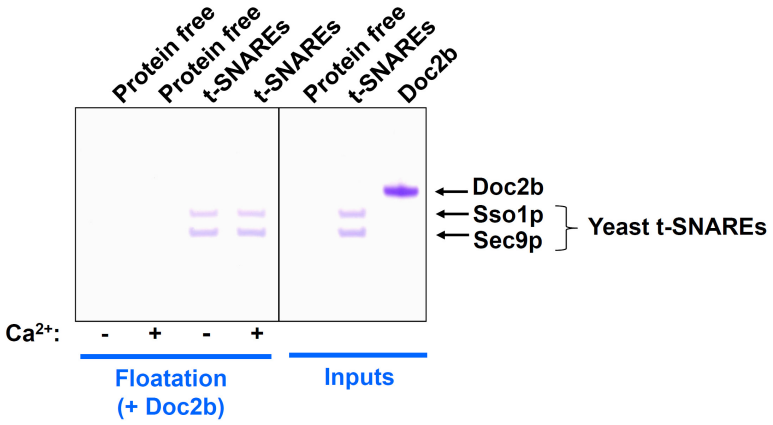

Figure S1. Doc2b does not interact with yeast exocytic t-SNAREs in the liposome co-flotation assay. Left: coomassie blue-stained gel showing the binding of Doc2b to protein-free or yeast SNARE liposomes. Yeast exocytic t-SNAREs, heterodimer of Sso1p and Sec9p, were reconstituted into liposomes composed of the neutral PC lipid. SUMO-Doc2b (5 μM) was incubated with protein-free or yeast t-SNARE liposomes at 4 ^{o}C for 1 hour in the presence of 1 mM $CaCl_2$ or EGTA. The samples were subsequently floated up on a Nycodenz gradient. SNAREs were added to the final concentration of 5 μM in the binding reactions. Right: coomassie blue-stained gel showing the input liposomes and recombinant SUMO-Doc2b.

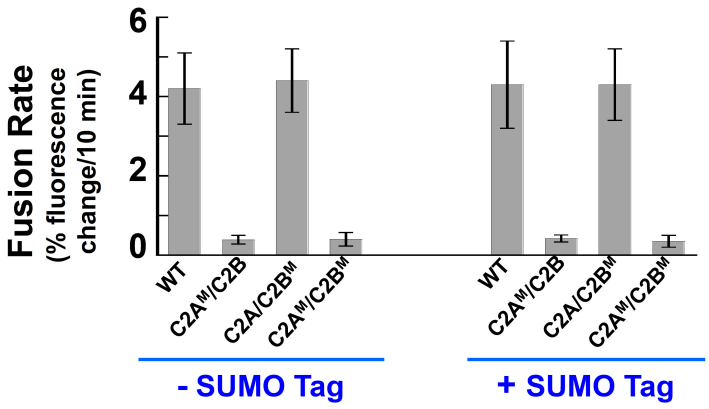

Figure S2. The SUMO tag does not affect the biological activity of Doc2b. Doc2b proteins (with or without the SUMO tag) were added to the reconstituted SNARE-mediated fusion reaction in the presence of 1 mM $CaCl_2$. The Doc2b mutants are depicted in Figure 7. Each fusion reaction contained 5 μM t-SNAREs and 1.5 μM v-SNARE. Data are presented as percentage of fluorescence change per 10 min. Error bars indicate standard deviation.

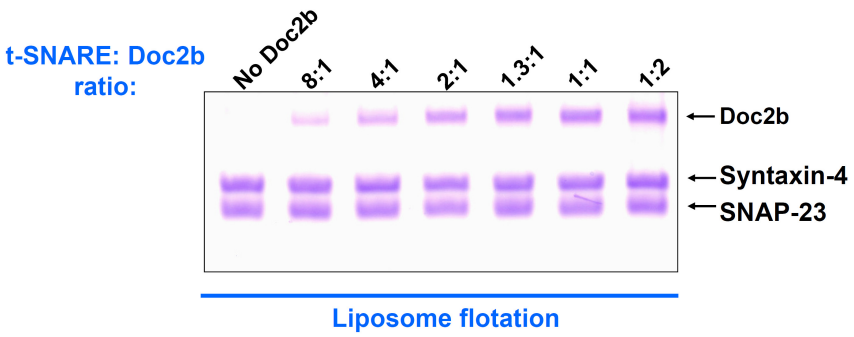

Figure S3. Stoichiometric interaction between Doc2b and GLUT4 exocytic SNAREs. In a liposome co-flotation assay, t-SNARE liposomes reconstituted with the neutral PC lipid were incubated with the indicated amounts of SUMO-Doc2b at 4 ^{o}C for 1 hour in the presence of 1 mM $CaCl_2$. The samples were subsequently floated up on a nycodenz gradient. Each binding reaction contained 5 μM t-SNAREs. The calculations took into account that about 1/3 of the SNAREs faced the lumenal side of the liposomes (Scott *et al.*, 2003).

