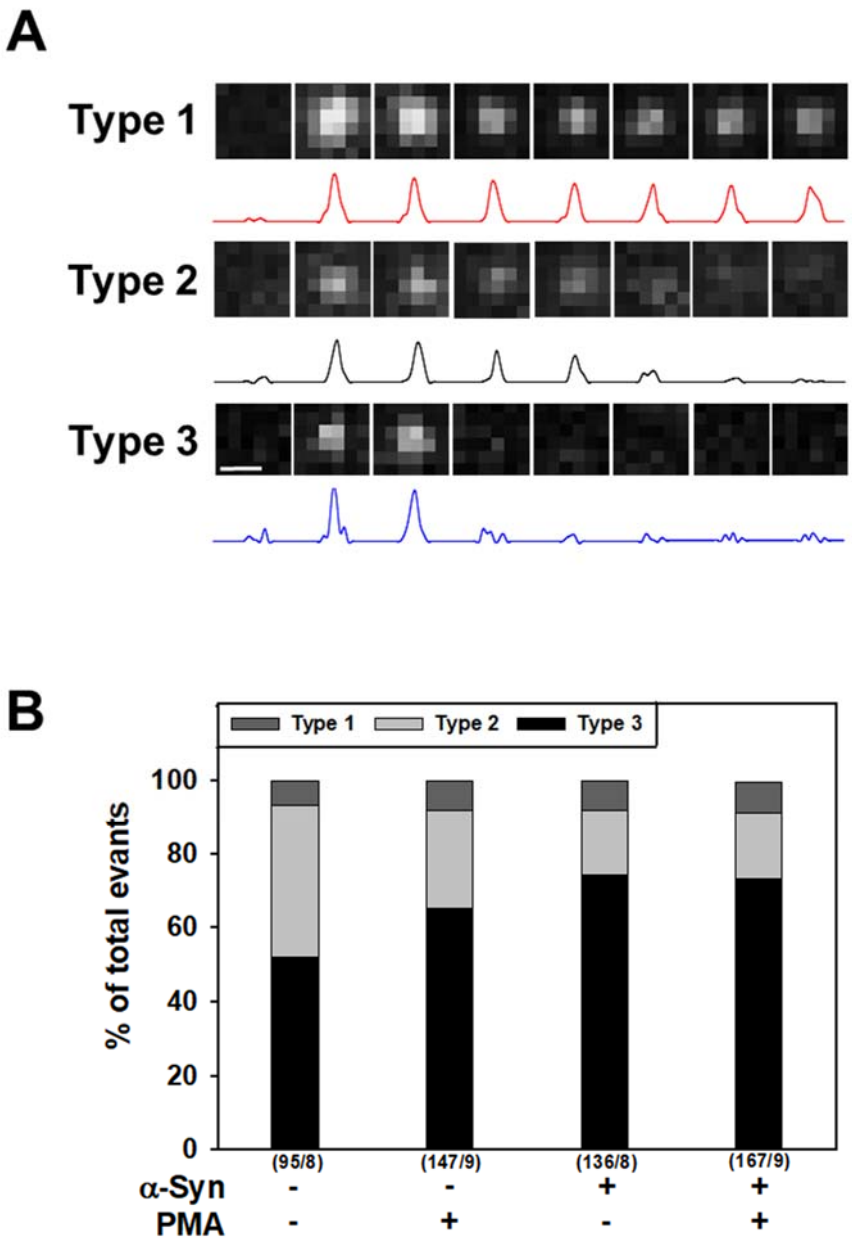

Figure S1: PMA and α-synuclein enhances exocytosis of NPY-pHluorin-labeled vesicles. PC12 cells were co-transfected with pECFP-C1 or pECFP-α-synuclein (α-Syn) and pNPY-pHluorin to monitor vesicle secretion. Experiment conditions for PMA treatment, ATP stimulation, TIRFM imaging and image analysis are similar to that described in the legend to Fig. 1. (A) The ATP-triggered fluorescence changes of the NPY-pHluorin-labeled vesicles can be divided into three patterns. Representative

time-lapse images of single vesicles show the different patterns of fluorescence changes. Traces under each image show the fluorescence intensity of the individual pixels across the center of the vesicle. (B) The distributions of the three patterns of NPY-pHluorin release are shown as a percentage of total exocytotic events. The number shown below each column represents the number of vesicles released events in 2 minutes and cells analyzed. The data were obtained from at least three independent transfections.

