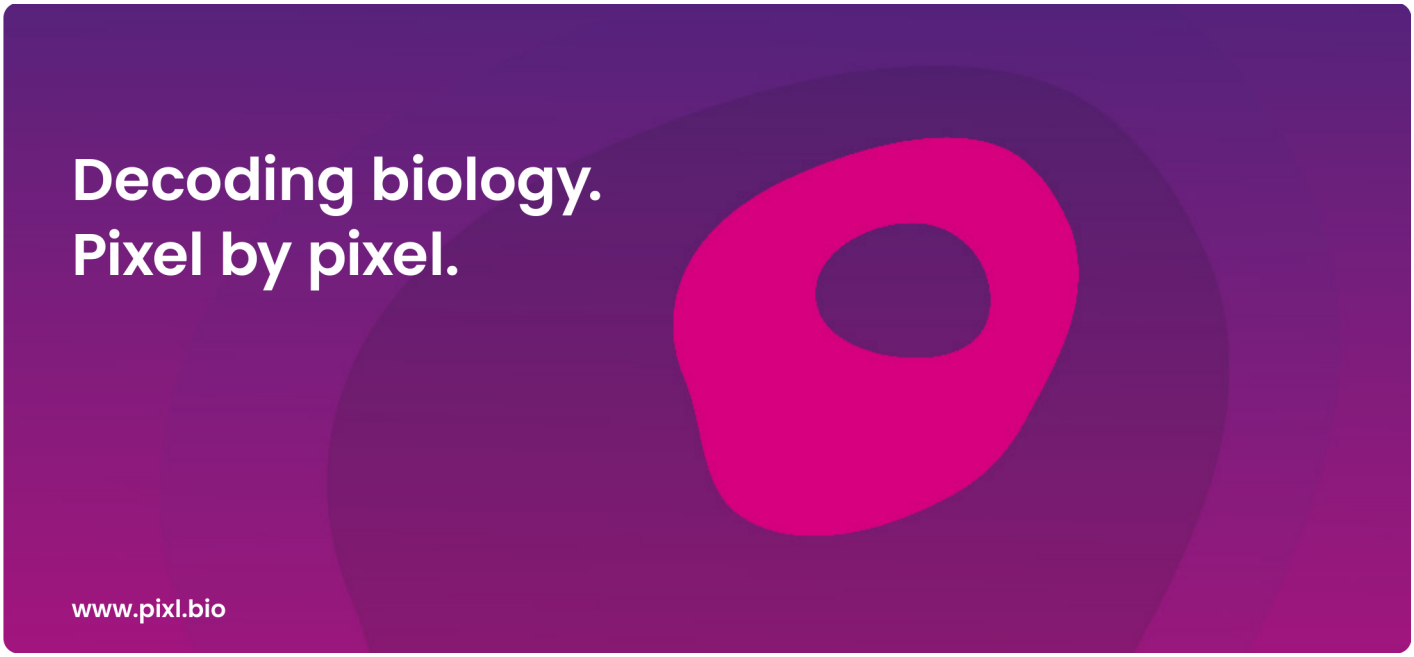
pixlbio



pixHep™: metabolically competent human hepatocytes for modelling MASLD progression

Metabolic dysfunction-associated steatotic liver disease (MASLD) is among the most prevalent chronic liver diseases worldwide, yet drug-discovery programmes remain constrained by the limited availability of models that accurately recapitulate human liver biology. Animal models often differ from humans in key metabolic and disease-related pathways; immortalised cell lines, such as HepG2, lack many features of mature hepatocyte function; additionally, primary human hepatocytes (PHH) are limited by donor-to-donor variability, restricted availability, and the rapid loss of phenotype during extended culture. An effective in vitro MASLD model must therefore be built on metabolically competent human hepatocytes and reproduce key stages of disease progression, from early steatosis and metabolic dysfunction to inflammatory and fibrogenic features associated with advanced metabolic dysfunction-associated steatohepatitis (MASH). At pixlbio, we evaluated pixHep™, our iPSC-derived hepatocyte platform, against these requirements by assessing hepatocyte maturity, lipid, and carbohydrate metabolism, insulin responsiveness, and its capacity to model both early- and advanced-stage MASLD. Advanced disease was modelled in a multicellular co-culture system combining pixHep™ with pixStellate™, our iPSC-derived hepatic stellate cells.

