Supplemental Information

Supplemental Figures

Figure S1

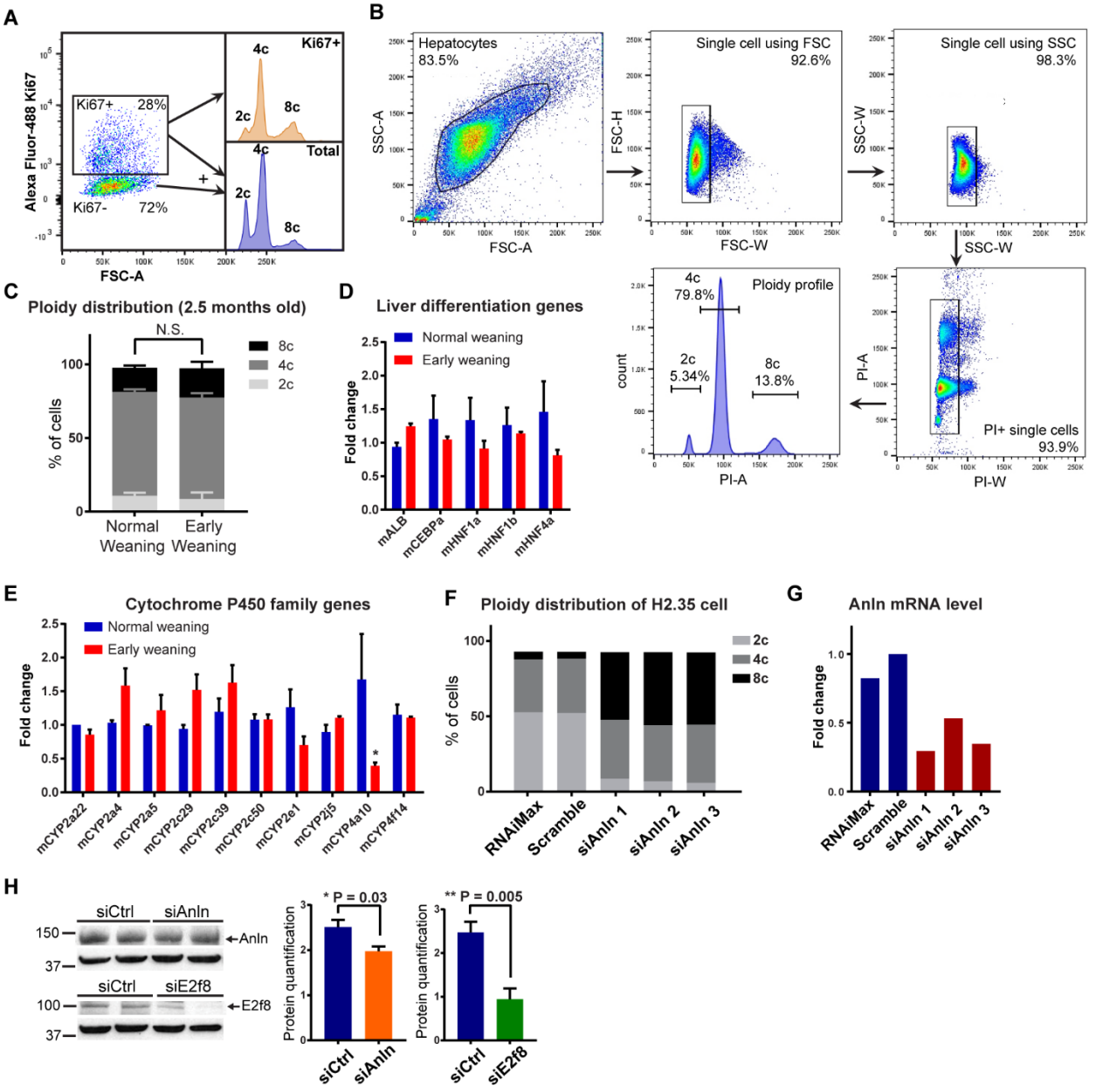

Figure S1. *Anln* and *E2f8* siRNA knockdown altered ploidy *in vitro* and *in vivo*. Related to Figure 1.

(A) Primary hepatocytes from a P29 WT B6 mouse were stained with Ki67 and PI to detect the ploidy distribution of the proliferating Ki67+ population (orange histogram) and the total population (blue histogram). The Ki67+ population contains a clear group of 8c cells, demonstrating that 4c tetraploid polyploid hepatocytes are also proliferating.

(B) Complete gating strategy for determining ploidy distribution of hepatocytes.

(C) The ploidy distribution of differentially weaned livers at the age of 2.5 months old (n = 5), as analyzed by flow cytometry with PI staining.

(D) qPCR for liver differentiation genes in differentially weaned livers (n = 2 mice per group).

(E) qPCR for Cytochrome P450 family genes in normally and early weaned livers (n = 2 mice per group).

(F) The ploidy distribution of H2.35 immortalized hepatocytes transfected with *Anln* siRNA, as analyzed by flow cytometry with PI staining.

(G) *Anln* mRNA levels in H2.35 cells transfected with *Anln* siRNA, as detected by qPCR.

