Supporting Information

Genetic evidence for an inhibitory role of tomosyn in insulin-stimulated GLUT4 exocytosis

(Running title: Inhibitory role of tomosyn in GLUT4 exocytosis)

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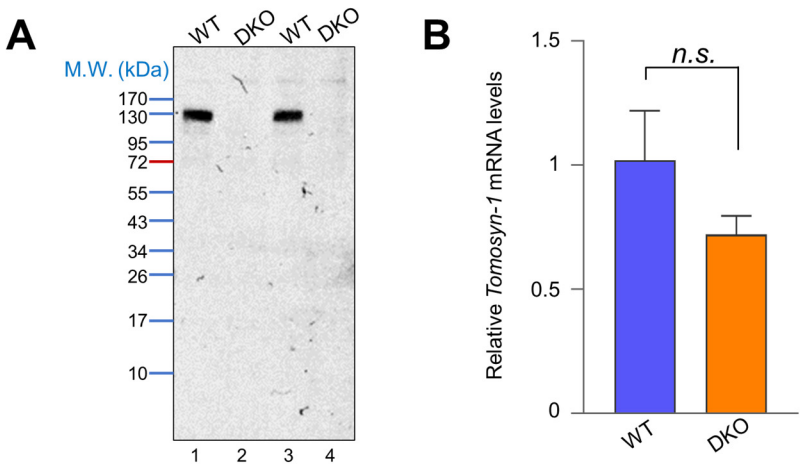

Figure S1. Analysis of tomosyn expression in wild-type (WT) and *Tomosyn-1/2* double knockout (DKO) cells. (A) Immunoblot showing the expression of tomosyn-1/2 proteins in WT and *Tomosyn-1/2* DKO preadipocytes (lanes 1-2) and adipocytes (lanes 3-4). The anti-tomosyn antibodies recognize both tomosyn-1 and tomosyn-2. A cropped area of the blot is shown in Figure 1B. **(B)** The fold changes of *Tomosyn-1/Stxbp5* gene expression in WT and *Tomosyn-1/2* DKO preadipocytes. The mRNA expression levels were determined by qRT-PCR using *Gapdh* as a reference gene. A Student's t-test was used to calculate statistical significance between WT and DKO preadipocytes. *n.s.*, $P>0.05$.

