In [1]:
```
import scanpy as sc
import anndata as ad
import matplotlib.pyplot as plt
import celltypist
from celltypist import models
import pandas as pd
import numpy as np
import seaborn as sns
import matplotlib.pyplot as plt
```

Load in the samples

In [2]:
```
Case1_YF=sc.read_10x_mtx('samples/GSM5910784')
Case1_ZY=sc.read_10x_mtx('samples/GSM5910785')
Case2_ZC=sc.read_10x_mtx('samples/GSM5910786')
Case2_YF=sc.read_10x_mtx('samples/GSM5910787')
Case2_ZY=sc.read_10x_mtx('samples/GSM5910788')
Case3_YF=sc.read_10x_mtx('samples/GSM5910789')
Case3_ZY=sc.read_10x_mtx('samples/GSM5910790')
Case4_ZY=sc.read_10x_mtx('samples/GSM5910791')
```

In [3]:
```
# create dictionary of samples for easy mapping

samples_dict = {
    "Case1_YF": Case1_YF,
    "Case1_ZY": Case1_ZY,
    "Case2_ZC": Case2_ZC,
    "Case2_YF": Case2_YF,
    "Case2_ZY": Case2_ZY,
    "Case3_YF": Case3_YF,
    "Case3_ZY": Case3_ZY,
    "Case4_ZY": Case4_ZY
}
```

Calculate pre filtering counts

In [4]:
```
before_counts = {}
before_genes = {}
for sample_name, ad in samples_dict.items():
    before_counts[sample_name] = ad.n_obs  # number of cells before fi
    before_genes[sample_name] = ad.n_vars # number of genes before fil
```

Quality Control

This quality control step identifies and flags genes associated with mitochondrial activity (MT-), ribosomal proteins (RPS, RPL), and hemoglobin (HB excluding pseudogenes) for each sample. Using these flags,

scanpy.pp.calculate_qc_metrics computes per-cell metrics such as the percentage of total counts derived from these gene categories, which are essential for filtering out low-quality or stressed cells during preprocessing.

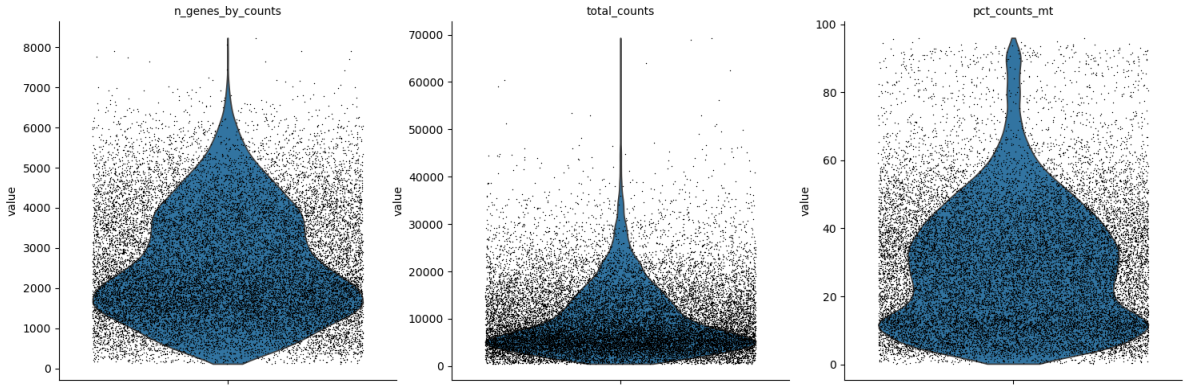
In [5]:
```
for sample in samples_dict.values():
    sample.var["mt"] = sample.var_names.str.startswith("MT-")
    sample.var["ribo"] = sample.var_names.str.startswith(("RPS", "RPL"
    sample.var["hb"] = sample.var_names.str.contains("^HB[^(P)]")
    sc.pp.calculate_qc_metrics(sample, qc_vars=["mt", "ribo", "hb"], i
```

Inspect violin plots of some of the computed QC metrics:

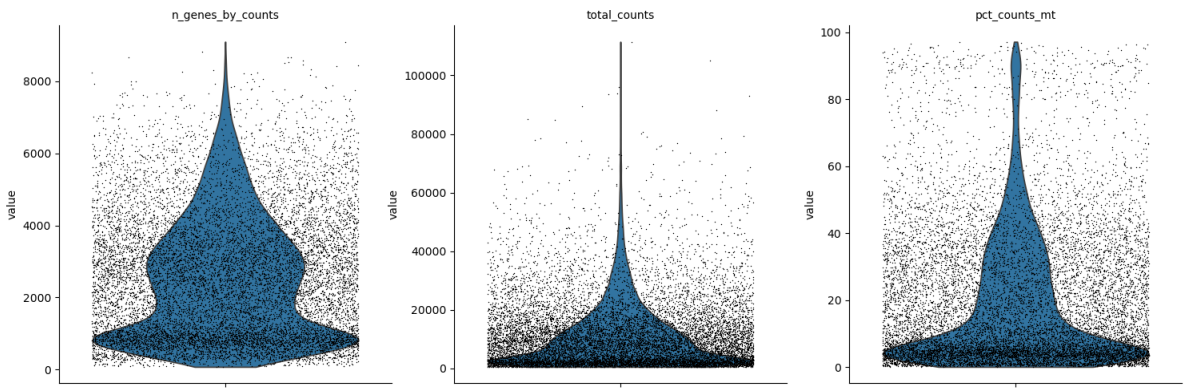
the number of genes expressed in the count matrix, the total counts per cell, the percentage of counts in mitochondrial genes

In [6]:
```
for sample_name, sample in samples_dict.items():
    print(f"Generating QC violin plot for {sample_name}")
    sc.pl.violin(
        sample,
        ["n_genes_by_counts", "total_counts", "pct_counts_mt"],
        jitter=0.4,
        multi_panel=True,
    )
```

```
... storing 'feature_types' as categorical
Generating QC violin plot for Case1_YF
```

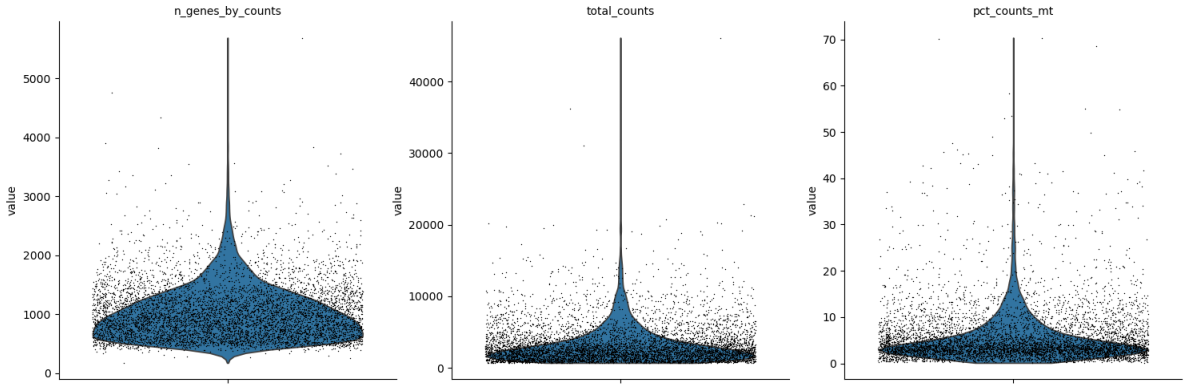



```
... storing 'feature_types' as categorical
Generating QC violin plot for Case1_ZY
```



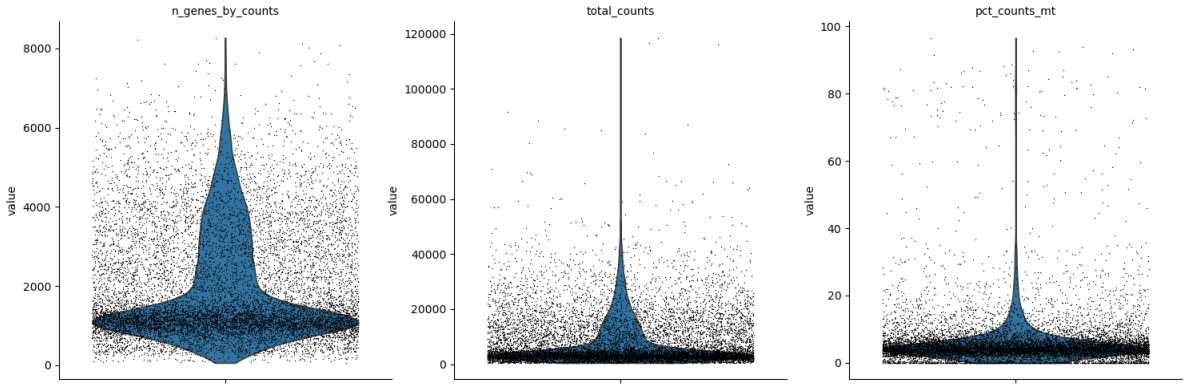
```
... storing 'feature_types' as categorical
```

```
Generating QC violin plot for Case2_ZC
```



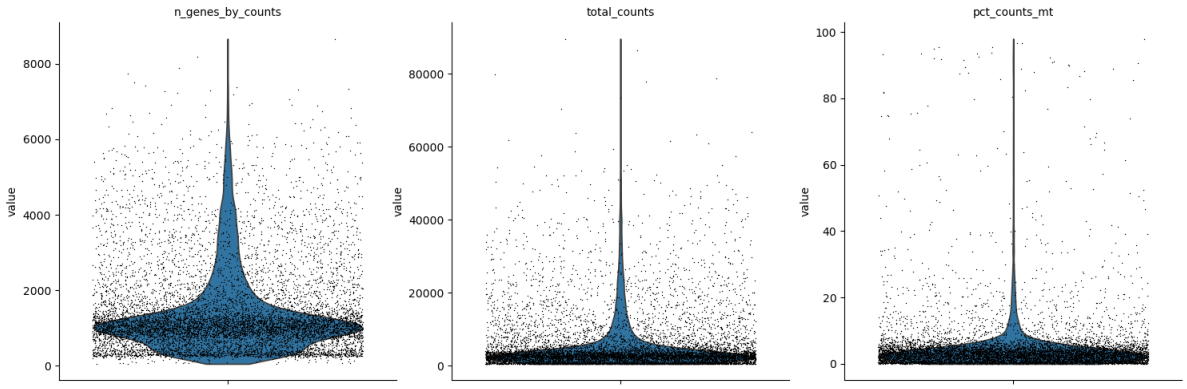

```
... storing 'feature_types' as categorical
```

```
Generating QC violin plot for Case2_YF
```



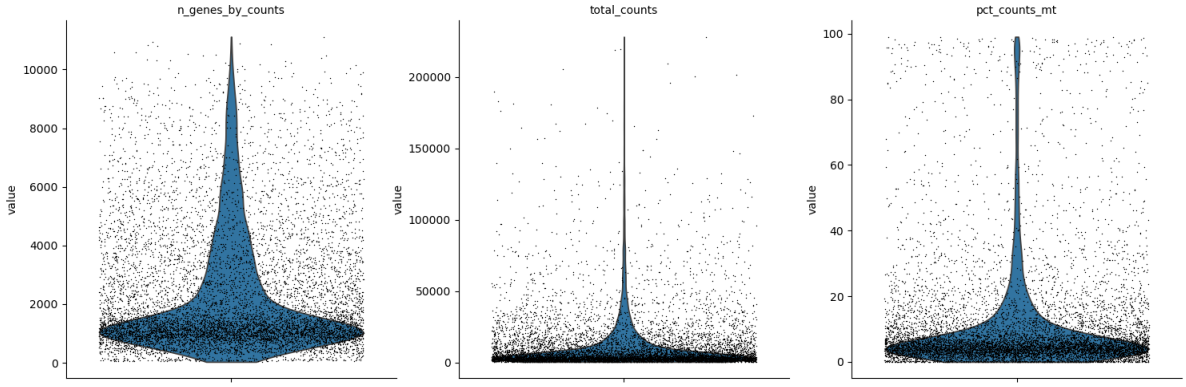

```
... storing 'feature_types' as categorical
```

```
Generating QC violin plot for Case2_ZY
```



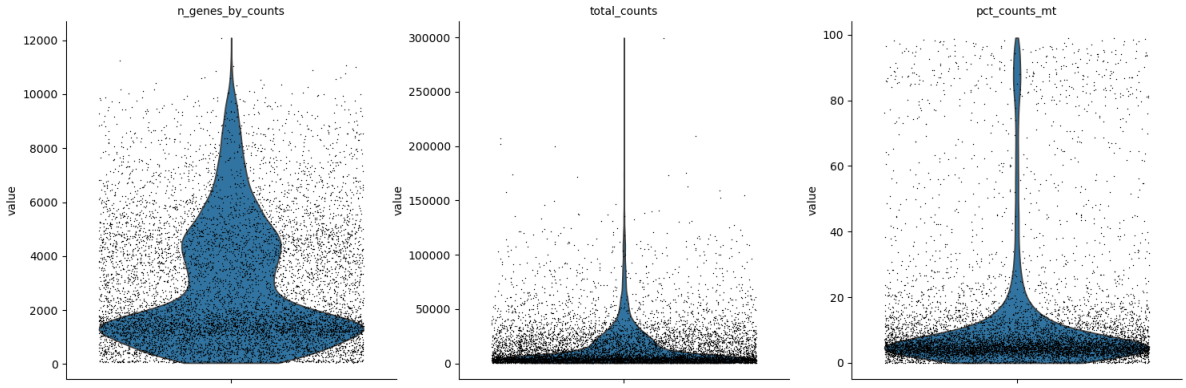

```
... storing 'feature_types' as categorical
```

```
Generating QC violin plot for Case3_YF
```



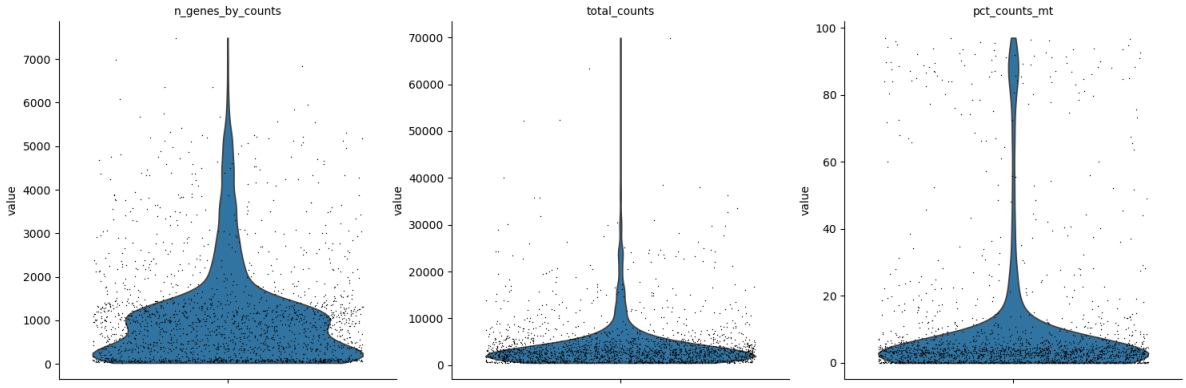

```
... storing 'feature_types' as categorical
```

```
Generating QC violin plot for Case3_ZY
```



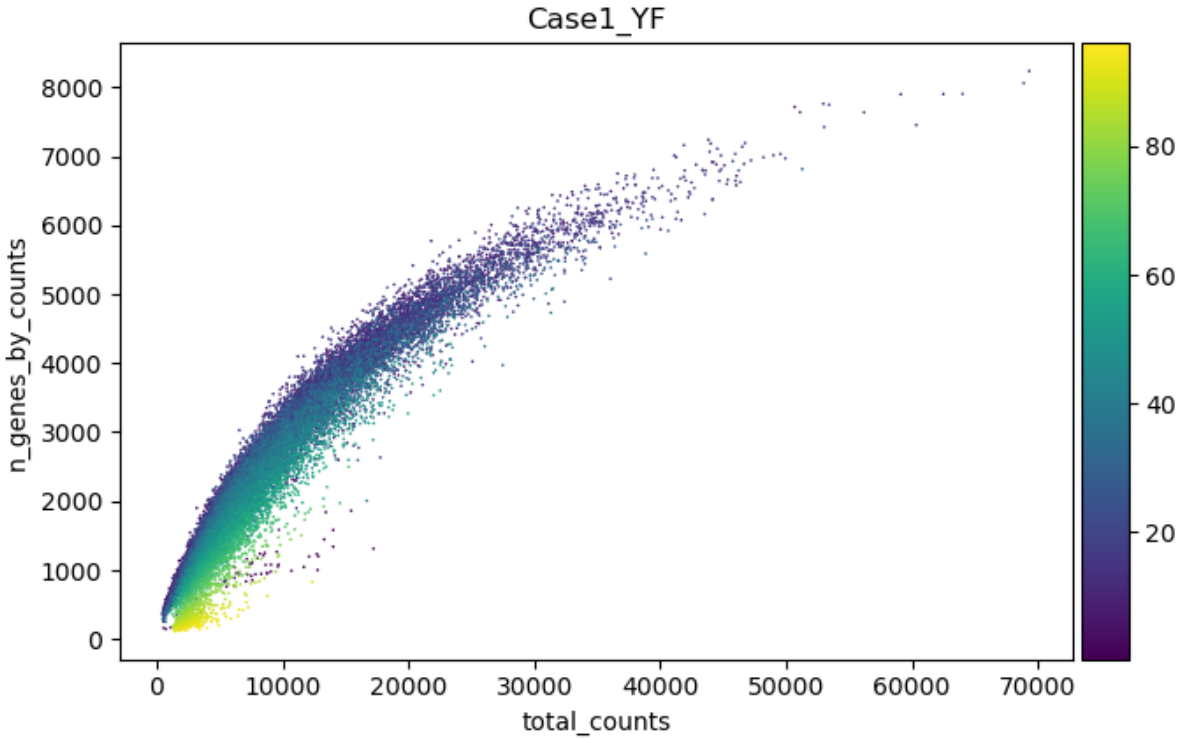

```
... storing 'feature_types' as categorical
```

```
Generating QC violin plot for Case4_ZY
```

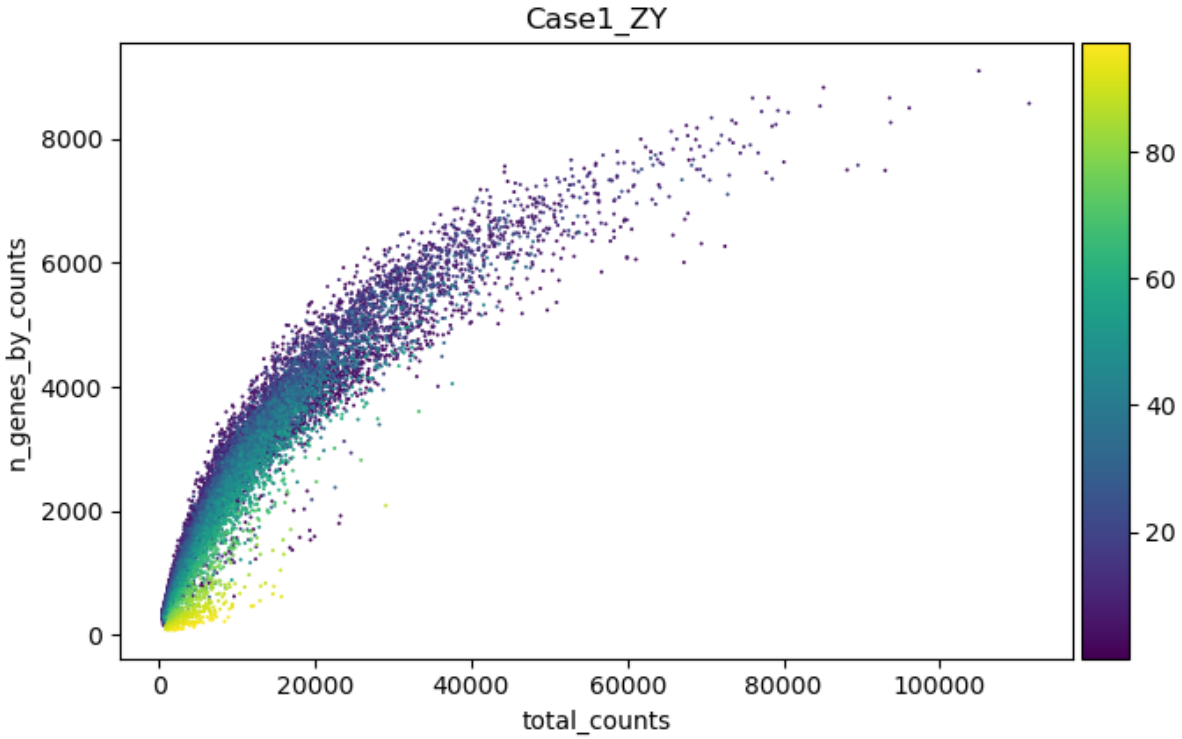


In [7]:
```python
for sample_name, sample in samples_dict.items():
    print(f"Generating scatter plot for {sample_name}")
    sc.pl.scatter(
        sample,
        x="total_counts",
        y="n_genes_by_counts",
        color="pct_counts_mt",
        title=sample_name,
        show=True
    )
```

