Summary

How to set up Jupyter Notebook on your terminal:

- 1. Open up your terminal interface - this is where you'll be entering most of the following commands
- 2. initialise the terminal by typing:

```
gcc
```

- 3. install the command line tools
- 4. install Mac Hombrew by going to google and typing "docs.brew.sh", this should open up the Homebrew webpage
- 5. search up and install python on H.B. (Homebrew) - this is the coding language we will be using
- 6. install the virtual environment with H.B.
- 7. go back to the terminal and type:

```
python3 -m venv ~/Documents/projects/venv
```

- 8. now activate the virtual environment by typing these commands into the console:

```
cd ~/Documents/projects
```

```
source venv/bin/activate
```

- 9. now install the interactive notebook "Jupyter Notebook" by typing

```
python -m install jupyter
```

```
python -m ipykernel install --name=venv
```

- 10. now install scanpy by typing:

```
pip install scanpy
```

- 11. install leiden:

```
pip install leidenalg
```

- 12. install numpy :

```
pip install numpy
```

- 13. install pandas:

```
pip install pandas
```

- 14. finally, start jupyter notebook by typing this into the console:

```
jupyter notebook
```

- 15. if you want to deactivate your virtual environment, type this into the console:

```
deactivate
```

Preprocessing and clustering 3k PBMCs

In [1]:

```

import numpy as np
import pandas as pd
import scanpy as sc
```

In [2]:

```
sc.settings.verbosity = 3

sc.logging.print_header()
sc.settings.set_figure_params(dpi=80, facecolor = "white")
```

```
scanpy==1.9.1 anndata==0.8.0 umap==0.5.3 numpy==1.21.6 scipy==1.8.
1 pandas==1.5.0 scikit-learn==1.1.2 statsmodels==0.13.2 python-igr
aph==0.10.1 pynndescent==0.5.7
```

In [3]:

```
results_file = "write/pbmc3k.h5ad"
```

In [4]:

```
adata = sc.read_10x_mtx(
"filtered_gene_bc_matrices/hg19/",
    var_names = "gene_symbols",
    cache = True
)
```

```
... reading from cache file cache/filtered_gene_bc_matrices-hg19-m
atrix.h5ad
```

In [5]:

```
adata.var_names_make_unique()
```

In [6]:

```
adata
```

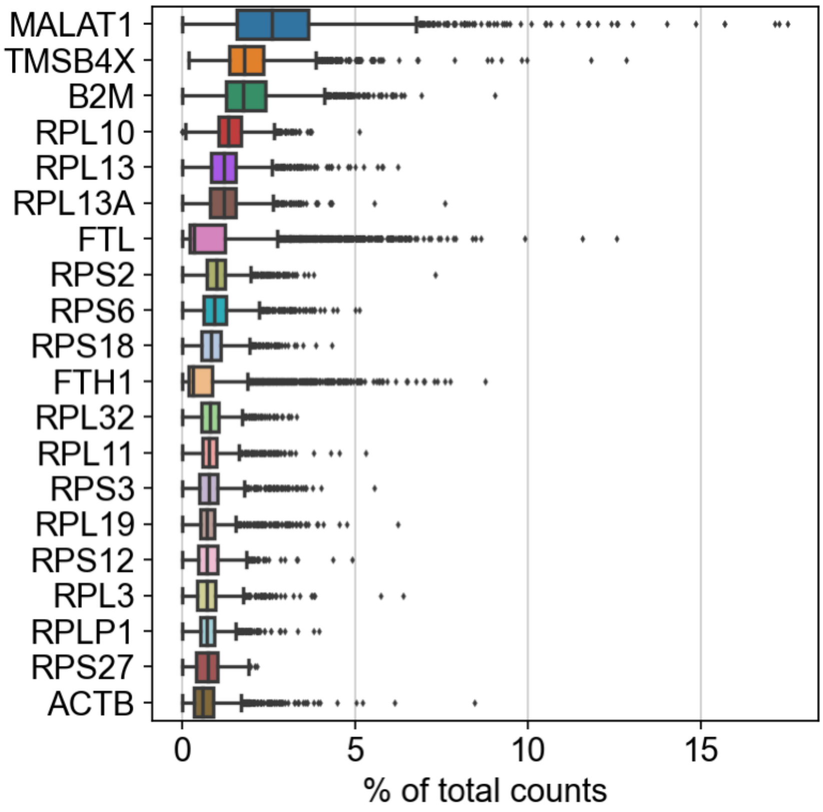
Out[6]:

```
AnnData object with n_obs × n_vars = 2700 × 32738
    var: 'gene_ids'
```

This shows the genes with the highest fraction of counts in each cell, across all cells

In [7]:
```
sc.pl.highest_expr_genes(adata, n_top=20, )
```

```
normalizing counts per cell
    finished (0:00:00)
```

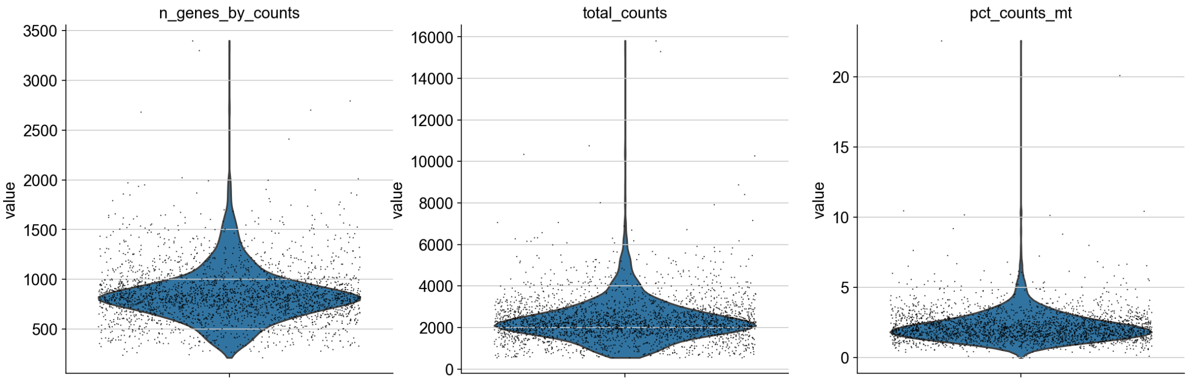


In [8]:
```
sc.pp.filter_cells(adata, min_genes=200)
sc.pp.filter_genes(adata, min_cells = 3)
```

```
filtered out 19024 genes that are detected in less than 3 cells
```

Filtered cell outliers based on genes expressed and counts

In [9]:
```
adata.var["mt"] = adata.var_names.str.startswith("MT-")
sc.pp.calculate_qc_metrics(adata, qc_vars = ["mt"], percent_top = N
In [10]: sc.pl.violin(adata, ["n_genes_by_counts", "total_counts", "pct_coun
```

