

The summary of scripts for the 3D genome and H2AZ paper in adipocyte thermogenesis

(Rongbin Zheng & Yang Zhang, et al.)

All scripts of the manuscript and instructions of implementation have been deposited into

https://github.com/zhengrongbin/brown_adipo_loop_H2AZ_paper

- RAW sequencing data, BigWig files, peak BED files, HiC files, and expression matrix can be found at NCBI GEO with the following accession numbers:

GSE261410	ATAC-Seq (mouse)	6
GSE261412	ChIP-Seq (mouse: H3K27ac, H3K4me3, H2AZ, Input, IgG)	24
GSE261413	RNA-Seq (mouse)	9
GSE261416	Micro-C (mouse)	24
GSE301359	RNA-Seq (human)	9
GSE301360	CUT&RUN (mouse: H2AZ in vivo, IgG)	10
GSE301361	PRO-seq (mouse)	6
GSE301366	ChIP-Seq (mouse: CTCF, SMC1, MED1, Input)	24
GSE301368	ChIP-Seq (human: H2AZ, H3K27ac, Input)	10
GSE301370	Micro-C (human)	12

- The RAW fastq file can be used for data pre-processing. The command lines for this task for each data type can be found on the GitHub, please also refer to manuscript methods section for some detailed parameters.
 - **ChIP-seq, CUT&RUN, and ATAC-seq:**
https://github.com/zhengrongbin/brown_adipo_loop_H2AZ_paper/tree/master/ChIP_ATAC_process. All ChIP-seq and CUT&RUN data were pre-processed using CHIPs pipeline (see script files named startswith “chips”). IgG or Input samples were used as background control. For H3K4me3 and H3K27ac in mouse, we performed both IgG and Input background control. Union peaks were used in the downstream analysis to improve peak detection. For other ChIP samples, either the Input or the IgG was used, depending on sample availability. For H2A.Z and ATAC-seq in both human and mouse, DANPOS2 was used to call peaks from BAM files generated by the CHIPs pipeline (https://github.com/zhengrongbin/brown_adipo_loop_H2AZ_paper/blob/master/ChIP_ATAC_process/danpos_run.sh). The extension size was 200bp for DANPOS running.
 - **Micro-C:** fastq files from different replicates were processed separately, including mapping and read pair identification, using https://github.com/zhengrongbin/brown_adipo_loop_H2AZ_paper/blob/master/MicroC/MicroC_data_preprocessing_cmd.sh, which generates pair files. The pair files of each replicate will be passed to a script for further processing, including read pair filtering, Hic file generation, COOL file generation, Compartment identification, and loop identification, using https://github.com/zhengrongbin/brown_adipo_loop_H2AZ_paper/blob/master/MicroC/MicroC_hic_cool_TAD_compartment_loop.sh. Specifically, compartment identification was done using https://github.com/zhengrongbin/brown_adipo_loop_H2AZ_paper/blob/master/MicroC/compartment

[ment.py](#), and the command lines have been included in the `MicroC_hic_cool_TAD_compartment_loop.sh`.

- **RNA-seq:** The fastq files can be used for pre-processing using https://github.com/zhengrongbin/brown_adipo_loop_H2AZ_paper/blob/master/RNAseq_process/RNAseq_data_preprocessing_cmd.sh. The count matrix will be used for differential gene expression analysis using https://github.com/zhengrongbin/brown_adipo_loop_H2AZ_paper/blob/master/RNAseq_process/diffexp_analysis.R. The way to call this R script can be found at https://github.com/zhengrongbin/brown_adipo_loop_H2AZ_paper/blob/master/RNAseq_process/diffexp_run.sh.
- Please check https://github.com/zhengrongbin/brown_adipo_loop_H2AZ_paper/blob/master/README.md and the manuscript methods for more details about software dependencies.
- Downstream analysis on further integration and generating figures based on the preprocessed data. The scripts for these analyses were summarized as follows. The Python script file may begin with shared or dependent code for loading necessary packages and datasets, as well as pre-computing for later analysis in the same category.
 - **Compartment_TAD_analysis_03012024.py:** The Python script for Compartment and TAD downstream analysis using the outputs from the preprocessing steps.
 - **Mouse_MicroC_ChIP_updated_2025.py:** The Python script for mouse loop analysis and integration with and between ChIP-seq (H2AZ, H3K27ac, CTCF, SMC1, and MED1), ATAC-seq, and RNA-seq using the outputs from preprocessing steps.
 - **human_MicroC_analysis_2025.py:** The Python script for human loop analysis and integration with and between ChIP-seq (H2AZ, H3K27ac), RNA-seq, and GWAS data using the outputs from preprocessing steps.
 - **exp_analysis_02292024.py:** The Python script for RNA-seq data downstream analysis using the outputs from preprocessing steps
 - **PROseq_analysis_2025.py:** The Python script for PRO-seq data downstream analysis using the outputs from preprocessing steps. The PRO-seq data was preprocessed by the core facility using their pipeline at (https://github.com/AdelmanLab/NIH_scripts)
- The script for generating figures for each panel is listed below. The line-number labels for the scripts generating the panel. Please note that they may depend on script sections in the same py file.
 - Fig 1b:
 - `Mouse_MicroC_ChIP_updated_2025.py`, line 873-891
 - `Mouse_MicroC_ChIP_updated_2025.py`, line 900-1119
 - Fig 1c: `Mouse_MicroC_ChIP_updated_2025.py`, line 1354-1378
 - Fig 1d: `Mouse_MicroC_ChIP_updated_2025.py`, line 1330-1350
 - Fig 1e: `Mouse_MicroC_ChIP_updated_2025.py`, line 2589-2611
 - Fig 2d: `exp_analysis_02292024.py`, line 147-175
 - Fig 2e: `exp_analysis_02292024.py`, line 147-175
 - Fig 3b:
 - `Mouse_MicroC_ChIP_updated_2025.py`, line 873-891
 - `Mouse_MicroC_ChIP_updated_2025.py`, line 900-1119
 - Fig 3c: `Mouse_MicroC_ChIP_updated_2025.py`, line 1792-1846
 - Fig 3d: `Mouse_MicroC_ChIP_updated_2025.py`, line 2618-2649
 - Fig 4a: `Mouse_MicroC_ChIP_updated_2025.py`, line 2119-2122
 - Fig 4b: `Mouse_MicroC_ChIP_updated_2025.py`, line 2232-2270, line 2410-2209

