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## Loupe Browser Walkthrough Visium HD Spatial Gene Expression Library, Human Colorectal Cancer (FFPE)

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Jennifer Becker<sup>1</sup>

<sup>1</sup>University of Rochester Medical Center



Jennifer Becker

University of Rochester Medical Center

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**Protocol status:** Working

**We use this protocol and it's working**



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**Keywords:** hosted visium hd ffpe colorectal cancer dataset, visium hd ffpe colorectal cancer dataset, visium hd spatial gene expression library, 10x genomics loupe browser, human colorectal cancer, gene, loupe browser walkthrough, more information on this dataset, ffpe, dataset

## Abstract

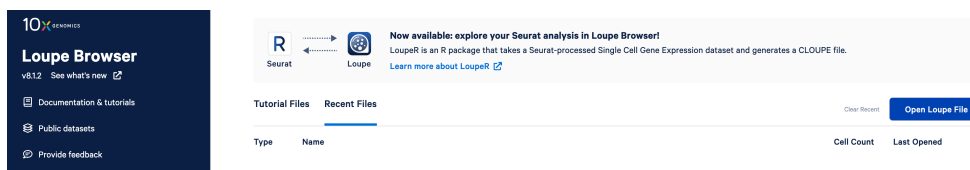
This protocol is intended to serve as an example of the capabilities of 10X Genomics Loupe Browser within their hosted Visium HD FFPE Colorectal Cancer dataset. More information on this dataset can be found at <https://www.10xgenomics.com/datasets/visium-hd-cytassist-gene-expression-libraries-of-human-crc>.

## Introduction

- 1 This protocol is intended to serve as an example of the capabilities of 10X Genomics Loupe Browser within their hosted Visium HD FFPE Colorectal Cancer dataset. More information on this dataset can be found at <https://www.10xgenomics.com/datasets/visium-hd-cytassist-gene-expression-libraries-of-human-crc>.

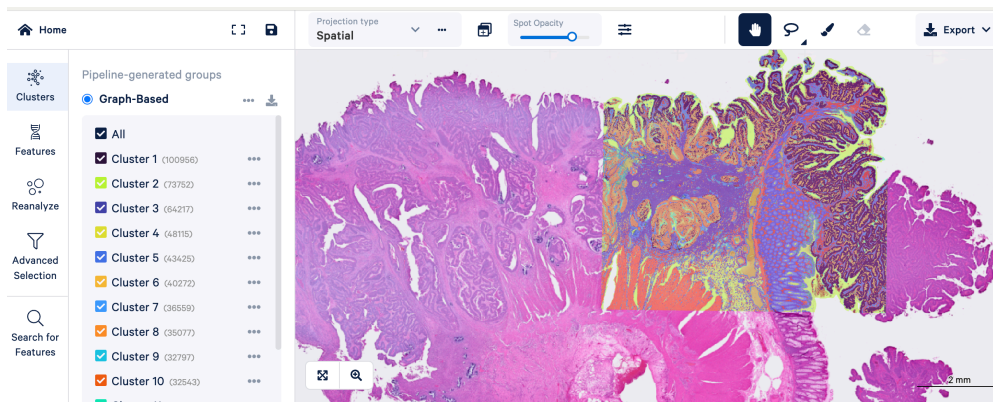
## Importing Data

- 2 Importing data into Loupe browser is as easy as navigating to your cloupe file!

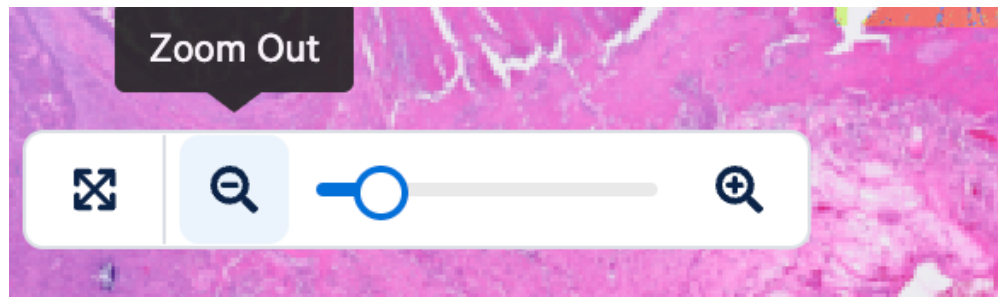


## Visualize Capture Area and Clustering

- 3 Once you've loaded the cloupe file the microscope image (in this case, a brightfield H&E image) and HD gene expression data are visualized together in the center of the screen.

