Supporting Information

Alpha-synuclein oligomers stabilize pre-existing defects in supported bilayers and propagate membrane damage in a fractal-like pattern

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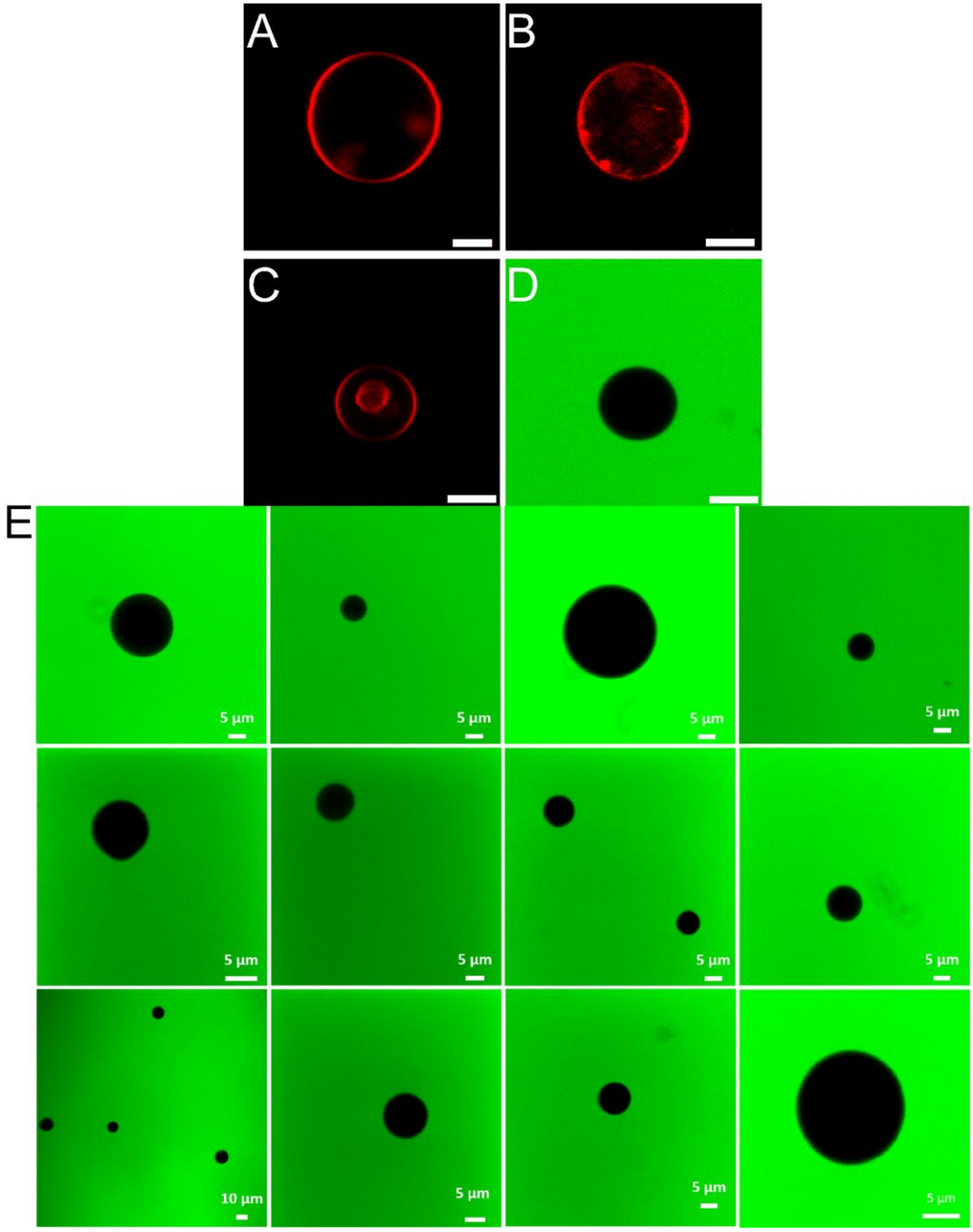

Figure S1: Representative images of immobilized POPC/POPS GUVs doped with Rh-DOPE (red) incubated with αS oligomers. POPC/POPS GUVs containing biotinylated lipids were immobilized on streptavidin-coated cover-slips. GUVs remain stable in the absence of αS oligomers (panel A) and even when incubated with 2.5 µM αS oligomers at room temperature for 20 hours (panel B). Scale bars are 5 µm in panel A and B. Panels C (red channel, GUV label) and D show images of GUVs incubated with 10 µM αS oligomer and calcein in the external solution at room temperature. Scale bars are 10 µm in panel C and D. Even after 24 hours of incubation with αS oligomers, vesicles remained intact and no influx of calcein was observed (Panel E).

