A validated, transitional and translational porcine model of hepatocellular carcinoma

SUPPLEMENTARY MATERIALS

Supplementary Figure 1: Linear fit plot of SC xenografted tumor growth. Scatter plot of SC xenografted tumor growth indicative of relatively linear growth kinetics. A line fit plot is a scatter plot for the actual data points along with the fitted regression line.
Supplementary Figure 2: Reproducibly of pHCC gene expression profiles. Each panel shows the Pearson’s correlation of gene expression profiles between: (A) pPH1 and pPH2 cells; (B) pPH1 and pPH3 cells; (C) pPH2 and pPH3 cells; (D) pHCC1 and pHCC2 cell lines; (E) pHCC1 and pHCC3 cell lines; (F) pHCC2 and pHCC3 cell lines; (G) pPH1 cells and pHCC1 cell lines; (H) pPH2 cells and pHCC2 cell lines; (I) pPH3 cells and pHCC3 cell lines. Each blue dot represents an individual gene.

Supplementary Table 1: Elevated pHCC cell lines gene expression relative to pPH cells.

See Supplementary File 1

Supplementary Table 2: Reduced pHCC cell line gene expression relative to pPH cells.

See Supplementary File 1
**Supplementary Table 3: Master regulators of genes with increased expression in Oncopig pHCC cell lines**

| Transcription factors | Number of upregulated target genes |
|-----------------------|-----------------------------------|
| *KLF4*                | 1,217                             |
| *MZFL1*               | 927                               |
| *AGGF1*               | 767                               |
| *ZNF503*              | 751                               |
| *ZCCHC14*             | 734                               |
| *NFE2L1*              | 409                               |

The number of target genes with elevated expression for each transcription factor is indicated for each cell line.

**Supplementary Table 4: Master regulators of genes with increased expression across 18 human HCC cell lines.**

See Supplementary File 1
Supplementary Table 5: Validation of RNA-seq results

| Gene symbol | qRT-PCR (log2 FC) | RNA-seq (log2 FC) |
|-------------|-------------------|-------------------|
| **ADAM8**   | 2.326             | 4.991             |
| **FZD7**    | 1.167             | 2.341             |
| **HIF1A**   | 1.421             | 2.601             |
| **NFE2L3**  | 1.697             | 2.977             |
| **NR4A2**   | 1.416             | 2.726             |
| **SDCBP2**  | 2.395             | 5.539             |
| **BHMT**    | -5.225            | -5.177            |
| **A1BG**    | -5.373            | -5.511            |
| **KLK3**    | -4.209            | -3.794            |
| **MST1**    | -4.053            | -4.174            |
| **ACKR1**   | -4.407            | -4.194            |
| **F2**      | -4.823            | -4.764            |
| **UBD**     | -5.264            | -5.282            |
| **GATM**    | -3.958            | -3.980            |
| **NKG7**    | -5.024            | -5.015            |
| **EXTL1**   | -3.921            | -4.045            |

Genes displaying increased (*ADAM8, FZD7, HIF1A, NFE2L3, NR4A2, SDCBP2*) and reduced expression (*BHMT, A1BG, KLK3, MST1, ACKR1, F2, UBD, GATM, NKG7, EXTL1*) in pHCC cell lines based on RNA-seq analysis. Relative transcript levels (qRT-PCR (log2 FC) fold change) under two different conditions was determined by qRT-PCR by measuring Ct values of each gene in the pHCC cell lines and the pPH cells and that of a housekeeping gene, *GAPDH*. The difference between the Ct value of a gene for pHCC cell lines and pPH cell lines was normalized to the Ct value of *GAPDH* ($\Delta\Delta$Ct) and expressed as $2^{\Delta\Delta\text{Ct}}$ to give the log2 FC. RNA-seq (log2 FC) is the ratio of the transcript levels (fold change) expressed as FPKM of a gene under two different conditions as determined by RNA-seq analysis and converted to logarithm base 2.

Supplementary Table 6: Sequences of qPCR primers used.

See Supplementary File 1

Supplementary Table 7: RNA-seq sequencing depths for each cell line

| Oncopig | Cells               | Number of reads |
|---------|---------------------|-----------------|
| 1       | pH cells            | 28, 385, 664    |
|         | pHCC cell lines     | 31, 047, 086    |
| 2       | pH cells            | 30, 286, 514    |
|         | pHCC cell lines     | 32, 296, 259    |
| 3       | pH cells            | 23, 391, 879    |
|         | pHCC cell lines     | 29, 239, 571    |