

## Unbiased integration of single cell multi-omics data

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## 1 Abstract

2 Acquiring accurate single-cell multiomics profiles often requires performing unbiased *in silico*  
3 integration of data matrices generated by different single-cell technologies from the same  
4 biological sample. However, both the rows and the columns can represent different entities in  
5 different data matrices, making such integration a computational challenge that has only been  
6 solved approximately by existing approaches. Here, we present bindSC, a single-cell data  
7 integration tool that realizes simultaneous alignment of the rows and the columns between data  
8 matrices without making approximations. Using datasets produced by multiomics technologies as  
9 gold standard, we show that bindSC generates accurate multimodal co-embeddings that are  
10 substantially more accurate than those generated by existing approaches. Particularly, bindSC  
11 effectively integrated single cell RNA sequencing (scRNA-seq) and single cell chromatin  
12 accessibility sequencing (scATAC-seq) data towards discovering key regulatory elements in  
13 cancer cell-lines and mouse cells. It achieved accurate integration of both common and rare cell  
14 types (<0.25% abundance) in a novel mouse retina cell atlas generated using the 10x Genomics  
15 Multiome ATAC+RNA kit. Further, it achieves unbiased integration of scRNA-seq and 10x  
16 Visium spatial transcriptomics data derived from mouse brain cortex samples. Lastly, it  
17 demonstrated efficacy in delineating immune cell types via integrating single-cell RNA and  
18 protein data. Thus, bindSC, available at <https://github.com/KChen-lab/bindSC>, can be applied in  
19 a broad variety of contexts to accelerate discovery of complex cellular and biological identities and  
20 associated molecular underpinnings in diseases and developing organisms.

## 21 **Introductions**

22 Advances in high-throughput single-cell technology such as single-cell RNA-sequencing (scRNA-  
23 seq)<sup>1</sup> and mass cytometry<sup>2</sup> have enabled systematic delineation of cell types based on thousands  
24 to millions of cells sampled from developing organisms or patient biopsies. For example, recent  
25 application of combinatorial indexing based technology has generated the transcriptomic and  
26 chromatin accessibility profiles of millions of cells in developing human fetus samples<sup>3</sup>. Rare cell  
27 types and complex cellular states, however, remain challenging to discover, which necessitates the  
28 development of multiomics technologies to simultaneously measure other cellular features,  
29 including DNA methylation<sup>4,5</sup>, chromatin accessibility<sup>6-8</sup> and spatial positions<sup>9,10</sup> in the same  
30 cells. Although available single-cell multiomics technologies<sup>8,11-14</sup> can profile thousands to  
31 millions of cells per experiment, the cost of the experiments is still quite high<sup>15</sup>; and the data  
32 generated are often of lower throughput than those generated by unimodal technologies. These  
33 restrictions necessitate the development of computational approaches that can accurately integrate  
34 multiple data matrices generated by different technologies from the same biological samples to  
35 acquire an accurate characterization of cellular identity and function.

36

37 However, different technologies create data matrices of different rows and columns, which  
38 correspond to different sets of cells and different types of features. How to align cells and features  
39 simultaneously across matrices is a core computational challenge. When the two sets of cells are  
40 sampled uniformly from the same biological sample, it is safe to assume that there exists an optimal  
41 way to align together cells of similar identities and features associated with these identities. This  
42 is mathematically challenging, however, as there are many possible ways to simultaneously align  
43 a large number of cells and features. To address this challenge, existing computational approaches

44 followed two directions: 1) aligning features empirically before aligning cells <sup>16-19</sup>; 2) obtaining  
45 separate embeddings for each modality, followed by performing unsupervised manifold alignment  
46 <sup>20-22</sup>. Taking integration of scRNA-seq and singe cell assay for transposase accessible chromatin  
47 sequencing (scATAC-seq) as an example, the first category of methods require constructing a  
48 “gene activity matrix” from scATAC-seq data by counting DNA reads aligned near and within  
49 each gene <sup>23</sup>. This strategy considers only the basic cis-regulatory relations and ignores long-range,  
50 trans-regulatory relationship established via other regulatory elements such as enhancers <sup>6</sup>, which  
51 are often critical to decipher cell identities. It also substantially simplifies (or loses) multifactorial  
52 relations between transcription factors (TF) and target genes <sup>24</sup>. Based on pre-aligned features  
53 generated by such empirical rules, Seurat applies canonical correlation analysis (CCA) and mutual  
54 nearest neighbors (MNNs) to identify cells anchoring the two data matrices <sup>17</sup>; LIGER uses an  
55 integrative non-negative matrix factorization (iNMF) to delineate shared and dataset-specific  
56 features <sup>19</sup>; Harmony projects cells onto a shared embedding using principle components analysis  
57 (PCA) and removes batch effects iteratively <sup>18</sup>. All these programs suffer from the aforementioned  
58 limitations and thereby cannot yield a comprehensive, unbiased gene regulatory network,  
59 particularly when chromatin changes are asynchronous from RNA transcriptions in cells  
60 undergoing state transitions <sup>25</sup>. The second category of methods <sup>20-22</sup> do not require prior feature  
61 alignment and are fully unsupervised. However, they depend heavily on the assumption that  
62 feature variation across cells is driven by a few latent variables in both modalities <sup>22</sup>. This  
63 assumption can get violated easily in datasets of complex biology involving dynamic processes  
64 such as differentiation, reprogramming and transdifferentiation <sup>22</sup>.

65

66 In this study, we develop a novel computational tool called bindSC (bi-order integration of single-  
67 cell data). The key algorithm implemented in bindSC is called bi-CCA (bi-order canonical  
68 correlation analysis). Bi-CCA learns the optimal alignment among rows and columns from two  
69 data matrices generated by two different experiments. The alignment matrix derived from bi-CCA  
70 can thereby be utilized to derive *in silico* multiomics profiles from aligned cells.

71

72 We assess our method on several challenging multimodality integration tasks between 1)  
73 transcriptomic and chromatin accessibility data, 2) transcriptomic and spatial transcriptomic data,  
74 and 3) transcriptomic and proteomic data. We validate scRNA-seq and scATAC-seq integration  
75 accuracy using datasets obtained directly from multiomics technologies, including a novel mouse  
76 retina cell atlas created by the 10x Genomics Multiome ATAC+RNA kit. We show that bindSC  
77 enables comprehensive characterization of epigenetic regulatory states in a lung adenocarcinoma  
78 cell-line A549 in response to dexamethasone treatment. And bindSC can align mouse retina cell  
79 types accurately, for multi-subtype bipolar cells and rare horizontal cells. Moreover, bindSC  
80 enables unbiased integration of spatial transcriptomics data with scRNA-seq data on mouse brain  
81 cortex samples, as well as single-cell RNA data with protein data from peripheral blood  
82 mononuclear cells. BindSC is implemented as an open-source R package available at  
83 <https://github.com/KChen-lab/bindSC>.

84

## 85 **Results**

86

### 87 **Bi-order integration of multi-omics data**

88 BindSC takes as input two single-cell data matrices (**X** and **Y**) generated uniformly from the same  
89 cell population by two different technologies (**Fig. 1a**). In most single-cell multi-omics integration  
90 tasks, neither the alignment between the cells in **X** and those in **Y**, nor the alignment between the  
91 features in **X** and those in **Y** is known. BindSC employs a bi-CCA algorithm developed in this  
92 study to address this challenge (**Fig. 1b**). Briefly, bi-CCA introduces a gene score matrix **Z** to link  
93 **X** and **Y**. The gene score matrix has the same rows as does **X** and the same columns as does **Y**. To  
94 reduce computational cost, **Z** can be initialized based on prior knowledge. Taking integration of  
95 scRNA-seq and scATAC-seq as an example, the gene score matrix can be initialized using the  
96 “gene activity matrix” estimated by other programs such as Seurat. Bi-CCA then iteratively  
97 updates **Z** to find an optimal solution which maximizes the correlation between **X** and **Z** and  
98 between **Y** and **Z** in the latent space simultaneously. Details about this iterative procedure can be  
99 found in **Methods** and **Supplementary Fig. 1a**.

100

101 Bi-CCA outputs canonical correlation vectors (CCVs), which project cells from two datasets onto  
102 a shared latent space (referring below as “co-embedding”). A K-nearest neighbor (KNN) graph is  
103 constructed based on Euclidean distances observed in the latent space, followed by modularity  
104 optimization techniques to partition the KNN into highly interconnected subgraphs, each of which  
105 corresponds to a putative cell type or state (**Fig. 1c**). Within each cluster, sub-clustering using  
106 similar strategies is further performed to derive what we call pseudo-cells (**Methods**). Each  
107 pseudo-cell encloses tens of cells from both datasets and thus has a consensus multiomic profile  
108 summarized from constituting cells (**Fig. 1c-d**). The joint multiomic profiles thus enable 1)  
109 characterizing gene and chromatin-accessibility relations from aligned scRNA-seq and scATAC-  
110 seq data; 2) associating transcriptomic profiles with spatial locations from aligned scRNA-seq and

111 spatial transcriptomic data; 3) associating transcriptomic profiles with proteomic profiles from  
112 aligned scRNA-seq and CyTOF data, and so on (**Fig. 1e**).

113

114 **Benchmarking bindSC performance on simulation datasets**

115 Existing integration methods such as Seurat, LIGER, and Harmony require pre-aligning features  
116 across modalities, i.e., compressing cell-peak matrices obtained from scATAC-seq onto cell-gene-  
117 activity matrices based on reference genome annotations. BindSC overcomes that restriction: its  
118 generic mathematical formulations allow free alignment amongst features to be established from  
119 data.

120

121 Under our formulation (**Methods**), **Z** has features (rows) aligned with **X** and cells (columns)  
122 aligned with **Y**. The introduction of **Z** enables bi-order alignment of the cells and the features,  
123 respectively.

124

125 To quantify how much this step matters to overall integration accuracy, we performed a set of  
126 simulation experiments. We started by creating a dataset **X** consisting of 3 cell clusters (types),  
127 each having 333 cells and 1,000 genes using Splatter<sup>26</sup> (**Supplementary Fig. 2a**). We created a  
128 second dataset **Y** and made it identical to **X**: **X** = **Y**. We then constructed a gene score matrix **Z**  
129 from **Y** by permuting a fraction of features (rows), termed misalignment rate (MR), into different  
130 orders. The features between **Z** and **Y** are perfectly aligned if MR equals 1 and are independent if  
131 MR equals 0. We further added white noise on all the entries of **Z** at a given signal-noise-ratio  
132 (SNR) level.

133

134 We then provide  $(\mathbf{X}, \mathbf{Z})$  as input to the other methods (**Supplementary Fig. 2b**), mimicking how  
135 they perform integration, while provide both  $(\mathbf{X}, \mathbf{Z})$  and  $(\mathbf{Y}, \mathbf{Z})$  to bindSC (**Supplementary Fig.**  
136 **2c**). As described, rather than taking  $\mathbf{Z}$  as it is from the input, bindSC will iteratively update  $\mathbf{Z}$   
137 until reaching convergence.

138

139 Since we know the true cell type and dataset origin of the cells in these experiments, we can assess  
140 the integration performance in terms of cell type classification accuracy and dataset alignment  
141 accuracy in the co-embeddings. It is necessary to measure both types of accuracy, as a high cell  
142 type classification accuracy can be achieved by simply projecting cells onto local clusters without  
143 achieving uniform mixing of the two datasets. Similarly, a high dataset alignment accuracy can be  
144 achieved by uniformly mixing cells from the datasets, regardless of their cellular identity. We used  
145 Silhouette score for measure cell type classification accuracy and alignment mixing score to  
146 measure the dataset alignment accuracy (**Methods**). We compared bindSC, CCA, Seurat, LIGER  
147 and Harmony under default settings (**Supplementary Note 1**).

148

149 We obtained results from a range of MRs under  $\text{SNR} = 0.25$  (**Fig. 2**). When there was no feature  
150 misalignment ( $\text{MR} = 0$ ), all methods achieved good performance. Even under this ideal scenario,  
151 bindSC achieved the highest Silhouette score ( $> 0.75$ ) (**Fig. 2a**). The worse performance of other  
152 methods can be explained by the noise introduced to distort the manifold structures between  $\mathbf{X}$  and  
153  $\mathbf{Z}$ . CCA showed better performance than Seurat, which may be due partly to label transferring  
154 errors introduced by Seurat's empirical anchor-based alignment approach. As MR increased from  
155 0 to 0.9, the Silhouette score for bindSC remained stable ( $> 0.7$ ), while all the other methods  
156 showed a decreasing trend, especially for LIGER and Harmony. Harmony worked well when MR

157  $\leq 0.15$  (**Fig. 2a-b**) but had a substantial drop on Silhouette score ( $< 0.1$ ) when  $MR > 0.15$ . In  
158 addition, its alignment mixing score dropped to 0 when  $MR > 0.2$ , with no mixing of cells from **X**  
159 and **Z** in the co-embedding UMAP (**Fig. 2b**;  $MR = 0.5$ ). Harmony takes cell coordinates from a  
160 reduced dimensional PCA space and runs an iterative algorithm to adjust for dataset-specific  
161 effects. When  $MR > 0.15$ , cells from **X** and **Z** already formed two dis-joint groups, which made  
162 the downstream integration impossible for Harmony. The Silhouette score of LIGER showed  
163 fluctuations but was always lower than 0.4. LIGER utilizes an integrated nonnegative matrix  
164 factorization (iNMF) method to identify shared and dataset-specific metagenes across two datasets.  
165 If it worked as designed, the errors caused by feature misalignment should be contained within  
166 dataset-specific modules. However, variance explained by the data-specific modules appeared to  
167 be small ( $< 1\%$ ). When  $MR \geq 0.95$ , all methods including bindSC failed to achieve reasonable  
168 integration. That was expected as **X** and **Z** (as well as **Y** and **Z**) became nearly independent.

