Pranke et al., http://www.jcb.org/cgi/content/full/jcb.201011118/DC1

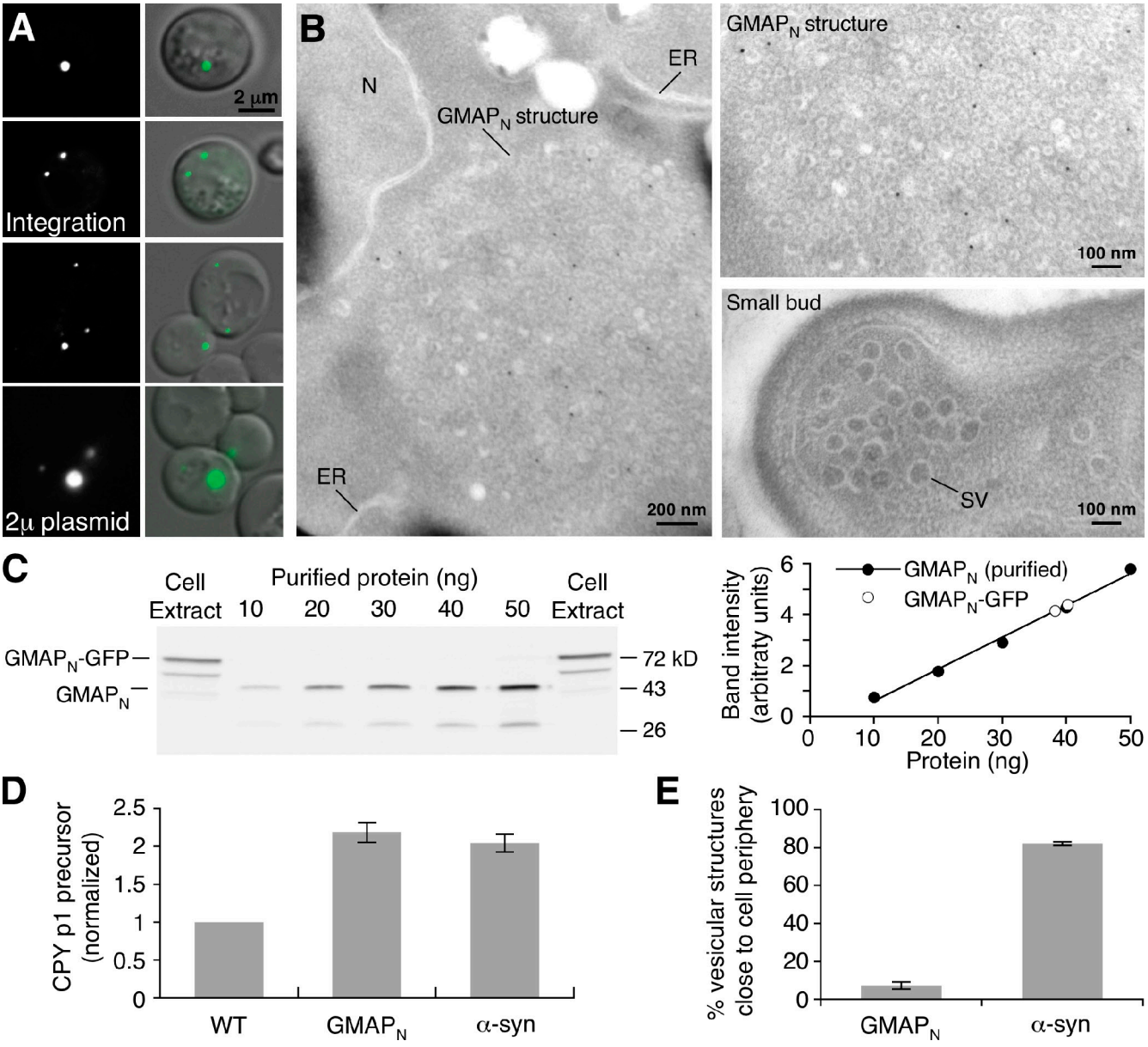

Figure S1. **Expression of GMAP$_N$-GFP and α-synuclein–GFP in yeast.** (A) GMAP$_N$-GFP expression in strain IPY4 URA3::TetO$_2$-GMAP$_N$-GFP GAL1::GMAP$_N$-GFP (top, Integration) and BY4742/pIP7 (2μ TetO$_2$-GMAP$_N$-GFP; bottom, 2μ plasmid). Approximately 25% of BY4742 and SEY6210 cells carrying pIP7 showed visible GFP puncta, with a variable number and size of puncta per cell. IPY4 had approximately twice as many cells with visible puncta and a more homogeneous distribution in these cells. The forth-row (bottom) fluorescence images were not subjected to deconvolution to represent more closely the actual size of the structures. (B) Immunogold labeling of BY4742/pIP7 cells after overnight induction of GMAP$_N$-GFP using GFP antibodies. (top right) A magnification of a portion of the GMAP$_N$ structure in the left is shown compared with a cell containing secretory vesicles (SV; bottom right). N, nucleus. (C) Quantification of GMAP$_N$-GFP in yeast cells. (left) Total cell extracts from two separate cultures of IPY4 GMAP$_N$-GFP cells (~1.10^6 cells of each) were loaded onto the first and seventh lane. In the second to the sixth lane, the indicated quantity of purified GMAP$_N$ was loaded to generate a standard curve (right, solid circles). The quantity of GMAP$_N$-GFP in cell extracts was determined (right, open circles) based on quantifications of the Western blot shown (left). Results shown are representative of four independent experiments. (D) SEY6210 (wild type [WT]), IPY4 (GMAP$_N$), and IPY5 (α-synuclein [α-syn]) were grown under inducing conditions overnight, and cells were prepared for Western blot analysis using CPY antibodies. p1 precursor and mature CPY levels were quantified as described in Materials and methods, and the fraction of p1 precursor to total CPY for IPY4 and IPY5 was normalized to the amount in wild-type cells. (E) BY4742/pIP7 (GMAP$_N$-GFP) and BY4742/pIP29 (α-synuclein–GFP) cells grown overnight under inducing conditions. For each experiment, 20 electron micrographs for each strain were scored for the presence of vesicular structures within 50 nm of the cytoplasmic side of the cell wall. Results show means and SDs of at least three independent experiments.

