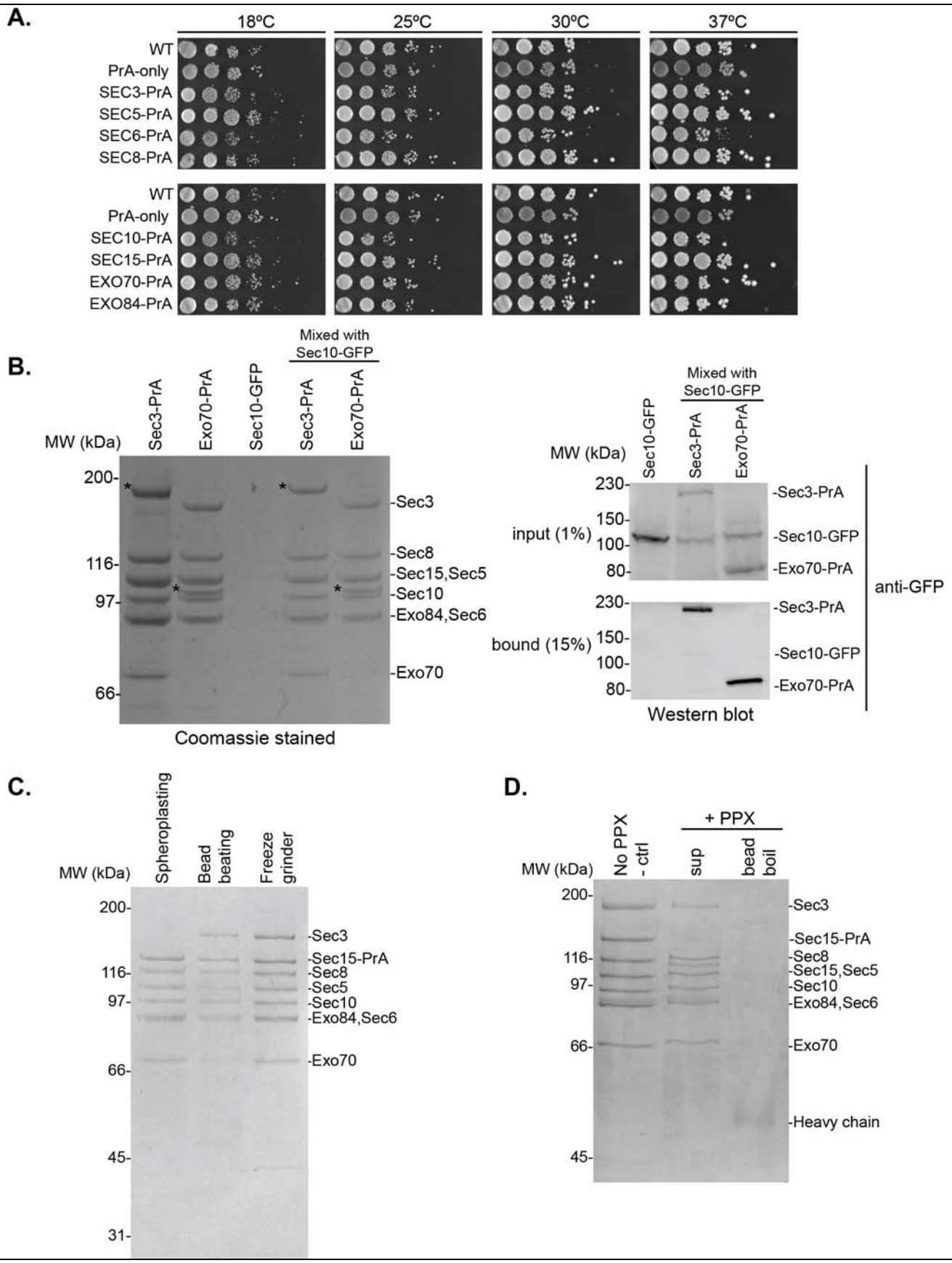
