Supplementary material

Egr1 confers protection against drug-induced hepatotoxicity via transcriptional upregulating of Acaa2

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Supplementary Tables and Figures

Table S1

Characteristics and Clinical data of the DILI patients for IHC

	DILI
Sex	
Male	10
Female	14
Age	53.13±10.13
Implicated drugs in causing DILI	
TCM/HDS	17
NSAID	6
Ibuprofen	1
Diclofenac	1
APAP	3
Details unknown	1
Cold medication (details unknown)	1
Latency (day)	36.67±27.93
Liver biochemistry at onset of DILI	
ALT (U/L)	1445.73±2686.27
AST (U/L)	637.22±598.63
ALP (U/L)	148.63±80.98
TBIL (μmol/L)	115.5±107.26

HDS, herbal and dietary supplements; TCM, traditional Chinese medicines; Age, latency and liver biochemistry are shown as mean ± SEM

Table S2

Characteristics and Clinical data of the DILI patients for ELISA

	DILI
Sex	
Male	9
Female	12
Age	54±16.02
Implicated drugs in causing DILI	
TCM/HDS	13
Antituberculosis drugs	2
Antitumor drug	3
Fluose fine hydrochloride	1
Cefaclor	1
Cyclosporine	1
Latency (day)	44.29±33.43
Liver biochemistry at onset of DILI	
ALT (U/L)	728.77±525.38

AST (U/L)	394.62±321.40
ALP (U/L)	198.38±166.53
TBIL (μmol/L)	20.63±13.10

HDS, herbal and dietary supplements; TCM, traditional Chinese medicines; Age, latency and liver biochemistry are shown as mean ± SEM

Table S3

Primer sets used for qPCR

Species	**Gene**	**Forward (Sequence 5'-3')**	**Reverse (Sequence 5'-3')**
mouse	*36b4*	GGGCATCACCACGAAAATCTC	CTGCCGTTGTCAAACACCT
mouse	*Gapdh*	TGAAGGTCGGTGTGAACGG	CGTGAGTGGAGTCATACTGGAA
mouse	*Egr1*	GTCCTTTTCTGACATCGCTCTGA	CGAGTCGTTTGGCTGGGATA
mouse	*Acaa2*	AAGAAAGGCAAACAGACCA	AGAACTGAGGGGCAAAAGC
mouse	*ND-1*	CCGGCCCATTCGCGTTATTCTTTA	AAGCGTGGATAGGATGCTCGGATT
mouse	*Egr1 exon2*	CCAACAGCCCTTTCACTTA	TTATGCCAACTTGATGGTCTA

