Vignette Type: Experimental Design (w/Optional Coding)

Specific Example: Whole Human Brain

User:

Career: Undergraduate Students | Graduate Students | **Post-Docs** | **Senior Scientists/PI** | Teachers

Experience of Cell Types: Novice| Advanced Beginner | **Intermediate** | Expert

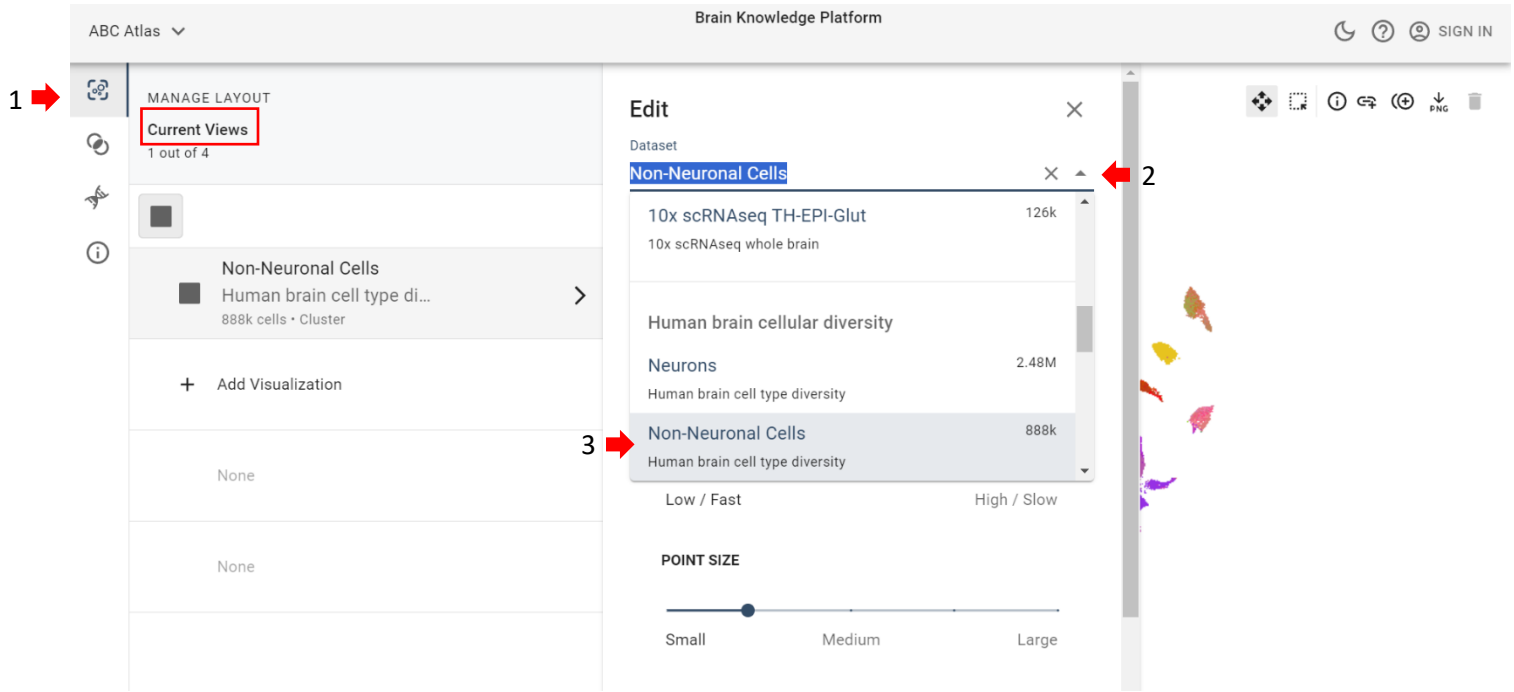
Research: Basic | **Translational**

Research Type: **Computational** | **Molecular** | Behavior

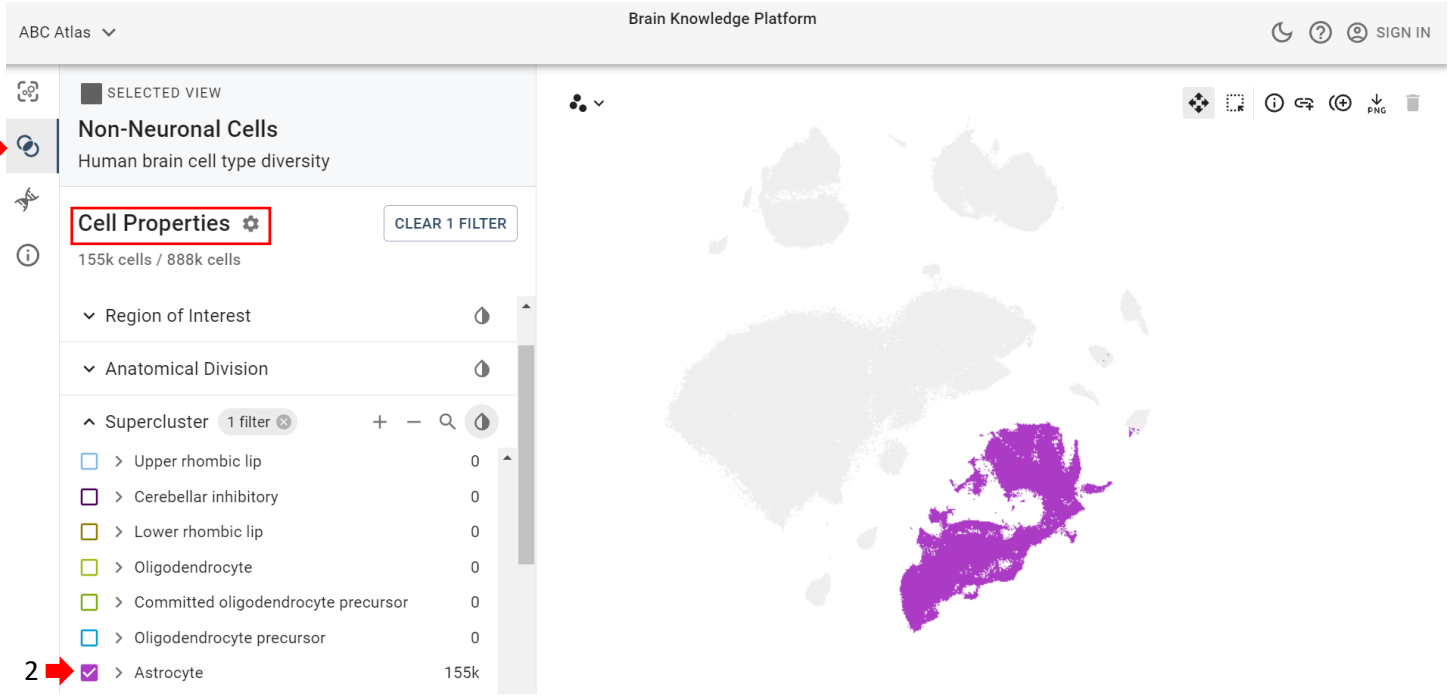
Experimental Model: Mouse | Rat | Non-Human Primate | **Human** | Invertebrate | Non-Traditional Vertebrate

