# Identification of a distal enhancer that determines the expression pattern of acute phase marker C-reactive protein

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**C-reactive protein (CRP) is a major acute phase protein and inflammatory marker, the expression of which is largely liver specific and highly inducible. Enhancers are regulatory elements critical for the precise activation of gene expression, yet the contributions of enhancers to the expression pattern of *CRP* have not been well defined. Here, we identify a constitutively active enhancer (E1) located 37.7 kb upstream of the promoter of human *CRP* in hepatocytes. By using chromatin immunoprecipitation, luciferase reporter assay, *in situ* genetic manipulation, CRISPRi, and CRISPRa, we show that E1 is enriched in binding sites for transcription factors STAT3 and C/EBP-β and is essential for the full induction of human *CRP* during the acute phase. Moreover, we demonstrate that E1 orchestrates with the promoter of *CRP* to determine its varied expression across tissues and species through surveying activities of E1-promoter hybrids and the associated epigenetic modifications. These results thus suggest an intriguing mode of molecular evolution wherein expression-changing mutations in distal regulatory elements initiate subsequent functional selection involving coupling among distal/proximal regulatory mutations and activity-changing coding mutations.**

Human C-reactive protein (CRP) is a marker of inflammation presumably functioning as a soluble pattern recognition receptor in host defense (1–4). Liver is the major organ that produces human CRP with its production in hepatocytes regulated primarily at the transcriptional level (5–7). The circulating concentrations of human CRP at the basal state are less than 1 μg/ml but can rapidly increase to several hundred micrograms per milliliter at the acute phase caused by infection or tissue injury (5–7). IL-6 and IL-1β have been identified as chief cytokines that act synergistically to induce the acute phase expression of human *CRP via* activation of transcription factors (TFs) STAT3, NF-κB, and C/EBP-β (8–13). The sequential binding of STAT3 and NF-κB to the proximal promoter of human *CRP* (8, 9, 11, 13) initiates DNA demethylation to enhance the recruitment of C/EBP-β (14), which is necessary and sufficient for the induction. The constitutive expression of human *CRP* at the basal state, however, is maintained by promoter binding of TFs, including HNF-1, HNF-3, and OCT-1 (11, 15). Therefore, the expression pattern of human *CRP* appears to be determined largely by the proximal promoter.

Nevertheless, an *in vivo* study examining the expression of human *CRP* transgenes in mice has suggested a critical contribution of distal regulatory elements (16). Enhancers are distal regulatory elements with key roles in controlling tissue-specific spatiotemporal gene expression programs (17, 18). Interestingly, an enhancer downstream the coding sequence of human *CRP* has been identified to be involved in the induction by TNF-α (19). The effects of this enhancer, however, is at best moderate, and whether this or additional enhancers also contribute to the acute phase induction by IL-6 and IL-1β remains unclear. In the present study, an upstream enhancer termed E1 with major impact on the acute phase induction of human *CRP* is identified. Further analysis suggests that variations in E1 may also partly underlie the different expression patterns of *CRP* across tissues and species.

## Results

### *Proximal promoter alone is insufficient for the acute phase induction of human* CRP

Previous studies have demonstrated that the core promoter, that is −157 to −1 bp, is sufficient to mediate the synergistic induction of human *CRP* by IL-6 and IL-1β in Hep3B, a human hepatic cell line used widely to study the acute phase responses (8–12). Consistent with these results, treating Hep3B cells with IL-6 and IL-1β induced strong expression of both endogenous *CRP* and a pGL2.0 luciferase reporter carrying the 157 bp core promoter (Fig. 1, *A* and *B*). Unexpectedly, though, no induction of the 157 bp core promoter by IL-6 and IL-1β could be observed when new

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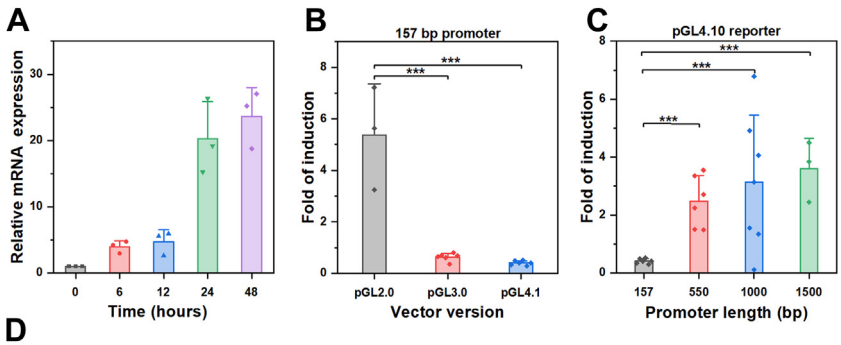


D

| | Similarity | | TF binding sites | | | Acute phase induction |
|---|---|---|---|---|---|---|
| | Identities | Coverage | NF-κB | STAT3 | C/EBP-β | |
| Human | 100% | 100% | 1 | 3 | 5 | Strong |
| Rabbit | 83% | 96% | 1 | 3 | 2 | Strong |
| Rat | 72% | 89% | 2 | 3 | 3 | Weak |
| Mouse | 75% | 89% | 2 | 4 | 2 | Weak |

