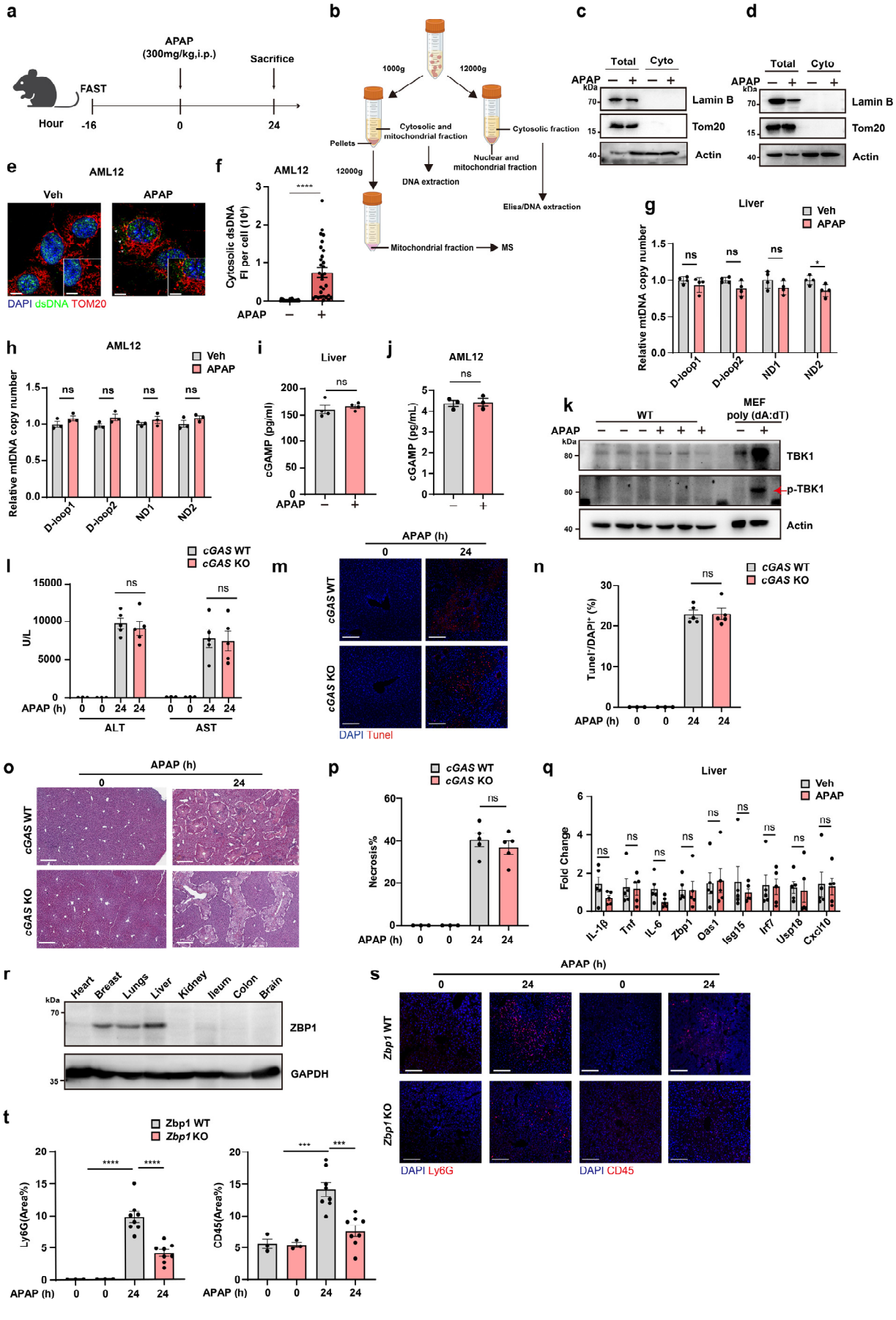
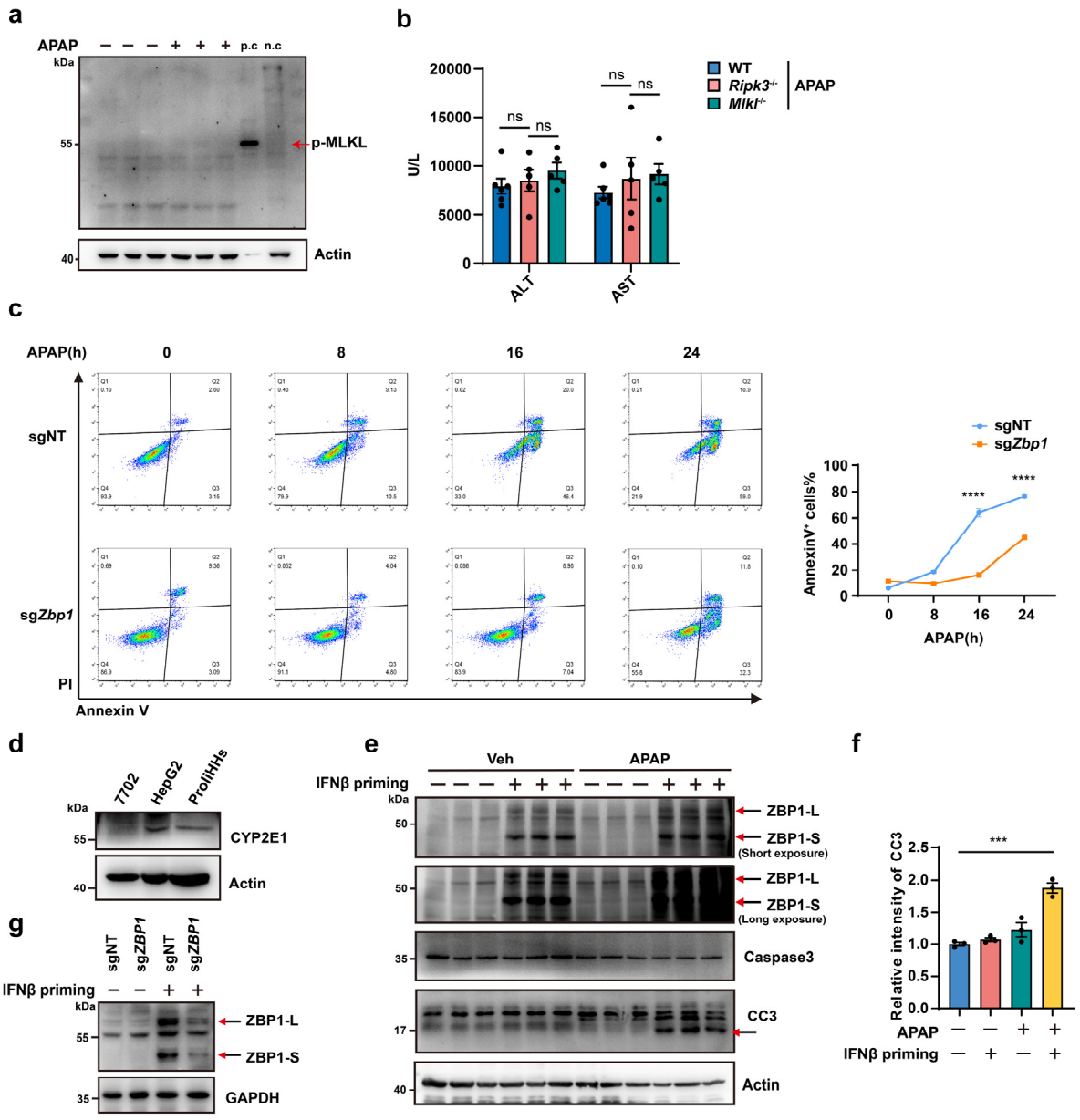

