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Supplemental information

The M domain in UNC-13 regulates the probability of neurotransmitter release

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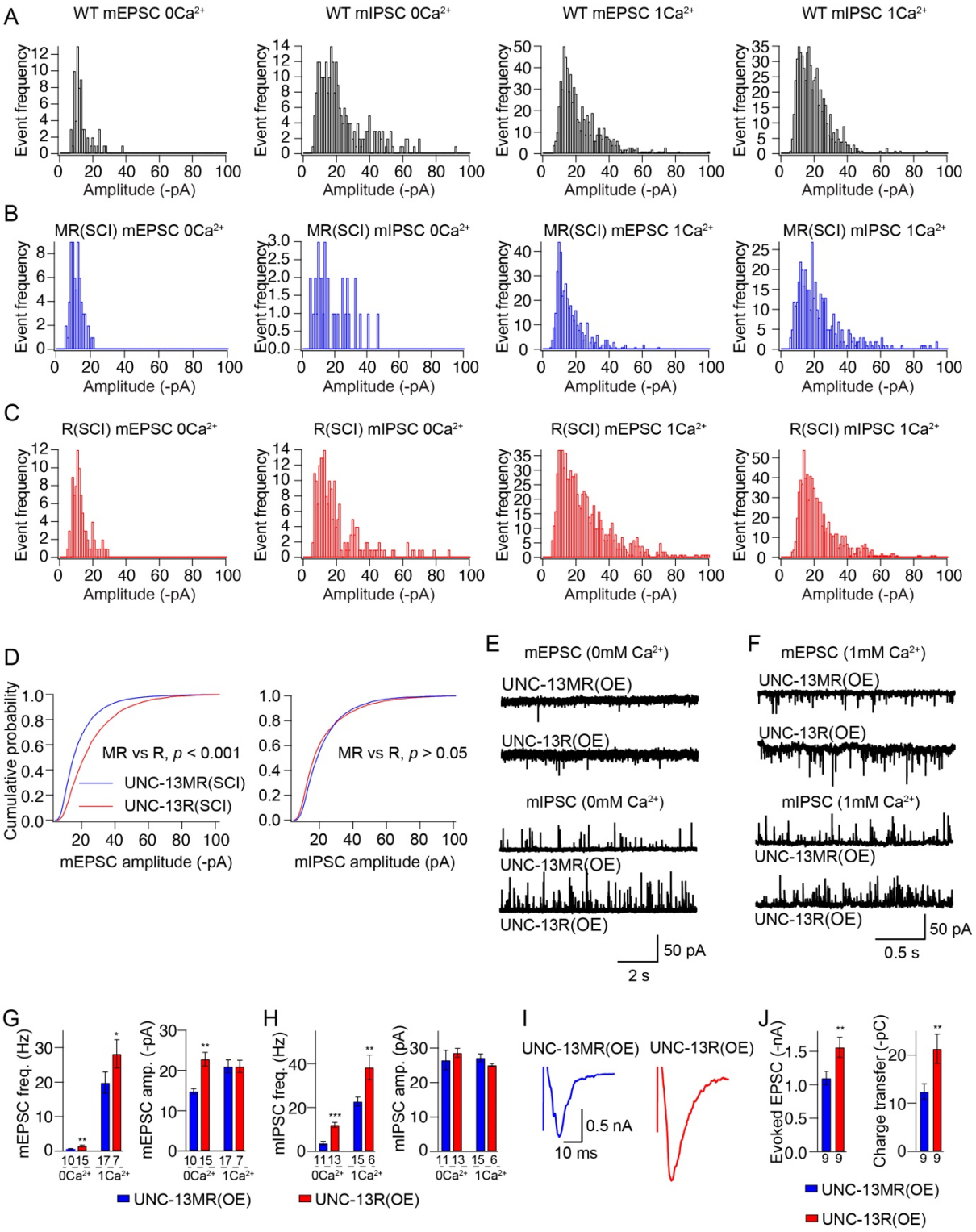

Figure S1. Amplitude distributions of spontaneous release, and M domain inhibition in overexpression rescue worms. Related to Figure 1.

(A-C) Representative peak amplitude distribution of mEPSCs and mIPSCs in 0mM and 1mM Ca^{2+} from Wild type (A), UNC-13MR(SCI) rescued animals (B), and UNC-13R(SCI) rescued animals (C). (D) Cumulative probability distributions of mEPSC and mIPSC amplitudes (in 1mM Ca^{2+}) for UNC-13MR(SCI) and UNC-13R(SCI) rescued animals (Kolmogorov–Smirnov test). (E, F) Representative mEPSC and mIPSC traces (recorded in 0mM and 1mM Ca^{2+}) from UNC-13MR and UNC-13R overexpression rescue (OE) animals. (G, H) Quantification of the frequency and amplitude of the mEPSCs and mIPSCs from the same genotypes as in E and F. (I) Example traces of stimulus-evoked EPSCs recorded from UNC-13MR and UNC-13R overexpression

rescue animals. (J) Quantification of the evoked EPSC amplitude and charge transfer. Data are mean ± SEM (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ when compared to UNC-13MR rescue animals; student's t-test). The number of worms analyzed for each genotype is indicated under the bar graphs.

