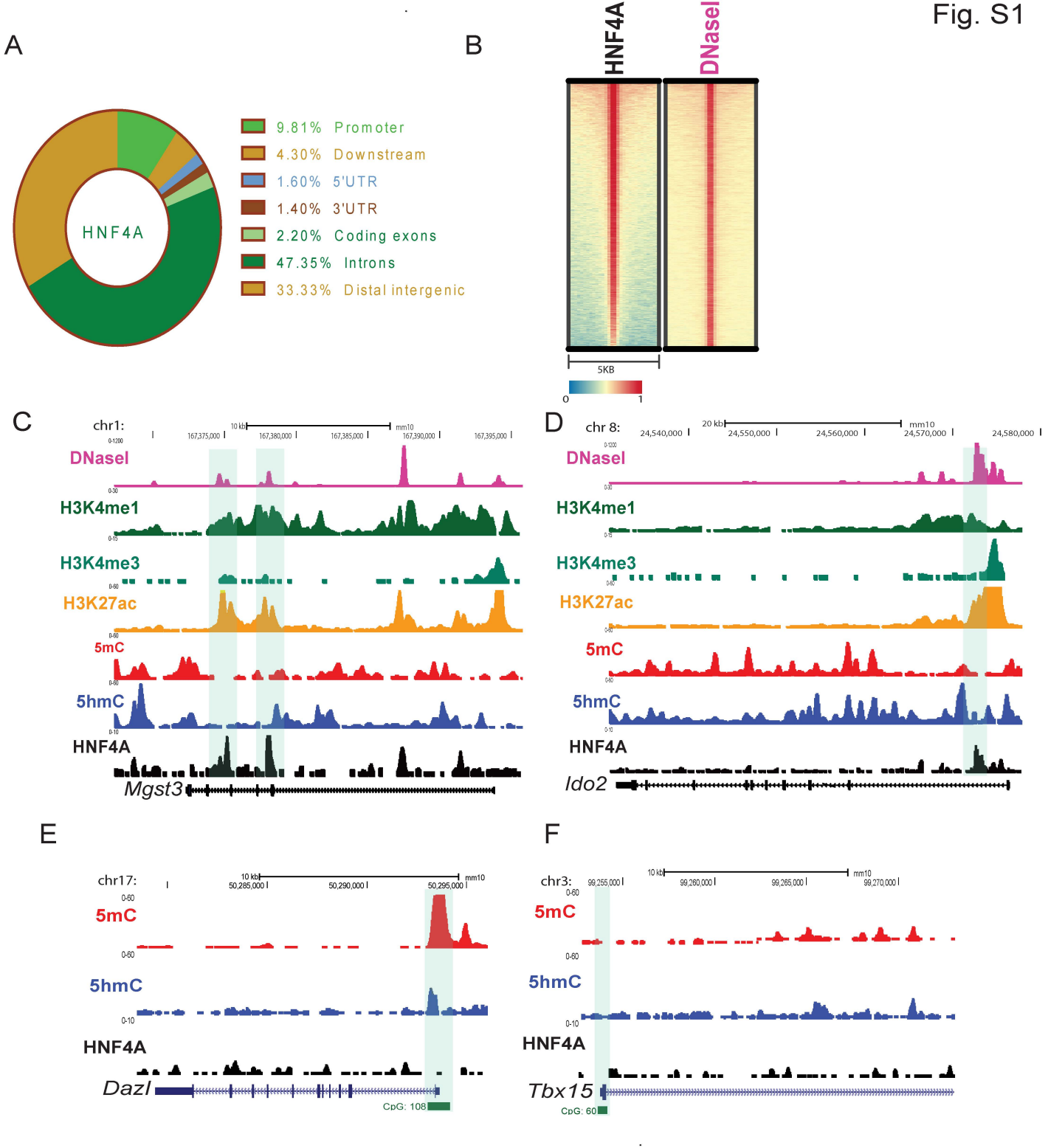
