



Multimodal chromatin profiling using nanobody-based single-cell CUT&Tag

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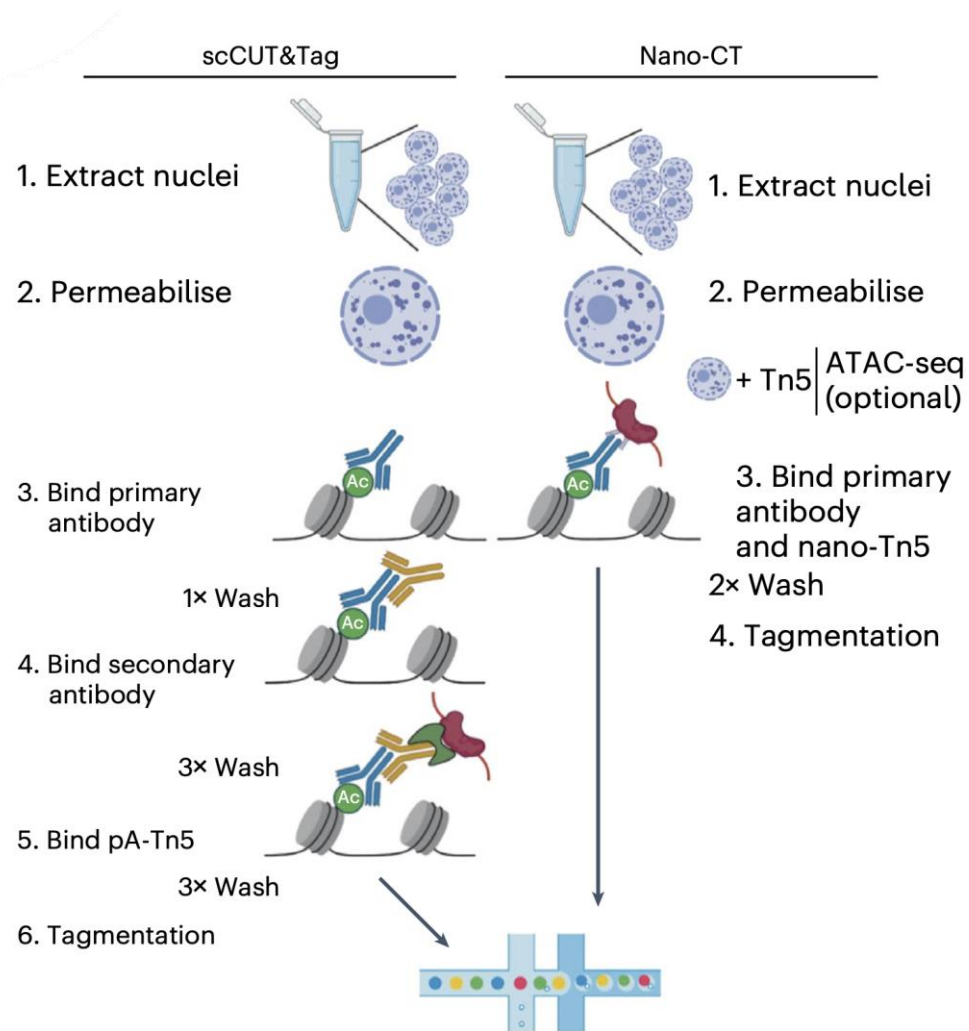


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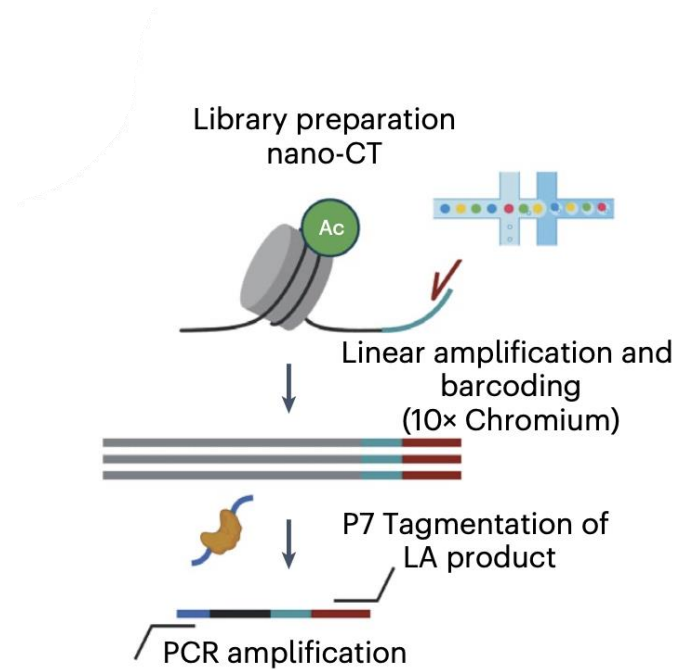
Nov 21, 2025

Overview

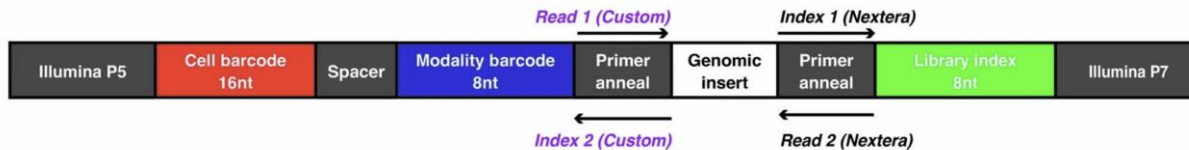


- Distinct adapter barcodes for multimodal profiling
- More nuclei recovered as a result of fewer washes: 28-68% increase in yield
- Possible to profile low-input samples, starting from 25,000 cells
- Up to 3 modalities (2 histone marks + ATAC)

