Electronic Supplementary Information for:

Dip-pen Nanolithography of Optically Transparent Cationic Polymers to Manipulate Spatial Organization of Proteolipid Membranes

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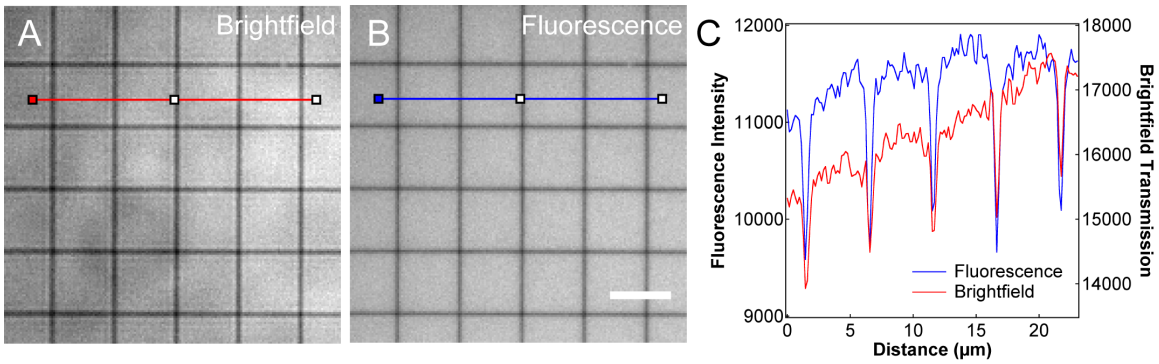

Figure S1. E-beam patterned chromium grid with 5 μm squares. The substrate was prepared with a 100 mol% DOPC bilayer followed by BSA blocking to prevent non-specific binding. The grid was imaged in a 1 μM fluorescein solution using (A) brightfield illumination and (B) epifluorescence. (C) Line scans taken from (A) and (B) reveal a steep drop-off in measured brightfield transmission and fluorescence intensity in areas where chromium was deposited. Scale bar = 5 μm.

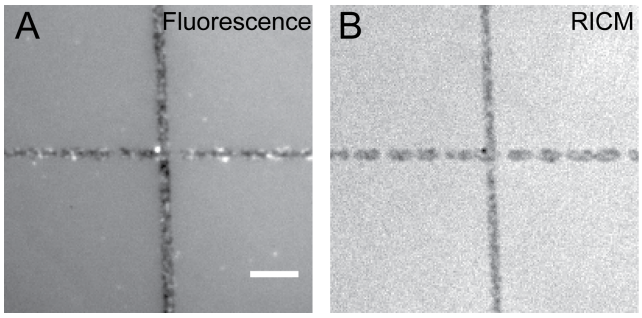

Figure S2. Deposition of partially formed PDAC lines. (A) Fluorescence image of NBD lipids surrounding intersecting polymer lines. (B) RICM image showing clear gaps in the patterned horizontal line. However, the vertical line contains no breaks. Scale bar = 5 μm.

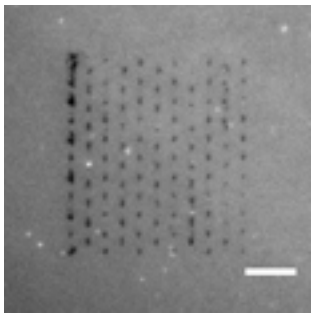

Figure S3. Representative fluorescence image of NBD-tagged lipid membrane formed on a PDAC dot array patterned glass coverslip. Patterning conditions were the same as those in Fig. 3. Scale bar = 5 μm.

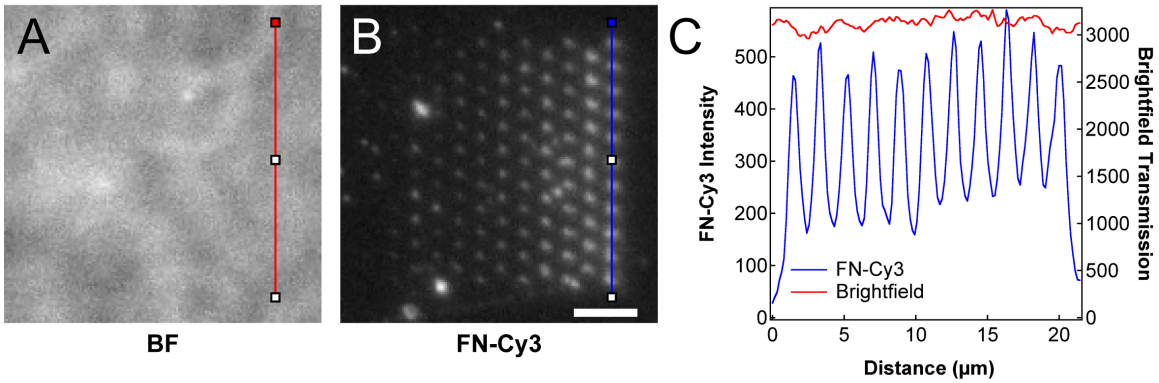

Figure S4. Images of a nanopatterned array of PDAC dots that verify optical transparency. The deposited polymer is transparent in brightfield (A) but visible in the Cy3 channel (B) where FN-Cy3 has selectively adhered. (C) A line scan through a column of dots is provided. Scale bar = 5 μm.

