Thermoplasmonic nano-rupture of cells reveals Annexin V function in plasma membrane repair

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Supporting Information Available

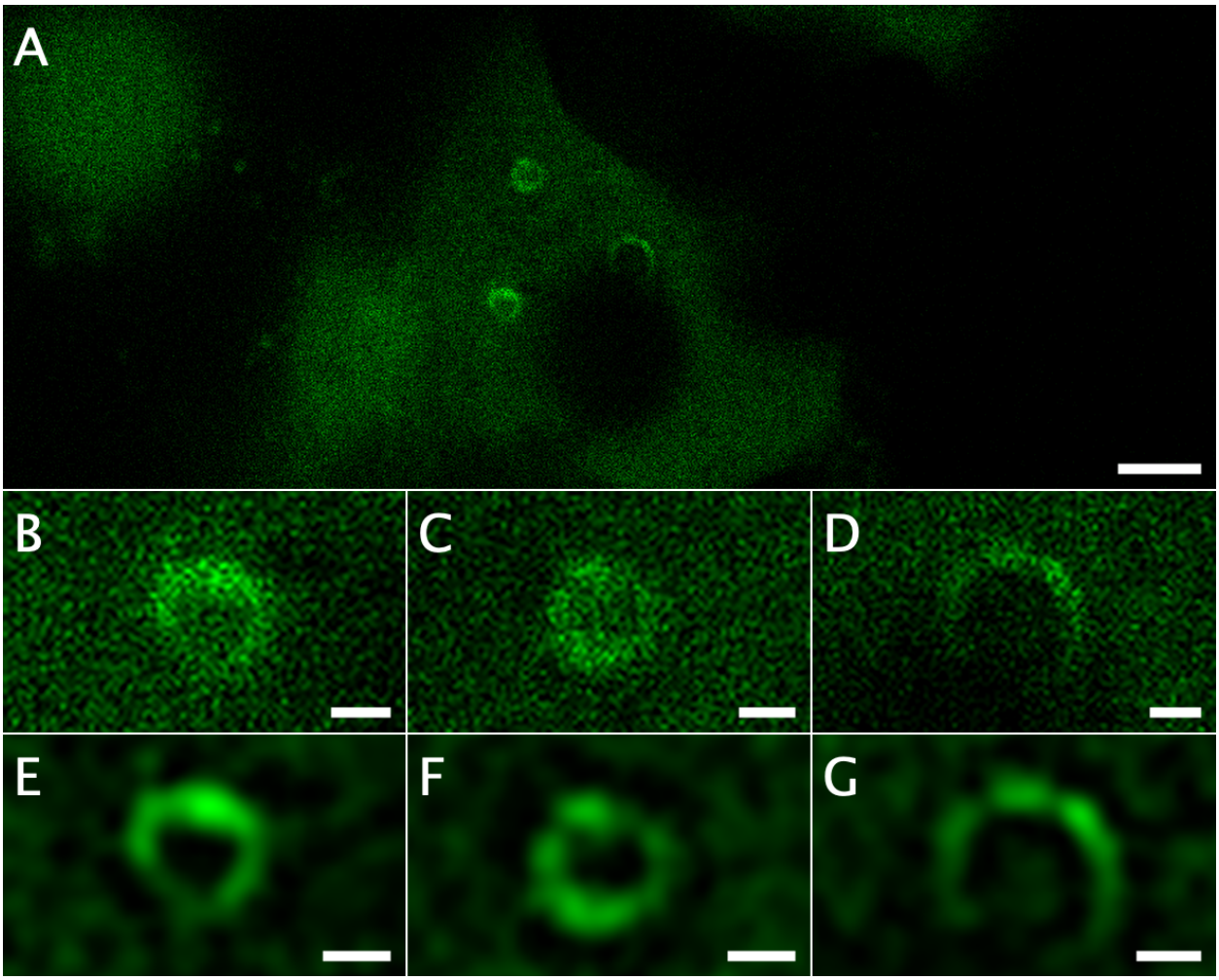

Fig. S 1: Membrane puncturing of a HEK293T cell. The cell is able to survive puncturing several times by activation of the PMR machinery. A) Cell with 3 punctures. Scale bar is 5 μm B), C) and D,) show the unedited rings from A. E), F) and G) show edited images using a Difference of Gaussian (DoG) filter of the images above. All scale bars, except for A), are 2 μm.

