## Supplemental Materials Molecular Biology of the Cell

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**Supplemental Figure 1**. Frequency of microvesicle fusion events, and radial spread of VAChT-pH following exocytosis, in PC12 cells. (A) Histogram showing number of fusion events before and after application of stimulation buffer (KCl). (B) Average normalized radial line scans of VAChT-pH during fusion. Contrast has been adjusted to show areas with lower intensity. Vertical dotted lines indicate the time-points corresponding to linescan intensity plots (Right). Standard errors are plotted as shaded areas around the average traces. Data was obtained from cells co-expressing VAChT-pH and farnesyl-mCherry. (C) Average time-lapse traces of normalized fluorescence intensities for VAChT-pHuji (magenta) and NPY-GFP (green). Individual event traces were time-aligned to 0 s, which corresponds to the fusion frame in the red channel. Standard errors are plotted as shaded areas around the average traces of cDNA, and the number of events and cells analyzed for each protein. \*Truncated CMV promoter.

**Supplemental Figure 2.** Dynamics of Rab proteins during SLMV fusion. (A) Average normalized radial line scans of the Rab proteins during microvesicle exocytosis. Bar, 1  $\mu$ m. Vertical dotted lines indicate the time-points corresponding to linescan intensity plots (Right). Standard errors are plotted as shaded areas around the average traces.

**Supplemental Figure 3.** Dynamics of SNARE proteins during fusion. (A) Average normalized radial line scan analysis of SNAREs and control mCherryproteins during microvesicle exocytosis. Bar, 1  $\mu$ m. Vertical dotted lines indicate the time-points corresponding to linescan intensity plots (Right). Standard errors are plotted as shaded areas around the average traces.

**Supplemental Figure 4**. Synaptotagmin1 and Muncprotein behavior at SLMV fusion sites. (A-C) Average time-lapse traces of normalized fluorescence intensities for: (A) synaptotagmin1-mCherry (266 events, 5 cells), (B) mCherry-Munc18a (250 events, 11 cells), and(C) Munc13-mCherry (132 events, 5 cells). Individual event traces were time-aligned to 0 s (vertical black line), which corresponds to the fusion frame in the green channel. Standard errors are plotted as shaded areas around the average traces.\*p $\leq$ 0.05, ns – not significant, when compared with baseline.

**Supplemental Figure 5.** Dynamics of SNARE modulators during SLMV fusion. (A) Average normalized radial line scans of accessory proteins during microvesicle exocytosis.Bar, 1  $\mu$ m. Vertical dotted lines indicate the time-points corresponding to linescan intensity plots (Right). Standard errors are plotted as shaded areas around the average traces.

**Supplemental Figure 6.** Dynamics of WT and mutant amphiphysin1 and syndapin2, and diffusion of VAChT-pH, during SLMV fusion. (A) Average normalized radial line scans of WT and mutant amphiphysin1 and syndapin2 proteins during microvesicle exocytosis. Bar, 1  $\mu$ m. (B) Average normalized radial line scans of VAChT-pH during fusion in PC12 cells co-expressing syndapin2-BAR. Contrast has been adjusted to show areas with lower intensity. Vertical dotted lines indicate the time-points corresponding to linescan

intensity plots (Right). Standard errors are plotted as shaded areas around the average traces.

**Supplemental Figure 7.** Endophilins are localized at SLMV fusion sites during exocytosis. (A-C) (Left) Average time-lapse traces of normalized fluorescence intensities for: (A) endophilinA1-mCherry (258 events, 13 cells), (B) endophilinA2-mCherry (207 events, 11 cells), and (C) endophilinB1-mCherry (213 events, 11 cells). Individual event traces were time-aligned to 0 s (vertical black line), which corresponds to the fusion frame in the green channel.\*p $\leq$ 0.05, \*\*p $\leq$ 0.01, \*\*\*p $\leq$ 0.0001, ns – not significant, when compared with baseline. (Middle) Average normalized radial line scans of endophilins during fusion. Bar, 1 µm. Vertical dotted lines indicate the time-points corresponding to linescan intensity plots (Right). Standard errors are plotted as shaded areas around the average traces.

**Supplemental Figure 8.**Effect of BAR domain proteins on loss of VAChT-pH from SLMV fusion sites. (A-D) Average time-lapse traces of normalized VAChT-pH fluorescence intensities in cells co-expressing VAChT-pH and the indicated BAR domain or control mCherry proteins. Individual traces were time-aligned to 0 s, which corresponds to the frame of fusion. Standard errors are plotted as shaded areas around the average traces.