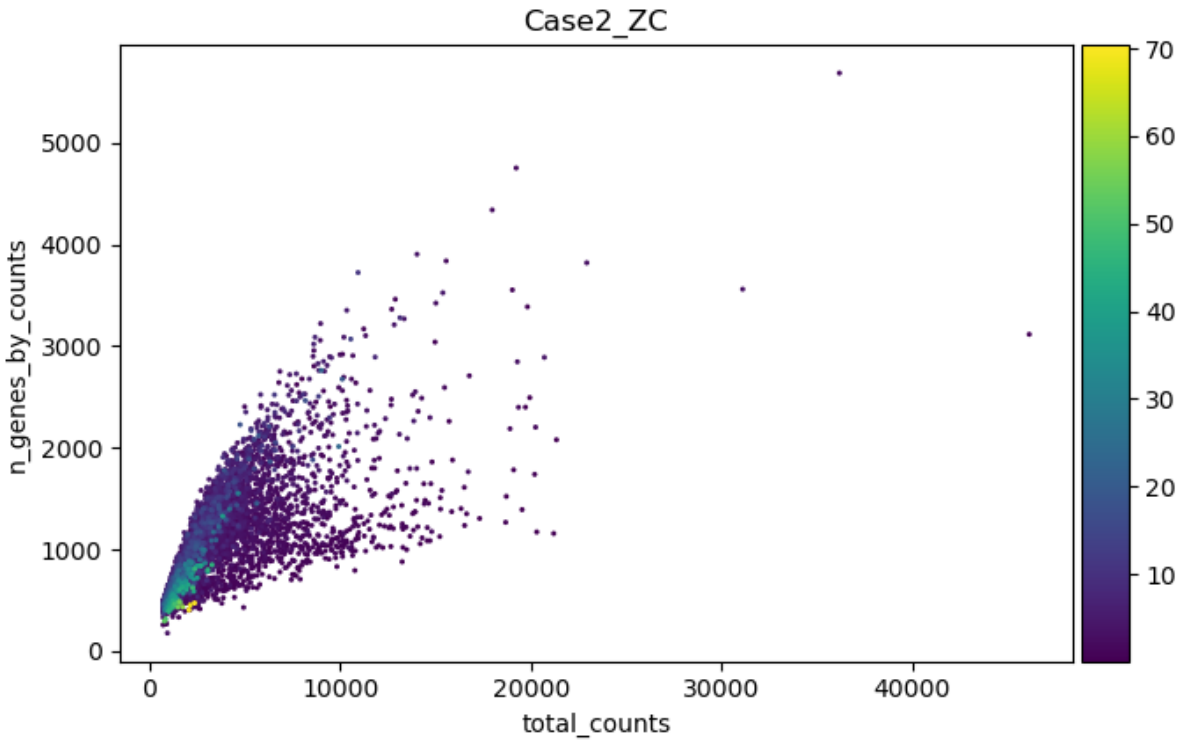
```
Generating scatter plot for Case1_YF
```



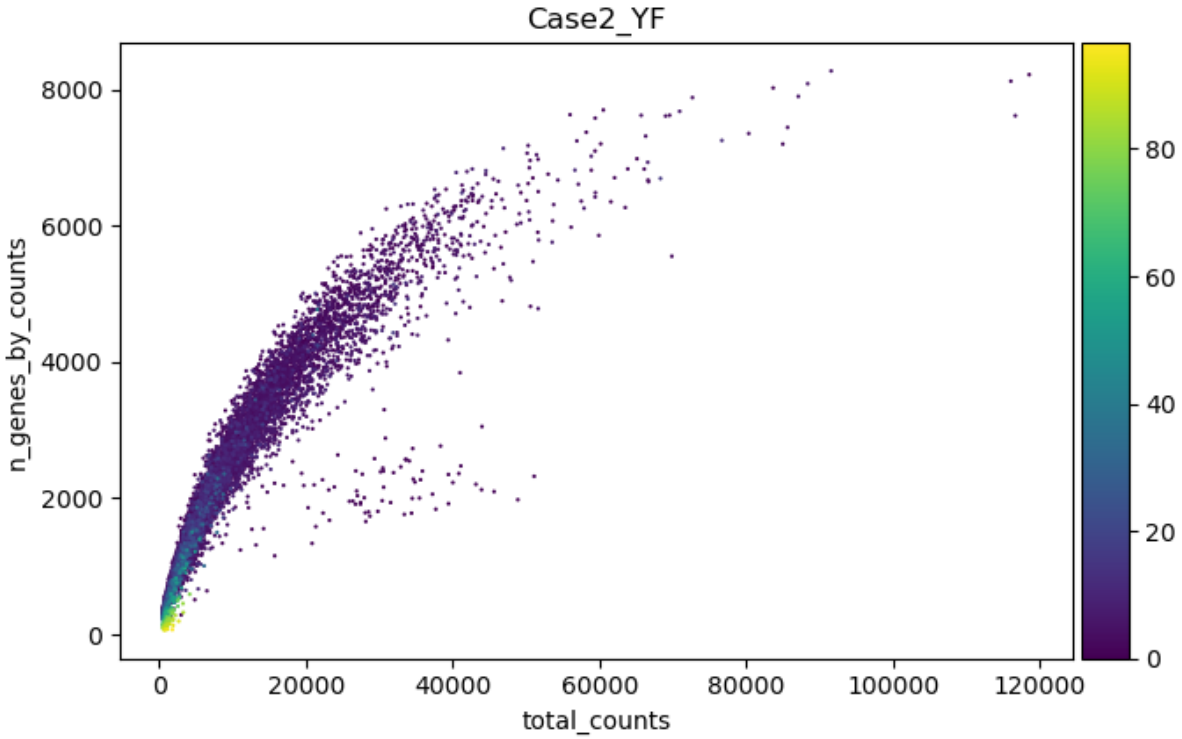

```
Generating scatter plot for Case1_ZY
```



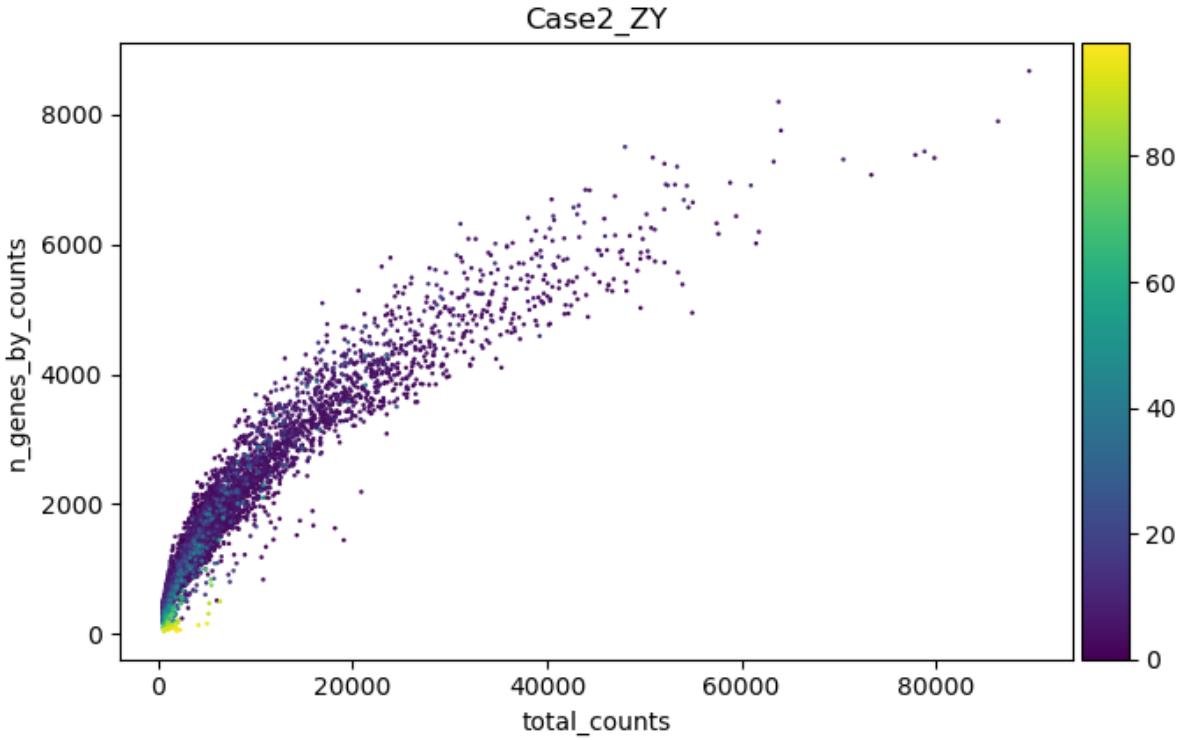

```
Generating scatter plot for Case2_ZC
```



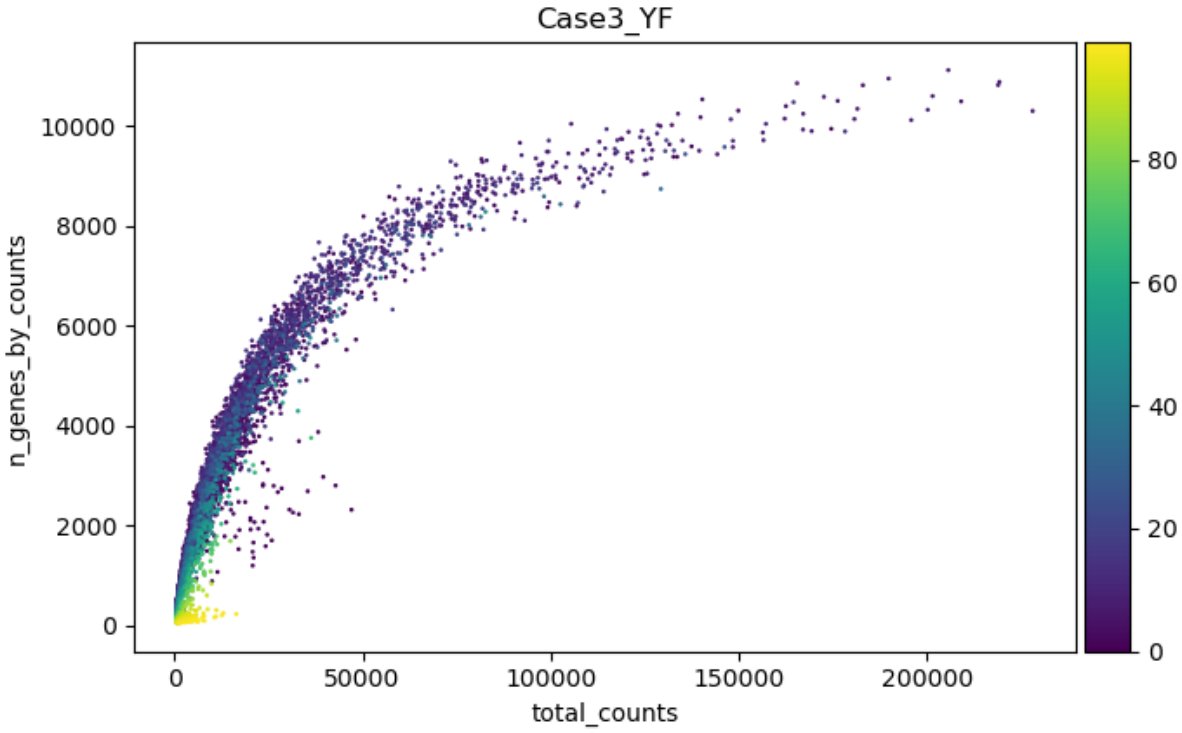

```
Generating scatter plot for Case2_YF
```



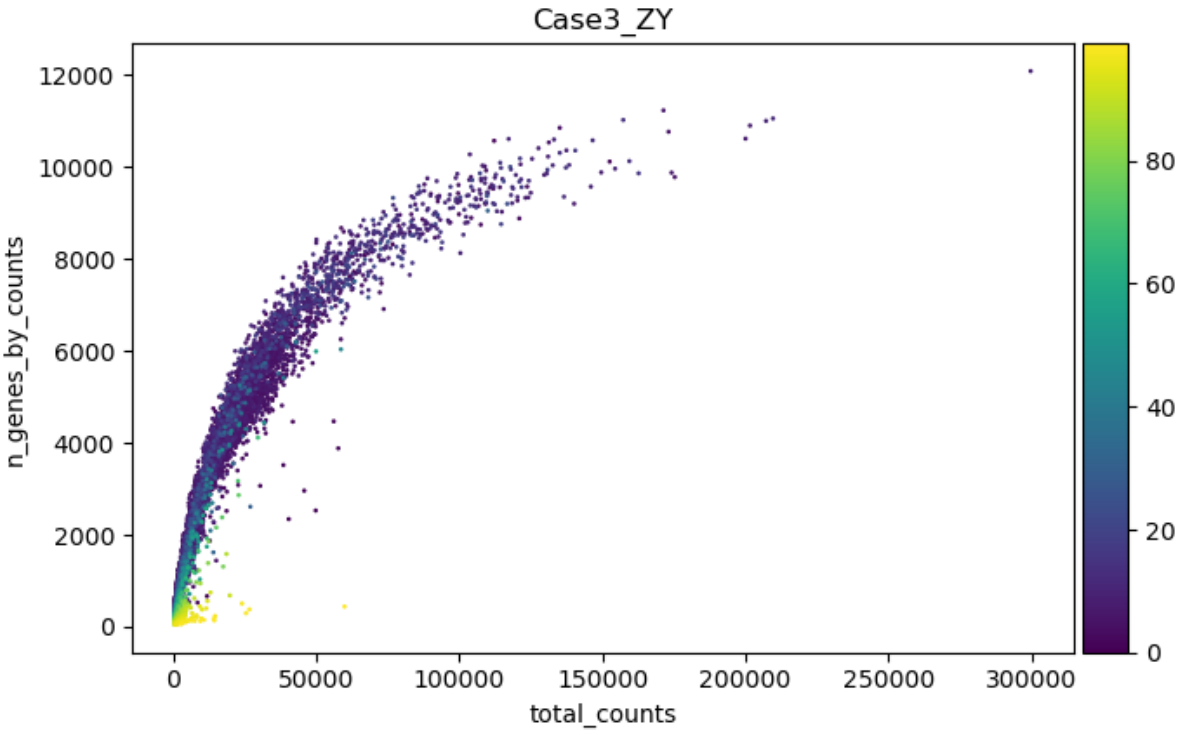

```
Generating scatter plot for Case2_ZY
```



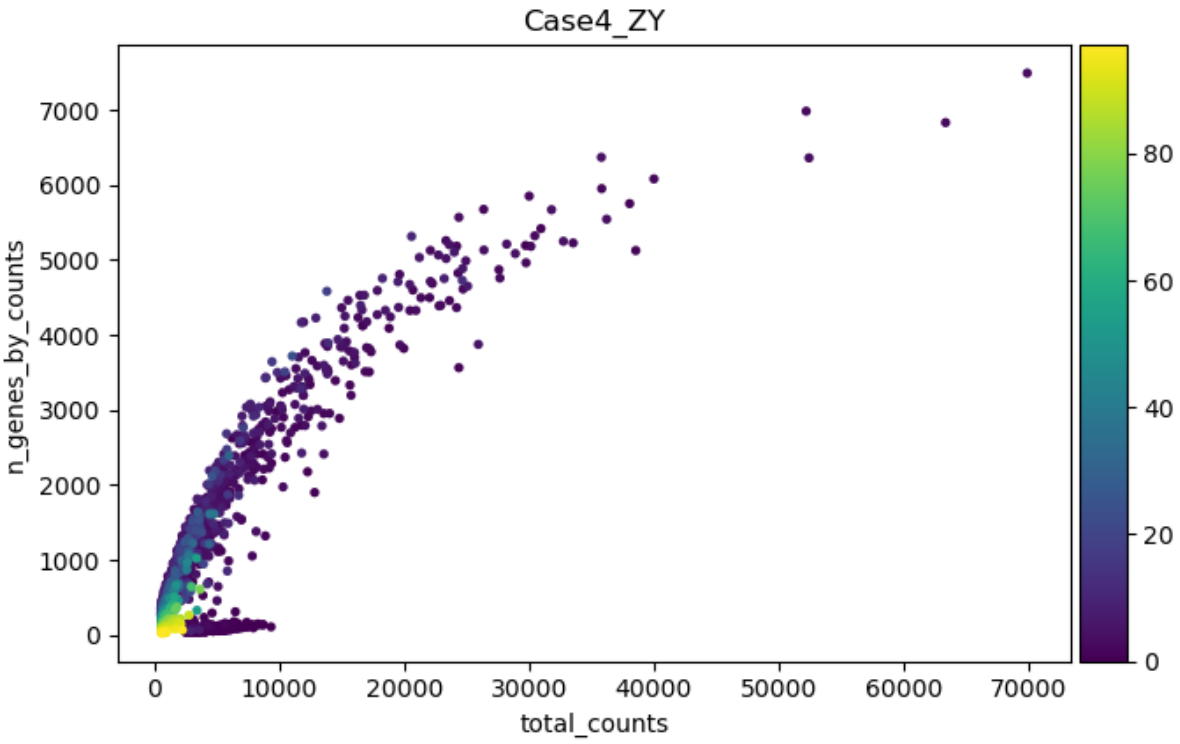

```
Generating scatter plot for Case3_YF
```




```
Generating scatter plot for Case3_ZY
```




```
Generating scatter plot for Case4_ZY
```



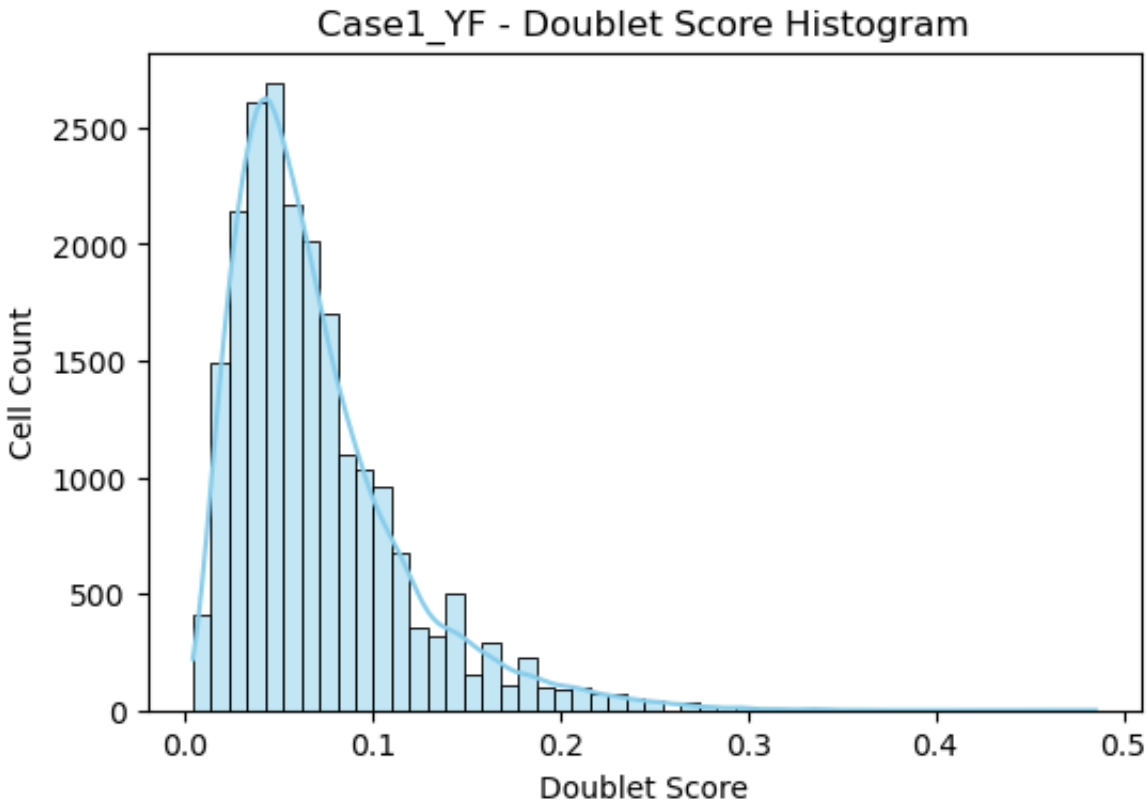
These scatter plots visualizes the relationship between the total number of molecules detected (total_counts) and the number of unique genes (n_genes_by_counts) for each cell in the dataset. Points are colored by the percentage of reads that map to mitochondrial genes (pct_counts_mt). This integrated view helps identify low-quality cells, doublets, or cells under stress.

