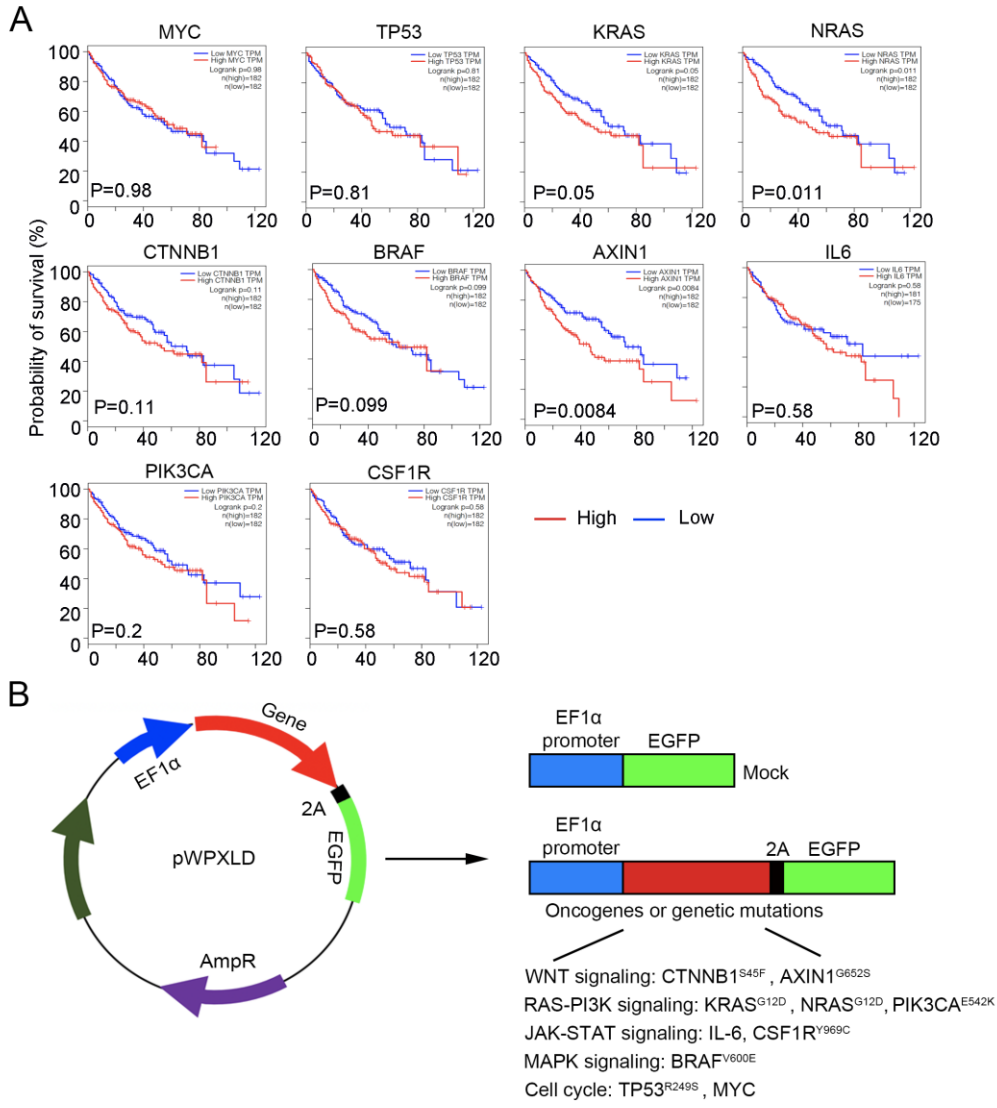
Appendix for: Transforming primary human hepatocytes to hepatocellular carcinoma with genetically defined factors.

