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Supplementary Materials for

Light-activated communication in synthetic tissues

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The PDF file includes:

Supplementary Figures

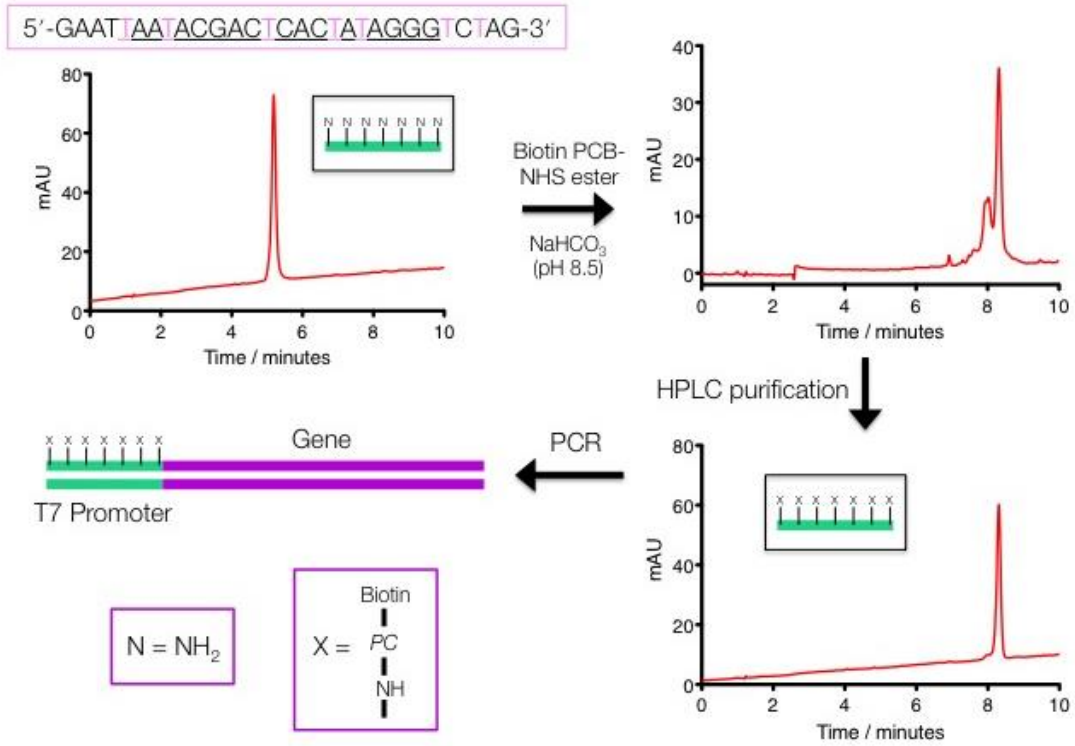


fig. S1. Synthesis of LA-DNA from an amino-T7 primer. The oligo containing seven C6-amino-dT modifications was reacted with the NHS-ester PC Biotin and purified by HPLC. The Oligo PC Biotin conjugate was used as a PCR primer to create a DNA template to express a gene of interest. HPLC signal is 260 nm absorbance.