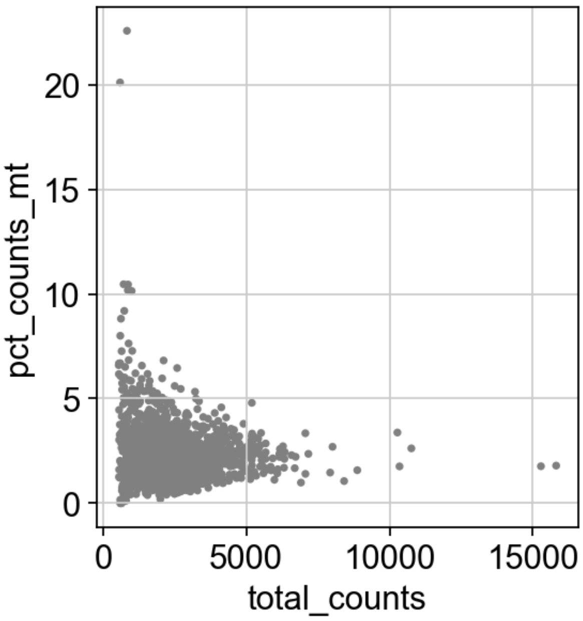


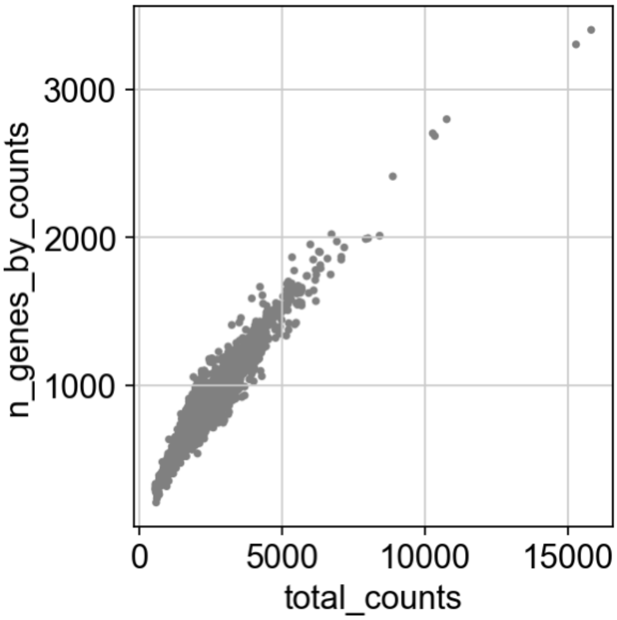
Violin plot measuring:

- the number of genes expressed in the count matrix
- total counts per cell
- the percentage of counts in mitochondrial genes

In [11]:

```
sc.pl.scatter(adata, x = "total_counts", y = "pct_counts_mt")
sc.pl.scatter(adata, x = "total_counts", y = "n_genes_by_counts")
```





Removing cells thats have too many mitochondrial genes expressed or too many counts by slicing the AnnData object

In [12]:

```
adata = adata[adata.obs.n_genes_by_counts <2500, :]
adata = adata[adata.obs.pct_counts_mt < 5, :]
```

Manipulate data so that counts become comparable amongst cells

In [13]:
```
sc.pp.normalize_total(adata, target_sum = 1e4)
```

```
normalizing counts per cell
    finished (0:00:00)
```

```
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/preprocessing/_normalization.py:170: UserWarning: Re
ceived a view of an AnnData. Making a copy.
  view_to_actual(adata)
```

Logarithmize the data so we can gather more from a graph

In [14]:
```
sc.pp.log1p(adata)
#np.log1p(adata.X)
```

Identify highly-variable genes

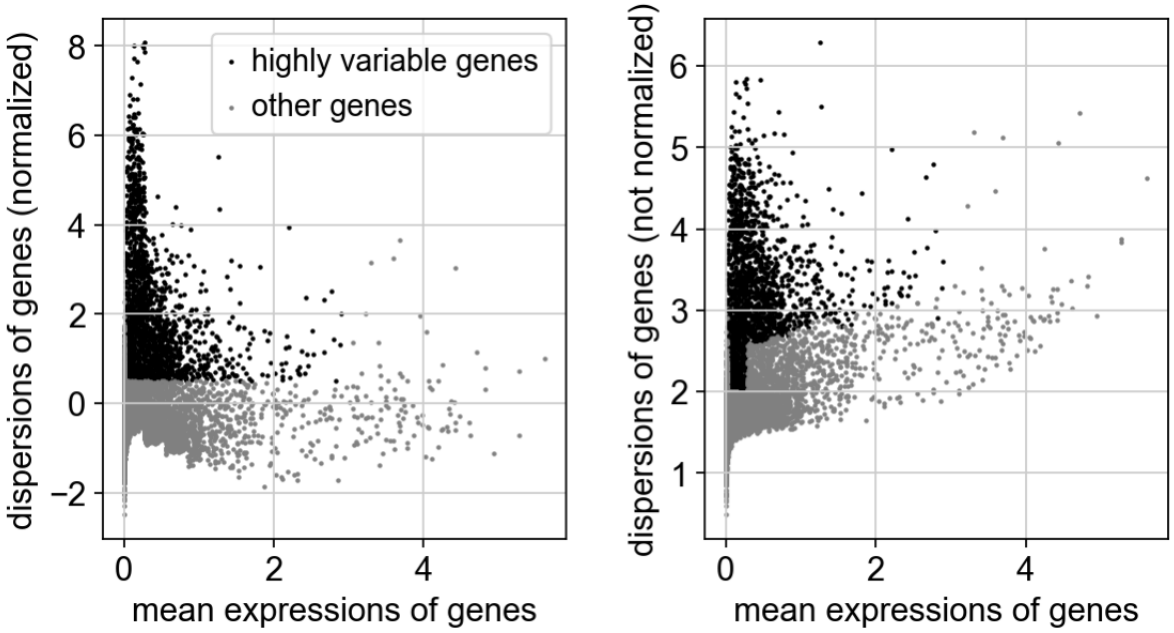
In [15]:
```
sc.pp.highly_variable_genes(adata, min_mean = 0.0125, max_mean = 3,
```

```
extracting highly variable genes
```

```
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/preprocessing/_highly_variable_genes.py:208: Runtime
Warning: invalid value encountered in log
  dispersion = np.log(dispersion)
```

```
    finished (0:00:00)
--> added
    'highly_variable', boolean vector (adata.var)
    'means', float vector (adata.var)
    'dispersions', float vector (adata.var)
    'dispersions_norm', float vector (adata.var)
```

```
In [16]: sc.pl.highly_variable_genes(adata)
```



Slice the data so what's left is just the highly-variable genes

```
In [17]: adata = adata[:, adata.var.highly_variable]
```