(H) The *in vivo* knockdown efficiency of *Anln* and *E2f8* siRNAs on protein levels as assessed by western blotting. Liver tissues were collected four days after the last in vivo siRNA injection. The band intensities were quantified on the right.

Figure S2

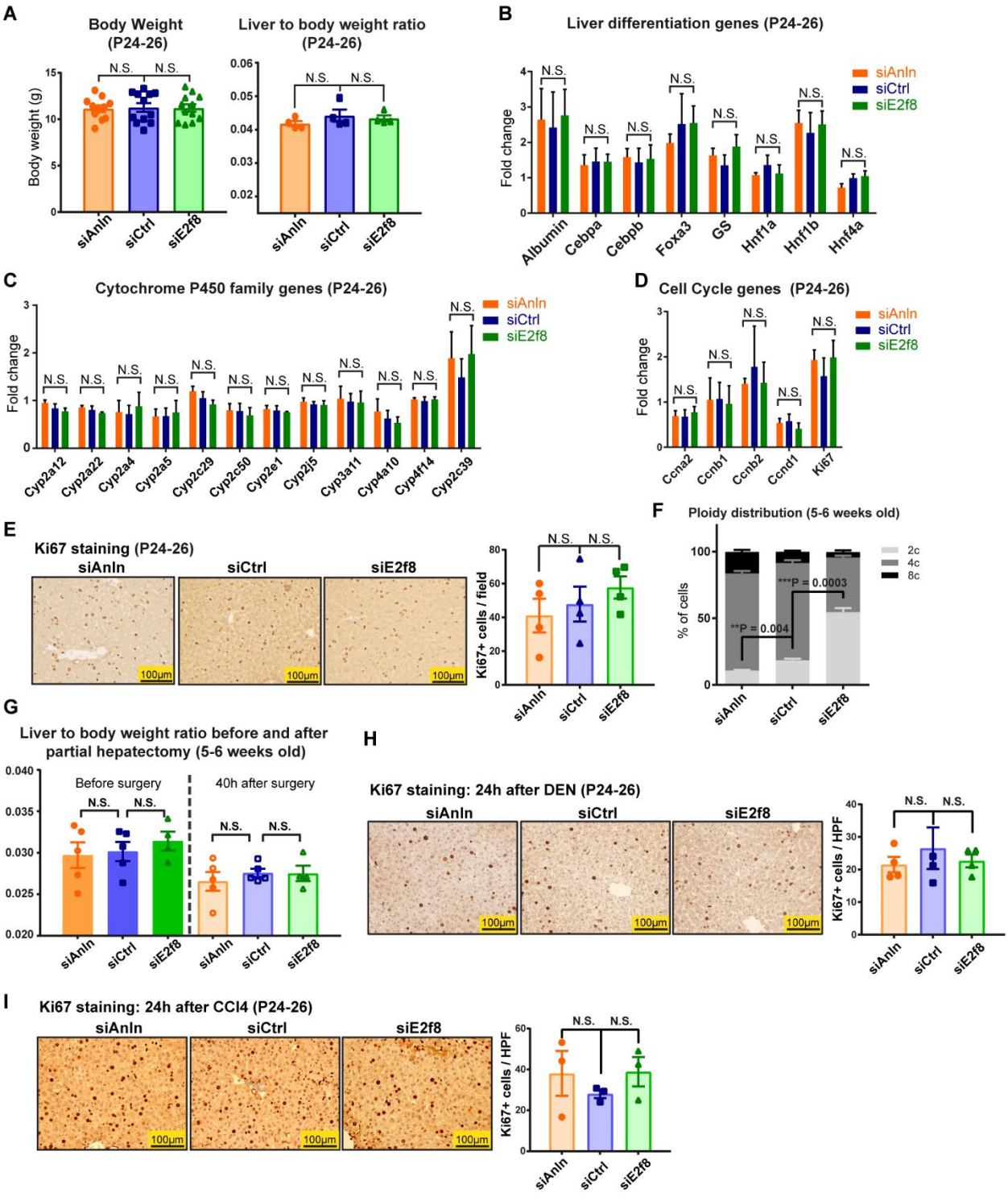
A
Body Weight (P24-26)
Body weight (g)
N.S.
siAnln
siCtrl
siE2f8
Liver to body weight ratio (P24-26)
B
Liver differentiation genes (P24-26)
Fold change
Albumin
Cebpa
Cebpb
Foxa3
GS
Hnf1a
Hnf1b
Hnf4a
C
Cytochrome P450 family genes (P24-26)
Cyp2a12
Cyp2a22
Cyp2a4
Cyp2a5
Cyp2c29
Cyp2c50
Cyp2e1
Cyp2j5
Cyp3a11
Cyp4a10
Cyp4f14
Cyp2c39
D
Cell Cycle genes (P24-26)
Ccna2
Ccnb1
Ccnb2
Ccnd1
Ki67
E
Ki67 staining (P24-26)
100μm
Ki67+ cells / field
F
Ploidy distribution (5-6 weeks old)
% of cells
2c
4c
8c
***P = 0.0003
**P = 0.004
G
Liver to body weight ratio before and after partial hepatectomy (5-6 weeks old)
Before surgery
40h after surgery
H
Ki67 staining: 24h after DEN (P24-26)
Ki67+ cells / HPF
I
Ki67 staining: 24h after CCl4 (P24-26)

Figure S2. siRNA treatment and altered ploidy did not impact overall liver development and regeneration. Related to Figure 2.

