Interspecies regulatory landscapes and elements revealed by novel joint systematic integration of human and mouse blood cell epigenomes

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Running Title: Integrated epigenomic profiles between species

Key words: epigenetics, gene regulatory elements, dimensional reduction, regulatory potential

Abstract

Knowledge of locations and activities of *cis*-regulatory elements (CREs) is needed to decipher basic mechanisms of gene regulation and to understand the impact of genetic variants on complex traits. Previous studies identified candidate CREs (cCREs) using epigenetic features in one species, making comparisons difficult between species. In contrast, we conducted an interspecies study defining epigenetic states and identifying cCREs in blood cell types to generate regulatory maps that are comparable between species, using integrative modeling of eight epigenetic features jointly in human and mouse in our **V**al**i**dated **S**ystematic **I**ntegrati**on** (VISION) Project. The resulting catalogs of cCREs are useful resources for further studies of gene regulation in blood cells, indicated by high overlap with known functional elements and strong enrichment for human genetic variants associated with blood cell phenotypes. The contribution of each epigenetic state in cCREs to gene regulation, inferred from a multivariate regression, was used to estimate epigenetic state Regulatory Potential (esRP) scores for each cCRE in each cell type, which were used to categorize dynamic changes in cCREs. Groups of cCREs displaying similar patterns of regulatory activity in human and mouse cell types, obtained by joint clustering on esRP scores, harbored distinctive transcription factor binding motifs that were similar between species. An interspecies comparison of cCREs revealed both conserved and species-specific patterns of epigenetic evolution. Finally, we showed that comparisons of the epigenetic landscape between species can reveal elements with similar roles in regulation, even in the absence of genomic sequence alignment.

Introduction

The morphology and functions of different cell types are determined by the expression of distinctive sets of genes in each. This differential gene expression is regulated by the interplay of transcription factors (TFs) binding to *cis*-regulatory elements (CREs) in the genomic DNA, such as promoters and enhancers, forging interactions among the CREs and components of transcriptional apparatus and ultimately leading to patterns of gene activation and repression characteristic of each cell type (Maston et al. 2006; Hamamoto and Fukaya 2022). Epigenetic features such as accessibility of DNA and modifications of histone tails in chromatin have pronounced impacts on the ability of TFs to bind to CREs, and furthermore, they serve as a molecular memory of transcription and repression (Strahl and Allis 2000; Ringrose and Paro 2004). Frequently co-occurring sets of chromatin features define epigenetic states, which are associated with gene regulation and expression (Ernst and Kellis 2010; Hoffman et al. 2013; Zhang et al. 2016). Genome-wide assignment of DNA intervals to epigenetic states (annotation) provides a view of the regulatory landscape that can be compared across cell types, which in turn leads to insights into the processes regulating gene expression (Libbrecht et al. 2021).

Comprehensive mapping of CREs within the context of the regulatory landscape in different cell types is needed to achieve a broad understanding of differential gene expression. Maps of candidate CREs (cCREs) provide guidance in understanding how changes in cCREs, including single nucleotide variants and indels, can lead to altered expression (Hardison 2012), and they can inform approaches for activation or repression of specific genes in potential strategies for therapies (Bauer et al. 2013). Indeed, most human genetic variants associated with common traits and diseases are localized in or near cCREs (Hindorff et al. 2009; Maurano et al. 2012; The_ENCODE_Project_Consortium 2012). Thus, knowledge of the activity and epigenetic state

of cCREs in each cell type can facilitate understanding the impact of trait-associated genetic variants on specific phenotypes. Furthermore, genome editing approaches in somatic cells have recently been demonstrated to have promise as therapeutic modalities (Frangoul et al. 2021), and a full set of cCREs annotated by activity and state can help advance similar applications.

The different types of blood cells in humans and mice are particularly tractable systems for studying many aspects of gene regulation during differentiation. The striking differences among mature cell types result from progressive differentiation starting from a common hematopoietic stem cell (HSC) (Kondo et al. 2003). While single cell analyses reveal a pattern of ostensibly continuous expression change along each hematopoietic lineage (Laurenti and Göttgens 2018), intermediate populations of multi-lineage progenitor cells with decreasing differentiation potential have been defined, which provide an overall summary and nomenclature for major stages in differentiation. These stem, progenitor, and mature cell populations can be isolated using characteristic cell surface markers (Spangrude et al. 1988; Payne and Crooks 2002), albeit with dramatically fewer cells in progenitor populations. In addition to the primary blood cells, several immortalized cell lines provide amenable systems for intensive study of various aspects of gene regulation during differentiation and maturation of blood cells (Weiss et al. 1997).

The VISION project aims to produce a **V**al**i**dated **S**ystematic **I**ntegrati**on** of hematopoietic epigenomes, harvesting extensive epigenetic and transcriptomic datasets from many investigators and large consortia into concise, systematically integrated summaries of regulatory landscapes and cCREs (Hardison et al. 2020). We previously published the results of these analyses for progenitor and mature blood cell types from mouse (Xiang et al. 2020). In the current study, we generated additional epigenetic datasets and compiled data from human blood cells to expand the integrative analyses to include data from both human and mouse.

Importantly, the systematic integrative analysis of epigenetic features across blood cell types was conducted jointly in both species to learn epigenetic states, generate concise views of epigenetic landscapes, and predict regulatory elements that are comparable in both species. This joint modeling enabled further comparisons using approaches that were not dependent on DNA sequence alignments between species, including a demonstration of the role of orthologous transcription factors in cell type-specific regulation in both species. An exploration of comparisons of epigenetic landscapes between species showed that they were informative for inferring regulatory roles of elements in lineage-specific (i.e., non-aligning) DNA. Together, this work provides valuable community resources that enable researchers to leverage the extensive existing epigenomic data into further mechanistic regulatory studies of both individual loci and genome-wide trends in human and mouse blood cells.

Results

Extracting and annotating epigenetic states by modeling epigenomic information jointly in human and mouse

A large number of data sets of epigenetic features related to gene regulation and expression (404 data sets, 216 in human and 188 in mouse; Fig. 1, Supplemental Material “Data generation and collection”, Supplemental Tables S1 and S2) served as the input for our joint integrative analysis of human and mouse regulatory landscapes across progenitor and mature blood cell types. The features included chromatin accessibility, which is a hallmark of almost all regulatory elements, occupancy by the structural protein CTCF, and histone modifications associated with gene activation or repression. After normalizing and denoising these diverse data sets (Supplemental Fig. S1), we conducted an iterative joint modeling to discover epigenetic states, i.e., sets of epigenetic features commonly found together, in a consistent manner for both

human and mouse blood cells (Fig. 2). The joint modeling took advantage of the Bayesian framework of the Integrative and Discriminative Epigenomic Annotation System, or IDEAS (Zhang et al. 2016; Zhang and Hardison 2017), to iteratively learn states in both species. The joint modeling proceeded in four steps: initial training on randomly selected regions in both species, retaining the 27 epigenetic states that exhibit similar combinatorial patterns of features in both human and mouse, using these 27 states as prior information to sequentially run the IDEAS genome segmentation on the human and mouse data sets, and removal of two heterogenous states (Fig. 2A and Supplemental Figs. S2, S3, S4, and S5). This procedure ensured that the same set of epigenetic states was learned and applied for both species. Previously, the segmentation and genome annotation (Libbrecht et al. 2021) method chromHMM (Ernst and Kellis 2012) was used to combine data between species by concatenating the datasets for both human and mouse cell types (Yue et al. 2014). This earlier approach produced common states between species, but it did not benefit from the positional information and automated approach to handling missing data that are embedded in IDEAS.

The resulting model with 25 epigenetic states (Fig. 2B) was similar to that obtained from mouse blood cell data (Xiang et al. 2020). The states captured combinations of epigenetic features characteristic of regulatory elements such as promoters and enhancers, transcribed regions, repressed regions marked by either Polycomb (H3K27me3) or heterochromatin (H3K9me3), including states that differ quantitatively in the contribution of specific features to each state. For example, H3K4me1 is the predominant component of states E1 and E, but E1 has a lower contribution of that histone modification. Similar proportions of the genomes of human and mouse were covered by each state (Fig. 2B).

Assigning all genomic bins in human and mouse to one of the 25 states in each hematopoietic cell type produced an annotation of blood cell epigenomes that gave a concise view of the

178 epigenetic landscape and how it changes across cell types, using labels and color conventions
179 consistently for human and mouse. The value of this concise view can be illustrated in
180 orthologous genomic intervals containing genes expressed preferentially in different cell
181 lineages as well as genes that are uniformly expressed (Fig. 2C, D). For example, the gene
182 *SLC4A1*/*Slc4a1*, encoding the anion transporter in the erythrocyte plasma membrane, is
183 expressed in the later stages of erythroid maturation (Dore and Crispino 2011). The epigenetic
184 state assignments across cell types matched the differential expression pattern, with genomic
185 intervals in the gene and its flanking regions, including a non-coding gene located upstream (to
186 its right, *Bloodlinc* in mouse), assigned to states indicative of enhancers (yellow and orange)
187 and promoters (red) only in erythroid cell types, with indications of stronger activation in the
188 more mature erythroblasts (region boxed and labeled E in Fig. 2 C, D). A similar pattern was
189 obtained in both human and mouse. Those genomic intervals assigned to the enhancer- or
190 promoter-like states contain candidates for regulatory elements, an inference that was
191 supported by chromatin binding data including occupancy by the transcription factor GATA1 (Xu
192 et al. 2012; Pimkin et al. 2014) and the co-activator EP300 (ENCODE datasets ENCSR000EGE
193 and ENCSR982LJQ) in erythroid cells. Similarly, the gene and flanking regions for *GRN*/*Grn*,
194 encoding the granulin precursor protein that is produced at high levels in granulocytes and
195 monocytes (Jian et al. 2013), and *ITGA2B*/*Itga2b*, encoding the alpha 2b subunit of integrin that
196 is abundant in mature megakaryocytes (van Pampus et al. 1992; Pimkin et al. 2014), were
197 assigned to epigenetic states indicative of enhancers and promoters in the expressing cell types
198 (boxed regions labeled G and MK, respectively). In contrast, genes expressed in all the blood
199 cell types, such as *UBTF*/*Ubtf*, were assigned to active promoter states and transcribed states
200 across the cell types. We conclude that these concise summaries of the epigenetic landscapes
201 across cell types showed the chromatin signatures for differential or uniform gene expression
202 and revealed discrete intervals as potential regulatory elements, with the consistent state

assignments often revealing similar epigenetic landscapes of orthologous genes in human and mouse.

While these resources are useful, some limitations should be kept in mind. For example, IDEAS used data from similar cell types to improve state assignments in cell types with missing data, but the effectiveness of this approach may be impacted by the pattern of missing data. In particular, the epigenetic data on human stem and progenitor cell types were largely limited to ATAC-seq data, whereas histone modification data and CTCF occupancy were available for the analogous cell types in mouse (Fig. 1). Thus, the state assignments for epigenomes in human stem and progenitor cells may be less robust compared to those for similar cell types in mouse. Another limitation is the broad range of quality in the data sets that cannot be completely adjusted by normalization, which leads to over- or under-representation of some epigenetic signals in specific cell types (Supplemental Fig. S5). Despite these limitations, the annotation of blood cell epigenomes after normalization and joint modeling of epigenetic states produced a highly informative painting of the activity and regulatory landscapes across the genomes of human and mouse blood cells.

Candidate *cis*-regulatory elements in human and mouse

We define a candidate *cis*-regulatory element, or cCRE, as a DNA interval with a high signal for chromatin accessibility in any cell type (Xiang et al. 2020). We utilized a version of the IDEAS methodology to combine peaks of accessibility across different cell types, running it in the signal intensity state (IS) mode only on chromatin accessibility signals (Xiang et al. 2021), which helps counteract excessive expansion of peak calls when combining them (Supplemental Fig. S6).

Employing the same peak-calling procedure to data from human and mouse resulted in 200,342 peaks of chromatin accessibility for human and 96,084 peaks for mouse blood cell types

(Supplemental Table S3). Applying the peak caller MACS3 (Zhang et al. 2008) on the same human ATAC-seq data generated a larger number of peaks, but those additional peaks tended to have low signal and less enrichment for overlap with other function-related genomic datasets (Supplemental Fig. S7).