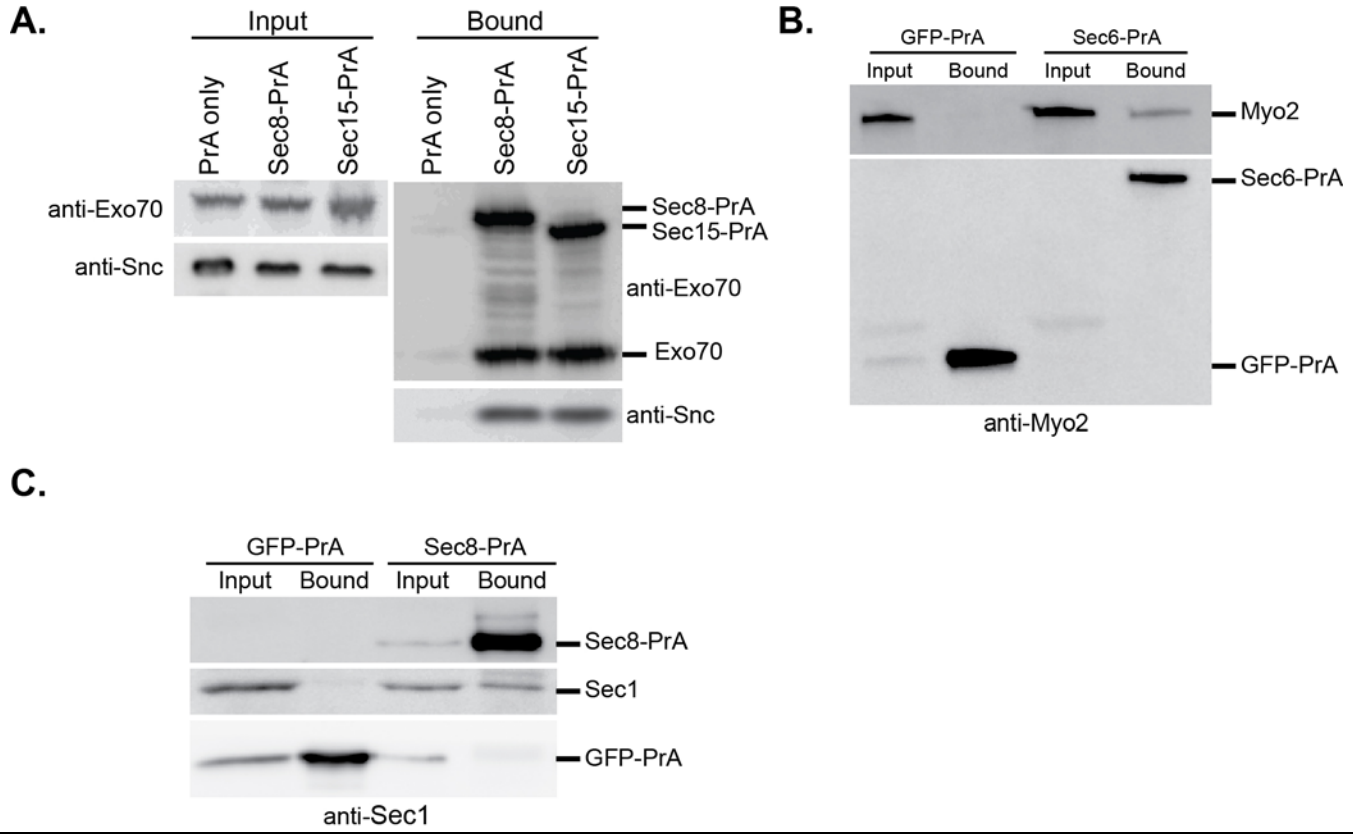