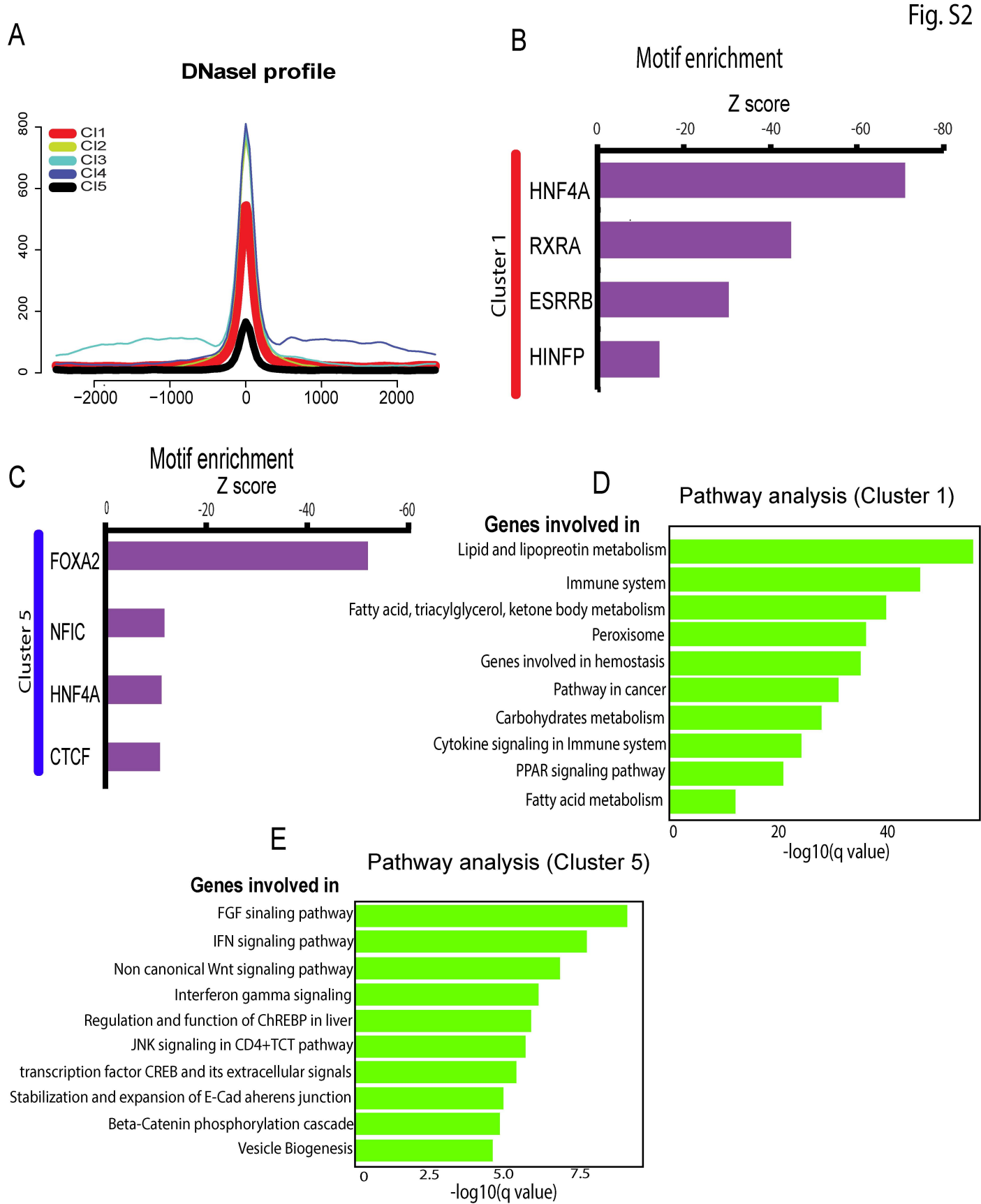
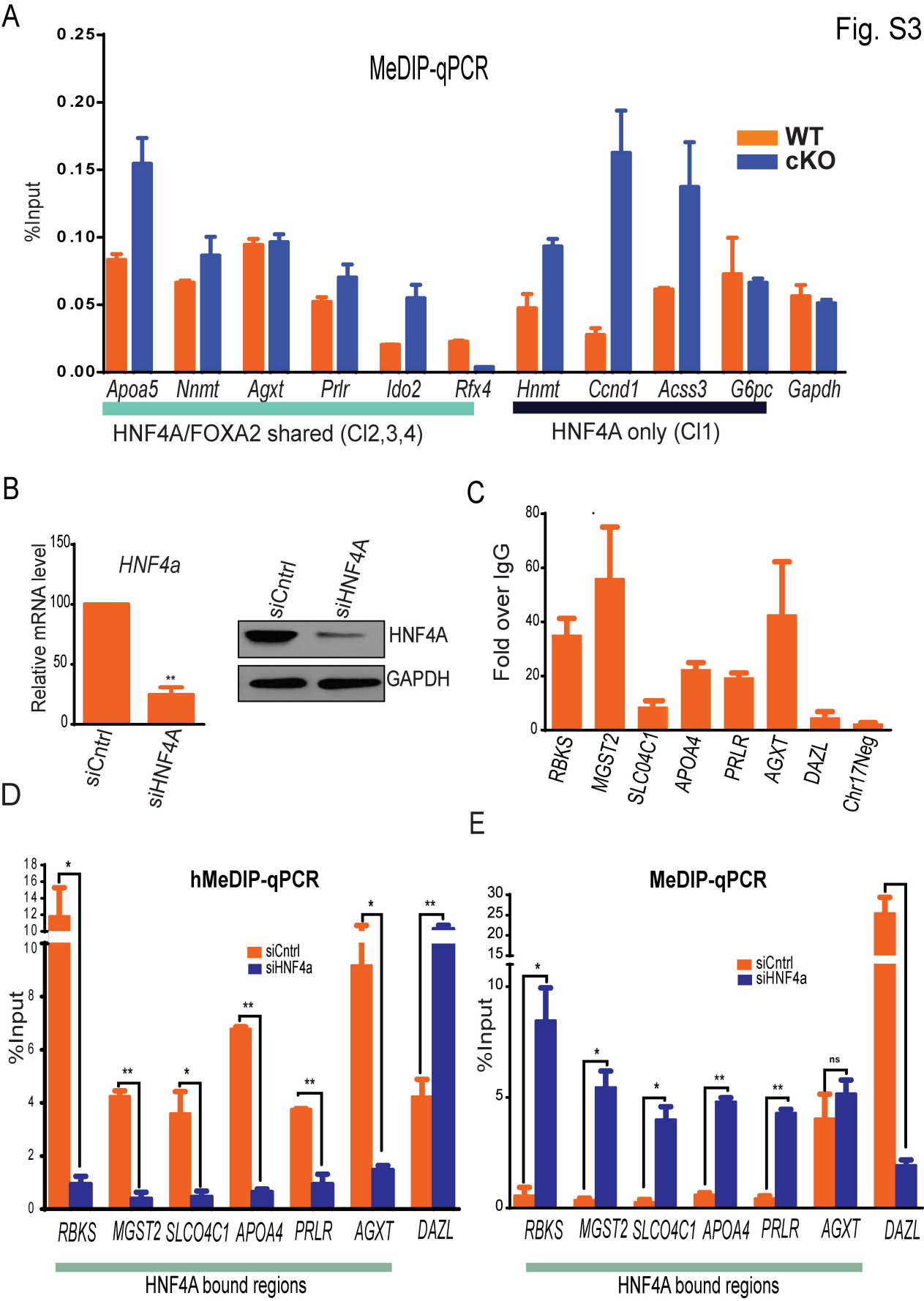