pixHep™ reach a mature, primary-like hepatocyte state

The quality of a metabolic disease model depends fundamentally on the phenotype and functional competence of its constituent cells. pixHep™ are generated using an optimised three-stage direct-differentiation platform and acquire key features of mature human hepatocytes at the transcriptional, protein and functional levels. Mature hepatocyte markers, including albumin, hepatocyte nuclear factor 4 alpha (HNF4A) and alpha-1 antitrypsin (A1AT), are strongly expressed, whereas alpha-fetoprotein (AFP), a marker associated with foetal or incompletely differen tiated hepatocytes, remains low. Immunofluorescence analysis confirms robust cytoplasmic albumin expression and nuclear localisation of HNF4A, with staining patterns comparable to those observed in primary human hep atocytes. Functional hepatocyte characteristics are also maintained, including albumin secretion and cell-surface localisation of the hepatocyte-specific asialoglycoprotein receptor 1 (ASGR1). Together, these findings demon strate that pixHep™ provide a mature and metabolically competent cellular foundation for modelling liver meta bolism and disease.

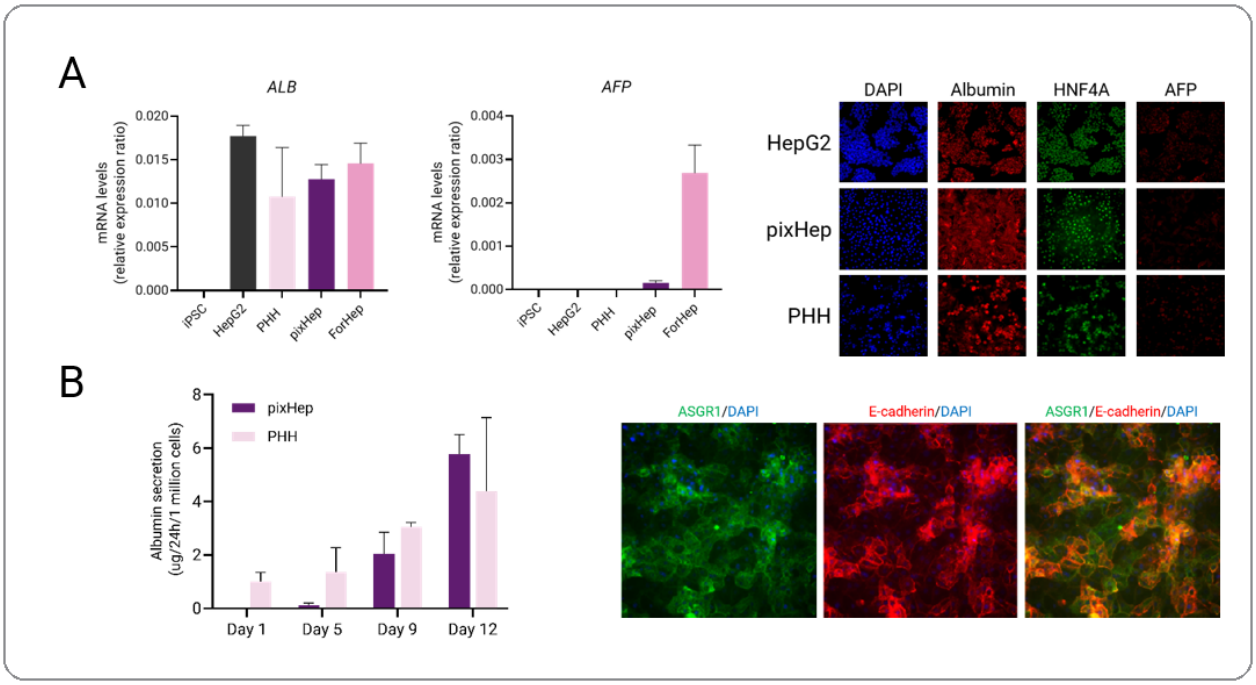


Figure 1. A) pixHep™ express the mature markers albumin and HNF4A at levels comparable to PHH, while the foetal marker AFP remains low. Immunofluorescence for DAPI (nuclei), albumin, HNF4A and AFP in HepG2, pixHep™, and PHH. **B)** pixHep™ secrete higher levels of albumin following 12 days of culture compared to PHH, in line with en hanced stability and function over time. **C)** pixHep™ demonstrate cell-surface localisation of the hepatocyte-spe cific asialoglycoprotein receptor 1 (ASGR1). PHH: Primary Human Hepatocytes; ForHep: Forward-reprogrammed iPSC-derived Hepatocytes

pixHep™ run the core metabolic programmes of the liver

Beyond hepatocyte identity and maturity, pixHep™ exhibit metabolic functions that are directly relevant to MASLD biology. Genome-wide transcriptional profiling demonstrated broad expression of genes involved in lipid metabol ism, carbohydrate metabolism, and insulin signalling across key metabolic pathways. This transcriptional profile was supported by functional activity: pixHep™ express glucose-6-phosphatase catalytic subunit (G6PC), which is further induced following metabolic stimulation, and perform de novo gluconeogenesis, releasing glucose in a regulated and substrate-dependent manner. The combination of a mature metabolic gene-expression profile and measurable metabolic output enables pixHep™ to respond appropriately to metabolic perturbation, providing a physiologically relevant foundation for inducible MASLD modelling.

