



Interactive dot plot/heat map viewer

This manual provides usage information for the interactive dot plot and heat map viewer at prohits-viz.lunenfeld.ca. This tool is designed to interactively display dot plots and heat maps generated from other tools on Prohits-viz.

Browser compatibility

Optimal performance of this tool is achieved with Chrome (tested in versions 47 - 55) although it is fully compatible and supported in Firefox (tested in versions 43 - 50) and Safari (tested in versions 7 - 9). For optimal performance with very large images, close unnecessary applications and browser tabs. In some cases fonts may appear too large in Firefox due to Firefox setting a hard cap on the minimum font size. This can be disabled by following the instructions [here](#).

Any other issues should be addressed to jknight@lunenfeld.ca.

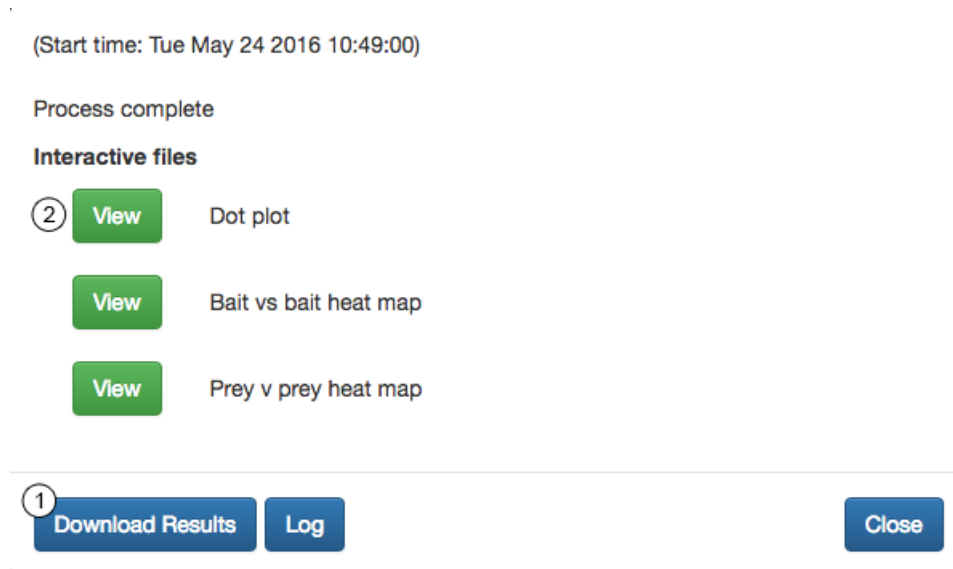
Sample input files

Input files are available for download from the “sample input files” hyperlink on the main page. These files were generated using the Dot plot Generator and Correlation tool using BioID data from the following paper:

Couzens, A.L., Knight, J.D.R., Kean, M.J., Teo, G., Weiss, A., Dunham, W.H., Lin, Z.Y., Bagshaw, R.D., Sicheri, F., Pawson, T., Wrana, J., Choi, H., Gingras, A.-C. (2013) Protein interaction network of the mammalian Hippo pathway reveals mechanisms of kinase-phosphatase interactions. *Sci Signal*, **6**: rs15. [PMID: 24255178](https://pubmed.ncbi.nlm.nih.gov/24255178/)

Inputting data

Currently, compatible output is produced from the following tools: Correlation Analysis and the Dot plot Generator. After running these tools, results can be downloaded (1) and interactive files for this viewer will be found in the folder *InteractiveFiles*. Alternatively, results can be passed directly from these tools by clicking on the *Interactive files* links (2) after the task is complete.

Figure 1

The input interactive file is simply a plain tab-delimited text file with between four to five columns ordered first by row and then by column as shown in **Figure 2**. The *score* column is optional for heat maps, while the column names *row*, *column*, *value* and *params* are required in all cases. Entries in the *params* column will dictate how the viewer displays and handles the input data. This column will be generated automatically by the tools on Prohits-viz but can be edited as desired. The first row in *params* must be one of the available display types, either “dot plot” or “heat map”. The second row specifies the type of filter that should be applied, with “0” indicating a score that functions as a “less-than” filter, i.e. the lower the score the better (e.g. FDR), while “1” indicates a score that functions in the opposite way, a “greater-than” filter. The third and fourth rows specify the primary and secondary filters to use for coloring the circle edge on dot plots. The fifth column indicates the name for the “score” being used, while the sixth column indicates the type of “value”, in this example “Spectral count”. These names are used simply for labeling the legend. For heat maps, only the first and sixth rows need to be specified.

