Supporting Information

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SI Methods

Construction of ZBTB20 Targeting Vector. A 14.5-kb genomic clone including exon 6 of ZBTB20 was isolated from a 129/SvEv mouse genomic library (Stratagene) with a probe containing full-length cDNA of human ZBTB20 and used to construct the targeting vector. A 2.1-kb *Bam*HI/*Eco*RI fragment containing intact exon 6 was subcloned between two loxP sites downstream of the neomycin resistance (Neo) cassette, and the resultant loxP-Neo-loxP-exon 6-loxP cassette was flanked by a 1.2-kb short arm and a 6.5-kb long arm for homologous recombination.

Generation of LZB20KO Mice. ES cells (129/SvJae background) were electroporated with *Pvu*I-linearized targeting vector and selected with G418 and ganciclovir. Homologous recombination at the ZBTB20 locus was confirmed by Southern blotting with the 5′ probe, which was located outside the targeting construct. The targeted ES cells carrying the ZBTB20$^{neo\text{-}flox}$ allele were injected into C57BL/6 blastocysts to produce chimeric mice. The resulting male chimeras transmitted the ZBTB20$^{neo\text{-}flox}$ allele to the progeny. Heterozygous ZBTB20$^{neo\text{-}flox}$ mice were bred with protamine promoter-*cre* transgenic mice (The Jackson Laboratory, stock number 003328) to remove the neomycin resistance cassette in the germ line. The removal was confirmed by PCR primers P3 and P4 flanking the neomycin resistance cassette. Liver-specific ZBTB20 knockout mice (LZB20KO) were generated by crossing ZBTB20^{flox} mice with Alb-cre transgenic mice *B6.Cg-Tg*(*Alb-cre*)*21Mgn*/*J* (The Jackson Laboratory, stock number 003574), which express *Cre* recombinase under control of the rat albumin promoter. All animals were housed in specific pathogen-free facilities under controlled temperature and light and fed with normal chow.

PCR Genotyping. Cre-mediated deletion of exon 6 in LZB20KO mice was assessed by 3-primer PCR including primers P1, P2, and P3, in which 0.8 kb product amplified by primer P1 and P2 indicated the presence of floxed exon 6 and a 1.2 kb product amplified by primer P3 and P2 indicated the deletion of exon 6 by Cre-mediated homologous recombination. PCR primers for genotyping are available on request. The PCR parameters used included 35 cycles of 95 °C for 5 min, 94 °C for 30 s, 57 °C for 30 s, and 72 °C for 90 s followed by a single cycle of 72°C for 5 min. Ten microliters of the PCR was run on 1% agarose gel for 30 min at 120 V.

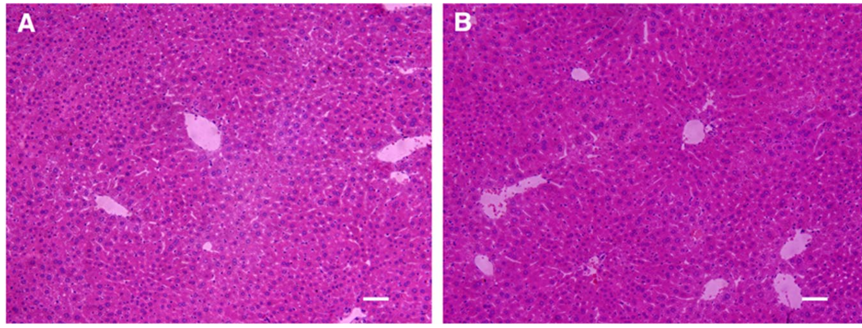

Fig. S1. Hematoxylin-eosin staining of the livers from ZBTB20$^{flox/flox}$ (*A*) and LZB20KO (*B*) mice at the age of 2 months. (Scale bar, 50 μm.)

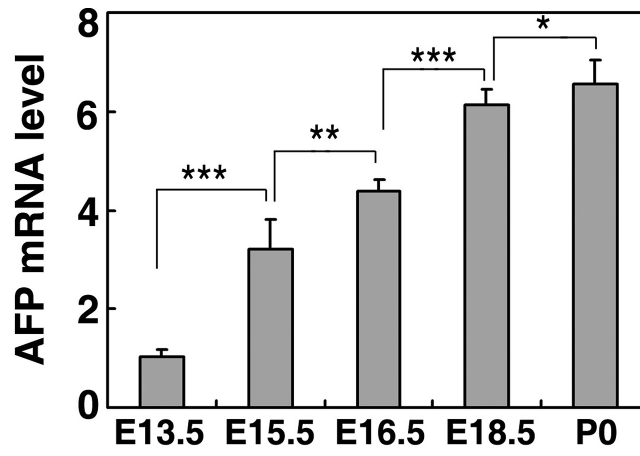

Fig. S2. Real-time RT-PCR analysis of AFP mRNA expression levels in mouse liver at different embryonic stages. The AFP mRNA level is represented by an arbitrary unit after normalization with the internal control 36B4. *, $P > 0.05$; **, $P < 0.001$; ***, $P < 0.0001$.

PNAS PNAS PNAS PNAS

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-172 gtggtctttgtccttgaacataggatacaagtgacccctgctctgttaat -123
-122 tattggcaaattgcctaacttcaacgtaaggaaatagagtcatatgtttg -73
-72  ctcactgaaggttactagttaacaggcatcccttaaacaggatataaaag -23
                          +1 →
-22  gacttcagcaggactgctcgaaACATCCCACTTCCAGCACTGCCTGCGG  +27
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Fig. S3. Demonstration of ZBTB20-binding sequence in mouse AFP promoter. Double-stranded oligonucleotide probes corresponding to −172/−104, −108/−53, and −56/+27 of the AFP promoter were used to test their binding to the ZBTB20 protein. ZBTB20 binds to the AFP promoter at −108/−53 (underlined).

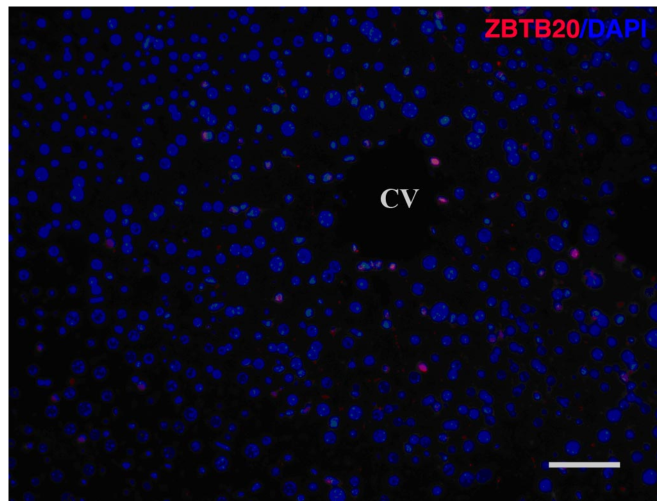

Fig. S4. Immunostaining of ZBTB20 in adult LZB20KO liver. ZBTB20 is undetectable in the vast majority of hepatocytes from liver-specific ZBTB20 knockout mice but was detected in a few non-hepatocyte cells in the liver. CV, central vein. (Scale bar, 50 μm.)