SUPPLEMENTARY INFORMATION

Spatially resolved epigenome-transcriptome co-profiling of mammalian tissues at the cellular level

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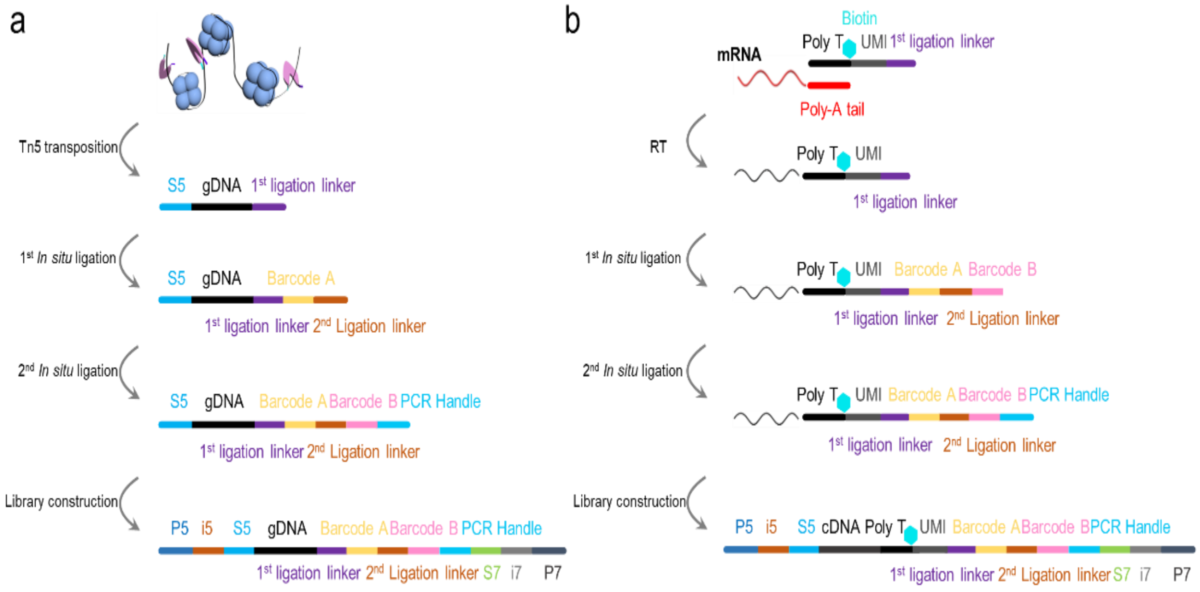

Fig. S1. Chemistry workflow of spatial-ATAC-RNA-seq. a, ATAC in spatial-ATAC-RNA-seq. **b,** RNA in spatial-ATAC-RNA-seq.

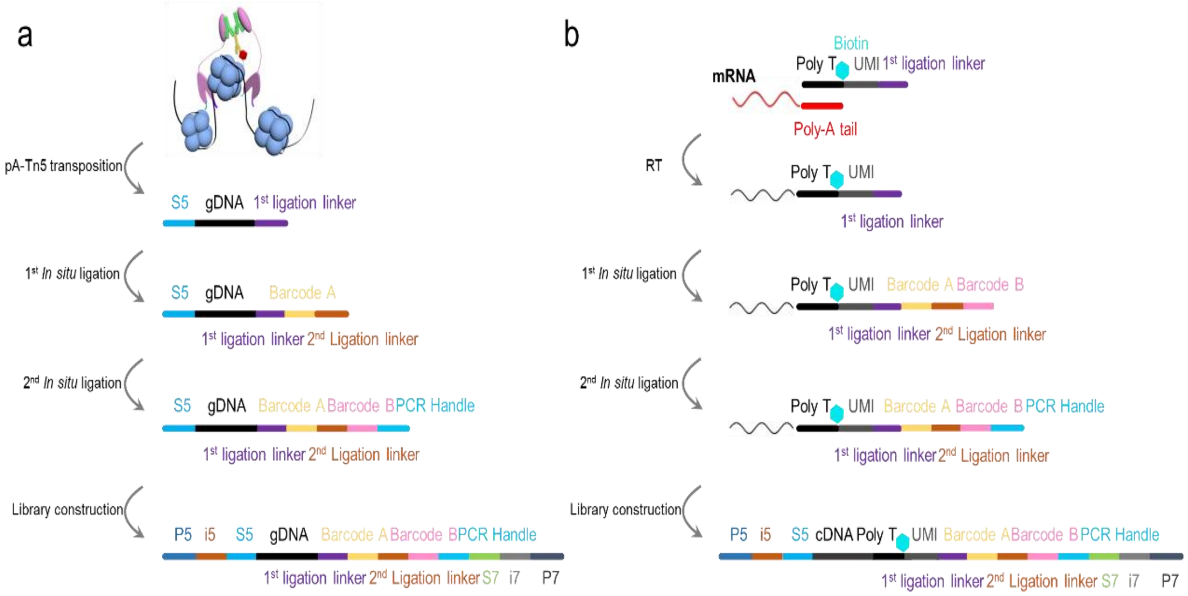

Fig. S2. Chemistry workflow of spatial-CUT&Tag-RNA-seq. a, CUT&Tag in spatial-CUT&Tag-RNA-seq. **b,** RNA in spatial-CUT&Tag-RNA-seq.

