SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure SF1: Nuclear DFz2C/LamC foci and muscle/NMJ structure and function

A-Single confocal slice through a larval muscle nucleus labeled with LamC and DFz2C antibodies.

B- A single slice of a structured illumination microscopy reconstruction of the same nucleus shown in A.

C- Single confocal slice (deconvolved) of a salivary gland nucleus labeled with LamC and DFz2C antibodies. Right panels are enlarged images of the boxed area on left panels showing the framework-like structure formed by LamC around DFz2C granules (arrowheads).

D, E- Confocal images of larval muscle microtubules, labeled with anti-tyrosinated tubulin antibodies, in

D- wild type control and

E- *lamC* mutant muscles, demonstrating that the microtubule network is normal at the light level in *lamC* null mutants in muscle 6/7 segments A2 and A3. Note the presence of a muscle nucleus (n), outlined by a white oval, normally enveloped by MTs in both control and *lamC* mutants.

F, G- Confocal images of F-actin at the contractile apparatus labeled with Texas Red conjugated phalloidin in

F- wild type and

G- *lamC* mutants, showing normal F-actin organization in *lamC* null mutants.

H, I- Transmission electron micrograph of a transversal larval muscle section, in

H- wild type and

I- *lamC* mutant, showing normal thick and thin filament spacing and thickness.

J- Single confocal slice (deconvolved) showing localization of a DFz2C/LamC focus to areas devoid of DNA, as revealed by labeling with propidium iodide (PI).

K- Bouton volume in *lamC* variants. N (number of boutons) is 263 for wild type, 108 for *LamC*, and 64 for *LamC* muscle rescue.

L- Muscle area in *lamC* genetic variants. N (number of animals) is 18 for wild type, 31 for *LamC*, 15 for UAS-LamC-RNAi-muscle, and 15 for $lamC^{GFP\text{-}trap}$/+.

M-Representative traces of mEJPs in the indicated genotypes.

N-Representative traces of evoked EJPs in the indicated genotypes.

Calibration bar is 3 µm for **A** , **B**, 7 µm for **C** (left), **F**, **G**, 2.5 µm for **C** (right), 6 µm for **D**, **E**, 60 nm for **H**, **I**, and 2 µm for **J**.

Supplemental Figure SF2: DFz2C foci contain membrane.

A- Deconvolved image of a muscle nucleus in a transgenic larva expressing the membrane reporter UAS-mCD8-GFP driven by the muscle BG487-Gal4 driver. Boxed area highlights a DFz2C focus at the nuclear periphery that is enriched for membrane staining.

B- High magnification views of the boxed area in A, showing that the DFz2C focus is “wrapped” by membrane (arrows).

Calibration bar is 4µm for **A**, and 2µm for **B**.

Supplemental Figure SF3: Evagination of the nuclear envelope resemblying HSV egress and controls for immunoelectron microscopy.

A, B- micrographs of foci in larval body wall muscle preparations processed for immunoelectron microscopy, in which the primary antibody was omitted, using

A- 18 nm gold conjugated second antibody and

B- 12 nm gold conjugated second antibody.

C-Electron micrograph of an S2 cell nucleus labeled with DFz2C antibody using 18 nm gold conjugated secondary antibody.

D- Electron micrograph of a larval muscle nucleus region exhibiting a double-walled evagination (arrow) containing two RNA granules (asterisks). The evagination displays a “neck” possibly for pinching off or driving scission of the INM (see model in **G**: step 2-3 in blue pathway).

E- Another double-walled evagination (arrow) appearing to have undergone INM scission (see model in F: step 2 in blue pathway). A RNA granule can be seen nearby in the cytoplasm (arrowhead).

F- A double-walled evagination near a granule (g)-containing INM invagination.

G- View of an abnormal focus shown at (**G1**) low and (**G2**) high magnification in a *lamC*$^{GFP\text{-}trap}$/+ preparation. Note the presence of electron dense amorphous material associated with the nuclear lamina (arrow).

H- Cartoon depicting a model for DFz2C import, packaging into mRNPs, and two potential pathways for the release of granules from the nucleus, based on mechanisms of HSV egress and our observations in this article. The two nuclear egress pathways (red and blue annotations) are not mutually exclusive, and while the end result of either pathway is the release of membrane-free granules to the cytoplasm, they are morphologically different.

I- A single slice through a SIM reconstruction of a muscle nucleus showing a possible egression event.

C=cytoplasm, N=nucleus, h=heterochromatin, NP=nuclear pore complex.

Calibration Bar is 0.3 µm for **A**, **D**-**F**, 0.5 µm for **B**, **G2**, 0.2 µm for **C**, 1 µm for **G1**, **I4**, 5 µm for **I1**-**3**.

Supplemental Figure SF4: Localization of endogenous PABP2 and its dependency on DFz2C.

A- Deconvolved confocal image of a muscle nucleus labeled with LamC and PABP2 antibodies showing intense PABP2 foci, above nucleoplasmic levels of PABP2, localized adjacent to LamC foci.

B,C- Single confocal slice through the nucleus of larva expressing nls-GFP in muscle cells, at (**B**) low magnification and (**C**) high magnification.

n=nucleus; c=cytoplasm .Calibration bar is 2 µm for **A**, **C**, and 7 µm for **B**.

D- Number of DFz2C foci versus number of PABP2-GFP foci in muscle nuclei. Numbers below each data point represent the number of nuclei found at each value.

Supplemental Figure SF5: Controls for PKC-substrate antibody, *par6* FISH and oligo-dT FISH.

