Supplemental Materials and methods

In vitro epigenetic reporter system: 293T cells (B4) containing the epigenetic reporter

Methylation status assessment

Bisulfite conversion

200ng of genomic DNA was treated with sodium bisulfite at 64^{o}C for 2.5 hours in the dark, followed by desulfanation and purification on a column using an EZ DNA Methylation Gold Kit (Zymo Research Corporation, CA, USA).

Methylation studies

Methylation at CTCF binding site 1 and 2 (Supplemental Figure 4A) was studied by conventional sequencing and cloning as previously described (5). A nested (or semi-nested) PCR was performed in 1.1X PCR Master Mix (1.5mM $MgCl_2$) (ABgene; AB-0575) including 250nM primer at total reaction volume of 20 µl; 1 µl of the first PCR products was used for the second PCR as a template. PCR conditions were 95°C 15min, (95°C 40sec, 50°C 40sec, 72°C 1min) X 10 cycles, (95°C 30sec, 50°C 30sec, 72°C 1min) X 30 cycles, 72°C 5min.

The methylation levels at CTCF binding sites 3 and 4 were studied by pyrosequencing (Supplemental Figures 4B-C); PCR amplified templates generated with 10ng of bifulfite converted DNA in a total reaction volume of 25µl with 1.5mM MgCl2, 200uM dNTPs, 200nM forward/reverse primers, 0.5U Platinum Taq, and 1x PCR buffer (200mM Tris-HCl pH8.4, 500mM KCl) (Invitrogen Corporation, Carlsbad, CA, USA). The primers used in the PCR were: for CTCF binding site 3, 5'- TGGAATATTTGTGTTTTTGGAGG-3' (forward) and 5'- TTACCACCCCTATAAATCCCTATT-3' (reverse) (CTCF 3 primers), and for CTCF

binding site 4, 5'- TAATGATTTATAAGGGTTATGGGG-3' (forward) and 5'-ACACAAATACCTAATCCCTTTATTAA-3' (reverse) (CTCF 4 primers). The CTCF 3 reverse primer and CTCF 4 forward primer were biotinylated at the 5' end. The amplification protocol was for 49 cycles at 95^{o}C for 20 seconds, and annealing of 50^{o}C with CTCF binding site 3 primer pair or 52^{o}C with CTCF binding site 4 primer pair for 20 seconds, then 72^{o}C for 20 seconds, and final extension at 72^{o}C for 5 minutes. 10 µl of the biotinylated PCR product was bound to 0.2uL of the Streptavidin Sepharose High Performance (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) with 38 µl of binding buffer (10mM Tris-HCl, 2M NaCl, 1mM EDTA, 0.1% Tween 20; pH7.6) in total volume of 80 µl by shaking together for 5 minutes. A Vacuum Prep Tool (Biotage, Uppsala, Sweden) was used to capture the Streptavidin sepharose beads - biotinylated PCR product complex and prepare single stranded pyrosequencing templates for use on PSQ HS 96 System and PyroMark MD Systems (Biotage, Uppsala, Sweden) with Pyro Gold Reagents (Biotage, Uppsala, Sweden) as previously described (3). The sequencing primers used were 5'-AAGTTGTTATGTGTAATAAG -3' for sequencing 6 CpGs at CTCF binding site 3 and 5'-CCTAAAATACTCAAAACTTT -3' for 4 CpGs at CTCF binding site 4. Methylation was quantitatively analysed by PSQ HS 96A SNP Software and Pyro Q-CpG Software (Biotage, Uppsala, Sweden).

Southern Blot Procedures

DNA was extracted from cells using the DNeasy tissue kit (Qiagen). Southern blot transfer and hybridisation was performed after restriction endonuclease digestion of 10µg of DNA with *PflfI* and either *BamHI* or *NdeI* using Amersham Nucleobond membranes according to the manufacturers instructions (Amersham Nucleobond). The membranes were hybridized

with a radioactively labelled probe of 1.87 kb overlapping with the *H19* ICR region (from *XbaI* to *AflII*). Probe labelling and purification was done with a Statagene Primer II kit according to manufacturers instructions.

Mass – spectrometry

To investigate which peptide(s) in CTCF are modified by PARlation, the His-tagged CTCF protein was produced in the baculovirus system and purified using the His-tag- affinity and ion-exchange chromatography as described previously (2). The purified CTCF protein was PARlated *in vitro* as reported earlier (7), and used for analysis of modifications of the peptides. It is known that, in the alkaline conditions, poly(ADP-ribose)-protein bonds break by hydrolysis (1) . We therefore performed hydrolysis of PARlated CTCF with NaOH to map the modified sites.

In these experiments, the band corresponding to CTCF was cut from the gel and subjected to in-gel tryptic digestion using routine procedures (2). For alkaline hydrolysis, the extracted tryptic peptides were dissolved in 2M NaOH and incubated at 56^0C for 1hr. The samples were then cooled and the solution of formic acid was added at 2M final to neutralise pH. Finally, both treated and non-treated tryptic peptides were desalted using a C18 cartridge, dried, dissolved in 0.1% trifluoroacetic acid (TFA) and loaded onto the MALDI target plate. Mass spectra acquisition was performed on Reflex IV Bruker Daltonics MALDI-TOF in both linear and reflectron modes. The spectra were analysed using Bruker FlexAnalysis Version 2.0 software.

Multiple species protein sequence alignment

CTCF protein sequences were obtained from NCBI, references as follows Mouse: AAAH24382, Rat: NP114012, Human: AAB07788, Cattle: AAI23742, Platypus: AB47392, Tammar: CB47391, Chicken: NP990663, Bearded Dragon: ACB47393, Xenopus: AAH57697, Zebrafish: AAI29308, Drosophila: AAL78208 and Mosquito: EDS31858. Multiple protein sequence alignment was performed using EMBL-EBI- Clustal W2.

PARylation –dependent CTCF insulator function: an endogenous system

Cell culture

A911M (maternal) and A911P (paternal), mouse human hybrid cells (4) were maintained in Dulbecco's modified Eagle's supplemented with L-Glutamine, Glucose Pyridoxine-HCL, $NaHCO_3$ (PAA) in the presence of Blasticidin S (Melford). The media was supplemented with 10% FBS (PAA) and 50µg/ml gentamicin (Invitrogen).

Purification of RNA, reverse transcription and Real Time PCR

Total RNA was isolated with Trisure (Bioline) as described by the manufacturer. RNA was treated with Turbo-DNA-free kit (Ambion). 1 µg of total RNA was used for reverse transcription with Superscript III (Invitrogen).

Real Time PCR was carried out in duplicate using a 20 µl PCR mix which consisted of 2x *POWER*SYBR Green PCR Master Mix (Applied Biosystems), 2µl of cDNA, 200nM of each primer. PCR conditions were as manufacturer's instructions. Amplification, data acquisition and analysis were carried out using Chromo4 Real Time PCR (Bio-Rad). The comparative Ct method was used to assess relative changes in *mRNA* levels (6). Primers used for Real Time PCR were as follows.

IGF2: For- 5' TTCACCCCTCTTGCACACG 3'

Rev- 5' CTGGCTGTTCAGAATTGAGGTAA 3'

GAPDH: For-5' –ACCACAGTCCATGCCATCAC 3'

Rev-5' –TCCACCACCCTGTTGCTGTA 3'

In Situ Proximity Ligation Assay (ISPLA) in HCT116 cells

HCT116 cells were grown in McCoy's 5A medium supplemented with 10% FCS and 1% Pencillin Streptomycin. The proximity ligation assay ((SPLA) was performed as described in the Materials and Methods section in the main text.

Supplemental Figure legends

Supplemental Figure 1

The poly (ADP-ribosyl)ated peptide is mapped to the N-terminal domain of CTCF and it is conserved between species.

A: Identification of the poly (ADP-ribosyl)ated peptide in CTCF by mass-spectrometry. The His-tagged CTCF protein was produced and purified as described under "Supplementary Materials and Methods" and used for analysis of modifications of the peptides. Peptides should loose the mark after alkaline hydrolysis if they were modified by PARlation resulting in the appearance of a peak. Mass spectra of the proteins are shown in red for a sample subjected to alkaline hydrolysis and blue for the untreated control sample. The peptide demonstrating differences in the mass is indicated by an arrow. The sequence of the identified peptide, positioned between amino acids 212-260 in human CTCF, is shown below; glutamic acid residues (the sites of possible PARlation) are highlighted in yellow.

B: Alignment of the peptide aa 212-260 shows conservation of this regions between species.

Alignment of the CTCF protein sequence from different species was performed using ClustalW2. The peptide sequence within the CTCF N-terminal and observed to be modified by poly(ADP-ribosyl)ation (human 212-260), in this study, shows sequence homology between species. Glutamic acid residues are highlighted in yellow.

Supplemental Figure 2

The PARP-1 inhibitor, PJ34, is not toxic for cells at 10µM.

The cytotoxicity of PJ 34 was tested in all cells lines by counting live cells for three days after addition of 10µM PJ34. Each experimental point shows an average of three experiments performed in triplicate. Error bars indicate standard deviations. A representative example (293T cells) is presented, but similar results were obtained with MDA435 and HeLa cells (data not shown).

Supplemental Figure 3

Selection of the 293T transgenic cell line (B4) containing stably integrated luciferase reporter construct

A: *A schematic diagram of the genotyping strategy for selection of stably integrated luciferase reporter construct in the 293T transgenic cell line.* Horizontal blue bars indicate genomic fragments that hybridize with 1.87 kb probe in Southern blot analysis (Supplemental Figure 3B). Horizontal red bars indicate overlapping fragments covering the construct that were amplified by long range PCR (Supplemental Figure 3C). Details of the construct were given in Figure 4A.

B: *Genotyping of transgenic cell lines using Southern blot analysis*. DNA from several stably transfected cell lines was digested with the restriction endonuclease *PflfI* and either *BamHI* or *NdeI* and Southern blots were hybridized with a radioactively labelled probe of 1.87 kb overlapping with the H19 ICR region (from *XbaI* to *AflII*). Single-copy single-site integration cell lines (*e.g.* B4) contain two fragments, a fragment of 2.4 kb and a fragment

larger than 3.5 kb, depending on a distance to the first upstream *BamHI/NdeI* site in the targeted locus. Multiple-copy multiple-site integration cell lines (*e.g.* Y18) contain more than two fragments.

C: *Genotyping of transgenic cell lines using long-range PCR*. DNA from several stably transfected cell lines was isolated and six overlapping fragments were amplified using long-range PCR (Roche). Single-copy cell lines (*e.g.* B4) are indicated by the amplification of fragments 1-5 and a failure to amplify fragment 6 (Supplemental Figure 3A). A 1 kb DNA ladder was run in the outermost lanes of each gel.