The ENCODE Project released regulatory element predictions in a broad spectrum of cell types in the Index of DHSs (Meuleman et al. 2020) and the SCREEN cCRE catalog (The_ENCODE_Project_Consortium et al. 2020), using data that were largely different from those utilized for the VISION analyses. Almost all the VISION cCRE calls in human blood cells were included in the regulatory element predictions from ENCODE (Supplemental Fig. S8A), supporting the quality of the VISION cCRE calls. Furthermore, as expected from its focus on blood cell types, the VISION cCRE catalog shows stronger enrichment for regulatory elements active in blood cells (Supplemental Fig. S8B, Supplemental Table S4).

Enrichment of the cCRE catalog for function-related elements and trait-associated genetic variants

Having generated catalogs of cCREs along with an assignment of their epigenetic states in each cell type, we characterized the human cCREs further by connecting them to orthogonal (not included in VISION predictions) datasets of DNA elements implicated in gene regulation or in chromatin structure and architecture (termed structure-related) (Fig. 3A, Supplemental Fig. S9, Supplemental Table S5). About two-thirds (136,664 or 68%) of the VISION human cCREs overlapped with elements in the broad groups of CRE-related (97,361 cCREs overlapped) and structure-related (83,327 cCREs overlapped) elements, with 44,024 cCREs overlapping elements in both categories (Fig. 3A, B). In contrast, ten sets of randomly chosen DNA intervals, matched in length and GC-content with the human cCRE list, showed much less overlap with the orthogonal sets of elements (Fig. 3B). Of the CRE-related superset, the enhancer-related

group of datasets contributed the most overlap with VISION cCREs, followed by SuRE peaks, which measure promoter activity in a massively parallel reporter assay (van Arensbergen et al. 2017), and CpG islands (Fig. 3C). Compared to overlaps with the random matched intervals, the VISION cCREs were highly enriched for overlap with each group of CRE-related datasets (Fig. 3C). Of the structure-related superset, the set of CTCF occupied segments (OSs) contributed the most overlap, followed by chromatin loop anchors, again with high enrichment relative to overlaps with random matched sets (Fig. 3D). Considering the VISION cCREs that intersected with both structure- and CRE-related elements, major contributors were the cCREs that overlap with enhancers and CTCF OSs or loop anchors (Supplemental Fig. S10). Furthermore, the VISION cCREs captured known blood cell CREs (Supplemental Table S4) and CREs demonstrated to impact a specific target gene in a high throughput analysis (Gasperini et al. 2019) (Fig. 3E). We conclude that the intersections with orthogonal, function- or structure-related elements lent strong support for the biological significance of the VISION cCRE calls and added to the annotation of potential functions for each cCRE.

The catalog of VISION human blood cell cCREs showed a remarkable enrichment for genetic variants associated with blood cell traits, further supporting the utility of the catalog. We initially observed a strong enrichment by overlap with variants from the NHGRI-EBI GWAS Catalog (Buniello et al. 2019) associated with blood cell traits (Supplemental Fig. S11). We then analyzed the enrichments while considering the haplotype structure of human genomes, whereby association signals measured at assayed genetic markers likely reflect an indirect effect driven by linkage disequilibrium (LD) with a causal variant (that may or may not have been genotyped). We employed stratified linkage disequilibrium score regression (sLDSC, Finucane et al. 2015) to account for LD structure and estimate the proportion of heritability of each trait explained by a given genomic annotation, quantifying the enrichment of heritability in 587 traits from the UK Biobank (UKBB) GWAS (Ge et al. 2017 and http://www.nealelab.is/uk-

biobank/) within the VISION cCREs relative to the rest of the genome (Supplemental Material section “Stratified linkage disequilibrium score regression”). Importantly, the traits encompassed 54 “blood count” traits that measure properties including size and counts of specific blood cell types, 60 “blood biochemistry” traits that measure lipid, enzyme, and other molecular concentrations within whole blood samples, and 473 non-blood-related traits, allowing us to assess the specific relevance of the cCREs to regulation of blood-related versus other phenotypes. At a 5% FDR threshold, we discovered 53 traits for which cCREs were significantly enriched in heritability (Fig. 3F). Strikingly, 52 (98%) of these traits were blood-related, of which 50 were blood count traits, representing 93% of all UKBB blood count traits included in our analysis. The remaining 2 significant traits pertained to blood biochemistry, specifically, the male and female glycated hemoglobin concentrations. These metrics and observations together lend support to the VISION cCRE annotation being composed of informative genomic regions associated with regulation of genes involved in development of blood cell traits.

Estimates of regulatory impact of cCREs during differentiation

The epigenetic states assigned to cCREs can reveal those that show changes in apparent activity during differentiation. Inferences about the activity of a cCRE in one or more cell types can be based on whether the cCRE was actuated, i.e., was found in a peak of chromatin accessibility, and which epigenetic state was assigned to the actuated cCRE. Those states can be associated with activation (e.g., enhancer-like or promoter-like) or repression (e.g., associated with polycomb or heterochromatin). In addition to these categorical state assignments, quantitative estimates of the impact of epigenetic states on expression of target genes are useful, e.g., to provide an estimate of differences in inferred activity when the states change. Previous work used signals from single or multiple individual features such as chromatin accessibility or histone modifications in regression modeling to explain gene expression (e.g., Karlić et al. 2010; Dong et al. 2012), and we applied a similar regression

307 modeling using epigenetic states as predictor variables to infer estimates of regulatory impact of
308 each state on gene expression (Xiang et al. 2020).
309
310 We used state assignments of cCREs across cell types in a multivariate regression model to
311 estimate the impact of each state on the expression of local genes (Supplemental Material,
312 “Estimation of the impact of epigenetic states and cCREs on gene expression”). That impact
313 was captured as β coefficients, which showed the expected strong positive impact for promoter
314 and enhancer associated states and negative impacts from heterochromatin and polycomb
315 states (Fig. 4A). The β coefficients were then used in further analysis, such as estimating the
316 change in regulatory impact as a cCRE shifts between states during differentiation (difference
317 matrix to the left of the β coefficient values in Fig. 4A). The β coefficient values also were used
318 to generate an epigenetic state Regulatory Potential (esRP) score for each cCRE in each cell
319 type, calculated as the β coefficient values for the epigenetic states assigned to the cCRE
320 weighted by the coverage of the cCRE by each state (Fig. 4B). These esRP scores were the
321 basis for visualizing the collection of cCREs and how their regulatory impact changed across
322 differentiation (Supplemental Fig. S12 and Supplemental movie S1). Comparison of the
323 integrative esRP scores with signal intensities for single features (ATAC-seq and H3K27ac)
324 showed all were informative for visualizations, and esRP performed slightly better than the
325 single features in differentiating cCREs based on locations within gene bodies (Supplemental
326 Fig. S13).
327
328 In addition, we explored the utility of the esRP scores for clustering the cCREs into groups with
329 similar activity profiles across blood cell types in both human and mouse. Focusing on the esRP
330 scores in 12 cell types shared between human and mouse along with the average across cell
331 types, we identified clusters jointly in both species. The clustering proceeded in three steps,
332 specifically finding robust K-means clusters for the combined human and mouse cCREs,

identifying the clusters shared by cCREs in both species, and then further grouping those shared K-means clusters hierarchically to define fifteen joint metaclusters (JmCs) (Supplemental Fig. S14). Each cCRE in both mouse and human was assigned to one of the fifteen JmCs, and each JmC was populated with cCREs from both mouse and human.

These JmCs established discrete categories for the cCREs based on the cell type distribution of their inferred regulatory impact (Fig. 4C). The clusters of cCREs with high esRP scores across cell types were highly enriched for promoter elements (Supplemental Fig. S15A). The cell type-restricted clusters of cCREs showed enrichment both for selected enhancer catalogs and for functional terms associated with those cell types (Supplemental Fig. S15A and B). Furthermore, clustering of human genes by the JmC assignments of cCREs in a 100kb interval centered on their TSS (Supplemental Material section “Enrichment of JmCs assigned to cCREs in gene loci”) revealed a strong enrichment for JmCs with high activity in the cell type(s) in which the genes are expressed (Fig. 4D). Examples include *IFNG* showing enrichment for JmC 12, which has high esRP scores in T and NK cells, *CSF1R* showing enrichment for JmC 15, which has high scores in monocytes, and *GATA1* showing enrichment for JmC 10, which has high scores in erythroid cells and megakaryocytes. Moreover, running sLDSC on cCREs in individual JmCs showed enrichment for heritability of blood cell-related traits in some specific JmCs (Supplemental Fig. S16).

As expected from previous work (e.g., Heintzman et al. 2009; Meuleman et al. 2020), similar metaclusters of cCREs were generated based on single signals from the histone modification H3K27ac or chromatin accessibility across cell types (Supplemental Fig. S17). Clustering based any of the three features better resolved individual cell types when larger numbers of clusters were considered, prior to collapsing the shared robust clusters into JmCs (Supplemental Fig. S18).

In summary, we show that the β coefficients and esRP scores provide valuable estimates of regulatory impacts of states and cCREs, respectively. The esRP-driven joint metaclusters provide refined subsets of cCREs that should be informative for investigating cell type-specific and general functions of cCREs. We also built self-organizing maps as a complementary approach to systematic integration of epigenetic features and RNA data across cell types (Supplementary Figure S19, Jansen et al. 2019).

Motif enrichment in joint metaclusters of human and mouse cCREs

We examined the sets of cCREs in each JmC to ascertain enrichment for transcription factor binding site (TFBS) motifs because these enriched motifs suggest the families of transcription factors that play a major role in regulation by each category of cCREs. Furthermore, having sets of cCREs determined and clustered for comparable blood cell types in human and mouse provided the opportunity to discover which TFBS motifs were shared between species and whether any were predominant in only one species.

To find TFBS motifs associated with each JmC, we calculated enrichment for all non-redundant motifs in the Cis-BP database (Weirauch et al. 2014) using Maelstrom from GimmeMotifs (Bruse and van Heeringen 2018) (Supplemental Material “Enrichment for transcription factor binding site motifs in joint metaclusters of cCREs”). The results confirmed previously established roles of specific TFs in cell lineages and showed little evidence for novel motifs (Fig. 4E). For example, TFBS motifs for the GATA family of transcription factors were enriched in JmCs 2 and 10, which have high esRP scores in progenitor and mature cells in the erythroid and megakaryocytic lineages, as expected for the known roles of GATA1 and GATA2 in this lineage (Blobel and Weiss 2009; Fujiwara et al. 2009). The GATA motif was also enriched in JmC 14, as expected for the role of GATA3 in natural killer (NK) and T cells (Rothenberg and

Taghon 2005). Furthermore, motifs for the known lymphoid transcription factors TBX21, TCF7L1, and LEF1 (Chi et al. 2009) were enriched in cCREs with high esRP scores in NK and T cells (JmCs 9 and 12), and motifs for myeloid-determining transcription factors CEBPA and CEBPB (Graf and Enver 2009) and the myeloid transcription factor PU.1 (Tenen et al. 1997) were enriched in cCREs that are active in progenitor cells and monocytes (JmCs 3 and 15). TFBS motifs for promoter-associated transcription factors such as E2F2 and SP1 (Dynan and Tjian 1983; Kaczynski et al. 2003) were enriched in broadly active cCREs (JmCs 1 and 4). These patterns of motif enrichments in the JmCs fit well with the expectations from previous studies of transcription factor activity across lineages of blood cells, and thus, they lend further credence to the value of the cCRE calls and the JmC groupings for further studies of regulation in the blood cell types.

