SUPPLEMENTARY MATERIALS for Xu et al., "Synaptotagmin-1 functions as Ca^{2+}-sensor for spontaneous release"

Supplementary Table 1

List of all electrophysiological results organized according the figures and supplementary figures of the paper. All data are from at least three independent neuronal cultures; if not specifically mentioned, statistical assessments were done by Student's t-test in Origin; Two-way ANOVA is done by Graphpad Prism

Figure	Genotype	Parameter	Mean Value	SEM	P value	Cell #
Fig.1 b	WT+2mM Ca	mIPSC Frequency (Hz)	1.782	0.184	P<0.001, compared with WT+0mM Ca; P<0.01, compared with WT+0mM Ca+Thapsigargin; P<0.001, compared with others;	13
	WT+0mM Ca	mIPSC Frequency (Hz)	0.499	0.052	P<0.001, compared with all others;	11
	WT+0mM Ca+BAPTA-AM	mIPSC Frequency (Hz)	0.08	0.024	P=0.8139, compared with WT+0mM Ca+Thapsigargin+BAPTA-AM	9
	WT+0mM Ca+Thapsigargin	mIPSC Frequency (Hz)	3.395	0.475	P<0.001, compared with all others;	13
	WT+0mM Ca+Thapsigargin+BAPTA-AM	mIPSC Frequency (Hz)	0.071	0.029	See above	12
Fig.1 d	WT	mIPSC Frequency (Hz)	1.926	0.173	P=0.8057 compared with ST1 KO+ST1 rescue	38
	ST1 KO	mIPSC Frequency (Hz)	15.257	1.114	P<0.001 compared with the others	35
	ST1 KO + ST1 Rescue	mIPSC Frequency (Hz)	2.005	0.297	see above	19
	ST1 KO+BAPTA-AM	mIPSC Frequency (Hz)	0.087	0.042	P<0.001 compared with the others	24
Fig.1 f	WT	RRP, Charge transfer (nF)	10.723	0.776	P=0.9043 compared with WT+BAPTA-AM; P=0.8637 compared with ST1 KO; P=0.2732 compared with ST1 KO+BAPTA-AM	20
	WT+BAPTA-AM	RRP, Charge transfer (nF)	10.564	1.093	P=0.9249 compared with ST1 KO; P=0.3933 compared with ST1 KO+BAPTA-AM	12
	ST1 KO	RRP, Charge transfer (nF)	10.414	1.395	P=0.5546 compared with ST1 KO+BAPTA-AM	14
	ST1 KO+BAPTA-AM	RRP, Charge transfer (nF)	9.34	0.796	see above	10
Fig.2 b	WT + 0.01mM Ca	mIPSC Frequency (Hz)	0.069	0.023	Two-way ANOVA, P<0.001, compared with ST1 KO, for mIPSC Frquency	10
	WT + 0.2mM Ca	mIPSC Frequency (Hz)	0.111	0.024		10

	WT + 0.5mM Ca	mIPSC Frequency (Hz)	0.433	0.072		10
	WT + 1mM Ca	mIPSC Frequency (Hz)	1.354	0.172		10
	WT + 2mM Ca	mIPSC Frequency (Hz)	3.288	0.365		10
	WT + 5mM Ca	mIPSC Frequency (Hz)	6.019	0.552		10
	WT + 10mM Ca	mIPSC Frequency (Hz)	6.896	0.664		10
	ST1 KO + 0.01mM Ca	mIPSC Frequency (Hz)	0.481	0.092		14
	ST1 KO + 0.2mM Ca	mIPSC Frequency (Hz)	2.151	0.465		14
	ST1 KO + 0.5mM Ca	mIPSC Frequency (Hz)	6.516	0.799		14
	ST1 KO + 1mM Ca	mIPSC Frequency (Hz)	8.521	0.965		14
	ST1 KO + 2mM Ca	mIPSC Frequency (Hz)	12.309	1.015		14
	ST1 KO + 5mM Ca	mIPSC Frequency (Hz)	13.246	0.982		14
	ST1 KO + 10mM Ca	mIPSC Frequency (Hz)	13.954	0.961		14
Fig. 2 c	WT + 0.1mM Ca	mEPSC Frequency (Hz)	0.037	0.009	Two-way ANOVA, P<0.001, compared with ST1 KO, for mEPSC Frquency	9
	WT + 0.2mM Ca	mEPSC Frequency (Hz)	0.061	0.007		9
	WT + 0.5mM Ca	mEPSC Frequency (Hz)	0.194	0.032		9
	WT + 1mM Ca	mEPSC Frequency (Hz)	0.525	0.074		9
	WT + 2mM Ca	mEPSC Frequency (Hz)	1.829	0.199		9
	WT + 5mM Ca	mEPSC Frequency (Hz)	3.481	0.359		9
	WT + 10mM Ca	mEPSC Frequency (Hz)	4.231	0.4		9
	ST1 KO + 0.01mM Ca	mEPSC Frequency (Hz)	0.379	0.079		12
	ST1 KO + 0.2mM Ca	mEPSC Frequency (Hz)	1.864	0.318		12
	ST1 KO + 0.5mM Ca	mEPSC Frequency (Hz)	6.173	0.584		12
	ST1 KO + 1mM Ca	mEPSC Frequency (Hz)	11.659	0.923		12
	ST1 KO + 2mM Ca	mEPSC Frequency (Hz)	16.235	1.18		12
	ST1 KO + 5mM Ca	mEPSC Frequency (Hz)	18.85	0.891		12
	ST1 KO + 10mM Ca	mEPSC Frequency (Hz)	20.08	1.055		12
Fig.2 d	WT, mIPSC	Cooperativity	1.89	0.05	P<0.001, compared with ST1 KO	10
	ST1 KO, mIPSC	Cooperativity	1.52	0.03	see above	14
	WT, mIPSC	Kd (mM)	2.24	0.07	P<0.001, compared with ST1 KO	10
	ST1 KO, mIPSC	Kd (mM)	0.71	0.06	see above	14