**Supplemental Figure 9.** Effect of syndapin2 knock-down on VAChT-pH loss from fusion sites. (A) Western blots showing expression of syndapin2 in PC12 cells, that is reduced following treatment with siRNA. (Top) Blot probed with anti-syndapin2 antibody. (Bottom) Same blot re-probed with anti- $\alpha$ -actin. (B) Average time-lapse traces of normalized VAChT-pH fluorescence intensities in cells treated with control siRNA (219 events, 15 cells) or sisyndapin2 (184 events, 16 cells). Individual traces were time-aligned to 0 s, which corresponds to the fusion frame. Standard errors are plotted as shaded areas around the average traces.

**Supplemental Figure 10.** Recruitment of WT and mutant dynamin to SLMV fusion sites, and effect of BAR domain proteins and dynamin on VAChT decay. (A-F) (Left) Average time-lapse traces of normalized fluorescence intensities for: (A) Dynamin1-WT (250 events, 14 cells), (B) Dynamin-K44A (309 events, 12 cells), (C) Dynamin1- $\Delta$ Amph1 (365 events, 17 cells), (D) Dynamin1- $\Delta$ Synd2 (341 events, 17 cells), (E) Dynamin2-WT (260 events, 12 cells), and (F) Dynamin2- $\Delta$ PRD (252 events, 14 cells). Individual event traces were time-aligned to 0 s (vertical black line), which corresponds to the fusion frame in the green channel. \*p $\leq$ 0.05, \*\*p $\leq$ 0.01, \*\*\*p $\leq$ 0.0001, ns – not significant, when compared with baseline. (Middle) Average normalized radial line scans of WT and mutant dynamin proteins during microvesicle exocytosis. Bar, 1 µm. Vertical dotted lines indicate the time-points corresponding to linescan intensity plots (Right). Standard errors are plotted as shaded areas around the average traces. (G) Single exponential decay constants for VAChT-pH when co-expressed with the indicated constructs, or treated with the indicated siRNA.

**Supplemental Figure 11.** Dynamics of other endocytic proteins at SLMV fusion sites. (A-D) (Left) Average time-lapse traces of normalized fluorescence intensities for: (A) N-WASP-mCherry (197 events, 8 cells), (B) ClathrinLC-mCherry (209 events, 5 cells), (C) AP2- $\mu$ 2-mCherry (153 events, 5 cells), and (D) mCherry-Intersectin (122 events, 5 cells). Individual event traces were time-aligned to 0 s (vertical black line), which corresponds to the fusion frame in the green channel. \*p $\leq$ 0.05, \*\*p $\leq$ 0.01, ns – not significant, when compared with baseline. (Middle) Average normalized radial line scan analysis. Bar, 1  $\mu$ m. Vertical dotted lines indicate the time-points corresponding to linescan intensity plots (Right). Standard errors are plotted as shaded areas around the average traces.

**Supplemental Figure 12**. Plasma membrane recruitment of amphiphysin1 following stimulation. (A) Images of a PC12 cell expressing VAChT-pH (top) and Amph1-mCherry (bottom) at indicated time-points, with '0 s' being the start of the experiment. Stimulation buffer (KCl) was applied from ~ 20 s to 60 s. Bar, 5  $\mu$ m. (B) Time-lapse traces show background-subtracted and normalized mean fluorescence intensities for VAChT-pH (green) and amphiphysin1-mCherry (magenta) from a region around the whole cell.



