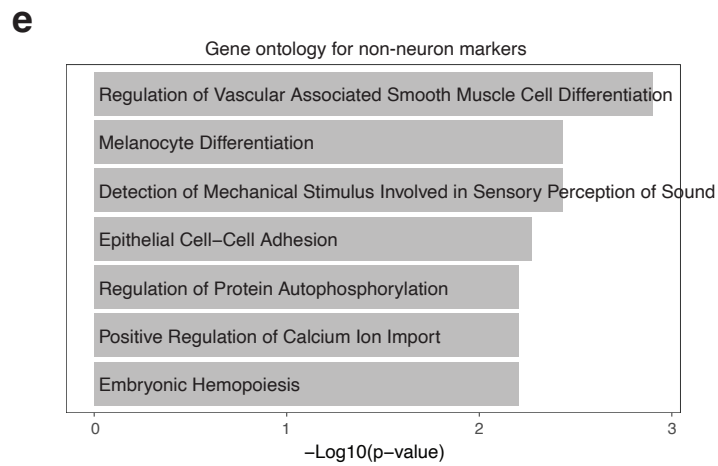
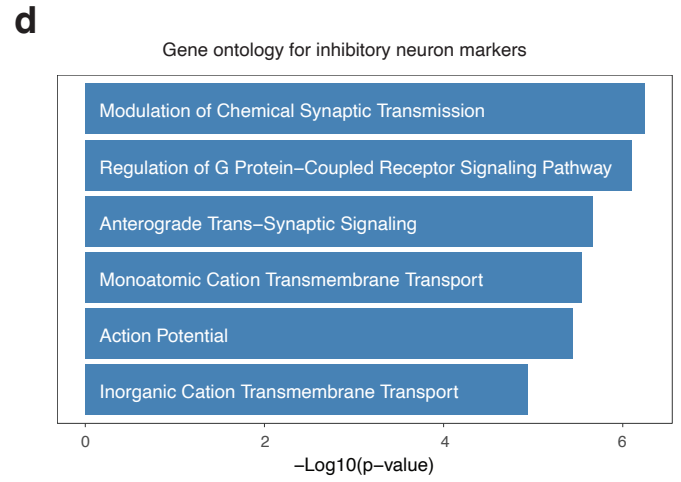
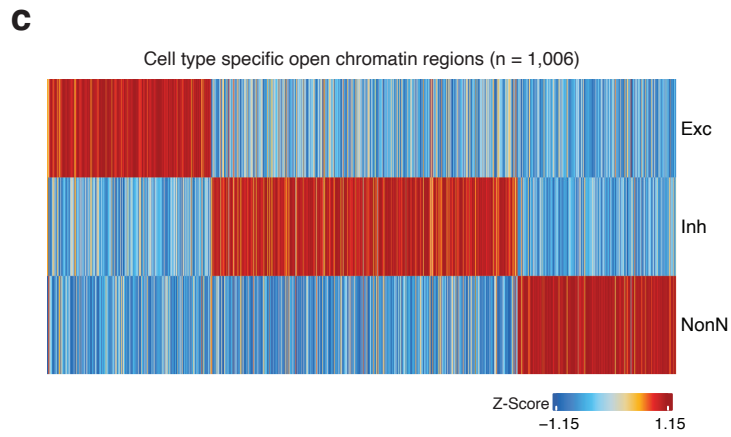
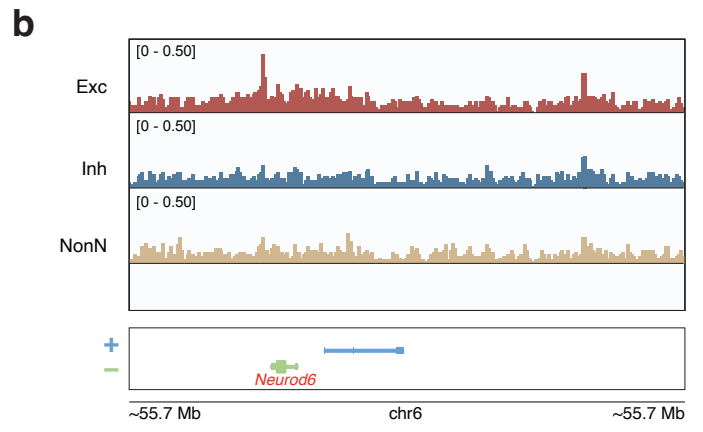
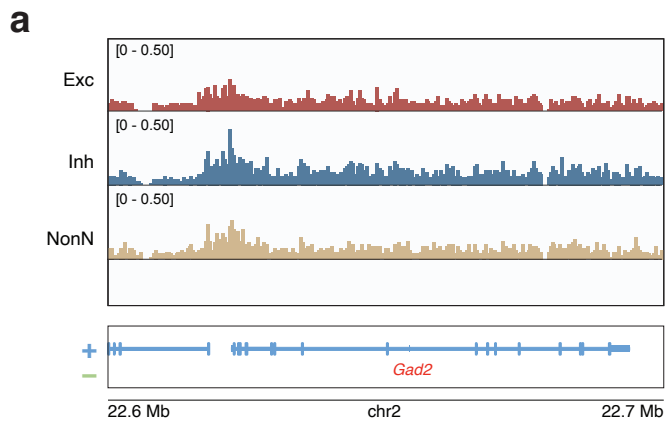


