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

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In the format provided by the authors and unedited

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## Supplementary sequence 1. Anti-mouse nanobody-Tn5 fusion protein sequence

>anti-Mouse-Tn5\_protein\_sequence

MHHHHHS SGVDLGT ENLYFQ SG DYKDHDGDYKDHDIDYKDDDDK GGGG QVQLVESGGGWVQPGGSLR  
LSCAASGFTFSDTAMMWVRQAPGKGREWVAIDTGGGYTYYADSVKGRFTISRDNAKNTLYLQMNSLK  
PEDTARYYCAKTYSGNYYSNYTVANYGTTGRGTLVTVSS GGGGSGGGGSGGGGSGGGGSHMITSALHR  
AADWAKSVFSSAALGDPRTARLVNVAQLAKYSGKSITISSEGSKAMQEGAYRFIRNPVSAEAIK  
AGAMQTVKLAQEFPELLAIEDTTSLSYRHQVAEELGKLSIQDKSRGWVHVSVLLLEATTFRTVGLLH  
QEWWMRPDDPADADEKESGKWLAAAATSRLRMGSMMSNVIAVCDREADIHAYLQDKLAHNERFVVRSK  
HPRKDVESGLYLYDHLKNQPELGGYQISIPQKGVVDKRGKRKNRPARKASLSLRSGRITLKQGNITLN  
AVLAEIINPPKGETPLKWLTLTSEPVESLAQALRVIDIYTHRWRIEFHKAWKTGAGAERQRMEEPDN  
LERMVSILSFVAVRLLQLRESFTPPQALRAQGLLKEAEHVESQSAETVLTLPDECQLLGYLDKGRKRYK  
EKAGSLQWAYMAIARLGGFMDSKRTGIASWGALWEGWEALQSKLDGFLLAAKDLMAQGIKI

6x HIS tag

TEV cleavage site

3xFLAG tag

Anti-mouse nanobody

4x GGGGS linker

Tn5 transposase

>anti-Mouse-Tn5\_DNA\_Sequence

ATGCACCACCACCATCACCATAGTAGCGGAGTCGACTTGGGCACCGAGAATCTGTACTTTCAAAG  
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ACAAGGGCGGTGGTTCACAAGTCCAATTGGTTGAGAGTGGCGGTGGTTGGGTTCAACCGGGTGGT  
TCATTGCGTCTCAGTTGTGCCGCGAGCGGCTTTACATTTTCAGACACCGCCATGATGTGGGTACG  
TCAAGCTCCGGCAAGGGCGGTGAGTGGGTGGCTGCCATCGACACTGGTGGCGGCTATACTTACT  
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TTACTACAGTAATTACACGGTTGCTAACTACGGGACGACCGGTCGTGGAACCTTGGTTACGTTTT  
CAAGCGGTGGTGGTGAAGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGTGGT  
CACATGATTACGTCAGCGTTGCACCGTGTGCGGACTGGGCGAAGAGCGTGTCTCAAGCGCAGC  
ACTTGGTGATCCTCGTCGTACGGCCCGCTGGTGAATGTTGCGGCCCAACTGGCCAAATACAGTG  
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CGTAATCCCAACGTTAGTGCAGGAAGCCATTCGCAAGGCAGGCGCGATGCAGACAGTGAAATTAGC  
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CGGAAGAACTGGGCAAATTAGGGTCAATCCAGGATAAAAAGCCGTGGTTGGTGGGTGCATTCAGTT  
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GCGGCTTTTATGGATTGCAAACGCACCGGCATTGCAAGCTGGGGCGCTCTGTGGGAGGGTTGGGAA  
GCGCTGCAGAGTAAGCTGGATGGTTTTTTTTGGCTGCTAAAGACTTGATGGCTCAGGGAATCAAAAT  
CTAA