**Figure 1. Proximal promoter alone is insufficient for the acute phase induction of human *CRP*.** *A*, mRNA levels of human *CRP* in Hep3B cells treated with 10 ng/ml IL-6 and 1 ng/ml IL-1β for the indicated times after normalization to that of the basal state (at 0 h). *B*, fold of induction of luciferase activities driven by the 157 bp proximal promoter of human *CRP* in the pGL2.0, 3.0, or 4.10 vector in Hep3B cells treated with 10 ng/ml IL-6 and 1 ng/ml IL-1β for 24 h. *C*, fold of induction of luciferase activities driven by the 157, 550, 1000, or 1500 bp proximal promoter of human *CRP* in the pGL4.10 vector in Hep3B cells treated with 10 ng/ml IL-6 and 1 ng/ml IL-1β for 24 h. *D*, sequence similarity and numbers of binding sites for transcription factor within the 300 bp promoter of human, rabbit, rat, and mouse *CRP* estimated by JASPAR. Of note, the estimated binding sites are not functionally validated and were simply used for bioinformatic comparison. ***$p < 0.001$ (*t* test).

versions of luciferase reporters (*i.e.*, pGL3.0 and 4.10) were used (Fig. 1*B*). In the new versions of reporters, optimizations are made to eliminate potential binding sites for NF-κB, which are critical to human *CRP* induction and may otherwise introduce unintended side effects in the context of pGL2.0. Further extending the promoter to −1500 bp only moderately rescued the defective induction of the new reporters (Fig. 1*C*), suggesting that the promoter alone is insufficient to mediate the acute phase induction of human *CRP*. Indeed, although the core promoter of *CRP* is evolutionarily conserved across species (Fig. 1*D*), CRP *per se* is not a major acute phase reactant in mice and rats.

### *Distal enhancer E1 mediates the acute phase induction of human* CRP

To examine the possible involvement of distal regulatory elements in the expression of human *CRP*, we determined the interactions between its promoter and flanking noncoding sequences (−60 kb–+120 kb) by chromosome conformation capture (Fig. 2*A*). Significant interactions with *CRP* promoter were detected at twelve distal elements. Of those elements, three were found to rescue the acute phase induction when inserted upstream of human *CRP* promoter in the pGL4.1 reporter (Fig. 2*B*). Further analysis revealed that only the most potent one of the three elements, termed E1 with a length of 1.2 kb, manifests chromatin features of active enhancers, including histone modifications of H3K4me1 and H3K27ac and DNase I hypersensitivity, in both human liver tissues and Huh7 hepatic cell line (Fig. 2*C*). E1 in Hep3B cells was also enriched of H3K4me1 and H3K27ac modifications, and interestingly, neither these modifications nor the promoter interactions of E1 differed between the basal state and acute phase (Fig. 2, *D* and *E*). Therefore, E1 appears to be a constitutively active enhancer in spatial proximity to the promoter of human *CRP*.

To further clarify its functional contributions, we examined the effects of *in situ* genetic manipulations of E1 on the acute phase induction of human *CRP* in Hep3B cells. Cas9-mediated heterozygous deletions within E1 markedly impaired (Fig. 3, *A* and *B*) and dCas9-mediated targeting of transcriptional repressor KRAB to E1 nearly abrogated the acute phase induction of human *CRP* (Fig. 3*C*). By contrast, dCas9-mediated targeting of transcriptional activator VP64 to E1 drastically enhanced the acute phase induction (Fig. 3*D*). Targeting KRAB or VP64 to distal elements other than E1, however, showed little effect. Importantly, these *in situ* manipulations did not affect the induction of another major human acute phase protein, that is, *serum amyloid A* (*SAA*) (20), thereby excluding possible off-target artifacts. We thus conclude that E1 is essential in mediating the acute phase induction of human *CRP*. Nevertheless, the basal expression of human *CRP* appeared to be independent of E1 even upon enforced recruitment of VP64 (Fig. 3*D*). Moreover, though E1 could confer acute phase induction to nonacute phase proteins, the extents of induction were much less pronounced (Fig. 3*E*). Therefore, the regulation of E1 is specific to both promoters and *trans*-acting factors activated during the acute phase.

### *The regulation of E1 is mediated by recruitment of specific TFs to the core sequence*

To understand the regulatory mechanisms of E1, we first mapped the functional core within its sequences. A panel of tiled E1 fragments was then tested for their ability to drive the acute phase induction of promoter activities of human *CRP*, locating the functional core to a 150 bp fragment (Fig. 4*A*). This core fragment is enriched of binding sites for various TFs,