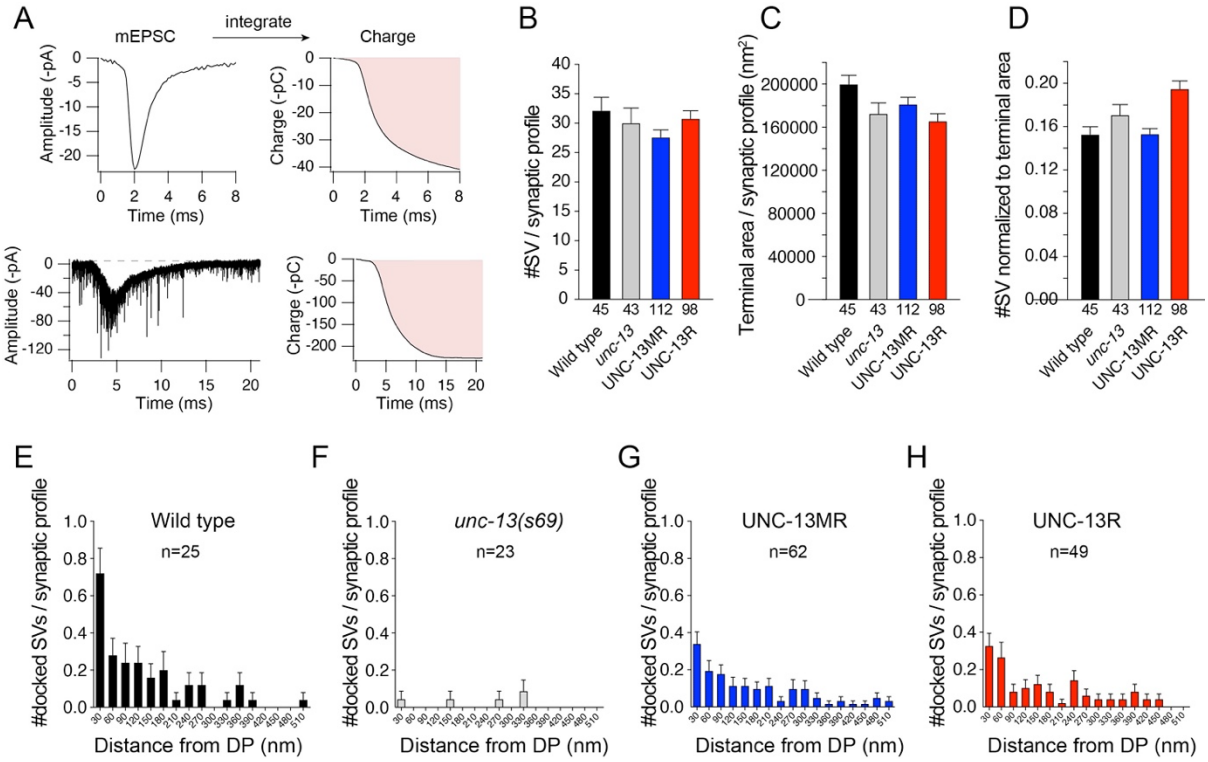

Figure S2. The M domain is not required for SV docking. Related to Figure 2.
(A) Example trace of an averaged mEPSC (upper) and sucrose current from wild-type animal. Total charge transfer included the mEPSC superimposed on the sucrose current were calculated by integrating the current over time (the pink area). (B-D) Quantification of number of synaptic vesicles (SVs) per synaptic profile, presynaptic terminal area per synaptic profile, and number of SVs normalized to the presynaptic terminal area. (E-H) Distribution of docked SVs plotted as distance from the dense projection. Data are mean ± SEM.

