

Comparative analysis of 3D-genome organization in the human and mouse brain

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Abstract

The 3D organization of the genome in cell nuclei is implicated in the determination of cell state, the mechanisms of cellular function, and the dysregulation in disease processes. Our prior work has characterized the 3D-genome organization at genome-wide scale in the mouse primary motor cortex, profiling over 40,000 cells using joint RNA and DNA multiplexed error-robust fluorescence *in situ* hybridization (MERFISH) [1]. Here we apply RNA MERFISH and genome-wide DNA MERFISH to the characterization of 3D-genome organization in the human brain. We profile over 30,000 cells in the human primary motor cortex, imaging 259 genes with RNA MERFISH and 2740 genomic loci with DNA MERFISH. In our comparative analysis of human and mouse motor cortex 3D-genome organization, we find conservation of 3D-genome features characteristic to neurons and non-neurons, including for nuclear organization, chromatin scaling, and radial positioning. On the other hand, we observe stronger patterns of nuclear volume differentiation between inhibitory and excitatory neurons in humans as compared to mice. To analyze corresponding 3D-genome regions between human and mouse, we present a genome-aligned approach for cross-species contact map comparison. Through this, we extend our prior study comparing the human and mouse brain using RNA MERFISH [2] and build toward a comprehensive understanding of the molecular organization in the mammalian brain.