169  
170 As expected, increasing SNR level worsened the integration performance for most of the methods  
171 except bindSC. For example, both CCA and Seurat had acceptable performance under  $MR = 0.5$   
172 and  $SNR = 0$  (**Supplementary Fig. 3a**), but Seurat failed to separate cell type 2 and 3 accurately  
173 when  $SNR = 0.25$  or  $0.5$  (**Supplemental Fig. 4a; Fig. 2**). For  $SNR = 0.5$ , Harmony failed in both  
174 alignment mixing ( $< 0.2$ ) and classification ( $= 0$ ) accuracy, even when  $MR$  was as low as 0.1  
175 (**Supplementary Fig. 4**).  
176

177 We repeated the above experiments by increasing the number of cells to 5,000 and 10,000,  
178 respectively. Similarly, bindSC showed robust performance regardless of  $MR$  and  $SNR$  levels,  
179 which was not achieved by other methods (**Supplementary Tables S2-3**). Overall, the simulation

180 results demonstrated that bindSC is robust to bias introduced by noise in the data and via pre-  
181 aligning features, thanks to its ability to align both cells and features simultaneously.

182

### 183 **Integrating single cell epigenomic data with single cell transcriptomic data**

184 Integrating single cell epigenomic data with single cell transcriptomic data obtained from  
185 unimodal technologies provides an opportunity to decipher epigenetic regulatory mechanisms  
186 underpinning cell transcriptomic identity. We examined the performance of bindSC in integrating  
187 the scRNA-seq and scATAC-seq data derived from lung adenocarcinoma (A549) cells after 0, 1,  
188 and 3 hours of dexamethasone (DEX) treatment <sup>6</sup>. This dataset was generated using a  
189 combinatorial indexing-based coassay (sci-CAR), which enabled jointly measurement of  
190 chromatin accessibility and transcriptome in the same cells. In this dataset, 6,005 cells have sci-  
191 RNA-seq profiles and 3,628 cells have sci-ATAC-seq profiles. Among them, 1,429 cells have both  
192 RNA-seq and ATAC-seq profiles, which can be used as a gold standard for evaluating integration  
193 accuracy of various methods (**Methods**).

194

195 For comparison, we ran the 4 methods on the same data and derived *in silico* co-embeddings. There  
196 was relatively clear separation between cells acquired at 0 hour and those at 1 or 3 hours in the co-  
197 embeddings (**Fig. 3a**). In terms of classifying cells by time, bindSC achieved the highest Silhouette  
198 score and Harmony the second, whereas Seurat had the lowest score with many sub-clusters in its  
199 co-embedding (**Fig. 3a-b**). As to alignment accuracy, bindSC and Harmony had similar scores,  
200 whereas Seurat received a relatively low score (**Fig. 3b**). Similar trends were observed in a  
201 previous study analyzing the same dataset <sup>27</sup>. As suggested by simulation, the low alignment  
202 mixing score of Seurat was likely attributable to bias introduced in its anchor-based integration.

203

204 A perfect integration method would place the two instances of the 1,429 co-assayed cells onto  
205 identical locations in the co-embeddings. We leveraged this expectation to compare the accuracy  
206 of various methods. We defined a metric, called anchoring distance that measures the normalized  
207 Euclidean distance between the two instances of a co-assayed cell in the co-embeddings  
208 (**Methods**). BindSC achieved substantially shorter anchoring distances than the other methods ( $p$   
209  $< 2.2\text{e-}16$ ; Student t-test; **Fig. 3b**).

210

211 We further compared how accurately TF (or peak) -gene correlations can be inferred from the co-  
212 embeddings produced by each method. For a fair comparison, we applied the same bindSC  
213 workflow to derive pseudo-cells for the 4 methods (**Methods; Supplementary Note 2**).

214

215 For each TF-gene (and peak-gene) pair, we calculated a Spearman rank correlation coefficient  
216 (SRCC) between the TF activity (and normalized peak) level and the gene expression level in the  
217 pseudo-cells (**Methods**). We repeated the same calculation in the co-assayed cells to create a gold  
218 standard. For each of the 4 methods in 3 types of relations: TF-gene, cis- peak-gene and trans-  
219 peak-gene, we calculated a summary SRCC between the SRCCs obtained from the pseudo-cells  
220 and the SRCCs obtained from the co-assayed cells. The summary SRCCs resulting from bindSC  
221 were consistently higher than those obtained from Seurat, LIGER and Harmony in all the  
222 categories of comparison, indicating that the bindSC multiomic profile had the highest accuracy.

223

224 We further examined the peak-gene association identified from the co-assayed cell profiles and  
225 found 585 trans- peak-gene pairs being supported by isogenic Hi-C data generated in an

226 independent study<sup>28</sup>. Compared with other approaches, bindSC derived peak-gene SRCCs of the  
227 highest level of agreement with those observed in the co-assayed cells (**Supplementary Fig. 5**).  
228 Among the 585 trans- peaks, 470 appeared more strongly correlated with the corresponding gene  
229 expression levels than did the corresponding cis- peaks. One example was the gene *CFLAR* and a  
230 trans- peak at chr2:201,770,437-201,770,992, which is 200-kb upstream of *CFLAR* transcription  
231 start site, spanning over three genes (**Fig. 3e**). The SRCC of this pair was 0.32 in the co-assayed  
232 cells. It was lower but comparable (0.23) in the bindSC pseudo-cells, however, became  
233 substantially lower (< 0.11) in the pseudo-cells generated by the other methods (**Supplementary**  
234 **Fig. 5**).

235  
236 The DEX treatment specifically targets the glucocorticoid receptor encoded by *NR3C1*, a TF that  
237 activates the mRNA transcription of a handful of downstream genes. BindSC accurately  
238 reconstructed the gene expression and TF activity kinetics of *NR3C1* (**Fig. 3d**), consistent with  
239 what was depicted in the original study<sup>6</sup> using the co-assayed cells: the *NR3C1* expression level  
240 decreased over time while its activity level increased; Even the slowing down trend of *NR3C1*  
241 activity was captured.

242  
243 We further evaluated the performance of bindSC in integrating scRNA-seq and scATAC-seq on  
244 another available multi-omics dataset generated recently by SHARE-seq technology<sup>14</sup>. There were  
245 a total of 37,774 cells from mouse skin tissues that had paired RNA and ATAC profiles. Compared  
246 with other methods, bindSC again achieved significantly shorter anchoring distances  
247 (**Supplementary Fig. 7; Supplementary Note 4**).

248

249 **Comprehensive evaluation using a novel mouse retinal cell atlas**

250 For comprehensive evaluation and comparison, we generated a novel multi-omics dataset from  
251 single nuclei of wild type mouse retina. Mouse retina is heterogeneous, composed of multiple  
252 neuronal and non-neuronal cell types, including five major neuron classes: photoreceptors (rods  
253 and cones), retinal ganglion cells (RGC), horizontal cells (HC), bipolar cells (BC), amacrine cells  
254 (AC), and a non-neuronal Müller glial cell (MG)<sup>4,29,30</sup>. While we<sup>29</sup> and others<sup>31-33</sup> have provided  
255 high-resolution single cell transcriptomic profiles of whole retina or specially sorted cell types on  
256 mouse and human retina tissue, little is known on the single-cell chromatin landscape of mouse  
257 retina tissue. Numerous studies<sup>34-36</sup> demonstrate the importance of transcription factors (TFs) on  
258 establishing or maintaining the chromatin landscapes that define retina cell identity. Therefore,  
259 integration of ATAC and RNA profiles at single cell resolution provides an exciting opportunity  
260 to comprehensively characterize cell types and rare cell subtypes in mouse retina.

261

262 We applied the newly released 10x Genomics Multiome ATAC+RNA kit on nuclei suspension  
263 acquired from adult mice retina samples. After performing standard quality control, we obtained  
264 an atlas of 9,383 nuclei of high-quality ATAC+RNA profiles. To define cell types, we first  
265 clustered the RNA and the ATAC data individually. Nineteen (19) clusters were identified from  
266 the RNA data alone, which included all the known major cell types with some subtypes identified:  
267 rod, BC (BC1~BC10), AC, RGC, cone, HC, MG and retina progenitor cells (RPC) (**Fig 4a and**  
268 **Supplementary Fig. 8**). Nineteen (19) clusters were also identified from the peak files of the  
269 ATAC data alone (**Fig. 4b**). Although known cell types appeared to be well separated in both  
270 modalities, there were some noticeable differences. For example, RGC cells and rod cells were  
271 separated clearly in the RNA data but partly blended together in the ATAC data, whereas ACs and

272 RGC cells were blended in the RNA data but well separated in the ATAC data. Interestingly, all  
273 the 10 BC cell subtypes, defined based on RNA expression levels, were well separated in the  
274 ATAC data except for BC1 and BC6. However, after reducing ATAC data to gene level in a gene  
275 activity matrix, the cell types became considerably harder to delineate (**Fig. 4c**).

276

277 To obtain *in silico* multiomics profiles, we ran bindSC together with three other methods on the  
278 data without using the known cell correspondence. As shown in the co-embedding UMAP  
279 (**Fig. 4d-e**), bindSC successfully aligned cell types across modalities, with most cell types well  
280 separated out (**Fig. 4d-f**). Interestingly, bindSC successfully aligned the HCs, which is quite rare  
281 in the dataset (23 cells, <0.25% abundance). None of the other methods aligned the HCs correctly  
282 as it was already difficult to separate the HCs from the ACs in the gene-level chromatin profiles  
283 (**Fig. 4c and Supplementary Fig. 9**), the input to the other methods. Overall, the anchoring  
284 distances in the co-embeddings generated by bindSC were considerably smaller than those  
285 generated by the other methods in all the cell types assessed (**Fig. 4f**).

286

287 Note that bindSC aligned the 10 BC subtypes reasonably well (**Fig. 4g**), although separations in  
288 the ATAC modality were not as clean as they were in the RNA modality. In comparison, Seurat  
289 and LIGER failed to generate meaningful alignments among the BC subtypes (**Fig. 4h-j** and  
290 **Supplementary Fig. 9**) while Harmony aligned a few subtypes successfully. These were due  
291 partly to the fact that these methods used the low precision gene-level chromatin accessibility  
292 profiles as the input (**Fig. 4c**).

293

294 Overall, our study demonstrated the power of multiomics in delineating rare cell types and proves  
295 that bindSC can generate *in silico* multiomics profiles that are considerably more accurate than do  
296 existing tools.

297

## 298 **Integrating scRNA-seq data with spatial transcriptomics (ST) data**

299 BindSC can integrate scRNA-seq data with spatial transcriptomics data to 1) assign spatial  
300 locations to cells in the scRNA-seq data and 2) associate additional RNA features to the spatial  
301 data for higher resolution delineation. For demonstration, we applied bindSC to integrate the  
302 SMART-Seq2 data with the *in situ* spatial transcriptomics data generated by 10x Visium from the  
303 same mouse frontal cortex tissue. These two datasets differ widely in number of cells: 1,072 spots  
304 in the ST data versus 14,249 cells in the scRNA-seq data (**Supplementary Fig. 10a**). The spots  
305 on the Visium assay are at ~50 um resolution and each spot can contain tens of cells. There were  
306 6 clusters identified from the ST data alone, which linked to distinct layers in the corresponding  
307 histology images (**Supplementary Fig. 10b-c**) and 23 cell types from the scRNA-seq data alone  
308 (**Supplementary Fig. 10d**).

309

310 We used bindSC and other programs to derive co-embeddings containing datapoints from both  
311 datasets (**Fig. 5a**). BindSC achieved evidently higher alignment mixing scores than the other  
312 programs (**Supplementary Fig. 11c**) while the Silhouette scores were similar (**Supplementary**  
313 **Fig. 11b**). For each pseudo-cell in the scRNA-seq data, we calculated its probability to map to a  
314 spatial location in the histology image. We then overlaid these cells on the histology image  
315 coloring by their probability scores (**Methods**). Noticeably, several cell types in the scRNA-seq  
316 data mapped to distinct spatial layers in the histology image, which is consistent with the known

317 cellular anatomy of mouse cortex, particularly for the laminar excitatory neuron cell types such as  
318 L2.3 IT, L4, L5.IT, L5.PT, L6.IT, L6.CT, L6B and NP (**Fig. 5b**). Consistent with previous  
319 observations, the oligodendrocyte-rich white matter (oligo cells) was mapped below the cortex.  
320 BindSC and Seurat were also able to map inhibitory clear cell types such as Lamp5, Vip, Pvalb  
321 and Sst in the scRNA-seq data to the histology image, but these cell types did not form distinct  
322 spatial patterns. LIGER and Harmony, which had worse alignment mixing scores (**Supplementary**  
323 **Fig. 11c**), failed to map these cells (**Supplementary Figs. 13-14**), especially the Vip cells. The  
324 poor mapping of the inhibitory cells may also be attributable to the limited resolution of the Visium  
325 technology.

326

327 Given that each spot in the ST data may encompass multiple cells from multiple cell types, we  
328 hypothesized that the probability scores calculated from the co-embeddings can reveal the  
329 composition of the cell types at each spot. **Fig. 5c** showed the relationship between cell type  
330 abundance in the scRNA-seq data and abundance estimated based on probability scores in the ST  
331 data. Results from bindSC achieved the best correlation (Pearson's  $R = 0.9$ ). L6.IT, Sst and Vip  
332 cell types were the top 3 most abundant cell types in both the scRNA-seq data and the ST data.  
333 Seurat also performed reasonably well (Pearson's  $R = 0.83$ ) while LIGER and Harmony performed  
334 worse. Note that Lamp5 was the cell type that showed the largest discordance in the bindSC result.  
335 In examining the spatial distributions of Lamp5 specific gene expressions such as *Lsp1*, *Npy2r*,  
336 and *Dock5*, we could not find any spatial patterns (**Supplementary Fig. 11 d-e**). This finding may  
337 indicate that Lamp5 does not have a characteristic spatial distribution.

