Supplementary Information

Lipid Phase Separation and Protein-Ganglioside Clustering in Supported Bilayers Are Induced by Photorelease of Ceramide[‡]

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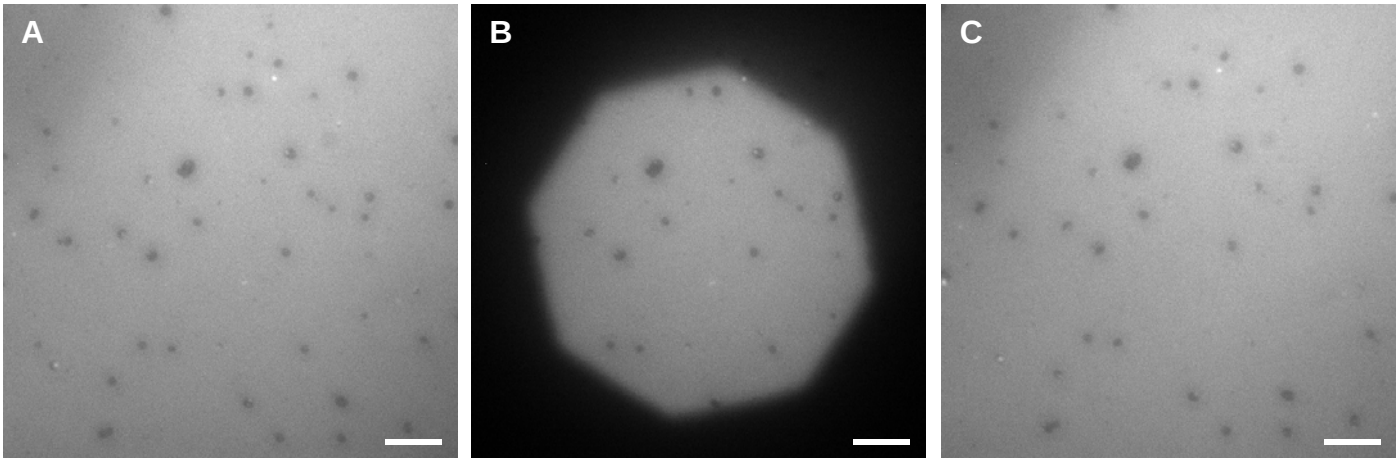
Electronic Supplementary Material (ESI) for Soft Matter

Fig. S1. Supported bilayers prepared from POPC/C16-Cer (9:1 molar ratio, labeled with 0.5 mol % DiI-C_{20}) are stable to UV irradiation. Fluorescence images were recorded: (A) before UV irradiation; (B) aperture adjusted to irradiate a localized area of the bilayer; (C) after 10 min UV irradiation. Scale bar = 5 μm.



B

Fig. S2. Photolysis of **1** in POPC SUVs was conducted with 350 nm irradiation. Consumption of **1** was monitored by HPLC analysis of the caged coumarin's fluorescence. (A) POPC/**1** in 9:1 a molar ratio; (B) POPC/**1** in a 98:2 molar ratio.

