

Supplementary Information

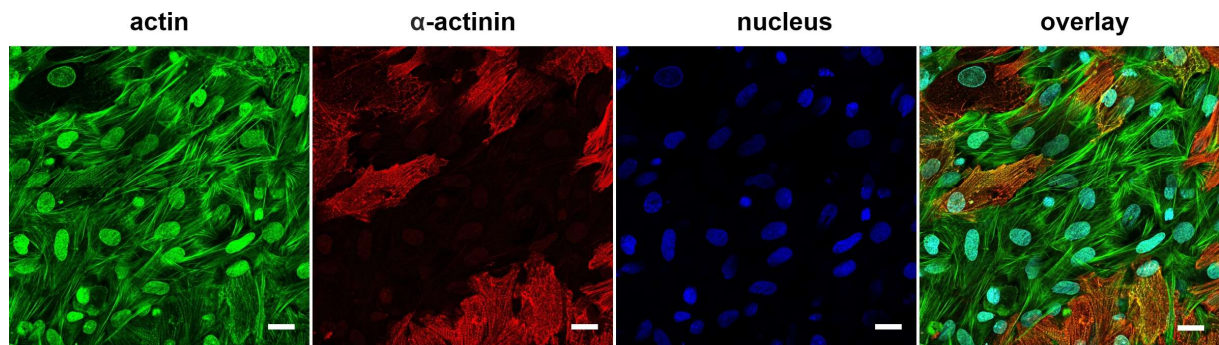
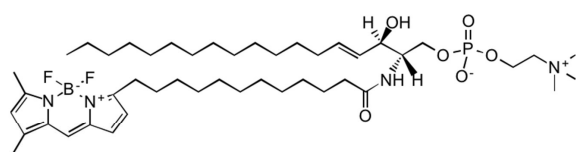
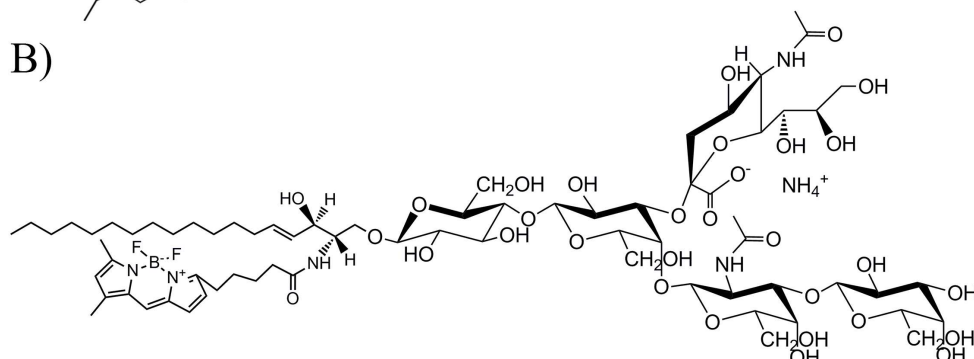


Figure S1 Identification of cardiac myocytes and myofibroblast in primary culture. The two cell types were clearly distinguished based on the immune staining of actin cytoskeleton with Alexa488-phalloidin (green), and the α -actinin staining with anti- α -actinin (red). Cell nuclei were visualized using NucBlue (blue). Myocytes were identified by a co-localized actin-cytoskeleton network and α -actinin (yellow). Scale bar, 20 μ m.

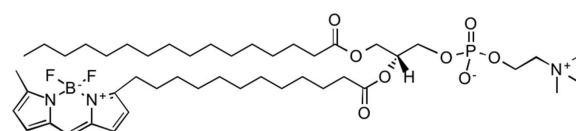
A)



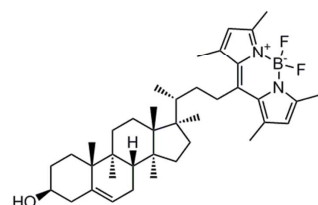
B)



C)



D)



E)

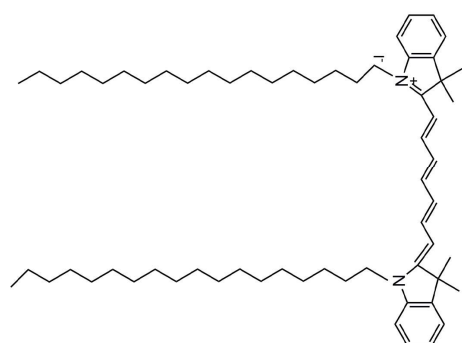


Figure S2 Fluorescent components incorporated into the PM of cardiac myofibroblasts.

