Supplementary Materials

Supplementary Materials and Methods

Lentiviral Transduction

Lentiviral particles for ELAVL1 knockdown (shELAVL1), overexpression (Lv-ELAVL1), HMGCR knockdown (shHMGCR), and overexpression (oeHMGCR) were generated using HEK293T cells. Target cells were infected with lentivirus with polybrene (8 μg/mL) and selected with puromycin (2 μg/mL) for 7 days. Non-targeting shRNA (shNC) or empty vector (Lv-Ctrl) was used as a control.

Subcellular Fractionation and Western Blotting

Cytoplasmic and nuclear protein extracts were prepared using a commercial kit (Beyotime). Total protein lysates were extracted with RIPA buffer additionally containing protease and phosphatase inhibitors. Proteins were separated by SDS-PAGE, transferred to PVDF membranes, and probed with primary antibodies against ELAVL1, HMGCR, SOAT1, DGAT1, DHCR7, CPT1A, ACSL1, PLIN2, ATGL, Bcl-2, Bax, cleaved caspase-3/9, TIMM23, TOM20, β-actin, and histone H3. HRP-conjugated secondary antibodies were used, and signals were visualized using ECL substrate (Pierce).

Immunohistochemistry (IHC) and Immunofluorescence (IF)

Paraffin-embedded tissue sections were first deparaffinized and rehydrated, followed by antigen retrieval. The sections were then incubated with the indicated primary

antibodies at 4 °C overnight. After washing, HRP-linked secondary antibodies were applied, and immunoreactivity was visualized using DAB. Finally, the slides were counterstained with hematoxylin.

For immunofluorescence analysis, cells or tissue samples were fixed in 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and blocked with 5% BSA prior to antibody incubation. Samples were then incubated with primary antibodies against ELAVL1, TIP47, or Tom20, followed by Alexa Fluor-labeled secondary antibodies. Nuclei were counterstained with DAPI or YO-PRO-1, and lipid droplets were detected using BODIPY 493/503. Fluorescence images were captured with a Zeiss LSM 800 confocal laser scanning microscope.

Lipid Staining and Quantification

Neutral lipids in cultured cells or frozen tissue sections were stained with Oil Red O (0.5% in isopropanol) for 15 min. After washing, images were captured under a light microscope. For quantitative detection, stained lipids were extracted with isopropanol, and absorbance was recorded at 510 nm. Cellular and tissue concentrations of total cholesterol (TC) and triglyceride (TG) were determined by commercially available assay kits (Nanjing Jiancheng Bioengineering Institute) following the manufacturer’s protocols.

RNA Extraction and qRT-PCR

Total RNA was extracted with TRIzol reagent (Invitrogen) according to the manufacturer's instructions and reverse-transcribed into cDNA using the ReverTra Ace

qPCR RT Kit (Toyobo). Quantitative PCR was carried out with UltraSYBR Mixture (Cwbio) on a Bio-Rad CFX96 real-time PCR system. Gene expression levels were normalized to GAPDH and analyzed using the 2^−ΔΔCt method

Cell Viability and Apoptosis Assays

Cell proliferation was assessed using the CCK-8 assay at 0, 24, 48, and 72 h. For apoptosis analysis, cells were stained with Annexin V-FITC and propidium iodide (PI) and analyzed by flow cytometry (BD FACSCanto II). Data were processed using FlowJo software.

Electron Microscopy (EM)

Cells were fixed in 2.5% glutaraldehyde and subsequently post-fixed with 1% osmium tetroxide. After dehydration through a graded ethanol series, samples were embedded in epoxy resin. Ultrathin sections were prepared, stained with uranyl acetate and lead citrate, and observed using a Hitachi HT7700 transmission electron microscope.

Live Observation of Lipid Droplets

Following the designated treatments, fluorescence images of the cells were acquired using a BX53 microscope. Dynamic changes in lipid droplets were then continuously recorded over a 12-h period using both the Axio Observer 7 live-cell imaging system and the Nanolive label-free 3D holographic imaging system.