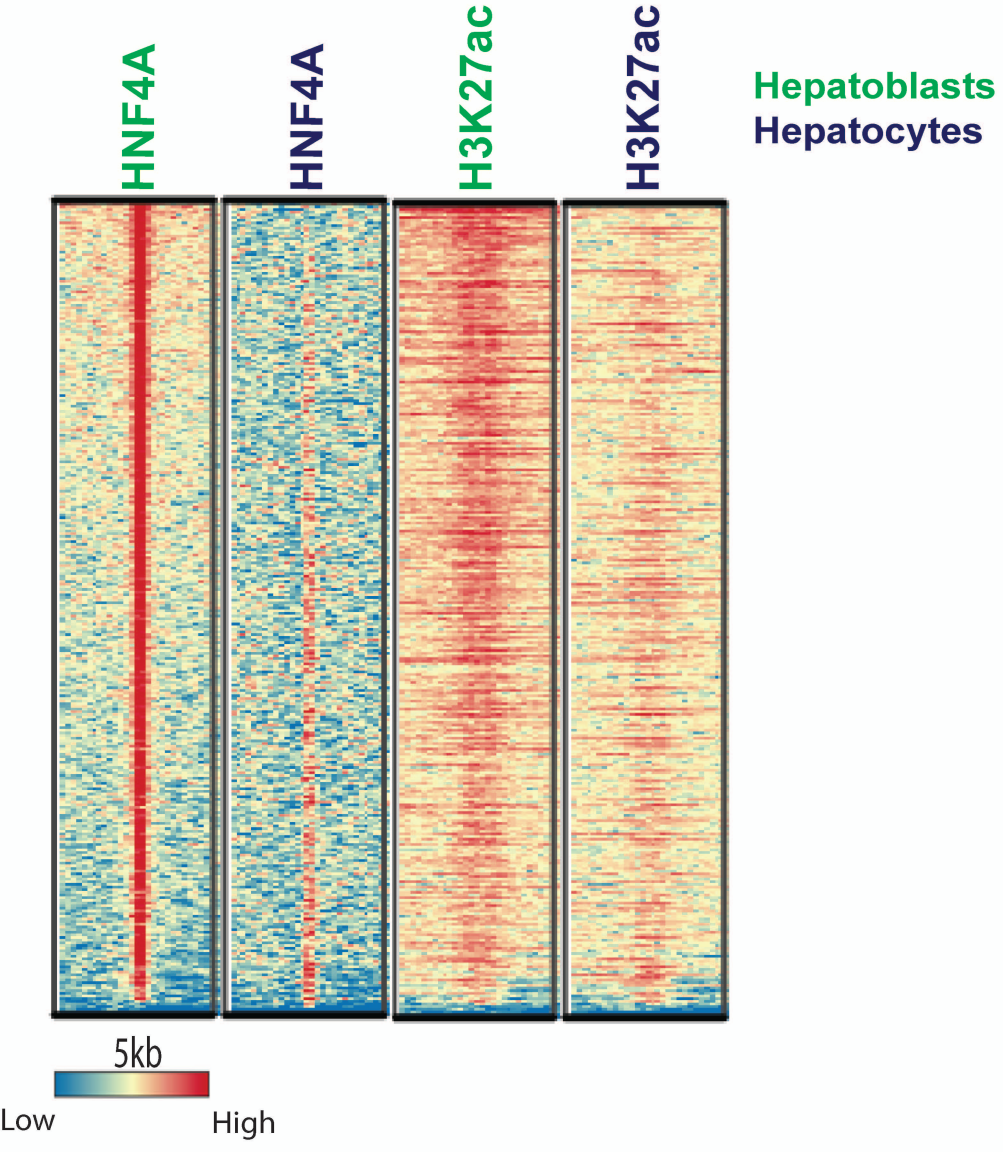