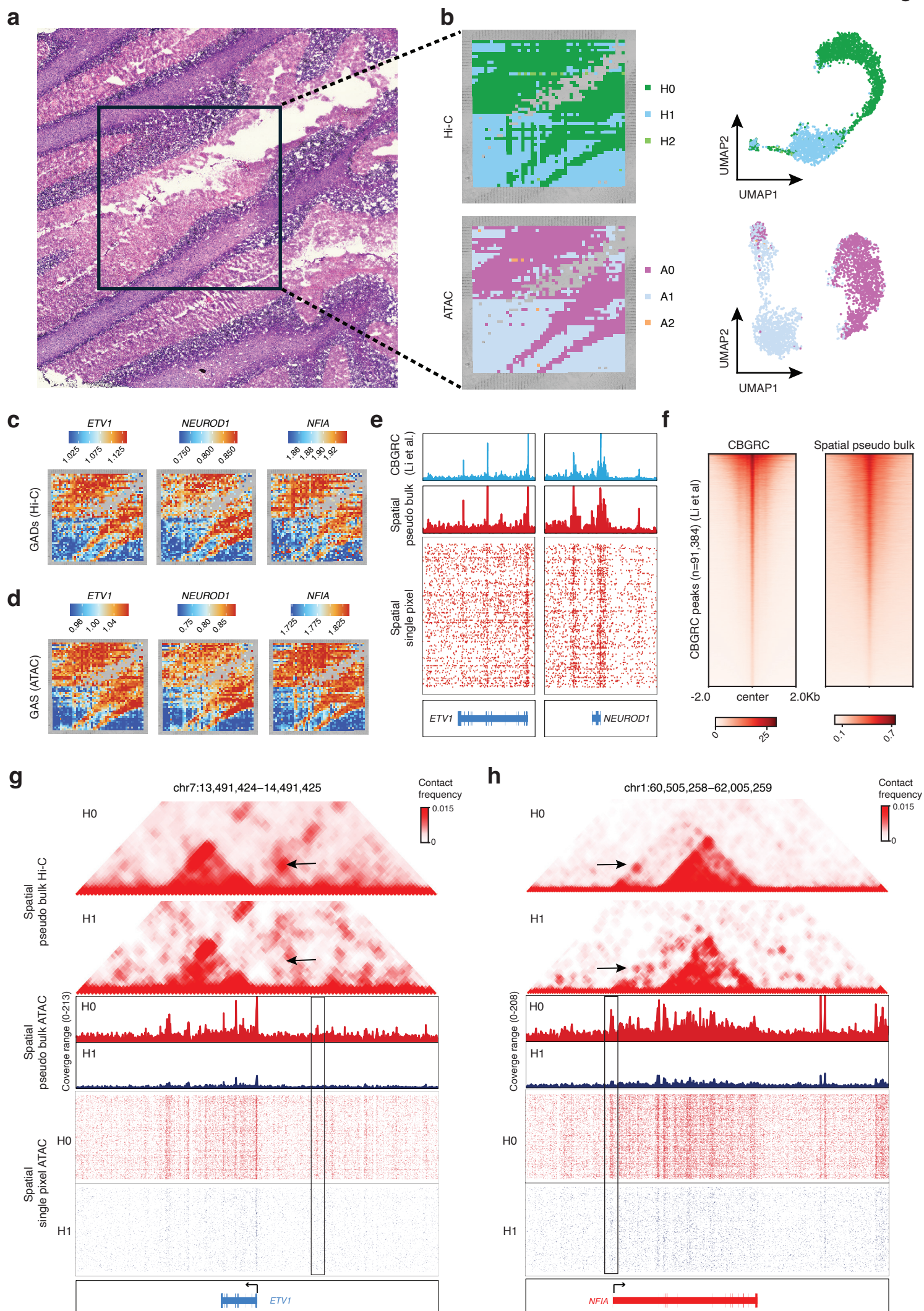
Spatial chromatin architecture and accessibility co-profiling of mammalian tissues

In the format provided by the
authors and unedited

Supplementary Figure 1	Analysis of spatial ATAC-seq in mouse brain R6
Supplementary Figure 2	Spatial 3D genome and epigenome co-profiling of human brain
Supplementary Figure 3	Analysis of spatial Hi-C and ATAC-seq in Glioblastoma sample NU03747
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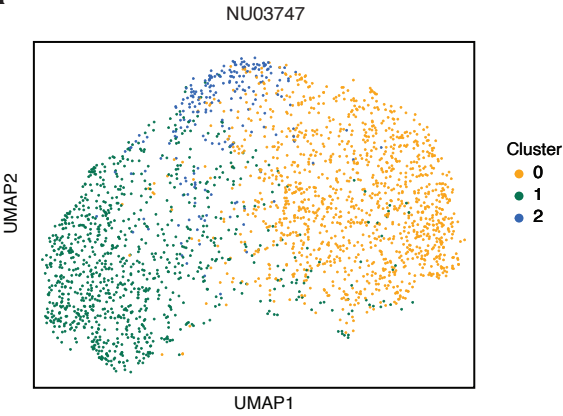
Supplementary Figure 1. Analysis of spatial ATAC-seq in mouse brain R6. a-b. Pseudo-bulk ATAC-seq tracks at *Gad2* (a) and *Neurod6* (b) regions for each cell type in mouse brain R6 sample. **c.** Heatmap showing cell-type-specific open chromatin regions in mouse brain R6 sample. The number of cell-type-specific open chromatin regions were labeled on top of the heatmap. **d-e.** GO term enrichment of inhibitory neuron (d) and non-neuron (e) marker genes. The statistical significance of GO term enrichment in d and e was assessed using Fisher's exact test.