Figure 2

row	column	value	score	params
gene A	gene X	4	0.06	dot plot
gene A	gene Y	5	0.06	0
gene A	gene Z	76	0.01	0.01
gene B	gene X	34	0	0.05
gene B	gene Y	26	0	FDR
gene B	gene Z	13	0.02	Spectral count
gene C	gene X	0	1	
gene C	gene Y	41	0	
gene C	gene Z	91	0	

Global view

After loading an image into the viewer there are several navigation features available from the side panel *Options* tab (≡ 1). If axis labels are too small, ✕ (2) can be enabled to enlarge them as shown in **Figure 4**. As the user scrolls down the page the x-axis will follow the scroll by default, although this can be disabled by de-selecting ⏸ (3). Select ☐ (4) to remove empty rows and columns on zoomed areas, i.e. rows and columns that contain no data points. The legend can be toggled with 📖 (5). Images on ProHits-viz will only be stored temporarily. Clicking the archive 📁 (6) button will store the image in our database with a permanent URL. The input at (7) is used for gene searches. Searches are case insensitive. Matches will be highlighted with a green circle on the appropriate axis (both if applicable) and these can be removed by ✂ (8). Finally, the image can be exported using (9) either as an SVG or PNG. When saving very large images (heat maps with more than 200K cells), we recommend saving first as a PNG before attempting an SVG export as this operation can often be too memory intensive. Long gene names will be truncated in the axes labels, but on hovering the full name will be displayed (10). The full name will be shown in an exported file.

