Analysis of Mouse Adipose Data Set

Table of contents:

Load Packages and Set Global Variables

Imports and Settings

In [1]:

```
import numpy as np
import scanpy.api as sc
import sys
import pandas as pd
import seaborn as sb
import matplotlib.pyplot as plt
```

```
/Users/david.fischer/opt/miniconda3/lib/python3.7/site-packages/an
ndata/_core/anndata.py:21: FutureWarning: pandas.core.index is dep
recated and will be removed in a future version.  The public class
es are available in the top-level namespace.
  from pandas.core.index import RangeIndex
/Users/david.fischer/opt/miniconda3/lib/python3.7/site-packages/sc
anpy/api/__init__.py:6: FutureWarning:

In a future version of Scanpy, `scanpy.api` will be removed.
Simply use `import scanpy as sc` and `import scanpy.external as sc
e` instead.

  FutureWarning,
```

In [2]:

```
base_dir = '/Users/david.fischer/phd/datasets/2019_Ussar_adipocytes
/MUC8387/'
dir_out = '/Users/david.fischer/phd/data/PreadipocytesBrown/results
/'

sc.settings.verbosity = 3 # amount of output
dir_in = base_dir+'mm10_ensrel94/'
dir_tables = dir_out+'tables/'
sc.settings.figdir = dir_out+'panels/'
dir_adata = dir_out+'anndata/'
sc.logging.print_versions()
sc.settings.set_figure_params(dpi=80, scanpy=True)
print (sys.version)
```

```
scanpy==1.4.5.1 anndata==0.7.1 umap==0.3.10 numpy==1.18.1 scipy==1
.4.1 pandas==1.0.1 scikit-learn==0.22.2.post1 statsmodels==0.11.1
python-igraph==0.8.0 louvain==0.6.1
3.7.4 (default, Aug 13 2019, 15:17:50)
[Clang 4.0.1 (tags/RELEASE_401/final)]
```

Global Variables

All embeddings and clusterings can be saved and loaded into this script. Be carful with overwriting cluster caches as soon as cell type annotation has started as cluster labels may be shuffled.

Set whether anndata objects are recomputed or loaded from cache.

```
In [3]: bool_recomp = False
```

Set whether clustering is recomputed or loaded from saved .obs file. Loading makes sense if the clustering changes due to a change in scanpy or one of its dependencies and the number of clusters or the cluster labels change accordingly.

```
In [4]: bool_recluster = False
```

Set whether cluster cache is overwritten. Note that the cache exists for reproducibility of clustering, see above.

```
In [5]: bool_write_cluster_cache = False
```

Set whether to produce plots, set to False for test runs.

```
In [6]: bool_plot = True
```

Set whether observations should be calculated. If false, it is necessary to read cacheed file that contains the necssary information. It then shows the the distributions of counts and genes, as well as mt_frac after filtering. Set to true in order to see the data before filtering and follow the decisions for cutoffs.

```
In [7]: bool_create_observations = True
```

Loading Data, Quality Control and Preprocessing

Read the data in:

```
In [8]: adata_raw = sc.read(dir_in+'raw_gene_bc_matrices.h5ad')
```

Quality Control

Summary of steps performed here: Only cells with at least 500 UMIs are kept. Counts per cell are cell library depth normalized. The gene (feature) space is reduced with PCA to 50 PCs. A nearest neighbour graph and umap are computed based on the PC space. Cell are clustered with louvain clustering based on the nearest neighbour graph. Graph abstraction is computed based on the louvain clustering.

```
In [9]: adata_raw.shape
```

```
Out[9]: (737280, 31125)
```

The data contains 737280 observations with 31125 different genes. Due to dropouts, some of the observations might not show any counts and genes. In order to calculate the fraction of mitochondrial RNA in the next steps, each observations without counts must be filtered out to prevent NaN from emerging.

```
In [10]: print('Total number of cells: {:d}'.format(adata_raw.n_obs))

         sc.pp.filter_cells(adata_raw, min_counts = 1)
         print('Number of cells after min count filter: {:d}'.format(adata_raw.n_obs))

         adata_raw.shape
```

```
Total number of cells: 737280
filtered out 352632 cells that have less than 1 counts
Number of cells after min count filter: 384648
```

```
Out[10]: (384648, 31125)
```

From the 737280 observations, only 384648 are left with counts greater than 0, which is ~52%.

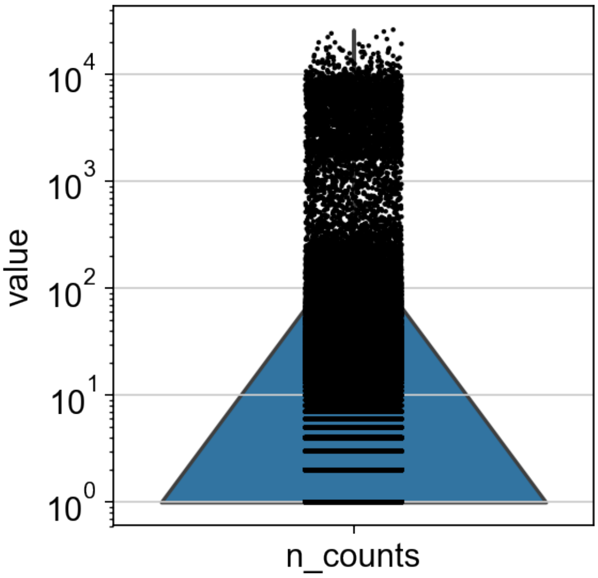
Gene numbers and counts with and without mitochondrial RNA

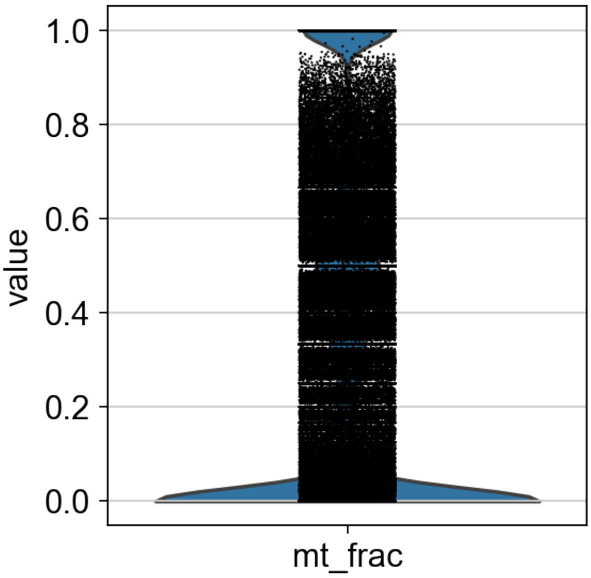
Create necessary obs:

In [11]:
```
adata_qc = adata_raw.copy()
adata_qc.obs['n_genes'] = (adata_qc.X > 0).sum(1)
mt_gene_mask = [gene.startswith('mt-') for gene in adata_qc.var_nam
es]
temp_mt_sum = adata_qc[:,mt_gene_mask].X.sum(1)
temp_mt_sum = np.squeeze(np.asarray(temp_mt_sum))
temp_n_counts = adata_qc.obs['n_counts']
adata_qc.obs['mt_frac'] = temp_mt_sum/adata_qc.obs['n_counts']
```

Plot n_counts and mt_frac:

In [12]:
```
if bool_plot == True:
    t1 = sc.pl.violin(adata_qc, 'n_counts', size =2 ,log=True, cut
= 0)
    t2 = sc.pl.violin(adata_qc, 'mt_frac')
```

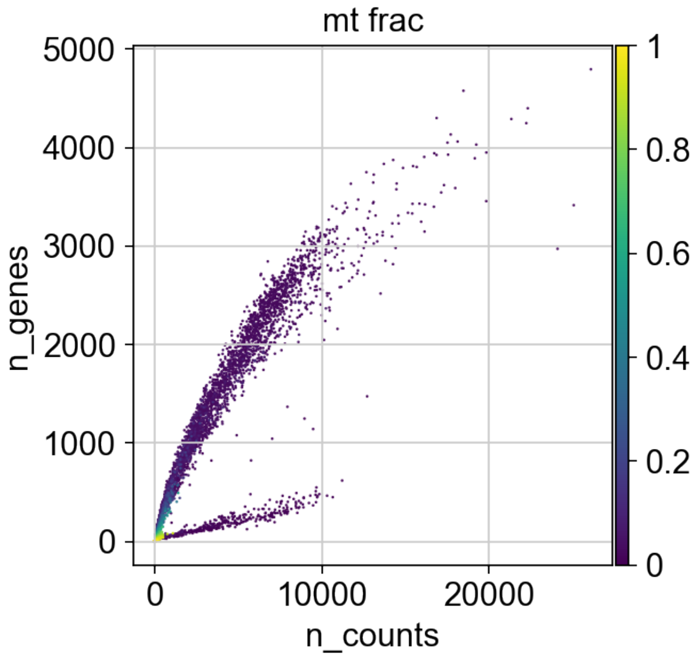


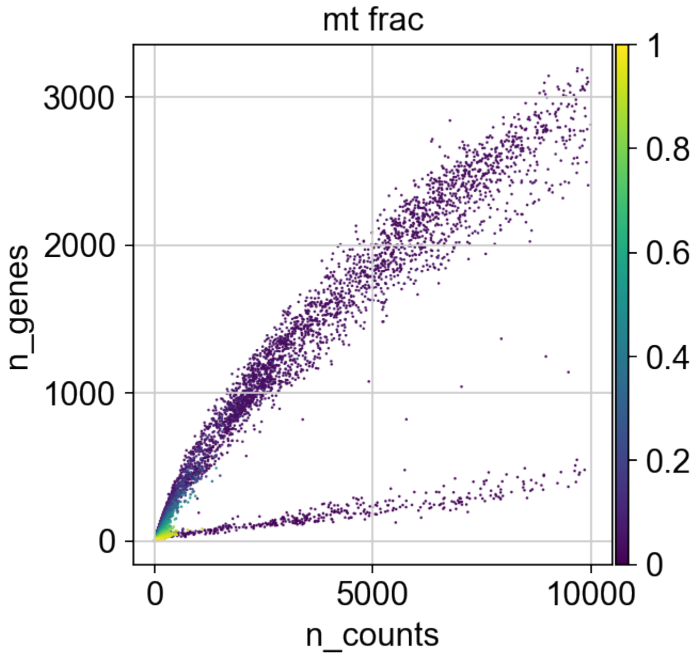


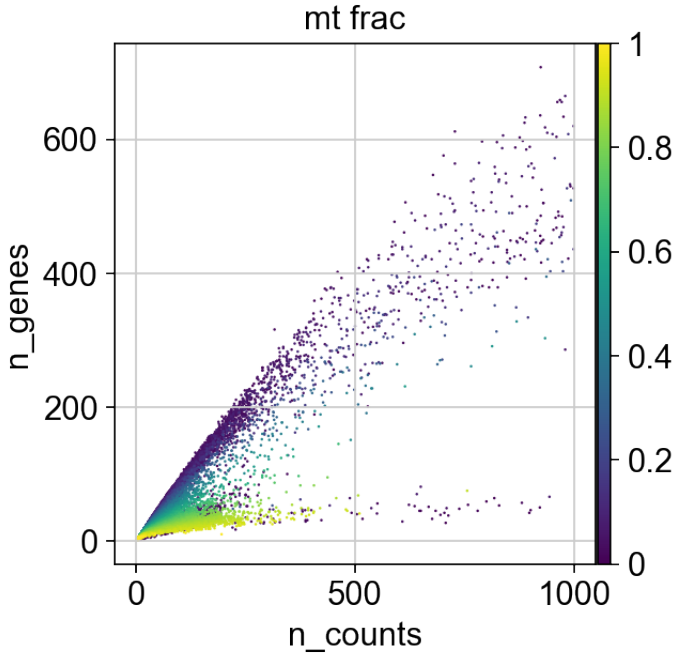
Overall, the data contains a lot of observations with high fractions of mitochondrial RNA. Additionally, most observations show counts below 100, suggesting poor data quality. To further investigate the distributions counts over genes per observations, scatterplots are created:

Number of Genes versus Number of Counts

```
In [13]: if bool_plot == True:
             p1 = sc.pl.scatter(adata_qc, 'n_counts','n_genes',color='mt_frac', size = 5)
             p2 = sc.pl.scatter(adata_qc[adata_qc.obs['n_counts']<10000],'n_counts','n_genes',color = 'mt_frac', size = 5)
             p3 = sc.pl.scatter(adata_qc[adata_qc.obs['n_counts']<1000],'n_counts','n_genes',color = 'mt_frac', size = 5)
```

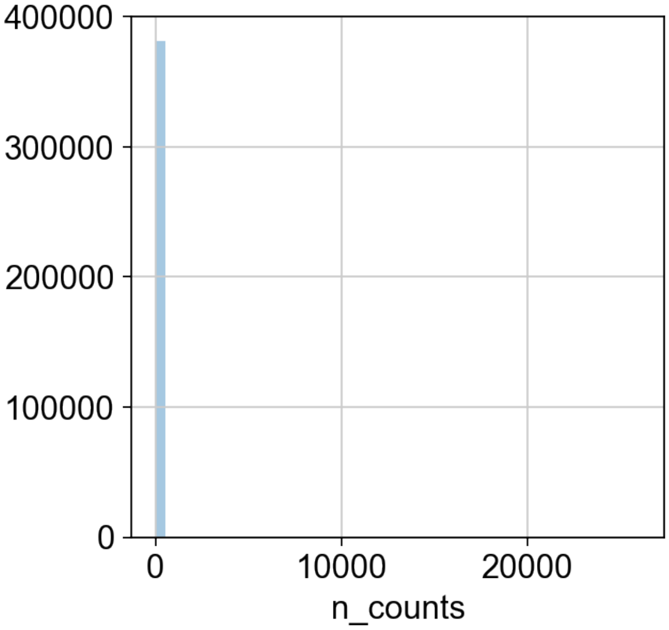


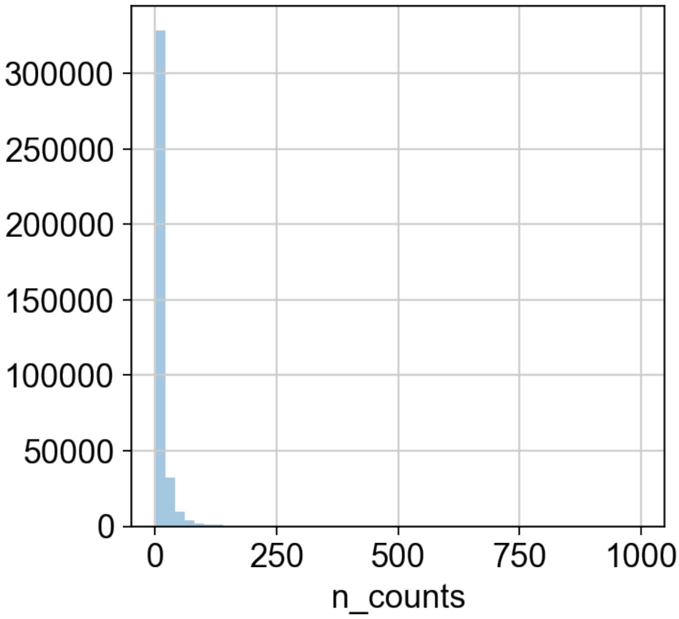


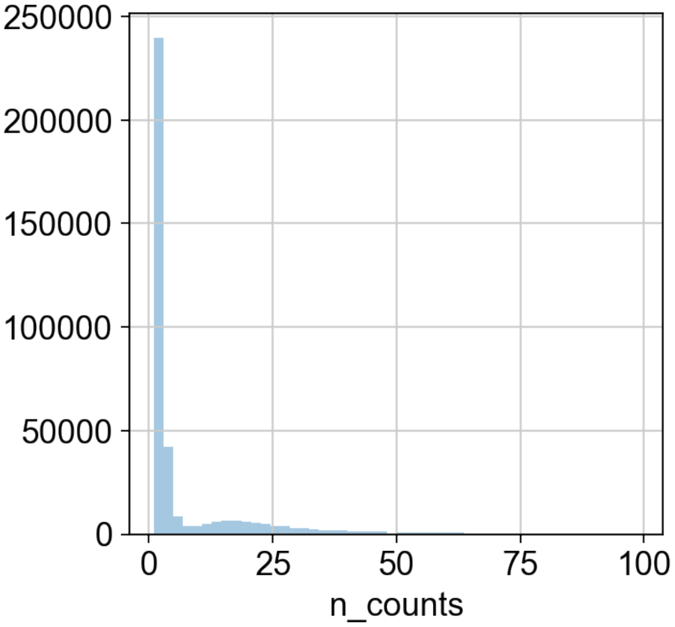


Distribution of Counts and Genes

In [14]:
```
if bool_plot == True:
    p6 = sb.distplot(adata_qc.obs['n_counts'], kde = False)
    plt.show()
    p7 = sb.distplot(adata_qc.obs['n_counts'][adata_qc.obs['n_count
s']<1000], kde = False)
    plt.show()
    p8 = sb.distplot(adata_qc.obs['n_counts'][adata_qc.obs['n_count
s']<100], kde = False)
    plt.show()
```

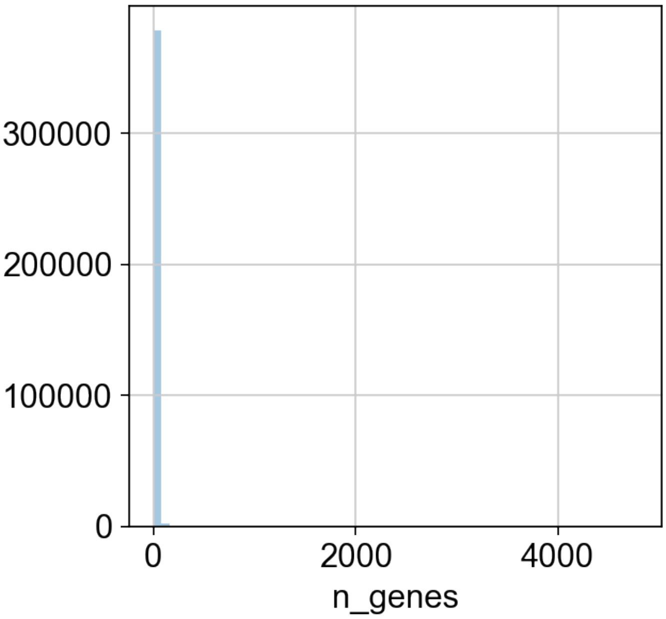

400000
300000
200000
100000
0
0
10000
20000
n_counts

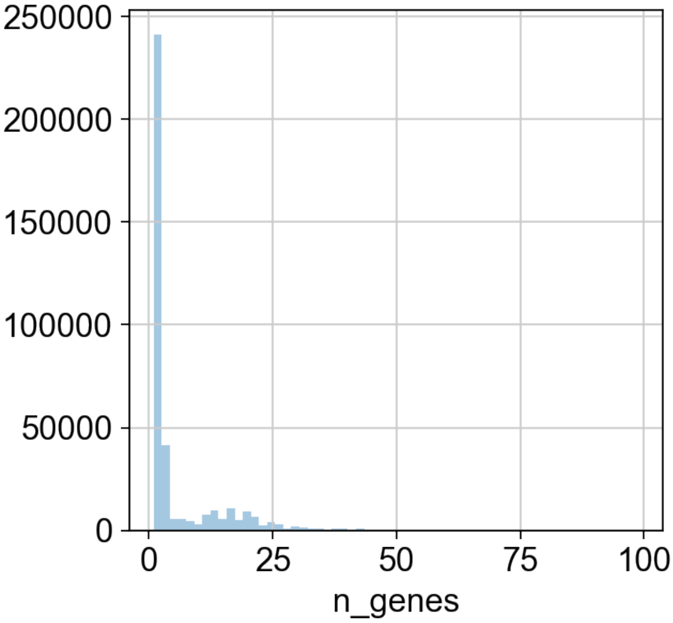

300000
250000
200000
150000
100000
50000
0
0
250
500
750
1000
n_counts



In [15]:

```
if bool_plot == True:
    p9 = sb.distplot(adata_qc.obs['n_genes'],kde = False, bins=60)
    plt.show()
    p10 = sb.distplot(adata_qc.obs['n_genes'][adata_qc.obs['n_genes
']<100],kde = False, bins=60)
    plt.show()
```


300000
200000
100000
0
0
2000
4000
n_genes


250000
200000
150000
100000
50000
0
0
25
50
75
100
n_genes

Filtering

In [16]:

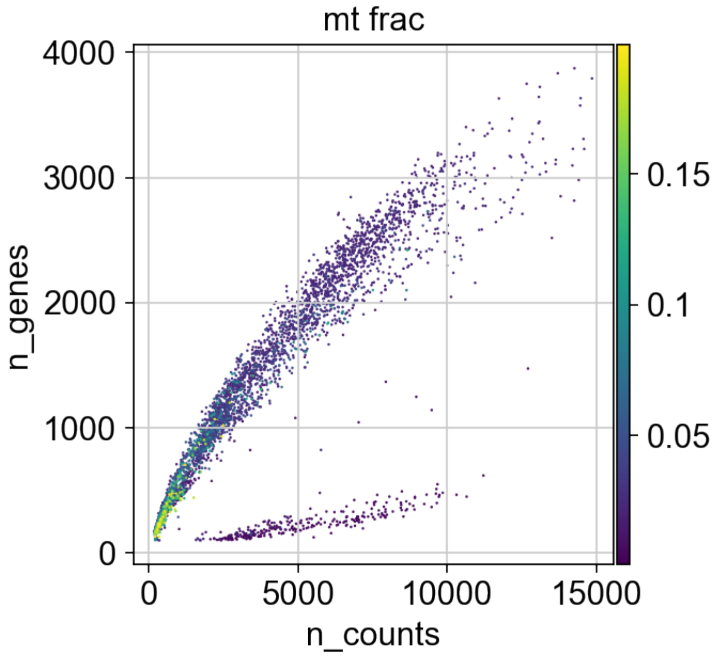
```
# Filter cells according to identified QC thresholds:
print('Total number of cells: {:d}'.format(adata_qc.n_obs))

sc.pp.filter_cells(adata_qc, min_counts = 200)
print('Number of cells after min count filter: {:d}'.format(adata_qc.n_obs))

sc.pp.filter_cells(adata_qc, max_counts = 15000)
print('Number of cells after max count filter: {:d}'.format(adata_qc.n_obs))

adata_qc = adata_qc[adata_qc.obs['mt_frac'] < 0.2]
print('Number of cells after MT filter: {:d}'.format(adata_qc.n_obs))

sc.pp.filter_cells(adata_qc, min_genes = 100)
print('Number of cells after gene filter: {:d}'.format(adata_qc.n_obs))
```

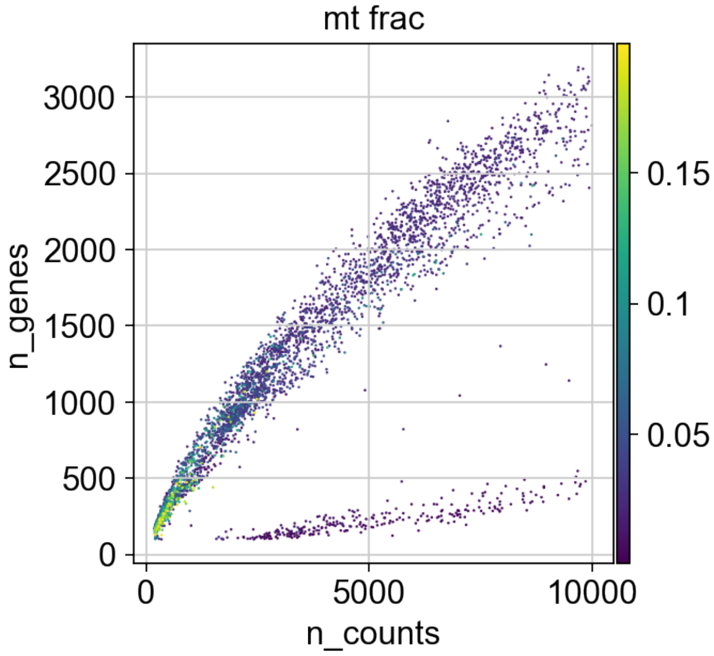
```
Total number of cells: 384648
filtered out 380040 cells that have less than 200 counts
Number of cells after min count filter: 4608
filtered out 32 cells that have more than 15000 counts
Number of cells after max count filter: 4576
Number of cells after MT filter: 4163
filtered out 150 cells that have less than 100 genes expressed

Trying to set attribute `.obs` of view, copying.

Number of cells after gene filter: 4013
```

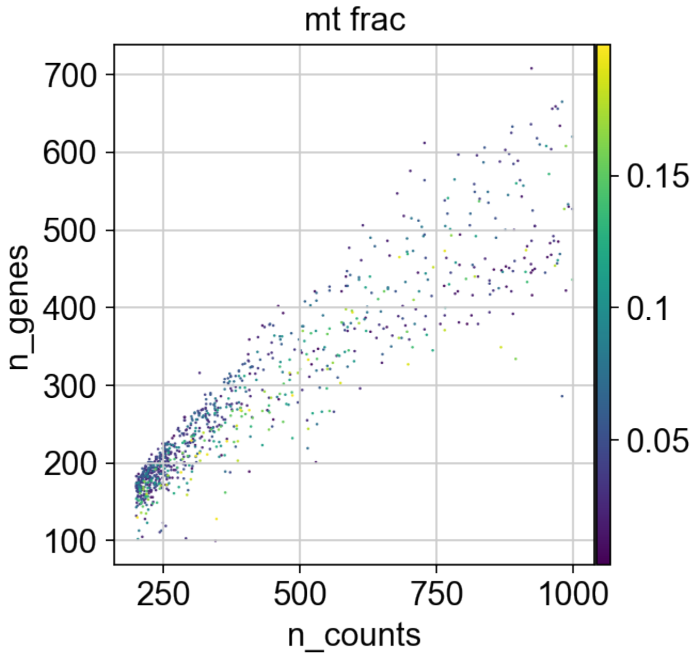
Only 4013 observations are left that pass the filters.

In [17]:

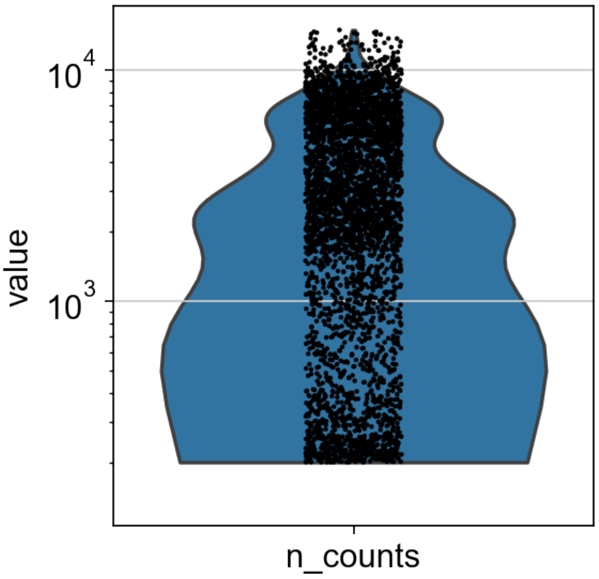
```
if bool_plot == True:
    p12 = sc.pl.scatter(adata_qc, 'n_counts','n_genes',color='mt_frac', size = 5)
    p22 = sc.pl.scatter(adata_qc[adata_qc.obs['n_counts']<10000],'n_counts','n_genes',color = 'mt_frac', size = 5)
    p52 = sc.pl.scatter(adata_qc[adata_qc.obs['n_counts']<1000],'n_counts','n_genes',color = 'mt_frac', size = 5)
```

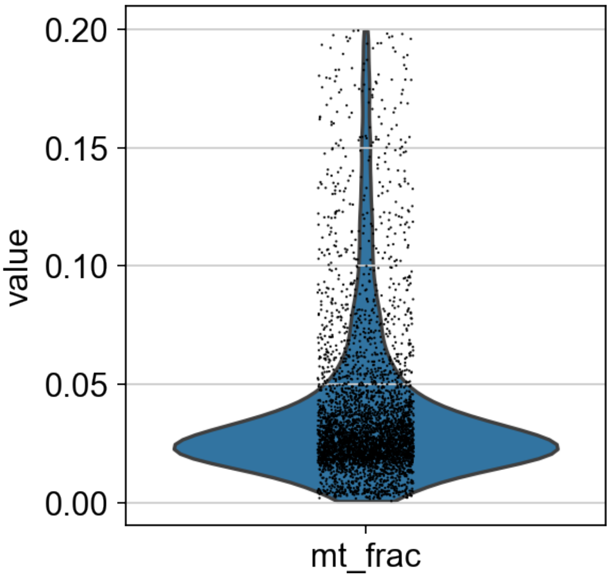

mt frac
n_genes
n_counts
0
1000
2000
3000
4000
0
5000
10000
15000
0.05
0.1
0.15


mt frac
n_genes
n_counts
0
500
1000
1500
2000
2500
3000
0
5000
10000
0.05
0.1
0.15



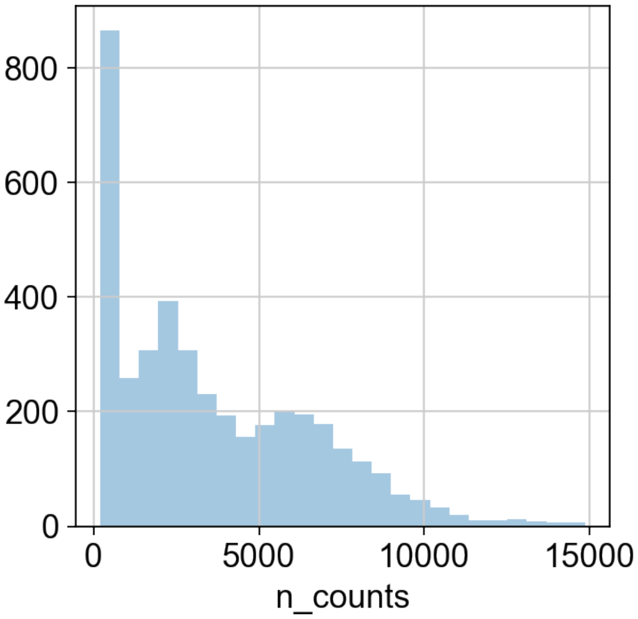
In [18]:
```
if bool_plot == True:
    sc.pl.violin(adata_qc, 'n_counts', size=2, log=True, cut=0)
    sc.pl.violin(adata_qc,'mt_frac')
```

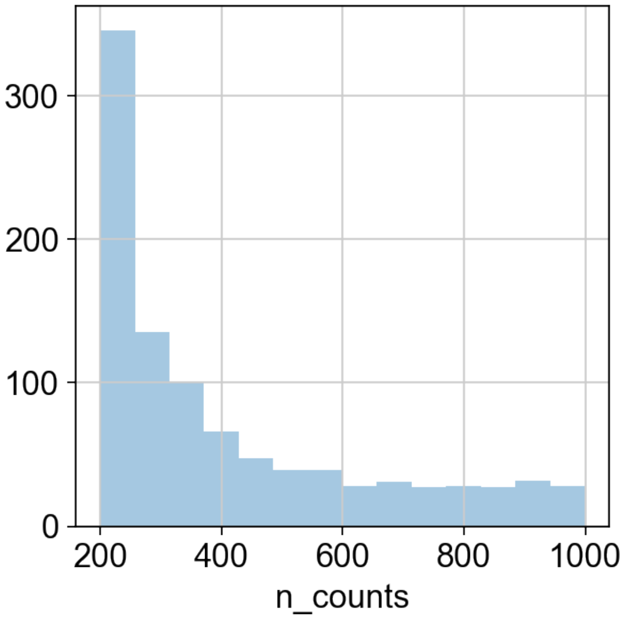




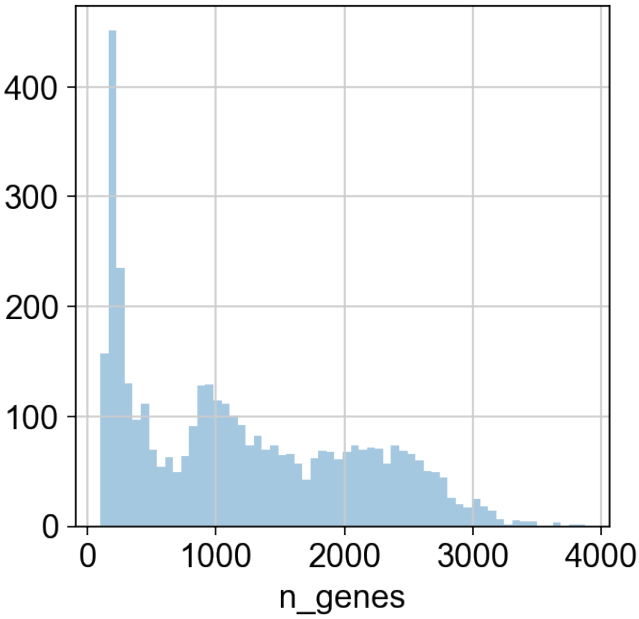
For the remaining observations, the fraction of mitochondrial RNA is generally very low and at most 20%