Fig.2 e	WT, mEPSC	Cooperativity	1.992	0.087	P<0.001, compared with ST1 KO	9
	ST1 KO, mEPSC	Cooperativity	1.61	0.02	see above	12
	WT, mEPSC	Kd (mM)	2.329	0.116	P<0.001, compared with ST1 KO	9
	ST1 KO, mEPSC	Kd (mM)	0.84	0.02	see above	12
Fig. 3 c	ST1-D2N WT + 0.5mM Ca	eIPSC amplitude (nA)	0.169	0.029	According to Two-way ANOVA, compare ST1-D2N WT with ST1-D2N, for eIPSC amplitude, P<0.001;	10
	ST1-D2N WT + 1mM Ca	eIPSC amplitude (nA)	0.791	0.087		10
	ST1-D2N WT + 2mM Ca	eIPSC amplitude (nA)	3.255	0.163		10
	ST1-D2N WT + 5mM Ca	eIPSC amplitude (nA)	5.109	0.241		10
	ST1-D2N WT + 10mM Ca	eIPSC amplitude (nA)	5.765	0.272		10
	ST1-D2N + 0.5mM Ca	eIPSC amplitude (nA)	0.9	0.075	According to Two-way ANOVA, compare ST1-D2N WT with ST1-D8N, for eIPSC amplitude, P<0.001; compare ST1-D2N WT with ST1-R3Q, for eIPSC amplitude, P<0.001;	9
	ST1-D2N + 1mM Ca	eIPSC amplitude (nA)	2.599	0.024		9
	ST1-D2N + 2mM Ca	eIPSC amplitude (nA)	5.669	0.251		9
	ST1-D2N + 5mM Ca	eIPSC amplitude (nA)	6.81	0.288		9
	ST1-D2N + 10mM Ca	eIPSC amplitude (nA)	7.227	0.248		9
	ST1-D8N WT + 0.5mM Ca	eIPSC amplitude (nA)	0.129	0.028	According to Two-way ANOVA, compare ST1-D8N WT with ST1-D8N, for eIPSC amplitude, P<0.001;	10
	ST1-D8N WT + 1mM Ca	eIPSC amplitude (nA)	0.726	0.108		10
	ST1-D8N WT + 2mM Ca	eIPSC amplitude (nA)	3.296	0.379		10
	ST1-D8N WT + 5mM Ca	eIPSC amplitude (nA)	5.451	0.277		10
	ST1-D8N WT + 10mM Ca	eIPSC amplitude (nA)	6.117	0.248		10
	ST1-D8N + 0.5mM Ca	eIPSC amplitude (nA)	0.161	0.041	According to Two-way ANOVA, compare ST1-D8N WT with ST1-R3Q, for eIPSC amplitude, P<0.001;	10
	ST1-D8N + 1mM Ca	eIPSC amplitude (nA)	0.724	0.135		10
	ST1-D8N + 2mM Ca	eIPSC amplitude (nA)	1.805	0.077		10
	ST1-D8N + 5mM Ca	eIPSC amplitude (nA)	4.101	0.139		10
	ST1-D8N + 10mM Ca	eIPSC amplitude (nA)	4.624	0.192		10
	ST1-R3Q WT + 0.5mM Ca	eIPSC amplitude (nA)	0.198	0.026	According to Two-way ANOVA, compare ST1-R3Q WT with ST1-R3Q, for eIPSC amplitude, P<0.001;	10
	ST1-R3Q WT + 1mM Ca	eIPSC amplitude (nA)	0.898	0.171		10

	ST1-R3Q WT + 2mM Ca	eIPSC amplitude (nA)	3.347	0.193		10
	ST1-R3Q WT + 5mM Ca	eIPSC amplitude (nA)	4.641	0.329		10
	ST1-R3Q WT + 10mM Ca	eIPSC amplitude (nA)	5.895	0.343		10
	ST1-R3Q + 0.5mM Ca	eIPSC amplitude (nA)	0.159	0.027	See above	10
	ST1-R3Q + 1mM Ca	eIPSC amplitude (nA)	0.459	0.08		10
	ST1-R3Q + 2mM Ca	eIPSC amplitude (nA)	1.362	0.125		10
	ST1-R3Q + 5mM Ca	eIPSC amplitude (nA)	2.819	0.224		10
	ST1-R3Q + 10mM Ca	eIPSC amplitude (nA)	3.807	0.296		10
Fig.3 d	ST1-D2N WT	Kd for Ca^{2+}(mM)	1.843	0.111	P<0.01, compared with ST1-D2N; P=0.885, compared with ST1-D8N WT; P<0.01, compared with ST1-D8N; P=0.8420, compared with ST1-R3Q WT; P<0.001, compared with ST1-R3Q	9
	ST1-D2N	Kd for Ca^{2+}(mM)	1.173	0.134	P<0.001, compared with ST1-D8N WT; compared with ST1-D8N; P=0.0248, compared with ST1-R3Q WT; P<0.001, compared with ST1-R3Q;	10
	ST1-D8N WT	Kd for Ca^{2+}(mM)	1.844	0.098	P<0.01, compared with ST1-D8N; P=0.941, compared with ST1-R3Q WT; P<0.001, compared with ST1-R3Q;	10
	ST1-D8N	Kd for Ca^{2+}(mM)	2.483	0.181	P=0.1331, compared with ST1-R3Q WT; P=0.0227<0.05, compared with ST1-R3Q	10
	ST1-R3Q WT	Kd for Ca^{2+}(mM)	1.871	0.344	P<0.01, compared with ST1-R3Q;	10
	ST1-R3Q	Kd for Ca^{2+}(mM)	3.518	0.374	see above	10
Fig. 4 b	ST1-D2N WT + 0.2mM Ca	mIPSC Frequency (Hz)	0.118	0.014	According to Two-way ANOVA, compare ST1-D2N WT with ST1-D2N, for mIPSC amplitude, P=0.0011<0.01;	8
	ST1-D2N WT + 0.5mM Ca	mIPSC Frequency (Hz)	0.435	0.066		8
	ST1-D2N WT + 1mM Ca	mIPSC Frequency (Hz)	1.3	0.112		8
	ST1-D2N WT + 2mM Ca	mIPSC Frequency (Hz)	3.003	0.454		8
	ST1-D2N WT + 5mM Ca	mIPSC Frequency (Hz)	6.315	0.899		8
	ST1-D2N WT + 10mM Ca	mIPSC Frequency (Hz)	7.19	1.039		8
	ST1-D2N + 0.2mM Ca	mIPSC Frequency (Hz)	0.136	0.0226	According to Two-way ANOVA, compare ST1-D2N with ST1-D8N, for mIPSC amplitude, P=0.0011<0.01; According to Two-way ANOVA, compare ST1-D2N with ST1-R3Q, for mIPSC amplitude, P<0.001;	9
	ST1-D2N + 0.5mM Ca	mIPSC Frequency (Hz)	0.798	0.12		9
	ST1-D2N + 1mM Ca	mIPSC Frequency (Hz)	2.477	0.215		9

