Supplemental Materials

Molecular Biology of the Cell

Dunsing et al.

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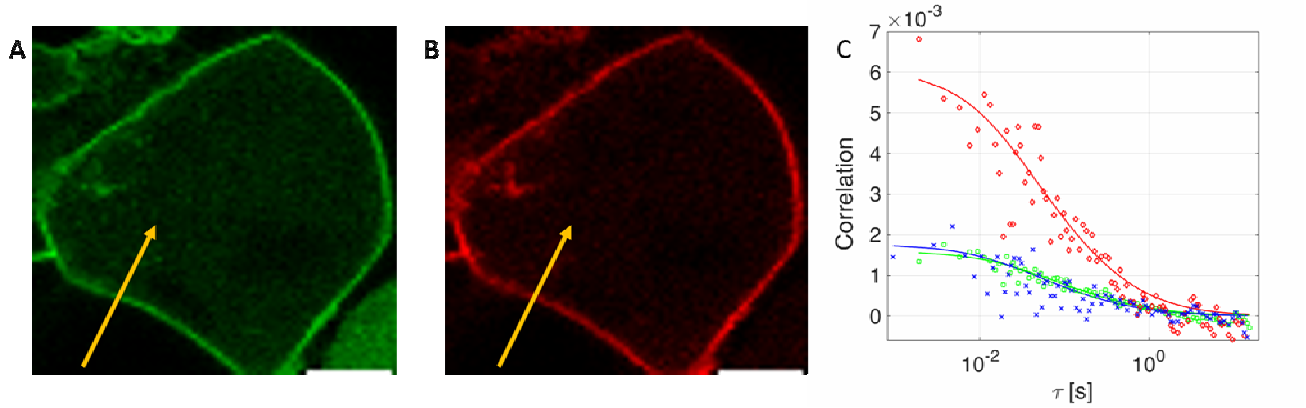

Figure S1: sFCS cross-correlation positive control. A, B: Representative image of a HEK cell expressing a myr-palm-YFP-Card tandem. YFP is detected in the green channel (A) and Card in the red channel (B). The yellow arrow represents a typical two-color sFCS line scan. Scale bar is 5 µm. C: Representative ACFs and CCF from two-color sFCS analysis of the cell represented in panels A and B. Red: ACF for the red channel (Card), green: ACF for the green channel (YFP), blue: CCF calculated between the two spectral channels. Solid lines represent the fit of a two-dimensional diffusion model to the data. 14 cells from three independent measurements were analyzed. All measurements were performed at room temperature.

