Cell Reports Methods, Volume *2*

Supplemental information

Interactive, integrated analysis of single-cell transcriptomic and phylogenetic data with PhyloVision

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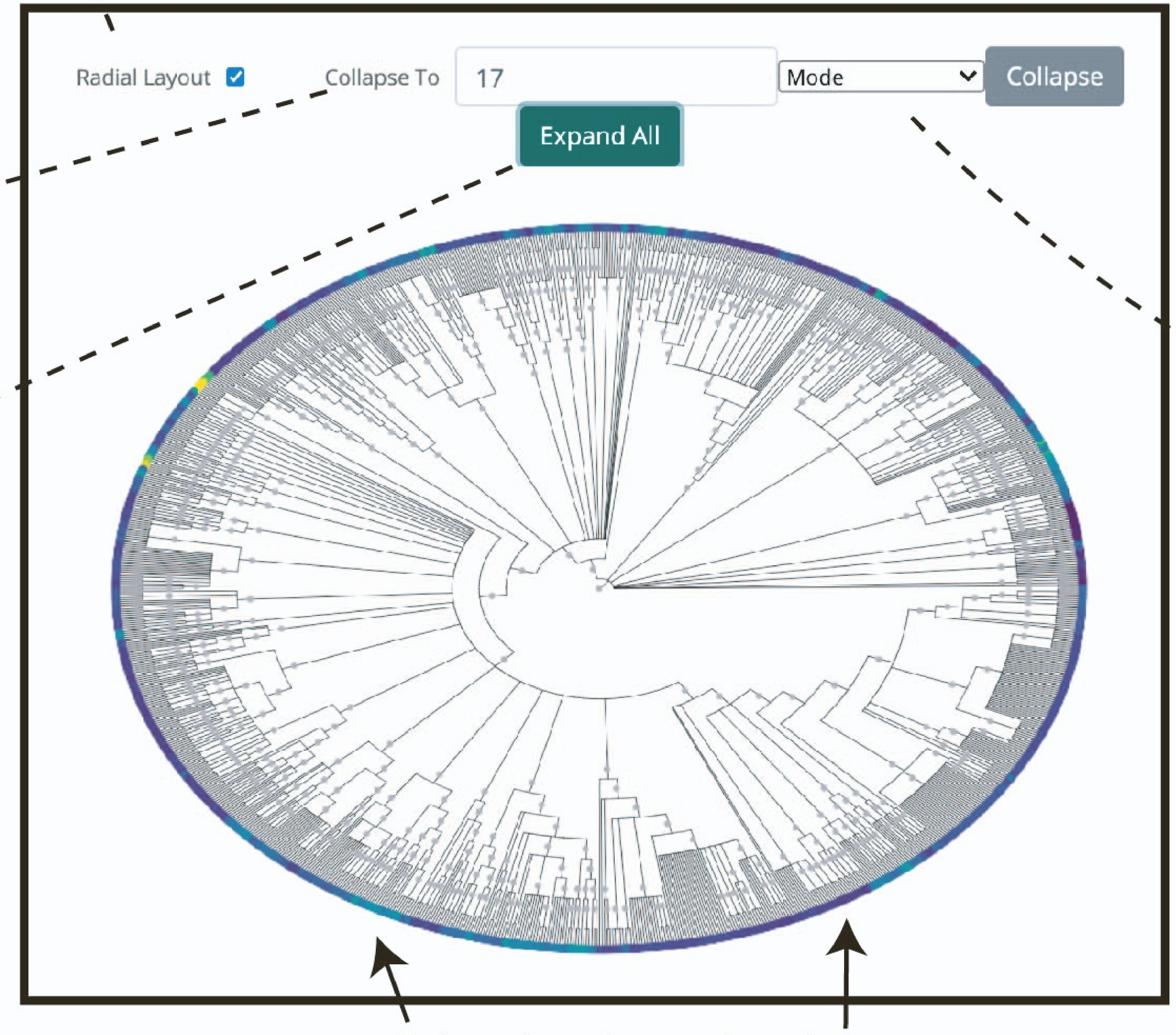
SUPPLEMENTARY FIGURES AND LEGENDS

Toggling layouts allows users to view the tree radially (as shown), or linearly.

Users can specify a depth at which to collapse the tree. For example, collapsing to depth 3 would collapse all edges to an LCA of depth 3 from the root.

Selecting “Expand All” uncollapses all edges.

