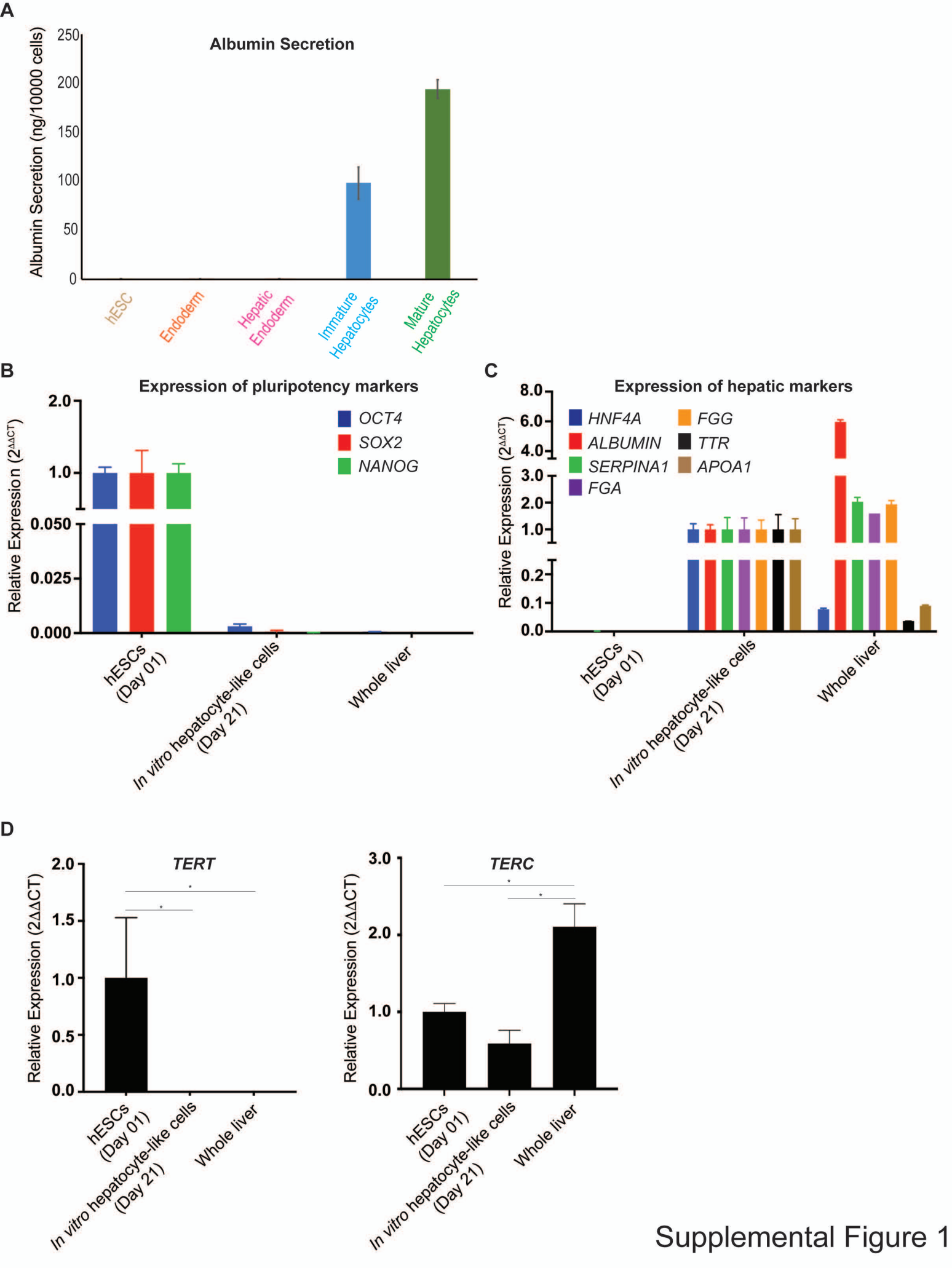