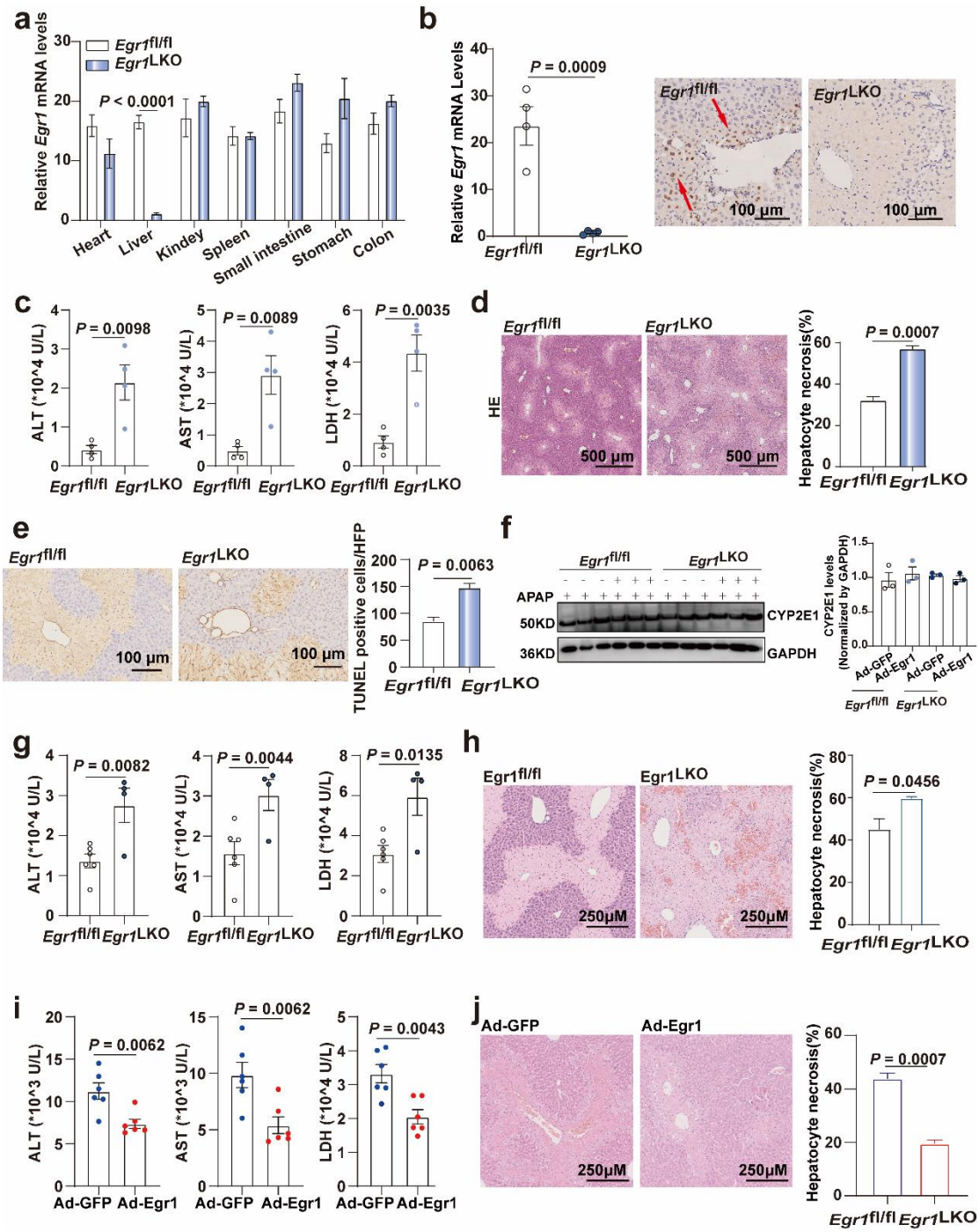

Fig. S1

a Relative *Egr1* mRNA levels in different tissues of *Egr1*^{LKO} mice (n=3 mice/group, t test).

b Relative *Egr1* mRNA levels in liver tissue of *Egr1*$^{fl/fl}$ and *Egr1*^{LKO} AILI mice (n=4 mice/group, t test). Immunohistochemical staining images of Egr1 in the liver tissue of

AILI *Egr1*$^{fl/fl}$ and *Egr1*^{LKO} mice (scale bar = 100 μm). Red arrows represent positive staining.

c–**e** *Egr1*$^{fl/fl}$ and *Egr1*^{LKO} mice were treated with 300 mg/kg APAP. After 12 h, liver and serum samples were collected.

c Serum ALT, AST, and LDH levels in Egr1$^{fl/fl}$ and Egr1^{LKO} mice after challenge with APAP for 12 h (n = 4 mice/group, *t* test).

d Liver sample obtained from AILI *Egr1*$^{fl/fl}$ and *Egr1*^{LKO} mice were stained with H&E, followed by quantified the area of hepatocyte necrosis (scale bars = 500 μm, *t* test).

e Liver sample obtained from *Egr1*$^{fl/fl}$ and *Egr1*^{LKO} AILI mice were stained with TUNEL, followed by quantified the numbers of TUNEL positive cells (scale bars = 500 μm, *t* test).

f Western blot analysis of CYP2E1 levels in the liver tissues of Ad-Egr1 or Ad-GFP pretreated AILI *Egr1*$^{fl/fl}$ and *Egr1*^{LKO} mice, followed by quantified the protein levels (n=3 mice/group, one-way ANOVA).

g–**h** *Egr1*$^{fl/fl}$ and *Egr1*^{LKO} mice were treated with 300 mg/kg APAP. After 24 h, liver and serum samples were collected.

g Serum ALT, AST, and LDH levels in *Egr1*$^{fl/fl}$ and *Egr1*^{LKO} mice after challenge with APAP for 24 h (n = 6 mice/*Egr1*$^{fl/fl}$ group, n=4 mice/*Egr1*^{LKO} group, *t* test).