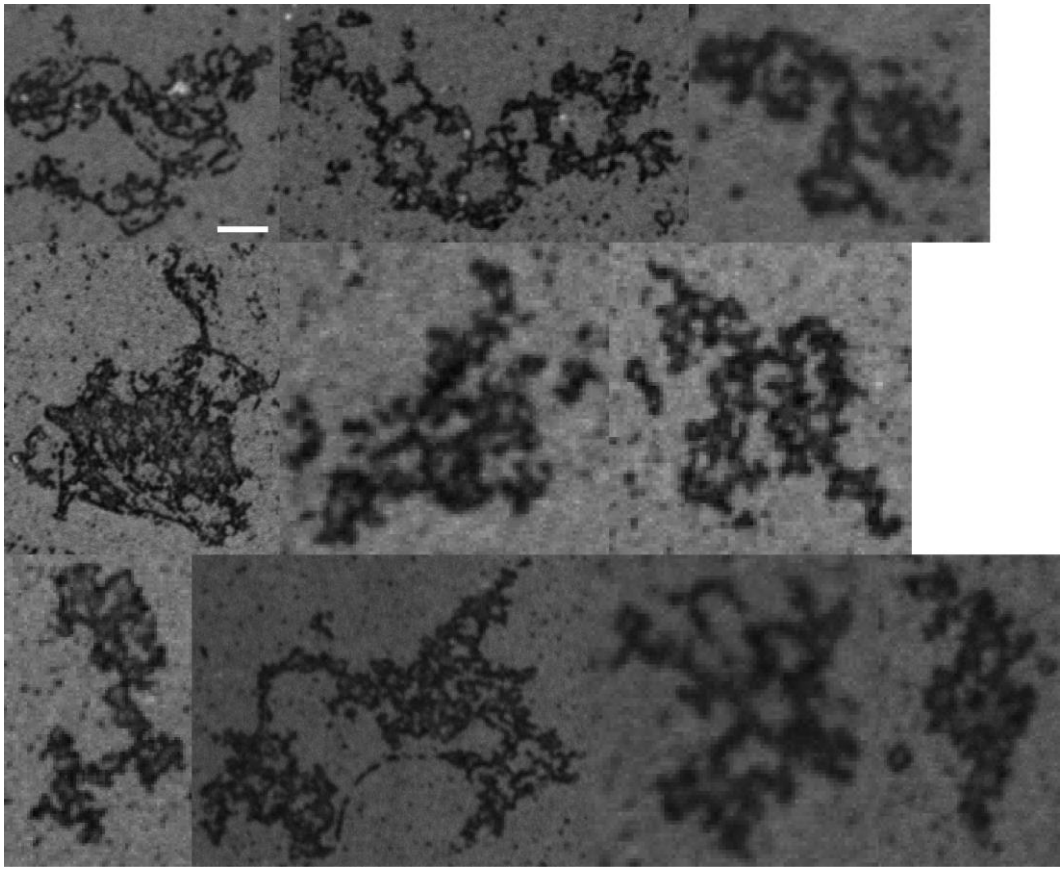

Figure S2: Analysis of αS oligomer induced membrane damage pattern by fluorescence confocal microscopy. (A) Representative gray-scale images showing damage patterns in Rhod-PE labeled POPC/POPS SLBs at room temperature acquired after 6 hours of incubation of 5 μM αS oligomers from two independent measurements. The SLBs were buffered with 10 mM Tris-Cl, 100 mM NaCl at pH 7.4. The scale bar is 2 μm.

