Supplementary Materials

Molecular Biology of the Cell

Orr *et al*.

Supplemental Figure S1. (A) The kinetics of fusion is shown for one replicate from Figure 5. Fusion of Jx swapped proteoliposomes is rescued by Sec17/Sec18/ATPgS even when zippering is arrested by Qc3Δ. (B) *Trans*-SNARE complexes form despite the swapped Jx regions of R and Qa, the presence or absence of Sec17/Sec18/ ATPgS, and despite arrested SNARE zippering imposed by the truncated Qc SNARE.

Supplemental Figure S2. (A) The kinetics of fusion are shown from one replicate of Figure 6. Having integral Qb on the membrane along with the Jx-swapped Qa relieves the block to fusion in the absence of Sec17. (B) A representative Western blot image is shown, indicating that differences in fusion are not due to differences in *trans*-SNARE complex assembly.

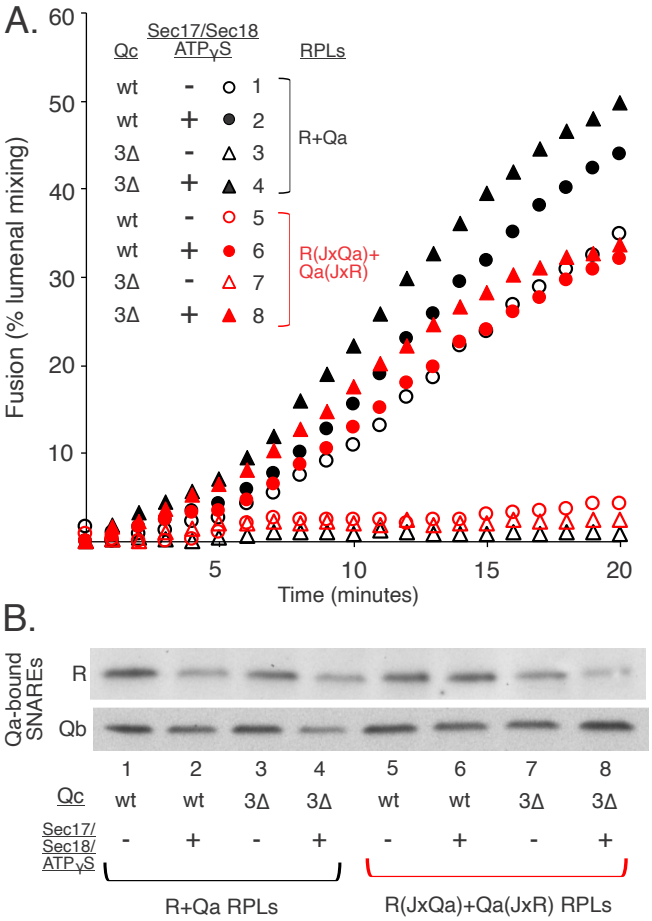
Supplemental Figure S3. Lowered levels of bound Qb do not *per se* render fusion sensitive to Jx swap. One of the 3 sets of fusion reactions shown in Figure 7.

Supplemental Figure S4. The absence of a Qb membrane anchor renders fusion sensitive to Jx swap between R and Qa. One of the 3 sets of fusion reactions from Figure 8 is shown.