A researcher studies the role of astrocytes in the development of multiple sclerosis (M.S.), using postmortem spinal cord samples from humans with and without M.S. They are beginning to design studies for experiments in other commonly affected brain areas, such as the cerebellum. Therefore, they are interested in learning more about astrocyte diversity in other brain areas in the non-M.S. brain.

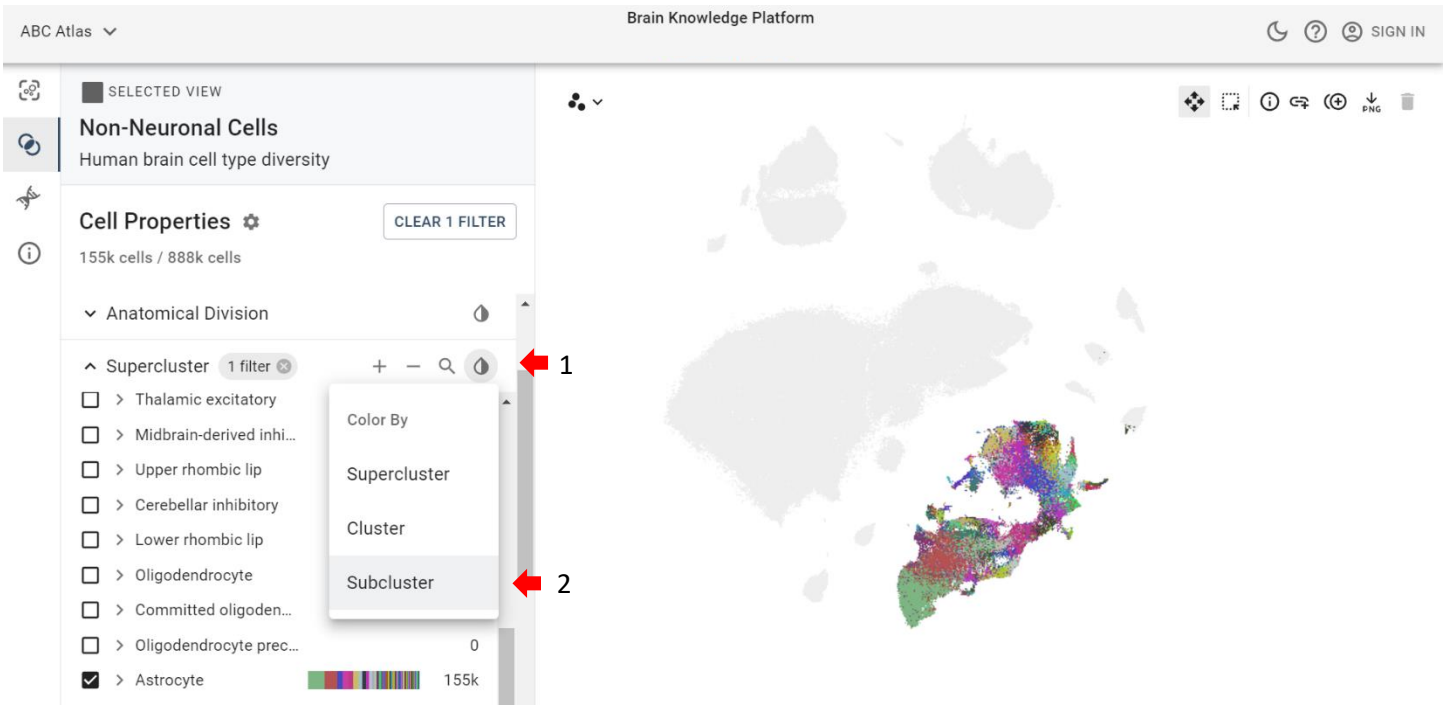
1. To view the non-neuronal nuclei in the dataset, the researcher clicks on the "Current Views" tab (step 1), clicks on the arrow next to "Dataset" (step 2) and clicks on "Non-Neuronal Cells" (step 3) under "Human brain cellular diversity". Link to view in ABC Atlas