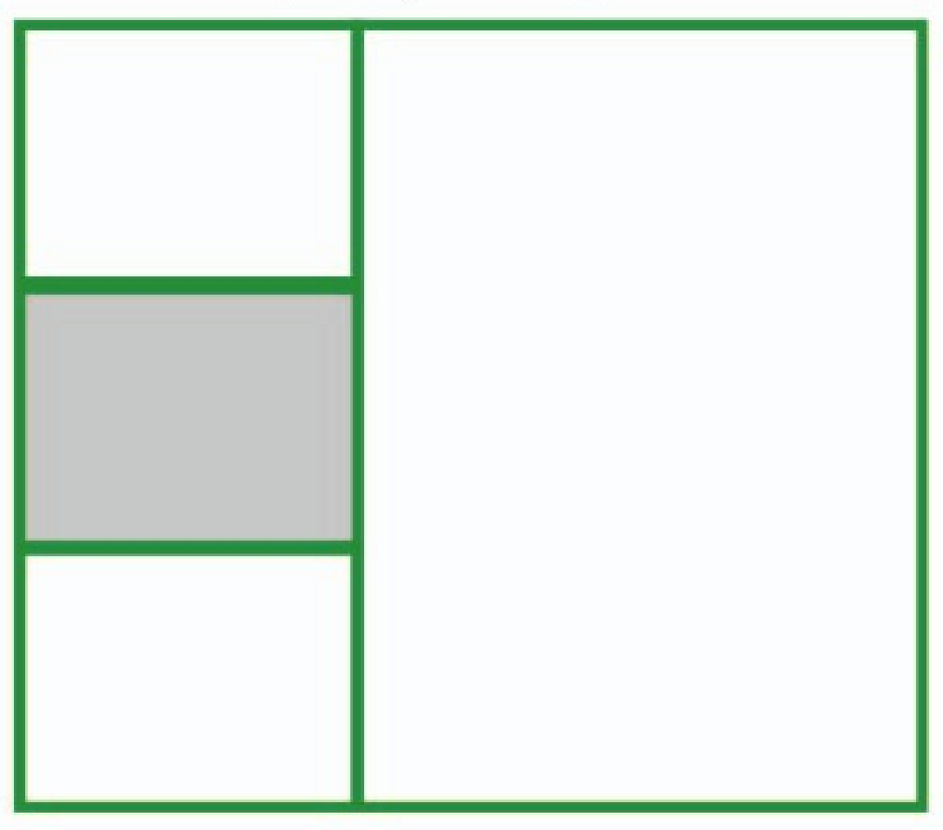


Radial Layout ☑

Collapse To 17

Mode

Display Legend



Users can specify how to compute a value for a collapsed node. Users can choose between mode, arithmetic mean, geometric mean, and median.

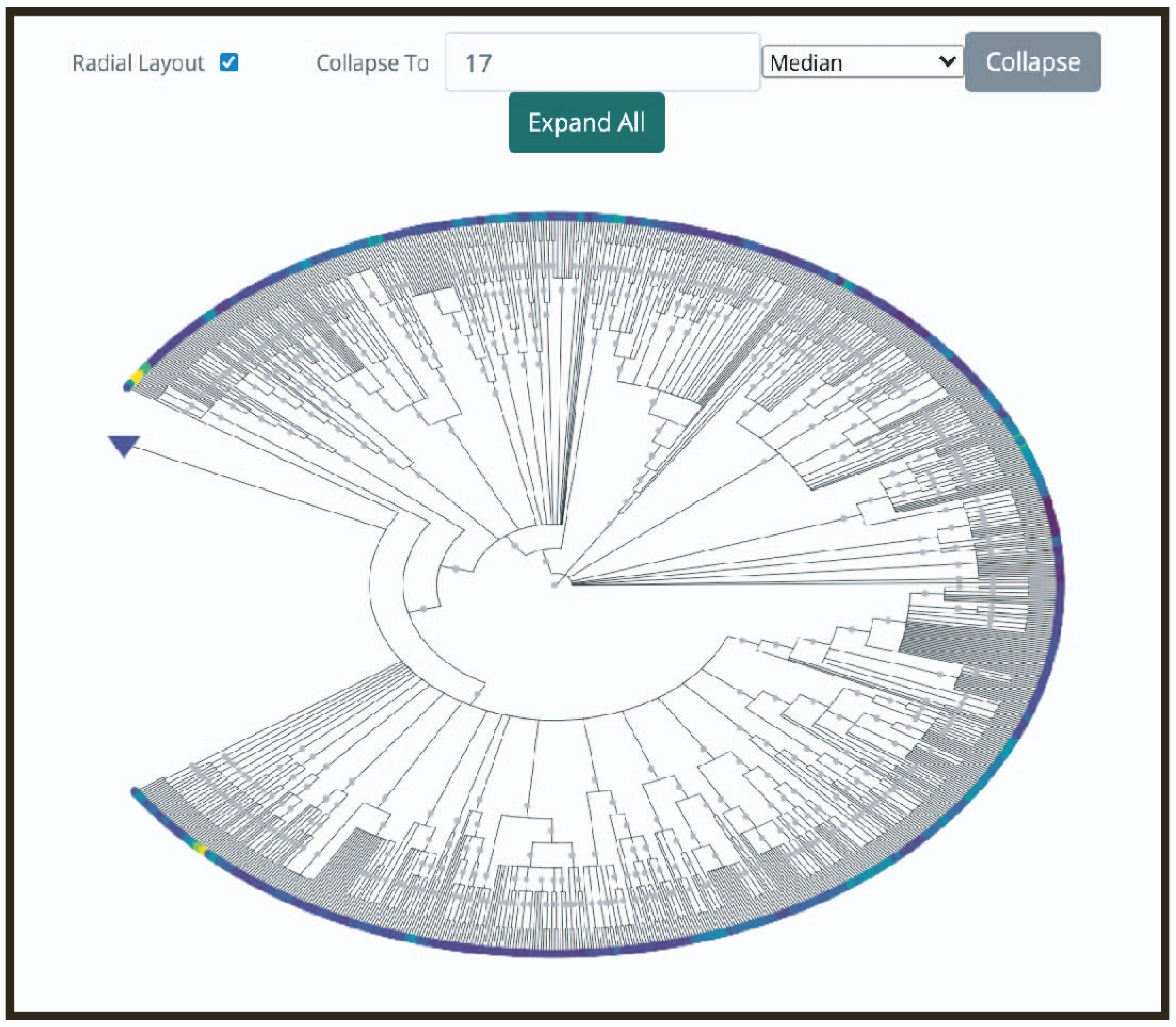
Colors plotted on each node correspond to the selected variable in the control panel