Overview

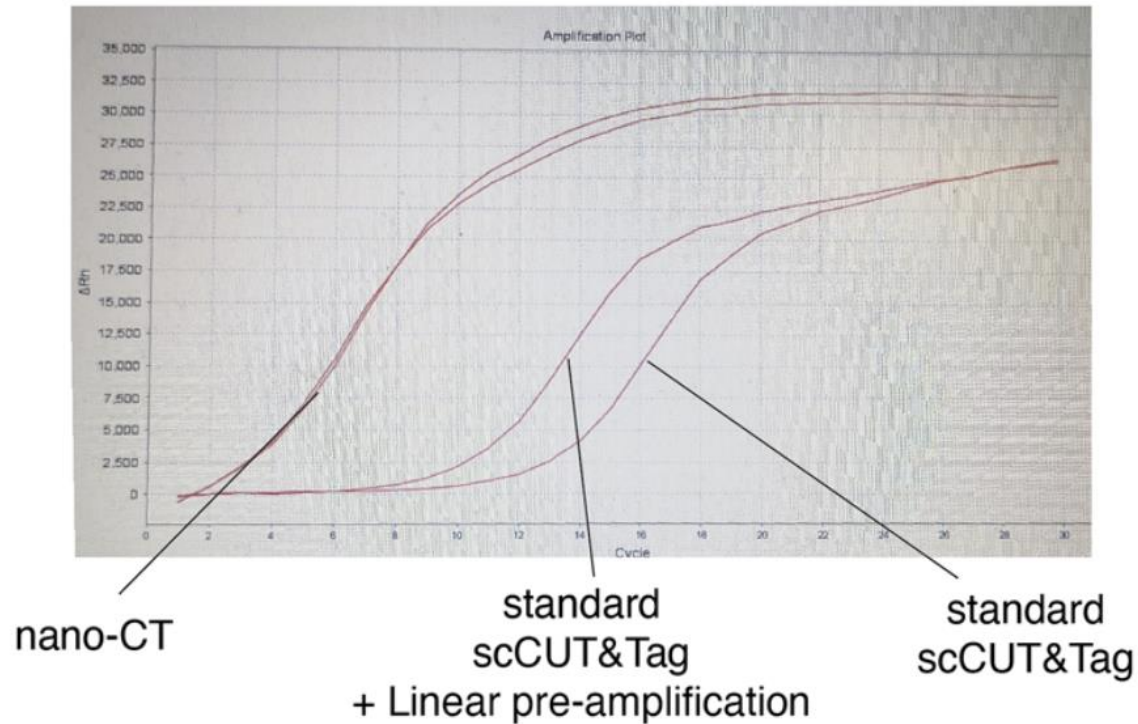


- Nuclei loaded onto 10x genomics chromium chip
- scATAC kit used



Nano-CT vs CUT&TAG

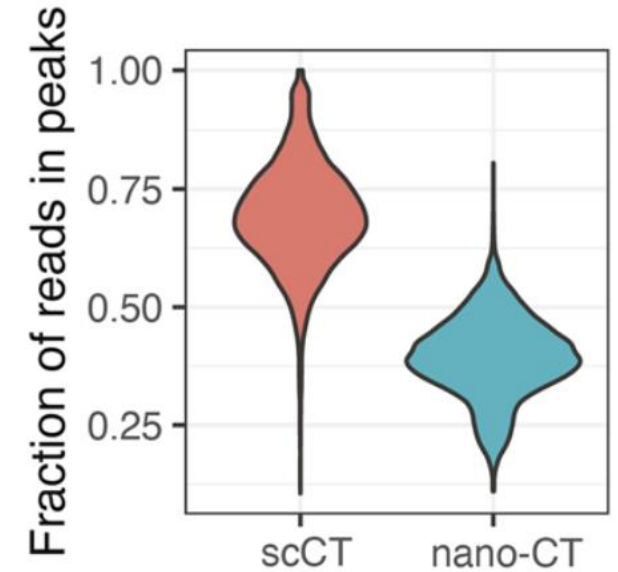
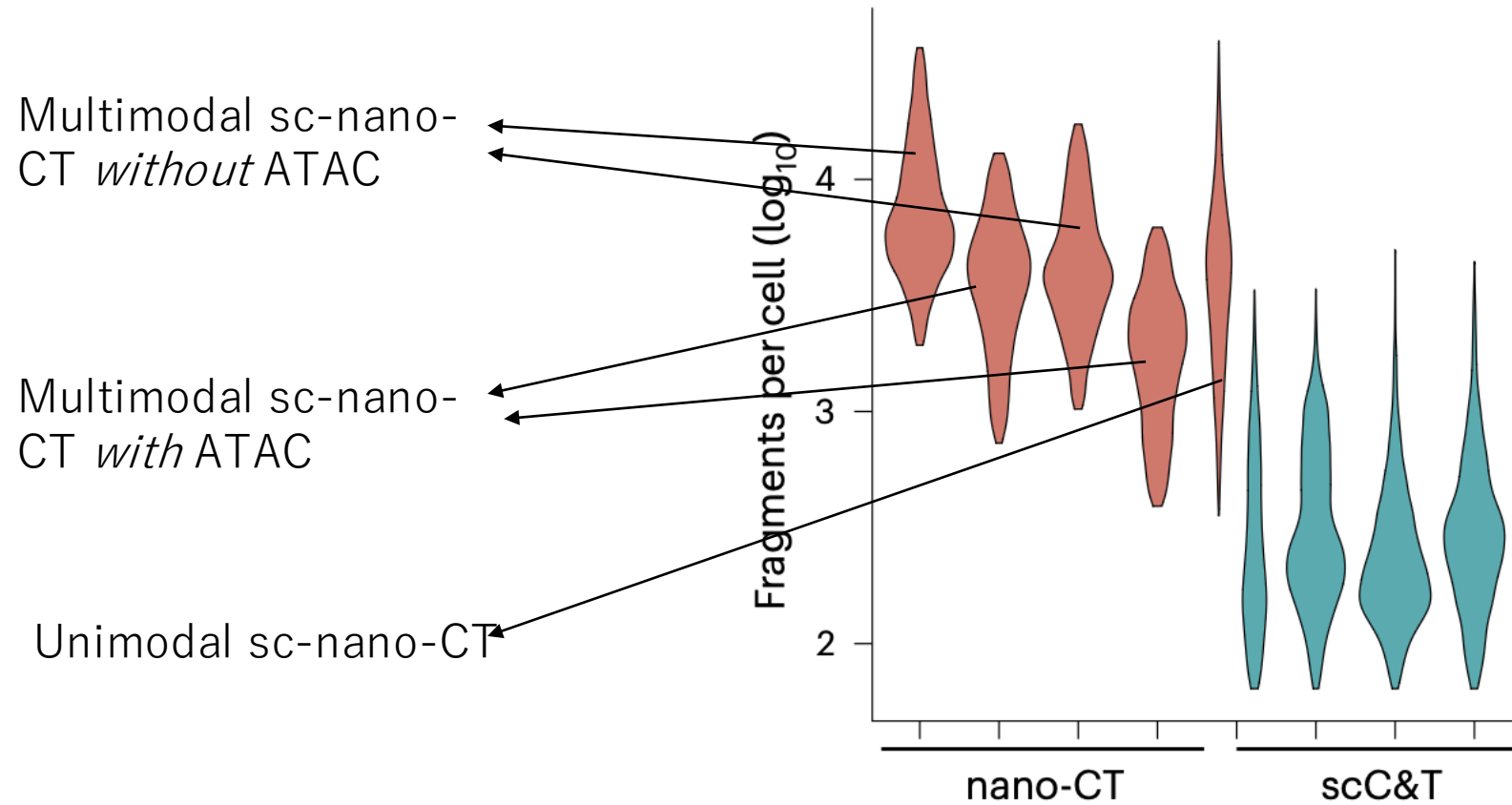
qPCR amplification curve using
same amount of input material



- Higher capture efficiency, fewer PCR cycles compared to CUT&TAG, not only explained by the linear amplification

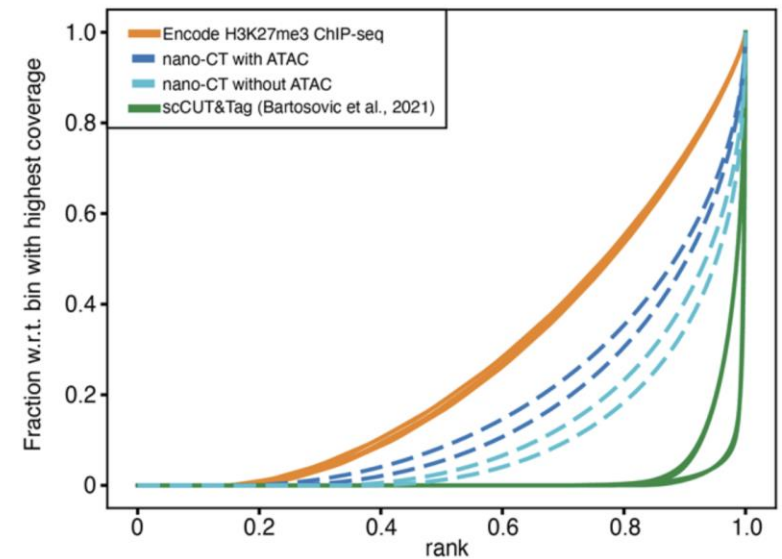
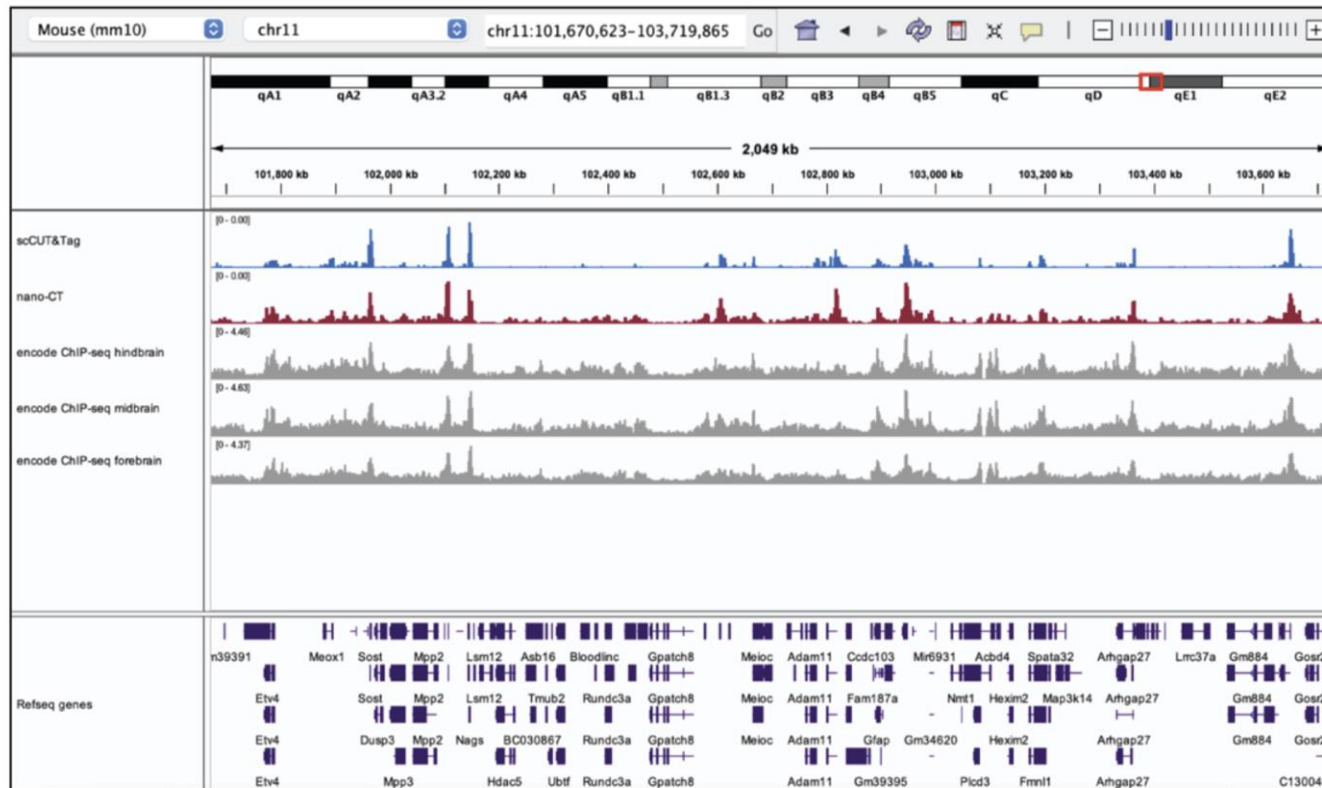
Unimodal single-cell nano-CT vs scCUT&TAG

unique reads per cell
(H3K27me3 in P19 mouse brain)

