

# Supplementary Information

Molecular organization of the COG vesicle tethering complex

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## Supplementary Figure Legends

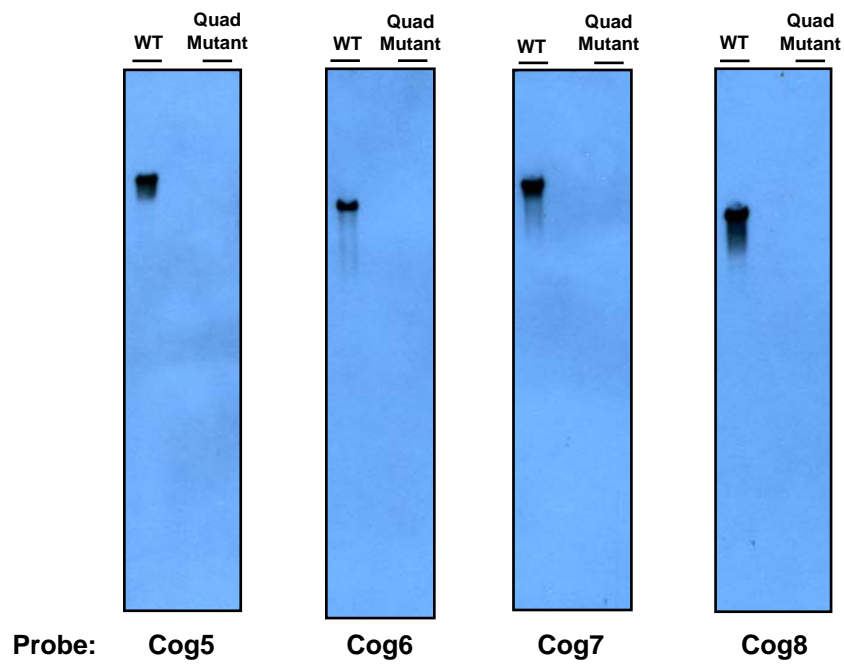
**Supplementary Figure 1.** Southern blots to confirm absence of COG ORFs from quadruple deletion yeast strain. Genomic DNA samples from wild-type and mutant strains were digested with BamHI and Sall and equal digest amounts separated on four separate 1% agarose gels. Denatured and depurinated samples were transferred from the gels to positively-charged nylon membranes by capillary transfer. Blots were probed with the indicated DIG-labeled probes, each covering an entire COG ORF sequence. Detection was performed with alkaline phosphatase-conjugated anti-DIG antibodies and CSPD chemiluminescence reagent.

**Supplementary Figure 2.** The 200 class averages obtained for purified Cog2-4 complex (side length = 45 nm). The number in the bottom right-hand corner of each panel indicates the number of particles in the class average.

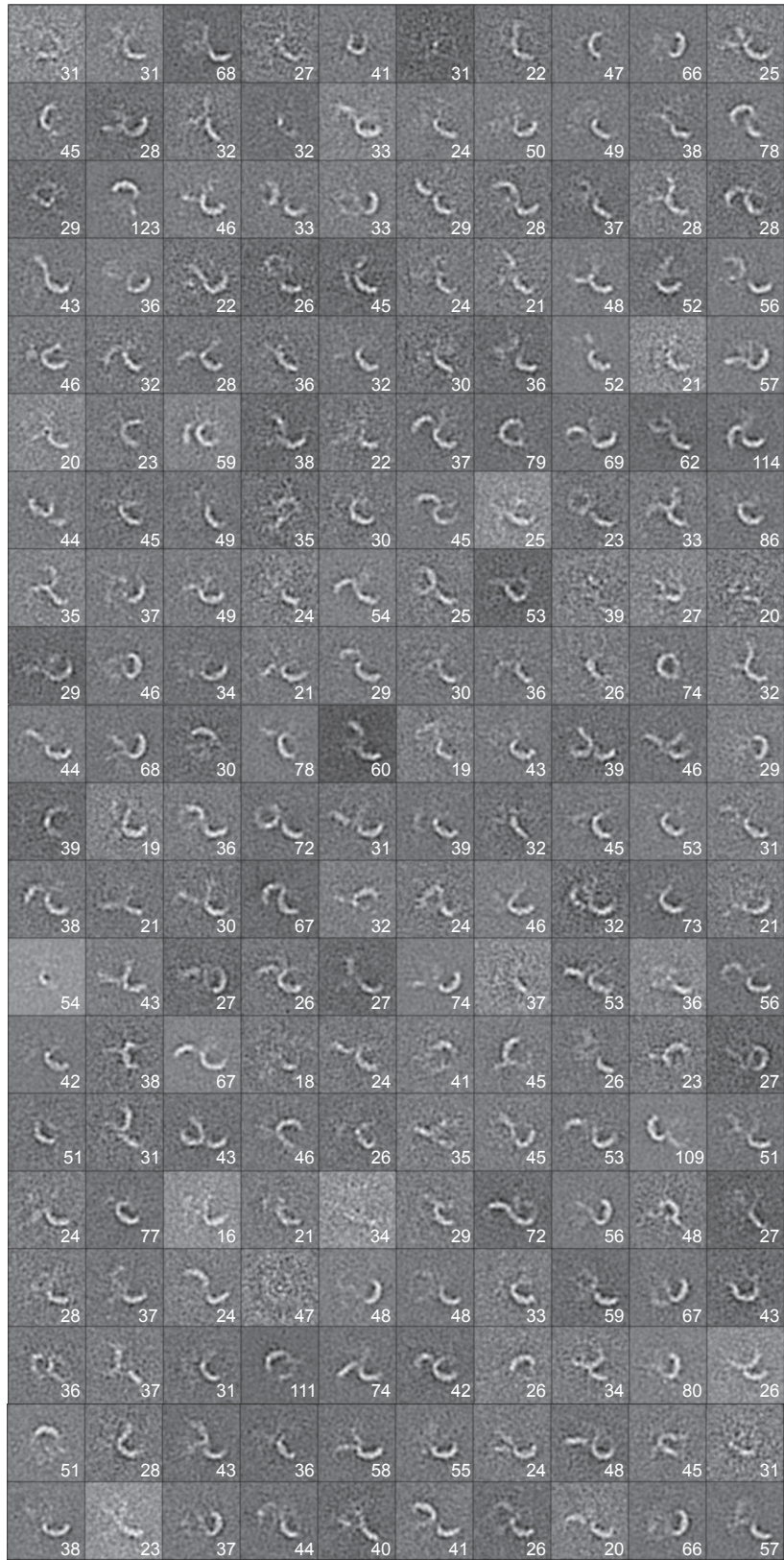
**Supplementary Figure 3.** The 200 class averages obtained for purified Cog1-4 complex (side length = 45 nm). The number in the bottom right-hand corner of each panel indicates the number of particles in the class average.

