

Supplementary Materials

C/EBPβ Coupled with E2F2 Promoted the Proliferation of hESC-Derived Hepatocytes through Direct Binding to the Pro-moter Regions of Cell-Cycle-Related Genes

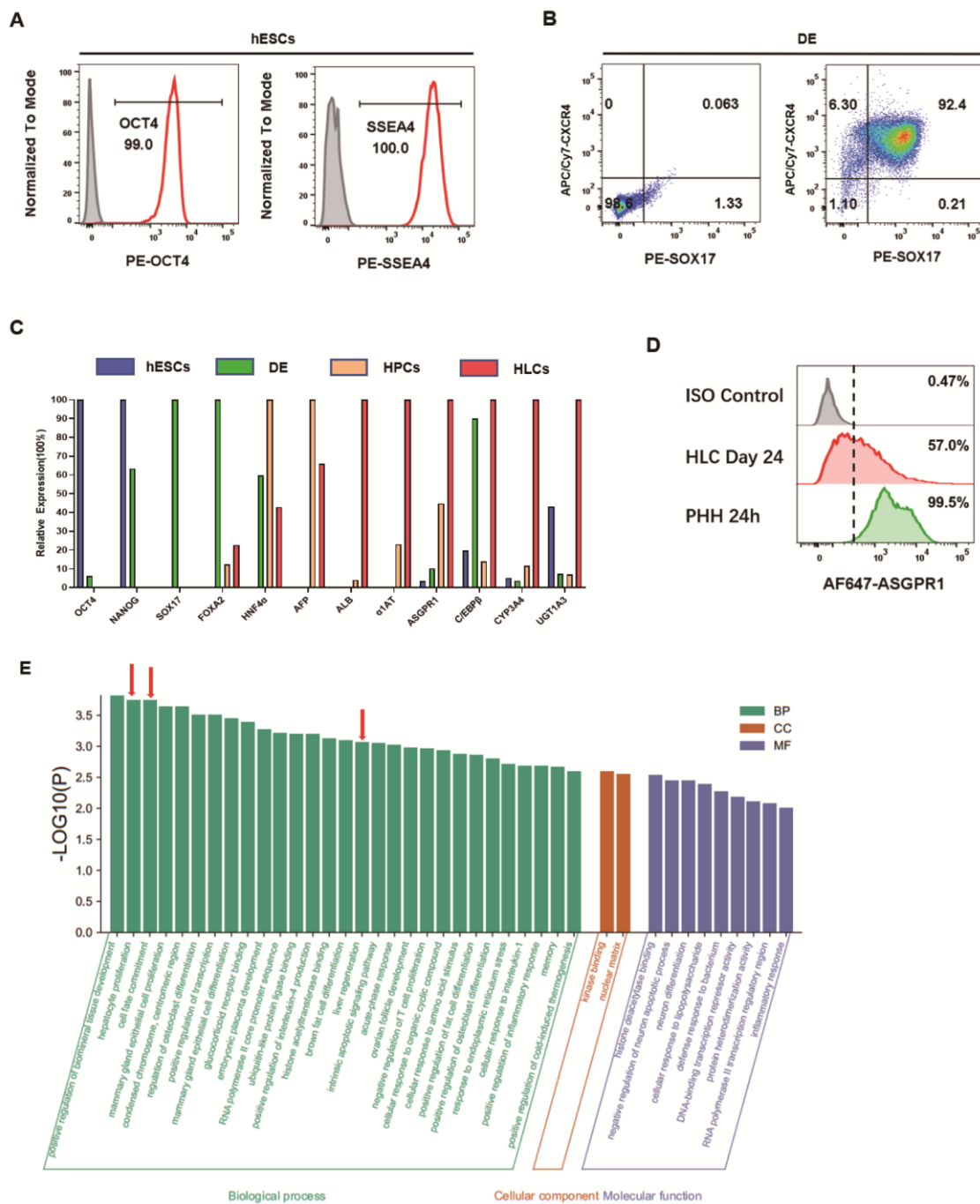


Figure S1. C/EBPβ was expressed in hepatocytes and involved in hepatocyte differentiation. (A,B) Flow cytometry analysis for the expression of specific markers at different stages of hESCs (OCT4, SSEA-4), DE cells (SOX17, CXCR4). (C) The relative expression of specific markers at different stages during the differentiation was determined by qPCR. (D) Flow cytometry analysis for the expression of mature hepatocyte marker ASGPR1. (E) GO terms were statistically and significantly associated

with the input gene C/EBP β , and enrichment analyses were available by using the online website KOBAS-intelligence.

