Supporting Information

Lipid Bilayer Membrane-Triggered Presynaptic Vesicle Assembly

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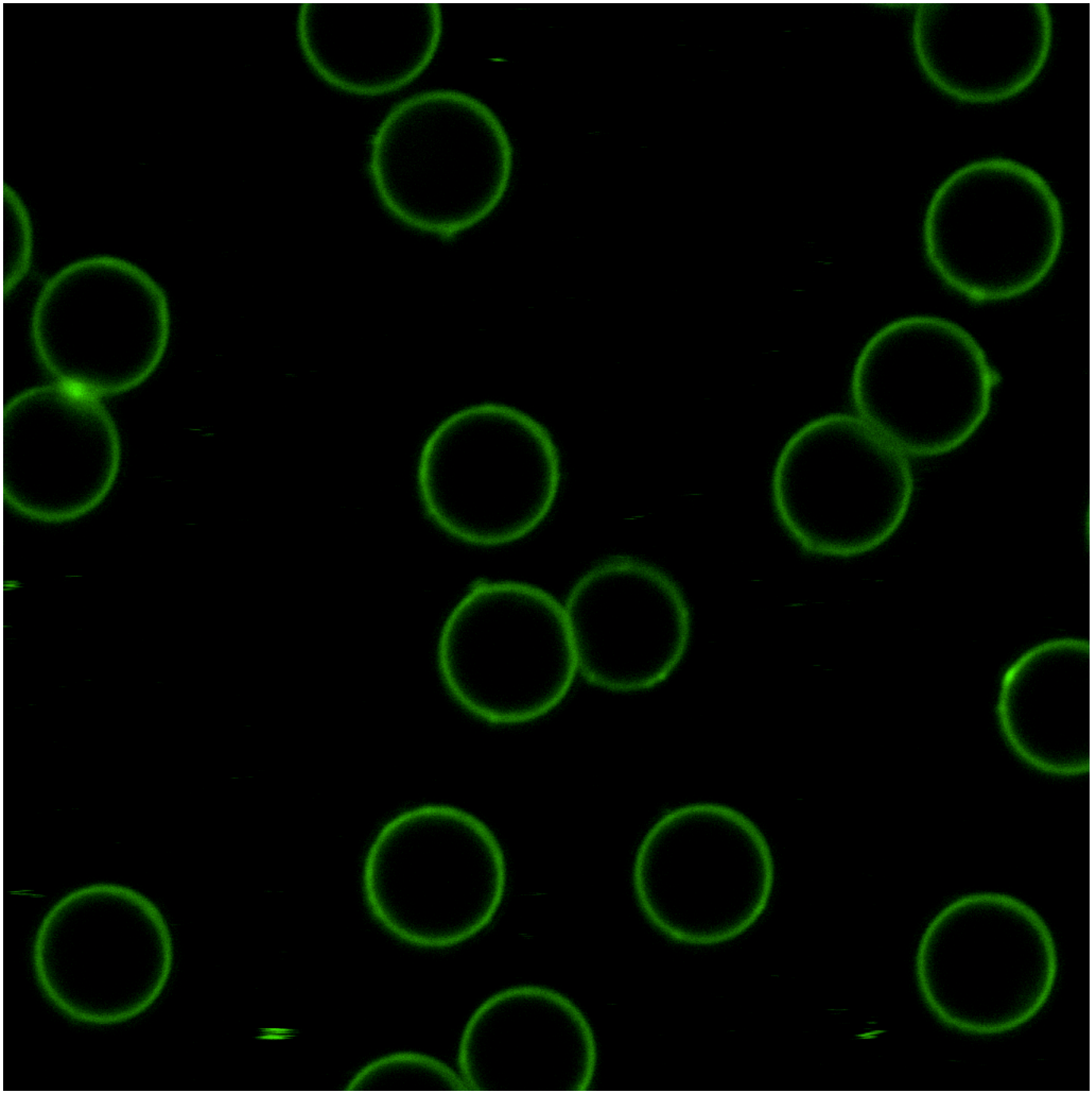

Figure SI.1. A representative confocal cross-sectional image of SS-BLM/beads composed of DOPC:DOTAP:DPPE (25:25:50). In this case, the fluorescence is derived from 0.1 mol% of phosphatidylethanolamine-NBD lipid. The image depicts uniform fluorescence around the beads, which supports formation of continuous bilayer lipid membranes around the beads' surface.