Regress out the effects of total count per cell and the percentage of mitochondrial genes expressed then scale said data to unit variance

```
In [18]: sc.pp.regress_out(adata, ["total_counts", "pct_counts_mt"])
```

```
regressing out ['total_counts', 'pct_counts_mt']
    sparse input is densified and may lead to high memory use
    finished (0:00:06)
```

Scale each gene to unit variance. Remove values exceeding a standard devation of 10 as if they do, they're most likely sickly cells

```
In [19]: sc.pp.scale(adata, max_value = 10)
```

Principle Component Analysis (Reducing Dimensionality)

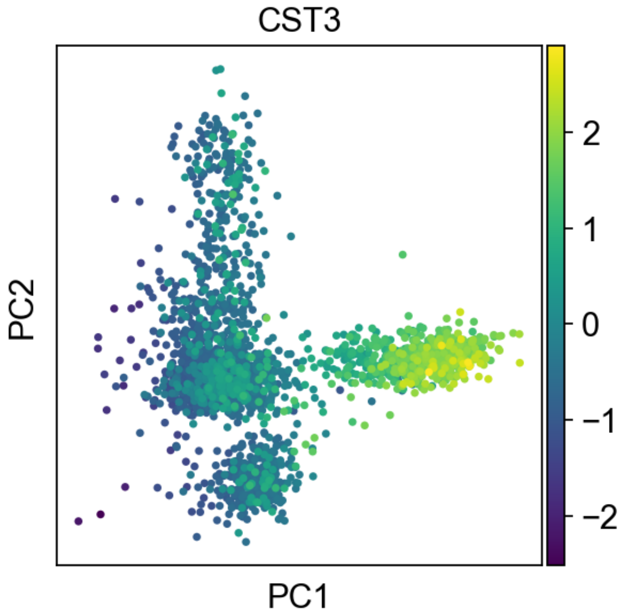
Denoise the data by using PCA (Principle Component Analysis) to focus on the main axes of variation

```
In [20]: sc.tl.pca(adata, svd_solver = "arpack")
```

```
computing PCA
    on highly variable genes
    with n_comps=50
    finished (0:00:00)
```

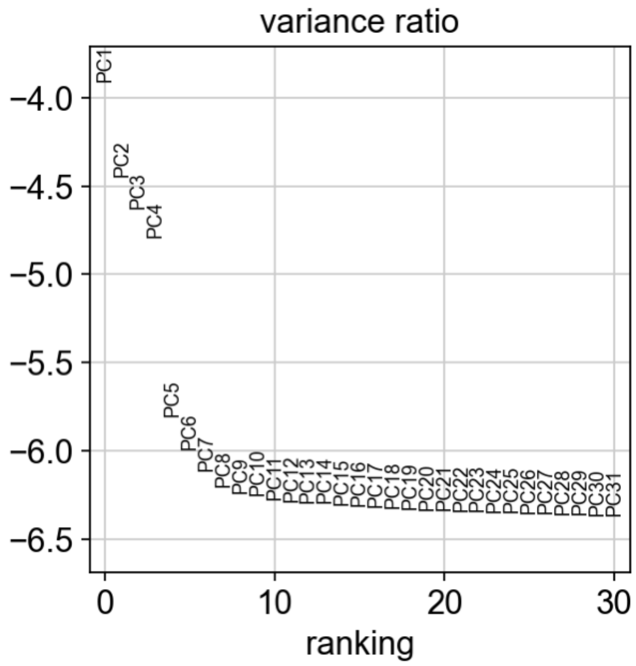
Create a colour co-ordinated scatter graph of the values

```
In [21]: sc.pl.pca(adata, color = "CST3")
```



Inspect the contrabution of single PCs to the total variance in data. This will tell us about how many PCs we should consider in order to compute the neighbouhood

In [22]:
```
sc.pl.pca_variance_ratio(adata, log = True)
```



Save the result

In [23]:
```
adata.write(results_file)
```

In [24]:
```
adata
```

Out[24]:
```
AnnData object with n_obs × n_vars = 2638 × 1838
    obs: 'n_genes', 'n_genes_by_counts', 'total_counts', 'total_co
unts_mt', 'pct_counts_mt'
    var: 'gene_ids', 'n_cells', 'mt', 'n_cells_by_counts', 'mean_c
ounts', 'pct_dropout_by_counts', 'total_counts', 'highly_variable'
, 'means', 'dispersions', 'dispersions_norm', 'mean', 'std'
    uns: 'log1p', 'hvg', 'pca'
    obsm: 'X_pca'
    varm: 'PCs'
```

Computing the neighbourhood graph

Now we will compute the graph using our PCA representation of the data array (matrix). We will use the following values used below

In [25]:

```
sc.pp.neighbors(adata, n_neighbors = 10, n_pcs = 40)
```

```
computing neighbors
    using 'X_pca' with n_pcs = 40
    finished: added to `.uns['neighbors']`
    `.obsp['distances']`, distances for each pair of neighbors
    `.obsp['connectivities']`, weighted adjacency matrix (0:00:02)
```

Embedding the neighbourhood graph

Here we will embed the graph into two dimensions using UMAP

In [26]:

```
## run leiden before
```

In [27]:

```
sc.tl.leiden(adata)
```

```
running Leiden clustering
    finished: found 9 clusters and added
    'leiden', the cluster labels (adata.obs, categorical) (0:00:00
)
```

In [28]:

```
sc.tl.paga(adata)
sc.pl.paga(adata, plot = False)
sc.tl.umap(adata, init_pos = "paga")
```

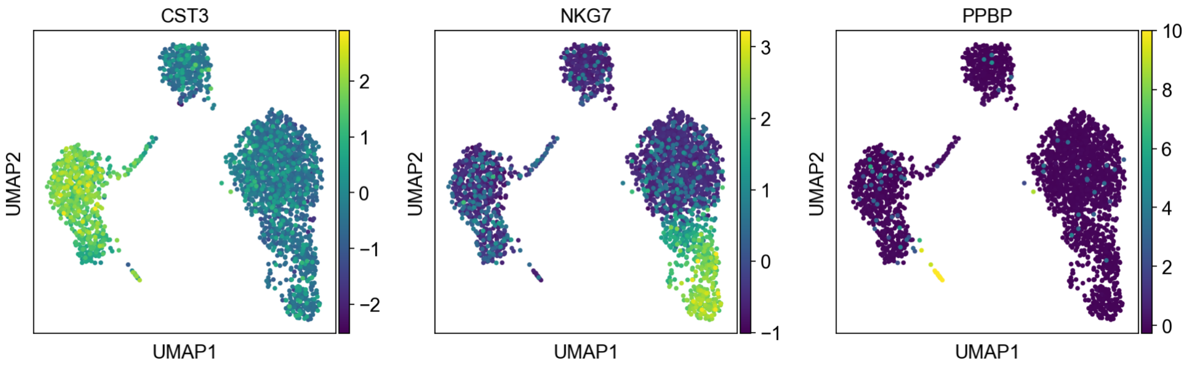
```
running PAGA
    finished: added
    'paga/connectivities', connectivities adjacency (adata.uns)
    'paga/connectivities_tree', connectivities subtree (adata.uns)
(0:00:00)
--> added 'pos', the PAGA positions (adata.uns['paga'])
computing UMAP
    finished: added
    'X_umap', UMAP coordinates (adata.obsm) (0:00:04)
```

