Supplemental Data

Supplemental Figure 1. α-Synuclein expressed in COS cells changes from "closed" to "open" conformation on membrane binding.

(A) Lysates prepared from COS cells transfected with fluorescent α-synuclein fusion constructs were immunoblotted for GFP. Although all constructs undergo some degradation, this remains a small proportion of the total. (B) Transfected COS cell lysates (20 μg/ml) were incubated with either 1 mM binding or control membranes, and the FRET measured. YsynC shows reduced FRET in the presence of membranes containing acidic but not control lipids. There was no intermolecular FRET between CFP (C) and YFP (Y), or between Ysyn and synC. The values indicate mean ± SEM. n=3-15 wells per group. (C) YsynC and YβsynC show a similar reduction in FRET in the presence of membranes containing acidic but not control lipids. Ysyn100C exhibits greater baseline FRET than YsynC, but shows a similar reduction with membranes. In contrast, the control Y4EBP1C has a lower baseline FRET than Ysyn100C, and does not change on membrane binding. The values indicate mean ± SEM. n=6-8 wells per group. (D) Ysyn80C but not A30P-Ysyn80C shows reduced FRET in the presence of membranes containing acidic but not control lipids. The direct fusion YC shows high FRET not affected by membranes. The values indicate mean ± SEM. n=3-15 wells per group.

Supplemental Figure 2. α-Synuclein exhibits a "closed" conformation in living neurons.

(A) In neurons expressing YsynC, the direct fusion YC and the intermolecular pairs synC and synY, and synC and Ysyn, CFP donor fluorescence was measured from individual cells before and after acceptor photobleaching. This data was used to generate Figures 5A-D. Only the CFP

fluorescence of cells expressing YsynC or YC consistently increases after photobleaching. (B) Scatter plot of FRET values for the individual cells shown in (A).

Supplemental Figure 3. Concentration does not significantly change the "closed" conformation of α-synuclein.

(A) The FRET signal of increasing concentrations of FsynC was measured. Although there was a significant relationship between FRET and increasing concentration ($p<0.0001$ by one way ANOVA), the extent of increase was very small. The values indicate mean ± SEM. n=8 wells per group.

(B) In neurons expressing YsynC or the direct fusion YC, linear regression was performed between the initial YFP value (as a surrogate for extent of expression) and FRET. There was no significant correlation between the two variables, in either the cell body or bouton. r^2 is the square of the correlation coefficient, and represents the proportion of variability in FRET that is accounted for by the initial YFP value. This data was used to generate Figures 5A-D.

Supplemental Figure 4. Brain mitochondria contain endogenous α-synuclein and purity of mitochondrial fractions.

(A) Purified rat brain mitochondria (BM), synaptosomal fractions LP1 (synaptic plasma membrane), LP2 (synaptic vesicles) and LS2 (synaptic cytosol), and the starting homogenate were immunoblotted for endogenous α-synuclein as well as the plasma membrane Na/K ATPase, mitochondrial ATP synthase and the synaptic vesicle protein synaptophysin. A constant fraction of the total yield for each group was loaded (1/1000 for all groups except total

homogenate which was 1/15,000). In addition to a large soluble pool in LS2, endogenous α-synuclein again shows preferential enrichment in BM and LP1, which contain ATP synthase, over LP2, which does not, consistent with the localization of α-synuclein to brain mitochondria. (B) Brain mitochondria, liver mitochondria and liver endoplasmic reticulum fractions were immunoblotted for calreticulin (a marker for the endoplasmic reticulum) and cytochrome C (a protein in the mitochondrial intermembrane space). Brain and liver mitochondria fractions have small and moderate amount of contamination with endoplasmic reticulum, respectively, while endoplasmic reticulum fractions have very little mitochondrial content.