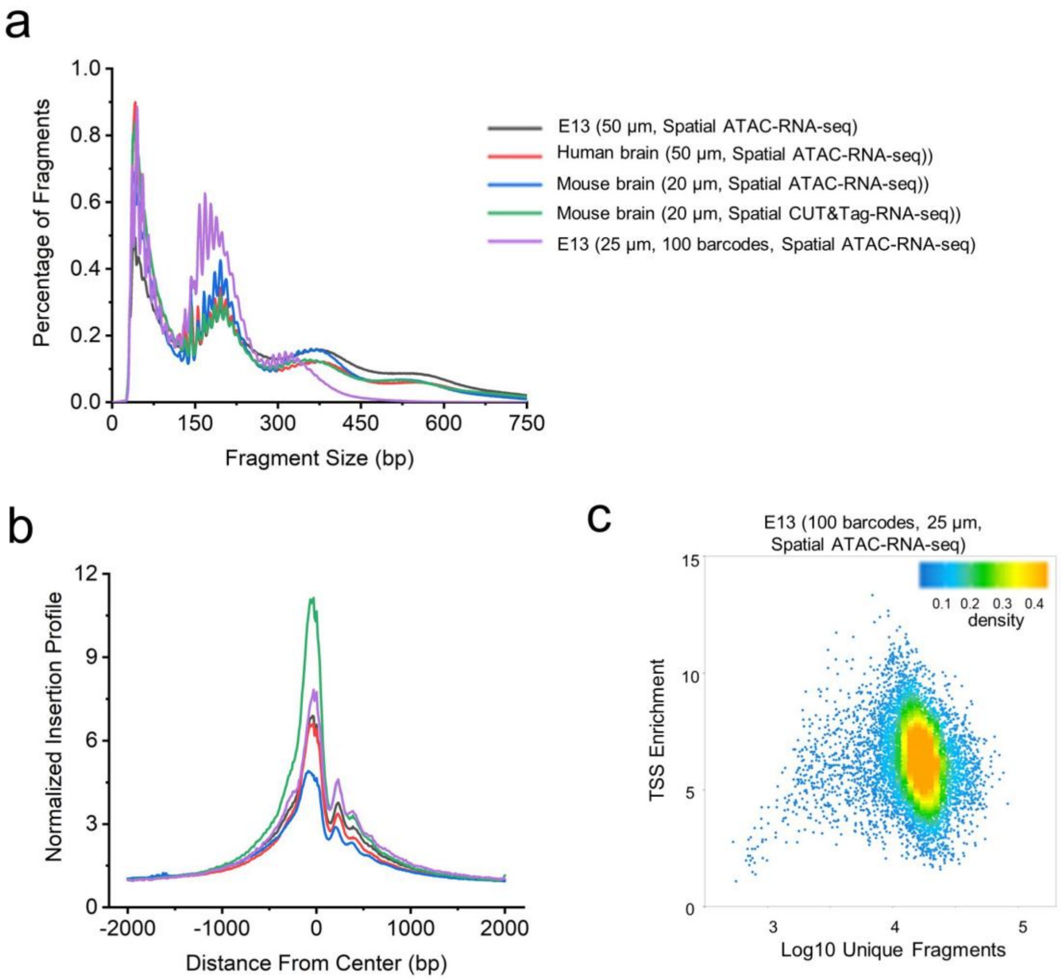

Fig. S3. Quality control metrics for spatial-ATAC-RNA-seq datasets. a, Comparison of insert size distribution of ATAC fragments between spatial-ATAC-RNA-seq and spatial-CUT&Tag-RNA-seq. **b,** Comparison of enrichment of ATAC reads around TSSs between spatial-ATAC-RNA-seq and spatial-CUT&Tag-RNA-seq. **c,** Scatterplot showing the TSS enrichment score vs unique nuclear fragments per cell in mouse embryo (100 x 100 barcodes).

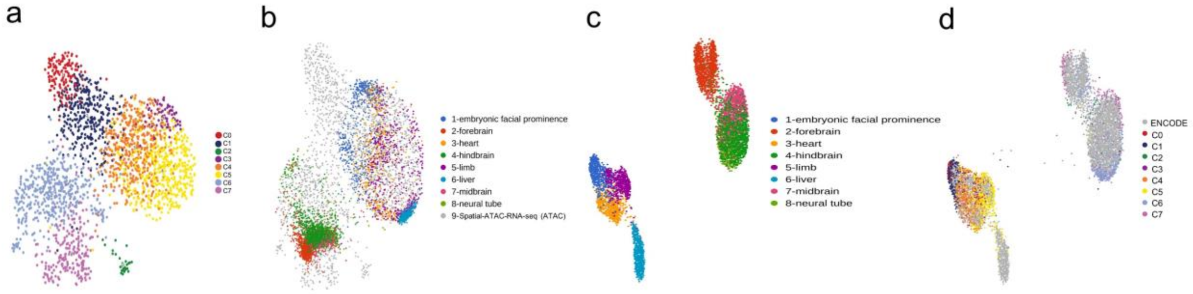

Fig. S4. Validation of chromatin accessibility in spatial-ATAC-RNA-seq on E13 mouse embryo with ENCODE reference data. a, UMAP embedding of unsupervised clustering analysis for ATAC in spatial-ATAC-RNA-seq. **b,** LSI projection of ENCODE bulk ATAC-seq data from diverse cell types of the E13.5 mouse embryo dataset onto the ATAC data in spatial-ATAC-RNA-seq embedding. **c,** UMAP embedding of unsupervised clustering analysis for ENCODE bulk ATAC-seq data from diverse cell types of the E13.5 mouse embryo dataset. **d,** LSI projection of the ATAC data in spatial-ATAC-RNA-seq data onto ENCODE bulk ATAC-seq embedding.

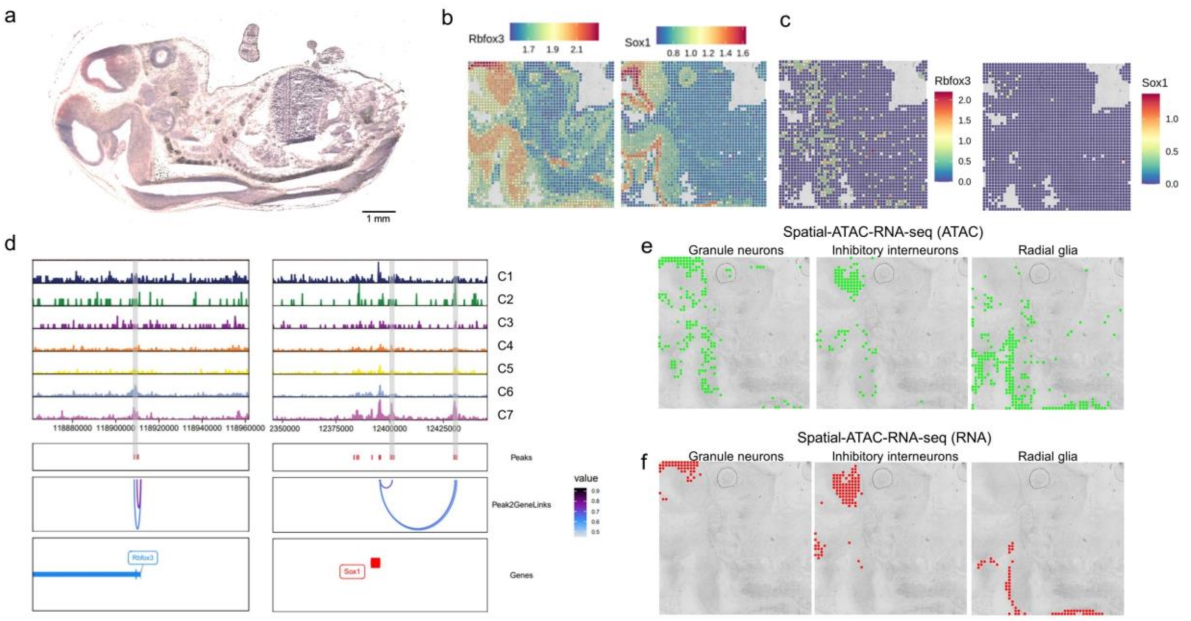

Fig. S5. H&E staining and spatial mapping of gene scores, cell types, and peak-to gene links in E13 mouse embryo. a, H&E image from an adjacent tissue section for spatial-ATAC-RNA-seq (50 µm pixel size). **b,** Spatial mapping of gene scores for selected marker genes for ATAC in spatial-ATAC-RNA-seq. **c,** Spatial mapping of gene scores for selected marker genes for RNA in spatial-ATAC-RNA-seq. **d,** Dynamics of chromatin accessibility of individual regulatory elements at *Rbfox3*, and *Sox1*. **e,** Spatial mapping of cell types identified by label transfer from scRNA-seq to ATAC in spatial-ATAC-RNA-seq. **f,** Spatial mapping of cell types identified by label transfer from scRNA-seq to RNA in spatial-ATAC-RNA-seq.

