Supplemental Protocol S4: Nuclei isolation.

Materials:

- CP02 cryoPREP automated dry pulverizer (Covaris 500001)
- Eppendorf thermomixer C (EP 5382000015)
- 2mL glass tissue grinder and pestle (Kimble chase, 885301-0002, 8853000002)
- 70 µm strainer (Fisher 501457900)
- Celltrics, 20 µm, 30µm cell strainer (Fisher NC9682496, Fisher NC9699018)
- Fisherbrand Sterile Plastic Culture (FACS) Tubes 149563C
- RNase Inhibitor (Thermofisher N8080119)
- Eppendorf Protein Lobind 1.5 mL tubes (Eppendorf 022431081)
- Eppendorf Protein Lobind 2.0 mL tubes (Eppendorf 022431102)

Buffers should be made fresh each time.

Wash Buffer	Stock	Final	4 ml
Tris-HCl 7.4 pH	1M	10mM	40ul
NaCl	5M	10mM	8ul
MgCl2	1M	3mM	12ul
BSA	10%	1%	400ul
Tween-20	10%	0.1%	40ul
DTT	1000mM	1mM	4ul
RNase Inhibitor	40U/ul	2U/ul	200ul
Water			3.3ml
Lysis Buffer	Stock	Final	2ml
Tris-HCl 7.4 pH	1M	10mM	20ul
NaCl	5M	10mM	4ul
MgCl2	1M	3mM	6ul

Tween-20	10%	0.1%	20ul
NP40	10%	0.1%	20ul
Digitonin	5%	0.01%	4ul
BSA	10%	1%	200ul
DTT	1000mM	1mM	2ul
RNase Inhibitor	40U/ul	2U/ul	100ul
water			1.62ml

Samples: This protocol is designed for 4 samples. Please scale up or down based on the number of samples.

All the steps have to be performed on ice or at 4°C.

All the 2 mL and 1.5 mL tubes used in this protocol are Protein LOBIND tubes from EPPENDORF.

Nuclei extraction:

1. Frozen tissue was pulverized into fine powder while cold (dry ice and LN2) using an automated dry pulverizer CP02 cryoPREP.

2. Pulverized frozen tissue was suspended in 1 mL of ice-cold 1x PBS in a 1.5 mL tube (Eppendorf 022431081) and centrifuged at 2000g for 3 min at 4°C. The supernatant was removed and the pellet was resuspended in 1 mL Lysis Buffer (1.5mL tube).

3. The tissue was lysed by rocking the tubes in Eppendorf thermomixer C (EP 5382000015) at 4°C at 300 rpm for 5 min.

4. Each sample was transferred into a prechilled 2 mL glass dounce homogenizer, and homogenized with 10 strokes of loose pestle A, and 20 strokes of tight pestle B and then transferred to 1.5 mL tube and centrifuged at 2000g for 5 min at 4°C.

5. The supernatant was aspirated and the pellet was resuspended in 1 mL of ice cold Wash Buffer and centrifuged at 100g for 1 min at 4°C.

6. The supernatant was collected, discarding the loose debris pellet.

7. All the filters were prewet; 70 µm, 30 µm and 20 µm filters, with 200 µL of Wash Buffer each and previously collected supernatant was sequentially filtered through 70 µm, 30 µm and 20 µm filters respectively.

8. The supernatant was filtered through 70 µm strainer and the filtrate was collected into a 50 mL conical tube.

9. The collected filtrate was passed through 30 µm celltrix strainer and collected into a 2mL tube.

10. The collected filtrate was passed through 20 µm celltrix strainer and collected into a 2mL tube.

11. The filtrate was transferred to a 1.5mL tube, and centrifuged at 350 x g for 10 min at 4°C (be careful of the tube direction). The supernatant was aspirated (the supernatant was saved as a precaution) with flexi tip gel loading tip (were very careful not to disturb the pellet) and the nuclei was resuspended in 500 µL of Wash Buffer.

12. The nuclei suspension was centrifuged again at 350 x g for 10 min at 4°C (be careful of the tube direction). The supernatant was aspirated very carefully (the supernatant was saved as a precaution) with flexi tip gel loading tip and the nuclei was resuspended in the appropriate volume of 1X diluted nuclei buffer (20x buffer supplied by 10X Genomics).

13. The nuclei were counted with cell counter (Trypan blue stains nuclei; typically 4-9 µm) and submitted for joint snATAC-seq and snRNA-seq.