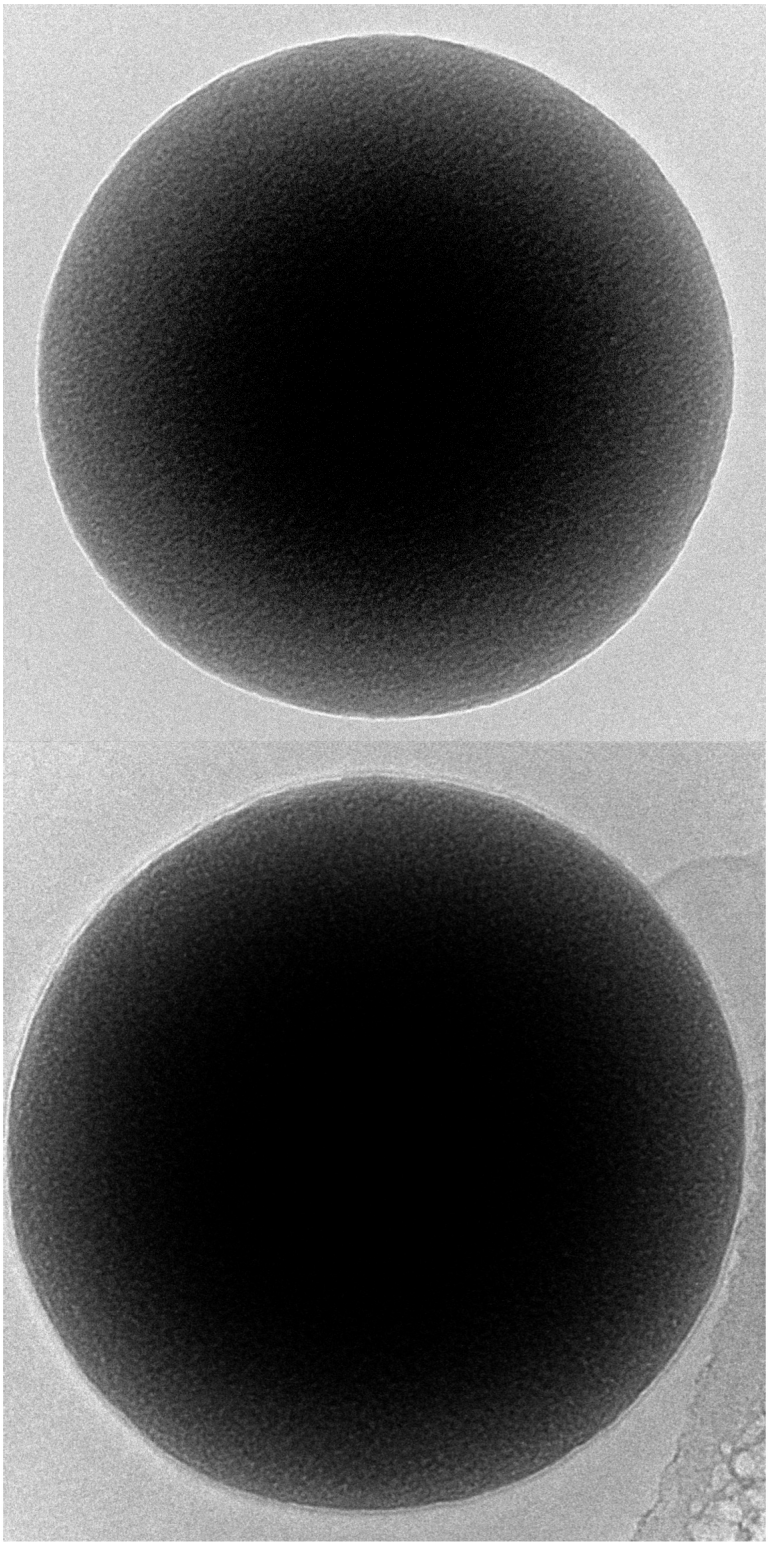

Figure SI.2. Enlarged view of the Cryo-TEM images shown in figure 1 c&d. The top image shows an uncoated bead whereas the bottom image shows an SS-BLM/bead. The bottom image clearly depicts the uniformity of the bilayer around the surface.

