

Reorganization of 3D Genome Structure May Contribute to Gene Regulatory Evolution in Primates



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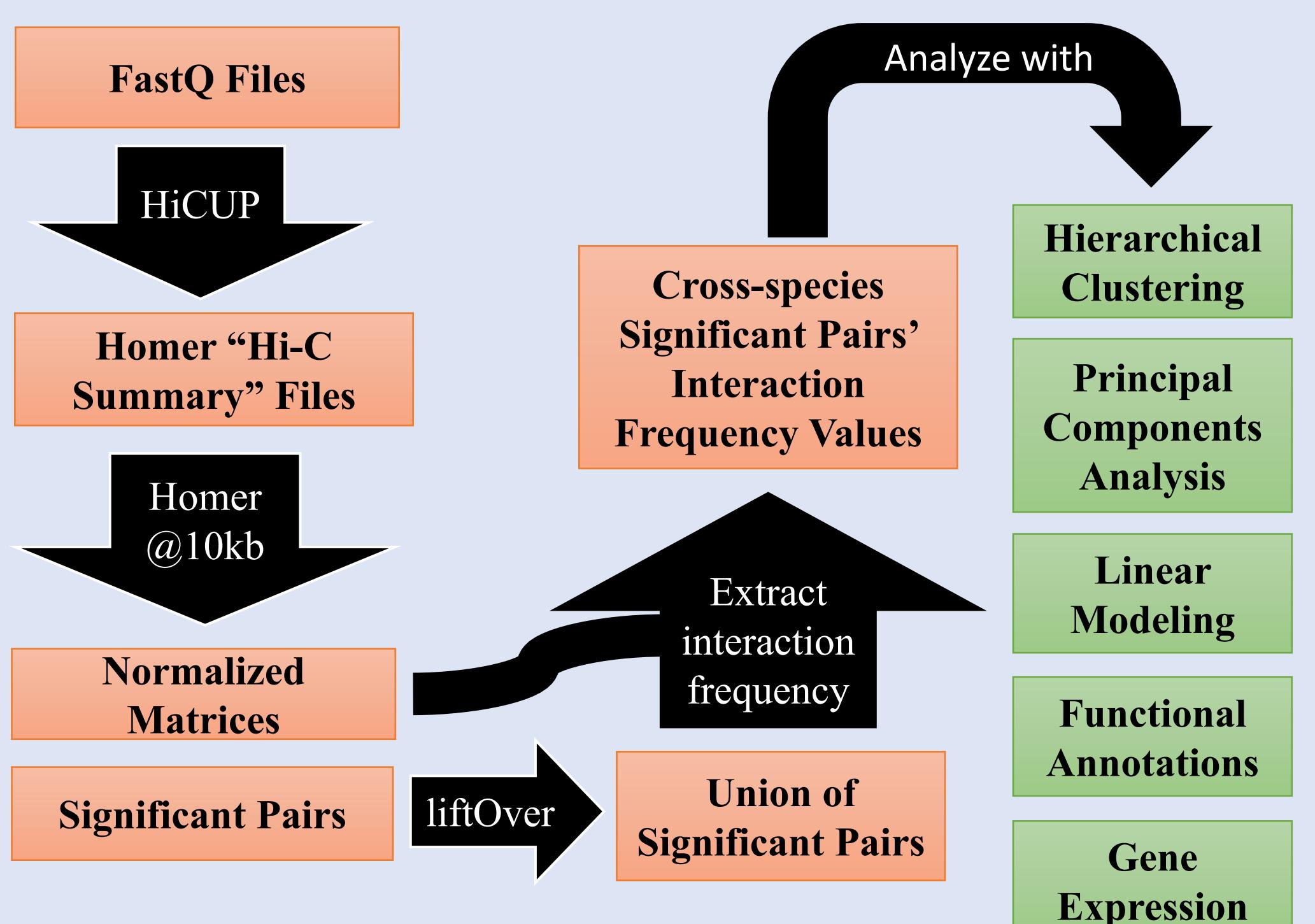
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We gratefully acknowledge the support of:
Genetics and Regulation Training Grant (T32 GM07197)
University of Chicago Research Computing Center
University of Chicago Genomics Facility