Spatially resolved single-cell 3D-genome profiling

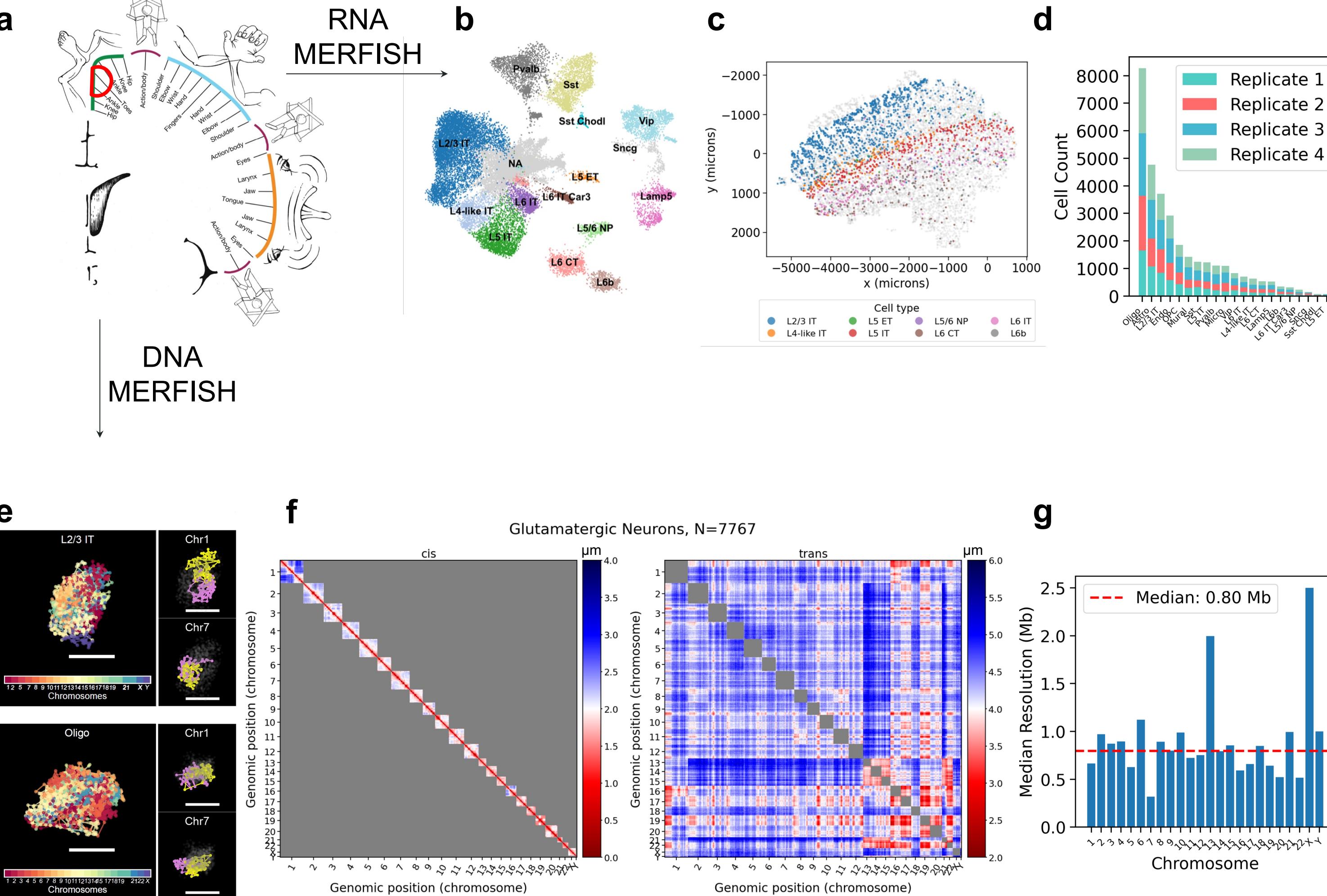


Figure 1. Joint RNA and DNA MERFISH profiling of 3D-genome organization in 32,026 cells of the human brain. (a) Integrate-isolate homunculus model schematic of the primary motor cortex [3], with a red outline around the specific region where imaging is performed. (b) Uniform manifold approximation and projection (UMAP) plot of primary motor cortex cell types determined by RNA MERFISH over 259 genes. (c) Spatial map of cell types in a coronal slice of the primary motor cortex, with each cell shown by its x-y spatial position in microns. Excitatory neurons are shown in color. (d) Cell type composition of four technical replicates based on RNA MERFISH. (e) 3D renderings of imaged chromosomes in cell nuclei for an excitatory neuron (top) and an oligodendrocyte (bottom). (f) Heatmap of pairwise median distances between 2740 loci for excitatory neurons within chromosomes (left) and between chromosomes (right). (g) A bar plot showing the median resolution of DNA MERFISH imaging for each chromosome.

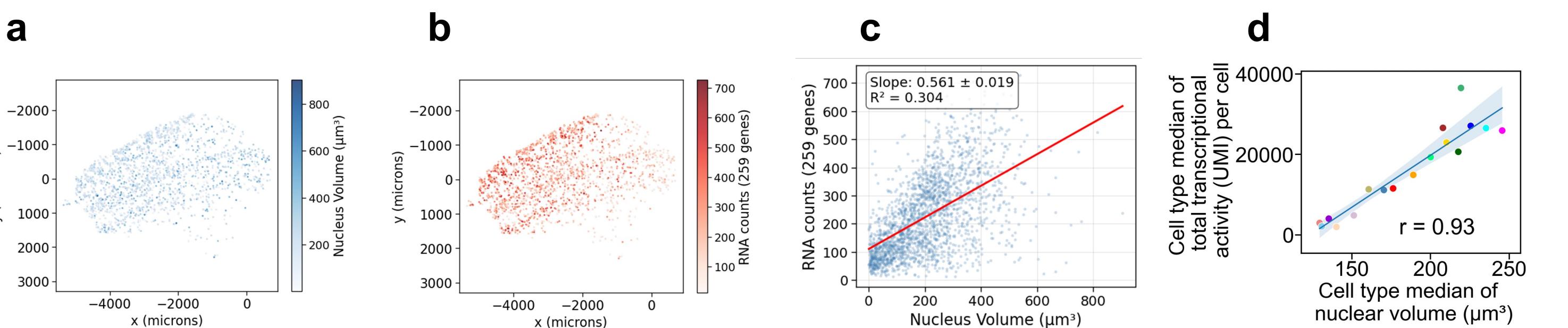


Figure 2. Spatial organization of overall cell activity markers in human excitatory neurons. Shown is a coronal slice (one technical replicate) of the primary motor cortex with the color of each excitatory neuron given by nuclear size (a) and estimated transcriptional activity from MERFISH RNA counts (b). (c) A scatterplot of excitatory neurons in this replicate (2084 cells) showing the correlation between nuclear volume and 259-gene transcriptional activity for excitatory neurons at the single-cell level. (d) Correlation at the cell type level between nuclear volumes measured by MERFISH (x-axis) and transcriptional activity measured by RNA sequencing [4] (y-axis).

