# Expanded View Figures

**Figure EV1. iHCC derived from PHHs by oncogenic candidate transduction.**

A Commercial cryopreserved PHHs (PHH1, PHH4, and PHH5) were seeded in 6-well plates and immunoassayed with antibodies that recognize ALB and KRT19. Scale bars, 20 μm.

B Representative FACS analysis of PHHs that were transduced with a cocktail of 10 OC lentiviruses (right) or mock lentivirus (left).

C qPCR assays measuring the transduction efficiencies of individual oncogenes using the genomic DNA samples of OC-transduced PHHs. Relative copy numbers of DNA of oncogenes to the DNA of GFP. Data are presented as the mean ± SD ($n = 3$). *N*-numbers refer to biological replicates.

D The levels of albumin secretion in PHHs and OC-transduced PHHs were measured by ELISA ($n = 3$), $P = 0.0378$ with unpaired *t*-test, $^{*}P \leq 0.05$. Data are presented as the mean ± SD. *N*-numbers refer to biological replicates.

E The levels of APOA1 secretion in PHHs and OC-transduced PHHs were measured by ELISA ($n = 3$), $P > 0.05$ with unpaired *t*-test, data are presented as the mean ± SD, N.S. indicates non-statistical significance. *N*-numbers refer to biological replicates.

F Glycogen storage was quantified by colorimetric in PHHs and OC-transduced PHHs ($n = 3$ ), $P > 0.05$ with unpaired *t*-test, data are presented as the mean ± SD. N.S. indicates non-statistical significance. *N*-numbers refer to biological replicates.

G qPCR results of expression levels of hepatic genes in PHHs, OC-transduced PHHs, and HepG2 cells on day 2 post-transduction in culture ($n = 3$), data are presented as the mean ± SD. *N*-numbers refer to biological replicates.

H The expression levels of endogenous (WT) and mutant (Mut) TP53 in OC-transduced PHHs ($n = 5$, $P < 0.0001$ with one-way ANOVA by Tukey's multiple comparison test), $^{***}P \leq 0.001$, data are presented as the mean ± SD. *N*-numbers refer to biological replicates.

I Mice with advanced intrahepatic tumor exhibited swollen and ascites-containing abdomens, allowing detection of tumor burden by palpation. Tumors were pointed with white arrows.

J–L Primary iHCC samples were harvested from mice from the OC group and were transplanted. $1 \times 10^6$ iHCC cells were subjected for serial transplantations into NSI mice subcutaneously or splenically. Tumors were reconstituted post-three rounds of subcutaneous transplantations of iHCC (J); Bright-field and EGFP imaging of an explanted liver from a tumor-bearing NSI mouse post splenic injection of iHCC cells. Scale bars, 0.5 cm (K); The anti-GFP immunofluorescence and H&E staining of tumor dissection from a tumor-bearing NSI mouse post splenic transplantation of iHCC. Scale bars, 20 μm (L).