a
APAP
(300mg/kg,i.p.)
Sacrifice
FAST
Hour
-16
0
24
b
1000g
12000g
Pellets
Cytosolic and
mitochondrial fraction
Cytosolic fraction
Nuclear and
mitochondrial fraction
DNA extraction
Elisa/DNA extraction
12000g
Mitochondrial fraction
MS
c
Total
Cyto
APAP
kDa
70
15
40
Lamin B
Tom20
Actin
d
Total
Cyto
APAP
kDa
70
15
40
Lamin B
Tom20
Actin
e
AML12
Veh
APAP
DAPI dsDNA TOM20
f
AML12
****
Cytosolic dsDNA
FI per cell (10⁴)
APAP
g
Liver
Veh
APAP
Relative mtDNA copy number
ns
*
D-loop1
D-loop2
ND1
ND2
h
AML12
Veh
APAP
Relative mtDNA copy number
ns
D-loop1
D-loop2
ND1
ND2
i
Liver
cGAMP (pg/ml)
ns
APAP
j
AML12
cGAMP (pg/mL)
ns
k
WT
MEF
poly (dA:dT)
APAP
kDa
80
80
40
TBK1
p-TBK1
Actin
l
cGAS WT
cGAS KO
U/L
ns
APAP (h)
ALT
AST
m
APAP (h)
cGAS WT
cGAS KO
DAPI Tunel
n
cGAS WT
cGAS KO
Tunel⁺/DAPI⁺ (%)
ns
APAP (h)
o
APAP (h)
cGAS WT
cGAS KO
p
cGAS WT
cGAS KO
Necrosis%
ns
APAP (h)
q
Liver
Veh
APAP
Fold Change
ns
IL-1β
Tnf
IL-6
Zbp1
Oas1
Isg15
Irf7
Usp18
Cxcl10
r
Heart
Breast
Lungs
Liver
Kidney
Ileum
Colon
Brain
kDa
70
35
ZBP1
GAPDH
s
APAP (h)
Zbp1 WT
Zbp1 KO
DAPI Ly6G
DAPI CD45
t
Zbp1 WT
Zbp1 KO
Ly6G(Area%)
CD45(Area%)
APAP (h)

Supplementary Fig. S1. Cytoplasmic mtDNA activates ZBP1, not cGAS, to drive hepatocyte death

a, The scheme of APAP treatment. **b**, Schematic of the cellular fractionation procedure for liver tissue and AML12 cells. **c**, **d**, Immunoblot analysis validating the purity of cytosolic fractions from liver tissue (**c**) or AML12 cells (**d**). **e**, Immunofluorescence staining of dsDNA (green) and TOM20 (red) in AML12 cells treated with 10 mM APAP as indicated. The white arrow indicates the released mtDNA. Scale bar, 10 μm. **f**, Quantification of the fluorescence intensity of dsDNA in (**e**). n=10 cells per group, repeated 3 times independently with similar results. **g**, **h**, Quantitative PCR analysis of the copy number of mtDNA in liver (**g**) or AML12 (**h**) treated with vehicle or APAP. **i**, cGAMP production was measured using an enzyme-linked immunosorbent assay (ELISA) in liver of mice treated with APAP. n=4 mice per group. **j**, cGAMP production was measured using ELISA in AML12 treated with APAP. n=3 independent samples. **k**, Western blotting analysis of liver proteins from wildtype (WT) mice treated with or without APAP. n=3 mice per group. Protein sample from poly(dA:dT)-treated MEFs was used as the positive control. **l**, Serum ALT and AST levels were determined in *cGAS* WT and *cGAS* KO mice. 0 h: n=3 mice per genotype, 24 h: n=5 mice per genotype. **m**, **n**, Representative images (**m**) and quantification analysis (**n**) of TUNEL staining in liver tissue from *cGAS* WT and *cGAS* KO mice. 0 h: n=3 mice, 24 h: n=5 mice. Scale bar, 100 μm. **o**, Hematoxylin and eosin (H&E) staining of the liver sections from *cGAS* WT and *cGAS* KO mice. Scale bar, 300 μm. **p**, Necrotic areas were encircled and quantified. 0 h: n=3 mice, 24 h: n=5 mice. **q**, qPCR analysis of interferon-stimulated genes, pro-inflammatory genes in the liver of WT mice treated with or without APAP. n=5 per group. **r**, Immunoblot analysis of the ZBP1 expression across multiple tissue from mice. **s**, Immunofluorescence staining for Ly6G and CD45 in *Zbp1* WT and *Zbp1* KO mice treated with APAP. Scale bar, 100 μm. **t**, Quantification analysis of the fluorescence area of Ly6G and CD45 in (**s**). 0 h: n=3 mice per genotype, 24 h: n=8 mice per genotype.

Data are mean ± s.e.m. Statistical analysis was performed using unpaired two-tailed Student's *t*-test (**f-j**, **l**, **n**, **p**, **q**), one-way analysis of variance (ANOVA) post hoc Dunnett's tests (**t**); ns, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. For gel source data, see Supplementary Data S1.

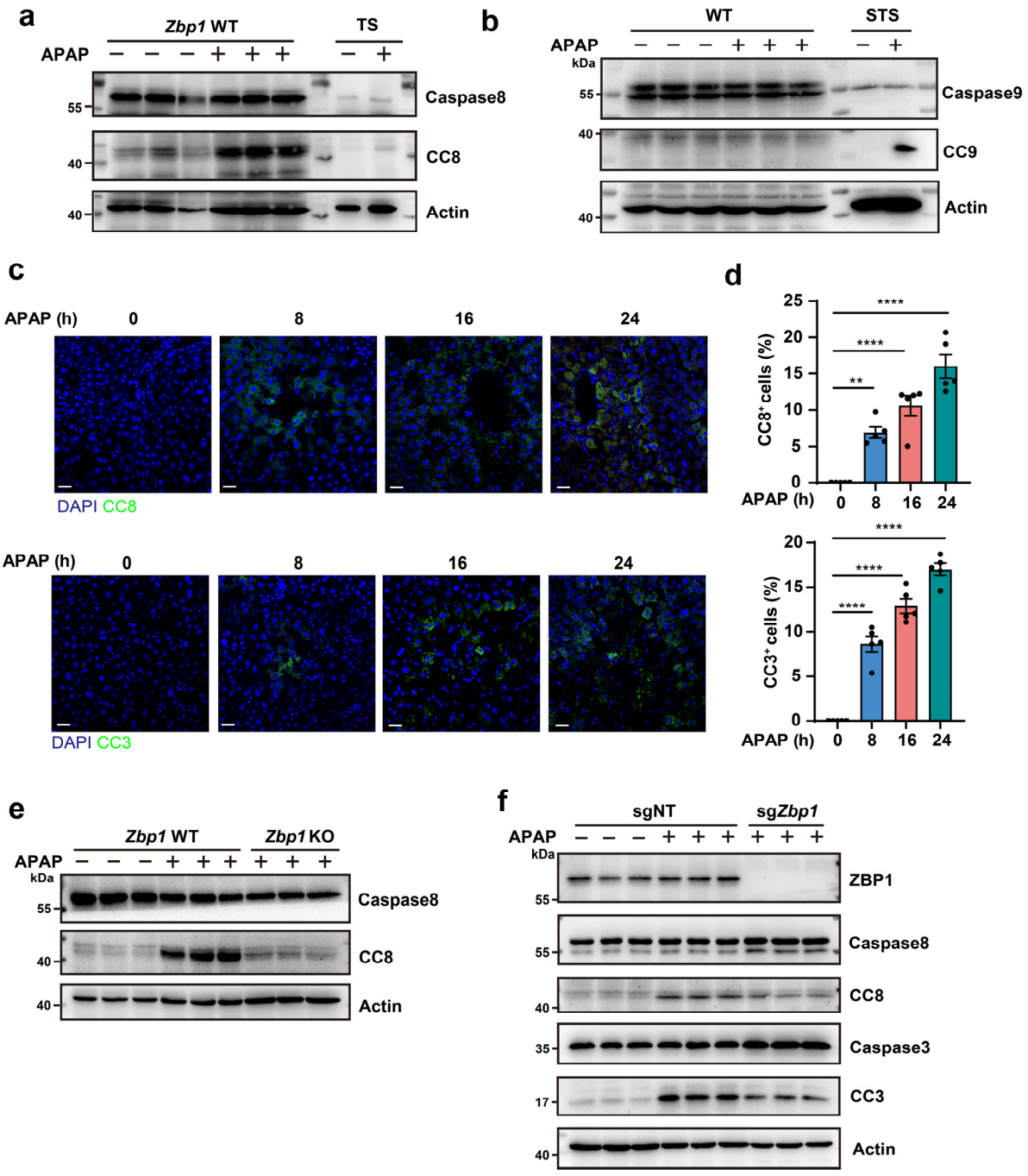


Supplementary Fig. S2. Apoptosis, but not necroptosis, mediates APAP-induced hepatocyte death

