In this Supplementary Information we provide further information regarding the study design, materials and methods, and additional data. The materials and methods section provide detailed description of the collection, dissection methods, and quality control assessments of postmortem human brain tissue used in this study. We provide technical descriptions of data generation and analyses.

# Tissue procurement

This study was conducted using postmortem human brain specimens from tissue collections at the Department of Neurobiology at Yale University School of Medicine and the Clinical Brain Disorders Branch of the National Institute of Mental Health. Additional specimens were procured from the Human Fetal Tissue Repository at the Albert Einstein College of Medicine (AECOM), the Brain and Tissue Bank for Developmental Disorders at the University of Maryland, the Birth Defects Research Laboratory at the University of Washington, Advanced Bioscience Resources Inc. and the MRC-Wellcome Trust Human Developmental Biology Resource at the Institute of Human Genetics, University of Newcastle, UK. Tissue was collected after obtaining parental or next of kin consent and with approval by the institutional review boards at the Yale University School of Medicine, the National Institutes of Health, and at each institution from which tissue specimens were obtained. Tissue was handled in accordance with ethical guidelines and regulations for the research use of human brain tissue set forth by the NIH (http://bioethics.od.nih.gov/humantissue.html) and the WMA Declaration of Helsinki (http://www.wma.net/en/30publications/10policies/b3/index.html).

All available non-identifying information was recorded for each specimen. 41 postmortem brain specimens ranging in age from 8 postconception weeks (PCW) to 40 years (Y) (Supplementary Table 1) were included in this study. Fetal age was extrapolated based on the date of the mother’s last menstruation, characteristics of the fetus noted upon ultrasonography scanning, foot length of the fetus, and visual inspection. The postmortem interval (PMI) was defined as hours between time of death and time when tissue samples were frozen.

# Brain regions and neocortical areas definition

Brain development is a highly dynamic process during which each region undergoes distinct organizational and maturational changes. Thus, we created a structural ontology that contains brain structures (e.g., NCX areas, HIP, AMY, STR, MD, CBC) that are well defined throughout most of time periods, and several transient structures (e.g., MGE, LGE, CGE, URL). In total, 10 regions for 8 - 9 PCW (window 1) specimens, and up to 16 regions for all ages after 9 PCW (windows 2- 9) – 15. Regions and areas were as described in Kang et al but are noted here again for comprehensive description of methods. Below we describe this ontology and anatomical definition of sampled brain regions and NCX areas (Extended Fig. 1) based on histological verification.

## Cerebrum

### Neocortex (NCX)

Samples collected from 8 – 9 PCW specimens contained the entire thickness of the cerebral wall. Samples collected from 12 – 22 PCW specimens contained the marginal zone, cortical plate, and part of the underlying subplate. Samples from 35 PCW – 40 Y specimens were dissected such that the entire gray matter (layer 1-6) and part of the underlying subplate ( 4- 12 M) or white matter (1 – 40 Y) were collected (Extended Fig. ). Nissl staining of the neighboring thin block was used to histologically verify the identity of the dissected area and to microscopically evaluate tissue. Neocortical cytoarchitecture of each sample was compared to areal cytoarchitectonic maps to distinguish Brodmann areas (BA)57. Samples with incorrect cytoarchitecture or abnormal microscopical appearance were excluded from the study. Neocortical areas (see below) were grouped according to the lobes from which they were sampled.

#### Frontal cortex

For 8 – 9 PCW specimens, the sampled area corresponded to different parts (orbital (OFC), dorsolateral (DFC), ventrolateral (VFC), and medial (MFC) of the anterior part of telencephalic vesicle (cerebral wall) corresponding to prospective FC. In addition, paracentral region corresponding approximately to the prospective motor and parietal somatosensory (M1C/S1C) cerebral wall was dissected as one sample (MSC).

For 12 – 22 PCW specimens, prior to the appearance of all gyri and sulci, multiple areas of the FC were sampled as follows:

• Orbital prefrontal cortex (OFC) was sampled from the middle part of the orbital surface of the cerebral hemisphere, immediately next to the prospective gyrus rectus.

• Dorsolateral prefrontal cortex (DFC) was sampled from the middle third of the dorsolateral surface of anterior third of the cerebral hemisphere.

• Ventrolateral prefrontal cortex (VFC) was sampled from the posterior part of the frontal operculum, above the lateral sulcus and prospective insula.

• Medial prefrontal cortex (MFC) was sampled from the perigenual and subgenual region of the medial surface.

• Primary motor cortex (M1C), prior to the appearance of the central sulcus, was sampled from the anterior third of the middle third of the cerebral hemisphere, medial third and upper part of the lower third of the dorsolateral surface. The striatum at the septal level was used as the landmark between the anterior and middle one third of the dorsolateral cortical surface. In some cases, M1C and S1C areas were sampled as single area and termed it motor-somatosensory cortex (M1C/S1C) due to the lack of clear anatomical and histological boundaries between immature M1C and S1C. After the appearance of the central sulcus M1C was sampled in front of the central sulcus from the middle and upper part of the lower third of the dorsolateral surface of the hemisphere.

For 35 PCW – 40 Y specimens, sampled areas were as follows:

• OFC was sampled from the anterolateral two thirds of the orbital gyri. OFC corresponds approximately to BA 11.

• DFC was sampled from approximate border between the anterior and middle third of the medial frontal gyrus. DFC corresponds approximately to BA 9 and 46.

• VFC was sampled from the posterior third of the inferior frontal gyrus, corresponding to the opercular and triangular part of the inferior frontal gyrus. VFC corresponds approximately to BA 44 and 45.

• MFC was sampled from perigenual and subgenual parts of the anterior cingulate gyrus and the anteromedial part of the superior frontal gyrus. MFC corresponds approximately to BA 24, 32 and 33.

• M1C was sampled from the ventrolateral part of the precentral gyrus, corresponding most closely to the orofacial region of M1C. M1C corresponds to BA4.

#### Parietal cortex

For 8 – 9 PCW specimens, the sampled areas included the paracentral region corresponding approximately to the prospective motor and parietal somatosensory (M1C/S1C) cerebral wall, and the posterior half of the dorsal middle third of the cerebral wall corresponding approximately to the prospective inferior parietal cortex (IPC).

For 12 – 22 PCW specimens, prior to the appearance of gyri and sulci, multiple areas of the PC were sampled as follows:

• Primary somatosensory cortex (S1C), prior to the appearance of the central sulcus, was sampled immediately caudal to the M1C (see M1C description above). After the appearance of the central sulcus, S1C was sampled behind the central sulcus from the middle and upper part of the lower third of the dorsolateral surface of the cerebral hemisphere adjacent to the M1C area.

• Posterior inferior parietal cortex (IPC) was sampled from the lower posterior part of the dorsolateral surface of the middle third of the cerebral hemisphere adjacent to the end of the lateral sulcus.

For 35 PCW – 40 Y specimens, sampled areas were as follows (Extended data Fig. ):

• S1C was sampled from the ventrolateral part of the postcentral gyrus adjacent to the M1C area. S1C corresponds to BA 1–3.

• IPC was sampled from the posterior half of the supramarginal gyrus. IPC corresponds approximately to BA 40.

#### Temporal cortex

For 8 – 9 PCW specimens, the sampled areas included the posterior two thirds of TC corresponding approximately to the prospective auditory and superior temporal cortex (A1C/STC) cerebral wall, and the anterior third corresponding approximately to the prospective inferior temporal cortex (ITC).

For 12 – 22 PCW specimens, prior to the appearance of gyri and sulci, multiple areas of the TC were sampled as follows:

• Primary auditory cortex (A1C) was sampled from the upper part of the temporal bank of the lateral sulcus.

• Posterior superior temporal cortex (STC) was sampled from the upper part of the superior third of the temporal lobe adjacent to the lateral sulcus and A1C area.

• Inferior temporal cortex (ITC) was sampled from the lower part of the inferior third of the temporal lobe adjacent to the temporal lobe pole.

For 35 PCW – 40 Y specimens, sampled areas were as follows:

• A1C was sampled from the planum temporale and the transverse temporal gyri. A1C corresponds to BA 41.