338

339 **Integrating single-cell RNA with protein data**

340 Complex interplay exists between mRNAs and proteins<sup>37</sup>. Single-cell proteomic methods such as  
341 mass cytometry (CyTOF)<sup>2,38</sup> measure abundance of a small set of (often 10-50) surface proteins  
342 (epitopes) and provide functional quantification of various cell populations. Integrating single-cell  
343 RNA and protein data from the same sample can potentially achieve higher resolution  
344 characterization and enable discovery of novel cellular states and associated features. BindSC can  
345 be applied for such a task. Notice that this task cannot be achieved by any of the existing tools  
346 because the mRNA and protein expression levels derived from the same genes are not well  
347 correlated, due to complex post-transcriptional modifications and technological limitations<sup>39</sup>.  
348 CITE-seq<sup>40</sup> performs jointly profiling of epitope and mRNA levels in the same cells and can be  
349 used to evaluate the results of *in silico* integration.

350  
351 We used a CITE-seq dataset consisting of 30,672 human bone marrow cells with a panel of 25  
352 antibodies<sup>17</sup>. We split the data into an RNA matrix and a protein matrix. Unsupervised clustering  
353 of the RNA matrix revealed cell types largely consistent with those in the protein matrix, except  
354 for some noticeable differences (**Fig. 6a-b**). CD8+ and CD4+ T cells were partly blended together  
355 in the RNA data but separated clearly in the protein data. On the other hand, conventional dendritic  
356 cells (cDC2) were separated from other clusters in the RNA data but were intermixed with other  
357 cell types in the protein data. In contrast, unsupervised clustering of the gene expression levels of  
358 the 25 protein-homologous RNAs could not yield meaningful classification (**Fig. 6c**).  
359 Consequently, Seurat, LIGER and Harmony, which work with only data matrix of 25 homologous  
360 features, failed to produce meaningful co-embeddings (**Supplementary Fig. 15**): the cells from  
361 the protein data were well clustered, but those from the RNA data were not meaningfully  
362 distributed in the co-embeddings.

363

364 We then tested bindSC on this task. The matrix **X** was set as the protein matrix, **Y** the RNA matrix  
365 of 3,000 highly variable genes, and **Z** the RNA matrix containing only the 25 protein-homologous  
366 genes. Remarkably, the majority of the cells from the two modalities became well aligned in the  
367 co-embedding (**Fig. 6d-e**), as they are expected to be. Similar to our previous experiments, we  
368 calculated the anchoring distance between the protein and the RNA cells deriving from the same  
369 original cells in the co-embeddings. The overall anchoring distance for bindSC was significantly  
370 lower than those obtained by Seurat, LIGER, Harmony, or random guesses (p-value < 2.2e-16;  
371 Student t-test; **Fig. 6f**). Notably, the bulk of CD4+ and CD8+ T cells in the RNA data became well  
372 separated in the co-embedding (**Fig. 6d-e**), thanks to the power of integration. Moreover, the  
373 anchoring distances revealed the extent of differences between the levels of the RNAs and those  
374 of the homologous proteins in individual cell types (**Fig. 6g**). Interestingly, relatively rare cell  
375 types such as HSC, Prog/NK, LMPP, and CD16+ Mono appeared relatively well anchored,  
376 whereas relatively common cell types such as CD8 naïve, CD8 memory, B progenitor, Treg, etc.  
377 appeared less well anchored. This indicates that there are higher degrees of post-transcriptional  
378 heterogeneity in cell types conducting adaptive immune surveillance <sup>41</sup>.

379

## 380 **Discussion**

381 Despite the ground-breaking advances in single-cell technologies, including multiomics  
382 technologies, there always exists a need to computationally integrate multiple data matrices of  
383 different modalities from the same biological samples to derive a more comprehensive  
384 characterization of cellular identities and functions.

385

386 Our method bi-CCA and tool bindSC appeared to have addressed this important analytical  
387 challenge without compromising biological complexity in the data. In our experiments, bindSC  
388 successfully integrated data obtained from a wide variety of vastly different technologies covering  
389 transcriptomes, epigenomes, spatial-transcriptomes and proteomes, and clearly outperformed  
390 existing tools such as Seurat, LIGER and Harmony, when being evaluated objectively using true  
391 single-cell multiomics data derived from the same cells. In particular, Seurat, LIGER, and  
392 Harmony are essentially first-order solutions that can be applied to only rows or columns but not  
393 both simultaneously. That approach introduced biases in the results and restricted the utility of  
394 those tools in discovering complex cell-type relations and molecular interactions. For instance,  
395 they consider only the basic *cis*-regulatory relations and ignores *trans*-regulatory relations<sup>6</sup>  
396 established via distal enhancers, as exemplified in the interaction between *CFLAR* and a 200 kbps  
397 upstream putative enhancer site discovered by bindSC and validated by Hi-C in the DEX-treated  
398 A549 data. Other scATAC-seq analysis pipelines such as MAESTRO<sup>16</sup> and ArchR<sup>42</sup> have similar  
399 restrictions.

400

401 Similarly, bindSC was able to meaningfully associate the expression levels of mRNAs with those  
402 of the surface proteins, a very challenging task due to complexity in post-transcriptional  
403 modification. The resulting co-embedding offered deeper biological insights than embeddings  
404 derived from single modality or by using other existing approaches. For example, CD4+ T cells  
405 became evidently separated from CD8+ T cells and so did pDC cells from other cell types.

406

407 BindSC also achieved meaningful mapping of scRNA-seq data to spatial locations in the brain  
408 cortex samples, after integrating with the ST data. Even though the two datasets were not both at  
409 single-cell resolution, bindSC was still able to achieve a meaningful integration.

410

411 Bi-CCA made two assumptions: 1) the two sets of cells are sampled uniformly from the same  
412 biological sample; 2) the features of the two datasets are linearly correlated. These two  
413 assumptions are met under many scenarios of current investigations, however, could be violated  
414 when there are insufficient number of cells obtained from a rapidly developing cell population.  
415 Consequently, the accuracy of the co-embedding could vary, depending on the sampling density  
416 and the complexity of the population. We measured accuracy with respect to data complexity in  
417 the simulation experiments, however, accuracy on a real dataset could be complex to gauge *a*  
418 *priori* and will require case by case investigation in the context of a specific study, followed by  
419 necessary experimental validation. Nonetheless, in this study we clearly proved based on objective  
420 ground truth data that bi-CCA substantially avoided bias introduced by existing methods and that  
421 bindSC is a robust implementation that can be applied to derive meaningful results on most recent  
422 datasets containing thousands to tens of thousands cells (**Supplementary Table 1**).

423

424 BindSC is efficiently implemented in R. The major computational cost for bindSC is from  
425 calculating cell/feature co-embedding coordinates using singular value decomposition (SVD)  
426 (**Methods**); It typically requires  $O(MNL)$  floating-point operations to construct  $MN$  cell-cell  
427 distance matrix as input to SVD decomposition, where  $M$  and  $N$  are cell number of the two  
428 modalities, respectively. To address this computational challenge, bindSC implements the “divide-  
429 and-conquer eigenvalue algorithm”. The divide part first splits cells into different blocks specified

430 by users, which can be solved in parallel with lower memory usage (**Supplementary Fig. 1b**). The  
431 conquer part then merges results from each block recursively. Therefore, the maximal memory  
432 usage of bindSC is independent of the total cell number.

433

434 Taken together, we believe that bindSC is likely the first tool that has achieved unbiased integration  
435 of data matrices generated by different technologies and can be applied in broad settings. In the  
436 single-cell domain, bindSC can clearly be applied to align cells and features simultaneously, which  
437 are important for ongoing investigations in the Human Cell Atlas <sup>43</sup>, the NIH HubMap <sup>44</sup>, the  
438 Human Tumor Cell Network <sup>45</sup> and on remodeling of tumor microenvironment <sup>46</sup>. Further, bindSC  
439 can potentially be applied to other domains, such as integrating patient sample mRNA profiles  
440 with cell-line drug-sensitivity data <sup>47</sup>.

441 **Methods**

442 **BindSC workflow**

443 BindSC workflow for creating *in silico* single cell multi-omics embeddings consists of five steps:

444 1. individual dataset preprocessing including variable feature selection and cell clustering,

445 2. initializing feature matching across modalities (i.e., constructing gene score matrix),

446 3. identifying cell correspondence using the bi-CCA algorithm,

447 4. jointly clustering cells between two modalities in the co-embedding latent space and

448 constructing pseudo-cell level multi-omics profiles, and

449 5. downstream analysis for various integration tasks.

450 We formulate our method for the case of two modalities. Let  $\mathbf{X} \in \mathbb{R}^{M \times K}$  be a single-cell dataset of

451 features  $g_1, g_1, \dots, g_M$  by cells  $c_1, c_1, \dots, c_K$  and  $\mathbf{Y} \in \mathbb{R}^{N \times L}$  be a single-cell dataset of feature

452  $p_1, p_2, \dots, p_N$  by cells  $d_1, d_1, \dots, d_L$ .  $M$  and  $N$  are the numbers of features (e.g., gene expression,

453 chromatin accessibility, protein abundance level) in the two datasets.  $K$  and  $L$  are the number of

454 cells in the two datasets. Without loss of generality, we assume that features

455  $g_1, g_1, \dots, g_M$  represent the gene expression levels and  $M \leq N$ . The important component of each

456 step is described as follows.

457

458 **1. Individual modality preprocessing**

459 For each modality, we follow standard processing pipeline, which includes variable feature

460 selection and unsupervised cell clustering. The cluster information derived from each modality is

461 used for downstream parameter optimization.

462

463 **2. Initializing feature matching across modalities**

464 Because features in the two datasets are generally different, bindSC requires one additional  
465 transition matrix  $\mathbf{Z} \in \mathbb{R}^{M \times L}$  as input for bridging the integration of  $\mathbf{X}$  and  $\mathbf{Y}$ . The transition matrix  
466  $\mathbf{Z}$  can be understood as the projection of  $\mathbf{Y}$  to the feature space of the first dataset  $\mathbf{X}$ . Taking the  
467 integration of scRNA-seq and scATAC-seq as an example, the matrix  $\mathbf{Z}$  can be derived from  
468 scATAC-seq profiles by summing reads in gene bodies<sup>17,19,23</sup>. This can also be input from the  
469 regulatory potential (RP) model in MAESTRO<sup>16</sup>. In a simpler case where  $\mathbf{X}$  and  $\mathbf{Y}$  have matched  
470 features, the integration tasks fall into two categories: 1) batch correction for scRNA-seq data  
471 across individuals, species, or technologies; 2) integration of scRNA-seq with spatial  
472 transcriptome data. In those cases, the transition matrix  $\mathbf{Z}$  is initialized as  $\mathbf{Y}$ . In bi-CCA,  $\mathbf{Z}$  is  
473 updated iteratively. In the following text, the initial value of  $\mathbf{Z}$  is denoted by  $\mathbf{Z}^{(0)}$ .

474

475 **3. Bi-order canonical correlation analysis (Bi-CCA)**

476 The key algorithm implemented in bindSC is Bi-CCA, the concept of which extends traditional  
477 CCA<sup>17,24,48</sup> to both rows and columns to enable capturing of correlated variables in cells and  
478 features simultaneously. Bi-CCA introduces two cell-level projection matrices  $\mathbf{U} \in \mathbb{R}^{K \times E}$ ,  $\mathbf{S} \in$   
479  $\mathbb{R}^{L \times E}$  such that the correlations between indices  $\mathbf{XU}$  and  $\mathbf{ZS}$  are maximized, and two feature-level  
480 projection matrices  $\mathbf{T} \in \mathbb{R}^{M \times E}$ ,  $\mathbf{V} \in \mathbb{R}^{N \times E}$  such that the correlations between indices  $\mathbf{Z'T}$  and  $\mathbf{Y'V}$   
481 are maximized. The optimization framework can be formulated as:

$$\underset{\mathbf{U}, \mathbf{S}, \mathbf{T}, \mathbf{V}, \mathbf{Z}}{\operatorname{argmax}} \operatorname{tr}\{(\mathbf{XU})' \mathbf{ZS} + (\mathbf{Z'T})' \mathbf{Y'V}\} \quad (1)$$

483 subject to  $(\mathbf{XU})' \mathbf{XU} = \mathbf{I}$ ,  $(\mathbf{ZS})' \mathbf{ZS} = \mathbf{I}$ ,  $(\mathbf{Z'T})' \mathbf{Z'T} = \mathbf{I}$ ,  $(\mathbf{Y'V})' \mathbf{Y'V} = \mathbf{I}$ .

484

485 If the transition matrix  $\mathbf{Z}$  is known, the objective (1) can be divided into two disjoint traditional  
486 canonical correlation analysis (CCA) problems. The left term is performed to identify cells of

487 similar (aligned) features, while the right term is performed to identify features shared by the  
488 (aligned) cells, each of which can be solved in the CCA framework. However, it is difficult to  
489 update transition matrix  $\mathbf{Z}$  in equation (1) even when matrices  $\mathbf{U}, \mathbf{S}, \mathbf{T}, \mathbf{V}$  are available. This is  
490 because: a) left optimization problem requires  $\mathbf{Z}$  as input and the right optimization problem  
491 requires  $\mathbf{Z}'$  as input, leading (1) to a non-linear optimization problem; b) transition matrix  $\mathbf{Z}$  shows  
492 up in constraints.