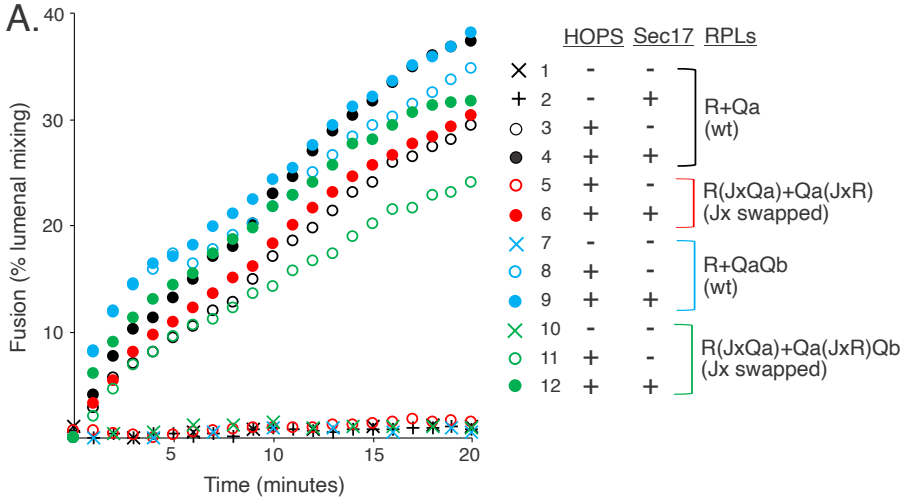
Supplemental Figure S5. Kinetics of fusion between R and QaQbQc proteoliposomes, with either sQb or wild-type, membrane-anchored Qb incorporated into the 3Q-SNARE complex during proteoliposome preparation, and with either wild-type or swapped R and Qa Jx

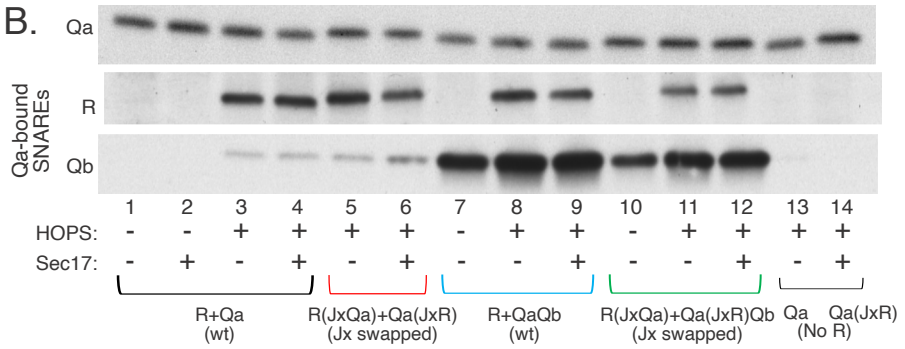
domains. Kinetic data from one of the 3 repeat assays for Figure 9 is shown.

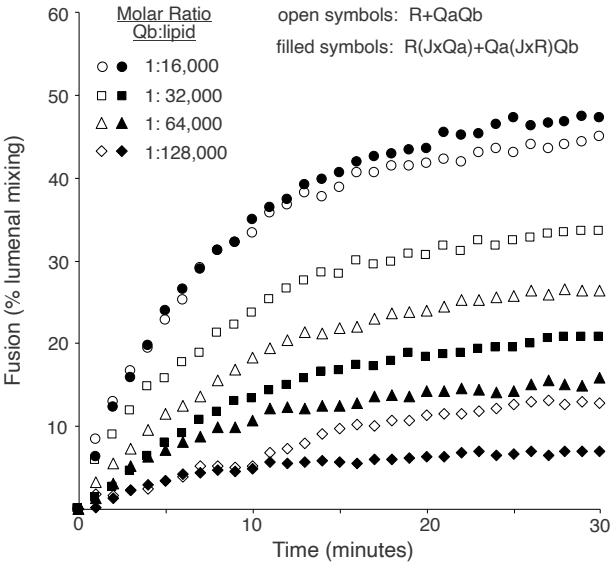
Supplemental Figure S6. Kinetics of fusion of one of three repeat assays is shown for Figure 10. (A) Fusion of Jx-swapped R+Qa RPLs remains sensitive to the addition of Sec17 with its hydrophobic loop even in the presence of Sec18/ATP. (B) Sec17 (wild-type or FSMS) is not needed for fusion with Jx-swapped RPLs with 2 anchored Q-SNAREs in the presence of Sec18/ATP.


