

Supplementary Table 1.

For statistical comparisons for diameters of vesicles and pits, *p*-values were calculated using Student's *t*-test. For number of vesicles and pits, we used Mann-Whitney U tests because of the skewed distribution of the data. The skewness was determined using Pearson's skewness test. The confidence level was set at 0.05 in each case. For Fig. 1f, 2c, 2d, and 3i, we applied the Bonferroni correction to reduce the chance of obtaining false positive in multiple comparisons on a single dataset. Therefore, the confidence level is 0.006 in those cases. The gray shade in the P-value columns suggest the difference observed is statistically significant.

Number of vesicles and pits in Channelrhodopsin transfected cells

	No stimulation		No Chr2 100 ms		
N (synaptic profiles)	193		174		
	Mean	SEM	Mean	SEM	P value
Exocytic pits (Fig. 1f)	0.01	0.01	0.03	0.01	0.12
Docked vesicles (Fig. 2c)	1.7	0.1	1.7	0.1	0.58
Tethered vesicles (Fig. 2d)	2.2	0.1	2	0.1	0.08
Shallow endocytic pits (Fig. 3i)	0.02	0.01	0.02	0.01	0.88
Deep endocytic pits (Fig. 3i)	0.01	0.01	0.02	0.01	0.34
Large vesicles (Fig. 3i)	0.06	0.01	0.05	0.01	0.68

	No stimulation		15 ms		
N (synaptic profiles)	193		193		
	Mean	SEM	Mean	SEM	P value
Exocytic pits (Fig. 1f)	0.01	0.01	0.19	0.03	<.0001
Docked vesicles (Fig. 2c)	1.7	0.1	1	0.1	<.0001
Tethered vesicles (Fig. 2d)	2.2	0.1	2.1	0.1	0.26
Shallow endocytic pits (Fig. 3i)	0.02	0.01	0.01	0.01	0.18
Deep endocytic pits (Fig. 3i)	0.01	0.01	0	0.01	0.57
Large vesicles (Fig. 3i)	0.06	0.01	0.07	0.01	0.68

	No stimulation		30 ms		
N (synaptic profiles)	193		189		
	Mean	SEM	Mean	SEM	P value

Exocytic pits (Fig.1f)	0.01	0.01	0.13	0.02	<.0001
Docked vesicles (Fig. 2c)	1.7	0.1	1	0.1	<.0001
Tethered vesicles (Fig. 2d)	2.2	0.1	2.2	0.1	0.67
Shallow endocytic pits (Fig. 3i)	0.02	0.01	0.01	0.01	0.43
Deep endocytic pits (Fig. 3i)	0.01	0.01	0.01	0.01	0.63
Large vesicles (Fig. 3i)	0.06	0.01	0.07	0.01	0.78

	No stimulation		50 ms		
N (synaptic profiles)	193		191		
	Mean	SEM	Mean	SEM	P value
Exocytic pits (Fig.1f)	0.01	0.01	0.05	0.02	0.03
Docked vesicles (Fig. 2c)	1.7	0.1	0.9	0.1	<.0001
Tethered vesicles (Fig. 2d)	2.2	0.1	2.4	0.1	0.49
Shallow endocytic pits (Fig. 3i)	0.02	0.01	0.13	0.01	<.0001
Deep endocytic pits (Fig. 3i)	0.01	0.01	0.04	0.01	0.05
Large vesicles (Fig. 3i)	0.06	0.01	0.1	0.01	0.18

	No stimulation		100 ms		
N (synaptic profiles)	193		191		
	Mean	SEM	Mean	SEM	P value
Exocytic pits (Fig.1f)	0.01	0.01	0.02	0.01	0.4
Docked vesicles (Fig. 2c)	1.7	0.1	1	0.1	<.0001
Tethered vesicles (Fig. 2d)	2.2	0.1	2.2	0.1	0.92
Shallow endocytic pits (Fig. 3i)	0.02	0.01	0.07	0.02	0.01
Deep endocytic pits (Fig. 3i)	0.01	0.01	0.14	0.02	<.0001
Large vesicles (Fig. 3i)	0.06	0.01	0.22	0.02	<.0001