A.
18ºC
25ºC
30ºC
37ºC
WT
PrA-only
SEC3-PrA
SEC5-PrA
SEC6-PrA
SEC8-PrA
WT
PrA-only
SEC10-PrA
SEC15-PrA
EXO70-PrA
EXO84-PrA
B.
Mixed with Sec10-GFP
Sec3-PrA
Exo70-PrA
Sec10-GFP
Sec3-PrA
Exo70-PrA
MW (kDa)
200-
116-
97-
66-
Sec3
Sec8
Sec15,Sec5
Sec10
Exo84,Sec6
Exo70
Coomassie stained
Mixed with Sec10-GFP
Sec10-GFP
Sec3-PrA
Exo70-PrA
MW (kDa)
230-
150-
100-
80-
input (1%)
bound (15%)
Sec3-PrA
Sec10-GFP
Exo70-PrA
anti-GFP
Western blot
C.
Spheroplasting
Bead beating
Freeze grinder
MW (kDa)
200-
116-
97-
66-
45-
31-
Sec3
Sec15-PrA
Sec8
Sec5
Sec10
Exo84,Sec6
Exo70
D.
+ PPX
No PPX - ctrl
sup
bead boil
MW (kDa)
200-
116-
97-
66-
45-
Sec3
Sec15-PrA
Sec8
Sec15,Sec5
Sec10
Exo84,Sec6
Exo70
Heavy chain

