**Supporting Information**

# The Sec1/Munc18 protein VPS33B forms a uniquely bidirectional complex with VPS16B

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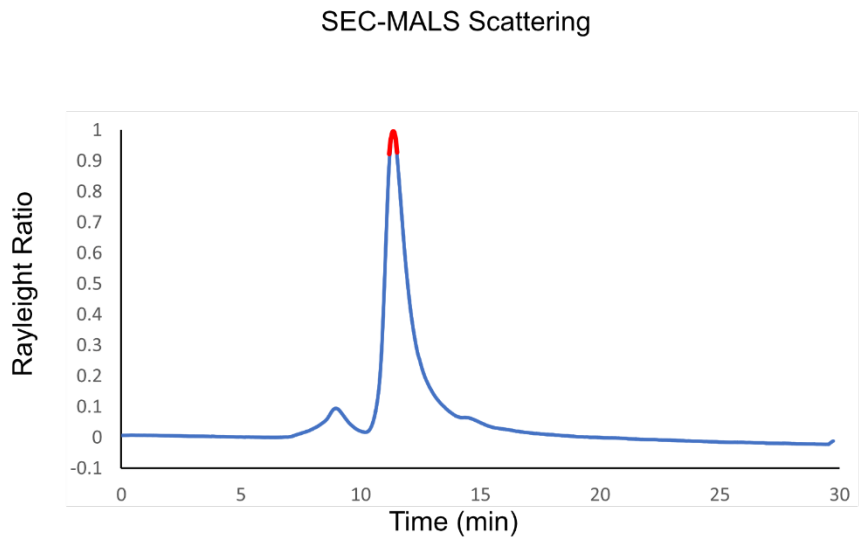
**Table S1. Mass spectrometry analysis of VPS33B/VPS16B-3xFLAG complex.** Recombinant human VPS33B/VPS16B-3xFLAG complex purified from yeast was recovered from the ~480 kDa band on a stained BN-PAGE gel (Fig. 3C) and subjected to mass spectroscopy.

| Protein Name | Species | Molecular weight (Da) | Exclusive unique peptide count | Exclusive spectrum count | % coverage |
|---|---|---|---|---|---|
| VPS33B | *Homo Sapiens* | 70,588.50 | 57 | 318 | 61.90% |
| VPS16B-3xFLAG | *Homo Sapiens* | 60,802.70 | 61 | 213 | 65.20% |
| YFR016C | *Saccharomyces cerevisiae* | 137,698.70 | 2 | 2 | 2.35% |

**Table S2. Mass spectrometry analysis of ~70 kDa band from Figure 8A, VPS33B L30P variant lane.**

| Protein Name | Species | Molecular weight (Da) | Exclusive unique peptide count | Exclusive spectrum count | % coverage |
|---|---|---|---|---|---|
| SSA2 | *Saccharomyces cerevisiae* | 69,471.80 | 64 | 285 | 73.4 |
| SSB2 | *Saccharomyces cerevisiae* | 66,595.70 | 40 | 132 | 66.9 |
| SSA1 | *Saccharomyces cerevisiae* | 69,659.40 | 17 | 49 | 78.5 |

**A**




**B**

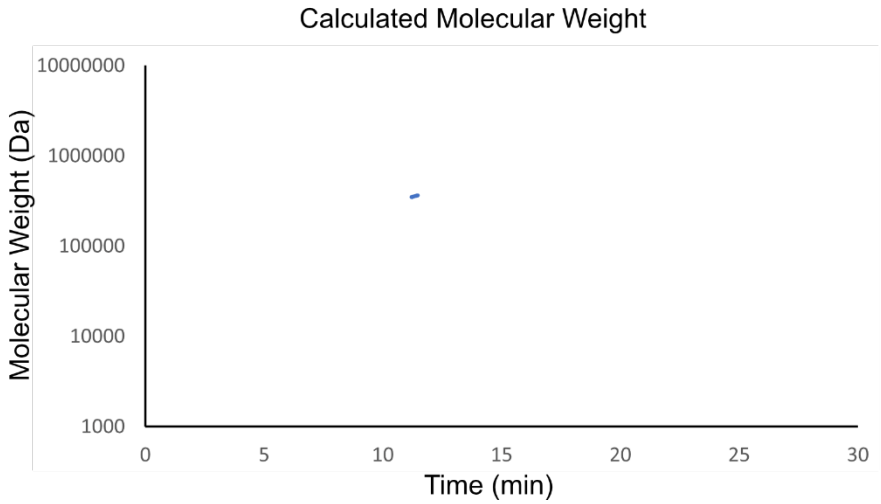



**Figure S1. Molecular weight determination of VPS33B/VPS16B-3xFLAG complex by SEC-MALS.** (A) Representative light scattering data from SEC-MALS analysis of purified VPS33B/VPS16B-3xFLAG (see Figure 3E for SEC results). B) Data for the complex peak (red) were used to calculate the molecular weight of the complex (logarithmic scale); estimates from 3 independent purifications yielded a mean value of 315 kDa.