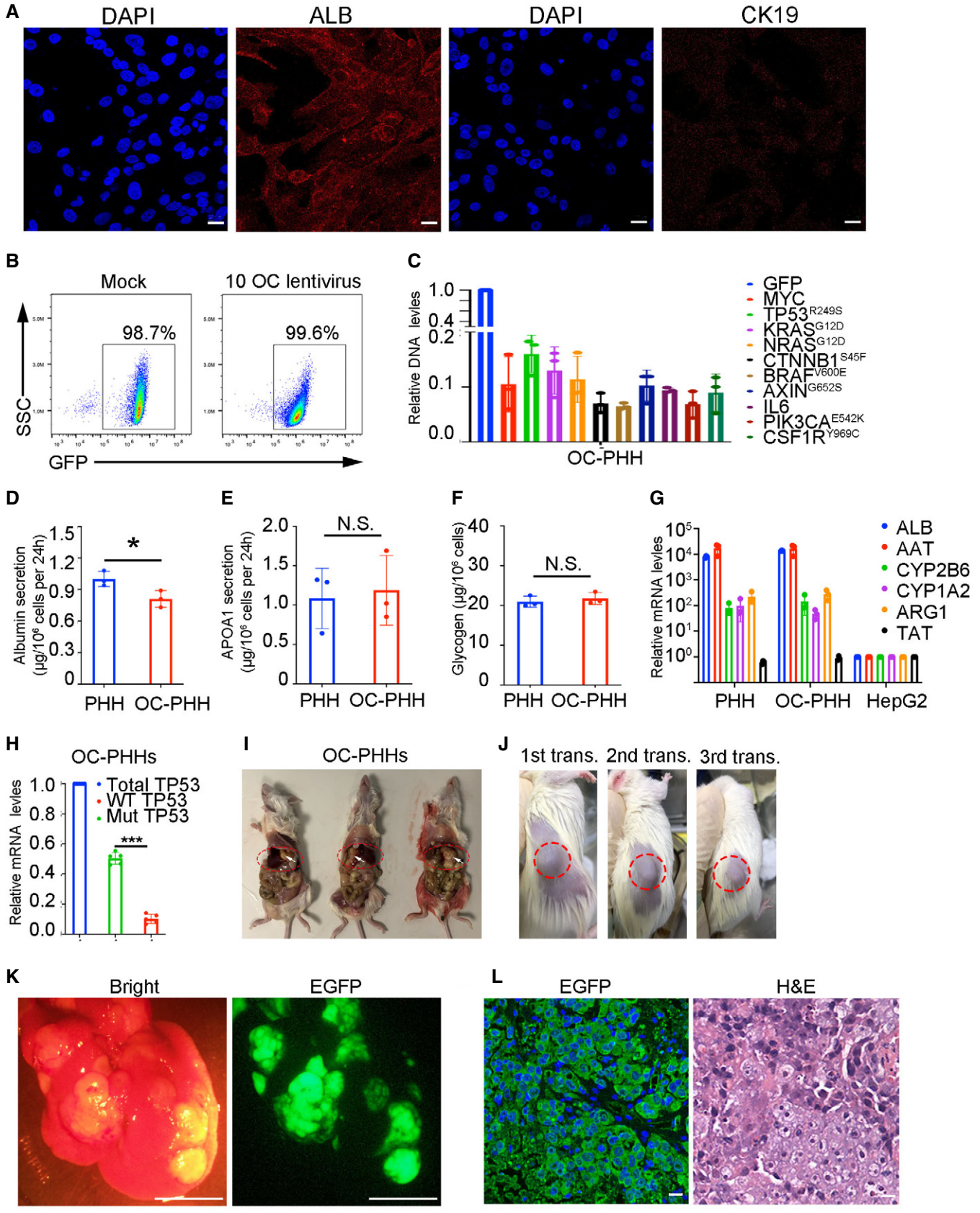

A
DAPI
ALB
DAPI
CK19
B
Mock
10 OC lentivirus
98.7%
99.6%
SSC
GFP
C
Relative DNA levles
1.0
0.4
0.2
0.1
0.0
OC-PHH
GFP
MYC
TP53R249S
KRASG12D
NRASG12D
CTNNB1S45F
BRAFV600E
AXING652S
IL6
PIK3CAE542K
CSF1RY969C
D
Albumin secretion (μg/10⁶ cells per 24h)
1.5
1.2
0.9
0.6
0.3
0
*
PHH OC-PHH
E
APOA1 secretion (μg/10⁶ cells per 24h)
2.0
1.5
1.0
0.5
0
N.S.
PHH OC-PHH
F
Glycogen (μg/10⁶ cells)
40
30
20
10
0
N.S.
PHH OC-PHH
G
Relative mRNA levles
10⁵
10⁴
10³
10²
10¹
10⁰
PHH OC-PHH HepG2
ALB
AAT
CYP2B6
CYP1A2
ARG1
TAT
H
OC-PHHs
Relative mRNA levles
1.0
0.8
0.6
0.4
0.2
0.0
Total TP53
WT TP53
Mut TP53
***
I
OC-PHHs
J
1st trans.
2nd trans.
3rd trans.
K
Bright
EGFP
L
EGFP
H&E