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Supplementary Figures

Figure S1

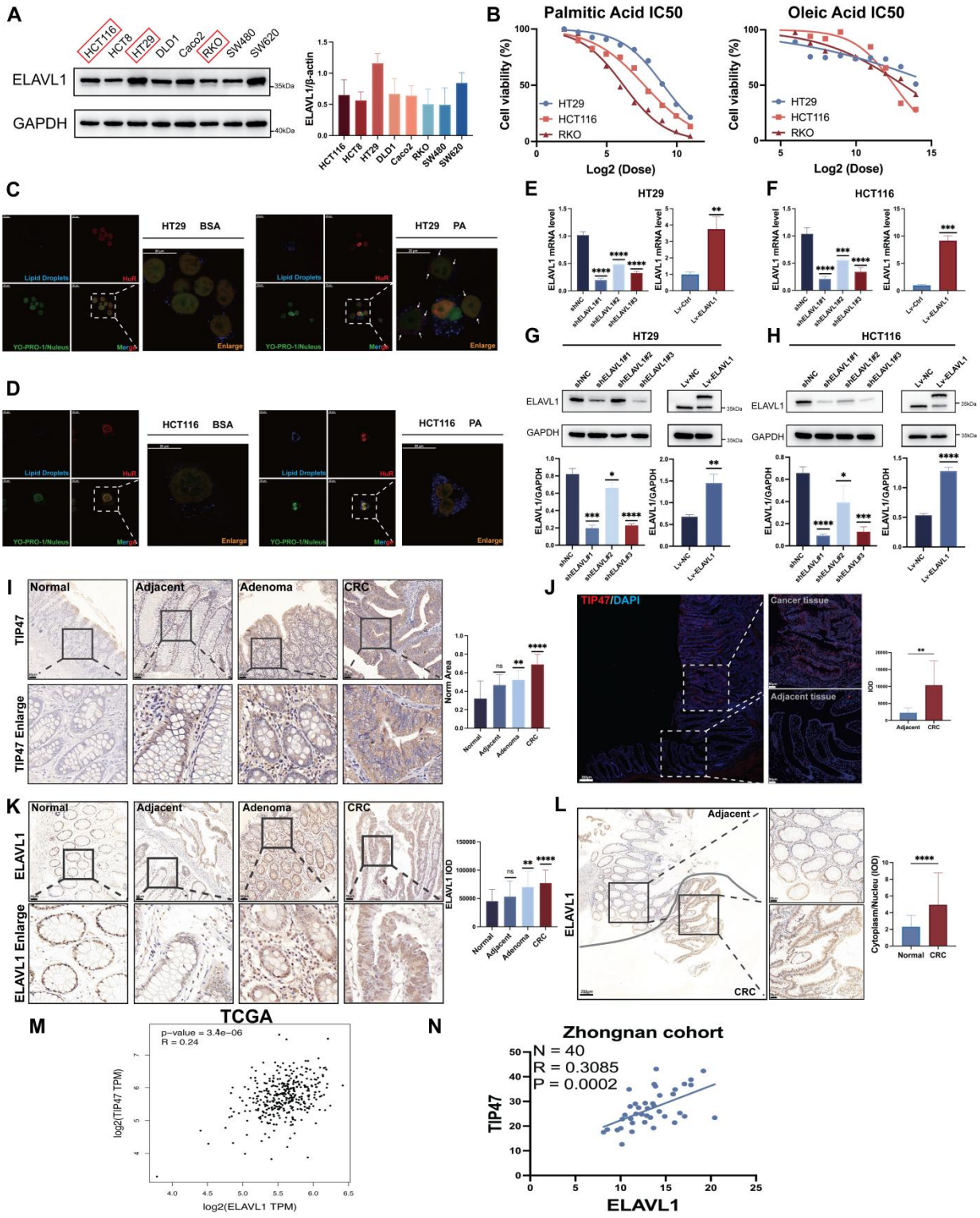


Figure S1.

A. Basal ELAVL1 protein in CRC cell lines. n = 3.

B. IC50 of PA and OA in HT29, HCT116, and RKO cells.

C–D. Lipid-droplet staining after PA treatment in HT29 (C) and HCT116 (D). Scale bar, 20 μm.

E–H. qPCR and WB validation of ELAVL1 KD and OE in HT29 (E, F) and HCT116 (G, H). n = 3.

I–J. TIP47 IHC (I) and IF (J) in human normal, adenoma and CRC tissues. Scale bars 50 μm and 200 μm. n = 10.

K–L. ELAVL1 IHC in CRC TMAs. Cytoplasmic/nuclear IOD ratio quantified. Scale bars 200 μm and 50 μm.