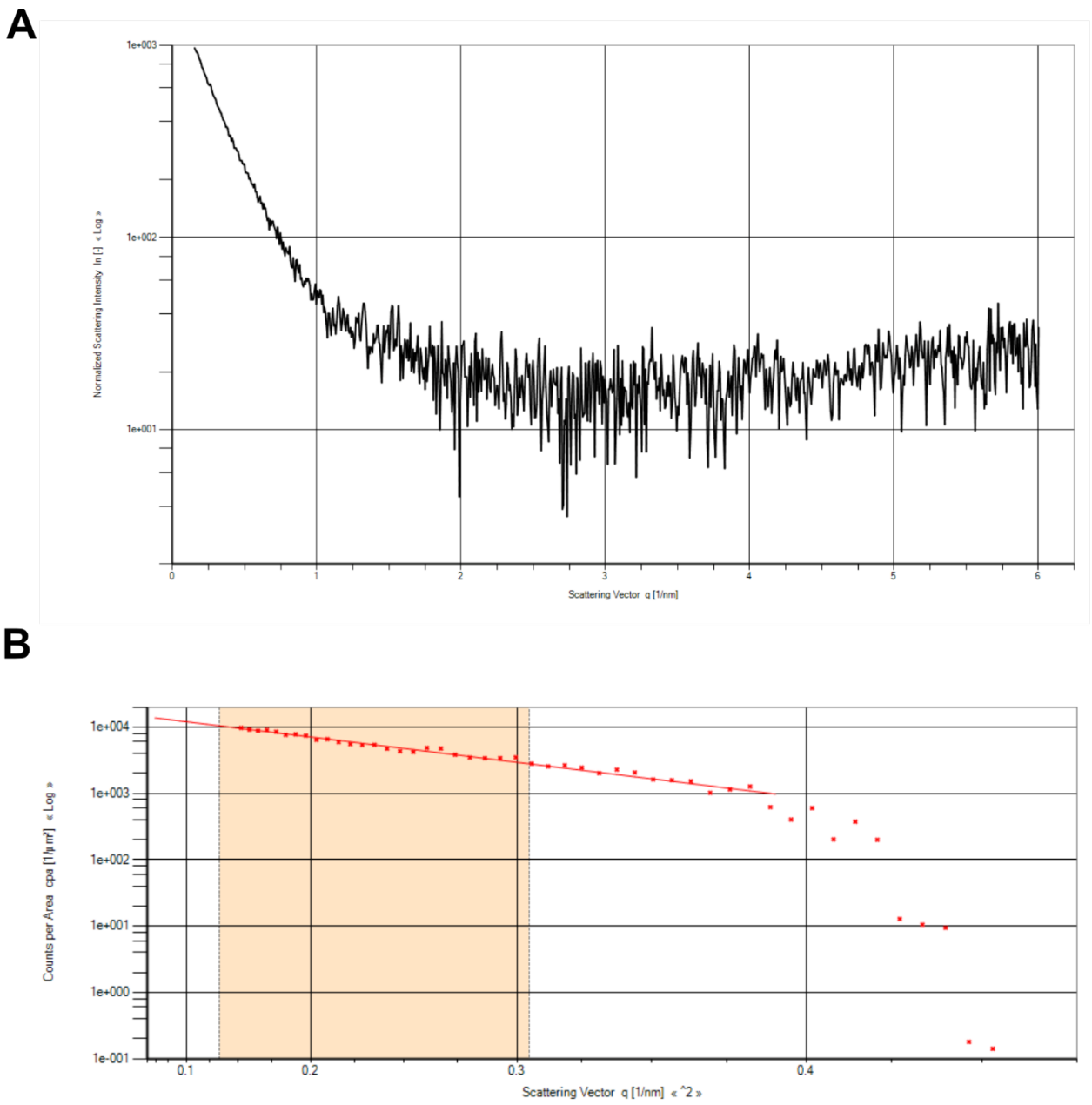
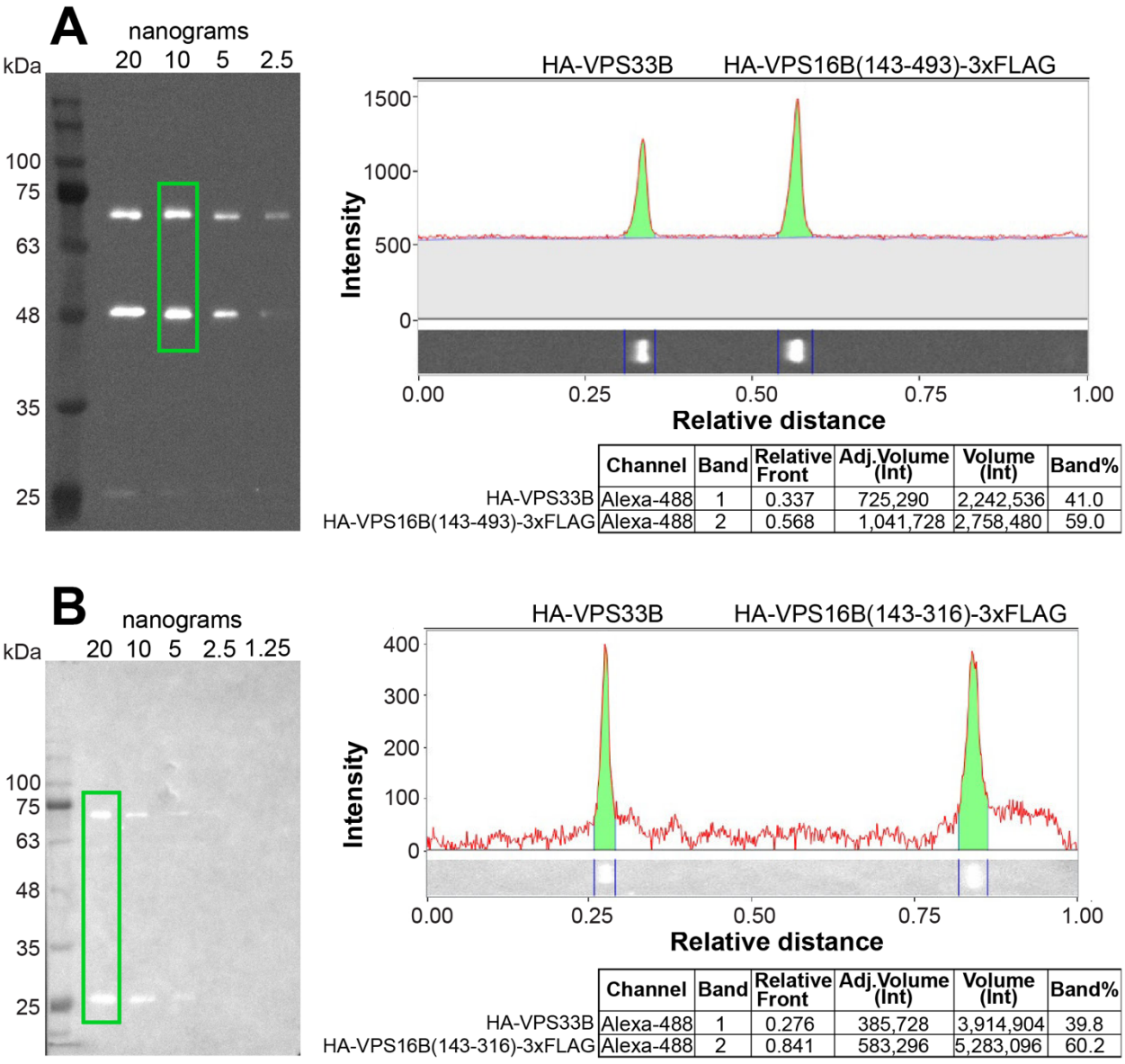



