pixlbio

CASE STUDY

Decoding biology.
Pixel by pixel.

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Modeling Urea cycle disorders through CRISPR-derived OTCD pixHep

Urea cycle disorders (UCDs) are a group of liver metabolic disorders caused by defects in the enzymes responsible for detoxifying nitrogen waste into urea. Deficiency in any of these enzymes leads to ammonia accumulation and is associated with symptoms ranging from developmental delay and cerebral oedema to coma and, ultimately, death. Among UCDs, Ornithine Transcarbamylase Deficiency (OTCD) and Citrullinemia Type 1 (ASS1 deficiency) are the most common. However, due to the lack of suitable in vitro disease models for drug efficacy screening, no licensed treatments are currently available. At pixlbio, we have developed an iPSC-derived UCD model to enable the study and identification of novel treatments.

Advantages
Functional urea cycle pathway with high expression levels of all urea cycle enzymes
Disease circuit verified carrying the D175V or G390R mutations in the OTC and ASS1 genes, respectively
Optimized bioassays measuring protein expression and urea secretion as endpoint assays in pixHep
Suitable *in vitro* platforms for screening of compound and gene therapy systems
Standardized cell products containing iPSC-derived human hepatocytes generating reproducible and biologically relevant data

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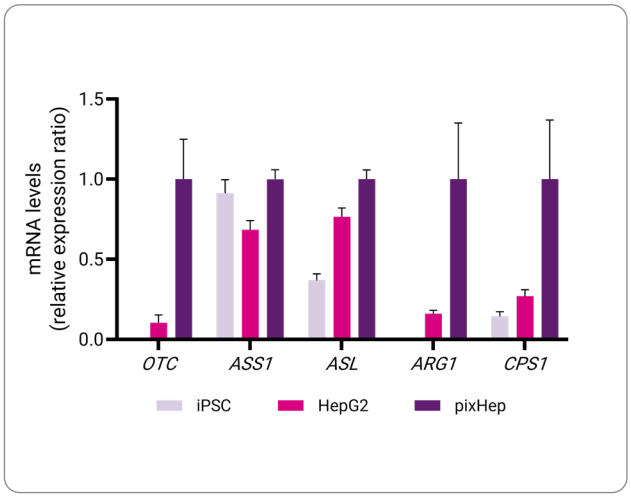
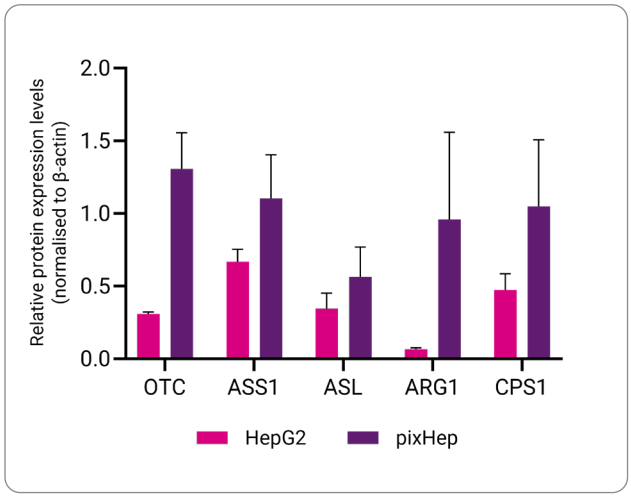
pixlbio pixHep demonstrate a functional urea cycle pathway