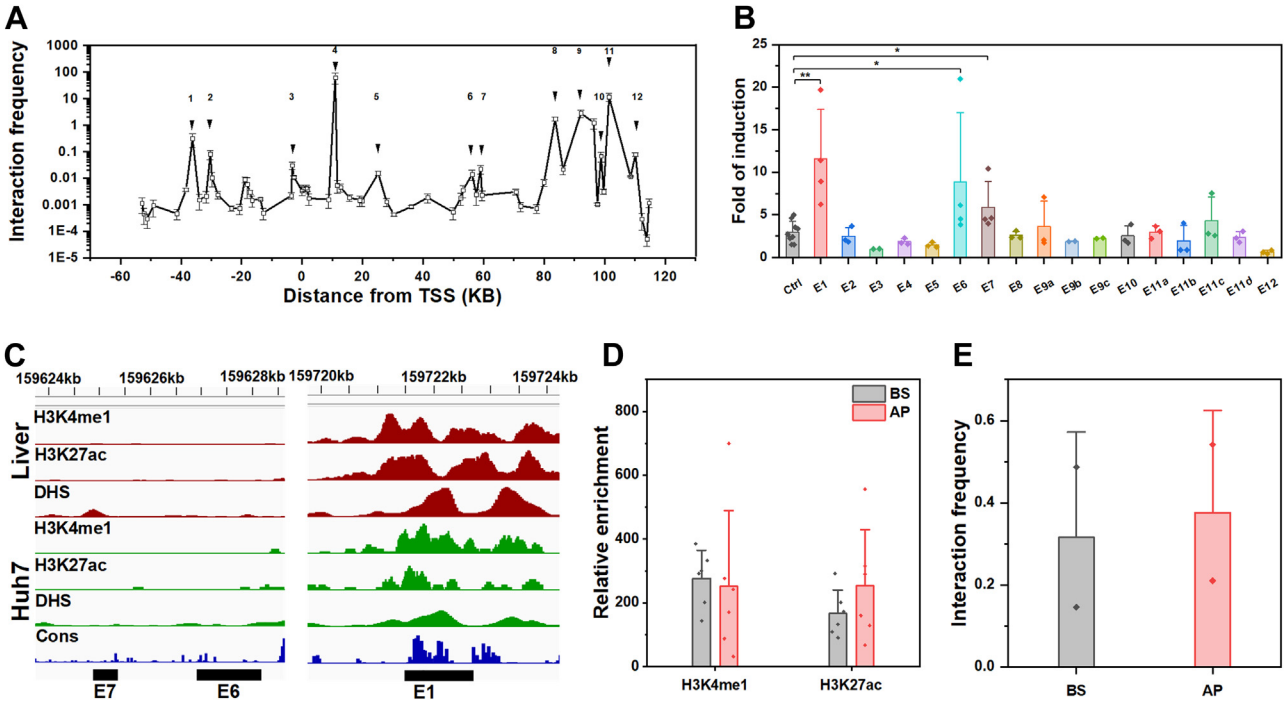


**Figure 2. E1 is an active enhancer regulating the expression of human *CRP*.** *A*, interaction frequencies of indicated distal elements with promoter of human *CRP* in Hep3B cells treated with 10 ng/ml IL-6 and 1 ng/ml IL-1β for 24 h. *B*, fold of induction of luciferase activities driven by the 550 bp promoter of human *CRP* without (ctrl) or with indicated distal elements inserted upstream in the pGL4.10 vector in Hep3B cells treated with 10 ng/ml IL-6 and 1 ng/ml IL-1β for 24 h. *C*, histone modifications, DNase I hypersensitive sites (DHS), and conservation score (across 100 vertebrates) of E1, E6, and E7 in human normal liver tissues and a human hepatic cell line Huh7. *D*, H3K4me1 and H3K27ac modifications of E1 in Hep3B cells treated without (basal state, BS) or with 10 ng/ml IL-6 and 1 ng/ml IL-1β for 24 h (acute phase, AP). *E*, interaction frequencies of E1 with the promoter of human *CRP* in Hep3B cell treated without or with 10 ng/ml IL-6 and 1 ng/ml IL-1β for 24 h. *$p < 0.05$; **$p < 0.01$ (*t* test).

including those activated by IL-6 and IL-1β (Fig. 4*B*). Disruption of the binding sites for STAT3, C/EBP-β, or USF1/2 within the core fragment markedly impaired E1-mediated acute phase induction of promoter activities of human *CRP*, whereas disruption of binding sites for NF-κB and TCF7L2 showed little effect (Fig. 4*C*). We further confirmed the recruitment of STAT3, C/EBP-β, and USF1/2 to E1 in Hep3B cells selectively upon IL-6 and IL-1β treatment (Fig. 4*D*), which was associated with greatly enhanced sensitivities of the targeted sequences to DNase I (Fig. 4*E*). These results thus indicate that the regulation of E1 on the acute phase induction of human *CRP* is primarily mediated by its functional core, which recruits specific TFs activated by IL-6 and IL-1β.

### *E1 contributes to varied expression of* CRP *across tissues and species*

Besides the acute phase induction, human *CRP* also manifests tissue-specific expression with the liver as the major tissue of production albeit its extrahepatic production has also been reported (21–30). Interestingly, the hallmark of active enhancers, that is, histone modifications of H3K27ac and H3K4me1, are significantly more enriched at E1 in the liver than in other human tissues (Fig. 5*A*). This would suggest that the epigenetic inactivation of E1 might partly account for the lack of induction of human *CRP* in nonhepatic tissues. In line with this suggestion, when inserted upstream of human *CRP* promoter, E1 could still enhance the reporter activities in various types of nonhepatic cells (Fig. 5*B*), albeit not as effective as in hepatic cells likely due to cell type–specific signaling evoked by IL-6 and IL-1β.

We next asked whether E1 contributes to species-specific induction of *CRP*. Human CRP is a major acute phase reactant, but mouse CRP is not. Interestingly, mouse E1 almost completely loses the markers of active enhancers in liver tissues (Fig. 5*C*) and failed to drive the acute phase induction of promoter activities of mouse *CRP* in Hep3B cells (Fig. 5*D*). By contrast, mouse E1 could still drive the acute phase induction of promoter activities of human *CRP* albeit to a weaker extent and *vice versa*. These results suggest that the loss of acute phase induction of mouse *CRP* is caused by abrogated crosstalk between E1 and the promoter but not by loss of their respective functions. As such, the expression pattern of mouse *CRP* appeared to be largely determined by the promoter, even when the core fragment of mouse E1 was replaced entirely or partially with that of human E1 (Fig. 5*E*).