The genome-wide collection of cCREs across many blood cell types in human and mouse provided an opportunity for an unbiased and large-scale search for indications of transcription factors that may be active specifically in one species for a shared cell type. Prior studies of transcription factors have shown homologous transcription factors used in analogous cell types across species (e.g., Carroll 2008; Noyes et al. 2008; Schmidt et al. 2010; Cheng et al. 2014; Villar et al. 2014), but it is not clear if there are significant exceptions. In our study, we found that for the most part, the motif enrichments were quite similar between the human and mouse cCREs in each JmC. Note that these similarities were not forced by requiring sequence matches between species; the cCREs were grouped into JmCs based on their pattern of activity, as reflected in the esRP scores, across cell types, not by requiring homologous sequences. This similarity between species indicates that the same transcription factors tend to be active in similar groups of cell types in both mouse and human. An intriguing potential exception to the sharing of motifs between species was the enrichment of TFBS motifs for CTCF and ZBTB7A in some JmCs, suggestive of some species selectivity in their binding in the

411 context of other TFs (Supplemental Figs. S20 and S21). These indications of conditional,
412 preferential usage of these TFs in human or mouse could serve as the basis for more detailed
413 studies in the future.
414
415 In summary, after grouping the cCREs in both human and mouse by their inferred regulatory
416 impact across blood cell in a manner agnostic to DNA sequence or occupancy by TFs, the
417 enrichment for TFBS motifs within those groups recapitulated known activities of TFs both
418 broadly and in specific cell lineages. The results also showed considerable sharing of inferred
419 TF activity in both human and mouse.
420
421 **Evolution of sequence and inferred function of cCREs**
422 The human and mouse cCREs from blood cells were assigned to three distinct evolutionary
423 categories (Fig. 5A). About one-third of the cCREs were present only in the reference species
424 (39% for human, 28% for mouse), as inferred from the failure to find a matching orthologous
425 sequence in whole-genome alignments with the other species. We refer to these as
426 nonconserved (N) cCREs. Of the two-thirds of cCREs with an orthologous sequence in the
427 second species, slightly over 30,000 were also identified as cCREs in the second species. The
428 latter cCREs comprise the set of cCREs conserved in both sequence and inferred function,
429 which we call SF conserved (SF) cCREs. Almost the same number of cCREs in both species
430 fall into the SF category; the small difference resulted from interval splits during the search for
431 orthologous sequences (Supplemental Fig. S22). The degree of chromatin accessibility in
432 orthologous SF cCREs was positively correlated between the two species (Supplemental Fig.
433 S23). The remaining cCREs (91,000 in human and 36,000 in mouse) were conserved in
434 sequence but not in an inferred function as a regulatory element, and we call them S conserved
435 (S) cCREs. The latter group could result from turnover of regulatory motifs or acquisition of
436 different functions in the second species.

437

438 The distributions of epigenetic states assigned to the blood cell cCREs in each of the three
439 evolutionary categories were similar between human and mouse, but those distributions differed
440 dramatically among evolutionary categories, with significantly more SF cCREs assigned to
441 promoter-like states than were S or N cCREs (Supplemental Fig. S24). Indeed, the SF cCREs
442 tended to be close to or encompass the TSSs of genes, showing a substantial enrichment in
443 overlap with TSSs compared to the overlap observed for all cCREs (Fig. 5B). Many of the S and
444 N cCREs were assigned to enhancer-like states (Supplemental Fig. S24D), giving a level of
445 enrichment for overlap with enhancer datasets comparable to that observed for the full set of
446 cCREs (Fig. 5B).

447

448 For both human and mouse, the level of sequence conservation, estimated by the maximum
449 phyloP score (Pollard et al. 2010), was higher in the collection of cCREs than in sets of
450 randomly chosen genomic intervals matching the cCREs in length and G+C content (Fig. 5C).
451 Among the evolutionary categories of cCREs, the distribution of phyloP scores for SF cCREs
452 was significantly higher than the distribution for S cCREs, which in turn was higher than that for
453 N cCREs, for both species (Fig. 5C). The whole genome alignments underlying the phyloP
454 scores are influenced by proximity to the highly conserved coding exons (King et al. 2007), and
455 the high phyloP scores of the promoter-enriched SF cCREs could reflect both this effect as well
456 as strong constraint on conserved function (Supplemental Fig. S25). In all three evolutionary
457 categories, the distribution of phyloP scores was higher for promoter-proximal cCREs than for
458 distal ones, but the relative levels of inferred conservation were the same for both, i.e., SF>S>N
459 (Supplemental Fig. S26).

460

In summary, this partitioning of the cCRE catalogs by conservation of sequence and inferred function revealed informative categories that differed both in evolutionary trajectories and in types of functional enrichment.

Comparison of epigenetic states around orthologous genes in human and mouse

The consistent state assignments from the joint modeling facilitated epigenetic comparisons between species. Such comparisons are particularly informative for orthologous genes with similar expression patterns but some differences in their regulatory landscapes. For example, the orthologous genes *GATA1* in human and *Gata1* in mouse each encode a transcription factor with a major role in regulating gene expression in erythroid cells, megakaryocytes, and eosinophils (Ferreira et al. 2005), with a similar pattern of gene expression across blood cell types in both species (Supplemental Fig. S27). The human and mouse genomic DNA sequences aligned around these orthologous genes, including their promoters and proximal enhancers; the alignments continued through the genes downstream of *GATA1*/*Gata1* (Fig. 6A). An additional, distal regulatory element located upstream of the mouse *Gata1* gene, which was bound by GATA1 and EP300 (Fig. 6A), was found only in mouse (Valverde-Garduno et al. 2004). The DNA sequences of the upstream interval harboring the mouse regulatory element did not align between mouse and human except in portions of the *GLOD5*/*Glod5* genes (Fig. 6A). Thus, the interspecies sequence alignments provide limited information about this distal regulatory element.

This limitation to sequence alignments led us to explore whether comparisons of epigenetic information would be more informative, utilizing the consistent assignment of epigenetic states in both human and mouse, which do not rely on DNA sequence alignment. In the large genomic regions (76kb and 101kb in the two species) encompassing the orthologous human *GATA1* and mouse *Gata1* genes and surrounding genes, we computed the correlation for each genomic bin

between the epigenetic state assignments across cell types in one species and that in the other species for all the bins (Supplemental Fig. S28). This local, all-versus-all comparison of the two loci yielded a matrix of correlation values showing similarities and differences in profiles of epigenetic states in the two species (Fig. 6B). The conserved promoter and proximal enhancers of the *GATA1*/*Gata1* genes were highly correlated in epigenetic states across cell types between the two species, in a region of the matrix that encompassed the aligning DNA sequences (labeled Px in Fig. 6B). In contrast, whereas the mouse-specific distal regulatory element did not align with the human DNA sequence, the epigenetic states annotating it presented high correlations with active epigenetic states in the human *GATA1* locus (labeled D in Fig. 6B).

The complexity of the correlation matrix (Fig. 6B) indicated that multiple epigenetic trends could be contributing to the patterns. To systematically reduce the high dimensionality of the matrix to a set of simpler matrices, we employed nonnegative matrix factorization (NMF) because of its interpretability (Stein-O'Brien et al. 2018; Lee and Roy 2021). The decomposed matrices from NMF revealed a set of factors, each of which (represented by each column in the mouse matrix and each row in the human matrix in Fig. 6C) captures a group of highly correlated elements in the original matrix that show a pattern distinct from the rest of the elements. The complex correlation matrix was decomposed into six distinct factors, as determined by the number of factors at which an “elbow” was found in the BIC score (Supplemental Fig. S29). Each factor encapsulated a specific epigenetic regulatory machinery or process exhibiting consistent cross-cell type patterns in both humans and mice (Supplemental Fig. S30). For example, the correlation matrices reconstructed by using signals from factor 3 exclusively highlighted the positive regulators for the *GATA1*/*Gata1* gene loci; these regulatory elements were evident in reconstructed correlation matrices between species (Fig. 6D) and within individual species (Fig. 6E). By applying a Z-score approach to identify peak regions in the factor 3 signal vector (with

FDR < 0.1; Supplemental Material), we pinpointed regions in both species showing an epigenetic regulatory machinery exhibiting positive regulatory dynamics for *GATA1*/*Gata1* gene loci, particularly in the ERY and MK cell types. In contrast, the correlation matrices reconstructed from the signals for factor 6 (Fig. 6F and G) highlighted regions marked by the transcription elongation modification H3K36me3 (epigenetic states colored green, Fig. 6G). The correlations in the factor 6 elongation signature were observed, as expected, between the human/mouse orthologous gene pairs *GATA1* and *Gata1* as well as between human *HDAC6* and mouse *Hdac6* (green rectangles in Fig. 6F). The factor 6 correlations were also observed between the *GATA1*/*Gata1* and *HDAC6*/*Hdac6* genes (black rectangles in Fig. 6F and G), showing a common process, specifically transcriptional elongation, at both loci. A similar analysis for other factors revealed distinct regulatory processes or elements, such as active promoters (factor 2), exhibiting unique cross-cell type patterns (Supplemental Fig. 30). The patterns captured by NMF factors 3 and 6 were robust to the choice of k in the NMF (Supplemental Fig. 31). Overall, these results underscore this method's capability to objectively highlight regulatory regions with analogous epigenetic patterns across cell types in both species. This method could aid in extracting additional information about similar epigenetic patterns between human and model organisms such as mice, for which only a portion of their genome aligns with human.

This comparison of epigenetic state profiles across cell types also provided a means to categorize cCREs between species that did not require a match in the underlying genomic DNA sequence (Supplemental Figs. S32 and S33). That approach revealed indications that certain cCREs were potentially involved in regulation of orthologous genes, even for cCREs with DNA sequences that did not align between species.

In summary, the IDEAS joint modeling on the input data compiled here and the consistent state assignments in both mouse and human confirmed and extended previous observations on known regulatory elements, and they revealed both shared and distinctive candidate regulatory elements and states between species. Correlations of state profiles between species provided a comparison of chromatin landscapes even in regions with DNA sequences that were not conserved between species.

Discussion

In this paper, the VISION consortium introduces a set of resources describing the regulatory landscapes of both human and mouse blood cell epigenomes. A key, novel aspect of our work is that the systematic integrative modeling that generated these resources was conducted jointly across the data from both species, which enabled robust comparisons between species without being limited by sequence alignments, allowing comparisons in non-conserved and lineage-specific genomic regions.

One major resource is the annotation of the epigenetic states across the genomes of progenitor and mature blood cells of both species. These state maps show the epigenetic landscape in a compact form, capturing information from the input data on multiple histone modifications, CTCF occupancy, and chromatin accessibility, and they use a common set of epigenetic states to reveal the patterns of epigenetic activity associated with gene expression and regulation both across cell types and between species. A second major resource is a catalog of cCREs actuated in one or more of the blood cell types in each species. The cCREs are predictions of discrete DNA segments likely involved in gene regulation, based on the patterns of chromatin accessibility across cell types, and the epigenetic state annotations suggest the type of activity

for each cCRE in each cell type, such as serving as a promoter or enhancer, participating in repression, or inactivity. A third major resource is a quantitative estimate of the regulatory impact of human and mouse cCREs on gene expression in each cell type, i.e., an esRP score, derived from multivariate regression modeling of the epigenetic states in cCREs as predictors of gene expression. The esRP scores are a continuous variable capturing not only the integration of the input epigenetic data, but also the inferred impacts on gene expression. Those impacts may be manifested as activation or repression during regulation or as transcriptional elongation. They are useful for many downstream analyses, such as determining informative groups of cCREs by clustering analysis. These resources along with browsers for visualization and tools for analysis are provided at our project website, http://usevision.org. Among these tools is cCRE_db, which records the several dimensions of annotation of the cCREs and provides a query interface to support custom queries from users.

Our human blood cell cCRE catalog should be valuable for mechanistic interpretations of trait-related human genetic variants. Human genetic variants associated with traits intrinsic to blood cells were significantly enriched in the VISION cCRE catalog, whereas variants associated with a broad diversity of other traits were not enriched. We expect that the extensive annotations in our cCRE catalog combined with information about TFBS motifs and TF occupancy should lead to specific, refined hypotheses for mechanisms by which a variant impacts expression, such as alterations in TF binding, which can be tested experimentally in further work.

The jointly learned state maps and cCRE predictions allowed us to extend previous work on the evolution of regulatory elements between mouse and human. Several previous studies focused on transcription factor (TF) occupancy, e.g. examining key TFs in one tissue across multiple species (Schmidt et al. 2010; Ballester et al. 2014; Villar et al. 2014) or a diverse set of TFs in multiple cell types and in mouse and human (Cheng et al. 2014; Yue et al. 2014; Denas et al.