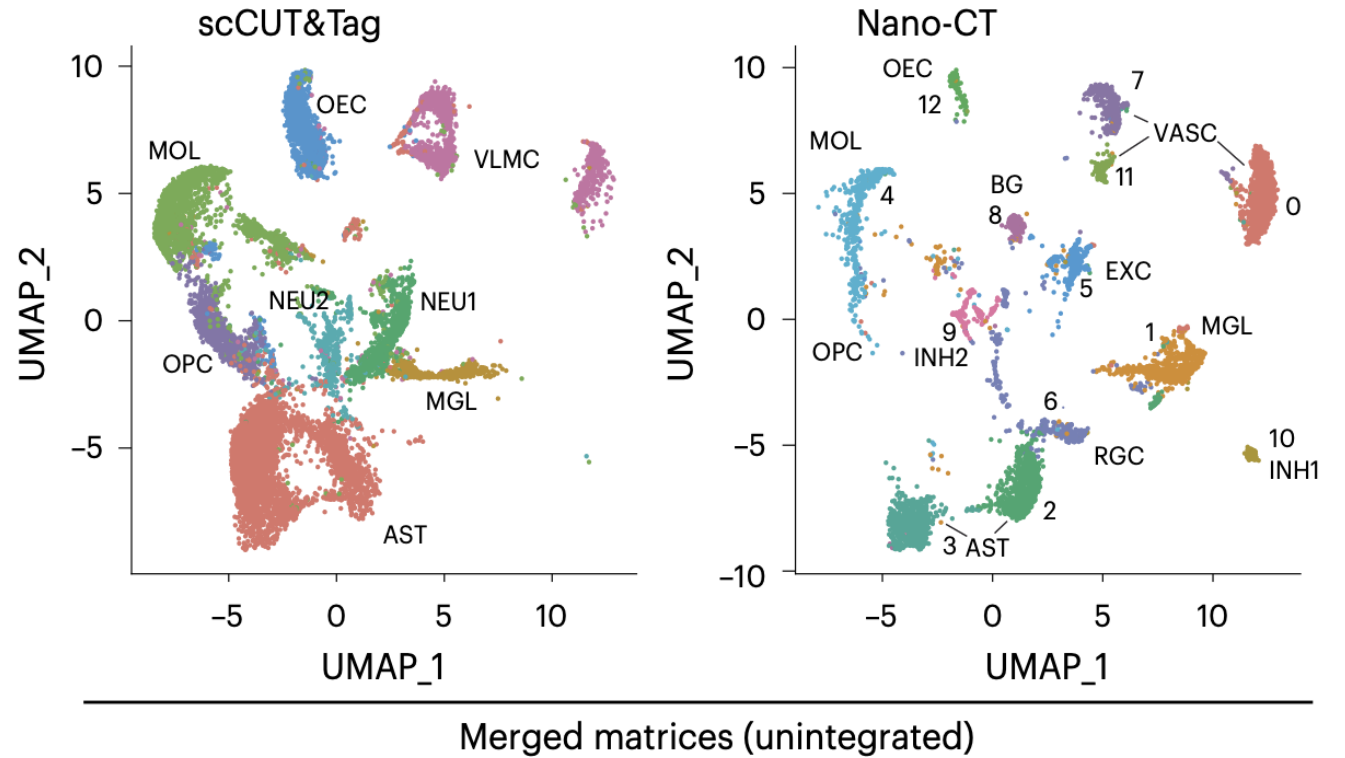
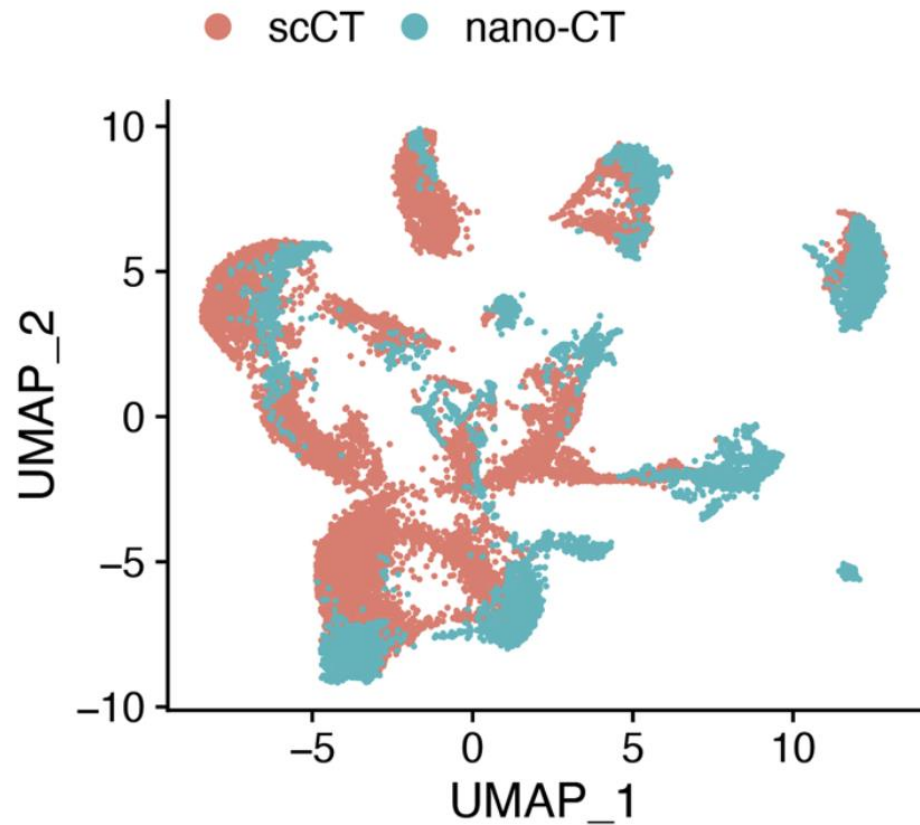