A- Larval body wall muscle nucleus from a larva expressing aPKC-RNAi in muscle, labeled with the PKC substrate antibody, showing no label enrichement at the nucleus..

B-C- *par6* FISH of larval body wall muscles double labeled with LamC antibodies in

A- wild type, showing *par6* FISH signal, and

B- a larva expressing Par6-RNAi-muscle, showing lack of *par6* FISH signal.

D- Quantification of muscle surface area in larvae expressing aPKC-RNAi or Baz-RNAi in body wall muscles. ***=p<0.0001, N= 6 animals for all genotypes.

E- *wg*-FISH to larval body wall muscles in a preparation double labeled with LamC antibodies showing absence of signal at the foci (arrows).

F- FISH in the absence of probe, in a preparation double labeled with LamC antibodies, showing absence of signal at the foci

Calibration bar is 4 µm for **A**, **E**, **F** and 7 µm for **B**, **C**.

Supplemental Figure SF6: Polyribosomes are present at the postsynaptic region of the NMJ.

A,B- Electron micrographs of NMJ regions, showing the presence of ribosomes (arrows) at the SSR. b=presynaptic bouton, gg=glycogen granule, sv=synaptic vesicle, em=extracellular matrix.

Calibration bar is 0.4µm.

Supplemental Movie 1: RNA granule leaving the foci.

This movie represents a single confocal slice through a body wall muscle nucleus stained with E36 dye. The entire movie represents 3 minutes, 36 seconds in real-time (18 seconds per time-point), or 12 frames, played back at 3 frames per second. Arrowhead points to exiting granule.

N=Nucleus, nu=nucleolus

Supplemental Movie 2: RNA granule leaving the foci.

This movie represents a projection of 3 confocal slices (~1.5 µm) through a body wall muscle nucleus stained with E36. The movie represents 2 minutes, 8 seconds in real-time (17 seconds per timepoint), or 8 frames, played back at 3 frames per second. Arrowhead points to exiting granule.

N=Nucleus, nu=nucleolus.

SUPPLEMENTAL TABLES

Supplemental Table 1: GFP-trap lines screened

Trap ID	symbol	Name; function	Gene ID
CA06921	Hrb98DE	Heterogeneous nuclear ribonucleoprotein at 98DE	CG9983
CA06961	pUf68	poly(U) binding factor 68kD	CG12085
CA07692	Spt6	chromatin binding	CG12225
CB02119	Rm62	RNA interference	CG10279
CB02655	sqd	Squid; mRNA binding	CG16901
CB03028	SF2	nuclear mRNA splicing	CG6987
CB03248	xl6	nuclear mRNA splicing	CG10203
CB04769	eIF3-S9	translation initiation factor	CG4878
CC00233	sm	Smooth; mRNA binding	CG9218
CC00236	shep	Alan Shepard; mRNA binding	CG32423
CC00479	pum	Pumilio	CG9755
CC00511	CG32062	Ataxin-2 binding protein 1; transcription factor binding	CG32062
CC00645	CG7185	alternative nuclear mRNA splicing,	CG7185
CC00737	Tudor-SN	transcription coactivator activity	CG7008
CC01220	CG33123	leucine-tRNA ligase activity	CG33123
CC01391	CG11266	CAPER; alternative nuclear mRNA splicing	CG11266
CC01563	Hrb98DE	Heterogeneous nuclear ribonucleoprotein at 98DE	CG9983
CC01925	tra2	transformer 2; regulation of nuclear mRNA splicing	CG10128
CC02043	Aats-glupro	Glutamyl-prolyl-tRNA synthetase	CG5394
CC06033	Hrb87F	Heterogeneous nuclear ribonucleoprotein at 87F	CG12749
CC06119	CG9809	Spargel; mRNA binding	CG9809
G00108	CG32423	Alan Shepard; mRNA binding	CG32423
G00261	shep	Alan Shepard; mRNA binding	CG32423
P00002	sqd	Squid; mRNA binding	CG16901
YB0052LE	Rm62	RNA interference	CG10279
YB0060	sqd	Squid; mRNA binding	CG16901
YB0077	Rm62	RNA interference	CG10279
YB0256	Rm62	RNA interference	CG10279
YC0015	Hrb98DE	Heterogeneous nuclear ribonucleoprotein at 98DE	CG9983
YC0023	sm	Smooth; mRNA binding	CG9218
YD0623	Rm62	RNA interference	CG10279
ZCL0588	Hrb98DE	Heterogeneous nuclear ribonucleoprotein at 98DE	CG9983
ZCL0734	sqd	Squid; mRNA binding	CG16901
ZCL2020	Pabp2	poly(A) RNA binding.	CG2163