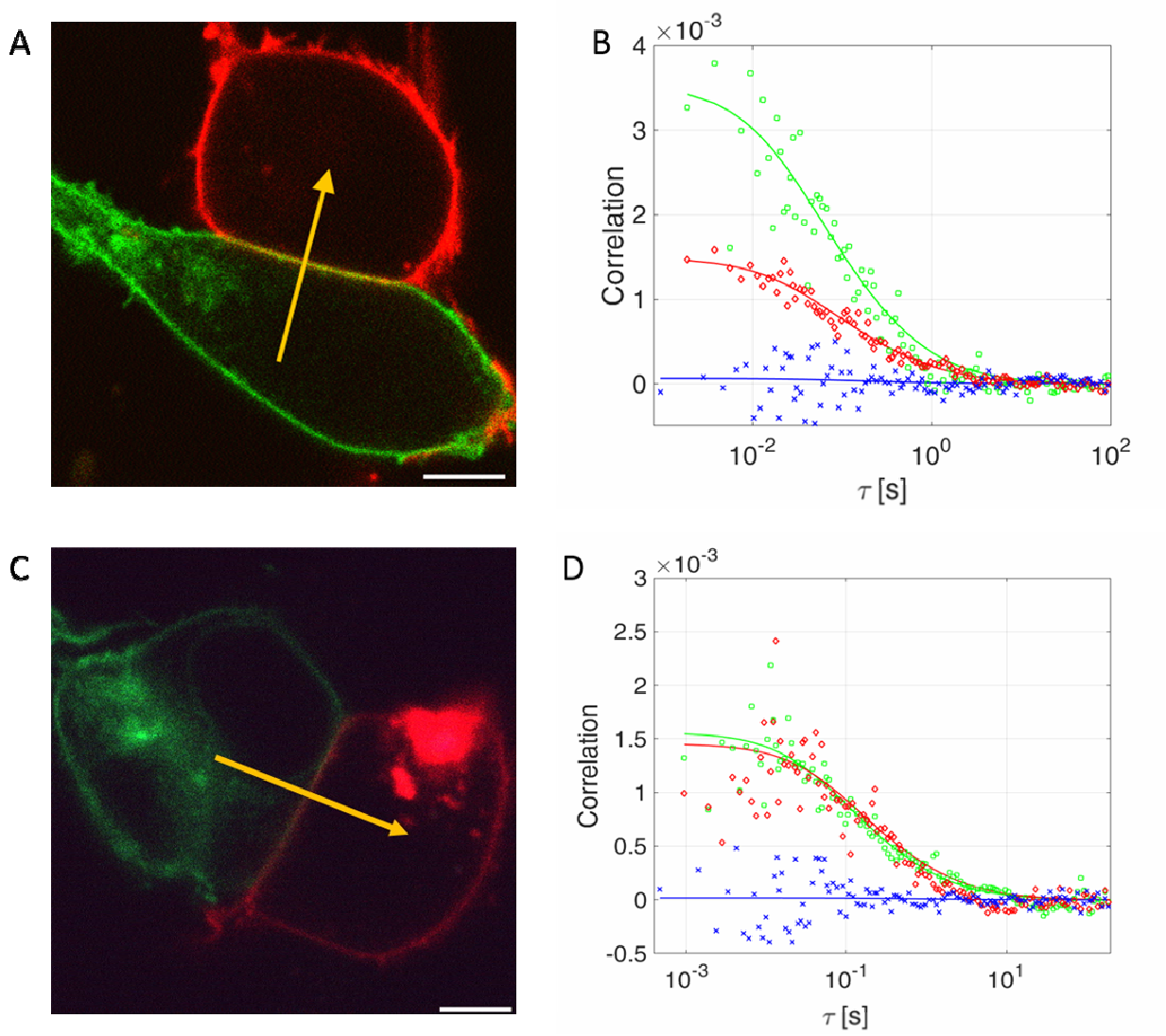

Figure S2: sFCS cross-correlation negative controls for *trans* binding. A: Representative image of HEK cells expressing myr-palm-YFP (green) or myr-palm-Card (red). The yellow arrow represents a typical two-color sFCS line scan. Scale bar is 5 µm. B: Representative ACFs and CCF from two-color sFCS analysis of cells represented in panel A. Red: ACF for the red channel (myr-palm-Card), green: ACF for the green channel (myr-palm-YFP), blue: CCF calculated between the two spectral channels. Solid lines represent the fit of a two-dimensional diffusion model to the data. 17 cells from three independent measurements were analyzed.

As an additional control, we monitored junctions between cells expressing APLP1-Card or CD86-YFP. CD86 is a transmembrane protein that is supposed to have a diffusion coefficient similar to that of APLP1. Also, no *trans* interaction is expected between CD86 and APLP1. C: Representative image of HEK cells expressing CD86-YFP (green) or APLP1-Card (red). The yellow arrow represents a typical two-color sFCS line scan. In this experiment, the fastest available scan speed (945.45µs for both lines) was used. Scale bar is 5 µm. D: Representative

ACFs and CCF from two-color sFCS analysis of cells represented in panel C. Red: ACF for the red channel (APLP1-Card), green: ACF for the green channel (CD86-YFP), blue: CCF calculated between the two spectral channels. Solid lines represent the fit of a two-dimensional diffusion model to the data. 12 cells from two independent measurements were analyzed. The average relative cross-correlation was 0.04±0.06 and the diffusion coefficient of CD86-YFP was 0.29±0.15 μm²/s (mean±s.d.). The diffusion coefficient of CD86-YFP is indeed comparable to that measured for APLP1-YFP (0.25±0.09 $\mu m^2/s$, see Main Text). All measurements were performed at room temperature.