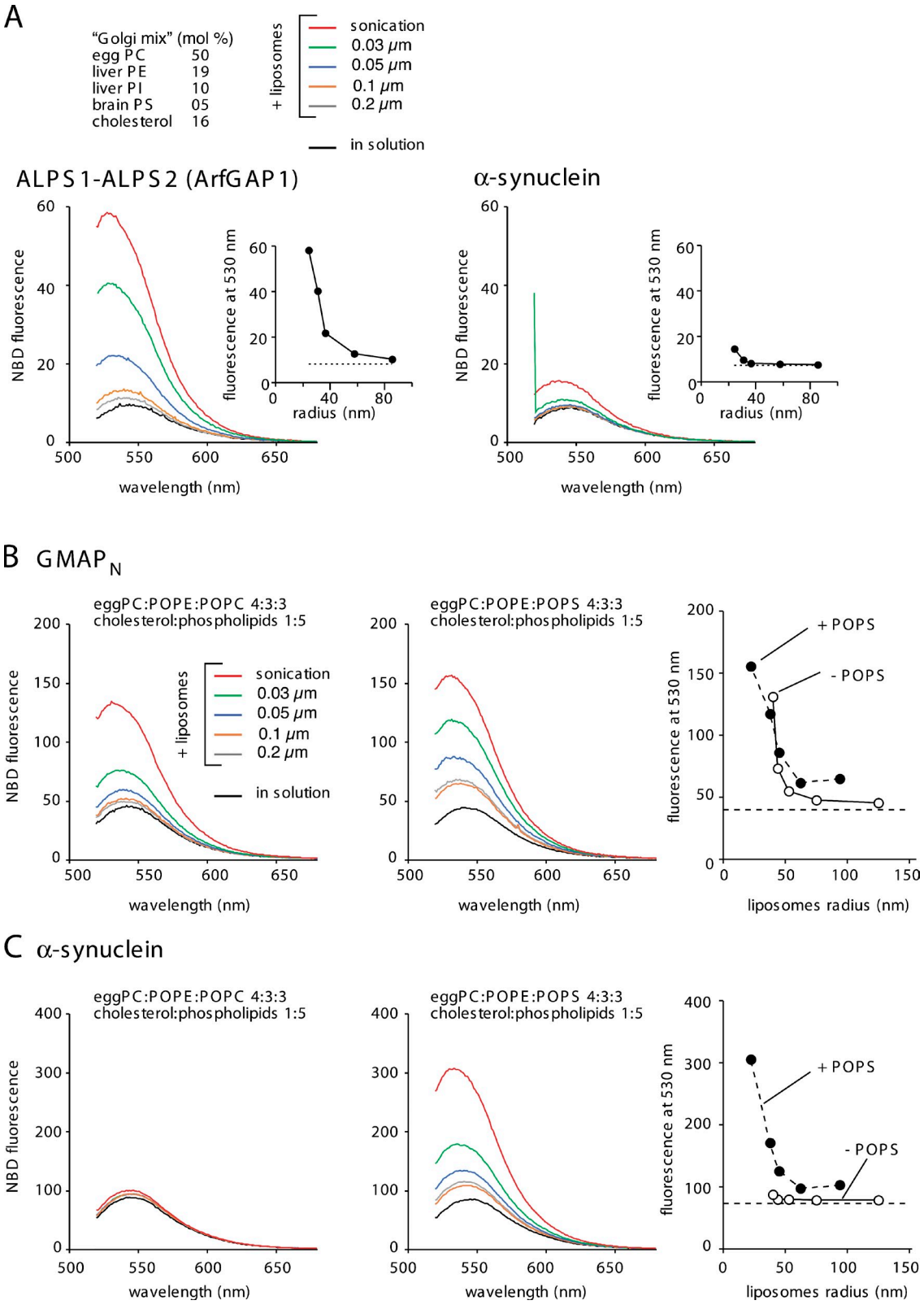

Figure S2. **α-Synuclein, but not ALPS motifs, are sensitive to liposome charge.** (A) Binding of the ALPS1-[NBD]-ALPS2 tandem (Mesmin et al., 2007) of ArfGAP1 (250 nM; left) or 250 nM [NBD]α-synuclein (right) to Golgi mix liposomes of defined size (150 μM lipids) as determined by NBD fluorescence. (B and C) Binding of 125 nM [NBD]GMAP$_N$ (B) or 250 nM [NBD]α-synuclein (C) to neutral liposomes (egg PC/POPE/POPC = 4:3:3; cholesterol/phospholipids = 1:5; phospholipid concentration 100 μM) or anionic liposomes (egg PC/POPE/POPS = 4:3:3; cholesterol/phospholipids = 1:5; phospholipid concentration 100 μM). The plots show the fluorescence level at 530 nm as a function of liposome radius (as determined by dynamic light scattering). The horizontal dashed lines indicate the fluorescence level in the absence of liposomes. The experiments in B and C were performed with larger excitation and emission bandwidths and in a different fluorescence cuvette than that used in the other panels and figures, hence the difference in fluorescence intensity.