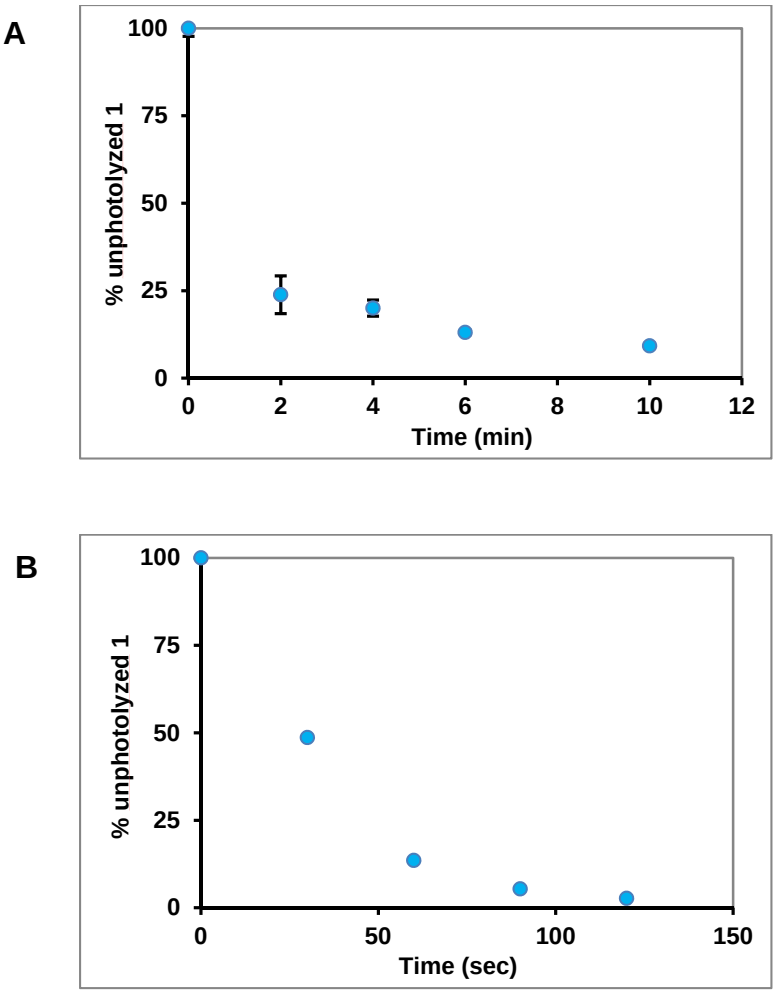


Fig. S3. A POPC/**1** supported bilayer labeled with 0.5 mol % DiI-C_{20} shows no phase separation before photolysis. (A) $T_{0\ min}$ fluorescence image and (B) AFM scan. (C) $T_{20\ min}$ post-AFM image. The bright features due to adsorbed vesicles show a different pattern after the AFM scan. Scale bar = 5 μm.

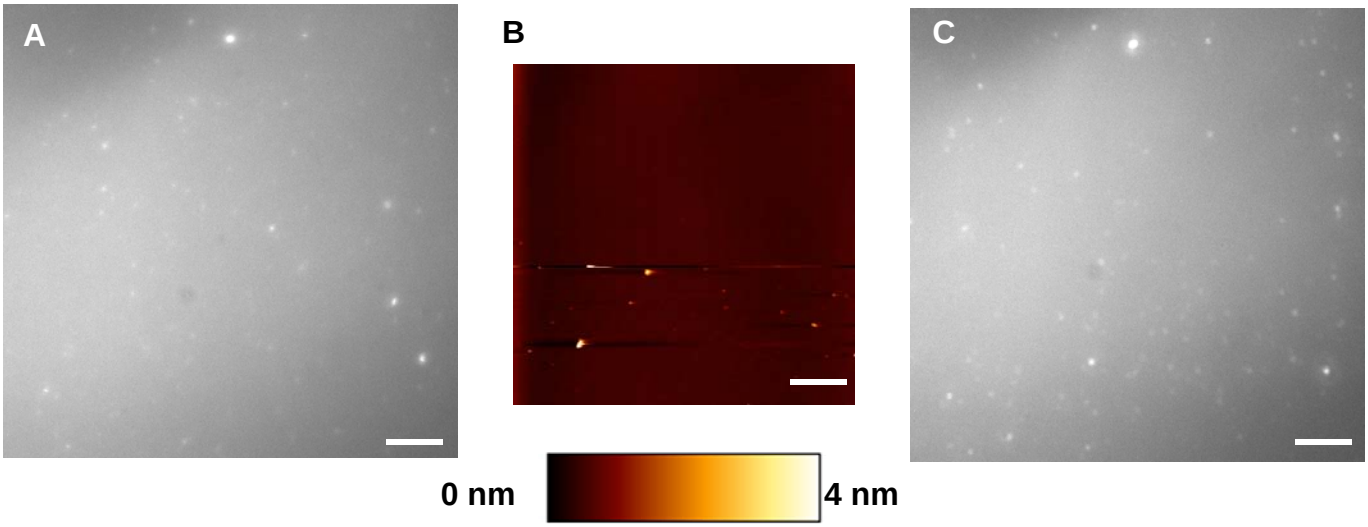


Fig. S4. Domain disappearance as a function of pattern size. Each region of the irradiated bilayer shown in Figure 4 was re-imaged 5 min after the end of the photolysis. The images shown below are the post-UV images for the following irradiation times: (A) 2.5 s; (B) 5 s; (C) 15 s; (D) 30 s; (E) 1 min. The rate of domain disappearance increases with increasing irradiation time. Scale bar = 10 μm.