	ST1-D2N + 2mM Ca	mIPSC Frequency (Hz)	5.272	0.384		9
	ST1-D2N + 5mM Ca	mIPSC Frequency (Hz)	7.2124	0.549		9
	ST1-D2N + 10mM Ca	mIPSC Frequency (Hz)	8.283	0.673		9
	ST1-D8N WT + 0.2mM Ca	mIPSC Frequency (Hz)	0.112	0.023	According to Two-way ANOVA, compare ST1-D8N WT with ST1-D8N, for mIPSC amplitude, P<0.001;	10
	ST1-D8N WT + 0.5mM Ca	mIPSC Frequency (Hz)	0.408	0.043		10
	ST1-D8N WT + 1mM Ca	mIPSC Frequency (Hz)	1.456	0.164		10
	ST1-D8N WT + 2mM Ca	mIPSC Frequency (Hz)	3.456	0.278		10
	ST1-D8N WT + 5mM Ca	mIPSC Frequency (Hz)	6.148	0.365		10
	ST1-D8N WT + 10mM Ca	mIPSC Frequency (Hz)	6.76	0.381		10
	ST1-D8N + 0.2mM Ca	mIPSC Frequency (Hz)	0.094	0.025	According to Two-way ANOVA, compare ST1-D8N with ST1-R3Q, for mIPSC amplitude, P<0.01;	10
	ST1-D8N + 0.5mM Ca	mIPSC Frequency (Hz)	0.426	0.107		10
	ST1-D8N + 1mM Ca	mIPSC Frequency (Hz)	0.994	0.064		10
	ST1-D8N + 2mM Ca	mIPSC Frequency (Hz)	2.096	0.312		10
	ST1-D8N + 5mM Ca	mIPSC Frequency (Hz)	4.609	0.285		10
	ST1-D8N + 10mM Ca	mIPSC Frequency (Hz)	5.424	0.344		10
	ST1-R3Q WT + 0.2mM Ca	mIPSC Frequency (Hz)	0.085	0.015	According to Two-way ANOVA, compare ST1-R3Q WT with ST1-R3Q, for mIPSC amplitude, P<0.001;	8
	ST1-R3Q WT + 0.5mM Ca	mIPSC Frequency (Hz)	0.385	0.042		8
	ST1-R3Q WT + 1mM Ca	mIPSC Frequency (Hz)	1.1575	0.106		8
	ST1-R3Q WT + 2mM Ca	mIPSC Frequency (Hz)	3.175	0.145		8
	ST1-R3Q WT + 5mM Ca	mIPSC Frequency (Hz)	5.678	0.355		8
	ST1-R3Q WT + 10mM Ca	mIPSC Frequency (Hz)	6.795	0.514		8
	ST1-R3Q + 0.2mM Ca	mIPSC Frequency (Hz)	0.053	0.009	see above	10
	ST1-R3Q + 0.5mM Ca	mIPSC Frequency (Hz)	0.264	0.041		10
	ST1-R3Q + 1mM Ca	mIPSC Frequency (Hz)	0.587	0.083		10
	ST1-R3Q + 2mM Ca	mIPSC Frequency (Hz)	1.367	0.116		10
	ST1-R3Q + 5mM Ca	mIPSC Frequency (Hz)	3.324	0.221		10
	ST1-R3Q + 10mM Ca	mIPSC Frequency (Hz)	4.241	0.237		10

Fig.4 c	ST1-D2N WT	Kd for Ca^{2+}(mM)	2.383	0.076	P<0.001, compared with ST1-D2N;	8
	ST1-D2N	Kd for Ca^{2+}(mM)	1.624	0.076	P<0.001, compared with ST1-D8N; P<0.001, compared with ST1-R3Q	9
	ST1-D8N WT	Kd for Ca^{2+}(mM)	2.024	0.065	P<0.01, compared with ST1-D8N;	10
	ST1-D8N	Kd for Ca^{2+}(mM)	2.686	0.208	P<0.01, compared with ST1-R3Q;	10
	ST1-R3Q WT	Kd for Ca^{2+}(mM)	2.351	0.139	P<0.001, compared with ST1-R3Q;	8
	ST1-R3Q	Kd for Ca^{2+}(mM)	3.409	0.128	see above	10
Fig.5 b	ST1-D2N WT	mIPSC Frequency (Hz), Slices	2.735	0.458	P=0.0276<0.05, compared with ST1-D2N;	10
	ST1-D2N	mIPSC Frequency (Hz), Slices	4.783	0.739	P<0.001, compared with ST1-R3Q; P<0.001, compared with ST1-D8N;	9
	ST1-D8N WT	mIPSC Frequency (Hz), Slices	2.353	0.095	P<0.001, compared with ST1-D8N;	15
	ST1-D8N	mIPSC Frequency (Hz), Slices	1.521	0.133	P=0.0118<0.05, compared with ST1-D8N;	23
	ST1-R3Q WT	mIPSC Frequency (Hz), Slices	2.69	0.277	P<0.001, compared with ST1-R3Q;	20
	ST1-R3Q	mIPSC Frequency (Hz), Slices	1.114	0.069	See above	21
Fig. 5 d	ST1-D2N WT, before Nicotine application	mIPSC Frequency(Hz), brain slice	2.573	0.162	P<0.001, compared with ST1-D2N WT, after Nicotine application; P<0.01, compared with ST1-D2N, before Nicotine application;	9
	ST1-D2N WT, after Nicotine application	mIPSC Frequency(Hz), brain slice	4.492	0.163	P<0.001, compared with ST1-D2N, after Nicotine application	9
	ST1-D2N , before Nicotine application	mIPSC Frequency(Hz), brain slice	3.985	0.339	P<0.001, compared with ST1-D2N, after Nicotine application	13
	ST1-D2N , after Nicotine application	mIPSC Frequency(Hz), brain slice	6.349	0.305	see above	13
	ST1-D8N WT, before Nicotine application	mIPSC Frequency(Hz), brain slice	2.671	0.103	P<0.001, compared with ST1-D8N WT, after Nicotine application; P<0.001, compared with ST1-D8N, before Nicotine application	10
	ST1-D8N WT, after Nicotine application	mIPSC Frequency(Hz), brain slice	4.033	0.181	P<0.001, compared with ST1-D8N, after Nicotine application	10
	ST1-D8N , before Nicotine application	mIPSC Frequency(Hz), brain slice	1.801	0.092	P<0.001, compared with ST1-D8N, after Nicotine application	17
	ST1-D8N , after Nicotine application	mIPSC Frequency(Hz), brain slice	2.94	0.143	see above	17
	ST1-R3Q WT, before Nicotine application	mIPSC Frequency(Hz), brain slice	2.817	0.275	P<0.01, compared with ST1-R3Q WT, after nicotine application; P=0.0349<0.05, compared with ST1-R3Q, before Nicotine	9