h Liver sample obtained from AILI *Egr1*$^{fl/fl}$ and *Egr1*^{LKO} mice were stained with H&E, followed by quantified the area of hepatocyte necrosis (scale bars = 250 μm, *t* test).

i–**j** C57BL/6J mice were injected with adenovirus encoding *Egr1* (Ad-Egr1) or control (Ad-GFP) via tail vein prior to 300 mg/kg APAP administration. After 24 h, liver and

serum samples were collected.

i Serum ALT, AST, and LDH levels of Ad-Egr1 and Ad-GFP mice after challenge with APAP for 24 h (n = 6 mice/ group, *t* test).

j Liver sample obtained from Ad-Egr1 and Ad-GFP mice were stained with H&E, followed by quantified the area of hepatocyte necrosis (scale bars = 250 μm, *t* test).

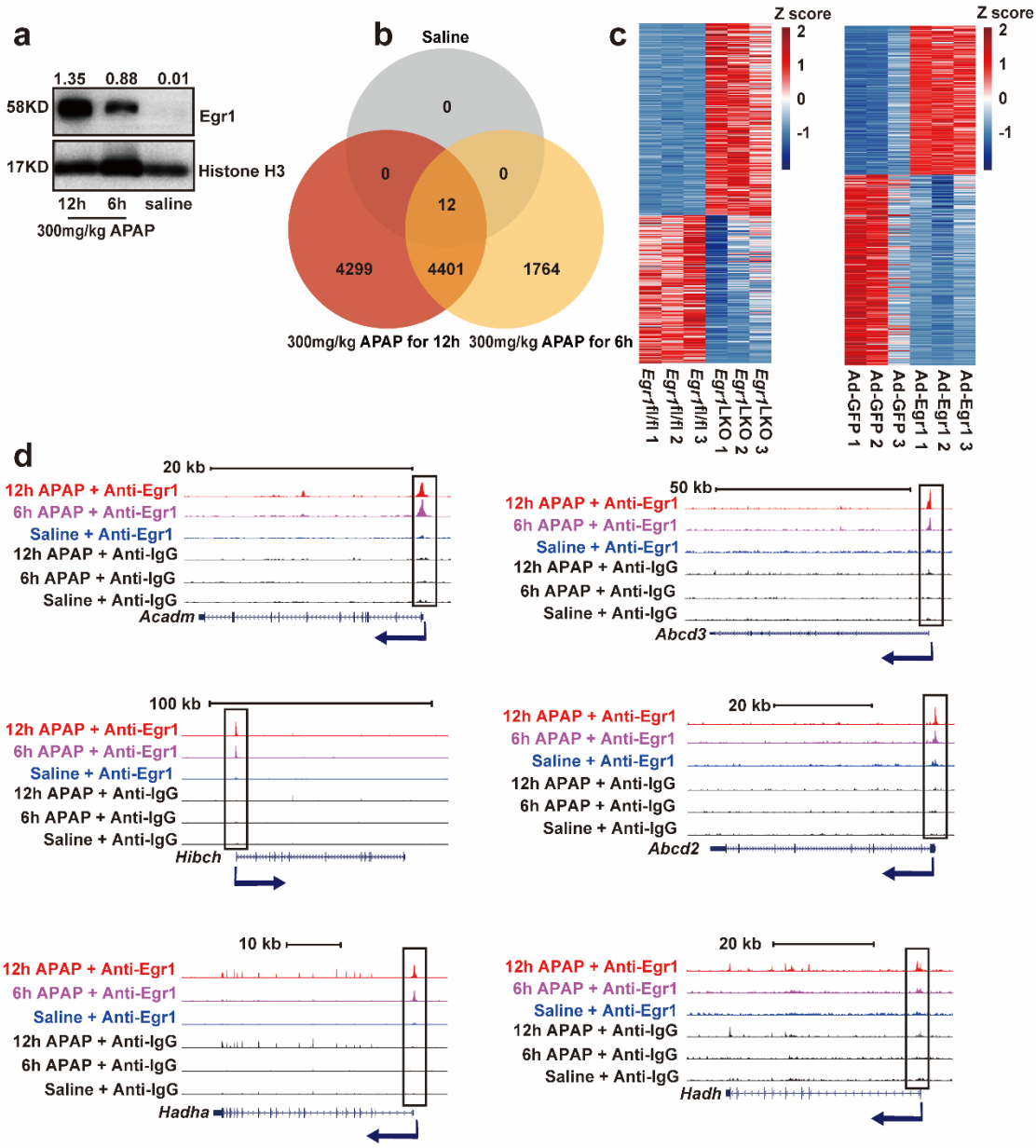

Fig. S2

a Western blot analysis of Egr1 nucleoprotein levels in the liver tissue of the mouse treated with saline for 6h, APAP for 6 h, or APAP for 12 h, the numbers above represent the quantified of the protein levels.

