

SUPPLEMENTARY INFORMATION

S1 Experimental methods

OVERVIEW OF METHODS

Experimental procedures: HeLa and IMR90 cells were obtained from ATCC and cultured under recommended conditions. Passage 32 H1 cells were grown in mTeSR1¹ medium on Matrigel (BD Biosciences, San Jose, California), for 5 passages. 15 X 10cm² dishes were grown using standard mTeSR1 culture conditions and 20 X 10cm² dishes were cultured in mTeSR1 supplemented with 200ng/ml BMP4 (RND systems, Minneapolis, MN). 5 days post passage, when cells were approximately 70% confluent, H1 p32 cells grown in unmodified mTeSR1 were crosslinked. To crosslink, 2.5ml of crosslinking buffer (5M NaCl, 0.5M EDTA, 0.5M EGTA, 1M HEPES pH 8, 37% fresh formaldehyde) as added to 10ml culture medium and incubated at 37°C for 30 minutes, 1.25ml of 2.5M Glycine was added to stop the crosslinking reaction. Cells were removed from culture dish with a cell scraper, and collected by centrifugation for 10 minutes at 2500 rpm at 4C. Cells were washed three times with cold PBS. After the final spin, cells were pelleted and flash frozen using liquid nitrogen. BMP4-treated cells were subjected to the same procedure after 6 days of exposure. GM06990 (#GM06990) B-lymphocyte cells were acquired from Coriell (www.ccr.coriell.org) and grown to a density of 2.5x10⁵ cells/mL in RPMI 1640 medium with 2mM L-glutamine containing 15% fetal bovine serum at 37°C, 5% CO₂. K562 (#CCL-243) cells were acquired from ATCC (www.atcc.org) and grown to a density of 2.5x10⁵ cells/mL in Iscove's modified Dulbecco's medium with 4 mM L-glutamine containing 1.5 g/L sodium bicarbonate, and 10% fetal bovine serum at 37°C, 5% CO₂. Chromatin

preparation, ChIP, DNA purification, and LM-PCR were performed as previously described²⁻⁴, using commercially available antibodies (α -H3K27ac, Abcam ab4729; α -H3K4me1, Abcam ab8895; α -H3K4me3, Upstate 07-473; α -p300, Santa Cruz sc-585; α -MED1, Santa Cruz sc-5334; α -STAT1, Santa Cruz sc-345). CTCF ChIP was performed with a previously described antibody³. ChIP samples were hybridized to the NimbleGen genome-wide tiling microarray set (NimbleGen Systems, Inc.) as previously described^{2,3} and to custom condensed enhancer microarrays (NimbleGen Systems, Inc.) using standard methods. The condensed enhancer microarrays consisted of tiled 10 kb windows around each of 38716 primary predicted enhancers and standard controls. DNase-chip was performed and the data analyzed as previously described⁵. Cloning and reporter assays were performed essentially as previously described⁴ and a fragment was designated as active if its relative luciferase value was greater than 2.33 standard deviations ($p = 0.01$) above the median random activity. The regions cloned were chosen in a semi-random fashion based on several selection criteria that were determined by computational and/or visual inspection: overlap with at least one additional marker of activation (DHS, p300, MED1, H3K27ac), exclusion of conflicting marks (CTCF, H3K4me3), and sequence content amenable to cloning by our PCR-based method (low abundance of highly repetitive sequences in the candidate region, sequences that permit design of compatible PCR primers).

ChIP-chip data analysis and chromatin signature-based predictions: Data were analyzed using standard methods, and ChIP-chip targets for CTCF, p300, MED1, and STAT1 were selected with the Mpeak program. Enhancers were predicted as previously described⁶, with slight modifications to account for probe spacing on genome-wide array platforms. Genome-wide enhancer predictions in HeLa cells were considered to be verified if their averaged H3K4me1 and H3K4me3 enrichment profiles on the condensed enhancer microarrays were sufficiently

correlated to known enhancer chromatin signatures, resulting in verification of 36589 enhancers out of 38716 primary predictions (94.5% verified). In K562 cells, we performed ChIP-chip on Nimblegen HD2 genome-wide tiling arrays (12 array set, hg18). We normalized the raw data from each array using MA2C⁷, and mapped the normalized data to hg17 coordinates using the UCSC liftOver tool. We predicted K562 enhancers using the H3K4me1 and H3K4me3 profiles as in HeLa, with the following changes: we could not simply map the HeLa training set used in Heintzman et al to the K562 dataset, as the cell types are different. Instead, we used the unaltered training set from Heintzman et al. ROC analysis (data not shown) indicates that a correlation cutoff of 10% and an intensity cutoff of 1×10^{-12} yields the best overlap with K562 predictions in the ENCODE regions. K-means clustering, intersection analysis, evolutionary conservation analysis, and other computational comparisons (to UCSC Known Genes, ChIP-chip target lists, etc.) of the prediction sets were performed as previously described⁶. Specifically, to assess the overlap of predicted enhancers with genome-wide transcription factor binding site (TFBS) data sets, we counted the number of experimentally determined TFBS within 2.5 kb of the enhancers. To determine the significance of this overlap, we compared this statistic to the distribution of statistics for 100 random sets of putative enhancers, which was approximated by a normal. Each random set had the same number of elements as the putative enhancer set. Our enhancer predictions were limited to regions on the ChIP-chip array. Similarly, each random enhancer was placed uniformly at random in a sample space consisting of well-represented regions on the ChIP-chip microarray. The chromosomal distribution of each of the sets was kept constant. This careful placement of random sites ensures we do not artificially inflate the significance of the overlaps.

Gene expression and entropy analysis: Gene expression in the various cell lines was analyzed using HGU133 Plus 2.0 microarrays (Affymetrix) as described⁶. Specificity of expression was determined using a function of Shannon entropy as described⁸ and the top, middle, and bottom 1000 genes from this analysis were designated as HeLa-specific expressed, non-specific expressed, and HeLa-specific repressed genes, respectively (Figure S5), for evaluation of enhancer enrichment in the insulator-defined domains containing the promoters for these classes of genes (as in Figure 3A), where insulators were defined by CTCF binding sites as described below. When counting enhancers around these promoters, we included all enhancers within 200 kb of a promoter as long as they were still within the same insulator-defined domain as described above. Random distributions were generated by averaging the enrichment profiles around promoters of 100 iterations of randomly selected enhancer sets of 36589 elements. To assess enhancer and gene expression specificity between HeLa and K562 cells (as in Figure 3E), we use the MAS5 algorithm from the Bioconductor R package to generate gene expression Present/Absent calls from each cell type. Since we have two biological replicates of K562 expression, to merge calls for these replicates, probes called differently in the two replicates are labeled as Marginal. To eliminate biases from genes not expressed in either HeLa or K562, we only consider a probe if it is called Present in either HeLa or K562. We map Affymetrix probes to gene identifiers using the knownToU133Plus2 table from the UCSC Genome Browser, and then map the identifiers to genomic coordinates (hg17, NCBI build 35) using the knownGene table from the UCSC Genome Browser. To reduce redundancy, we keep only the first gene when multiple Affymetrix probes map to the same annotated gene. The result from this filtering and mapping is a set of 11783 genes. For each such gene, we count the number of enhancers predicted in each cell type within that gene's CTCF-domain. We sort the genes by differential

(HeLa – K562) gene expression (as defined by the RMA algorithm from the Bioconductor R package) and use a sliding window of 1000 genes to generate a profile of the average number of enhancers for each cell type as a function of average differential gene expression. This gives two profiles: one using HeLa enhancers and one using K562 enhancers. To normalize, we repeat this analysis for each cell type using 100 sets of random enhancers (placed uniformly at random on the tiling microarrays), giving 100 random enhancer-expression profiles. We then define the enhancer enrichment profile as the ratio between the number of enhancers in the observed profile and the expected number of enhancers in the averaged random profile.

Motif analysis: Enhancer regions were defined as 2 kb windows centered on each prediction, and promoter regions were defined as 1 kb windows upstream from annotated TSS. Promoter regions were excluded from enhancer regions; repeats, exons and transposons were excluded from both. Motif conservation in each region was evaluated relative to the genomes of opossum, tenrec, elephant, armadillo, cow, dog, rabbit, rat and mouse, extracted from UCSC Genome Browser and used with permission. The mammalian tree, along with branch lengths, was computed using DNAML (PHYLIP package)⁹ with the F84 nucleotide model of evolution in ~500kb of randomly selected exon sequence. Known and novel motifs were discovered as previously described¹⁰, with the primary difference that instances were not required to have perfect conservation and were considered conserved if they were found across a number of species spanning at least 50% of the total branch length of the mammalian tree (Branch-Length-Score > 50%)^{11,12}. We ranked motifs based on their over-conservation, measured as the probability of observing a substantially increased number of conserved motif instances compared to that expected for motifs of identical composition, and selected all motifs with $P < 1 \times 10^{-3}$. We evaluated a motif's enrichment as its over-abundance, or the hypergeometric probability of

observing a substantially increased number of occurrences in the intergenic and intronic regions of the human genome (regardless of evolutionary conservation) compared to motifs of identical composition, with a cutoff of $P < 1 \times 10^{-3}$.

Supplementary data for the microarray experiments has been formatted for viewing in the UCSC genome browser via <http://bioinformatics-renlab.ucsd.edu/enhancer>

S1.1 ChIP-chip

We performed ChIP-chip analysis¹³ to determine the chromatin modification patterns along 44 human loci selected by the ENCODE Consortium as common targets for genomic analysis⁶, totaling 30 Mbp or 1% of the human genome. We investigated the patterns of six specific histone modifications: acetylated histone H3 lysine 9, 18 and 27 (H3K9Ac, H3K18Ac and H3K27Ac), and mono-, di- and tri-methylated histone H3 lysine 4 (H3K4Me1, H3K4Me2, and H3K4Me3), as well as the insulator-binding protein, CTCF. ChIP samples were amplified, labeled, and hybridized to tiling oligonucleotide microarrays and data were analyzed as previously described¹³, generating high-resolution maps of histone modifications and CTCF binding in 5 cell lines: cervical carcinoma HeLa, immortalized lymphoblast GM06690 (GM), leukemia K562, embryonic stem cells (ES), and BMP4-induced ES cells (dES).

ChIP-chip procedure and antibodies against p300, TAF1, histone H3, H3K4Me1, H3K4Me2, H3K4Me3, and CTCF were previously described^{2,3,13}. Additional antibodies are commercially available (α -H3K9Ac Abcam ab4441; α -H3K18Ac Abcam ab1191; and α -H3K27Ac Abcam ab4729). All ChIP-chip experiments were completed in triplicate, except for with normal and BMP4-treated ES cells (Figure S8). All ChIP-DNA samples were hybridized to NimbleGen

ENCODE HG17 microarrays (NimbleGen Systems). DNA was labeled according to NimbleGen Systems' protocol. Samples were hybridized at 42°C for 16 hours on a MAUI 12-bay hybridization station (BioMicro Systems). Microarrays were washed, scanned and stripped for re-use following protocols from NimbleGen Systems. Gene expression data for HeLa, K562, and GM cells were obtained using HU133 Plus 2.0 microarrays (Affymetrix).

We also performed ChIP-chip of histone modifications H3K4Me1 and H3K4Me3 in HeLa cells on Nimblegen genome-wide tiling arrays (38 array set, hg17). We normalized the raw data from each array using both the median and loess algorithms from the Bioconductor R package (treating each probe equally). For each array, we chose the normalization method that gave the most balanced distribution of random probes about a log ratio of 0. We performed ChIP-chip of histone modifications H3K4Me1, H3K4Me3, and H3K27Ac in K562 cells on Nimblegen HD2 genome-wide tiling arrays (12 array set, hg18). We normalized the raw data from each array using MA2C⁷, and mapped the normalized data to hg17 coordinates using the UCSC Genome Browser liftOver tool.

S1.2 Gene Expression Analysis for ES and dES cells

The Human Whole Genome Expression arrays containing ~385,000 60-mer probes was manufactured by NimbleGen Systems (<http://www.nimblegen.com>). This array design tiles transcripts from approximately 36,000 human locus identifiers for the hg17 (UCSC) assembly with typically 10 or 11 probes per transcript. For gene expression analysis, we isolated the total RNA from H1 ES cells or BMP4-treated cells using Trizol (Invitrogen, Carlsbad, CA) according to the manufacturer's recommendations. Total RNA was enriched for the polyA fraction using

Oligotex mRNA Mini Kit (Qiagen). Enriched mRNA (250 ng) was primed using random hexamers and reverse transcribed using Superscript III (Invitrogen) in the presence of 5-(3-aminoallyl)-dUTP (Ambion). The purified product was coupled to Cy5-NHS ester (Amersham). Similarly, sonicated genomic DNA (2 μ g) was primed with random octamers and labeled using Klenow in the presence of 5-(3-aminoallyl)-dUTP. The resulting product was coupled to Cy3-NHS ester (Amersham). Cy3-labeled genomic DNA (4.5 μ g) was used as a reference and added along with the Cy5-labeled mRNA sample (2 μ g) onto each array. Hybridizations were performed in 3.6X SSC buffer with 35% formamide and 0.07% SDS at 42°C overnight. Arrays were then washed, dried, and scanned using a GenePix 4000B scanner. We set the expression level of genes in undifferentiated cells as 1 and calculated the relative fold change of individual genes in the dES cells.

S2 Data analysis

S2.1 Expression array analysis

We use the GCRMA package¹⁴ to normalize Affymetrix mRNA expression arrays for HeLa, GM, and K562 cell types. For every pair of these cell types, we also use GCRMA to find differentially expressed and repressed genes using a p-value cutoff of 0.01 in conjunction with a fold change cutoff of 2.0. The expression data for ES and dES cell types was done using the Nimblegen platform, and thus are not directly comparable to the Affymetrix expression data. As such, we can only use this expression data to compare ES and dES cell types. As a conservative measure of differential expression, we use a fold-change cutoff of 2.

S2.2 Gene Expression Data Analysis for ES and dES cells

Gene expression raw data were extracted using NimbleScan software v2.1. Considering that the signal distribution of the RNA sample is distinct from that of the gDNA sample, the signal intensities from RNA channels in all eight arrays were normalized with the Robust Multiple-chip Analysis (RMA) algorithm¹⁴. Separately, the same normalization procedure was performed on those from the gDNA samples. For a given gene, the median-adjusted ratio between its normalized intensity from the RNA channel and that from the gDNA channel was then calculated as follows:

Ratio = intensity from RNA channel / (intensity from gDNA channel + median intensity of all genes from the gDNA channel).

We have found that this median-adjusted ratio gives the most consistent results when compared to other published human ES cell expression data, such as SAGE library information available from the Cancer Genome Anatomy Project (CGAP). Consequently, we used this median-adjusted ratio as the measurement for the gene expression level

S2.3 Expanded maps of histone modifications

Previously, we demonstrated enhancers could be determined by distinct chromatin signatures of H3K4Me1 and H3K4Me3 at these functional elements¹³. Focusing on HeLa cells, we found that three additional histone modification marks, namely H3K9Ac, H3K18Ac and H3K27Ac are also part of the chromatin patterns at promoters and enhancers. All three acetylation marks localize to

active transcription start sites (TSSs), and remain absent, as do other chromatin modifications, at inactive promoters (Figure S9A). These results agree with individual promoter studies observing acetylation or hyper-acetylation at active promoters^{15,16}, as well as with large-scale histone modification studies in yeast^{17,18}. Distal p300 binding sites show clear enrichment of H3K18Ac and H3K27Ac, while H3K9Ac is much reduced (Figure S9B). These results suggest that H3K9Ac is preferentially associated with active promoters, while H3K18Ac and H3K27Ac are associated with both promoters and enhancers.

S2.4 Identification of CTCF and p300 binding sites

The Mpeak program can reliably detect binding sites of transcription factors, and has worked well in previous studies to identify TAF1, CTCF, and p300 binding sites^{2,3,13,19}. We use the Mpeak program to determine binding sites of CTCF³ and p300¹³ peaks. Specifically, we call a CTCF peak if there is a stretch of 4 probes separated by at most 300 bp that are at least 2.5 standard deviations above the mean. For p300, we use a simple FDR cutoff of 0.0001 to define peaks as in Heintzman et al¹³. We used different parameters for consistency with previous publications, but swapping these parameters does not vary the results significantly.

S2.5 Most human promoters are universally associated with a set of active chromatin marks in different cell types

Modulation of chromatin state is a key component of tissue-specific gene expression programs^{17,20}. Given the diversity of these five cell lines and the critical role of promoters in regulating gene expression, we hypothesized that the chromatin modifications at promoters would uniquely define each cell type, but we actually observed the opposite. At promoters of 414

genes in the ENCODE regions, we found that the chromatin signatures at promoters are remarkably similar across all cell types (Figure 1A). To quantify this, we defined a cell type's enrichment profile as the sum of the log ratio enrichment values of H3K4Me1, H3K4Me3, and H3K27Ac for each promoter. We then calculated the Pearson correlation coefficient between enrichment profiles from different cell types (Figure S1A). The enrichment profiles are highly correlated between all pairs of cell types, with an average correlation coefficient of 0.71. This observation also holds at the larger set of Gencode promoters (Figure S2). The generally invariant nature of the chromatin marks at promoters suggest that epigenetic features at this class of regulatory element are not the dominant drivers of cell type-specific gene expression patterns.