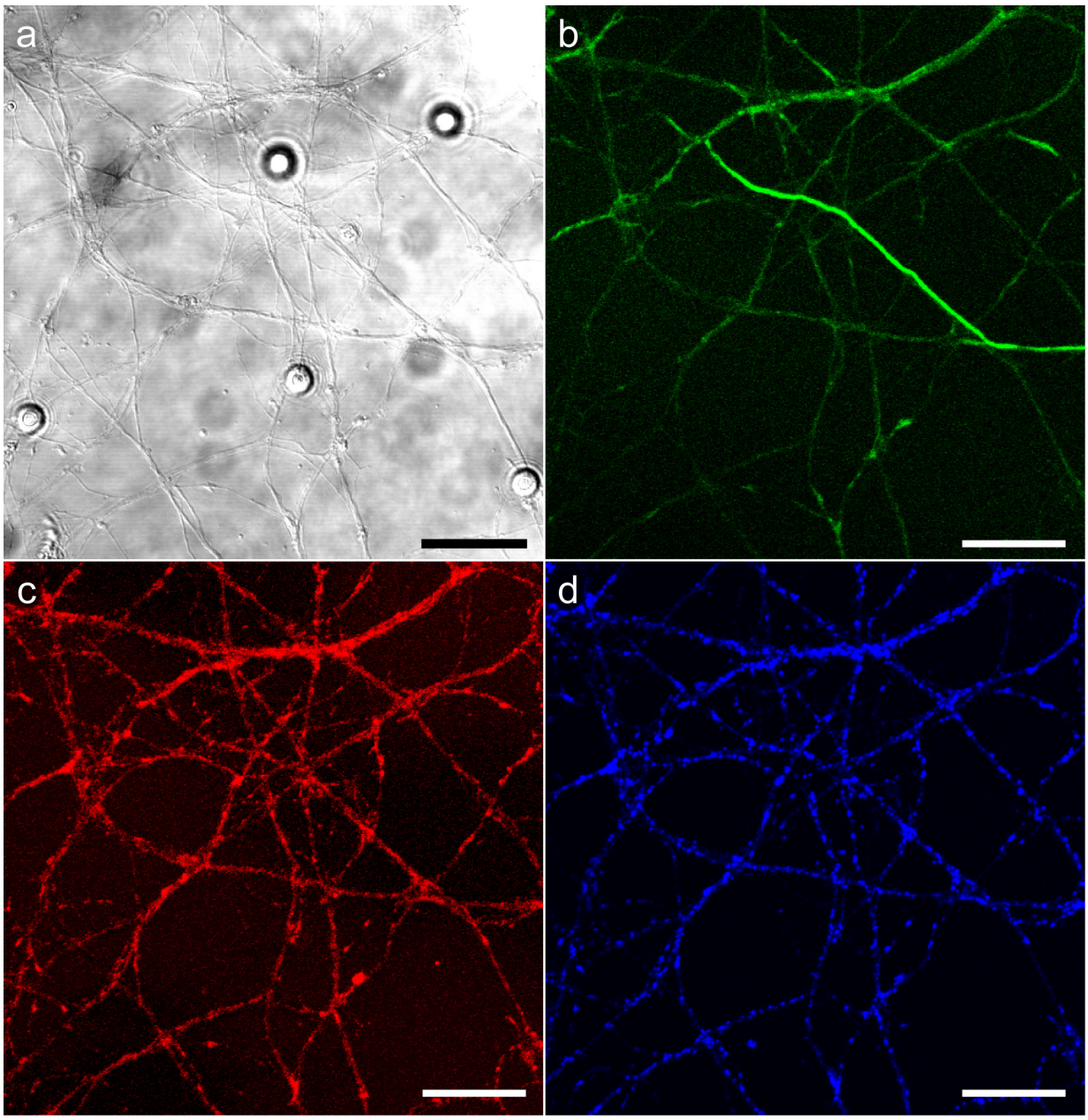

Figure SI.3. Representative confocal cross-section images (b-d) showing the absence of synaptic proteins at the bead-axon contact points. This control experiment was performed using SS-BLM/beads containing of DOPC:DOTAP/ 75:25 in the bilayer when co-cultured with hippocampal neurons (DIV 14). The fluorescence clustering is only seen along the hippocampal neurons. Unlike in figures 3 and 4, no enhanced clustering is observed at the bead-axon contacts. The cells are fixed and incubated with (b) phalloidin-Alexa-488 (green channel) for actin filaments, (c) anti-synaptophysin /α-rabbit-Alexa-543 (red channel) for synaptophysin as well as (d) anti-bassoon/α-mouse-Cy-5 (blue channel) for bassoon. DIC channel (a) shows the exact locations of SS-BLM/beads in the culture. Scale bars are 25 µm.