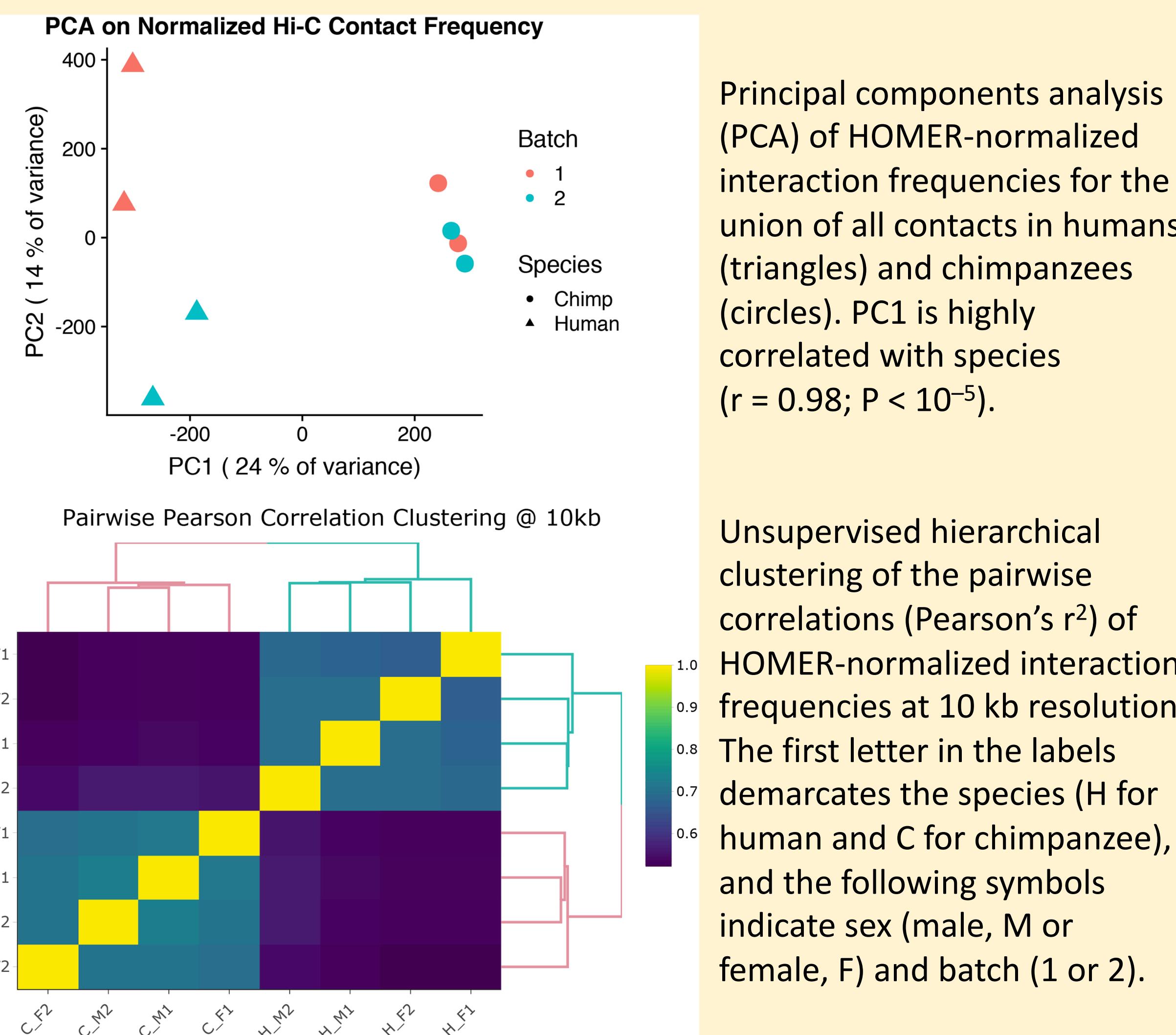
ABSTRACT

A growing body of evidence supports the notion that variation in gene regulation plays a crucial role in both speciation and adaptation. However, a comprehensive functional understanding of the mechanisms underlying regulatory evolution remains elusive. In primates, one of the crucial missing pieces of information towards a better understanding of regulatory evolution is a comparative annotation of interactions between distal regulatory elements and promoters. Chromatin conformation capture technologies have enabled genome-wide quantifications of such distal 3D interactions. However, relatively little comparative research in primates has been done using such technologies. To address this gap, we used Hi-C to characterize 3D chromatin interactions in induced pluripotent stem cells (iPSCs) from humans and chimpanzees. We also used RNA-seq to collect gene expression data from the same lines. We generally observed that lower-order, pairwise 3D genomic interactions are conserved in humans and chimpanzees, but higher order genomic structures, such as topologically associating domains (TADs), are not as conserved. Inter-species differences in 3D genomic interactions are often associated with gene expression differences between the species. To provide additional functional context to our observations, we considered previously published chromatin data from human stem cells. We found that inter-species differences in 3D genomic interactions, which are also associated with gene expression differences between the species, are enriched for both active and repressive marks. Overall, our data demonstrate that, as expected, an understanding of 3D genome reorganization is key to explaining regulatory evolution.

