Expanded View Figures

Figure EV1. p53 restricts cholesterol synthesis.

A *p53* wild-type and knockout mice were examined by genotyping. $p53^{+/+}$ product size: 281 bp. $p53^{-/-}$ product size: 441 bp (left). Representative mice photos are shown (right).

B Weekly body weight gain of mice is shown.

C, D Cholesterol concentration of SK-HEP-1 (C) and BEL-7402 (D) cells treated with control or two sets of *p53* siRNA for 72 h.

E, F Differential expression genes from RNA-seq of mice livers from $p53^{+/+}$ and $p53^{-/-}$ mice fed with normal (E) or HFD (F) diet were analyzed by REACTOME Database Enrichment analysis. Dot plots show the 20 most significantly enriched gene biological process. Dot sizes represent counts of enriched differential expressed genes. Dot color scale changes from red to blue, with blue indicating lower adjusted *P*-value (*P*adj) for the category.

Data information: (C, D) Bars represent mean ± s.d., $^{**}P < 0.01$; $^{***}P < 0.001$; (B) $n = 6$-8 biologically independent samples; (C, D) $n = 3$ biologically independent samples; statistical significance was determined by two-tailed unpaired *t*-test.

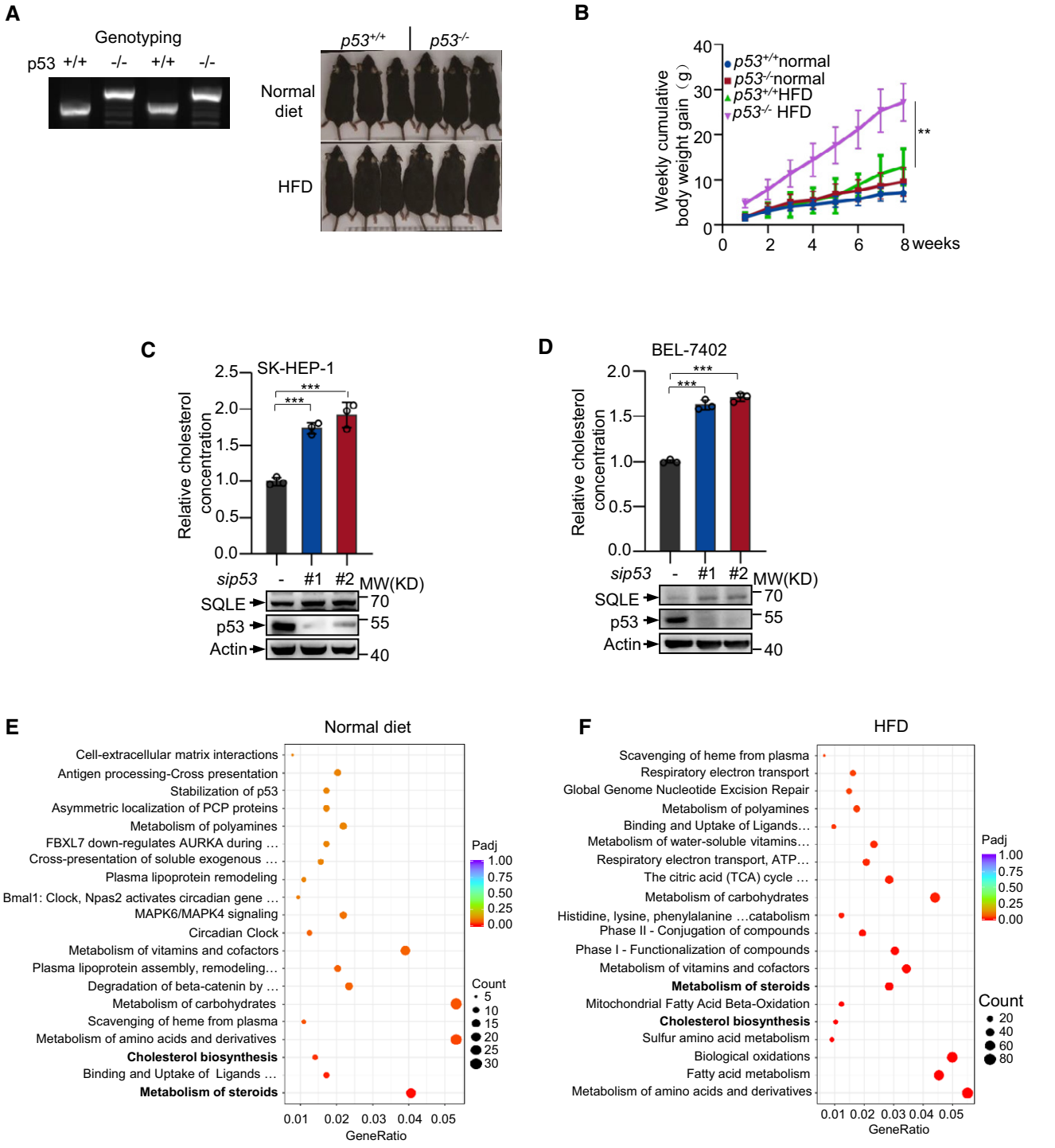
A
Genotyping
p53 +/+ -/- +/+ -/-
p53+/+
p53-/-
Normal diet
HFD
B
Weekly cumulative body weight gain (g)
p53+/+normal
p53-/-normal
p53+/+HFD
p53-/- HFD
**
weeks
C
SK-HEP-1
Relative cholesterol concentration
***
***
sip53 - #1 #2
MW(KD)
SQLE
p53
Actin
70
55
40
D
BEL-7402
Relative cholesterol concentration
***
***
sip53 - #1 #2
MW(KD)
SQLE
p53
Actin
70
55
40
E
Normal diet
Cell-extracellular matrix interactions
Antigen processing-Cross presentation
Stabilization of p53
Asymmetric localization of PCP proteins
Metabolism of polyamines
FBXL7 down-regulates AURKA during …
Cross-presentation of soluble exogenous …
Plasma lipoprotein remodeling
Bmal1: Clock, Npas2 activates circadian gene …
MAPK6/MAPK4 signaling
Circadian Clock
Metabolism of vitamins and cofactors
Plasma lipoprotein assembly, remodeling…
Degradation of beta-catenin by …
Metabolism of carbohydrates
Scavenging of heme from plasma
Metabolism of amino acids and derivatives
Cholesterol biosynthesis
Binding and Uptake of Ligands …
Metabolism of steroids
Padj
Count
GeneRatio
F
HFD
Scavenging of heme from plasma
Respiratory electron transport
Global Genome Nucleotide Excision Repair
Metabolism of polyamines
Binding and Uptake of Ligands…
Metabolism of water-soluble vitamins…
Respiratory electron transport, ATP…
The citric acid (TCA) cycle …
Metabolism of carbohydrates
Histidine, lysine, phenylalanine …catabolism
Phase II - Conjugation of compounds
Phase I - Functionalization of compounds
Metabolism of vitamins and cofactors
Metabolism of steroids
Mitochondrial Fatty Acid Beta-Oxidation
Cholesterol biosynthesis
Sulfur amino acid metabolism
Biological oxidations
Fatty acid metabolism
Metabolism of amino acids and derivatives
Padj
Count
GeneRatio