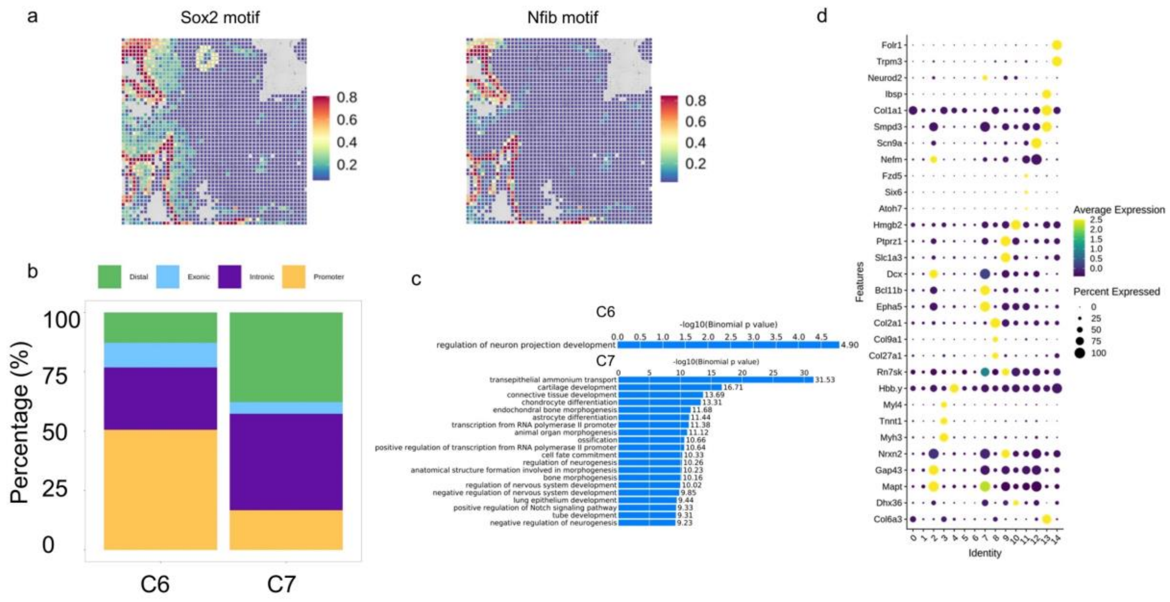

Fig. S6. Motif enrichment analysis for E13 mouse embryo. a, Spatial mapping of selected TF motif deviation scores. **b**, Annotation of marker peaks across clusters. **c**, GREAT enrichment analysis of marker peaks across clusters. **d,** The expression level and the percentage of cells in all clusters (marker genes for each cluster) for RNA data.

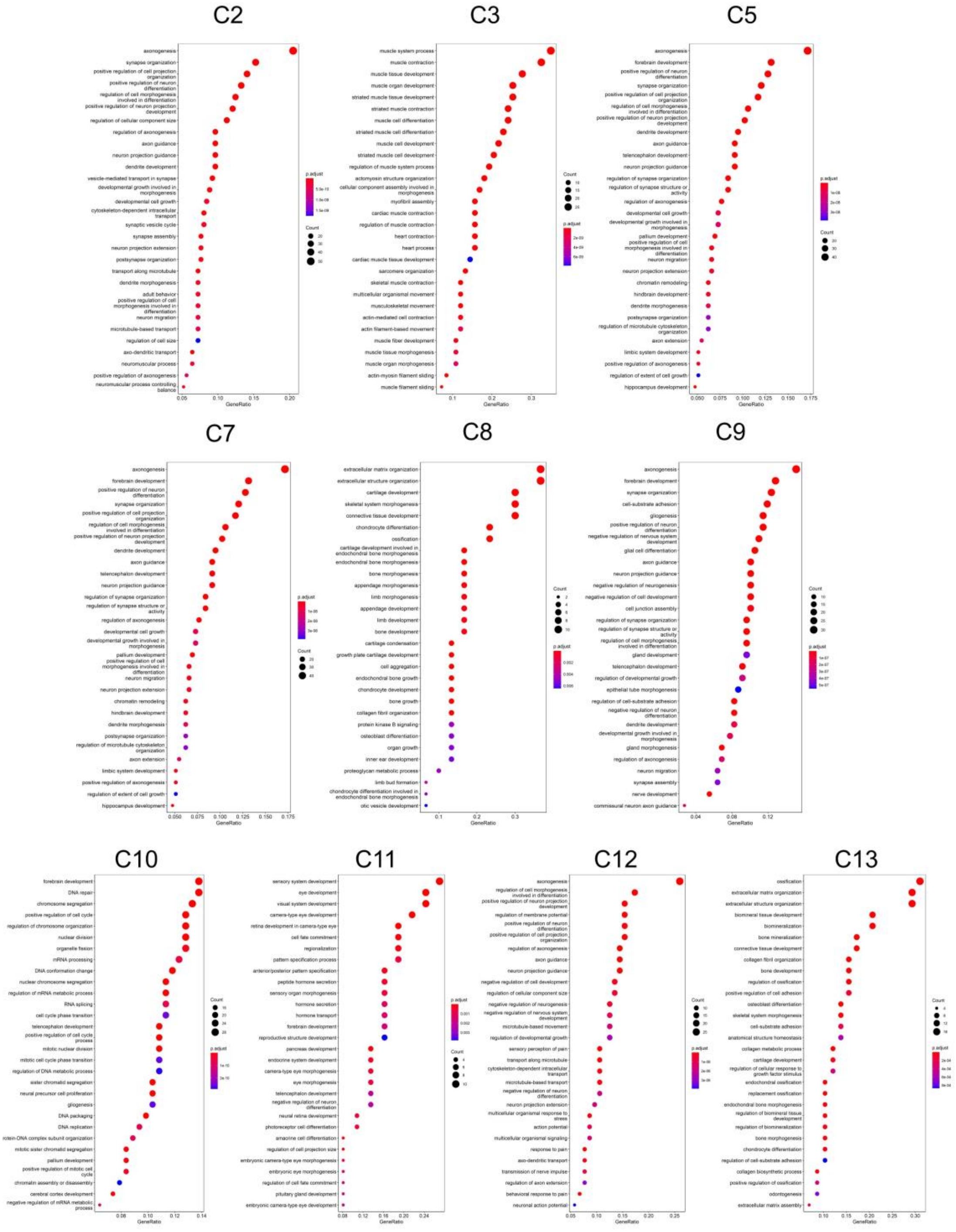

Fig. S7. GO enrichment analysis of differentially activated genes in selected clusters of E13 mouse embryo.

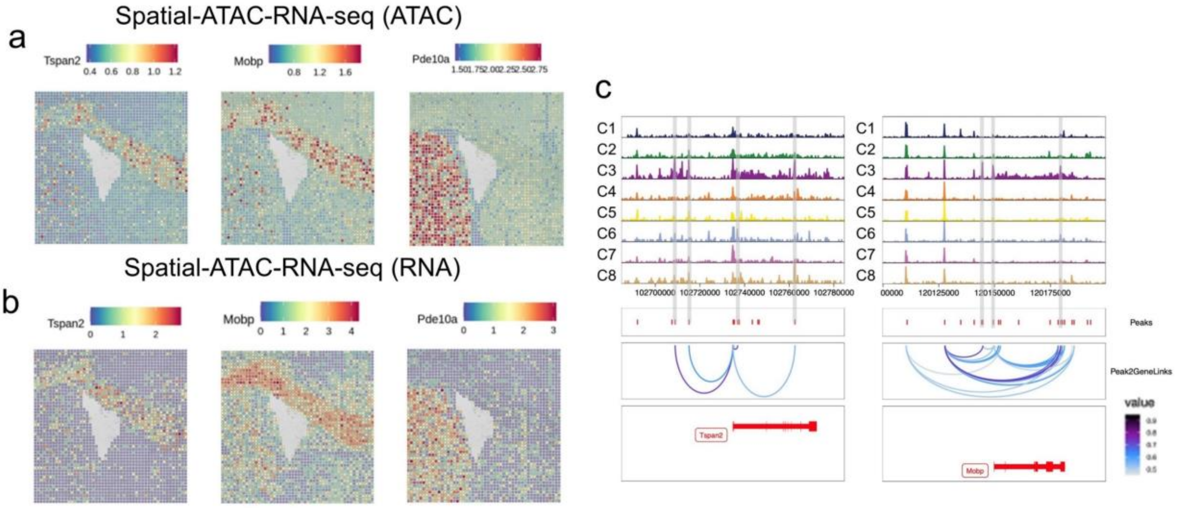

Fig. S8. Spatial mapping of gene scores, peak-to gene links, and chromatin states in P21 mouse brain. a, Spatial mapping of gene scores for selected marker genes for ATAC in spatial-ATAC-RNA-seq. **b,** Spatial mapping of gene scores for selected marker genes for RNA in spatial-ATAC-RNA-seq. **c,** Dynamics of chromatin accessibility of individual regulatory elements at *Tspan2, and Mobp*.