• STC was sampled from the posterior third of the superior temporal gyrus. STC corresponds approximately to BA 22.

• ITC was sampled from the anterior third of the inferior temporal gyrus. ITC corresponds approximately to BA 20.

#### Occipital cortex

For 8 – 9 PCW specimens, sampled tissue corresponded to the posterior (occipital) part of the cerebral wall.

For 12 – 22 PCW specimens, prior to the appearance of gyri and sulci, sampled tissue corresponded to prospective primary visual cortex (V1C). Prior to the appearance of the calcarine fissure, V1C was sampled from the posterior third of the medial wall of the prospective occipital lobe. After appearance of the calcarine fissure, V1C was sampled as described below.

For 35 PCW – 40 Y specimens, V1C was sampled from the area surrounding the calcarine fissure. Only samples in which the stria of Gennari could be recognized were included. V1C corresponds to BA 17. Small pieces of the neighbouring BA18 could have been occasionally present in the sample, but the majority of the sample corresponded to BA17.

### Hippocampus (HIP)

For 8 – 9 PCW specimens, HIP was sampled from the hippocampal anlage, located on the ventromedial side of the cerebral hemisphere.

For 12 PCW – 40 Y, HIP was sampled from the middle third of the retrocommissural hippocampal formation, located on the medial side of the temporal lobe. Sampled areas always contained dentate gyrus and the Ammon’s horn. Samples dissected from the frozen tissue may contain small quantities of the neighboring choroid plexus.

### Amygdala (AMY)

For 12 PCW – 40 Y specimens, at the aim was to dissect the whole AMY. Potentially very small quantities of surrounding white matter and other surrounding structures in the basal telencephalon were included in samples.

### Striatum (STR)

For 8 – 9 PCW specimens, the Medial ganglionic eminence (MGE), Lateral ganglionic eminence (LGE) and caudal ganglionic eminence (CGE) were sampled separately. Small quantities of surrounding tissue may be included in the samples.

For 12 PCW – 40 Y specimens, striatum (STR) was sampled as follows: the anterior part of striatum containing the head of the caudate nucleus and the putamen were dissected, separated by the internal capsule and ventrally connected to the nucleus accumbens. Small quantities of surrounding white matter are included in the samples.

## Thalamus

For 8 – 9 PCW specimens, the sampled region corresponds to the dorsal part of the thalamic anlage (DTH).

For 12 PCW – 40 Y specimens, the whole mediodorsal nucleus of the thalamus (MD) was sampled from the dorsal and medial thalamus. Small quantities of surrounding thalamic nuclei could be present in the samples.

## Cerebellar cortex (CBC)

For 8 – 9 PCW specimens, region corresponding to the upper (rostral) rhombic lip and adjacent tissue located above the upper rhomboid fossa were dissected.

For 12 PCW – 40 Y specimens, CBC was sampled from the lateral part of the posterior lobe. The sampled area contained all three layers of cerebellar cortex and underlying white matter but not the deep cerebellar nuclei. CBC approximately corresponds to the lateral pontocerebellum.

# Tissue dissection

Depending on the condition and period of the procured specimens, four different dissection methods were used. Photos and/or video were used to document dissections using digital cameras. Regions of interest were matched between different specimens, ages, and hemispheres of each brain. Specific dissection protocol depended upon the period of the specimen and the method by which it was preserved. For all brain specimens procured at Yale University School of Medicine and the Human Fetal Tissue Repository at AECOM, brain regions and NCX areas of interest were collected from fresh tissue. For all other specimens, regions/areas were collected from frozen tissue slabs or whole specimens stored at -80 ºC. To ensure consistency between specimens, all dissections were performed by the same person. Small samples of fresh or frozen CBC were used to measure tissue pH.

## Tissue dissection methods

Different dissection procedures were used for each specimen, depending upon the period of the brain (see below). Our pilot experiments indicated that the quality of RNA and DNA was largely unaffected by variation between the dissection methods used.

### Regional sampling from fresh brain specimens

Brains were chilled on ice for 15–30 minutes prior to sectioning. Brains were placed ventral side up onto a chilled aluminium plate (1 cm thick) on ice. The brainstem and cerebellum were removed from the cerebrum by making a transverse cut at the junction between the diencephalon and midbrain. Next, the cerebrum was divided into left and right hemispheres by cutting along the midline using a Tissue-Tek Accu-Edge trimming blade, 260 mm. The cerebellum was separated from the brainstem by cutting directly posterior to the brainstem, along the cerebellar peduncles. The regions of interest were dissected using a scalpel blade and immediately frozen in liquid nitrogen. Dissected samples were either immediately processed for RNA extraction or stored at -80 °C for later RNA extraction. The remaining brain tissue was cut to obtain 1 cm (specimens from 16 PCW) or 0.5 cm (12 – 13 PCW specimens) thick serial, coronal sections. The tissue slabs were snap frozen in isopentane (J.T. Baker)/dry ice at -30 to -40 ºC and stored at -80 ºC.

### Regional sampling from frozen brain specimens

All previously frozen 12 PCW – 40 Y specimens and tissue slabs were microscopically inspected and the desired region was demarcated, then dissected using a dental drill (AnyXing, 300D) and a Lindemann Bone Cutter H162A.11.016 or diamond disk saw (Dental Burs USA; r=11 mm) on a 1 cm thick aluminium plate over dry ice. Dissected tissue samples were stored at -80 ºC prior to further processing.

### Regional sampling from specimens processed in RNAlater ICE

8 – 9 PCW frozen specimens were sectioned coronally at approximately 2 mm, beginning at the frontal pole, using a dental diamond disk saw. For gradual thawing, tissue slabs were transferred from -80 °C storage to overnight storage in RNAlater ICE (Ambion) at -20 °C. Tissue slabs were visually inspected for gross anatomical neuropathological abnormalities. Next, regions of interest were sampled under a dissection microscope at 4 °C and stored in Buffer RLT Plus from the RNeasy Plus Mini Kit (Qiagen) at 4 °C. RNA was immediately extracted.

## Histological verification of tissue sampling

To verify that the region or NCX area of interest is properly and consistently sampled, we also collected small tissue blocks, from both frozen and fresh brain specimens, adjacent to the tissue sample dissected for the RNA extraction. We have done this for the majority of M1C, S1C, IPC, A1C and V1C samples, which in our experience were hard to match across different specimens but can be histologically verified using Nissl method in postnatal specimens due to cytoarchitectonic differences. This method was also occasionally used for other regions or NCX areas. These tissue blocks were then fixed in 4% paraformaldehyde for 48 h, sectioned at 50 µm thickness using a vibratome, and Nissl stained to verify the identities of dissected adjacent tissue.

## Dissection scoring

We developed a scoring system to evaluate the precision of how well the sampled region/area was represented at the same position of corresponding samples of the same period.

Score Sample description

1 or 2 : The region/area of interest was absent (1) or largely absent (2) and thus not collected.

3 : The region/area of interest was not complete but was of suitable quality to collect.

4 : The region/area of interest was largely intact but was not histologically verified or could not be collected at precisely the same position from which the corresponding contralateral sample was collected.

5 : The region/area of interest was fully intact, verified by gross inspection or Nissl staining (NCX areas), and collected at precisely the same position as corresponding samples of the same period.

## Tissue pulverization

To ensure proper representation of the region of interest, frozen tissue samples were pulverized in liquid nitrogen using a ceramic mortar and pestle (Fisher Scientific, cat# 12-961C and 12-961-5C). Pulverized samples were transferred to chilled wide-mouth cryogenic vials (Nalgene, cat# 03-337-7B) and stored at -80 ºC until used for RNA extraction.

