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

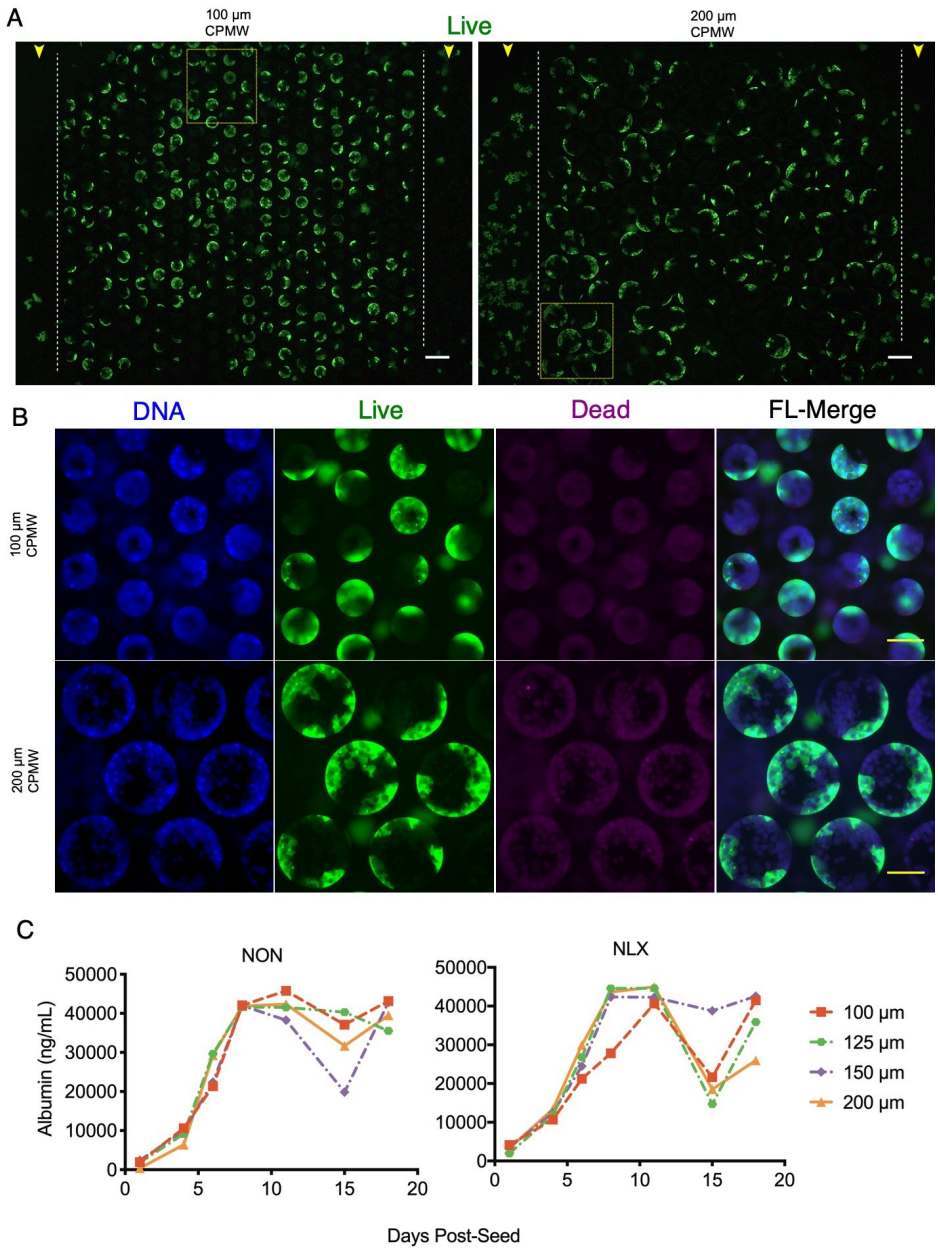

Fig. S1 Characterization of primary human hepatocytes (PHHs) in long-term PDMS-µHEP culture. (A) Live PHHs stained with Calcein AM (green) and imaged with a 10x objective following three weeks of culture in PDMS-µHEP slides with either 100 or 200µm-diameter Close-Packed Microwells (CPMWs). Dotted white line outlines edge of CPMW array, yellow arrows represent hepatocyte on raised shelf outside of CMPW array. Yellow box indicated the area imaged with a 40x objective and shown in in part (B), which includes a panel of dead hepatocyte stained with ethidium homodimer (magenta). Yellow bar represents 100 µm, white bar represents 200 µm. (C) Albumin production of two different PHH lots (NON and NLX) over three week of PDMS-µHEP culture in CPMWs of four different diameters. All four diameters of CPMW supported long-term albumin production and none of the diameters appeared superior for supporting albumin production (Repeated Measure ANOVA with alpha = 0.05).