A
HNF4A
9.81% Promoter
4.30% Downstream
1.60% 5'UTR
1.40% 3'UTR
2.20% Coding exons
47.35% Introns
33.33% Distal intergenic
B
HNF4A
DNaseI
5KB
0
1
C
chr1:
DNaseI
H3K4me1
H3K4me3
H3K27ac
5mC
5hmC
HNF4A
Mgst3
D
chr 8:
DNaseI
H3K4me1
H3K4me3
H3K27ac
5mC
5hmC
HNF4A
Ido2
E
chr17:
5mC
5hmC
HNF4A
Dazl
CpG: 108
F
chr3:
5mC
5hmC
HNF4A
Tbx15
CpG: 60

Fig. S2

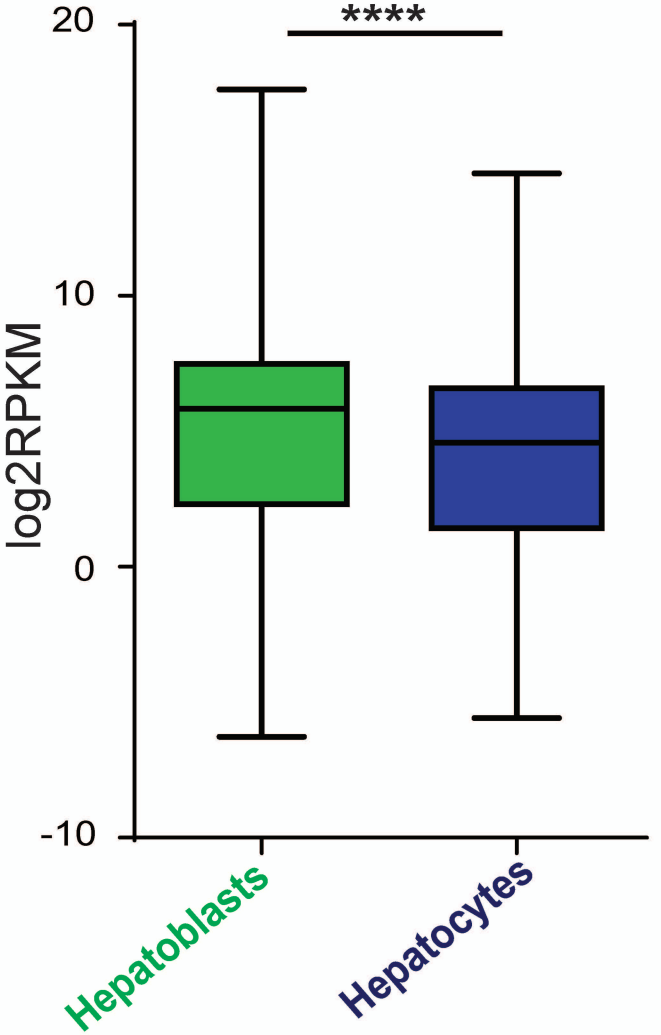

A
DNaseI profile
CI1
CI2
CI3
CI4
CI5
800
600
400
200
0
−2000
−1000
0
1000
2000
B
Motif enrichment
Z score
0
-20
-40
-60
-80
Cluster 1
HNF4A
RXRA
ESRRB
HINFP
C
Motif enrichment
Z score
0
-20
-40
-60
Cluster 5
FOXA2
NFIC
HNF4A
CTCF
D
Pathway analysis (Cluster 1)
Genes involved in
Lipid and lipopreotin metabolism
Immune system
Fatty acid, triacylglycerol, ketone body metabolism
Peroxisome
Genes involved in hemostasis
Pathway in cancer
Carbohydrates metabolism
Cytokine signaling in Immune system
PPAR signaling pathway
Fatty acid metabolism
0
20
40
-log10(q value)
E
Pathway analysis (Cluster 5)
Genes involved in
FGF sinaling pathway
IFN signaling pathway
Non canonical Wnt signaling pathway
Interferon gamma signaling
Regulation and function of ChREBP in liver
JNK signaling in CD4+TCT pathway
transcription factor CREB and its extracellular signals
Stabilization and expansion of E-Cad aherens junction
Beta-Catenin phosphorylation cascade
Vesicle Biogenesis
0
2.5
5.0
7.5
-log10(q value)

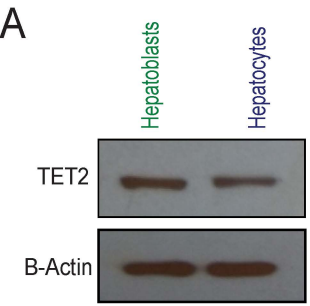

A
MeDIP-qPCR
%Input
0.25
0.20
0.15
0.10
0.05
0.00
WT
cKO
Apoa5
Nnmt
Agxt
Prlr
Ido2
Rfx4
Hnmt
Ccnd1
Acss3
G6pc
Gapdh
HNF4A/FOXA2 shared (Cl2,3,4)
HNF4A only (Cl1)
B
Relative mRNA level
HNF4a
siCntrl
siHNF4A
HNF4A
GAPDH
C
Fold over IgG
RBKS
MGST2
SLC04C1
APOA4
PRLR
AGXT
DAZL
Chr17Neg
D
hMeDIP-qPCR
%Input
siCntrl
siHNF4a
RBKS
MGST2
SLCO4C1
APOA4
PRLR
AGXT
DAZL
HNF4A bound regions
E
MeDIP-qPCR
%Input
siCntrl
siHNF4a
ns
RBKS
MGST2
SLCO4C1
APOA4
PRLR
AGXT
DAZL
HNF4A bound regions

