

**Table S1. Absolute values for normalized data and additional statistics.** The figure and panel, genotype, and conditions used are noted. For electrophysiological recordings, average values for mEPSP, EPSP, quantal content, resting membrane potential, input resistance, number of data samples (*n*), P values, and significance are shown. For confocal imaging analysis, average values for intensity levels of different synaptic markers and other relevant parameters such as numbers and area are shown. Standard error values are noted in parentheses. Rows highlighted in blue are the respective controls or baseline values for the particular experiment being referenced. amp, amplitude; freq, frequency; QC, quantal content.

Figure 1 (Muscle 6) 0.4 mM Ca <sup>2+</sup>	Genotype	mEPSP (mV)	EPSP (mV)	QC	mEPSP (Hz)	Resting Potential (mV)	Input Resistance (MΩ)	n	P value (significance) (mEPSP amp, EPSP, QC, mEPSP freq)
1D,E,F,G	<i>w<sup>1118</sup></i>	0.958 (0.029)	32.3 (1.215)	33.84 (1.147)	2.805 (0.156)	-66.7 (1.307)	9.259 (0.787)	13	
1D,E,F,G	<i>w;endo<sup>1</sup>/endo<sup>A4</sup></i>	1.35 (0.036)	31.59 (0.845)	23.61 (1.07)	2.337 (0.091)	-57.454 (1.171)	14.213 (1.236)	10	0.0001 (****), 0.9998 (ns), 0.0003 (**), 0.1126 (ns)
1D,E,F,G	<i>w;ok6-Gal4;UAS-endo<sup>RNAi</sup></i>	1.388 (0.056)	30.6 (0.693)	22.17 (1.034)	2.707 (0.145)	-67.598 (1.519)	7.790 (0.614)	12	0.0001 (****), 0.9235 (ns), <0.0001 (****), 0.9824 (ns)
1D,E,F,G	<i>c155-Gal4;endo<sup>1</sup></i>	1.048 (0.054)	33.54 (0.774)	33.19 (1.879)	3.009 (0.148)	-68.889 (1.820)	11.113 (0.909)	10	0.5076 (ns), 0.4368 (ns), 0.9997 (ns), 0.1224 (ns)
1D,E,F,G	<i>w;UAS-vGlut/ok371-Gal4</i>	1.426 (0.055)	32.89 (1.262)	23.38 (1.291)	2.175 (0.112)	-64.861 (2.328)	8.407 (0.781)	9	0.0001 (****), 0.9572 (ns), 0.0021 (**), 0.3172 (ns)

Muscle 4 0.8 mM Ca <sup>2+</sup>	Genotype	mEPSP (mV)	EPSP (mV)	QC	mEPSP (Hz)	Resting Potential (mV)	Input Resistance (MΩ)	n	P value (significance) (mEPSP amp, EPSP, QC, mEPSP freq)
	<i>w<sup>1118</sup></i>	0.917 (0.025)	28.76 (1.108)	31.36 (1.257)	2.834 (0.136)	-61.39 (0.907)	9.259 (0.787)	10	
	<i>w;endo<sup>1</sup>/endo<sup>A4</sup></i>	1.27 (0.028)	26.49 (0.807)	20.86 (1.178)	2.237 (0.091)	-53.474 (1.201)	16.225 (1.038)	7	0.0001 (****), 0.9998 (ns), 0.0003 (**), 0.0136 (*)
	<i>w;rab3<sup>rup</sup></i>	1.021 (0.016)	31.98 (0.813)	31.32 (0.934)	2.917 (0.175)	-62.527 (1.327)	7.595 (0.466)	9	0.0001 (****), 0.9235 (ns), <0.0001 (****), 0.9739 (ns)

Figure 1	Genotype	Bouton #/NMJ	Bouton area (μm <sup>2</sup> )	n	P value (significance) (number, area)
1B	<i>w<sup>1118</sup></i>	38.53 (2.676)	7.84 (0.431)	15	
1B	<i>w;endo<sup>1</sup>/endo<sup>A4</sup></i>	73.77 (4.491)	05.32 (0.309)	13	<0.0001 (****), 0.0002 (**)
1B	<i>w;ok6-Gal4;UAS-endo<sup>RNAi</sup></i>	64.15 (3.214)	6.09 (0.361)	13	<0.0001 (****), 0.0168 (*)
1B	<i>c155-Gal4;endo<sup>1</sup></i>	45.31 (2.811)	7.433 (0.347)	13	0.5115 (ns), 0.9432 (ns)
1B	<i>w;UAS-vGlut/ok371-Gal4</i>	41.87 (1.737)	7.574 (0.424)	15	0.9266 (ns), 0.9860 (ns)