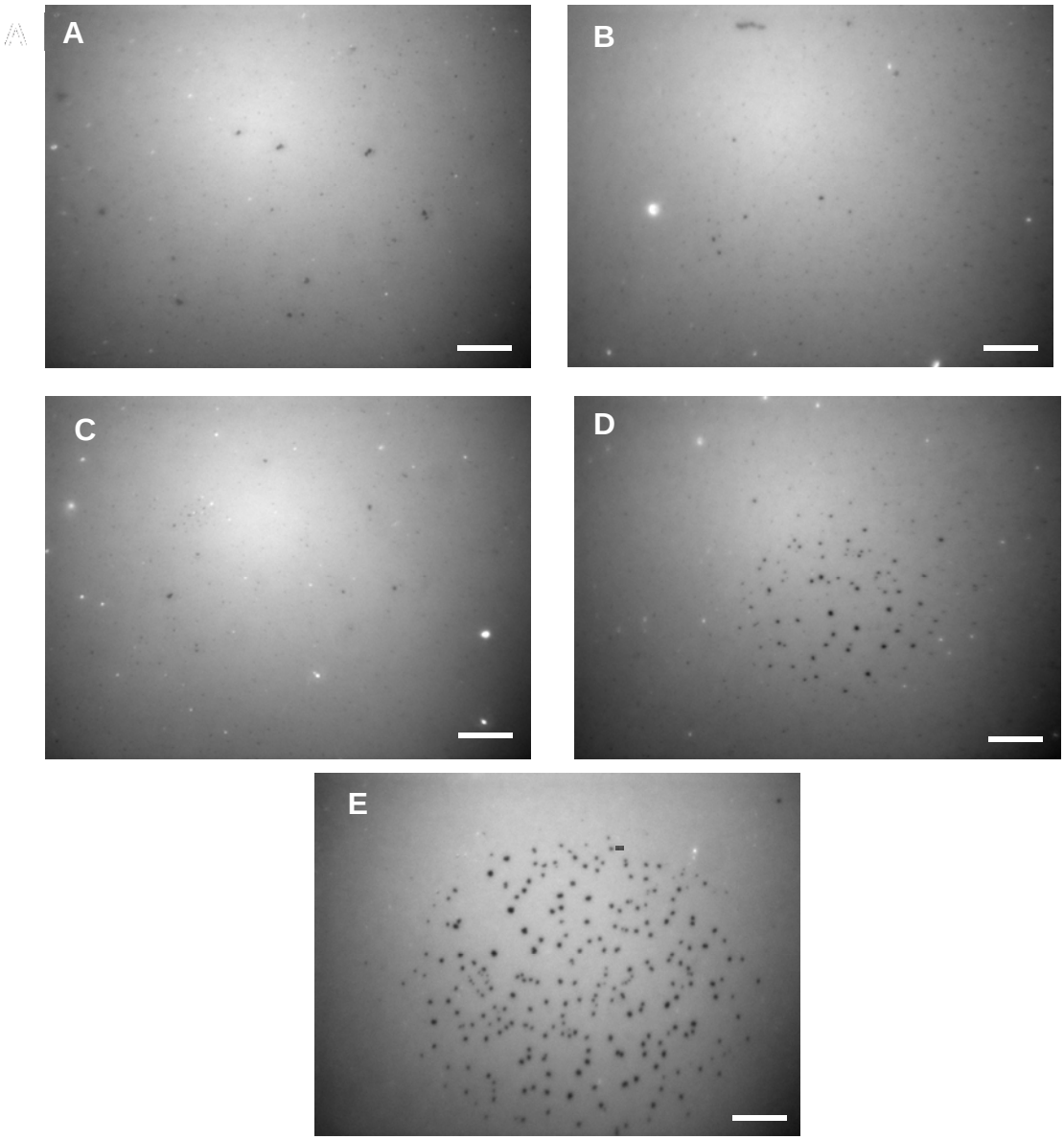


Fig. S5. Photorelease of C16-Cer in a POPC/**1** supported bilayer (95/5 molar ratio + 0.5 mol% DiI-C_{20}) also results in the formation of ceramide-rich domains. (A) $T_{0\ min}$ before UV; (B) closed iris showing the area to be irradiated; (C) following 5 s UV exposure; (D) $T_{5\ min}$ post-UV. Scale bar = 5 μm.