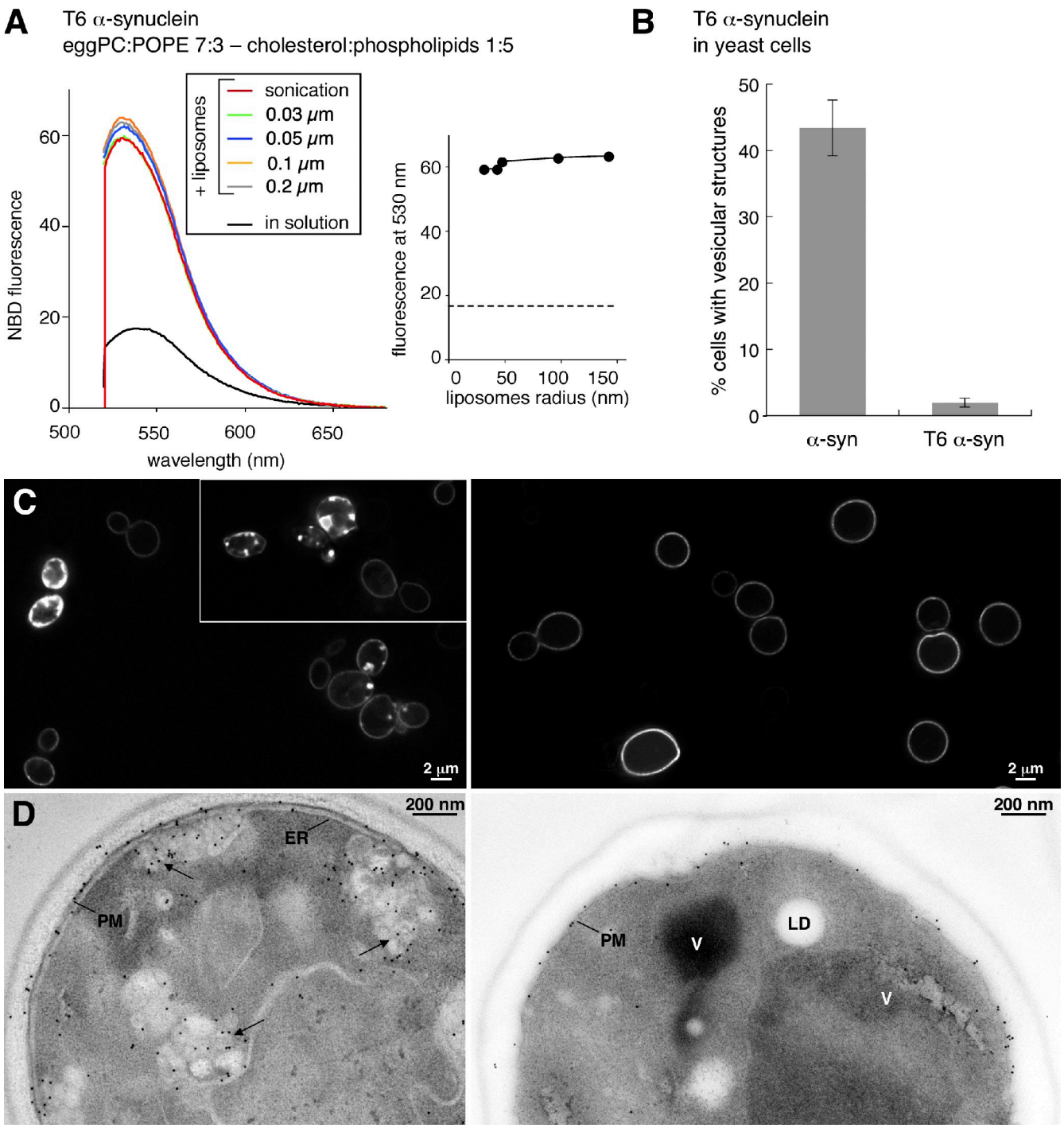

Figure S3. **The sextuple T6 mutant of α-synuclein binds to liposomes in a curvature-independent manner and does not cause accumulation of vesicular structures in yeast cells.** (A, left) Emission fluorescence spectra of the sextuple T6 mutant of α-synuclein (α-syn; 125 nM) with or without calibrated neutral liposomes obtained by extrusion or sonication (150 μM phospholipids; egg PC/POPE = 7:3; cholesterol/phospholipids = 1:5). This experiment was performed with the same liposomes as those used in Fig. 2 B. (right) Fluorescence level at 530 nm as a function of liposome radius (as determined by dynamic light scattering). The horizontal dashed line indicates the fluorescence level in the absence of liposomes. Note that the T6 mutant binds to these liposomes regardless of their size in marked contrast to wild-type α-synuclein, which does not bind at all, and $GMAP_N$, which binds in a curvature-dependent manner (see Fig. 2 B). (B–D) BY4742 cells carrying plasmid pIP29 α-synuclein (left) or a derivative encoding the α-synuclein T6 mutant protein (right) were grown overnight under inducing conditions and imaged by confocal microscopy (B and C) or prepared for immuno-EM using GFP antibodies (D). Wild-type and T6 α-synuclein proteins were expressed to equivalent levels. Inset in C shows a different field of cells at the same magnification. Arrows point to vesicular structures. PM, plasma membrane; V, vacuole; LD, lipid droplet.

