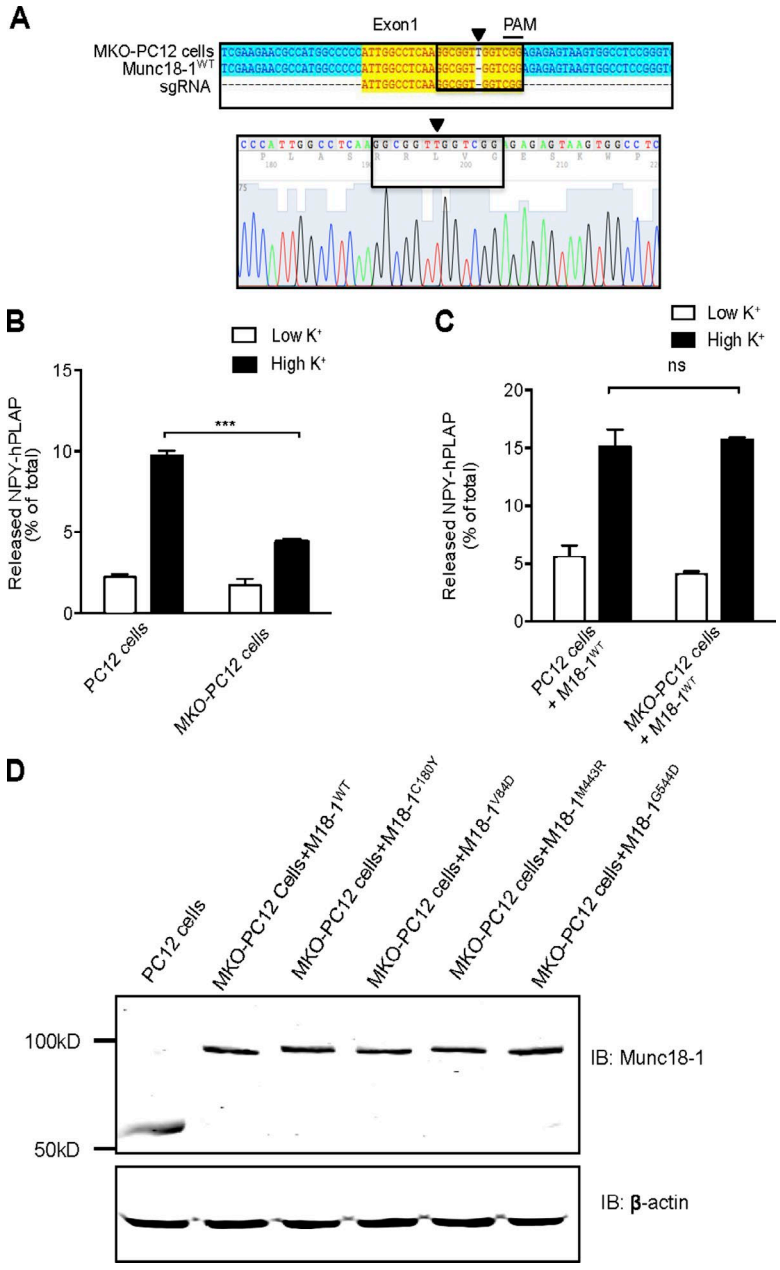

Figure S1. **Functional validation of MKO-PC12 cells and rescue of Munc18-1 expression levels.** (A) Sequence alignment of exon1 of the *Rattus norvegicus* Munc18-1 sequence and MKO-PC12 cells identified by genomic PCR. The complementary strand sequences corresponding to the 20-nt target and 3-nt PAM are highlighted in yellow. The inserted base is indicated in black and the sequence chromatogram of the inserted base region is shown. PC12 cells and MKO-PC12 cells were cotransfected with empty vector (B) or Munc18-1^{WT}-emGFP (C) and NPY-hPLAP washed and incubated in low K^+ or high K^+ (depolarizing) buffer for 15 min. The supernatant was assayed and expressed as the percentage total amount of hPLAP. Data represent mean ± SEM (n = 3 independent experiments; ***, $P < 0.001$; n.s, not significant, unpaired Student's t test). (D) MKO-PC12 cells were transfected with Munc18-1^{WT}-emGFP and Munc18-1 EIEE4-causing mutants, and the level Munc18-1 expression was probed by Western blotting with an anti–Munc18-1 antibody and compared with that of endogenous Munc18-1 in PC12 cells (loading control probed with anti–β-actin antibody).

