

Expanded View Figures

Figure EV1. Relative ternary SNARE complex formation bearing the syntaxin-1 linker region mutations in the presence of Munc18-1 and/or the MUN domain as monitored by native gel electrophoresis.

Integrated intensities of the bands of the Munc18-1/ syntaxin-1 (M18/Syx) complex were used to determine relative ternary SNARE complex formation: The band of the M18/Syx complex gradually disappears with ternary SNARE complex formation in the presence of SNAP-25, synaptobrevin-2, and the MUN domain. The upper panel shows representative Coomassie brilliant blue-stained native electrophoresis gels from one of three independent experiments. Data are processed by ImageJ (NIH) and shown as means \pm SEM; n.s., not significant (P > 0.05); ***P < 0.001, using Student's *t*-test with n = 3 technical replicates.





Figure EV2. The R151A, I155A mutations of syntaxin-1 have no effect on ternary SNARE complex formation with syntaxin-1, SNAP-25, and synaptobrevin-2 (i.e. in the absence of Munc18-1 and the MUN domain).

A, B Ternary SNARE complex formation as monitored by ensemble FRET efficiency between fluorescent dye-labeled SNAP-25 and the cytoplasmic domain of synaptobrevin-2 (A) and quantification of the results (B). Neg. ctrl. (negative control): The experiment was performed in the absence of syntaxin-1. Shown are means \pm SD for n = 3 technical replicates.

	H _c helix					linker
	معمعمعم	مممممممم	وووو	موموموموموم	للالالا	٥٥٥٥٥
	120	130	140	150	*	160
P32851 Rn_syx1a	QHSTLSRK	FVEVMSEYNA	TQSD.	YRERCKGRIQR	QLEITGR.	
O35526 Mm syx1a	QHSTLSRK	FVEVMSEYNA	TQSD	YRERCKGRIQR	QLEITGR.	TTTSEELE
Q16623 Hs_syx1a	QHSTLSRK	FVEVMSEYNA	TQSD	YRERCKGRIQR	QLEITGR.	
X1WC57 Dr_syx1a	QHSTLSRK	FVEVMSEYNA.	AQSE	YRERCKGRIQR	QLEITGR.	
Q24547 Dm_syx1a	QHSTLSRK	FVEVMTEYNR	TQTD	YRERCKGRIQR	QLEITGR.	PTNDDELE
O46345 Dp_s-syx	QYSTISRK	FVEVMSDYNT	TQID	.YRDRCKARIKR.	. QMEITGR.	TTTNEELE
O16000 Ce_unc64	QHSTLSRR	FVEVMTDYNK	TQTD	YRERCKGRIQR	QLDIAGK.	QVGDEDLE
P61265 Rn_syx1b	QHSTLSRK	FVEVMTEYNA	TQSK	.YRDRCKDRIQR.	. QLEITGR.	
P50279 Rn_syx2	QHSVLSRK	FVDVMTEYNE.	AQIL	FRERSKGRIQR	QLEITGR.	
Q08849 Rn_syx3	QHSVLSRK	FVEVMTKYNE.	AQVD	FRERSKGRIQR	. QLEITGK.	KTTDEELE
Q08850 Rn_syx4	QHGVLSQQ	FVELINKCNS:	MQSE	.YREKNVERIRR.	QLKITNA.	
Q08851 Rn_syx5	KLA <mark>SMS</mark> ND	FKSVLEVRTE:	NLKQ	.QRNRREQFSR.	APVSALP.	LAPNNLGGGPIVLGGES
Q63635 Rn_syx6	STRQIVRD	MKDQMSASSV	QALA	ERKNRQALL.	G	· · · · · · · · · · · · · · · · · · ·
O70257 Rn_syx7	QKDRLVAE	FTTALTNFQK	VQRQ	.AAEREKEFVAR.	VRASSRVS	GG.FPEDSSKE
Q9Z2Q7 Rn_syx8	RERLLLAS	FKNEG <mark>SE</mark> PD.		LIRSSLM.	SE.E.	AK
G3V7P1 Rn_syx12	QKERLMND	FSSALNNFQV	VQRK	.VSEKEKESIAR.	ARAGSRLS	SAE.DRQREEQL
Q9Z158 Rn_syx17	AAAAATAE	FLQLHLESVE	ELKK	QVKNEEALL.	QPSLTRSI	
P32867 Sc_sso1	QAENSRQR	FLKLIQDYRI	VDSN	.YKEENKEQAKR.	QYMIIQP.	E.ATEDEVEA
Q12241 Sc_vam3	QNGKLSAD	FKNLKTKYQS	LQQS	.YNQRKSLFPLK.	TPISPGTS	K.ERKDIHPRTEAVRQDPESSYISIKV
Q08144 Sc_tlg2	KIQTESNK	FRVLQNNYLK	FLNKDDI	LKPIRNKASAEN.	TLLLDDEE	CEEAAREKREGL
Q01590 Sc_sed5	QMKNISGS	FKDVLEERQR	LEMA	.NKDRWQKLTTD1	GHAPADDQTQ	SNHAADLTTYNNSNPFMTSLLDESSEK
Relative accessability						

