Supporting Information

Sandovici et al. 10.1073/pnas.1019007108

SI Methods

Animals. All procedures were conducted in accordance with the 1986 British Home Office Animals Act. Pregnant and lactating Wistar rats were maintained on a 20% wt/wt protein - C or isocaloric 8% wt/wt protein - LP (Arie Blok). Litters were randomly reduced to eight pups at 3 d of age. All offspring were weaned at 21 d onto a standard diet containing 20% wt/wt protein (LAD1; Special Diet Services) and remained on this diet until the end of the study. Four male rats from each litter were killed at 3M or 15M (±3 d) after overnight fasting. Each sample was composed of a pool of two to four animals from a single litter.

Islet Isolation. Rat islets were isolated by ductal collagenase distension and Histopaque gradient separation (Sigma). Pancreata were perfused with HBSS buffer (Sigma) containing 1 mg/mL Collagenase P (Roche), excised, and incubated at 37 °C for 15 min. After digestion, islets were washed and then purified using a Histopaque discontinuous gradient. Human pancreatic islets were obtained through the Nordic Network for Clinical Islet Transplantation and the Human Tissue Laboratory at Lund University Diabetes Centre from deceased nondiabetic donors (6 female and 12 male) aged 31–73 y, with a body mass index ranging from 17.6–29.1 kg/m^2. On admission to intensive care unit, the donor, or his or her relatives, had given consent to donate organs, and the local ethics committees approved the protocols. The islets were isolated using a modified semiautomated digestion filtration method, cultured for an average of 4 d in CMRL (Connaught Medical Research Laboratories) medium 1066, and then snap-frozen and stored at −80 °C until use.

RNA Extraction. Frozen liver and kidney samples were utilized for RNA extraction using the TRI reagent (Sigma), followed by purification using the RNeasy mini kit (Qiagen) and DNaseI treatment. Freshly collected pancreatic islets were used for RNA extraction with the RNeasy mini kit following the manufacturer's instructions, with an additional DNaseI treatment step. RNA quantification was performed using a NanoDrop spectrophotometer (Thermo Fisher Scientific). RNA integrity was assessed using the Agilent Bioanalyser (Agilent Technologies). One microgram of RNA was used to synthesize cDNA using oligo-dT primers and M-MLV reverse transcriptase (Promega). The rat *Hnf4a* probe detects α1, α2, α4, α5, α7, and α8 isoforms (Santa Cruz Biotechnology), and rat and human P1 and P2 probes or primers detect only promoter-specific isoforms. Quantification of gene expression was performed using the ABI Prism 7900 system (Applied Biosystems). Fold changes in gene expression were calculated using the 2-ΔΔ cycle threshold method (1) and normalized against cyclophilin A (*Ppia* or human *PPIA*). Additional corrections using the Pffafl equation (2) were applied for sets of primers with unequal efficiencies. Using additional absolute quantification measurements, we found that *Ppia* was not differentially expressed between C and LP islets and did not change during aging.

Protein Extraction and Quantification. Two hundred microliters of lysis buffer (250 mM Hepes, 3 M NaCl, 500 mM NaF, 100 mM $Na_4P_3O_7$, 100 mM Na_3VO_4, 500 mM EDTA, 20% vol/vol Triton X-100, and fresh protease inhibitors) was added to 50–100 frozen islets on ice. Islets were sonicated at 4 °C for 10 s, and supernatants were retained and stored at −80 °C until use. Protein concentration was determined using a 1:50 dilution of copper sulfate in bicinchoninic acid solution. Samples were measured in duplicate by comparison with suitable BSA standards. For Western blot analysis, protein lysates were diluted to 2 μg/μL to standardize protein loading.

Western Blot Analysis. Forty micrograms of protein extracts was loaded onto 10% vol/vol acrylamide gels, along with a protein molecular weight standard (Amersham), and separated by electrophoresis. Proteins were transferred to a nitrocellulose membrane (Millipore), and the membranes were then incubated overnight at 4 °C in blocking buffer. HNF-4α protein expression was detected using an antibody raised in goat against the C-terminal end of the rat HNF-4α protein sequence, which detects isoforms α1, α2, α4, α5, α7, and α8. The α-tubulin antibody (Sigma) was used as an internal control for protein loading. The intensities of the bands were quantified using AlphaEase spot densitometry software (AlphaInnotech).

Cell Culture. INS-1 cells were obtained from Frank Reimann (Metabolic Research Laboratories, Institute of Metabolic Science, University of Cambridge, Cambridge, United Kingdom), and BRIN-BD11 cells were provided by GlaxoSmithKline. All cells were cultured at 37 °C and in 5% vol/vol CO_2 in a humidified atmosphere and were passaged every 5 d using 0.05% trypsin-EDTA. The INS-1 cell culture medium was RPMI-1640 with 11.1 mmol/L D-glucose supplemented with 10% vol/vol FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, 10 mmol/L Hepes, 2 mmol/L L-glutamine, 1 mmol/L sodium pyruvate, and 50 μmol/L β-mercaptoethanol (Sigma). BRIN cell culture medium was RPMI-1640 with 11.1 mmol/L D-glucose supplemented with 10% vol/vol FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mmol/L L-glutamine (Sigma).

Luciferase Assays and in Vitro Methylation Assays. P2 promoter fragments obtained by PCR were cloned into the pGL4.10 luciferase vector (Promega). The *Hnf4a* enhancer region was inserted upstream of minimal P2 promoter into the pGL4.10 vector (primer sequences are provided in Table S1). Each independent measurement was performed in triplicate. For the in vitro methylation assays, the minimal P2 promoter fragment was excised from the pGL4.10 vector and cloned into the CpG-free luciferase vector pCpGL (3). The P2-pCpGL vector was then methylated with M.SssI, M.HpaII, or M. HhaI methylase (New England Biolabs). Before transfection, INS-1 cells were seeded onto 24-well plates in 500 μL of medium (4×10^5 cells per well) and incubated overnight. Cells were then transfected with 400 ng of plasmid DNA + 50 ng of pGL4.74 Renilla vector (Promega) as a control for transfection efficiency, 0.5 μL of PLUS reagent (Invitrogen), and 1μL of Lipofectamine LTX (Invitrogen) in 100 μL of serum-free medium following the manufacturers' instructions. Cells were incubated for 24 h before a Dual-Luciferase assay was used (Promega). One hundred microliters of 1× PLB (Promega lysis buffer) was used to lyse the cells, and 50 μL of the lysate was transferred to a 96-well plate. One hundred microliters of LARII reagent was added to detect the luciferase signal, and 100 μL of Stop & Glo reagent (Promega) was then used to detect the Renilla signal utilizing the Centro LB 960 Luminometer (Bad Wildbad).