2015). Other studies focused on discrete regions of high chromatin accessibility in multiple cell types and tissues between mouse and human (Stergachis et al. 2014; Vierstra et al. 2014). These previous studies revealed that only a small fraction of elements was conserved both in genomic sequence and in inferred function. A notable fraction of elements changed considerably during mammalian diversification, including turnover of TF binding site motifs and repurposing of elements (Schmidt et al. 2010; Cheng et al. 2014; Stergachis et al. 2014; Denas et al. 2015). These prior studies focused primarily on regions of the genome with sequences that aligned between human and mouse, with the non-aligning regions used to infer that some elements were lineage-specific and that many were derived from transposable elements and endogenous retroviruses (Bourque 2009; Rebollo et al. 2012; Jacques et al. 2013; Sundaram et al. 2014).

Our evolutionary analyses confirmed the previous observations, e.g., finding about one-third of cCREs are conserved in both sequence and inferred function between human and mouse, and further showing that this evolutionary category was highly enriched for proximal regulatory elements. Going beyond the prior studies, our jointly learned epigenetic state maps generated a representation of multiple epigenetic features, not just TF occupancy or chromatin accessibility, and they are continuous in bins across genomes of both species. Thus, they provided a basis for comparisons of the epigenetic profiles between species. These epigenetic comparisons were a strong complement to genomic sequence alignments, allowing us to find elements with similar epigenetic profiles even in genomic regions in which the DNA sequence does not align between species. In the current work, we used both a correlation between profiles of epigenetic states and joint clusterings of cCREs between species by esRP scores as initial explorations of these epigenetic comparisons. Previous work compared epigenetic profiles across species, such as the phylo-HMGP method to find different evolutionary states in multi-species epigenomic data (Yang et al. 2018) and the LECIF scores to find evidence of conservation from functional

615 genomic data (Kwon and Ernst 2021). These approaches are powerful but limited to the
616 genomic regions with DNA sequences that align between the species. Importantly, our
617 approach of correlating epigenetic states is agnostic to the underlying DNA sequence
618 alignments (or absence of them), and thus it complements traditional approaches that rely of
619 DNA sequence alignments to find similar elements. Our inter-species comparisons of loci
620 surrounding pairs of orthologous genes included both DNA segments that align between human
621 and mouse and those that do not. Our detection, even in segments of DNA that do not align
622 between species, of epigenetic similarity indicative of a common role in gene regulation
623 suggests that processes or structures, such as chromatin interactions, chromatin complexes, or
624 molecular condensates, may be conserved between species in a manner that is not fully
625 revealed by comparisons of genome sequences. Hence, further studies of this apparent
626 epigenetic dimension of regulatory conservation may be productive.
627
628 Several innovations were developed to produce the resources introduced here. A major
629 innovation was to extend the IDEAS framework (Zhang et al. 2016) to jointly learn epigenetic
630 states and assign them to annotate the epigenomes in human and mouse blood cells. The
631 IDEAS method employs a Bayesian approach to the modeling to learn the states, which we
632 utilized to bring in states learned from the data in one species as priors for learning states in the
633 data from the second species. Another extension of the IDEAS framework was to learn states
634 based on one feature, specifically ATAC-seq data, defining discrete signal intensity states. This
635 approach was used for calling cCREs, implemented as the IDEAS-IS method (Xiang et al.
636 2021). The approach is relatively simple and benefits from joint modeling across the input
637 datasets. Other methods for predicting cCREs based on chromatin accessibility across many
638 cell types prevented excessive expansion of the summary calls for overlapping peaks by
639 employing a centroid determination for the DNase hypersensitive sites (DHS) index (Meuleman
640 et al. 2020) or by choosing the highest signal peak for the ENCODE cCRE catalog

(The_ENCODE_Project_Consortium et al. 2020). The ENCODE cCRE catalog paired DHS peaks with individual chromatin modifications or CTCF occupancy, which led to complications when data on diagnostic features were missing from some cell types. The IDEAS framework used for the VISION cCRE sets leveraged data in related cell types to ameliorate the impact of missing data.

While the resources introduced here are valuable for many applications, it is prudent to acknowledge their limitations. First, the quality of the products of integrated analyses are limited by the quality and completeness of the input, raw data. We endeavored to reduce the impact of variances in the input data by normalization. The S3V2 procedure (Xiang et al. 2021) systematically normalized the input data to adjust for differences in signal-to-noise and variance in signal across the datasets. Some epigenetic features were not determined in some cell types, and we used the IDEAS method in part because it is able to assign an epigenetic state even in the context of missing data by learning patterns from local similarities in cell types for which the data are present (Zhang and Mahony 2019). However, these approaches cannot completely overcome all issues with variance in input data, and further developments in these directions (such as Shahraki et al. 2023; Xiang et al. 2023) may help to improve integrative resources. Second, the resolution of both the epigenetic state assignments and the cCRE inference is limited to 200 bp, which is the window size we utilized in the IDEAS analyses. Other resources, such as DHS calls (Meuleman et al. 2020), DNase footprints (Vierstra et al. 2020), and motif instances (Weirauch et al. 2014), achieve a higher resolution. Indeed, one can use these higher resolution datasets to derive further information about cCREs, such as families of TFs that are likely to be binding to them. Regarding esRP scores, a third limitation is that we do not make explicit assignments for target genes of cCREs. Predictions of a large number of target gene-cCRE pairs were made in our prior work (Xiang et al. 2020); these assignments cover large genomic intervals around each gene and are most useful when used with further filtering, such

as restricting cCREs and target genes to the same topologically associated domains. On-going work is examining other models and approaches for assigning likely target genes to cCREs. A fourth limitation is that our inference of repression-related cCREs apply only to those with stable histone modifications. Elements that had been involved in initiation of repression but eventually were packaged into quiescent chromatin, e.g., via a hit-and-run mechanism (Shah et al. 2019), would not be detected. A fifth limitation concerns the scale of the studies of epigenetic conservation by correlations of epigenetic states. Our current approach is limited to individual examination of specific genetic loci, since we used orthologous genes as the initial anchors, and it is likely that a direct application to whole chromosomes or genomes would generate high false discovery. Exploring ways to expand the scale of the analytical approach is a goal of future research. Finally, the work presented here was restricted to blood cell types. In future work, extension of the approaches developed in this study to a broader spectrum of cell types would expand the utility of the resulting resources.

In conclusion, we present several important new resources to enable further and more detailed studies of gene regulation in human and mouse blood cells both during normal differentiation and in pathological contexts. The patterns of epigenetic states in cCREs across cell types show value in developing an understanding of how genetic variants impact blood cell traits and diseases. Furthermore, the joint modeling between species opens avenues for further exploration of comparisons of epigenetic landscapes in addition to sequence alignments for insights into evolution and function of regulatory elements between species.

Methods

Data generation, collation, normalization, and integration

692 The data sets used as input, including the ones generated for the work reported here (with
693 methods), are described in Supplemental Material section “Data generation and collection” and
694 Supplemental Tables S1 and S2. The S3V2 approach (Xiang et al. 2021) was used for
695 normalization and denoising the data sets prior to integration. The data sets were integrated to
696 find and assign epigenetic states using IDEAS (Zhang et al. 2016; Zhang and Hardison 2017);
697 the extension of this approach to joint learning and annotation between species is described in
698 Supplemental Material sections “Data normalization” and “Joint systematic integration of human
699 and mouse blood cell epigenomes by IDEAS”.
700
701 **Prediction, annotation, and estimation of regulatory impact of cCREs**
702 The identification of cCREs as peaks of chromatin accessibility employed IDEAS in the signal
703 intensity state (IS) mode (Xiang et al. 2021). This approach and comparisons with MACS peaks
704 (Zhang et al. 2008) are described in Supplemental Material section “Prediction of VISION
705 cCREs using IDEAS-IS”. The cCREs are provided in Supplemental Table S3. Annotation of
706 potential cCRE functions used intersections with orthogonal data sets of elements implicated in
707 regulation or chromatin structure (Supplemental Table S5). Enrichment of genetic variants
708 associated with blood cell traits used stratified linkage disequilibrium score regression (sLDSC,
709 Finucane et al. 2015). The impact of epigenetic states in cCREs on regulation of gene
710 expression used a multivariate linear regression approach like one described previously (Xiang
711 et al. 2020). Methods and supplementary results on these analyses are presented in detail in
712 the Supplemental Material.
713
714 **Identification of clusters of cCREs based on epigenetic regulatory potential scores**
715 The sets of human and mouse cCREs were placed jointly into groups based on their epigenetic
716 regulatory potential (esRP) scores using a series of K-means clustering steps, as described in
717 detail in Supplemental Material and Supplementary Fig. S14. Methods and results for

718 enrichment of the resulting joint meta-clusters (JmCs) for orthogonal sets of regulatory elements
719 and SNPs associated with blood cell traits, along with comparisons of clusters based on
720 chromatin accessibility and H3K27ac signal, are described in Supplemental Material and
721 Supplementary Figs. S15 - S18. Motifs that were differentially enriched across JmCs were
722 identified using the Maelstrom tool in the GimmeMotifs suite (v0.17.1) (Bruse and van
723 Heeringen 2018) and SeqUnwinder (Kakumanu et al. 2017), as described in detail in
724 Supplemental Material and Supplementary Fig. S21.
725
726 **Partitioning cCREs to evolutionary categories based on DNA sequence alignments and**
727 **cCRE calls between species**
728 The human and mouse cCREs were assigned to three evolutionary categories using the
729 following procedure. The set of human cCREs was mapped to mouse genome assembly mm10
730 using the liftOver tool at the UCSC Genome Browser (Hinrichs et al. 2006). Human cCREs that
731 failed to map to mm10 were grouped as N cCREs. Matches to mouse cCREs for the human
732 cCREs that could be mapped by liftOver to mm10 were determined using the intersect tool in
733 BEDTools (Quinlan and Hall 2010). Human cCREs that overlapped with mouse cCREs were
734 labeled as SF cCREs, while human cCREs that mapped to mm10 but did not match mouse
735 cCREs were labeled as S cCREs. A similar process was performed on the set of mouse cCREs
736 using liftOver to map to human genome build GRCh38/hg38.
737
738 **Calculation of pairwise correlation coefficients for epigenetic landscapes between**
739 **human and mouse**
740 A bin-to-bin pairwise correlation analysis was used to quantify the similarity of epigenetic
741 landscapes between two DNA regions in human and mouse. For each 200bp bin in one cell
742 type in one species, the assigned epigenetic state was replaced by a vector of mean signals of
743 8 epigenetic features in the IDEAS state model. After replacing the states in all 15 matched cell

744 types (14 analogous cell types and one pseudo-cell type with average values for all cell types)
745 in the two species, the original two categorical state vectors with 15 elements were converted
746 into two numeric vectors with 120 numbers (Supplemental Fig. S28). The similarity of cross-cell
747 type epigenetic landscape between two bins in the two species was defined as the correlation
748 coefficient between each pair of numeric vectors with 120 numbers. When calculating the
749 correlation coefficients, we added random noise (mean=0, sd=0.2) to the raw values to avoid
750 high correlation coefficients created between regions with states that have low signals. The
751 complex correlation matrix was decomposed into distinctive factors using Nonnegative Matrix
752 Factorization (Lee and Seung 1999). Methods and supplementary results on these analyses are
753 presented in detail in the Supplemental Material.
754

755 ## Data access

756 All raw and processed sequencing data generated in this study have been submitted to the
757 NCBI Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo/) under accession
758 number GSE229101 and the NCBI BioProject database
759 (https://www.ncbi.nlm.nih.gov/bioproject/) under accession number PRJNA952902. Resources
760 developed in the VISION project are available at the website https://usevision.org; the data can
761 be viewed via a track hub at the UCSC Genome Browser or any compatible browser by using
762 this URL: https://usevision.org/data/trackHub/hub.txt or by clicking the track hubs link at
763 usevision.org. The database cCRE db supports flexible user queries on extensive annotation of
764 the cCREs, including epigenetic states and esRP scores across cell types, chromatin
765 accessibility scores across cell types, membership in JmCs, and evolutionary categories. Code
766 developed for this study is in the Supplemental Material and at these GitHub repositories:
767 https://github.com/guanjue/Joint_Human_Mouse_IDEAS_State for the joint human-mouse
768 IDEAS pipeline and https://github.com/usevision/cre_heritability for the sLDSC analysis.

Competing interest statement

The authors declare no competing interests.