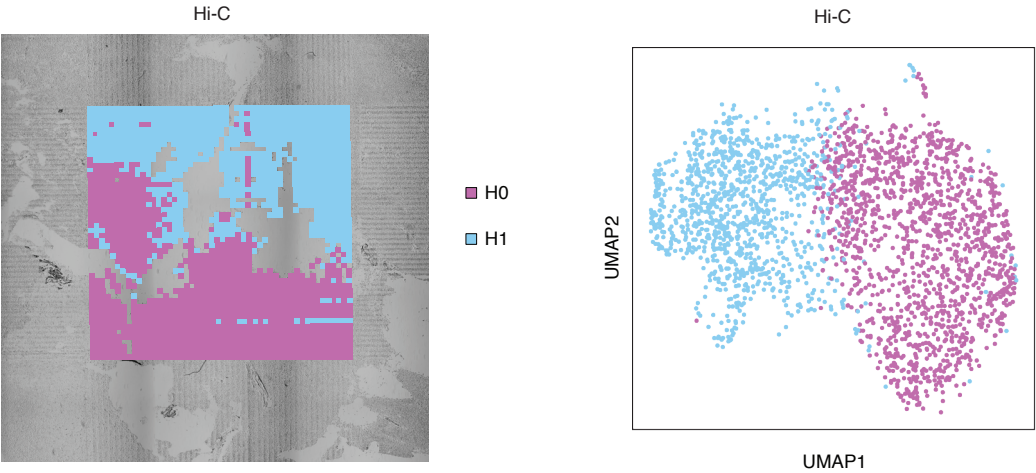
Supplementary Figure 2. Spatial 3D genome and epigenome co-profiling of human brain.

a. H&E staining of human cerebellum. Region selected for the spatial experiment is highlighted in the black square box. **b.** Spatial distribution (left panel) and UMAP (right panel) of all clusters based on Hi-C (upper panel) and ATAC-seq (lower panel) in the human cerebellum. **c-d.** Spatial distribution of GADs (**c**) and GAS (**d**) for selected marker genes. **e.** Comparison of spatial pseudo-bulk ATAC-seq track (middle panel) and single pixel tracks (lower panel) with public single cell pseudo-bulk ATAC-seq CBGRC track⁷³ (upper panel) at *ETV1* and *NEUROD1* regions. **f.** Signal enrichment of CBGRC pseudo-bulk scATAC-seq data (left panel) and Spatial-ATAC-Hi-C pseudo-bulk ATAC-seq data (right panel) at CBGRC peak regions. **g-h.** Pseudo-bulk Hi-C maps (upper panels), pseudo-bulk ATAC-Seq tracks (middle panels) and single-pixel ATAC-Seq tracks (bottom panels) from different Hi-C clusters at gene *ETV1* (**g**) and *NFIA* (**h**) regions. Differential chromatin interactions between clusters are highlighted with arrows.