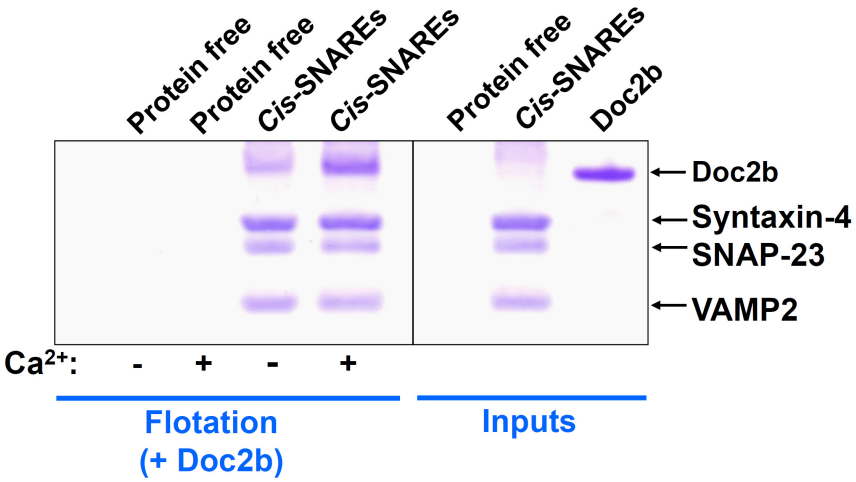

Figure S4. Doc2b interacts with the *cis*-SNARE complex in a liposome co-flotation assay. Left: coomassie blue-stained SDS-PAGE gel showing the binding of SUMO-Doc2b to protein-free or *cis*-SNARE liposomes (PC-only) in the presence of 1 mM EGTA or $CaCl_2$. The *cis*-SNARE complex was composed of syntaxin-4, SNAP-23, and VAMP2. Doc2b was added to a final concentration of 5 μM.

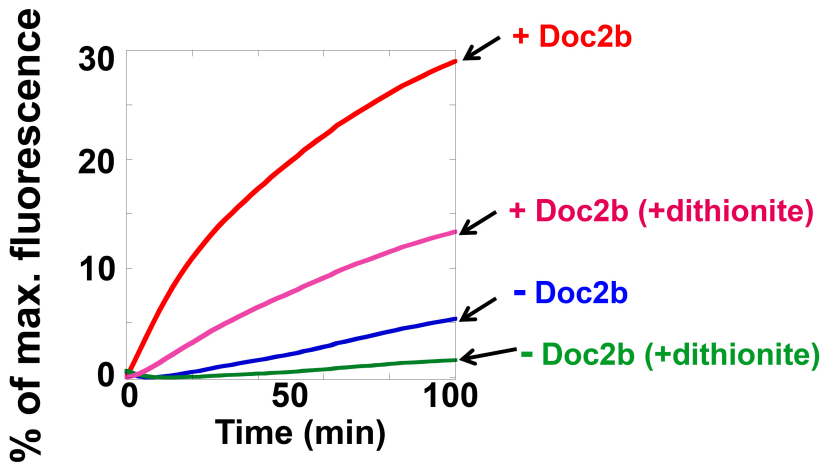

Figure S5. Doc2b promotes full liposome fusion. To examine whether Doc2b promoted full fusion or hemifusion, dithionite quenching experiments were performed as previously described (Xu *et al.*, 2005). v-SNARE liposomes were treated with 10 mM dithionite to remove fluorophores on the outer leaflet of the bilayer, thereby revealing the lipid mixing signals from the inner leaflet. Fusion of untreated and treated liposomes was monitored using FRET-based lipid mixing as in standard fusion reactions. The binding reactions contained 1 mM $CaCl_2$. Each fusion reaction contained 5 μM t-SNAREs and 1.5 μM v-SNARE. Doc2b was added to a final concentration of 5 μM.