Supplementary Figure 1

Characterization of purified yeast exocyst complexes.

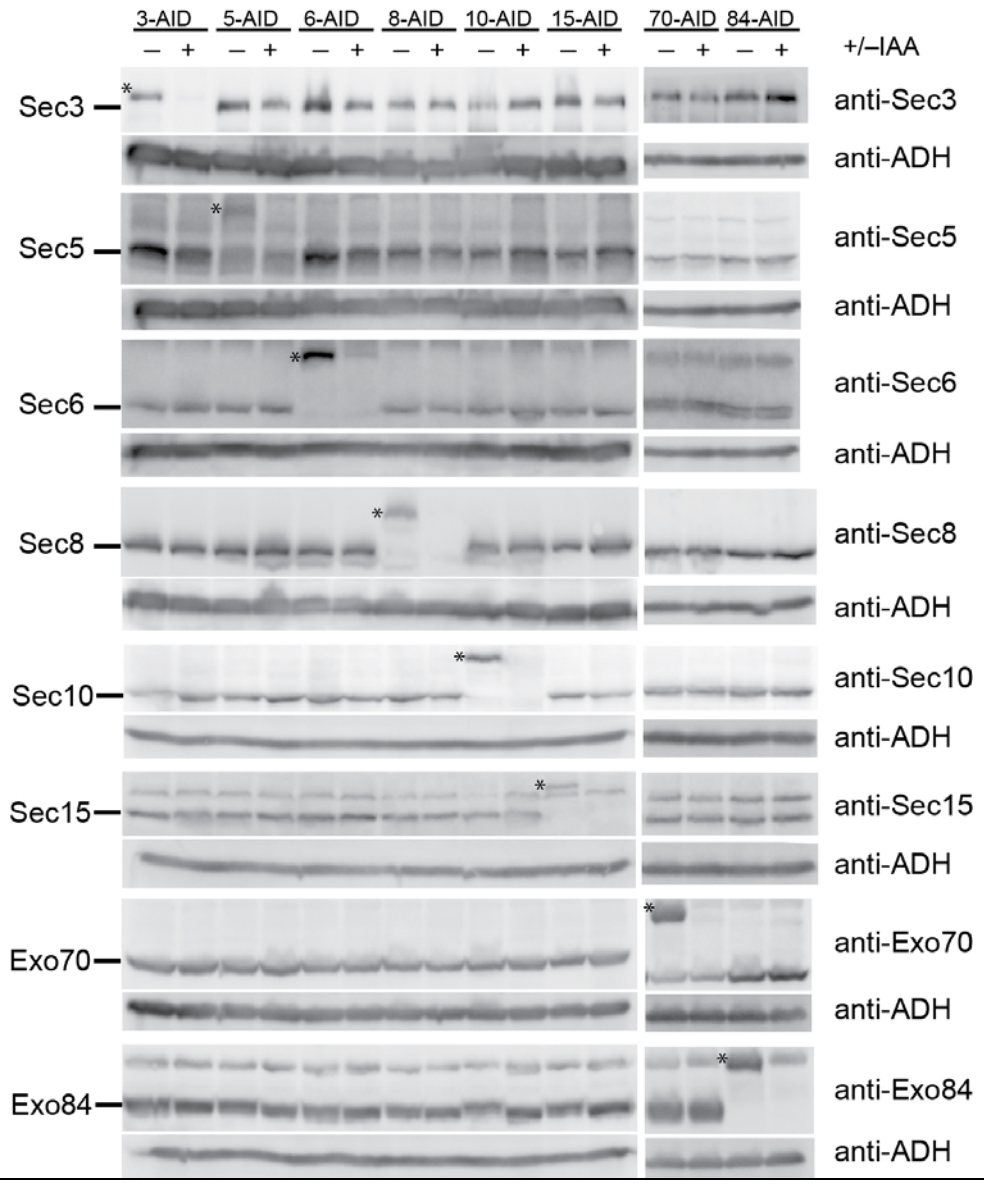
(**a**) Yeast strains with C-terminally PrA-tagged exocyst subunits show normal growth compared to wild-type (WT) by serial dilution at all temperatures tested. PrA-only corresponds to yeast expressing PrA tag alone. (**b**) Lysate mixing reveals exocyst complexes are not disassembling and reassembling during purification. Sec3-PrA or Exo70-PrA lysate powders were each mixed individually with lysate powder from Sec10-GFP. Exocyst complexes were subsequently purified from the mixed lysates (after 60 min binding at 4°C in 20 mM PIPES pH 6.8, 300 mM KCl) and run on SDS-PAGE for Coomassie staining (left) and Western blot (right). GFP antibody also recognizes exocyst PrA tag. Asterisks indicate PrA-subunit on Coomassie gel. (**c**) Cryogenic ball mill grinding improves yield and complex integrity. Protein concentrations (mg/ml) were measured using BioRad protein assay and all beads were incubated with the same total protein in the same volume. Purified complexes were separated by SDS-PAGE and visualized by Coomassie staining. (**d**) Native elution from IgG-beads using a PreScission Protease site engineered between the C-terminus of each exocyst subunit and the PrA tag. Sec15-PrA tagged exocyst complexes bound to IgG-beads were incubated with PPX for 60 minutes at 4°C to elute native, intact complexes into buffer 20 mM PIPES pH 6.8, 300 mM NaCl (sup). IgG-beads were boiled in SDS/DTT loading buffer to release any undigested complexes (bead boil). Heavy chain of Rabbit IgG is indicated.