## Discussion

The proximal promoter has long been thought to be the major determinant of the expression pattern of human *CRP*, though possible involvement of distal regulatory elements was suggested decades ago (16). That might be partly due to older reporter constructs used in previous studies (8–12). These suboptimized constructs harbor additional binding sites for TFs such as NF-κB, which might unintendedly amplify

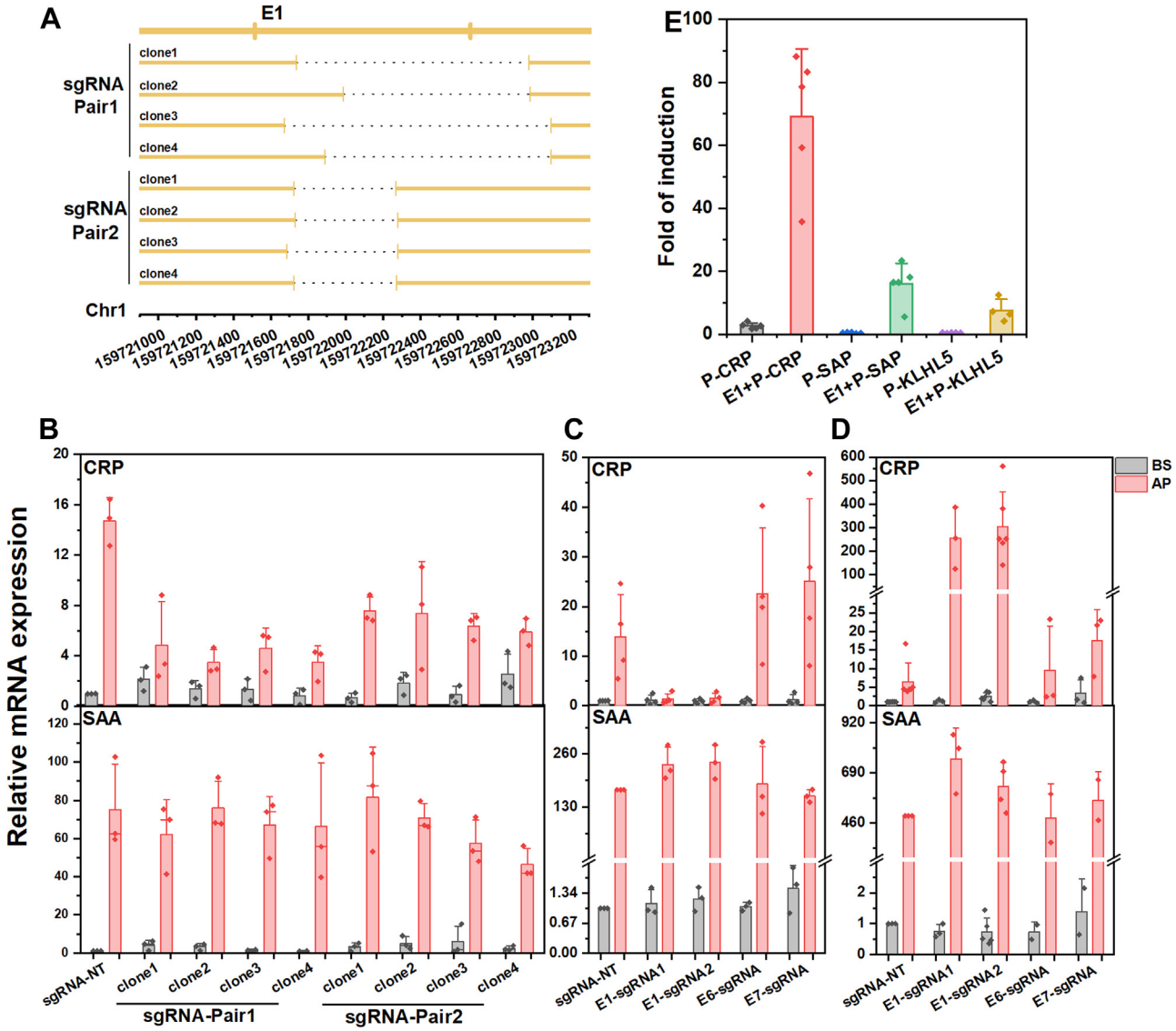


**Figure 3. E1 enhancer is essential to the acute phase induction of human *CRP*.** *A*, sequences deleted in E1 by CRISPR/Cas9 using two distinct pairs of sgRNAs in eight clones of Hep3B cells. *B*, mRNA levels of human *CRP* and *SAA* in the eight E1 deletion clones of Hep3B cells treated without (basal state, BS) or with 10 ng/ml IL-6 and 1 ng/ml IL-1β for 24 h (acute phase, AP). Hep3B cells edited by CRISPR/Cas9 using nontargeting sgRNAs (sgRNA-NT) served as control. *C*, mRNA levels of human *CRP* and *SAA* in Hep3B cells without (sgRNA-NT) or with dCas9-KRAB mediated E1 inactivation at the basal state or acute phase. *D*, mRNA levels of human *CRP* and *SAA* in Hep3B cells without (sgRNA-NT) or with dCas9-VP64 mediated E1 activation at the basal state or acute phase. *E*, fold of induction of luciferase activities driven by the promoter of human *CRP* (P-CRP), *serum amyloid P* (P-SAP), or *KLHL5* (P-KLHL5) without (ctrl) or with E1 inserted upstream in the pGL4.10 vector in Hep3B cells at the basal state or acute phase. SAP is a paralog of CRP but is not an acute phase protein in humans (1–4). sgRNA, single guide RNA.

activities of the tested promoters, leading to the overlook of contributions of distal regulatory elements. The present study, however, by using the optimized new construct, reveals an essential contribution of E1 enhancer, a distal regulatory element located 37.7 kb upstream the promoter. Importantly, the function of E1 is further established by complementary *in situ* genetic manipulations targeting E1 in its original chromatin context. As the potency of E1 in driving gene expression also varies with promoters, it becomes clear that the expression pattern of human *CRP* is determined by the concerted regulation of E1 and the proximal promoter.