**Figure EV1.**

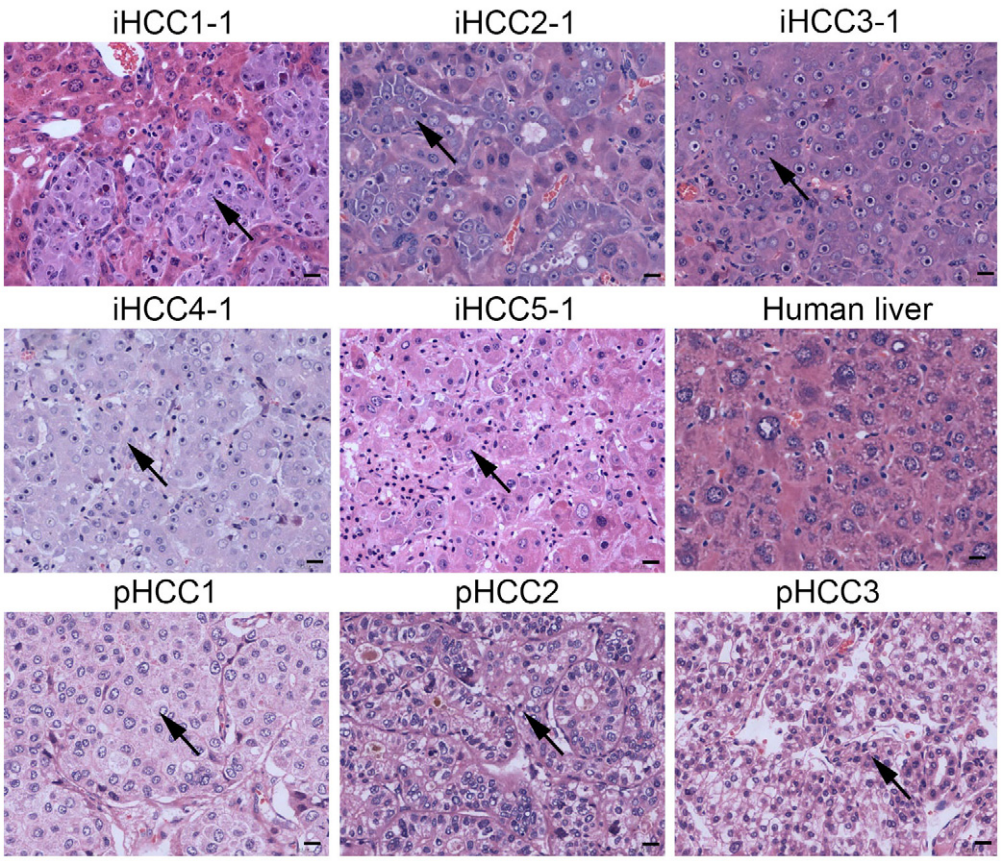



**Figure EV2. Morphologic characteristics of iHCC.**

Representative H&E-stained sections of iHCC tissues (iHCC1-5) from tumor-bearing mice transplanted with PHHs from 5 different donors (PHH1~5) transduced with the combination of *MYC*, *TP53*$^{R249S}$, and *KRAS*$^{G12D}$ overexpression lentiviruses, normal human liver tissue (Human liver), and primary HCC tissues from three different patients (pHCC1~3). Tumor cells are pointed with black arrows. Scale bar, 20 μm.

**Figure EV3. iHCC samples express HCC markers.**

A Representative IHC images of four iHCC samples (iHCC1-1, iHCC2-1, iHCC3-1, and iHCC4-1) from NSIF mice transplanted with MTK-transduced PHHs from four different PHH donors (PHH1~4) and an HCC tissue from a patient (pHCC2). Anti-HLA-ABC, anti-ALB, anti-AFP, anti-GPC3 and anti-KRT19 antibodies were used. Scale bars, 20 μm.