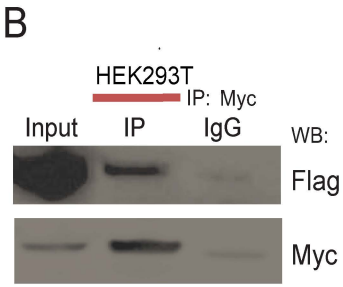
A

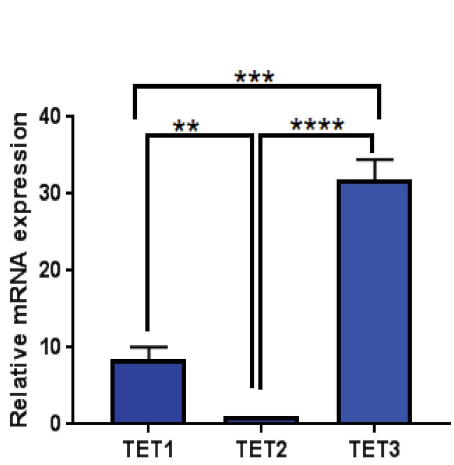

HNF4A
HNF4A
H3K27ac
H3K27ac
Hepatoblasts
Hepatocytes
5kb
Low
High

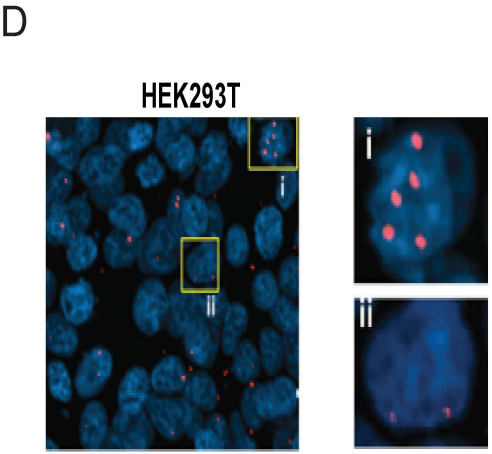
B

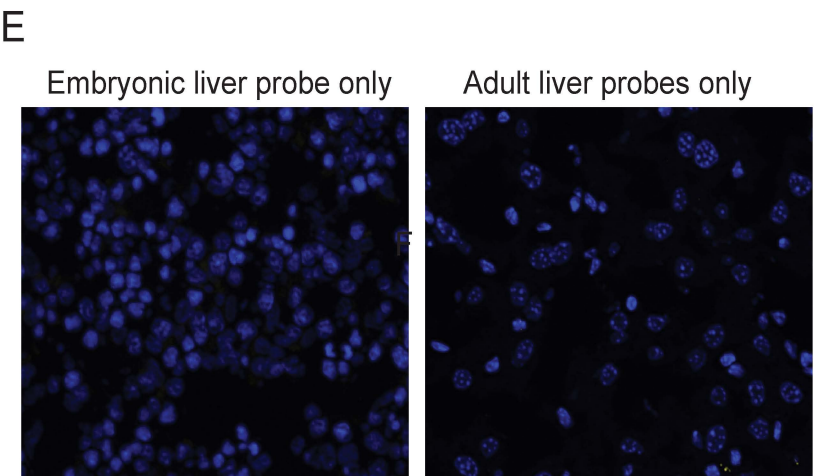

****
20
10
0
-10
log2RPKM
Hepatoblasts
Hepatocytes


A
Hepatoblasts
Hepatocytes
TET2
B-Actin

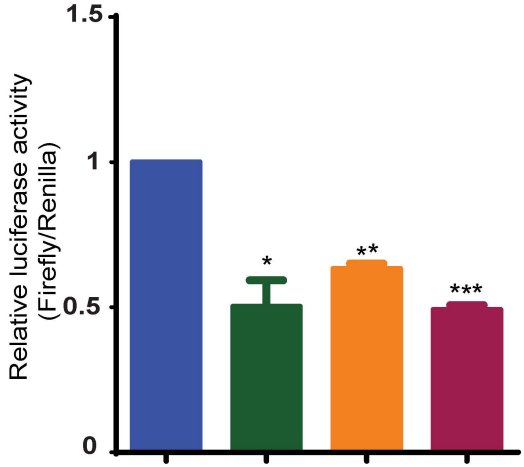

B
HEK293T
IP: Myc
Input
IP
IgG
WB:
Flag
Myc


C
***
**
****
Relative mRNA expression
40
30
20
10
0
TET1
TET2
TET3


D
HEK293T
i
ii
i
ii

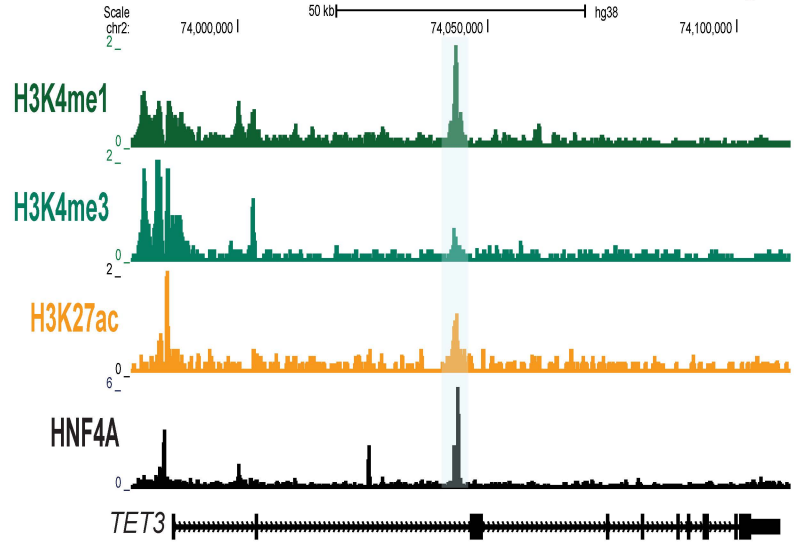

E
Embryonic liver probe only
Adult liver probes only

A

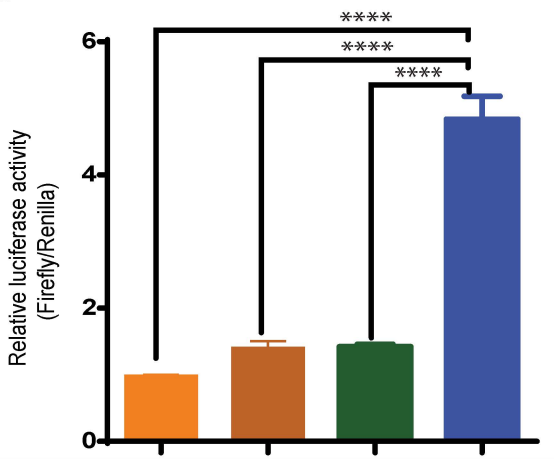


PGL3EIBP-mTET3	+	-	-	-
PGL3-EIBP-mTET3Δ1	-	+	-	-
PGL3-EIBP-mTET3Δ2	-	-	+	-
PGL3-EIBP-mTET3Δ1Δ2	-	-	-	+
PDGT-HNF4A	+	+	+	+

B

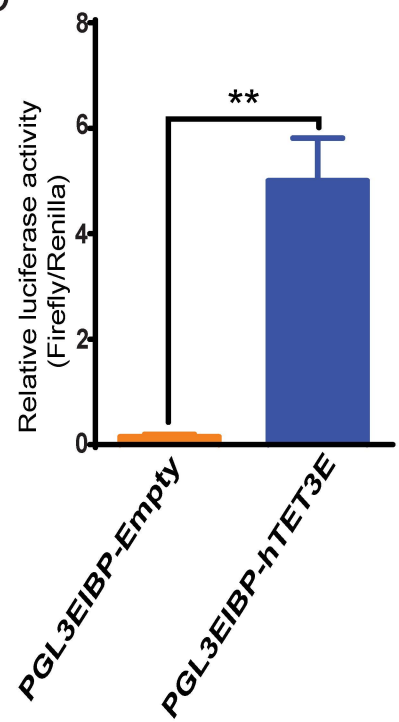


C



PGL3-EIBP-Empty	+	-	+	-
PGL3-EIBP-hTET3E	-	+	-	+
PDGT-Empty	+	+	-	-
PDGT-HNF4A	-	-	+	+

D



SUPPLEMENTARY MATERIALS AND METHODS

Cell culture and reagents

HepG2 and HEK293T cells were purchased from American Type Culture Collection (Manassas, VA). Cells were grown in DMEM media (STEMCELL Technologies) supplemented with 10% fetal bovine serum (Gibco). HPPL cells were generated and maintained as previously described [(1)]. All cells were maintained in a humidified incubator at 37°C and 5% CO_2. For knockdowns, cells were seeded on 6 well plates and transfected with HNF4A and TET3 siRNA (Dharmacon) using RNAiMAX (ThermoFisher) for 72 hrs. siRNA used in this study are presented in Table S2.

Bioinformatic analysis

ChIP-seq analysis: Single-end ChIP-seq and corresponding DNA Input short read sequence data from multiple sources with read lengths ranging 27bp to 50bp were uniformly processed to generate genome-wide wiggle-format files (https://genome.ucsc.edu/FAQ/FAQformat.html#format6) and bigWig files for further analysis and visualization with the help of UCSC genome browser. First, short reads were aligned to mm10 (NCBI build 38) mouse reference genome using BWA aligner (v.0.5.9) 