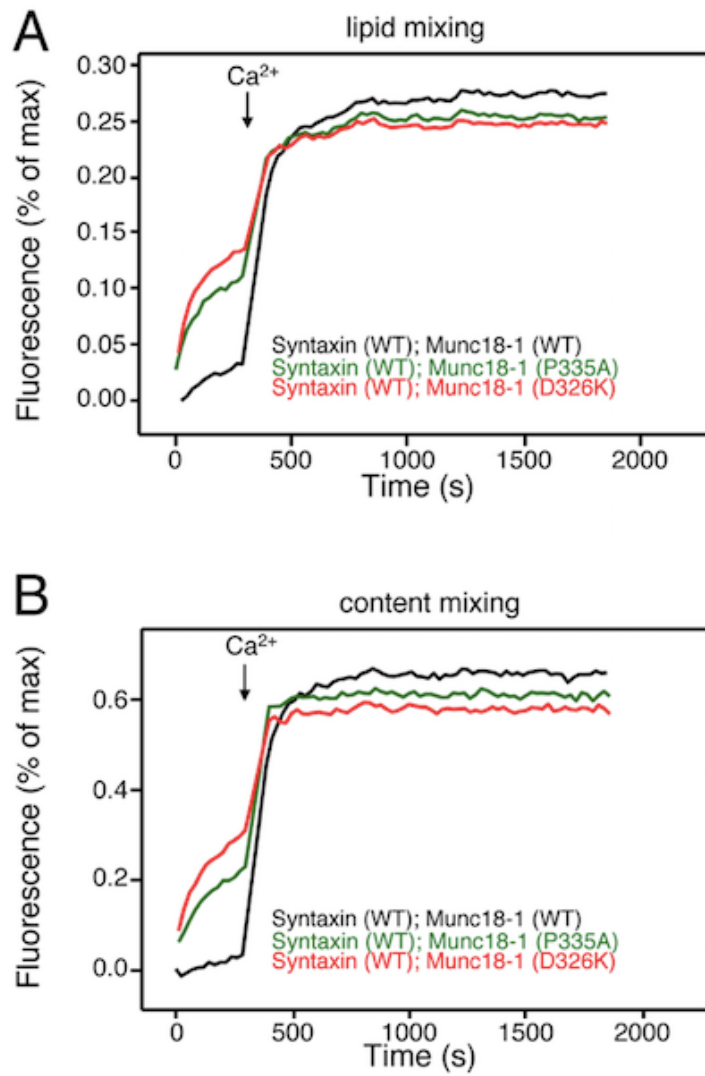


## SUPPLEMENTARY INFO



**Supplementary Figure 1.** Stimulatory effects of the P335A and D326K Munc18-1 mutations on Ca<sup>2+</sup>-independent liposome fusion.

(A, B) Lipid mixing (A) between V-liposomes and S-liposomes containing WT syntaxin-1 was monitored from the fluorescence de-quenching of Marina Blue lipids, and content mixing (B) was monitored from the increase in the fluorescence signal of Cy5-streptavidin trapped in the V-liposomes caused by FRET with PhycoE-biotin trapped in the S-liposomes upon liposome fusion. Assays were performed in the presence of NSF,  $\alpha$ SNAP, Munc13-1C and WT, P335A or D326K Munc18-1 as indicated by the color code. Experiments were started in the presence of 100 mM EGTA and 5 mM streptavidin, and  $\text{Ca}^{2+}$  (600 nM) was added at 300s.