# RNA sample preparation and sequencing

## RNA extraction

RNA was extracted using RNeasy Plus Mini Kit (Qiagen) for mRNA and mirVana kit (Ambion) for small RNA. Either approximately 30 mg of pulverized tissue (12 PCW – 40 Y specimens) or entire amount of dissected brain piece (8 – 9 PCW, smaller than 30 mg) was processed. Tissue was pulverized with liquid nitrogen in a chilled mortar and pestle and transferred to a chilled safe-lock microcentrifuge tube (Eppendorf). Per tissue mass, equal mass of chilled stainless steel beads (Next Advance, cat# SSB14B) along with two volumes of lysis buffer were added. Tissue was homogenized for 1 min in Bullet Blender (Next Advance) at speed 6 and incubated at 37°C for 5 min. Lysis buffer up to 0.6 ml was again added, tissue homogenized for 1 min and incubated at 37°C for 1 min. Extraction was further carried out according to manufacturer’s protocol. Genomic DNA was removed by a proprietary column provided in RNeasy Plus Mini Kit (Qiagen) or by DNase treatment using TURBO DNA-free Kit (Ambion/ Life technologies). 260:A280 ratio and RNA Integrity Number (RIN) were determined for each sample with NanoDrop (Thermo Scientific) and Agilent 2100 Bioanalyzer system, respectively.

## mRNA-sequencing library preparation

The mRNA-sequencing (mRNA-seq) Sample preparation Kit (Illumina) was used to prepare cDNA libraries per manufacturer instructions with some modifications. Briefly, polyA RNA was purified from 1 to 5 µg of total RNA using (dT) beads. Quaint-IT RiboGreen RNA Assay Kit (Invitrogen) was used to quantitate purified mRNA with the NanoDrop 3300. Following mRNA quantitation, 2.5 µl spike-in master mixes, containing five different types of RNA molecules at varying amounts (2.5 × 10-7 to 2.5 × 10-14 mol), were added per 100 ng of mRNA53. Spike-in RNAs were synthesized by the External RNA Control Consortium (ERCC) by *in vitro* transcription of de novo DNA sequences or DNA derived from *B. subtilis* or the deep-sea vent microbe *M. jannaschii* and were a generous gift of Dr. Mark Salit at The National Institute of Standards and Technology (NIST). Each sample was tagged by adding two spike-in RNAs unique to the region from which the sample was taken and three common spike-ins (**Supplementary Table 2**). Spike-in sequences are available at <http://archive.gersteinlab.org/proj/brainseq/spike_in/spike_in.fa>.The purpose of spike-in RNAs is explained under section 8.4.1.

The mixture of mRNA and spike-in RNAs was subjected to fragmentation, reverse transcription, end repair, 3’ end adenylation, and adapter ligation to generate libraries of short cDNA molecules, followed by PCR amplification. The PCR enriched product was assessed for its size distribution and concentration using Bioanalyzer DNA 1000 Kit.

## smallRNA-sequencing library preparation

The TruSeq Small RNA Sample Kit (Illumina) was used to prepare libraries for small RNA-sequencing (smallRNA-seq) per manufacturer instructions. Briefly, 1 µg of total RNA was ligated with 3’- and 5’- adapters, followed by reverse transcription and PCR amplification. Unique index sequence tags were introduced during PCR to enable multiplexed sequencing. Each library was assessed for the presence of desired micro RNA population and quantity by Bioanalyzer High Sensitivity DNA Kit. 21 samples (16 samples from one brain and 5 technical replicates) were pooled and size selected for fragments between 145-160 bp, including the ligated 5’ and 3’ adapters, by gel excision. The extracted product was assessed for its size distribution and concentration using Bioanalyzer DNA 1000 Kit.

## Sequencing

mRNA-sequencing (mRNA-seq) was carried out on Illumina Genome Analyzer IIx (GAIIx), loading one sample per lane. Each flow cell was run for 76 cycles using a single-read recipe (v4 sequencing kits) according to the manufacturer's instructions.

Small RNA-sequencing (smRNA-seq) was done on Illumina HiSeq 2000 by loading 21 samples per lane. The HiSeq flow cell was run for 50 cycles using a single-read recipe (v2 sequencing kit) according to the manufacturer's instructions.

Total of 607 samples, 16 brain regions from 41 post-mortem individuals, which passed stringent tissue quality measures, including postmortem interval (PMI) = 12.9 ± 10.4 (hours); tissue pH = 6.5 ± 0.3; and RNA integrity number (RIN) = 8.8 ± 1 (**Supplementary Table 3** ) were subjected to profiling by mRNA- and smRNA-seq. In total, it was generated 16 billion uniquely mapped mRNA-seq reads (26.5 ± 8.6 million per sample) (**Supplementary Table 3** ).

# DNA methylation

## DNA extraction

Genomic DNA was isolated using the DNeasy Blood and Tissue kit (Qiagen). In detail, approximately 25 mg of brain tissue from each brain region was lysed in Buffer ATL supplemented with proteinase K at 56˚C for 3 to 4 hours. If necessary, tissue was homogenized with a pellet pestle motor homogenizer (Kontes) before adding Proteinase K. Genomic DNA was purified from the lysate by passing it through the column provided in the kit. The DNA was eluted with 200ul of AE buffer. The concentration of the DNA was measured using the PicoGreen dsDNA assay kit (Invitrogen) on a NanoDrop (Thermo Scientific). DNA quality was assessed by visualization on 2% agarose gels.

## Bisulfite conversion

Bisulfite treatment of the DNA using Zymo Research EZ DNA Methylation kit (D5001) was carried out at the USC Epigenome Center. Briefly, 500 ng of DNA was bisulfite treated by incubating in the dark at 50˚C for 12-16 hours with CT Conversion Reagent provided in the kit. Each sample was purified on the column provided in the kit. C to T converted DNA was eluted with 10 μl of M-Elution Buffer in the kit.

## Illumina Infinium HumanMethylation450 BeadChip

Further steps of DNA amplification, fragmentation, hybridization, single base extension and signal detection were performed at the USC core facility, according to standard Illumina protocols. The signals were imported into Illumina’s GenomeStudio software. Two samples were discarded at this stage because of detectable drop-outs (an indicator suggestive of poor quality) as measured by standard Illumina metrics.

# ChIP and sequencing

## Chromatin isolation

DFC and CBC were used for ChIP-seq. Due to tissue limitations, tissues from the right and left hemispheres, when available, were pooled. Dissected tissue was pulverized in liquid nitrogen using a ceramic mortar and pestle and cross-linked with formaldehyde at a final concentration of 1% for 15 minutes at room temperature. Cross-linking was quenched by adding glycine to a final concentration of 125 mM and tissue was washed twice in ice-cold 1X Dulbecco’s PBS (Sigma-Aldrich, P5368). Tissue was then dounced in ice-cold 1X-PBS and centrifuged to obtain a pellet of single cells. To isolate nuclei, the cell pellet was first incubated in Lysis buffer 1 (50 mM HEPES-KOH, pH 7.5; 140 mM sodium chloride; 1 mM EDTA, pH 8; 10% glycerol; 0.5% NP-40; 0.25% TritonX-100; 1X protease inhibitor cocktail) on a rotator at 4 C, followed by incubation in Lysis buffer 2 (200 mM sodium chloride; 1 mM EDTA, pH 8; 0.5 mM EGTA, pH 8; 10 mM Tris-HCl, pH 8; 1X protease inhibitor cocktail) at RT, with a centrifugation step after each incubation. Nuclei in second pellet were lysed by suspending in Lysis buffer 3 (1 mM EDTA, pH 8; 0.5 mM EGTA, pH 8; 10 mM Tris-HCl, pH 8; 20% Sarkosyl; 1X protease inhibitor cocktail). Chromatin was sonicated to sizes between 200 bp and 500 bp with Misonix 4000 (S-4000).

## Immunoprecipitation

Antibodies validated and used in ENCODE studies51,52 were used in this study: Millipore - H3K4me3 (07-743), H3K27me3 (07-449), CTCF (07-729), and Abcam – H3K27ac (ab4729). For each ChIP reaction, 50ul of washed Dyna Protein G beads (Invitrogen, 10004D) were incubated with 5 μl of the respective antibody for 12 h at 4 ˚C. Beads were washed thrice with ice-cold BSA solution (5 mg/ ml BSA in 1X PBS), once with 1X TE, and then suspended in 50 μl of ice-cold BSA solution. 50 μg of the sheared chromatin was added to beads and the mixture was incubated for at least 16 h at 4 ˚C. Dynabeads were washed at room temperature eight times with RIPA buffer (50 mM HEPES, pH 8; 1 mM EDTA, pH 8; 0.7% deoxycholate; 1% NP-40; 0.5 M lithium chloride; 1X protease inhibitor cocktail) and once with 1X TE. Immunoprecipitated chromatin was eluted in 200 μl of Elution buffer (20% SDS in 1X TE) at 65˚C for 15 minutes, with occasional vortexing. The eluted protein-chromatin complex was reverse cross-linked by overnight incubation at 65˚C. ChIPped and input DNA were treated with RNaseA (Sigma-Aldrich, R4875), Proteinase K (Denville Scientific Inc, CB3210-7) and purified with a Qiagen purification kit (Qiagen, 28104).