Supplemental Table 2: Transcripts tested for their localization at LamC foci

Gene	Probe ID	Forward Primer	Reverse Primer	Signal at LamC foci
Evenness Interrupted/Wntless	Evi/Wls	AAGGTCACACTGCTTTGTTG	ACAGAAAGAGGAAATAAATGGCTGC	no
Atypical Protein Kinase C	aPKC	TTTAAGACAGAGGCCGCACG	ACACATCTTCCAGGCCAAGC	no
dCASK/Caki	dCASK	AGTTTCAGAAGAACACGGAC	AAGAAGTGACCGTATAGCTG	yes
Saxophone	Sax	GGCCCAGTAAACGCAATACG	CTATCACGCAGGAGCCGTTC	no
Mothers against Dpp	Mad	ATGGATTTCAATCCAACAGG	GTCTCCCATCGCAAGGGTCT	no
Wishful thinking	Wit	GCTTTGTAGGGAGGTGTTGC	ACACTTTCTGTTTCACCATC	no
Thick vein	Tkv	CACACCCAAGCTGACCACAC	TACATCATCCTCCTGCCAGC	yes
Shibire	Shi	TTGGCGTGGTGAATAGATCC	CCATCGGGACTAAATAAAGC	no
Par6	Par6 probe 1	GTCGAAGAACAAGATAAACACAACG	GCAGCACTCCATCCTTGACATC	yes
Par6	Par6 probe 2	GGATTAACCCGGCGATACAG	GACGAGTATTGGACACAATGAC	yes
Bazooka/Par3	Baz	CCAGCGCCTCCCATTCCGGT	GATAAGCAGCGCCGTGTTGC	no
Discs-Large	DLG	ATGGCGATGATAGCTGGTTATACG	CTCTTGGTCGCTGCCATCTTC	no
Actin 5C	Act5C	AAATGTGTGACGAAGAAGTTGCTG	TTAATACGCTGGAACCACACAAC	no
Fasciclin 2	FasII	GCTCGAAATGATCGGAATAG	CACAGCAAGAGGCAAACCAG	no
dPak	dPak	TCCAAGAAGCCAGTGGAGAAG	TATTCTTGTCCAGCGTCGTCG	yes
Beta Spectrin	ßspec	TATGTGGATATGCGGGATGG	ATAACGCTCCGATTCCAGTTC	no
Ca^{2+}/ Calmodulin-dependent protein kinase II	CamKII	TGTACGCGTTTTTCGGACAATTACG	CGACTGTAGTACTGCGATCAACGG	yes
Membrane associated guanylate kinase inverted	Magi	GCCAATAATCACGGCCACGAC	TCCCGTCACTTCCCACAATC	yes
Glutamate Receptor IIC	GluRIIC	AGTTCGACGAGGATGGACAG	GGTCAACACCTTCCAGATTGTC	no
Wingless	Wg	CATTGCCAAGGTCGGCG	GCCGGTATCGACGAATTCC	no

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Fly Strains

All flies were reared on standard *Drosophila* medium at temperatures between 18-29 ˚C, depending on the nature of the experiment. Flies were reared in low density to avoid decreased crawling behavior which results from overcrowding (Sigrist et al., 2003). For quantitative evaluation of nuclear DFz2C/LamCfoci, larvae were reared in 5 ml freshly made food in 28mm vials. Parents where allowed to lay eggs in agar plates supplemented with a drop of live yeast paste, and 50 just hatched first instar larvae where transferred to the food vials, which were maintained at 25˚C at constant 60% humidity and with a 12:12 hrs lights on: lights off. The following strains were used: Canton S (CS) (*wild type*); *w;LamC*^{EX296} */ w;LamC*^{EX265}, *P*-element excisions predicted to be protein nulls (Schulze et al., 2005); *w;;dfz2*^{C1} *FRT2A /Df(3L)dfz2* (Mathew et al., 2005), *w;LamC*^{EX265} */ BG487, LamC*^{EX296}*; UAS-LamC-3 /* +, with BG487-Gal4 driving expression in muscle 6/7 in an antero-posterior gradient (Gorczyca et al., 2007), the hyperexcitable mutant *eag*^{1},*Sh*^{133} (Budnik et al., 1990), and hs-PKM (Drier et al., 2002). The following UAS-RNAi and UAS-transgenes were utilized: UAS-DFz2-RNAi (ID – 44391; Vienna Drosophila RNAi Center [VDRC])(Dietzl et al., 2007), UAS-LamC-RNAi built in the pWiz vector (see below) and UAS-aPKC-RNAi (Ramachandran et al., 2009). UAS-Par6-RNAi (ID-19371, VDRC), UAS-Baz-RNAi (Ramachandran et al., 2009), UAS-DFz2 (Mathew et al., 2005), UAS-PKM (Ruiz-Canada et al., 2004). RNAi knockdown and muscle transgene expression was carried out using the muscle-specific Gal4 strain C57-Gal4

(Budnik et al., 1996), unless otherwise indicated. We also used the GFP traps: PABP2-GFP (ZCL2020) and *lamC*$^{GFP\text{-}trap}$/+ (G00158) ((Yale FlyTrap collection); Morin et al., 2001), containing GFP fused in frame to the endogenous loci (Buszczak et al., 2007; Kelso et al., 2004; Morin et al., 2001).

Immunocytochemistry and Fluorescent Dye Labeling

The following antibodies and fluorescent labels were used: mouse anti-LamC, 1:30 (LC28.26; Developmental Studies Hybridoma Bank (DSHB))(Riemer et al., 1995), rabbit anti-DFz2-C, 1:500(Mathew et al., 2005), mouse anti-nuclear pore complex mAb414, 1:300 (ab24609; Covance)(Aris and Blobel, 1989), rabbit anti-PABP2, 1:1500 (Benoit et al., 1999), rabbit anti-Baz, 1:600 (Wodarz et al., 1999), rabbit anti-aPKC, 1:2000 (Sigma), rabbit anti-PKC substrate, 1:2000 (Cell Signaling Technology), mouse anti-GluRIIA, 1:10 (8B4D2; DSHB), mouse anti-FITC, 1:700 (Sigma), rabbit anti-DLG, 1:40,000 (Koh et al 1999), goat anti-Horseradish Peroxidase (HRP), 1:200 comjugated to Dylight 488, 594, or 649 (Jackson Immunoresearch), Alexa Fluor 488 conjugated-Conconavalin-A (ConA) 50 µg/ml (Molecular Probes), donkey anti-mouse Peroxidase, 1:5000 (Jackson), mouse anti-rabbit (IgG light chain) Peroxidase , 1:2000 (Jackson), donkey anti-mouse FITC, 1:200 (Jackson), Propidium Iodide (PI) 10 µg/ml (Sigma), Hoechst 3342, 100 µg/ml, texas red (TxR)-conjugatged dextran, 50 µg/ml (70kD; Molecular Probes), and E36 RNA dye(Li et al., 2006), 10µM in HL3 saline and 0.2% DMSO.

