Project PXD005835- CELL LYSATE DATA only from “Selective stalling of human translation through small molecule engagement of the ribosome nascent chain”

In [33]:
```
import scanpy as sc
from sklearn.model_selection import train_test_split
from sklearn.preprocessing import StandardScaler
from sklearn.impute import SimpleImputer
from sklearn.ensemble import RandomForestClassifier
import pandas as pd
import numpy as np
import anndata as ad
from scipy.sparse import csr_matrix
```

In [ ]:
```
# Load expression matrix from CSV file
expression_matrix = pd.read_csv('/content/drive/MyDrive/BIO/expression_matr
```

In [ ]:
```
expression_matrix.head()
```

Out[ ]:

	AAACTCCGTTGGATCT.1	AAATGCTTCATGGCCG.1	AAATTCGCAATTCA
ENSG00000238009	0.0	0.0	
ENSG00000239945	0.0	0.0	
ENSG00000241860	0.0	0.0	
ENSG00000241599	0.0	0.0	
ENSG00000290385	0.0	0.0	

5 rows × 713 columns

In [ ]:

```
expression_matrix.info()
```

```
<class 'pandas.core.frame.DataFrame'>
Index: 29112 entries, ENSG00000238009 to ENSG00000277196
Columns: 713 entries, AAACTCCGTTGGATCT.1 to TGTGTTGAGGTTAGTT.1
dtypes: float64(713)
memory usage: 158.6+ MB
```

In [37]:

```
counts = csr_matrix(expression_matrix.values).T
```

In [38]:

```
a_data = ad.AnnData(counts)
```

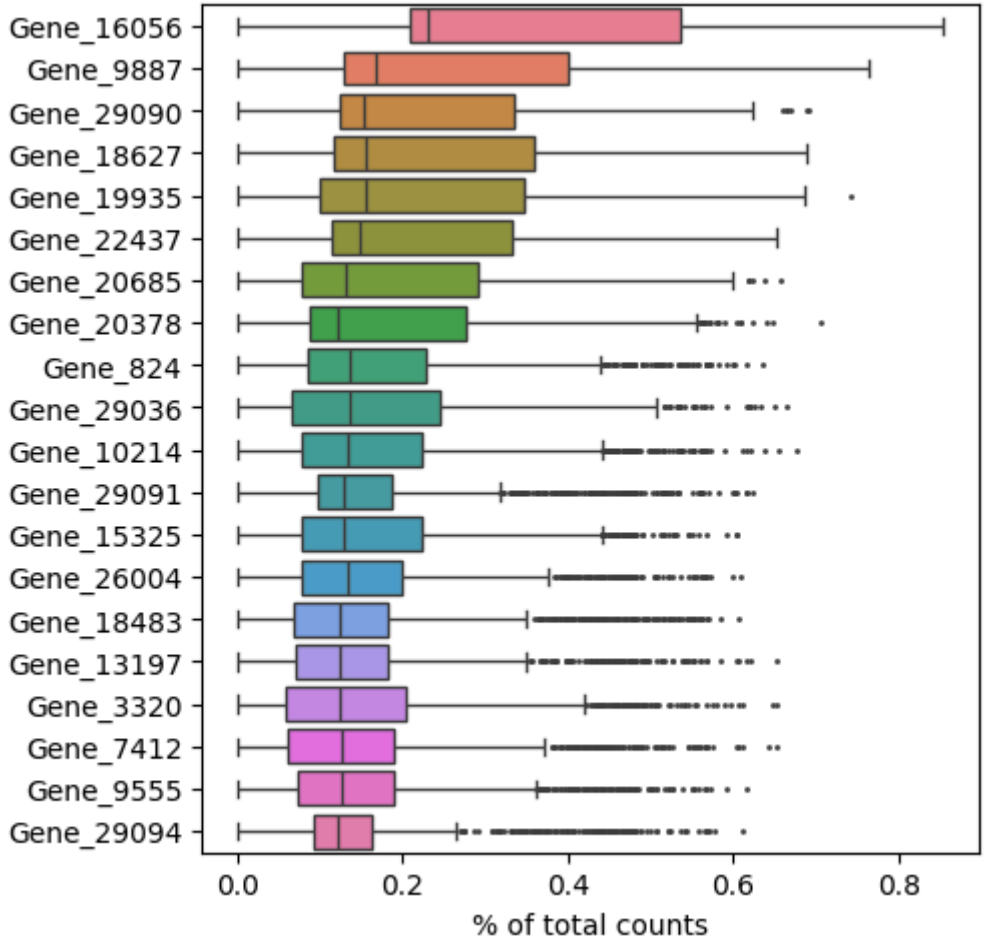
In [39]:
```
a_data.X
```

Out[39]:
```
<713x29112 sparse matrix of type '<class 'numpy.float64'>'
        with 982555 stored elements in Compressed Sparse Column format>
```

In [40]:
```
a_data.obs_names = [f"Cell_{i:d}" for i in range(a_data.n_obs)]
a_data.var_names = [f"Gene_{i:d}" for i in range(a_data.n_vars)]
print(a_data.obs_names[:10])
```

```
Index(['Cell_0', 'Cell_1', 'Cell_2', 'Cell_3', 'Cell_4', 'Cell_5', 'Cell_6',
       'Cell_7', 'Cell_8', 'Cell_9'],
      dtype='object')
```

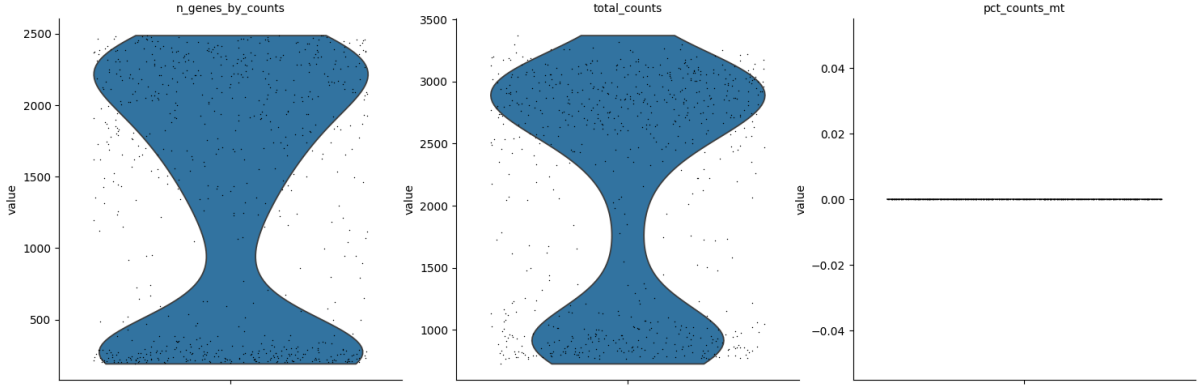
In [41]:
```
sc.pl.highest_expr_genes(a_data, n_top=20)
```


Gene_16056
Gene_9887
Gene_29090
Gene_18627
Gene_19935
Gene_22437
Gene_20685
Gene_20378
Gene_824
Gene_29036
Gene_10214
Gene_29091
Gene_15325
Gene_26004
Gene_18483
Gene_13197
Gene_3320
Gene_7412
Gene_9555
Gene_29094
0.0
0.2
0.4
0.6
0.8
% of total counts