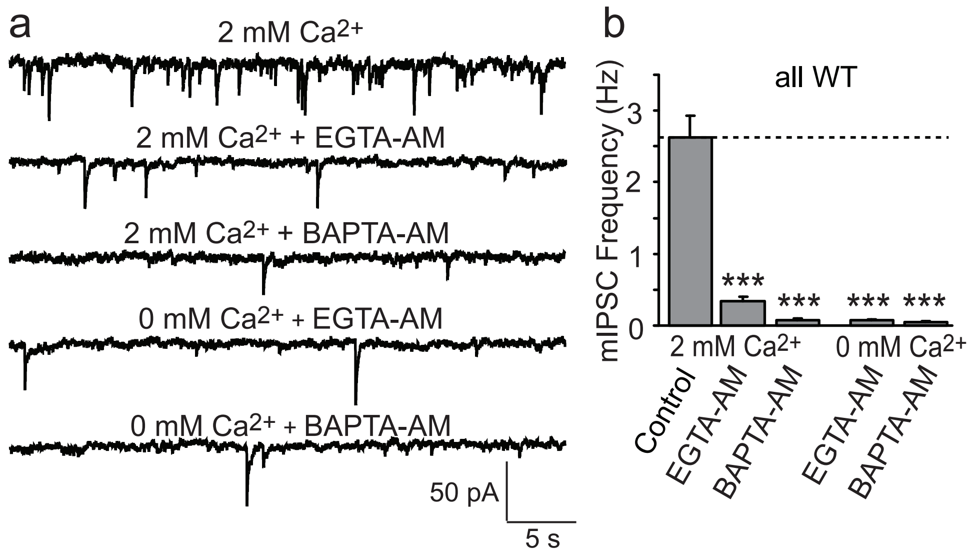
					application;	
	ST1-R3Q WT, after Nicotine application	mIPSC Frequency(Hz), brain slice	4.143	0.327	P=0.0696, compared with ST1-R3Q, after Nicotine application	9
	ST1-R3Q , before Nicotine application	mIPSC Frequency(Hz), brain slice	1.898	0.314	P=0.0179<0.05, compared with ST1-R3Q, after Nicotine application	12
	ST1-R3Q , after Nicotine application	mIPSC Frequency(Hz), brain slice	3.148	0.374	see above	12
Fig. 6 b	WT	eIPSC amplitude (nA)	3.034	0.222	P=0.763, compared with Syt1 rescue; P<0.001, compared all the rest	14
	Syt1 KO	eIPSC amplitude (nA)	0.085	0.018	P<0.001, compared with Syt1 rescue and Syt1-C2A-3DA rescue; P=0.0677, compared with Syt1-C2B-3DA rescue; P=0.0978, compared with Syt1-C2AB-6DA rescue	14
	Syt1 KO + Syt1 rescue	eIPSC amplitude (nA)	2.955	0.133	P<0.001, compared with all other rescues	14
	Syt1 KO + Syt1 C2A-3DA rescue	eIPSC amplitude (nA)	1.42	0.208	P<0.001, compared with Syt1-C2B-3DA rescue and Syt1-C2AB-6DA rescue	17
	Syt1 KO + Syt1 C2B-3DA rescue	eIPSC amplitude (nA)	0.173	0.045	P=0.7165, compared with Syt1-C2AB-6DA rescue	13
	Syt1 KO + Syt1 C2AB-6DA rescue	eIPSC amplitude (nA)	0.15	0.037	see above	10
Fig. 6 c	WT	mIPSC Frequency (Hz)	2.092	0.158	P<0.001, compared with Syt1 KO; P=0.7891 compared with Sy1 rescue; P<0.001, compared with others	18
	Syt1 KO	mIPSC Frequency (Hz)	14.646	1.307	P<0.001, compared with Syt1 rescue; P=0.0215<0.05, compared with Syt1-C2A-3DA rescue; P<0.001, compared with Syt1-C2B-3DA rescue; P=0.8134, compared with Syt1-C2AB-6DA rescue	29
	Syt1 KO + Syt1 rescue	mIPSC Frequency (Hz)	2.001	0.296	P<0.001 compared with other three rescues	19
	Syt1 KO + Syt1 C2A-3DA rescue	mIPSC Frequency (Hz)	11.099	0.7	P<0.001, compared with Syt1-C2B-3DA rescue; P<0.01, compared with Syt1-C2AB-6DA rescue	28
	Syt1 KO + Syt1 C2B-3DA rescue	mIPSC Frequency (Hz)	6.421	0.776	P<0.001, compared with Syt1-C2AB-6DA rescue	28
	Syt1 KO + Syt1 C2AB-6DA rescue	mIPSC Frequency (Hz)	14.296	0.6	see above	28
Fig. 6 d	WT + 0.01mM Ca	mIPSC Frequency (Hz)	0.058	0.012	According to Two-way ANOVA, compare WT with syt1 KO, for mIPSC frequency, P<0.001; According to Two-way ANOVA, compare WT with syt1 C2A3DA rescue, for mIPSC frequency, P<0.001; According to Two-way ANOVA, compare WT with syt1 C2B3DA rescue, for mIPSC frequency, P<0.001;	8

	WT + 0.2mM Ca	mIPSC Frequency (Hz)	0.098	0.017		8
	WT + 0.5mM Ca	mIPSC Frequency (Hz)	0.369	0.031		8
	WT + 1mM Ca	mIPSC Frequency (Hz)	1.269	0.128		8
	WT + 2mM Ca	mIPSC Frequency (Hz)	3.184	0.252		8
	WT + 5mM Ca	mIPSC Frequency (Hz)	5.975	0.424		8
	WT + 10mM Ca	mIPSC Frequency (Hz)	6.776	0.485		8
	Syt1 KO + 0.01mM Ca	mIPSC Frequency (Hz)	0.428	0.115	According to Two-way ANOVA, compare syt1 KO with syt1C2A3DA rescue, for mIPSC frequency, P<0.001; According to Two-way ANOVA, compare WT with syt1 C2B3DA rescue, for mIPSC frequency, P<0.001;	8
	Syt1 KO + 0.2mM Ca	mIPSC Frequency (Hz)	2.845	0.767		8
	Syt1 KO + 0.5mM Ca	mIPSC Frequency (Hz)	7.576	0.863		8
	Syt1 KO + 1mM Ca	mIPSC Frequency (Hz)	10.534	0.886		8
	Syt1 KO + 2mM Ca	mIPSC Frequency (Hz)	14.204	1.101		8
	Syt1 KO + 5mM Ca	mIPSC Frequency (Hz)	15.337	1.124		8
	Syt1 KO + 10mM Ca	mIPSC Frequency (Hz)	15.897	1.212		8
	Syt1 KO + Syt1-C2A-3DA rescue + 0.01mM Ca	mIPSC Frequency (Hz)	0.131	0.014	According to Two-way ANOVA, compare syt1 KO with syt1C2A3DA rescue, for mIPSC frequency, P<0.001;	8
	Syt1 KO + Syt1-C2A-3DA rescue + 0.2mM Ca	mIPSC Frequency (Hz)	1.014	0.132		8
	Syt1 KO + Syt1-C2A-3DA rescue + 0.5mM Ca	mIPSC Frequency (Hz)	3.17	0.336		8
	Syt1 KO + Syt1-C2A-3DA rescue + 1mM Ca	mIPSC Frequency (Hz)	6.138	0.375		8
	Syt1 KO + Syt1-C2A-3DA rescue + 2mM Ca	mIPSC Frequency (Hz)	9.309	0.502		8
	Syt1 KO + Syt1-C2A-3DA rescue + 5mM Ca	mIPSC Frequency (Hz)	11.499	0.59		8
	Syt1 KO + Syt1-C2A-3DA rescue + 10mM Ca	mIPSC Frequency (Hz)	12.299	0.646		8

