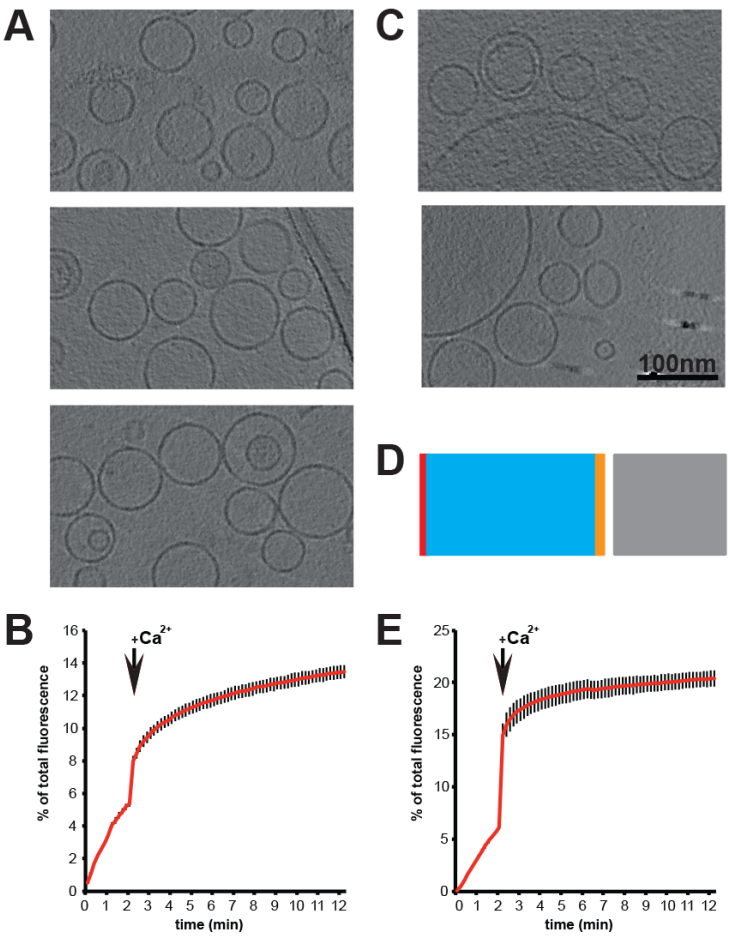

Figure S4. Protrusion formation is not detected in SUVs and occurs preferentially on the t-SNARE GUV membrane.

(A) Tomographic slices from a reaction similar to the one shown in Figure 1C, except that both v-SNARE and t-SNARE proteins were reconstituted into SUVs. (B) Kinetics of the fusion reaction monitored by a plate reader assay for lipid mixing. Error bars indicate S.E.M. (n=3). (C) Tomographic slices of the same *in vitro* system as in Figure 1C, except that VAMP2 and synaptotagmin 1 were reconstituted into GUVs and syntaxin1/SNAP-25 were reconstituted into SUVs. (D) Quantification of the cryoET data from one experiment showed that almost all of the docked t-SNARE SUVs (93.1% of the docked SUVs, see Table S3) were unable to induce a protrusion in the v-SNARE GUVs. Colours as in Figure 3 (E) Kinetics of the fusion reaction monitored by a plate reader assay for lipid mixing. This reaction displays fast response to Ca^{2+} activation. Error bars indicate S.E.M. (n=3).