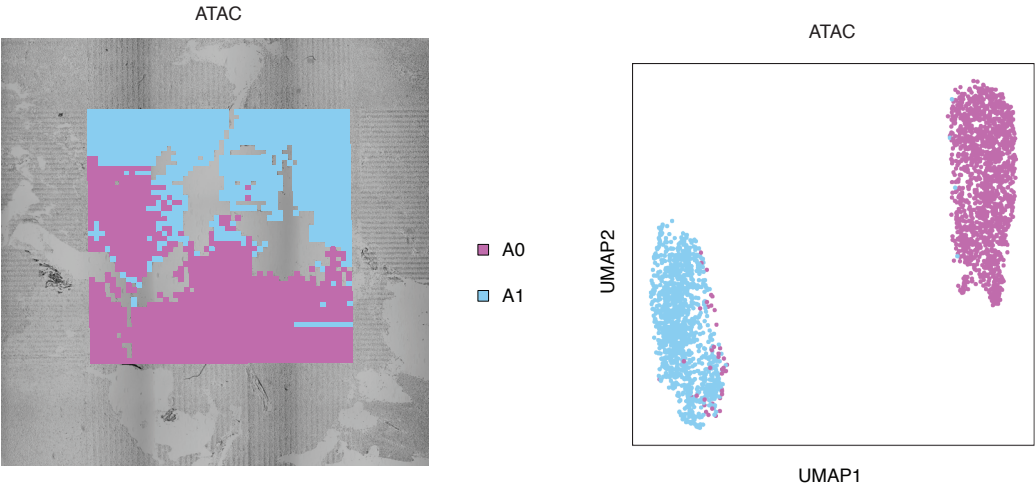
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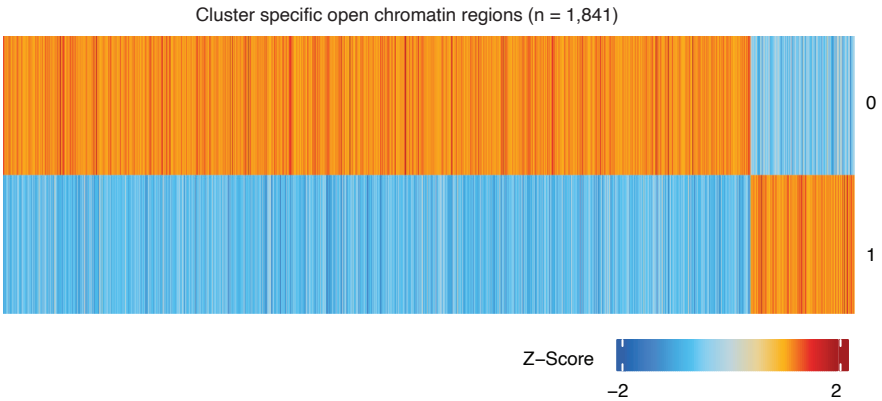
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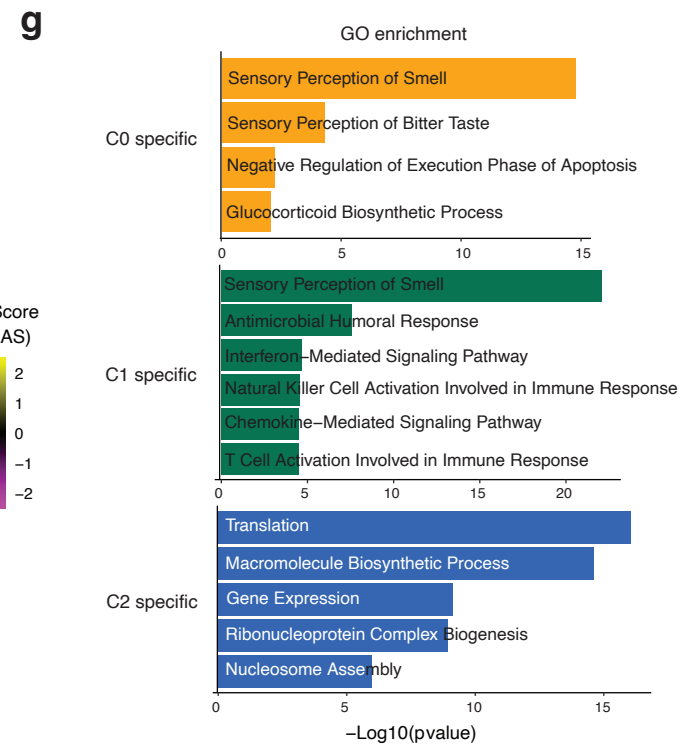
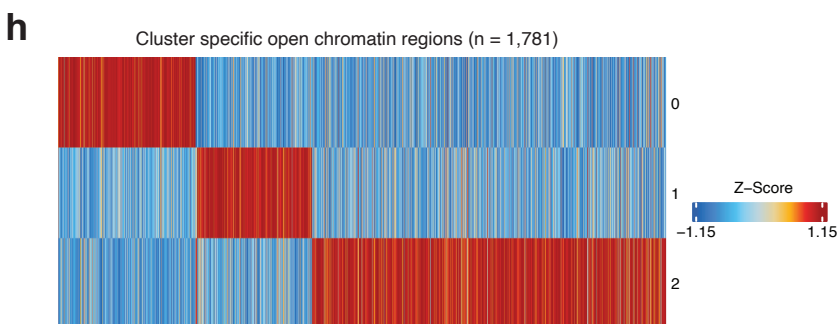
