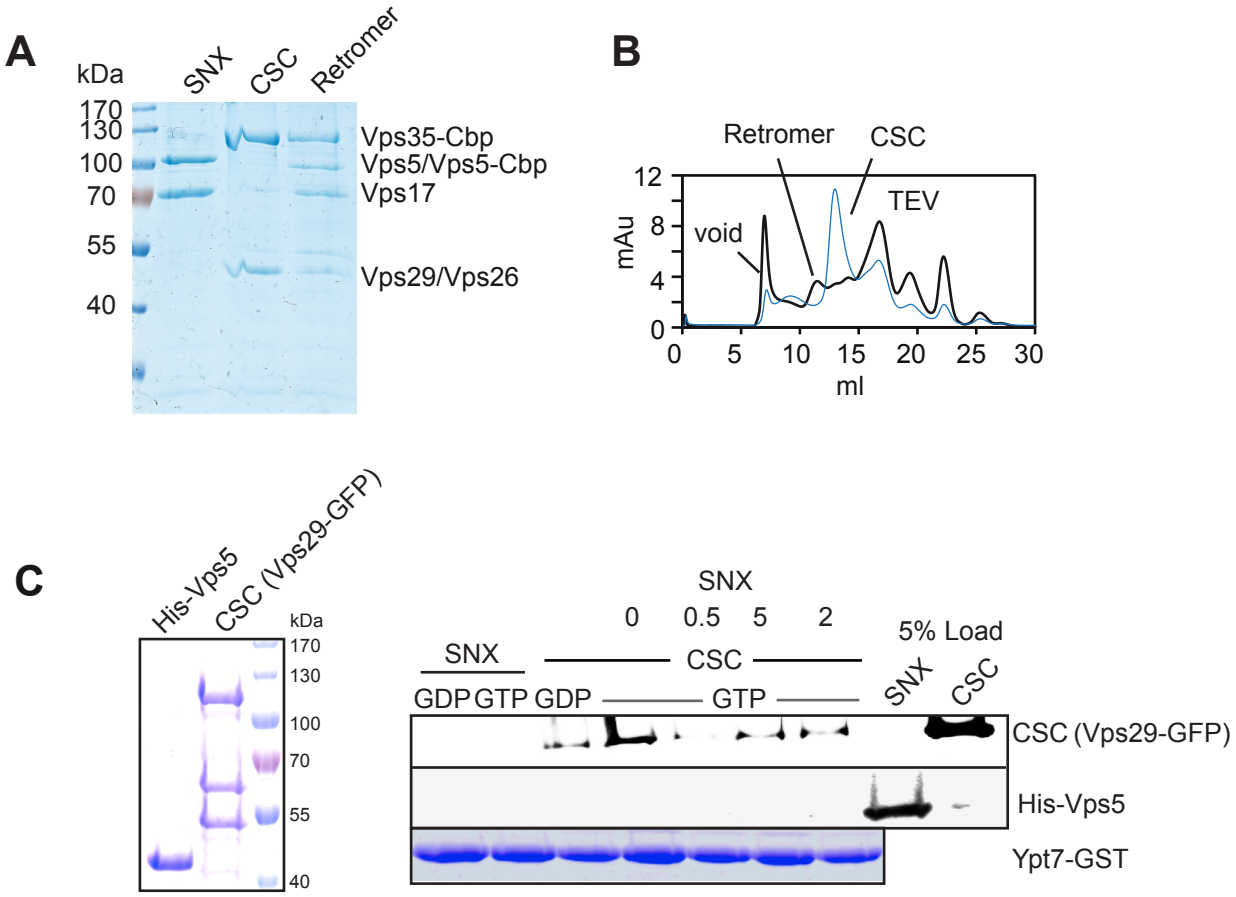
Supplemental Materials

Molecular Biology of the Cell

Purushothaman et al.

Figure S1: Further biochemical analyses of retromer complex and assembly. (A) Analysis of the purified retromer subcomplex. Overproduced CSC, SNX-BAR, and SNX-BAR retromer complexes were purified as in Figure 1. Complexes were analyzed via SDS-PAGE, and gels were stained with Coomassie. **(A, B)** Gel filtration of retromer. TEV-eluates from IgG columns was applied to gel filtration as described in Methods. TEV indicates the migration of the tobacco etch virus protease used to elute the protein from IgG beads. The overall profile for the purified CSC (Figure 1D) is displayed in blue. (C) Competition of the N-terminal fragment of Vps5 with Ypt7. Vps5 was purified via an N-terminal His-tag (shown to left on an SDS-PAGE that was stained with Coomassie). Note that the fragment runs higher than its expected molecular weight. The protein was added at the indicated molar ratio relative to CSC to GST-Ypt7 as described in Figure 5A and Methods.


A
kDa
SNX
CSC
Retromer
170
130
100
70
55
40
Vps35-Cbp
Vps5/Vps5-Cbp
Vps17
Vps29/Vps26
B
Retromer
CSC
TEV
void
mAu
12
8
4
0
0
5
10
15
20
25
30
ml
C
His-Vps5
CSC (Vps29-GFP)
kDa
170
130
100
70
55
40
SNX
0
0.5
5
2
5% Load
SNX
CSC
GDP GTP GDP
GTP
SNX
CSC
CSC (Vps29-GFP)
His-Vps5
Ypt7-GST