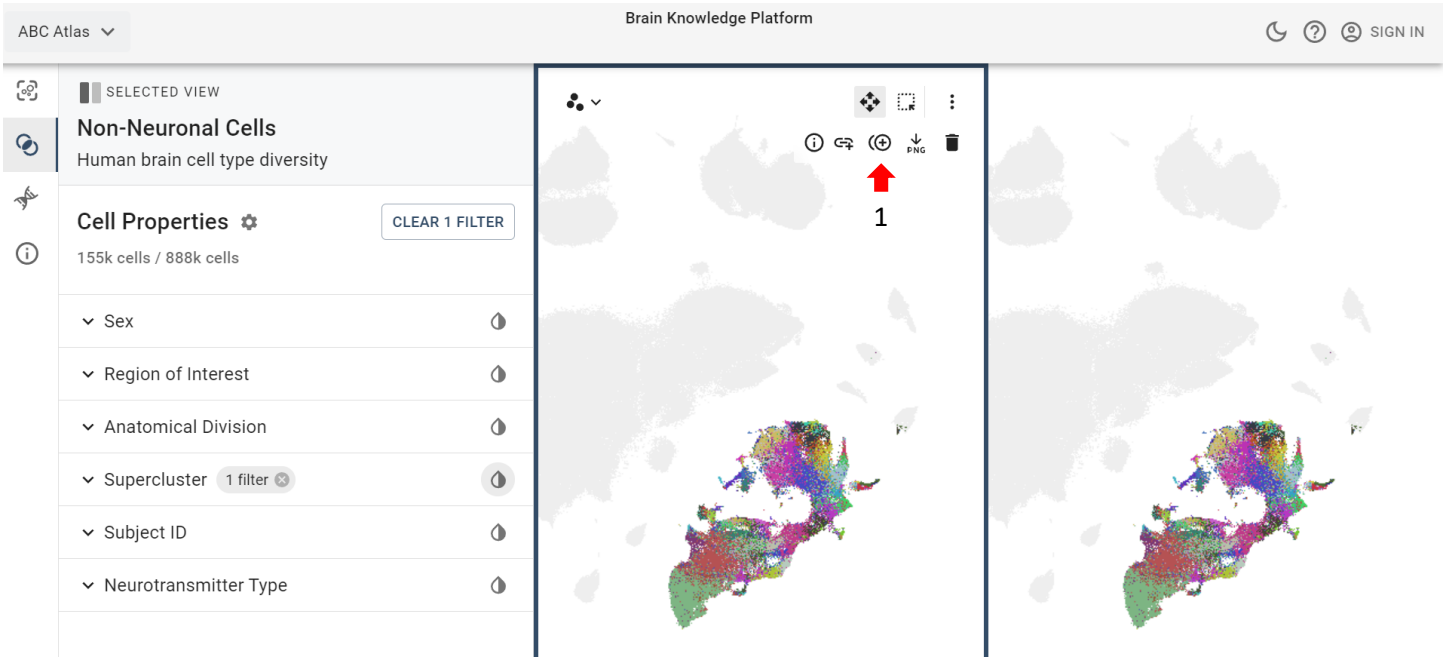
2. To select only astrocytes, the researcher clicks on the “Cell Properties” tab (step 1), then clicks on the arrow next to “Supercluster” and checks the box next to “Astrocyte” (step 2). Link to view in ABC Atlas



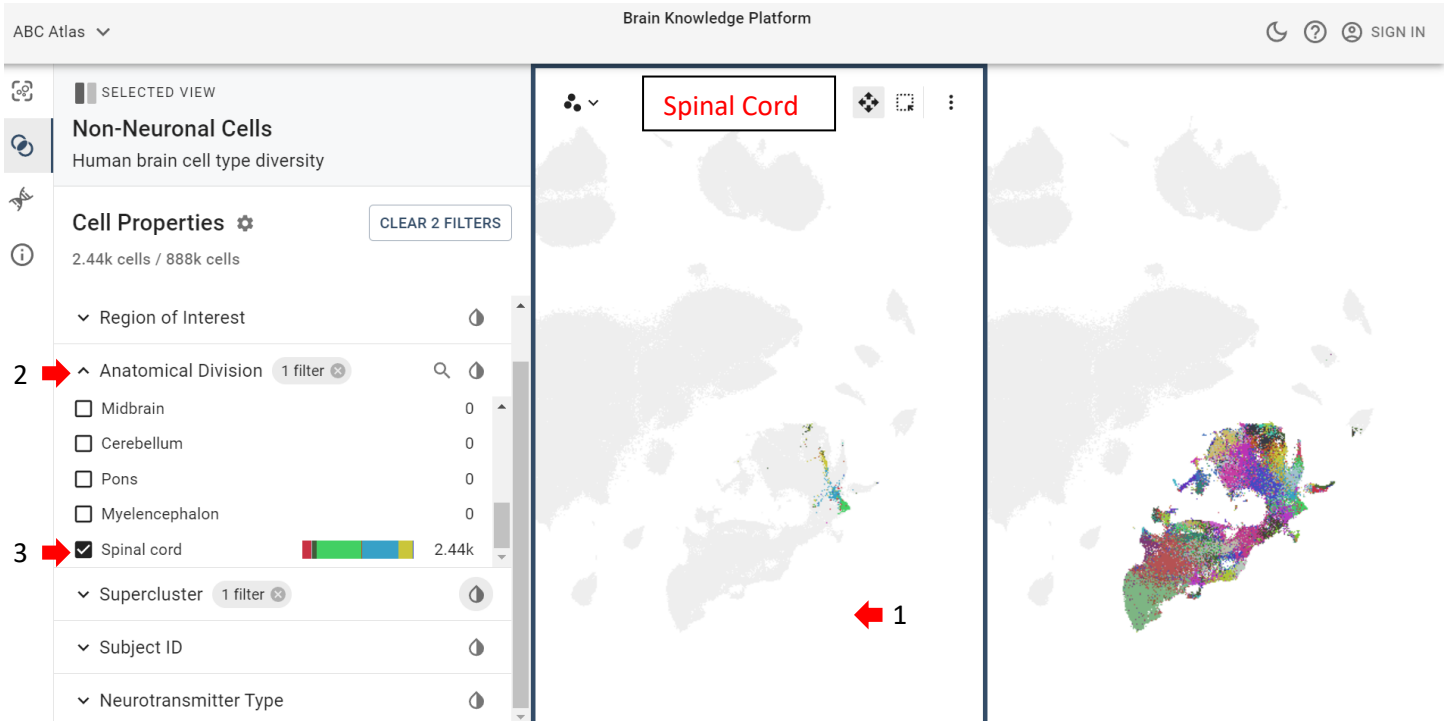
3. To view the subclusters within the Astrocyte supercluster, the researcher clicks on the ink drop symbol (step 1) next to “Supercluster” and clicks on “Subcluster” on the pop-up menu (step 2). Link to view in ABC Atlas