In [29]:

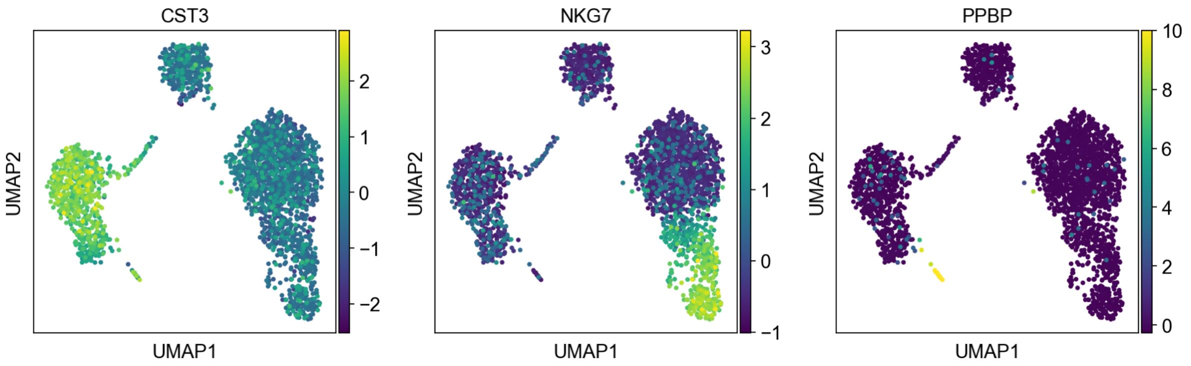
```
sc.tl.umap(adata)
```

```
computing UMAP
    finished: added
    'X_umap', UMAP coordinates (adata.obsm) (0:00:03)
```

In [30]:
```
sc.pl.umap(adata, color = ["CST3", "NKG7", "PPBP"])
```

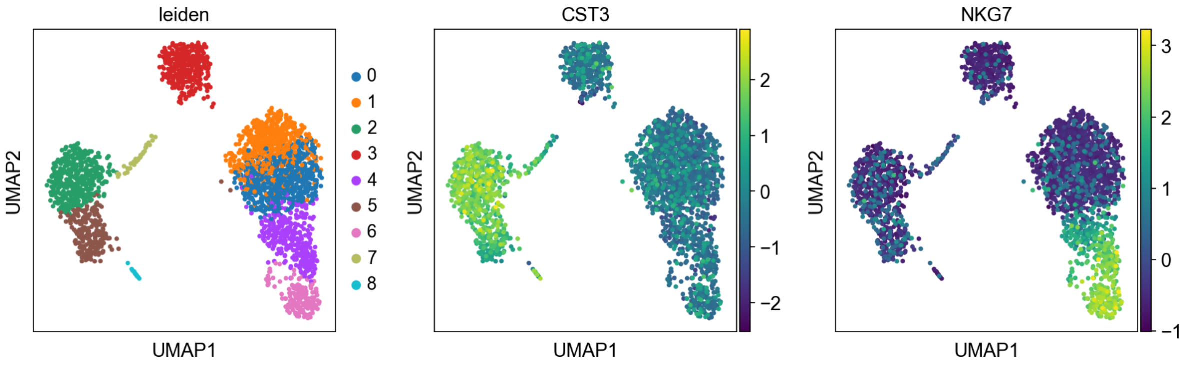


In [31]:
```
sc.pl.umap(adata, color = ["CST3", "NKG7", "PPBP"], use_raw = False
```



In [32]:
```
sc.pl.umap(adata, color = ["leiden", "CST3", "NKG7"])
```

```
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/plotting/_tools/scatterplots.py:392: UserWarning: No
data for colormapping provided via 'c'. Parameters 'cmap' will be
ignored
  cax = scatter(
```



In [33]:
```
adata.write(results_file)
```

In [34]:

```
sc.tl.rank_genes_groups(adata, "leiden", method = "t-test")
sc.pl.rank_genes_groups(adata, n_genes = 25, sharey = False)
```

```
ranking genes
    finished: added to `.uns['rank_genes_groups']`
    '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)

/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
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/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
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/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
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/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
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/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
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/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
```

