Figure S2

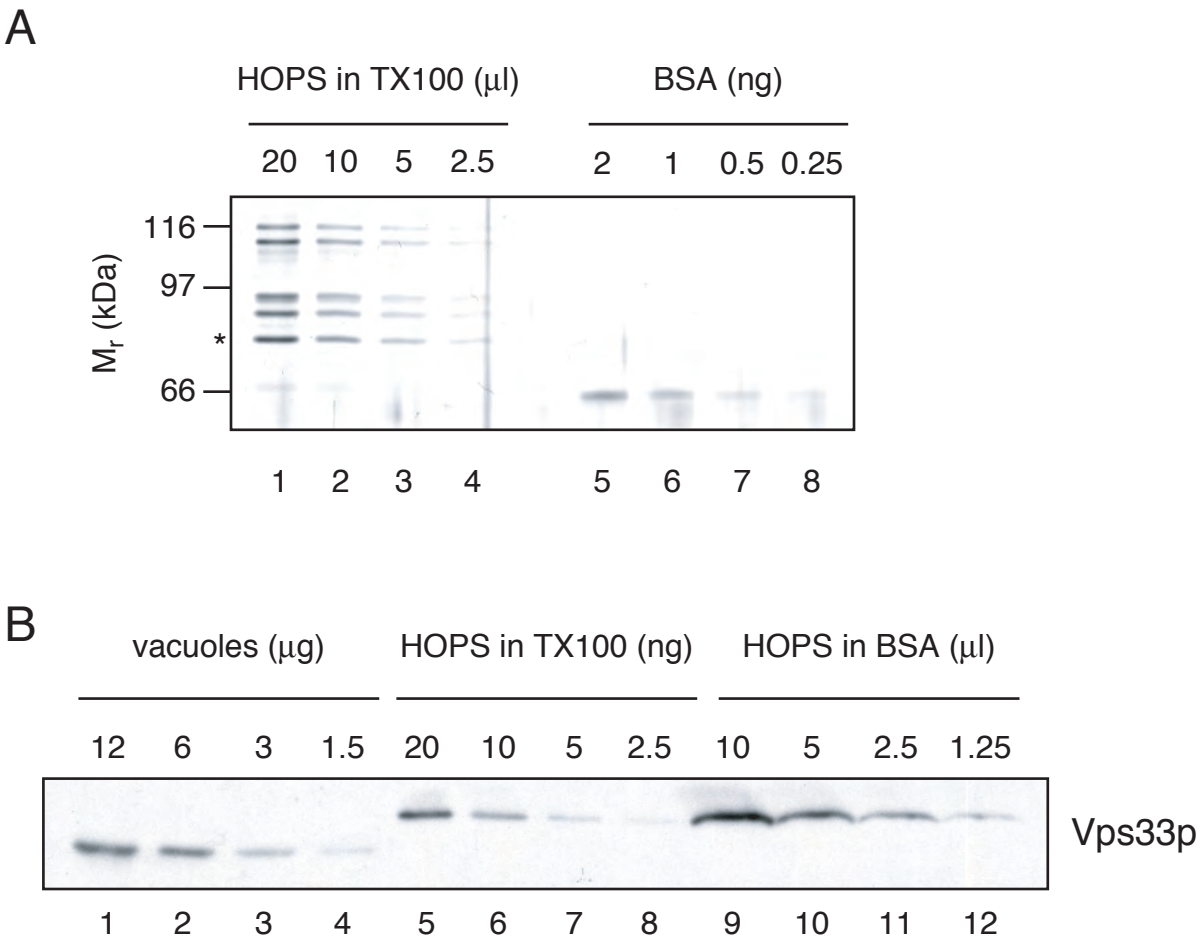
A
HOPS in TX100 (µl)
BSA (ng)
20 10 5 2.5
2 1 0.5 0.25
116
97
*
66
M_r (kDa)
1 2 3 4 5 6 7 8
B
vacuoles (µg)
HOPS in TX100 (ng)
HOPS in BSA (µl)
12 6 3 1.5
20 10 5 2.5
10 5 2.5 1.25
Vps33p
1 2 3 4 5 6 7 8 9 10 11 12

Figure S2 Quantification of pure HOPS complex by comparison of HOPS in lysis buffer containing Triton X-100 and in HOPS buffer containing BSA. **(A)** Pure HOPS complex in lysis buffer (lane 1, 20 μl; lane 2, 10 μl; lane 3, 5 μl; lane 4, 2.5 μl) and BSA (lane 5, 2 ng; lane 6, 1 ng; lane 7, 0.5 ng; lane 8, 0.25 ng) were subjected to SDS-PAGE and silver staining. The concentration of Vps33p (marked by an asterisk) was estimated by densitometry to be 0.13 ng/μl. If we assume that the HOPS complex contains one copy of each subunit, the concentration of HOPS in lysis buffer is 1.0 ng/μl, or 1.6 nM. **(B)** Vacuoles (lane 1, 12 μg; lane 2, 6 μg; lane 3, 3 μg; lane 4, 1.5 μg), pure HOPS complex in lysis buffer (lane 5, 20 ng; lane 6, 10 ng; lane 7, 5 ng; lane 8, 2.5 ng), and pure HOPS complex in HOPS buffer with BSA (lane 9, 10 μl; lane 10, 5 μl; lane 11, 2.5 μl; lane 12, 1.25 μl) were subjected to SDS-PAGE and immunoblotting for Vps33p. The concentration of Vps33p in the HOPS complex in HOPS buffer was estimated by densitometry to be 1.1 ng/μl. Again assuming that HOPS contains one copy of each subunit, the concentration of HOPS complex in HOPS buffer is 8.3 ng/μl, or 13 nM. The amount of Vps33p in 1 μg of vacuole extract was estimated by densitometry to be 0.22 ng, corresponding to 1.7 ng of HOPS complex per μg of vacuole extract, using the assumption that HOPS contains one copy of each subunit. Thus, a standard fusion reaction without added HOPS would contain approximately 10 ng HOPS complex. In our standard HOPS fusion assay (containing 5 μl of added pure HOPS complex) we therefore added approximately 42 ng pure HOPS, 4 times the amount of HOPS present in a standard fusion reaction.