Figure 2	Genotype	neuronal surface area ( $\mu\text{m}^2$ )	BRP puncta density (#/ $\mu\text{m}^2$ )	BRP puncta #/NMJ	BRP puncta area ( $\mu\text{m}^2$ )	n	P value (significance) (neuronal area, density, , #, area)
2B,C,D,E	<i>w<sup>1118</sup></i>	269 (12.91)	1.131 (0.046)	278.5 (12.61)	0.174 (0.004)	22	
2B,C,D,E	<i>w;endo<sup>1</sup>/endo<sup>A4</sup></i>	489.5 (23.1)	1.012 (0.061)	480.4 (19.07)	0.113 (0.004)	17	<0.0001 (****), 0.9917 (ns), 0.0001 (****), 0.0003 (**)
2B,C,D,E	<i>w;ok6-Gal4;UAS-endo<sup>RNAi</sup></i>	448.8 (31.57)	1.085 (0.088)	457.7 (22.36)	0.139 (0.003)	15	<0.0001 (****), 0.9989 (ns), <0.0001 (****), 0.0003 (**)
2B,C,D,E	<i>c155-Gal4;endo<sup>1</sup></i>	301.6 (14.05)	1.127 (0.07)	328.5 (18.03)	0.165 (0.006)	12	0.7614 (ns), 0.7767 (ns), 0.8251 (ns), 0.9799 (ns)
2B,C,D,E	<i>w;UAS-vGlut/ok371-Gal4</i>	259 (17.39)	0.933 (0.077)	228.5 (16.18)	0.185 (0.005)	16	0.5587 (ns), 0.6519 (ns), 0.4119 (ns), 0.7198 (ns)

Figure 3	Genotype	mEPSP (mV)	EPSP (mV)	QC	mEPSP (Hz)	Resting Potential (mV)	Input Resistance (M $\Omega$ )	n	P value (significance) (mEPSP amp, EPSP, QC, mEPSP freq)
3E	<i>w<sup>1118</sup></i>	0.952 (0.041)	31.25 (1.33)	33.31 (1.794)	3.105 (0.106)	-68.07 (2.257)	6.553 (0.587)	11	
3E	<i>w;rab3<sup>rup</sup></i>	0.967 (0.051)	29.99 (1.564)	31.412 (1.985)	2.957 (0.088)	-72.404 (3.122)	7.293 (0.636)	7	0.9879 (ns), 0.9106 (ns), 0.9993 (ns), 0.8126 (ns)
3E	<i>c155-Gal4/+;UAS-rab3<sup>RNAi/+</sup></i>	0.933 (0.042)	30.6 (0.928)	33.605 (2.152)	2.767 (0.169)	-73.508 (2.229)	8.590 (0.714)	10	0.7891 (ns), 0.9800 (ns), 0.8875 (ns), 0.9824 (ns)
3E	<i>w; rab3<sup>rup</sup>,endo<sup>1</sup>/rab3<sup>rup</sup>,endo<sup>A4</sup></i>	1.297 (0.035)	33.51 (1.872)	25.899 (1.529)	2.591 (0.248)	-60.759 (2.131)	14.123 (1.099)	5	<0.0001 (****), 0.7080 (ns), 0.0224 (*), 0.1224 (ns)

Figure 3	Genotype	neuronal surface area ( $\mu\text{m}^2$ )	BRP puncta density (#/ $\mu\text{m}^2$ )	BRP puncta #/NMJ	BRP puncta area ( $\mu\text{m}^2$ )	Bouton #/NMJ	n	P value (significance) (neuronal area, density, #, area, bouton #/NMJ)
3B,C,F,G	<i>w<sup>1118</sup></i>	306.5 (13.57)	1.021 (0.024)	281.8 (11.52)	0.165 (0.004)	33.43 (2.856)	13	
3B,C,F,G	<i>w;rab3<sup>rup</sup></i>	298.77 (21.99)	0.411 (0.019)	111.878 (10.089)	0.356 (0.003)	31.65 (2.018)	15	>0.9999 (ns), <0.0001 (****), <0.0001 (****), 0.0003 (**), >0.9999 (ns)
3B,C,F,G	<i>c155-Gal4/+;UAS-rab3<sup>RNAi/+</sup></i>	290.112 (20.229)	0.501 (0.03)	136.11 (0.031)	0.297 (0.002)	36.77 (2.54)	11	0.9109 (ns), <0.0001 (****), <0.0001 (****), <0.0001 (****), 0.9987 (ns)
3B,C,F,G	<i>w; rab3<sup>rup</sup>,endo<sup>1</sup>/rab3<sup>rup</sup>,endo<sup>A4</sup></i>	354.185 (29.998)	1.046 (0.003)	340.198 (30.871)	0.179 (0.011)	48.43 (4.259)	4	0.0419 (*), 0.9917 (ns), 0.0391 (*), 0.0411 (*)