a, Immunoblot analysis of liver lysates from WT mice treated as indicated for 24 h. Phosphorylated MLKL (p-MLKL) was detected in the RIPA insoluble fraction of liver lysates. Sample from TNFα+Smac+zVAD (TSZ)-treated MEFs was used as positive control for p-MLKL. n=3 mice per group. n.c: negative control; p.c: positive control. **b**, Serum ALT and AST levels were determined in WT, *Ripk3* KO, *Mlkl* KO mice treated with APAP. n=5 mice per group. **c**, (Left) Representative

Annexin V/PI flow cytometry plots of AML12 cells treated with APAP (10 mM) for the indicated time periods. (Right) Quantification of Annexin V-positive cells. **d**, Immunoblot analysis of CYP2E1 expression in human hepatocyte cell line 7702 and HepG2, or proliferating human hepatocytes (ProliHHs). **e**, **f**, Immunoblot (**e**) and quantification analysis (**f**) of cleaved caspase-3 (CC3) of HepG2 cells pretreated with or without IFNβ for 30 h, followed by treatment with vehicle or APAP (20 mM) for 24 h. **g**, Immunoblot analysis of ZBP1 protein level in sgNT and sg*ZBP1* HepG2 cells treated with or without IFNβ for 30 h.

Data are mean ± s.e.m. Statistical analysis was performed using two-way analysis of variance (ANOVA) post hoc Bonferroni's tests (**c**), one-way analysis of variance (ANOVA) post hoc Dunnett's tests (**b**, **f**); ns, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. For gel source data, see Supplementary Data S1.

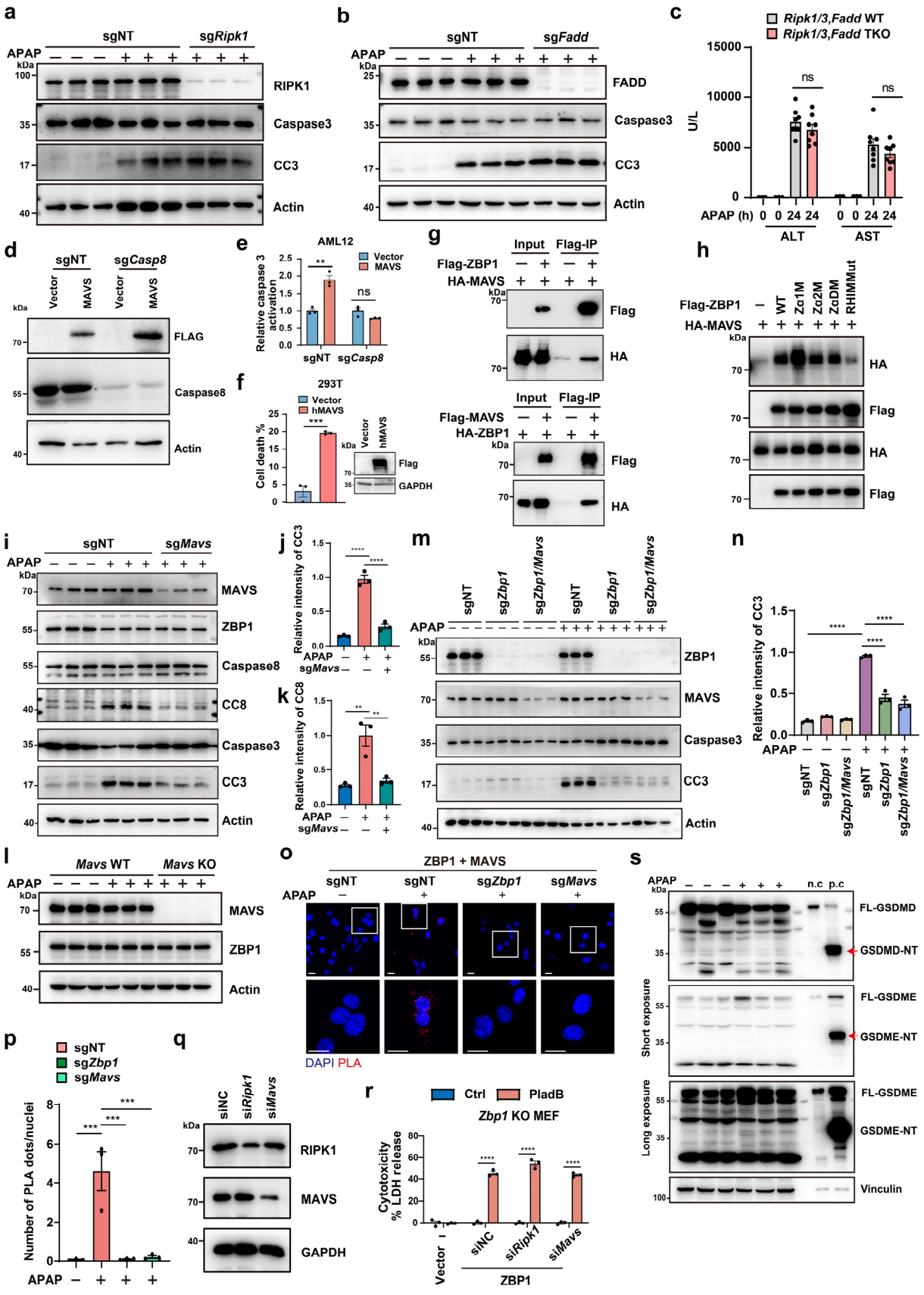


Supplementary Fig. S3. ZBP1 activates caspase-8-dependent apoptosis upon APAP stimulation

a, **b**, Immunoblot analysis of caspae-8 (**a**) and caspase-9 (**b**) in liver lysates from WT mice treated with APAP for 24 h. TNFα plus Smac mimetic (TS) and staurosporine (STS) treatments served as positive controls for caspase-8 and caspase-9 cleavage, respectively. **c**, **d**, Representative images (**c**) and quantification analysis (**d**) of cleaved caspase-8 (CC8) and cleaved caspase-3 (CC3) staining in liver

treated with APAP as indicated. n=5 mice per group. Scale bar, 25 μm. **e**, Immunoblot analysis of liver proteins from *Zbp1* WT and *Zbp1* KO mice treated with or without APAP for 24 h. n=3 mice per group. **f**, Immunoblot analysis of proteins from sgNT and sg*Zbp1* AML12 cells treated with or without APAP for 24 h.

