Johnson and Chapman, http://www.jcb.org/cgi/content/full/jcb.201002089/DC1

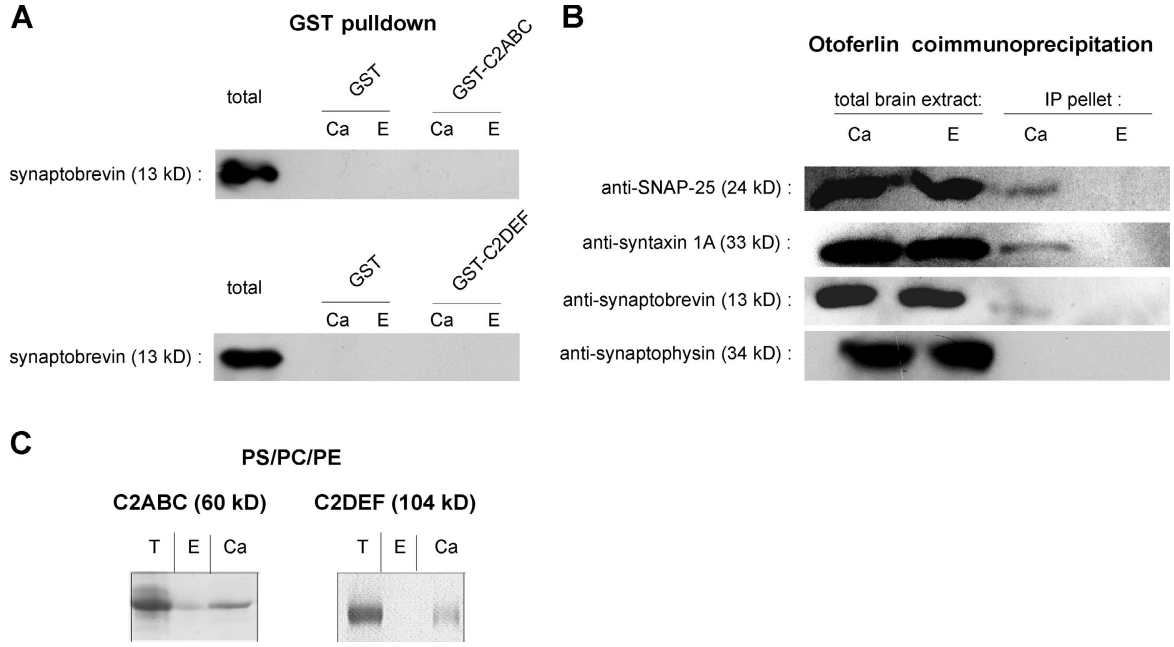

Figure S1. **Otoferlin binds to ternary SNARE complexes but does not bind to isolated synaptobrevin 2.** (A) Otoferlin does not directly bind to synaptobrevin 2. GST pull-down assays were performed using 10 µg immobilized otoferlin C2ABC and C2DEF fragments and 30 µM full-length synaptobrevin 2 in 1% Triton X-100. Assays were conducted in the presence of 0.1 mM EGTA (E) or 1 mM calcium (Ca). GST alone was included as a negative control. 20% of the pellets from the pull-down samples were subjected to SDS-PAGE, and bound protein was visualized by immunoblot analysis. Total indicates 5% of the supernatant used in the pull-down assays. (B) Immunoblot of rat brain detergent extract immunoprecipitated (IP) with an anti-otoferlin monoclonal antibody and probed with anti–syntaxin 1, anti–SNAP-25, anti-synaptobrevin, and anti-synaptophysin antibodies. 15% of the immunoprecipitated material was loaded into each lane. Total brain extract corresponds to 1 µg brain extract. (C) Coflotation assays were performed using the C2ABC and C2DEF fragments of otoferlin and protein-free liposomes (15% PS, 55% PC, and 30% PE) in the presence of 1 mM calcium or 0.1 mM EGTA. 30% of each sample was resolved by SDS-PAGE and stained with Coomassie blue. T corresponds to the sample before floatation.

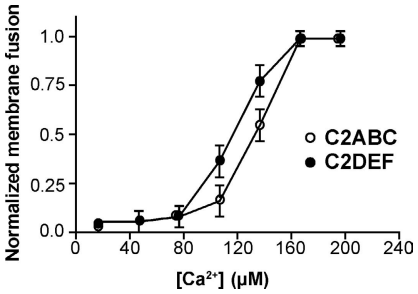

Figure S2. **Calcium dose-response for otoferlin-regulated SNARE-mediated membrane fusion.** Fusion assays were performed using 10 µM of each otoferlin fragment. The $[Ca^{2+}]_{1/2}$ values for the C2ABC and C2DEF fragments were 109 µM and 131 µM, respectively. Error bars represent SD (n = 3).

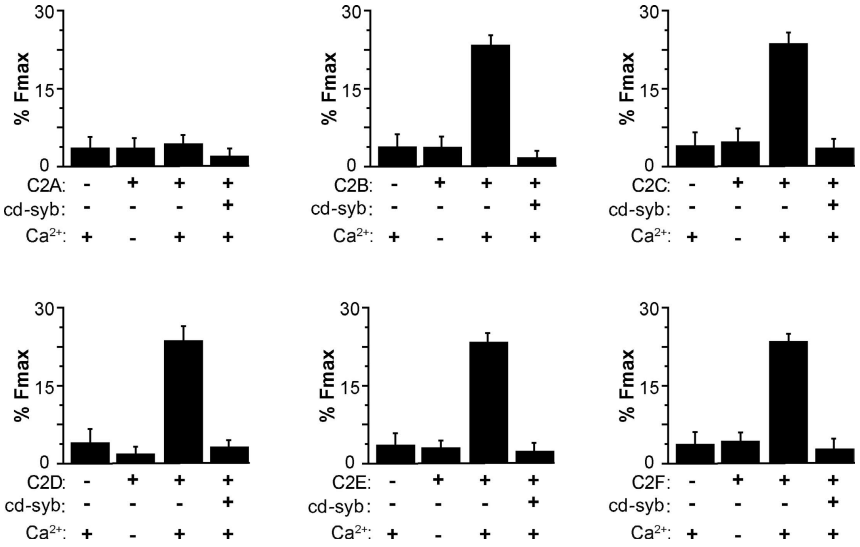

Figure S3. **Quantitation of otoferlin C2 domain–stimulated SNARE-mediated membrane fusion.** Stimulated fusion was calcium dependent, and the stimulatory effects of the 30 µM isolated otoferlin C2 domains were abrogated by cd-syb. Error bars represent SD (n = 3).

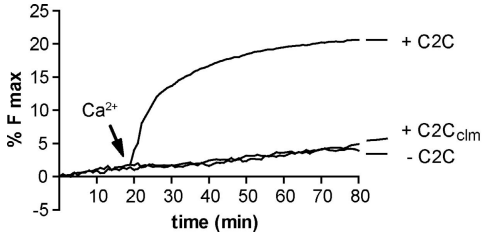

Figure S4. **Neutralization of the putative calcium ligands D515 and 517 in the C2C domain of otoferlin abolishes calcium-stimulated fusion.** 30 µM WT otoferlin C2C or 30 µM of the calcium ligand mutant (C2Cclm) were analyzed in the in vitro fusion assay as described in Fig. 6. As a control, a fusion assay without any C2 domain is also shown.