ANALYSIS PIPELINE



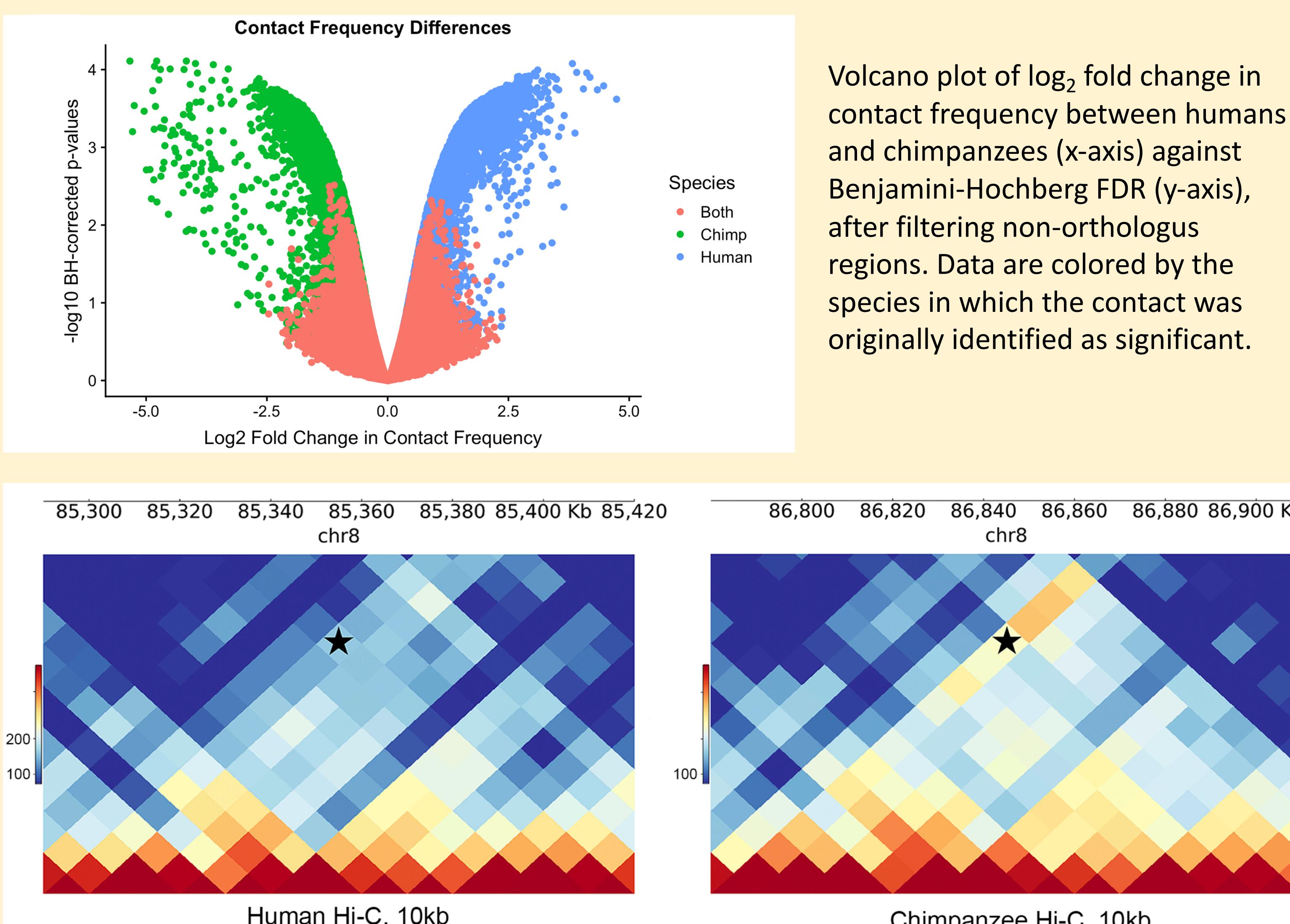
GENOMIC REGULATORY LANDSCAPES SEPARATE OUT BY SPECIES



Principal components analysis (PCA) of HOMER-normalized interaction frequencies for the union of all contacts in humans (triangles) and chimpanzees (circles). PC1 is highly correlated with species ($r = 0.98$; $P < 10^{-5}$).

