## SUPPLEMENTARY MATERIALS for Xu et al., "Synaptotagmin-1 functions as Ca<sup>2+</sup>-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
1 19.1 2	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 1 wo-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 <sup>2+</sup> (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 <sup>2+</sup> (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 <sup>2+</sup> (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 <sup>2+</sup> (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 <sup>2+</sup> (mM)	1.871	0.344	P<0.01, compared with ST1-R3Q:	10
	ST1-R3Q	Kd for Ca <sup>2+</sup> (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	8 283	0.673		9
		0.200	0.010	According to Two-way ANOVA, compare ST1-D8N WT with ST1-	
ST1-D8N WT + 0.2mM Ca	mIPSC Frequency (Hz)	0.112	0.023	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 <sup>2+</sup> (mM)	2.383	0.076	P<0.001, compared with ST1-D2N;	8
	ST1-D2N	Kd for Ca <sup>2+</sup> (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 <sup>2+</sup> (mM)	2.024	0.065	P<0.01, compared with ST1-D8N;	10
	ST1-D8N	Kd for Ca <sup>2+</sup> (mM)	2.686	0.208	P<0.01, compared with ST1-R3Q;	10
	ST1-R3Q WT	Kd for Ca <sup>2+</sup> (mM)	2.351	0.139	P<0.001, compared with ST1- R3Q;	8
	ST1-R3Q	Kd for Ca <sup>2+</sup> (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