'aln' and 'samse' options. Duplicated reads are marked by Picard (Picard tools 1.52) and collapsed (only one copy of the reads from a collection of the reads that have the same alignment position is retained). Reads mapped to the multiple locations are removed by applying alignment quality threshold QA>5. The average DNA fragment length was evaluated for both ChIP-Seq and DNA Input using self-correlation technique. Then, for each library the fragment coverage genomic profile was calculated using directional extension of all aligned reads with estimated average fragment length (in the range 150-200bp). Further a custom BAM2WIG java tool (http://www.epigenomes.ca/tools-and-software, M. Bilenky, unpublished) was used to generate UCSC browser wig files. 'wigToBigWig' program from UCSC tools suit (http://hgdownload.soe.ucsc.edu/downloads.html#source_downloads) was used to convert wig

files into bigwig files https://genome.ucsc.edu/goldenPath/help/bigWig.html). ENCODE adult mouse liver was downloaded from https://www.encodeproject.org/experiments/

hmeDIP-seq/meDIP-seq analyses: Sequencing reads were aligned to mm10 using Burrows-Wheeler Aligner (BWA, version 0.5.7) [2] and converted to BAM format using Samtools (version 0.1.19) [3]. PCR duplicates were marked using Picard (version 1.52). Deeptools bamCoverage (version 2.5.0.1) [4] was used to normalize library coverage to RPKM based on bin sizes of 20bp, and the resulting BIGWIG file was used for downstream analyses and visualization. BWA mapping quality scores less than 5 were discarded and duplicate reads were only counted once. Heatmaps and K-means clustering were generated using ChAsE [5]. CEAS [6] determined genomic distribution. Profile plots of histone modifications and DNA modifications were generated using SitePro within the Galaxy/Cistrome toolbox [6]. Motif analysis was performed using SeqPos in Cistrome [7]. GREAT was used for gene association [8]. EMBL ENA accession number for the data reported in this paper is PRJEB31004.