Figure 3	Genotype	Goodness of Fit ( $R^2$ ) with ideal tuning curve	Degrees of Freedom
3H	<i>w<sup>1118</sup></i>	0.7524	22
3H	<i>w;endo<sup>1</sup>/endo<sup>A4</sup></i>	0.6915	17

3H	<i>w;ok6-Gal4/ok6-Gal4;UAS-endo<sup>RNAi</sup>/ UAS-endo<sup>RNAi</sup></i>	0.7209	15
3H	<i>w;UAS-vGlut/ok371-Gal4</i>	0.6425	16
3H	<i>w;rab3<sup>rup</sup></i>	0.5573	15
3H	<i>w; rab3<sup>rup</sup>,endo<sup>1</sup>/ rab3<sup>rup</sup>,endo<sup>A4</sup></i>	0.3197	3

Figure 4	Genotype	BRP puncta mean intensity (% wild type)	Unc 13A puncta mean intensity (% wild type)	BRP puncta sum intensity (% wild type)	Unc13A puncta sum intensity (% wild type)	n	P value (significance) (BRP mean, Unc13A mean, BRP sum, Unc13A sum)
4B,C	<i>w<sup>1118</sup></i>	100 (1.264)	100 (3.004)	100 (2.39)	100 (4.439)	15	
4B,C	<i>w;endo<sup>1</sup>/endo<sup>A4</sup></i>	91.95 (4.092)	105.1 (4.897)	60.81 (4.093)	60.62 (2.439)	17	0.1201 (ns), 0.7429 (ns), <0.0001 (****), 0.0172 (*)
4B,C	<i>w;UAS-vGlut/ok371-Gal4</i>	102.5 (2.035)	101 (1.962)	110.6 (4.839)	108.9 (9.642)	22	0.8810(ns), 0.9955 (ns), 0.3633 (ns), 0.8560 (ns)
4B,C	<i>w;rab3<sup>rup</sup></i>	110.8 (3.5)	113 (4.021)	199.4 (8.876)	174.4 (15.22)	18	0.0148 (*), 0.0341 (*), <0.0001(****), <0.0001 (****)

Figure 4	Genotype	BRP total intensity/NMJ (% wild type)	Unc13A total intensity/NMJ (% wild type)	n	P value (significance) (BRP, Unc13A)
4D	<i>w<sup>1118</sup></i>	100 (4.812)	100 (7.029)	15	
4D	<i>w;endo<sup>1</sup>/endo<sup>A4</sup></i>	104.5 (6.111)	99.69 (10.19)	17	0.9157 (ns), >0.9999 (ns)
4D	<i>w;UAS-vGlut/ok371-Gal4</i>	89.85 (4.519)	107.1 (7.336)	22	0.4301 (ns), 0.8947 (ns)
4D	<i>w;rab3<sup>rup</sup></i>	92.48 (4.158)	109.8 (6.885)	18	0.4116 (ns), 0.9998 (ns)

Figure 4	Genotype	BRP puncta mean intensity (% wild type)	Cac per BRP mean intensity (% wild type)	BRP puncta sum intensity (% wild type)	Cac per BRP sum intensity (% wild type)	n	P value (significance) (BRP mean, Cac mean, BRP sum, Cac sum)
4B,C	<i>cac<sup>sfGFP-N</sup></i>	100 (7.628)	100 (7.241)	100 (8.910)	100 (7.643)	17	
4B,C	<i>cac<sup>sfGFP-N</sup>;endo<sup>1</sup>/endo<sup>A4</sup></i>	91.009 (8.24)	95.51 (7.241)	60.44 (5.431)	55.44 (5.73)	8	0.8896 (ns), 0.9998 (ns), 0.008 (**), 0.0091 (**)
4B,C	<i>cac<sup>sfGFP-N</sup>;UAS-vGlut/ok371-Gal4</i>	104.85 (6.861)	108.71 (8.318)	108.11 (10.641)	109.11 (9.642)	18	0.7165(ns), 0.2136 (ns), 0.3389 (ns), 0.3251 (ns)
4B,C	<i>cac<sup>sfGFP-N</sup>;rab3<sup>rup</sup></i>	112.44 (10.271)	107.891 (9.078)	259.35 (14.661)	218.5 (17.22)	14	0.0715 (ns), 0.1198 (ns), <0.0001(****), 0.005 (**)