Acknowledgments

This work was supported by grants from the National Institutes of Health: R24DK106766 to RCH, GAB, MJW, YZ, FY, JT, MS, DB, DH, JRH, BG; R01DK054937 to GAB; R01GM121613 to YZ and SM; R01GM109453 to QL; R35GM133747 to RCM; F31HG012900 to DJT; R01HG011139; National Science Foundation DBI CAREER 2045500 to SM, and intramural funds from the National Human Genome Research Institute. We dedicate this paper to the memory of JT.

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1015 **Figure Legends**

1016 **Figure 1. Cell types and data sets used for systematic integration of epigenetic features**
1017 **of blood cells. (A)** The tree on the left shows the populations of stem, progenitor, and mature
1018 blood cells and cell lines in human. The diagram on the right indicates the epigenetic features
1019 and transcriptomes for which genome-wide data sets were generated or collected, with
1020 distinctive icons for the major sources of data, specifically the Blueprint project (Martens and

Stunnenberg 2013; Stunnenberg et al. 2016), Corces et al. (2016), abbreviated CMB, and St. Jude Children's Research Hospital (SJCRH, Cheng et al. 2021; Qi et al. 2021). (B) Cell types and epigenetic data sets in mouse, diagrammed as for panel A. Sources were described in Xiang et al. (2020) and Supplemental Table S1. Abbreviations for blood cells and lines are: HSC = hematopoietic stem cell, MPP = multipotent progenitor cell, LMPP = lymphoid-myeloid primed progenitor cell, CMP = common myeloid progenitor cell, MEP = megakaryocyte-erythrocyte progenitor cell, K562 = a human cancer cell line with some features of early megakaryocytic and erythroid cells, HUDEP = immortalized human umbilical cord blood-derived erythroid progenitor cell lines expressing fetal globin genes (HUDEP1) or adult globin genes (HUDEP2), CD34_E = human erythroid cells generated by differentiation from CD34+ blood cells, ERY = erythroblast, RBC = mature red blood cell, MK = megakaryocyte, GMP = granulocyte monocyte progenitor cell, EOS = eosinophil, MON = monocyte, MONp = primary monocyte, MONc = classical monocyte, NEU = neutrophil, CLP = common lymphoid progenitor cell, B = B cell, NK = natural killer cell, TCD4 = CD4+ T cell, TCD8 = CD8+ T cell, LSK = Lin-Sca1+Kit+ cells from mouse bone marrow containing hematopoietic stem and progenitor cells, HPC7 = immortalized mouse cell line capable of differentiation in vitro into more mature myeloid cells, G1E = immortalized mouse cell line blocked in erythroid maturation by a knockout of the *Gata1* gene and its subline ER4 that will further differentiate after restoration of *Gata1* function in an estrogen inducible manner (Weiss et al. 1997), MEL = murine erythroleukemia cell line that can undergo further maturation upon induction (designated iMEL), CFUE = colony forming unit erythroid, FL = designates ERY derived from fetal liver, BM = designates ERY derived from adult bone marrow, CFUMK = colony forming unit megakaryocyte, iMK = immature megakaryocyte, MK_fl = megakaryocyte derived from fetal liver.

Figure. 2. Genome segmentation and annotation jointly between human and mouse using IDEAS. (A) Workflow for joint modeling. (1) Initial epigenetic states from 100 randomly selected

regions separately in human and mouse hematopoietic cell types were identified in IDEAS runs. (2) States that were reproducible and shared in both species were retained. (3a and 3b) The profile of epigenetic feature contribution to each of the reproducible states was sequentially refined by applying IDEAS across the full genomes of human and of mouse, updating the state model after each IDEAS run. (4) Two heterogeneous states were removed to generate the final joint epigenetic states in the two species. **(B)** The 25 joint epigenetic states for human and mouse hematopoietic cell types. The average signal of the epigenetic features for each state are shown in the heatmap. The corresponding state colors, the state labels based on the function, and the average proportions of the genome covered by each state across cell types are listed on the right-side of the heatmap. **(C)** Annotation of epigenetic states in a large genomic interval containing *SLC4A1* and surrounding genes across human blood cell types. The genomic interval is 210kb, GRCh38 chr17:44,192,001-44,402,000, with gene annotations from GENCODE V38. Binding patterns for selected transcription factors are from the VISION project ChIP-seq tracks (CTCF and GATA1 in adult erythroblasts, signal tracks from MACS, track heights 100 and 80, respectively) or from the ENCODE data portal (EP300 in K562 cells, experiment ENCSR000EGE, signal track is fold change over background, track height is 50). The epigenetic state assigned to each genomic bin in the different cell types is designated by the color coding shown in panel (B). The replicates in each cell type examined in Blueprint are labeled by the id for the donor of biosamples. Genes and regulatory regions active primarily in erythroid (E), granulocytes (G), and megakaryocytes (MK) are marked by gray rectangles. **(D)** Annotation of epigenetic states in a large genomic interval containing *Slc4a1* and surrounding genes across mouse blood cell types. The genomic interval is 198kb, mm10 chr11:102,290,001-102,488,000, with gene annotations from GENCODE VM23. Binding patterns for selected transcription factors are from the VISION project ChIP-seq tracks (CTCF in adult erythroblasts, GATA1 and EP300 from the highly erythroid fetal liver, signal tracks from MACS, track heights 200, 200, and 150, respectively; the EP300 track was made by re-mapping reads from

ENCODE experiment ENCSR982LJQ). The tracks of epigenetic states and highlighted regions are indicated as in panel (C).

Figure. 3. Overlaps of VISION cCREs with other catalogs and enrichment for variants associated with blood cell traits. (A) Venn diagram showing intersections of human VISION cCREs with a combined superset of elements associated with nuclear structure (CTCF OSs, loop anchors, and TAD boundaries) and with a combined superset of DNA intervals associated with *cis*-regulatory elements (CREs), including TSSs, CpG islands, peaks from a massively parallel promoter and enhancer assay, and enhancers predicted from enhancer RNAs, peaks of binding by EP300, and histone modifications in erythroblasts (see Supplemental Material, Supplemental Fig. S9, and Supplemental Table S5). **(B)** The proportions of cCREs and randomly selected, matched sets of intervals in the overlap categories are compared in the bar graph. For the random sets, the bar shows the mean, and the dots show the values for each of ten random sets. **(C)** The UpSet plot provides a higher resolution view of intersections of VISION cCREs with the four groups of CRE-related elements, specifically enhancer-related (Enh), transcription start sites (TSS), Survey of Regulatory Elements (SuRE), and CpG islands (CpG). The enrichment for the cCRE overlaps compared to those in randomly selected, matched sets of intervals are shown in the boxplots below each overlap subset, with dots for the enrichment relative to individual random sets. **(D)** Overlaps and enrichments of VISION cCREs for three sets of structure-related elements, specifically CTCF OSs (CT), loop anchors (LA), and TAD boundary elements. **(E)** Overlaps of VISION cCREs with two sets of experimentally determined blood cell cCREs. **(F)** Enrichment of SNPs associated with blood cell traits from UK Biobank in VISION cCREs. Results of the sLDSC analysis of all cCREs are plotted with enrichment of the cCRE annotation in heritability of each trait on the x-axis, and the significance of the enrichment on the y-axis. The analysis covers 292 unique traits with GWAS results from both males and females and 3 traits with results only from males. The vertical dotted line

indicates an enrichment of 1, and the horizontal dotted line delineates the 5% FDR significance threshold. Points and labels in red represent traits for which there was significant enrichment of SNPs associated with the VISION cCREs. Traits with a negative enrichment were assigned an arbitrary enrichment of 0.1 for plotting and appear as the column of points at the bottom left of the plot. The shape of the point indicates the sex in which the GWAS analysis was performed for each trait.

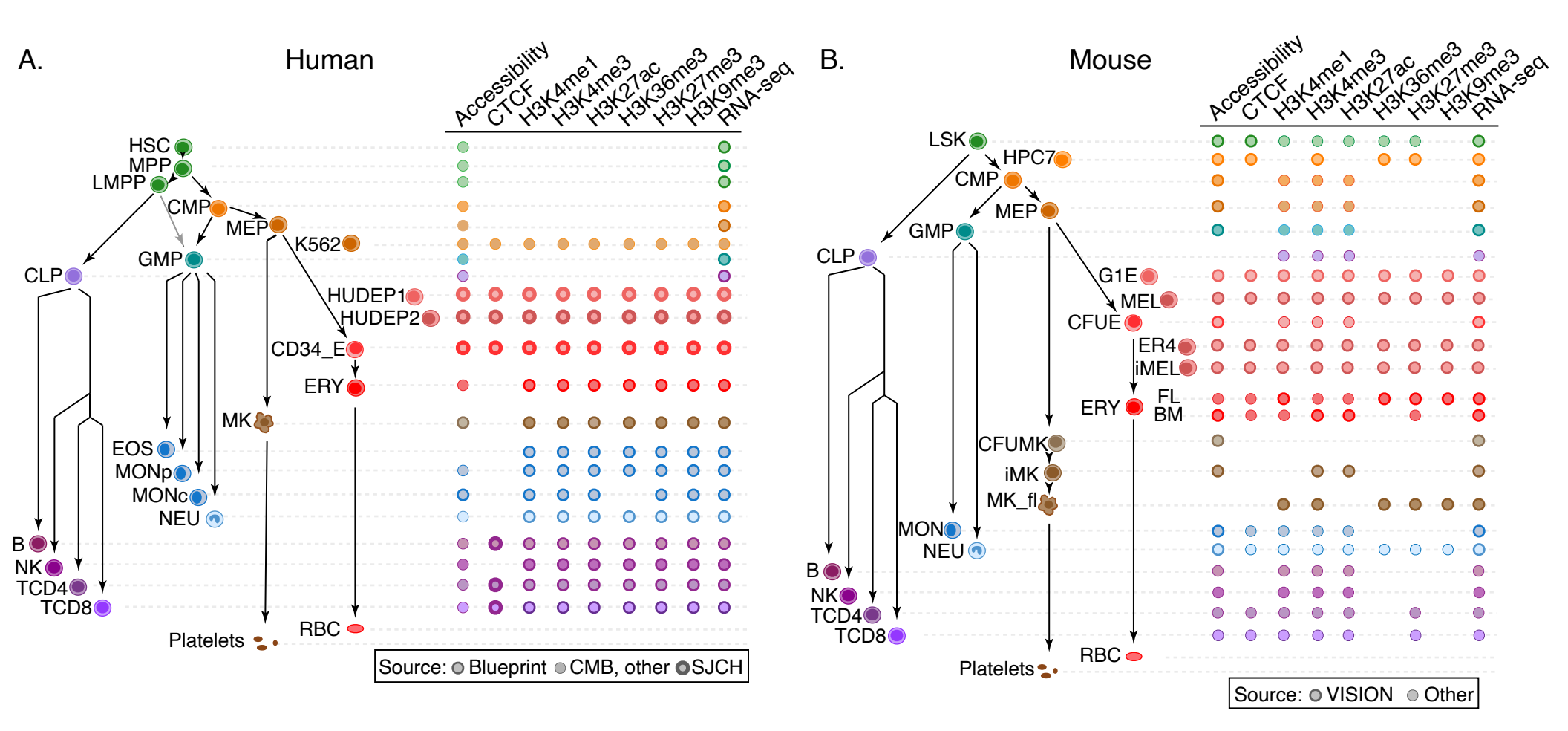
Figure 4. Beta coefficients of states, esRP scores of cCREs, joint human-mouse metaclusters of cCREs based on esRP scores, and enrichment for TFBS motifs. (A) Beta coefficients and the difference of beta coefficients of the 25 epigenetic states. The vertical columns on the right show the beta coefficients along with the ID, color, and labels for the 25 joint epigenetic states. The triangular heatmap shows the difference of the beta coefficients between two states in the right columns. Each value in the triangle heatmap shows the difference in beta coefficients between the state on top and the state below based on the order of states in the right columns. **(B)** An example of calculating esRP score for a cCRE in a cell type based on the beta coefficients of states. For a cCRE covering more than one 200bp bin, the esRP equals the weighted sum of beta coefficients of states that covers the cCRE, where the weights are the region covered by different states. **(C)** The average esRP score of all cCREs in JmCs across blood cell types shared by human and mouse. The right column shows the number of human cCREs in each JmC. **(D)** The average enrichment of JmCs in 15 homologous gene clusters. The genes are clustered based on the JmCs' enrichments by K-means. **(E)** Motifs enriched in joint metaclusters. The top heatmap shows the enrichment of motifs in the cCREs in each JmC in human (H) and mouse (M) as a Z-score. The logo for each motif is given to the right of the heat map, labeled by the family of transcription factors that recognize that motif. The heatmap below is aligned with the motif enrichment heatmap, showing the mean esRP score for the cCREs in each JmC for all the common cell types examined

between human and mouse. A summary description of the cell types in which the cCREs in each JmC are more active is given at the bottom.

