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# Supplemental Information

# Synaptic PI(3,4,5)$P_3$ Is Required for Syntaxin1A Clustering and Neurotransmitter Release

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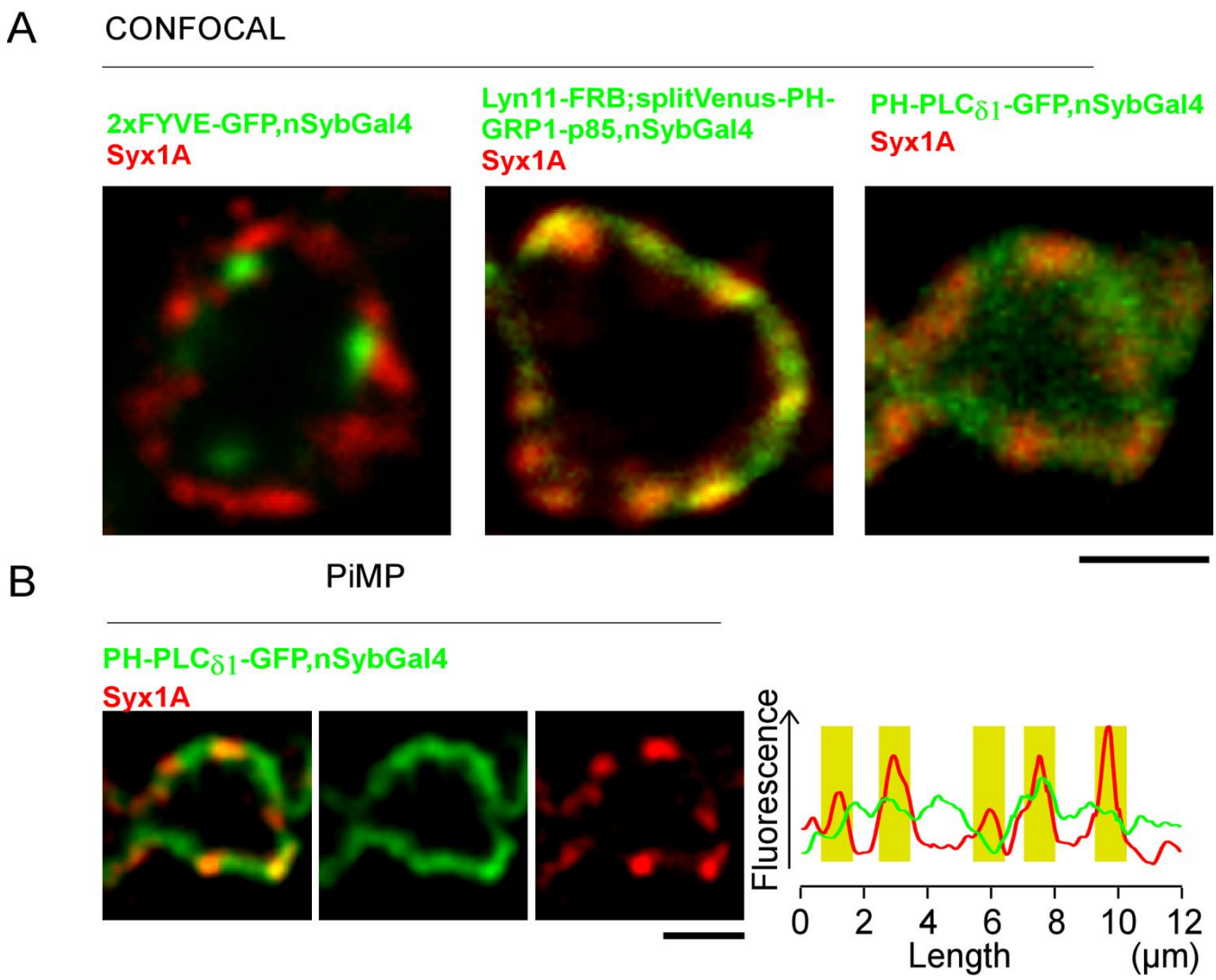
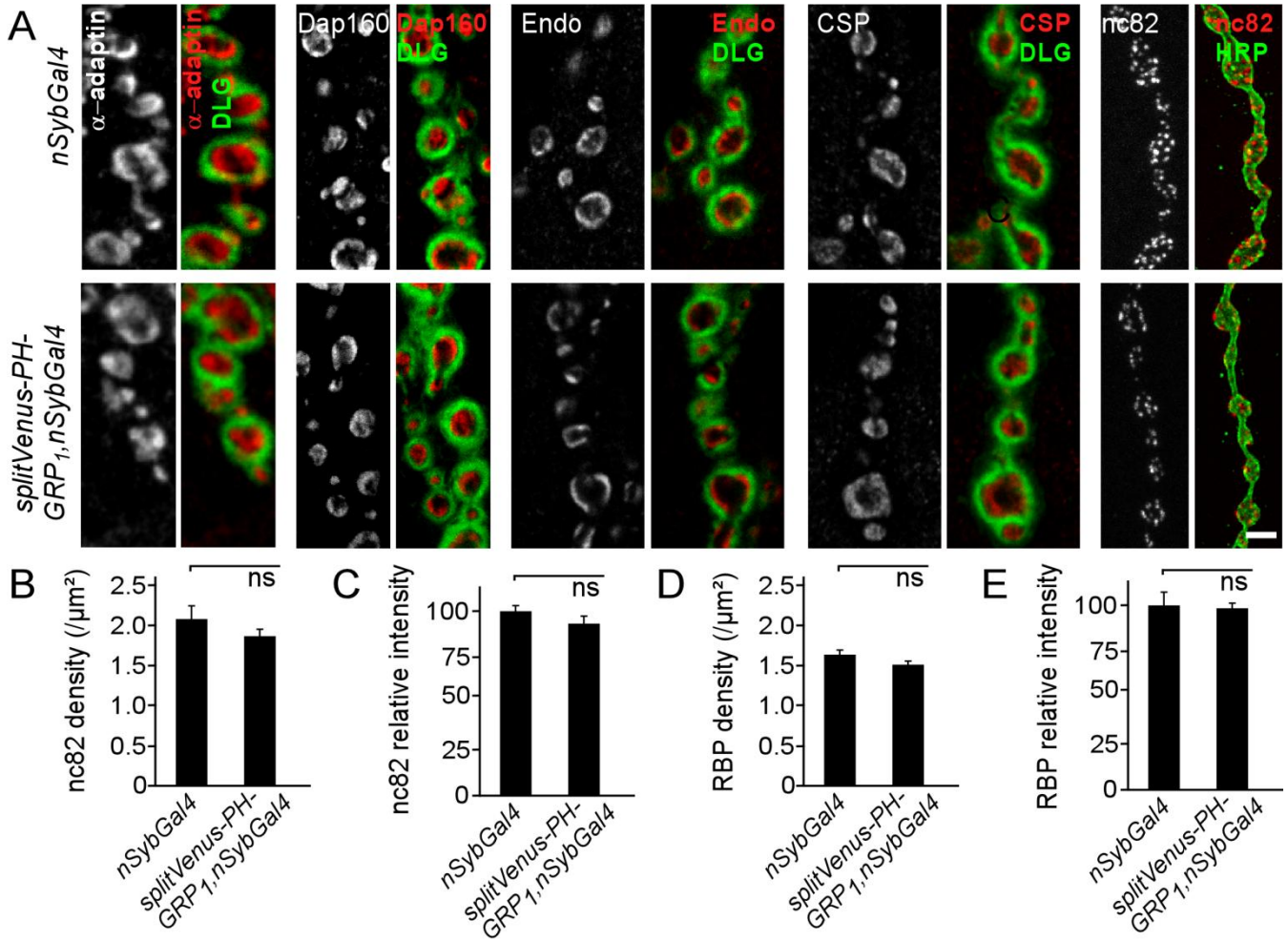



