1. **Fluorescent microscope imaging of microtissues**
   1. To visualize HK in the microtissues, cells were first transduced with an adenoviral vector expressing GFP1 (Ad-eGFP - Vector Biolabs) by spinfection.
   2. Freshly thawed HK were mixed with Ad-GFP (MOI: 25) and centrifuged at 100 x *g*, 4 °C for 50 min.
   3. Cells were washed and then seeded into the MPS with PHH.
   4. Following culture scaffolds were fixed for 15 min in 4% paraformaldehyde and then washed with PBS before imaging using a Nikon Ti Eclipse fluorescence microscope with FITC filter.
2. **Pre-qualification assessment of cells**
   1. PHHs are validated in-house following a standardized protocol and are cultured in 3D under flow in LC12 plates for 5 days. Several functional hepatic biomarkers are assayed (LDH, urea, albumin and CYP3A4) at end of experiment, and thresholds set for each in part are:

LDH pass <2 × 106 cells

Urea pass >40g/106/day

Albumin >4 g/106 cells/day

CYP3A4 >1.5 pmol/106/min

* 1. HKCs cells are pre-validated in-house prior to use in experiments and are co-cultured with validated in 3D under flow in LC12 plates for 7 days PHHs. HKCs must have low levels of post-thaw activation; this is assessed by measuring biomarkers IL-6 and TNF-alpha. LPS stimulation is also performed; significant difference to stimulation is excepted between monoculture of PHHs and co-cultures.

**REFERENCES:**

1. Kostrzewski, T. et al*.* A microphysiological system for studying nonalcoholic steatohepatitis. *Hepatology Communications*. **4** (1), 77–91 (2020).