ChIP. Histone modification analysis was performed using the native ChIP assay (4). Briefly, 1 g of frozen liver (−80 °C) was first disaggregated using the Medimachine system (BD Biosciences) in cold PBS supplemented with 5 mM sodium butyrate, 0.1 mM PMSF, and protease inhibitors. The release of nuclei was then obtained by passing the homogenate through 50-μm Filcons (BD Biosciences). For pancreatic islets, INS-1 and BRIN cells (~10^7 cells) were used in each experiment. A single-cell suspension was obtained

by stirring with a magnetic flea for 10–15 min on ice using the same buffer as described above. Nuclei were then released with a Dounce all-glass homogenizer with a "tight" pestle (GPE Scientific) using 10 strokes (on ice). This results in a 75–80% yield of intact nuclei as determined by counting under the microscope. Nuclei were pelleted (1,250 × *g*, 7 min, 4 °C), resuspended in 10 mL of 0.32 M sucrose buffer [10 mM Tris·HCl (pH 7.5), 0.32 M sucrose, 5 mM $MgCl_2$, 0.2 mM PMSF], pelleted again (1,800 × *g*, 10 min, 4 °C), and resuspended in 5 mL of digestion buffer [50 mM Tris·HCl (pH 7.5), 0.32 M sucrose, 4 mM $MgCl_2$, 1 mM $CaCl_2$, 0.1 mM PMSF]. DNA concentration was estimated by spectrophotometric measurement at A_{260}, and samples were adjusted to a final concentration of 250 ng/μL. We mixed aliquots of 1 mL of chromatin solution with 50 IU of micrococcal nuclease (Pharmacia) and incubated the mixture for 7 min at 30 °C (for liver) and 5 min at 28 °C (for pancreatic islets and INS-1 and BRIN cells). Digested samples were pelleted at 1,800 × *g* for 5 min, and the supernatant was removed (fraction S1). The pellet was resuspended in 1 mL of lysis buffer [2 mM Tris·HCl (pH 7.5), 0.2 mM EDTA, 5 mM sodium butyrate, 0.2 mM PMSF], dialyzed overnight at 4 °C against lysis buffer, and centrifuged the next morning (1,800 × *g*, 10 min) to obtain fraction S2. Fractions S1 and S2 were then analyzed by agarose gel electrophoresis. If the chromatin was satisfactory (one to six nucleosomes), S1 and S2 were combined and used for immunoprecipitation. For each immunoprecipitation, we used 25 μg of chromatin (liver) or 2 μg of chromatin (pancreatic islets and INS-1 and BRIN cells) with appropriate amounts of antibody as recommended by the manufacturers. The following antibodies were used: Acetyl-Histone H3 (H3Ac; Upstate–Millipore), Histone 3H (trimethyl K4) (H3K4me3; Abcam), Histone 3H (monomethyl K4) (H3K4me1; Abcam), Histone 3H (dimethyl K9) (H3K9me2; Abcam), and Histone 3H (trimethyl K27) (H3K27me3; Abcam). After overnight incubation with the antibody at 4 °C, the mixture was incubated for 4 h with Protein-A Sepharose beads (Invitrogen). The chromatin–antibody complexes were then eluted from the beads, and the DNA was purified by phenol-chloroform extraction, followed by ethanol precipitation. The DNA from each fraction was then quantified using the NanoDrop spectrophotometer and utilized for quantification. Quantification was performed by qPCR with an ABI Prism 7700 system (Applied Biosystems) using the SYBR-Green system (Sigma) and 5 ng of DNA from each fraction as template (primers are listed in Table S1).

Analysis of CTCF and Rad21 binding was performed using ChIP on formaldehyde-fixed chromatin, as previously described (5). Data on CTCF occupancy in rat liver were obtained by ChIP-Seq (data kindly provided by D. Schmidt and D. Odom, Cancer Research Institute, LiKaShing Centre, Cambridge, UK) and validated by ChIP. In rat liver, we confirmed all 10 predicted CTCF peaks (Fig. S5). Freshly collected samples (liver or islets) were washed twice in cold PBS, fixed in 1% formaldehyde (in 50 mM Hepes-KOH (potassium hydroxide), 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA) at 37 °C for 10 min, washed again in cold PBS, snap-frozen in liquid N_2, and stored at −80 °C until use. Frozen fixed tissue was washed three times in cold PBS and then mechanically disrupted in cold PBS using a Dounce tissue grinder (tight pestle) and filtered through 100-μm cell strainers to retain connective tissue. Cells were then lysed in three successive ice-cold lysing buffers [LB1: 50 mM Hepes-KOH (pH 7.5), 140 mM NaCl, 1 mM EDTA, 10% vol/vol glycerol, 0.5% Nonidet P-40, 0.25% Triton X-100; LB2: 10 mM Tris·HCl (pH 8), 200 mM NaCl, 1 mM EDTA, 0.5 mM EGTA; LB3: 10 mM Tris·HCl (pH 8), 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA, 0.1% Na-deoxycholate, 0.5% *N*-lauroylsarcosine), all supplemented with protease inhibitors (Complete, EDTA-free, no. 11873580001; Roche). After lysis, nuclei were sonicated in LB3 buffer for 10 min using cycles of 30 s on/30 s off at 30-W power (Bioruptor; Diagenode) to obtain chromatin fragments between 200 and 500 bp (efficiency of sonication was checked using a small aliquot de–cross-linked overnight and run in an agarose gel). For each immunoprecipitation, we used 100 μg of chromatin for liver and 20 μg of chromatin for islets. One hundred microliters of Dynabeads (Invitrogen) per immunoprecipitation was washed and preincubated the night before with 10 μL of antibody (anti-CTCF, 04-1497; Upstate–Millipore and anti-Rad21, ab992; Abcam) in 0.05% BSA in PBS. To reduce the unspecific binding further, we performed preclearing of the chromatin for 2 h at 4 °C before the immunoprecipitation was performed overnight at 4 °C. No-antibody control tubes (beads-only) were used in each experiment. After overnight immunoprecipitation, the beads were separated from the supernatant using a magnetic stand and were washed seven times in cold RIPA buffer [50 mM Hepes-KOH (pH 7), 500 mM LiCl, 1 mM EDTA, 1% Nonidet P-40, 0.7% Na-deoxycholate] and once in TBS [20 mM Tris·HCl (pH 7.6), 150 mM NaCl], eluted in 200 μL of elution buffer [50 mM Tris·HCl (pH 8), 10 mM EDTA, 1% SDS] and de–cross-linked overnight at 65 °C. The samples were then digested with RNase A at 37 °C for 30 min and with proteinase K at 55 °C for 2 h, and the DNA was purified by phenol-chloroform precipitation, followed by ethanol precipitation. Finally, the DNA was dissolved in 30 μL of 10 mM Tris·HCl (pH 8), and the DNA concentration was quantified using the Picogreen system (Invitrogen) before being utilized for qPCR.

q3C. For q3C, cells from freshly collected tissues were cross-linked with 1% formaldehyde. The fixed cells were then washed in cold PBS, snap-frozen in liquid nitrogen, and stored at −80 °C until use. Frozen fixed cells were washed three times in cold PBS and then lysed on ice in lysis buffer [50 mM Tris·HCl (pH 8), 1% SDS, 10 mM EDTA, 0.4% Nonidet P-40) for 30 min using additional mechanical disruption with a Dounce all-glass homogenizer (tight pestle) for liver and 26-gauge syringes for pancreatic islets. Nuclei were recovered by centrifugation and resuspended in *Hind*III digestion buffer (New England Biolabs) supplemented with Triton X-100 to a final concentration of 1.8% vol/vol and incubated for 1 h at 37 °C. A total of 1.5 × 10^6 nuclei were digested overnight with 1,000 U of *Hind*III (New England Biolabs) in a 300-μL reaction volume. Digestion efficiency at each *Hind*III restriction site within the locus was assessed by qPCR across each restriction site. The percentage of digestion was determined by comparing template amplification of digested and undigested fractions (not religated) after normalizing to copy number (primers are listed in Table S1). Only samples with at least a 70% digestion level at each restriction site were further used. Ligation was carried out overnight at 16 °C using 2.5 ng/μL digested chromatin in a 1.5-mL reaction volume of T4 ligase buffer containing 3,200 U of T4 ligase (New England Biolabs). A further digestion step with 1,000 U of *Bgl*II (which cuts outside the hybrid religated products) was incorporated before reversal of cross-links, phenol chloroform purification, and ethanol precipitation. This step is necessary to remove possible qPCR biases caused by size differences in the religated products.

q3C PCR primers flanking restriction sites were designed to have similar melting temperatures and sizes appropriate for qPCR analysis (Table S2). The PCR efficiency of each primer combination was then assessed by qPCR on a PCR standard template. The PCR standard template was prepared as previously described (6). Quantitative determination of association frequencies was done as previously described (7), and the results were normalized against the circularization frequencies of the *Actb* and *Tubb3* loci, which were used as internal controls. The q3C primers and the combinations used are listed in Tables S1 and S2. The frequencies of the random ligations decrease exponentially the further a restriction site is away from the anchor, whereas specific associations occur as "peaks" above the random ligation curves (7).