**Figure S1, related to Figure 1. PH- $PLC_{\delta 1}$-GFP that binds $PI(4,5)P_2$ does not extensively colocalize with Syntaxin1A**

(A) Single confocal section of third instar larval *Drosophila* NMJ boutons that express 2xFYVE-GFP (*yw; UAS-2xFYVE-GFP/+; nSybGal4/+*) of a bouton expressing FRB-Lyn11, FKBP-p85 and splitVenus PH-$GRP_1$ (*yw; UAS-Lyn11-FRB ; UAS-VenusC-PH-$GRP_1$ UAS-VenusN-PH-$GRP_1$ UAS-FKBP-p85 nSybGal4*) on rapamycin, and of a bouton expressing PH-$PLC_{\delta 1}$-GFP (*yw; UAS- PH-$PLC_{\delta 1}$-GFP nSybGal4*) labeled with anti-Syntaxin1A$^{8C3}$ (Syx1A, red). GFP or Venus fluorescence is in green.

(B) PiMP super resolution imaging of the bouton expressing PH-PLC$_{\delta 1}$-GFP shown in (A) and fluorescence intensity plot (arbitrary units) along the circumference of the bouton on the left adjusted to the total bouton circumference of anti-Syntaxin labeling intensity and of PH-PLC$_{\delta 1}$-GFP fluorescence intensity. Yellow highlighted sections mark peaks of labeling. Scale bar in A and B, 5 µm.


A
nSybGal4
splitVenus-PH-GRP1,nSybGal4
α–adaptin
α–adaptin DLG
Dap160
Dap160 DLG
Endo
Endo DLG
CSP
CSP DLG
nc82
nc82 HRP
B
nc82 density (/μm²)
ns
C
nc82 relative intensity
ns
D
RBP density (/μm²)
ns
E
RBP relative intensity
ns
nSybGal4
splitVenus-PH-GRP1,nSybGal4

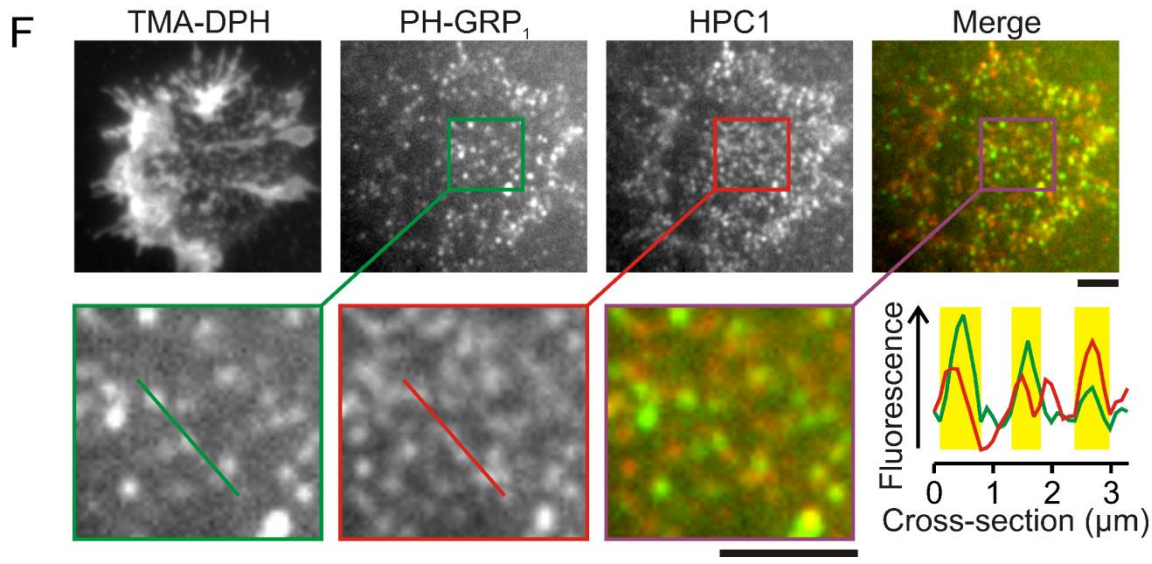

F
TMA-DPH
PH-GRP1
HPC1
Merge
Fluorescence
Cross-section (μm)
0
1
2
3

**Figure S2, related to Figure 2. PH-GRP$_1$, labeling PI(3,4,5)P$_3$ colocalizes with Syntaxin1A in PC12 cells**

(A) Single confocal sections of third instar larval *Drosophila* NMJ boutons of controls (top, *nSybGal4*) and of splitVenus-PH-GRP$_1$ expressing animals (bottom, *yw; UAS-VenusC-PH-GRP$_1$ UAS-VenusN-PH-GRP$_1$ nSybGal4*) labeled with anti-Alpha-adaptin, anti-Dap160, anti-Endophilin, anti-CSP and anti-BRP (nc82) (in Red) as well as with anti-DLG or anti-HRP (both synaptic markers, Green). Scale bar: 3 µm.