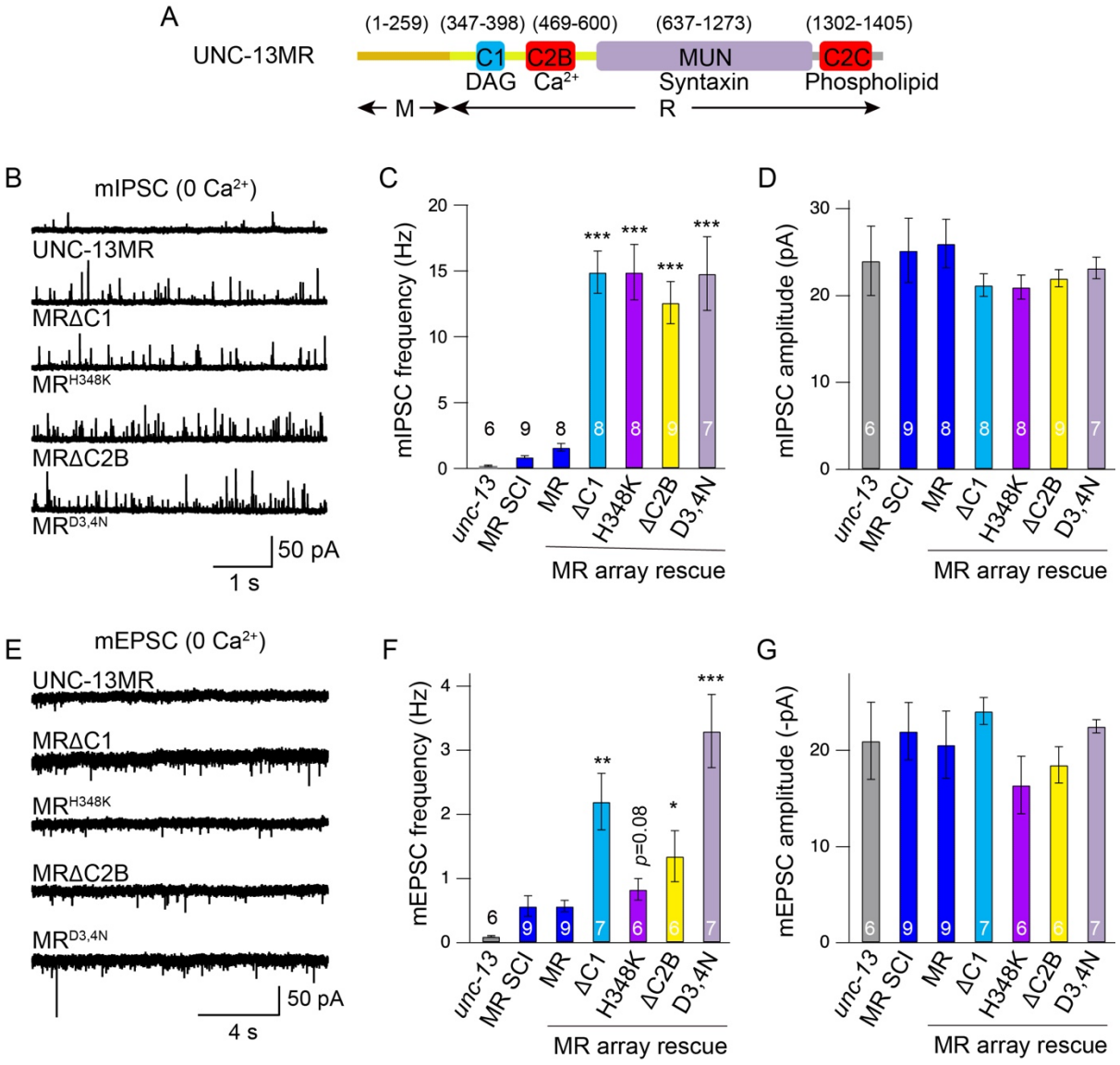

Figure S3. The HK and DN mutations in the C1 and C2B domains in UNC-13MR cause a dramatic increase in spontaneous release. Related to Figure 3.

(A) Cartoon depicting the HK and DN mutations in UNC-13MR. (B) Representative mIPSC traces (recorded in 0mM Ca^{2+}) from the indicated genotypes. (C, D) Quantification of the mIPSC frequency and amplitude from the same genotypes as in B. (E) Representative mEPSC traces (recorded in 0mM Ca^{2+}) from the indicated genotypes. (F, G) Quantification of the mEPSC frequency and amplitude from the same genotypes as in E. Data are mean ± SEM (*, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$ when compared to UNC-13MR rescue; one-way ANOVA test for data in D, G, one-way ANOVA following Kruskal-Wallis test for data in C, F). The number of worms analyzed for each genotype is indicated in the bar graphs.