(A) Body and liver to body weight ratios of siRNA treated mice.

(B) qPCR for liver differentiation genes in siRNA treated livers (n = 4 mice per group).

(C) qPCR for Cytochrome P450 family genes in siRNA treated livers (n = 4 mice per group).

(D) qPCR for cell cycle genes in siRNA treated livers (n = 4 mice per group).

(E) Ki67 staining in siRNA treated livers (left panel), quantification on the right.

(F) The ploidy distribution of siAnln, siCtrl or siE2f8 treated mice livers at 5-6 weeks of age (n = 3), as analyzed by flow cytometry with PI staining

(G) Liver to body weight ratios before and 40 hours after partial hepatectomy. siRNA treated mice underwent 70% partial hepatectomy at six weeks of age and remnant livers were harvested and analyzed 40 hours after surgery.

(H) Ki67 staining of siRNA treated livers given DEN (left panel). Four days after the last siRNA injection, these mice received one dose of DEN (75μg/g of mouse) and were euthanized 24 hours later. Ki67+ cells were quantified in the right panel.

(I) Ki67 staining of siRNA treated livers given CCl4 (left panel). Four days after the last siRNA injection, these mice received one dose of CCl4 (100μL total, 10% CCl4 by volume) and livers were harvested 24 hours later. Ki67+ cells were quantified in the right panel.

Figure S3

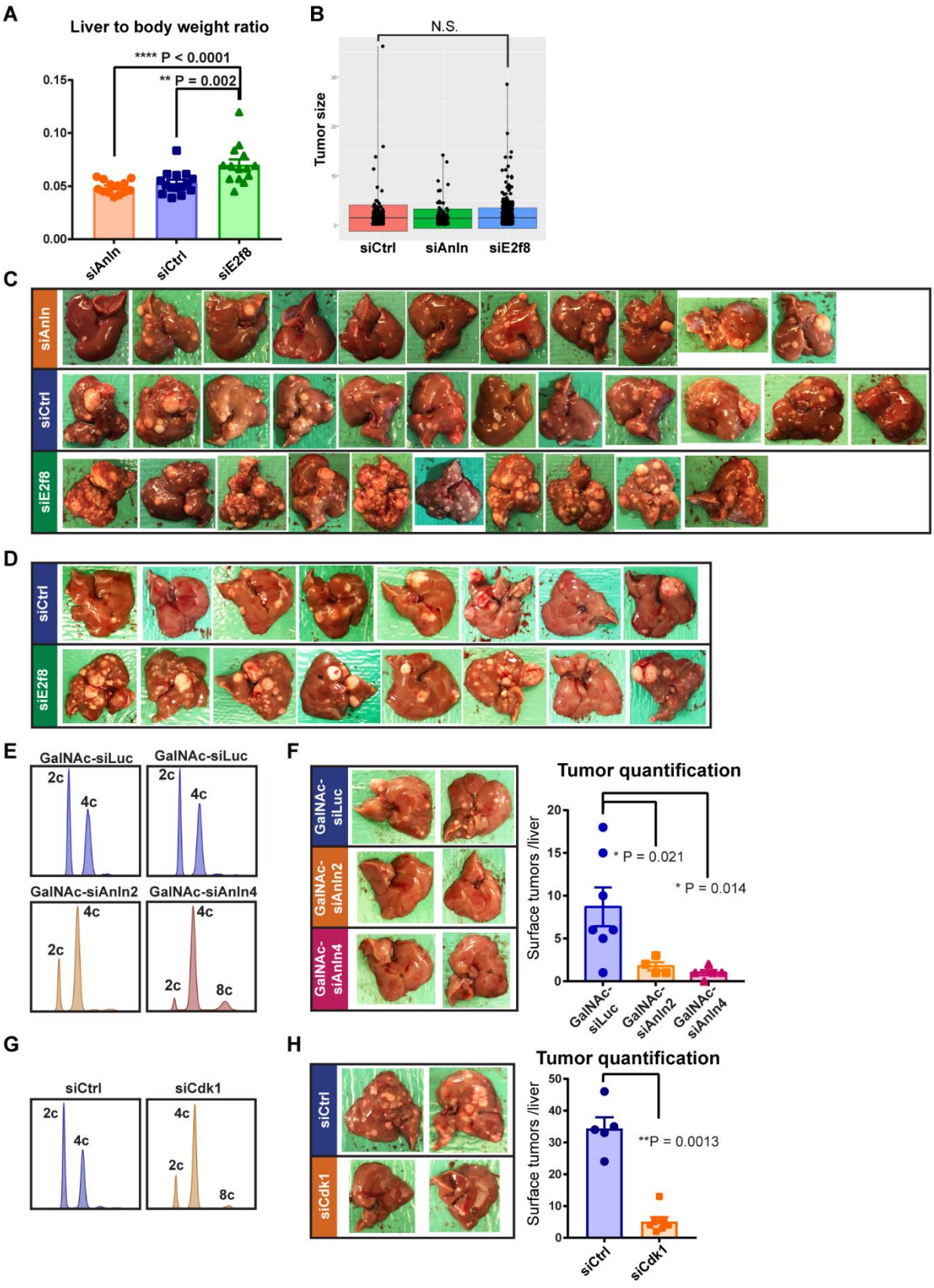
A
Liver to body weight ratio
**** P < 0.0001
** P = 0.002
0.15
0.10
0.05
0.00
siAnln
siCtrl
siE2f8
B
N.S.
Tumor size
siCtrl
siAnln
siE2f8
C
siAnln
siCtrl
siE2f8
D
siCtrl
siE2f8
E
GalNAc-siLuc
GalNAc-siLuc
2c
4c
2c
4c
GalNAc-siAnln2
GalNAc-siAnln4
4c
2c
4c
2c
8c
F
GalNAc-siLuc
GalNAc-siAnln2
GalNAc-siAnln4
Tumor quantification
Surface tumors /liver
* P = 0.021
* P = 0.014
GalNAc-siLuc
GalNAc-siAnln2
GalNAc-siAnln4
G
siCtrl
siCdk1
2c
4c
4c
2c
8c
H
siCtrl
siCdk1
Tumor quantification
Surface tumors /liver
**P = 0.0013
siCtrl
siCdk1

