SUPPLEMENTAL INFORMATION

Related to figure S2

To validate our FRAP settings on polarized MDCK cells grown upside-down on filters, we first defined the behaviour of GFP-FR (raft-associated) and P75-GFP (non raft-associated) at room temperature and at 37°C. We found that at room temperature, the mobile fraction of the raft-associated protein (GFP-FR) is 100% while the mobile fraction for non raft-associated protein (P75-GFP) is only partial (74%) (with a statistical significance of $p<0.003$) (figure S2A). Furthermore, the apparent diffusion coefficient (D) of P75-GFP is significantly lower compared to GFP-FR ($p<0.0001$) (figure S2B). When we performed the same analysis at 37°C, the mobile fraction became 100% also for the non raft-associated protein, however the difference in the apparent diffusion coefficient between these proteins remained significant (D Raft> D Non Raft, $p<0.0001$). These data are in agreement with the results of Meder and collaborators [30], therefore validating our FRAP settings. In addition, we observed the same range of differences between the apparent diffusion coefficient of raft- and non raft-associated protein at both temperatures and also reported that the increase of the apparent diffusion coefficient, at 37°C, is more important for raft-associated protein than for non raft-associated protein as previously published [30]. These results are indicative of differences in the environment surrounding raft- and non raft-associated protein at the level of the apical membrane of polarized MDCK cells and confirm that FRAP analysis is a powerful tool to investigate the close proteins environment in membranes of living cells.

FIGURES LEGENDS

Fig. S1 : Polarized MDCK cells stably expressing the apical raft-associated protein GFP-FR at the level of the plasma membrane (A) or in the Golgi (B) before (a, c) and after the photobleaching (b, d). The red circle is the region of interest (ROI) photobleached.

Fig. S2 : FRAP analysis at the apical pole of MDCK cells at 25°C and at 37°C.
Analysis by FRAP of the mobile fraction (A) and of the apparent diffusion coefficients (B) of two apical proteins, a raft-associated protein (GFP-FR) and a non-raft-associated protein (P75-GFP) at room temperature (filled boxes) or at 37°C (dashed boxes). These experiments have been performed at least three independent times, $n>15$. Error bars are the mean +/- SDs, $**p<0.003$, $*p<0.0005$.

Fig. S3 : Methyl β cyclodextrin affects the apparent diffusion coefficient of both raft- and not raft-associated proteins at plasma membrane.

Apparent diffusion coefficients (D) of our studied proteins at steady state (filled boxes) or upon treatment with methyl β cyclodextrin (dashed boxes). Experiments were performed at least three independent times, n>15.

Fig. S4: Addition of cholesterol does not affect the DRM-association of P75-GFP in MDCK cells.

Analysis by sucrose gradient of DRM-association of P75-GFP protein at steady state and upon loading of cholesterol. Flotilin-1 was used as control for DRM-associated protein.

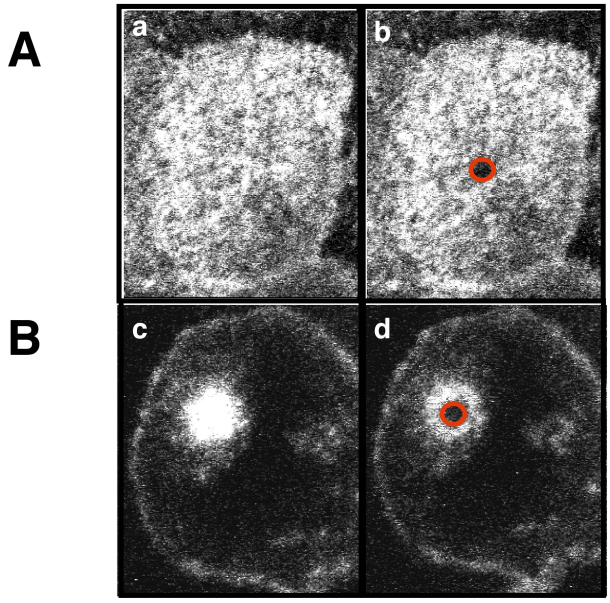
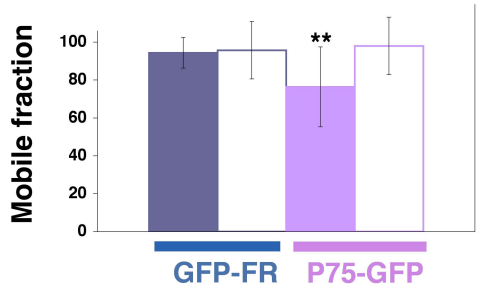


