**SUPPLEMENTARY FIGURE LEGENDS:**

**Supplementary Figure S1: *dPak*$^{11}$ and *dlg*$^{m52}$ mutants have reduced Synd and muscle T-tubules do not contain syndapin.**

**(A-A'')** Projected confocal image of wild-type third instar larval NMJ, costained for PAK (green) and Synd (red).

**(B-B'')** Projected confocal image of *dPak*$^{11}$ third instar larval NMJ, costained for PAK (green) and Synd (red).

**(B''')** Bar graph showing quantification of synaptic syndapin in dPAK$^{11}$ mutants. Number in bar graphs represents number of boutons quantified. The average value of syndapin fluorescence was 127.5 $\pm$ 7.1 for control and 50.14 $\pm$ 1.96 for Dlg$^{m52}$; P< 0.001.

**(C-C")** Projected confocal image of wild-type third instar larval NMJ, costained for Dlg (green) and Synd (red).

**(D-D'')** Projected confocal image of *dlg*$^{m52}$ third instar larval NMJ, costained for Dlg (green) and Synd (red).

**(D''')** Bar graph showing quantification of synaptic syndapin in Dlg$^{m52}$ mutants. Number in bar graphs represents number of boutons quantified. The average value of syndapin fluorescence was 126.5 $\pm$ 8.8 for control and 20.3 $\pm$ 2.8 for dPAK$^{11}$; P< 0.001.

**(E-E'')** Projected confocal image of wild-type third instar larval muscle costained for Amphiphysin (green) and F-actin (red).

**(F-F'')** Projected confocal image of wild-type third instar larval muscle, costained for Amphiphysin (green) and Synd (red). Note that Amph and Synd do not localize to T-

tubules. Even at very high laser power, syndapin could not be detected at muscle T-tubules.

Scale bar in D'' represents 25 μm for A-D''. Scale bar in F'' represents 6 μm for E-F''. Error bar represents standard error of the mean (s.e.m) calculated using two-tailed t-test.

**Supplementary Figure S2: Dynamin is not enriched in SSR and overexpressed syndapin specifically gets targeted to type I boutons.**

**(A-C)** Single confocal image of wild-type third instar larval NMJ costained for Dlg (green) and dynamin (red).

**(D)** Magnified single section confocal image of a bouton costained for Dlg (green) and dynamin (red).

**(D')** Magnified single section confocal image of a bouton costained for Dlg (green) and dynamin (red). Presynapitc dynamin signal was saturated in this image to detect any possible dynamin signal. Note that no specific dynamin signal above background could be detected in the SSR.

**(E-G)** Single section confocal image (0.4 μm) of type I synaptic boutons at muscle 6/7 co-stained with GluRIIA (green) and Synd (red) antibodies. Note that GluRIIA and syndapin do not colocalize.

**(H,I)** Muscles 12 and 13 of a third instar wild-type larvae (H) or syndapin overexpressing animals in muscles (I) double-labelled with anti-Synd (red) and anti-HRP (green) antibodies. Note that the ectopically expressed syndapin is correctly targeted to type I synapses and is not detectable at type II and III synapses.

Scale bar in C represents 5 μm for (A-C) and 0.8 μm for D and D'. Scale bar in G represents 4 μm for (E-G) and 30 μm for H and I.

**Supplementary Figure S3: Syndapin induces synaptic and extrasynaptic SSR but has no effect on synaptic physiology.**

**(A-C)** An electron micrograph of type 1s motor terminals of (A) *Mef2-Gal4/ UAS-syndapin* animals overexpressing syndapin in muscle; (B) electron micrograph of a syndapin overexpressing muscle showing dense extrasynaptic SSR without associated presynaptic boutons; (C) A magnified electron micrograph of a syndapin overexpressing muscle showing that syndapin induced extrasynaptic SSR is composed of tubulolamellar structures.

**(D, E)** Representative traces of (D) mEJP from control and syndapin overexpressing NMJ; (E) evoked synaptic potentials from control and Synd overexpressing animals.

(F) Bar graphs showing average mEJP frequency (left) and average mEJP amplitudes (right) for the indicated genotypes. mEJP frequency was 3.65 $\pm$ 0.2 in controls compared to 3.0 $\pm$ 0.28 in *Mef2-Gal4*; *UAS-Synd*, P> 0.096; and mEJP amplitude was 0.77 $\pm$ 0.04 mV in controls compared to 0.89 $\pm$ 0.55 mV in *Mef2-Gal4*; *UAS-Synd*, P> 0.109.