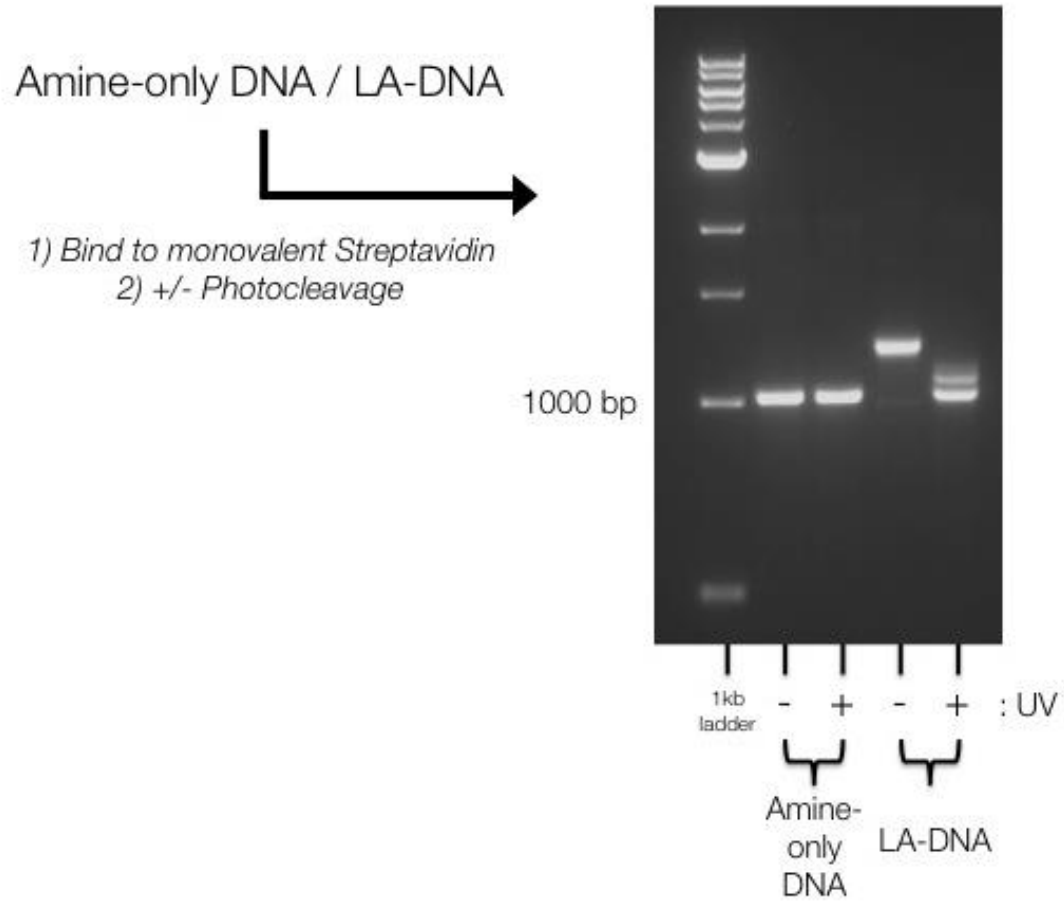


fig. S2. Binding of monovalent streptavidin and photocleavage of LA-DNA. Amine-only DNA and LA-DNA were both incubated with monovalent streptavidin. Monovalent streptavidin only bound to the LA-DNA as seen in the gel shift. Upon UV illumination, the LA-DNA was reduced to the same apparent mass as the amine-only DNA, by photocleavage of the PC/biotin/streptavidin from the DNA.

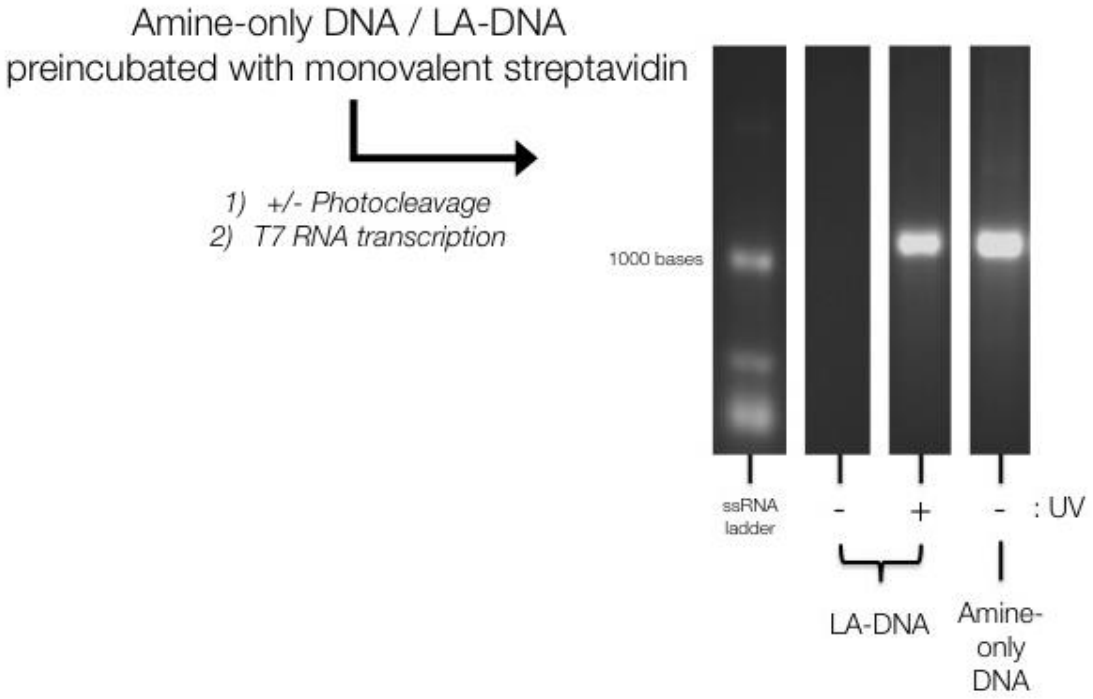


fig. S3. T7 RNA transcription from LA-DNA. Amine-only DNA and LA-DNA were pre-incubated with monovalent streptavidin. Each DNA was used as a template for T7 RNA transcription. Without UV light, no RNA was produced from LA-DNA. With UV light, similar amounts of RNA were observed for LA-DNA and amine-only DNA.