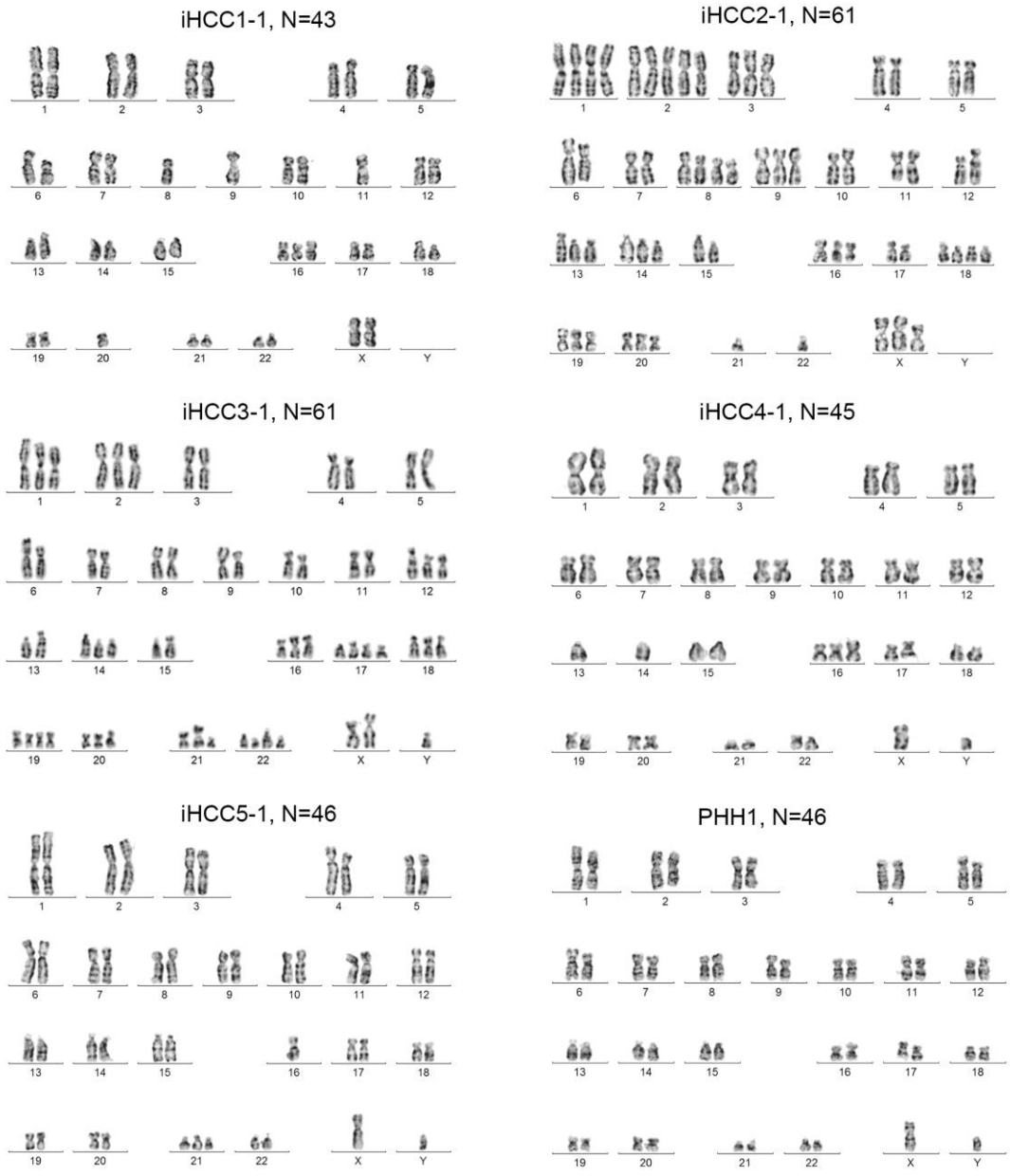
Table of content (TOC)