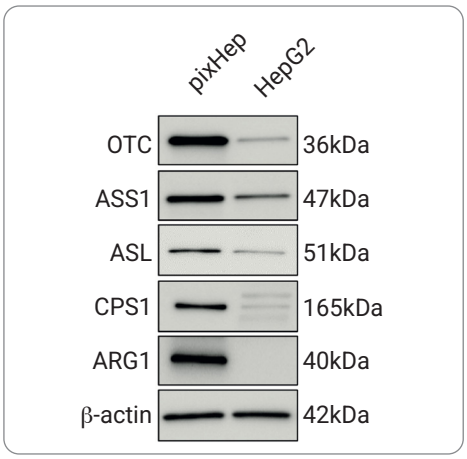
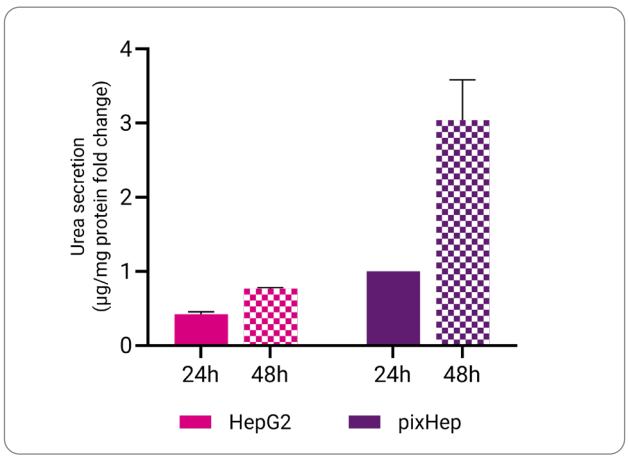


Figure 1: mRNA expression levels of the five urea cycle genes in wild-type induced pluripotent stem cells (iPSC), liver carcinoma HepG2 cells, and pixHeps (upper left). Protein expression levels of the five urea cycle enzymes in liver carcinoma HepG2 cells and pixHeps (upper right and lower left). mRNA data were normalized to 18S rRNA and are presented as mean±SEM of n=3 biological replicates. Protein data were normalized to β-actin and are presented as mean±SEM of n=3 biological replicates.

pixlbio secrete urea in a time-dependent manner and respond to urea cycle substrates



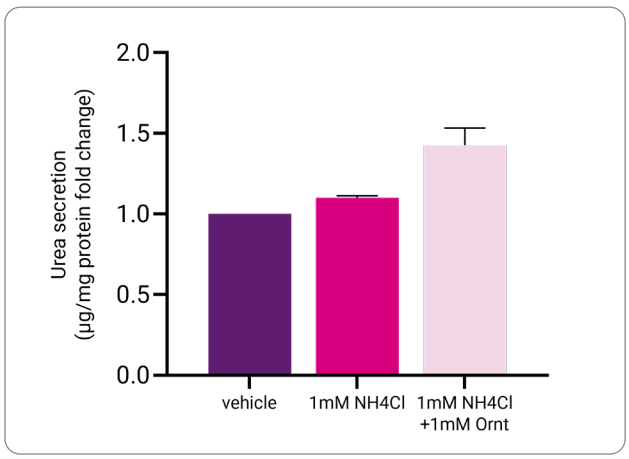


Figure 2: 24- and 48-hour urea secretion levels in liver carcinoma HepG2 cells and pixHeps (left). Urea secretion levels in pixHeps cultured in the presence of either vehicle, 1mM NH4Cl, or 1mM NH4Cl +1mM Ornithine for 24 hours, suggestive of functional OTC activity (right). Urea secretion data were normalized to total cell number and are presented as mean±SEM fold change of n=3 biological replicates.

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pixlbio CRISPR-derived OTCD iPSCs carry the D175V mutation without any effect in pluripotency status

