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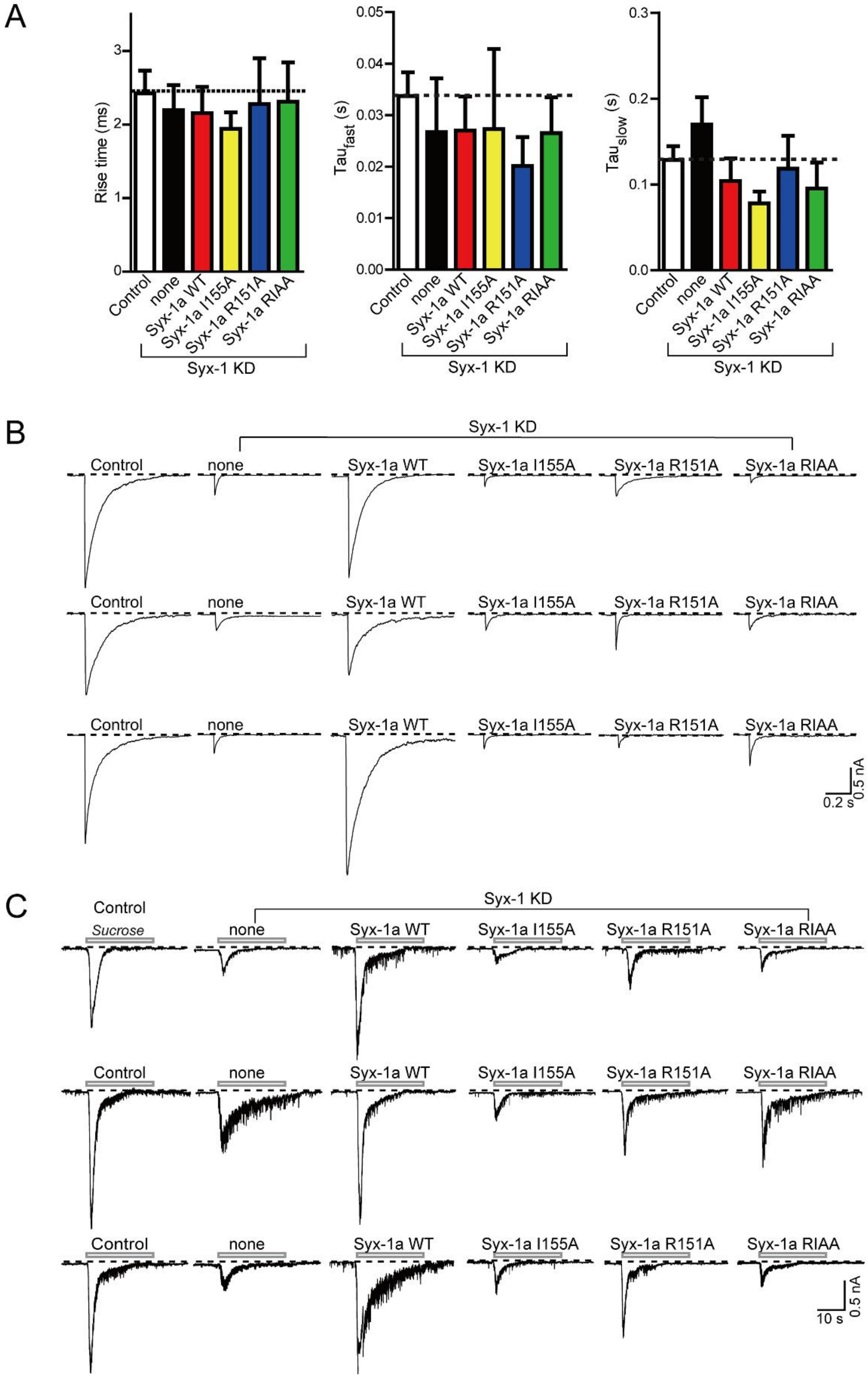
Appendix Table S2: Data summary table for results shown in Figure 4 for syntaxin-1-CC.

