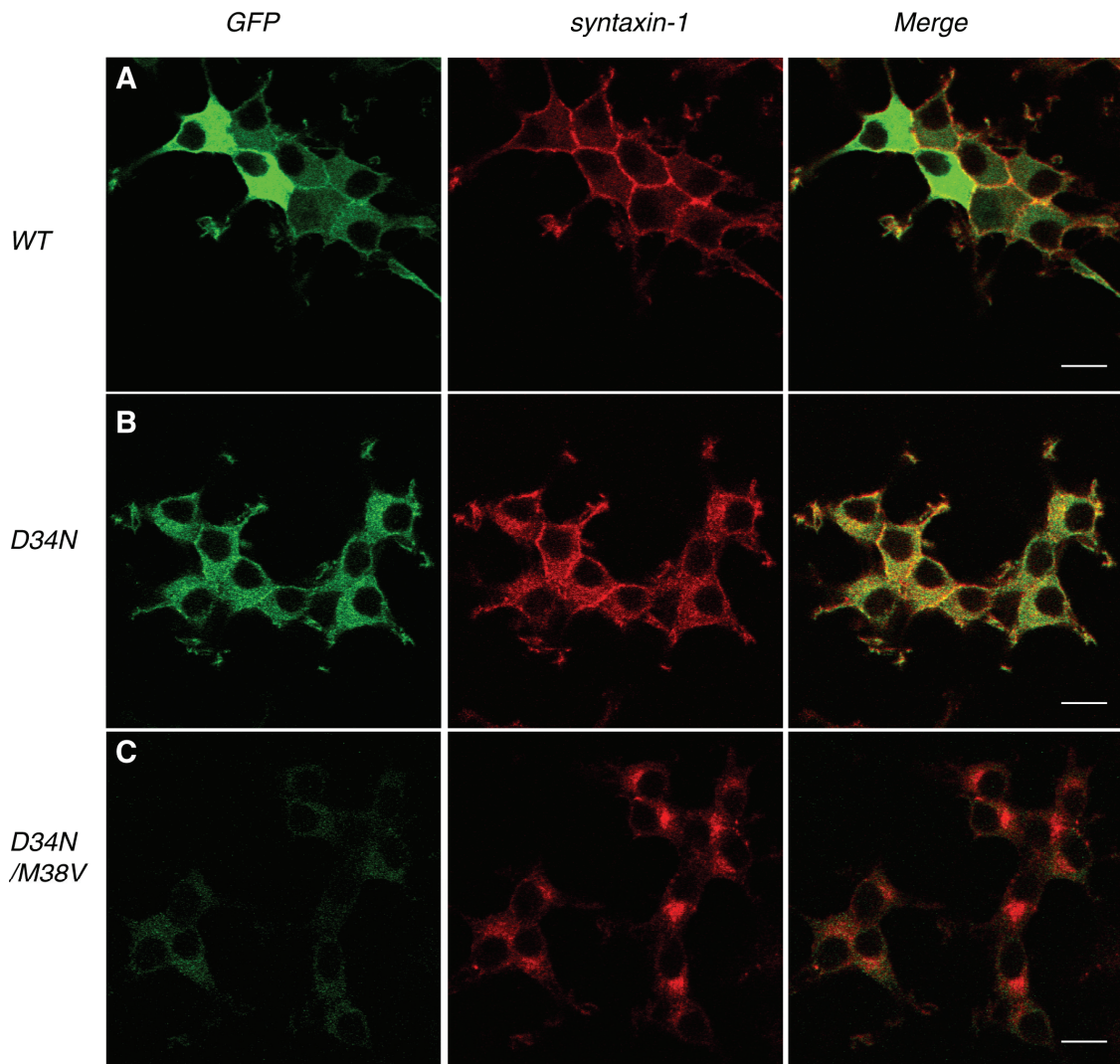


Supplemental Figure 1

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**Supplemental Figure 1. Differential rescue of syntaxin-1 localization in Munc18-1/-2 double knockdown clone (D7) upon reintroduction of Munc18-1 variants.** Confocal images of D7 cells infected with lentiviruses that express (A) negative control EmGFP alone, (B) wild-type Munc18-1-EmGFP, (C) K46E-EmGFP, (D) E59K-EmGFP and (E) K46E/E59K-EmGFP (left). These cells were stained with anti-syntaxin-1 antibodies followed by red-x-conjugated anti-mouse antibodies (middle). Right panels are merged pictures. Bar, 10  $\mu$ m.



Supplemental Figure 2

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**Supplemental Figure 2. Syntaxin-1 localization in Munc18-1/-2 double knockdown clone (D7) upon reintroduction of D34N and D34N/M38V Munc18-1 variants.**

Confocal images of D7D16 cells infected with lentiviruses that express (A) wild-type Munc18-1-EmGFP, (B) D34N-EmGFP, and (C) D34N/M38V-EmGFP (left). These cells were stained with anti-syntaxin-1 antibodies followed by red-x-conjugated anti-mouse antibodies (middle). Right panels are merged. Bar, 10  $\mu$ m.