Supplemental Figure 4

Methylation analysis of the transgenic ICR

A: *Alignment of Bisulphite sequencing for CTCF binding sites 1 and 2.* Upper track is the bisulphite sequence, lower track is the reference H19 sequence. After bisulphite treatment, all CpG sites in the region (highlighted in yellow) as well as those CpGs within CTCF binding sites 1 and 2 are converted to TpG indicating that the ICR is unmethylated. The chromatograms CTCF Sites 1 and 2 are shown below.

B: *Pyrosequencing analysis of CTCF binding site 3.* Pyrosequencing analysis of CTCF binding site 3 showing that all 6 CpGs are unmethylated in this region. The average methylation of 6 CpGs analyzed is 3.7%.

C: *Pyrosequencing analysis of CTCF binding site 4.* Pyrosequencing analysis of CTCF binding site 4, showing that all 7 CpGs are unmethylated in this region. The average methylation of 7 CpGs is 4%.

Supplemental Figure 5: complementary images of the ChIP DNA resolved in agarose gels (1%); typical examples are shown.

A: (complementary images to Fig 4D): *Endogenous CTCF is associated with the CTCF binding Sites 1 and 2 at the H19 ICR in B4 cells non-treated and treated with PJ34.* The B4 cells ($5x10^6$) were either treated or not treated with 10μM PJ34 for 24 hours, cells were cross-linked with formaldehyde and the standard ChIP assay was performed to assess the *in vivo* CTCF occupancies at the DNA. To separate two CTCF binding sites, Site 1 and Site 2, the DNA/ protein complexes were digested overnight with *Apo1* restriction endonuclease followed by immunoprecipitation with the anti-CTCF antibody. The PCR amplification was carried out using primers situated within Site 1 and Site 2.

B: (complementary images to Fig 4E): *Exogenous CTCF is associated with the CTCF binding Sites 1 and 2 at the H19 ICR in B4 cells transfected with CTCF Wt and CTCF Mut.* The B4 (5×10^6) cells were transfected with either the His-Tagged CTCF Wt or CTCF Mut4 expressing plasmids. 48 hours post-transfection the cells were crosslinked with formaldehyde and the standard ChIP assay was performed to assess the in vivo occupancies at the DNA. To separate two CTCF binding sites, Site 1 and Site 2, the DNA/ protein complexes were digested overnight with *Apo1* restriction endonuclease followed by immunoprecipitation with the anti-His-Tag antibody. PCR amplification was carried out using primers situated within Site 1 and Site 2.

C: PARylation-dependent CTCF insulator function: an endogenous model

A911M and A911P cells were either treated with PJ34 or transfected with EV, CTCF Wt or CTCF Mut 4. The level of IGF2 mRNA, in all samples, was determined by RT- PCR. The

relative expression was calculated using the comparative CT method, normalised to GAPDH expression. The AP11P cells, not treated with PJ34, were used as the control and designated a relative expression value of one.

Supplemental Figure 6

(complementary images to Fig 5D): *PARP-1 is associated with both CTCF Wt and CTCF Mut4 at CTCF binding sites 1 and 2 at the H19 ICR in B4 cells, as shown by ChIP and Re-ChIP assay.*

The B4 ($5x10^{6)}$ cells were transfected with either pCi EV, CTCF Wt or CTCF Mut4 and crosslinked with formaldehyde. A standard ChIP assay was performed followed by an additional elution step and re-ChIP to assess the *in vivo* occupancies by PARP-1, CTCF Wt and CTCF Mut4 at the DNA target sites. To separate two CTCF binding sites, Site 1 and Site 2, the DNA/ protein complexes were digested overnight with *Apo1* restriction endonuclease followed by immunoprecipitation with either anti-PARP-1 or anti-His-Tag antibody. An anti- Tubulin antibody was used as the negative control in both the ChIP and re-ChIP experiments. PCR amplification was carried out using primers situated within Site 1 and Site 2.

Supplemental Figure 7

ISPLA –CTCF and PARP-1 co-localisation

A:*ISPLA controls in MCF7 cells*

DAPI merge images with the following ISPLA controls are shown (left hand a-i).

(a) ISPLA without primary CTCF antibody counterstained with fluorescent anti-PARP1.

(b) ISPLA without primary PARP1 antibody counterstained with fluorescent anti-CTCF. (c) ISPLA without both primary antibodies, only secondary antibody. (d) ISPLA without any antibodies, only solutions used. (e) ISPLA without linker and backbone.

(f) Oligo detector alone added. (g) ISPLA without secondary anti-Rabbit antibody.

(h) ISPLA without secondary anti-Mouse antibody. (i) ISPLA without both secondary antibodies.

B.*ISPLA- CTCF and PARP-1 co-localisation in HCT116 cells*

The In situ Proximity Ligation Assay was carried out using HCT116 cells. DAPI staining is shown (a). (b) The ISPLA image using CTCF and PARP-1 antibodies. The red positive signal indicates that the two proteins are within close proximity, a maximum of 40nm or overlapping. (c) The merge of the DAPI and ISPLA signals.

Supplemental Figures 8 (panels A-N)

Genome-wide co-localisation of CTCF and PARP-1 is revealed by bioinformatics analysis. A comparison was made of the micro array data obtained from two independent ChIP-ChIP experiments studying CTCF and PARP-1, deposited on the GEO (Gene Expression Omnibus) database. The identified CTCF and PARP-1 sites of co-localisation ('hotspot'/'hotspot cluster') within the human genome are shown for each identified chromosome (except chromosome 11- 5 hotspots) as detailed in Figures 6 D and E. The approximate position on the chromosome is shown (top). The bottom diagram is the identified 'chromosome region in detail', the approximate position of hotspot/ hotspot clusters shown with a red arrow (bottom). The data was obtained using Ensembl genome browser.

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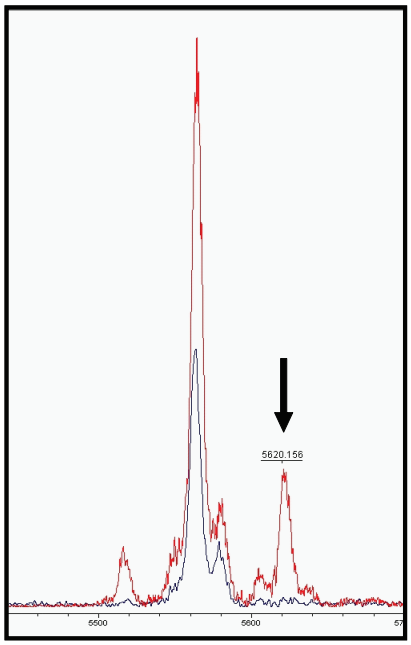
Supplemental Figure 1

A



Peptide match

Masses : 5620.156
Position: 212-260
Sequence: LRYTEEGKDVDVSVYDFEEEQQEGLLSEVNAEKVVGNKPPKPTKIKKK

B

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Mouse            PDYQPPAKKTKKTKKSKLRYTEEG-KDVDVSVYDFEEEQQEGLLSEVNAEKVVGNMKPPK 253
Rat              PDYQPPAKKTKKTKKSKLRYTEEG-KDVDVSVYDFEEEQQEGLLSEVNAEKVVGNMKPPK 253
Human            PDYQPPAKKTKKTKKSKLRYTEEG-KDVDVSVYDFEEEQQEGLLSEVNAEKVVGNMKPPK 253
Cattle           PDYQPPAKKTKKTKKSKLRYTEEG-KDVDVSVYDFEEEQQEGLLSEVNAEKVVGNMKPPK 253
Platypus         PDYQPPAKKTKKTKKSKLRYTEEG-KDVDVSVYDFEEEQQEGLLSEVNAEKVVGNMKPPK 253
Tammar           PDYQPPAKKTKKTKKSKLRYTEEG-KDVDVSVYDFEEEQQEGLLSEVNAEKVVGNMKPPK 253
Chicken          PDYQPPAKKTKKNKKSKLRYTEEG-KDVDVSVYDFEEEQQEGLLSEVNAEKVVGNMKPPK 253
Bearded Dragon   PDYQPPAKKTKKTKKSKLRYTEEG-KDVDVSVYDFEEEQQEGLLSEVNAEKVVGNMKPPK 253
Xenopus          PDYVPPMKKSKKTKKSKLRYTEEG-KDVDVSVYDFEEEQQEGLLSDVNAEKVVGNMKPPK 251
Zebrafish        PDYTPPVKKVKKTKKSKLRYNTEGDKDMDVSVYNFEEEQQEGLLSEVNAEKVVGNMKPPK 293
Drosophila       LSDYTVDEAAVEAATATLTPNEAEVYEFEDNATTEDENADKKDVDFVLSNKEV-KLKTAS 281
Mosquito         -----------EVGVGEDGDDDENIYEFEDGEFSQAEDDKSS------VSKVT-KFKTQS 163
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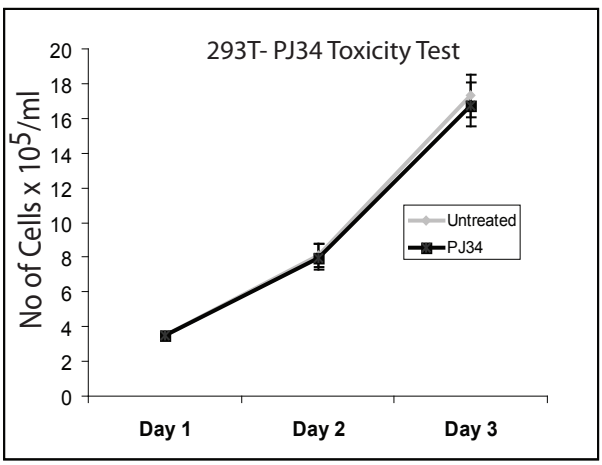
Supplemental Figure 2



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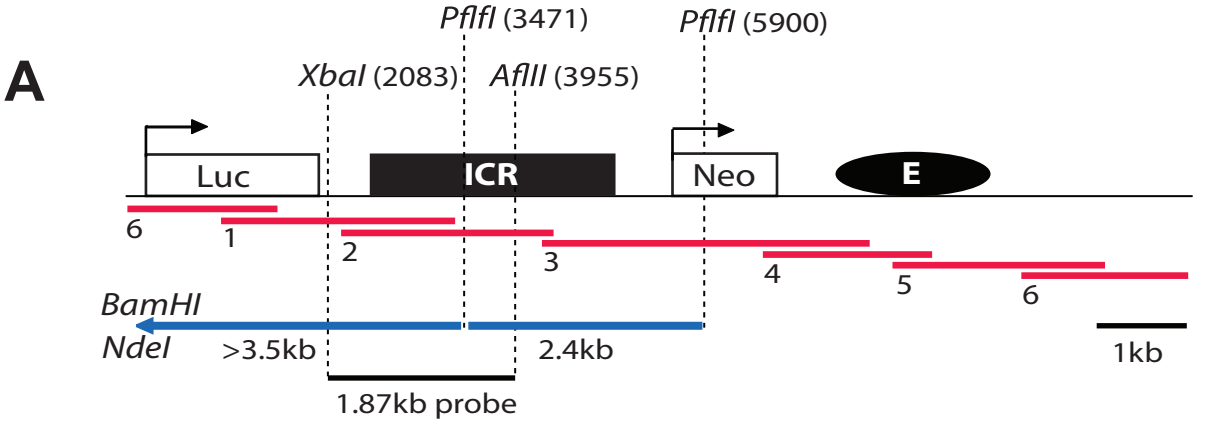