Image: second						application;	
Image: split and split							
STI-R3Q WT, after Noctine application         mIPSC Frequency(Hz), brain slice         P=0.0666, compared with STI- R3Q, after Nicotine application         9           STI-R3Q, perfore Nicotine         mIPSC Frequency(Hz), application         1.898         0.314         P=0.0759-0.05, compared with STI- rapplication         12           STI-R3Q, after Nicotine         mIPSC Frequency(Hz), application         1.898         0.314         application         12           Fig. 6 b         WT         effect         0.334         csea above         12           Fig. 6 b         WT         effect         0.334         0.222         rest         7.0001, compared with Sy11           Sy11 KO + Sy11 rescue         effect         0.001         compared with Sy11- rescue and Sy11-C2A-3DA rescue; P=0.0978, compared with Sy11- C2B-3DA rescue; P=0.0978, compared with Sy11- C2B-3DA rescue; and Sy11-C2AB-5DA         14           Sy11 KO + Sy11 rescue         effect         0.001 rescue and Sy11-C2AB-5DA         17           Sy11 KO + Sy11 rescue         effect         0.016         compared with Sy11- C2B-3DA rescue         17           Sy11 KO + Sy11 C2B-3DA         effect         0.015         0.037         see above         10           Fig. 6 c         WT         miPSC         1.42         0.208         EDA rescue         13           Sy11 K							
ST1-R3Q WT, after Nicitive application         miPSC brain silee         4.143         0.327         Ps0.0886, compared with ST1- R3Q, after Nicotine application         9           st1-R3Q, before Nicotine application         miPSC frequency(Hz), brain silee         1.988         0.314         application         12           st1-R3Q, after Nicotine application         miPSC frequency(Hz), brain silee         3.148         0.374         see above         12           Fig. 6 b         WT         elPSC amplitude (nA)         3.034         0.222         rest         14           Fig. 6 b         WT         elPSC amplitude (nA)         0.086         compared with Syt1- c2B-SDA rescue: Po.0087, compared with Syt1- C2B-SDA rescue         14           Syt1 KO + Syt1 C2A-3DA applitude (nA)         1.42         0.036         Pc.001, compared with Syt1- C2B-SDA rescue         14           Syt1 KO + Syt1 C2A-3DA amplitude (nA)         1.42         0.228         Frequency Hz)         14           Syt1 KO + Syt1 C2A-3DA amplitude (nA)         0.172         0.08         Pc.001, compared with Syt1- C2B-SDA rescue         14           Syt1 KO + Syt1 C2A-3DA amplitude (nA)         0.172         0.045         C2A6=GDA rescue         16			1700				
Nicotine application         brain site         4.143         0.327         R30, after Nicotine application         9           ST1-R30, after Nicotine application         mIPSC brain site         1.898         0.314         P=0.0179-0.05, compared with ST1-R30, after Nicotine application         12           Fig. 6 b         T1-R30, after Nicotine application         eIPSC requency(Hz), brain site         3.148         0.374         see above         12           Fig. 6 b         WT         eIPSC amplitude (nA)         3.034         0.222         result - Po.078, compared with Syt1 rescue - Po.007, compared with Syt1 rescue - Po.007, compared with Syt1 rescue - Po.0078, compared with Syt1 rescue - Po.0071, com		ST1-R3Q WT. after	Frequency(Hz).			P=0.0696. compared with ST1-	
STI-R3Q, before Nicotine         ImPSC Frequency(Hz), brain silce         ImPSC 1.898         P=0.0179-0.05, compared with application         12           STI-R3Q, after Nicotine application         miPSC Frequency(Hz), brain silce         1.898         0.314         P=0.763, compared with Syl1 rescue P=0.001, compared with Syl1 rescue P=0.001, compared with Syl1 rescue P=0.001, compared with Syl1 rescue P=0.007, compared with Syl1 rescue P=0.001, co		Nicotine application	brain slice	4.143	0.327	R3Q, after Nicotine application	9
ST1-R30, abore Nicotine         Frequency(Hz), application         ST1-R30, atter Nicotine         12           ST1-R30, atter Nicotine         miPSC Frequency(Hz), brain silce         1.988         0.374         see above         12           Fig. 6 b         wT         eIPSC encuency(Hz), brain silce         1.488         0.374         see above         12           Fig. 6 b         wT         eIPSC encuency(Hz), brain silce         3.148         0.374         see above         12           Fig. 6 b         wT         eIPSC encuency(Hz), brain silce         3.034         0.222         rescue: P-0.001, compared with Syt1 rescue: P-0.007, compared with Syt1- C2B-3DA rescue: P-0.007, emplitude (nA)         0.085         0.018         rescue: P-0.007, compared with Syt1- c2B-3DA rescue: P-0.007, emplitude (nA)         0.045         C2B-3DA rescue and Syt1-C2AB- emplitude (nA)         14           Syt1 KO + Syt1 C2A-3DA rescue         eIPSC emplitude (nA)         0.173         0.045         C2AB-DA rescue and Syt1- C2B-3DA rescue and Syt1- C2B-3DA rescue         13           Fig. 6 c         eIPSC encue         eIPSC emplitude (nA)         0.173         0.045         C2AB-DA rescue         14           Fig. 6 c         eIPSC encue         eIPSC emplitude (nA)         0.173         0.045         C2AB-DA rescue         17           Syt1 KO + Syt1 C2AB- fDA rescue <td></td> <td></td> <td>mIPSC</td> <td></td> <td></td> <td>P=0.0179&lt;0.05, compared with</td> <td></td>			mIPSC			P=0.0179<0.05, compared with	
application         Data since         1:080         0:011         application         1/2           ST1-R3Q, after Nicotine application         IPSC Frequency(Hz), brain silce         3:148         0.374         see above         12           Fig. 6 b         WT         eIPSC amplitude (nA)         3:034         0.222         rescue ?P-0.001, compared with Syrt rescue ?P-0.0073, compared with Syrt rescue and Syrt-C2A-3DA         14           Syrt KO         eIPSC amplitude (nA)         0.085         0.018         rescue?P-0.0073, compared with Syrt- rescue?         14           Syrt KO + Syrt rescue         eIPSC amplitude (nA)         0.085         0.018         rescue rescue?P-0.0073, compared with Syrt- rescue         14           Syrt KO + Syrt rescue         eIPSC amplitude (nA)         1.42         0.208         6DA rescue         14           Syrt KO + Syrt C2A-3DA rescue         eIPSC amplitude (nA)         1.42         0.208         6DA rescue         17           Syrt KO + Syrt C2A-3DA rescue         eIPSC amplitude (nA)         0.15         0.037         see above         10           Fig. 6 c         WT         mIPSC Frequency (Hz)         2.092         0.158         6DA rescue         13           Syrt KO + Syrt C2A-3DA rescue         Frequency (Hz)         2.092         0.158         Frequency		ST1-R3Q, before Nicotine	Frequency(Hz),	1 909	0.214	ST1-R3Q, after Nicotine	10