Figure 4	Genotype	BRP total intensity/NMJ (% wild type)	Cac total intensity/NMJ (% wild type)	n	P value (significance) (BRP, Cac)
4D	<i>cac</i> <sup>sfGFP-N</sup>	100 (10.67)	100 (9.613)	17	
4D	<i>cac</i> <sup>sfGFP-N</sup> ; <i>endo</i> <sup>1</sup> / <i>endo</i> <sup>A4</sup>	89.331 (8.773)	95.781 (9.88)	8	0.5091 (ns), 0.9998 (ns)
4D	<i>cac</i> <sup>sfGFP-N</sup> ; <i>UAS-vGlut</i> ok371- <i>Gal4</i>	109.718 (10.641)	110.11 (9.642)	18	0.4119 (ns), 0.4506 (ns)
4D	<i>cac</i> <sup>sfGFP-N</sup> ; <i>rab3</i> <sup>rup</sup>	108.85 (9.876)	109.78 (10.22)	14	0.5691 (ns), 0.5197 (ns)

Figure 5	Genotype	BRP ring diameter (nm)	Unc13A ring diameter (nm)	BRP:Unc13A ratio	n	P value (significance) (BRP, unc13A, ratio)
5B,C,D	<i>w</i> <sup>1118</sup>	223.4 (11.84)	176.6 (9.019)	1.106 (0.026)	112	
5B,C,D	<i>w;ok6-Gal4</i> , <i>UAS-Dcr</i> +/+; <i>UAS-endo</i> <sup>RNAi</sup> /+	141.9 (5.132)	129.7 (4.493)	1.097 (0.015)	159	<0.0001 (****), <0.0001 (****), 0.7614 (ns)
5B,C,D	<i>w;rab3</i> <sup>rup</sup>	457.7 (26.83)	341.1 (17.25)	1.153 (0.023)	53	<0.0001 (****), <0.0001 (****), 0.1097 (ns),

Figure 5	Genotype	BRP modules/AZ	Unc13A modules/AZ	BRP:unc13A module # ratio	n	P value (significance) (BRP, unc13A, ratio)
5F,G,H	<i>w</i> <sup>1118</sup>	5.98 (0.235)	3.74 (0.112)	1.113 (0.091)	112	
5F,G,H	<i>w;ok6-Gal4</i> , <i>UAS-Dcr</i> +/+; <i>UAS-endo</i> <sup>RNAi</sup> /+	3.957 (0.132)	2.409 (0.093)	1.065 (0.061)	159	<0.0001 (****), <0.0001 (****), 0.8835 (ns)
5F,G,H	<i>w;rab3</i> <sup>rup</sup>	11.21 (0.541)	7.623 (0.443)	0.798 (0.037)	53	<0.0001 (****), <0.0001 (****), 0.0382 (*)

Figure 6	Genotype	BRP puncta #/NMJ	BRP puncta intensity (% wild type)	BRP total intensity/NMJ (% wild type)	vGlut total intensity/NMJ (% wild type)	EPSP	n (stain,EPSP)	P value (significance) (BRP puncta #, puncta intensity, BRP total, vGlut total, EPSP)
6C	<i>w</i> <sup>1118</sup>	266.35 (10.14)	100 (2.75)	100 (3.92)	100 (6.5)	29.26 (1.23)	17,12	
6C	<i>w;arl8</i> <sup>e00336</sup>	80.12 (4.99)	108.53 (3.00)	32.99 (1.79)	49.31 (3.06)	17.86 (0.85)	17,12	<0.0001 (****), 0.0442 (ns), <0.0001 (****), <0.0001 (****), <0.0001 (****)
6C	<i>w;ok6-Gal4</i> , <i>UAS-arl8-GFP</i>	294.29 (18.2)	128.01 (3.25)	118.47 (5.44)	137.9 (6.26)	41.09 (0.67)	14,15	0.1711 (ns), <0.0001 (****), 0.0088 (**), 0.0004 (**), <0.0001 (****)
6F	<i>w;ok6-Gal4</i> , <i>UAS-arl8-GFP</i> , <i>endo</i> <sup>RNAi</sup>	395.47 (12.08)	70.27 (3.76)	98.88 (4.51)	90.88 (3.11)	31.72 (1.16)	15, 9	
6F	<i>w;ok6-Gal4</i> , <i>UAS-arl8-GFP</i> , <i>endo</i> <sup>RNAi</sup>	287.14 (12.58)	90.75 (4.01)	118.37 (6.18)	110.34 (6.09)	37.86 (1.31)	14, 7	<0.0001 (****), 0.0009 (**), 0.0124 (*), 0.0159 (*), 0.0102 (*)