Radial Layout ☑

Collapse To 17

Median

Clades can be selected by clicking with the left-mouse button on an internal node. These selections can be propagated to the cell projection viewer or the differential expression analysis tool.



Radial Layout ☑

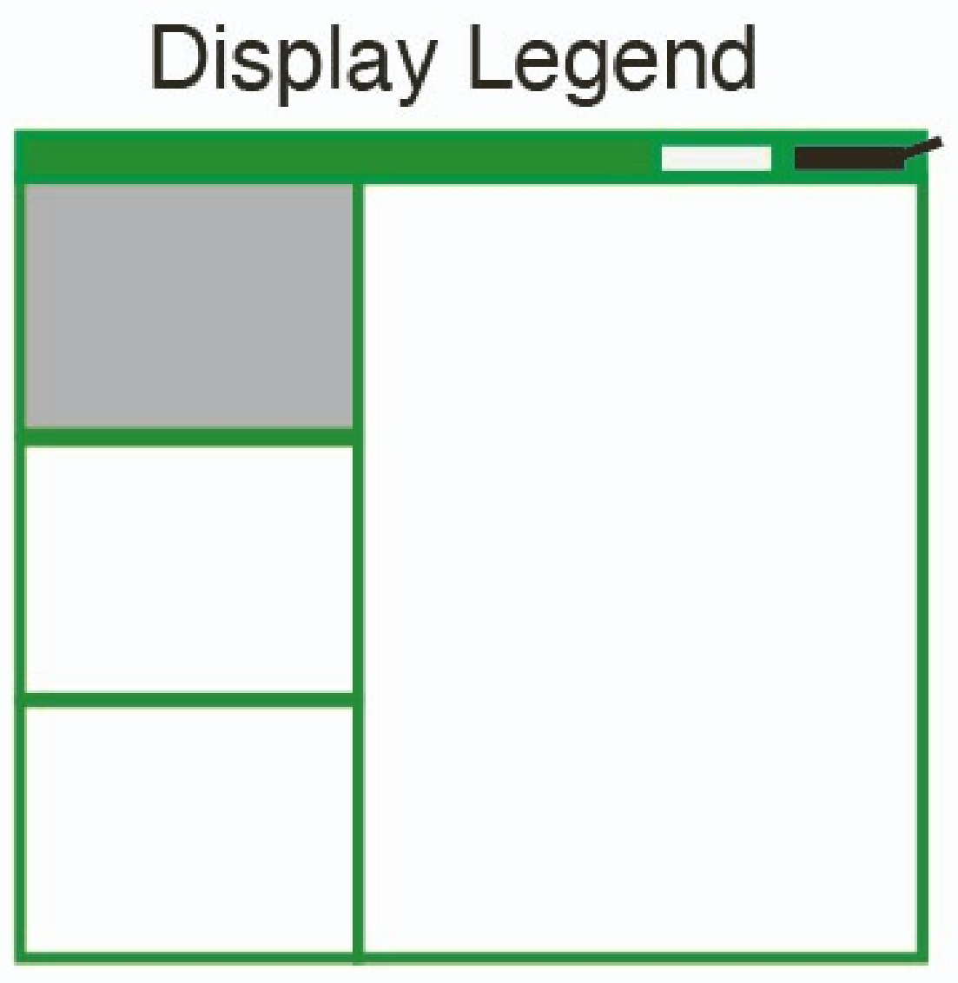
Collapse To 17

Median

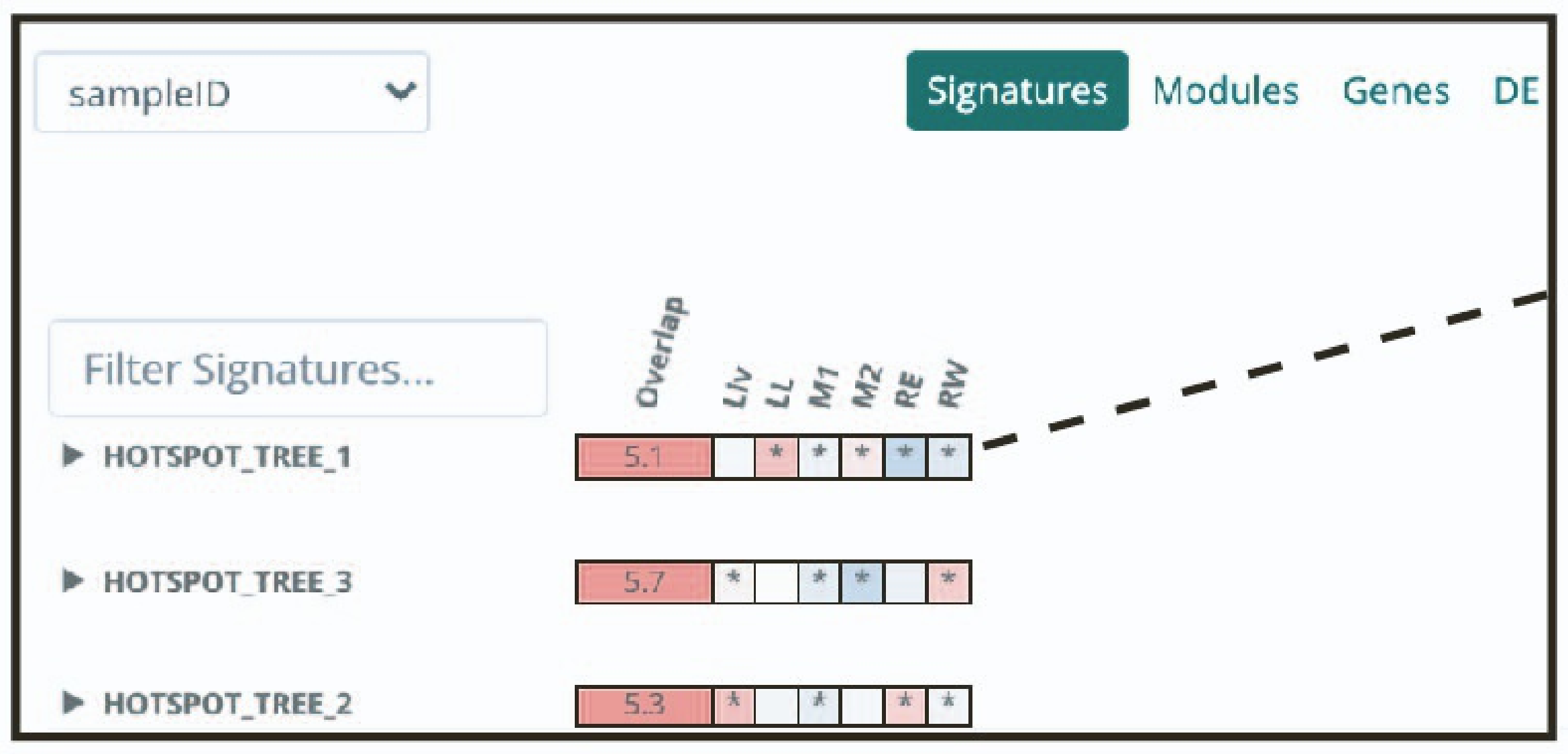
Clades can be collapsed individually by clicking with the right-mouse button on an internal node. Values displayed will be computed using the specified function (e.g., median)

Figure S1: Elements of the PhyloPlot interface, related to Figure 1. A tutorial over the features of the PhyloPlot.js interface used in the *PhyloVision* web-based report. Screenshots indicate how to interact with the object, including how to select, collapse, and expand clades.