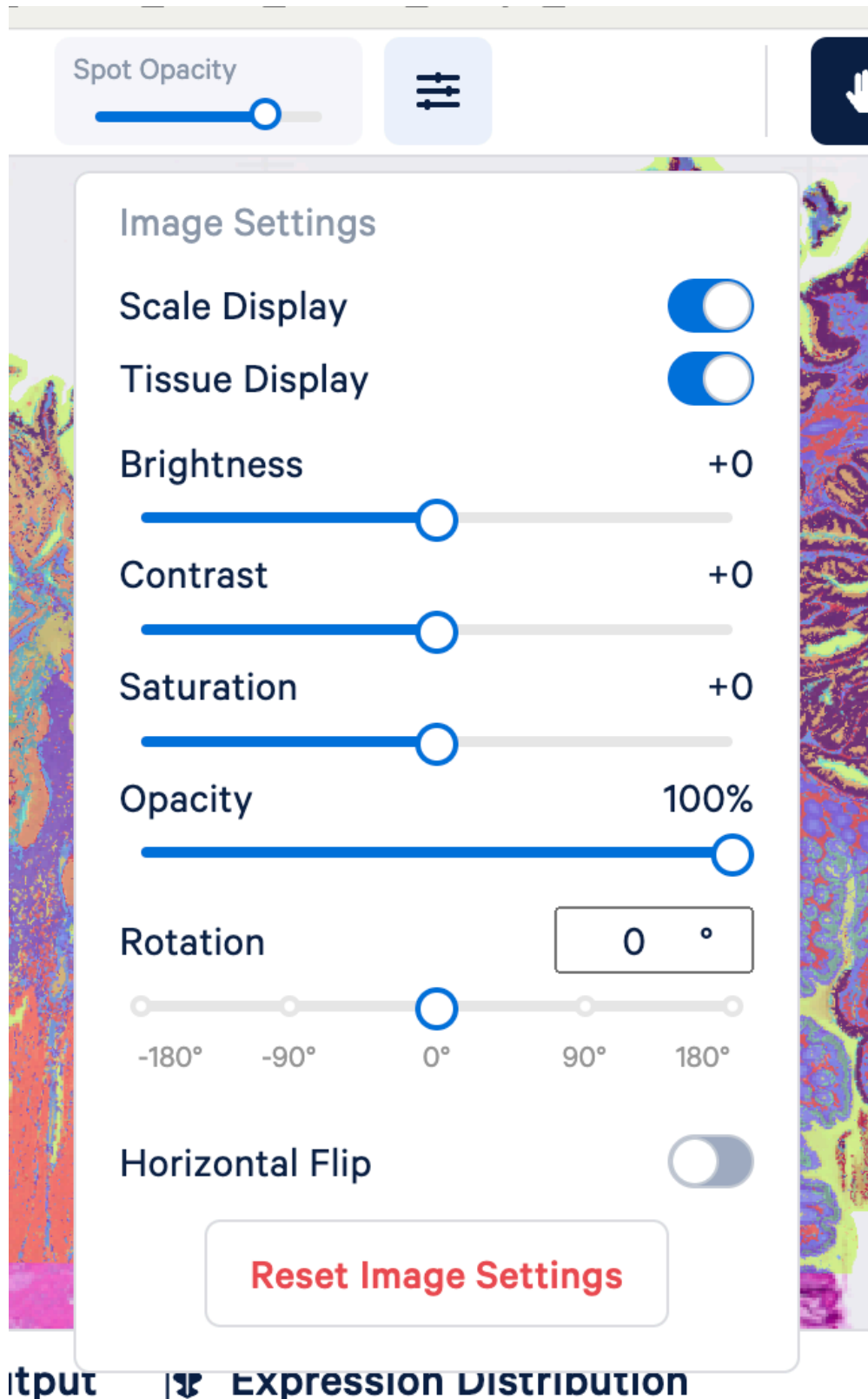


- 4 You can easily zoom in and out by clicking on the magnifying glass or simply scrolling with your mouse. The scale bar in the lower right hand corner is adjusted accordingly.



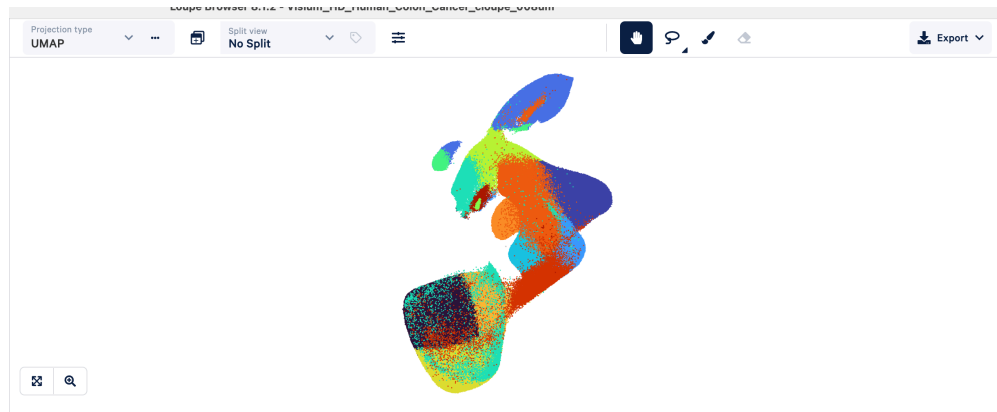
- 5 We can also adjust the projection settings to optimize visualization.



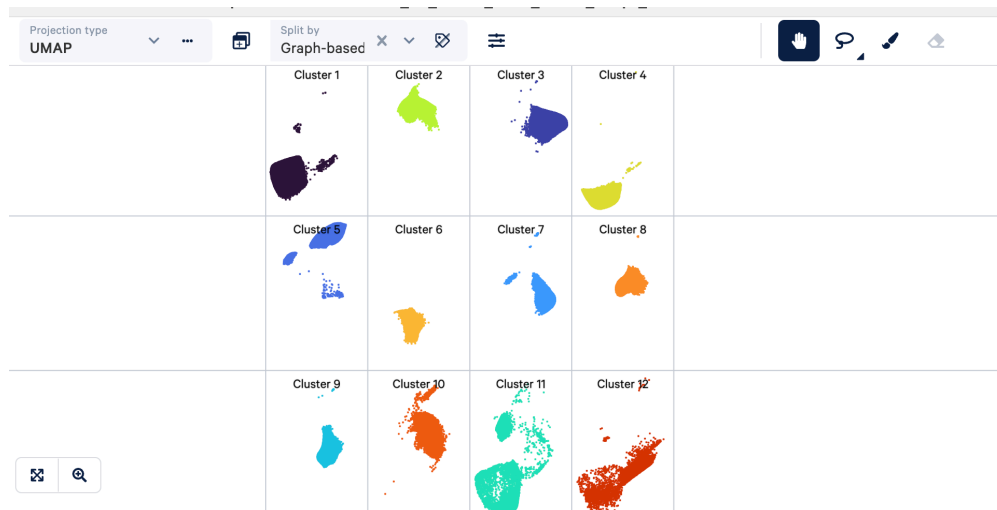


Decreasing the saturation can help highlight the capture area.

## 6 You can change the projection type to view clusters as a UMAP



And change the view to see clusters plotted individually



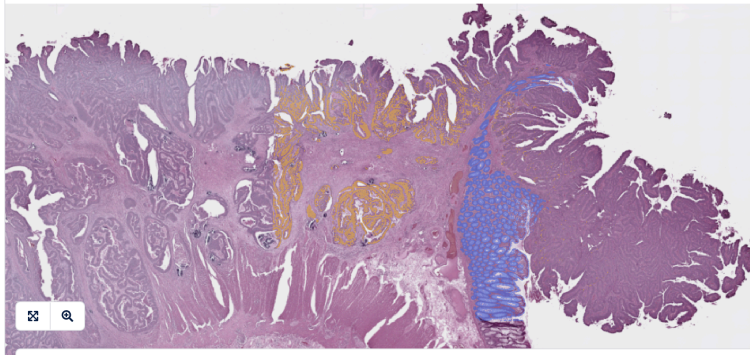
You can visualize Graph-Based or K-Means clusters for both Spatial and UMAP projects.

## Customize Cluster Visualization

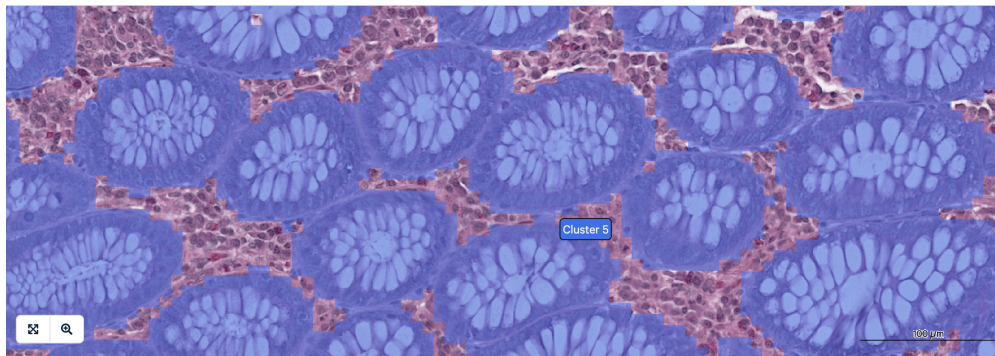
### 7 View as many or as few clusters as you like by checking/un-checking the boxes in the sidebar. We will take a look at cluster 5.