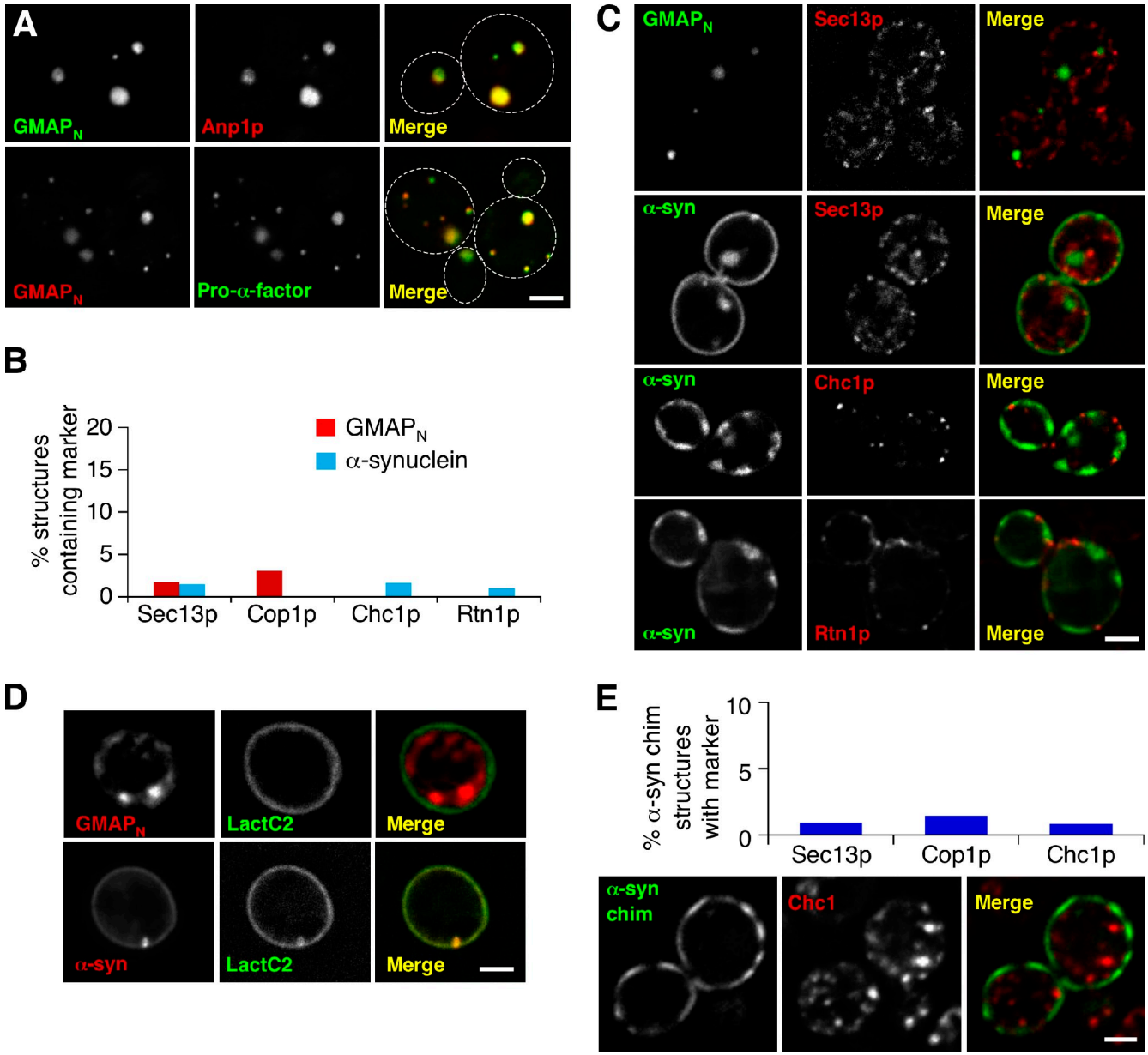

Figure S4. **Confocal microscopy analysis of GMAP$_N$, α-synuclein, and α-synuclein chimera coexpressed with different markers.** (A) Anp1-mRFP cells expressing GMAP$_N$-GFP (top) and SEY6210 cells expressing GMAP$_N$-mCherry and pro–α-factor–citrine (bottom) were induced overnight and imaged. Quantifications are shown in Fig. 4 A. Outlines of cells are indicated by dashed lines. (B) Sec13p-mRFP, Cop1p-mRFP, Chc1p-mRFP, and Rtn1p-mRFP strains were induced overnight for expression of GMAP$_N$-GFP or α-synuclein and imaged. For GMAP$_N$-GFP, 60 structures (and for α-synuclein-GFP, between 120 and 170 structures) from cells imaged in three separate experiments were scored for the presence or absence of the mRFP-tagged coat subunit, and the percentage with coat was calculated. (C) Representative images of cells expressing GMAP$_N$-GFP or α-synuclein–GFP with the indicated markers. (D) BY4742 cells coexpressing GMAP$_N$-mCherry or α-synuclein–mCherry with LactC2-GFP were grown overnight under inducing conditions and imaged. (E, top) The α-synuclein chimera (α-syn chim) expressed in Sec13p-mRFP, Cop1p-mRFP, or Chc1p-mRFP cells was induced overnight. α-synuclein chimera–GFP structures (between 320 and 370) from cells imaged in three separate experiments were scored for the presence or absence of the mRFP-tagged coat subunit, and the percentage with coat was calculated. (bottom) Representative images are shown. Bars, 2 µm.

