Supplementary Information to:

A FLUORESCENT MEMBRANE TENSION PROBE

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SUPPLEMENTARY METHODS

Cells culture.

For microscopy, MDCK and Hela cells were spread on 35 mm glass bottom dishes (Mattek Corporation, P35G-0.170 14-C) and grew in DMEM medium (Gibco, 10013CM) and MEM medium (Gibco, 11090099) respectively, enriched with 10%FBS, 1%PS and NEEA, for 72 h until they reached about 70% confluence. For imaging, the medium was replaced with the same medium containing 3 µM of **FliptR** probe and kept for the following days.

Osmotic shock measurements were carried out using a sterilized coverslip (24x60mm) assembled with a flow chamber (Sticky-Slide VI, 0.4, Ibidi, USA 80608). MDCK and Hela cells were then injected into the chamber and grown until 70% confluency, as described above. Before imaging, the medium was replaced by medium without phenol red (Leibovitz's Medium, life technology 21083027) and containing 3 µM of **FliptR**. One entry of the flow chamber was connected to a syringe pump (Aladdin, World Precision Instruments, Sarasota, FL, USA), and the other left open for sequential introduction of osmotic shock solutions.

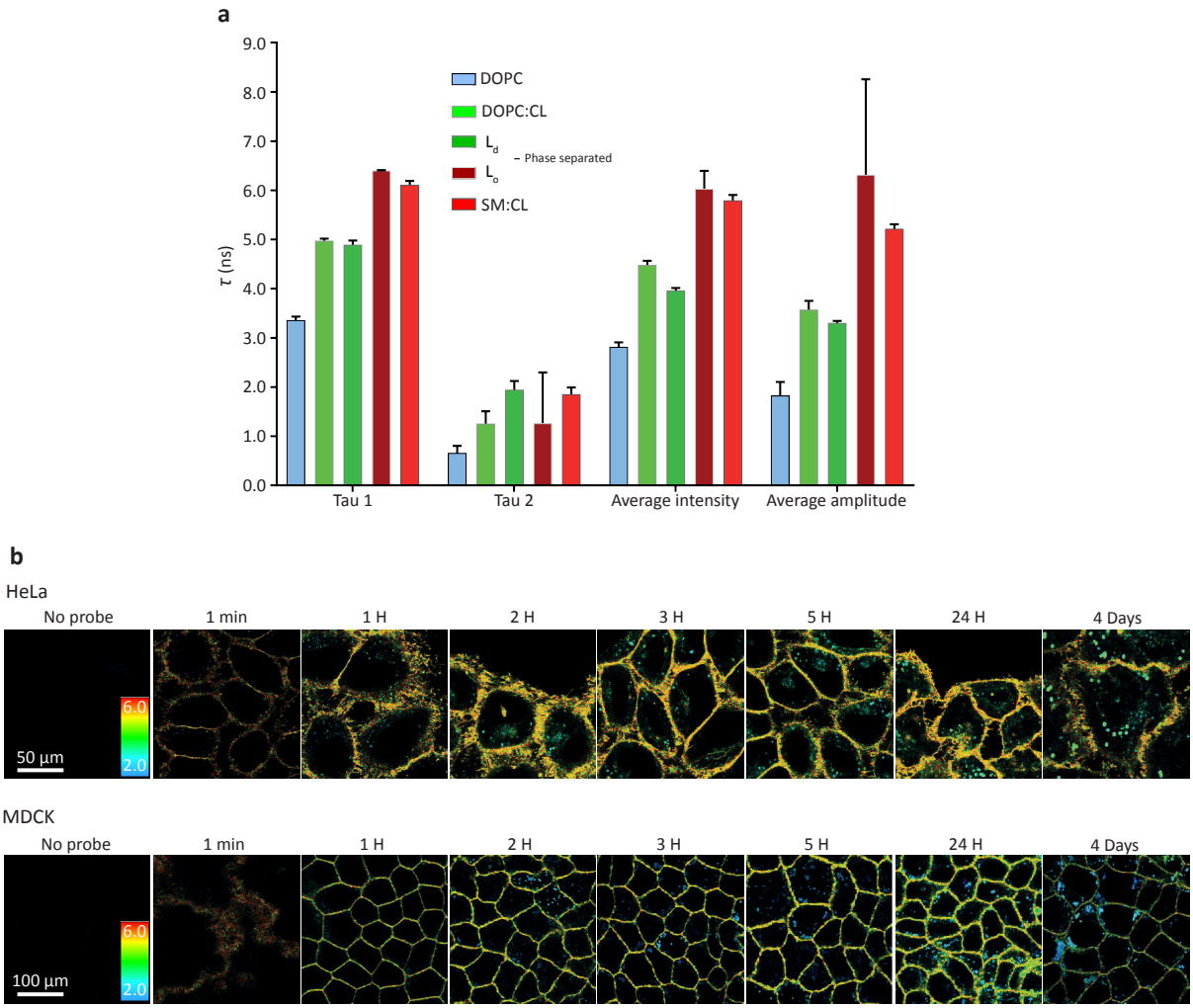
Primary cultures of cortical neurons were prepared as follow: Cortices were dissected from E18 mouse embryos, treated with trypsin-EDTA 0.25% supplemented with 15mM glucose, and disrupted by ten to fifteen cycles of aspiration and ejection through a micropipette tip. Dissociated cortical cells were seeded at the density of 100 000 cell/cm^2 on P35 glass bottom culture dishes (MatTek corporation), pre-coated overnight with 50ug/ml poly-d-lysine (Sigma), in filled with seeding medium (DMEM supplemented with 10% heat-inactivated horse serum and 0.5mM glutamine). Neurons were maintained in water-saturated 95% air/5% CO_2 at 37°C. The seeding medium was replaced after 20 h with serum free neuronal culture medium (Neurobasal medium containing D27 supplement, penicillin/streptomycin, and 0.5mM glutamine). Neurons were culture for 15 days in vitro prior to imaging.

