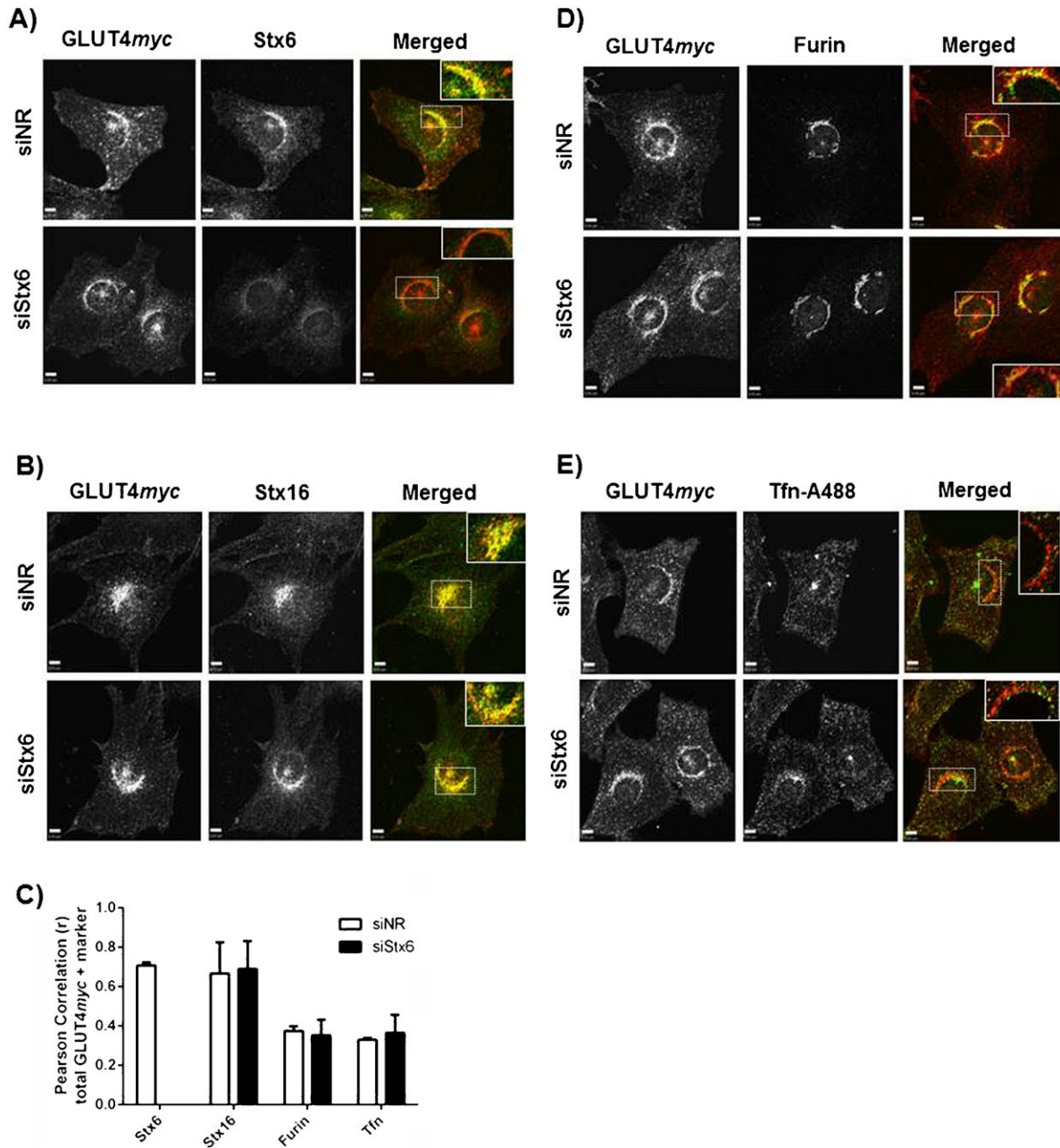


## Supplementary Material

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**Fig. S1. Stx6 depletion does not alter the steady-state localization of GLUT4.** Cells were fixed, permeabilized, and labeled for GLUT4myc (red) and Stx6 (green) (A) or GLUT4myc (red) and Stx16 (green) (B). Inset = single optical slices of the perinuclear regions. (C) GLUT4myc co-localization with markers of endomembrane compartments was quantified using Pearson correlations. Single cells were selected and Pearson correlations calculated using Velocity software (N=2). 8–15 cells were quantified in each experiment. (D) Cells were fixed, permeabilized, and labeled for GLUT4myc (red) and furin (green). Inset = single slice of perinuclear region. (E) Cells were loaded with Tfn-A488 for 30 min before being fixed, permeabilized, and labeled for GLUT4myc (red). Inset = single optical slice of the perinuclear region. Scale bars: 6  $\mu$ m.