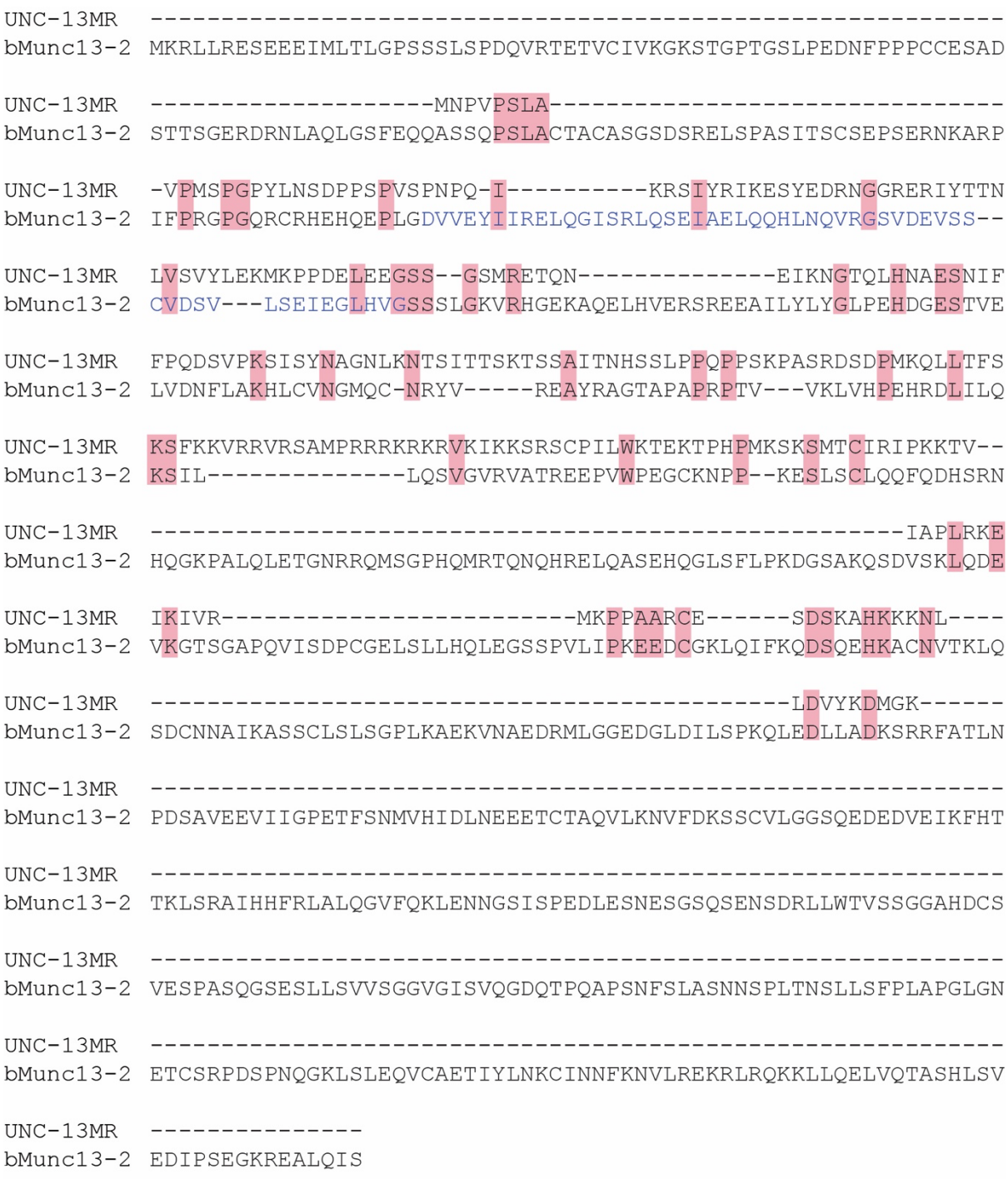

Figure S4. Sequence alignment between the UNC-13 M domain and the bMunc13-2 N terminus. Related to Figure 7.
The small stretch region in bMunc13-2 is indicated by blue.

