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A

SBO Adapter ------------P7-PCR-handle----------|---UMI(8N)--|--Sample barcode (18bp)--|------Universal sequence-----|----------CST-------------

5'-CCTTGGCACCCGAGAATTCCANNNNNNNNTGAATTGCGTGATGCACTCTACTCTGCGTTGATACCAGACGCTGCCGACGA-3'

SNuBar-ATAC chemistry

Step 1 Nuclei tagmentation & barcoding (in tube)

```
5-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-----DNA insert--  CTGTCTCTTATACACATCT-3
3-AGCAGCCGTCGCA TCTACACATATTCTCTGTC   ---DNA insert----GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5
               GACCATAGTTGCGTCTCATCTCACGTAGTGCGTTAAGT-N8-ACCTTAAGAGCCCACGGTTCC-5'
```

Step 2 Gap filling (in droplets)

ATAC fragments
```
5-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-----DNA insert----CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-3
3-AGCAGCCGTCGCAGTCTACACATATTCTCTGTC-----DNA insert----GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5
```

Sample barcode fragments
```
5'-CCTTGGCACCCGAGAATTCCA-N8-TGAATTGCGTGATGCACTCTACTCTGCGTTGATACCAGACGCTGCCGACGA-3'
```

Step 3 Single primer linear amplification (in droplets)

Cell barcodes primer
```
5-AATGATACGGCGACCACCGAGATCTACAC-N16-TCGTCGGCAGCGTC-3
```

ATAC
```
5-AATGATACGGCGACCACCGAGATCTACAC-N16-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG--DNA--CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-3
3-TTACTATGCCGCTGGTGGCTCTAGATGTG-N16-AGCAGCCGTCGCAGTCTACACATATTCTCTGTC--DNA--GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5
```

Sample barcode
```
5-AATGATACGGCGACCACCGAGATCTACAC-N16-TCGTCGGCAGCGTCTGGTATCAACGCAGAGTAGAGTGCATCACGCAATTCA-N8-TGGAATTCTCGGGTGCCAAGG-3
3-TTACTATGCCGCTGGTGGCTCTAGATGTG-N16-AGCAGCCGTCGCAGACCATAGTTGCGTCTCATCTCACGTAGTGCGTTAAGT-N8-ACCTTAAGAGCCCACGGTTCC-5
```

Step 4 Emulsion breaking and dual primer exponential PCR amplification (in tube)

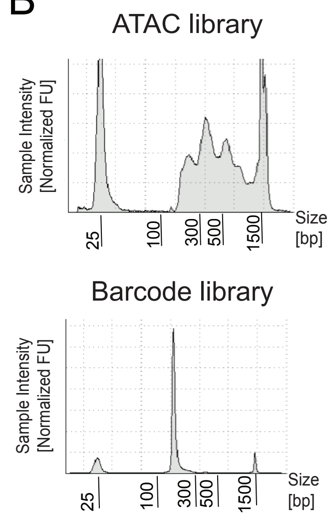
ATAC library primers
```
5-AATGATACGGCGACCACCGAGA-3
5-CAAGCAGAAGACGGCATACGAGAT-I8-GTCTCGTGGGCTCGG-3
```

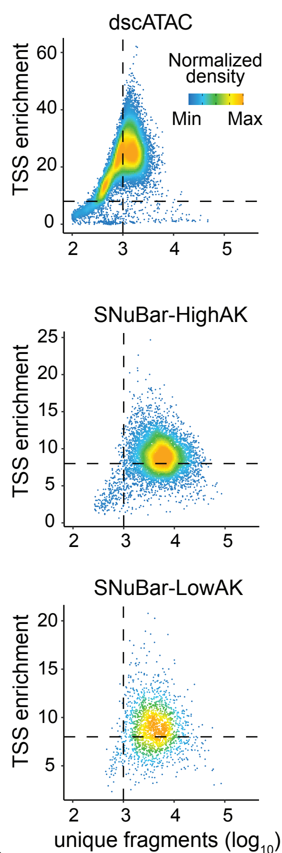
Barcode library primers
```
5-AATGATACGGCGACCACCGAGATCTACAC-3
5-CAAGCAGAAGACGGCATACGAGAT-I6-GTGACTGGAGTTCCTTGGCACCCGAGAATTCCA-3
```

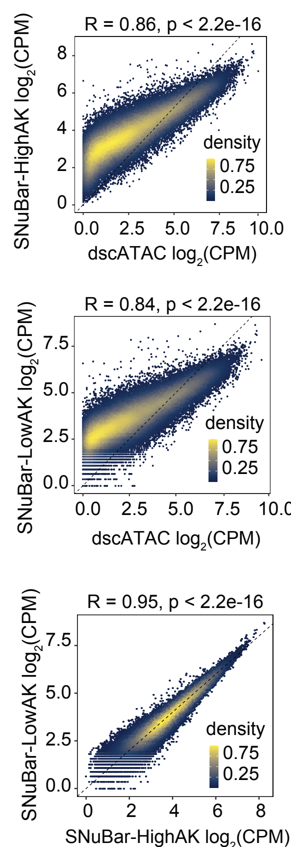
Final ATAC library
```
5-AATGATACGGCGACCACCGAGATCTACAC-N16-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG--DNA--CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-I8-ATCTCGTATGCCGTCTTCTGCTTG-3
3-TTACTATGCCGCTGGTGGCTCTAGATGTG-N16-AGCAGCCGTCGCAGTCTACACATATTCTCTGTC--DNA--GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-I8-TAGAGCATACGGCAGAAGACGAAC-5
```

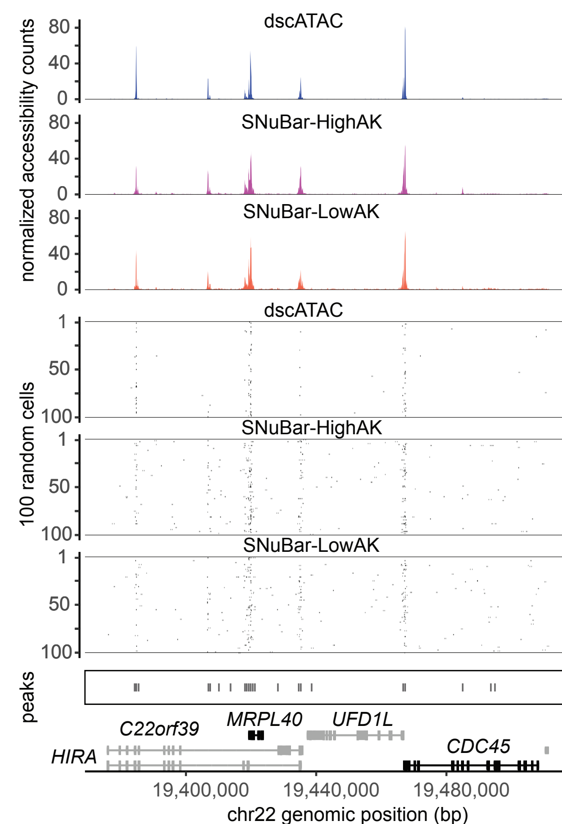
Final barcode library
```
5-AATGATACGGCGACCACCGAGATCTACAC-N16-TCGTCGGCAGCGTCTGGTATCAACGCAGAGTAG
3-TTACTATGCCGCTGGTGGCTCTAGATGTG-N16-AGCAGCCGTCGCAGACCATAGTTGCGTCTCATC
    AGTGCATCACGCAATTCA-N8-TGGAATTCTCGGGTGCCAAGGTTGAGGTCAGTG-I6-ATCTCGTATGCCGTCTTCTGCTTG-3
    TCACGTAGTGCGTTAAGT-N8-ACCTTAAGAGCCCACGGTTCCAACTCCAGTCAC-I6-TAGAGCATACGGCAGAAGACGAAC-5
```

```
-N16-: 16bp Cell Barcode   -I6-: 6bp index
-N8- : 8bp UMI             -I8-: 8bp index
```









