SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. 1. Co-localization of tomosyn and synaptotagmin-1 in hippocampus. Adult mouse hippocampal sections were double immunostained with anti-tomosyn pAb and anti-synaptotagmin-1 mAb. Insets are enlarged images of the boxed areas. The arrowheads indicate the representative areas where tomosyn colocalizes with synaptotagmin-1. SR, stratum radiatum; SL, stratum lucidum; SP stratum pyramidale; SO, stratum oriens. Bars, 30 μm.

Supplemental Fig. 2. Affinities for the binding of tomosyn with t-SNAREs. *A*, Estimation of the binding affinity of the N-terminal WD40 repeats of tomosyn to syntaxin-1 in the presence of Ca^{2+}. MBP-tomosyn-N (100 pmol) was immobilized on amylose beads and reacted with the indicated amounts of syntaxin-1-ΔTM in the presence of Ca^{2+} in the same manner as described in **Fig. 3*B***. The *right panel* shows immunoblotting with the anti-syntaxin-1 mAb. The results shown are representative of three independent experiments. The asterisk indicates degraded MBP-tomosyn-N. *B*, Estimation of the binding affinity of the N-terminal WD40 repeats of tomosyn to SNAP-25 in the presence of Ca^{2+}. The assay was carried out in the same manner as described in *A*, except that SNAP-25 was used instead of syntaxin-1-ΔTM. The *right panel* shows immunoblotting with the anti-SNAP-25 mAb. The results shown are representative of three independent experiments. The asterisk indicates degraded MBP-tomosyn-N. *C*, Estimation of the binding affinity of full-length tomosyn to t-SNAREs in the presence of Ca^{2+}. MBP-tomosyn (100 pmol) was immobilized on amylose beads and reacted with the indicated amounts of syntaxin-1-ΔTM and SNAP-25 in the presence of Ca^{2+} in the same manner as described in *A*. Bound and free syntaxin-1-ΔTM and SNAP-25 were quantified and plotted in the *right panel*. The results shown are representative of three independent experiments. The apparent K_d value was calculated by Scatchard analysis. The asterisk indicates degraded MBP-tomosyn.

Supplemental Fig. 3. Comparable bindings of synaptotagmin-1 and t-SNAREs to tomosyn. MBP-tomosyn immobilized on amylose beads was reacted with equal amounts of ΔTM-synaptotagmin-1, syntaxin-1-ΔTM, and full-length SNAP-25 at various concentrations in the presence of Ca^{2+}. After being washed, bound proteins were eluted with SDS sample buffer, and subjected to SDS-PAGE followed by CBB staining. The result shown is representative of three independent experiments. The asterisk indicates degraded MBP-tomosyn.

Supplemental Fig. 4. No inhibition of synaptotagmin-1 functions by tomosyn-C. *A*, Effect of tomosyn-C on the SNARE-mediated membrane fusion. The t-SNARE vesicles were incubated with or without ΔTM-synaptotagmin-1 alone, MBP-tomosyn-C alone, or both ΔTM-synaptotagmin-1 and MBP-tomosyn-C in the presence of Ca^{2+}, and SNARE-mediated liposome fusion was measured as described in the legend for **Fig. 4*A***. The result shown is representative of three independent experiments. The *right panel* shows the relative level of liposome fusion at 60 min. Error bars represent S.D. *B*, Effect of tomosyn-C on the membrane bending activity of synaptotagmin-1. Folch liposomes were reacted with ΔTM-synaptotagmin-1 alone, MBP-tomosyn-C alone, or both ΔTM-synaptotagmin-1 and MBP-tomosyn-C in the presence of Ca^{2+}, and the samples were analyzed as described in the legend for **Fig. 4*B***. As a negative control, Folch liposomes were simply stained with uranyl acetate. Bar, 200 nm. The results shown are representative of three independent experiments. *C*, Additive effects of ΔTM-synaptotagmin-1 on inhibition of neurotransmitter release by tomosyn-C. Evoked EPSPs were recorded every 10 sec. MBP-tomosyn-C, ΔTM-synaptotagmin-1, or a mixture of MBP-tomosyn-C and ΔTM-synaptotagmin-1 was

microinjected into presynaptic SCG neurons at time=0 (50 μM each in the pipette). *Ca*, Change in EPSP traces with MBP-tomosyn-C, ΔTM-synaptotagmin-1, or the mixture of MBP-tomosyn-C and ΔTM-synaptotagmin-1 from one representative experiment. *Cb*, The EPSP amplitudes were processed in the same manner as those shown in **Fig. 5*Bb***. *Cc*, Decrease in EPSP amplitude at 20 min after the injection. Error bars represent S.E.M. The graphs of the ΔTM-synaptotagmin-1 are repetitions of **Figs. 5*Ba***, **5*Bb***, and **5*Bc***.

Supplemental Fig. 5. Co-localization of tomosyn and synaptotagmin-1 in SCG neurons. The SCG neurons were double immunostained with anti-tomosyn pAb and anti-synaptotagmin-1 mAb. DIC, differential interference contrast image (cell nuclei borders are marked with dashed lines). Insets are enlarged images of boxed areas. N, nucleus. The arrowheads indicate the representative areas where tomosyn colocalizes with synaptotagmin-1. Bar, 20 μm.