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

A high-throughput microfluidic microphysiological system (PREDICT-96) to recapitulate hepatocyte function in dynamic, re-circulating flow conditions

Supplementary Information

Materials and methods:

Calcein AM staining: Cell viability post-seeding was determined using calcein AM staining (Thermo, Cat No. C3100MP). A solution of 10 μM calcein AM dye in HMM was introduced into the bottom channel of the device and incubated for 10 mins. Cells in the microfluidic MPS were imaged using FITC filter. Average area coverage of green fluorescence was determined using image J software.

Cell seeding optimization using fluorescent microspheres: Fluoro-Max Dyed green fluorescent microspheres (10 μm diameter, Thermo, Cat No. CDG1000) were used to optimize methods for seeding PHH in the PREDICT-96 array. Fluorescent microspheres were sonicated for 30 seconds and solutions of 0.1, 0.25, 2.5, 5 million microspheres/mL were prepared in PBS. Microspheres were introduced into the channels through the inlet and outlet ports of the top channel and allowed to settle for 10 min. Bead coverage in channels was imaged using a fluorescence microscope. Bead density in the channels was estimated using Image J software.

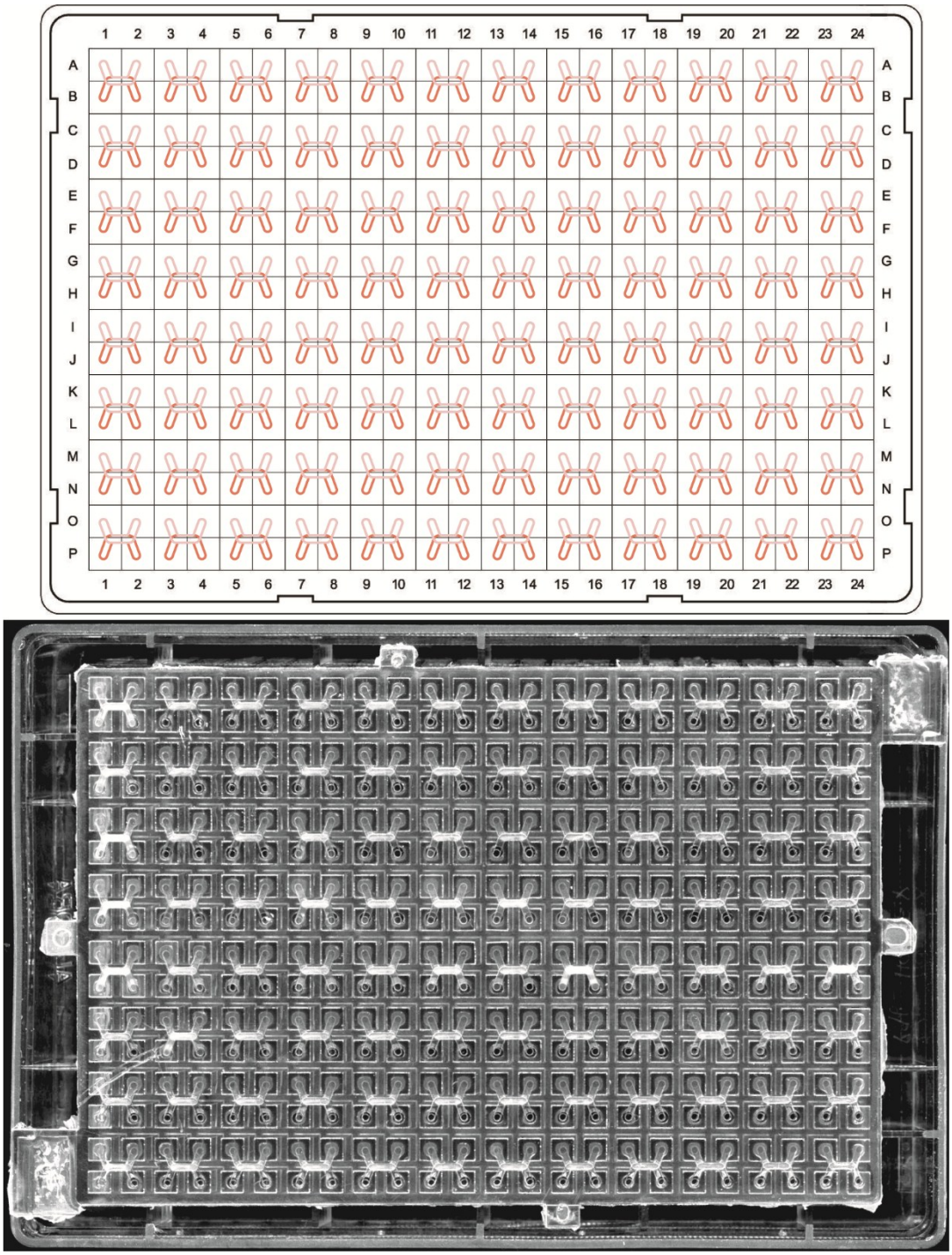

Figure S1. PREDICT-96 array. Schematic showing arrangement of 96 microfluidic MPS in an array format. Each microfluidic MPS occupies 4 wells of a 384 well plate. Wells act as reservoirs for media circulation using pumps.

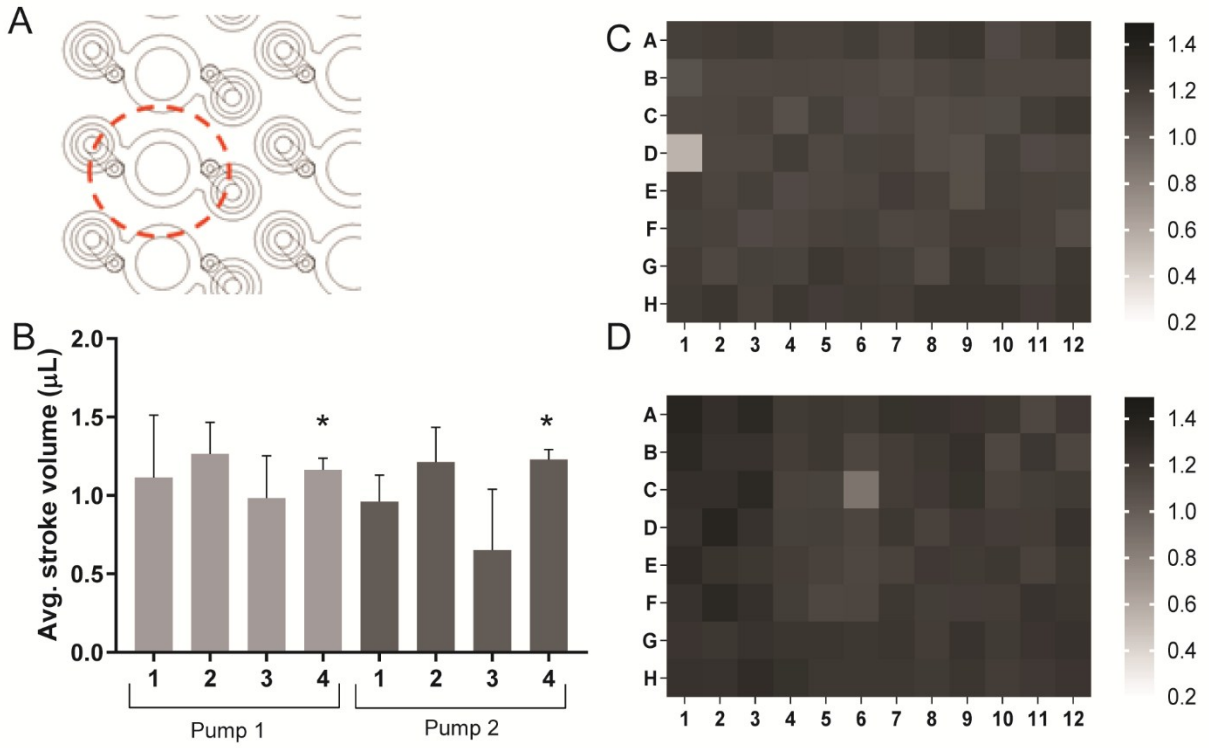

Figure S2. PREDICT-96 pump. A) Footprint of a single pneumatic-driven pump in comparison with 96-well plate (shown in red), B) Average stroke volume of pumps measured prior to introduction of flow in PHH culture. Two pump arrays were used for independent experiments (1-4). Stroke volume was determined for n = 96 pumps on each pump array. Integrity of pumps and stroke volume were maintained over multiple experiments. Data shown as mean ± SD. Detailed heatmap from 4th calibration (denoted by *) showing stroke volume of C) Pump 1 and D) Pump 2