Appendix Figure S1



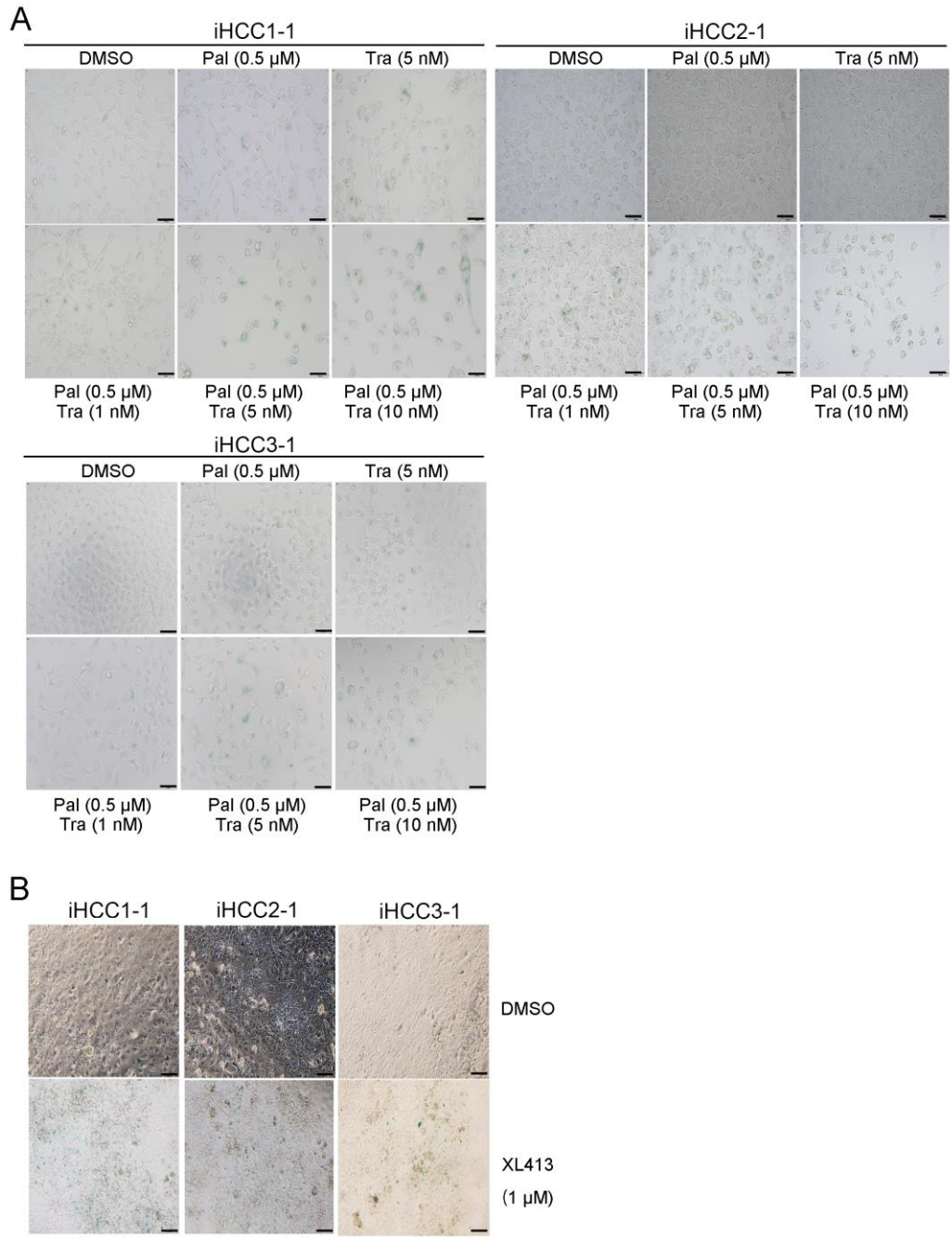
Appendix Figure S1. Ten oncogenic genes and its association with HCC prognosis. (A) Kaplan-Meier analysis of TCGA-LIHC cohorts based on the expression levels of the indicated genes in the cohort samples (n = 364, high expression in red, low expression in black, the number of patients were indicated in figures, Statistical significance was determined using a log-rank test.); (B) The pWPXLD vector was used for overexpression of the 10 selected oncogenic candidates (OC) individually. Candidates and EGFP, separated by 2A, were driven by a EF1α promoter. These OC are associated with the WNT, PI3K, JAK-STAT, MAPK, and cell cycle signaling pathways. The mock vector does not contain any candidate genes but EGFP.

Appendix Figure S2



Appendix Figure S2. Karyotype analysis of iHCC. Representative images of iHCC cell chromosomes in metaphase used for ploidy analysis. iHCC1-1, iHCC2-1, iHCC3-1, iHCC4-1, and iHCC5-1 were derived from tumors in NSIF mice that were transplanted MTK-transduced PHHs and were cultured for 2-3 passages before karyotype analysis. Un-transduced PHHs from PHH1 were used as normal controls.

Appendix Figure S3



Appendix Figure S3. Trametinib, Palbociclib, and XL413 treatment induced senescence in iHCC. (A, B) SA-β-Gal staining of iHCC cells from iHCC1-1, iHCC2-1, and iHCC3-1 treated with a MEK inhibitor (trametinib) and/or a CDK4/6 inhibitor (palbociclib) (A) and cell division cycle 7 homolog (CDC7) kinase inhibitor (XL413) (B). The data shown are representative of three biological replicates. Scale bars, 50 μm.

Appendix Table S1. Information of ALB levels, repopulation rates and survival duration of PHH-transplanted NSIF mice.

PHH-transplanted NSIF mice No.	ALB (μg/ml)	Repopulation[1] (%)	Survival duration (Days)
1	1.84	6.32	28
2	0.66	7.21	30
3	2.04	9.63	32
4	1.2	5.51	35
5	1.21	10.33	38
6	110.26	27.64	54
7	83.24	24.22	62
8	416.28	20.55	66
9	165.23	15.68	66
10	61.78	20.32	72
11	650.24	35.20	80
12	1330.24	46.20	85
13	1614.15	36.02	90
14	2124.38	44.77	93
15	1205.23	57.17	104

Repopulation[1]: Quantification of the repopulation efficiency was calculated based on the percentages of hALB-positive staining cells in reconstituted livers at indicated time points post transplantation with ImageJ software.