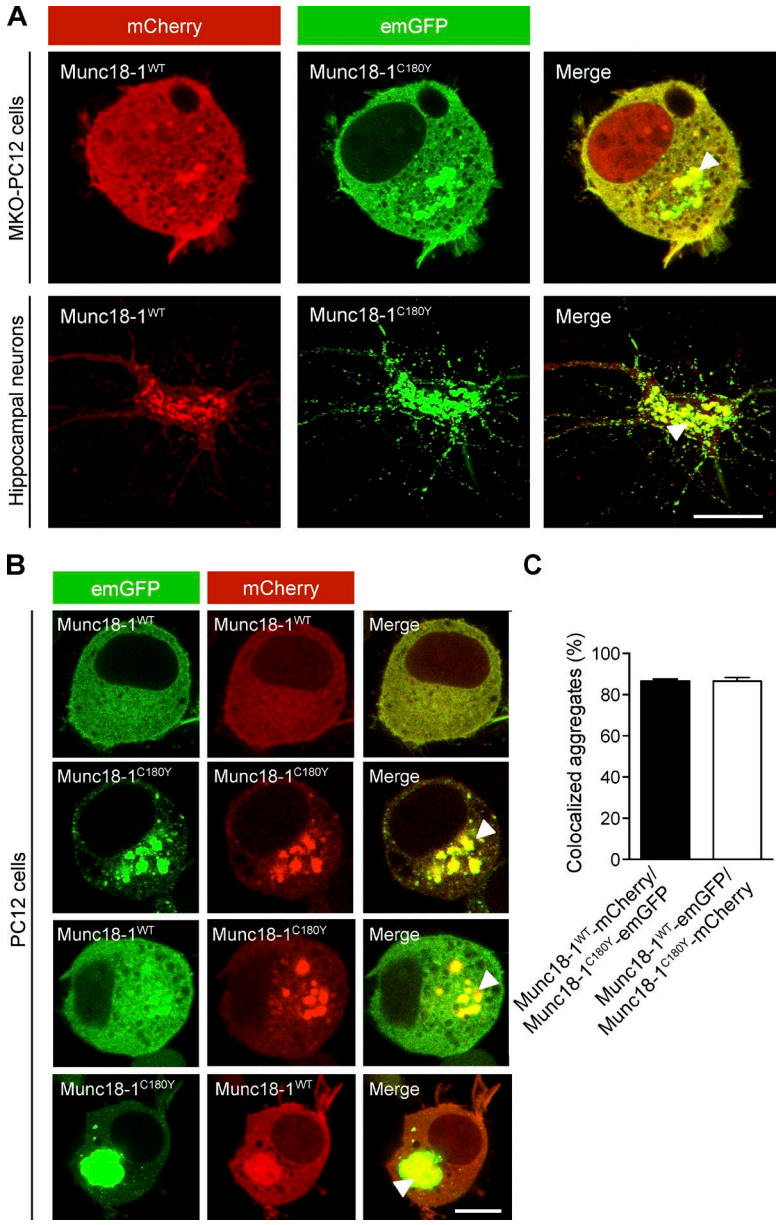

Figure S2. **The Munc18-1^{C180Y} mutant coaggregates with Munc18-1^{WT} in MKO-PC12 cells and hippocampal neurons.** PC12 cells were cotransfected with Munc18-1^{WT}-emGFP and Munc18-1^{WT}-mCherry (top) or Munc18-1^{C180Y}-emGFP and Munc18-1^{C180Y}-mCherry (middle) or either Munc18-1^{WT}-emGFP and Munc18-1^{C180Y}-mCherry or Munc18-1^{WT}-mCherry and Munc18-1^{C180Y}-emGFP (bottom). Note that the tags used here have been switched with those used in Fig. 2 (C and F). (A) Representative images showing coaggregates positive for Munc18-1^{WT} and Munc18-1^{C180Y} detected in MKO-PC12 cells and hippocampal neurons. Bar, 20 µm. Arrowheads indicate the colocalized aggregates. (B) Representative images showing coaggregates positive for Munc18-1^{WT} and Munc18-1^{C180Y} detected in PC12 cells. Bar, 20 µm. Arrowheads indicate the colocalized aggregates. (C) Percentage of colocalized aggregates per cell. Data represent mean ± SEM; 15–20 cells were analyzed for each independent experiment (n = 5).