493

494 Therefore, we modify equation (1) in a much more practical way. First, we standardize  $\mathbf{X}$  to let it  
495 have  $\mathbf{X}'\mathbf{X} = \mathbf{I}$ , and standardize  $\mathbf{Y}$  so that  $\mathbf{Y}\mathbf{Y}' = \mathbf{I}$ . The standardization process can be seen in  
496 **Algorithm 1**. Thus, equation (1) could be simplified as

497 
$$\underset{\mathbf{U}, \mathbf{S}, \mathbf{T}, \mathbf{V}, \mathbf{Z}}{\operatorname{argmax}} \operatorname{tr}\{(\mathbf{X}\mathbf{U})'\mathbf{Z}\mathbf{S} + (\mathbf{Z}'\mathbf{T})'\mathbf{Y}'\mathbf{V}\} \quad (2)$$

498 subject to  $\mathbf{U}'\mathbf{U} = \mathbf{I}$ ,  $(\mathbf{Z}\mathbf{S})'\mathbf{Z}\mathbf{S} = \mathbf{I}$ ,  $(\mathbf{Z}'\mathbf{T})'\mathbf{Z}'\mathbf{T} = \mathbf{I}$ ,  $\mathbf{V}'\mathbf{V} = \mathbf{I}$ .

499

500 To eliminate transition matrix  $\mathbf{Z}$  from constraints, we introduce two transition matrices  $\mathbf{Z}_l \in \mathbb{R}^{M \times L}$   
501 and  $\mathbf{Z}_r \in \mathbb{R}^{M \times L}$  and optimize the following problem:

502 
$$\underset{\mathbf{U}, \mathbf{S}, \mathbf{T}, \mathbf{V}, \mathbf{Z}_l, \mathbf{Z}_r}{\operatorname{argmax}} \operatorname{tr}\{(\mathbf{X}\mathbf{U})'\mathbf{Z}_l\mathbf{S} + (\mathbf{Z}_r'\mathbf{T})'\mathbf{Y}'\mathbf{V}\} + \|\mathbf{Z}_l - \mathbf{Z}_r\|_2 \quad (3)$$

503 subject to  $\mathbf{U}'\mathbf{U} = \mathbf{I}$ ,  $\mathbf{S}'\mathbf{S} = \mathbf{I}$ ,  $\mathbf{T}'\mathbf{T} = \mathbf{I}$ ,  $\mathbf{V}'\mathbf{V} = \mathbf{I}$ ,  $\mathbf{Z}_l\mathbf{Z}_l' = \mathbf{I}$ ,  $\mathbf{Z}_r'\mathbf{Z}_r = \mathbf{I}$ .

504

505 To solve equation (3), we also standardize  $\mathbf{Z}^{(0)}$  to let  $\mathbf{Z}^{(0)'}\mathbf{Z}^{(0)} = \mathbf{I}$ , and initialized with  $\mathbf{Z}_l := \mathbf{Z}^{(0)}$ .  
506 The standard singular value decomposition (SVD) can be implemented to obtain the canonical  
507 correlation vectors (CCVs) at cell levels. We used a user-defined number ( $E$ ) of singular vectors  
508 that approximate the CCVs (**Algorithm 2**). Here we term  $E$  to represent the cell-level

509 “dimensionality” in the latent space, which is a parameter required to be optimized (Details seen  
510 in **Parameter optimization**).

511 
$$(\mathbf{U}, \mathbf{S}) := \underset{\mathbf{U}, \mathbf{S}}{\operatorname{argmax}} \operatorname{tr}(\mathbf{U}' \mathbf{X}' \mathbf{Z}_l \mathbf{S}) \text{ subject to } \mathbf{U}' \mathbf{U} = \mathbf{I}, \mathbf{S}' \mathbf{S} = \mathbf{I}. \quad (4)$$

512

513 Having CCV pair  $(\mathbf{U}, \mathbf{S})$  obtained, we have cell correspondence in the latent space between two  
514 datasets. The left transition matrix  $\mathbf{Z}_l$  can be updated by:

515 
$$(\mathbf{Z}_l) := \underset{\mathbf{Z}_l}{\operatorname{argmax}} \operatorname{tr}(\mathbf{U}' \mathbf{X}' \mathbf{Z}_l \mathbf{S}) \text{ subject to } \mathbf{Z}_l \mathbf{Z}_l' = \mathbf{I}. \quad (5)$$

516

517 The details of solving optimization problem (5) is in **Algorithm 2**.

518

519 We then set

520 
$$(\mathbf{Z}_r) := \mathbf{Z}_l. \quad (6)$$

521 The similar SVD algorithm (**Algorithm 2**) is used to approximate CCVs:

522

523 
$$(\mathbf{T}, \mathbf{V}) := \underset{\mathbf{T}, \mathbf{V}}{\operatorname{argmax}} \operatorname{tr}(\mathbf{T}' \mathbf{Z}_r \mathbf{Y}' \mathbf{V}) \text{ subject to } \mathbf{T}' \mathbf{T} = \mathbf{I}, \mathbf{V}' \mathbf{V} = \mathbf{I}. \quad (7)$$

524

525 Once CCV pairs  $(\mathbf{T}, \mathbf{V})$  are obtained, the features are matched in the latent space between two  
526 datasets. The right transition matrix  $\mathbf{Z}_r$  could be updated as:

527

528 
$$(\mathbf{Z}_r) := \underset{\mathbf{Z}_r}{\operatorname{argmax}} \operatorname{tr}\{(\mathbf{Z}_r' \mathbf{T})' \mathbf{Y}' \mathbf{V}\} \text{ subset to } \mathbf{Z}_r' \mathbf{Z}_r = \mathbf{I}. \quad (8)$$

529 Next, we set

530 
$$(\mathbf{Z}_l) := \mathbf{Z}_r, \quad (9)$$

531 The update process (4) ~ (9) are repeated until convergence. Because each of the subproblems is  
532 convex with respect to the block variables being optimized, the algorithm is guaranteed to  
533 converge to a fixed point (local minimum).

534

535 In the above framework, the transition matrix  $\mathbf{Z}$  (represented by  $\mathbf{Z}_l$  and  $\mathbf{Z}_r$ ) is updated based on  
536 original observed matrices  $\mathbf{X}$  and  $\mathbf{Y}$ . In practice, we introduce the couple coefficient  $\alpha$  ( $0 \leq \alpha \leq 1$ )  
537 to assign weights on initialized matrix  $\mathbf{Z}^{(0)}$  on transition process (6) and (9).

538 
$$(\mathbf{Z}_r) := (1 - \alpha)\mathbf{Z}^{(0)} + \alpha\mathbf{Z}_l \quad (10)$$

539 and

540 
$$(\mathbf{Z}_l) := (1 - \alpha)\mathbf{Z}^{(0)} + \alpha\mathbf{Z}_r \quad (11)$$

541 The couple coefficient  $\alpha$  can reflect the contribution of initial  $\mathbf{Z}^{(0)}$  on linking two modalities.  
542 Equations (10) and (11) will be reduced to Equations (6) and (9) if  $\alpha = 1$ . The bi-CCA algorithm  
543 will be reduced to traditional CCA if  $\alpha = 0$ . Selection of coefficient  $\alpha$  can be seen in **Parameter**  
544 **optimization**. Notably, the final  $\mathbf{Z}_r$  and  $\mathbf{Z}_l$  will be converged to different matrices if  $\alpha < 1$ . The  
545 workflow of the iterative process is shown in **Supplementary Fig. 1a**.

546

547 **Jointly clustering cells across datasets in shared latent space and constructing pseudo-cell  
548 level multi-omics profiles**

549 Equation (4) projects cells of two datasets into a correlated  $E$ -dimensional space with cell  
550 coordinates  $\mathbf{U} = (\mathbf{u}_1, \mathbf{u}_2, \dots, \mathbf{u}_K)$  and  $\mathbf{S} = (\mathbf{s}_1, \mathbf{s}_2, \dots, \mathbf{s}_L)$ , respectively. L2-normalization is  
551 performed to remove global differences in scale, therefore

552 
$$\hat{\mathbf{u}}_i = \mathbf{u}_i / \|\mathbf{u}_i\|_2, i = 1, 2, \dots, K, \quad (12)$$

553 
$$\hat{\mathbf{s}}_i = \mathbf{s}_i / \|\mathbf{s}_i\|_2, i = 1, 2, \dots, L.$$

554

555 The shared nearest neighbor (SNN) graph is constructed by calculating the  $l$ -nearest neighbors (20  
556 by default) based on the Euclidean distance of L2-normlized latent space. The fraction of shared  
557 nearest neighbors between the cell and its neighbors is used as weights of the SNN graph. The  
558 modularity optimization technique Leiden algorithm <sup>49</sup> is used to group cells into interconnected  
559 clusters (termed meta-cluster) based on constructed SNN graph with a resolution parameter setting  
560 by users (default 0.5).

561

562 To understand the molecular-level interaction among modalities, we construct the pseudo-cell  
563 level multi-omics profiles. Briefly, for cells in each meta-cluster identified, the Leiden algorithm  
564 is further performed based on SNN graph with a higher resolution (default = 2). In this way, cells  
565 in each meta-cluster are further grouped into highly interconnected sub-clusters. We term such  
566 sub-clusters as pseudo-cells. Only pseudo-cells that consist of at least  $n$  cells (default = 10) are  
567 kept for downstream analysis, while the others are considered data-specific and discarded. Profiles  
568 of the pseudo-cells are constructed by aggregating the cells included. We denote by  $\mathbf{X}^{pseudo} \in$   
569  $\mathbb{R}^{M \times P}$  be pseudo-cell profiles of feature  $g_1, g_1, \dots, g_M$  and  $\mathbf{Y}^{pseudo} \in \mathbb{R}^{N \times P}$  be pseudo-cell profiles  
570 of feature  $p_1, p_2, \dots, p_N$ .  $P$  is the number of pseudo-cells.

571

## 572 **Algorithm 1. Standardizing inputs**

573 For input matrix  $\mathbf{X}$ , we denote  $\Sigma_{\mathbf{X}'\mathbf{X}}^{-1}$  as the generalized inverse of matrix  $\mathbf{X}'\mathbf{X}$ , and redefine  $\mathbf{X} :=$   
574  $\Sigma_{\mathbf{X}'\mathbf{X}}^{-1/2} \mathbf{X}$ . For input matrix  $\mathbf{Y}$ , we denote  $\Sigma_{\mathbf{Y}'\mathbf{Y}}^{-1}$  as the generalized inverse of matrix  $\mathbf{Y}'\mathbf{Y}$ , and redefine  
575  $\mathbf{Y} := \mathbf{Y}\Sigma_{\mathbf{Y}'\mathbf{Y}}^{-1/2}$ . The standardization of  $\mathbf{Z}_r$  and  $\mathbf{Z}_l$  is the same as above.

576

577 **Algorithm 2. Calculating CCVs using SVD**

578 Take subproblem from the Equation (4) as an example, the goal of this module is to find projection  
579 matrix  $\mathbf{U} \in \mathbb{R}^{K \times E}$  and  $\mathbf{S} \in \mathbb{R}^{L \times E}$  such that the correlations between two indices  $\mathbf{XU}$  and  $\mathbf{Z}_l\mathbf{S}$  are  
580 maximized.

581 
$$\underset{\mathbf{U}, \mathbf{S}}{\operatorname{argmax}} \operatorname{tr}(\mathbf{U}' \mathbf{X}' \mathbf{Z}_l \mathbf{S}) \text{ subject to } \mathbf{U}' \mathbf{U} = \mathbf{I}, \mathbf{S}' \mathbf{S} = \mathbf{I}. \quad (\text{A1})$$

582 We define  $\Sigma_{\mathbf{X}' \mathbf{Z}_l} := \mathbf{X}' \mathbf{Z}_l$ . Let  $\mathbf{U} \in \mathbb{R}^{K \times D}$  and  $\mathbf{S} \in \mathbb{R}^{K \times D}$  be the matrices of the first  $E$  left- and  
583 right singular vectors of  $\Sigma_{\mathbf{X}' \mathbf{Z}_l}$ . Then the optimum in Equation (A1) is solved.

584

585 **Algorithm 3. Updating transition matrix with orthogonality constraints**

586 Take subproblem from the Equation (5) as an example, the goal of this module is to optimize  $\mathbf{Z}_l$ .

587 
$$(\mathbf{Z}_l) := \underset{\mathbf{Z}_l}{\operatorname{argmax}} \operatorname{tr}(\mathbf{U}' \mathbf{X}' \mathbf{Z}_l \mathbf{S}), \text{ subject to } \mathbf{S}' \mathbf{Z}_l' \mathbf{Z}_l \mathbf{S} = \mathbf{I} \quad (\text{A2})$$

588 Equation (A2) is maximized when  $\mathbf{Z}_l \mathbf{S} = \mathbf{XU}$ . Therefore, we can update  $\mathbf{Z}_l$  as

589 
$$(\mathbf{Z}_l) := \mathbf{XU} \mathbf{S}' \Sigma_{\mathbf{SS}'}^{-1}, \quad (\text{A3})$$

590 where  $\Sigma_{\mathbf{SS}'}^{-1}$  denotes the generalized inverse of matrix  $\mathbf{SS}'$ .

