SUPPLEMENTARY FIGURE LEGENDS

Figure S1. (A) Anti-syntaxin 5 immunoblot of cell lysates from the experiment in Figure 1A and B. Both syntaxin 5 isoforms were more than 90% depleted from the NRK cell population. (B) Intensity parameters used to calculate the transport index in the experiment in Figure 1D. Note that the differences between experimental conditions are less robust and the error bars are larger when the individual intensity parameters are compared, as opposed to the transport index. This is because calculation of the transport index inherently normalizes for differences in VSV-G-GFP expression, cell size and morphology. Error bars represent standard errors. (C) Comparison of α-synuclein expression levels in total extracts of transiently transfected NRK cells (as would normally be used in our transport assays) and whole rat brain lysate. Immunoblotting was carried out with a monoclonal antibody that recognizes human and rat α-synuclein.

Figure S2. (A) 3-Methyl adenine treatment amplifies α-synuclein particles. NRK cells expressing α-synuclein A53T were treated for 3 hours with the carrier DMSO, 10 mM 3-methyl adenine (3-MA), or 10 μM MG132 prior to fixation and anti-synuclein immunostaining. Shown are widefield epifluorescence images. (B) Quantification of the number of α-synuclein particles per cell in 50 randomly imaged cells. Intensity thresholds in Openlab software were used to objectively define and count the particles. (C) NRK cells transfected with α-synuclein A53T contain significantly more LC3-positive bodies than mock-transfected cells. See Methods for quantification details. Serum starved cells are included as a positive control for induction of macroautophagy. Error bars represent standard errors. Where indicated, p values were generated using a two-tailed Student's t-test. (D) α-Synuclein A53T is not visible in LC3-positive structures by immunofluorescence. Occasionally overlapping objects were seen (not shown), however, whether these were significant is unknown. Shown is a merged image of matching individual focal planes of deconvolved widefield images.

Figure S3. Subcellular distributions of α-synuclein A53T and the unperturbed endogenous cellular transport machinery. (A) Comparison between α-synuclein A53T and sec22b staining patterns. (B) Comparison of α-synuclein A53T and sec31. (C) Comparison of α-synuclein A53T and p115. (D) Comparison of α-synuclein A53T and p24. In each block shown, green asterisks mark the nucleus of an α-synuclein A53T-expressing cell and red asterisks mark the nucleus of an untransfected cell for comparison. Shown are individual focal planes of deconvolved widefield images. Dashed blue squares denote the regions of the top images in each block that are digitally magnified in the lower images.

Figure S4. Representative anti-myc-VSV-G immunoblot quantified in the experiment shown in Figure 8A.

Figure S5. (A) Glutathione beads preloaded with GST or GST-α-syn A53T were incubated with purified soluble SNAREs as described in the legend to Figure 9A. Panel shows the Ponceau staining of the same bead binding reactions that are immunoblotted in Figure 9A. (B) Glutathione beads preloaded with GST-membrin were incubated with the soluble proteins listed above the blots as described in the legend to Figure 9C. Panel shows the Ponceau staining of the same bead pellets that are immunoblotted in Figure 9C, top blot. The yield of immobilized GST-membrin is consistent. (C) Purified soluble sec22b, syntaxin 5, membrin, and rbet1 were co-incubated with either purified GST or GST/α-syn A53T as detailed in the Figure 9E legend and the Methods. Just prior to injection onto the Superdex 200 column, small samples of the soluble incubation mixtures were saved and subsequently immunoblotted for each of the four SNAREs as indicated along the left, to ascertain whether α-synuclein significantly affected the amount of soluble SNARE proteins. Shown are the results for the same experiment as Figure 9E; other experiments showed similar small effects on protein yield.