## ChIP-seq library preparation and sequencing

For adult samples, 5 ng of the purified immunoprecipitated DNA was used to prepare sequencing libraries for the Illumina sequencing platform. Sequencing libraries were generated using the ChIP-seq DNA sample prep kit (Illumina, IP-102-1001) according to the manufacturer’s protocol with some modifications, as follows. DNA fragments were end-repaired and adenylated, as per manufacturer’s instructions. In order to reduce the free adaptor dimers that can be preferentially amplified during PCR, a 1:40 dilution of the adaptor was used instead of the recommended 1:10 dilution. The recommended gel purification step was performed only after DNA fragments were enriched with PCR amplification. DNA fragments between 250bp and 350bp were size selected by gel electrophoresis and band excision. During gel extraction, the gel was melted at room temperature, instead of 50˚C. For multiplexing, index primers from the Multiplexing Sample Preparation Oligonucleotide Kit (Illumina, PE-400-1001) were used.

For fetal and infant samples, 5 ng of the purified immunoprecipitated DNA was used to prepare sequencing libraries using the TruSeq ChIP Library Prep Kit (Illumina, IP-202-1012) according to the manufacturer’s protocol with some modifications. The recommended gel purification step was performed after DNA fragments were enriched with PCR amplification.

Sequencing of the libraries was carried out at the Yale Center for Genome Analysis using either an Illumina Genome Analyzer IIx (GAIIx) or an Illumina HiSeq 2000 (adult samples) and an Illumina HiSeq 2500 (fetal/ infant samples).

# ChIP validation

## ChIP-qPCR

Independent ChIP was carried out as described above. Selected regions were tested for enrichment by droplet digital PCR (QX100™ Droplet Digital™ PCR System, Bio-Rad). The primer-probe pairs used are listed in Supplementary Table 4 . Each reaction included 500 pg of immunoprecipitated DNA or input DNA, 500 nM each primer, 250 nM of the probe and 1X ddPCR Master mix (Bio-Rad). Droplets were generated and DNA was PCR amplified as follows: 95ºC/10 minutes, (95 ºC/30 s, 60 ºC/ 1 min) x 40 cycles, 98 ºC/ 2 minutes. The PCR plate was read in a droplet reader (Bio-Rad) and the absolute values obtained by QuantaSoft analysis software. Enrichment was calculated as percentage of input (%IP). Three independent PCR reactions were carried out for each target region and the error bars indicate the standard error of the mean.

## 5’RACE

To confirm the presence of novel TSS identified by the enrichment of H3K4me3, 5’ RACE was carried out by the SMARTer RACE cDNA Amplification Kit (Clontech) as per the manufacturer's instructions. Briefly, RACE-Ready cDNA was synthesized with 5’-CDS Primer A. Regions of interest were then PCR amplified using gene specific primers (Supplementary table 4), gel extracted, and cloned using TOPO TA cloning kit (Invitrogen) and sequenced.

# mRNA-seq data analyses

mRNA-seq data processing is based on the RSEQtools framework (ref) (<http://archive.gersteinlab.org/proj/rnaseq/rseqtools/>, version 0.5) and the workflow is shown in **Supplementary Fig. 1**. Details of each processing step are given below.

## Sequence alignment

mRNA-seq reads were aligned to the reference human genome hg38/GRCh38. The FASTA files of the reference genome were retrieved from the UCSC genome browser (ref). From the full reference genome, minor haplotypes, random and unknown sequences were excluded. Hence, only 22 chromosomes, chromosomes X, Y and M were considered for the alignment. In addition to the genome, the sequences of spike-in RNAs were added to the reference. The FASTA file of the spike-in RNAs is available at <http://archive.gersteinlab.org/proj/brainseq/spike_in/spike_in.fa>

Alignment of the reads was performed by STAR (version 2.4.0e) (ref). Command line"--runMode genomeGenerate" was used to build the sequence index for both human genome and spike-in sequences. To improve the mapping quality of splice junction reads, human gene annotation retrieved from the GENCODE project (version 21, http://www.gencodegenes.org/releases/21.html) (ref) was additionally provided. Command line "--sjdbOverhang 74" was used to construct splice junction library.

As an example, the alignment of Sample A is given below

--runMode alignReads --readFilesIn A.fq --outFileNamePrefix A.out --genomeDir hg38ANDSpikein --runThreadN 8 --outSAMattributes All --outSAMtype BAM SortedByCoordinate --limitBAMsortRAM 62000000000 --quantMode TranscriptomeSAM --outFilterMismatchNoverLmax 0.1 --alignSJoverhangMin 8 --alignSJDBoverhangMin 1 --outSAMunmapped Within --outFilterType BySJout --alignMatesGapMax 500 --outFilterMultimapNmax 50 --alignEndsType Local --outSAMstrandField intronMotif --outFilterIntronMotifs RemoveNoncanonical

where,"hg38ANDSpikein" is the indexed human genome and spike-in sequences. More details about the parameters are available <https://github.com/alexdobin/STAR/releases>. Some default parameters were changed to obtain high confidence alignment. Options "--outSAMstrandField intronMotif" and "--outFilterIntronMotifs RemoveNoncanonical" were used to make the STAR alignment compatible with Cufflinks in the downstream splicing analyses.

Finally, the entire mRNA-seq information were wrapped in the BAM format alignments.

## Measuring expression

After the reads were mapped to the reference sequences, the expression level of genes, exons, and spike-in RNAs were measured in the commonly used units of RPKM (reads per kilobase of exon model per million mapped reads) ref. SAMtools ref (<http://samtools.sourceforge.net/>) and RSEQtools ref (<http://info.gersteinlab.org/RSEQtools>) software packages were used to perform this task.

First, the BAM format alignment was converted into SAM format alignment by using the "view" function in SAMtools, and then the "sam2mrf" function in RSEQtools was used to convert the SAM format to Mapped Read Format (MRF). Analyses further downstream were carried out using only uniquely mapped reads because of the uncertainty of the reads mapped to multiple sites. In addition, mitochondrial reads were excluded due to their large variability across different individuals. After filtering, RPKM values were computed using "*mrfQuantifier*" function in RSEQtools. This program required an annotation set, which includes elements whose expression level is to be measured. Due to the presence of multiple transcripts for each gene in the human genome, assignment of reads to specific transcript is not straightforward. Hence, a composite model of a gene, which is a union of all exonic nucleotides across all of its transcripts, was defined. "*mergeTranscript*" function in RSEQtools was used to generate the composite gene model from GENCODE (version 21, [http://www.gencodegenes.org/releases/21.html](http://www.gencodegenes.org/releases/10.html)). From the composite model of genes, the composite model exons were also extracted. The *mrfQuantifier* calculated an expression value for each annotation entry by counting reads from all nucleotides that overlap that annotation entry. Subsequently, this value was normalized per million mapped nucleotides and the length of the annotation item per kb (RPKM).

In summary, the steps to compute the expression levels for sample A:

Convert BAM format alignment to SAM format alignment:

$ samtools view A.bam > A.sam

Convert SAM format to MRF:

$sam2mrf < A.sam > A.mrf

Build composite model of gene:

$mergeTranscripts knownIsoforms.txt transcript.interval compositeModel > geneComposite.interval

where "knownIsoforms.txt" determines which transcript isoforms belong together; "*transcript.interval*" is the interval format annotation of transcript isoforms; "*geneComposite.interval*" is the interval format annotation of gene composite model, from which the interval format annotation of exon composite model is extracted;

Calculate expression of any assigned element:

$mrfQuantifier annotation.interval singleOverlap < A.mrf > A.expression

where "*annotation.inteval*" should be the interval format annotations for gene composite model, exon composite model and spike-in RNAs; "*singleOverlap*" means reads that overlap with multiple annotated features were excluded.