1. Livak KJ, Schmittgen TD (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 25:402–408.
2. Pfaffl MW (2001) A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 29:e45.
3. Klug M, Rehli M (2006) Functional analysis of promoter CpG methylation using a CpG-free luciferase reporter vector. *Epigenetics* 1:127–130.
4. O'Neill LP, Turner BM (2003) Immunoprecipitation of native chromatin: NChIP. *Methods* 31:76–82.
5. Schmidt D, et al. (2009) ChIP-seq: Using high-throughput sequencing to discover protein-DNA interactions. *Methods* 48:240–248.
6. Nativio R, et al. (2009) Cohesin is required for higher-order chromatin conformation at the imprinted IGF2-H19 locus. *PLoS Genet* 5:e1000739.
7. Hagège H, et al. (2007) Quantitative analysis of chromosome conformation capture assays (3C-qPCR). *Nat Protoc* 2:1722–1733.

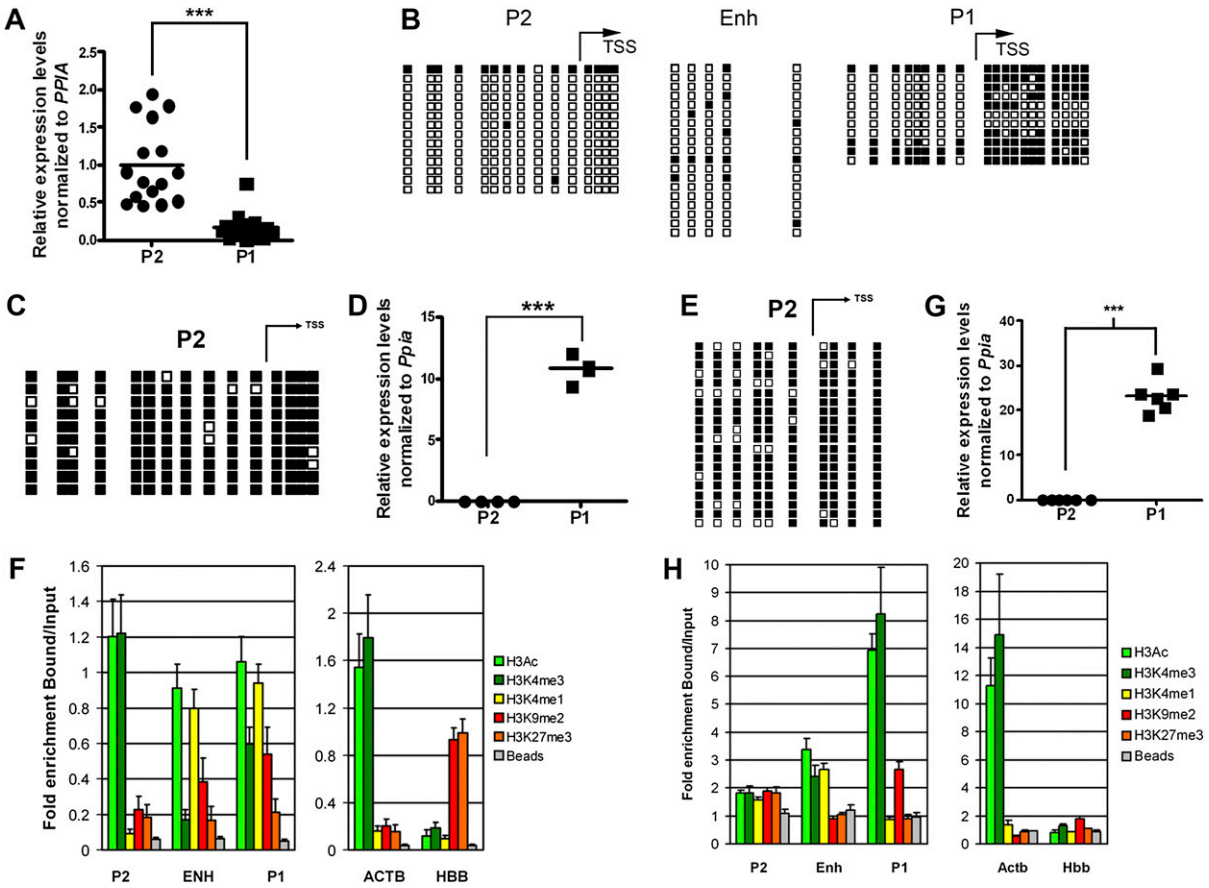

Fig. S1. Expression and epigenetic patterns at the *Hnf4a* locus in human and rat tissues. mRNA expression analysis by qRT-PCR in human islet (*A*), rat kidney (*D*), and rat liver (*G*). Data were normalized against cyclophilin A (*Ppia*) (n = 16 human islets, n = 4 rat kidney, and n = 6 rat liver). Note that P1 expression showed considerable heterogeneity in human islets, with some individuals exhibiting almost no expression, whereas others expressed significant levels. Bisulphite sequencing analysis of DNA methylation in human islets (*B*), blood (*C*), and rat kidney (*E*) (n = 2 for each tissue). In human islets, the P1 promoter showed low levels of DNA methylation upstream of TSS and high levels of DNA methylation downstream. Filled squares represent methylated CpG dinucleotides. TSS, transcription start site. Native ChIP analysis of histone marks in human islets (*F*, n = 6) and rat liver (*H*, n = 4). *Actb* and *Hbb* promoter regions were used as controls. Error bars indicate SEM. ***$P < 0.001$.