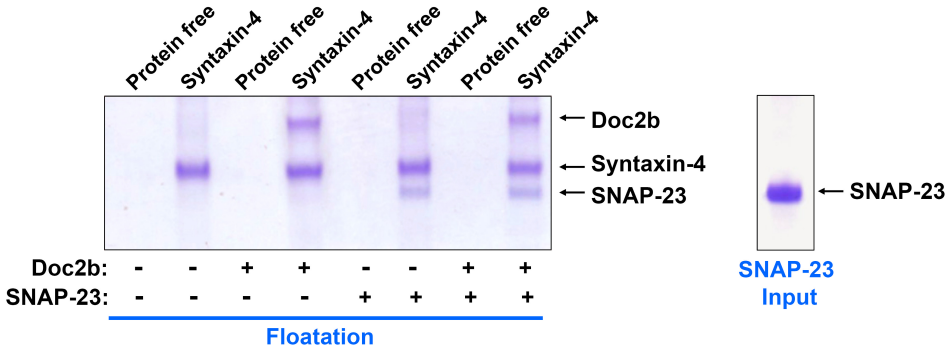

Figure S6. The Doc2b-syntaxin-4 heterodimer does not affect the assembly of the GLUT4 exocytic t-SNARE complex. Left: coomassie blue-stained gel showing the formation of the t-SNARE complex in a liposome floatation assay. Syntaxin-4 liposomes reconstituted with the neutral PC lipid were incubated with or without Doc2b at 4 ^{o}C for 1 hour, followed by the addition of SNAP-23. After two hours of incubation at 4 ^{o}C, the samples were floated up on a nycodenz gradient. The binding reactions contained 1 mM $CaCl_2$. SNAREs and Doc2b were added to the final concentrations of 5 μM. Right: coomassie blue-stained gel showing the recombinant SNAP-23 protein. Other input materials including the t-SNAREs liposomes and Doc2b protein proteins are shown in Figure 1.

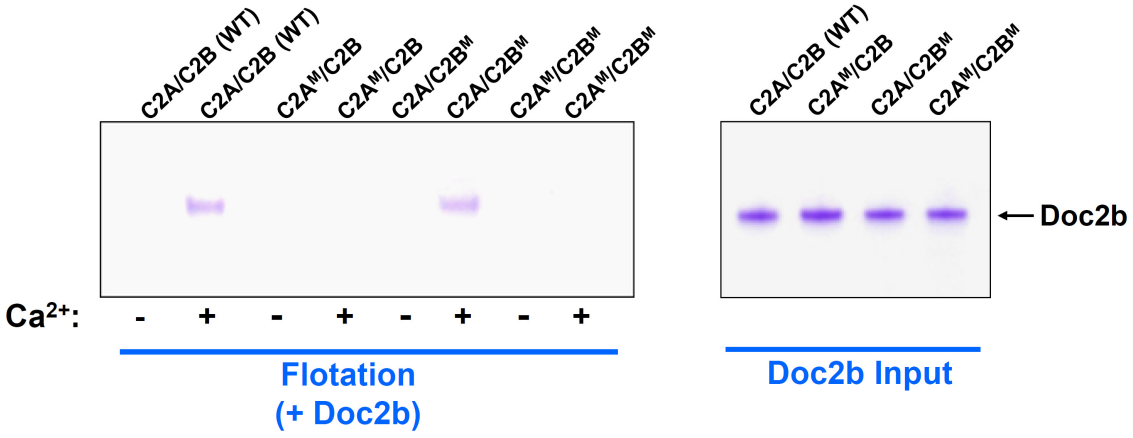

Figure S7. Binding of Doc2b mutants to the lipid bilayer. Left: coomassie blue-stained gel showing the binding of WT and mutant Doc2b proteins to protein-free liposomes in a liposome flotation assay. Protein-free liposomes (containing PS lipid) were incubated with WT or mutant Doc2b (5 μM) at 4 ^{o}C for 1 hour, in the presence of 1 mM EGTA or $CaCl_2$. The samples were subsequently floated up on a nycodenz gradient. Right: coomassie blue-stained gel showing the recombinant Doc2b proteins. Diagrams of the Doc2b mutants are shown in Figure 7.