The regulatory effects of E1 appear to be mediated by STAT3, C/EBP-β, and USF1/2. Among them, USF1/2 is of particular interest, as the first two are canonical TFs activated by IL-6 and IL-1β and work together also at the proximal promoter. By contrast, USF1/2 is recruited selectively to E1 but not to the promoter of human *CRP* at the acute phase. Previous studies have demonstrated that USF1 and USF2 mainly act as a heterodimer and play important roles in regulating responses to stresses (31, 32). Future studies are warranted to illustrate which isoform of USF1/2 plays a major role and how it acts in concert with other TFs at E1 to drive the induction.

E1 appears to also contribute to the evolutionarily varied expression of *CRP*. It is well known that, in contrast to human CRP, mouse CRP is only a minor acute phase reactant, the circulating level of which increases just twofold to threefold in response to inflammatory insults (33, 34). Somewhat unexpectedly, the regulatory functions of mouse E1 and mouse *CRP*

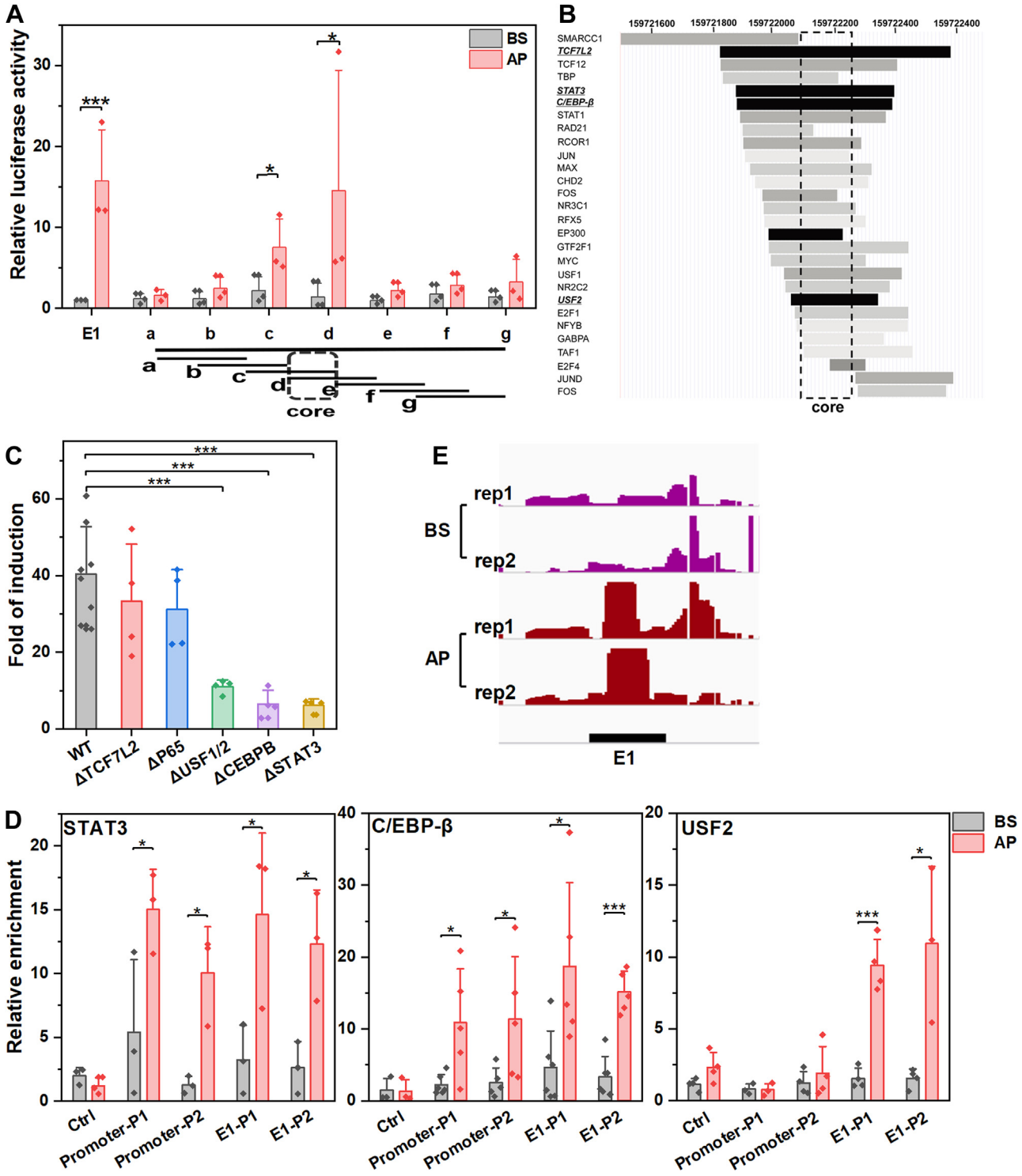


**Figure 4. The regulation of E1 enhancer is mediated by transcription factor (TF) binding to the core sequence.** *A*, relative luciferase activities driven by the 550 bp promoter of human *CRP* with subsegments of E1 inserted upstream in the pGL4.10 vector in Hep3B cells treated without (basal state, BS) or with 10 ng/ml IL-6 and 1 ng/ml IL-1β for 24 h (acute phase, AP). *B*, binding sites for TFs within E1 obtained from ENCODE. Of note, USF1 and 2 act as a heterodimer (31, 32). *C*, fold of induction of luciferase activities driven by the 550 bp promoter of human *CRP* with upstream E1 of WT or mutants lacking binding sites for the indicated TF in the pGL4.10 vector in Hep3B cells treated with 10 ng/ml IL-6 and 1 ng/ml IL-1β for 24 h. *D*, relative enrichment of indicated TFs at the promoter and E1 of human *CRP* in Hep3B cells at the basal state or acute phase. P1 and P2 denote two different primer pairs used to evaluate the enrichment of TFs. *E*, DNase I hypersensitive sites of E1 in Hep3B cells at the basal state and acute phase. $^{*}p < 0.05$; $^{***}p < 0.001$ (*t* test).