Pipeline-generated groups

● Graph-Based

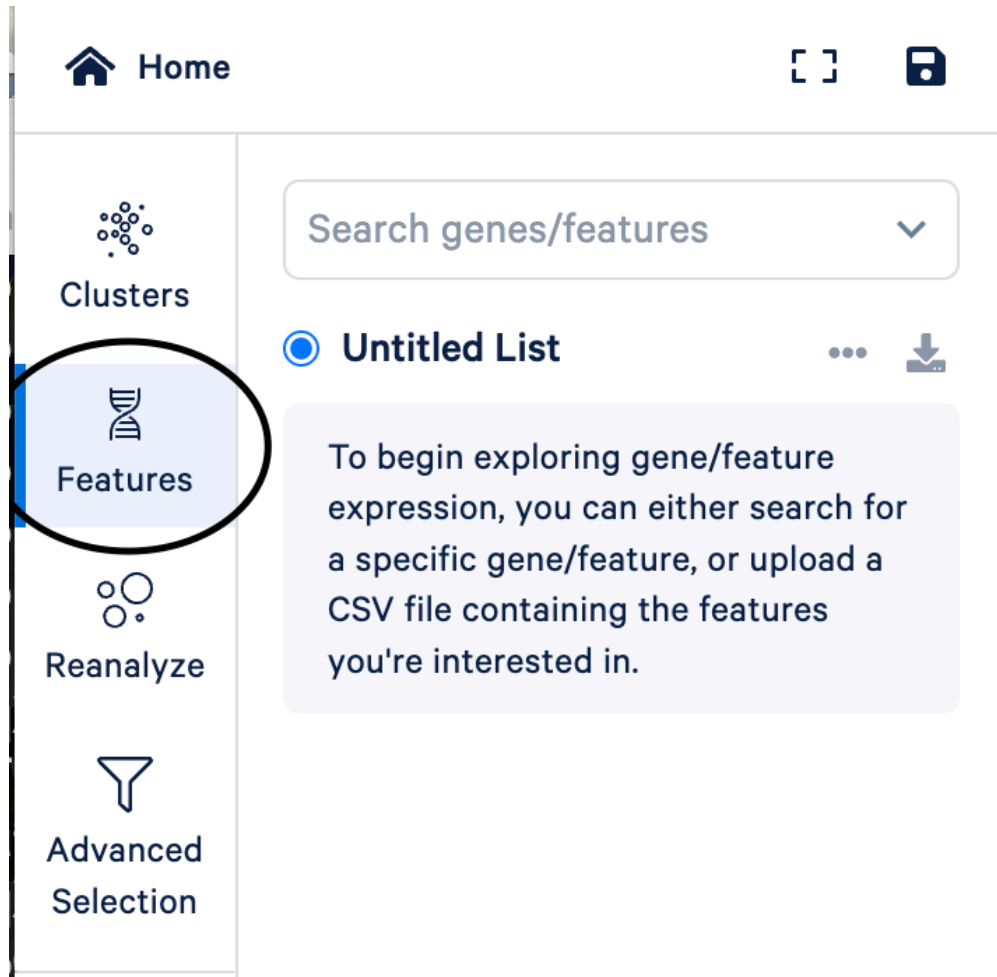
☐ All☐ Cluster 1 (100956)☐ Cluster 2 (73752)☐ Cluster 3 (64217)☐ Cluster 4 (48115)☒ Cluster 5 (43425)☐ Cluster 6 (40272)☐ Cluster 7 (36559)☐ Cluster 8 (35077)☐ Cluster 9 (32797)☐ Cluster 10 (32543)☐ Cluster 11 (17614)

8 Zoom in to see how closely clustering corresponds with the tissue morphology



## Transcript Localization and Mapping Cell Types

9 We can utilize the features tab to identify cell types using genetics markers.



- 10 Let's begin by viewing marker genes for goblet cells, which can be found in normal colon mucosal tissue. We can start creating a gene list.

Home

Clusters

Features

Reanalyze

Advanced Selection

Search genes/features

Untitled List

...