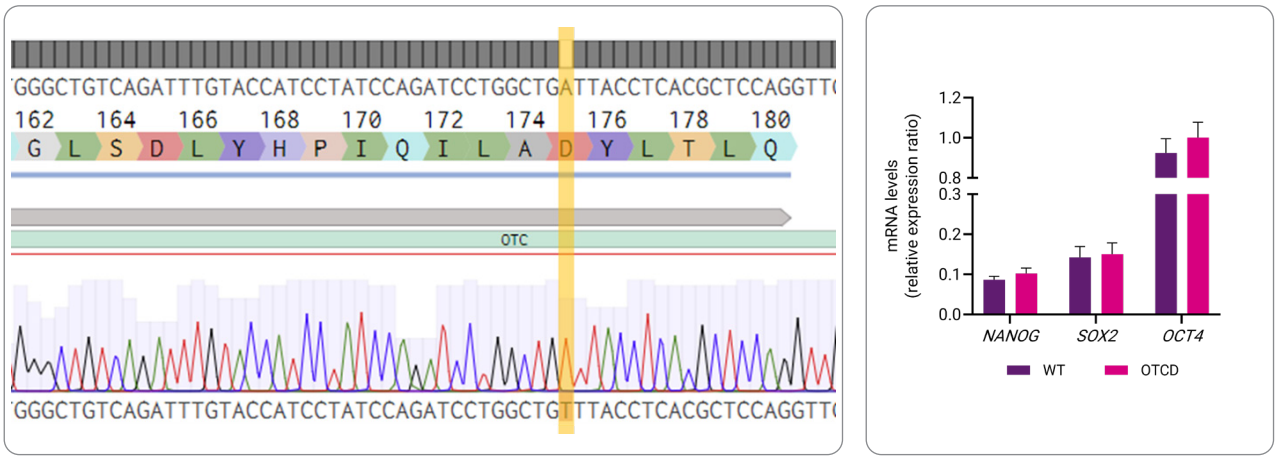


Figure 3: Sanger sequencing showing wild-type (top sequence) and mutated iPSCs (bottom sequence) carrying the D175V mutation (GAT>GTT) in the OTC gene. The codon change is highlighted with yellow (left). mRNA expression levels of the key pluripotency markers NANOG, SOX2, and OCT4 in wild-type (WT) and CRISPR-derived OTCD pixHeps. mRNA data were normalized to GAPDH and are presented as mean±SEM of n=3 biological replicates.

CRISPR-derived OTCD iPSCs successfully differentiate to pixHep

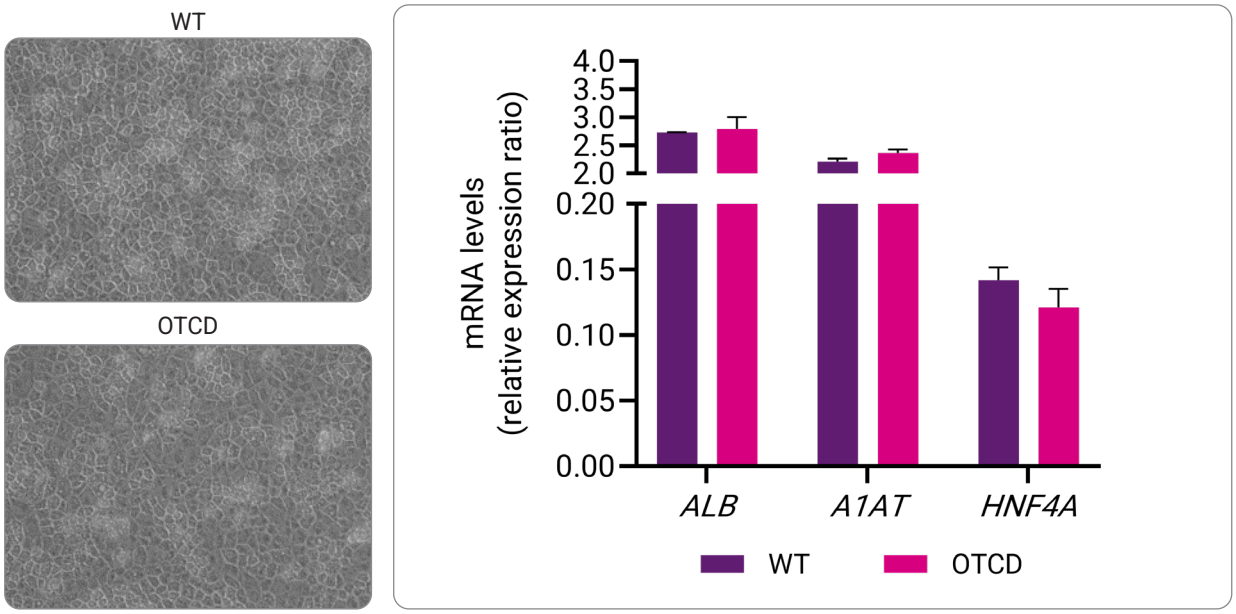
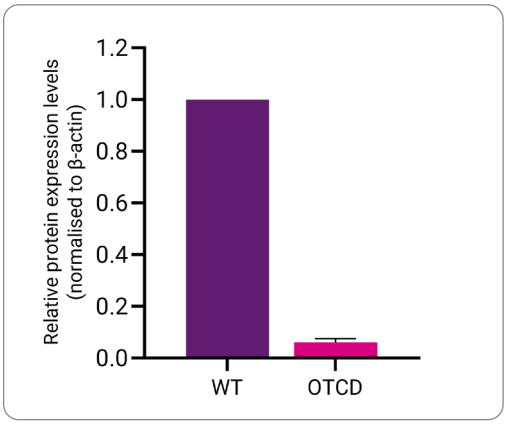
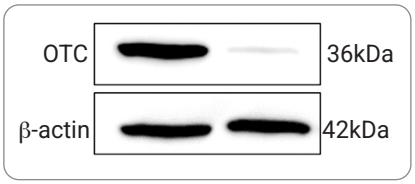