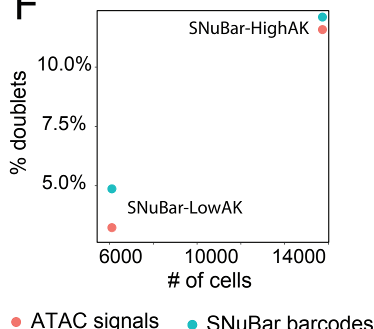
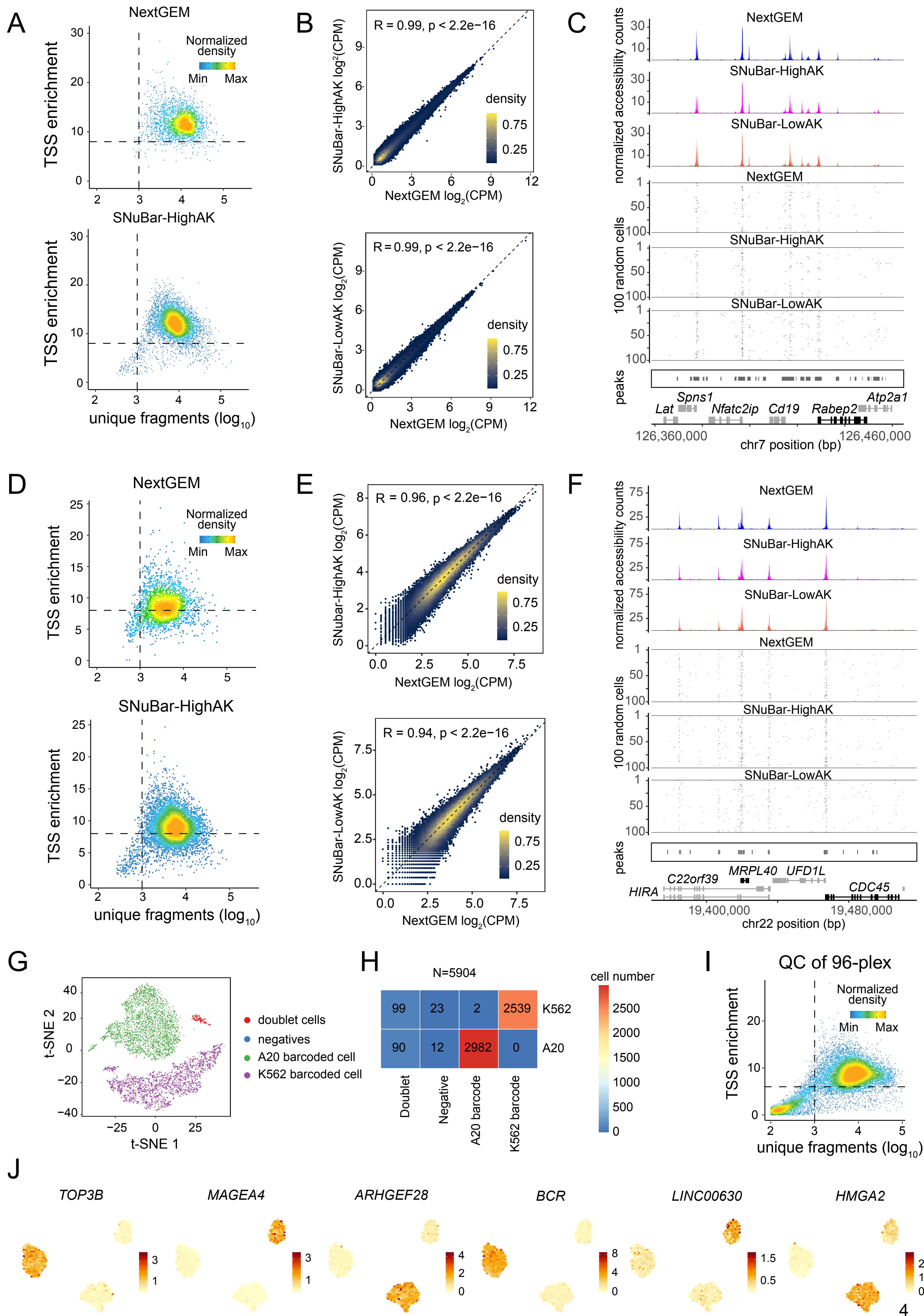
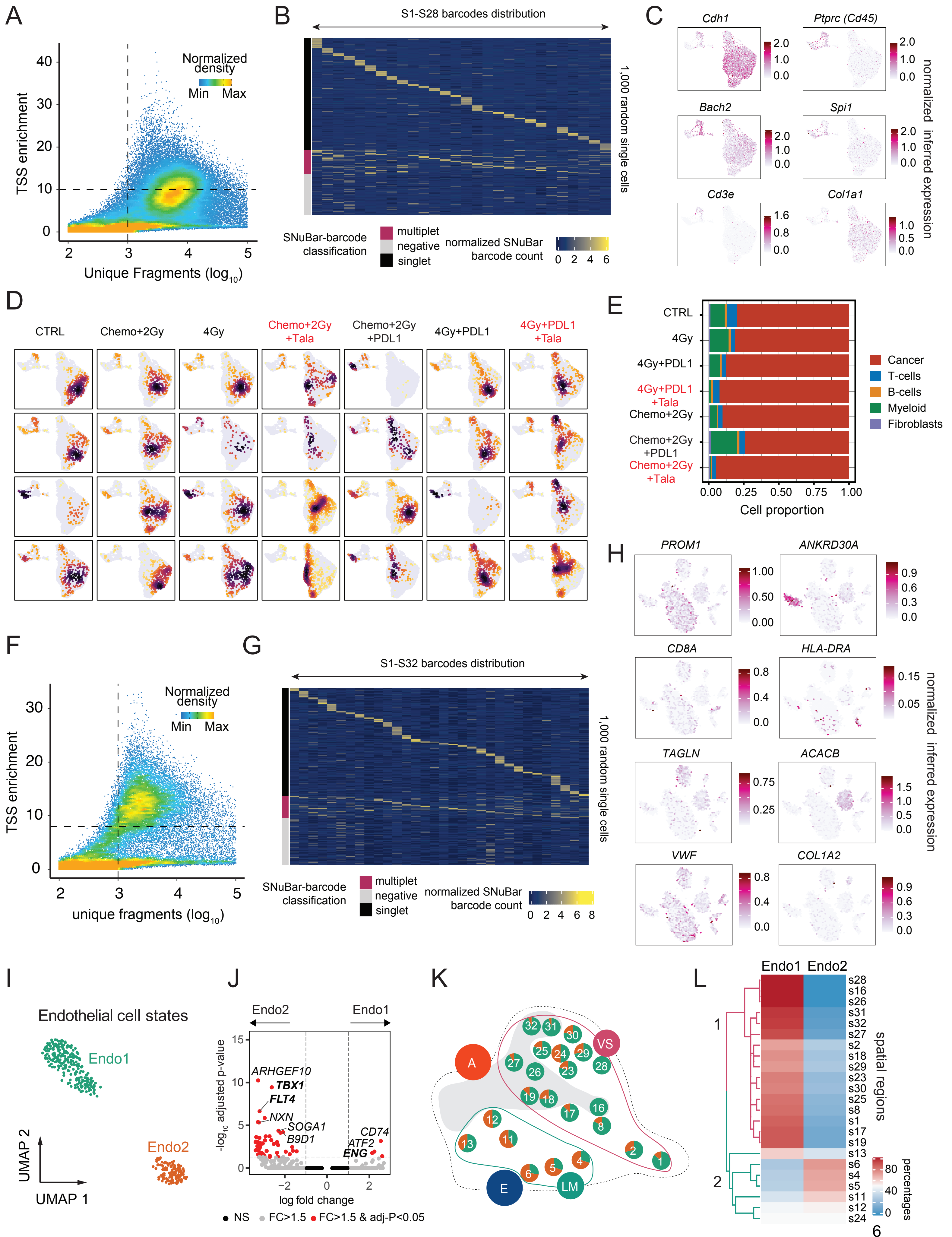


Figure S1. SNuBar-ATAC technical performance (related to Figure 1).
(A) Molecular structure of the SNuBar barcode oligonucleotide (SBO) sequence and the chemistry of the SNuBar-ATAC workflow.
(B) DNA fragment size traces of the ATAC and barcoded libraries for representative SNuBar-ATAC experiments.
(C) Quality control of K562 cells in the dscATAC, SNuBar-HighAK and SNuBar-LowAK data.
(D) Comparison of the aggregated fragment counts within peaks for K562 cells in the dscATAC, SNuBar-HighAK, and SNuBar-LowAK experiments. Pearson's R correlation and p-values are indicated.
(E) Comparison of scATAC profiles of the K562 cells from the dscATAC, SNuBar-HighAK, and SNuBar-LowAK data in a genomic region on chromosome 22. Upper panels show the aggregated fragments of all cells and lower panels show fragment present in 100 random single cells.
(F) Comparison of the doublet rates determined by species-specific ATAC fragments or SNuBar barcode classifications in the SNuBar-HighAK and SNuBar-LowAK experiments.



	Doublet	Negative	A20 barcode	K562 barcode
K562	99	23	2	2539
A20	90	12	2982	0

Figure S2. SNuBar compatibility and scalability (related to Figure 1).
(A) SNuBar was used to barcode A20 and K562 cell lines, followed by nuclei pooling and loading for scATAC-seq using the Chromium NextGEM Single Cell ATAC platform (10X Genomics). Quality control plot of A20 cells in SNuBar-NextGEM (upper panel) and SNuBar-HighAK (lower panel).
(B) Comparison of the aggregated and log-normalized fragments within peaks for A20 cells using the NextGEM platform and standard version (10X Genomics) with Pearson's R correlation and p-values labelled.
(C) Comparison of scATAC profiles of the A20 cells from the NextGEM platform and standard version shown for a region on chromosome 7. Upper panels show aggregated signals of all cells, while lower panels show fragments in each single cell from 100 random cells.
(D) Quality control plot of K562 cells in SNuBar-NextGEM (upper panel) and SNuBar-HighAK (lower panel).
(E) Comparison of the aggregated and log-normalized fragments within peaks for K562 cells using the NextGEM platform and standard version, with Pearson's R correlation and p-values labelled.
(F) Comparison of scATAC profiles of the K562 cells from the NextGEM platform and standard version experiments on a region of chromosome 22.
(G) t-SNE projection of the SNuBar-ATAC profiles colored by singlets (K562 or A20), doublets, or negatives as determined by SNuBar barcode classifications**.**
(H) Heatmap showing the number of singlets and doublets determined by either barnyard analysis (rows) or the SNuBar barcode classification (columns).
(I) Quality control of three mixed cell lines (MDA-MB-231, MDA-MB-436, K562) for a SNuBar-ATAC 96-plex experiment.
(J) Feature UMAP plots of scATAC data shown in **Figure 1H** in which dots (cells) are colored by the normalized inferred RNA expression of cell line specific marker genes.