Unimodal single-cell nano-CT vs scCUT&TAG

nano-CT shows more background noise than scCUT&TAG but less than encode ChIP-seq

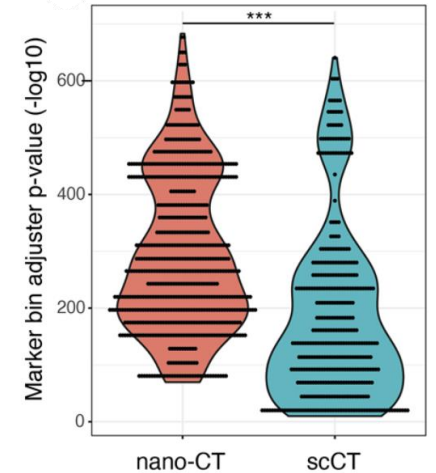
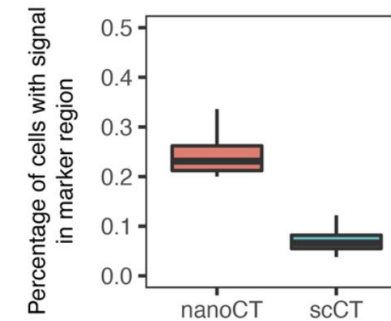
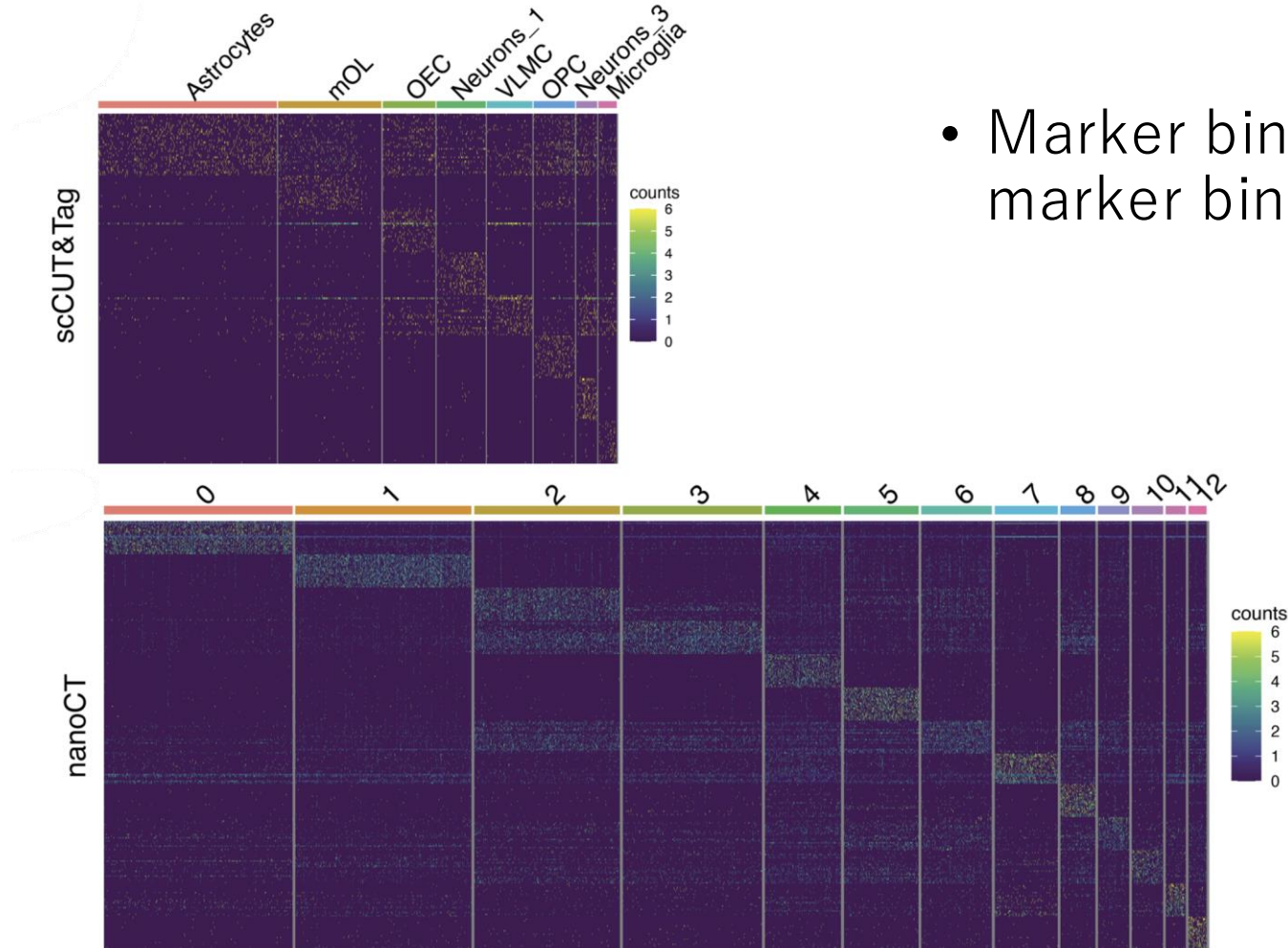


Unimodal single-cell nano-CT vs scCUT&TAG

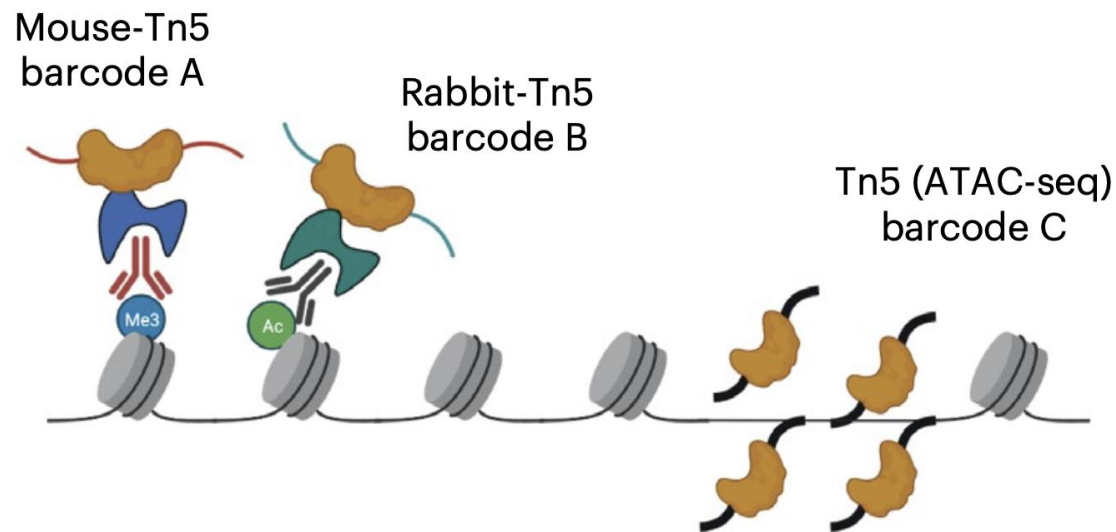


Unimodal single-cell nano-CT vs scCUT&TAG

- Marker bins identified by sc-nano-CT vs marker bins identified by scCUT&TAG

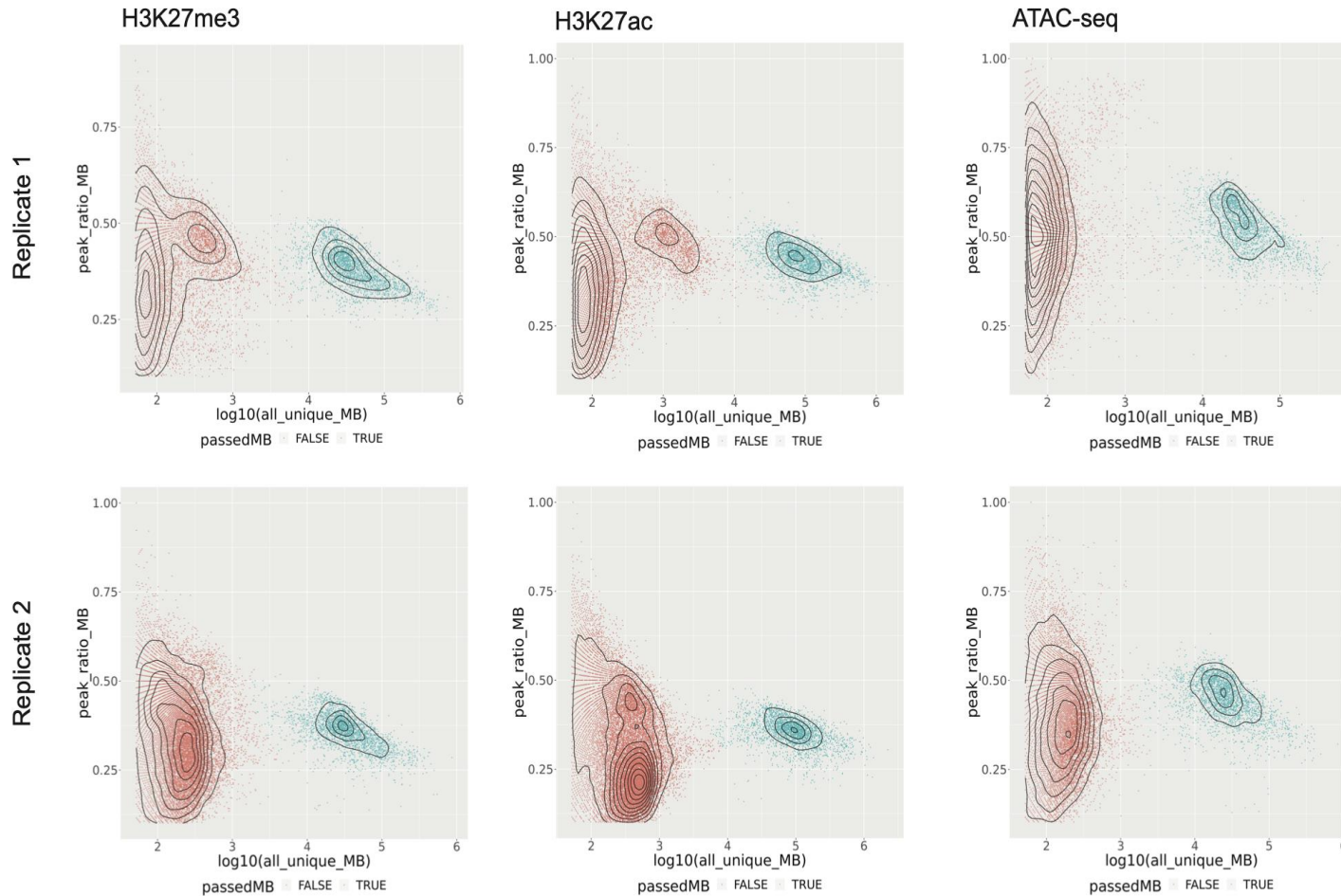


Multimodal sc-nano-CT in the mouse brain

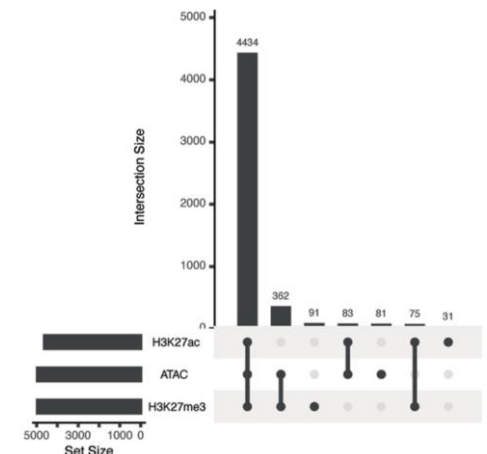


- sc-nano-CT on the 10x Genomics Chromium ATAC-seq v1.1
- Fresh mouse brain tissue from P19
- H3K27ac, H3K27me3, ATAC
- 2 replicates
- Needed at least 200,000 cells for using 3 modalities
- Demultiplexed libraries using **cellranger**