	Syt1 KO + Syt1-C2B-3DA rescue + 0.01mM Ca	mIPSC Frequency (Hz)	0.223	0.027	see above	8
	Syt1 KO + Syt1-C2B-3DA rescue + 0.2mM Ca	mIPSC Frequency (Hz)	1.156	0.114		8
	Syt1 KO + Syt1-C2B-3DA rescue + 0.5mM Ca	mIPSC Frequency (Hz)	3.276	0.362		8
	Syt1 KO + Syt1-C2B-3DA rescue + 1mM Ca	mIPSC Frequency (Hz)	5.38	0.454		8
	Syt1 KO + Syt1-C2B-3DA rescue + 2mM Ca	mIPSC Frequency (Hz)	7.559	0.444		8
	Syt1 KO + Syt1-C2B-3DA rescue + 5mM Ca	mIPSC Frequency (Hz)	8.573	0.711		8
	Syt1 KO + Syt1-C2B-3DA rescue + 10mM Ca	mIPSC Frequency (Hz)	9.29	0.611		8
Fig. 6 e	WT	Ca^{2+} cooperativity	1.987	0.072	P=0.0343<0.05, compared with Syt1 KO; P<0.01, compared with Syt1-C2A-3DA rescue and Syt1-C2B-3DA rescue;	8
	Syt1 KO	Ca^{2+} cooperativity	1.567	0.164	P=0.872, compared with Syt1-C2A-3DA rescue; P=0.5573, compared with Syt1-C2B-3DA rescue;	8
	Syt1 KO + Syt1-C2A-3DA	Ca^{2+} cooperativity	1.538	0.065	P=0.405, compared with Syt1-C2B-3DA rescue	8
	Syt1 KO + Syt1-C2B-3DA	Ca^{2+} cooperativity	1.462	0.06	see above	8
Fig. 6 f	WT	Kd (mM)	2.244	0.078	P<0.001, compared with all the rest	8
	Syt1 KO	Kd (mM)	0.629	0.102	P<0.01, compared with Syt1-C2A-3DA rescue; P=0.121, compared with Syt1-C2B-3DA	8
	Syt1 KO + Syt1-C2A-3DA	Kd (mM)	1.044	0.031	P<0.001, compared with Syt1-C2B-3DA rescue	8
	Syt1 KO + Syt1-C2B-3DA	Kd (mM)	0.806	0.033	see above	8
Fig. 7 c	WT + 0.5mM Ca	eIPSC amplitude (nA)	0.18642	0.02147	According to Two-way ANOVA, compare ST1-D2N WT with ST1-D2N, for eIPSC amplitude, P<0.001;	8
	WT + 1mM Ca	eIPSC amplitude (nA)	0.85865	0.09933		8
	WT + 2mM Ca	eIPSC amplitude (nA)	3.39997	0.18793		8
	WT + 5mM Ca	eIPSC amplitude (nA)	5.15096	0.24384		8
	WT + 10mM Ca	eIPSC amplitude (nA)	6.05015	0.21035		8
	ST1-2xLinker + 0.5mM Ca	eIPSC amplitude (nA)	0.23692	0.06206		12
	ST1-2xLinker + 1mM Ca	eIPSC amplitude (nA)	0.38952	0.0602		12
	ST1-2xLinker + 2mM Ca	eIPSC amplitude (nA)	1.88884	0.16747		12

	ST1-2xLinker + 5mM Ca	eIPSC amplitude (nA)	3.45591	0.36635		12
	ST1-2xLinker + 10mM Ca	eIPSC amplitude (nA)	3.77317	0.50218		12
Fig. 7 e	WT	mIPSC frequency (Hz)	2.052	0.17236	P=0.5142, compared with Syt1-2Xlinker	35
	Syt1-2xlinker	mIPSC frequency (Hz)	1.85832	0.25242		21
Suppl Fig. 1 b	WT+2mM Ca	mIPSC Frequency (Hz)	2.625	0.304	P<0.001 compared with other four genotypes	15
	WT+EGTA-AM+2mM Ca	mIPSC Frequency (Hz)	0.338	0.0617	P<0.001 compared with other four genotypes	14
	WT+BAPTA-AM+2mM Ca	mIPSC Frequency (Hz)	0.0764	0.0167	P<0.001 compared with WT+EGTA-AM+2mM Ca; P=0.8311 compared with WT+BAPTA-AM-0mM Ca; P=0.1580 compared with WT+EGTA-AM+0mM Ca	14
	WT+EGTA-AM+0mM Ca	mIPSC Frequency (Hz)	0.0429	0.0136	P=0.2488 compared with WT+BAPTA-AM+0mM Ca	10
	WT+BAPTA-AM+0mM Ca	mIPSC Frequency (Hz)	0.0715	0.0141	see above	26
Suppl Fig. 2b	WT Con	mEPSC Frequency (Hz)	2.613	0.159	P<0.001, compared with WT Con+ Caffeine	15
	WT Con + Caffeine	mEPSC Frequency (Hz)	5.545	0.356	see above	11
Suppl Fig. 4a	ST1-D2N WT	Apparent Ca^{2+}-cooperativity	2.769	0.424	P=0.4534, compared with ST1-D2N; P=0.8006, compared with ST1-D8N WT; P=0.1947, compared with ST1-R3Q WT; P=0.0153<0.05, compared with ST1-R3Q	9
	ST1-D2N	Apparent Ca^{2+}-cooperativity	2.436	0.153	P=0.2970, compared with ST1-D8N WT; P=0.2901, compared with ST1-D8N; P=0.6805, compared with ST1-R3Q WT; P<0.001, compared with ST1-R3Q	10
	ST1-D8N WT	Apparent Ca^{2+}-cooperativity	2.923	0.426	P=0.1213, compared with ST1-D8N; P=0.2663, compared with ST1-R3Q WT; P<0.01, compared with ST1-R3Q;	10
	ST1-D8N	Apparent Ca^{2+}-cooperativity	2.133	0.233	P=0.7764, compared with ST1-R3Q WT; P=0.0731, compared with ST1-R3Q;	10
	ST1-R3Q WT	Apparent Ca^{2+}-cooperativity	2.263	0.386	P=0.1318, compared with ST1-R3Q;	10
	ST1-R3Q	Apparent Ca^{2+}-cooperativity	1.615	0.142	see above	10
Suppl Fig. 4b	ST1-D2N WT	Apparent Ca^{2+}-cooperativity	1.813	0.058	P=0.3707, compared with ST1-D2N;	8
	ST1-D2N	Apparent Ca^{2+}-cooperativity	2.012	0.196	P=0.4458, compared with ST1-D8N; P=0.3798, compared with ST1-R3Q	9
	ST1-D8N WT	Apparent Ca^{2+}-cooperativity	2.008	0.077	P=0.2378, compared with ST1-D8N;	10
	ST1-D8N	Apparent Ca^{2+}-cooperativity	1.839	0.115	P=0.9240, compared with ST1-R3Q;	10
	ST1-R3Q WT	Apparent Ca^{2+}-cooperativity	1.877	0.112	P=0.7157, compared with ST1-R3Q;	8
	ST1-R3Q	Apparent Ca^{2+}-cooperativity	1.825	0.088	see above	10