A
Normalized density
Min Max
TSS enrichment
Unique Fragments (log10)
B
S1-S28 barcodes distribution
1,000 random single cells
SNuBar-barcode classification
multiplet
negative
singlet
normalized SNuBar barcode count
C
Cdh1
Ptprc (Cd45)
Bach2
Spi1
Cd3e
Col1a1
normalized inferred expression
D
CTRL
Chemo+2Gy
4Gy
Chemo+2Gy +Tala
Chemo+2Gy +PDL1
4Gy+PDL1
4Gy+PDL1 +Tala
E
CTRL
4Gy
4Gy+PDL1
4Gy+PDL1 +Tala
Chemo+2Gy
Chemo+2Gy +PDL1
Chemo+2Gy +Tala
Cell proportion
Cancer
T-cells
B-cells
Myeloid
Fibroblasts
F
Normalized density
Min Max
TSS enrichment
unique fragments (log10)
G
S1-S32 barcodes distribution
1,000 random single cells
SNuBar-barcode classification
multiplet
negative
singlet
normalized SNuBar barcode count
H
PROM1
ANKRD30A
CD8A
HLA-DRA
TAGLN
ACACB
VWF
COL1A2
normalized inferred expression
I
Endothelial cell states
Endo1
Endo2
UMAP 2
UMAP 1
J
Endo2
Endo1
-log10 adjusted p-value
ARHGEF10
TBX1
FLT4
NXN
SOGA1
B9D1
CD74
ATF2
ENG
log fold change
NS
FC>1.5
FC>1.5 & adj-P<0.05
K
A
VS
E
LM
L
Endo1 Endo2
spatial regions
percentages

Figure S3. Multiplexing of murine lung cancer treatment samples and human breast tissue spatial regions (related to Figures 2 and 3).
(A) SNuBar-ATAC quality control plots for drug treatment screening experiments, showing the number of unique ATAC-seq fragments in each single droplet compared to the TSS enrichment score of all fragments in that droplet.
(B) Heatmap showing the normalized SBO counts from 1,000 random single cells with SNuBar sample identity classifications from 28 mice.
(C) UMAPs of scATAC data for the murine drug treatment screening experiments showing normalized inferred RNA levels of cell type specific markers.
(D) UMAP projection of scATAC-seq profiles showing cells per sample as kernel densities.
(E) Barplot showing the tumor and microenvironment cell type percentages in each treatment group.
(F) SNuBar-ATAC quality control plots for HBCA spatial SNuBar-ATAC experiment.
(G) Heatmap showing the normalized SBO counts from 1,000 random single cells with SNuBar sample identity classifications of 32 spatial regions.
(H) UMAPs of scATAC data from the human normal breast tissue spatial SNuBar-ATAC experiments, showing the normalized inferred RNA expression of different cell type specific markers across the single cells.
(I) UMAP of the two endothelial cell state clusters.
(J) Volcano plot showing the DAPs between two endothelial cell states.
(K) Pie charts of the endothelial cell states frequencies across the 32 spatial regions of the normal breast tissue.
(L) Clustered heatmap of cell states percentages, showing a dendrogram that is labelled and colored by cluster.

A

CST PCR handle Sample barcode Poly A tail

SBOC oligo 5'-CGAGCCCACGAGACCCTTGGCACCCGAGAATTCCAAGTATGCTCCTTCCGTCCAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3'

Step 1 Nuclei tagmentation & barcoding (in tube)

ATAC + Sample barcode
```
5-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-----DNA insert--  CTGTCTCTTATACACATCT CGAGCCCACGAGACCCTTGGCACCCGAGAATTCCAAGTATGCTCCTTCCGTCC
                 3-TCTACACATATTCTCTGTC  ---DNA insert----GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5
                                                                                                        AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3'
```
RNA
```
5 ————————————————————————AAAAAAAAAAAAAAAAAAAAAAAAAAAAAA-3
```

Step 2 Barcode attaching: Ligation + Reverse Transcription (in droplets)

ATAC
```
5-AATGATACGGCGACCACCGAGATCTACAC-N16-CGCGTCTGTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-----DNA--   CTGTCTCTTATACACATCT-3
                                    3-GCGCAGACAGCAGCCGTCGCA TCTACACATATTCTCTGTC    --DNA-----GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5
```
RNA
```
5-CTACACGACGCTCTTCCGATCT-N16-N12-T30-VN-----cDNA_Insert-----CCCATGTACTCTGCGTTGATACCACTGCTT-3
```
Sample barcode
```
5-CTACACGACGCTCTTCCGATCT-N16-N12-T30-VNGGACGGAAGGAGCATACTTGGAATTCTCGGGTGCCAAGGGTCTCGTGGGCTCGCCCATGTACTCTGCGTTGATACCACTGCTT-3
```

Step 3 Pre-amplification (in tube)

ATAC
```
5-AATGATACGGCGACCACCGAGATCTACAC-N16-CGCGTCTGTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-----DNA-----CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-3
3-TTACTATGCCGCTGGTGGCTCTAGATGTG-N16-GCGCAGACAGCAGCCGTCGCAGTCTACACATATTCTCTGTC-----DNA-----GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-5
```
RNA
```
5-CTACACGACGCTCTTCCGATCT-N16-N12-T30-VN-----cDNA_Insert-----CCCATGTACTCTGCGTTGATACCACTGCTT-3
3-GATGTGCTGCGAGAAGGCTAGA-N16-N12-A30-VN-----cDNA_Insert-----GGGTACATGAGACGCAACTATGGTGACGAA-5
```
Sample barcode
```
5-CTACACGACGCTCTTCCGATCT-N16-N12-T30-VNGGACGGAAGGAGCATACTTGGAATTCTCGGGTGCCAAGG-3
3-GATGTGCTGCGAGAAGGCTAGA-N16-N12-A30-VNCCTGCCTTCCTCGTATGAACCTTAAGAGCCCACGGTTCC-5
```
```
-N16-: 16bp Cell Barcode
-N12-: 12bp UMI
-T30-: 30bp T
-I10-: 10bp library index
```

Step 4 Final library structure (in tube)

Final ATAC library
```
5-AATGATACGGCGACCACCGAGATCTACAC-N16-CGCGTCTGTCGTCGGCAGCGTCAGATGTGTATAAGAGACAG
3-TTACTATGCCGCTGGTGGCTCTAGATGTG-N16-GCGCAGACAGCAGCCGTCGCAGTCTACACATATTCTCTGTC
DNA--CTGTCTCTTATACACATCTCCGAGCCCACGAGAC-I8-ATCTCGTATGCCGTCTTCTGCTTG-3
DNA--GACAGAGAATATGTGTAGAGGCTCGGGTGCTCTG-I8-TAGAGCATACGGCAGAAGACGAAC-5
```
Final RNA library
```
5-AATGATACGGCGACCACCGAGATCT-I10-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-N16-N12-T30-VN
3-TTACTATGCCGCTGGTGGCTCTAGA-I10-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-N16-N12-A30-VN
cDNA--AGATCGGAAGAGCACACGTCTGAACTCCAGTCAC-I10-ATCTCGTATGCCGTCTTCTGCTTG-3
cDNA--TCTAGCCTTCTCGTGTGCAGACTTGAGGTCAGTG-I10-TAGAGCATACGGCAGAAGACGAAC-5
```
Final barcode library
```
5-AATGATACGGCGACCACCGAGATCT-I10-ACACTCTTTCCCTACACGACGCTCTTCCGATCT-N16-N12-T30-VN
3-TTACTATGCCGCTGGTGGCTCTAGA-I10-TGTGAGAAAGGGATGTGCTGCGAGAAGGCTAGA-N16-N12-A30-VN
GGACGGAAGGAGCATACTTGGAATTCTCGGGTGCCAAGGAACTCCAGTCAC-I8-ATCTCGTATGCCGTCTTCTGCTTG-3
CCTGCCTTCCTCGTATGAACCTTAAGAGCCCACGGTTCCTTGAGGTCAGTG-I8-TAGAGCATACGGCAGAAGACGAAC-5
```

B

SNuBarARC-ATAC

TSS enrichment

Normalized density

Min Max

SNuBar-HighAK

TSS enrichment

unique fragments ($\log_{10}$)

C

R=0.97, p<2.2e-16

SNuBar-HighAK $\log_2$(CPM)

density 0.75 0.25

SNuBarARC-ATAC $\log_2$(CPM)