A) *N*-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-dodecanoyl)sphingosyl phosphocholine (BFL-SM), B) *N*-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-pentanoyl)ganglioside (BFL-GM1), C) 2-(4,4-difluoro-5,7-dimethyl-4-bora-3a,4a-diaza-*s*-indacene-3-dodecanoyl)-1-hexadecanoyl-*sn*-glycero-3-phosphocholine (BFL-PC), D) 23-(dipyrrrometheneboron difluoride)-24-norcholesterol (TopChol), and E) 1,1'-di-octadecyl-3,3,3',3'-tetramethylindotricarbocyanine iodide (DiIC₁₈(7)).

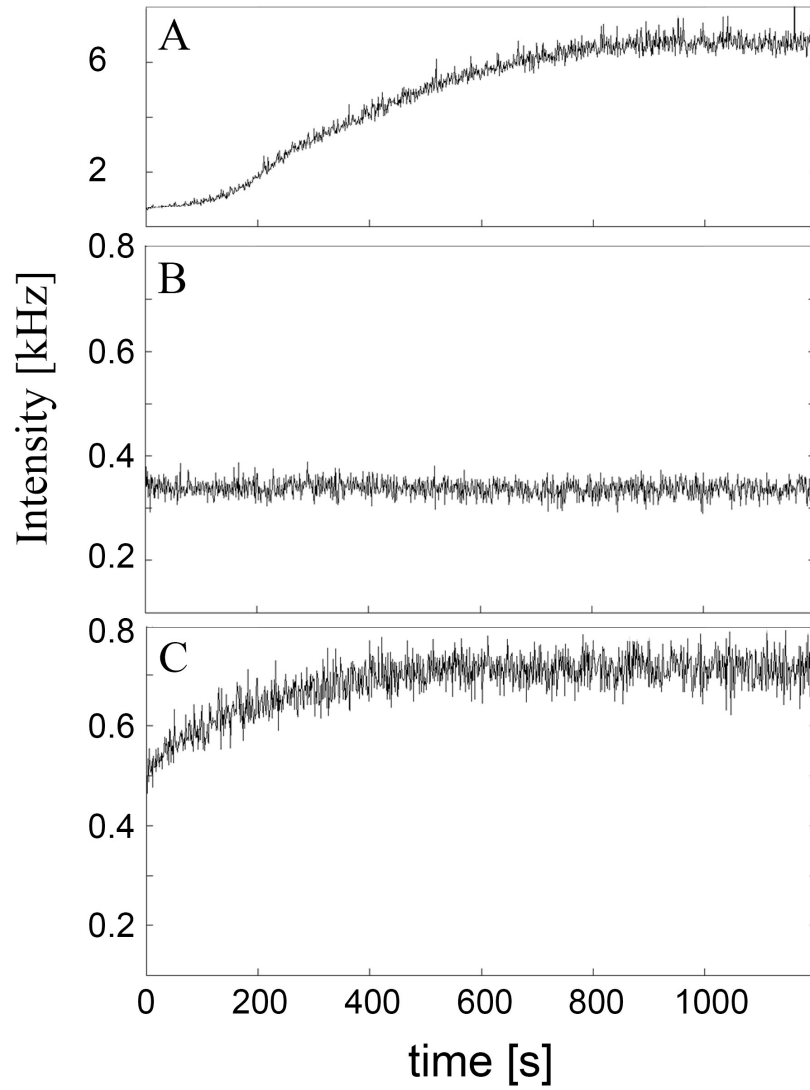


Figure S3 Increase of background signal when using BFGI-GM1 in cells and model systems. The fluorescence intensity was measured 50 μm above A) adherent cells treated with fusogenic vesicles, B) a supported lipid bilayer (DPPC/BFL-GM1 1/0.002 w/w) covered with PBS and C) a supported lipid bilayer (DPPC/BFL-GM1 1/0.002 w/w) covered with PBS + 10% fetal calf serum. Intensities were recorded for 20 min and averaged over 1 μs periods.