For PI staining, fixed body wall muscle preparations were first treated with 500 µg/ml RNase A for 20 minutes and then incubated with PI for 20 minutes. Hoechst 3342 staining was applied to preparations for 1.5 hours.

Fixable texas red (TxR)-conjugatged dextran was pressure injected into the muscle using a PV380 Pneumatic PicoPump and beveled sharp electrodes. Briefly, sharp electrodes (~4-5 megaohm) were pulled on a Flaming/Brown micropipette puller and then beveled at a15˚ angle to a final resistance of ~2 megaohm. Dextran dye was resuspended in an internal patch clamp solution (Yoshihara et al., 2000) and passed through a 0.2 µm filter via centrifugation. Muscles 6 or 7 from dissected larval body wall muscle preparations bathed in 0.1 mM Ca^{2+} HL3 saline were pressure injected using 4 ms pulses of ~ 8-10 lbs until sufficient dye was injected as determined under epifluorescence. After injection, the dye was typically given ~ 40 minutes to diffuse before fixation. All fixations were performed at room temperature with ice-cold, freshly made, 4% paraformaldehyde for 15 minutes.

Image acquisition and analysis

Confocal images were acquired using a Zeiss LSM5 Pascal confocal microscope equipped with a Zeiss 63x Plan-Apochromat 1.4 NA DIC oil immersion objective at a digital zoom of 3x which met the Nyquist sampling requirements (pixel size equals 51 nm in XY) and Z step of 170 nm, unless otherwise indicated. Hoechst 3342 images were captured with a spinning disk confocal microscope equipped with a CoolSNAP^{TM} HQ camera (1392x1040) and a Nikon 60x Plan-apochromatic, NA 1.4 objective. FISH images were captured

on a spinning disk confocal microscope equipped with a Zeiss 63x Plan-Apochromat 1.4 NA DIC oil immersion objective and a Hamamatsu C9100-13 EM-CCD camera (512x512), with a total magnification of 150X to the CCD (pixel size = 100 nm in XY) and a 200 nm step.

For deconvolution, as noted in figure legends, images were deconvolved using measured point spread functions (PSF) and the iterative deconvolution algorithm in the image analysis software package Volocity (Perkin Elmer). Briefly, a PSF was obtained for each acquisition channel (laser lines 488, 543 and 633) by drying the appropriate wavelength Molecular Probes PS-Speck beads (505/515, 540/560 and 633/660) onto the back of a coverslip and imaging the beads under the same conditions as for the experimental images, with the exception of the laser power and PMT/CCD gain. To check for changes in the PSF at depth in the muscle, beads were injected into the muscle and imaged as they lay on top of the actin contractile apparatus, roughly the same plane at which nuclei lie. No obvious differences were seen with the PSF from these beads and those at the back of the coverslip, and deconvolution using the two PSFs gave similar results. Thus, PSF measurements from the back of the coverslip were utilized for the remainder of the studies. In Volocity, an iteration limit of 13 was set on the deconvolution process. Registration corrections were made to images when imaging 100 nm tetraspeck beads revealed channel alignment issues. Deconvolved images were exported as TIFFs from Volocity and opened in Photoshop for image construction.

Structured illumination images were acquired using a Zeiss ELYRA PS.1 equipped with a 63x 1.4 NA PlanApo lens. Five grid rotations per channel per Z-plane were captured (Voxel size = 39 x 39 x 90 nm). Images were reconstructed in Zen 2010 and channel alignment was performed based on images from 100 nm tetraspeck beads.

Quantification of foci, ghost boutons, and bouton volume

Nuclear foci were counted at muscles 6 & 7 from abdominal segments A2 and A3 of wandering third instar larvae. All nuclei in each of these muscles were quantified. The average number of foci per nucleus was calculated by dividing the total number of foci by the total number of nuclei quantified. The number of foci/nucleus was normalized to simultaneously processed wild type controls. Number of nuclei analyzed is: wild type=2596, *eag Sh*=530, DFz2-RNAi-muscle=593, UAS-DFz2-muscle=617, $dfz2^{C1}$/Def=302, $LamC^{EX265}$/ $LamC^{EX296}$=413, C57/+= 328, aPKC-RNAi-muscle=532, wild type with chelerythrine=726, PKM-muscle=677, wild type heat shock=713, hs-PKM no heat shock=601, hs-PKM with heat shock=595, Baz-RNAi-muscle=652. Total number of boutons was quantified in 3rd instar larval preparations double labeled with antibodies to HRP and GluRIII or DLG, at segments A3 muscles 6-7. The number of ghost boutons was assessed by counting HRP immunoreactive boutons that were devoid of GluRIII or DLG immunoreactivity. Number of NMJs analyzed: wild type=12, $LamC^{EX265}$/ $LamC^{EX296}$ =12, $LamC^{EX265}$/ $LamC^{EX296}$ muscle rescue=18).. Number of NMJs analyzed is: wild type=17, $LamC^{EX265}$/ $LamC^{EX296}$ =25, LamC-RNAi-muscle=16, $lamC^{GFP\text{-}trap}$/+=16, Baz-RNAi-

