Supplementary Information

Figure S1. Determination of the surface density of freely diffusing CD59-mGFP. Fluorescence images of a ROI in a representative patterned T24 CD59-mGFP cell before **(A)** and 5 minutes after **(B)** bleaching an area defined by a field stop (indicated by the yellow rectangle). The mean brightness of "ON" and "OFF" areas after photobleaching was 5625 ± 267 (s.d.) and 5575 ± 163 (s.d.), indicating that the surface density of freely diffusing CD59-mGFP recovering into the bleached area is essentially the same in "ON" and "OFF" areas. Scale bars are 3 μm.
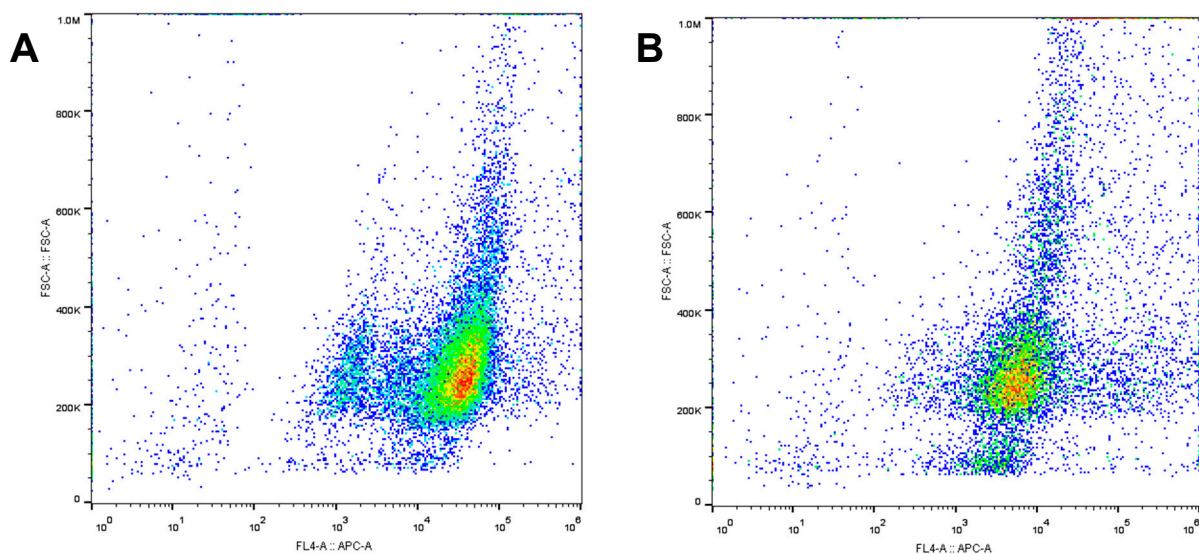

Figure S2. Estimation of the relative expression levels of endogenous CD59 in T24 CD59-mGFP cells. **(A)** Flow cytometry data of T24 CD59-mGFP and **(B)** T24 wildtype cells labeled with anti-CD59-Alexa Fluor™647. The fluorescence intensity in the red color channel is plotted vs. the forward scattering. The mean fluorescence intensities per cell were 3.7×10^4 for the T24 CD59-mGFP cells and 6.6×10^3 for T24 wildtype cells. Based on these values we estimate the fraction of "dark" endogenous CD59 in CD59-mGFP-positive cells to be lower than 1/5 of total CD59.
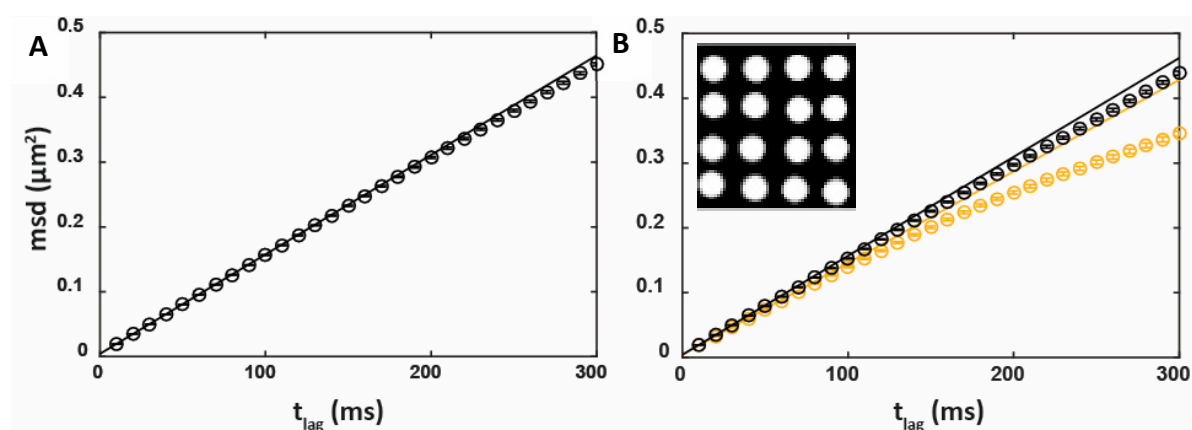
Figure S3. Effect of application of a selection mask on msd vs. t_{lag} plots. Msd vs. t_{lag} plots of Chol-PEG-KK114 diffusion recorded in a non-patterned cell without **(A)** and with **(B)** application of a typical selection mask generated from CD59-mGFP patterns to separate "ON" (yellow) and "OFF" (black) trajectories. The selection mask is shown as an insert in **(B)**; white areas in the mask correspond to "ON" areas, black to "OFF" areas. The diffusion coefficients determined from the first two data points without selection mask, in "ON" and in "OFF" areas were 0.38 ± 0.01 $\mu m^2/s$, 0.37 ± 0.01 $\mu m^2/s$ and 0.38 ± 0.01 $\mu m^2/s$, respectively.

Table S1. Diffusion of lipids in patterned and non-patterned cells. Diffusion of lipids was recorded and determined in "OFF" areas in patterned cells and unpatterned cells. Standard errors of the mean are indicated.

	Chol–PEG-KK114	DOPE–PEG-KK114
Unpatterned cells	0.343 ± 0.036[1]	0.405 ± 0.036
"OFF" areas	0.379 ± 0.028	0.411 ± 0.032

[1] Diffusion constant in $\mu m^2/s$.

Table S2. Apparent lipid/CD59-mGFP sizes d_{app}. Apparent sizes of tracer/obstacle pairs were determined by fitting data from Figures 2B and 3B-D with Eq.2. Standard errors (SE) are indicated; the 95% confidence interval is given by $d_{app} \pm (1.96 \times SE)$.

	untreated cells	chol depleted	untreated cells soft disc model	incomplete chromophore maturation
Chol-PEG-KK114	4.9 ± 0.4[1]	5.5 ± 0.6	5.2 ± 0.4	4.4 ± 0.4
DOPE-PEG-KK114	5.0 ± 0.5	5.0 ± 0.9	5.3 ± 0.5	4.5 ± 0.4

[1] d_{app} is given in nm.