Figure 3

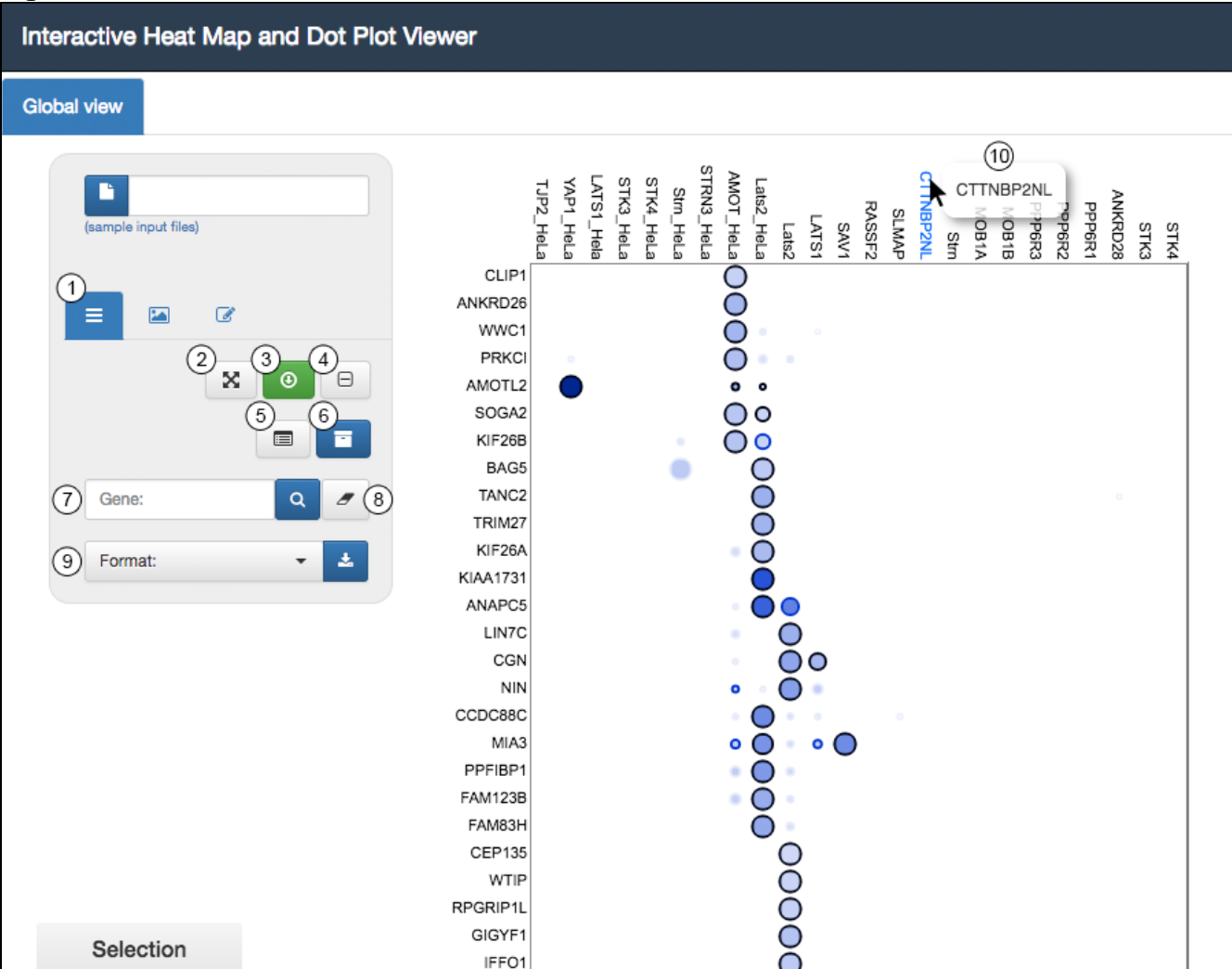
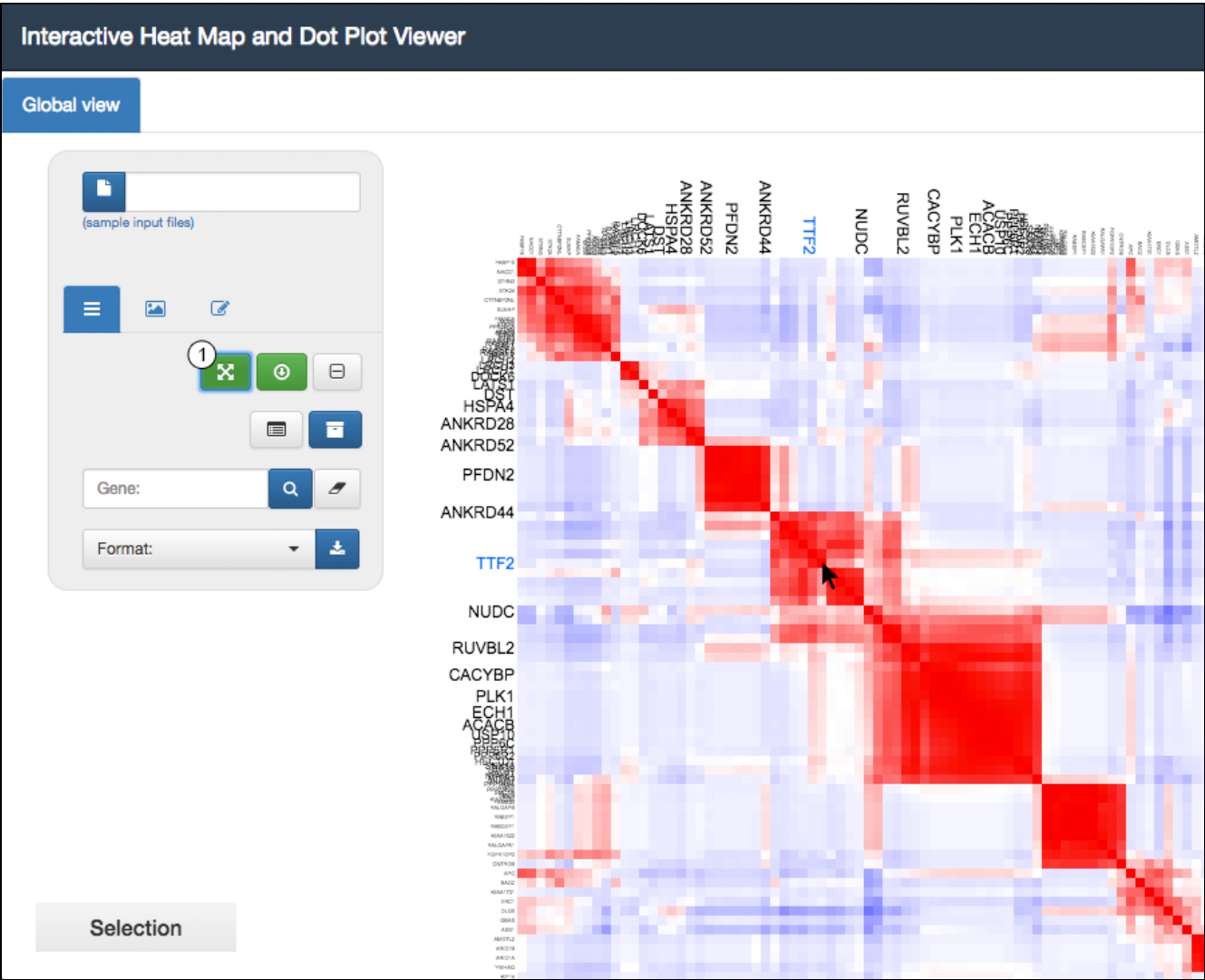


Image navigation

Axis labels that are too small can be selectively enlarged relative to the cursor position by enabling  (1). The cell over which the cursor is positioned will have its labels colored blue.