MeDIP and hMeDIP-qPCR

MeDIP and hMeDIP for locus-specific qPCR analysis were performed as described [9] with several modifications. DNA from mouse hepatocytes, hepatoblasts, whole livers (WT and HNF4A KO) and HepG2 cells was isolated using Quick DNA MiniPrep kit (Zymo Reseach). DNA was sonicated to achieve fragments size of 300-500bp. 800ng of sonicated DNA (40ul) was added to siliconized tubes, denatured at 95°C for 10 minutes, and cooled for 2 minutes on ice. Samples were incubated with 2µg of anti-5mC or anti-5hmC antibodies (Table S2) for 2 hours. Next, 20ul of Dynabeads™ Protein G (ThermoFisher Scientific) was added to samples and incubated for 4 hours at 4°C followed by three washes with 0.05% Triton X-100 in PBS. DNA was eluted by heating samples at 65°C for 10 minutes with elution buffer (1% SDS in TE). The total eluent was treated with proteinase K (Invitrogen) and RNase A (Invitrogen) overnight at 55°C. DNA was purified by phenol-chloroform extraction. 3µl of enriched and input DNA were

used for qPCR using loci-specific primers (Table S1) to determine enrichment of target loci normalised to input.

RNA sequencing

We used our previously published RNA-seq data analysis pipeline as described [10]. RPKM values were used to generate box plots indicating global gene expression using GraphPad Prism software. For individual genes RPKM values were plot using Microsoft Excel.

RT-qPCR:

RNA was extracted from cells or tissues using TRIzol (Invitrogen). cDNA was synthesized using Transcriptor First Strand cDNA synthesis kit (Roche). qRT-PCR was carried out using the SYBR Green Master Mix (Roche) on the StepOnePlus Real-Time PCR System (Applied Biosystems). Relative mRNA quantification was calculated using Ct values ($2^{-\Delta\Delta Ct}$). mRNA expression levels were normalised to *Gapdh* or *β-actin.* RT-qPCR primers are presented in Table S1.

Western blot

Cells were lysed with ice-cold lysis buffer (Cell Signalling Technology) with added protease inhibitor cocktail (Sigma Aldrich). Proteins were quantified using the Pierce BCA Protein Assay kit (Thermo Fisher Scientific). An equal amount of protein was loaded onto SDS-polyacrylamide gels and transferred onto PVDF membranes. After blocking for one hour with 5% non-fat milk in TBS, membranes were incubated with primary antibodies overnight at 4°C (Table S2). The membranes were washed with TBS and incubated with HRP-conjugated secondary antibodies at room temperature for 1 hour. HRP activity was detected with Pierce ECL Western Blotting Substrate (Thermo Fisher Scientific). Antibodies used for westerns are listed in Table S2.

Luciferase assay

Enhancer regions were PCR amplified and clones in PGL3 basic vector under the control of EIB promoter. HNF4A binding motifs were deleted using site-directed mutagenesis. Luciferase constructs with RLTK (Promega) were transfected using PEI and lipofectamine in HEK293T and HepG2 cells respectively. Cells were harvested 48 hours post-transfection and luciferase assays were performed using the Dual-Luciferase Reporter System (Promega) and presented as Firefly/Renilla ratio. Primers used for enhancer cloning and mutagenesis are listed in Table S1.

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10. Alder O, Cullum R, Lee S, Kan AC, Wei W, Yi Y, et al. Hippo signaling influences HNF4A and FOXA2 enhancer switching during hepatocyte differentiation. Cell Rep. 2014;9:261–271.

SUPPLEMENTARY TABLES

Table S1: PRIMERS USED

		ChIP, MeDIP and hMeDIP-qPCR	
Name	**Species**	**Forward 5'to 3'**	**Reverse 5' to 3'**
Cdc42bpb	Mouse	GAGTTCCTTCTGGGCCGTAA	CCTGCGCAGACCTTGAAATC
Mgst3	Mouse	AACTCGAAGTGGGACAAGGG	GCAAGTGGAGCGGGAAAATC
Tet3	Mouse	GATCCCAGGCCAACTCCAAA	ATCTACCGATCTGGCTCCCT
Ido2	Mouse	CGAGGAGCAAAGTTCCATAGAGACAC	TTGGGAAAGAACAGTGGGTTTGGC
Rfx4	Mouse	ACAGGAGCCAGGAAAGGAACAGAA	AGGCATGCTTTGTTTAGCCTGAGC
Tbx15	Mouse	TCCCCCTTCTCTTGTGTCAG	CGGAAGCAAGTCTCAGATCC
IGd	Mouse	CCCTCTGGCCCTGAATTTAT	CACCCAGCAATGCTTCAGT
Dazl	Mouse	CTAAGGCCTGCTCCTACGG	AGCACAGAGAAGTGGGGAGA
Apoa5	Mouse	ACCCAAATCAATCCTCCCTCCTCT	ACTTGCAGGCATTCTCCAGTCTCT
Nnmt	Mouse	ACCCTCATTACATGGCTTGTGACC	GCAGTGGGCAGAACATCCAAAGAA
Agxt	Mouse	ATCAGCAAGTGAAGGCCACGAAAC	TGCAACTTGAGAAGCTGGCCATTG
Prlr	Mouse	TCCCTTCATCTCGTCGGTGTTTGA	GCGGTATCATTGCTGTGTGGCATT
Hnmt	Mouse	GTACAGCCAGCACAAGACCA	ACCATCCATCGAGGAATCAC
Ccnd1	Mouse	TCCAGAAGGGCTTCAATCTG	CTGGCAGCTCTCACTGGATT
Acss3	Mouse	TCAAAATTGAGGATGCGATG	GGGAAAGAGGGGAGAGTGTT
G6pc	Mouse	TCCAAGAGCTGACATCCTCAC	TCCCACCACGTGGGTATTGA
Gapdh	Mouse	GGGTTGAATTGGAGGAGGCT	GAAAGGGGCAGTGTCTCCTA
Six1	Mouse	CTATCTTTGTGCCGGGTGTT	GGTCCCATTCTCCCTTGTTA
RBKS	Human	AGTAACTGGCCCCGGACTAA	AGGAGTGGCGTCTCTGGTTA