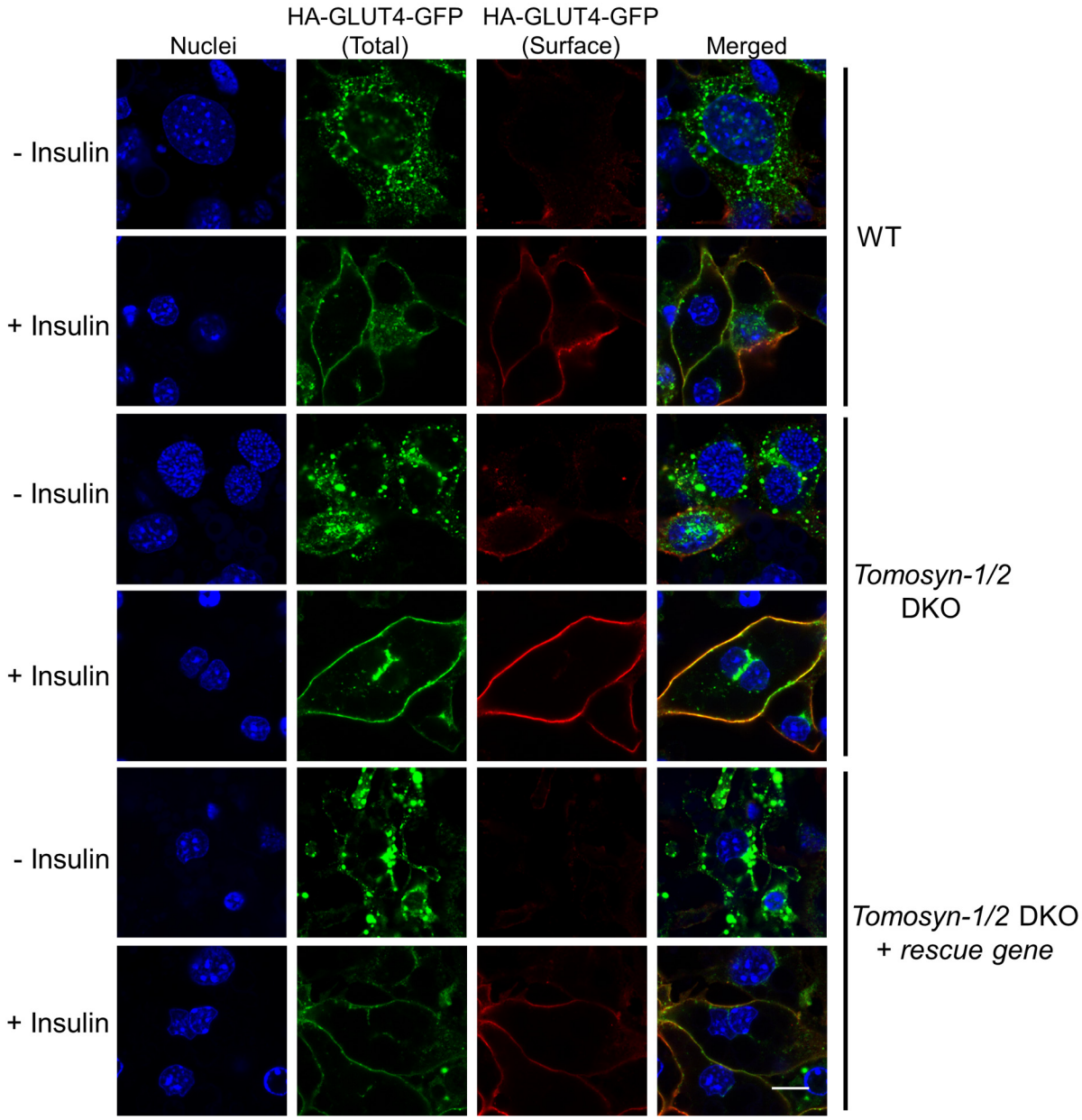

Figure S2. Insulin-stimulated GLUT4 translocation is elevated in *Tomosyn-1/2* DKO adipocytes. Preadipocytes expressing the HA-GLUT4-GFP reporter were cultured and differentiated as described in Figure 2A. The cells were either untreated or treated with 100 nM insulin for 30 minutes. GLUT4 reporters on the surfaces of non-permeabilized cells were labeled using anti-HA antibodies and Alexa Fluor 568-conjugated secondary antibodies. Nuclei were stained with Hoechst 33342. The images were captured using a 100× oil immersion objective on a Nikon A1 Laser Scanning confocal microscope. Representative images are shown. Scale bar: 25 μm.

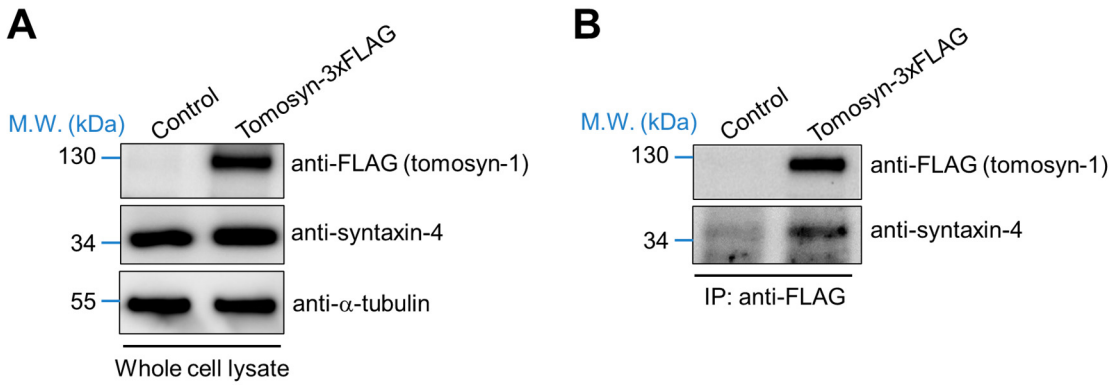

Figure S3. Tomosyn interacts with syntaxin-4 in the cell. (A) Immunoblots showing the expression of the indicated proteins in *Tomosyn-1/2* DKO preadipocytes expressing an empty vector (control) or the human *TOMOSYN-1* rescue gene. **(B)** Immunoblots showing the interaction of 3xFLAG-tagged tomosyn-1 (encoded by the rescue gene) with endogenous syntaxin-4. Tomosyn-1 was immunoprecipitated using anti-FLAG antibodies and the presence of syntaxin-4 and tomosyn-1 in the immunoprecipitates was detected using anti-syntaxin-4 and anti-FLAG antibodies, respectively.