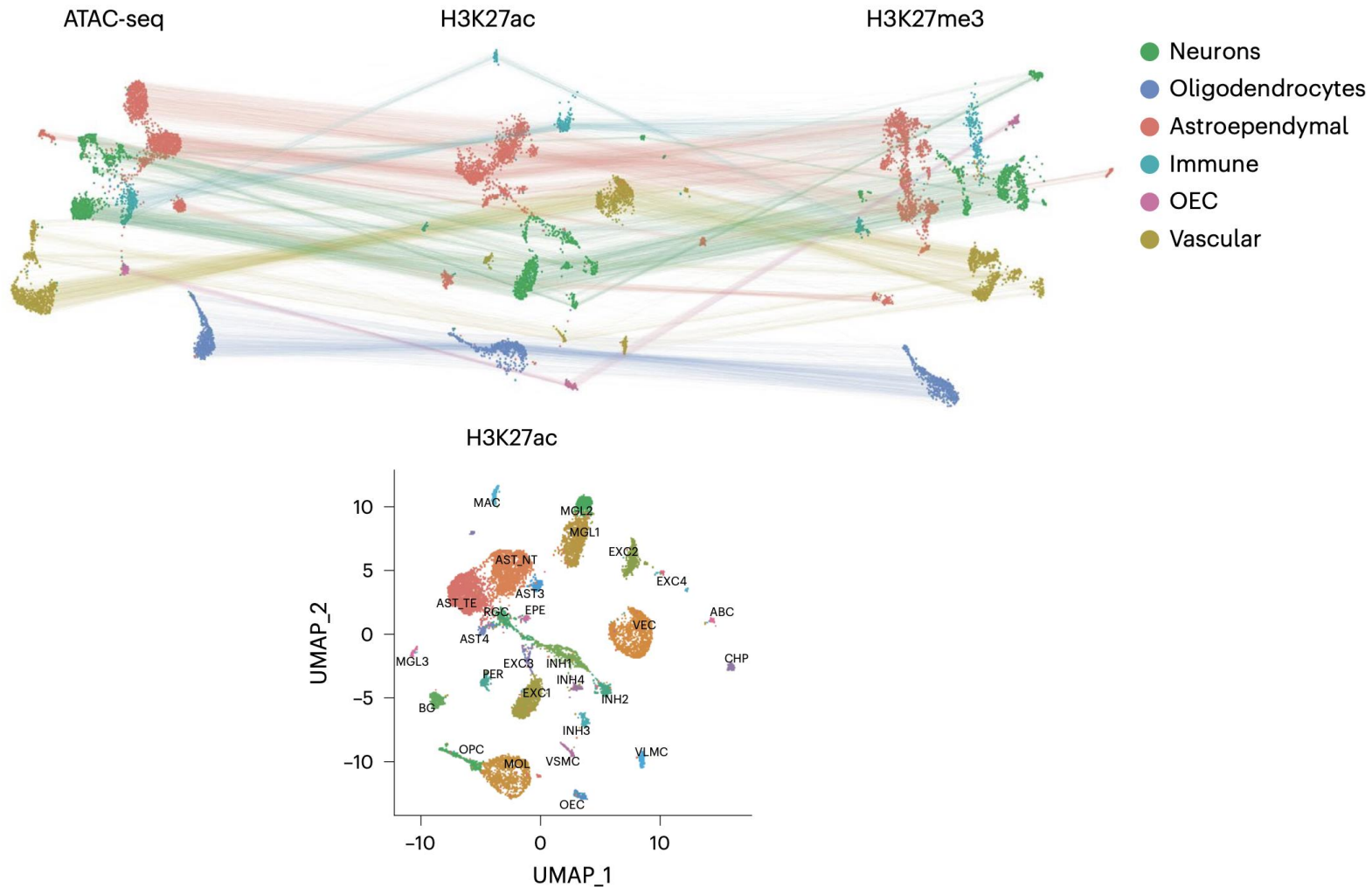
Multimodal sc-nano-CT in the mouse brain



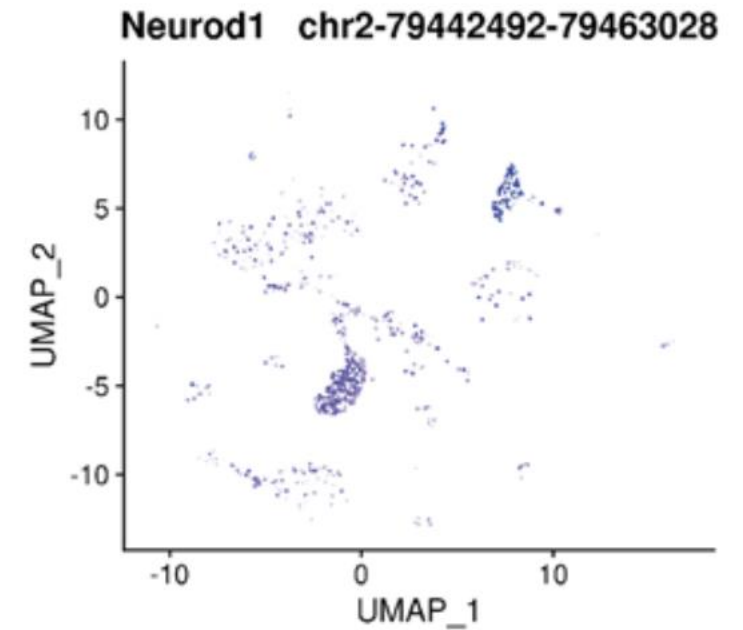
- Filter out low quality cells by looking at fraction of reads in peak per cell vs number of reads per cell
- Used a Gaussian mixture model to do so
- Out of 5,157 cells 4,434 (85.9%) passed QC in all modalities



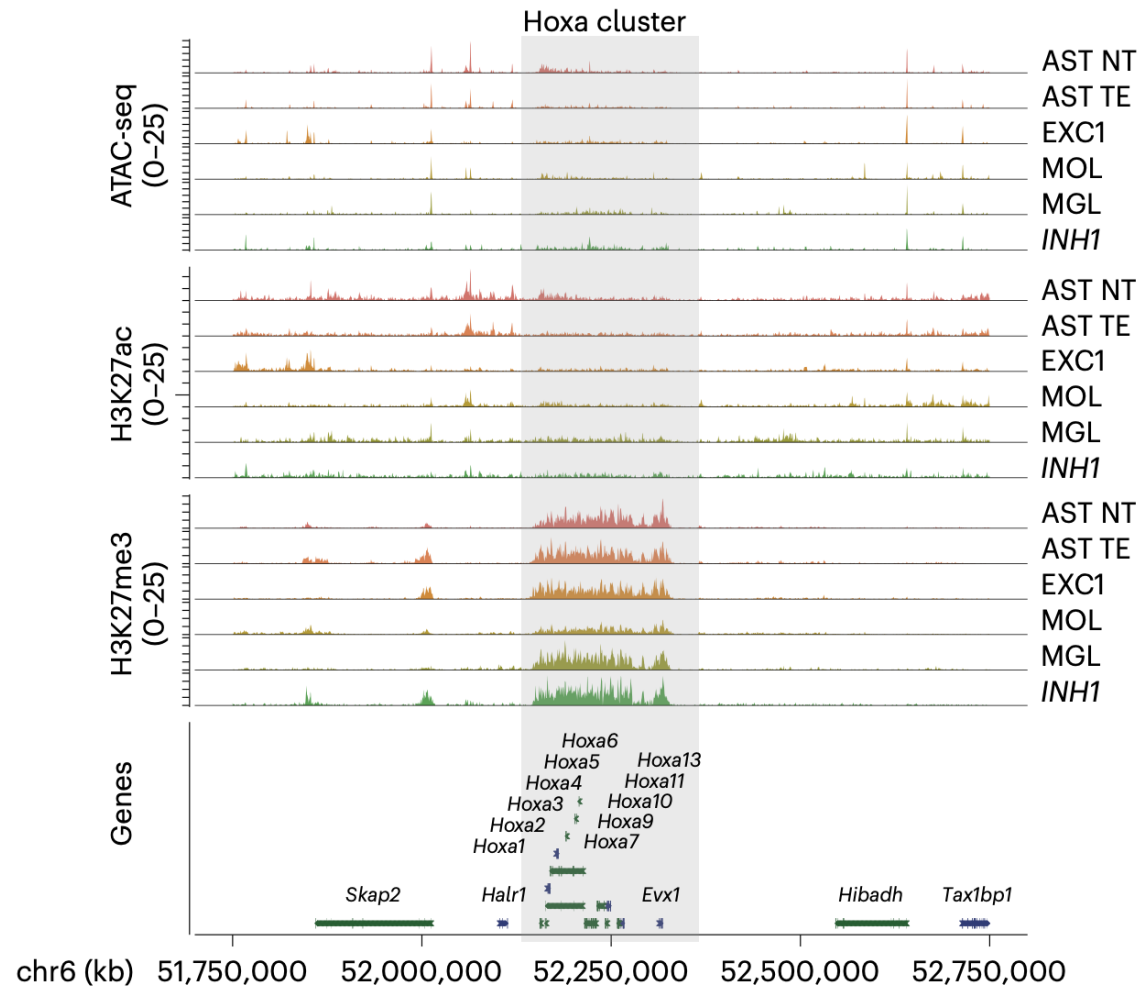
Multimodal sc-nano-CT in the mouse brain



