

Supplemental Fig. S1. **Munc18-1 with truncated domains has no binding to Syntaxin1a.** *A*, Munc18-1 representation showing the location of Phe115Glu (F115E) mutation and truncation of Munc18-1 domains (Munc18-1-dom1 and Munc18-1-dom2/3). *B*, Munc18-1 with truncated domains binding to Sx1a-His tethered on  $\text{Co}^{2+}$ -affinity beads was analysed with Coomassie-stained SDS-PAGE. Truncation in Munc18-1 domains abolished its binding to Sx1a compared to Munc18-1-WT. Munc18-1-F115E mutant has reduced binding to Sx1a compared to Munc18-1-WT.

Supplemental Fig. S2. **Expression of Munc18-1-F115E-E132A rescues plasma membrane Syntaxin1a localization in Munc18-1 knockdown PC12 KD43 cells.** *A*, Confocal images of Munc18-1 knockdown PC12 KD43 cells transfected with pCMV5-Munc18-1-F115E-E132A and immunolabeled for Munc18-1 (green) and Sx1a (red). *B*, *C*, Intensity profile analysis outlined in *A*, shows higher Sx1a fluorescence staining at plasma membrane in the cell expressing Munc18-1-F115E-E132A as compared that of untransfected cell.