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Supplemental Information

**Human Stem Cell Resources Are an Inroad to Neandertal DNA
Functions**

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SUPPLEMENTARY INFORMATION

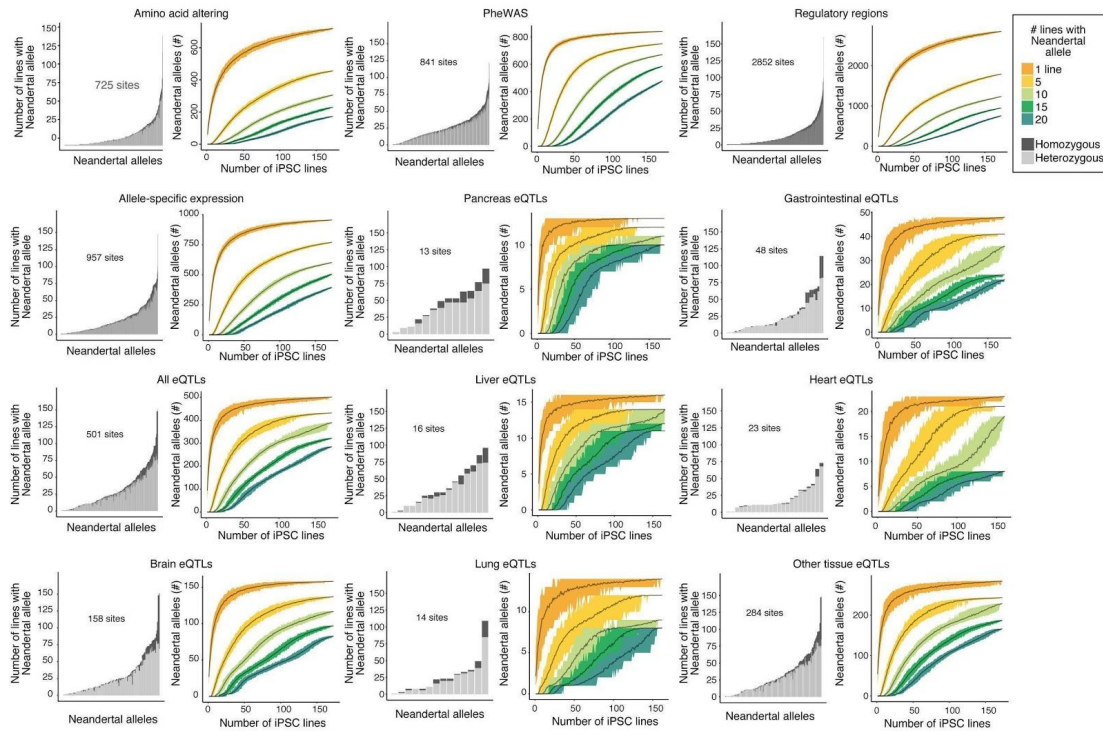


Figure S1 : Power analyses for multiple classes of functional alleles. Related to Figure 2.

The number of iPSC lines that contain each introgressed allele is shown for multiple different categories of functional alleles, colored by homozygosity (dark) or heterozygosity (light) (left). For each category, a power analysis shows how many introgressed alleles are present in a random sample of X lines (x-axis) (right).

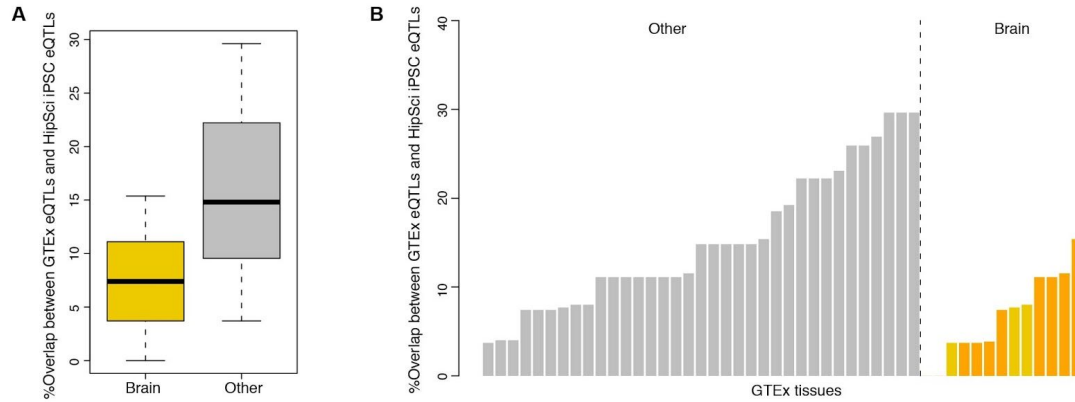


Figure S2: Presence of iPSC Neandertal-associated eQTLs from the HipSci resource in other GTEx tissues. Related to Figure 4.