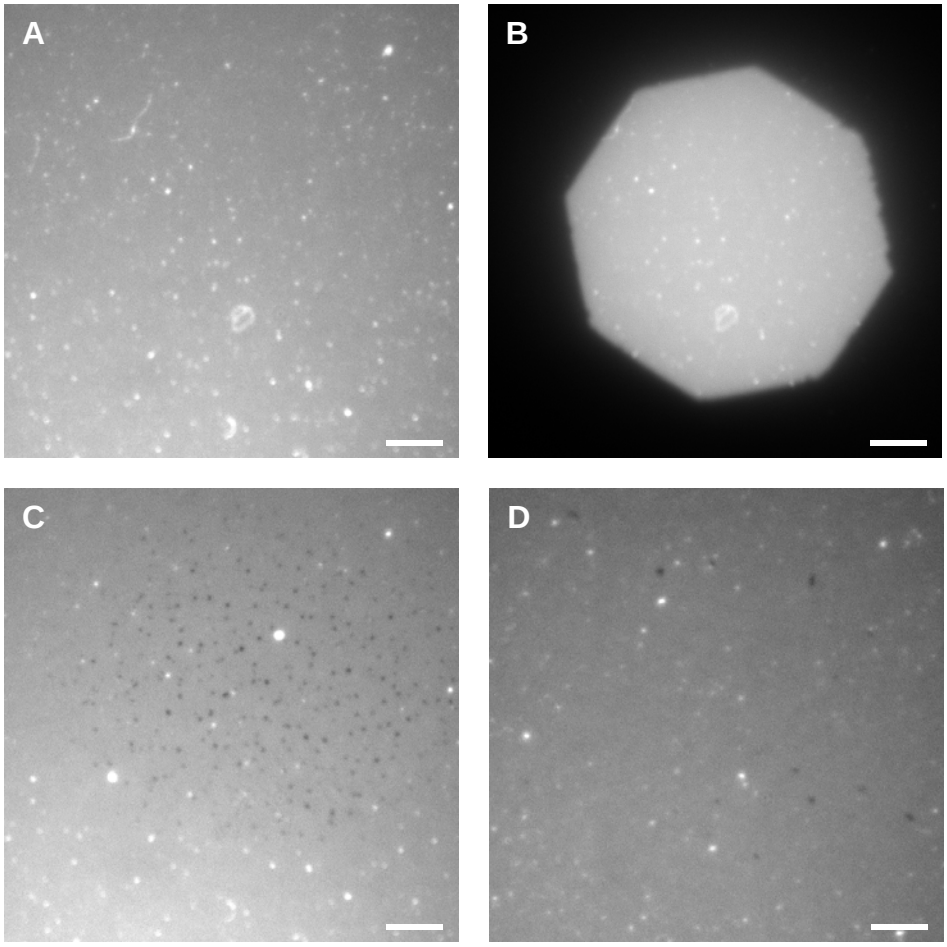


Fig. S6. Photorelease of C16-Cer in a POPC/**1** supported bilayer (98/2 molar ratio + 0.5 mol% DiI-C_{20}) does not afford ceramide-rich domains. (A) $T_{0\ min}$ before UV; (B) closed iris showing the area to be irradiated; (C) following 5 s UV exposure; (D) 3 min post-UV. Scale bar = 5 μm.