M–N. ELAVL1 expression in CRC metabolic subtypes from TCGA and Zhongnan Hospital cohorts.

ns $P \geq 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

Figure S2

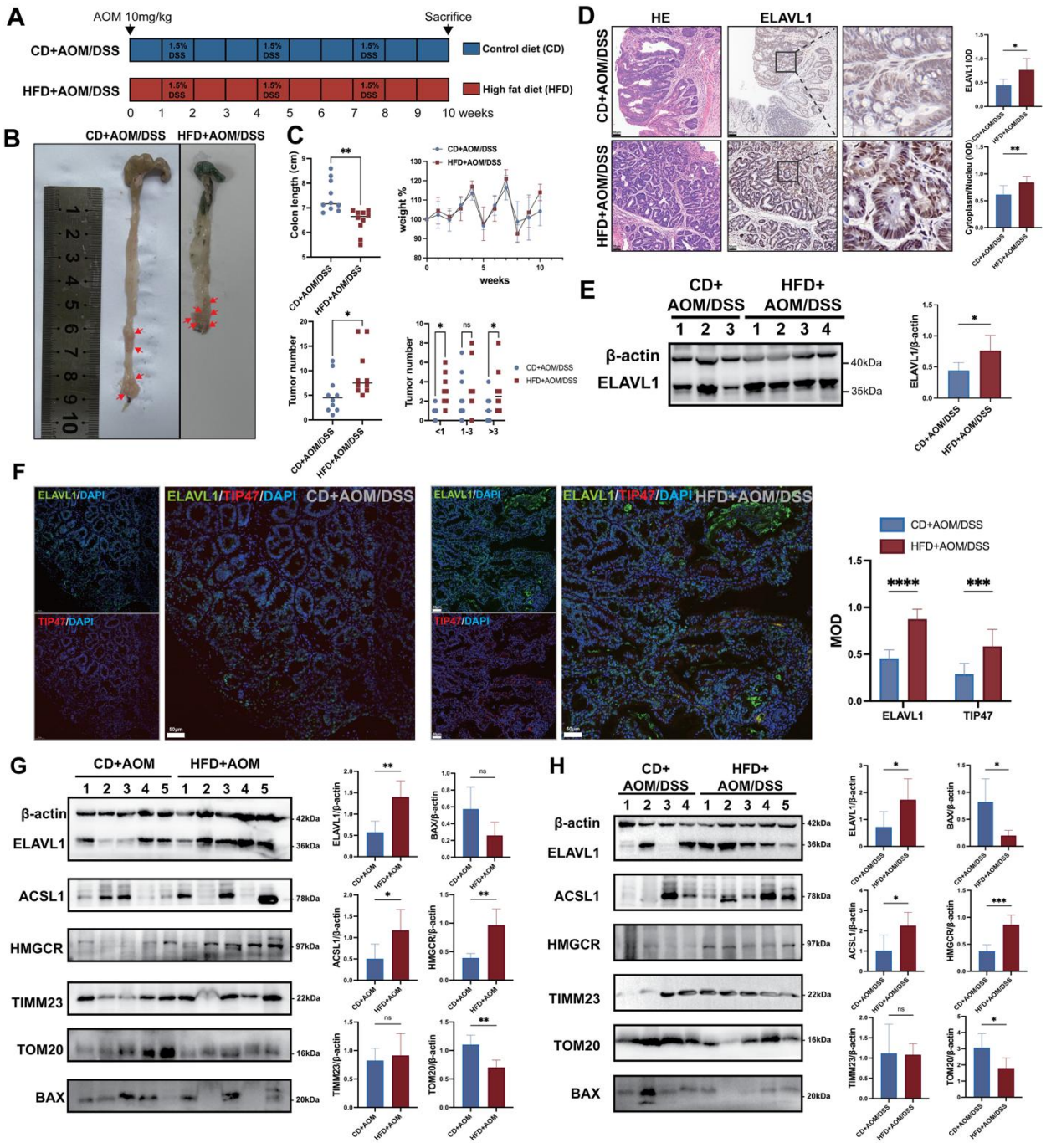

A
AOM 10mg/kg
Sacrifice
CD+AOM/DSS
HFD+AOM/DSS
1.5% DSS
Control diet (CD)
High fat diet (HFD)
0 1 2 3 4 5 6 7 8 9 10 weeks
B
CD+AOM/DSS
HFD+AOM/DSS
C
Colon length (cm)
weight %
weeks
Tumor number
<1
1-3
>3
D
HE
ELAVL1
ELAVL1 IOD
Cytoplasm/Nucleu (IOD)
E
CD+ AOM/DSS
HFD+ AOM/DSS
β-actin
ELAVL1
40kDa
35kDa
ELAVL1/β-actin
F
ELAVL1/DAPI
TIP47/DAPI
ELAVL1/TIP47/DAPI
MOD
ELAVL1
TIP47
G
CD+AOM
HFD+AOM
ACSL1
HMGCR
TIMM23
TOM20
BAX
H

Figure S2.