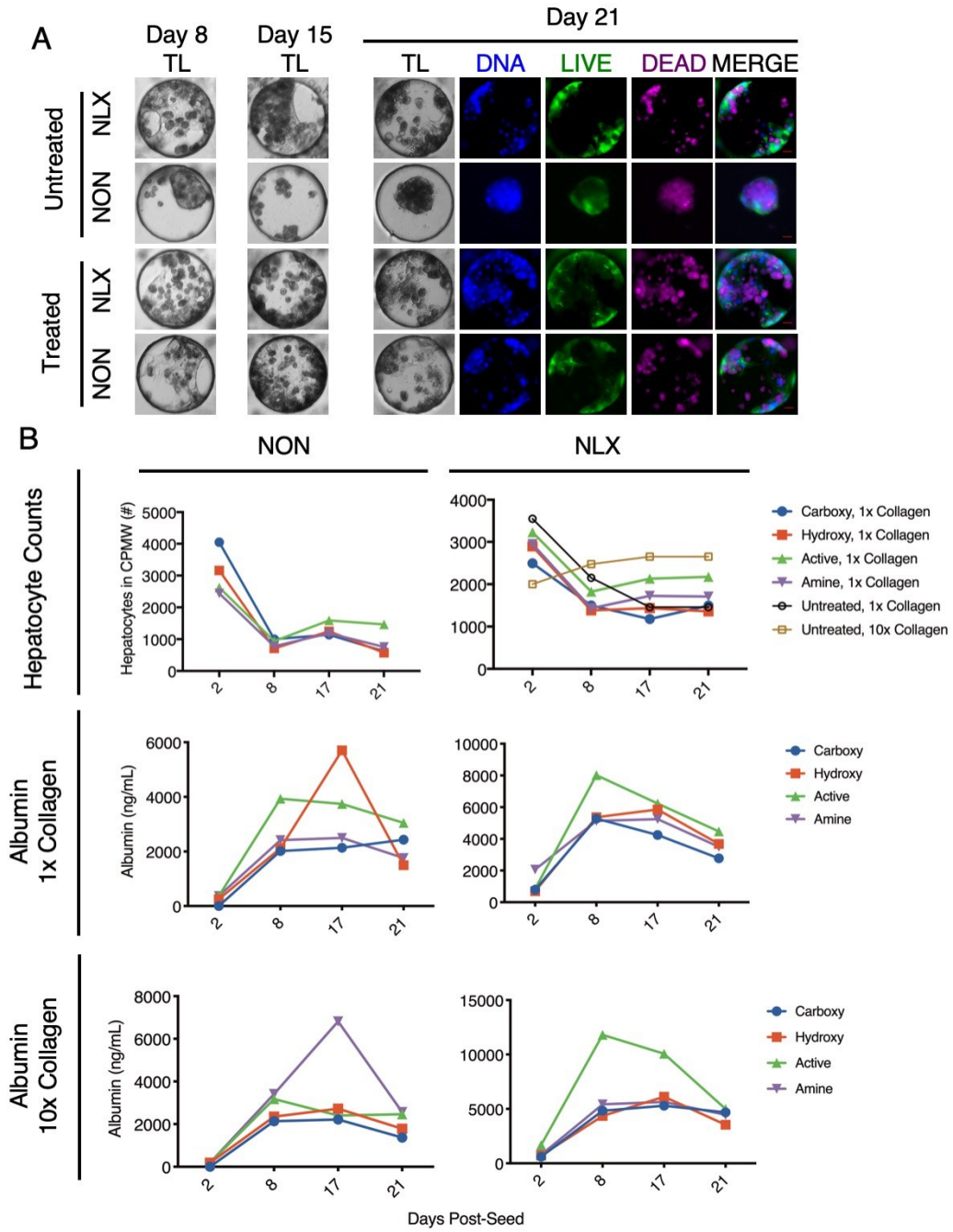

Fig. S2 Characterization of surface treatments and collagen coating in PS-µHEP slides. (A) Phase images and live/dead staining after 21-day culture of NON and NLX hepatocytes in untreated and hydroxy-surface treated CPMW-µHEPs. Green: calcein AM (live cells), Magenta: ethidium homodimer (dead cells) stained after 21 days. Red bar represents 25 µm. (**B**) Hepatocyte counts and albumin production on either 1x (5 µg/cm^2) or 10x (50 µg/cm^2) collagen coated surfaces over 21 days of CPMW-µHEPs culture; matrixed with 4 surface treatments. All four treatments supported long-term albumin production and none of the treatments appeared superior for supporting albumin production (Repeated Measure ANOVA with alpha = 0.05).