Appendix Table S2. Screening oncogenes contributing to the transformation of PHHs into HCC.

Hepatocytes[1]	PHH1 (AKB)		PHH4 (HVN)		PHH5 (QBU)		In total
Oncogenes	Mock	OC[2]	Mock	OC	Mock	OC	
Transduction rates (%)	98.7	99.6	97.1	97.4	99.3	98.5	98.4
Tumorigenesis rate[3]	0/3	5/18	0/3	1/6	0/3	2/6	8/30
MYC[4]	0/3	5/5	0/3	1/1	0/3	2/2	8/8
$TP53^{R249S}$	0/3	5/5	0/3	1/1	0/3	2/2	8/8
$KRAS^{G12D}$	0/3	5/5	0/3	1/1	0/3	1/2	7/8
$NRAS^{G12D}$	0/3	0/5	0/3	0/1	0/3	0/2	0/8
$CTNNB1^{S45F}$	0/3	0/5	0/3	0/1	0/3	0/2	0/8
$BRAF^{V600E}$	0/3	0/5	0/3	0/1	0/3	0/2	0/8
$AXIN1^{G652S}$	0/3	0/5	0/3	0/1	0/3	0/2	0/8
IL6	0/3	0/5	0/3	0/1	0/3	0/2	0/8
$PIK3CA^{E542K}$	0/3	0/5	0/3	0/1	0/3	0/2	0/8
$CSF1R^{Y969C}$	0/3	0/5	0/3	0/1	0/3	0/2	0/8

Hepatocytes[1]: PHHs from three donors, including PHH1 (AKB, female, 39 years old), PHH4 (HVN, male, 33 years old), and PHH5 (QBU, male, 50 years old), were purchased from Bioreclamation IVT (Baltimore, MD, USA) and were used in the experiment.

OC[2]: A cocktail of lentivirus containing oncogenic candidates (*MYC,* $TP53^{R249S}$, $KRAS^{G12D}$, $NRAS^{G12D}$, $CTNNB1^{S45F}$, $BRAF^{V600E}$, $AXIN1^{G652S}$, *IL6,* $PIK3CA^{E542K}$, *and* $CSF1R^{Y969C}$) were transduced into PHHs from different donors.

Tumorigenesis rate[3]: The ratios of mice that developed iHCC in the OC-PHHs transplanted group. For example, 5 out of 18 NSIF mice in the OC-PHHs group in which PHHs were derived from the PHH1 donor were observed bearing tumours in livers. In contrast, none of nine mice in the mock-PHHs group in which PHHs were from the three donors developed iHCC.

MYC[4]: The ratios of mice, in which tumours contained the indicated oncogenic candidate, in all tumour bearing mice. For example, all tumour samples from these 5 mice contained the lentiviral transduced MYC detected by PCR, within the 5 mice bearing iHCC in the OC-PHHs group (Donor: PHH1). In contrast, only one mouse whose tumour sample contained the lentiviral transduced $KRAS^{G12D}$ in the two iHCC-bearing mice (Donor: PHH5).

Appendix Table S3. Information of iHCC samples from *MYC, TP53^{R249S}, and KRAS^{G12D}* transduced PHHs.

iHCC[1]	Donor	Lot No.	Gender of donor	Age of donor	OC[2]	Survival[3] (Months)
iHCC1-1	PHH1	AKB	Female	39	MTK	4
iHCC1-2						4
iHCC1-3						4.5
iHCC1-4						4.5
iHCC1-5						6
iHCC1-6						8
iHCC1-7					MT	8
iHCC2-1	PHH2	XSM	Female	59	MTK	3
iHCC2-2						3
iHCC2-3						3
iHCC2-4						4
iHCC2-5					MT	6
iHCC3-1	PHH3	ANG	Male	0.3	MTK	3.5
iHCC3-2						4
iHCC3-3						5
iHCC3-4						5
iHCC4-1	PHH4	HVN	Male	33	MTK	3.5
iHCC5-1	PHH5	QBU	Male	50	MTK	6

Hepatocytes[1]: PHHs from three donors, including PHH1 (AKB, female, 39 years old), PHH2 (XSM, female, 59 years old), PHH3 (ANG, male, 3 months old), PHH4 (HVN, male, 33 years old), and PHH5 (QBU, male, 50 years old), were purchased from Bioreclamation IVT (Baltimore, MD, USA) and were used in the experiment.

