

# visAPPprot Usage Instructions

visAPPprot provides alternative visualizations for common omics (e.g., RNAseq; Genomic or Proteomic Microarrays) outputs. In the following pages we detail instructions for using our omics visualization application visAPPprot.

Excluding installation of the application, this document should take about 50 minutes to complete. The estimate for time required to complete each section is noted at the beginning of each section.

## 1. Run visAPPprot

visAPPprot is run through a Chrome browser at localhost:8888. You should see the screen below. **Make sure your Chrome browser is at full screen and your window is in “normal” mode AKA not incognito.**

visAPPprot

\*If you need to compute an expression matrix select the column of values to use. Skip if you have prepared an expression matrix already.

Block

**Compute ExpMat**

Inputs required for all processes:

\*Dataset: PatientCharacter1.csv

\*Expression matrix: ExpMat1.csv

\*Analysis method type: Generalized Linear Model (GLM)

\*Level 1: Control

\*Level 2: Disease

**Volcano Plot** **Pathway Map** **Heatmap**

## 2. Omics Visualization

Two demo autoantibody (protein) microarray datasets are provided for you that consists of control samples and disease samples. In this document we refer to Toy Dataset 1, which includes ExpMat1.csv and PatientCharacter1.csv. Since you are given a pre-computed expression matrix for the examples in this document, you will not need the “Compute ExpMat” option. Follow the instructions under the bold **TODO**.

## 3. Compute Expression Matrix

The “Compute ExpMat” button allows you to compute an expression matrix based on any column in your microarray data. The columns of your microarray data are listed in the dropdown menu above the “Compute ExpMat” button. After your expression matrix is computed, it will show up in the dropdown list for “Expression matrix.” The “Compute ExpMat” option is an alternative to providing your own expression matrix; if you already have an expression matrix CSV file it will appear in the dropdown list for “Expression matrix.”

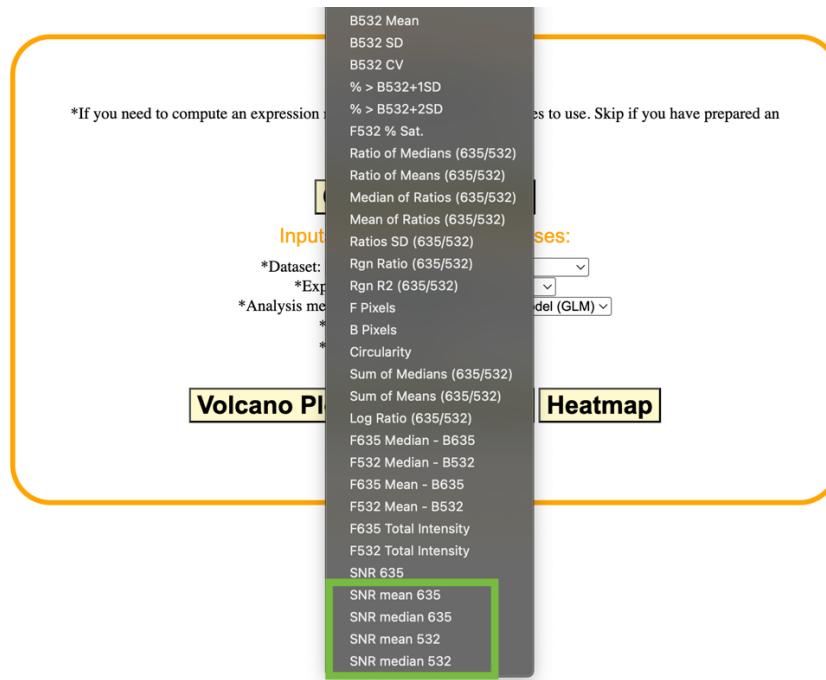
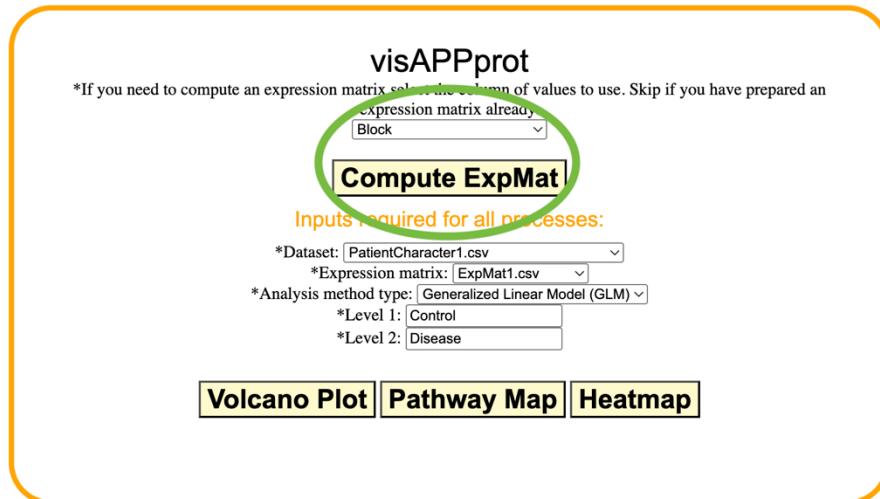
The steps below detail how to compute an expression matrix using columns from your own microarray data.

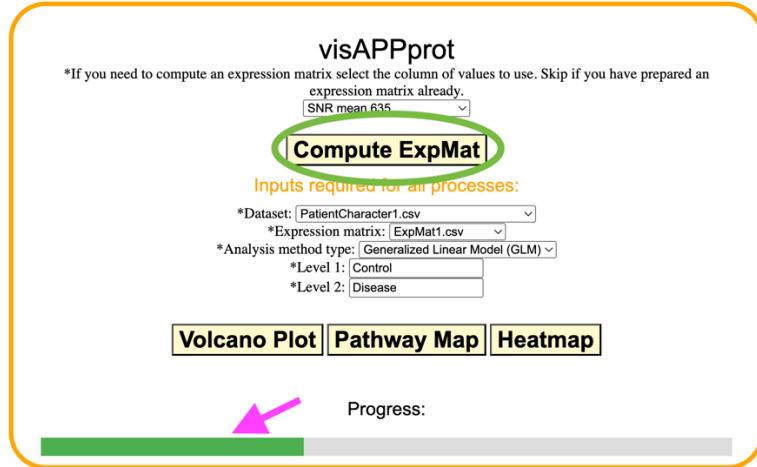
**TODO:** On the application home page please do the following:

- Click field above the “Compute ExpMat” button to display a dropdown menu with all columns of the microarray data. If your *microarray\_data/* folder is empty and contains no microarray data files, then the dropdown menu will be disabled.
- Select any of the columns you would like to use to compute an expression matrix. If your microarray data contains both F635 and B635 columns, you will also see options for “SNR median 635” and “SNR

mean 635." If your microarray data contains both F532 and B532 columns, you will also see options for "SNR median 532" and "SNR mean 532."

- Click the yellow button for "Compute ExpMat." A green progress bar is displayed at the bottom of the window to indicate how much work remains in this process.
- When the expression matrix is computed successfully, you will be shown a button to go "Home" to the application home page again. Now, when you click on the dropdown menu for *Expression matrix* you should see your newly computed expression matrix, named with the column of the data you selected.





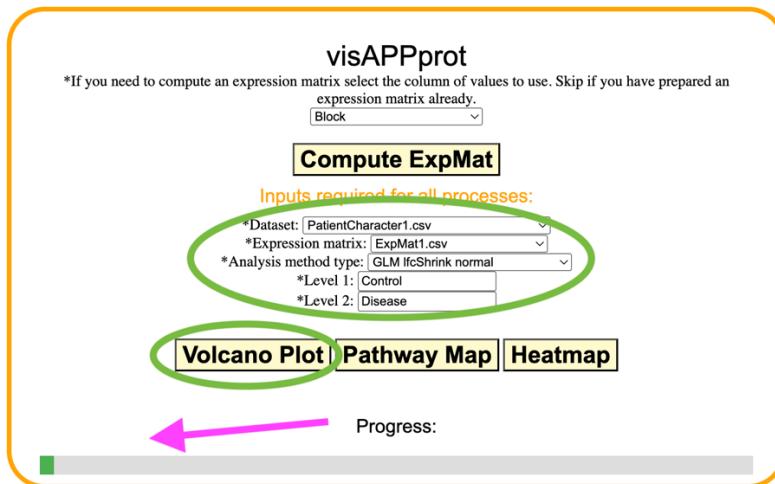
Since you are given a pre-computed expression matrix for the examples in this document, you will not need to use the “Compute ExpMat” functionality to complete the examples in this manual. The instructions above are for when you wish to compute expression matrices on your own dataset.

## 4.1 Volcano Plot

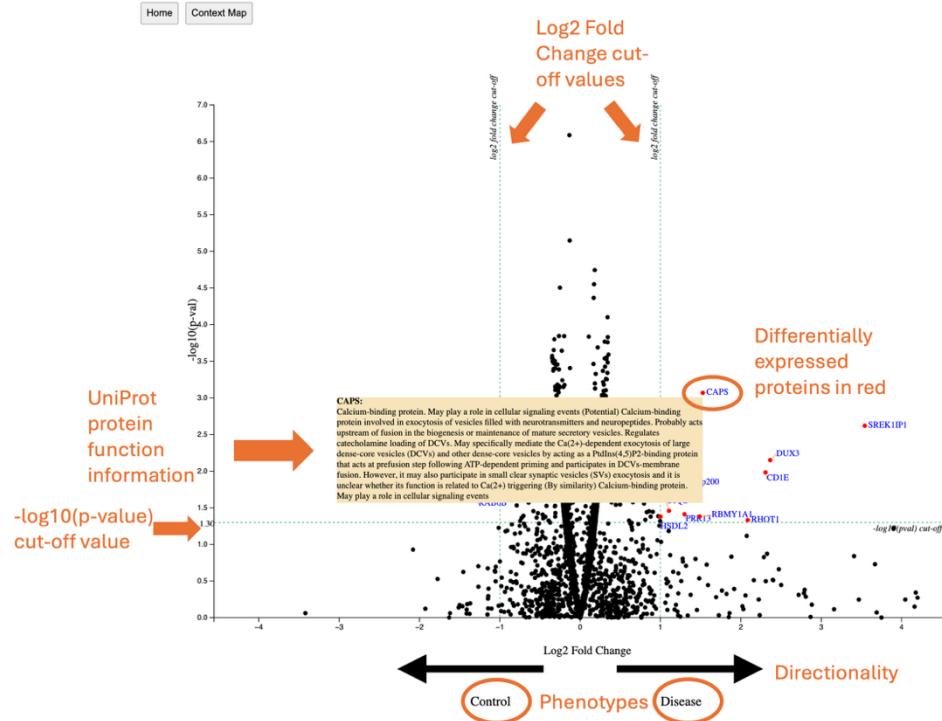
*(Time Estimate: 3 minutes)*

**TODO 1:** On the application home page please do the following:

- Select your desired dataset and corresponding expression matrix. In this example, we use Toy Dataset 1, which include PatientCharacter1.csv and ExpMat1.csv
- Specify which analysis method you wish to use. In this example, we use GLM IfcShrink normal. (Note that not all datasets are compatible with all analysis methods; you will have to determine the appropriate method for your own datasets.)
- Specify names of phenotypes (levels) to be compared to evaluate differential expression. For this demo dataset, the two phenotypes (levels) are Level 1 = Control and Level 2 = Disease. Type these names in the appropriate boxes shown below.
- Click the yellow button for “Volcano Plot.” A green progress bar is displayed at the bottom of the window to indicate how much work remains in this process.



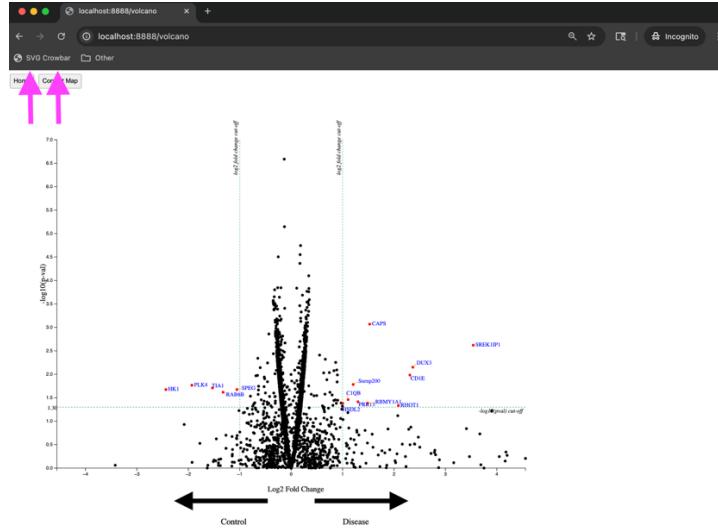
When the process is complete, the volcano plot will be displayed. The volcano plot may take up to 5 seconds to load once the web page changes.



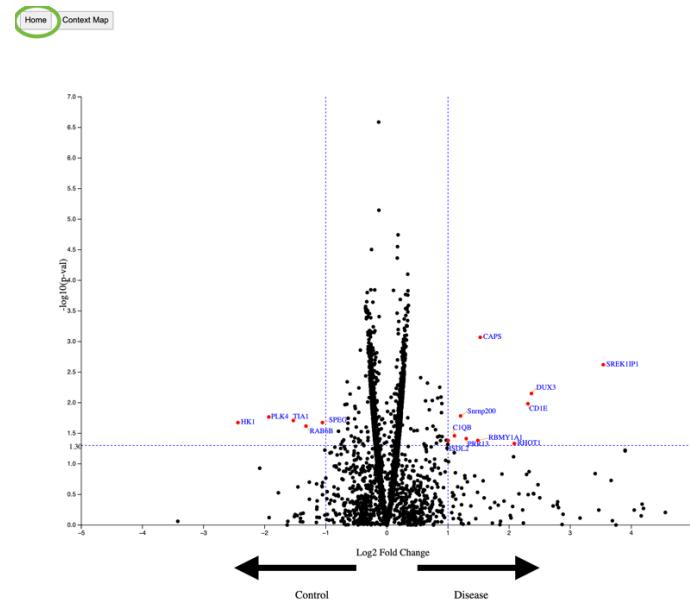
### Volcano plot features:

- Differentially expressed autoantibodies are marked with a red dot and labeled in blue with the corresponding gene names instead of protein names as is a common practice in the field.
- Phenotypes are labeled on the X-axis along with directionality of the expression. Autoantibodies that are expressed higher in the Control samples than the Disease samples are located to the left of 0.0 (i.e., expressed as negative log2-fold change). Autoantibodies that are expressed higher in the Disease samples than the Control samples are located to the right of 0.0 (i.e., expressed as positive log2-fold change).
- Y-axis represents -log<sub>10</sub> of p-value with a cutoff value of p<0.05 and the X-axis represents the log<sub>2</sub> fold change with a threshold of the absolute value of 1. Dashed blue vertical and horizontal lines mark the thresholds.
- Clicking on a differentially expressed autoantibody (red dot) will display its function information from the UniProt database.

**TODO 2:** Download figure:



- Click on SVG-Crowbar in bookmarks tab, shown by the arrows in the figure below.
- Click on the *static/download\_imgs\_PatientCharacter1* folder under your *visAPPprot\_mac*/ directory (if using Mac) or your *visAPPprot\_windows*/ directory (if using Windows) and drag the *volcano\_PatientCharacter1\_0.svg* file to your Chrome browser. The file will display in the Chrome browser. It will be a copy of the same image that was in the visAPPprot. When you are finished examining the volcano plot svg file, close only the tab containing the svg file for the volcano plot.
- Return to the localhost:8888 tab. Click the Home button on the top left of the visAPPprot volcano plot page to go back to the main visAPPprot page. You may have to scroll up to the top of the page to see the Home button. Please see figure below.



## 4.2 Pathway Map

(Time Estimate: 10 minutes)

**TODO 3:** On the application home page please do the following:

- Select your desired dataset and corresponding expression matrix. In this example, we use Toy Dataset 1, which include PatientCharacter1.csv and ExpMat1.csv

- Specify which analysis method you wish to use. In this example, we use GLM IfcShrink normal. (Note that not all datasets are compatible with all analysis methods; you will have to determine the appropriate method for your own datasets.)
- Click the yellow button for “Pathway Map.” Additional input will appear: these are all the individual pathways you can include in your pathway map. Click a single pathway to select it. Hold the “Cmd” key (if using Mac) or the “Ctrl” key (if using Windows) while clicking on sequential pathways to select multiple pathways. To select all pathways as shown in the following image, click on a single pathway and then hold down the “Cmd” + “A” keys (if using Mac) or the “Ctrl” + “A” keys (if using Windows) on your keyboard. You should see all the pathways highlighted now.
- Click the Submit button and a progress bar will appear to indicate how much of the process remains.
- Note that Level 1 and Level 2 are automatically filled in for you, after you specified it for the previous visualization.

**visAPPprot**

\*If you need to compute an expression matrix select the column of values to use. Skip if you have prepared an expression matrix already.