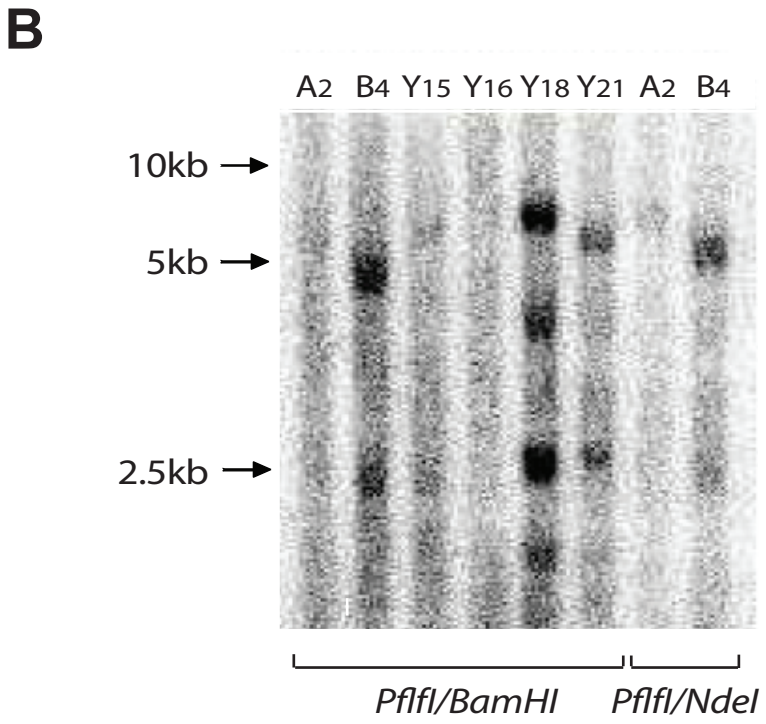

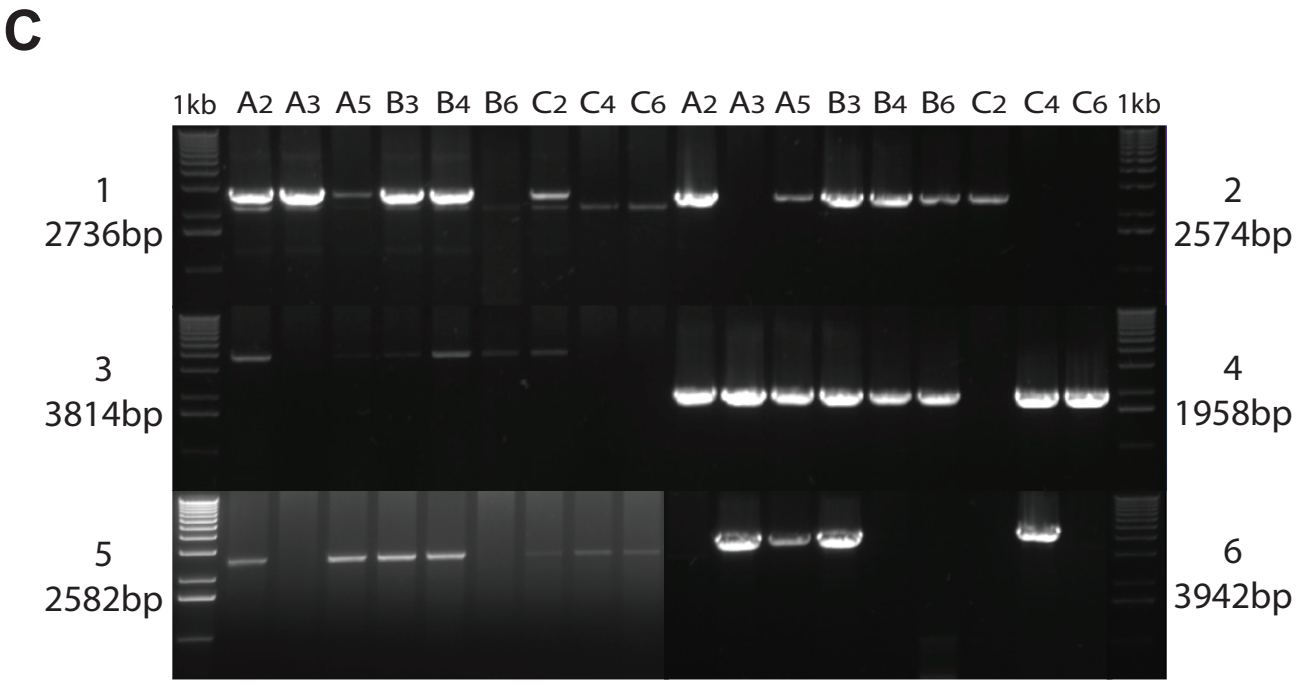

Supplemental Figure 4

A

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H19ICR_bisSeq      ----------TTGAGMGCCTYGSTGYWCAM----------CGAAMTRTGGGAGTTGTTG 39
H19                ATGCCCTATTCTTGGACGTCT-GCTGAATCAGTTGTGGGGTTTATACGCGGGAGTTGCCG 299
                             ***   * ** * **                 *     ********  *
                                                                          Site 1
H19ICR_bisSeq      TGTGGTGGTAGTAAAATTGATTGTKTWAAATTTAAAGAGTTTTTTTATTTTTGGTATTGG 99
H19                CGTGGTGGCAGCAAAATCGATTGCGCCAAACCTAAAGAGCCCCCCCACCCCTGGTATTGG 359
                    ******* ** ***** *****    ***  *******       *    *********
                            Site 1
H19ICR_bisSeq      AATTTATAAATGGTAATGTTGTGGGTTATTTAAGTTTAGTATTTTAGGGGGGTTATAAAT 159
H19                AATTCACAAATGGCAATGCTGTGGGTCACCCAAGTTCAGTACCTCAGGGGGGTCACAAAT 419
                   **** * ****** **** ******* *   ***** ****  * ******** * ****

H19ICR_bisSeq      GTTATTAGGGGGGTAGGATATATGTATTTTTTAGGTTGGTATTTTGTGGATTTGGATTTT 219
H19                GCCACTAGGGGGGCAGGACACATGCATTTTCTAGGCTGGTACCTCGTGGACTCGGACTCC 479
                   *  * ******** **** * *** ***** **** *****  * ***** * *** *

H19ICR_bisSeq      TAAATTAATAAGGTTGGTTTATTTTTTGTAAAGAATTTTTTGTGTGTAAAGATTAGGGTT 279
H19                CAAATCAACAAGGTCGGCTTACTCTCTGCAAAGAATCCTTTGTGTGTAAAGACCAGGGTT 539
                    **** ** ***** ** *** * * ** *******  **************  ******

H19ICR_bisSeq      GTTGTATGGTGGTAGTGAAGTTTTGTATATTGTAGTTTTAAAATGGATTGTAATTGATTG 339
H19                GCCGCACGGCGGCAGTGAAGTCTCGTACATCGCAGTCCTAAAACGGATTGCAACTGATTG 599
                   *  * * ** ** ******** * *** ** * ***  ***** ****** ** ******
                            Site 2
H19ICR_bisSeq      AGTTTTTTTTTTTWTTWTTWTTTWTRATTTTATAGTTATGGKTTTTWTKAG--------- 390
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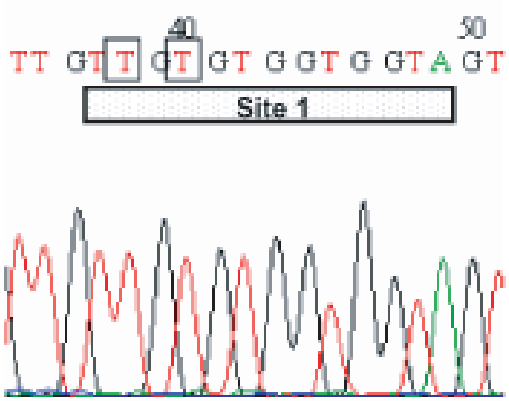
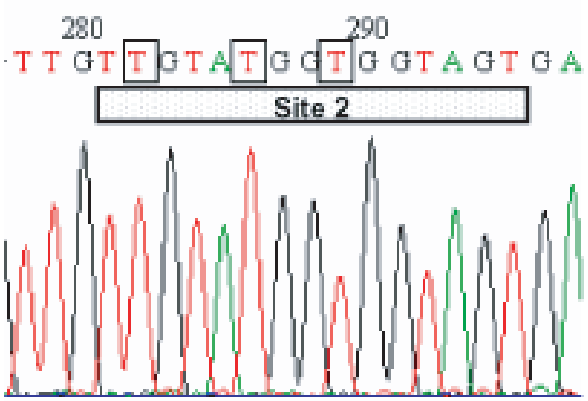
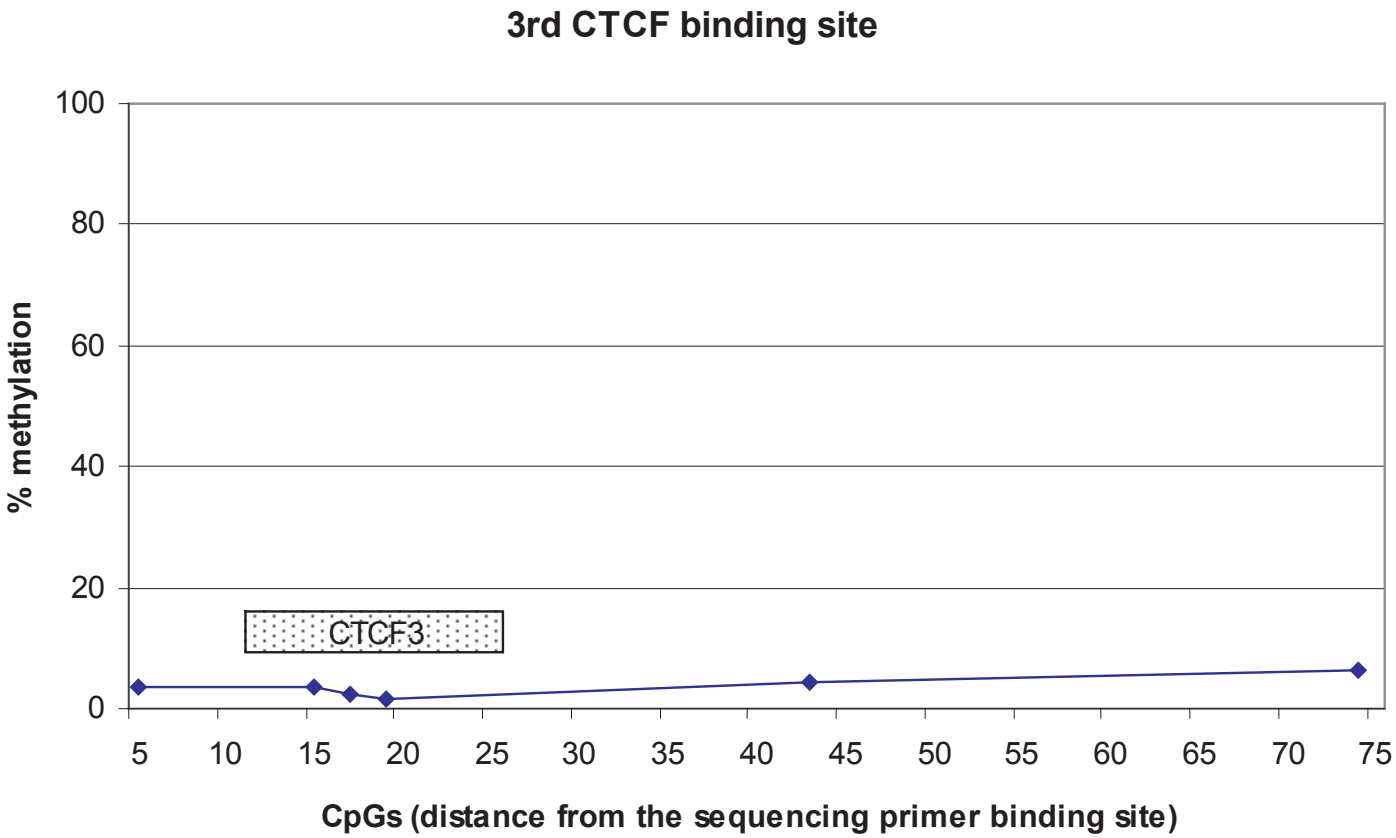