OC[2]: A cocktail of lentivirus containing different combinations of oncogenic candidates (M for *MYC,* T for *TP53^{R249S},* and K for *KRAS^{G12D}*) were transduced into PHHs from different donors.

Survival[3]: The length of survival of NSIF mice post transplantation of PHHs that were transduced with different combinations of oncogenic candidates. Mice were euthanatized for further analysis after showing severe weight loss and fatigue.

Appendix Table S4. Primers and sgRNAs were used in this study.

Genes	Forward primer (5' - 3')	Reverse primer (5' - 3')	Amplicon (bp)
MYC	CAGGCTCCTGGCAAAAGGTCA	ACGTCGCCGTCCAGCTCGAC	684
$TP53^{R249S}$	CCTATGAGCCGCCTGAGGTT	ACGTCGCCGTCCAGCTCGAC	685
$KRAS^{G12D}$	ATGACTGAATATAAACTTGT	ACGTCGCCGTCCAGCTCGAC	725
$NRAS^{G12D}$	ATGACTGAGTACAAACTGGT	ACGTCGCCGTCCAGCTCGAC	728
$CTNNB1^{S45F}$	TGCTTTATTCTCCCATTGAA	ACGTCGCCGTCCAGCTCGAC	700
$BRAF^{V600E}$	TCACAGTAAAAATAGGTGAT	ACGTCGCCGTCCAGCTCGAC	697
$AXIN1^{G652S}$	CTTCATCCAAGACCCCACCAT	ACGTCGCCGTCCAGCTCGAC	684
IL6	ATTCCAAAGATGTAGCCGCC	ACGTCGCCGTCCAGCTCGAC	694
$PIK3CA^{E542K}$	TATATGATGCAGCCATTGAC	ACGTCGCCGTCCAGCTCGAC	700
$CSF1R^{Y969C}$	ACTTCGGGCTGGCTAGGGAC	ACGTCGCCGTCCAGCTCGAC	691
Fah	ATAGCTTGTGAGCATTGATT	CAGGCAGCCAGACAGCCAAG	430
ALB	GAGACCAGAGGTTGATGTGATG	CTTTGGCAACAGGCAGGCAG	196
AAT	ATGCTGCCCAGAAGACAGATA	AGAGCATTGCAAAGGCTGTA	177
TAT	TGCCGGGAAAAATGAAAGGC	CAGGGTCTGTAGGCAGGTTTC	186
ARG1	GTGGAAACTTGCATGGACAAC	TCAAAATGTAGTGTTCCCCAGG	162
CYP1A2	CTGGGCACTTCGACCCTTAC	AGGTAGCGAAGGATGGGGAAG	187
CYP2B6	GCACTCCTCACAGGACTCTTG	CCCAGGTGTACCGTGAAGAC	185

Total-TP53	GAGGTTGGCTCTGACTGTACC	CGGAGATTCTCTTCCTCTGTGC	200
Mutant TP53	ACTCAAGGATGCCCAGGCTGG	TGGACCTGGATTGCTTTCTACATCC	220
WT TP53	ACTCAAGGATGCCCAGGCTGG	AAGGGTTCAAAGACCCAAAACCC	224
MYC	AAGAGGACTTGTTGCGGAAACGA	GCTTTCTACATCCCCAGCCAG	146
$TP53^{R249S}$	AAGGGTCAGTCTACCTCCCG	GCTTTCTACATCCCCAGCCAG	128
$KRAS^{G12D}$	CAGCAAAGACAAGACAGGGTG	GCTTTCTACATCCCCAGCCAG	196
$NRAS^{G12D}$	CCAAGACCAGACAGGGTGTTGA	GCTTTCTACATCCCCAGCCAG	196
$CTNNB1^{S45F}$	CACCACCCTGGTGCTGACT	GCTTTCTACATCCCCAGCCAG	185
$BRAF^{V600E}$	ATTCACCGCAGTGCATCAGAA	GCTTTCTACATCCCCAGCCAG	194
$AXIN1^{G652S}$	GTTTGAGGAGGTTCGAGAGGACG	GCTTTCTACATCCCCAGCCAG	144
IL6	CCTGCTGACGAAGCTGCAG	GCTTTCTACATCCCCAGCCAG	180
$PIK3CA^{E542K}$	ATGATGCACATCATGGTGGCT	GCTTTCTACATCCCCAGCCAG	139
$CSF1R^{Y969C}$	CTGGAGGAGGAGAGCTCTAGTG	GCTTTCTACATCCCCAGCCAG	158
GFP	CTGGCTGGGGATGTAGAAAGC	TTGTGGCCGTTTACGTCGC	119
sgTP53	CAGTGACCCGGAAGGCAGTC		20