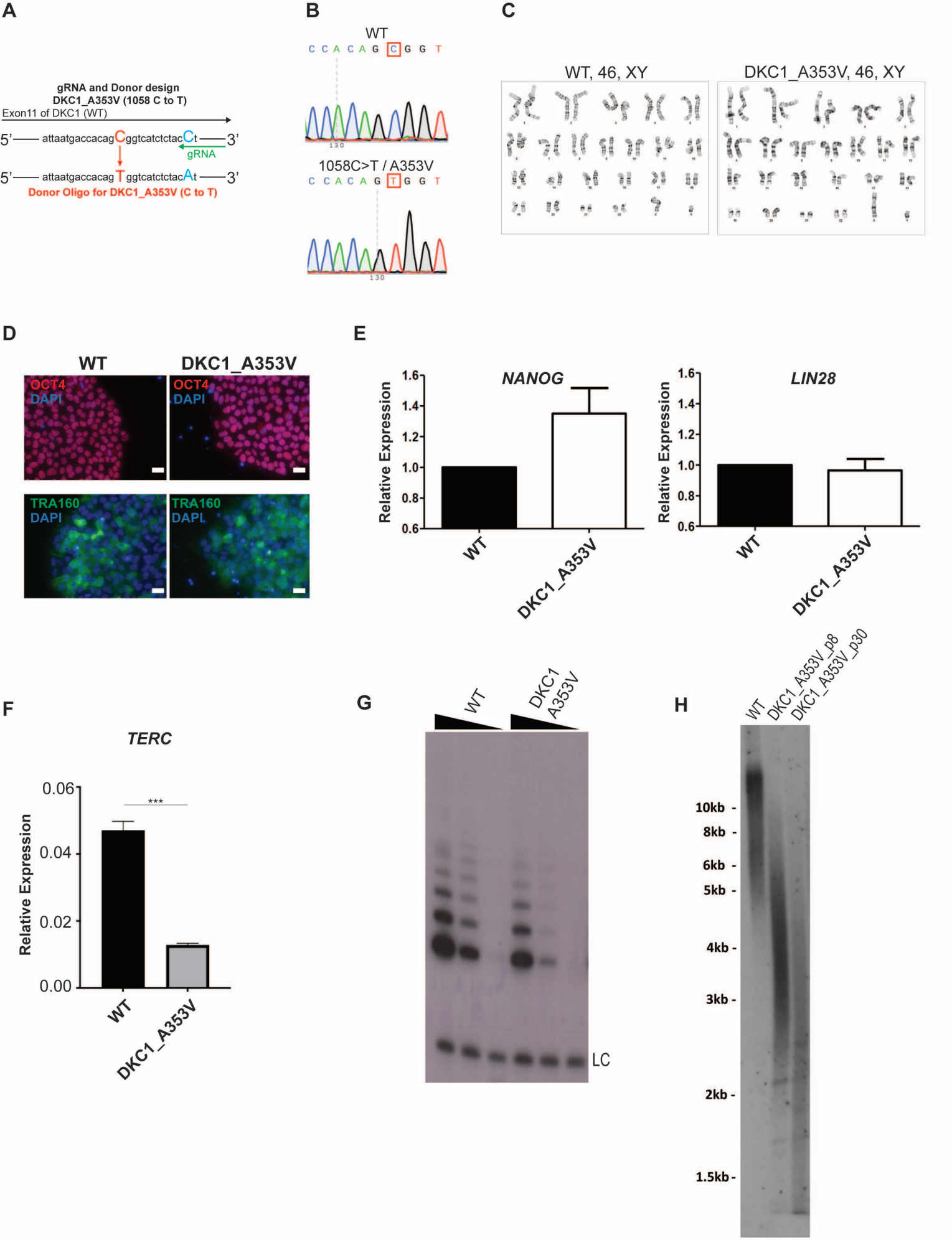
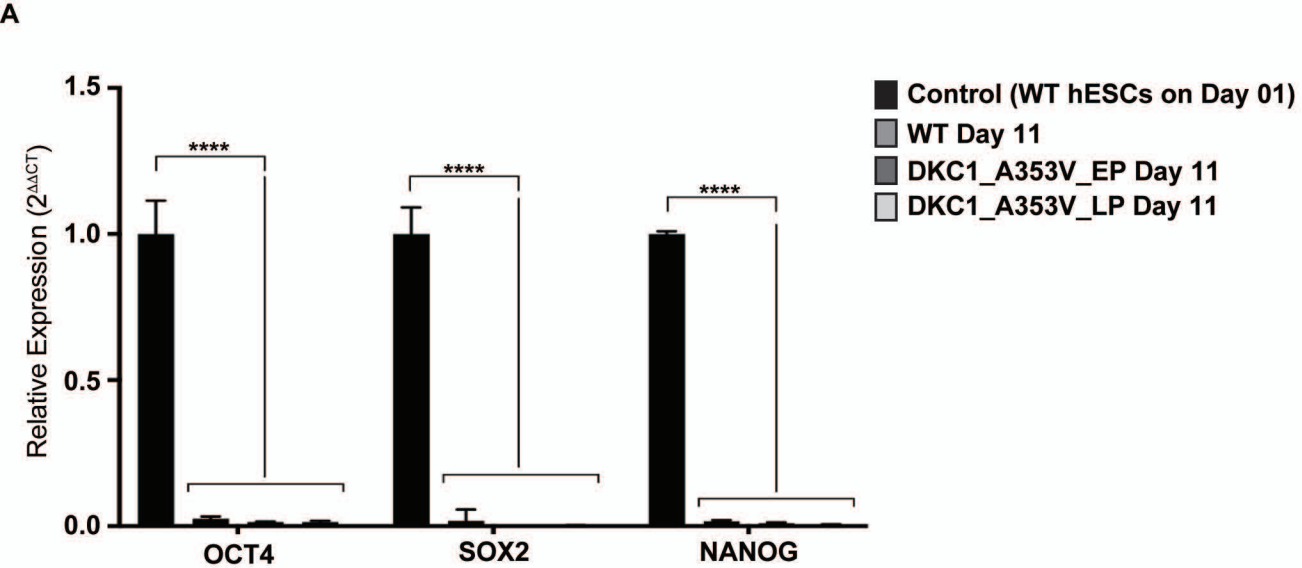
