1. Download and import libraries

```
!pip install scanpy dask louvain leidenalg

!python -m pip uninstall matplotlib
!pip install matplotlib==3.1.3

import numpy as np
import pandas as pd
import scanpy as sc
import urllib
import os
import louvain
import matplotlib.pyplot as plt
%matplotlib inline
```

2. Obtain the scRNA-seq data (GSM3215435)

```
!mkdir -p data

!wget
https://ftp.ncbi.nlm.nih.gov/geo/samples/GSM3215nnn/GSM3215435/suppl/
GSM3215435_ldlr_ko_barcodes.tsv.gz
!mv GSM3215435_ldlr_ko_barcodes.tsv.gz data/barcodes.tsv.gz
!gunzip data/barcodes.tsv.gz

!wget
https://ftp.ncbi.nlm.nih.gov/geo/samples/GSM3215nnn/GSM3215435/suppl/
GSM3215435_ldlr_ko_genes.tsv.gz
!mv GSM3215435_ldlr_ko_genes.tsv.gz data/genes.tsv.gz
!gunzip data/genes.tsv.gz

!wget
https://ftp.ncbi.nlm.nih.gov/geo/samples/GSM3215nnn/GSM3215435/suppl/
GSM3215435_ldlr_ko_matrix.mtx.gz
!mv GSM3215435_ldlr_ko_matrix.mtx.gz data/matrix.mtx.gz
!gunzip data/matrix.mtx.gz

adata = sc.read_10x_mtx('data', var_names='gene_symbols', cache=True)
adata

AnnData object with n_obs × n_vars = 3781 × 27998
    var: 'gene_ids'
```

3. Filter out bad genes and cells

```
sc.pp.filter_cells(adata, min_genes=10)
sc.pp.filter_genes(adata, min_cells=10)
adata
```

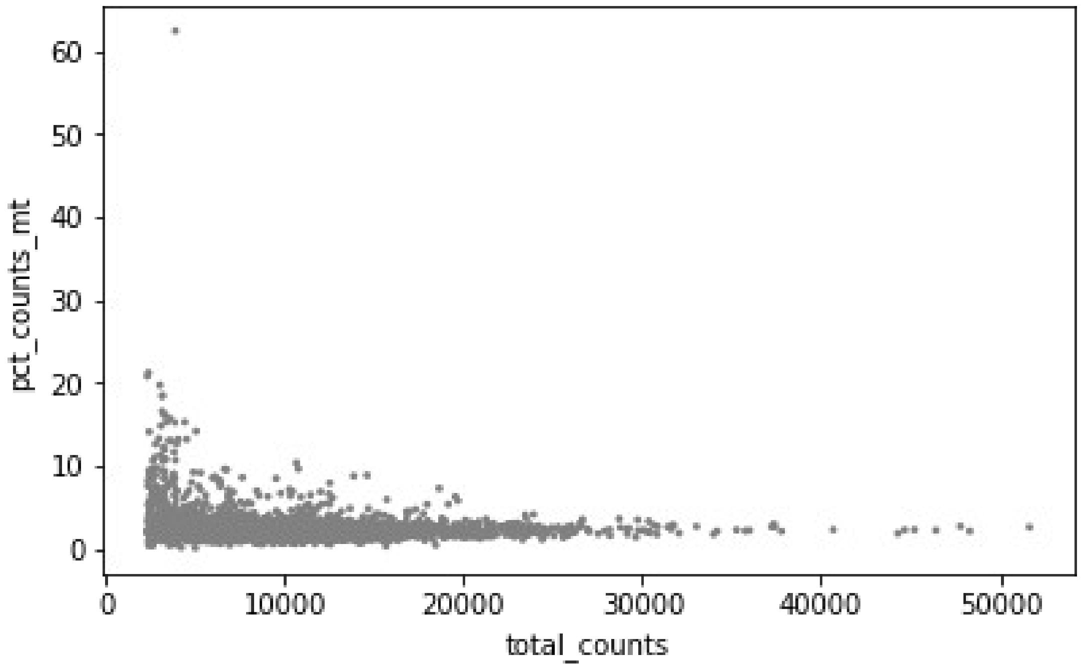
```
AnnData object with n_obs × n_vars = 3781 × 13105
    obs: 'n_genes'
    var: 'gene_ids', 'n_cells'
```

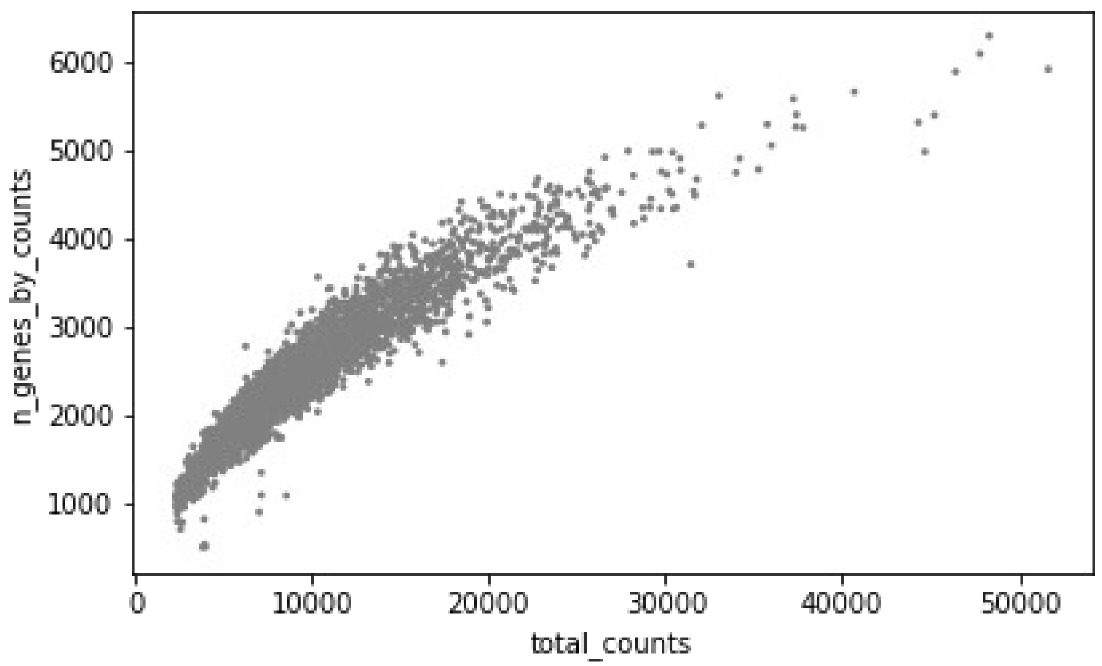
```
adata.var['mt'] = adata.var_names.str.startswith('mt-')  # annotate
the group of mitochondrial genes as 'mt'
adata.var['mt'].value_counts()
```