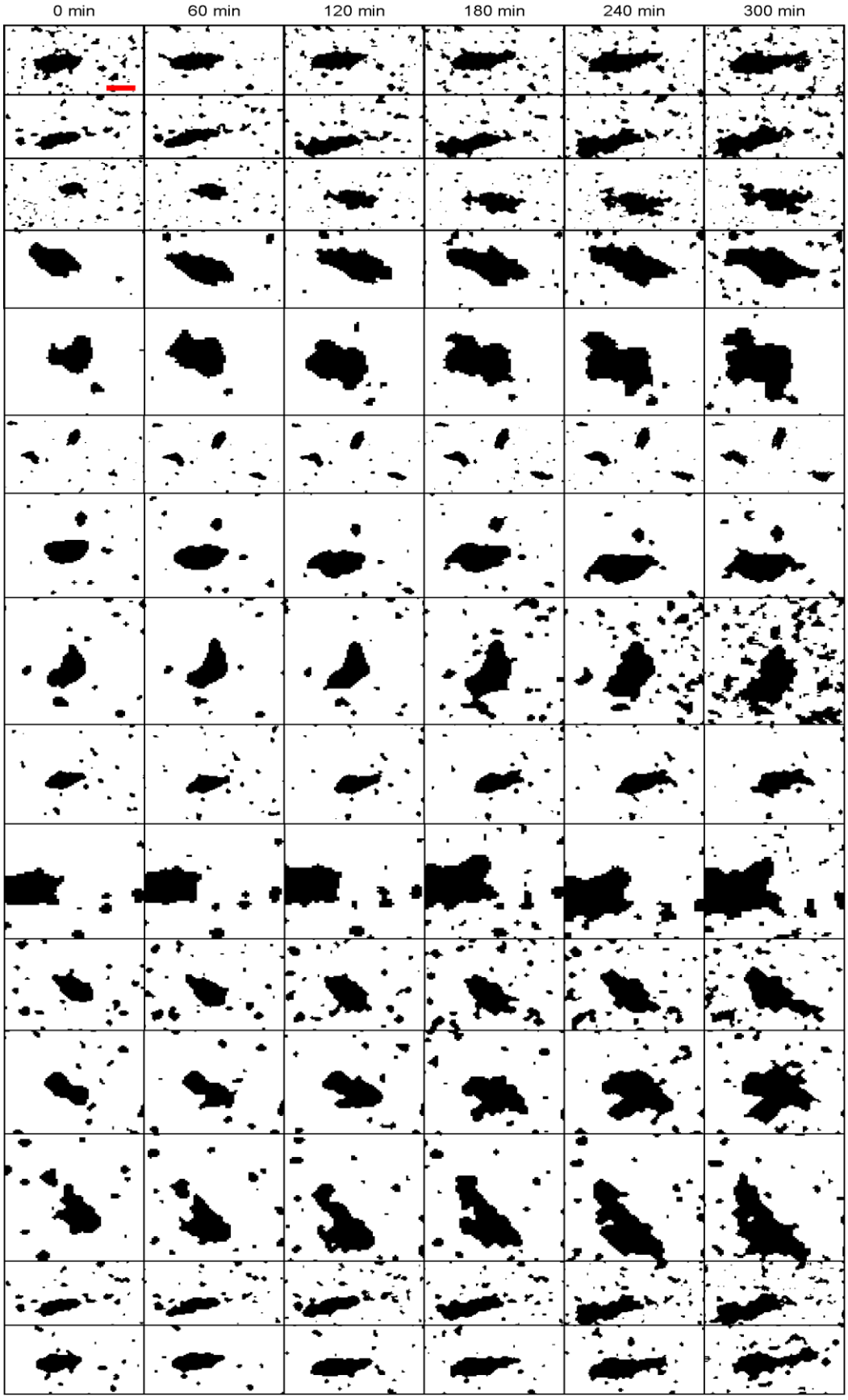

Figure S3: Time-lapse confocal images of evolution of damage patterns on POPC:POPS (1:1) SLBs on a PEG surface upon addition of αS oligomers. All time-lapse images from the lipid channel are converted to binary scale to aid visualization. The red scale bar is 2 μm.