Figure 4: Representative images demonstrating the characteristic hepatocyte cobblestone morphology in wild-type (WT) and OTCD pixHep (left). mRNA expression levels of the hepatocyte maturity markers albumin (ALB), alpha-1-antitrypsin (A1AT), and hepatocyte nuclear factor 4A (HNF4A) in wild-type (WT) and OTCD pixHep (right). mRNA data were normalized to PPIA and are presented as mean±SEM of n=2 biological replicates. Magnification: 10x.

pixlbio CRISPR-derived OTCD pixHep demonstrate decreased OTC protein expression and urea secretion compared to wild-type pixHep





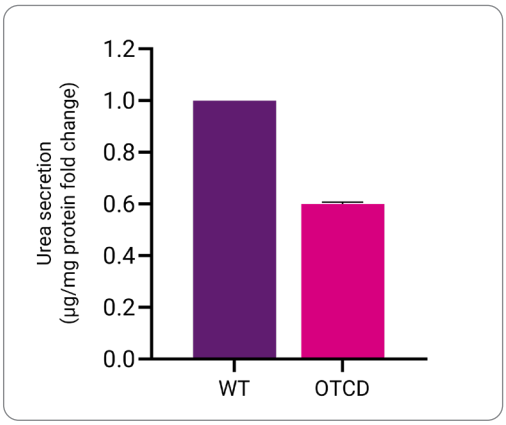
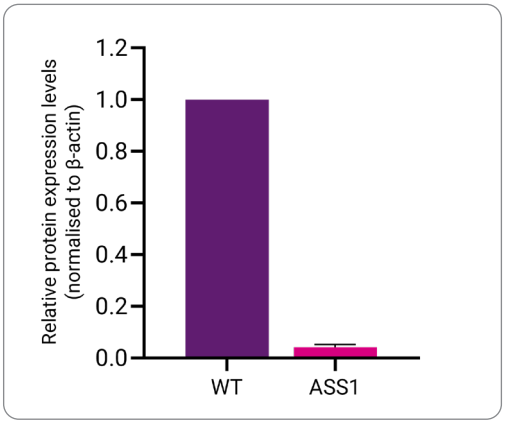
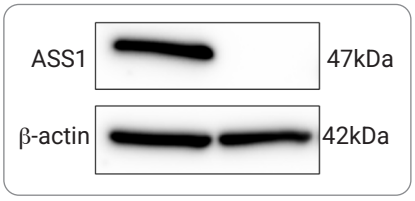


Figure 5: Protein expression levels of OTC in wild-type (WT) and CRISPR-derived OTCD pixHep (upper left and upper right). 48-hour urea secretion levels in wild-type (WT) and CRISPR-derived OTCD pixHep (lower left). Protein data were normalized to β-actin and are presented as mean±SEM of n=3 biological replicates. Urea secretion data were normalized to total cell number and are presented as mean±SEM fold change of n=2 biological replicates.