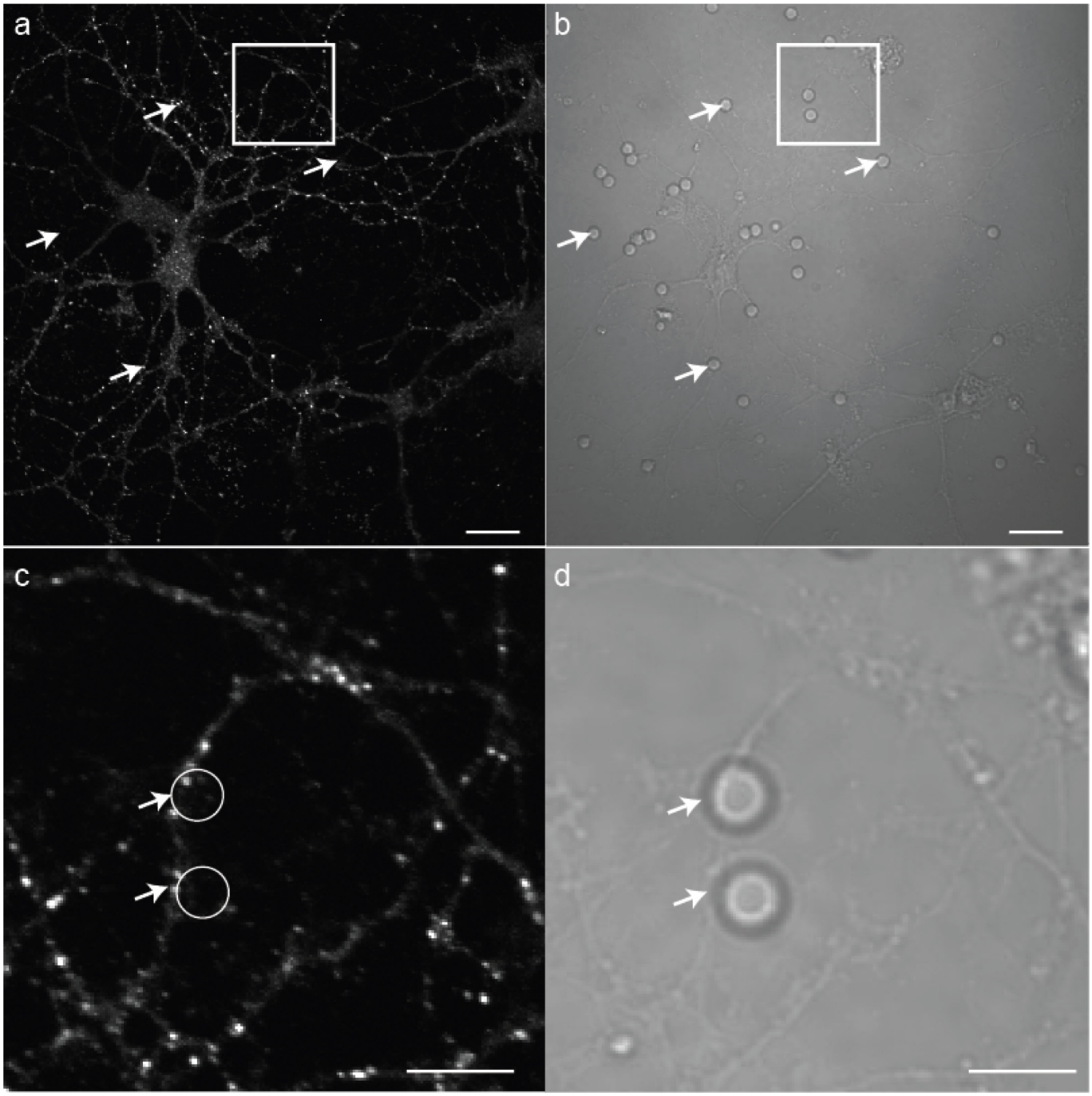

Figure SI.4. Representative confocal cross-section images of fluorescence (a) and DIC (b) channels showing post-synaptic staining of hippocampal neurons (DIV 23) when co-cultured with 25:25:50/ DOPC:DOTAP:DPPE SS-BLMs. The white boxes in images a&b represents the magnified area shown in c&d. The location of beads are encircled in white in image (c). Though post-synapses are present in culture as revealed by the presence of fluorescent boutons, there is virtually no clustering/ fluorescence enhancement at the bead-axon contacts. The fluorescent boutons (similar in size and intensity as seen everywhere in the culture) seen at the bead-axon contacts (indicated by arrows) are simple sticking of the post-synapses. The cells are fixed and incubated with anti-PSD-95/α-mouse-Cy-5 antibodies. Scale bars are 25 μm (a&b) and 10 μm (c&d).