Figure EV1.

Figure EV2. p53 inhibits SQLE expression.

A–C mRNA and protein expression of SQLE in BEL-7402 (A) and SK-HEP-1 (B and C) treated with control or *p53* siRNA for 48 h.

D SQLE protein expression and mRNA levels in MCF-7 cells stably expressing control or *p53* shRNA.

E $p53^{+/+}$ and $p53^{-/-}$ HCT116 cells were treated with Fatostatin (10 μM) for 24 h. mRNA and protein expression were analyzed by qRT–PCR and Western blotting respectively.

F H1299 p53-inducible cells treated with or without doxycycline (1 μg/ml) were transfected with control siRNA or *SREBP2* siRNA for 48 h. mRNA and protein expression were examined as indicated.

G, H Control and *SREBP2* knockout MCF-7 cells (G) or SK-HEP-1 cells (H) using sgRNA CRISPR/Cas9 were treated with control siRNA or *p53* siRNA for 48 h. mRNA and protein expression were examined as indicated.

I mRNA and protein expression of SQLE and p53 in MCF-7 (wild-type p53), MDA-MB-468 (mutant p53 R273H), SK-BR-3 (mutant p53 R175H), and MDA-MB-435s (mutant p53 G266E) cells treated with *p53* or control siRNA for 48 h.

J H1299 cells were transfected with increasing amounts of control plasmid or plasmid expressing wild-type (PRK5-flag p53) or mutant p53 (PRK5-flag-p53-G266E, PRK5-HA-p53-R273H, and PRK5-HA-p53-R175H) for 24 h as indicated. Cell lysates were analyzed by Western blotting. Relative SQLE/actin ratios are shown below. Data represent three independent experiments.