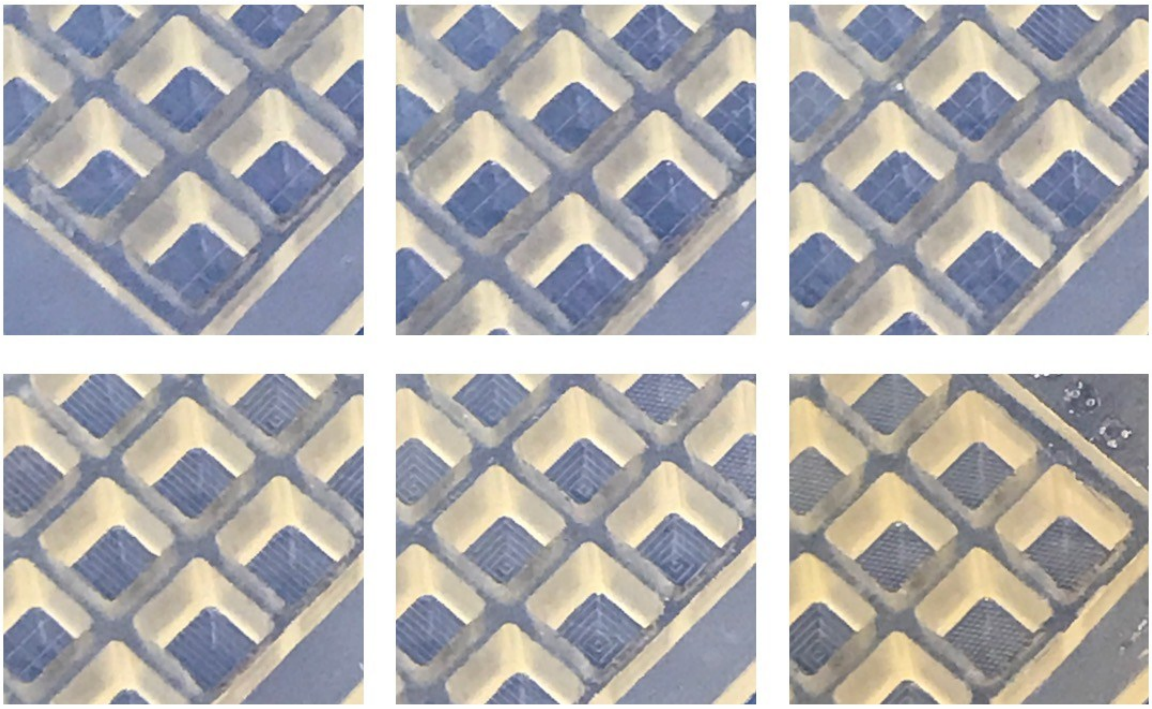

Fig. S3 Microfeature geometries in a PS-µHEP slide. Top, Left to Right: 2x2, 3x3, 4x4 microfeatures geometries. Bottom Left to Right: Channels, Concentric Squares (CSQ), Close-Packed Microwells (CPMW). Each of six geometries is arrayed in a 2x4 well format (eight wells each) on a single 48-well slide to directly compare the effect of geometry on cell phenotypes.