R=0.94, p<2.2e-16

SNuBar-LowAK $\log_2$(CPM)

density 0.75 0.25

SNuBarARC-ATAC $\log_2$(CPM)

D

normalized accessibility counts

SNuBarARC-ATAC

SNuBar-HighAK

SNuBar-LowAK

100 random cells

SNuBarARC-ATAC

SNuBar-HighAK

SNuBar-LowAK

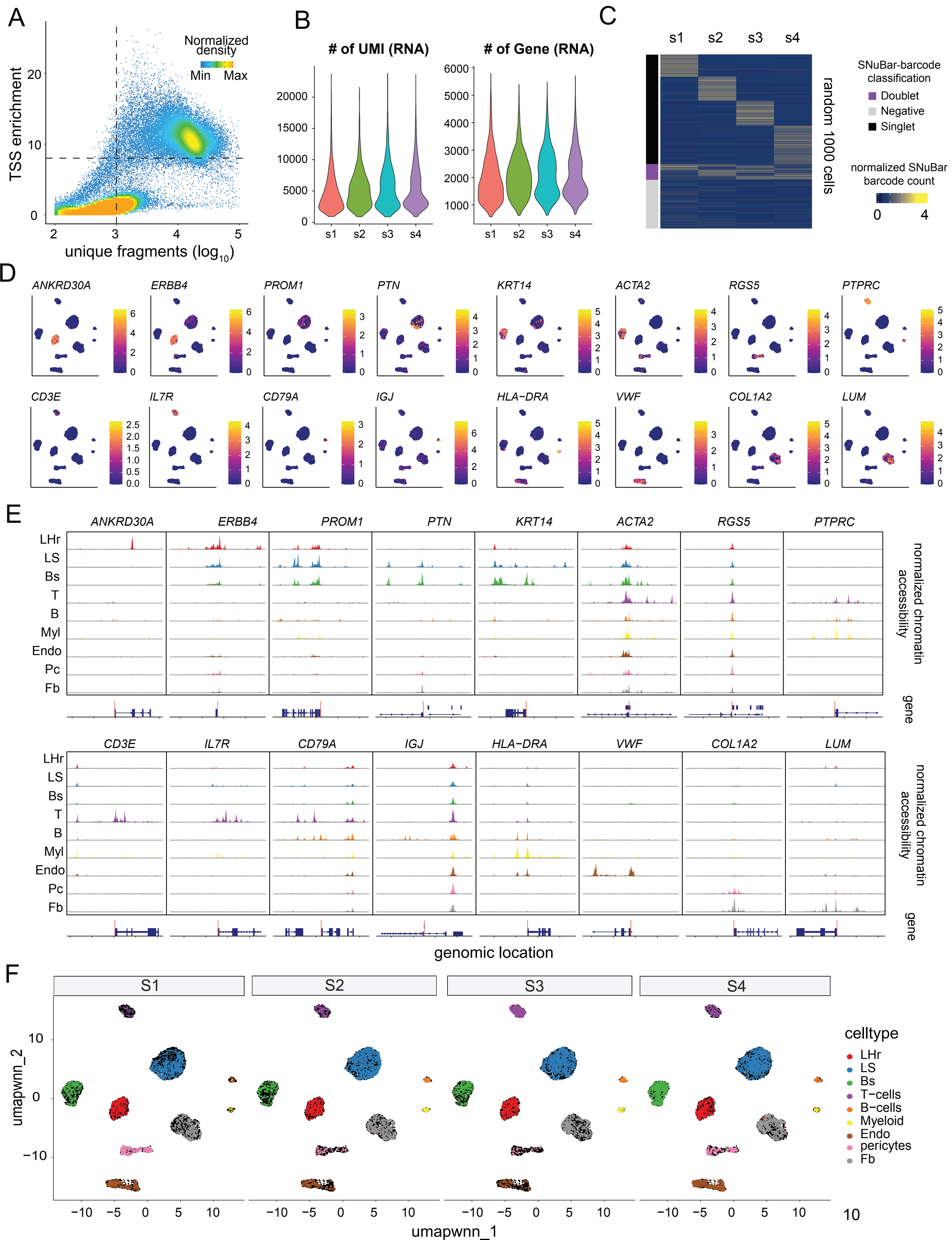
peaks

MRPL40 UFD1L C22orf39 HIRA CDC45

19,400,000 19,480,000

chr22 position (bp)

Figure S4. Technical performance of the SNuBar ATAC&RNA co-assay (related to Figure 4).
(A) Molecular structure of the SNuBar barcode oligonucleotide co-assay (SBOC) sequence and the chemistry of the SNuBar-ARC workflow.
(B) Quality control for ATAC signals from K562 cells using the SNuBar-ARC co-assay and SNuBar-HighAK experiments, showing the number of unique ATAC-seq nuclear fragments in each single cell (dots) compared to the TSS enrichment of all fragments in that cell.
(C) Plots of log-normalized reads in ATAC-seq fragments in K562 cells from the SNuBar-ARC co-assay, SNuBar-HighAK and SNuBar-LowAK experiments, with Pearson's R correlation and p-values labelled.
(D) Comparison of single cell chromatin accessibility profiles of K562 cells from SNuBar-ARC co-assay, SNuBar-HighAK, and SNuBar-LowAK experiments for a region on chromosome 22 with upper panels showing the aggregated profiles of all cells in each dataset, while lower panels show the presence of fragments in each of the 100 random cells from each dataset.



A
B
C
D
E
F
Normalized density
Min
Max
TSS enrichment
unique fragments (log10)
# of UMI (RNA)
# of Gene (RNA)
s1
s2
s3
s4
random 1000 cells
SNuBar-barcode classification
Doublet
Negative
Singlet
normalized SNuBar barcode count
ANKRD30A
ERBB4
PROM1
PTN
KRT14
ACTA2
RGS5
PTPRC
CD3E
IL7R
CD79A
IGJ
HLA−DRA
VWF
COL1A2
LUM
LHr
LS
Bs
T
B
Myl
Endo
Pc
Fb
normalized chromatin accessibility
gene
genomic location
S1
S2
S3
S4
umapwnn_2
umapwnn_1
celltype
T−cells
B−cells
Myeloid
pericytes

Figure S5. Multiplexing of 4 human normal breast tissues using SNuBar-ARC (related to Figure 5).
(A) Quality control plots for chromatin accessibility of SNuBar-ARC in normal breast tissue, showing the number of unique ATAC-seq fragments in each single droplet compared to the TSS enrichment score of all fragments in that droplet.
(B) UMI and gene number per cell for 4 different patients (s1-s4) measured by the RNA assay of SNuBar-ARC.
(C) Heatmap showing the normalized SBO counts from 1,000 random single cells with SNuBar-ARC classifications of 4 different patients.
(D) UMAP projection of canonical expression markers of different cell types.
(E) Gene plots showing the normalized chromatin accessibility counts aggregated by the cell types within TSS-flanking regions of the canonical cell type markers of (D).
(F) Distribution of cells from different patients in high dimensional space.