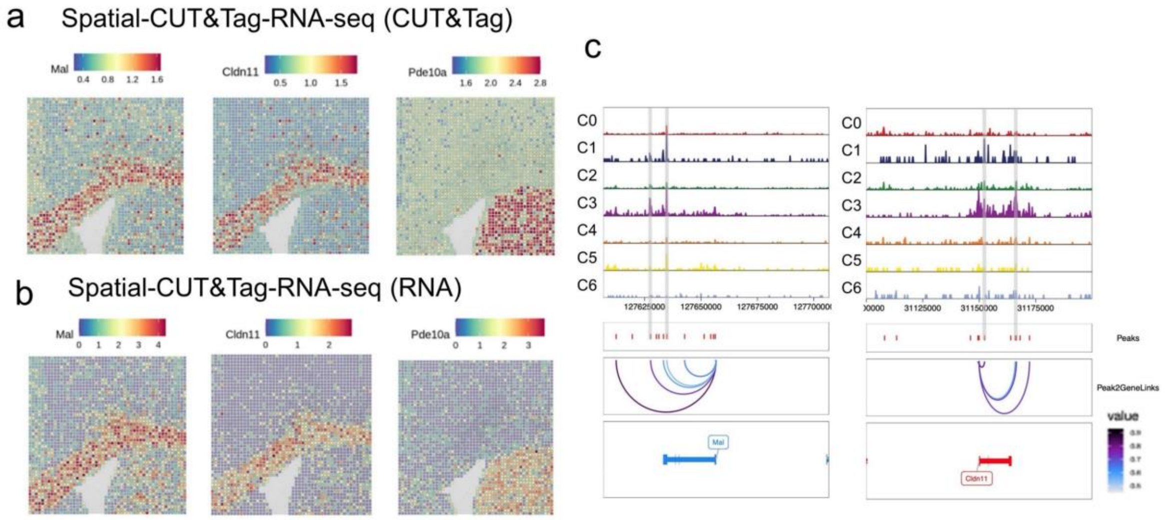

Fig. S9. Spatial mapping of gene scores, peak-to gene links, and chromatin states in P21 mouse brain. **a**, Spatial mapping of gene scores for selected marker genes for CUT&Tag in spatial- CUT&Tag-RNA-seq. **b,** Spatial mapping of gene scores for selected marker genes for RNA in spatial-CUT&Tag-RNA-seq. **c,** Dynamics of chromatin accessibility of individual regulatory elements at *Mal, and Cldn11*.

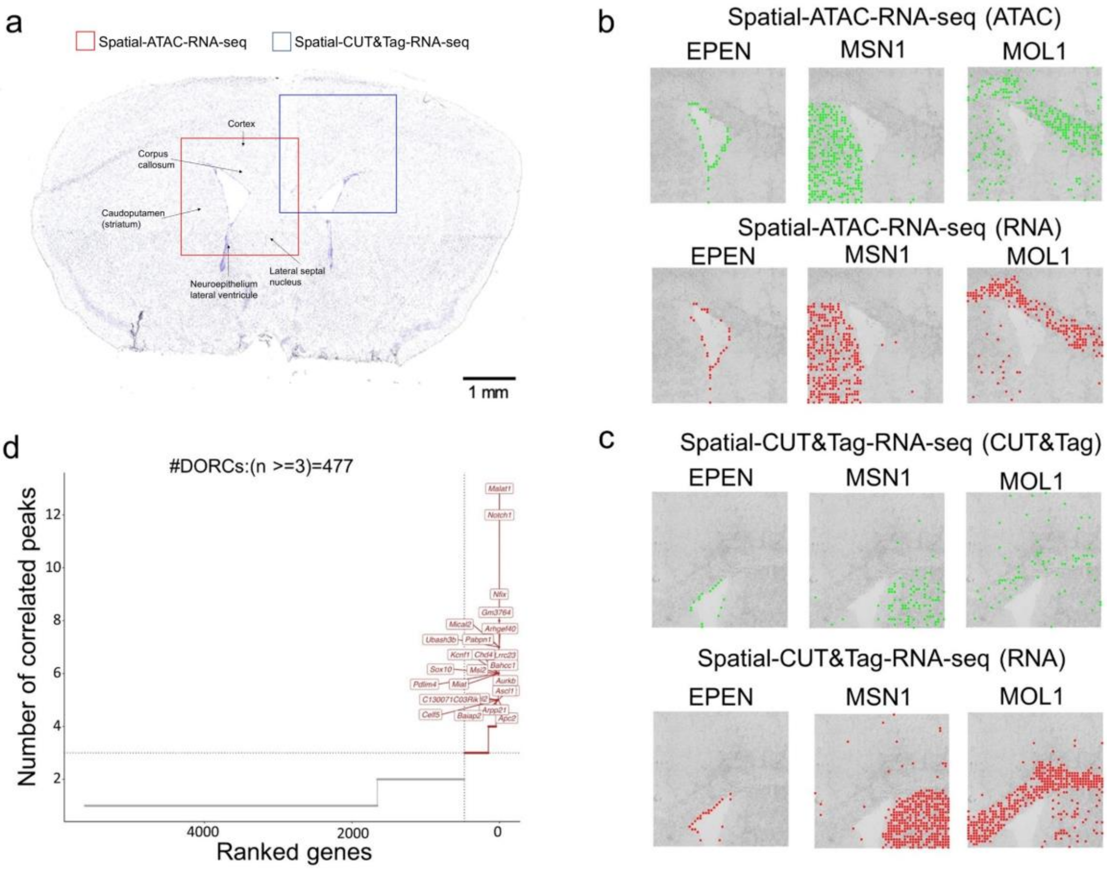

Fig. S10. H&E staining, spatial mapping of cell types, and DORC in P21 mouse brain. a, Nissl-stained image from an adjacent tissue section of P21 mouse brain. **b,** Spatial mapping of cell types identified by label transfer from scRNA-seq to ATAC and RNA in spatial-ATAC-RNA-seq. **c,** Spatial mapping of cell types identified by label transfer from scRNA-seq to CUT&Tag and RNA in spatial-CUT&Tag-RNA-seq. **d,** The number of significantly correlated peaks for each gene.

