

Table S1: Absolute and additional values for normalized and presented data.

Figure	Label	Genotype	Picδ1-PH-GFP Intensity (% wild type)	n	P Value (significance)
3A,B	Control	<i>w¹¹¹⁸; OK6-Gal4/+; UAS-PLCδ-PH-GFP/+</i>	100 (5.121)	11	
3A,B	<i>Esy</i> t	<i>w¹¹¹⁸; OK6-Gal4/+; Esyt¹/Esy^{t2}; UAS-PLCδ-PH-GFP</i>	119.211 (8.986)	11	0.0780 (ns)
3A,B	<i>Esy</i> t-OE	<i>w¹¹¹⁸; OK6-Gal4/+; UAS-PLCδ1-PH-GFP/UAS-Esy^{tFlag};</i>	137.201 (17.707)	12	0.0658 (ns)
Figure	Label	Genotype	GFP-myc-2xFYVE Intensity (% wild type)	n	P Value (significance)
3C,D	Control	<i>w¹¹¹⁸; OK6-Gal4/UAS-GFP-myc-2xFYVE</i>	100 (3.618)	10	
3C,D	<i>Esy</i> t	<i>w¹¹¹⁸; OK6-Gal4/UAS-GFP-myc-2xFYVE; Esyt¹/Esy^{t2}</i>	100.167 (5.491)	11	0.9804 (ns)
3C,D	<i>Esy</i> t-OE	<i>w¹¹¹⁸; OK6-Gal4/UAS-GFP-myc-2xFYVE; UAS-Esy^{tFlag}/+</i>	109.861 (4.552)	13	0.1209 (ns)

Figure	Label	Genotype	Bouton Number	Neuronal Membrane Surface Area (μm ²)	BRP #/NMJ	BRP Density (μm ²)	BRP Area (μm ²)	n	P Value (significance: Bouton #, Membrane Surface Area, BRP #/NMJ, BRP Density, BRP Area)
4A-F	wild type	<i>w¹¹¹⁸</i>	31.750 (2.972)	280.067 (25.205)	337.500 (26.839)	1.234 (0.054)	59.578 (4.766)	12	
4A-F	<i>Esy</i> t	<i>w¹¹¹⁸; Esyt¹/Esy^{t2}</i>	32.818 (2.423)	313.736 (24.891)	338.091 (20.662)	1.137 (0.094)	63.103 (4.353)	11	0.7856 (ns), 0.3539 (ns), 0.9864 (ns), 0.3764 (ns), 0.7695 (ns)
4A-F	<i>Esy</i> t-OE	<i>w¹¹¹⁸; OK6-Gal4/UAS-Esy^{tFlag}</i>	46.000 (2.799)	445.615 (32.373)	486.167 (26.754)	1.110 (0.044)	80.273 (6.931)	12	0.0021 (**), 0.0006 (***), 0.0007 (***), 0.0912 (ns), 0.3622 (ns)
Not shown	NA	<i>w¹¹¹⁸; OK6-Gal4/UAS-Esy^{tMCh}</i>	49.700 (3.114)	473.291 (37.492)	512.800 (38.086)	1.102 (0.051)	86.131 (6.687)	10	0.0005 (***), 0.0003 (***), 0.0010 (***), 0.0956 (ns), 0.5455 (ns)

Figure	Label	Genotype	[Ca ²⁺]	mEPSC amplitude (pA)	mEPSC frequency (Hz)	EPSC (nA)	QC	Input Resistance (MΩ)	Leak Current (nA)	n	P Value (significance: mEPSC amp, mEPSC freq, EPSC, QC)
5A-D	wild type	<i>w¹¹¹⁸</i>	0.4	-521.184 (34.236)	2.740 (0.314)	-48.786 (13.471)	96.867 (28.767)	9.375 (0.375)	-3.033 (0.493)	7	
5A-D	<i>Esy</i> t	<i>w¹¹¹⁸; Esyt¹/Esy^{t2}</i>	0.4	-539.886 (19.112)	2.877 (0.228)	-21.445 (5.505)	40.805 (10.995)	7.455 (0.638)	-2.646 (0.711)	9	0.6120 (ns), 0.7274 (ns), 0.0458 (*), 0.0489 (*)
5A-D	<i>Esy</i> t rescue	<i>w¹¹¹⁸; OK6-Gal4/UAS-Esy^{tFlag}; Esyt¹/Esy^{t2}</i>	0.4	-436.698 (9.558)	2.667 (0.553)	-41.852 (5.251)	96.388 (12.850)	8.286 (0.787)	-4.482 (2.851)	9	0.0105 (*), 0.9053 (ns), 0.5761 (ns), 0.9862 (ns)
5A-D	<i>Esy</i> t-OE	<i>w¹¹¹⁸; OK6-Gal4/UAS-Esy^{tFlag}</i>	0.4	-453.664 (16.148)	2.019 (0.180)	-45.744 (7.691)	93.523 (18.061)	8.667 (0.575)	-1.760 (0.309)	15	0.0518 (ns), 0.0450 (*), 0.8458 (ns), 0.9233 (ns)

