Inventory of Supplemental Information; Muller et al., "RIM-Binding Protein Links Synaptic Homeostasis to the Stabilization and Replenishment of High Release Probability Vesicles"

We have included one supplemental figure and one supplemental table.

Supplemental Figure 1 relates to Figure 3 and Figure 6 in our manuscript.
Supplemental Table 1 provides raw values for normalized data presented in Figures 1, 3, 4, 8.

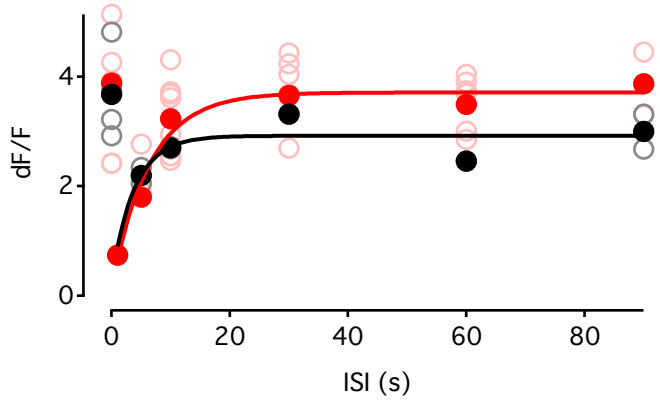

Figure S1. Presynaptic Ca^{2+} Influx during 60-Hz Stimulation

Spatially-averaged Ca^{2+} transient peak amplitudes at the end (last 5 stimuli) of 60-Hz stimulation (30 stimuli) at baseline (ISI=0, after a 30s period of rest), and during recovery from synaptic depression (a second 60-Hz train was applied at ISI=1,5,10,30,60, 90s; 1mM $[Ca^{2+}]_e$; OGB-1; 0.5 μM NASP was applied to prevent muscle contractions; *rbp*: n=3 cells, red data; and wild type: n=2 cells, black data; light data show amplitudes of single transients). Note the similar peak dF/F peak amplitude at the end of 60-Hz stimulation during baseline conditions (ISI=0s), and during recovery from synaptic depression. Exponential fits to the data revealed a slight difference in Ca^{2+} transient amplitude recovery kinetics between *rbp* (τ=6.4s) and wild type (τ=3.9s). Based on this experiment we conclude that *i.* Ca^{2+} transient amplitudes at the end of 60-Hz stimulation are similar between wild type and *rbp* (at least as far as we can resolve it), *ii.* that the pronounced slowing of EPSC recovery (4-fold) is not a secondary consequence of reduced Ca^{2+} influx during the 60-Hz stimulation, or during recovery from synaptic depression, and *iii.* that Ca^{2+} transient peak amplitude is not saturated by single AP stimulation, because we can obtain dF/F values of almost 4.

Figure	Genotype	Condition	mEPSP ampl. (mV)	EPSP or EPSC ampl.	Quantal Content	n
1	w^{1118}	- PhTX	1.08 ± 0.1	36.3 ± 1.5 mV	36.1 ± 2.6	8
	w^{1118}	+ PhTX	0.56 ± 0.01	33.4 ± 1.5 mV	59.6 ± 2.4	7
	drbp	- PhTX	1.14 ± 0.06	26.2 ± 1.1 mV	24.5 ± 1.4	16
	drbp	+ PhTX	0.54 ± 0.04	12.4 ± 1.3 mV	23.5 ± 2.0	17
3	w^{1118}	- EGTA (0.1 Hz)		222.3 ± 12.0 nA		7
	w^{1118}	+ EGTA (0.1 Hz)		196.4 ± 12.3 nA		6
	drbp	- EGTA (0.1 Hz)		41.7 ± 7.5 nA		8
	drbp	+ EGTA (0.1 Hz)		12.0 ± 2.2 nA		8
	w^{1118}	- EGTA (1 Hz)		139.6 ± 8.4 nA		7
	w^{1118}	+ EGTA (1 Hz)		196.4 ± 12.3 nA		6
	drbp	- EGTA (1 Hz)		27.2 ± 4.6 nA		8
	drbp	+ EGTA (1 Hz)		11.6 ± 1.7 nA		8
	w^{1118}	- EGTA (0.1 Hz)	(0.45 Ca)	41.1 ± 7.4 nA		8
	w^{1118}	+ EGTA (0.1 Hz)	(0.45 Ca)	40.7 ± 4.7 nA		10
8	w^{1118}	- PhTX	1.05 ± 0.06	31.4 ± 3.0 mV	30.3 ± 3.3	6
	w^{1118}	+ PhTX	0.5 ± 0.03	29.3 ± 3.2 mV	59.3 ± 5.7	6
	rim^{103}/+	- PhTX	0.86 ± 0.14	28.1 ± 1.5 mV	35.0 ± 6.2	7
	rim^{103}/+	+ PhTX	0.39 ± 0.04	23.5 ± 2.1 mV	57.1 ± 7.5	5
	drbp/+	- PhTX	1.05 ± 0.04	27.6 ± 1.7 mV	52 ± 3	10
	drbp/+	+ PhTX	0.48 ± 0.03	27.3 ± 2.3 mV	27 ± 2	19
	drbp/rim^{103}	- PhTX	0.86 ± 0.06	6.6 ± 1.8 mV	7.6 ± 1.7	12
	brp^{69}/+; *drbp/+*	- PhTX	1.26 ± 0.09	23.7 ± 1.7 mV	19.5 ± 1.7	10
	w^{1118}	- PhTX (1 Ca)	0.86 ± 0.11	52.7 ± 6.8 mV	113.8 ± 15.9	8
	w^{1118}	+ PhTX (1 Ca)	0.48 ± 0.03	53.8 ± 4.2 mV	316.6 ± 40.2	8
	rim^{103}/+; *drbp/+*	- PhTX (1 Ca)	0.78 ± 0.04	30.2 ± 4.1 mV	67.5 ±13.6	12
	rim^{103}/+; *drbp/+*	+ PhTX (1 Ca)	0.37 ± 0.02	27.8 ± 4.3 mV	126.5 ± 23.7	12