STAR, SAMtools format conversion, and measuring expression by RSEQtools were driven by in-house Perl scripts and were run at the Yale High Performance Computing clusters. This processing also compared the region spike-in pairing detected in each sample with the brain region nominally associated with the sample and generated a report that identified any inconsistencies.

## Normalization and batch correction

Gene expression values were further processed to remove unwanted technical variates. First, the mitochondrial genes were excluded from further analyses because high inter-individual variability was observed and their expression values also strongly correlated to PMI values (Spearman correlation coefficient = 0.62). Second, conditional quantile normalization was performed using CQN Bioconductor (ref) to correct the global distortion and to exclude GC-content bias. Finally, to remove batch effects due to processing at two different sequencing centers, ComBat (ref) was used.

## Quality control assessments

Several quality control measures were implemented throughout sample preparation and data analysis steps. Samples that failed to pass the quality control measures were resequenced, when possible, or removed from analyses. A work flow of all quality control steps are shown in the Supplementary figure 1

### Spike-in RNAs and sequencing error rate

Multiple spike-in RNAs were used to tag different samples (**Supplementary Table 2**). A pair of spike-in RNAs unique to the region from which the sample was taken was used to identify any possible sample swaps from library preparation to sample loading on the Genome Analyzer. In addition, three common spike-in RNAs were also added. All five spike-in RNAs were used to calculate sequence error rate. Since the sequence of the spike-in RNAs was known, mismatches between the sequenced reads and the reference spike-in RNAs was used to estimate sequencing error rate of Genome Analyzer. The dependence between the percentage of mismatches and the sequencing cycles were plotted and mostly low sequencing error rates (median values <5%) were found. As expected, a higher error rate was observed towards last sequencing cycles (Supplementary figure 2)

### Reads from mitochondrial DNA, rRNA and tRNA

Data from all samples were assessed for the number of reads or percentage of reads that mapped to mitochondrial, rRNA and tRNA (Supplementary Table 3).

### Read distribution across gene length

All samples were analyzed to detect any bias in read distribution along the body of each annotated gene. The composite exon-model gene was split into 100 equal segments, from 5'-end to 3'-end and RPKM of each segment was calculated. Then a ratio of the RPKM of each segment relative to the median RPKM value of the gene was taken. The median ratios of all genes in all samples (represented as a flat black line in the Supplementary Figure 2) around zero indicated uniform read distribution along genes. There was only a slight trend of 5’ underrepresentation and 3’ overrepresentation.

## Clustering analyses

### AC-PCA: PCA adjusting for individual variation

Strong individual effects were observed in mRNA-seq data. Given the small differences observed between neocortical areas in the standard PCA, the first several principal components likely represent individual variation rather than inter-areal variation. To adjust for individual variation, AC-PCA was implemented. Within a time window, suppose there are *K* individuals. For every individual, samples from *B* regions were taken. For every sample, the expression levels of *G* genes were measured. Let *Xi* denote the *B×G* matrix for individual *i*. Let *w* denote a *G×1* vector, representing the loading for the principal components. AC-PCA maximized the following objective function:

where is a tuning parameter. To encourage the coordinates for the same brain region across individuals to be similar, a penalty term was added in the objective function of standard PCA. The spatial pattern of the brain regions is quite robust to the choice of , and was chose. To gain robustness, the rank of RPKM across samples was used instead of RPKM itself. Furthermore, genes that have median RPKM less than 2 across all samples were filtered out in the analysis. Full implementation details for AC-PCA will be submitted in a separate manuscript soon.

For mRNA-Seq, the median gene expression levels (log2 RPKM) were calculated across samples and genes with median expression greater than 2 were selected. This step retained 10, 952 genes. To gain robustness, the genes across samples were ranked and the rank used for dimension reduction. For exon array: the median gene expression levels were calculated across samples and genes with median expression greater than 5.5 were selected. This step retained 10, 689 genes. The pairwise Euclidean distance between brain regions/neocortical areas were calculated for each brain based on the first two PCs. The average pairwise distance was calculated for each brain and plotted by time window.

### Multiple dimension scaling (MDS)

The multiple dimension scaling (MDS) was analyzed by using all expressed genes from 607 samples. The Euclidean distance between any pair of samples were calculated to construct distance matrix with dimension 607 x 607. The R function *cmdscale* was used to calculate two-dimensional MDS. The returned two vectors were used as coordinates x and y for each of the 618 samples to make a scatter plot in a 2- dimensional plane. The 607 data points, each representing one sample, were plotted and colored by window and brain region (Extend data Figure) and their correlation with covariates, e.g., window, period, day, region were shown in heatmap with correlation coefficients inside.

## Identification of temporally/ spatially differentially expressed genes

Differentially expressed (DEX) genes were computed for every pair of 16 brain regions and 9 time windows (1-9). This means the comparisons were separately performed in two different dimensions, of which the spatial dimension was the pairwise region comparison in each time window and the temporal dimension was the pairwise windows comparison for each region.

The DESeq2 (version 1.4.5) package was utilized to perform the differential expression (DEX) analyses. The GC content correction from CQN package were incorporated to DESeq2. Besides, the two sequencing sites (Yale and USC) were set as a covariates to reduce the batch effects. Statistical tests for differential expression were based on a model using the negative binomial distribution. The reported statistical significances corrected for multiple testing using the Benjamini-Hochberg procedure (ref) with a false discovery rate less than 0.01. In addition, to be called DEX genes were required to have RPKM > 1, reads count > 10 in at least one condition, and fold change > 2.

## Identification of genes differentially expressed by sex

For this analysis, low- and non-expressed transcripts with mean counts <10 in all sample sets as defined by region, period, and sex, were filtered out leaving a total of 27,896 (46.4%) transcripts for analysis. Log2-transformed counts were adjusted for processing site using ComBat {REF}, and for sample-level quantitative variables (RIN, PMI, and sequencing depth) using linear regression. By the nature of the collection the male and female samples were not closely matched for developmental stage, however these stages are a strong contributor to the variance in gene expression (Figure X) and strongly correlated (R2=\_\_\_) with PC1 from all 27,896 expressed transcripts. To account for the differences in developmental stage between male and females linear interpolation (‘approx’ function in R) was used to estimate the expression of each transcript at a given developmental period for males and females. This interpolation was performed separately for the male and female samples within each of 16 brain regions (Table X) for all periods from 4 (13 PCW) to 13 (40 years). Post-interpolated counts were estimated at three points per period (25% of period, midpoint, and 75%). Conditional quantile normalization (CQN) {REF} was then applied to the interpolated counts to correct for transcript-level variables GC-content and gene length.

Differential expression analysis was assessed using DESeq2 {REF} on the interpolated counts and normalization factors from CQN within each of the 16 brain regions and 10 time periods for a total of 160 tests. To identify the top 100 genes expressed more highly in males (male-DEX), and the top 100 genes expressed more highly in females (female-DEX), from each region-period, the mean -log10(p-value) and log2(FD) for 9 protein-coding, Y-chromosome genes (KDM5D, DDX3Y, ZFY, PCDH11Y, USP9Y, RPS4Y1, TMSB4Y, NLGN4Y, EIF1AY) representing a gold standard for sex-DEX genes were calculated. Starting at this point, and moving along the slope towards the intersection of the x-axis (-log10(p-value) = 0) and y-axis (log2(FD) = 0), all the genes were ranked in the order they passed one of these thresholds to select the top 100 male-DEX genes. For females the negative log2(FD) value estimated from the gold standard was used instead.

