Supplemental Materials

Molecular Biology of the Cell

Zlatic et al.

Figure S1. Co-fractionation of AP-3 β3A in Clathrin Coated Vesicle Fractions is Not Affected by Bloc1s5.

A) Coomassie stain of fractions from clathrin coated vesicle isolations in SHSY5Y cells expressing control shRNA (Lanes 1-6) or shRNA directed against the BLOC1 subunit Muted (Bloc1s5, Lanes 1'-6'). B) Densitometry analysis of CCV fractions from A. C) Western blot of clathrin coated vesicle fractions from A. H, Homogenate; P1, Pellet 1; S1, Supernatant 1; P2, Pellet 2; S2, Supernatant 2; CCV, Clathrin Coated Vesicle fraction; IB, Immunoblot.

Figure S2. Acute Perturbation of Clathrin Imaged with Confocal Microscopy As Compared To Control.

Microscopy of PC12 cells stably expressing mCh-FKBP-CLC. Cells were treated for two hours with either vehicle control (top panel) or the drug AP20187 (lower panel). Middle column shows three-fold increase in magnification of boxed regions for the respective panels. Far-right panel depicts percent colocalization of AP-3 δ and clathrin light chain signal in PC12 cells stably expressing mCh-FKBP-CLC. Columns 1 and 2 depict the analysis of 10 cells.

Figure S3. High-Resolution Deconvolution and 3-Dimensional Surface Reconstructions of EGFP-Rab5-Q79L Expressing Cells With Acute Clathrin Perturbation As Compared To Control.

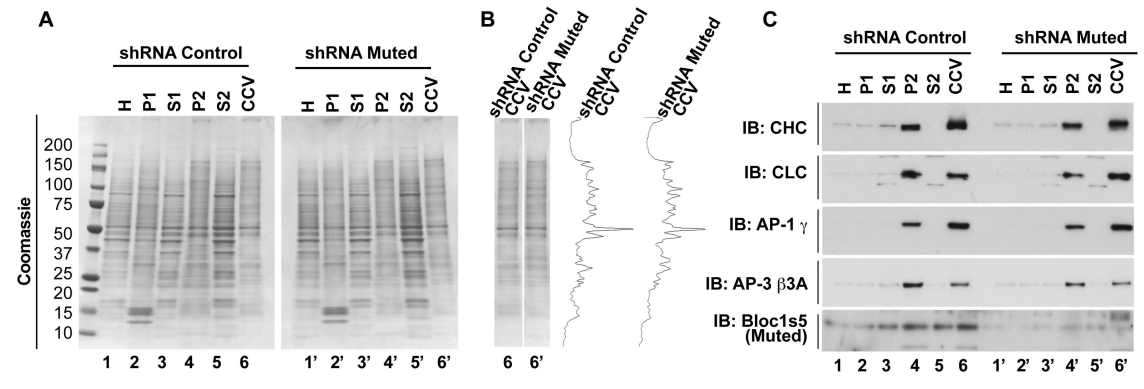
PC12 cells stably expressing mCh-FKBP-CLC and transiently expressing EGFP-Rab5-Q79L and treated for two hours with either vehicle control (A and B) or the drug AP20187 (C and D) which acutely perturbs clathrin function in these cells. A) High-resolution deconvolution microscopy through a single z-plane. Scale bar is 10 μm. B) 3-Dimensional surface reconstruction of thresholded signals through 10 z-planes. Scale bar is 10 μm. B1) Enlargement of boxed region in B. Scale bar is 1 μm. B2) 180° rotation of the structure in B1 around a vertical axis. C) High-resolution deconvolution microscopy through a single z-plane. Scale bar is 10 μm. D) 3-Dimensional surface reconstruction of threshold signals through 10 z-planes. D1) Enlargement of boxed region in D. Scale bar is 1 μm. D2) 180° rotation of the structure in D1 around a vertical axis.