Supplemental Figure 4

B



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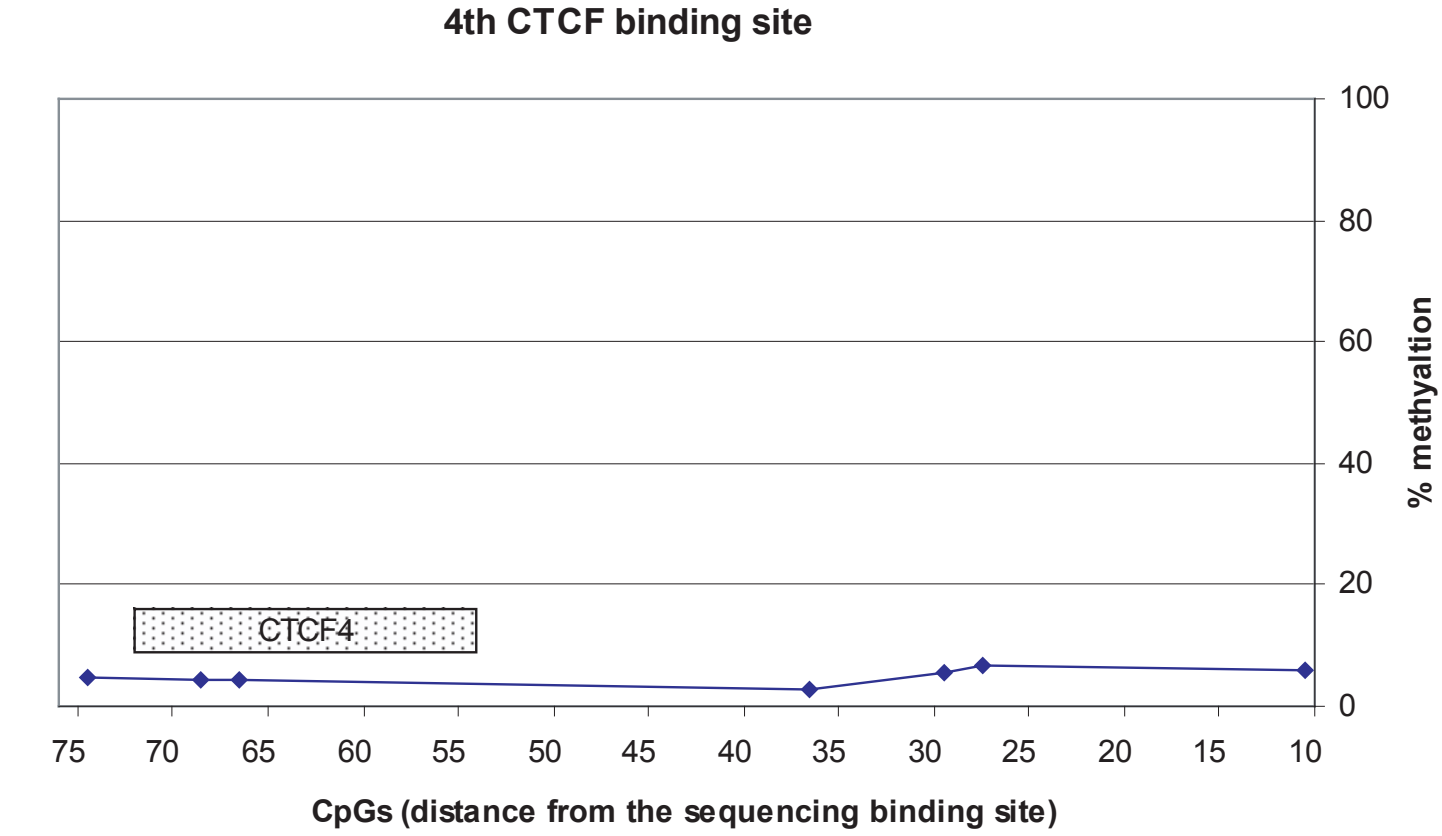
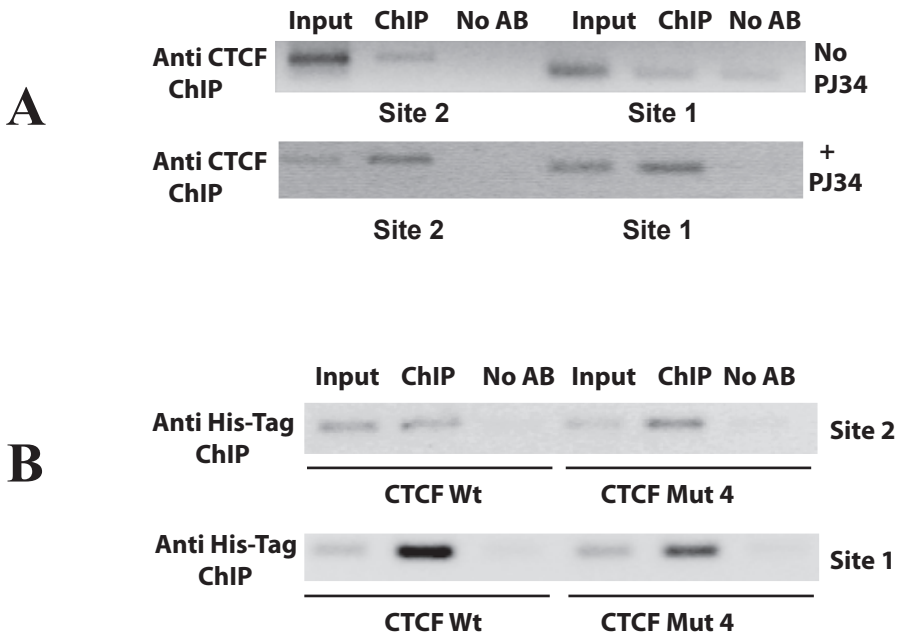
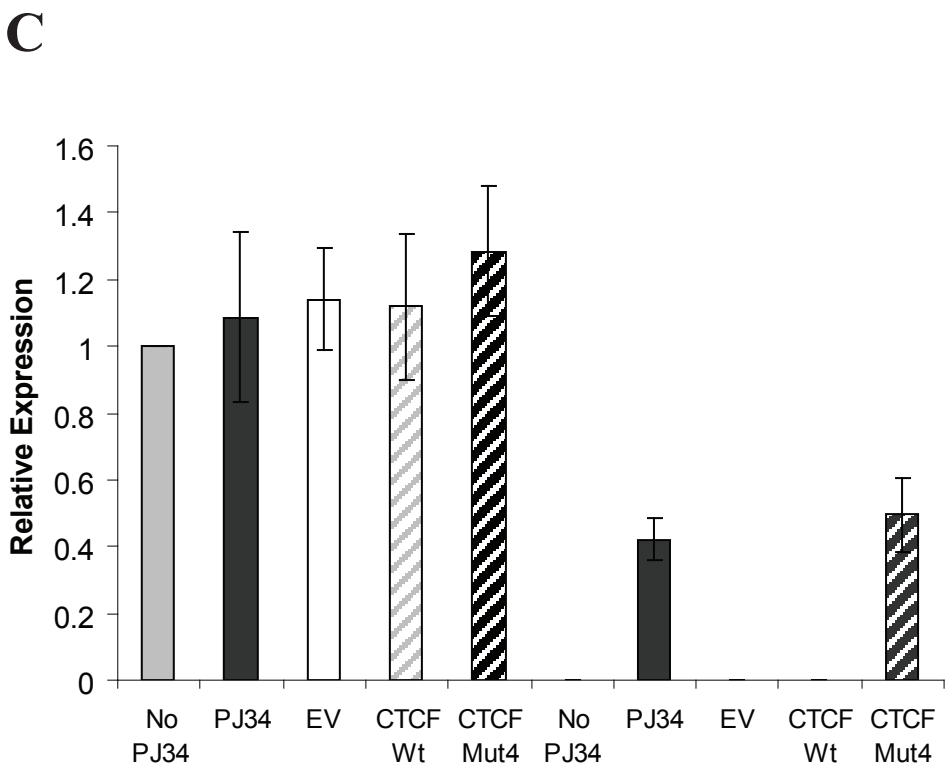
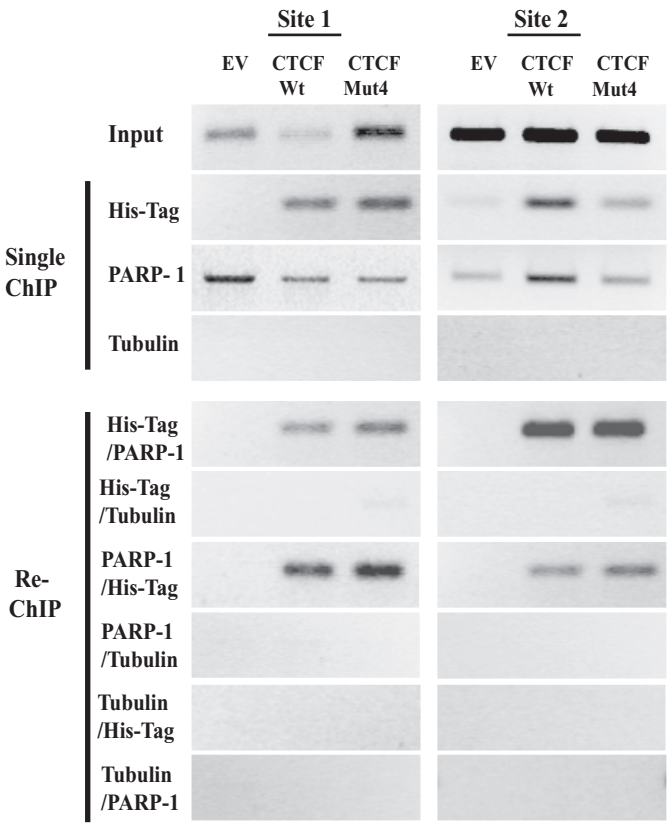