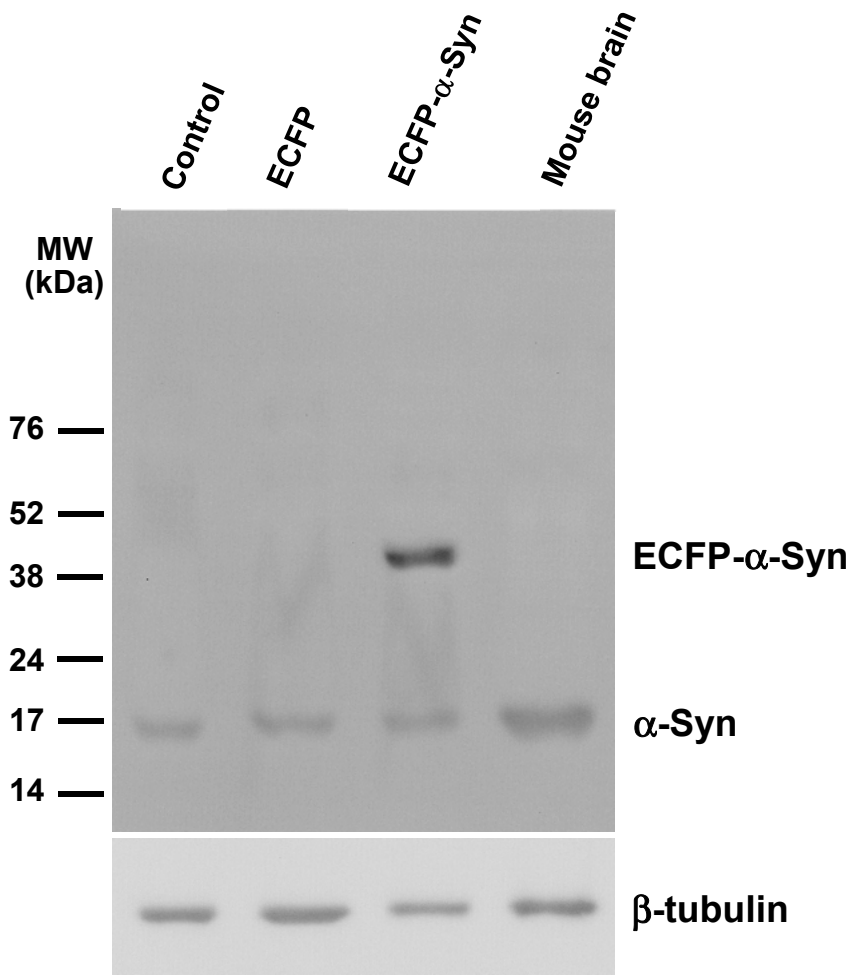

Figure S2: Endogenous and overexpression of α-Synuclein are monomeric. PC12 cells were co-transfected with pCI-puro (control) and pECFP-C1 (ECFP) or pECFP-α-synuclein (ECFP-α-Syn). After 24 hours, the transfected cells were treated with 5 µg/ml puromycin for 72 hours to select for α-synuclein-overexpressing cells. In the transfected cells, the expression of α-Synuclein was analyzed by Western blotting using α-Synuclein polyclonal antibodies. β-tubulin is shown as an internal control.