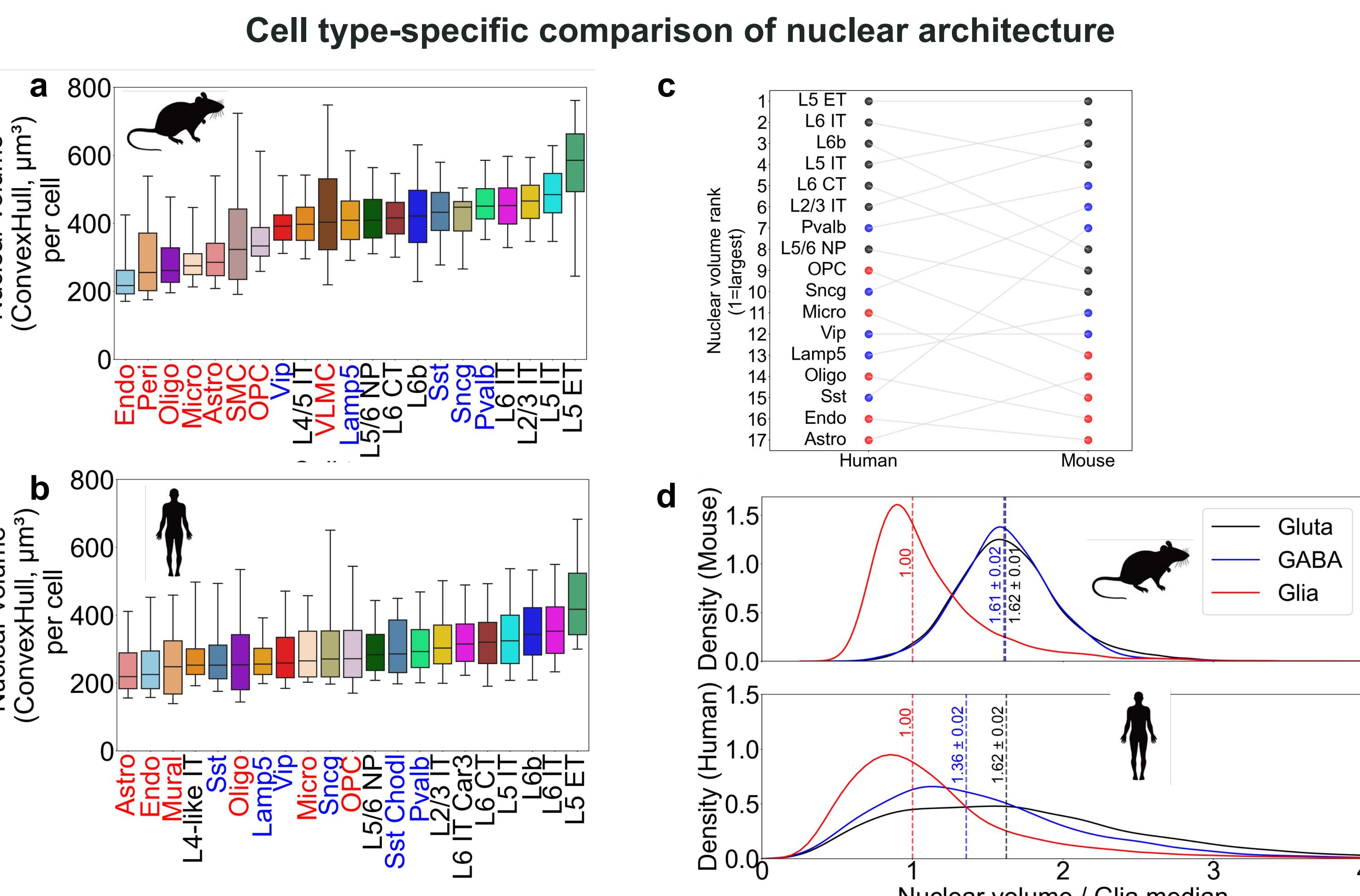


Figure 3. Nuclear volume shows stronger patterns of differentiation between excitatory and inhibitory neurons in human. (a) Boxplot of nuclear volume distributions across cells by cell type in the mouse primary motor cortex. (b) Boxplot of nuclear volume distributions across cells by cell type in the human primary motor cortex. (c) Comparison of the ordering of cell types by median nuclear volume in human and mouse primary. (d) Distributions of the nuclear volumes of individual cells within the populations of excitatory neurons, inhibitory neurons, and glial cells for human (top) and mouse (bottom), normalized by median non-neuron nuclear volume. Vertical lines show the normalized median nuclear volume for each population of cells.