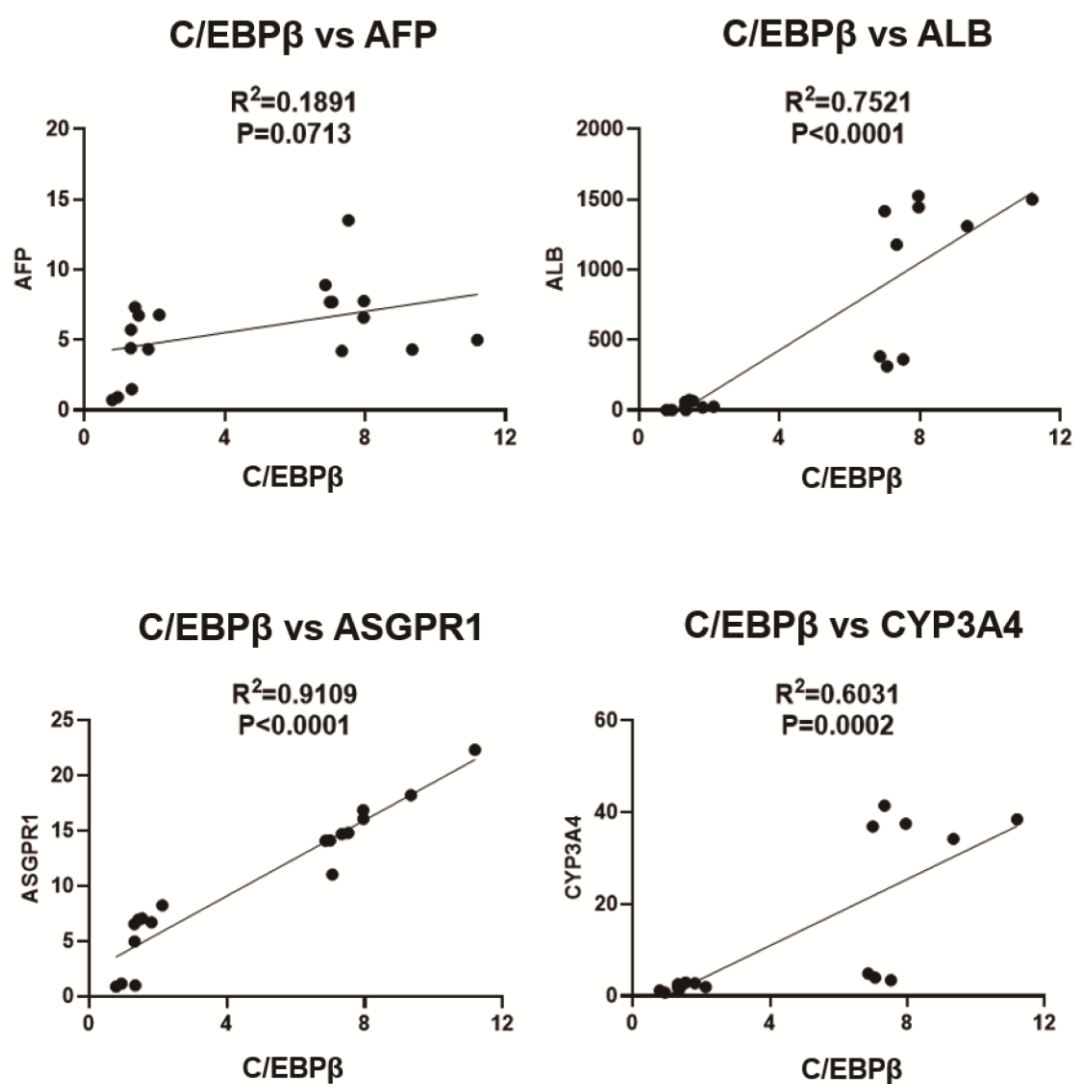


Figure S2. Correlation analysis of C/EBP β gene expression with AFP, ALB, ASGPR1 and CYP3A4 during the differentiation of hepatocyte-like cells (HLCs) from hESCs.