S2.6 CTCF binding in the genome is generally cell-type invariant

Insulator elements play key roles in restricting enhancers from activating inappropriate promoters, thereby defining the boundaries of gene regulatory domains²¹. Nearly all insulators that have been experimentally defined in the mammalian genome require the insulator binding protein CTCF to function²². Our previous genome-wide location analysis of the insulator binding protein CTCF in human fibroblasts indicated that predicted insulators (those sites in the genome bound by CTCF) are closely correlated with the distribution of genes, and are highly conserved throughout evolution, consistent with their key role in transcription regulation³. Intriguingly, the overlap of predicted insulators in two cell lines in that study (IMR90 lung fibroblast and U937 hematopoietic progenitor cells) was a remarkable 67%, suggesting cell-type invariance. To further investigate this possibility, we used ChIP-chip to identify CTCF binding sites in the ENCODE regions in each of the five cell types. On average, 517 predicted insulators were recovered in each cell type, with a remarkable average of 82.8% shared between pairs of cell

types (Table S1). Indeed, the CTCF enrichment profiles at 729 non-redundant CTCF binding sites are nearly identical across all five cell types studied here and IMR90 cells (Figure S1E), and the average Pearson correlation coefficient between all pairs of profiles is 0.72 (Figure S1B), comparable to the value observed at promoters. The consistency of CTCF binding appears to extend to the entire genome (Figure S6). These results support insulators as being largely cell-type invariant, to a greater degree than previously appreciated. Additionally, none of the histone modifications that we examined were consistently present at predicted insulators (Figure S11) – further experiments are necessary to determine a chromatin signature for insulators, if one exists.

S2.7 p300 binding sites are cell-type specific

As an alternative method to predicting enhancers based on chromatin modifications, we use the stringent criteria of defining enhancers to be binding sites of p300, a histone acetyltransferase and co-activator protein. While the presence of p300 is sufficient to indicate an enhancer, p300 is not necessarily found at all enhancers¹³. Using the Mpeak program¹⁹, we identified a total of 411 TSS-distal p300 binding sites in HeLa, GM, and K562 cell lines. We observe that, unlike CTCF and chromatin modifications at promoters, the localization of p300 binding sites appears unique to each cell-type in the three cell types where p300 ChIP-chip analysis was performed (Figure 1F). The cell-type specificity of p300 binding sites is supported by the extremely low correlations observed: the average pair wise Pearson correlation coefficient at p300 binding sites is -0.11 (Figure S1C), compared to the much higher correlations 0.71 and 0.72 observed at promoters and insulators, respectively. More strikingly, p300 binding sites are largely cell-type specific: of the 411 distal peaks recovered from the three cell types, the vast majority (378, 92.9%) are unique to a single cell type, 29 (7.1%) are shared among exactly two cell types, and 4

(1.0%) are common among all three cell types. These results support the findings for enhancers defined by chromatin-based signatures.

S2.8 Enhancer prediction method

The procedure used to predict enhancers follows closely to that in Heintzman et al¹³. We first bin the tiling ChIP-chip data into 100 bp bins, averaging multiple probes that fall into the same bin. Using a sliding window on H3K4Me1 and H3K4Me3, we scan for chromatin signatures resembling a training set of enhancer patterns defined by the p300 binding sites in HeLa cells, keeping only those windows that correlate most with the training sets and that have significant enrichment of chromatin modifications. We use a discriminative filter to keep only those predictions that correlate with an averaged enhancer training set more than the promoter training set. Finally, we apply a descriptive filter, keeping only those remaining predictions having a correlation of at least 0.5 with an averaged training set.

In both ENCODE and genome-wide predictions of this study, we made predictions of active promoters and enhancers as in Heintzman et al¹³, with the following modifications:

- Repetitive regions of the genome are not covered by the probes on tiling arrays, contributing to gaps in coverage. In Heintzman et al, we interpolated through all gaps. But this can lead to false positive predictions or biasing of the underlying background distributions when there are many gaps. To remove these concerns, here we interpolate only through gaps smaller than 1000 bp.
- In the prediction algorithm, we slide a 10 kb window across the tiled regions and compute 2 statistics for each window: the correlation with a training set and the sum of the absolute

values of intensities of the middle 2 kb region of the window. The correlation part has remained unchanged in this study. In Heintzman et al, the intensity statistic appeared normally distributed, and as such we approximated it with a Gaussian distribution. In light of the larger datasets in this study, this normal assumption did not appear entirely correct. Here, we change our intensity statistic to the sum of squares of the normalized intensities in the 2 kb region. A normalized intensity is an intensity subtracted from the mean array intensity and divided by the standard deviation of the array intensity. Since each array is properly normalized to follow a Gaussian distribution, by definition, this statistic follows a Chi-squared distribution with 42 degrees of freedom (for each window, each of the 2 modifications has 21 normalized intensities squared: 10 in each direction and one at 0).

The training set used here contained the same six groups of training sets used in Heintzman et al, with the exception that the HeLa enhancer predictions used data derived from the genome-wide H3K4Me1 and H3K4Me3 arrays.

In the ENCODE regions, as in Heintzman et al, we keep predictions in the top 10% of the intensity distribution and top 1% of the correlation distribution. For the genome-wide enhancer predictions in HeLa, a ROC analysis (data not shown) indicates that a correlation cutoff of 1% and an intensity cutoff of 1% yields the best overlap with our previously published predictions in the ENCODE regions. Similarly, for the genome-wide predictions for the MA2C-normalized K562 data, ROC analysis suggests using a correlation cutoff of 10% and an intensity cutoff of $1e-12$.

S2.9 Validation of predicted enhancers in the ENCODE regions

Several lines of evidence suggest that the histone-modification-based predictions of enhancers are truly enhancers. First, we compare the predicted enhancers to DNase I hypersensitive (HS) sites, as hypersensitivity is a hallmark of enhancers. Using a recently published set of HS sites²³ mapped in HeLa, GM, K562, and H9 ES cells, we computed the percentage of predicted enhancers within 2.5 kb of HS sites (Figure S3A-D). For comparison, we also computed the overlap percentage of 100 sets of randomly placed enhancers restricted to regions on the ChIP-chip microarray. We notice that predicted enhancers in HeLa (53.0% overlap, Z-score = 20.4, $p = 3.2E-93$), GM (38.2% overlap, Z-score = 14.4, $p = 5.1E-47$), K562 (overlap = 62.6%, Z-score = 22.7, $p = 3.9E-114$), and ES (59.2% overlap, Z-score = 18.0, $p = 1.0E-72$) are enriched in HS sites in their respective cell types. Thus, the predicted enhancers are supported by HS data.

Second, enhancers are defined to be regions in the genome bound to transcription factors and co-activators. To verify the predicted enhancers, we compare their overlap with p300 binding sites. For every cell line where we mapped p300 binding, we observe significant enrichment of predicted enhancers at p300 binding sites (HeLa: 86.4% overlap, Z-score = 27.7, $p = 2.9E-169$; GM: 79.2% overlap, Z-score = 35.7, $p = 4.6E-279$; K562: 63.6% overlap, Z-score = 23.3, $p = 1.7E-120$) (Figure S3E-G), again supporting the predicted enhancers as being real. To further validate the predicted enhancers in the ES cell line, we rely on the definition of enhancers as binding sites for transcription factors and compare the predicted enhancers with previously mapped binding sites for the ES-specific transcription factors Oct4, Sox2, and Nanog²⁴ (Figure S4). Compared to predicted enhancers from other cell types, we notice greater than 2-fold enrichment of the predicted ES enhancers with these ES-specific factors. Although we do not

have the corresponding functional data for the dES cell type, several lines of evidence suggest that they are also real. First, like the other cell types, the histone modification patterns at predicted dES enhancers are enriched in H3K4Me1 and H3K27Ac, but lack H3K4Me3. Second, there is a significant enrichment of dES enhancers at HS sites and p300 binding sites from the other cell types, indicating that at least some of these dES enhancers are real.

S2.10 Validation of genome-wide predictions of enhancers

The features of enhancers in the HeLa genome are consistent with what we observed across cell types in the ENCODE regions. Most predicted enhancers (23686, 64.7%, $p = 6.6e-208$) exhibit strong evolutionary conservation (PhastCons ≥ 0.8 , see Methods). The genomic distribution of the predicted enhancers are distinct from those of promoters: except for a small fraction that overlap with Known Gene 5'-ends, CAGE tags, or CpG islands, the predicted enhancers are distal to promoters, with predominantly intronic (37.9%) or intergenic (56.3%) localization (Figure 2C). Most predicted enhancers (61.4%) are marked by moderate or high levels of acetylation of H3K27 (Figure 2A). The co-activator p300 and Mediator component MED1, known to bind enhancers, are found at 10741 (29.4%) and 5764 (15.8%) enhancer predictions, respectively (see Methods). Additionally, 19776 (54.1%) of the predicted enhancers exhibit significant DNaseI hypersensitivity. Collectively, we found that 23722 (64.8%) predicted enhancers are supported by some combination of DHS and/or binding of p300 and/or MED1 (Figure 2D). Further, the predicted enhancers seem to be distinct from other distal regulatory elements. Only 2666 (8.0%) enhancers are found near a collection of 23267 TSS-distal CTCF sites called in HeLa, IMR90, and CD4 T cells^{3,25,26} (1.53-fold enrichment, $p = 7.81e-120$). Comparison to a genome-wide binding profile of the repressor NRSF/REST²⁷ (which binds

mainly transcriptional silencer elements) revealed that only 39 (0.11%) predicted enhancers overlap with distal NRSF/REST binding sites, significantly lower than that expected at random (3.23-fold depletion, $p = 3.21 \times 10^{-12}$). These findings indicate that our map of predicted enhancers is strongly enriched for true enhancer elements.

S2.11 Identification of novel sequence motifs in enhancers

We examined the predicted HeLa genome-wide enhancers for the presence of DNA sequence elements that may guide the establishment and maintenance of chromatin structure or the recruitment of regulatory factors. We reasoned that if such functional motifs are abundant within enhancers, they could show increased evolutionary conservation across related mammals. Indeed, we found increased conservation of motif-like sequence patterns in enhancer regions, evaluated using several hundred shuffled TRANSFAC motifs across 10 mammals in a phylogenetic framework that tolerates motif movement, partial motif loss, and sequencing or alignment discrepancies. Enhancers showed conservation for 4.3% of instances (at Branch-Length-Score > 50%), which is substantially greater than for the remaining intergenic regions (2.9%, $p < 1 \times 10^{-100}$), and even promoter regions (3.9%, $p = 1 \times 10^{-57}$). This suggests that these predicted enhancer regions may indeed contain functional regulatory motifs.

Consequently, we asked whether motifs for known transcriptional regulators show increased abundance and conservation in enhancer regions. We tested a list of 123 unique TRANSFAC motifs as reported previously¹⁰ and found that 67 (54%) of these motifs are over-conserved in enhancers, and 39 (32%) are enriched in enhancers (Table S12). This suggests that many known motifs for well-studied transcriptional regulators at promoters are likely to also play roles in

enhancers, implying strongly shared regulatory mechanisms between these two classes of elements at the DNA sequence level. Indeed, of the 67 known motifs over-conserved in enhancers and the 65 over-conserved in promoters, 54 (83%) are over-conserved in both. The enriched motifs include known sequence motifs for binding of transcription factors involved in diverse cellular processes.

Additionally, we searched for evidence of unique enhancer-specific sequence motifs that have previously remained elusive due to the lack of genome-wide knowledge of enhancers. We performed de novo motif discovery in enhancer regions using multiple alignments of ten mammalian genomes, revealing 41 enhancer motifs, of which 19 match known transcription factor motifs while 22 are novel (Table S13). These motifs show conservation rates between 7% and 22% in enhancers (median 9.3%), compared to only 1.1% for control shuffled motifs of identical composition. Even without taking conservation into account, 27 (65%) of these motifs show significant enrichment in human enhancers. Further, over 90% of these motifs appear to be unique to enhancers, as only 4 motifs are enriched in promoter regions and 12 are in fact depleted in promoters (Table S13). In contrast, shuffled versions of these motifs show significantly reduced enrichment in enhancer regions (only 12% of shuffled motifs, a 5-fold reduction) and also reduced depletion in promoters (22%, a 2-fold increase). This indicates that although enhancer regions contain many known promoter motifs, they also contain unique regulatory sequences that may be specific to enhancer function.

S2.12 CTCF knockdown analysis

Most of the predicted enhancers (92%) are located greater than 10 kb from the nearest transcription start site (TSS), posing a challenge in assigned enhancers to their appropriate target genes. We partly resolved the enhancer/target gene relationship by using genome-wide location data for the insulator binding protein CTCF^{3,25,26}. To determine if CTCF binding sites can be used to define the boundaries of regulatory domains within which enhancers and gene promoters may interact, we examined the effects of the loss of CTCF on global gene expression. A recent study showed that siRNA-mediated CTCF depletion in HeLa cells resulted in upregulation of 1062 genes and downregulation of 1130 genes (at least 50% change)²⁵. We hypothesized that upregulation of these genes was caused by increased interactions of their promoters with nearby enhancers that had been blocked by CTCF prior to its depletion (Figure S14, upper panel), in line with the current understanding of CTCF function. If so, we expect to find more predicted enhancers in the vicinity of upregulated genes and fewer enhancers near genes whose expression is unchanged or downregulated. To test this hypothesis, first we identified insulator-delineated domains in the genome, defining our set of insulators as the union of published CTCF binding sites from IMR90, HeLa, and CD4+ T cells^{3,25,26}, since we observed consistent CTCF enrichment at nearly all putative insulators across cell types in the ENCODE regions and genome-wide (Figure S1E, Figure S14). We then created three sets of genes: the 1000 most up-regulated upon siCTCF treatment, the 1000 most down-regulated, and the median 1000 unchanged genes. Then we counted predicted enhancers within five insulator-delineated domains (between CTCF binding sites) adjacent to subsets of genes that were upregulated, downregulated, or unchanged by depletion of CTCF in HeLa cells. To generate a random distribution, we also repeated this analysis for 100 sets of 1000 random genes. To obtain enhancer enrichment, we divided the observed counts with the averaged random counts. Finally,

to assess significance, we assumed the random counts followed a normal distribution. Indeed, we observed on average a 2.2-fold enrichment of enhancers within domains adjacent to upregulated genes compared to downregulated genes (Figure S14), and a 1.4-fold depletion of enhancers in domains adjacent to downregulated genes relative to genes whose expression is unchanged by CTCF depletion (see Supplemental Materials). These results support the use of CTCF binding sites as boundaries of regulatory domains on a global scale.

S2.13 Enhancers are clustered

To obtain a coarse view of the localization pattern of enhancers, we first examined the distribution of distances between adjacent enhancers. We observed that enhancers are more highly clustered than expected at random (Wilcoxon $p < 1E-300$) (Figure 2E), as has also been observed in *Drosophila*²⁸. Similar analysis from multiple cell types in the ENCODE regions supports this conclusion (Wilcoxon $p = 1.1E-27$) (Figure S12A). In comparison, we observed an enrichment of small TSS-TSS distances, indicative of clustering of TSSs (Wilcoxon $p = 0$, Matlab) (Figure S11A), which is also consistent with previous studies. However, the same cannot be said of CTCF-CTCF distances, which appear indistinguishable from what is expected by a random placement of sites (Wilcoxon $p = 0.1268$) (Figure S12B).

S2.14 Predicted enhancers in the ENCODE regions are enriched near cell-type specific genes

To expand our investigation across additional cell lines, we focused on differentially expressed genes between pairs of cell lines in the ENCODE regions. We counted the number of enhancers near the differentially expressed genes in the neighboring domains defined by CTCF sites. We

found that enhancers are enriched near differentially expressed genes as compared to the same genes that are differentially repressed in another cell type, and this enrichment is largely confined within CTCF binding sites that directly flank the gene's TSS (Figure S13B). On average within this block, there are 0.82 enhancers per differentially down-regulated gene, while there are 1.83 enhancers per differentially up-regulated gene (Figure S13C). This 2.2-fold difference suggests that cell-type specific expression is influenced by enhancers and that the action of enhancers is distance-dependent and favoring proximal promoters. When we focused only on the enhancer closest to the differentially expressed gene rather than all enhancers within a CTCF block, we find smaller difference between the distributions of enhancers in up- and down-regulated genes (Figure S13D). The smaller 1.76-fold difference observed here further emphasizes that multiple enhancers, and not just the single closest enhancer, are likely required to regulate differential gene expression of a single promoter.

S2.15 Histone modification-based prediction of promoters on a genome-scale

Using the genome-wide ChIP-chip enrichment profiles of H3K4me1 and H3K4me3, we used our histone modification-based prediction method to make 13116 promoter predictions (Figure S10A). We found that 9835 (75%) predicted promoters overlap with 5'-ends of UCSC Known Genes²⁹ (Figure S10B). We also compared the promoter predictions to the RIKEN human CAGE data set³⁰ and observed that 11001 (83.9%) overlap with multiple CAGE tags. Further, our prediction model correctly located 76% of active RefSeq transcription start sites (TSS) (Figure S10C) and even 31.5% of inactive TSS, consistent with recent studies demonstrating the presence of similar chromatin landmarks at most promoters in the human genome³¹. We also examined the overlap of predicted promoters with CpG islands (as annotated at the UCSC

genome browser³²), sequence elements conventionally understood to be associated with many promoters¹³. The vast majority of promoter predictions (11186, 85.1%) overlap CpG islands, representing almost half (43.3%) of the genome's CpG islands (Figure S9D). These findings agree with our previous genome-wide promoter analysis² and are comparable to the specificity and sensitivity of our prediction model in the ENCODE regions¹³.

S2.16 P-values

The p-values for correlations were obtained from using the Matlab corr function. This p-value measures the probability that there is no correlation between the two variables, against the alternative that the correlation is non-zero. The p-values for Wilcoxon rank sum tests were obtained from the Matlab ranksum function.