**Compute ExpMat**

**Inputs required for all processes:**

\*Dataset:   
 \*Expression matrix:   
 \*Analysis method type:   
 \*Level 1:   
 \*Level 2:

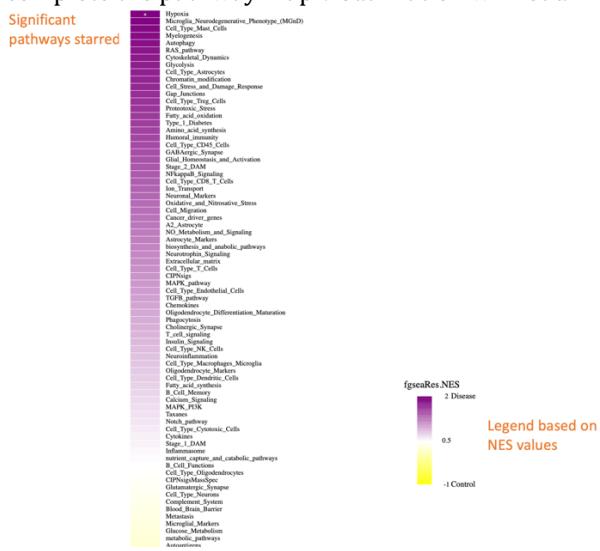
**Volcano Plot** **Pathway Map** **Heatmap**

**Additional input required for pathway map:**

Pathway data:

**Submit**

When the process is complete the pathway map visualization will load.



#### Pathway map features:

- Normalized enrichment scores (NES) values are presented in the pathway map. Pathways that are enriched in Disease patients are in ranges of purple and those that are enriched in Control patients are

in ranges of yellow. \*p<0.05, \*\*p<0.01, \*\*\*p<0.001, \*\*\*\*p<0.0001.

- Scroll to see full image.

**TODO 4:** Download figure:

- Click on SVG-Crowbar in bookmarks tab.
- Click on the *static/download\_imgs\_PatientCharacter1* folder under your *visAPPprot\_mac/* directory (if using Mac) or your *visAPPprot\_windows/* directory (if using Windows) and drag the *pathway\_PatientCharacter1\_0.svg* file to your Chrome browser. The file will display in the Chrome browser. It will be a copy of the same image that was in the visAPPprot. When you are finished examining the pathway map svg file, close only the tab containing the svg file for the pathway map.
- Return to the localhost:8888 tab. Click the Home button on the top left of the visAPPprot pathway map page to go back to the main visAPPprot page. You may have to scroll up to the top of the page to see the Home button.

## 4.3 Heatmap

The tasks for the heatmap will require repeating the same procedure 2 times, with slightly different input options each time.

### 4.3.1 Normalized data heatmap, Row and Column clustering

(Time Estimate: 8 minutes)

**TODO 5:** On the application home page please do the following:

- Select your desired dataset and corresponding expression matrix. In this example, we use Toy Dataset 1, which include PatientCharacter1.csv and ExpMat1.csv
- Specify which analysis method you wish to use. In this example, we use GLM IfcShrink normal. (Note that not all datasets are compatible with all analysis methods; you will have to determine the appropriate method for your own datasets.)
- Note that Level 1 and Level 2 are automatically filled in for you, after you specified it for the previous visualization.
- Click on the yellow “Heatmap” button to see input options for generating the heatmap.
- Select the *Normalized* option under “Use Raw or Normalized data?”
- Select *Both Rows and Columns* from the “Choose clustering type” dropdown.
- Click yellow button for “Submit”. The heatmap will take time to load. Progress can be monitored on the progress bar.

The screenshot shows the visAPPprot application interface. At the top, there is a note: "\*If you need to compute an expression matrix select the column of values to use. Skip if you have prepared an expression matrix already." Below this is a dropdown menu labeled "Block". A large orange box encloses the "Compute ExpMat" section. This section contains the following fields:

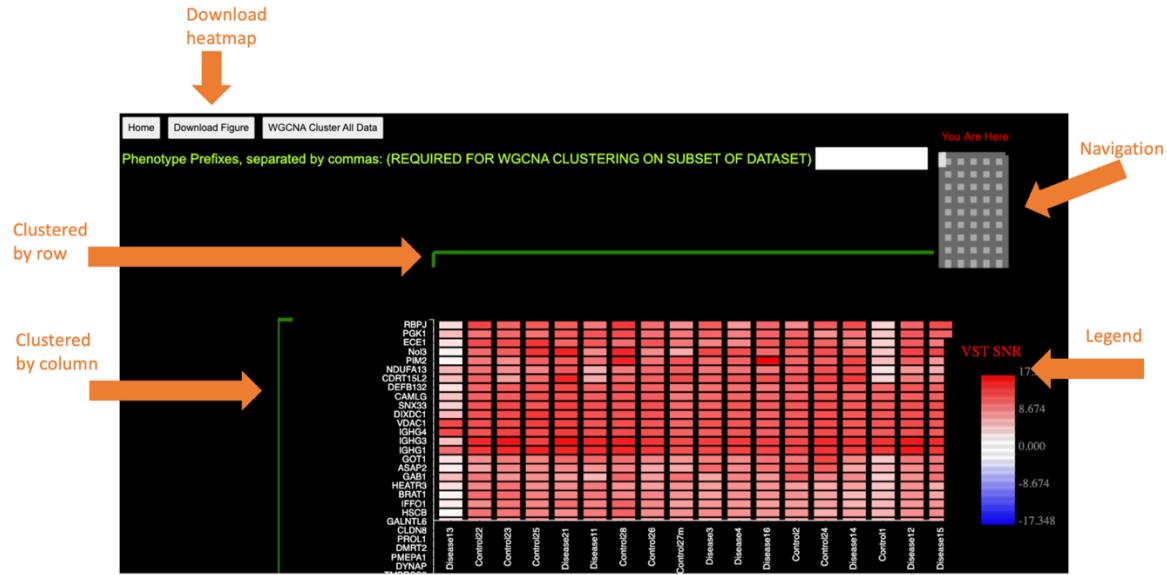
- \*Dataset: PatientCharacter1.csv
- \*Expression matrix: ExpMat1.csv
- \*Analysis method type: GLM IfcShrink normal
- \*Level 1: Control
- \*Level 2: Disease

Below this section, there are three tabs: Volcano Plot, Pathway Map, and Heatmap. The Heatmap tab is highlighted with a green oval. Underneath the tabs, there is a note: "Additional input required for heatmap:". This section includes:

- Use Raw or Normalized data?  
○ Raw  
● Normalized (this option is selected)
- Choose clustering type: Both Rows and Columns

A large green oval encircles the "Submit" button at the bottom of this section.

## Heatmap:



### General Heatmap features:

- **Heatmap**: displays all autoantibodies for each patient in the dataset. This dataset contains 14,396 autoantibodies and 18 patients. The data is presented as raw expression or as a normalized expression using a standard variance-stabilizing transformation (VST) for normalization, depending on the option selected on the home page.
- **Axes**: Y-axis shows individual autoantibodies and X-axis shows individual patients. Axes will float with your window as you scroll.
- **Clustering**: a vertical green bar is displayed to the left of the heatmap if “cluster by row” was selected on the Home page, and a horizontal green bar is displayed above the heatmap if “cluster by column” was selected on the Home page.
- **Navigation**: A gray and white grid on the right-hand side labeled “You Are Here” shows your current location in the larger heatmap. The white rectangular marker within the grid moves as you scroll.
- **Legend**: legend on the right-hand side shows the range of values and associated colors in the heatmap.
  - Notes:
    - \* For raw data, the range of values is much larger than the median. Therefore, we used the max value to display the 99th percentile of the actual values.
    - \* For normalized data, the significant values are those outside of the range of the absolute value of 1. Therefore, all values between -1 and +1 are displayed as 0.
- **Autoantibody Search**: Use “Cmd key+F” (if using Mac) or “Ctrl key+F” (if using Windows) to search for specific autoantibodies. Again, note that the gene nomenclature is used instead of protein names. Therefore, use the gene name to search for the protein of interest.

### TODo 6: Explore the heatmap:

- Scroll down and across the heatmap. Notice the elements of the heatmap that move across the window as you scroll. Notice the marker on the Navigation map move as you scroll.
- Explore patterns of autoantibody expression in the heatmap. Recommend using zooming out of the image to reduce the size from 100% to 50% to see the expanse of the heatmap which will better highlight the patterns.
- Search for your favorite autoantibodies using “Cmd key+F” (if using Mac) or “Ctrl key+F” (if using Windows). Remember to use the gene name as the surrogate for the protein.



#### **TODO 7:** Download heatmap:

- Go back to the localhost:8888 tab in the Chrome browser. Scroll up the top of the page if you have not already done so. Click the Download Figure button until it turns yellow. The figure will be saved to the *static/download\_imgs\_PatientCharacter1* folder as *heatmap\_PatientCharacter1\_normalized\_0.svg*. When the download is complete, the page will reload the heatmap.
- Open the svg. It should be similar to the image that you just saw in the application. When you are finished examining the heatmap svg file, close the tab containing the svg file.
- Return to the localhost:8888 tab. Click the Home button on the top left of the visAPPprot heatmap page to go back to the main visAPPprot page. You may have to scroll up to the top of the page to see the Home button.

#### **4.3.2 Raw data heatmap, Row clustering**

(Time Estimate: 5 minutes)

#### **TODO 8:** On the application home page please do the following:

- Select your desired dataset and corresponding expression matrix. In this example, we use Toy Dataset 1, which include PatientCharacter1.csv and ExpMat1.csv
- Specify which analysis method you wish to use. In this example, we use GLM lfcShrink normal. (Note that not all datasets are compatible with all analysis methods; you will have to determine the appropriate method for your own datasets.)
- Note that Level 1 and Level 2 are automatically filled in for you, after you specified it for the previous visualization.
- Select the *Raw* option under “Use Raw or Normalized data?”
- Select *Rows* from the “Choose clustering type” dropdown.
- Click yellow button for “Submit”. The heatmap will take time to load. Progress can be monitored on the progress bar.

**Heatmap features:** described above.

**TODO 9:** Download heatmap:

- Click the Download Figure button until it turns Yellow. The figure will be saved to the *static/download\_imgs\_PatientCharacter1* folder as *heatmap\_PatientCharacter1\_unnormalized\_1.svg*. When the download is complete, the page will reload the heatmap.
- Open the svg. It should be similar to the image that you just saw in the application. When you are finished examining the heatmap svg file, close the tab containing the svg file.
- Return to the localhost:8888 tab. **PLEASE STAY ON THIS TAB.**

## 4.4 WGCNA Clustering

In the following tasks we will explore weighted gene (or protein) co-expression network analysis (WGCNA) of the dataset. First, pairwise correlation is determined for each possible autoantibody pair in the expression dataset. These pairwise correlations are then represented as network modules defined using clustering analysis. In essence the modules consist of groups of autoantibodies that are co-expressed.

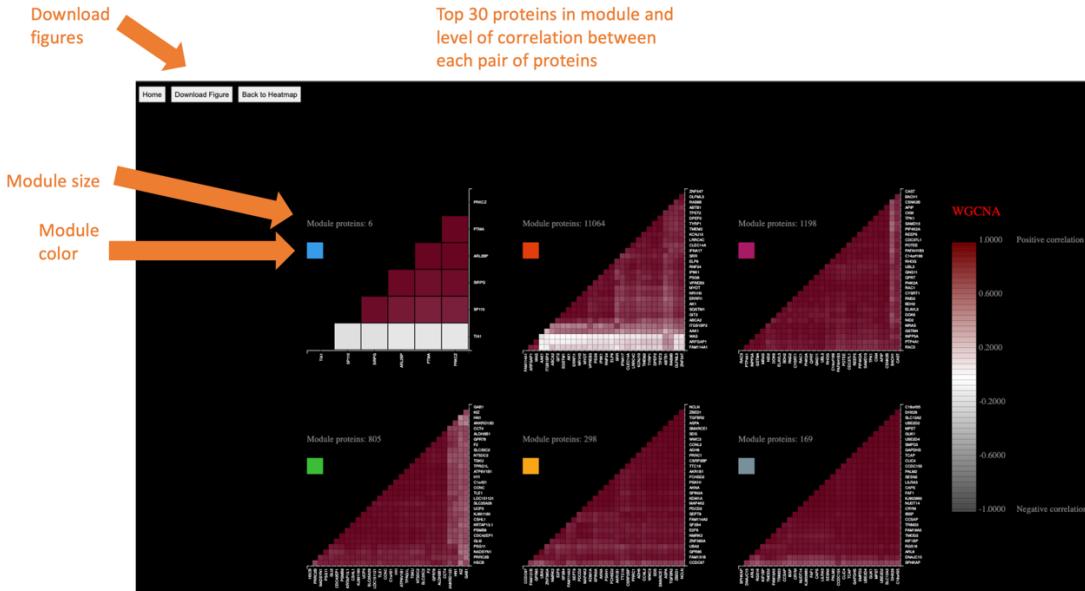
### 4.4.1 Clustering over entire dataset

(Time Estimate: 11 minutes)

**TODO 10:** From the current unnormalized (i.e., raw) heatmap clustered by row *visAPPprot* page, click on the "WGCNA Cluster All Data" button on the top left of the page. The button will turn Yellow. Progress can be monitored on the progress bar.

There are 3 parts to the WGCNA clustering visualizations: (1) modules, (2) dendrogram, and (3) heatmap. The 3 figures are related by the color-encoding of the modules. Below is a description of features of the WGCNA figures.

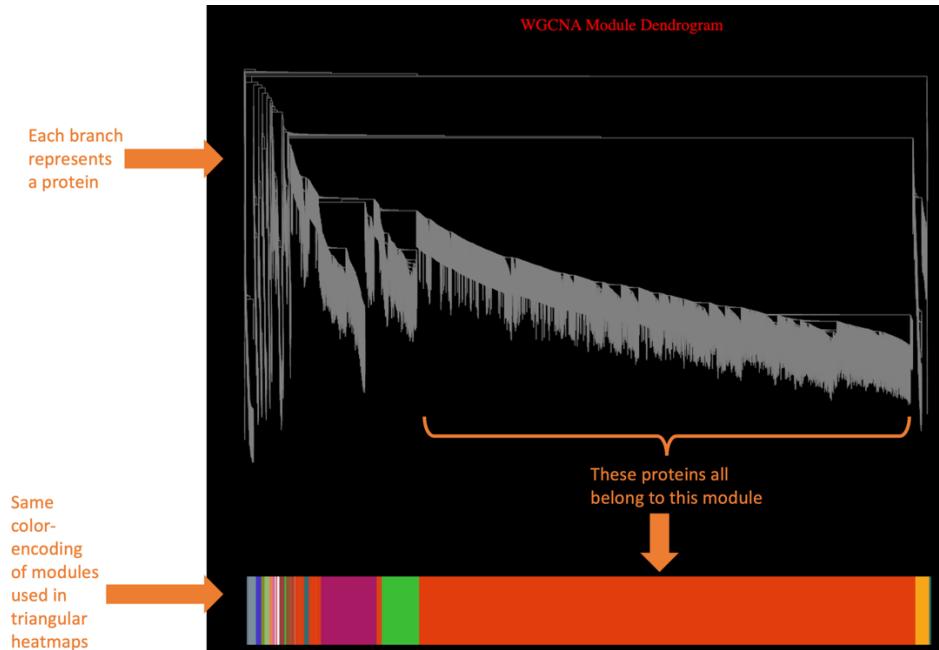
#### Modules



Each upper triangular heatmap shows the top 30 most significant autoantibodies using Pearson correlation for that module and any clustering within the module. The module color is shown in a square to the left of the triangular heatmap, as well as the total number of autoantibodies in the module. [Heatmap Legend](#): dark grey = high negative correlation, white = no correlation, and deep red = high positive correlation.  
 Note that not all modules will be composed of 30 autoantibodies; for modules with under 30 autoantibodies the total number of autoantibodies in the module will be displayed in the triangular heatmap.

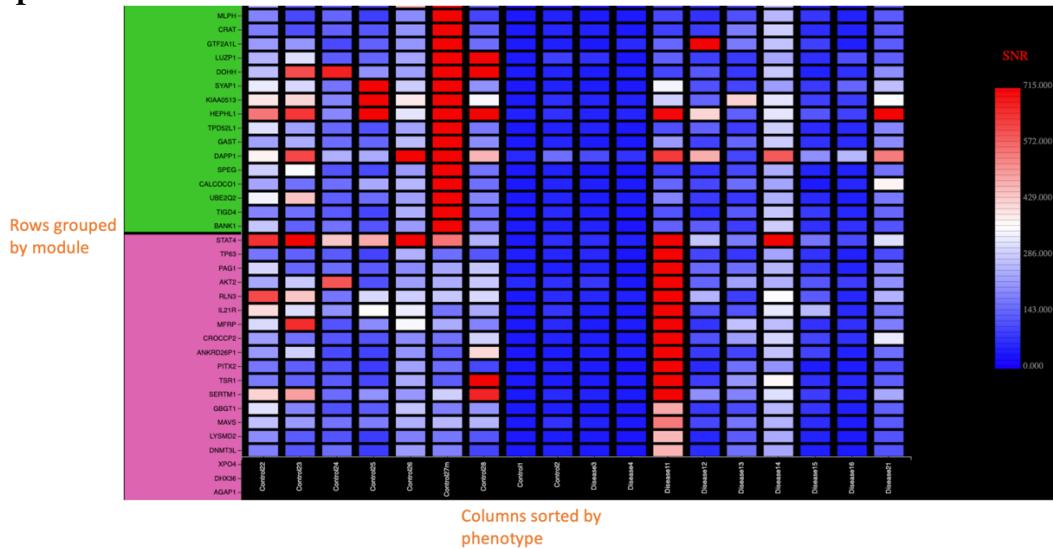
The top 12 largest modules are displayed on the page.

## Dendrogram



A hierarchical clustering dendrogram displaying a child-less branch for each autoantibody in this WGCNA clustering process. Groups of autoantibodies belonging to the same module are clustered under a shared parent branch and the color bar below the dendrogram indicates the module in which the autoantibody belongs. The width of each color in the color bar correlates with the size of the module, shown in the *Modules* section of the page.