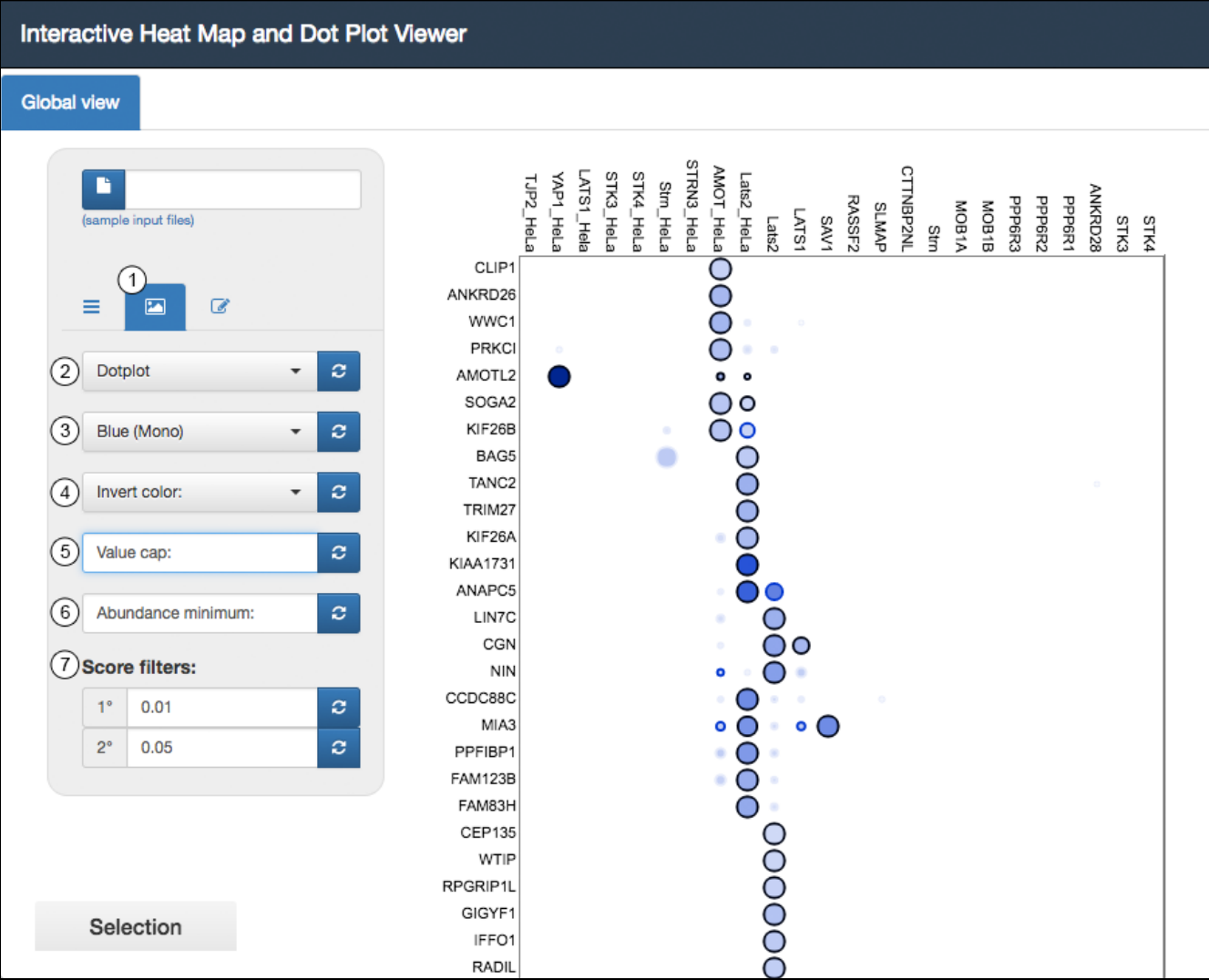
Figure 4



Display options

The image that is shown on the *Global view* tab is static in that rows and columns cannot be re-ordered, however superficial display changes can be made from the *Display options* side panel tab  (1). The image can be switched between a dot plot and heat map by using the select box at (2) and hitting refresh . When an interactive file is loaded into the viewer, a dot plot will be displayed by default if an *score* column is present. If a *score* column is not present and the user switches from a heat map to a dot plot, it will be assumed that all entries have passed the 1° filter and will be displayed accordingly. A variety of color schemes are available from (3) although monochromatic (*Mono*) color schemes will only be available for data with values ≥ zero. The color scheme can be inverted with (4). For example, for data with positive and negative values a color scheme of red-green can be selected where red would be used for negative values and green for positive, and the *Invert color* option allows this to be reversed. The *Value cap* (5) specifies the color display cap and by default is set to the maximum value in the dataset or 50, whichever is lower, although the user can select a different value. Any values above this cap will be displayed in the image with the same value as the cap. This is too ensure there is sufficient dynamic display range for lower values in the dataset. Data points below a certain minimum can be removed from the image with (6). The coloring of edges in dot plots is controlled by the filter settings in (7). The 1° filter dictates the darkest edge color, while the 2° filter dictates the medium intensity edge color; values above this secondary cutoff will be displayed with the lightest edge color.