Figure S3. The polyploid state protected against DEN-induced HCC development. Related to Figure 2.

(A) Liver to body weight ratios of different ploidy groups 6 months after DEN induction. The liver mass includes tumor burden and normal liver tissue.

(B) Quantified individual tumor sizes from three ploidy groups. Tumor sizes were measured using ImageJ.

(C) All livers from the DEN experiment (n = 11 for siAnln group, n = 12 for siCtrl group, n = 10 for siE2f8 group) are shown.

(D) All livers treated with DEN 14 days after siRNA delivery. 14 days after four doses of siRNAs, one dose of DEN (100μg/g) was given. 7.5 months later, tumor burden was assessed (n = 8 in each group).

(E) Ploidy distribution of *GalNAc-siLuc, GalNAc-siAnln#2* and *GalNAc-siAnln#4* treated livers at P26, analyzed by flow cytometry with PI staining.

(F) Representative gross tumor burden from the *GalNAc-siRNA* DEN experiment: C3H mice were subcutaneously treated with three doses of 4.0mg/kg of *GalNAc-siLuc, GalNAc-siAnln#2* or *GalNAc-siAnln#4* starting at P8-10, given 4 days apart. DEN (75ug/g) was given at P26. Five months after DEN, tumors on the liver surface were quantified (right).

(G) Ploidy distribution of siCtrl and siCdk1 treated livers at P26, analyzed by flow cytometry with PI staining.

(H) Representative gross tumor burden from the siCdk1 DEN experiment: *Cdk1* and scramble siRNAs (siCtrl) were injected into WT C3H mice starting at P10. Four total injections (two intraperitoneal and two retro-orbital) were performed twice per week. At P26, mice were injected with DEN (75μg/g). Tumors on the liver surface were quantified at 6 months (right).