## Supplementary sequence 2. Anti-mouse nanobody-Tn5 fusion protein sequence

>anti-Rabbit-Tn5\_protein\_sequence

MHHHHHSSGVDLGTENLYFQSGDYKDHDGDYKDHDIDYKDDDDKGGGSQVQLVESGGGLVQAGDSLRLSCVASGRSLDGATMRWYRQAPGKEREVAGIFWDEIGTEYADTAKGRFTISRDNKNTIYLQMTNLRSEDTAMYCNGLVFGGEYWGQGTQVTVSSGGGSGGGSGGGSGGGSGGGSHMITSALHRAADWAKSVFSSAALGDPRTARLVNVAQLAKYSGKSITISSEGSKAMQEGAYRFIRNPVSAEAIKAGAMQTVKLAQEFPELLAIEDTTSLSYRHQVAEELGKLGSIQDKSRGWVHVSVLLLEATTFRTVGLLHQEWWMRPDDPADADEKESGKWLAAAATSRLRMGSMSNVIAVCDREADIHAYLQDKLAHNERFVVRSKHPRKDVESGLYLYDHLKNQPELGGYQISIPQKGVVDKRGKRKNRPARKASLSLRSGRITLKQGNITLNAVLAEEINPPKGETPLKWLTTSEPVESLAQALRVIDIYTHRWRIEEFHKAWKTGAGAERQRMEEPDLNLERMVSILSFVAVRLLQLRESFTPPQALRAQGLLKEAEHVESQSAETVLTTPDECQLLGYLDKGRKRKEKAGSLQWAYMAIARLGGFMSKRTGIASWGALWEGWEALQSKLDGFLAAKDLMAQGIKI