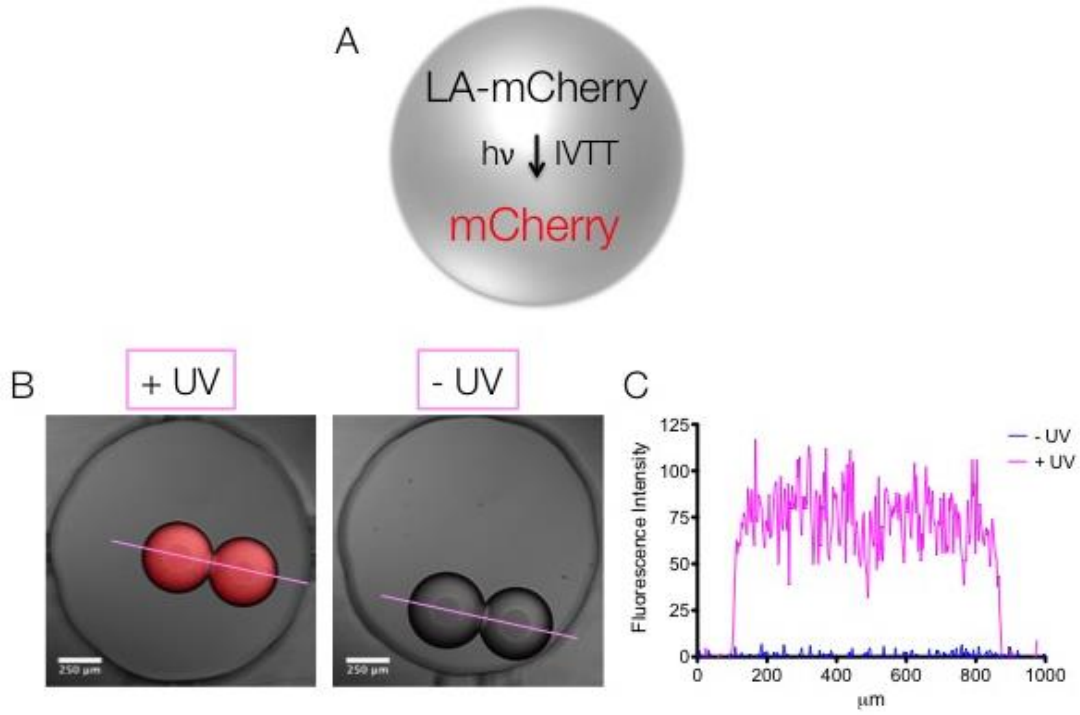


fig. S4. Light-activated expression from LA-mCherry DNA in synthetic cells. (A) Schematic of a synthetic cell that will express mCherry protein upon light-activation. (B) Synthetic cells containing LA-mCherry DNA expressed mCherry upon light-activation. (C) Fluorescence intensity line profiles from B.