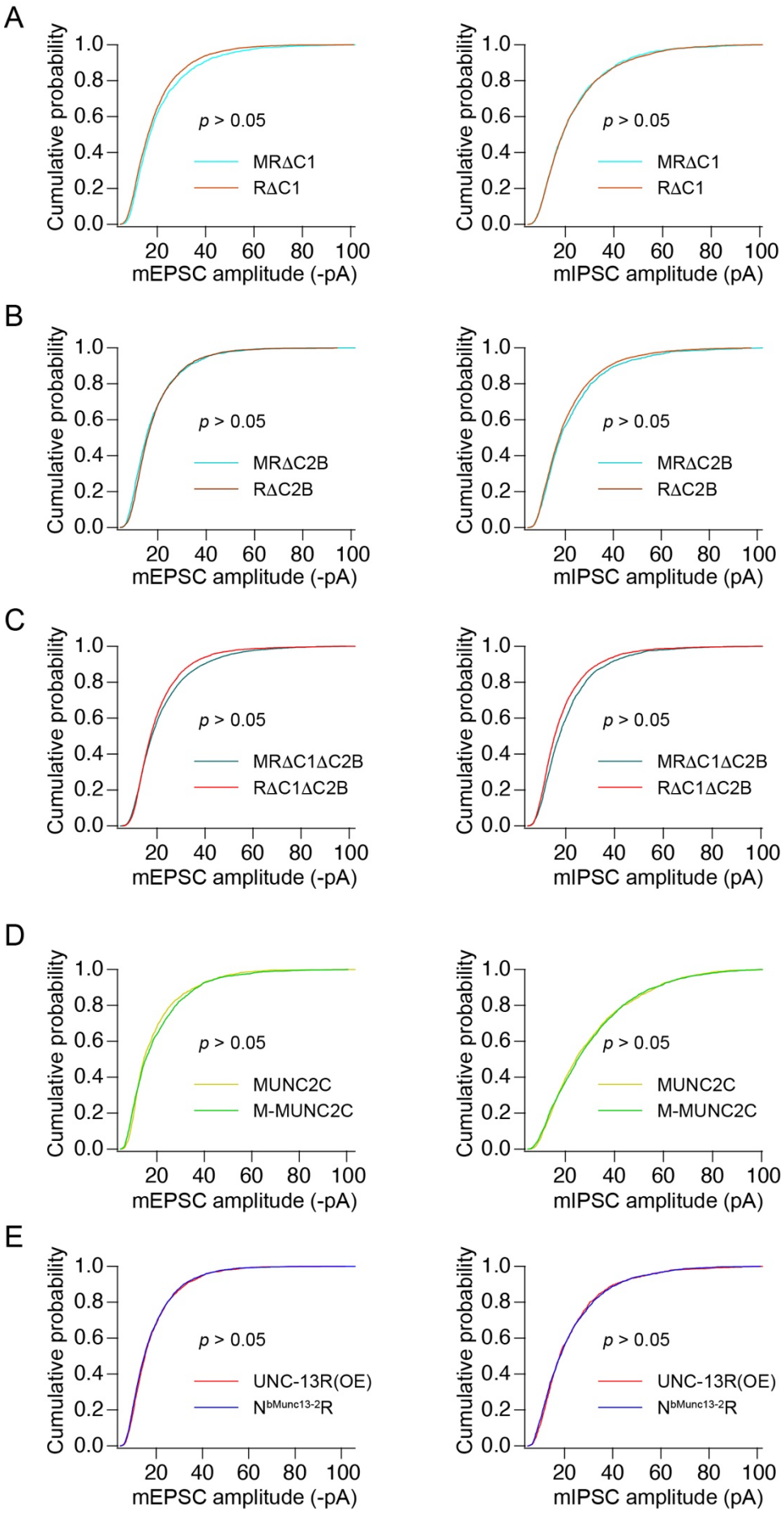
A
Cumulative probability
1.0
0.8
0.6
0.4
0.2
0.0
20 40 60 80 100
mEPSC amplitude (-pA)
p > 0.05
MRΔC1
RΔC1
mIPSC amplitude (pA)
B
MRΔC2B
RΔC2B
C
MRΔC1ΔC2B
RΔC1ΔC2B
D
MUNC2C
M-MUNC2C
E
UNC-13R(OE)
NbMunc13-2R

