SUPPLEMENTARY MATERIALS for Khvotchev et al. "Dual Modes of Munc18-1/SNARE Interactions Are Coupled by Functionally Critical Binding to syntaxin-1 N-terminus"

SUPPLEMENTARY METHODS

Analytical ultracentrifugation (AUC). Sedimentation equilibrium experiments were performed with a Beckman Optima XL-I analytical ultracentrifuge using a 4-position An60Ti rotor and absorbance optical system. Each cell has a 6-channel carbon-epon centerpiece with two quartz windows giving an optical path length of 1.2 cm. The sample channels and reference channels were filled with 100 µL proteins and 110 µL buffers, respectively. Each cell was scanned stepwise (0.002 cm steps) at a wavelength of 280 nm and absorbance monitored relative to buffer. Samples were centrifuged at 13000 rpm, 15000 rpm and 23000 rpm at 4°C until equilibrium had been reached. After equilibrium was reached, overspeed runs at 42000 rpm were carried out to obtain baseline values of absorbance which were used in subsequent fits. The partial specific volume of Munc18-1, Syntaxin-1A\(^{2-243}\), Syntaxin-1A\(^{2-243}\) 'LE' mutant, Syntaxin-1A\(^{10-243}\), and Syntaxin-1A\(^{10-243}\) 'LE' mutant, at 4°C were calculated from their amino acid composition to be 0.7332 cm\(^3\)·g\(^{-1}\), 0.7164 cm\(^3\)·g\(^{-1}\), 0.7161 cm\(^3\)·g\(^{-1}\), 0.7167 cm\(^3\)·g\(^{-1}\), and 0.7164 cm\(^3\)·g\(^{-1}\), and their calculated monomeric molecular weights are 68952.2 Da, 28205.4 Da, 28105.2 Da, 27178.2 Da, and 27078.1 Da, respectively. The solvent density was calculated to be 1.006 g·mL\(^{-1}\) at 4°C. Data sets for the Munc18-1/Syntaxin-1A complexes were globally fitted to hetero-association model using UltraScan 7.1. Global analysis was applied to data sets obtained at different molar ratios (1:1 and 1:2) of interacting proteins and/or different rotor speeds (13000 rpm, 15000 rpm and 23000 rpm). Figures were replotted using Origin 6.03.

Isothermal Titration Calorimetry (ITC). ITC experiments were performed using a VP-ITC system (MicroCal) at 20°C in PBS buffer containing 2 mM TCEP at pH=7.3. 90-120 µM Syntaxin-1A\(^{2-243}\) or Syntaxin-1A\(^{10-243}\) solution was injected 25 times in 8 µL aliquots into the 1.4 mL sample cell containing recombinant Munc18-1 at 5-7 µM concentration. The data were fitted using a nonlinear least squares routine with a single-site binding model using Origin 7.
Crosslinking experiments. Crosslinking was performed in lysates prepared from transfected HEK293 cells in buffer containing 150 mM NaCl, 25 mM HEPES, pH 7.6, 1% Triton X-100, 1 mM EDTA and protease inhibitors. All samples were treated with 5 mM EDC (Pierce) for 2 hours on ice. Excess of crosslinker was quenched by adding β-mercaptoethanol.

Miscellaneous procedures. Immunoprecipitations, SDS PAGE, immunoblotting, confocal microscopy and other procedures were performed using standard protocols.
### Supplementary Table 1

**Apparent affinities of the Munc18-1 complexes with syntaxin 1A<sup>2-243</sup> and syntaxin 1A<sup>10-243</sup>**

<table>
<thead>
<tr>
<th>Method</th>
<th>Munc18-1/Syntaxin 1A&lt;sup&gt;2-243&lt;/sup&gt;</th>
<th>Munc18-1/Syntaxin 1A&lt;sup&gt;10-243&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Analytical ultracentrifugation</strong></td>
<td>Kd = 20.0 ± 5.0 nM (n=2)</td>
<td>Kd = 18.0 ± 4.0 nM (n=2)</td>
</tr>
<tr>
<td><strong>Isothermal calorimetry</strong></td>
<td>Kd = 11.8 ± 2.0 nM (n=3)</td>
<td>Kd = 16.6 ± 2.0 nM</td>
</tr>
</tbody>
</table>
**SUPPLEMENTARY FIGURE LEGENDS**

