# Supplemental Information

# SNAREpin Assembly by Munc18-1 Requires Previous Vesicle Docking by Synaptotagmin1

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## Supplemental Figures

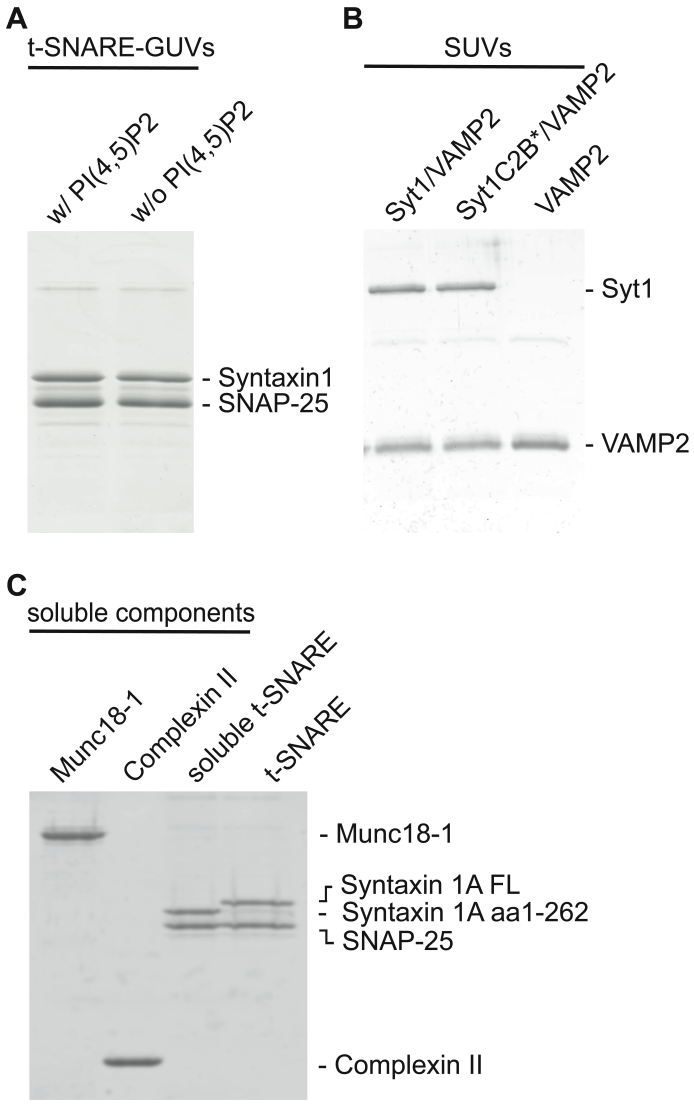
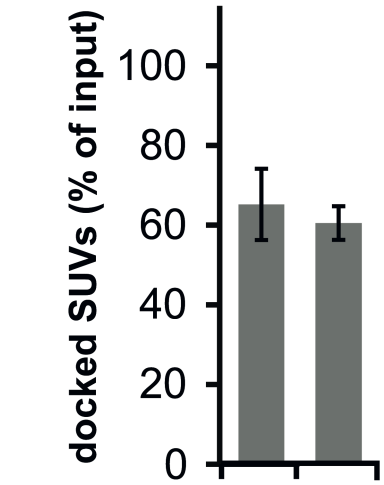



**Figure S1.** Purity of components used in this study. (A) The presence of 2 mol% PI(4,5)P2 does not effect t-SNARE reconstitution into GUVs. Same lipid amounts of reconstituted t-SNARE-GUVs were analyzed by SDS-PAGE and Coomassie Blue staining. Quantification of protein intensities with ImageJ confirms comparable amounts of proteins in each GUV population. (B) Same protein to lipid ratios were reconstituted into different SUVs. SUVs containing the same lipid amounts, but distinct protein compositions were analyzed by SDS-PAGE and Coomassie Blue staining. Quantification of protein intensities with ImageJ confirms comparable amounts of proteins in each SUV population. (C) CpxII, Munc18-1, soluble and full-length t-SNARE are purified to a high degree. 1 $\mu$g of each purified protein was analyzed by SDS-PAGE and Coomassie Blue staining.




| | | |
|---|---|---|
| VAMP2 | + | + |
| Syt1 | + | + |
| t-SNARE | + | - |
| $PI(4,5)P_2$ | + | + |
| BoNT/C | + | - |

**Figure S2.** $PI(4,5)P_2$ clusters are not required for Syt1/$PI(4,5)P_2$-mediated vesicle docking. GUVs were prepared with or without t-SNARE and subjected to BoNT/C treatment. Possible clustering of PI(4,5)P2 induced by BoNT/C cleaved syntaxin1 remnants has no effect on Syt1-mediated docking. Left bar reproduced from Fig. 1. Error bars are 95% confidence intervals (n=3).