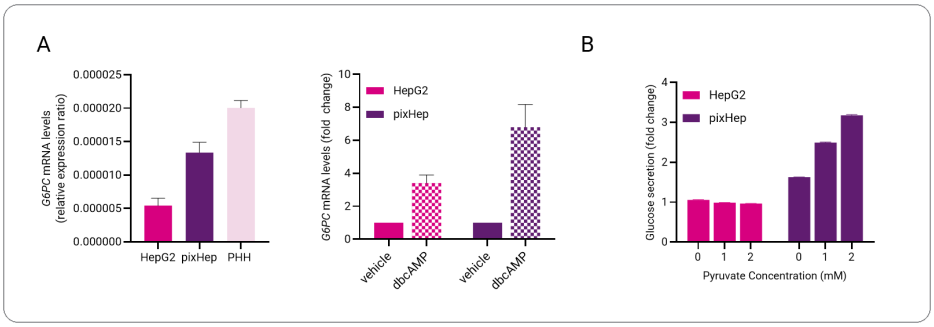


Figure 2. A) G6PC mRNA expression across HepG2, pixHep™ and PHH, and induction of G6PC on stimulation. **B)** de novo gluconeogenesis - glucose secretion by pixHep™ increases with substrate (pyruvate) concentration, confirming functional, regulated hepatic glucose production.

Table 1. Hepatocyte competency summary. pixHep™ reproduce the identity and metabolic profile of primary human hepatocytes.

Endpoint	pixHep™	Benchmark (PHH)
Albumin (identity)	Expressed	Comparable
HNF4A (identity)	Expressed	Comparable
A1AT (identity)	Expressed	Comparable
AFP (foetal marker)	Low	Low
Metabolic gene expression	Lipid / carb / insulin pathways	Concordant
De novo gluconeogenesis	Functional & inducible	Retained

Modelling early MASLD: steatosis, insulin resistance and inflammation

The metabolic competence of pixHep™ enables them to respond to lipotoxic and glucotoxic stress by developing key features associated with early-stage MASLD. Exposure to elevated fatty acids and glucose induces dose-dependent intracellular lipid accumulation, consistent with hepatocellular steatosis, as demonstrated by lipid staining. This is accompanied by impaired insulin signalling, evidenced by reduced AKT phosphorylation following insulin stimulation, together with an inflammatory response characterised by increased expression of pro-inflammatory cytokines, including IL-1β, IL-6, IL-8 and TNF-α. Collectively, these responses reproduce three important features of early MASLD: steatosis, insulin resistance and inflammation, within a single, human-relevant and quantifiable in vitro system suitable for mechanistic studies and compound screening.

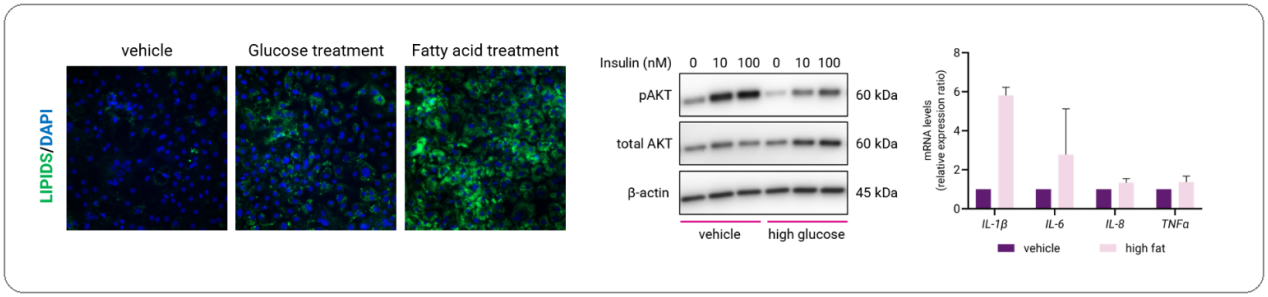


Figure 3. Lipid staining (LIPIDS / DAPI) in pixHep™ under vehicle, glucose and fatty-acid treatment. High-glucose-treated pixHep™ show reduced insulin-stimulated AKT phosphorylation (10–100 nM), indicating impaired insulin signalling. Intracellular lipid accumulation increases increases expression of IL-1β, IL-6, IL-8 and TNF-α, recapitulating lipotoxicity, a key feature of hepatic steatosis.