Figure S4

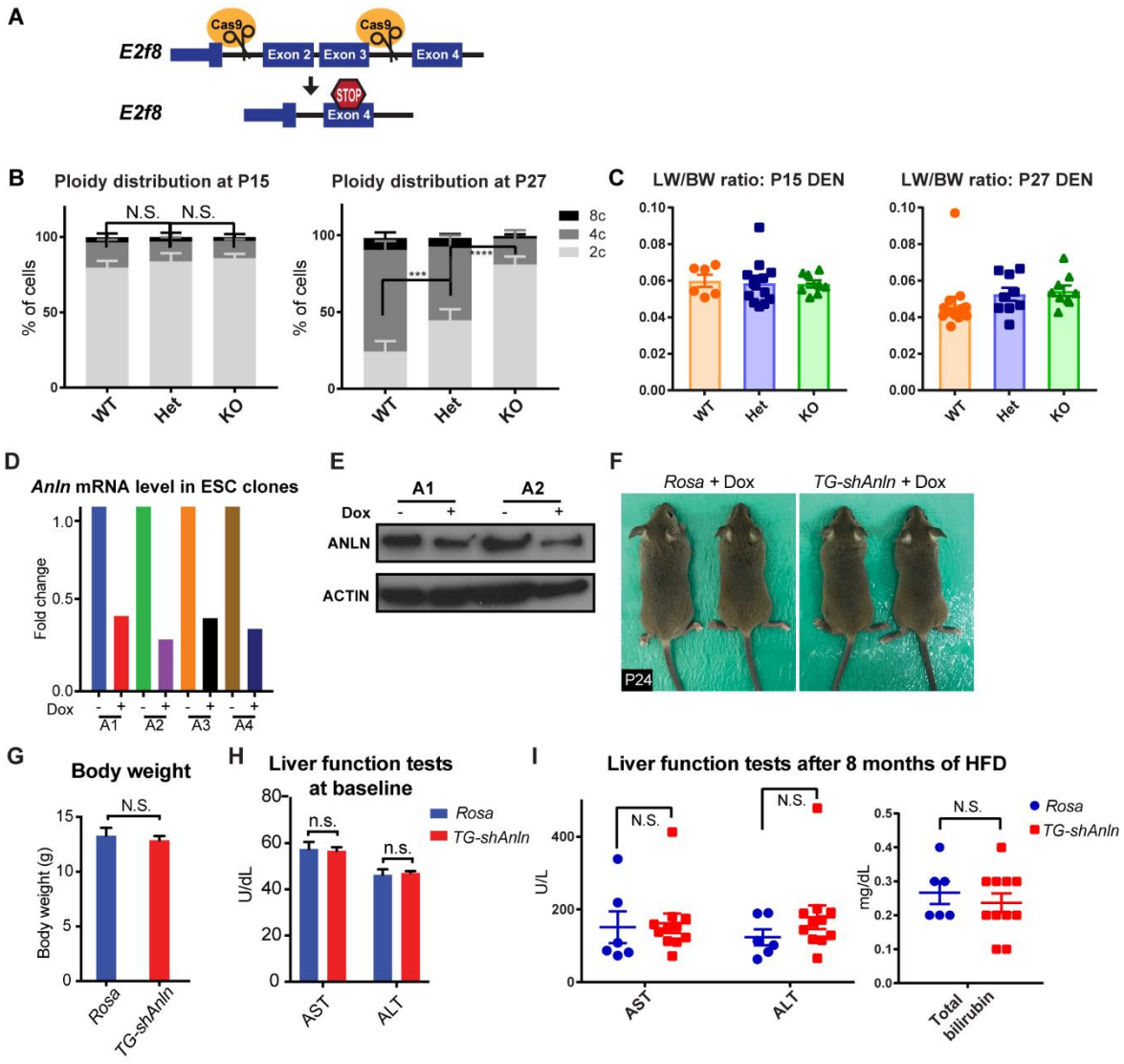
A
E2f8
Cas9
Exon 2
Exon 3
Exon 4
STOP
B
Ploidy distribution at P15
Ploidy distribution at P27
N.S.
% of cells
8c
4c
2c
WT
Het
KO
C
LW/BW ratio: P15 DEN
LW/BW ratio: P27 DEN
D
Anln mRNA level in ESC clones
Fold change
Dox
A1
A2
A3
A4
E
ANLN
ACTIN
F
Rosa + Dox
TG-shAnln + Dox
P24
G
Body weight
Body weight (g)
Rosa
TG-shAnln
H
Liver function tests at baseline
U/dL
n.s.
AST
ALT
I
Liver function tests after 8 months of HFD
U/L
mg/dL
Total bilirubin

Figure S4. Design and characterization of the *E2f8* KO and *TG-shAnln* mouse models. Related to Figure 3 and Figure 4.

(A) CRISPR/Cas9 was used to generate whole-body *E2f8* knockout (KO) mice. Two guide RNAs were designed to target introns 1 and 3 in order to delete exon 2 and 3. Successful deletion was predicted create a premature stop codon in exon 4.

(B) Ploidy distribution of *E2f8* WT, Het and KO livers at P15 (n = 3) or P27 (n = 6 for WT, n = 7 for Het, n = 4 for KO), analyzed by flow cytometry with PI staining.

(C) The liver to body weight ratios of DEN treated *E2f8* WT, Het, and KO mice at six months of age. The mice were given DEN at P15 or at P27, and euthanized 4.5-5 months later.

(D) Inducible *TG-shAnln* embryonic stem cells showing *Anln* expression levels after dox induction for 72 hours.

(E) Western blot of Anln protein levels in embryonic stem cell clones after dox induction.

(F) *Rosa* or *TG-shAnln* transgenic mice exposed to dox water from P0-P20 develop normally.

(G) Body weight of the *Rosa* and *TG-shAnln* mice (n = 3) after dox water treatment from P0-P20.

(H) AST/ALT serum liver function tests at base line in *Rosa* and *TG-shAnln* mice (n = 3) after dox treatment.

(I) The AST/ALT and total bilirubin levels of *Rosa* and *TG-shAnln* mice after 8 months of HFD feeding.