(B-E) Quantification of anti-BRP (nc82) and anti-RBP labeling intensity (C, E) and the number of dots per area (B, D) at third instar synaptic boutons of controls and of animals neuronally expressing splitVenus-PH-GRP$_1$. Data is mean ± SEM; n (the number of animals) >5; t-test: ns: not significant.

(F) PC12 membrane sheet immunostained with an Atto647N-labeled primary antibody raised against Syntaxin1A (HPC1) and stained with PH-GRP$_1$ genetically fused to mCherry. Sheets were imaged with TMA-DPH, a generic membrane dye. A representative image is shown from 16 sheets of 2 independent preparations. Bottom right, fluorescence intensity plot of PH-GRP$_1$-Cherry fluorescence (green) and Syntaxin1A labeling intensity (red) along the line shown. Yellow highlighted sections mark peaks of labeling. Scale bar, 3 µm.

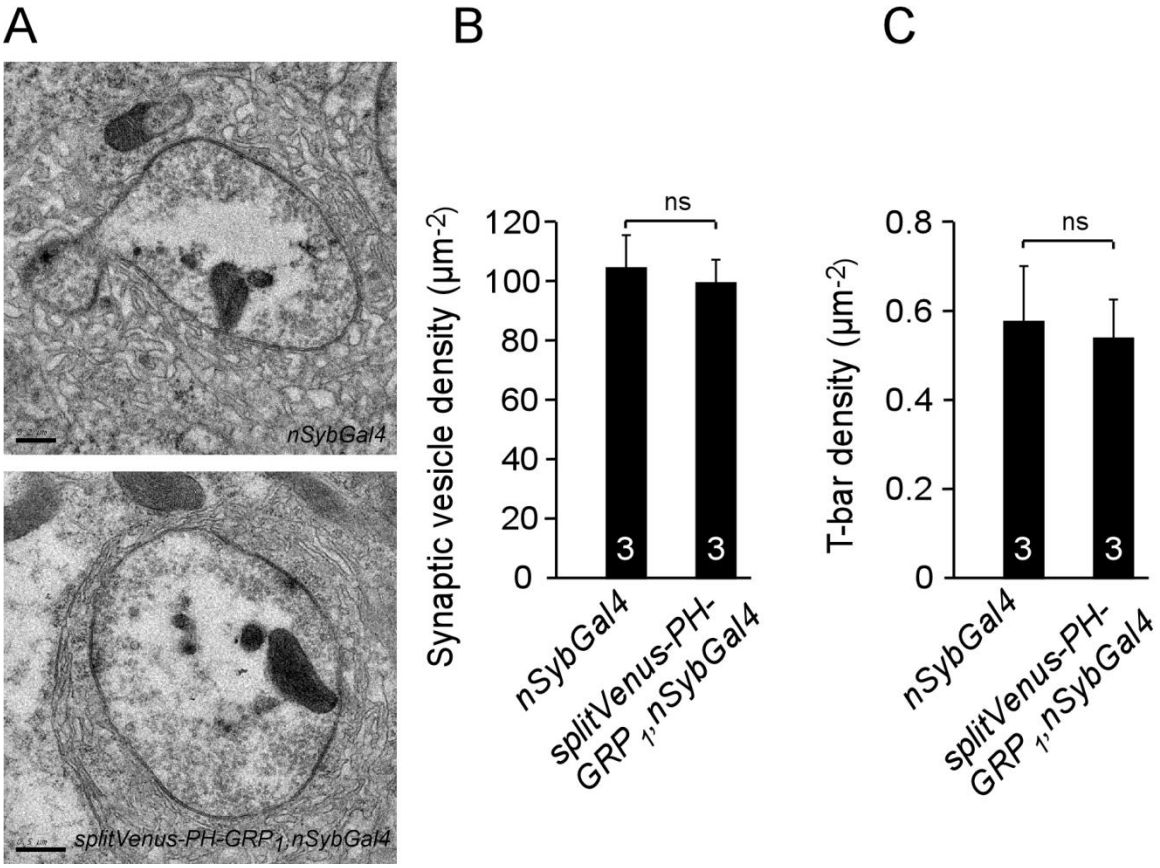



**Figure S3, related to Figure 5. Normal ultrastructure of synaptic boutons in flies neuronally expressing splitVenus-PH-GRP$_1$.**

(A) Electron micrographs of type Ib boutons of control (*nSybGal4*) (top) and animals expressing splitVenus-PH-GRP$_1$ (*yw; UAS-VenusC-PH-GRP$_1$ UAS-VenusN-PH-GRP$_1$ nSybGal4*, bottom).