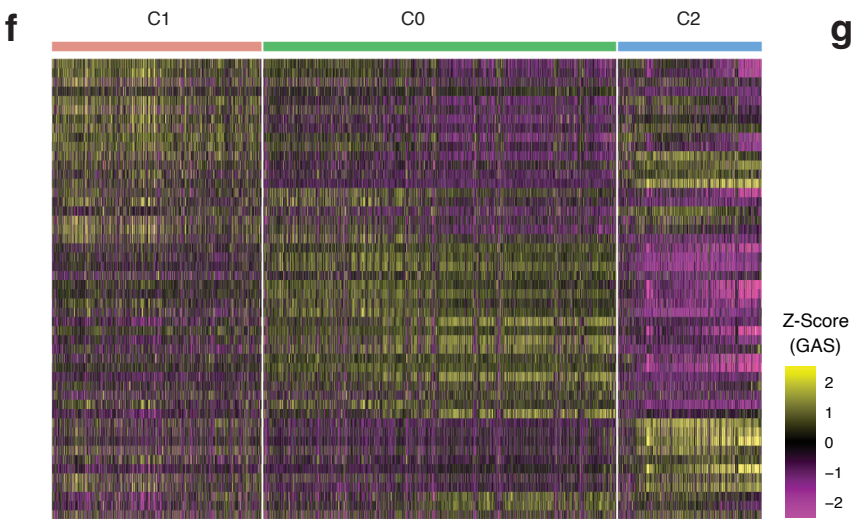
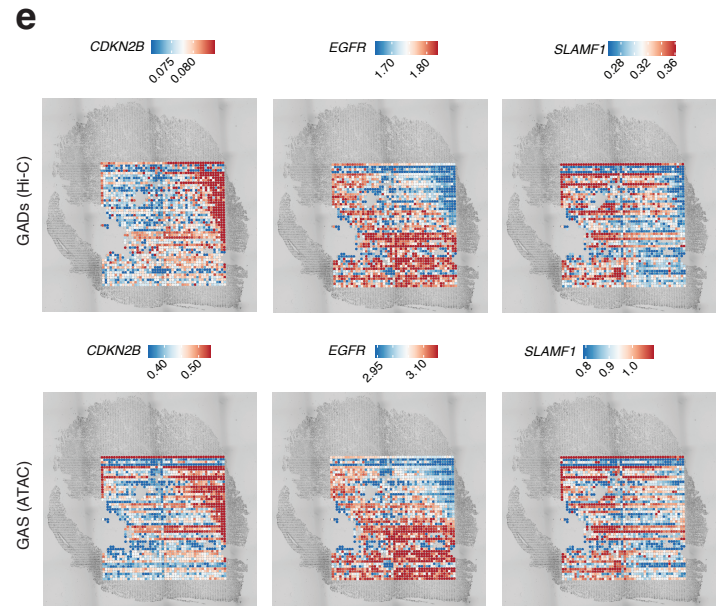
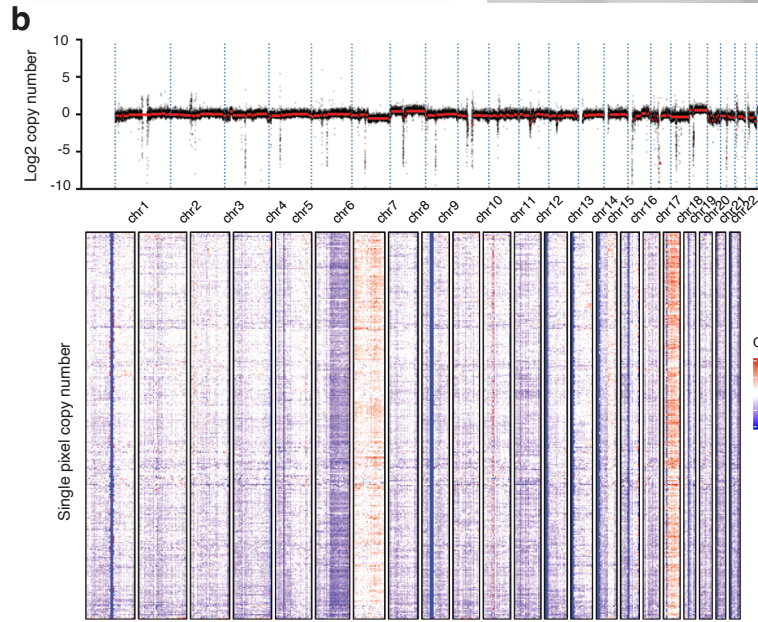
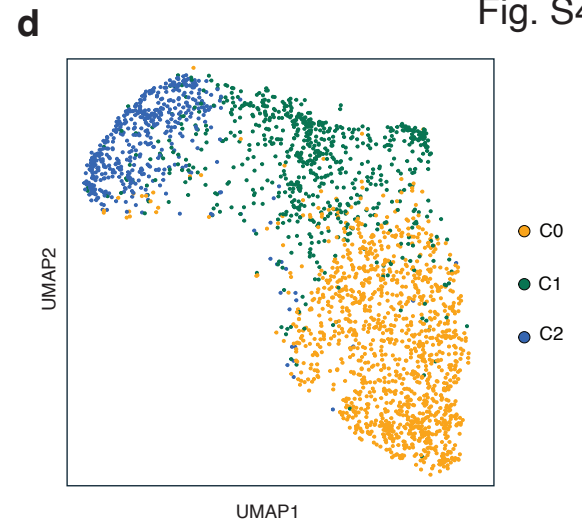
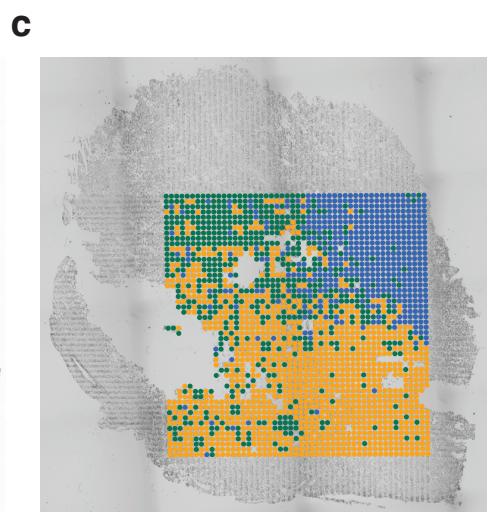
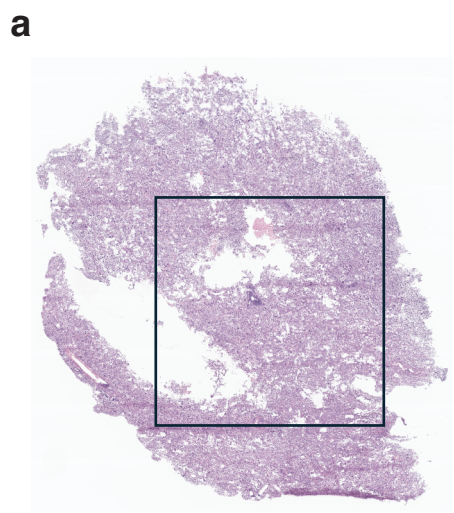
c



