**Supplemental Table 1. Summary of genotypes and statistics for all experiments in this study.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Figure/****Experiment** | **Genotype/Conditions** | **N** | **Statistical Test(s)** |
| 1A-D GFP::ActinGMALifeact::Ruby | C155-GAL4/+ or Y; UAS-GFP::actin/+C155-GAL4/+ or Y; UAS-GMA/+C155-GAL4/+ or Y; UAS-lifeact::Ruby/+ | 823 patches/9 NMJ/5 larvae819 patches/15 NMJ/6 larvae363 patches/7 NMJ/3 larvae | NA |
| 1E-FArp3BRP | C155-GAL4/UAS-Arp3::GFP; UAS-lifeact::Ruby/+C155-GAL4/+; UAS-lifeact::Ruby/BRP::GFP[MIMIC] | 12 NMJ/3 larvae13 NMJ/3 larvae | Kruskal-Wallis + Dunn’s test(with Fig 7C) |
| 1G-IControlWASp | C155-GAL4/+ or Y; UAS-GMA/+C155-GAL4/+ or Y; wsp1,e,UAS-GMA/wsp1,e | 832 patches/18 NMJ/8 larvae532 patches/15 NMJ/6 larvae | Kolmogorov-Smirnoff (G) Welch's t test (H) |
|  |  |  |  |
|  |  |  |  |
| 1S1A-B | C155-GAL4/+ or Y; UAS-GMA/+ | 1606 patches/20 NMJ/8 larvae | Kolmogorov-Smirnoff |
| 1S1C-EControl*wsp* RNAi | C155-GAL4/Y; UAS-GMA/UAS-luciferaseRNAiC155-GAL4/Y; UAS-GMA/UAS-WASpRNAi | 709 patches/15 NMJ/6 larvae286 patches/14 NMJ/5 larvae | Kolmogorov-Smirnoff (H) Mann-Whitney (I)t test (J) |
| 1S1FControl*wsp* RNAi | C155-GAL4/Y; UAS-myc::WASp/+; /UAS-mCherryRNAi/+C155-GAL4/Y; UAS-myc::WASp/+; /UAS-*wsp* RNAi/+ | 18NMJ/3 larvae24NMJ/4 larvae | t-test |
|  |  |  |  |
| 1S2 | Same experiment as Fig 1 G-I |  |  |
|  |  |  |  |
| 2A | Nwk::GFP[MIMIC] |  |  |
| 2B | Vglut-GAL4/Y; CD8::RFP/+; Nwk::GFP/+ |  |  |
| 2C-D | C155-GAL4/Y; UAS-WASp::myc/+; UAS-GMA/+ | 14 NMJ/3 larvae | NA |
| 2E-F | C155-GAL4/Y; UAS-WASp::myc/+; UAS-GMA/+ | 12 NMJ/3 larvae | NA |
|  |  |  |  |
| 3BGST-XNwk1-731 | 5μg3μg | 3 independent mixtures per group | NA |
| 3BGST-CDNwk1-428Nwk1-731 | 1.6μM CD, 1.2μM C/D1.5μM0.8μM | 3 independent mixtures per group | NA |
| 3DDapFLDapΔDDapΔCDDapRNAi | rescues=C155-GAL4/+; dap160Δ1/Df3450; X X=UAS-DapFL::mCherry/+ X=UAS-DapΔD/+ X=UAS-DapΔCD/+C155-GAL4, UAS-Dcr2/Y; UAS Dap160-RNAi/+ | 18 NMJ/3 larvae18 NMJ/3 larvae17 NMJ/3 larvae16 NMJ/3 larvae | ANOVA+Tukey’s multiple comparison test |
| 3EDapFLDapΔDDapΔCD | rescues= C155-GAL4/+; dap160Δ1/Df3450; X X=UAS-DapFL::mCherry/+ X=UAS-DapΔD/+ X=UAS-DapΔCD/+ | 18 NMJ/3 larvae18 NMJ/3 larvae15 NMJ/3 larvae | Kruskal-Wallis + Dunn’s multiple comparison test |
|  |  |  |  |
|  |  |  |  |
| **Figure/****Experiment** | **Genotype/Conditions** | **N** | **Statistical Test(s)** |
| 3 S1ANwk607-731 | 7μM |  |  |
| 3 S1BGST-XNwk1-428 | .375mg/mL (CD=8.5μM, C/D=11μM)1.5μM | 3 independent mixtures per group | ANOVA+Tukey’s multiple comparison test |