b Venn diagram showed the distinct peaks in liver tissue of the mouse treated with saline for 6h, APAP for 6 h, or APAP for 12 h.

c Heatmaps of the whole metabolomic profile of *Egr1*$^{fl/fl}$ AILI mice and *Egr1*^{LKO} AILI mice, and of Ad-Egr1 or Ad-GFP pretreated AILI *Egr1*^{LKO} mice.

d Genome-browser screenshots of *Acadm*, *Abcd3*, *Hibch*, *Abcd2*, *Hadha,* and *Hadh* occupancy at *Egr1* gene loci.

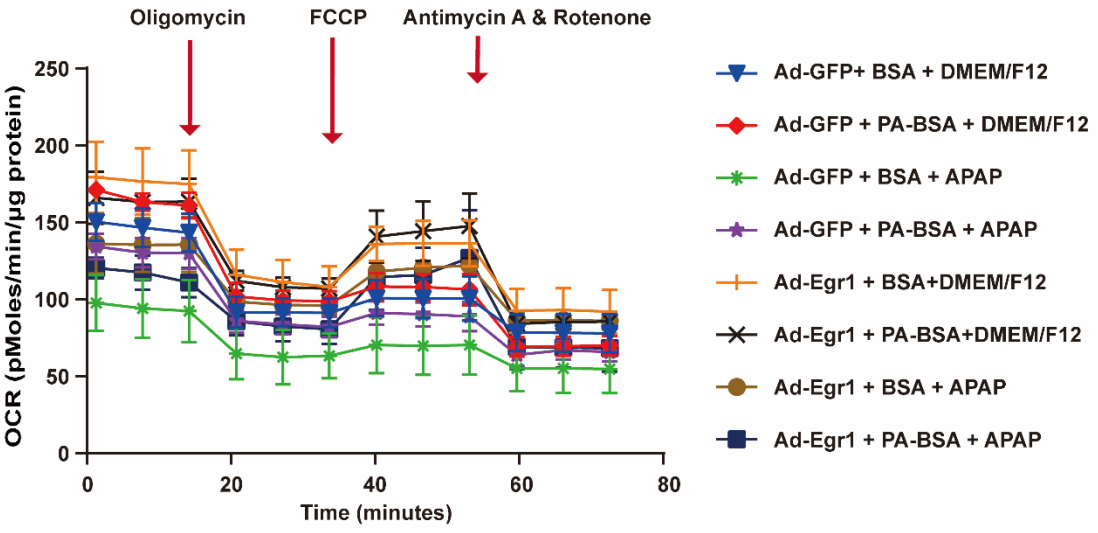

Fig. S3

AML12 cells were treated with Ad-Egr1 and Ad-CON for 48 h and then challenged with 10 mM APAP for 6 h, followed by palmitate-BSA treated for 1 h. Palmitate oxidation stress OCRs were measured using Seahorse XF96 analyzer (n = 4/group). BSA was used as a control for PA-BSA.

