

Supplemental Table 1.

Harris, K.P., Littleton, J.T., & Stewart, B.A.

Postsynaptic Syntaxin 4 negatively regulates the efficiency of neurotransmitter release.

Sample size (n), mean, SEM, and pairwise statistical comparisons for all Figures.

Figure 1A: EJP amplitude

Genotype	n	mean	SEM
Control	12	6.40	0.62
<i>Syx4</i> ⁷³	12	15.44	1.70
<i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	12	6.54	0.86
<i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	10	14.72	1.22
24B-GAL4>UAS-Syx4	10	6.90	0.58

ANOVA (Tukey's multiple comparisons test)	Summary	Adjusted P Value
Control vs. <i>Syx4</i> ⁷³	***	< 0.0001
Control vs. <i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	ns	> 0.999
Control vs. <i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	***	< 0.0001
Control vs. 24B-GAL4>UAS-Syx4	ns	0.998
<i>Syx4</i> ⁷³ vs. <i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	***	< 0.0001
<i>Syx4</i> ⁷³ vs. <i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	ns	0.991

Resting Membrane Potential

Genotype	n	mean	SEM
Control	17	-56.92	1.62
<i>Syx4</i> ⁷³	15	-57.50	1.69
<i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	11	-57.42	1.67
<i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	11	-58.10	2.29
24B-GAL4>UAS-Syx4	10	-56.70	1.80

ANOVA (Tukey's multiple comparisons test)	Summary	Adjusted P Value
Control vs. <i>Syx4</i> ⁷³	ns	0.999
Control vs. <i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	ns	>0.999
Control vs. <i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	ns	0.991
Control vs. 24B-GAL4>UAS-Syx4	ns	>0.999
<i>Syx4</i> ⁷³ vs. <i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	ns	>0.999
<i>Syx4</i> ⁷³ vs. <i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	ns	0.999

Figure 1C: Mini Frequency

Genotype	n	Mean	Std. Error of Mean
Control	17	2.62	0.31
<i>Syx4</i> ⁷³	15	3.42	0.32
<i>Syx4</i> ⁷³ 24B>UAS-Syx4	11	2.57	0.18
<i>Syx4</i> ⁷³ elav>Syx4	11	2.88	0.15
24B>UAS-Syx4	10	2.70	0.25

ANOVA (Tukey's multiple comparisons test)	Summary	Adjusted P Value
Control vs. <i>Syx4</i> ⁷³	ns	0.172
Control vs. <i>Syx4</i> ⁷³ 24B>UAS-Syx4	ns	>0.999
Control vs. <i>Syx4</i> ⁷³ elav>Syx4	ns	0.959
Control vs. 24B>UAS-Syx4	ns	>0.999

Figure 1D: Mini Amplitude

Genotype	n	mean	SEM
Control	17	0.77	0.08
<i>Syx4</i> ⁷³	15	0.78	0.06
<i>Syx4</i> ⁷³ 24B>UAS- <i>Syx4</i>	11	0.79	0.10
<i>Syx4</i> ⁷³ elav> <i>Syx4</i>	11	0.72	0.04
24B>UAS- <i>Syx4</i>	10	0.79	0.06

ANOVA (Tukey's multiple comparisons test)	Summary	Adjusted P Value
Control vs. <i>Syx4</i> ⁷³	ns	>0.999
Control vs. <i>Syx4</i> ⁷³ 24B>UAS- <i>Syx4</i>	ns	0.883
Control vs. <i>Syx4</i> ⁷³ elav> <i>Syx4</i>	ns	0.989
Control vs. 24B>UAS- <i>Syx4</i>	ns	0.925

Input resistance

Genotype	n	mean	SEM
Control	17	6.48	0.31
<i>Syx4</i> ⁷³	15	6.8	0.29
<i>Syx4</i> ⁷³ 24B>UAS- <i>Syx4</i>	11	6.73	0.48
<i>Syx4</i> ⁷³ elav> <i>Syx4</i>	11	6.91	0.43
24B>UAS- <i>Syx4</i>	10	6.79	0.38

ANOVA (Tukey's multiple comparisons test)	Summary	Adjusted P Value
Control vs. <i>Syx4</i> ⁷³	ns	0.956
Control vs. <i>Syx4</i> ⁷³ 24B>UAS- <i>Syx4</i>	ns	0.986
Control vs. <i>Syx4</i> ⁷³ elav> <i>Syx4</i>	ns	0.915
Control vs. 24B>UAS- <i>Syx4</i>	ns	0.976