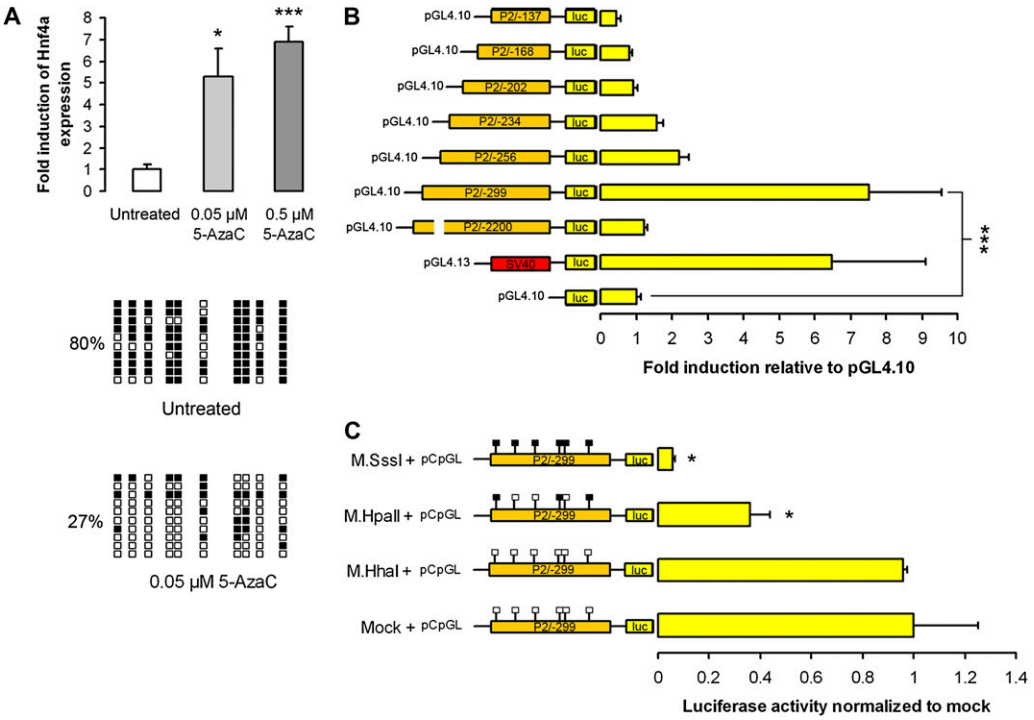

Fig. S2. Control of *Hnf4a* P2 promoter activity by DNA methylation in insulin-secreting cells. (*A*) mRNA and DNA methylation levels in BRIN cells after 72 h of treatment with 0.05 μM or 0.5 μM 5-AzaC. mRNA qPCR data were normalized against *Ppia* and presented relative to the mean level of P2 transcripts in untreated cells, arbitrarily set to 1. Filled squares represent methylated CpG dinucleotides. (*B*) P2 promoter luciferase reporter assays in INS-1 cells. Promoter activity was measured as fold induction relative to the promoterless pGL4.10. The minimal promoter is a 299-bp fragment upstream of the transcription start site that shows the highest luciferase activity. (*C*) Methylation of the P2 promoter using the CpG-free vector pCpGL. *M.Sss*I and *M.Hpa*II enzymes were used to methylate the minimal P2 promoter. *M.Sss*I methylates all six CpG dinucleotides, and *M.Hpa*II methylates three of six CpGs. Data are shown relative to the unmethylated minimal P2 promoter, pCpGL + Mock. All data represent the mean of $n = 3$ experiments ± SEM. $^{*}P < 0.05$; $^{***}P < 0.001$.

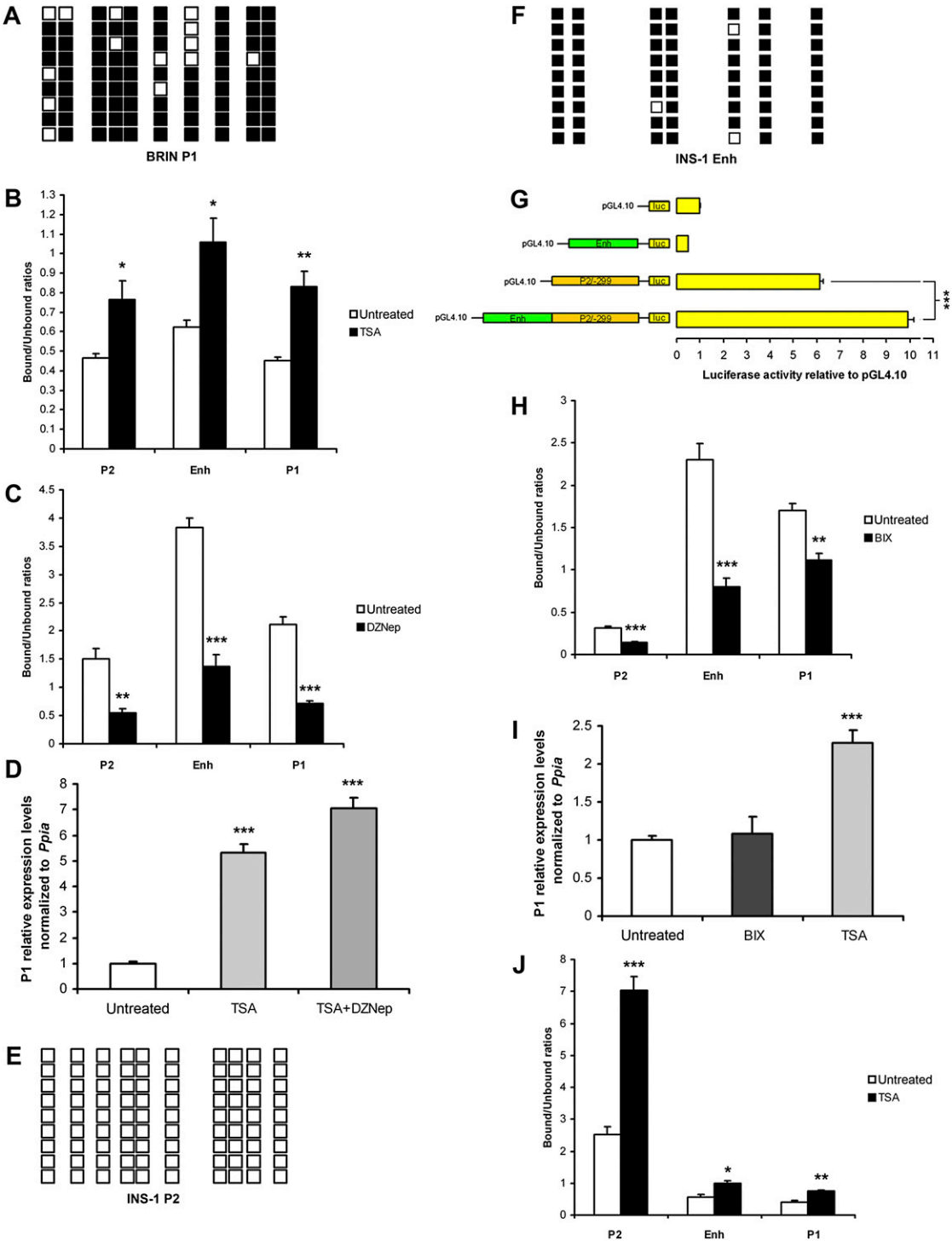

Fig. S3. In vitro pharmacological modulation of epigenetic marks in insulin-secreting cell lines. Bisulphite sequencing analysis of DNA methylation (n = 2) at the P1 promoter in BRIN cells (*A*), enhancer region (*E*), and the P2 promoter in INS-1 cells (*F*). Filled squares represent methylated CpG dinucleotides. (*B*) H3Ac levels in untreated and TSA-treated BRIN cells. (*C*) H3K27me3 levels in untreated and DZNep-treated BRIN cells. (*D*) qRT-PCR analysis of P1 mRNA levels in BRIN-treated cells. (*G*) Enhancer luciferase assays (n = 3). A luciferase vector with the enhancer cloned upstream of the minimal P2 promoter was transfected in INS-1 cells. Enhancer activity was measured relative to the empty pGL4.10 vector. (*H*) H3K9me2 levels in untreated and BIX-treated INS-1 cells. (*I*) qRT-PCR analysis of P1 mRNA levels in INS-1–treated cells. (*J*) H3Ac levels in untreated and TSA-treated INS-1 cells. In *D* and *I*, data were normalized against *Ppia* and are shown relative to untreated cells, arbitrarily set to 1. n = 4–8 for all treatment experiments. Error bars indicate SEM. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