**Supplementary Figure 1**

‘Open’ and ‘closed’ Syntaxin-1A fragments truncated up to residue 243 but not shorter fragments form SDS-resistant SNARE complexes with SNAP-25 and synaptobrevin. HEK293 cells were co-transfected with SNAP-25A, synaptobrevin 2 and empty vector (control) or various Syntaxin-1A fragments. Cell lysates were analyzed by denaturing SDS-PAGE to visualize SNARE complexes.

**Supplementary Figure 2**

Sedimentation equilibrium analytical ultracentrifugation analysis of Munc18-1/Syntaxin-1A heterodimerization. Munc18-1 was mixed in 1:1 (○) or 1:2 (△) molar ratios with Syntaxin-1A<sub>2-243</sub> and Syntaxin-1A<sub>2-243</sub> 'LE' mutant (A) or Syntaxin-1A<sub>10-243</sub> and Syntaxin-1A<sub>10-243</sub> 'LE' mutant (B). Samples were centrifuged at 13000 rpm (black), 15000 rpm (red), 23000 rpm (green) at 4°C until equilibrium had been reached. The data shown in panels a and c are the fitting to the 2-component hetero-associating equilibrium model and the residuals between data and fitted curves for Munc-18/Syntaxin complex, respectively. The data shown in panels b and d are the fitting to the 2-component hetero-associating equilibrium model and the residuals between data and fitted curves for the Munc-18/Syntaxin 'LE' mutant complex, respectively. The fitted curves are colored in blue. Calculated K<sub>d</sub> value for the Munc18-1/Syntaxin-1A<sub>2-243</sub> complex is 0.020 ± 0.005 µM, for the Munc18-1/Syntaxin-1A<sub>2-243</sub> 'LE' mutant complex is 2.20 ± 0.3 µM, for the Munc18-1/Syntaxin-1A<sub>10-243</sub> complex is 0.018 ± 0.004 µM, and for the Munc18-1/Syntaxin-1A<sub>10-243</sub> 'LE' mutant complex is 2.0 ± 0.4 µM.

**Supplementary Figure 3**

Mutations in the SNARE motif of Syntaxin-1A<sub>1-243</sub> that impair SNARE complex formation (L205D and I209D) do not block Munc18-1 binding. HEK293 cells were co-transfected with Munc18-1 and empty vector (control) or various Syntaxin-1A fragments. Cell lysates were treated with ‘zero spacer’ crosslinker EDC and analyzed by SDS-PAGE and immunoblotting. Arrows indicate position of Syntaxin-1A fragments and crosslinked adducts of Munc18-1 and Syntaxin-1A.

**Supplementary Figure 4**

The isolated SNARE motif of Syntaxin-1A forms stable SNARE complexes. GFP-fused SNARE motif of Syntaxin-1A forms SDS-resistant SNARE complexes. HEK293 cells were co-transfected with SNAP-25A, synaptobrevin 2 and Syntaxin-1A<sub>1-243</sub> fragment or Syntaxin-1A<sub>172-243</sub> GFP fusion protein. GFP was added to stabilize unfolded SNARE motif in cells. Cell lysates were processed by boiling in SDS-PAGE loading buffer or left untreated and analyzed by SDS-PAGE and immunoblotting.
Supplementary Figure 5

Immunoprecipitation analysis of SM/Syntaxin protein complexes: dependence on the N-terminus of Syntaxin. A. Syntaxin-1A binds to Munc18-1 independent of the N-terminus. HEK293 cells were co-transfected with myc-tagged Munc18-1 or Sly I (as a control) and Syntaxin-1A or Syntaxin-3 (as a control) fragments as indicated. Immunoprecipitations were performed in cell lysates using myc monoclonal antibody. Co-immunoprecipitated Syntaxin-1A fragments were visualized by western blotting. B. Critical importance of the Syntaxin-4 N-terminus for the interaction with Munc18-3. Experiments were performed as described in A. For visualization Syntaxin-4 fragments were fused to Venus GFP.