Figure S5. Amplitude distributions of spontaneous release. Related to Figures 3, 5, and 7.
(A-E) Cumulative probability distributions of mEPSC and mIPSC amplitudes (in 1mM Ca^{2+}) for indicated genotypes (Kolmogorov–Smirnov test).

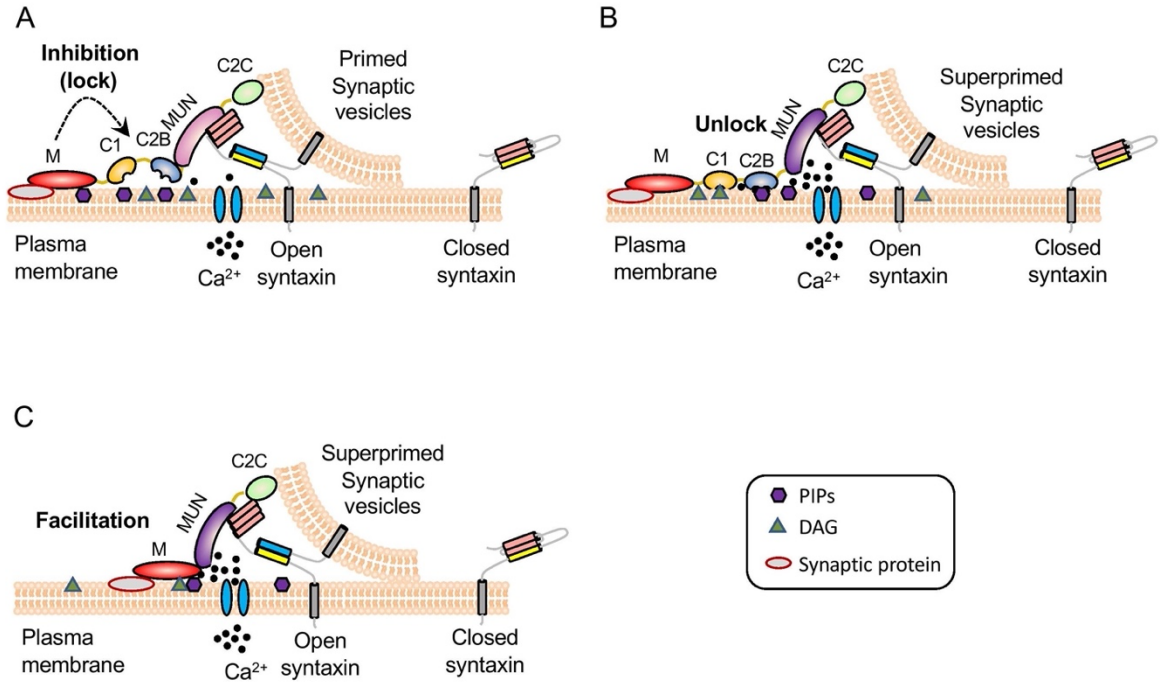

Figure S6. Models for the M domain function in UNC-13. Related to Figures 1-7. (A) The M domain locks the C1 and C2B domains in an autoinhibitory conformation under low Ca^{2+} conditions, limiting UNC-13MR triggered membrane fusion. The red oval represents potential synaptic protein that binds to the M domain. (B) Ca^{2+} increase activates the C1 and C2B domains and unlocks from the M domain. The release of the C1 and C2B domains in turn bind to the plasma membrane and superprime synaptic vesicles. (C) The M domain potentially interacts with the plasma membrane or other unknown membrane proteins establishing an UNC-13 bridge with the synaptic vesicle. This stabilizes the MUN domain and enhances SV fusion.

Table S1. Summary of all tonic release

0mM Ca^{2+}

	mEPSC Frequency (Hz)	mEPSC Amplitude (-pA)	N	mIPSC Frequency (Hz)	mIPSC Amplitude (pA)	N
Wild type	1.9±0.6 (2.2)	27.3±1.7 (26.8)	18	11.0±0.9 (10.8)	25.9±0.9 (25.8)	20
unc-13(s69)						
UNC-13MR (SCI)	0.57±0.1 (0.4)	15.2±0.6 (15.3)	9	0.9±0.1 (0.9)	27.7±2.5 (30.6)	9
UNC-13R (SCI)	1.0±0.2 (0.9)	20.5±1.8 (19.7)	6	14.4±1.2 (15.3)	24.2±1.3 (23.8)	6
UNC-13MR	0.62±0.1 (0.5)	14.8±0.6 (14.3)	10	3.7±0.8 (3.3)	26.5±2.8 (26.1)	11
UNC-13R	1.49±0.2 (1.4)	22.8±1.6 (20.4)	15	12.1±1.1 (11.6)	28.6±1.2 (28.9)	13
MRΔC1	2.2±0.4 (2.1)	24.1±1.3 (22.9)	7	14.9±1.5 (15.1)	21.2±1.2 (20.7)	8
RΔC1	3.7±0.5 (3.5)	20.7±1.1 (18.7)	9	20.7±1.6 (20.2)	25.6±0.5 (26.1)	7
MRΔC2B	1.1±0.3 (1.0)	18.5±1.9 (18.1)	8	11.6±1.6 (9.9)	22.3±1 (21.6)	8
RΔC2B	2.1±0.4 (2.0)	23.2±1.1 (22.2)	8	17.4±1.6 (16.7)	24.4±1 (24.2)	8
MRΔC1ΔC2B	6.6±1.1 (5.9)	23.9±1.4 (22.3)	10	18.8±3.9 (16.8)	24.8±1.4 (25.8)	8
RΔC1ΔC2B	4.1±1.2 (3.5)	23.6±2.2 (22.9)	6	12.2±1.1 (13.1)	21.4±0.7 (20.6)	7
MR^{HKDN}	1.4±0.2 (1.7)	18.8±1.1 (17.9)	6	13.2±1.65(11.5)	22.6±0.4 (22.2)	6
R^{HKDN}	1.8±0.8 (1.2)	18.4±0.7 (18.4)	5	14±1.6 (13.2)	23.9±0.9 (24.0)	5
MUNC2C (SCI)						
M-MUNC2C (SCI)						
MUNC2C	1.9±0.3 (1.4)	19.8±1.3 (17.5)	11	4.5±0.5 (4.4)	25.2±1.1 (23.1)	13
M-MUNC2C	1.6±0.4 (1.2)	23.4±1.6 (22.3)	7	4.2±0.4 (3.9)	27.8±1.9 (29.7)	7
$N^{bMunc13-2}$ R	0.9±0.1 (0.7)	23.8±2.3 (23.1)	12	9.9±1.4 (8.6)	25.9±1.4 (26.2)	12
$N^{bMunc13-2}$-MUNC2C	1.4±0.3 (1.4)	23.4±1.9 (22.7)	7	4.5±1.2 (3.9)	24.1±1.3 (19.8)	8

0.25mM Ca^{2+}

	mEPSC Frequency (Hz)	mEPSC Amplitude (-pA)	N	mIPSC Frequency (Hz)	mIPSC Amplitude (pA)	N
Wild type	42±4.2 (40.6)	23±1.6 (22.4)	10	49.7±6.2 (44.2)	26.2±1.1 (25.7)	12
unc-13(s69)						
UNC-13MR (SCI)	6.4±0.8 (7.1)	18.8±0.6 (18.9)	13	5.4±1.4 (2.6)	24.7±1.7 (22.6)	13
UNC-13R (SCI)	15.6±2.8 (16.5)	20.1±0.8 (19.3)	6	18.4±1.4 (18.8)	24±1 (23.6)	7
UNC-13MR						
UNC-13R						
MRΔC1						
RΔC1						
MRΔC2B						
RΔC2B						
MRΔC1ΔC2B	35.5±4.8 (34.6)	24.5±2.1 (23.9)	6	23.7±1.6 (23.2)	24.3±1.1 (24.1)	6
RΔC1ΔC2B	36.6±5.2 (33.7)	20.6±2.2 (20.1)	6	22.9±2.8 (21.7)	24.3±1.5 (23.8)	5
MR^{HKDN}						
R^{HKDN}						
MUNC2C (SCI)						
M-MUNC2C (SCI)						
MUNC2C						
M-MUNC2C						
$N^{bMunc13-2}$ R						
$N^{bMunc13-2}$-MUNC2C						