591

592 **Parameter optimization**

593 There are two key hyperparameters when running bindSC for integration. The first one is the  
594 dimensionality  $E$  in the latent space and the second one is the couple coefficient  $\alpha$ . Similar with  
595 previous integration methods, the number of dimension  $E$  is very important on cell type  
596 classification. We provide heuristics to guide the selection of  $E$  based on integration metrics  
597 defined below, though sometimes helpful, are not substitute for biological insights. As a general  
598 suggestion, we recommend starting with a value of  $E$  the same with the minimal number of

599 principle components (PCs) used on single modality clustering. The selection of couple coefficient  
600  $\alpha$  depends on whether initialized  $Z^{(0)}$  can represent the gene score of  $Y$ . We devise two metrics to  
601 aid in selecting  $\alpha$ , which measure integration performance on accuracy (no mixing of cell type)  
602 and alignment (mixing of datasets) as defined below.

603

604 **1) Silhouette score**

605 To measure integration accuracy, we use the Silhouette score. Cluster for each cell is defined  
606 using the cell type labels assigned from single dataset clustering. The Silhouette score assesses the  
607 separation of cell types, where a high score suggests that cells of the same cell type are close  
608 together and far from cells of a different type. The Silhouette score  $s(i)$  for each cell is calculated  
609 as following. Let  $a(i)$  be the average distance of cell  $i$  to all other cells within  $i$ 's cluster and  $b(i)$   
610 the average distance of  $i$  to all cells in the nearest cluster, to which cell  $i$  does not belong. Cell-cell  
611 distance is computed in the L2-normalized co-embeddings (**Equation 12**).  $s(i)$  can be computed  
612 as:

$$s(i) = \begin{cases} 1 - \frac{a(i)}{b(i)} & \text{if } a(i) < b(i) \\ 0 & \text{if } a(i) = b(i) \\ \frac{b(i)}{a(i)} - 1 & \text{if } a(i) > b(i) \end{cases}$$

613  
614 Notably, given accurate correspondence between two modalities unknown, calculating  $s(i)$  for cell  
615  $i$  in above equation only includes cells from the same dataset. We average values across all cells  
616 to obtain an overall silhouette score for integration task.

617

618 **2) Alignment mixing score**

619 To measure integration mixing level, we use an alignment mixing score similar to those of previous  
620 studies <sup>50</sup>. We first build a 20-nearest neighbor graph for each cell from L2-normalized co-  
621 embeddings (**Equation 12**). For cell  $i$ , assuming proportions of cells from two modalities are  $p_{1i}$   
622 and  $p_{2i}$ , respectively, the alignment mixing score is calculated as

623 
$$H(i) = -p_{1i} \log_2 p_{1i} - p_{2i} \log_2 p_{2i}$$

624 This corresponds to a mixing metric per cell, and we average values across all cells to obtain an  
625 overall mixing metric.

626

627 We run bindSC by ranging  $a$  from 0 to 1 (with step size 0.1). Silhouette score and alignment  
628 mixing score is calculated for each scenario. We select appropriate  $a$  that generally has best  
629 performance in Silhouette score and alignment mixing score. Parameter values used in this study  
630 can be seen in **Supplementary Table S1**.

631

## 632 **Performance and benchmarking**

633 In our evaluation, in addition to Silhouette score and alignment mixing score, we also consider  
634 anchoring distance for evaluation datasets from multi-omics technologies, in which each cell has  
635 paired profiles. For cell  $i$  from the first data, we calculate its distance (Euclidean distance) with all  
636 cells in the second data as  $\mathbf{D}_i$ , and its distance with cell  $i$  in the second data as  $d_i$ . The anchoring  
637 distance for cell  $i$  is calculated as  $2d_i/\max(\mathbf{D}_i)$ . We then average anchoring distance across all  
638 cells to obtain an overall anchor distance metric. The anchoring distance of cell  $i$  is 0 when it is  
639 anchored correctly. The overall anchoring distance is 1 if we randomly layout cells on co-  
640 embeddings.

641

642 **Simulation dataset**

643 We generated simulation dataset to evaluate method performance in integrating two modalities  
644 assuming accurate feature matching unknown. We used Splatter tool <sup>26</sup> to simulate dataset  $\mathbf{X}$  with  
645 1,000 genes and cells with different sizes (from 1,000 to 10,000). The whole population is  
646 consisted of three batches (cell types). To mimic the feature unmatching case, we first generated  
647 dataset  $\mathbf{X}_1$  by randomly permutating genes of  $\mathbf{X}$  under specified misalignment rates (MR). MR  
648 ranges from 0 to 1 with step size being 0.05 in this study.  $\mathbf{X}_1$  is the same as  $\mathbf{X}$  if MR = 0. Half of  
649 genes are matched between  $\mathbf{X}_1$  and  $\mathbf{X}$  if MR = 0.5. No genes are matched between  $\mathbf{X}_1$  and  $\mathbf{X}$  if MR  
650 = 1. Then we generated matrix  $\mathbf{Z}$  by adding  $\mathbf{X}_1$  with white noise at certain level (i.e., Signal-Noise-  
651 Ratio; SNR). SNR is set to be three levels (0, 0.25 and 0.5).

652

653 For method comparison, previous methods including traditional CCA, Seurat <sup>17</sup>, Liger <sup>19</sup>, and  
654 Harmony tools <sup>18</sup> take  $\mathbf{X}$  and  $\mathbf{Z}$  as input assuming that cell correspondence between them is  
655 unknown. bindSC takes two parts as input: 1)  $\mathbf{X}$  and  $\mathbf{Z}$  with cell correspondence unknown; 2)  $\mathbf{X}$   
656 and  $\mathbf{Z}$  with feature-level matching unknown (**Supplementary Fig. 2**).

657

658 **Preparation of dexamethasone (DEX) treated A549 cell dataset**

659 To investigate the ability of bindSC in integrating scRNA-seq and scATAC-seq profiles, we  
660 explored the DEX-treated A549 dataset generated from sci-CAR technology, which uses  
661 combinatorial indexing-based assay to jointly profile chromatin accessibility and mRNA on same  
662 cell <sup>6</sup>. In the A549 dataset, DEX is a synthetic corticosteroid which activates glucocorticoid  
663 receptor (GR), binds to thousands of locations, and alternates the expression of hundreds of genes  
664 <sup>51</sup>. The human lung adenocarcinoma derived A549 cells after 0, 1, or 3 hours of 100nM DEX

665 treatment are assayed. The sci-RNA-seq dataset was from GSE117089  
666 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE117089>) and sci-ATAC-seq data was  
667 from GSM3271041 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM3271041> ). The  
668 original A549 data includes sci-RNA-seq profiles for 6,150 cells and sci-ATAC-seq profiles for  
669 6,260 cells. There are 1,429 cells co-assayed. Following Cao et al., pre-processing pipeline  
670 ([https://github.com/KChen-lab/bindSC/blob/master/vignettes/A549/A549\\_preprocess.ATAC.Rmd](https://github.com/KChen-lab/bindSC/blob/master/vignettes/A549/A549_preprocess.ATAC.Rmd)), we binarized peak count  
671 matrix for cells from both ATAC-seq only and co-assay. Loci present in less than 5 cells and cells  
672 with less than 300 accessible loci were removed. Peaks within 1kb were merged and reads in  
673 merged peaks were aggregated to generate a merged peak matrix, leading to 3,628 cells with  
674 32,791 loci. Each locus' accessibility in each cell was calculated by dividing the cell's raw read  
675 count by cell specific size factor using *estimateSizeFactors* function in *Monocle 2*<sup>52</sup>. For RNA-  
676 seq data, cells with expression counts less than 500 and more than 9100 were removed. The gene  
677 expression in each cell was also calculated by dividing the cell's raw read count by cell specific  
678 size factor, followed by *log2* normalization. Genes with no variation in expression across cells  
679 were further removed. The gene activity matrix was collapsed from the peak matrix by summing  
680 all counts with the gene body plus 2kb upstream using *CreateGeneActivityMatrix* function in  
681 Seurat3<sup>17</sup>. We then picked top 10,000 variable genes in both sci-RNA-seq data and gene activity  
682 data and used the overlapped 4,759 genes between them for integration. Finally, the sci-RNA-seq  
683 matrix was composed of 6,005 cells with 4,759 genes, the gene activity matrix was composed of  
684 3,628 cells with 4,758 genes, and the sci-ATAC-seq matrix was composed of 3,628 cells with  
685 24,953 loci. There were 1,429 cells co-assayed.

687

688 **Preparation of the mouse skin cell data**

689 We examined the performance of bindSC in integrating the scRNA-seq and scATAC-seq data  
690 derived from mouse skin tissue. This dataset was generated using SHARE-seq (3) which included  
691 34,774 cells that have joint profiles of RNA and ATAC profiles. The RNA data was downloaded  
692 from <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM4156608>. The ATAC data was  
693 downloaded from <https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSM4156597>. The final  
694 ATAC-seq matrix includes 25,594 cells on 74,161 peaks after quality control (including removing  
695 cells with less than 350 genes expressed; peaks that exist in less than 500 cells). In addition, 4,894  
696 genes were identified that were highly variable in both gene expression and gene activity profiles.  
697 For this evaluation, we only focused on the third metric (e.g., anchoring distance) that represents  
698 the chance for the two instances of a co-assayed cell to appear in the co-embeddings.

699

700 **Preparation of the mouse retina 10x Genomics Multiome ATAC+RNA data**

701 One mouse retina was dissociated by papain-based enzymatic digestion as described previously<sup>53</sup>  
702 with slight modifications. Briefly, 45 U of activated papain solution (with 1.2 mg L-cysteine  
703 (Sigma) and 1200U of DNase I (Affymetrix) in 5ml of HBSS buffer) was added to the tissue and  
704 incubated at 37 °C for 20 minutes to release live cells. Post-incubation, papain solution was  
705 replaced and deactivated with ovomucoid solution (15 mg ovomucoid (Worthington biochemical)  
706 and 15 mg BSA (Thermo Fisher Scientific) in 10 ml of MEM (Thermo Fisher Scientific)). The  
707 remaining tissue clumps were further triturated in the ovomucoid solution and filtered through a  
708 20nm nylon mesh. After centrifugation at 300g 10min at 4C, the singe cells were resuspended PBS  
709 with 0.04% BSA and checked for viability and cell count. About 1 million cells were pelleted and  
710 resuspend in chilled lysis buffer (10x Genomics), incubate for 2 minutes on ice while monitored

711 under microscope. 1ml of chilled wash buffer (10x Genomics) was added and sample was spun  
712 down at 500g 5min at 4C and washed before resuspended in Diluted Nuclei Buffer (10x Genomics).  
713 Nuclei concentration was determined using countess and proceed with transposition according to  
714 manufacturer's recommendation (10x Genomics). After incubation for one hour at 37C, the  
715 transposed nuclei were combined with barcoded gel beads, RT mix and partition oil on Chromium  
716 to generate gel beads in Emulsion (GEMs). Single cell ATACseq library and 3'RNAseq library  
717 were subsequently generated following recommended protocol from 10x Genomics. Libraries  
718 were quantified and loaded on Novaseq 6000 and run with the following parameter: 151, 8, 8,  
719 151bp. Data was analyzed using bcl2fastq (to generate fastq files) and cellranger pipeline (10x  
720 Genomics).

721

## 722 **Preparation of the mouse frontal cortex cell data**

723 We investigate bindSC ability in integrating spatially resolved transcriptomic (ST) with  
724 dissociated scRNA-seq. For the ST dataset, we used sagittal mouse brain slices generated from the  
725 Visium v1 chemistry. The dataset was downloaded from <https://support.10xgenomics.com/spatial->  
726 [gene-expression/datasets](#). The pre-processing workflow was guided by the Seurat3  
727 ([https://satijalab.org/seurat/v3.2/spatial\\_vignette.html](https://satijalab.org/seurat/v3.2/spatial_vignette.html)). Briefly, cells were subset from anterior  
728 region, followed by *sctransform*<sup>54</sup>. We then proceed to run dimensionality reduction and clustering  
729 using standard workflow as did for scRNA-seq. Cluster ID 1,2,3,5,6,7 was extracted, followed by  
730 segment based on exact position (Details in **Subset out anatomical regions** part in Seurat3  
731 tutorial), leading to 1,072 cortical cells left for the ST data. One cortical scRNA-seq data composed  
732 of ~14,000 adult mouse cortical cell taxonomy from the Allen Institute was collected  
733 ([https://www.dropbox.com/s/cuowvm4vrf65pvq/allen\\_cortex.rds?dl=1](https://www.dropbox.com/s/cuowvm4vrf65pvq/allen_cortex.rds?dl=1)). This dataset was

734 generated using the SMART-Seq2 protocol <sup>55</sup>. The *sctransform* normalization was performed  
735 based on 3,000 variable genes. We used the cell type annotation provided by published meta data  
736 available. There was a total of 14,294 cortical cells with 34,617 genes for the scRNA-seq data.  
737 Integration of scRNA-seq and ST is based on 2,316 variable genes overlapped between two  
738 datasets.

739  
740 To predict locations of each cell type from scRNA-seq in the histological images, we built a  
741 support vector machine (SVM) that trained on cell profiles from scRNA-seq data. In the training  
742 model, features were identified as cell coordinates in co-embeddings and labels were  
743 corresponding cell types. The trained SVM was applied to ST data and output predicted probability  
744 of each cell type at each spot. The *SpatialFeaturePlot* function in Seurat3 was used to overlay  
745 predicted probabilities for each cell type on top of tissue histology.