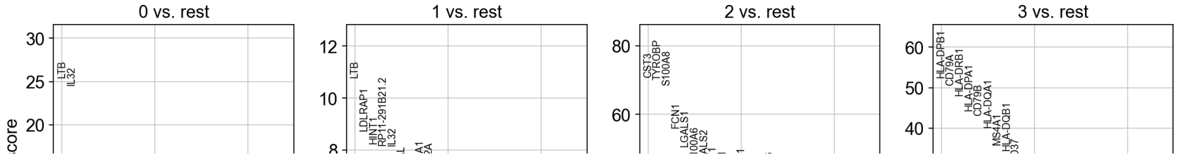

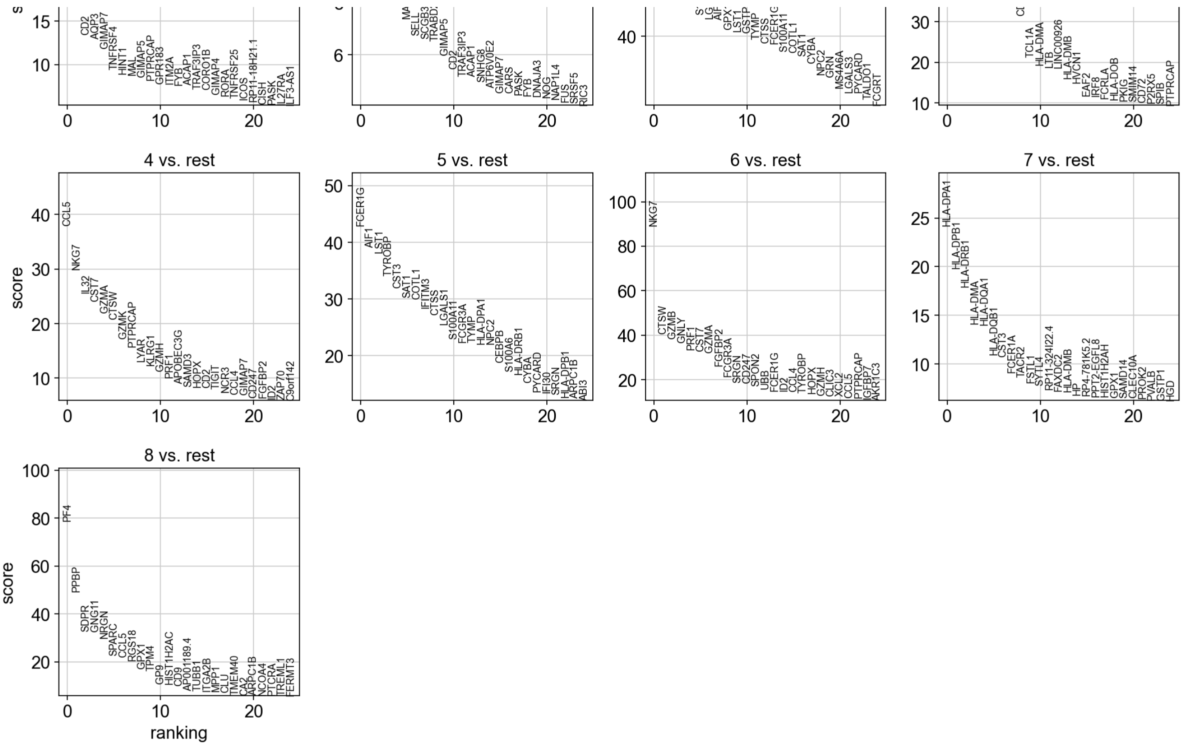

In [35]:
```
sc.settings.verbosity = 2
```

In [36]:
```
sc.tl.rank_genes_groups(adata, "leiden", method = "wilcoxon")
sc.pl.rank_genes_groups(adata, n_genes = 25, sharey = False)
```

```
ranking genes
    finished (0:00:00)

/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
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/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
```
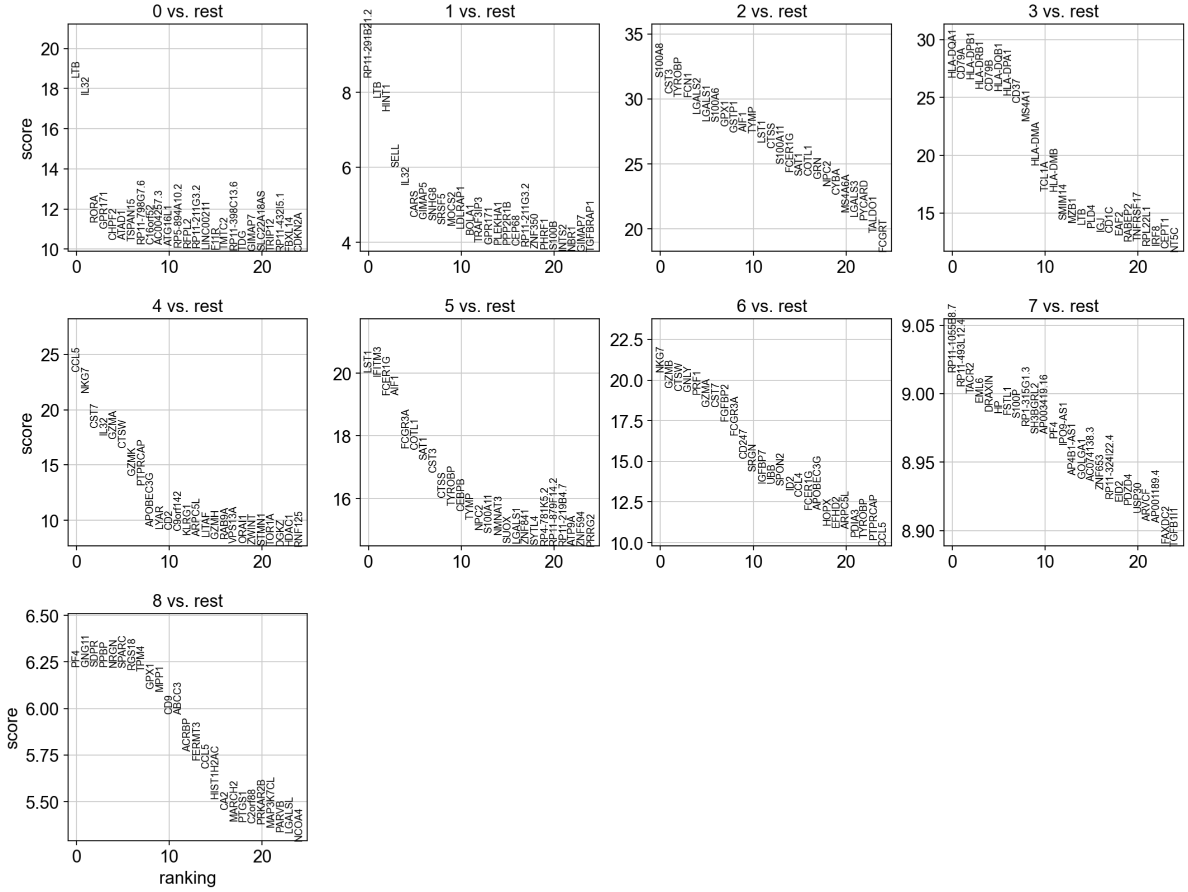
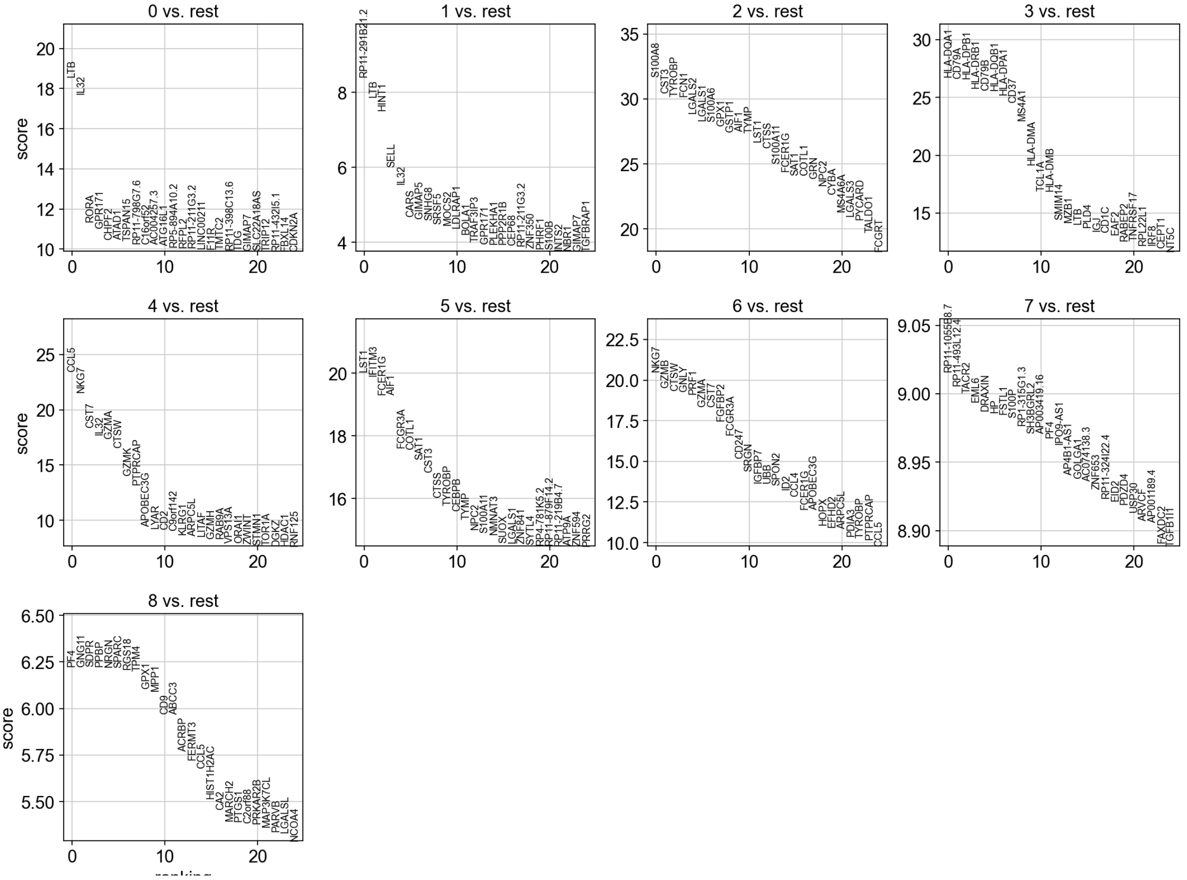