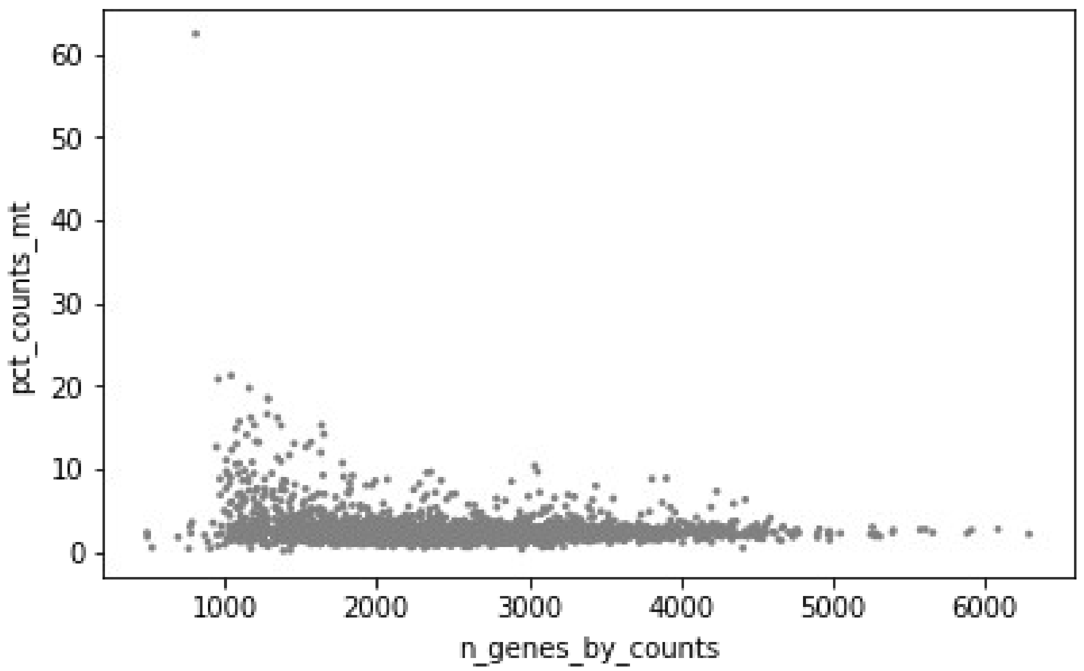
```
False    13092
True        13
Name: mt, dtype: int64
```

```
sc.pp.calculate_qc_metrics(adata, qc_vars=['mt'], percent_top=None,
log1p=True, inplace=True)
```

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





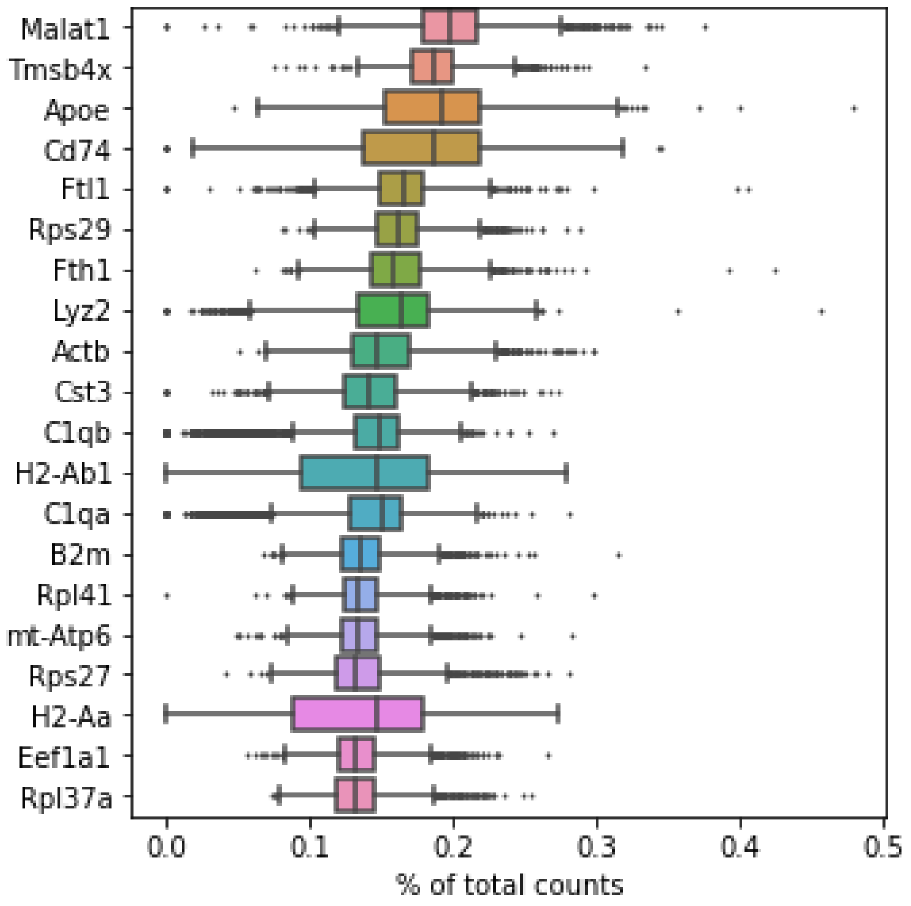


```
adata = adata[adata.obs.n_genes_by_counts > 1000, :].copy()
adata = adata[adata.obs.pct_counts_mt < 5, :].copy()

adata

AnnData object with n_obs × n_vars = 3600 × 13105
    obs: 'n_genes', 'n_genes_by_counts', 'log1p_n_genes_by_counts',
'total_counts', 'log1p_total_counts', 'total_counts_mt',
'log1p_total_counts_mt', 'pct_counts_mt'
```

```
    var: 'gene_ids', 'n_cells', 'mt', 'n_cells_by_counts',
'mean_counts', 'log1p_mean_counts', 'pct_dropout_by_counts',
'total_counts', 'log1p_total_counts'
```