746  
747 **Preparation of human bone marrow cell dataset**  
748 We examined the performance of bindSC in integrating the single-cell RNA and protein data  
749 derived from human bone marrow tissue. This dataset was generated using the CITE-seq  
750 technology <sup>40</sup>, which included 30,672 cells that have joint profiles of RNA and a panel of 25  
751 antibodies. The dataset was downloaded from  
752 [https://satijalab.org/seurat/v4.0/weighted\\_nearest\\_neighbor\\_analysis.html](https://satijalab.org/seurat/v4.0/weighted_nearest_neighbor_analysis.html). We extracted the 25  
753 protein-homologous gene expression profile from the RNA data and kept cells that have total  
754 expression count > 2. The final protein matrix includes 28,609 cells with 25 protein abundance  
755 levels. The gene expression matrix includes 28,609 cells with 3,000 genes. The protein-  
756 homologous RNA matrix includes 28,609 cells with the RNA levels of the 25 genes homologous

757 to the 25 proteins. To measure anchoring accuracy for each cell type, we used the third metric,  
758 anchoring distance, which measures the distance of protein and gene expression for each cell in  
759 co-embeddings.

760

### 761 **Motif-based Transcription Factors (TFs) activity estimation**

762 To estimate transcription factor activity from scATAC-seq data, we used default settings in  
763 chromVAR<sup>56</sup> package. This approach quantifies accessibility variation across single cells by  
764 aggregating accessible regions containing a specific TF motif. It calculated motif-based TF activity  
765 by comparing the observed accessibility of all the peaks containing a TF motif to a background set  
766 of peaks normalizing against known technical confounders.

767

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780 **Author contributions**

781 K.C. conceptualized and supervised the project. J.D. designed the bindSC tool, implemented the  
782 software and performed analysis. R.C., Y. L., X. C., S.K., J.C., contributed to mouse retina 10x  
783 Genomics ATAC+RNA data generation, curation. V.M. contributed to data interpretation. J.D.,  
784 S.L. and K.C. drafted the manuscript. All authors reviewed, edited, and approved the manuscript.

785

786 **Competing interests**

787 The authors declare no competing interests.

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- 907

908 **Figure Legends**

909 **Fig. 1 Overview of bindSC.** BindSC takes as input two data matrices produced by different modalities  
910 from the same cell population **(a)**. The modalities may include transcriptomes, epigenomes, spatial  
911 transcriptomes and proteomes. Bi-order integration of two modalities (**X** and **Y**) with unpaired cells and  
912 unmatched features using Bi-CCA algorithm **(b)**. In the data matrices, each row represents one gene/locus,  
913 and each column represents one cell. The gene score matrix **Z** that links the first modality with the second  
914 one is initialized by prior gene activity modeling (see **Methods**). Bi-CCA algorithm aims to update gene  
915 score matrix **Z** iteratively by maximizing the correlation of between **X** and **Z** and between **Y** and **Z**  
916 simultaneously. Based on canonical correlation vectors (CCVs) in the derived latent space, K-nearest  
917 neighbor (KNNs) clustering is performed to define cell types in both modalities **(c)**. Within each cell-type  
918 cluster, KNN clustering is further performed at a higher resolution to define pseudo-cells consisting of 10s  
919 cells from both modalities. *In silico* multimodal profiles are constructed from cells assigned to the same  
920 pseudo-cell **(d)**. The color in each box indicates the relative level of each feature, with white corresponding  
921 to missing values. The multiomics feature profiles enable us to 1) link genes to regulatory elements, 2) map  
922 RNA expressions to spatial locations and 3) delineate cells by both RNA and protein signatures **(e)**.

923

924 **Fig. 2 Benchmarking bindSC performance on simulation datasets.** Comparison of bindSC to CCA,  
925 Seurat, LIGER, and Harmony based on Silhouette score and alignment mixing score **(a)**. The dataset  
926 contains 1,000 genes and 1,000 cells equally distributed in 3 cell types. Signal-to-noise ratio (SNR) was set  
927 at 0.25. X-axes denote the misalignment rates (MR) between features in the two datasets, which ranges  
928 from 0 to 1. The features between two datasets have perfect match if MR = 0 and are unrelated if MR = 1.  
929 UMAP views of the co-embeddings generated by bindSC, CCA, Seurat, LIGER, and Harmony **(b)**. From  
930 top to bottom are results with MR = 0.1, 0.5, and 0.9, respectively. Each point denotes one cell that is  
931 colored based on its true cell type label (red, green, or cyan).

932

933 **Fig.3 Benchmarking bindSC performance on the DEX-treated A549 cell-line data.** UMAP of cells  
934 from DEX-treated A549 cell-line data for bindSC, Seurat, LIGER and Harmony respectively, colored by  
935 collection time (red: 0 hour, green: 1 hour and blue: 3 hour) on the top panel and by technologies (grey: sci-  
936 RNA and brown: sci-ATAC) on the bottom panel **(a)**. Comparison of the 4 methods based on Silhouette  
937 score (top), alignment mixing score (middle) and anchoring distance (bottom) **(b)**. Also included for  
938 comparison are metrics from randomly selected cells. Accuracy of *in silico* multiomics profile in pseudo-  
939 cells **(c)**. The TF-gene correlation is quantified in each pseudo-cell (top panel) by calculating for each TF-  
940 gene pair (1.8 million pairs total) a Spearman's rank-order correlation coefficient (SRCC) between the TF  
941 activity level, inferred based on motif enrichment in the ATAC data, and the gene expression level in the  
942 RNA data. The peak-gene correlation is quantified by calculating for each peak-gene pair a Spearman's  
943 rank-order correlation coefficients (SRCC) between a normalized ATAC peak level and a gene expression  
944 levels for the cis-peaks (middle panel, 7,833 pairs) and the trans-peaks (bottom panel, 118.7 million pairs),  
945 respectively. X-axes are the SRCCs estimated from the co-assayed cells, which serve as the gold standard,  
946 while Y-axes are the SRCCs estimated from the pseudo-cells generated by each method. The overall  
947 concordance between X and Y are further quantified using a single SRCC shown on the up-left corner of  
948 each subfigure. Cis is defined as gene bodies plus 2,000 bps upstream. Reconstructing the gene expression  
949 and the TF activity level (Y-axes) of *NR3C1* using bindSC pseudo-cells **(d)**. X-axis is the averaged  
950 treatment time of the cells in each pseudo-cell. A genome browser view showing putative regulatory  
951 relations between an accessible distal site chr2:201770437-201770992 and the gene *CFLAR* **(e)**. The 6  
952 tracks at the top show ATAC peak levels and gene expression levels at six time points. The track in the  
953 middle shows chromatin interactome from published Hi-C data. The bottom track shows the *NR3C1* binding  
954 targets (ChIP-Seq) peaks published in an independent study <sup>28</sup>.

955  
956 **Fig. 4 Integrating single-cell RNA-seq and ATAC-seq on a mouse retinal cell atlas.** UMAP views of  
957 9,383 mouse retina cells based on gene expression levels in the RNA-seq data **(a)**, chromatin accessibility  
958 peak profiles in the ATAC-seq data **(b)**, gene-level collapsed chromatin accessibility profiles **(c)**. The cells

959 are colored by cell types annotated based on RNA expression levels (**Supplementary Fig. 8**). BindSC co-  
960 embeddings for the cells in the RNA-seq data (**d**) and those in the ATAC-seq data (**e**). Anchoring distances  
961 resulting from bindSC, Seurat, LIGER and Harmony (**f**). The median anchoring distance for each cell type  
962 was highlighted as a bold horizontal bar in the middle of each box in each panel. The dotted line denotes the  
963 anchoring distance based on random guesses. Zoomed out UMAP views for the BC cells in the co-  
964 embeddings generated by bindSC (**g**), Seurat (**h**), LIGER (**i**), and Harmony (**j**). Integration results for all  
965 the cell types can be seen in **Supplementary Fig. 9**. RGC: retinal ganglion cells; HC: horizontal cells; BC:  
966 bipolar cells; AC: amacrine cells; MG: Müller glial cell.

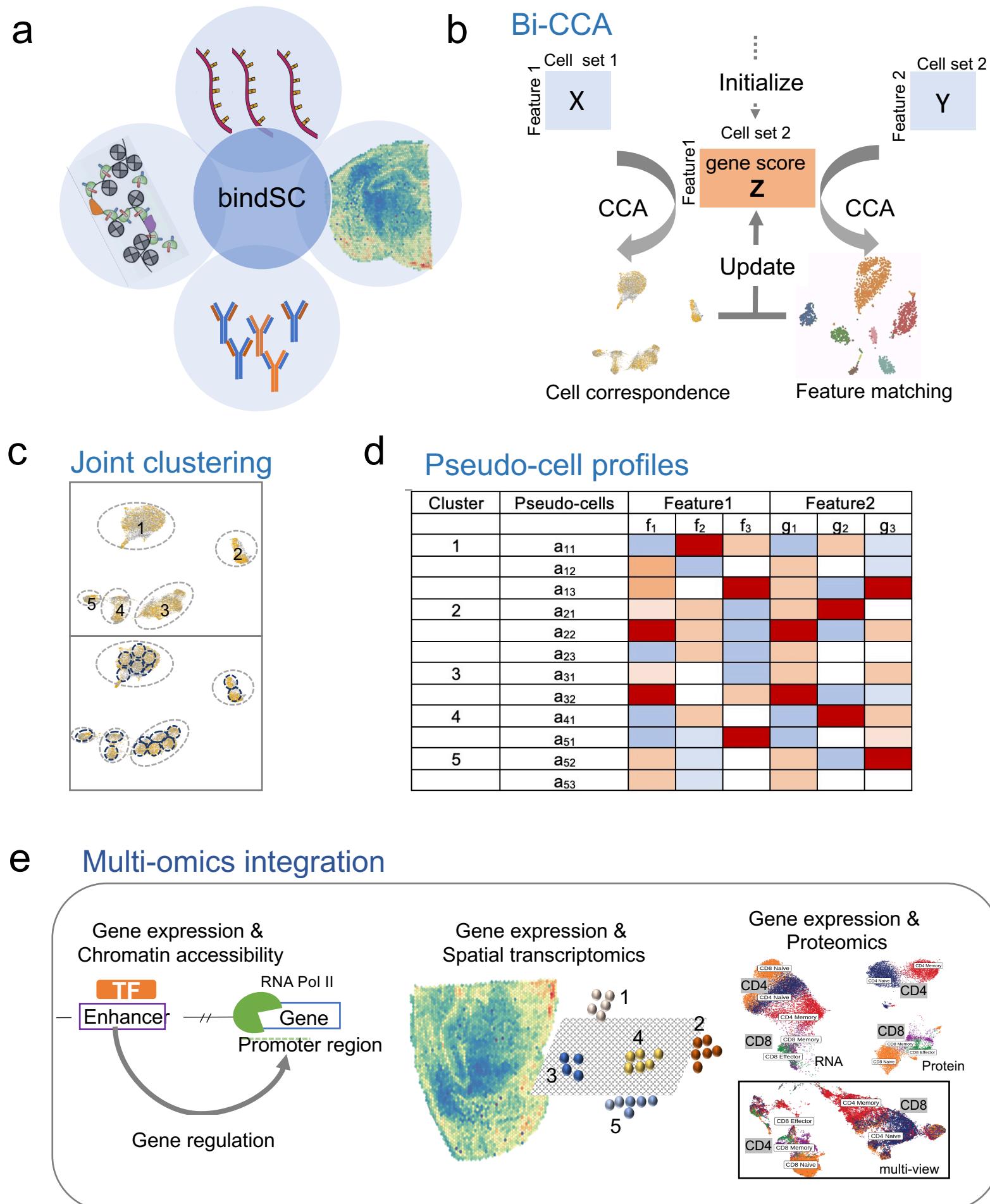
967

968 **Fig. 5 Integrating spatially resolved transcriptomic (ST) data with scRNA-seq data from mouse**  
969 **frontal cortex.** UMAPs of the gene expression levels for the 14,249 cells profiled by SMART-Seq2 and  
970 for the 1,072 spots profiled by the 10x Visium technology (**a**). Cell type labels are from the original  
971 publication<sup>57</sup>. Predicted locations of each cell type in the histological images (**b**). Color gradient  
972 corresponds to the probability score of a cell being mapped to a particular spatial location. Comparison of  
973 cell type frequencies estimated from the ST data (Y-axis) to those estimated from the scRNA-seq data (X-  
974 axis) (**c**). Correlation coefficients (R) and P values are calculated based on Pearson's correlation analysis.  
975 Each dot corresponds to a cell-type (labeled in different colors). The blue line and the grey shade represent  
976 regression lines and 95% confidence intervals from performing linear regressions.

977

978 **Fig. 6 Integrating single-cell RNA with protein data produced by a CITE-seq assay.** The UMAP of  
979 30,672 human bone marrow cells based on 25 surface protein levels (**a**), 3,000 highly variable gene  
980 expression levels (**b**) and 25 protein-homologous gene expression levels (**c**). The cell type labels are from  
981 the original study<sup>17</sup>. UMAP of the protein (**d**) and the RNA (**e**) expression data in the co-embedding  
982 generated by bindSC. Comparison of anchoring distances generated by bindSC, Seurat, LIGER and  
983 Harmony (**f**). The red dotted line denotes the anchoring distance from random guesses. Anchoring distances  
984 for each cell type in the bindSC co-embedding (**g**).