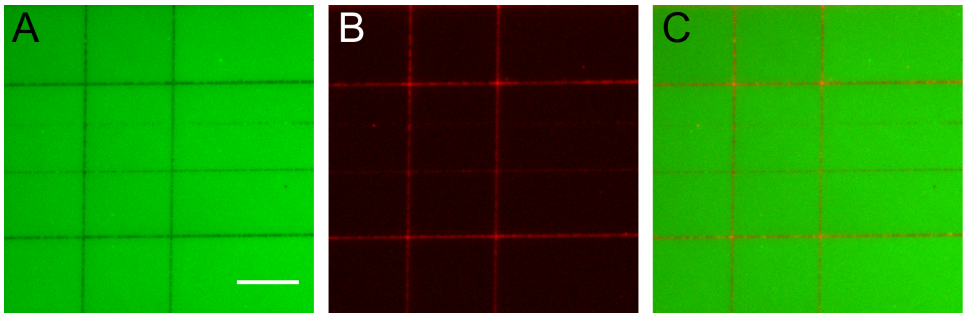

Figure S5. Fluorescence images demonstrating the selective localization of BSA-Cy3 onto PDAC lines. (A) NBD lipids on a glass surface patterned with a PDAC grid. Scale bar = 25 μm. (B) BSA-Cy3 was selectively adsorbed onto the polymer lines. (C) Image overlay of (A) and (B), which shows that BSA-Cy3 adheres specifically to the polymer regions and not the lipid corrals.

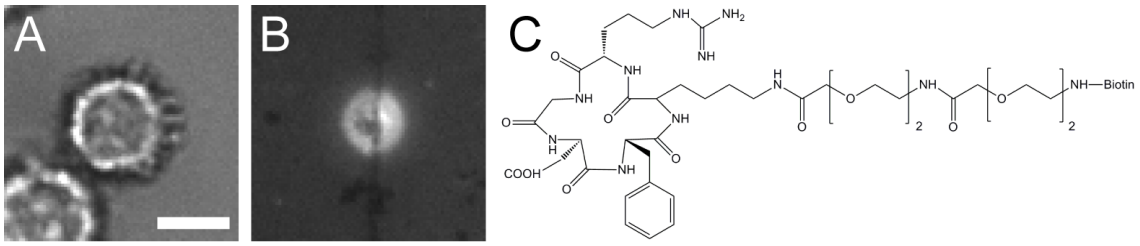

Figure S6. Image of live cells on a biotinylated lipid surface functionalized with Alexa Fluor 647 labeled streptavidin (Streptavidin-647). Biotinylated cyclic RGD (cRGD) was placed onto the surface, and the cells were incubated onto the supported lipid membrane for 30 min. (A) Brightfield image of a cell that adhered to a DPN-generated PDAC line. Scale bar = 10 μm. (B) Epifluorescence image revealing the location of the streptavidin-647 and cRGD. (C) Chemical structure of biotinylated cRGD.

Tip Array	PDAC (by wt)	Ethanol	Water	Ethylene Glycol	Relative Humidity	Pattern
F Type	1%	57.9%	21.1%	20%	Ambient	Lines
F Type	5%	49.3%	25.7%	20%	Ambient	Lines
F Type	10%	38.6%	31.4%	20%	Ambient	Lines
F Type	20%	6.5%	48.5%	20%	Ambient	Lines
F Type	10%	38.6%	31.4%	20%	75%	Lines
F Type	1%	10%	69%	20%	Ambient	Lines
F Type	5%	10%	65%	20%	Ambient	Lines
F Type	10%	10%	60%	20%	Ambient	Lines
F Type	5%	10%	65%	20%	75%	Lines
M Type	15.8%	10%	29.2%	45%	Ambient	Dots
M Type	10%	10%	60%	20%	Ambient	Dots

Table 1. Summary of the different PDAC ink compositions tested for this study including the type of tip array used and the pattern written.

Determination of pY levels from representative images:

To quantify activation levels, a region of interest (ROI) was selected around each cell based on the bright field image. Using the fluorescence signal from the physisorbed fibronectin, the PDAC dots were located using the particle analysis function in ImageJ, as seen in (1). The integrated signal from the pY channel excluding the dots was measured and divided by the total pY intensity under the cellular ROI (2). This allowed for a direct measurement of EGFR that was activated by fluid, membrane-bound EGF.