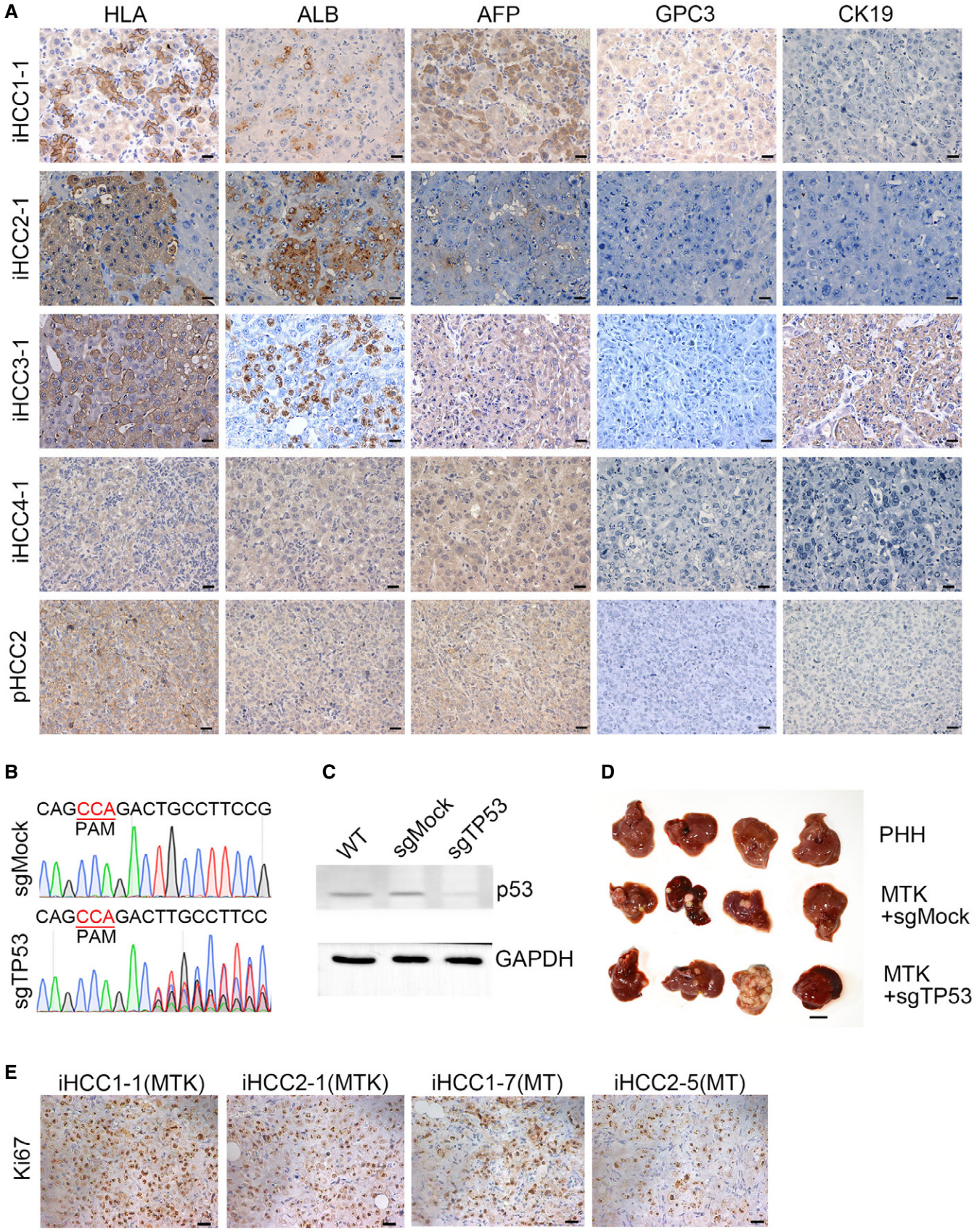
B DNA sequencing confirmed mutations of TP53 in the genomic DNA of sgTP53 transduced PHH cells.

C Expression of p53 was detected in the PHH cells transduced with sgMock or sgTP53 by Western blotting.

D Representative images of in situ liver carcinomas derived from PHHs transduced with a combination of MTK with or without deletion of TP53 in NSIF mice. 3 out of 4 mice harbored tumor in both MTK+ sgMock and MTK+ sgTP53 groups after 11 weeks ($n$ = 4 per group). *N*-numbers refer to biological replicates.

E Representative IHC images Ki67 staining of liver sections from NSIF mice transplanted with PHHs that were transduced with the combinations of *MYC*/*TP53*$^{R249S}$/*KRAS*$^{G12D}$ (MTK) or *MYC*/*TP53*$^{R249S}$ (MT) lentiviruses. Scale bars, 20 μm.

Source data are available online for this figure.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HLA
ALB
AFP
GPC3
CK19
iHCC1-1
iHCC2-1
iHCC3-1
iHCC4-1
pHCC2
B
sgMock
CAGCCAGACTGCCTTCCG
PAM
sgTP53
CAGCCAGACTTGCCTTCC
PAM
C
WT
sgMock
sgTP53
p53
GAPDH
D
PHH
MTK +sgMock
MTK +sgTP53
E
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iHCC2-1(MTK)
iHCC1-7(MT)
iHCC2-5(MT)
Ki67