VAChT-pHluorin     D. Clapham (Janelia)       dCMV-mCherry-SNAP25"     97     8     J. Taraska (NHLBJ/NIH)       VAMP2-mCherry-SNAP25"     97     8     J. Taraska (NHLBJ/NIH)       VAMP2-mCherry     172     7     R. Scheller (Stanford U.)       mCherry     274     8     Clontech       Farnesyl-mCherry     166     9     J. Taraska (NHLBJ/NIH)       Complexin2-mCherry     202     7     Biobasic synthesis       CAPS-mKate2     232     5     T. Martin (UW, Madison)       NSF-mCherry     274     5     P. Hanson (Wash U.)       Synaptotagmin1-mCherry     266     5     J. Taraska (NHLBJ/NIH)       mCherry-Munc18a     250     11     Biobasic synthesis       mCherry-Rabz7A     103     4     W. Westroek (NIH)       mRFP-Rab3A     196     5     M. Fukuda (Tohoku U.)       mCherry-Rabpilin3A     198     6     I. Macara (Vanderbilt U.)       mCherry-Rabz7B     73     3     J. Taraska (NHLBJ/NIH)       Amphiphysin1-mCherry     214     13     J. Taraska (NHLBJ/NIH) <	DNA Construct	No. of Events	No. of Cells	Source of cDNA
dCMV-mCherry-Syntaxin1a*     76     4     W. Almers (OHSU)       dCMV-mCherry-SNAP25*     97     8     J. Taraska (NHLBI/NIH)       VAMP2-mCherry     172     7     R. Scheller (Stanford U.)       mCherry-Dherry     274     8     Clontech       Farnesyl-mCherry     202     7     Biobasic synthesis       CAPS-mKate2     232     5     T. Martin (UW, Madison)       mCherry-Tomosyn     2289     9     Biobasic synthesis       NSF-mCherry     274     5     P. Hanson (Wash U.)       Synaptotagmin1-mCherry     266     5     J. Taraska (NHLBI/NIH)       mCherry-Rubratina     2250     11     Biobasic synthesis       Munc13-mCherry     132     5     Biobasic synthesis       mCherry-Rab27A     103     4     W. Westbroek (NIH)       mCherry-Rab3A     196     5     M. Fukuda (Tohoku U.)       mCherry-Rab3A     196     6     C. Merrifield (Addgene 27681)       mCherry-Rab45A     276     6     C. Merrifield (Addgene 27682)       mCherry-Rab5A     273     3	VAChT-pHluorin			D. Clapham (Janelia)
dCMV-mCherry-SNAP25*     97     8     J. Taraska (NHLB/NIH)       VAMP2-mCherry     172     7     R. Scheller (Stanford U.)       mCherry     274     8     Clontech       Farnesyl-mCherry     202     7     Biobasic synthesis       CAPS-mKate2     232     5     T. Martin (UW, Madison)       mCherry-Tomosyn     289     9     Biobasic synthesis       Synaptotagmin1-mCherry     266     5     J. Taraska (NHLB/NIH)       mCherry-Munc18a     250     11     Biobasic synthesis       Munc13-mCherry     132     5     Biobasic synthesis       Munc13-mCherry     132     5     Biobasic synthesis       Munc13-mCherry     132     5     Biobasic synthesis       Munc13-mCherry-Rab27A     103     4     W. Westbroek (NIH)       mCherry-Rab27A     103     4     W. Westbroek (NIH)       mCherry-Rab27B     73     3     J. Taraska (NHLB/NIH)       mCherry-Rab27B     73     3     J. Taraska (NHLB/NIH)       Amphiphysin1-mCherry     371     9     C. Merrifield	dCMV-mCherry-Syntaxin1a*	76	4	W. Almers (OHSU)
VAMP2-mCherry     172     7     R. Scheller (Stanford U.)       mCherry     274     8     Clonlech       FarnesyI-mCherry     166     9     J. Taraska (NHLBI/NIH)       Complexin2-mCherry     202     7     Biobasic synthesis       CAPS-mKate2     232     5     T. Martin (UW, Madison)       mCherry-Tomosyn     289     9     Biobasic synthesis       Synaptotagmin1-mCherry     274     5     P. Hanson (Wash U.)       Synaptotagmin1-mCherry     266     5     J. Taraska (NHLBI/NIH)       mCherry-Munc18a     250     11     Biobasic synthesis       Munc13-mCherry     132     5     Biobasic synthesis       mCherry-Rab27A     103     4     W. Westbroek (NIH)       mRFP-Rab3A     196     5     M. Fukuda (Tohoku U.)       mCherry-Rab27B     73     3     J. Taraska (NHLBI/NIH)       Amphiphysin1-MCherry     276     6     C. Merrifield (Addgene 27681)       MCherry-Rab27B     73     3     J. Taraska (NHLBI/NIH)       Amphiphysin1-ABAR-mCherry     174     9	dCMV-mCherry-SNAP25*	97	8	J. Taraska (NHLBI/NIH)
mCherry     274     8     Clontech       Farnesyl-mCherry     166     9     J. Taraska (NHLBI/NIH)       Complexin2-mCherry     202     7     Biobasic synthesis       CAPS-mKate2     232     5     T. Martin (UW, Madison)       mCherry-Tomosyn     289     9     Biobasic synthesis       NSF-mCherry     274     5     P. Hanson (Wash U.)       Synaptotagmin1-mCherry     274     5     P. Hanson (Wash U.)       Synaptotagmin1-mCherry     274     5     Biobasic synthesis       Munc13-mCherry     276     6     5     J. Taraska (NHLBI/NIH)       mCherry-Rab27A     103     4     W. Westbroek (NIH)       mCherry-Rab27B     73     3     J. Taraska (NHLBI/NIH)       Amphiphysin1-MCherry     214     13     J. Taraska (NHLBI/NIH)       Amphiphysin1-ABAR-mCherry     117     8	VAMP2-mCherry	172	7	R. Scheller (Stanford U.)