(A-B) Boxplot (A) shows the distribution of and barplots (B) show the proportion of 76 significant eQTLs in the HipSci iPSC expression data (FDR<0.05) (Kilpinen et al., 2017) that are tagged by a Neandertal haplotype associated SNP in 48 GTEx tissues (brain tissues are shown in yellow, brain cortex tissues in orange and all other non-brain tissues in grey). Significant GTEx eQTLs were defined as $P < 0.01$ (Dannemann et al., 2017).

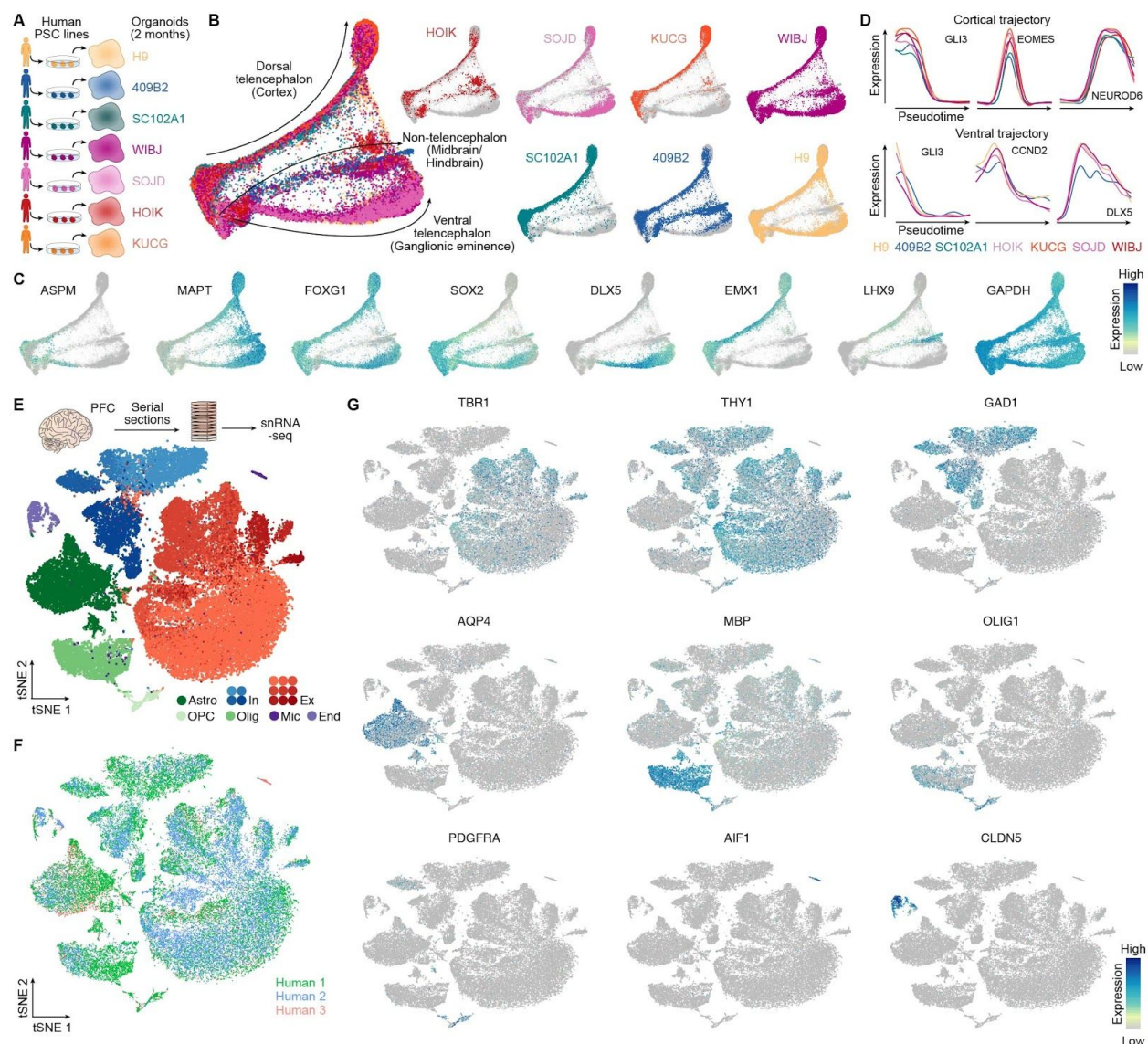


Figure S3: Single-cell and single-nucleus RNA-seq analysis of human cerebral organoids and adult human cortex. Related to Figure 4.