Table S1

Sample barcode oligonucleotides of SNuBar-ATAC	
Name	Sequences
SNuBarATAC-I5N-bc-1	CCTTGGCACCCGAGAATTCCANNNNNNNNNTCTATCTAGACCTAGATGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-2	CCTTGGCACCCGAGAATTCCANNNNNNNNNGAGGCGTACACTACAACCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-3	CCTTGGCACCCGAGAATTCCANNNNNNNNNGGTAGGGATCAGGACTCCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-4	CCTTGGCACCCGAGAATTCCANNNNNNNNNGACGCATTCCCGTGAACTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-5	CCTTGGCACCCGAGAATTCCANNNNNNNNNCGCTATAGTGACGACGCGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-6	CCTTGGCACCCGAGAATTCCANNNNNNNNNCAACGGATGGGATAGAGCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-7	CCTTGGCACCCGAGAATTCCANNNNNNNNNGCGCCACTCTTTAGGTAGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-8	CCTTGGCACCCGAGAATTCCANNNNNNNNNTGAGGCACTCTGTTGGGACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-9	CCTTGGCACCCGAGAATTCCANNNNNNNNNCATTGGTTCCGTACTCGACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-10	CCTTGGCACCCGAGAATTCCANNNNNNNNNTCATGCACGACGTTAGGGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-11	CCTTGGCACCCGAGAATTCCANNNNNNNNNCTCGAACGTCACTATGACCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-12	CCTTGGCACCCGAGAATTCCANNNNNNNNNTGATTGTTTAATGTCCGTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-13	CCTTGGCACCCGAGAATTCCANNNNNNNNNTCATAAACGCGTCATCCTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-14	CCTTGGCACCCGAGAATTCCANNNNNNNNNGTGTTACTTGAAGTGATGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-15	CCTTGGCACCCGAGAATTCCANNNNNNNNNCTCAGTGTTCGGTATATTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-16	CCTTGGCACCCGAGAATTCCANNNNNNNNNGCGTTAGAAGTAACAGGGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-17	CCTTGGCACCCGAGAATTCCANNNNNNNNNCGAGTACAAGGTACATACCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-18	CCTTGGCACCCGAGAATTCCANNNNNNNNNACCTATCGCCTCGGCACCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-19	CCTTGGCACCCGAGAATTCCANNNNNNNNNTTGCGAACATGGTGCACACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-20	CCTTGGCACCCGAGAATTCCANNNNNNNNNCGATATTTCATCAACCTACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-21	CCTTGGCACCCGAGAATTCCANNNNNNNNNTAGGAGCCGCGTCATTTGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-22	CCTTGGCACCCGAGAATTCCANNNNNNNNNCATACTCGGATGCCGCGTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-23	CCTTGGCACCCGAGAATTCCANNNNNNNNNAGATACCCAGCGCAAGATCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-24	CCTTGGCACCCGAGAATTCCANNNNNNNNNCTTTAATTAGAGGCATCGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-25	CCTTGGCACCCGAGAATTCCANNNNNNNNNGTCCGCGGCGCTCAGATGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-26	CCTTGGCACCCGAGAATTCCANNNNNNNNNCACCGTGCTAGAAGTCAACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-27	CCTTGGCACCCGAGAATTCCANNNNNNNNNCGTGTCCATAGAAGCTGTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-28	CCTTGGCACCCGAGAATTCCANNNNNNNNNGAGCCTCAAAGTGATGGGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-29	CCTTGGCACCCGAGAATTCCANNNNNNNNNCAGAATTCACCTCCAGTACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-30	CCTTGGCACCCGAGAATTCCANNNNNNNNNTCTAGCGTTAGAATGCGACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-31	CCTTGGCACCCGAGAATTCCANNNNNNNNNTCCACGCGTAGGACTAGTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-32	CCTTGGCACCCGAGAATTCCANNNNNNNNNGCCCACTTTCGCCGTAGTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-33	CCTTGGCACCCGAGAATTCCANNNNNNNNNTGAAACTCCTGTCTCGCGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-34	CCTTGGCACCCGAGAATTCCANNNNNNNNNTGACGCTCGTCTATAGAGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-35	CCTTGGCACCCGAGAATTCCANNNNNNNNNATGGGCTGAATGTCACACCTACTCTGCGTTGATACCAGACGCTGCCGACGA

SNuBarATAC-I5N-bc-36	CCTTGGCACCCGAGAATTCCANNNNNNNNNTGGCCGCGAATCCTGCACCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-37	CCTTGGCACCCGAGAATTCCANNNNNNNNNACCCGGCGTAACTAGATACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-38	CCTTGGCACCCGAGAATTCCANNNNNNNNNAGATGCTGTGAGACCGCACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-39	CCTTGGCACCCGAGAATTCCANNNNNNNNNCCATAGTTAATGCGCTTCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-40	CCTTGGCACCCGAGAATTCCANNNNNNNNNCAGCGTTGGTGAAGCCGGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-41	CCTTGGCACCCGAGAATTCCANNNNNNNNNCCGTGCGCGTAAGTCGATCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-42	CCTTGGCACCCGAGAATTCCANNNNNNNNNGGGTCACTTGCGTCGTTGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-43	CCTTGGCACCCGAGAATTCCANNNNNNNNNTAACGTTTAGGATGGGCGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-44	CCTTGGCACCCGAGAATTCCANNNNNNNNNCCGCATAGAGCTGAGTAACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-45	CCTTGGCACCCGAGAATTCCANNNNNNNNNCATCCACGTGAATGCTAACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-46	CCTTGGCACCCGAGAATTCCANNNNNNNNNAGGTCTAACCGTTCGTACCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-47	CCTTGGCACCCGAGAATTCCANNNNNNNNNACATAGACGCCGTCTCGGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-48	CCTTGGCACCCGAGAATTCCANNNNNNNNNCGACTAAGTATGTAGCGCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-49	CCTTGGCACCCGAGAATTCCANNNNNNNNNAGCAACTTTGAGCAAGATCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-50	CCTTGGCACCCGAGAATTCCANNNNNNNNNCATCATCTTACAAGAAAGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-51	CCTTGGCACCCGAGAATTCCANNNNNNNNNACGTCGTTATGTGTTACCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-52	CCTTGGCACCCGAGAATTCCANNNNNNNNNAGGCTAATTAGCACGCGACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-53	CCTTGGCACCCGAGAATTCCANNNNNNNNNGGTACGAGCTAATTCGCACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-54	CCTTGGCACCCGAGAATTCCANNNNNNNNNGTATTGGTGCAGCCTGCTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-55	CCTTGGCACCCGAGAATTCCANNNNNNNNNAGGTGTGCCGCGTCTCTACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-56	CCTTGGCACCCGAGAATTCCANNNNNNNNNGGCCATACTAATGTGTGGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-57	CCTTGGCACCCGAGAATTCCANNNNNNNNNCCGGATACGTAGTCCTCACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-58	CCTTGGCACCCGAGAATTCCANNNNNNNNNACAAGAATTGACAAGATCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-59	CCTTGGCACCCGAGAATTCCANNNNNNNNNTCGCATGTTAACCCGACGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-60	CCTTGGCACCCGAGAATTCCANNNNNNNNNCTAGCGGCGTTGAGCGTTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-61	CCTTGGCACCCGAGAATTCCANNNNNNNNNCCTCACGCCGGACGAGTTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-62	CCTTGGCACCCGAGAATTCCANNNNNNNNNGCGATTACCTATGATTTGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-63	CCTTGGCACCCGAGAATTCCANNNNNNNNNCTGTGAGTTTAATACGCCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-64	CCTTGGCACCCGAGAATTCCANNNNNNNNNCATACGCTATCTCCGCCCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-65	CCTTGGCACCCGAGAATTCCANNNNNNNNNCCCGGGTACTTTCGCGAACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-66	CCTTGGCACCCGAGAATTCCANNNNNNNNNTCCATCTCTGGGTGCGACCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-67	CCTTGGCACCCGAGAATTCCANNNNNNNNNGATTTCAGGCACTACGCGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-68	CCTTGGCACCCGAGAATTCCANNNNNNNNNGTACGTGAAAGGGAGACTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-69	CCTTGGCACCCGAGAATTCCANNNNNNNNNTTGGAGTCAACATGTGCCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-70	CCTTGGCACCCGAGAATTCCANNNNNNNNNTTGACCACACTATACTTCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-71	CCTTGGCACCCGAGAATTCCANNNNNNNNNGACCTCAGATCCCTGGACCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-72	CCTTGGCACCCGAGAATTCCANNNNNNNNNCAGTGCTCTTATGTCGTTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-73	CCTTGGCACCCGAGAATTCCANNNNNNNNNTGCTTATCGGGACCAGAACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-74	CCTTGGCACCCGAGAATTCCANNNNNNNNNACAAAGAGACGTTAATAGCTACTCTGCGTTGATACCAGACGCTGCCGACGA

SNuBarATAC-I5N-bc-75	CCTTGGCACCCGAGAATTCCANNNNNNNNCCTGCGTACAAGGCTACACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-76	CCTTGGCACCCGAGAATTCCANNNNNNNNTTGCTAAATCTAACCGCCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-77	CCTTGGCACCCGAGAATTCCANNNNNNNNTCCGGAGCGAACACGATTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-78	CCTTGGCACCCGAGAATTCCANNNNNNNNTCGGCGCGTTATGTCTAACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-79	CCTTGGCACCCGAGAATTCCANNNNNNNNGAAACTGCATCCTCGACTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-80	CCTTGGCACCCGAGAATTCCANNNNNNNNTGGTCGCTAGTACGTACCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-81	CCTTGGCACCCGAGAATTCCANNNNNNNNACGCGGGTCCTGAGGTCACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-82	CCTTGGCACCCGAGAATTCCANNNNNNNNTCGATATGGTGATGCCTACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-83	CCTTGGCACCCGAGAATTCCANNNNNNNNTAATGTACGCGCTTCGACCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-84	CCTTGGCACCCGAGAATTCCANNNNNNNNATGAGGTGGGCGCCCAATCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-85	CCTTGGCACCCGAGAATTCCANNNNNNNNCTGGTTGACACTTCTTCCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-86	CCTTGGCACCCGAGAATTCCANNNNNNNNACCAATCTCTAATCTCGTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-87	CCTTGGCACCCGAGAATTCCANNNNNNNNGGTCTCTTAGCCGCGCTACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-88	CCTTGGCACCCGAGAATTCCANNNNNNNNGGATTTAGCCGGCCCTCGCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-89	CCTTGGCACCCGAGAATTCCANNNNNNNNCGCCAGCCCTGGTCCGATCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-90	CCTTGGCACCCGAGAATTCCANNNNNNNNACCCGTTTCGCCAGGCATCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-91	CCTTGGCACCCGAGAATTCCANNNNNNNNTAACTGCGTTACGCGATTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-92	CCTTGGCACCCGAGAATTCCANNNNNNNNAGTCATAGGGAGCCGCCTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-93	CCTTGGCACCCGAGAATTCCANNNNNNNNATATCGAAGTTTGGGTTTCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-94	CCTTGGCACCCGAGAATTCCANNNNNNNNCCGTTCGAGCGTTCGCTACTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-95	CCTTGGCACCCGAGAATTCCANNNNNNNNTTTCAACCCGAGGAAGTCCTACTCTGCGTTGATACCAGACGCTGCCGACGA
SNuBarATAC-I5N-bc-96	CCTTGGCACCCGAGAATTCCANNNNNNNNTCTCCCTATAGACTCTGTCTACTCTGCGTTGATACCAGACGCTGCCGACGA

Table S1. SNuBar-ATAC barcodes for 96-plex experiments (related to STAR Methods). 96 sample barcodes used for 96-plex SNuBar-ATAC experiments. Each barcode consists of a 21 bp P7-PCR handle, 8bp unique molecular identifier (UMI), 18bp sample barcode, 19bp universal sequence, and 14bp complementary sequence of the transposome universal tail (CST).