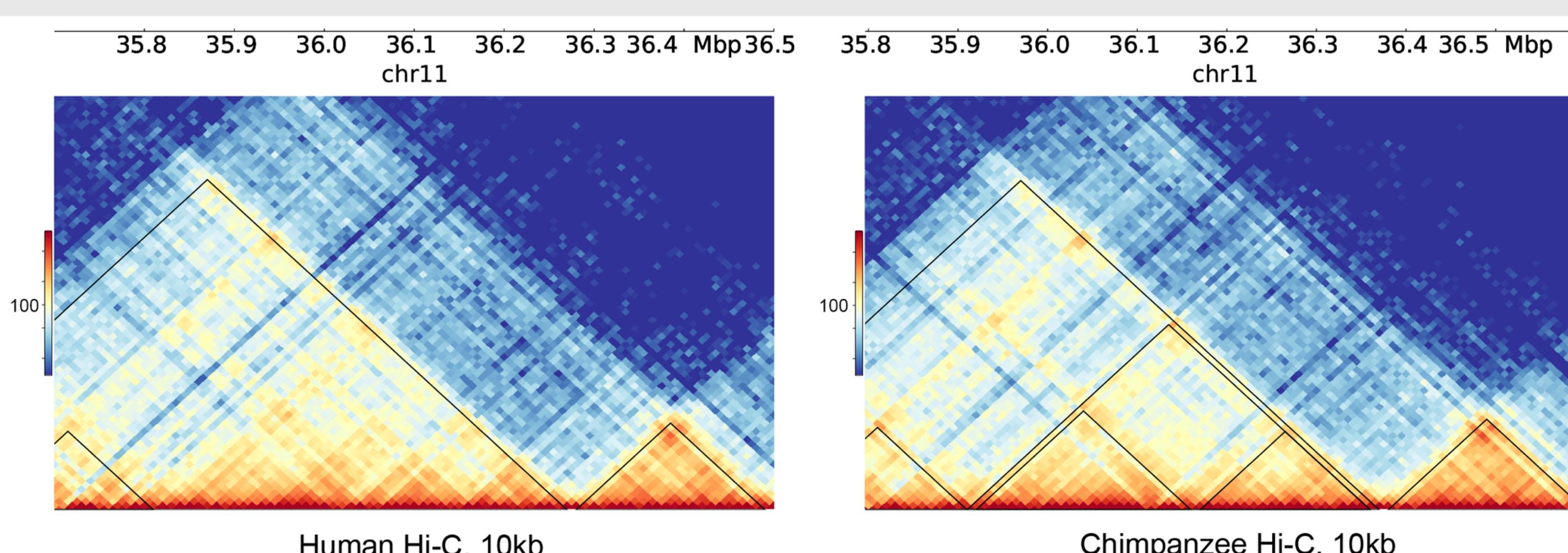
Unsupervised hierarchical clustering of the pairwise correlations (Pearson's r^2) of HOMER-normalized interaction frequencies at 10 kb resolution. The first letter in the labels demarcates the species (H for human and C for chimpanzee), and the following symbols indicate sex (male, M or female, F) and batch (1 or 2).