In [37]:
```
adata.write(results_file)
```

In [38]:
```
sc.tl.rank_genes_groups(adata, "leiden", method = "wilcoxon")
sc.pl.rank_genes_groups(adata, n_genes = 25, sharey = False)
```

```
ranking genes
    finished (0:00:00)

/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
```

```
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
```



ranking

In [39]:
```
marker_genes = ["CD79A", "MS4A1", "LGALS3", "S100A8", "GNLY", "NKG7
```

In [40]:
```
adata = sc.read(results_file)
```

In [41]:
```
pd.DataFrame(adata.uns["rank_genes_groups"]["names"]).head(5)
```

Out[41]:

	0	1	2	3	4	5	6	7	8
0	LTB	RP11-291B21.2	S100A8	HLA-DQA1	CCL5	LST1	NKG7	RP11-1055B8.7	PF4
1	IL32	LTB	CST3	CD79A	NKG7	IFITM3	GZMB	RP11-493L12.4	GNG11
2	RORA	HINT1	TYROBP	HLA-DPB1	CST7	FCER1G	CTSW	TACR2	SDPR
3	GPR171	SELL	FCN1	HLA-DRB1	IL32	AIF1	GNLY	EML6	PPBP
4	CHPF2	IL32	LGALS2	CD79B	GZMA	FCGR3A	PRF1	DRAXIN	NRGN

In [42]:
```
result = adata.uns["rank_genes_groups"]
groups = result["names"].dtype.names
pd.DataFrame(
    {group + "_" + key[:1]: result[key][group]
     for group in groups for key in ["names", "pvals"]}).head(5)
```

Out[42]:

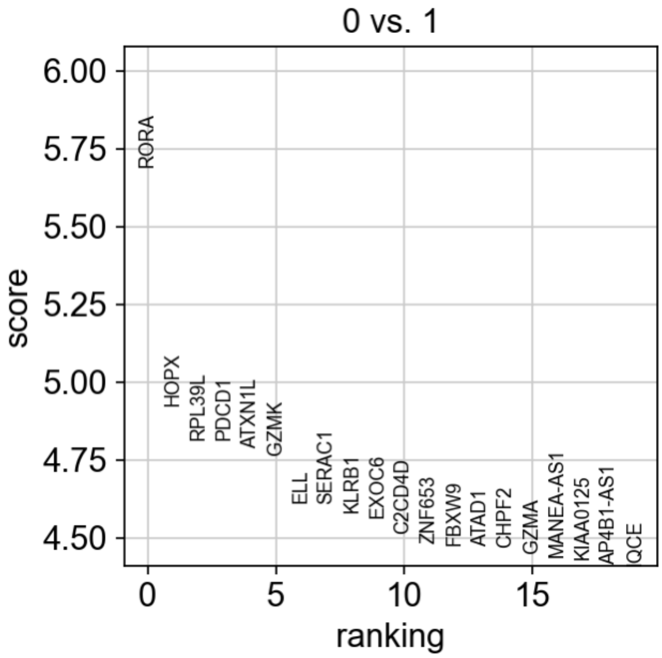
	0_n	0_p	1_n	1_p	2_n	2_p	3_n	3_p	4
0	LTB	3.234600e-77	RP11-291B21.2	3.777929e-17	S100A8	7.776926e-221	HLA-DQA1	2.979480e-158	CC
1	IL32	2.221225e-70	LTB	3.307598e-15	CST3	6.631019e-204	CD79A	1.658759e-156	NK
2	RORA	5.439522e-30	HINT1	5.195510e-14	TYROBP	1.641608e-200	HLA-DPB1	1.699735e-156	CS
3	GPR171	1.531700e-28	SELL	1.727438e-09	FCN1	3.313601e-200	HLA-DRB1	3.802586e-147	IL
4	CHPF2	1.079645e-25	IL32	2.644750e-08	LGALS2	4.611816e-184	CD79B	4.576998e-145	GZ

In [43]:
```
sc.pp.log1p(adata)
```

```
WARNING: adata.X seems to be already log-transformed.

/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/preprocessing/_simple.py:352: RuntimeWarning: invali
d value encountered in log1p
  np.log1p(X, out=X)
```