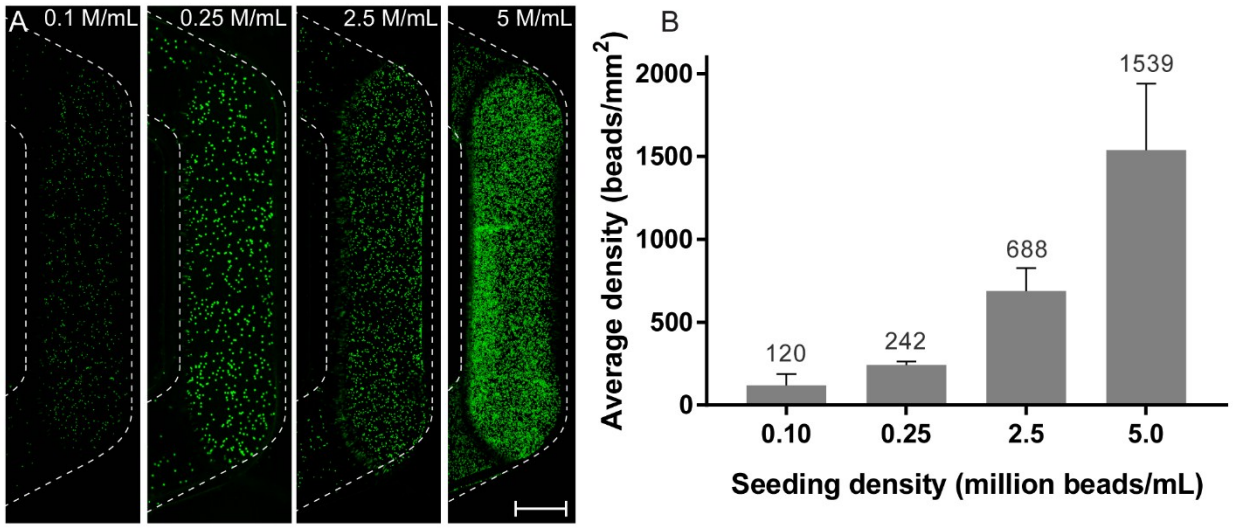

Figure S3. Seeding optimization using fluorescent beads in PREDICT-96 array. A) Fluorescent bead (10μm diameter) seeding in the top channel at various densities (scale bar = 500 μm), B) Average density obtained in the top channel (mean ± SD).