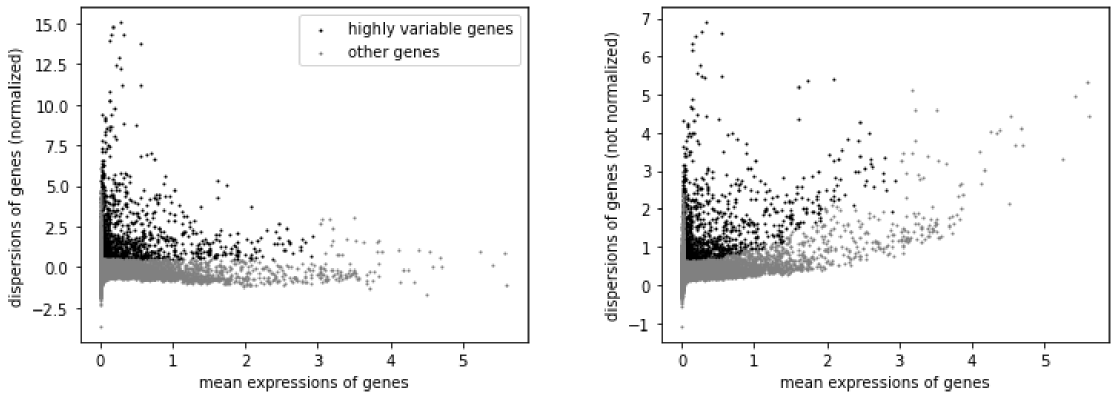
4. Data normalization

```
sc.pp.normalize_total(adata, target_sum=1e4) # Total-count normalize
(library-size correct) the data matrix X to 10,000 reads per cell, so
that counts become comparable among cells.
sc.pp.log1p(adata) # log-normalization

sc.pl.highest_expr_genes(adata, n_top=20, )
```



5. Get highly variable genes

```
sc.pp.highly_variable_genes(adata, min_mean=0.0125, max_mean=3,
min_disp=0.5)
gca = sc.pl.highly_variable_genes(adata, show=False, log=False) # can
not scale only x
```

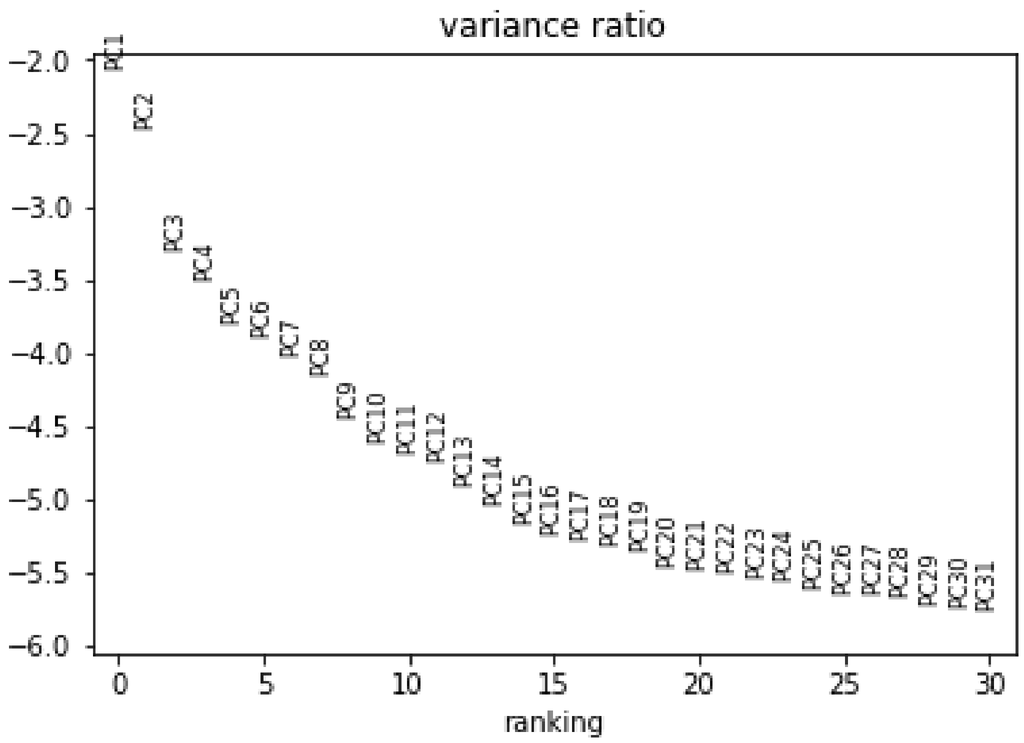


```
adata.var['highly_variable'].value_counts()
```

```
False    11878
True      1227
Name: highly_variable, dtype: int64
```