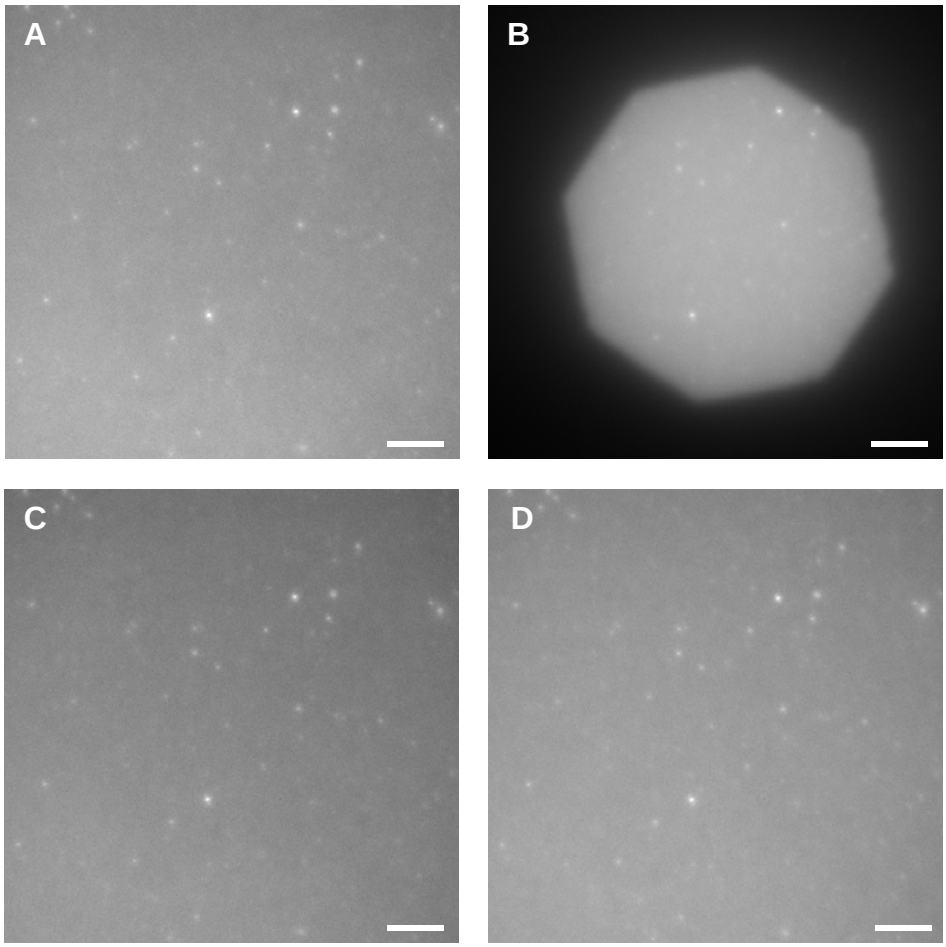


Fig. S7. Correlated fluorescence and AFM imaging of a POPC/**1** supported bilayer labeled with 0.5 mol % DiI-C_{20} after photolysis of the entire membrane surface confirms that the dye-excluded domains are stable. (A) $T_{0\ min}$ before photolysis; (B) $T_{52\ min}$ a second area of the bilayer following 30 min UV exposure of the entire sample; (C) $T_{62\ min}$ AFM confirms the presence of gel-phase domains; (D) $T_{75\ min}$ a fluorescence image taken following two consecutive AFM scans indicates a stable domain pattern. Scale bar = 5 μm.

