Supplementary Notes

We observed repressive histone marks H3K9me3 and H3K27me3 at genes either silenced ubiquitously or just in pan-neuronal cells (**Fig. 3**, group I, II-a and II-b). Developmental processes related genes driving specification of major cell types (neuron and non-neuron cells) were selectively repressed by H3K27me3 (group II), and olfactory and vomeronasal receptor gene clusters (group I) were repressed by H3K9me3 heterochromatin. However, we did not observe patterned distributions for these two repressive histone marks for genes selectively expressed in one or few closely related cell types (group III). These genes might be de-repressed by hypo-trimethylation of H3K27 and H3K9 and selectively activated by cell-type-specific active chromatin states (H3K4me1 and H3K27ac). Other mechanisms such as DNA methylation may further contribute to the maintenance of the cell-type-specific active/inactive states of these genes[28,66].

Sequence-specific transcription factors, together with histone proteins, play critical roles in development and cellular gene expression in response to extracellular signaling. Here we obtained DNA-binding profiles of modified histone proteins by washing out non-specific bound proteinA-Tn5 (open chromatin signal) but with histone proteins largely unaffected; TF proteins weakly bound to DNA will be removed together with non-specific bound proteinA-Tn5 if using the same conditions. By performing optimizations that can reduce non-specific signals but keep those from proteinA-Tn5 specifically targeted to TF proteins, such as to improve buffer conditions or use mild crosslinked nuclei, the single-cell multi-omics strategy here may also be expanded to the joint analysis of transcriptional regulator binding, such as Pol II and CTCF[14], with gene expression in complex tissues.

Recently, the CUT&Tag strategy was combined with 10X scATAC-seq platform and enabled high-throughput CUT&Tag analysis in a time-efficient manner[67,68]. However, the reads per nuclei are relatively low: only median # of 98 (H3K36me3) and 453 (H3K27ac) unique reads were captured per nuclei[67], while the 10X platform was able to capture more than 20,000 unique ATAC fragments per nuclei. That may due to the incompatibility of commercially kits with customized CUT&Tag enzymes and buffers. While for Paired-seq and Paired-Tag, we can obtain even more histone reads (e.g.,

~20k/nuclei for H3K4me1) compared to Paired-seq (~5k/nuclei ATAC reads) as well as with comparable RNA profiles. Based on these data, we may also anticipate low-quality histone profiles by simply combining CUT&Tag with 10X multiome ATAC+RNA kits.

sci-CAR is the first published high-throughput joint ATAC+RNA assay[29]. In their design, reverse transcription is performed prior to tagmentation reaction, which is incompatible with immuno-staining steps required by CUT&Tag. By swapping the order of ATAC and RT reaction will enable the inclusion of immuno-staining; however, sci-CAR only have 2-round of barcoding (Tn5 tagmentation and PCR indexing), thus 96 tagmentation reactions (instead of 12 in Paired-Tag) will be needed to give a sufficient number of barcode combinations, which further increased the cost and time for immuno-staining and washing steps required by CUT&Tag.

SNARE-seq[30], which is based on droplet-barcoding system will be another optimal platform. Immuno-staining and washing can be performed in bulk and single-nuclei were then barcoded within droplets in a time-efficient way: the “open-source” feature of SNARE-seq may allow customized optimizations to increase the compatibilities of droplet-based platforms with CUT&Tag.

Various computational methods have recently been developed to integrate datasets from multiple modalities[69] (e.g. RNA and ATAC). Algorithms include canonical correlation analysis or non-negative matrix factorization and so on, using scRNA-seq as a common reference. These strategies should work for active marks such as H3K4me1 and H3K27ac. However, for repressive histone marks, it is nearly impossible to convert the repressive information to active gene expression states (de-repressed do not necessarily mean active); thus, it is very still challenging to integrate different histone states computationally.

References:

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