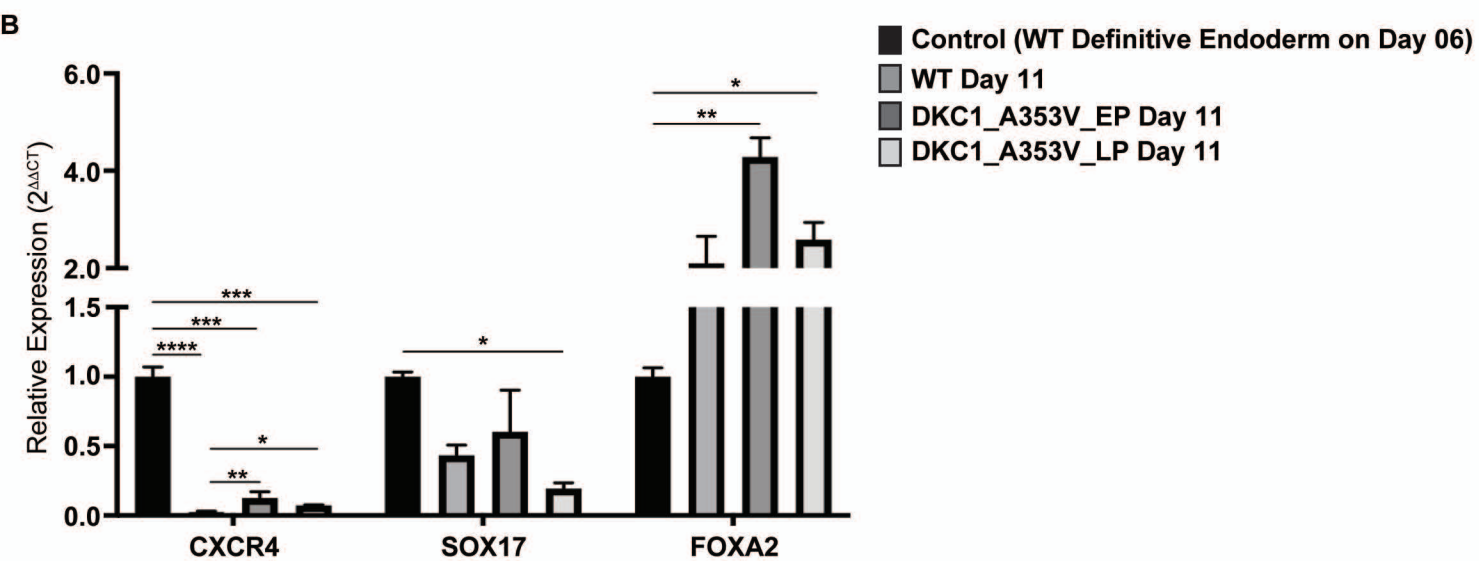


