**Supplementary Data**

# Complex lipid requirements for SNARE- and SNARE chaperone-dependent membrane fusion*

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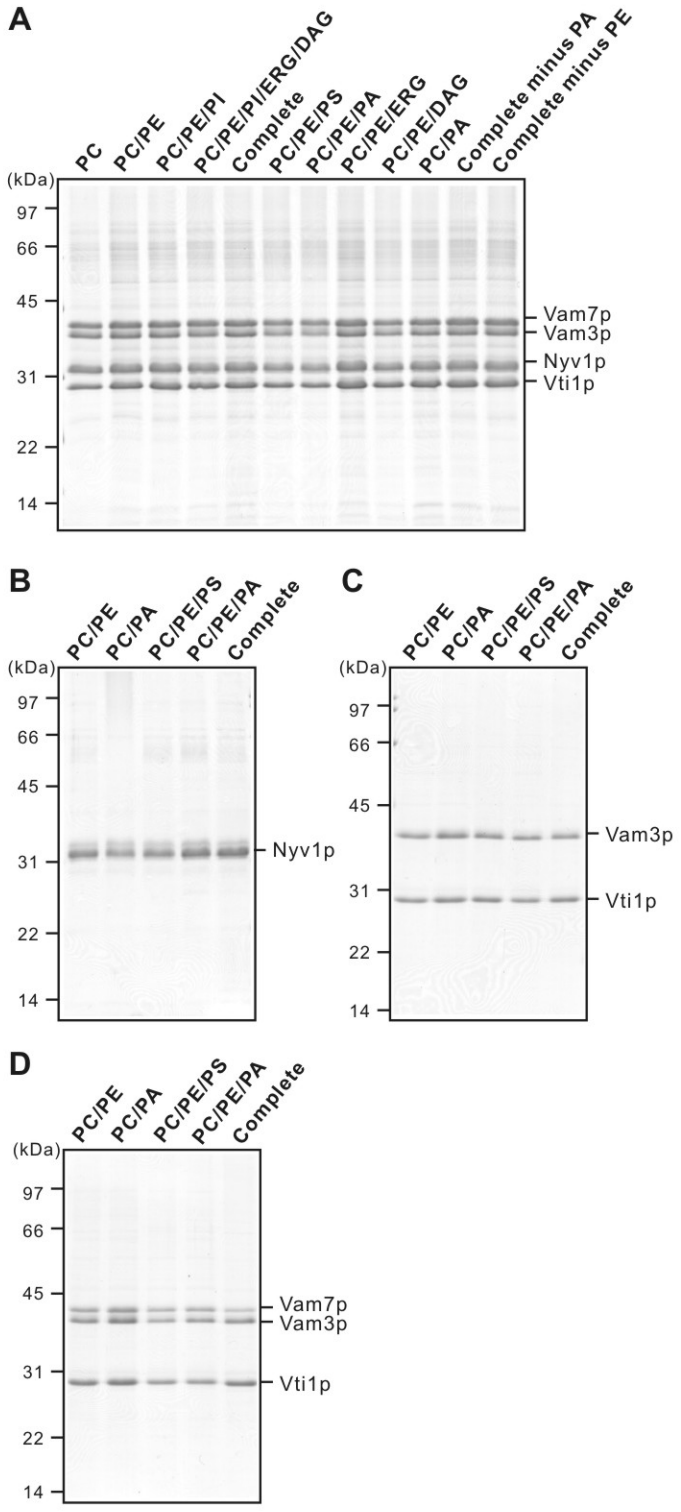
**Supplementary Figure legends**

**FIGURE S1. Coomassie-stained gels of reconstituted proteoliposomes (RPLs) bearing yeast vacuolar SNAREs used in this study.** The RPLs were reconstituted with 4SNAREs (*A*), 1R-SNARE (Nyv1p) (*B*), 2Q-SNAREs (Vam3p and Vti1p) (*C*), and 3Q-SNAREs (Vam3p, Vti1p, and Vam7p) (*D*). Donor and acceptor RPLs were used in (*A*, *B*) and (*C*, *D*), respectively. See TABLE 1 for the detailed lipid compositions of the RPLs.

**FIGURE S2. Sec18p and HOPS dependence of vacuolar SNARE RPL lipid mixing.** Vacuolar 4-SNARE RPLs with complete vacuolar lipid composition (Table 1) were used. (A) Sec18p dependence. Lipid mixing was assayed in RB150 with 4-SNARE RPLs (450 μM total lipids, 300-750 nM each SNARE), ATP (1mM), $MgCl_2$ (2 mM), diC8-PI(3)P (90 μM), Sec17p (1 μM), HOPS (45 nM), and Sec18p (1, 100, 300, or 900 nM). (B) HOPS dependence. Lipid mixing was assayed as in (A) but with Sec18p (900 nM) and HOPS (0, 5, 15, or 45 nM).