d



Supplementary Figure 3. Analysis of spatial Hi-C and ATAC-seq in Glioblastoma sample NU03747. **a.** UMAP of clusters based on copy numbers inferred from spatial Hi-C data in GBM sample NU03747. **b-c.** Spatial distribution (left panel) and UMAP (right panel) of all clusters based on Hi-C (**b**) and ATAC-seq (**c**) in GBM sample NU03747. **d.** Heatmap showing cluster-specific open chromatin regions in GBM sample NU03747.



Supplementary Figure 4. Spatial 3D genome and epigenome co-profiling in Glioblastoma sample NU03766. **a.** H&E staining from an adjacent tissue section of sample NU03766. Region selected for the spatial experiment is highlighted in the black square box. **b.** Copy number profiles inferred from pseudo-bulk spatial Hi-C data in GBM sample (upper panel). Heatmap (lower panel) shows copy numbers in each pixel. **c-d.** spatial distribution (**c**) and UMAP (**d**) of clusters based on copy numbers inferred from spatial Hi-C data. **e.** Spatial distribution of GADs (top panel), GAS (bottom panel) for *CDKN2B*, *EGFR* and *SLAMF1*. **f.** Heatmap showing the top cluster-specific protein coding genes. **g.** GO term enrichment of cluster-specific genes. **h.** Heatmap showing cluster-specific open chromatin regions. The statistical significance of GO term enrichment in **g** was assessed using Fisher's exact test.

Supplementary Table 1. DNA oligos used for library preparation

Tn5ME-A	5'-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3'
Tn5MErev	5'-/5Phos/CTGTCTCTTATACACATCT-3'
Tn5ME-B	5'-/5Phos/CATCGGCGTACGACTAGATGTGTATAAGAGA CAG-3'
N501	AATGATACGGCGACCAACGAGATCTACACTAGATCGCTCGTCGGCAGCG TCAGATGTGTATAAGAGACAG
N701	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTCTCGTGGGCTCGGAG ATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
N702	CAAGCAGAAGACGGCATACGAGATCTAGTACGGTCTCGTGGGCTCGGAG ATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
N703	CAAGCAGAAGACGGCATACGAGATTTCTGCCTGTCTCGTGGGCTCGGAG ATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
N704	CAAGCAGAAGACGGCATACGAGATGCTCAGGAGTCTCGTGGGCTCGGA GATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
N705	CAAGCAGAAGACGGCATACGAGATAGGAGTCCGTCTCGTGGGCTCGGA GATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
N706	CAAGCAGAAGACGGCATACGAGATCATGCCTAGTCTCGTGGGCTCGGAG ATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT
N707	CAAGCAGAAGACGGCATACGAGATGTAGAGAGGTCTCGTGGGCTCGGA GATGTGTATAAGAGACAGCAAGCGTTGGCTTCTCGCATCT