pixStellate™ add the fibrogenic cell biology of advanced-stage disease

Progression to advanced MASLD is characterised by the development of hepatic fibrosis, driven primarily by the activation of hepatic stellate cells. pixlbio's iPSC-derived hepatic stellate cells, pixStellate™, provide this essential cellular component of the disease model. They express established markers of hepatic stellate cell identity and recapitulate the transition from a quiescent to an activated, fibrogenic phenotype. In their quiescent state, pixStellate™ retain vitamin A within characteristic retinoid-containing lipid droplets, whereas activation promotes the production and deposition of collagen and other extracellular-matrix components that contribute to hepatic scarring. Incorporating pixStellate™ into the platform therefore extends the model beyond hepatocyte-intrinsic meta-

bolic dysfunction, enabling investigation of the multicellular and fibrogenic processes associated with progressive MASH.

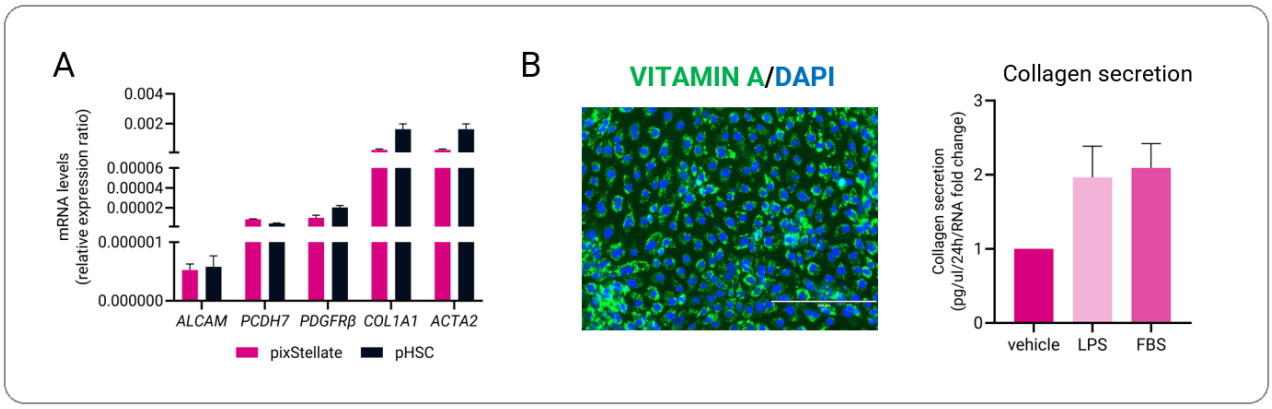


Figure 4. A) mRNA expression of Hepatic Stellate Cell (HSC) markers in 2-week differentiated pixStellate™ and primary HSCs. **B)** vitamin A / DAPI staining in quiescent pixStellate™, showing retinoid storage characteristic of resting hepatic stellate cells. Collagen secretion increases on stellate-cell activation, the hallmark pro-fibrotic response.

Modelling late MASLD / MASH in multicellular co-culture

Co-culture of pixHep™ with pixStellate™ generates a multicellular model of MASH in which disease-associated phenotypes are induced through a defined metabolic and inflammatory challenge. The system captures key features of progressive disease, including hepatocellular lipid accumulation, inflammatory cytokine induction and increased collagen production. Importantly, these phenotypes are pharmacologically responsive, with treatment reducing both steatotic and fibrogenic readouts. The model therefore supports not only mechanistic investigation of MASH progression, but also quantitative assessment of candidate therapeutic interventions and their ability to ameliorate disease-associated phenotypes.