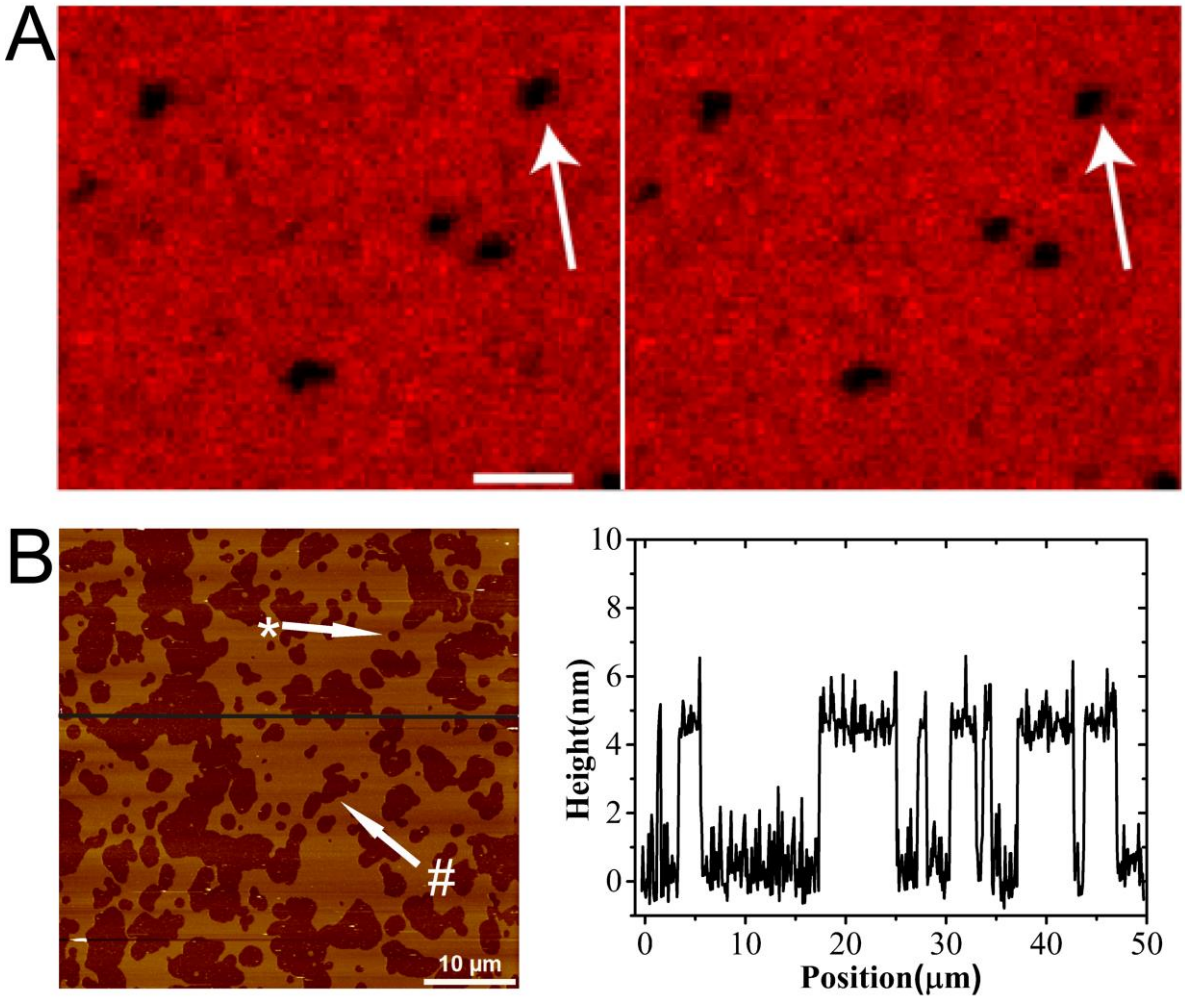

Figure S4: A) Confocal images of POPC:POPS (1:1) SLBs before (left) and after (right) WT-αS monomer addition. The panel on the right was acquired after 6 hours of incubation of 10 µM αS monomers and show slight decrease in defect areas (see white arrow). The quantification of the changes in defect area is shown in Figure 2A of the main text. The SLBs were buffered with 10 mM Tris-Cl, 100 mM NaCl at pH 7.4. The scale bar is 1 µm. B) AFM image of a defect containing POPC/POPS SLB in the absence of αS oligomers (left panel). Height profile of the SLB along the black line shown in right top panel. The height differences in the profile of ~5 nm correspond to the typical thickness of a lipid bilayer. White arrow indicates nearly circular defects (indicated by *) and several rounded defects merging (indicated by #).