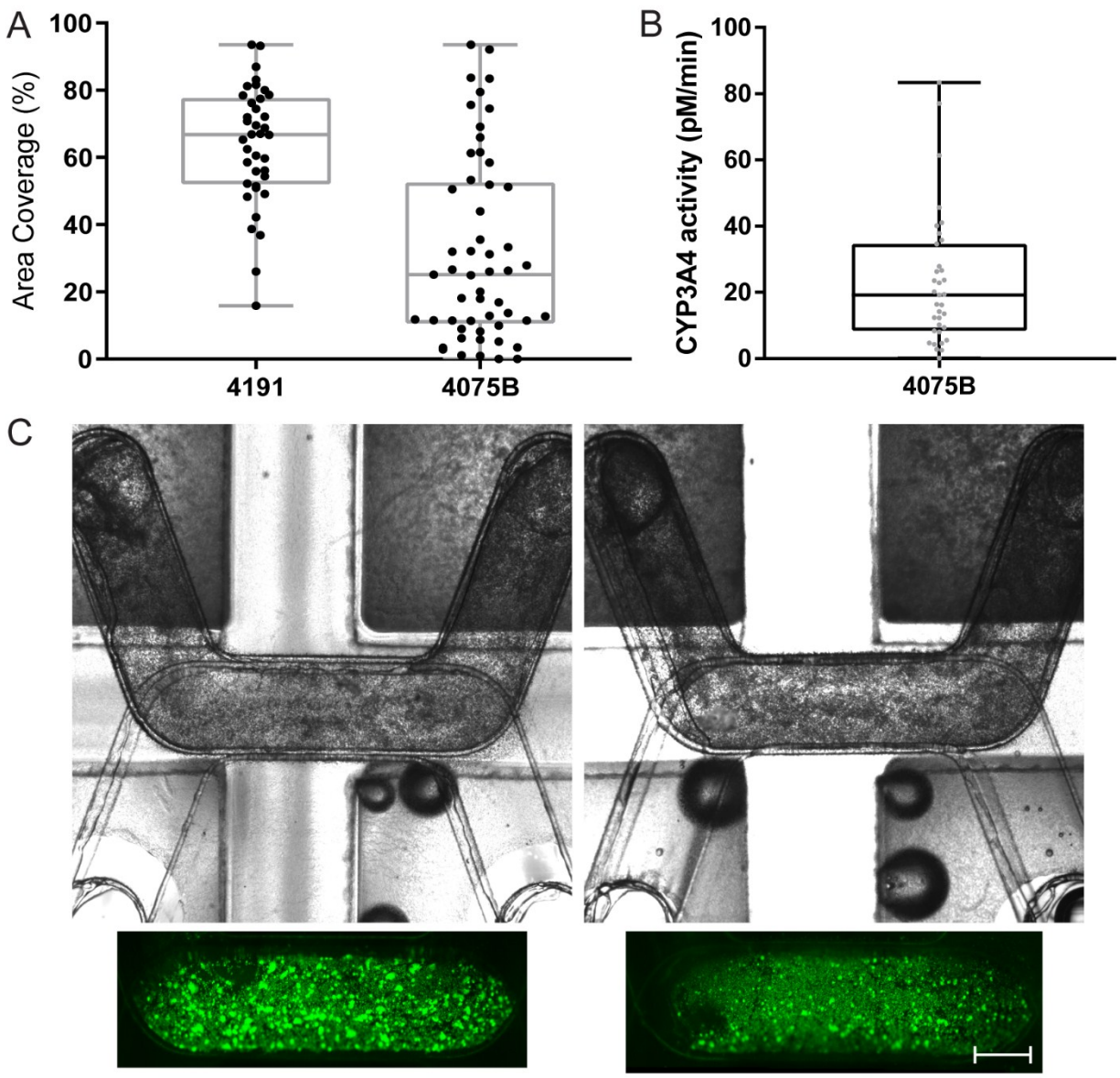

Figure S4. PHH seeding in PREDICT-96 array. A) Average surface coverage of PHH in PREDICT-96 array calculated using calcein AM staining (n = 38 for Lot 4191, n = 53 for Lot 4075B), B) Day 3 CYP activity of lot 4075B seeded in PREDICT-96 array (n=36), C) Representative brightfield images and calcein AM staining of matched devices with PHH (Lot 4075B). Scale bar = 500 μm.

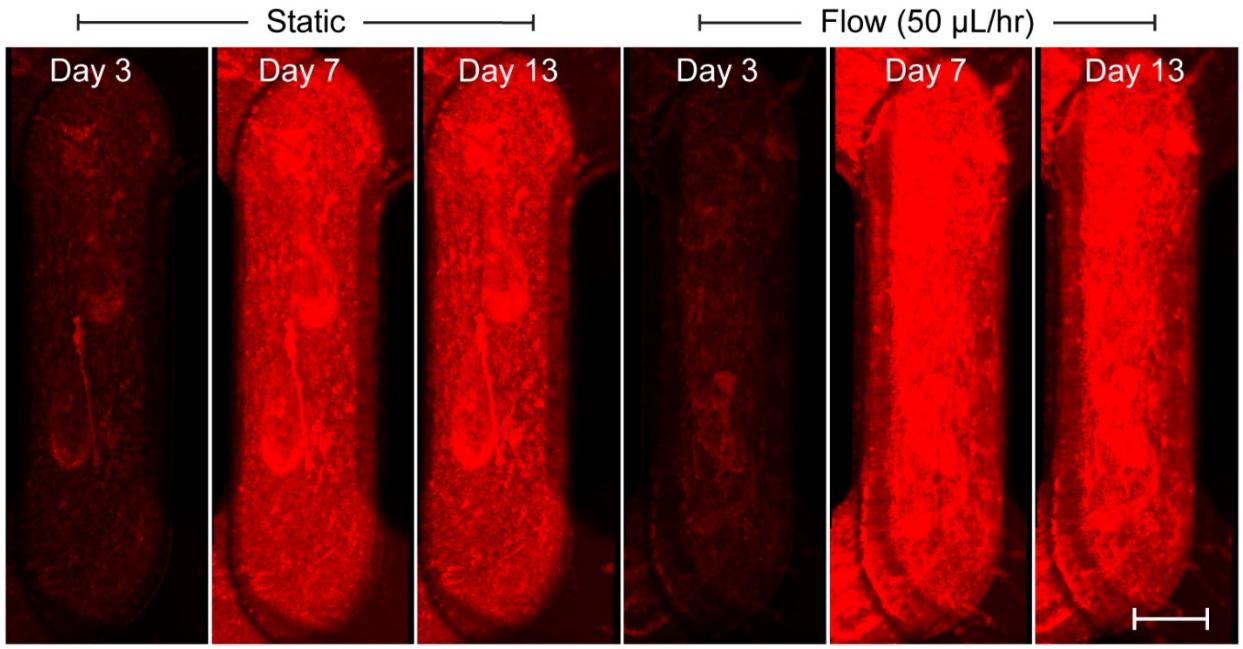

Figure S5. Tdtomato-CNA35 probe to monitor *in situ* collagen production by PHH under static and flow conditions. Collagen production in PREDICT-array visualized using tdTomato-CNA35 probe in static and flow cultures (scale bar = 500 µm).