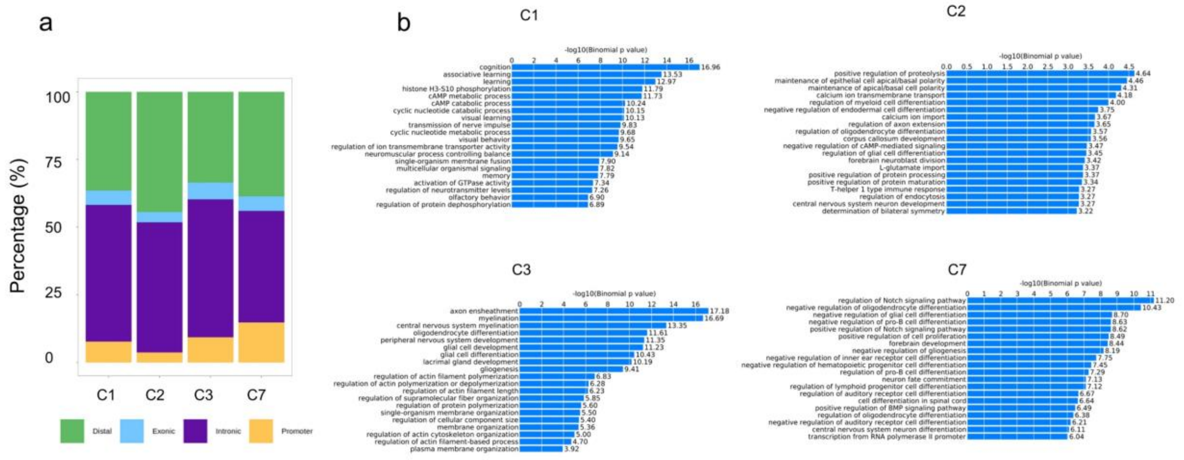

Fig. S11. Motif enrichment analysis for P21 mouse brain in spatial-ATAC-RNA-seq. a, Annotation of marker peaks across clusters. **b**, GREAT enrichment analysis of marker peaks across clusters.

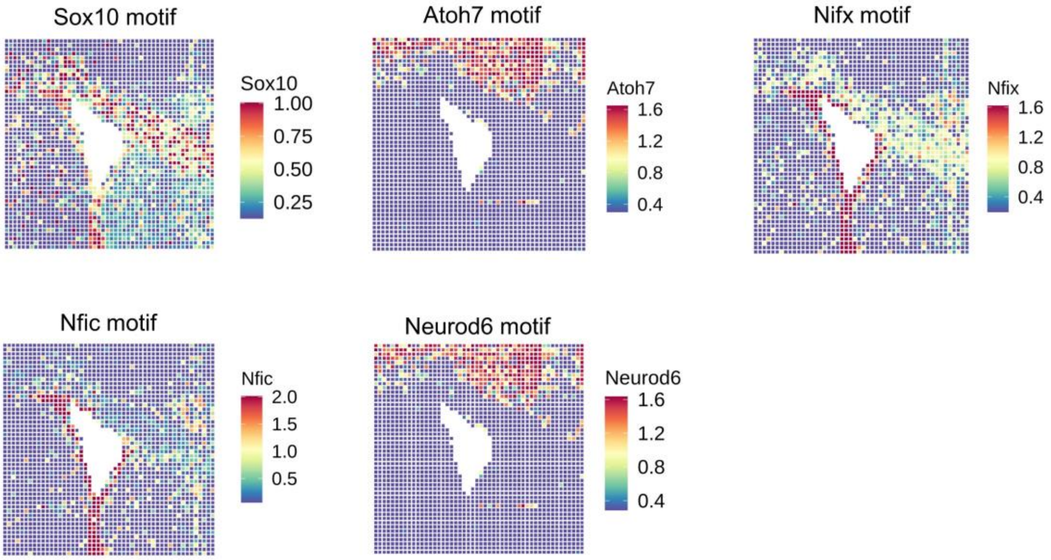

Fig. S12. Spatial mapping of selected TF motif deviation scores in spatial-ATAC-RNA-seq of mouse brain.

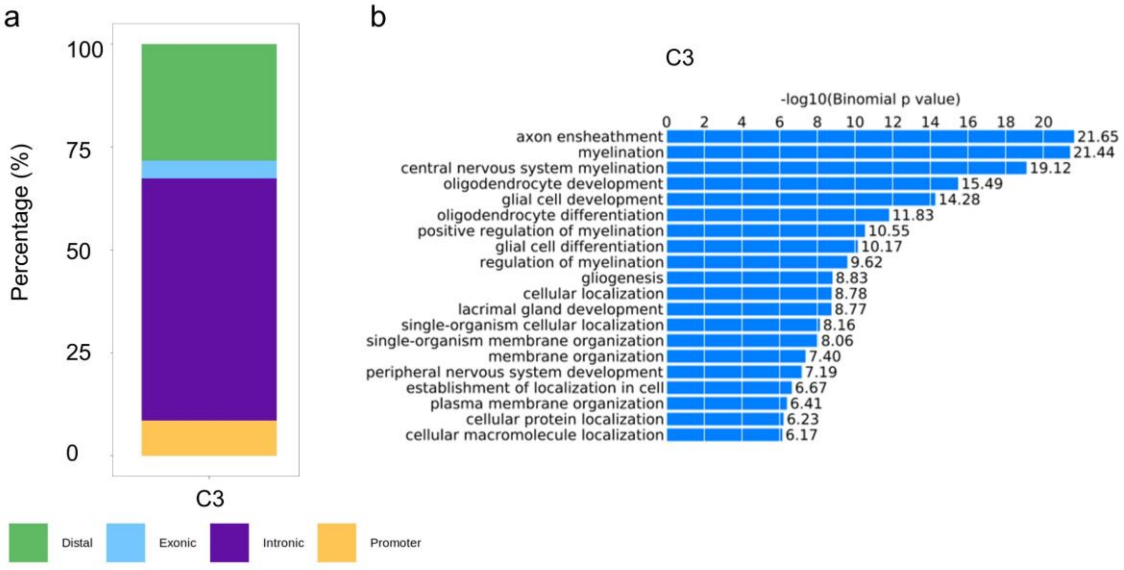

Fig. S13. Motif enrichment analysis for P21 mouse brain in spatial-CUT&Tag-RNA-seq. a, Annotation of marker peaks. **b**, GREAT enrichment analysis of marker peaks.

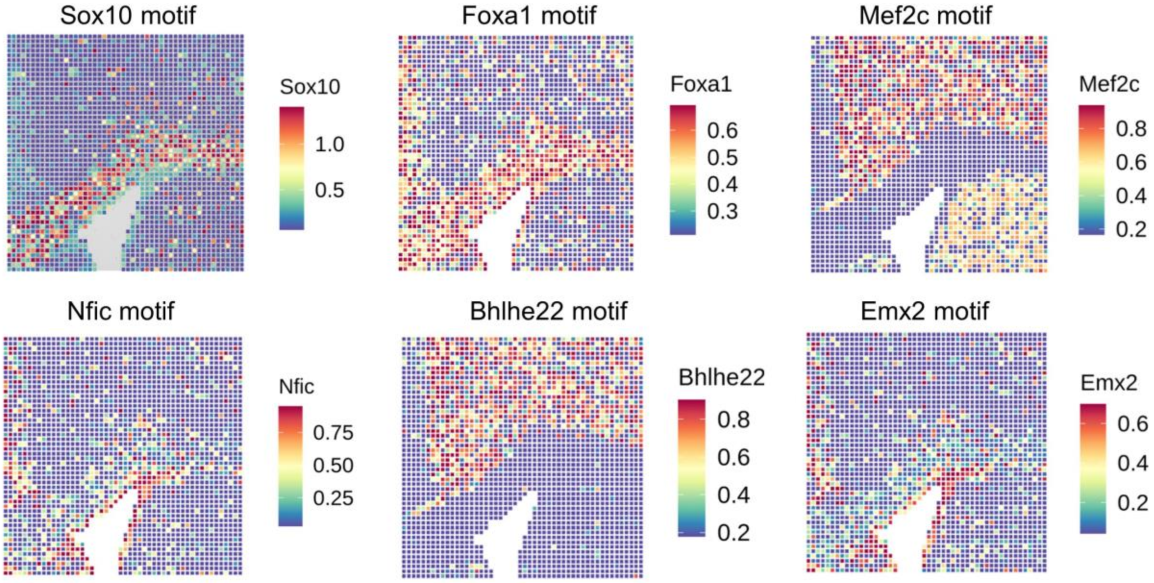

Fig. S14. Spatial mapping of selected TF motif deviation scores in spatial-CUT&Tag-RNA-seq of mouse brain.

