PieParty: Visualizing cells from scRNA-seq data as pie charts

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Abstract

Single cell RNA sequencing (scRNA-seq) has been a transformative technology in many research fields. Dimensional reduction techniques such as UMAP and tSNE are used to visualize scRNA-seq data in two or three dimensions in order for cells to be clustered in biologically meaningful ways. Subsequently, gene expression is frequently mapped onto these plots to show the distribution of gene expression across the plots, for instance to distinguish cell types. However, plotting each cell with only one color leads to repetitive and unintuitive representations. Here, we present Pie Party, which allows scRNA-seq data to be plotted such that every cell is represented as a pie chart, and every slice in the pie charts corresponds to the gene expression of individual genes. This allows for the simultaneous visualization of the expression of multiple genes and gene networks. The resulting figures are information dense, space efficient and highly intuitive. PieParty is publicly available on GitHub at https://github.com/harbourlab/PieParty.

Introduction

Gene expression data at single-cell resolution has brought a lot of opportunity to gain detailed understanding of heterogenous cell populations, but also many challenges regarding its processing and visualization. Principle component analysis (PCA) was initially used to reduce dimensions on single-cell RNA sequencing (scRNA-seq) datasets and to plot them in two or three dimensions. t-SNE and UMAP were developed subsequently, offering superior and global resolution, and are currently the most commonly used dimensional reduction techniques for scRNA-seq[1,2]. For most applications, gene expression is mapped onto t-SNE or UMAP plots, for instance to highlight different cell types or cell states. The common and only approach nowadays is to color the respective cell dots in UMAP or tSNE plots, where the intensity of the color correlates with how high the gene is expressed. However, this only allows to plot one gene per cell, which is inefficient, and results in many repetitive illustrations when expression of multiple different marker genes needs to be shown. This not only consumes valuable figure space, but is

also not intuitive in many cases. Here we present PieParty, a python script that allows users to display each cell in t-SNE, UMAP, or any other single-cell plots with coordinates, as pie charts. Each pie chart can be used to visualize expression of multiple genes at once, and can be customized using different colors or color palettes. PieParty also offers additional settings for normalization and customized plotting.

Results

The basic principle of the PieParty visualization is to generate pie charts for every cell in a single cell sequencing plot, like t-SNE and UMAP plots. The user provides a gene list of all genes that they want to visualize, and PieParty will generate pie charts where each slice in a pie chart represents the normalized gene expression of one individual gene in the list. Each gene (slice) can be assigned a unique color, or a color palette can be chosen to auto-assign unique colors for every gene. Choosing a color palette is useful for larger gene lists. As an example, we analyzed scRNA-seq data from human testis, and clustered the cells with UMAP[3]. The analysis reveals a developmental trajectory, ranging from stem cells to differentiated sperm, in very defined cell stages. Figure 1 and Supplementary Figure 1 shows PieParty plots, where every cell (n = 15,479) is represented by a pie chart, with 145 different differentiation markers plotted per pie chart. Each differentiation marker is automatically assigned a unique color on a color map, sorted from early markers in dark violet to markers of differentiation in yellow. The resulting plot shows that even plotting a large number of genes in pie charts still yields a very informative and intuitive UMAP plot, showing that the expression of these differentiation markers forms a continuum along the differentiation axis from stem cells to differentiated sperm cells.

Besides merely plotting a single list of genes, PieParty also allows for more complex visualizations. As an example, we used scRNA-seq data from uveal melanoma (UM) tumors to demonstrate a more complex application[4]. There are two main classes of UM tumors, class 1, which rarely metastasize, and class 2, which frequently metastasize and have a high mortality