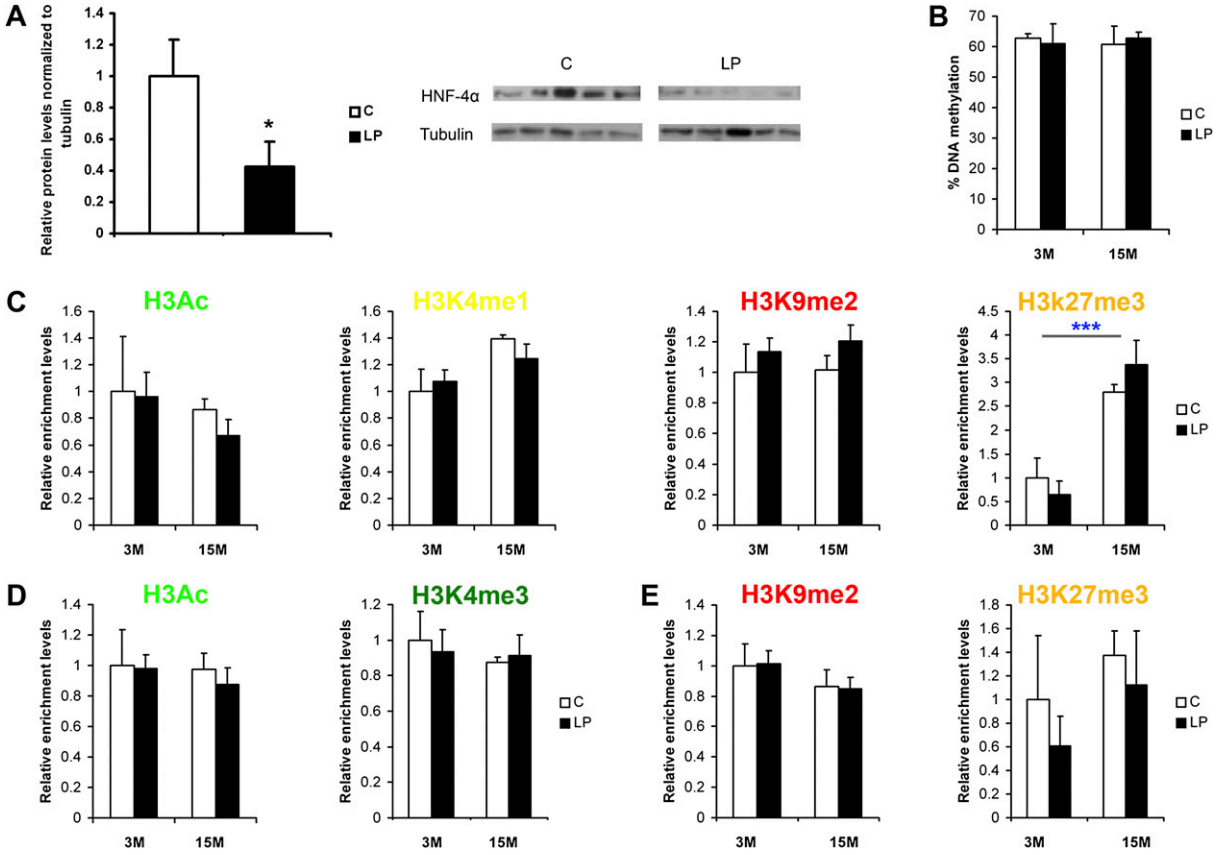

Fig. S4. Maternal diet- and aging-associated changes at the rat *Hnf4a* locus. (*A*) HNF-4α protein levels in 3M C and LP islets measured by Western blotting ($n = 10$ per group). Data were normalized against tubulin and are shown relative to 3M C samples, arbitrarily set to 1. Blots from representative examples are presented. (*B*) Quantitative measurement of DNA methylation levels at the *Hnf4a* enhancer region in 3M and 15M C and LP islets by MassArray analysis ($n = 6$ per group). Quantitative measurement of histone marks at the *Hnf4a* P1 promoter (*C*), *Actb* promoter (*D*), and *Hbb* promoter (*E*) in 3M and 15M C and LP islets by ChIP (3M, $n = 7$ per group; 15M, $n = 6$ per group). Data are presented relative to 3M C samples, arbitrarily set to 1. Error bars indicate SEM. $^{*}P < 0.05$; $^{***}P < 0.001$.

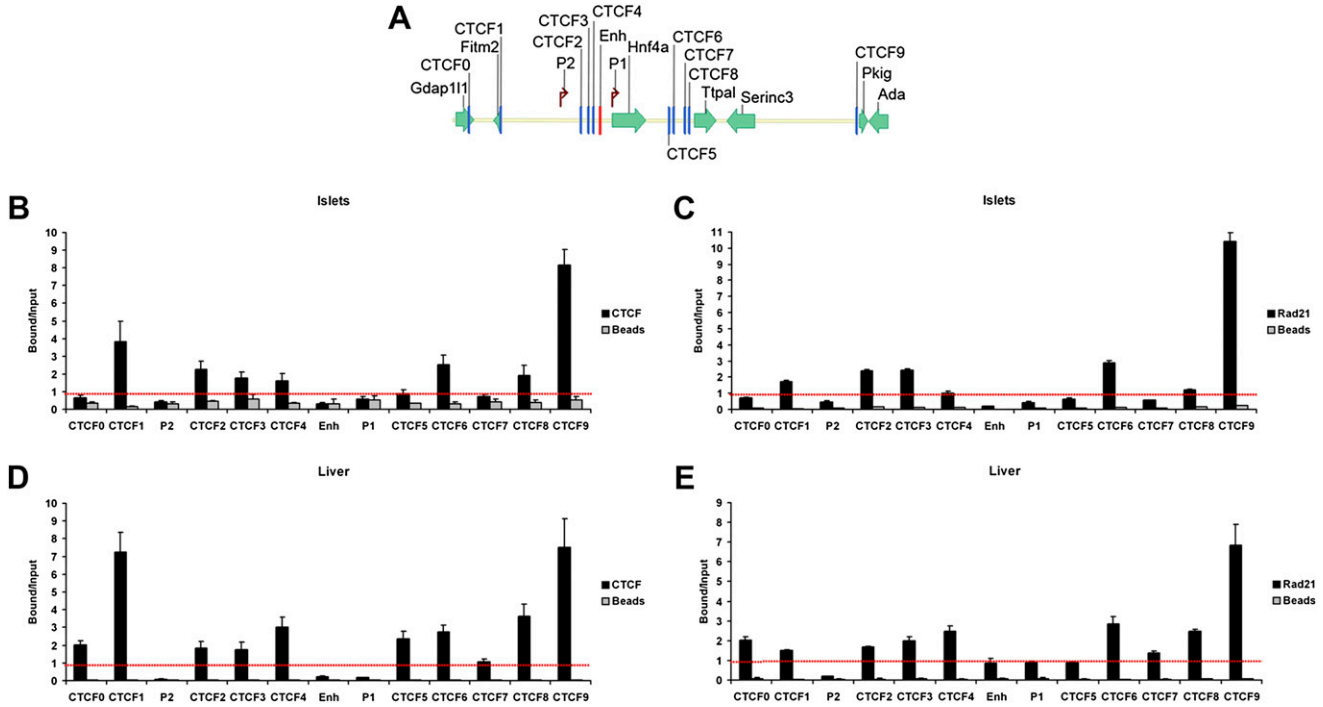

Fig. S5. CTCF and Rad21 binding at the rat *Hnf4a* locus. (*A*) Schematic representation of a 300-kb region on rat chromosome 3 containing the *Hnf4a* locus. Green arrows indicate the transcriptional orientation of the genes within this region. Blue vertical bars indicate CTCF/cohesin-binding sites identified by ChIP-Seq in rat liver and used for validation assays. (*B*) CTCF-ChIP in rat islets ($n = 4$). (*C*) Rad21-ChIP in rat islets ($n = 3$). (*D*) CTCF-ChIP in rat liver ($n = 4$). (*E*) Rad21-ChIP in rat liver ($n = 4$). Regions were classified as enriched or depleted using the arbitrary bound/input ratio of 1 (red dotted line) as the threshold. Error bars represent SEM.