In [43]:
```
sc.pp.filter_cells(a_data, min_genes=200)
sc.pp.filter_genes(a_data, min_cells=3)
```

In [44]:
```
# annotate the group of mitochondrial genes as "mt"

a_data.var["mt"] = a_data.var_names.str.startswith("MT-")
sc.pp.calculate_qc_metrics(
    a_data, qc_vars=["mt"], percent_top=None, log1p=False, inplace=True
)
```

In [45]:
```
sc.pl.violin(
    a_data,
    ["n_genes_by_counts", "total_counts", "pct_counts_mt"],
    jitter=0.4,
    multi_panel=True,
)
```



In [46]:
```
# filtering ..

a_data = a_data[a_data.obs.n_genes_by_counts < 2500, :]
a_data = a_data[a_data.obs.pct_counts_mt < 5, :].copy()
```

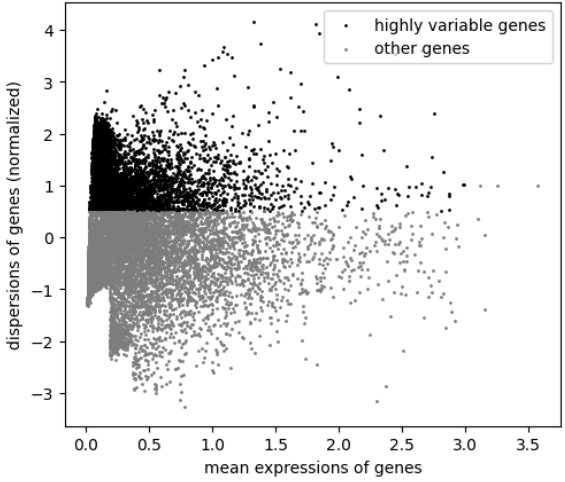
In [47]:
```
# Total-count normalize (library-size correct) the data matrix
# to 10,000 reads per cell, so that counts become comparable among cells.

sc.pp.normalize_total(a_data, target_sum=1e4)
```

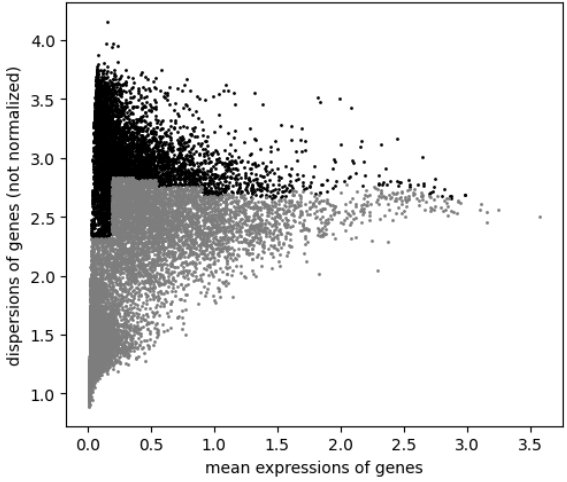
In [49]:
```
# Log. the data:
sc.pp.log1p(a_data)
```

```
# Identifying highly-variable genes
sc.pp.highly_variable_genes(a_data, min_mean=0.0125, max_mean=3, min_disp=0
```

In [50]:

```
#plot them

sc.pl.highly_variable_genes(a_data)
```





In [51]:

```
a_data.raw = a_data
```

In [52]:

```
# more filtering..
a_data = a_data[:, a_data.var.highly_variable]


# Regress out effects of total counts per cell and the % of mit. genes expr
# Scaling then the data to unit variance.

sc.pp.regress_out(a_data, ["total_counts", "pct_counts_mt"])
```

```
/usr/local/lib/python3.10/dist-packages/scanpy/preprocessing/_simple.py:641:
UserWarning: Received a view of an AnnData. Making a copy.
  view_to_actual(adata)
```

In [53]:

```
# so, scaling data and clipping values exceeding standard deviation 10

sc.pp.scale(a_data, max_value=10)
```

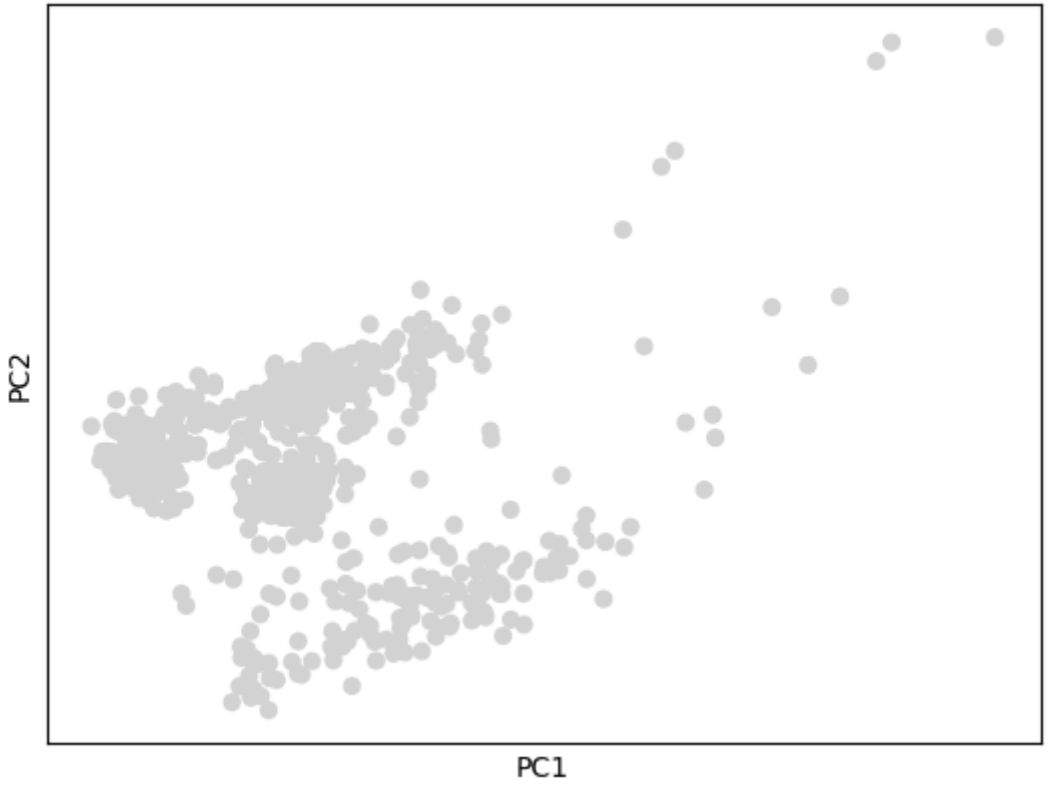
In [54]:

```
#########

#######   PCA

sc.tl.pca(a_data, svd_solver="arpack")
```

In [56]:

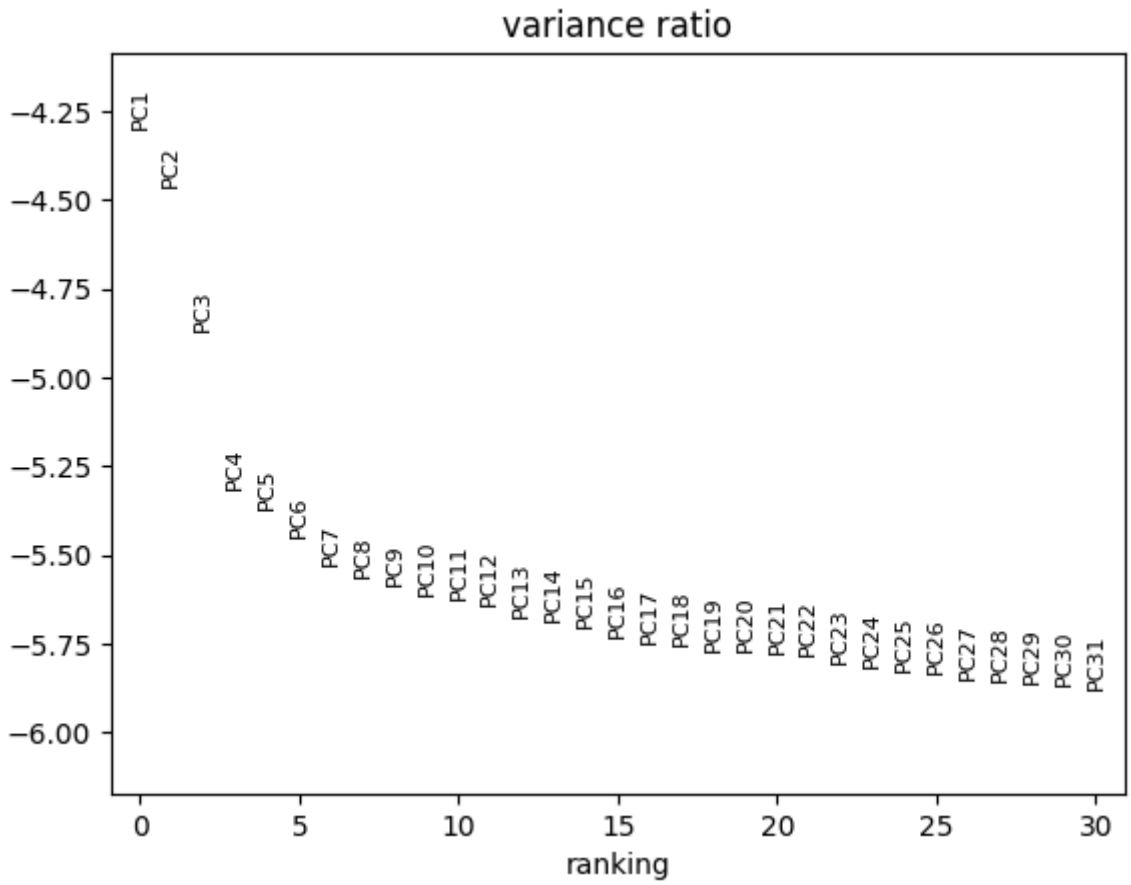
```

```

```
sc.pl.pca(a_data)
```



In [57]:

```
sc.pl.pca_variance_ratio(a_data, log=True)
```


variance ratio
−4.25
−4.50
−4.75
−5.00
−5.25
−5.50
−5.75
−6.00
PC1
PC2
PC3
PC4
PC5
PC6
PC7
PC8
PC9
PC10
PC11
PC12
PC13
PC14
PC15
PC16
PC17
PC18
PC19
PC20
PC21
PC22
PC23
PC24
PC25
PC26
PC27
PC28
PC29
PC30
PC31
0
5
10
15
20
25
30
ranking

In [59]:
```
# save results

a_data.write('results_file.hdf5')
```

In [60]:
```
a_data
```

Out[60]:
```
AnnData object with n_obs × n_vars = 713 × 4798
    obs: 'n_genes', 'n_genes_by_counts', 'total_counts', 'total_counts_mt',
'pct_counts_mt'
    var: 'n_cells', 'mt', 'n_cells_by_counts', 'mean_counts', 'pct_dropout_
by_counts', 'total_counts', 'highly_variable', 'means', 'dispersions', 'dis
persions_norm', 'mean', 'std'
    uns: 'log1p', 'hvg', 'pca'
    obsm: 'X_pca'
    varm: 'PCs'
```

In [ ]:
```

```

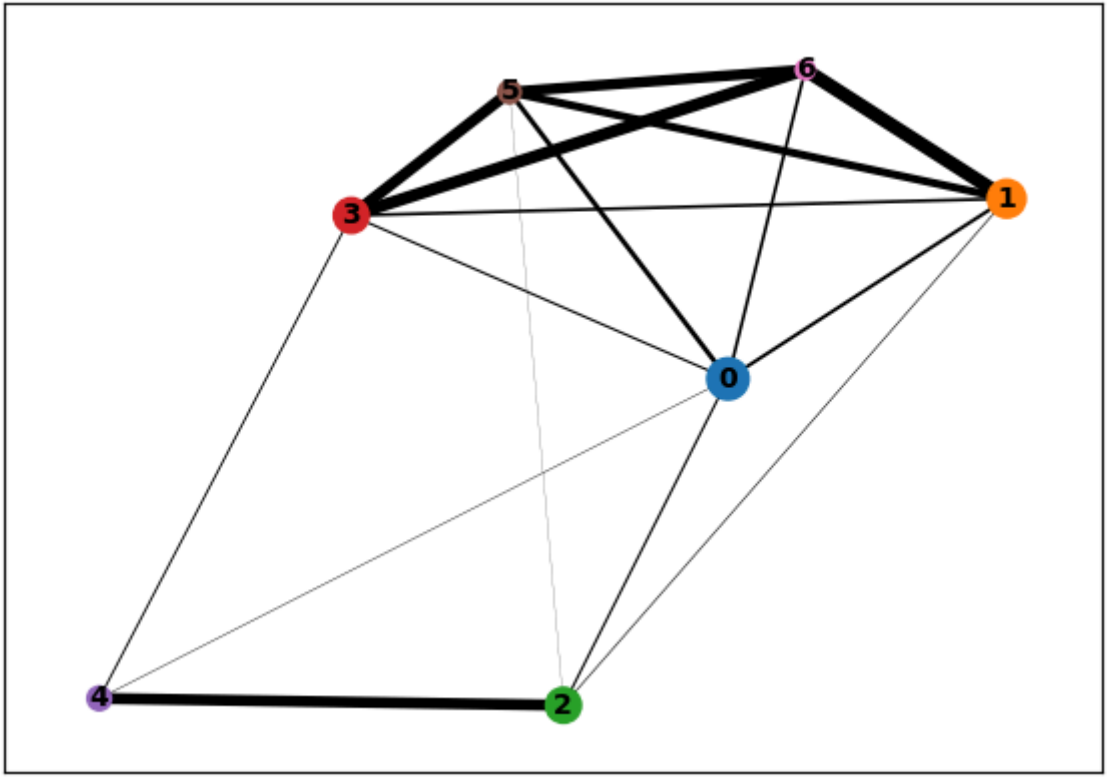
In [68]:
```
sc.tl.louvain(a_data)
```

In [69]:
```
## Computing the neighborhood graph of cells using
##  the PCA repr. of the data matrix