- Fig 4c: Mouse_MicroC_ChIP_updated_2025.py, line 2140-2209
- Fig 4d: Mouse_MicroC_ChIP_updated_2025.py, line 2418-2461
- Fig 4e: Mouse_MicroC_ChIP_updated_2025.py, line 3322-3361
- Fig 4f: Mouse_MicroC_ChIP_updated_2025.py, line 3294-3305
- Fig 4g: Mouse_MicroC_ChIP_updated_2025.py, line 3307-3319
- Fig 6b:
 - human_MicroC_analysis_2025.py, line 819-837
 - human_MicroC_analysis_2025.py, line 941-1137
- Fig 6c: human_MicroC_analysis_2025.py, line 1361-1383
- Fig 6e:
 - human_MicroC_analysis_2025.py, line 914-933
 - human_MicroC_analysis_2025.py, line 941-1137
- Fig 6f: human_MicroC_analysis_2025.py, line 1492-1519
- Fig 6i: human_MicroC_analysis_2025.py, line 1528-1720
- Extend Fig 1a: RNAseq_process/diffexp_analysis.R
- Extend Fig 1b: exp_analysis_02292024.py, line 47-69
- Extend Fig 2a: Mouse_MicroC_ChIP_updated_2025.py, line 3322-3432
- Extend Fig 2b: PROseq_analysis_2025.py, line 86-99
- Extend Fig 3a: Compartment_TAD_analysis_03012024.py, line 175-189
- Extend Fig 3b: Compartment_TAD_analysis_03012024.py, line 148-169
- Extend Fig 3c: Compartment_TAD_analysis_03012024.py, line 230-274
- Extend Fig 3d: Compartment_TAD_analysis_03012024.py, line 280-315
- Extend Fig 5d: PROseq_analysis_2025.py, line 540-591
- Extend Fig 5e: exp_analysis_02292024.py, line 97-144
- Extend Fig 6a: Mouse_MicroC_ChIP_updated_2025.py, line 3322-3432
- Extend Fig 6b: Mouse_MicroC_ChIP_updated_2025.py, line 1470-1481
- Extend Fig 6c: Mouse_MicroC_ChIP_updated_2025.py, line 1503-1645
- Extend Fig 6d: Mouse_MicroC_ChIP_updated_2025.py, line 1680-1785
- Extend Fig 6e: Mouse_MicroC_ChIP_updated_2025.py, line 3476-3525
- Extend Fig 6f: Mouse_MicroC_ChIP_updated_2025.py, line 2656-2715
- Extend Fig 6h: PROseq_analysis_2025.py, line 504-538
- Extend Fig 7a: Mouse_MicroC_ChIP_updated_2025.py, line 3581-3671
- Extend Fig 7d: Mouse_MicroC_ChIP_updated_2025.py, line 3528-3578
- Extend Fig 7f: Mouse_MicroC_ChIP_updated_2025.py, line 3088-3112
- Extend Fig 7g: Mouse_MicroC_ChIP_updated_2025.py, line 3650-3751
- Extend Fig 7h: Mouse_MicroC_ChIP_updated_2025.py, line 3131-3206
 - Mouse_MicroC_ChIP_updated_2025.py, line 3131-3163
 - Mouse_MicroC_ChIP_updated_2025.py, line 3170-3206
- Extend Fig 9c: exp_analysis_02292024.py, line 177-207
- Extend Fig 9f: human_MicroC_analysis_2025.py, line 550-664
- Extend Fig 9g: human_MicroC_analysis_2025.py, line 941-1137
- Extend Fig 10b: Mouse_MicroC_ChIP_updated_2025.py, line 2866-2910
- Extend Fig 10c: Mouse_MicroC_ChIP_updated_2025.py, line 2916-2986
- Extend Fig 10d: Mouse_MicroC_ChIP_updated_2025.py, line 2992-3011
- Extend Fig 10e: Mouse_MicroC_ChIP_updated_2025.py, line 2724-2856