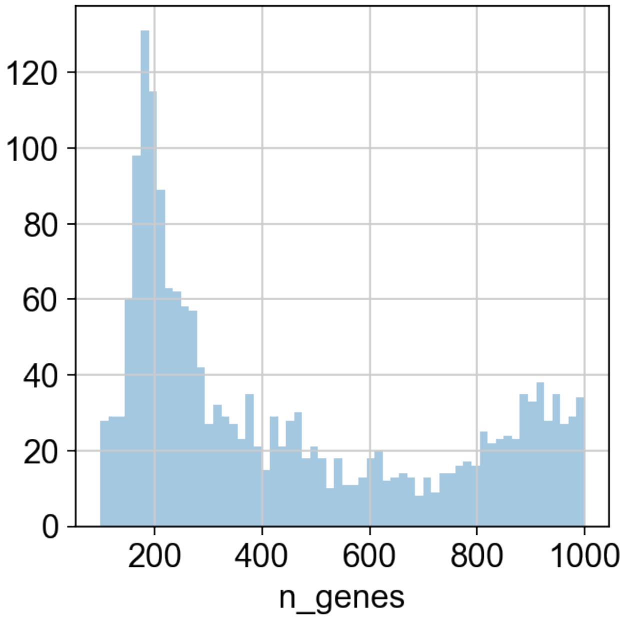
```
In [19]: if bool_plot == True:
             p62 = sb.distplot(adata_qc.obs['n_counts'], kde = False)
             plt.show()
             p72 = sb.distplot(adata_qc.obs['n_counts'][adata_qc.obs['n_coun
         ts']<1000], kde = False)
             plt.show()
```





In [20]:
```
if bool_plot == True:
    p92= sb.distplot(adata_qc.obs['n_genes'],kde = False, bins=60)
    plt.show()
    p100 = sb.distplot(adata_qc.obs['n_genes'][adata_qc.obs['n_genes']<1000],kde = False, bins=60)
    plt.show()
```





In [21]:

```
#Filter genes:
print('Total number of genes: {:d}'.format(adata_qc.n_vars))

# Min 20 cells - filters out 0 count genes
sc.pp.filter_genes(adata_qc, min_cells=20)
print('Number of genes after cell filter: {:d}'.format(adata_qc.n_vars))
```

```
Total number of genes: 31125
filtered out 18501 genes that are detected in less than 20 cells
Number of genes after cell filter: 12624
```

Normalization and Clustering with highly variable genes

In [22]:

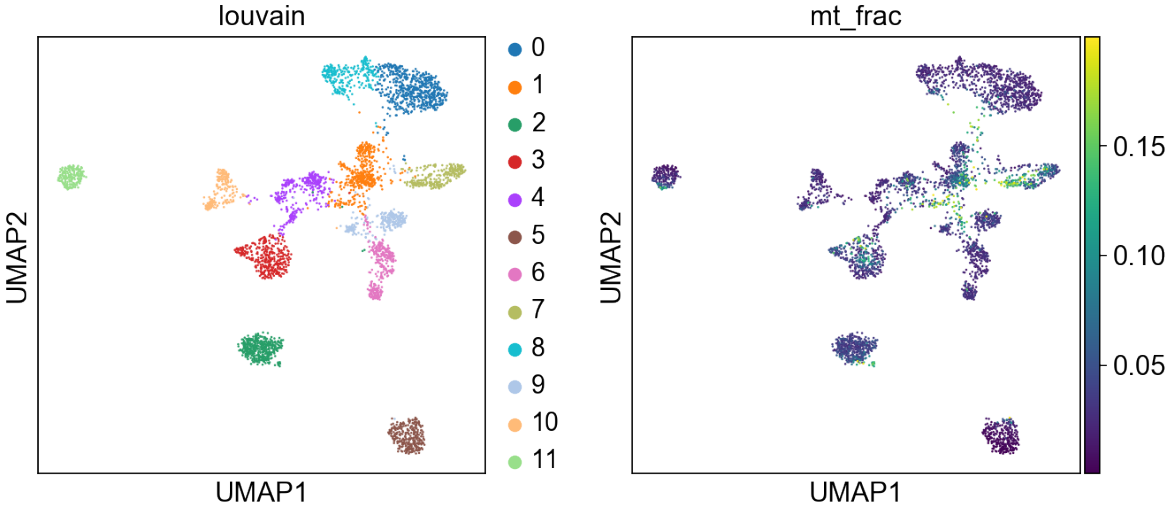
```
if bool_recomp == True:

    adata_proc = adata_qc.copy()
    adata_proc.raw = adata_proc
    sc.pp.normalize_per_cell(adata_proc)
    sc.pp.log1p(adata_proc)
    sc.pp.highly_variable_genes(adata_proc, flavor='cell_ranger',n_top_genes=4000)
    sc.pl.highly_variable_genes(adata_proc)

    sc.pp.pca(adata_proc, n_comps=50, random_state=0, use_highly_variable=True, svd_solver='arpack')
    sc.pp.neighbors(adata_proc, n_neighbors=100, knn=True, method='umap', n_pcs=50, random_state=0)
    #sc.tl.tsne(adata_proc, n_jobs=3)
    sc.tl.umap(adata_proc)
    if bool_recluster == True:
        sc.tl.louvain(adata_proc, resolution=1, flavor='vtraag', random_state=0)
        pd.DataFrame(adata_proc.obs).to_csv(path_or_buf =dir_adata+"obs_adata_proc.csv")
    else:
        obs = pd.read_csv(dir_adata+'obs_adata_proc.csv')
        adata_proc.obs['louvain']=pd.Series(obs['louvain'].values, dtype = 'category')
    sc.write(dir_adata+'adata_proc.h5ad',adata_proc)
else:
    adata_proc = sc.read(dir_adata+'adata_proc.h5ad')
sc.tl.paga(adata_proc)
```

```
running PAGA
    finished: added
    'paga/connectivities', connectivities adjacency (adata.uns)
    'paga/connectivities_tree', connectivities subtree (adata.uns)
(0:00:00)
```

Produce some summarizing plots that show the global characteristics of the data.

```
In [23]: if bool_plot == True:
             sc.pl.umap(adata_proc, color=['louvain','mt_frac'], size=5, sav
         e="_all_louvain.pdf", use_raw = False)
```

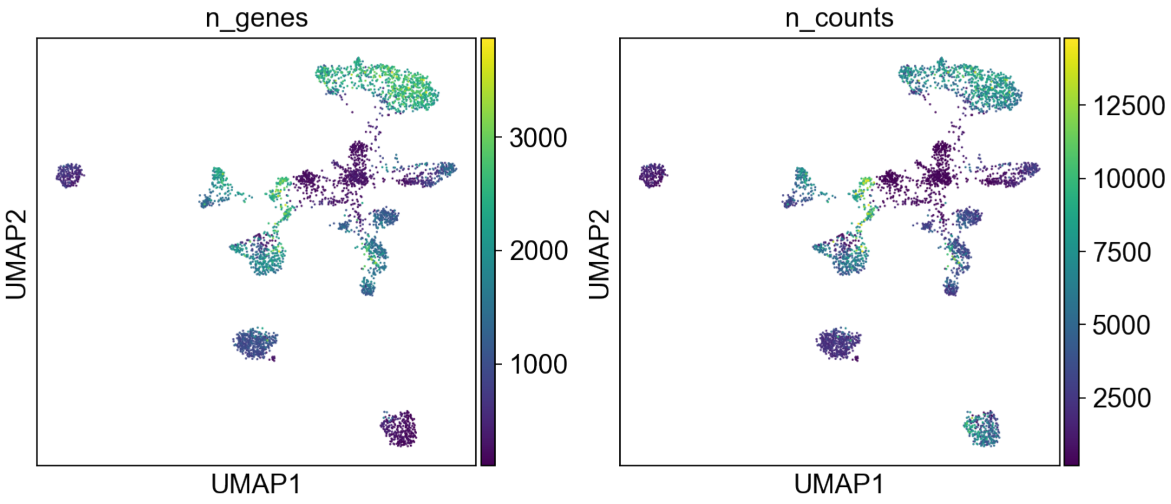
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_all_louvain.pdf
```



A high fraction of mitochondrial RNA is in cluster 7 and around the central cluster 2.

```
In [24]: if bool_plot == True:
             sc.pl.umap(adata_proc, color=['n_genes','n_counts'], size=5, sa
         ve="_all_n_counts.pdf", use_raw = False)
```

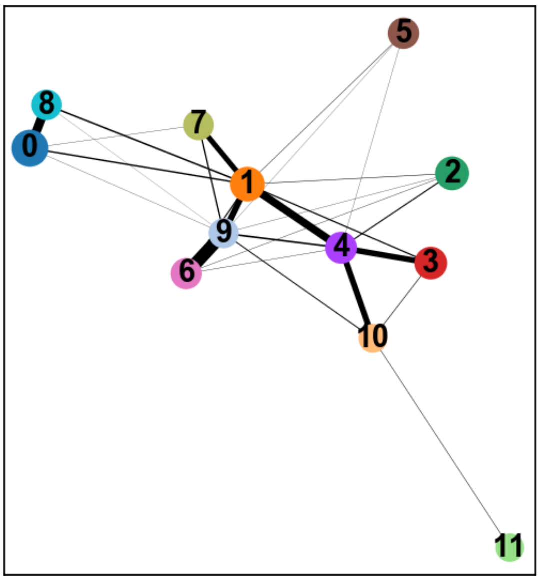
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_all_n_counts.pdf
```



Given the number of genes and number of counts, cluster 0 and 8 could contain preadipocytes.

```
In [25]: if bool_plot == True:
             sc.pl.paga(adata_proc, save="_all.pdf")
```

```
--> added 'pos', the PAGA positions (adata.uns['paga'])
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/paga_all.pdf
```



Number of cells in each cluster:

```
In [26]: adata_proc.obs["louvain"].value_counts()
```

```
Out[26]: 0      614
         1      473
         2      422
         3      366
         4      328
         5      304
         6      299
         7      272
         8      270
         9      254
         10     207
         11     204
         Name: louvain, dtype: int64
```

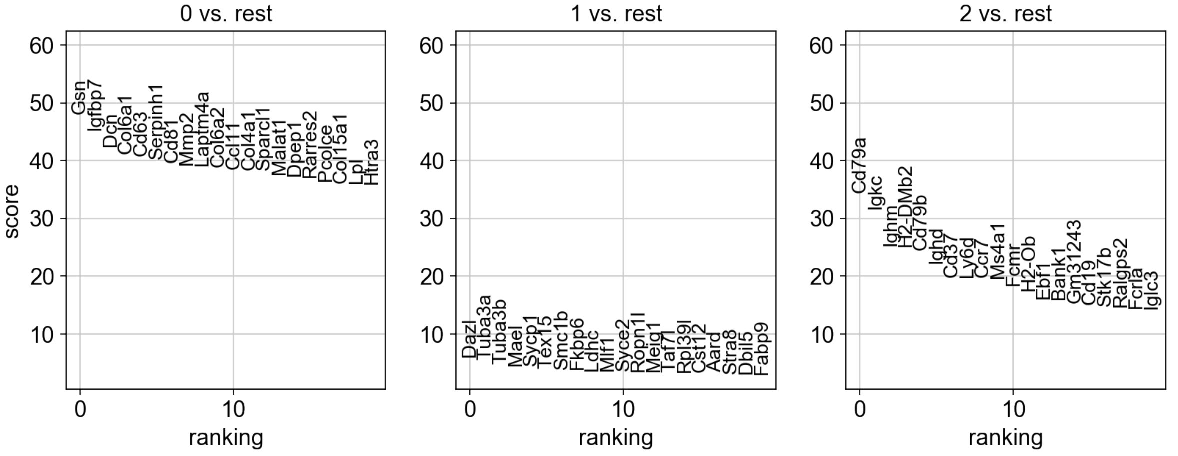
Define Cell Types

DE Genes

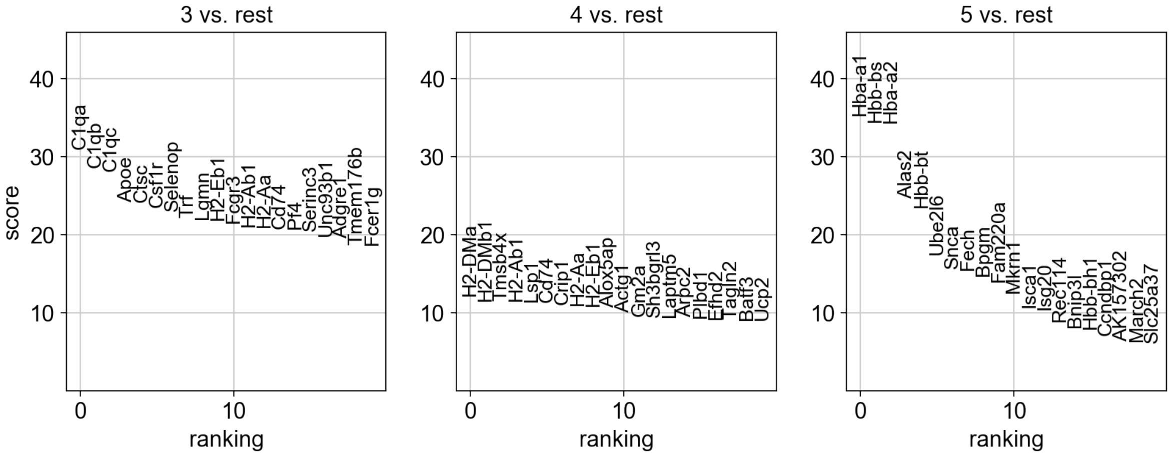
In [27]:

```python
sc.tl.rank_genes_groups(adata_proc, groupby='louvain', key_added='rank_genes')
sc.pl.rank_genes_groups(adata_proc, key='rank_genes', groups=['0','1','2'], fontsize=12, save="_adata_proc_genes_1")
sc.pl.rank_genes_groups(adata_proc, key='rank_genes', groups=['3','4','5'], fontsize=12, save="_adata_proc_genes_2")
sc.pl.rank_genes_groups(adata_proc, key='rank_genes', groups=['6','7','8'], fontsize=12, save="_adata_proc_genes_2")
sc.pl.rank_genes_groups(adata_proc, key='rank_genes', groups=['9','10'], fontsize=12, save="_adata_proc_genes_2")
```

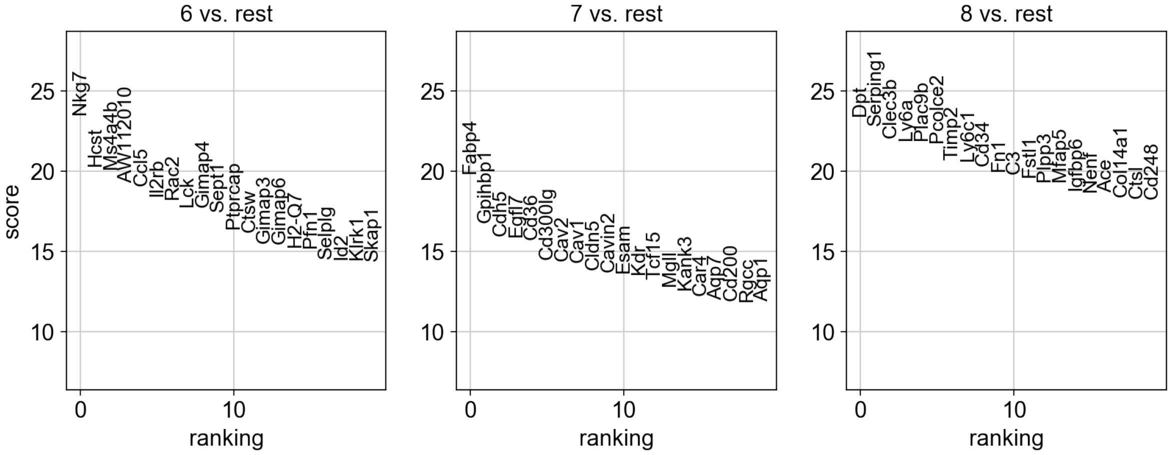
```
ranking genes

/Users/david.fischer/opt/miniconda3/lib/python3.7/site-packages/scanpy/tools/_rank_genes_groups.py:237: RuntimeWarning: overflow encountered in expm1
  foldchanges = (expm1_func(mean_group) + 1e-9) / (expm1_func(mean_rest) + 1e-9)  # add small value to remove 0's

    finished: added to `.uns['rank_genes']`
    'names', sorted np.recarray to be indexed by group ids
    'scores', sorted np.recarray to be indexed by group ids
    'logfoldchanges', sorted np.recarray to be indexed by group ids
    'pvals', sorted np.recarray to be indexed by group ids
    'pvals_adj', sorted np.recarray to be indexed by group ids (0:00:00)
WARNING: saving figure to file /Users/david.fischer/phd/data/PreadipocytesBrown/results/panels/rank_genes_groups_louvain_adata_proc_genes_1.pdf
```



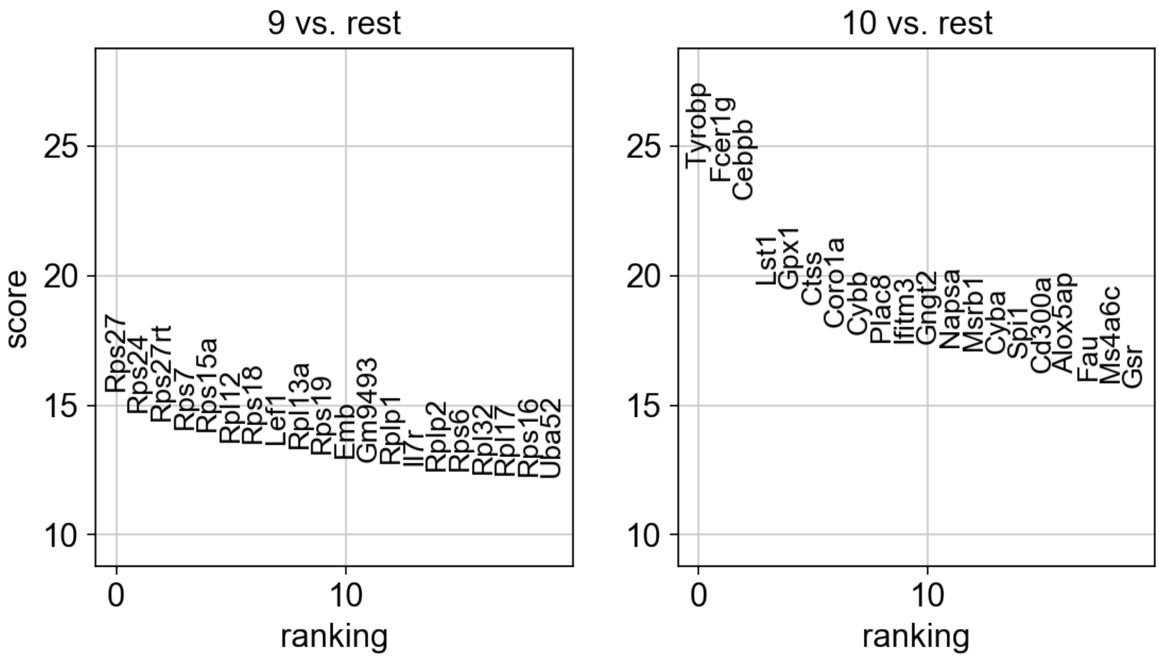
```
WARNING: saving figure to file /Users/david.fischer/phd/data/PreadipocytesBrown/results/panels/rank_genes_groups_louvain_adata_proc_genes_2.pdf
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/rank_genes_groups_louvain_adata_proc_
genes_2.pdf
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/rank_genes_groups_louvain_adata_proc_
genes_2.pdf
```



Define Marker Sets

Define marker sets for some of the expected cell types and add DE genes.

In [28]:

```
# Leukocyte markers:
leukocyte_markers = ['Ptprc','Tcf25']
tc_markers = ['Cd3d','Cd3e','Cd3g','Cd4','Cd8a1','Cd8b','Ms4a4b','Trbc2']
nk_markers = ['Nkg7','Il2rb', 'Ncr1', 'Klrd1', 'Klrb1b', 'Klrb1f']
myeloid_markers = ['Cd79a', 'Itgax', 'Itgam', 'Fcgr3','S100a8', 'S100a9']
mp_markers = ['Adgre1', 'Lyz2','C1qa','Pf4']
dc_markers = ['Cd74', 'Anpep' , 'Cd33', 'Cd80', 'Cd83', 'Cd86']
bc_markers = ['Cd19','Igkc','Ighm','Cd79a']
adipocyte_markers = ['Fgf10','Bmp4','Zfp423','Psmb8','Pdgfra', 'Slc7a10', 'Pparg', 'Cd34', 'Dlk1', 'Dpp4', 'Fabp4', 'Ucp1','P2rd5', 'Slc36a2', 'Tmem26','Adipoq','Prdm16','Ppargc1a','Tcf25','Bin1','Eif5','Slc2a4','Cebpa','Lpl','Pnpla2','Plin1'] # 'Slc7a10' is 'Asc1'; Dlk1 is Pref1; Cd29 is Itgb1
megakaryocyte_markers = ['Ppbp']
erythrocyte_markers = ['Gypa','Hba-a2','Hbb-bt','Alas2']
go_adip_dev = ['Aacs', 'Acat1', 'Arid5b','Arrdc3', 'Atf2', 'Bbs4', 'Bdh1',
 'Csf1', 'Dgat2','Dyrk1b', 'Ebf2', 'Fam123b', 'Fto','Hgmcs2', 'Id2',
 'Lep', 'Lrp5', 'Nampt', 'Oxct1', 'Paxip1', 'Pik3ca', 'Ppard', 'Pppargc1a',
 'Rorc', 'Sh3pxd2b', 'Slc25a25',  'Sox8', 'Spg20', 'Tbl1xr1', 'Umold1', 'Xbp1', 'Znf516']
```

Only keep markers occurring in data set.

In [29]:

```
leukocyte_markers = np.array([x for x in leukocyte_markers if x in adata_proc.var_names])
tc_markers = np.array([x for x in tc_markers if x in adata_proc.var_names])
nk_markers = np.array([x for x in nk_markers if x in adata_proc.var_names])
myeloid_markers = np.array([x for x in myeloid_markers if x in adata_proc.var_names])
mp_markers = np.array([x for x in mp_markers if x in adata_proc.var_names])
dc_markers = np.array([x for x in dc_markers if x in adata_proc.var_names])
bc_markers = np.array([x for x in bc_markers if x in adata_proc.var_names])
megakaryocyte_markers = np.array([x for x in megakaryocyte_markers if x in adata_proc.var_names])
erythrocyte_markers = np.array([x for x in erythrocyte_markers if x in adata_proc.var_names])
adipocyte_markers = np.array([x for x in adipocyte_markers if x in adata_proc.var_names])
go_adip_dev = np.array([x for x in go_adip_dev if x in adata_proc.var_names])
```

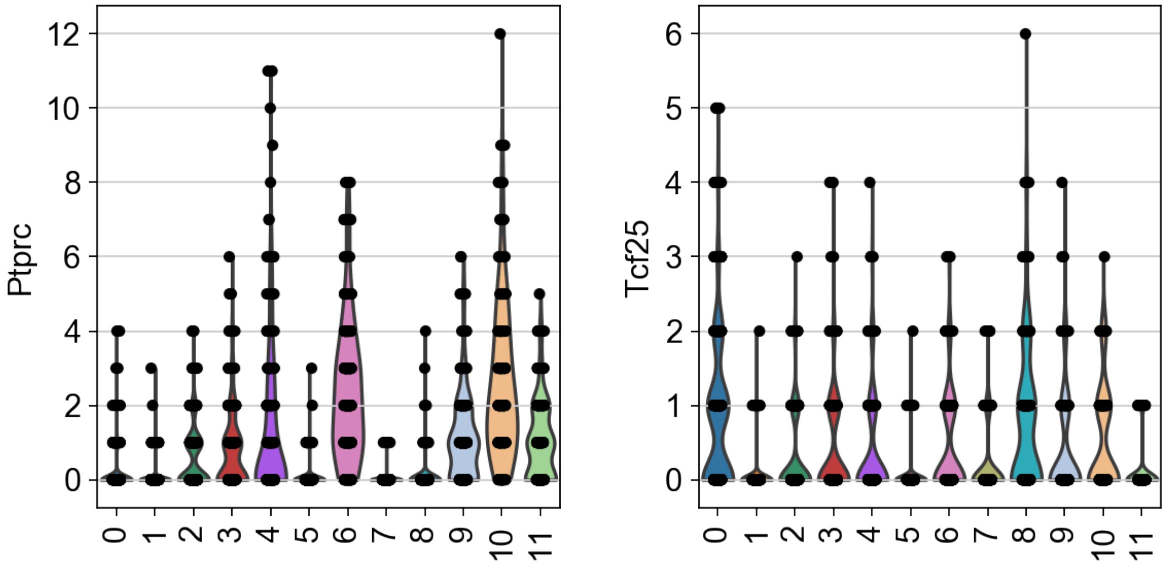
Plotting Routines for Markers Gene Sets:

```
In [30]: def plot_violin_marker(adata, markers, save=None, use_raw=True):
             for i in range(len(markers) // 2 + len(markers) % 2):
                 if save is not None:
                     sc.pl.violin(
                         adata,
                         groupby='louvain',
                         keys=markers[(2*i):np.min([2*(i+1), len(markers)])]
         ,
                         use_raw=use_raw,
                         rotation=90, size=5,
                         save=save+"_"+str(i)+".pdf"
                     )
                 else:
                     sc.pl.violin(
                         adata,
                         groupby='louvain',
                         keys=markers[(2*i):np.min([2*(i+1), len(markers)])]
         ,
                         use_raw=use_raw,
                         rotation=90, size=5,
                     )
         def plot_umap_marker(adata, markers, size=3, save=None, use_raw=Tru
         e):
             for i in range(len(markers) // 2 + len(markers) % 2):
                 print(markers[(2*i):np.min([2*(i+1), len(markers)])])
                 if save is not None:
                     sc.pl.umap(
                         adata,
                         color=markers[(2*i):np.min([2*(i+1), len(markers)])
         ],
                         size=size,
                         use_raw=use_raw,
                         save=save+"_"+str(i)+".pdf"
                     )
                 else:
                     sc.pl.umap(
                         adata,
                         color=markers[(2*i):np.min([2*(i+1), len(markers)])
         ],
                         size=size,
                         use_raw=use_raw
                     )
```

Leukocyte Markers

```
In [31]: if bool_plot == True:
             plot_violin_marker(adata_proc, leukocyte_markers.tolist(), save
         ="_all_markers_leukocyte")
```

```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_leukocyte_0.pdf
```

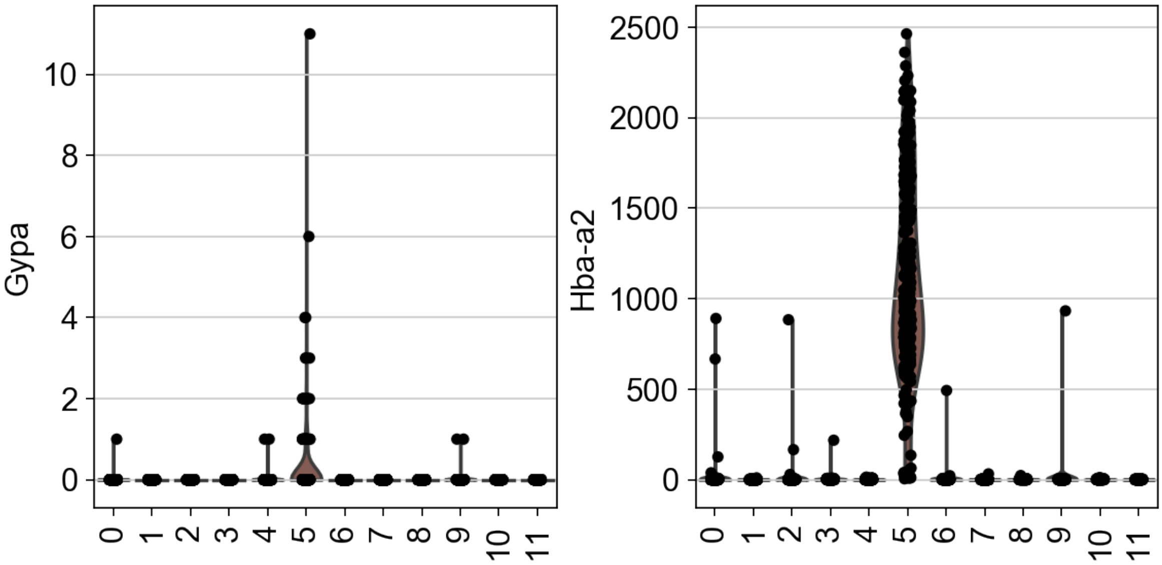


Cluster 1,9 and 10 show expression for the leukocyte marker Ptprc. Tcf25 found in many clusters.

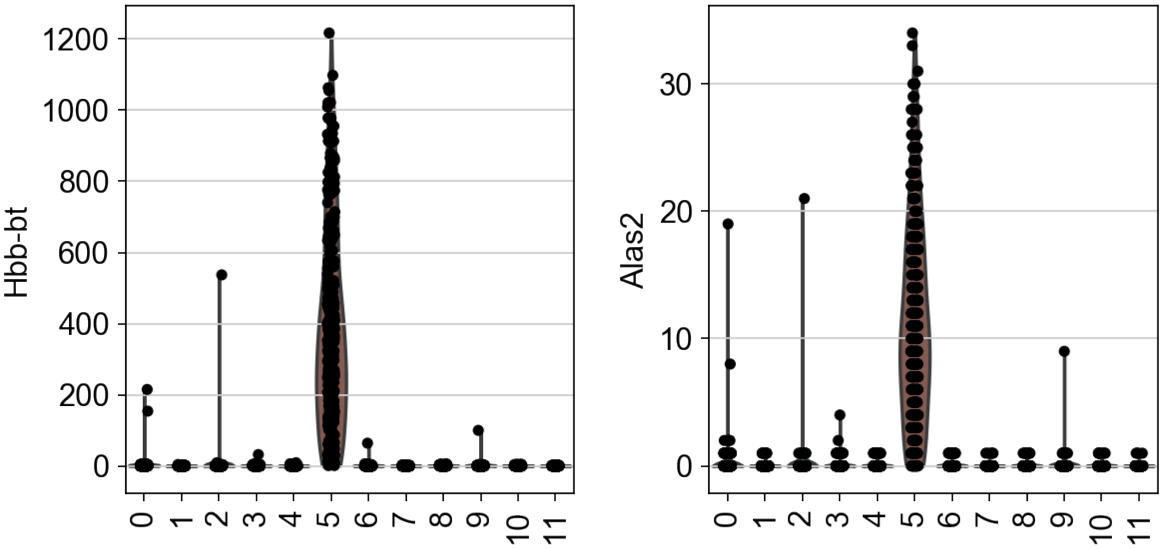
Erythrocyte Markers

```
In [32]: if bool_plot == True:
             plot_violin_marker(adata_proc, erythrocyte_markers.tolist(), sa
         ve="_all_markers_erythrcytes")
```

```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_erythrcytes_0.pdf
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_erythrcytes_1.pdf
```



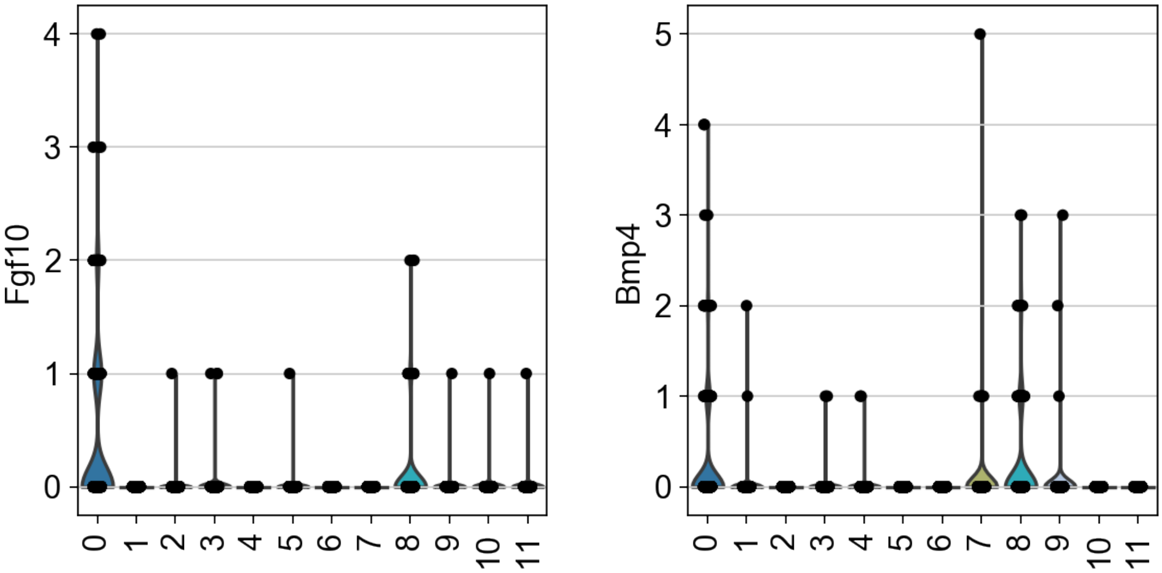
The haemoglobin markers and Alas2 show high counts in cluster 5 indicating that cluster 5 corresponds to erythrocytes.