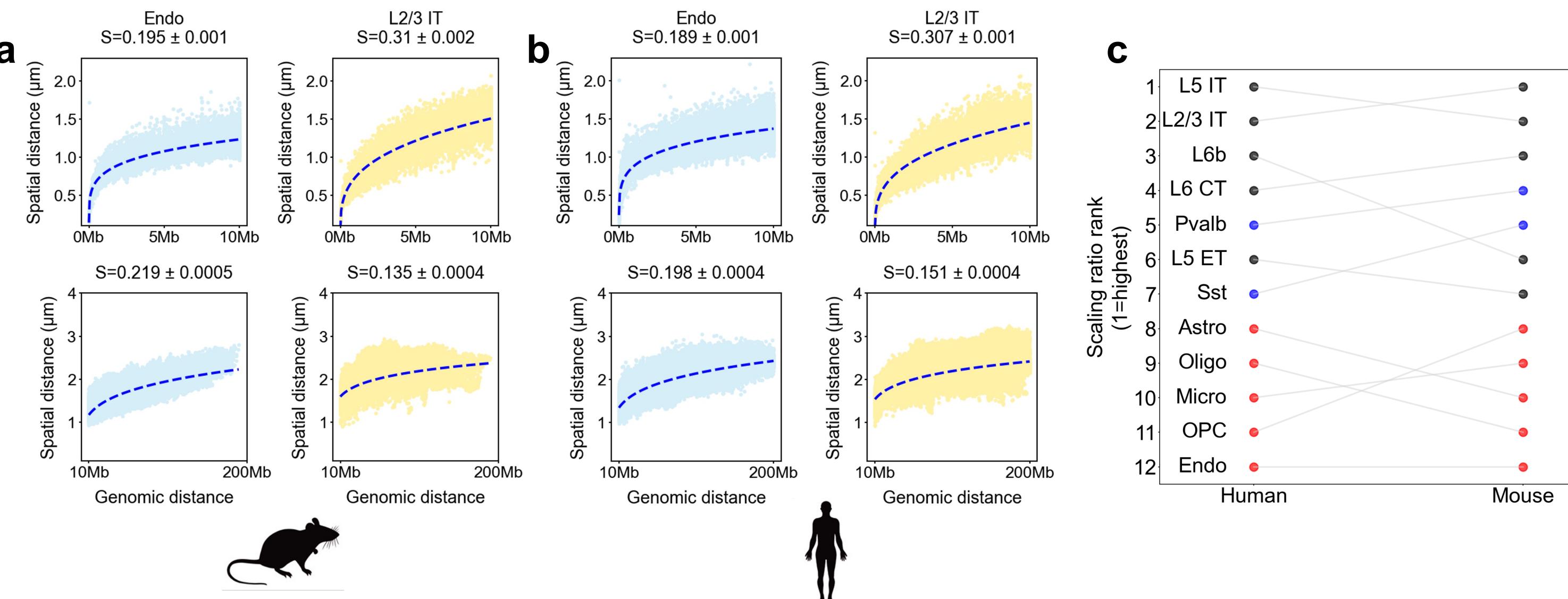


Figure 4. Chromatin scaling. (a, b) We show short-range (top) and long-range (bottom) scaling patterns for endothelial cells (left) and L2/3 excitatory neurons (right). We compare mouse (a) and human (b). In each scatterplot, all individual locus pairs in the given cell type with the shown genomic distance range are plotted by their physical spatial distance. Plots are labeled by their exponential scaling power. (c) A comparative ranking of cell types by their short-range to long-range scaling ratios, where the scaling ratio for each cell type is calculated by first dividing the short-range and long-range exponential scaling powers for each chromosome then taking a median over chromosomes.