K Human HCC TCGA datasets were analyzed to determine whether tumors bearing wild-type p53 correlate with lower expression of SQLE. Patients were classified by p53 status (wild-type versus mutant). SQLE exhibited higher expression levels in p53 mutant (mut, $n = 220$) HCC tumors compared with wild-type ($n = 508$) p53 tumors.

L SQLE expression in p53 wild-type ($n = 307$) and p53 mutant (mut, $n = 795$) human breast tumors (BRCA) of the TCGA database.

Data information: (A–I) Bars represent mean ± s.d., $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $n = 3$ biologically independent samples; statistical significance was determined by two-tailed unpaired *t*-test.

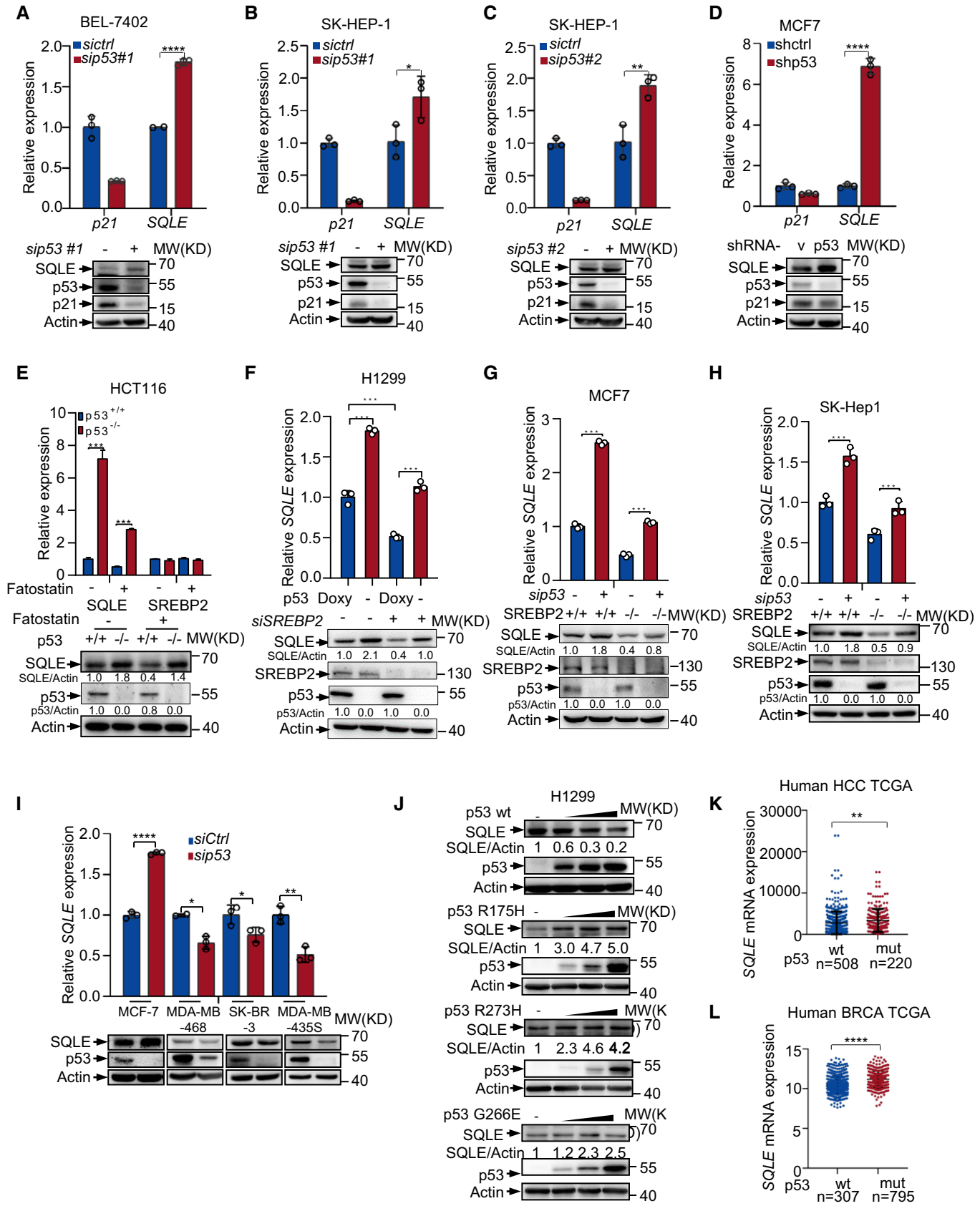
A
BEL-7402
sictrl
sip53#1
Relative expression
p21
SQLE
sip53 #1 - + MW(KD)
SQLE
p53
p21
Actin
70
55
15
40
B
SK-HEP-1
sictrl
sip53#1
Relative expression
p21
SQLE
sip53 #1 - + MW(KD)
SQLE
p53
p21
Actin
70
55
15
40
C
SK-HEP-1
sictrl
sip53#2
Relative expression
p21
SQLE
sip53 #2 - + MW(KD)
SQLE
p53
p21
Actin
70
55
15
40
D
MCF7
shctrl
shp53
Relative expression
p21
SQLE
shRNA- v p53 MW(KD)
SQLE
p53
p21
Actin
70
55
15
40
E
HCT116
p53 +/+
p53 -/-