Farnesyl-mCherry     166     9     J. Taraska (NHLBI/NIH)       Complexin2-mCherry     202     7     Biobasic synthesis       CAPS-mKate2     232     5     T. Martin (UW, Madison)       mCherry-Tomosyn     289     9     Biobasic synthesis       Synaptotagmin1-mCherry     266     5     J. Taraska (NHLBI/NIH)       mCherry-Aburc18a     250     11     Biobasic synthesis       Munc13-mCherry     132     5     Biobasic synthesis       Munc13-mCherry     132     5     Biobasic synthesis       mCherry-Rab27A     103     4     W. Westbroek (NIH)       mRFP-Rab3A     196     5     M. Fukuda (Tohoku U.)       mCherry-Rab27B     73     3     J. Taraska (NHLBI/NIH)       mCherry-Rab5A     276     6     C. Merrifield (Addgene 27681)       mCherry-Rab7B     73     3     J. Taraska (NHLBI/NIH)       Amphiphysin1-abRA-mCherry     169     4     J. Taraska (NHLBI/NIH)       Amphiphysin1-abRA-mCherry     117     8     J. Taraska (NHLBI/NIH)       Amphiphysin1-abAR-mCherry     116	mCherry	274	8	Clontech
Complexin2-mCherry     202     7     Biobasic synthesis       CAPS-mKate2     232     5     T. Martin (UW, Madison)       mCherry-Tomosyn     289     9     Biobasic synthesis       NSF-mCherry     274     5     P. Hanson (Wash U.)       Synaptotagmin1-mCherry     266     5     J. Taraska (NHLBI/NIH)       mCherry-Munc18a     250     11     Biobasic synthesis       Munc13-mCherry     132     5     Biobasic synthesis       Munc13-mCherry     132     5     Biobasic synthesis       Munc13-mCherry     132     5     Biobasic synthesis       MCherry-Rab27A     103     4     W. Westbroek (NIH)       mRFP-Rab3A     196     5     M. Fukuda (Tohoku U.)       mCherry-Rab27B     73     3     J. Taraska (NHLBI/NIH)       Amphiphysin1-SH3-mCherry     214     13     J. Taraska (NHLBI/NIH)       Amphiphysin1-SH3-mCherry     214     13     J. Taraska (NHLBI/NIH)       Amphiphysin1-SH3-mCherry     214     13     J. Taraska (NHLBI/NIH)       Amphiphysin1-SH3-mCherry     214	Farnesyl-mCherry	166	9	J. Taraska (NHLBI/NIH)
CAPS-mKate2     232     5     T. Martin (UW, Madison)       mCherry-Tomosyn     289     9     Biobasic synthesis       NSF-mCherry     274     5     P. Hanson (Wash U.)       Synaptotagmin1-mCherry     266     5     J. Taraska (NHLBI/NIH)       Munc13-mCherry     132     5     Biobasic synthesis       Munc13-mCherry     132     5     Biobasic synthesis       mCherry-Rab27A     103     4     W. Westbroek (NIH)       mCherry-Rab27A     196     5     M. Fukuda (Tohoku U.)       mCherry-Rab27B     73     3     J. Taraska (NHLBI/NIH)       Amphiphysin1-MCherry     169     4     J. Taraska (NHLBI/NIH)       Amphiphysin1-Sh3-mCherry     117     8     J. Taraska (NHLBI/NIH)       Amphiphysin1-Sh3-mCherry     117     8     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ABAR     136	Complexin2-mCherry	202	7	Biobasic synthesis
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NSF-mCherry     274     5     P. Hanson (Wash U.)       Synaptotagmin1-mCherry     266     5     J. Taraska (NHLBI/NIH)       mCherry-Munc18a     250     11     Biobasic synthesis       Munc13-mCherry     132     5     Biobasic synthesis       mCherry-Rab27A     103     4     W. Westbroek (NIH)       mRFP-Rab3A     196     5     M. Fukuda (Tohoku U.)       mCherry-Rabphilin3A     198     6     I. Macara (Vanderbilt U.)       mCherry-Rab27B     73     3     J. Taraska (NHLBI/NIH)       Amphiphysin1-mCherry     371     9     C. Merrifield (Addgene 27681)       Amphiphysin1-ABAR-mCherry     169     4     J. Taraska (NHLBI/NIH)       Amphiphysin1-SH3-mCherry     117     8     J. Taraska (NHLBI/NIH)       Amphiphysin1-SH3-mCherry     117     8     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-BAR     136     11     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-BAR     136     1     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-BAR     136     11     DNASU HSCD00005501       E	mCherry-Tomosyn	289	9	Biobasic synthesis
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Incherry-Rabphilin3A     198     6     Information (on the second	mREP-Rab3A	196	5	M. Fukuda (Tohoku U.)
Internet	mCherry-Rabphilin3A	198	6	L Macara (Vanderbilt U.)