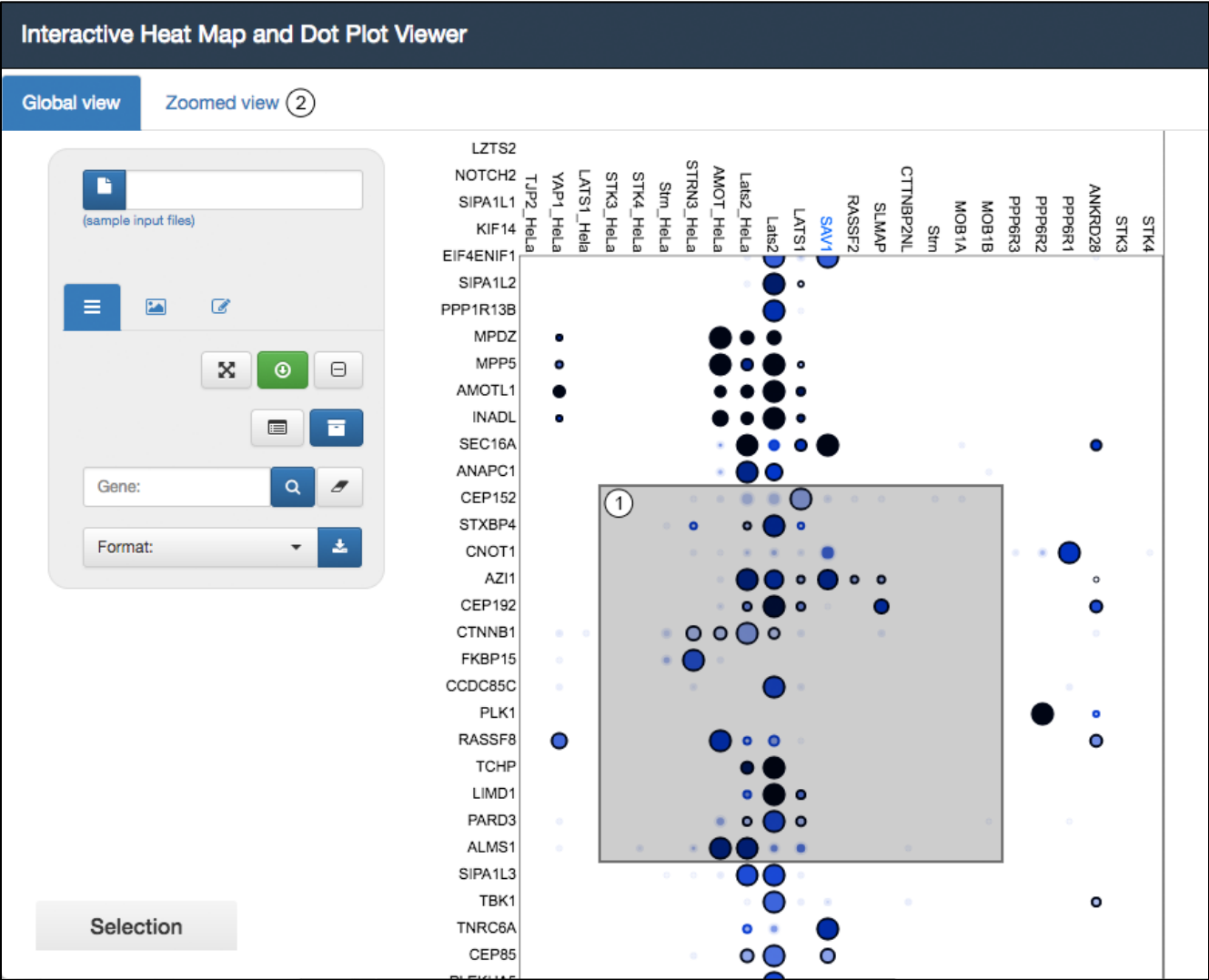
Figure 5



Selections

From the *Global view* tab regions can be selected for zooming and further analysis by dragging and selecting a region with the mouse (1). The selected region will be passed to the *Zoomed view* tab (2).