```

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

In [70]:

```
# embedding the graph in two dimensions using UMAP

sc.tl.paga(a_data)
sc.pl.paga(a_data)
sc.tl.umap(a_data, init_pos='paga')
```

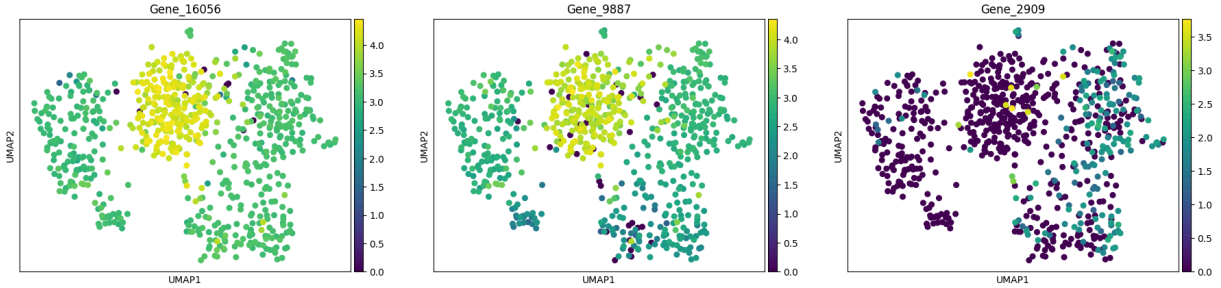


In [77]:

```
sc.tl.umap(a_data)
```

In [79]:

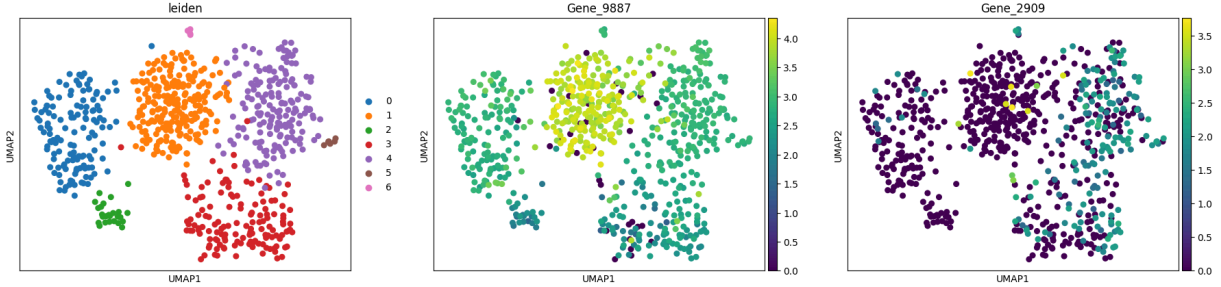
```
sc.pl.umap(a_data, color=["Gene_16056", "Gene_9887", "Gene_2909"])
```



In [80]:

```
# Clustering the neighborhood graph

sc.tl.leiden(
    a_data,
    resolution=0.9,
    random_state=0,
    flavor="igraph",
    n_iterations=2,
    directed=False,
)
```

In [82]:
```
sc.pl.umap(a_data, color=["leiden", "Gene_9887", "Gene_2909"])
```

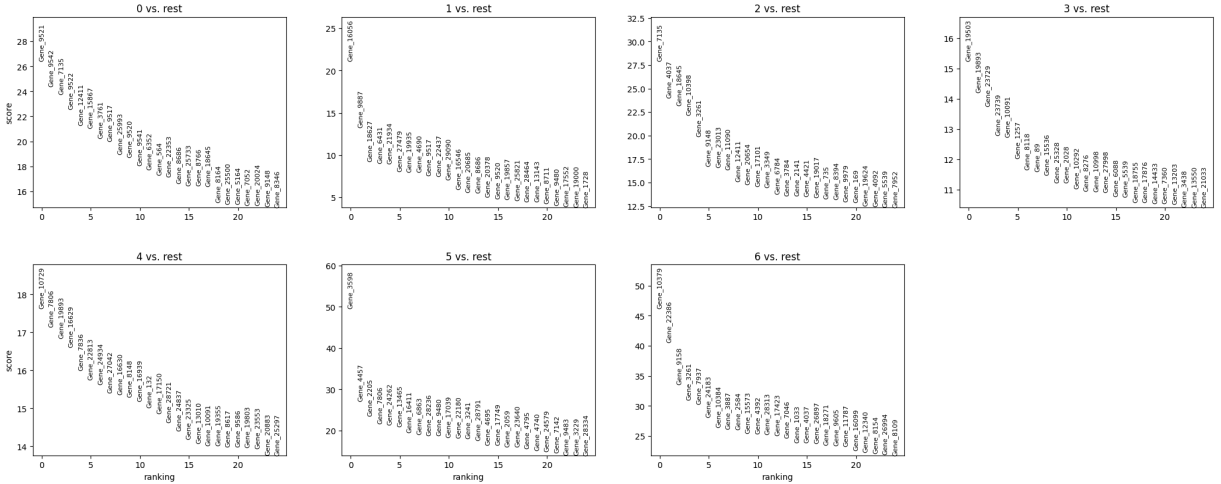


In [84]:
```
a_data.write('results.hdf5')
```

Finding marker genes computing a ranking for the highly differential genes in each cluster. .raw attribute of AnnData is used by default in case it has been initialized before. The simplest and fastest method is the t-test.

In [85]:
```
sc.tl.rank_genes_groups(a_data, "leiden", method="t-test")
```

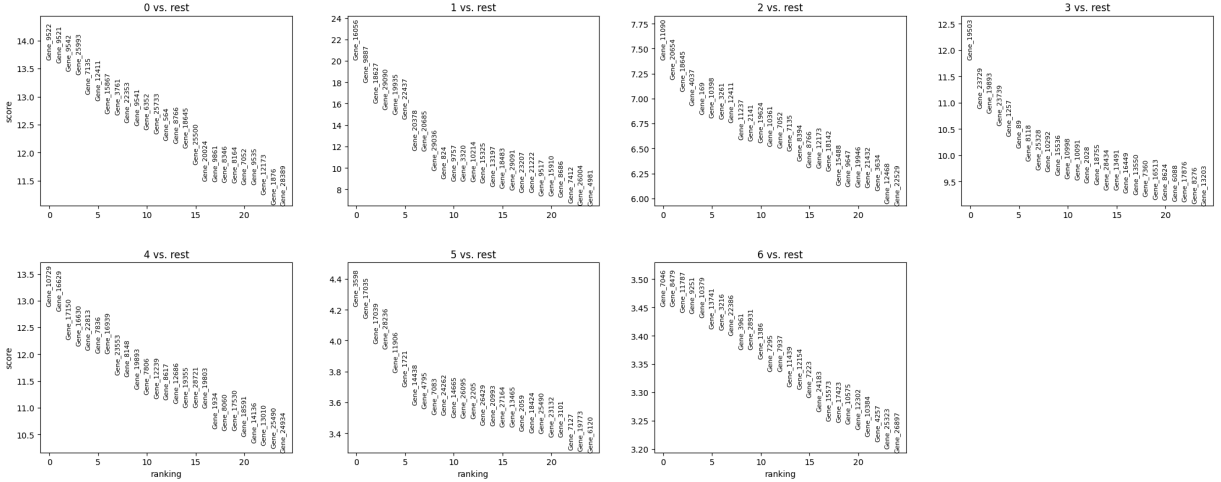
```
sc.pl.rank_genes_groups(a_data, n_genes=25, sharey=False)
```