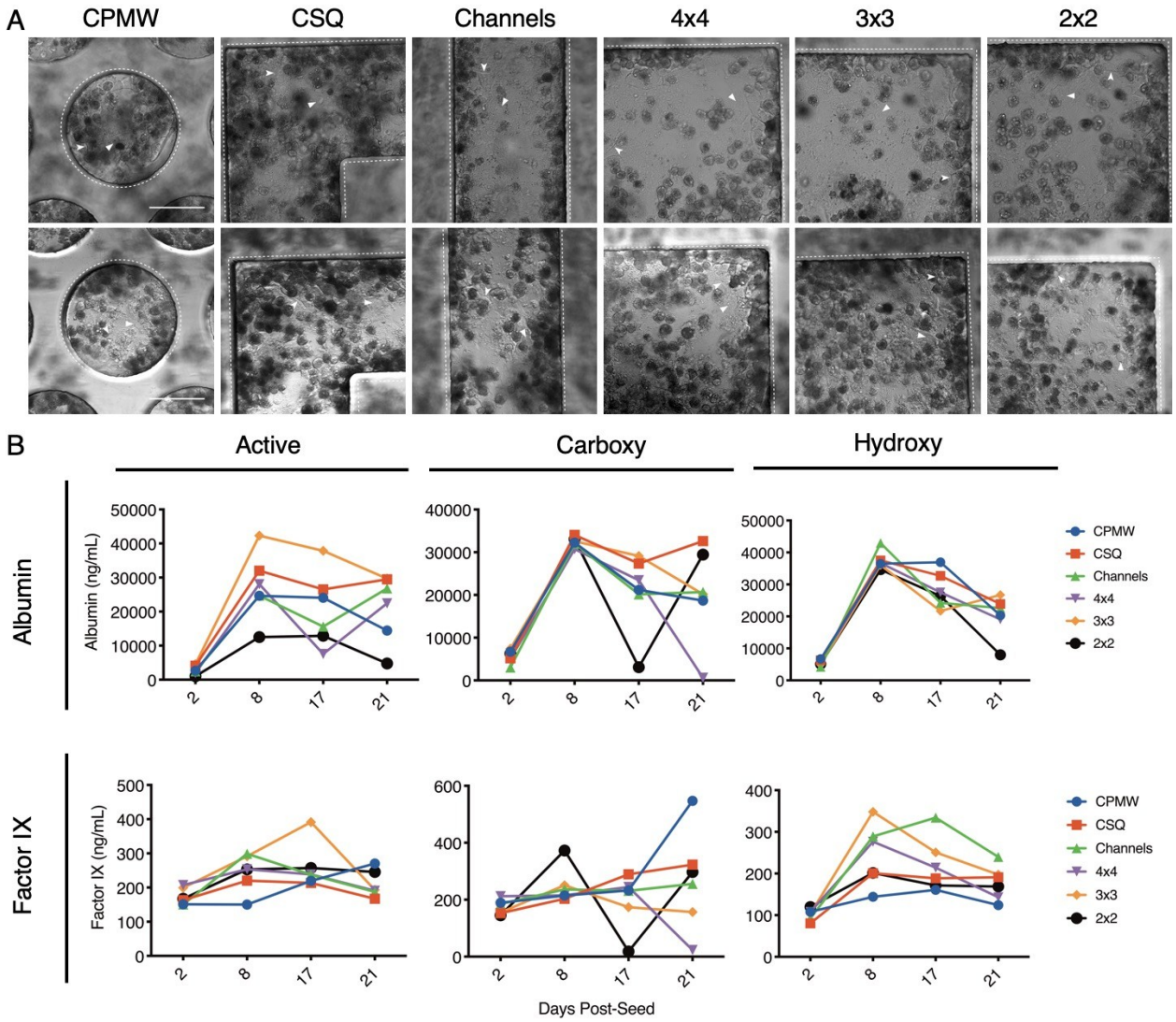

Fig. S4 Hepatic phenotype screening with multiple microfeature geometries and surface treatments (A) Phase images of primary human hepatocytes (PHHs) in active-treated µHep slides of indicated geometry at 1 week (top) and 2 weeks (bottom) post-seed (CPMW: Close-Packed Microwells, CSQ: Concentric Squares). Full arrows indicate a hepatic nucleus, open arrows indicate cell-cell boundaries, dotted lines indicate microfeature edge, scale bars represent 100 µm. B) Phenotypic characterization of PHHs cultured in various PS-µHEP geometries and surface treatments over three weeks. While all conditions resulted in stable phenotypes, no significantly different conditions were noted (Repeated Measure two-way ANOVA with alpha = 0.05).

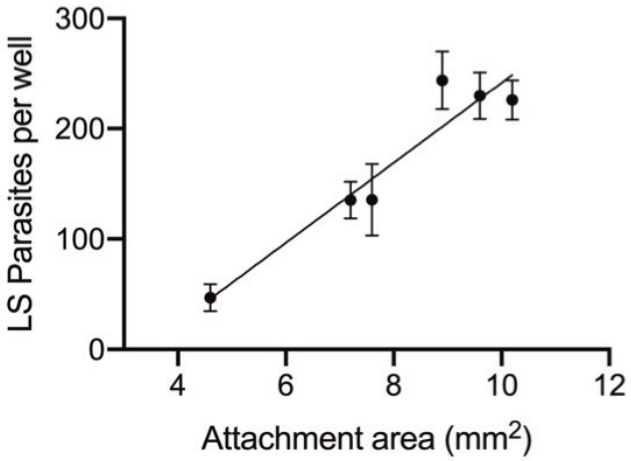

Fig. S5. Infection efficiency versus attachment area. LS parasite infections per well correlate to the available intra-feature attachment area within each geometry. Bars represent S.D. (n=4), line represents a linear regression, Y = 36.25*X - 121.0, R^2 = 0.8460.