Supplementary Table 2. Spatial DNA barcodes used for library preparation

Barcode A	Sequence
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Barcode A-2	/5Phos/AGGCCAGAGCATTCTGAAACATCGGTGGCCGATGTTTCG
Barcode A-3	/5Phos/AGGCCAGAGCATTCTGATGCCTAAGTGGCCGATGTTTCG
Barcode A-4	/5Phos/AGGCCAGAGCATTCTGAGTGGTCAGTGGCCGATGTTTCG
Barcode A-5	/5Phos/AGGCCAGAGCATTCTGACCACTGTGTGGCCGATGTTTCG
Barcode A-6	/5Phos/AGGCCAGAGCATTCTGACATTGGCGTGGCCGATGTTTCG
Barcode A-7	/5Phos/AGGCCAGAGCATTCTGCAGATCTGGTGGCCGATGTTTCG
Barcode A-8	/5Phos/AGGCCAGAGCATTCTGCATCAAGTGTGGCCGATGTTTCG
Barcode A-9	/5Phos/AGGCCAGAGCATTCTGCGCTGATCGTGGCCGATGTTTCG
Barcode A-10	/5Phos/AGGCCAGAGCATTCTGACAAGCTAGTGGCCGATGTTTCG
Barcode A-11	/5Phos/AGGCCAGAGCATTCTGCTGTAGCCGTGGCCGATGTTTCG
Barcode A-12	/5Phos/AGGCCAGAGCATTCTGAGTACAAGGTGGCCGATGTTTCG
Barcode A-13	/5Phos/AGGCCAGAGCATTCTGAACAACCAAGTGGCCGATGTTTCG
Barcode A-14	/5Phos/AGGCCAGAGCATTCTGAACCGAGAGTGGCCGATGTTTCG
Barcode A-15	/5Phos/AGGCCAGAGCATTCTGAACGCTTAGTGGCCGATGTTTCG
Barcode A-16	/5Phos/AGGCCAGAGCATTCTGAAGACGGAGTGGCCGATGTTTCG
Barcode A-17	/5Phos/AGGCCAGAGCATTCTGAAGGTACAGTGGCCGATGTTTCG
Barcode A-18	/5Phos/AGGCCAGAGCATTCTGACACAGAAGTGGCCGATGTTTCG
Barcode A-19	/5Phos/AGGCCAGAGCATTCTGACAGCAGAGTGGCCGATGTTTCG
Barcode A-20	/5Phos/AGGCCAGAGCATTCTGACCTCCAAGTGGCCGATGTTTCG
Barcode A-21	/5Phos/AGGCCAGAGCATTCTGACGCTCGAGTGGCCGATGTTTCG
Barcode A-22	/5Phos/AGGCCAGAGCATTCTGACGTATCAGTGGCCGATGTTTCG
Barcode A-23	/5Phos/AGGCCAGAGCATTCTGACTATGCAGTGGCCGATGTTTCG
Barcode A-24	/5Phos/AGGCCAGAGCATTCTGAGAGTCAAGTGGCCGATGTTTCG
Barcode A-25	/5Phos/AGGCCAGAGCATTCTGAGATCGCAGTGGCCGATGTTTCG
Barcode A-26	/5Phos/AGGCCAGAGCATTCTGAGCAGGAAGTGGCCGATGTTTCG
Barcode A-27	/5Phos/AGGCCAGAGCATTCTGAGTCACTAGTGGCCGATGTTTCG
Barcode A-28	/5Phos/AGGCCAGAGCATTCTGATCCTGTAGTGGCCGATGTTTCG
Barcode A-29	/5Phos/AGGCCAGAGCATTCTGATTGAGGAGTGGCCGATGTTTCG
Barcode A-30	/5Phos/AGGCCAGAGCATTCTGCAACCACAGTGGCCGATGTTTCG
Barcode A-31	/5Phos/AGGCCAGAGCATTCTGGACTAGTAGTGGCCGATGTTTCG
Barcode A-32	/5Phos/AGGCCAGAGCATTCTGCAATGGAAGTGGCCGATGTTTCG
Barcode A-33	/5Phos/AGGCCAGAGCATTCTGCACCTCGAGTGGCCGATGTTTCG
Barcode A-34	/5Phos/AGGCCAGAGCATTCTGCAGCGTTAGTGGCCGATGTTTCG
Barcode A-35	/5Phos/AGGCCAGAGCATTCTGCATACCAAGTGGCCGATGTTTCG
Barcode A-36	/5Phos/AGGCCAGAGCATTCTGCCAGTTCAGTGGCCGATGTTTCG
Barcode A-37	/5Phos/AGGCCAGAGCATTCTGCCGAAGTAGTGGCCGATGTTTCG
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Barcode A-39	/5Phos/AGGCCAGAGCATTCTGCCTCCTGAGTGGCCGATGTTTCG
Barcode A-40	/5Phos/AGGCCAGAGCATTCTGCGAACTTAGTGGCCGATGTTTCG
Barcode A-41	/5Phos/AGGCCAGAGCATTCTGCGACTGGAGTGGCCGATGTTTCG
Barcode A-42	/5Phos/AGGCCAGAGCATTCTGCGCATACAGTGGCCGATGTTTCG
Barcode A-43	/5Phos/AGGCCAGAGCATTCTGCTCAATGAGTGGCCGATGTTTCG
Barcode A-44	/5Phos/AGGCCAGAGCATTCTGCTGAGCCAGTGGCCGATGTTTCG
Barcode A-45	/5Phos/AGGCCAGAGCATTCTGCTGGCATAGTGGCCGATGTTTCG
Barcode A-46	/5Phos/AGGCCAGAGCATTCTGGAATCTGAGTGGCCGATGTTTCG
Barcode A-47	/5Phos/AGGCCAGAGCATTCTGCAAGACTAGTGGCCGATGTTTCG
Barcode A-48	/5Phos/AGGCCAGAGCATTCTGGAGCTGAAGTGGCCGATGTTTCG
Barcode A-49	/5Phos/AGGCCAGAGCATTCTGGATAGACAGTGGCCGATGTTTCG
Barcode A-50	/5Phos/AGGCCAGAGCATTCTGGCCACATAGTGGCCGATGTTTCG