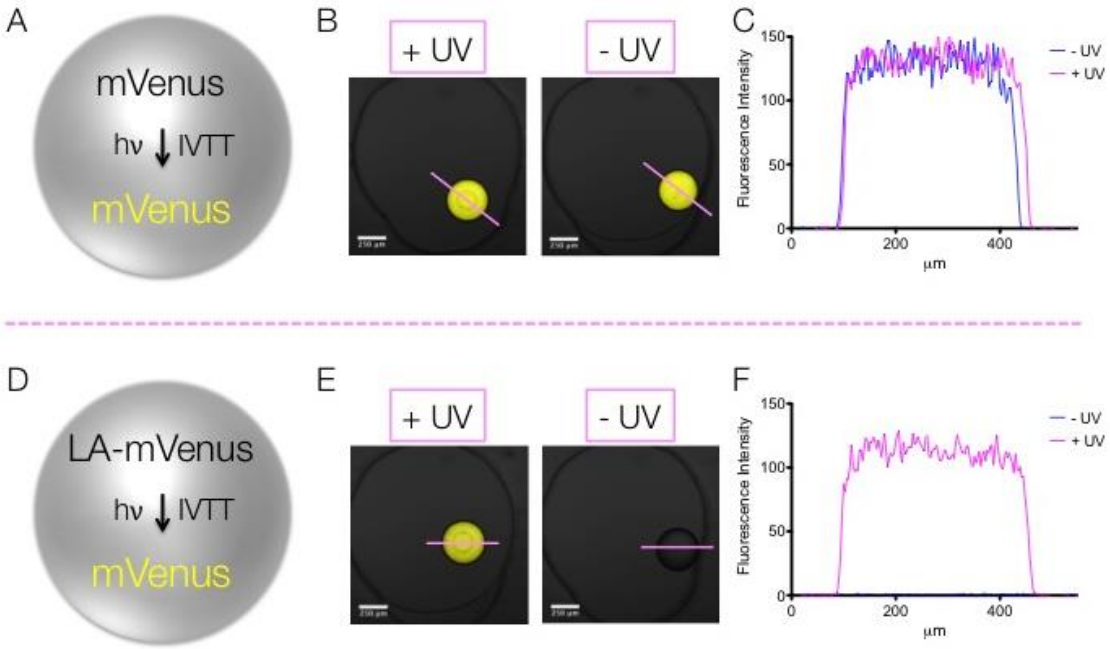


fig. S5. Expression from amine-only mVenus DNA and LA-mVenus DNA in synthetic cells. (A) Schematic of a synthetic cell containing amine-only mVenus DNA. (B) Synthetic cells containing amine-only DNA express protein with or without light-activation. (C) Fluorescence intensity line profiles from B. (D) Schematic of a synthetic cell containing LA-mVenus DNA. (E) Synthetic cells containing LA-mVenus DNA expressed mVenus upon light-activation. (F) Fluorescence intensity line profiles from E.

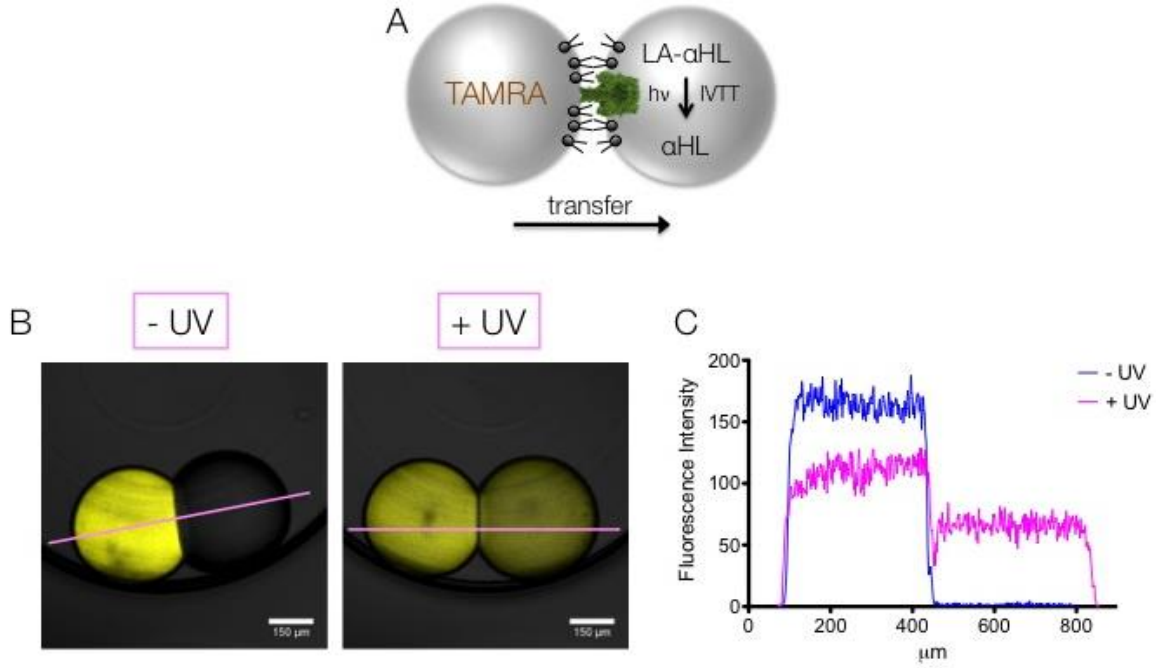


fig. S6. Light-activated transfer of a small-molecule fluorophore between synthetic cells. (A) Schematic of the synthetic cell pair. One cell contained LA-αHL DNA, the other contained the small-molecule fluorophore TAMRA. TAMRA diffused across the bilayer only when αHL was expressed and permeabilized the membrane. (B) Diffusion of TAMRA across the bilayer was only observed after light-activation. (C) Fluorescence intensity line profiles from B.