Incherry-Rab27B     T3     3     J. Taraska (NHLBI/NIH)       Amphiphysin1-mCherry     371     9     C. Merrifield (Addgene 27692)       Amphiphysin1-ΔBAR-mCherry     169     4     J. Taraska (NHLBI/NIH)       Amphiphysin1-ΔSH3-mCherry     214     13     J. Taraska (NHLBI/NIH)       Amphiphysin1-SH3-mCherry     117     8     J. Taraska (NHLBI/NIH)       Mcherry-Syndapin2     228     6     C. Merrifield (Addgene 27681)       mCherry-Syndapin2     228     6     C. Merrifield (Addgene 27681)       mCherry-Syndapin2-ΔBAR     136     11     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔBAR     136     11     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔBAR     184     9     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔSH3     184     9     J. Taraska (NHLBI/NIH)       mCherry     207     11     DNASU HsCD00000899       EndophilinA1-mCherry     258     13     DNASU HsCD00042012       Dynamin1-McHerry     250     14     C. Merrifield (Addgene 27697)       Dynamin1-McHerry     309     12     J. Taraska	mCherry-Rab5A	276	6	C Merrifield (Addgene 27681)
Internet     10     0 <th0< td=""><td>mCherry-Rab27B</td><td>73</td><td>3</td><td>J Taraska (NHI BI/NIH)</td></th0<>	mCherry-Rab27B	73	3	J Taraska (NHI BI/NIH)
Amphiphysin1-ΔBAR-mCherry     169     4     J. Taraska (NHLBI/NIH)       Amphiphysin1-ΔBAR-mCherry     214     13     J. Taraska (NHLBI/NIH)       Amphiphysin1-ΔSH3-mCherry     117     8     J. Taraska (NHLBI/NIH)       Amphiphysin1-SH3-mCherry     117     8     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2     228     6     C. Merrifield (Addgene 27681)       mCherry-Syndapin2-ΔBAR     136     11     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔBAR     418     13     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔBAR     418     13     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔSH3     184     9     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔSH3     184     9     J. Taraska (NHLBI/NIH)       mCherry     207     11     DNASU HsCD0000899       EndophilinA1-mCherry     213     11     DNASU HsCD000042012       Dynamin1-mCherry     250     14     C. Merrifield (Addgene 27697)       Dynamin1-K374E/S778E-mCherry     309     12     J. Taraska (NHLBI/NIH)       Dynamin2-MCherry     260     12 <t< td=""><td>Amphiphysin1-mCherry</td><td>371</td><td>9</td><td>C Merrifield (Addgene 27692)</td></t<>	Amphiphysin1-mCherry	371	9	C Merrifield (Addgene 27692)
Amphiphysin1-ΔSH3-mCherry     214     13     J. Taraska (NHLBI/NIH)       Amphiphysin1-SH3-mCherry     117     8     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2     228     6     C. Merrifield (Addgene 27681)       mCherry-Syndapin2-ΔBAR     136     11     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔBAR     136     11     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔBAR     418     13     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔSH3     184     9     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔPRO     258     13     DNASU HscD00000589       EndophilinA2-mCherry     207     11     DNASU HscD000042012       Dynamin1-mCherry     250     14     C. Merrifield (Addgene 27697)       Dynamin1-mCherry     250     14     C. Merrifield (Addgene 27697)       Dynamin1-833-838A-mCherry     365     1	Amphiphysin1-ABAR-mCherry	169	4	J Taraska (NHI BI/NIH)
Amphiphysin1-SH3-mCherry     117     Ref     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2     228     6     C. Merrifield (Addgene 27681)       mCherry-Syndapin2-ΔBAR     136     11     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔBAR     136     11     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔBAR     418     13     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔSH3     184     9     J. Taraska (NHLBI/NIH)       mCherry     207     11     DNASU HscD0000899       EndophilinA1-mCherry     207     11     DNASU HscD000042012       Dynamin1-mCherry     250     14     C. Merrifield (Addgene 27697)       Dynamin1-mCherry     309     12     J. Taraska (NHLBI/NIH)       Dynamin1-833-838A-mCherry     365     17     J. Taraska (NHLBI/NIH)       Dynamin1-S774E/S778E-mCherry     260     12     C.	Amphiphysin1-ASH3-mCherry	214	13	J Taraska (NHI BI/NIH)