64 rate. Eight biomarkers (EIF1B, FXR1, ID2, LMCD1, LTA4H, MTUS1, ROBO1, SATB1) are
65 expressed in class 1 tumors, and four biomarker genes are up-regulated in the high-risk class 2
66 tumors (CDH1, ECM1, HTR2B, RRAB31). The classical way of visualizing this is by showing 12
67 individual UMAP plots. Supplementary Figure 2 shows all 12 genes plotted separately, which is
68 very space consuming, and while still possible for 12 genes, not immediately comprehensible.
69 For these and other cases, it is further useful to plot class 1 and class 2 tumor cells separately,
70 which is depicted in Supplemental Figures 3 and 4. However, with 24 total plots this consumes
71 even more precious space within a figure using the traditional way. PieParty offers several options
72 to visualize this complex relationship of class 1 and class 2 genes. One consideration to make is
73 that plotting two or more gene lists with different number of genes, in this case eight genes for
74 class 1 versus four genes for class 2, comes with a bias which needs to be corrected for. If one
75 gene list is twice the size of a second, but every gene is expressed in the same amount, the pie
76 would be colored more with colors of the longer gene list. Hence, PieParty allows to normalize for
77 that fact, and weighs gene sets equally by applying a normalization factor to account for the
78 difference in number of genes in each gene list. This normalization technique was applied for all
79 following plots, and the color intensity of the individual pie slices was set to correlate with gene
80 expression, in addition to the size of the pies itself.
81
82 Figure 2a depicts the tSNE plots of 11 UM tumor samples, (3 class 1, 8 class 2, n = 59,915 cells)
83 plotted with PieParty and a simple blue/red color scheme. Class 1 and Class 2 tumor cells were
84 plotted individually, as well as combined in one big plot. It is immediately evident, that the tumor
85 cell markers are not only found in tumor cells, but also in immune cells. Class 2 tumor markers
86 are predominantly enriched in macrophages and monocytes, whereas class 1 tumor markers are
87 present in other immune cells including Lymphocytes, NK cells, T-Cells. Especially interesting is
88 the fact that in this scRNA-seq dataset one class 1 tumor marker is expressed in some class 2
89 immune cells. While all this information is technically visible when all genes are plotted individually

90 in 24 plots (see Supplemental Figures 3 and 4), the PieParty plots are intuitively readable and
91 visualize these relationships in a space efficient way. The information density can be further
92 increased by assigning individual colors to each gene, which PieParty can do automatically.
93 Figure 2b shows the same dataset with color palettes applied automatically to the two gene lists.
94 All labels indicating which gene was assigned which colors are generated by PieParty. This
95 visualization shows that for class 2 tumors the biomarkers expressed by immune cells are
96 different from the genes expressed by tumor cells, whereas for class 1 tumors there is overlap.
97 Together, visualizing this dataset with PieParty, proportional expression of biomarkers, as well as
98 expression amount for each gene are all assessable in one single plot.
99 Another field of application for PieParty is distinguishing cell types and identifying rare cell
100 populations. As an example, we visualized different immune cells from the UM dataset. Figure 3a
101 shows the macrophage population extracted from the complete dataset (n = 8,048 cells), where
102 three M1 macrophage markers assigned the color red, and eleven M2 macrophage markers were
103 assigned blue. As mentioned above, PieParty normalized by gene numbers in the different marker
104 lists, which permits for lists with very different number of genes to be utilized like in this case.
105 Figure 3a shows the clear visualization of M1 and M2 macrophages on the right side of the plot,
106 strongly expressing the indicated markers. Interestingly, there is an equally big population of cells
107 present that express mainly M2 markers but weakly, which cluster to the left of the M1 and M2
108 cells. This provides a striking and clear visualization of macrophage activation heterogeneity and
109 phenotype diversity within the tumor immune microenvironment, Hence, this visualization allows
110 to distinguish these very similar cell types in one plot in a data-driven way, and display the
111 landscape of the expression data. This approach can also be applied to highlight rare cell
112 populations. As an example, we extracted macrophages, monocytes, and undetermined
113 lymphocytes form the UM scRNA-seq dataset (n = 5,111 cells) (Figure 3b). In this dataset T-cells
114 are a rare cell population, comprising only 1.1% of the total cells. We distinguished T-cells by
115 expression of CD3G, CD4, CD8A, and CD8B from monocytes and macrophages, which express