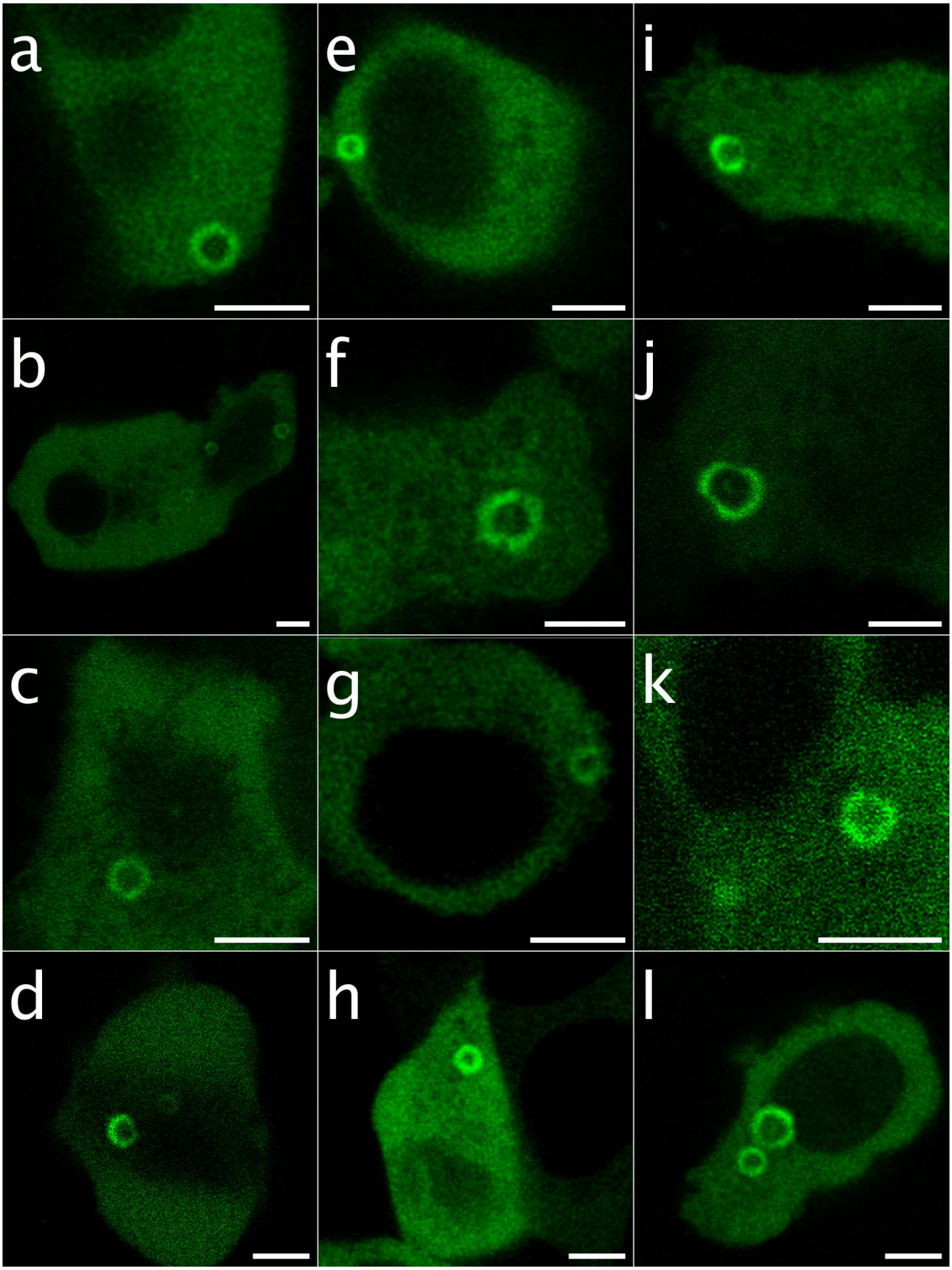

Fig. S 2: Examples of punctured HEK293T cells expressing ANXA5-gfp. All cells were punctured by plasmonic gold nanoparticles located between a coverglass and an adhering cell. Laser powers ranged from (add approximate laser levels). Scale bars are all 5 μm.