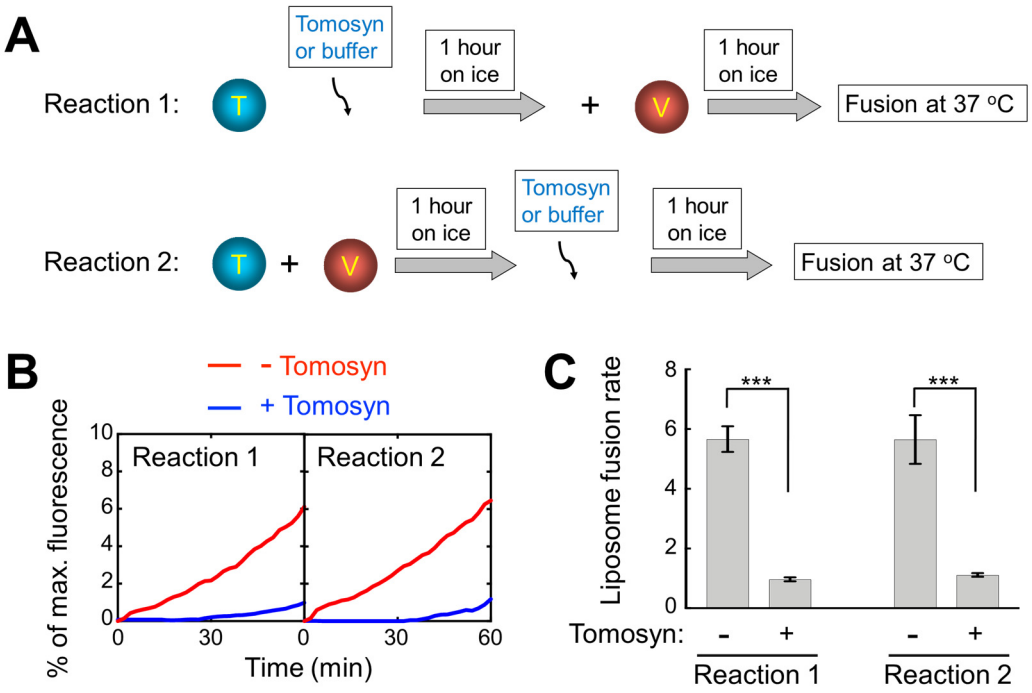

Figure S4. Tomosyn inhibits the fusion of preincubated SNARE liposomes. (A) Diagrams illustrating the liposome fusion reactions. The t-SNARE liposomes containing syntaxin-4 and SNAP-23 were directed to fuse with VAMP2-bearing liposomes with or without preincubation. The fusion reactions were carried out in the absence or presence of 5 μM tomosyn. All fusion reactions contained 100 mg/mL Ficoll 70 as the crowding agent. **(B)** Fusion reactions depicted in A were measured using a FRET-based lipid-mixing assay. **(C)** Lipid-mixing rates of the liposome fusion reactions in B. Data are presented as mean ± SD. n = 3. P values were calculated using Student's t-test. *** $P<0.001$.

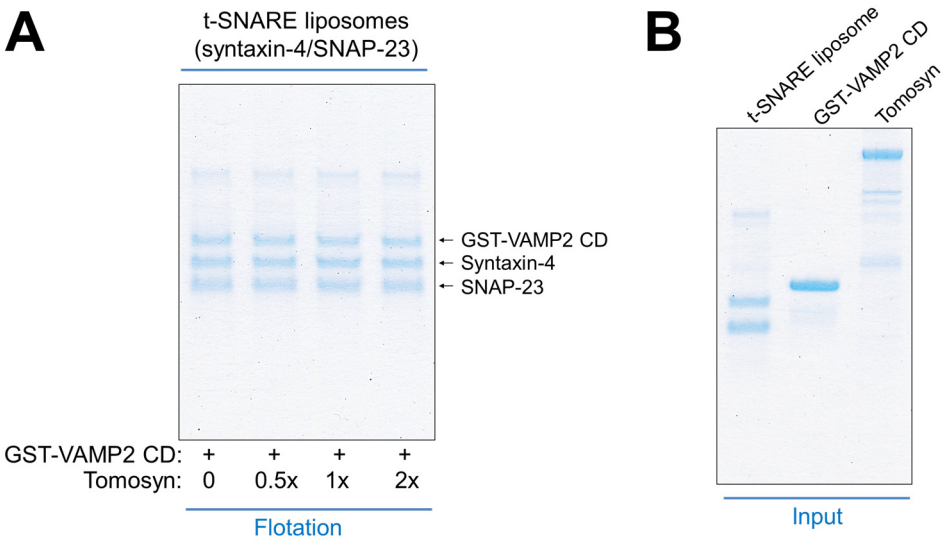

Figure S5. Tomosyn is unable to displace VAMP2 from fully assembled cis-SNARE complexes. (A) Coomassie blue-stained SDS-PAGE gel showing membrane-anchored cis-SNARE complexes with or without incubation with tomosyn. Cis-SNARE liposomes were prepared by incubating t-SNARE liposomes containing syntaxin-4 and SNAP-23 with GST-VAMP2 cytoplasmic domain (CD) for one hour at room temperature. Tomosyn was added to cis-SNARE liposomes at the indicated molar ratios (relative to SNAREs). After another hour of incubation at room temperature, liposomes and associated proteins were isolated using Nycodenz flotation. **(B)** Coomassie blue-stained SDS-PAGE gel showing input liposomes and proteins.