Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

Multiplexed patterning of hybrid lipid membrane and protein arrays for cell signaling study

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Fig. S1 – Fig. S8

Captions for supporting movies

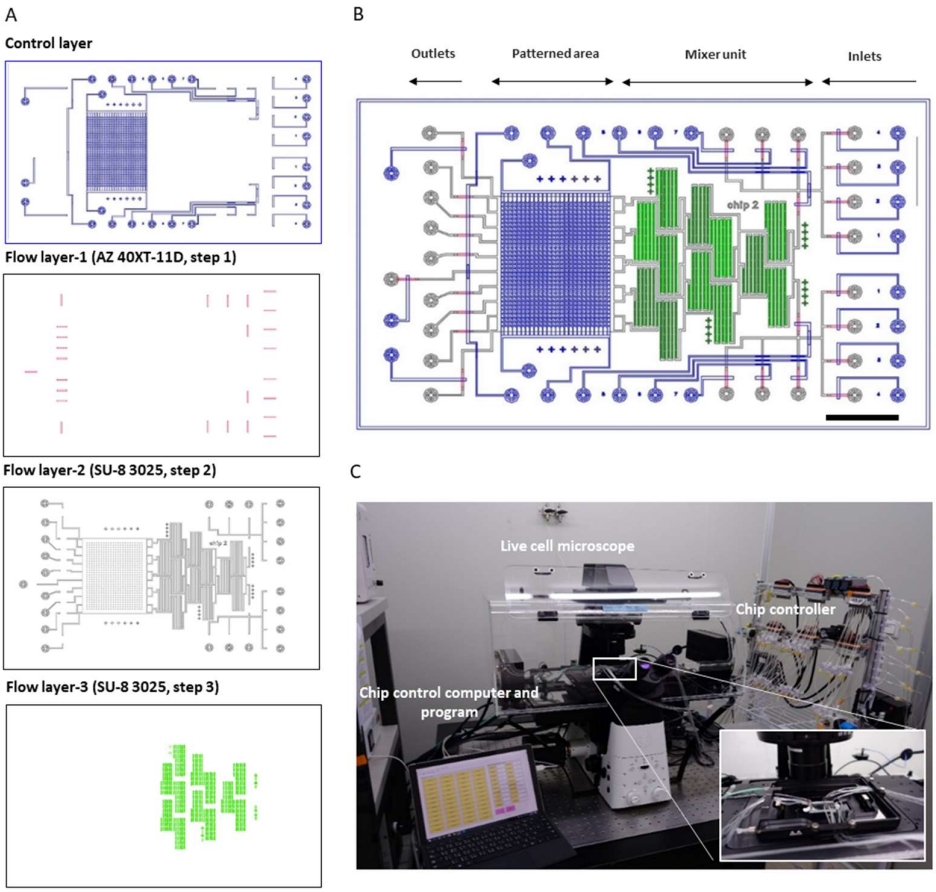

Fig. S1 The design layout of the SLB patterning chip. (A) The control layer used 1 photomask while the flow layer used 3 photomasks for the mold fabrication. (B) A combined view of the patterning chip. Scale bar is 5 mm in B. (C) The chip control system on the fluorescence microscopy equipped with a live cell incubator system. The inlet shows a zoom-in image of a mounted chip.