H3K27ac on select enhancer

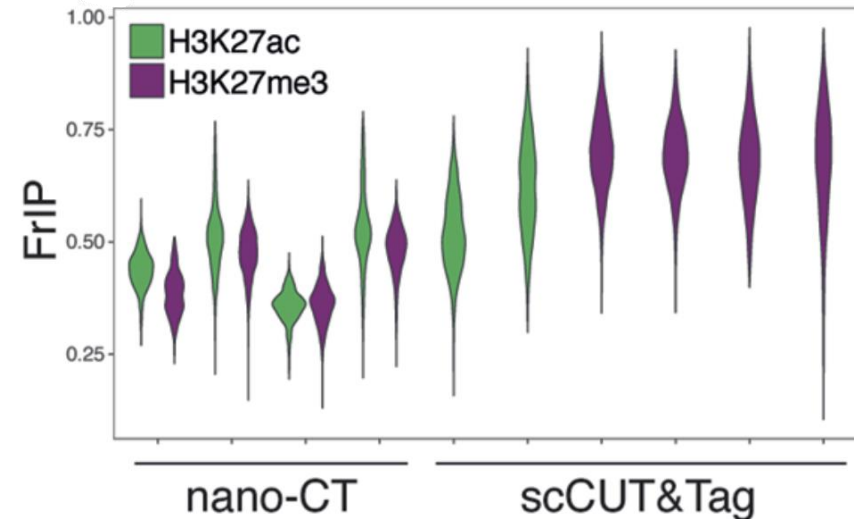
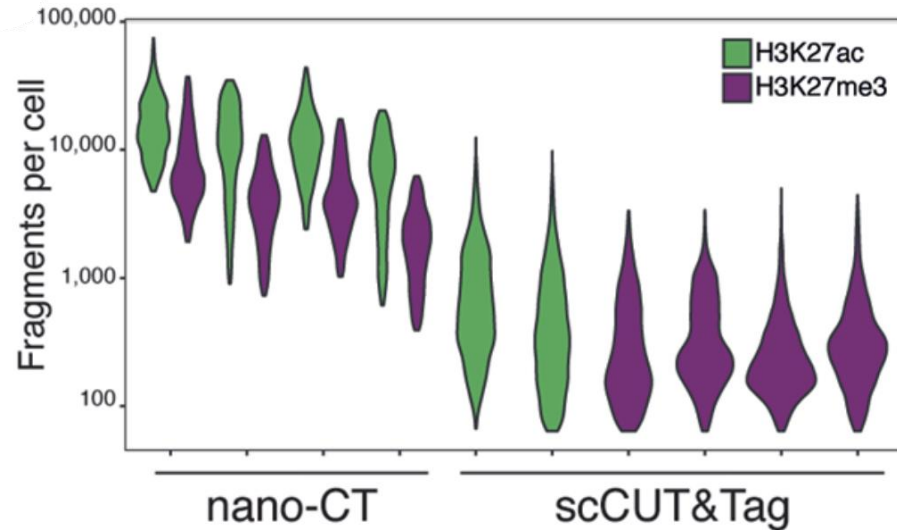


Specificity of H3K27me3 modality at HoxA locus

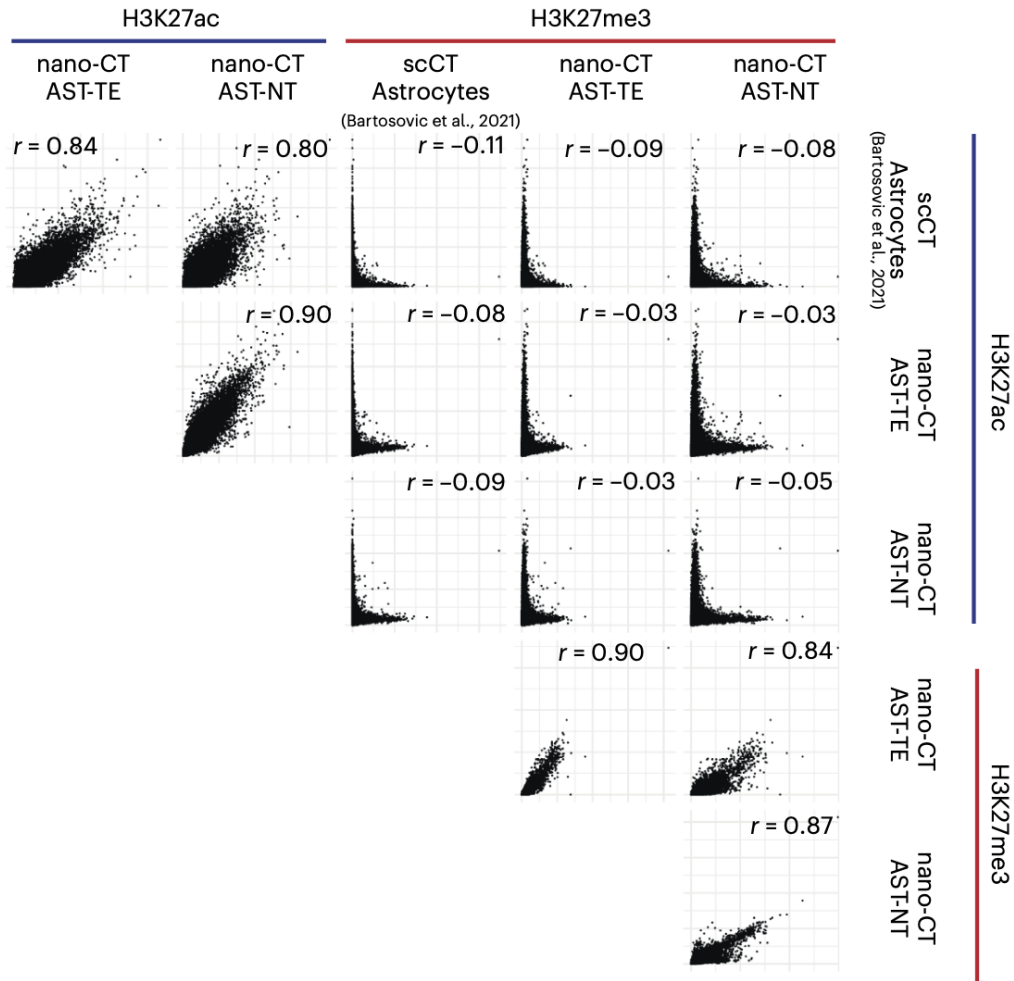


- No ATAC or H3K27Ac, only H3K27me3 expected
- Similarly for HoxB, HoxC, and HoxD