## Heatmap



This is the same heatmap representation as the previous heatmap page. This heatmap is a reference to the autoantibodies selected for this WGCNA clustering process. However, there are 2 updates made to this heatmap. First, the columns are grouped by their phenotypes. Second, the rows are grouped and color-coded by the modules they belong to as a result of the WGCNA clustering. Note that the color coding is the same as the dendrogram and module triangular heatmaps.

### **TODO 11:** Explore the WGCNA figures:

- Examine the triangular heatmaps for the modules. Notice where there are clusters within the modules.
- Examine the dendrogram. Notice how the sizes of the modules correlate to the number of autoantibodies per module indicated above the triangular heatmaps.
- Examine the heatmap at the bottom of the page. Notice how the horizontal axis and legend move with the window as you scroll. Notice the color-encoding of the modules.
- Search for your favorite autoantibodies using “Cmd key+F” (if using Mac) or “Ctrl key+F” (if using Windows). Remember to use the gene name as the surrogate for the protein.

### **TODO 12:** Download figures:

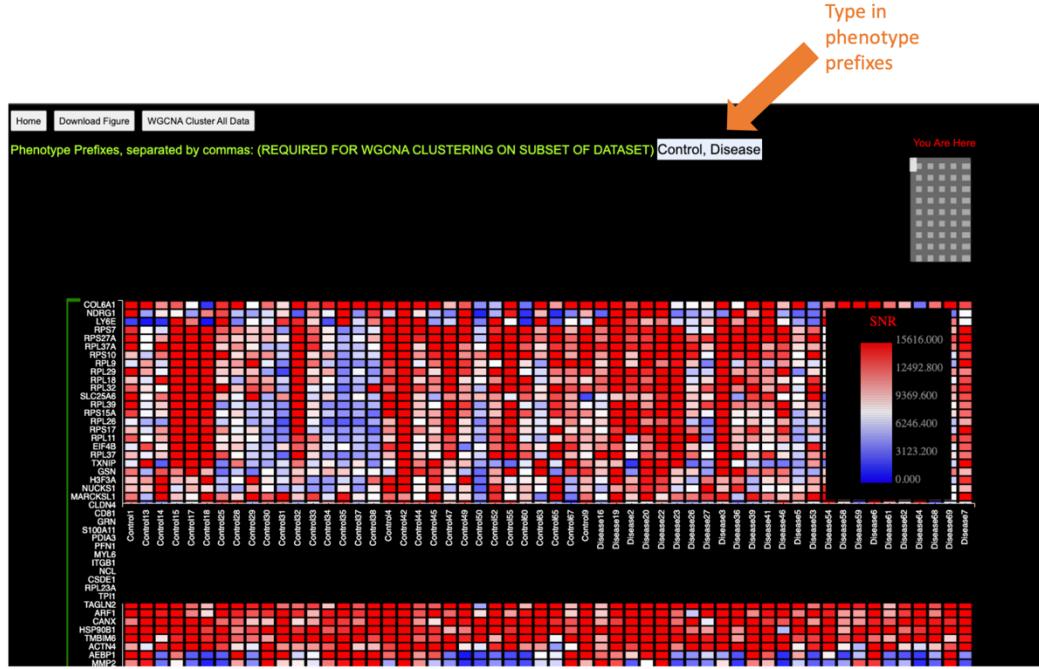
- Click the Download Figure button until it turns Yellow. Two separate files will be downloaded.
- The first figure will be saved to *static/download\_imgs\_PatientCharacter1* folder as *figures\_PatientCharacter1\_wgcna\_0.svg* and will contain the triangular heatmaps showing each WGCNA module as well as the dendrogram.
- The second figure will be saved as *heatmap\_PatientCharacter1\_wgcna\_0.svg* and will contain the heatmap sorted and color-coded by their modules.
- Open the svg. It should be similar to the image that you just saw in the application. When you are finished examining the heatmap svg file, close the tab containing the svg file.
- Return to the localhost:8888 tab. Note that after WGCNA figures are downloaded, *visAPPprot* automatically goes back to the previous large heatmap.

### 4.4.2 Clustering over subset of data

(Time Estimate: 4 minutes)

**TODO 13:** From the current unnormalized (i.e., raw) heatmap clustered by rows *visAPPprot* page, at the top of the page in the text field for *Phenotype Prefixes*, enter the phenotype prefixes as follows:

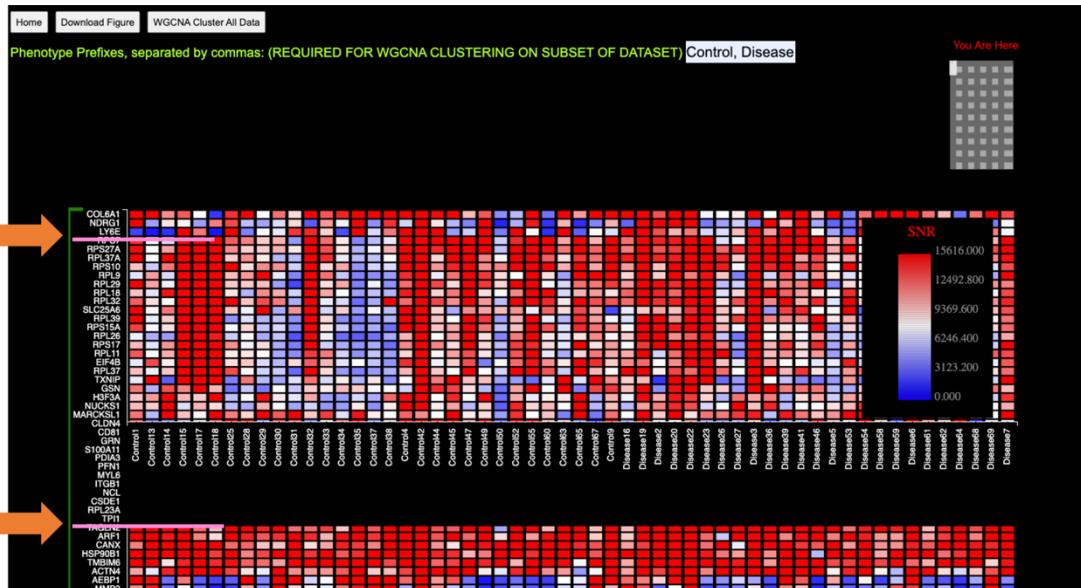
- Phenotype prefix is the set of letters in the sample name that indicates the phenotype of the sample.
  - Examples: the prefix of “Control65” is Control, the prefix of “Disease87” is Disease.
- Example of phenotype prefix input to text field: **Control, Disease** (please see image below)



**TODO 14:** Select subset range for WGCNA clustering: **READ THROUGH STEPS 1 - 5 BEFORE START.**

1. Scroll through the heatmap and determine which rows of the heatmap to use for the clustering analysis. It is suggested to zoom out to 33% so that you can see the full width of the heatmap. Remember to use the grey Navigation Map to get your bearings on your location in the heatmap.
  - It is strongly suggested that you choose a section of autoantibodies with significantly different expression levels, i.e., your section should include rows with both bright red and bright blue autoantibody expression.
  - It is strongly suggested that your section contain 100s of autoantibodies to increase the chance of finding clusters. To be safe, scroll down the page (i.e., swipe the scroll wheel on your mouse) 4 or 5 times between the top and bottom bounds of your section.
2. Press the option key (if using Mac) or the ALT key (if using Windows) **ONLY ONCE** on your keyboard to go into *select mode*. Your cursor should change to a crosshair icon indicating this is *select mode*.
3. Click above your desired topmost row to select the upper boundary for the subset you wish to use. You should see a pink line appear where you clicked to indicate the upper bound.
4. Then click below the bottom most row to select the lower boundary for the subset. You should again see a pink line appear to indicate the lower bound.
5. Press the option key (if using Mac) or the ALT key (if using Windows) **ONLY ONCE** on your keyboard to submit the process.

Note that the figure below is an example indicating what specifying boundaries for WGCNA clustering looks like on the interface. **THIS FIGURE IS ONLY FOR ILLUSTRATION PURPOSES PLEASE DO NOT PICK SUCH A SMALL SECTION FOR YOUR CLUSTERING.**



After your clustering operation is complete, you should see a page of WGCNA figures similar to the previous process, but with fewer modules since there are fewer autoantibodies involved in the clustering.

#### **TODO 15:** Explore the WGCNA figures:

- Examine the triangular heatmaps for the modules. Notice where there are clusters within the modules.
- Examine the dendrogram. Notice how the sizes of the modules correlate to the number of autoantibodies per module indicated in the above triangular heatmaps.
- Examine the heatmap at the bottom of the page. Notice how the horizontal axis and legend move with the window as you scroll. Notice the color-encoding of the modules.
- Search for your favorite autoantibodies using “Cmd key+F” (if using Mac) or “Ctrl key+F” (if using Windows). Remember to use the gene name as the surrogate for the protein.

#### **TODO 16:** Download figures:

- Click the Download Figure button until it turns yellow. Two separate files will be downloaded.
- The first figure will be saved to the *static/download\_imgs\_PatientCharacter1* folder as *figures\_PatientCharacter1\_wgcna\_1.svg* and will contain the triangular heatmaps showing each WGCNA module as well as the dendrogram.
- The second figure will be saved as *heatmap\_PatientCharacter1\_wgcna\_1.svg* and will contain the heatmap sorted and color-coded by their modules.
- Open the *svg*. It should be similar to the image that you just saw in the application. When you are finished examining the heatmap *svg* file, close the tab containing the *svg* file.

## 4.5 Context Map

(Time Estimate: 10 minutes)

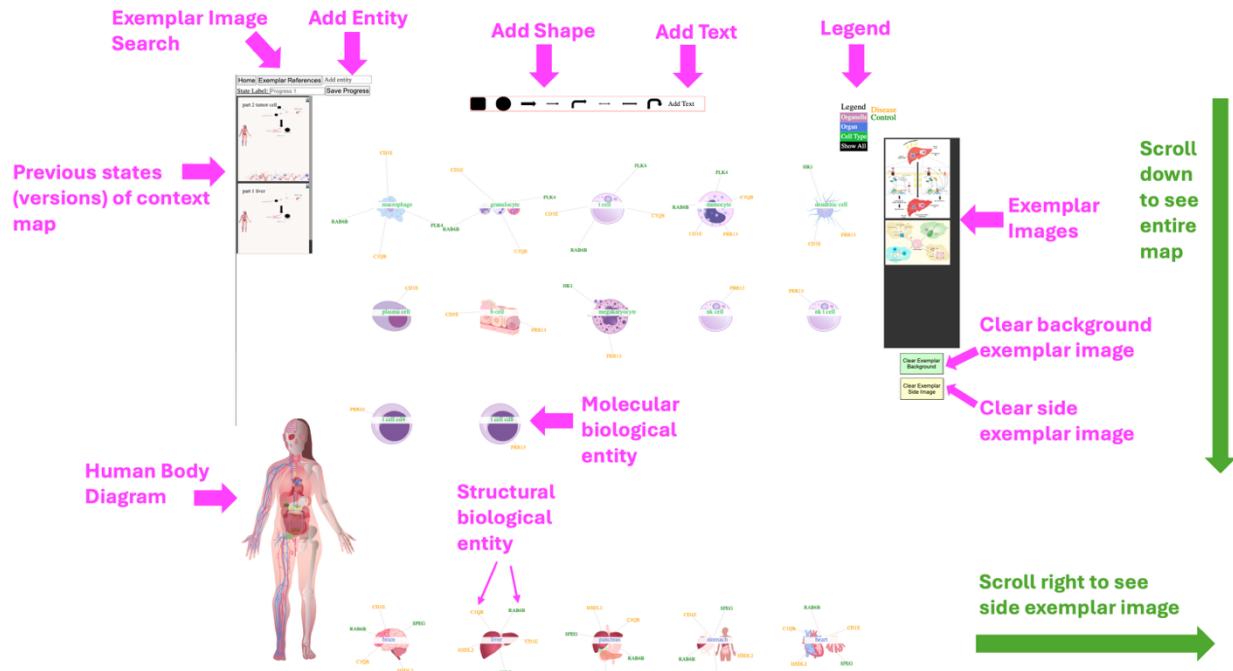
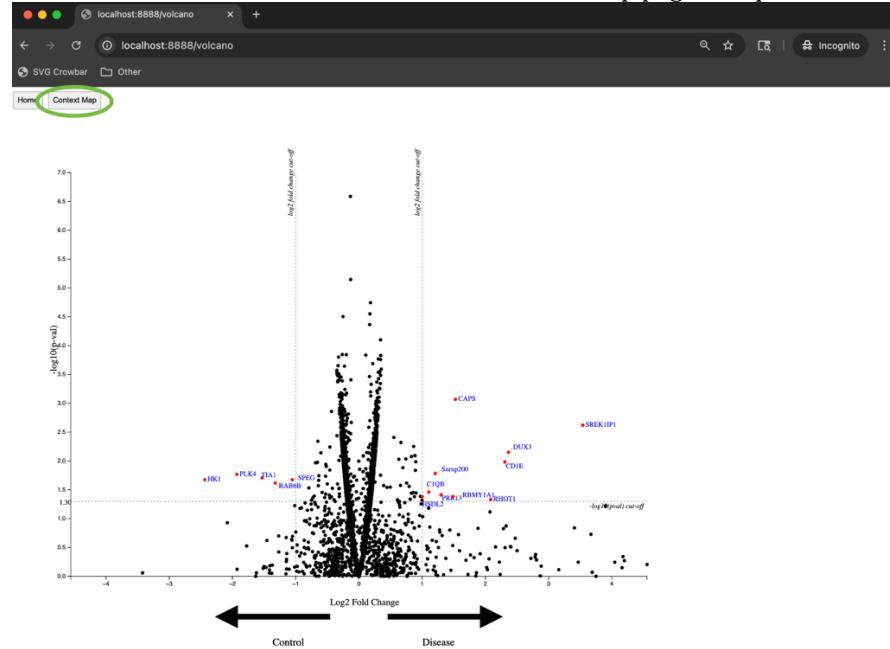
In this section we are going to create a context map for the autoantibodies and related biological entities in this dataset. A context map is a diagram containing the autoantibodies from an input dataset and the biological entities related to those autoantibodies. It demonstrates how the biological entities are related via arrows and other shapes.

We arrive at the Context Map page through the Volcano Plot page.

#### **TODO 17:** On the application home page please do the following:

- Specify names of phenotypes (levels) to be compared to evaluate differential expression. For this demo dataset, the two phenotypes (levels) are Level 1 = Control and Level 2 = Disease.

- Click the yellow button for “Volcano Plot.”
- Once the Volcano Plot page loads, click on the “Context Map” button in the top left.
- It is recommended to scale the screen to 33% on the context map page for optimal viewing.



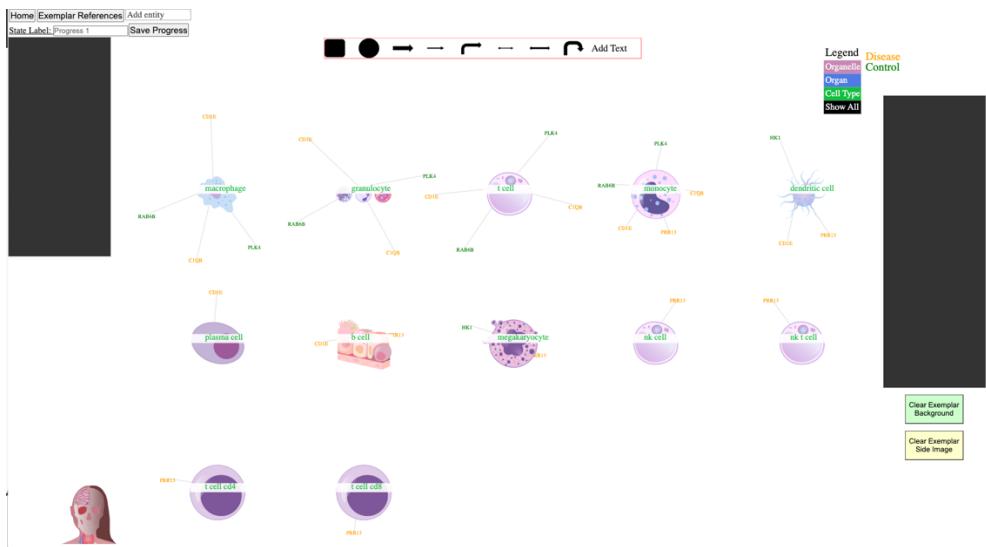
#### Context Map features:

- **Legend:** Categorizes the 3 major types of biological entities: Organelle, Organ, Cell Type. The molecular biological entities in the context map are color-coded by their category. Clicking on a category will highlight the category and filter the context map so that only molecular biological entities of that type are displayed. To once again display all molecular biological entities, click on the “Show All” bar in the legend. Autoantibodies (structural biological entities) that are in the **Control** and **Disease** groups are also labeled with their respective colors.