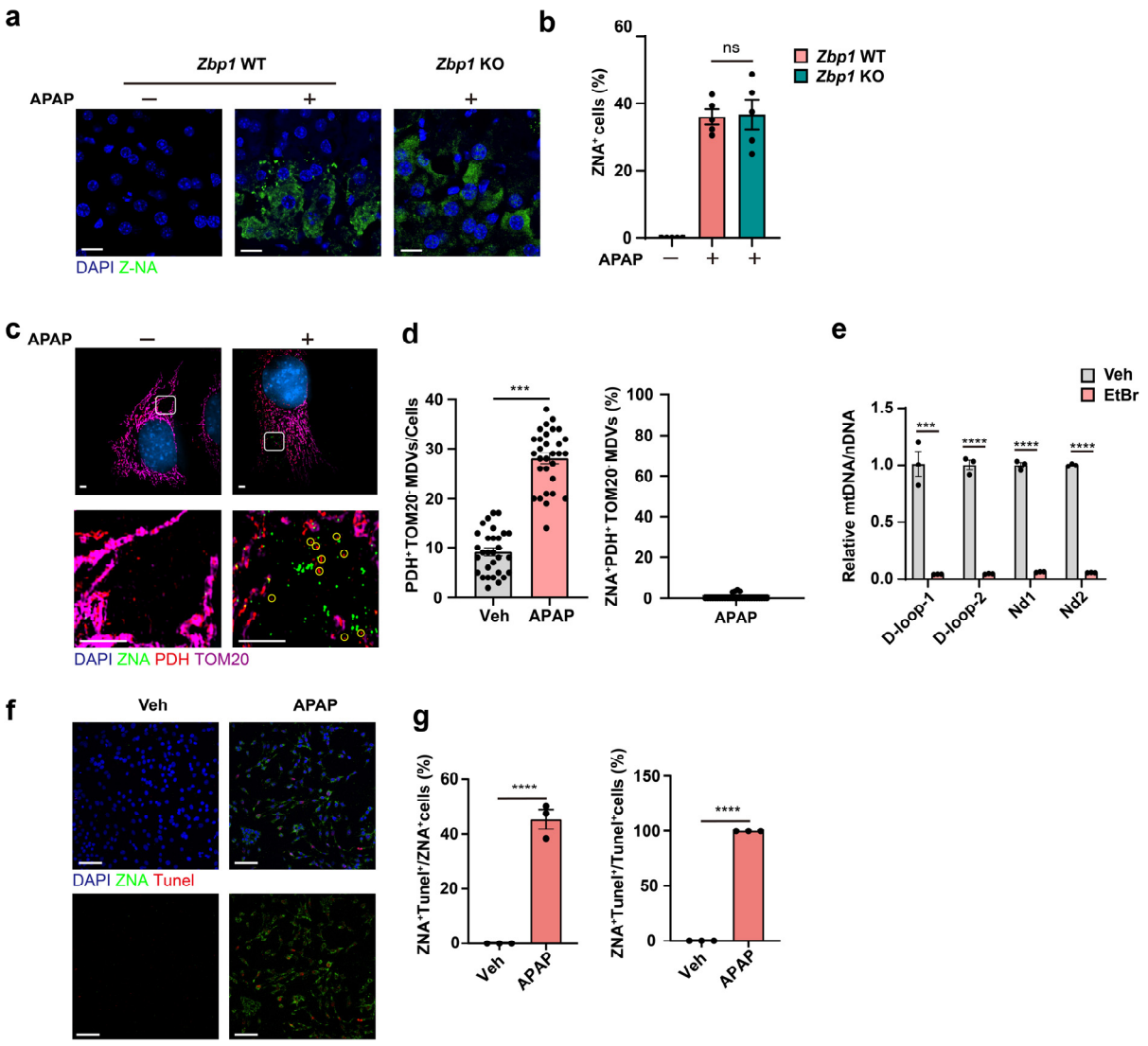
Data are mean ± s.e.m. Statistical analysis was performed using one-way analysis of variance (ANOVA) post hoc Dunnett's tests (**d**), ns, not significant; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$. For gel source data, see Supplementary Data S1.


a
sgNT
sgRipk1
APAP
kDa
100
RIPK1
35
Caspase3
17
CC3
40
Actin
b
sgNT
sgFadd
APAP
kDa
25
FADD
35
Caspase3
17
CC3
40
Actin
c
Ripk1/3,Fadd WT
Ripk1/3,Fadd TKO
15000
10000
5000
0
U/L
ns
ns
APAP (h) 0 0 24 24 0 0 24 24
ALT
AST
d
sgNT
sgCasp8
Vector
MAVS
Vector
MAVS
kDa
70
FLAG
55
Caspase8
40
Actin
e
AML12
Vector
MAVS
Relative caspase 3 activation
2.5
2.0
1.5
1.0
0.5
0.0
**
ns
sgNT
sgCasp8
f
293T
Vector
hMAVS
Cell death %
25
20
15
10
5
0
***
kDa
Vector
hMAVS
70
Flag
35
GAPDH
g
Input
Flag-IP
Flag-ZBP1
HA-MAVS
kDa
70
Flag
70
HA
Input
Flag-IP
Flag-MAVS
HA-ZBP1
kDa
70
Flag
70
HA
h
Flag-ZBP1
WT
Zα1M
Zα2M
ZαDM
RHIMMut
HA-MAVS
kDa
70
HA
70
Flag
70
HA
70
Flag
i
sgNT
sgMavs
APAP
kDa
70
MAVS
55
ZBP1
55
Caspase8
40
CC8
35
Caspase3
17
CC3
40
Actin
j
Relative intensity of CC3
1.5
1.0
0.5
0.0
****
****
APAP
sgMavs
k
Relative intensity of CC8
1.5
1.0
0.5
0.0
**
**
APAP
sgMavs
m
sgNT
sgZbp1
sgZbp1/Mavs
sgNT
sgZbp1
sgZbp1/Mavs
APAP
kDa
55
ZBP1
70
MAVS
35
Caspase3
17
CC3
40
Actin
n
Relative intensity of CC3
1.5
1.0
0.5
0.0
****
****
****
APAP
sgNT
sgZbp1
sgZbp1/Mavs
sgNT
sgZbp1
sgZbp1/Mavs
l
Mavs WT
Mavs KO
APAP
kDa
70
MAVS
55
ZBP1
40
Actin
o
ZBP1 + MAVS
sgNT
sgNT
sgZbp1
sgMavs
APAP
DAPI PLA
s
APAP
kDa
n.c
p.c
55
FL-GSDMD
35
GSDMD-NT
Short exposure
55
FL-GSDME
35
GSDME-NT
Long exposure
55
FL-GSDME
35
GSDME-NT
100
Vinculin
p
sgNT
sgZbp1
sgMavs
Number of PLA dots/nuclei
8
6
4
2
0
***
***
***
APAP
q
siNC
siRipk1
siMavs
kDa
70
RIPK1
70
MAVS
40
GAPDH
r
Ctrl
PladB
Zbp1 KO MEF
Cytotoxicity % LDH release
80
60
40
20
0
****
****
****
Vector
siNC
siRipk1
siMavs
ZBP1

Supplementary Fig. S4. ZBP1 activates caspase-8 via a MAVS-dependent, RIPK1/FADD-independent pathway in APAP hepatotoxicity