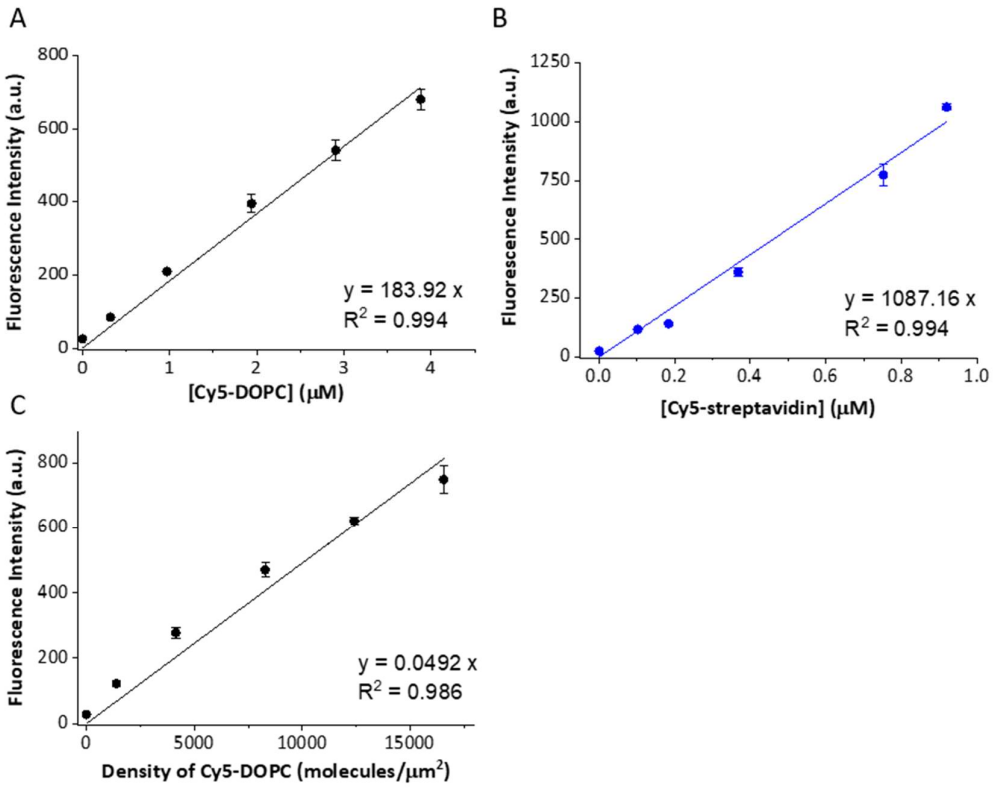

Fig. S2 The density of Cy5-streptavidin on the SLB was measured using the quantitative fluorescence microscopy. (A) Cy5-doped vesicles were used as a bulk calibration standard. (B) A calibration plot showing fluorescence intensities of solutions containing labeled Cy5-streptavidin. (C) A series of Cy5-DOPC doped SLB were used as a surface density calibration standard.

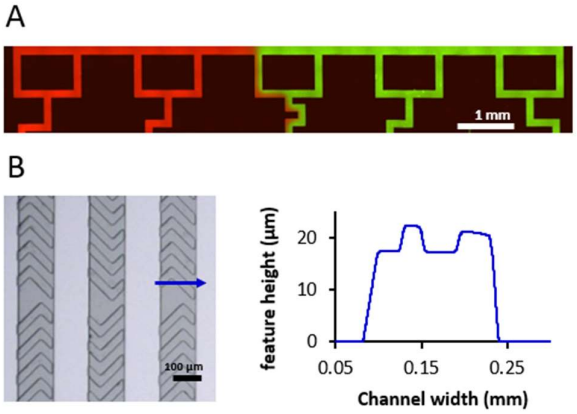

Fig. S3 Optimization of the SUVs mixing by adding the staggered herringbone mixers (SHMs). (A) A fluorescence image of incompletely SUVs mixing in the absence of SHMs. (B) A bright-field image (left) and the characterized height of SHMs at ~4-5 μm measured by a stylus profilometry.

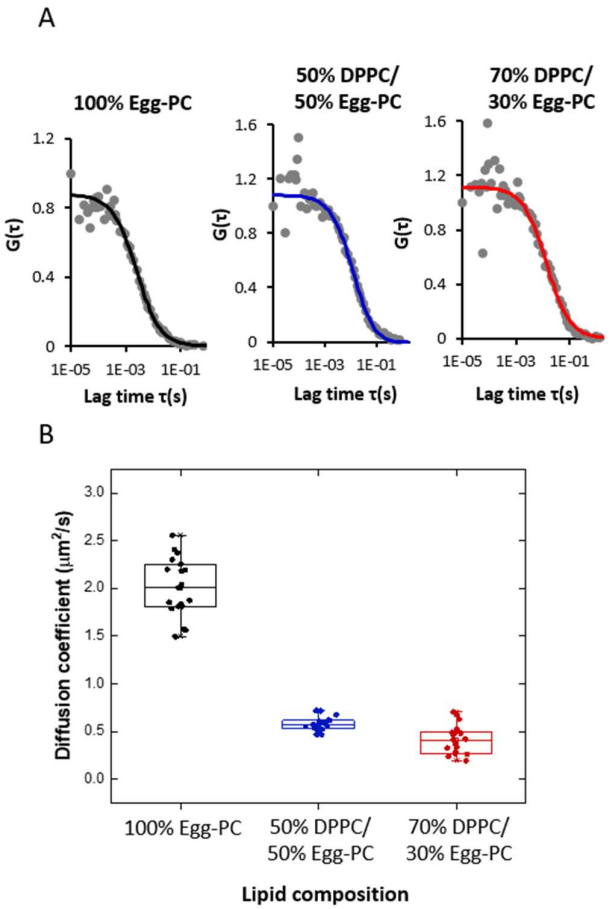

Fig. S4 (A) Autocorrelation curves measured by the FCS and (B) resulting diffusion coefficients for SLB samples with lipid compositions of 100% Egg-PC (left), 50% DPPC/50% Egg-PC (middle) and 70%DPPC/30% Egg-PC (right) respectively.

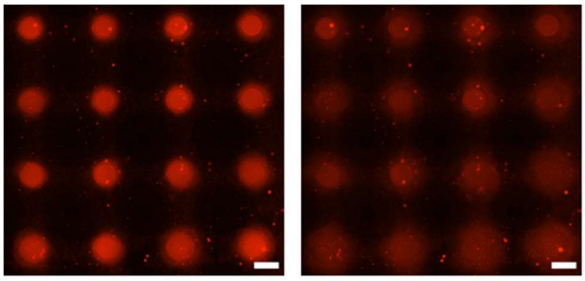

Fig. S5 Significant lipid exchanges between patterned SLB was observed in the absence of the diffusion barrier. Fluorescence images of button SLB taken immediately (left) and 1 hours after (right) SLB formation; Lipid composition for the exterior area: 50% DPPC/50% Egg-PC, and for button area: 100% Egg-PC (doped with 0.01% Texas Red DHPE for visualization). Scale bar is 100 μm.