ST1-R3Q, after Nicotine         miPSC Fig. 6 b         0.374         ase above         12           Fig. 6 b         wT         amplitude (nA)         3.148         0.374         ase above         12           Fig. 6 b         wT         amplitude (nA)         3.034         0.222         rescue: P-0.001; compared with Syl1 rescue: P-0.0077; compared with Syl1 rescue: P-0.0077; compared with Syl1 rescue: P-0.0077; compared with Syl1- c28-3DA rescue; P-0.0077; compared with Syl1-C2A-3DA         14           Syl1 KO         amplitude (nA)         0.085         0.018         rescue: P-0.0077; compared with Syl1-C2A-3DA         14           Syl1 KO + Syl1 C2A-3DA         eIPSC amplitude (nA)         2.955         0.133         P<0.001; compared with Syl1- rescue         14           Syl1 KO + Syl1 C2A-3DA         eIPSC amplitude (nA)         1.42         0.208         PA:0.001; compared with Syl1- rescue         17           Syl1 KO + Syl1 C2A-3DA         eIPSC amplitude (nA)         0.173         0.045         C2AB-6DA rescue         10           Fig. 6 c         wT         miPSC Frequency (Hz)         2.092         0.158         0.037         see above         10           Fig. 6 c         mIPSC Frequency (Hz)         2.092         0.158         0.037         see above         10           Syl1 KO + Syl1 C2AB- Syl1 KO		application		1.090	0.314		12
application         brain slice         3.148         0.374         see above         12           Fig. 6 b         WT         eIPSC amplitude (nA)         3.034         0.222         rescue; P-0.001, compared with Syrt rescue; P-0.007, compared with Syrt compared with Syrt- C2B-3DA rescue; P-0.007, compared with Syrt- c2B-3DA rescue; P-0.001, compared with Syrt- rescue; P-0.001, compared with Syrt- c2B-3DA rescue; P-0.001,		ST1-R30 after Nicotine	mIPSC Frequency(Hz)				
Fig. 6 b         WT         eIPSC amplitude (nA)         3.034         P-0.07(3), compared with Syl1 rescue; PR-0.01, compared with Syl1 rescue; PA:0.01, compared with Syl1 rescue and Syl1-C2A.9DA rescue; P=0.0877, compared with Syl1- C2B-3DA rescue; P=0.0878, compared with Syl1-C2AB-6DA           Syl1 KO         eIPSC amplitude (nA)         0.085         0.018         rescue and Syl1-C2A.9DA rescue; P=0.0877, compared with Syl1- C2B-3DA rescue; P=0.0878, compared with Syl1-C2AB-6DA         14           Syl1 KO + Syl1 rescue         eIPSC amplitude (nA)         1.42         P<0.001, compared with Syl1- C2B-3DA rescue and Syl1-C2AB-6DA         14           Syl1 KO + Syl1 C2A-3DA rescue         eIPSC amplitude (nA)         1.42         P<0.001, compared with Syl1- C2B-3DA rescue         17           Syl1 KO + Syl1 C2B-3DA rescue         eIPSC amplitude (nA)         0.173         0.045         P=0.0165, compared with Syl1- C2B-3DA rescue         13           Syl1 KO + Syl1 C2B-3DA eiDA rescue         eIPSC amplitude (nA)         0.173         0.045         P=0.001, compared with Syl1 rescue; P=0.001, comp		application	brain slice	3.148	0.374	see above	12
Fig. 6 b         WT         amplitude (nA) amplitude (nA)         3.034         0.222 resile         FC.00.1, compared with Syt1 rescue and Syt1-C2A-3DA rescue; P=0.0677, compared with Syt1- (C2B-3DA rescue; P=0.0978, compared with Syt1-C2A-6DA         14           Syt1 KO         amplitude (nA)         0.085         0.018         rescue         14           Syt1 KO + Syt1 rescue         eIPSC amplitude (nA)         0.085         0.013         rescues         14           Syt1 KO + Syt1 rescue         eIPSC amplitude (nA)         2.955         0.133         rescue         14           Syt1 KO + Syt1 C2A-3DA rescue         eIPSC amplitude (nA)         0.173         0.045         C2AB-6DA rescue         17           Syt1 KO + Syt1 C2A-3DA rescue         eIPSC amplitude (nA)         0.173         0.045         C2AB-6DA rescue         13           Syt1 KO + Syt1 C2A-3DA rescue         eIPSC amplitude (nA)         0.173         0.045         C2AB-6DA rescue         10           Fig. 6 c         WT         mIPSC Syt1 KO         erscue         2.092         0.158         otherscue rescue; P=0.016, compared with Syt1 rescue; P=0.016, compared with Syt1- C2B-3DA rescue; P=0.016, compared with Syt1- C2B-3DA rescue; P=0.017, compared with Syt1- C2B-3DA rescue; P=0.0101, compared with Syt1- C2B-3DA rescue; P=0.0101, co						P=0.763, compared with Syt1	
Fig. 6 c         Syt1 KO         amplitude (nA)         0.085         0.018         rescue and Syt1-C2A-BD A rescue; P=0.087, compared with Syt1-C2A-BD A rescue         14           Syt1 KO + Syt1 rescue         eIPSC amplitude (nA)         2.955         0.133         Pe.0.001, compared with Syt1-C2A-BD A rescue; P=0.087, compared with Syt1-C2A-BD A rescue and Syt1-C2A-BD A rescue         14           Syt1 KO + Syt1 C2A-3DA rescue         eIPSC amplitude (nA)         0.173         0.045         C2AB-6DA rescue         17           Syt1 KO + Syt1 C2A-3DA rescue         eIPSC amplitude (nA)         0.173         0.045         C2AB-6DA rescue         10           Fig. 6 c         WT         miPSC rescue         rescue         P=0.001, compared with Syt1 rescue         18           Fig. 6 c         WT         miPSC requency (Hz)         2.092         0.158         others rescue         19           Syt1 KO + Syt1 C2A-3DA rescue, P=0.001, compared with Syt1-C2A-3DA rescue, P=0.001, compared with Syt1-	Fig. 6 b	WT	amplitude (nA)	3.034	0.222	rest	14
Fig. 6 c         WT + 0.01mM Ca         mIPSC Frequency (Hz)         0.085 0.01         0.085 0.01         Peol.007, compared with Syt1- C2B-3DA rescue; mompared with Syt1- rescue; mompared with Syt1- c2B-3DA rescue; mompared with Syt1- rescue; mompared with Syt1- c2B-3DA rescue; mompared with Syt1- rescue; mompared with Syt1- c2B-3DA rescue; mompared with	-					P<0.001, compared with Syt1	
Sy11 KO         eIPSC amplitude (nA)         0.085         0.018         rescue         P=0.0978. compared with Sy1-C2AB-6DA           Sy11 KO + Sy11 rescue         eIPSC amplitude (nA)         0.085         0.113         rescue         14           Sy11 KO + Sy11 rescue         eIPSC amplitude (nA)         2.955         0.133         rescues         14           Sy11 KO + Sy11 C2A-3DA rescue         eIPSC amplitude (nA)         0.173         0.045         P=0.001, compared with Sy11- C2B-3DA rescue and Sy11-C2AB- for rescue         17           Sy11 KO + Sy11 C2A-3DA rescue         eIPSC amplitude (nA)         0.173         0.045         P=0.7165, compared with Sy11- C2B-3DA rescue         13           Sy11 KO + Sy11 C2AB- gDA rescue         eIPSC amplitude (nA)         0.15         0.037         see above         10           Fig. 6 c         WT         Frequency (Hz)         2.092         0.158         P=0.001, compared with Sy11 rescue; P=0.001, compared with Sy11- c2B-3DA rescue; P=0.001, com						rescue and Syt1-C2A-3DA rescue; P=0.0677 compared with Syt1-	