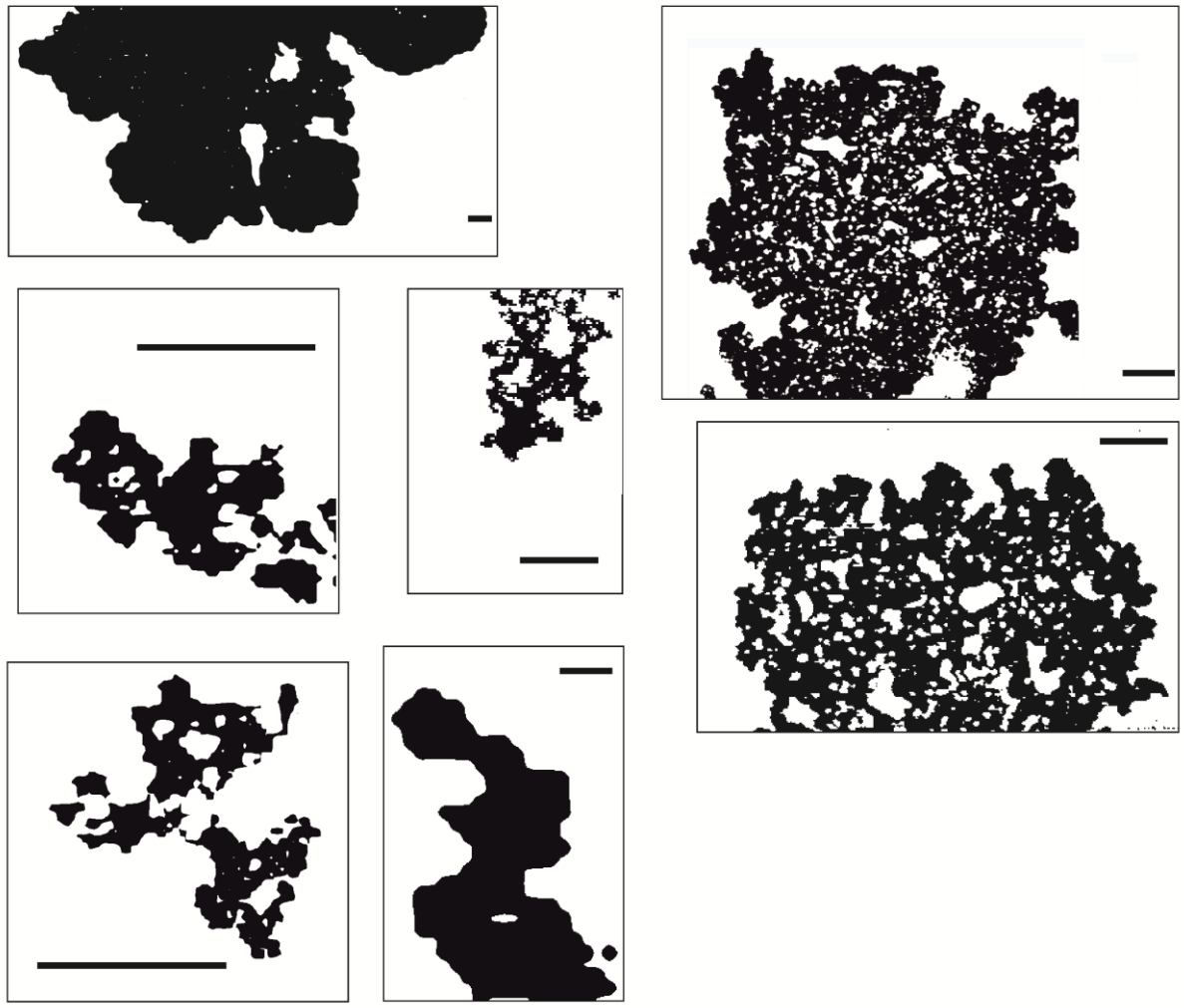

Figure S5: Overview of membrane damage patterns obtained from AFM images on POPC:POPS (1:1) SLBs on mica. All time-lapse images are converted to binary scale to aid visualization. The SLBs were buffered with 10 mM Tris-Cl, 100 mM NaCl at pH 7.4. Scale bar is 0.5 µm.