Relative expression
Fatostatin - + - +
SQLE SREBP2
Fatostatin - +
p53 +/+ -/- +/+ -/- MW(KD)
SQLE
SQLE/Actin 1.0 1.8 0.4 1.4
p53
p53/Actin 1.0 0.0 0.8 0.0
Actin
70
55
40
F
H1299
Relative SQLE expression
p53 Doxy - Doxy -
siSREBP2 - - + + MW(KD)
SQLE
SQLE/Actin 1.0 2.1 0.4 1.0
SREBP2
p53
p53/Actin 1.0 0.0 1.0 0.0
Actin
70
130
55
40
G
MCF7
Relative SQLE expression
sip53 - + - +
SREBP2 +/+ +/+ -/- -/- MW(KD)
SQLE
SQLE/Actin 1.0 1.8 0.4 0.8
SREBP2
p53
p53/Actin 1.0 0.0 1.0 0.0
Actin
70
130
55
40
H
SK-Hep1
Relative SQLE expression
sip53 - + - +
SREBP2 +/+ +/+ -/- -/- MW(KD)
SQLE
SQLE/Actin 1.0 1.8 0.5 0.9
SREBP2
p53
p53/Actin 1.0 0.0 1.0 0.0
Actin
70
130
55
40
I
Relative SQLE expression
siCtrl
sip53
MCF-7
MDA-MB -468
SK-BR -3
MDA-MB -435S
MW(KD)
SQLE
p53
Actin
70
55
40
J
H1299
p53 wt
MW(KD)
SQLE
SQLE/Actin 1 0.6 0.3 0.2
p53
Actin
70
55
40
p53 R175H
MW(KD)
SQLE
SQLE/Actin 1 3.0 4.7 5.0
p53
Actin
70
55
40
p53 R273H
MW(K
SQLE
SQLE/Actin 1 2.3 4.6 4.2
p53
Actin
70
55
40
p53 G266E
MW(K
SQLE
SQLE/Actin 1 1.2 2.3 2.5
p53
Actin
70
55
40
K
Human HCC TCGA
SQLE mRNA expression
p53
wt n=508
mut n=220
L
Human BRCA TCGA
SQLE mRNA expression
p53
wt n=307
mut n=795