FIGURE S1

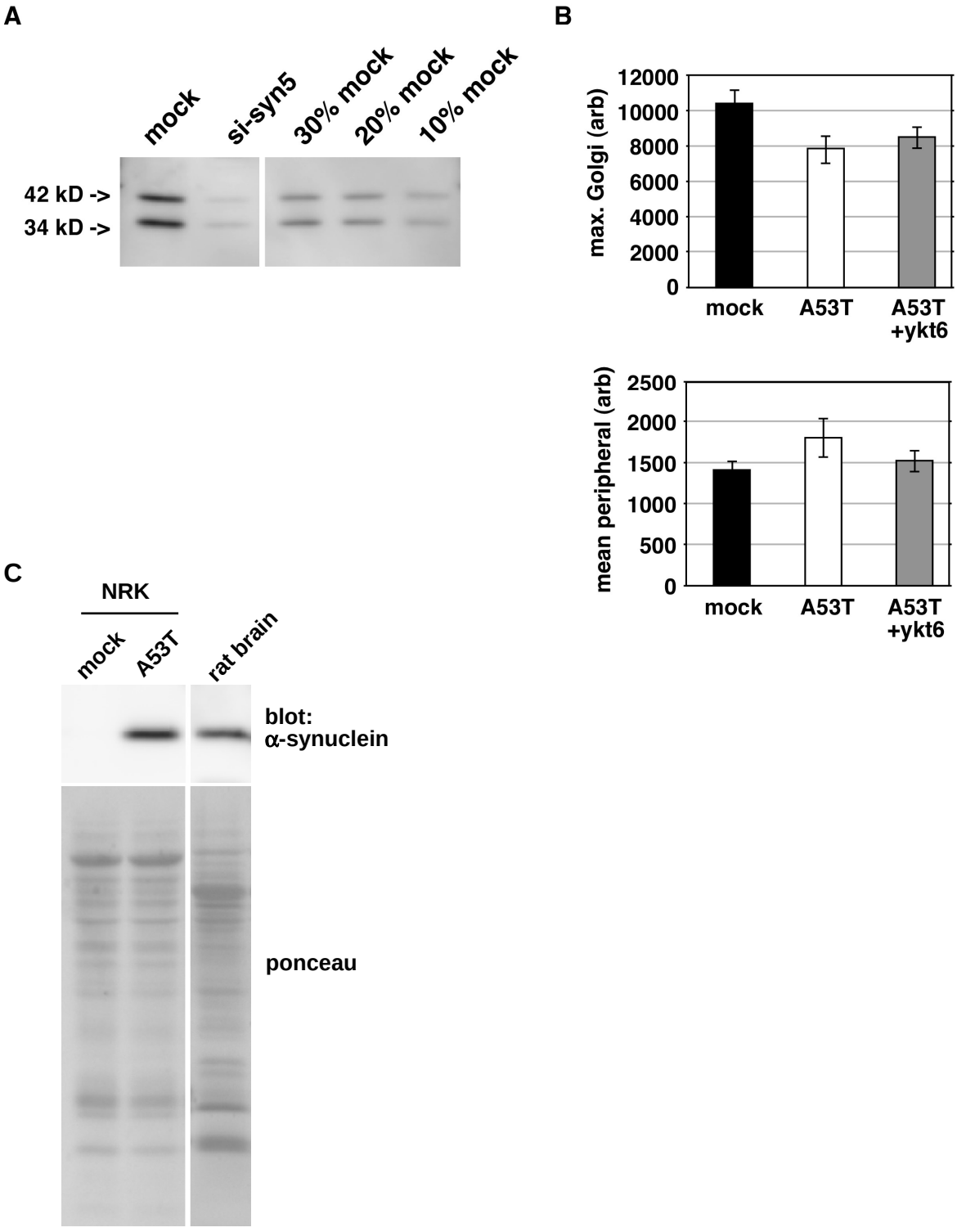
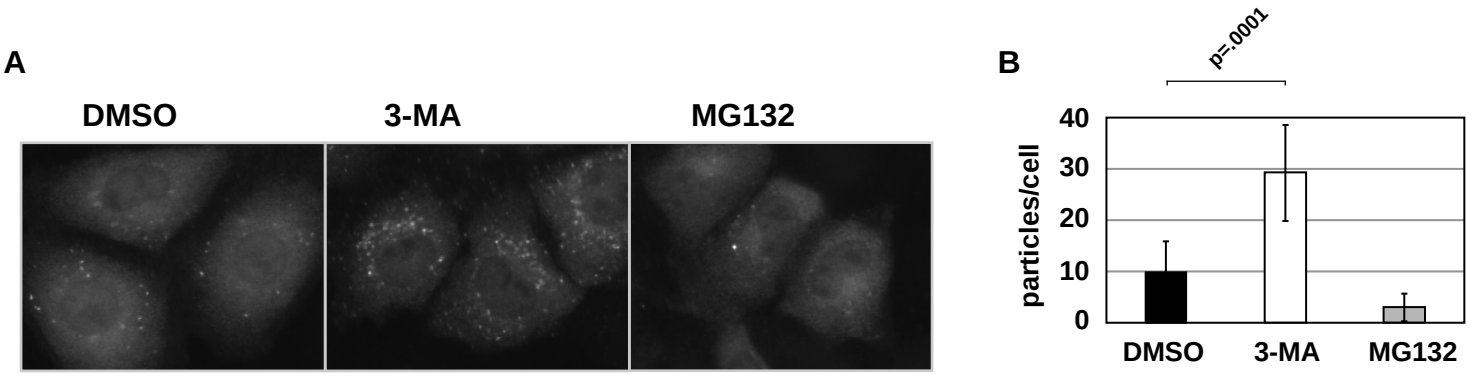