Instruction     Instruction     Instruction       mCherry-Syndapin2     228     6     C. Merrifield (Addgene 27681)       mCherry-Syndapin2-ΔBAR     136     11     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔBAR     418     13     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔSH3     184     9     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔSH3     184     9     J. Taraska (NHLBI/NIH)       EndophilinA1-mCherry     258     13     DNASU HsCD0000899       EndophilinA2-mCherry     207     11     DNASU HsCD00005501       EndophilinB1-mCherry     213     11     DNASU HsCD00042012       Dynamin1-mCherry     250     14     C. Merrifield (Addgene 27697)       Dynamin1-K44A-mCherry     309     12     J. Taraska (NHLBI/NIH)       Dynamin1-K44A-mCherry     365     17     J. Taraska (NHLBI/NIH)       Dynamin1-S774E/S778E-mCherry     365     17     J. Taraska (NHLBI/NIH)       Dynamin2-MCherry     260     12     C. Merrifield (Addgene 27689)       Dynamin2-MCherry     252     14     J. Taraska (NHLBI/NIH) <td>Amphiphysin1-SH3-mCherry</td> <td>117</td> <td>8</td> <td>J Taraska (NHI BI/NIH)</td>	Amphiphysin1-SH3-mCherry	117	8	J Taraska (NHI BI/NIH)
mCherry-Syndapin2-ΔBAR     136     11     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔBAR     418     13     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔSH3     184     9     J. Taraska (NHLBI/NIH)       mCherry-Syndapin2-ΔSH3     184     9     J. Taraska (NHLBI/NIH)       EndophilinA1-mCherry     258     13     DNASU HsCD0000899       EndophilinA2-mCherry     207     11     DNASU HsCD000042012       Dynamin1-mCherry     250     14     C. Merrifield (Addgene 27697)       Dynamin1-mCherry     309     12     J. Taraska (NHLBI/NIH)       Dynamin1-K44A-mCherry     309     12     J. Taraska (NHLBI/NIH)       Dynamin1-S774E/S778E-mCherry     365     17     J. Taraska (NHLBI/NIH)       Dynamin2-mCherry     260     12     C. Merrifield (Addgene 27689)       Dynamin2-MCherry     252     14     J. Taraska (NHLBI/NIH)       mCherry-N-WASP     197     8     H. Yamada (Okayama U.)       AP2-µ2-mCherry     153     5     C. Merrifield (Addgene 27672)       mCherry-Intersectin     122     5     Peter McPherson	mCherry-Syndapin2	228	6	C. Merrifield (Addgene 27681)
Internet	mCherry-Syndapin2-ABAR	136	11	J Taraska (NHI BI/NIH)
Initial of the second secon	mCherry-Syndapin2-BAR	418	13	J. Taraska (NHI BI/NIH)
Initial Content	mCherry-Syndapin2-ASH3	184	9	J Taraska (NHI BI/NIH)
EndophilinA2-mCherry     207     11     DNASU HscD00005501       EndophilinB1-mCherry     213     11     DNASU HscD00042012       Dynamin1-mCherry     250     14     C. Merrifield (Addgene 27697)       Dynamin1-K44A-mCherry     309     12     J. Taraska (NHLBI/NIH)       Dynamin1-833-838A-mCherry     365     17     J. Taraska (NHLBI/NIH)       Dynamin1-S774E/S778E-mCherry     341     17     J. Taraska (NHLBI/NIH)       Dynamin2-mCherry     260     12     C. Merrifield (Addgene 27689)       Dynamin2-MCherry     260     12     C. Merrifield (Addgene 27689)       Dynamin2-MCherry     260     12     C. Merrifield (Addgene 27689)       Dynamin2-MCherry     252     14     J. Taraska (NHLBI/NIH)       mCherry-N-WASP     197     8     H. Yamada (Okayama U.)       AP2-μ2-mCherry     153     5     C. Merrifield (Addgene 27672)       mCherry-Intersectin     122     5     Peter McPherson (McGill U.)       Clathrin Light Chain-mCherry     209     5     W. Almers (OHSU)       Total     8249     324     Average	EndophilinA1-mCherry	258	13	DNASU HsCD0000899
EndophilinB1-mCherry     213     11     DNASU HscD00042012       Dynamin1-mCherry     250     14     C. Merrifield (Addgene 27697)       Dynamin1-mCherry     309     12     J. Taraska (NHLBI/NIH)       Dynamin1-833-838A-mCherry     365     17     J. Taraska (NHLBI/NIH)       Dynamin1-S774E/S778E-mCherry     341     17     J. Taraska (NHLBI/NIH)       Dynamin2-mCherry     260     12     C. Merrifield (Addgene 27689)       Dynamin2-MCherry     260     12     C. Merrifield (Addgene 27689)       Dynamin2-MCherry     260     12     C. Merrifield (Addgene 27689)       Dynamin2-MCherry     252     14     J. Taraska (NHLBI/NIH)       mCherry-N-WASP     197     8     H. Yamada (Okayama U.)       AP2-μ2-mCherry     153     5     C. Merrifield (Addgene 27672)       mCherry-Intersectin     122     5     Peter McPherson (McGill U.)       Clathrin Light Chain-mCherry     209     5     W. Almers (OHSU)       Total     8249     324     Average     217.08     8.53	EndophilinA2-mCherry	207	11	DNASU HsCD00005501