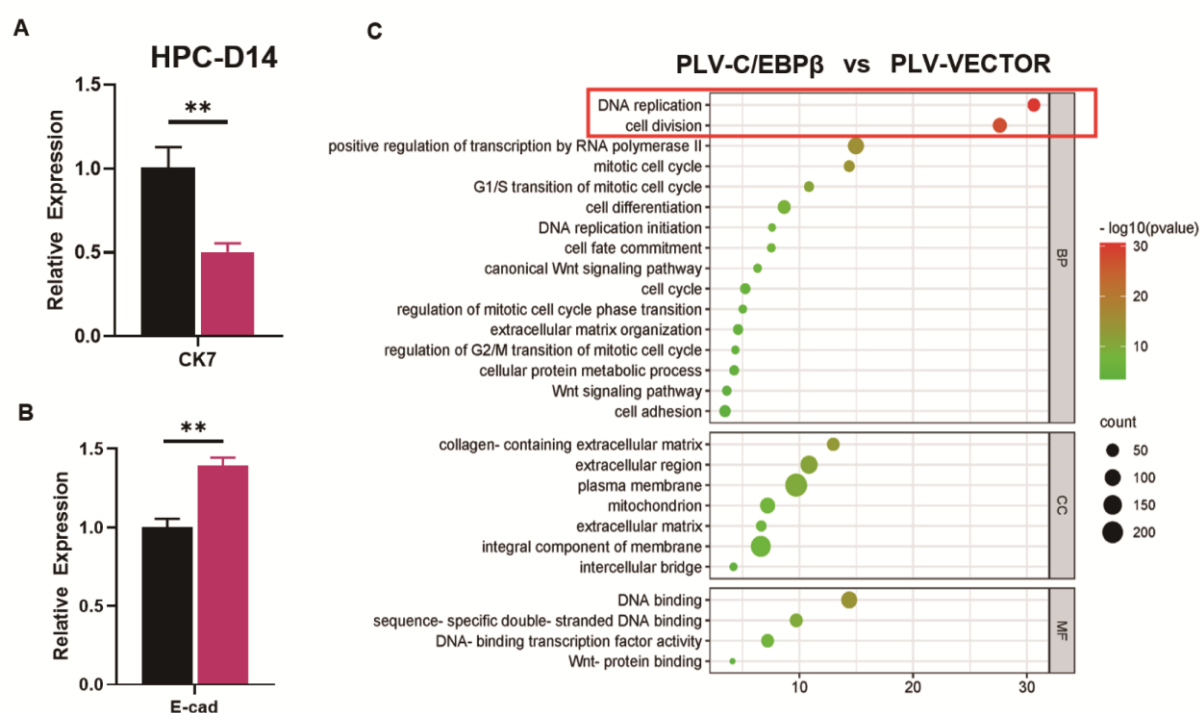


Figure S3. Enhanced expression of C/EBP β promoted hepatic differentiation and cell cycle progression in HPCs. (**A,B**) Relative expression analysis of bile duct gene CK7 (**A**) and epithelia marker E-cad (**B**) were determined by qPCR. (**C**) GO analysis of the differentially expressed genes (DEGs), and the GO categories included BP and CC as well as MF respectively. Highly enriched GO terms showed that DNA replication and cell division were ranked at the top in the lists.