a, **b**, Immunoblot analysis of proteins from sg*Fadd* (**a**) and sg*Ripk1* (**b**) AML12 cells treated with or without APAP for 24 h. **c**, Serum ALT and AST levels were determined in *Ripk1&Ripk3&Fadd* WT and *Ripk1*&*Fadd*&*Ripk3* triple-knockout (TKO) mice. 0 h: n=3 mice per genotype, 24 h: n=8 mice per genotype. **d**, **e**, sgNT (non-target control) and sg*Casp8* AML12 cells were overexpressed with empty vector or Flag-tagged mouse MAVS as indicated (**d**), followed by measurement of caspase-3 activity (**e**). n=3 independent samples. **f**, Cell death was measured by ATP assay in 293T cell overexpressed with empty vector or Flag-tagged human MAVS-RFP. n=3 independent samples. **g**, **h**, Immunoblot analysis of anti-Flag immunoprecipitates and inputs from 293T cells co-expressing ZBP1 and MAVS as indicated. **i-k**, Immunoblot (**i**) and quantification analysis of cleaved caspase-3 (CC3) (**j**) and cleaved caspase-8 (CC8) (**k**) of proteins from sgNT and sg*Mavs* AML12 cells treated with or without APAP for 24 h. **l**, Immunoblot analysis of liver proteins from *Mavs* WT and *Mavs* KO mice treated with or without APAP for 24 h. n=3 mice per group. **m**, **n**, Western blotting (**m**) and quantitative analysis (**n**) of sgNT (non-target control), sg*Zbp1* or sg*Zbp1*+*Mavs* AML12 cells treated with or without APAP for 24 h. n=3 independent samples. **o**, **p**, Representative image of PLA of ZBP1-MAVS interaction in sgNT, sg*Zbp1* or sg*Mavs* AML12 cells treated with or without APAP (10 mM) for 24 h (**o**). Scale bars, 20 μm. Quantification is shown in (**p**). n=3 independent samples, n>40 cells per sample. **q**, **r** *Zbp1* KO MEF cells reconstituted with ZBP1 were transfected with siRNAs targeting a non-coding control (NC), *Ripk1*, or *Mavs*. Knockdown efficiency was verified by immunoblotting (**q**), and cell death (**r**) was measured by LDH release assay 14 hours after treatment with PladB (50 nM). **s**, Immunoblot analysis of liver lysates from WT mice treated as indicated for 24 h. n=3 mice per group. n.c: negative control; p.c: positive control. Protein sample from TNFα+Smac (TS)-treated MC38 cells was used as the positive control for GSDME-NT. Protein sample from LPS+Nigericin-treated RAW264.7 cells was used as the positive control for GSDMD-NT.

Data are mean ± s.e.m. Statistical analysis was performed using one-way analysis of variance (ANOVA) post hoc Dunnett's tests (**j**, **k**, **n** and **p**), and unpaired two-tailed Student's *t*-test (**c**, **e**, **f**, **r**). ns, not significant; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$; $****P < 0.0001$. For gel source data, see Supplementary Data S1.

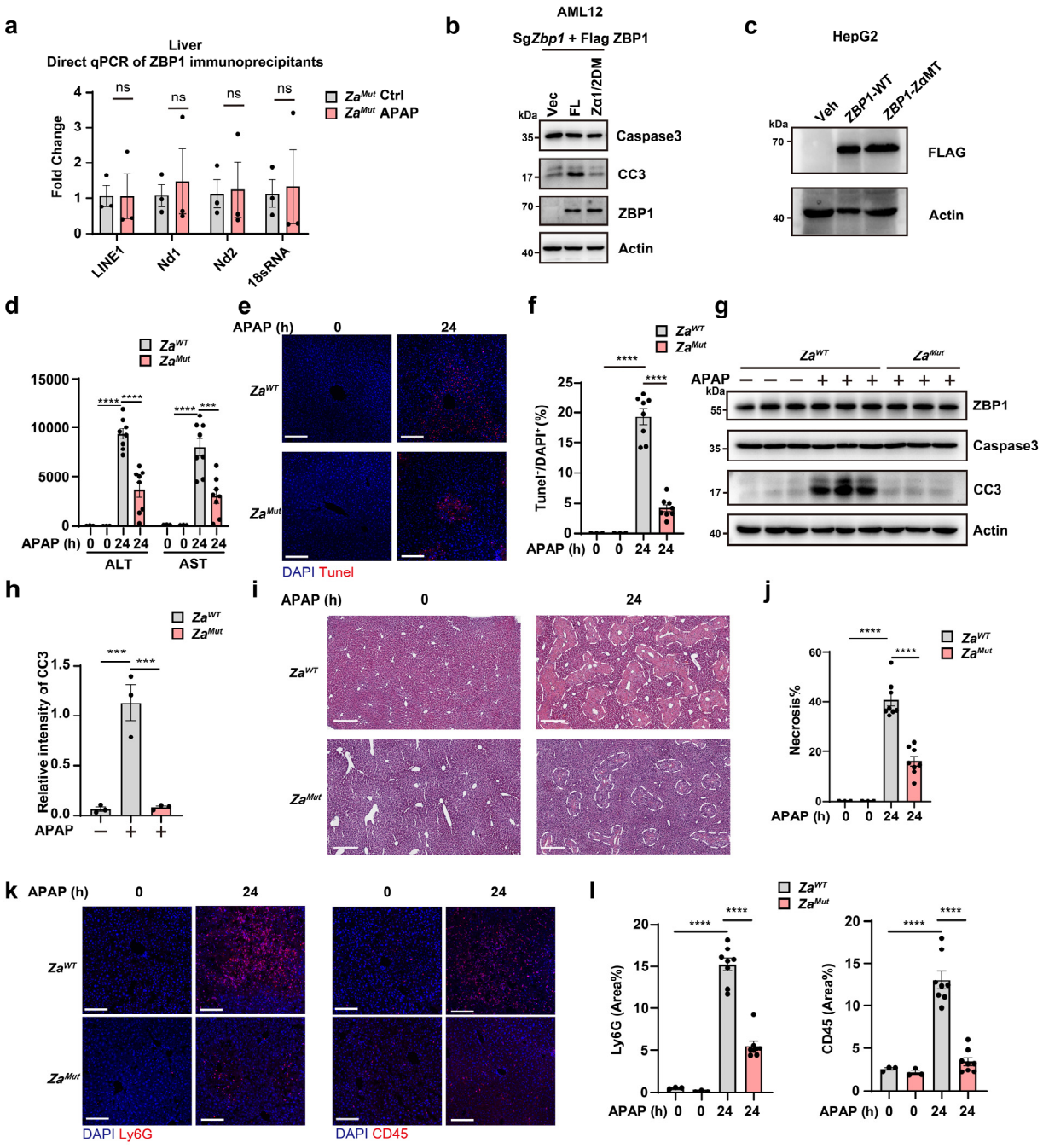


Supplementary Fig. S5. ZBP1 binds to Z-DNA derived from released mitochondrial DNA

a, Immunofluorescence staining of ZNA (green) in the liver of *Zbp1* WT and *Zbp1* KO mice treated with or without APAP (300 mg/kg) for 24 h. Scale bars, 15 μm. **b**, Quantification of the fluorescence area of ZNA signals in (**a**). n=5 mice per group. **c**, **d**, Representative images (**c**) and quantitative analysis of percentage of ZNA positive MDVs (PDH^+TOM20^-) (**d**) in AML12 cells treated with APAP. Scale bar, 3 μm. **e**, Quantitative PCR analysis of cytosolic mtDNA release in cytosol fraction of AML12 cells with or without mtDNA depletion by EtBr (400 ng/ml) treatment for 6 days and stimulated with Vehicle or APAP (10 mM) for 24 h. **f**, **g**, Representative images (**f**) and quantification (**g**) of ZNA and TUNEL double-positive AML12 cells after APAP treatment. Scale bar, 100 μm. n=3

independent samples.

Data are mean ± s.e.m. Statistical analysis was performed using unpaired two-tailed Student's *t*-test (**b**, **d**, **e** and **g**); ns, not significant; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.