Syrt KO         amplitude (n)         0.085         0.018         rescue         14           Syrt KO + Syrt rescue         amplitude (nA)         2.955         0.133         P<0.001, compared with all other rescues         14           Syrt KO + Syrt C2A-3DA rescue         amplitude (nA)         1.42         0.208         6DA rescue         17           Syrt KO + Syrt C2B-3DA rescue         eIPSC amplitude (nA)         1.42         0.208         6DA rescue         17           Syrt KO + Syrt C2B-3DA rescue         eIPSC amplitude (nA)         0.173         0.045         C2AB+6DA rescue         13           Syrt KO + Syrt C2AB- 6DA rescue         eIPSC amplitude (nA)         0.15         0.037         see above         10           Fig. 6 c         WT         Frequency (Hz)         2.092         0.158         rescue; P<0.001, compared with Syrt rescue; P<0						C2B-3DA rescue; P=0.0978,	
Optimize         ainplitude (nA)         Code         Code <thcode< th="">         Code         <thcode< th=""> <thcode< th="">         Code</thcode<></thcode<></thcode<>		Sut1 KO	eIPSC	0.085	0.018	compared with Syt1-C2AB-6DA	14
Syrt KO + Syrt rescue         amplitude (nA)         2.955         0.133         rescues         14           Syrt KO + Syrt C2A-3DA         eIPSC         amplitude (nA)         1.42         0.208         DA rescue and Syrt-C2AB- C2B-3DA rescue and Syrt-C2AB- C2B-3DA rescue and Syrt-C2AB- amplitude (nA)         1.42         0.208         DA rescue         17           Syrt KO + Syrt C2B-3DA         eIPSC amplitude (nA)         0.173         0.045         C2AB-6DA rescue         17           Syrt KO + Syrt C2B-3DA         eIPSC amplitude (nA)         0.173         0.045         C2AB-6DA rescue         10           Syrt KO + Syrt 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 Syrt rescue; P=0.0215<0.05, compared with Syrt -C2AB-6DA rescue; P=0.0215<0.05, compared with Syrt - C2B-3DA rescue; P=0.031, compared with Syrt - C2B-3DA rescue; P=0.031, compared with Syrt - C2B-3DA rescue; P=0.031, compared with Syrt - C2B-3DA rescue; P=0.01, compared with Syrt - C2B-3DA rescue         28           Syrt KO + Syrt C2A-3DA rescue         mIPSC Frequency (Hz)         0.126         P<0.001, compared with Syrt - C2B-3DA rescue; P=0.01, compared with Syrt - C2B-3DA rescu		- Syll KO		0.005	0.018		14
Syt1 KO + Syt1 C2A-3DA rescueeIPSC amplitude (nA)1.42P-0.001, compared with Syt1- C2B-3D rescue and Syt1-C2AB- D2B0 Freque and Syt1-C2AB- C2AB-6DA rescue17Syt1 KO + Syt1 C2A-B- 6DA rescueeIPSC amplitude (nA)0.1730.045P=0.7165, compared with Syt1- C2AB-6DA rescue13Syt1 KO + Syt1 C2AB- 6DA rescueeIPSC amplitude (nA)0.150.037see above10Fig. 6 cWTmIPSC Frequency (Hz)2.0920.158others18Fig. 6 cWTmIPSC Frequency (Hz)2.0920.168others18Fig. 7 Syt1 KOSyt1 KOmIPSC Frequency (Hz)2.0920.168others18Syt1 KOmIPSC Frequency (Hz)14.6461.307rescue; P=0.011, compared with Syt1 rescue; P=0.011, compared with Syt1- C2B-3DA rescue; P=0.01, compared with Syt1- C2B-3DA rescue; P=		Syt1 KO + Syt1 rescue	amplitude (nA)	2.955	0.133	rescues	14
Syrt IK 0 + Syrt C2A-3DA rescue         eIPSC amplitude (nA)         1.42         0.208         6DA rescue         17           Syrt IK 0 + Syrt C2B-3DA rescue         eIPSC amplitude (nA)         0.173         0.045         P=0.7165, compared with Syrt- C2AB-6DA rescue         13           Syrt IK 0 + Syrt C2AB- 6DA rescue         eIPSC amplitude (nA)         0.173         0.045         C2AB-6DA rescue         10           Fig. 6 c         WT         Frequency (Hz)         2.092         0.158         0.037         see above         10           Fig. 6 c         WT         Frequency (Hz)         2.092         0.158         0.037         see above         10           Fig. 6 c         WT         Frequency (Hz)         2.092         0.158         0.037         see above         10           Fig. 6 c         WT         Frequency (Hz)         2.092         0.158         others         18           Fig. 6 c         WT         Frequency (Hz)         14.646         1.307         rescue         29           Syt1 KO         Frequency (Hz)         14.646         1.307         rescue         29           Syt1 KO + Syt1 C2A-3DA rescue         mIPSC Frequency (Hz)         11.099         0.77         rescue         29           Syt1 KO						P<0.001, compared with Svt1-	
rescue         amplitude (nA)         1.42         0.208         6DA rescue         17           Syt1 K0 + Syt1 C2B-3DA rescue         eIPSC amplitude (nA)         0.173         0.045         C2AB-6DA rescue         13           Syt1 K0 + Syt1 C2AB- 6DA rescue         eIPSC amplitude (nA)         0.175         0.037         see above         10           Fig. 6 c         WT         mIPSC Frequency (Hz)         2.092         0.158         0.037         see above         10           Fig. 6 c         WT         mIPSC Frequency (Hz)         2.092         0.158         Pe0.001, compared with Syt1 rescue; P.0.001, compared with Syt1 rescue; P.0.001, compared with Syt1 rescue; P.0.0125-0.05, compared with Syt1-C2AB-3DA rescue; Pe0.001, compared with Syt1- C2B-3DA rescue; Pe0.013-00 rescue; Pe0.001, compared with Syt1- C2B-3DA rescue; Pe0.013-00 rescue; Pe0.001, compared with Syt1- C2B-3DA rescue; Pe0.013-00 rescue; Pe0.001, compared with Syt1- C2B-3DA rescue; Pe0.015- compared with Syt1-C2AB-6DA         29           Syt1 KO + Syt1 rescue         mIPSC Frequency (Hz)         14.646         1.307         rescue         29           Syt1 KO + Syt1 C2A-3DA rescue         mIPSC Frequency (Hz)         11.099         0.7         rescue         28           Syt1 KO + Syt1 C2A-3DA rescue         mIPSC Frequency (Hz)         11.099         0.7         rescue         28           Syt1 KO + Syt1 C2AB-3DA rescue		Syt1 KO + Syt1 C2A-3DA	eIPSC			C2B-3DA rescue and Syt1-C2AB-	
Syft K0 + Syft C2B-3DA rescue         eIPSC amplitude (nA)         0.173         0.045         C2AB-6DA rescue         13           Syft K0 + Syft C2AB- 6DA rescue         eIPSC amplitude (nA)         0.15         0.037         see above         10           Fig. 6 c         WT         mIPSC Frequency (Hz)         0.15         0.037         see above         10           Fig. 6 c         WT         mIPSC Frequency (Hz)         2.092         0.158         0.037         see above         10           Fig. 6 c         WT         mIPSC Frequency (Hz)         2.092         0.158         0.037         see above         10           Fig. 6 c         WT         mIPSC Frequency (Hz)         2.092         0.158         P-0.001; compared with Syft rescue; P-0.0215         0.500; compared with Syft rescue; P-0.0215         0.001; compared with Syft rescue; P-0.0215         2.001         0.296         Proteorpared with Syft rescue         29           Syft KO + Syft C2A-3DA rescue         mIPSC Frequency (Hz)         14.646         1.307         rescue         29           Syft KO + Syft C2A-3DA rescue         mIPSC Frequency (Hz)         2.001         0.296         Proteorpared with Syft rescue         29           Syft KO + Syft C2A-3DA rescue         mIPSC Frequency (Hz)         11.099         0.77         re		rescue	amplitude (nA)	1.42	0.208	6DA rescue	17