(B-C) Quantification of vesicle density (B) and T-bar density (C) in control and animals expressing splitVenus-PH-GRP$_1$. Data is mean ± SEM; n (the number of animals) is indicated in the bars; Tukey's test: ns: not significant.

**Table S1, related to Figure 3. Conservation of positively charged juxtamembrane domain residues in Syntaxin1A**

| ***Species*** | ***juxtamembrane domain sequence and basic stretch (highlight)*** |
|---|---|
| *Drosophila melanogaster* | KKALKYQSKARRKKIMILICLT |
| *Xenopus (Silurana) tropicalis* | KKAVKYQSKARRKKIMIIICCV |
| *Rattus norvegicus* | KKAVKYQSKARRKKIMIIICCV |
| *Homo sapiens* | KKAVKYQSKARRKKIMIIICCV |

**Table S2, related to the experimental procedures. Primers used**

| **Primer** | **Sequence** |
|---|---|
| **VenusN** | |
| VenusN-F | CTCCGCGGCCGCGTCGACACAAAATGGTGAGCAAGGGCGAGG |
| VenusN-R | CTCGAGATCTGAGTCCGGAGGCGGTGATATAGACGTTG |
| **VenusC** | |
| VenusC-F | CTCCGGATCCGCGGCCGCGTCGACACAAAATGGACAAGCAGA AGAACGGCATC |
| VenusC-R | CTCGAGATCTGAGTCCGGACTTGTACAGCTCGTCCATGC |
| **Lyn11-FRB** | |
| Lyn11-F | CTCCGCGGCCGCACAAAATGGGATGTATAAAATCAAAAG |
| Lyn11-R | CTTAGCGGCCGCGGTACCCTTATGCGTAGTCTGGTACG |
| **FKBP-p85** | |
| p85-F | CTCCGCGGCCGCGTCGACACAAAATGGTGAGCAAGGGCGAGG |
| p85-R | CTTAGCGGCCGCGGTACCCTCACGTGCGCTCCTCGTGG |

| **HA-syntaxin1A$^{WT}$** | |
|---|---|
| pFL44S AscI Syx1A F | AGCGTCAGCGGGTTCTCGACGGTCACGGCGGGCATGTCGAAGG CGCGCCGGTGAAAACGTGCTGATTTG |
| Syx1A Part1 R | GTTCTCCTGTTGAAAACTCACACAAAAGTAGCCTCATCACTCAC ACACTCACAACACCGATGAAGAAGAG |
| Syx1A Part2 F | CTCTTCTTCATCGGTGTTGTGAGTGTGTGAGTGATGAGGCTACT TTTGTGTGAGTTTTCAACAGGAGAAC |
| HA syx1A Part2 R | GATAATACCCCGACACAAAGATGTACCCCTACGACGTGCCCGA CTACGCCACTAAAGACAGATTAGCCGC |
| HA syx1A Part3 F | GCGGCTAATCTGTCTTTAGTGGCGTAGTCGGGCACGTCGTAGG GGTACATCTTTGTGTCGGGGTATTATC |
| Syx1A Part3 R | TGCGTCCCTCTCAAACACACACCCTGAATCGGCGCCGACGACG CGTACGCAACAAACGAAAATAAAACGC |
| Syx1A Part4 F | GCGTTTTATTTTCGTTTGTTGCGTACGCGTCGTCGGCGCCGATT CAGGGTGTGTGTTTGAGAGGGACGCA |
| pFL44S PacI Syx1A R | CAAAAATGGGTTTTATTAACTTACATACATACTAGAATTCACCT TAATTAACTCCAATAGGACCAGTGTTG |
| **HA-syntaxin1A$^{KARRAA}$** | |
| pFL44S AscI Syx1A F | AGCGTCAGCGGGTTCTCGACGGTCACGGCGGGCATGTCGAAGG CGCGCCGGTGAAAACGTGCTGATTTG |
| Syx1A Part1 R | GTTCTCCTGTTGAAAACTCACACAAAAGTAGCCTCATCACTCAC ACACTCACAACACCGATGAAGAAGAG |
| Syx1A Part2 F | CTCTTCTTCATCGGTGTTGTGAGTGTGTGAGTGATGAGGCTACT |