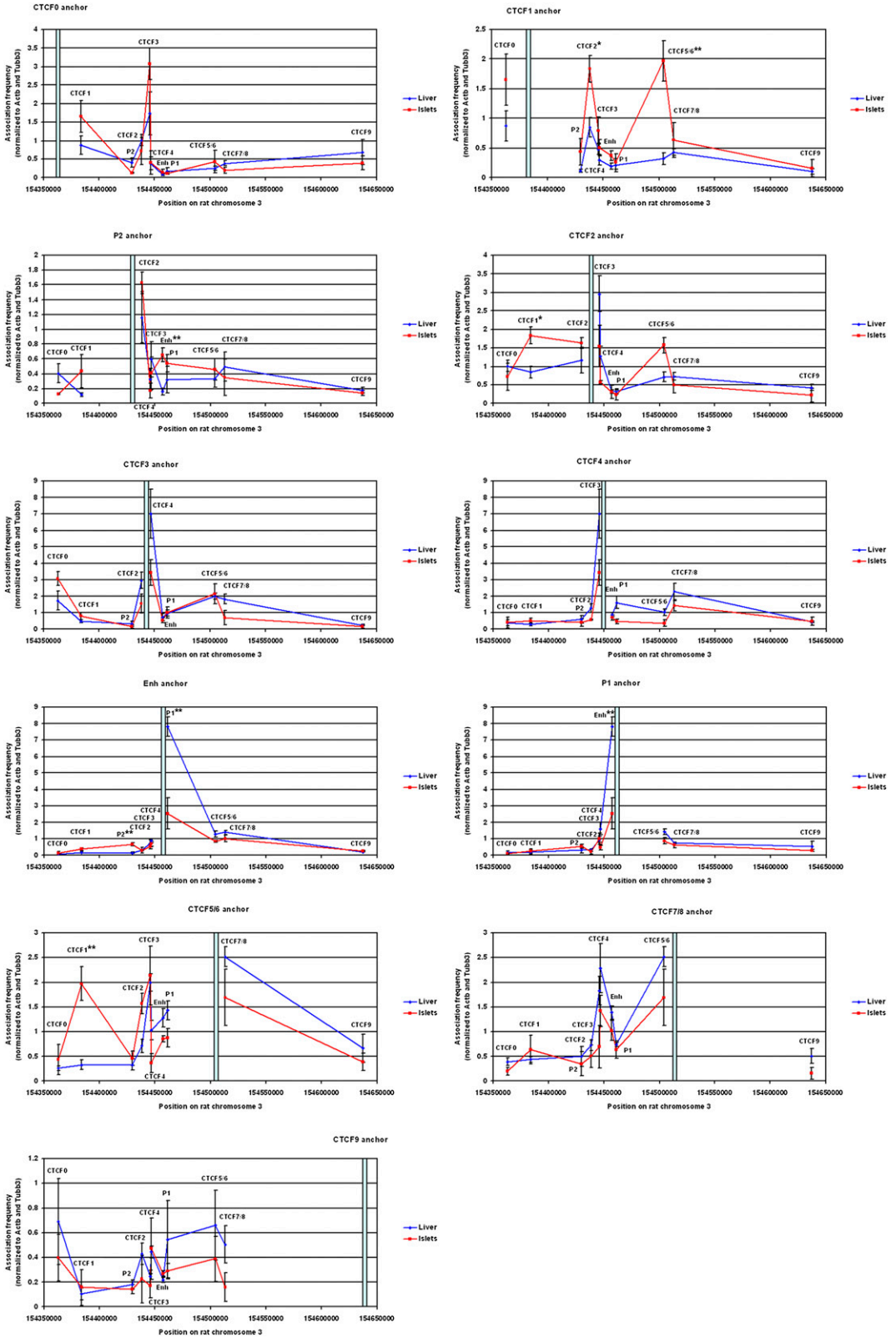

Fig. S6. Quantitative measurement by q3C of long-distance interactions around the rat *Hnf4a* locus in liver ($n = 4$) and islets ($n = 3$). The light blue vertical bars represent positions of anchors along chromosome 3. The data were normalized relative to the average signal for *Actb* and *Tubb3*. Error bars represent SEM. $^{*}P < 0.05$; $^{**}P < 0.01$.

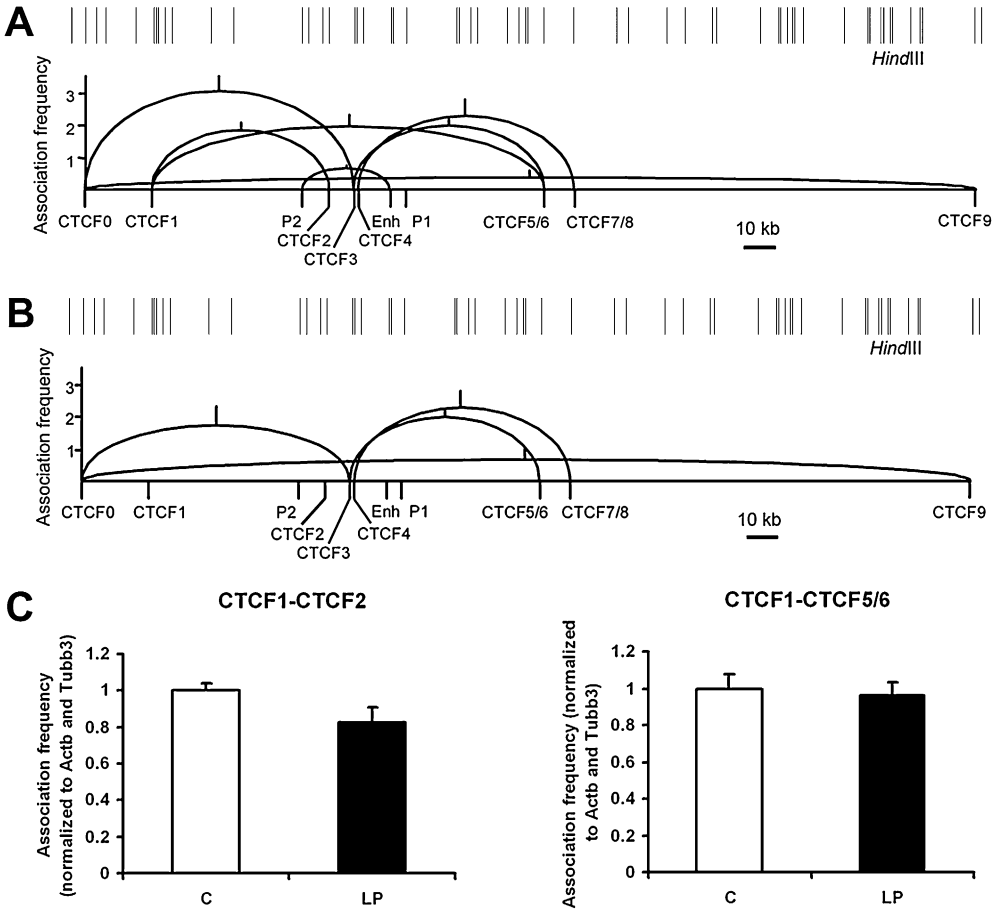

Fig. S7. Long-distance interactions at the rat *Hnf4a* locus. Summary of long-distance interactions analyzed in Fig. S6 in islets (*A*) and liver (*B*). Specific interactions are depicted by black lines, with the height of the curve corresponding to the cross-linking frequency and plotted relative to the signal for *Actb* and *Tubb3*. (*C*) Comparative CTCF1-CTCF2 and CTCF1-CTCF5/6 association frequencies in 3M C (n = 4) and LP (n = 5) islets. Data are shown relative to the 3M C, arbitrarily normalized to 1. Error bars represent SEM.