Supplemental Figure 1



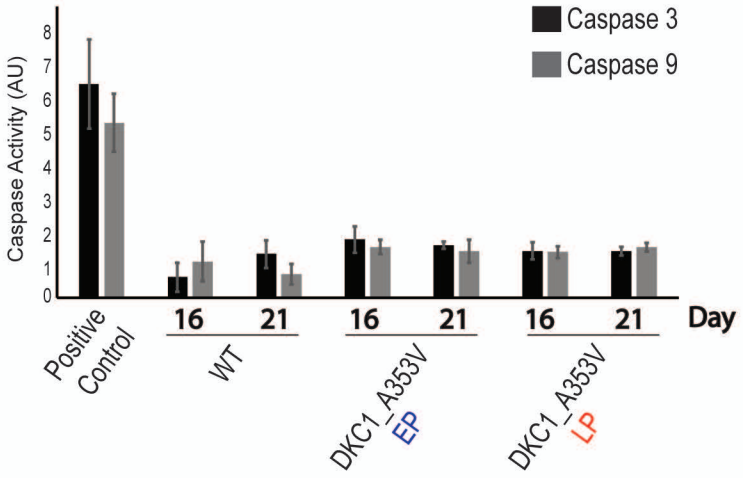
Supplemental Figure 2





Supplemental Figure 3

A



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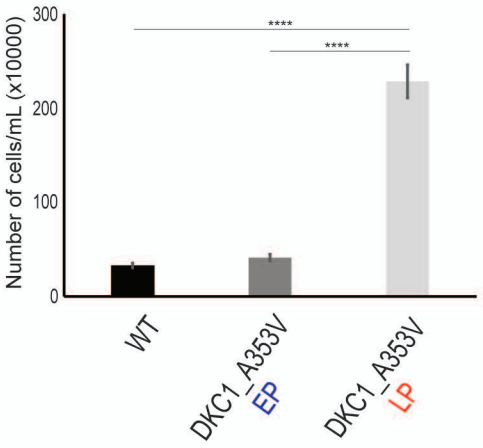
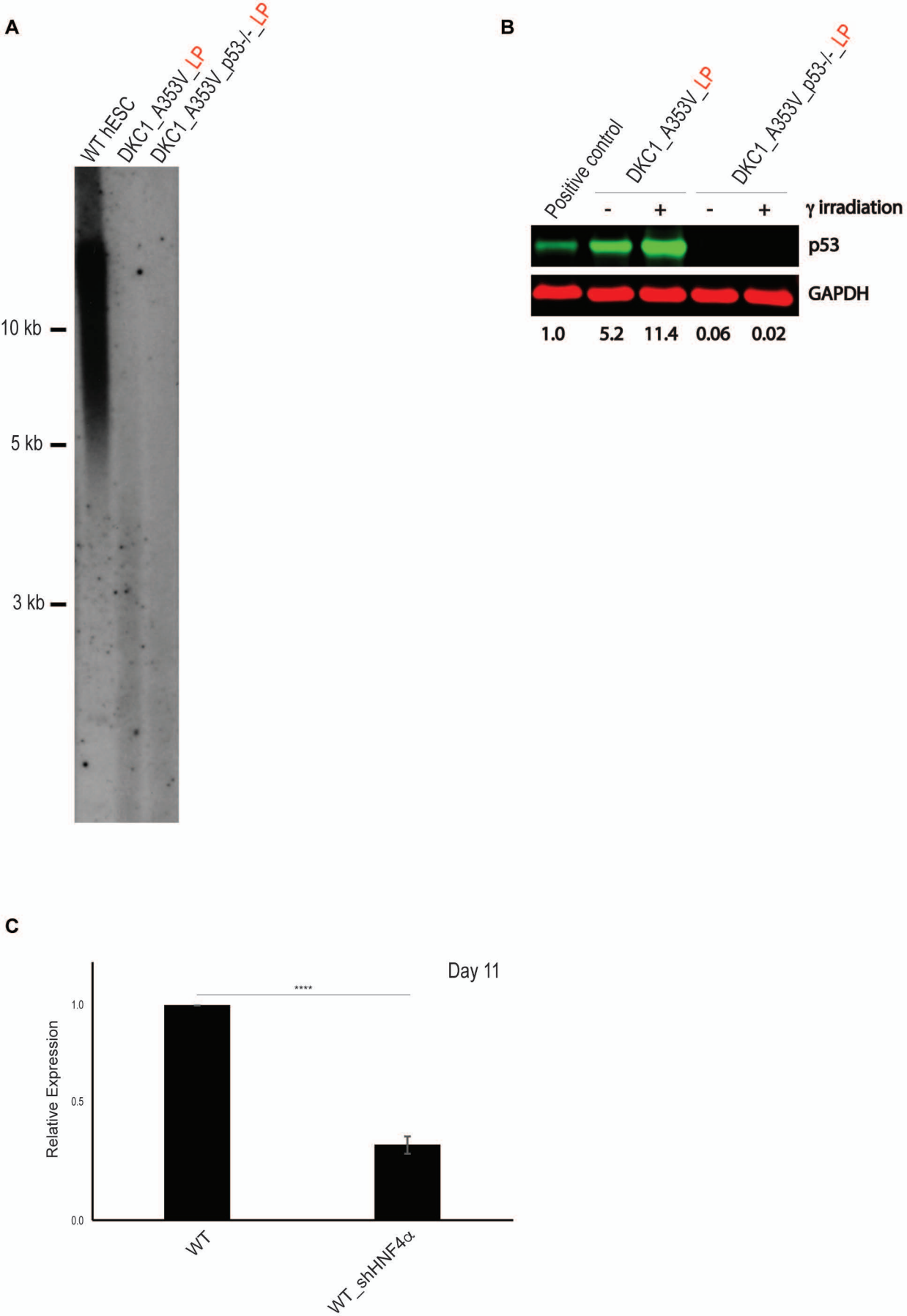