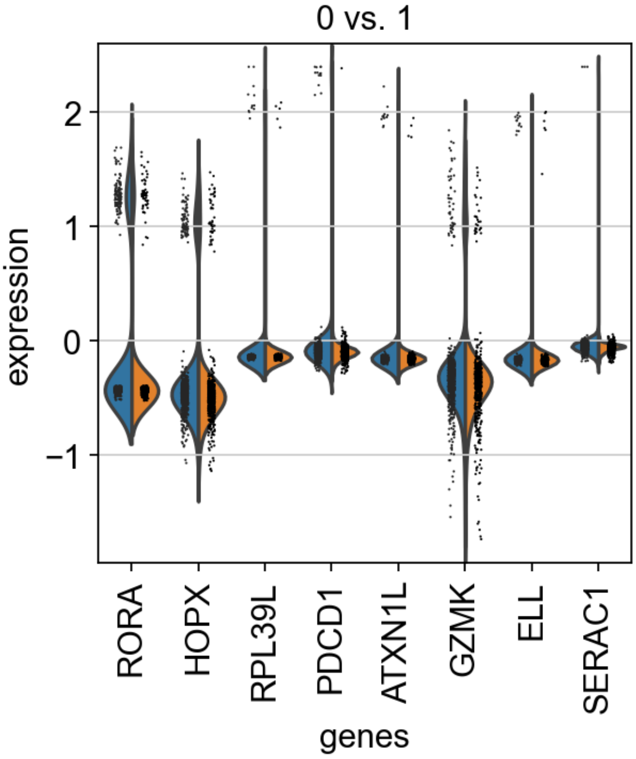
In [44]:
```
sc.tl.rank_genes_groups(adata, "leiden", groups = ["0"], reference
sc.pl.rank_genes_groups(adata, groups = ["0"], n_genes = 20)
```

```
ranking genes
    finished (0:00:00)

/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/tools/_rank_genes_groups.py:420: RuntimeWarning: inv
alid value encountered in log2
  self.stats[group_name, 'logfoldchanges'] = np.log2(
```



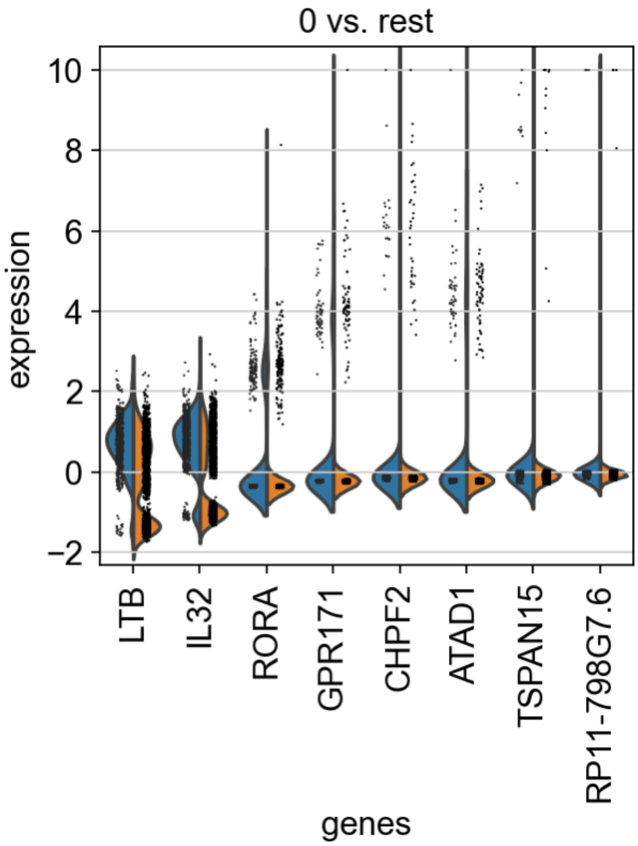
```
In [45]: sc.pl.rank_genes_groups_violin(adata, groups = "0", n_genes = 8)
```

```
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/seaborn/categorical.py:166: FutureWarning: Setting a gradie
nt palette using color= is deprecated and will be removed in versi
on 0.13. Set `palette='dark:black'` for same effect.
  warnings.warn(msg, FutureWarning)
```

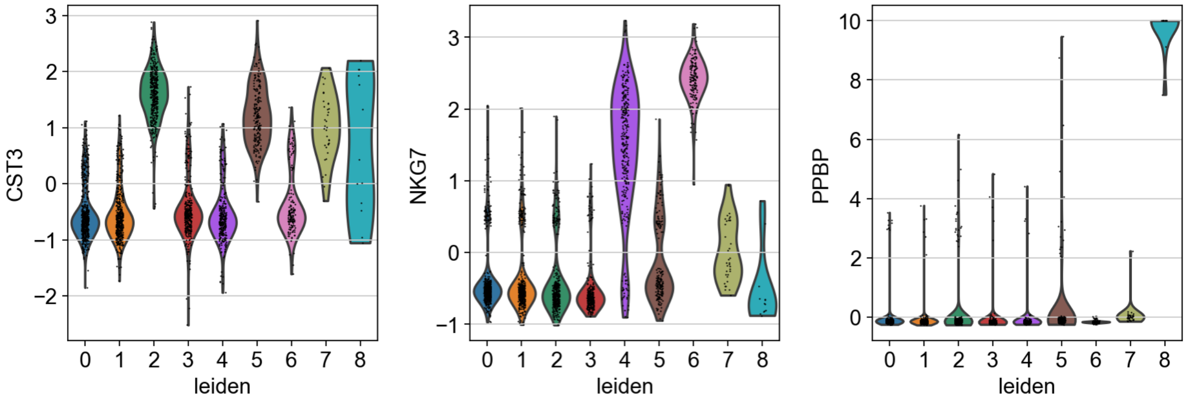


```
In [46]: adata = sc.read(results_file)
In [47]: sc.pl.rank_genes_groups_violin(adata, groups = "0", n_genes = 8)
```

```
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/seaborn/categorical.py:166: FutureWarning: Setting a gradie
nt palette using color= is deprecated and will be removed in versi
on 0.13. Set `palette='dark:black'` for same effect.
  warnings.warn(msg, FutureWarning)
```



```
In [48]: sc.pl.violin(adata, ["CST3", "NKG7", "PPBP"], groupby = "leiden")
```



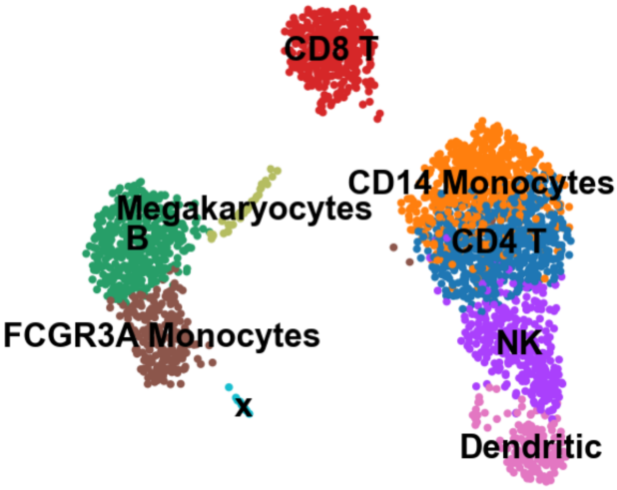
```
In [49]: new_cluster_names = [
             "CD4 T", "CD14 Monocytes", "B", "CD8 T", "NK", "FCGR3A Monocyte
         adata.rename_categories("leiden", new_cluster_names)
```

```
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/anndata/_core/anndata.py:1160: FutureWarning: The `inplace`
parameter in pandas.Categorical.rename_categories is deprecated an
d will be removed in a future version. Removing unused categories
will always return a new Categorical object.
  self.obs[key].cat.rename_categories(categories, inplace=True)
```

```
In [50]: sc.pl.umap(adata, color = "leiden", legend_loc = "on data", title =
```