Itescue     aniplicade (IN)     0.113     0.043     C2ADF0LAT rescue     13       Sy11 KO + Sy11 C2AB- 6DA rescue     eIPSC amplitude (IA)     0.15     0.037     see above     10       Fig. 6 c     WT     mIPSC Frequency (Hz)     2.092     0.158     others     18       Sy11 KO     Frequency (Hz)     14.646     1.307     rescue     29       Sy11 KO     Frequency (Hz)     14.646     1.307     rescue     29       Sy11 KO + Sy11 rescue     mIPSC Frequency (Hz)     2.001     0.296     three rescues     19       Sy11 KO + Sy11 C2A-3DA rescue     mIPSC Frequency (Hz)     11.099     0.7     rescue     28       Sy11 KO + Sy11 C2A-3DA rescue     mIPSC Frequency (Hz)     14.296     0.6     see above     28       Sy11 KO + Sy11 C2AB- 6DA rescue     mIPSC Frequency (Hz)     14.296     0.6     see above     28       Sy11 KO + Sy11 C2AB- 6DA rescue     mIPSC		Syt1 KO + Syt1 C2B-3DA	eIPSC	0 172	0.045	P=0.7165, compared with Syt1-	12
Syt1 KO + Syt1 C2AB- 6DA rescueeIPSC amplitude (nA)0.150.037see above10Fig. 6 cWTFrequency (Hz)2.0920.158P<0.001, compared with Syt1 rescue; P<0.001, compared with Syt1 rescue29Syt1 KOmIPSC Frequency (Hz)14.6461.307rescueSyt1 KO + Syt1 C2A-3DA rescuemIPSC Frequency (Hz)2.0010.296P<0.001, compared with Syt1- rescue28Syt1 KO + Syt1 C2A-3DA rescuemIPSC Frequency (Hz)11.0990.77rescue28Syt1 KO + Syt1 C2A-3DA rescuemIPSC Frequency (Hz)0.680.66see above28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)0.640.66see above28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)0.680.66see above28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)0.680.66see above28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)0.68 <td< td=""><td></td><td>Tescue</td><td></td><td>0.175</td><td>0.045</td><td>CZAB-ODA Tescue</td><td>15</td></td<>		Tescue		0.175	0.045	CZAB-ODA Tescue	15
Bit No. Log / Log		Svt1 KO + Svt1 C2AB-	eIPSC				
Fig. 6 cWTmIPSC Frequency (Hz)2.0920.158P=0.001, compared with Sy1 rescue; P=0.001, compared with sy1 rescue; P=0.001, compared with sy1 rescue; P=0.0215 C.2B-3DA rescue; P=0.001, compared with sy1- c2B-3DA rescue; P=0.001, compared with sy1- c2B-3DA rescue; P=0.01118Sy11 KOSy11 KO + Sy11 rescuemIPSC Frequency (Hz)14.6461.307rescue29Sy11 KO + Sy11 rescuemIPSC Frequency (Hz)0.296P<0.001 compared with Sy1- c2B-3DA rescue; P=0.6134, rescue29Sy11 KO + Sy11 rescuemIPSC Frequency (Hz)0.016P<0.001 compared with Sy1- c2B-3DA rescue; P=0.6134, rescue19Sy11 KO + Sy11 C2A-3DA rescuemIPSC Frequency (Hz)0.016P<0.001 compared with Sy1- c2B-3DA rescue; P=0.01, compared with Sy1- c2B-3DA rescue28Sy11 KO + Sy11 C2A-3DA rescuemIPSC Frequency (Hz)0.776P<0.001, compared with Sy1- C2B-3DA rescue28Sy11 KO + Sy11 C2AB- DA rescuemIPSC Frequency (Hz)0.776P<0.001, compared with Sy1- C2B-3DA rescue28Sy11 KO + Sy11 C2AB- DA rescuemIPSC Frequency (Hz)0.66see above28Sy11 KO + Sy11 C2AB- DA rescuemIPSC Frequency (Hz)0.66see above28Sy11 KO + Sy11 C2AB- DA rescuemIPSC Frequency (Hz)0.66see above28Sy11 KO + Sy11 C2AB- DA rescuemIPSC Frequency (Hz)0.67rescue, for mIPSC C2BA rescue28Sy11 KO + Sy11 C2AB- DA rescuemIPSC Frequency (Hz)0.		6DA rescue	amplitude (nA)	0.15	0.037	see above	10
Fig. 6 cWTmIPSC Frequency (Hz)2.0920.158others18Fig. 6 cWTFrequency (Hz)2.0920.158others18Fig. 6 cWTFrequency (Hz)2.0920.158others18Fig. 6 cWTFrequency (Hz)2.0920.158others18Fig. 6 cWTFrequency (Hz)14.6461.307rescue; P=0.8134, compared with Syt1-C2A-B6DASyt1 KOFrequency (Hz)14.6461.307rescue29Syt1 KO + Syt1 rescueFrequency (Hz)2.0010.296three rescues19Syt1 KO + Syt1 C2A-3DA rescueFrequency (Hz)11.0990.77rescue28Syt1 KO + Syt1 C2B-3DA rescuemIPSC Frequency (Hz)11.0990.77rescue28Syt1 KO + Syt1 C2B-3DA 6DA rescuemIPSC Frequency (Hz)P<0.001, compared with Syt1- C2B-3DA rescue28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)0.776C2AB-6DA rescue28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)0.66see above28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)14.2960.6see above28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)14.2960.6see above28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)14.2960.6see above28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)0.012 <td></td> <td></td> <td></td> <td></td> <td></td> <td>P&lt;0.001, compared with Syt1 KO;</td> <td></td>						P<0.001, compared with Syt1 KO;	
Fig. 6 c         WT         Frequency (Hz)         2.092         0.158         others         18           Fig. 6 c         WT         Frequency (Hz)         2.092         0.158         others         18           P<0.001, compared with Syt1			mIPSC			rescue; P<0.001, compared with	
Fig. 6.d       WT + 0.01mM Ca       mIPSC       P<0.001, compared with Sy11- cscue; P=0.0215-0.05, compared with Sy11-C2A-3DA rescue; P<0.001, compared with Sy11- C2B-3DA rescue; P<0.8134, compared with Sy11-C2AB-6DA       29         Sy11 KO       Frequency (Hz)       14.646       1.307       rescue; P<0.01 compared with other	Fig. 6 c	WT	Frequency (Hz)	2.092	0.158	others	18
Syt1 KO       mIPSC       P<0.001, compared with Syt1-C2A-3DA rescue;						rescue: P=0.0215<0.05, compared	
Syt1 KOmIPSC Frequency (Hz)14.6461.307P<0.001, compared with Syt1- C2B-3DA rescue; P=0.8134, compared with Syt1-C2AB-6DASyt1 KO + Syt1 rescuemIPSC Frequency (Hz)0.296P<0.001 compared with Syt1- c2B-3DA rescue; P<0.01, compared with Syt1- C2B-3DA rescue; P<0.01; compared with Syt1- C2B-3DA rescue; P<0.01; 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 C2B-3DA rescue, for mIPSC requency, P<0.001; According to Two-way ANOVA, compare WT with syt1 C2B-3DA rescue, for mIPSC requency, P<0.001; According to Two-way ANOVA, compare WT with syt1 C2B-3DA rescue, for mIPSC requency, P<0.001; According to Two-way ANOVA, compare WT with syt1 C2B-3DA rescue, for mIPSC requency, P<0.001; According to Two-way ANOVA, compare WT with syt1 C2B-3DA rescue, for mIPSC requency, P<0.001; According to Two-way ANOVA, compare WT with syt1 C2B-3DA rescue, for mIPSC requency, P<0.001; According to Two-way ANOVA, compare WT with syt1 C2B-3DA rescue, for mIPSC requency, P<0.001; According to Two-way ANOVA, compare WT with syt1 C2B-3DA rescue, for mIPSC<						with Syt1-C2A-3DA rescue;	
Syt1 KOmIPSC Frequency (Hz)14.6461.307compared with Syt1-C2AB-6DA rescue29Syt1 KO + Syt1 rescuemIPSC Frequency (Hz)0.296P<0.001 compared with other three rescues19Syt1 KO + Syt1 C2A-3DA rescuemIPSC Frequency (Hz)0.296P<0.001, compared with Syt1- C2B-3DA rescue; P<0.01, compared with Syt1-C2AB-6DA19Syt1 KO + Syt1 C2A-3DA rescuemIPSC Frequency (Hz)11.0990.7rescue28Syt1 KO + Syt1 C2B-3DA rescuemIPSC Frequency (Hz)6.4210.776C2AB-6DA rescue28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)6.4210.776C2AB-6DA rescue28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)14.2960.6see above28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)0.0580.012rescue, for mIPSC frequency, P<0.001; According to Two-way ANOVA, compare WT with syt1 C2B3DA rescue, for mIPSC C2B3DA rescue, for mIPSC8						P<0.001, compared with Syt1- C2B-3DA rescue: P=0.8134	