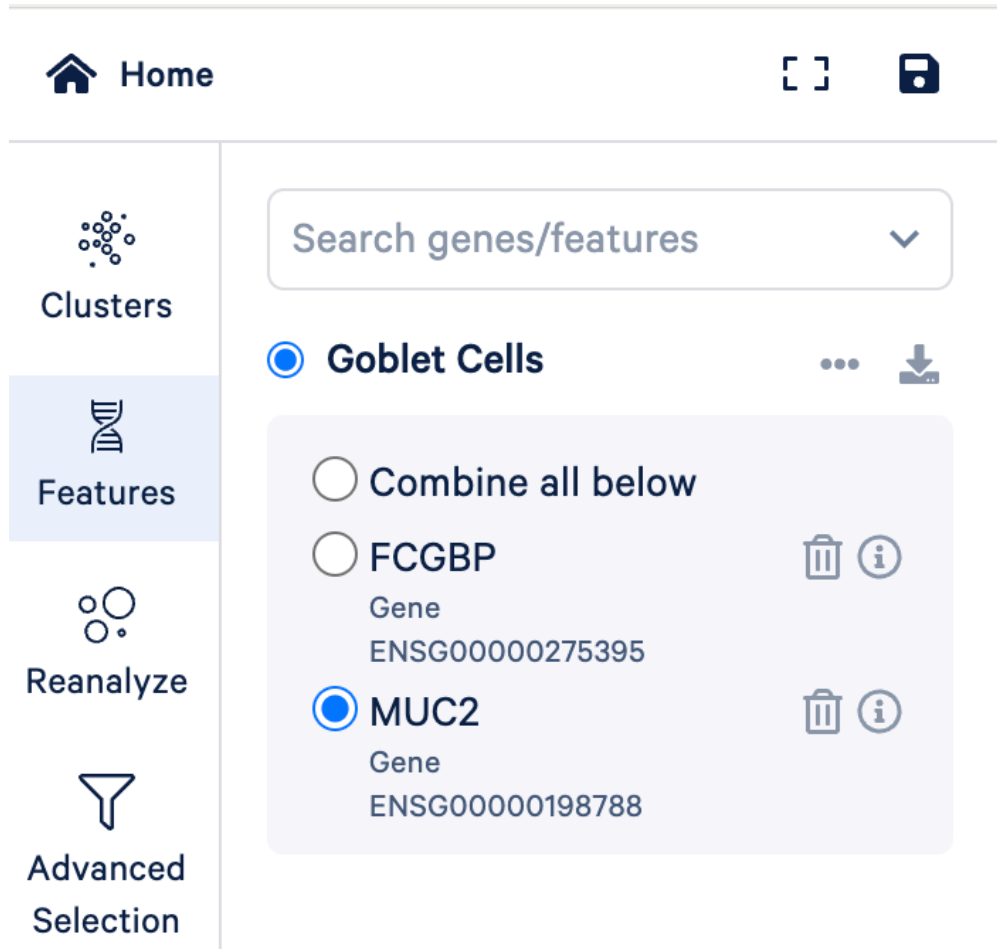
Edit list name

Delete list

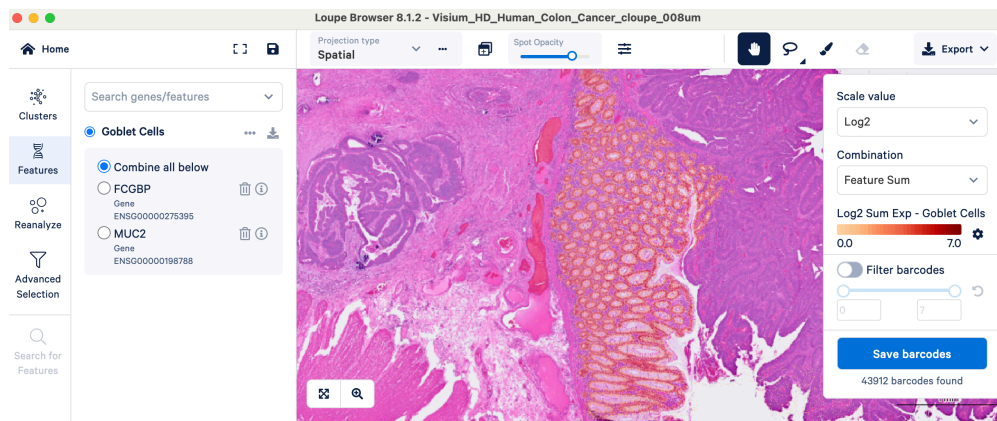
To begin exploring gene/feature expression, you can either search for a specific gene/feature, or upload a CSV file containing the features you're interested in.

Projection type  
Spatial

In the search bar type FCGBP and MUC2 to add them to our Goblet Cell marker list.

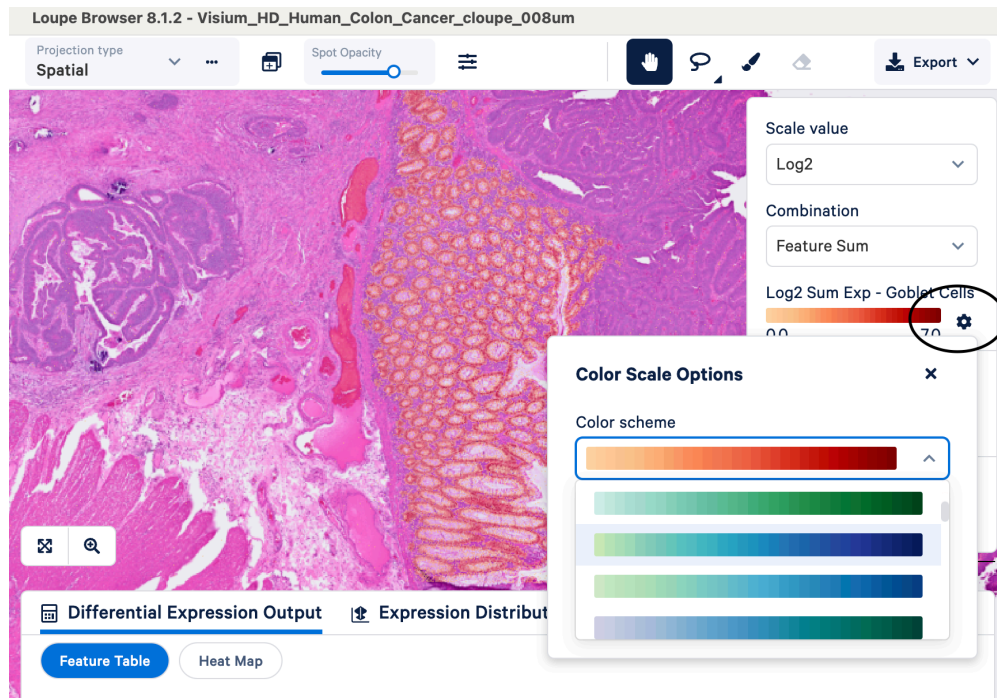


11 We can view each gene individually or combined.



To optimize visualization we can change the color scheme





We can also change the scale value to Linear to display UMI counts.

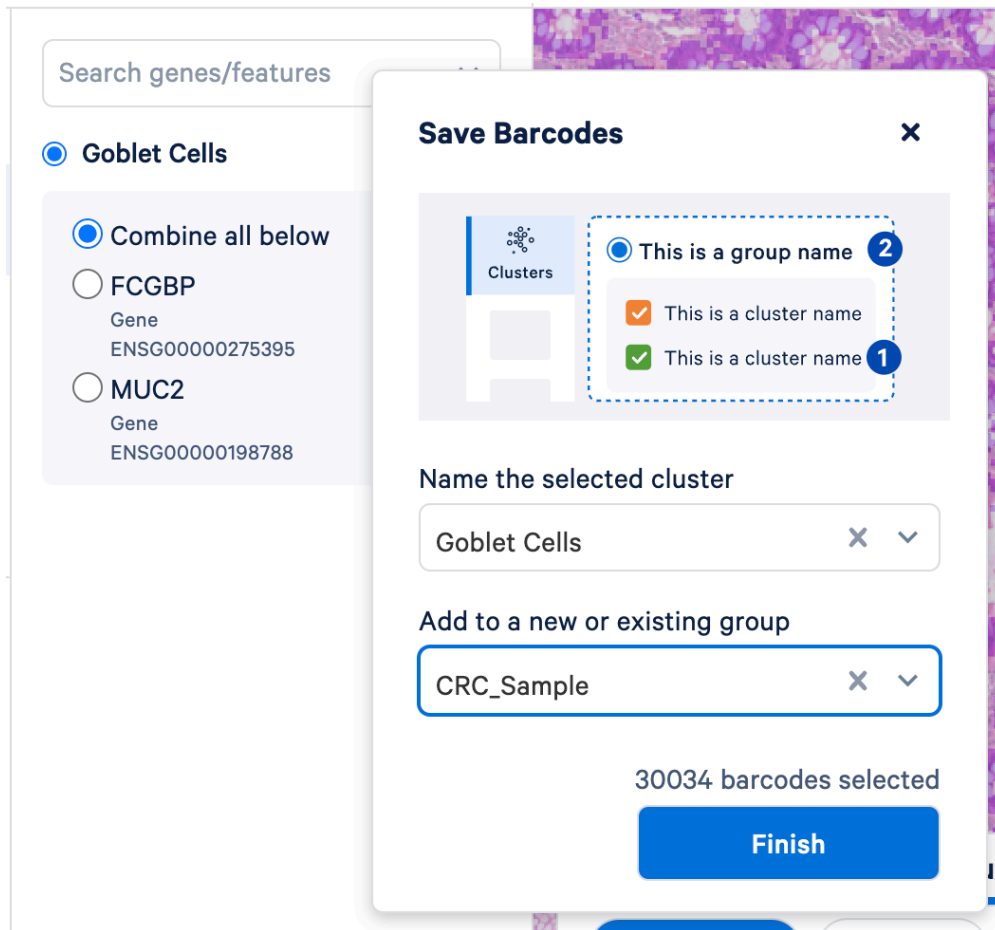


We can see that there are some bins scattered outside of the glandular tissue. We can filter barcodes to exclude bins with low UMI counts. If we filter barcodes by a minimum UMI count of 2, we can eliminate these bins.