Figure EV2.

Figure EV3. Murine p53 transcriptionally regulates mouse SQLE.

A $p53^{+/+}$ and $p53^{-/-}$ HepG2 cells were treated with PFTα (20 μM) for 24 h. mRNA and protein expression were analyzed by qRT–PCR and Western blotting respectively.
B mRNA and protein expression of H1299 p53-inducible cells (wild-type versus R175H mutant) treated with or without doxycycline (1 μg/ml).
C $p53^{+/+}$ and $p53^{-/-}$ HepG2 cells treated with or without PFTα (20 μM) for 24 h were analyzed by chromatin immunoprecipitation (ChIP) assay using normal IgG and anti-p53 antibody as indicated.
D Schematic representation of mouse *SQLE* genomic structure. Shown are the exon/intron organization and two potential p53 binding sites (BS1 and BS2) and the corresponding mutant binding sites.
E $p53^{+/+}$ and $p53^{-/-}$ mice livers were analyzed by ChIP assay using normal IgG and anti-p53 antibody.
F $p53^{+/+}$ mice livers from Normal or HFD mice were analyzed by ChIP assay using normal IgG and anti-p53 antibody.
G Luciferase reporter constructs containing mouse *SQLE* potential binding sites BS1 and BS2 were transfected into HEK293T cells together with control or mouse p53 expression vector for 48 h. Renilla vector pRL-CMV was used as a transfection internal control. Relative levels of luciferase are shown. Protein expression is shown.
H Luciferase reporter constructs containing mouse *SQLE* potential binding sites BS1, BS2, and their corresponding mutant binding sites (BS1 mut and BS2 mut) were transfected into HEK293T cells together with control or mouse p53 expression vector for 48 h. Renilla vector pRL-CMV was used as a transfection internal control. Relative levels of luciferase are shown. Protein expression is shown.