|  |  |  |  |
| 3S1C | Same experiment as Figure 3B | Same experiment as Fig 3B | ANOVA+Tukey’s multiple comparison test |
|  |  |  |  |
| 3S2B | Same experiment as Figure 3D | Same experiment as Fig 3D | ANOVA+Tukey’s multiple comparison test |
| 3S2C | Same experiment as Figure 3E | Same experiment as Figure 3E | ANOVA+Tukey’s  |
| 3S2DControlDap160RNAi | vglut/Y; UAS-Dcr2/+; UAS-Nwk::GFPMiMIC/UAS-luciferaseRNAivglut/Y; UAS-dcr2/+; Nwk::GFPMiMIC/Dap160RNAi | 11NMJ/3larvae11NMJ/3larvae | t test |
|  |  |  |  |
| 4AActinArp2/3WASpNwk1-731DapCDDapC | 2μM50nM50nM500nM2μM 2μM | (1) 2 replicates (2) 2 replicates(3) 3 replicates(4) 3 replicates(5) 5 replicates(6) 2 replicatesReplicates are independent mixtures | ANOVA+ Tukey’s multiple comparison test |
| 4BActinArp2/3WASpNwk1-731DapCDPI(4,5)P2 | 2μM50nM50nM100nM500nM 2μM 10% PI(4,5)P2 liposomes | (1) 2 replicates (2) 2 replicates(3) 3 replicates(4) 3 replicates(5) 3 replicatesReplicates are independent mixtures | ANOVA+ Tukey’s multiple comparison test |
| 4CNwk1-633Nwk1-731+DapCD | 2μM OG-actin, 50nM Arp2/3, 50nM WASp500nM Nwk1-633::SNAP549500nM Nwk1-731::SNAP549+2μM Dap160SH3CD | 41 droplets 22 droplets | NA |
|  |  |  |  |
|  |  |  |  |
| 5ANwk1-428Nwk1-754DapCD | DOPC/DOPE/DOPS/PI(4,5)P2 = 70/15/5/103μM1.125μM1.69-6.75μM | 3 independent mixtures per group | ANOVA+ Tukey’s multiple comparison test |
| 5BNwk1-XXXDap160X | DOPC/DOPE/DOPS/PI(4,5)P2 = 80-x/15/5/x2μM 6μM | 3 independent mixtures per group; except 2 replicates for: 2.5%PIP2-Nwk1-4282.5%-Nwk1-731+DapSH3C | ANOVA+Tukey’s multiple comparison test |
| 5CNwk1-731WASpDap160CD | DOPC/DOPE/DOPS/PI(4,5)P2 = 70/15/5/101μM1μM3μM | 3 independent mixtures per group | ANOVA+ Tukey’s multiple comparison test |
| **Figure/****Experiment** | **Genotype/Conditions** | **N** | **Statistical Test(s)** |
| 5DNwk1-731Nwk/WASp/Dap | 5% PI(4,5)P2 GUVs 500nM Nwk1-731::SNAP549250nM Nwk1-731::SNAP549, 250nM WASp, 1.25μM Dap160SH3CD | Representative from:11 GUVs imaged12 GUVs imaged | NA |
| 5E-FControlDap160RNAi | vglut/Y; UAS-Dcr2/+; UAS-Nwk::GFPMiMIC/UAS-luciferaseRNAivglut/Y; UAS-dcr2/+; Nwk::GFPMiMIC/Dap160RNAi | 9 NMJs/4 larvae10 NMJs/5 larvae | Two step association curve |
|  |  |  |  |
| 5S1A-BNwk1-633WASpDap160CD | DOPC/DOPE/DOPS/PI(4,5)P2 = 75/15/5/51µM2 µM3µM | 3 independent mixtures per group | ANOVA+ Tukey’s multiple comparison test |
| 5S1CNwk1-731WASpDap160CD | DOPC/DOPE/DOPS/PI(4,5)P2 = 70/15/5/101μM1μM3μM | 3 independent mixtures per group | ANOVA+ Tukey’s multiple comparison test |