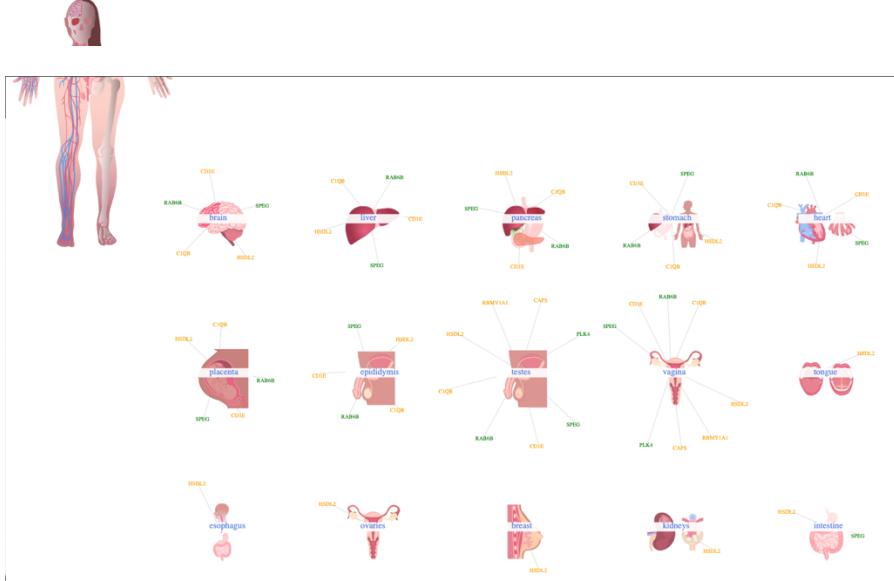
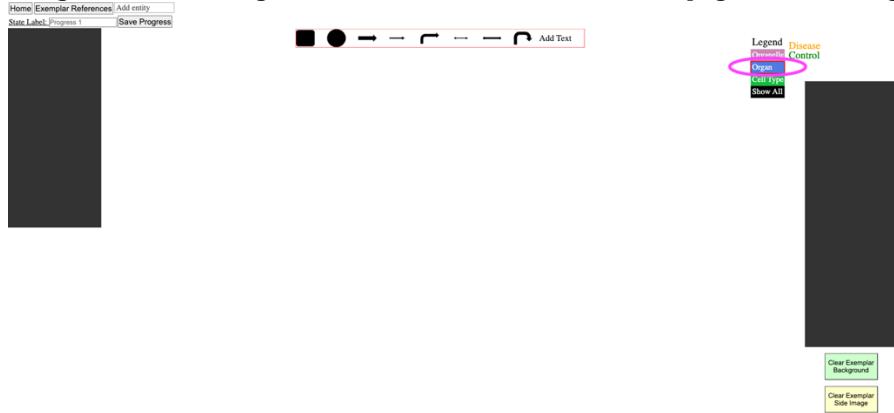
- [Human body diagram](#): Hovering over each organ will highlight the organ and display its name above the human body diagram. Clicking on the organ will filter the context map so that only the molecular biological entities with autoantibodies associated with the selected organ will be shown. The autoantibodies (structural biological entities) that are associated with the selected organ will be underlined and highlighted in **red text**. To once again display all molecular biological entities and autoantibodies, click on the “Show All” bar in the legend.
- [Add shape](#): Clicking on any shape will add it to the middle of the page. You may have to scroll down to see the newly added entity.
- [Add text](#): Add text to the context map. After clicking on “Add Text”, a text box will appear in the middle of the page. You will enter the text you wish to add and hit the *Enter* key on your keyboard. You may have to scroll down to see the newly added text.
- [Add entity](#): List of biological entities that can be added to your context map. You can search for the name of an entity in the search bar that appears after clicking on the “Add Entity” dropdown. Clicking on an entity will add it to the middle of the page.
- [Modify shape/text/entity](#): All shapes, text, and entities you add can be dragged by moving your cursor over the shape/text/entity, clicking, holding the left mouse button, and dragging the shape/text/entity across the page. For more modification options, hold the *option* key (if using Mac) or the ALT key (if using Windows) and left-click your mouse while your cursor is over the shape/text/entity. A red box will appear around the text/shape/entity along with an “X” at one corner, an “R” at another corner, and an “S” at the third corner. Left-clicking on the “X” will remove the shape/text/entity from the context map. Left-clicking and holding the “R” and dragging the mouse will rotate the shape/text/entity. Finally, left-clicking and holding the “S” and dragging the mouse will change the size of the shape/text/entity.
- [Save state](#): Save the current entities in your context map and their positions. After a state is saved, its thumbnail will be added to the scroll panel on the lefthand side. Please name the state with words separated by spaces, eg. “Progress 1”.
- [Load state](#): Hovering over each thumbnail in the scroll panel on the lefthand side will show the context map in that state. Click on the thumbnail to load that version of the context map and continue working on it.
- [Exemplar image search](#): Clicking on this button will open a new tab. You can input your own query into the search bar and download images of interest images by checking the box to the right of the image(s) and clicking the button “Add Exemplar References to Context Map”. The purpose of this page is to provide relevant pathway maps that can inspire your own context map.
- [Exemplar image options](#): This panel shows available exemplar images to use for organizing the entities in your context map. Hovering over an image will show a larger version of the image for ease of viewing. Clicking on the image will display the image in the background of the context map and to the right of the exemplar images panel, as well as automatically place any entities from your context map that are also in present in the exemplar image in positions similar to those in the exemplar image. Feel free to use either the background exemplar image or the side exemplar image as reference when creating your context map. Additionally, you can filter the exemplar images using the same legend categories used to filter the entities in the context map: clicking on a legend category will display only the exemplar images that include entities in that category. Finally, clicking the green “Clear Exemplar Background” button below this panel will remove the exemplar image from the background of your context map and clicking the yellow “Clear Exemplar Side Image” button will remove the exemplar image from the right side of the page.

*The following tasks will familiarize you with each of the features in the Context Map. This tutorial involves live image search from the Internet. Because search engine results are continually updating, the specific figures and their resolutions may be different from what is shown in the images below; this variability is normal. If you don't see the exact results as in the screenshots provided, find the closest one. The goal is to practice using the system, not necessarily achieve identical visualizations. (Exemplar image figures belong to their original authors and publishers, which are cited at the end of this document.)*

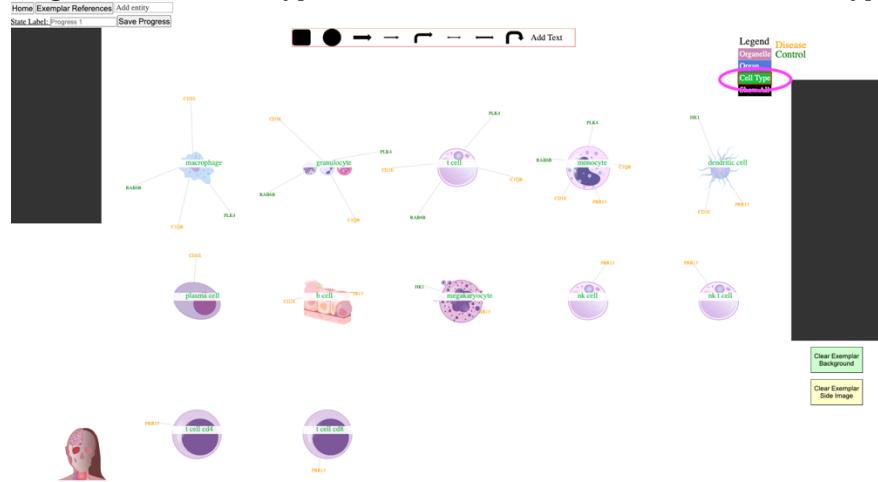
The image below shows the initial interface you should see.



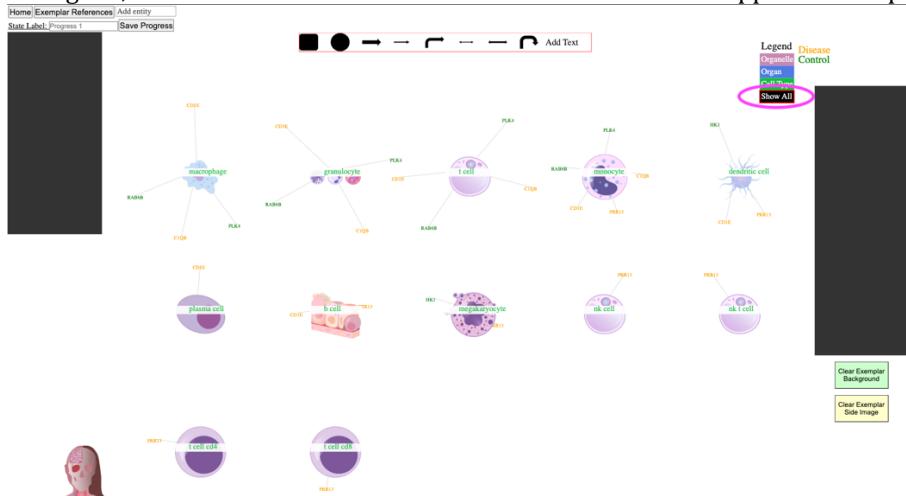
**TODO 18:** In the legend, click on “Organ”. You will have to scroll down the page to see the organ entities.



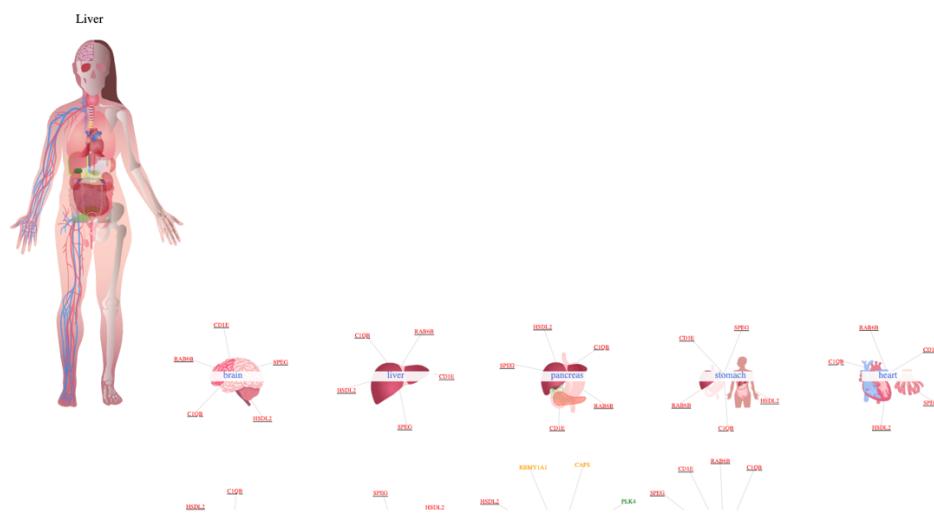
**TODO 19:** In the legend, click on “Cell Type”. You will see all the entities that are of a cell type on the page.



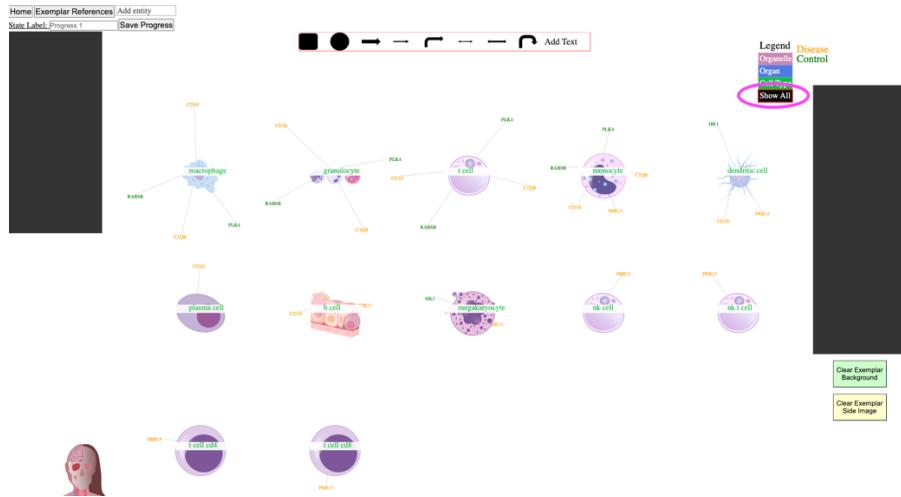
**TODO 20:** In the legend, click on “Show All”. You should see all the entities reappear on the page.



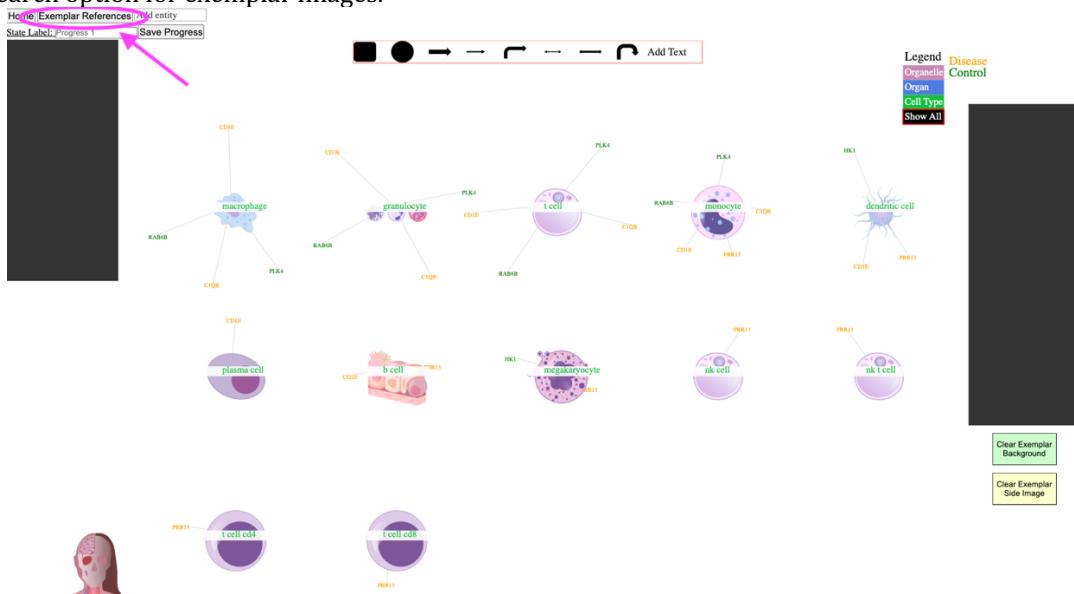
**TODO 21:** Hover over the human body diagram until you see the liver. Click on the liver organ. You should see only the liver on the page, and all the autoantibodies associated with the liver highlighted in red.



**TODO 22:** In the legend, click on “Show All”. You will be able to see all the entities on the page again.



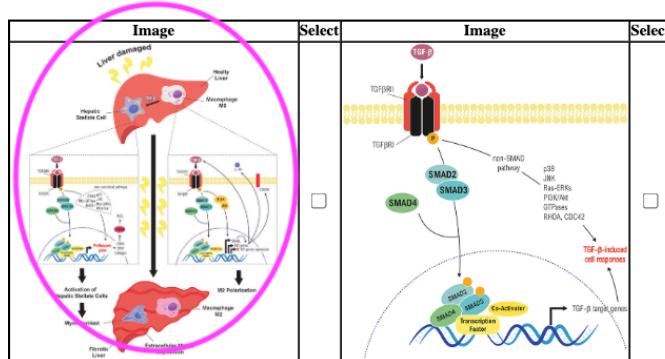
**TODO 23:** Then click on the “Exemplar References” button at the top left of the page to open the Exemplar Image Search option for exemplar images.



**TODO 24:** In the search field paste in the following text: "role of TGF- $\beta$ " "cell plasticity of hepatic stellate cells" "liver fibrosis"

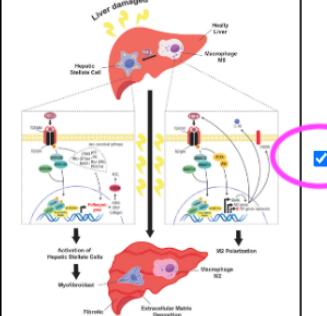
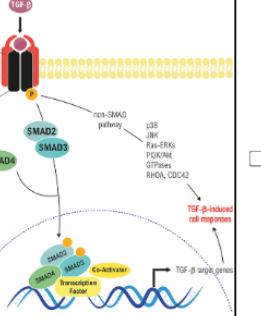
Click the Search button. Scroll down the page of results until you see the circled image below. If you don't see the image below, click the “Next” button to view the next page of image results.