Cell culture for side view

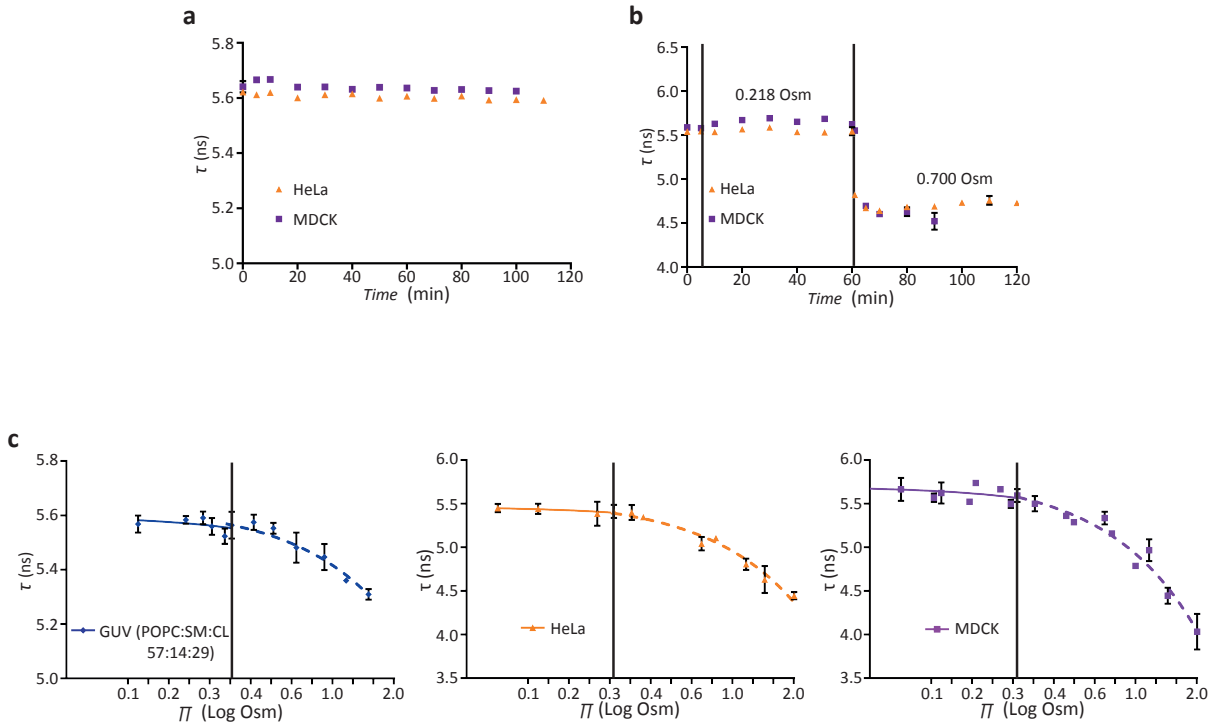
PDMS (Polydimethylsiloxane) structures were introduced on cell growth substrates in order to easily access to the side view of MDCK polarized monolayers. Sylgard 184 silicone elastomer (Dow Corning, 10 wt% of curing agent) was poured in a homogeneous master, degassed to remove any bubbles, and cured 15 min at 100 °C in an oven. PDMS was then cut