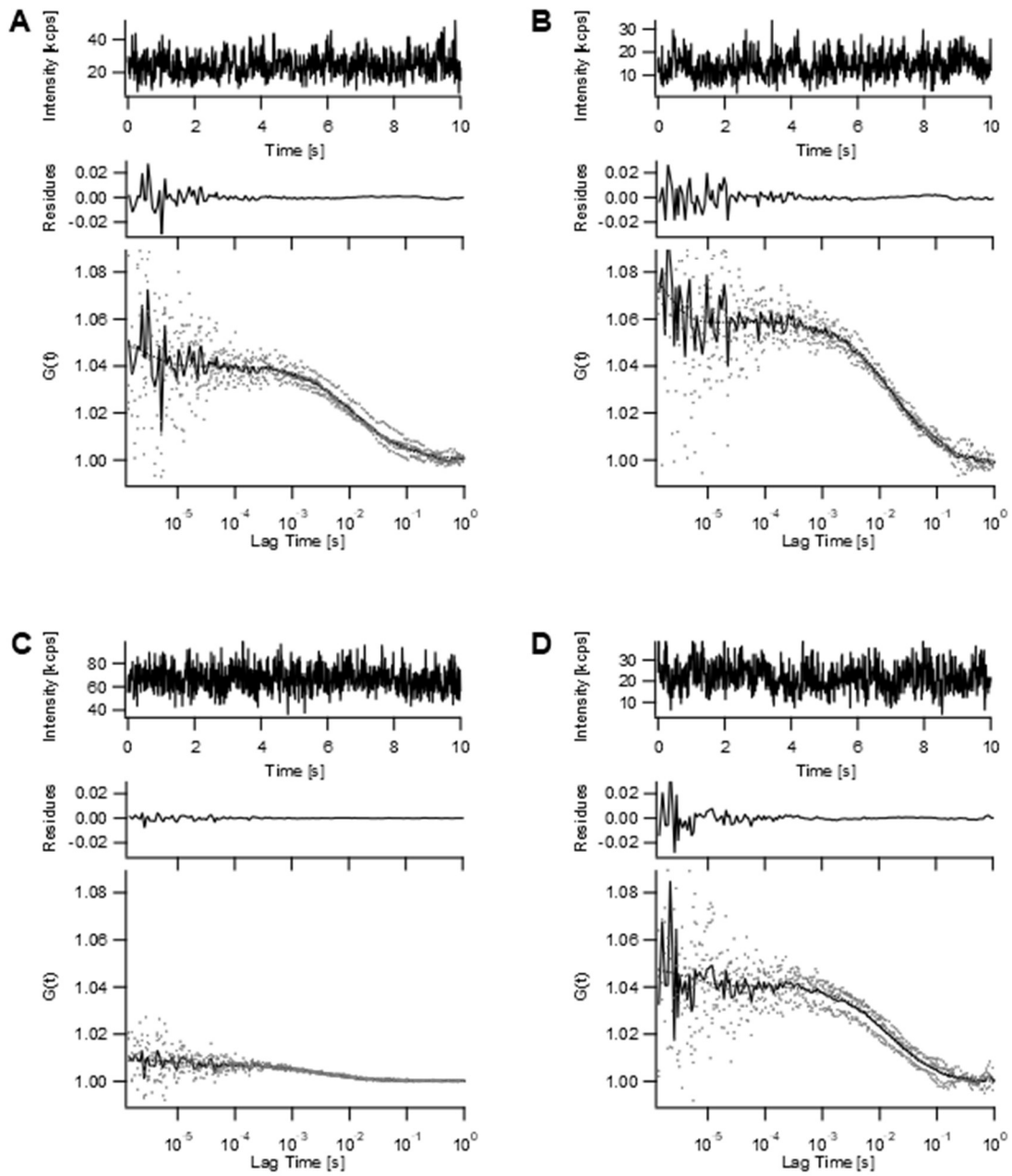


FIGURE S4 Diffusion analyses of sphingomyelin in different membrane systems. Each panel shows (from top to bottom) raw intensity readings in 1 ms intervals, differences between averaged correlogram and fit result, and the correlograms (grey dots: individual data, solid line: averages of repeated measurements, dotted line: result of fit with Eq. 1). A) Free area of the plasma membrane. Fit results in $D=1.3 \mu\text{m}^2/\text{s}$ and $N=25$. B) Focal adhesion. Fit results in $D=0.97 \mu\text{m}^2/\text{s}$ and $N=17$. C) Liquid disordered phase of a GUV. Fit results in $D=5.1 \mu\text{m}^2/\text{s}$ and $N=144$. D) Liquid ordered phase of a GUV. Fit results in $D=1.1 \mu\text{m}^2/\text{s}$ and $N=25$. Fits of the individual measurements showed that the noticeable splitting of individual measurements in A and D was due to widely varying contributions of triplet state decay at very short lag times while diffusion constants and particle numbers were consistent in all repeats.