**Figure EV3.**

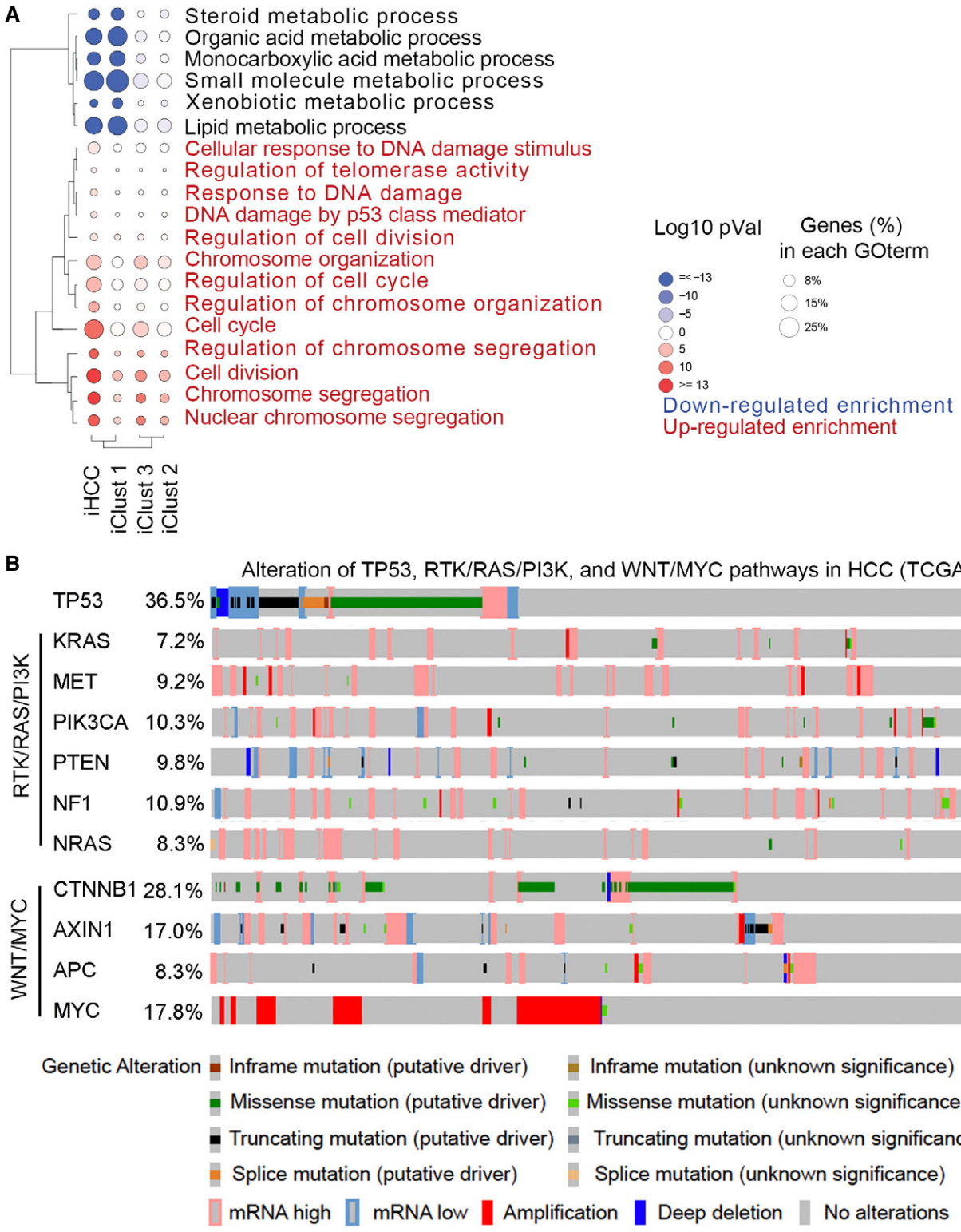


**Figure EV4. Mutational signatures in liver hepatocellular carcinoma(TCGA-LIHC).**

A Gene ontology (GO) analysis of the pathways enriched with DEGs (fold change ≥ 2 and *P* value≤ 0.05) in iHCC cells and clinical patient HCC (iClust 1, iClust 2 and iClust 3).

B Alteration signatures of TP53 mutations, and mutations in the RTK/RAS/PI3K and WNT/MYC pathways in clinical HCC samples obtained from the TCGA-LIHC database.

**Figure EV5. Cell surface markers associated with poor prognosis and the design of CAR molecules.**

A Kaplan-Meier analysis of the TCGA-HCC (TCGA-LIHC) cohorts based on the expression levels of *SLC34A2, FBN2, FOLR1,* and *SLC39A10* in the cohort samples ($n = 364$, high expression in red, low expression in black, the number of patients were indicated in figures, Statistical significance was determined using a log-rank test).

B, C Representative IHC staining of FAP and MUC1 in a normal liver (PHH), primary HCC (pHCC) and five MTK-transduced iHCC tissues (iHCC1-1, iHCC2-1, iHCC3-1, iHCC4-1, and iHCC5-1) that were derived from five different donors (PHH1~5). Scale bars, 20 μm.