A. HFD+AOM/DSS protocol schematic.

B. Gross intestine images of CD+AOM/DSS and HFD+AOM/DSS groups.

C. Colon length, body weight, tumour counts, and size of CD+AOM/DSS and HFD+AOM/DSS groups. n = 10.

D. ELAVL1 IHC with cytoplasmic/nuclear IOD ratio of CD+AOM/DSS and HFD+AOM/DSS groups. Scale bar, 50 μm.

E. WB of ELAVL1 proteins in CD+AOM/DSS and HFD+AOM/DSS groups. n = 3.

F. IF of ELAVL1 and TIP47 of CD+AOM/DSS and HFD+AOM/DSS groups. Scale bar, 50 μm.

G–H. WB profiling (ELAVL1, ACSL1, HMGCR, TIMM23, TOM20, BAX) in HFD+AOM (G) and HFD+AOM/DSS (H) models. n = 5.

ns $P \geq 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

Figure S3

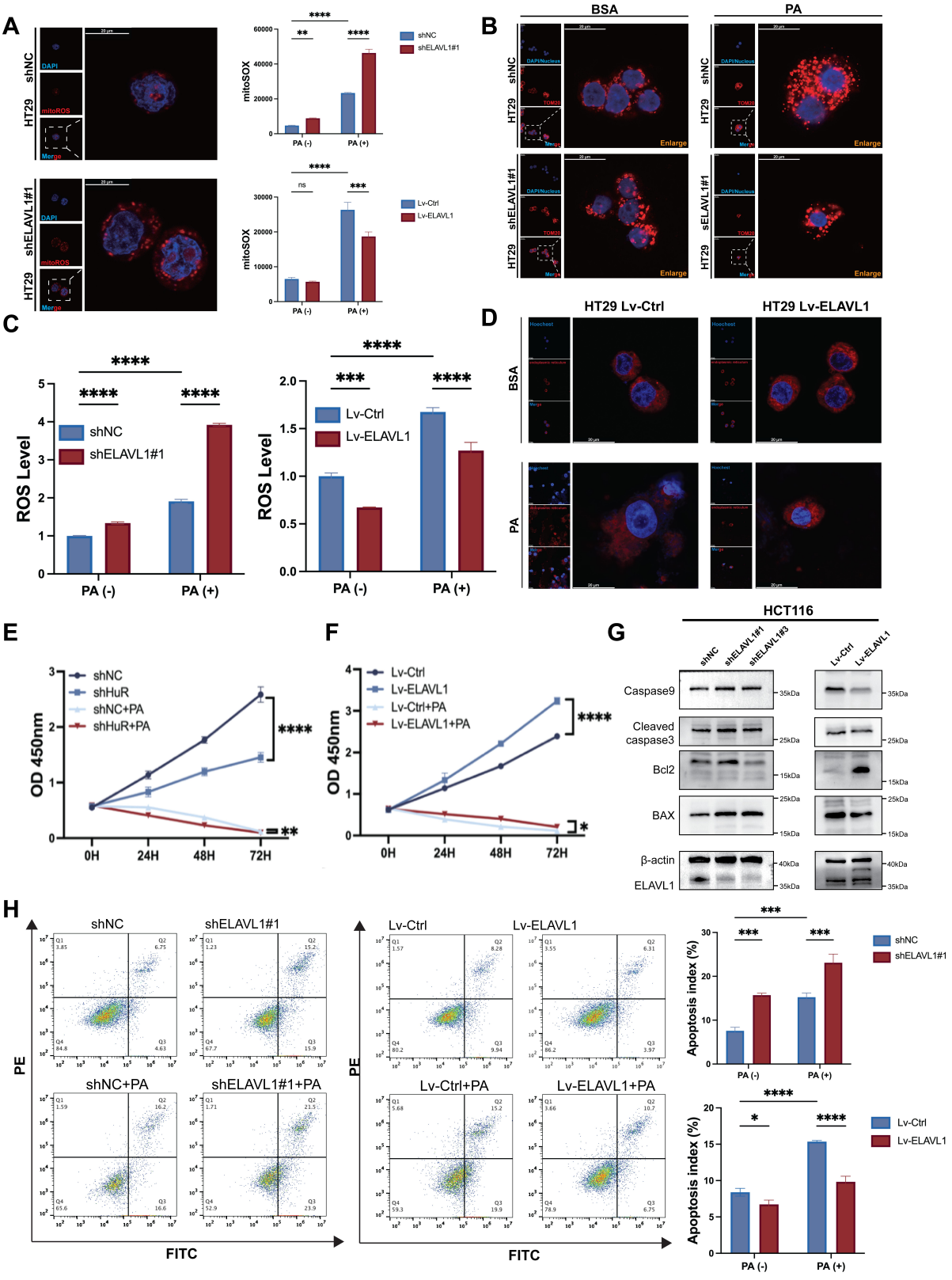

A
shNC
shELAVL1#1
HT29
DAPI
mitoROS
Merge
mitoSOX
shNC
shELAVL1#1
Lv-Ctrl
Lv-ELAVL1
PA (-)
PA (+)
B
BSA
PA
DAPI/Nucleus
TOM20
Enlarge
C
ROS Level
D
HT29 Lv-Ctrl
HT29 Lv-ELAVL1
Hoechest
E
shNC
shHuR
shNC+PA
shHuR+PA
OD 450nm
0H
24H
48H
72H
F
Lv-Ctrl
Lv-ELAVL1
Lv-Ctrl+PA
Lv-ELAVL1+PA
G
HCT116
shNC
shELAVL1#1
shELAVL1#3
Lv-Ctrl
Lv-ELAVL1
Caspase9
Cleaved caspase3
Bcl2
BAX
β-actin
ELAVL1
35kDa
25kDa
15kDa
40kDa
H
shNC
shELAVL1#1
shNC+PA
shELAVL1#1+PA
Lv-Ctrl
Lv-ELAVL1
Lv-Ctrl+PA
Lv-ELAVL1+PA
PE
FITC
Apoptosis index (%)

Figure S3.

A. Mito-SOX staining and flow cytometry. n = 3.

B. Mitochondrial morphology by fluorescence microscopy. Scale bar, 20 μm.

C. Total ROS flow cytometry.

D. ER-Tracker staining in HT29 cells.

E-F. Growth curves of HCT116 cells ± PA and ELAVL1 KD.

G. Apoptosis-related proteins in HCT116 cells.

H. Apoptosis rate in HT29 cells under PA stress.

ns $P \geq 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.