Appendix Table S3: Data summary table for results shown in Figure 4 for syntaxin-1-MN.

Appendix Supplementary Figures and Figure legends

Appendix Figure S1



Appendix Figure S1

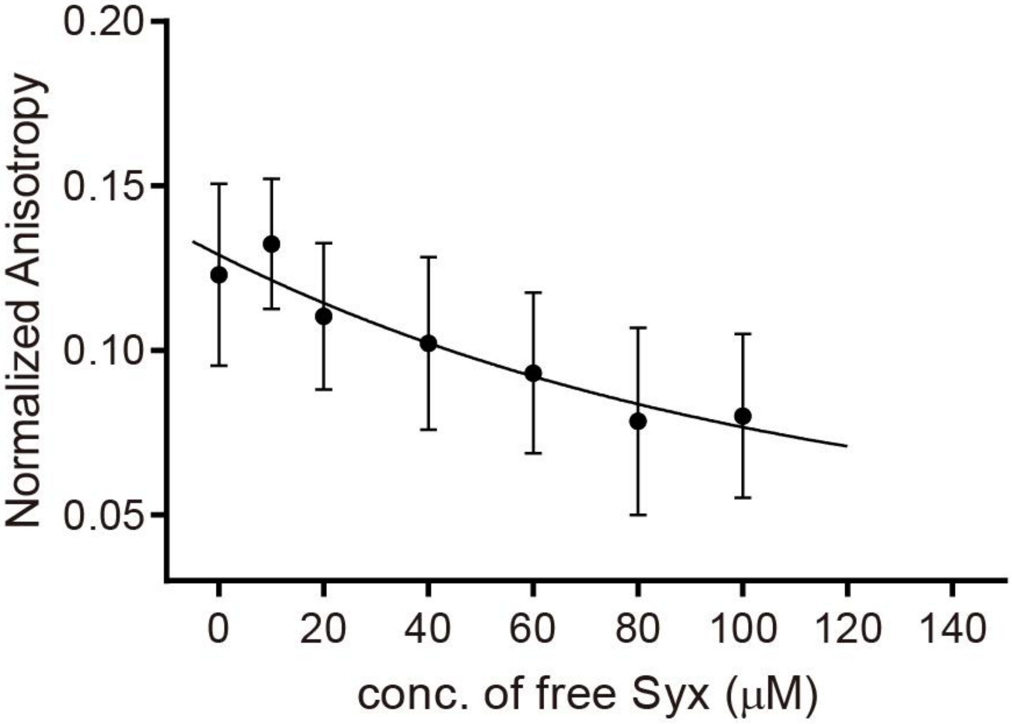
The syntaxin-1 R151A, I155A mutations do not influence the release kinetics.

A. Summary graphs of rise times (left), decay times τ_{fast} (center), and decay times τ_{slow} (right) of the evoked-IPSC experiments shown in Fig 2B.

B. Three additional example recordings illustrate the variability of the evoked-IPSC experiments shown in Fig 2B.

C. Three additional example recordings illustrate the variability of RRPs experiments shown in Fig 2C. Data are presented as means ± SEM.