Preadipocyte Markers

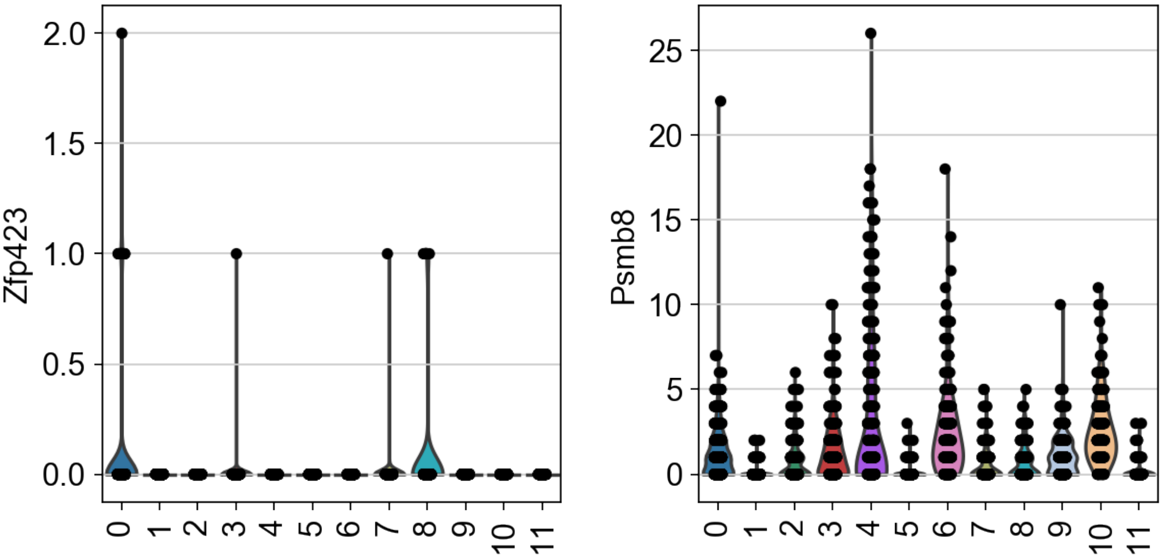
In [33]:

```
if bool_plot == True:
    plot_violin_marker(adata_proc, adipocyte_markers.tolist(), save
="_all_markers_preadipocytes")
```

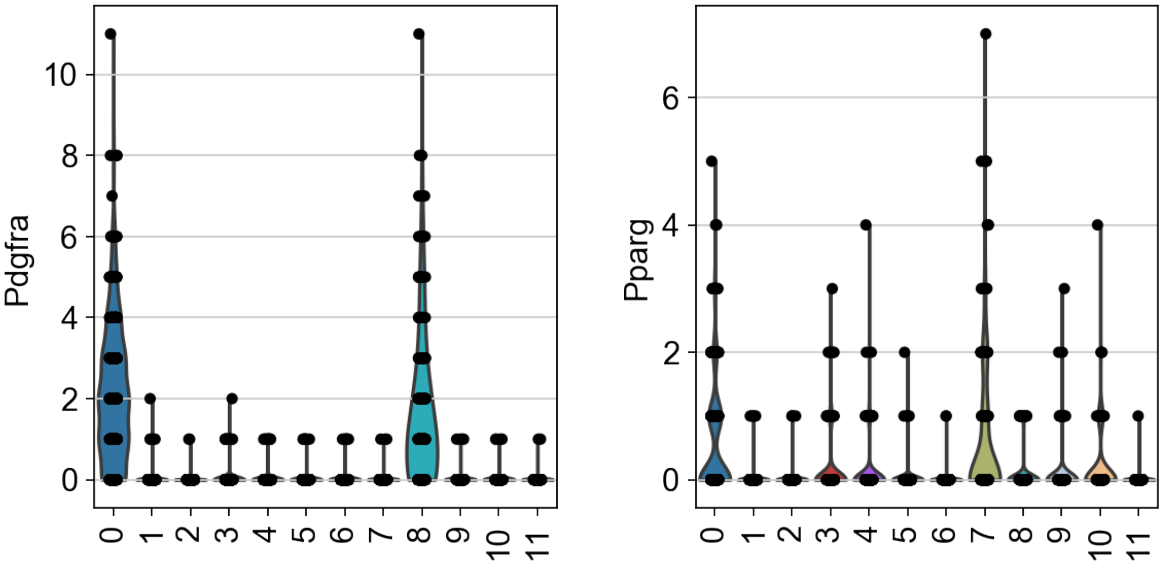
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_preadipocytes_0.pd
f
```



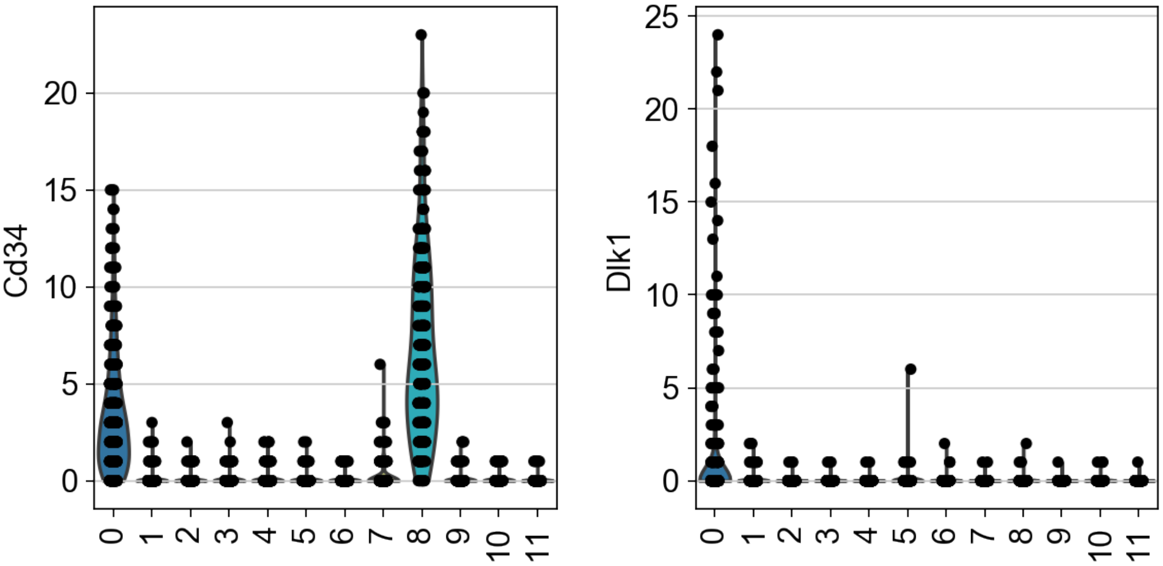
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_preadipocytes_1.pd
f
```



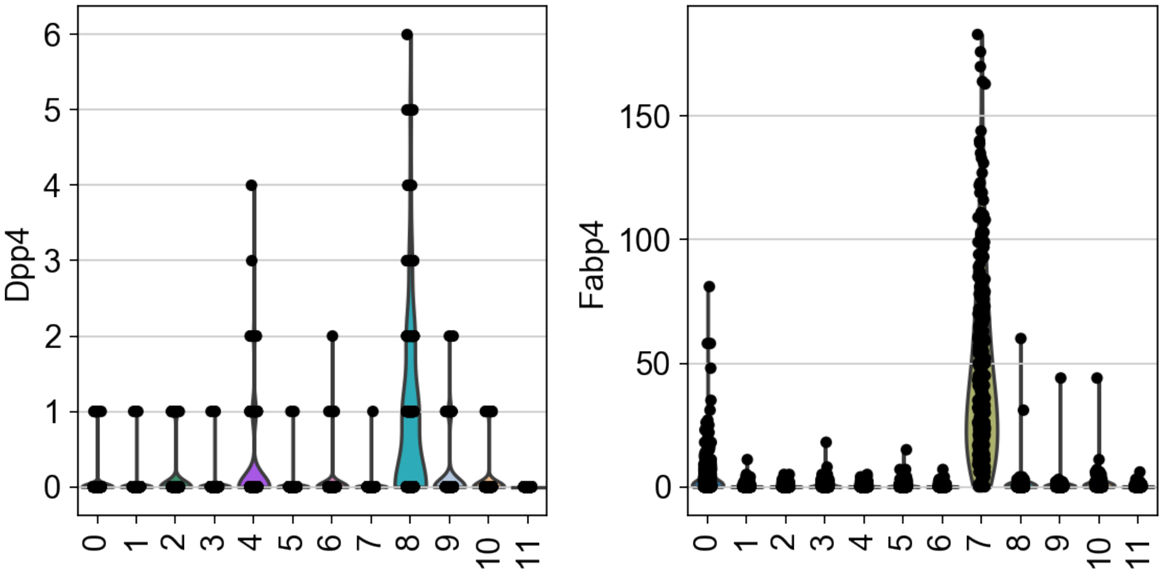
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_preadipocytes_2.pd
f
```



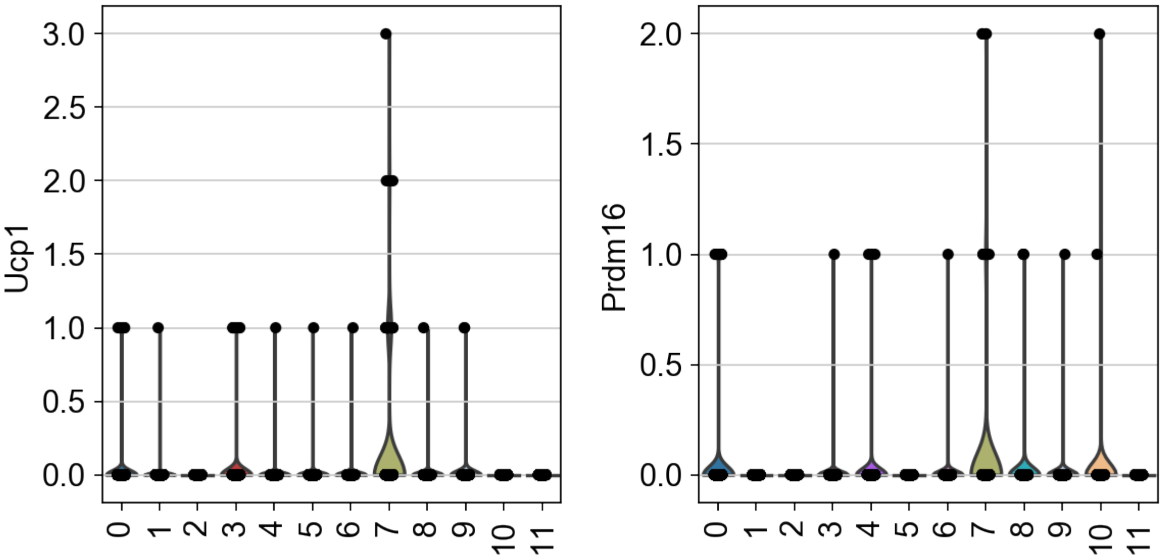
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_preadipocytes_3.pd
f
```



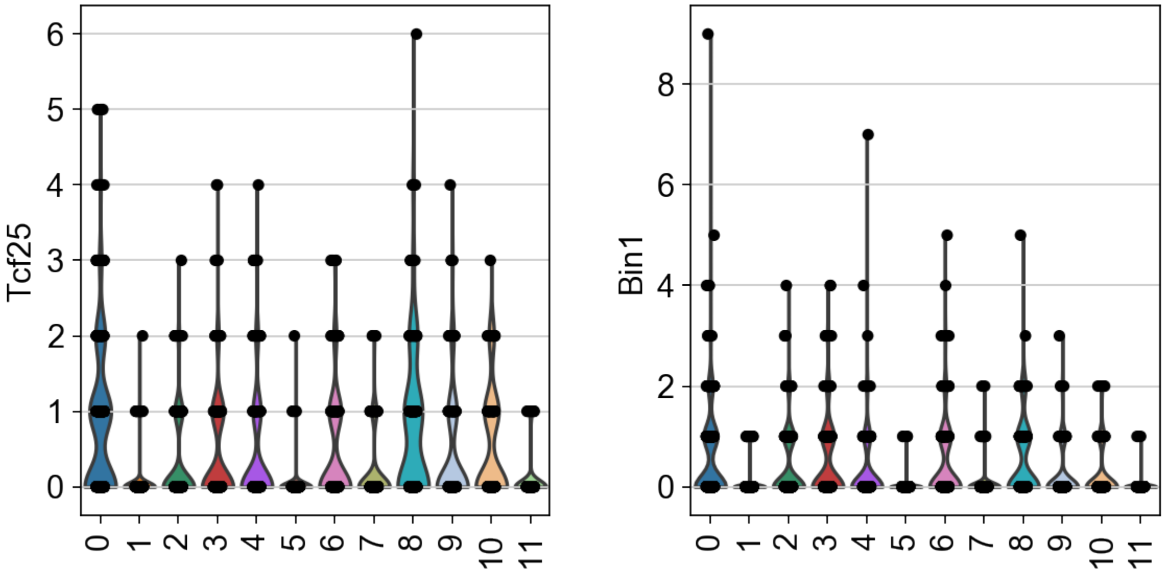
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_preadipocytes_4.pd
f
```



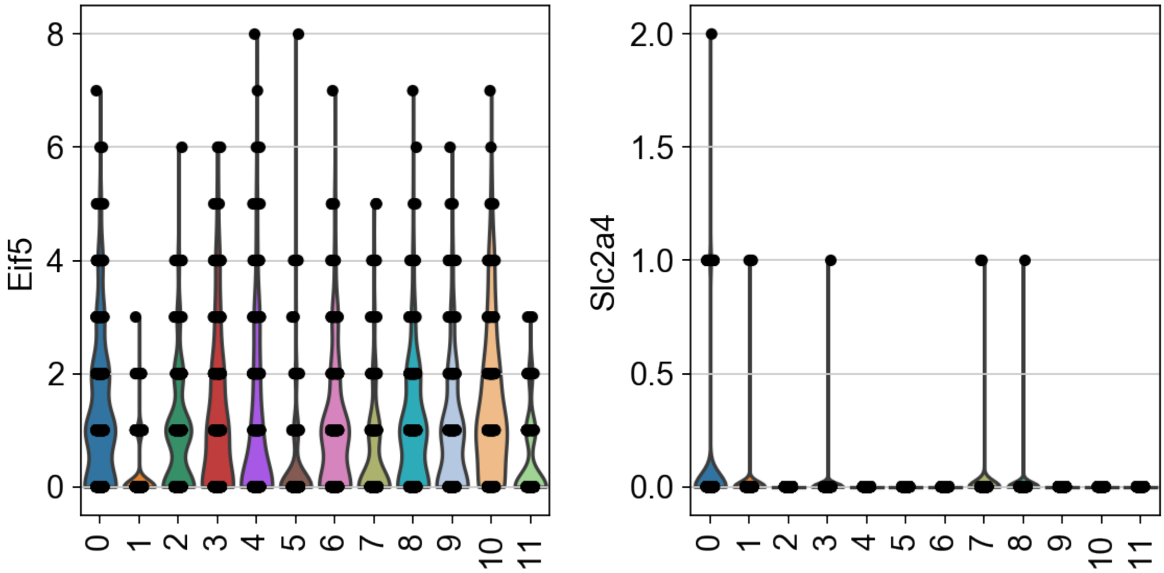
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_preadipocytes_5.pd
f
```



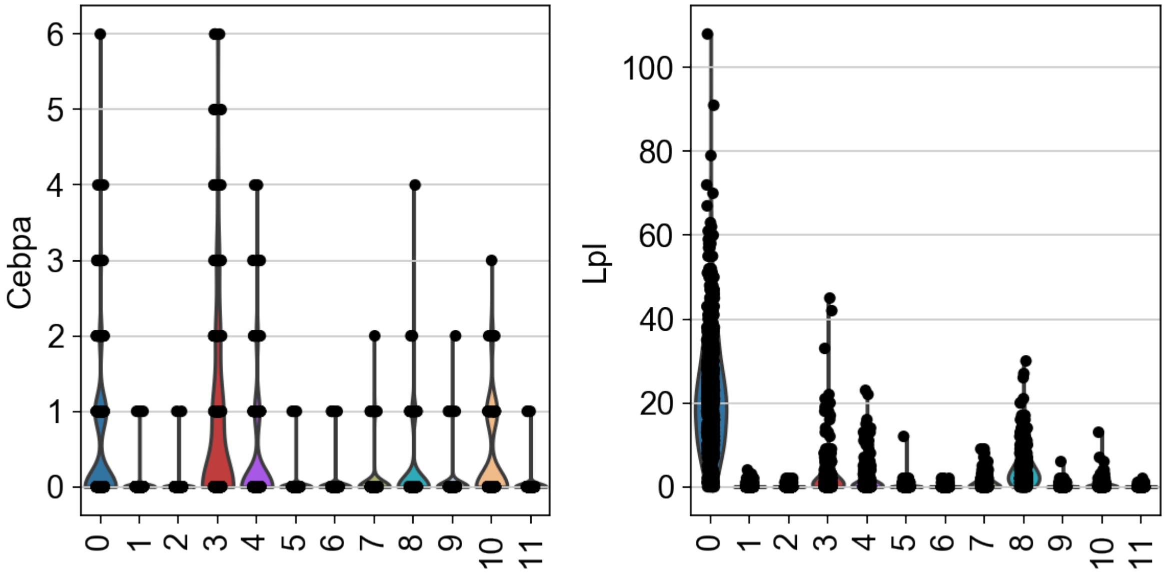
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_preadipocytes_6.pd
f
```



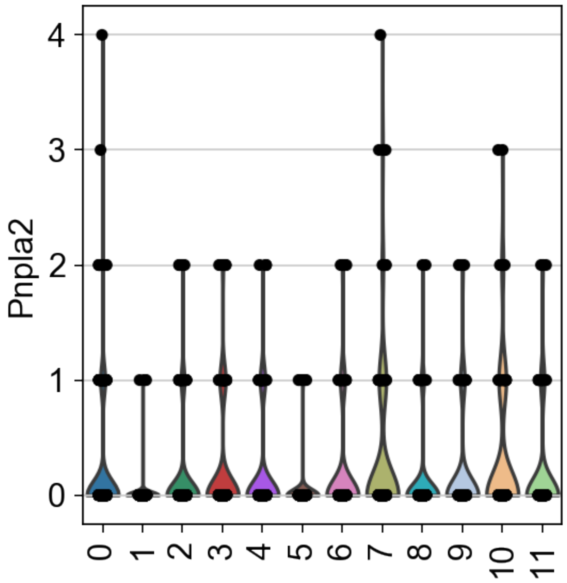
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_preadipocytes_7.pd
f
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_preadipocytes_8.pd
f
```



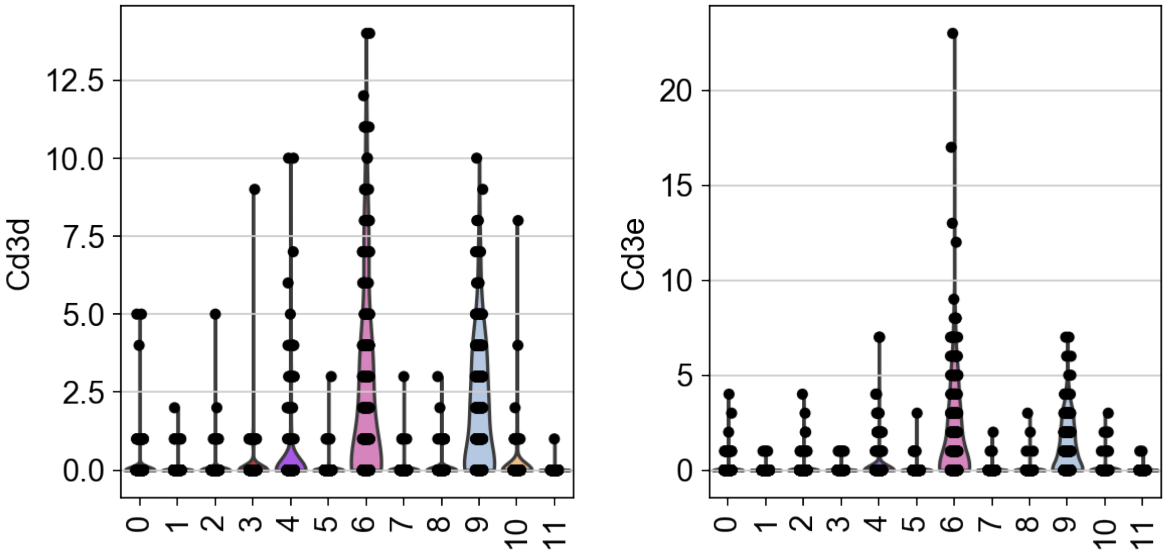
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_preadipocytes_9.pd
f
```



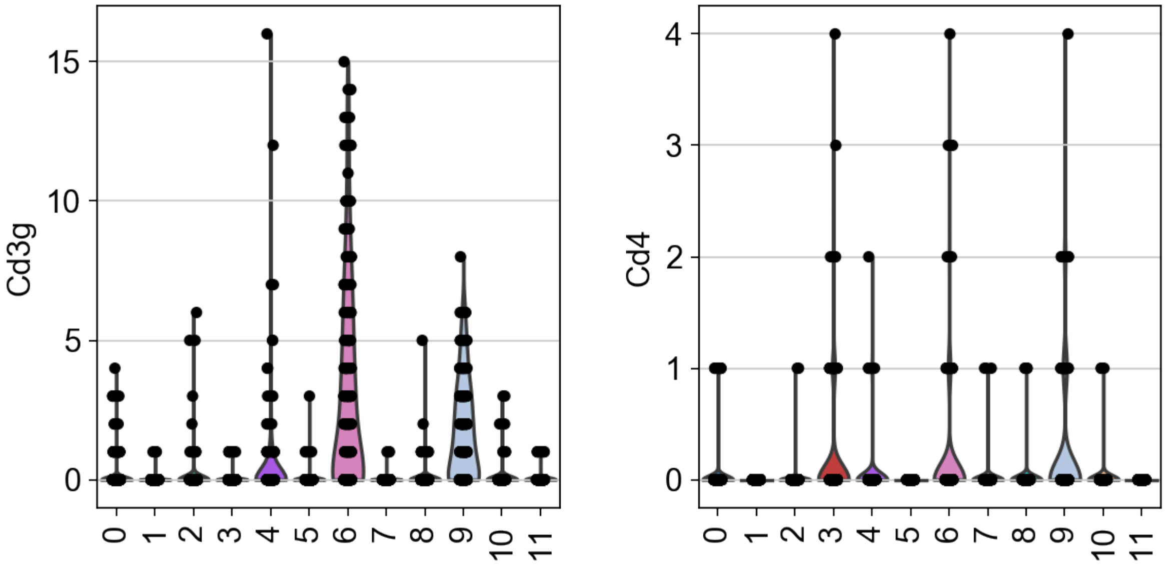
T-Cell Markers

In [34]:
```
if bool_plot == True:
    plot_violin_marker(adata_proc, tc_markers.tolist(), save="_all_
markers_tcells")
```

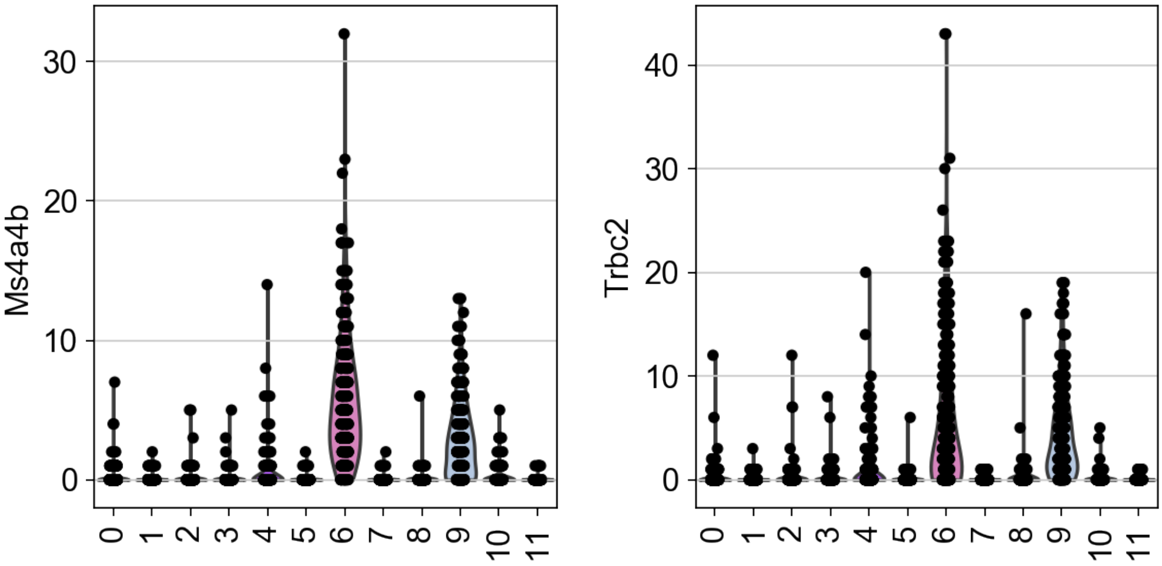
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_tcells_0.pdf
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_tcells_1.pdf
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_tcells_2.pdf
```

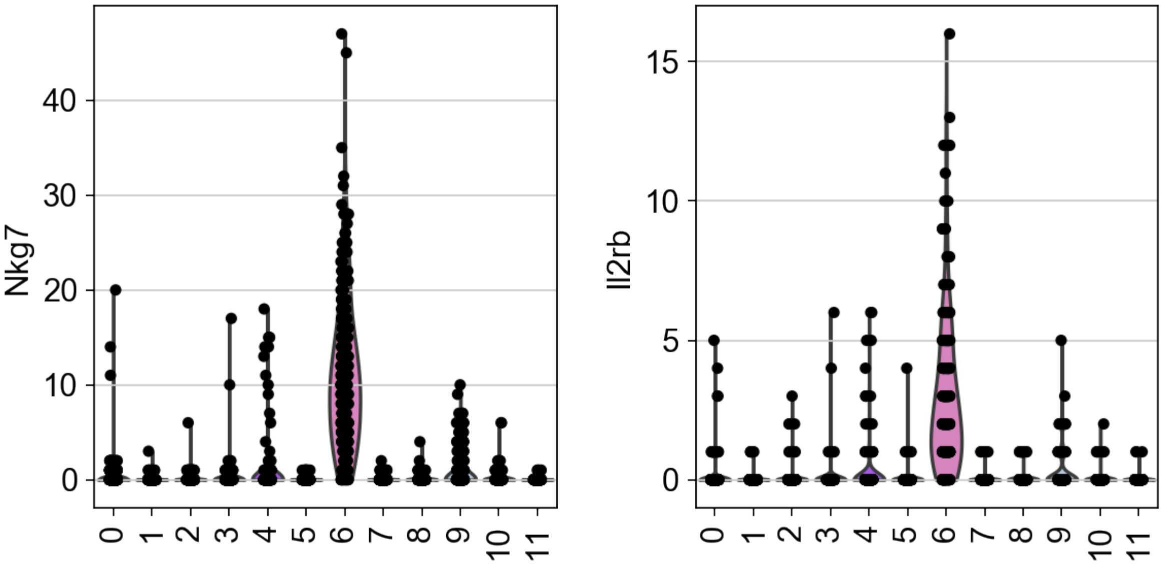


T-cell markers are strongly expressed in cluster 6.

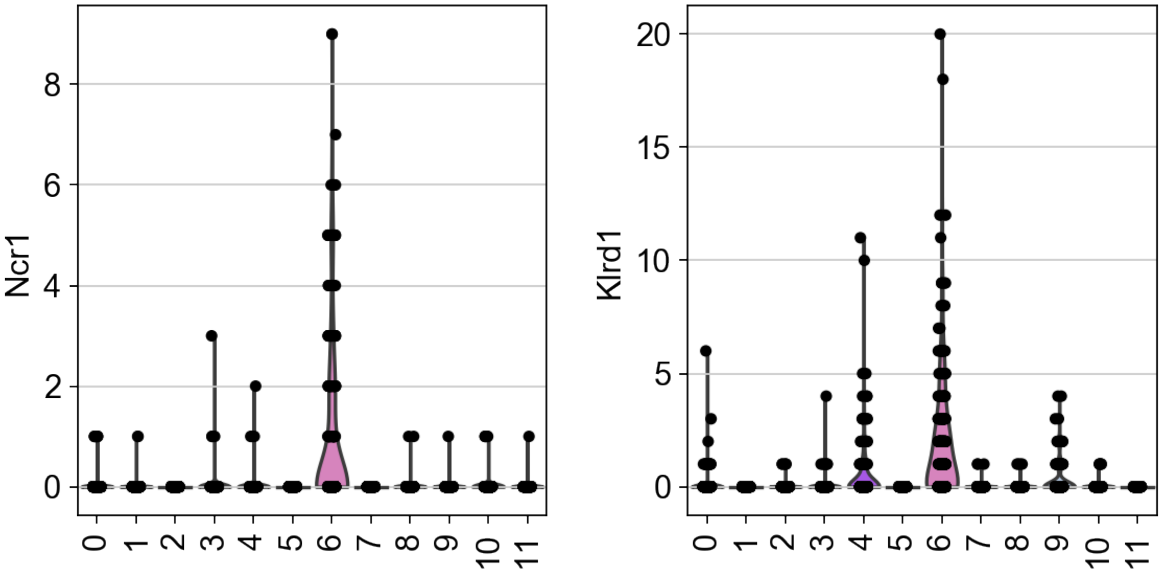
Natural Killer Cells Markers

```
In [35]: if bool_plot == True:
             plot_violin_marker(adata_proc, nk_markers.tolist(), save="_all_markers_nk")
```

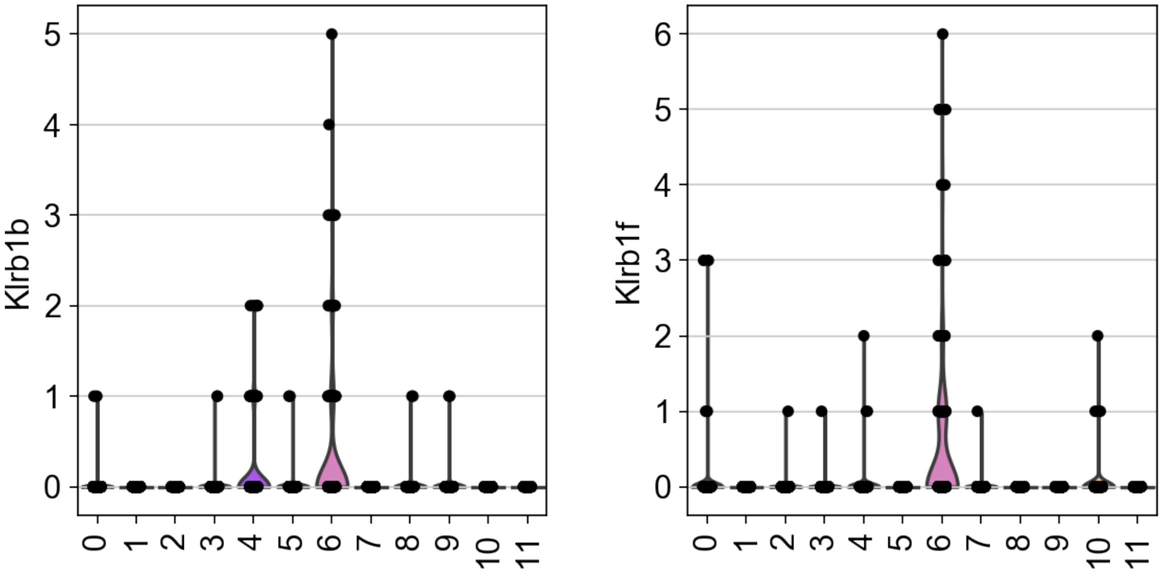
```
WARNING: saving figure to file /Users/david.fischer/phd/data/PreadipocytesBrown/results/panels/violin_all_markers_nk_0.pdf
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/PreadipocytesBrown/results/panels/violin_all_markers_nk_1.pdf
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_nk_2.pdf
```

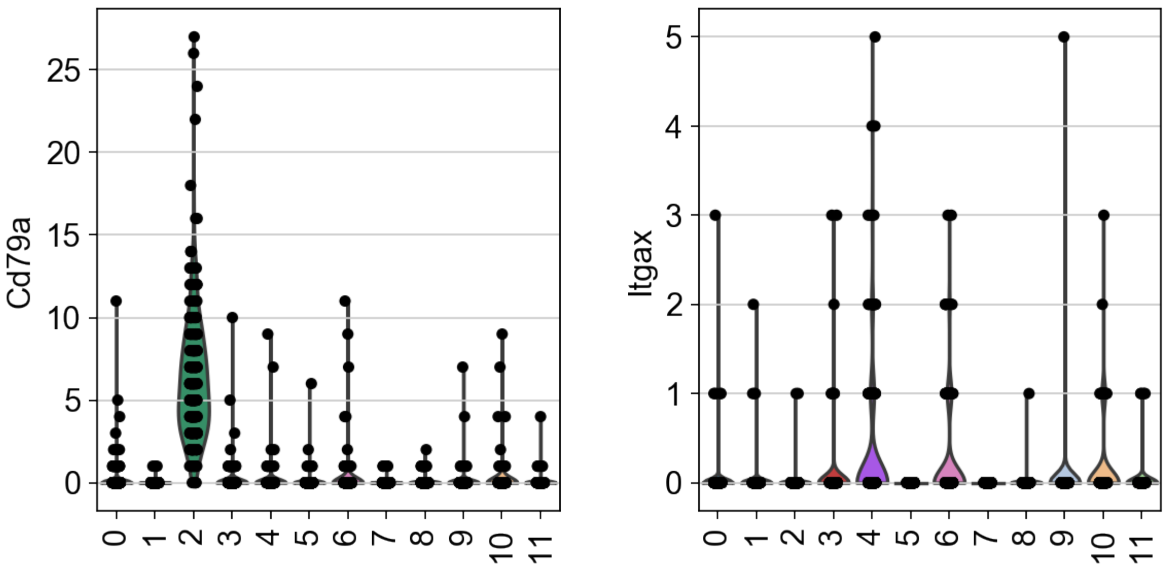


NK cell markers are expressed in cluster 6, which is inline with their transcriptomic similarity to T cells which are also found in cluster 6.