Supplemental Figure 5



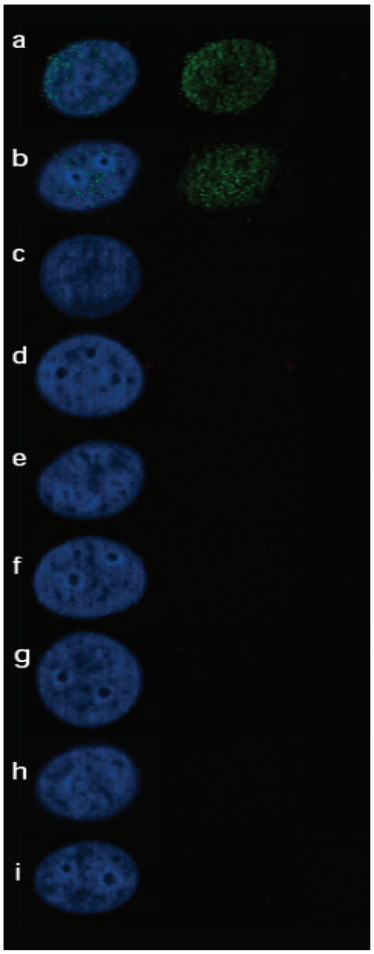


Supplemental Figure 6



Supplemental Figure 7

A



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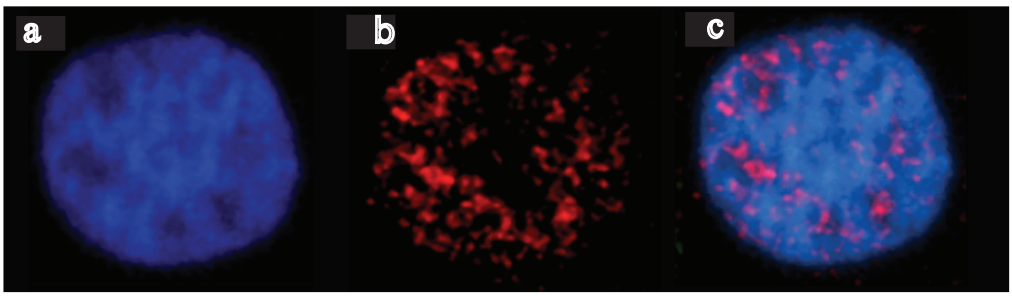
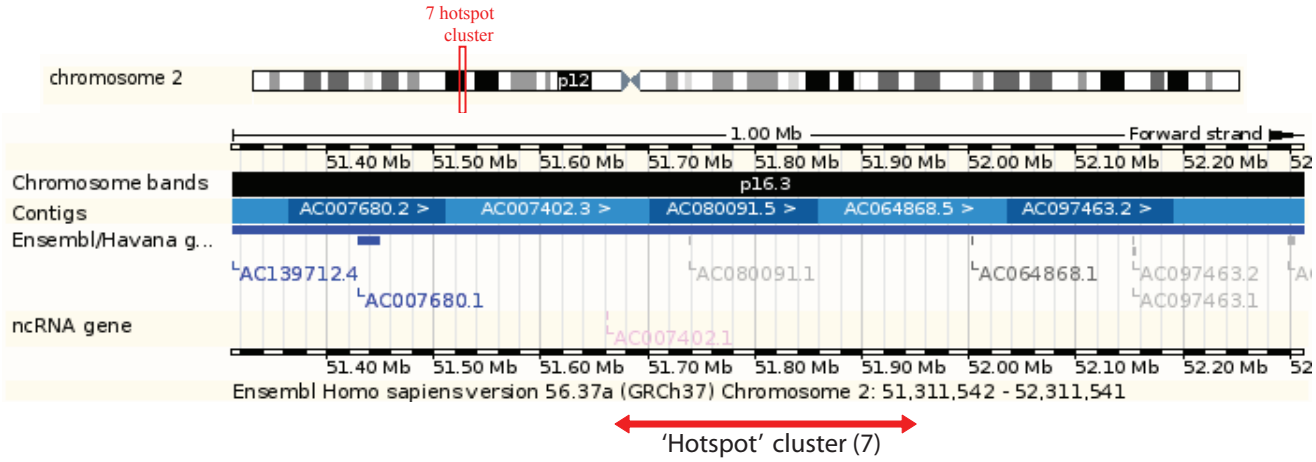