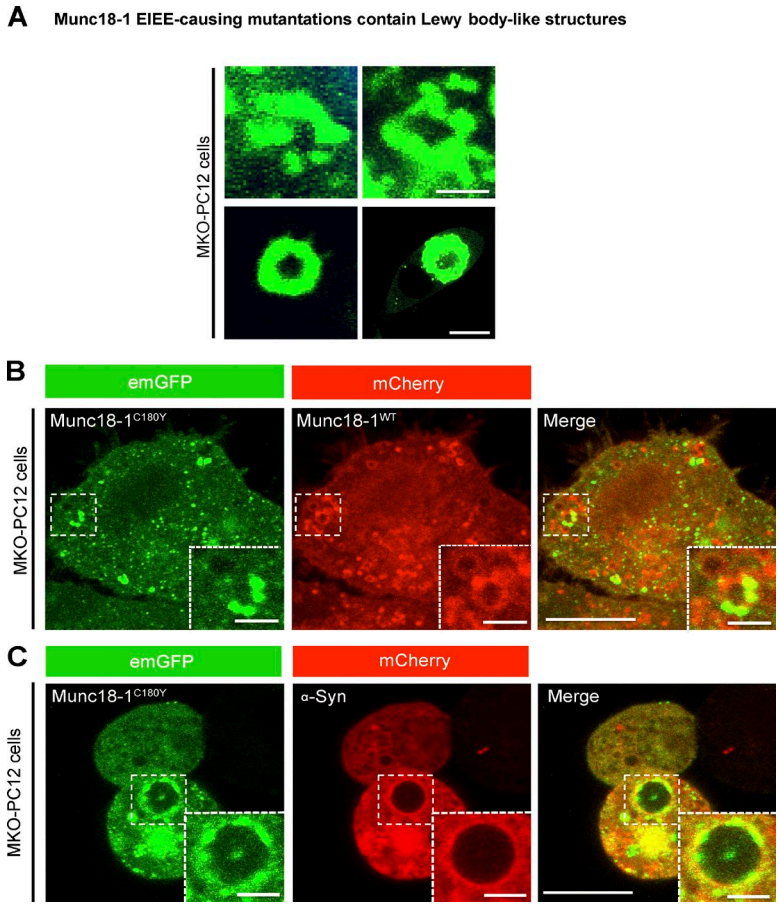

Figure S3. **Munc18-1^{C180Y} mutants cause Lewy body–like structures positive for Munc18-1^{WT} and α-Syn.** MKO-PC12 cells were transfected with Munc18-1^{C180Y} and imaged by confocal microscopy. (A) Representative images showing Lewy body–like structures from MKO-PC12 cells expressing Munc18-1^{C180Y}-emGFP. Bars: (top) 2 µm; (bottom) 10 µm. (B) Representative images showing Lewy body–like structures from MKO-PC12 cells cotransfected with Munc18-1^{C180Y}-emGFP and Munc18-1^{WT}-mCherry. Munc18-1^{C180Y}-emGFP aggregates were colocalized with Munc18-1^{WT}-mCherry in Lewy body–like structures. Bars: (main) 20 µm; (zoom) 2 µm. (C) Representative images showing Lewy body–like structures in MKO-PC12 cells cotransfected with Munc18-1^{C180Y}-emGFP and α-Syn^{WT}-mCherry. Bars: (main) 20 µm; (zoom) 5 µm. Munc18-1^{C180Y}-emGFP aggregates were colocalized with α-Syn^{WT}-mCherry Lewy body–like structures.