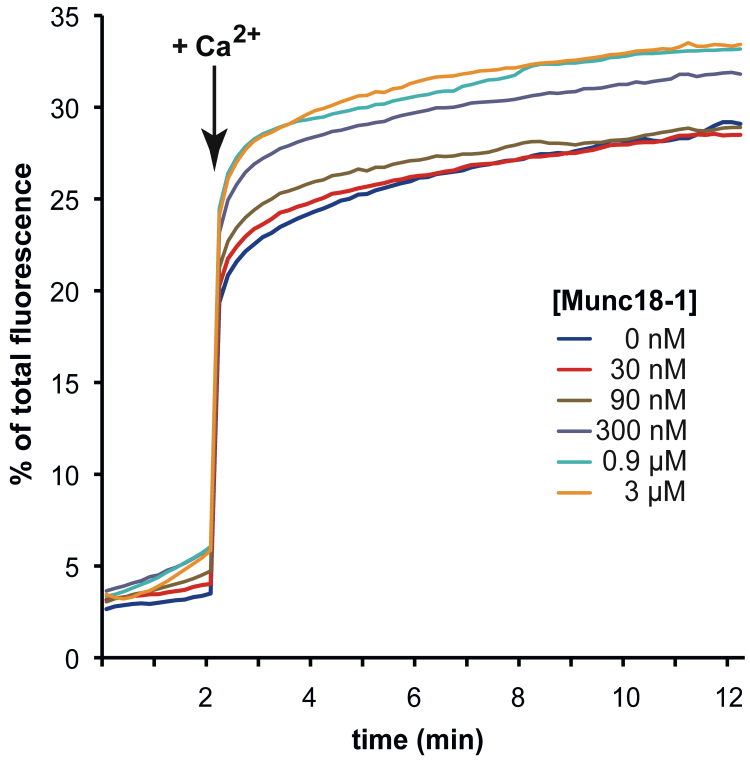



**Figure S3.** Effect of increasing Munc18-1 concentrations on lipid mixing kinetics in the presence of 6 $\mu$M CpxII. Munc18-1 was added at the indicated concentrations to syntaxin1/SNAP-25-GUVs 10 min prior to the addition of VAMP2/Syt1-SUVs. After 5 min of co-incubation of the SUV/GUV mixture at 4 °C to ensure sufficient docking, samples were heated to 37 °C and lipid mixing was measured. Saturation of the Munc18-1 stimulatory effect is reached at 0.9 $\mu$M. Lipid mixing reactions were monitored and analyzed as described in Experimental Procedures.