DAPI **CTCF/PARP-1 ISPLA** **MERGE**

Supplemental Figure 8

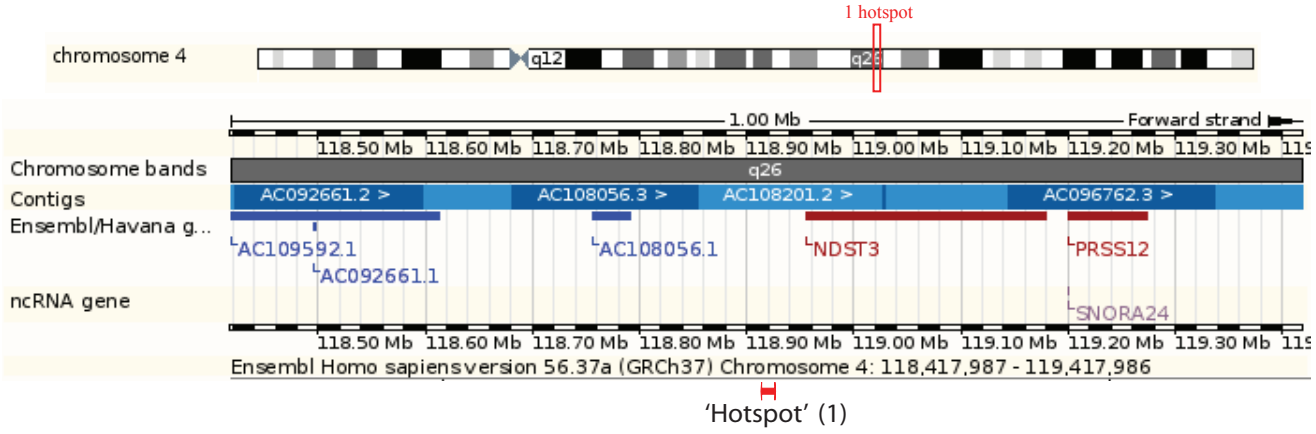
A

Chromosome 2



B

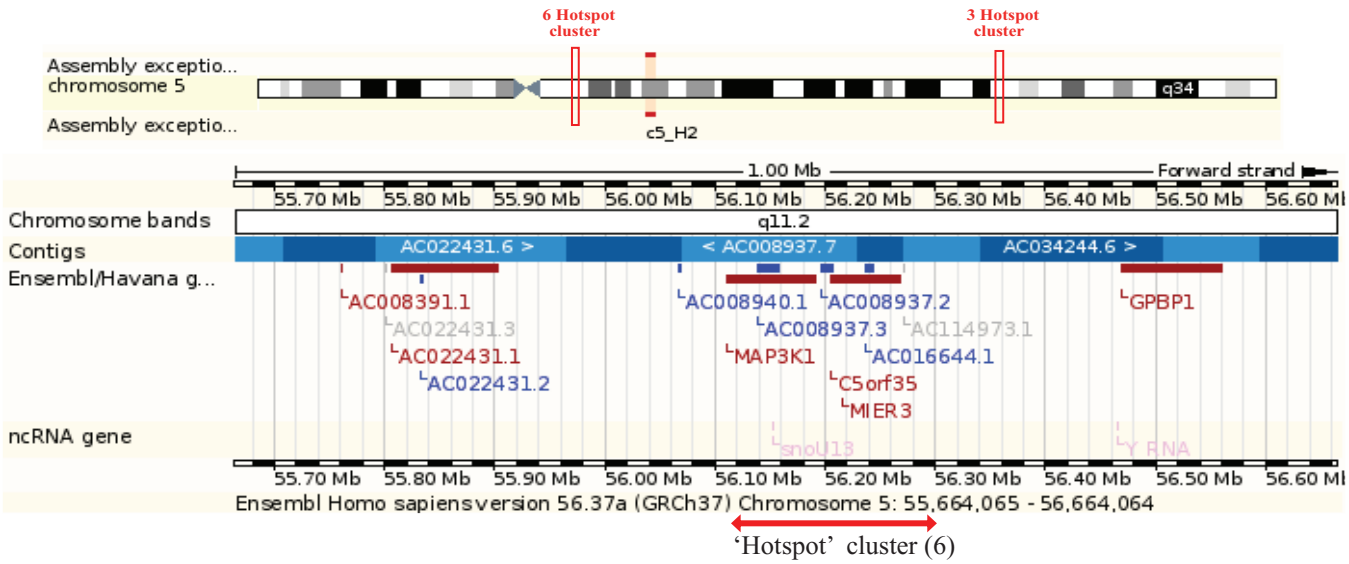
Chromosome 4



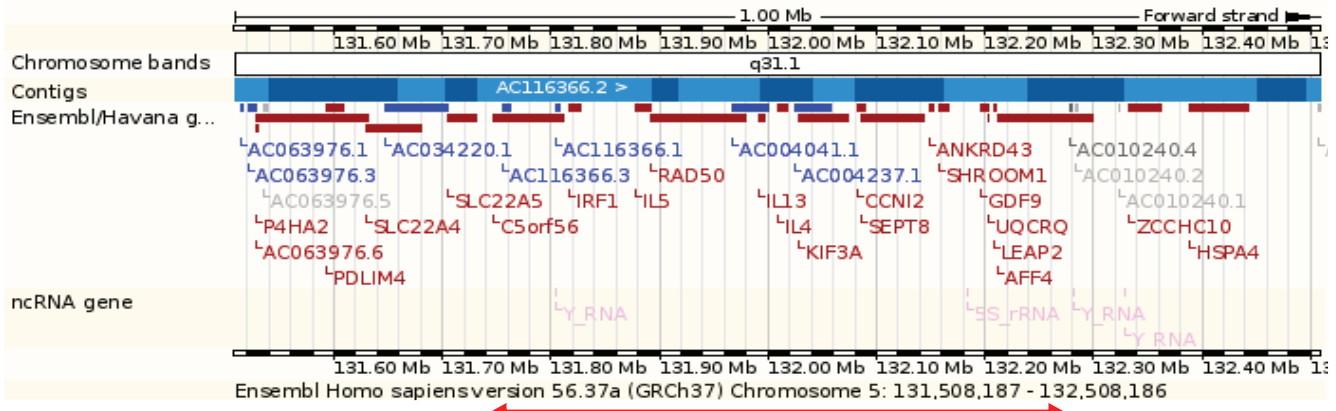
Supplemental Figure 8

C

Chromosome 5



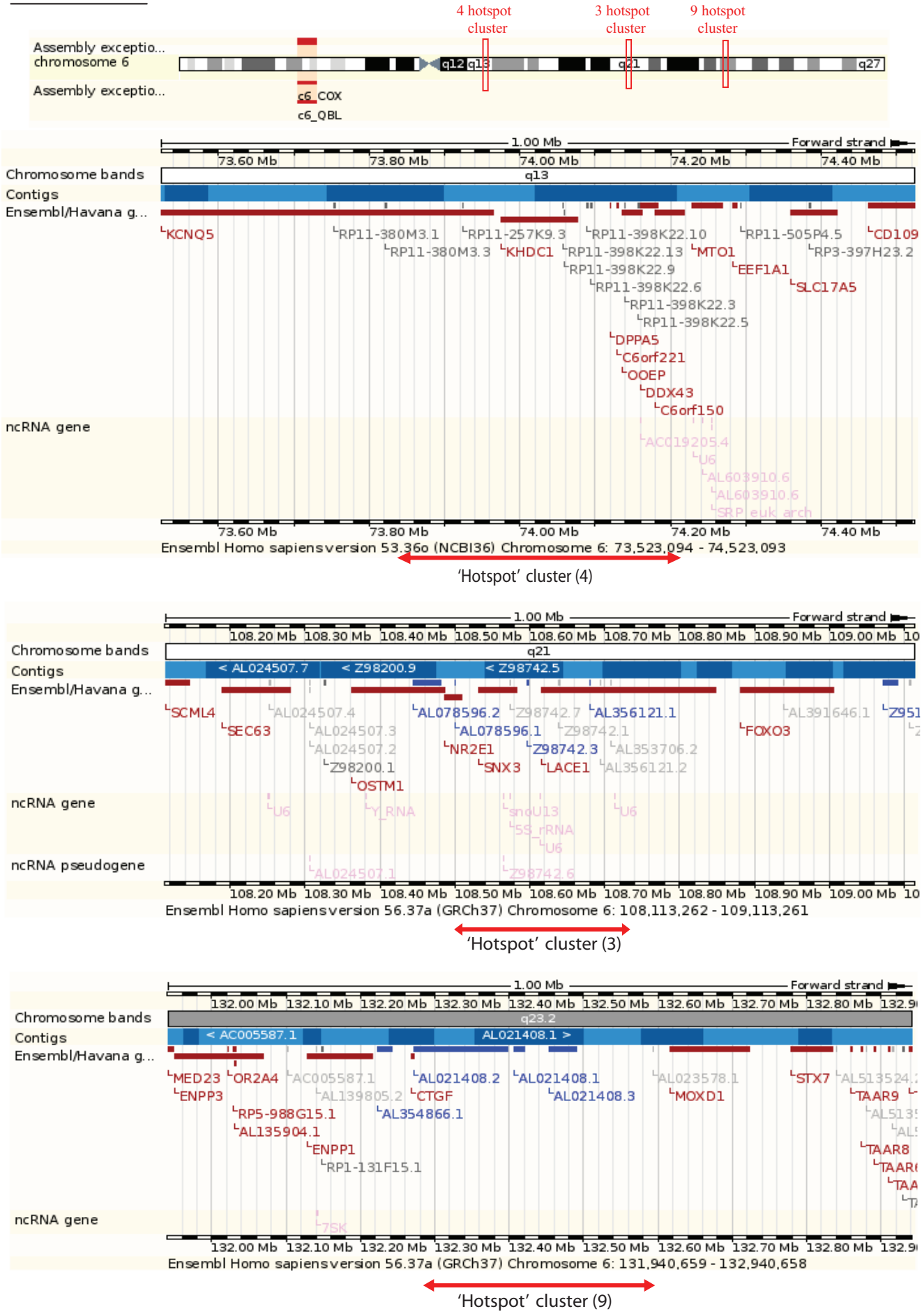
'Hotspot' cluster (6)



'Hotspot' cluster (3)