**(G)** Bar graphs showing average EJP amplitude (left) and average quantal content (obtained by dividing average EJP amplitude by average mEJP amplitude) (right) for the indicated genotypes. EJP amplitude was 42.8 $\pm$ 2 mV in controls compared to 37.5 $\pm$ 1.5 mV in *Mef2-Gal4*; *UAS-Synd*, P> 0.06; and quantal content was 56.22 $\pm$ 3.58 in controls compared to 51.1 $\pm$ 4.36 in *Mef2-Gal4*; *UAS-Synd*, P> 0.37.

**(H)** Representative traces of EJPs at indicated time points during 10 Hz stimulation in 1.5 mM $Ca^{2+}$ containing HL3 saline.

(**I**) Normalized EJP amplitudes in control (blue lines) and *Mef2-Gal4*; *UAS-Synd* (black lines) animals stimulated at 10 Hz for 5 minutes. No significant difference in EJP amplitude was observed at any time points.

Scale bar in (C) represents 1.2 μm for (A), 2.4 μm for (B) and 600 nm for (C). Numbers in histogram indicate number of animals analyzed. Error bar represents standard error of the mean (s.e.m) calculated using two-tailed t-test.

**Supplementary Figure S4:**

(**A**) **Sequence alignment of F-BAR domain proteins reveals that positively charged residues required for membrane binding are conserved in syndapin** Sequence alignment of *Drosophila* syndapin F-BAR with F-BAR domains of human FBP17 and human CIP4 proteins. Note the conserved positively charged amino acid residues (marked with yellow box) in hFBP17, hCIP4 and *Drosophila* syndapin.

(**B**) **Muscle expression of Nwk does not induce SSR formation**. Projected confocal image of (upper panel, left) wild-type third instar larva double stained for HRP (green) and Nwk (red). Projected confocal image of (upper panel, right) third instar larval NMJ overexpresing Nwk in muscles double stained (upper panel, right) for HRP (green) and Nwk (red). Note that the overexpressed protein does not gets targeted to NMJs. Projected confocal image (lower panel, left) of muscle 6/7 of larva overexpresing Nwk in muscles, stained with membrane labeling dye, FM1-43. Projected confocal image (lower panel, right) of muscle 6/7 of larva overexpresing syndapin in muscles, stained with membrane labeling dye, FM1-43. Note that synaptic and extrasynaptic region is strongly labeled with FM1-43.

Scale bar represents 18 μm for all panels in B.

**Supplemental Movies**

**Supplemental Movie M1**

Time-lapse movie of a cell expressing EYFP-syndapin. Note that syndapin induces very small number of tubules.

**Supplemental movie M2**

Time-lapse movie of a cell expressing FCH domain of syndapin (EYFP-Synd$^{1-150}$).

**Supplemental Movie M3**

Time-lapse movie of a cell expressing F-BAR domain of syndapin (EYFP-Synd$^{1-300}$). A massive tubulation is observed in these cells.