Data information: (A, B, C, E, F, G, H) Bars represent mean ± s.d., *$P < 0.05$; ***$P < 0.001$; ****$P < 0.0001$; $n = 3$ biologically independent samples; statistical significance was determined by two-tailed unpaired *t*-test.

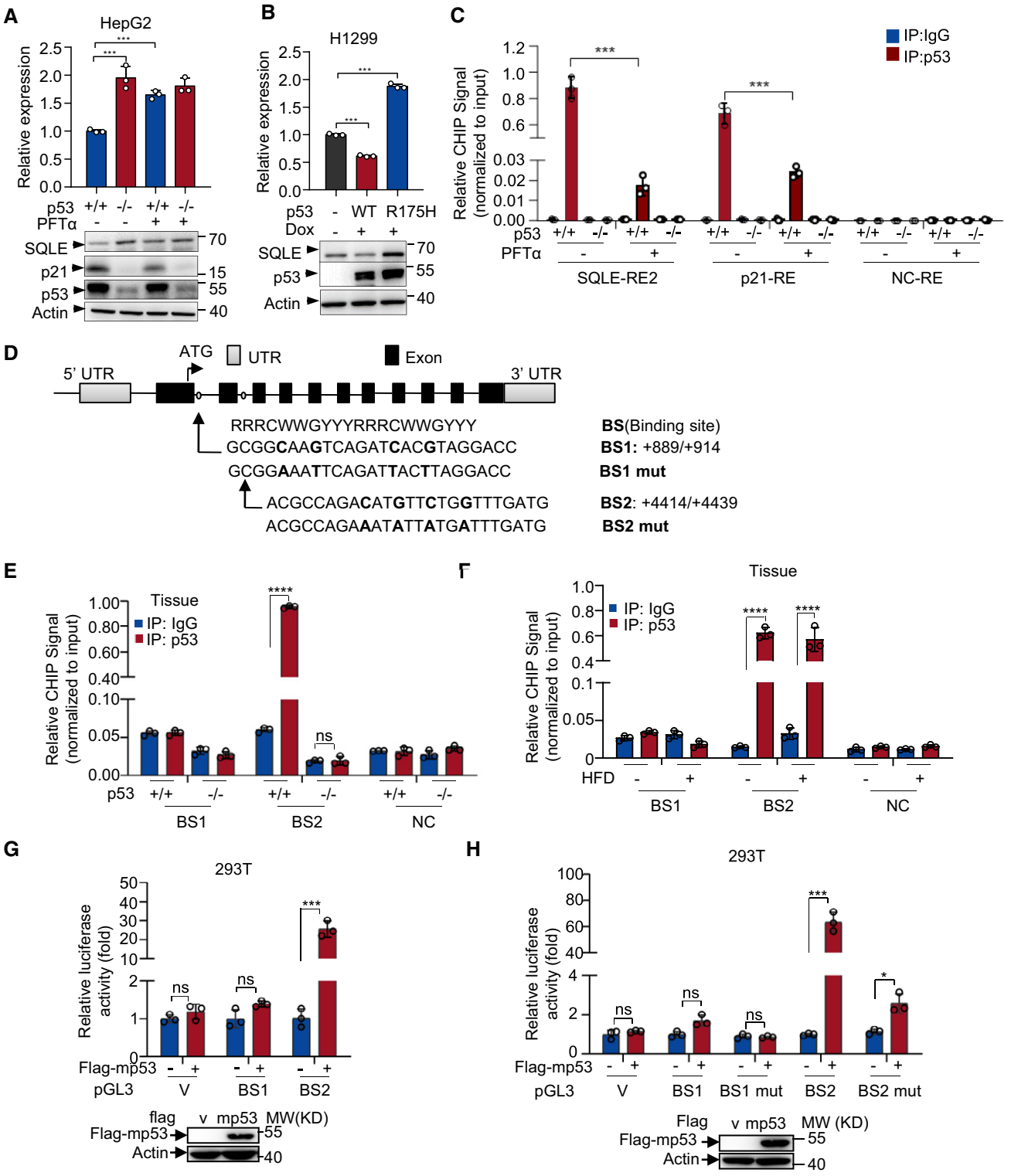
A
HepG2
Relative expression
p53 +/+ -/- +/+ -/-
PFTα - - + +
SQLE
p21
p53
Actin
70
15
55
40
B
H1299
Relative expression
p53 - WT R175H
Dox - + +
SQLE
p53
Actin
70
55
40
C
IP:IgG
IP:p53
Relative CHIP Signal (normalized to input)
p53 +/+ -/- +/+ -/- +/+ -/- +/+ -/- +/+ -/- +/+ -/-
PFTα - + - + - + - + - +
SQLE-RE2
p21-RE
NC-RE
D
5' UTR
ATG
UTR
Exon
3' UTR
RRRCWWGYYYRRRCWWGYYY
GCGGCAAGTCAGATCACGTAGGACC
GCGGAAATTCAGATTACTTAGGACC
ACGCCAGACATGTTCTGGTTTGATG
ACGCCAGAAATATTATGATTTGATG
BS(Binding site)
BS1: +889/+914
BS1 mut
BS2: +4414/+4439
BS2 mut
E
Tissue
IP: IgG
IP: p53
Relative CHIP Signal (normalized to input)
p53 +/+ -/- +/+ -/- +/+ -/-
BS1
BS2
NC
ns
F
Tissue
IP: IgG
IP: p53
Relative CHIP Signal (normalized to input)
HFD - + - + - +
BS1
BS2
NC
G
293T
Relative luciferase activity (fold)
Flag-mp53 - + - + - +
pGL3
V
BS1
BS2
flag v mp53 MW(KD)
Flag-mp53
Actin
55
40
H
293T
Relative luciferase activity (fold)
Flag-mp53 - + - + - + - + - +
pGL3
V
BS1
BS1 mut
BS2
BS2 mut
Flag v mp53 MW (KD)
Flag-mp53
Actin
55
40