promoter appear to be largely intact as they, individually, could still drive apparent yet impaired acute phase induction when acted with the promoter or E1 of human *CRP*. Therefore, the loss of acute phase induction of mouse *CRP* should be the result of highly coupled mutations in both E1 and the promoter that abrogate their crosstalk. Such a tight coupling

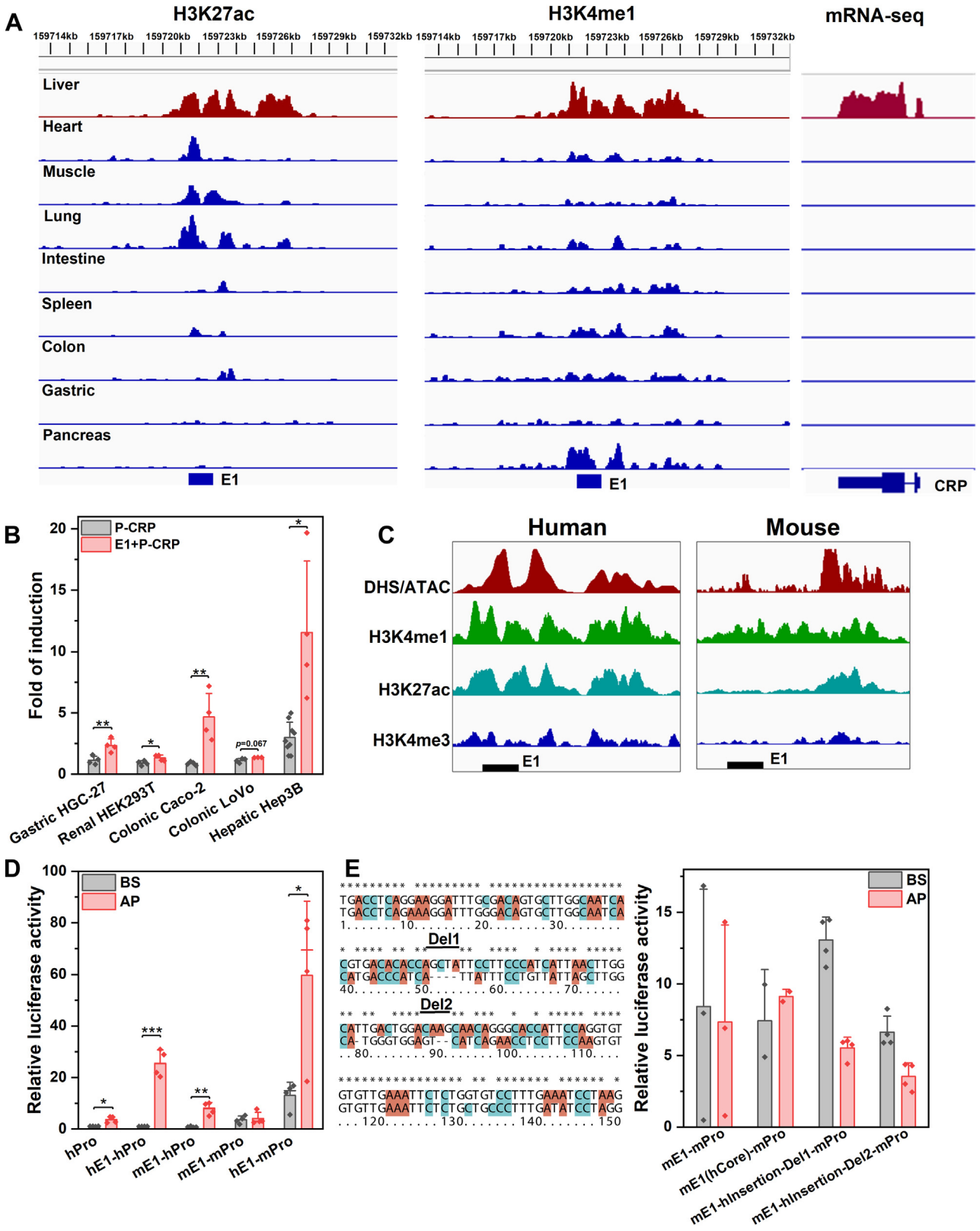


**Figure 5. E1 enhancer regulates tissue- and species-specific expression of *CRP*.** *A*, histone modifications and mRNA levels of *CRP* in different human tissues. *B*, fold of induction of luciferase activities driven by the 550 bp promoter of human *CRP* with upstream E1 in the pGL4.10 vector in the indicated cell lines treated without (basal state, BS) or with 10 ng/ml IL-6 and 1 ng/ml IL-1β for 24 h (acute phase, AP). *C*, histone modifications and DNase I hypersensitive sites (DHS) of E1 in human and mouse liver tissues. *D*, relative luciferase activities driven by the 550 bp promoter of human or mouse *CRP* (hPro or mPro) with human or mouse E1 (hE1 or mE1) inserted upstream in the pGL4.10 vector in Hep3B cells at the basal state and acute phase. *E*, relative luciferase activities driven by the 425 bp promoter of mouse *CRP* (mPro, homologous to human 550 bp promoter) with the WT or mutated mouse E1 inserted upstream in the pGL4.10 vector in Hep3B cells at the basal state and acute phase. mE1(hCore): the core sequence of mouse E1 was replaced with that of human E1. mE1-hInsertion-Del1/2: the core sequences present only in human E1 were inserted into mouse E1 at the corresponding positions. $*p < 0.05$; $**p < 0.01$; $***p < 0.001$ (*t* test).

would also argue that the epigenetic inactivation of mouse E1 is a late event occurred after the loss of acute phase induction.