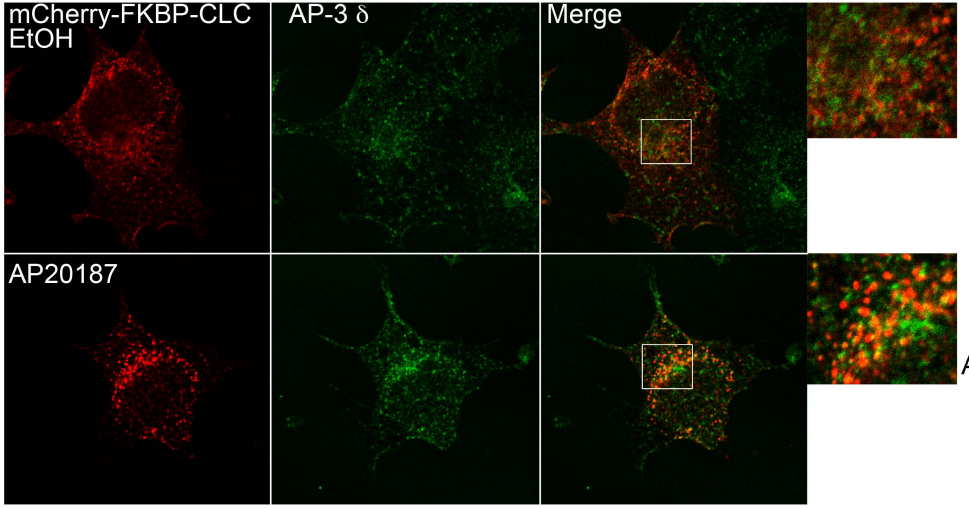
Figure S4. AP-3 δ Present in EGFP-Rab5-Q79L Endosomes Overlaps with mCh-FKBP-CLC.