In [86]:
```
sc.settings.verbosity = 2
```

In [87]:
```
sc.tl.rank_genes_groups(a_data, "leiden", method="wilcoxon")
sc.pl.rank_genes_groups(a_data, n_genes=25, sharey=False)
```

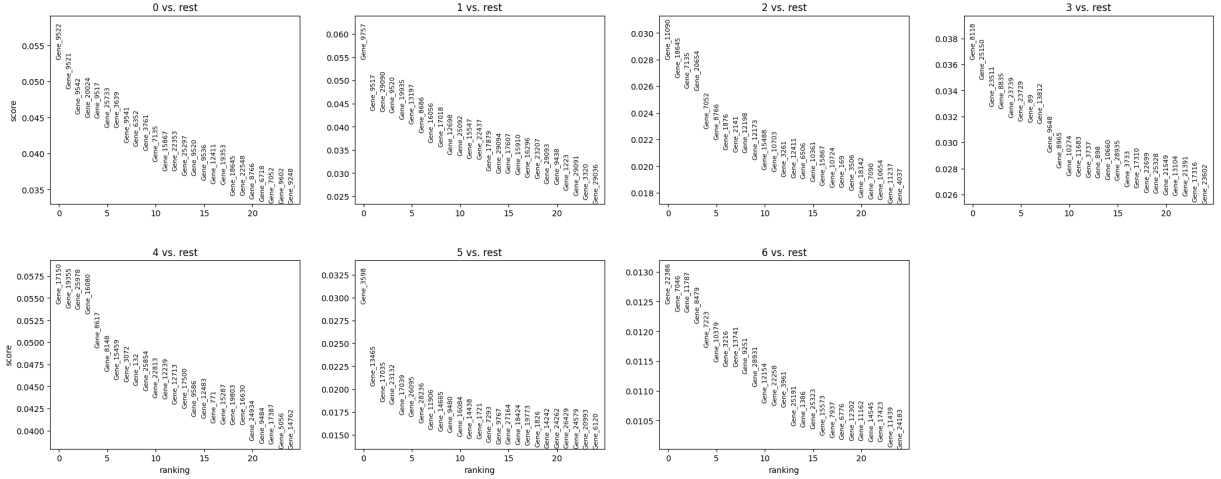
```
ranking genes
    finished (0:00:01)
```



In [88]:
```
a_data.write('results.hdf5')
```

In [89]:
```
sc.tl.rank_genes_groups(a_data, "leiden", method="logreg", max_iter=1000)
sc.pl.rank_genes_groups(a_data, n_genes=25, sharey=False)
```

```
ranking genes
    finished (0:01:00)
```



In [111…

```
marker_genes = [
    *["Gene_9522", "Gene_16056",        "Gene_11090",   "Gene_19503",   "Ge
]
```

In [96]:

```
a_data = sc.read_h5ad('/content/results.hdf5')
```

In [97]:

```
pd.DataFrame(a_data.uns["rank_genes_groups"]["names"]).head(5)
```

Out[97]:

	0	1	2	3	4	5	
0	Gene_9522	Gene_16056	Gene_11090	Gene_19503	Gene_10729	Gene_3598	Gene_
1	Gene_9521	Gene_9887	Gene_20654	Gene_23729	Gene_16629	Gene_17035	Gene_
2	Gene_9542	Gene_18627	Gene_18645	Gene_19893	Gene_17150	Gene_17039	Gene_
3	Gene_25993	Gene_29090	Gene_4037	Gene_23739	Gene_16630	Gene_28236	Gene_
4	Gene_7135	Gene_19935	Gene_169	Gene_1257	Gene_22813	Gene_11906	Gene_

In [98]:

```
result = a_data.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)
```

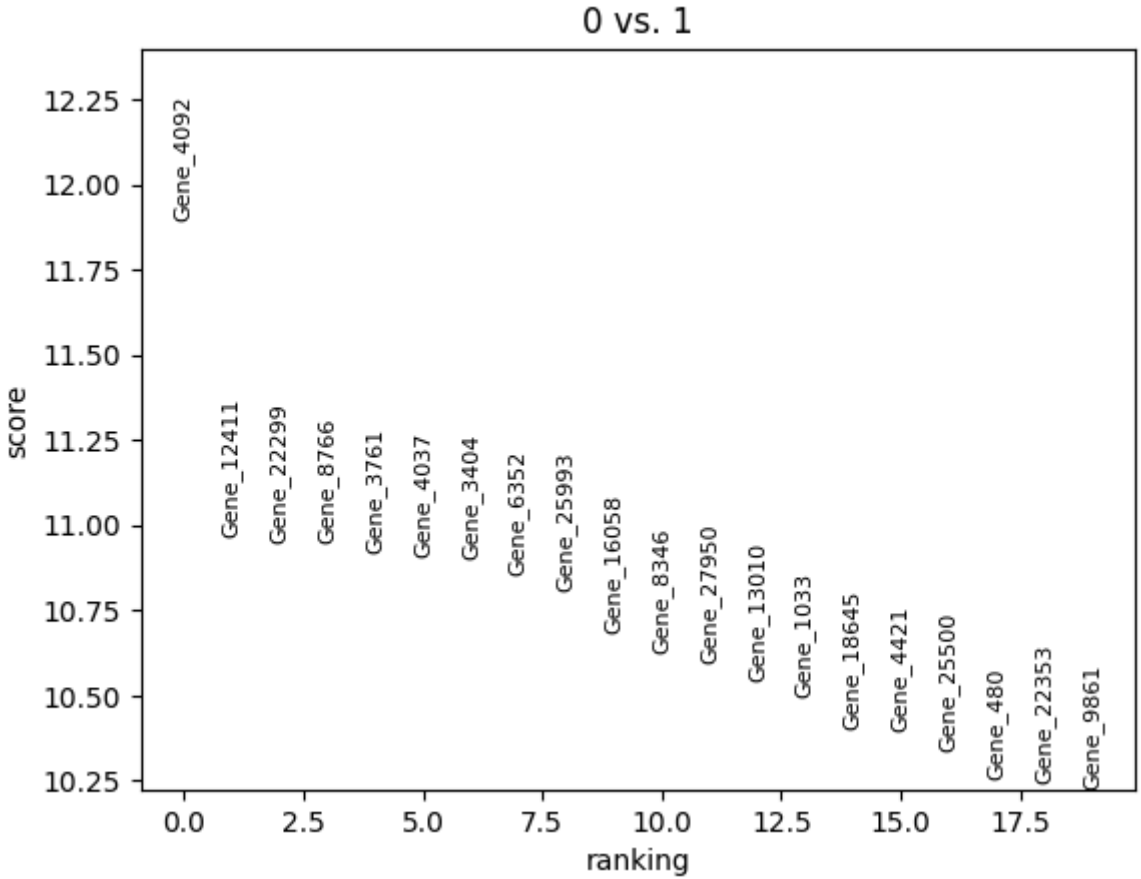
Out[98]:

	0_n	0_p	1_n	1_p	2_n	2_p	3_ı
0	Gene_9522	1.681611e-42	Gene_16056	4.207777e-90	Gene_11090	1.446218e-13	Gene_1950
1	Gene_9521	3.655379e-42	Gene_9887	1.994549e-72	Gene_20654	6.232277e-13	Gene_2372
2	Gene_9542	2.805685e-41	Gene_18627	3.342711e-58	Gene_18645	1.487036e-12	Gene_1989
3	Gene_25993	6.925274e-41	Gene_29090	5.091573e-54	Gene_4037	4.081295e-12	Gene_2373
4	Gene_7135	6.628745e-39	Gene_19935	7.580512e-51	Gene_169	7.627725e-12	Gene_125

In [99]:

```
sc.tl.rank_genes_groups(a_data, "leiden", groups=["0"], reference="1", meth
sc.pl.rank_genes_groups(a_data, groups=["0"], n_genes=20)
```

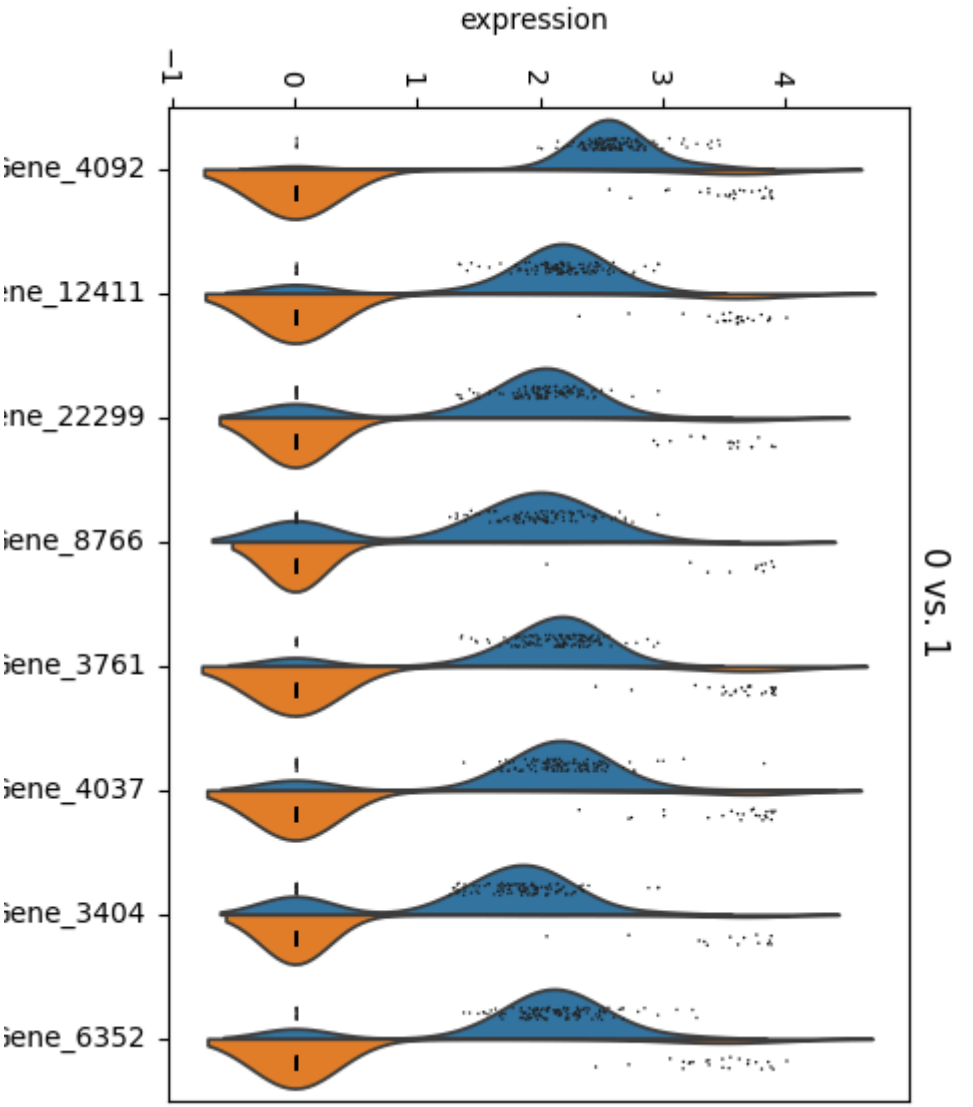
```
ranking genes
    finished (0:00:00)
```

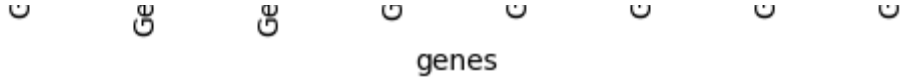

0 vs. 1
score
ranking
12.25
12.00
11.75
11.50
11.25
11.00
10.75
10.50
10.25
0.0
2.5
5.0
7.5
10.0
12.5
15.0
17.5
Gene_4092
Gene_12411
Gene_22299
Gene_8766
Gene_3761
Gene_4037
Gene_3404
Gene_6352
Gene_25993
Gene_16058
Gene_8346
Gene_27950
Gene_13010
Gene_1033
Gene_18645
Gene_4421
Gene_25500
Gene_480
Gene_22353
Gene_9861

In [100…

```
sc.pl.rank_genes_groups_violin(a_data, groups="0", n_genes=8)
```

```
/usr/local/lib/python3.10/dist-packages/scanpy/plotting/_tools/__init__.py:13
03: UserWarning: FixedFormatter should only be used together with FixedLocato
r
  _ax.set_xticklabels(new_gene_names, rotation="vertical")
```


expression
−1
0
1
2
3
4
Gene_4092
ene_12411
ene_22299
Gene_8766
Gene_3761
Gene_4037
Gene_3404
Gene_6352
0 vs. 1