LINEAR MODELING REVEALS INTER-SPECIES DIFFERENCES IN 3D GENOMIC INTERACTIONS



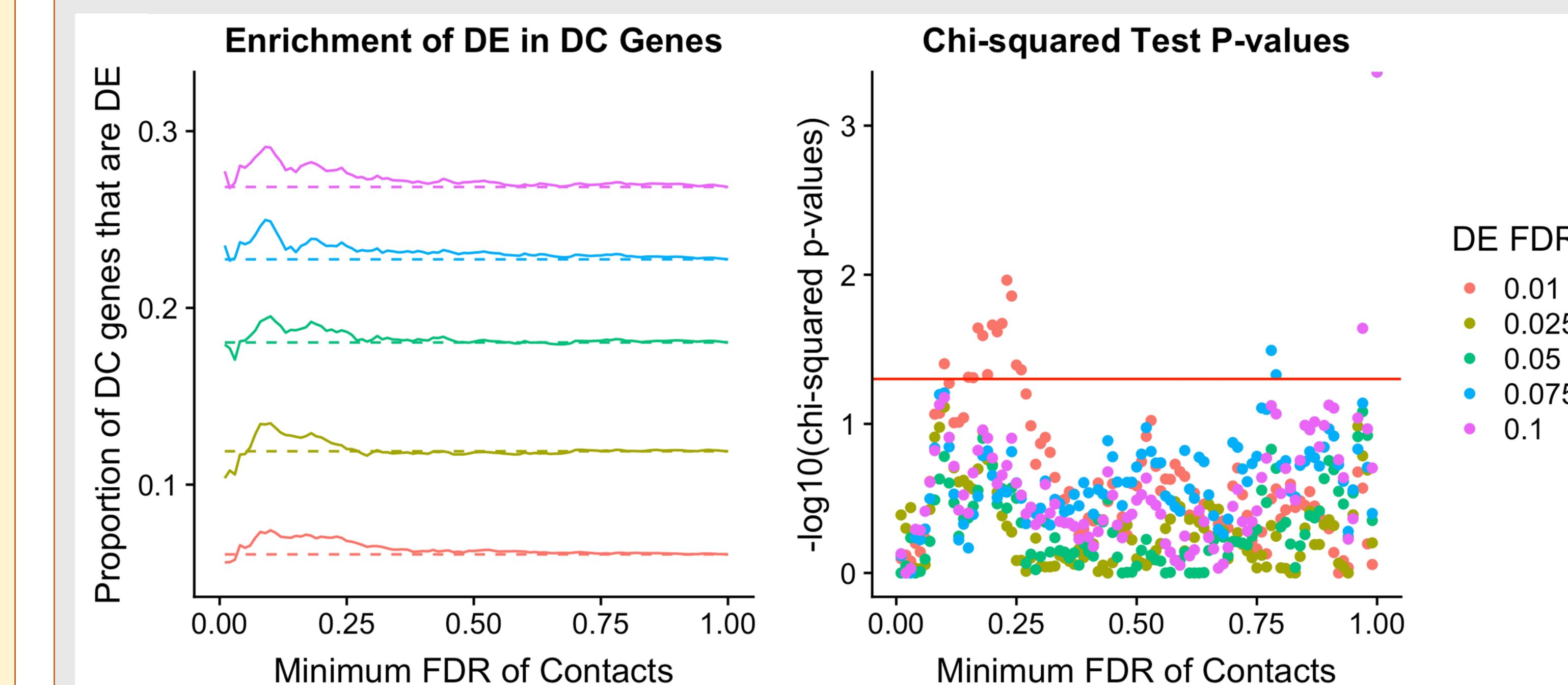
Visual representation of local Hi-C heatmaps in humans and chimpanzees, with contact frequency between loci ranging from weak (blue) to strong (red). Specifically shown are PyGenomeTracks¹ plots of a chromosome 8 interaction between bins 130kb, with black stars indicating a contact inferred to be differential between species.