A
mock
si-syn5
30% mock
20% mock
10% mock
42 kD ->
34 kD ->
B
max. Golgi (arb)
12000
10000
8000
6000
4000
2000
0
mock
A53T
A53T +ykt6
mean peripheral (arb)
2500
2000
1500
1000
500
0
mock
A53T
A53T +ykt6
C
NRK
mock
A53T
rat brain
blot: α-synuclein
ponceau

FIGURE S2


A
DMSO
3-MA
MG132
B
p=.0001
particles/cell
40
30
20
10
0
DMSO
3-MA
MG132

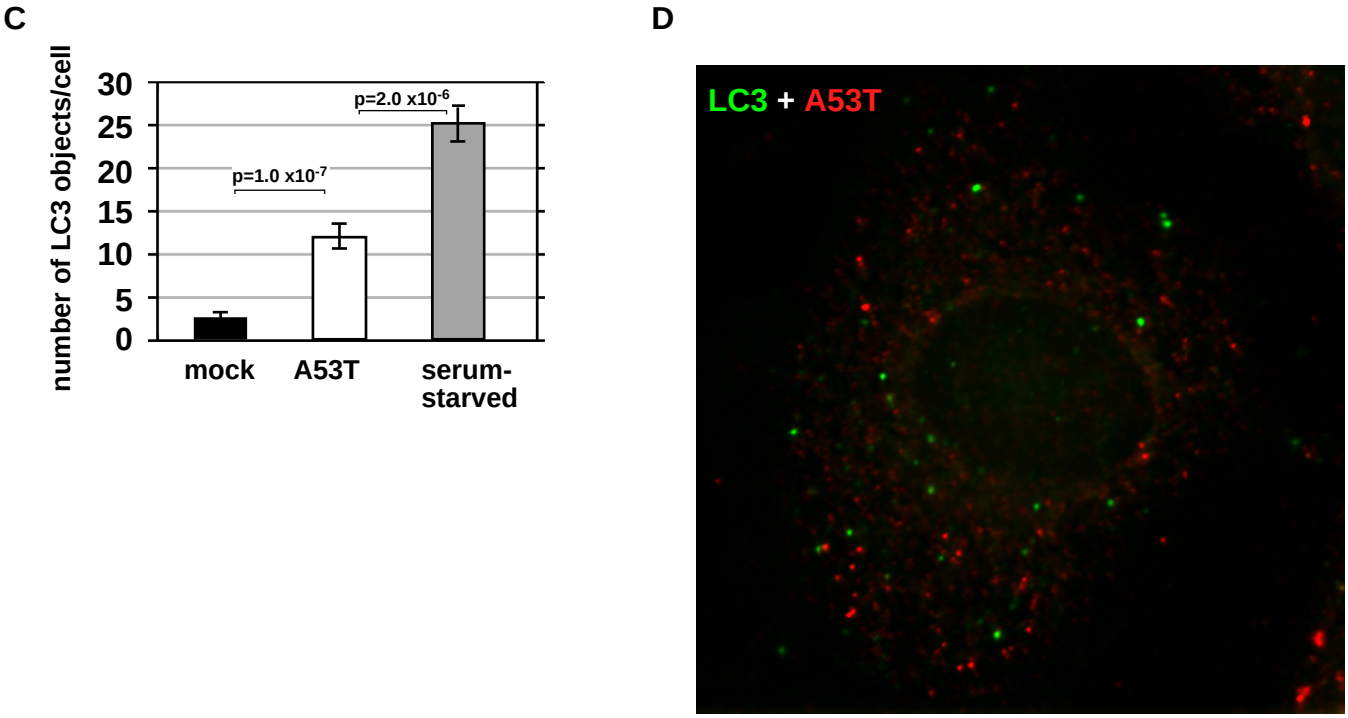

C
number of LC3 objects/cell
30
25
20
15
10
5
0
p=1.0 x10-7
p=2.0 x10-6
mock
A53T
serum-starved
D
LC3 + A53T