116 CD14, FCGR3A, FCGR1A, CD68, TFRC, CCR5, and ITGAM. The PieParty plot shows how a
117 rare cell population can be clearly distinguished directly in a data-driven way, in contrast to relying
118 on a manually labeled cell cluster.
119 Another functionality in PieParty is to plot the average gene expression per cell cluster. Figure 4
120 depicts the "plot clusters" functionality, which generates one pie chart per cell cluster, with the pie
121 chart size correlating with the number of cells in the respective cluster. This plotting style can
122 greatly simplify complex datasets, and allow for an intuitive assessment of the expression of
123 different markers in the clusters.
124
125 **Discussion**
126 Here we present PieParty, which allows single cells from scRNA-seq data to be represented as
127 pie charts instead of single-colored dots. PieParty plots are far more information rich, allowing
128 multi-dimensional single cell sequencing data to be represented in an intuitive and space-efficient
129 way, and for the identification of previously unrecognized transcriptional heterogeneity. We have
130 presented use cases including gene lists with large number of genes to display expression of
131 differentiation markers in sperm differentiation (Figure 1), discern tumor risk classes (Figure 2),
132 characterize immune cell infiltrates (Figure 3a), as well as highlight rare cell populations (Figure
133 3b). With the public availability of various gene sets, this new visualization technique can be
134 utilized to visualize various cell types, cell states, pathways, cell cycle, senescence, gene
135 networks and many more[5]. Together, PieParty can provide deeper insights into biology derived
136 from single cell sequencing data by allowing for multidimensional visualization of high-density
137 datasets.
138
139

140 **Methods**

141 scRNA-seq data for testis and UM are publicly available [3,4], and were downloaded, analyzed with
142 Seurat (Version 3.2.2) as previously described[4]. The pure macrophage population was generated
143 by extracting cells with CD68 expression > 1. The macrophages, monocytes, and undetermined
144 lymphocytes population used for rare cell population identification was generated by extracting
145 cell clusters based on cell type classifications generated from the original analysis[4].
146 PieParty plots were generated using PieParty 1.4 - 1.8, with standard settings. For Figure 1a
147 "lighten colors", "-lc" was set to "False".
148

149 **Author contribution statement**

150 SK developed PieParty, and prepared the manuscript. JJD helped with design, data acquisition,
151 analysis, and manuscript preparation. MAD helped with design and manuscript preparation. JWH
152 helped with overall design, data interpretation, and manuscript preparation.
153

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165

166 **References**

167 1 van der Maaten, L. & Hinton, G. Viualizing data using t-SNE. *Journal of Machine Learning*

168 *Research* **9**, 2579-2605 (2008).

169 2 McInnes, L., Healy, J. & Melville, J. Umap: Uniform manifold approximation and projection

170 for dimension reduction. *arXiv preprint arXiv:1802.03426* (2018).

171 3 Guo, J. *et al.* The adult human testis transcriptional cell atlas. *Cell Res* **28**, 1141-1157,

172 doi:10.1038/s41422-018-0099-2 (2018).

173 4 Durante, M. A. *et al.* Single-cell analysis reveals new evolutionary complexity in uveal

174 melanoma. *Nat Commun* **11**, 496, doi:10.1038/s41467-019-14256-1 (2020).

175 5 Subramanian, A. *et al.* Gene set enrichment analysis: a knowledge-based approach for

176 interpreting genome-wide expression profiles. *Proc Natl Acad Sci U S A* **102**, 15545-15550,

177 doi:10.1073/pnas.0506580102 (2005).

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179

180 **Figure legends**

181 **Figure 1:** PieParty plot depicting scRNA-seq data from 5,479 testis cells. Around 150
182 differentiation markers were sorted from early (blue) to late (yellow), and auto-assigned with a
183 color.

184

185 **Figure 2:** (a) Class 1 and Class 2 tumor cells plotted separately, with class 1 associated genes
186 in blue, and class 2 associated genes in red. The big plot on the right side of (a) combines all
187 class 1 and class 2 cells ("All cells") (b) Same plot as in Figure 2a, but with auto-assigned color
188 palettes, giving each gene a unique color. Color intensity of each slice correlates with gene
189 expression, in addition to slice size.