MGST2	Human	TGCTCTGATCCCAGTCAGTG	TGCTTCTTGCAGGTTTTCTGT
SLCO4C1	Human	GGTGCTTCAGAGCAGCAGA	CTGCGAGCTGGAGTAGGAAG
APOA4	Human	CCCAGCAACTCAATGCCCT	CCTTCAGTTTCTCCGAGTCCT
PRLR	Human	CGCCATGGGTACTCACTTTT	GCACCGAGTTGAGCTTCTTC
AGXT	Human	CTGCAGTCCCAATCTCACCT	GACTTCTCCCCGAAGTCCTC
DAZL	Human	CTCCTTTGACCACTCGAAGC	CACCCACGAGTGAAGACTCC
Chr17Neg	Human	GCAGGCACATAAGGGTGTTT	CCGTGTTAGCCAAGATGGTC
		Primers used for qRT-PCR	
Tet3	Mouse	CAAACCACCCAAGGAAAAGA	CCTGGACCTGGATTTCTTGA
Hnf4a	Mouse	AGCGTGAGGAAGAACCACAT	AGCCCGGAAGCACTTCTTA
Gapdh	Mouse	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
B-Actin	Mouse	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT
HNF4A	Human	CGAAGGTCAAGCTATGAGGACA	ATCTGCGATGCTGGCAATCT
TET1	Human	CAGAACCTAAACCACCCGTG	TGCTTCGTAGCGCCATTGTAA
TET2	Human	GATAGAACCAACCATGTTGAGGG	TGGAGCTTTGTAGCCAGAGGT
TET3	Human	TCCAGCAACTCCTAGAACTGAG	AGGCCGCTTGAATACTGACTG
BACTIN	Human	TCCCTGGAGAAGAGCTACGA	AGCACTGTGTTGGCGTACAG
		Cloning and mutagenesis	
mTET3E	Mouse	CGACGCGTTTCTCGGGAACAGGCAGTGG	CCGCTCGAGTAAGTCCACACCACCTGA
mTET3EΔ1	Mouse	GGATCAAGGCGGGAGGGGAG	CTCCCCTCCCGCCTTGATCC
mTET3EΔ2	Mouse	GGGTCCATAGCACAGGCAGA	TCTGCCTGTGCTATGGACCC
hTET3E	Human	CCGGAGCTCTCATTTGAGGCCAGAAGT	CCGCTCGAGGGGTATGTGAAGTCCCAA

Table S2: ANTIBODIES, siRNA AND PLA PROBES

Antibodies used				
Antibody	**Company**	**Catalogue**	**Species**	**Application**
Anti-5-Methylcytosine Antibody	Eurogenetec	BI-MECY-1000	Mouse	MeDIP-sequencing
5-Hydroxymethylcytosine antibody	Active Motif	39769	Rbbit	hMeDIP-sequencing
Anti-5-Methylcytosine Antibody	Epigentek	A1014	Mouse	MeDIP-qPCR
5-Hydroxymethylcytosine Antibody	Active Motif	39769	Rbbit	hMeDIP-qPCR
H3K27ac	Abcam	ab4729	Rabbit	ChIP-seq and ChIP-qPCR
TET3	GeneTex	121453	Rabbit	Western Blot/PLA
TET2	Active Motif	6389	Rbbit	Western Blot
HNF4A	Santa Cruz	SC-8987	Rabbit	ChiP-seq/ChIP-qPCR/CO-IP/Western Blot
HNF4A	Santa Cruz	SC-6556	Goat	Co-IP/Western Blot/PLA
Myc	COVANCE	MMS-150P	Mouse	Co-IP/Western Blot/PLA
Myc	Sigma	C3956	Rabbit	Co-IP/Western Blot/PLA
Flag	Sigma	F3165	Mouse	Co-IP/Western Blot/PLA
Flag	Sigma	F7425	Rabbit	Co-IP/Western Blot/PLA
Normal Mouse IgG	Santa Cruz Biotechnology	sc-2025	Mouse	Co-IP

Normal Rabbit IgG	Santa Cruz Biotechnology	sc-2027	Rabbit	Co-IP/ChIP-qPCR
siRNA used				
Name	**Company**	**Catalogue**		
ON-TARGETplus Non-targeting siRNA	Dharmacon	D-001810-01-055		
ON-TARGETplus Human HNF4A siRNA-SMARTpool	Dharmacon	L-003406-00-0005		
ON-TARGETplus Human TET3 siRNA-SMARTpool	Dharmacon	L-022722-02		
ON-TARGETplus Mouse HNF4A siRNA-SMARTpool	Dharmacon	L-065463-00		
ON-TARGETplus Mouse Tet3-siRNA-SMARTpool	Dharmacon	L-054156-01		
PLA probes used				
Name	**Company**	**Catalogue**		
Anti mouse Plus	Sigma/Duolink	DUO92001		
Anti Rabbit minus	Sigma/Duolink	DUO92005		
Anti Goat Plus	Sigma/Duolink	DUO92003		