Based on the logic that a truly sex-DEX gene would show a degree of sexual dimorphism in neighboring time periods (from different individuals) the probability of observing a gene in the top 100 for each sex in neighboring time periods by chance was estimated. By randomizing the assignment of sex (18 random females and 21 random males, as in the true data) to each of the samples, 100 permutations of the experiment, including the interpolation, differential expression, and selection of 100 top “male”-DEX and “female”-DEX genes were run. By determining the proportion of genes that appeared in N consecutive time periods across all regions, time periods, and permutations the false discovery rate (q-value) was estimated for a given value of N. All genes with q-values ≤0.05, which represents a threshold of two or more neighboring time periods, are reported and characterized.

## Gene co-expression network analyses and module characterization

Weighted gene co-expression network analysis (WGCNA) (ref) was performed using R package WGCNA to investigate the spatio-temporal dynamics of gene expression. The normalized and batch corrected gene RPKM values were first log2 transformed. Next, *pickSoftThreshold* function (with 10 as soft-threshold power) was used to analyze the network topology. In order to do automatic network construction and module detection, *blockwiseModules* function was used. Modules with fewer than 10 genes were merged to their closest larger module. For each module, WGCNA generated an eigengene to represent the module. To check the reliability of detected modules, custom R scripts were used to calculate the correlation between a gene and modular eigengene; genes were re-assigned to another module if the gene had the largest correlation coefficient with that module’s eigengene than its own. Overall, only a small number of genes were re-assigned to other modules. The *moduleEigengenes* function was used to re-calculate the eigengene for the changed modules. To illustrate the modular feature, the smoothed fitted trajectories of the modular eigengenes were plotted using *lowess* function in R (Extended data Figure 9).

## Alternative splicing analyses

Cufflinks (version 2.2.1) and RSEM (version 1.2.19) (ref), two different tools using different statistical models and assumptions were employed to predict the expression of the GENCODE v21 annotated transcripts (196,327 in total).

As per both developers’ suggestion, multiple mapping reads were also included to improve the accuracy. Many transcripts were found to be not expressed or had very low expression and were excluded. Only transcripts that met the following criteria were considered 1) Fragments Per Kilobase of transcript per Million mapped reads (FPKM) ≥ 1 in at least 5 samples, 2) at least 5 brains having at least 1 region with FPKM ≥1, 3) Cufflinks prediction confidential intervals (CI) < 1 in at least 5 samples. The correlation analysis showed that many transcripts were consistently predicted by two tools (Extended data Figure 8). Transcripts that were inconsistently predicted (with Pearson correlation coefficient less than 0.5) were excluded. Finally, 92,824 transcripts were included in the analysis.

The expression quantification of transcripts may also be affected by GC content and sequencing batch. The same normalizationprocedures using CQN and ComBat packages (see normalization and batch correction) were also carried out for transcript quantification. Due to higher uncertainty of transcripts expression over gene expression prediction, “tsoutliers” function in "forecast" R-package was additionally used to filter out and fix some prediction outliers. Since the samples in a given region and developmental period are supposed to be similarly expressed (see biological reproducibility), the samples in the adjacent periods should change smoothly. A fitted smooth curve along developmental period for each region would represent their changing trend. The outliers would be far from the curve, and were fixed by using the corresponding values on the curve. After fixing, the expression filtering was redone, and resulted in 73,902 transcripts.

One gene generally has more than one predicted transcripts. The relative expression of different transcripts in different situations may dramatically change, e.g., the low expressed transcript changed to high expression while the high expressed transcript changed to low expression. To quantify the different composition of transcripts, we introduced gene entropy, Ei = -∑Pij log2(Pij), where Pi were the ratio of j transcript in i gene (sum of all transcripts). Gene with more diverse transcripts is expected to have larger value of entropy.

## Identification of novel transcriptionally active regions (nTARs)

Transcriptionally active regions (TARs) are locations on the genome that show evidence of being transcribed. The goal was to identify novel TARs (nTARs), *i.e.*, regions that have not been previously reported, and characterize their expression profile across brain regions. Our approach to achieve this was as follows: (1) Reads that mapped to AceView 2010 (converted to hg38 using liftOver [ref]), GENCODE v21 composite model, and UCSC knownGenes were removed from each sample. To be conservative, a 1-bp overlap to an annotated entry was considered sufficient to exclude the read. All remaining reads were combined, resulting in 835,436,409 reads mapped to un-annotated regions. This represents our “novel sample”. (2) The combined reads were converted into “signal” tracks/BedGraph files through RSEQtools. Specifically, *mrf2bgr* was used to generate BedGraph files that depicted for each position the read coverage, normalized by the total number of reads. Then, the signal track was segmented using the max-gap, min-run algorithm. Briefly, nucleotides with a normalized read coverage greater than 0.5 were identified. Then, regions that were less than 100 nucleotides (max-gap) apart were joined together and the resulting regions, at least 150 nucleotide long (min-run), were kept. To determine these thresholds for segmentation, we reasoned that nTARs should be similarly characterized as lncRNAs and genes annotated in GENCODE v21. We determined the most reasonable values by exploring their distribution density curve in the GENCODE v21 database. (3)The result of the first two steps could lead to the generation of artificially fragmented nTARs. For example, assume there is a 1 Kb nTAR. If in the middle of that region there is a 200 nucleotide long repetitive element. When selecting the reads in novel regions, those mapped to that repetitive element will be excluded, leaving a gap of at least 200 nucleotides and identifying two nTARs in this region. To reduce this issue, we counted the mappability of the intervals between the adjacent nTARs. If they were far less than 1 and no other annotated gene fully contained, this gap was filled and the nTARs were merged. (4) The nTARs were then classified as intergenic, intronic, 5’UTR, or 3’UTR according to their distance from the nearest GENCODE gene. If there were no annotated genes within a 2 Kb window flanking the nTAR, it was labeled as intergenic. (5)The known ESTs were retrieved from UCSC Table Browser and overlapped with nTARs, providing additional evidence of transcription. (6) The PhastCon score of nTAR was calculated to estimate the phylogenetic conservation of the sequence. (7) nTARs were overlapped with epigenetic marks H3K4me3 and H3K27ac.

## Interspecies transcriptome comparison

To identify genes with conserved or differential patterning between species, three data sets were compared against gene RPKM expression values from the six adult brains in this study:

1) microarray intensity expression levels from 6 human brains from the Allen Human Brain Atlas (http://human.brain-map.org/; Hawrylycz et al 2012);

2) RNA-Seq RPKM expression levels from 5 adult macaque brains (Nenad's group to update); and

3) "expression energy" (defined as fraction of stained volume \* average intensity of stain) of mouse in situ hybridization data from the Allen Mouse Brain Atlas (http://mouse.brain-map.org/; Lein et al 2007; Lee et al 2008). More specifically, coronal data for 2651 mouse genes with reproducible coronal and sagittal series and with orthologs expressed in adult human brain were included for direct comparison between species, and sagittal ISH were included in the figure to show cortical and cerebellar expression in a single section.

16 homologous brain regions were sampled from human (BrainSpan) and macaque. Expression levels for substructures of these 16 sampled regions were averaged in the HBA data set. In mouse, brain structures were carefully matched to these 16 structures and expression energies of the 250 most highly expressed voxels in each region were averaged for comparison with macaque and human. Expression levels from all primate data sets were log2 transformed (in the two RNA-Seq data sets after offsetting RPKM values by 1). Additional ISH validations for macaque were included from the NIH Blueprint Non-Human Primate Atlas (<http://www.blueprintnhpatlas.org/>)

Differentially expressed genes in human (current data set) and macaque were defined as those with significant repeated measures ANOVA p-values (p<0.01; Bonferroni corrected; samples matched by brain) across all 16 brain regions. The 3113 "regionally patterned" genes meeting this criterion in both species were then compared between species by calculating the Pearson correlation across the 16 regions, and the results were plotted as a density plot. As a within-species control, the same set of genes were similarly compared between all 20 possible pairs of human trios. Nearly all genes (99.976%) were correlated within human with R>0.5, so this value was used as the cutoff to assess whether or not a gene is conserved between species, and the results were presented in pie charts. To compare human and macaque with mouse, the same analysis was performed, with the exception that only the 862 regionally patterned genes that were included in the 2651 high-confidence mouse gene list could be considered. Furthermore, at the time of the analysis the mouse brain atlas was only annotated to the level of cortical lobes, and so the average expression in 9 comparable regions was compared in this analysis. In total ISH data was available in whole mouse and macaque brain for 35 of the 3113 regionally patterned genes, from which three of the genes best representing conserved and non-conserved patterning were chosen to present as ISH validation.