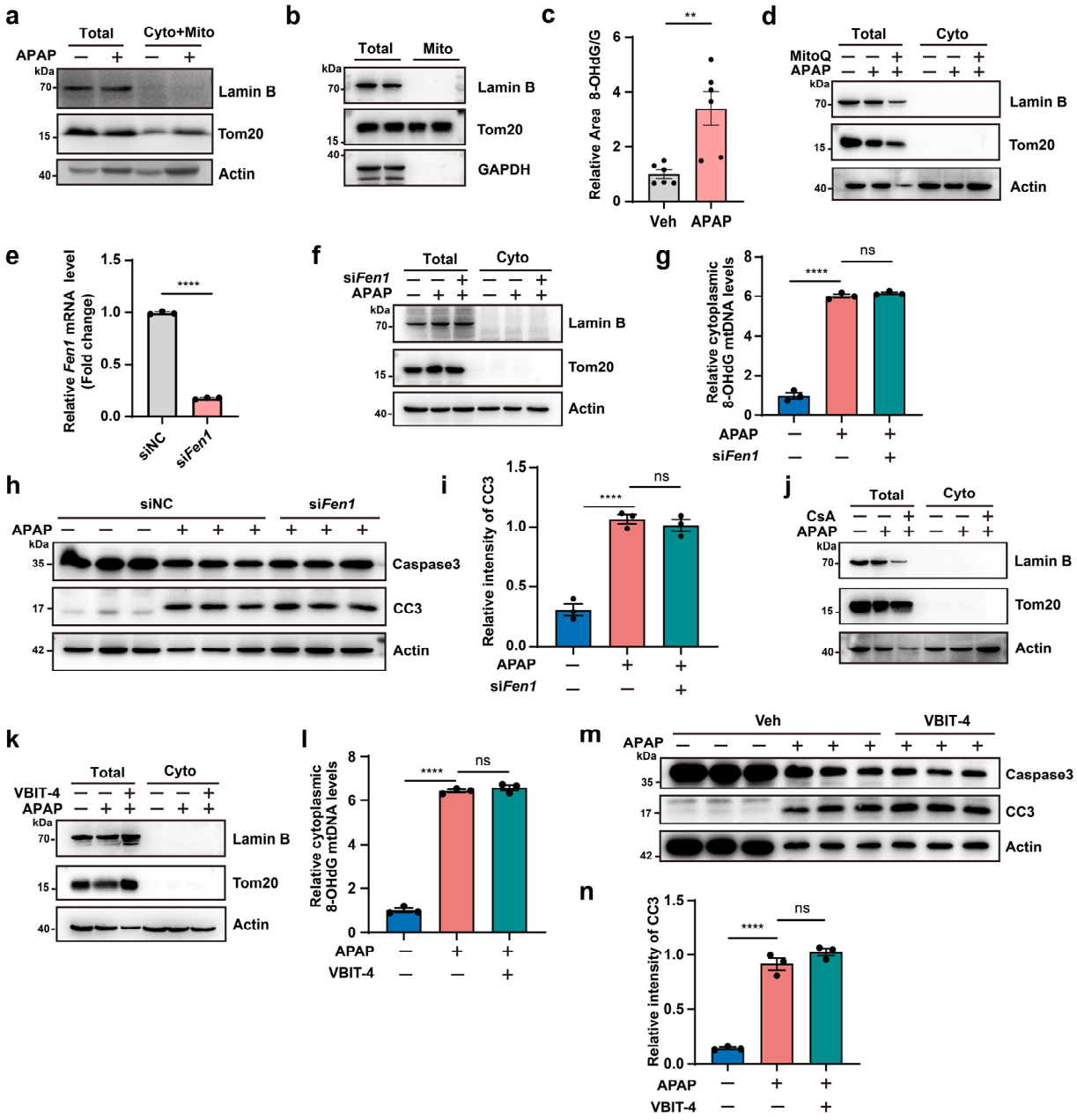


Supplementary Fig. S6. Zα-mediated recognition of Z-DNA by ZBP1 plays a pivotal role in APAP-induced hepatocyte death and liver injury

a, Quantitative qPCR analysis of ZBP1-binding DNAs enriched by anti-ZBP1 immunoprecipitation in liver of Za^{WT} and Za^{Mut} mice treated with APAP (300 mg/kg) for 24 h. n=3 mice per group. **b**, Immunoblot analysis of cleaved caspase-3 in *Zbp1* knockout AML12 cells reconstituted with WT or

Zα mutant ZBP1. **c**, Immunoblot analysis of ZBP1 expression in HepG2 cells reconstituted with empty vector, human WT ZBP1 or Zα mutant ZBP1. **d**, Serum ALT and AST levels were determined in Za^{WT} and Za^{Mut} mice treated with APAP as indicated. 0 h: n=3 mice per genotype; 24 h: n=8 mice per genotype. **e**, **f**, Representative images (**e**) and quantification analysis (**f**) of TUNEL staining in liver tissue from Za^{WT} and Za^{Mut} mice treated with APAP as indicated. 0 h: n=3 mice; 24 h: n = 8 mice. Scale bar, 100 μm. **g**, **h**, Western blotting (**g**) and quantification (**h**) analysis of liver proteins from Za^{WT} and Za^{Mut} mice treated with APAP as indicated. n=3 mice per group. **i**, Hematoxylin and eosin (H&E) staining of the liver sections from Za^{WT} and Za^{Mut} mice treated with APAP as indicated. Scale bar, 300 μm. **j**, Necrotic areas were encircled and quantified. 0 h: n=3 mice; 24 h: n=8 mice. **k**, Immunofluorescence staining for Ly6G and CD45 in *Zbp1* WT and *Zbp1* KO mice treated with APAP. Scale bar, 100 μm. **l**, Quantification analysis of the fluorescence area of Ly6G and CD45 in (**k**). 0 h: n=3 mice; 24 h: n=8 mice.

Data are mean ± s.e.m. Statistical analysis was performed using unpaired two-tailed Student's *t*-test (**a**), one-way analysis of variance (ANOVA) post hoc Dunnett's tests (**d**, **f**, **h**, **j** and **l**); ns, not significant; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. For gel source data, see Supplementary Data S1.

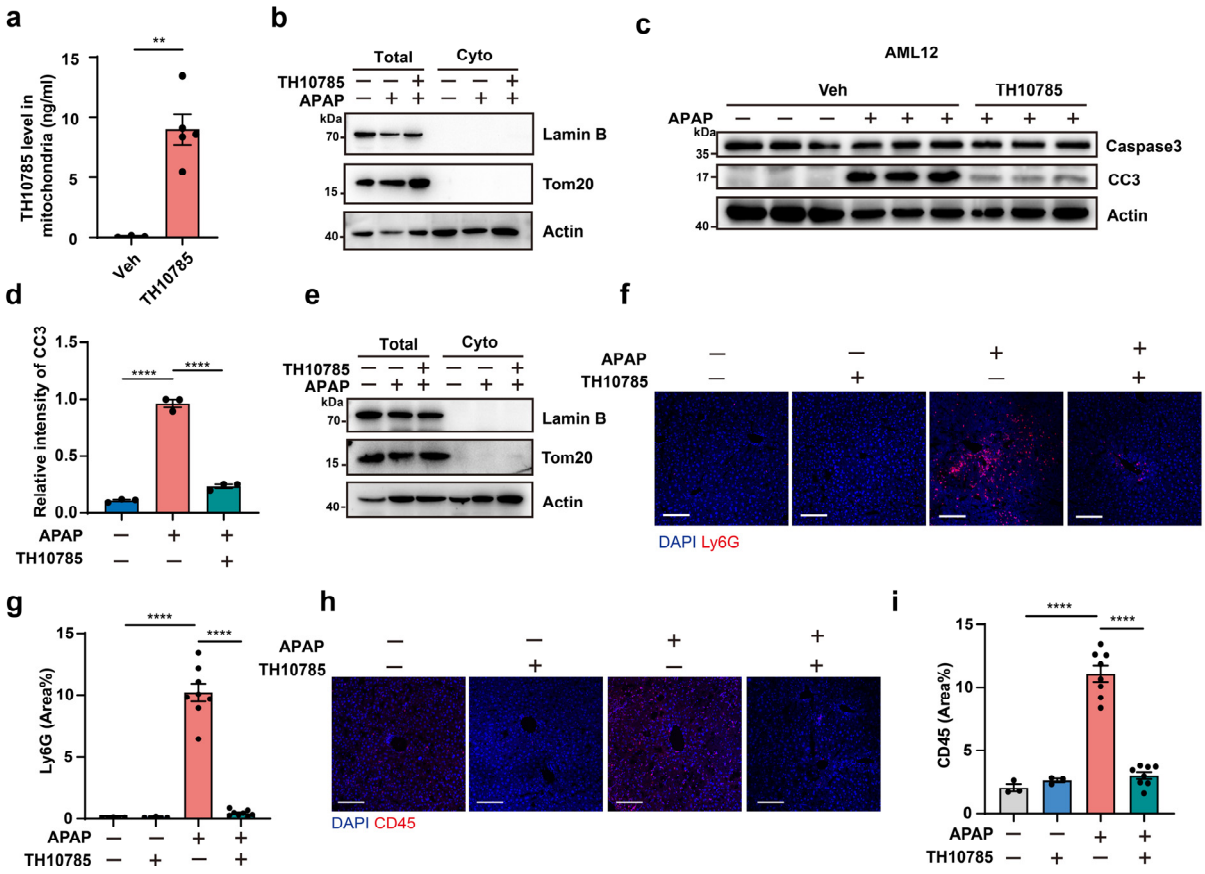


Supplementary Fig. S7. FEN1 and VDAC1 are dispensable for APAP-induced mtDNA fragmentation and cytoplasmic leakage