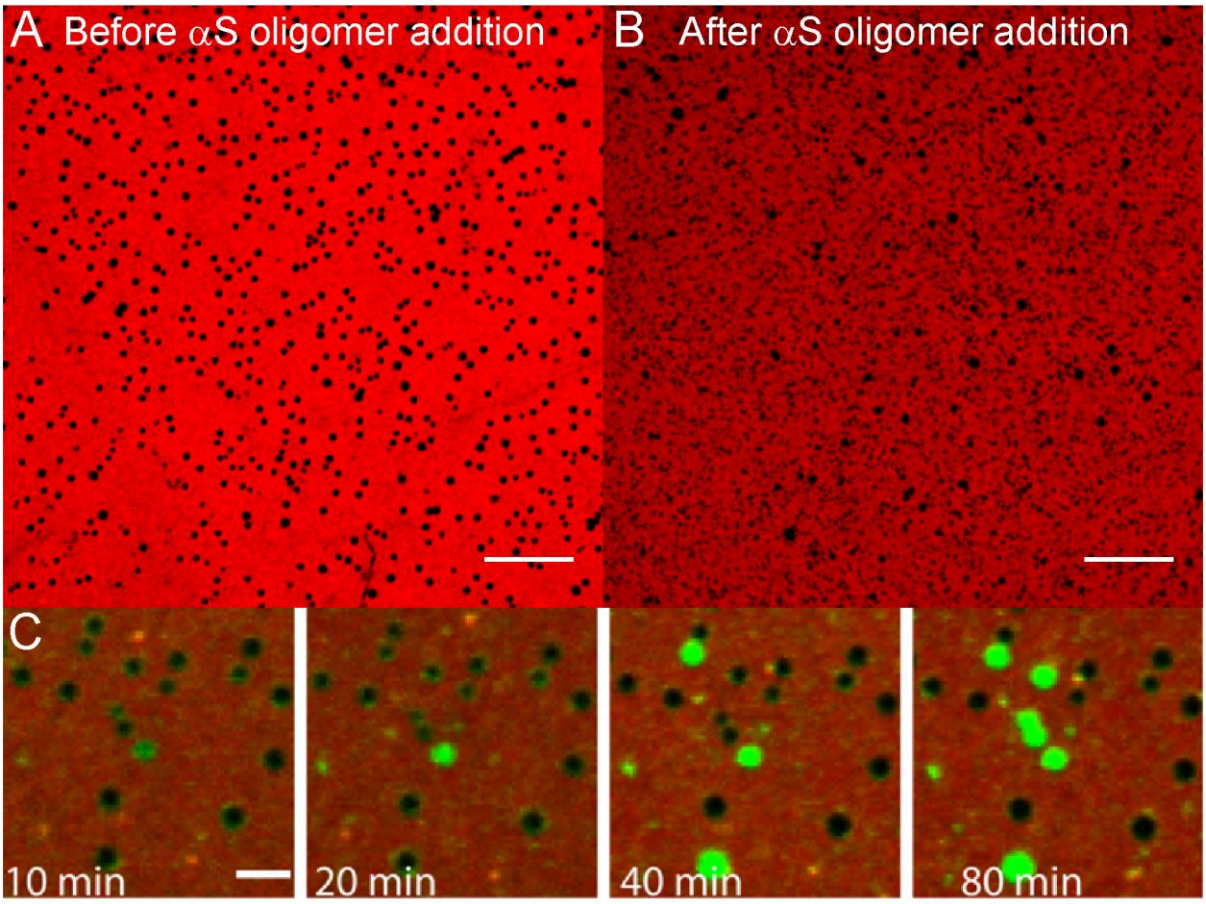

Figure S6: Rounded defects (panel A) in SLBs on glass substrate in absence of αS oligomers and irregularly shaped defects (panel B) in presence of αS oligomers. The scale bar is 10 μm. **C) αS oligomers accumulate at membrane defect edges.** POPC:POPS (1:1) SLBs were doped with 0.5 mol% Rhod-PE (red above). 5 μM of αS oligomers (10% AlexaFluor488 labeled, green above) was added to SLBs with preexisting defects. A green fluorescence ring is seen along the defect edges before it filles up αS oligomers from the solution. The SLBs were buffered with 10 mM Tris-Cl, 100 mM NaCl at pH 7.4. All measurements were conducted at room temperature. The scale bar is 2.5 μm.