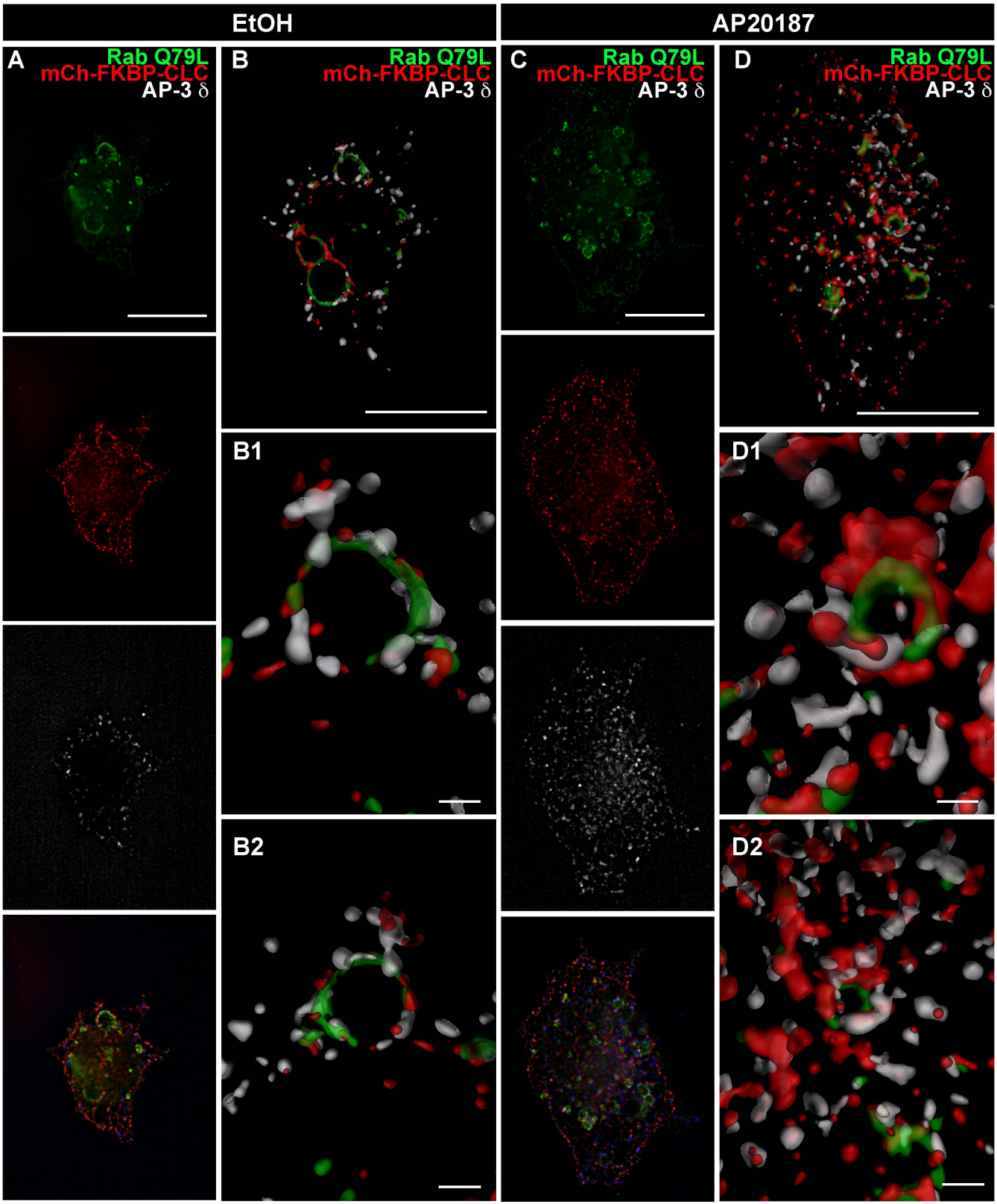
A) Percent colocalization of AP-3 δ, clathrin light chain and EGFP-Rab5-Q79L signal in PC12 cells stably expressing mCherry-FKBP-CLC and transiently expressing EGFP-Rab5-Q79L. These cells were treated with vehicle or AP20187 to acutely perturb clathrin function. Each dot represents a z-slice through an EGFP-Rab5-Q79L profile. B) Dot plot shows no significant difference in AP-3/mCh-FKBP-CLC colocalization as a function of total clathrin light chain content in an EGFP-Rab5-Q79L profile. $p = 0.276$ Fisher r-to-z transformation. C) Dot plot shows no significant difference in AP-3/mCh-FKBP-CLC colocalization as a function of EGFP-Rab5-Q79L profile perimeter. $p = 0.116$. D) Probability plot shows a significant difference in EGFP-Rab5-Q79L profile perimeter between vehicle (EtOH) and AP20187 treated cells. $p < 0.0001$ Kolmogorov-Smirnov Test. A-D present analysis of 445 organelles in 24 cells treated with vehicle (EtOH, blue) and 626 organelles in 28 cells treated with AP20187 (red).