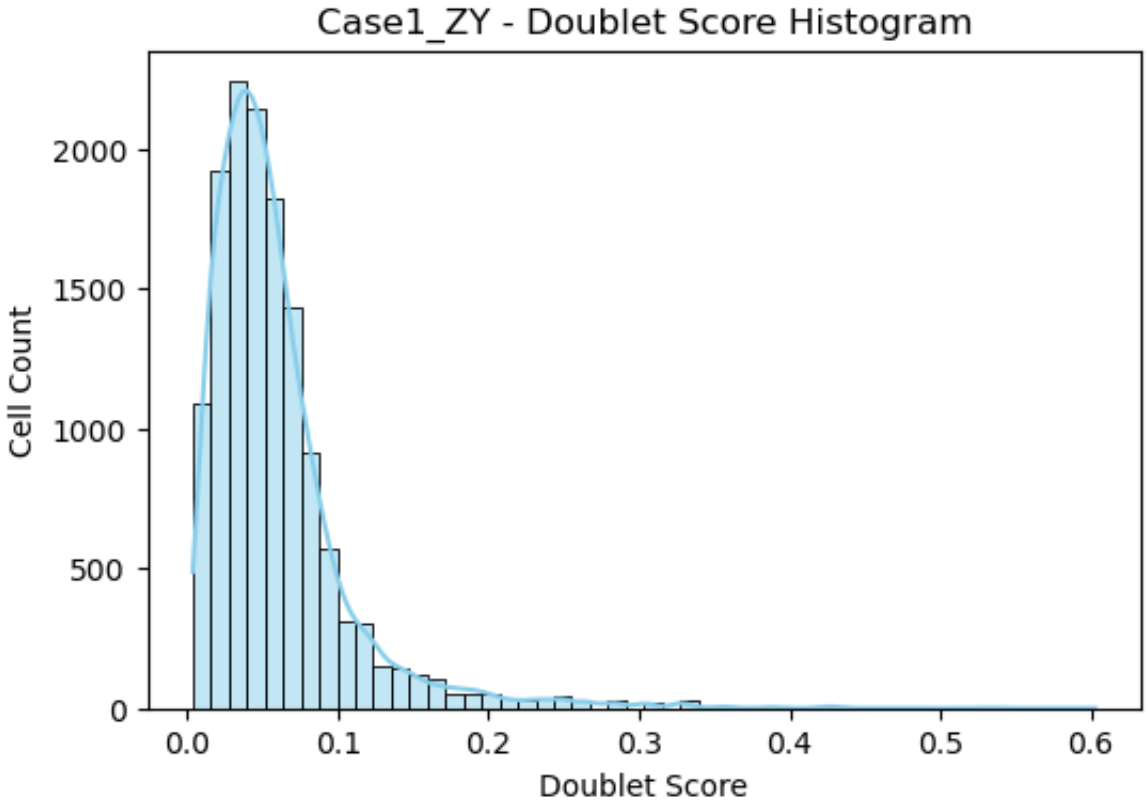
Filter samples by a minimum of 100 genes and a minimum of 3 cells

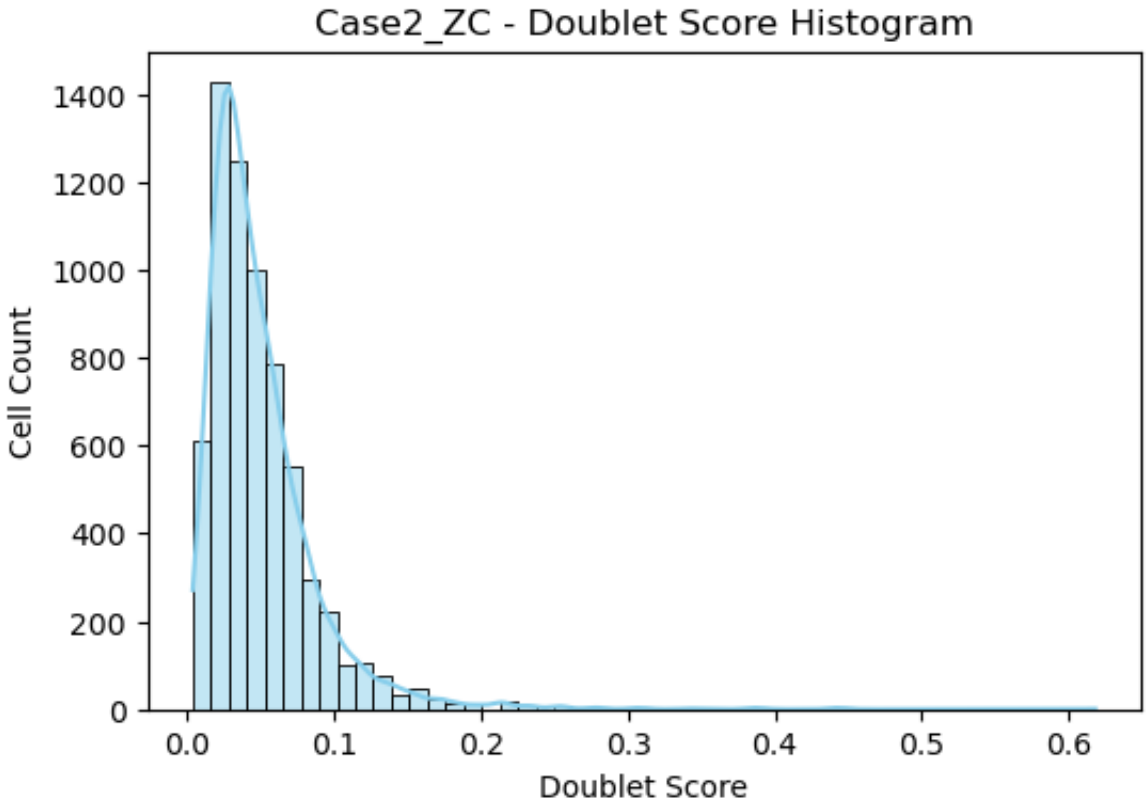
In [8]:
```
for sample_name, sample in samples_dict.items():
    sc.pp.filter_cells(sample, min_genes=100)
    sc.pp.filter_genes(sample, min_cells=3)
```

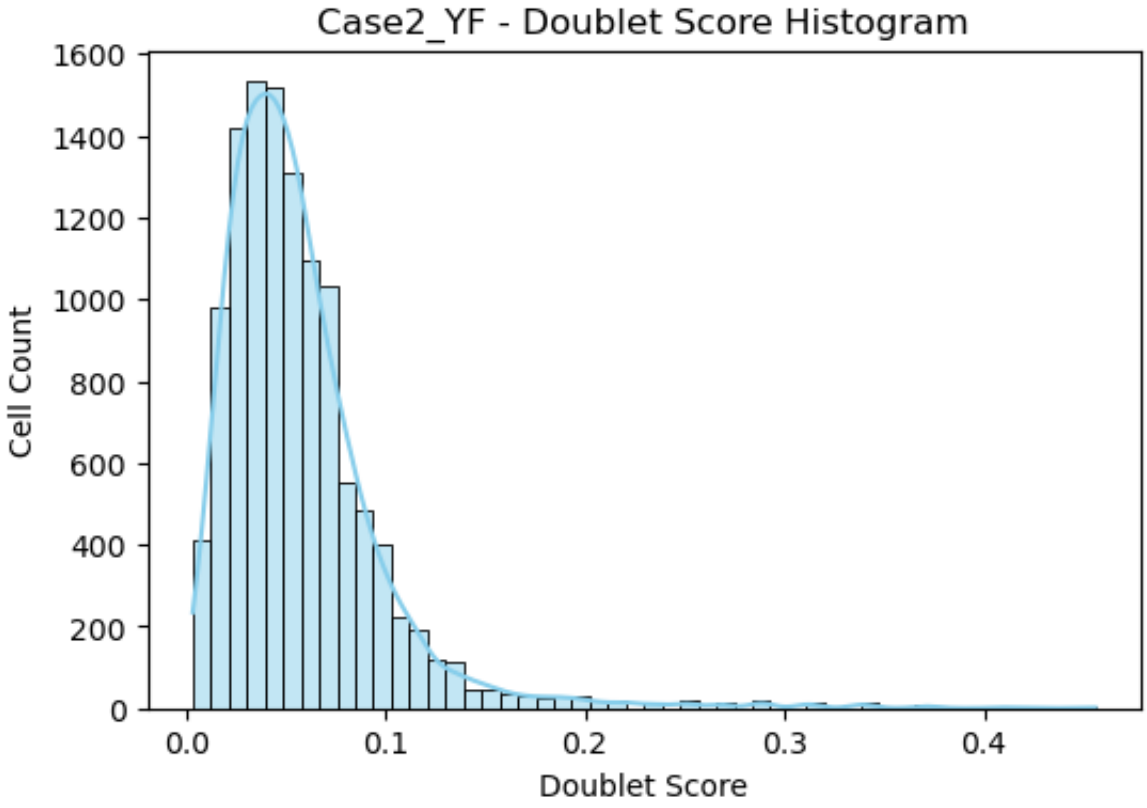
Perform scrublet and display doublet detection results

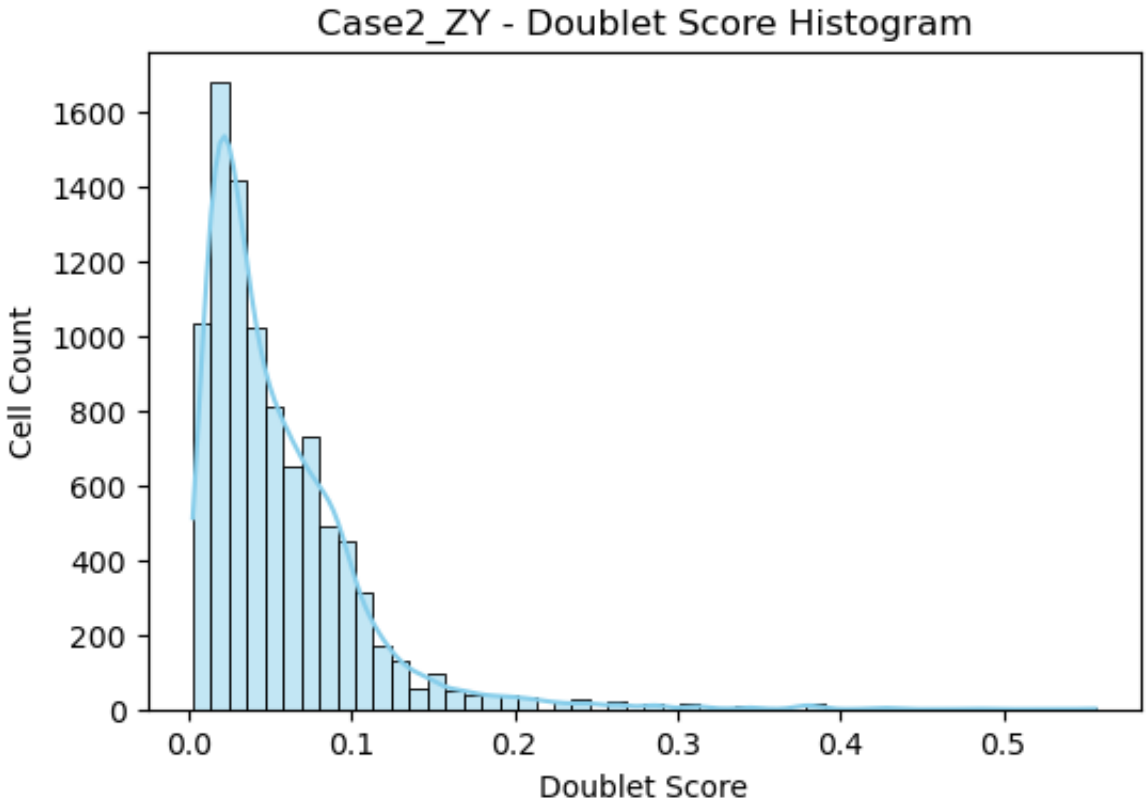
In [9]:
```
for sample_name, sample in samples_dict.items():
    sc.pp.scrublet(sample)

    # Create histogram to display doublet results
    plt.figure(figsize=(6, 4))
    sns.histplot(sample.obs['doublet_score'], bins=50, color='skyblue'
    plt.title(f'{sample_name} - Doublet Score Histogram')
    plt.xlabel('Doublet Score')
    plt.ylabel('Cell Count')
    plt.show()
```

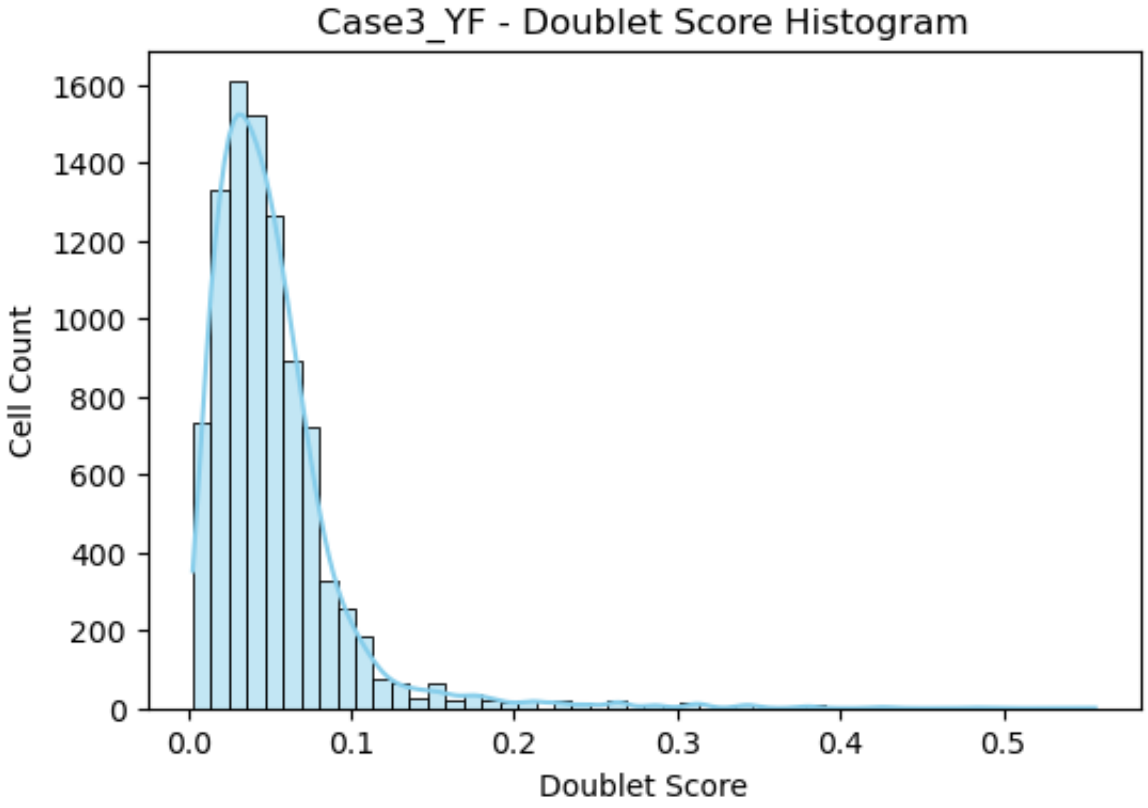


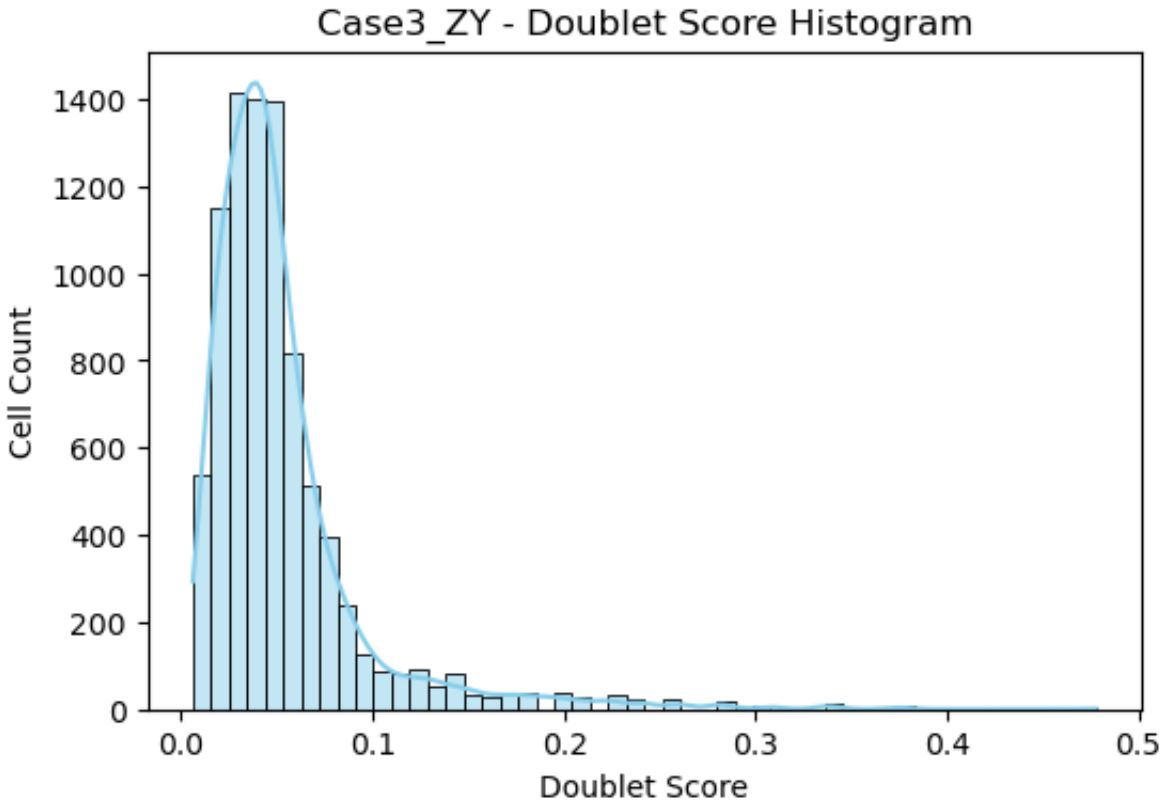

Case1_ZY - Doublet Score Histogram
Cell Count
Doublet Score
0
500
1000
1500
2000
0.0
0.1
0.2
0.3
0.4
0.5
0.6