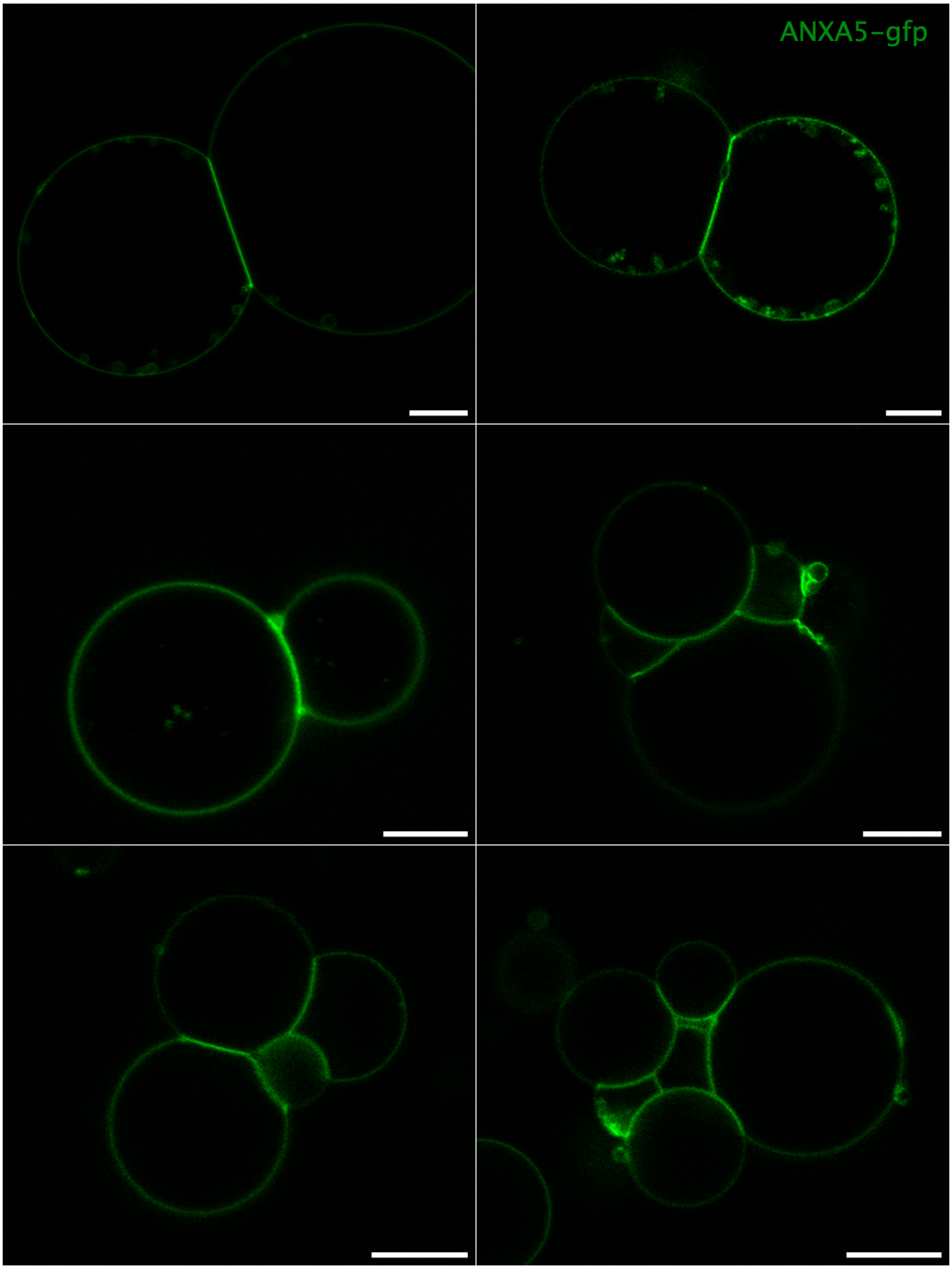

Fig. S 3: Interaction of C-terminal in between ANXA5 interacting at the interface between apposing vesicles. These are examples of GUVs prepared using electroformation (DOPC 90% + DOPS 10%) in a CA2+ buffer of 2mM. Purified ANXA5-gfp was added in solution causing strong binding of GUVs. Scale bars are all 10 μm.