**Supplementary Figure 4.** Cog1-4 complex subunit stoichiometry. 1.5  $\mu\text{g}$  of Cog1-4 complex with a GFP tag at the N-terminus of Cog4 was analyzed on a 12% SDS-PAGE gel. Band intensities are consistent with a 1:1:1:1 subunit ratio for each particle.

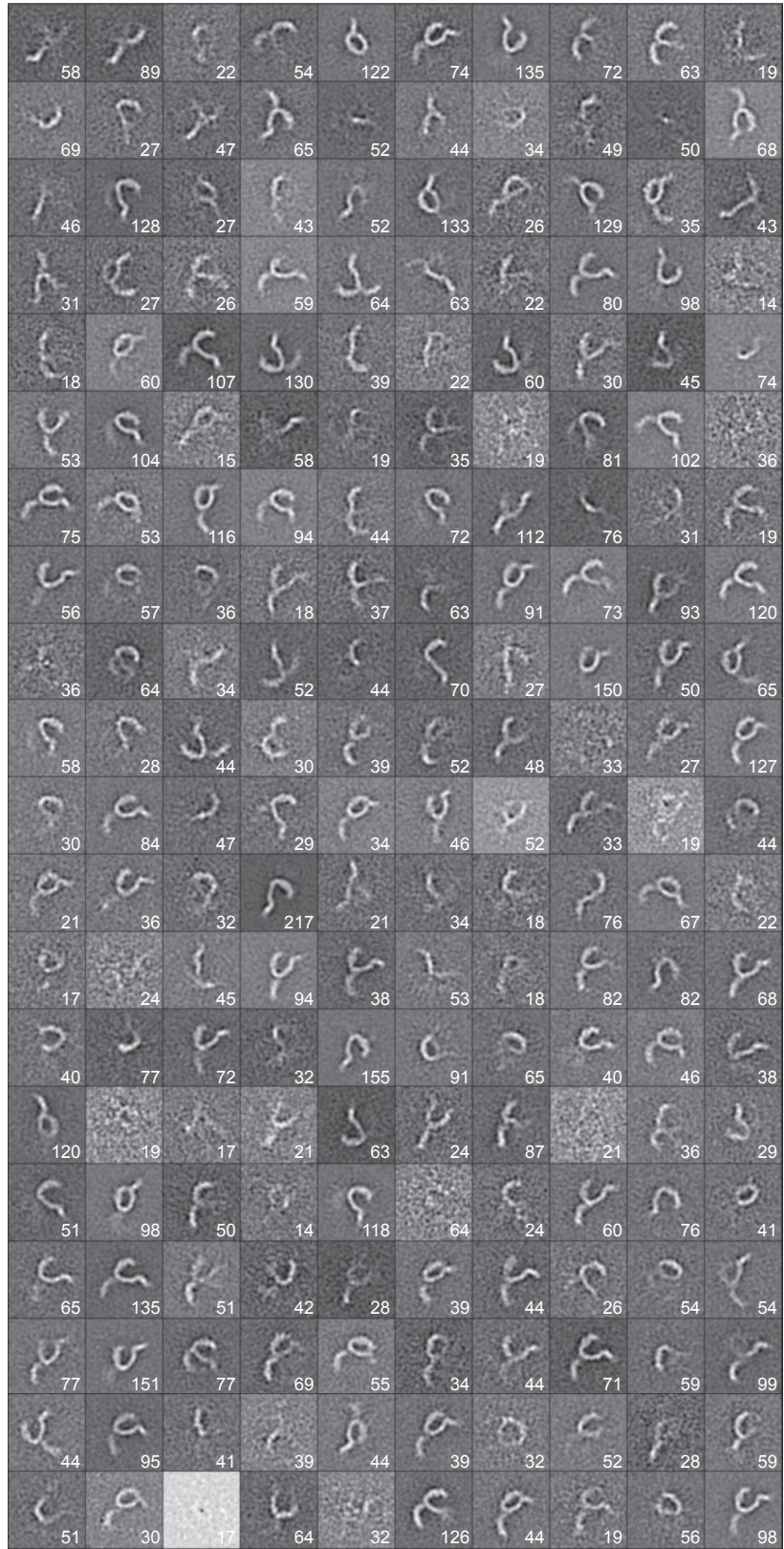
**Supplementary Figure 5.** Purification of the Cog1/2/3/4/8 complex. Cog1/7xHis-Cog2/Cog3/GFP-Cog4 complex was incubated with a 3.5-fold molar excess of 6xHis-Cog8 for 3 hours at 4°C. The sample was loaded on a Superdex 200 10/300 GL size exclusion column and peak fractions analyzed by SDS-PAGE. A peak fraction containing pentameric complex is shown in the inset. Excess Cog8 forms the second, smaller peak.