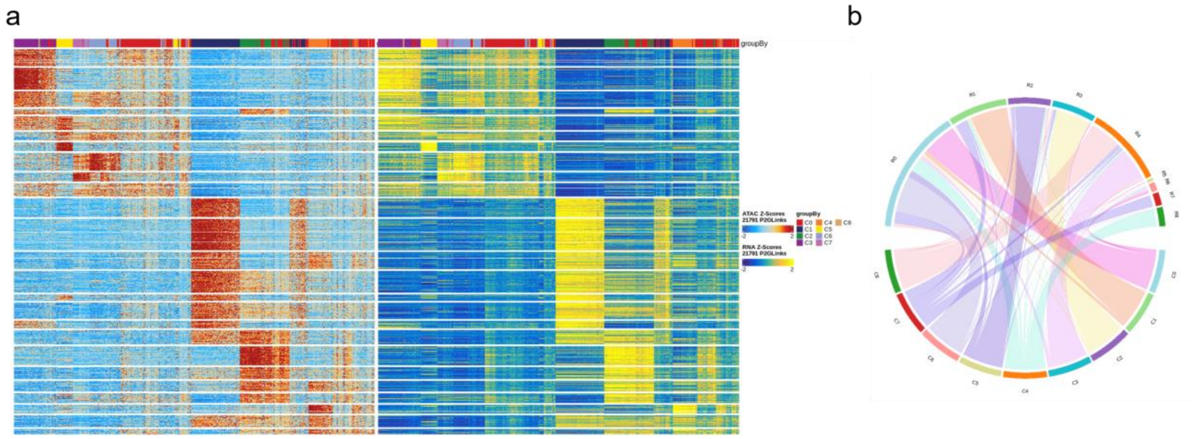

Fig. S15. Heatmaps of peak-to-gene links and Ribbon plot for P21 mouse brain with spatial-ATAC-RNA-seq. a, Heatmaps of peak-to-gene links for ATAC and RNA in spatial-ATAC-RNA-seq. **b,** Ribbon plot of ATAC (C0-C8) and RNA (R0-R8) clusters in spatial-ATAC-RNA-seq.

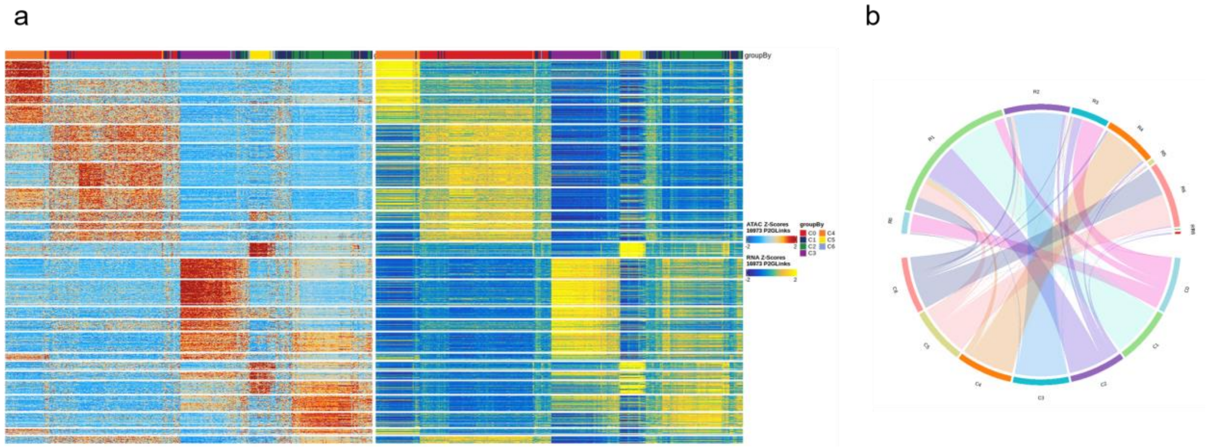

Fig. S16. Heatmaps of peak-to-gene links and Ribbon plot for P21 mouse brain with spatial-CUT&Tag-RNA-seq. a, Heatmaps of peak-to-gene links for CUT&Tag and RNA in spatial-CUT&Tag-RNA-seq. **b,** Ribbon plot of CUT&Tag (C0-C6) and RNA (R0-R8) clusters in spatial-CUT&Tag-RNA-seq.

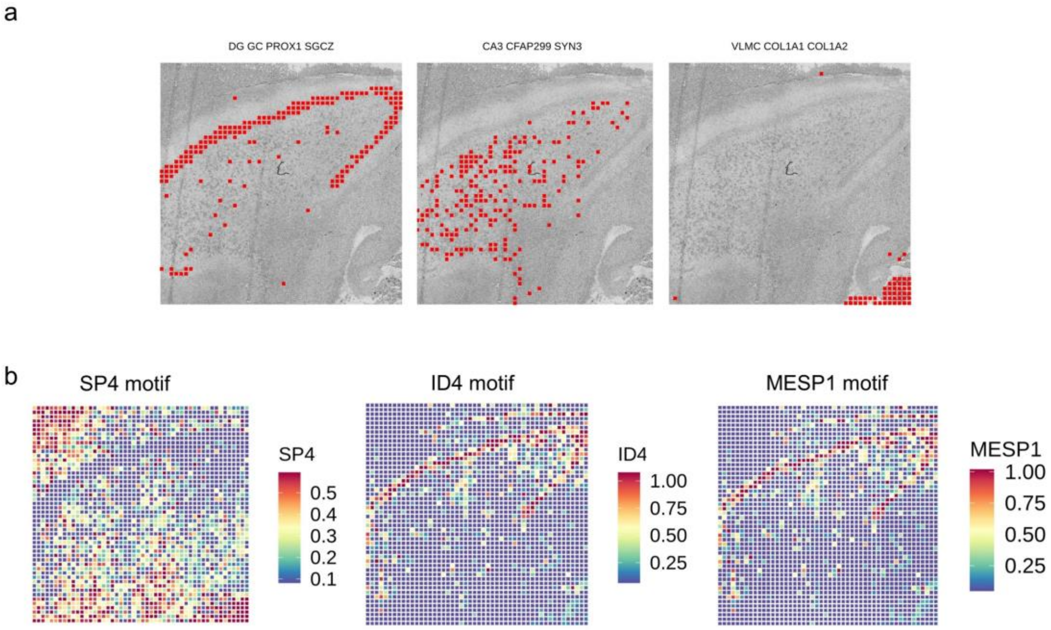

Fig. S17. Spatial mapping of cell types and TF motifs. a, Spatial mapping of cell types identified by label transfer from scRNA-seq to RNA data in spatial-ATAC-RNA-seq for human hippocampus. **b,** Spatial mapping of selected TF motif deviation scores in spatial-ATAC-RNA-seq of human hippocampus.