Figure. 5. Evolutionary and epigenetic comparisons of cCREs. (A) Workflow to partition blood cell cCREs in human and mouse into three evolutionary categories. N=nonconserved, S=conserved in sequence but not inferred function, SF=conserved in both sequence and inferred function as a cCRE, y=yes, n=no. **(B)** Enrichment of SF-conserved human cCREs for TSSs. The number of elements in seven sets of function-related DNA intervals that overlap with the 32,422 SF human cCREs was determined, along with the number that overlap with three subsets (32,422 each) randomly selected from the full set of 200,342 human cCREs. The ratio of the number of function-related elements overlapping SF-cCREs to the number overlapping a randomly chosen subset of all cCREs gave the estimate of enrichment plotted in the graph. The mean for the three determinations of enrichment is indicated by the horizontal line for each set. Results are also shown for a similar analysis for the S and N cCREs. **(C)** Distribution of PhyloP scores for three evolutionary categories of cCREs in human and mouse. The maximum phyloP score for each genomic interval was used to represent the score for each cCRE, using genome sequence alignments of 100 species with human as the reference (phyloP100) and alignments of 60 species with mouse as the reference (phyloP60). The distribution of phyloP scores for each group are displayed as a violin plot. All ten random sets had distributions similar to the one shown. The asterisk (*) over brackets indicates comparison for which the P values for Welch's t-test is less than 2.2e-16.

Figure. 6. Epigenetic comparisons of regulatory landscapes and cCREs. (A and B) DNA sequence alignments and correlations of epigenetic states in human *GATA1* and mouse *Gata1* genes and flanking genes. **(A)** Dot-plot view of chained blastZ alignments by PipMaker (Schwartz et al. 2000) between genomic intervals encompassing and surrounding the human

GATA1 (GRCh38 chrX:48,760,001-48,836,000; 76kb) and mouse *Gata1* (mm10 chrX:7,919,401-8,020,800; 101.4kb, reverse complement of reference genome) genes. The axes are annotated with gene locations (GENCODE), predicted *cis*-regulatory elements (cCREs), and binding patterns for GATA1 and EP300 in erythroid cells. **(B)** Matrix of Pearson correlation values between epigenetic states (quantitative contributions of each epigenetic feature to the assigned state) across 15 cell types analogous for human and mouse. The correlation is shown for each 200bp bin in one species with all the bins in the other species, using a red-blue heat map to indicate the value of the correlation. Axes are annotated with genes and cCREs in each species. **(C)** Decomposition of the correlation matrix (panel **B**) into six component parts or factors using nonnegative matrix factorization. **(D-G)** Correlation matrices for genomic intervals encompassing *GATA1*/*Gata1* and flanking genes, reconstructed using values from NMF factors. **(D and E)** Correlation matrices using values of NMF factor 3 between human and mouse (panel **D**) or within human and within mouse (panel **E**). The red rectangles highlight the positive regulatory patterns in the *GATA1*/*Gata1* genes (labeled Px), which exhibit conservation of both DNA sequence and epigenetic state pattern. The orange rectangles denote the distal positive regulatory region present only in mouse (labeled D), which shows conservation of epigenetic state pattern without corresponding sequence conservation. Beneath the correlation matrices in panel **E** are maps of IDEAS epigenetic states across 15 cell types, followed by a graph of the score and peak calls for NMF factor 3 and annotation of cCREs (thin black rectangles) and genes. **(F and G)** Correlation matrices using values of NMF factor 6 between human and mouse (panel **F**) or within human and within mouse (panel **G**). The green rectangles highlight the correlation of epigenetic state patterns within the same gene, both across the two species and within each species individually, while the black rectangles highlight the high correlation observed between the two genes *GATA1* and *HDAC6*.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H3K4me3
H3K27ac
H3K36me3
H3K27me3
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HSC
MPP
LMPP
CMP
MEP
K562
GMP
CLP
HUDEP1
HUDEP2
CD34_E
ERY
MK
EOS
MONp
MONc
NEU
B
NK
TCD4
TCD8
Platelets
RBC
Source: Blueprint CMB, other SJCH
B.
Mouse
LSK
HPC7
CMP
MEP
GMP
CLP
G1E
MEL
CFUE
ER4
iMEL
ERY
FL
BM
CFUMK
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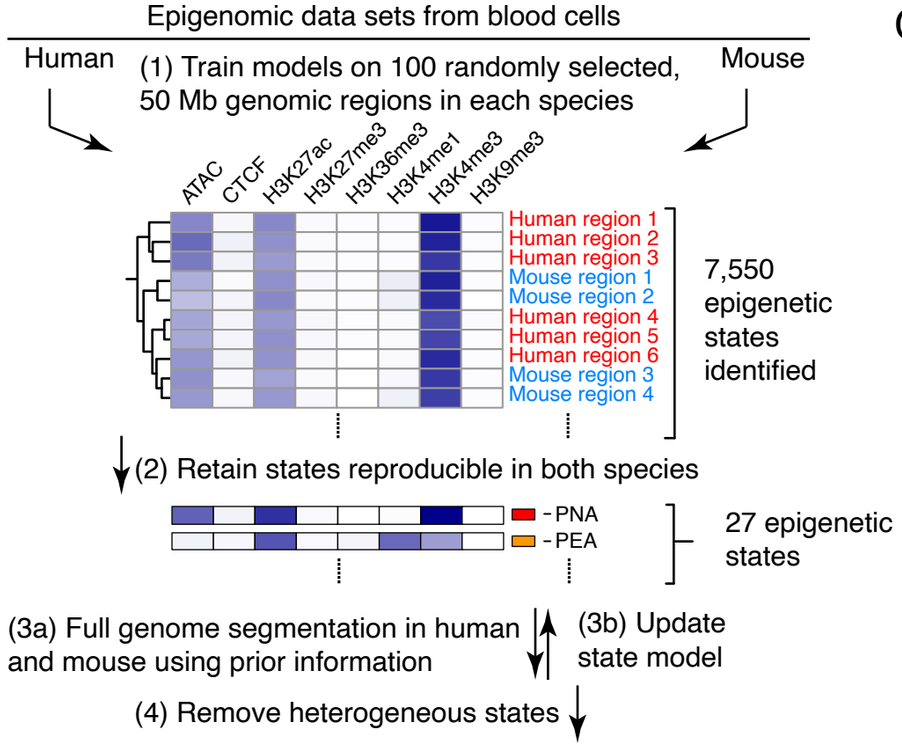
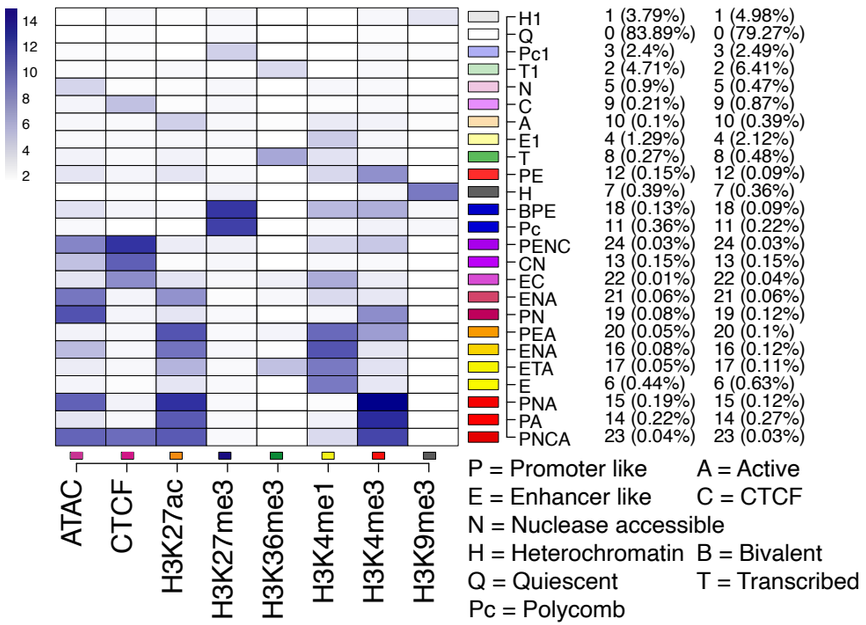
Platelets
RBC
Source: VISION Other

A.



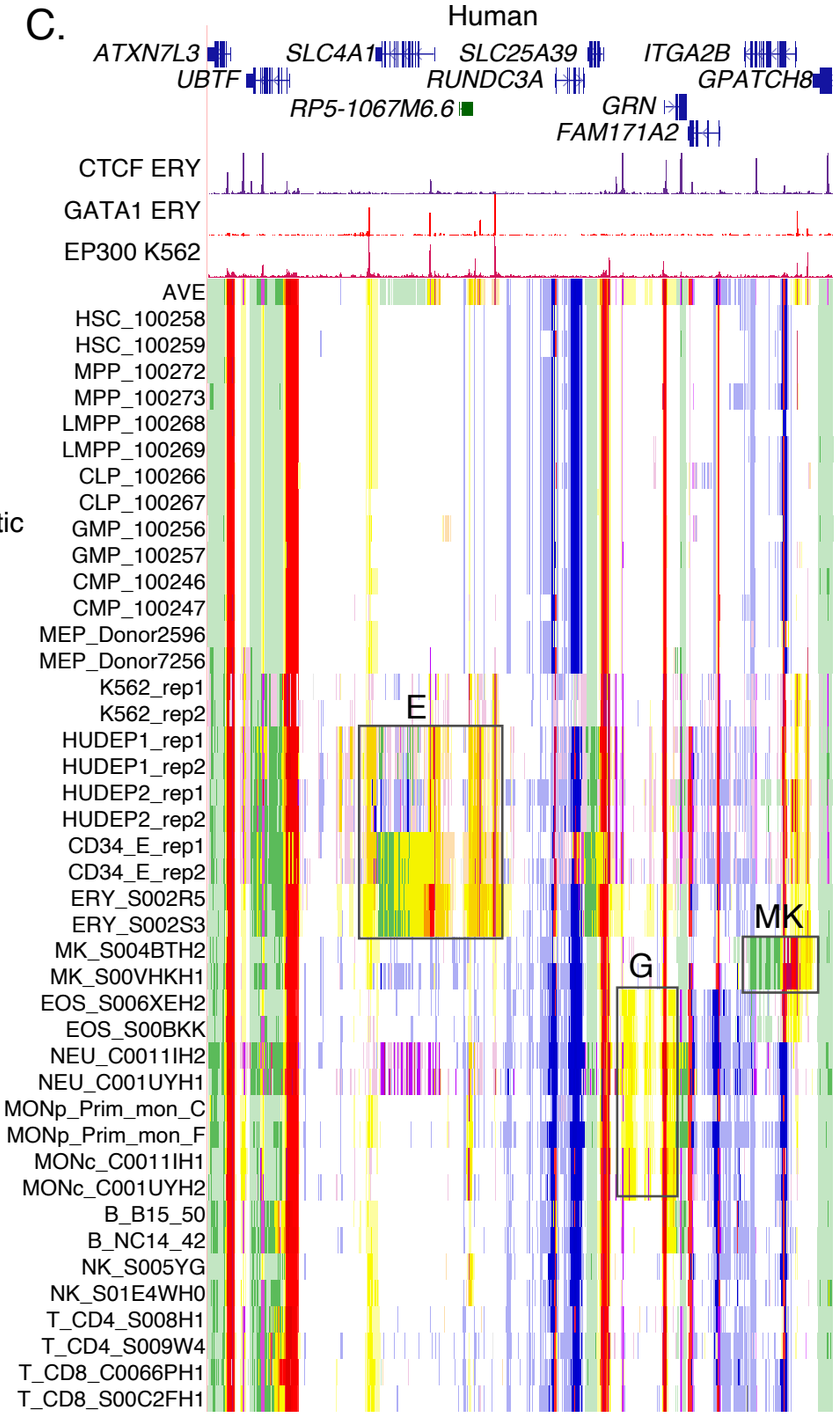
B. 25 epigenetic states extracted jointly from human and mouse



