Supporting Online Material

Additional details on the photoactivation process

The effect of photoactivation on 1:1:1 lipid mixture was tested using TLC. The BODIPY<sub>FL</sub>-C<sub>5</sub>-HPC probe was previously (J Solon and P Bassereau, in preparation) shown to generate superoxide ions during photoactivation that could oxidize surrounding molecules. TLC analysis showed that a small amount of cholesterol was oxidized after strong photoactivation of 1:1:1 Small Unilamellar vesicles (SUVs) (Fig. S2A). The amount of oxidized cholesterol decreased with light intensity, and was estimated to be more than 10 times less for a 400-fold decrease in light intensity (Fig. S2C). GUVs made of this strongly photoactivated mixture were segregated and no further photoactivation was possible (Fig. S2B). As expected, lipids extracted from non-photoactivated SUVs produced homogeneous and photoactivable GUVs (Fig. S2B). Oxidized forms of cholesterol probably interact with sphingolipids in a different way than cholesterol itself. The induction of phase separation by photoactivation was not observable with NBD-C<sub>5</sub>-HPC, a fluorescent probe that does not generate superoxide ions (data not shown). We further tested if phase separation was due to cholesterol depletion, or to the apparition of new species (i.e. oxidized cholesterol). To discriminate between the two hypotheses, cholesterol was added to the photoactivated lipid mixture. The addition of 2% cholesterol to the photoactivated lipid mixture did not restore homogeneity, whereas 6% cholesterol did. Photoactivation of these GUVs induced phase separation with a small delay compared to non-photoactivated 1:1:1 mixture, probably due to a slightly higher concentration of cholesterol (Fig. S2B). We concluded
that photoactivation has the same effect than a depletion of cholesterol. Adding cholesterol could compensate for this depletion and thus, we can estimate to a few percent the amount of cholesterol oxidized by strong photoactivation.