Figure	Label	Genotype	[Ca ²⁺]	mEPSC amplitude (pA)	EPSC (nA)	QC	Input Resistance (MΩ)	Leak Current (nA)	n	P Value (significance: QC)
5D	wild type	<i>w¹¹¹⁸</i>	1.5	-450.666 (24.021)	-121.851 (13.781)	275.466 (33.501)	10.286 (1.728)	-3.471 (1.284)	7	
5D	<i>Esyt</i>	<i>w¹¹¹⁸; Esyt¹/Esys²</i>	1.5	-412.004 (9.141)	-74.990 (8.278)	187.452 (25.777)	11.375 (1.165)	-1.865 (0.997)	14	0.0212 (*)
5D	Esyt-OE	<i>w¹¹¹⁸; OK6-Gal4/UAS-Esyt^{Flag}</i>	1.5	-424.308 (12.000)	-122.506 (7.006)	288.902 (15.404)	8.500 (1.000)	-4.233 (0.952)	8	0.7095 (ns)
5D	wild type	<i>w¹¹¹⁸</i>	3.0	-468.450 (16.126)	-235.727 (12.185)	507.770 (29.561)	11.200 (1.526)	-3.682 (0.332)	10	
5D	<i>Esyt</i>	<i>w¹¹¹⁸; Esyt¹/Esys²</i>	3.0	-543.625 (21.252)	-175.172 (18.352)	323.801 (34.036)	7.375 (0.680)	-4.522 (0.577)	8	0.0008 (***)
5D	Esyt rescue	<i>w¹¹¹⁸; OK6-Gal4/UAS-Esyt^{Flag}; Esyt¹/Esys²</i>	3.0	-432.083 (9.491)	-232.206 (20.847)	537.128 (46.699)	7.625 (0.532)	-3.046 (0.282)	8	0.5882 (ns)
5D	Esyt-OE	<i>w¹¹¹⁸; OK6-Gal4/UAS-Esyt^{Flag}</i>	3.0	-463.322 (27.241)	-243.366 (16.005)	541.470 (57.371)	7.625 (0.800)	-3.612 (0.502)	7	0.5780 (ns)

Figure	Label	Genotype	[Ca ²⁺]	EPSC 1 (nA)	EPSC 2 (nA)	EPSC 3 (nA)	EPSC 4 (nA)	PPR (EPSC4/EPSC1)	n	P Value (significance: PPR)
5E,F	wild type	<i>w¹¹¹⁸</i>	0.4	-48.786 (13.471)	-58.458 (15.204)	-59.546 (15.006)	-64.168 (13.139)	1.494 (0.192)	8	
5E,F	<i>Esyt</i>	<i>w¹¹¹⁸; Esyt¹/Esys²</i>	0.4	-21.445 (5.505)	-25.338 (4.967)	-33.088 (4.871)	-39.937 (4.998)	2.746 (0.495)	9	0.0405 (*)
5E,F	<i>Esyt</i> + neuronal rescue	<i>w¹¹¹⁸; OK6-Gal4/UAS-Esyt^{Flag}; Esyt¹/Esys²</i>	0.4	-41.852 (5.251)	-50.389 (5.596)	-53.090 (6.214)	-56.064 (6.297)	1.358 (0.050)	9	0.3932 (ns)
5E,F	Esyt-OE	<i>w¹¹¹⁸; OK6-Gal4/UAS-Esyt^{Flag}</i>	0.4	-45.744 (7.692)	-53.576 (7.062)	-59.997 (6.989)	-67.920 (6.600)	1.915 (0.216)	15	0.3013 (ns)
5G,H	wild type	<i>w¹¹¹⁸</i>	3.0	-235.728 (12.186)	-176.158 (14.671)	-158.895 (13.545)	-145.829 (12.728)	0.613 (0.032)	10	
5G,H	<i>Esyt</i>	<i>w¹¹¹⁸; Esyt¹/Esys²</i>	3.0	-175.173 (18.352)	-156.200 (14.530)	-157.531 (12.401)	-142.820 (10.817)	0.859 (0.076)	8	0.0053 (**)
5G,H	<i>Esyt</i> + neuronal rescue	<i>w¹¹¹⁸; OK6-Gal4/UAS-Esyt^{Flag}; Esyt¹/Esys²</i>	3.0	-232.206 (20.848)	-161.540 (14.482)	-150.038 (13.173)	-131.543 (11.281)	0.569 (0.013)	8	0.2539 (ns)
5G,H	Esyt-OE	<i>w¹¹¹⁸; OK6-Gal4/UAS-Esyt^{Flag}</i>	3.0	-243.367 (16.005)	-196.454 (11.530)	-184.588 (12.270)	-169.124 (12.732)	0.691 (0.028)	7	0.1013 (ns)