Figure S5

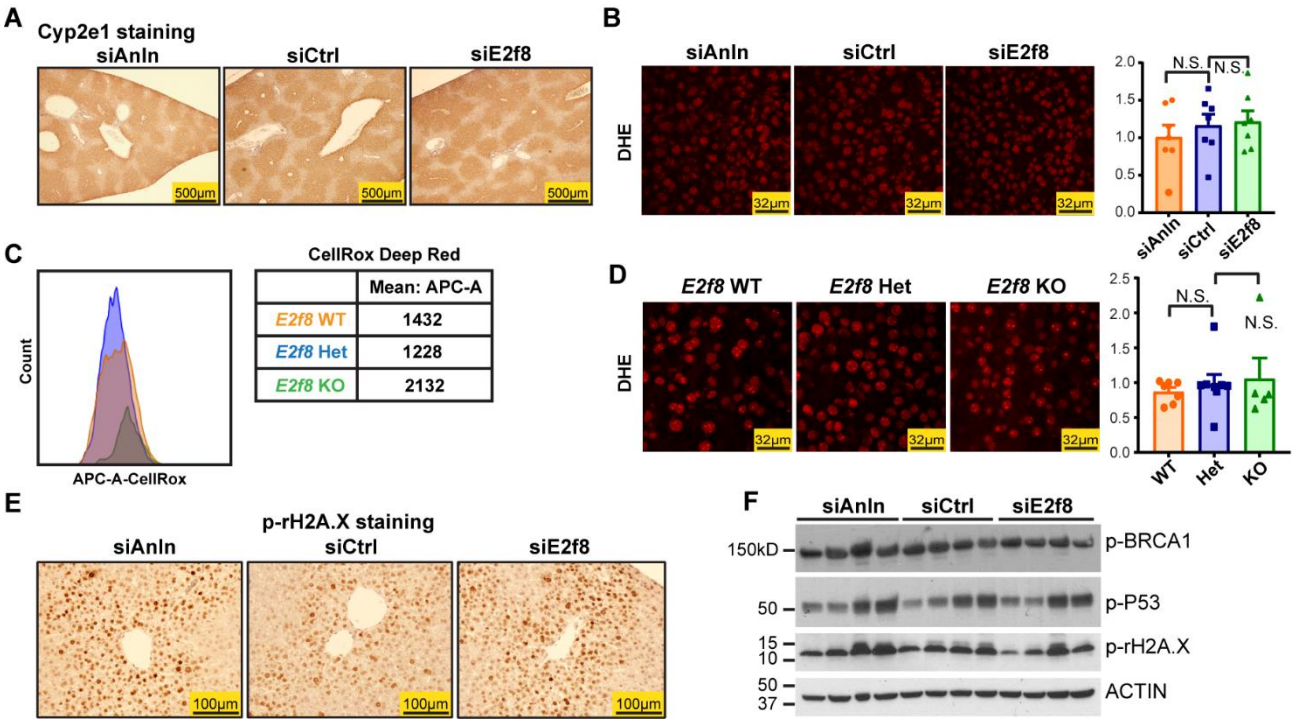

Figure S5. After DEN, livers with altered ploidy did not exhibit differential xenobiotic metabolism, oxidative stress, or DNA damage response. Related to Figure 5.

(A) CYP2E1 staining in siRNA treated livers with altered ploidy.

(B) Dihydroethidium (DHE) staining of siAnln, siCtrl or siE2f8 treated livers at P26. Staining intensities are quantified on the right, 20 cells from 5 fields in each mouse were measured and averaged.

(C) CellRox Deep Red staining for ROS in *E2f8* WT, Het, and KO livers, as analyzed by flow cytometry. The table on the right shows the mean values for the APC-A channel, which detects CellRox.

(D) DHE staining of *E2f8* WT, Het and KO livers at P30. Staining intensities are quantified on the right, 20 cells from 5 fields in each mouse were measured and averaged.

(E) Four days after the last siRNA injection, one dose of DEN (75μg/g) was given. 24 hours later, livers were stained for p-γH2A.X.

(F) The DNA damage response proteins, p-Brca1, p-p53, and p-γH2A.X, as assessed by western blots.