"role of TGF- $\beta$ " "cell plasticity of hepatic stellate cells" "liver fibrosis"  Add Exemplar References to Context Map



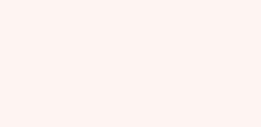
**TODO 25:** Check the box **to the right** of the image to indicate selection of this image. Then click the “Add Exemplar Reference to Context Map” button at the top of the page. Your image is now being downloaded. It should take approximately 5 minutes to download. The words “Loading” will flash on the page during the 5 minutes. **Please leave the browser alone during the download!!! Please do not click on anything.**

"role of TGF- $\beta$ " "cell plasticity of hepatic stellate cells" "liver fibrosis" | Search | Add Exemplar References to Context Map |  | [Prev](#) [Next](#)

| Image   | Select                              | Image  | Select                   |
|---|-------------------------------------|--|--------------------------|
|  | <input checked="" type="checkbox"/> |  | <input type="checkbox"/> |

"role of TGF- $\beta$ " "cell plasticity of hepatic stellate cells" "liver fibrosis" | Search | Add Exemplar References to Context Map | [Prev](#) [Next](#)

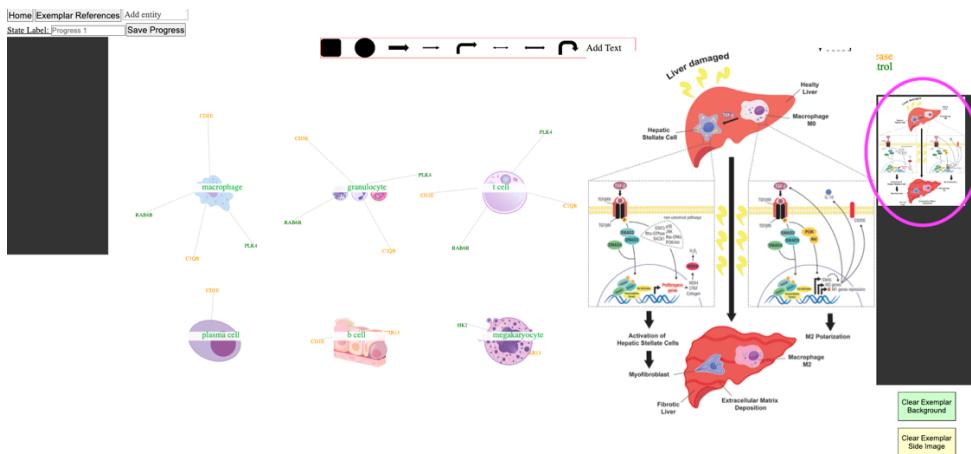
**Loading**

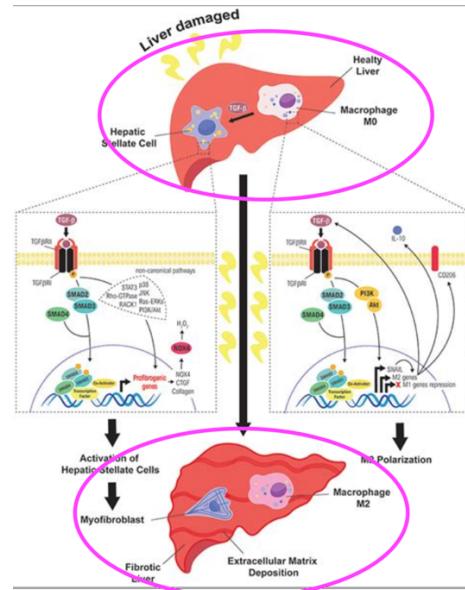
| Image  | Select                              | Image   | Select                   |
|--|-------------------------------------|---|--------------------------|
|  | <input checked="" type="checkbox"/> |  | <input type="checkbox"/> |

**TODO 26:** In the exemplar image options panel on the righthand side of the page, hover over the first image with the liver to display a larger version of the image. We will use this image as a guide to create our context map. We will incorporate the “Healthy Liver” and “Fibrotic Liver” elements of the image.

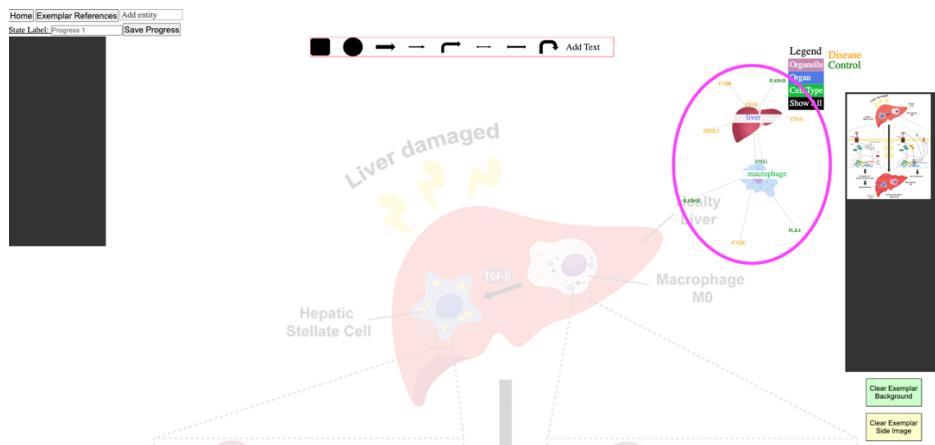
Home | Exemplar References | Add entity | State Label | Progress 1 | Save Progress

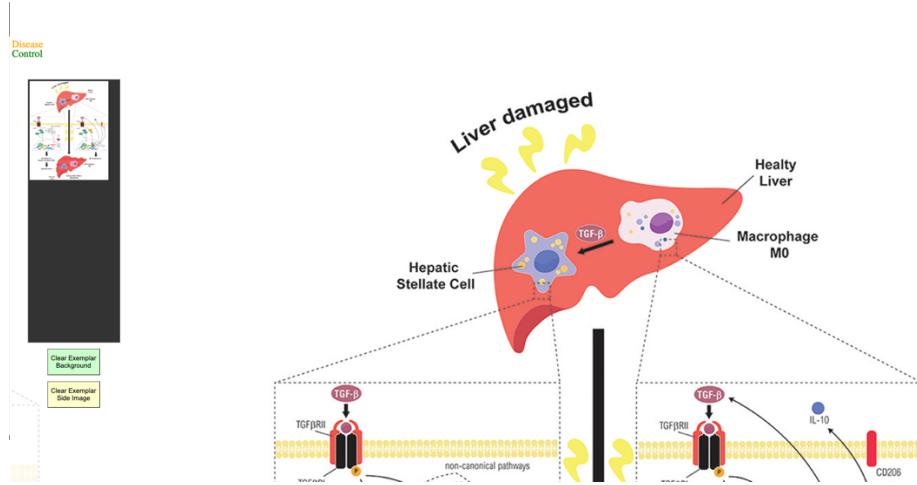
Add Text |



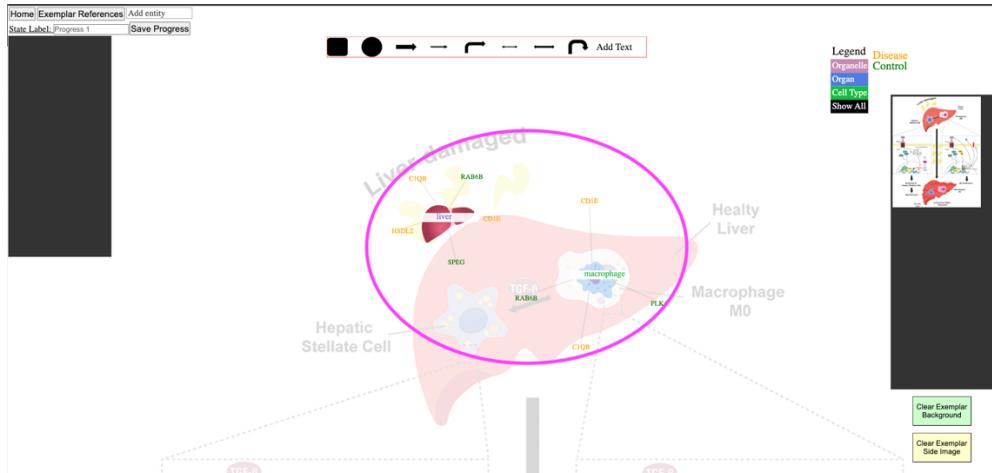


**TODO 27:** Click on the image in the exemplar image options panel. The image should show up both in the background of the context map, and also to the right of the exemplar images panel when you scroll right on the page. You can reference either image as you continue to create your context map. On the context map canvas, the liver and macrophage entities are included in the image so they should be placed near the top of the page, while all the remaining biological entities that are not included in the image are placed at the bottom of the page so they are out of the way. Note that the liver entity is placed close to the words "Healthy Liver" from the background image and the macrophage entity is placed close to the words "Macrophage M0" from the background image.

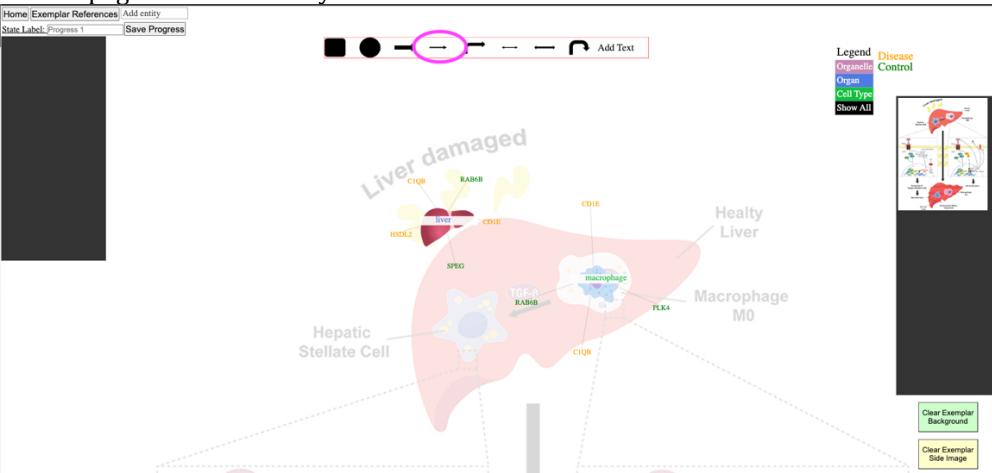


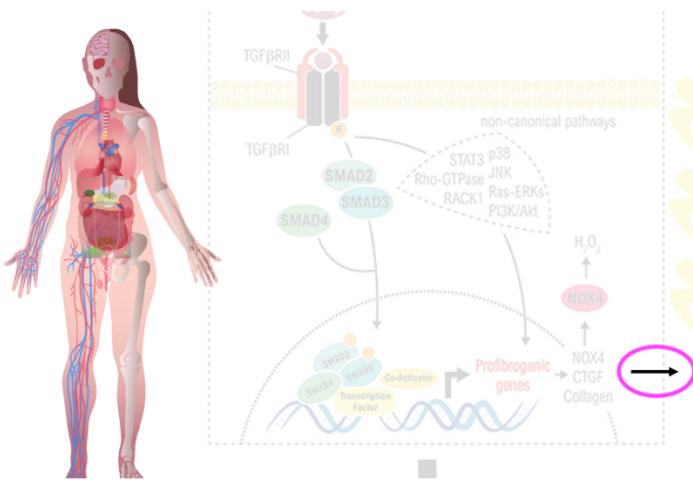


**TODO 28:** To avoid overlap of entities, drag the liver entity to the left so that it is now underneath the “Liver Damaged” words from the background image and drag the macrophage entity so that it sits on top of the macrophage in the background image.

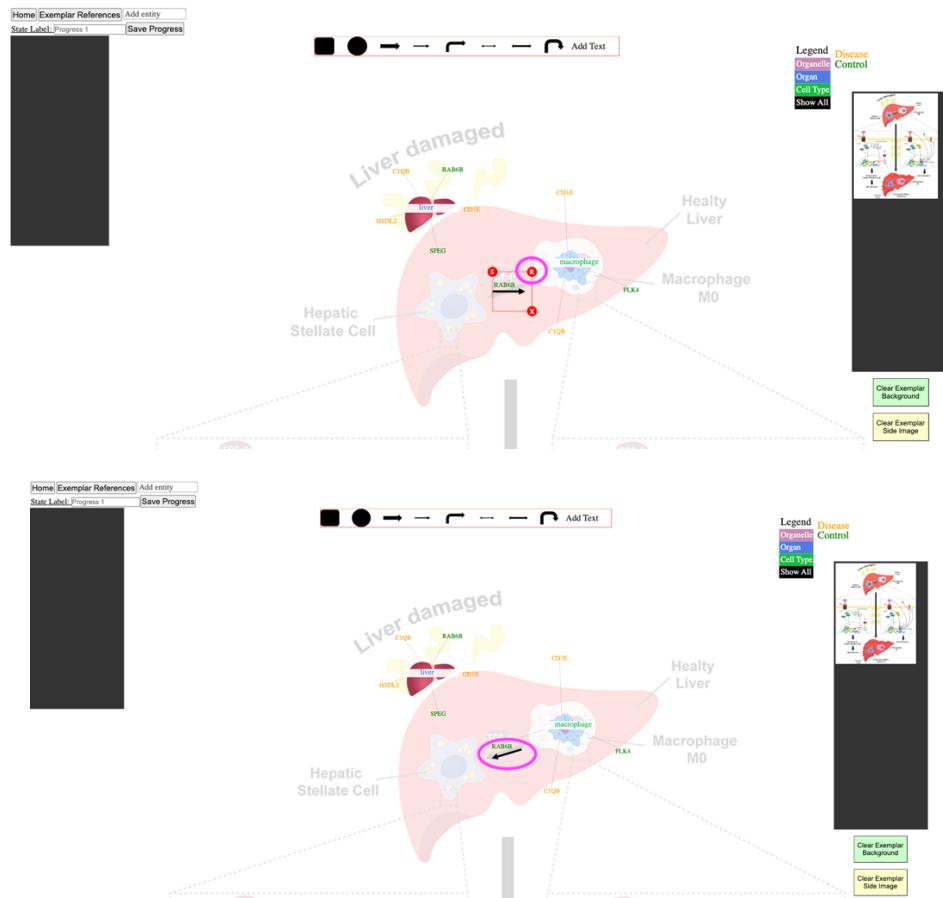


**TODO 29:** Click on the thin arrow icon to add an arrow to the middle of the page. You will have to scroll down to the middle of the page to see the newly added arrow.

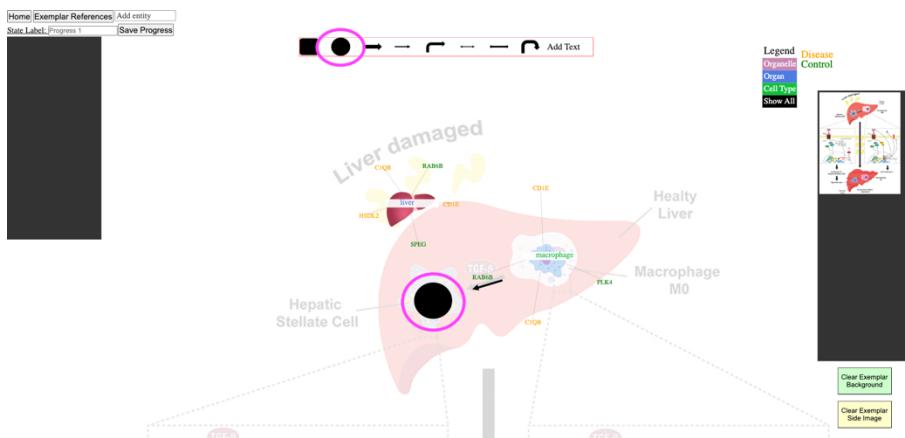




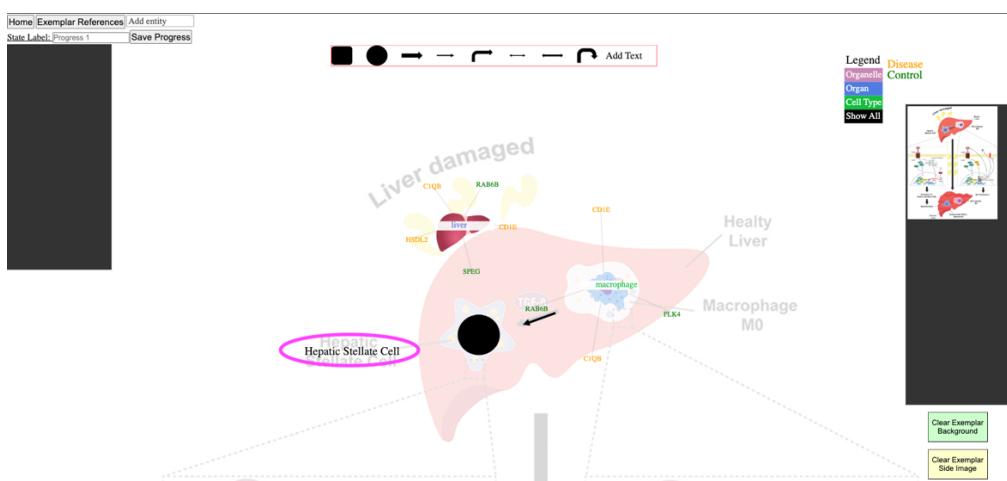
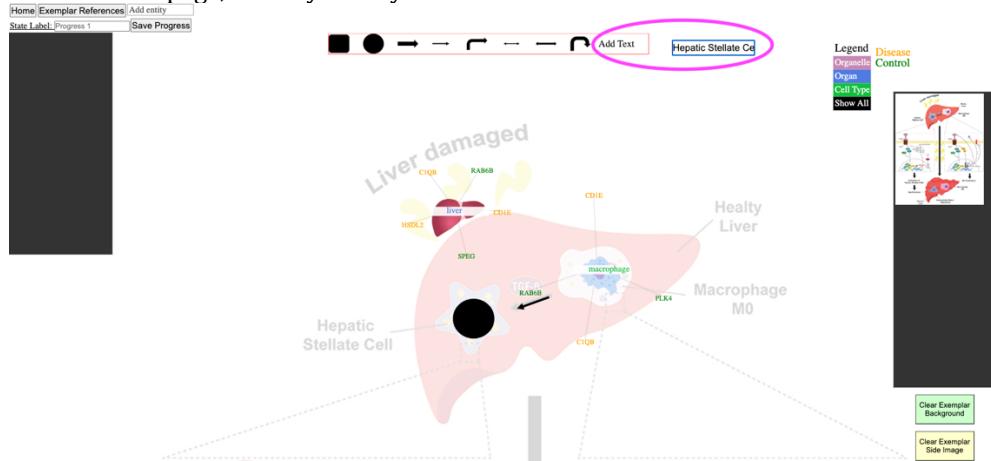
**TODO 30:** Drag the arrow to the left of the macrophage, and place it so that it sits over the arrow next to the macrophage in the background image. Next, hold the option key (if using Mac) or the ALT key (if using Windows) while clicking on the arrow to show the edit options. Click and drag the “R” on the top right of the edit options to rotate the arrow so that it points to the left. When you are satisfied with the direction of your arrow, hold down the option key (if using Mac) or the ALT key (if using Windows) while clicking on the arrow again to hide the edit options.