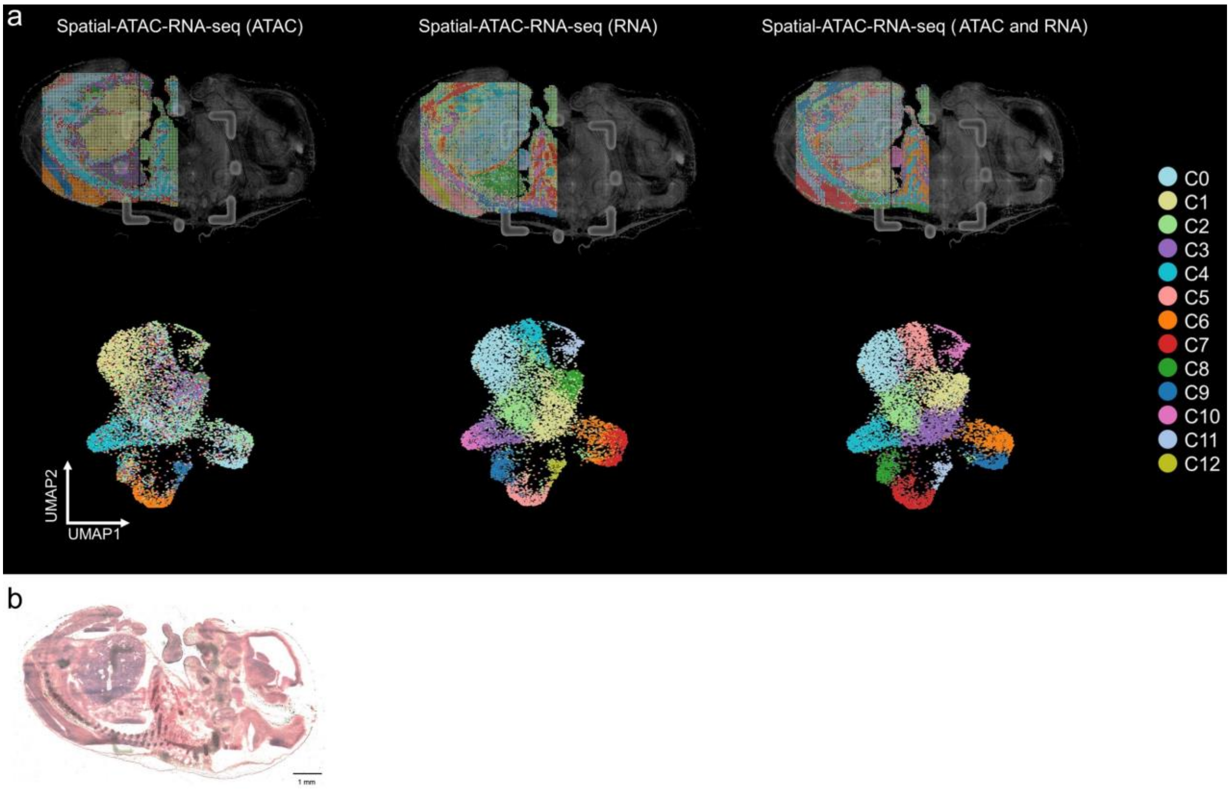

Fig. S18. Spatial chromatin accessibility and transcriptome co-sequencing of E13 mouse embryo with 100 x 100 barcodes. a, Spatial map and UMAP of all the clusters for ATAC, RNA, and joint clustering of ATAC and RNA data (25 µm pixel size). Overlay of clusters with the tissue image reveals that the spatial clusters precisely match the anatomic regions. **b**, H&E staining of an adjacent tissue of E13 mouse embryo.

Table S1. DNA oligos used for PCR and preparation of sequencing library.

RT primer	/5Phos/CATCGGCGTACGACTNNNNNNNNNN/iBiodT/TTTTTTTTTTTTTTTVN
Ligation linker 1	AGTCGTACGCCGATGCGAAACATCGGCCAC
Ligation linker 2	CGAATGCTCTGGCCTCTCAAGCACGTGGAT
PCR Primer 1	CAAGCGTTGGCTTCTCGCATCT
PCR Primer 2	AAGCAGTGGTATCAACGCAGAGT
N501	AATGATACGGCGACCACCGAGATCTACACTAGATCGCTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
N701	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
N702	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
N703	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
N704	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
N705	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
N706	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
N707	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT

Table S2. DNA barcode A sequences.

Barcode A	**Sequence**
Barcode A-1	/5Phos/AGGCCAGAGCATTCGAACGTGATGTGGCCGATGTTTCG
Barcode A-2	/5Phos/AGGCCAGAGCATTCGAAACATCGGTGGCCGATGTTTCG
Barcode A-3	/5Phos/AGGCCAGAGCATTCGATGCCTAAGTGGCCGATGTTTCG
Barcode A-4	/5Phos/AGGCCAGAGCATTCGAGTGGTCAGTGGCCGATGTTTCG
Barcode A-5	/5Phos/AGGCCAGAGCATTCGACCACTGTGTGGCCGATGTTTCG
Barcode A-6	/5Phos/AGGCCAGAGCATTCGACATTGGCGTGGCCGATGTTTCG
Barcode A-7	/5Phos/AGGCCAGAGCATTCGCAGATCTGGTGGCCGATGTTTCG
Barcode A-8	/5Phos/AGGCCAGAGCATTCGCATCAAGTGTGGCCGATGTTTCG
Barcode A-9	/5Phos/AGGCCAGAGCATTCGCGCTGATCGTGGCCGATGTTTCG
Barcode A-10	/5Phos/AGGCCAGAGCATTCGACAAGCTAGTGGCCGATGTTTCG
Barcode A-11	/5Phos/AGGCCAGAGCATTCGCTGTAGCCGTGGCCGATGTTTCG
Barcode A-12	/5Phos/AGGCCAGAGCATTCGAGTACAAGGTGGCCGATGTTTCG
Barcode A-13	/5Phos/AGGCCAGAGCATTCGAACAACCAGTGGCCGATGTTTCG
Barcode A-14	/5Phos/AGGCCAGAGCATTCGAACCGAGAGTGGCCGATGTTTCG
Barcode A-15	/5Phos/AGGCCAGAGCATTCGAACGCTTAGTGGCCGATGTTTCG
Barcode A-16	/5Phos/AGGCCAGAGCATTCGAAGACGGAGTGGCCGATGTTTCG
Barcode A-17	/5Phos/AGGCCAGAGCATTCGAAGGTACAGTGGCCGATGTTTCG
Barcode A-18	/5Phos/AGGCCAGAGCATTCGACACAGAAGTGGCCGATGTTTCG
Barcode A-19	/5Phos/AGGCCAGAGCATTCGACAGCAGAGTGGCCGATGTTTCG
Barcode A-20	/5Phos/AGGCCAGAGCATTCGACCTCCAAGTGGCCGATGTTTCG
Barcode A-21	/5Phos/AGGCCAGAGCATTCGACGCTCGAGTGGCCGATGTTTCG
Barcode A-22	/5Phos/AGGCCAGAGCATTCGACGTATCAGTGGCCGATGTTTCG
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Barcode A-46	/5Phos/AGGCCAGAGCATTCGGAATCTGAGTGGCCGATGTTTCG
Barcode A-47	/5Phos/AGGCCAGAGCATTCGCAAGACTAGTGGCCGATGTTTCG
Barcode A-48	/5Phos/AGGCCAGAGCATTCGGAGCTGAAGTGGCCGATGTTTCG
Barcode A-49	/5Phos/AGGCCAGAGCATTCGGATAGACAGTGGCCGATGTTTCG
Barcode A-50	/5Phos/AGGCCAGAGCATTCGGCCACATAGTGGCCGATGTTTCG

Table S3. DNA barcode B sequences.