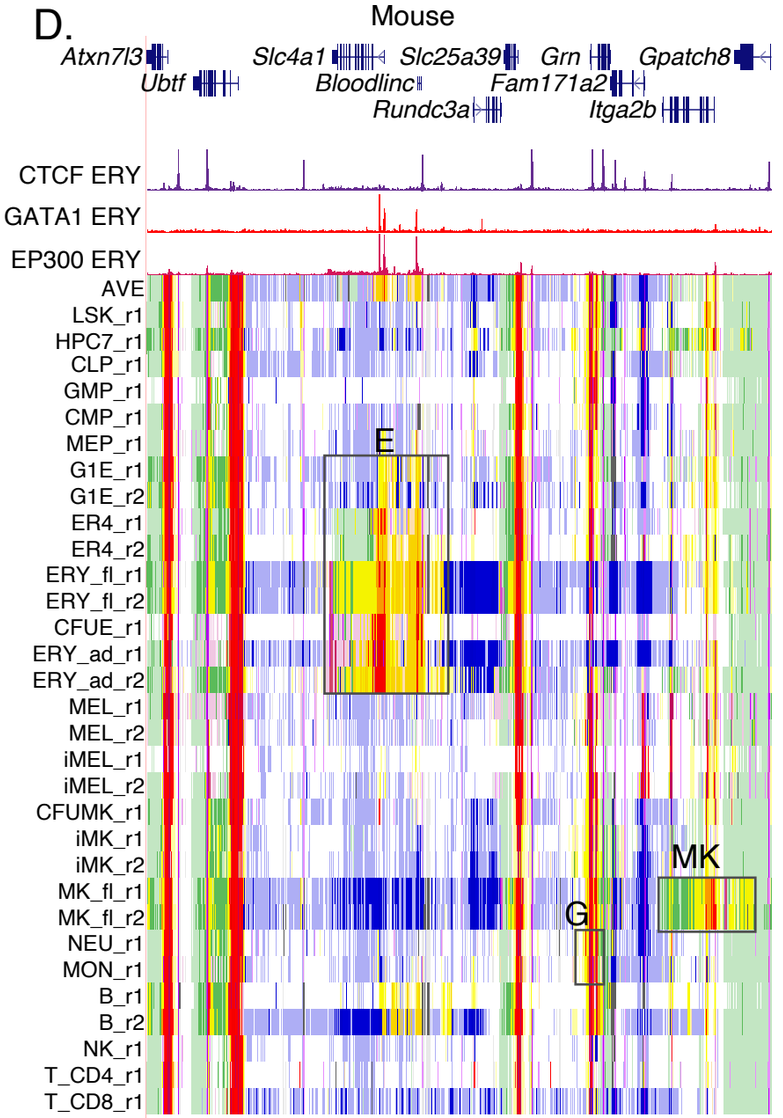
	Human	Mouse
H1	1 (3.79%)	1 (4.98%)
Q	0 (83.89%)	0 (79.27%)
Pc1	3 (2.4%)	3 (2.49%)
T1	2 (4.71%)	2 (6.41%)
N	5 (0.9%)	5 (0.47%)
C	9 (0.21%)	9 (0.87%)
A	10 (0.1%)	10 (0.39%)
E1	4 (1.29%)	4 (2.12%)
T	8 (0.27%)	8 (0.48%)
PE	12 (0.15%)	12 (0.09%)
H	7 (0.39%)	7 (0.36%)
BPE	18 (0.13%)	18 (0.09%)
Pc	11 (0.36%)	11 (0.22%)
PENC	24 (0.03%)	24 (0.03%)
CN	13 (0.15%)	13 (0.15%)
EC	22 (0.01%)	22 (0.04%)
ENA	21 (0.06%)	21 (0.06%)
PN	19 (0.08%)	19 (0.12%)
PEA	20 (0.05%)	20 (0.1%)
ENA	16 (0.08%)	16 (0.12%)
ETA	17 (0.05%)	17 (0.11%)
E	6 (0.44%)	6 (0.63%)
PNA	15 (0.19%)	15 (0.12%)
PA	14 (0.22%)	14 (0.27%)
PNCA	23 (0.04%)	23 (0.03%)

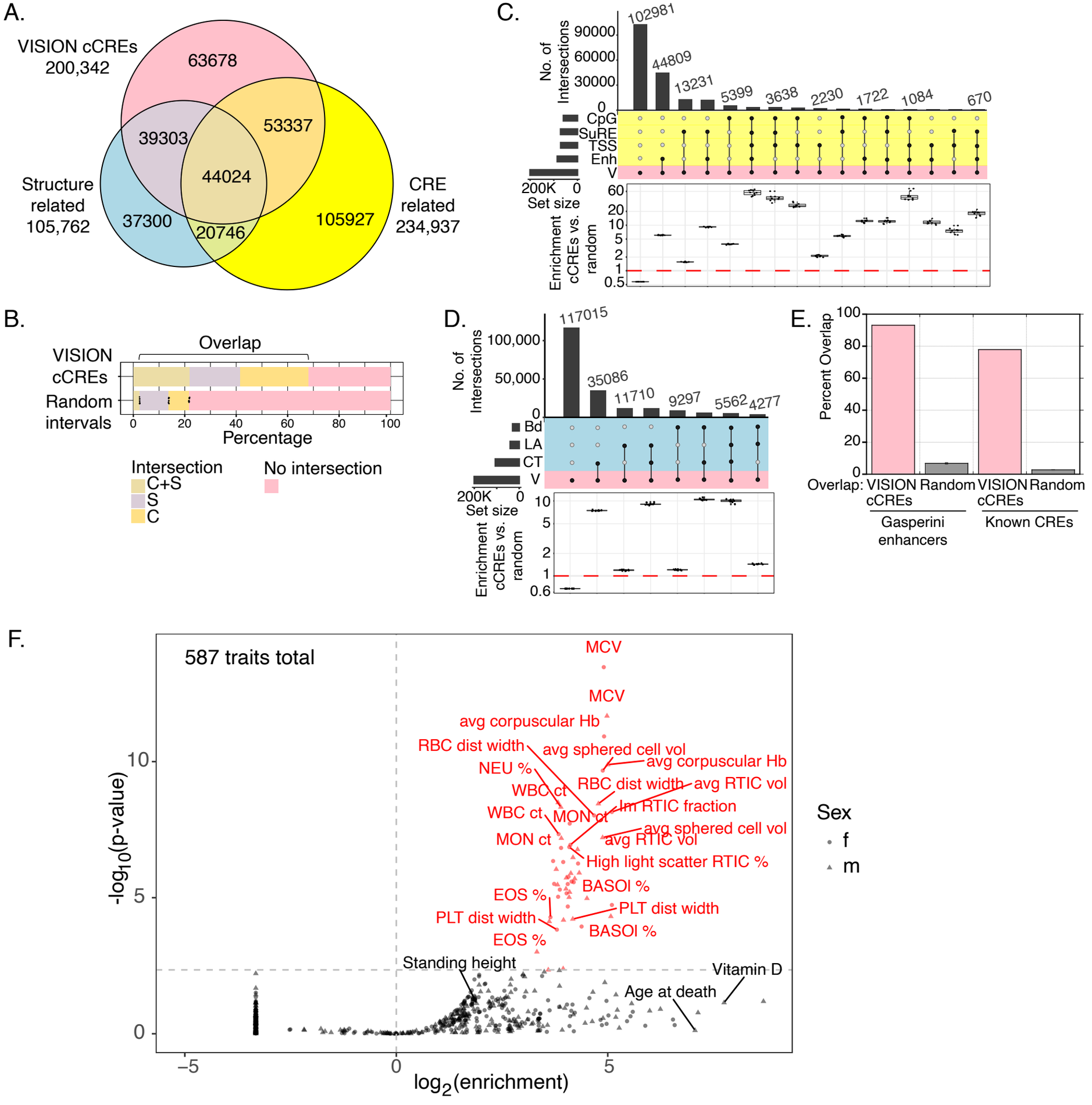
P = Promoter like
E = Enhancer like
N = Nuclease accessible
H = Heterochromatin
Q = Quiescent
Pc = Polycomb
A = Active
C = CTCF
B = Bivalent
T = Transcribed

C.



D.