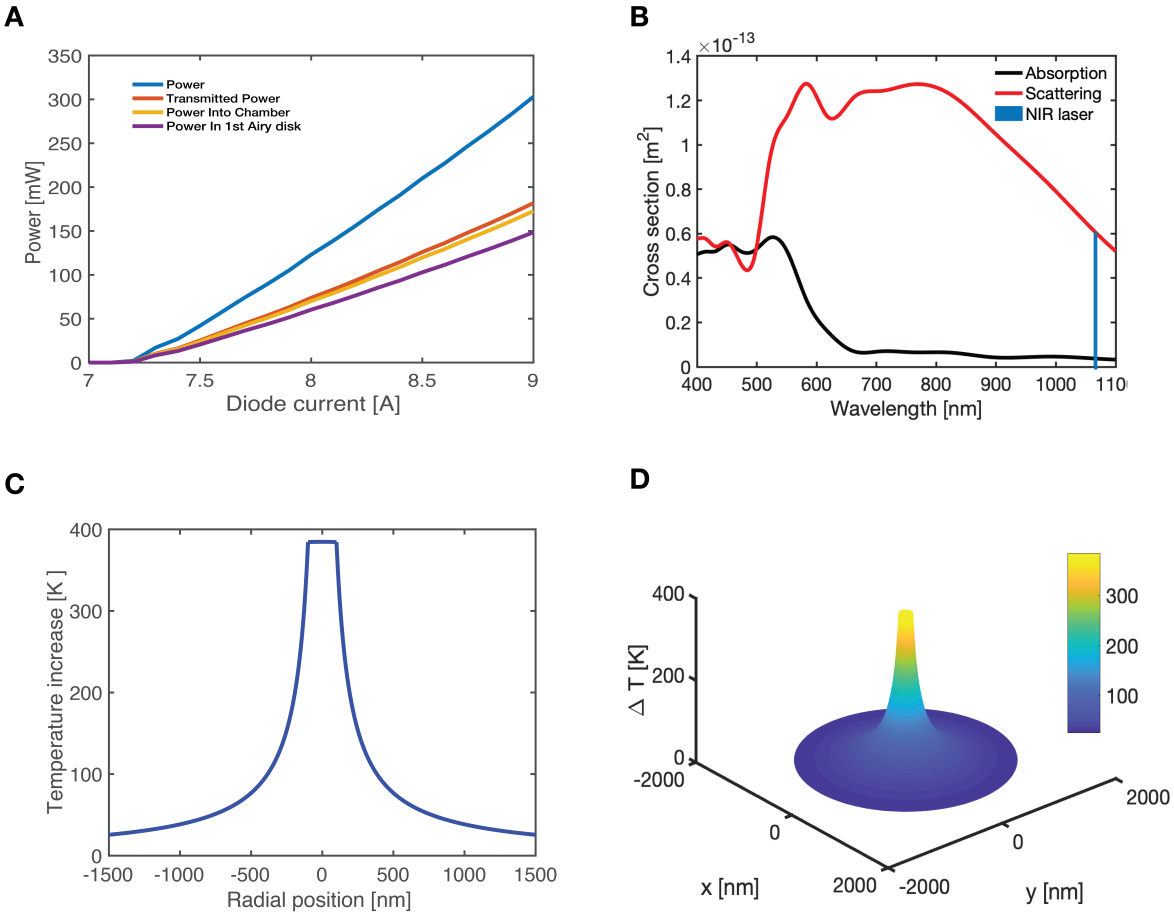

Fig. S 4: Calculations of thermoplasmonic heating for an irradiated 200 nm gold nanoparticle. (A) Power measurements of the experimental set up at different points of the NIR laser path. Blue, right after the fiber. Red and orange are before and after the objective respectively. Purple is the power measured in a sample. (B) Absorption and scattering cross sections calculated for a AuNP of 200 nm diameter. (C) Calculation of temperature increase around a 200 nm irradiated gold nanoparticle based on parameters shown in (A) and (B) and using a current of 8A. (D) 3D render of the same heating profile presented in (C).