D Design of the pWPXLD-CAR vector. pWPXLD-CAR contains an anti-FAP/MUC1 single-chain fragment variation (scFv), CD28 transmembrane and endodomain, TLR2 endodomain, CD3ζ domain, and EGFP.

E The transduction efficiency of pWPXLD-CAR is shown. Data are presented as the mean ± SD. *N*-numbers refer to biological replicates.

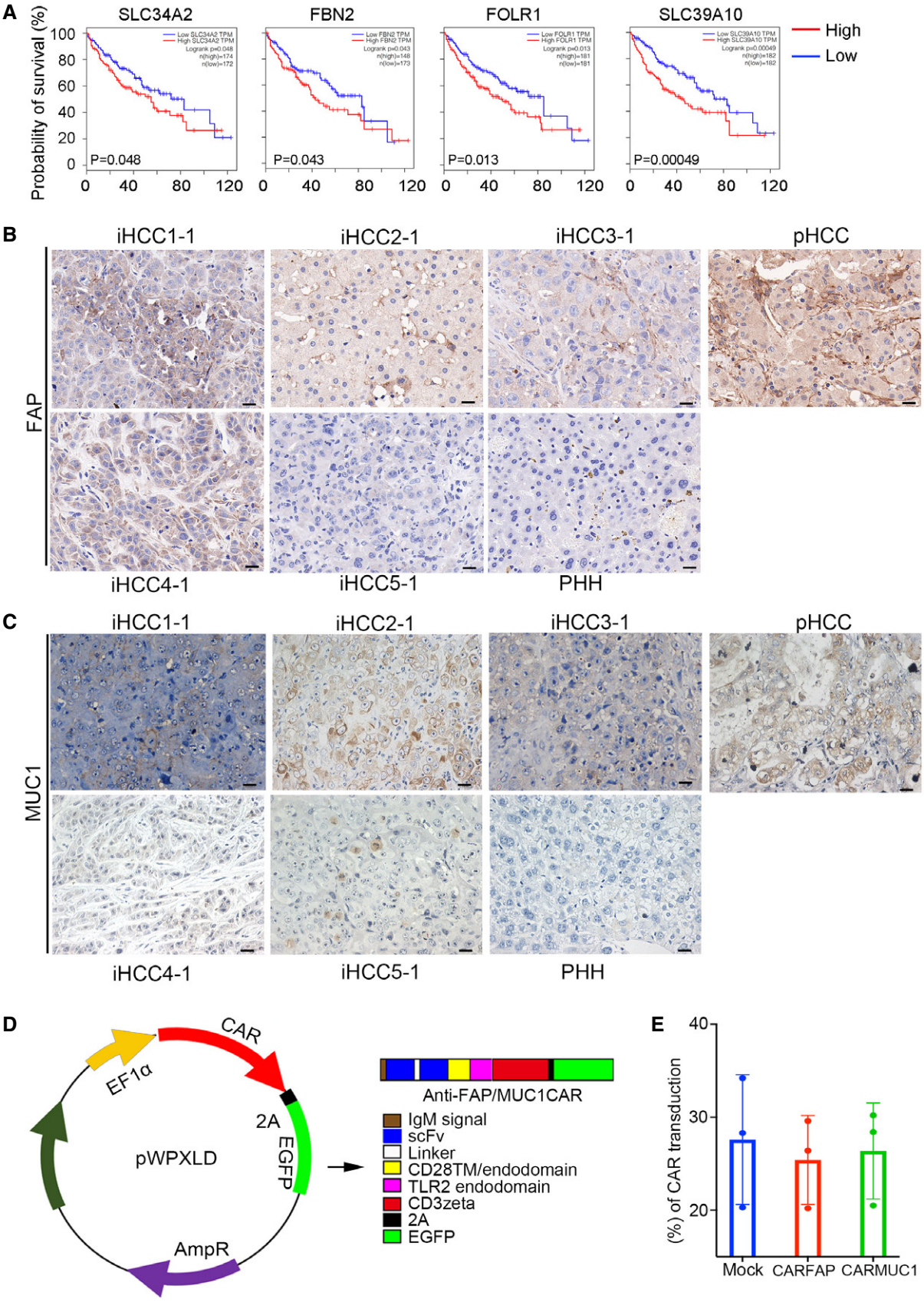


**Figure EV5.**