Though the acute phase induction of *CRP* in human and mouse manifests strong differences, we have shown recently that their functional phenotypes in acute inflammation are nevertheless consistent (35). Such an expression–function mismatch appears to be readily explained by corresponding changes in the hidden activities of CRP (36). As noncoding regulatory sequences are less constrained than coding sequences during the evolution, we propose that noncoding mutations in regulatory sequences might precede the selection on coding mutations tuning hidden activities of CRP. Regarding noncoding mutations, we favor the possibility that E1 mutations occurred before promoter mutations, as the function of mouse *CRP* promoter is less impaired than that of E1. These together imply a cascade of molecular evolution wherein expression-changing mutations in distal regulatory elements initiate subsequent functional selection orchestrated by crosstalk among distal/proximal regulatory mutations and activity-changing coding mutations.

## Experimental procedures

### *Primers and single guide RNAs*

Detailed information of primers and single guide RNAs (sgRNAs) used in this study is listed in Tables S1 and S2. sgRNAs targeting E1 were designed with CRISPRscan (37).

### *Chromosome conformation capture*

Chromosome conformation capture experiments were performed according to the published protocol (19). Briefly, Hep3B cells were treated with or without IL-6 (10 ng/ml; R&D Systems; catalog number: 206-IL-010) and IL-1β (1 ng/ml; R&D Systems; catalog number: 201-LB-010) in serum-free media for 24 h followed by crosslinking with 2% formaldehyde for 10 min at room temperature (RT). Crosslinking was stopped by adding glycine to a final concentration of 0.125 M. Cells were then washed and lysed on ice for 10 min in 10 mM Tris, 10 mM NaCl, 0.2% NP-40, and 1 mM PMSF, pH 8.0. Released nuclei were washed and resuspended in 1× H buffer (Takara) containing 0.1% SDS for 30 min at 37 °C, followed by addition of 1 % Triton X-100 and 1500U Bgl II in 500 μl volume (Takara; catalog number: 1021B) overnight. Digested fragments were ligated with 800U T4 DNA Ligase (Takara; catalog number: 2011B) in about 7 ml volume for 4 h at 16 °C and then sequentially treated with 100 μg/ml of proteinase K (Takara; catalog number: 9034) for overnight at 65 °C and further 0.5 μg/ml of RNase A (Takara; catalog number: 2158) for 30 min at 37 °C. DNA was finally extracted as the sample library. The interaction frequencies were determined with quantitative PCR (qPCR) using primer pairs derived from *CRP* promoter and the indicated distal elements, respectively, and normalized by over 20 random interaction frequencies measured in the gene desert region (ENCODE region Enr313) (38). We also generated a control library using BAC clones RP11-60O15 spanning the CRP locus (Children's Hospital Oakland Research Institute) to normalize differences in primer efficiency.

### *Chromatin immunoprecipitation*

Chromatin immunoprecipitation (ChIP) experiments were performed according to the instruction of CHIP Kit (Abcam; ab500). Briefly, Hep3B cells were treated with or without IL-6 (10 ng/ml) and IL-1β (1 ng/ml) for 24 h followed by crosslinking with 1.1% formaldehyde for 10 min at RT. Nuclear fractions were collected after cell lysis and subjected to sonification (CPX750, Cole-Parmer Instruments, Microtip, 30 cycles of 1 s pulses/3 s stop and repeated ten times with 2 min rest at 40% amplitude output). Immunoprecipitation was performed with 5 μg of anti-STAT3 (Abcam; catalog number: ab267373; lot number: GR3380060-7), anti-USF2 (Abcam; catalog number: ab125184; lot number: GR276142-6), anti-C/EBP-b (Invitrogen; catalog number: PA5-27244; lot number: WG3339777), anti-H3K27ac (Abcam; catalog number: ab4729; lot number: GR288393-1), anti-H3K4me1 (Abcam; catalog number: ab8895), or negative control of rabbit IgG for overnight at 4 °C. Then, DNA fragments were extracted, reversed crosslink, and then assayed by qPCR using relevant primers.

### *Luciferase reporter assay*

The promoter fragments of *CRP* (+3 to −157 bp, +3 to −550 bp, +3 to −1000 bp, or +3 to −1500 bp), *SAP* (−1 to −1000 bp), or *KLHL5* (−1 to −1500 bp) were cloned into pGL2.0, pGL3.0, or pGL4.10 vector (Promega; catalog numbers: E1541, E1751, or E6651). Enhancer fragments were inserted upstream the promoter fragments. Hep3B cells (cell bank of Chinese Academy of Sciences) were cotransfected with 1.5 μg of pGL vector and 0.075 μg of phRL-TK (Promega; catalog number: E6241) using X-tremeGENE 9 DNA transfection reagent (Roche; catalog number: 06365787001) for 16 h. Cells were then treated with or without IL-6 (10 ng/ml) and IL-1β (1 ng/ml) for 24 h followed by measurement of luciferase activities with Dual-Luciferase Reporter Assay System (Promega; catalog number: E1960) by a Synergy HTX Multi-Mode Microplate Reader (BioTek). Activities of firefly luciferase driven by inserted promoters were normalized with activities of cotransfected Renilla luciferase.