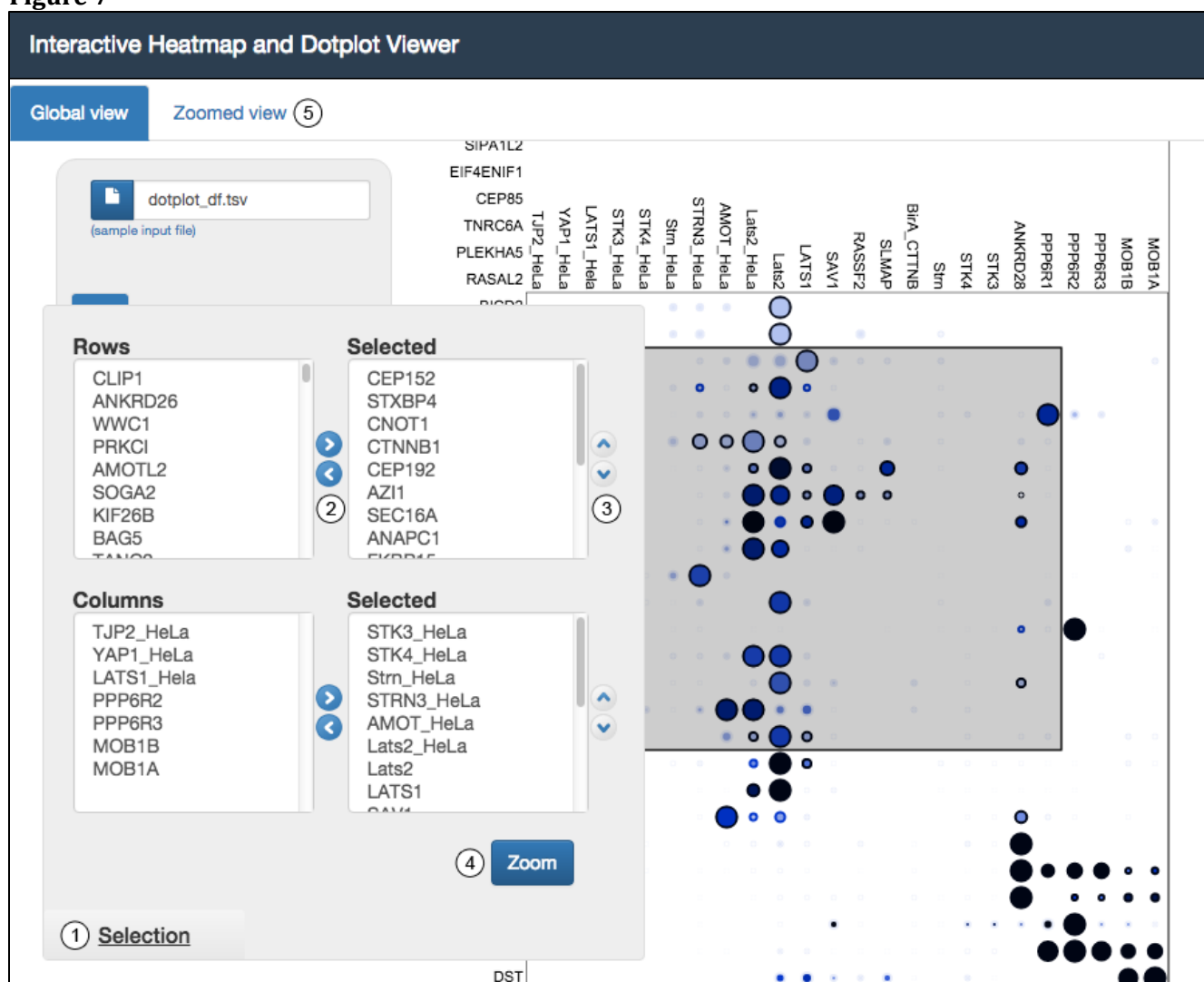
Figure 6



Selections continued

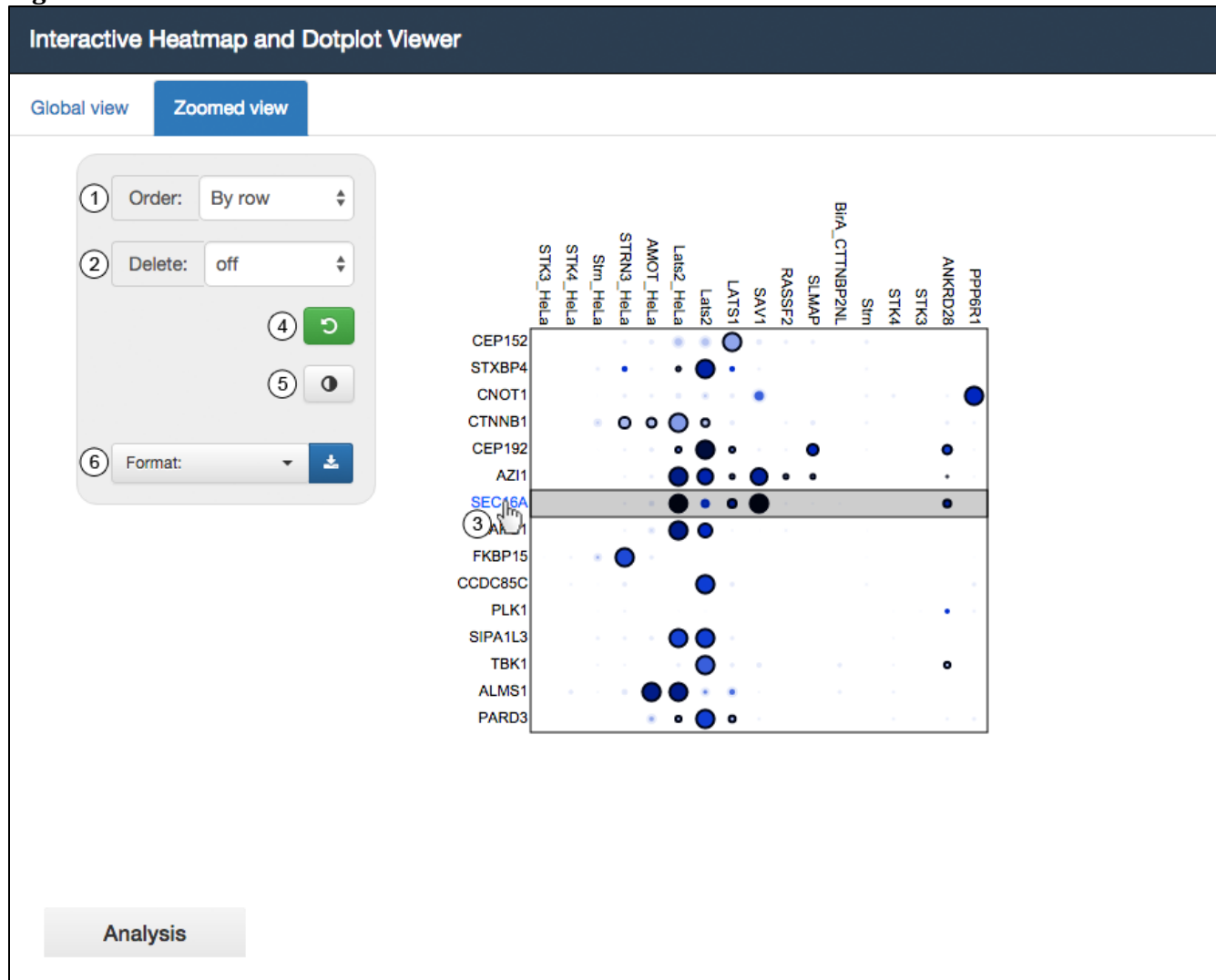
Alternatively, genes or regions can be selected use the *Selection* panel (1), shown in **Figure 7**. Here, row and column names will be listed based on their order in the input file. If clustering has been performed using the Dot plot generator, the order will reflect the order post-clustetering. They can be chosen and passed to the *Selected* boxes using the arrows shown at (2). The arrows at (3) can be used to reorder the genes. On hitting *Zoom* (4), the selected genes will be displayed in the chosen order on the *Zoomed view* tab (5). Note: if rows have been selected but no columns, all columns will be selected by default when hitting *Zoom*, and vice versa.

Figure 7



Zoomed view

The *Zoomed view* tab is used for exploring zoomed regions, generating custom images and performing analysis. The *Zoomed view* is a dynamic image in that rows and columns can be re-ordered and/or deleted. To re-order or delete a row or column, select the appropriate entry (from (1) or (2)). When re-ordering, clicking on a row or column in the image (3) will highlight it while dragging will reposition it. Deleting a row or column is done by simply clicking on it after selecting the desired action from the “Delete” box (2). The most recent action can be undone by pressing ↶ (4); pressing this button again will undo the next most recent action and so on. On dot plots, the circle size indicates the relative abundance of a row gene across the columns. On the *Zoomed view* tab this size will still be in reference to the entire data set by default. However, ● (5) can be toggled to make this size relative to the currently selected region. Finally, the image can be exported as a PNG, SVG or PDF using 📄 (6).