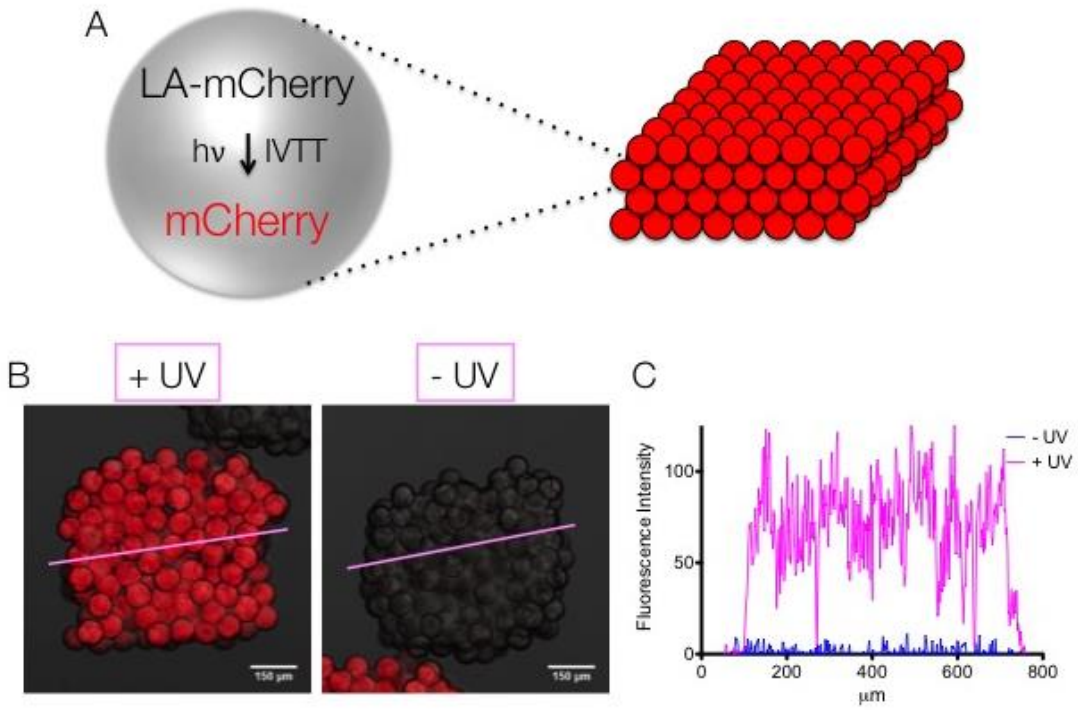


fig. S7. Light-activated expression from LA-mCherry DNA in synthetic tissues. (A) Schematic of a synthetic cell that will express mCherry after light-activation. (B) 3D-printed synthetic tissues containing LA-mCherry DNA express mCherry upon light-activation. (C) Fluorescence intensity line profiles from B.

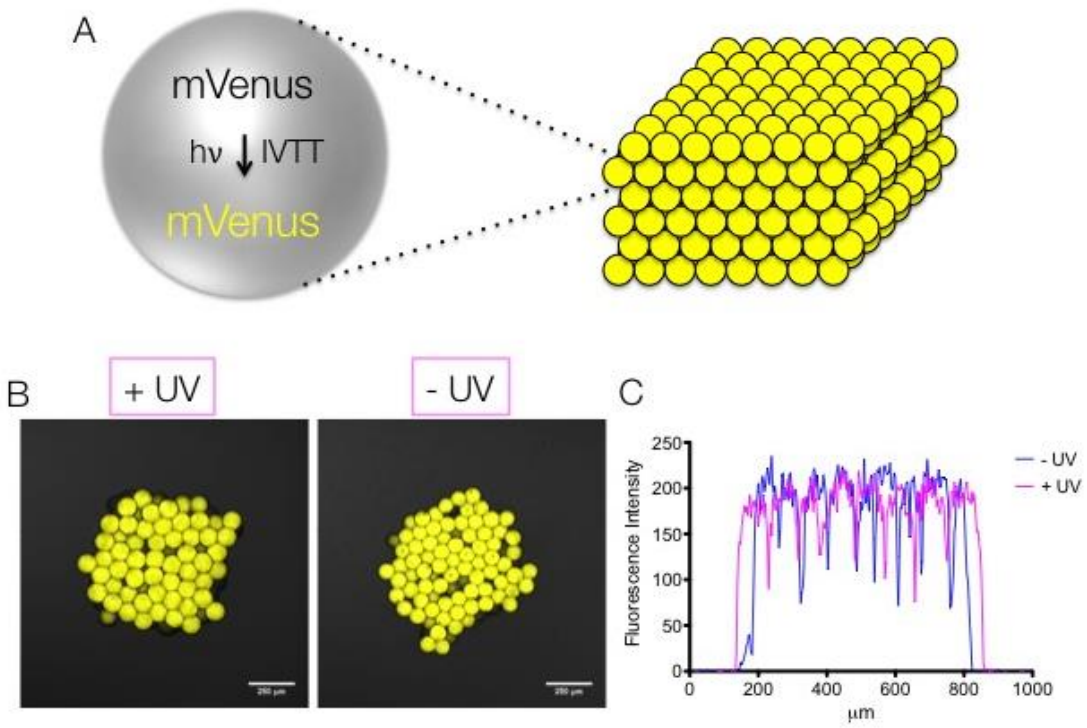


fig. S8. Expression from amine-only mVenus DNA in synthetic tissues. (A) Schematic of synthetic cells that will express mVenus protein with or without light-activation. (B) Synthetic tissues containing amine-only mVenus DNA express mVenus with or without light-activation. (C) Fluorescence intensity line profiles from B.