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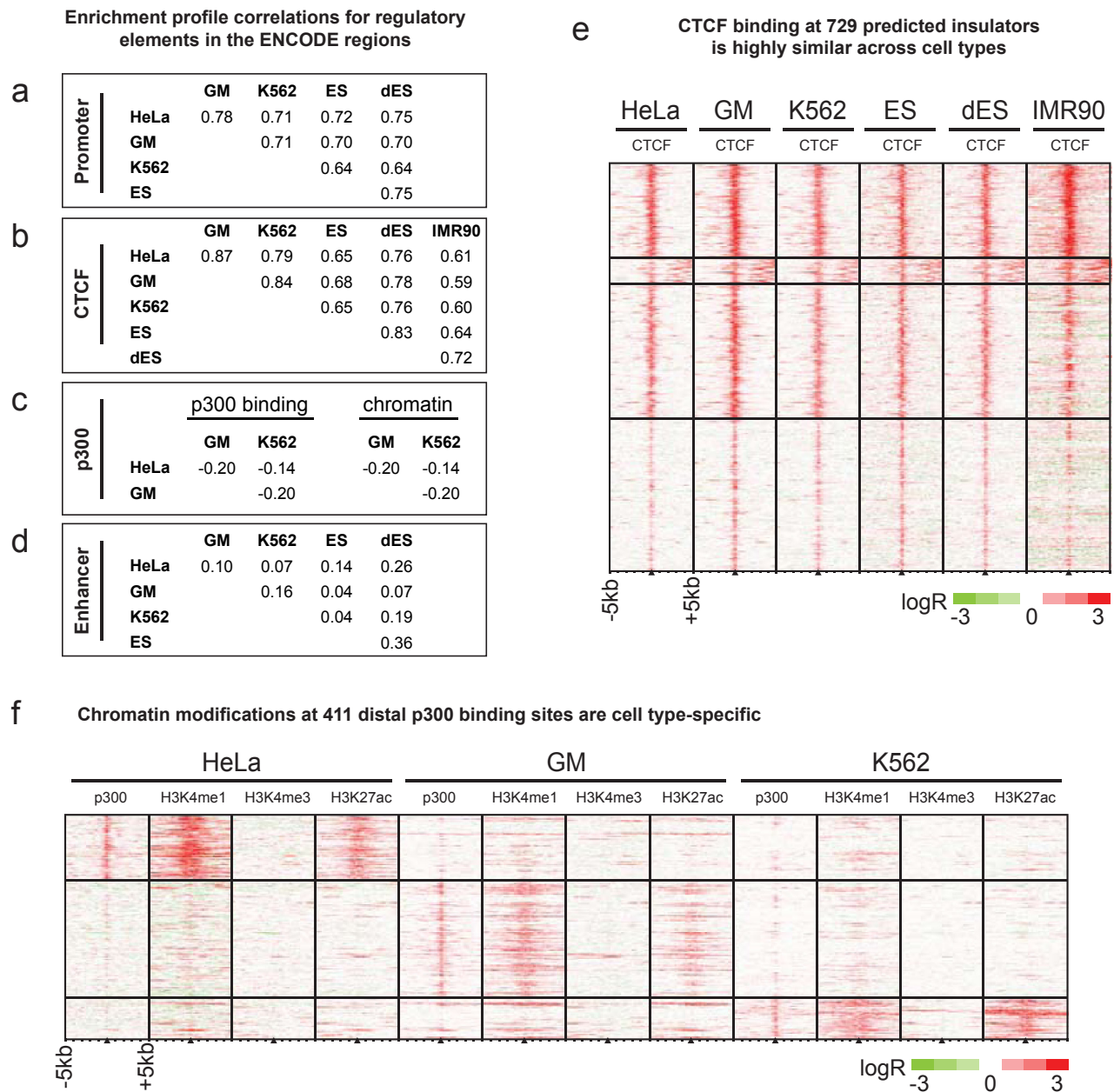


Figure S1: Quantitative comparison of regulatory elements in the ENCODE regions

Pearson correlation coefficients for ChIP-chip enrichment profiles (see Supplementary

Information) calculated between cell types for (A) chromatin modifications at promoters, (B)

CTCF binding at putative insulators, (C) p300 binding and chromatin modifications at putative

enhancers, and (D) chromatin modifications at predicted enhancers. (E) We performed k-means

clustering on CTCF enrichment at 729 non-redundant CTCF binding sites found by Mpeak. For

comparison we have also shown the enrichment patterns from a genome-wide study in IMR90

cells³, which supports the cell type-invariant nature of CTCF binding. (F) We clustered the

chromatin modifications at 411 non-redundant p300 binding sites in HeLa, GM, and K562 cells.

Enrichment of p300 binding, which was not a criteria in the clustering, confirms the cell type-

specificity of the chromatin marks at enhancers.

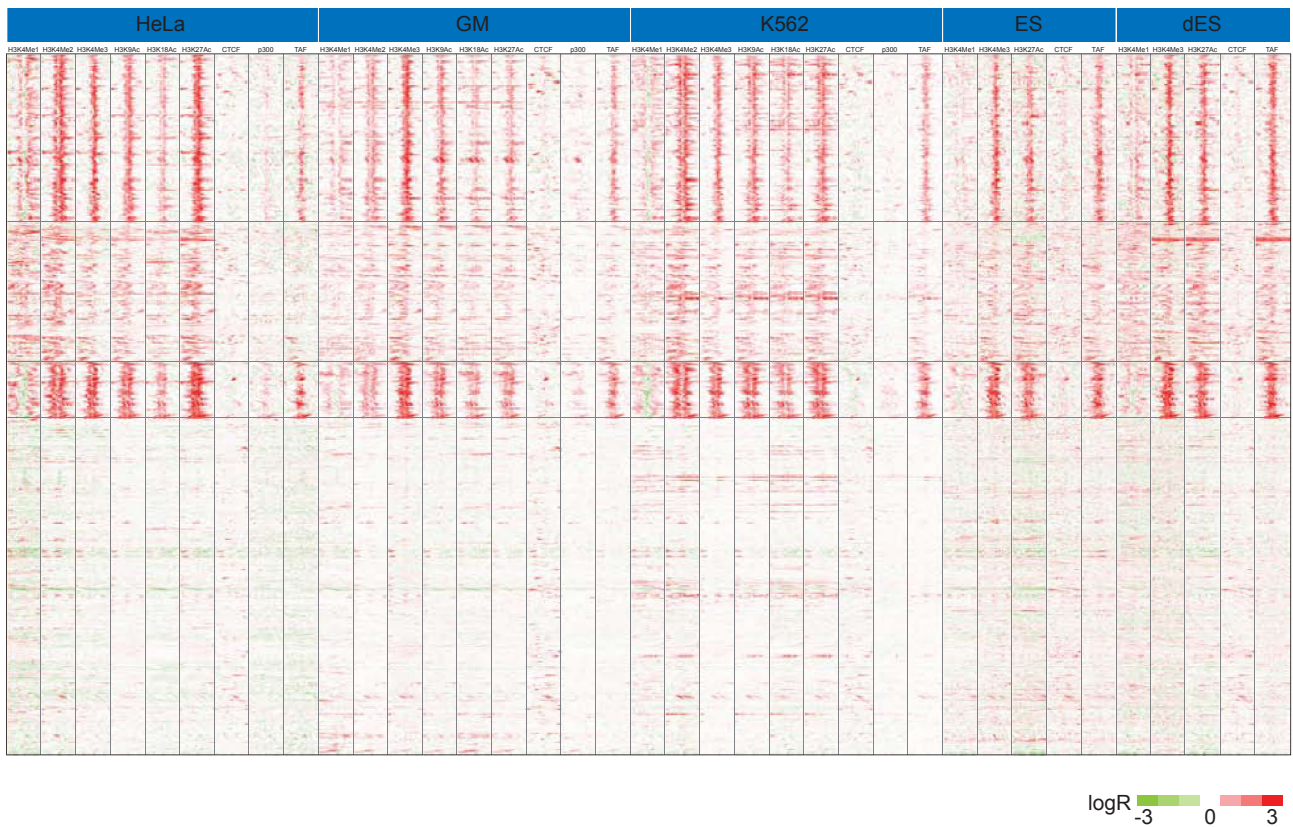


Figure S2: ChIP-chip enrichment profiles at Gencode promoters in the ENCODE regions

ChIP-chip enrichment data for all marks in all cell types are displayed as 10 kb windows centered on the TSS of all Gencode genes in the ENCODE regions.

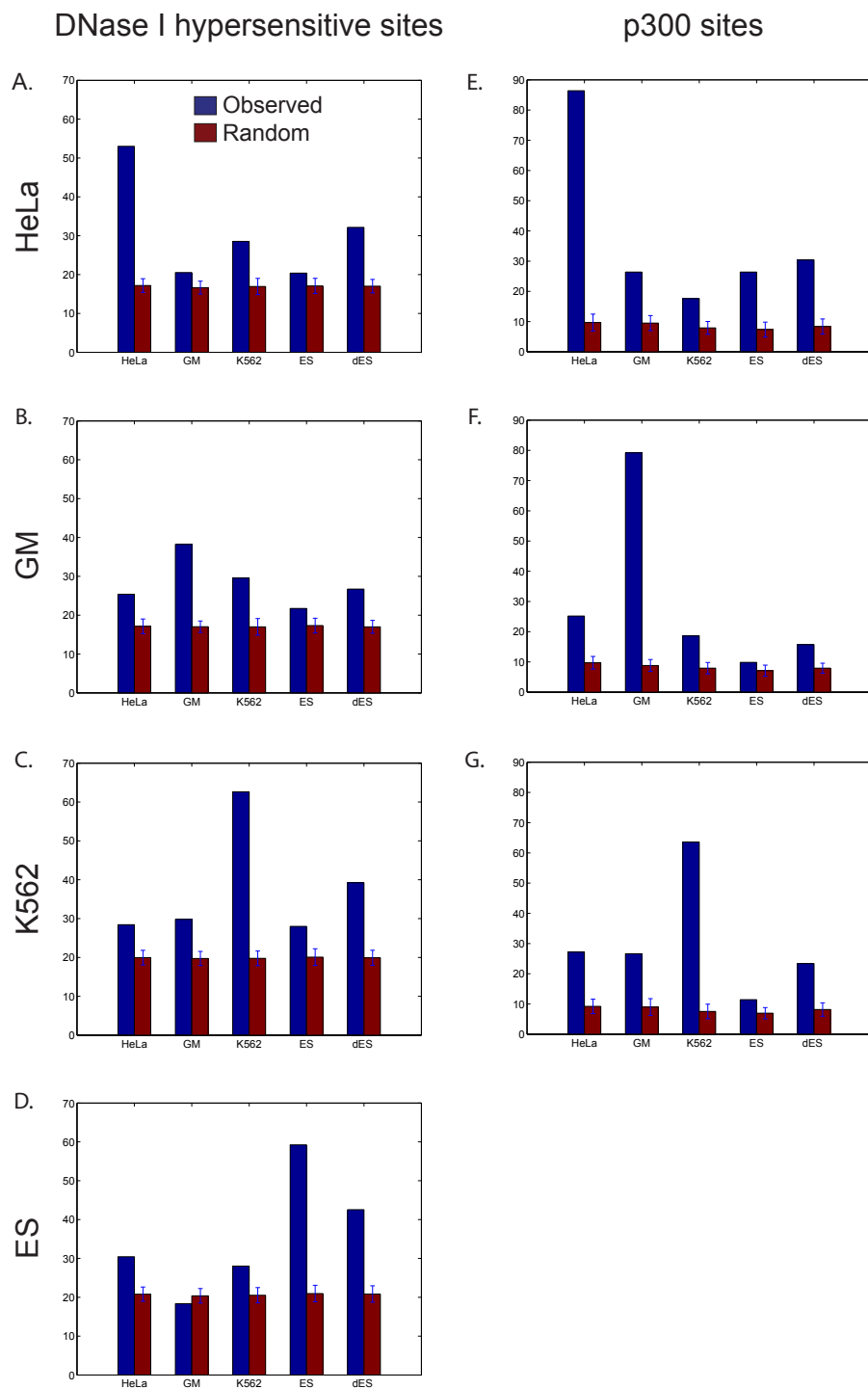


Figure S3: Verification of histone modification-based prediction of enhancers in the ENCODE regions

(A-D) The percentage of predicted enhancers within 2.5 kb of hypersensitive sites in HeLa, GM, K562, and ES cells as previously defined²³. (E-G) The percentage of p300 sites mapped in HeLa, GM, and K562 cell lines within 2.5 kb of predicted enhancers (see Supplementary Information).

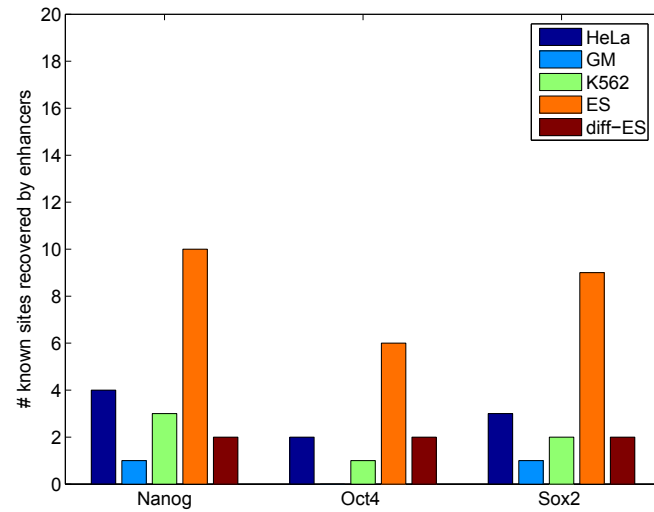
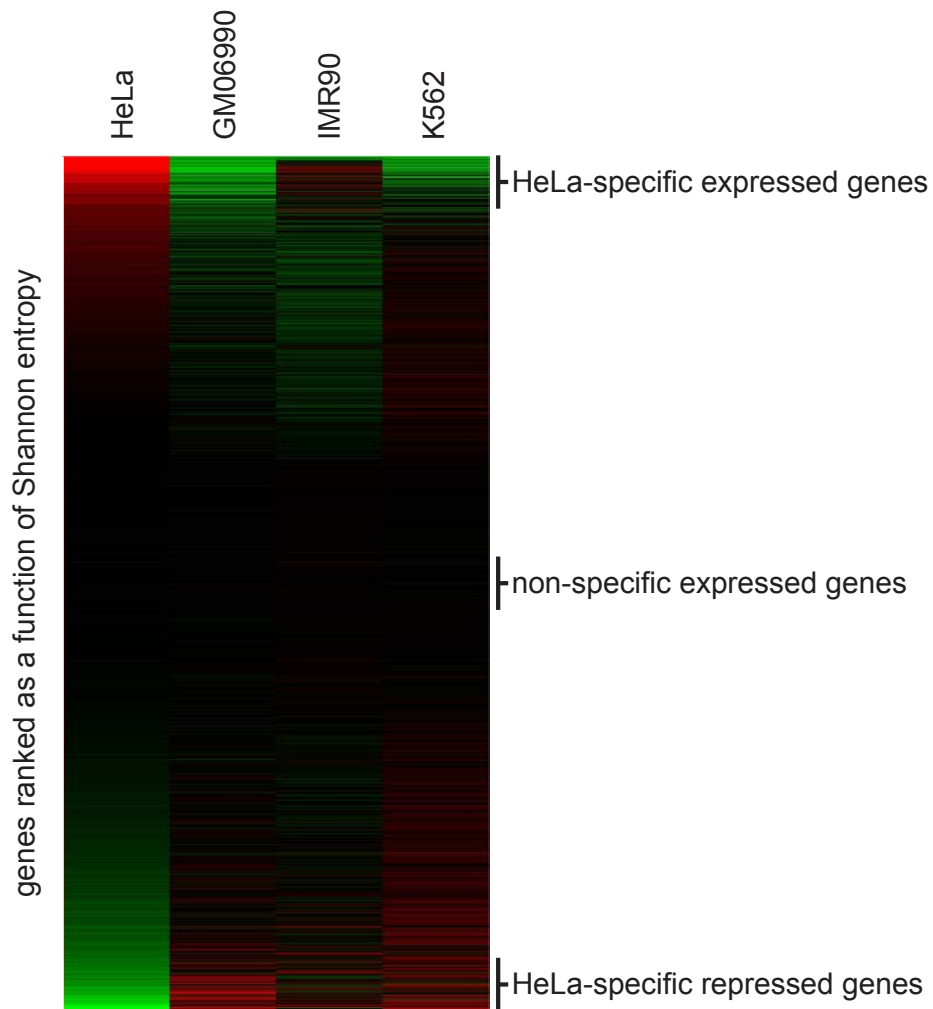


Figure S4: Predicted ES enhancers are enriched in known ES-specific transcription factors

Known NANOG, OCT4, and SOX2 binding sites at predicted enhancers in the ENCODE regions (see Supplementary Information).

FigureS5

**Figure S5: Comparison of cell type-specific gene expression in four cell types**

We used Shannon entropy (see Supplementary Information) to rank genes by the specificity of their expression levels in HeLa as compared to three other cell lines (K562, GM06990, and IMR90 cells, representing leukemia, lymphoblast, and fibroblast lineages, respectively). The most HeLa-specific expressed genes are found at the top of the cluster (represented in red in Figure 3B), while genes that are specifically repressed in HeLa cells are found at the bottom (green in Figure 3B). Genes in the middle portion of the cluster (black in Figure 3B) have expression levels that are similar in all four cell lines.

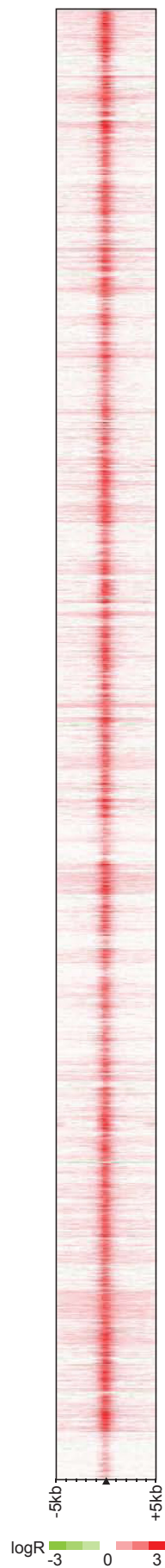


Figure S6: CTCF enrichment at genome-wide putative insulators in IMR90 cells

Experimentally-determined binding sites published for CTCF in IMR90, HeLa, and CD4+ T cells^{3,25,26} were combined into one set of binding sites, and the ChIP-chip enrichment data at all of these sites from IMR90 cells are visualized as 10 kb windows centered at the CTCF binding sites as described above, organized by genomic position as in Figure 2A. These data support the consistency of CTCF binding across cell types.