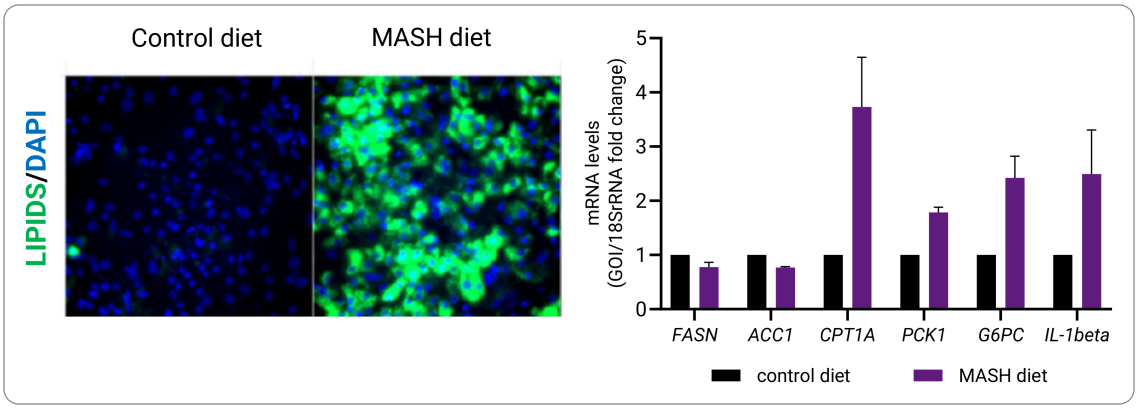


Figure 5. Hepatocyte/stellate-cell co-culture under control and MASH-inducing conditions. The MASH diet increases lipid accumulation and alters metabolic and inflammatory gene expression, consistent with a MASH-like phenotype.

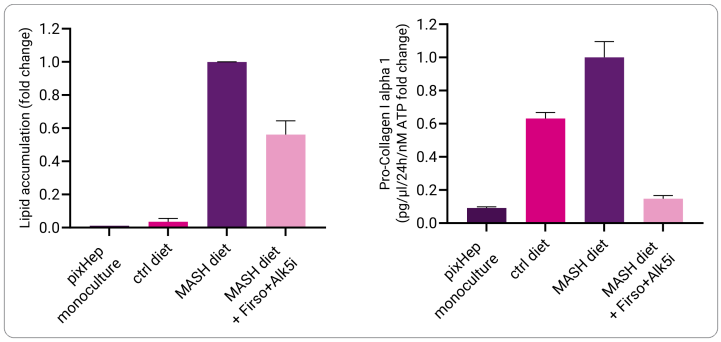


Figure 6. Reversibility of disease endpoints in co-culture: lipid accumulation (left) and collagen secretion (right) are elevated under the MASH diet and reduced by pharmacological treatment, demonstrating a measurable, reversible efficacy readout.

Table 2. MASLD disease features modelled across the pixHep™ and pixStellate™ platform, from early steatosis to reversible, late-stage MASH.

MASLD feature	How it is modelled	Result
Steatosis	Fatty-acid / glucose challenge	Lipid accumulation ↑
Insulin resistance	Insulin stimulation ± lipotoxic stress	AKT phosphorylation ↓
Inflammation	Lipotoxic challenge	IL-1β, IL-6, IL-8, TNFα ↑
Stellate activation / fibrosis	pixStellate™ activation	Collagen secretion ↑
Late-stage MASH	pixHep™ + pixStellate™ co-culture, MASH diet	Full phenotype recapitulated
Pharmacological reversibility	Compound treatment in co-culture	Steatosis & fibrosis reversed ✓

Why metabolic competency matters

Together, these findings demonstrate that our pixHep™ cells model key stages of MASLD progression rather than lipid accumulation alone. Their mature metabolic gene-expression profile and functional gluconeogenic capacity provide a physiologically relevant foundation, while metabolic and lipotoxic challenge induces steatosis, impaired insulin signalling and inflammatory responses. Co-culture with pixStellate™ further enables modelling of multicellular and fibrogenic features associated with progressive MASH, including pharmacologically responsive disease endpoints. As a consistent and scalable iPSC-derived platform - available as wild-type, disease-associated and CRISPR-edited lines, including MASLD risk variants such as PNPLA3 and TM6SF2 - the system supports target identification, mechanism-of-action studies, and efficacy and safety assessment across key stages of MASLD.

Conclusions

pixHep™ are metabolically competent human hepatocytes that reproduce key features of MASLD in vitro. Their mature hepatocyte phenotype and functional lipid, carbohydrate and insulin metabolism enable induction of early disease-associated features, including steatosis, impaired insulin signalling and inflammation. When co-cultured with pixStellate™, the platform extends to multicellular and fibrogenic features of progressive MASH, with quantifiable and pharmacologically responsive endpoints. Together, pixHep™ and pixStellate™ provide a scalable, human-relevant system for metabolic-disease research, toxicology and liver modelling across key stages of MASLD progression.

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