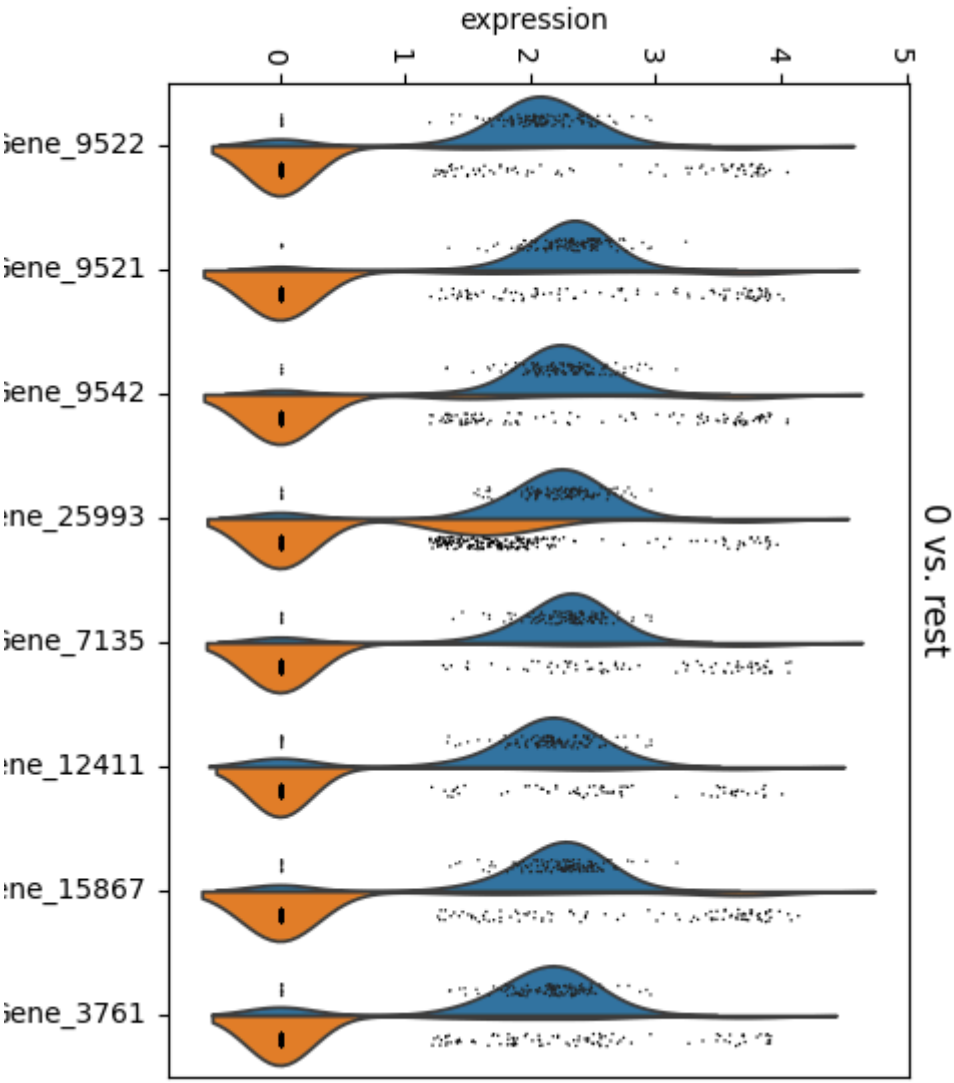
In [101…

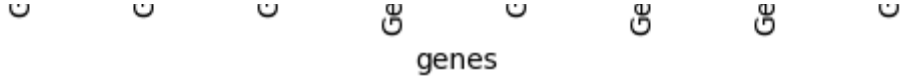
```
a_data = sc.read_h5ad('/content/results.hdf5')
```

In [102…

```
sc.pl.rank_genes_groups_violin(a_data, groups="0", n_genes=8)
```

```
/usr/local/lib/python3.10/dist-packages/scanpy/plotting/_tools/__init__.py:1303: UserWarning: FixedFormatter should only be used together with FixedLocator
  _ax.set_xticklabels(new_gene_names, rotation="vertical")
```


expression
0
1
2
3
4
5
Gene_9522
Gene_9521
Gene_9542
ene_25993
Gene_7135
ene_12411
ene_15867
Gene_3761
0 vs. rest



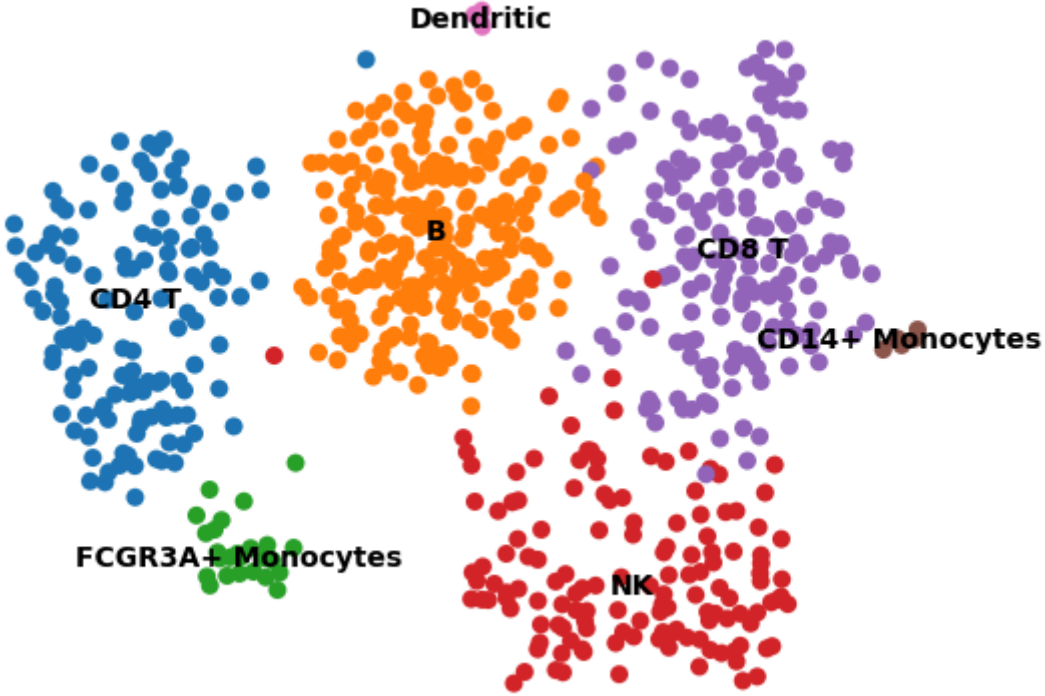
In [104…

```
new_cluster_names = [
    "CD4 T",
    "B",
    "FCGR3A+ Monocytes",
    "NK",
    "CD8 T",
    "CD14+ Monocytes",
    "Dendritic",
    #"Megakaryocytes",
]
a_data.rename_categories("leiden", new_cluster_names)
```

In [105…

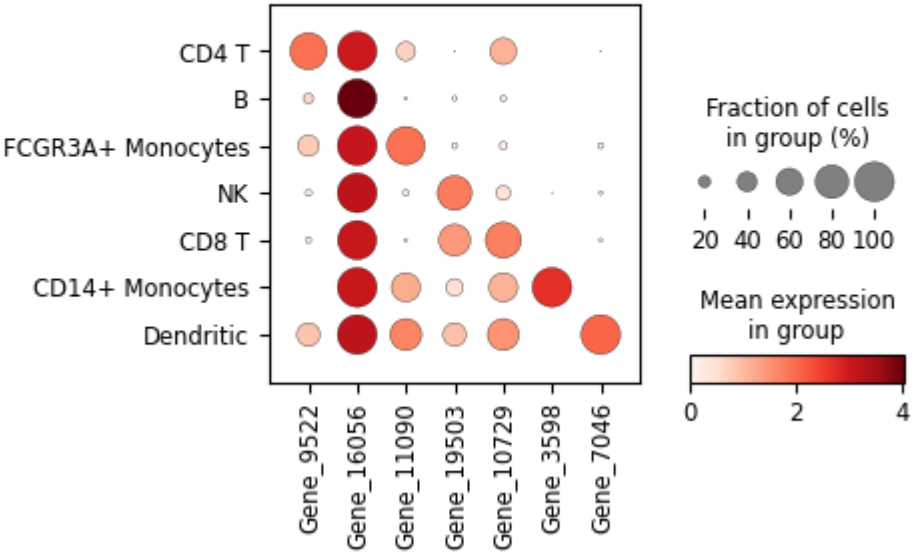
```
sc.pl.umap(
    a_data, color="leiden", legend_loc="on data", title="", frameon=False,
)
```