Functional activity of predicted enhancers in HeLa cells

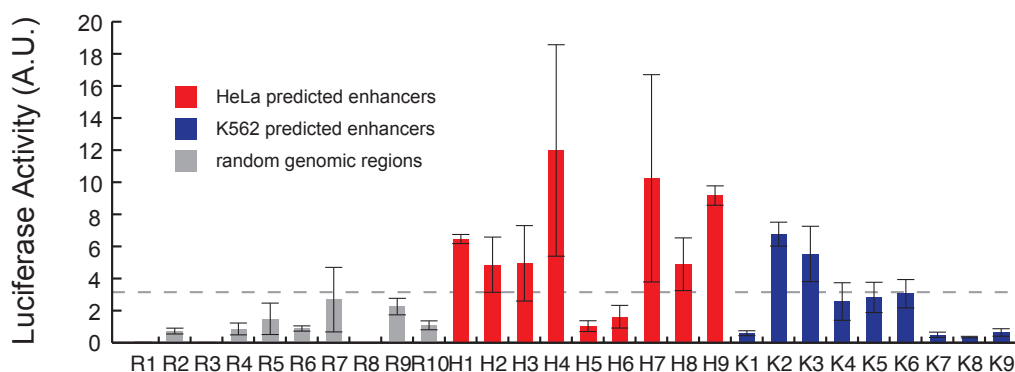


Figure S7: Cell type-specificity of predicted enhancer activity in reporter assays

In addition to the HeLa-specific enhancers and random regions assayed in Figure 2F, additional K562-specific enhancers were cloned and assayed for reporter activity in HeLa cells. Enhancers predicted specifically in K562 cells (blue bars) were much less likely to be active in HeLa cells than the HeLa-specific enhancers (red), and the median activity is not significantly different from random regions (gray). The dashed line represents a significance threshold of $p = 0.01$ as in Figure 2F.

Figure S8: ENCODE Replicates

	HeLa	GM	K562	ES	dES
H3K9Ac	3	3	3		
H3K18Ac	3	3	3		
H3K27Ac	3	3	3	1	1
H3K4Me1	3	3	3	1	1
H3K4Me2	3	3	3		
H3K4Me3	3	3	3	1	1
p300	3	3	3		
TAF	3	3	3	1	1
CTCF	3	3	3	1	1
Affy expression	1	2	2		
Relative expression				1	1

Figure S8: Summary of ChIP-chip and expression experiments.

The number of biological replicates for each cell-type and antibody pair.

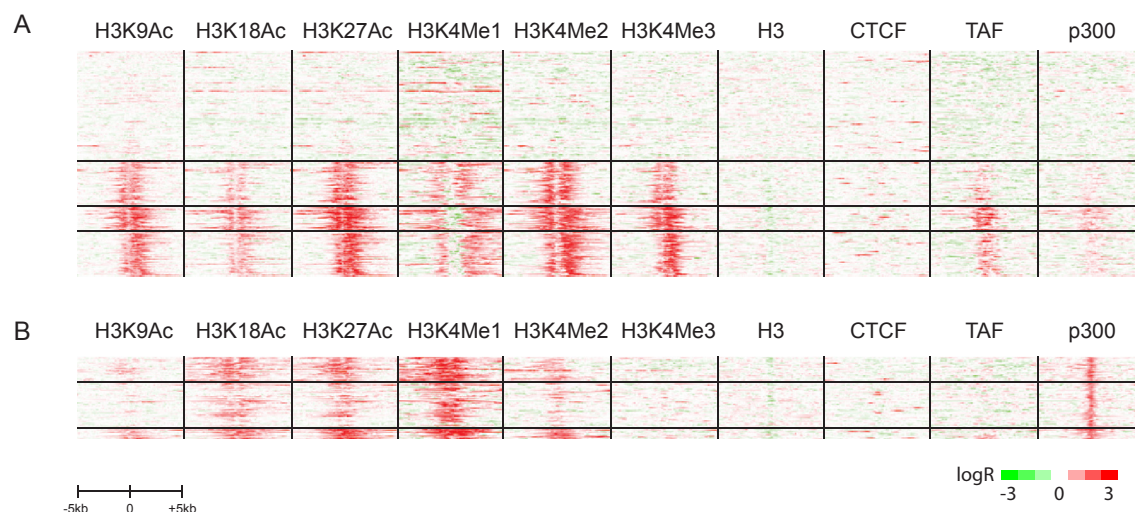


Figure S9: Chromatin acetylation features at promoters and enhancers in the ENCODE regions.

ChIP-chip was performed on the acetylated histones H3K9Ac, H3K18Ac, and H3K27Ac, and the enrichment was compared to the (A) promoter and (B) p300 clusters from Heintzman et al in HeLa cells¹³. Each horizontal line details the ChIP-chip enrichment of various chromatin modifications and transcription factors in 10 kb windows. For consistency in comparison, we clustered the data in the same order as Heintzman et al.¹³, which used k-means clustering. All three active promoter clusters P2, P3, and P4 are highly enriched in all three acetylated histones, whereas the enhancer clusters are mostly enriched in H3K18Ac and H3K27Ac, but have only weak H3K9Ac enrichment. Average profiles of log enrichment ratios for promoters or p300 binding sites in each cluster are shown at the bottom of each panel.

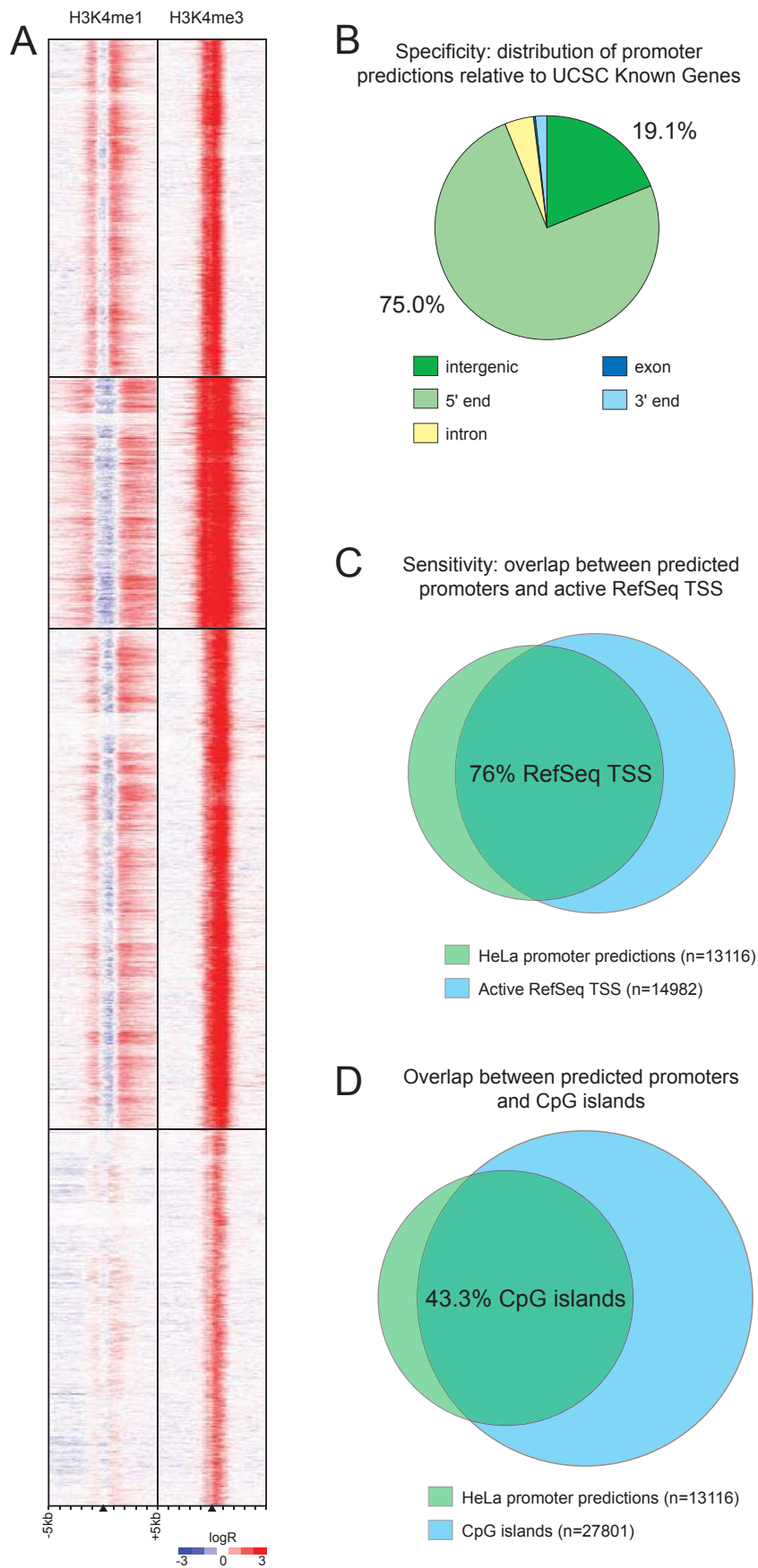


Figure S10: Active promoter predictions in the human genome.

(A) We predict 13116 active promoters in HeLa cells based on chromatin signatures for

H3K4me1 and H3K4me3 as determined by ChIP-chip using genome-wide tiling microarrays.

(B) 75% of promoter predictions map to 5' ends of UCSC Known Genes, indicating a high degree of specificity. (C) 76% of active promoters (defined as RefSeq TSS for expressed transcripts) are correctly predicted, indicating a high degree of sensitivity. (D) 85.1% of promoter predictions overlap with CpG islands (defined by UCSC Genome Browser), accounting for close to half of the CpG islands in the genome.

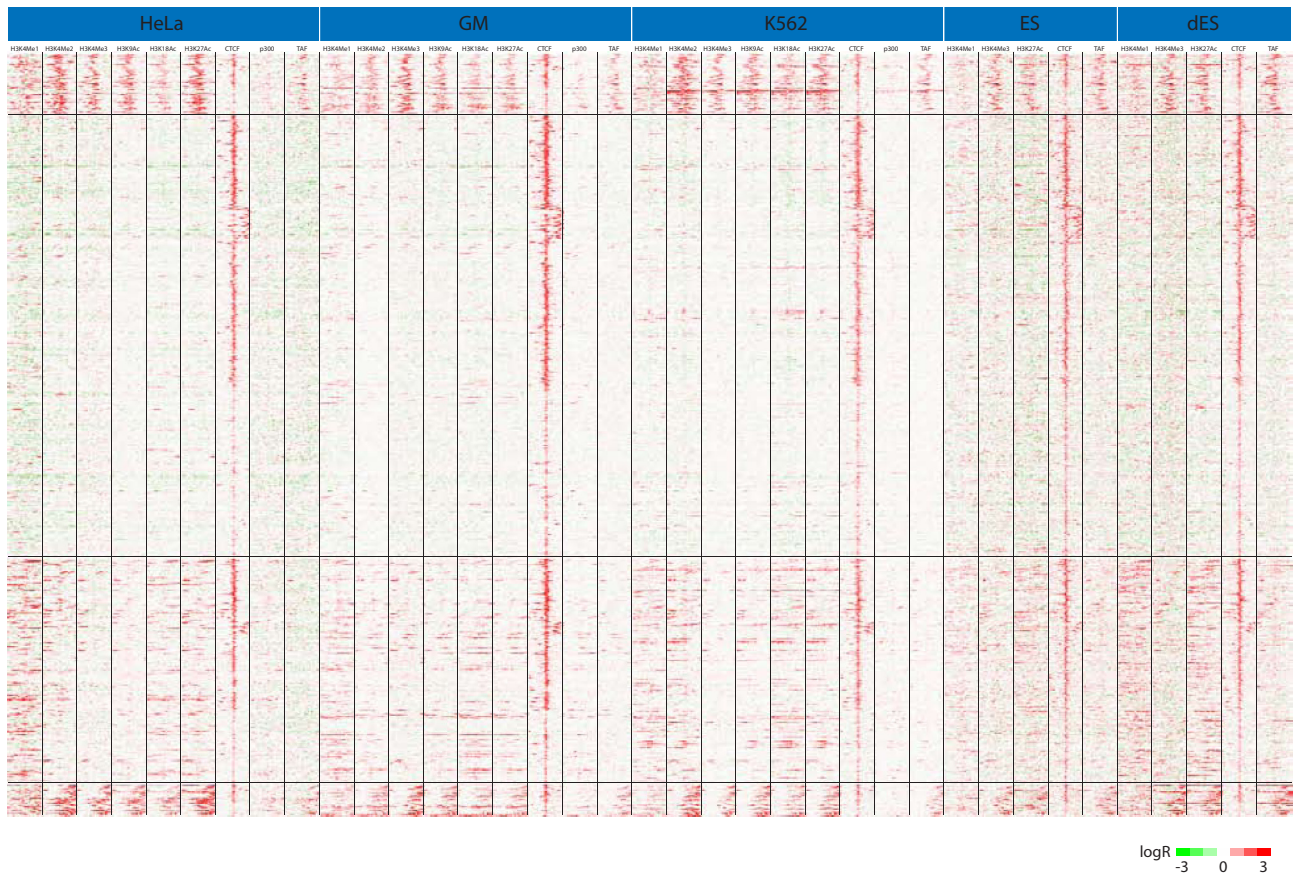


Figure S11: Chromatin features at CTCF binding sites.

Heat-map of the chromatin (H3K4me1, H3K4me2, H3K4me3, H3K9ac, H3K18ac, H3K27ac) and transcriptional (p300, TAF1, CTCF) features within a 10-kb window of 729 consensus CTCF binding sites obtained from merging CTCF sites called for the five cell types in the ENCODE regions. This heat-map is the result of performing k-means clustering ($k=4$).

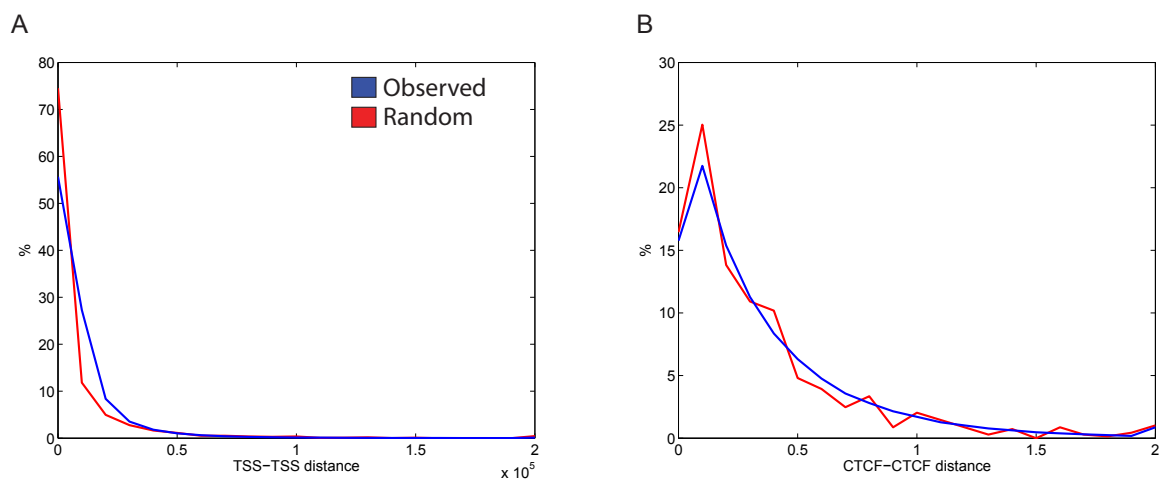


Figure S12: Distribution of promoters and insulators in the ENCODE regions.

(A) The distribution of adjacent TSS-TSS distances (red) for Gencode TSSs, as compared to a random placement of sites (blue). (B) The distribution of adjacent CTCF-CTCF distances (red), as compared to a random placement of sites (blue).