**TODO 31:** To represent the Hepatic Stellate Cell from the background image, use a circle by clicking the circle icon from the top of the page. Drag the circle to be placed over the icon for the hepatic stellate cell in the background image. Again, the circle will initially appear in the middle of the page, which you may need to scroll down to see.

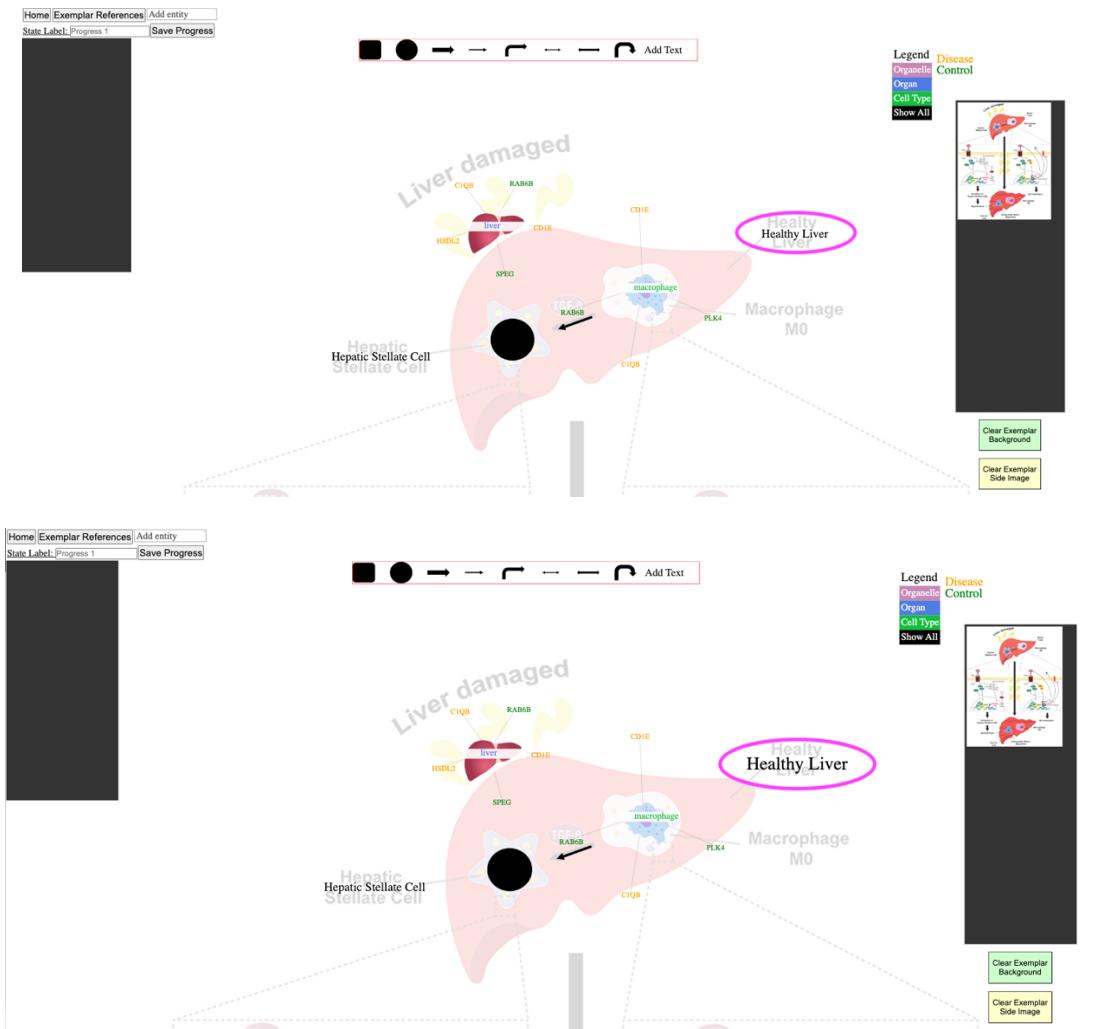


**TODO 32:** Label the Hepatic Stellate Cell using the Add Text option from the top of the page. Drag your "Hepatic Stellate Cell" text to be placed over the same text from the background image. The text will initially be added to the middle of the page, which you may have to scroll down to see.

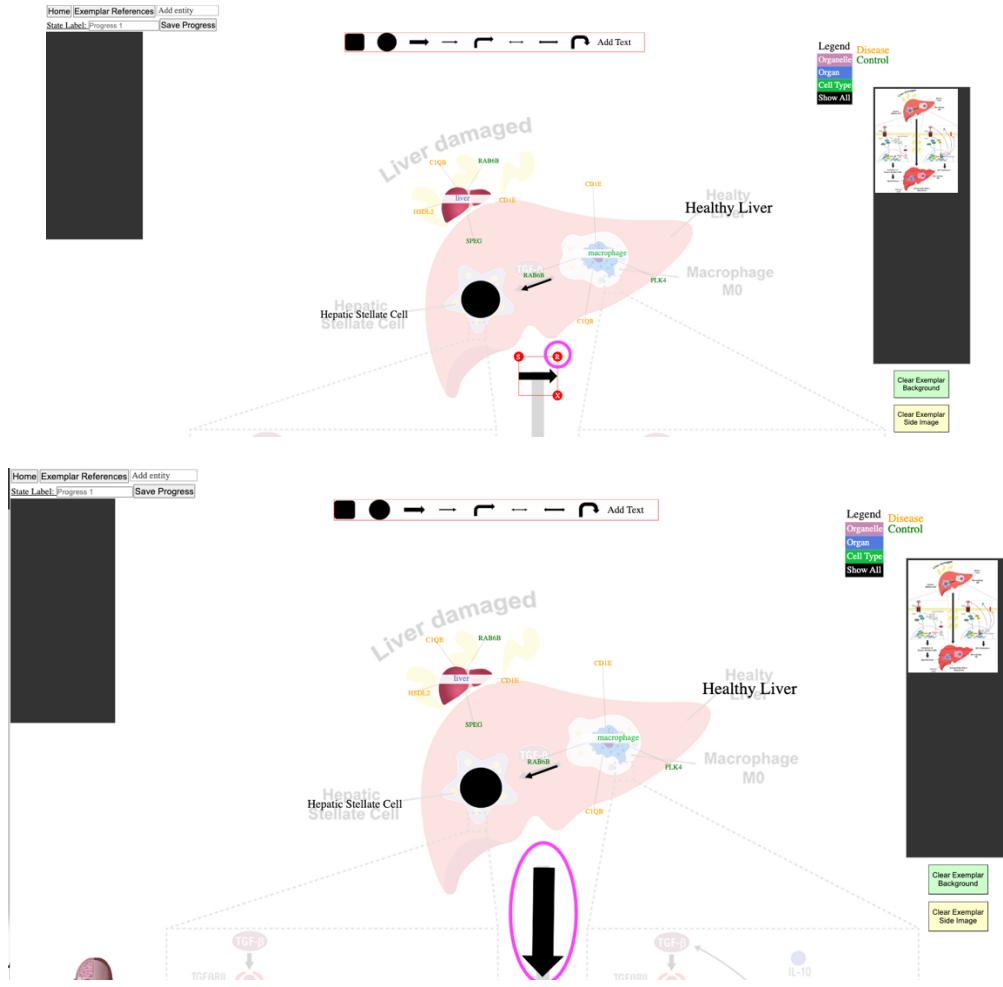


**TODO 33:** Label the entire section we just worked on "Healthy Liver". Use the Add Text option near the top of the page to add the words "Healthy Liver" to the page and drag the text to be placed over where the same text is located in the background image. Scale the size of the text up by using the edit options: hold down the option key (if using Mac) or the ALT key (if using Windows) and click the text "Healthy Liver" to show the edit options. Click the "S" at the top left of the edit options and drag to increase the size of the text. When you are satisfied with the

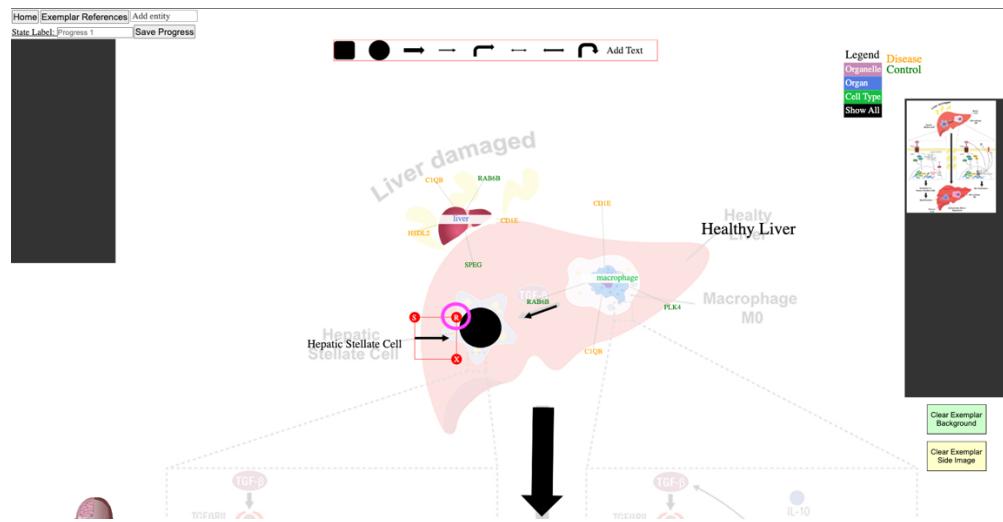
size of your text, hold down the option key (if using Mac) or the ALT key (if using Windows) while clicking on the text again to hide the edit options.

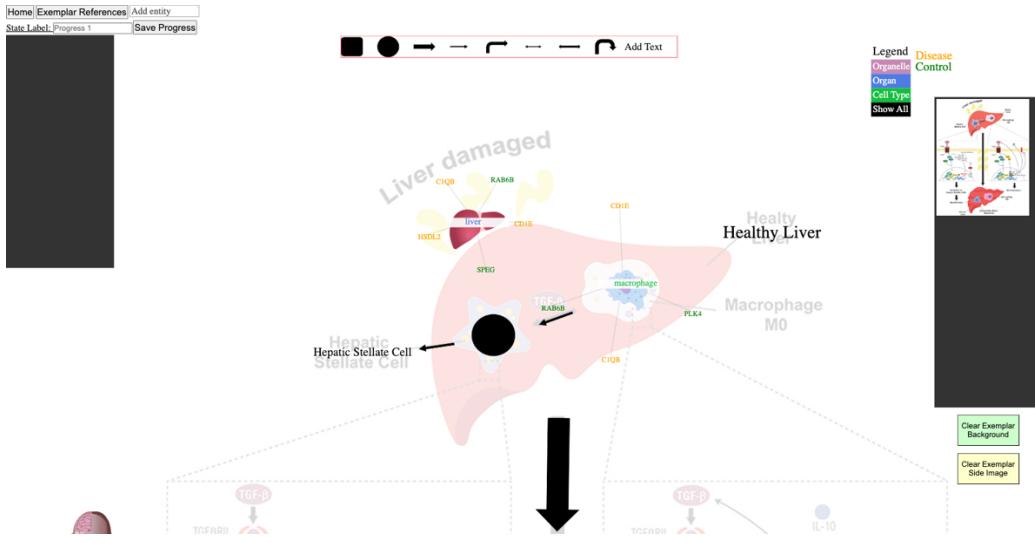


**TODO 34:** Add a wide arrow to the page. Drag the arrow to be placed at the top of the long arrow from the background image. Use the edit options to rotate the arrow so that it points downward: hold down the option key (if using Mac) or the ALT key (if using Windows) and click the arrow you added to show the edit options. Click the "R" at the top right of the edit options and drag to rotate the direction of the arrow. Then click and drag the "S" to increase the size of the arrow. When you are satisfied with the size and direction of your arrow, hold down the option key (if using Mac) or the ALT key (if using Windows) while clicking on the arrow again to hide the edit options.

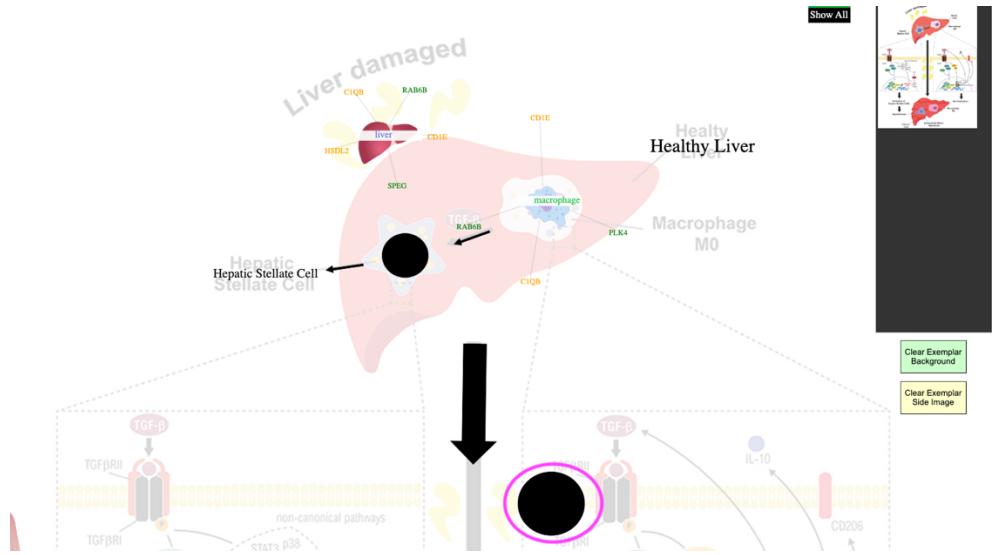


**TODO 35:** Add another thin arrow to point the “Hepatic Stellate Cell” text to the circle icon, for clarity. Rotate the arrow so that it points from the circle towards the text: hold down the option key (if using Mac) or the ALT key (if using Windows) and click the arrow you added to show the edit options. Click the “R” at the top right of the edit options and drag rotate the direction of the arrow. When you are satisfied with the direction of your arrow, hold down the option key (if using Mac) or the ALT key (if using Windows) while clicking on the arrow again to hide the edit options.

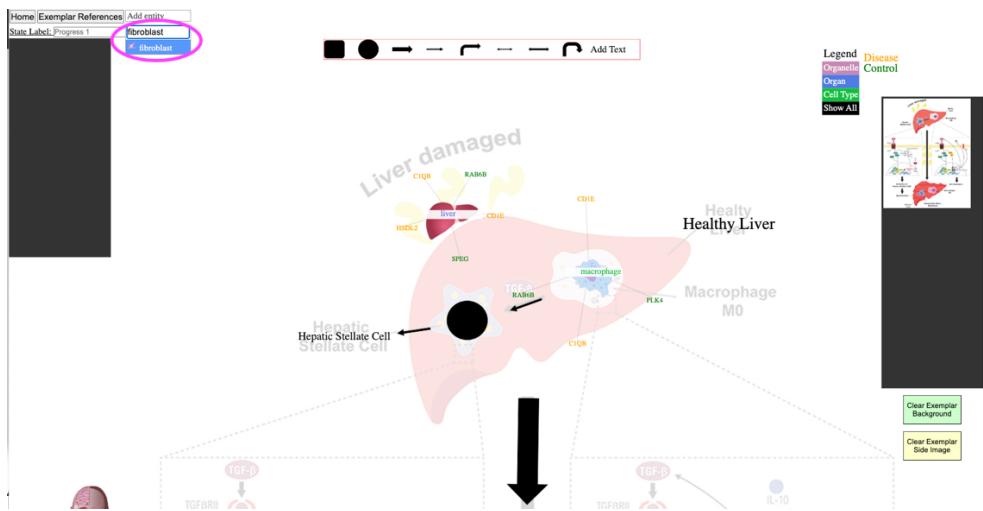




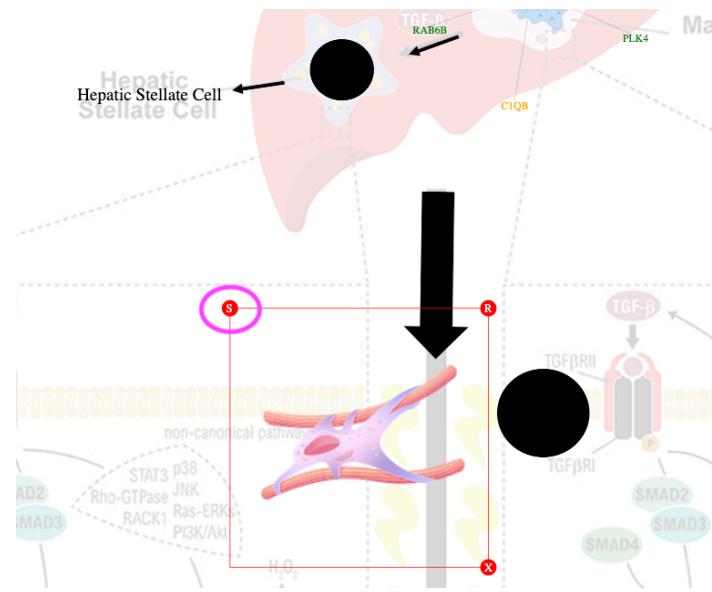
**TODO 36:** We now want to incorporate the Fibrotic Liver aspect into our context map. Start by adding a circle to the context map, to substitute for the Macrophage M2 icon from the background image. Increase the size of the circle using the edit options for the shape mentioned previously. (The image below is cropped but you should scroll down on your context map page to see the entire background image use it as a reference to decide where to place your shapes and text.)