**Supplemental Movie M4**

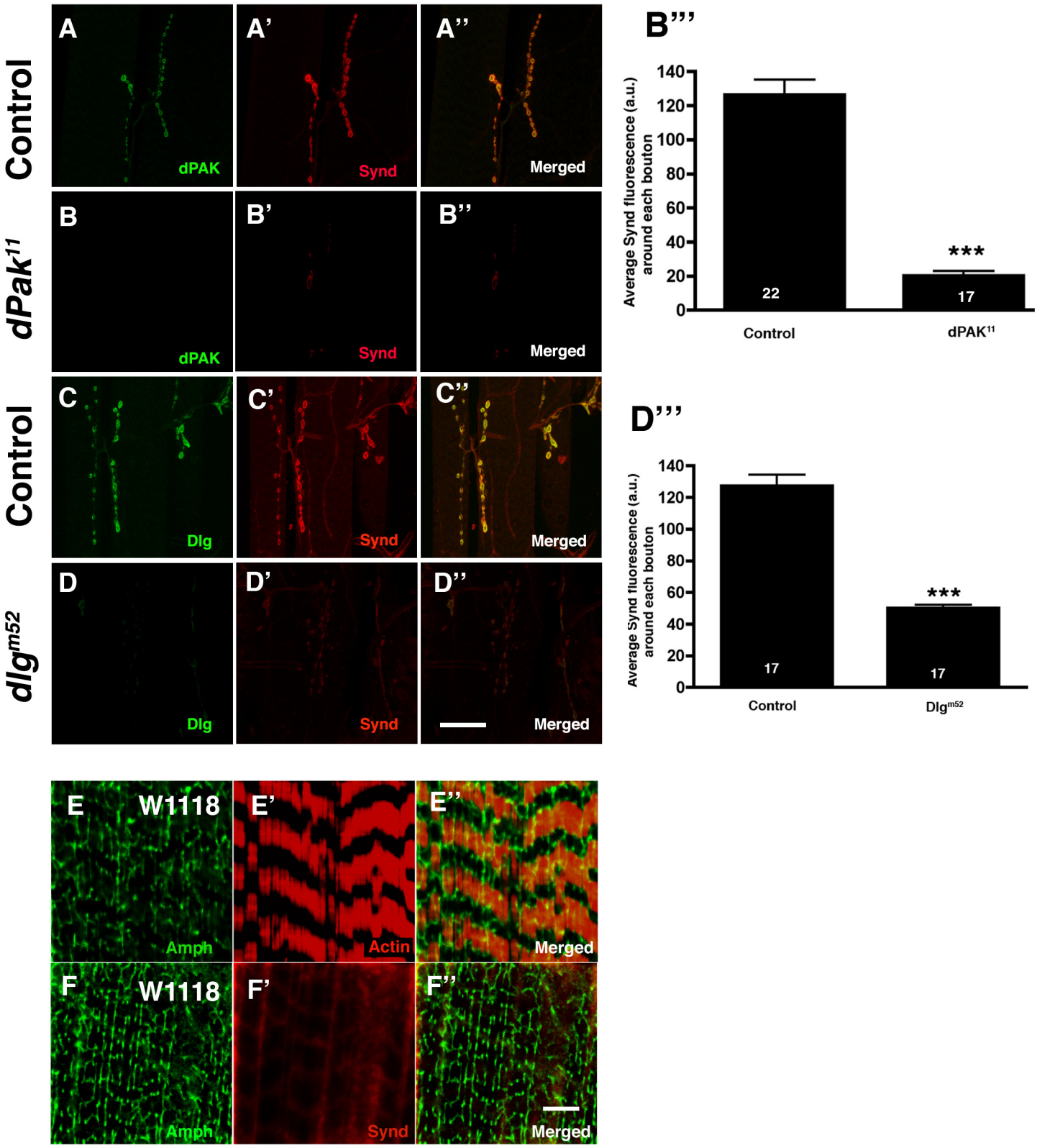
Time-lapse movie of a cell expressing K63EK64 mutants of syndapin (EYFP-Synd$^{K63EK64E}$). These mutants abolish the ability of F-BAR domain of syndapin to induce membrane tubulation in S2 cells.

**Supplemental Movie M5**

Time-lapse movie of a cell expressing R129EK130E mutants of syndapin (EYFP-Synd$^{R129EK130E}$). These mutants abolish the ability of F-BAR domain of syndapin to induce membrane tubulation in S2 cells.