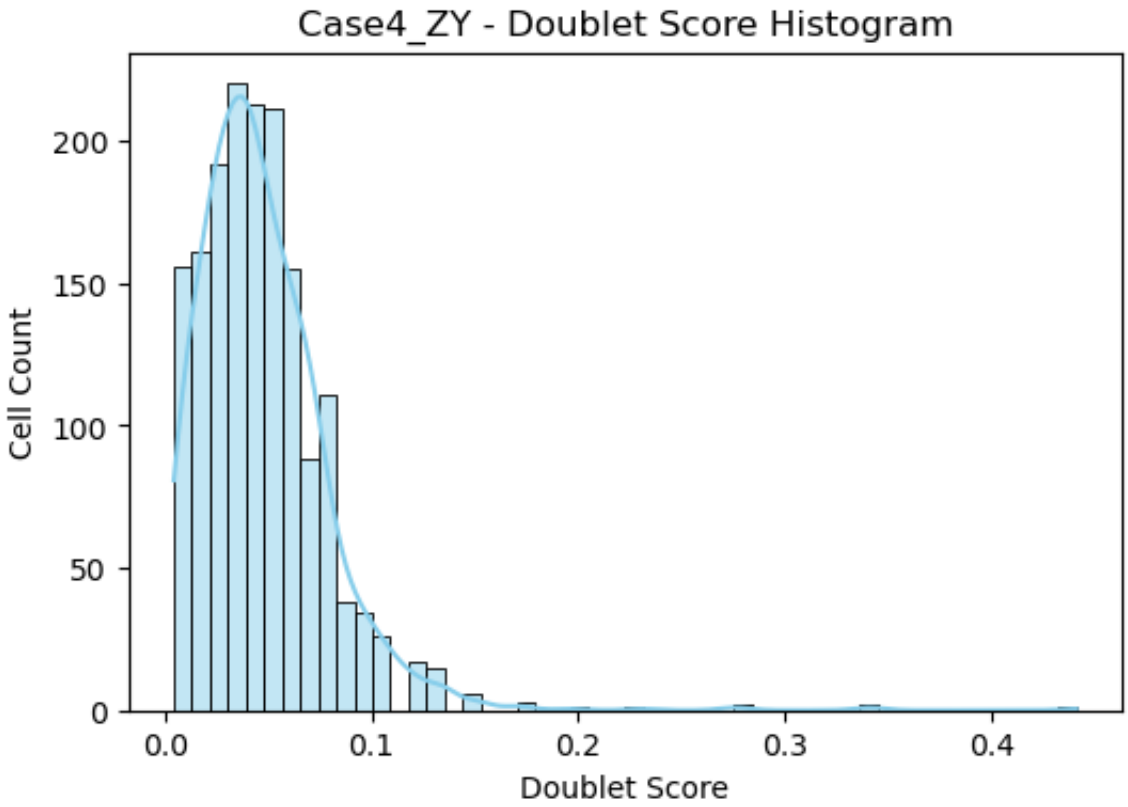

Case2_ZC - Doublet Score Histogram
Cell Count
Doublet Score
0
200
400
600
800
1000
1200
1400
0.0
0.1
0.2
0.3
0.4
0.5
0.6


Case2_YF - Doublet Score Histogram
Cell Count
Doublet Score
0
200
400
600
800
1000
1200
1400
1600
0.0
0.1
0.2
0.3
0.4


Case2_ZY - Doublet Score Histogram
Cell Count
Doublet Score
0
200
400
600
800
1000
1200
1400
1600
0.0
0.1
0.2
0.3
0.4
0.5


Case3_YF - Doublet Score Histogram
Cell Count
Doublet Score
0
200
400
600
800
1000
1200
1400
1600
0.0
0.1
0.2
0.3
0.4
0.5


Case3_ZY - Doublet Score Histogram
Cell Count
Doublet Score
0
200
400
600
800
1000
1200
1400
0.0
0.1
0.2
0.3
0.4
0.5



In [10]:
```
after_counts = {}
after_genes = {}
for sample_name, ad in samples_dict.items():
    # Save filtered cell count
    after_counts[sample_name] = ad.n_obs
    after_genes[sample_name] = sample.n_vars
```

In [11]:
```
# Create a combined summary DataFrame for cells and genes
summary = []

# Totals
total_cells_before = total_cells_after = 0
total_genes_before = total_genes_after = 0

for sample in before_counts:
    # Cells
    cbefore = before_counts[sample]
    cafter  = after_counts.get(sample, 0)
    cdiff   = cbefore - cafter
    cperc   = (cafter / cbefore) * 100 if cbefore > 0 else 0

    # Genes
    gbefore = before_genes[sample]
    gafter  = after_genes.get(sample, 0)
    gdiff   = gbefore - gafter
    gperc   = (gafter / gbefore) * 100 if gbefore > 0 else 0

    # Accumulate totals
```

```
    total_cells_before += cbefore
    total_cells_after  += cafter
    total_genes_before += gbefore
    total_genes_after  += gafter

    summary.append({
        "Sample":            sample,
        "Cells Before":      cbefore,
        "Cells After":       cafter,
        "Cells Removed":     cdiff,
        "% Cells Retained":  f"{cperc:.1f}%",
        "Genes Before":      gbefore,
        "Genes After":       gafter,
        "Genes Removed":     gdiff,
        "% Genes Retained":  f"{gperc:.1f}%"
    })

# Add a total row
total_cdiff = total_cells_before - total_cells_after
total_cperc = (total_cells_after / total_cells_before) * 100 if total_
total_gdiff = total_genes_before - total_genes_after
total_gperc = (total_genes_after / total_genes_before) * 100 if total_

summary.append({
    "Sample":            "Total",
    "Cells Before":      total_cells_before,
    "Cells After":       total_cells_after,
    "Cells Removed":     total_cdiff,
    "% Cells Retained":  f"{total_cperc:.1f}%",
    "Genes Before":      total_genes_before,
    "Genes After":       total_genes_after,
    "Genes Removed":     total_gdiff,
    "% Genes Retained":  f"{total_gperc:.1f}%"
})

# Convert to DataFrame and sort so 'Total' is last
filter_summary_df = (
    pd.DataFrame(summary)
      .sort_values(by="Sample",
                   key=lambda x: x.map(lambda y: (y != "Total", y)))
)

filter_summary_df
```

Out[11]:

	Sample	Cells Before	Cells After	Cells Removed	% Cells Retained	Genes Before	Genes After	Genes Removed	F
8	Total	83283	82648	635	99.2%	268304	131984	136320	
0	Case1_YF	21560	21560	0	100.0%	33538	16498	17040	
1	Case1_ZY	13685	13672	13	99.9%	33538	16498	17040	
3	Case2_YF	11786	11774	12	99.9%	33538	16498	17040	
2	Case2_ZC	6606	6606	0	100.0%	33538	16498	17040	
4	Case2_ZY	9406	9388	18	99.8%	33538	16498	17040	
5	Case3_YF	9379	9291	88	99.1%	33538	16498	17040	
6	Case3_ZY	8837	8704	133	98.5%	33538	16498	17040	
7	Case4_ZY	2024	1653	371	81.7%	33538	16498	17040	

Preprocessing Discussion

What filtering thresholds did you choose and how did you decide on them?:

Cells were filtered to retain only those with at least 100 detected genes, which helps remove low-quality cells or empty droplets. Additionally, genes were required to be expressed in at least three cells to avoid including extremely rare transcripts that could introduce noise. Doublets were identified using the Scrublet algorithm. These thresholds were chosen based on standard practices established in the single-cell RNA-seq literature

How many cells / genes are present before and after implementing your filtering thresholds?

Before applying quality control filters, the dataset consisted of approximately 83,283 cells and 33,538 genes. After implementing the filtering thresholds, which included removing low-quality cells, extremely rare genes, and predicted doublets, the dataset was reduced to approximately 82,648 cells and 16,498 genes. This filtering process primarily eliminated low-quality observations while retaining the vast majority of biologically informative cells for downstream analysis.

Look in the literature, what are some potential strategies to set thresholds that don't rely on visual inspection of plots?

In addition to visual inspection of QC plots, several data-driven strategies exist for setting filtering thresholds in single-cell RNA-seq analysis. Statistical approaches, such as removing cells outside 1.5× the interquartile range for key metrics (e.g., gene counts, total counts, mitochondrial percentage), can be applied systematically. Model-based methods, including Gaussian Mixture Models, can probabilistically distinguish high- from low-quality cells. Automated tools like scater, Scrublet, or CellBender also provide thresholding without manual visual inspection. These approaches help improve reproducibility and minimize subjective bias in quality control.

Combine both samples together using Anndata concat

In [12]:
```
import anndata as ad  # make sure you have this

adata = ad.concat(
    list(samples_dict.values()),
    join='outer',
    label='sample',
    keys=list(samples_dict.keys())
)
adata_celltypist = adata.copy()
```

In [13]:
```
adata
```

Out[13]:
```
AnnData object with n_obs × n_vars = 82648 × 25875
    obs: 'n_genes_by_counts', 'log1p_n_genes_by_counts', 'total_counts', 'log1p_total_counts', 'pct_counts_in_top_50_genes', 'pct_counts_in_top_100_genes', 'pct_counts_in_top_200_genes', 'pct_counts_in_top_500_genes', 'total_counts_mt', 'log1p_total_counts_mt', 'pct_counts_mt', 'total_counts_ribo', 'log1p_total_counts_ribo', 'pct_counts_ribo', 'total_counts_hb', 'log1p_total_counts_hb', 'pct_counts_hb', 'n_genes', 'doublet_score', 'predicted_doublet', 'sample'
```

BREAK THIS where the fix is supposed to go

Count Normalization

For count normalization, I applied total count normalization followed by log-transformation, following best practices from the Scanpy workflow and general scRNA-seq processing guidelines. First, the raw counts were normalized to a common median total count per cell using sc.pp.normalize_total(). This step accounts for differences in sequencing depth between cells, ensuring that comparisons across cells are meaningful. I then applied a log1p transformation using sc.pp.log1p() to stabilize variance across the dynamic range of expression values. This normalization strategy is widely used because it is simple, interpretable, and preserves the biological structure of the data while mitigating the influence of highly expressed genes. Additionally, saving the raw counts in adata.layers["counts"] ensures that untransformed data remain accessible for downstream analyses, such as differential expression testing.

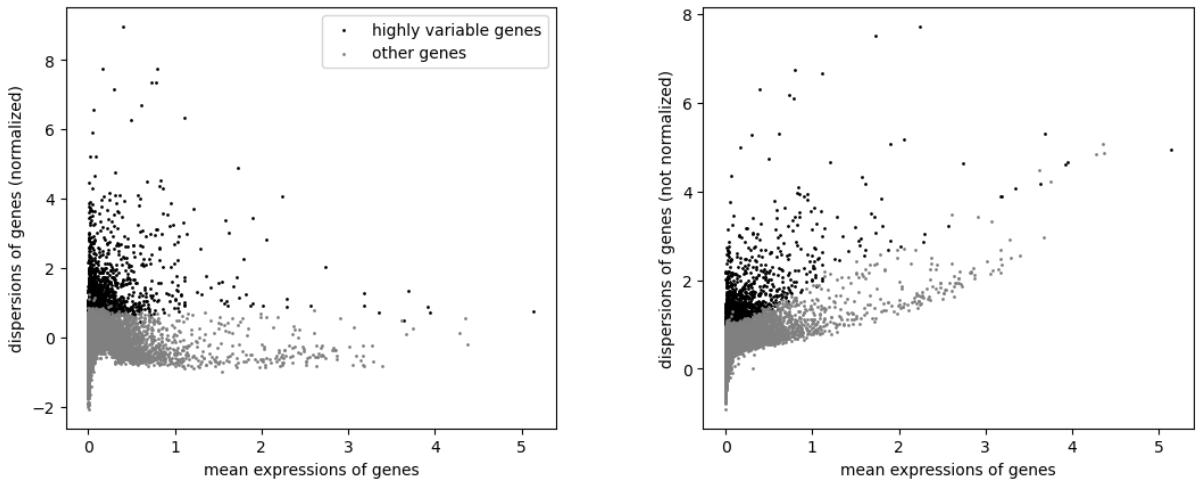
In [14]:
```
# Saving count data
adata.layers["counts"] = adata.X.copy()
```

In [15]:
```
# Normalizing to median total counts
sc.pp.normalize_total(adata)
# Logarithmize the data
sc.pp.log1p(adata)
```

Feature Selection

In [16]:
```
sc.pp.highly_variable_genes(adata, n_top_genes=2000, batch_key="sample
```

In [17]:
```
sc.pl.highly_variable_genes(adata)
```



Determine number of highly variable genes

In [18]:
```
n_hvg = np.sum(adata.var["highly_variable"])
n_not_hvg = np.sum(~adata.var["highly_variable"])

print(f"Highly variable genes: {n_hvg}")
print(f"Not highly variable genes: {n_not_hvg}")
```

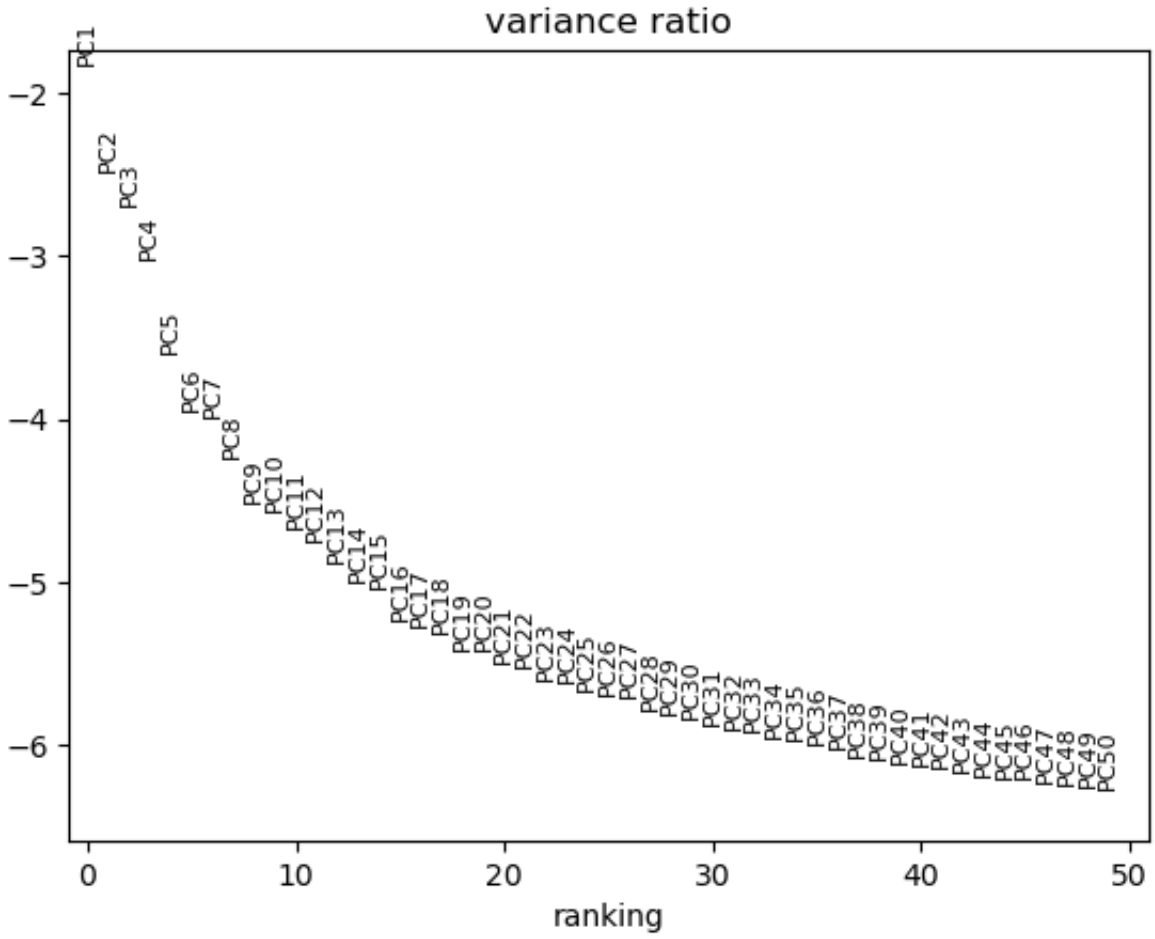
```
Highly variable genes: 2000
Not highly variable genes: 23875
```

Feature Selection Discussion

For feature selection, I used Scanpy's highly_variable_genes function to identify the most informative genes across all cells. I selected the top 2,000 highly variable genes (HVGs) while correcting for batch effects using the batch_key="sample" parameter to account for differences across samples. This method is based on calculating the mean and dispersion (variance) of each gene, selecting genes that are highly variable relative to their mean expression. Out of a total of approximately 27,183 genes, 2,000 were selected as highly variable features for downstream dimensionality reduction. The remaining genes were considered less informative for capturing biological variation in the dataset.

PCA

In [19]:
```
sc.tl.pca(adata)
sc.pl.pca_variance_ratio(adata, n_pcs=50, log=True)
```



Justification for 40 principal components

After performing principal component analysis (PCA) on the highly variable genes, I evaluated the variance explained by the first 50 principal components using a log-transformed variance ratio plot. The plot showed a sharp decline in variance explained across the first several PCs, with the curve beginning to flatten around PC 40. Based on the location of the “elbow” and the goal of capturing as much biological signal as possible without introducing excessive noise, I chose to retain the first 40 PCs for downstream analyses. This choice ensures that meaningful variability in the dataset is preserved while minimizing the inclusion of technical noise. The variance explained beyond PC 40 was minimal, justifying the cutoff.