6. Perform PCA

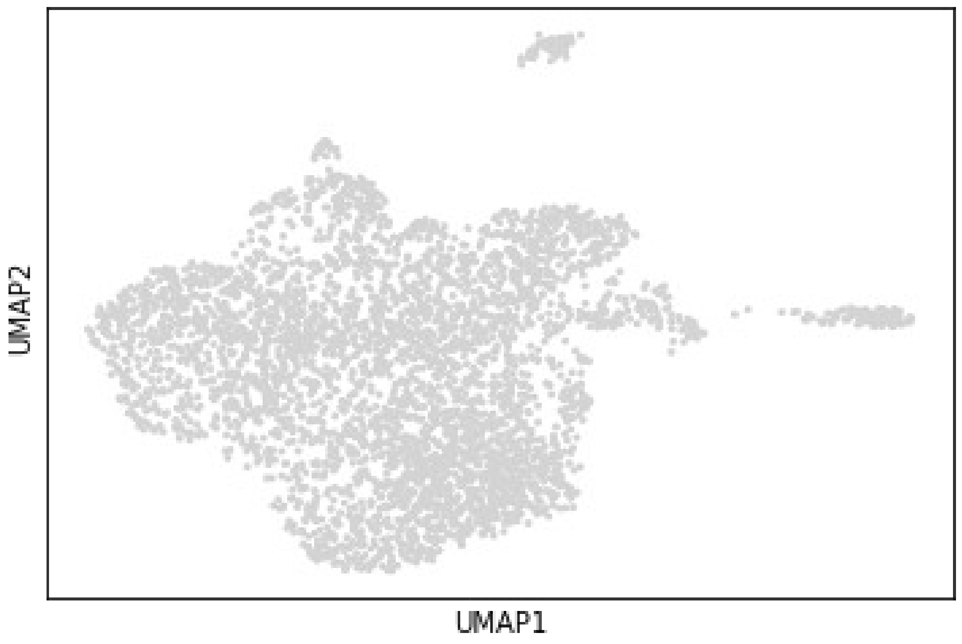
```
sc.tl.pca(adata, svd_solver='arpack')
sc.pl.pca_variance_ratio(adata, log=True)
```



7. Get a 2D visualization with UMAP

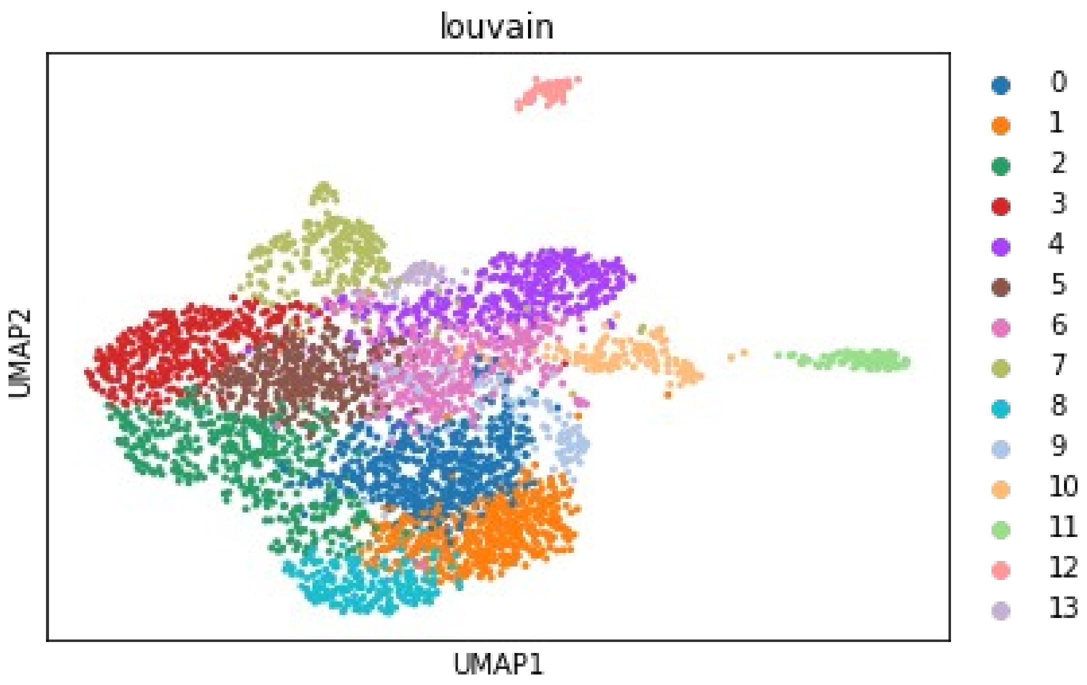
```
sc.pp.neighbors(adata, n_neighbors=30, n_pcs=30)
sc.tl.umap(adata)
sc.pl.umap(adata)
```

```
/usr/local/lib/python3.7/dist-packages/numba/np/ufunc/parallel.py:363:
NumbaWarning: The TBB threading layer requires TBB version 2019.5 or
later i.e., TBB_INTERFACE_VERSION >= 11005. Found
TBB_INTERFACE_VERSION = 9107. The TBB threading layer is disabled.
  warnings.warn(problem)
```