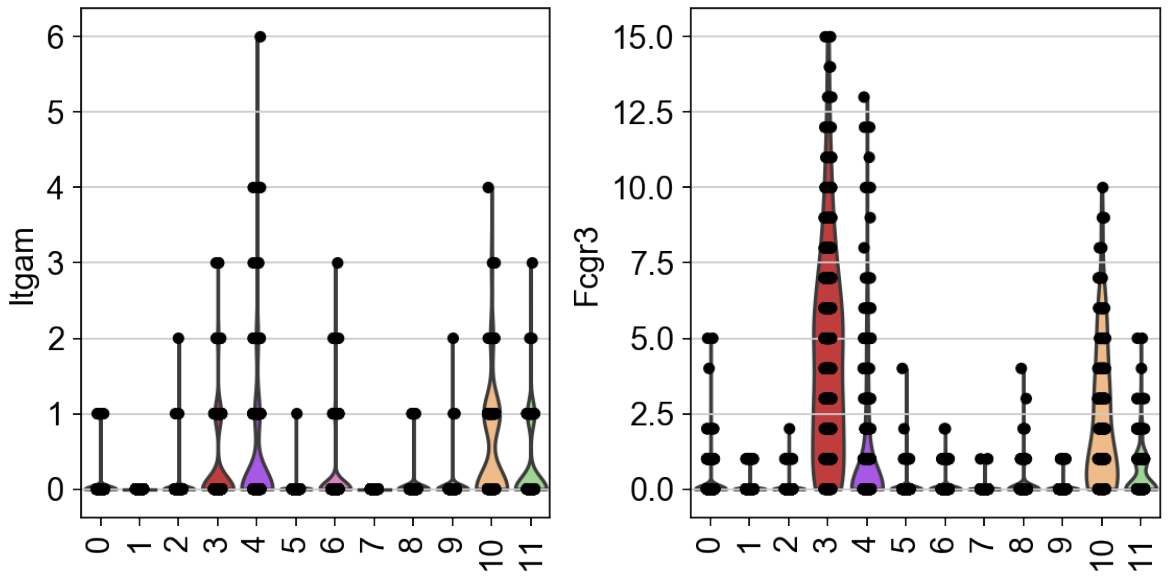
Myeloid Cell Markers

In [36]:
```
if bool_plot == True:
    plot_violin_marker(adata_proc, myeloid_markers.tolist(), save="
_all_markers_myeloid")
```

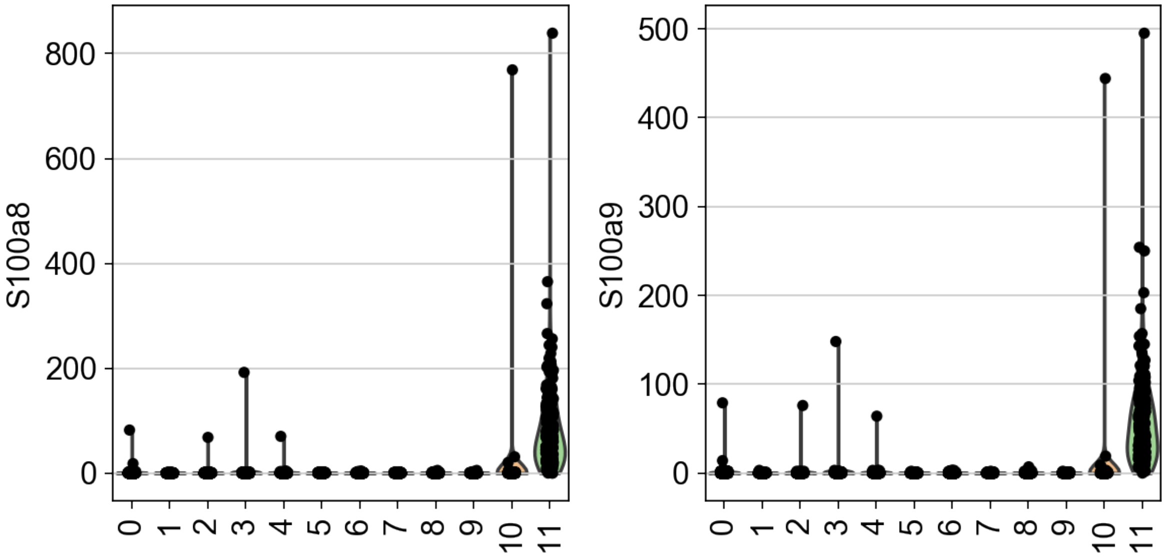
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_myeloid_0.pdf
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_myeloid_1.pdf
```



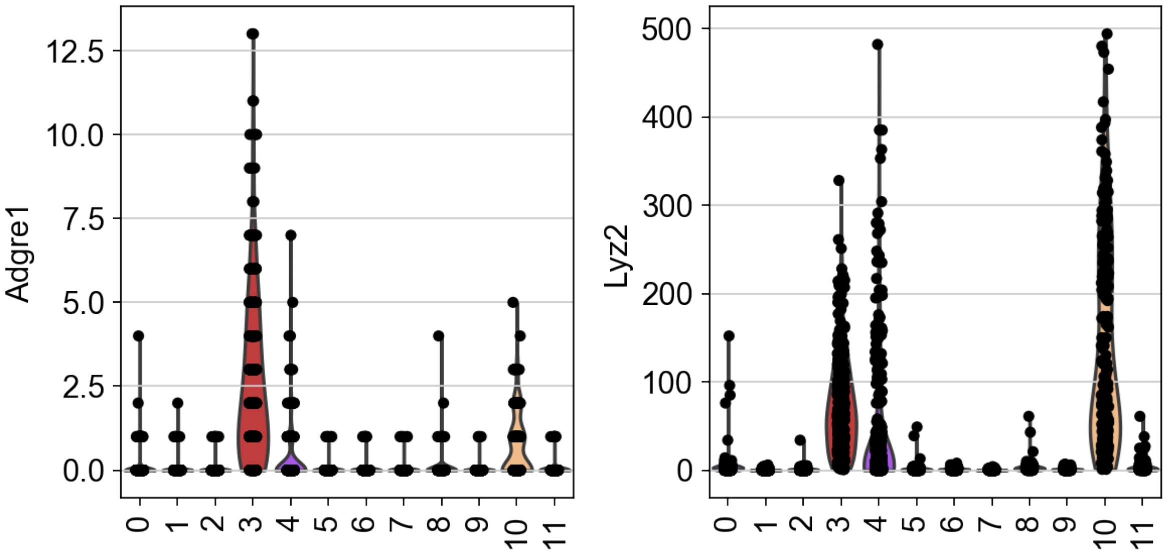
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_myeloid_2.pdf
```



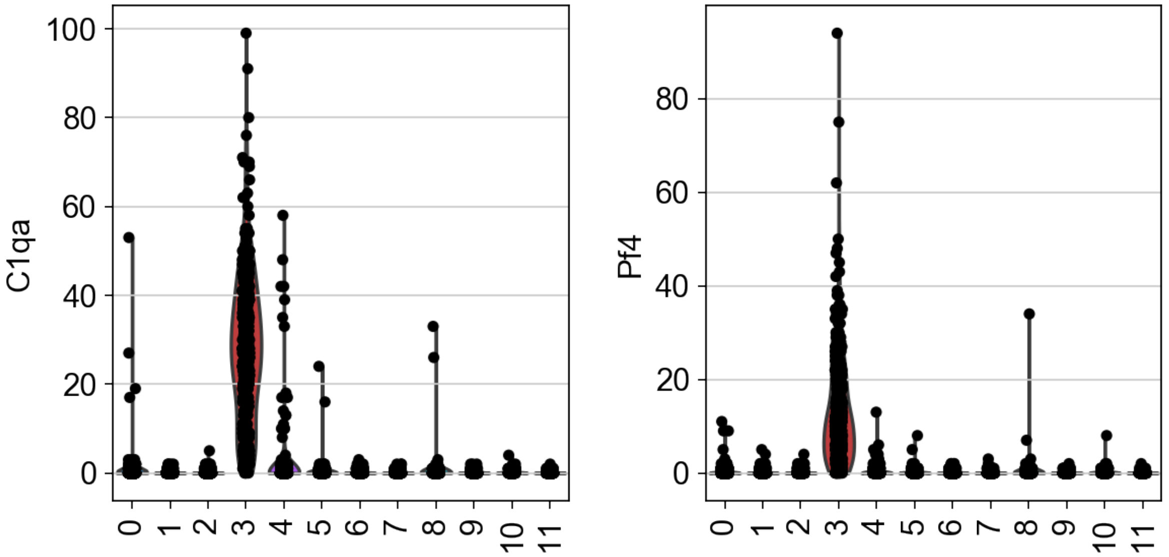
Macrophage Markers

In [37]:
```python
if bool_plot == True:
    plot_violin_marker(adata_proc, mp_markers.tolist(), save="_all_markers_macrophages")
```

```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_macrophages_0.pdf
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_macrophages_1.pdf
```

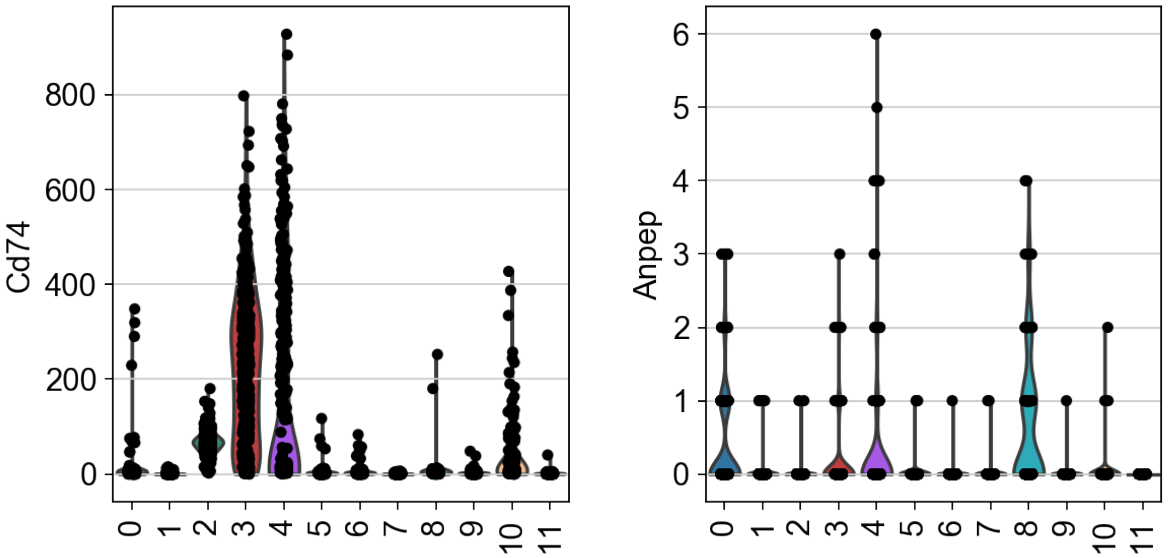


The macrophage markers are strongly expressed in cluster 3.

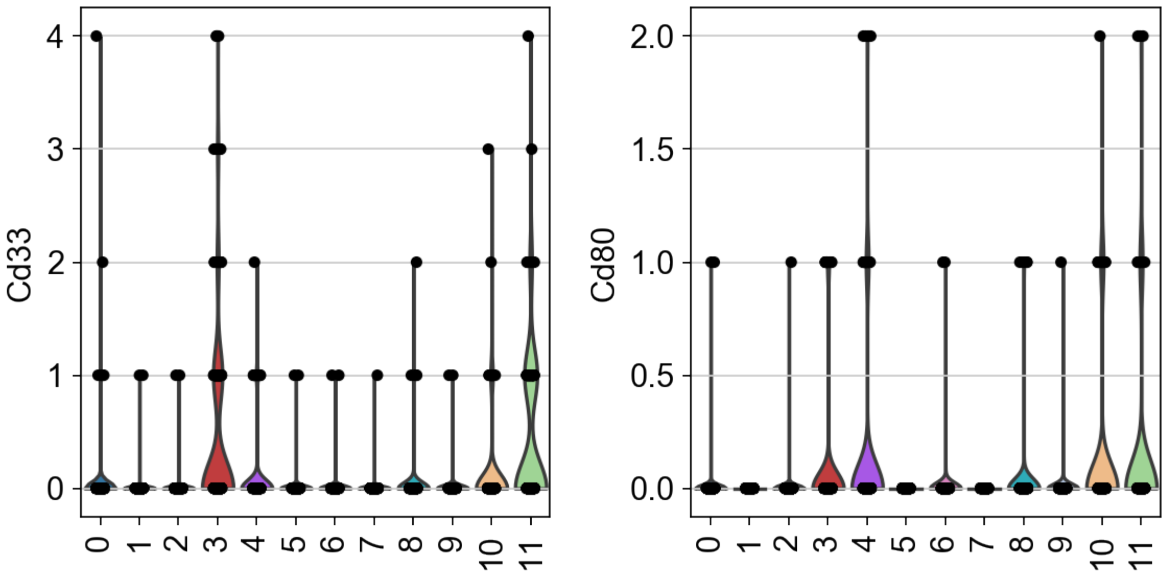
Dendritic cell Markers

```
In [38]: if bool_plot == True:
             plot_violin_marker(adata_proc, dc_markers.tolist(), save="_all_
         markers_dendritic")
```

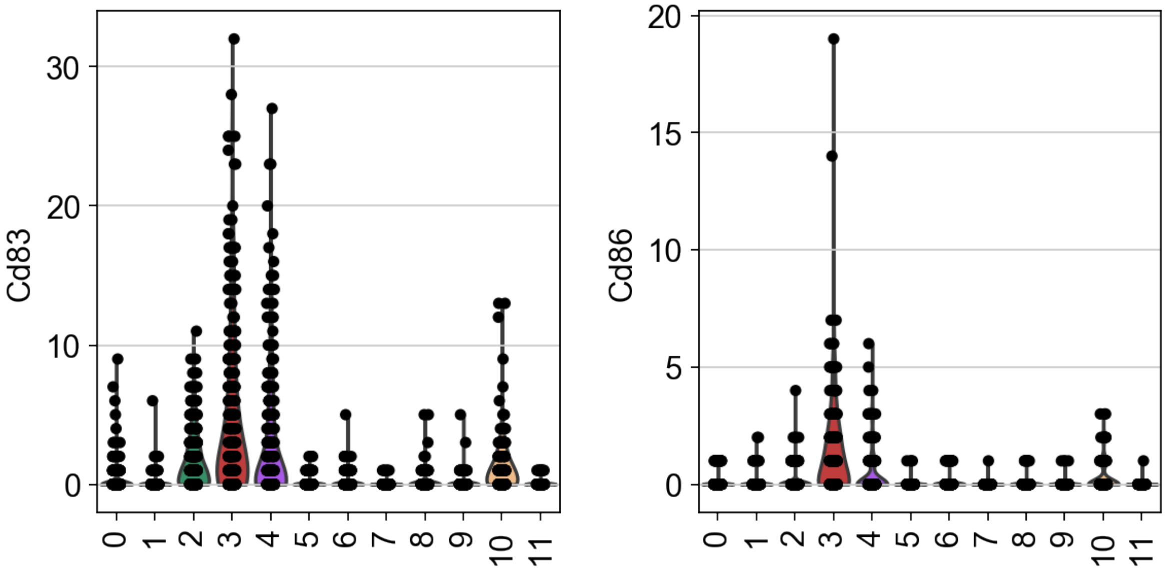
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_dendritic_0.pdf
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_dendritic_1.pdf
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_dendritic_2.pdf
```

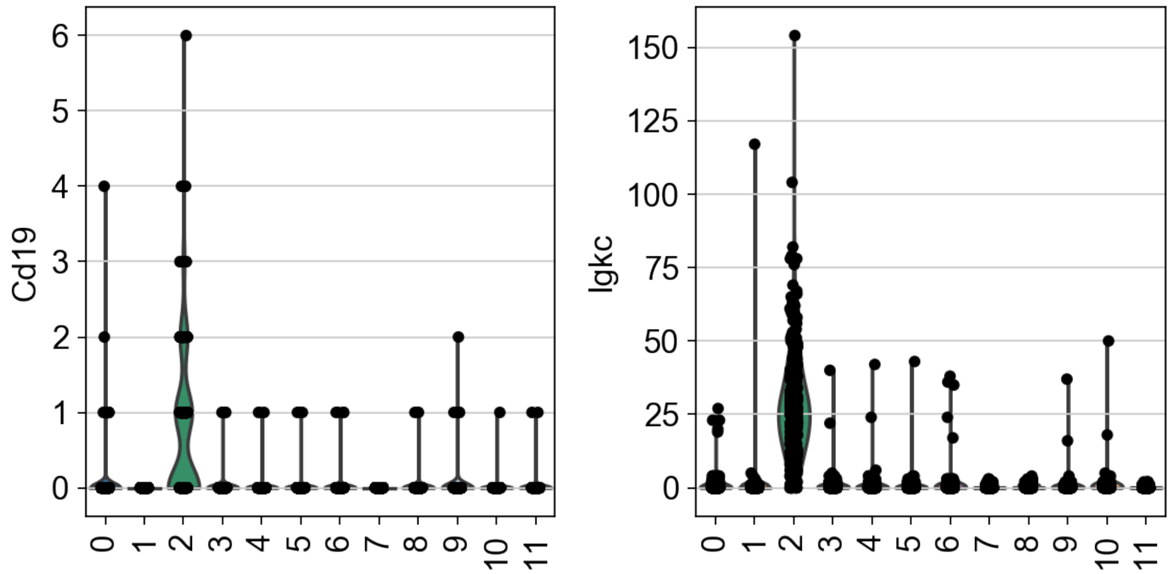


Dendritic cells markers are stongly expressed in cluster 3 and 4.

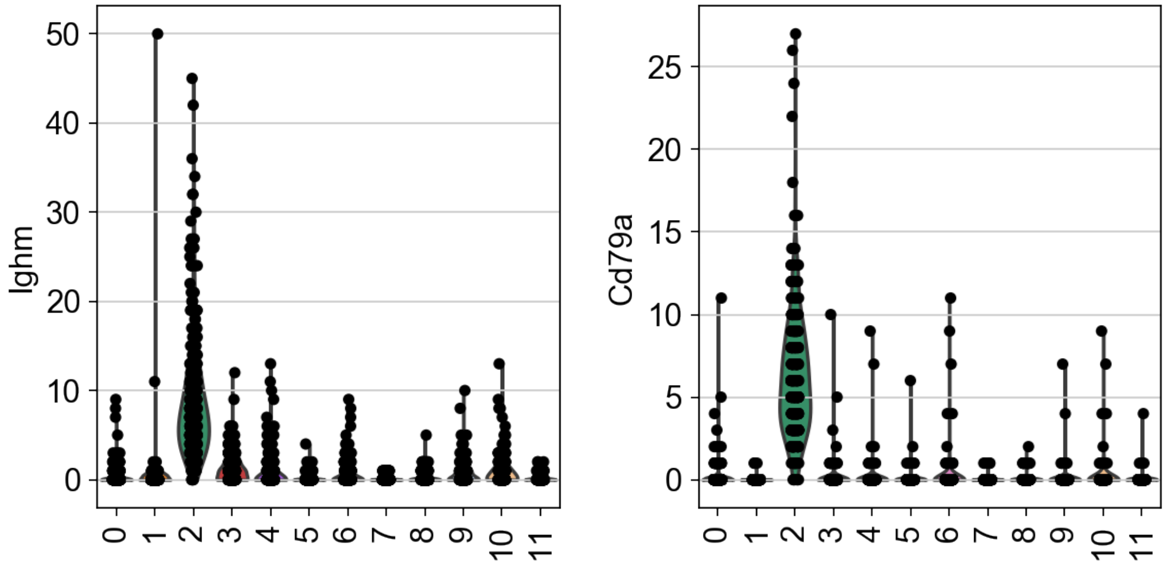
B-Cell Markers

In [39]:
```
if bool_plot == True:
    plot_violin_marker(adata_proc, bc_markers.tolist(), save="_all_markers_bcells")
```

```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_bcells_0.pdf
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_all_markers_bcells_1.pdf
```



B cell markers are highly expressed in cluster 2.

Summary Heatmap for cluster assignments

In [40]:

```
selected_leukocyte_markers = ['Ptprc']
selected_tc_markers = ['Cd3d', 'Cd3g','Cd3e']
selected_nk_markers = ['Nkg7','Klrd1','Ncr1','Il2rb']
selected_myeloid_markers = ['Fcgr3','S100a8']
selected_mp_markers = ['Adgre1', 'C1qa','Pf4']
selected_dc_markers = ['Cd74', 'Cd83','Cd86']
selected_bc_markers = ['Cd19','Igkc','Ighm','Cd79a']
selected_adipocyte_markers = ['Pdgfra','Cd34', 'Dlk1','Fabp4','Fgf1
0','Bmp4','Pparg','Ucp1','Prdm16','Lpl'] # 'Slc7a10' is 'Asc1'; Dlk
1 is Pref1; Cd29 is Itgb1
#selected_megakaryocyte_markers = ['Ppbp']
selected_erythrocyte_markers = ['Gypa','Hba-a2','Hbb-bt','Alas2']
```

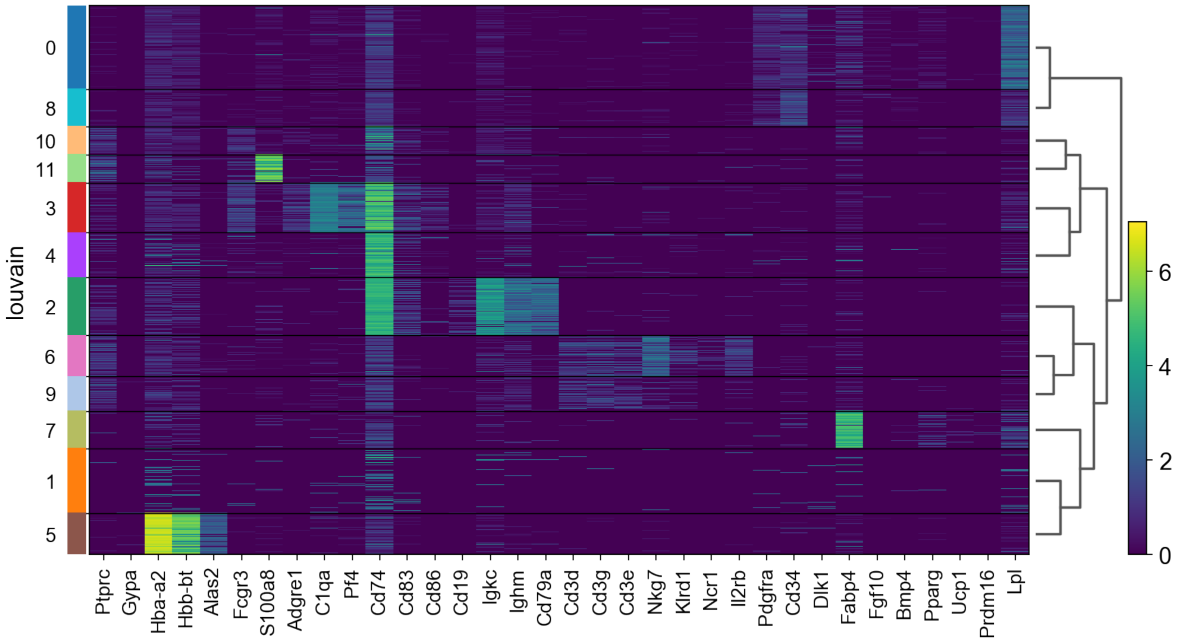
In [41]:

```
selected_cell_markers = selected_leukocyte_markers + \
selected_erythrocyte_markers + \
selected_myeloid_markers + \
selected_mp_markers + \
selected_dc_markers + \
selected_bc_markers + \
selected_tc_markers + \
selected_nk_markers + \
selected_adipocyte_markers
```

In [42]:

```
if bool_plot==True:
    sc.pl.heatmap(
        adata=adata_proc,
        var_names=selected_cell_markers,
        groupby="louvain",
        use_raw=False,
        log=False,
        dendrogram=True,
        var_group_rotation=90,
        show_gene_labels=True,
        show=True,
        save="_all_markers_celltypes.pdf"
    )
```

```
WARNING: dendrogram data not found (using key=dendrogram_louvain).
Running `sc.tl.dendrogram` with default parameters. For fine tunin
g it is recommended to run `sc.tl.dendrogram` independently.
    using 'X_pca' with n_pcs = 50
Storing dendrogram info using `.uns['dendrogram_louvain']`
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/heatmap_all_markers_celltypes.pdf
```

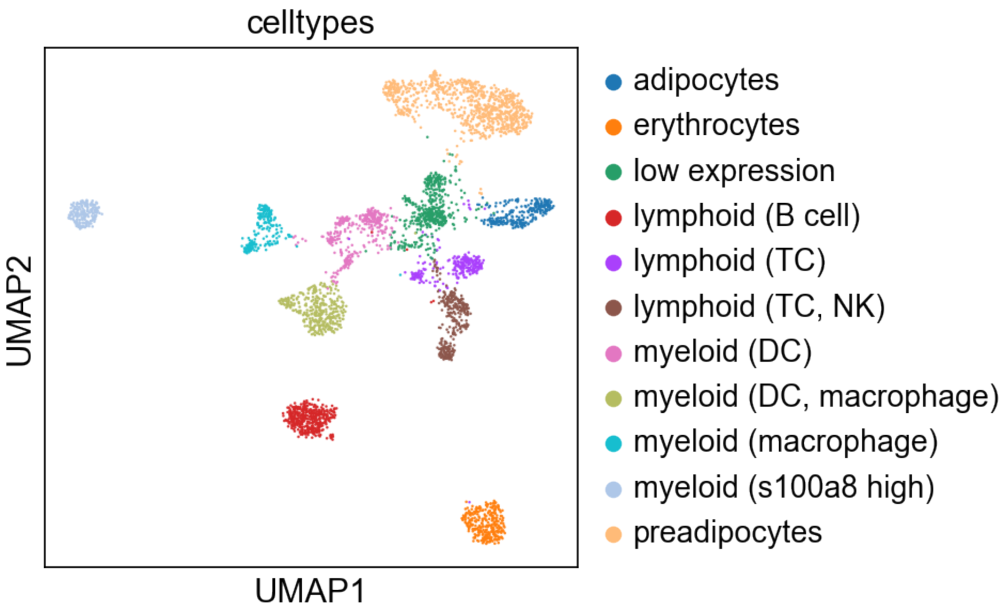


UMAP with assigned cell types

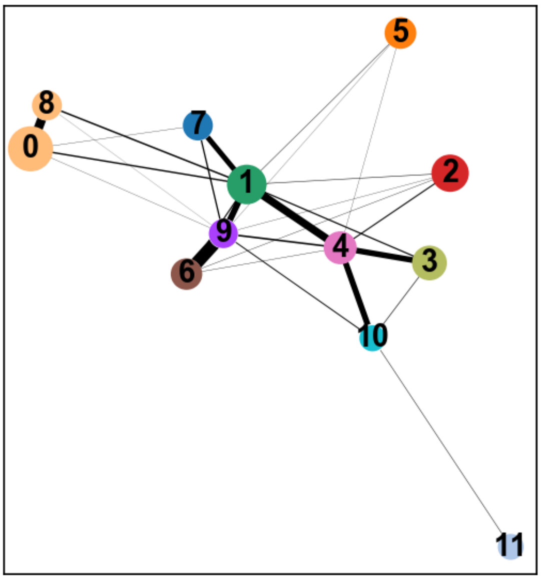
In [43]:

```
new_cluster_names = {
    '0': "preadipocytes",
    '1': "low expression",
    '2': "lymphoid (B cell)",
    '3': "myeloid (DC, macrophage)",
    '4': "myeloid (DC)",
    '5': "erythrocytes",
    '6': "lymphoid (TC, NK)",
    '7': "adipocytes",
    '8': "preadipocytes",
    '9': "lymphoid (TC)",
    '10': "myeloid (macrophage)",
    '11': "myeloid (s100a8 high)"
}
adata_proc.obs['celltypes'] = [new_cluster_names[x] for x in  adata_proc.obs['louvain']]
if bool_plot == True:
    sc.pl.umap(adata_proc, size=5, color=['celltypes'], save="_all_louvain_named.pdf")
    sc.pl.paga(adata_proc, color=['celltypes'], save="_all_named.pdf")
    #sc.pl.umap(adata_proc, size=5, color=adipocyte_markers)
```

```
... storing 'celltypes' as categorical

WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_all_louvain_named.pdf
```



```
--> added 'pos', the PAGA positions (adata.uns['paga'])
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/paga_all_named.pdf
```



Adipocytes only

Embedding and Clustering

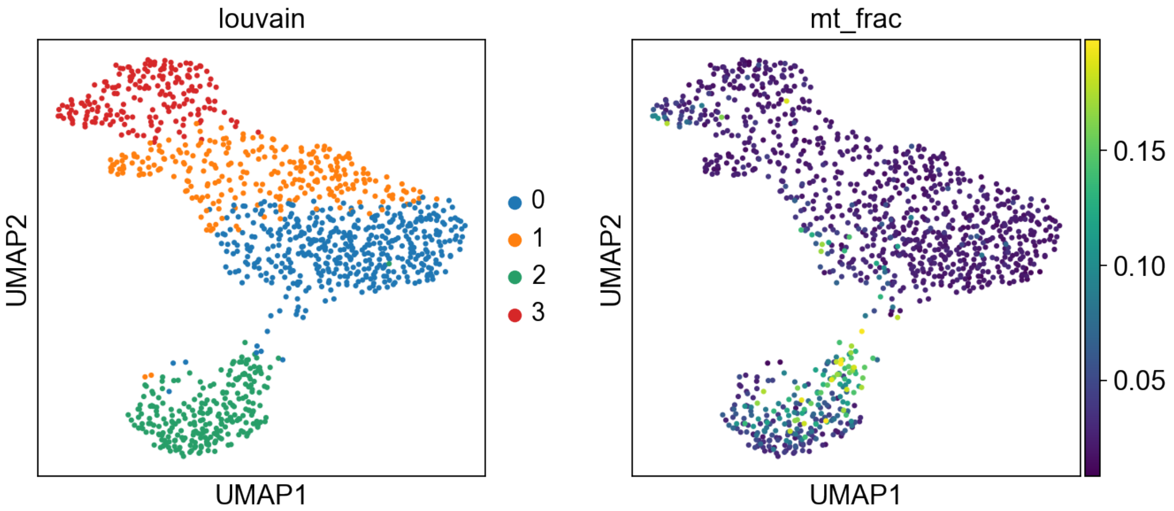
In [44]:

```
if bool_recomp == True:
    cell_ids_adip = np.asarray(adata_proc.obs_names)[
        [x in ['preadipocytes','adipocytes']
         for x in np.asarray(adata_proc.obs['celltypes'].values)]
    ]
    adata_adip = adata_raw[cell_ids_adip,:].copy()
    adata_adip.obs['n_genes'] = (adata_adip.X > 0).sum(1)
    mt_gene_mask = [gene.startswith('mt-') for gene in adata_adip.var_names]
    temp_mt_sum = adata_adip[:,mt_gene_mask].X.sum(1)
    temp_mt_sum = np.squeeze(np.asarray(temp_mt_sum))
    temp_n_counts = adata_adip.obs['n_counts']
    adata_adip.obs['mt_frac'] = temp_mt_sum/adata_adip.obs['n_counts']
    adata_adip.raw = adata_adip
    sc.pp.normalize_per_cell(adata_adip)
    sc.pp.log1p(adata_adip)
    sc.pp.highly_variable_genes(adata_adip,n_top_genes=4000)
    sc.pl.highly_variable_genes(adata_adip)
    sc.pp.pca(adata_adip, n_comps=50, use_highly_variable = True, random_state=0, svd_solver='arpack')
    sc.pp.neighbors(adata_adip, n_neighbors=100, knn=True, method='umap', n_pcs=50, random_state=0)
    sc.tl.umap(adata_adip)
    if bool_recluster == True:
        sc.tl.louvain(adata_adip, resolution=1, flavor='vtraag', random_state=0)
        pd.DataFrame(adata_adip.obs).to_csv(path_or_buf=dir_adata+'obs_adata_adip.csv')
    else:
        obs = pd.read_csv(dir_adata+'obs_adata_adip.csv')
        adata_adip.obs['louvain']=pd.Series(obs['louvain'].values, dtype = 'category')
    sc.write(dir_adata+'adata_adip.h5ad',adata_adip)
else:
    adata_adip = sc.read(dir_adata+'adata_adip.h5ad')
sc.tl.paga(adata_adip)
```

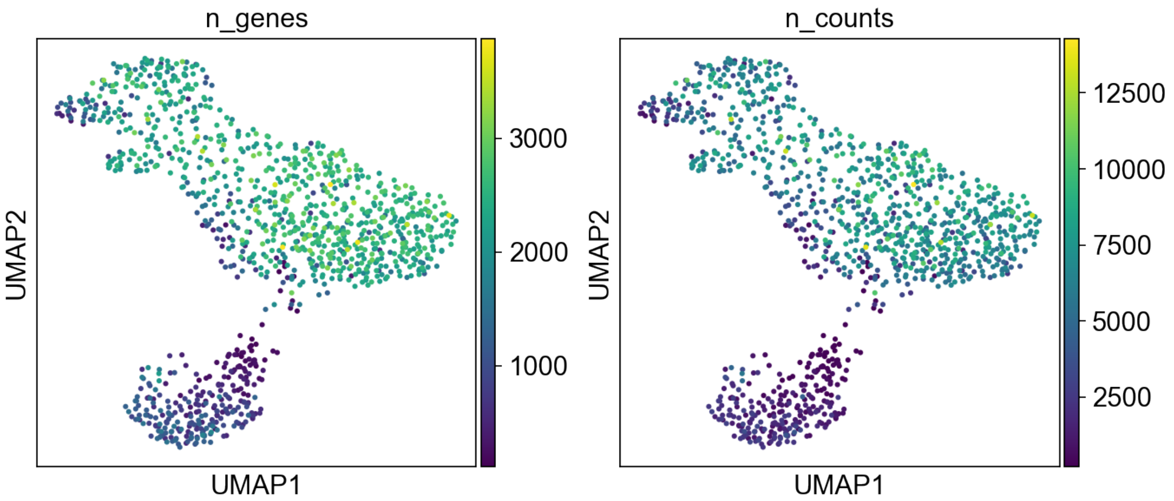
```
running PAGA
    finished: added
    'paga/connectivities', connectivities adjacency (adata.uns)
    'paga/connectivities_tree', connectivities subtree (adata.uns)
(0:00:00)
```