A.
VISION cCREs
200,342
63678
39303
53337
44024
Structure
related
105,762
37300
20746
105927
CRE
related
234,937
B.
Overlap
VISION
cCREs
Random
intervals
0
20
40
60
80
100
Percentage
Intersection
C+S
S
C
No intersection
C.
No. of
Intersections
90000
60000
30000
0
102981
44809
13231
5399
3638
2230
1722
1084
670
CpG
SuRE
TSS
Enh
V
200K
0
Set size
Enrichment
cCREs vs.
random
60
20
10
5
2
1
0.5
D.
No. of
Intersections
100,000
50,000
0
117015
35086
11710
9297
5562
4277
Bd
LA
CT
V
200K
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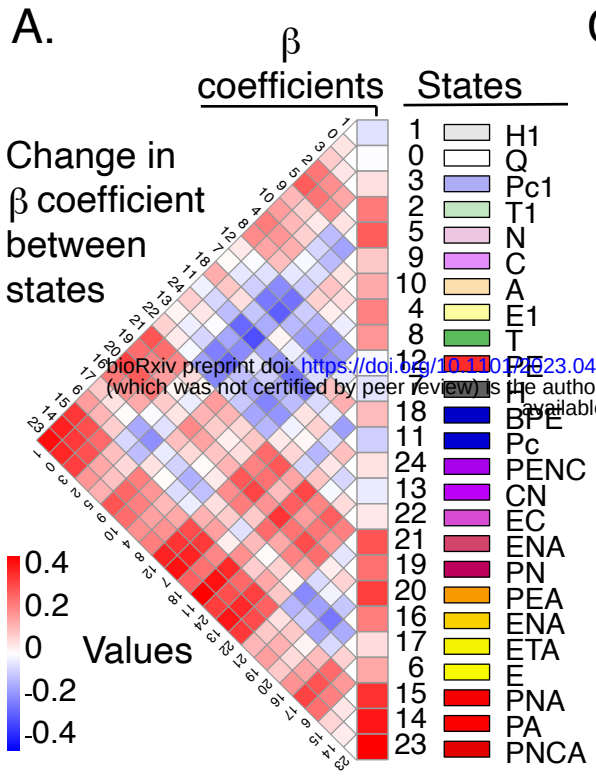
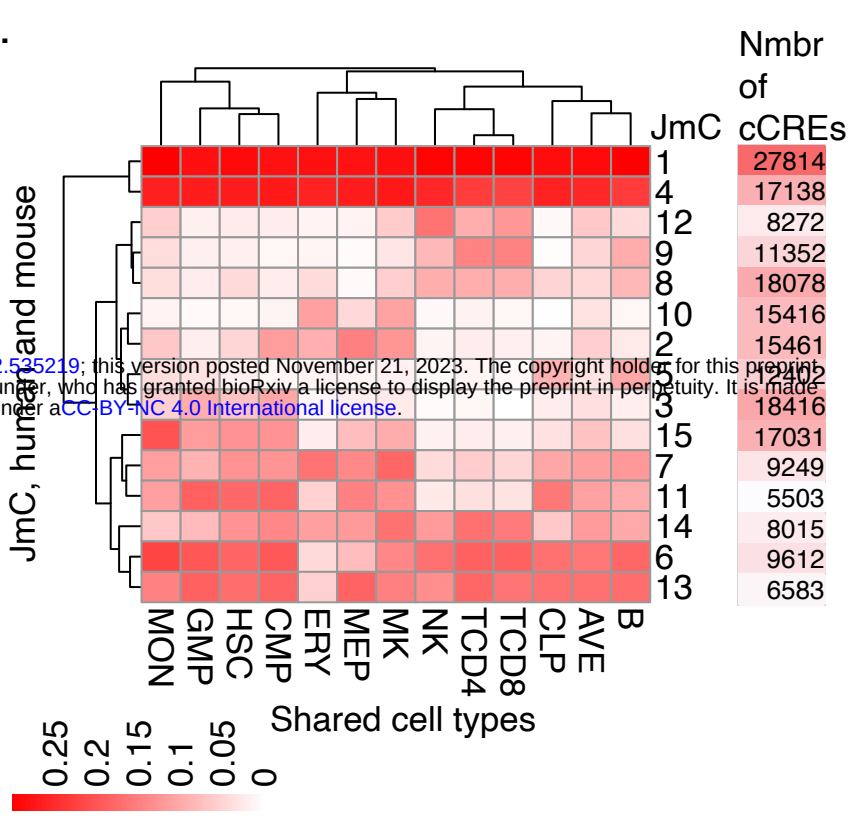
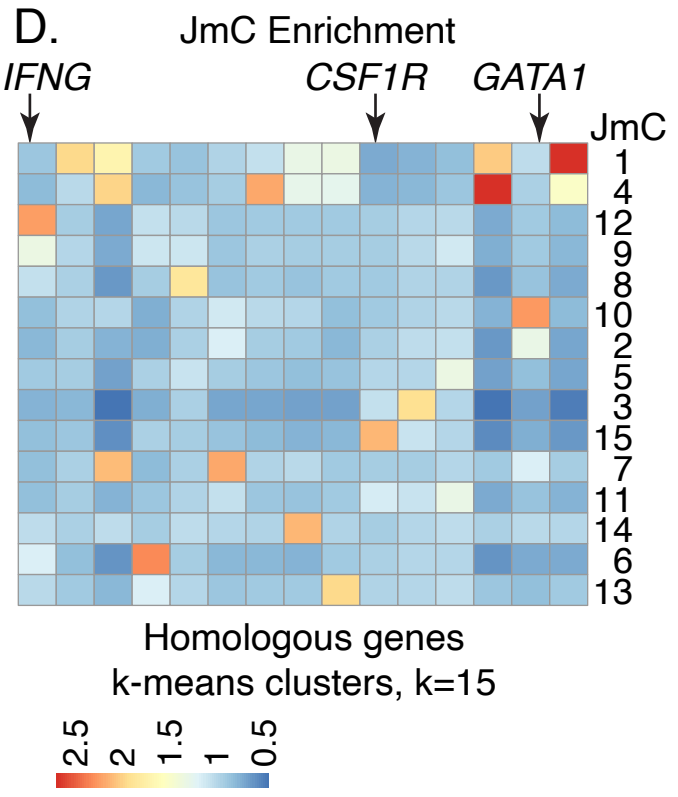
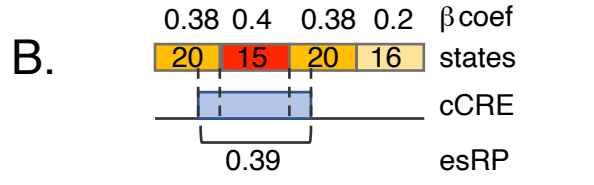
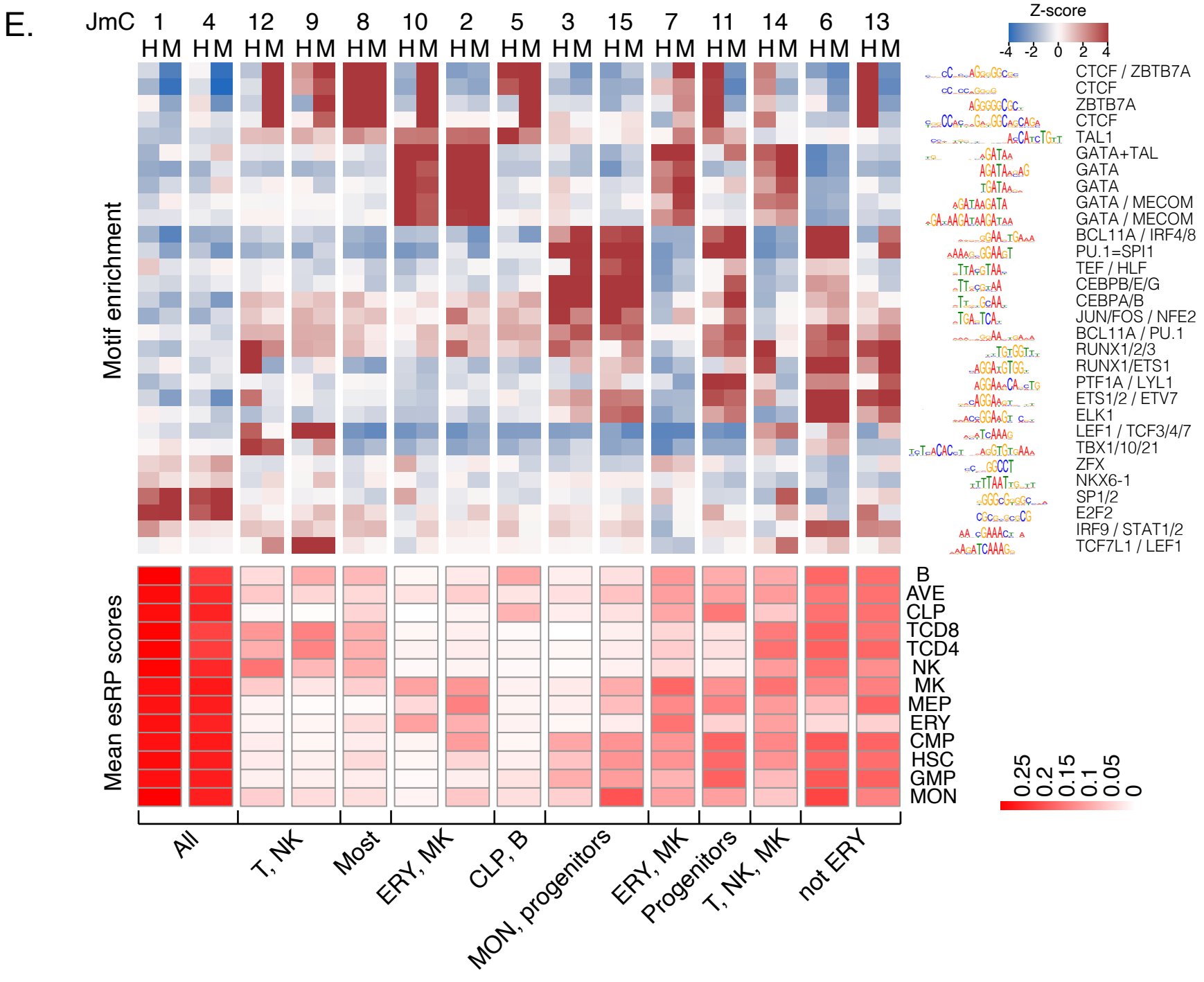
Set size
Enrichment
cCREs vs.
random
10
5
2
1
0.6
E.
Percent Overlap
100
80
60
40
20
0
Overlap: VISION
cCREs
Random
VISION
cCREs
Random
Gasperini
enhancers
Known CREs
F.
587 traits total
MCV
MCV
avg corpuscular Hb
RBC dist width
avg sphered cell vol
avg corpuscular Hb
NEU %
RBC dist width
avg RTIC vol
WBC ct
Im RTIC fraction
WBC ct
MON ct
avg sphered cell vol
MON ct
avg RTIC vol
High light scatter RTIC %
BASOI %
EOS %
PLT dist width
PLT dist width
BASOI %
EOS %
Standing height
Vitamin D
Age at death
10
5
0
-log10(p-value)
−5
0
5
log2(enrichment)
Sex
f
m

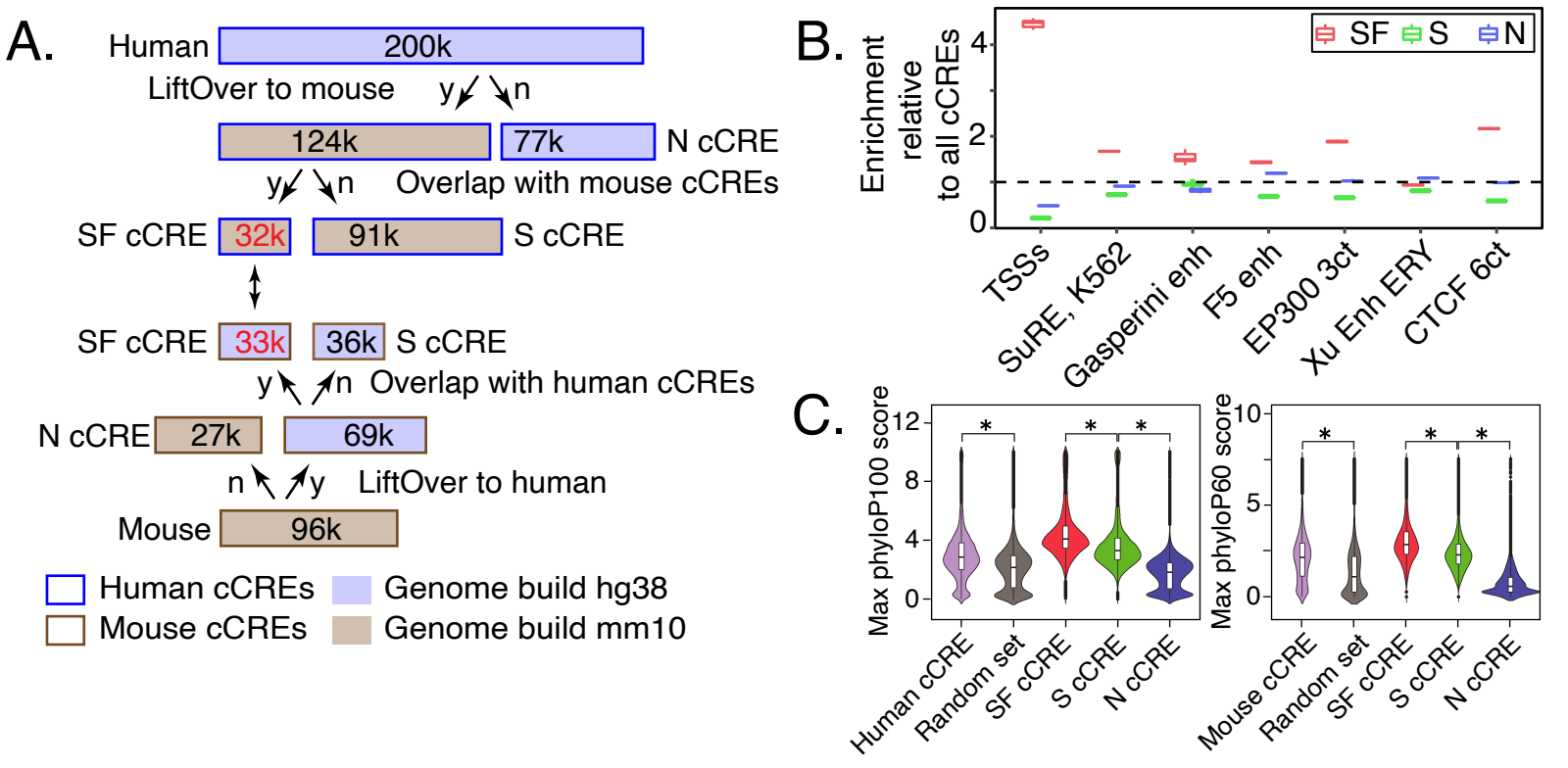











A.
Human
200k
LiftOver to mouse
y
n
124k
77k
N cCRE
Overlap with mouse cCREs
SF cCRE
32k
91k
S cCRE
SF cCRE
33k
36k
S cCRE
Overlap with human cCREs
N cCRE
27k
69k
LiftOver to human
Mouse
96k
Human cCREs
Mouse cCREs
Genome build hg38
Genome build mm10
B.
Enrichment relative to all cCREs
SF
S
N
TSSs
SuRE, K562
Gasperini enh
F5 enh
EP300 3ct
Xu Enh ERY
CTCF 6ct
C.
Max phyloP100 score
Max phyloP60 score
Human cCRE
Random set
SF cCRE
S cCRE
N cCRE
Mouse cCRE