in approximately 1 cm long right prisms to get out-of-plane surfaces as support to grow cells: 2 of these PDMS structures were bonded on a 35 glass bottom dish (Mattek Corporation, P35G-0.15-20-C) by previous surface activation (O_2 plasma for 45 s). Right after, this final structured substrate was functionalized for 45 min at 37 °C in a CO_2 incubator with Fibronectin (Life Technologies, #33016015) coating (2 µg/cm^2). Finally, MDCK cells were seeded on the substrate with a concentration of 10^5 cells/cm^2. After about 4 h, samples were washed with fresh DMEM medium (Dulbecco's Modified Eagle's medium – Life Technologies, 61965-026) supplemented with 10% v/v FBS (Fetal Serum Bovine, Life Technologies, 10270-106), 1% v/v NEAA (MEM Non-Essential Amino Acids, Life Technologies, #11140-035) and 1% v/v antibiotics (penicillin-streptomycin 10000 U·mL^{-1}, Life Technologies, #15140-122) in order to remove non-adhesive cells. Cells were then maintained in humidified atmosphere containing 5% CO_2 at 37 °C for 3 days to allow cell monolayer formation and cell polarization. 1h before imaging, the medium was replaced by medium containing 3 µM of FliptR and then by medium without phenol red (Leibovitz's medium, Life Technologies, #2108302 supplemented with 10% v/v FBS) for live imaging observations. Cell monolayer images were acquired along the vertical PDMS surface, were the cell apical-basal section results parallel to the optical focal plane.