Supplementary Figure 2

Exocyst complexes purified under physiological conditions interact with known binding partners.

Exocyst complexes were purified from yeast lysate (using 50 mM Hepes pH 7.4, 150 mM NaCl as resuspension buffer), run on SDS-PAGE, and western blotted to look for co-purification of known exocyst interacting partners when compared to a negative control (PrA-expressing strain in (**a**) and GFP-PrA expressing strain in (**b**) and (**c**)). 0.5% lysate input samples were run for the Sec1 and Myo2 binding experiments and 0.4% input for the Snc binding blot. 100% of bound samples were used in all cases. (**a**) Sec8-PrA and Sec15-PrA were each used as purification handles to co- purify Snc. We blotted our pull-downs for Exo70 to show that we are pulling down assembled exocyst complexes with Sec8-PrA and Sec15-PrA. The rabbit antibody reacts with both Exo70 and the PrA tag. (**b**) Sec6-PrA was purified and Myo2 binding was detected. (**c**) Sec8-PrA was purified and Sec1 binding was detected. There was some bleed over of the GFP-PrA bound lane into the input lane of Sec8-PrA.



Supplementary Figure 3

Degradation of one exocyst subunit does not affect the protein levels of the remaining exocyst subunits.

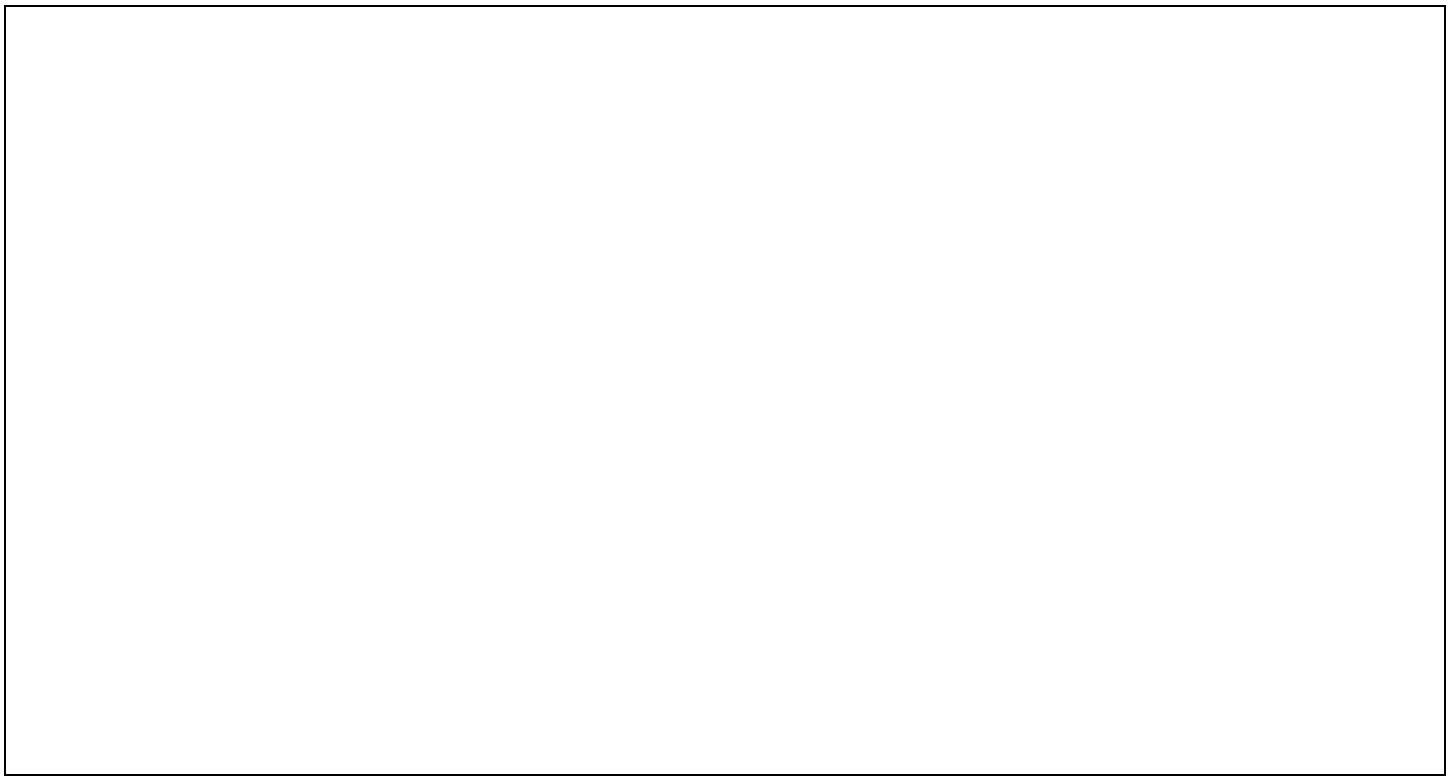
Exocyst-AID strains were grown in YPD 30°C and treated with IAA for 60 minutes. Degradation of the AID-tagged exocyst subunit was confirmed by western blot of yeast lysates from NaOH/SDS lysis. The protein levels for the remaining subunits were blotted in the same strain (same column in the western blot). (–) denotes untreated and (+) treated with IAA. The positions of the untagged exocyst subunits are indicated to the left of the blots and the AID-tagged subunit is marked with (*). All lysates were blotted for ADH as a loading control.