**Figure S2. SAXS analysis of VPS33B/VPS16B-3xFLAG.** (A) Scattering profile of VPS33B/VPS16B-3xFLAG complex. (B) Guinier plot showing linear fit (red line) and region (orange shading) used to calculate the radius of gyration of the complex.




| | Channel | Band | Relative Front | Adj.Volume (Int) | Volume (Int) | Band% |
|---|---|---|---|---|---|---|
| HA-VPS33B | Alexa-488 | 1 | 0.337 | 725,290 | 2,242,536 | 41.0 |
| HA-VPS16B(143-493)-3xFLAG | Alexa-488 | 2 | 0.568 | 1,041,728 | 2,758,480 | 59.0 |

| | Channel | Band | Relative Front | Adj.Volume (Int) | Volume (Int) | Band% |
|---|---|---|---|---|---|---|
| HA-VPS33B | Alexa-488 | 1 | 0.276 | 385,728 | 3,914,904 | 39.8 |
| HA-VPS16B(143-316)-3xFLAG | Alexa-488 | 2 | 0.841 | 583,296 | 5,283,096 | 60.2 |

**Figure S3. Quantitative immunoblots of VPS33B/VPS16B complexes containing truncated VPS16B subunits show same component ratio as wild type complex.** SDS-PAGE immunoblots of serially-diluted purified complex were probed with monoclonal anti-HA primary antibody and Alexa Fluor 488-conjugated anti-mouse secondary antibody. Total protein (nanograms) loaded indicated at top of each lane; lanes used for quantification outlined in green. Blots were imaged and quantified as described in Figure 4 for wild type complex. (A) Results for 10 ng complex containing full-length HA-VPS33B and HA-VPS16B(143-493)-3xFLAG show these components account for 41% and 59% of signal respectively, indicating 2 and 3 copies of each in the complex. (B) Results for 20 ng of complex containing HA-VPS33B and HA-VPS16B(143-316)-3xFLAG also indicate presence of 2 and 3 copies respectively.