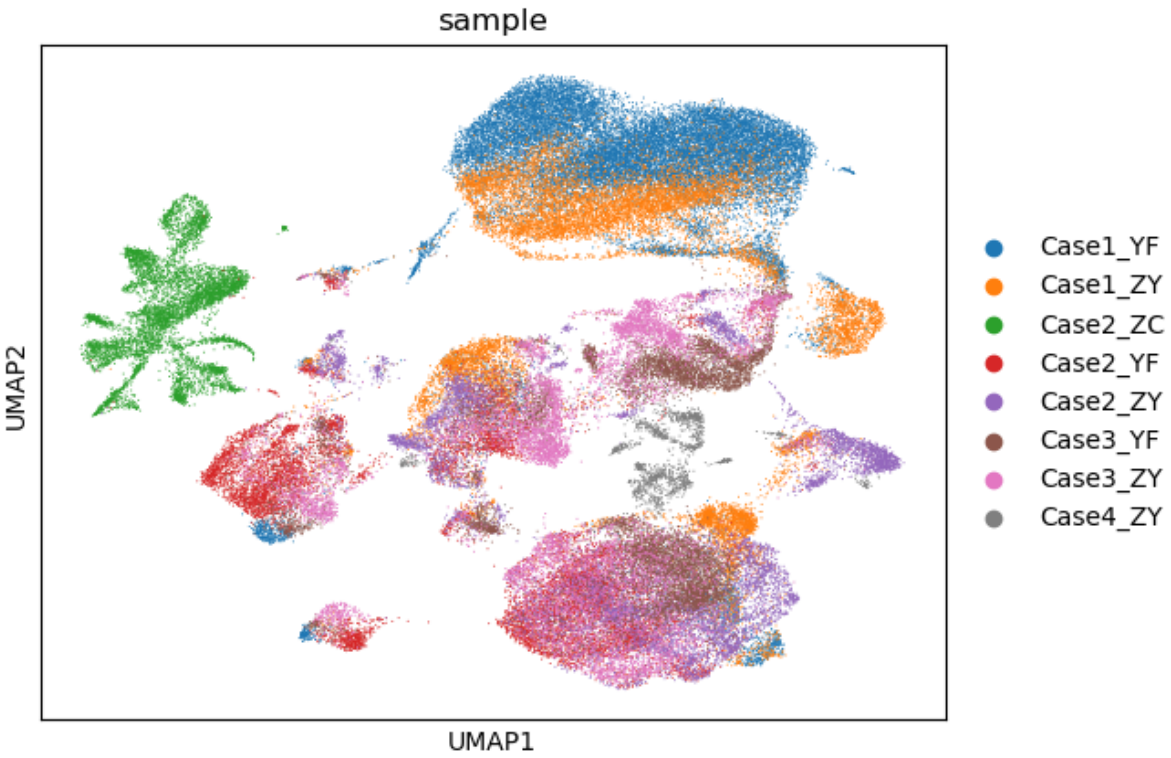
Nearest neighbor graph constuction and visualization

In [20]:
```
sc.pp.neighbors(adata, n_pcs=40)
sc.tl.umap(adata)
```

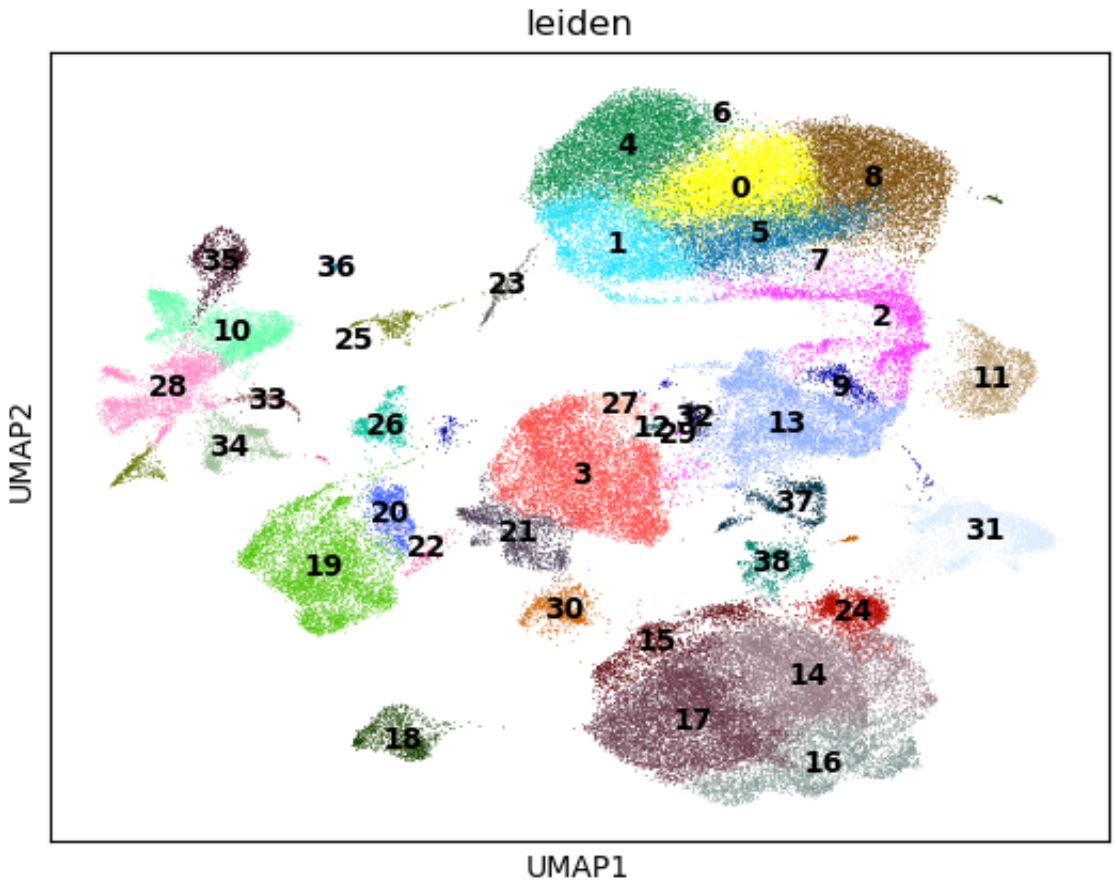
```
/projectnb/bf528/students/npetruni/.conda/envs/finalproj_scrnaseq/lib/python3.12/site-packages/tqdm/auto.py:21: TqdmWarning: IProgress not found. Please update jupyter and ipywidgets. See https://ipywidgets.readthedocs.io/en/stable/user_install.html
  from .autonotebook import tqdm as notebook_tqdm
```

Clustering

In [21]:
```
sc.pl.umap(
    adata,
    color="sample",
    # Setting a smaller point size to get prevent overlap
    size=2,
)
```



In [22]:
```
# Using the igraph implementation and a fixed number of iterations can
sc.tl.leiden(adata, flavor="igraph", n_iterations=2)
sc.pl.umap(adata, color=["leiden"], legend_loc='on data')
```



In [23]:
```
n_clusters = adata.obs["leiden"].nunique()
print(f"Number of clusters: {n_clusters}")
```

```
Number of clusters: 39
```

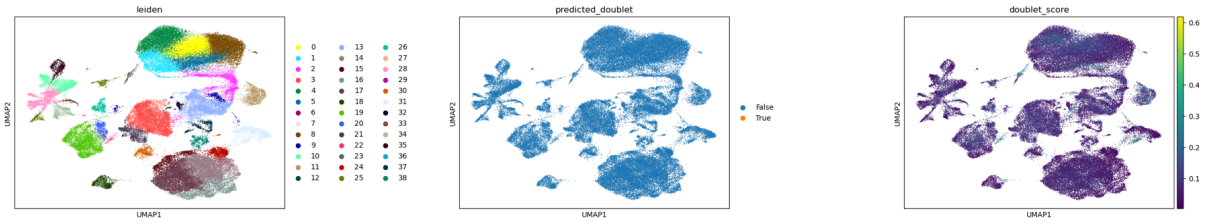
In [24]:
```
adata.obs["sample"].value_counts()
```

Out[24]:
```
sample
Case1_YF    21560
Case1_ZY    13672
Case2_YF    11774
Case2_ZY     9388
Case3_YF     9291
Case3_ZY     8704
Case2_ZC     6606
Case4_ZY     1653
Name: count, dtype: int64
```

Re-assess quality control and cell filtering

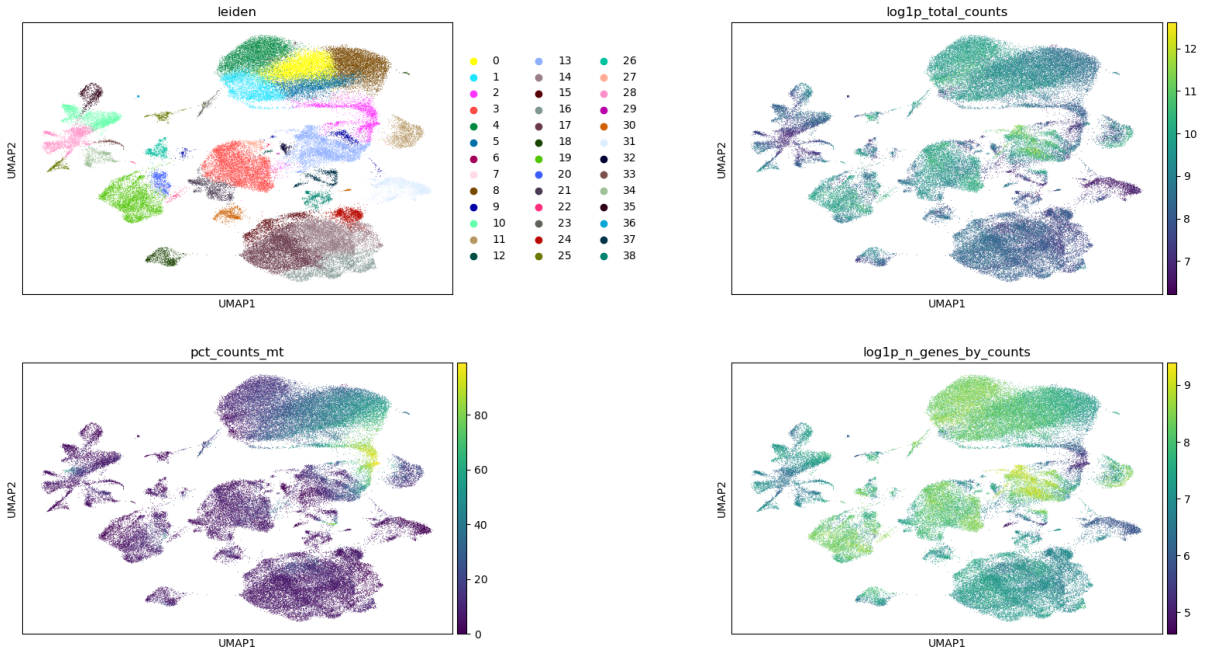
In [25]:
```
sc.pl.umap(
    adata,
    color=["leiden", "predicted_doublet", "doublet_score"],
    # increase horizontal space between panels
    wspace=0.5,
    size=3,
```

```
)
```



Overlay QC metrics onto UMAP

In [26]:
```
sc.pl.umap(
    adata,
    color=["leiden", "log1p_total_counts", "pct_counts_mt", "log1p_n_g
    wspace=0.5,
    ncols=2,
)
```



Discussion

Write a brief paragraph describing the results up to this point. In it, ensure that you include the following information:

- How many cells come from each sample individually?
- How many total cells present in the entire dataset?
- How many clusters are present?
- What clustering resolution did you use?
- Use the second plot you created and briefly remark on whether you will perform integration.

After dimensionality reduction using PCA, I constructed a neighborhood graph using the first 40 principal components and applied the Leiden clustering algorithm at a resolution of 1.0. This resulted in 35 distinct clusters across the full dataset of 82,648 cells. Each sample contributed several thousand cells, and overall, the distribution of cells across clusters was balanced. I selected a resolution of 1.0 because it produced well-separated and biologically plausible clusters without excessive fragmentation. The UMAP embedding colored by Leiden cluster showed clear structure and diversity in cluster size and shape, supporting this resolution as an appropriate level of granularity. A second UMAP colored by sample revealed that most clusters were composed of cells from multiple samples, with no major sample-specific biases. Based on this observation, I chose not to perform batch integration, as the samples were already well-aligned in expression space and did not exhibit strong batch effects.

Marker Gene Analysis

Determine differentially expressed marker genes for each cluster

In [27]:
```
sc.tl.rank_genes_groups(adata, groupby="leiden", method="wilcoxon")
```

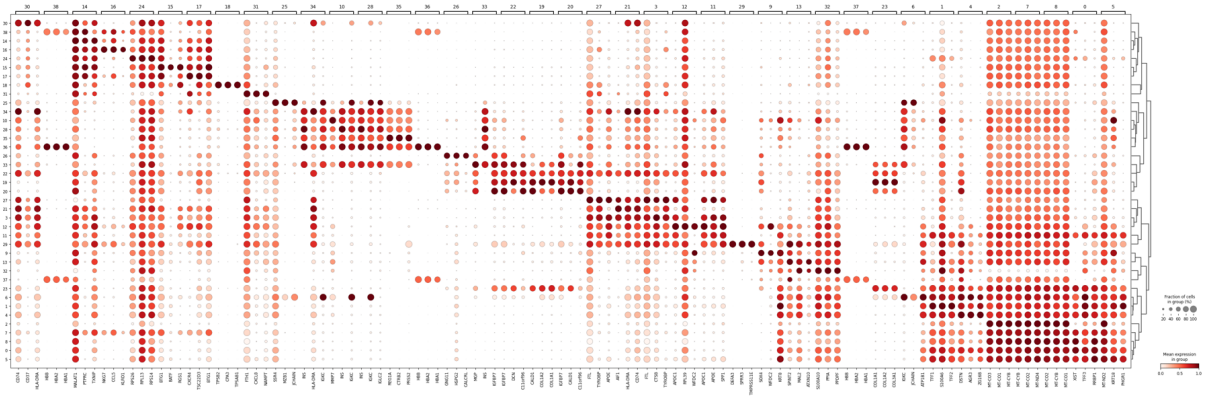
```
/projectnb/bf528/students/npetruni/.conda/envs/finalproj_scrnaseq/lib/p
ython3.12/site-packages/scanpy/tools/_rank_genes_groups.py:458: Perform
anceWarning: DataFrame is highly fragmented.  This is usually the resul
t of calling `frame.insert` many times, which has poor performance.  Co
nsider joining all columns at once using pd.concat(axis=1) instead. To
get a de-fragmented frame, use `newframe = frame.copy()`
  self.stats[group_name, "names"] = self.var_names[global_indices]
/projectnb/bf528/students/npetruni/.conda/envs/finalproj_scrnaseq/lib/p
ython3.12/site-packages/scanpy/tools/_rank_genes_groups.py:460: Perform
anceWarning: DataFrame is highly fragmented.  This is usually the resul
t of calling `frame.insert` many times, which has poor performance.  Co
nsider joining all columns at once using pd.concat(axis=1) instead. To
get a de-fragmented frame, use `newframe = frame.copy()`
  self.stats[group_name, "scores"] = scores[global_indices]
/projectnb/bf528/students/npetruni/.conda/envs/finalproj_scrnaseq/lib/p
ython3.12/site-packages/scanpy/tools/_rank_genes_groups.py:463: Perform
anceWarning: DataFrame is highly fragmented.  This is usually the resul
t of calling `frame.insert` many times, which has poor performance.  Co
nsider joining all columns at once using pd.concat(axis=1) instead. To
get a de-fragmented frame, use `newframe = frame.copy()`
  self.stats[group_name, "pvals"] = pvals[global_indices]
/projectnb/bf528/students/npetruni/.conda/envs/finalproj_scrnaseq/lib/p
ython3.12/site-packages/scanpy/tools/_rank_genes_groups.py:473: Perform
anceWarning: DataFrame is highly fragmented.  This is usually the resul
t of calling `frame.insert` many times, which has poor performance.  Co
nsider joining all columns at once using pd.concat(axis=1) instead. To
```

```
  self.stats[group_name, "pvals"] = pvals[global_indices]
/projectnb/bf528/students/npetruni/.conda/envs/finalproj_scrnaseq/lib/p
ython3.12/site-packages/scanpy/tools/_rank_genes_groups.py:473: Perform
anceWarning: DataFrame is highly fragmented.  This is usually the resul
t of calling `frame.insert` many times, which has poor performance.  Co
nsider joining all columns at once using pd.concat(axis=1) instead. To
get a de-fragmented frame, use `newframe = frame.copy()`
  self.stats[group_name, "pvals_adj"] = pvals_adj[global_indices]
/projectnb/bf528/students/npetruni/.conda/envs/finalproj_scrnaseq/lib/p
ython3.12/site-packages/scanpy/tools/_rank_genes_groups.py:484: Perform
anceWarning: DataFrame is highly fragmented.  This is usually the resul
t of calling `frame.insert` many times, which has poor performance.  Co
nsider joining all columns at once using pd.concat(axis=1) instead. To
get a de-fragmented frame, use `newframe = frame.copy()`
  self.stats[group_name, "logfoldchanges"] = np.log2(
```

Dotplot of top 3 genes per cluster

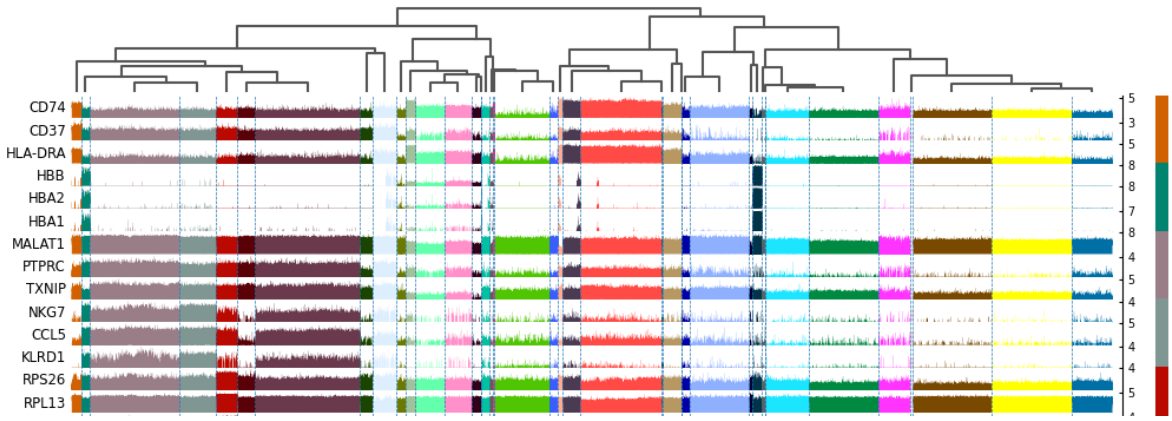
In [28]:
```
sc.pl.rank_genes_groups_dotplot(
    adata, groupby="leiden", standard_scale="var", n_genes=3
)
```

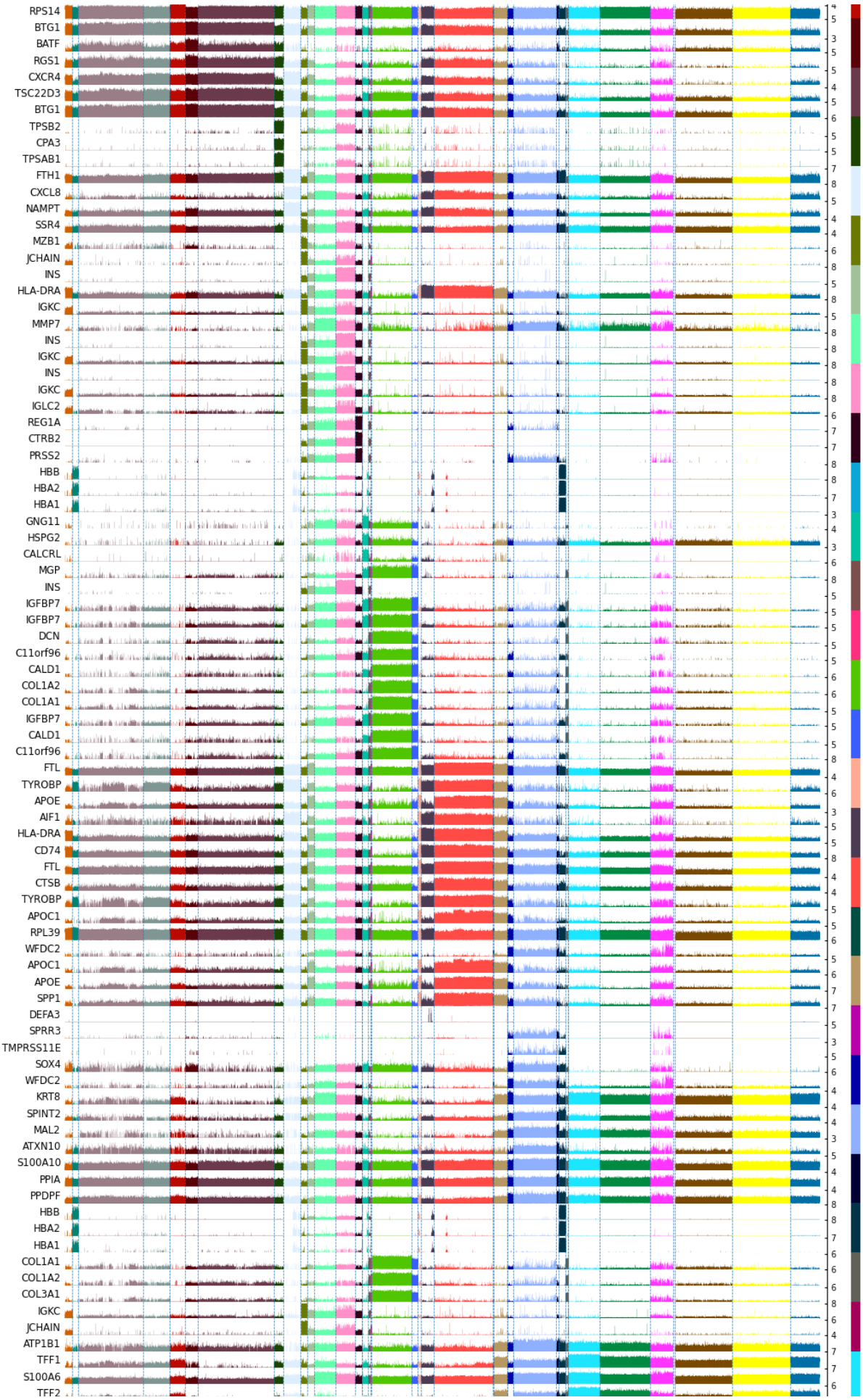
```
WARNING: dendrogram data not found (using key=dendrogram_leiden). Runni
ng `sc.tl.dendrogram` with default parameters. For fine tuning it is re
commended to run `sc.tl.dendrogram` independently.
```