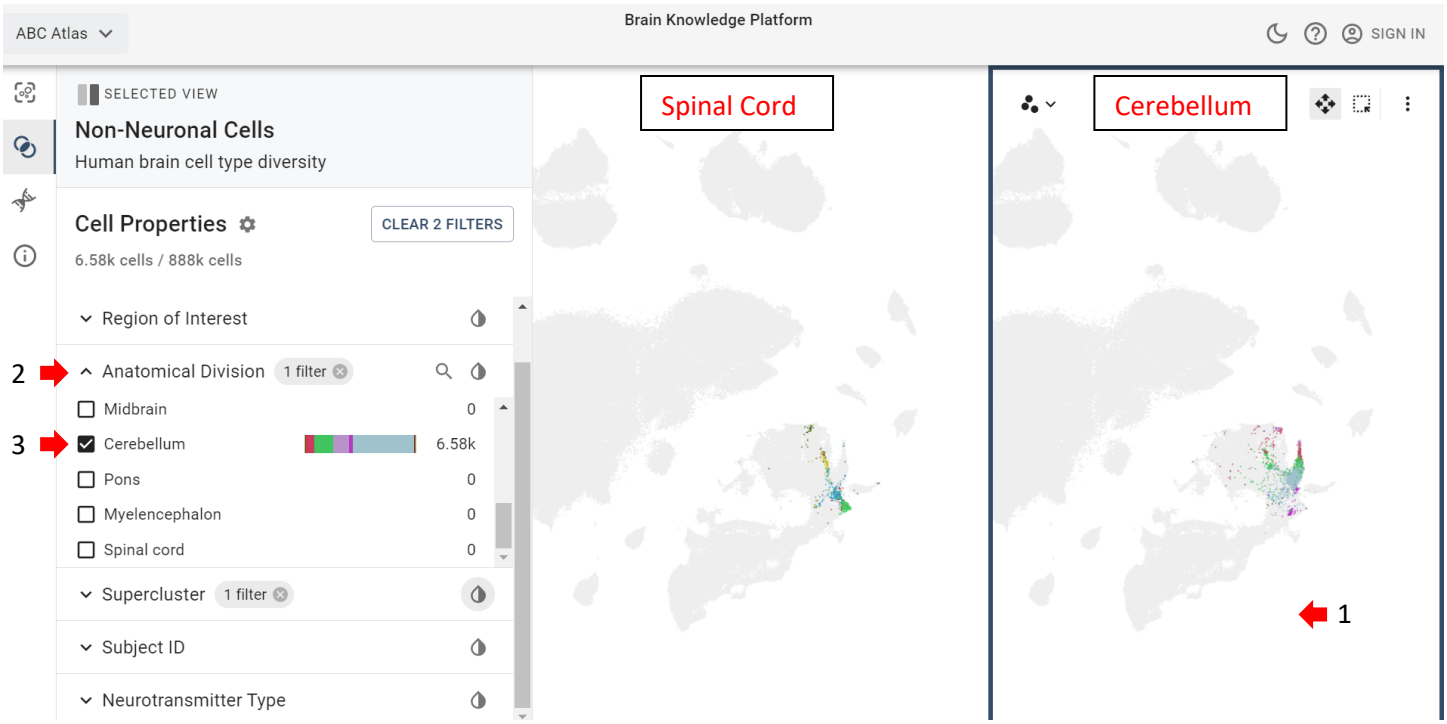
4. To duplicate the view and look at two t-SNE plots at once, the researcher clicks the “Duplicate View” button (step 1). [Link to view in ABC Atlas]



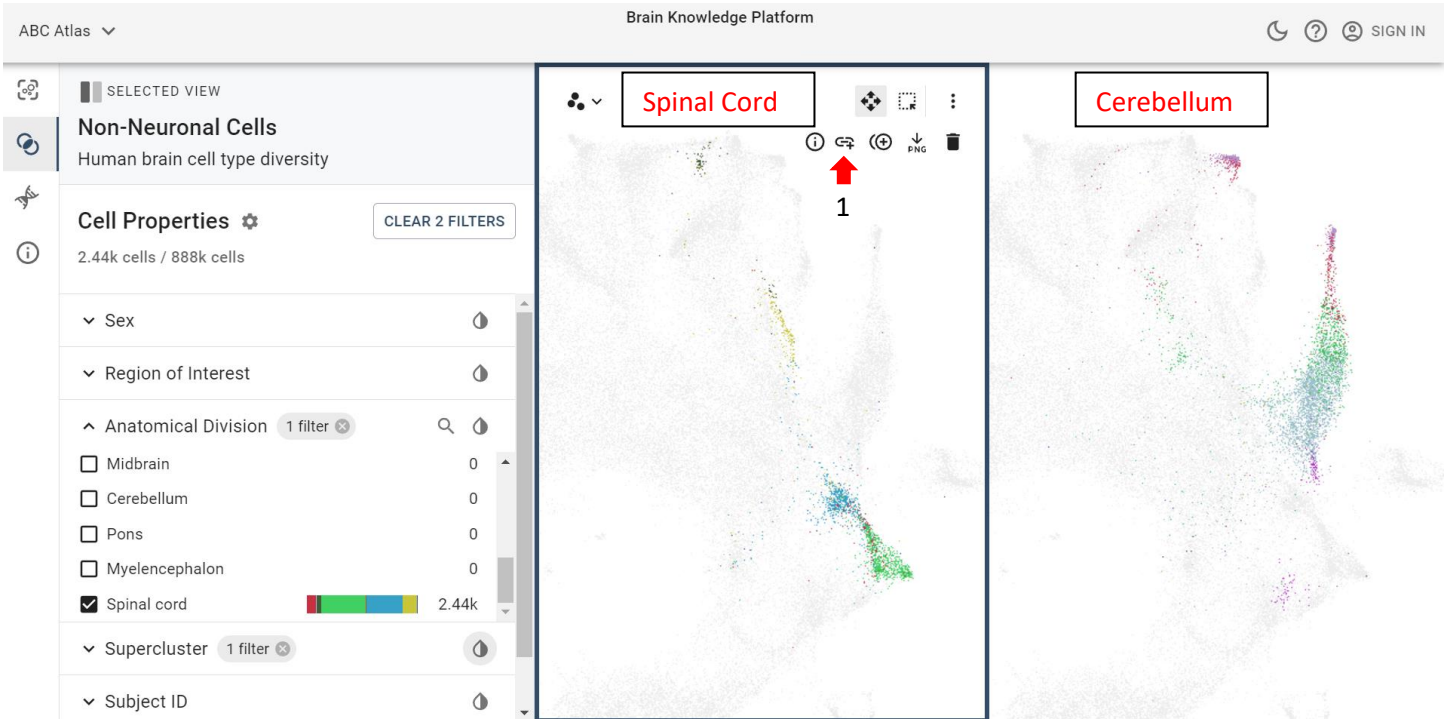
5. To look at just the spinal cord in the lefthand t-SNE, the researcher clicks on the lefthand t-SNE (step 1), clicks on the arrow next to “Anatomical Division” (step 2), and checks the box next to “Spinal cord” (step 3). [Link to view in ABC Atlas]