6I	<i>w;ok6-Gal4; UAS-rab3<sup>RNAi</sup></i>	165.11 (7.97)	149.45 (10.78)	92.44 (6.62)	96.14 (4.83)	30.09 (0.94)	17, 10	
6I	<i>w;rab3<sup>rup</sup>;arl8<sub>e00336</sub></i>	42.1 (2.54)	93.6 (4.78)	15.81 (0.96)	51.36 (3.6)	13 (0.54)	10, 9	<0.0001 (***) , 0.2215 (ns), <0.0001 (***) , <0.0001 (***) , <0.0001 (***)
6I	<i>w;ok6-Gal4; UAS-arl8-GFP; UAS-rab3<sup>RNAi</sup></i>	258.05 (15.71)	179.64 (12.6)	110.71 (4.89)	115.57 (4.4)	38.44 (1.01)	14, 9	0.0002 (***) , 0.1089 (ns), 0.0316 (*) , 0.0087 (**), 0.012 (*)

Figure 7	Genotype	PhTx	mEPSP (mV)	EPSP (mV)	QC	mEPSP (Hz)	Resting Potential (mV)	Input Resistance (MΩ)	n	P value (significance) (mEPSP amp, EPSP, QC, mEPSP freq)
7B,C	<i>w<sup>1118</sup></i>	-	0.979 (0.028)	32.576 (1.417)	33.785 (2.027)	2.849 (0.126)	-63.958 (1.006)	6.995 (0.414)	10	
7B,C	<i>w<sup>1118</sup></i>	+	0.52 (0.028)	29.141 (1.412)	57.578 (4.686)	2.285 (0.154)	-66.762 (1.696)	7.109 (0.391)	10	<0.0001 (***) , 0.1192 (ns), <0.0001 (***) , 0.009 (**)
7B,C	<i>w;endo<sup>1</sup>/endo<sup>44</sup></i>	-	1.292 (0.048)	28.301 (1.522)	21.976 (1.165)	2.575 (0.255)	-61.808 (1.078)	12.259 (0.529)	6	
7B,C	<i>w;endo<sup>1</sup>/endo<sup>44</sup></i>	+	0.679 (0.025)	26.183 (1.273)	39.097 (2.923)	1.877 (0.208)	-58.117 (1.65)	13.117 (1.206)	7	<0.0001 (***) , 0.3048 (ns), <0.0001 (***) , 0.0559 (ns)
7B,C	<i>w;rab3<sup>rup</sup>, UAS-vGlut/rab3<sup>rup</sup>, ok6-Gal4</i>	-	1.285 (0.065)	29.672 (1.08)	23.745 (1.565)	2.634 (0.142)	-64.833 (2.139)	7.185 (0.329)	11	
7B,C	<i>w;rab3<sup>rup</sup>, UAS-vGlut/rab3<sup>rup</sup>, ok6-Gal4</i>	+	0.685 (0.044)	28.49 (1.092)	42.677 (3.191)	1.212 (0.161)	-69.561 (2.971)	8.099 (0.619)	9	<0.0001 (***) , 0.4349(ns), <0.0001 (***), <0.0001 (***) ,

Figure 7	Genotype	PhTx	BRP-GFP puncta sum intensity (% baseline)	vGlut puncta sum intensity (% baseline)	n	P value (significance) (paired t-test :BRP GFP, vGlut-GFP, unpaired t-test: :BRP GFP, vGlut-GFP)
7F,G,H	<i>w<sup>1118</sup></i>	-	100 (2.429)	100 (3.988)	135	
7F,G,H	<i>w<sup>1118</sup></i>	+	150.8 (4.481)	116.4 (4.348)	72	<0.0001 (***) , <0.0001 (***) , <0.0001 (***) , 0.006 (**)