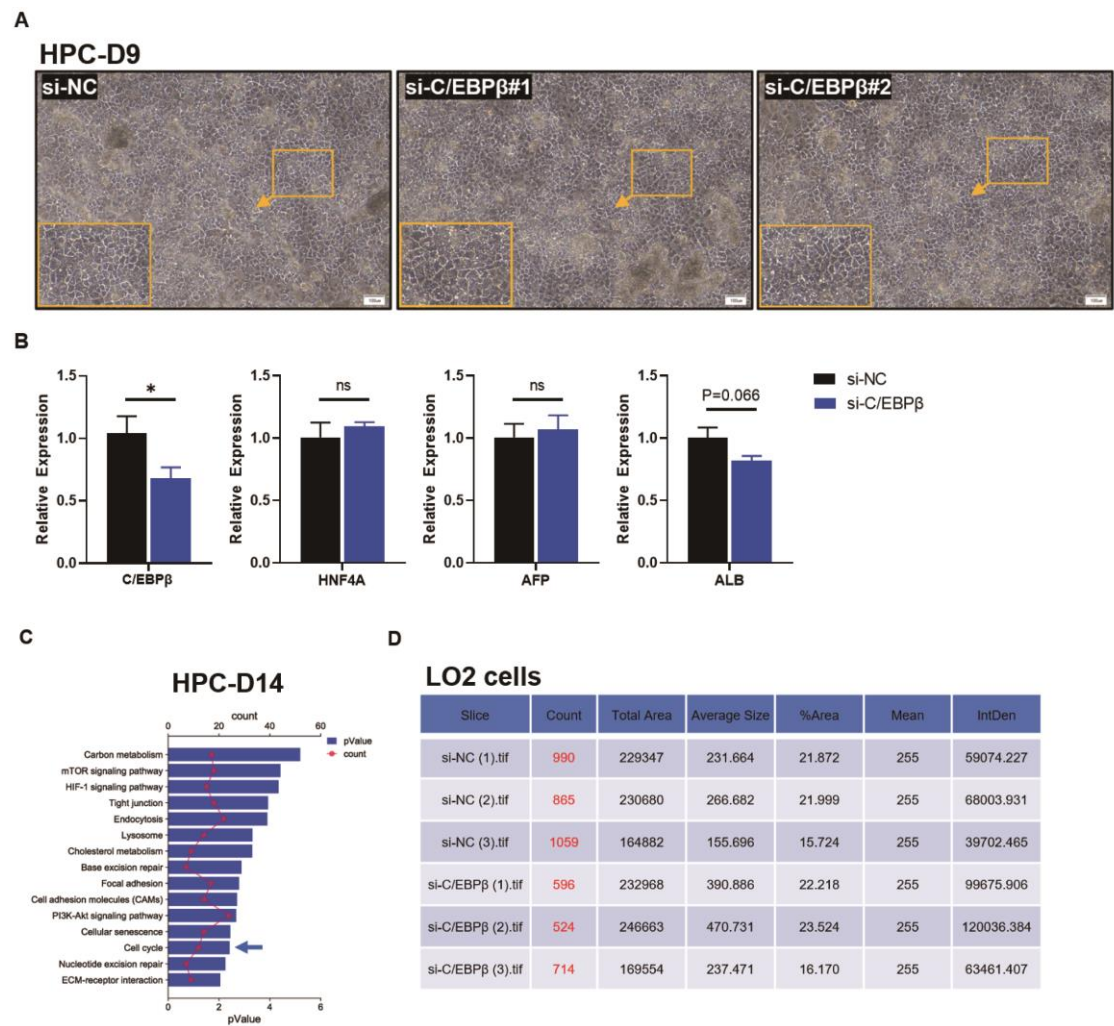


Figure S4. C/EBPβ Knockdown at the early stage had little impact on the hepatic differentiation from hESCs. **(A)** Representative images of cell morphologies 2 days after the treatment with 2 pairs of si-RNA at day 7 during the hepatic differentiation. **(B)** The expression of transcription factors (C/EBPβ and HNF4A) and hepatocyte genes (AFP and ALB) were detected by qRT-PCR at day 9 after the treatment with siRNAs at day 7. **(C)** The KEGG pathways for the differentially expressed genes in HPCs treated with si-NC or with si-C/EBPβ at day 14. **(D)** si-RNA mediated C/EBPβ knock-down were conducted in LO2 cells, and DAPI staining was performed, then cell numbers were defined by ImageJ software.