Figure 2A: Paired-pulse facilitation

Genotype (interval)	n	mean	SEM
Control (25)	10	1.33	0.15
Control (50)	10	1.46	0.11
Control (75)	10	1.30	0.07
Control (100)	10	1.25	0.04
<i>Syx4</i> ⁷³ (25)	8	0.93	0.03
<i>Syx4</i> ⁷³ (50)	8	1.09	0.02
<i>Syx4</i> ⁷³ (75)	8	1.12	0.02
<i>Syx4</i> ⁷³ (100)	8	1.09	0.01
<i>Syx4</i> ⁷³ 24B-GAL4>UAS- <i>Syx4</i> (25)	8	1.58	0.09
<i>Syx4</i> ⁷³ 24B-GAL4>UAS- <i>Syx4</i> (50)	8	1.73	0.12
<i>Syx4</i> ⁷³ 24B-GAL4>UAS- <i>Syx4</i> (75)	8	1.50	0.07
<i>Syx4</i> ⁷³ 24B-GAL4>UAS- <i>Syx4</i> (100)	8	1.51	0.09
<i>Syx4</i> ⁷³ elav-GAL4>UAS- <i>Syx4</i> (25)	8	1.05	0.03
<i>Syx4</i> ⁷³ elav-GAL4>UAS- <i>Syx4</i> (50)	8	1.04	0.01
<i>Syx4</i> ⁷³ elav-GAL4>UAS- <i>Syx4</i> (75)	8	1.06	0.01
<i>Syx4</i> ⁷³ elav-GAL4>UAS- <i>Syx4</i> (100)	8	1.00	0.01
24B-GAL4>UAS- <i>Syx4</i> (25)	6	1.27	0.16
24B-GAL4>UAS- <i>Syx4</i> (50)	6	1.42	0.16
24B-GAL4>UAS- <i>Syx4</i> (75)	6	1.28	0.10

24B-GAL4>UAS-Syx4 (100)	6	1.25	0.03
-------------------------	---	------	------

ANOVA (Tukey's multiple comparisons test)	Summary	Adjusted P Value
25 ms		
Control vs. <i>Syx4</i> ⁷³	*	0.040
Control vs. <i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	ns	0.480
Control vs. <i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	ns	0.363
Control vs. 24B-GAL4>UAS-Syx4	ns	0.996
<i>Syx4</i> ⁷³ vs. <i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	**	0.004
<i>Syx4</i> ⁷³ vs. <i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	ns	0.948
50 ms		
Control vs. <i>Syx4</i> ⁷³	*	0.047
Control vs. <i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	ns	0.291
Control vs. <i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	*	0.021
Control vs. 24B-GAL4>UAS-Syx4	ns	0.998
<i>Syx4</i> ⁷³ vs. <i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	**	0.001
<i>Syx4</i> ⁷³ vs. <i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	ns	0.997
75 ms		
Control vs. <i>Syx4</i> ⁷³	ns	0.254
Control vs. <i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	ns	0.116
Control vs. <i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	*	0.041
Control vs. 24B-GAL4>UAS-Syx4	ns	>0.999
<i>Syx4</i> ⁷³ vs. <i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	**	0.002
<i>Syx4</i> ⁷³ vs. <i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	ns	0.944
100 ms		
Control vs. <i>Syx4</i> ⁷³	*	0.030
Control vs. <i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	**	0.002
Control vs. <i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	**	0.002
Control vs. 24B-GAL4>UAS-Syx4	ns	> 0.999
<i>Syx4</i> ⁷³ vs. <i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	****	< 0.0001
<i>Syx4</i> ⁷³ vs. <i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	ns	0.702

Figure 3D: Cac-GFP/Brp

Genotype	n	mean	SEM
Control	16	1.05	0.05
<i>Syx4</i> ⁷³	16	1.33	0.09
<i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	10	0.94	0.05
<i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	13	1.32	0.13
24B-GAL4>UAS-Syx4	12	0.93	0.10

ANOVA (Tukey's multiple comparisons test)	Summary	Adjusted P Value
Control vs. <i>Syx4</i> ⁷³	*	0.033
Control vs. <i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	ns	0.923
Control vs. <i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	*	0.045
Control vs. 24B-GAL4>UAS-Syx4	ns	0.864
<i>Syx4</i> ⁷³ vs. <i>Syx4</i> ⁷³ 24B-GAL4>UAS-Syx4	*	0.030
<i>Syx4</i> ⁷³ vs. <i>Syx4</i> ⁷³ elav-GAL4>UAS-Syx4	ns	> 0.999