| | |
|---|---|
| | TTTGTGTGAGTTTTCAACAGGAGAAC |
| 20bp before AA 20bp after R | ACCAGAGTAAAGCCCGACGAGCCGCCATCATGATACTGATCTGCCT |
| 20bp before AA 20bp after F | AGGCAGATCAGTATCATGATGGCGGCTCGTCGGGCTTTACTCTGGT |
| HA syx1A Part2 R | GATAATACCCCGACACAAAGATGTACCCCTACGACGTGCCCGACTACGCCACTAAAGACAGATTAGCCGC |
| HA syx1A Part3 F | GCGGCTAATCTGTCTTTAGTGGCGTAGTCGGGCACGTCGTAGGGGTACATCTTTGTGTCGGGGTATTATC |
| Syx1A Part3 R | TGCGTCCCTCTCAAACACACACCCTGAATCGGCGCCGACGACGCGTACGCAACAAACGAAAATAAAACGC |
| Syx1A Part4 F | GCGTTTTATTTTCGTTTGTTGCGTACGCGTCGTCGGCGCCGATTCAGGGTGTGTGTTTGAGAGGGACGCA |
| pFL44S PacI Syx1A R | CAAAAATGGGTTTTATTAACTTACATACATACTAGAATTCACCTTAATTAACTCCAATAGGACCAGTGTTG |

**Table S3, related to the experimental procedures. Genotypes of flies used**

| **Abbreviation** | **Genotype** |
|---|---|
| *nSybGal4* | *yw; nSybGal4* |
| $yv^{1}$*/+;nSybGal4/+* | $yv^{1}$*/yw;nSybGal4/+* |
| $w^{1118}$*/+;nSybGal4>DCR-2/+* | $w^{1118}$*/yw UAS-DCR-2;nSybGal4/+* |
| *splitVenus-PH-GRP$_1$ nSybGal4* | *yw; UAS-VenusC-PH-GRP$_1$ UAS-VenusN-PH-GRP$_1$ nSybGal4* |

| | |
|---|---|
| *Lyn11-FRB ;splitVenus-PH-GRP$_1$ nSybGal4,* | *yw; UAS-Lyn11-FRB/+ ; UAS-VenusC-PH-GRP$_1$, UAS-VenusN-PH-GRP$_1$ nSybGal4* |
| *Lyn11-FRB ;splitVenus-PH-GRP$_1$ FKBP-p85 nSybGal4* | *yw; UAS-Lyn11-FRB/+ ; UAS-VenusC-PH-GRP$_1$ UAS-VenusN-PH-GRP$_1$ UAS-FKBP-p85 nSybGal4/ UAS-VenusC-PH-GRP$_1$ UAS-VenusN-PH-GRP$_1$ nSybGal4* |
| *nSybGal4> TRIP PI3K92E$^{35798 \text{ or } 27690}$* | *yv$^1$/yw;UAS-RNAi(TRIP PI3K92E$^{35798 \text{ or } 27690}$ /nSybGal4* |
| *nSybGal4>DCR-2, RNAi PI4P5K$^{47027}$* | *w$^{1118}$/yw UAS-DCR-2;UAS-RNAi (PI4P5K$^{47027}$)/nSybGal4* |
| *yv$^1$/+;nSybGal4>DCR-2/+* | *yv$^1$/yw UAS-DCR-2;nSybGal4/+* |
| *nSybGal4>DCR-2, TRIP Syx1A$^{25811}$* | *yv$^1$/yw UAS-DCR-2; UAS-RNAi(TRIP Syx1A$^{25811}$/nSybGal4* |
| *HA-syx1A$^{WT}$* | *yw;P{CaryP}attP40 (syx1A$^{wt}$)* |
| *HA-syx1A$^{KARRAA}$* | *yw;P{CaryP}attP40 (syx1A$^{KARRAA}$)* |
| *HA-syx1A$^{WT}$; syx1A$^{\Delta 229}$/+* | *yw/+;P{CaryP}attP40 (syx1A$^{wt}$); syx1A$^{\Delta 229}$/+* |
| *HA-syx1A$^{KARRAA}$; syx1A$^{\Delta 229}$/+* | *yw/+;P{CaryP}attP40 (syx1A$^{KARRAA}$); syx1A$^{\Delta 229}$/+* |