a, **b**, **d**, **f**, **j**, **k**, Immunoblot analysis of the purity of certain cellular fractions for liver tissue (**a**) or AML12 cells (**b**, **d**, **f**, **j**, **k)** treated as indicated. **c**, Quantitative analysis of relative level of 8-oxodG in mtDNA of AML12 cells treated with or without APAP, measured by mass spectrometry. **e**, Quantitative RT-PCR analysis of *Fen1* in AML12 cells transfected with siRNAs targeting the indicated genes or non-target control (NC). n=3 independent samples. **g**, Quantification of the amounts of cytosolic Ox-

mtDNA in the AML12 cells transfected with siRNAs targeting the indicated genes or NC and treated with or without APAP (10 mM) for 24 h. n=3 independent samples. **h**, **i**, Western blotting (**h**) and quantification analysis (**i**) of the AML12 cells transfected with siRNAs targeting the indicated genes or NC and treated with or without APAP (10 mM) for 24 h. n=3 independent samples. **l**, Quantification of the amounts of cytosolic Ox-mtDNA in the AML12 cells treated with or without APAP (10 mM) for 24 h, in the presence or absence of VBIT-4 (10 μM). n=3 independent samples. **m**, **n**, Western blotting (**m**) and quantification (**n**) analysis of the AML12 cells treated with or without APAP (10 mM) for 24 h, in the presence or absence of VBIT-4 (10 μM). n=3 independent samples.

Data are mean ± s.e.m. Statistical analysis was performed using unpaired two-tailed Student's *t*-test (**c**, **e**) and one-way analysis of variance (ANOVA) post hoc Dunnett's tests (**g**, **i**, **l** and **n**); ns, not significant; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. For gel source data, see Supplementary Data S1.

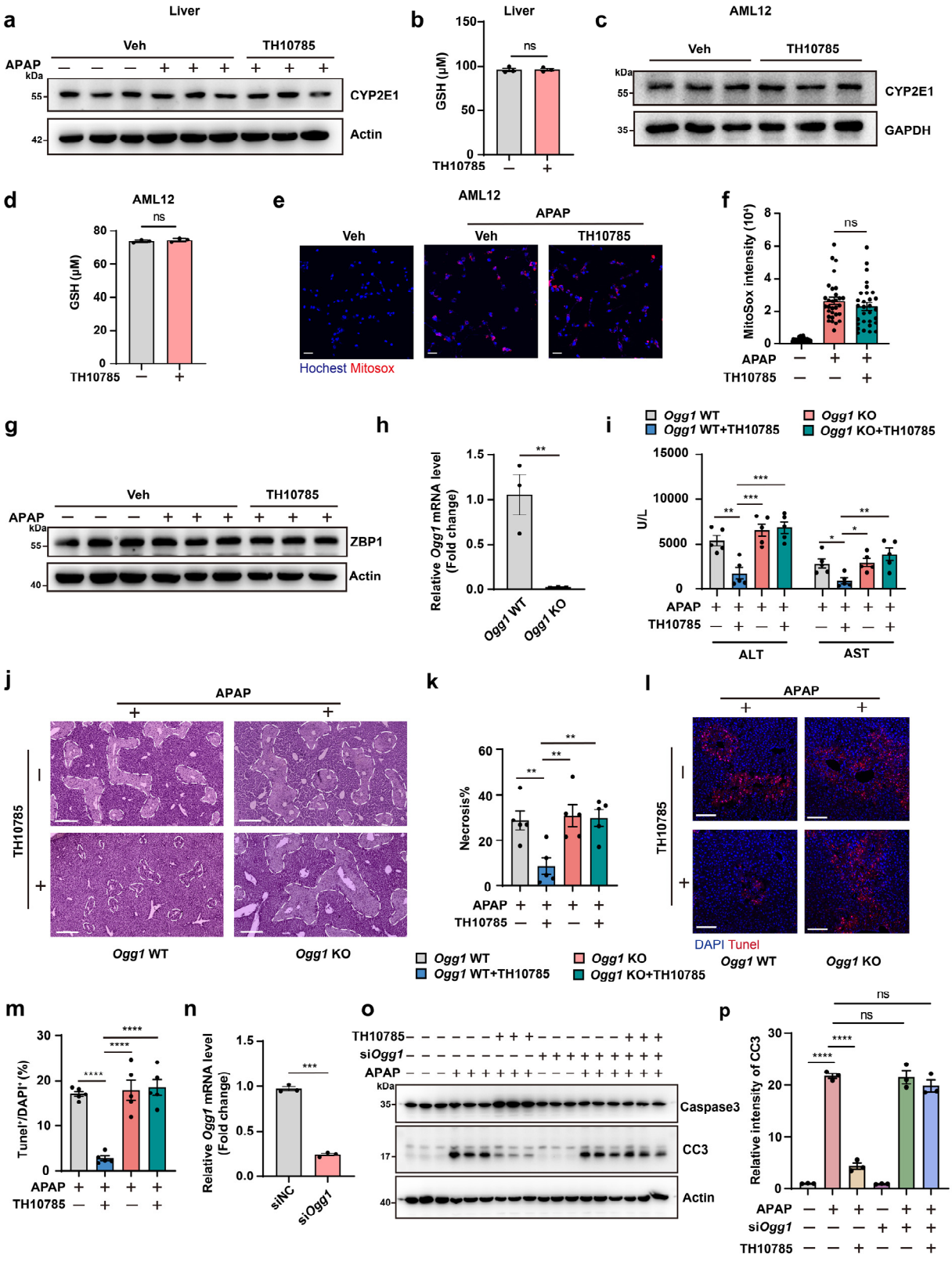


Supplementary Fig. S8. Deoxidation by OGG1 agonist TH10785 protects against APAP-induced cell death and liver failure

a, Mass spectrometry analysis confirming the presence of TH10785 in isolated mitochondria. Veh group: n=3; TH10785 group: n=5 **b, e,** Immunoblot analysis of the purity of certain cellular fractions as indicated. **c**, **d**, Western blotting (**c**) and quantification (**d**) analysis of the AML12 cells treated with or without APAP (10 mM) for 24 h, in the presence or absence of TH10785 (10 μM). n=3 independent samples. **f**, **g**, Immunofluorescence staining (**f**) and quantification (**g**) of Ly6G (red) in the liver of WT mice treated with or without APAP (300 mg/kg) for 24 h in the presence or absence of TH10785 (5 μg/g). Scale bars, 100 μm. **h**, **i**, Immunofluorescence staining (**h**) and quantification (**i**) of CD45 (red) in the liver of WT mice treated with or without APAP (300 mg/kg) for 24 h in the presence or absence of TH10785 (5 μg/g). **f-i**, WT mice only treated with or without TH10785: n=3; WT mice treated with or without APAP in the presence or absence of TH10785: n=8. Scale bars, 100 μm.

Data are mean ± s.e.m. Statistical analysis was performed using unpaired two-tailed Student's *t*-test

(**a**), and one-way analysis of variance (ANOVA) post hoc Dunnett's tests (**d**, **g** and **i**); ns, not significant; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. For gel source data, see Supplementary Data S1.