muscle=12, aPKC-RNAi-muscle=12, UAS-PKM-muscle=12. Muscle surface was measured by multiplying the width by the length of muscle 6 and 7 in segment A3, determined using a scale within the ocular of the epifluorecence microscope. Number muscles measured is: wild type=18, $LamC^{EX265}$/ $LamC^{EX296}$ =31, UAS-LamC-RNAi-muscle=15, $lamC^{GFP\text{-}trap}$/+=15, Baz-RNAi-muscle=12, aPKC-RNAi-muscle=12, UAS-PKM-muscle=12. Bouton volume was quantified using Volocity by cropping the image to individual boutons based on HRP staining, and then using the software to measure volume. Number of boutons analyzed is: wild type= 263, $LamC^{EX265}$/ $LamC^{EX296}$=108, $LamC^{EX265}$/ $LamC^{EX296}$ muscle rescue=64. Glutamate receptor quantification was also performed using Volocity software, where volume and total intensity of each cluster was measured. Total intensity was normalized to wild type levels.

For the analysis of PABP2-GFP foci vs. F2zC foci, the number of PABP2-GFP and dFz2C foci in a given nuclei was counted and then plotted against each other (N=113 nuclei) (Fig. SF4B). As this data did not follow a normal distribution a Spearman correlation analysis in Prism5 was run, which suggested there is a significant positive correlation between PABP2 and DFz2C foci (Spearman R=0.52). Analysis of the data with a Generalized Extreme Studentized Deviate (ESD) test suggested there were multiple outliers in the data set (these outliers were not excluded from the graph in Fig. SF4B). To ensure that these were not eliciting an erroneous correlation, the outliers were removed and the analysis performed again. This analysis also suggested a significant positive correlation ($p<0.0001$) between the two foci (Spearman R= 0.44). The number of PABP2-

GFP foci was quantified by placing the PABP2-GFP trap into the two experimental genetic backgrounds. Number of nuclei is: control=229, *LamC*^{EX265}/ *LamC*^{EX296} =175, *dfz2*^{C1}/Def= 301.

Chelerythrine feeding

Wild type larvae were raised under ideal density (50 just hatched larvae per 5 ml medium) in a 25-degree humidity controlled incubator. When larvae were mid-third instar (selected based on size) they were placed on food plates containing, freshly mixed, either 15µL 26mM Chelerythrine (in water) or 15µL water (control) per 4 ml media and incubated at 25 ˚C for 21 hours prior to fixing. Plates were wrapped with aluminum foil as Chelerythrine is light sensitive.

Lambda Phosphatase treatment

Wild type larvae were dissected and fixed and then divided between two 0.5mL tubes, both containing 400 µL of 1X NEBuffer for PMP with 1mM $MnCl_2$. One tube contained 10,000 Units of Lambda protein phosphatase (New England Biolabs) and the other an equal volume of water. Both tubes were incubated on a rotating shaker at 37°C for 1 hour. Samples were then washed in 0.2% Phosphate buffered triton and processed for immunocytochemistry.

Heat shock protocol

Food plates were made as above, but with no drug added. Mid-late third instar larvae were collected from wild type and hs-PKM vials and either left at room temperature (control) or shifted to 30°C for 30 minutes. Then, they were returned to room temperature for a two-hour recovery period prior to dissection.

Statistical Analysis

Statistical analysis was performed on raw data (i.e.- not normalized or transformed). Unpaired two-tailed Student's t-tests were run for comparisons of experiments where a single experimental sample was processed in parallel with a wild type control. If the variance between the samples was significant, an unpaired t-test with Welch's correction was performed. In cases where multiple experimental groups were compared to a single control, a one-way ANOVA was performed, with either a Tukey (when comparing samples to each other) or Dunnet (when comparing samples to a control) post-hoc tests. All statistical analysis was carried out in Prism 5 (Graphpad Software, Inc.). Error bars in all graphs represent ±SEM.

E36 RNA dye staining and live imaging

Larvae were grown at 25˚C at low to mid density. One hour prior to dissection and imaging, larvae were placed for 1 hour at 29˚C to increase activity. Single larvae were dissected in 0.1 mM Ca^{++}-containing HL3 saline, leaving body wall muscles and CNS intact. Then, the preparation was incubated in 100 µM E36 for approximately 20 minutes at room temperature, washed, and the nuclei imaged by time-lapse microscopy for approximately 20 min at 150X magnification using a 40X 1.2NA water immersion objective, using an Improvision spinning disk confocal microscope. Image acquisition was carried out through a Z-stack spanning the entire volume of the nuclei, including fiduciary markers, such as trachea, neighboring nuclei and nucleoli. After imaging, samples were fixed and immunostained with antibodies to LamC and/or DFz2C.

Samples were imaged again to identify the foci and images scaled to time-lapse images using the fiduciary markers. For analysis of movies, individual granules were tracked across Z-sections by hand. The movie shown Suppl. Movie 1 is a single confocal slice and represents 3 minutes, 36 seconds (18 seconds per frame), while Suppl. Movie 2 is a Z-stack of 3 slices, as the granule moved across these focal planes and represents 2 minutes, 8 seconds (17 seconds per frame).