**Figure S1**


A
PC
PC/PE
PC/PE/PI
PC/PE/PI/ERG/DAG
Complete
PC/PE/PS
PC/PE/PA
PC/PE/ERG
PC/PE/DAG
PC/PA
Complete minus PA
Complete minus PE
(kDa)
97
66
45
31
22
14
Vam7p
Vam3p
Nyv1p
Vti1p
B
PC/PE
PC/PA
PC/PE/PS
PC/PE/PA
Complete
(kDa)
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Nyv1p
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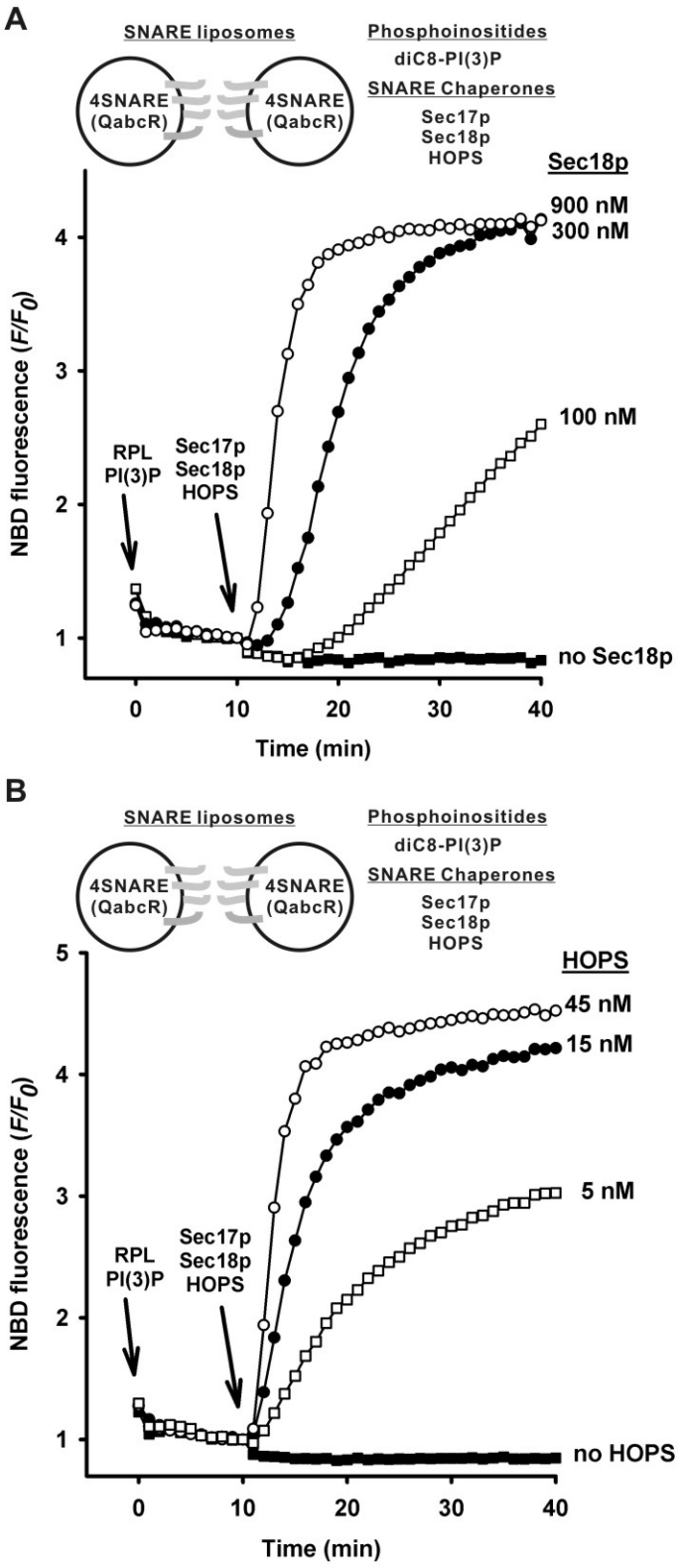
Figure S2


A
SNARE liposomes
4SNARE (QabcR)
4SNARE (QabcR)
Phosphoinositides
diC8-PI(3)P
SNARE Chaperones
Sec17p
Sec18p
HOPS
Sec18p
900 nM
300 nM
100 nM
no Sec18p
RPL PI(3)P
Sec17p Sec18p HOPS
NBD fluorescence (F/F0)
Time (min)
B
SNARE liposomes
4SNARE (QabcR)
4SNARE (QabcR)
Phosphoinositides
diC8-PI(3)P
SNARE Chaperones
Sec17p
Sec18p
HOPS
HOPS
45 nM
15 nM
5 nM
no HOPS
RPL PI(3)P
Sec17p Sec18p HOPS
NBD fluorescence (F/F0)
Time (min)