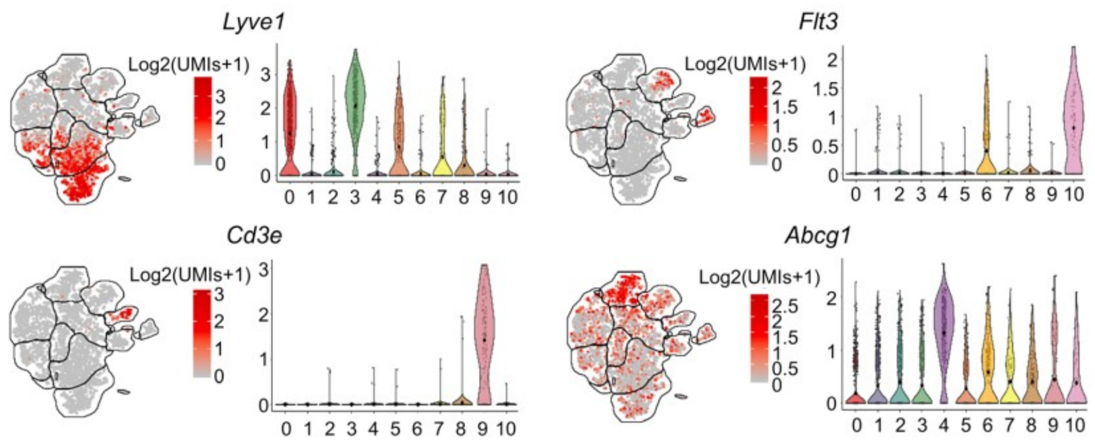
8. Get clusters

```
sc.tl.louvain(adata, resolution=1.2)
sc.pl.umap(adata, color='louvain')
```

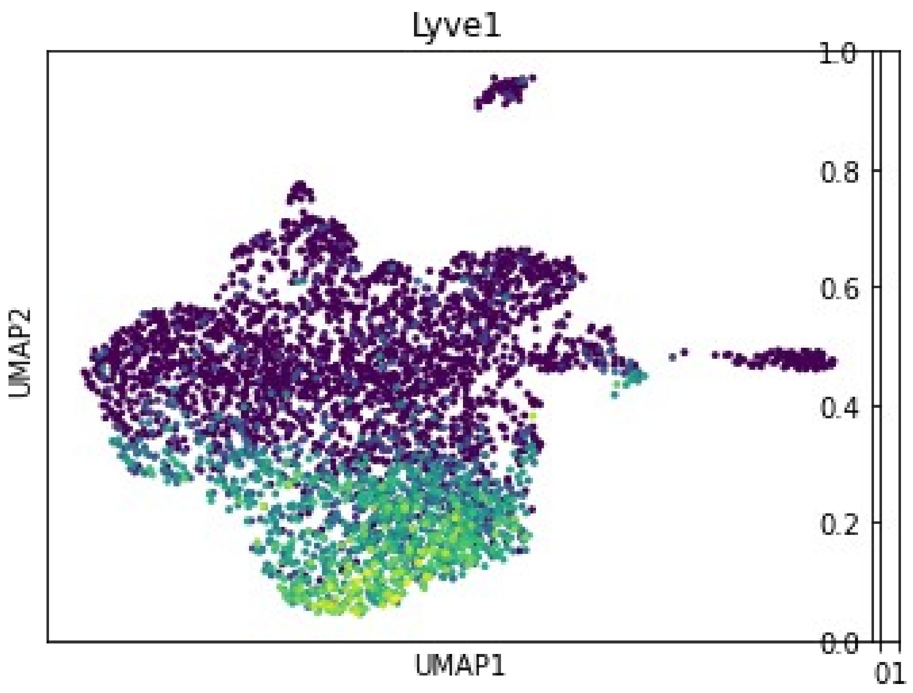


9. Check for some of the known markers

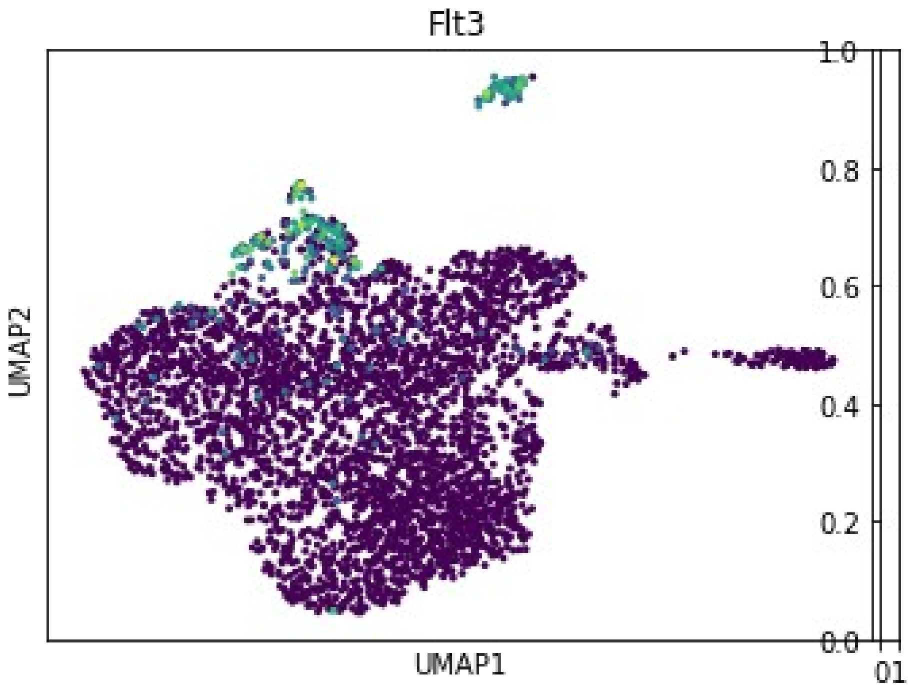
Now let's see whether known markers are clustered together, like in the referenced paper.



```python
try:
    sc.pl.umap(adata, color=["Lyve1"])
except Exception:
    pass
```