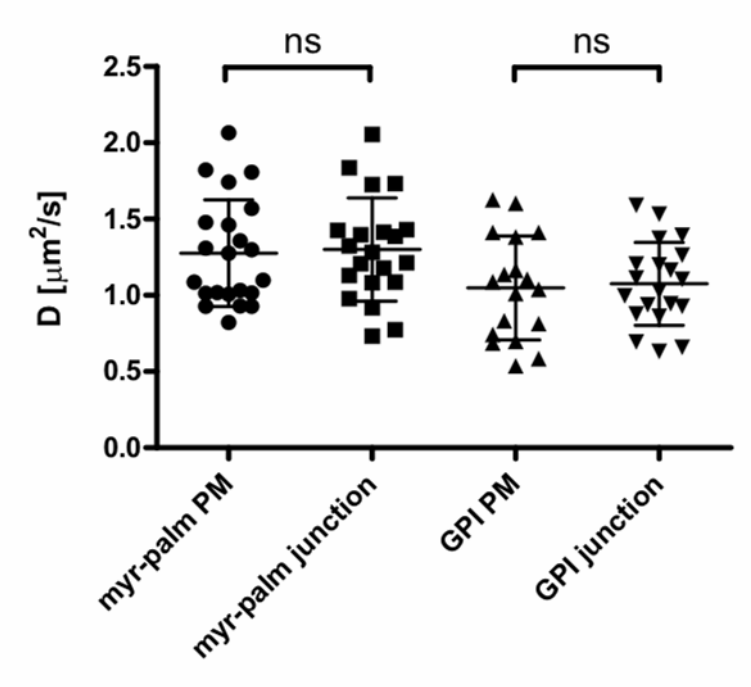

Figure S3: Diffusion coefficents of simple model membrane proteins, within or outside cell-cell junctions. Diffusion coefficients of myr-palm-YFP and GPI-YFP measured with one-color sFCS on 18-22 cells each, in two independent experiments. “ns” indicates that no statistically significant difference could be determined with a Welch’s two-sided t-test. All measurements were performed at room temperature.

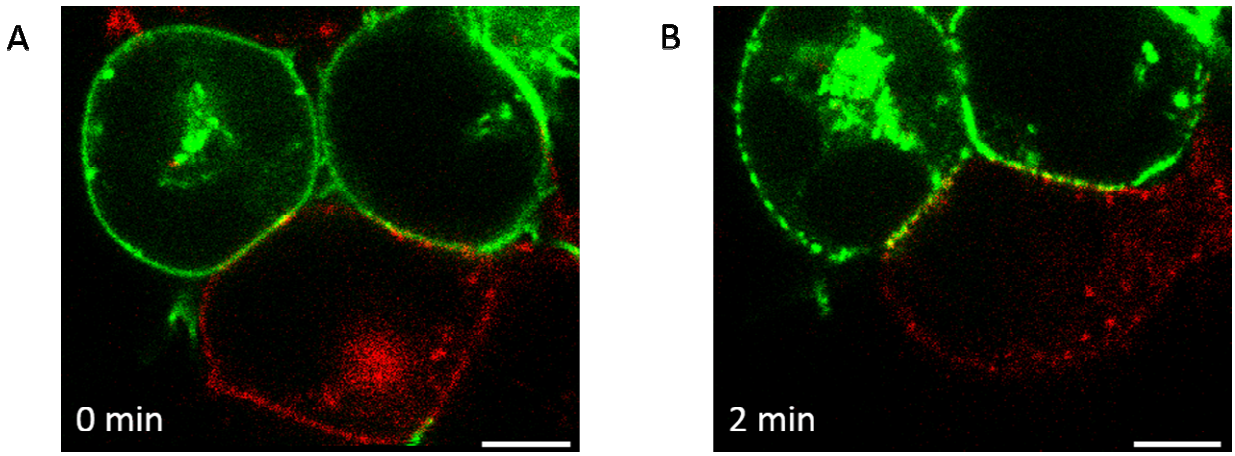

Figure S4: Zinc induces the rapid formation of APLP1 clusters. HEK cells were transfected with APLP1-Card (red) or APLP1-YFP (green). A: Representative image of HEK cells expressing the two fluorescent APLP1 constructs in the absence of zinc. The buffer was then supplemented with 50 µM $ZnCl_2$ and time-series images were acquired. B: Representative image of the same HEK cells in panel A, 2 min after the addition of zinc. These results indicate that zinc induces the formation of both *cis* and *trans* APLP1 clusters already within ~2 min. Scale bar is 5 µm. All measurements were performed at room temperature. The experiment was replicated at least twice using independent samples.