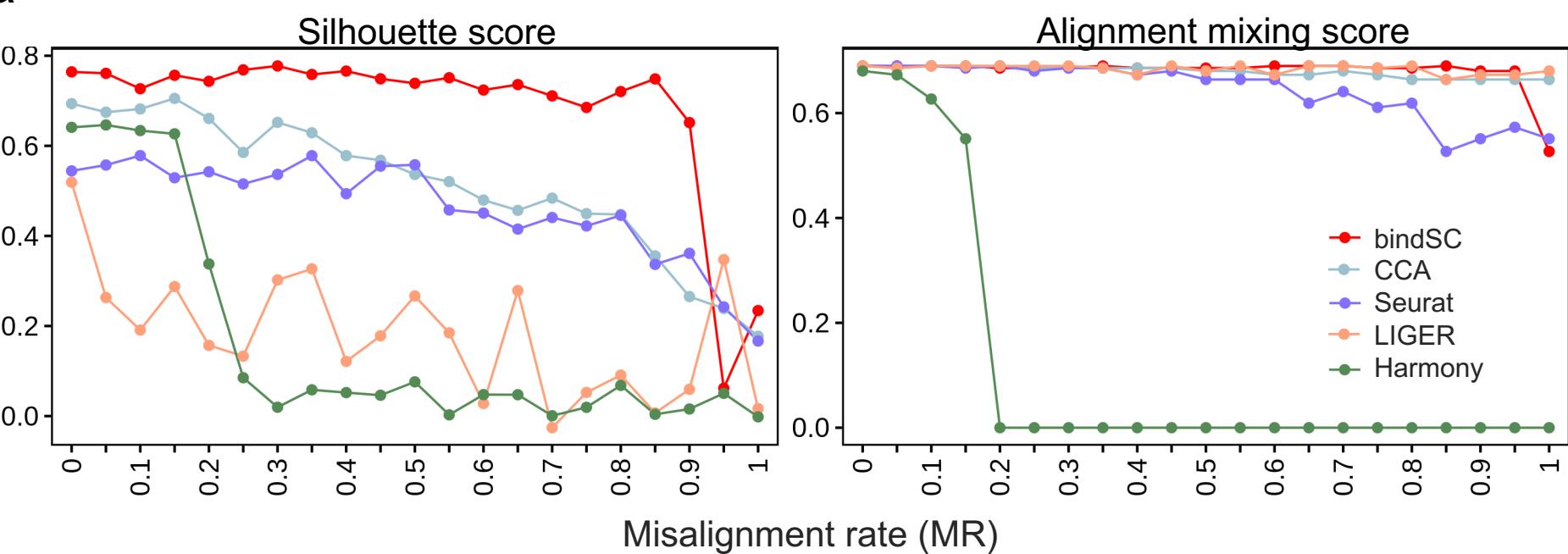
# Fig. 1



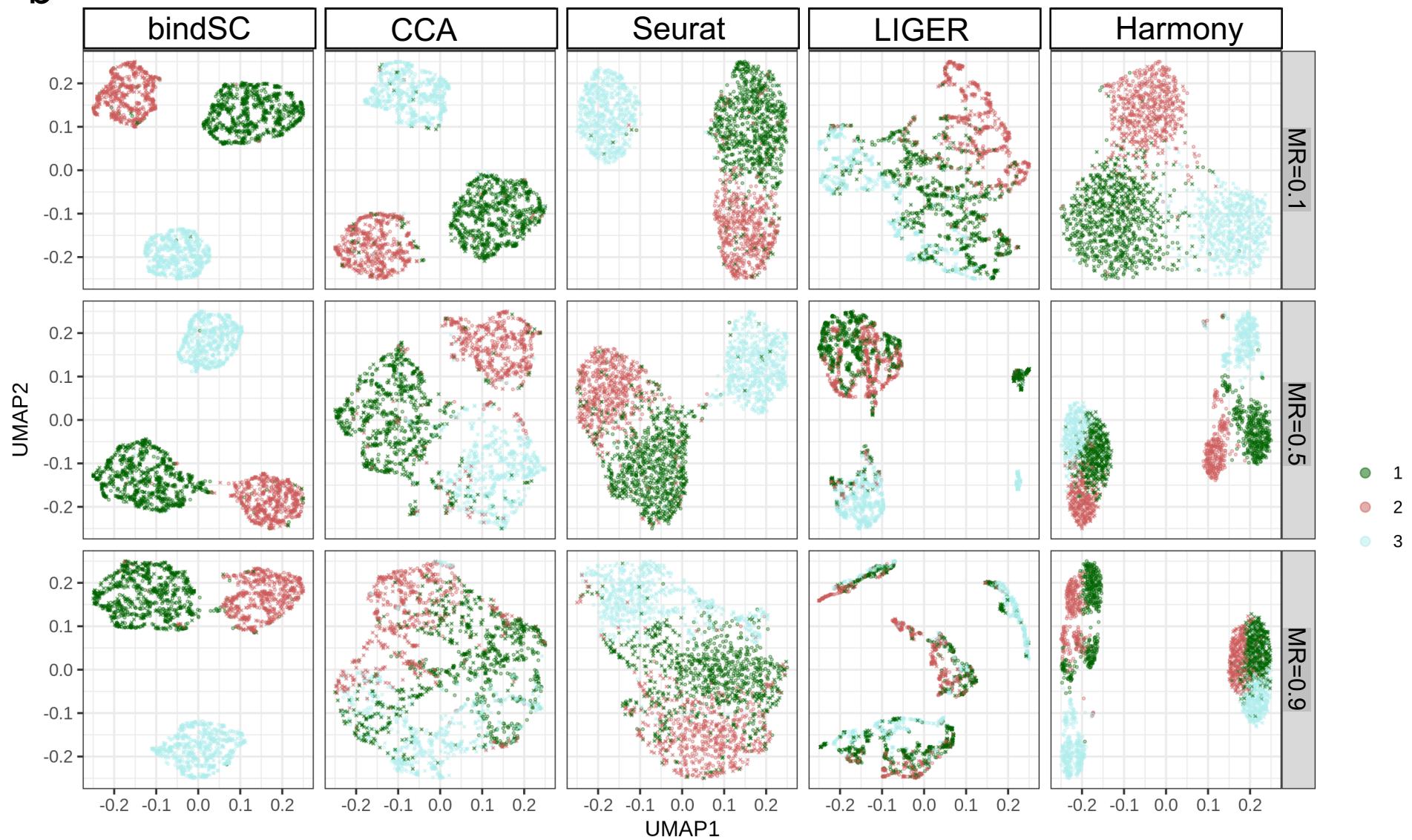
**Fig. 1 Overview of bindSC.** BindSC takes as input two data matrices produced by different modalities from the same cell population (a). The modalities may include transcriptomes, epigenomes, spatial transcriptomes and proteomes. Bi-order integration of two modalities (**X** and **Y**) with unpaired cells and unmatched features using Bi-CCA algorithm (b). In the data matrices, each row represents one gene/locus, and each column represents one cell. The gene score matrix **Z** that links the first modality with the second is initialized by prior gene activity modeling (see **Methods**). Bi-CCA algorithm aims to update gene score matrix **Z** iteratively by maximizing the correlation of between **X** and **Z** and between **Y** and **Z** simultaneously. Based on canonical correlation vectors (CCVs) in the derived latent space, K-nearest neighbor (KNNs) clustering is performed to define cell types in both modalities (c). Within each cell-type cluster, KNN clustering is further performed at a higher resolution to define pseudo-cells consisting of 10s cells from both modalities. *In silico* multimodal profiles are constructed from cells assigned to the same pseudo-cell (d). The color in each box indicates the relative level of each feature, with white corresponding to missing values. The multiomics feature profiles enable us to 1) link genes to regulatory elements, 2) map RNA expressions to spatial locations and 3) delineate cells by both RNA and protein signatures (e).

Fig. 2

a

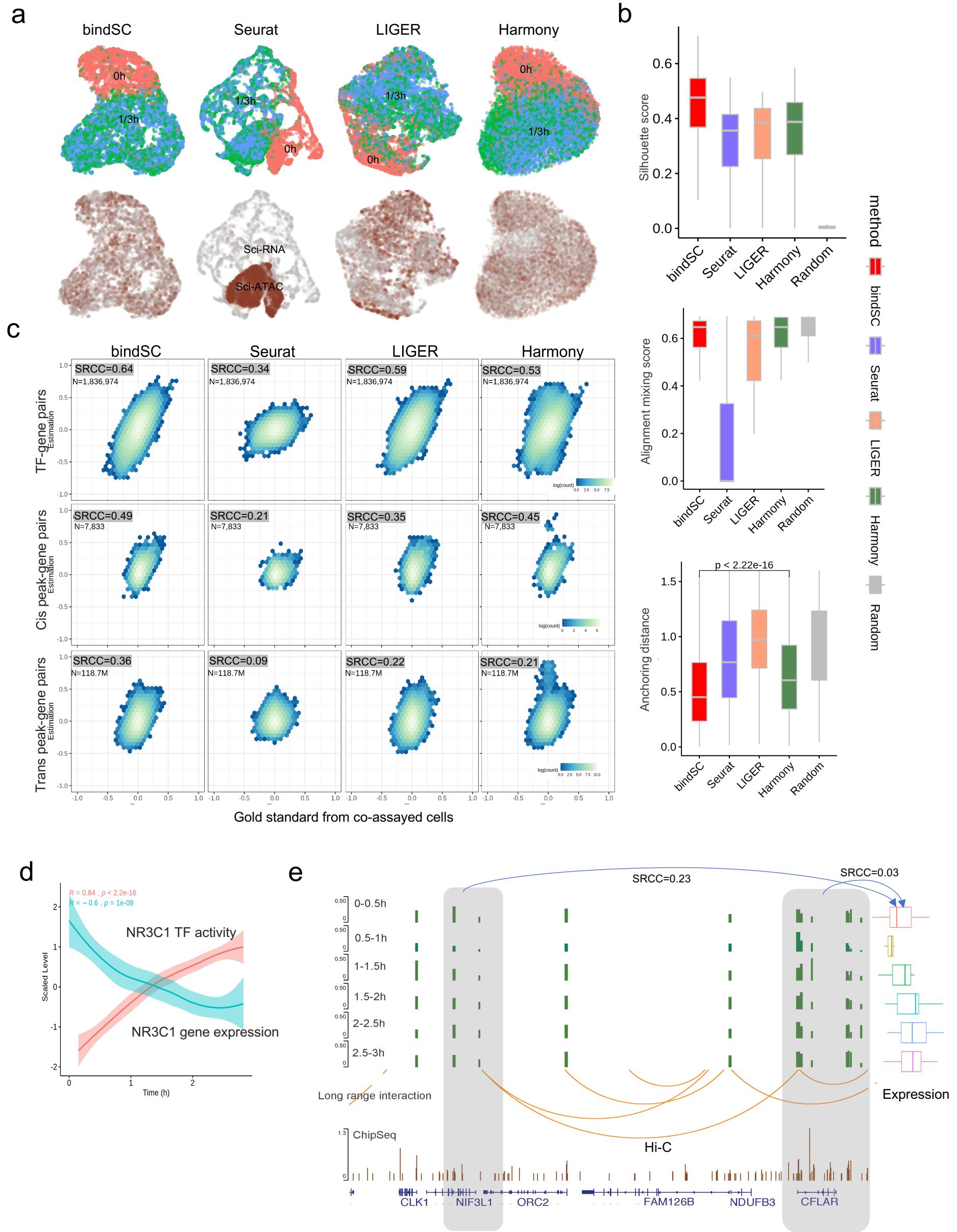


b



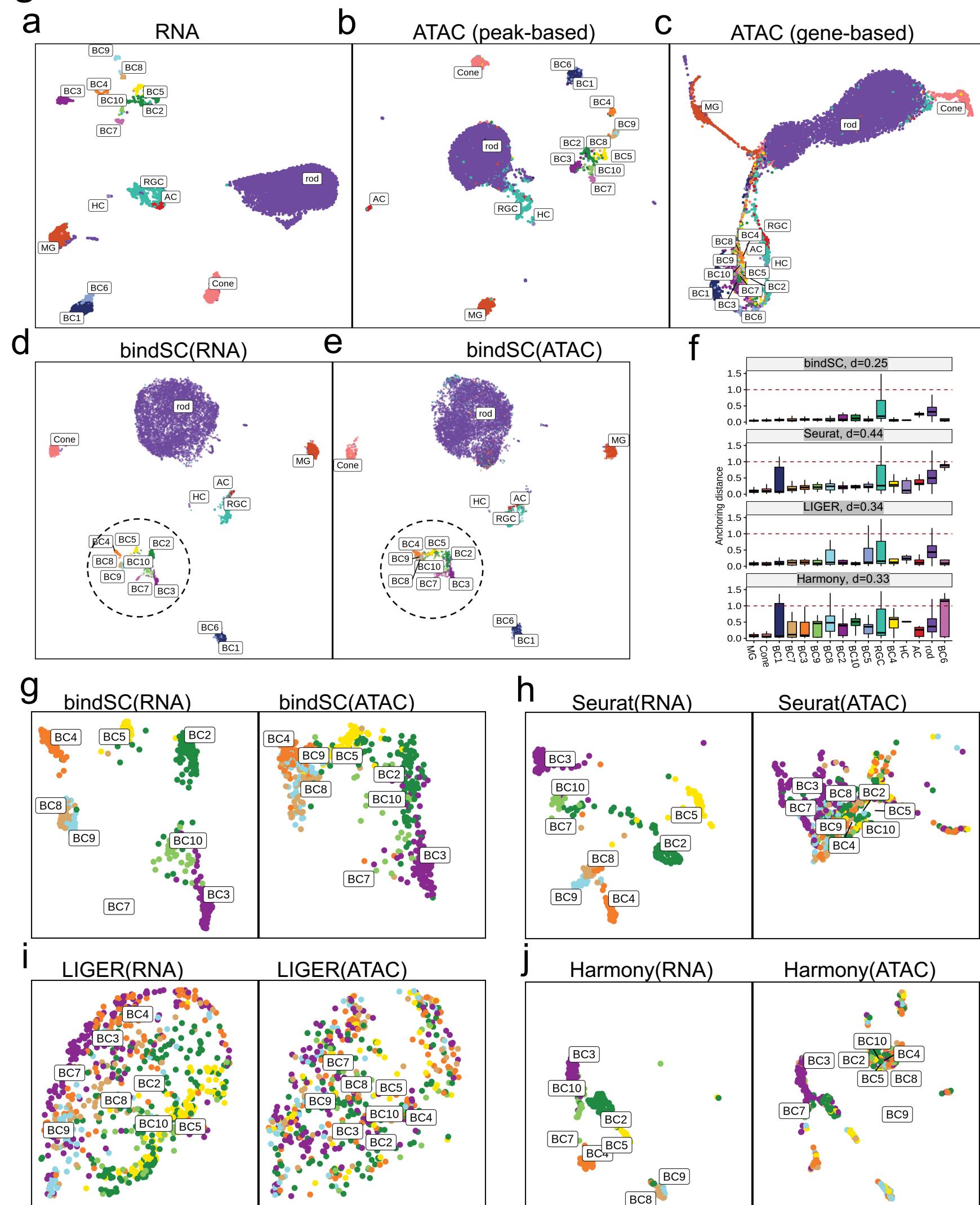
**Fig. 2 Benchmarking bindSC performance on simulation datasets.** Comparison of bindSC to CCA, Seurat, LIGER, and Harmony based on Silhouette score and alignment mixing score (a). The dataset contains 1,000 genes and 1,000 cells equally distributed in 3 cell types. Signal-to-noise ratio (SNR) was set at 0.25. X-axes denote the misalignment rates (MR) between features in the two datasets, which ranges from 0 to 1. The features between two datasets have perfect match if  $MR = 0$  and are unrelated if  $MR = 1$ . UMAP views of the co-embeddings generated by bindSC, CCA, Seurat, LIGER, and Harmony (b). From top to bottom are results with  $MR = 0.1$ ,  $0.5$ , and  $0.9$ , respectively. Each point denotes one cell that is colored based on its true cell type label (red, green, or cyan).