Figure S1

A


Mobile fraction
100
80
60
40
20
0
**
GFP-FR
P75-GFP

B

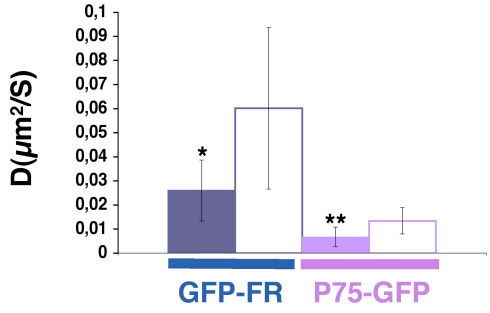

D(μm²/S)
0,1
0,09
0,08
0,07
0,06
0,05
0,04
0,03
0,02
0,01
0
*
**
GFP-FR
P75-GFP

Figure S2

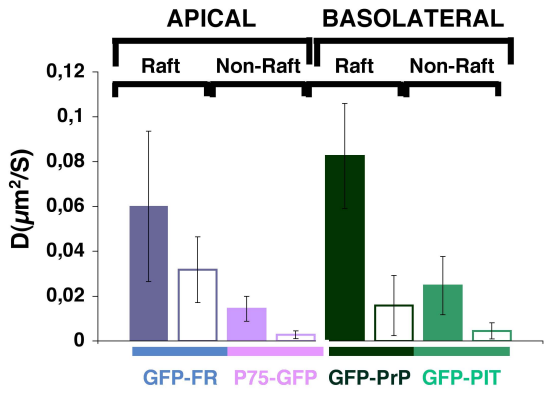

APICAL
BASOLATERAL
Raft
Non-Raft
Raft
Non-Raft
0,12
0,1
0,08
0,06
0,04
0,02
0
D(μm²/S)
GFP-FR
P75-GFP
GFP-PrP
GFP-PIT

Figure S3

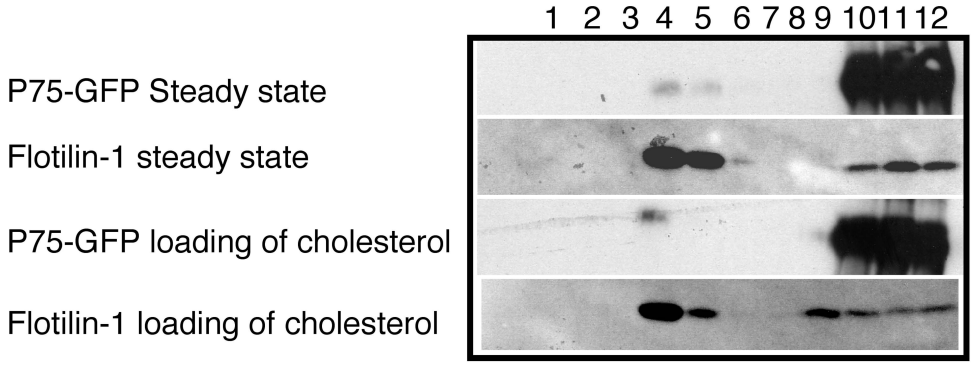

1 2 3 4 5 6 7 8 9 10 11 12
P75-GFP Steady state
Flotilin-1 steady state
P75-GFP loading of cholesterol
Flotilin-1 loading of cholesterol

Figure S4