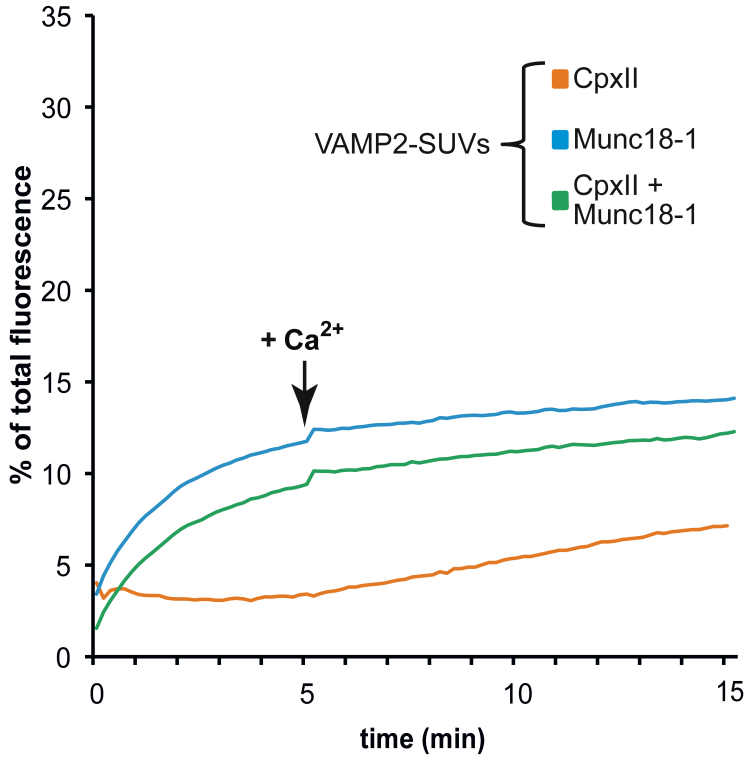
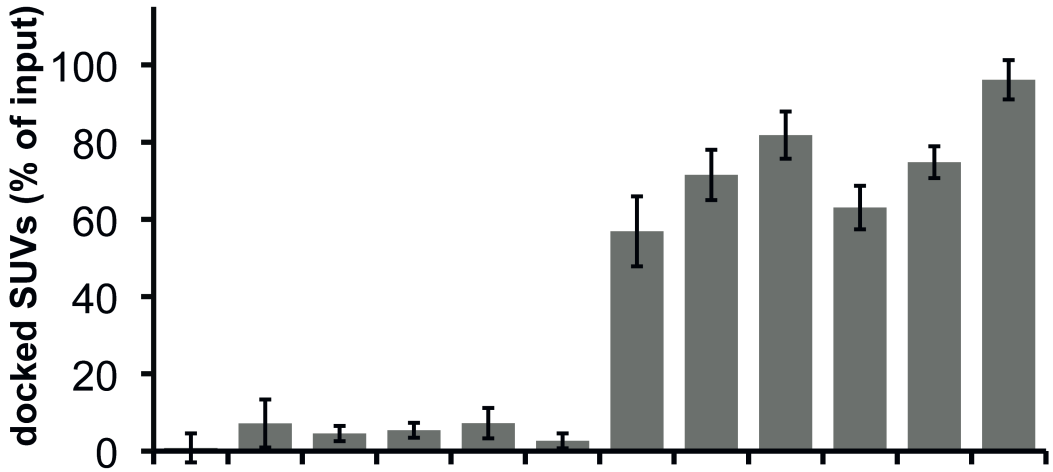
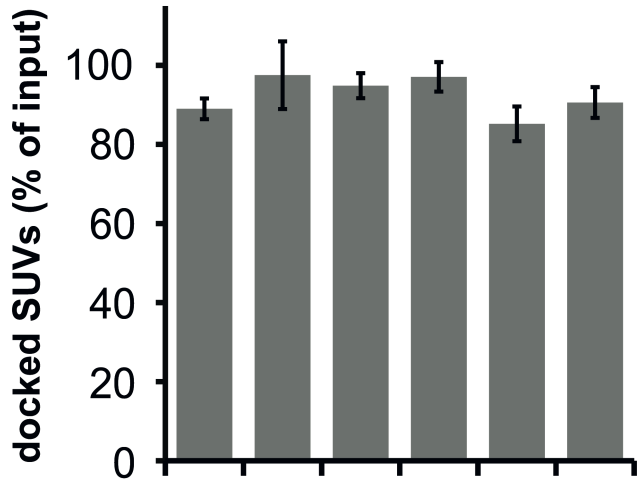



**Figure S4.** Preincubation of VAMP2-SUVs with syntaxin1/SNAP-25-GUVs in the presence of Munc18-1 for 1 h on ice greatly accelerates initial lipid mixing rates. Liposomes were preincubated in the presence of 0.9 $\mu$M Munc18-1 and/or 6 $\mu$M CpxII for 1 h, subsequently were transferred to a 96 well-plate at room temperature (instead of the usual 37 °C pre-heated 96-well plates), and lipid mixing was monitored and analyzed as described in Experimental Procedures.




| | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VAMP2 | + | + | + | + | - | + | - | - | - | + | + | + |
| Syt1 | - | - | - | - | + | + | + | + | + | + | + | + |
| t-SNARE | - | + | - | + | - | - | + | - | + | + | - | + |
| $PI(4,5)P_2$ | - | - | + | + | - | - | - | + | + | - | + | + |
| CpxII | + | + | + | + | + | + | + | + | + | + | + | + |

**Figure S5.** CpxII does not further enhance Syt1-mediated docking. GUVs were pre-incubated with 6 $\mu$M CpxII for 5 min on ice prior to SUV addition followed by a co-incubation of SUVs and GUVs for an additional 5 min. GUVs and associated SUVs were isolated and analyzed as described in Experimental Procedures. Where indicated (-) VAMP2 or t-SNAREs were cleaved by BoNTs. (Fourth bar reproduced from Figure 3A.) Error bars are 95% confidence intervals (n=3).




| | | | | | | |
|---|---|---|---|---|---|---|
| VAMP2 | + | + | + | + | - | - |
| Syt1 | + | + | + | + | + | + |
| t-SNARE | + | + | + | + | + | + |
| $PI(4,5)P_2$ | + | + | + | + | + | + |
| Munc18-1 | + | + | + | + | + | + |
| CpxII | + | + | - | - | + | + |
| Co-incubat. | 5 ‘ | 1 h | 5 ‘ | 1 h | 5 ‘ | 1 h |

**Figure S6.** Munc18-1 does not further enhance Syt1-mediated liposome docking. GUVs containing the indicated proteins/lipids were pre-incubated with 0.9 $\mu$M Munc18-1 (10 min) and 6 $\mu$M CpxII (5 min) prior to SUV addition. SUVs and GUVs were co-incubated for 5 min on ice and analyzed as described in Experimental Procedures. Where indicated (-) VAMP2 or t-SNAREs were cleaved by BoNTs. Error bars are 95% confidence intervals (n=3).