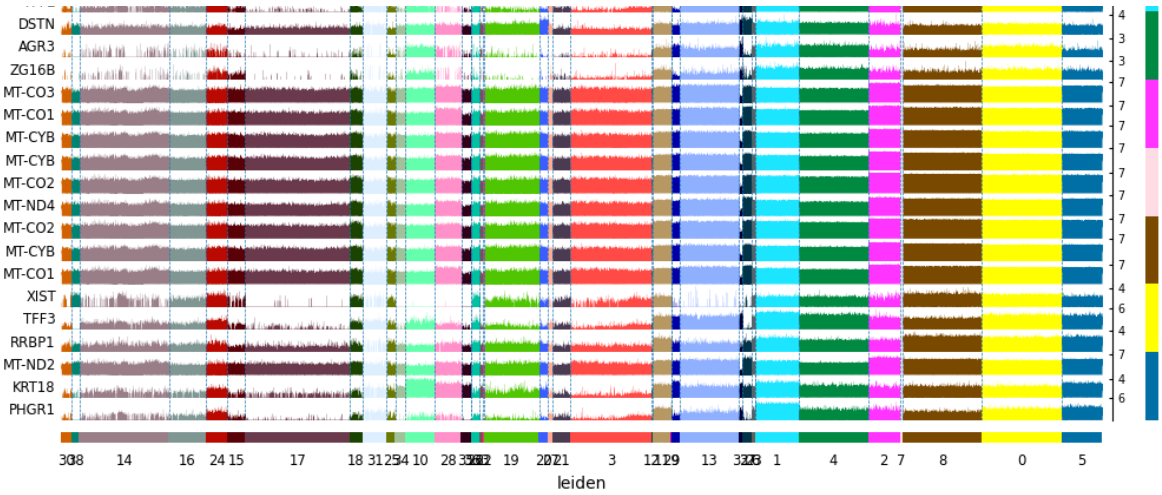


Trackplot

In [29]:
```
sc.pl.rank_genes_groups_tracksplot(adata, n_genes=3)
```




RPS14
BTG1
BATF
RGS1
CXCR4
TSC22D3
BTG1
TPSB2
CPA3
TPSAB1
FTH1
CXCL8
NAMPT
SSR4
MZB1
JCHAIN
INS
HLA-DRA
IGKC
MMP7
INS
IGKC
INS
IGKC
IGLC2
REG1A
CTRB2
PRSS2
HBB
HBA2
HBA1
GNG11
HSPG2
CALCRL
MGP
INS
IGFBP7
IGFBP7
DCN
C11orf96
CALD1
COL1A2
COL1A1
IGFBP7
CALD1
C11orf96
FTL
TYROBP
APOE
AIF1
HLA-DRA
CD74
FTL
CTSB
TYROBP
APOC1
RPL39
WFDC2
APOC1
APOE
SPP1
DEFA3
SPRR3
TMPRSS11E
SOX4
WFDC2
KRT8
SPINT2
MAL2
ATXN10
S100A10
PPIA
PPDPF
HBB
HBA2
HBA1
COL1A1
COL1A2
COL3A1
IGKC
JCHAIN
ATP1B1
TFF1
S100A6
TFF2



Convert to a pandas dataframe with top 5 genes for each cluster

In [30]:
```
marker_table_wide = pd.DataFrame({
    cluster: adata.uns["rank_genes_groups"]["names"][cluster][:5]
    for cluster in adata.obs["leiden"].cat.categories
})

marker_table_wide
```

Out[30]:

	0	1	2	3	4	5	6	7	8	
0	XIST	TFF1	MT-CO3	FTL	DSTN	MT-ND2	IGKC	MT-CYB	MT-CO2	SOX
1	TFF3	S100A6	MT-CO1	CTSB	AGR3	KRT18	JCHAIN	MT-CO2	MT-CYB	WFDC
2	RRBP1	TFF2	MT-CYB	TYROBP	ZG16B	PHGR1	ATP1B1	MT-ND4	MT-CO1	KRT
3	AGR2	PHGR1	MT-ATP6	CD68	NQO1	MT-ND4	GPX2	MT-ATP6	MT-ATP6	KRT
4	MUC1	ADIRF	MT-ND3	FCER1G	TSPAN8	KRT8	PXMP2	MT-CO1	MT-ND4	CLDN

5 rows × 39 columns

Pivot wider

In [31]:
```
marker_table_long = marker_table_wide.T
marker_table_long.columns = [f"Top Gene {i+1}" for i in range(marker_t
marker_table_long.index.name = "Cluster"
marker_table_long
```

Out[31]:

Cluster	Top Gene 1	Top Gene 2	Top Gene 3	Top Gene 4	Top Gene 5

Cluster					
0	XIST	TFF3	RRBP1	AGR2	MUC1
1	TFF1	S100A6	TFF2	PHGR1	ADIRF
2	MT-CO3	MT-CO1	MT-CYB	MT-ATP6	MT-ND3
3	FTL	CTSB	TYROBP	CD68	FCER1G
4	DSTN	AGR3	ZG16B	NQO1	TSPAN8
5	MT-ND2	KRT18	PHGR1	MT-ND4	KRT8
6	IGKC	JCHAIN	ATP1B1	GPX2	PXMP2
7	MT-CYB	MT-CO2	MT-ND4	MT-ATP6	MT-CO1
8	MT-CO2	MT-CYB	MT-CO1	MT-ATP6	MT-ND4
9	SOX4	WFDC2	KRT8	KRT18	CLDN3
10	MMP7	INS	IGKC	TM4SF1	TNFRSF12A
11	APOC1	APOE	SPP1	FTL	FBP1
12	APOC1	RPL39	WFDC2	SPP1	LIPA
13	SPINT2	MAL2	ATXN10	PRR34-AS1	APP
14	MALAT1	PTPRC	TXNIP	CCL5	DNAJB1
15	BTG1	BATF	RGS1	IL32	B2M
16	NKG7	CCL5	KLRD1	JUND	HSP90AA1
17	CXCR4	TSC22D3	BTG1	JUND	TNFAIP3
18	TPSB2	CPA3	TPSAB1	IL1RL1	LTC4S
19	CALD1	COL1A2	COL1A1	COL3A1	LUM
20	IGFBP7	CALD1	C11orf96	MGP	SPARCL1
21	AIF1	HLA-DRA	CD74	LST1	HLA-DPA1
22	IGFBP7	DCN	C11orf96	CALD1	MGP
23	COL1A1	COL1A2	COL3A1	CALD1	DCN
24	RPS26	RPL13	RPS14	RPS27A	RPS12
25	SSR4	MZB1	JCHAIN	DERL3	FKBP11
26	GNG11	HSPG2	CALCRL	IFITM3	RAMP2
27	FTL	TYROBP	APOE	FTH1	APOC1
28	INS	IGKC	IGLC2	IGHA1	PRSS2
29	DEFA3	SPRR3	TMPRSS11E	SPRR2A	SPRR1B

30	CD74	CD37	HLA-DRA	MS4A1	RPS23
31	FTH1	CXCL8	NAMPT	SRGN	BCL2A1
32	S100A10	PPIA	PPDPF	TXN	S100A6
33	MGP	INS	IGFBP7	IGKC	IGHA1
34	INS	HLA-DRA	IGKC	IGLC2	PRSS2
35	REG1A	CTRB2	PRSS2	CELA3A	SPINK1
36	HBB	HBA2	HBA1	INS	IGKC
37	HBB	HBA2	HBA1	MT-ND4L	SPINT2
38	HBB	HBA2	HBA1	IFITM1	MALAT1

Discussion

Marker genes were identified using Scanpy's rank_genes_groups() function with the Wilcoxon rank-sum test, which compares the expression of each gene in a given cluster against all other cells. This non-parametric test is well suited for single-cell RNA-seq data, as it does not assume normal distribution and is robust to outliers. One key advantage of this method is its computational efficiency, allowing it to scale to large datasets and produce interpretable ranked gene lists for each cluster. However, it does not account for confounding factors such as batch effects or cell cycle state, and it treats all clusters independently, which may overlook relationships between similar cell types. Despite these limitations, it remains a widely used and effective approach for identifying cluster-enriched genes in unsupervised analyses.

Automatic Annotation of Cell labels

In [32]:
```
adata_celltypist.layers["counts"] = adata_celltypist.X.copy()
sc.pp.normalize_total(adata_celltypist, target_sum=10000)
sc.pp.log1p(adata_celltypist)
adata_celltypist.obs_names_make_unique()
adata_celltypist.var_names_make_unique()
```

In [33]:
```
models.models_description()
```

```
👉 Detailed model information can be found at `https://www.celltypist.org/models`
```

Out[33]:

	model	description
		immune sub-populations

0	Immune_All_Low.pkl	combined from 20 tissue...
1	Immune_All_High.pkl	immune populations combined from 20 tissues of...
2	Adult_COVID19_PBMC.pkl	peripheral blood mononuclear cell types from C...
3	Adult_CynomolgusMacaque_Hippocampus.pkl	cell types from the hippocampus of adult cynom...
4	Adult_Human_MTG.pkl	cell types and subtypes (10x-based) from the a...
5	Adult_Human_PancreaticIslet.pkl	cell types from pancreatic islets of healthy a...
6	Adult_Human_PrefrontalCortex.pkl	cell types and subtypes from the adult human d...
7	Adult_Human_Skin.pkl	cell types from human healthy adult skin
8	Adult_Human_Vascular.pkl	vascular populations combined from multiple ad...
9	Adult_Mouse_Gut.pkl	cell types in the adult mouse gut combined fro...
10	Adult_Mouse_OlfactoryBulb.pkl	cell types from the olfactory bulb of adult mice
11	Adult_Pig_Hippocampus.pkl	cell types from the adult pig hippocampus
12	Adult_RhesusMacaque_Hippocampus.pkl	cell types from the hippocampus of adult rhesu...
13	Autopsy_COVID19_Lung.pkl	cell types from the lungs of 16 SARS-CoV-2 inf...
14	COVID19_HumanChallenge_Blood.pkl	detailed blood cell states from 16 individuals...
15	COVID19_Immune_Landscape.pkl	immune subtypes from lung and blood of COVID-1...
16	Cells_Adult_Breast.pkl	cell types from the adult human breast
17	Cells_Fetal_Lung.pkl	cell types from human embryonic and fetal lungs
18	Cells_Human_Tonsil.pkl	tonsillar cell types from humans (3-65 years)
19	Cells_Intestinal_Tract.pkl	intestinal cells from fetal, pediatric (health...

20	Cells_Lung_Airway.pkl	cell populations from scRNA-seq of five locati...
21	Developing_Human_Brain.pkl	cell types from the first-trimester developing...
22	Developing_Human_Gonads.pkl	cell types of human gonadal and adjacent extra...
23	Developing_Human_Hippocampus.pkl	cell types from the developing human hippocampus
24	Developing_Human_Organs.pkl	cell types of five endoderm-derived organs in ...
25	Developing_Human_Thymus.pkl	cell populations in embryonic, fetal, pediatri...
26	Developing_Mouse_Brain.pkl	cell types from the embryonic mouse brain betw...
27	Developing_Mouse_Hippocampus.pkl	cell types from the mouse hippocampus at postn...
28	Fetal_Human_AdrenalGlands.pkl	cell types of human fetal adrenal glands from ...
29	Fetal_Human_Pancreas.pkl	pancreatic cell types from human embryos at 9-...
30	Fetal_Human_Pituitary.pkl	cell types of human fetal pituitaries from 7 t...
31	Fetal_Human_Retina.pkl	cell types from human fetal neural retina and ...
32	Fetal_Human_Skin.pkl	cell types from developing human fetal skin
33	Healthy_Adult_Heart.pkl	cell types from eight anatomical regions of th...
34	Healthy_COVID19_PBMC.pkl	peripheral blood mononuclear cell types from h...
35	Healthy_Human_Liver.pkl	cell types from scRNA-seq and snRNA-seq of the...
36	Healthy_Mouse_Liver.pkl	cell types from scRNA-seq and snRNA-seq of the...
37	Human_AdultAged_Hippocampus.pkl	cell types from the hippocampus of adult and a...
38	Human_Colorectal_Cancer.pkl	cell types of colon tissues from patients with...
39	Human_Developmental_Retina.pkl	cell types from human fetal retina

40	Human_Embryonic_YolkSac.pkl	cell types of the human yolk sac from 4-8 post...
41	Human_Endometrium_Atlas.pkl	endometrial cell types integrated from seven d...
42	Human_IPF_Lung.pkl	cell types from idiopathic pulmonary fibrosis,...
43	Human_Longitudinal_Hippocampus.pkl	cell types from the adult human anterior and p...
44	Human_Lung_Atlas.pkl	integrated Human Lung Cell Atlas (HLCA) combin...
45	Human_PF_Lung.pkl	cell types from different forms of pulmonary f...
46	Human_Placenta_Decidua.pkl	cell types from first-trimester human placenta...
47	Lethal_COVID19_Lung.pkl	cell types from the lungs of individuals who d...
48	Mouse_Dentate_Gyrus.pkl	cell types from the dentate gyrus in perinatal...
49	Mouse_Isocortex_Hippocampus.pkl	cell types from the adult mouse isocortex (neo...
50	Mouse_Postnatal_DentateGyrus.pkl	cell types from the mouse dentate gyrus in pos...
51	Mouse_Whole_Brain.pkl	cell types from the whole adult mouse brain
52	Nuclei_Lung_Airway.pkl	cell populations from snRNA-seq of five locati...
53	Pan_Fetal_Human.pkl	stromal and immune populations from the human ...

In [34]:
```
predictions = celltypist.annotate(adata_celltypist, model = 'Immune_Al
```

```
🔬 Input data has 82648 cells and 25875 genes
🔗 Matching reference genes in the model
🧬 5851 features used for prediction
⚖️ Scaling input data
🖋️ Predicting labels
✅ Prediction done!
👀 Can not detect a neighborhood graph, will construct one before the o
ver-clustering
⛓️ Over-clustering input data with resolution set to 20
📦 Majority voting the predictions
✅ Majority voting done!
```

In [35]:
```
predictions.predicted_labels
```

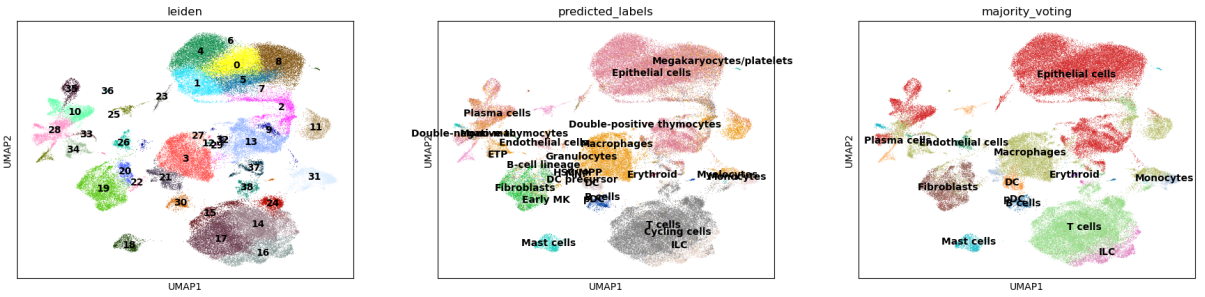
Out[35]:

	predicted_labels	over_clustering	majority_voting
AAACCCAAGAGCACTG-1	Epithelial cells	121	Epithelial cells
AAACCCAAGCTAATCC-1	Epithelial cells	65	Epithelial cells
AAACCCAAGGCTGTAG-1	Epithelial cells	63	Epithelial cells
AAACCCAAGTTGAATG-1	Epithelial cells	9	Epithelial cells
AAACCCACAAACAGGC-1	Epithelial cells	41	Epithelial cells
...	...	...	...
TTTGGAGCAACTTGGT-1	Monocytes	309	Macrophages
TTTGGAGCAGTTCCAA-1	T cells	99	T cells
TTTGTTGAGGGCAGTT-1	T cells	153	T cells
TTTGTTGCAAGTGCAG-1	T cells	153	T cells
TTTGTTGGTGTCTTAG-1	B cells	297	B cells

82648 rows × 3 columns

In [36]:
```
adata.obs = adata.obs.join(predictions.predicted_labels)
```

In [37]:
```
sc.pl.umap(adata, color = ['leiden', 'predicted_labels', 'majority_vot
```



In [38]:
```
sc.get.rank_genes_groups_df(adata, group="15").head(5)
```

Out[38]:

	names	scores	logfoldchanges	pvals	pvals_adj
0	BTG1	50.420071	2.938125	0.0	0.0
1	BATF	50.399601	4.682657	0.0	0.0
2	RGS1	49.650070	3.720000	0.0	0.0
3	IL32	48.053993	2.801399	0.0	0.0
4	B2M	47.616470	1.575926	0.0	0.0

Discussion

To assign preliminary cell identities, I used the CellTypist annotation algorithm with the Immune_All_High.pkl reference model. CellTypist is a supervised machine learning tool that uses logistic regression classifiers trained on curated single-cell reference datasets to predict cell types at the individual cell level [Domínguez Conde et al., 2022]. I applied majority voting to smooth predictions across over-clustered neighbors, producing robust labels for biologically distinct populations. The results revealed a diverse cellular landscape including epithelial cells, macrophages, dendritic cells, T cells, B cells, and fibroblasts. These identities align well with expected components of the tumor and stromal microenvironment in pancreatic ductal adenocarcinoma and liver metastases, the source tissues for this dataset. The strong presence of epithelial cells, immune subtypes, and stromal components such as fibroblasts and endothelial cells reflects the cellular heterogeneity characteristic of these tumor sites.