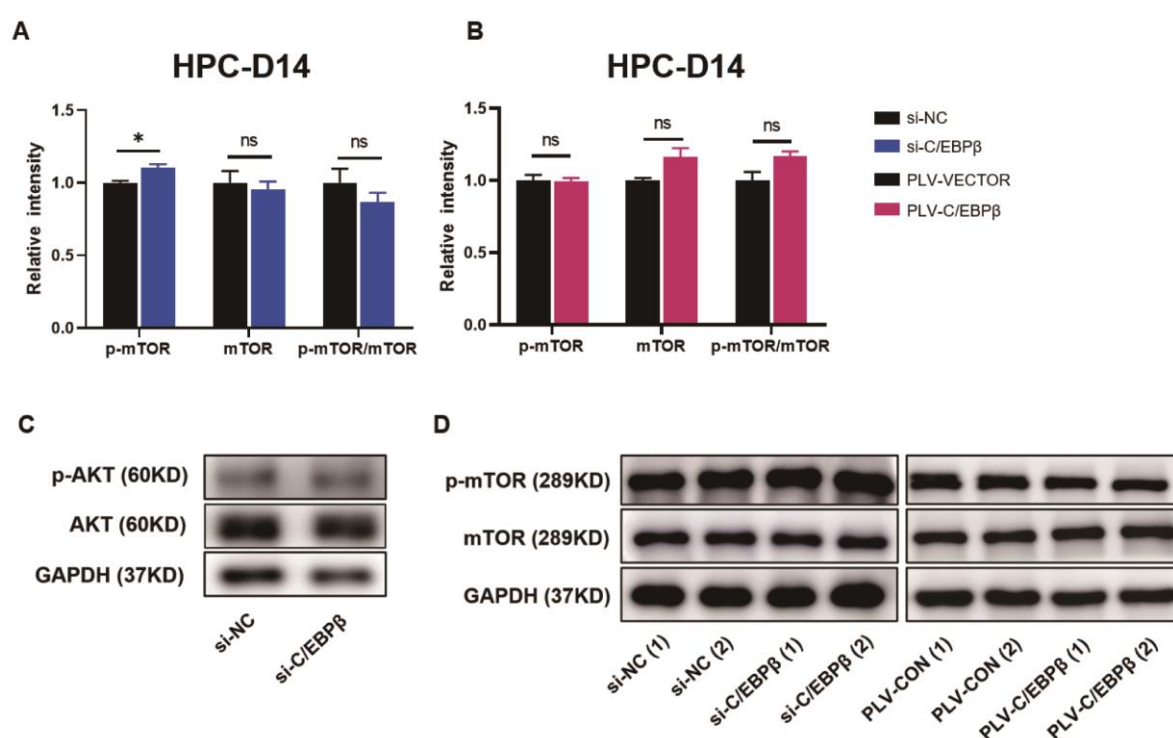


Figure S5. C/EBPβ did not orchestrate the PI3K/AKT/mTOR signaling pathway to promote cell proliferation. (A,B) The statistical results of mTOR/p-mTOR protein expressions in Fig. S5D. (C) Western blot analysis of the AKT/p-AKT after the knockdown of C/EBPβ at day 14 of HPCs. (D) Western blot analysis of the PI3K/AKT/mTOR signaling pathway in knockdown (left panel) or overexpression (right panel) of C/EBPβ on day 14 of HPCs.