Table S1. Sequences of primers and TaqMan probes used

Primer sequence	Annealing temperature
Primers for qRT-PCR analysis of gene expression	
Rat-*Hnf4a*	56 °C
F: 5′-GAGCCATCACCACCATCGTC-3′	
R: 5′-TGAGCCAGCAGAAGCCTCAC-3′	
P: 5′-CCAAGCATCACCAAGCAGGAAGCCATC-3′	
Rat-P1	56 °C
F: 5′-ACCCTCGCCGACATGGACAT-3′	
R: 5′-TTGCCCATGGTCAACACCTG-3′	
P: 5′-ACAGTGCTGCCTTGGACCCAGCCTACAC-3′	
Rat-P2	56 °C
F: 5′-TCCATGCTCCCCAGTGTCCA-3′	
R: 5′-ATAAGGACTCGCCACTGGAG-3′	
P: 5′-TGGTCATGGTCAGTGTGAACGCGCCCCT-3′	
Rat-*Ppia*	56 °C
F: 5′-TGAGAACTTCATCCTGAAGCATACA-3′	
R: 5′-CATTTGTGTTTGGTCCAGCATT-3′	
Human-P1	60 °C
F: 5′-TTGAGAATGTGCAGGTGTTGA-3′	
R: 5′-CTCGAGGCACCGTAGTGTTT-3′	
Human-P2	60 °C
F: 5′-TCCAGTGGAGAGTTCTTACG-3′	
R: 5′-GGAGTACATGTGGTTCTTCC-3′	
Primers used for DNA methylation analyses by bisulphite sequencing or MassArray	
Bisulphite sequencing Rat-P1	56 °C
F: 5′-ATAGAGGAAGGTAGAGAAGGGATTT-3′	
R: 5′-CCCATTTCCTAATAAACAAAACAAC-3′	
Bisulphite sequencing Rat-P2	55 °C
F: 5′-TAGAATTTAAGATTGGGTTTGAGTG-3′	
R: 5′-TAATCCTACCCTATCCAACCATC-3′	
Bisulphite sequencing Rat-Enh	56 °C
F: 5′-GTATTTTTTTTAAGATTTTTTGGTTTGGAT-3′	
R: 5′-AACTAATAACTAAACCCCATCCCTAAAAC-3′	
Sequenom Rat-P2	59 °C
F: 5′-AGGAAGAGAGTAGAATTTAAGATTGGGTTTGAGTG-3′	
R: 5′-CAGTAATACGACTCACTATAGGGAGAAGGCTTAATCCTACCCTATCCAACCATC-3′	
Sequenom Rat-Enh	59 °C
F: 5′-AGGAAGAGAGGTATTTTTTTTAAGATTTTTTGGTTTGGAT-3′	
R: 5′-CAGTAATACGACTCACTATAGGGAGAAGGCTAACTAATAACTAAACCCCATCCCTAAAAC-3′	
Bisulphite sequencing Human-P1	52 °C
F: 5′-GTGAGTTATGATGTTTGTTTTGTATA-3′	
R: 5′-ACTAAATCCAATACAACACTATAATC-3′	
Bisulphite sequencing Human-P2	56 °C
F: 5′-GAGTGGATTTTAGGTTTGGTTAGGT-3′	
R: 5′-TAAAAACTCTCCACTAAAACCCC-3′	
Bisulphite sequencing Human-Enh	56 °C
F: 5′-AGGGGGAATAAGTAGATTGTTGATT-3′	
R: 5′-TAATAACTAAACCCCATCCCTAAAC-3′	
Sequenom Human-P1	52 °C
F: 5′-AGGAAGAGAGGTGAGTTATGATGTTTGTTTTGTATA-3′	
R: 5′-CAGTAATACGACTCACTATAGGGAGAAGGCTACTAAATCCAATACAACACTATAATC-3′	
Sequenom Human-P2	56 °C
F: 5′-AGGAAGAGAGGAGTGGATTTTAGGTTTGGTTAGGT-3′	
R: 5′-CAGTAATACGACTCACTATAGGGAGAAGGCTTAA AAACTCTCCACTAAAACCCC-3′	
Sequenom Human-Enh	56 °C
F: 5′-AGGAAGAGAGAGGGGGAATAAGTAGATTGTTGATT-3′	
R: 5′-CAGTAATACGACTCACTATAGGGAGAAGGCTTA ATAACTAAACCCCATCCCTAAAC-3′	
Primers for PCR amplification of fragments used in luciferase assays*	

Table S1. Cont.

Primer sequence	Annealing temperature
P2 promoter	60 °C
−137F: 5′-CAATAAGATAACCGGGCGGTG-3′	
−168F: 5′-GTATCCACCCACCTTGGGT-3′	
−202F: 5′-CACAGGTTCCCTAAGTGACTG-3′	
−234F: 5′-CTGTTGCCATGACAAAAGCGAC-3′	
−256F: 5′-GACCTCCGGTCTAGCCAG-3′	
−299F: 5′-TTCAGGCTCCCCTAACCC-3′	
−382F: 5′-CATGATGGTCTCTCCACAGC-3′	
−516F: 5′-TCAACTCCCAGTATCCAGTCTG-3′	
−2200F: 5′-CAGGGGTACATTAAGATCCTATC-3′	
R: 5′-GACCAAGTATACCCAGCTGGA-3′	
Enhancer	60 °C
F: 5′-CTCGAGCTACCTTTATCTCCC-3′	
R: 5′-CTCGAGGAGCCTGTTGGTCTT-3′	
Primers used for qPCR analysis of the ChIP data	
Rat-*Hnf4a*-P2	65 °C
F: 5′-GTTATGGAGCCTACAGGACGACAGTATG-3′	
R: 5′-CACAGCCATCCCCTTGTCAGAG-3′	
Rat-*Hnf4a*-ENH	61 °C
F: 5′-GCTACCTTTATCTCCCTGTGG-3′	
R: 5′-GATTTGTCTCACCCCTAACCC-3′	
Rat-*Hnf4a*-P1	62 °C
F: 5′-ATCTTTGGCACTGGACTTTGG-3′	
R: 5′-GGTCTCGGTCACCCAGAATATC-3′	
Rat-*Actb*	66 °C
F: 5′-ATCATTATTCCCGTGACATCCAC-3′	
R: 5′-ATGAAGAGTTTGGCGATGGGT-3′	
Rat-*Hbb*	61 °C
F: 5′-ATCCAGGGAGAGATATGCTTGTCATC-3′	
R: 5′-ATCCCGCCCCACCTTTTATG-3′	
Human-*HNF4A*-P2	61 °C
F: 5′-CATGGAGACAGCAACAGTCC-3′	
R: 5′-CGCCCGGTTATCTTATTGATT-3′	
Human-*HNF4A*-Enh	64 °C
F: 5′-TTTTTAAGGCAAAATCAACAGG-3′	
R: 5′-GCAACATTCATGTGGTTAACTG-3′	
Human-*HNF4A*-P1	64 °C
F: 5′-TTGATAACTGAACATCGGTGAG-3′	
R: 5′-GTGGGGGTTAATGGTTAATCG-3′	
Human-*ACTB*	64 °C
F: 5′-CATAAAAGGCAACTTTCGGAAC-3′	
R: 5′-CCTCTTCCTCAATCTCGCTCT-3′	
Human-*HBB*	64 °C
F: 5′-ATATATCTCTTGGCCCCATACC-3′	
R: 5′-CAATATGCTTACCAAGCTGTGA-3′	
Primers used for qPCR analysis of the CTCF and Rad21 ChIP data	
ChIP-CTCF0	64 °C
F: 5′-AAGCCTACAGTTTTGGGTGATG-3′	
R: 5′-CCCTCCCTCACTTTCTCTTTCT-3′	
ChIP-CTCF1	64 °C
F: 5′-CTGGTACCGACACATCCCTCT-3′	
R: 5′-GGTGGATGTAGACTTGCACTCTG-3′	
ChIP-CTCF2	64 °C
F: 5′-TTGTGGTCATTGTTGGTGTTTT-3′	
R: 5′-ATTCCAGCACTTGGGAGGTAAT-3′	
ChIP-CTCF3	64 °C
F: 5′-GTTGATTGGACTCAGATCGTCA-3′	
R: 5′-TCAAAGAGACAGGCAGACAAAA-3′	
ChIP-CTCF4	64 °C
F: 5′-CAGTGGGCTGTGACTGGATTAC-3′	
R: 5′-ATCCAGCATTGAGATTTGGTCT-3′	

Table S1. Cont.