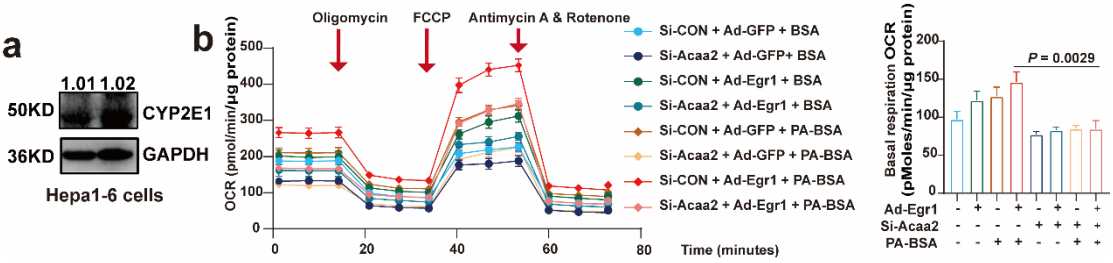

Fig. S4

a Western blot analysis of CYP2E1 levels in Hepa1-6 cells, the numbers above represent the quantified of the protein levels.

b Acaa2 was knocked down in Hepa1-6 cells at 24 h, then overexpressed Egr1 for 48 h and followed by 10 mM APAP treatment for 3 h, finally PA-BSA or BSA treated for 1 h. Palmitate oxidation stress OCRs were measured using Seahorse XF96 analyzer. Basal respiration was calculated according to instruction (n = 5–6/group, one-way ANOVA). BSA was used as a control for PA-BSA.

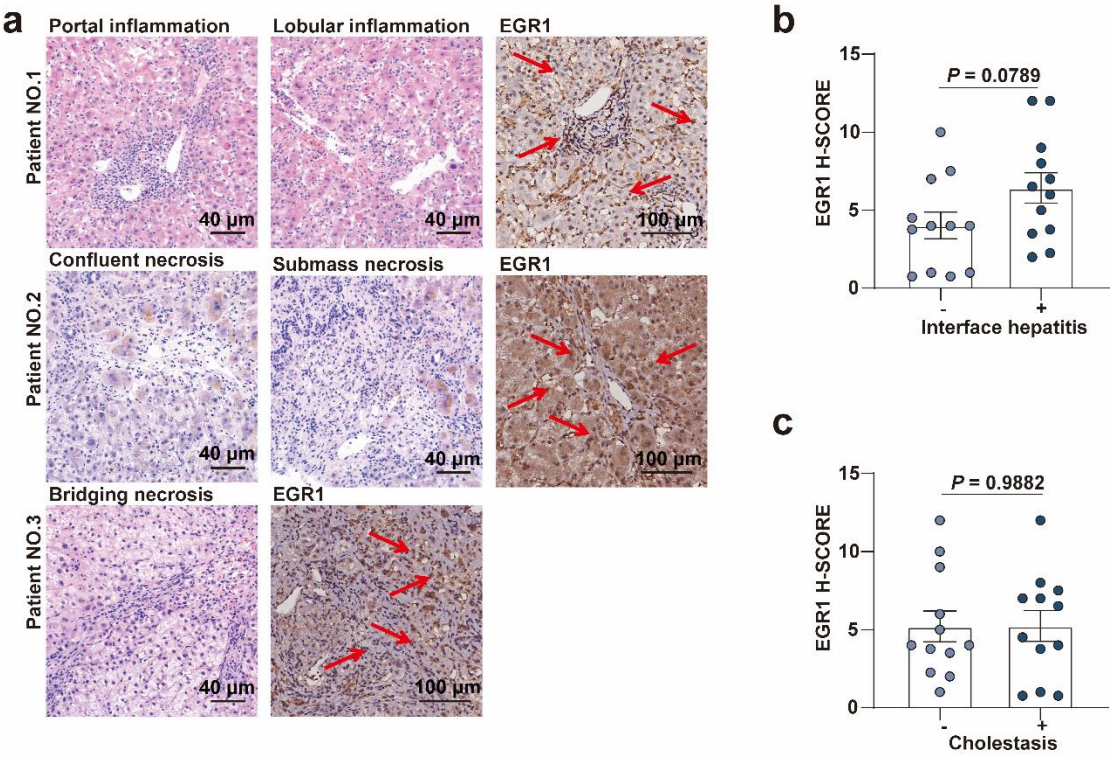

Fig. S5

a Representative images of histological features (scale bar = 40 μm) and corresponding EGR1 staining patterns (scale bar = 100 μm) in DILI patients. Red arrows indicated positive staining.

b Distribution of interface hepatitis and corresponding EGR1 H-scores in patients with DILI (*t* test).

c Distribution of cholestasis and corresponding EGR1 H-scores in patients with DILI (*t* test).