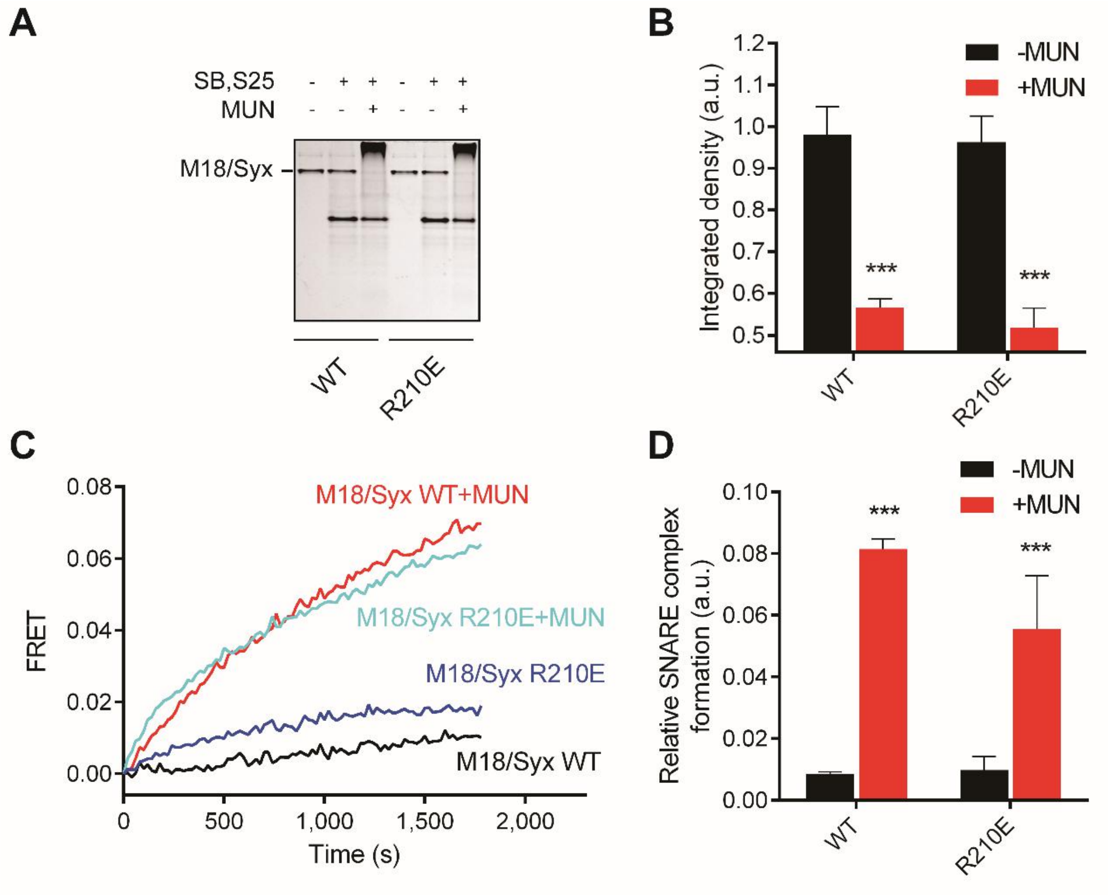
Appendix Figure S2



Appendix Figure S2

Competitive effect of the cytoplasmic domain of syntaxin-1 (residues 2–253) in the binding between the fluorescent dye-labeled syntaxin-1 linker peptide (residues 148–162) and the MUN-BC fragment. The concentrations of the MUN-BC fragment and the rhodamine B-labeled syntaxin peptide are 60 μM and 50 nM, respectively. Measurements are shown as means ± SEM (n = 10). A single exponential decay curve was fit to the data (solid line).

Appendix Figure S3



Appendix Figure S3

The syntaxin-1 R210E mutant does not influence the transit of syntaxin-1 from the Munc18-1/syntaxin-1 complex to the ternary SNARE complex mediated by the MUN domain.

A, B. Measurements of the transition from the Munc18-1/syntaxin-1 complex to the SNARE complex by native gel experiments. Representative Coomassie brilliant blue-stained native electrophoresis gels from one of three independent experiments are shown. The data were processed by Image J (NIH).

C, D. Measurements of the transit of syntaxin-1 from the Munc18-1/syntaxin-1 complex to the ternary SNARE complex using an ensemble FRET assay. Measurements are shown as means ± SD and the significance was assessed by the Student's t-test using n = 3 technical replicates.

Appendix Tables

Appendix Table S1

Data summary table for the results shown in Figure 4 for the specified mutants of uncomplexed syntaxin-1-CC and syntaxin-1-CC (RIAA: R151A/I155A, LEAA: L165A/E166A, LEAA+RIAA: R151A/I155A/L165A/E166A)*

	FRET efficiency (state 1)	**FRET efficiency (state 2)**	**% population of state 1**
WT	0.311 ± 0.025	0.584 ± 0.006	33.07 ± 6.83
RIAA	0.278 ± 0.003	0.592 ± 0.015	29.11 ± 13.5
LEAA	0.347 ± 0.002	0.646 ± 0.013	90.15 ± 0.49
LEAA+RIAA	0.338 ± 0.027	0.649 ± 0.013	87.60 ± 4.38

* Shown are means ± SD.