Multimodal sc-nano-CT vs scCUT&TAG

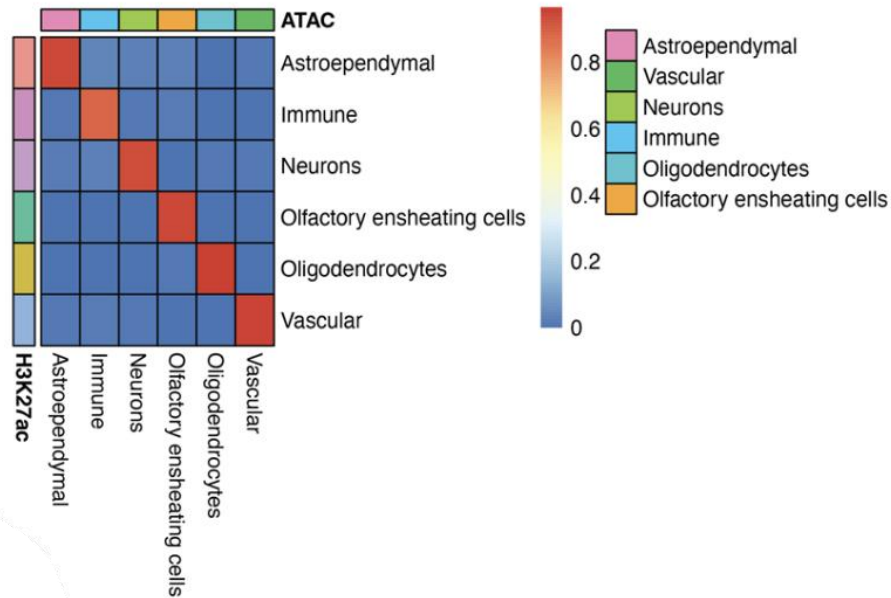


Multimodal sc-nano-CT vs scCUT&TAG

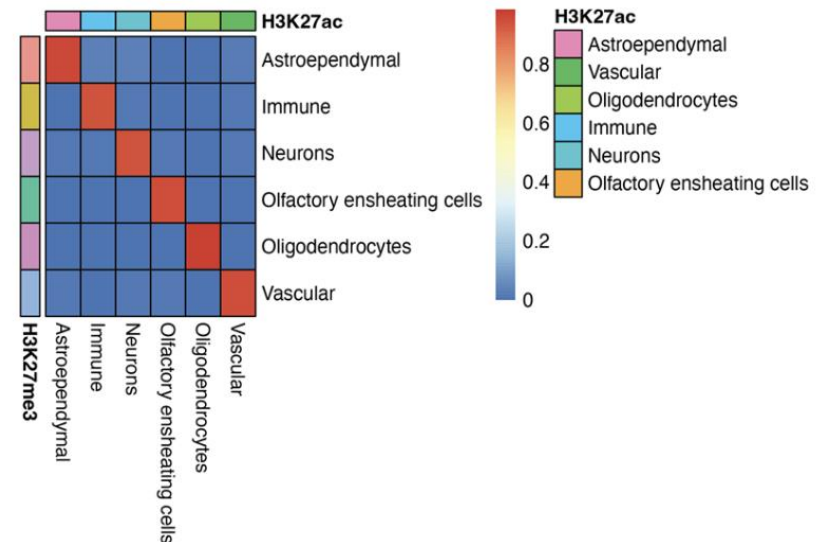
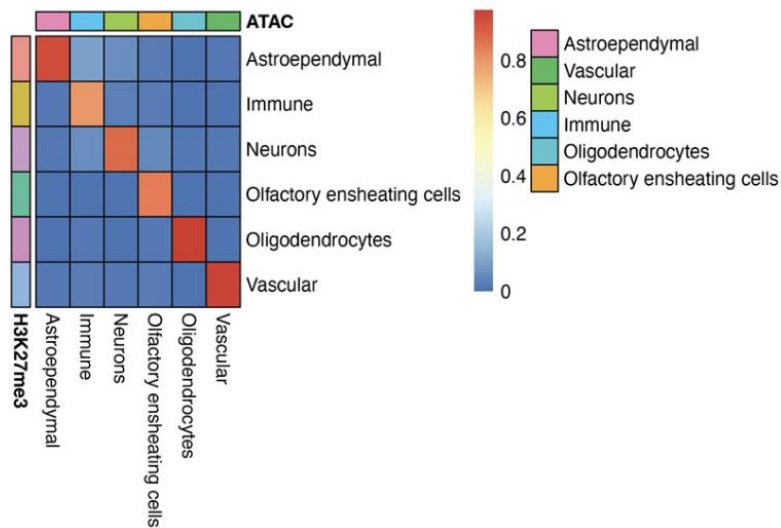


- H3K27ac and H3K27me3 in astrocytes
- Pearson correlation
- Correlation between different technologies on the same mark
- AST-TE: astrocytes telencephalon
AST-NT: astrocytes non-telencephalon

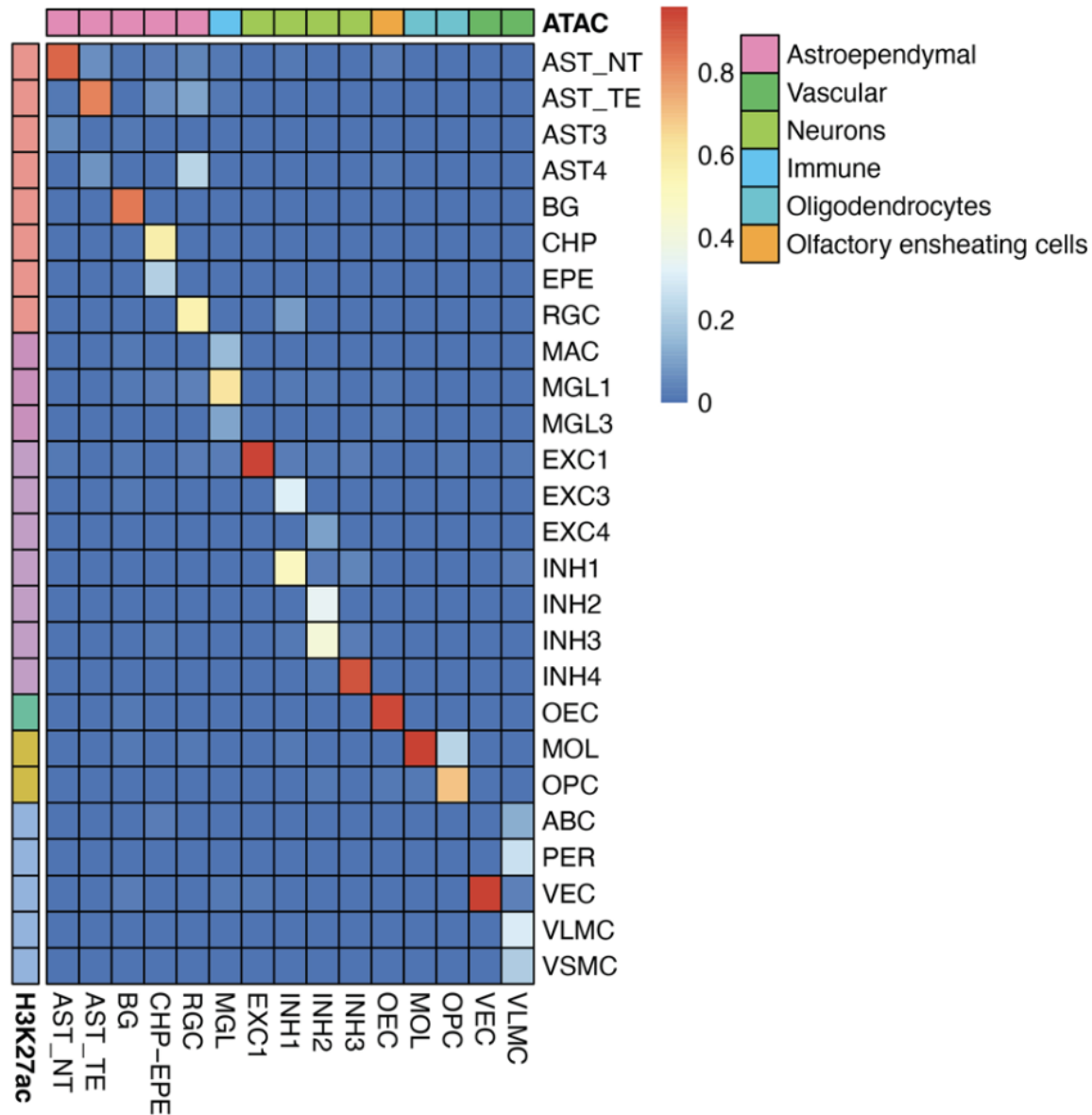
How well do cell identities agree across modalities



- Color represents proportion of cells in a given cluster
- Overall, agreement across major cell types