Syt1 KOFrequency (Hz)14.6461.307rescue29Syt1 KO + Syt1 rescuemIPSC Frequency (Hz)2.0010.296P<0.001 compared with other three rescues19Syt1 KO + Syt1 C2A-3DA rescuemIPSC Frequency (Hz)P<0.001, compared with Syt1- C2B-3DA rescue; P<0.01, compared with Syt1-C2AB-6DA rescue28Syt1 KO + Syt1 C2B-3DA rescuemIPSC Frequency (Hz)0.77P<0.001, compared with Syt1- C2AB-6DA rescue28Syt1 KO + Syt1 C2B-3DA rescuemIPSC Frequency (Hz)6.4210.776P<0.001, compared with Syt1- C2AB-6DA rescue28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)6.4210.776C2AB-6DA rescue28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)14.2960.6see above28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)14.2960.6see above28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)14.2960.6see above28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)0.68see above 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 mIPSC8Fig. 6 dWT + 0.01mM CamIPSC Frequency (Hz)0.0580.012frequency rescue, for mIPSC			mIPSC			compared with Syt1-C2AB-6DA	
Syt1 K0 + Syt1 rescuemIPSC Frequency (Hz)2.0010.296Hree rescues19Syt1 K0 + Syt1 C2A-3DA rescuemIPSC Frequency (Hz)0.296Nescue; P<0.01, compared with Syt1- C2B-3DA rescue; P<0.01, compared with Syt1-C2AB-6DA28Syt1 K0 + Syt1 C2B-3DA rescuemIPSC Frequency (Hz)0.776P<0.001, compared with Syt1- C2B-3DA rescue28Syt1 K0 + Syt1 C2B-3DA rescuemIPSC Frequency (Hz)0.776P<0.001, compared with Syt1- C2AB-6DA rescue28Syt1 K0 + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)0.66see above28Syt1 K0 + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)0.676off See above28Syt1 K0 + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)0.676see above28Syt1 K0 + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)0.0580.012see above28		Syt1 KO	Frequency (Hz)	14.646	1.307	rescue	29
Syt1 K0 + Syt1 C2A-3DA rescue       mIPSC Frequency (Hz)       11.09       0.7       P<0.001, compared with Syt1- C2B-3DA rescue; P<0.01, compared with Syt1-C2AB-6DA         Syt1 K0 + Syt1 C2B-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 K0 + Syt1 C2B-3DA rescue       mIPSC Frequency (Hz)       6.421       0.776       P<0.001, compared with Syt1- C2AB-6DA rescue       28         Syt1 K0 + Syt1 C2AB- 6DA rescue       mIPSC Frequency (Hz)       14.296       0.6       see above       28         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; According to Two-way ANOVA, compare WT with syt1 C2B3DA rescue, for mIPSC		Svt1 KO + Svt1 rescue	mIPSC Frequency (Hz)	2 001	0 296	P<0.001 compared with other	19
Syt1 KO + Syt1 C2A-3DA rescue       mIPSC Frequency (Hz)       11.09       0.7       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)       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         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       mIPSC         Fig. 6.d       WT + 0.01mM Ca       mIPSC Frequency (Hz)       0.058       0.012       frequency, P<0.001; C2B3DA rescue, for mIPSC       8				2.001	0.200	P<0.001, compared with Syt1-	
rescue       Frequency (Hz)       11.099       0.7       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)       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         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       mIPSC         Fin 6 d       WT + 0.01mM Ca       mIPSC Frequency (Hz)       0.058       0.012       frequency P<0.001;		Sv#1 KO + Sv#1 C2A 2DA	mIPSC			C2B-3DA rescue; P<0.01,	
Syt1 KO + Syt1 C2B-3DA rescuemIPSC Frequency (Hz)6.4210.776P<0.001, compared with Syt1- C2AB-6DA rescue28Syt1 KO + Syt1 C2AB- 6DA rescuemIPSC Frequency (Hz)14.2960.6see above28According 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 C2A3DA rescue, 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 C2B3DA rescue, for mIPSC According to Two-way ANOVA, compare WT with syt1 C2B3DA rescue, for mIPSC C2B3DA rescue, for mIPSC8		rescue	Frequency (Hz)	11.099	0.7	rescue	28
rescue       Frequency (Hz)       6.421       0.776       C2AB-6DA rescue       28         Syt1 KO + Syt1 C2AB- 6DA rescue       mIPSC Frequency (Hz)       number of the secue       28       28         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 C2A3DA rescue, for mIPSC frequency, P<0.001; According to Two-way ANOVA, compare WT with syt1 C2B3DA rescue, for mIPSC         Fin 6 d       WT + 0.01mM Ca       mIPSC Frequency (Hz)       0.058       0.012       frequency, P<0.001; According to Two-way ANOVA, compare WT with syt1 C2B3DA rescue, for mIPSC       8		Syt1 KO + Syt1 C2B-3DA	mIPSC			P<0.001, compared with Syt1-	
Syt1 KO + Syt1 C2AB- 6DA rescue       mIPSC Frequency (Hz)       14.296       0.6       see above       28         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         Fin 6 d       WT + 0.01mM Ca       mIPSC Frequency (Hz)       0.058       0.012       frequency, P<0.001; According to Two-way ANOVA, compare WT with syt1       8		rescue	Frequency (Hz)	6.421	0.776	C2AB-6DA rescue	28
Syt1 KO + Syt1 C2AB- 6DA rescue       mIPSC Frequency (Hz)       14.296       0.6       see above       28         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         Fin 6 d       WT + 0.01mM Ca       mIPSC Frequency (Hz)       0.058       0.012       frequency, P<0.001; According to Two-way ANOVA, compare WT with syt1       8							
BibA rescue       Prequency (Hz)       14.290       0.0       see above       28         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 C2A3DA rescue, for mIPSC frequency, P<0.001; According to Two-way ANOVA, compare WT with syt1		Syt1 KO + Syt1 C2AB-	mIPSC	14 206	0.6		20
Fig. 6 d       WT + 0.01mM Ca       mIPSC       0.058       0.012       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		UDA IESCUE		14.290	0.0	According to Two-way ANOVA.	20
Fig. 6 d       WT + 0.01mM Ca       Frequency (Hz)       0.058       0.012       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         Eig. 6 d       WT + 0.01mM Ca       Frequency (Hz)       0.058       0.012       frequency, P<0.001;						compare WT with syt1 KO, for	
Fig. 6 d       WT + 0.01mM Ca       Frequency (Hz)       0.058       0.012       frequency         Fig. 6 d       WT + 0.01mM Ca       Frequency (Hz)       0.058       0.012       frequency						According to Two-way ANOVA.	
Fig. 6 d       WT + 0.01mM Ca       Frequency (Hz)       0.058       0.012       frequency of mIPSC						compare WT with syt1 C2A3DA	
Fig. 6 d WT + 0.01mM Ca Frequency (Hz) 0.058 0.012 frequency P<0.001: 8						rescue, for mIPSC frequency, P<0.001: According to Two-way	
Fig. 6 d WT + 0.01mM Ca Frequency (Hz) 0.058 0.012 frequency P<0.001: 8						ANOVA, compare WT with syt1	
(	Fig 6 d	WT + 0.01mM Ca	mIPSC Frequency (Hz)	0.058	0 012	C2B3DA rescue, for mIPSC	R