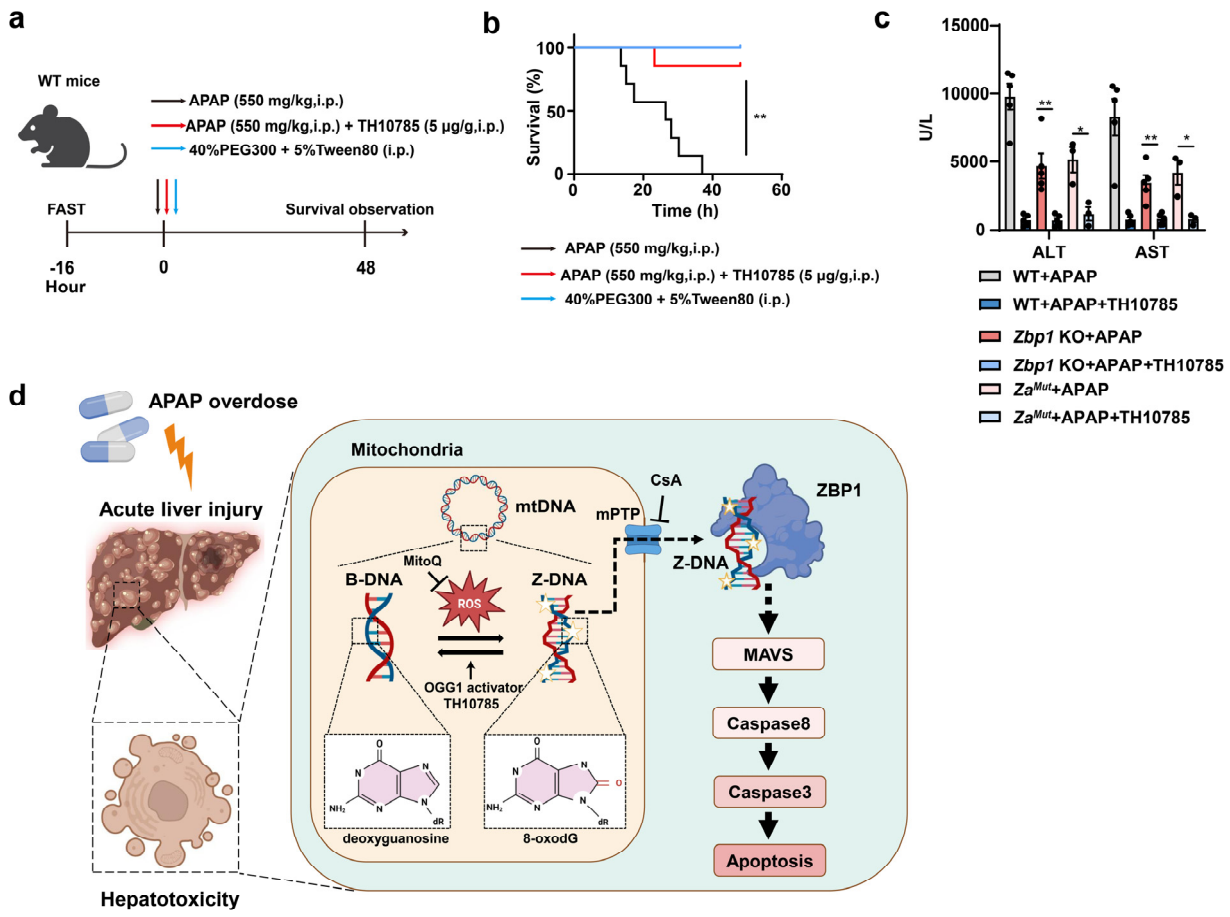


Supplementary Fig. S9. OGG1 agonist TH10785 functions on-target

a, Western blotting analysis of CYP2E1 in the liver from WT mice treated with or without APAP (300

mg/kg) for 24 h, in the presence or absence of TH10785 (10 μM). n=3 independent samples. **b**, Quantification analysis of GSH level in the liver from WT mice treated with or without TH10785 (10 μM) for 24 h. **c**, Western blotting analysis of CYP2E1 in AML12 cells treated with or without TH10785 (10 μM) for 24 h. n=3 independent samples. **d**, Quantification analysis of GSH level in AML12 cells treated with or without TH10785 (10 μM) for 24 h. n=3 independent samples. **e**, Representative images of mitoSOX Red+ cells indicating mitochondrial ROS production in AML12 cells treated with or without APAP (10 mM) for 24 h, in the presence or absence of TH10785 (10 μM). Scale bars, 50 μm. **f**, Quantification analysis of the fluorescence intensity of MitoSOX Red per cell in (**e**). n=10 cells per group, repeated 3 times independently with similar results. **g**, Western blotting analysis of ZBP1 in the AML12 cells treated with or without APAP for 24 h in the presence or absence of TH10785 (10 μM). n=3 independent samples. **h**, qPCR analysis of relative mRNA level of *Ogg1* in WT and *Ogg1* KO mice. n=3 mice per group. **i**, Serum ALT and AST levels were determined in *Ogg1* WT and *Ogg1* KO mice treated with APAP (300 mg/kg) in the presence or absence of TH10785 (5 μg/g) for 24 h. n=5 mice per group. **j**, Hematoxylin and eosin (H&E) staining of the liver sections from (**i**). **k**, Necrotic areas were encircled and quantified. Scale bar, 300 μm. **l**, **m**, Representative images (**l**) and quantification analyses (**m**) of TUNEL staining in liver tissues from (**i**). Scale bar, 100 μm. **n**, Quantitative RT-PCR analysis of *Ogg1* in AML12 cells transfected with siRNAs targeting the indicated genes or non-target control (NC). n=3 independent samples. **o**, **p**, Immunoblot analysis (**o**) and quantification analysis (**p**) of the AML12 cells in (**n**) treated with or without APAP (10 mM) in the presence or absence of TH10785 (10 μM) for 24 h.

Data are mean ± s.e.m. Statistical analysis was performed using unpaired two-tailed Student's *t*-test (**b**, **d**, **f**, **h** and **n**) and one-way analysis of variance (ANOVA) post hoc Dunnett's tests (**i**, **k**, **m** and **p**); ns, not significant; $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$. For gel source data, see Supplementary Data S1.



Supplementary Fig. S10. Deoxidation by OGG1 agonist TH10785 protects against APAP-induced mortality

a. The scheme of APAP treatment in the presence or absence of TH10785. **b**, Survival curves of WT mice treated with a lethal dose of APAP (550 mg/kg, i.p.) in the presence or absence of TH10785 (5 μg/g), n=7 mice per group. **c**, Serum ALT and AST levels were determined in *Zbp1* WT, *Zbp1* KO and *Zα*^{mut} mice treated with or without APAP (300 mg/kg) in the presence or absence of TH10785 (5 μg/g) for 24 h. *Zbp1* WT and *Zbp1* KO: n=5 mice per group; *Zα*^{mut}: n=3 mice per group. **d**, Proposed Model of APAP-Induced Hepatotoxicity. This schematic illustrates our proposed mechanism for APAP-induced liver injury: (i) APAP triggers the generation of oxidized mitochondrial DNA (mtDNA), which adopts a Z-DNA conformation; (ii) cytosolic Z-DNA is sensed by ZBP1 via its Zα domains, initiating a signaling cascade; (iii) ZBP1 engages MAVS to activate caspase-8-dependent apoptosis, a critical driver of pathology; and (iv) therapeutic repair of oxidized DNA by OGG1 activation (e.g., via TH10785) facilitates the reversion of Z-DNA to the B-form, rescuing liver failure even beyond the

standard therapeutic window. Schematic was created with BioRender.com.

Data are mean ± s.e.m. Statistical analysis was performed using Mantel−Cox tests (**b**) and unpaired two-tailed Student's *t*-test (**c**); ns, not significant; **P* < 0.05; ***P* < 0.01; ****P* < 0.001; *****P* < 0.0001.