Barcode B	Sequence
Barcode B-1	CAAGCGTTGGCTTCTCGCATCTAACGTGATATCCACGTGCTTGAG
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
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Barcode B-11	CAAGCGTTGGCTTCTCGCATCTCTGTAGCCATCCACGTGCTTGAG
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Barcode B-18	CAAGCGTTGGCTTCTCGCATCTACACAGAAATCCACGTGCTTGAG
Barcode B-19	CAAGCGTTGGCTTCTCGCATCTACAGCAGAATCCACGTGCTTGAG
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Barcode B-21	CAAGCGTTGGCTTCTCGCATCTACGCTCGAATCCACGTGCTTGAG
Barcode B-22	CAAGCGTTGGCTTCTCGCATCTACGTATCAATCCACGTGCTTGAG
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Barcode B-26	CAAGCGTTGGCTTCTCGCATCTAGCAGGAAATCCACGTGCTTGAG
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Barcode B-29	CAAGCGTTGGCTTCTCGCATCTATTGAGGAATCCACGTGCTTGAG
Barcode B-30	CAAGCGTTGGCTTCTCGCATCTCAACCACAATCCACGTGCTTGAG
Barcode B-31	CAAGCGTTGGCTTCTCGCATCTGACTAGTAATCCACGTGCTTGAG
Barcode B-32	CAAGCGTTGGCTTCTCGCATCTCAATGGAAATCCACGTGCTTGAG
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Barcode B-34	CAAGCGTTGGCTTCTCGCATCTCAGCGTTAATCCACGTGCTTGAG
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Barcode B-37	CAAGCGTTGGCTTCTCGCATCTCCGAAGTAATCCACGTGCTTGAG
Barcode B-38	CAAGCGTTGGCTTCTCGCATCTCCGTGAGAATCCACGTGCTTGAG
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Barcode B-41	CAAGCGTTGGCTTCTCGCATCTCGACTGGAATCCACGTGCTTGAG
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Barcode B-46	CAAGCGTTGGCTTCTCGCATCTGAATCTGAATCCACGTGCTTGAG
Barcode B-47	CAAGCGTTGGCTTCTCGCATCTCAAGACTAATCCACGTGCTTGAG
Barcode B-48	CAAGCGTTGGCTTCTCGCATCTGAGCTGAAATCCACGTGCTTGAG
Barcode B-49	CAAGCGTTGGCTTCTCGCATCTGATAGACAATCCACGTGCTTGAG
Barcode B-50	CAAGCGTTGGCTTCTCGCATCTGCCACATAATCCACGTGCTTGAG

Supplementary Table 3. Summary of metrics for in-situ Hi-C, Spatial-no-barcoding Hi-C and pseudo-bulk Hi-C in Spatial-ATAC-Hi-C

SampleID	# of total read pairs	# of read pairs with spatial barcodes	# of mapped Pairs	# of uniquely mapped read pairs	# of HiC cis-contacts (hg38 or mm10)	# of HiC long-range cis-contacts >=20kb	Ratio of HiC long-range cis-contacts >=20kb
<i>In-situ</i> Hi-C	494,536,845	NA	354,662,640	311,291,126	267,926,106	142,483,787	0.46
Spatial-no-barcoding	781,108,863	NA	585,545,074	248,397,034	220,337,966	67,441,178	0.27
MouseBrain (R6)	881,106,933	823,093,569	469,031,923	181,231,943	162,842,395	45,066,448	0.25
MouseBrain (R8)	1,093,753,070	920,259,820	517,874,604	212,419,318	191,425,012	54,780,734	0.26
HumanBrain (Cerebellum)	514,752,302	396,983,624	182,824,782	121,916,478	106,393,846	27,468,253	0.23
HumanBrain (Cortex)	528,487,966	405,578,312	207,358,244	85,623,513	76,531,703	18,453,945	0.22
NU04041A (Astrocytoma)	523,561,200	334,232,442	198,135,119	105,141,777	92,554,894	26,419,599	0.25
NU03766 (GBM21)	590,046,003	456,505,473	246,898,297	157,253,943	139,391,757	43,316,688	0.28
NU03747 (GBM6)	571,065,078	504,618,202	283,567,115	140,868,476	126,113,211	31,316,756	0.22

Supplementary Table 7. Number of ATAC-seq peaks, A/B compartment, TAD boundaries and chromatin loops for each subtype/cluster in each sample

Sample	cell type	pixel #	intra reads #	ATAC-Seq peaks #	A/B compartment # (100kb resolution)	TAD boundary # (25kb resolution, boundary_strength > 0.5)	loop # (25kb resolution)
mousebrain R6	Excitatory neuron	733	93,413,053	4,223	11,025/13,468	3,424	4,335
mousebrain R6	Inhibitory neuron	710	104,028,060	5,964	10,689/13,749	3,386	4,648
mousebrain R6	Non-neuron	523	46,809,596	4,401	10,812/13,688	3,636	1,767
mousebrain R8	Excitatory neuron	1,238	218,447,101	4,752	10,999/13,402	3,302	6,882
mousebrain R8	Inhibitory neuron	278	28,266,938	4,453	11,243/13,351	3,972	960
mousebrain R8	Non-neuron	808	55,881,687	2,963	11,152/13,459	3,586	2,397
human cerebellum	C0	1,187	26,255,899	61,835	12,764/15,165	3,231	3,424
human cerebellum	C1	1,102	4,274,581	22,903	12,321/15,461	4,943	148
human cerebellum	C2	6	NA	NA	NA	NA	NA
NU03747 (GBM6)	C0	1,053	83,740,251	59,670	11,918/15,749	3,574	4,424
NU03747 (GBM6)	C1	784	74,878,074	38,846	11,478/16,018	3,455	4,647
NU03747 (GBM6)	C2	164	N/A	30,237	N/A	N/A	N/A
NU03766 (GBM21)	C0	1,124	66,180,137	9,230	12,568/14,973	3,814	4,548
NU03766 (GBM21)	C1	628	41,474,582	8,468	12,761/14,755	3,867	2,734
NU03766 (GBM21)	C2	497	25,030,993	8,462	13,252/14,392	4,070	1,547