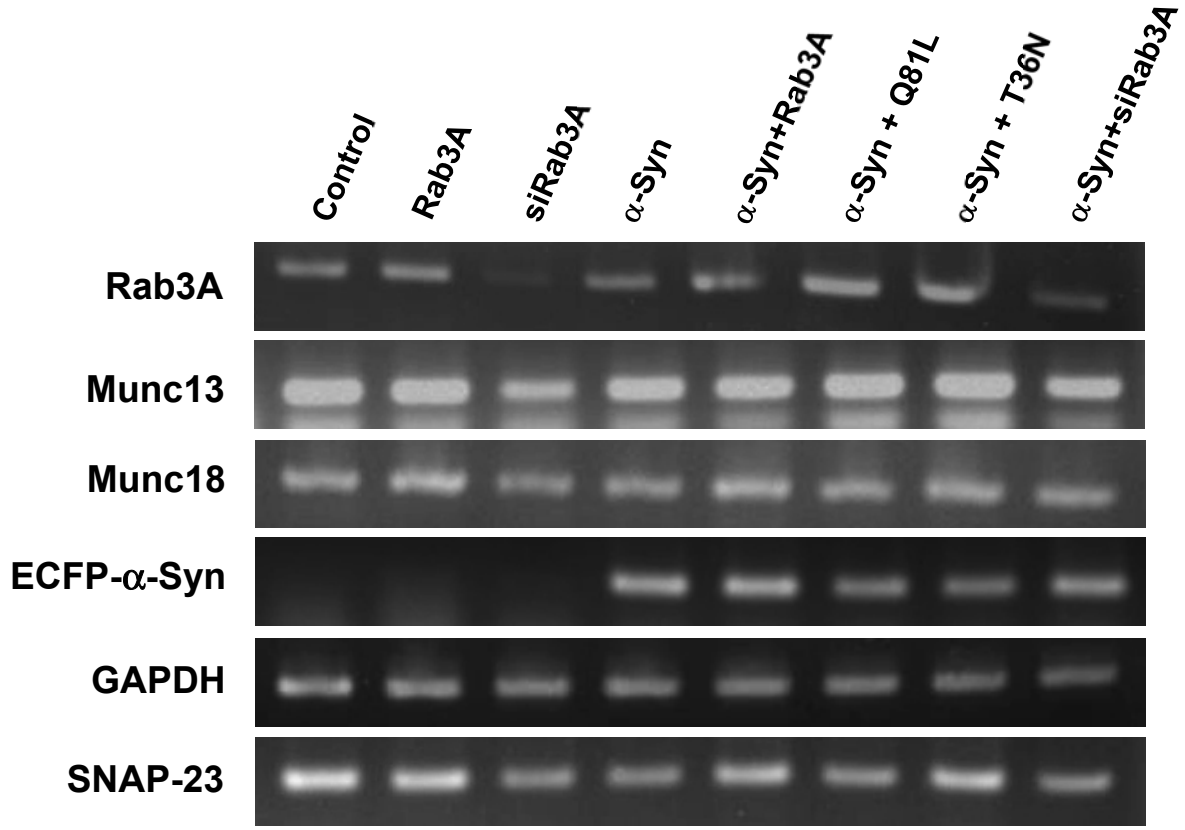

Figure S3: The mRNA level of the Rab3A/Munc13-1/Munc18-1/ECFP-α-Syn in PC12 cells transfected with various Rab3A mutant genes. PC12 cells were co-transfected with pCI-puro and pECFP-Rab3A (Rab3A), pECFP-Rab3A-Q81L (Q81L), pECFP-Rab3A-T36N (T36N), pSiliencer-Rab3A (siRab3A), or pECFP-α-Synuclein (α-Syn) as indicated. The transfected cells were selected by puromycin. The mRNA was extracted and used to perform RT-PCR using primers specific targeting the various mRNAs (Table S2). GAPDH and SNAP-23 were used as control.