Figure EV3.

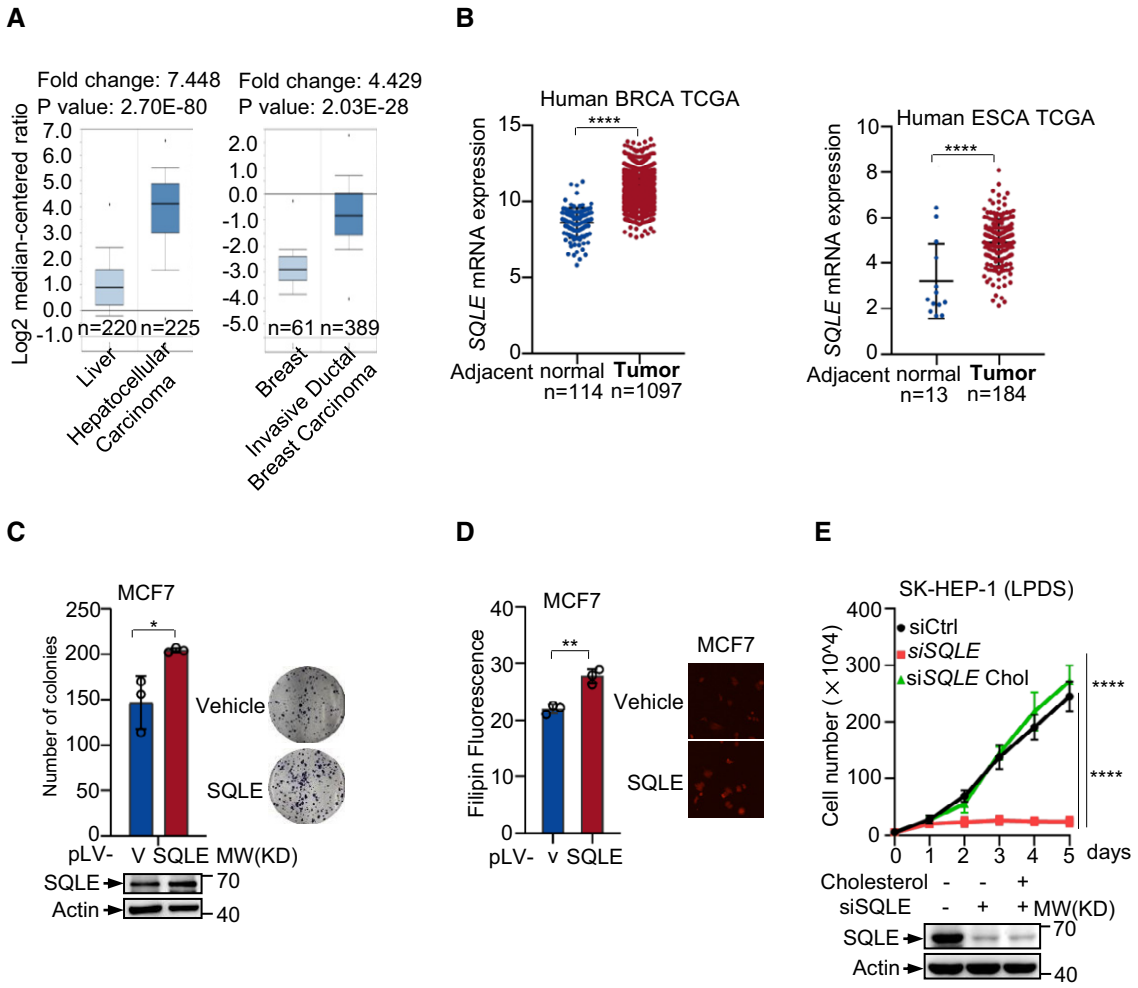

Figure EV4. SQLE is critical for tumor cell growth.

A Box plot comparing *SQLE* transcript levels in hepatocellular carcinoma, invasive ductal breast carcinoma, and their normal counterparts. The graphs were derived from ONCOMINE database. The differential expression data are centered on the median of expression levels and plotted on a log2 scale. Whiskers indicate minimum and maximum data values that are not outliers. The *P* value was calculated using a two-sample *t*-test. The number of samples (*n*) in each class is shown in below.

B *SQLE* gene expression in breast cancer (BRCA) and esophageal cancer (ESCA) were compared with adjacent normal tissue. Data were derived from TCGA-Breast cancer and TCGA-Esophageal cancer. Number of each sample (*n*, biological replicates) is shown. Bars represent mean ± s.d.

C Colony formation assay of MCF-7 cells stably overexpressing SQLE (pLV-SQLE) or control vector (pLV-v) as indicated (left). Representative images of stained colonies were shown (right). Protein expressions are shown by Western blotting.

D Cholesterol concentrations of stably overexpressing SQLE or control vector MCF-7 cells were determined by Filipin III staining.

E SK-HEP-1 cells transfected with control or *SQLE* siRNA were cultured in LPDS medium containing 5 μg cholesterol as indicated. Cell proliferation is shown. Protein expression was analyzed after transfection 48 h.

Data information: (C, D, E) Bars represent mean ± s.d., $*P < 0.05$; $**P < 0.01$; $****P < 0.0001$; $n = 3$ biologically independent samples; statistical significance was determined by two-tailed unpaired *t*-test.

Figure EV5. p53 modulates tumor cell growth through SQLE and cholesterol.

A Proliferation of $p53^{+/+}$ and $p53^{-/-}$ HCT116 cells treated with control or *SQLE* siRNA. Protein expression was examined after transfection 48 h.

B Growth of $p53^{+/+}$ and $p53^{-/-}$ HepG2 cells (left panel) and HCT116 cells (right panel) treated with DMSO or 30 μm Terbinafine.

C Growth of $p53^{+/+}$ and $p53^{-/-}$ HCT116 cells transfected with or without exogenous SQLE (HA-SQLE) in the LPDS medium. Protein expression is shown.

D Colonies number of $p53^{+/+}$ and $p53^{-/-}$ HCT116 cells expressing control or *SQLE* siRNA. A number of colonies with a diameter greater than 10 μm were quantified.

E Cell migration of $p53^{+/+}$ and $p53^{-/-}$ HCT116 cells expressing control or *SQLE* siRNA for 48 h.

F, G Cholesterol concentration, protein expression (F), and cell proliferation (G) of $p53^{+/+}$ and $p53^{-/-}$ HCT116 cells in LPDS medium in presence or absence of 5 μg cholesterol.

H Percentage of senescence-associated β-galactosidase (SA-β-gal)-positive cells of $p53^{+/+}$ and $p53^{-/-}$ HCT116 cells expressing control or *SQLE* siRNA for 48 h. Protein expression is shown below.

I Numbers of Promyelocytic leukemia nuclear bodies (PML-NBs) in $p53^{+/+}$ and $p53^{-/-}$ HepG2 cells treated with control or *SQLE* siRNA for 48 h (top). Representative images are shown (bottom). Scale bar, 100 μm.