Dynamin1-mCherry     250     14     C. Merrifield (Addgene 27697)       Dynamin1-mCherry     309     12     J. Taraska (NHLBI/NIH)       Dynamin1-833-838A-mCherry     365     17     J. Taraska (NHLBI/NIH)       Dynamin1-S774E/S778E-mCherry     341     17     J. Taraska (NHLBI/NIH)       Dynamin2-mCherry     260     12     C. Merrifield (Addgene 27689)       Dynamin2-mCherry     260     12     C. Merrifield (Addgene 27689)       Dynamin2-MCherry     260     12     C. Merrifield (Addgene 27689)       Dynamin2-MCherry     252     14     J. Taraska (NHLBI/NIH)       mCherry-N-WASP     197     8     H. Yamada (Okayama U.)       AP2-μ2-mCherry     153     5     C. Merrifield (Addgene 27672)       mCherry-Intersectin     122     5     Peter McPherson (McGill U.)       Clathrin Light Chain-mCherry     209     5     W. Almers (OHSU)       Total     8249     324     4	EndophilinB1-mCherry	213	11	DNASU HscD00042012
Dynamin1-K44A-mCherry     309     12     J. Taraska (NHLBI/NIH)       Dynamin1-K44A-mCherry     365     17     J. Taraska (NHLBI/NIH)       Dynamin1-833-838A-mCherry     365     17     J. Taraska (NHLBI/NIH)       Dynamin1-S774E/S778E-mCherry     341     17     J. Taraska (NHLBI/NIH)       Dynamin2-mCherry     260     12     C. Merrifield (Addgene 27689)       Dynamin2-ΔPRD-mCherry     252     14     J. Taraska (NHLBI/NIH)       mCherry-N-WASP     197     8     H. Yamada (Okayama U.)       AP2-μ2-mCherry     153     5     C. Merrifield (Addgene 27672)       mCherry-Intersectin     122     5     Peter McPherson (McGill U.)       Clathrin Light Chain-mCherry     209     5     W. Almers (OHSU)       Total     8249     324     Average     217.08     8.53	Dynamin1-mCherry	250	14	C. Merrifield (Addgene 27697)
Dynamin1-833-838A-mCherry     365     17     J. Taraska (NHLBI/NIH)       Dynamin1-S774E/S778E-mCherry     341     17     J. Taraska (NHLBI/NIH)       Dynamin2-mCherry     260     12     C. Merrifield (Addgene 27689)       Dynamin2-MCherry     252     14     J. Taraska (NHLBI/NIH)       mCherry-N-WASP     197     8     H. Yamada (Okayama U.)       AP2-μ2-mCherry     153     5     C. Merrifield (Addgene 27672)       mCherry-Intersectin     122     5     Peter McPherson (McGill U.)       Clathrin Light Chain-mCherry     209     5     W. Almers (OHSU)       Total     8249     324     453	Dynamin1-K44A-mCherry	309	12	J. Taraska (NHLBI/NIH)
Dynamin1-S774E/S778E-mCherry     341     17     J. Taraska (NHLBI/NIH)       Dynamin2-mCherry     260     12     C. Merrifield (Addgene 27689)       Dynamin2-ΔPRD-mCherry     252     14     J. Taraska (NHLBI/NIH)       mCherry-N-WASP     197     8     H. Yamada (Okayama U.)       AP2-μ2-mCherry     153     5     C. Merrifield (Addgene 27672)       mCherry-Intersectin     122     5     Peter McPherson (McGill U.)       Clathrin Light Chain-mCherry     209     5     W. Almers (OHSU)       Total     8249     324     4	Dynamin1-833-838A-mCherry	365	17	J. Taraska (NHI BI/NIH)
Dynamin2-mCherry26012C. Merrifield (Addgene 27689)Dynamin2-DPRD-mCherry25214J. Taraska (NHLBI/NIH)mCherry-N-WASP1978H. Yamada (Okayama U.)AP2-µ2-mCherry1535C. Merrifield (Addgene 27672)mCherry-Intersectin1225Peter McPherson (McGill U.)Clathrin Light Chain-mCherry2095W. Almers (OHSU)Total8249324324Average217.088.535	Dynamin1-S774E/S778E-mCherry	341	17	J Taraska (NHI BI/NIH)
Dynamin2-ΔPRD-mCherry     252     14     J. Taraska (NHLBI/NIH)       mCherry-N-WASP     197     8     H. Yamada (Okayama U.)       AP2-μ2-mCherry     153     5     C. Merrifield (Addgene 27672)       mCherry-Intersectin     122     5     Peter McPherson (McGill U.)       Clathrin Light Chain-mCherry     209     5     W. Almers (OHSU)       Total     8249     324     453	Dynamin2-mCherry	260	12	C. Merrifield (Addgene 27689)
mCherry-N-WASP1978H. Yamada (Okayama U.)AP2-μ2-mCherry1535C. Merrifield (Addgene 27672)mCherry-Intersectin1225Peter McPherson (McGill U.)Clathrin Light Chain-mCherry2095W. Almers (OHSU)Total8249324Average217.088.53	Dynamin2-ΔPRD-mCherry	252	14	J. Taraska (NHLBI/NIH)
AP2-μ2-mCherry1535C. Merrifield (Addgene 27672)mCherry-Intersectin1225Peter McPherson (McGill U.)Clathrin Light Chain-mCherry2095W. Almers (OHSU)Total8249324Average217.088.53	mCherry-N-WASP	197	8	H. Yamada (Okayama U.)
mCherry-Intersectin1225Peter McPherson (McGill U.)Clathrin Light Chain-mCherry2095W. Almers (OHSU)Total8249324Average217.088.53	AP2-u2-mCherry	153	5	C. Merrifield (Addgene 27672)
Clathrin Light Chain-mCherry2095W. Almers (OHSU)Total8249324Average217.088.53	mCherry-Intersectin	122	5	Peter McPherson (McGill U.)
Total     8249     324       Average     217.08     8.53	Clathrin Light Chain-mCherry	209	5	W. Almers (OHSU)
Average 217.08 8.53	Total	8249	324	
	Average	217.08	8.53	