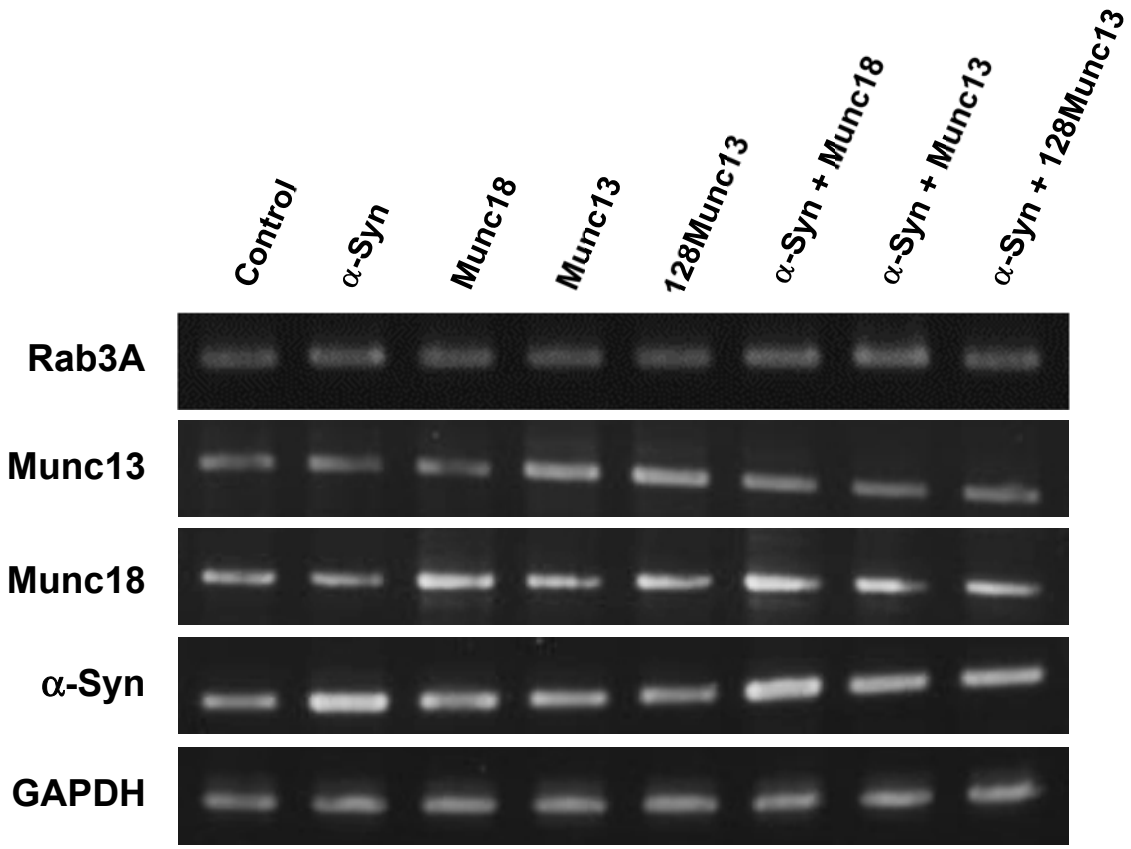

Figure S4: The mRNA level of the Rab3A/Munc13-1/Munc18-1/ECFP-α-Syn in PC12 cells transfected with various genes of Munc and mutants. PC12 cells were co-transfected with pCI-puro and pECFP-Munc18-1 (Munc18), pECFP-Munc13 (Munc13), pcDNA3.1-128-Munc13-1 (128Munc13), or pECFP-α-Synuclein (α-Syn) as indicated. The transfected cells were selected by puromycin. The mRNA was extracted and used to perform RT-PCR using primers specific targeting the various mRNAs (Table S2). GAPDH was used as a control.

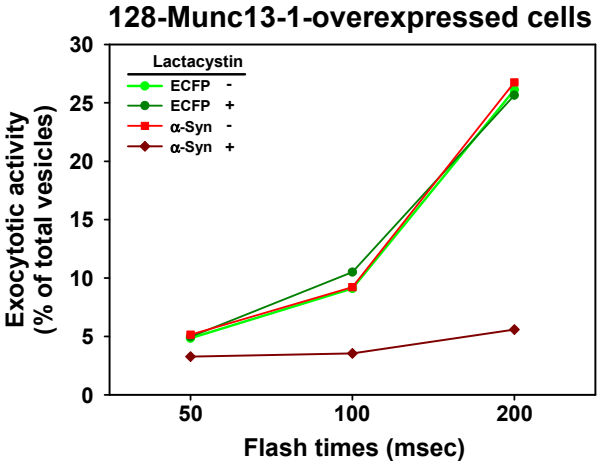

Figure S5: Effect of caged-calcium induced vesicle secretion in α-Synuclein expressed PC12 cells. PC12 cells were co-transfected with pNPY-EGFP, pcDNA3.1-128-Munc13-1 and pECFP-C1 (ECFP) or pECFP-α-Synuclein (α-Syn). After 48 hours, the transfected cells were loaded with 50 μM NP-EGTA AM at 37°C 45 minutes. Different uncaging flash times were used to increase Ca^{2+} release in order to induce secretion. Fluorescence changes affecting the NPY-EGFP-labeled vesicles upon flash photolysis were monitored by TIRF microscope. The exocytotic activities of the cells are shown as percentages of total NPY-EGFP-labeled vesicles in the evanescent field that were secreted. Data shown are the means and were obtained from at least three independent transfections (n=8~10).

The effect of PMA on morphological docked vesicle in evanescent field

α-synuclein	**-**	**+**
Control buffer	113.0 ± 7.9	109.5 ± 9.0
PMA	105.7 ± 9.6	106.1 ± 11.9

Table S1. PMA and α-synuclein have no effect on morphological docked vesicle in evanescent field. NPY-EGFP overexpressing PC12 cells were pre-treated with either control buffer or 100 nM PMA for 10 minutes. The numbers of NPY-EGFP-labeled vesicles in the evanescence field before ATP stimulation were monitored by TIRF microscope and analyzed as described in Materials and Methods. The data shown are the means ± s.e.m. from the number of cells as indicated in the parenthesis of Fig. 1B.

Gene		Primer sequence (5'→3')	Size
Rab3A	F	ATGGCTTCCGCCACAGACT	604
	R	ACCGGTGACCGCAGGGTCTGC	
Munc13-1	F	AGAACAGAATGATGATCCC	329
	R	TCATCCAGCTCTGAGCCCTG	
Munc18-1	F	GAGGATGAATACTGGAGAG	597
	R	GTGCTGAAGGATGCGGAAG	
α-Synuclein	F	CATGAAAGGACTTTCAAAGG	309
	R	GCTCCTTCTTCATTCTTG	
ECFP-αSyn	F	GATCACATGGTCCTGCT	452
	R	GCTCCTTCTTCATTCTTG	

Table S2. Primers used for various genes. Primers are shown in 5′ to 3′ direction.