Figure 7	Genotype	PhTx	BRP puncta sum intensity (% baseline)	vGlut puncta sum intensity (% baseline)	BRP total intensity/NMJ (% baseline)	vGlut total intensity/NMJ (% baseline)	n	P value (significance) (BRP puncta, vGlut puncta, total BRP, total vGlut)
7H,I,J	<i>w<sup>1118</sup></i>	-	100 (8.807)	100 (7.041)	100 (9.971)	100 (11.004)	15	
7H,I,J	<i>w<sup>1118</sup></i>	+	170.04 (12.665)	127.9 (6.441)	172.418 (15.89)	127.901 (10.481)	11	.0007 (**), 0.003 (**), 0.0008 (**), 0.0098 (**),
7H,I,J	<i>w;endo<sup>1</sup>/endo<sup>44</sup></i>	-	100 (10.891)	100 (9.954)	100 (10.098)	100 (8.914)	6	
7H,I,J	<i>w;endo<sup>1</sup>/endo<sup>44</sup></i>	+	154.61 (13.64)	130.15 (9.441)	171.7 (18.609)	125.85 (10.871)	14	0.0003 (**), 0.0099 (**), 0.0007 (**), 0.0031 (**)
7H,I,J	<i>w;rab3<sup>rup</sup>, UAS-vGlut/rab3<sup>rup</sup>, ok6-Gal4</i>	-	100 (7.452)	100 (6.338)	100 (9.679)	100 (9.551)	12	

7H,I,J	<i>w;rab3<sup>up</sup>, UAS-vGlut/rab3<sup>up</sup>, ok6-Gal4</i>	+	165.19 (13.991)	117.76 (4.581)	159.76 (15.619)	115.71 (4.711)	14	0.0002 (**), 0.0108 (*), 0.0008 (**), 0.07 (*)
--------	---	---	--------------------	-------------------	--------------------	-------------------	----	--

Figure 8	Genotype	PhTx	mEPSP (mV)	EPSP (mV)	QC	mEPSP (Hz)	Resting Potential (mV)	Input Resistance (MΩ)	n	P value (significance) (mEPSP amp, EPSP, QC, mEPSP freq)
8E,F	<i>w<sup>1118</sup></i>	-	0.924 (0.03)	32.791 (1.339)	35.748 (1.799)	2.73 (0.168)	-66.412 (1.494)	6.915 (0.414)	9	
8E,F	<i>w<sup>1118</sup></i>	+	0.569 (0.015)	31.407 (1.583)	55.374 (3.065)	2.144 (0.166)	-69.488 (2.514)	7.110 (0.492)	7	<0.0001 (**), 0.5978 (ns), <0.0001 (**), 0.0289 (*)
8E,F	<i>w;gluRIIA<sup>sp16</sup></i>	-	0.486 (0.027)	27.663 (1.374)	57.964 (4.103)	2.063 (0.201)	-69.292 (2.098)	8.02 (0.55)	7	<0.0001 (**), 0.0674 (ns), <0.0001 (**), 0.0253 (*)
8E,F	<i>w;arl8<sup>e00336</sup></i>	-	1.043 (0.046)	18.295 (0.744)	17.871 (0.909)	1.135 (0.015)	-51.974 (1.526)	16.019 (0.805)	18	
8E,F	<i>w;arl8<sup>e00336</sup></i>	+	0.551 (0.02)	17.64 (0.814)	31.36 (1.288)	0.940 (0.013)	-50.998 (1.414)	16.109 (0.764)	13	<0.0001 (**), 0.9955 (ns), <0.0001 (**), 0.7804 (ns)
8E,F	<i>w;gluRIIA<sup>sp16</sup>, arl8<sup>e00336</sup></i>	-	0.41 (0.027)	7.14 (0.857)	17.779 (2.438)	0.8263 (0.071)	-69.292 (2.098)	18.02 (0.55)	7	0.0029(**), <0.0001(**), 0.0587(ns), 0.0345(*)