**Supplemental Movie M6**

Time-lapse movie of a cell expressing SH3 domain of syndapin (EYFP-Synd$^{SH3}$). These mutants do not induce membrane tubulation in S2 cells.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Control
dlgm52
A
A'
A''
dPAK
Synd
Merged
B
B'
B''
dPAK
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Merged
C
C'
C''
Dlg
Synd
Merged
D
D'
D''
Dlg
Synd
Merged
B'''
Average Synd fluorescence (a.u.) around each bouton
140
120
100
80
60
40
20
0
***
22
17
Control
dPAK11
D'''
Average Synd fluorescence (a.u.) around each bouton
140
120
100
80
60
40
20
0
***
17
17
Control
Dlgm52
E
W1118
E'
E''
Amph
Actin
Merged
F
W1118
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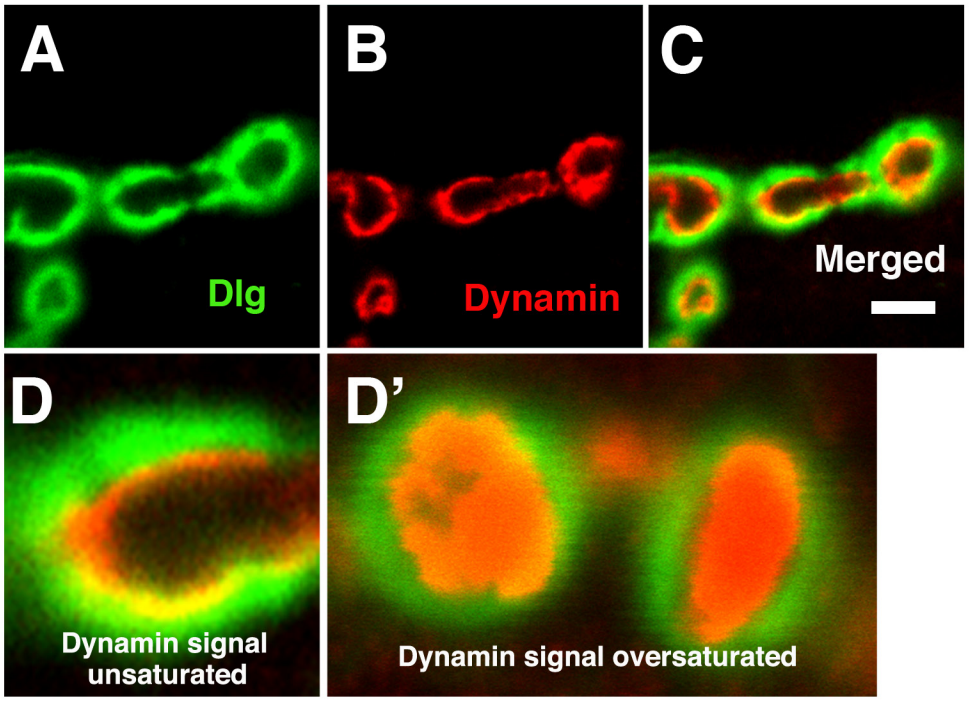
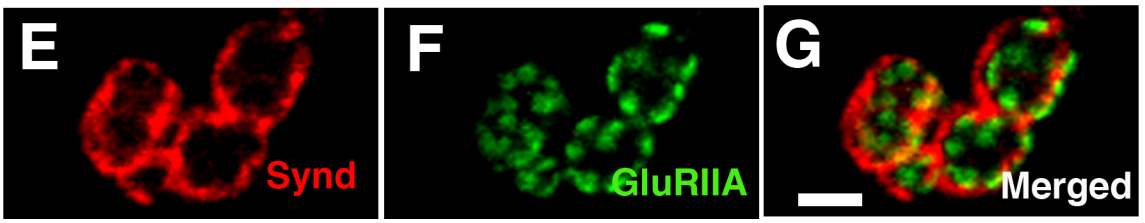
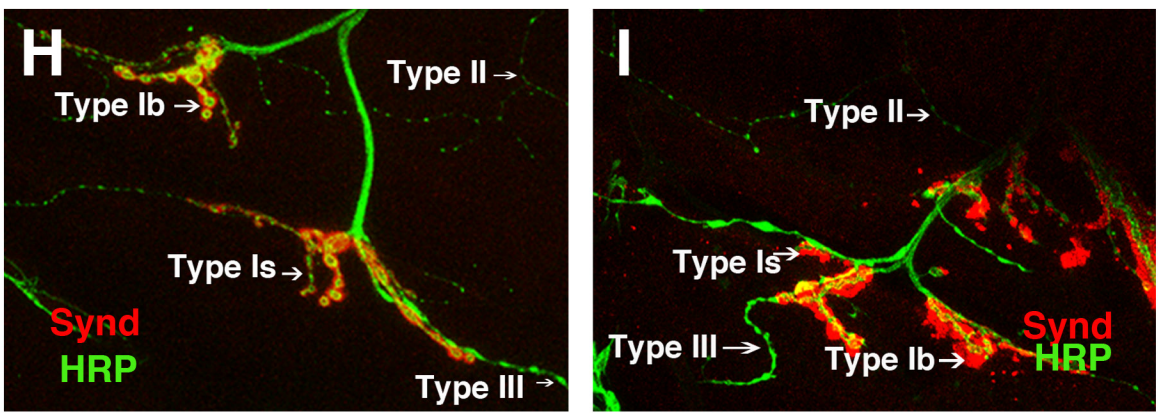
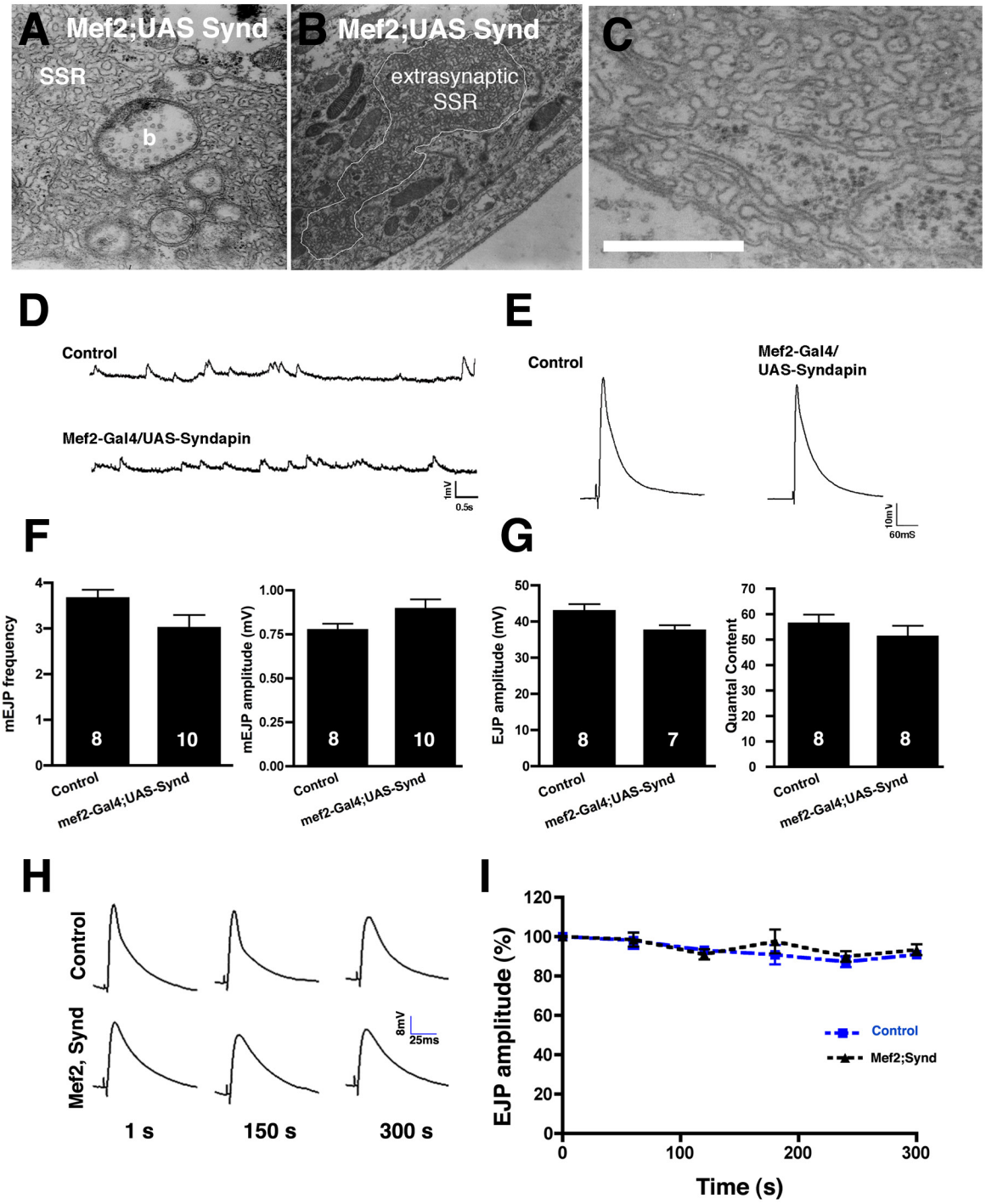
**Figure S1**