Figure S6

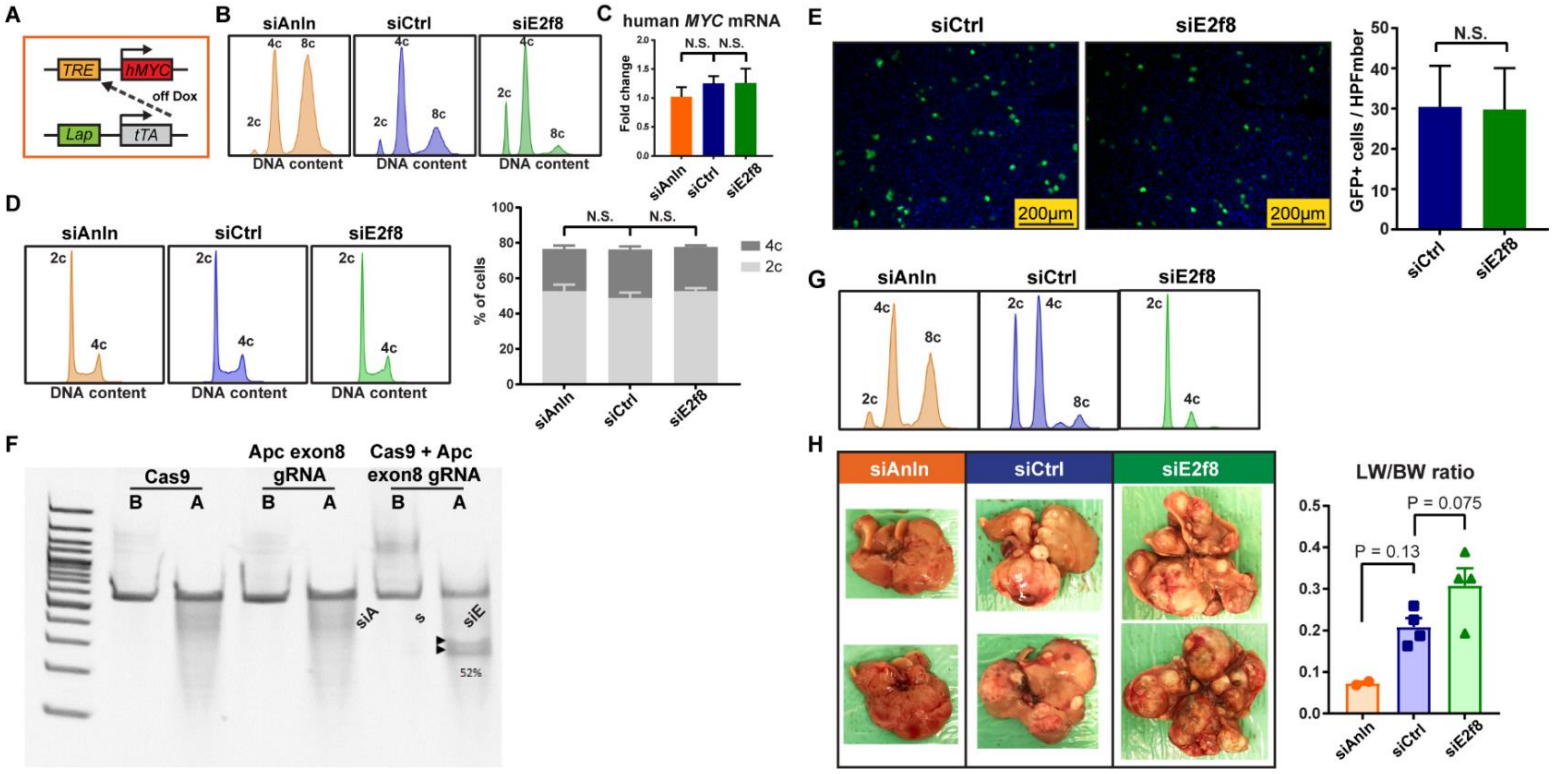
A
TRE
hMYC
off Dox
Lap
tTA
B
siAnln
siCtrl
siE2f8
4c
8c
2c
DNA content
C
human MYC mRNA
Fold change
N.S.
siAnln
siCtrl
siE2f8
D
siAnln
siCtrl
siE2f8
2c
4c
DNA content
% of cells
N.S.
4c
2c
E
siCtrl
siE2f8
200μm
GFP+ cells / HPFmber
N.S.
F
Cas9
Apc exon8 gRNA
Cas9 + Apc exon8 gRNA
B
A
siA
s
siE
52%
G
siAnln
siCtrl
siE2f8
H
LW/BW ratio
P = 0.075
P = 0.13

Figure S6. Polyploids were protected from TSG loss but not oncogene activation. Related to Figure 5.

(A) The *LAP-tTA; TRE-MYC* liver cancer model. Withdrawing doxycycline drives human *MYC* expression under the control of a liver specific promoter (*LAP*).

(B) Representative cellular ploidy of *LAP-tTA; TRE-MYC* livers at P30, analyzed by flow cytometry with PI staining.

(C) Relative human *MYC* mRNA expression in *LAP-tTA; TRE-MYC* livers with different ploidies as measured by qPCR. Expression was analyzed at P30 in non-tumor tissues.

(D) Representative cellular ploidy of the MYC tumors from different ploidy groups, analyzed by flow cytometry with PI staining. 6 tumors were analyzed from each group and the ploidy is summarized on the right.

(E) GFP expression (left panel) in siCtrl and siE2f8 treated livers 5 days after Adenovirus-GFP (1.4×10^8 pfu/mouse) injection. GFP+ cells were quantified in the right panel.

(F) The surveyor assay was used to test the efficiency of the *Apc* exon 8 sgRNA in the H2.35 cell line. B: before surveyor enzyme digestion; A: after surveyor enzyme digestion. Cutting efficiency (52%) was quantified by comparing the intensity of the cut bands.

(G) Representative cellular ploidy of FVB livers treated with siAnln, siCtrl or siE2f8, analyzed by flow cytometry with PI staining.

(H) Gross tumor burden from the Ad-*Cas9-gPten* plus AAV-*KPL* experiment: four doses of *Anln, E2f8* and scramble siRNAs (siCtrl) were injected into WT FVB strain mice starting at P10. One week later, the mice were injected with Ad-*Cas9-sgPten* (10^9 pfu/mouse) and AAV-*KPL* (10^{12} pfu/mouse). Tumor burden 2 months after virus injection is shown. Liver to body weight ratio (right).