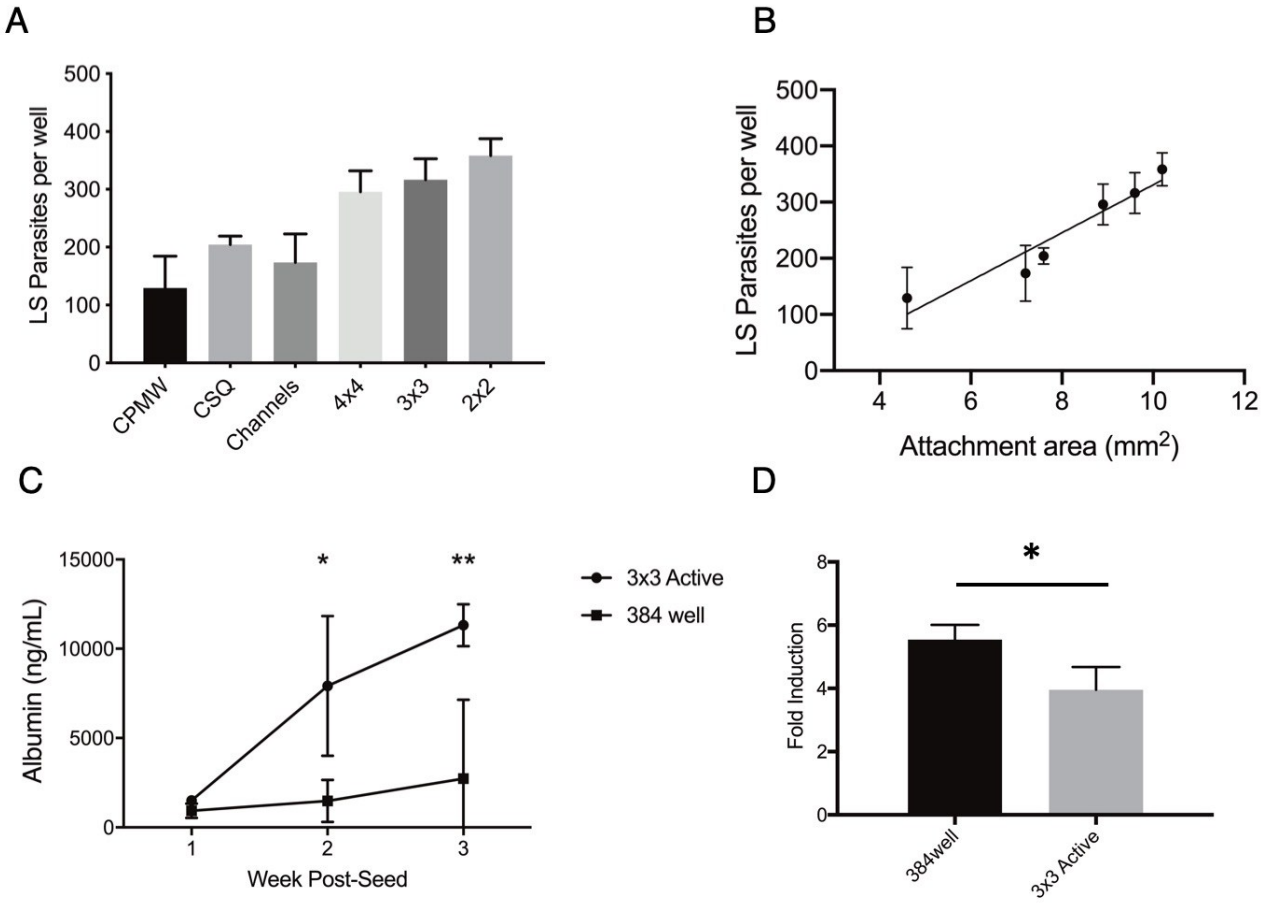

Fig. S6 Correlation of infection rate to attachment area and long-term culture of primary human hepatocytes (PHHs) in 3x3 active-treated PS-µHEP slides. (A) Following selection of 3x3 as the geometry of choice and the active treatment as the surface treatment of choice, PS-µHEP slides were fabricated, seeded with primary human hepatocytes (PHHs) and infected with *P. vivax* parasites. (B) The data from (A) charted by the available attachment area (from Fig. 2B), demonstrating a strong correlation of liver stage parasite formation rate to the area available for cell attachment. Bars represent S.D. (n=3), line represents a linear regression, Y = 42.64*X - 95.63, R^2=0. 7984. (C,D) Additional active-treated 3x3 PS-µHEP slides and a collagen-coated Corning 384-well plate were seeded with PHHs and cultured for three weeks. (C) Albumin production in both devices is sustained for three weeks, but at slightly higher levels in µHEP culture. (D) CYP3A4 induction measured at the end of three weeks of culture is slightly higher in the commercial plate but sustained in both devices. (C,D) error bars indicate S.D. of replicate wells (n=4). Significance determined using unpaired t test with alpha = 0.05, *P < 0.05, **P < 0.01.