Figure 3E: Brp/HRP

Genotype	n	Mean	SEM
Control	20	72.55	2.83
Syx4 ⁷³	20	71.95	2.93
Syx4 ⁷³ 24B-GAL4>UAS-Syx4	20	75.56	2.14
Syx4 ⁷³ elav-GAL4>UAS-Syx4	20	72.53	2.89
24B-GAL4>UAS-Syx4	20	74.11	3.32

ANOVA (Tukey's multiple comparisons test)	Summary	Adjusted P Value
Control vs. Syx4 ⁷³	ns	>0.999
Control vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.945
Control vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	> 0.999
Control vs. 24B-GAL4>UAS-Syx4	ns	0.995
Syx4 ⁷³ vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.898
Syx4 ⁷³ vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	> 0.999

HRP fluorescence intensity

Genotype	n	Mean	SEM
Control	20	49.14	2.94
Syx4 ⁷³	20	43.47	4.27
Syx4 ⁷³ 24B-GAL4>UAS-Syx4	20	46.03	2.32
Syx4 ⁷³ elav-GAL4>UAS-Syx4	20	46.26	5.85
24B-GAL4>UAS-Syx4	20	43.97	2.51

ANOVA (Tukey's multiple comparisons test)	Summary	Adjusted P Value
Control vs. Syx4 ⁷³	ns	0.800
Control vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.977
Control vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	0.983
Control vs. 24B-GAL4>UAS-Syx4	ns	0.870
Syx4 ⁷³ vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.984
Syx4 ⁷³ vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	> 0.999

Figure 4A: EJP versus [Ca2+]

Genotype ([Ca2+])	n	mean	SEM
Control (0.3)	6	2.84	0.37
Control (0.5)	6	6.73	1.76
Control (0.7)	6	23.20	2.07
Control (1.0)	6	31.85	1.08
Syx4 ⁷³ (0.3)	6	6.55	0.63
Syx4 ⁷³ (0.5)	6	15.20	1.74
Syx4 ⁷³ (0.7)	6	31.25	2.88
Syx4 ⁷³ (1.0)	6	32.31	1.74
Syx4 ⁷³ 24B-GAL4>UAS-Syx4 (0.3)	6	2.32	0.40
Syx4 ⁷³ 24B-GAL4>UAS-Syx4 (0.5)	6	5.87	0.67
Syx4 ⁷³ 24B-GAL4>UAS-Syx4 (0.7)	6	16.00	3.03
Syx4 ⁷³ 24B-GAL4>UAS-Syx4 (1.0)	5	30.27	3.07
Syx4 ⁷³ elav-GAL4>UAS-Syx4 (0.3)	6	6.43	0.63
Syx4 ⁷³ elav-GAL4>UAS-Syx4 (0.5)	6	16.07	0.97

Syx4 ⁷³ elav-GAL4>UAS-Syx4 (0.7)	5	32.34	1.63
Syx4 ⁷³ elav-GAL4>UAS-Syx4 (1.0)	6	30.81	1.67
24B-GAL4>UAS-Syx4 (0.3)	6	2.34	0.46
24B-GAL4>UAS-Syx4 (0.5)	6	7.29	0.96
24B-GAL4>UAS-Syx4 (0.7)	5	18.93	1.92
24B-GAL4>UAS-Syx4 (1.0)	6	32.71	2.19

ANOVA (Tukey's multiple comparisons test)	Summary	Adjusted P Value
0.3 mM Ca2+		
Control vs. Syx4 ⁷³	***	0.0002
Control vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.949
Control vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	***	0.0004
Control vs. 24B-GAL4>UAS-Syx4	ns	0.954
Syx4 ⁷³ vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	****	< 0.0001
Syx4 ⁷³ vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	>0.999
0.5 mM Ca2+		
Control vs. Syx4 ⁷³	***	0.001
Control vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.990
Control vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	***	0.0003
Control vs. 24B-GAL4>UAS-Syx4	ns	0.998
Syx4 ⁷³ vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	***	0.0003
Syx4 ⁷³ vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	0.989
0.7 mM Ca2+		
Control vs. Syx4 ⁷³	*	0.043
Control vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.607
Control vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	*	0.039
Control vs. 24B-GAL4>UAS-Syx4	ns	0.960
Syx4 ⁷³ vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	**	0.002
Syx4 ⁷³ vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	>0.999
1.0 mM Ca2+		
Control vs. Syx4 ⁷³	ns	>0.999
Control vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.981
Control vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	0.995
Control vs. 24B-GAL4>UAS-Syx4	ns	0.998
Syx4 ⁷³ vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.953
Syx4 ⁷³ vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	0.981