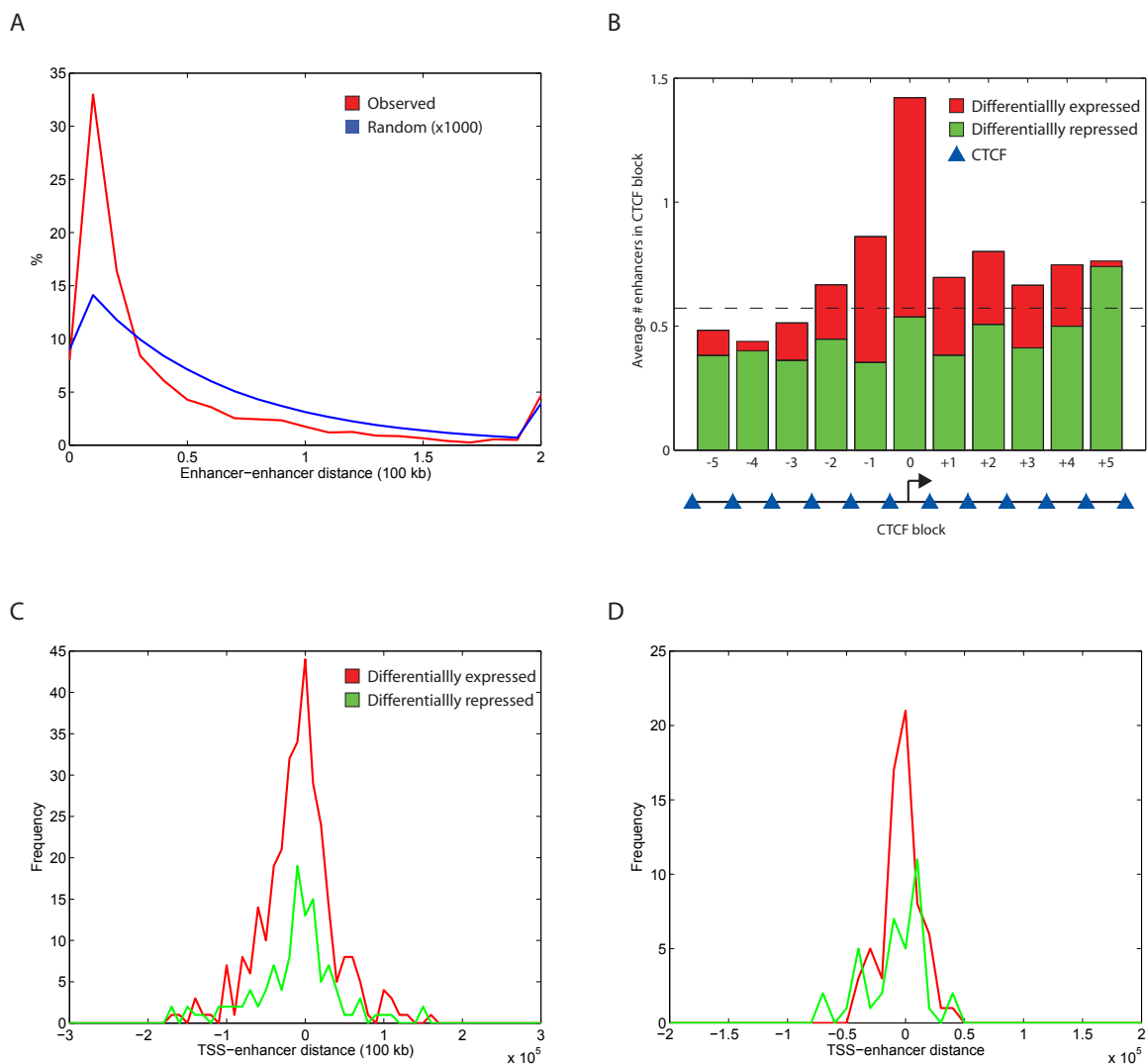
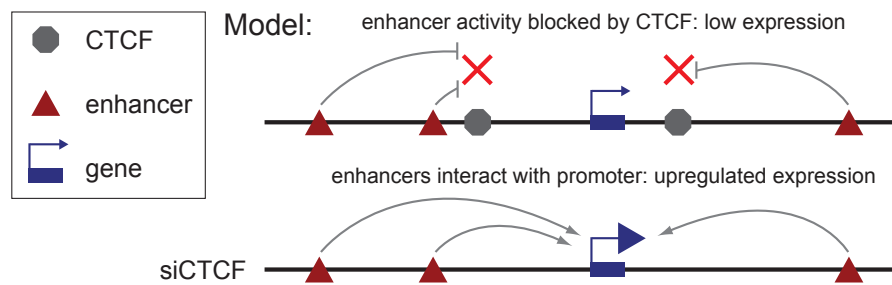


Figure S13: ENCODE Enhancers are clustered at differentially expressed genes.

(A) To show that enhancers are clustered, we compute the distance between adjacent enhancers and examine the distribution of these distances. The distribution of adjacent enhancer-enhancer distances (red), as compared to 1000 sets of randomly placed sites (blue), indicates that enhancers are highly clustered. (B) A CTCF block is defined by flanking CTCF binding sites. Using the 729 consensus CTCF binding sites to define CTCF blocks, we count the average number of enhancers found in blocks relative to the TSSs of differentially expressed and repressed genes. For a given TSS, CTCF block 0 is defined by the CTCF binding sites immediately flanking the TSS, CTCF block -1 is the block immediately upstream of CTCF block 0, CTCF block +1 is the block immediately downstream of CTCF block 0, etc. Differentially expressed genes are enriched in enhancers when compared to differentially repressed genes, with the strongest enrichment found in CTCF block 0. The dotted line indicates the expected average

number of enhancers in a CTCF block. For HeLa, GM, and K562, differential expression is defined by an RMA p-value cutoff of 0.01 and a fold change cutoff of 2.0. (C) A detailed view of the distribution of enhancers in CTCF block 0. Here, we show the distribution of enhancer-TSS distances all enhancers within this CTCF block. Negative distances indicate upstream enhancers, while positive distances indicate downstream enhancers. Enhancers are more concentrated to differentially expressed genes relative to differentially repressed genes. (D) Rather than examining the distribution of all enhancer-TSS distances in a differentially expressed/repressed gene's CTCF block, we examined only the closest one here. While we do observe enrichment in differentially expressed genes, the effect is smaller than that observed when we consider all enhancer-TSS distances.

CTCF depletion by siRNA permits increased interactions between promoters and predicted enhancers



Predicted enhancers are enriched near upregulated genes after depletion of CTCF in HeLa cells

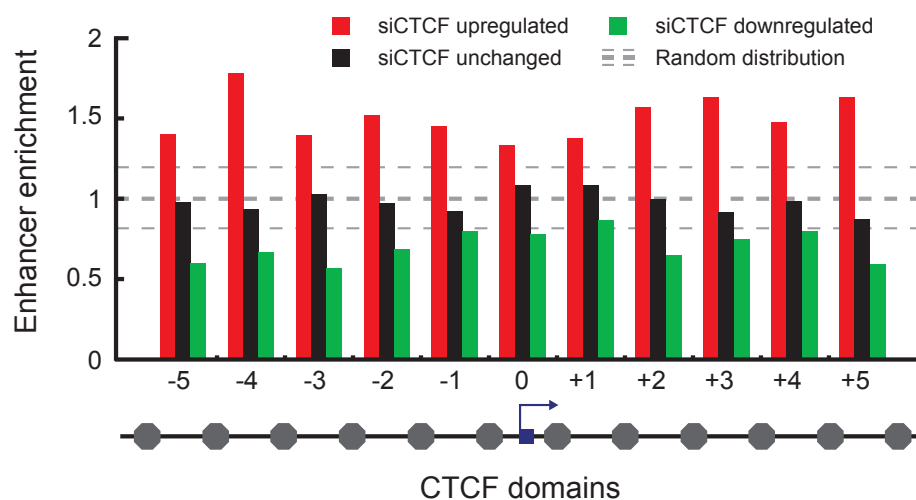


Figure S14: CTCF sites may serve as domain boundaries for promoter-enhancer interactions

Insulators bound by CTCF are thought to block promoter-enhancer interactions that would otherwise occur in the absence of CTCF (upper panel), a model supported by the enrichment of predicted enhancers in domains adjacent to genes that are upregulated in response to CTCF-depletion by siRNA (lower panel, red bars). Enhancers are depleted in domains adjacent to downregulated genes (green bars) relative to unchanged genes (black bars) and a random distribution (gray lines). Gene expression data are from a recently published study²⁵. Domains are defined as the regions between CTCF sites as recently reported^{3,25,26}; enhancers were counted in the five domains adjacent to each gene, upstream and downstream, and summed across respective domains to calculate enrichment relative to a random distribution.

TABLE CAPTIONS**Table S1: CTCF binding sites in five cell types in the ENCODE regions**

Coordinates are listed in hg17 for 729 non-redundant CTCF binding sites identified in HeLa, GM, K562, ES, and dES cells (see Supplementary Information).

Table S2: p300 binding sites in HeLa cells in the ENCODE regions

Coordinates are listed in hg17 for p300 binding sites identified in HeLa cells (see Supplementary Information).

Table S3: p300 binding sites in GM cells in the ENCODE regions

Coordinates are listed in hg17 for p300 binding sites identified in GM cells (see Supplementary Information).

Table S4: p300 binding sites in K562 cells in the ENCODE regions

Coordinates are listed in hg17 for p300 binding sites identified in K562 cells (see Supplementary Information).

Table S5: Predicted enhancers in HeLa cells in the ENCODE regions

Coordinates are listed in hg17 for enhancers predicted in HeLa cells based on chromatin signatures (see Supplementary Information).

Table S6: Predicted enhancers in GM cells in the ENCODE regions

Coordinates are listed in hg17 for enhancers predicted in GM cells based on chromatin signatures (see Supplementary Information).

Table S7: Predicted enhancers in K562 cells in the ENCODE regions

Coordinates are listed in hg17 for enhancers predicted in K562 cells based on chromatin signatures (see Supplementary Information).

Table S8: Predicted enhancers in ES cells in the ENCODE regions

Coordinates are listed in hg17 for enhancers predicted in ES cells based on chromatin signatures (see Supplementary Information).

Table S9: Predicted enhancers in dES cells in the ENCODE regions

Coordinates are listed in hg17 for enhancers predicted in dES cells based on chromatin signatures (see Supplementary Information).

Table S10: Genome-wide predicted enhancers in HeLa cells

Coordinates are listed in hg17 for 36589 enhancers predicted in HeLa cells based on chromatin signatures (see Supplementary Information).

Table S11: Clone information for reporter assays

Coordinates (hg17) and primers used to amplify regions containing predicted enhancers in HeLa (H1-H9) and K562 (K1-K9) cells for cloning and reporter assays, as well as random regions selected as controls (R1-R10).

Table S12: Known motifs in predicted enhancers

Enrichment of motifs in enhancers was analyzed as described^{11,12}. Over-conservation and Enrichment are calculated as the excess conservation and overabundance, respectively, of a motif in enhancers or promoters relative to that expected for a random motif of identical composition. All significance values are expressed as *Z*-scores, corresponding to the number of standard deviations away from the mean of a normal distribution.

Table S13: De novo motifs enriched in predicted enhancer regions

Known Match score represents the shared information content between novel and known motif^{1,2}. Over-conservation is calculated as the excess conservation of a motif in enhancers or promoters relative to that expected for a random motif of identical composition. Enrichment is calculated as the over-abundance of a motif in enhancers or promoters relative to that expected for a random motif of identical composition. Enhancer-specific motifs are those lacking significant promoter enrichment. All significance values are expressed as Z-scores, corresponding to the number of standard deviations away from the mean of a normal distribution.

Table S14: Genome-wide predicted enhancers in K562 cells

Coordinates are listed in hg17 for 24566 enhancers predicted in K562 cells based on chromatin signatures (see Supplementary Information).

Table S15: Overlap of predicted enhancers in HeLa with transcription factor binding sites in other cell types

Coordinates are listed in hg17 for each HeLa predicted enhancer with notation of overlap with experimentally determined transcription factor binding sites (see Supplementary Information).

Table S16: STAT1 binding sites in the genome of IFN γ -treated HeLa cells

Coordinates are listed in hg17 for 1969 STAT binding sites as determined by ChIP-chip.

Table S1. Combined list of CTCF sites recovered from all five cell types. Coordinates are in hg17.

ENCODE region	Position	Chr
ENm001	115407271	chr7
ENm001	115414358	chr7
ENm001	115460547	chr7
ENm001	115469971	chr7
ENm001	115484183	chr7
ENm001	115493018	chr7
ENm001	115575725	chr7
ENm001	115760158	chr7
ENm001	115817880	chr7
ENm001	115822972	chr7
ENm001	115825043	chr7
ENm001	115825974	chr7
ENm001	115832244	chr7
ENm001	115833004	chr7
ENm001	115867869	chr7
ENm001	115905698	chr7
ENm001	116020610	chr7
ENm001	116036912	chr7
ENm001	116043904	chr7
ENm001	116164763	chr7
ENm001	116218951	chr7
ENm001	116222561	chr7
ENm001	116247109	chr7
ENm001	116353680	chr7
ENm001	116361983	chr7
ENm001	116391889	chr7
ENm001	116447464	chr7
ENm001	116546967	chr7
ENm001	116619851	chr7
ENm001	116634424	chr7
ENm001	116693229	chr7
ENm001	116715174	chr7
ENm001	116739589	chr7
ENm001	116903958	chr7
ENm001	116908081	chr7
ENm001	116950223	chr7
ENm001	116985240	chr7
ENm001	117015868	chr7
ENm001	117025957	chr7

ENm001	117063672	chr7
ENm001	117227452	chr7
ENm002	131366628	chr5
ENm002	131371910	chr5
ENm002	131426725	chr5
ENm002	131428663	chr5
ENm002	131540782	chr5
ENm002	131543860	chr5
ENm002	131601867	chr5
ENm002	131636561	chr5
ENm002	131679539	chr5
ENm002	131749212	chr5
ENm002	131750751	chr5
ENm002	131783317	chr5
ENm002	131790670	chr5
ENm002	131828233	chr5
ENm002	131864979	chr5
ENm002	131890534	chr5
ENm002	131954887	chr5
ENm002	132041223	chr5
ENm002	132078729	chr5
ENm002	132110858	chr5
ENm002	132117280	chr5
ENm002	132118743	chr5
ENm002	132139928	chr5
ENm002	132149048	chr5
ENm002	132165977	chr5
ENm002	132171164	chr5
ENm002	132184160	chr5
ENm002	132193508	chr5
ENm002	132229931	chr5
ENm002	132246993	chr5
ENm003	115981740	chr11
ENm003	115991316	chr11
ENm003	116017802	chr11
ENm003	116067962	chr11
ENm003	116091332	chr11
ENm003	116108679	chr11
ENm003	116118901	chr11
ENm003	116167522	chr11
ENm003	116212134	chr11
ENm003	116216371	chr11
ENm003	116252775	chr11

ENm003	116256005	chr11
ENm003	116271471	chr11
ENm003	116303315	chr11
ENm004	30172556	chr22
ENm004	30216864	chr22
ENm004	30277455	chr22
ENm004	30282015	chr22
ENm004	30349978	chr22
ENm004	30354671	chr22
ENm004	30386211	chr22
ENm004	30470210	chr22
ENm004	30539731	chr22
ENm004	30567452	chr22
ENm004	30668000	chr22
ENm004	30672826	chr22
ENm004	30691142	chr22
ENm004	30698229	chr22
ENm004	30801114	chr22
ENm004	30801817	chr22
ENm004	30824427	chr22
ENm004	30848690	chr22
ENm004	30861686	chr22
ENm004	30896760	chr22
ENm004	30923208	chr22
ENm004	30927844	chr22
ENm004	30986782	chr22
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Table S2. p300 binding sites in HeLa. Coordinates are in hg17.

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ENm006	152688801	chrX
ENm006	153147920	chrX
ENm006	153334030	chrX

ENm006	153392496	chrX
ENm006	153522596	chrX
ENm006	153592064	chrX
ENm006	153856990	chrX
ENm007	59037366	chr19
ENm008	317326	chr16
ENm009	5251051	chr11
ENm010	26928234	chr7
ENm010	26949248	chr7
ENm012	113736717	chr7
ENm012	113764343	chr7
ENm012	113887805	chr7
ENm012	114053827	chr7
ENm012	114164369	chr7
ENm012	114169043	chr7
ENm012	114369949	chr7
ENm012	114463695	chr7
ENm012	114465177	chr7
ENm013	89620759	chr7
ENm013	89853243	chr7
ENm013	89869431	chr7
ENm014	126116795	chr7
ENm014	126171356	chr7
ENr111	29810695	chr13
ENr111	29813431	chr13
ENr111	29814647	chr13
ENr122	59459522	chr18
ENr122	59477154	chr18
ENr122	59499878	chr18
ENr122	59501512	chr18
ENr123	38666972	chr12
ENr123	38887828	chr12
ENr131	234390742	chr2
ENr131	234447604	chr2
ENr131	234665621	chr2
ENr132	112414204	chr13
ENr133	39244796	chr21
ENr133	39278768	chr21
ENr133	39279908	chr21
ENr133	39287508	chr21
ENr212	142234526	chr5
ENr212	142362890	chr5
ENr213	23853739	chr18

ENr213	23899719	chr18
ENr213	24038495	chr18
ENr221	55947564	chr5
ENr221	55957292	chr5
ENr221	56363854	chr5
ENr222	132301101	chr6
ENr222	132314363	chr6
ENr222	132317859	chr6
ENr222	132342901	chr6
ENr222	132486009	chr6
ENr222	132599287	chr6
ENr222	132697213	chr6
ENr223	74287664	chr6
ENr231	147983813	chr1
ENr231	147986055	chr1
ENr231	148280631	chr1
ENr231	148328435	chr1
ENr231	148332539	chr1
ENr231	148334553	chr1
ENr232	128981861	chr9
ENr232	129176041	chr9
ENr233	41904256	chr15
ENr311	52994981	chr14
ENr312	130797711	chr11
ENr312	130799003	chr11
ENr312	130853381	chr11
ENr321	118929328	chr8
ENr321	119018970	chr8
ENr321	119059820	chr8
ENr321	119090790	chr8
ENr321	119100138	chr8
ENr321	119107320	chr8
ENr321	119111272	chr8
ENr321	119160026	chr8
ENr321	119189020	chr8
ENr321	119190274	chr8
ENr323	108722998	chr6
ENr324	122821641	chrX
ENr331	220379440	chr2
ENr332	63944182	chr11
ENr333	33363284	chr20
ENr333	33793748	chr20
ENr334	41546634	chr6

Table S3. p300 binding sites in GM. Coordinates are in hg17.