pixlbio CRISPR-derived ASS1 deficiency pixHep demonstrate decreased ASS1 protein expression compared to wild-type pixHep





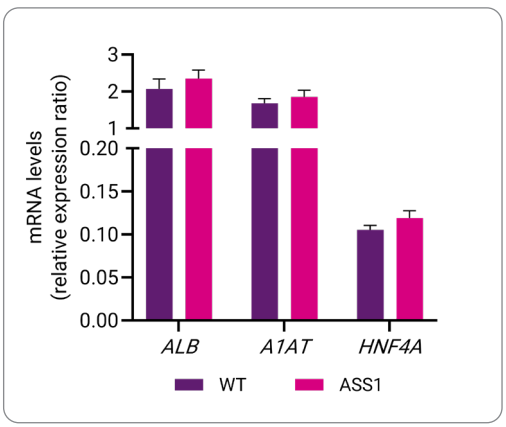


Figure 6: Protein expression levels of ASS1 in wild-type (WT) and CRISPR-derived ASS1 pixHep (upper left and right).mRNA expression levels of the hepatocyte maturity markers albumin (ALB), alpha-1-antitrypsin (A1AT), and hepatocyte nuclear factor 4A (HNF4A) in wild-type (WT) and ASS1 pixHep (lower left). mRNA data were normalized to PPIA and are presented as mean±SEM of n=3 biological replicates. Protein data were normalized to β-actin and are presented as mean±SEM of n=3 biological replicates.

pixlbio CRISPR-derived ASS1 iPSCs carry the G390R mutation without any effect in pluripotency status

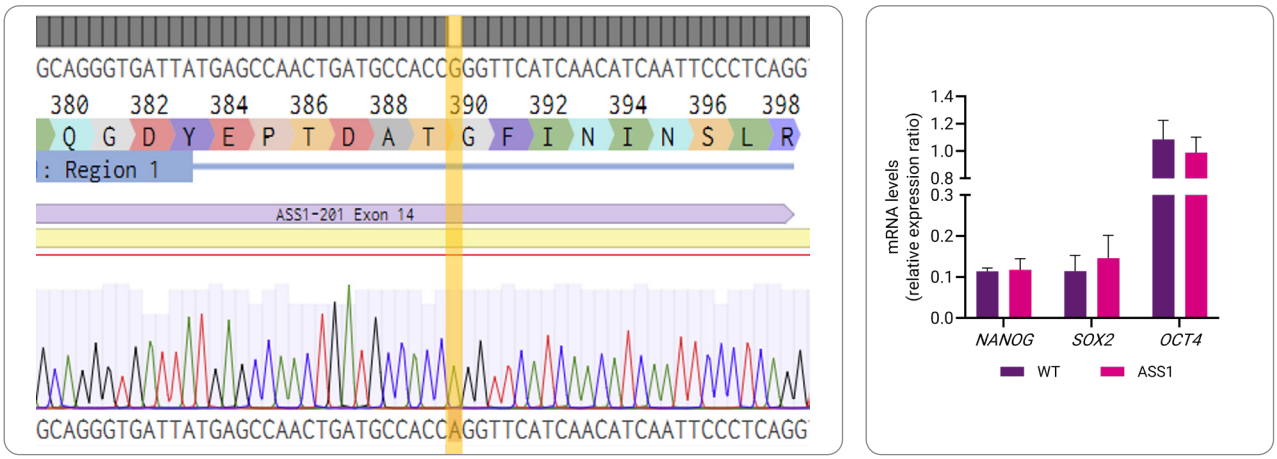


Figure 7: Sanger sequencing showing wild-type (top sequence) as well as mutated iPSCs (bottom sequence) carrying the G390R mutation (GGG>AGG) in the ASS1 gene. The codon change is highlighted with yellow (left). mRNA expression levels of the key pluripotency markers NANOG, SOX2, and OCT4 in wild-type (WT) and CRISPR-derived ASS1 pixHep (right). mRNA data were normalized to GAPDH and are presented as mean±SEM of n=3 biological replicates.

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