```
In [45]: if bool_plot == True:
             sc.pl.umap(adata_adip, color=['louvain','mt_frac'], size=30, save="_preadip_louvain_0.pdf")
             sc.pl.umap(adata_adip, color=['n_genes','n_counts'], size=30, save="_preadip_louvain_1.pdf")
             sc.pl.paga(adata_adip, color=['louvain','n_counts'], save="_preadip_louvain.pdf")
```

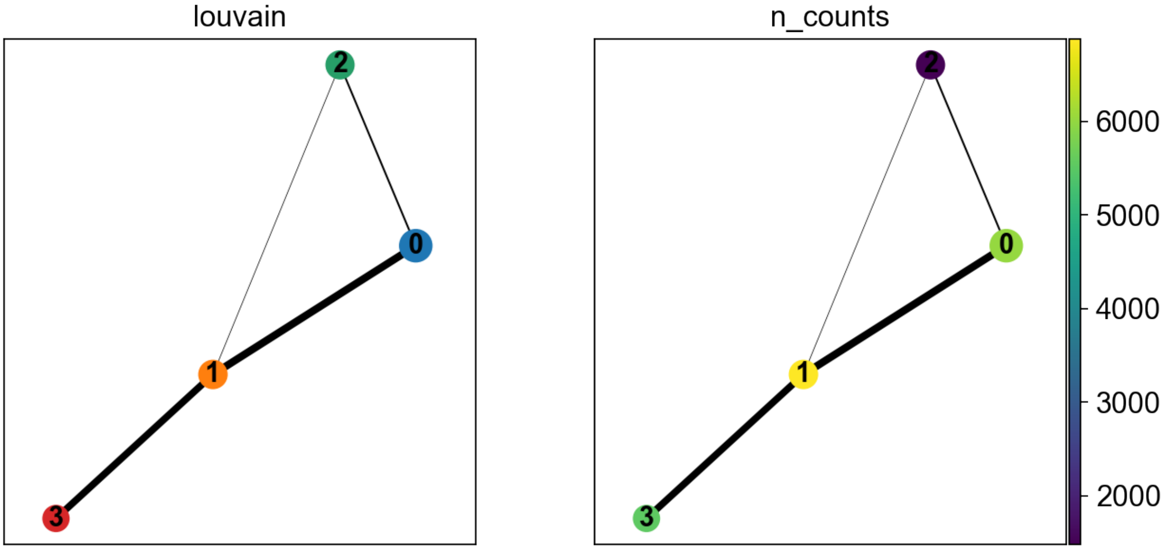
```
WARNING: saving figure to file /Users/david.fischer/phd/data/PreadipocytesBrown/results/panels/umap_preadip_louvain_0.pdf
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/PreadipocytesBrown/results/panels/umap_preadip_louvain_1.pdf
```



```
--> added 'pos', the PAGA positions (adata.uns['paga'])
WARNING: saving figure to file /Users/david.fischer/phd/data/PreadipocytesBrown/results/panels/paga_preadip_louvain.pdf
```

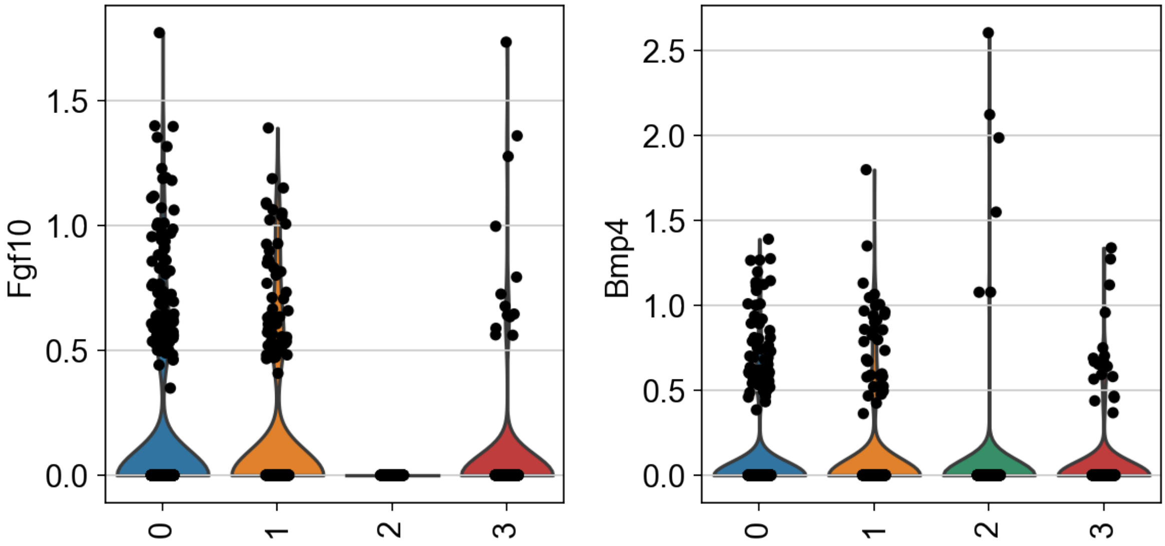


Cluster 0,1,3 (top) shows many more counts than cluster 2 (bottom).

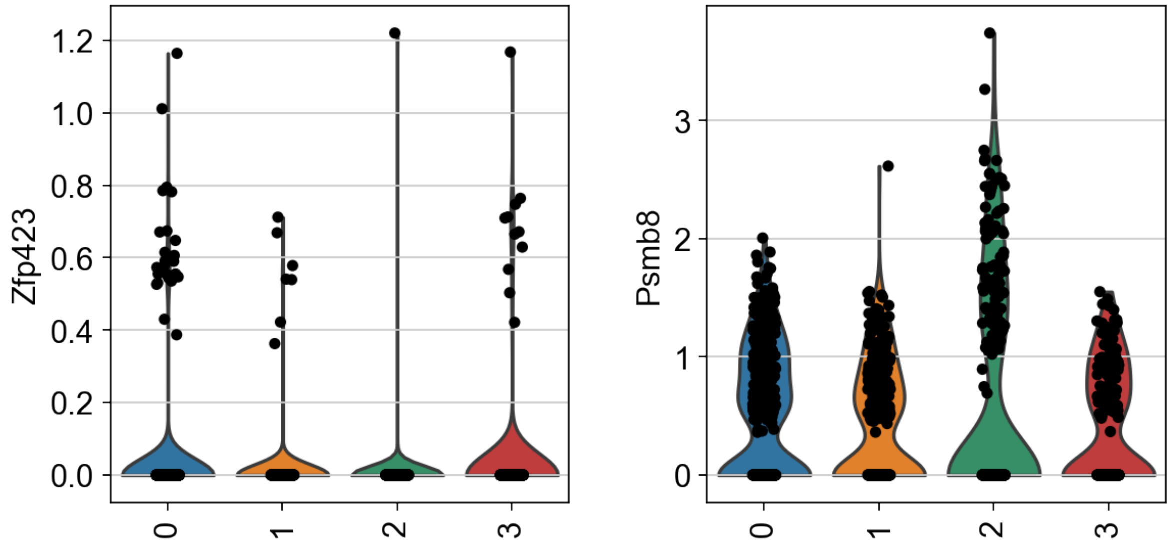
Adipocyte Marker Analysis

```
In [46]: if bool_plot == True:
             plot_violin_marker(adata_adip, adipocyte_markers.tolist(), save
         ="_preadip_markers_preadipcytes", use_raw=False)
```

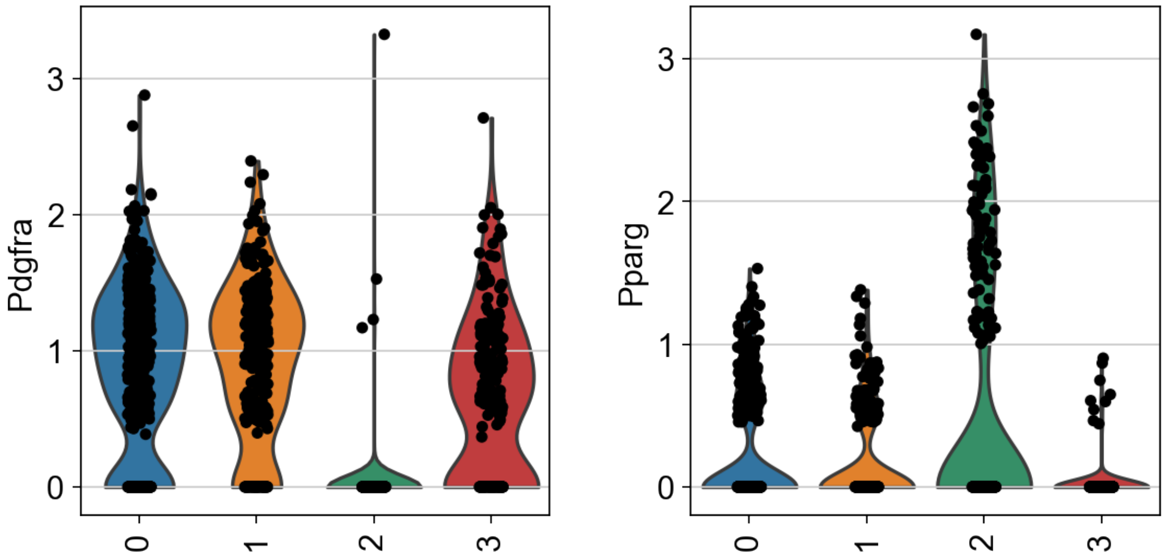
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_preadip_markers_preadipcytes_0
.pdf
```



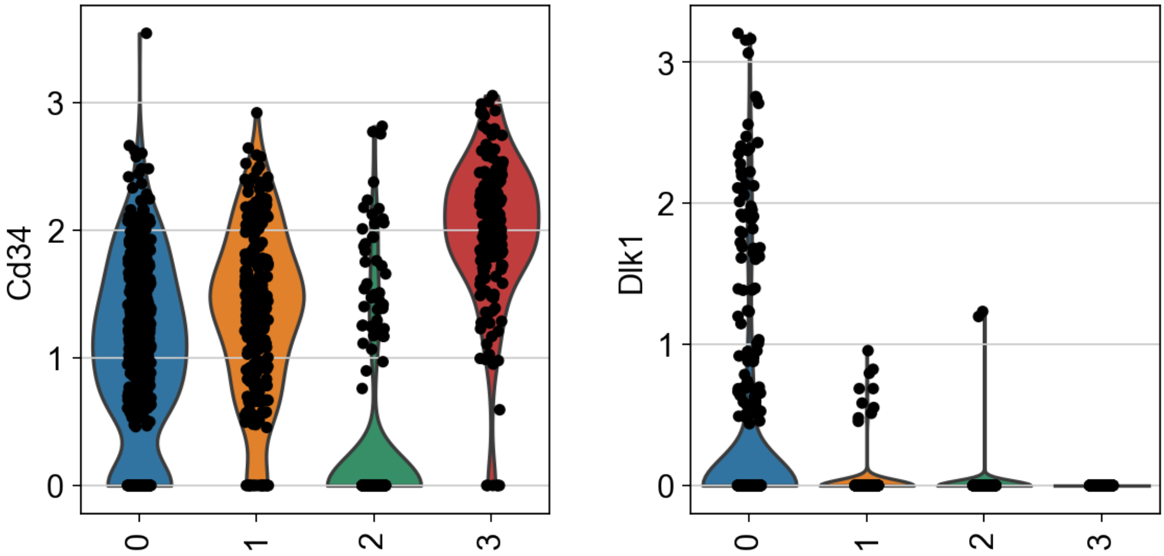
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_preadip_markers_preadipcytes_1
.pdf
```



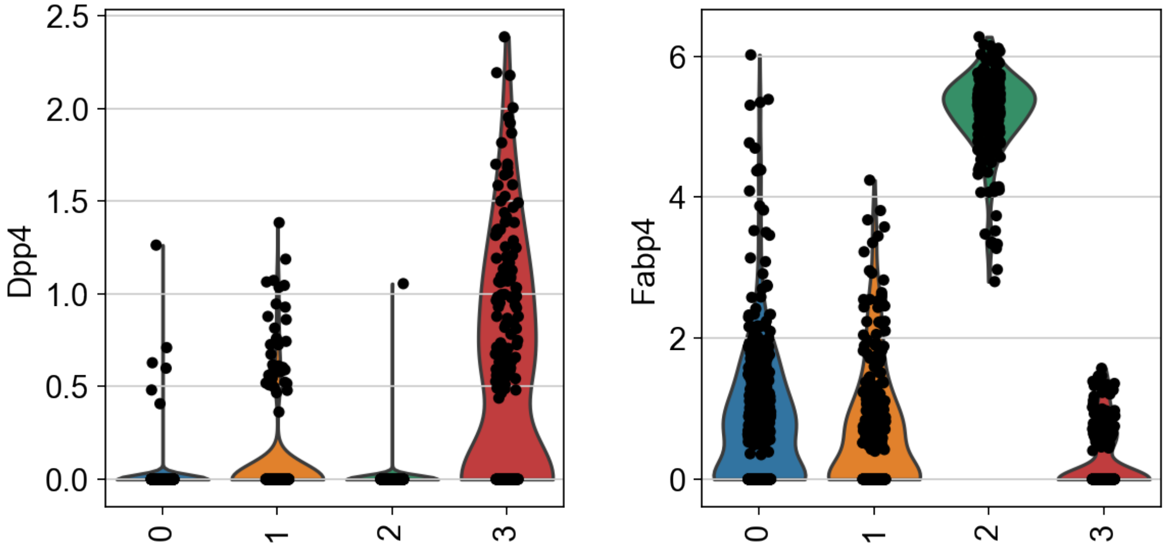
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_preadip_markers_preadipcytes_2
.pdf
```



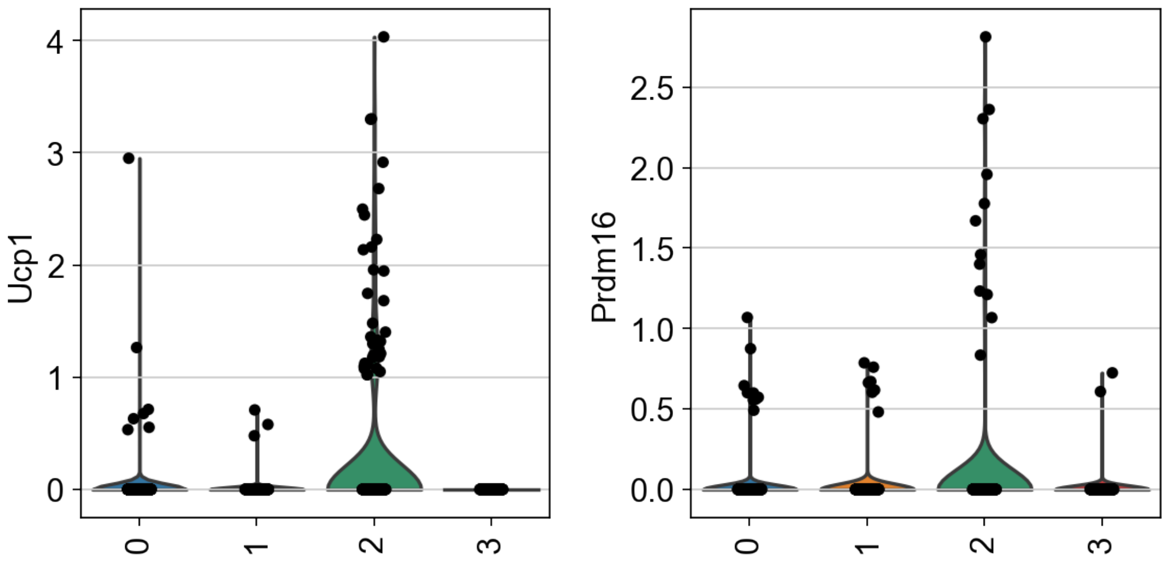
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_preadip_markers_preadipcytes_3
.pdf
```



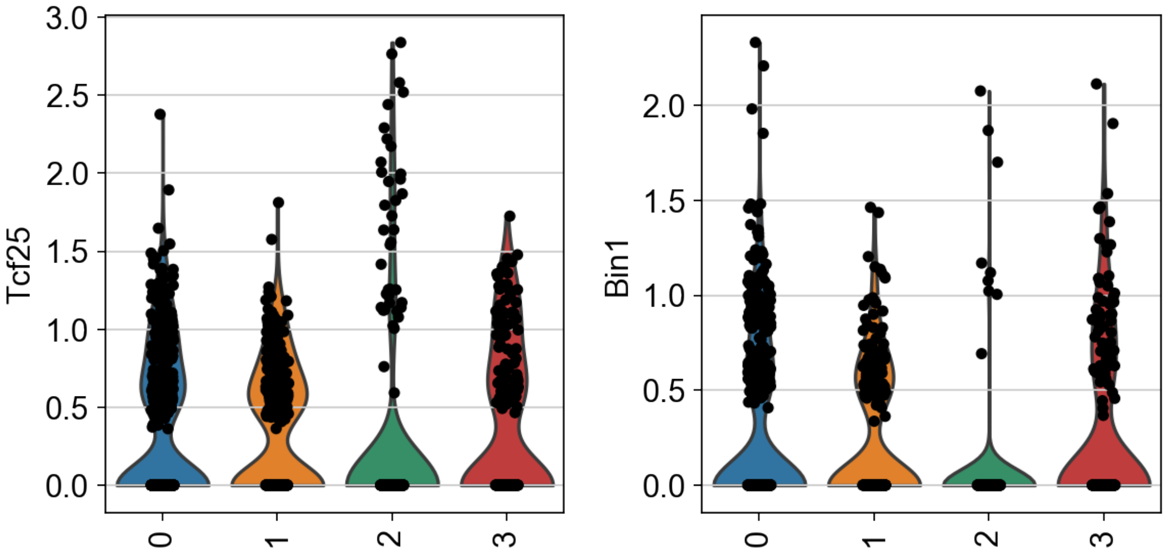
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_preadip_markers_preadipcytes_4
.pdf
```



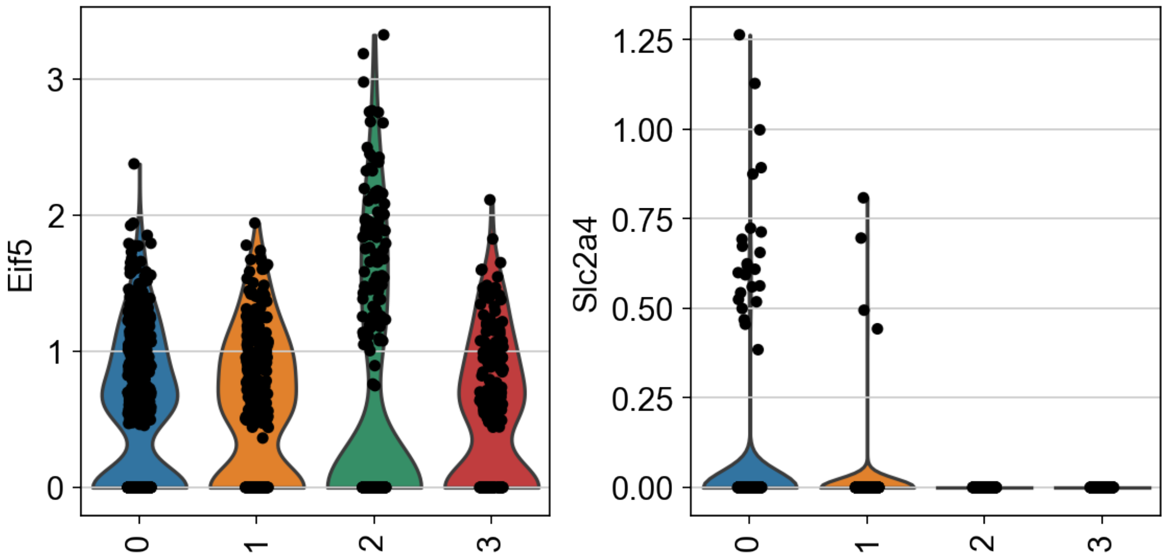
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_preadip_markers_preadipcytes_5
.pdf
```



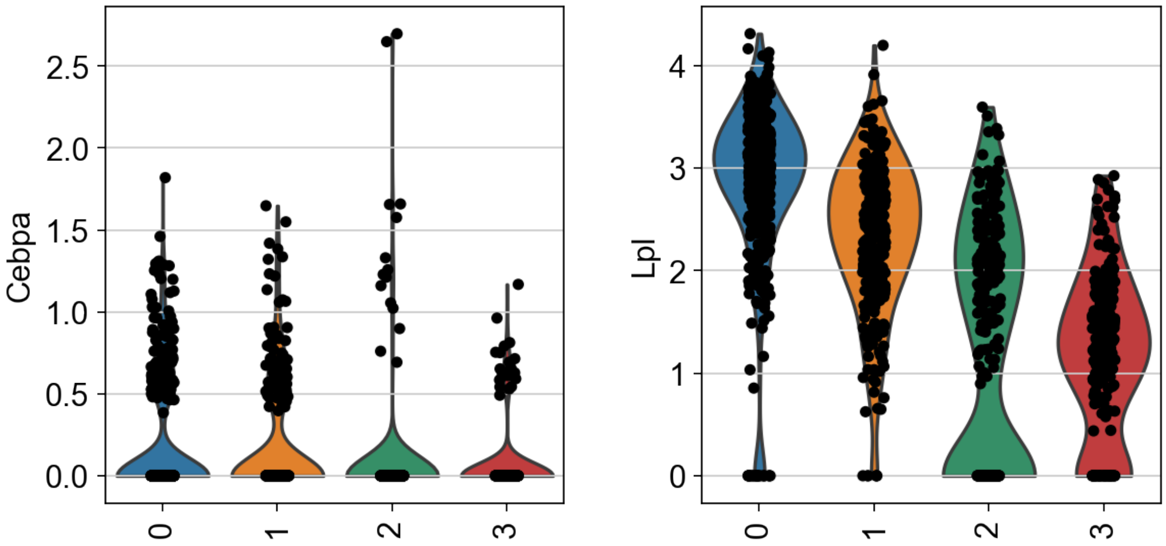
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_preadip_markers_preadipcytes_6
.pdf
```



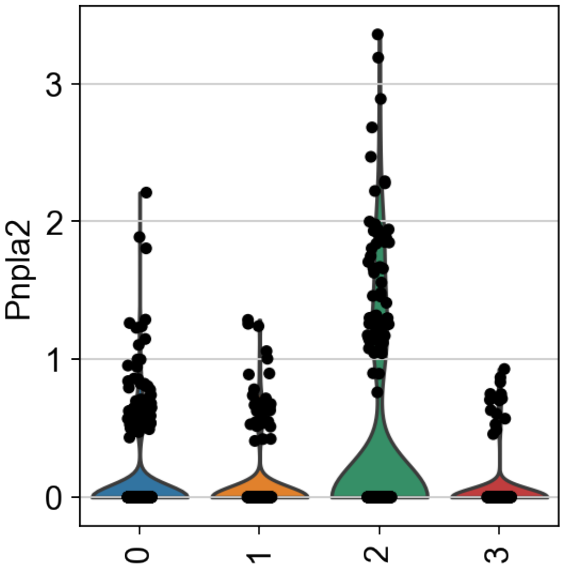
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_preadip_markers_preadipcytes_7
.pdf
```



```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_preadip_markers_preadipcytes_8
.pdf
```



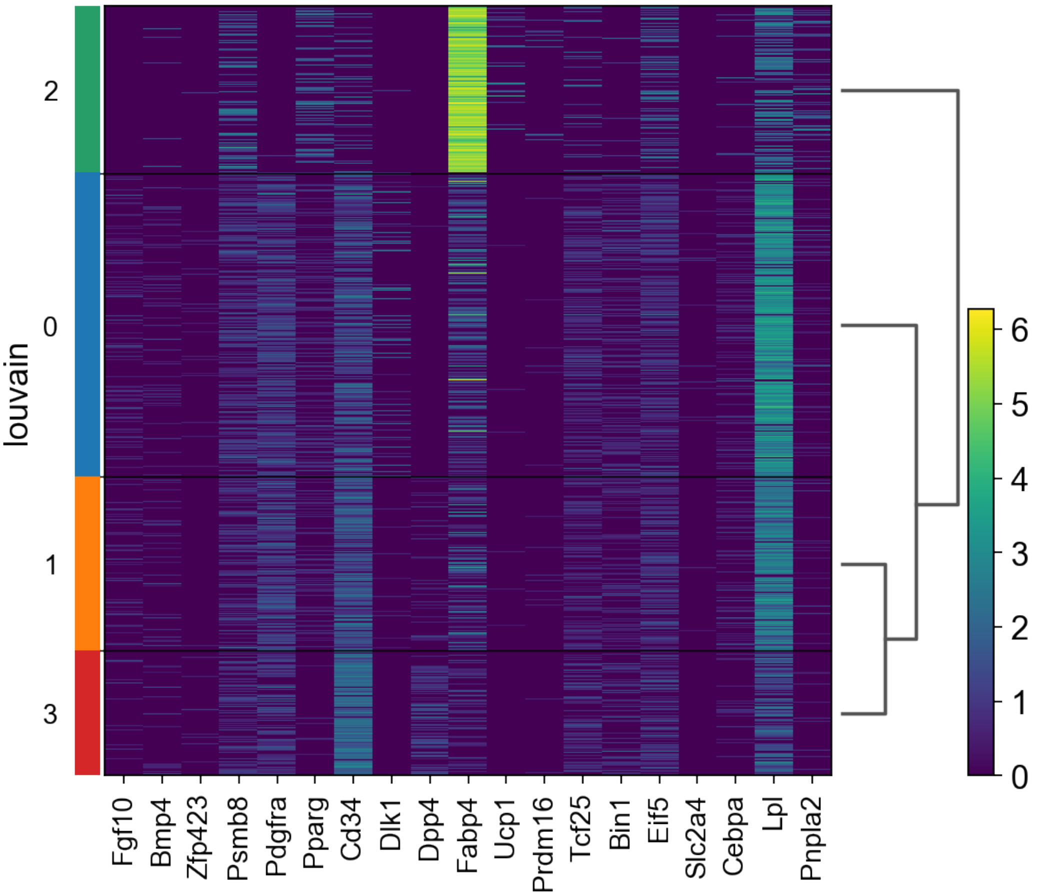
```
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/violin_preadip_markers_preadipcytes_9
.pdf
```



FABP4 is present only in cluster 2. Together with Pparg expression in cluster 2, it can be concluded that cluster 2 represents mature adipocytes instead of preadipocytes. Clusters 0,1 and 3 express multiple preadpocyte markers.

In [47]:
```
if bool_plot==True:
    sc.pl.heatmap(
        adata=adata_adip,
        var_names=adipocyte_markers,
        groupby="louvain",
        use_raw=False,
        log=False,
        dendrogram=True,
        var_group_rotation=90,
        show_gene_labels=True,
        show=True,
        save="_adipocyte_markers_celltypes.pdf"
    )
```

```
WARNING: dendrogram data not found (using key=dendrogram_louvain).
Running `sc.tl.dendrogram` with default parameters. For fine tunin
g it is recommended to run `sc.tl.dendrogram` independently.
    using 'X_pca' with n_pcs = 50
Storing dendrogram info using `.uns['dendrogram_louvain']`
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/heatmap_adipocyte_markers_celltypes.p
df
```

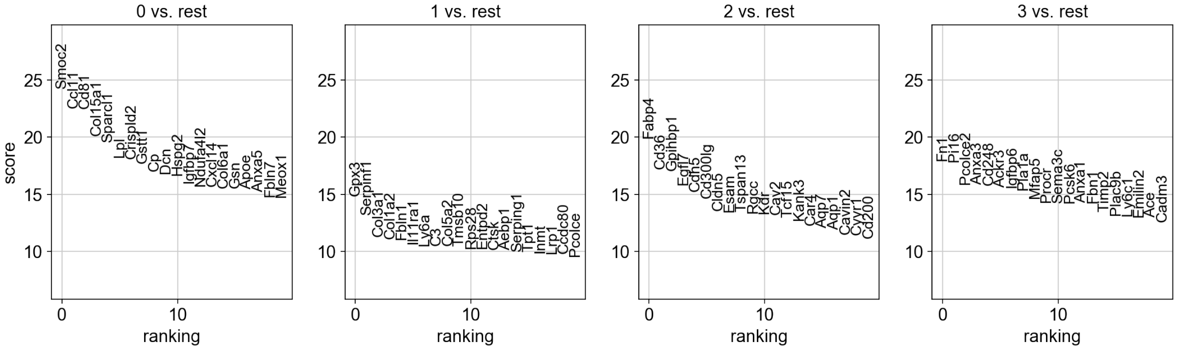


Top ranking DE Genes

In [48]:

```
sc.tl.rank_genes_groups(adata_adip, groupby='louvain', key_added='rank_genes')
if bool_plot == True:
    sc.pl.rank_genes_groups(adata_adip, key='rank_genes', groups=['0','1','2','3'], fontsize=12, save="_adip_genes")
```

```
ranking genes
    finished: added to `.uns['rank_genes']`
    'names', sorted np.recarray to be indexed by group ids
    'scores', sorted np.recarray to be indexed by group ids
    'logfoldchanges', sorted np.recarray to be indexed by group ids
    'pvals', sorted np.recarray to be indexed by group ids
    'pvals_adj', sorted np.recarray to be indexed by group ids (0:00:00)
WARNING: saving figure to file /Users/david.fischer/phd/data/PreadipocytesBrown/results/panels/rank_genes_groups_louvain_adip_genes.pdf
```

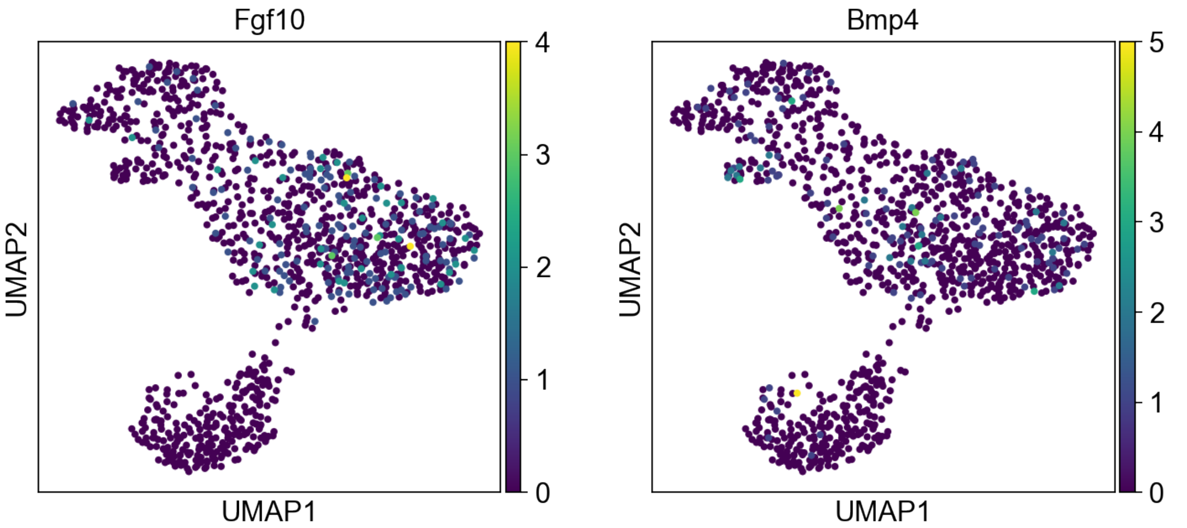


Marker Counts Raw and Normalized

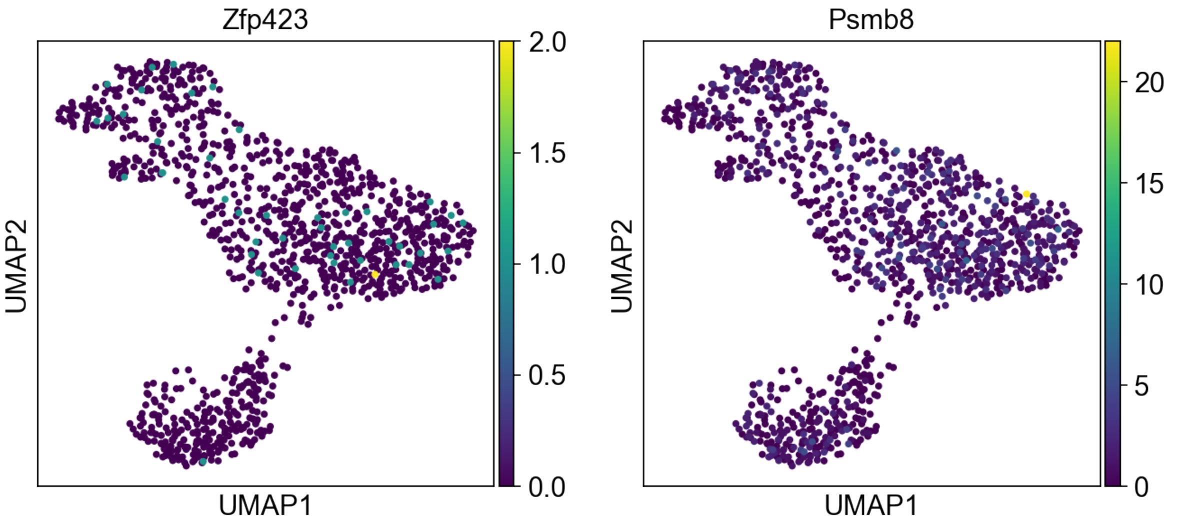
In [49]:

```
if bool_plot == True:
    plot_umap_marker(adata_adip, adipocyte_markers.tolist(), size=50, save="_preadip_markers_preadipcytes_raw", use_raw=True)
```

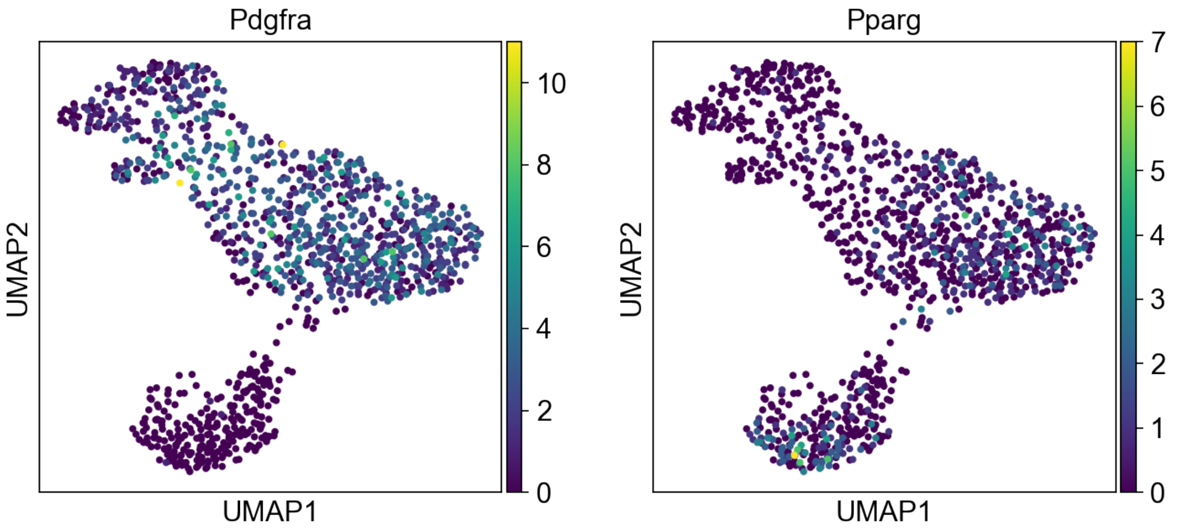
```
['Fgf10', 'Bmp4']
WARNING: saving figure to file /Users/david.fischer/phd/data/PreadipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_raw_0.pdf
```



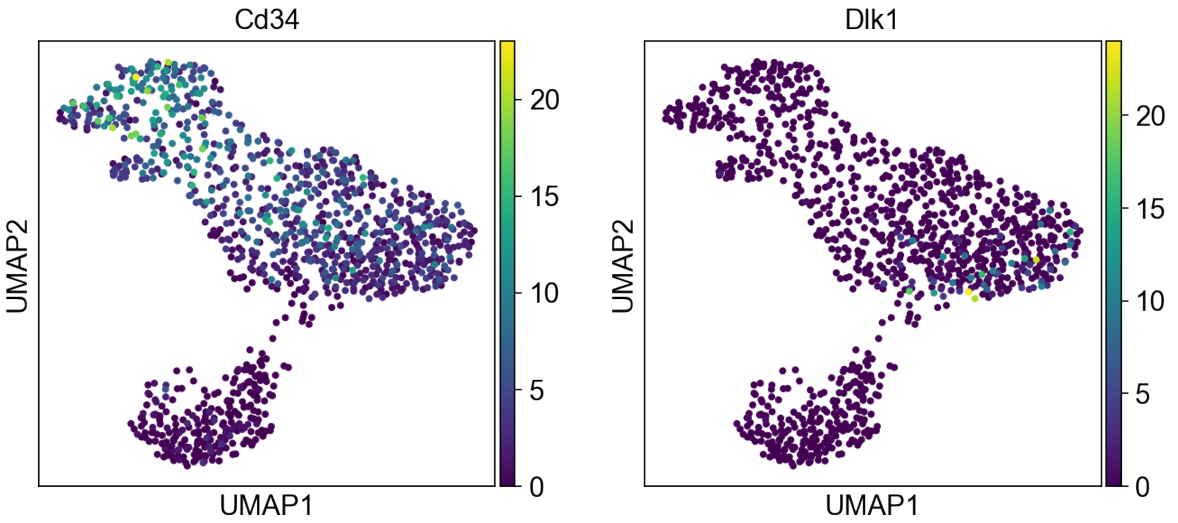
```
['Zfp423', 'Psmb8']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_raw
_1.pdf
```



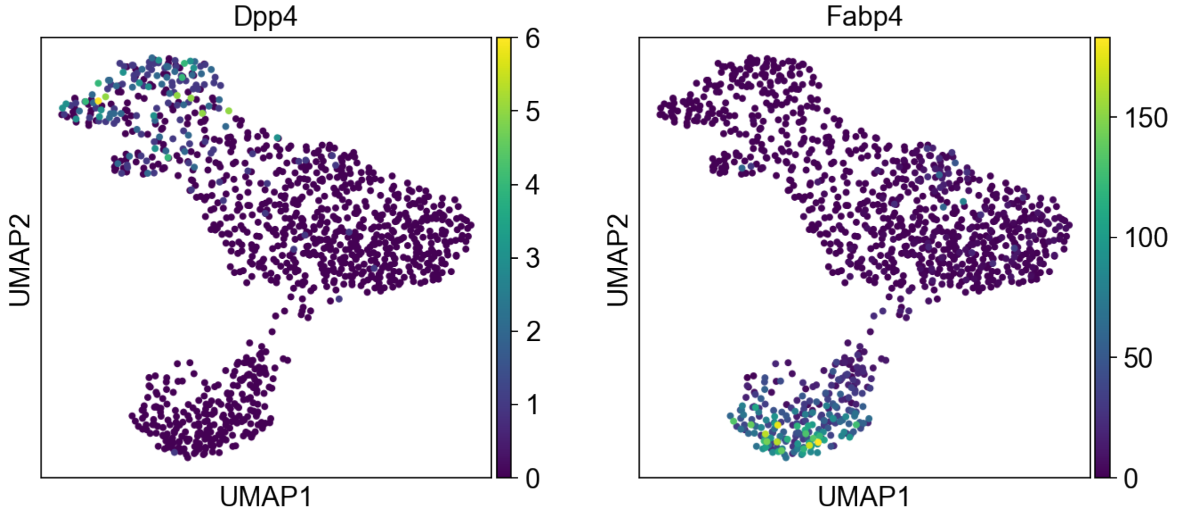
```
['Pdgfra', 'Pparg']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_raw
_2.pdf
```



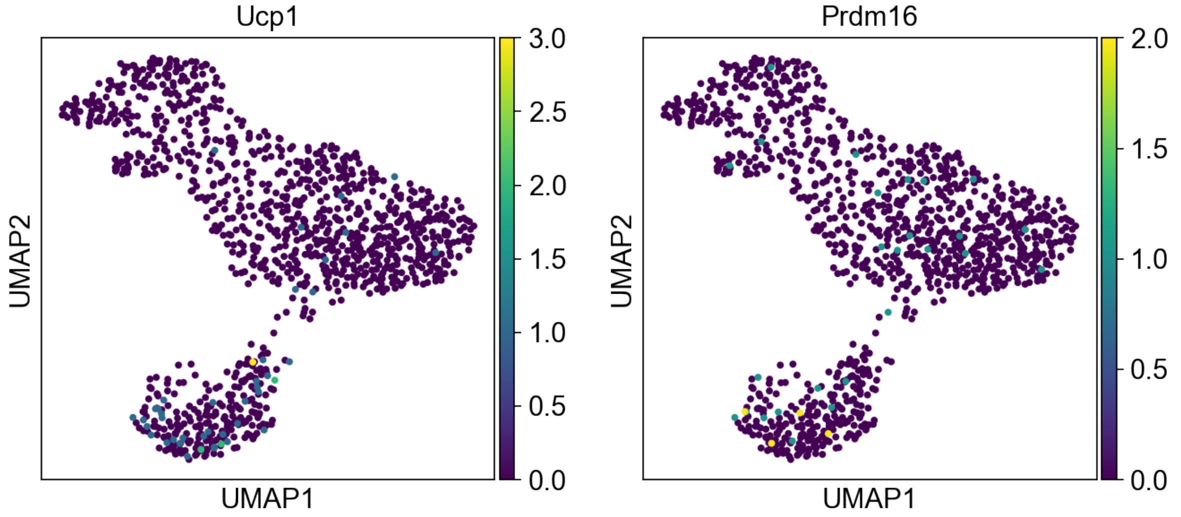
```
['Cd34', 'Dlk1']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_raw
_3.pdf
```



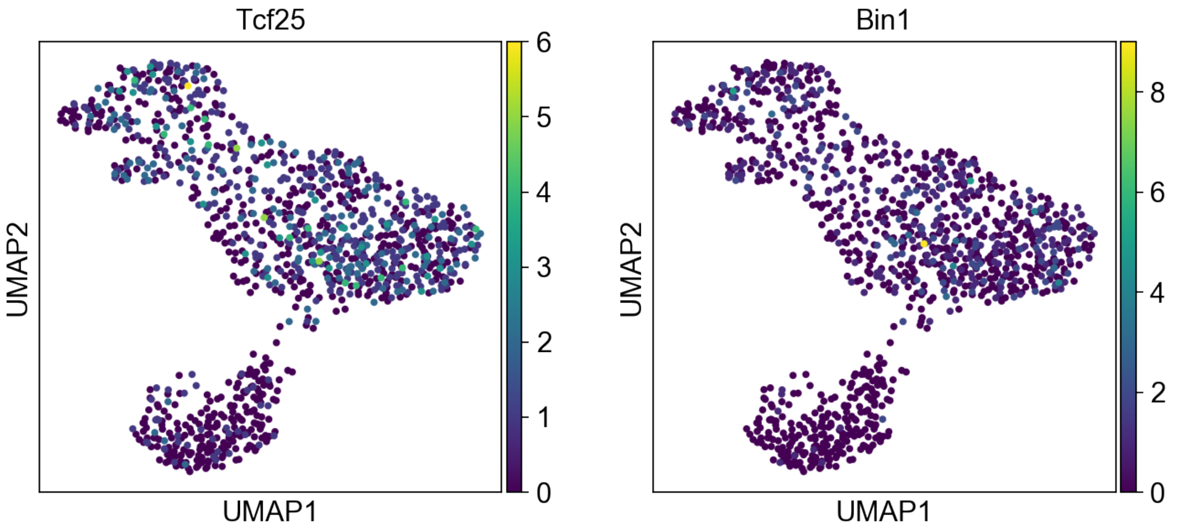
```
['Dpp4', 'Fabp4']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_raw
_4.pdf
```



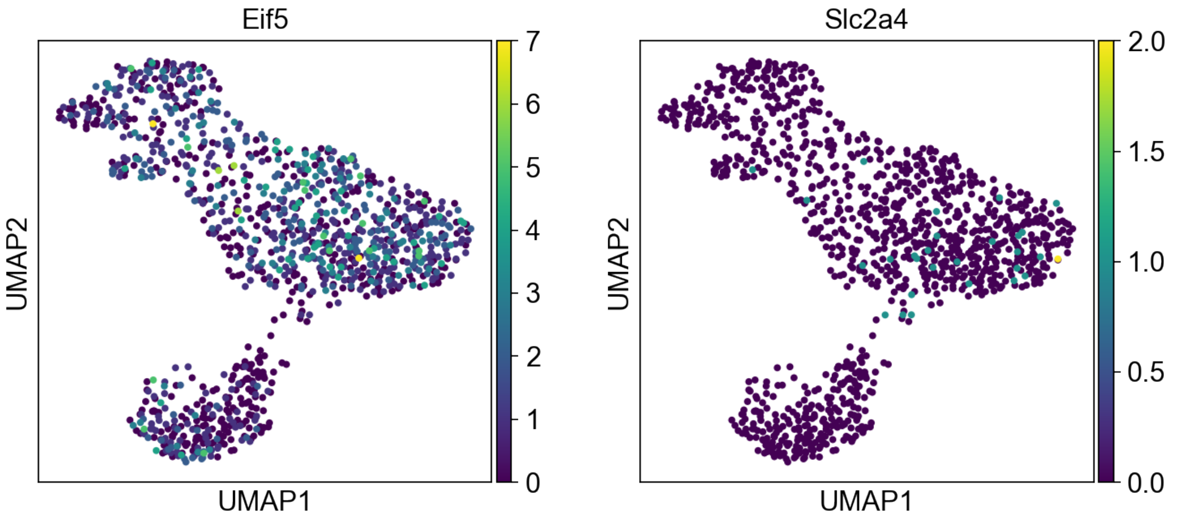
```
['Ucp1', 'Prdm16']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_raw
_5.pdf
```



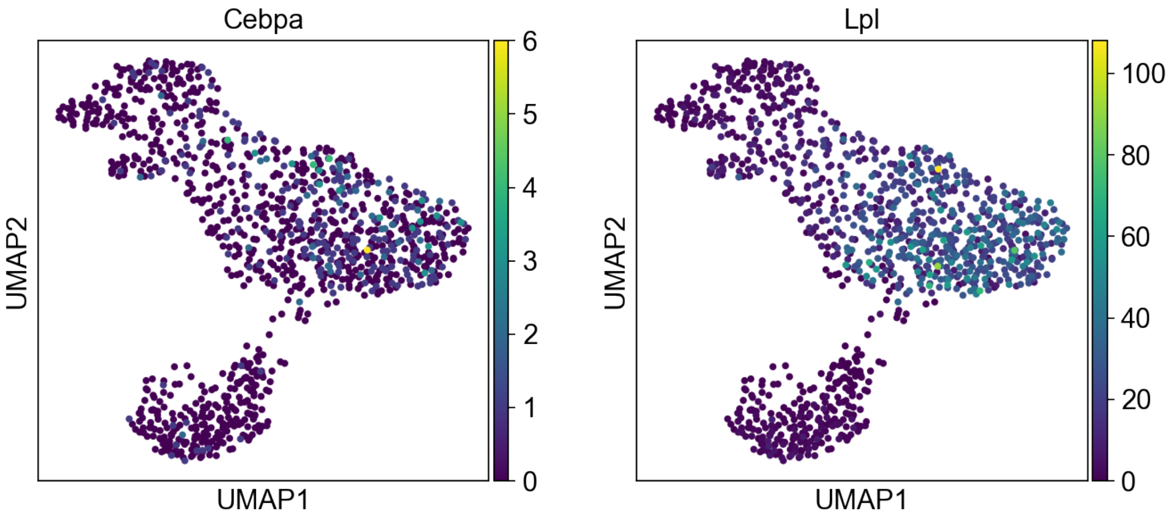
```
['Tcf25', 'Bin1']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_raw
_6.pdf
```



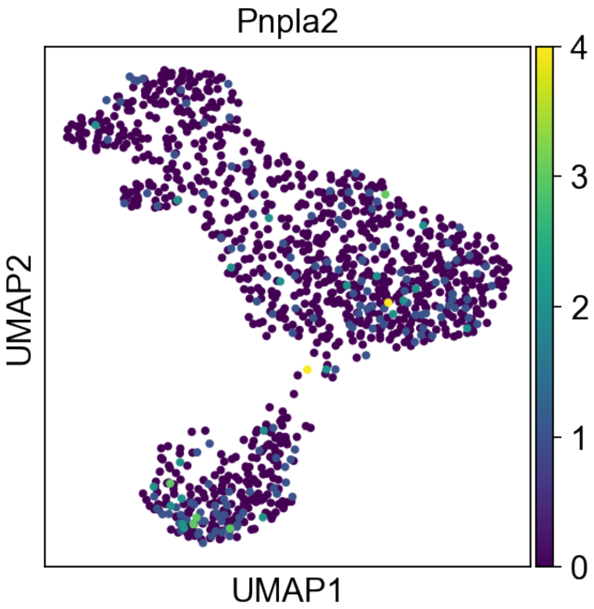
```
['Eif5', 'Slc2a4']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_raw
_7.pdf
```



```
['Cebpa', 'Lpl']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_raw
_8.pdf
```

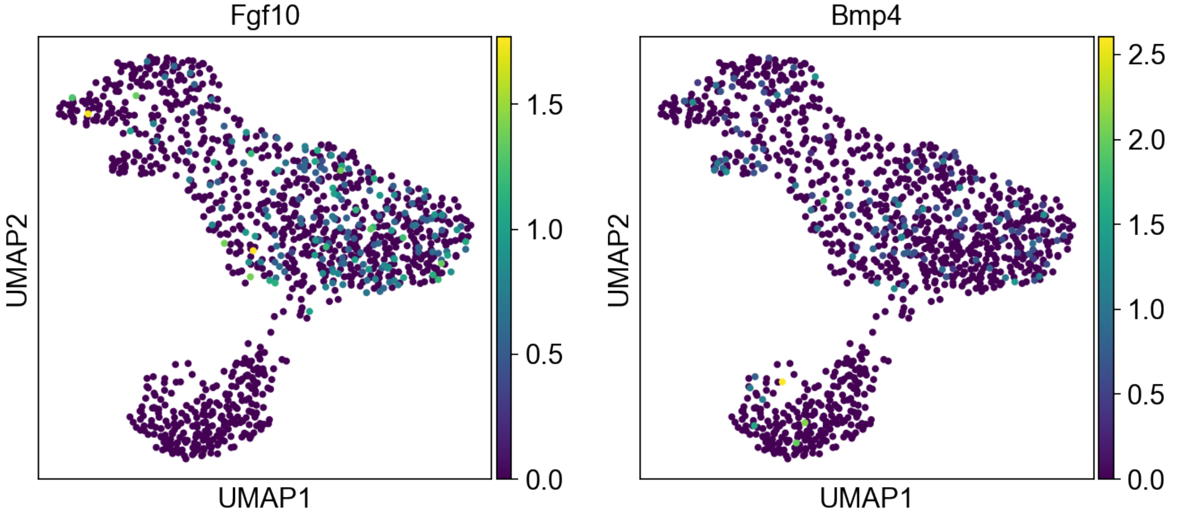


```
['Pnpla2']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_raw
_9.pdf
```

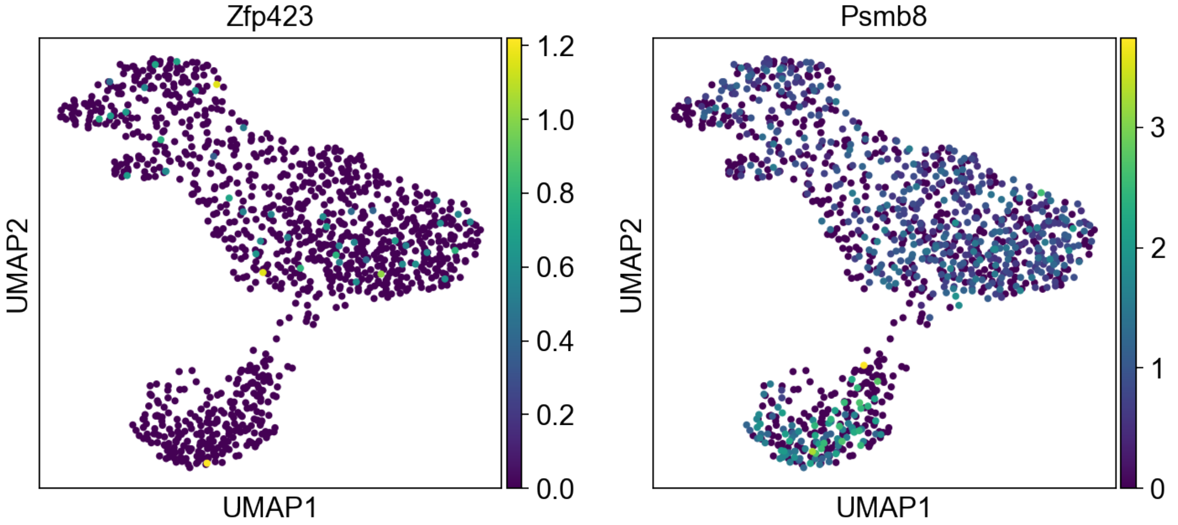


```
In [50]: if bool_plot == True:
             plot_umap_marker(adata_adip, adipocyte_markers.tolist(), size=5
         0, save="_preadip_markers_preadipcytes_norm", use_raw=False)
```

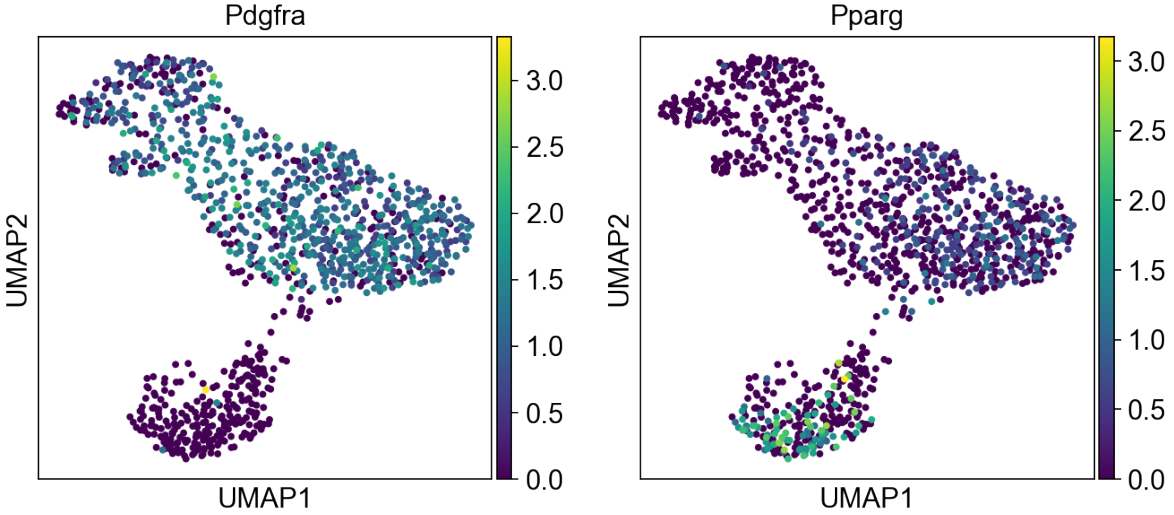
```
['Fgf10', 'Bmp4']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_0.pdf
```



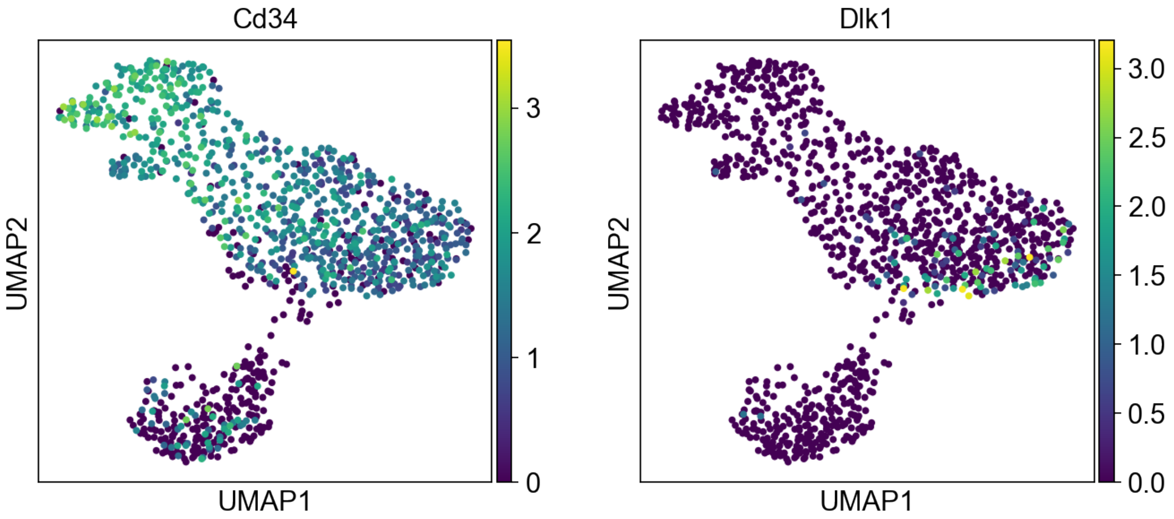
```
['Zfp423', 'Psmb8']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_1.pdf
```



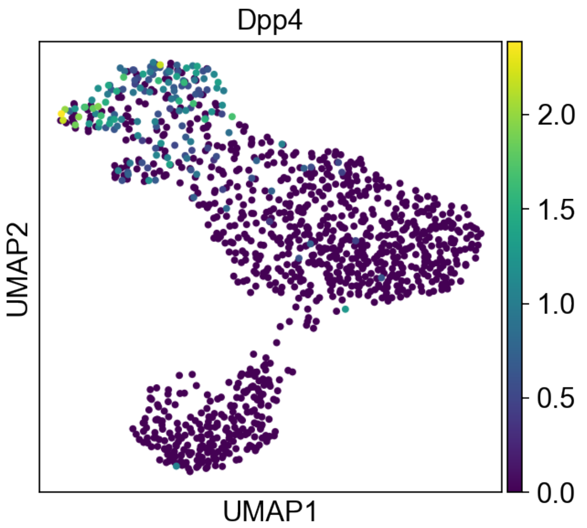
```
['Pdgfra', 'Pparg']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_2.pdf
```

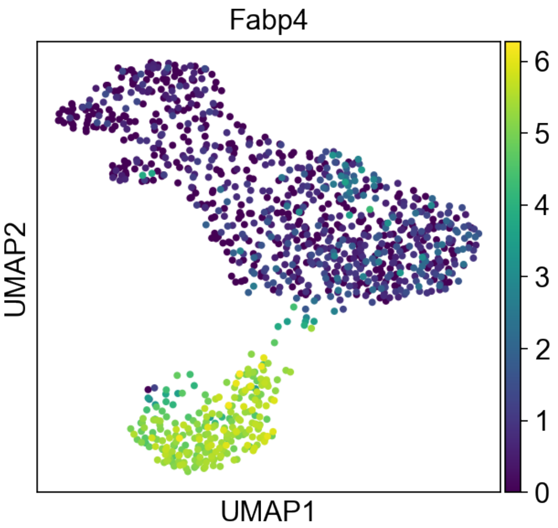


```
['Cd34', 'Dlk1']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_3.pdf
```

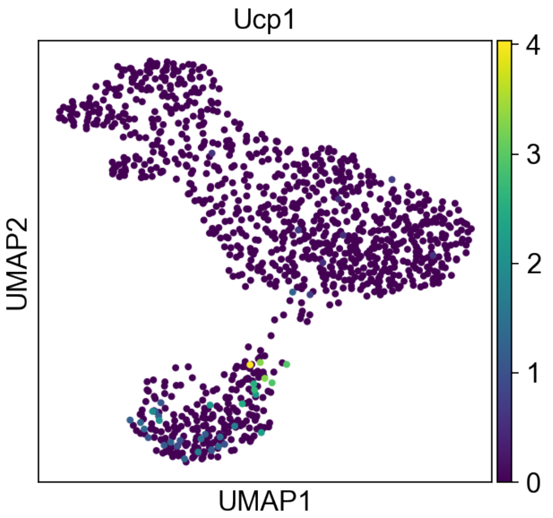


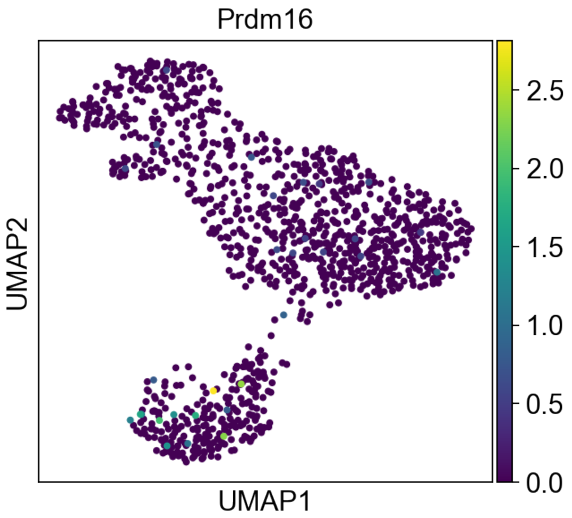
```
['Dpp4', 'Fabp4']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_4.pdf
```



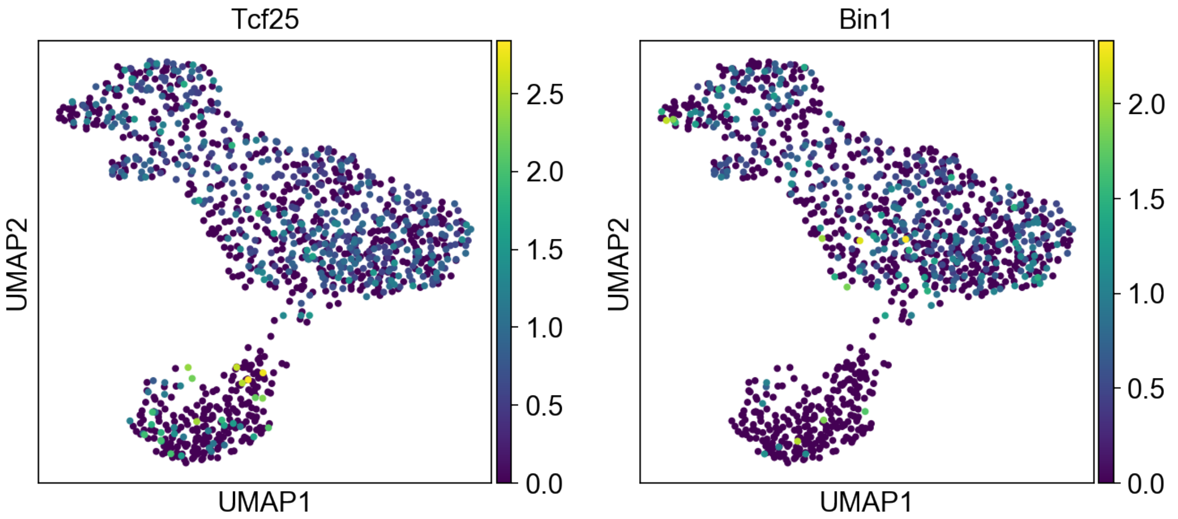


```
['Ucp1', 'Prdm16']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_5.pdf
```

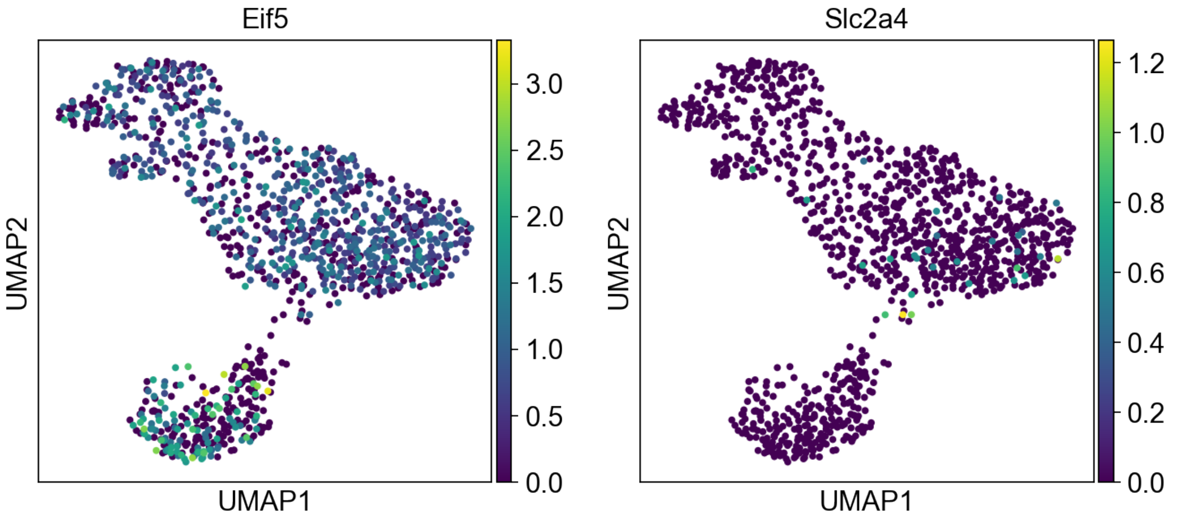




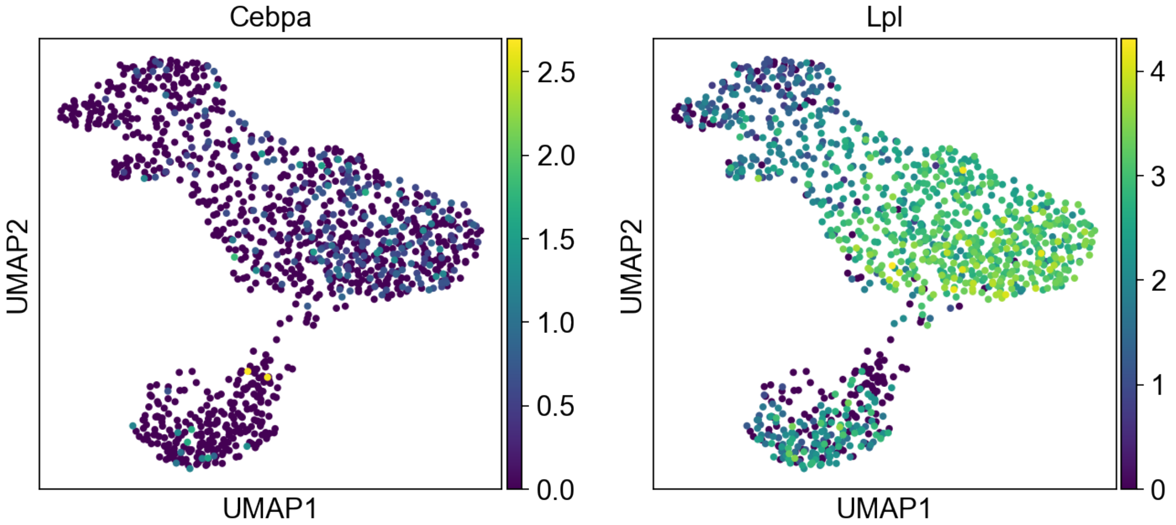
```
['Tcf25', 'Bin1']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_6.pdf
```



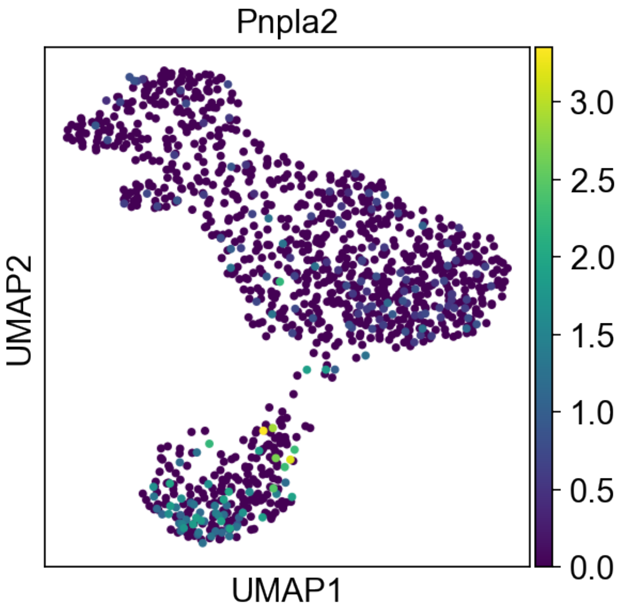
```
['Eif5', 'Slc2a4']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_7.pdf
```