12 Now that we have defined our goblet cells we can assign these barcodes to a cluster.

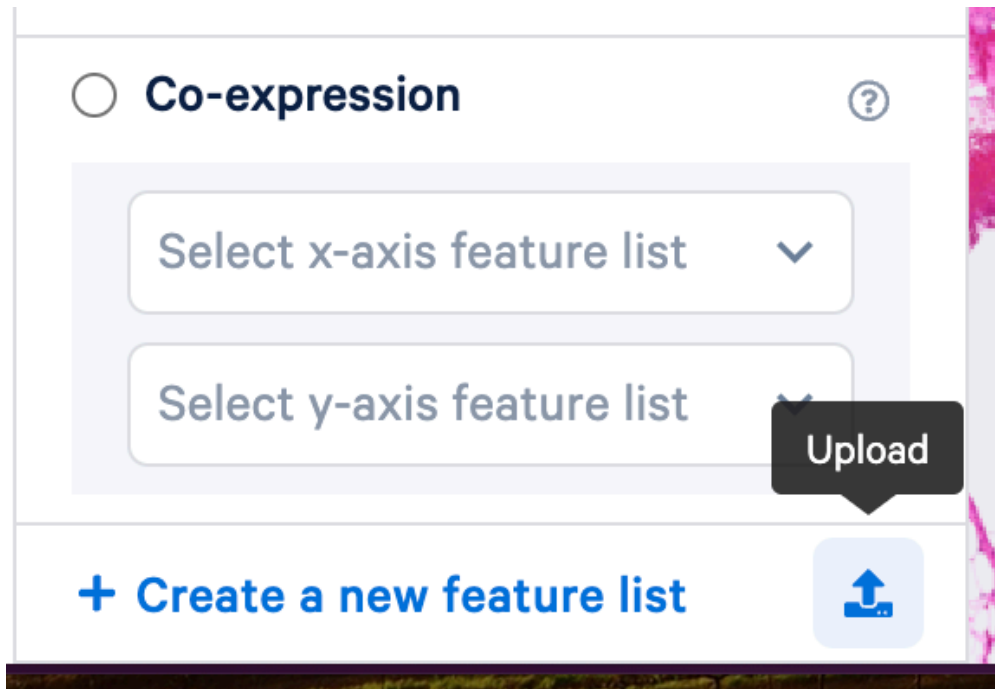




We now have a Goblet Cell cluster!



- 13 Let's identify another cell type based on a list of marker genes. This time, instead of searching for genes individually we will import a list from a csv file. Heading back to the Features tab we can select the upload option in **Create a new feature list**.



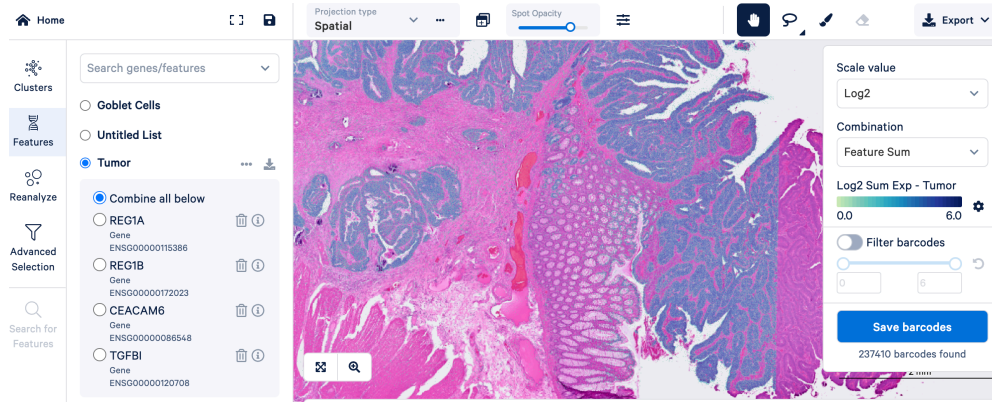
We will select a list of tumor markers to identify the tumor cells in our sample (this csv file is located in the same directory as the cloupe file)

```
\\SMDNAS02.urmc-  
sh.rochester.edu\GRC_Workshop\Visium_HD_Colorectal_Cancer_FFPE
```

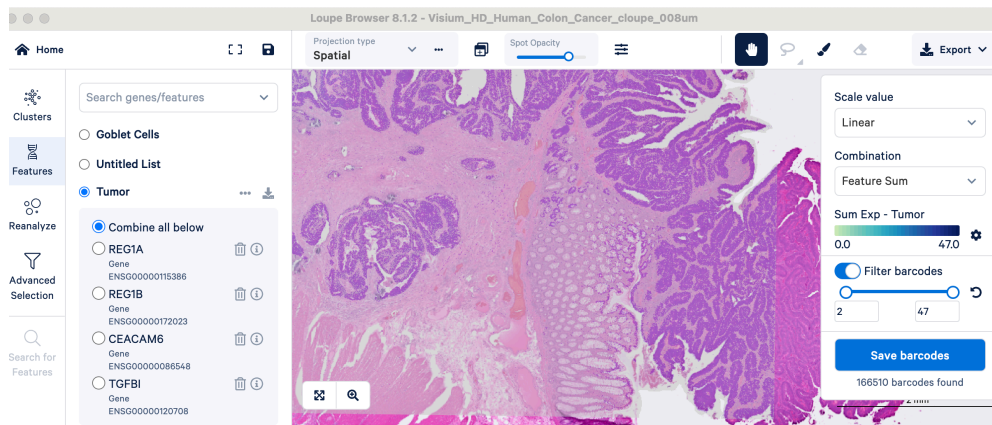
Or can be downloaded from the following link:

<https://cdn.10xgenomics.com/raw/upload/v1710907742/software-support/Spatial-GEX/LB-v8.0/hd-tutorial/Tumor.csv>