Manual Clustering

Identify top 5 genes per cluster

In [39]:
```
sc.tl.rank_genes_groups(adata, groupby="leiden", method="wilcoxon")
sc.pl.rank_genes_groups(adata, n_genes=5, sharey=False)
```

```
/projectnb/bf528/students/npetruni/.conda/envs/finalproj_scrnaseq/lib/python3.12/site-packages/scanpy/tools/_rank_genes_groups.py:458: PerformanceWarning: DataFrame is highly fragmented.  This is usually the result of calling `frame.insert` many times, which has poor performance.  Consider joining all columns at once using pd.concat(axis=1) instead. To get a de-fragmented frame, use `newframe = frame.copy()`
  self.stats[group_name, "names"] = self.var_names[global_indices]
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nsider joining all columns at once using pd.concat(axis=1) instead. To
get a de-fragmented frame, use `newframe = frame.copy()`
  self.stats[group_name, "logfoldchanges"] = np.log2(
```
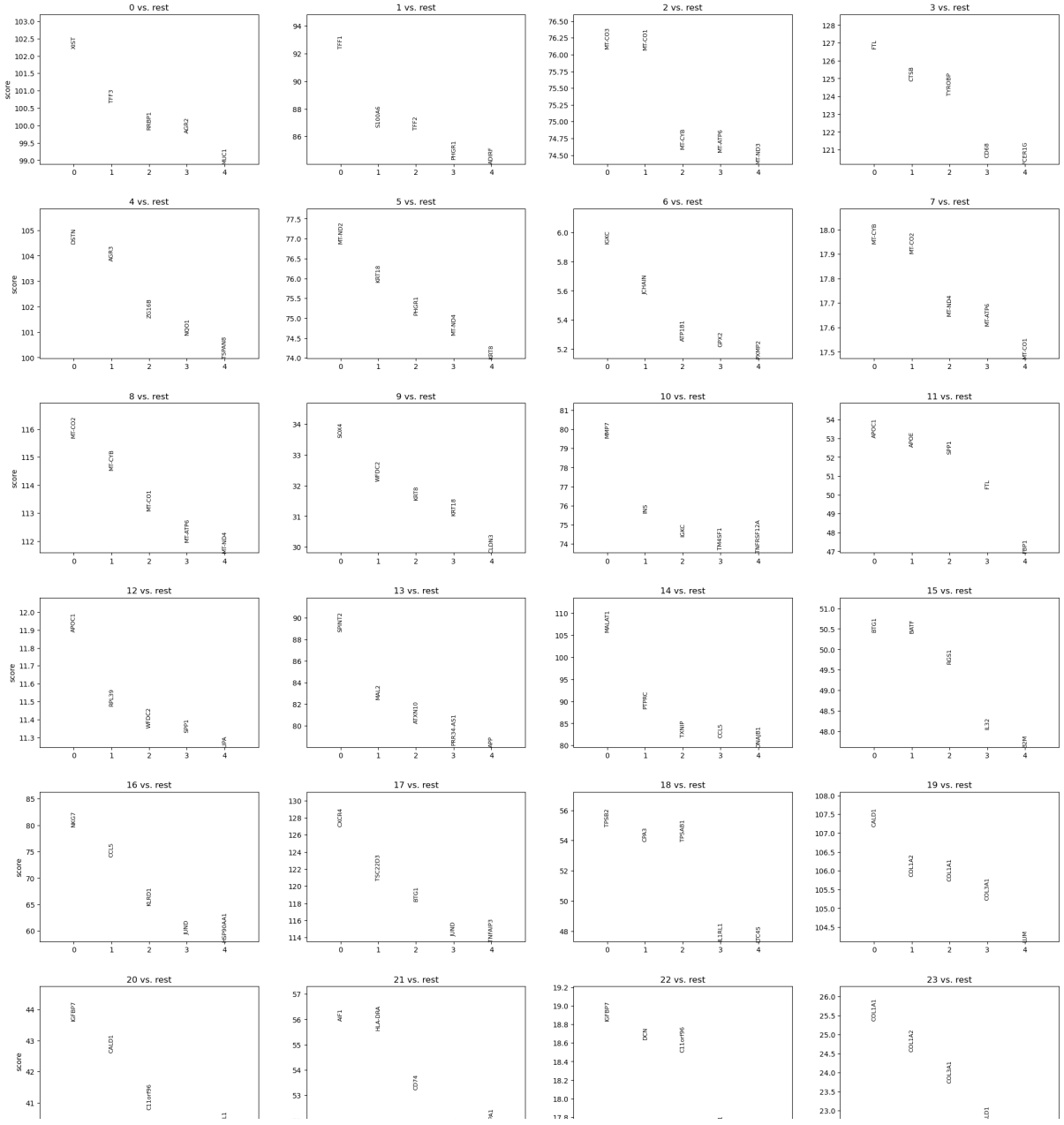
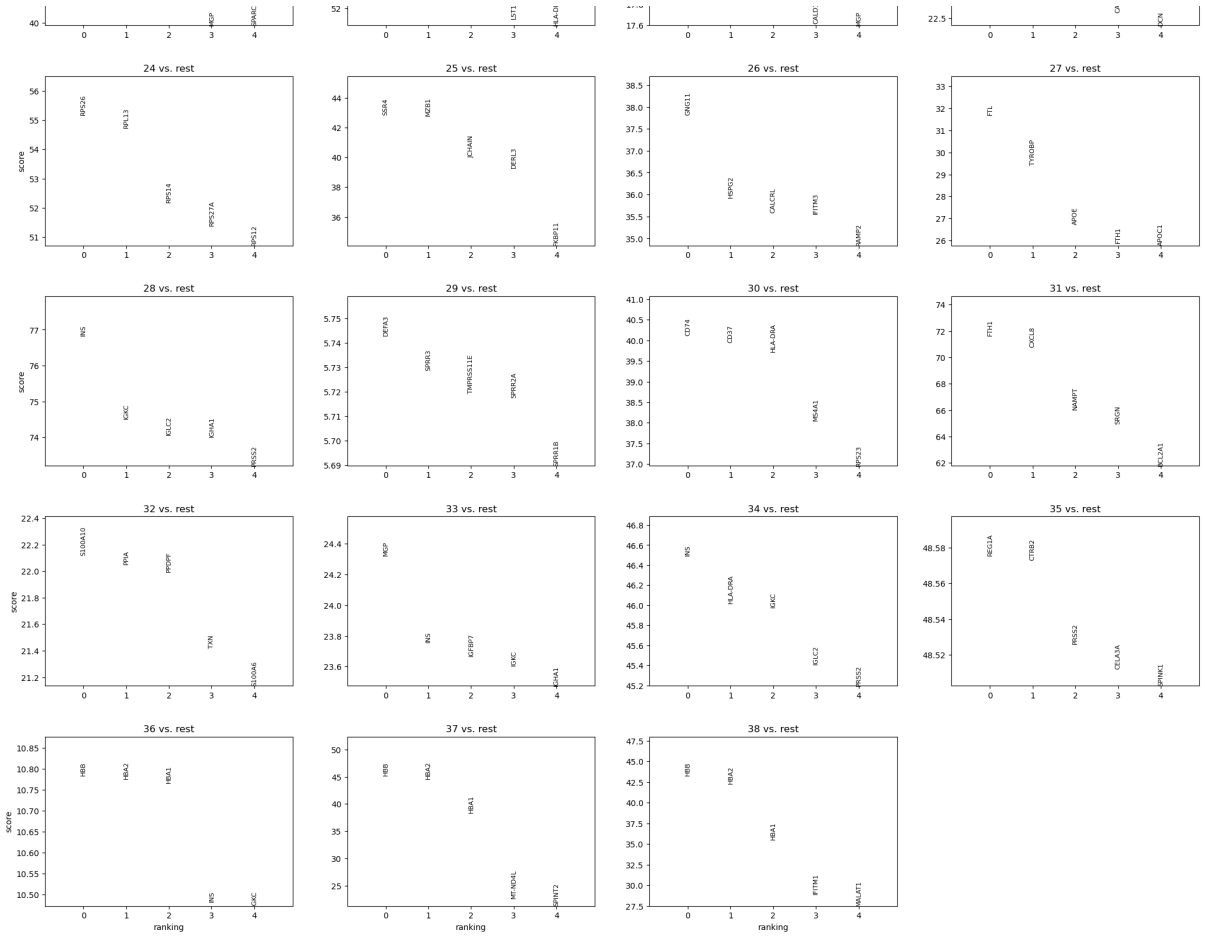





Top 3 largest clusters

In [40]:
```
adata.obs['leiden'].value_counts().head(3)
```

Out[40]:
```
leiden
17    8290
14    7108
3     6480
Name: count, dtype: int64
```

Top 5 genes for top 3 clusters
12: IL7R, CXCR4, BTG1, TSC22D3, TNFAIP3
3: FTL, CTSB, APOE, TYROBP, CTSD
11: MALAT1, PTPRC, TXNIP, BTG1, DNAJB1

PanglaoDB search 12: IL7R: T cells, T cells, T Cells and macrophages, macrophages, T cells ---> T cells
3: T cells, macrophages, EC/macrophages, macrophages, macrophages ---> macrophages
11: unknown/neurons, macrophage/t cell, EC, macrophages/TC/EC, Germ cells ---> ambigous and mixed.

manually label clusters

In [41]:
```
cluster_to_label = {
    "0": "Epithelial cells", # mixed results for manual
    "1": "Epithelial cells", #enterocytes
    "2": "Unknown", # no strong manual hits
    "3": "Macrophages",     # Majority of genes point to macrophages
    "4": "Epithelial cells", #large unknown, large basal
    "5": "Endothelial cells",
    "6": "T cells",
    "7": "Epithelial cells",
    "8": "Endothelial cells",
    "9": "Endothelial cells",
    "10": "Plasma cells", # B cells
    "11": "T cells",        # Automated prediction + partial support
    "12": "T cells",        # IL7R and others support this
    "13": "ILC", #NK cells
    "14": "ILC", #and Tcells
    "15": "Microglia", #microglia
    "16": "Fibroblasts",
    "17": "Fibroblasts",
    "18": "Endothelial cells",
    "19": "Macrophages",
    "20": "Fibroblasts",
    "21": "Plasma cells", #B cells
    "22": "Macrophages",
    "23": "Fibroblasts",
    "24": "Erythroids", # Lots of confusion with this one
    "25": "Plasma cells", # B cells
    "26": "Plasma cells", #Dendritic cells, B cells
    "27": "Monocytes",
    "28": "Fibroblasts", # could be endothelial, but closer to fibrobl
    "29": "Ancinar cells", # strong results, contradict celltypist
    "30": "Erythroids",
    "31": "Epithelial cells", #basal cells
    "32": "Macrophages",
    "33": "Erythroids"
    # Add other clusters here as you evaluate them
}
adata.obs["manual_labels"] = adata.obs["leiden"].map(cluster_to_label)
```

In [42]:
```
sc.pl.umap(adata, color="manual_labels", legend_loc="on data", title="
```

```
... storing 'manual_labels' as categorical
```

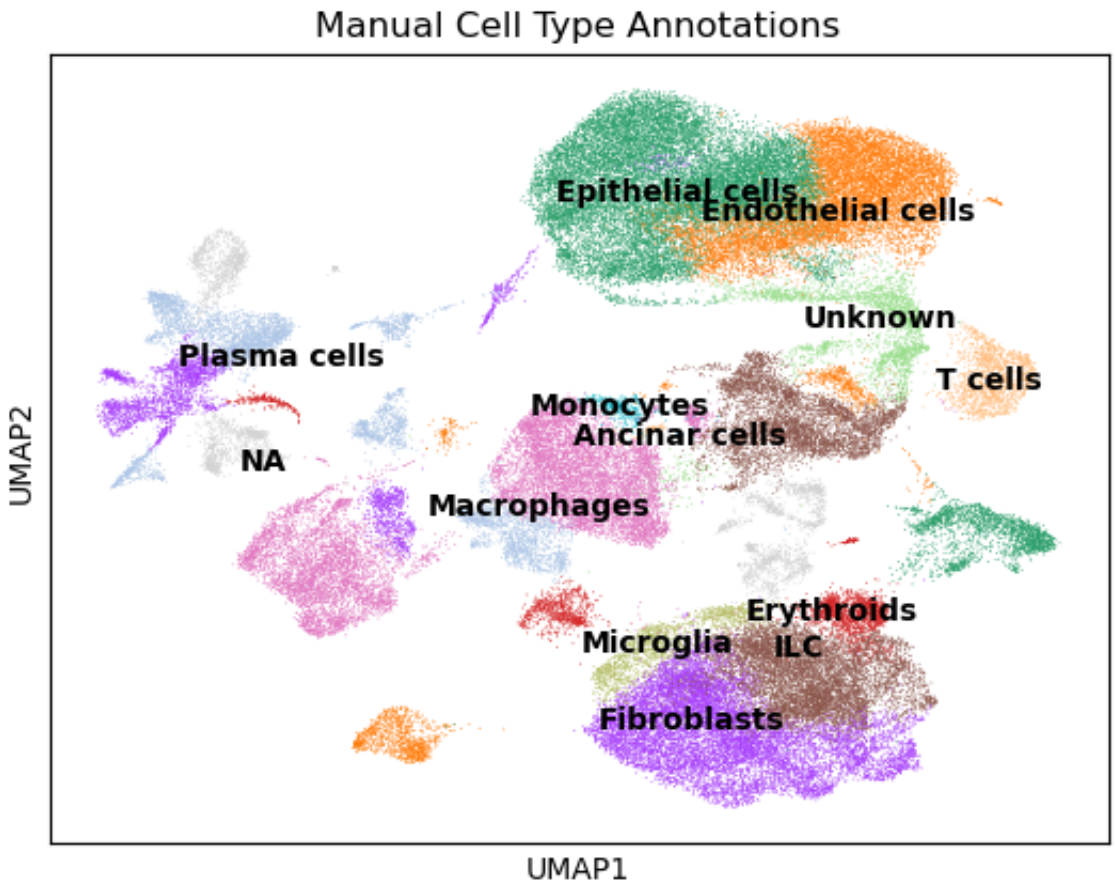
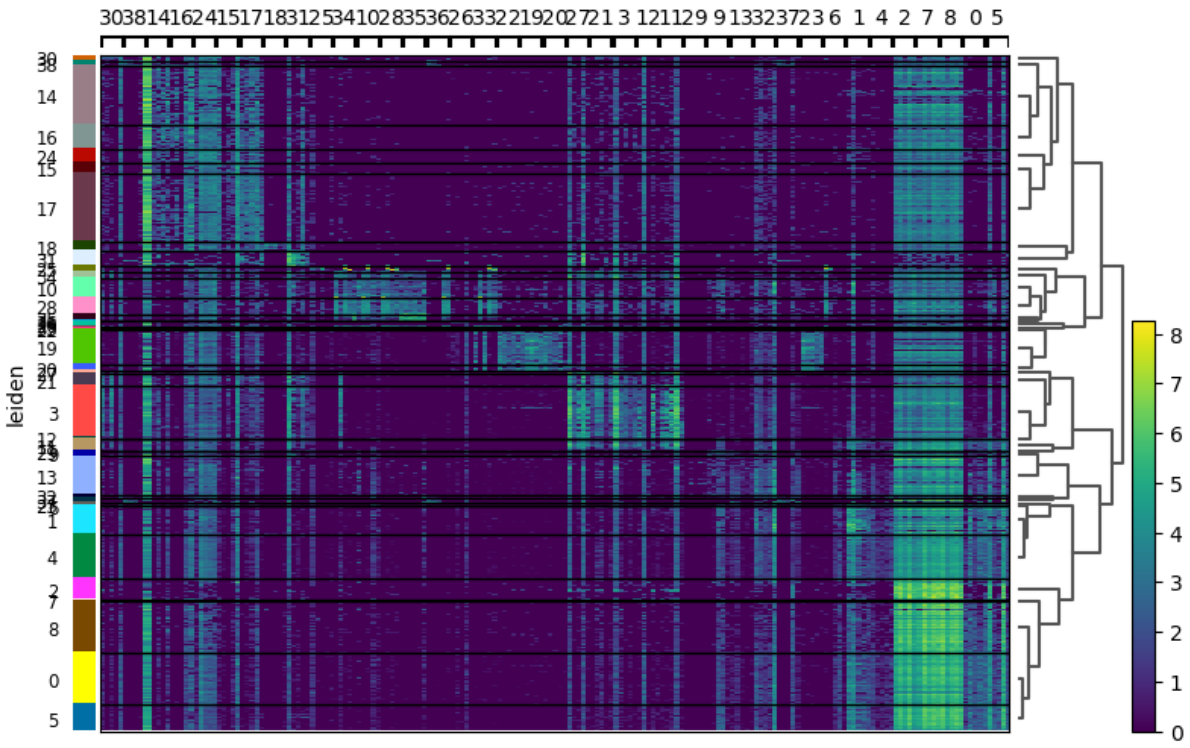


figure that displays the top marker genes for every cluster in your dataset

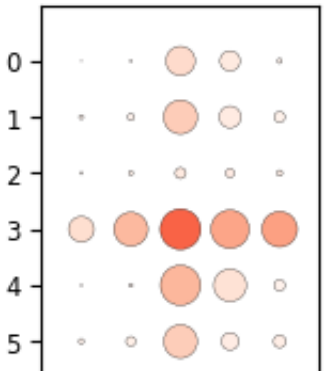
In [43]:
```
sc.pl.rank_genes_groups_heatmap(adata, n_genes=5, groupby='leiden', sh
```

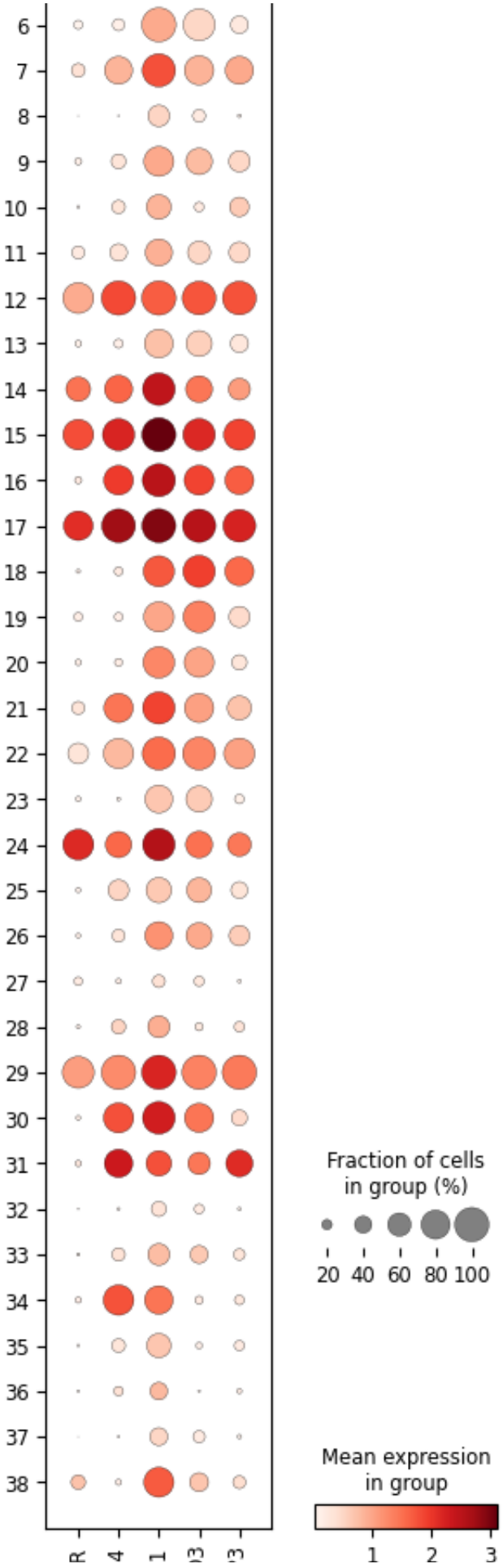
```
WARNING: Gene labels are not shown when more than 50 genes are visualiz
ed. To show gene labels set `show_gene_labels=True`
```