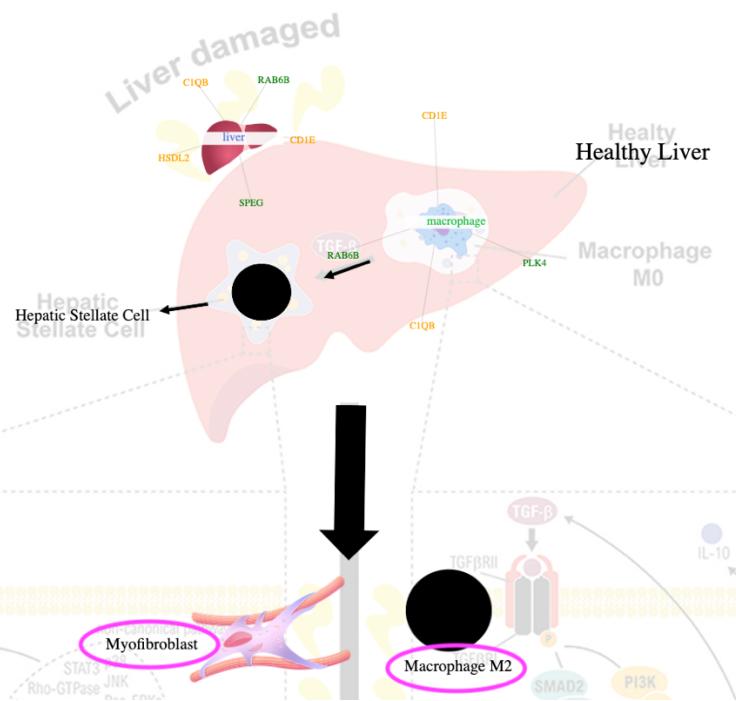
**TODO 37:** For the Myofibroblast in the background image, we will look for “fibroblast” in the Add Entity dropdown at the top left of the page.



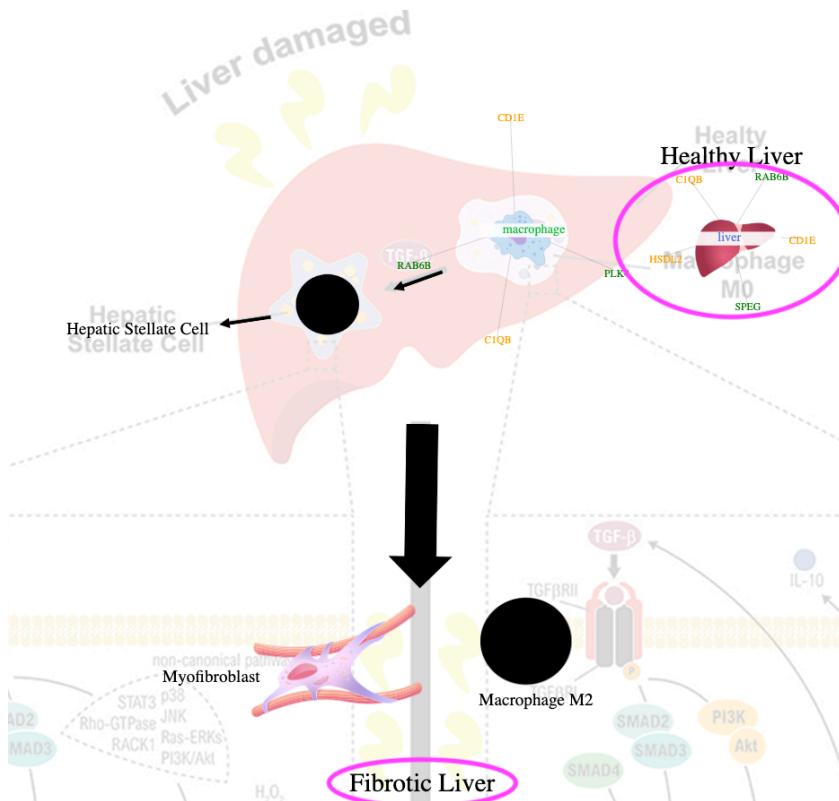
**TODO 38:** Scale the size of the fibroblast to your liking with the edit options as mentioned previously. Drag the fibroblast so that it is to the left of the Macrophage M2, similar to the background image.



**TODO 39:** Label the Myofibroblast and Macrophage M2 with text. Reference your background image to determine where to place your text.

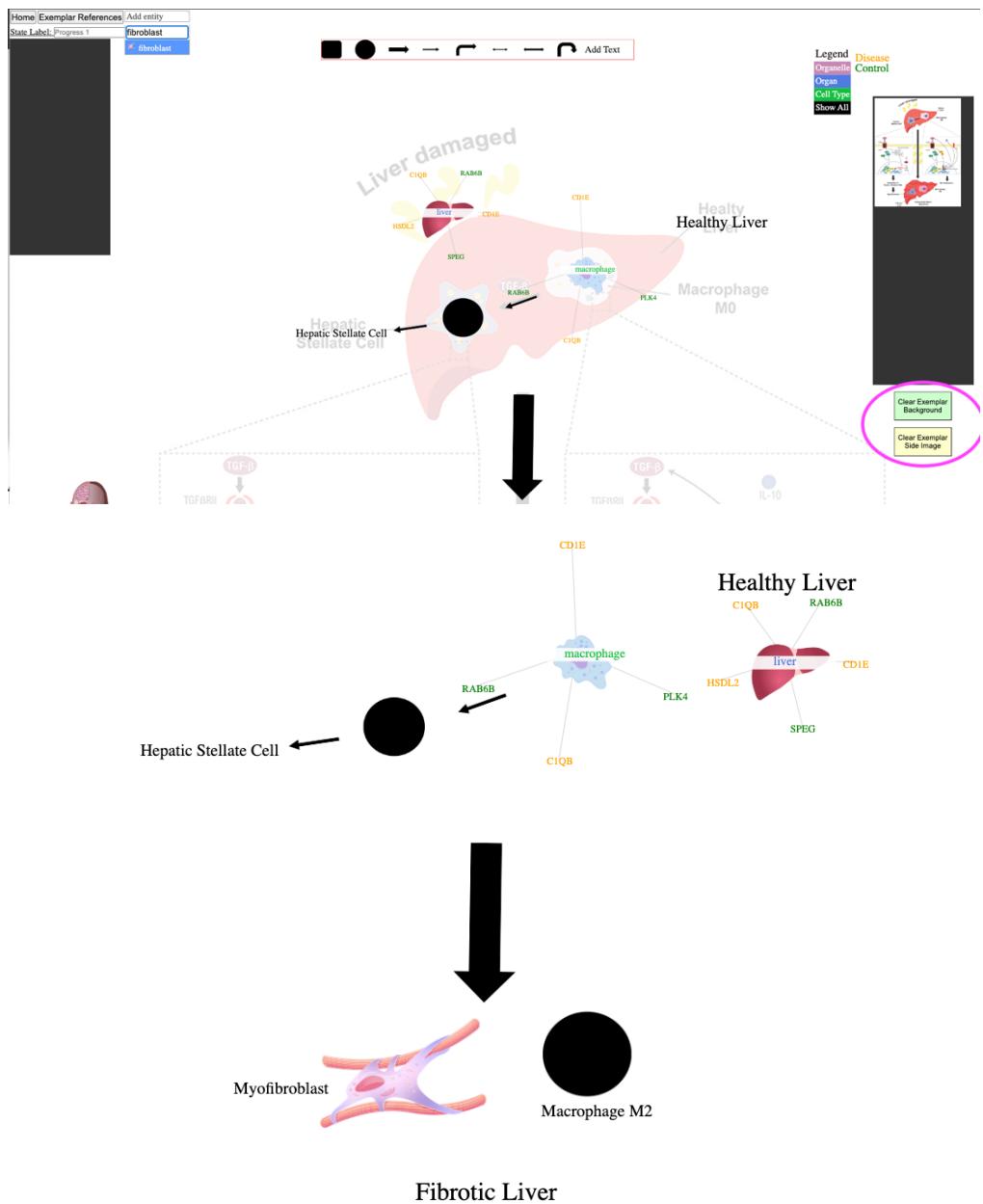


**TODO 40:** Similarly to the background image, label the bottom half of our context map creation with "Fibrotic Liver". Increase the size of the text using the edit options mentioned previously. Also move the liver entity to be under the "Healthy Liver" text.



**TODO 41:** Click the button "Clear Exemplar Background" below the exemplar image options panel on the righthand side of the page. The background image will disappear, leaving only your context map creation. Then

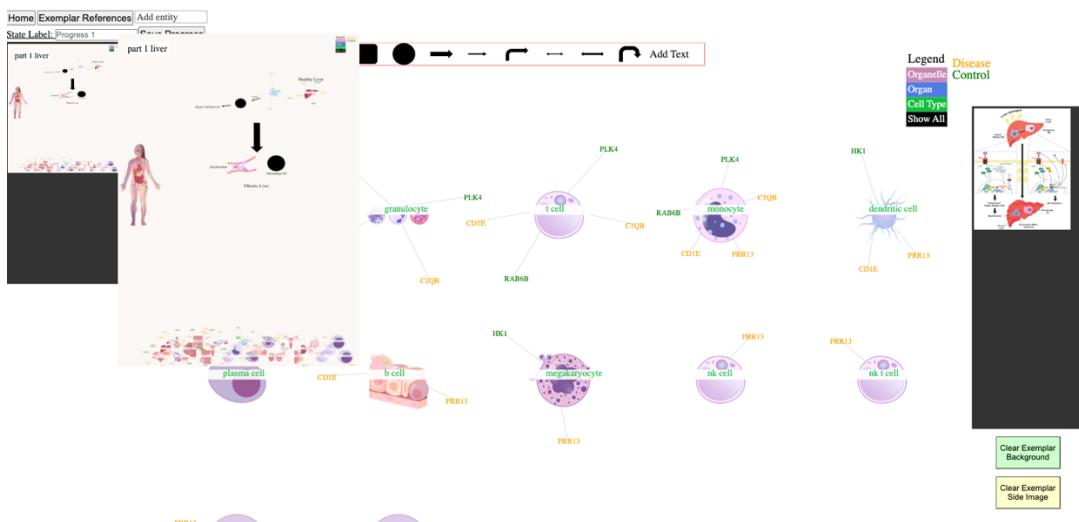
click on the “Clear Exemplar Side Image” button to clear the exemplar image from the right of the page.



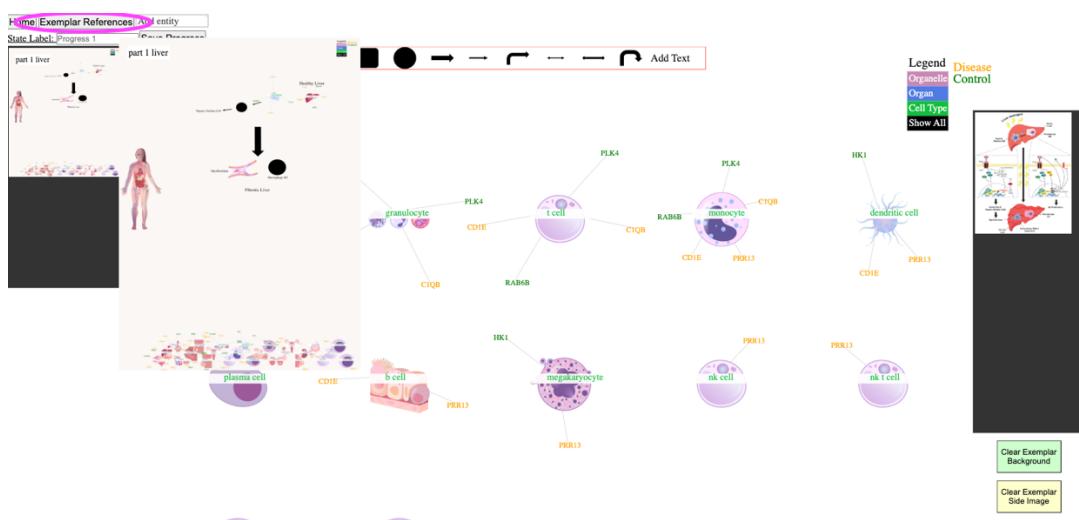
**TODO 42:** Save your work: find the “State Label” text box at the top left of the page. Type “part 1 liver” and click “Save Progress”. The page will reload.

|                                  |                                     |                               |
|----------------------------------|-------------------------------------|-------------------------------|
| <a href="#">Home</a>             | <a href="#">Exemplar References</a> | fibroblast                    |
| State Label: <b>part 1 liver</b> |                                     | <a href="#">Save Progress</a> |

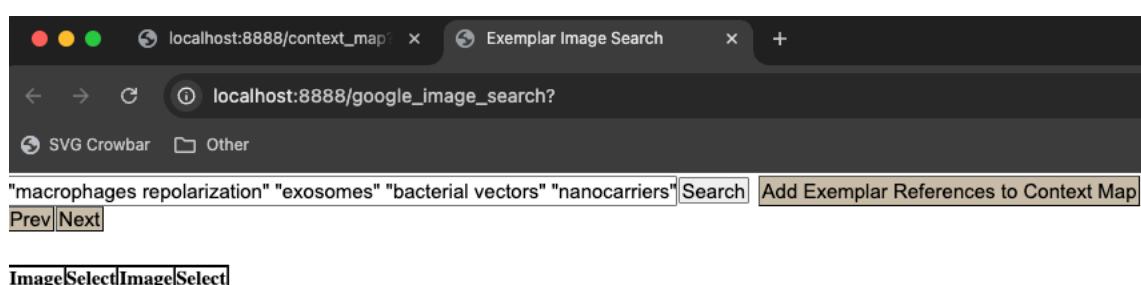
**TODO 43:** Now a new thumbnail should appear in the progress panel on the lefthand side of the page. Hover over the thumbnail labeled “part 1 liver” to see a larger version of the image.



**TODO 44:** After you have verified that you have found the thumbnail for the work you just saved, click on the thumbnail to load your work. Then click on the “Exemplar References” button at the top left of the page to open the Exemplar Image Search option for exemplar images.

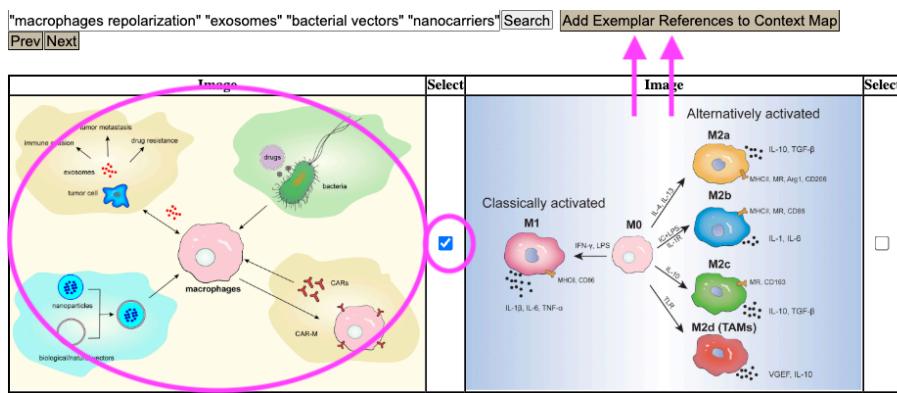


**TODO 45:** In the search field paste in the following text: "*macrophages repolarization*" "*exosomes*" "*bacterial vectors*" "*nanocarriers*". Click the Search button.