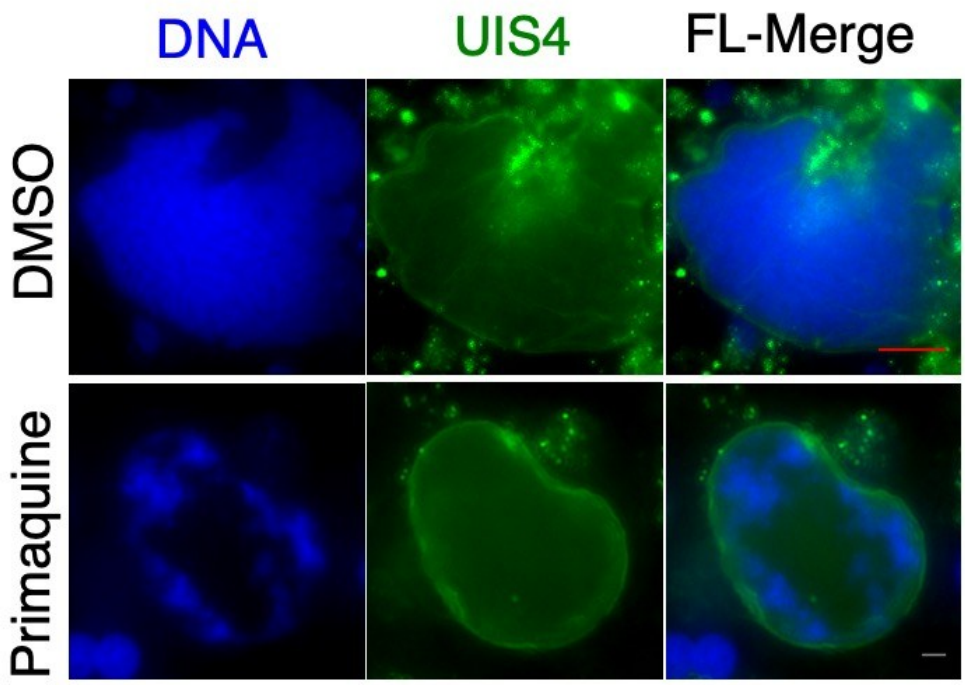

Fig. S7 Non-clearance of parasites treated with primaquine in μHEP culture. Cultures were treated in Radical Cure mode with 10 μM primaquine and LS parasites quantified by HCI using UIS4 signal, localizing to the parasite's parasitophorous vacuole membrane. This membrane material persists in primaquine-treated parasites, however, nuclear replication and morphology are aberrant compared to DMSO-treated controls, indicating parasites are likely not viable. Red scale bar represents 25 μm, grey scale bar represents 5 μm.

Contact Angles, Unpackaged

Treatment	15 Days	30 Days	60 Days	180 Days
Carboxy	10	10	11.3	10
Hydroxy	10.9	16.1	17.1	69.4
Active	60.8	62.8	60.1	65.6
Amine	62.7	67.6	69.9	77.1

Contact Angles, Air-Tight Packaging

Treatment	Initial		22 Days		75 Days	
Storage	RT	4°C	RT	4°C	RT	4°C
Carboxy	<10	<10	11.4	<10	<10	10.6
Hydroxy	<10	<10	11.3	<10	12.5	11.1
Active	15.4	15.4	47.1	48.2	65.3	51.6

Table S1. Water surface contact angle measurements with different surface treatments and storage conditions.