J Percentage of SA-β-gal-positive cells of $p53^{+/+}$ and $p53^{-/-}$ HepG2 cells cultured in LPDS medium in the presence or absence of cholesterol (top). Representative images are shown (bottom). Scale bar, 100 μm.

Data information: (A–J) Bars represent mean ± s.d., *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$; (A–D, F, G, I, J) $n = 3$ biologically independent samples; (E, H) $n = 5$ biologically independent samples; statistical significance was determined by two-tailed unpaired *t*-test.

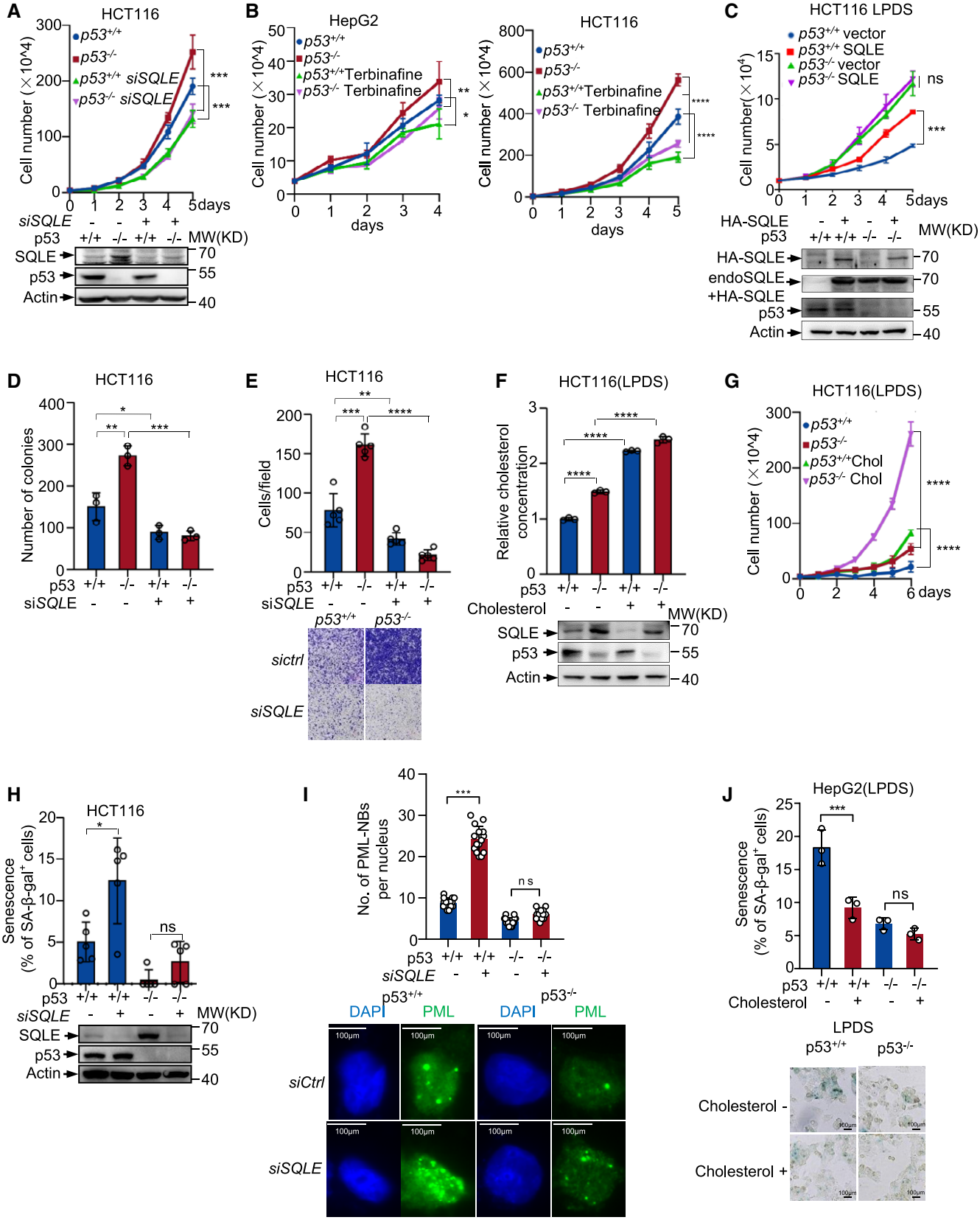

Figure EV5.