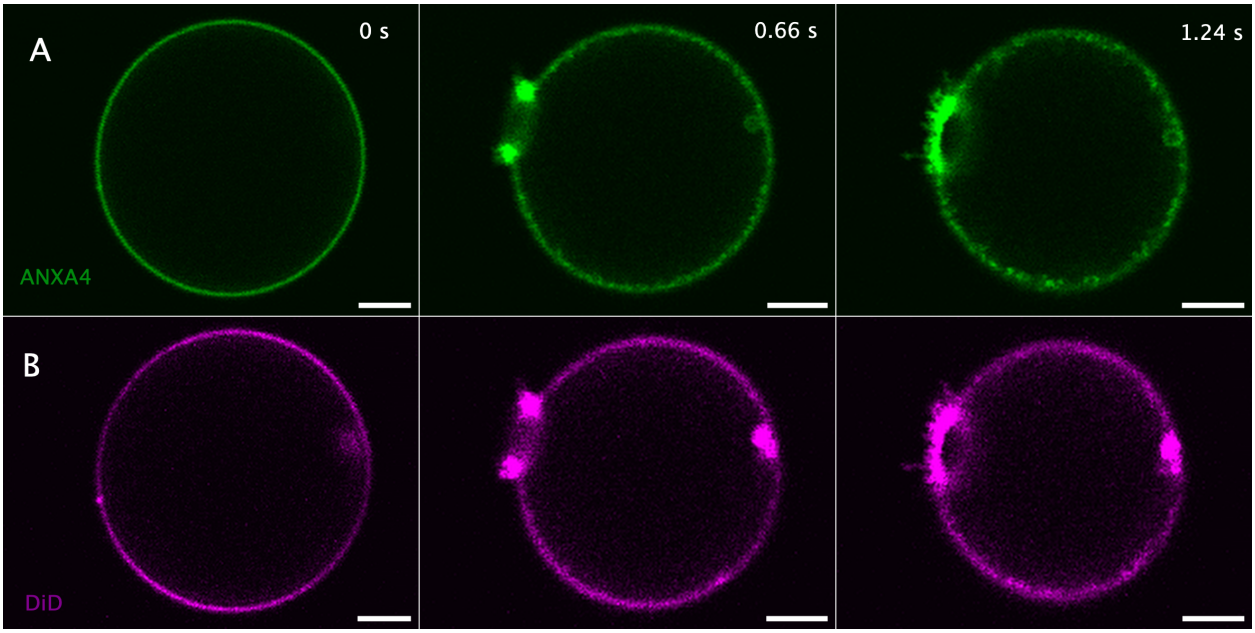

Fig. S 5: Example of punctured GUV with bound ANXA4-gfp and membrane DiD labeling. A) GFP-ANXA4 fluorescence channel with time interval in between frames. B) DiD fluorescence channel. The time interval in between frames is the same as for panel A). Scale bars are all 5 μm.

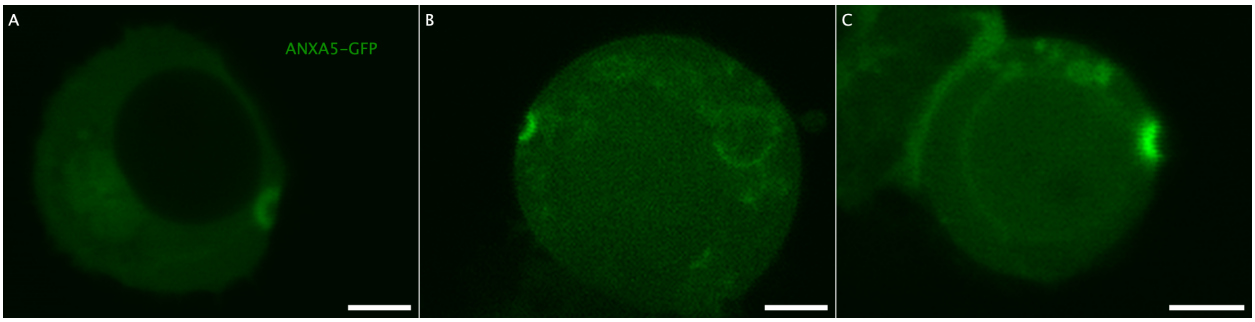

Fig. S 6: HEK293T cells expressing ANXA5 punctured from one side. Scale bars are all 5 μm.