Figure 8	Genotype	PhTx	BRP puncta sum intensity (% baseline)	vGlut puncta sum intensity (% baseline)	BRP total intensity/NMJ (% baseline)	vGlut total intensity/NMJ (% baseline)	n	P value (significance) (BRP puncta, vGlut puncta, total BRP, total vGlut)
8B,C	<i>w<sup>1118</sup></i>	-	100 (8.807)	100 (7.041)	100 (9.971)	100 (11.004)	15	
8B,C	<i>w<sup>1118</sup></i>	+	170.04 (12.665)	127.9 (6.441)	172.418 (15.89)	127.901 (10.481)	11	<0.0001 (**), 0.0052 (**), 0.0038 (**), 0.0075 (**),
8B,C	<i>w;gluRIIA<sup>sp16</sup></i>	-	152.5 (11.79)	125.2 (8.401)	124.4 (6.48)	124.2 (6.324)	12	0.0011 (**), 0.0064 (**), 0.0089 (**), 0.0071 (**) (compared to w <sup>1118</sup> )
8B,C	<i>w;arl8<sup>e00336</sup></i>	-	100 (7.801)	100 (6.904)	100 (5.091)	100 (7.884)	14	
8B,C	<i>w;arl8<sup>e00336</sup></i>	+	93.05 (5.941)	102.6 (2.944)	90.27 (8.577)	87.74 (7.011)	18	0.4182 (ns), 0.6783 (ns), 0.3863 (ns), 0.1937 (ns)
8B,C	<i>w;gluRIIA<sup>sp16</sup>, arl8<sup>e00336</sup></i>	-	80.79 (6.782)	85.18 (9.007)	84.43 (4.746)	66.67 (7.05)	7	0.0536 (ns), 0.1870 (ns), 0.0816 (ns), 0.0028 (**) (compared to arl8)

Figure S1	Genotype	Synaptic vesicle diameter (nm)	Synaptic vesicle density (#/nm <sup>2</sup> )	n	P value (significance) (diameter, density)
S1B,C	<i>w<sup>1118</sup></i>	31.42 (0.902)	0.0764 (0.004)	65	
S1B,C	<i>w;endo<sup>1</sup>/endo<sup>44</sup></i>	44.97 (2.731)	0.0118 (0.0005)	70	<0.0001 (**), <0.0001 (**)
S1B,C	<i>w;UAS-vGlut/ok371-Gal4</i>	51.62 (2.672)	0.0702 (0.0022)	81	<0.0001 (**), 0.4107 (ns)

Figure S1	Genotype	vGlut puncta mean intensity (%WT)	Synapsin puncta mean intensity (%WT)	Syntaptotagmin puncta mean intensity (%WT)	n	P value (significance) (vGlut, Syn, Syt)
S1F,G,H	<i>w<sup>1118</sup></i>	100 (1.846)	100 (3.661)	100 (4.019)	13	
S1F,G,H	<i>w;endo<sup>1</sup>/endo<sup>A4</sup></i>	107.60 (5.063)	92.572 (7.422)	104.989 (4.422)	7	0.7959 (ns), 0.7023 (ns), 0.8916 (ns)
S1F,G,H	<i>w;ok6-Gal4;UAS-endo<sup>RNAi</sup></i>	102.195 (4.497)	99.048 (3.688)	97.455 (5.465)	13	0.9889 (ns), 0.9984 (ns), 0.9722 (ns)
S1F,G,H	<i>w;UAS-vGlut/ok371-Gal4</i>	167.307 (6.372)	105.554 (3.986)	107.743 (2.961)	13	<0.0001 (***) , 0.7687 (ns), 0.5545 (ns)

Figure S2	Genotype	total quanta released	n	P value (significance)
S2D	<i>w<sup>1118</sup></i>	424868.594 (33989.487)	8	
S2D	<i>w;UAS-vGlut/ok371-Gal4</i>	277238.453 (33268.614)	7	0.0091 (**)

Figure S3	Genotype	GluRIII puncta density (#/ $\mu\text{m}^2$ )	GluRIII puncta #/NMJ	GluRIII puncta area ( $\mu\text{m}^2$ )	BRP-GluRIII apposition	n	P value (significance) (density, , #, area, apposition)
S3B,C,D,E	<i>w<sup>1118</sup></i>	0.515 (0.024)	157 (7.329)	1.287 (0.077)	96.109 (2.451)	22	
S3B,C,D,E	<i>w;endo<sup>1</sup>/endo<sup>A4</sup></i>	0.581 (0.027)	200 (12.35)	0.776 (0.027)	95.415 (3.191)	17	0.9004 (ns), <0.0001 (***) , 0.004 (**), 0.9871 (ns)
S3B,C,D,E	<i>w;ok6-Gal4;UAS-endo<sup>RNAi</sup></i>	0.509 (0.032)	211 (11.181)	0.765 (0.043)	94.198 (3.709)	15	0.6989 (ns), <0.0001 (***) , 0.003 (**), 0.8711 (ns)
S3B,C,D,E	<i>c155-Gal4;endo<sup>1</sup></i>	0.498 (0.031)	139 (9.526)	1.153 (0.066)	93.819 (2.718)	12	0.8199 (ns), 0.6166 (ns), 0.7755 (ns), 0.7919 (ns)
S3B,C,D,E	<i>w;UAS-vGlut/ok371-Gal4</i>	0.481 (0.039)	138 (7.98)	1.21 (0.061)	97.719 (2.551)	16	0.5109 (ns), 0.5908 (ns), 0.9071 (ns), 0.8999 (ns)