ENCODE region	Position	Chr
ENm001	115446183	chr7
ENm001	115447969	chr7
ENm001	115551063	chr7
ENm001	115746117	chr7
ENm001	115811591	chr7
ENm001	115817861	chr7
ENm001	115864943	chr7
ENm001	115867793	chr7
ENm001	116188019	chr7
ENm001	116189957	chr7
ENm001	116220965	chr7
ENm001	116244791	chr7
ENm001	116245969	chr7
ENm001	116249845	chr7
ENm001	116257673	chr7
ENm001	116275305	chr7
ENm001	116344655	chr7
ENm001	116464621	chr7
ENm001	116475261	chr7
ENm001	116485483	chr7
ENm001	116658421	chr7
ENm001	116669669	chr7
ENm001	116822581	chr7
ENm001	116965765	chr7
ENm001	117007907	chr7
ENm001	117015013	chr7
ENm001	117274857	chr7
ENm002	131383823	chr5
ENm002	131462977	chr5
ENm002	131464497	chr5
ENm002	131467461	chr5
ENm002	131569909	chr5
ENm002	131632875	chr5
ENm002	131721909	chr5
ENm002	131777199	chr5
ENm002	131778605	chr5
ENm002	131784647	chr5
ENm002	131786623	chr5
ENm002	131789359	chr5

ENm002	131790765	chr5
ENm002	131801101	chr5
ENm002	131820063	chr5
ENm002	131822609	chr5
ENm002	131830399	chr5
ENm002	131889033	chr5
ENm002	131920573	chr5
ENm002	131987377	chr5
ENm002	131993343	chr5
ENm002	131999651	chr5
ENm002	132003679	chr5
ENm002	132023895	chr5
ENm002	132027885	chr5
ENm002	132038829	chr5
ENm002	132052699	chr5
ENm002	132115361	chr5
ENm003	116109325	chr11
ENm003	116229671	chr11
ENm003	116301909	chr11
ENm003	116304531	chr11
ENm003	116307799	chr11
ENm003	116398619	chr11
ENm003	116439089	chr11
ENm003	116453757	chr11
ENm004	30209207	chr22
ENm004	30211031	chr22
ENm004	30214793	chr22
ENm004	30216579	chr22
ENm004	30257999	chr22
ENm004	30267575	chr22
ENm004	30367249	chr22
ENm004	30372455	chr22
ENm004	30373899	chr22
ENm004	30382297	chr22
ENm004	30473953	chr22
ENm004	30510775	chr22
ENm004	31128085	chr22
ENm004	31137623	chr22
ENm004	31196523	chr22
ENm004	31351943	chr22
ENm005	33066236	chr21
ENm005	33498410	chr21
ENm005	33509962	chr21

ENm005	33525162	chr21
ENm005	33532762	chr21
ENm005	33534168	chr21
ENm005	33559932	chr21
ENm005	33560958	chr21
ENm005	33563162	chr21
ENm005	33603138	chr21
ENm005	33618376	chr21
ENm005	33619592	chr21
ENm005	33668574	chr21
ENm005	33677694	chr21
ENm005	33699468	chr21
ENm005	33729184	chr21
ENm005	33736670	chr21
ENm005	33836078	chr21
ENm005	33837370	chr21
ENm005	34215014	chr21
ENm005	34241804	chr21
ENm005	34242868	chr21
ENm005	34245984	chr21
ENm005	34270646	chr21
ENm005	34278322	chr21
ENm005	34314878	chr21
ENm005	34317348	chr21
ENm005	34341212	chr21
ENm006	152791115	chrX
ENm006	153092619	chrX
ENm006	153137954	chrX
ENm006	153147768	chrX
ENm006	153677980	chrX
ENm006	153819530	chrX
ENm007	59310928	chr19
ENm007	59396922	chr19
ENm007	59403952	chr19
ENm007	59485576	chr19
ENm007	59549644	chr19
ENm007	59574154	chr19
ENm007	59577346	chr19
ENm007	59581792	chr19
ENm007	59700922	chr19
ENm007	59721822	chr19
ENm007	59724406	chr19
ENm007	59735730	chr19

ENm007	59754768	chr19
ENm007	59810514	chr19
ENm007	59858052	chr19
ENm007	59864170	chr19
ENm008	16176	chr16
ENm008	177106	chr16
ENm008	225214	chr16
ENm008	230230	chr16
ENm009	5603007	chr11
ENm009	5662553	chr11
ENm009	5667493	chr11
ENm009	5668785	chr11
ENm011	1831041	chr11
ENm011	1832979	chr11
ENm012	113789005	chr7
ENm012	113864815	chr7
ENm012	114033915	chr7
ENm012	114067165	chr7
ENm012	114100149	chr7
ENm012	114151601	chr7
ENm012	114154375	chr7
ENm012	114156389	chr7
ENm012	114158099	chr7
ENm012	114160113	chr7
ENm012	114162469	chr7
ENm012	114165851	chr7
ENm012	114168207	chr7
ENm012	114243219	chr7
ENm013	89447213	chr7
ENm013	89454357	chr7
ENm013	89517057	chr7
ENm013	89620759	chr7
ENm013	89677759	chr7
ENm013	89699077	chr7
ENm013	89871217	chr7
ENm013	89879235	chr7
ENm013	89887215	chr7
ENm013	89891585	chr7
ENm013	89983469	chr7
ENm013	90000455	chr7
ENm013	90011247	chr7
ENm013	90050159	chr7
ENm013	90051299	chr7

ENm013	90090933	chr7
ENm013	90206491	chr7
ENm014	125681077	chr7
ENm014	125684413	chr7
ENm014	125893270	chr7
ENm014	125935302	chr7
ENm014	125936372	chr7
ENm014	126015197	chr7
ENm014	126648104	chr7
ENr111	29592613	chr13
ENr111	29613247	chr13
ENr111	29614615	chr13
ENr111	29618187	chr13
ENr111	29629929	chr13
ENr111	29664851	chr13
ENr111	29666067	chr13
ENr111	29676859	chr13
ENr111	29713871	chr13
ENr111	29785539	chr13
ENr111	29842463	chr13
ENr111	29844059	chr13
ENr111	29846035	chr13
ENr111	29848923	chr13
ENr111	29867809	chr13
ENr111	29894789	chr13
ENr111	29903377	chr13
ENr111	29913751	chr13
ENr111	29916221	chr13
ENr121	118288419	chr2
ENr122	59726016	chr18
ENr122	59728372	chr18
ENr122	59755352	chr18
ENr122	59767398	chr18
ENr122	59769222	chr18
ENr122	59794036	chr18
ENr122	59846514	chr18
ENr123	38787736	chr12
ENr123	38849220	chr12
ENr123	38889158	chr12
ENr123	38905308	chr12
ENr123	38906638	chr12
ENr123	38910400	chr12
ENr123	38942814	chr12

ENr123	38994342	chr12
ENr131	234598763	chr2
ENr132	112469988	chr13
ENr132	112505290	chr13
ENr132	112530598	chr13
ENr132	112575894	chr13
ENr133	39279756	chr21
ENr133	39481042	chr21
ENr212	142359128	chr5
ENr212	142362738	chr5
ENr212	142364220	chr5
ENr212	142369008	chr5
ENr212	142379724	chr5
ENr213	24002243	chr18
ENr213	24008817	chr18
ENr221	55920774	chr5
ENr221	56001676	chr5
ENr221	56018434	chr5
ENr221	56024324	chr5
ENr221	56148964	chr5
ENr221	56150370	chr5
ENr222	132553383	chr6
ENr223	73943840	chr6
ENr223	74217630	chr6
ENr223	74282230	chr6
ENr223	74288082	chr6
ENr231	148067261	chr1
ENr231	148133229	chr1
ENr231	148243809	chr1
ENr231	148250611	chr1
ENr231	148261099	chr1
ENr231	148280555	chr1
ENr231	148421459	chr1
ENr232	128982051	chr9
ENr232	128983419	chr9
ENr232	129018075	chr9
ENr232	129112809	chr9
ENr233	41814652	chr15
ENr233	41822822	chr15
ENr233	41823962	chr15
ENr233	41826508	chr15
ENr233	41855806	chr15
ENr233	41869106	chr15

ENr233	41878682	chr15
ENr233	41880088	chr15
ENr233	41889588	chr15
ENr233	41904180	chr15
ENr311	53079645	chr14
ENr311	53081963	chr14
ENr311	53107043	chr14
ENr311	53108715	chr14
ENr311	53150211	chr14
ENr311	53157811	chr14
ENr311	53191859	chr14
ENr311	53233317	chr14
ENr311	53237915	chr14
ENr311	53418301	chr14
ENr311	53419327	chr14
ENr311	53425255	chr14
ENr311	53428371	chr14
ENr311	53431107	chr14
ENr312	131019593	chr11
ENr313	61230581	chr16
ENr321	118963072	chr8
ENr321	118997614	chr8
ENr321	119060162	chr8
ENr321	119071144	chr8
ENr321	119080074	chr8
ENr321	119082164	chr8
ENr321	119190046	chr8
ENr321	119340640	chr8
ENr321	119361160	chr8
ENr321	119363478	chr8
ENr323	108631494	chr6
ENr323	108689596	chr6
ENr323	108722770	chr6
ENr323	108776654	chr6
ENr324	122592805	chrX
ENr324	122659685	chrX
ENr324	122689743	chrX
ENr324	122820577	chrX
ENr324	122821641	chrX
ENr331	220288164	chr2
ENr331	220591594	chr2
ENr332	64296784	chr11
ENr332	64375520	chr11

ENr332	64383652	chr11
ENr332	64389124	chr11
ENr332	64393494	chr11
ENr332	64400296	chr11
ENr332	64401550	chr11
ENr332	64403526	chr11
ENr332	64404818	chr11
ENr333	33794280	chr20
ENr334	41410024	chr6
ENr334	41550548	chr6
ENr334	41564684	chr6
ENr334	41568218	chr6
ENr334	41607776	chr6
ENr334	41621570	chr6
ENr334	41782044	chr6
ENr334	41789454	chr6
ENr334	41792494	chr6
ENr334	41793406	chr6
ENr334	41796142	chr6
ENr334	41799448	chr6
ENr334	41808834	chr6

Table S4. p300 binding sites in K562. Coordinates are in hg17.

ENCODE region	Position	Chr
ENm001	115414529	chr7
ENm001	115446145	chr7
ENm001	115452263	chr7
ENm001	115573787	chr7
ENm001	115589101	chr7
ENm001	115598145	chr7
ENm001	115652409	chr7
ENm001	115654081	chr7
ENm001	115673195	chr7
ENm001	115699985	chr7
ENm001	115724039	chr7
ENm001	115726737	chr7
ENm001	115735819	chr7
ENm001	115745433	chr7
ENm001	115747257	chr7
ENm001	116043581	chr7
ENm001	116111449	chr7
ENm001	116183383	chr7
ENm001	116186157	chr7
ENm001	116206145	chr7
ENm001	116212301	chr7
ENm001	116248401	chr7
ENm001	116252277	chr7
ENm001	116253949	chr7
ENm001	116255203	chr7
ENm001	116358867	chr7
ENm001	117026413	chr7
ENm001	117175905	chr7
ENm002	131358401	chr5
ENm002	131360187	chr5
ENm002	131462103	chr5
ENm002	131467271	chr5
ENm002	131478215	chr5
ENm002	131541485	chr5
ENm002	131543879	chr5
ENm002	131677753	chr5
ENm002	131679349	chr5
ENm002	131687063	chr5
ENm002	131786433	chr5

ENm002	131823217	chr5
ENm002	131893137	chr5
ENm002	131921029	chr5
ENm002	132152069	chr5
ENm002	132154273	chr5
ENm002	132237037	chr5
ENm003	116149719	chr11
ENm003	116152683	chr11
ENm003	116448665	chr11
ENm004	30305879	chr22
ENm004	30307057	chr22
ENm004	30347337	chr22
ENm004	30485543	chr22
ENm004	30588029	chr22
ENm004	30612083	chr22
ENm004	30616377	chr22
ENm004	30622267	chr22
ENm004	30658139	chr22
ENm004	31203477	chr22
ENm004	31260325	chr22
ENm004	31594307	chr22
ENm004	31601451	chr22
ENm005	32840516	chr21
ENm005	33007374	chr21
ENm005	33493090	chr21
ENm005	33506086	chr21
ENm005	33595690	chr21
ENm005	33599072	chr21
ENm005	33729146	chr21
ENm005	33805260	chr21
ENm005	33836002	chr21
ENm005	33837218	chr21
ENm005	33838358	chr21
ENm005	33871988	chr21
ENm005	33977704	chr21
ENm005	34242032	chr21
ENm005	34270988	chr21
ENm005	34300856	chr21
ENm006	152640162	chrX
ENm006	152878002	chrX
ENm006	152879067	chrX
ENm006	152882304	chrX
ENm006	152977237	chrX

ENm006	153014355	chrX
ENm006	153148186	chrX
ENm006	153334030	chrX
ENm006	153509986	chrX
ENm006	153541028	chrX
ENm006	153543682	chrX
ENm006	153576347	chrX
ENm006	153591190	chrX
ENm006	153592330	chrX
ENm006	153593836	chrX
ENm006	153608468	chrX
ENm006	153790538	chrX
ENm006	153814581	chrX
ENm006	153939473	chrX
ENm007	59404104	chr19
ENm007	59426372	chr19
ENm007	59805460	chr19
ENm007	59858128	chr19
ENm007	59883740	chr19
ENm008	95254	chr16
ENm008	103652	chr16
ENm008	110188	chr16
ENm008	133102	chr16
ENm008	447020	chr16
ENm009	4860031	chr11
ENm009	4870025	chr11
ENm009	5102281	chr11
ENm009	5107221	chr11
ENm009	5109805	chr11
ENm009	5128121	chr11
ENm009	5129603	chr11
ENm009	5131845	chr11
ENm009	5150047	chr11
ENm009	5182879	chr11
ENm009	5203019	chr11
ENm009	5212519	chr11
ENm009	5220119	chr11
ENm009	5223273	chr11
ENm009	5225401	chr11
ENm009	5226503	chr11
ENm009	5228137	chr11
ENm009	5230227	chr11
ENm009	5231405	chr11

ENm009 5232963 chr11
ENm009 5234749 chr11
ENm009 5245047 chr11
ENm009 5248125 chr11
ENm009 5253673 chr11
ENm009 5258575 chr11
ENm009 5262451 chr11
ENm009 5265909 chr11
ENm009 5267847 chr11
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ENm009 5662325 chr11
ENm009 5669431 chr11
ENm010 26884572 chr7
ENm013 89620303 chr7
ENm013 89677759 chr7
ENm014 125933210 chr7
ENm014 125935302 chr7
ENm014 126109659 chr7
ENm014 126123314 chr7
ENm014 126596851 chr7
ENm014 126599600 chr7
ENm014 126601315 chr7
ENm014 126617452 chr7
ENm014 126623464 chr7
ENr111 29701217 chr13
ENr111 29780827 chr13
ENr111 29835243 chr13
ENr121 118484537 chr2
ENr123 38779604 chr12
ENr131 234391008 chr2
ENr131 234548508 chr2
ENr132 112403678 chr13
ENr132 112414432 chr13
ENr132 112416066 chr13
ENr133 39250914 chr21

ENr133	39279756	chr21
ENr133	39297882	chr21
ENr133	39389120	chr21
ENr133	39641592	chr21
ENr133	39716452	chr21
ENr211	25824906	chr16
ENr221	56095574	chr5
ENr221	56143872	chr5
ENr221	56315746	chr5
ENr223	74052406	chr6
ENr223	74282420	chr6
ENr223	74287892	chr6
ENr231	148067033	chr1
ENr231	148097319	chr1
ENr231	148244037	chr1
ENr231	148381939	chr1
ENr231	148453873	chr1
ENr233	41520532	chr15
ENr233	41549450	chr15
ENr233	41573390	chr15
ENr233	41588666	chr15
ENr233	41593568	chr15
ENr233	41674964	chr15
ENr233	41749216	chr15
ENr233	41904484	chr15
ENr323	108418466	chr6
ENr323	108723112	chr6
ENr324	122588473	chrX
ENr324	122592653	chrX
ENr324	122662991	chrX
ENr324	122821603	chrX
ENr324	122822819	chrX
ENr324	122848241	chrX
ENr331	220189706	chr2
ENr331	220191910	chr2
ENr331	220287784	chr2
ENr331	220415122	chr2
ENr331	220419720	chr2
ENr332	64413254	chr11
ENr333	33378446	chr20
ENr333	33395660	chr20
ENr333	33792152	chr20
ENr333	33794280	chr20

ENr334 41894486 chr6

Table S5. Predicted enhancers in HeLa. Coordinates are in hg17.

ENCODE region	Position	Chr
ENr122	59459451	chr18
ENr122	59477151	chr18
ENr122	59501151	chr18
ENr122	59544751	chr18
ENr122	59556151	chr18
ENr122	59593551	chr18
ENr122	59637351	chr18
ENr122	59685951	chr18
ENr122	59700751	chr18
ENr132	112354915	chr13
ENr132	112414315	chr13
ENr132	112427415	chr13
ENr132	112575215	chr13
ENr132	112786815	chr13
ENr334	41505745	chr6
ENr334	41520245	chr6
ENr334	41540745	chr6
ENr334	41547545	chr6
ENr334	41565045	chr6
ENr334	41579445	chr6
ENr334	41581145	chr6
ENr334	41607245	chr6
ENr334	41654545	chr6
ENr334	41701445	chr6
ENr334	41774545	chr6
ENr334	41781945	chr6
ENr334	41788545	chr6
ENr334	41790845	chr6
ENr334	41799145	chr6
ENr334	41807745	chr6
ENr334	41843245	chr6
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ENr322	98915974	chr14
ENr322	98917974	chr14
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ENr111	29785366	chr13
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ENr111	29810566	chr13
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ENr111	29902866	chr13
ENr231	148098184	chr1
ENr231	148156884	chr1
ENr231	148280784	chr1

ENr231	148298284	chr1
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ENr123	38906127	chr12
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ENm014	126171357	chr7
ENm014	126222257	chr7
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ENr324	122684300	chrX
ENr324	122690100	chrX
ENr324	122728600	chrX
ENr324	122765000	chrX
ENr324	122782500	chrX
ENr324	122830700	chrX
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ENr324	122840300	chrX

Table S6. Predicted enhancers in GM. Coordinates are in hg17.