A.
Fusion (% lumenal mixing)
Time (minutes)
Qc
Sec17/Sec18 ATPγS
RPLs
wt - 1
wt + 2
3Δ - 3
3Δ + 4
R+Qa
wt - 5
wt + 6
3Δ - 7
3Δ + 8
R(JxQa)+ Qa(JxR)

B.
Qa-bound SNAREs
R
Qb
1 2 3 4 5 6 7 8
Qc
wt wt 3Δ 3Δ wt wt 3Δ 3Δ
Sec17/ Sec18/ ATPγS
- + - + - + - +
R+Qa RPLs
R(JxQa)+Qa(JxR) RPLs


A.
Fusion (% lumenal mixing)
40
30
20
10
5
10
15
20
Time (minutes)
HOPS Sec17 RPLs
1 - -
2 - +
3 + -
4 + +
5 + -
6 + +
7 - -
8 + -
9 + +
10 - -
11 + -
12 + +
R+Qa (wt)
R(JxQa)+Qa(JxR) (Jx swapped)
R+QaQb (wt)
R(JxQa)+Qa(JxR)Qb (Jx swapped)

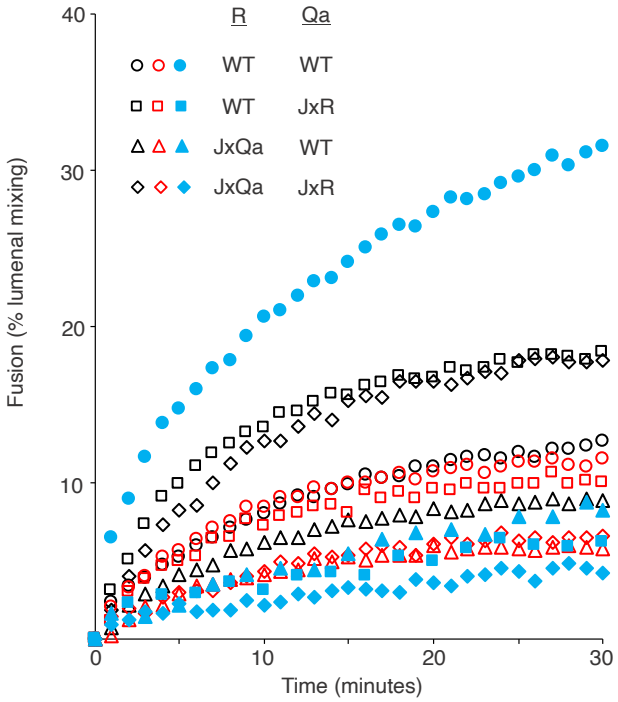

B.
Qa
R
Qb
Qa-bound SNAREs
1 2 3 4 5 6 7 8 9 10 11 12 13 14
HOPS: - - + + + + - + + - + + + +
Sec17: - + - + - + - - + - - + - +
R+Qa (wt)
R(JxQa)+Qa(JxR) (Jx swapped)
R+QaQb (wt)
R(JxQa)+Qa(JxR)Qb (Jx swapped)
Qa (No R)
Qa(JxR)