Figure 4B: Quantal content versus [Ca2+]

Genotype ([Ca2+])	n	mean	SEM
Control (0.3)	6	2.89	0.38
Control (0.5)	6	8.73	1.94
Control (0.7)	6	24.37	2.56
Control (1.0)	6	38.84	1.45
Syx4 ⁷³ (0.3)	6	6.79	0.67
Syx4 ⁷³ (0.5)	6	16.59	2.00
Syx4 ⁷³ (0.7)	6	37.77	3.95
Syx4 ⁷³ (1.0)	6	39.27	2.70
Syx4 ⁷³ 24B-GAL4>UAS-Syx4 (0.3)	6	2.35	0.41

Syx4 ⁷³ 24B-GAL4>UAS-Syx4 (0.5)	6	6.08	0.71
Syx4 ⁷³ 24B-GAL4>UAS-Syx4 (0.7)	6	17.98	3.96
Syx4 ⁷³ 24B-GAL4>UAS-Syx4 (1.0)	5	36.60	4.32
Syx4 ⁷³ elav-GAL4>UAS-Syx4 (0.3)	6	6.68	0.69
Syx4 ⁷³ elav-GAL4>UAS-Syx4 (0.5)	6	17.61	1.15
Syx4 ⁷³ elav-GAL4>UAS-Syx4 (0.7)	5	38.61	2.10
Syx4 ⁷³ elav-GAL4>UAS-Syx4 (1.0)	6	36.33	2.43
24B-GAL4>UAS-Syx4 (0.3)	6	2.37	0.47
24B-GAL4>UAS-Syx4 (0.5)	6	7.60	1.05
24B-GAL4>UAS-Syx4 (0.7)	5	21.23	2.51
24B-GAL4>UAS-Syx4 (1.0)	6	40.07	3.02

ANOVA (Tukey's multiple comparisons test)	Summary	Adjusted P Value
0.3 mM Ca2+		
Control vs. Syx4 ⁷³	***	0.0002
Control vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.995
Control vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	***	0.0003
Control vs. 24B-GAL4>UAS-Syx4	ns	0.960
Syx4 ⁷³ vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	****	< 0.0001
Syx4 ⁷³ vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	>0.999
0.5 mM Ca2+		
Control vs. Syx4 ⁷³	**	0.007
Control vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.704
Control vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	**	0.0020
Control vs. 24B-GAL4>UAS-Syx4	ns	0.981
Syx4 ⁷³ vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	***	0.0003
Syx4 ⁷³ vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	0.987
0.7 mM Ca2+		
Control vs. Syx4 ⁷³	ns	0.153
Control vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.238
Control vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	0.105
Control vs. 24B-GAL4>UAS-Syx4	ns	0.745
Syx4 ⁷³ vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	**	0.001
Syx4 ⁷³ vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	0.998
1.0 mM Ca2+		
Control vs. Syx4 ⁷³	ns	>0.999
Control vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.986
Control vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	0.967
Control vs. 24B-GAL4>UAS-Syx4	ns	0.998
Syx4 ⁷³ vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.965
Syx4 ⁷³ vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	ns	0.943

Figure 4C

Linear regression		
Genotype	Slope	SEM
Control	2.64	0.22
Syx4 ⁷³	1.65	0.20
Syx4 ⁷³ 24B-GAL4>UAS-Syx4	2.42	0.26

Syx4 ⁷³ elav-GAL4>UAS-Syx4	1.67	0.24
24B-GAL4>UAS-Syx4	2.58	0.27

ANOVA (Tukey's multiple comparisons test)	Summary	Adjusted P Value
Control vs. Syx4 ⁷³	*	0.039
Control vs. Syx4 ⁷³ 24B-GAL4>UAS-Syx4	ns	0.969
Control vs. Syx4 ⁷³ elav-GAL4>UAS-Syx4	*	0.045
Control vs. 24B-GAL4>UAS-Syx4	ns	>0.999