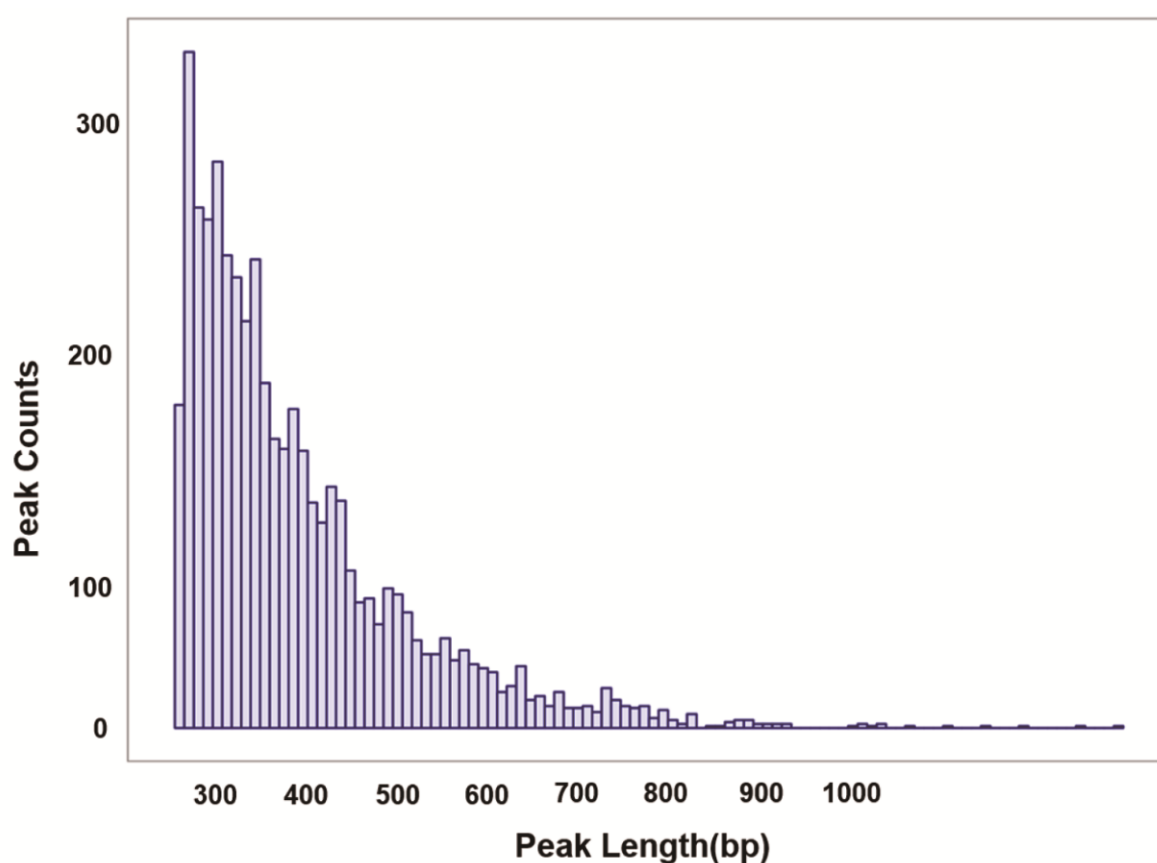


Figure S6. Length distribution of peaks in Cut&Tag analysis for efficient C/EBP β binding, the abscissa is the length of peaks (bp), and the ordinate is the number of corresponding peaks.

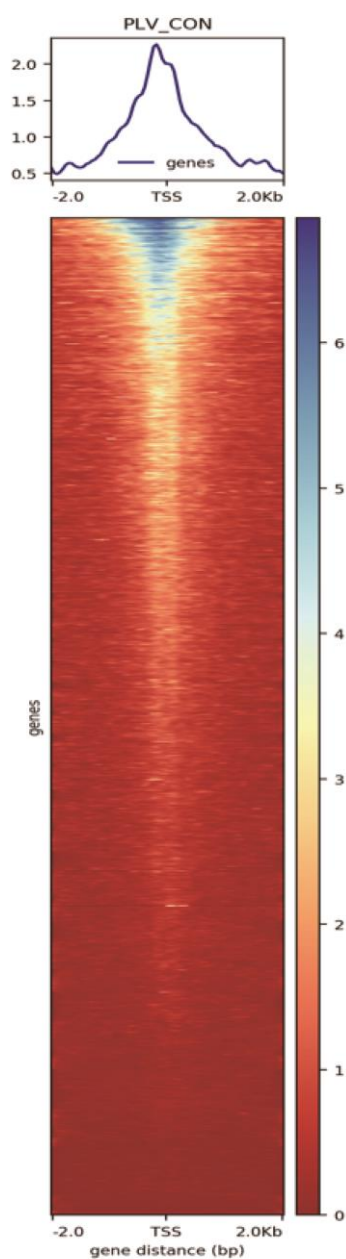


Figure S7. Heat map around genomic sites called from C/EBP β binding profile in Cut&Tag analysis for efficient C/EBP β binding.