**Supplementary Figure S2**




# Supplementary Figure S3

**A**

```
                1        10        20        30        40        50        60        70        75
                |--------+---------+---------+---------+---------+---------+---------+----|
hFBP17                   MSWGTELWD--QFDNLEKHTQWGIDILEKYIKFYKERTEIELSYAKQLRNLSKKYQPKKNSKEEE
hCIP4                    MDWGTELWD--QFEVLERHTQWGLDLLDRYVKFYKERTEVEQAYAKQLRSLVKKYLPKRPAKDDP
dSynd           MSHHSDDQLLQAGSDSFWEPGNYKRTTKRIEDGYKLCNDLQQLIQERADIEKGYAKSLRTWSKKWGELIEKGPEY
Consensus       ..........m.wgtelW#..#%..lekht#wG.dll#.y.kf!kERt#!E..YAKqLR.lsKKy.pk...k.#.

                76       85        95        105       115       125       135       145  150
                |--------+---------+---------+---------+---------+---------+---------+----|
hFBP17          EYKYTSCKAFISNLNEMNDYAGQ-HEVISENMASQIIV-DLARYVQELKQ--ERKSNFHDGRKAQQHIETCWKQL
hCIP4           ESKFSQQQSFVQILQEVNDFAGQ-RELVAENLSVRYCL-ELTKYSQEMKQ--ERKMHFQEGRRAQQQLENGFKQL
dSynd           GTTEAAWKGVLTESERISDVHMKIKDNLCNDVNSQIKTWQKENYHHTLMQIKERKDLEDLFKKAQKPWAKLLAKV
Consensus       e.k....k.f...l#e.nD.agq..#...##..sq!...#l..Y.qe$kQ..ERK..f..grkAQq..e...kql

                151      160       170       180       190       200       210       220  225
                |--------+---------+---------+---------+---------+---------+---------+----|
hFBP17          ESSKRRFERDCKEADRAQQYFEKMDADINVTKADVEKARQQAQIRHQMAEDSKADYSSILQKFNHEQHEYYHTHI
hCIP4           ENSKRKFERDCREAEKAAQTAERLDQDINATKADVEKAKQQAHLRSHMAEESKNEYAAQLQRFNRDQAHFYFSQM
dSynd           EKAKADYHSACKTERSATNQERNANADSSLSPDQVKKMHDRVQKTKDQVQKCREKYEQAIAEITK-YNSVYIEDM
Consensus       E.sKr.%erdCkea..A.#..e..#aDin.tka#VeKa.#qaq.r..ma#.sk..Y...lq.fn..q...Y...m

                226      235       245       255       265       275       285       295  300
                |--------+---------+---------+---------+---------+---------+---------+----|
hFBP17          PNIFQKIQEMEERRIVRMGES-MKTYAEVDRQVIPIIGKCLDGIVKAAESIDQKNDSQLVIEAYKSGFEPPGDIE
hCIP4           PQIFDKLQDMDERRATRLGAG-YGLLSEAELEVVPIIAKCLEGMKVAANAVDPKNDSHVLIELHKSGFARPGDVE
dSynd           TSVFEKCQTFEKTRLQFFKEILFNVHSCLDLTKVQSLPQIYEEFSHTINNADQQKDLKWWSNNHGINMAMNWP-S
Consensus       p.!F#K.Q.m#erR..r.ge.....se.#l.v!pii.kcl#g...aa#..DqknDs...i#.hksgfa.pgd.e
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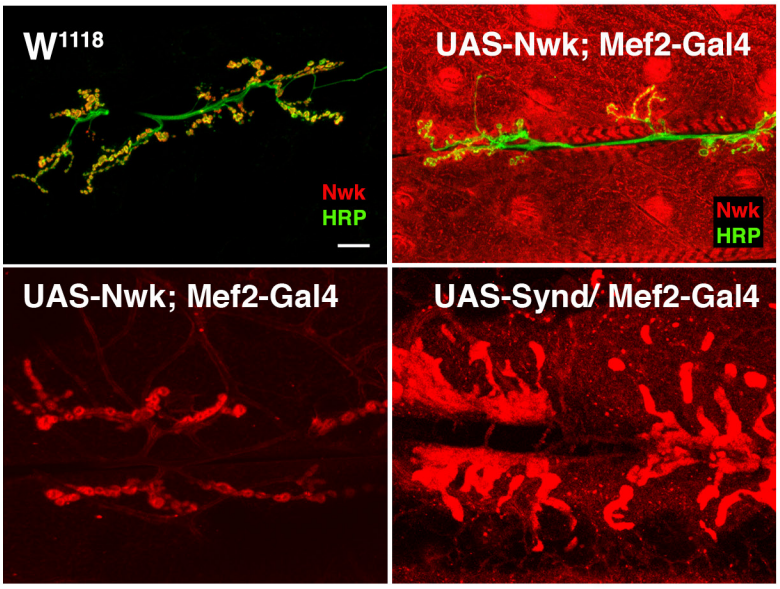
**B**




**Supplementary Figure S4**