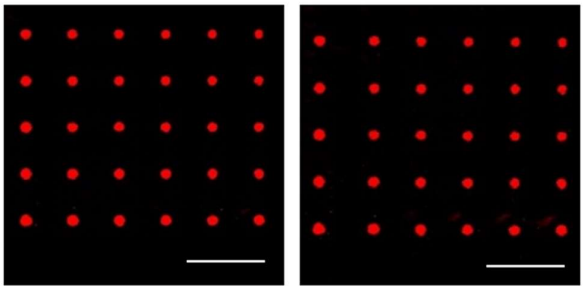

Fig. S6 Lipid exchange between SLB patterns can be blocked in the absence of a diffusion barrier by using DPPC as constituent lipids. Fluorescence images of button SLB taken immediately (left) and 10 hours after (right) SLB formation; Lipid composition for the exterior area: 50% DPPC/50% Egg-PC, and for button area: 100% DPPC (doped with 0.01% Texas Red DHPE for visualization). Scale bar is 500 µm.

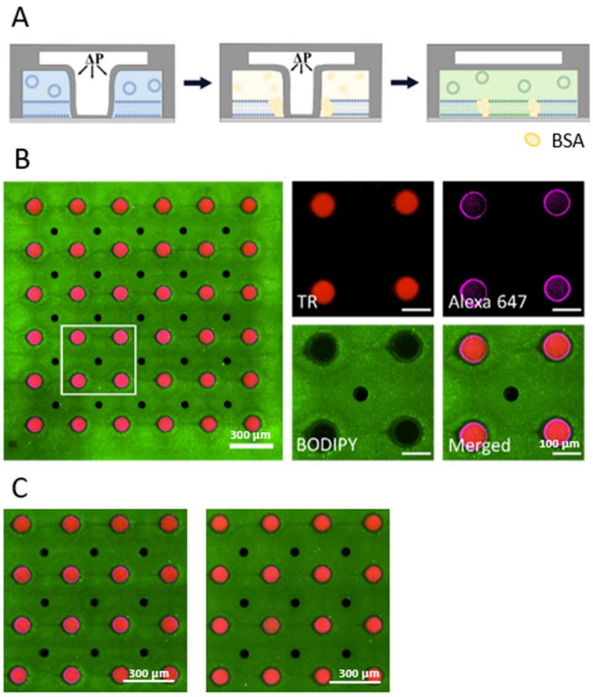

Fig. S7 A long-term stable SLB array could be obtained by introducing the BSA as diffusion barriers. (A) An illustration showing steps to form the hybrid SLB arrays with BSA rings. (B) A merged fluorescence image of patterned SLB with BSA ring (left). The exterior region is composed of 50% DPPC/50% Egg-PC labeled with BODIPY-DHPE, and the button area is composed of 99.9% Egg-PC doped with 0.01% Texas Red-DHPE. The diffusion barrier is prepared by using BSA-AF647 for visualization (magenta). Showing on the right are magnified images for each channel. (C) Fluorescence images acquired immediately (left) and 5 hours (right) after the hybrid SLB formation. The result indicates that the BSA ring can maintain the long-term stability of the hybrid array.

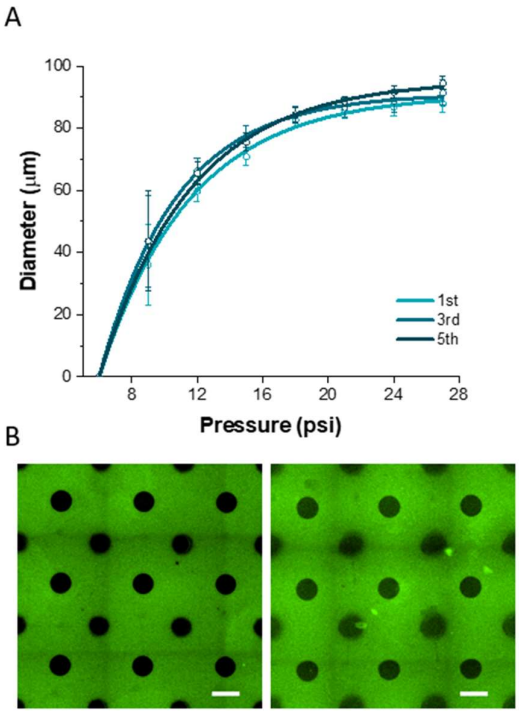

Fig. S8 The reusability analysis of the SLB patterning chip. (A) A relation between applied pressures and average button diameters of the pattern after 5 consecutive uses of the same chip. (B) Fluorescence images of the hybrid SLB array formed using a fresh (left) and a reused chip (right). Scale bar is 100 μm.

Captions for supporting movies

Movie S1. A time-lapse video showing the chip operation and mixing of two food dye solution (yellow and green). Control lines for patterning buttons and chip operation are shown in red and blue respectively.

Movie S2. The immune NF-κB response of a cell upon its encounter to the LPS-functionalized hybrid SLBs array.