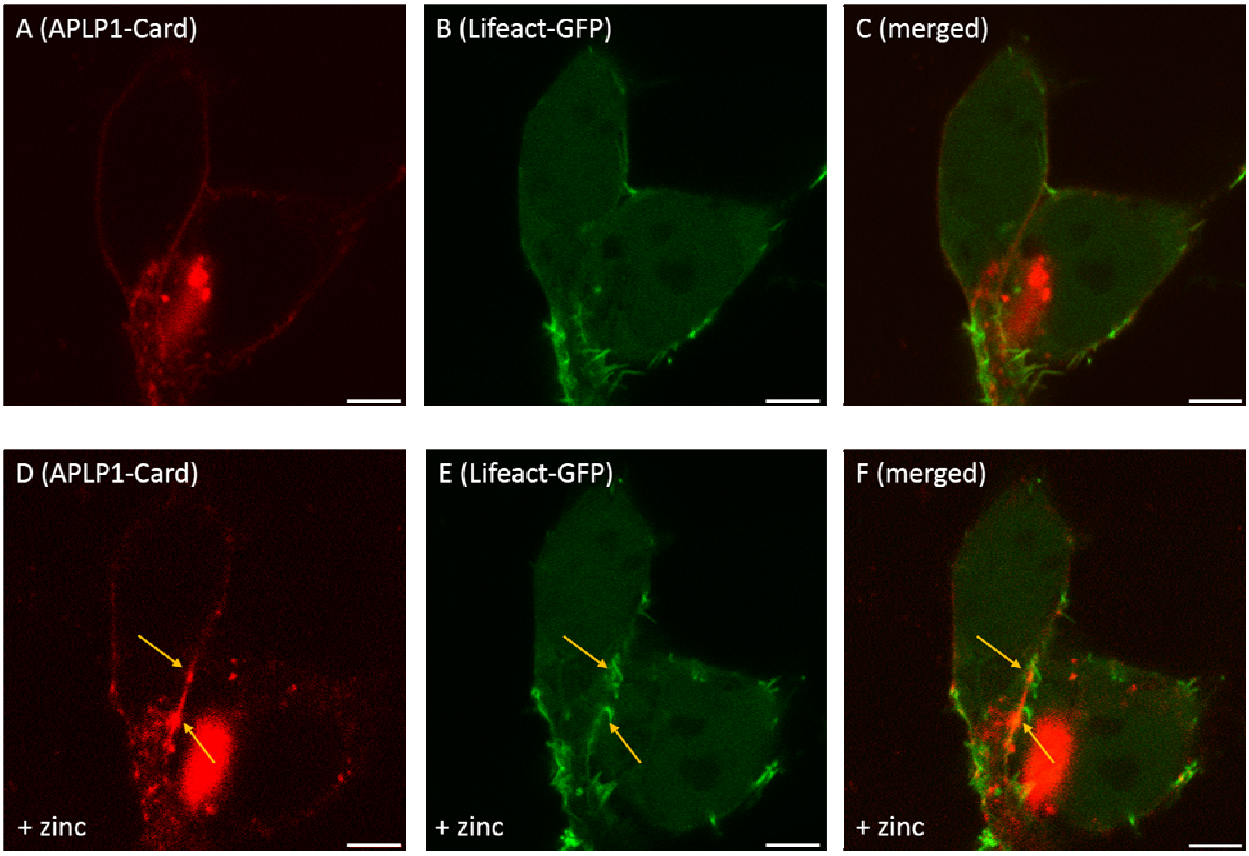

Figure S5: Filamentous actin accumulates in correspondence of zinc-induced APLP1 clusters at cell-cell junctions. A, B: Representative images of HEK cells expressing APLP1-Card (A) and Lifeact-GFP (B) forming a junction in the absence of zinc. APLP1-Card is distributed homogeneously in the PM. The actin-labelling probe is visible throughout the cytoplasm and enriched in certain regions of the PM, but not at the cell-cell junction. C: Merged channels from panels A and B. The buffer was next supplemented with 50 µM $ZnCl_2$ and, after 20 min, the same cells were imaged (D-F). The yellow arrows indicate APLP1-Card clusters forming at the cell-cell junction. Enrichment of filamentous actin can be observed in close proximity of APLP1 clusters, thus suggesting an involvement of actin in APLP1-APLP1 interaction. Scale bar is 5 µm. All measurements were performed at room temperature. The experiment was replicated at least twice using independent samples.