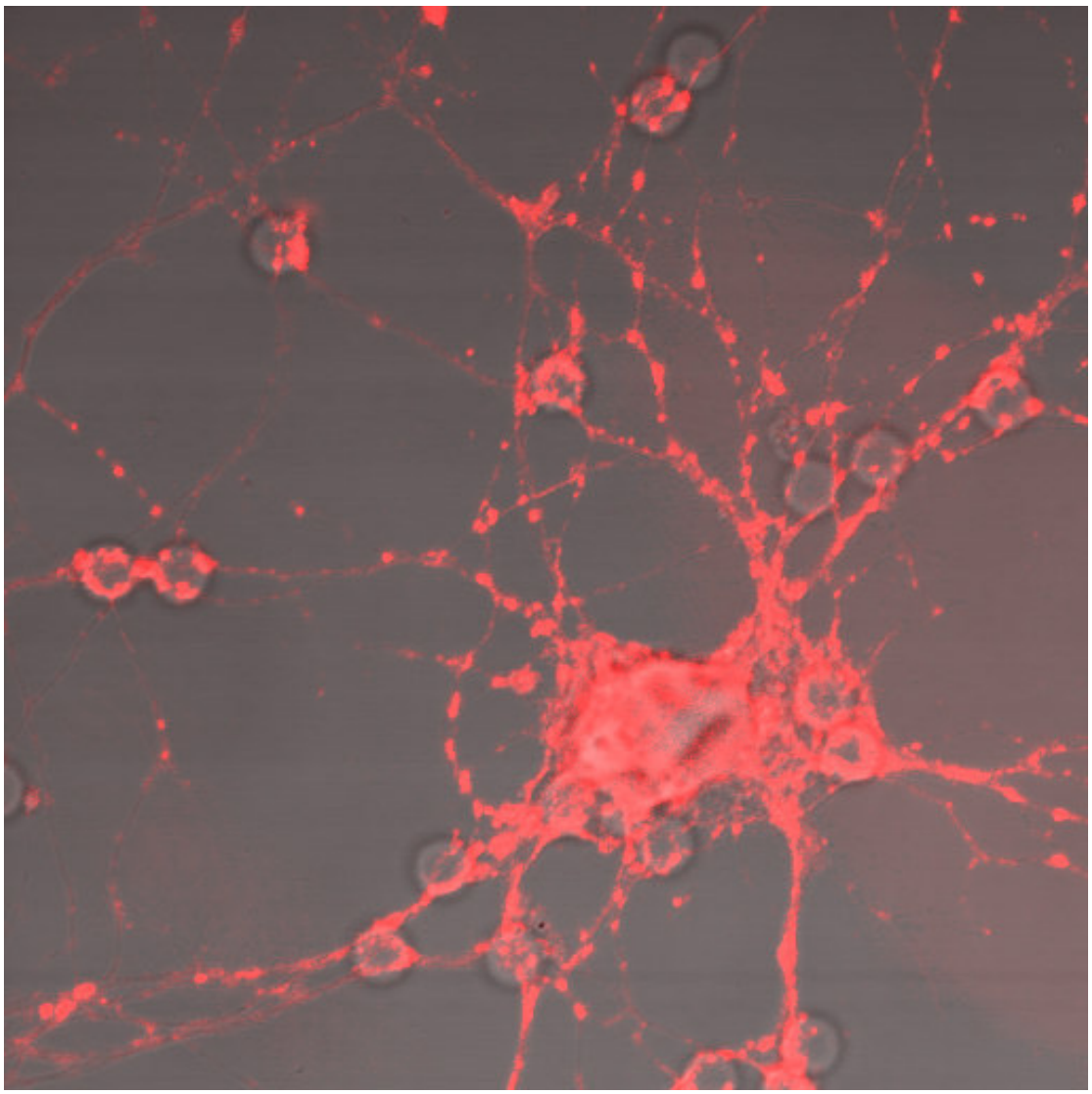

Figure SI.5. Representative confocal cross-section image (overlay of fluorescence and DIC channels) showing the influence of 25:25:50/ DOPC:DOTAP:DOPE SS-BLMs on hippocampal neurons. The fluorescence clustering is considerably low compared to the DPPE version of the SS-BLMs. The cells are fixed and incubated with synaptophysin antibodies, which were subsequently stained using Alexa-543 tagged secondary antibodies.