```
['Cebpa', 'Lpl']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_8.pdf
```



```
['Pnpla2']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_9.pdf
```



Count distribution for Tcf25, Bin1 and Eif5

Single Double and Triple Positive Counts

Define booleans for single, double and triple positive counts of bin1, eif5 and tcf25

In [54]:

```
non_boolean_int = np.array((adata_adip[:,'Bin1'].X.todense()<=0) & (adata_adip[:,'Eif5'].X.todense()<=0) & (adata_adip[:,'Tcf25'].X.todense()<=0), dtype=int)

bin1_single_boolean = (adata_adip[:,'Bin1'].X.todense()>0) & (adata_adip[:,'Eif5'].X.todense()<=0) & (adata_adip[:,'Tcf25'].X.todense()<=0)
eif5_single_boolean = (adata_adip[:,'Eif5'].X.todense()>0) & (adata_adip[:,'Bin1'].X.todense()<=0) & (adata_adip[:,'Tcf25'].X.todense()<=0)
tcf25_single_boolean = (adata_adip[:,'Tcf25'].X.todense()>0) & (adata_adip[:,'Eif5'].X.todense()<=0) & (adata_adip[:,'Bin1'].X.todense()<=0)
single_boolean_int = np.array((bin1_single_boolean | eif5_single_boolean | tcf25_single_boolean), dtype=int)*1

bin1_eif5_double_boolean = (adata_adip[:,'Bin1'].X.todense()>0) & (adata_adip[:,'Eif5'].X.todense()>0) & (adata_adip[:,'Tcf25'].X.todense()<=0)
bin1_tcf25_double_boolean = (adata_adip[:,'Eif5'].X.todense()<=0) & (adata_adip[:,'Bin1'].X.todense()>0) & (adata_adip[:,'Tcf25'].X.todense()>0)
tcf25_eif5_double_boolean = (adata_adip[:,'Tcf25'].X.todense()>0) & (adata_adip[:,'Eif5'].X.todense()>0) & (adata_adip[:,'Bin1'].X.todense()<=0)
double_boolean_int = np.array((bin1_eif5_double_boolean | bin1_tcf25_double_boolean | tcf25_eif5_double_boolean), dtype=int)*2

triple_boolean = (adata_adip[:,'Tcf25'].X.todense()>0) & (adata_adip[:,'Eif5'].X.todense()>0) & (adata_adip[:,'Bin1'].X.todense()>0)
triple_boolean_int = np.array(triple_boolean, dtype=int)*3
non_boolean_int *=0
```

In [55]:

```
bin1_single_pos = adata_adip[:,'Bin1'].X[bin1_single_boolean]
eif5_single_pos = adata_adip[:,'Eif5'].X[eif5_single_boolean]
tcf25_single_pos = adata_adip[:,'Tcf25'].X[tcf25_single_boolean]

print('Bin1 Single Positive ',len(bin1_single_pos))
print('eif5 Single Positive ',len(eif5_single_pos))
print('tcf25 Single Positive ',len(tcf25_single_pos))

bin1_eif5_double_pos = adata_adip[:,'Bin1'].X[bin1_eif5_double_bool
ean]
bin1_tcf25_double_pos = adata_adip[:,'Eif5'].X[bin1_tcf25_double_bo
olean]
tcf25_eif5_double_pos = adata_adip[:,'Tcf25'].X[tcf25_eif5_double_b
oolean]

print('Bin1/Eif5 Double Positive ',len(bin1_eif5_double_pos))
print('Bin1/Tcf25 Double Positive ',len(bin1_tcf25_double_pos))
print('Tcf25/Eif5 Double Positive ',len(tcf25_eif5_double_pos))

triple_pos = adata_adip[:,'Bin1'].X[triple_boolean]
print('Triple Positive ',len(triple_pos))
```

```
Bin1 Single Positive  1
eif5 Single Positive  1
tcf25 Single Positive  1
Bin1/Eif5 Double Positive  1
Bin1/Tcf25 Double Positive  1
Tcf25/Eif5 Double Positive  1
Triple Positive  1
```

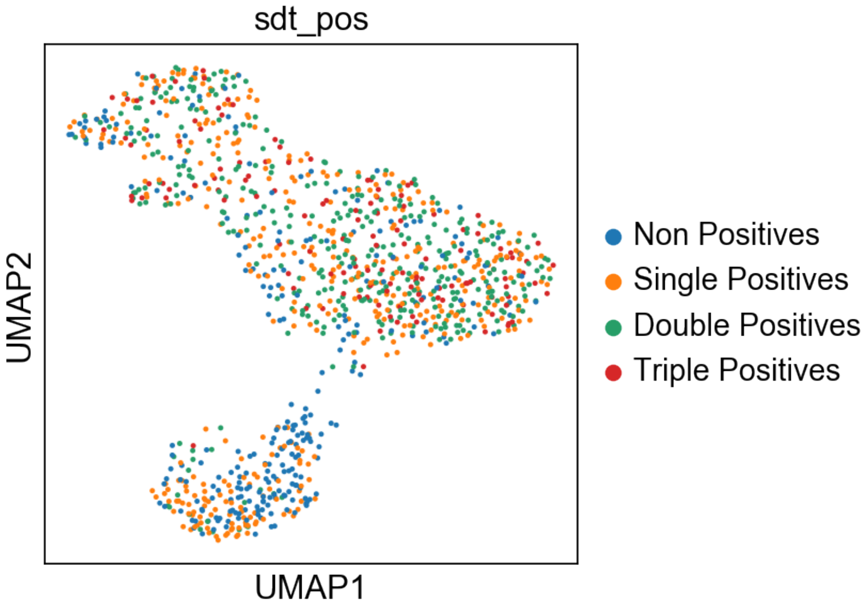
Define observations for single, double and triple positive counts of bin1, eif5 and tcf25

In [56]:

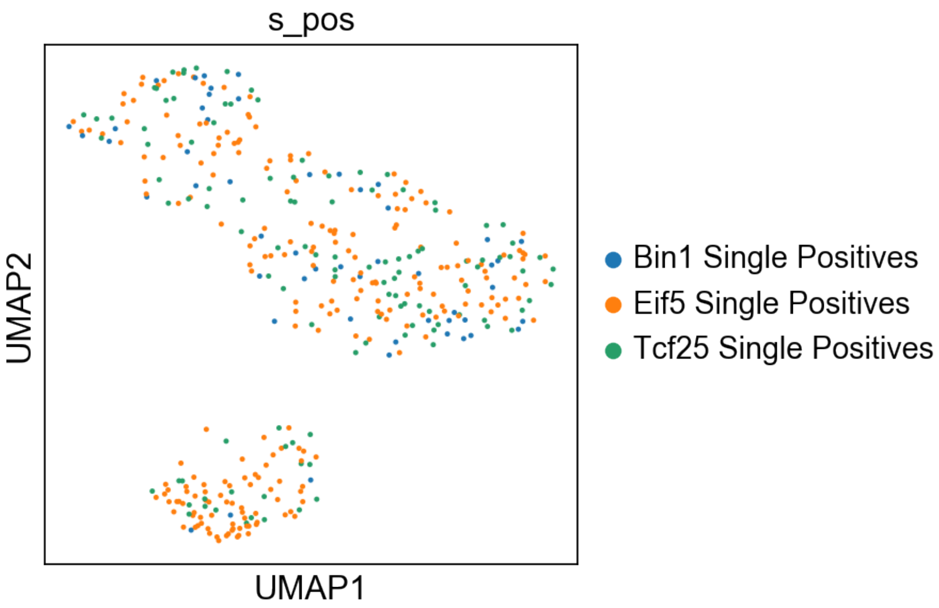
```
adata_adip.obs['sdt_pos'] = np.array((non_boolean_int + single_bool
ean_int + double_boolean_int + triple_boolean_int), dtype=str)
adata_adip.obs['s_pos'] = np.array((np.array(bin1_single_boolean, d
type=int)*1)+(np.array(eif5_single_boolean, dtype=int)*2)+(np.array
(tcf25_single_boolean, dtype=int)*3), dtype=str)
adata_adip.obs['d_pos'] = np.array((np.array(bin1_eif5_double_boole
an, dtype=int)*1)+(np.array(bin1_tcf25_double_boolean, dtype=int)*2
)+(np.array(tcf25_eif5_double_boolean, dtype=int)*3), dtype=str)
adata_adip.obs['t_pos'] = np.array(triple_boolean, dtype=str)
# make them categorical
adata_adip.obs['sdt_pos'] = pd.Series(adata_adip.obs['sdt_pos'], dt
ype="category")
adata_adip.obs['s_pos'] = pd.Series(adata_adip.obs['s_pos'], dtype=
"category")
adata_adip.obs['d_pos'] = pd.Series(adata_adip.obs['d_pos'], dtype=
"category")
adata_adip.obs['t_pos'] = pd.Series(adata_adip.obs['t_pos'], dtype=
"category")
```

In [57]:

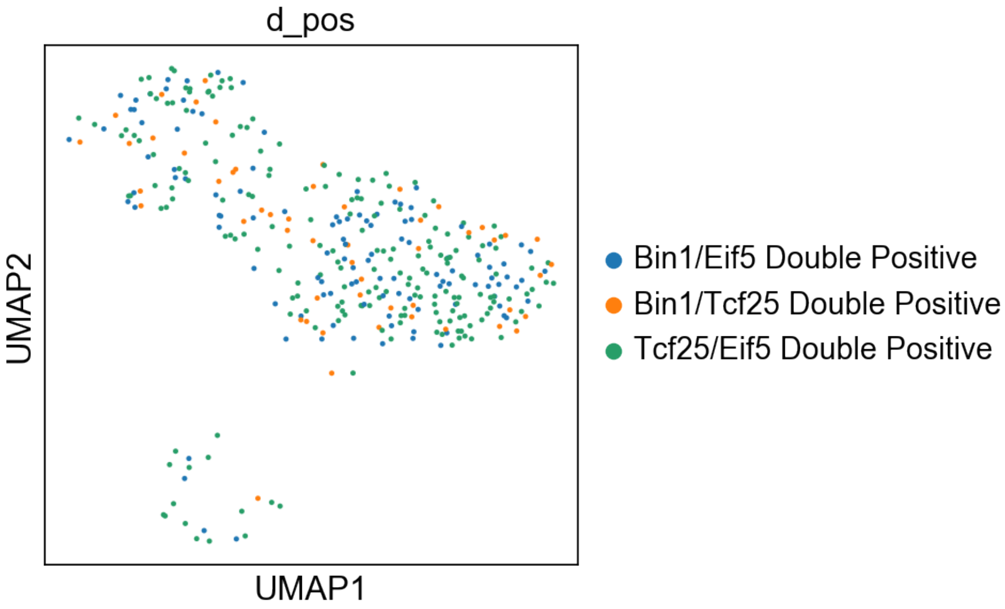
```
if bool_plot == True:
    new_cluster_names = ['Non Positives','Single Positives','Double Positives','Triple Positives']
    adata_adip.rename_categories('sdt_pos', new_cluster_names)
    sc.pl.umap(adata_adip, color=['sdt_pos'], size=20)
    new_cluster_names = ['Non Single Positives','Bin1 Single Positives','Eif5 Single Positives','Tcf25 Single Positives']
    adata_adip.rename_categories('s_pos', new_cluster_names)
    sc.pl.umap(adata_adip[adata_adip.obs['s_pos']!='Non Single Positives'], color=['s_pos'], size=20)
    new_cluster_names = ['Non Double Positives','Bin1/Eif5 Double Positive','Bin1/Tcf25 Double Positive','Tcf25/Eif5 Double Positive']
    adata_adip.rename_categories('d_pos', new_cluster_names)
    sc.pl.umap(adata_adip[adata_adip.obs['d_pos']!='Non Double Positives'], color=['d_pos'], size=20)
    new_cluster_names = ['Non Triple Positives','Triple Positives']
    adata_adip.rename_categories('t_pos', new_cluster_names)
    sc.pl.umap(adata_adip[adata_adip.obs['t_pos']!='Non Triple Positives'], color=['t_pos'], size=20)
```



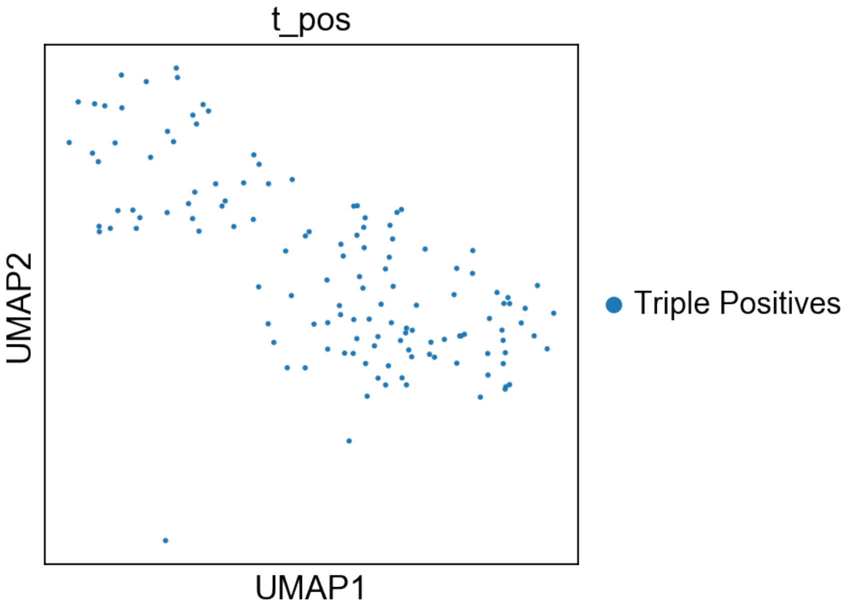
```
Trying to set attribute `.uns` of view, copying.
```



```
Trying to set attribute `.uns` of view, copying.
```



```
Trying to set attribute `.uns` of view, copying.
```



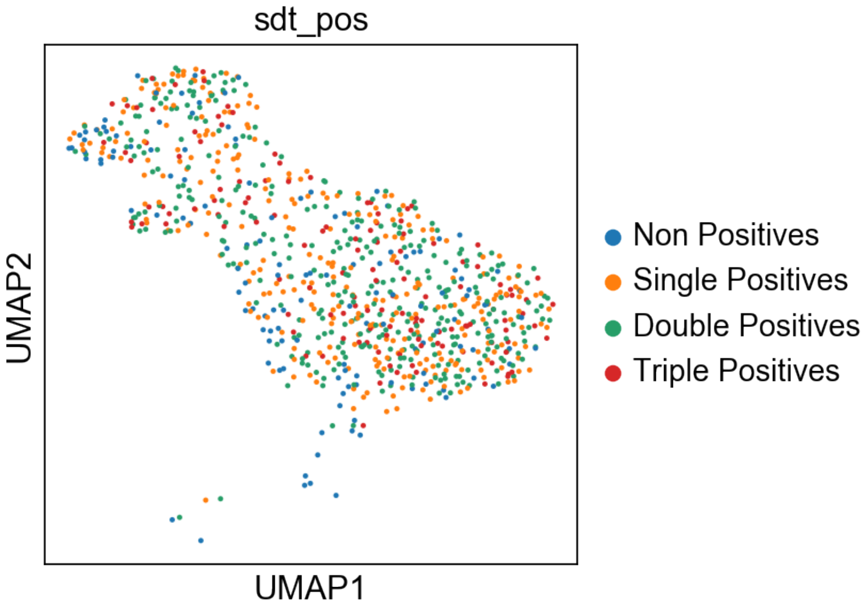
Single positives are found in all 4 clusters. Double positives are mostly in the clusters 0,1 and 3 (preadipocyte clusters) and far less present in cluster 2 (mature adipocyte cluster). Triple positives are found only in clusters 0,1 and 3 with only one exception.

Analysis separately for preadipocytes and mature adipocytes

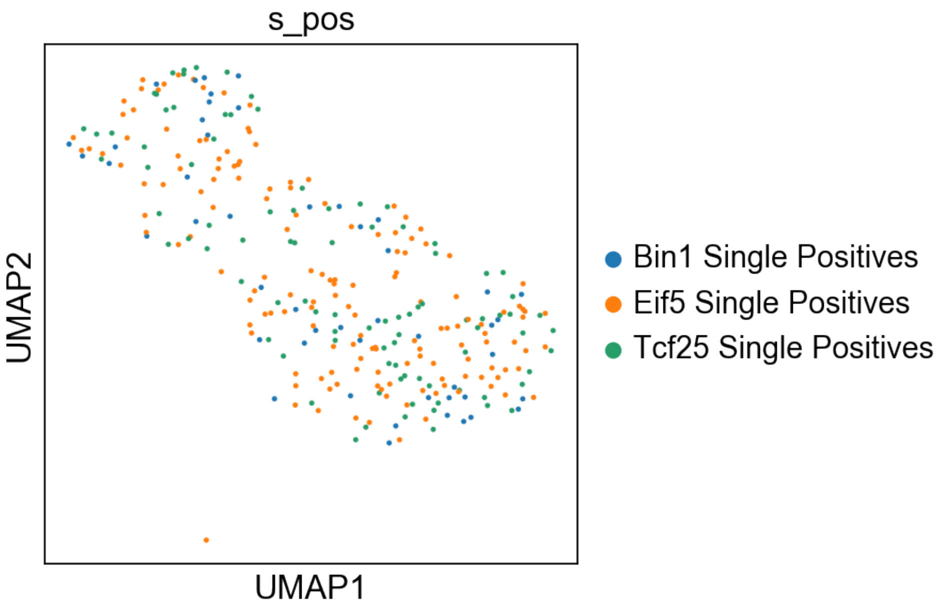
```
In [58]: adata_adip.obs['louvain'].head()
         adata_preadip = adata_adip[adata_adip.obs['louvain']!='2']
         adata_matadip = adata_adip[adata_adip.obs['louvain']=='2']
```

Preadipocyte clusters (0,1,3)

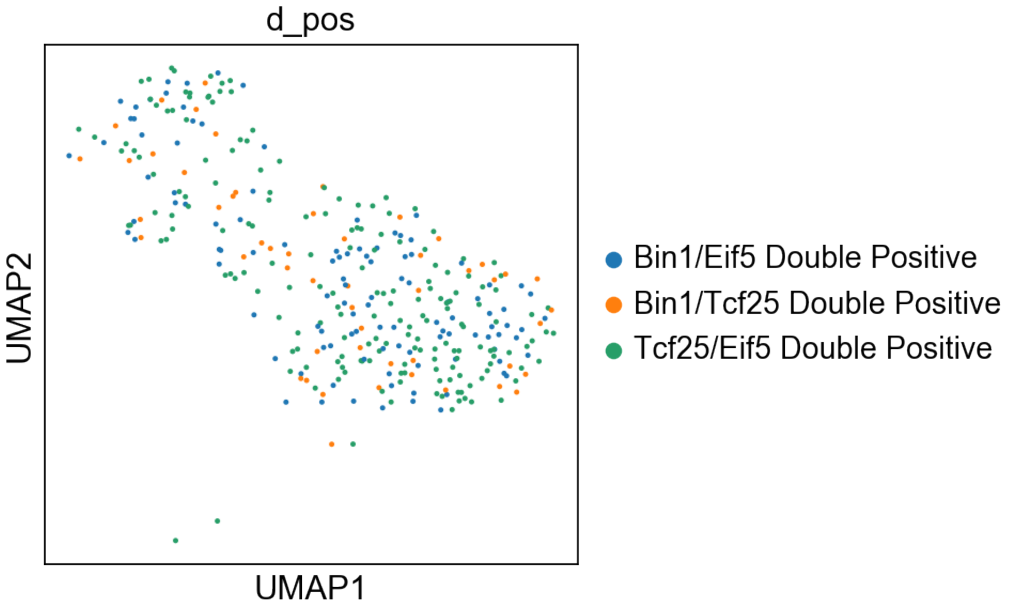
```
In [59]: if bool_plot == True:
             sc.pl.umap(adata_preadip, color=['sdt_pos'], size=20)
             sc.pl.umap(adata_preadip[adata_preadip.obs['s_pos']!='Non Singl
         e Positives'], color=['s_pos'], size=20)
             sc.pl.umap(adata_preadip[adata_preadip.obs['d_pos']!='Non Doubl
         e Positives'], color=['d_pos'], size=20)
             sc.pl.umap(adata_preadip[adata_preadip.obs['t_pos']!='Non Tripl
         e Positives'], color=['t_pos'], size=20)
```



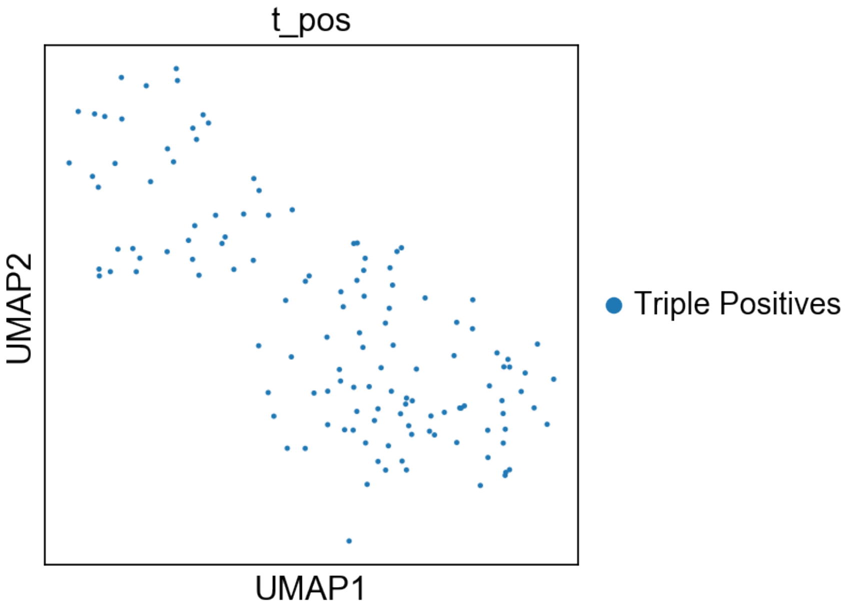
```
Trying to set attribute `.uns` of view, copying.
```



```
Trying to set attribute `.uns` of view, copying.
```

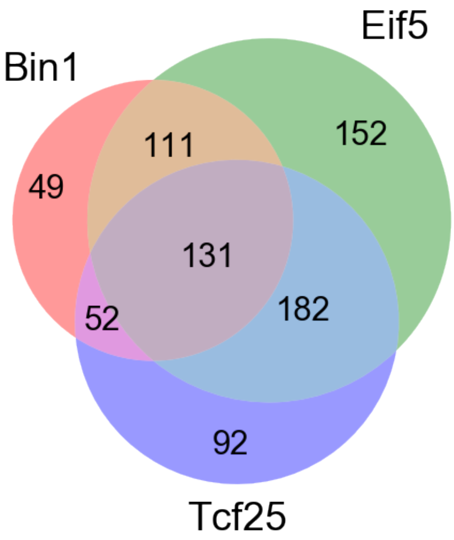


```
Trying to set attribute `.uns` of view, copying.
```



In [76]:

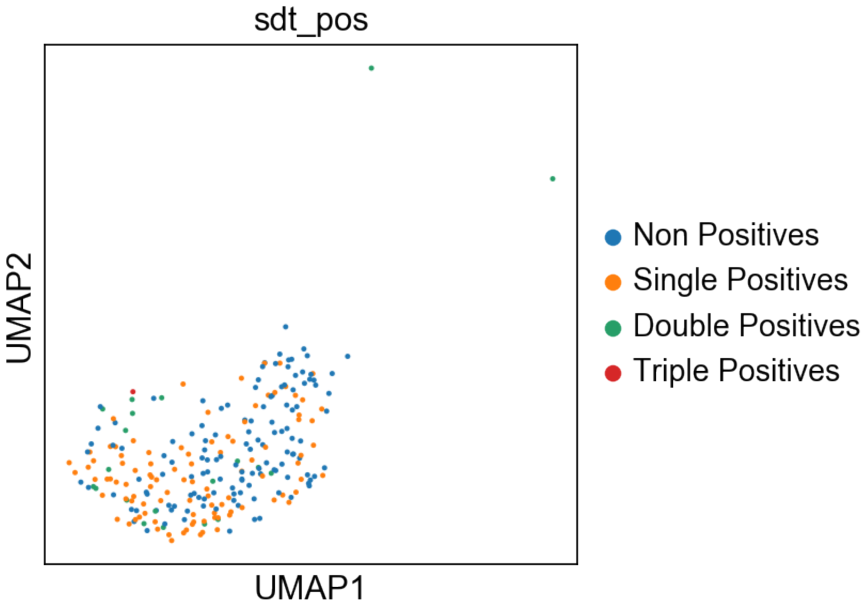
```
import matplotlib_venn

bin1_pos = adata_preadip.obs_names[np.asarray(adata_preadip[:,'Bin1'].X.todense()).flatten()>0]
eif5_pos = adata_preadip.obs_names[np.asarray(adata_preadip[:,'Eif5'].X.todense()).flatten()>0]
tcf25_pos = adata_preadip.obs_names[np.asarray(adata_preadip[:,'Tcf25'].X.todense()).flatten()>0]

matplotlib_venn.venn3([
    set(bin1_pos),
    set(eif5_pos),
    set(tcf25_pos)
], set_labels = ("Bin1", "Eif5", "Tcf25"))
plt.savefig(dir_out + "panels/venn_target_positives_preadipocytes.pdf")
```



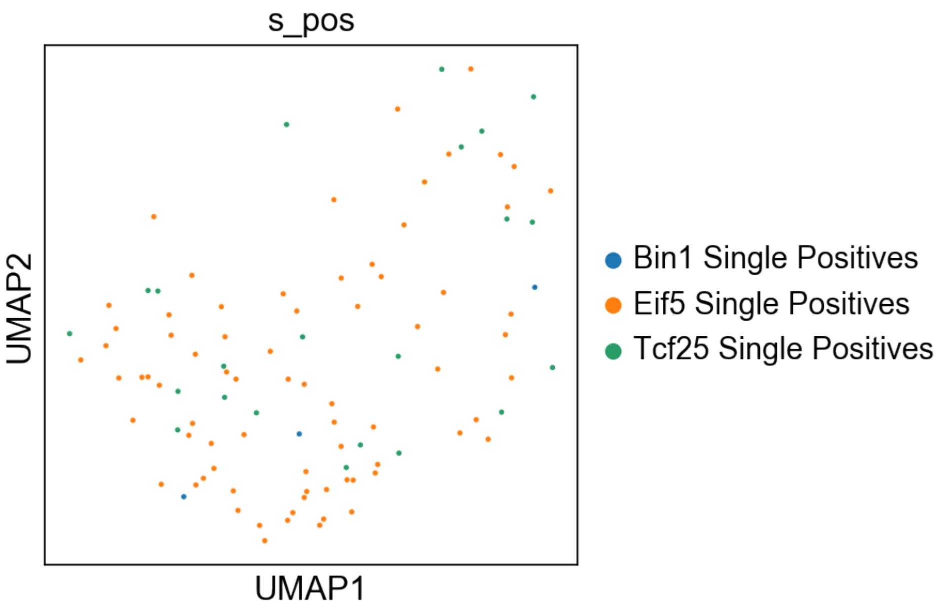
Mature adipocyte cluster

In [60]:

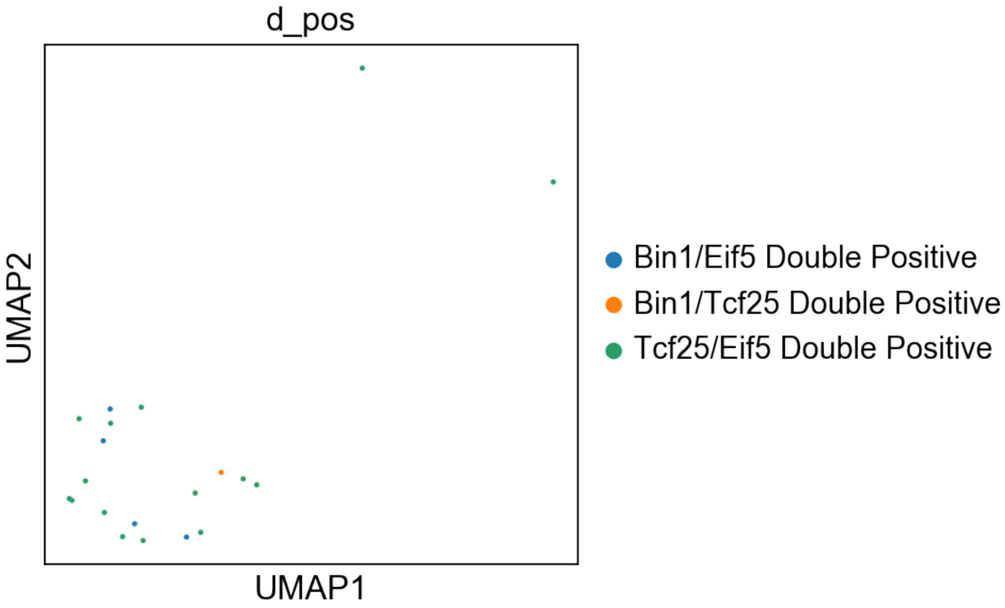
```
if bool_plot == True:
    sc.pl.umap(adata_matadip, color=['sdt_pos'], size=20)
    sc.pl.umap(adata_matadip[adata_matadip.obs['s_pos']!='Non Single Positives'], color=['s_pos'], size=20)
    sc.pl.umap(adata_matadip[adata_matadip.obs['d_pos']!='Non Double Positives'], color=['d_pos'], size=20)
    sc.pl.umap(adata_matadip[adata_matadip.obs['t_pos']!='Non Triple Positives'], color=['t_pos'], size=20)
```



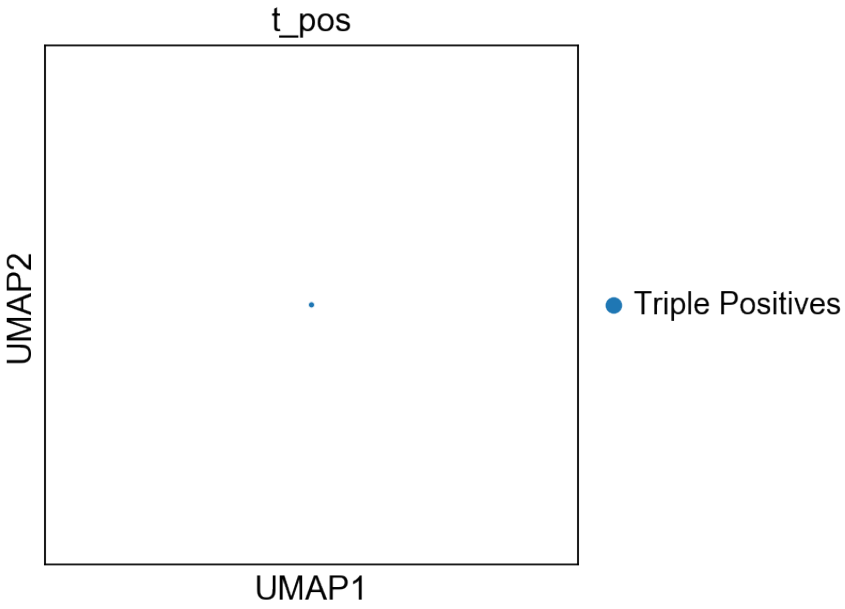
```
Trying to set attribute `.uns` of view, copying.
```



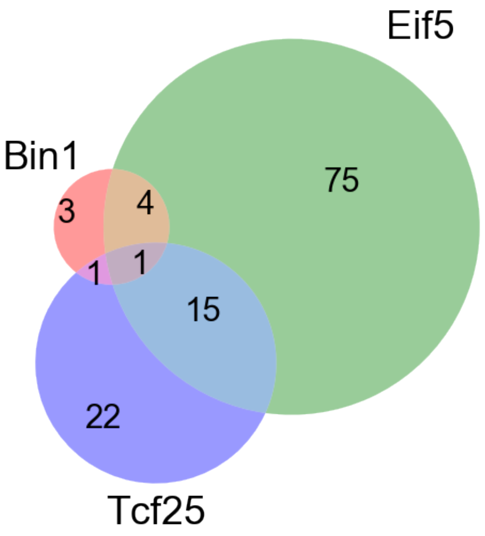
```
Trying to set attribute `.uns` of view, copying.
```