190

191 **Figure 3:** PieParty plots of immune cell populations of uveal melanoma tumors. (a) M1 (red)
192 and M2 (blue) macrophage markers are plotted to distinguish M1 and M2 macrophages. (b) Use
193 pf PieParty to plot rare cell populations distinguished by multiple markers. T-cell markers
194 (green) are combined to highlight this rare cell population and distinguish them from monocytes
195 and macrophages. Color intensities correlate with gene expression levels.

196

197 **Figure 4:** PieParty cluster plot. Each cell cluster is assigned one pie chart depicting the average
198 gene expression in the respective cluster. The chart sizes correlate with the number of cells in
199 the cluster. Class 1 tumor genes were auto-assigned colors from the colormap "winter" (blue-
200 green), and class 2 tumor genes were colored with "autumn" (red-yellow).

201

202 **Supplementary Figure 1:** Different color maps applied to the data shown in Figure 1. The
203 colors are automatically assigned to individual genes.

204

205 **Supplementary Figure 2:** tSNE plots for twelve uveal melanoma biomarker genes.

206 **Supplementary Figure 3:** tSNE plots for twelve uveal melanoma biomarker genes in class 1

207 uveal melanoma cells extracted from the entire dataset.

208

209 **Supplementary Figure 4:** tSNE plots for twelve uveal melanoma biomarker genes in class 2

210 uveal melanoma cells extracted from the entire dataset.