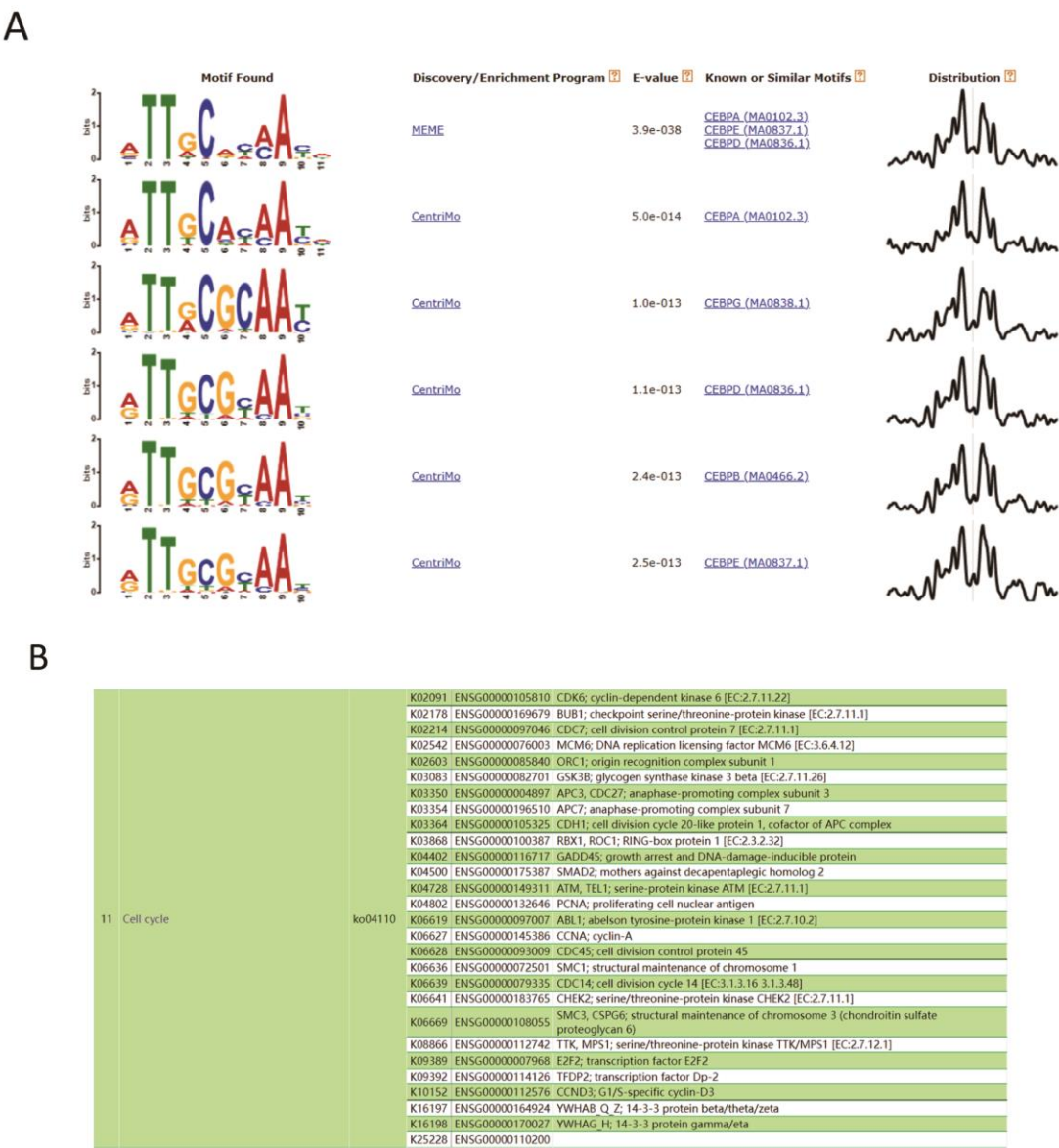


Figure S8. Cut&Tag analysis for efficient C/EBPβ binding. **(A)** The top 6 motif sequences associated with C/EBPβ binding showed high similarity to known protein-binding motifs in CEBP family. **(B)** Gene lists from KEGG enrichment in Fig. 6D, which showed that cell cycle signaling pathway was significantly enriched.

Table S1. The antibodies used for Flow cytometry in this study.

Antigen	Cojugate	Company	Catalog number	Clone
OCT4	PE	STEMCELL	60093PE	3A2A20
TRA-1-81	PE	STEMCELL	60065PE	TRA-1-81
CXCR4	APC/Cy7	Biolegend	306528	12G5
SOX17	PE	BD Pharmingen	561591	P7-969
AFP	PE	BD Pharmingen	563002	C3/AFP
ALB	PE	R&D	IC1455P	/
α1-AT	FITC	Bethyl	A80-122F	/
Isotype (IgG1,κ)	PE	BD Pharmingen	556650	/
Isotype (IgG1,κ)	APC/Cy7	BD Pharmingen	557873	/
Isotype (IgG2b,κ)	PE	BD Pharmingen	555742	/

Table S2. The primers used for real-time quantitative PCR in this study.