Supplemental Figure 4



Supplemental Figure 5

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: Characterization of hepatocyte differentiation *in vitro*. (A) Quantification of albumin secretion (by ELISA) at different stages of hepatocyte development in WT cells. hESC (Day 01); Endoderm (Day 06); Hepatic Endoderm (Day 11); Immature Hepatocytes (Day 16); Mature Hepatocytes (Day 21). **(B)** Relative gene expression analysis by real-time quantitative PCR of different pluripotency markers (*OCT4, SOX2* and *NANOG*) in hESCs (Day 01 of differentiation protocol), *in vitro* derived hepatocyte-like cells (Day 21 of differentiation protocol), and RNA extracted from human whole liver samples. **(C)** Relative gene expression analysis by real-time quantitative PCR of different hepatic markers (*HNF4α, ALBUMIN, SERPINA1, FGA, FGG, TTR and APOA1*) on hESCs (Day 01 of differentiation protocol), *in vitro* derived hepatocyte-like cells (Day 21 of differentiation protocol), and RNA extracted from human whole liver samples. **(D)** Relative gene expression analysis of *TERT* and *TERC* in hESCs (Day 01 of differentiation protocol), *in vitro* derived hepatocyte-like cells (Day 21 of differentiation protocol), and RNA extracted from human whole liver samples. n=3, mean ± SEM, $p \leq 0.05$. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

Supplemental Figure 2: Construction of telomerase mutant DKC1_A353V hESCs. (A) Strategy for introduction of disease-specific mutations in DKC1. Guide RNAs (gRNAs) targeting Exon 11 (DKC1) were used with in combination with specific ssDNA donor oligo templates for introduction of DKC1 (A353V; *C>T*). In blue, silent mutations introduced to facilitate CRISPR/Cas9 mediated genome modification. **(B)** DNA sequencing confirms correct C>T

modification in DKC1_A353V hESCs. **(C)** G-band analysis in wild-type and DKC1_A353V hESCs. No chromosomal abnormalities were detected. **(D)** Immunofluorescence analysis confirming normal expression of the pluripotency markers OCT4 and TRA160 in DKC1_A353V hESCs. Scale Bars represent 20 μM. **(E)** Real-time quantitative PCR analysis of different pluripotency markers in WT and DKC1_A353V hESCs. **(F)** Real-time quantitative PCR analysis of *TERC* in WT and DKC1_A353V hESCs. **(G)** Telomerase activity by TRAP in WT and DKC1_A353V mutants. Range of concentrations represent four-fold serial dilutions. LC: loading control. **(H)** Telomere length analysis by Telomere Restriction Fragment (TRF) of wild-type and DKC1_A353V hESCs at different cell passages, demonstrating progressive telomere shortening in mutant cells. Molecular weight (in kb) is shown. n=3, mean ± SEM, ***p≤0.001. Statistical analysis was performed using unpaired t-test.

Supplemental Figure 3: Gene expression analysis during hepatic endoderm specification. (A) Real-time quantitative PCR analysis of different pluripotency markers (*OCT4*, *SOX2* and *NANOG*) from WT, DKC1_A353V_EP and DKC1_A353V_LP cells on Day 11 of differentiation (hepatic endoderm stage). Expression is shown in relation to WT hESCs on Day 01 of differentiation (hESC stage). **(B)** Real-time quantitative PCR analysis of endoderm markers (*CXCR4* and *SOX17)* and *FOXA2* from WT, DKC1_A353V_EP and DKC1_A353V_LP cells on Day 11 of differentiation (hepatic endoderm stage). Expression is shown in relation to WT hESCs on Day 06 of differentiation (definitive endoderm stage). n=3, mean ± SEM, *p≤0.05; **p≤0.01; ***p≤0.001; ****p≤0.0001. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test for each gene.