(A) We previously performed scRNA-seq on 2-month-old human organoids from 6 iPSC lines and 1 ESC (H9) line (Kanton et al., 2019). (B) Developmental trajectories were reconstructed using the SPRING algorithm (Weinreb et al., 2018) based on the reference similarity spectrum (RSS) (Kanton et al., 2019) of each organoid cell to bulk human brain RNA-seq BrainSpan data from Allen Brain Atlas. Plots are colored by cell line with the major trajectories labeled. (C) SPRING plot with cells colored by expression level of marker genes. (D) Expression of marker genes are shown for each cell line over the dorsal (top) and ventral (bottom) telencephalon

trajectories. (E) Single-nucleus RNA-seq was performed on sliced tissue cubes dissected from adult human, prefrontal cortex tissue from three individuals. Projection of integrated data shows different clusters of major cell classes. The data and annotations are from Kanton et al. (Kanton et al., 2019) Ex, excitatory neuron; In, Inhibitory neuron; Astro, astrocyte; Olig, oligodendrocyte; OPC, oligodendrocyte precursor cell; Mic, microglia; End, endothelial cell. (F-G) Nuclei are colored based on the individual of origin (B) or marker gene (G).

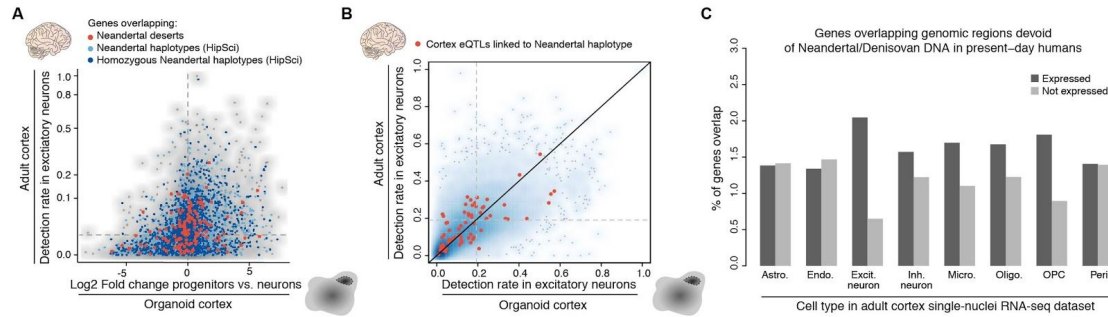


Figure S4: Gene expression in the adult and organoid brain. Related to Figure 4.

(A) The expression difference between progenitors and neurons in organoids (x axis) and the detection rate in excitatory neurons in the adult cortex (y axis) are shown for protein coding genes. Genes that are located in genomic regions devoid of Neandertal and Denisovan DNA (Sankararaman et al., 2016; Vernot et al., 2016) are highlighted in red and genes that overlap Neandertal haplotypes detected in the HipSci cohort are shown in blue (heterozygous haplotypes: light blue, homozygous haplotypes: dark blue). (B) Scatterplot shows the detection rate of protein coding genes in organoid (x axis) and adult (y axis) excitatory neurons. Genes with previously reported Neandertal-linked eQTLs in GTEx cortex tissues (Dannemann et al., 2017) are highlighted in red. (C) The proportion of the sets of expressed (dark grey) and not expressed (light grey) genes in 8 cell types from the adult cortex overlapping with genomic regions that devoid of Neandertal and Denisovan DNA (Sankararaman et al., 2016; Vernot et al., 2016).

Table S1: Neandertal ancestry estimates and putative functional alleles in HipSci resource. Related to Table 1.

Inferred Neandertal haplotypes in HipSci resource based on Vindija and Altai Neandertal.

Tables for each HipSci individual with all inferred haplotypes including genomic locations, FDRs for tests of compatibility of incomplete lineage sorting and inferred Neandertal alleles (with chromosomal location, hg19 and status of Neandertal allele; 0|1: heterozygous, 1|1: homozygous) are available at <https://bioinf.eva.mpg.de/stemcellbrowser/> using the links “Download annotation Vindija maps” and “Download annotation Altai maps”.