6x HIS tag

TEV cleavage site

3xFLAG tag

Anti-rabbit nanobody

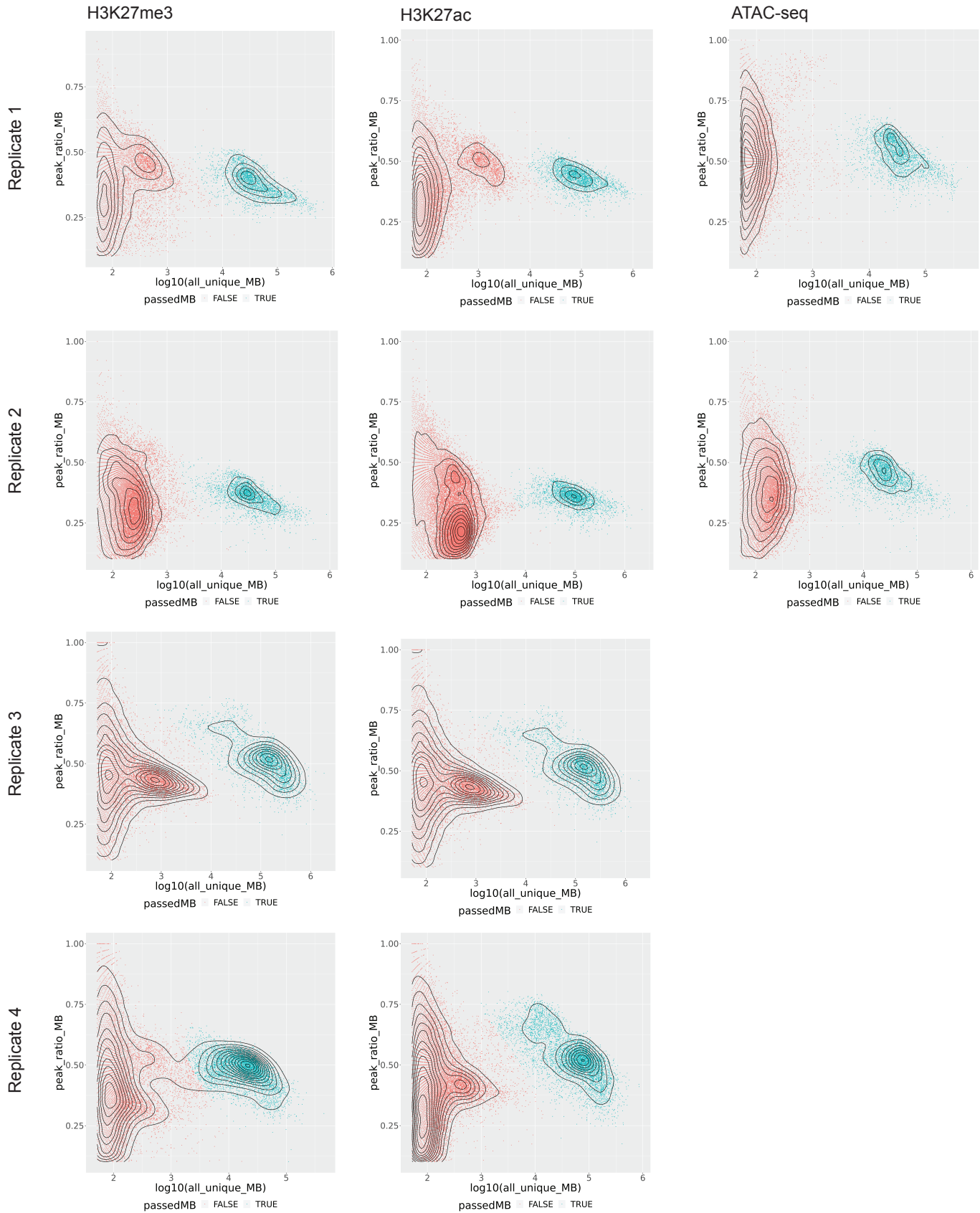
4x GGGGS linker

Tn5 transposase

>anti-Rabbit-Tn5\_DNA\_Sequence

ATGCATCACCACCACCACCATTTCTTCTGGCGTCGACTTGGGTACGGAGAATCTGTATTTTCAATC  
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GCAAAGCCATGCAAGAGGGTGCATCGTTTCATCCGCAATCCGAATGTATCGGCTGAAGCAATT  
CGTAAAGCCGGGGCTATGCAGACCGTGAAACTTGCCCAAGAATTTCTGAACTCCTGGCAATCGA  
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GACGAATGTCAGTTGTTGGGGTATCTGGATAAAGGCAAACGCAAACGGAAAGAGAAAGCCGGCTC  
CCTCCAATGGGCGTACATGGCAATCGCACGCTTGGGCGGCTTCATGGATAGCAAACGTACCGGTA  
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## Supplementary figure 1

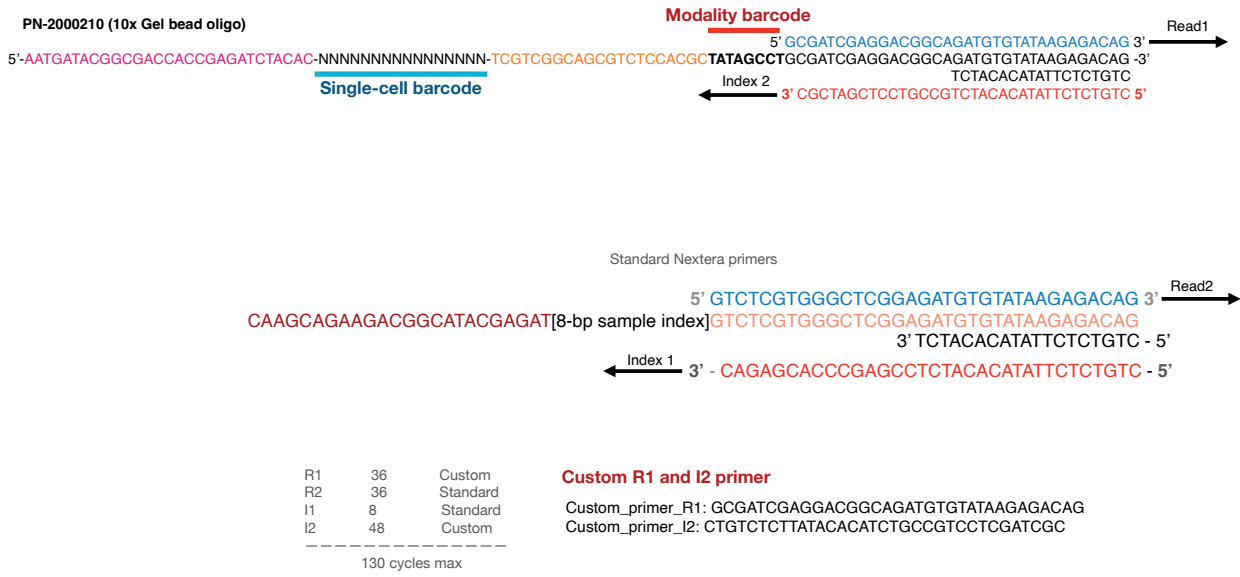


**Supplementary data figure 1. a.** Scatter plot showing number of reads per cell (x-axis) versus fraction of reads in peak regions (y-axis). Gaussian mixed model clustering was used to identify clusters of cells and cluster with the highest median of number of reads was selected as valid cells. Valid cells are labeled with cyan, while cells that do not pass QC are red. Contour lines depict density of points within regions.



# Supplementary figure 2

a



## Supplementary data figure 2

a. Structure of the nano-CT library with highlighted barcode regions.