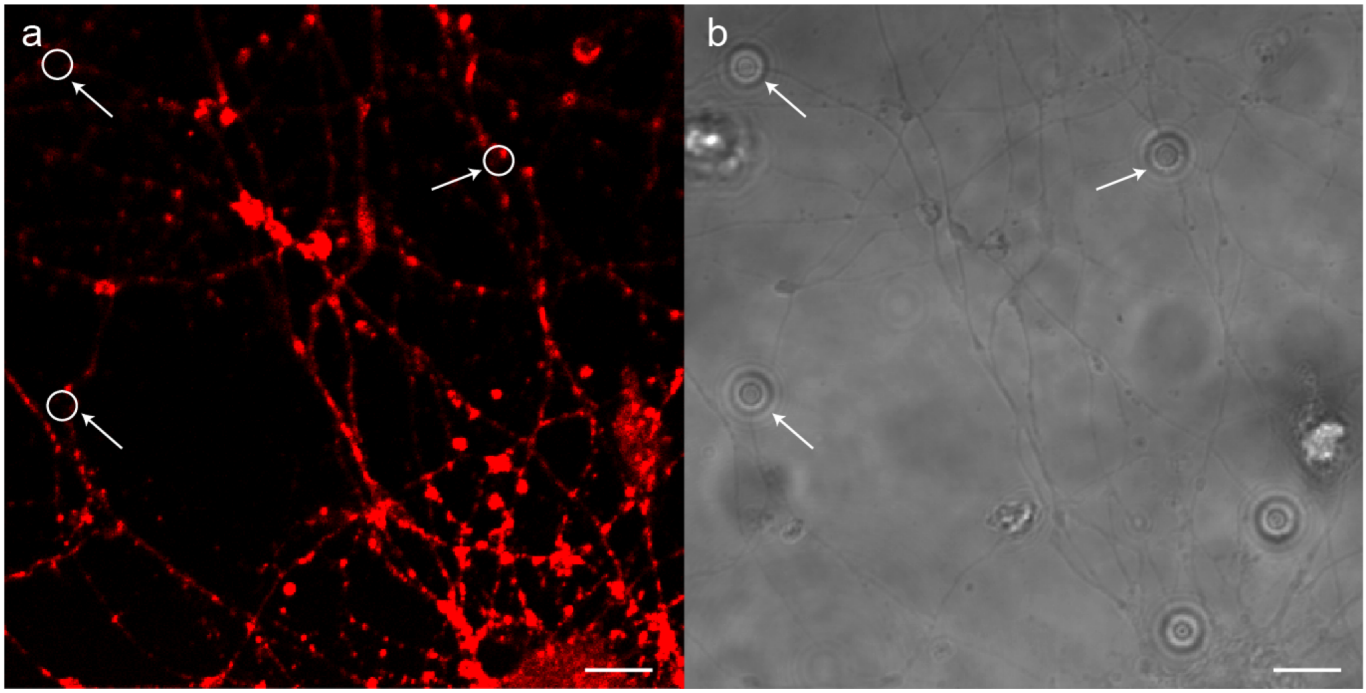

Figure SI.6. Representative confocal cross-section fluorescence (a) and corresponding DIC (b) images showing the absence of synaptophysin at the bead-axon contact points. This control experiment was performed using SS-BLM/beads containing of DOPC:DOTAP:DPPE-PEG-2000/ 25:25:50 in the bilayer when co-cultured with hippocampal neurons (DIV 16). We have tried varying compositions of DPPE-PEG-2000 in the SS-BLMs ranging from 1 to 50% and the results were all negative in terms of presynapse formation. The exact location of the beads are shown in white circles in image (a). The fluorescence