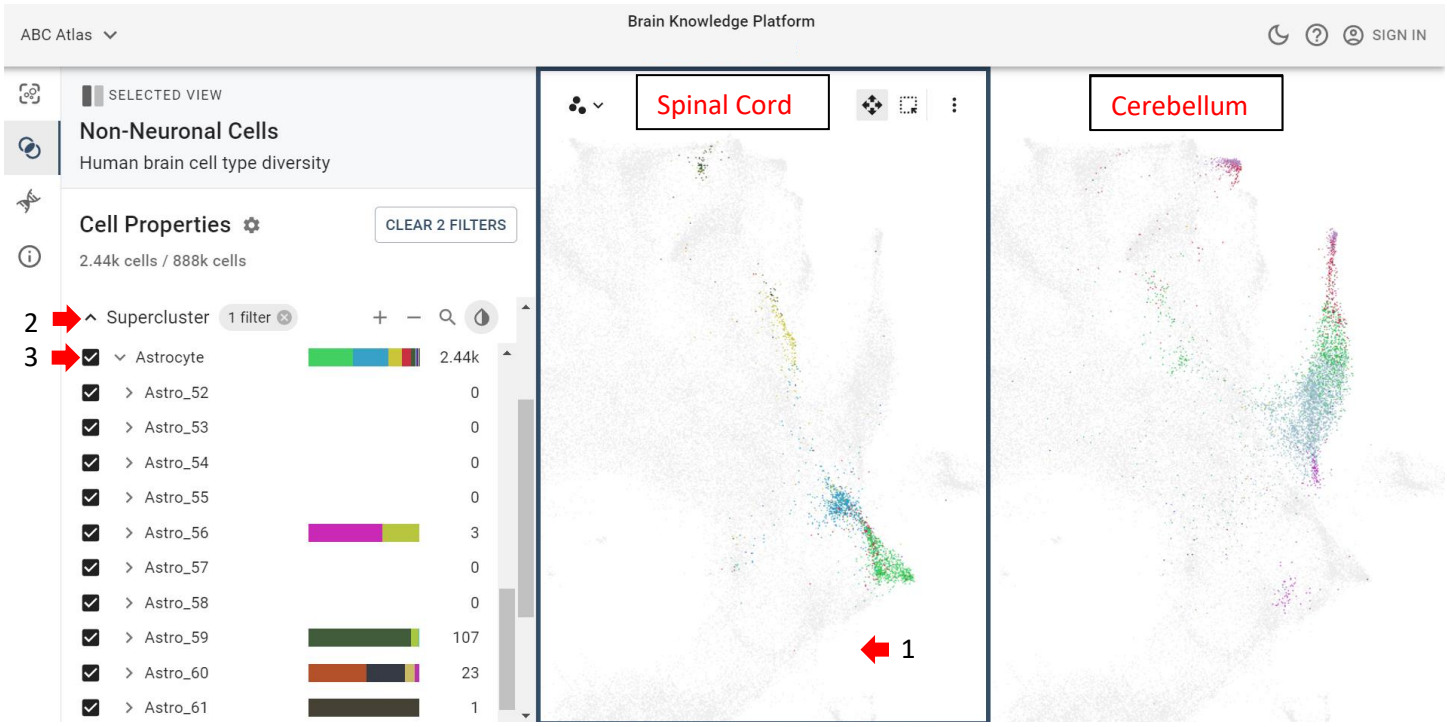
6. To look at just the cerebellum in the righthand t-SNE, the researcher clicks on the righthand t-SNE (step 1), clicks on the arrow next to “Anatomical Division” (step 2), and checks the box next to “Cerebellum” (step 3). [Link to view in ABC Atlas]



7. To zoom in on both t-SNE plots at the same time, the researcher clicks the “Enable Zoom & Pan Sync” button (accessible by clicking on the three dots) on both t-SNE plots to link them together (step 1) and then zooms in by scrolling their mouse. [Link to view in ABC Atlas]