-10

0

10

20

Time (s)

30

40

50



Distance (µm)

1.65

1.65

1.65









G

VAChT-pH co-expressed or treated with	Tau (s)
Amphiphysin1-mCherry	2.39 ± 0.04
Amphiphysin1-ΔBAR-mCherry	1.36 ± 0.06
Amphiphysin1-ΔSH3-mCherry	3.12 ± 0.05
Amphiphysin1-SH3-mCherry	1.88 ± 0.08
mCherry-Syndapin2	2.28 ± 0.04
mCherry-Syndapin2-∆BAR	2.63 ± 0.05
mCherry-Syndapin2-∆SH3	3.43 ± 0.07
mCherry-Syndapin2-BAR	2.94 ± 0.04
EndophilinA1-mCherry	3.24 ± 0.04
EndophilinA2-mCherry	3.07 ± 0.06
EndophilinB1-mCherry	4.16 ± 0.07
Dynamin1-mCherry	3.47 ± 0.05
Dynamin1-K44A-mCherry	2.09 ± 0.08
Dynamin1-833-838A-mCherry	3.68 ± 0.05
Dynamin1-S774E/S778E-mCherry	3.93 ± 0.07
Dynamin2-mCherry	3.56 ± 0.09
Dynamin2-∆PRD-mCherry	2.69 ± 0.06
mCherry-N-WASP	2.64 ± 0.05
mCherry	2.07 ± 0.1
Farnesyl-mCherry	2.33 ± 0.04
siControl	3.15 ± 0.07
siSvndapin2	$4.01 \pm 0.06$





Time(s)