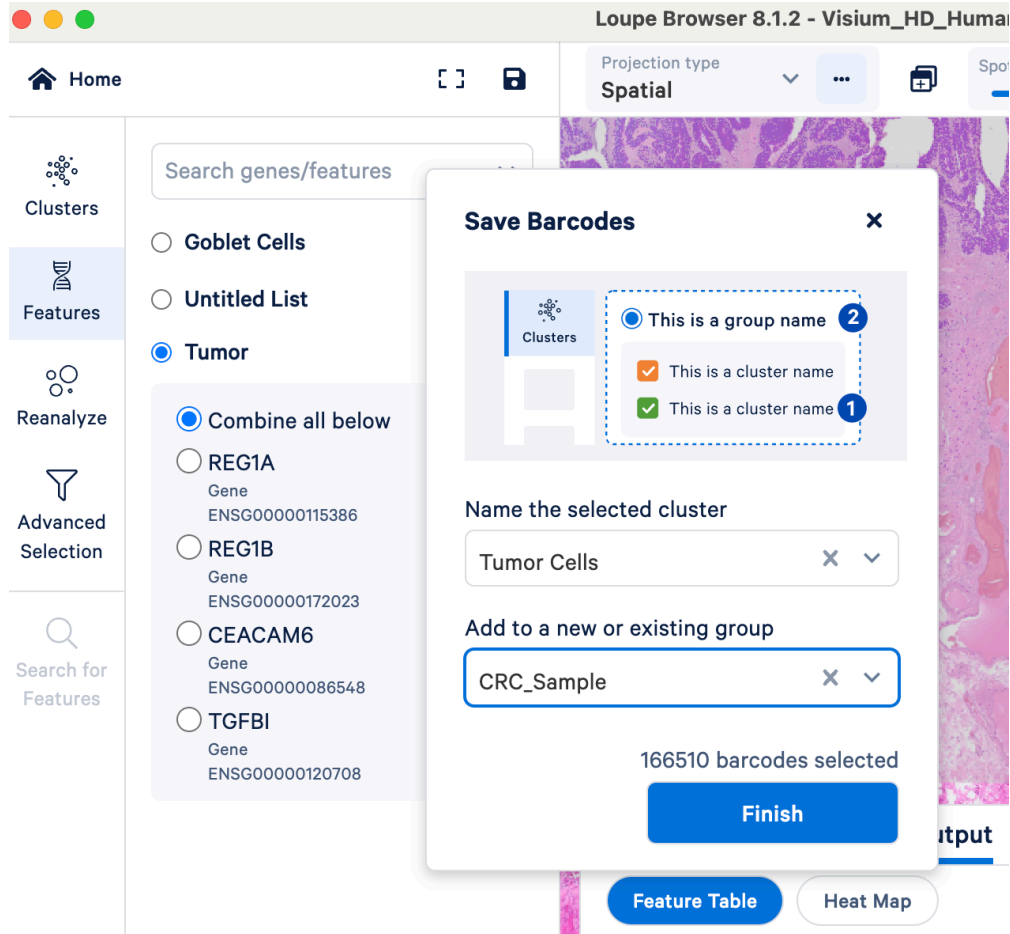
We can now see the areas that contain high levels tumor marker expression.



Let's change to the linear scale and filter barcodes.



Let's assign these barcodes to new cluster "Tumor Cells" within our CRC\_Sample group.



This step can be repeated to define additional cell types.

## Differential Expression and Co-expression

- 14 We can run differential expression between our tumor cell and goblet cell clusters by returning to the **Clusters** tab.
- 15 Unfortunately we cannot change the order of the clusters listed in our CRC\_Sample group. Since we typically want to see our differential expression results in context to our experimental group, let's quickly create a new group with the clusters listed as:
  1. Tumor Cells
  2. Goblet Cells

We will click on "Create a new group" and name it "Differential Expression".

 Home



Clusters



Features



Reanalyze



Advanced  
Selection



Search for  
Features

## Pipeline-generated groups

☐ Graph-Based

☐ K-Means

## Custom groups

☐ CRC\_Sample

☒ Differential Expression

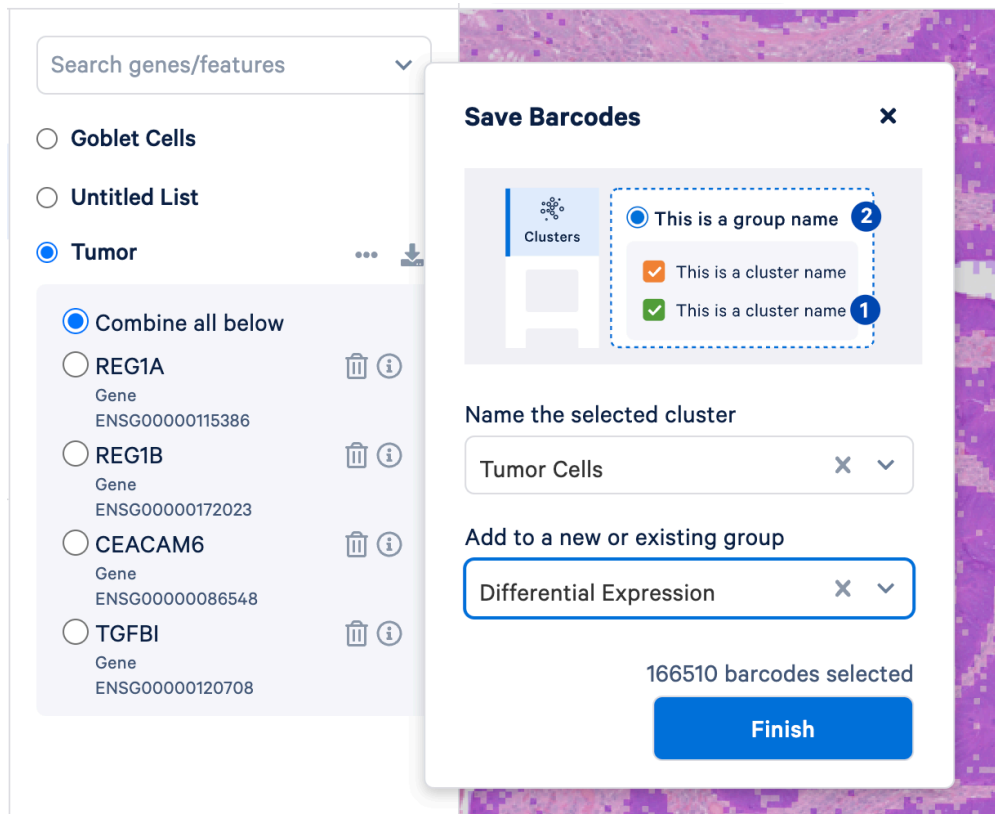


There are no cells assigned to this group at the moment.

[+ Create a new group](#)



- 16 Return to the Features tab, select the Tumor gene list, filter barcodes and save to the "Differential Expression" group.



Repeat the process with the Goblet cells.

- 17 With both the tumor and goblet cell clusters selected click on "Run Differential Expression" and then select the option to compare "Between selected cluster(s) themselves". Lastly click "Start analysis"



**Differential Expression Settings**

For more meaningful results, we recommend selecting specific clusters, and then comparing between the selected clusters themselves.

Compare all selected clusters in **Differential Expression**:

Between selected cluster(s) themselves

Feature type

Gene

**Start analysis**

**Differential Expression Output** **Expression Distribution**

**Feature Table** **Heat Map**

To perform an analysis to identify differentially expressed features:

1. From the "Clusters" tab, select the clusters that you wish to analyze.
2. Click on the "Run Differential Expression" button at the bottom and choose how you want to compare the results.
3. View the output in this panel.

We can now view the differential expression results to view genes that are upregulated in the Tumor cell cluster.