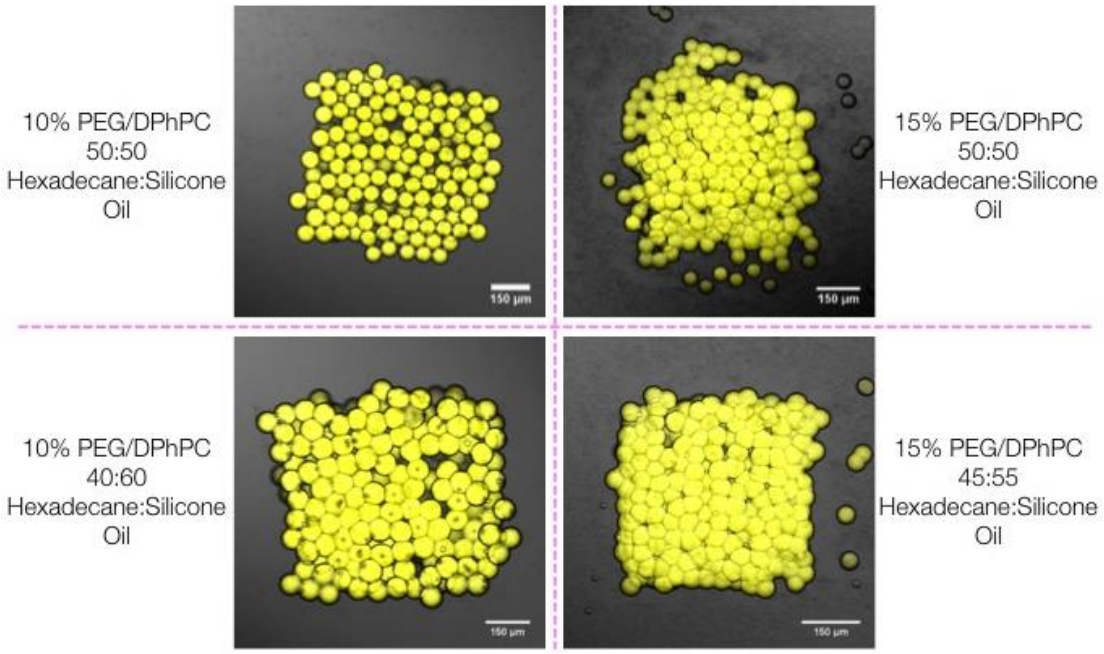


fig. S9. Tuning the packing of synthetic cells inside synthetic tissues by altering the bilayer size between synthetic cells. By increasing the fraction of DPPE-mPEG2000 lipid or the silicone oil proportion, the bilayer size increases which causes the droplets in the synthetic tissue to pull each other closer together. The conditions shown in the bottom left panel (10% PEG, 40:60 hexadecane:silicone oil) were chosen for all further experiments, because of the close packing observed and because less leakage of the solution from the capillary occurred during printing.

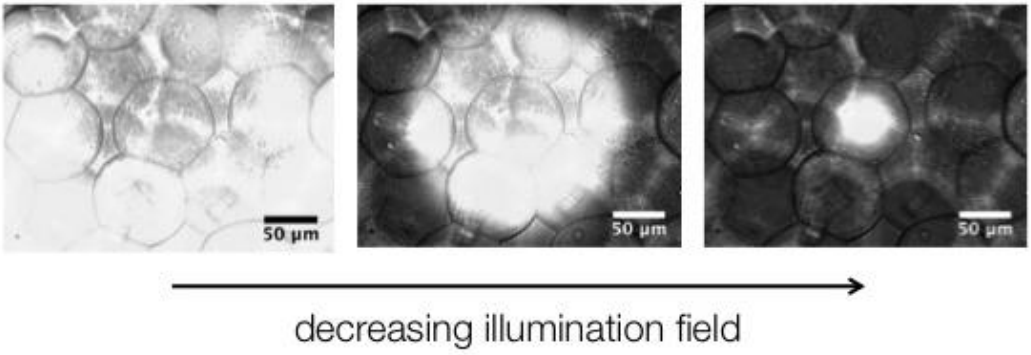


fig. S10. Restricted microscope illumination of a synthetic tissue. A fluorescence microscope with a field diaphragm (see methods) was used to illuminate a large area (illumination field setting 3), small area (illumination field setting 2) and a single droplet (illumination field setting 1) within a synthetic tissue, where all droplets contain mVenus protein.