Table S2

	Load #*	Estimated number of cells (hg19/mm10)	Pass QC #	Median fragments per cell (hg19/mm10)	Fraction of fragments overlapping any targeted region (hg19/mm10)	Total number of read pairs	Fraction of read pairs with a valid barcode	Q30 bases	Fraction of total read pairs mapped confidently to genome (>30 mapq)
SNuBar HighAK	21,420	9,005/8,301	10,810	6,250/8,882	77.2%/65.7%	376,835,243	97.60%	94.63%	63.80%
SNuBar LowAK	4,000	2,038/4,078	4,598	4,222/13,179	78.6%/67.4%	189,635,626	98.10%	94.60%	73.40%
SNuBar-96plex	17,160	11,674	10,922	7,259	85.20%	354,762,107	98.30%	95.48%	79.70%
SNuBar-mouse1	24,480	14,094	5,900	5,834	56.90%	497,553,244	98.20%	94.60%	68.60%
SNuBar-mouse2	24,480	14,609	6,040	5,657	57.40%	519,951,301	97.90%	94.30%	71.20%
SNuBar-mouse3	24,480	13,310	5,733	5,732	56.80%	470,126,740	96.60%	93.48%	68.60%
SNuBar-NextGem	12,000	2,821/3,242	4,503	3,572/10,618	79.3%/66.5%	232,235,816	97.30%	95.40%	65.10%
SNuBar-HBCA1	12,800	10,681	8,096	2,914	70.80%	639,812,415	97.20%	93.40%	80.70%
SNuBar-HBCA2	8,000	6,687	5,012	2,295	71.90%	299,369,771	97.40%	93.73%	81.00%
SNuBar-CoAssay-ATAC	17,000	5,123/5,411	9,128	15,737/21,276	82.0%/70.0%	497,226,584	98.40%	94.23%	82.80%
SNuBar-CoAssay-HBCA-ATAC	40,000	24,373	10,331	13,139	83.90%	1,022,880,813	98.00%	94.80%	84.40%

*: the number counted by Countess^{TM} II (Applied Biosystem), this number is a rough estimation of loading amount, probably not accurate.

Table S2. Basic quality control metrics for SNuBar (related to Figures 1-5).

Methods S1. Step by step protocols for SNuBar-ATAC and SNuBar-ARC (related to Figures 1 and 4)

SNuBar-ATAC

1. Prepare reagent

a. Wash Buffer: 10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.1% Tween-20, and 1% BSA
b. Diluted Nuclei Buffer: dilute the 10X nuclei buffer (PN-2000153, 10X Genomics) to 1X
c. Lysis buffer for cell suspension (1X lysis buffer): 10mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.1% Tween-20, 0.1% Nonidet P40 Substitute, 0.01% Digitonin, and 1% BSA
d. Lysis buffer for tissue (0.1X lysis buffer): 10mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.01% Tween-20, 0.01% Nonidet P40 Substitute, 0.001% Digitonin, and 1% BSA

2a. Nuclei Isolation from cell suspension according to the Nuclei Isolation for Single Cell ATAC Sequencing Protocol (CG000169 Rev D, 10X Genomics)

a. Add 500k-1M cells of each sample to different microcentrifuge tubes. Centrifuge at 300 rcf for 5 min at 4°C. Remove ALL the supernatant without disrupting the cell pellet.

For each tube:

b. Add 100 μl chilled 1X Lysis Buffer. Pipette mix 10x.
c. Incubate for 5 min on ice. (Incubation time may vary by tissue.)
d. Add 1 ml chilled Wash Buffer to the lysed cells. Pipette mix 5x.
e. Centrifuge at 500 rcf for 5 min at 4°C. Remove the supernatant without disrupting the nuclei pellet.
f. Resuspend the cells in 50 μl PBS.
g. Determine the nuclei concentration using a Countess II FL Automated Cell Counter.

2b. Nuclei isolation from tissues according to the Nuclei Isolation from Mouse Brain Tissue for Single Cell ATAC Sequencing Protocol (CG000212 Rev B, 10X Genomics)

a. Cut tissues into small pieces and transfer each piece to a 1.5 ml microcentrifuge tube.
b. Add 500 μl chilled 0.1X lysis buffer and homogenize tissues 15X using a pellet pestle. After 5 min incubation on ice, pipette mix 10X and incubate for an additional 10 min.
c. Add 500 μl chilled Wash Buffer to the lysed cells and pipette mix 5X.
d. Pass through 20 μm filter (pluriStrainer Mini) and centrifuge at 500 rcf for 5 min at 4°C. Remove supernatant without disrupting the nuclei pellet. Resuspended nuclei in 50 μl chilled PBS.
e. Determine the nuclei concentration using a Countess II FL Automated Cell Counter.

3. Tagmentation

a. Prepare PBS+1% BSA, PBS+0.5% BSA. Coat each 1.5ml tube with PBS+0.5% BSA, make sure to remove the coating reagent thoroughly after coating.
b. 35K cells were used for each sample. Add the following reagent in the coated tubes:

2X TD buffer	25 μl
0.5% digitonin	1 μl
Cells in PBS	14 μl
H_2O	5 μl
Tn5	5 μl
Total	**50 μl**

Condition: 37 °C, 850 rpm (15s mix and 15s pause).
Note: After 30-35 min tagmentation, add 1 µl 2 uM different SNuBar barcodes (SBOs) in each tube and perform tagmentation for another 30-35 min.

4. Wash and buffer exchange:

For each tube:

a. Add 500 µl chilled PBS+1% BSA and centrifuge at 500g for 5min at 4°C.
b. Remove supernatant. Then add 1mL chilled PBS+1% BSA (or 1mL diluted nuclei buffer) slowly (do not resuspend to allow buffer exchange), incubate on ice for 5-10 min, and centrifuge at 500g for 5min at 4°C. Remove the supernatant. Resuspend the nuclei with ~20 µl PBS+1% BSA (or diluted nuclei buffer).
c. Mix all cells from different tubes together. Centrifuge at 500g for 5min at 4°C. Remove supernatant (10~50 µl leftover).
d. Repeat b using diluted nuclei buffer for buffer exchange and sample resuspension.
a. Count nuclei number using a Countess II FL Automated Cell Counter. Nuclei concentration was adjusted using Diluted Nuclei Buffer to the desired capture number.
e. Load 8 µl nuclei (in diluted nuclei buffer) and 7 µl ATAC buffer for GEM generation.

Note: if sample number is less than 4, we recommend to duplicate each sample to get enough cells for GEM generation step.

5. GEM Generation & Barcoding (Follow 10X Genomics protocol, CG000168 Rev A for Chromium Single Cell ATAC Reagent Kits, or CG000209 Rev D for Chromium Next GEM Single Cell ATAC Library Kit v1.1. CG000168 protocol was used as example below)

a. Prepare Master Mix [61.5 µl Barcoding Reagent (PN: 2000124); 1.5 µl Reducing Agent B (PN: 2000087); 2 µl Barcoding Enzyme (PN: 2000125/2000139)].
b. Add 65 µl Master Mix to each tube containing Transposed Nuclei for a total of 80 µl in each tube.
c. Dispense 75 µl Master Mix + Transposed Nuclei, 40 µl Gel Beads and 240 µl Partition Oil into Chromium Chip E in a 10x Genomics Chip Holder.
d. Run the Chromium Controller and transfer GEMs into PCR tubes.
e. Perform PCR reaction:

72 °C	5min
98 °C	30s
98 °C	10s
59 °C	30s
72 °C	1min
	Go to step3, repeat 11X
15 °C	hold

6. Post GEM Incubation Cleanup & QC

a. Add 125 µl Recovery Agent (PN: 220016) to each sample and gently invert tube 10x to mix well. Then centrifuge briefly and slowly remove 125 µl Recovery Agent/Partitioning Oil from the biphasic mixture.
b. Vortex and add 200 µl Dynabeads Cleanup Mix [182 µl Cleanup Buffer (PN:2000088); 8 µl Dynabeads MyOne SILANE (PN: 2000048); 5 µl Reducing Agent B (PN:2000087); 5 µl Nuclease-free Water] to each sample.

c. Incubate 10min, and wash with 80% ethanol twice, then elute with 40.5 µl Elution Solution I (100 µl recipe: 98 µl Buffer EB, 1 µl 10% Tween 20 and 1 µl Reducing Agent B).
d. Post GEM Incubation Cleanup using SPRIselect reagent and separate the ATAC and barcode libraries. In detail:
   (1) Vortex the SPRIselect reagent until fully resuspended. Add 48 µl (1.2X) SPRIselect reagent. vortex mix thoroughly. Incubate 5 min at room temperature.
   (2) Centrifuge briefly. Place on the magnet•High until the solution clears.
   (3) Move the supernatant to another tube.