Fluorescence In-situ Hybridization (FISH)

The FISH protocol is an adaptation of (Tam et al., 2002). Briefly, larvae were dissected, pre-extracted for 5 min in RNase-free 0.1M Phosphate buffer containing 0.5% Triton-X100 (0.5% PBT) and 10mM Ribonucleoside-vanadyl complex (RVC, New England Biolabs), and then fixed in ice-cold 4% Paraformaldehyde (Sigma) in 0.1M phosphate buffer for 30 minutes. Preparations for synaptic FISH were then transferred directly to 0.2% PBT with 10mM RVC, while preparations for nuclear in situ were fixed for an additional 10 min in ice cold 100% Methanol, and then transferred to 0.2% PBT with 10mM RVC. Preparations were dissected quickly to minimize RNA degradation, with less than 30 min between the first and last preparation. RNase treated controls were treated for 30 minutes with 100µM RNase A for 30 minutes. Samples were incubated for a minimum of 20 min in 0.2% PBT, with one exchange of fresh 0.2% PBT with 10mM RVC after 10 min. Samples were then gradually exchanged into hybridization buffer (2X SSC, 10% Dextran Sulfate, 20mM RVC, 50% Formamide (except when using oligo-dT, in which case all conditions used

20% Formamide instead of 50%)), by first mixing with 50% hybridization buffer for 5 min, and then with 100% hybridization buffer for 10 min. Probes (see below) were mixed (equal parts digoxigenin-tagged probe and blocking probe) and heated to 80°C for 10 min, then combined with concentrated hybridization buffer (4X SSC, 20% Dextran Sulfate, 40mM RVC) to a final concentration of 2.5ng/µL probe and blocking probe, applied to samples, and incubated at 37°C for 3 hr (for synaptic FISH) or up to 18 hr (for nuclear FISH). Samples were then washed sequentially in 2X SSC with 50% formamide, 2X SSC, and 1X SSC for 15 min each. Samples were then exchanged back to 4X SSC, and then incubated in 1:100 Sheep anti-Digoxigenin (Sigma) with 20mM RVC for 1 hr at 37°C. Preparations were then washed in 4X SSC, 4X SSC with 0.1% Triton-X100, and 4X SSC for 10 min each, then fixed in 4% paraformaldehyde for 10 min, and washed 3 times in 0.2% PBT for 10 min each. Preparations were subsequently treated for 2 hours with 1:200 Donkey anti Sheep-FITC, washed 3 times in 0.2% PBT, and then overnight in 1:700 Rabbit anti-FITC (Invitrogen) and either 1:30 Mouse anti-LamC (DSHB) (for nuclear FISH) or anti-FITC alone (for synaptic FISH). Finally, preparations were incubated with fluorescently labeled quaternary antibodies labeled with Dylight 488, Dylight 594, or Dylight 649, and Goat anti HRP-Dylight 594 or Dylight 649 (Jackson Immunoresearch).

Probe preparation for FISH:

Probes were designed against 0.8-1.4 Kb regions of genes of interest (Suppl. Table 2) that showed minimal (less than 18 bp similarity) to no homology to other genes in the *Drosophila* genome. Probes were PCR amplified from

cDNA, purified, and then applied to a Bionick (Invitrogen) nick translation kit, along with digoxigenin-11-dUTP (Roche), incubated for 2.5 hr at 18°C, treated with 5% SDS plus 0.25M EDTA and heated to 65°C for 10 min. Probes were then mixed with 10µg salmon sperm DNA for every 1µg probe, and precipitated with ethanol and sodium acetate. Probes were resuspended in 100% formamide to a concentration of 10ng/µL, and stored at -20°C until needed. Blocking probe was prepared the same way as above, but with dTTP in place of dig-dUTP. PolyT and PolyA probes were 24 nucleotide oligos with digoxigenin tags on both the 3' and 5' ends (Integrated DNA Technologies).

Electron Microscopy

Transmission electron microscopy was carried out as in (Korkut et al., 2009). Immuno-gold labeling was accomplished by a modification of the heat induced antigen retrieval protocol by (Yamashita et al., 2009). Briefly, larvae were dissected and fixed with 4% formaldehyde containing 2.5 mM $CaCl_2$, 1.25 mM $MgCl_2$ in a 0.1 M HEPES buffer (pH 7.4) for 2 hrs and then with the same fixative in 0.1 M HEPES (pH 8.5) overnight at room temperature. Osmolarity of the fix solution was adjusted to ~ 330 mOsm by the addition of glucose. After fixation, specimens were rinsed in fresh HEPES buffer and dehydrated in a graded series (50%, 70%, 90%, and 100%) of dimethylformamide (DMF) on ice. Samples were infiltrated with DMF and LR White at ratios of 2:1 and 1:2 respectively for 30 min each and finally with pure LR White overnight at 40°C. Polymerization of the resin was carried out overnight at 55°C. Ultrathin sections were collected on Nickel grids and incubated with 20 mM Tris buffer (pH 9.0) for

2 hr at 95°C in a PCR cycler. After cooling, the grids were washed in 0.1M TBS (pH 7.2) for 30 min at room temperature. Subsequently the grids were incubated with LamC (1:50 to 1:5) and/or DFz2C (1:500 to 1:250) antibodies overnight at 4°C. Grids were then washed in 0.1 M TBS (pH 7.2) for 30 min at room temperature. Immuno-gold labeling was accomplished with 12 nm or 18 nm colloidal gold particles conjugated to secondary antibodies for 1-3 hrs. After washing in TBS for 30 min the grids were fixed with 2% glutaraldehyde containing 0.05% tannic acid in 0.1 M phosphate buffer (pH 5.5) for 5 min. Some grids were exposed to osmium vapor for 5- 10 min and then all grids were stained with uranyl acetate and lead citrate and viewed with a FEI EM 10 electron microscope at 80 kv.