ENCODE region	Position	Chr
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ENr122	59794451	chr18
ENr122	59799351	chr18
ENr122	59846351	chr18
ENr211	25817078	chr16
ENr211	25885678	chr16
ENr211	25887378	chr16
ENr132	112413315	chr13
ENr132	112427415	chr13
ENr132	112436215	chr13
ENr132	112470615	chr13
ENr132	112482915	chr13
ENr132	112506015	chr13
ENr132	112530615	chr13
ENr132	112552515	chr13
ENr132	112575315	chr13
ENr334	41419845	chr6
ENr334	41516345	chr6
ENr334	41550145	chr6
ENr334	41563645	chr6
ENr334	41570345	chr6
ENr334	41607545	chr6
ENr334	41614345	chr6
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ENr331	220535401	chr2
ENr331	220591901	chr2
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ENr322	98926174	chr14
ENr322	98948774	chr14
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ENr133	39279317	chr21

ENr133	39483317	chr21
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ENr133	39668317	chr21
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ENr324	122832300	chrX
ENr324	122838600	chrX
ENr324	122844500	chrX
ENr324	122850300	chrX
ENr324	122868600	chrX
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Table S7. Predicted enhancers in K562. Coordinates are in hg17.

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ENr334	41654345	chr6
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ENr334	41843945	chr6
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ENm002	131623964	chr5
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ENr331	220151101	chr2

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ENr331	220166101	chr2
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ENr331	220209201	chr2
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ENr131	234483175	chr2
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ENm001	116347022	chr7
ENm001	116443622	chr7
ENm001	116464222	chr7
ENm001	116514222	chr7
ENm001	117007922	chr7
ENm001	117026122	chr7
ENm007	59038335	chr19
ENm007	59084035	chr19
ENm007	59405735	chr19
ENm007	59419635	chr19
ENm007	59438935	chr19
ENm007	59722935	chr19
ENm007	59776035	chr19

ENm007	59858135	chr19
ENm007	59898735	chr19
ENr324	122585300	chrX
ENr324	122660300	chrX
ENr324	122677100	chrX
ENr324	122684500	chrX
ENr324	122729100	chrX
ENr324	122764700	chrX
ENr324	122828800	chrX
ENr324	122844300	chrX
ENr324	122852400	chrX
ENr324	122881500	chrX
ENr324	122932200	chrX

Table S8. Predicted enhancers in ES cells. Coordinates are in hg17.

ENCODE region	Position	Chr
ENr122	59836351	chr18
ENr122	59902051	chr18
ENr211	25804378	chr16
ENr211	25806278	chr16
ENr211	25844478	chr16
ENr211	25870178	chr16
ENr211	25938978	chr16
ENr211	25969078	chr16
ENr211	25974778	chr16
ENr211	25990278	chr16
ENr211	26052278	chr16
ENr211	26096178	chr16
ENr211	26125178	chr16
ENr211	26138678	chr16
ENr211	26200778	chr16
ENr132	112406515	chr13
ENr132	112415215	chr13
ENr132	112420815	chr13
ENr132	112428615	chr13
ENr132	112437715	chr13
ENr132	112445415	chr13
ENr132	112595115	chr13
ENr132	112604515	chr13
ENr132	112608115	chr13
ENr334	41483345	chr6
ENr334	41518945	chr6
ENr334	41531945	chr6
ENr334	41538545	chr6
ENr334	41547645	chr6
ENr334	41564145	chr6
ENr334	41566045	chr6
ENr334	41570545	chr6
ENr334	41630545	chr6
ENr334	41638445	chr6
ENr334	41646145	chr6
ENr334	41647945	chr6
ENr334	41654745	chr6
ENr334	41660945	chr6
ENr334	41676445	chr6

ENr334	41720245	chr6
ENr334	41756545	chr6
ENr114	55260469	chr10
ENm002	131338564	chr5
ENm002	131365964	chr5
ENm002	131686664	chr5
ENm002	131751064	chr5
ENm002	131801464	chr5
ENm002	131809164	chr5
ENm002	131838364	chr5
ENm002	132118864	chr5
ENm010	26758211	chr7
ENm010	26763611	chr7
ENm010	26843211	chr7
ENm010	27187811	chr7
ENr223	73843903	chr6
ENr223	73965203	chr6
ENr121	118026154	chr2
ENr121	118325054	chr2
ENr121	118494854	chr2
ENm009	4840846	chr11
ENm009	5115546	chr11
ENm009	5597546	chr11
ENm009	5668446	chr11
ENr331	220151101	chr2
ENr331	220176701	chr2
ENr331	220197501	chr2
ENr331	220203801	chr2
ENr331	220211301	chr2
ENr331	220251701	chr2
ENr331	220267801	chr2
ENr331	220361801	chr2
ENr331	220376101	chr2
ENr331	220389101	chr2
ENr331	220391601	chr2
ENr331	220398101	chr2
ENr331	220418401	chr2
ENr331	220592201	chr2
ENr322	98529874	chr14
ENr322	98637574	chr14
ENr322	98669374	chr14
ENr322	98724774	chr14
ENr322	98794674	chr14

ENr322	98800574	chr14
ENr322	98931174	chr14
ENr322	98949574	chr14
ENr133	39275117	chr21
ENr133	39282617	chr21
ENr133	39284817	chr21
ENr133	39316317	chr21
ENr133	39323317	chr21
ENr133	39376217	chr21
ENr133	39431717	chr21
ENr133	39724017	chr21
ENr131	234620175	chr2
ENr131	234665375	chr2
ENm004	30345858	chr22
ENm004	30368358	chr22
ENm004	30588058	chr22
ENm004	30681258	chr22
ENm004	31046458	chr22
ENm004	31057458	chr22
ENm004	31289358	chr22
ENm004	31343558	chr22
ENm004	31524758	chr22
ENm004	31532158	chr22
ENm004	31551258	chr22
ENm004	31707758	chr22
ENm004	31740958	chr22
ENm004	31783758	chr22
ENm004	31814458	chr22
ENm013	89498590	chr7
ENm013	89896790	chr7
ENm013	89983790	chr7
ENm013	89985590	chr7
ENm013	90000390	chr7
ENm013	90038590	chr7
ENm013	90166590	chr7
ENm013	90306590	chr7
ENm013	90317890	chr7
ENm013	90408590	chr7
ENr312	130621648	chr11
ENr312	130650448	chr11
ENr312	130665848	chr11
ENr312	130747548	chr11
ENr312	130783948	chr11

ENr312	130846348	chr11
ENr312	130966248	chr11
ENr312	130992848	chr11
ENr312	131015248	chr11
ENr312	131017148	chr11
ENr312	131037248	chr11
ENr212	141900901	chr5
ENr212	142034401	chr5
ENr212	142044501	chr5
ENr212	142077001	chr5
ENr212	142142901	chr5
ENr212	142157301	chr5
ENr212	142177301	chr5
ENr212	142186401	chr5
ENr212	142206301	chr5
ENr212	142208401	chr5
ENr212	142217101	chr5
ENr212	142223201	chr5
ENr212	142225001	chr5
ENr212	142232701	chr5
ENr212	142258701	chr5
ENr212	142342401	chr5
ENr323	108415547	chr6
ENr323	108418547	chr6
ENr323	108562247	chr6
ENm012	113648834	chr7
ENm012	113864434	chr7
ENm012	113893234	chr7
ENm012	113923534	chr7
ENm012	113943634	chr7
ENm012	114056534	chr7
ENm012	114058634	chr7
ENm012	114077434	chr7
ENm012	114164634	chr7
ENm012	114179334	chr7
ENm012	114206534	chr7
ENm012	114220634	chr7
ENm012	114236934	chr7
ENm012	114242634	chr7
ENm012	114266434	chr7
ENr233	41596239	chr15
ENr233	41610339	chr15
ENr233	42000139	chr15

ENr233	42007639	chr15
ENr233	42009339	chr15
ENr113	118877609	chr4
ENr113	119004909	chr4
ENm006	152649295	chrX
ENm006	152664895	chrX
ENm006	152727395	chrX
ENm006	152820295	chrX
ENm006	152821995	chrX
ENm006	152827895	chrX
ENm006	152837595	chrX
ENm006	152878095	chrX
ENm006	152960295	chrX
ENm006	152983495	chrX
ENm006	152997795	chrX
ENm006	153020895	chrX
ENm006	153035595	chrX
ENm006	153839395	chrX
ENr213	23735682	chr18
ENr213	23819382	chr18
ENr213	23879982	chr18
ENr213	23882882	chr18
ENr213	23898482	chr18
ENr213	23920882	chr18
ENr213	23944582	chr18
ENr213	23989082	chr18
ENr213	23994282	chr18
ENm008	321251	chr16
ENm008	447651	chr16
ENm008	473551	chr16
ENm008	481451	chr16
ENr112	51736606	chr2
ENr222	132263090	chr6
ENr222	132271090	chr6
ENr222	132300390	chr6
ENr222	132318190	chr6
ENr222	132495290	chr6
ENr222	132599490	chr6
ENr321	119002671	chr8
ENr321	119015971	chr8
ENr321	119023171	chr8
ENr321	119028971	chr8
ENr321	119070471	chr8

ENr321	119076671	chr8
ENr321	119092671	chr8
ENr321	119099771	chr8
ENr321	119106071	chr8
ENr321	119118771	chr8
ENr321	119134271	chr8
ENr321	119151671	chr8
ENr321	119154071	chr8
ENr321	119156171	chr8
ENr321	119157771	chr8
ENr321	119160271	chr8
ENr321	119162171	chr8
ENr321	119204571	chr8
ENr321	119291571	chr8
ENr321	119338571	chr8
ENm005	32721987	chr21
ENm005	32772287	chr21
ENm005	32775787	chr21
ENm005	32788087	chr21
ENm005	32806087	chr21
ENm005	32817887	chr21
ENm005	32899187	chr21
ENm005	33100787	chr21
ENm005	33247187	chr21
ENm005	33253887	chr21
ENm005	33259187	chr21
ENm005	33329687	chr21
ENm005	33370087	chr21
ENm005	33405587	chr21
ENm005	33407387	chr21
ENm005	33410487	chr21
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ENm005	33707287	chr21
ENm005	33950887	chr21
ENm005	33969187	chr21
ENm005	33978087	chr21
ENm005	33981287	chr21
ENm005	33990987	chr21
ENm005	34088287	chr21
ENm005	34153287	chr21
ENm005	34235887	chr21

ENm005	34241787	chr21
ENm005	34269687	chr21
ENm005	34316687	chr21
ENr311	53103726	chr14
ENr311	53108426	chr14
ENr311	53135826	chr14
ENr311	53278626	chr14
ENr311	53280626	chr14
ENr111	29430566	chr13
ENr111	29436966	chr13
ENr111	29492066	chr13
ENr111	29510466	chr13
ENr111	29524966	chr13
ENr111	29849366	chr13
ENr111	29867866	chr13
ENr231	148080384	chr1
ENr231	148157384	chr1
ENr231	148302484	chr1
ENm011	1750942	chr11
ENm011	1886442	chr11
ENm011	1982642	chr11
ENr123	38701527	chr12
ENr123	38712827	chr12
ENr123	38835427	chr12
ENr333	33363879	chr20
ENr333	33488679	chr20
ENr333	33612179	chr20
ENr333	33691379	chr20
ENr333	33693279	chr20
ENr333	33696479	chr20
ENr232	128913406	chr9
ENr232	128968806	chr9
ENr232	128982306	chr9
ENr232	128983906	chr9
ENr232	128986606	chr9
ENr232	129009106	chr9
ENr232	129119806	chr9
ENr232	129177406	chr9
ENr232	129246606	chr9
ENm003	116063866	chr11
ENm003	116079466	chr11
ENm003	116085266	chr11
ENm003	116091566	chr11

ENm003	116107566	chr11
ENm003	116307166	chr11
ENm003	116332866	chr11
ENm003	116366466	chr11
ENm003	116370366	chr11
ENm003	116449066	chr11
ENm003	116453766	chr11
ENr332	64083239	chr11
ENr332	64169039	chr11
ENr332	64235439	chr11
ENr332	64239239	chr11
ENr332	64250039	chr11
ENr332	64264439	chr11
ENr332	64285239	chr11
ENr332	64287939	chr11
ENr332	64412039	chr11
ENr221	55939657	chr5
ENr221	55947157	chr5
ENr221	55969057	chr5
ENr221	56001557	chr5
ENr221	56030657	chr5
ENr221	56046557	chr5
ENr221	56048657	chr5
ENr221	56158357	chr5
ENm014	125759157	chr7
ENm014	126116557	chr7
ENm014	126330357	chr7
ENm014	126348857	chr7
ENm014	126423557	chr7
ENm014	126769957	chr7
ENm001	115465422	chr7
ENm001	115486622	chr7
ENm001	115827322	chr7
ENm001	115849622	chr7
ENm001	115969722	chr7
ENm001	115971722	chr7
ENm001	116017122	chr7
ENm001	116036722	chr7
ENm001	116090422	chr7
ENm001	116138922	chr7
ENm001	116254822	chr7
ENm001	116352122	chr7
ENm001	116360022	chr7

ENm001	116374022	chr7
ENm001	116376022	chr7
ENm001	116383422	chr7
ENm001	116720322	chr7
ENm001	116886522	chr7
ENm001	116907622	chr7
ENm001	116910422	chr7
ENm001	117043022	chr7
ENm001	117047822	chr7
ENm001	117052922	chr7
ENm001	117069722	chr7
ENm001	117100822	chr7
ENm001	117122622	chr7
ENm001	117197322	chr7
ENm007	59037535	chr19
ENm007	59236635	chr19
ENm007	59470835	chr19
ENm007	59492935	chr19
ENm007	59528635	chr19
ENm007	59594535	chr19
ENm007	59639335	chr19
ENm007	59676535	chr19
ENr324	122685000	chrX
ENr324	122783000	chrX
ENr324	122838100	chrX
ENr313	60864100	chr16
ENr313	61020700	chr16
ENr313	61257100	chr16

Table S9. Predicted enhancers in BMP4 differentiated ES cells. Coordinates are in hg17.

ENCODE region	Position	Chr
ENr122	59798651	chr18
ENr211	25834378	chr16
ENr211	26108478	chr16
ENr211	26200778	chr16
ENr132	112354715	chr13
ENr132	112360615	chr13
ENr132	112405715	chr13
ENr132	112413815	chr13
ENr132	112415715	chr13
ENr132	112422515	chr13
ENr132	112431815	chr13
ENr132	112459315	chr13
ENr132	112483715	chr13
ENr132	112505815	chr13
ENr132	112596515	chr13
ENr132	112724915	chr13
ENr334	41448045	chr6
ENr334	41519545	chr6
ENr334	41541045	chr6
ENr334	41547745	chr6
ENr334	41564445	chr6
ENr334	41570445	chr6
ENr334	41580745	chr6
ENr334	41596345	chr6
ENr334	41645545	chr6
ENr334	41648045	chr6
ENr334	41654445	chr6
ENr334	41656345	chr6
ENr334	41700145	chr6
ENr334	41721045	chr6
ENr334	41732345	chr6
ENr334	41799445	chr6
ENr334	41816045	chr6
ENr334	41824045	chr6
ENr334	41843345	chr6
ENm002	131359064	chr5
ENm002	131360664	chr5
ENm002	131366464	chr5
ENm002	131569764	chr5

ENm002	131589964	chr5
ENm002	131598564	chr5
ENm002	131622564	chr5
ENm002	131629164	chr5
ENm002	131659264	chr5
ENm002	131665464	chr5
ENm002	131685464	chr5
ENm002	131752164	chr5
ENm002	131760164	chr5
ENm002	131791864	chr5
ENm002	131801264	chr5
ENm002	131809064	chr5
ENm002	131828764	chr5
ENm002	131839264	chr5
ENm002	131841264	chr5
ENm002	132123764	chr5
ENm002	132135264	chr5
ENm002	132152164	chr5
ENm002	132164164	chr5
ENm002	132171664	chr5
ENm002	132203864	chr5
ENm010	27024511	chr7
ENm010	27026711	chr7
ENm010	27039511	chr7
ENm010	27063811	chr7
ENm010	27070411	chr7
ENm010	27119011	chr7
ENm010	27159911	chr7
ENm010	27186411	chr7
ENm010	27188211	chr7
ENr223	73876903	chr6
ENr223	73968703	chr6
ENr121	118186854	chr2
ENr121	118200854	chr2
ENr121	118244554	chr2
ENm009	5668546	chr11
ENm009	5702146	chr11
ENr331	220143201	chr2
ENr331	220150901	chr2
ENr331	220160401	chr2
ENr331	220190201	chr2
ENr331	220202401	chr2
ENr331	220211301	chr2

ENr331	220217101	chr2
ENr331	220251901	chr2
ENr331	220267801	chr2
ENr331	220335501	chr2
ENr331	220353801	chr2
ENr331	220372001	chr2
ENr331	220393101	chr2
ENr331	220418601	chr2
ENr331	220562101	chr2
ENr322	98572274	chr14
ENr322	98669774	chr14
ENr322	98714074	chr14
ENr322	98752374	chr14
ENr322	98754074	chr14
ENr322	98767174	chr14
ENr322	98769474	chr14
ENr322	98777974	chr14
ENr322	98798374	chr14
ENr322	98937774	chr14
ENr322	98949374	chr14
ENr133	39252417	chr21
ENr133	39282217	chr21
ENr133	39288517	chr21
ENr133	39299117	chr21
ENr133	39315217	chr21
ENr133	39323317	chr21
ENr133	39390217	chr21
ENr133	39391917	chr21
ENr133	39431917	chr21
ENr133	39454717	chr21
ENr133	39724517	chr21
ENr131	234665175	chr2
ENm004	30305158	chr22
ENm004	30322758	chr22
ENm004	30345358	chr22
ENm004	30771558	chr22
ENm004	30779758	chr22
ENm004	31252458	chr22
ENm004	31254158	chr22
ENm004	31260758	chr22
ENm004	31267158	chr22
ENm004	31276258	chr22
ENm004	31277858	chr22