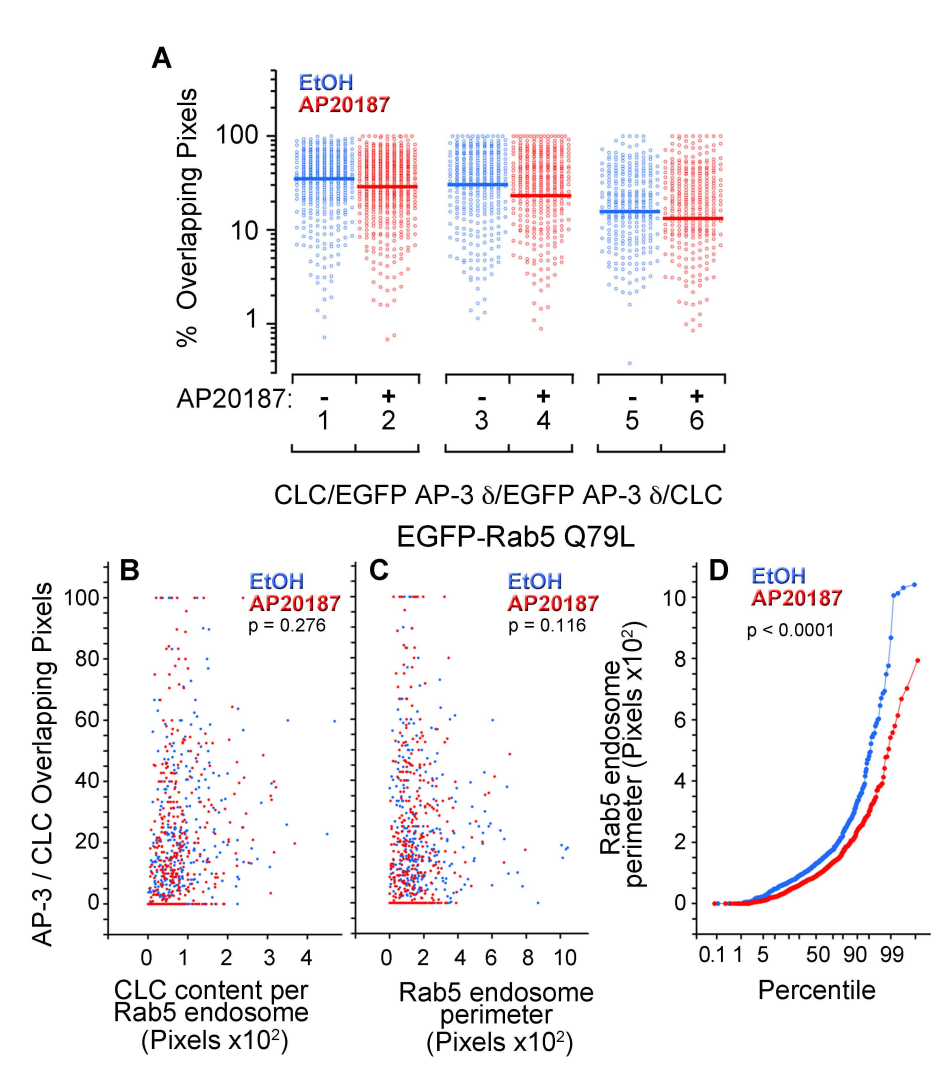
Figure S5. Synaptic Like Micro-Vesicle Formation Remains Sensitive to a Prolonged Brefeldin A Block.

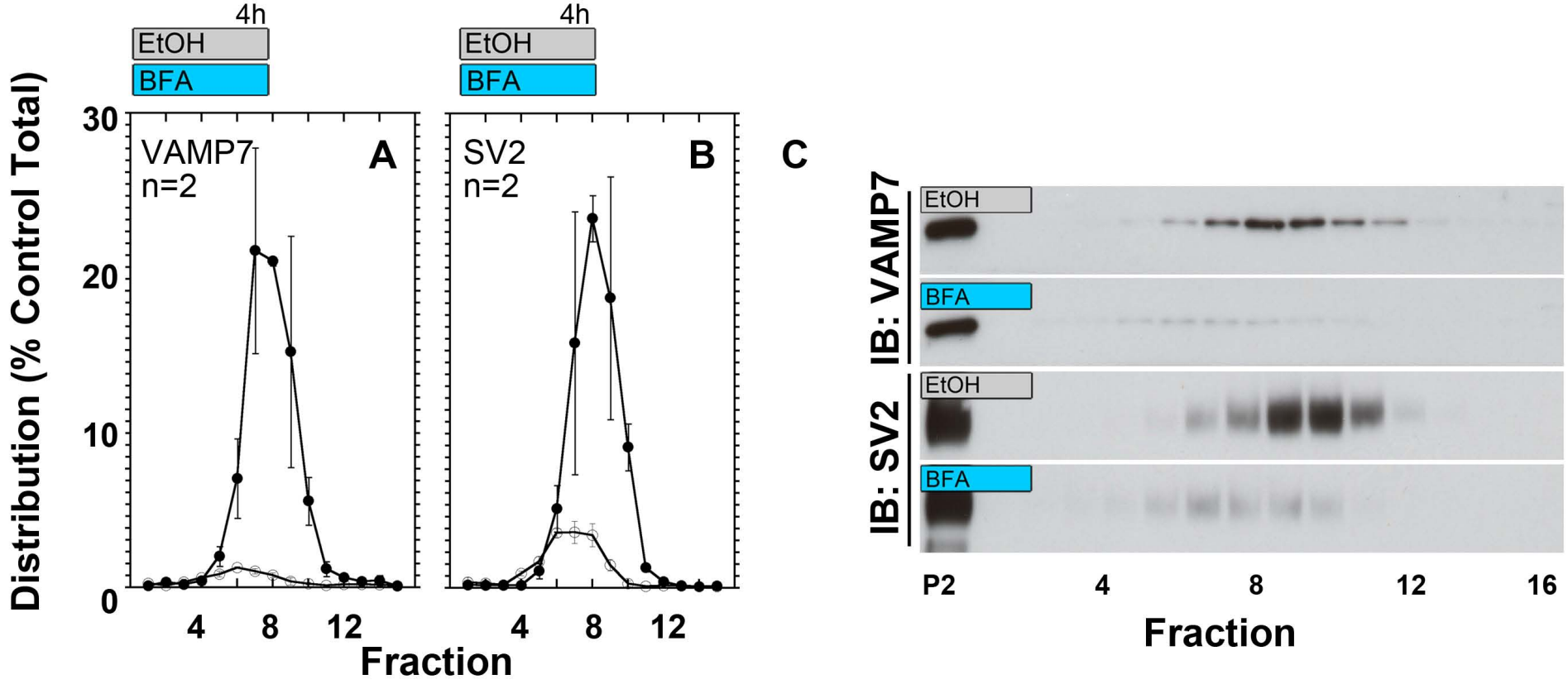
PC12 cells stably expressing mCh-FKBP-CLC were treated for four hours with Brefeldin A (BFA) or Vehicle Control (EtOH) at 37°C. Following this four hour treatment reactions were stopped at 4°C. Cells were homogenized and fractionated to isolate Synaptic Like Micro-Vesicles (SLMV), which sediment to the middle of 5% - 20% glycerol velocity gradients. Fractions from this gradient were loaded onto SDS-polyacrylamide gels for western blot and densitometry quantifications. Immunoblot (IB) for SV2 and VAMP7 was used to track SLMVs. Densitometry quantification of VAMP7 (A) and SV2 (B) fraction signal per total signal in BFA treated cells (white circles) as compared to EtOH only treated cells (black circles). C depicts blots of experiments quantified in A and B.