Barcode B	**Sequence**
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Barcode B-2	CAAGCGTTGGCTTCTCGCATCTAAACATCGATCCACGTGCTTGAG
Barcode B-3	CAAGCGTTGGCTTCTCGCATCTATGCCTAAATCCACGTGCTTGAG
Barcode B-4	CAAGCGTTGGCTTCTCGCATCTAGTGGTCAATCCACGTGCTTGAG
Barcode B-5	CAAGCGTTGGCTTCTCGCATCTACCACTGTATCCACGTGCTTGAG
Barcode B-6	CAAGCGTTGGCTTCTCGCATCTACATTGGCATCCACGTGCTTGAG
Barcode B-7	CAAGCGTTGGCTTCTCGCATCTCAGATCTGATCCACGTGCTTGAG
Barcode B-8	CAAGCGTTGGCTTCTCGCATCTCATCAAGTATCCACGTGCTTGAG
Barcode B-9	CAAGCGTTGGCTTCTCGCATCTCGCTGATCATCCACGTGCTTGAG
Barcode B-10	CAAGCGTTGGCTTCTCGCATCTACAAGCTAATCCACGTGCTTGAG
Barcode B-11	CAAGCGTTGGCTTCTCGCATCTCTGTAGCCATCCACGTGCTTGAG
Barcode B-12	CAAGCGTTGGCTTCTCGCATCTAGTACAAGATCCACGTGCTTGAG
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Barcode B-16	CAAGCGTTGGCTTCTCGCATCTAAGACGGAATCCACGTGCTTGAG
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Barcode B-22	CAAGCGTTGGCTTCTCGCATCTACGTATCAATCCACGTGCTTGAG
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Barcode B-24	CAAGCGTTGGCTTCTCGCATCTAGAGTCAAATCCACGTGCTTGAG
Barcode B-25	CAAGCGTTGGCTTCTCGCATCTAGATCGCAATCCACGTGCTTGAG
Barcode B-26	CAAGCGTTGGCTTCTCGCATCTAGCAGGAAATCCACGTGCTTGAG
Barcode B-27	CAAGCGTTGGCTTCTCGCATCTAGTCACTAATCCACGTGCTTGAG
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Barcode B-32	CAAGCGTTGGCTTCTCGCATCTCAATGGAAATCCACGTGCTTGAG
Barcode B-33	CAAGCGTTGGCTTCTCGCATCTCACTTCGAATCCACGTGCTTGAG
Barcode B-34	CAAGCGTTGGCTTCTCGCATCTCAGCGTTAATCCACGTGCTTGAG
Barcode B-35	CAAGCGTTGGCTTCTCGCATCTCATACCAAATCCACGTGCTTGAG
Barcode B-36	CAAGCGTTGGCTTCTCGCATCTCCAGTTCAATCCACGTGCTTGAG
Barcode B-37	CAAGCGTTGGCTTCTCGCATCTCCGAAGTAATCCACGTGCTTGAG
Barcode B-38	CAAGCGTTGGCTTCTCGCATCTCCGTGAGAATCCACGTGCTTGAG
Barcode B-39	CAAGCGTTGGCTTCTCGCATCTCCTCCTGAATCCACGTGCTTGAG

Barcode B-40	CAAGCGTTGGCTTCTCGCATCTCGAACTTAATCCACGTGCTTGAG
Barcode B-41	CAAGCGTTGGCTTCTCGCATCTCGACTGGAATCCACGTGCTTGAG
Barcode B-42	CAAGCGTTGGCTTCTCGCATCTCGCATACAATCCACGTGCTTGAG
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Barcode B-44	CAAGCGTTGGCTTCTCGCATCTCTGAGCCAATCCACGTGCTTGAG
Barcode B-45	CAAGCGTTGGCTTCTCGCATCTCTGGCATAATCCACGTGCTTGAG
Barcode B-46	CAAGCGTTGGCTTCTCGCATCTGAATCTGAATCCACGTGCTTGAG
Barcode B-47	CAAGCGTTGGCTTCTCGCATCTCAAGACTAATCCACGTGCTTGAG
Barcode B-48	CAAGCGTTGGCTTCTCGCATCTGAGCTGAAATCCACGTGCTTGAG
Barcode B-49	CAAGCGTTGGCTTCTCGCATCTGATAGACAATCCACGTGCTTGAG
Barcode B-50	CAAGCGTTGGCTTCTCGCATCTGCCACATAATCCACGTGCTTGAG

Table S4. Chemicals and reagents.

Name	**Catalog number**	**Vender**
Formaldehyde solution	PI28906	Thermo Fisher Scientific
HEPES pH 7.5	BBH-75-250	Boston BioProducts
Glycine	50046	Sigma-Aldrich
NaCl	AM9760G	Thermo Fisher Scientific
Digitonin	G9441	Promega
$MgCl_2$	AM9530G	Thermo Fisher Scientific
Spermidine	S0266	Sigma-Aldrich
EDTA-free Protease Inhibitor Cocktail	11873580001	Millipore Sigma
NP40	11332473001	Sigma-Aldrich
EDTA Solution pH 8.0	AB00502	AmericanBio
Bovine Serum Albumin (BSA)	A8806	Sigma-Aldrich
α-H3K27ac antibody	ab177178	Abcam
Secondary antibody	ABIN101961	Antibodies-Online
pA-Tn5 Transposase – unloaded	C01070002	Diagenode
Triton X-100	T8787	Sigma-Aldrich
T4 DNA Ligase	M0202L	New England Biolabs
T4 DNA Ligase Reaction Buffer	B0202S	New England Biolabs
NEBuffer 3.1	B7203S	New England Biolabs
DPBS	14190144	Thermo Fisher Scientific
Proteinase K	EO0491	Thermo Fisher Scientific
Ampure XP beads	A63880	Beckman Coulter
NEBNext High-Fidelity 2X PCR Master Mix	M0541L	New England Biolabs
SYBR Green I Nucleic Acid Gel Stain	S7563	Thermo Fisher Scientific
DNA Clean & Concentrator-5	D4014	Zymo Research
Tn5 Transposase - unloaded	C01070010	Diagenode
Tagmentation Buffer (2x)	C01019043	Diagenode
Sodium dodecyl sulfate	71736	Sigma-Aldrich
Maxima H Minus Reverse Transcriptase (200 U/L)	EP0751	Thermo Fisher Scientific
dNTP mix	R0192	Thermo Fisher Scientific
SUPERased In RNase Inhibitor	AM2694	Thermo Fisher Scientific
Ampure XP beads	A63880	Beckman Coulter
Dynabeads MyOne C1	65001	Thermo Fisher Scientific
RNase Inhibitor	Y9240L	Enzymatics
Kapa Hotstart HiFi ReadyMix	KK2601	Kapa Biosystems
Nextera XT DNA Preparation Kit	FC-131-1024	Illumina