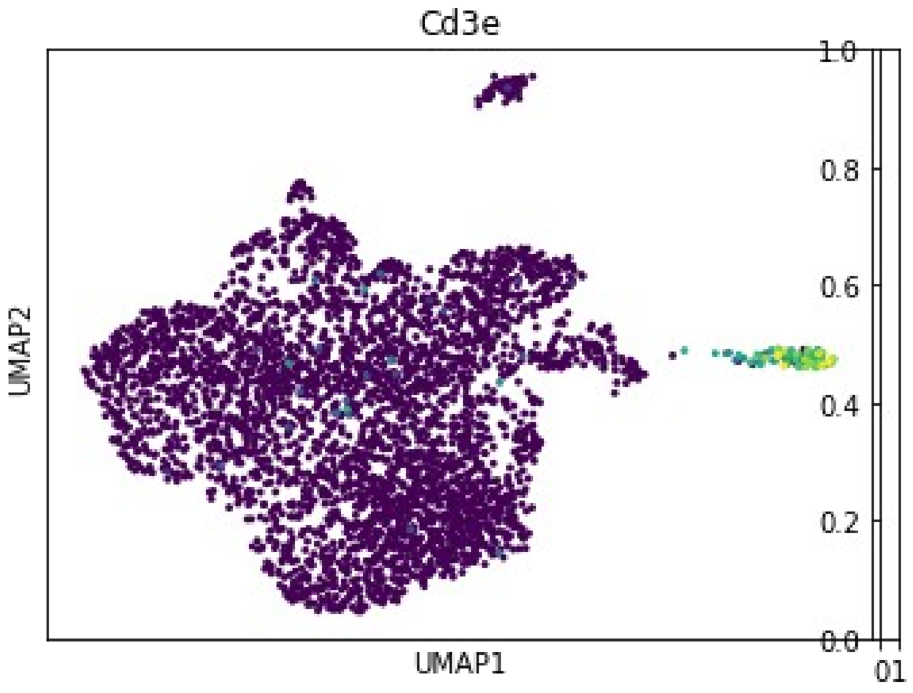


```python
try:
    sc.pl.umap(adata, color=["Flt3"])
except Exception:
    pass
```

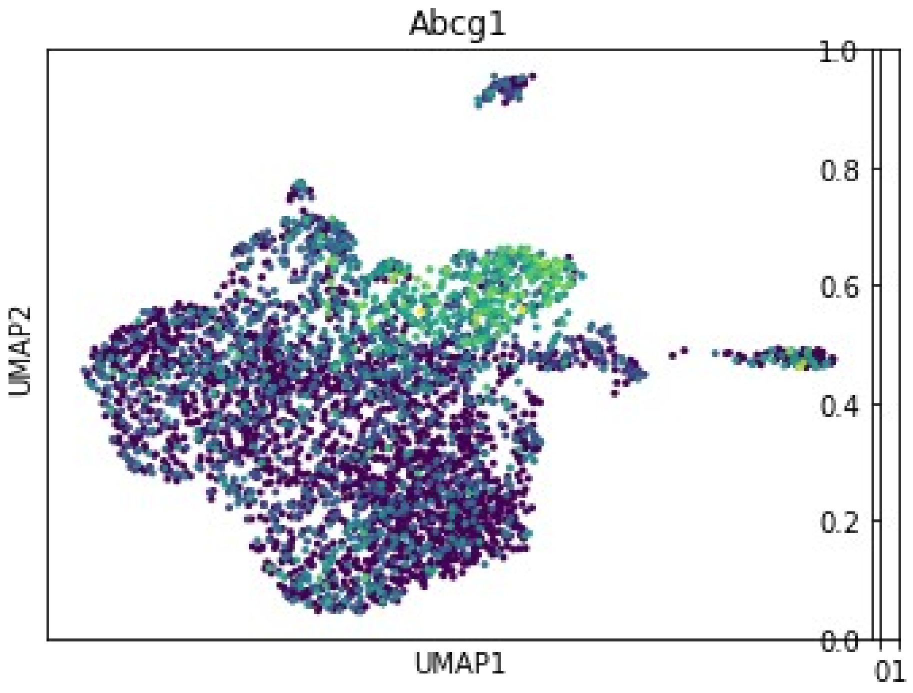


```python
try:
    sc.pl.umap(adata, color=["Cd3e"])
```

```
except Exception:
    pass
```



```
try:
    sc.pl.umap(adata, color=["Abcg1"])
except Exception:
    pass
```

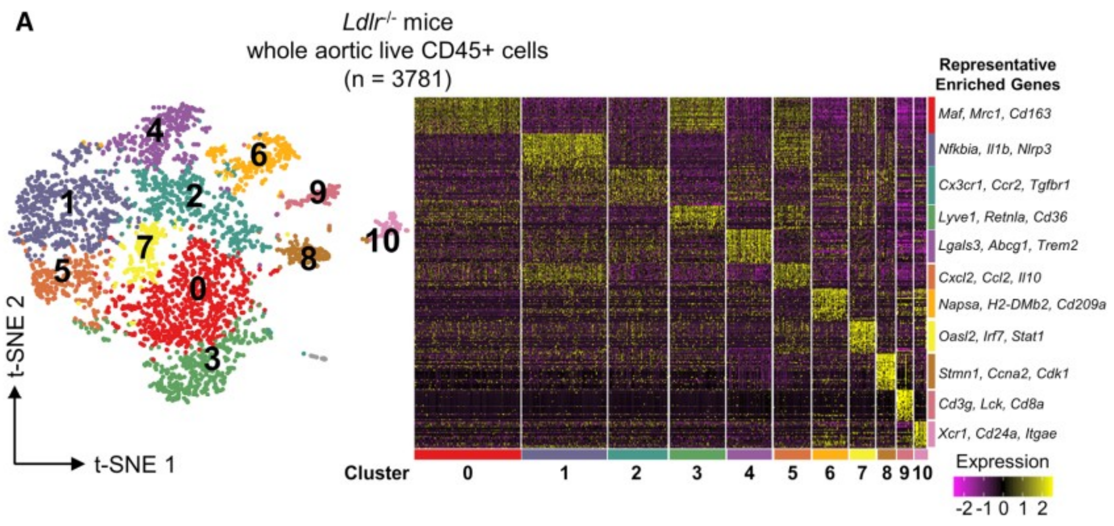


As we can see, markers do indeed group together.