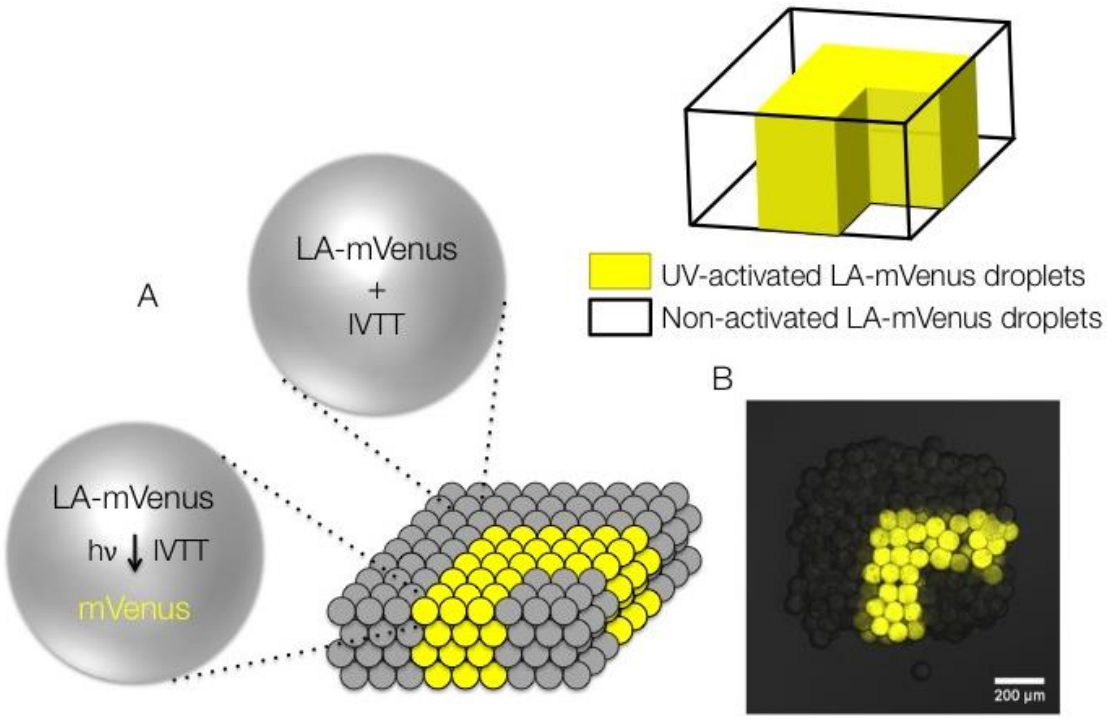


fig. S11. LA-mVenus protein expression in a light-patterned pathway. (A) Schematic showing a printed tissue with all droplets containing LA-mVenus DNA, but only those droplets that have been illuminated with the microscope express protein (yellow), while the rest do not (grey). (B) mVenus is only expressed in the light-activated droplets (yellow fluorescence) illuminated by the microscope.