FIGURE S3

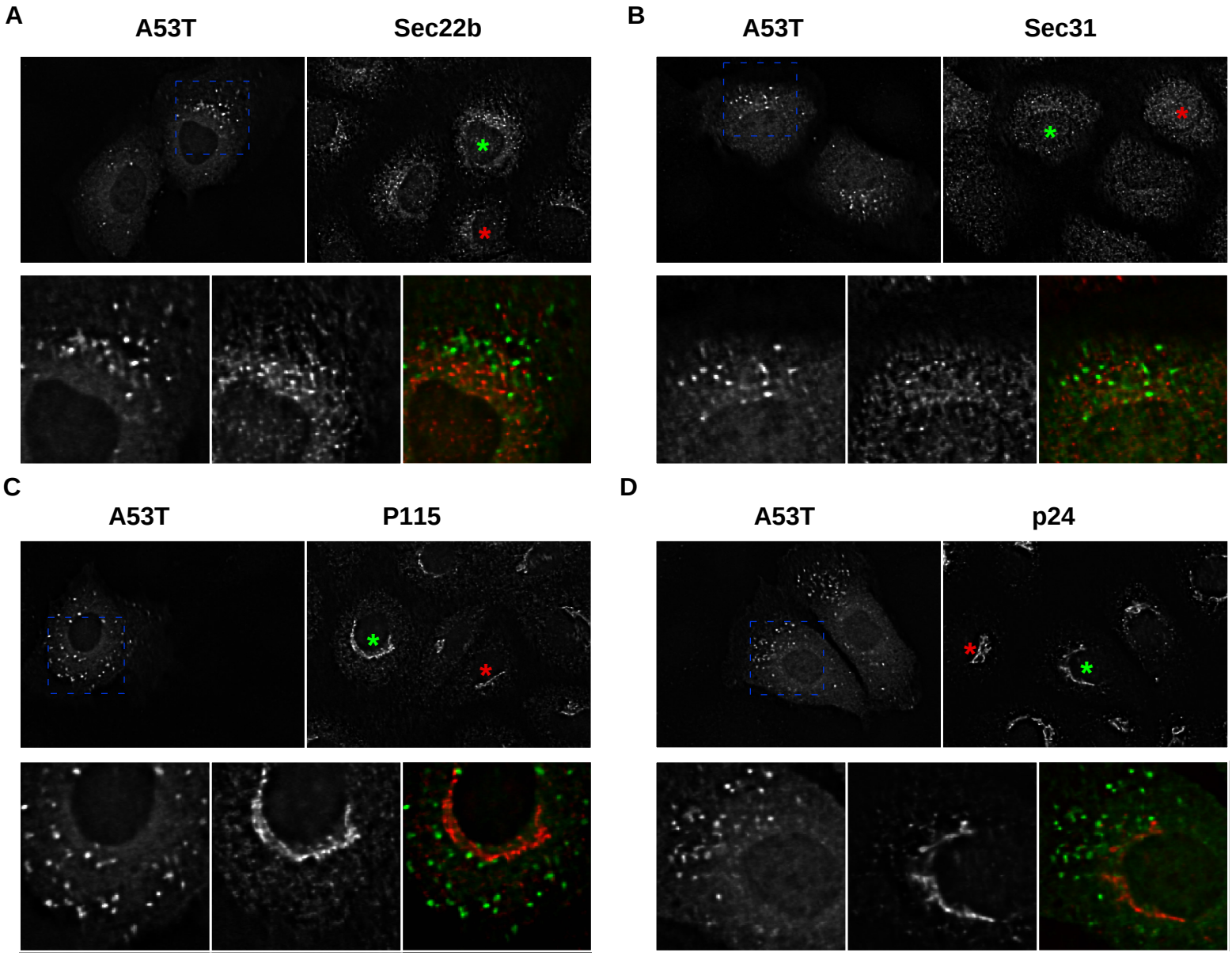


FIGURE S4

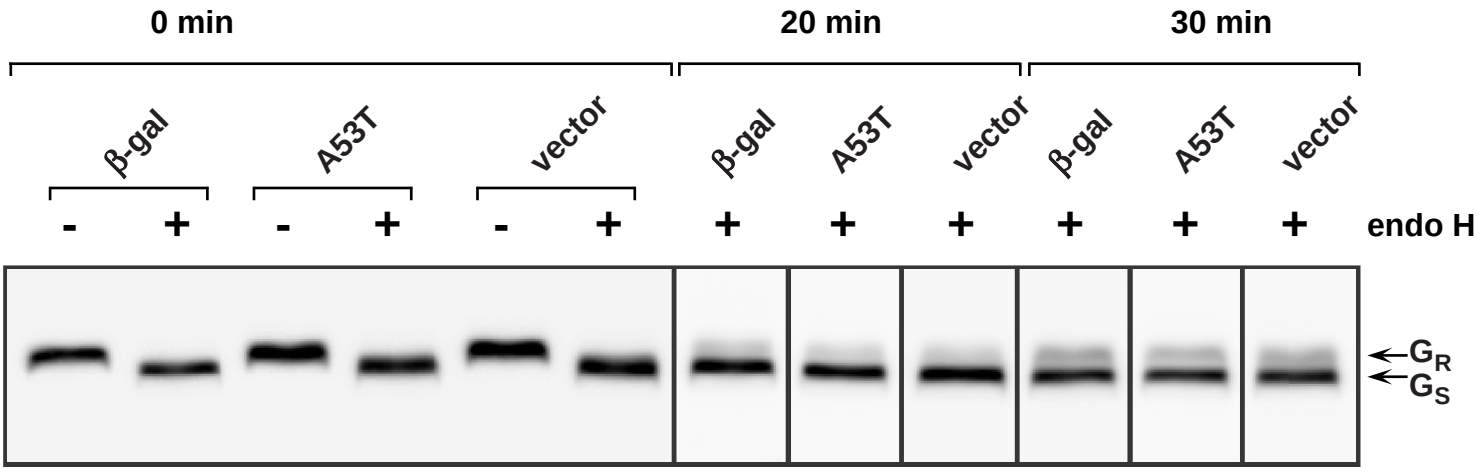
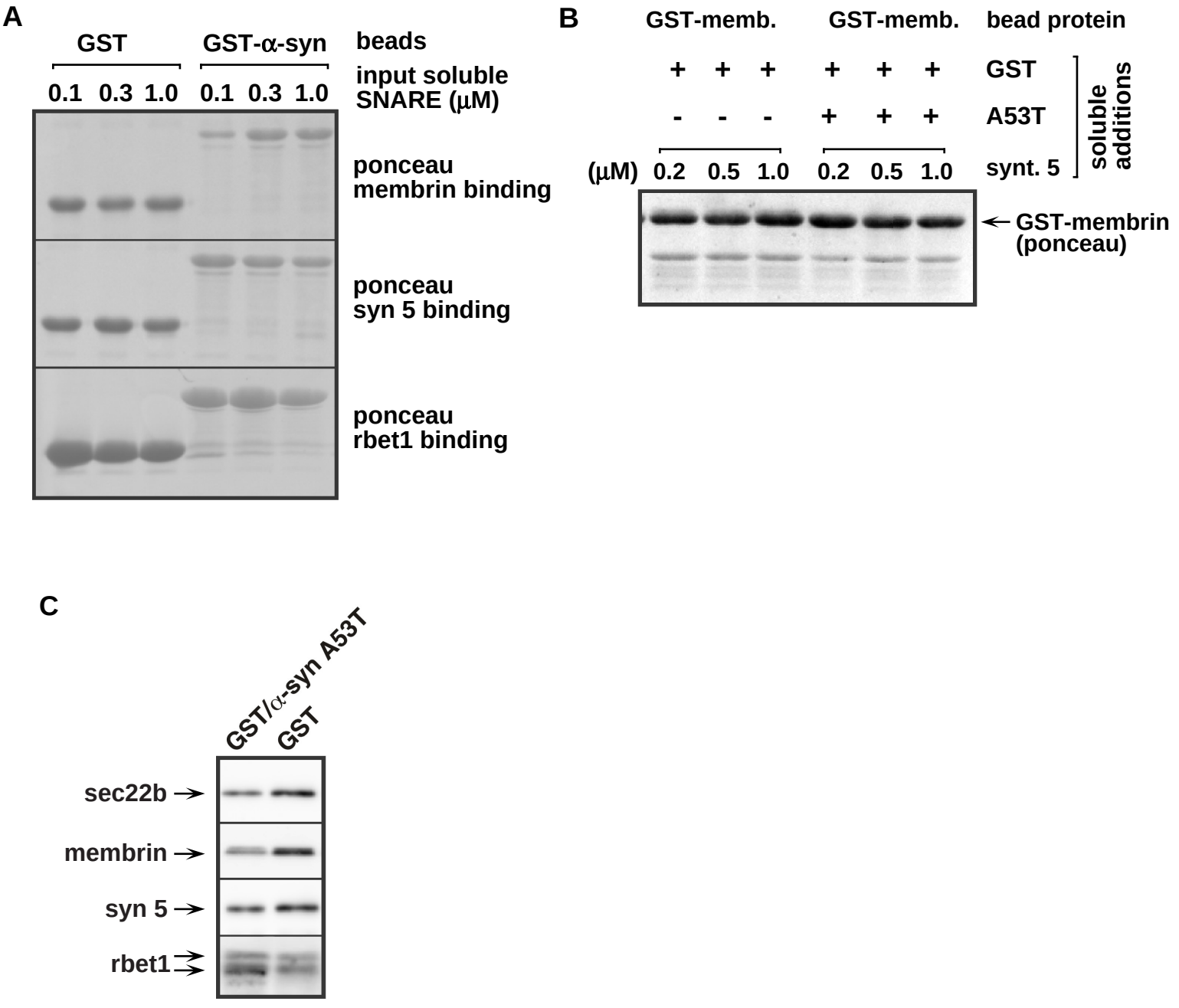

0 min
20 min
30 min
β-gal
A53T
vector
β-gal
A53T
vector
β-gal
A53T
vector
- + - + - + + + + + + +
endo H
G_R
G_S

FIGURE S5


A
GST
GST-α-syn
beads
0.1 0.3 1.0 0.1 0.3 1.0
input soluble SNARE (μM)
ponceau membrin binding
ponceau syn 5 binding
ponceau rbet1 binding
B
GST-memb.
GST-memb.
bead protein
+ + + + + + GST
- - - + + + A53T
soluble additions
(μM) 0.2 0.5 1.0 0.2 0.5 1.0 synt. 5
GST-membrin (ponceau)
C
GST/α-syn A53T
GST
sec22b
membrin
syn 5
rbet1