10. Find differential genes that are the most specific for each cluster

```
sc.tl.rank_genes_groups(adata, 'louvain', method='wilcoxon')
pd.DataFrame(adata.uns['rank_genes_groups']['names']).head(5)
```

```
/usr/local/lib/python3.7/dist-packages/statsmodels/tools/
_testing.py:19: FutureWarning: pandas.util.testing is deprecated. Use
the functions in the public API at pandas.testing instead.
  import pandas.util.testing as tm

         0      1     2      3       4       5       6        7      8
9  \
0   Hspa1a    Pf4  Ccl8  Ifrd1  Lgals3    Atf3    Ccr2    Napsa  Folr2
Zbp1
1    Txnip   Cbr2  Ier3   Cd83    Ctsz    Ccl4  Cx3cr1    Ifi30   Ccl6
Fcgr1
2   Hspa1b    Maf  Ccl7   Cd14    Ctsb     Ubc    Ctss  S100a11  Lyve1
Irf7
3  Serinc3  Stab1  Ccl2  Nlrp3    Ctsd  Nfkbia    Psap   S100a4  F13a1
Mnda
4      Pf4  Folr2  Ccl6   Il1b   Mpeg1  Nfkbiz   Mpeg1   Syngr2  Fxyd2
Stat1

      10        11       12      13
0  Stmn1      Cd3g    Cd24a   Plac8
1  Hmgb2    Rpl13a     Naaa  Tmsb10
2  Tubb5    Rps15a     Cst3   Msrb1
3  Top2a  AW112010   Tmsb10   H3f3a
4   Ptma      Cd3d     Ppt1   Napsa
```



As we can see, the most enriched genes for each clusters are very similar with the ones obtained in the referenced paper.

Clusters in paper correspond to my clusters then as written below:

Their cluster - my cluster (common enriched gene)

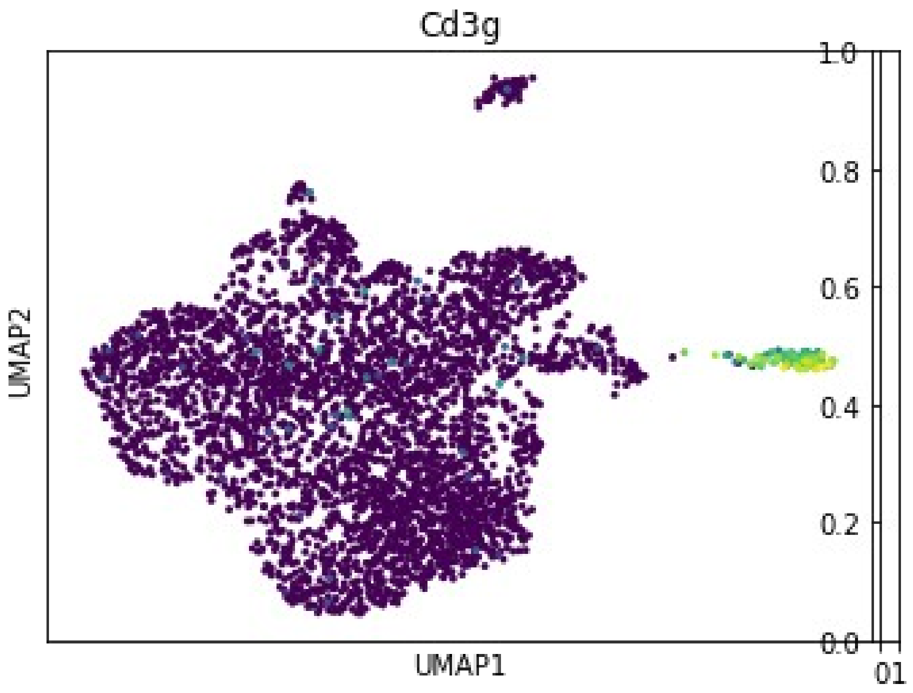
- 0 - 1 (Maf)
- 1 - 5 (Nfkbia)
- 2 - 6 (Ccr2)
- 3 - 8 (Lyve1)
- 4 - 4 (Lgals3)
- 5 - 2 (Ccl2)
- 6 - 7 (Napsa)
- 7 - 9 (Irf7)
- 8 - 10 (Stmn1)
- 9 - 11 (Cd3g)
- 10 - 12 (Cd24a)

However, I have some clusters left - 0, 3, 13.

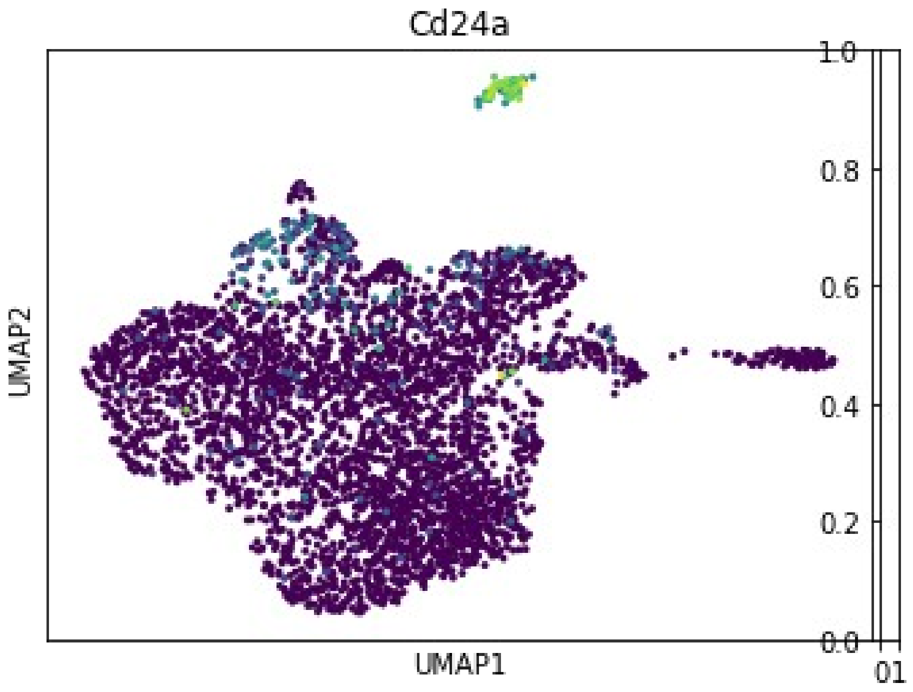
My cluster 3 has common top enriched genes with cluster 1 from the paper, 13 - with 6.