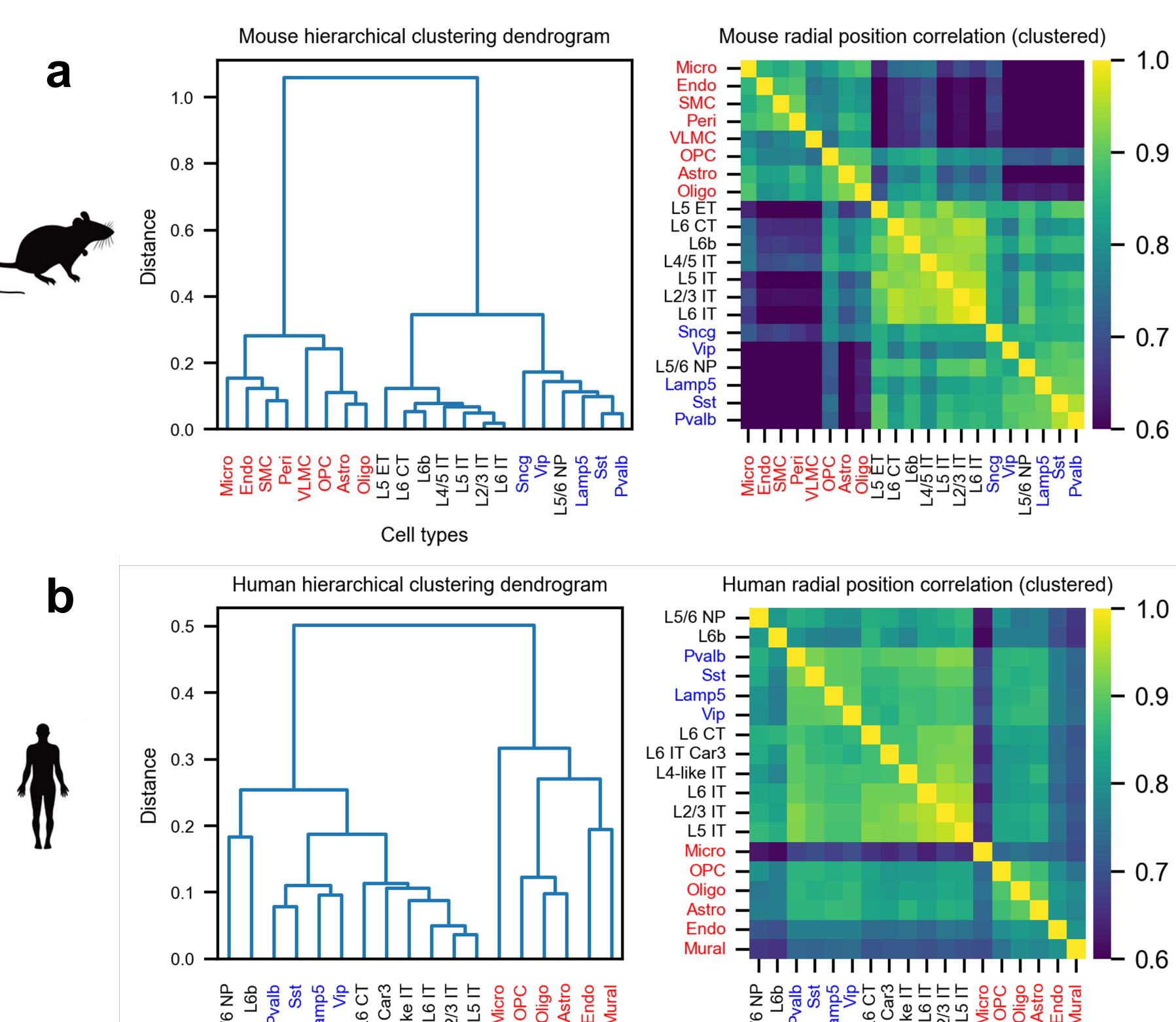


Figure 5. Radial positioning correlations. For mouse (a) and human (b), we show cell types clustered by their radial position correlations. Radial position profiles are calculated for each cell type using the median nuclear radial position for each genomic locus. Correlations between these profiles are then calculated between cell types. Ward clustering of cell types is performed using [distance = 1 - correlation].