HIGHER-ORDER CHROMOSOMAL STRUCTURE DIFFERS IN HUMANS AND CHIMPANZEES



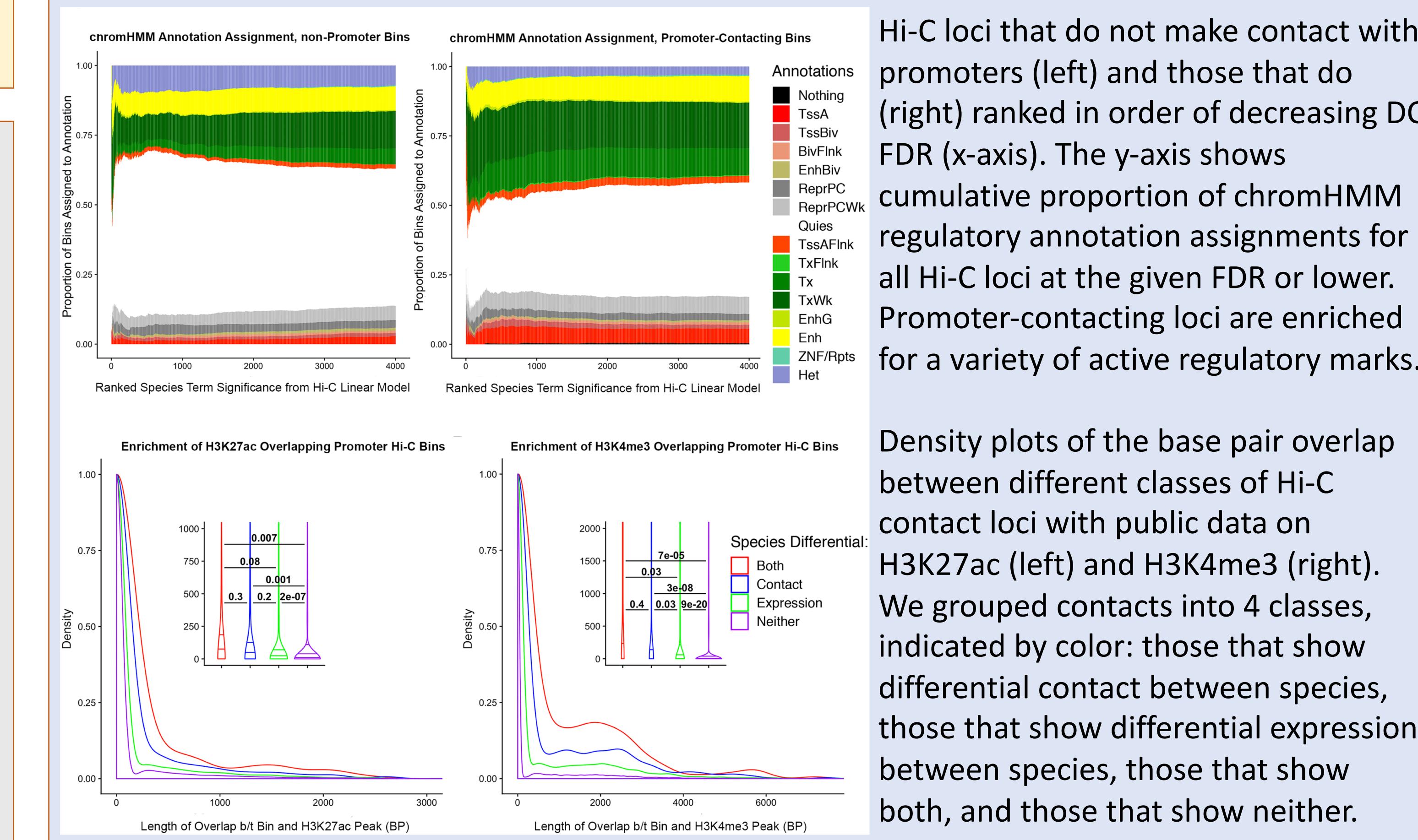
A region on chromosome 11, showing examples of conserved and divergent Arrowhead² inferences of Topologically Associating Domains (TADs, black lines). TADs represent larger-scale genomic structures, constraining local neighborhoods of regulation by promoting within-TAD interactions and inhibiting intra-TAD interactions. All the TADs seen in the human map (left) appear conserved in the chimpanzee map (right), whereas three smaller TADs inferred in the chimpanzee map are not found in the human map, suggesting divergence. Intriguingly, while algorithmic inference suggests divergence in TADs, manual visual examination suggests that actual conservation of these structures may be relatively high between the species.

