Nano-imaging of membrane topography affects interpretations in cell biology

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Supplementary figures and text:

| Supplementary Figure 1 | Reconstructed axial pointspread function imaging yields profiles of the basal cell membrane with nanometer resolution. |
Reconstructed axial pointspread function imaging yields profiles of the basal cell membrane with nm resolution. (a) Elevation of a small subresolution membrane fold (< 700 nm, cannot be directly distinguished from a flat area by conventional light microscopy. A 40 nm-spaced Z-stack was acquired, and Regions of Interest were drawn within (blue) and just outside (remainder) the fluorescent enrichment. For both ROIs, the average intensity was plotted against the Z-position, thereby reconstructing the axial pointspread functions of the two regions. The offset between the ROIs, determined by fitting, shows wrinkle elevation with nm precision. (b) Applying this approach on a pixel-by-pixel basis directly visualizes the membrane topography, and illustrates that at fluorescence enrichments the membrane is highly wrinkled. Time lapse RAP imaging showed membrane wrinkles to be highly dynamic. (c) Confocal images were taken from a membrane labeled with a phosphatidylinositol-4,5-bisphosphate probe GFP-PH(PLCδ1) (GFP-tagged pleckstin homology domain of PLCδ1) and a homogenously distributed lipid marker DiI. Scale bar represents 1 μm.