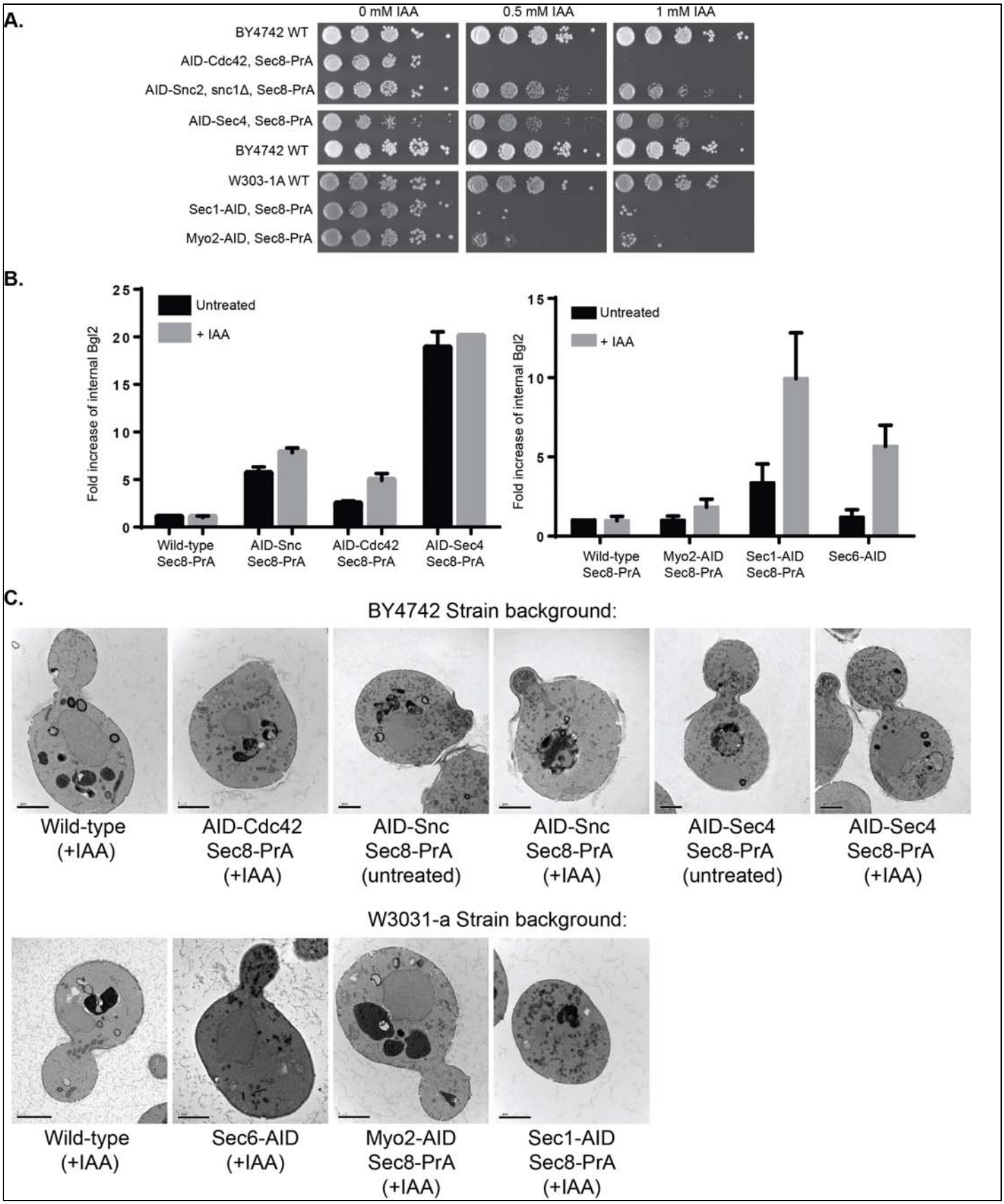

A.
0 mM IAA
0.5 mM IAA
1 mM IAA
OsTIR1
Sec3-AID, Sec8-PrA
Sec3-AID, Sec15-PrA
Sec5-AID, Sec8-PrA
Sec5-AID, Sec15-PrA
Sec6-AID, Sec8-PrA
Sec6-AID, Sec15-PrA
OsTIR1
Sec8-AID, Sec6-PrA
Sec8-AID, Sec15-PrA
Sec10-AID, Sec8-PrA
Exo70-AID, Sec8-PrA
Exo70-AID, Sec15-PrA
Exo84-AID, Sec8-PrA
Exo84-AID, Sec15-PrA
OsTIR1
OsTIR1, Sec6-PrA
OsTIR1, Sec8-PrA
OsTIR1, Sec15-PrA
B.
3-AID 8-PrA
3-AID 15-PrA
5-AID 8-PrA
5-AID 15-PrA
6-AID 8-PrA
6-AID 15-PrA
10-AID 8-PrA
8-AID 6-PrA
8-AID 15-PrA
70-AID 8-PrA
70-AID 15-PrA
84-AID 8-PrA
84-AID 15-PrA
– +
α-3
α-5
α-6
α-8
α-10
α-15
α-70
α-84

Supplementary Figure 4

Investigating connectivity and assembly determinants by combining PrA purification with IAA-induced subunit depletion.