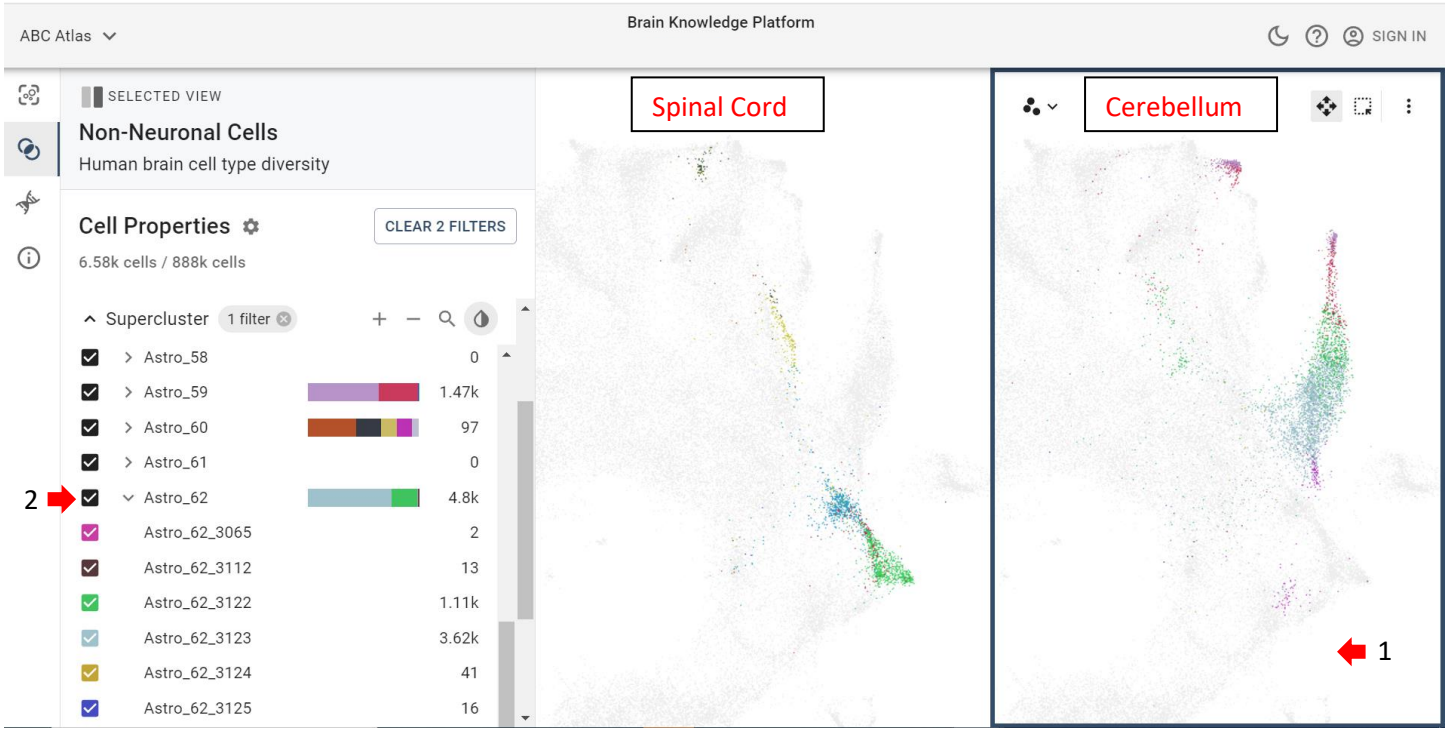


8. To view the clusters within the “Astrocyte” supercluster in the spinal cord, the researcher clicks on the lefthand t-SNE (step 1), clicks on the arrow next to Supercluster (step 2), then clicks on the arrow next to “Astrocyte” (step 3) to reveal the clusters. [Link to view in ABC Atlas]

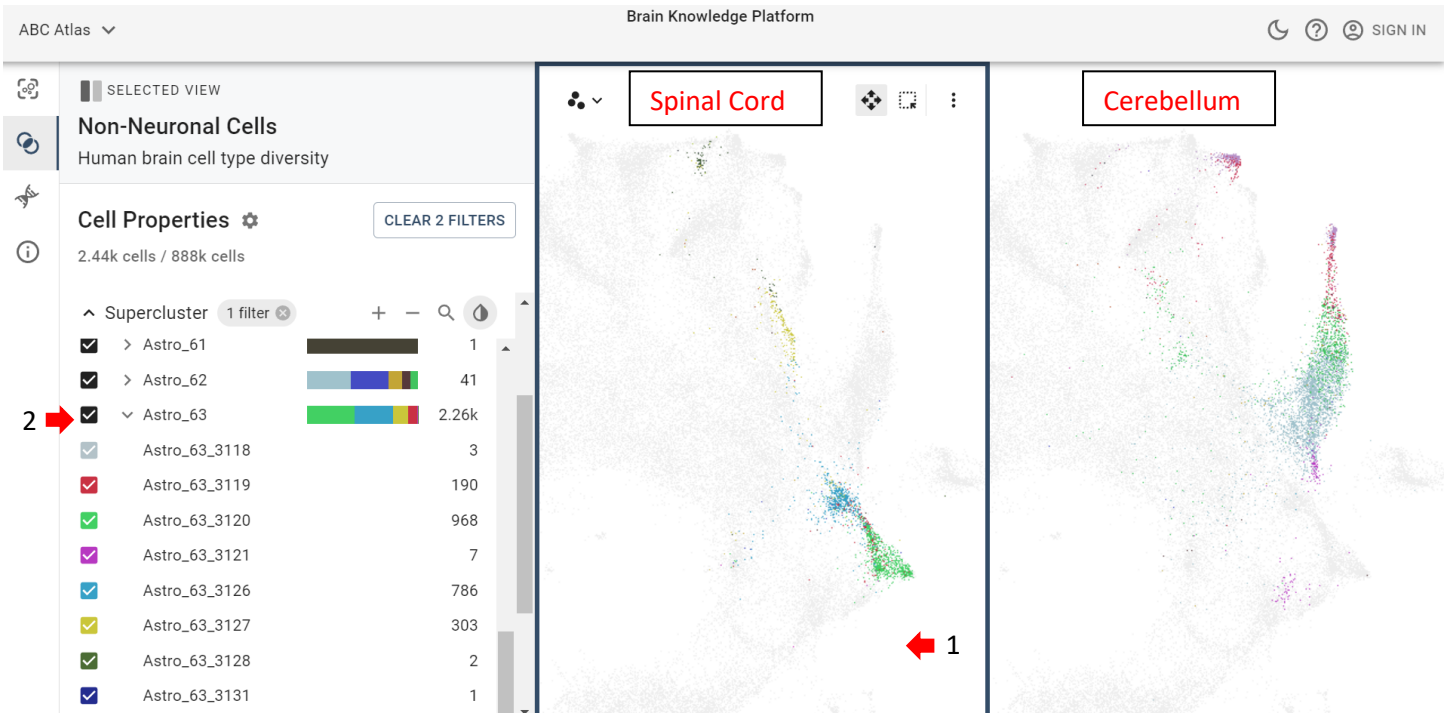


9. To view the clusters within the “Astrocyte” supercluster in the cerebellum, the researcher clicks on the righthand t-SNE (step 1), then click on the arrow next to Supercluster (step 2), then clicks on the arrow next to “Astrocyte” (step 3) to reveal the clusters. [Link to view in ABC Atlas]