| 5S1DNwk1-754WASpDap160SH3CD | DOPC/DOPE/DOPS/PI(4,5)P2 = 70/15/5/10250nM1μM250nM5% PI(4,5)P2 GUVs | Representative from:5 GUVs imaged10 GUVs imaged | NA |
|  |  |  |  |
|  |  |  |  |
| 6A-CControl*nwk*1/2 | C155-GAL4/+ or Y; UAS-GMA/+C155-GAL4/+ or Y; UAS-GMA, *nwk*2,*h*/ *nwk*1 | 1606 patches/20 NMJ/8 larvae1928 patches/22 NMJ/8 larvae | Mann-Whitney (B)Kolm.-Smirnoff (C) |
| 6D-FFLΔCD | C155-GAL4/+; dap160Δ1/Df3450; UAS-GMA/X X=UAS-DapFL::mCherry/+ X=UAS-DapΔCD/+ | 1279 patches/14 NMJ/7 larvae1937 patches/17 NMJ/7 larvae | Mann-Whitney (E)Kolm.-Smirnoff (F) |
|  |  |  |  |
|  |  |  |  |
| 6S1A-B  | Same experiment as 6A-C | As 6A-C |  |
|  |  |  |  |
| 6S2 | Same experiment as 6A-C | As 6A-C | t-test (B-C) |
|  |  |  |  |
| 7A-BClc::GFPBRP::GFP | C155-GAL4/+; UAS-lifeact::Ruby/+; UAS-Clc::GFP/+C155-GAL4/+; UAS-lifeact::Ruby/BRP::GFP[MIMIC] | 9 NMJ/3 larvae13 NMJ/3 larvae | Kruskal-Wallis + Dunn’s test (With Fig 1E-F) |
| 7C  | C155-GAL4/+; UAS-lifeact::Ruby/AP2::GFP | 14 NMJ/3 larvae | NA |
| 7D-EControl*shi*TS1 | C155-GAL4/Y; UAS-GMA/+C155-GAL4, shiTS1/Y; UAS-GMA/+ | 669 patches/16 NMJ/8 larvae901 patches/19 NMJ/8 larvae | Welch's t test (D) |
| 7F-GControl*nwk*1/2*shi*TS1 | C155-GAL4/Y; UAS-GFP/+C155-GAL4/Y; UAS-GFP/+; *nwk*1/*nwk*2*h*C155-GAL4, shiTS1/Y; UAS-GFP/+ | 23 NMJ/4 larvae24 NMJ/4 larvae23 NMJ/4 larvae | Kruskal-Wallis + Dunn’s multiple comparison test |
| 7H-IDapFLDapΔDDapΔCD*shi*TS! | rescues= C155-GAL4/+; dap160Δ1/Df3450; X X=UAS-DapFL::mCherry/+ X=UAS-DapΔD/+ X=UAS-DapΔCD/+C155, shiTS1/Y x UAS-RFP | 32 NMJs/8 larvae12 NMJs/3 larvae12 NMJs/3 larvae8 NMJs/2 larvae | Unpaired t-tests to dish-matched controls |
| **Figure/****Experiment** | **Genotype/Conditions** | **N** | **Statistical Test(s)** |
| 7S1AClc::GFPAP2::GFP | C155-GAL4/+; UAS-Clc::GFP/+AP2::GFPKI | 6 boutons7 boutons | NA |
| 7S1B-CAP2 | C155-GAL4/+; UAS-lifeact::Ruby/AP2::GFP | 14 NMJ/3 larvae | NA |
|  |  |  |  |
| Figure 7S2A-BDapRNAimChDapFLDapΔDDapΔCD | C155-GAL4, UAS-Dcr2/Y; UAS Dap160-RNAi/+C155-GAL4/+; UAS-CD8::RFP/+ X=UAS-DapFL::mCherry/+ X=UAS-DapΔD/+ X=UAS-DapΔCD/+ | 24 NMJ/6 larvae22 NMJ/6 larvae21 NMJ/6 larvae16 NMJ/5 larvae23 NMJ/6 larvae | ANOVA+Tukey’s multiple comparison test |
|  |  |  |  |
| 7S3A-CControl*nwk*1/2 | C155-GAL4/Y; UAS-GFP/+C155-GAL4/Y; UAS-GFP/+; *nwk*1/*nwk*2*h* | 15 NMJ/4 larvae19 NMJ/5 larvae | Mann-Whitney (B)t-test (C) |
| Figure 7S3D-FDapFLDapΔDDapΔCD | rescues= C155-GAL4/+; dap160Δ1/Df3450; X X=UAS-DapFL::mCherry/+ X=UAS-DapΔD/+ X=UAS-DapΔCD/+ | 16 NMJ/4 larvae16 NMJ/4 larvae16 NMJ/5 larvae | ANOVA+Tukey’s multiple comparison test |