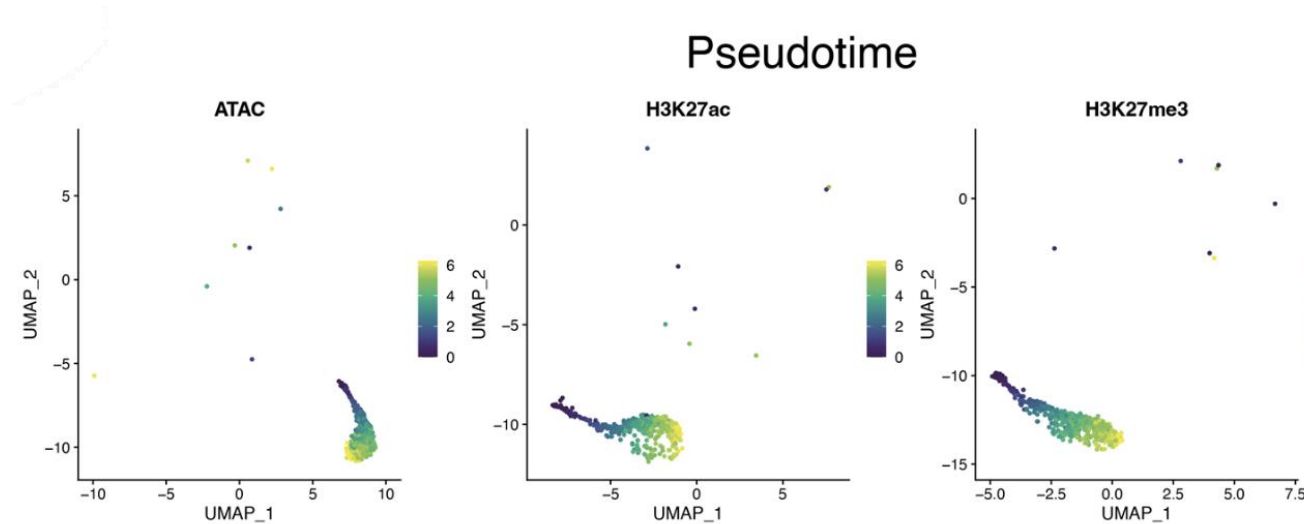


How well do cell identities agree across modalities



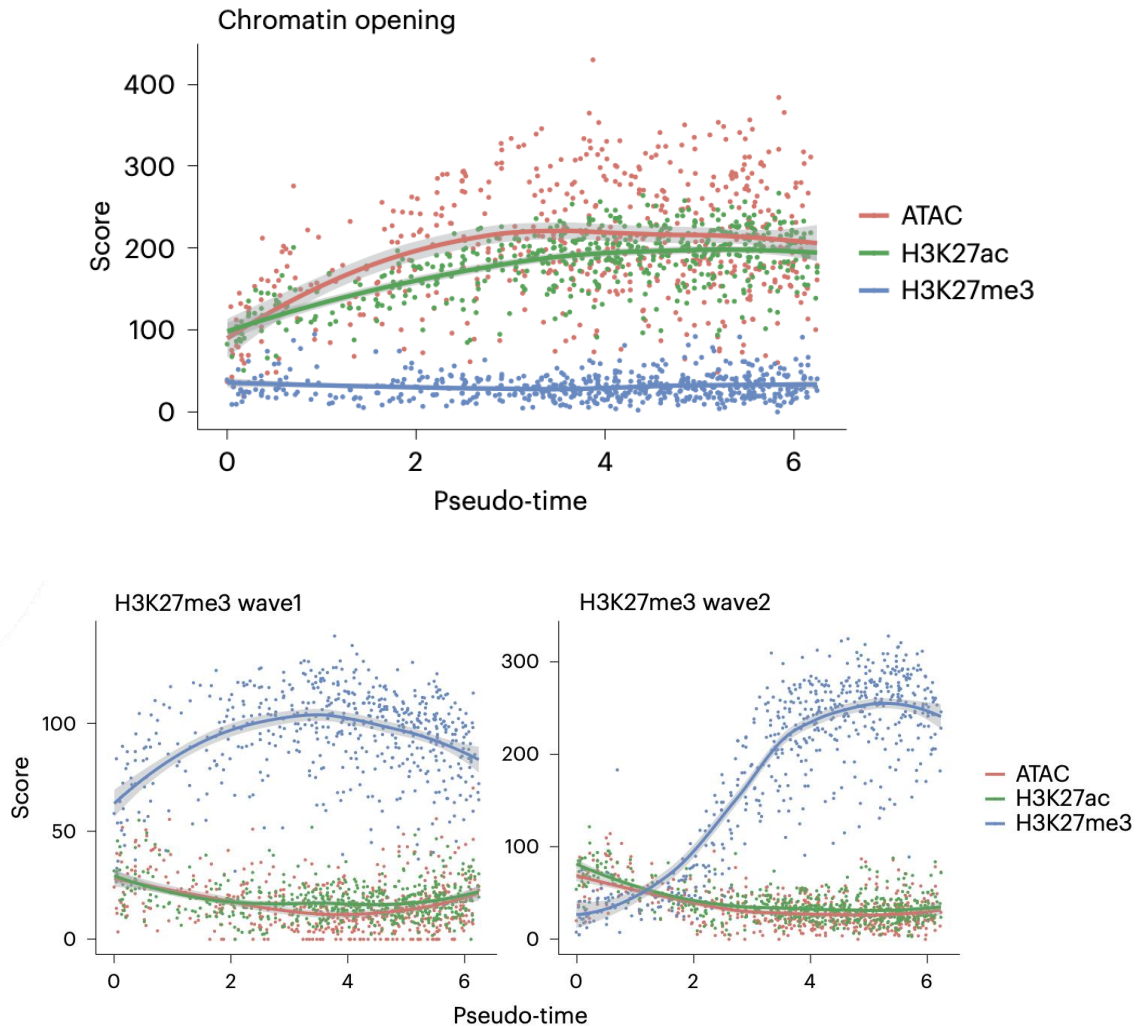
- Color represents proportion of cells in a given cluster
- Specific clusters in certain modalities

Following histone marks across differentiation



- Differentiation from oligodendrocyte progenitor cells towards mature oligodendrocytes
- WNN on joint modalities, and estimated pseudo-time on this using slingshot
- Individual modality trends align with estimated pseud-time

Chromatin dynamics across modalities



- Loci which are opening in the chromatin, acquire H3K27ac with a slight pseudo-time delay, but with overall little change in H3K27me3
- Similar but less prominent trend for loci gaining H3K27ac
- 2 waves of H3K27me3 deposition during oligodendrocyte differentiation:
 - 1: repression of genes expressed in neurons
 - 2: repression of neuronal and progenitor genes

Final Remarks

- Nanobody-Tn5 fusion not commercially available (made by the lab)
- Nice that nuclei can be loaded onto the 10x Genomics chromium chip for library preparation
- Fraction of reads in peaks for sc-nano-CT: is this acceptable noise level?
- Lower fraction of reads in peaks could be due to linear amplification of the tagmented DNA which also ensures that events with single integration are amplified
- Studying different chromatin changes simultaneously over time can show more information/insights that are not possible to find with transcriptomics only
- Future developments: targeting other histone marks and TFs

Overview of epigenomic single cell technologies

	Method	Fusion protein	Number of simultaneous histone modifications	ATAC-seq	Transcriptome	Fragments per cell	Input material	Throughput	Biological sample	single-cell indexing	other features/requirements
Ku et al., 2019	scChIC-seq	pA-MNase	1	No	No	131,000	1,000,000	~100	WBC	Plate-based	
Ku et al., 2021	iscChIC-seq	pA-MNase	1	No	No	9000-40,000	2,000,000	Thousands	WBC	Combinatorial indexing	
Harada et al., 2019	scChIL-seq	pA-Tn5	1	No	No	~15,000	<100	<100	Cell lines	Plate-based	
Wang et al., 2019	CoBATCH	pA-Tn5	1	No	No	~10,000	unknown	Thousands	Endothelial cells	Combinatorial indexing	
Carter et al., 2019	scACT-seq	pA-Tn5	1	No	No	2500	1,000,000	Thousands	Cell lines	Combinatorial indexing	
Rooijers et al., 2019	scDam&T-seq	Dam methylase fusion	1	No	Yes	~40,000	Hundreds	Hundreds	Cell lines	Plate-based	* Requires ectopic expression of Dam fusion proteins
Wu et al., 2021	scCUT&Tag	pA-Tn5	1	No	No	279-3,500	250,000-1,000,000	Thousands	Cell lines, PBMCs, Glioblastoma	Droplet-based (10x)	
Bartosovic et al., 2021	scCUT&Tag	pA-Tn5	1	No	No	98-597	150,000	Thousands	Mouse brain	Droplet-based (10x)	
Zhu et al., 2021	Paired-Tag	pA-Tn5	1	No	Yes	2,000-20,000	3,600,000	Thousands	Mouse brain	Combinatorial indexing	
Bartlett et al., 2021	scTIP-seq	pA-Tn5	1	No	No	unknown	<100	< Hundreds	Cell lines	1 cell per tube	
Bartlett et al., 2021	sciTIP-seq	pA-Tn5	1	No	No	~38,000	>100,000	Thousands	Cell lines	Combinatorial indexing	
Rang et al., 2022	EpiDamID	Dam methylase fusion	1	No	Yes	~45,000	Hundreds	Hundreds	Cell lines, zebrafish embryo	Plate-based	* Requires ectopic expression of Dam fusion proteins
Zhang et al., 2022	scCUT&Tag-pro	pA-Tn5	1	No	No	250-1,000	5,000,000	Thousands	PBMCs	Droplet-based (10x)	* Surface protein expression combined with scCUT&Tag
Gopalan et al., 2022	multi-CUT&Tag	pA-Tn5	3 in bulk, 2 in single-cell)	No	No	~250	200	Thousands	Cell lines	Droplet-based (10x)	
Tadesco et al., 2021	scGET-seq	pA-Tn5	1	Yes	No	unknown	unknown	Thousands	Cell lines, organoids	Droplet-based (10x)	
Stuart et al., 2022	NTT-Seq	nano-Tn5	3	No	No	300-1,100	1,000,000	Thousands	Bone marrow	Droplet-based (10x)	
Bartosovic et al., 2022	nano-CUT&Tag	nano-Tn5	2	Yes	No	1,800-14,000	25,000-200,000	Thousands	Mouse Brain	Droplet-based (10x)	