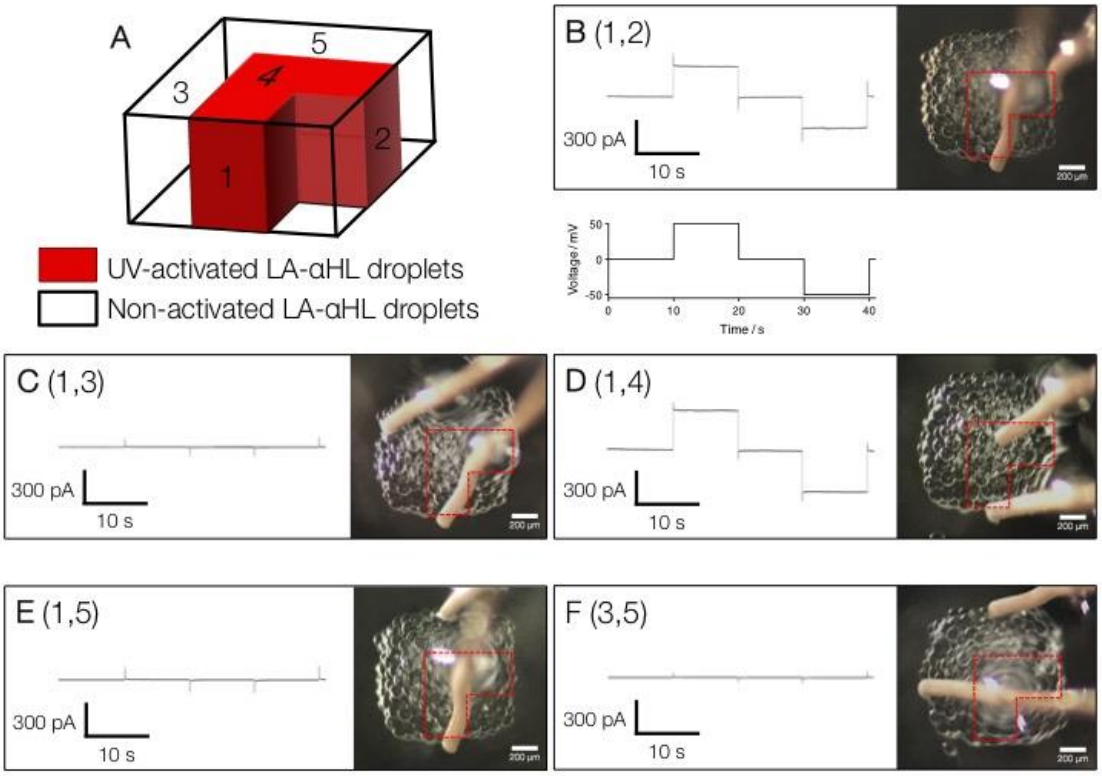
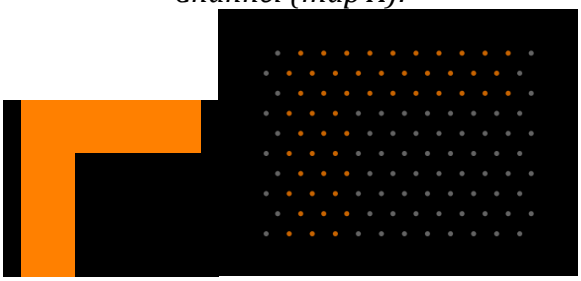
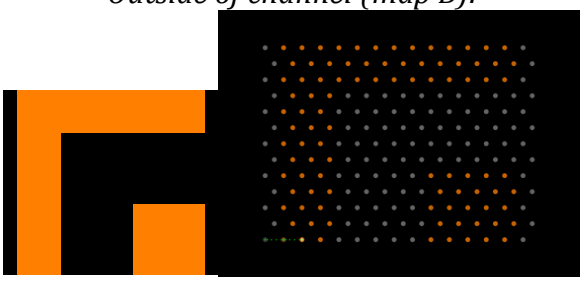


fig. S12. Electrical recordings from an L-shaped 2D pathway formed by light-patterning of an LA-αHL DNA 3D-printed synthetic tissue. (A) Schematic of the printed tissue where all droplets contain LA-αHL DNA, but only those illuminated with the microscope express protein (red), while the rest do not (clear region within black frame). Numbers represent sides of the cuboid where electrodes were placed to detect the conductive pathway. Electrical recordings detect a current when the electrodes are at positions 1 and 2 (B), based on the voltage protocol below, and at 1 and 4 (D). No electrical signal is observed when the electrodes are positioned at side 1 of the pathway and 3 (C) or 5 (E), or when both electrodes are positioned away from the pathway, 3 and 5 (F).

Channel (map A):



Outside of channel (map B):



Top of network (map C):

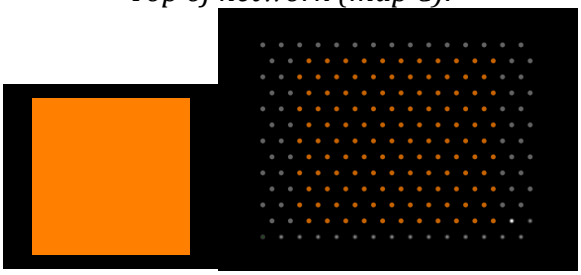


fig. S13. The three printing maps used to create the 3D printed pathway. Left images are the maps created in Microsoft Paint, where a single pixel equates to a single droplet. Right images are the maps recreated in the printing software. The orange pixels correspond to a printed droplet. The black pixels correspond to no printed droplet.

These 3D-printing pathway maps (left images) were created in Microsoft Paint where a single pixel equates to a single droplet. Also shown are the maps recreated in the printing software (fig. S13, right images) and demonstrate the dimensions and number of droplets in each layer of each map. The orange pixels correspond to a printed droplet. The black pixels correspond to no printed droplet.

Channel (map A): Length of 10 droplets and a width of 3 droplets.

Outside of channel (map B): A width of 3 droplets and a length of 13 droplets, along with a small square of 5 × 5 droplets. A gap of five droplets was left between both structures, for where the channel structure was printed.

Top of network (map C): An 11 × 11 droplet square. The gap around the outside was to ensure these droplets only landed on the top of the structure.