```
Trying to set attribute `.uns` of view, copying.
```



In [75]:
```
import matplotlib_venn

bin1_pos = adata_matadip.obs_names[np.asarray(adata_matadip[:,'Bin1'].X.todense()).flatten()>0]
eif5_pos = adata_matadip.obs_names[np.asarray(adata_matadip[:,'Eif5'].X.todense()).flatten()>0]
tcf25_pos = adata_matadip.obs_names[np.asarray(adata_matadip[:,'Tcf25'].X.todense()).flatten()>0]

matplotlib_venn.venn3([
    set(bin1_pos),
    set(eif5_pos),
    set(tcf25_pos)
], set_labels = ("Bin1", "Eif5", "Tcf25"))
plt.savefig(dir_out + "venn_target_positives_mature_adipocytes.pdf")
```



Mean Counts

Normalized

In [61]:
```
print('Bin1 mean counts: ',np.mean(adata_adip[:,'Bin1'].X))
print('Eif5 mean counts: ',np.mean(adata_adip[:,'Eif5'].X))
print('Tcf25 mean counts: ',np.mean(adata_adip[:,'Tcf25'].X))
```

```
Bin1 mean counts:  0.23998265
Eif5 mean counts:  0.6047478
Tcf25 mean counts:  0.37402338
```

Preadipocytes only

In [63]:

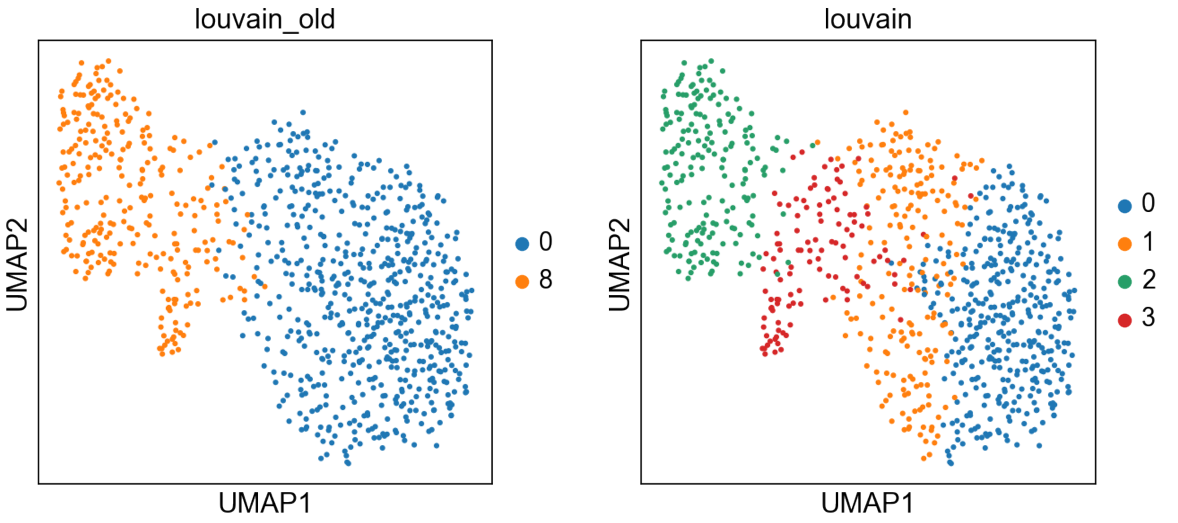
```
if bool_recomp == True:
    cell_ids_adip2 = np.asarray(adata_proc.obs_names)[
        [x in ['preadipocytes']
         for x in np.asarray(adata_proc.obs['celltypes'].values)]
    ]
    adata_adip2 = adata_raw[cell_ids_adip2,:].copy()
    adata_adip2.obs['n_genes'] = (adata_adip2.X > 0).sum(1)
    temp_n_counts2 = adata_adip2.obs['n_counts']
    adata_adip2.raw = adata_adip2
    adata_adip2.obs['louvain_old'] = adata_proc[cell_ids_adip2, :].
obs['louvain']
    sc.pp.normalize_per_cell(adata_adip2)
    sc.pp.log1p(adata_adip2)
    sc.pp.highly_variable_genes(adata_adip2,n_top_genes=4000)
    sc.pl.highly_variable_genes(adata_adip2)
    sc.pp.pca(adata_adip2, n_comps=50, use_highly_variable = True,
random_state=0, svd_solver='arpack')
    sc.pp.neighbors(adata_adip2, n_neighbors=100, knn=True, method=
'umap', n_pcs=50, random_state=0)
    sc.tl.umap(adata_adip2)
    if bool_recluster == True:
        sc.tl.louvain(adata_adip2, resolution=1, flavor='vtraag', r
andom_state=0)
        pd.DataFrame(adata_adip2.obs).to_csv(path_or_buf=dir_adata+
'obs_adata_adip2.csv')
    else:
        obs2 = pd.read_csv(dir_adata+'obs_adata_adip2.csv')
        adata_adip2.obs['louvain']=pd.Series(obs2['louvain'].values
, dtype = 'category')
    sc.write(dir_adata+'adata_adip2.h5ad',adata_adip2)
else:
    adata_adip2 = sc.read(dir_adata+'adata_adip2.h5ad')
sc.tl.paga(adata_adip2)
```

```
running PAGA
    finished: added
    'paga/connectivities', connectivities adjacency (adata.uns)
    'paga/connectivities_tree', connectivities subtree (adata.uns)
(0:00:00)
```

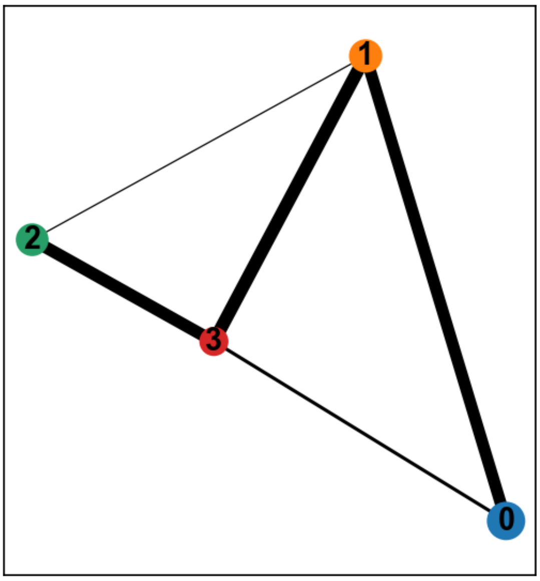
In [64]:

```
if bool_plot == True:
    sc.pl.umap(adata_adip2, color=['louvain_old', 'louvain'], size=30, save="_preadip2_louvain_0.pdf")
    sc.pl.paga(adata_adip2, color=['louvain'], save="_preadip2_louvain.pdf")
```

```
WARNING: saving figure to file /Users/david.fischer/phd/data/PreadipocytesBrown/results/panels/umap_preadip2_louvain_0.pdf
```



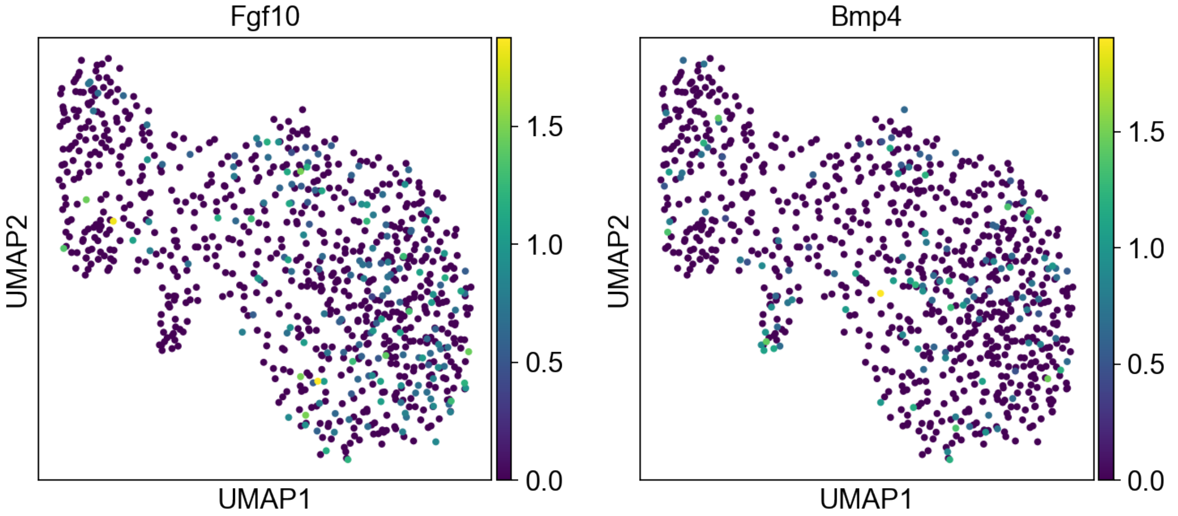
```
--> added 'pos', the PAGA positions (adata.uns['paga'])
WARNING: saving figure to file /Users/david.fischer/phd/data/PreadipocytesBrown/results/panels/paga_preadip2_louvain.pdf
```



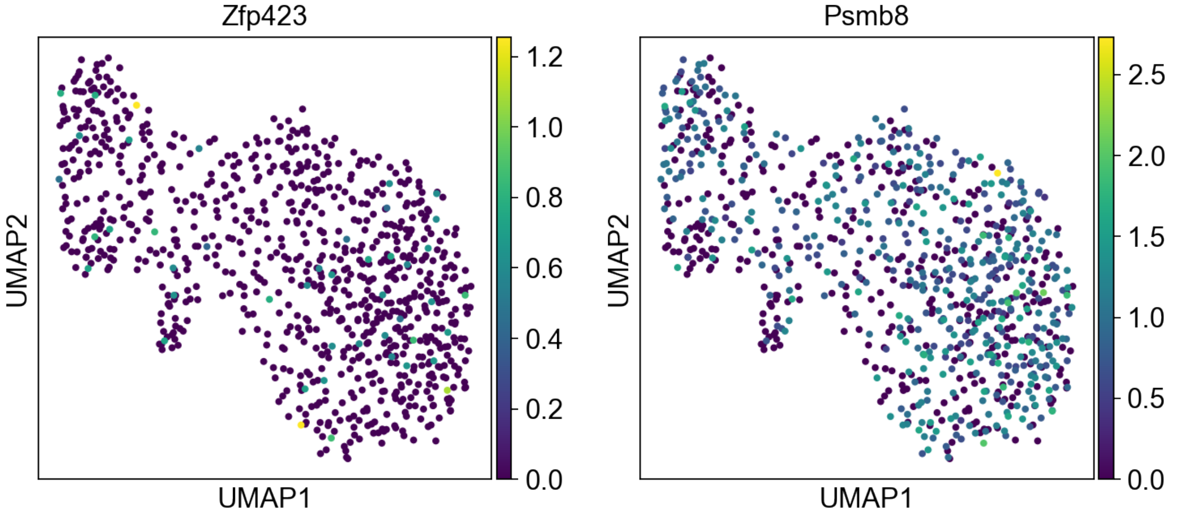
In [65]:

```
if bool_plot == True:
    plot_umap_marker(adata_adip2, adipocyte_markers.tolist(), size=50, save="_preadip_markers_preadipcytes_norm", use_raw=False)
```

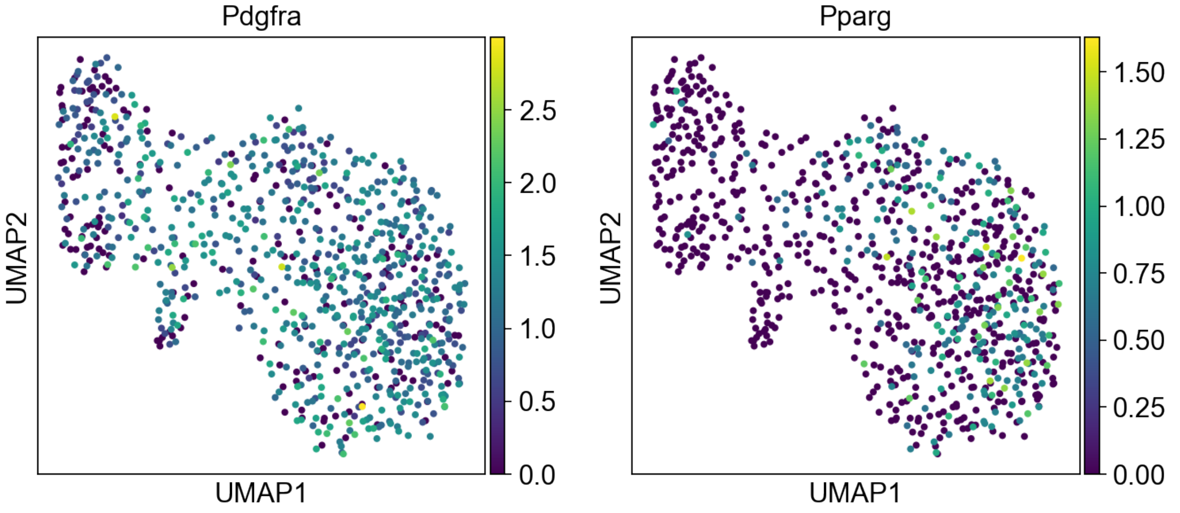
```
['Fgf10', 'Bmp4']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_0.pdf
```



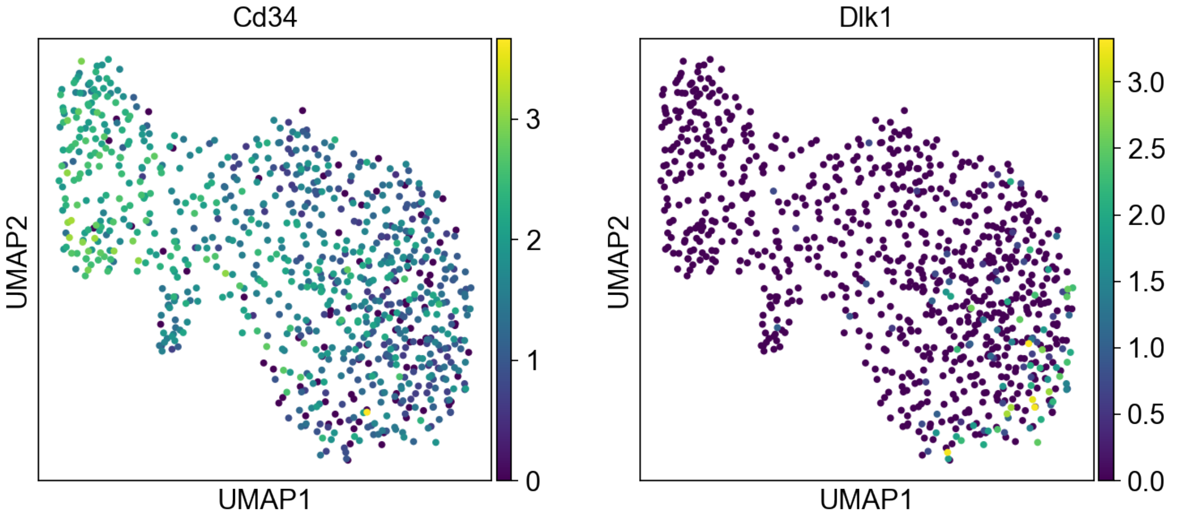
```
['Zfp423', 'Psmb8']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_1.pdf
```



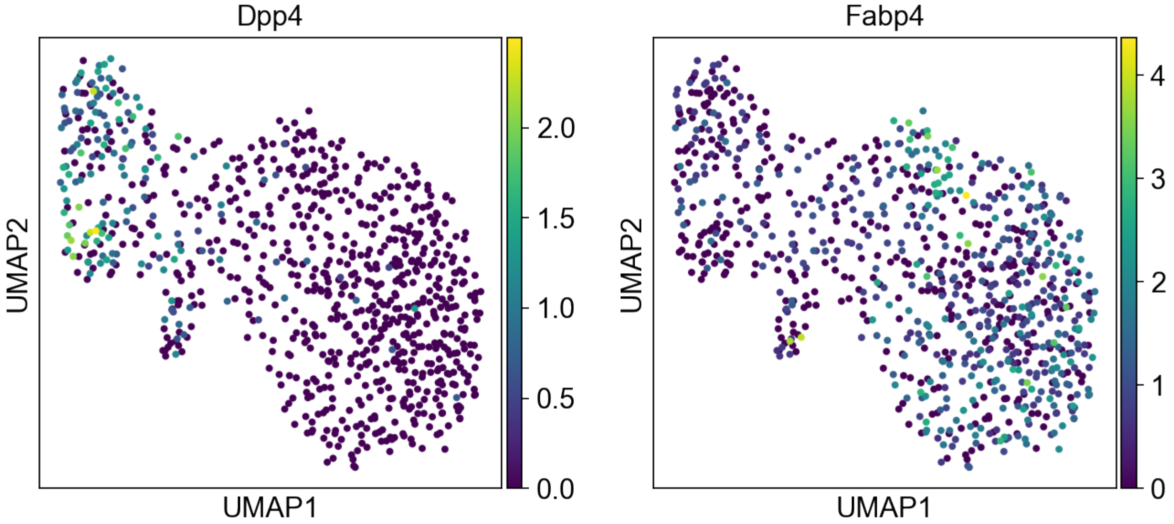
```
['Pdgfra', 'Pparg']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_2.pdf
```



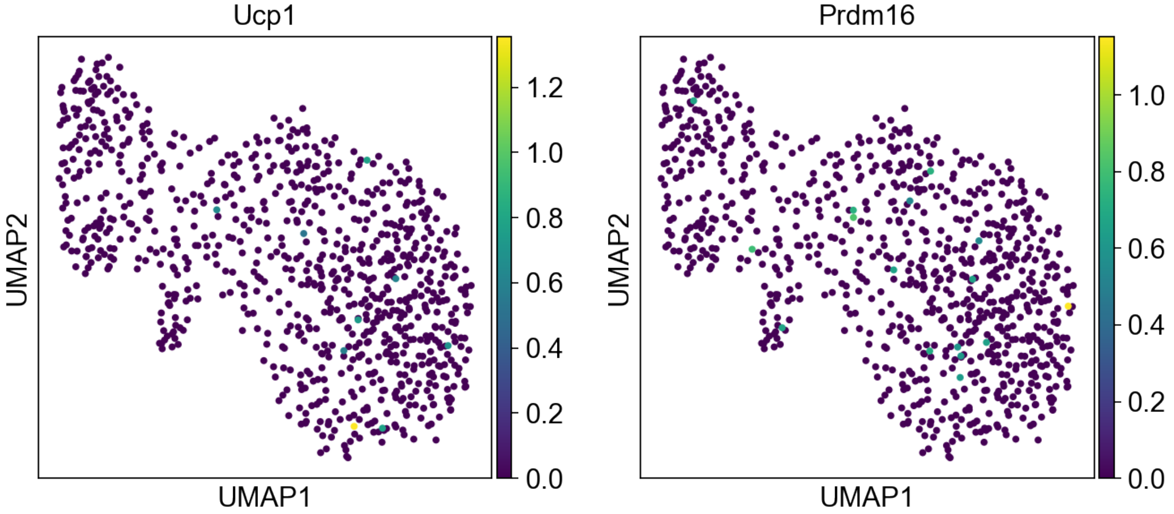
```
['Cd34', 'Dlk1']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_3.pdf
```



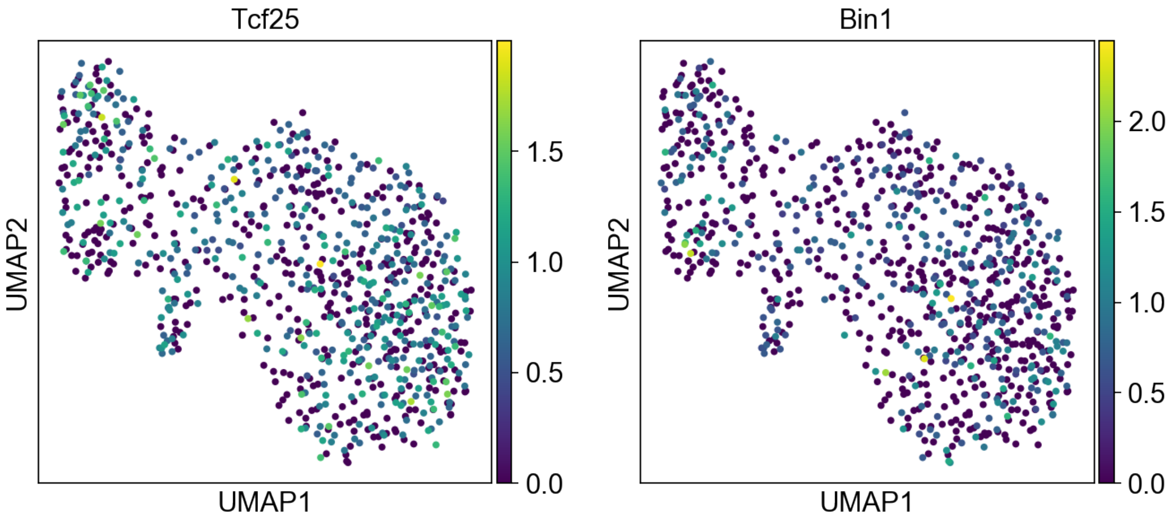
```
['Dpp4', 'Fabp4']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_4.pdf
```



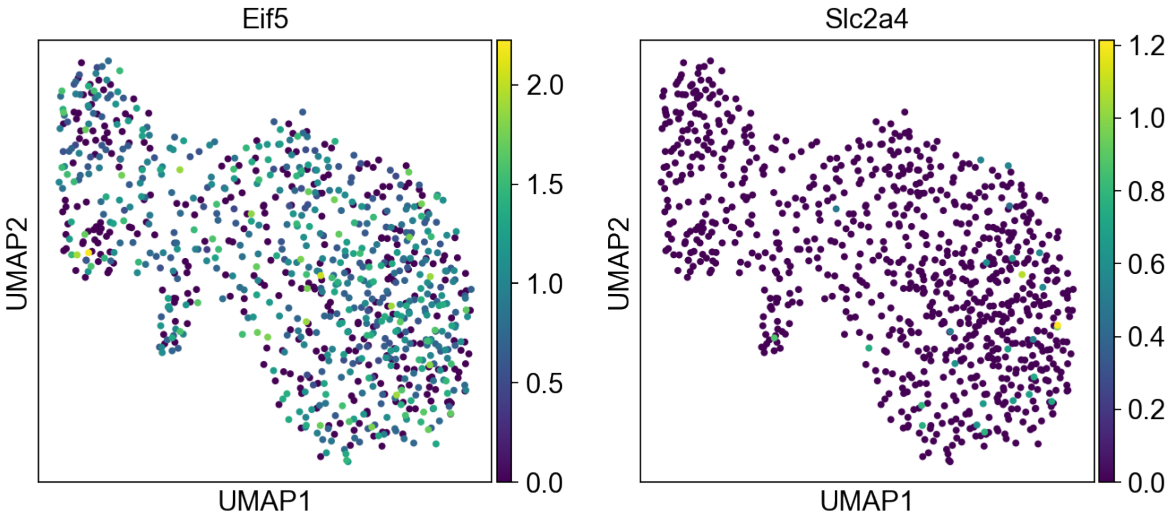
```
['Ucp1', 'Prdm16']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_5.pdf
```



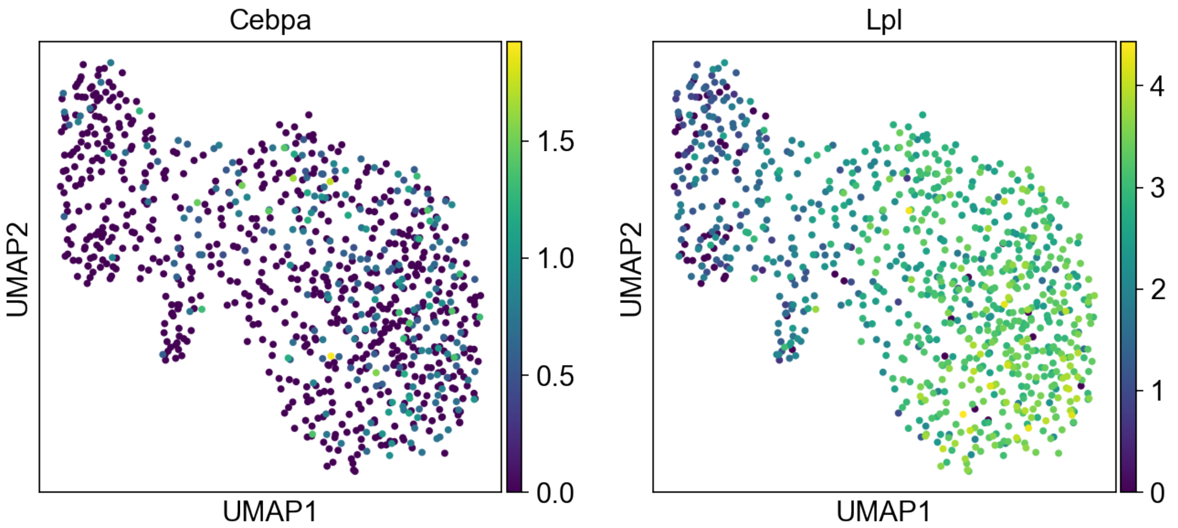
```
['Tcf25', 'Bin1']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_6.pdf
```



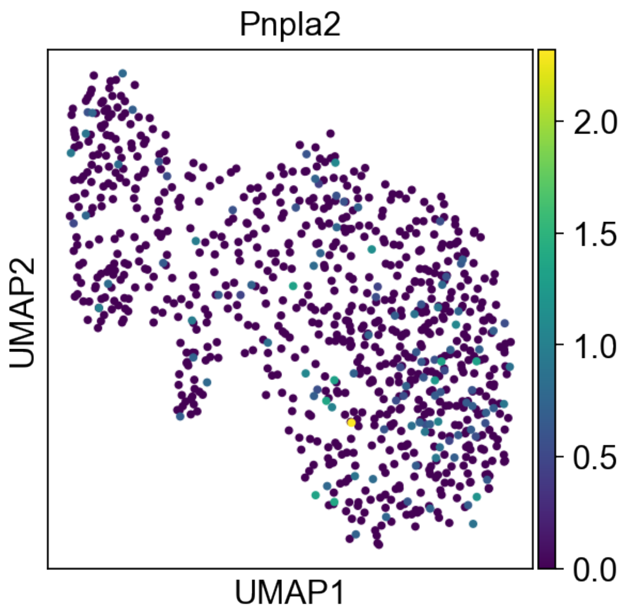
```
['Eif5', 'Slc2a4']
WARNING: saving figure to file /Users/david.fischer/phd/data/Preadipocytes Brown/results/panels/umap_preadip_markers_preadipcytes_norm_7.pdf
```



```
['Cebpa', 'Lpl']
WARNING: saving figure to file /Users/david.fischer/phd/data/Preadipocytes Brown/results/panels/umap_preadip_markers_preadipcytes_norm_8.pdf
```



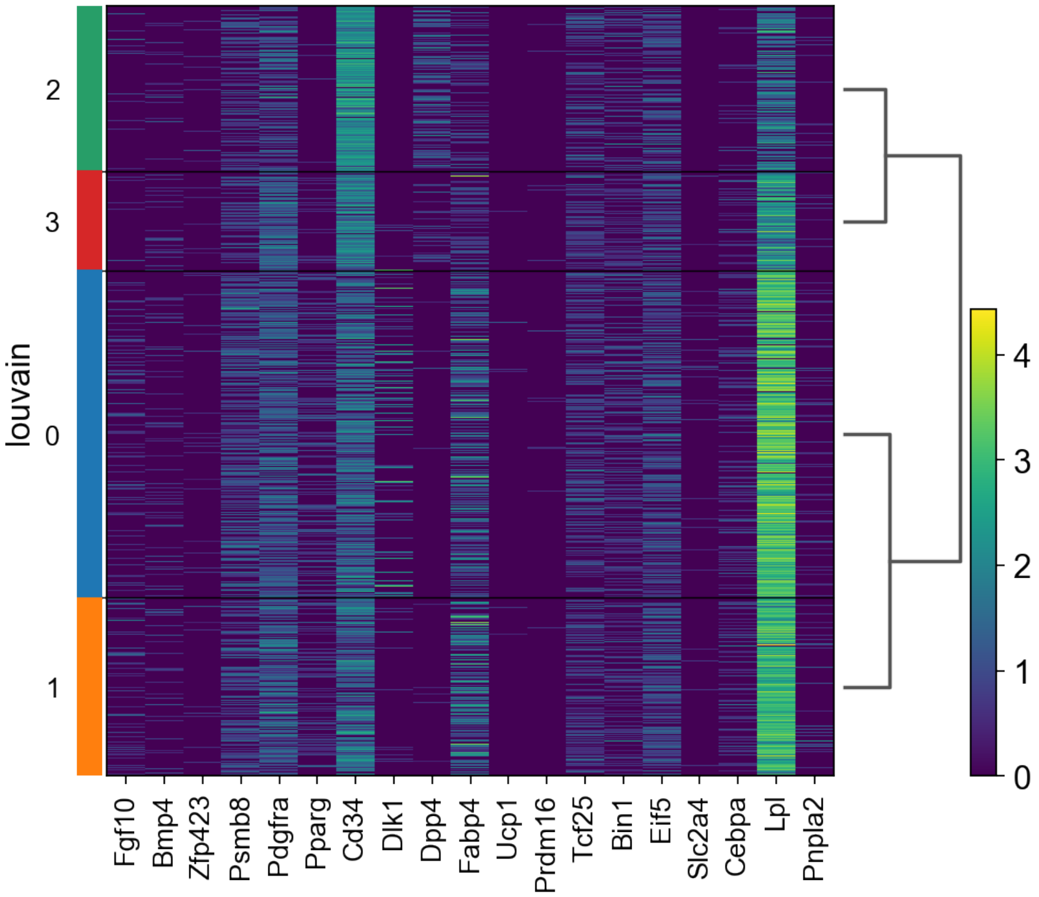
```
['Pnpla2']
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/umap_preadip_markers_preadipcytes_nor
m_9.pdf
```



In [66]:

```
if bool_plot==True:
    sc.pl.heatmap(
        adata=adata_adip2,
        var_names=adipocyte_markers,
        groupby="louvain",
        use_raw=False,
        log=False,
        dendrogram=True,
        var_group_rotation=90,
        show_gene_labels=True,
        show=True,
        save="_preadipocyte_markers_celltypes.pdf"
    )
```

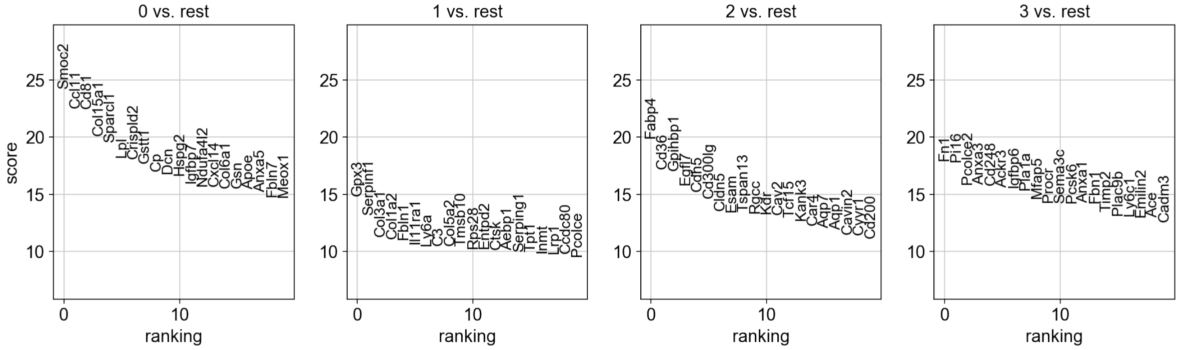
```
WARNING: dendrogram data not found (using key=dendrogram_louvain).
Running `sc.tl.dendrogram` with default parameters. For fine tunin
g it is recommended to run `sc.tl.dendrogram` independently.
    using 'X_pca' with n_pcs = 50
Storing dendrogram info using `.uns['dendrogram_louvain']`
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/heatmap_preadipocyte_markers_celltype
s.pdf
```



In [67]:

```
sc.tl.rank_genes_groups(adata_adip2, groupby='louvain', key_added='
rank_genes')
if bool_plot == True:
    sc.pl.rank_genes_groups(adata_adip, key='rank_genes', groups=['
0','1','2','3'], fontsize=12, save="_preadip_genes")
```

```
ranking genes
    finished: added to `.uns['rank_genes']`
    'names', sorted np.recarray to be indexed by group ids
    'scores', sorted np.recarray to be indexed by group ids
    'logfoldchanges', sorted np.recarray to be indexed by group id
s
    'pvals', sorted np.recarray to be indexed by group ids
    'pvals_adj', sorted np.recarray to be indexed by group ids (0:
00:00)
WARNING: saving figure to file /Users/david.fischer/phd/data/Pread
ipocytesBrown/results/panels/rank_genes_groups_louvain_preadip_gen
es.pdf
```


0 vs. rest
score
ranking
Smoc2
Ccl11
Cd81
Col15a1
Sparcl1
Lpl
Crispld2
Gstt1
Cp
Dcn
Hspg2
Igfbp7
Ndufa4l2
Cxcl14
Col6a1
Gsn
Apoe
Anxa5
Fbln7
Meox1
1 vs. rest
Gpx3
Serpinf1
Col3a1
Col1a2
Fbln1
Il11ra1
Ly6a
C3
Col5a2
Tmsb10
Rps28
Entpd2
Ctsk
Aebp1
Serping1
Tpt1
Inmt
Lrp1
Ccdc80
Pcolce
2 vs. rest
Fabp4
Cd36
Gpihbp1
Egfl7
Cdh5
Cd300lg
Cldn5
Esam
Tspan13
Rgcc
Kdr
Cav2
Tcf15
Kank3
Car4
Aqp7
Aqp1
Cavin2
Cyyr1
Cd200
3 vs. rest
Fn1
Pi16
Pcolce2
Anxa3
Cd248
Ackr3
Igfbp6
Pla1a
Mfap5
Procr
Sema3c
Pcsk6
Anxa1
Fbn1
Timp2
Plac9b
Ly6c1
Emilin2
Ace
Cadm3