10. When looking at the cerebellum astrocyte clusters in step 9, the researcher notices that the majority of nuclei (4.8k) belong to the Astro_62 cluster. To learn more about the Astro_62 cluster, the researcher clicks on the righthand t-SNE (step 1), then clicks on the arrow next to Astro_62 (step 2) to reveal the subclusters within the Astro_62 cluster. Here the researcher sees that the majority of the nuclei (3.62k) belong to the Astro_62_3123 subcluster. Link to view in ABC Atlas

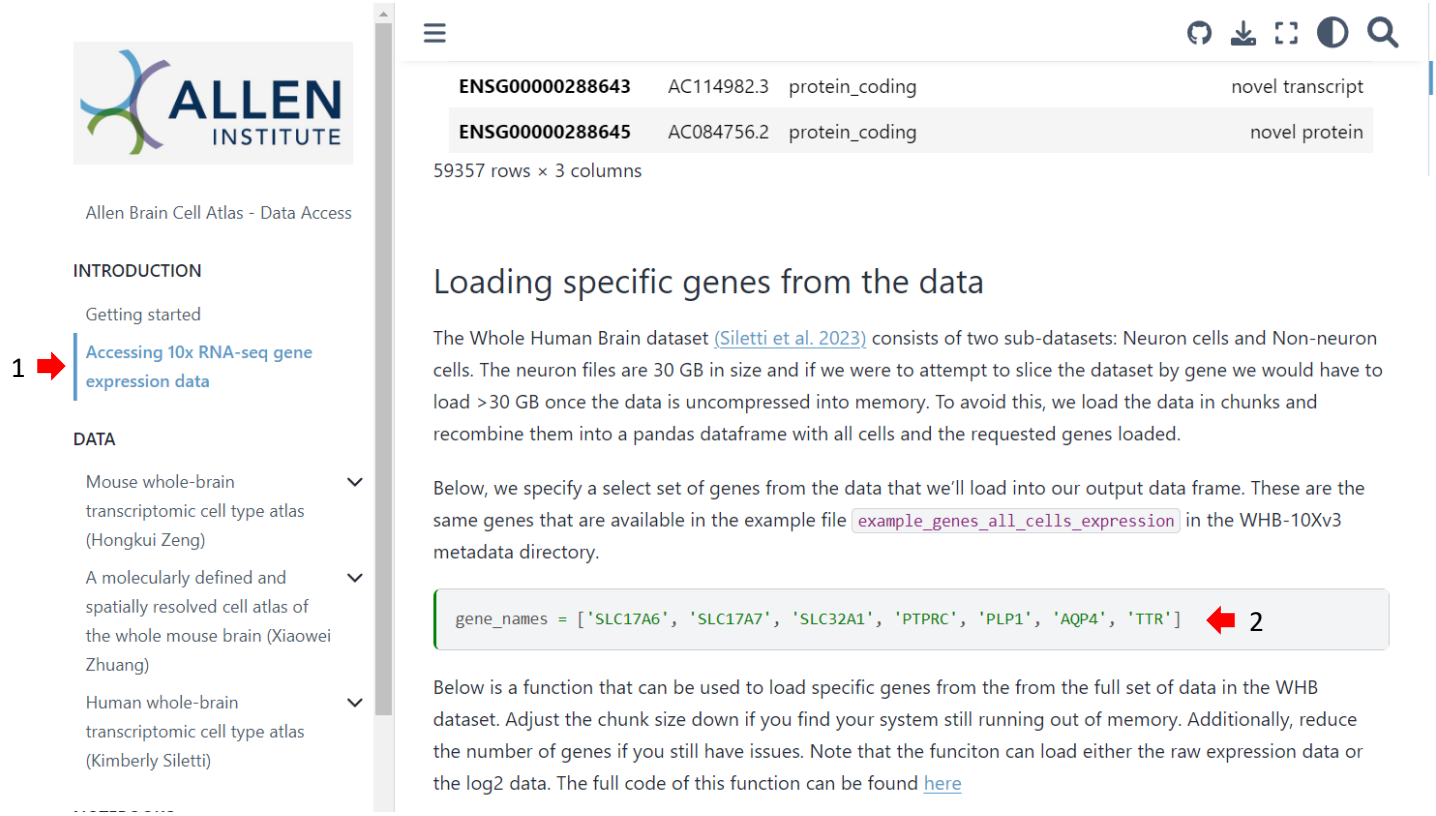


11. When looking at the spinal cord astrocyte clusters in step 8, the researcher notices that the majority of nuclei (2.26k) belong to the Astro_63 cluster. To learn more about the Astro_63 cluster, the researcher first clicks on the lefthand t-SNE (step 1), then clicks the arrow next to Astro_63 (step 2) to reveal the subclusters within the Astro_63 cluster. Here the researcher sees that the majority of the nuclei (968) belong to the Astro_63_3120 subcluster. Link to view in ABC Atlas



12. To learn more about the subcluster Astro_63_3120 (found in the spinal cord) and subcluster Astro_62_3123 (found in the cerebellum), the researcher goes to the subcluster annotation table to find the marker genes for these subclusters. In the table, the researcher finds the marker genes 'LINC01094' 'CD44' 'GFAP' 'ID3' 'APLNR' 'AL096709.1' 'AL627316.1' 'HSPB8' 'AC012405.1' 'FOS' for Astro_63_3120 and marker genes 'CPAMD8' 'GFAP' 'AC012405.1' 'AL627316.1' 'AC073941.1' 'CD44' 'AC097450.1' 'SLC14A1' 'PAX3' 'TNC' for Astro_62_3123.