### *CRISPR interference and activation*

The pLenti-dCas9-KRAB_Blast plasmid was constructed by replacing Cas9-BFP in pLentiCas9-BFP (Addgene; plasmid #78545) with dCas9-KRAB derived from pHR-SFFV-KRAB-dCas9-P2A-mCherry (Addgene; plasmid # 60954). pLenti-dcas9-vp64_Blast plasmid was obtained from Addgene (plasmid #61425). These plasmids were cotransfected with packaging plasmids pMD2.G (Addgene; plasmid #12259) and psPAX2 (Addgene; plasmid #12260) into HEK293T cells using a ratio of 4:3:1 by Lipofectamine 2000 (Thermo Fisher Scientific; catalog number: 11-668-019). Culture media containing virus particles were collected 48 or 72 h post transfection and used to infect Hep3B cells in the presence of 10 μg/ml polybrene for 6 h. Infected cells were selected 3 days later with

blasticidin S (Sigma–Aldrich; catalog number: 15205), followed by infection with pKLV-U6gRNA(BbsI)-PGKpuro2ABFP plasmid (Addgene; plasmid #50946) containing sgRNAs and selection with puromycin (Solarbio; catalog number: P8230). Positively selected cells were treated with or without IL-6 (10 ng/ml) and IL-1β (1 ng/ml) for 24 h, and mRNA levels of *CRP* and *SAA* were determined with qPCR.

### In situ *deletion within E1 enhancer*

Hep3B cells were infected with virus containing pLentiCas9-BFP (Addgene; plasmid #78545). Infected cells were then infected with virus containing pKLV-U6gRNA(BbsI)-PGKpuro2ABFP (Addgene; plasmid #50946). sgRNA pairs were designed to target sequences surrounding the E1 enhancer. The successful deletion within E1 in positively selected cell clones was validated by PCR and Sanger sequencing. These cells were treated with or without IL-6 (10 ng/ml) and IL-1β (1 ng/ml) for 24 h, and mRNA levels of *CRP* and *SAA* were determined with qPCR.

### *DNase-ChIP*

The experiments were performed according to the published protocol (39). Briefly, Hep3B cells were treated with or without IL-6 (10 ng/ml) and IL-1β (1 ng/ml) for 24 h. Nuclei were collected from lysed cells and lightly digested with DNase I (Takara; catalog number: 2270A). DNase-digested ends were repaired by T4 DNA polymerase (Takara; catalog number: 2040A), ligated to biotinylated linkers, sonicated to an average size of 300 to 700 bp, and purified with streptavidin beads (Sigma–Aldrich; catalog number: HY-K0208). The purified products were ligated to the second set of linkers, amplified, labeled, and hybridized to custom tiling arrays (Agilent; 4*44K). Probes (45–75 nt) covering *CRP* locus with a 2 bp resolution (chr1:159567616-159742333) were designed and detected by Shanghai Bio Corporation. Results were analyzed with ACME packages and visualized using Integrative Genomics Viewer.

### *Statistical analysis*

Experiments were performed in duplicate and repeated independently for at least three times. Data are presented as mean ± SD. Statistical analysis was performed by two-tailed Student's $t$ test. Differences were considered significant at values of $p < 0.05$.

## Data availability

H3K27ac (GSM1112808, GSM910557, GSM916064, GSM906395, GSM1013131, GSM1120338, GSM910559, GSM910555, GSM1013129), H3K4me1 (GSM537706, GSM915335, GSM621640, GSM910572, GSM956019, GSM910577, GSM910577, GSM910574, GSM910576), H3K4me3 (GSM537697) modifications and relevant mRNA-seq (GSM1067795, GSM1010970, GSM1101686, GSM1010946, GSM1120313, GSM1120316, GSM1010942, GSM1010960, GSM1120309) of human normal liver or other tissues were from NIH Roadmap Epigenomics Project (https://www.ncbi.nlm.nih.gov/geo/roadmap/epigenomics).

H3K27ac (GSM2360939) and H3K4me1 (GSM2360945) of hepatoma carcinoma cell line Huh7 were from GEO dataset GSE89212 (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE89212).

DNase hypersensitivity of human primary liver (GSM816663) and hepatoma carcinoma cell line Huh7 (GSM816641) were from ENCODE/Duke (http://genome.ucsc.edu/cgi-bin/hgTrackUi?db=hg19&g=wgEncodeOpenChromDnase).

H3K4me3 (GSM4579741) of mouse normal liver was from GSE151503. H3K4me1 (GSM5404605) and H3K27ac (GSM5404612) of mouse normal liver were from GSE179052. Merged ATAC-Seq data of mouse normal liver were from GSE191030.

The conservation among 100 vertebrates was estimated by PhastCons method and downloaded from UCSC Genome Browser (http://hgdownload.soe.ucsc.edu/goldenPath/hg19/)

*Supporting information*—This article contains supporting information.

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*Conflict of interest*—The authors declare that they have no conflicts of interest with the contents of this article.

*Abbreviations*—The abbreviations used are: ChIP, chromatin immunoprecipitation; qPCR, quantitative PCR; sgRNA, single guide RNA; TF, transcription factor.

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