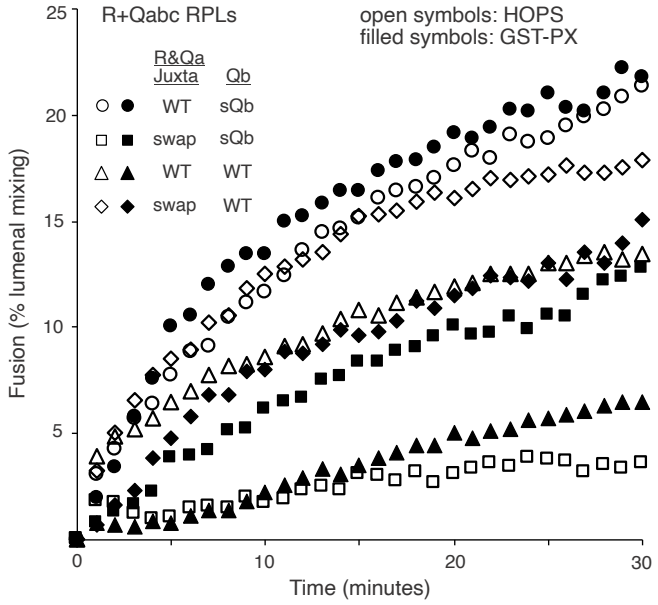

Molar Ratio
Qb:lipid
1:16,000
1: 32,000
1: 64,000
1:128,000
open symbols: R+QaQb
filled symbols: R(JxQa)+Qa(JxR)Qb
Fusion (% lumenal mixing)
Time (minutes)
0
10
20
30
10
20
30
40
50
60

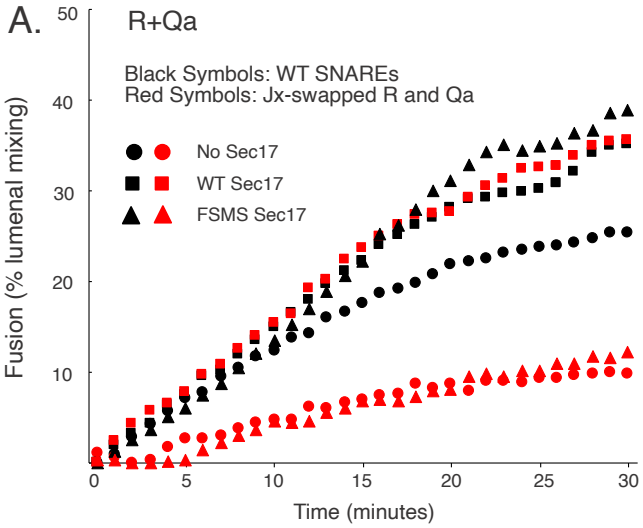
Qb:Lipid Molar ratio 1:64,000

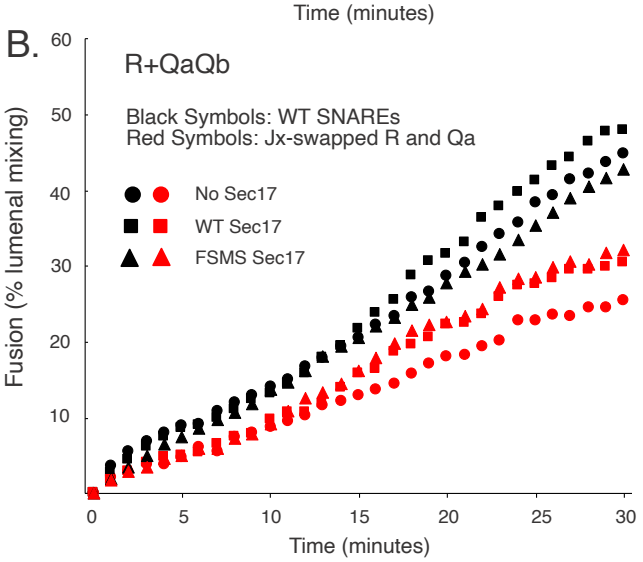
Qb:Lipid Molar ratio 1:128,000

sQb:Lipid Molar ratio 1:200,000




R+Qabc RPLs
open symbols: HOPS
filled symbols: GST-PX
R&Qa Juxta
Qb
WT sQb
swap sQb
WT WT
swap WT
Fusion (% lumenal mixing)
Time (minutes)
0
10
20
30
5
15
25


A.
R+Qa
Black Symbols: WT SNAREs
Red Symbols: Jx-swapped R and Qa
No Sec17
WT Sec17
FSMS Sec17
Fusion (% lumenal mixing)
Time (minutes)
0
5
10
15
20
25
30
10
20
30
40
50


B.
R+QaQb
Black Symbols: WT SNAREs
Red Symbols: Jx-swapped R and Qa
No Sec17
WT Sec17
FSMS Sec17
Fusion (% lumenal mixing)
Time (minutes)
0
5
10
15
20
25
30
10
20
30
40
50
60