0.5mM Ca^{2+}

	mEPSC Frequency (Hz)	mEPSC Amplitude (-pA)	N	mIPSC Frequency (Hz)	mIPSC Amplitude (pA)	N
Wild type	56.2±7.3 (58.4)	21.9±1.3 (21.5)	10	43.5±4.3 (41.3)	22.5±1.3 (22.0)	9
unc-13(s69)						
UNC-13MR (SCI)	10.6±1.7 (10.3)	20.3±1.5 (19.2)	9	12±2.7 (8.8)	28.1±1.9 (27.8)	10
UNC-13R (SCI)	28.9±5.2 (24.5)	24.2±1.3 (22.9)	5	29.2±5.6 (28.4)	23.4±1.1 (23.0)	7
UNC-13MR						
UNC-13R						
MRΔC1						
RΔC1						
MRΔC2B						
RΔC2B						
MRΔC1ΔC2B	40.2±6.2 (37.3)	20.8±1.7 (20.2)	7	35.6±2.7 (35.1)	25.3±0.8 (25.1)	8
RΔC1ΔC2B	44.7±7.6 (46.2)	21.3±1.6 (19.9)	7	31.7±4.0 (32.4)	22.8±0.6 (22.6)	8
MR^{HKDN}						
R^{HKDN}						
MUNC2C (SCI)						
M-MUNC2C (SCI)						
MUNC2C						
M-MUNC2C						
$N^{bMunc13-2}$ R						
$N^{bMunc13-2}$-MUNC2C						

1mM Ca^{2+}

	mEPSC Frequency (Hz)	mEPSC Amplitude (-pA)	N	mIPSC Frequency (Hz)	mIPSC Amplitude (pA)	N
Wild type	50.9±5.3 (48.3)	22.2±1.1 (21.0)	17	48±4.7 (47.3)	22.4±1.0 (21.5)	19
unc-13(s69)	0.5±0.09 (0.4)	16.8±1.75 (17.3)	14	0.1±0.05 (0.1)	26±2.51 (25.8)	5
UNC-13MR (SCI)	21.3±2.3 (22.0)	19.3±0.9 (23.0)	17	19.5±2.6 (17.7)	26.8±0.8 (26.5)	15
UNC-13R (SCI)	33.6±4.4 (26.1)	25.7±1.4 (27.1)	15	47±3.8 (47.3)	25.1±1.1 (24.2)	15
UNC-13MR	19.8±3.1 (18.5)	21±1.6 (20.2)	17	22.7±2.0 (21.8)	27.2±1.1 (26.9)	15
UNC-13R	28.2±4.1 (27.9)	21±1.5 (18.0)	7	38.3±5.6 (39.5)	25.1±0.4 (24.7)	6
MRΔC1	23.4±4.6 (23.0)	23.1±1.1 (22.4)	9	20.7±4.2 (19.7)	25.3±1.8 (23.8)	7
RΔC1	30.4±3.6 (29.9)	21.9±0.6 (21.4)	14	45.4±4.9 (47.6)	27.4±2.3 (28.7)	9
MRΔC2B	23.2±3.7 (22.5)	21.4±0.8 (22.7)	8	16.1±1.8 (15.7)	25.2±1.8 (26.4)	7
RΔC2B	24.7±2.8 (25.5)	20.9±0.9 (20.2)	7	44.3±5.1 (42.9)	23.9±1.6 (24.5)	7
MRΔC1ΔC2B	49±3.7 (48.6)	24.3±1.2 (23.9)	11	32±3.2 (31.5)	24.7±1.1 (25.1)	8
RΔC1ΔC2B	41.1±3.6 (40.8)	22.1±1.1 (22.0)	14	31.7±2.1 (30.6)	21.6±0.8 (21.3)	8
MR^{HKDN}	29.6±3.3 (27.9)	24.2±3.5 (23.2)	11	25.1±3.9 (16.9)	24.1±2.9 (24.9)	7
R^{HKDN}	29±3.6 (19.4)	22.9±2.7 (20.9)	7	32.3±4.5 (33.9)	27.6±4.5 (27.7)	7
MUNC2C (SCI)	0.2±0.06 (0.18)	21.5±3.3 (20.6)	8	0.13±0.05 (0.13)	26.2±2.3 (25.8)	8
M-MUNC2C (SCI)	2.6±0.7 (2.5)	22.6±2.2 (22.0)	6	1.7±0.4 (1.4)	25.5±3.1 (23.4)	6
MUNC2C	16.6±2.5 (18.2)	21.2±1.4 (19.8)	16	37.5±5.4 (35.5)	30.4±1.6 (31.4)	10
M-MUNC2C	22.3±4.3 (20.7)	22.7±1.2 (23.5)	10	30.9±2.8 (30.6)	33.5±2.0 (33.7)	8
$N^{bMunc13-2}$ R	20.5±2.3 (21.4)	20.5±0.8 (20.4)	12	24.4±4.4 (21.9)	25.7±1.5 (25.0)	8
$N^{bMunc13-2}$-MUNC2C	22.8±3.1 (21.6)	22±1.5 (24.2)	16	36.5±3.9 (37.3)	26.8±2.3 (23.9)	10

