Supplementary figures for:

Diffusion of lipids and GPI-anchored proteins in actin-free plasma membrane vesicles measured by STED-FCS

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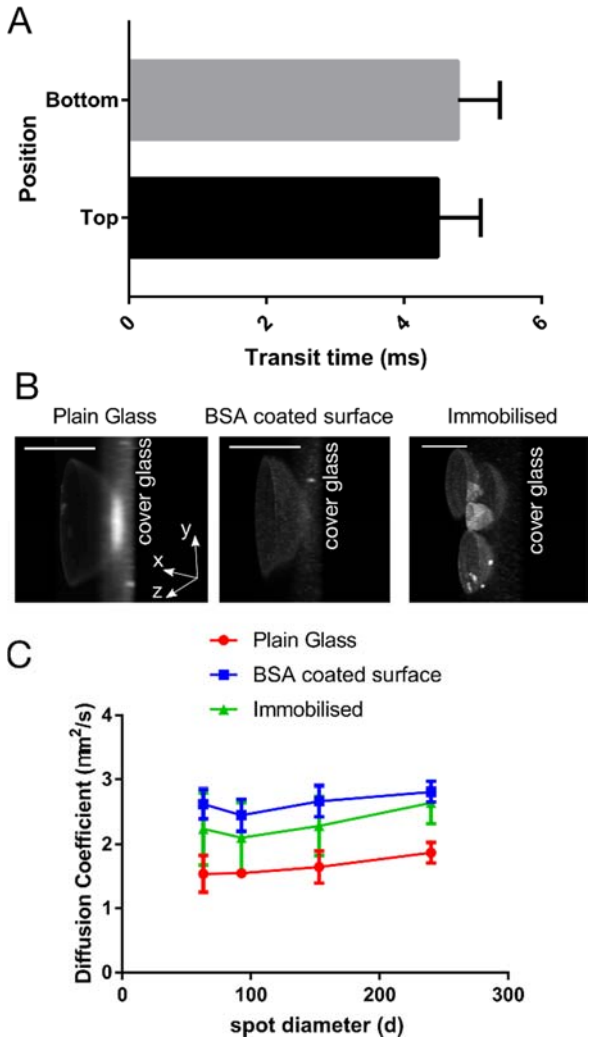

Figure S1. Impact of immobilization on diffusion in GPMVs. A) Results from confocal FCS measurements at the top and bottom membrane of immobilized GPMVs. Average transit times of Atto647N-DPPE were similar, confirming that the immobilisation of the GPMVs at the bottom membrane did not cause any additional hindrance in diffusion. FCS data were taken for at least five GPMVs, and error bars represent standard deviations. B, C) STED-FCS measurements on immobilized vs mobile GPMVs, B) 3D confocal images of GPMVs: (top) non-immobilized GPMVs on plain glass, (middle) non-immobilized GPMVs on BSA coated surfaces, and (bottom) GPMVs after immobilisation (scale bars 10 µm), C) Results from the STED-FCS recordings of Atto647N-DPPE on the basal membrane of the differently prepared GPMVs: Dependency of the apparent diffusion coefficient D on the observation spot diameter d for non-immobilized GPMVs on plain glass (red) and on BSA coated surfaces (blue), and for immobilized GPMVs. In all cases, Atto647N-DPPE was diffusing freely (straight line of the D(d) dependence). While overall diffusion is similar for non-immobilized GPMVs on BSA coated surfaces and immobilized GPMVs, it was significantly slower for the non-immobilised GPMVs on glass. We attribute the latter slow-down to the direct interaction between the membrane and the glass surface. STED-FCS data were recorded on at least five GPMVs, and error bars represent standard deviations.

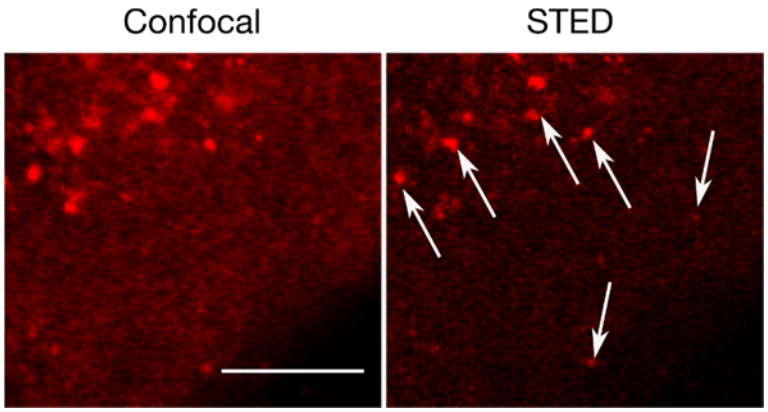

Figure S2. Confocal (left) and STED (right) images of live PtK2 cells expressing GPI-SNAP labelled with Abbberior Star Red, revealing bright immobile clusters (representative clusters are marked by arrows). Scale bar 10 µm.

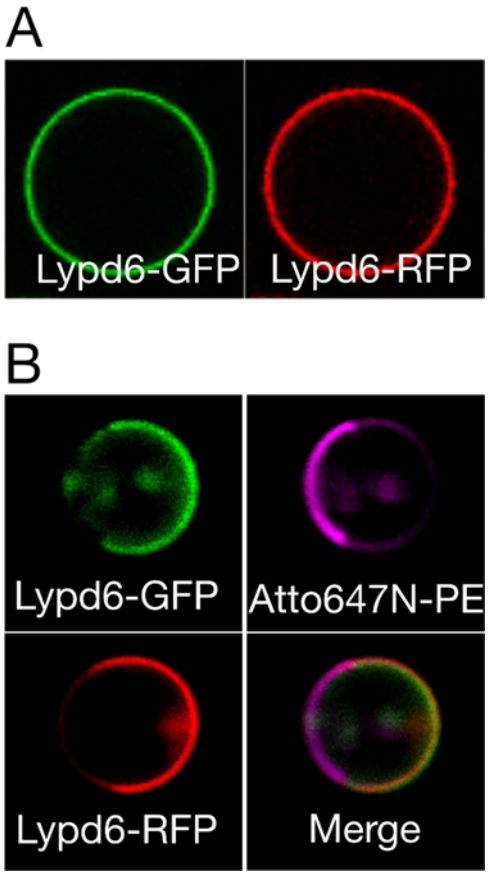

Figure S3. Confocal images of the equatorial plane of GPMVs prepared from cells expressing Lypd6-GFP and Lypd6-RFP. A) Confocal images at room temperature, revealing no visible clusters. B) Confocal images at 10 °C, where the plasma membrane of GPMVs phase-separates into a more ordered and a more disordered phases. Lypd6-GFP and Lypd6-RFP partition into the ordered domains. The disordered domains are marked by Atto647N-DOPE.

Supplementary Movie 1

Time-lapse imaging of PtK2 cells expressing Lypd6-GFP. Clusters are immobile during the imaging time (≈5 min).