DIFFERENTIALLY EXPRESSED (DE) GENES ARE ENRICHED FOR DIFFERENTIAL CONTACTS



Left: enrichment of inter-species differentially expressed (DE) genes with corresponding differences in Hi-C contact frequencies (DC) between the species. The proportion of DC genes that are significantly DE (y-axis) is shown across a range of DC FDRs (x-axis). Colors indicate different DE FDR thresholds, and dashed lines indicate the proportion of DE genes expected by chance alone. The observed enrichments suggest DC may be a driver of DE. Right: P values of Chi-squared tests of the null hypothesis that there is no difference in proportion of DE genes among DC genes (y-axis), shown for a range of DC FDRs (x-axis). In both panels, DC regions were chosen to have the minimum FDR supporting inter-species difference in contact frequency.

SPECIES DIFFERENTIAL CONTACTS (DC) ARE ENRICHED FOR ACTIVE REGULATORY MARKS



Hi-C loci that do not make contact with promoters (left) and those that do (right) ranked in order of decreasing DC FDR (x-axis). The y-axis shows cumulative proportion of chromHMM regulatory annotation assignments for all Hi-C loci at the given FDR or lower. Promoter-contacting loci are enriched for a variety of active regulatory marks.

Density plots of the base pair overlap between different classes of Hi-C contact loci with public data on H3K27ac (left) and H3K4me3 (right). We grouped contacts into 4 classes, indicated by color: those that show differential contact between species, those that show differential expression between species, those that show both, and those that show neither.

REFERENCES & PUBLICATION

1. Ramírez F, Bhardwaj V, Arrigoni L, Lam KC, Grünig BA, Villaveces J, et al. High-resolution TADs reveal DNA sequences underlying genome organization in flies. *Nat Commun.* 2018;9: 189. pmid:29335486
2. Durand NC, Shamim MS, Machol I, Rao SSP, Huntley MH, Lander ES, et al. Juicer Provides a One-Click System for Analyzing Loop-Resolution Hi-C Experiments. *Cell Syst.* 2016;3: 95–98. pmid:27467249

This work was recently published in PLOS Genetics:
Eres IE, Luo K, Hsiao CJ, Blake LE, Gilad Y. Reorganization of 3D genome structure may contribute to gene regulatory evolution in primates. *PLOS Genetics.* 2019;15. doi:10.1371/journal.pgen.1008278