	No stimulation		300 ms		
N (synaptic profiles)	193		188		
	Mean	SEM	Mean	SEM	P value
Exocytic pits (Fig.1f)	0.01	0.01	0.04	0.02	0.14
Docked vesicles (Fig. 2c)	1.7	0.1	1	0.1	<.0001
Tethered vesicles (Fig. 2d)	2.2	0.1	2.6	0.1	0.01
Shallow endocytic pits (Fig. 3i)	0.02	0.01	0.02	0.02	0.74

3i)					
Deep endocytic pits (Fig. 3i)	0.01	0.01	0.04	0.01	0.05
Large vesicles (Fig. 3i)	0.06	0.01	0.16	0.01	0.002

	No stimulation		1 s		
N (synaptic profiles)	193		179		
	Mean	SEM	Mean	SEM	P value
Exocytic pits (Fig. 1f)	0.01	0.01	0.07	0.02	0.002
Docked vesicles (Fig. 2c)	1.7	0.1	1.2	0.1	<.0001
Tethered vesicles (Fig. 2d)	2.2	0.1	2	0.1	0.18
Shallow endocytic pits (Fig. 3i)	0.02	0.01	0.07	0.02	0.02
Deep endocytic pits (Fig. 3i)	0.01	0.01	0.11	0.02	<.0001
Large vesicles (Fig. 3i)	0.06	0.01	0.13	0.01	0.03

	No stimulation		3 s		
N (synaptic profiles)	193		189		
	Mean	SEM	Mean	SEM	P value
Exocytic pits (Fig. 1f)	0.01	0.01	0.02	0.01	0.4
Docked vesicles (Fig. 2c)	1.7	0.1	1.3	0.1	0.003
Tethered vesicles (Fig. 2d)	2.2	0.1	1.8	0.1	0.003
Shallow endocytic pits (Fig. 3i)	0.02	0.01	0.02	0.01	0.73
Deep endocytic pits (Fig. 3i)	0.01	0.01	0.04	0.01	0.09
Large vesicles (Fig. 3i)	0.06	0.01	0.08	0.01	0.5

	No stimulation		10 s		
N (synaptic profiles)	193		178		
	Mean	SEM	Mean	SEM	P value
Exocytic pits (Fig. 1f)	0.01	0.01	0.04	0.02	0.07
Docked vesicles (Fig. 2c)	1.7	0.1	1.6	0.1	0.37
Tethered vesicles (Fig. 2d)	2.2	0.1	1.8	0.1	0.007
Shallow endocytic pits (Fig. 3i)	0.02	0.01	0.03	0.01	0.44
Deep endocytic pits (Fig. 3i)	0.01	0.01	0.01	0.01	0.94
Large vesicles (Fig. 3i)	0.06	0.01	0.06	0.01	0.99

Number of vesicles and pits in Munc13 DKO

	Munc13 DKO No stimulation		Munc13 DKO 30 ms		
N (synaptic profiles)	151		139		
	Mean	SEM	Mean	SEM	P value
Exocytic pits	0	0	0	0	N/A
Docked vesicles	0.1	0.03	0.1	0.03	0.68
Tethered vesicles	2.2	0.2	3	0.2	<.0001
Shallow endocytic pits (Fig. 3j)	0.01	0.01	0.01	0.01	0.95
Deep endocytic pits (Fig. 3j)	0	0	0	0	N/A
Large vesicles (Fig. 3j)	0.04	0.003	0.06	0.005	0.48

	Munc13 DKO No stimulation		Munc13 DKO 100 ms		
N (synaptic profiles)	151		140		
	Mean	SEM	Mean	SEM	P value
Exocytic pits	0	0	0	0	
Docked vesicles	0.1	0.03	0.1	0.02	0.22
Tethered vesicles	2.2	0.2	2.5	0.2	0.22
Shallow endocytic pits (Fig. 3j)	0.01	0.01	0	0	N/A
Deep endocytic pits (Fig. 3j)	0	0	0	0	N/A
Large vesicles (Fig. 3j)	0.04	0.003	0.06	0.005	0.35

Number of endocytic structures in tomograms

Fig. 4c	50 ms		100 ms		
N (tomograms)	8		35		
	Mean	SEM	Mean	SEM	P value
Shallow endocytic pits	0.41	0.13	0.17	0.06	
Deep endocytic pits	0.06	0.06	0.26	0.08	
LV <50 nm of memb	0.06	0.06	0.17	0.06	