Supplemental Table

Table S1. Sequences, efficiencies, and R^2 of qPCR primers used in the paper. Related to STAR Methods.

Primer name	Sequence	Efficiency	R^2
mAnln-F	TGACGCTCTGACATTTCCTAC	95.01%	0.9968
mAnln-R	GTAATAGCCTTGGACTTGGAGG		
mE2f8-F	TCTCAAAATTCTCCACCCGTC	99.90%	0.9968
mE2f8-R	TTGTAGTGCCCAGATCTTGC		
mActinB-F	CAGAAGGAGATTACTGCTCTGGCT	91.59%	0.9995
mActinB-R	TACTCCTGCTTGCTGATCCACATC		
mALB-F	GACCAGGAAGTGTGCAAGAA	95.20%	0.9967
mALB-R	CAAGTCTCAGCAACAGGGATAC		
mHNF1a-F	CCTTGGTGGAGGAGTGTAATAG	93.96%	0.9970
mHNF1a-R	GTTGGCAAACCAGTTGTAGAC		
mHNF1b-F	CCTCTCTCAACACCTCAACAAG	93.76%	0.9950
mHNF1b-R	GAGGATCTCCCGTTGCTTTC		
mHNF4a-F	TGCCTCAAAGCCATCATCTT	98.59%	0.9986
mHNF4a-R	GTAATCCTCCAGGCTCACTTG		
mFOXA3-F	GACATCGACACCTTCCAAACT	96.65%	0.9934
mFOXA3-R	TAAGCAGAGAGCGGGAATAGA		
mC/EBPa-F	CAAGAAGTCGGTGGACAAGAA	99.55%	0.9982
mC/EBPa-R	CGTTGCGTTGTTTGGCTTTA		
mC/EBPb-F	CGCGACAAGGCCAAGAT	90.18%	0.9967
mC/EBPb-R	GCTGCTCCACCTTCTTCTG		
mGS-F	AATGGACATGGTGAGCAACC	91.83%	0.9970
mGS-R	GGTAGTGAGCCTCCACGATG		
mCyp2e1-F	TTTCTGCAGGAAAGCGCG	96.95%	0.9995
mCyp2e1-R	CTGCCAAAGCCAATTGTAACAG		
mCyp2a12-F	GTCAGCTCCACACTACGATATG	93.69%	0.9989
mCyp2a12-R	GCCAATCACTCGGTCAATCT		
mCyp2a22-F	TGAGACAGTCAGCTCCTTACTA	98.31%	0.9998
mCyp2a22-R	GCCAATCACTCGGTCAATCT		
mCyp2a4-F	CTTCATCGACTCCTTCCTCATC	88.08%	0.9999
mCyp2a4-R	GTGCCAGCAAAGAAGAGATTTAG		
mCyp2a5-F	CAACCCAAAGCACTTCCTAGA	92.38%	0.9993
mCyp2a5-R	CCAGTCCTTCTCCGAAACAATA		
mCyp2c29-F	CCAATCCTTCACCAACTTCTCA	94.79%	0.9999
mCyp2c29-R	AGCTTCCTTCACTGCTTCATAC		
mCyp2c39-F	GAGGAAGCATTCCAATGGTAGA	92.23%	0.9998
mCyp2c39-R	TGAGTGTGAAGCGCCTAATC		
mCyp2c50-F	CAGAGACAACAAGCACAACAC	97.31%	0.9993
mCyp2c50-R	GCCGATCACATGCTCAATTTC		
mCyp2j5-F	CTGGTGGAAGCCATAAGAGAG	98.33%	0.9999
mCyp2j5-R	CCAAAGGTGACAGAGCAAATG		
mCyp3a11-F	ACCACCAGTAGCACACTTTC	96.95%	0.9991
mCyp3a11-R	CCAGGTATTCCATCTCCATCAC		

mCyp4a10-F	CCCTGATGGACGCTCTTTAC	95.39%	0.9993
mCyp4a10-R	GGGTCAAACACCTCTGGATT		
mCyp4f14-F	TCTGTCTCATCAGCATCTTTGG	93.34%	0.9991
mCyp4f14-R	CCGCCGAGAAGGGAATAAAT		
mCcna2-F	TGAATCACCACATGCTAT	88.60%	0.9945
mCcna2-R	TAACCTCCATTTCCCTAAG		
mCcnb1-F	CTCTGTAGTGAATATGTG	96.77%	0.9811
mCcnb1-R	CATCTGAACCTGTATTAG		
mCcnb2-F	TCTTGCCTGTCTCAGAAG	94.15%	0.9999
mCcnb2-R	CTCCATGTAGCCTGTGTAA		
mKi67-F	AGAAGTCCAGGTCTACAG	88.23%	0.9871
mKi67-F	TCGTTGCTATTGCTAAGG		
mCcnd1-F	TGCCATCCATGCGGAAA	94.37%	0.9994
mCcnd1-R	AGCGGGAAGAACTCCTCTTC		
hcMYC-F	ACCAGATCCCGGAGTTGGAA	99.55%	0.9984
hcMYC-R	CGTCGTTTCCGCAACAAGTC		