(**a**) We constructed yeast strains expressing double-tagged exocyst complexes: one subunit with a C-terminal AID-tag and another with a C-terminal PrA tag. All tags were integrated at the genomic loci under the endogenous promoter. Strains were serially diluted on standard YPD plates or YPD plates containing IAA and grown at 30°C. Exocyst complexes with both AID-tagged and PrA-tagged subunits are functional and yeast strains are inviable on IAA-containing YPD plates. (**b**) Western blot confirms composition of exocyst subcomplexes following depletion of individual subunits. Exocyst complexes were purified using the indicated PrA purification handle (blue) from yeast strains where one AID-tagged subunit (magenta) is degraded. Purified complexes were run on SDS-PAGE and visualized by Western blotting with antibodies specific to exocyst subunits (antibody indicated in inset box for each blot). (–) denotes untreated and (+) treated with IAA for 40 minutes. Exocyst subunits are denoted by their number. Magenta asterisks indicate the AID-tagged subunit, and green asterisks indicate the subunit whose co-purification is being monitored in that particular blot. Polyclonal antibodies also recognize PrA-tagged subunits, which in all cases is the band running higher than the subunit monitored (green asterisk), with the exception of Sec3, which runs above the PrA- tagged subunit.

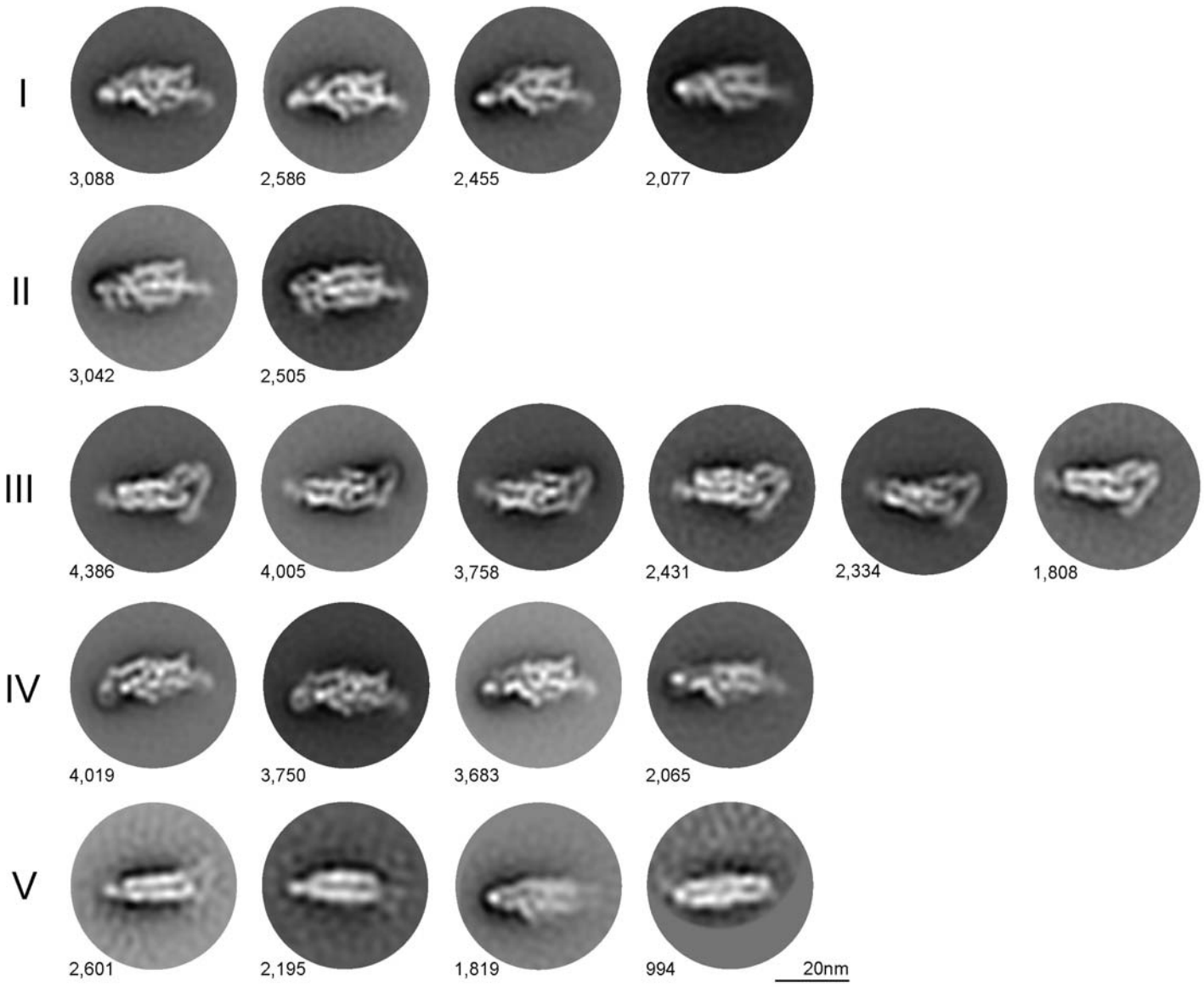



A.
0 mM IAA
0.5 mM IAA
1 mM IAA
BY4742 WT
AID-Cdc42, Sec8-PrA
AID-Snc2, snc1Δ, Sec8-PrA
AID-Sec4, Sec8-PrA
BY4742 WT
W303-1A WT
Sec1-AID, Sec8-PrA
Myo2-AID, Sec8-PrA
B.
Untreated
+ IAA
Fold increase of internal Bgl2
25
20
15
10
5
0
Wild-type Sec8-PrA
AID-Snc Sec8-PrA
AID-Cdc42 Sec8-PrA
AID-Sec4 Sec8-PrA
Untreated
+ IAA
Fold increase of internal Bgl2
15
10
5
0
Wild-type Sec8-PrA
Myo2-AID Sec8-PrA
Sec1-AID Sec8-PrA
Sec6-AID
C.
BY4742 Strain background:
Wild-type (+IAA)
AID-Cdc42 Sec8-PrA (+IAA)
AID-Snc Sec8-PrA (untreated)
AID-Snc Sec8-PrA (+IAA)
AID-Sec4 Sec8-PrA (untreated)
AID-Sec4 Sec8-PrA (+IAA)
W3031-a Strain background:
Wild-type (+IAA)