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



In [112…

```
# Visualize marker genes
sc.pl.dotplot(a_data, marker_genes, groupby='leiden')
```


CD4 T
B
FCGR3A+ Monocytes
NK
CD8 T
CD14+ Monocytes
Dendritic
Gene_9522
Gene_16056
Gene_11090
Gene_19503
Gene_10729
Gene_3598
Gene_7046
Fraction of cells in group (%)
20 40 60 80 100
Mean expression in group
0 2 4

In [113…

```
a_data
```

Out[113…

```
AnnData object with n_obs × n_vars = 713 × 4798
    obs: 'n_genes', 'n_genes_by_counts', 'total_counts', 'total_counts_mt', 'pct_counts_mt', 'louvain', 'leiden'
    var: 'n_cells', 'mt', 'n_cells_by_counts', 'mean_counts', 'pct_dropout_by_counts', 'total_counts', 'highly_variable', 'means', 'dispersions', 'dispersions_norm', 'mean', 'std'
    uns: 'hvg', 'leiden', 'leiden_colors', 'log1p', 'louvain', 'louvain_colors', 'louvain_sizes', 'neighbors', 'paga', 'pca', 'rank_genes_groups', 'umap'
    obsm: 'X_pca', 'X_umap'
    varm: 'PCs'
    obsp: 'connectivities', 'distances'
```

In [118…

```
library_sizes = np.sum(a_data.X, axis=1)
size_factors = library_sizes / np.median(library_sizes)
```

In [123…

```
encoder_louvain = LabelEncoder()
encoder_louvain.fit(a_data.obs['louvain'])

encoder_leiden = LabelEncoder()
encoder_leiden.fit(a_data.obs['leiden'])
```

Out[123…

```
LabelEncoder()
```

In a Jupyter environment, please rerun this cell to show the HTML representation or trust the notebook.
On GitHub, the HTML representation is unable to render, please try loading this page with nbviewer.org.

In [124…

```
encoders = {
    'obs': {
        'louvain': encoder_louvain.transform,
        'leiden': encoder_leiden.transform
    }
}
```

In [196…

```
import tensorflow as tf
from tensorflow.keras import layers, Model
import numpy as np

class CVAE(Model):
    def __init__(self, latent_dim, num_clusters, input_dim):
        super(CVAE, self).__init__()
        self.latent_dim = latent_dim
        self.num_clusters = num_clusters
        self.input_dim = input_dim
        self.encoder = self.build_encoder()
```

```
        self.decoder = self.build_decoder()

    def build_encoder(self):
        input_data = layers.Input(shape=(self.input_dim,))
        input_cluster = layers.Input(shape=(self.num_clusters,))
        x = layers.Concatenate()([input_data, input_cluster])
        x = layers.Dense(256, activation='relu')(x)
        z_mean = layers.Dense(self.latent_dim)(x)
        z_log_var = layers.Dense(self.latent_dim)(x)
        return Model(inputs=[input_data, input_cluster], outputs=[z_mean, z

    def build_decoder(self):
        latent_inputs = layers.Input(shape=(self.latent_dim,))
        input_cluster = layers.Input(shape=(self.num_clusters,))
        x = layers.Concatenate()([latent_inputs, input_cluster])
        x = layers.Dense(256, activation='relu')(x)
        output = layers.Dense(self.input_dim, activation='sigmoid')(x)
        return Model(inputs=[latent_inputs, input_cluster], outputs=output)

    def sample(self, z_mean, z_log_var):
        batch = tf.shape(z_mean)[0]
        dim = tf.shape(z_mean)[1]
        epsilon = tf.keras.backend.random_normal(shape=(batch, dim))
        return z_mean + tf.exp(0.5 * z_log_var) * epsilon

    def call(self, inputs):
        input_data, input_cluster = inputs
        z_mean, z_log_var = self.encoder([input_data, input_cluster])
```

```
        z = self.sample(z_mean, z_log_var)
        reconstructed = self.decoder([z, input_cluster])
        return reconstructed

data = np.array(x_data)
clusters = np.array(clusters_data)
size_factors = np.array(size_factors_data)

# Defining the dimensions
input_dim = data.shape[1]
latent_dim = 10
num_clusters = clusters.shape[1]

# Instantiating the CVAE model
cvae_model = CVAE(latent_dim, num_clusters, input_dim)

# optimizer
optimizer = tf.keras.optimizers.Adam()

#  loss function (e.g., Mean Squared Error)
mse_loss_fn = tf.keras.losses.MeanSquaredError()


# Compile
cvae_model.compile(optimizer=optimizer, loss=mse_loss_fn)

# Train
history = cvae_model.fit([data, clusters], data, epochs=20, batch_size=125)
```

```
Epoch 1/20
1/1 [==============================] - 1s 1s/step - loss: 0.9645
Epoch 2/20
1/1 [==============================] - 0s 23ms/step - loss: 0.8992
Epoch 3/20
1/1 [==============================] - 0s 31ms/step - loss: 0.8063
Epoch 4/20
1/1 [==============================] - 0s 18ms/step - loss: 0.6249
Epoch 5/20
1/1 [==============================] - 0s 21ms/step - loss: 0.7566
Epoch 6/20
1/1 [==============================] - 0s 19ms/step - loss: 0.4773
Epoch 7/20
1/1 [==============================] - 0s 20ms/step - loss: 0.4225
Epoch 8/20
1/1 [==============================] - 0s 16ms/step - loss: 0.6150
Epoch 9/20
1/1 [==============================] - 0s 18ms/step - loss: 0.3960
Epoch 10/20
1/1 [==============================] - 0s 18ms/step - loss: 0.5649
Epoch 11/20
1/1 [==============================] - 0s 18ms/step - loss: 0.3890
Epoch 12/20
1/1 [==============================] - 0s 19ms/step - loss: 0.5268
Epoch 13/20
1/1 [==============================] - 0s 20ms/step - loss: 0.3868
Epoch 14/20
1/1 [==============================] - 0s 17ms/step - loss: 0.3860
Epoch 15/20
```

```
1/1 [==============================] - 0s 19ms/step - loss: 0.4847
Epoch 16/20
1/1 [==============================] - 0s 17ms/step - loss: 0.3847
Epoch 17/20
1/1 [==============================] - 0s 20ms/step - loss: 0.3842
Epoch 18/20
1/1 [==============================] - 0s 17ms/step - loss: 0.3842
Epoch 19/20
1/1 [==============================] - 0s 20ms/step - loss: 0.4555
Epoch 20/20
1/1 [==============================] - 0s 17ms/step - loss: 0.3843
```