ENm004	31325858	chr22
ENm004	31327458	chr22
ENm004	31337458	chr22
ENm004	31343758	chr22
ENm004	31352858	chr22
ENm004	31364958	chr22
ENm004	31370758	chr22
ENm004	31404658	chr22
ENm004	31419158	chr22
ENm004	31433258	chr22
ENm004	31434958	chr22
ENm004	31456058	chr22
ENm004	31466158	chr22
ENm004	31474258	chr22
ENm004	31485458	chr22
ENm004	31506258	chr22
ENm004	31538958	chr22
ENm004	31551658	chr22
ENm004	31560458	chr22
ENm004	31601758	chr22
ENm004	31671858	chr22
ENm004	31698558	chr22
ENm004	31707158	chr22
ENm004	31714858	chr22
ENm004	31750258	chr22
ENm013	89847590	chr7
ENm013	89853590	chr7
ENm013	89897690	chr7
ENm013	89935390	chr7
ENm013	89952090	chr7
ENm013	89981590	chr7
ENm013	90001090	chr7
ENm013	90090990	chr7
ENr312	130699748	chr11
ENr312	130798948	chr11
ENr312	130840148	chr11
ENr312	130846748	chr11
ENr312	130933348	chr11
ENr312	131032348	chr11
ENr312	131045048	chr11
ENr212	141891101	chr5
ENr212	141903201	chr5
ENr212	141910501	chr5

ENr212	141927101	chr5
ENr212	141950001	chr5
ENr212	141959201	chr5
ENr212	141966601	chr5
ENr212	141981501	chr5
ENr212	141986301	chr5
ENr212	141996801	chr5
ENr212	142004701	chr5
ENr212	142045501	chr5
ENr212	142057801	chr5
ENr212	142094501	chr5
ENr212	142144501	chr5
ENr212	142205501	chr5
ENr212	142243301	chr5
ENr212	142261201	chr5
ENr212	142342001	chr5
ENr323	108393947	chr6
ENr323	108400347	chr6
ENr323	108418947	chr6
ENr323	108518647	chr6
ENr323	108546847	chr6
ENr323	108594347	chr6
ENr323	108680047	chr6
ENr323	108681847	chr6
ENr323	108705847	chr6
ENm012	113649134	chr7
ENm012	113893434	chr7
ENm012	114070334	chr7
ENm012	114076734	chr7
ENm012	114163434	chr7
ENm012	114221234	chr7
ENm012	114237434	chr7
ENm012	114321934	chr7
ENm012	114459134	chr7
ENm012	114465334	chr7
ENr233	41596339	chr15
ENr233	41692639	chr15
ENr233	41747839	chr15
ENr233	41959339	chr15
ENr233	41981139	chr15
ENr233	41993139	chr15
ENr233	42000739	chr15
ENr233	42010939	chr15

ENr113	118700409	chr4
ENm006	152665495	chrX
ENm006	152689295	chrX
ENm006	152727295	chrX
ENm006	152785995	chrX
ENm006	152806095	chrX
ENm006	152808395	chrX
ENm006	152826695	chrX
ENm006	152829595	chrX
ENm006	152837795	chrX
ENm006	152850495	chrX
ENm006	153298095	chrX
ENm006	153324795	chrX
ENm006	153332595	chrX
ENm006	153527095	chrX
ENm006	153529195	chrX
ENm006	153591595	chrX
ENm006	153679795	chrX
ENr213	23755982	chr18
ENr213	23898682	chr18
ENr213	23905582	chr18
ENr213	23912982	chr18
ENr213	23943482	chr18
ENr213	23949882	chr18
ENr213	23967782	chr18
ENr213	23969682	chr18
ENr213	23987182	chr18
ENr213	23993782	chr18
ENr213	23995882	chr18
ENm008	67251	chr16
ENm008	103351	chr16
ENm008	321551	chr16
ENm008	336051	chr16
ENm008	447451	chr16
ENm008	473751	chr16
ENm008	481951	chr16
ENr222	132447790	chr6
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Table S11. Coordinates (hg17) and primers used to amplify regions containing predicted enhancers in HeLa (H1-H9) and K562 (K1-K9) cells for cloning and reporter assays, as well as random regions selected as controls (R1-R10).

Enhancer ID	Chromosomal Location (Hg17)	Left Primer	Right Primer	Active (P < 1%)
Random R1	chr10:66880252-66881251	CTGCCAGGAAACACAAAAT	TGGCATGAGTCTCAACAAGG	No
R2	chr11:41402448-41403447	TGACATGGTTTGTGGCTTGT	TAGACGGAGGTCTTGCCAAC	No
R3	chr12:125703599-125704598	CCCAGTGTCTGTGGTCATTG	TGACCAAATGCACTTTCTGC	No
R4	chr21:19996324-19997323	TCAATTTCCAGTTGGCTTCC	TTGCGTGCCTTTGGTGTCTAT	No
R5	chr3:165054540-165055539	TGAAGCAACAGTGAAACCA	CCTGCTTAGGCTTTTGTTC	No
R6	chr4:59799610-59800609	GGGGAACTTGGTCCTAAGC	CAGCACAACCAGCAAGTGT	No
R7	chr14:83695494-83696493	CAAAGCGAGGCAAAAACAAT	TTTGCCAGAATTACCTGAGTTG	No
R8	chr2:125537487-125538486	TCCACGATAGCTGGTGACTG	TTGAGTGTGGAAGCTCATGG	No
R9	chr3:83292230-83293229	GCTTTCAAGCATGTTTCACG	TCTTCCTGCTTTTCTCTGC	No
R10	chr5:18441221-18442220	TGGAAGGCTTTGGCTCTTA	CAAGAGGCCCAAGAGAAGAA	No
HeLa-Specific H1	chr1:222249552-222251133	TACACCTGGTCACCAAGCAA	CCAGTGGTGCTGGAGGTAAT	Yes
H2	chr17:76107151-76108600	TGTTGGGTGGGTCCCTATTA	AGCCTCCTCAGCCTCACTCT	Yes
H3	chr3:12460699-12462100	CAAATACGGTGACTGTCCTTCA	GATGGAGGTGGGCAGTGAT	Yes
H4	chr21:39314751-39316050	GCTTCCCTCTCCCTTCAAC	GTTGCCAAGCATGTGAATGA	Yes
H5	chr16:4914351-4915550	ATGTCACGGGGCCAGTAAT	TTAAGAGCCCCAGTAACACTAAGG	No
H6	chr22:26195364-26196450	GCTTCCCTCAGCTCCCTTG	AGGGCACAGAGAGGCAGAT	No
H7	chr18:19553351-19554300	GCTCCAAGGTAAATGTAATAGGG	AAATGTTGCAGAGACTCAAGTG	Yes
H8	chr20:55780621-55781450	GCAGGGTCTTGACAACAG	CCTTTAAGTTCTTGCACTGGA	Yes
H9	chr10:128075851-128077369	CAGTGTGTGCAACCACGTT	GTGACAGAAAACAATGCTCCT	Yes
K562-Specific K1	chr16:20665043-20666000	CAGTCAACATGTGCTTAAAAACAGC	TCTCAAAGGAGTGGGAAGGAA	No
K2	chr8:38110846-38111845	TAGTGGCTGTTGGGAAATC	TTAAAGTAAGCTGGCCTTCCA	Yes
K3	chr16:23739868-23740868	ACTGTAGGGTGGTTTTTAAATGTAGC	TCAAGCAAATGAAGGGGAAA	Yes
K4	chr16:132622-133650	CCAGTAACCCTGGCACTCAT	GGTGTCCACTGGTGGTTTCT	No
K5	chr12:7166351-7167590	GGGGCTACAAGTTCCTGAG	GGGGCTACTGGCAGAGTCTT	No
K6	chr20:57022250-57023500	TGCTGCTTTCACCCTCTAAG	AGCAGTGCCTGCATTTAGGT	No
K7	chr4:145284959-145286250	TGTGTTGGCTCATCTCACAG	GCTTCCTGAAGGGAAGCTG	No
K8	chr5:126333651-126335000	TGTCCACTTAGGAAAGTGTCTTGA	GCAAGCATTCTACTTCTCTCAA	No
K9	chr8:91274651-91276001	TCTGAGTACCAAAGGCTTGAAA	AATCCACAGGCAAAGTCACTG	No

Table S12. Known motifs enriched in predicted enhancer regions.

Motifs are from Xie et al., Nature 2005

Name	Consensus	Enhancer over-conservation	Enhancer enrichment	Promoter over-conservation	Promoter enrichment
AP-2ALPHA	SCYNNNGGC	1.00E-300	1.00E-300	4.66E-05	
STAT5A	AWTTCY	1.00E-300	5.80E-220	3.83E-177	6.16E-31
TEF-1	GRRATG	1.00E-300	4.33E-65	5.75E-36	
IY	AWTTTCC	1.04E-279	2.06E-50	5.09E-36	9.98E-18
FOXO1	RWAAACAA	1.08E-270	6.63E-53	1.03E-43	
HNF-3	TRTTTRYTYW	1.12E-247	4.51E-50	3.64E-21	
MEIS1	TGACAG	3.92E-221	4.25E-05	1.88E-56	
MYOD	RNCAGGTG	3.84E-215	1.25E-94	4.19E-05	
NF-AT	WGGAAANW	1.57E-188	3.85E-60	7.83E-15	6.57E-24
TCF11	WNNATGAC	5.86E-181	5.69E-94	1.33E-31	
AP-1	CTGASTCA	9.09E-174	1.00E-300	2.31E-43	1.14E-05
SRY	KTWGTTT	3.09E-140	1.41E-10	2.05E-37	
AP-4	GCAGCTGNY	2.92E-123	5.07E-05	4.77E-48	
ER	RNNNTGACCT	6.28E-112	6.10E-15	3.00E-35	
GATA	WGATAR	1.43E-109			
PEA3	MGGAWGT	4.43E-105	3.71E-21	7.04E-282	6.15E-57
TCF-4	WTCAAAGS	7.02E-104	1.87E-11	1.71E-20	
AML1	ACCACA	1.76E-96	2.89E-48		
T3R	MNTGWCCT	2.48E-95	2.16E-27	9.79E-09	
TBP	TATAAATW	5.39E-80	4.88E-32		
NCX	GTAAKTNG	3.35E-74	2.10E-19		
SOX-5	ATTGTT	4.66E-74	4.02E-27	6.44E-17	
NKX6-2	WADTAAWTA	6.53E-74	7.96E-14		
DBP	GTDTGCT	1.21E-72	2.18E-07	1.09E-05	
NKX2-5	TYAAGTG	7.55E-72	4.13E-39	5.54E-12	
CHX10	GCTAATTA	8.62E-70	1.17E-12		
HOXA4	CYAATTWT	2.12E-69	9.41E-14	6.20E-05	
NF-1	TGGNNNNNGCCAA	1.45E-56	2.85E-99	1.39E-09	
IRF1	AAGTGAA	1.23E-50	8.26E-19	9.56E-12	1.50E-14
NKX2-5	CWTAATTG	2.56E-50	4.83E-09		
ELF-1	RNWMBAGGAART	1.87E-40	7.83E-07	7.46E-16	3.63E-07
TCF-1(P)	GKCRGKTT	1.52E-36	2.04E-07		
STAT1	CANTTCCS	9.86E-34	6.14E-05	3.26E-140	3.80E-119
C/EBPBETA	KNTTGCNYAAAY	9.27E-29	1.71E-32	1.12E-04	1.35E-04
OCTAMER	ATGCAAATNA	3.15E-28	1.21E-23	1.09E-09	
CRX	KGRGATTANNR	5.19E-28	2.22E-09	6.76E-04	
CREB	TGACGTMA	3.24E-27	5.64E-14	6.38E-123	5.41E-249
STAT	TCCMAGAA	4.13E-27	6.70E-11	2.94E-08	8.08E-07
SF-1	TGRCCTTG	2.14E-25	5.11E-17		
YY1	GCCATNTT	2.37E-21	2.65E-133		
AREB6	WCAGGTGWNW	1.16E-17	3.38E-08	4.09E-10	
NF-Y	YSATTGGYY	3.54E-17	1.00E-300	5.94E-209	
PBX-1	WTGATTGNT	7.86E-16	6.41E-06		
RFX1	GTTRCYWNGYNAC	3.03E-15	5.23E-27	4.52E-09	
C-REL	SGRNTTCC	1.63E-14	4.04E-11		
BACH2	SRTGAGTCANC	7.87E-14	1.56E-163	6.73E-09	
E4BP4	RTTACRTAAY	9.31E-14	7.43E-10		
TAL-1ALPHA/E47	AACAGATGKT	2.48E-12			
PU.1	WGAGGAAG	2.84E-12	1.37E-14		

POU3F2	ATTARCATAA	2.82E-10	1.42E-06		
POU1F1	ATGAATAAWT	1.59E-09	6.04E-06	1.19E-04	
RORALPHA2	TGACCTANWTW	2.93E-09			
P65	GGGRATTTCC	7.63E-09	3.37E-04	1.03E-09	5.33E-04
CAC-BP	GRGGSTGGG	4.39E-08			
HNF-6	HWAAATCAATAW	7.46E-08			
ELK-1	CCGGAART	1.27E-07	5.30E-165	1.00E-300	
RP58	TCCAGATGTT	2.58E-07			
MEF-2	YTAAAWATAGCY	1.19E-05	2.42E-05	2.16E-04	5.26E-04
AHR	TNGCGTG	1.48E-05	3.31E-04		
MYB	GNCNGTT	2.03E-05	2.93E-19		
AREB6	ABWCAGGTRNR	2.52E-05			
IPF1	KGTCATTANDC	3.98E-05			
ATF-1	TGACGTCARRG	4.76E-05	1.65E-06	6.91E-09	4.34E-30
BRN-2	YKNATTWYSNATG	1.24E-04			
MAZ	GGGGAGGG	1.78E-04	5.23E-06	4.87E-07	
LHX3	TTAATTAATT	3.50E-04			
LEF1	CTTTGA	1.02205884431E-310	4.34E-13	1.17E-66	

Table S13. De novo motifs enriched in predicted enhancer regions.

Name	Consensus	Known Match (score)	Enhancer over-conservation Z-score (stdev)	Enhancer enrichment Z-score (stdev)	Promoter over-conservation Z-score (stdev)	Promoter enrichment Z-score (stdev)	Promoter depletion Z-score (stdev)
M01	VTGABTCRC	AP-1 (80%)	36.0	37.1	15.7		
M02	TAATTGM	NKX2-5 (88%)	36.0	4.2	14.8		
M03	MAAKGTCR	SF-1 (80%)	35.7	20.4	11.7		
M04	CTTTGAW	TCF-4 (97%)	32.2	8.4	12.9		
M05	YGANTYRGC		31.1	26.8	13.1		4.6
M06	GGAARTGA	STAT1 (88%)	29.5	13.1	21.4	8.3	
M07	TAATTAC	CHX10 (80%)	27.2	4.3	11.0		
M08	YTGCCNNNNNKYCMR	NF-1 (82%)	25.5	30.2	11.3		13.7
M09	YCATTAGY		25.4	5.0	9.2		3.8
M10	ATYWGTCR		23.8	14.0	6.5		
M11	RCATTCCA	TEF-1 (79%)	20.9	21.5	8.2		
M12	RACAGMTGK	TAL-1ALPHA/E47 (86%)	20.3		8.8		9.3
M13	CNTRGCAAC		18.9	5.6	21.7		
M14	AAACCACA	AML1 (86%)	18.6	13.1	5.8		3.7
M15	TGASGTCR	CREB (85%)	18.4	12.7	18.6	15.7	
M16	TAAWTTA	POU6F1 (78%)	15.7				3.3
M17	GCCARGAA		15.7	7.9	5.3		13.9
M18	CACNAGNGGG		15.5		8.3		
M19	GCTAWWWWTAG	MEF-2 (83%)	15.3	8.6	9.0	4.2	
M20	CATNANTAAT		15.1	5.2	5.8		
M21	TGTYKACR		14.6	3.3	6.8		
M22	GCCARNNNAACA		12.0	15.1			
M23	TATTNNNNYYGGC		12.0	3.7			
M24	YGTCNRRACA		11.8	4.3			
M25	TAATGAGC	CHX10 (83%)	11.6		5.5		
M26	TAATTGGC	CHX10 (83%)	11.5		4.2		
M27	AGGTTAAT		11.5	3.7			
M28	ATTANNNNYGACR		10.5				
M29	GTCAGAC		10.3	4.4	4.1		
M30	YGTCRNNNNNATTA		10.3				
M31	CANYAGVTGGC		10.1		7.3		3.6
M32	YGTCRRTCA		9.8		9.7		
M33	SATCAATCR	PBX-1 (84%)	9.5				
M34	YGATTNNTGC		9.5	4.1	7.8	4.7	
M35	AGGCNNNNGCCAR		8.3	9.9	3.8		18.7
M36	GCCRRNNNNNATTA		7.5				8.6
M37	GGAAWTNCCC	P65 (94%)	7.4	5.5	4.5		
M38	CAKCTGGA	RP58 (85%)	7.3	4.9	4.4		12.9
M39	AGCAGCTGC	AP-4 (90%)	6.5		4.2		
M40	RCCATATGGY		4.7				8.4
M41	GTYNCCANRGNRAC		3.7		4.1		

Table S14. 24566 enhancer predictions in K562 cells.
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Table S15. Overlap of genome-wide enhancer predictions in steady-state HeLa cells with binding sites of ER in MCF7 cells, p53 in HCT116 cells, p63 in ME180 cells, and STAT1 in IFN γ -induced HeLa cells. Coordinates are in hg17.

Chr	Position	Overlap ER?	Overlap p53?	Overlap p63?	Overlap STAT1?
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chr7	1334750				
chr7	1350650				
chr7	1378450				
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chr7	2342850	ER			
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chr7	6198250	ER			
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