# Small RNA-seq Data Analyses

Raw sequence reads were obtained from an Illumina HiSeq2000 using the Illumina TruSeq Small RNA-seq protocol. The library was size-selected for cRNA fragments between 145-160 bp, including the ligated 5’ and 3’ adapters and samples were multiplexed using barcoding PCR primers that are implemented in the TruSeq protocol. Supplementary Figure shows a schematic of the workflow used for analysis. All samples passed quality control using FastQC (http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc/), considering a wide range of quality metrics including per-sequence and per-base quality, per-sequence and per-base GC content, per base N content, sequence duplication levels, and overrepresented sequences; an example plot of the per-base quality for one of the samples is provided in Supplementary Figure. Quality metrics were similar for all remaining samples (data not shown). Sequence reads were clipped to remove the Illumina small RNA 3' adapter (TGGAATTCTCGGGTGCCAAGG). A minimum of 5 adapter bases were required for clipping, resulting in approximately 90% of the reads being shortened. We experimented with using more stringent minimum barcode-nucleotides required for clipping, however values between 4-10 bases yielded very similar numbers and length distributions of clipped reads (data not shown). Clipped reads were collapsed into the multi-fasta format that contains only unique sequences and associated counts for each read for input into the pipeline constructed around the miRanalyzer miRNA analysis software described in ref .

For the analysis, trimmed reads between 16-36nt were selected and searched against mature and precursor/hairpin databases in miRBase (release 18) ref. Reads not mapped to known targets in the miRBase reference were searched, in the following order, against piwi-interacting RNA (piRNA) sequences in RNAdb (version 2)ref, enhancer RNA (eRNA) sequences from genomic coordinates defined in83 (converted from hg17 to hg19 using UCSC’s liftover tool), transfer RNA sequences in GtRNAdb84, mRNAs defined in GENCODE (version 10) annotation72, and entries in the Rfam database (version 10.1) 85. See Extended Data Fig. 10c for the fraction of total reads mapping to each of these entities. Reads mapping to more than 5 distinct entities in any of these data sources were discarded due to poor specificity. All remaining reads were mapped to the human genome (hg19) to identify read clusters corresponding to potential novel miRNAs. All read mapping to miRBase, RNAdb, GtRNAdb, GENCODE, Rfam, and the genomic reference was performed using Bowtie (version 0.12.7)57 using the '-best' and '-strata' arguments, in addition to a maximum 2-base mismatch in sequence alignment. As miRNAs are not a fixed size, a 17nt seed region is used (corresponding to the minimum length of a known miRNA) and the mapping information was post-processed to identify the best alignment to longer miRNA sequences.

Identification of novel miRNAs constituted the final task in the pipeline; a detailed description of this procedure is provided in ref. Briefly, this was performed in three stages:

1. Sequence reads were clustered into putative mature microRNAs;

2. Candidate pre-microRNAs were extracted from the genome (at the positions of the putative mature sequences from step one). This was done iteratively, extending the precursor sequence from a minimum length of 65 to a maximum of 135 bases in order to select the optimal candidate as defined by the one with the minimum free energy of RNA secondary-structure provided by the Vienna RNA package (version 2.0)ref;

3. The Weka software environment (version 3.5.3)87 was used to filter and assign confidence scores to each of the candidates on the basis of five different Random Forest models88.

A total of 74,202 candidate miRNA sequences were output from this procedure. These were further filtered to retain only candidates for which reads that mapped to the mature sequence had not been successfully mapped to any other RNA species earlier in the pipeline. The remaining 41,061 candidates were condensed by collapsing overlapping candidates, based on the genomic coordinates of the extrapolated putative novel miRNA precursor. The remaining 527 candidates were filtered based on their consistency across the technical replicate samples such that any candidate with more than 10 mapped reads on average were removed if the coefficient of variation in read-depth across the technical replicates exceeded 1. Further filtering was performed such that novel miRNA candidates were required to be detected, i.e., have >4 reads, in at least five of six biological replicate samples in at least one of the 16 brain regions. Expression data for the remaining 95 candidates were subsequently averaged over technical replicates and taken forward to assess differential expression between brain region pairs.

Known miRNA, novel miRNA, and piRNA data for all samples were normalized by the total number of mapped reads over all RNA species to reduce any potential bias from very highly [differentially] expressed RNAs; this method of normalization should result in less inflation of expression estimates for lower-expressing RNAs in tissues with fewer detected RNAs such as the CBC. The total number of small RNA species detected in our dataset is summarized in Extended Data Fig. 10c. An assessment of the similarity of the technical replicate library preparations was performed using Pearson correlation (Supplementary Figure).

Differential expression analyses were performed on the known miRNAs, novel miRNAs, and piRNAs using the DESeq (version 1.6.1) package ref, part of the bioconductor project (ref) in the statistical scripting language R (version 2.14.1) ref. Statistical tests for differential expression were based on a model using the negative binomial distribution and reported statistical significances corrected for multiple testing using the Benjamini-Hochberg procedure with a false discovery rate less than 0.0566 (Supplementary Figure?).

The 16 novel miRNA candidates found to be differentially expressed between at least one brain region-pair were input into the miRanda software (version 3.3a) and mapped against all human 3' UTR sequences obtained using the GENCODE v10 annotation72. Putative mRNA targets were filtered to include only the top 1-12% of interactions (dependent on the novel miRNA; mean=3.8%) achieving a score greater than 165 as reported by miRanda. For all remaining putative interactions, the vector of miRNA fold-changes across all tissue pairs was correlated (Pearson) with the same vector of mRNA fold-changes. Network visualization was performed using the edge-weighted force-directed layout in Cytoscape (version 2.82)89. Network edges were weighted and shaded according to the strength of the anti-correlation found between the miRNA/mRNA fold-changes over all brain region-pairs.

Details of the miRNA and piRNA analysis can be found in Supplementary Tables 5-6 in which the known miRNAs, novel miRNAs, differentially expressed known miRNAs, differentially expressed novel miRNAs, and differentially expressed piRNAs are presented.

# DNA methylation analyses

## Data normalization

The raw data from the Illumina 450K arrays were normalized by Fresco (<https://github.com/paulmanser/fresco>, manuscript in prep). Briefly, a group of loci from housekeeping genes whose CpG sites were judged very likely to be consistently highly methylated or unmethylated in all cell types within brain tissue were identified. Then a three-dimensional local regression surface to the deviations of each array from the average was fit, as a function of non-biological variables such as average signal intensities and probe CG content. This gave an estimate of the distortion due to technical factors on each array for each combination of our non-biological predictors. This estimated distortion was subtracted from raw signals and these adjusted signals were then used to obtain the β-value (Supplementary figure 3), an estimate of the proportion of cells methylated at each locus. It has to be noted that this process does not use any information about batches, but is nonetheless able to completely remove batch effects as measured by sample clustering and one-way ANOVAs for batch effect. After normalization, a high correlation was observed between biological replicates (Supplementary figure 4)

## Regional and inter-individual variation

In order to estimate the proportion of variation in methylation attributable to individual differences and to regional differences, sex chromosomes and CBC samples were first removed. For each CpG, a two-way analysis of variance (ANOVA) was fit using individual and brain region as two main factors and log2 of the post conception day as a covariate of the linear model. For each CpG, R2 values were computed using the sum of squares from the resulting ANOVA table by taking the ratio of sum of squares for each factor over the total sum of squares.  After computing R2 values for the two factors, individual R2 against regional R2 for sets of CpGs stratified by genomic location were plotted.

## Identification of cell type specific methylation sites

Cell-type specific methylation probes were defined as those CpG sites that exhibit 30% methylation level differences (a delta (β) ≥0.3) between the neuronal and non-neuronal cell samples from Kozlenkov, Alexey et al 2014. A total of 27,349 sites were identified.