WT + 0.2mM Ca	mIPSC Frequency (Hz)	0 098	0.017		8
WT + 0.5mM Co	mIPSC	0.260	0.021		0
WT + 0.5min Ca	mIPSC	0.309	0.031		0
WT + 1mM Ca	Frequency (Hz) mIPSC	1.269	0.128		8
WT + 2mM Ca	Frequency (Hz)	3.184	0.252		8
WT + 5mM Ca	Frequency (Hz)	5.975	0.424		8
WT + 10mM Ca	mIPSC Frequency (Hz)	6.776	0.485		8
Syd1 KO + 0.01mM Co	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
	mIPSC	0.420	0.110		0
Syt1 KO + 0.2mM Ca	Frequency (Hz) mIPSC	2.845	0.767		8
Syt1 KO + 0.5mM Ca	Frequency (Hz)	7.576	0.863		8
Syt1 KO + 1mM Ca	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 <sup>2+</sup> 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
J. J	Svt1 KO	Ca <sup>2+</sup>	1 567	0 164	P=0.872, compared with Syt1- C2A-3DA rescue; P=0.5573, compared with Syt1-C2B-3DA	8
	Syt1 KO + Syt1-C2A-3DA	Ca <sup>2+</sup> cooperativity	1.538	0.065	P=0.405, compared with Syt1- C2B-3DA rescue	8
	Syt1 KO + Syt1-C2B-3DA	Ca <sup>2+</sup> 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	elPSC	0 18642	0.02147	According to Two-way ANOVA, compare ST1-D2N WT with ST1- D2N, for eIPSC amplitude, B<0.001	8
1 ig. 7 C		elPSC	0.05005	0.02147	1 \0.001,	0
		eIPSC	2 20007	0.09933		<u></u>
	WT + 2mixi Ca	elPSC	3.39997	0.18793		8
	WT + 5mM Ca	amplitude (nA)	5.15096	0.24384		8
	WT + 10mM Ca	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
	Svt1-2xlinker	mIPSC frequency (Hz)	1.85832	0.25242		21
Suppl		mIPSC			P<0.001 compared with other four	
Fig. 1 b	WT+2mM Ca	Frequency (Hz)	2.625	0.304	genotypes	15
	WT+EGTA-AM+2mM Ca	Frequency (Hz)	0.338	0.0617	genotypes	14
	WT+BAPTA-AM+2mM Ca	mIPSC Frequency (Hz)	0.0764	0.0167	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		Apparent Ca <sup>2+</sup> -			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	
Fig. 4a	ST1-D2N WT	cooperativity	2.769	0.424	ST1-R3Q P=0 2970 compared with ST1-	9
		Apparent Ca <sup>2+</sup> -	2 426	0 153	D8N WT; P=0.2901, compared with ST1-D8N; P=0.6805, compared with ST1-R3Q WT; B<0.001, compared with ST1 P30	10
		Apparent Ca <sup>2+</sup> -	2.430	0.133	P=0.1213, compared with ST1- D8N; P=0.2663, compared with ST1-R3Q WT; P<0.01, compared	10
	ST1-D8N WT	cooperativity	2.923	0.426	with ST1-R3Q; P=0.7764, compared with ST1-	10
	ST1-D8N	Apparent Ca <sup>2+</sup> - cooperativity	2.133	0.233	R3Q WT; P=0.0731, compared with ST1-R3Q;	10
	ST1-R3Q WT	Apparent Ca <sup>2+</sup> -	2.263	0.386	P=0.1318, compared with ST1- R3Q:	10
	ST1-R3Q	Apparent Ca <sup>2+</sup> - cooperativity	1.615	0.142	see above	10
Suppl Fig. 4b	ST1-D2N WT	Apparent Ca <sup>2+</sup> -	1.813	0.058	P=0.3707, compared with ST1- D2N:	8
g	ST1-D2N	Apparent Ca <sup>2+</sup> -	2.012	0.196	P=0.4458, compared with ST1- D8N; P=0.3798, compared with ST1-R3Q	9
	ST1-D8N WT	Apparent Ca <sup>2+</sup> -	2.008	0.077	P=0.2378, compared with ST1- D8N;	10
	ST1-D8N	Apparent Ca <sup>2+</sup> - cooperativity	1.839	0.115	P=0.9240, compared with ST1- R3Q;	10
	ST1-R3Q WT	Apparent Ca <sup>2+</sup> - cooperativity	1.877	0.112	P=0.7157, compared with ST1- R3Q;	8
	ST1-R3Q	Apparent Ca <sup>2+</sup> - 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