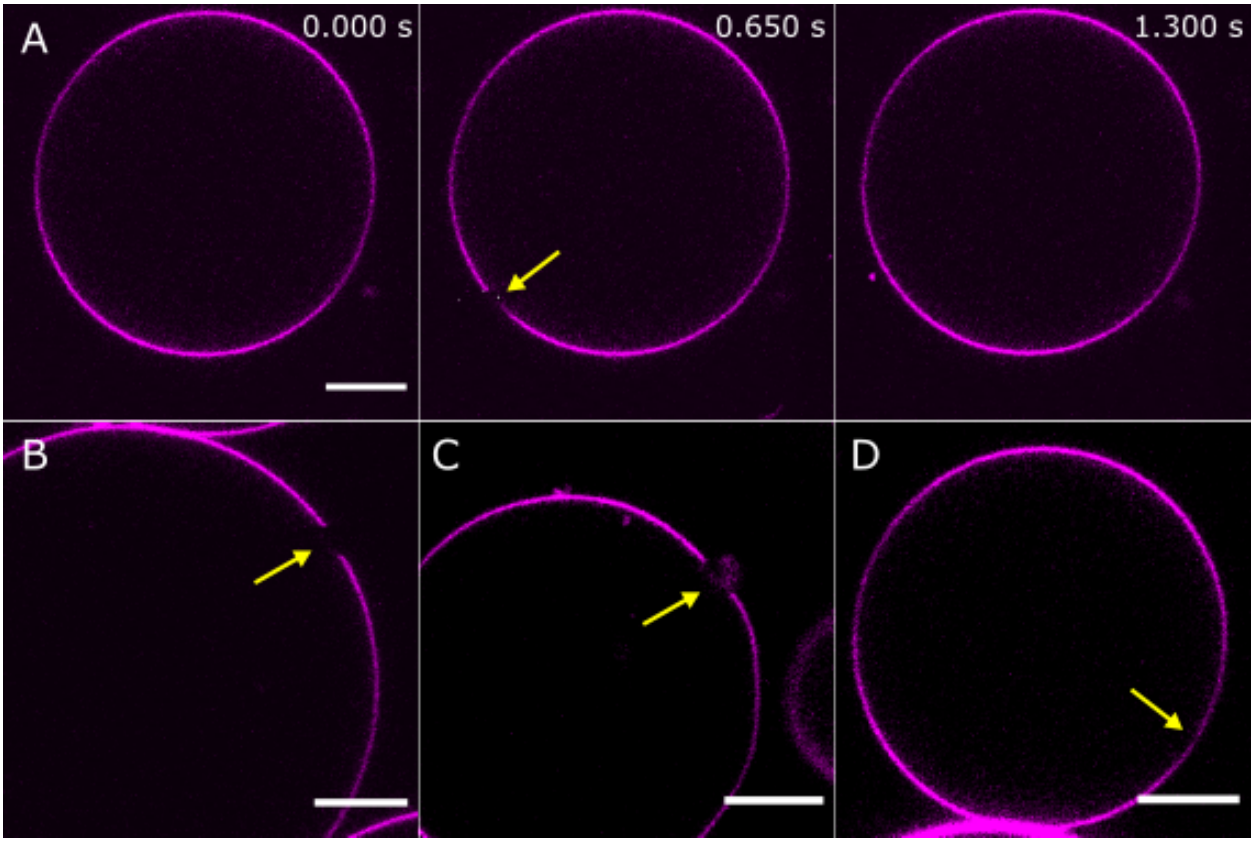

Fig. S 7: Control experiments with GUVs containing only labelled PM with DiD. We can not observe rolling, budding or rings-like structures when ANXAs are not present. Yellow arrows indicate the thermoplasmonic-induced puncture sites. A) Time sequence of a punctured whole and its repair. B) punctured whole C) Punctured whole D) Punctured whole. Scale bars are all 10 μm.