Figure	Label	Genotype	Total quanta released	n	P Value (significance)
6A,B	wild type	<i>w¹¹¹⁸</i>	170746.2 (13327.07)	7	
6A,B	<i>Esyt</i>	<i>w¹¹¹⁸; Esyt¹/Esys²</i>	185279.4 (20449.02)	6	0.5526 (ns)
6A,B	Esyt-OE	<i>w¹¹¹⁸; OK6-GAL4/UAS-Esyt^{Flag}</i>	286885.4 (7323.839)	7	<0.0001 (***)

Figure	Label	Genotype	Synaptic Vesicle Density (#/μm ²)	Synaptic Vesicle Number within 300nm of AZ	T Bar Length (nm)	Active Zone Length (nm)	n (SV Density, SV#, T bar length, AZ length)	P Value (significance: SV Density, SV # within 300nm of AZ, T Bar Length, AZ Length)
7A-E	wild type	<i>w¹¹¹⁸</i>	120.095 (18.270)	267.100 (13.934)	178.061 (17.930)	618.601 (48.895)	11,18,14,21	
7A-E	<i>Esyt</i>	<i>w¹¹¹⁸; Esyt¹/Esys²</i>	105.311 (17.145)	231.480 (12.717)	166.827 (12.947)	597.936 (30.003)	8,14,9,24	0.5769 (ns), 0.0762 (ns), 0.6548 (ns), 0.7131 (ns)

7A-E	Esy-OE	$w^{1118}; OK6-Gal4/UAS-Esyt^{Flag}$	94.309 (11.161)	238.215 (8.299)	183.306 (16.969)	507.527 (35.967)	10,30,14,31	0.2545 (ns), 0.0641 (ns), 0.8334 (ns), 0.0673 (ns)
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Figure	Label	Genotype	FM1-43 Intensity (% wild type)	n	P Value (significance)
7F,G	wild type	w^{1118}	100 (5.584)	10	
7F,G	<i>Esyt</i>	$w^{1118}; Esyt^1/Esyt^2$	102.545 (4.869)	11	0.7338 (ns)
7F,G	Esy-OE	$w^{1118}; OK6-Gal4/UAS-Esyt^{Flag}$	84.487 (4.990)	8	0.0606 (ns)

Figure	Label	Genotype	[Ca ²⁺]	PhTx	mEPSC (pA)	EPSC (nA)	QC	Leak Current (nA)	n	P Value (significance: mEPSC amp, EPSC, QC)
8A,B	wild type	w^{1118}	0.4	-	-521.184 (34.236)	-48.786 (13.471)	96.867 (28.767)	-3.033 (0.493)	7	
8A,B	wild type	w^{1118}	0.4	+	-247.505 (11.750)	-33.785 (1.855)	142.951 (11.613)	-6.737 (0.497)	17	
8A,B	<i>Esyt</i>	$w^{1118}; Esyt^1/Esyt^2$	0.4	-	-539.886 (19.112)	-21.445 (5.505)	40.805 (10.995)	-2.646 (0.711)	9	0.6120 (ns), 0.0458 (*), 0.0489 (*)
8A,B	<i>Esyt</i>	$w^{1118}; Esyt^1/Esyt^2$	0.4	+	-294.331 (13.337)	-21.815 (3.173)	74.209 (10.868)	-2.716 (0.485)	11	
8A,B	Esy-OE	$w^{1118}; OK6-Gal4/UAS-Esyt^{Flag}$	0.4	-	-453.664 (16.148)	-45.744 (7.691)	93.523 (18.061)	-1.760 (0.309)	15	0.0597 (ns), 0.8458 (ns), 0.9233 (ns)
8A,B	Esy-OE	$w^{1118}; OK6-Gal4/UAS-Esyt^{Flag}$	0.4	+	-240.749 (17.108)	-36.660 (3.297)	160.813 (17.770)	-1.719 (0.291)	12	

The figure and panel, genotype, and conditions used are noted (external calcium concentration as well as whether PhTx was applied or not). Average values with standard error values noted in parentheses are shown for all morphology data such as neuronal membrane surface area, bouton number, BRP number, BRP density, BRP area as well as intensity values. For electrophysiological experiments, all passive membrane properties (input resistance, leak current) and mEPSC, EPSC, QC, number of data samples (n), p-values from statistical test are shown, with standard error values noted in parentheses.