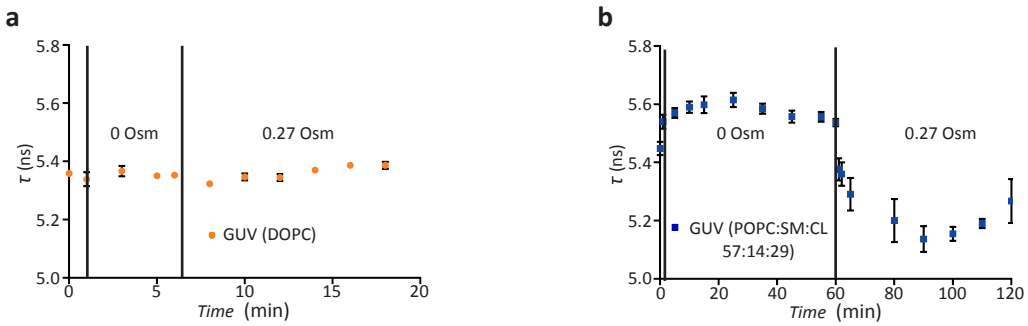
SUPPLEMENTARY FIGURES



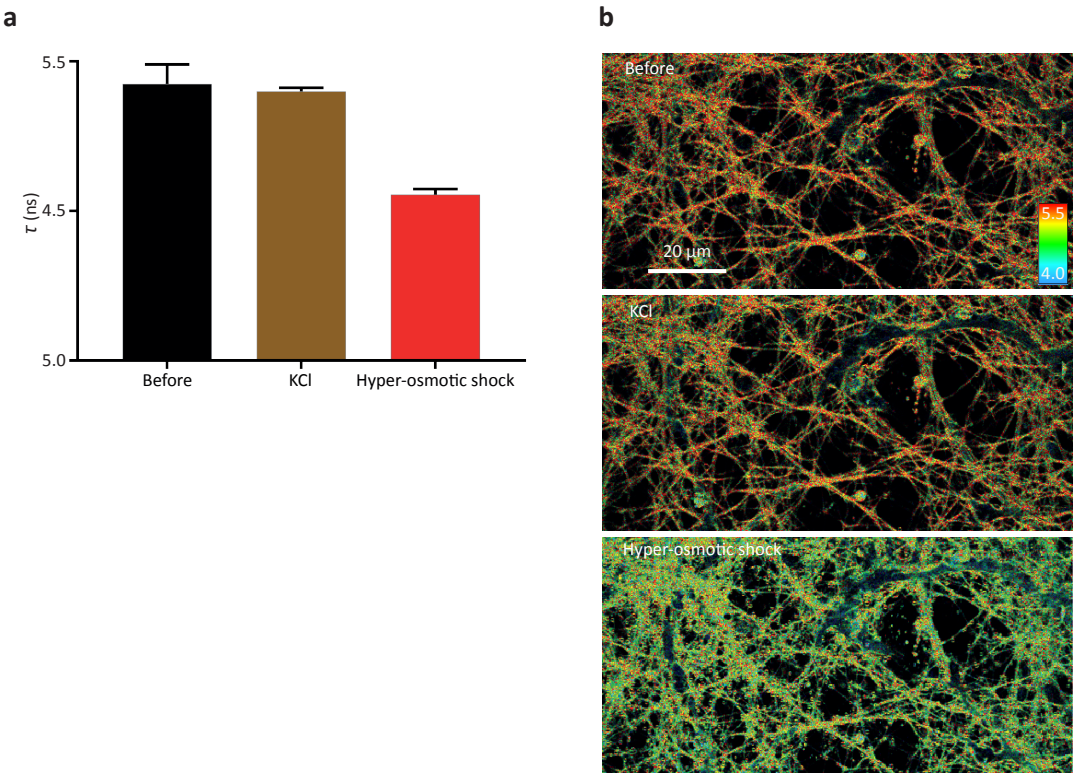
Supplementary Figure 1| The **FliptR** probe FLIM analysis and behavior on cells. (**a**) An example of the four components extracted from double fitted photon lifetime histograms**:** τ_1, τ_2, average intensity and corresponding average amplitudes of **FliptR** as a function of lipid composition in GUVs, from liquid-disordered membrane (L_d) to increasingly liquid-ordered membranes (L_o) (data are Mean ± SD). Compositions are: DOPC, DOPC:CL 60:40, phase-separated DOPC:SM:CL 25:58:17 and SM:CL 70:30. **(b)** Evolution over time of **FliptR** labelling on MDCK and HeLa cells. **FliptR** mostly stays in the plasma membrane. However, after 2h of incubation, bright spots with low lifetime values (blue colors) are seen, probably corresponding to endosomes.



Supplementary Figure 2 | FliptR stability and reaction. **(a)** Constant **FliptR** fluorescence lifetime τ_1 on isoosmotic (▲) Hela and (■) MDCK cells conditions (N=2 and R=21, N=2 and R=19). **(b)** Hela and MDCK cells labelled with **FliptR** changed and stayed stable after hypoosmotic and hyperosmotic shocks along the time (N=2 and R=14, N=2 and R=49). **(c)** Detail of τ_1 versus osmotic pressure Π on (◆) GUV (POPC:SM:CL 57:14:29) (▲) Hela and (■) MDCK cells. Plain lines and dash lines are linear fits to the data (be aware of the log scale) for hypo and hyper osmotic conditions respectively. The slopes for Hela (▲) are hypo= -0.27 ± 0.13 ns·Osm^{-1}, slope hyper: -0.59 ± 0.04 ns·Osm^{-1} (N=22), MDCK cells (■), slope hypo= -0.50 ± 0.33 ns·Osm^{-1}, slope hyper: -0.88 ± 0.07 ns·Osm^{-1} (N=20) and GUVs (◆) slope hypo= -0.19 ± 0.18 ns·Osm^{-1}, slope hyper: -0.22 ± 0.03 ns·Osm^{-1}). All data are represented as mean ± SD.



Supplementary Figure 3 | FliptR lifetime response in GUVs overtime. **(a) Lifetime** τ_1 on L_d GUVs (DOPC only) didn't change upon various changes of osmotic pressure Π (N=2 and R=14). **(b)** τ_1 of FliptR in GUVs made of POPC:SM:CL 57:14:29 changes upon osmotic pressure changes (N=1 and R=6). All data are represented as mean ± SD.



Supplementary Figure 4 | FliptR lifetime is independent of membrane depolarization. **(a) Lifetime** τ_1 of **FliptR** on neurons does not change after the addition of KCL 47mM, which depolarizes neuronal membranes. Furthermore, τ_1 decreased (red) upon hyperosmotic shock (700mOsm sucrose solution), (N=3). **(b)** Lifetime images of the three experimental cases shown in a). All data are represented as mean ± SD.

Video captions

Supplementary Video 1| Staining of cells with **FliptR.** Timelabs of HeLa cells along the injection of FliptR probe. In the second 14.08s FliptR is injected and in less than 1 minute the main part of the plasmatic membrane is stained. Scale bar: 25 µm.

Supplementary Video 2| Fast-FLIM time-lapse of HELA cells under hyperosmotic shock**.** 500ul of 0.5M of sucrose were added on the cells at time 2s. Images shown in Fig 5d are initial and final time of this video.