0-th cluster is quite small and is located between two other clusters - it is probably a statistical artifact that it clustered separately from them (or maybe it is correct and the paper is wrong.)

```python
try:
    sc.pl.umap(adata, color=["Cd3g"])
except Exception:
    pass
```

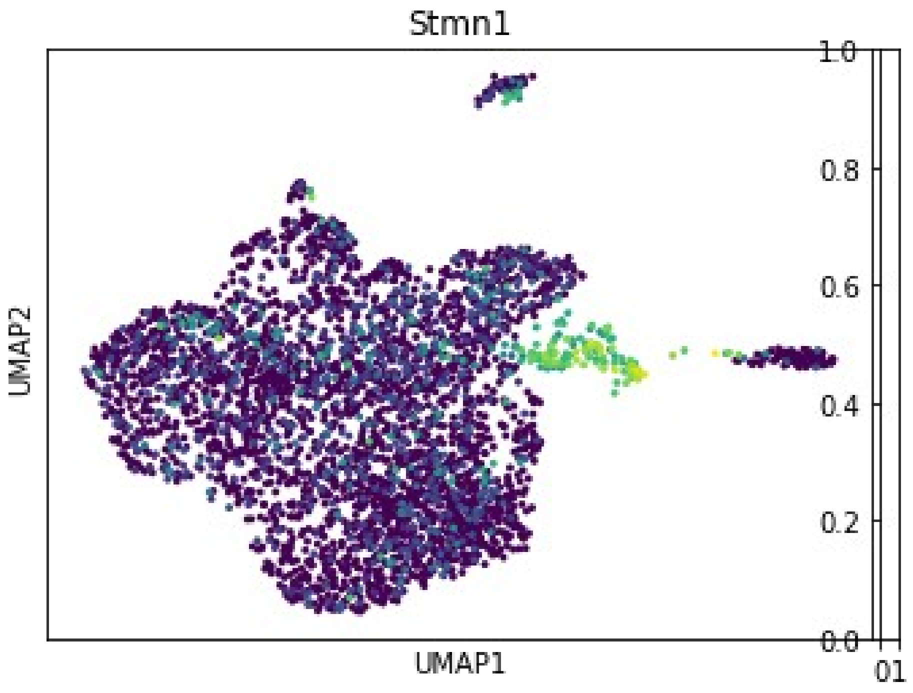


```
try:
    sc.pl.umap(adata, color=["Cd24a"])
except Exception:
    pass
```



```
try:
    sc.pl.umap(adata, color=["Stmn1"])
```

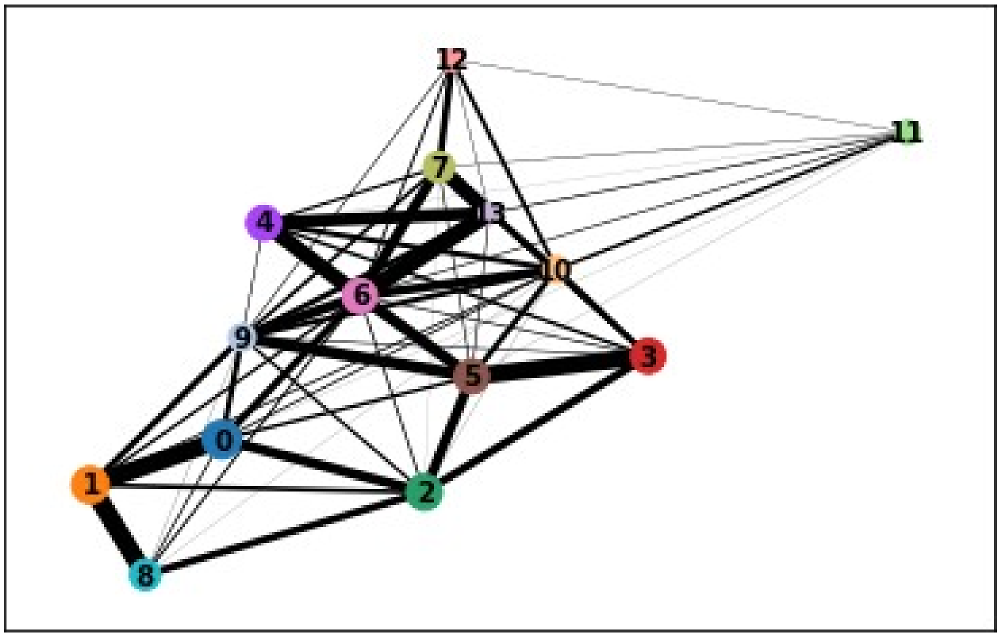
```
except Exception:
    pass
```



11. Trajectory inference

```
sc.tl.paga(adata, groups='louvain')
```

```
sc.pl.paga(adata, color=['louvain'])
```



12
11
7
13
4
10
6
9
3
5
0
1
2
8