# Fig. 3



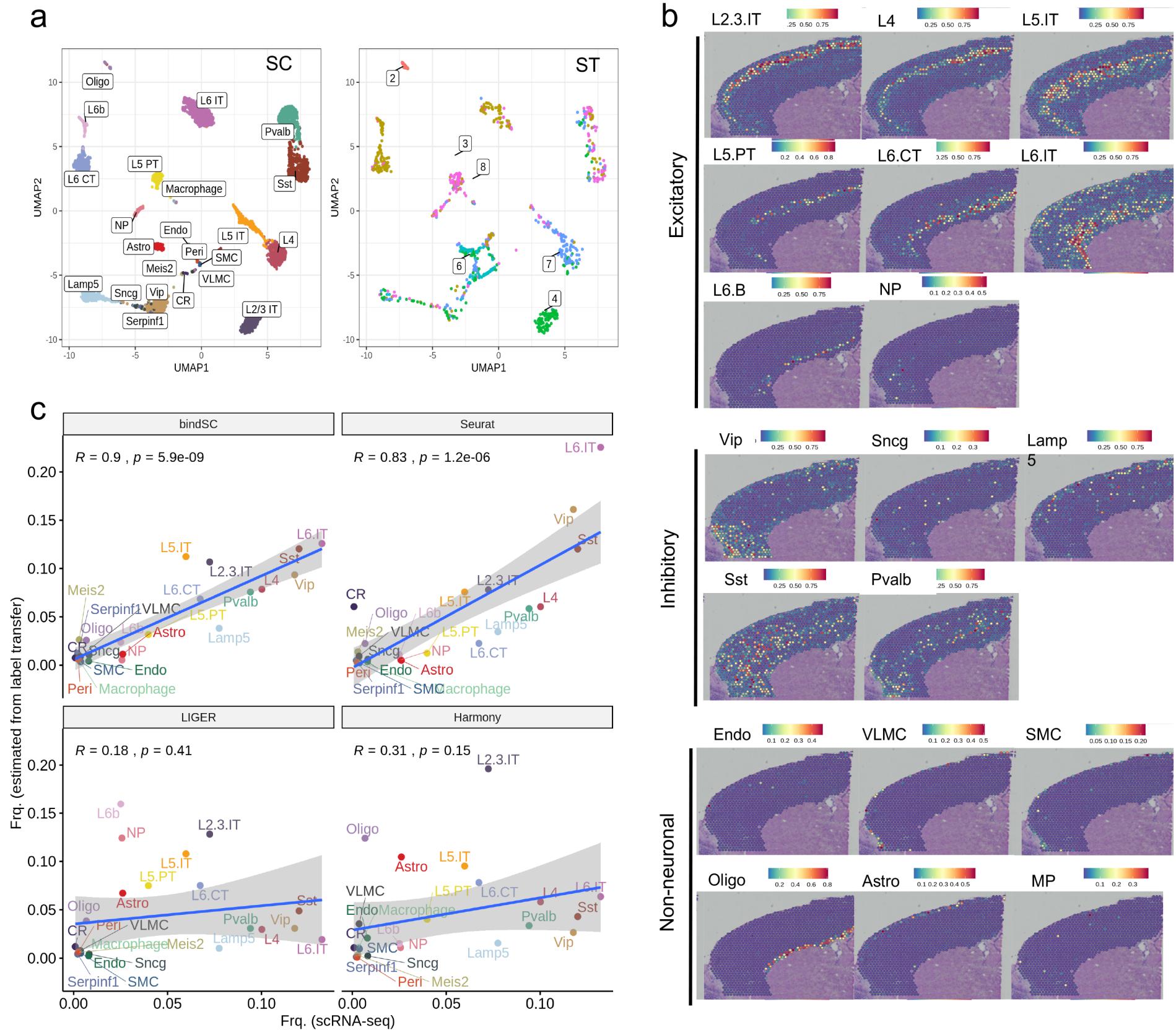
**Fig.3 Benchmarking bindSC performance on the DEX-treated A549 cell-line data.** UMAP of cells from DEX-treated A549 cell-line data for bindSC, Seurat, LIGER and Harmony respectively, colored by collection time (red: 0 hour, green: 1 hour and blue: 3 hour) on the top panel and by technologies (grey: sci-RNA and brown: sci-ATAC) on the bottom panel **(a)**. Comparison of the 4 methods based on Silhouette score (top), alignment mixing score (middle) and anchoring distance (bottom) **(b)**. Also included for comparison are metrics from randomly selected cells. Accuracy of *in silico* multiomics profile in pseudo-cells **(c)**. The TF-gene correlation is quantified in each pseudo-cell (top panel) by calculating for each TF-gene pair (1.8 million pairs total) a Spearman's rank-order correlation coefficient (SRCC) between the TF activity level, inferred based on motif enrichment in the ATAC data, and the gene expression level in the RNA data. The peak-gene correlation is quantified by calculating for each peak-gene pair a Spearman's rank-order correlation coefficients (SRCC) between a normalized ATAC peak level and a gene expression levels for the cis-peaks (middle panel, 7,833 pairs) and the trans-peaks (bottom panel, 118.7 million pairs), respectively. X-axes are the SRCCs estimated from the co-assayed cells, which serve as the gold standard, while Y-axes are the SRCCs estimated from the pseudo-cells generated by each method. The overall concordance between X and Y are further quantified using a single SRCC shown on the up-left corner of each subfigure. Cis is defined as gene bodies plus 2,000 bps upstream. Reconstructing the gene expression and the TF activity level (Y-axes) of *NR3C1* using bindSC pseudo-cells **(d)**. X-axis is the averaged treatment time of the cells in each pseudo-cell. A genome browser view showing putative regulatory relations between an accessible distal site chr2:201770437-201770992 and the gene *CFLAR* **(e)**. The 6 tracks at the top show ATAC peak levels and gene expression levels at six time points. The track in the middle shows chromatin interactome from published Hi-C data. The bottom track shows the *NR3C1* binding targets (ChIP-Seq) peaks published in an independent study<sup>28</sup>.

Fig. 4

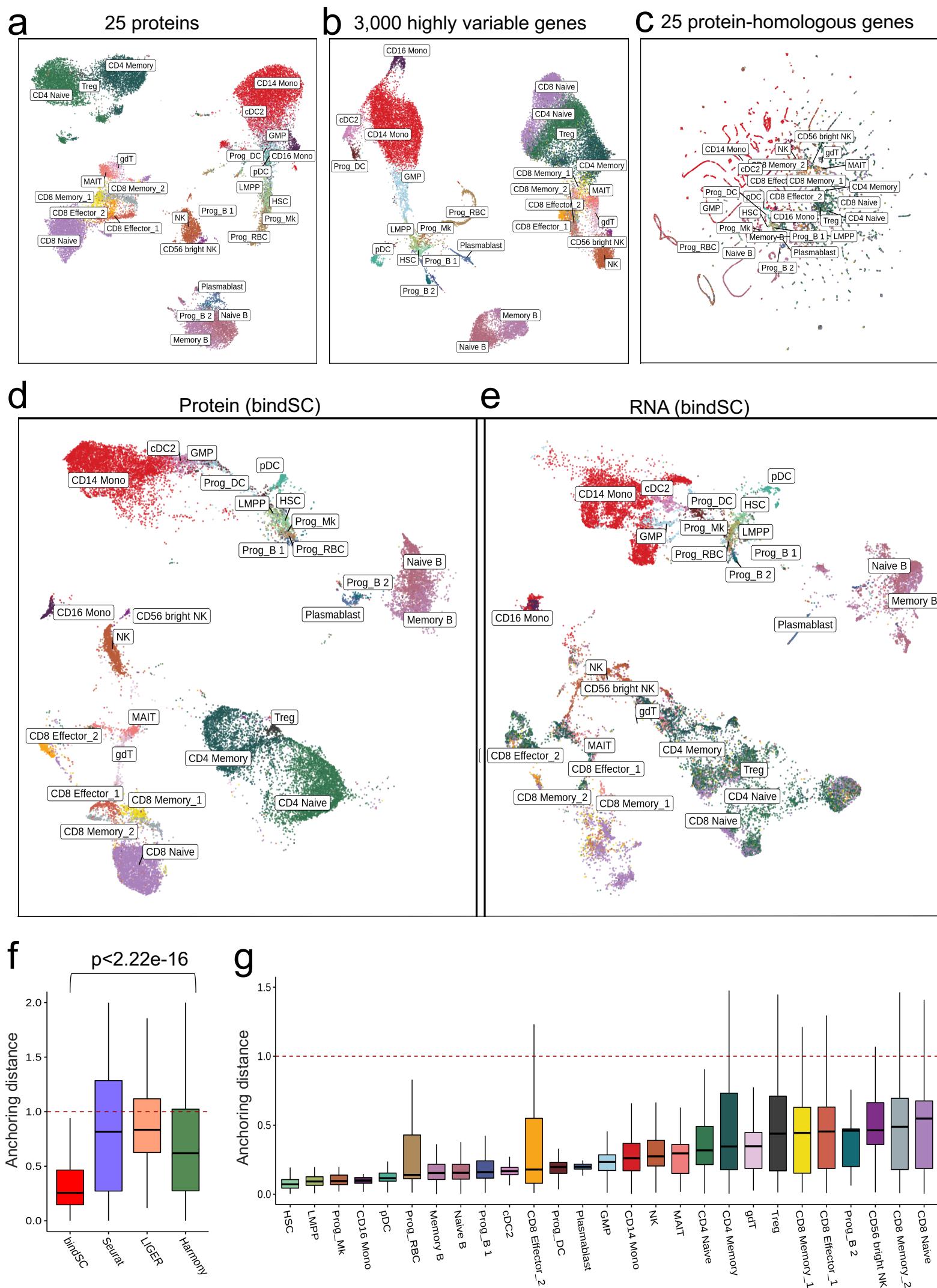


**Fig. 4 Integrating single-cell RNA-seq and ATAC-seq on a mouse retinal cell atlas.** UMAP views of 9,383 mouse retina cells based on gene expression levels in the RNA-seq data **(a)**, chromatin accessibility peak profiles in the ATAC-seq data **(b)**, gene-level collapsed chromatin accessibility profiles **(c)**. The cells are colored by cell types annotated based on RNA expression levels (**Supplementary Fig. 8**). BindSC co-embeddings for the cells in the RNA-seq data **(d)** and those in the ATAC-seq data **(e)**. Anchoring distances resulting from bindSC, Seurat, LIGER and Harmony **(f)**. The median anchoring distance for each cell type was highlighted as a bold horizontal bar in the middle of each box in each panel. The dotted line denotes the anchoring distance based on random guesses. Zoomed out UMAP views for the BC cells in the co-embeddings generated by bindSC **(g)**, Seurat **(h)**, LIGER **(i)**, and Harmony **(j)**. Integration results for all the cell types can be seen in **Supplementary Fig. 9**. RGC: retinal ganglion cells; HC: horizontal cells; BC: bipolar cells; AC: amacrine cells; MG: Müller glial cell.

# Fig. 5



# Fig. 6



**Fig. 6 Integrating single-cell RNA with protein data produced by a CITE-seq assay.** The UMAP of 30,672 human bone marrow cells based on 25 surface protein levels (a), 3,000 highly variable gene expression levels (b) and 25 protein-homologous gene expression levels (c). The cell type labels are from the original study <sup>17</sup>. UMAP of the protein (d) and the RNA (e) expression data in the co-embedding generated by bindSC. Comparison of anchoring distances generated by bindSC, Seurat, LIGER and Harmony (f). The red dotted line denotes the anchoring distance from random guesses. Anchoring distances for each cell type in the bindSC co-embedding (g).