Figure 8

Analysis

Genes displayed on the *Zoomed view* tab can be passed for analysis by selecting the *Analysis* panel (1) in **Figure 9**. Genes highlighted in the *Rows* and *Columns* lists can be passed to the *Selected* list using the arrows at (2). Available analysis options are displayed as tabs (3). Currently, three analysis options exist and more will be added on an ongoing basis. To learn more about the current analysis options, follow the hyperlink (4) next to “For help”. In this example, GO enrichment is performed through the site at biit.cs.ut.ee/gprofiler/ where the user can find help information and details about the analysis tool. Analysis begins by pressing *Start* (5) and the results will be available as a new tab (6) when complete.

Figure 9

The screenshot displays the 'Interactive Heatmap and Dotplot Viewer' interface. At the top, there are three tabs: 'Global view', 'Zoomed view', and 'GO Analysis' (labeled with a circled 6). Below the tabs, the 'Zoomed view' section contains two lists: 'Rows' (TBK1, ALMS1, PARD3) and 'Columns' (STK3_HeLa, STK4_HeLa, Strn_HeLa, STRN3_HeLa, AMOT_HeLa, Lats2_HeLa, Lats2, LATS1, GATC). Below these lists are three arrows (down, up, down) labeled with a circled 2. A 'Selected' list below contains CEP152, STXBP4, CNOT1, CTNNB1, CEP192, AZI1, SEC16A, ANAPC1, and EPCOR1. To the right of the 'Zoomed view' section, there are two tabs: 'GO enrichment' (labeled with a circled 3) and 'Domain Analysis'. Below the tabs, there is a 'Start' button (labeled with a circled 5) and a 'Complete' button. Below the buttons, there is an 'Options' section (labeled with a circled 4) with a link 'For help, see [g:Profiler](#)'. The 'Options' section includes a dropdown for 'Organism' (Homo sapiens), a 'Query databases' section with checkboxes for 'Gene Ontology' (checked), 'Biological process' (checked), 'Cellular component' (checked), and 'Molecular function' (checked). Below this, there are checkboxes for 'Biological pathways' (checked), 'KEGG' (checked), 'Reactome' (checked), 'Corum protein complexes' (checked), 'Human Phenotype Ontology' (checked), 'BioGRID' (checked), and 'Regulatory motifs in DNA' (checked), 'TRANSFAC TFBS' (checked), and 'miRBase' (checked). At the bottom of the 'Options' section is an 'Advanced options' button. At the bottom left of the interface, there is a button labeled 'Analysis' (labeled with a circled 1).

Analysis tabs

On an analysis tab, the user can view the results of their inquiry. At the top of every analysis tab is located an annotation text box (1) and an *Annotate* button (2). From here the user can annotate the original selection made on the *Global view* tab (3). For example, if the user thought “cell cycle phase transition” best characterized his/her chosen selection, that term could either be typed in the annotation box or passed directly to it by pressing the + button (4) beside the term. This term will be displayed over the selection on the *Global view* tab (Figure 11) after pressing Annotate (2).

Figure 10

Interactive Heatmap and Dotplot Viewer

Global view Zoomed view **GO Analysis**

1 cell cycle phase transition 2 Annotate Clear Export Table

Source	Term name	Term ID	No. of term genes (T)	No. of query genes (Q)
BP	•negative regulation of protein metabolic process +	GO:0051248	1049	11
BP	•negative regulation of cellular protein metabolic pr... +	GO:0032269	981	11
BP	negative regulation of protein modification proc... +	GO:0031400	597	11
BP	•cell cycle phase transition + 4	GO:0044770	502	11
BP	•microtubule organizing center organization +	GO:0031023	111	11
BP	centrosome organization +	GO:0051297	101	11
BP	•centrosome cycle +	GO:0007098	71	11
BP	•regulation of chromosome segregation +	GO:0051983	88	11
BP	regulation of sister chromatid segregation +	GO:0033045	72	11
mi	•MI:hsa-miR-569 +	MI:hsa-miR-569	571	11
re	•Mitotic G2-G2/M phases +	REAC:453274	116	10

Annotations

After annotating a selection from an analysis tab and returning to the global view, further customizations are available from the side panel *Annotations* tab (1). All selected regions will by default be delimited by a border (2). This automatic recording of selections can be enabled by (3). If selections are being recorded, they can be removed individually (in the reverse order from which they were added) by (4) or completely removed by (5). Text annotations will be placed in the middle of the selection box by default but these can be moved by selecting (6) and dragging the annotation to the desired position (7). Manual annotations can be added with (8). Similar removal options are available for annotations as were described for selection boxes. If any adjustments to the image are made on the side-panel *Display options* tab (as shown in **Figure 5**), for example changing from a dot plot to a heat map or changing the color scheme, all annotations and selection borders will remain on the image. If the image is exported as an SVG, all annotations will be editable in Adobe Illustrator. PDF images will also be editable, although text elements may be grouped in unexpected ways.

Figure 11

