**Material and Methods**

**STACKS Device Design and Fabrication:**

The 96 well STACKS device was modified from the STACKS1 to increase throughput and compatibility with automated microscope imaging. The device was designed to fit within a Nunc Omniray and has a culture area of 3.14 mm2 and a working volume of 12.56 µL for each layer. The device was then fabricated through standardized rapid prototyping methods using Micro-CNC milling techniques to fabricate each layer from sheets of polystyrene (4 mm, Goodfellow) using Autodesk's Fusion 360 CAD software and a Tormach PCNC 770 Mill2. Following milling, the devices were cleaned using ultrasound sonication in isopropanol for 10 minutes to remove any debris or particles.

**Holder Fabrication:**

Custom holders were designed and fabricated using Fused Deposition Modeling (FDM) 3D printing on a 5th generation Makerbot, with white polylactic acid filament (Matterhackers) extruded using the Makerbot software's coarse setting. The holders function as an aligner for securing STACKS layers on top of each other and as spacers to prevent device contamination when placed on a benchtop. The CAD files and dimensions for the STACKS device and custom holder are included with the **Supporting Information.**

1. Yu J, Berthier E, Craig A, de Groot TE, Sparks S, Ingram PN, Jarrard DF, Huang W, Beebe DJ, Theberge AB. Reconfigurable open microfluidics for studying the spatiotemporal dynamics of paracrine signalling. Nat Biomed Eng. 2019 Oct;3(10):830-841. doi: 10.1038/s41551-019-0421-4. Epub 2019 Aug 19. PMID: 31427781; PMCID: PMC7543914.
2. Guckenberger DJ, de Groot TE, Wan AM, Beebe DJ, Young EW. Micromilling: a method for ultra-rapid prototyping of plastic microfluidic devices. Lab Chip. 2015 Jun 7;15(11):2364-78. doi: 10.1039/c5lc00234f. PMID: 25906246; PMCID: PMC4439323.