Sec6-AID (+IAA)
Myo2-AID Sec8-PrA (+IAA)
Sec1-AID Sec8-PrA (+IAA)

Supplementary Figure 5

AID-tagged exocyst binding partners are functional and have varying levels of growth and secretion defects in IAA-containing media.

(a) N-terminally AID-tagged Cdc42, Snc2, and Sec4 combined with Sec8-PrA and OsTIR1 were tested for growth on YPD and YPD-IAA plates at 30°C relative to the wild-type (WT) parent strain (BY4742). AID- Sec4/Sec8-PrA demonstrated a mild growth defect on YPD plates and in liquid culture (data not shown); this growth defect was exacerbated slightly in the presence of IAA. AID-Snc2/ *snc1*Δ/Sec8-PrA showed a slight growth defect in the presence of IAA, and AID-Cdc42/Sec8-PrA was inviable on IAA plates. Sec1-AID/Sec8-PrA and Myo2-AID/Sec8-PrA showed no growth defects when compared to their parent strain (W303-1A) but were inviable on IAA plates. (b) Graphs depict the fold increase of internal Bgl2 levels in AID-tagged partner strains over internal Bgl2 levels in the appropriate WT untreated control strain. Sec1-AID and Sec6-AID showed severe secretion defects, while Myo2-AID and AID-Cdc42 showed minor defects consistent with previous reports[43,44,48]. AID-Snc and AID-Sec4 showed severe Bgl2 accumulation even before treatment, suggesting a partial loss of function due to the AID tag. Error bars indicate SEM for n=3-4 different treated or untreated yeast cultures. (c) Thin section EM confirms the vesicle accumulation defects observed in the Bgl2 assay. AID-Cdc42 cells also showed a loss of polarity and fewer budding cells. Scale bar=1 μm.



Supplementary Figure 6

The complete class gallery of the Sec15-GFP tagged exocyst complexes.

Class averages with similar orientations are shown in the same row. Each row starts with the most populated class and ends with the least populated class. The number of particles per class is shown near the lower left corners. Although the last row is labeled V as a different class from the others, we cannot rule out that these 2D averages belong to class III and the flexible ends were averaged out.