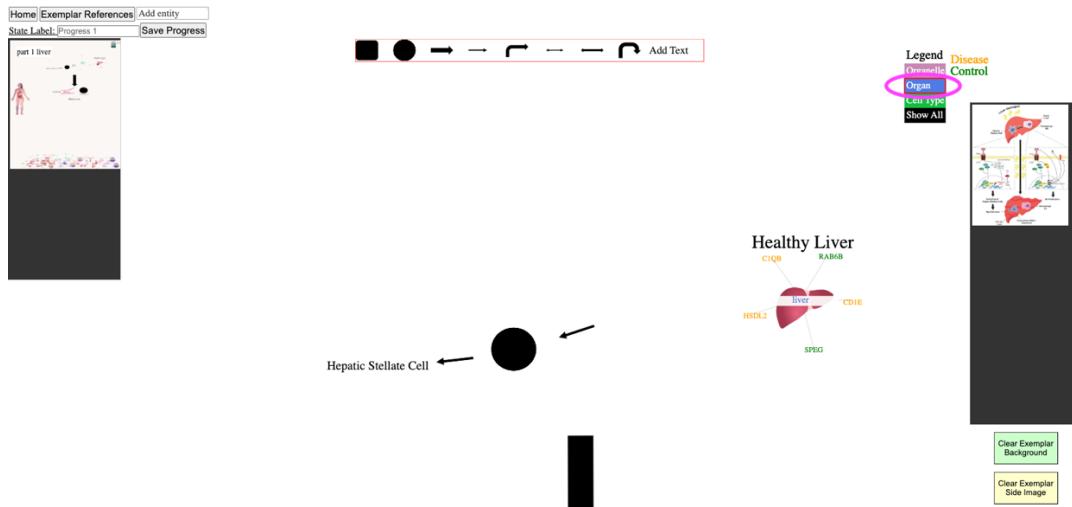


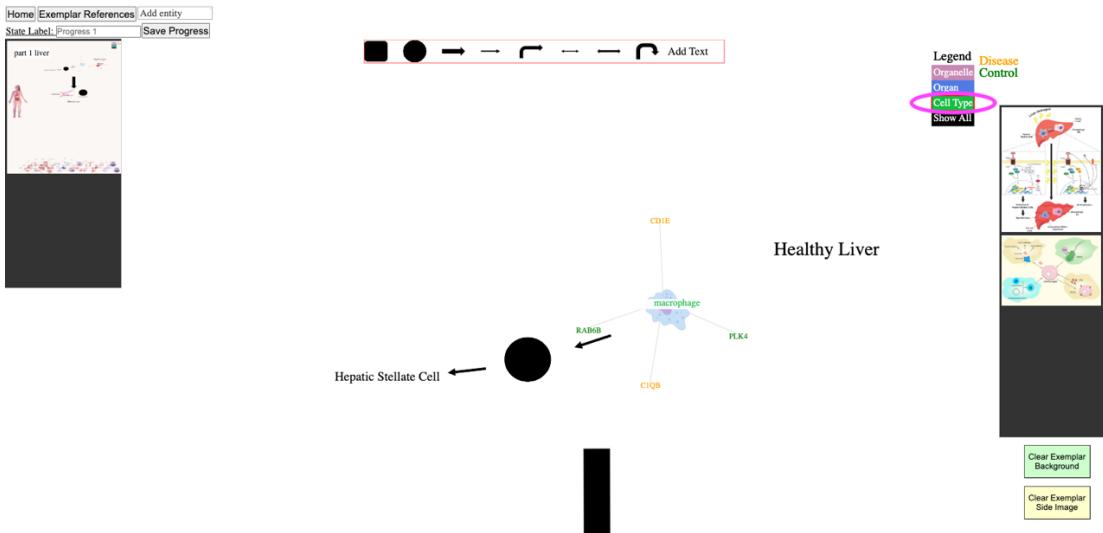
**TODO 46:** Scroll down the page of results until you see the circled image below. If you don't see the image below, click the “Next” button to view the next page of image results. Check the box **to the right** of the image to indicate selection of this image. Then click the “Add Exemplar Reference to Context Map” button at the top of the page. Your image is now being downloaded. It should take approximately 5 minutes to download. The words

"Loading" will flash on the page during the 5 minutes. **Please leave the browser alone during the download!!!**  
**Please do not click on anything.**

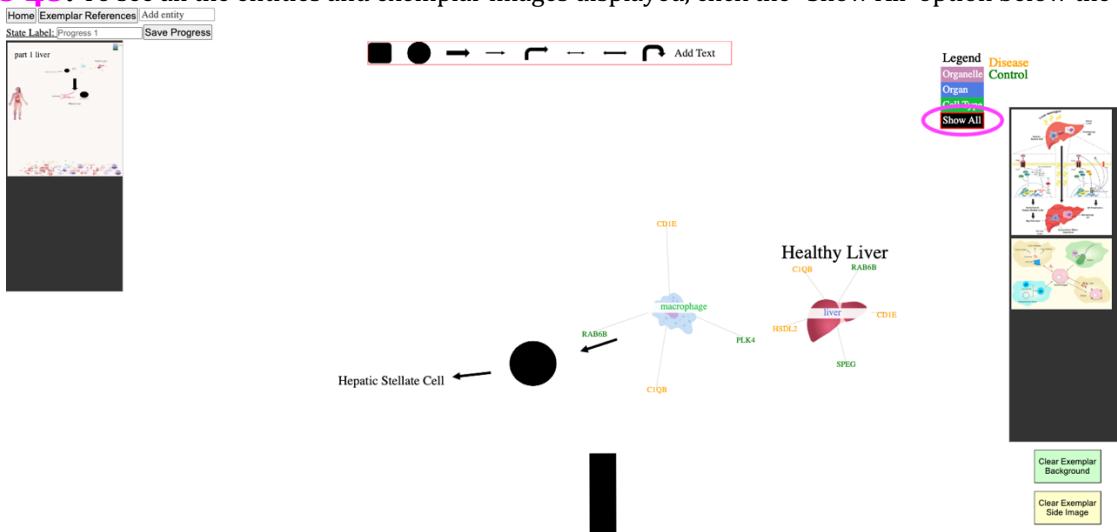


**TODO 47:** Once the image finishes downloading, the page will load back to the context map. The entities have reset their positions, so you will have to click on the "part 1 liver" thumbnail on the lefthand side panel to bring your work back to the page. After your work loads, notice that your newly downloaded image is now present on the exemplar image options panel on the righthand side of the page. In the legend, click on the "Organ" and "Cell Type" categories. Notice that when you click on the "Organ" category, only the liver entity is present on the context map AND the first liver exemplar image is displayed in the panel, because the liver is an organ. Notice that when you click on the "Cell Type" category, only the macrophage entity is present on the context map AND both the exemplar you just used and the newly downloaded image are displayed in the panel, because the macrophage is a cell type and the macrophage is included in both images.

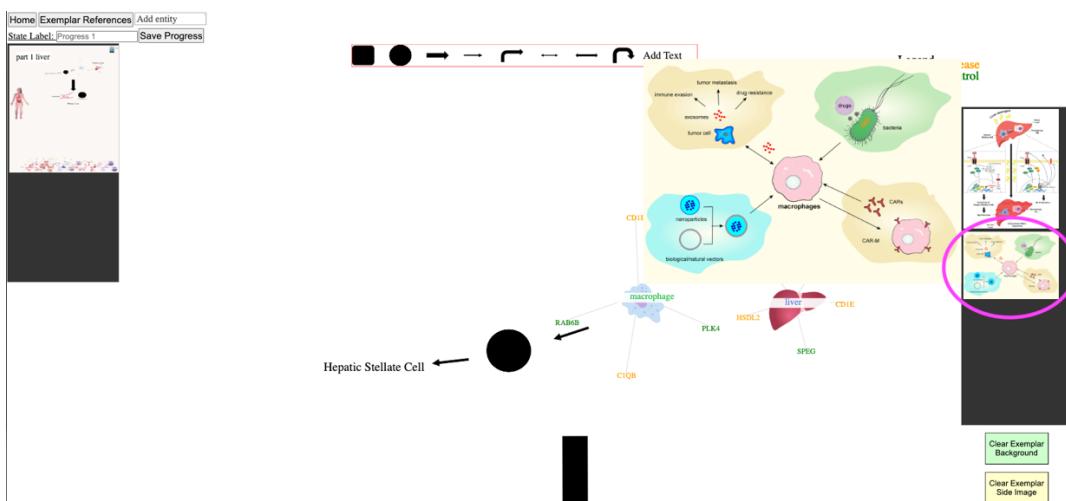




**TODO 48:** To see all the entities and exemplar images displayed, click the “Show All” option below the legend.

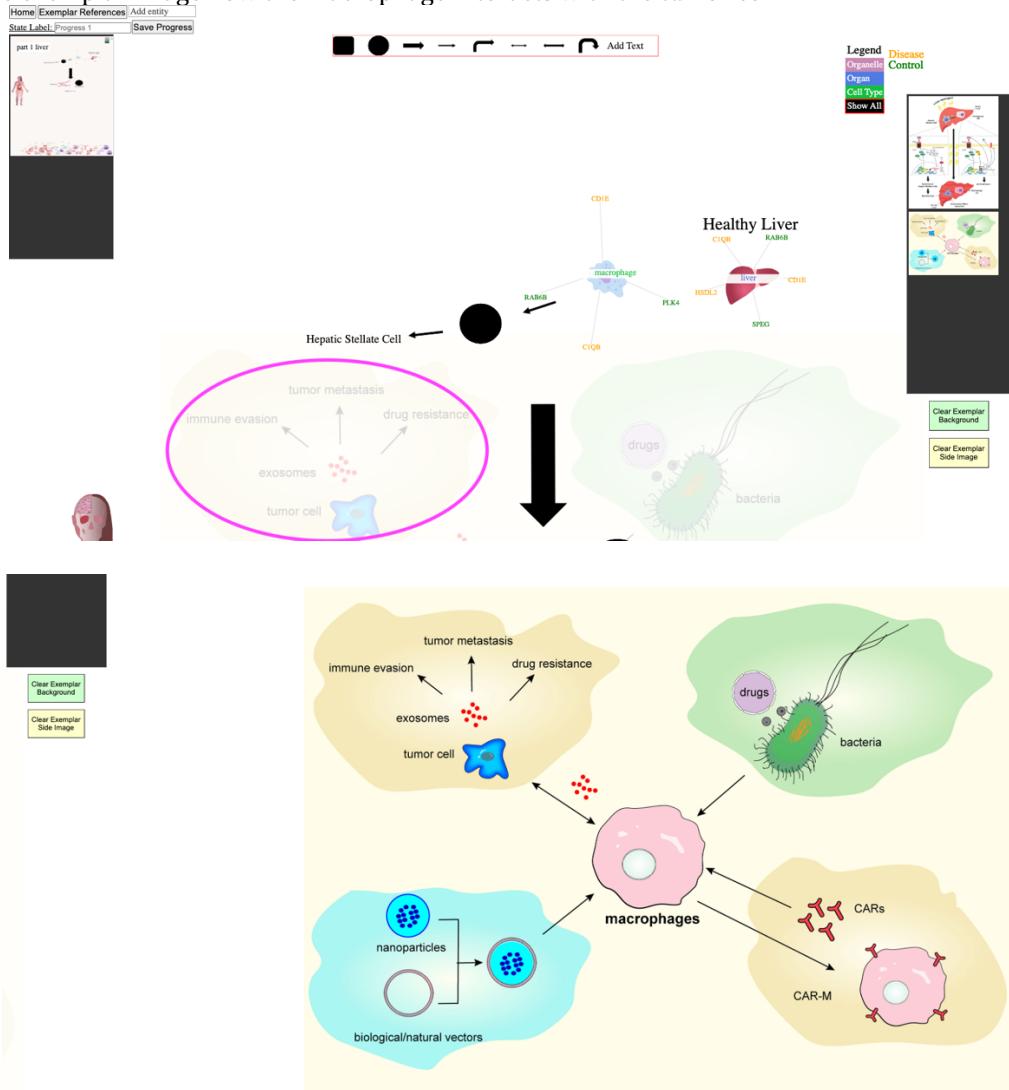


**TODO 49:** Hover over the newly downloaded exemplar image to show a larger version of the image.

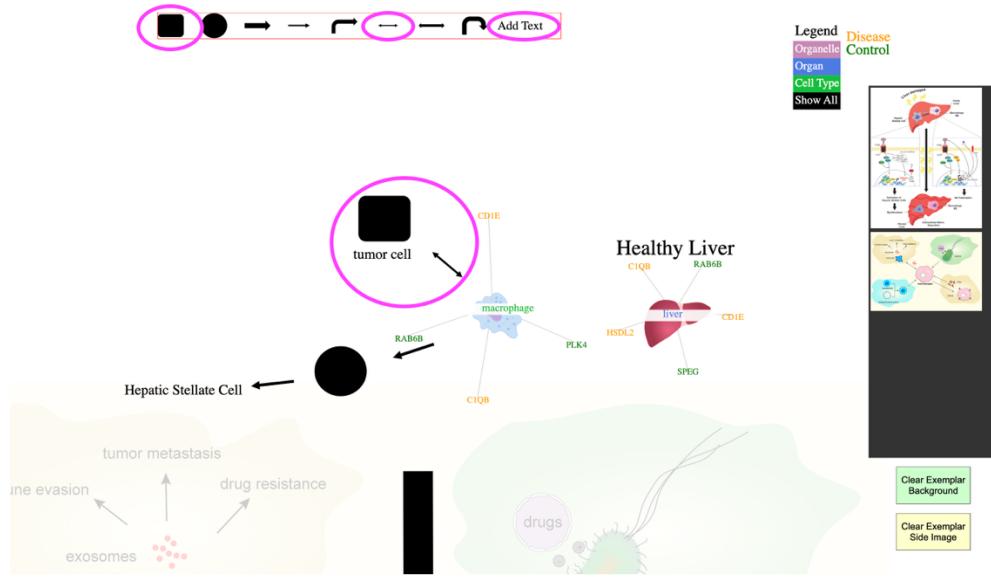


**TODO 50:** Click on the newly downloaded exemplar image. The image will show in the background of your context map, and to the right of the exemplar images panel. Feel free to use either one as reference when building

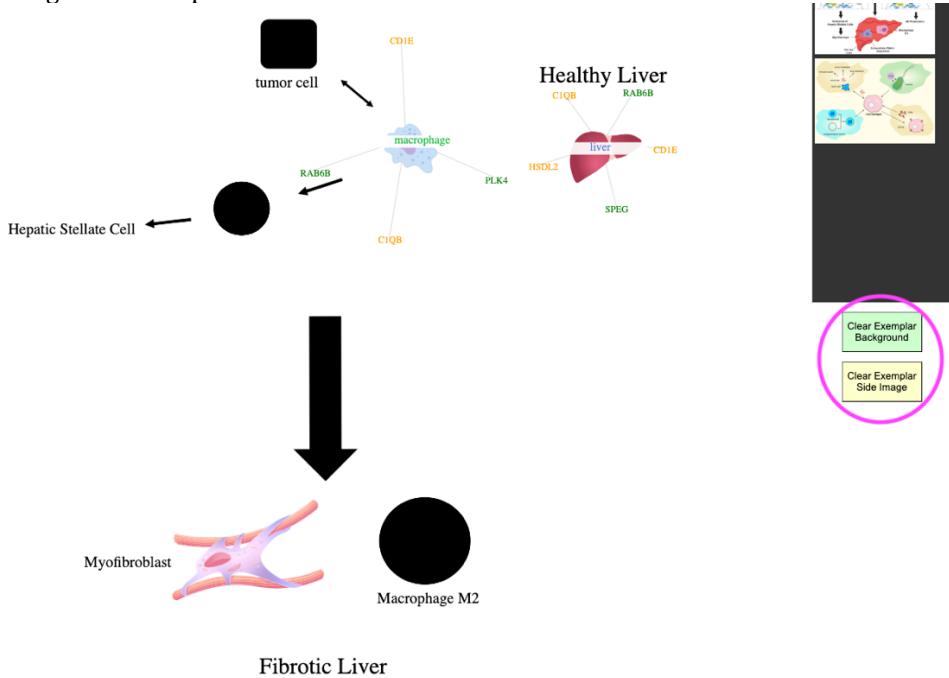
your context map. We will be layering information from this exemplar image to the context map we have already. Notice in the exemplar image how the macrophage interacts with the tumor cell.



**TODO 51:** We will replicate the tumor cell – macrophage interaction in our context map. Use the rectangle icon to substitute for the tumor cell. Use the double-ended arrow to relate the tumor cell to the macrophage entity, similarly to the exemplar image. Label the tumor cell with text.



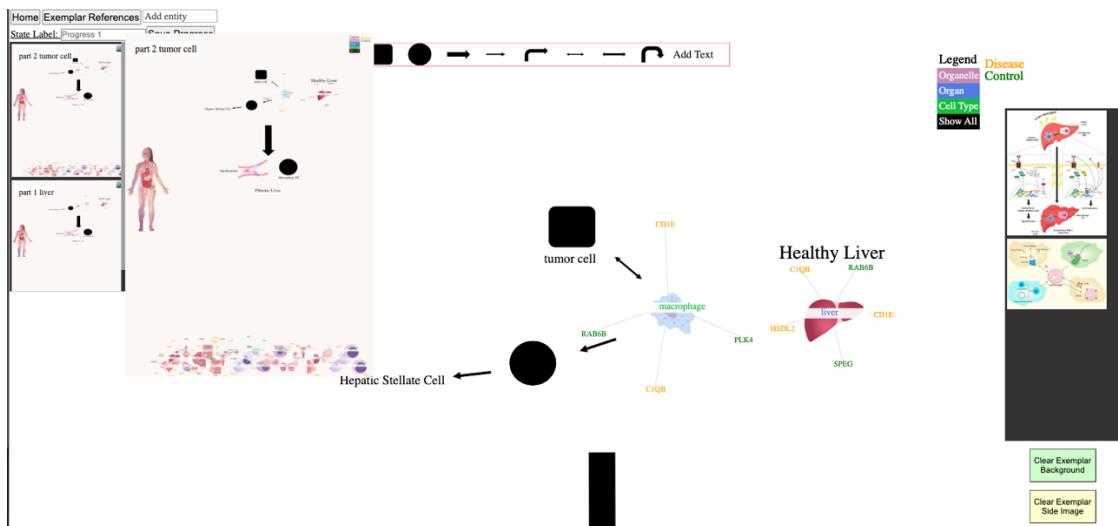
**TODO 52:** Click the “Clear Exemplar Background” button to remove the background image so that you are just left with your resulting context map.



**TODO 53:** Locate the State Label text box to the top left of the page. Enter in “part 2 tumor cell” and click the Save Progress button. The page will reload.

|                                |                     |               |
|--------------------------------|---------------------|---------------|
| Home                           | Exemplar References | Add entity    |
| State Label: part 2 tumor cell |                     | Save Progress |

**TODO 54:** In the scrollbar to the lefthand side of the page you will see your new work for “part 2 tumor cell”. Hover over the thumbnail to display the larger image and verify that this is the most recent version of your work. Click on the thumbnail to load “part 2 tumor cell” and see your work on your page.



You have finished exploring the context map!

## **References for Context Map Walkthrough Exemplar Images**

Fabregat I and Caballero-Díaz D (2018) Transforming Growth Factor- $\beta$ -Induced Cell Plasticity in Liver Fibrosis and Hepatocarcinogenesis. *Front. Oncol.* 8:357. doi: 10.3389/fonc.2018.00357

Gao J, Liang Y and Wang L (2022) Shaping Polarization Of Tumor-Associated Macrophages In Cancer Immunotherapy. *Front. Immunol.* 13:888713. doi: 10.3389/fimmu.2022.888713