Appendix Table S2

Data summary table for results shown in Figure 4 for syntaxin-1-CC*

	Conditions	FRET efficiency	Number of analyzed traces	Student's t-test (p value)
WT	**uncomplexed**	0.584 ± 0.006	90	0.007
	M18	0.747 ± 0.018	66	
	S25+SB	0.156 ± 0.027	77	0.002
	M18+S25+SB	0.742 ± 0.028	70	0.706
	M18+MUN	0.714 ± 0.008	57	0.131
	M18+S25+SB+MUN	0.217 ± 0.005	105	0.001
	M18+MUN NFAA	0.728 ± 0.011	65	0.319
	M18+S25+SB+MUN NFAA	0.729 ± 0.002	80	0.270
RIAA	**uncomplexed**	0.592 ± 0.015	99	0.015
	M18	0.764 ± 0.026	105	
	S25+SB	0.135 ± 0.029	93	0.002
	M18+S25+SB	0.729 ± 0.015	97	0.243
	M18+MUN	0.713 ± 0.024	136	0.179
	M18+S25+SB+MUN	0.718 ± 0.016	129	0.172
LEAA	**uncomplexed**	0.347 ± 0.002	104	0.002
	M18	0.701 ± 0.007	131	
	M18+MUN	0.693 ± 0.004	133	0.304
	M18+S25+SB	0.205 ± 0.007	90	0.0002
	M18+S25+SB+MUN	0.196 ± 0.012	102	0.0004
LEAA+RIAA	**uncomplexed**	0.338 ± 0.027	99	0.004
	M18	0.716 ± 0.023	116	
	M18+MUN	0.713 ± 0.010	98	0.879
	M18+S25+SB	0.203 ± 0.003	107	0.001
	M18+S25+SB+MUN	0.199 ± 0.017	104	0.002

* Shown are means ± SD.

Appendix Table S3
Data summary table for results shown in Figure 4 for syntaxin-1-MN*

	Conditions	**FRET efficiency**	**Number of analyzed traces**	**Student's t-test (p value)**
WT	**uncomplexed**	0.521 ± 0.001	79	0.003
	M18	0.603 ± 0.006	94	
	S25+SB	0.403 ± 0.010	92	0.002
	M18+S25+SB	0.617 ± 0.010	46	0.264
	M18+MUN	0.513 ± 0.010	80	0.008
	M18+S25+SB+MUN	0.412 ± 0.003	90	0.001
	M18+MUN NFAA	0.594 ± 0.004	82	0.221
	M18+S25+SB+MUN NFAA	0.596 ± 0.005	58	0.344
RIAA	**uncomplexed**	0.518 ± 0.009	93	0.007
	M18	0.599 ± 0.003	128	
	S25+SB	0.406 ± 0.001	153	0.0001
	M18+S25+SB	0.610 ± 0.004	70	0.078
	M18+MUN	0.613 ± 0.018	112	0.399
	M18+S25+SB+MUN	0.611 ± 0.016	105	0.396
LEAA	**uncomplexed**	0.441± 0.015	141	0.402
	M18	0.456 ± 0.013	58	
	M18+MUN	0.450 ± 0.020	91	0.746
	M18+S25+SB	0.437 ± 0.019	114	0.366
	M18+S25+SB+MUN	0.425 ± 0.016	103	0.161
LEAA+RIAA	**uncomplexed**	0.435 ± 0.013	76	0.670
	M18	0.444 ± 0.022	90	
	M18+MUN	0.450 ± 0.028	51	0.857
	M18+S25+SB	0.425 ± 0.014	117	0.400
	M18+S25+SB+MUN	0.435 ± 0.008	113	0.625

* Shown are means ± SD.