Regressive EDTA

Regressive EDTA was conducted as in (Monneron and Bernhard, 1969) Briefly, grids were stained with freshly prepared 5% uranyl acetate for 3 min, then rinsed in water for 3 min. Grids were then immersed in 0.2M EDTA pH-7 for 3 -4 hr, washed in water, stained with lead acetate and viewed with a Philips EM10 electron microscope.

Molecular Biology

The UAS-LamC-RNAi construct was subcloned in the *Drosophila* pWiz transformation vector (Lee and Carthew, 2003). Briefly, a 547 base pair fragment(1:ATCGATCTCAAGCTTGGCCTTCTCTTTGGCGGTCTCATCGAGCA GCTTGCGAGCGGCGGCCAGCTCCTTCTCATAGACCGCCTTCAGATTAGAGG TCTCCCTGTTGACGGTATCCTGGGCGAGATTCAGTTCCTGGGTGAGCCGGC

TGTTCTCGTTCTCCAGGTTGCGCATGCGATCGATGTAGCAGGCCAGGCGAT
CGTTCAAATGCTGCAGTTCCTCCTTCTCCTGCTGCCGGCTGGTGCGCGTGG
GCGAGGTGGGTGAGGTGGCGCCCACCCTTGACGAGGTGGATGCCCCGCC
CACCGGCGTGGAGGTGGAGGCGCGCGAAACGCGTGTGTTCAATGTGACGC
GGCGTGCTGACATTTTTGCAATGTGTTTTCTTTTCTTTTTGCGAGGTGCGAG
TTGCTAAGTTAAGTACGTAATCCTCTCAAGTCACTGTCAATATTTTTCCAGAC
GTTTGATTCTGAATTTTTTTTGCTTGTACGTCCGCCTGCTTTGACGACTAAAA
ATTGACTGAAACTTTGACTCGAAACGAACGGCTATCATCGAC:547) was cloned from a cDNA library and ligated in opposing orientations into the EcoR1/AvrII and NheI/XbaI sites of pWiz, which flank a consensus *Drosophila* intron from the *white* gene, thus creating a double stranded snapback RNA when expressed.

Western Blot and Lamin immunoprecipitations

Third instar body wall muscles were dissected and homogenized in lysis buffer with phosphatase inhibitors (Ramachandran et al., 2009) and 2-3 larval equivalents were loaded in each lane of a 8% acrylamide gel and transferred to a nitrocellulose membrane. The membrane was then blocked with 3% BSA, and probed with either Rabbit anti-PKC phosho-serine substrate (Cell signaling) or Mouse anti-LamC (DSHB). After second antibody incubation, using either Donkey anti-rabbit or anti-mouse secondary antibodies conjugated to peroxidase, a Chemiluminescent detection kit (GE life sciences) was used to detect the signal.

For LamC immunoprecipitation,, 30 larvae per genotype were dissected and homogenized in lysis buffer containing phosphatase inhibitors (as above) plus 2% SDS, and then diluted to 0.1% SDS with normal lysis buffer. The lysates were then precleared with Protein G beads (GE Life sciences), and then incubated with Protein G beads that had been pre-incubated with anti-LamC. The samples were then incubated for 4 hours, after which they were treated as the western blots above.

RNA immunoprecipitations

S2 cells were grown until 100% confluency in 25 mL T-flasks (Nunc), and then harvested at 800 x g for 1 minute at room temperature. Cells were then homogenized in lysis buffer containing protease inhibitors as well as Ribonucleoside Vanadyl complex (an RNase inhibitor). Lysate was then precleared on Protein G beads (GE Life sciences) and then incubated with Protein G beads, which were pre-incubated with anti-DFz2C antibodies. Samples were incubated overnight at 4ºC, beads were then washed and treated with proteinase K, to digest RNP particles. RNA was then extracted using Trizol (Invitrogen), and then precipitated using glycogen and isopropyl alcohol. RNA pellets were washed with 70% ethyl alcohol, and then resuspended in RNase free water. RNA concentration was assessed with a Nanodrop spectrometer, and then 0.5 µg of RNA was used for a reverse transcription (RT) reaction using the Superscript III Kit (Invitrogen) and random hexamer primers. PCR was then performed using the same primers used for probe generation (Supplemental Table 2).

Electrophysiology

Samples were processed as in Gorczyca et al 2007. Briefly third instar larval body wall muscles were dissected in 0.3 mM calcium HL3 saline (Stewart et al 1994). Muscle 6 segment A3, in all samples, was impaled with a sharpened glass electrode. Samples were recorded using an Axoclamp-2A (Axon Instruments) amplifier, recorded in Heka Pulse software, and analyzed using mini analysis software (Synaptosoft).

SUPPLEMENTAL REFERENCES

Aris, J.P., and Blobel, G. (1989). Yeast nuclear envelope proteins cross react with an antibody against mammalian pore complex proteins. J Cell Biol *108*, 2059-2067.

Benoit, B., Nemeth, A., Aulner, N., Kuhn, U., Simonelig, M., Wahle, E., and Bourbon, H.M. (1999). The Drosophila poly(A)-binding protein II is ubiquitous throughout Drosophila development and has the same function in mRNA polyadenylation as its bovine homolog in vitro. Nucleic Acids Res *27*, 3771-3778.