SCI, single copy insertion; If not indicated, all other strains represent overexpression rescue; N, number of worms analyzed; The median value is shown in the bracket following each number.

Table S1. Summary of all tonic release in this study. Related to Figures 1, 3, 4, 5, and 7.

Table S2. Summary of evoked EPSCs and sucrose charge

	Evoked EPSCs									Sucrose	
	0.25mM Ca^{2+}			0.5mM Ca^{2+}			1mM Ca^{2+}				
	Amplitude (-nA)	Charge (-pC)	N	Amplitude (-nA)	Charge (-pC)	N	Amplitude (-nA)	Charge (-pC)	N	Charge (-pC)	N
Wild type	0.28±0.029 (0.24)	2.75±0.61 (1.9)	6	0.97±0.14 (1.1)	9.8±1.21 (9.4)	7	2.01±0.1 (2.1)	17.2±1.19 (18)	14	221±14.6 (210)	20
unc-13(s69)							0.026±0.007 (0.025)	0.15±0.008 (0.014)	5	25.5±7.06 (24.2)	7
UNC-13MR (SCI)	0.067±0.013 (0.05)	0.18±0.06 (0.1)	10	0.25±0.045 (0.23)	1.98±0.41 (1.95)	8	1.26±0.1 (1.1)	14.2±1.4 (13.3)	15	216±20.2 (208)	17
UNC-13R (SCI)	0.18±0.032 (0.15)	1.54±0.3 (1.68)	9	0.79±0.15 (0.6)	9.75±2.6 (5.7)	9	1.52±0.1 (1.45)	20±1 (20.6)	14	323±32.2 (287)	11
UNC-13MR							1.1±0.1 (1.13)	12.4±1.62 (12.5)	9		
UNC-13R							1.56±0.15 (1.65)	21.3±3.0 (18.9)	9		
MRΔC1							2±0.2 (1.66)	21.4±2.86 (18.6)	8		
RΔC1							1.88±0.14 (1.89)	21.2±2.88 (20.6)	10		
MRΔC2B							1.97±0.12 (1.9)	27.4±2.63 (26.2)	7		
RΔC2B							2.45±0.17 (2.45)	32.5±3.63 (28.2)	15		
MRΔC1ΔC2B	0.55±0.14 (0.45)	7.1±2.1 (6.1)	6	1.76±0.26 (1.48)	27.4±4.95 (24.6)	6	3.29±0.16 (3.1)	62±6.09 (57.6)	13		
RΔC1ΔC2B	0.58±0.25 (0.31)	5.4±2.1 (3.3)	6	1.84±0.19 (1.69)	24.8±4.76 (18.3)	8	2.87±0.17 (2.9)	65±8.14 (59.9)	10		
$MR^{HK,DN}$							2.37±0.19 (2.51)	40.2±6.22 (37)	13		
$R^{HK,DN}$							2.71±0.25 (2.76)	48.2±6.57 (49.3)	14		
MUNC2C (SCI)							0.018±0.0068 (0.017)	0.22±0.15 (0.19)	6		
M-MUNC2C (SCI)							0.1±0.022 (0.09)	0.83±0.25 (0.9)	6		
MUNC2C	0.074±0.016 (0.06)	0.33±0.14 (0.14)	9	0.26±0.068 (0.17)	1.83±0.47 (1.8)	9	0.56±0.12 (0.55)	4.4±1.19 (3.7)	15	150±17.8 (142)	15
M-MUNC2C	0.1±0.016 (0.09)	0.74±0.2 (0.63)	12	0.65±0.1 (0.6)	5.8±1.1 (4.3)	9	1.5±0.11 (1.53)	13.2±1.18 (13.8)	8	153±13.1 (150)	9
$N^{bMunc13\text{-}2}R$							0.84±0.13 (0.82)	10.7±2.21 (9.8)	8		
$N^{bMunc13\text{-}2}$-MUNC2C							1.15±0.17 (1.1)	9.0±1.58 (10.0)	13		

SCI, single copy insertion; If not indicated, all other strains represent overexpression rescue; N, number of worms analyzed

Table S2. Summary of all evoked EPSCs and sucrose charge. Related to Figures 1, 2, 3, 4, 5, and 7.