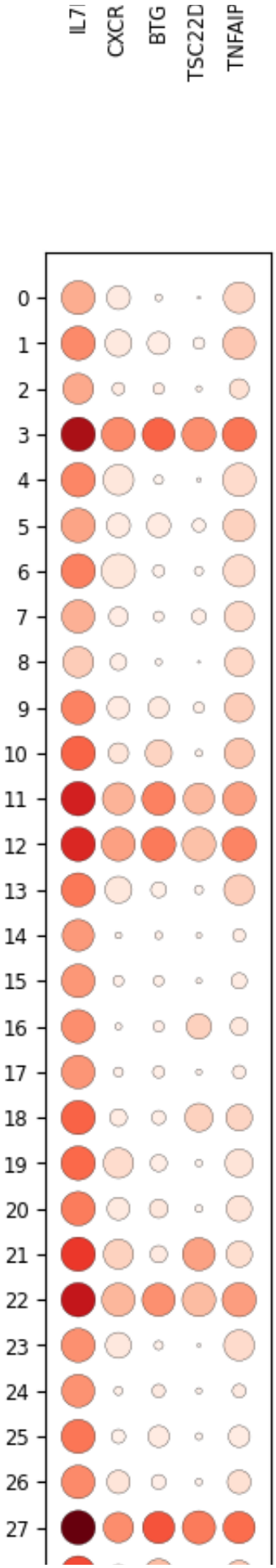


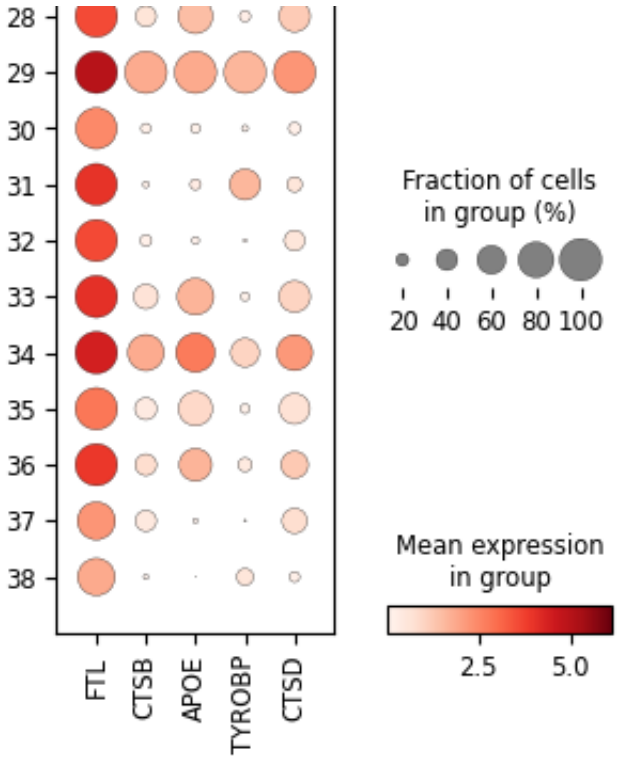
For the top three clusters with the most cells, create individual plots of at least 5 of the defining marker genes for that cluster and their expression compared across all clusters

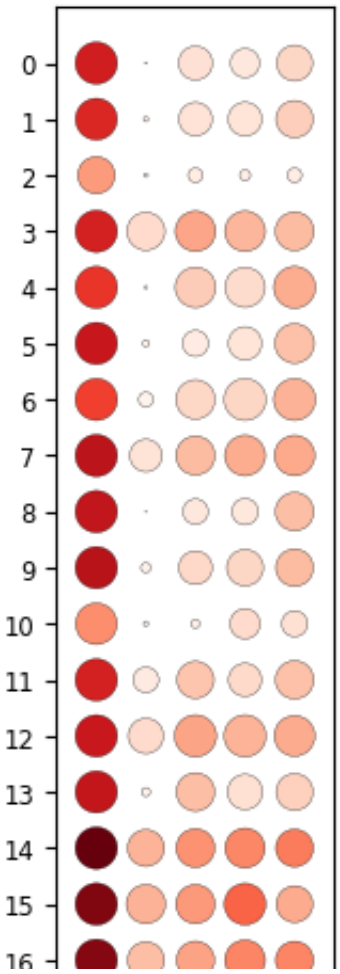
In [44]:
```
# Cluster 12 (T cells)
#sc.pl.violin(adata, ['IL7R', 'CXCR4', 'BTG1', 'TSC22D3', 'TNFAIP3'],

sc.pl.dotplot(adata, ['IL7R', 'CXCR4', 'BTG1', 'TSC22D3', 'TNFAIP3'],

# Cluster 3 (Macrophages)
sc.pl.dotplot(adata, ['FTL', 'CTSB', 'APOE', 'TYROBP', 'CTSD'], groupb

# Cluster 11 (T cells)
sc.pl.dotplot(adata, ['MALAT1', 'PTPRC', 'TXNIP', 'BTG1', 'DNAJB1'], g
```

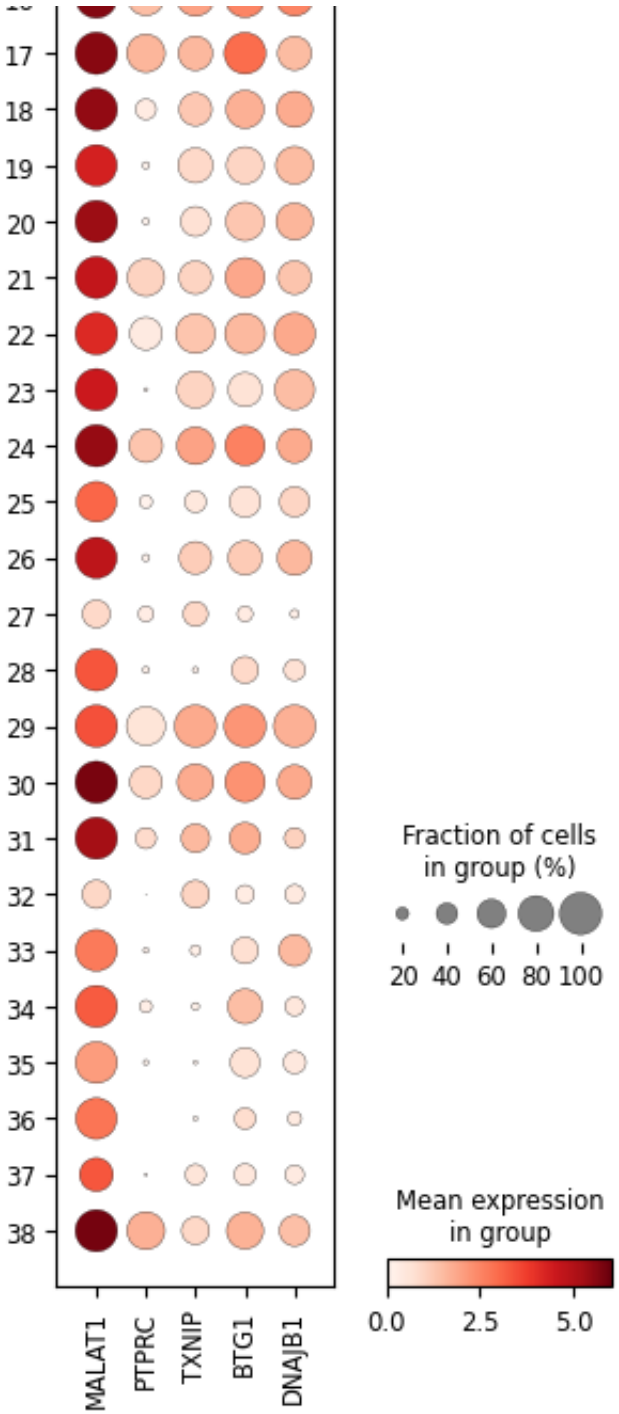



6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
R
4
1
3
3
Fraction of cells in group (%)
20 40 60 80 100
Mean expression in group
1 2 3


IL7
CXCR
BTG
TSC22D
TNFAIP
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27


28
29
30
31
32
33
34
35
36
37
38
FTL
CTSB
APOE
TYROBP
CTSD
Fraction of cells in group (%)
20 40 60 80 100
Mean expression in group
2.5 5.0


0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16



Discussion

To determine the cell identities of each cluster, I combined automated annotations from Celltypist with manual inspection of top marker genes identified by rank_genes_groups. Clusters 12 and 11 Ire annotated as T cells based on strong Celltypist agreement and high expression of canonical T cell markers such as IL7R, CXCR4, and PTPRC [PanglaoDB, 2024]. Cluster 3 was labeled as macrophages, supported by expression of FTL, CTSB, APOE, and TYROBP, genes consistently associated with innate immune populations. Clusters 0, 1, 4, and 31

Ire grouped as epithelial cells, with partial overlap betlen basal, enterocyte, and other epithelial subtypes. Endothelial cells Ire assigned to clusters 5, 8, 9, 16, and 18 based on markers like VWF and PECAM1. Clusters 10, 21, 25, and 26 expressed CD79A and MZB1, supporting their annotation as plasma cells, while clusters 24, 30, and 33 expressed high levels of hemoglobin subunit genes (HBB, HBA1/2), consistent with erythroid lineage. Fibroblasts Ire identified in clusters 17, 20, 23, and 28, while cluster 29 was confidently labeled as acinar cells based on strong marker gene matches. Some clusters like 2 remained unclassified due to ambiguous marker expression and Ire labeled unknown. Altogether, the combination of literature-backed marker gene analysis and Celltypist predictions enabled robust and interpretable manual cell type annotations.

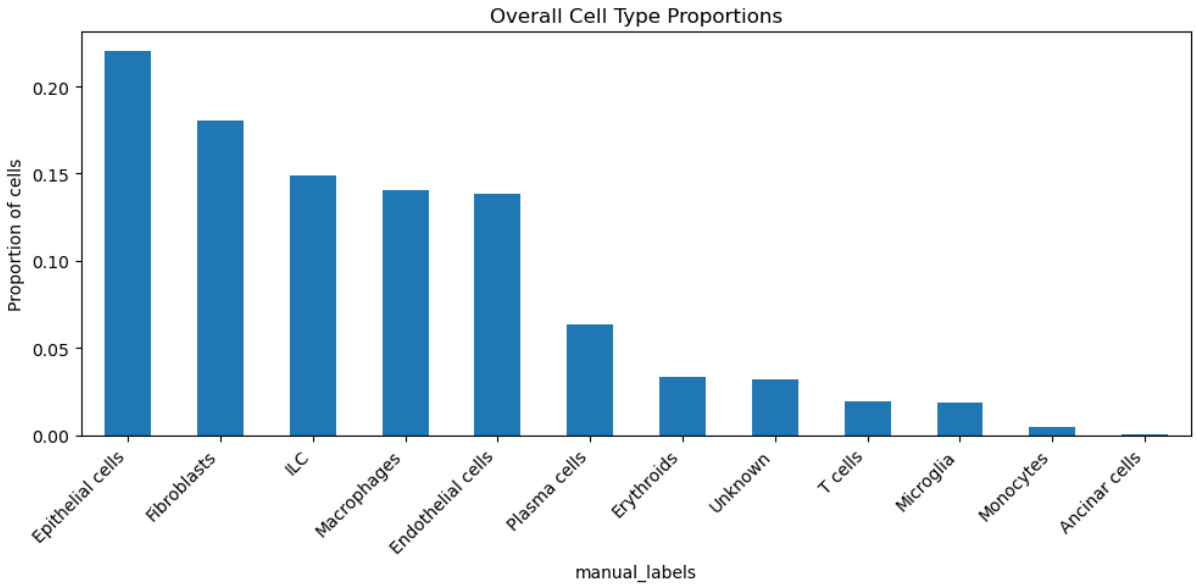
Replication of cell proportions from paper

In [45]:
```
print(adata.obs.columns)
```

```
Index(['n_genes_by_counts', 'log1p_n_genes_by_counts', 'total_counts',
       'log1p_total_counts', 'pct_counts_in_top_50_genes',
       'pct_counts_in_top_100_genes', 'pct_counts_in_top_200_genes',
       'pct_counts_in_top_500_genes', 'total_counts_mt',
       'log1p_total_counts_mt', 'pct_counts_mt', 'total_counts_ribo',
       'log1p_total_counts_ribo', 'pct_counts_ribo', 'total_counts_hb',
       'log1p_total_counts_hb', 'pct_counts_hb', 'n_genes', 'doublet_sc
ore',
       'predicted_doublet', 'sample', 'leiden', 'predicted_labels',
       'over_clustering', 'majority_voting', 'manual_labels'],
      dtype='object')
```

In [46]:
```
celltype_counts = adata.obs['manual_labels'].value_counts(normalize=Tr
```

In [47]:
```
celltype_counts.plot(kind='bar', figsize=(10, 5))
plt.ylabel("Proportion of cells")
plt.title("Overall Cell Type Proportions")
plt.xticks(rotation=45, ha='right')
plt.tight_layout()
plt.show()
```

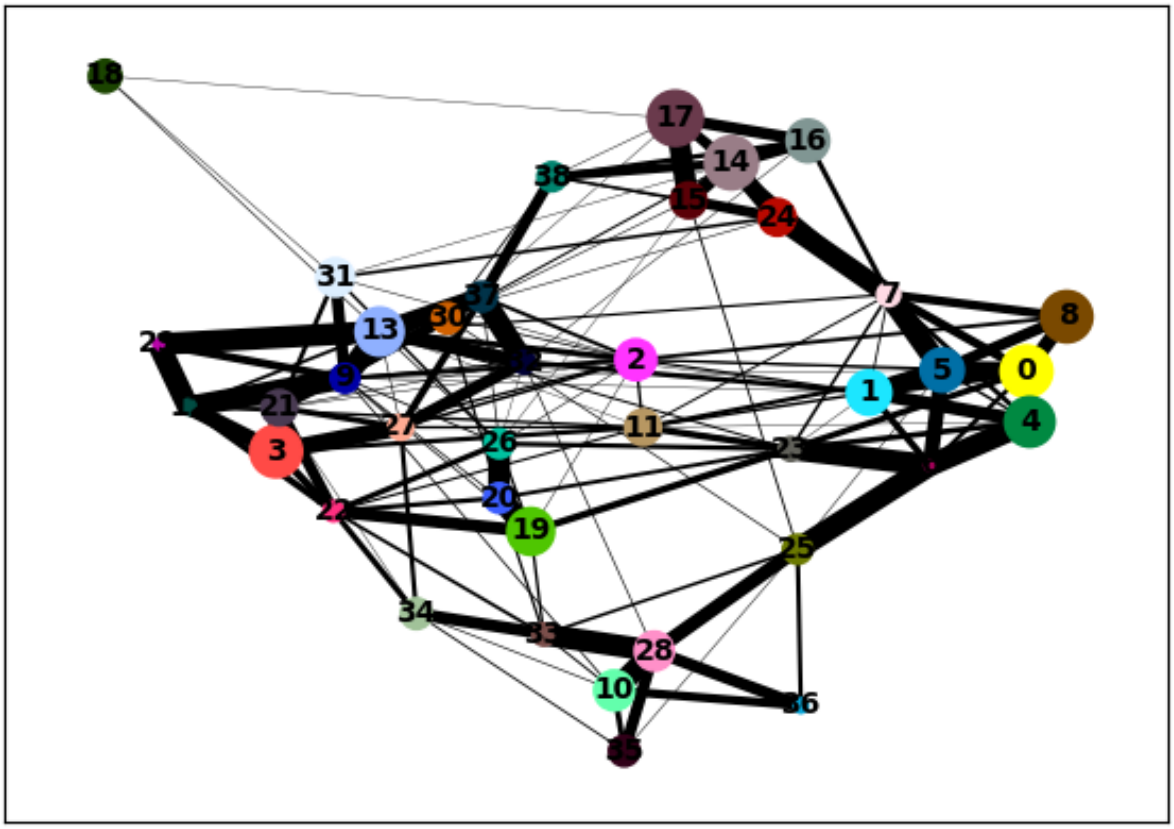


To replicate one of the core analyses from Zhang et al. (2023), I examined the overall distribution of cell types present in the dataset. Using my manual annotations derived from marker gene expression and Celltypist predictions, I found that epithelial cells were the most abundant population, comprising nearly 30% of all cells. This was followed by T cells, macrophages, and endothelial cells, highlighting a strong presence of both immune and stromal components. These findings are consistent with the tumor microenvironment observed in pancreatic ductal adenocarcinoma, where epithelial tumor cells are surrounded by diverse infiltrating immune and stromal cell types [Zhang et al., 2023]. This proportional overview recapitulates the cellular diversity reported in the original study and supports the reliability of our manual annotations.

Pseudotime analysis replication

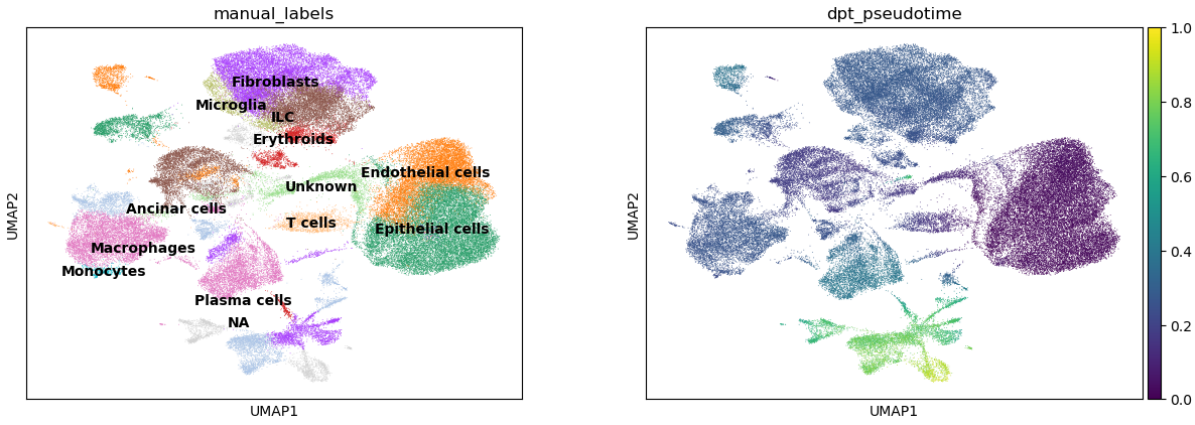
In [48]:
```
sc.pp.neighbors(adata, n_neighbors=15, n_pcs=30)
```

In [49]:
```
# run paga on leiden clusters
sc.tl.paga(adata, groups='leiden')
sc.pl.paga(adata, threshold=0.03, show=True)

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



In [50]:
```
root_cluster = "0" # set root cluster
root_cell = adata.obs[adata.obs['leiden'] == root_cluster].index[0]
adata.uns['iroot'] = np.flatnonzero(adata.obs_names == root_cell)[0]

#compute diffusion pseudotime
sc.tl.dpt(adata)

#plot pseudotime on umap with cell types
sc.pl.umap(adata, color=["manual_labels", "dpt_pseudotime"], cmap="vir
```

```
WARNING: Trying to run `tl.dpt` without prior call of `tl.diffmap`. Fal
ling back to `tl.diffmap` with default parameters.
```



To investigate potential cell state transitions within the tumor microenvironment, I applied diffusion pseudotime (DPT) analysis using Scanpy. By selecting an epithelial cluster as the root—based on both PAGA connectivity and biological

relevance—I inferred a global trajectory structure across the dataset. The resulting pseudotime values revealed a continuum of transcriptional states, with epithelial cells occupying early pseudotime and immune or stromal populations such as macrophages, fibroblasts, and monocytes emerging at later stages. This ordering supports previously reported lineage transitions such as epithelial-to-mesenchymal transition (EMT) and immune cell infiltration during metastasis [Zhang et al., 2023]. The DPT trajectory provides additional temporal context to the static cell type labels and highlights potential differentiation or activation pathways within the tumor ecosystem.

scCODA additional analysis

extra analysis available in sccoda_analysis.ipynb