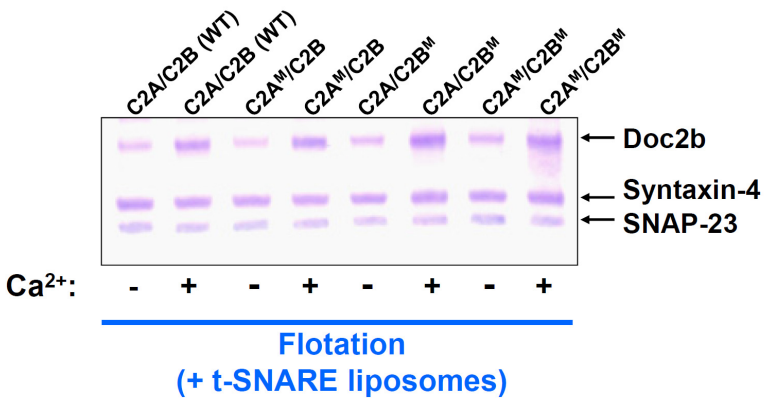

Figure S8. The binding of Doc2b mutants to the GLUT4 exocytic t-SNAREs remains intact. In a liposome flotation assay, t-SNARE liposomes reconstituted with the neutral PC lipid were incubated with WT or mutant Doc2b (5 μM) at 4 ^{o}C for 1 hour, in the presence of 1 mM EGTA or $CaCl_2$. The samples were subsequently floated up on a nycodenz gradient. Each binding reaction contained 5 μM t-SNAREs. Diagrams of the Doc2b mutants are shown in Figure 7.

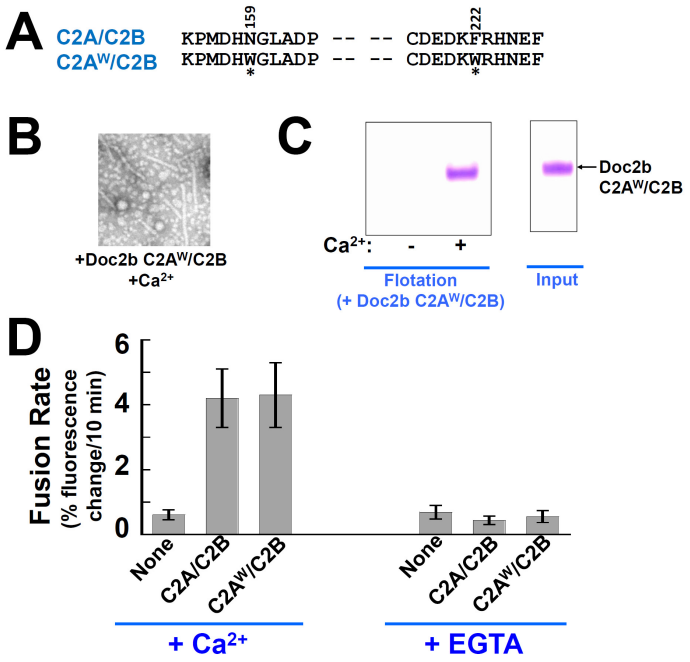

Figure S9. **Mutations of the C2A hydrophobic residues of Doc2b into tryptophans do not reduce its stimulatory activity. (A)** Sequences of the hydrophobic residues in the C2A domain of Doc2b that are predicted to embed in the lipid bilayer. Mutated residues are indicated with amino acid numbers on the top and asterisks at the bottom. **(B)** Electron micrograph showing the bending of Folch liposomes by Doc2b^{W}, in which the hydrophobic residues of the C2A domain were mutated into tryptophans. The reaction contained 1 mM $CaCl_2$. **(C)** Left: coomassie blue-stained gel showing the binding of Doc2b^{W} to protein-free liposomes in a liposome co-flotation assay. Protein-free liposomes (containing PS lipid) were incubated with Doc2b^{W} at 4 ^{o}C for 1 hour, in the presence of 1 mM EGTA or $CaCl_2$. The samples were subsequently floated up on a nycodenz gradient. Right: coomassie blue-stained gel showing the recombinant Doc2b^{W} protein. **(D)** Initial rates of the indicated fusion reactions in the presence of 1 mM EGTA or $CaCl_2$. Each fusion reaction contained 5 μM t-SNAREs and 1.5 μM v-SNARE. Data are presented as percentage of fluorescence change per 10 min. Error bars indicate standard deviation.