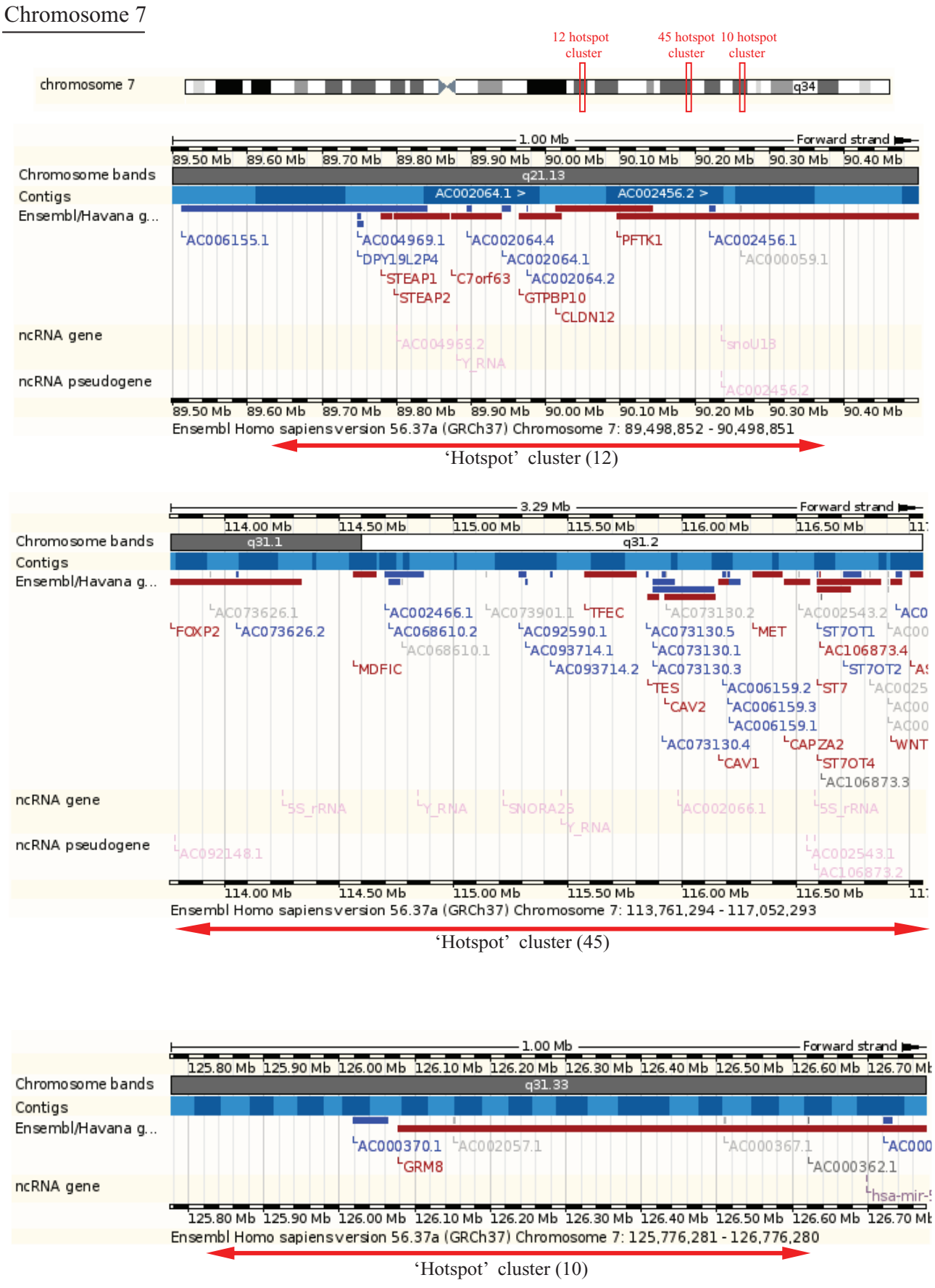
Supplemental Figure 8

D



Supplemental Figure 8

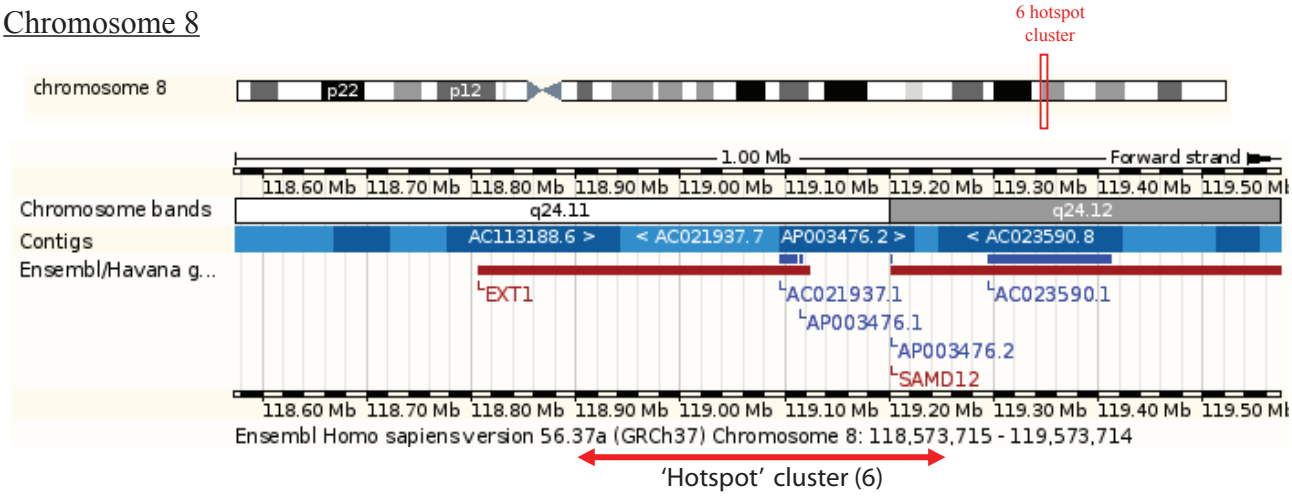
E



Supplemental Figure 8

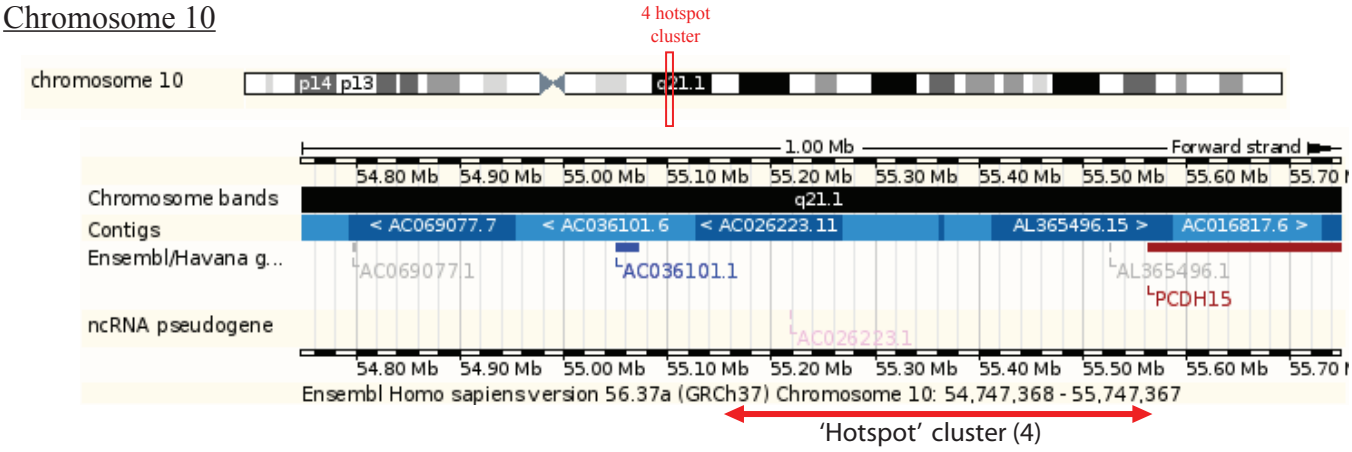
F

Chromosome 8



G

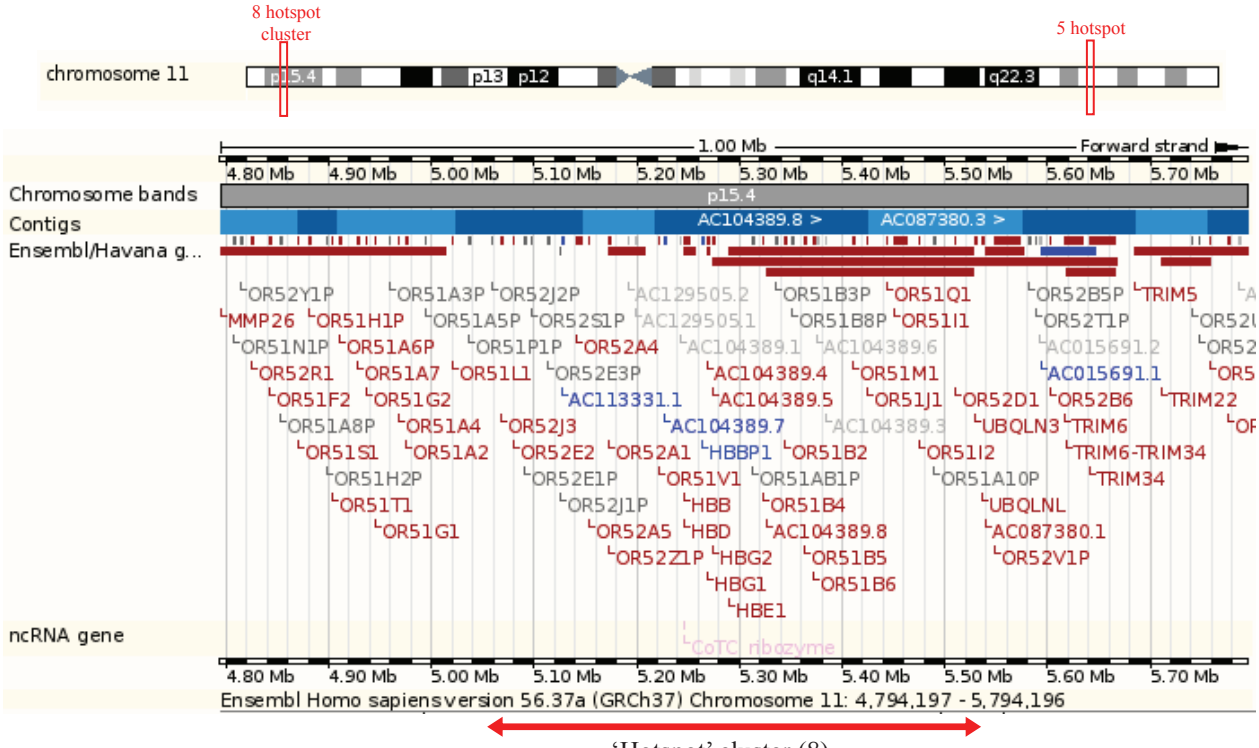
Chromosome 10



Supplemental Figure 8

H

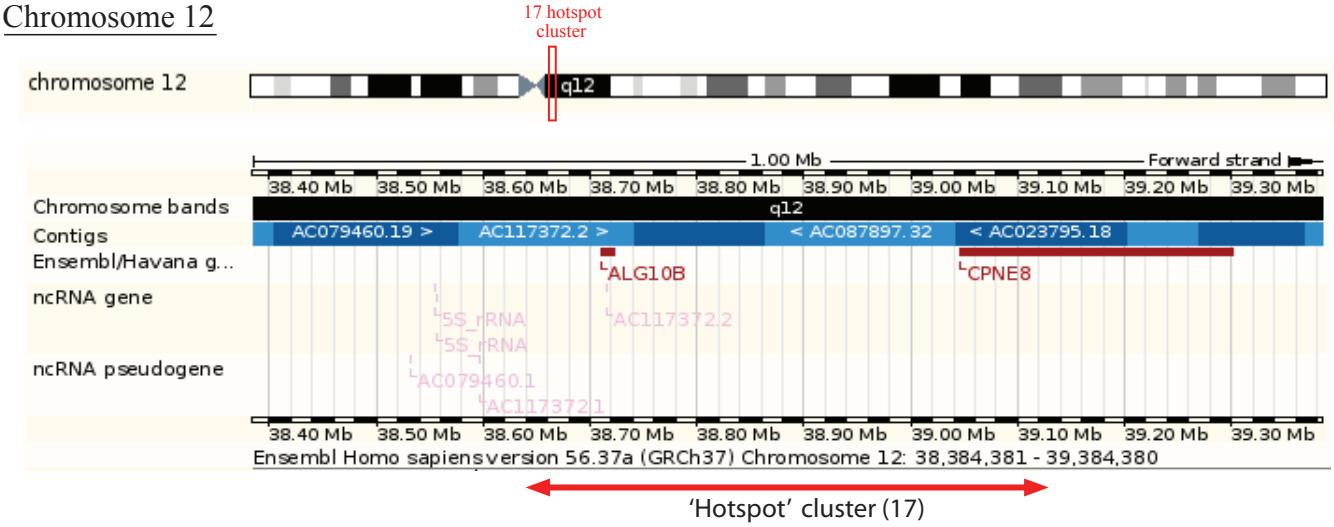
Chromosome 11



‘Hotspot’ cluster (8)