Primer sequence	Annealing temperature
ChIP-CTCFa	64 °C
F: 5′-AGCTGTCTTCAGACACACCAGA-3′	
R: 5′-TGTTCTTCCAAAGGTCCTGAGT-3′	
ChIP-CTCFb	64 °C
F: 5′-TTACCTCTTTCGCCTCTTATGC-3′	
R: 5′-CAGAAGCAACTTGTTCACCCTA-3′	
ChIP-CTCF5	64 °C
F: 5′-GGCACCTTAGCTACAGATGGTT-3′	
R: 5′-ATTGAGGGACTGCAGAGATGAC-3′	
ChIP-CTCF6	64 °C
F: 5′-GGTAGCATCTCTCCGTTGAAGA-3′	
R: 5′-AACGACTAAGCTTGGGTGAAAA-3′	
ChIP-CTCF7	64 °C
F: 5′-AATACTTTAGCAGCCCCTTGTG-3′	
R: 5′-CCCCAGATCGTCTAAGATAGTG-3′	
ChIP-CTCF8	64 °C
F: 5′-TGCCATCTCTAAACTTTCACTCG-3′	
R: 5′-AACCCATATGAACCTCTTACCG-3′	
ChIP-CTCF9	64 °C
F: 5′-GAGTTCTTTAGGTGGAGGCTCA-3′	
R: 5′-GCAAAATTCTGTGTGAGGGAGT-3′	
Primers used to quantify the efficiency of chromatin digestion with *Hind*III (first 11 primer sets) and the association frequencies during the q3C experiment	
q3C-CTCF0	64 °C
F: 5′-GGAAGGACAGAAGGACAGAGAA-3′	
R: 5′-AAGAGCAAAGGTAAGCAAATGG-3′	
q3C-CTCF1	64 °C
F: 5′-CTTGAGCCTCTCTGCGTCTACT-3′	
R: 5′-GCAGGTCTGATCTGTAGCACAC-3′	
q3C-P2	64 °C
F: 5′-CAAAGTATAGCCTTTCCCACCA-3′	
R: 5′-CACGCACACATTTAAAACAGAAA-3′	
q3C-CTCF2	64 °C
F: 5′-TTGTGGTCATTGTTGGTGTTTT-3′	
R: 5′-GTGTCTACATTCCAGCACTTGG-3′	
q3C-CTCF3	64 °C
F: 5′-TCACATGTAGTGGTGGTGATGA-3′	
R: 5′-AAAGTTAGCATGGAGATGTGAGC-3′	
q3C-CTCF4	64 °C
F: 5′-TCAGATCCAAGGTGCAAAAATA-3′	
R: 5′-CAGCGAGGAGTTGTTAGGAAAA-3′	
q3C-ENH	64 °C
F: 5′-TCTGACTGGCTAACGATCACAT-3′	
R: 5′-CACCACAGTGTTTGCCATATCT-3′	
q3C-P1	64 °C
F: 5′-AGGGTGTTTCCTTTGCCACT-3′	
R: 5′-AGCTGTGTCGTGGAGTCATAGA-3′	
q3C-CTCF5/6	64 °C
F: 5′-ATAACACTCCGGGGACTACAAA-3′	
R: 5′-AAAAATCACAATGAAATTCACCAA-3′	
q3C-CTCF7/8	64 °C
F: 5′-CGGTAAGAGGTTCATATGGGTTA-3′	
R: 5′-GCTGTTTAAATGGGCTCATAGG-3′	
q3C-CTCF9	64 °C
F: 5′-CTTGTAGAGCCGTTCCAGAAGT-3′	
R: 5′-CTTGCACTCTGACTCCACGTT-3′	
q3C-Internal	64 °C
F: 5′-GGCACCTTAGCTACAGATGGTT-3′	
R: 5′-ATTGAGGGACTGCAGAGATGAC-3′	
q3C-*Tubb3*	64 °C
F: 5′-TCAGGATAGAGGAGAGCTCAGG-3′	
R: 5′-TCCATGGTCAGCTCTGTAACAC-3′	

Table S1. Cont.

Primer sequence	Annealing temperature
q3C-*Actb*	64 °C
F: 5′-CCAACTTTACCTTGGCCACTAC-3′	
R: 5′-TTGGGCTAGGGTATCATAAAGC-3′	
q3C-P2-F2: 5′-CCTCCACACATCTTTCCATCTT-3′	64 °C
q3C-CTCF5/6-F2: 5′-CCAAGTGGTCACATTTTGTCAT-3′	64 °C

Enh, enhancer region; F, forward primer; P, TaqMan probe; R, reverse primer.
*Numbers preceding F represent the size of the PCR product cloned into the pGL4.10 vector.

Table S2. Combinations of primers used to quantify the association frequencies during the q3C experiment

Interaction interrogated	Primers used	PCR size, bp
CTCF0 + CTCF1	R + R	151
CTCF0 + P2	F + F2	125
CTCF0 + CTCF2	F + F	128
CTCF0 + CTCF3	F + F	115
CTCF0 + CTCF4	F + F	143
CTCF0 + Enh	R + R	119
CTCF0 + P1	R + R	189
CTCF0 + CTCF5/6	F + F2	113
CTCF0 + CTCF7/8	F + F	115
CTCF0 + CTCF9	F + F	101
CTCF1 + P2	R + R	97
CTCF1 + CTCF2	F + F	156
CTCF1 + CTCF3	F + F	143
CTCF1 + CTCF4	F + F	171
CTCF1 + Enh	F + F	154
CTCF1 + P1	F + F	116
CTCF1 + CTCF5/6	R + R	131
CTCF1 + CTCF7/8	F + F	143
CTCF1 + CTCF9	R + R	125
P2 + CTCF2	R + R	65
P2 + CTCF3	R + R	98
P2 + CTCF4	R + R	53
P2 + Enh	R + R	65
P2 + P1	R + R	135
P2 + CTCF5/6	R + R	94
P2 + CTCF7/8	R + R	110
P2 + CTCF9	F2 + F	122
CTCF2 + CTCF3	F + F	139
CTCF2 + CTCF4	F + F	167
CTCF2 + Enh	F + F	150
CTCF2 + P1	F + F	112
CTCF2 + CTCF5/6	F + F	117
CTCF2 + CTCF7/8	F + F	139
CTCF2 + CTCF9	F + F	125
CTCF3 + CTCF4	F + F	154
CTCF3 + Enh	F + F	137
CTCF3 + P1	F + F	99
CTCF3 + CTCF5/6	F + F	104
CTCF3 + CTCF7/8	F + F	126
CTCF3 + CTCF9	F + F	112
CTCF4 + Enh	F + F	165
CTCF4 + P1	F + F	127
CTCF4 + CTCF5/6	F + F	132
CTCF4 + CTCF7/8	F + F	154
CTCF4 + CTCF9	F + F	140
Enh + P1	F + F	110
Enh + CTCF5/6	F + F	115
Enh + CTCF7/8	F + F	137
Enh + CTCF9	F + F	123
P1 + CTCF5/6	F + F	77
P1 + CTCF7/8	F + F	99
P1 + CTCF9	F + F	85
CTCF5/6 + CTCF7/8	F + F	104
CTCF5/6 + CTCF9	F + F	90
CTCF7/8 + CTCF9	R + R	138
Tubb3	F + R	141
Actb	F + R	148
Internal	F + R	104

The sequences of primers used are listed in Table S1. Enh, enhancer region.