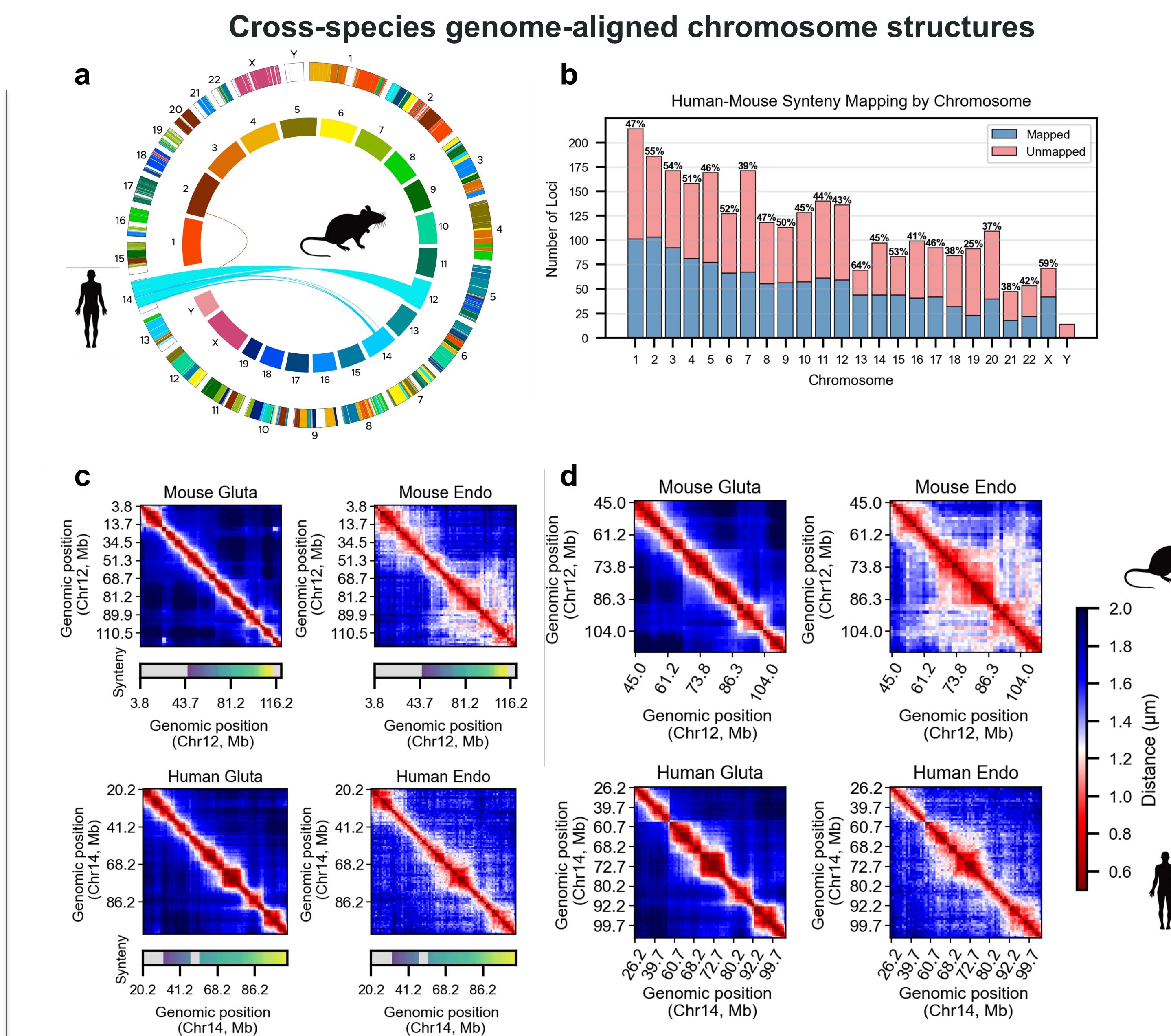


Figure 6. Chromosomal features analyzed with genome alignment. (a) A diagram showing syntenic blocks between the mouse (inside) and human (outside) genomes, with the extended synteny between human chromosome 14 and mouse chromosome 12 indicated [5]. (b) We map human genomic loci to loci we imaged in mouse based on synteny block alignment, enforcing a 1 Mb threshold between calculated mapping and closest imaged locus, keeping the closest-mapped human locus if multiple map to the same mouse locus. We are able to map 46% of 2740 human loci to 64% of 1982 mouse loci, with total of 1267 mapped loci. (c) We show pairwise distance maps in mouse chromosome 12 (top) and human chromosome 14 (bottom) for excitatory neurons (left) and endothelial cells (right).

Conclusions

- We profile 32,026 cells in the human primary motor cortex using 259-gene RNA MERFISH and 2740-locus DNA MERFISH imaging, deriving spatially resolved, cell type-specific 3D-genome organization in the human brain.
- We demonstrate the capacity of joint RNA and DNA MERFISH to show the spatial organization of 3D-genome features and transcriptional activity within intact tissue.
- We show conservation of neuron- and non-neuron- specific features for nuclear volume, chromatin scaling, and radial positioning between human and mouse. In both human and mouse, neurons have greater volume and show greater variation in chromatin scaling between small and large genomic distances.
- We find stronger patterns of differentiation between excitatory and inhibitory neurons by nuclear volume in the human primary motor cortex.
- We present an approach for studying region-specific 3D-genome organization across species, showing synteny block-aligned chromosomal contact maps for excitatory neurons and endothelial cells.
- In future work, we will improve the resolution, scale, and precision of our experimental methods, and further develop computational approaches to enhance image processing and better elucidate meaningful biological signal.

References

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