I

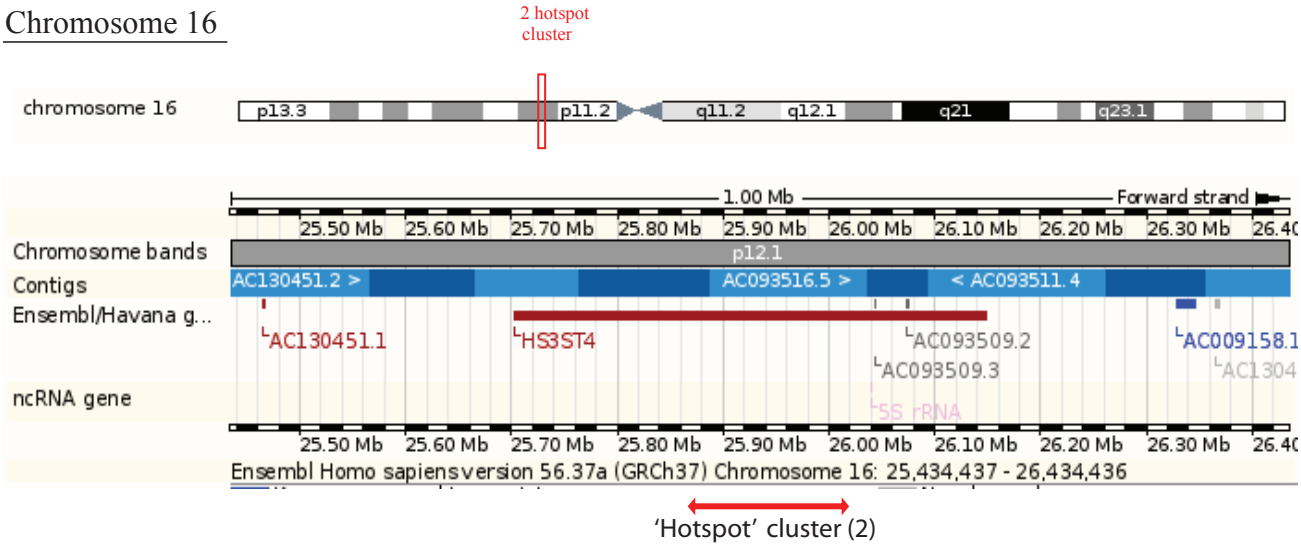
Chromosome 12



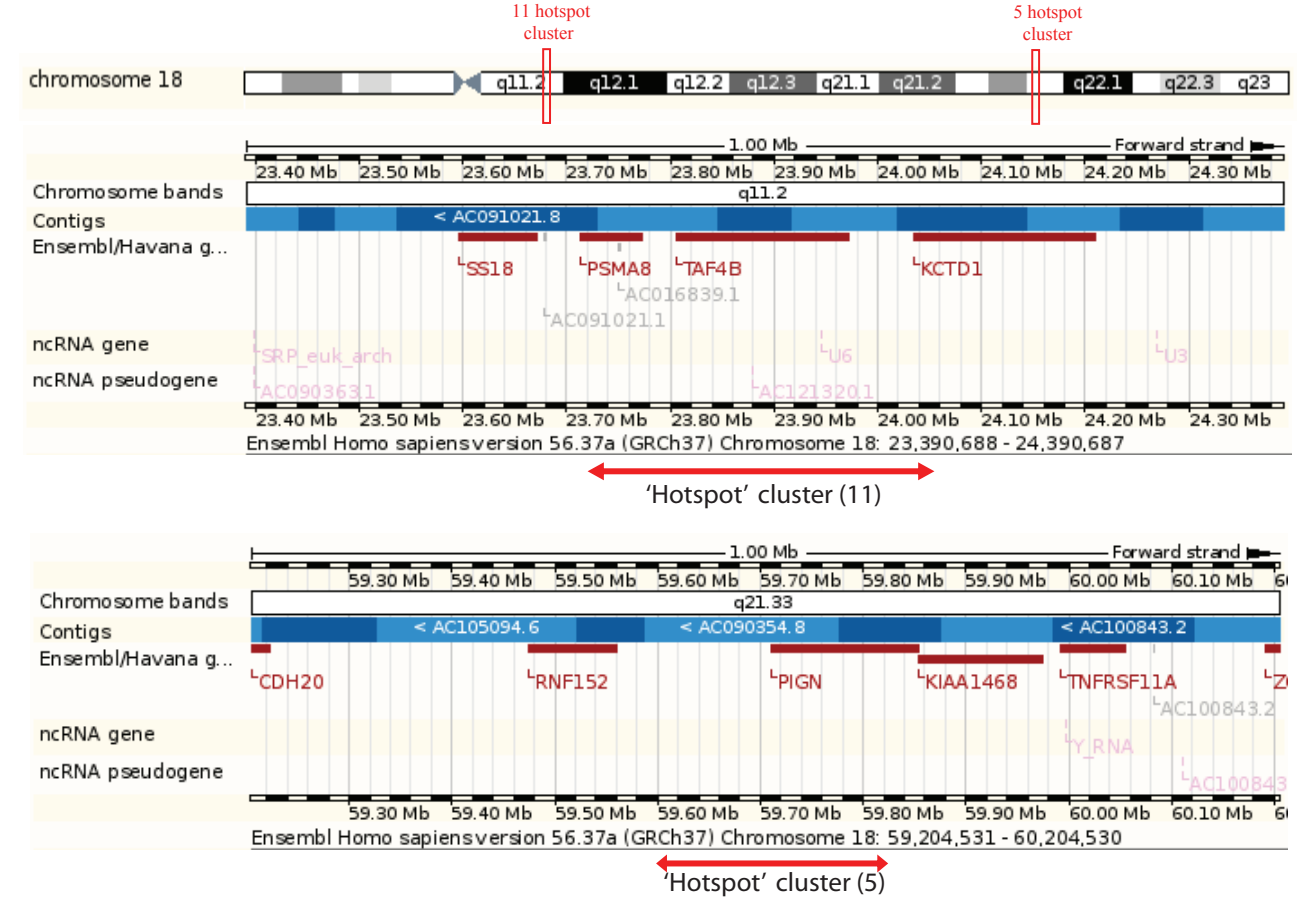
‘Hotspot’ cluster (17)

Supplemental Figure 8

J



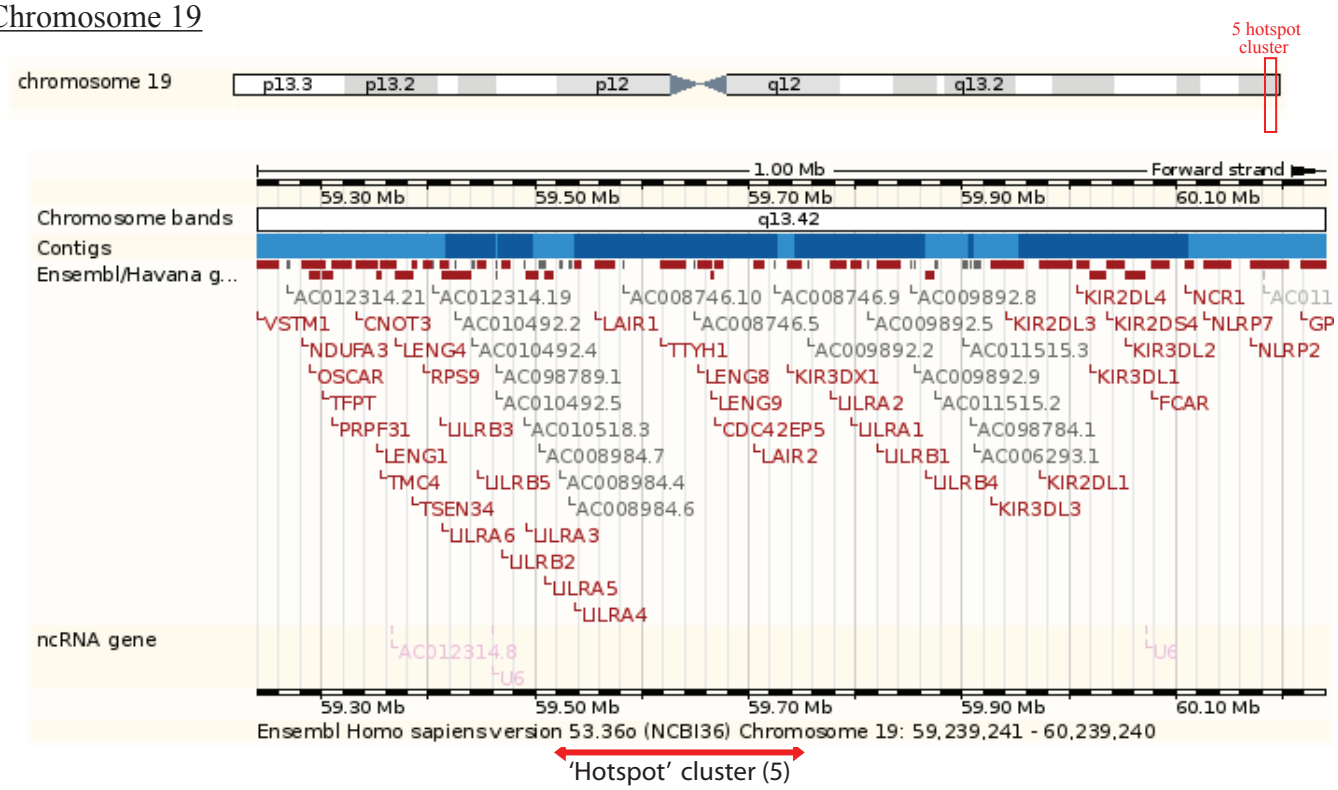
K



Supplemental Figure 8

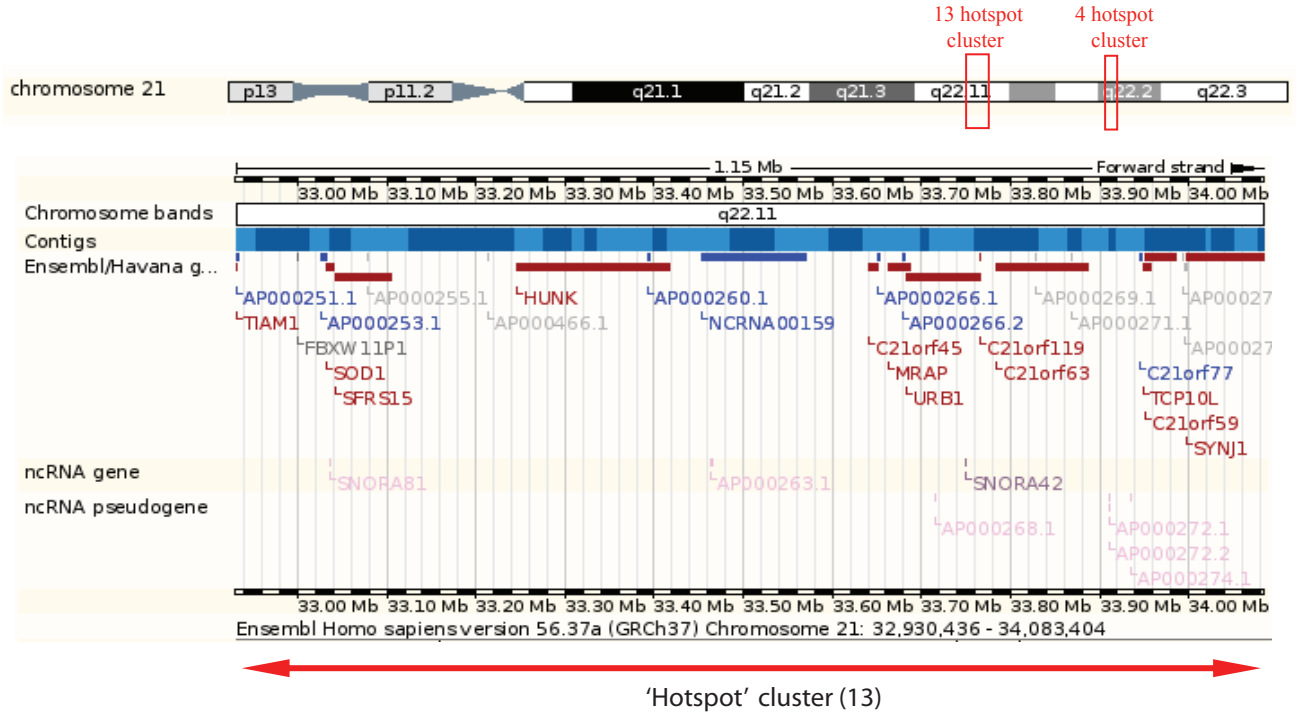
L

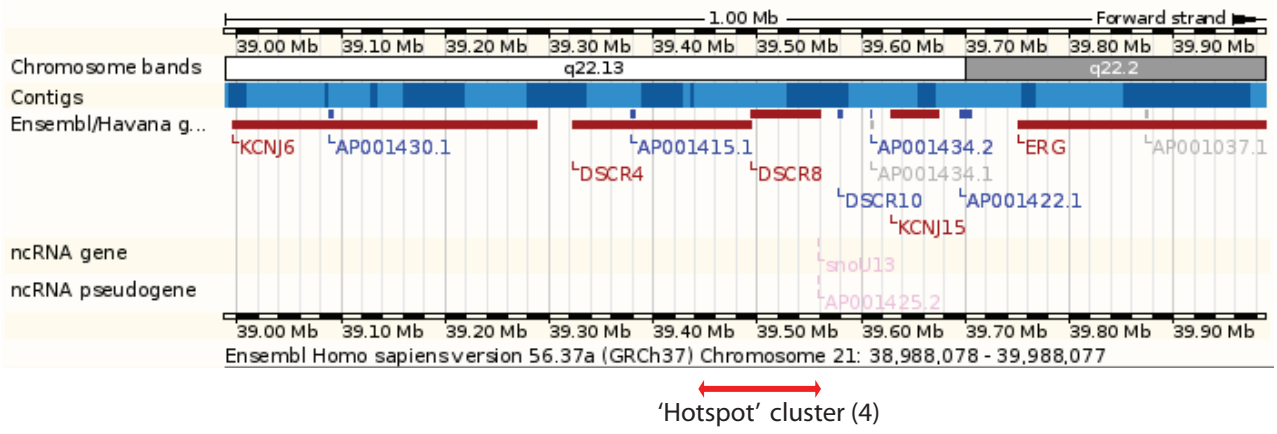
Chromosome 19



Supplemental Figure 8 M

Chromosome 21





Supplemental Figure 8 N

Chromosome 22