211

Figures

Figure 1

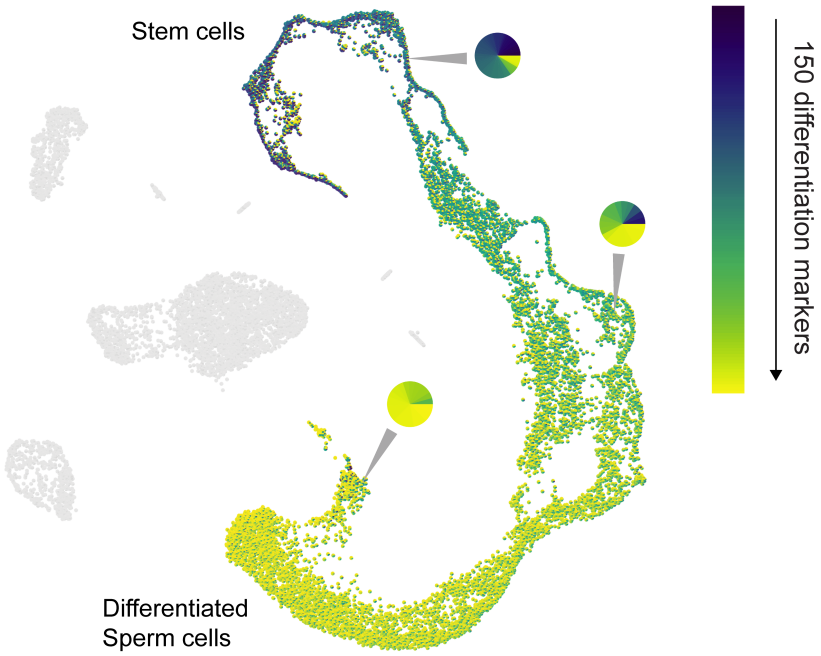


Figure 2

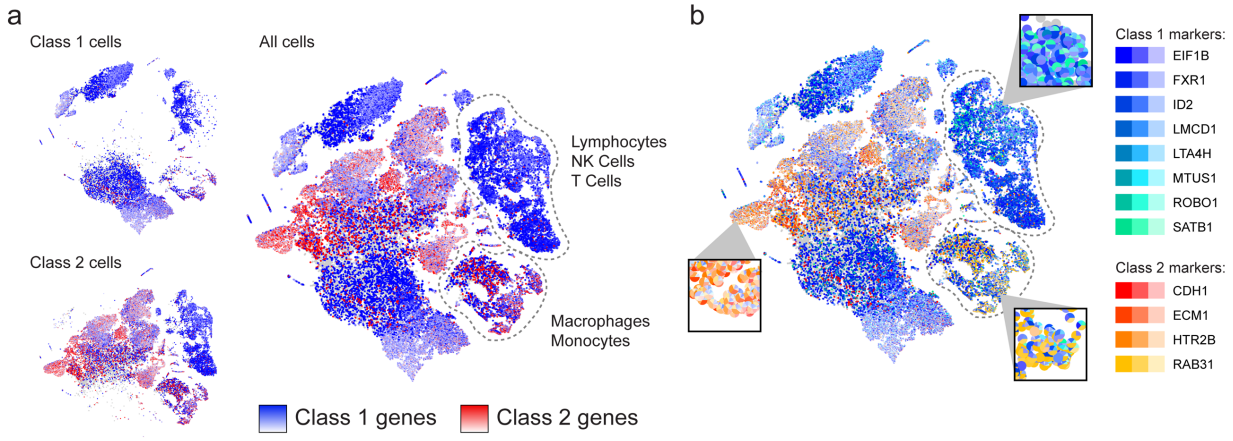


Figure 3