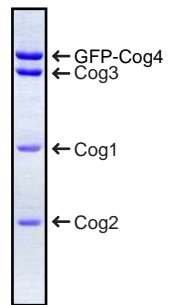
**Figure S1**



**Figure S2**



**Figure S3**



**Figure S4**

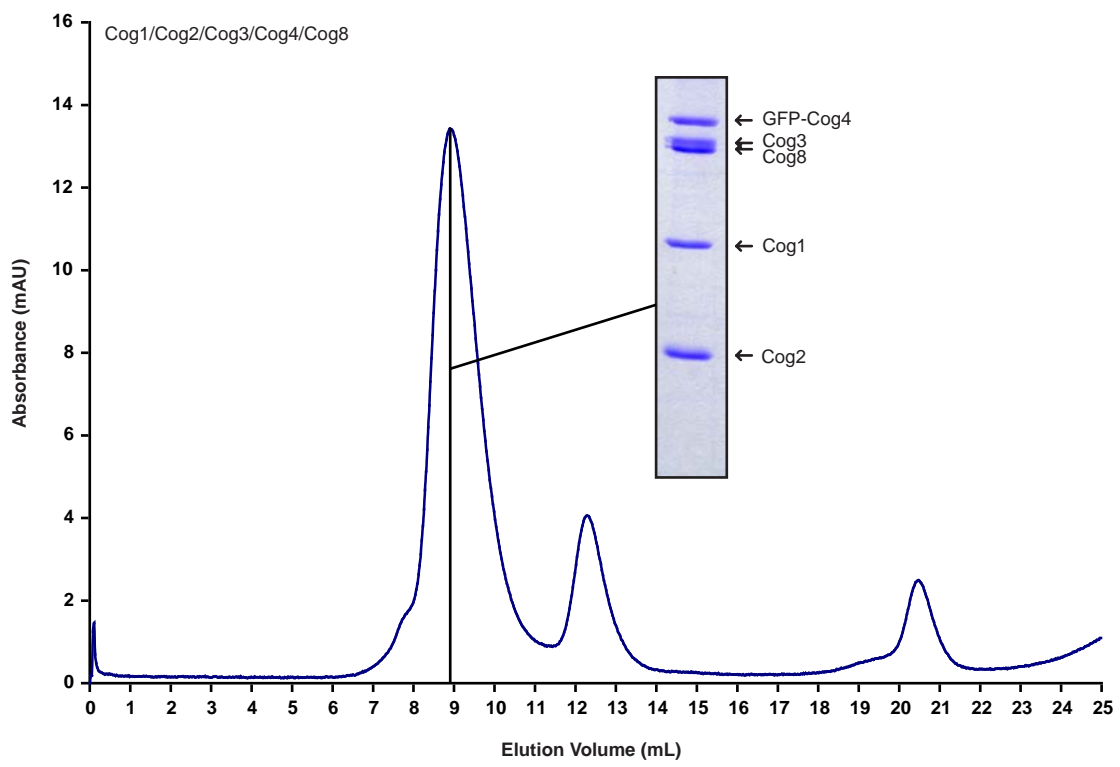


Figure S5