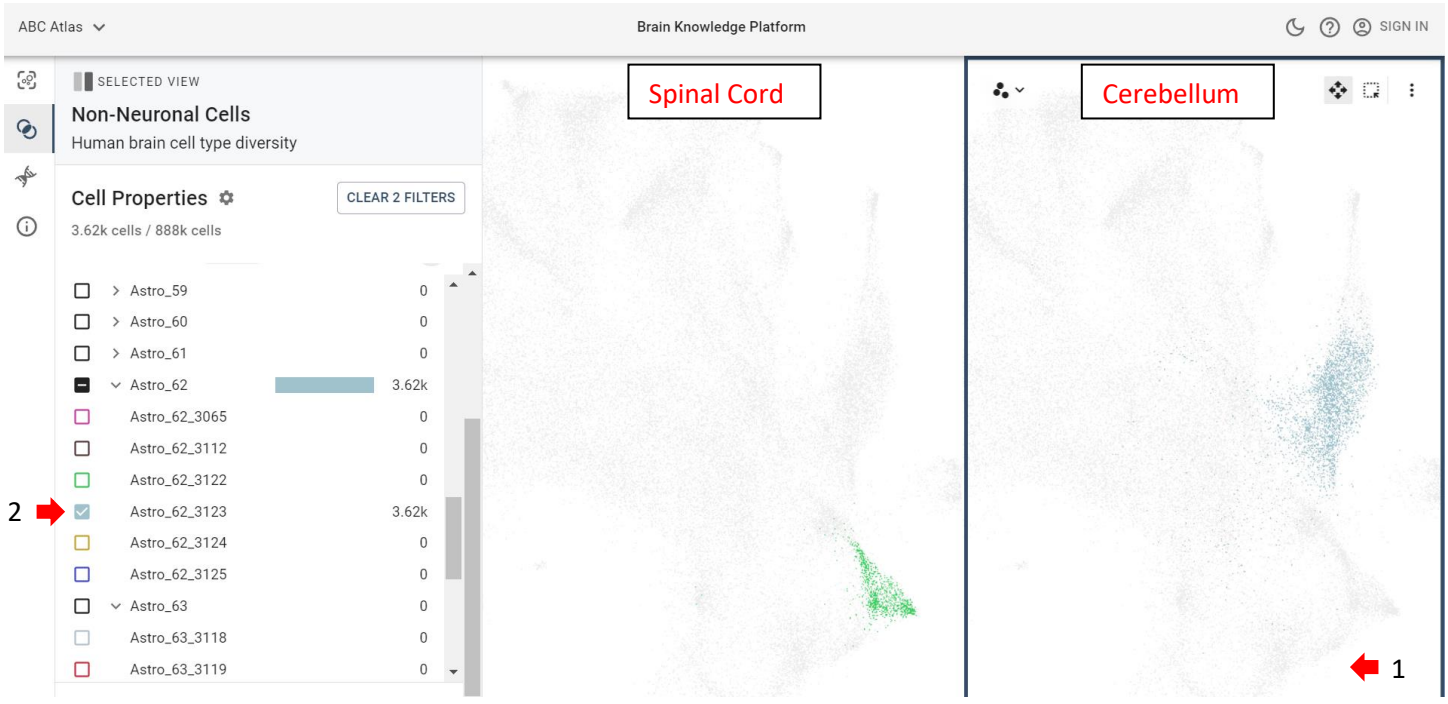
13. After finding marker genes from the subcluster annotation table, the researcher decides to compare the expression of these genes across the two subcluster populations. The researcher does this by accessing the WHB Jupyter Notebooks, and running the "Getting started" notebook. Afterwards, they click on "Accessing 10x RNA-seq gene expression data" notebook (step 1), scroll to "loading specific genes from the data," and replace the example gene names listed with the subcluster marker genes (step 2.) Then they combine with the "getting started" notebook and run the cells. Since the researcher is looking at non-neuronal data, they only need to download the non-neuron dataset.



14. To compare gene expression across subclusters, the researcher clicks on the "Whole Human Brain 10x RNA-seq gene expression" tab under notebooks and clicks on "part 2." Next, they change "example_cells_with_genes" to "gene_data" to use the genes they typed in for step 13, then combine with the notebook in the previous step and run the cells.

15. After looking at the heatmaps of 'LINC01094' 'CD44' 'GFAP' 'ID3' 'APLNR' 'AL096709.1' 'AL627316.1' 'HSPB8' 'AC012405.1' 'FOS' and 'CPAMD8' 'GFAP' 'AC012405.1' 'AL627316.1' 'AC073941.1' 'CD44' 'AC097450.1' 'SLC14A1' 'PAX3' 'TNC', the researcher sees that *PAX3* has higher expression in Astro_62_3123 than Astro_63_3120.

16. To isolate individual subclusters in the t-SNEs, the researcher then filters the lefthand spinal cord t-SNE by the Astro_63_3120 subcluster and the righthand cerebellum t-SNE by the Astro_62_3123 subcluster by clicking on the respective t-SNE plots (step 1) and then checking only those boxes under the "Supercluster" tab (step 2).
[Link to view in ABC Atlas]



17. To color the Astro_63_3120 (spinal cord t-SNE) and Astro_62_3123 (cerebellum t-SNE) subclusters by the *PAX3* gene, the researcher clicks on a t-SNE plot (step 1), clicks on the "Genes" tab (step 2), types in "PAX3" (step 3), and clicks on the ink drop symbol (step 4), and then repeats these steps with the second t-SNE plot.

    The resercher then zooms in on the two t-SNE plots individually by undoing the pan and zoom feature by clicking on the "Enable Zoom & Pan Sync" symbol (accessible by clicking the three dots in the upper right corner) on both of the t-SNE plots, and scrolls on each plot with their mouse to zoom. [Link to view in ABC Atlas]

18. Now the researcher begins to design experiments based on their new knowledge of astrocyte diversity in the spinal cord vs. cerebellum.