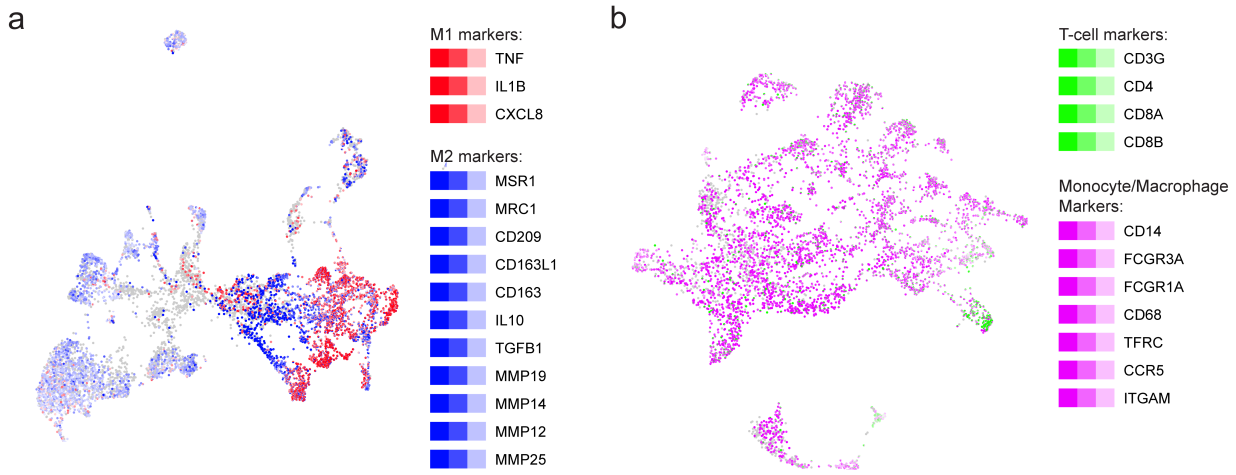
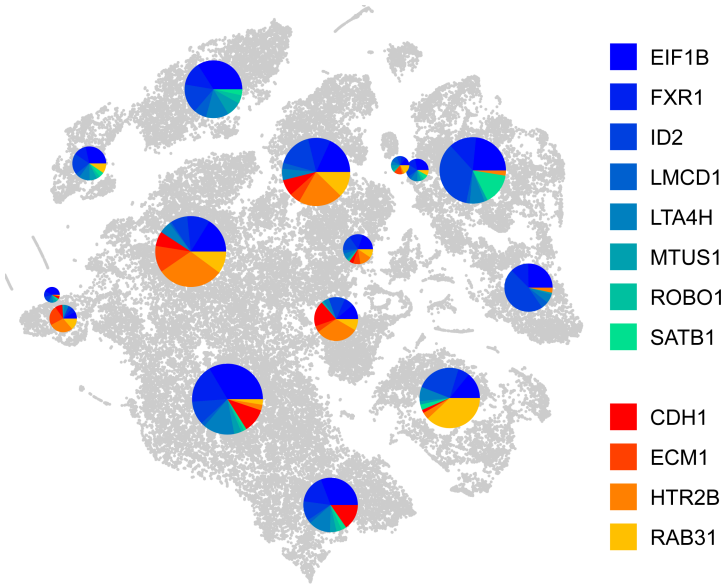
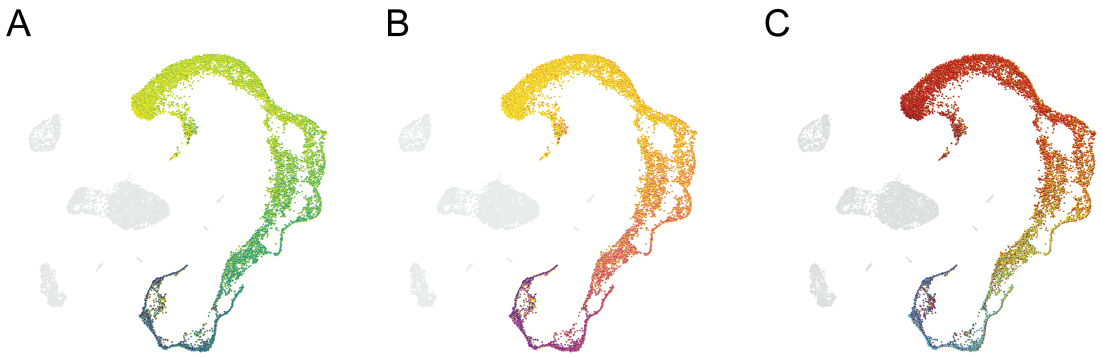