clustering is only seen along the neurites in culture. Unlike in figures 3 and 4, no enhanced clustering is observed at the bead-axon contacts. The cells are fixed and incubated with anti-synaptophysin /α-rabbit-Alexa-543. Scale bars are 10 µm.

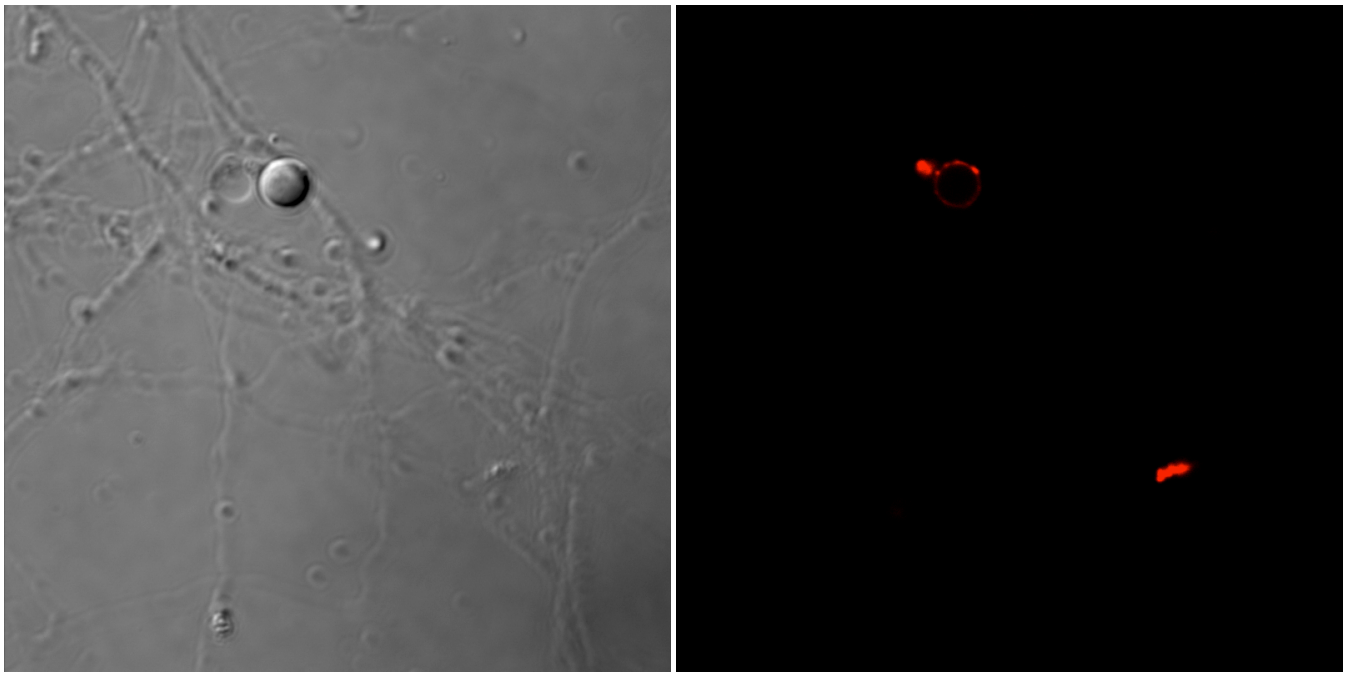

Figure SI.7. Representative confocal cross-section fluorescence image (right) and corresponding DIC image (left) showing the stability of bilayers when SS-BLM/beads were used on hippocampal neuronal cultures for 24 hrs. The fluorescence is from the TRITC-DHPE, a fluorescently labelled lipid that was used to label the SS-BLM membrane.