- For the ATAC library:
   (4) Add 200 µl 80% ethanol to the beads. Wait 30 sec. Remove the ethanol. Repeat this step one more time.
   (5) Centrifuge. Place on the magnet•Low. Remove any remaining ethanol.
   (6) Immediately add 40.5 µl Buffer EB. Vortex and incubate 2min at room temperature.
   (7) Centrifuge briefly. Place on the magnet•Low. Transfer 40 µl sample to a new tube.
- For the barcode library:
   (8) Add another 24 µl (1.8X) Amp beads to the supernatant of step (3). Pipette or vortex mix thoroughly. Incubate 5 min at room temperature.
   (9) Wash twice with 200 µl 80% ethanol to the beads. Wait 30 sec. Centrifuge briefly. Place on the magnet•Low. Remove ethanol.
   (10) Immediately add 20.5 µl H_2O. Vortex and incubate 2min at room temperature.
   (11) Centrifuge briefly. Place on magnet•Low. Transfer 20 µl barcode library to a new tube.

7. Library Construction

(1) ATAC library construction (Sample Index PCR) – Follow 10X Genomics protocol

a. Add 57.5 µl Sample Index PCR Mix [50 µl Amp Mix (PN: 2000047/2000103); 7.5 µl SI-PCR Primer B (PN: 2000128)] and 2.5 µl an individual Chromium i7 Sample Index N, Set A to 40 µl sample.
b. Incubate in a thermal cycler.

98 °C	45s
98 °C	20s
67 °C	30s
72 °C	20s
	Go to step2, repeat 9-12X
72 °C	1min
4 °C	hold

(2) Barcode library construction:

Add the following reagent in an 8-tube strip:

2X KAPA HiFi HotStart ReadyMix	25 µl
Product of 6.b.(11)	10 µl
10 µM TruSeq RPIX primer(5'-CAAGCAGAAGACGGCATACGAGATNNNNNNGTGACTGGAGTTCCTTGGCACCCGAGAATTCCA-3')	1.5 µl
10 µM P5 Adaptor (5'-AATGATACGGCGACCACCGAGATCTACAC-3')	1.5 µl
H_2O	12 µl

Total	**50 µl**

PCR condition:

98 °C	30s
98 °C	15s
60 °C	30s
72 °C	30s
	Go to step2, 8-10 PCR cycles
72 °C	1min
4 °C	hold

8. Post Sample Index Double Sided Size Selection – SPRIselect

For **ATAC** library, follow 10X Genomics protocol to do 0.4X and 1.2X double-sided selection. We recommend to do one more time 1.2X SPRIselect reagent purification to further enrich the target library.

9. Post PCR Selection of barcode library – SPRIselect

a. Vortex the SPRIselect reagent until fully resuspended. Add 75 µl (1.5X) SPRIselect reagent. Vortex mix thoroughly. Incubate 5 min at room temperature.
b. Centrifuge briefly. Place on the magnet•High until the solution clears.
c. Remove the supernatant. Add 200 µl 80% ethanol to the beads. Wait 30 sec. Remove the ethanol. Repeat once.
d. Centrifuge. Place on the magnet•Low. Remove any remaining ethanol.
e. Add 30.5 µl H_2O. Vortex and incubate 2min at room temperature.
f. Centrifuge briefly. Place on the magnet•Low. Transfer 30 µl sample to a new tube.

10. Final ATAC and barcode libraries were mixed at a ratio of 4:1 ~ 20:1 and sequenced on the Illumina NextSeq instrument.

Custom sequencing primers: Read 1 (5'- TCGTCGGCAGCGTCTGGTATCAACGCAGAGTAG-3') and I5 Index Primer (5'-CTACTCTGCGTTGATACCAGACGCTGCCGACGA-3') are spiked in to the sequencer for sequencing the barcode library. Read 1: 50bp, Read 2: 50bp, Index 1: 8bp, Index 2: 16bp.

SNuBar-ARC

1. Prepare reagent

a. Wash Buffer: 10 mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.1% Tween-20, 1% BSA, 1 mM DTT, and 1U/µl RNase Inhibitor
b. Lysis buffer: 10mM Tris-HCl pH 7.4, 10 mM NaCl, 3 mM $MgCl_2$, 0.1% Tween-20, 0.1% Nonidet P40 Substitute, 0.01% Digitonin, 1% BSA, 1 mM DTT, and 1U/µl RNase inhibitor
c. Diluted Nuclei Buffer: 1X Nuclei Buffer (PN-2000207, 10X Genomics), 1 mM DTT, and 1U/µl RNase inhibitor
d. Diluted Nuclei Suspension Buffer: 1X Nuclei Buffer (PN-2000207, 10X Genomics), 0.625 mM DTT, and 1 U/µl RNase Inhibitor

2. Nuclei Isolation according to the Nuclei Isolation for Single Cell Multiome ATAC + Gene Expression Sequencing Protocol (10X Genomics, CG000365 Rev A)

a. Add 500k-1M cells of each cell suspension to a 2 ml microcentrifuge tube. Centrifuge at 300 rcf for 5 min at 4°C. Remove ALL the supernatant without disrupting the cell pellet.

For each tube:

b. Add 100 µl chilled Lysis Buffer. Pipette mix 10x.
c. Incubate for 5 min on ice. (Since cell lines were used for this assay.)
d. Add 1 ml chilled Wash Buffer to the lysed cells. Pipette mix 5x.
e. Centrifuge at 500 rcf for 5 min at 4°C. Remove the supernatant without disrupting the nuclei pellet.
f. Repeat steps d-e two more times for a total of 3 washes.
g. Based on cell concentration step 2.a and assuming ~50% nuclei loss during cell lysis, resuspend in 20 µl Diluted Nuclei Buffer.
h. Determine the nuclei concentration using a Countess II FL Automated Cell Counter.

3a. Tagmentation using 10X Genomics standard Tn5 enzyme and buffer exchange

a. Transfer 16.1K cells of each cell sample in 8-tube strip. Follow the 10X Genomics protocol (total 15 µl).
b. Tagmentation reactions were incubated at 37 °C for 35 min in a thermal cycler.
c. Add 0.5 µl of 0.5 µM HPLC purified barcode oligonucleotides to different samples, and incubate at 37 °C for another 35 min with pipetting mixing every 10 mins.

Wash and buffer exchange:

d. Coat 1.5 ml tubes with PBS+0.5% BSA+1U/µl RNase inhibitor. Make sure to remove the coating reagent thoroughly after coating.
e. Add 300 µl PBS+1% BSA+1U/µl RNase inhibitor to resuspend the cells and transfer to the different coated 1.5 ml tubes. Use anther 200 µl PBS+1% BSA+1U/µl RNase inhibitor to wash the PCR tubes and transfer to the 1.5 ml tubes.
f. Centrifuge at 500g for 5 min at 4°C.
g. Remove supernatant. Then add 1000 µl Diluted Nuclei Suspension Buffer, incubate on ice for 8min, and centrifuge at 500g for 5min at 4°C.
h. Remove the supernatant and resuspend the nuclei with ~5 µl Diluted Nuclei Suspension Buffer.
i. Pool nuclei from different samples together and count using the Countess™ II Automated Cell Counter. Adjust nuclei concentration using Diluted Nuclei Buffer to the desired capture number.
j. Combine 8 µl of the adjusted nuclei solution with 7 µl ATAC buffer for GEM generation.

3b. Tagmentation using Illumina Tn5 enzyme and buffer exchange

a. Transfer 35K cells of each sample to 8-tube strip. Add the following reagent:

2X TD buffer	25 µl
0.5% digitonin	1 µl
Cells in Diluted Nuclei Suspension Buffer	14 µl
H_2O	5 µl
Tn5	5 µl
Total	**50 µl**

Note: Incubate at 37 °C. After 35 min tagmentation, add 2 µl 1 uM different SNuBar oligonucleotide co-assay (SBOC) barcodes in each tube and perform tagmentation for another 35 min.