Number of pits in Latrunculin-treated cells

Fig. 5e	No drug		0.1% DMSO		
N (synaptic profiles)	193		178		
	Mean	SEM	Mean	SEM	P value

Exocytic pits	0.02	0.01	0.06	0.02	0.12
Endocytic pits	0.21	0.03	0.17	0.03	0.41

Fig. 5e	No drug		10 μ M Latrunculin-a		
N (synaptic profiles)	193		185		
	Mean	SEM	Mean	SEM	P value
Exocytic pits	0.02	0.01	0.02	0.01	0.97
Endocytic pits	0.21	0.03	0	0	<.0001

Number of endocytic structures in dynasore-treated cells

Fig. 5f	control 0 ms		80 μ M Dynasore 0 ms		
N (synaptic profiles)	193		53		
	Mean	SEM	Mean	SEM	P value
Shallow endocytic pits	0.02	0.01	0.02	0.01	0.94
Deep endocytic pits	0.01	0.01	0.02	0.01	0.62
LV <5 nm of memb	0.02	0.01	0.04	0.01	0.48
LV <6-50 nm of memb	0.04	0.01	0.04	0.01	0.91

Fig. 5f	control 100 ms		80 μ M Dynasore 100 ms		
N (synaptic profiles)	191		50		
	Mean	SEM	Mean	SEM	P value
Shallow endocytic pits	0.07	0.02	0.06	0.03	0.75
Deep endocytic pits	0.14	0.02	0.16	0.04	0.67
LV <5 nm of memb	0.09	0.02	0.16	0.04	<.0001
LV <6-50 nm of memb	0.13	0.01	0.04	0.01	0.08

Fig. 5f	control 1 s		80 μ M Dynasore 1 s		
N (synaptic profiles)	179		223		
	Mean	SEM	Mean	SEM	P value
Shallow endocytic pits	0.07	0.02	0.08	0.02	0.94

Deep endocytic pits	0.11	0.02	0.08	0.02	0.38
LV <5 nm of memb	0.06	0.02	0.22	0.03	<.0001
LV <6-50 nm of memb	0.06	0.02	0.03	0.01	0.09

Number of ferritin-positive endocytic structures

Supplementary Fig. 5f	No ferritin 100 ms		ferritin 100 ms		
N (synaptic profiles)	191		73		
	Mean	SEM	Mean	SEM	P value
Shallow endocytic pits	0.07	0.02	0.07	0.02	
Deep endocytic pits	0.14	0.02	0.15	0.02	
LV <5 nm of memb	0.09	0.02	0.1	0.02	
LV <6-50 nm of memb	0.13	0.01	0.07	0.02	

Supplementary Fig. 5f	Ferritin no stim		ferritin 100 ms		
N (synaptic profiles)	100		73		
	Mean	SEM	Mean	SEM	P value
Shallow endocytic pits	0.01	0.01	0.07	0.02	
Deep endocytic pits	0	0	0.15	0.02	
LV <5 nm of memb	0	0	0.1	0.02	
LV <6-50 nm of memb	0.01	0.01	0.07	0.02	

Number of exocytic or endocytic pits in 2 mM calcium

2 mM calcium	No stimulation		30 ms		100 ms	
N (synaptic profiles)	193		174		174	
	Mean	SEM	Mean	SEM	Mean	SEM
Exocytic pits	0.02	0.01	0.08	0.02	0	0
Endocytic pits	0.03	0.01	0.01	0.01	0.12	0.03

Diameter of vesicles and pits

Fig. 1g	Synaptic vesicles	Exocytic pits	P value
Mean	41.1	40.9	0.71
SEM	0.09	1.16	

Fig. 3h	Large vesicles	Endocytic pits	P value
Mean	82.03	86.02	0.33
SEM	1.2	2.4	

Fig. 4d	Large vesicles	Endocytic pits	P value
Mean	78	76.3	0.56
SEM	3.3	1.9	

Supplementary Fig. 5g	No ferritin 100 ms		
	N	Mean	SEM
Endocytic pits diameter	40	76.7	1.9
LV diameter	572	82	1.2
	ferritin 100 ms		
	N	Mean	SEM
Endocytic pits diameter	16	77.3	3
LV diameter	51	86	2.4