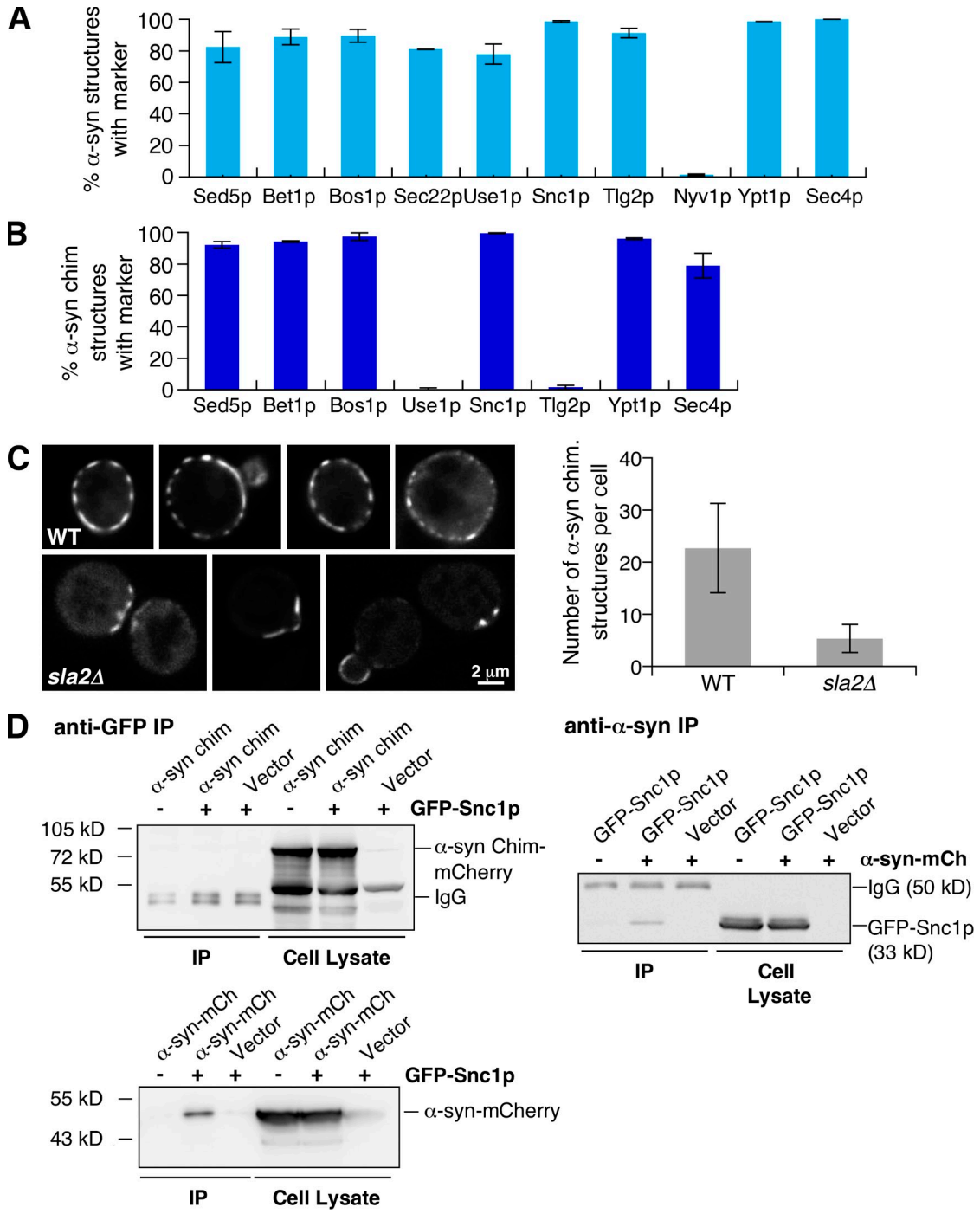

Figure S5. **Comparison of α-synuclein and α-synuclein chimera colocalizations and interactions, and effects in the *sla2Δ* endocytosis mutant.** (A and B) SEY6210 cells expressing α-synuclein–GFP (A) or the α-synuclein chimera–GFP (B) and the indicated protein N-terminally tagged with mCherry were grown overnight under inducing conditions and imaged. The percentages of GFP structures with the mCherry marker were determined. Means and SD of three independent experiments are shown. (C) *sla2Δ*, *sac6Δ*, *pil1Δ*, *lsp1Δ*, *sur7Δ*, *abp1Δ*, *syp1Δ*, *ede1Δ*, and wild-type (WT) BY4742 cells expressing the α-synuclein chimera (α-syn chim) were induced overnight and imaged. A significant difference in the α-synuclein chimera structures was seen in *sla2Δ* mutant cells compared with wild type (left). The number of α-synuclein chimera structures per cell was scored for each strain. Means and SDs of three independent experiments are shown. (D) Lysates of cells expressing GFP-Snc1p and either the α-synuclein chimera or α-synuclein (α-syn) fused to mCherry were immunoprecipitated with GFP antibodies. Immunoprecipitations (IP) were subjected to Western analysis using GMAP-210 (top left) or α-synuclein antibodies (bottom left). (right) Cell lysates expressing α-synuclein–mCherry (mCh) and GFP-Snc1p as indicated were immunoprecipitated with α-synuclein antibodies, and Western blotting was performed with GFP antibodies. In all three experiments, one half of the immunoprecipitations and 2.5% of total cell lysates were loaded where indicated.