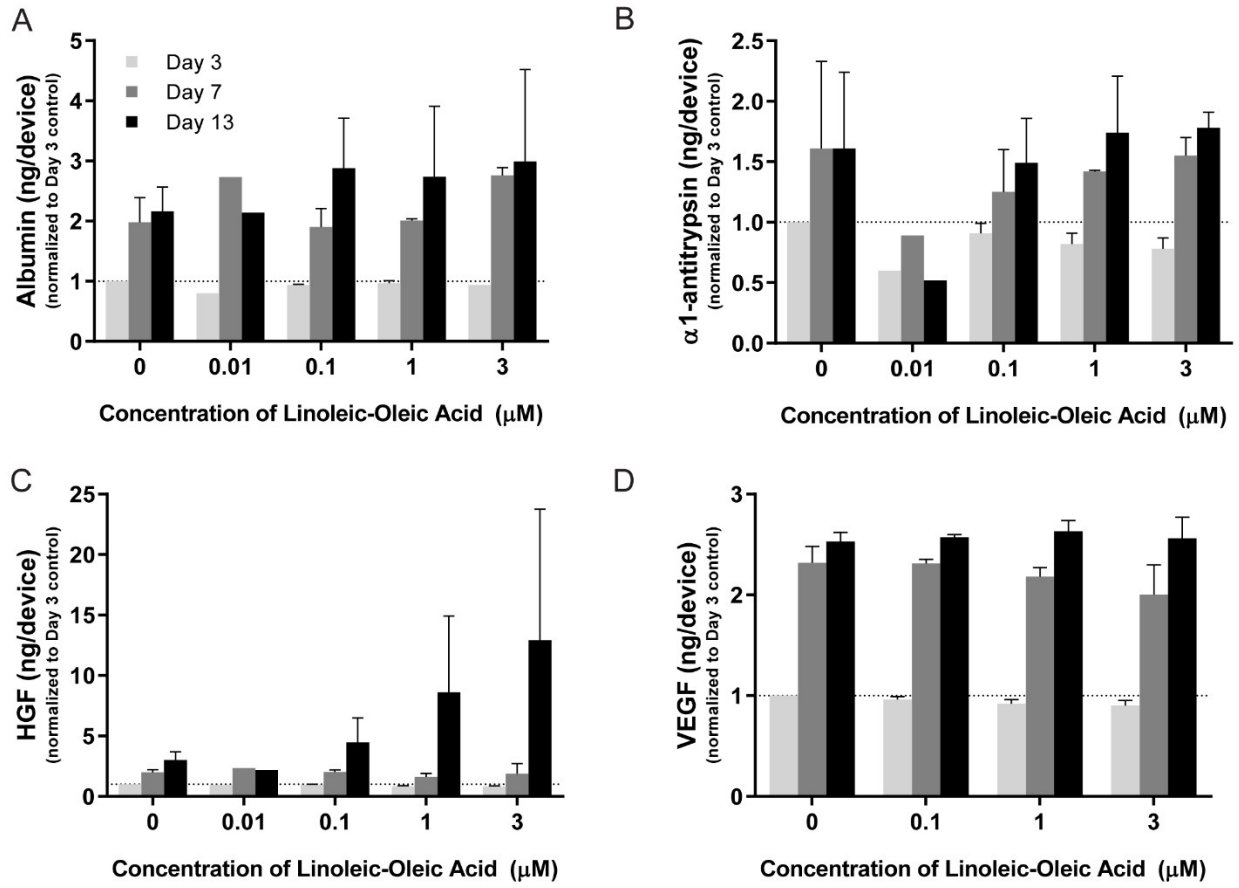

Figure S6. PHH secretions during chronic exposure to FFA. Time course analysis of A) albumin, B) α1-antitrypsin, C) HGF and D) VEGF secretions by PHH during exposure to LA-OA mixtures. Activity is reported as fold change over controls on day 3 within each independent experiment. Data is shown as mean fold change ± SEM (n=6 independent experiments with n=9-16 replicates per condition).