Figure 4



Supplementary Figure 1



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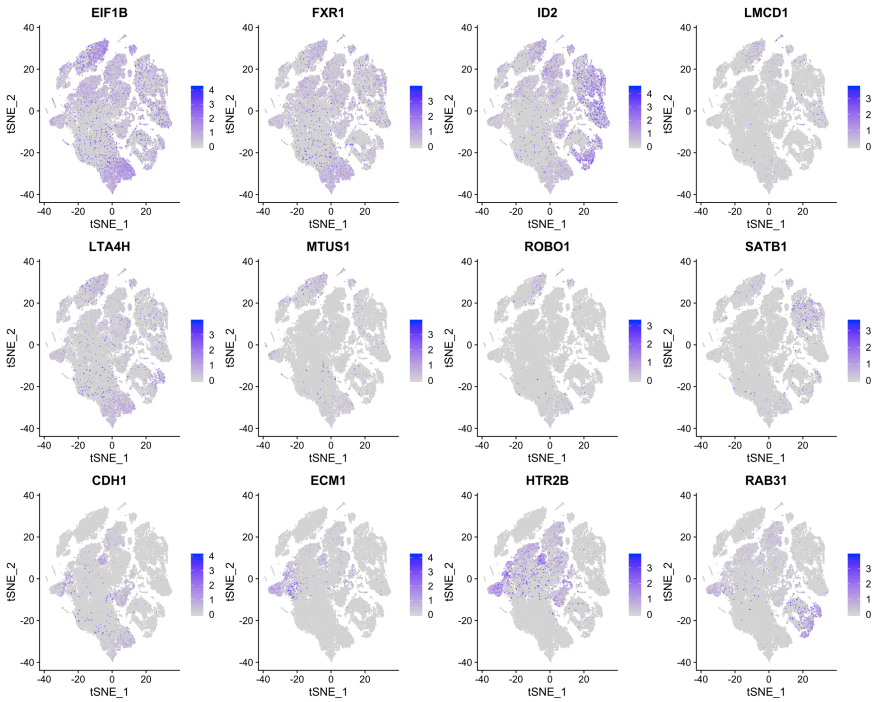
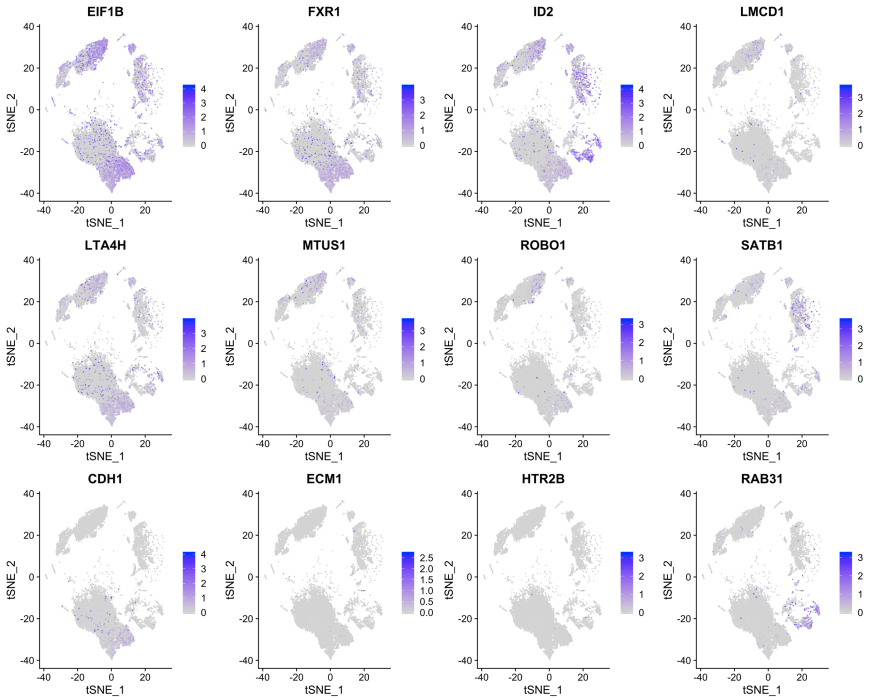
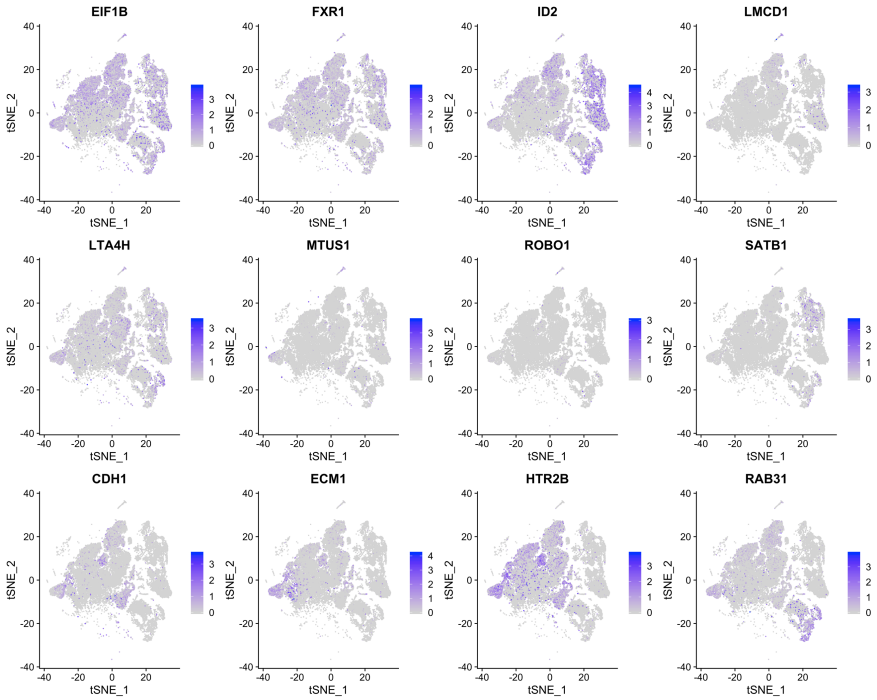


Supplementary Figure 3



Supplementary Figure 4