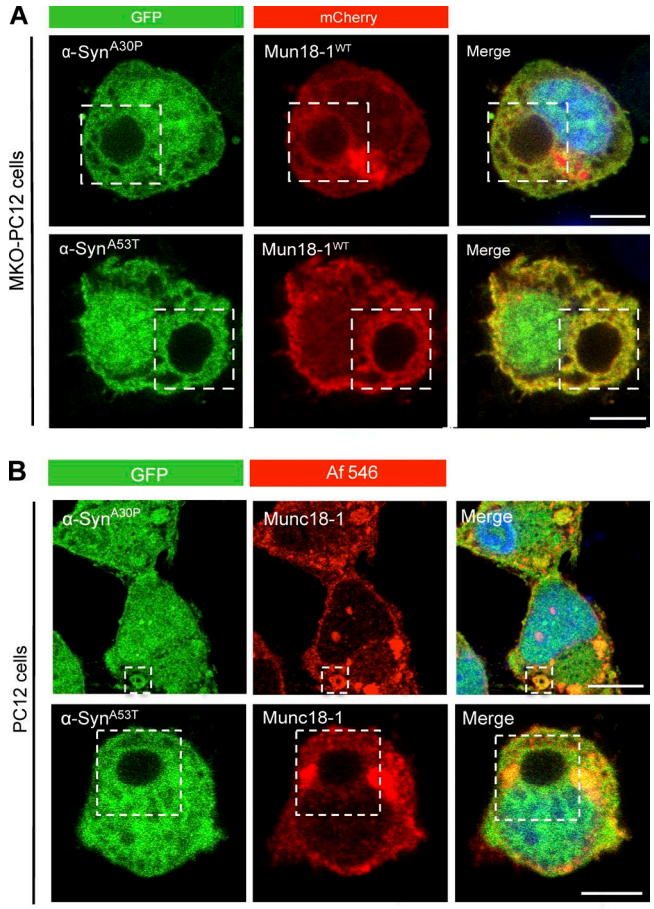

Figure S4. **PD-linked α-Syn mutants form Mun18-1^{WT} and endogenous Munc18-1–positive Lewy body–like structures.** (A) Representative images showing Lewy body–like structures from MKO-PC12 cells cotransfected with indicated GFP-tagged PD-linked α-Syn mutants and Munc18-1^{WT}-mCherry. Bar, 20 µm. (B) Representative images showing endogenous Munc18-1 present in Lewy body–like structures from PC12 cells transfected with indicated GFP-tagged PD-linked α-Syn mutants and immunolabeled with anti–Munc18-1. Bar, 20 µm.

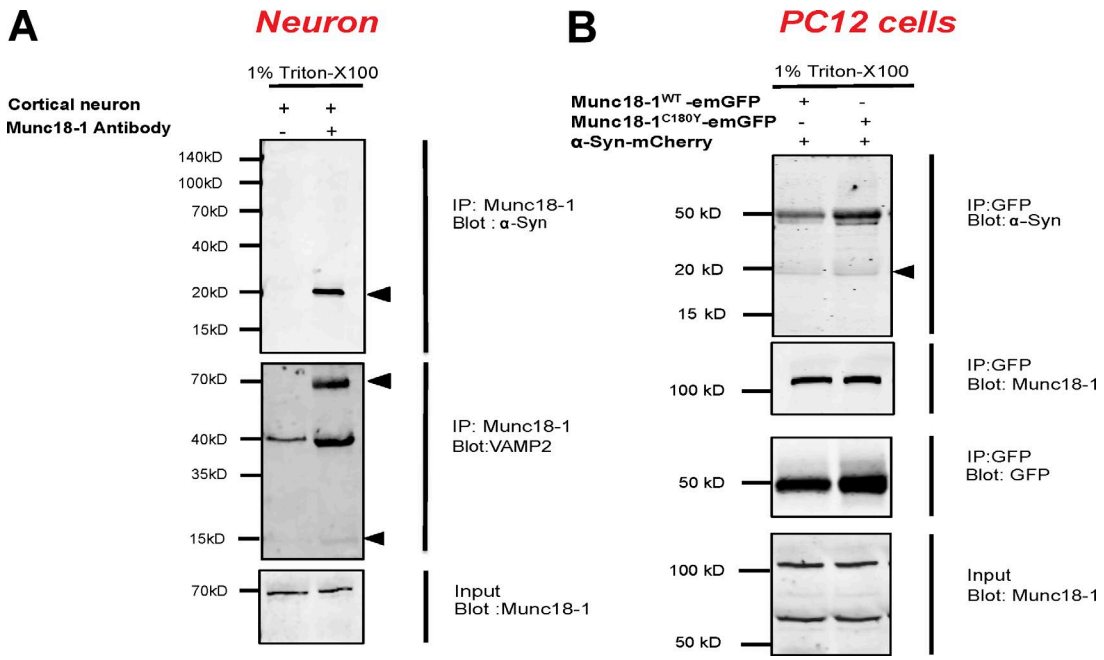

Figure S5. **Endogenous Munc18-1 interacts with endogenous α-Syn in cortical neurons and PC12 cells.** (A) Rat cortical neurons (19 d in vitro) were lysed, solubilized, and immunoprecipitated using an anti–Munc18-1 antibody. Bound proteins were analyzed by Western blotting and probed with anti–α-Syn and anti-VAMP2 antibodies. (B) PC12 cells were either cotransfected with Munc18-1^{WT}-emGFP and α-Syn-mCherry or Munc18-1^{C180Y}-emGFP and α-Syn-mCherry. Cells were lysed, solubilized, and immunoprecipitated with anti–Munc18-1 antibodies. Bound proteins were analyzed by Western blotting and probed with anti–α-Syn and anti–Munc18-1 antibodies.