Name	Primers (Forward ; 5' to 3')	Primers (Reverse ; 5' to 3')
C/EBP β	GCCCTCGCAGGTCAAGAGCA	TTGAACAAGTTCCGCAGGGTG
C/EBP β (EXO)	CGACGGAGAC- TACAAGGATCATGA	TTGCCGCCGTACGCAG- CAGCCAA
C/EBP α	CTCGAGGCTTGCCAGACCGT	GCGGGCTTGTCGGGATCTCAG
ALB	GAGACCAGAGGTTGATGTGATG	AGTTCCGGGG- CATAAAAGTAAG
AFP	GGGAGCGGCTGACATTAT	TGTTTCATCCACCACCAA
α 1-AT	TCGCTACAGCCTTTGCAATG	TTGAGGGTACGGAGGAGTTCC
ASGPR1	GAAGCAGTTCGTGTCTGACCTG	AGCGAGAGAACCAGTAG- CAGCT
HNF4 α	GGTGTCCATACGCATCCTTGAC	AGCCGCTTGATCTTCCCTGGAT
CYP3A7	AAGGGCTATTGGACGTTTGACA	ATCCCACTGGCCCGAAAG
CK7	TCCGCGAGGTCACCATTAAAC	GCTCTGTCAACTCCGTCTCAT
CK19	ACCAAGTTTGAGACGGAACAG	CCCTCAGCGTACTGATTTCT
CDC25C	AGAAGCCCATCGTCCCTTTGGA	GCAGGATACTGGTTCAGAGACC
CDC45L	TGGATGCTGTCCAAGGACCTGA	CAGGACACCAACATCAG- TCACG
PCNA	CAAGTAATGTCGATAAAGAG- GAGG	GTGTCACCGTTGAAGAGAG- TGG
E2F1	GGACCTGGAAACTGACCATCAG	CAGTGAGGTCTCATAGCGTGAC
E2F2	CTCTCTGAGCTTCAAGCACCTG	CTTGACGGCAATCACTGTCTGC
MCM3	CGAGACCTAGAAAATGGCAGCC	GCAGTGCAAAGCACAT- ACCGCA
GIN51	GCAAAGTCAGGTGGAC- GAAGTG	CTGATCCGAAGCAAGCGGTCAT
GAPDH	GAAGATGGTGATGGGATTTC	GAAGGTGAAGGTCGGAGTC

Table S3. The antibodies used for Western Blot in this study.

Antigen	Species Reactivity	Company	Catalog number	Dilution
C/EBP β	Mouse anti-human	Santa Cruz	sc-7962X	1:1000
CDC45L	Rabbit pAb	GeneTex	GTX110586	1:1000
PCNA	Rabbit pAb	GeneTex	GTX100539	1:1000
E2F2	Mouse anti-human	Santa Cruz	sc-9967	1:500
AKT(pan)	Rabbit pAb	CST	4691T	1:1000
p-AKT(T308)	Rabbit pAb	CST	13038T	1:1000
mTOR	Rabbit pAb	CST	2983T	1:1000
p-mTOR	Rabbit pAb	CST	5536T	1:1000
CyclinD1	Rabbit pAb	CST	55506T	1:1000
E-cad	Rabbit pAb	CST	3195S	1:1000
GAPDH	Rabbit pAb	GeneTex	GTX100118	1:5000
IgG (H+L)	Goat anti-mouse	Beyotime	A0216	1:2000
IgG (H+L)	Goat anti-rabbit	Beyotime	A0208	1:2000

Table S4. The siRNA(C/EBP β) used in this study.

Oligo Name	Primer	Sequence (5'-3')
hs-C/EBP β -si-1	F'	CCUGCCUUUAAAUCGAUGGdTdT
	R'	CCAUGGAUUUAAAGGCAGGdTdT
hs-C/EBP β -si-2	F'	CGACUCCUCUCCGACCUCdTdT

hs-C/EBP β -si-3	R'	GAGGUCGGAGAGGAAGUCGdTdT
	F'	GCACAGCGACGAGUACAAGdTdT
	R'	CUUGUACUCGUCGCUGUGCdTdT
si-NC	F'	UUCUCCGAACGUGUCACGUdTdT
	R'	ACGUGACACGUUCGGAGAAdTdT

Table S5. The shRNA(C/EBP β) target sequences in this study.

Name	Symbol	Location	Target Sequence
sh-con	/	/	ACAGAAGCGATTGTTGATC
sh-a	C/EBP β	505	GCCTGCCTTTAAATCCATGGA
sh-b	C/EBP β	1258	GCACAGCGACGAGTACAAGAT
sh-c	C/EBP β	1432	GCGGAACCTTGTTCAAGCAGCT