A
shRNA Control
shRNA Muted
H
P1
S1
P2
S2
CCV
Coomassie
200
150
100
75
50
37
25
20
15
10
1 2 3 4 5 6
1' 2' 3' 4' 5' 6'
B
shRNA Control
CCV
shRNA Muted
CCV
6 6'
C
shRNA Control
shRNA Muted
IB: CHC
IB: CLC
IB: AP-1 γ
IB: AP-3 β3A
IB: Bloc1s5 (Muted)
1 2 3 4 5 6 1' 2' 3' 4' 5' 6'


mCherry-FKBP-CLC
EtOH
AP-3 δ
Merge
AP20187
EtOH
AP20187
100
10
1
% Overlapping Pixels
AP20187:
-
+
1
2
AP-3 δ/CLC
Whole Cell


EtOH
AP20187
A
B
C
D
Rab Q79L
mCh-FKBP-CLC
AP-3 δ
B1
B2
D1
D2


A
EtOH
AP20187
% Overlapping Pixels
100
10
1
AP20187:
-
+
1
2
3
4
5
6
CLC/EGFP AP-3 δ/EGFP AP-3 δ/CLC
EGFP-Rab5 Q79L
B
EtOH
AP20187
p = 0.276
AP-3 / CLC Overlapping Pixels
CLC content per Rab5 endosome (Pixels x10²)
C
EtOH
AP20187
p = 0.116
Rab5 endosome perimeter (Pixels x10²)
D
EtOH
AP20187
p < 0.0001
Rab5 endosome perimeter (Pixels x10²)
0.1 1 5 50 90 99
Percentile


4h
EtOH
BFA
4h
EtOH
BFA
Distribution (% Control Total)
30
20
10
0
VAMP7
n=2
A
SV2
n=2
B
4
8
12
4
8
12
Fraction
C
IB: VAMP7
IB: SV2
EtOH
BFA
EtOH
BFA
P2
4
8
12
16
Fraction