Wash and buffer exchange:

b. Coat 1.5 ml tubes with PBS+0.5% BSA+1U/µl RNase inhibitor. Make sure to remove the coating reagent thoroughly after coating.
c. Add 300 µl PBS+1% BSA+1U/µl RNase inhibitor to resuspend the tagmented cells and transfer to the different coated 1.5 ml tubes. Use anther 200 µl PBS+1% BSA+1U/µl RNase inhibitor to wash the PCR tubes and transfer to the 1.5 ml tubes.
d. Centrifuge at 500g for 5 min at 4°C.
e. Remove supernatant. Then add 1000 µl PBS+1% BSA+1U/µl RNase inhibitor incubate on ice for 8min, and centrifuge at 500g for 5min at 4°C.
f. Remove the supernatant and resuspend the nuclei with ~5 µl PBS+1% BSA+1U/µl RNase inhibitor.
g. Pool nuclei from different samples together. Centrifuge at 500g for 5min at 4°C. Remove supernatant (20~50 µl leftover)
h. Add 1000 µl Diluted Nuclei Suspension Buffer, incubate on ice for 8min, and centrifuge at 500g for 5min at 4°C.
i. Remove the supernatant and resuspend the nuclei with ~5 µl Diluted Nuclei Suspension Buffer.
j. Count cells using the Countess™ II Automated Cell Counter. Adjust nuclei concentration using Diluted Nuclei Suspension Buffer to the desired capture number.
k. Combine 8 µl of the adjusted nuclei solution with 7 µl ATAC buffer for GEM generation.

4. GEM Generation & Barcoding [Follow 10X Genomics protocol (CG000338 Rev A for Chromium Next GEM Single Cell Multiome ATAC + Gene Expression)]

a. Prepare Master Mix [49.5 µl Barcoding Reagent (PN: 2000267); 1.1 µl Template Switch Oligo (PN: 3000228); 1.9 µl Reducing Agent B (PN: 2000087); 7.5 µl Barcoding Enzyme PN: (2000266/2000273)].
b. Add 60 µl Master Mix to each tube containing Transposed Nuclei for a total of 75 µl in each tube.
c. Dispense 70 µl Master Mix + Transposed Nuclei, 50 µl Gel Beads and 45 µl Partition Oil into Chromium Next GEM Chip J in a 10x Genomics Chip Holder.
d. Run the Chromium Controller and transfer GEMs into PCR tubes.
e. Perform PCR reaction:

37 °C	45min
25 °C	30min
15 °C	hold

f. Add 5 µl Quenching Agent to each sample to stop the reaction.

5. Post GEM Incubation Cleanup (Follow 10X Genomics protocol).

a. Add 125 µl Recovery Agent to each sample and gently invert tube 10x to mix well. Then centrifuge briefly and slowly remove 125 µl Recovery Agent/Partitioning Oil from the biphasic mixture.
b. Vortex and add 200 µl Dynabeads Cleanup Mix [182 µl Cleanup Buffer (PN:2000088); 13 µl Dynabeads MyOne SILANE (PN: 2000048); 5 µl Reducing Agent B (PN:2000087)] to each sample.
c. Incubate 10min, and wash with 80% ethanol twice, then elute with 50 µl Elution Solution I (100 µl recipe: 98 µl Buffer EB, 1 µl 10% Tween 20 and 1 µl Reducing Agent B).
d. Then purify above products with 1.8X SPRIselect reagent follow 10X Genomics protocol and elute with 46.5 µl Buffer EB.

6. Pre-Amplification PCR (Follow 10X Genomics protocol).

a. Add 54 µl Pre-Amplification Mix [50 µl Pre-Amp Mix (PN: 2000270/2000274); 4 µl Pre-Amp Primers (PN: 2000271)] to each sample. Pipette mix and centrifuge briefly.
b. Perform PCR reaction using below protocol, then purify with 1.6 X SPRIselect reagent.

72 °C	5min
98 °C	3min
98 °C	20s
63 °C	30s
72 °C	1min
	Go to step3, repeat 6X
72 °C	1min
4 °C	hold

7. ATAC Library construction (Follow 10X Genomics protocol).

a. Add 57.5 µl Sample Index PCR Mix [50 µl Amp Mix (PN: 2000047/2000103); 7.5 µl SI-PCR Primer B (PN: 2000128)] and 2.5 µl an individual Sample Index N, Set A to 40 µl sample.
b. Incubate in a thermal cycler.

98 °C	45s
98 °C	20s
67 °C	30s
72 °C	20s
	Go to step2, repeat 6-8X
72 °C	1min
4 °C	hold

c. Cleanup with SPRIselect reagent (0.6X-1.55X double selection).

8. cDNA Amplification

a. Add 65 µl cDNA Amplification mix [50 µl Amp Mix (PN: 2000047/2000103); 15 µl cDNA Primers (PN: 2000089)] into 35 µl pre-amplified sample from step 6, then add 1 µl 2.5µM barcode primer bcP. Incubate in a thermal cycler.

98 °C	3min
98 °C	15s
63 °C	20s
72 °C	1min
	Go to step2, repeat 5-8X
72 °C	1min
4 °C	hold

b. cDNA cleanup and Separate the cDNA and barcode – SPRIselect

(1) Vortex the SPRIselect reagent until fully resuspended. Add 60 µl (0.6X) SPRIselect reagent to the 100 µl sample product. vortex mix thoroughly. Incubate 5 min at room temperature.
(2) Centrifuge briefly. Place on the magnet•High until the solution clears.
(3) Move the supernatant to a new tube. Add another 120 µl (1.8X) Amp beads to the supernatant. Pipette or vortex mix thoroughly. --- this step is to get the barcode.

- For the cDNA targets:

(4) Add 200 µl 80% ethanol to the beads. Wait 30 sec. Remove the ethanol. Repeat this washing step to a total of 2 washes.
(5) Centrifuge briefly. Place on the magnet•Low. Remove any remaining ethanol. Air dry for 1 min. DO NOT exceed 2 min as this will decrease elution efficiency.
(6) Immediately add 40µl Elution Solution I. Vortex and incubate 2min at room temperature.
(7) Centrifuge briefly. Place on the magnet•Low. Transfer 40 µl sample to a new tube.

- For the barcode:

(8) Incubate 5 min at room temperature.
(9) Wash twice with 200 µl 80% ethanol to the beads. Wait 30 sec. Centrifuge briefly. Place on the magnet•Low. Remove ethanol.
(10) Immediately add 20.5 µl H_2O. Vortex and incubate 2min at room temperature.
(11) Centrifuge briefly. Place on magnet•Low. Transfer 20 µl sample to a new tube.

9. Barcode Library Construction

a. Add the following reagent in an 8-tube strip:

2X KAPA HiFi HotStart ReadyMix	25 µl
Product of step 8.b.(11)	10 µl
10 µM InPE 1 (5'-AATGATACGGCGACCACCGAGATCTACACTCT TTCCCTACACGACGCTCTTCCGATCT-3')	2 µl
TruSeq RPIX primer (5'-CAAGCAGAAGACGGCAT ACGAGATNNNNNNGTGACTGGAGTTCCTTGGCACCC GAGAATTCCA-3')	2 µl
H_2O	11 µl
Total	**50 µl**

PCR condition:

98 °C	30s
98 °C	15s

60 °C	30s
72 °C	30s
	Go to step2, repeat 5-7 PCR cycles
72 °C	1min
4 °C	hold

b. Post PCR barcode Selection – SPRIselect
   (1) Vortex the SPRIselect reagent until fully resuspended. Add 75 µl (1.5X) SPRIselect reagent. Vortex mix thoroughly. Incubate 5 min at room temperature.
   (2) Centrifuge briefly. Place on the magnet•High until the solution clears.
   (3) Remove the supernatant. Add 200 µl 80% ethanol to the beads. Wait 30 sec. Remove the ethanol. Repeat this washing step to a total of 2 washes.Centrifuge briefly.
   (4) Place on the magnet•Low. Remove any remaining ethanol.
   (5) Add 30.5 µl H_2O. Vortex and incubate 2min at room temperature.
   (6) Centrifuge briefly. Place on the magnet•Low. Transfer 30 µl sample to a new tube.

10. Follow standard 10X Genomics protocols to make RNA library.

a. Add 15 µl Fragmentation Mix [5 µl Fragmentation Buffer (PN: 2000091); 10 µl Fragmentation Enzyme (PN: 2000090)] and 25 µl Buffer EB 10 µl cDNA sample, and incubate at:

4 °C	hold
32 °C	5min
65 °C	30min
4 °C	hold

b. Cleanup with SPRIselect reagent (0.6X-0.8X double selection) and elute with 50.5 µl Buffer EB.
c. Add 50 µl Adaptor Ligation Mix [20 µl Ligation Buffer (PN: 2000092); 10 µl DNA Ligase (PN: 220110/220131), 20 µl Adaptor Oligos (PN: 2000094)] to 50 µl above sample, then incubate at:

20 °C	15min
4 °C	hold

d. Cleanup with SPRIselect reagent (0.8X) and elute with 30.5 µl Buffer EB.
e. Add 50 µl Amp Mix (PN-2000047/2000131) and 20 µl of an individual Dual Index TT Set A to 30 µl above eluted sample and incubate in a thermal cycler with the following protocol.

98 °C	45s
98 °C	20s
54 °C	30s
72 °C	20s
	Go to step2, repeat 4-15X
4 °C	hold

f. Cleanup with SPRIselect reagent (0.6X-0.8X double selection) and elute with 50.5 µl Buffer EB.

11. Final RNA and barcode libraries were mixed at a ratio of 4:1 ~ 20:1 and sequenced on Illumina NextSeq instrument follow 10X Genomics protocol. Final ATAC library was sequenced separately on the Illumina NextSeq instrument follow 10X Genomics protocol.