#### Effect of the Ca<sup>2+</sup>-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<sup>2+</sup>. Neurons were cultured from wild-type mice, and were preincubated with as indicated 10  $\mu$ M EGTA-AM or BATPA-AM at 37 °C for 1 hr in 2 or 0 mM extracellular Ca<sup>2+</sup> before recordings. Note that EGTA-AM is a much slower Ca<sup>2+</sup>-chelator than BAPTA-AM, and thus not as effective as BAPTA-AM in extracellular medium containing Ca<sup>2+</sup> because Ca<sup>2+</sup>-fluxes originating from Ca<sup>2+</sup>-channels or internal Ca<sup>2+</sup>-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).



#### 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).



#### 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.



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<sup>2+</sup>-binding sites.

a, Apparent Ca<sup>2+</sup>-cooperativity of evoked IPSCs, as determined by Hill equation fitting to the data shown in Fig. 3.

b, Apparent Ca<sup>2+</sup>-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).



## 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  $\mu$ M APV, 20  $\mu$ M CNQX, and 1  $\mu$ M TTX. In the BAPTA-AM treatment experiment, the neurons were pretreated with 10  $\mu$ M BAPTA-AM for 1 hr in a 37 <sup>O</sup>C incubator before recording; during the recordings, 10  $\mu$ M BAPTA-AM were added to the Ca<sup>2+</sup>-free ACSF in the bath to fully suppress the intracellular Ca<sup>2+</sup>.

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).



# 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).



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 synapto-tagmin-1 as indicated. Recordings were performed at the standard extracellular Ca<sup>2+</sup>-concentration.

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



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<sup>2+</sup>-binding site mutant of synaptotagmin-1, monitored at increasing concentrations of extracellular Ca<sup>2+</sup>.

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<sup>2+</sup> 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.



## Membrane proximity of Syt1 C2-domains does not alter apparent Ca<sup>2+</sup>-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<sup>2+</sup>-cooperativity and Ca<sup>2+</sup>-affinity of evoked IPSCs was determined by Hill function fitting to individual experiments titrating the IPSC size as a function of the extracellular Ca<sup>2+</sup>-concentration (see Figs. 7b and 7c). Data shown are means  $\pm$  SEMs (see Suppl. Table S1 for all numerical values).