# A 3D culture model facilitates mass production of *in vitro Plasmodium falciparum* haemolymph-like sporozoites

Kiara Hatzakis[1], Zachary MacMillen[1], Payton Kirtley[2], Maya Aleshnick[2], Thomas Martinson[2], Priya Gupta[4], Kristian E. Swearingen[3], Brandon K. Wilder[2], James W. Davie[1], Marion Avril[1]

1. MalarVx, Inc., 1551 Eastlake Ave, E Suite 100, Seattle WA 98102, Seattle WA 98102

2. 505 NW 185th Ave, RM 2224, OHSU West Campus, Beaverton, OR 97006.

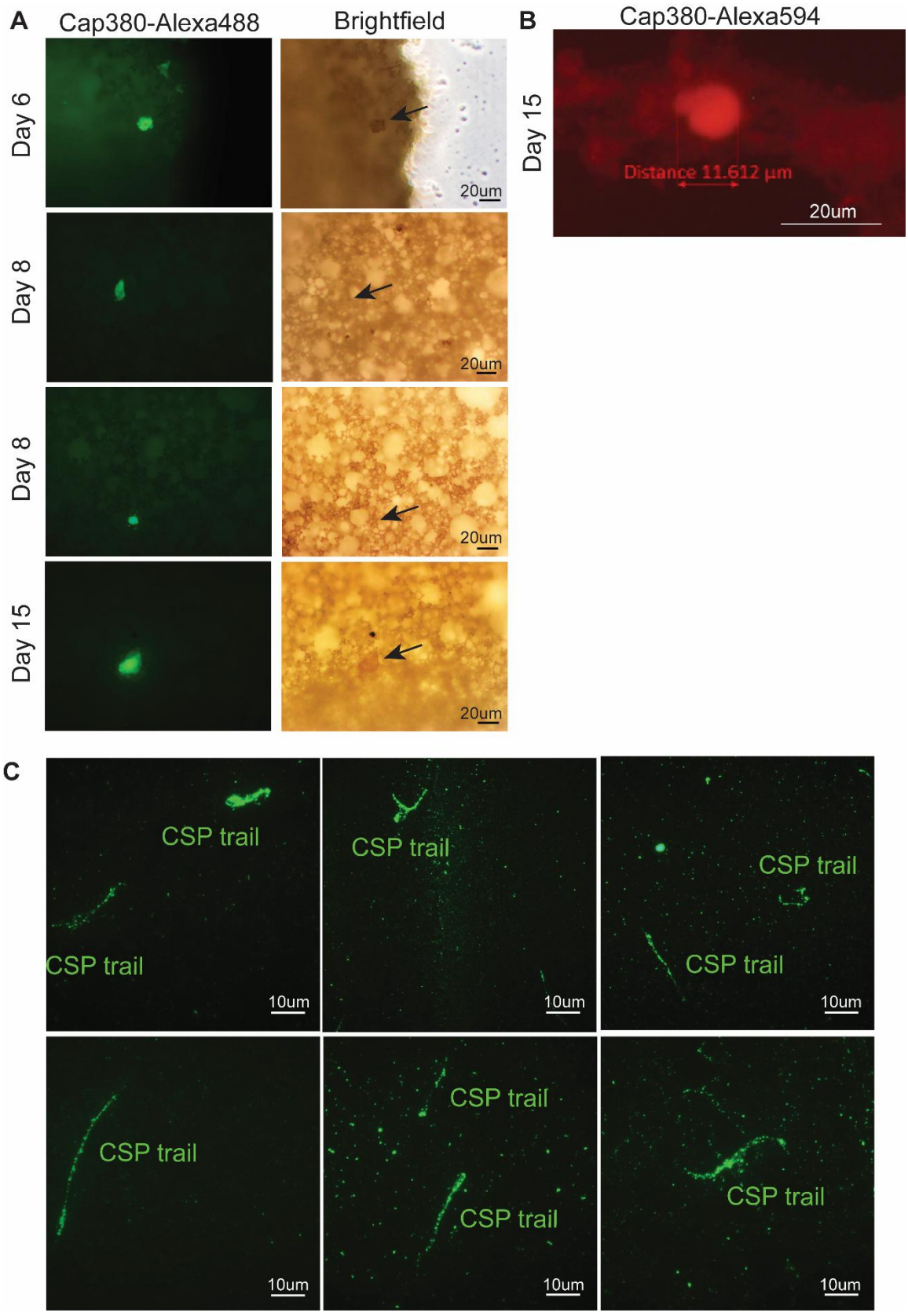
3. Institute for Systems Biology, Seattle, WA 98109.

4. Seattle Children's Research Institute, Seattle, WA 98109.

## ADDITIONAL FILES

**Additional File 1:** All datasets relating to the mass spectrometry analysis are available in the PRIDE repository (https://www.ebi.ac.uk/pride/login)

**Additional File 2: Alvetex Strata for oocyst growth and IVS gliding path.** A) IFA of PfNF54 GFP-Luc oocyst embedded in the Alvetex Strata. Strata were permeabilized and oocysts were detected by IFA on days 6, 8, and 15 with anti-Cap380 polyclonal rabbit antibodies with an anti-rabbit Alexa488-conjugated secondary. Field view at x40. B) Strata were permeabilized and oocysts were detected by IFA on day 15 with anti-Cap380 polyclonal rabbit antibodies with an anti-rabbit Alexa594 conjugated secondary. Field view at x100. C) Gliding assay of PfNF54 GFP-Luc IVS showing linear and curved CSP trails on coated slide with monoclonal Pf 2A10 antibodies.


A
Cap380-Alexa488
Brightfield
Day 6
Day 8
Day 8
Day 15
20um
B
Cap380-Alexa594
Day 15
Distance 11.612 µm
20um
C
CSP trail
10um

**Additional File 3: PfNF54 GFP-Luc exo-erythrocytic stages in HC04 cells**. PfNF54 GFP at an early liver stage were visualized live on confluent HC04 on day 0 to day 9 post-infection with IVS. Nuclei are stained with DAPI. Scale bar 10µm as indicated.