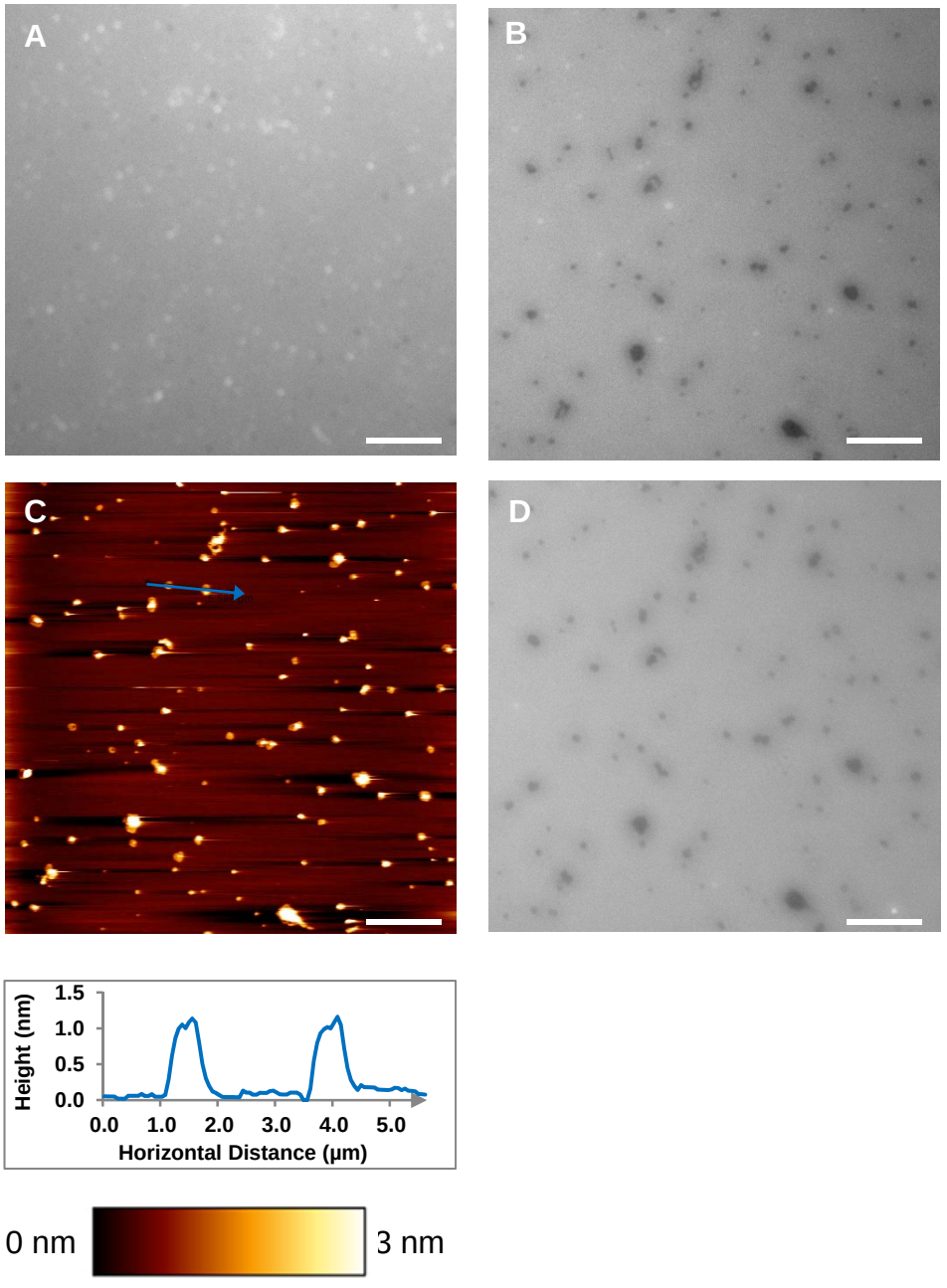


Fig. S8. Photolysis of a POPC/**1**/GM1 bilayer (90/10/1 molar ratio labeled with 0.5 mol % DiI-C_{20}) induces comparable morphological changes to those observed in the absence of GM1. (A) $T_{0\ min}$ before UV; (B) closed aperture identifying the area to be irradiated; (C) following 2.5 s of UV exposure small, dye-excluded Cer-rich domains are observed in the area exposed to UV light; (D) $T_{5\ min}$ the dark domains have completely disappeared; (E) a second 2.5 s photolysis in the same area recreates dark domains, most of which correspond in size and position to those in C. As before the domains disappear within minutes (not shown); (F) $T_{20\ min}$ a similar distribution of domains is reproduced after a third 2.5 s photolysis. Scale bar = 5 μm.