Figure S4	Genotype	AZ length (nm)	T-bar width (nm)	n (AZ length, T-bar width)	P value (significance) (AZ length, T-bar width)
S4B,C	<i>w<sup>1118</sup></i>	719 (40.63)	159.4 (15.52)	(79, 27)	
S4B,C	<i>w;endo<sup>1</sup>/endo<sup>A4</sup></i>	569.4 (29.35)	109 (7.108)	(64, 33)	0.0088 (**), <0.0001 (***) , 0.9765 (ns)
S4B,C	<i>w;UAS-vGlut/ok371-Gal4</i>	811.9 (44.95)	143.9 (7.382)	(54, 24)	0.1405 (ns), 0.1646 (ns), 0.5617 (ns)

Figure S5	Genotype	PhTx	mEPSP (mV)	EPSP (mV)	QC	mEPSP (Hz)	Resting Potential (mV)	Input Resistance (MΩ)	n	P value (significance) (mEPSP amp, EPSP, QC, mEPSP freq)
S5B,C	<i>w<sup>1118</sup></i>	-	0.924 (0.03)	32.791 (1.339)	35.748 (1.799)	2.73 (0.168)	-66.412 (1.494)	6.915 (0.414)	9	
S5B,C	<i>w<sup>1118</sup></i>	+	0.569 (0.015)	31.407 (1.583)	55.374 (3.065)	2.144 (0.166)	-69.488 (2.514)	7.110 (0.492)	7	<0.0001 (****), 0.5131 (ns), <0.0001 (****), 0.0289 (*)
S5B,C	<i>w;UAS-vGlut/ok371-Gal4</i>	-	1.273 (0.063)	30.504 (1.035)	24.275 (1.121)	2.135 (0.22)	-61.974 (1.526)	8.219 (0.795)	9	
S5B,C	<i>w;UAS-vGlut/ok371-Gal4</i>	+	0.602 (0.017)	32.742 (2.208)	54.231 (2.688)	2.408 (0.223)	-62.956 (1.624)	9.109 (0.774)	8	<0.0001 (****), 0.3312 (ns), <0.0001 (****), 0.7804 (ns)
S5B,C	<i>w;rab3<sup>rup</sup></i>	-	1.004 (0.058)	33.296 (0.995)	33.809 (1.619)	2.669 (0.175)	-66.754 (1.746)	7.195 (0.524)	10	
S5B,C	<i>w;rab3<sup>rup</sup></i>	+	0.486 (0.027)	27.663 (1.374)	57.964 (4.103)	2.263 (0.201)	-69.292 (2.098)	8.02 (0.55)	8	0.0029(**), 0.0022(**), 0.0587(ns), 0.9722 (ns)

Figure S5	Genotype	PhTx	BRP puncta sum intensity (% baseline)	vGlut puncta sum intensity (% baseline)	BRP total intensity/NMJ (% baseline)	vGlut total intensity/NMJ (% baseline)	n	P value (significance) (BRP puncta, vGlut puncta, total BRP, total vGlut)
S5E, F	<i>w<sup>1118</sup></i>	-	100 (8.807)	100 (7.041)	100 (9.971)	100 (11.004)	15	
S5E, F	<i>w<sup>1118</sup></i>	+	170.04 (12.665)	127.9 (6.441)	172.418 (15.89)	127.901 (10.481)	11	0.0007 (**), 0.003 (**), 0.0008 (**), 0.0098 (**),
S5E, F	<i>w;UAS-vGlut/ok371-Gal4</i>	-	100 (7.807)	100 (8.041)	100 (8.991)	100 (9.092)	14	
S5E, F	<i>w;UAS-vGlut/ok371-Gal4</i>	+	167.61 (15.271)	115.21 (7.556)	178.55 (17.071)	117.87 (9.951)	16	0.0002 (**), 0.0301 (*), 0.0005 (**), 0.0207 (*)
S5E, F	<i>w;rab3<sup>rup</sup></i>	-	100 (5.817)	100 (4.949)	100 (7.903)	100 (9.334)	12	
S5E, F	<i>w;rab3<sup>rup</sup></i>	+	165.66 (15.991)	120.766 (4.001)	180.62 (13.654)	121.72 (5.887)	15	0.0004 (**), 0.0091 (**), <0.0001 (****), 0.002 (**)