Differential Expression Output

Expression Distribution

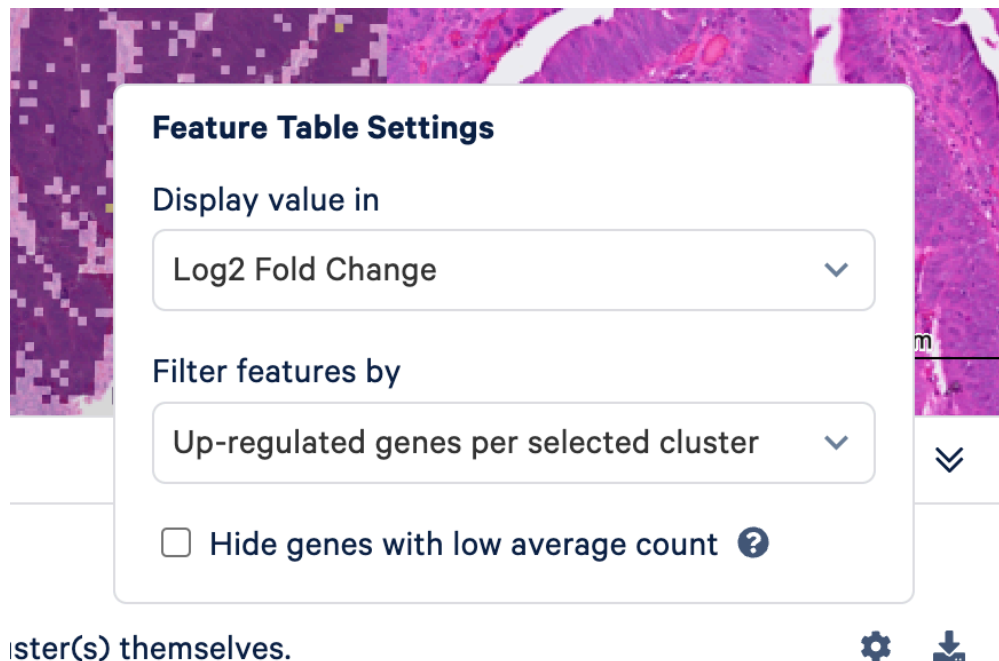
Feature Table

Heat Map

Up-regulated genes per selected cluster in group **Differential Expression**, compared between selected cluster(s) themselves.

| Feature  | Tumor Cells |           | Goblet Cells |  |
|----------|-------------|-----------|--------------|--|
| Name     | L2FC ⓘ      | P-Value ⓘ | L2FC ⓘ       |  |
| KRT23    | 6.02        | 1.27e-67  | -6.02        |  |
| SLC22A31 | 5.79        | 1.16e-60  | -5.79        |  |
| LY6G6D   | 5.60        | 7.22e-59  | -5.60        |  |
| ALPK3    | 5.41        | 8.55e-59  | -5.41        |  |
| NKX2-1   | 5.83        | 1.83e-58  | -5.83        |  |
| CKMT2    | 5.74        | 2.50e-56  | -5.74        |  |
| MME      | 5.25        | 5.00e-56  | -5.25        |  |
| SFTA3    | 6.08        | 6.59e-55  | -6.08        |  |
| GNG4     | 5.38        | 2.08e-54  | -5.38        |  |
| EREG     | 4.95        | 6.38e-54  | -4.95        |  |
| PTP4A3   | 4.88        | 2.38e-53  | -4.88        |  |
| TRIM72   | 5.94        | 8.67e-53  | -5.94        |  |
| NKD1     | 5.23        | 3.38e-51  | -5.23        |  |
| DPEP1    | 5.86        | 6.81e-51  | -5.86        |  |
| NRP2     | 4.85        | 7.49e-51  | -4.85        |  |
| NPSR1    | 5.70        | 2.21e-50  | -5.70        |  |
| IGF2BP1  | 5.78        | 6.46e-49  | -5.78        |  |
| STMN3    | 4.95        | 1.13e-48  | -4.95        |  |

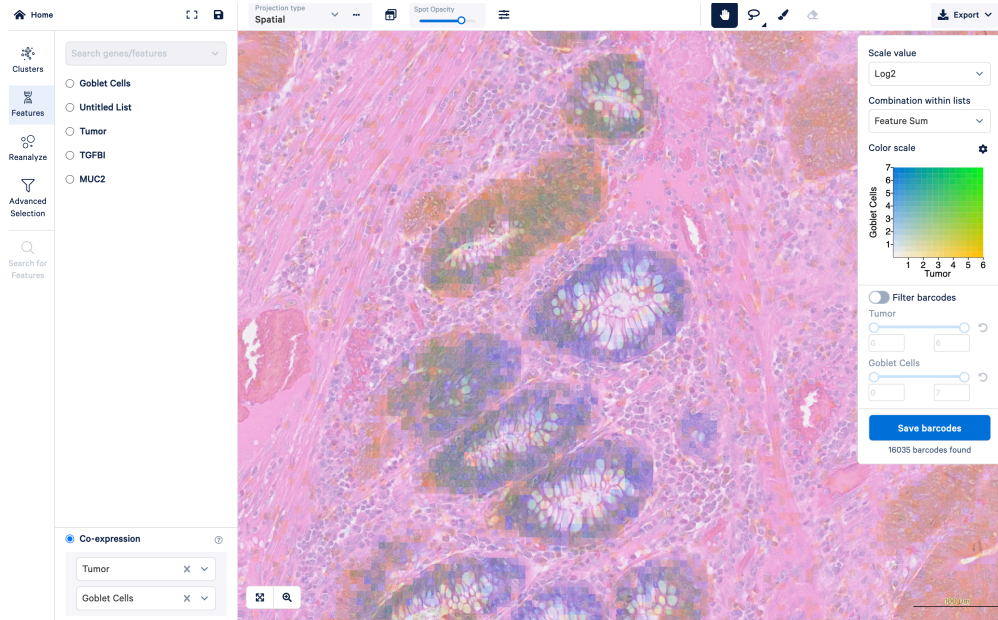
If desired you can change the Feature Table Settings by clicking on the gear icon.



You also have the option to export the Feature Table.



- 18 Next we will explore the Co-expression feature in Loupe Browser. Let's say we are interested in investigating areas of glandular tissue that have been infiltrated by tumor cells. We can look for bins where tumors markers and goblet cell markers are co-expressed.
- 19 Select the Co-expression feature and choose a cell type for each axis. We can now see the areas of glandular tissue that express high levels of Tumor and Goblet cell markers. The current view can be exported as a low resolution or high resolution image.



20 We can also display co-expression of specific genes. For example, say we are interested in identifying SPP1 + Macrophages. First we will need to add the individual genes in the Features tab. Click on "Create a new feature list", search for LYZ (a Macrophage marker) in the search bar and select. We can rename the list "LYZ". Repeat this process for SPP1.