## Comparison of DNA methylation trajectories in fetal vs post-natal development.

For each one of the 461,594 CpG probes analyzed, DNA methylation values were fitted to a multiple linear model with the log2 of the post-conception days and sex. Analysis was performed with data from all neocortical regions, independently testing pre-natal and post-natal samples to overcome any possible uncorrected batch effect and the fact that pre-natal samples came from an independent external dataset (Spiers et al., 2015). The probes were then classified in to three categories – those showing an increasing methylation trajectory, decreasing, or non-changing through age. Age-correlated categories required a Bonferroni corrected p-value <0.05 and absolute difference of beta (delta(β)) values between the first and the last fitted points predicted by the model to be greater than 0.1. Non-changing trajectories required nominal p-values <0.05 and delta(β) <0.1. All possible combinations of these three categories for pre and post-natal periods were then constructed to obtain the nine subcategories showed in the first two panels of Figure 5.

CpGs in each category were tested for enrichment of 5 different types of elements:

1) genomic elements related to genes. For defining genomic elements the GENCODE (v21) gene model in hg38 was used to map CpG sites to exons, introns, intergenic regions or promoters (defined as 1Kb regions around the TSS).

2) enhancers, defined as H3K27ac Chip-seq peaks in six DFC samples (3 fetal, 3 adult). Peaks overlapping TSS were excluded to avoid assessing promoters. Adult and fetal-specific enhancers, defined as those peaks that appeared in the three samples from one period and in none of the three samples from the other period were also tested.

3) cell-type specific methylation sites in brain (as described in section 11),

4) genes with cell-type specific expression in brain (as described in section xxx), and

5) age-related methylation sites in blood. For enrichment in age related methylation sites in blood, CpGs that differentiate newborn from nonagenarian blood samples from Heyn et al. 2012, were used.

All cited categories were compared to CpGs that do not change through age in pre or post-natal periods. When testing for enrichment in genes instead of CpGs genes related to each category's CpG according to GENCODE annotations were used. Enrichment p-values were obtained by means of a two-tailed Fisher exact test and a Bonferroni corrected p-value <0.05 was considered significant. Odd ratios were also calculated applying Haldane correction when needed. Odds ratios for each category are shown in the rightmost panel in Figure 5.

# ChIP-seq analyses

## Peak calling, annotation and normalization

Sequencing reads from each sample were mapped to human genome assembly hg38 (NCBI GRCh 38) using Bowtie 1.054 with parameter “ -m 1 -p 4 --phred33-quals -S –q”.

MACS 2.0 was used to detect peaks for H3K4me3, H3K27ac and CTCF. For these marks, aligned reads from all DFC and CBC samples were pooled and analyzed by MACS (FDR ≤ 0.05) to detect the peaks enriched in immunoprecipitated samples over the input. The peaks detected in this pooled analysis yielded a master list of peaks for the subsequent analyses. Each brain sample was then analyzed individually. If there was at least a 50 % overlap between a peak in an individual brain sample and a peak in the master list, that peak was scored as detected in that brain. A peak was defined as present if it was detected in multiple brains (2 out of 3 or 3 out of 4) in DFC or CBC. Enriched peaks in H3K27me3 were detected by SICER 1.156, with parameter settings of window size as 1000bp, gap size as 3000bp, and FDR ≤ 0.01. An approach similar to other marks was applied to construct a master list of H3K27me3 peaks and to score a present peak after comparing individual brain sample peaks. To compare biological replicates, peaks with FDR ≤ 0.1 were selected and plotted as scatter plots. The function 'kde2d' in R package was used to create a 2D density image for each comparison. A high correlation was observed between replicates (Supplementary Figure )

All peaks were annotated by our in-house Python scripts according to database GENCODEv21. We selected all transcripts in the database with types “protein\_coding”, “lincRNA”, “snoRNA”, “snRNA”, “miRNA”, “misc\_RNA”, and “rRNA” for our annotation. We matched each peak with transcripts in the database as follows. First, the peaks were matched with extended promoter regions, defined as TSS ± 1kb, in the database. If peaks were within the promoter regions, the genic location of the peaks was assigned as “TSS”. If peaks were outside the promoter regions, we then tested if they were matched with any transcripts. Where peaks matched multiple transcripts, we selected the longest one as the target transcript for further analysis. The genic location of a peak matching a transcript was assigned as “Exon” if that peak overlapped any exon. If the peak did not overlap with an exon it was assigned as an “Intron”. If peaks did not overlap any transcripts we assigned their genic location as “Intergenic”.

All ChIP-seq peaks in the master lists were normalized prior to any downstream analysis. For each ChIP-seq sample, signal values for each base pair in target peaks created by MACS were read and an average signal value calculated using the R package Conductor. Total signal value was also calculated from all peaks of each sample. The average signal value for each peak was normalized by the total signal value. These normalized average values were used for all subsequent inter-sample comparisons, including regional specificity analysis and PCA analysis, and for analysis of correlation between histone modification enrichment and gene expression.

## Differential enrichment of histone modifications and gene expression

To identify H3K27ac peaks that were differentially enriched between DFC and CBC first peaks were defined as promoter (if they were in the TSS) or enhancer peaks (all non-TSS peaks). For each group, differential enrichment was calculated as w=(SDFC – SCBC) / (SDFC + SCBC +1) , where SDFC and SCBC are average values of normalized signals in DFC and CBC, respectively. Based on w values, peaks were defined as DFC-enriched (w > 0.3), CBC-enriched (w < -0.3) and nonspecific (-0.3 to 0.3).

To compare differential enrichment of histone marks with gene expression, expression RPKM value of the gene that the peak overlaps with was considered. For each of the 3 groups defined above, the RPKM values for all genes were summed and a t-test was performed to check the expression difference between the groups.

## Identification of spatial and temporal enhancers

# Enrichment of cell type specific methylation sites and temporal specific enhancers in WGCNA modules

To identify the co-expression network modules enriched for cell-type specific methylation sites (identified as described in xxx) and temporal specific enhancers (identified as described in xx), the significance of the overlap between genes associated with epigenetic marks and all co-expression network modules pairwise was tested using the Hypergeometric test and the p-values are then adjusted for the multiple testing (FDR < 0.01).

# Enrichment of disease associated variants in regulatory regions

Six diseases, including 3 brain related disorders (schizophrenia, bipolar disorder and parkinsons disease) and 3 diseases ( type 2 diabetes, coronary artery disease and asthma) primarily affecting other non-brain tissues were included in this analysis. For schizophrenia, 1714 credible causal single nucleotide polymorphisms (SNPs) from the Psychiatric Genomics Consortium (PGC) GWAS data set (ref) were used. For each of the other five diseases, SNPs were obtained from GRASP: Genome-wide Repository of Associations between SNPs and Phenotypes. For these diseases, SNPs were ordered by ascending p values and top 1714 were included in the analysis.

The selected GWAS SNPs were mapped to H3K27ac (active promoter and active enhancer mark) and H3K4me3 (promoter mark) enriched regions identified in fetal and adult brains in this study, H3K27ac enriched regions identified in embryonic brains in xx et al study, and active enhancers identified in other tissues in the Roadmap epigenomics project (<http://www.roadmapepigenomics.org>). H3K27ac and H3K4me3 enriched regions in the fetal and adult brains were identified as described in Section 10.3. Embryonic brain data were also processed identically. H3K27ac enriched regions were further subcategorized by genomic region (Transcriptional start site [TSS], non-TSS [including exon, intron and intergenic regions] as defined by annotation described in Section 10.1), brain region (DFC, CBC) and developmental stage (embryonic, fetal, adult)

After mapping SNPs, enrichment was calculated with Fisher’s exact test by using 1000 Genomes project SNPs as background. –log10 p-value of 3.38 was chosen as cutoff by using Bonferroni correction.

The enrichment of the GWAS SNPs of six diseases in different DNA methylation trajectories (Figure 6) based on prenatal and postnatal methylation changes was also evaluated. CpGs mapped to 20 kb window centered on SNP were considered and enrichment was calculated by Fisher’s exact test using all CpGs in the array as the background.–log p-value of 2.98 was chosen as cutoff by using Bonferroni correction.