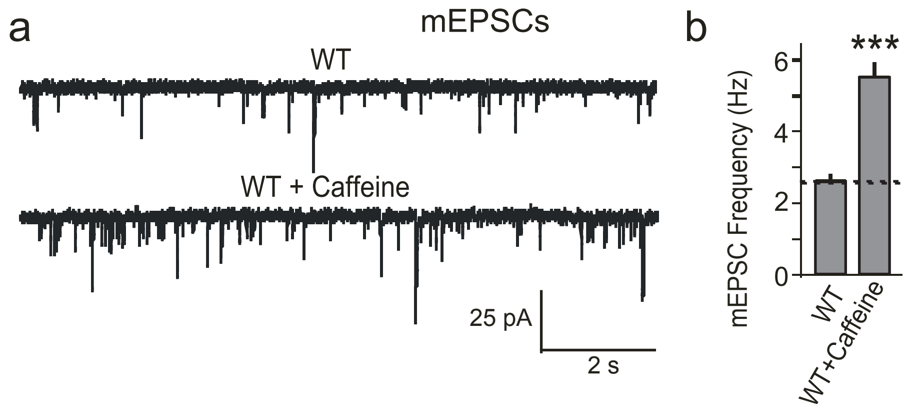
Suppl Fig.5 b	WT	mIPSC Frequency (Hz)	2.061	0.198	P<0.001, compared with ST1-D2N and all others;	10
	ST1-D2N	mIPSC Frequency (Hz)	5.63	0.606	P<0.001, compared with all others;	13
	WT+BAPTA-AM	mIPSC Frequency (Hz)	0.156	0.028	P<0.1505, compared with ST1-D2N+BAPTA-AM;	10
	ST1-D2N + BAPTA-AM	mIPSC Frequency (Hz)	0.232	0.039	see above	13
Suppl. Fig.9	WT	Kd for Ca2+(mM)	1.92581	0.09797		8
	Syt1 2xL	Kd for Ca2+(mM)	2.05325	0.12141		12
	WT	Apparent cooperativity	2.55757	0.15808		8
	Syt1 2xL	Apparent cooperativity	2.66923	0.17942		12



Supplementary Figure 1

Effect of the Ca^{2+}-chelators EGTA-AM and BAPTA-AM on the mini frequency in wild-type cortical neurons.

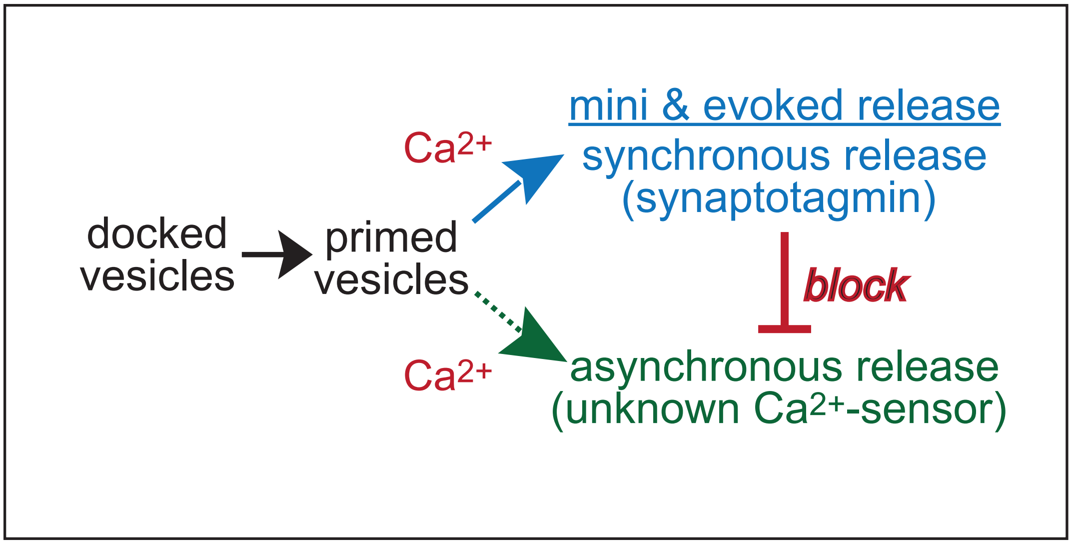
mIPSCs were monitored in cortical neurons in extracellular medium containing 2 mM or 0 mM Ca^{2+}. Neurons were cultured from wild-type mice, and were preincubated with as indicated 10 μM EGTA-AM or BATPA-AM at 37 ^{O}C for 1 hr in 2 or 0 mM extracellular Ca^{2+} before recordings. Note that EGTA-AM is a much slower Ca^{2+}-chelator than BAPTA-AM, and thus not as effective as BAPTA-AM in extracellular medium containing Ca^{2+} because Ca^{2+}-fluxes originating from Ca^{2+}-channels or internal Ca^{2+}-stores are not buffered away quickly.
a, Representative traces The calibration bars at the bottom apply to all traces.
b, Summary graphs (means ± SEMs; see Suppl. Table 1 for numerical parameters; ***=p<0.001 as determined by Student's t-test).



Supplementary Figure 2

Effect of caffeine on spontaneous mini release in wild-type cortical neurons.

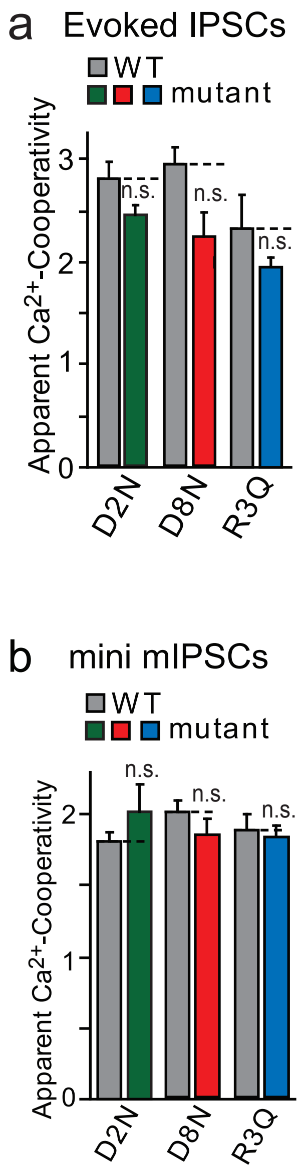
Caffeine was applied to cultured wild-type neurons, and mEPSCs were measured before and after the application.
a, Representative traces (calibration bars at the bottom apply to all traces above the bars).
b, Summary graphs (means ± SEMs; see Suppl. Table 1 for numerical parameters; ***=p<0.001 as determined by Student's t-test).