21 As in step 19, we can now choose the individual genes for the X and Y axis



 Home



Clusters



Features



Reanalyze



Advanced  
Selection



Search for  
Features

Search genes/features



☐ Goblet Cells

☐ Untitled List

☐ Tumor

☐ TGFBI

☐ MUC2

☒ **Co-expression**



TGFB1

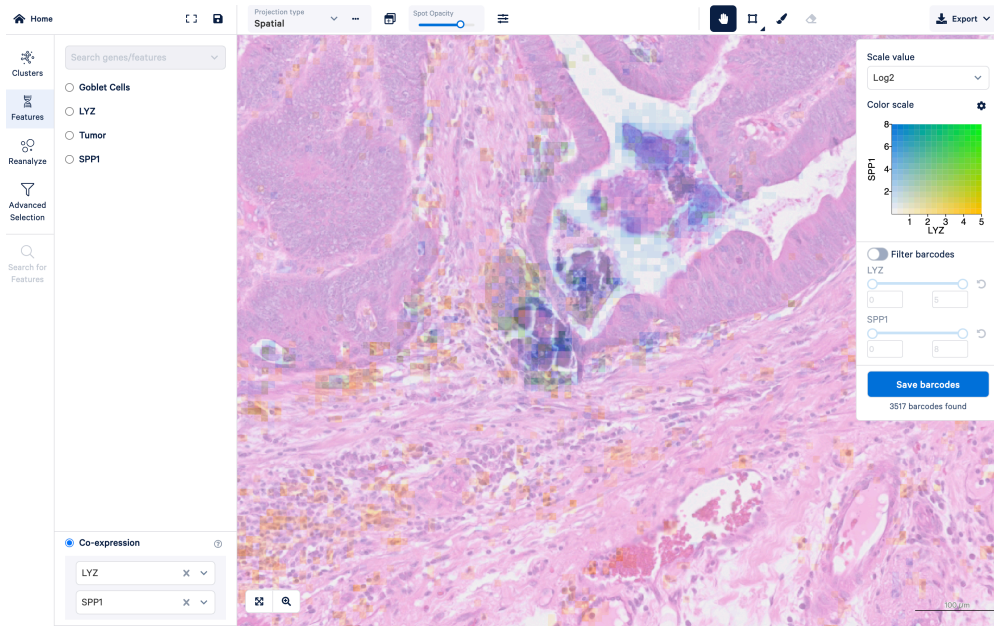


MUC2



**+ Create a new feature list**

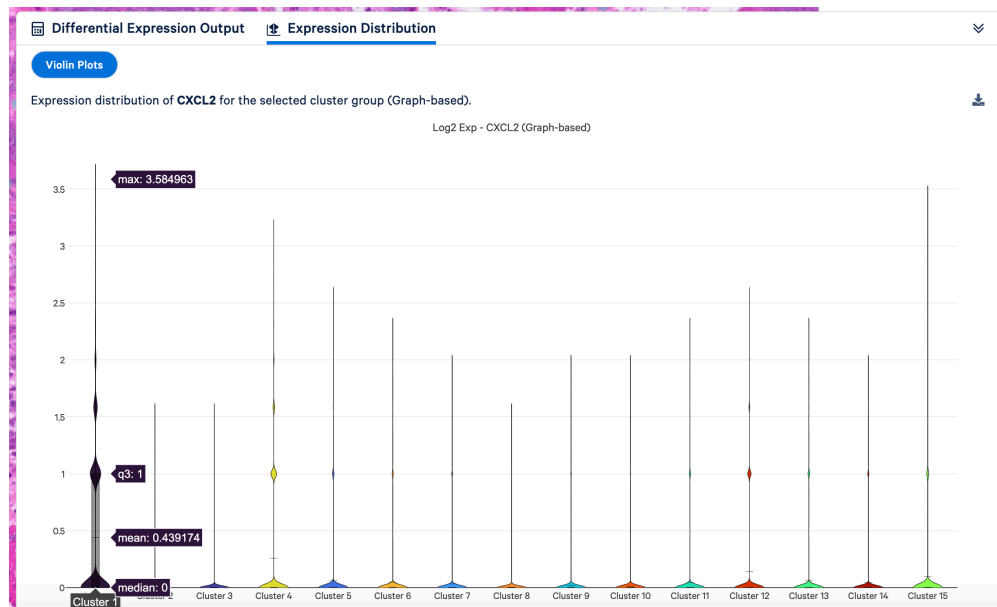




## Expression Distribution

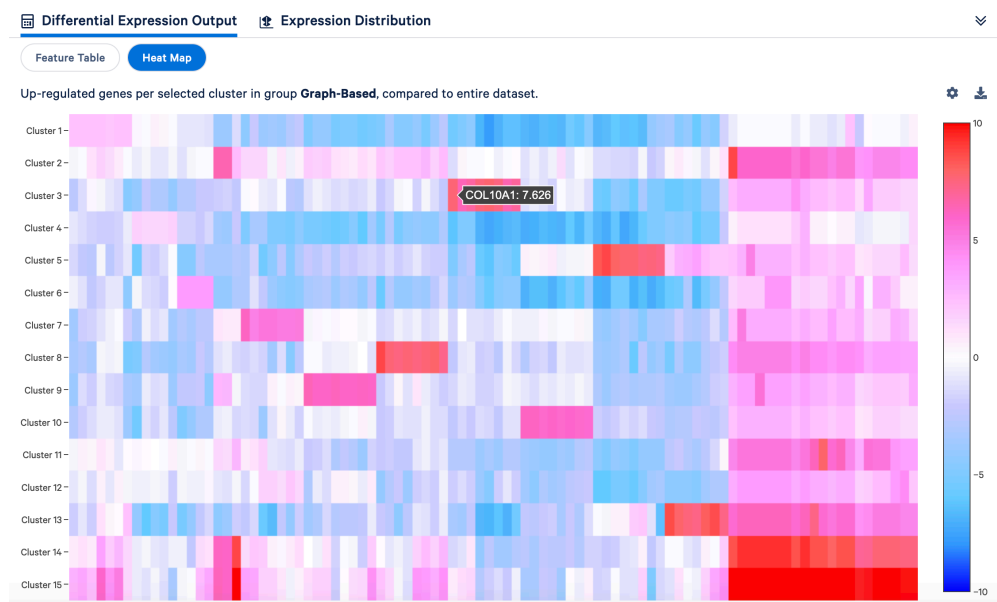
- 22 Lastly we will touch on visualizing expression distribution. In Loupe Browser we can create heat maps and violin plots that display the expression of a feature(s) across clusters.
- 23 To create a violin plot we can return to the Features tab, and create a new feature list and search for a gene of interest using the search bar. Today we will look at the expression distribution of CXCL2.





This plot is interactive and will display more detailed expression data when hovering over a cluster. This plot can also be exported as a high or low resolution image.

- 24 To create a heat map including several clusters, we will move back to the Cluster tab. With your clusters of interest selected simply click on the Heat Map button to view gene expression across these clusters. This plot is also interactive and will display the gene name when hovering over sections of the heat map.





You have the option to filter features by selecting the gear icon, and this plot can also be exported as a png or svg image.

## Advanced Features

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- 25 Assistance with the use of the **Reanalyze** and **Advanced Selection** features will be provided during the hands-on session.