Figure S4

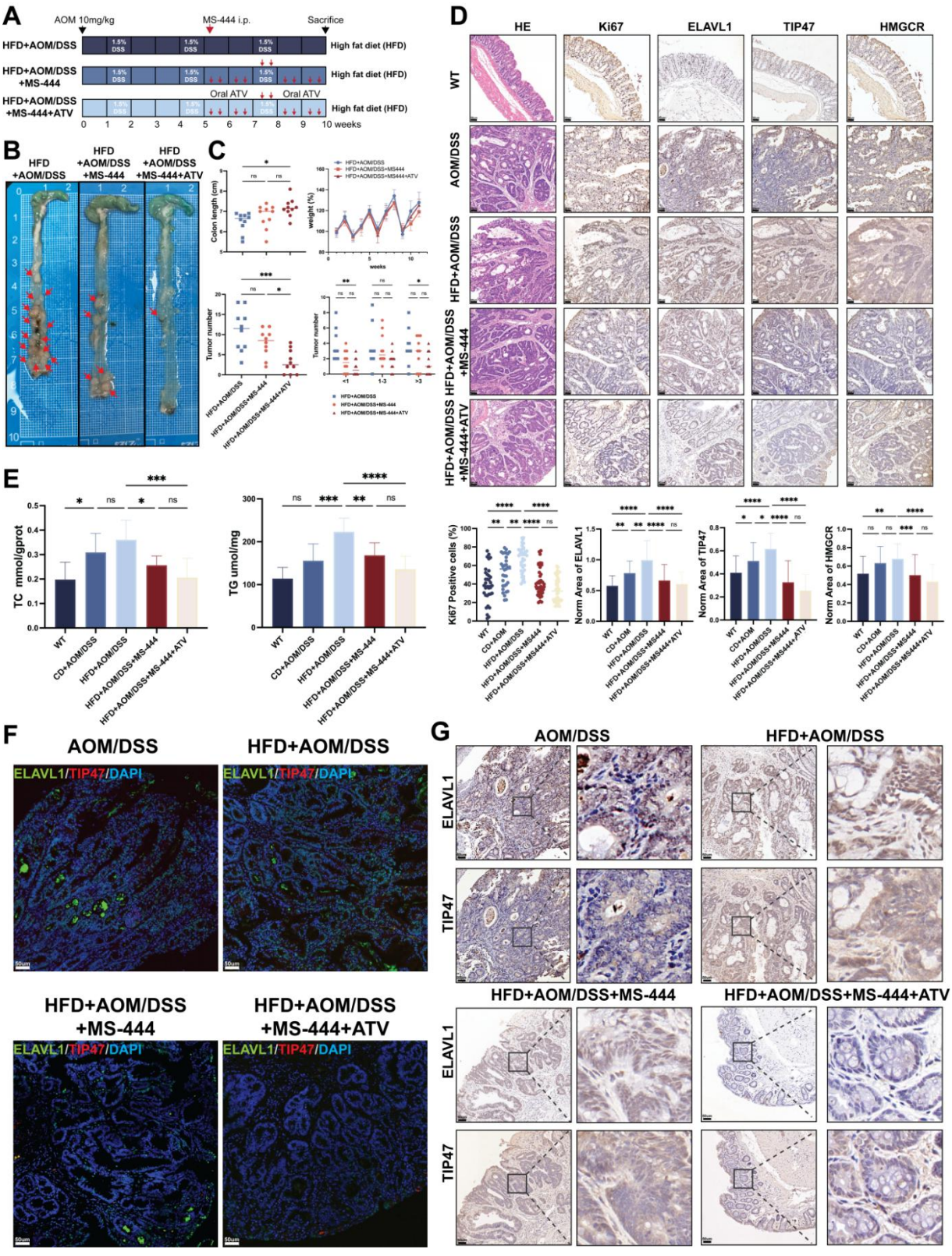

A
AOM 10mg/kg
MS-444 i.p.
Sacrifice
HFD+AOM/DSS
HFD+AOM/DSS +MS-444
HFD+AOM/DSS +MS-444+ATV
High fat diet (HFD)
Oral ATV
0 1 2 3 4 5 6 7 8 9 10 weeks
B
HFD +AOM/DSS
HFD +AOM/DSS +MS-444
HFD +AOM/DSS +MS-444+ATV
C
Colon length (cm)
weight (%)
weeks
Tumor number
D
HE
Ki67
ELAVL1
TIP47
HMGCR
WT
AOM/DSS
HFD+AOM/DSS
HFD+AOM/DSS +MS-444
HFD+AOM/DSS +MS-444+ATV
Ki67 Positive cells (%)
Norm Area of ELAVL1
Norm Area of TIP47
Norm Area of HMGCR
E
TC mmol/gprot
TG umol/mg
WT
CD+AOM/DSS
HFD+AOM/DSS
HFD+AOM/DSS+MS-444
HFD+AOM/DSS+MS-444+ATV
F
AOM/DSS
HFD+AOM/DSS
HFD+AOM/DSS +MS-444
HFD+AOM/DSS +MS-444+ATV
ELAVL1/TIP47/DAPI
50um
G
AOM/DSS
HFD+AOM/DSS
HFD+AOM/DSS+MS-444
HFD+AOM/DSS+MS-444+ATV
ELAVL1
TIP47

Figure S4.

A. HFD+AOM/DSS intervention scheme.

B. Gross intestinal images from HFD+AOM/DSS, HFD+AOM/DSS+MS444, HFD+AOM/DSS+MS444+ATV groups.

C. Colon length, body weight, tumour counts, and size of HFD+AOM/DSS, HFD+AOM/DSS+MS444, HFD+AOM/DSS+MS444+ATV groups.

D. H&E, Ki67, ELAVL1, TIP47, and HMGCR IHC of WT, AOM/DSS, HFD+AOM/DSS, HFD+AOM/DSS+MS444, HFD+AOM/DSS+MS444+ATV groups. The statistical chart is shown below. Scale bar, 50 μm.

E. Tissue TC and TG levels of WT, AOM/DSS, HFD+AOM/DSS, HFD+AOM/DSS+MS444, HFD+AOM/DSS+MS444+ATV groups. n = 5.

F–G. IF co-staining (F) and IHC (G) of ELAVL1 and TIP47 of AOM/DSS, HFD+AOM/DSS, HFD+AOM/DSS+MS444, HFD+AOM/DSS+MS444+ATV groups. Scale bar, 50 μm.

ns $P \geq 0.05$, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ****$P < 0.0001$.