Supplementary Figure 3

Hypothesis for a common mechanism of evoked and spontaneous mini release in synapses.

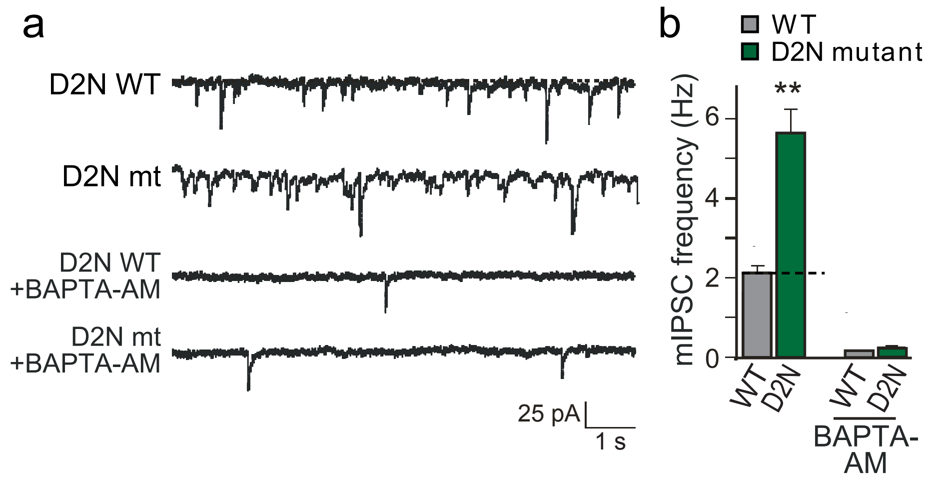
Both evoked and spontaneous release are proposed to be triggered by Ca^{2+}-binding to Syt1 which simultaneously blocks a second Ca^{2+}-sensor that triggers asynchronous release (dotted green arrow). Upon deletion of Syt1, the second Ca^{2+}-sensor that exhibits a higher apparent Ca^{2+}-affinity than Syt1 is activated, resulting in an increase in spontaneous release.



Supplementary Figure 4

Summary graphs of synaptic parameters observed in cortical neurons cultured from littermate wild-type control mice and synaptotagmin-1 knockin mice carrying mutations in the synaptotagmin Ca^{2+}-binding sites.

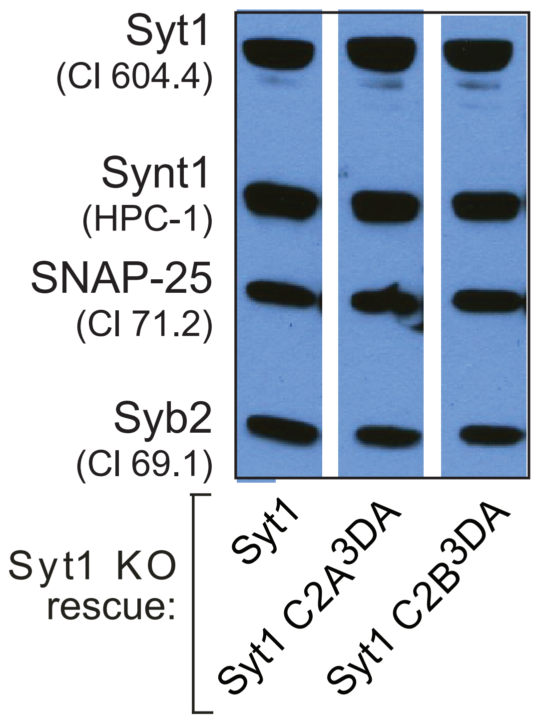
a, Apparent Ca^{2+}-cooperativity of evoked IPSCs, as determined by Hill equation fitting to the data shown in Fig. 3.
b, Apparent Ca^{2+}-cooperativity of spontaneous mIPSCs, as determined by Hill equation fitting to the data shown in Fig. 4.
Data shown are means ± SEMs; see Suppl. Table 1 for numerical parameters (**=p<0.001 as determined by Student's t-test; n.s. = non-significant).



Supplementary Figure 5

mIPSCs are increased in frequency in D2N-mutant synaptotagmin-1 knockin mice, but can be fully blocked by preincubation with BAPTA-AM.

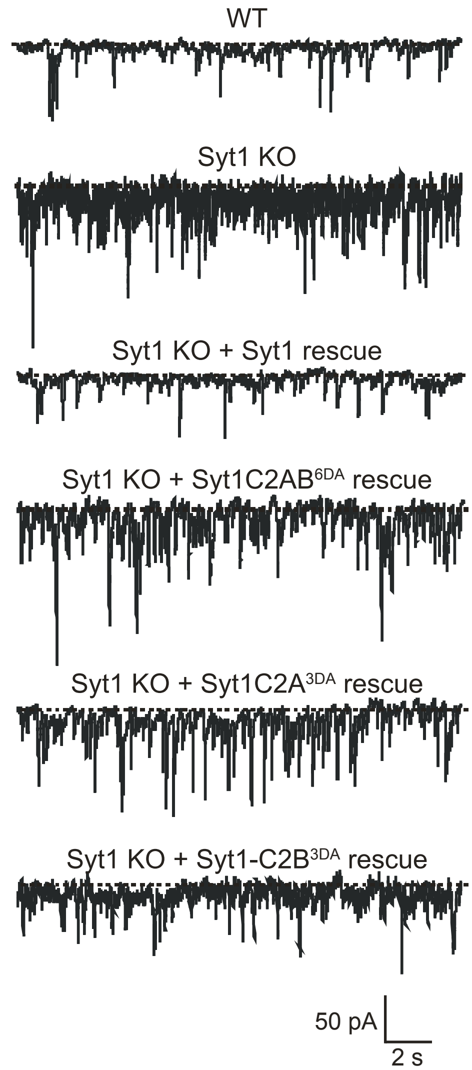
mIPSCs were monitored in cultured cortical neurons from Syt1 D2N and littermate wild-type control mice by whole-cell voltage-clamp recordings in the presence of 50 μM APV, 20 μM CNQX, and 1 μM TTX. In the BAPTA-AM treatment experiment, the neurons were pretreated with 10 μM BAPTA-AM for 1 hr in a 37 ^{O}C incubator before recording; during the recordings, 10 μM BAPTA-AM were added to the Ca^{2+}-free ACSF in the bath to fully suppress the intracellular Ca^{2+}.
a, Representative traces (calibration bars at the bottom apply to all traces above the bars)
b, Summary graphs (means ± SEMs; see Suppl. Table 1 for numerical parameters; ***=p<0.001 as determined by Student's t-test).