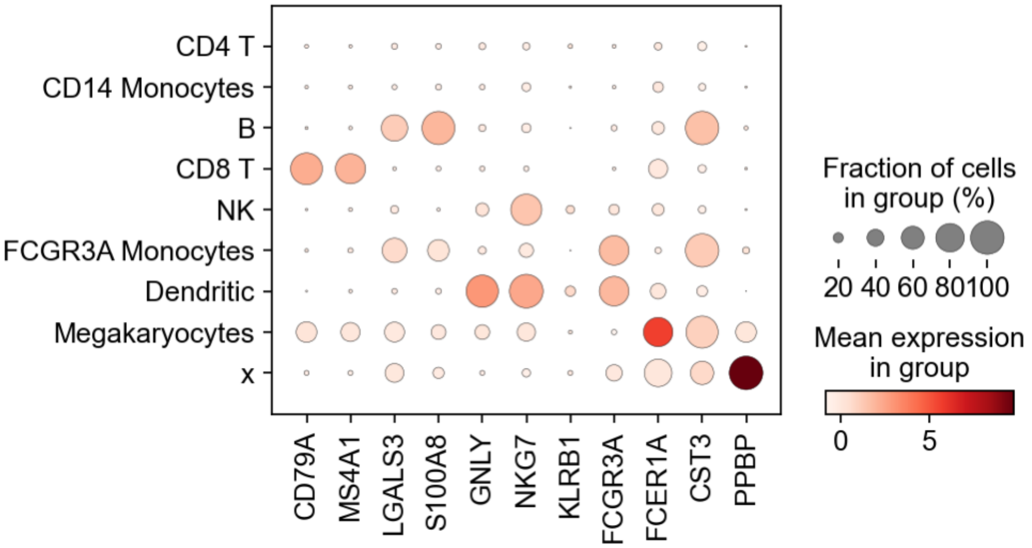
```
WARNING: saving figure to file figures/umap.pdf
```

```
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/plotting/_tools/scatterplots.py:392: UserWarning: No
data for colormapping provided via 'c'. Parameters 'cmap' will be
ignored
  cax = scatter(
```

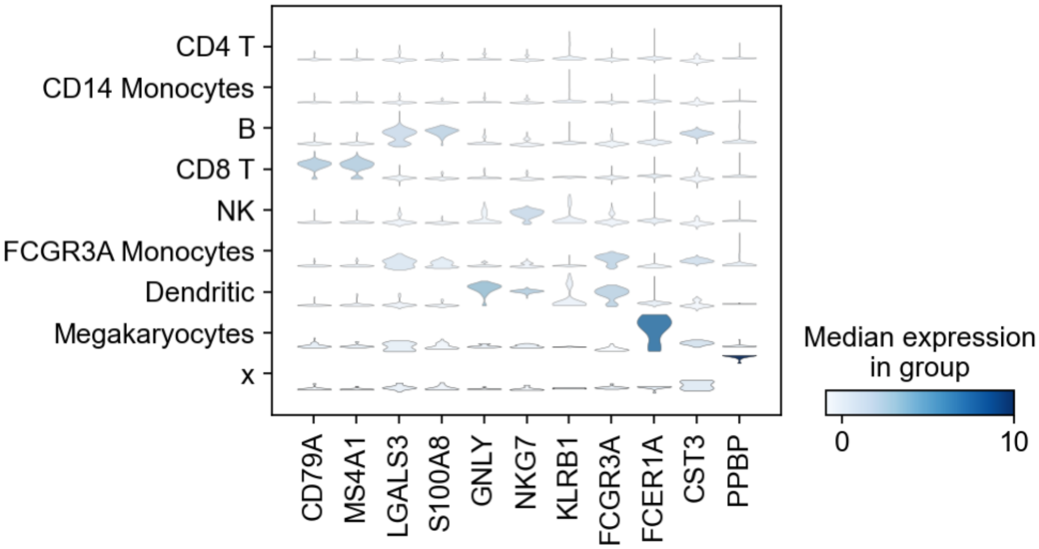


```
In [51]: sc.pl.dotplot(adata, marker_genes, groupby = "leiden");
```

```
/Users/workexperience/Documents/Project/venv/lib/python3.9/site-pa
ckages/scanpy/plotting/_dotplot.py:749: UserWarning: No data for c
olormapping provided via 'c'. Parameters 'cmap', 'norm' will be ig
nored
  dot_ax.scatter(x, y, **kwds)
```



```
In [52]: sc.pl.stacked_violin(adata, marker_genes, groupby = "leiden", rotat
```



In [53]:

```
adata
```

Out[53]:

```
AnnData object with n_obs × n_vars = 2638 × 1838
    obs: 'n_genes', 'n_genes_by_counts', 'total_counts', 'total_co
unts_mt', 'pct_counts_mt', 'leiden'
    var: 'gene_ids', 'n_cells', 'mt', 'n_cells_by_counts', 'mean_c
ounts', 'pct_dropout_by_counts', 'total_counts', 'highly_variable'
, 'means', 'dispersions', 'dispersions_norm', 'mean', 'std'
    uns: 'hvg', 'leiden', 'leiden_colors', 'leiden_sizes', 'log1p'
, 'neighbors', 'paga', 'pca', 'rank_genes_groups', 'umap'
    obsm: 'X_pca', 'X_umap'
    varm: 'PCs'
    obsp: 'connectivities', 'distances'
```

In [54]:

```
adata.write(results_file, compression = "gzip")
```

In [55]:

```
adata.raw.to.adata().write("./write/pbmc3k_withoutX.h5ad")
```

```
------------------------------------------------------------------
---------
AttributeError                            Traceback (most recent c
all last)
Cell In[55], line 1
----> 1 adata.raw.to.adata().write("./write/pbmc3k_withoutX.h5ad")

AttributeError: 'NoneType' object has no attribute 'to'
```

In [ ]:

```

```

In [ ]:

```
adata.var
```

Trouble shooting:

if the code is having issues running, try out these steps:

- 1. double check all your syntax and variables
- 2. check your files contain the same data
- 3. type these commands into the console to install these versions of the following softwares and re-run the program:

```
pip install numpy==1.21.6
pip install scipy==1.8.1
pip install pandas==1.5.0
pip install scikit-learn==1.1.2
pip install statsmodel==0.13.2
pip install statsmodels==0.13.2
pip install python-igraph==0.10.1
pip install pynndescent==0.5.7
```