Figure EV3. Sequence alignment of the syntaxin-1 linker region.

Sequence alignment was performed with Clustal Omega and interpreted with ESPript 3.0. Residues above 60% similarity are colored in red, and residues R151 and 1155 are colored in cyan and indicated by asterisks on the top of the sequences. Secondary structure and relative solvent accessibility were calculated based on the crystal structure of the Munc18-1/syntaxin-1 complex (PDB entry 3C98): white, buried; blue, exposed; cyan, partially exposed. UniProt entries of each of the sequences are on the left. Rn, *Rattus norvegicus*; Mm, *Mus musculus*; Hs, *Homo sapiens*; Dr, *Danio rerio*; Dm, *Drosophila melanogaster*; Dp, *Doryteuthis pealeii*; Ce, *Caenorhabditis elegans*; Sc, *Saccharomyces cerevisiae*.



Figure EV4. The NF residues in the MUN domain of Munc13-1 are critical for synaptic vesicle priming and neurotransmitter release.

- A Sample traces (left) and summary graphs (right) of mIPSCs recorded in WT hippocampal neurons that were infected with a control lentivirus (Control) or a lentivirus expressing only Munc13-1 shRNAs (none) or Munc13-1 shRNAs plus either full-length Munc13-1 (WT), or the C₁-C₂B-MUN fragment (C₁-C₂B-MUN) or the C₁-C₂B-MUN) or the C₁-C₂B-MUN) or the C₁-C₂B-MUN fragment containing the NFAA (N1128A, F1131A) mutations (C₁-C₂B-MUN NFAA), respectively.
- B Sample traces (left) and summary graphs (right) of action potential-evoked IPSCs recorded in the infected neuronal cultures described in panel (A).
- C Sample traces (left) and summary graphs (right) of IPSCs evoked by 0.5 M sucrose, recorded in the infected neuronal cultures described in panel (A).

Data information: Shown are means \pm SEM; numbers of cells/independent cultures analyzed are listed in the bars. Statistical assessments were performed by Student's *t*-test comparing each condition to control (*P < 0.05; **P < 0.01; ***P < 0.001).



Figure EV5. Effect of the MUN domain containing the NFAA (N1128A, F1131A) mutations on conformational changes of syntaxin-1 bound to Munc18-1. smFRET efficiency histograms and FRET efficiency values (bar chart) corresponding to the peak positions of the Gaussian functions fit to the smFRET efficiency histograms for the specified syntaxin-1 label pairs and conditions, using the same procedure as in Fig 4. The bar charts show mean values \pm SD for the two subsets of an equal partition of the data that are comprised of the observed FRET efficiency values for all molecules in each different condition (see Appendix Tables S2 and S3). Shown are smFRET efficiency histograms for the cytoplasmic domain of syntaxin-1 bound to Munc18-1, upon the addition of the NFAA mutant of the MUN domain, upon the simultaneous addition of SNAP-25, the cytoplasmic domain of synaptobrevin-2, and the NFAA mutant of the MUN domain. S25, SNAP-25; SB, synaptobrevin-2; M18, Munc18-1.