Supplementary Figure 6

Representative immunoblots of synaptotagmin-1 KO neurons infected with lentivirus expressing wild-type synaptotagmin-1 (Syt1), or mutant synaptotagmin-1 with the C2A- or C2B-domain 3DA mutations.

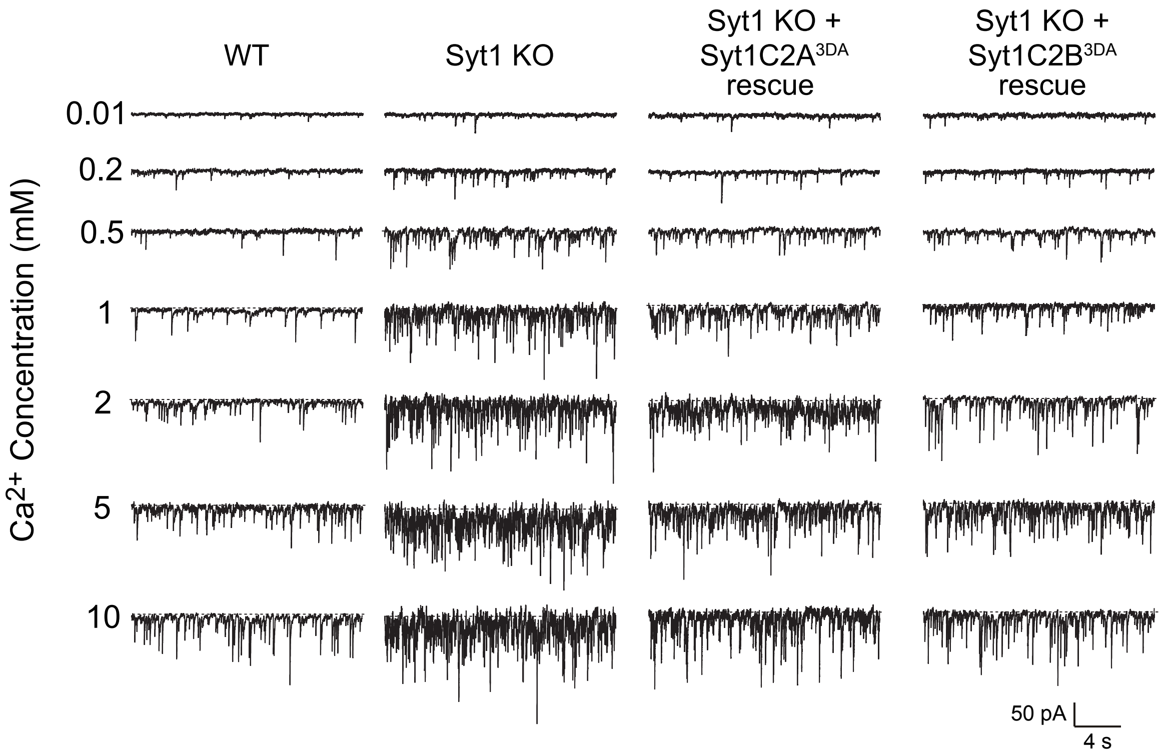
The image shows three lanes excised from the same blot of the neuronal proteins labeled with antibodies to synaptotagmin-1 (Syt1), syntaxin-1 (Synt1), SNAP-25, or synaptobrevin-2 (Syb2). Immunoreactive bands were visualized by ECL. Note that although the experiment shown does not represent a quantitative comparison, the image reveals that the exogenous synaptotagmin-1 forms are expressed at similar levels when compared to the endogenous synaptic proteins (syntaxin-1, synaptobrevin-2, and SNAP-25) analyzed on the same blots (see Fig. 6a for a description of the mutations).



Supplementary Figure 7

Representative traces of mIPSCs monitored in cortical neurons cultured from wild-type mice (WT) or from synaptotagmin-1 KO mice (Syt1 KO) that were either infected with control lentivirus, or with lentivirus expressing wild-type synaptotagmin-1 (Syt1 rescue) or various mutants of synaptotagmin-1 as indicated. Recordings were performed at the standard extracellular Ca^{2+}-concentration.

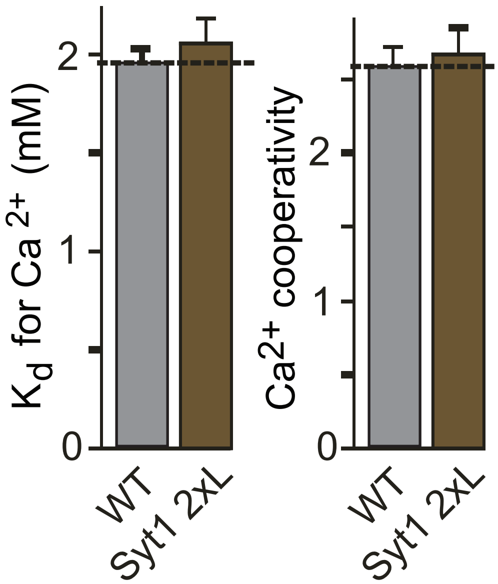
The calibration bars at the bottom apply to all traces above the bars.



Supplementary Figure 8

Representative traces of mIPSCs recorded in cortical neurons cultured from wild-type mice or synaptotagmin-1 KO mice either infected with a control lentivirus (Syt1 KO), or with lentivirus expressing the C2A- or C2B-domain Ca^{2+}-binding site mutant of synaptotagmin-1, monitored at increasing concentrations of extracellular Ca^{2+}.

Neurons were cultured from wild-type mice (WT) or from synaptotagmin-1 KO mice (Syt1 KO) that were either infected with control lentivirus, or with lentivirus expressing mutant synaptotagmin-1 unable to bind Ca^{2+} to its C2A-domain (Syt1-C2A3DA rescue) or to its C2B-domain (Syt1-C2B3DA rescue). The calibration bar at the bottom apply to all traces above the bar.



Supplementary Figure 9

Membrane proximity of Syt1 C2-domains does not alter apparent Ca^{2+}-affinity or -cooperativity of evoked release

IPSCs were measured in neurons from littermate wild-type mice and Syt-1 KO mice expressing mutant Syt1 in which the linker sequence was duplicated (see Fig. 7a). The mean apparent Ca^{2+}-cooperativity and Ca^{2+}-affinity of evoked IPSCs was determined by Hill function fitting to individual experiments titrating the IPSC size as a function of the extracellular Ca^{2+}-concentration (see Figs. 7b and 7c). Data shown are means ± SEMs (see Suppl. Table S1 for all numerical values).