Figure	Genotype	Condition	mEPSP ampl. (mV)	Cum. EPSC ampl. (nA)	n
4	w^{1118}	- PhTX	0.76 ± 0.04	650.5 ± 91.6	5
	w^{1118}	+ PhTX	0.29 ± 0.02	793.0 ± 71.4	7
	drbp	- PhTX	0.6 ± 0.06	766.5 ± 87.3	9
	drbp	+ PhTX	0.33 ± 0.02	384.5 ± 50.3	12

Table S1. Summary of raw data. Data are mean ± SEM, and 'n' refers to the number of synapses. All data are average peak amplitude values, and were obtained at an extracellular [Ca^{2+}] of 0.4 mM (Figure 8), 1.5mM (Figure 1), 3mM (Figure 3), and 15mM (Figure 4), unless otherwise noted. Quantal content values of data in Figure 1 were corrected for non-linear summation (Martin, 1955). Data from experimental and control groups were collected side by side, and all recordings were done at room temperature. See Experimental Procedures for further details.

Supplemental Materials and Methods

Electrophysiology

Two-electrode voltage clamp recordings were performed with an Axoclamp 2B amplifier. The extracellular HL3 saline contained (in mM): 70 NaCl, 5 KCl, 10 $MgCl_2$, 10 $NaHCO_3$, 115 sucrose, 4.2 trehalose, 5 HEPES, and various concentrations of $CaCl_2$ (see Results and Figures). For homeostatic challenge, larvae were incubated in Philanthotoxin-433 (PhTX ; 20 µM; Sigma-Aldrich) for 10 min (Frank et al., 2006). The average single AP-evoked EPSP amplitude (stimulus duration, 3 ms), or EPSC amplitude (stimulus duration, 1 ms) of each recording is based on 30 presynaptic stimuli. At least 100 mEPSPs were collected per recording to obtain a mean mEPSP amplitude value. Quantal content is given by the ratio of the average EPSP amplitude over average mEPSP amplitude of a recording, and then averaging recordings across all NMJs for a given genotype. The apparent size of the readily-releasable vesicle pool (RRP) was probed by the method of cumulative EPSC amplitudes (Schneggenburger et al., 1999), which was recently applied to the Drosophila NMJ (Hallermann et al., 2010c; Miśkiewicz et al., 2011; Müller et al., 2012; Weyhersmüller et al., 2011). Muscles were clamped to their resting membrane potential (Vm), or clamped to – 65 mV if the Vm was more positive than – 60 mV. Muscles with a Vm>55mV were discarded. Synapses were stimulated with 60-Hz trains (30 stimuli, at least 5 trains per synapse). EPSC amplitudes during a stimulus train were calculated as the difference between peak and baseline before stimulus onset of a given EPSC. The cumulative EPSC amplitude was obtained by back-extrapolating a line fit to the last 10 cumulative EPSC amplitude values of the 60 Hz train to time zero (Figure 3C and 4A). Relative changes in the apparent size of the readily-releasable vesicle pool after PhTX application (‘norm. RRP size’; Figure 4B) were calculated by normalizing the ratio of cumulative EPSC amplitude over the relative decrease in mEPSP amplitude to the respective baseline value in the absence of PhTX.

Ca^{2+} Imaging

Ca^{2+} imaging experiments were done as described in Müller and Davis (2012). Third instar larvae were dissected and incubated in ice cold, Ca^{2+}-free HL3 containing 5 mM Oregon-Green 488 BAPTA-1 (OGB-1; hexapotassium salt, Invitrogen) and 1 mM Alexa 568 (Invitrogen). After incubation for 10 minutes, the preparation was washed with ice cold HL3 for 10 – 15 minutes. This lead to an intraterminal OGB-1 concentration of approximately 50 mM (Müller and Davis, 2012). Single action-potential evoked spatially-averaged Ca^{2+} transients were measured in type-1b boutons synapsing onto muscle 6/7 of abdominal segments A2/A3 at an extracellular [Ca^{2+}] of 1 or3 mM using a confocal laser-scanning

system (Ultima, Prairie Technologies) at room temperature. Excitation light (488 nm) from an air-cooled krypton-argon laser was focused onto the specimen using a 60 × objective (1.0 NA, Olympus), and emitted light was detected with a gallium arsenide phosphide-based photocathode photomultiplier tube (Hamamatsu). Line scans across single boutons were made at a frequency of 313 Hz. Fluorescence changes were quantified as $\Delta F/F = (F(t)-Fbaseline)/(Fbaseline-Fbackground)$, where F(t) is the fluorescence in a region of interest (ROI) containing a bouton at any given time, Fbaseline is the mean fluorescence from a 300-ms period preceding the stimulus, and Fbackground is the background fluorescence from an adjacent ROI without any indicator-containing cellular structures. One synapse (4 – 12 boutons) was imaged per preparation. The average Ca^{2+} transient of a single bouton is based on 8 – 12 line scans. Experiments in which the resting fluorescence decreased by >15 %, and/or which had a Fbaseline > 650 a.u. were excluded from analysis. Data of experimental and control groups were collected side by side. The Ca^{2+} indicator was not saturated by single AP stimulation because repetitive stimulation induced a further increase in the peak $\Delta F/F$ amplitude.