Budnik, V., Koh, Y.H., Guan, B., Hartmann, B., Hough, C., Woods, D., and Gorczyca, M. (1996). Regulation of synapse structure and function by the Drosophila tumor suppressor gene dlg. Neuron *17*, 627-640.

Budnik, V., Zhong, Y., and Wu, C.F. (1990). Morphological plasticity of motor axons in Drosophila mutants with altered excitability. J Neurosci *10*, 3754-3768.

Buszczak, M., Paterno, S., Lighthouse, D., Bachman, J., Planck, J., Owen, S., Skora, A.D., Nystul, T.G., Ohlstein, B., Allen, A., *et al.* (2007). The carnegie protein trap library: a versatile tool for Drosophila developmental studies. Genetics *175*, 1505-1531.

Dietzl, G., Chen, D., Schnorrer, F., Su, K.C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S., *et al.* (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature *448*, 151-156.

Drier, E.A., Tello, M.K., Cowan, M., Wu, P., Blace, N., Sacktor, T.C., and Yin, J.C. (2002). Memory enhancement and formation by atypical PKM activity in Drosophila melanogaster. Nat Neurosci *5*, 316-324.

Gorczyca, D., Ashley, J., Speese, S., Gherbesi, N., Thomas, U., Gundelfinger, E., Gramates, L.S., and Budnik, V. (2007). Postsynaptic membrane addition depends on the Discs-Large-interacting t-SNARE Gtaxin. J Neurosci *27*, 1033-1044.

Kelso, R.J., Buszczak, M., Quinones, A.T., Castiblanco, C., Mazzalupo, S., and Cooley, L. (2004). Flytrap, a database documenting a GFP protein-trap insertion screen in Drosophila melanogaster. Nucleic Acids Res *32*, D418-420.

Korkut, C., Ataman, B., Ramachandran, P., Ashley, J., Barria, R., Gherbesi, N., and Budnik, V. (2009). Trans-synaptic transmission of vesicular Wnt signals through Evi/Wntless. Cell *139*, 393-404.

Lee, Y.S., and Carthew, R.W. (2003). Making a better RNAi vector for Drosophila: use of intron spacers. Methods *30*, 322-329.

Li, Q., Kim, Y., Namm, J., Kulkarni, A., Rosania, G.R., Ahn, Y.H., and Chang, Y.T. (2006). RNA-selective, live cell imaging probes for studying nuclear structure and function. Chem Biol *13*, 615-623.

Mathew, D., Ataman, B., Chen, J., Zhang, Y., Cumberledge, S., and Budnik, V. (2005). Wingless signaling at synapses is through cleavage and nuclear import of receptor DFrizzled2. Science *310*, 1344-1347.

Monneron, A., and Bernhard, W. (1969). Fine structural organization of the interphase nucleus in some mammalian cells. J Ultrastruct Res *27*, 266-288.

Morin, X., Daneman, R., Zavortink, M., and Chia, W. (2001). A protein trap strategy to detect GFP-tagged proteins expressed from their endogenous loci in Drosophila. Proc Natl Acad Sci U S A *98*, 15050-15055.

Ramachandran, P., Barria, R., Ashley, J., and Budnik, V. (2009). A critical step for postsynaptic F-actin organization: regulation of Baz/Par-3 localization by aPKC and PTEN. Dev Neurobiol *69*, 583-602.
Riemer, D., Stuurman, N., Berrios, M., Hunter, C., Fisher, P.A., and Weber, K. (1995). Expression of Drosophila lamin C is developmentally regulated: analogies with vertebrate A-type lamins. J Cell Sci *108 (Pt 10)*, 3189-3198.
Ruiz-Canada, C., Ashley, J., Moeckel-Cole, S., Drier, E., Yin, J., and Budnik, V. (2004). New Synaptic Bouton Formation Is Disrupted by Misregulation of Microtubule Stability in aPKC Mutants. Neuron *42*, 567-580.
Schulze, S.R., Curio-Penny, B., Li, Y., Imani, R.A., Rydberg, L., Geyer, P.K., and Wallrath, L.L. (2005). Molecular genetic analysis of the nested Drosophila melanogaster lamin C gene. Genetics *171*, 185-196.
Sigrist, S.J., Reiff, D.F., Thiel, P.R., Steinert, J.R., and Schuster, C.M. (2003). Experience-dependent strengthening of Drosophila neuromuscular junctions. J Neurosci *23*, 6546-6556.
Tam, R., Shopland, L.S., Johnson, C.V., McNeil, J., and Lawrence, J.B. (2002). Applications of RNA FISH for visualizing gene expression and nuclear architecture. In FISH Practical Approach, B.M.S.S.J. Beatty, ed. (New York, Oxford University Press), pp. 93-118.
Wodarz, A., Ramrath, A., Kuchinke, U., and Knust, E. (1999). Bazooka provides an apical cue for Inscuteable localization in Drosophila neuroblasts. Nature *402*, 544-547.
Yamashita, S., Katsumata, O., and Okada, Y. (2009). Establishment of a standardized post-embedding method for immunoelectron microscopy by applying heat-induced antigen retrieval. J Electron Microsc (Tokyo) *58*, 267-279.
Yoshihara, M., Suzuki, K., and Kidokoro, Y. (2000). Two independent pathways mediated by cAMP and protein kinase A enhance spontaneous transmitter release at Drosophila neuromuscular junctions. J Neurosci *20*, 8315-8322.