Table S1. ***Saccharomyces cerevisiae* strains used in this study**

Strain	Genotype	Reference
BY4742	*MATα his3Δ1 leu2Δ0 lys2Δ0 ura3Δ0*	Research Genetics
Anp1-mRFP	BY4742 *ANP1-mRFP::kanMX6*	Huh et al., 2003
Sec13-mRFP	BY4742 *SEC13-mRFP::kanMX6*	Huh et al., 2003
Cop1-mRFP	BY4742 *COP1-mRFP::kanMX6*	Huh et al., 2003
Chc1-mRFP	BY4742 *CHC1-mRFP::kanMX6*	Huh et al., 2003
Rtn1-mRFP	BY4742 *RTN1-mRFP::kanMX6*	This study
BY4742 *sla2Δ*	BY4742 *sla2Δ::kanMX4*	Research Genetics
BY4742 *sac1Δ*	BY4742 *sac1Δ::kanMX4*	Research Genetics
BY4742 *abp1Δ*	BY4742 *abp1Δ::kanMX4*	Research Genetics
BY4742 *ede1Δ*	BY4742 *ede1Δ::kanMX4*	Research Genetics
BY4742 *isp1Δ*	BY4742 *isp1Δ::kanMX4*	Research Genetics
BY4742 *pil1Δ*	BY4742 *pil1Δ::kanMX4*	Research Genetics
BY4742 *sur7Δ*	BY4742 *sur7Δ::kanMX4*	Research Genetics
BY4742 *syp1Δ*	BY4742 *syp1Δ::kanMX4*	Research Genetics
SEY6210	*MATα ura3-52 his3-Δ200 leu2-3,-112 trp1-Δ901 lys2-801 suc2-Δ9*	Robinson et al., 1988
IPY4	SEY6210 *URA3::TetO$_2$-GMAP$_N$-yEGFP GAL1::GMAP$_N$-yEGFP-His3MX6*	This study
IPY5	SEY6210 *URA3::TetO$_2$-α-synuclein-yEGFP GAL1::α-synuclein-yEGFP-His3MX6*	This study
IPY6	SEY6210 *URA3::TetO$_2$-α-syn chim-yEGFP GAL1::α-syn chim-yEGFP-His3MX6*	This study

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