Supplemental Figure 4: Analysis of cellular viability during hepatic differentiation. (A) Quantification of caspases 3 and 9 activation in early passage and late passage cells after 21 days of differentiation. Positive control: Ultraviolet Light irradiated (UV; 30J/m^2) WT hESCs. **(B)** Total number of cells after hepatic differentiation of WT and DKC1_A353V hESCs. Cells were collected on Day 21 and figure shows total number of cells found in each population (total numbers quantified by cell counter). n=3, mean ± SEM, *p≤0.05; **p≤0.0025; ***p≤0.001; ****p≤0.0001. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test.

Supplemental Figure 5: Generation of DKC1_A353V_p53-/- and WT_shHNF4α hESCs. (A) Telomere length analysis by Telomere Restriction Fragment (TRF) from WT and isogenic, CRISPR/Cas9 engineered, DKC1_A353V and DKC1_A353V_$p53^{-/-}$ hESCs. Telomeres in DKC1_A353V and DKC1_A353V_$p53^{-/-}$ hESCs are significantly shorter, as these cells reduced telomerase activity. Molecular weight (in kb) is shown. **(B)** Representative immunoblot analysis of p53 expression in DKC1_A353V and DKC1_A353V_$p53^{-/}$ hESCs. Samples were irradiated (+) or not (-) with 5Gy of gamma irradiation. Positive control: gamma irradiated (5Gy) WT hESCs. GAPDH is shown as loading control. Relative expression levels are indicated. **(C)** Efficient silencing of *HNF4α* expression in WT_sh HNF4α cells at the hepatic endoderm stage (Day 11). n=3, mean ± SEM, ****p≤0.0001. Statistical analysis was performed using unpaired t-test.

Sup. Table 1: Primer sequences used in this manuscript.

Primer	Forward sequence	Reverse sequence
β-actin	TTCCTTCCTGGGCATGGAGT	AATGCCAGGGTACATGGTGG
18S rRNA	GCTTAATTTGACTCAACACGGGA	AGCTATCAATCTGTCAATCCTGTC
β-2-microglobulin	TTCAGGTTTACTCACGTCATCC	AGACAAGTCTGAATGCTCCAC
TERC	CGCTGTTTTTCTCGCTGACT	GCTCTAGAATGAACGGTGGAA
TERT	CGAAAACCTTCCTCAGGACCC	GGCCGGCATCTGAACAAAAG
OCT4	GACAGGGGGAGGGGAGGAGCTAGG	CTTCCCTCCAACCAGTTGCCCCAAAC
SOX2	TTCACATGTCCCAGCACTACCAGA	TCACATGTGTGAGAGGGGCAGTGTGC
NANOG	TGAACCTCAGCTACAAACAG	TGGTGGTAGGAAGAGTAAAG
CXCR4	GGCAGCAGGTAGCAAAGTGA	TGAAGTGTATATACTGATCCCCTCC
SOX17	CTCCGGTGTGAATCTCCCC	CACGTCAGGATAGTTGCAGTAAT
FOXA2	GGAGCAGCTACTATGCAGAGC	CGTGTTCATGCCGTTCATCC
HNF4α	ATAGCTTGACCTTCGAGTGC	TGGACAAAGACAAGAGGAACC
AFP	GGCAGCCACAGCAGC ACTT	TGCAGCGCTACACCCTGAGC
FGA	CAGCCCCACCCTTAGAAAAG	CTCCTTCAGCTAGAAAGTCACC
FGG	CAAAGACACGGTGCAAATCC	TTCCAGACCCATCGATTTCAC
ALBUMIN	TGGCACAATGAAGTGGGTAA	CTGAGCAAAGGCAATCAACA
CYP1A1	CCCAACCCTTCCCTGAATG	TTCTTCTCCTGACAGTGCTCAATC
SERPINA1	AGCCAGGGAGACAGGGA	CTTAAATACGGACGAGGACAGG
TTR	CAGGTTTGCAGTCAGATTGG	CCATCCTGCCAAGAATGAGT
APOA1	CTTTGAGCACATCCACGTACA	GCCGTGCTCTTCCTGAC