“Hotspot” mode can be selected in the top right corner of the web-report

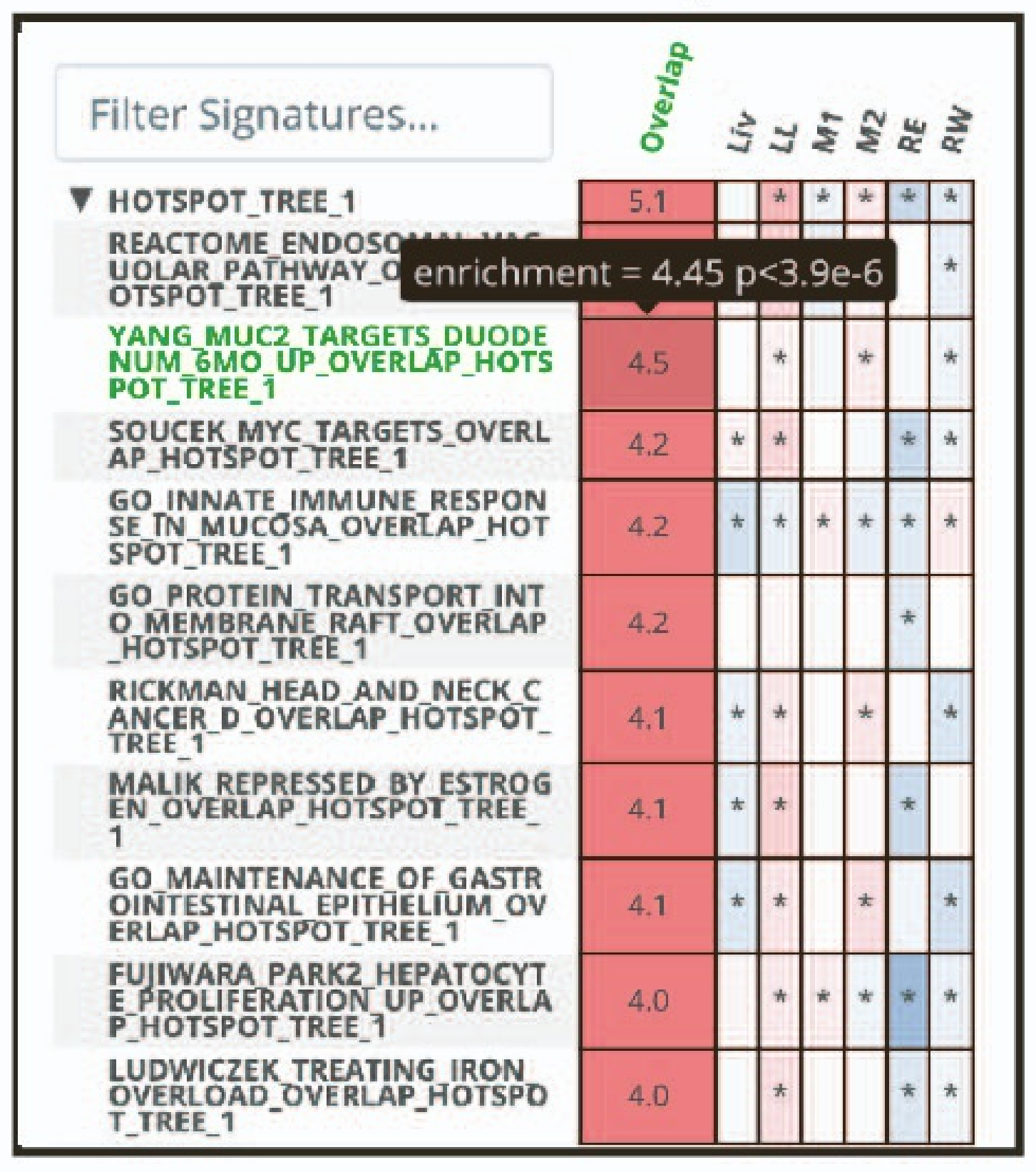


The control panel in “Hotspot” mode contains drop down panels for each Hotspot gene module identified.



Clicking on the arrow next to a module reveals a table with entries for each signature that significantly overlaps with the module

The values in the first column, labeled “Overlap” display the enrichment statistic between the genes in the Hotspot module and signature

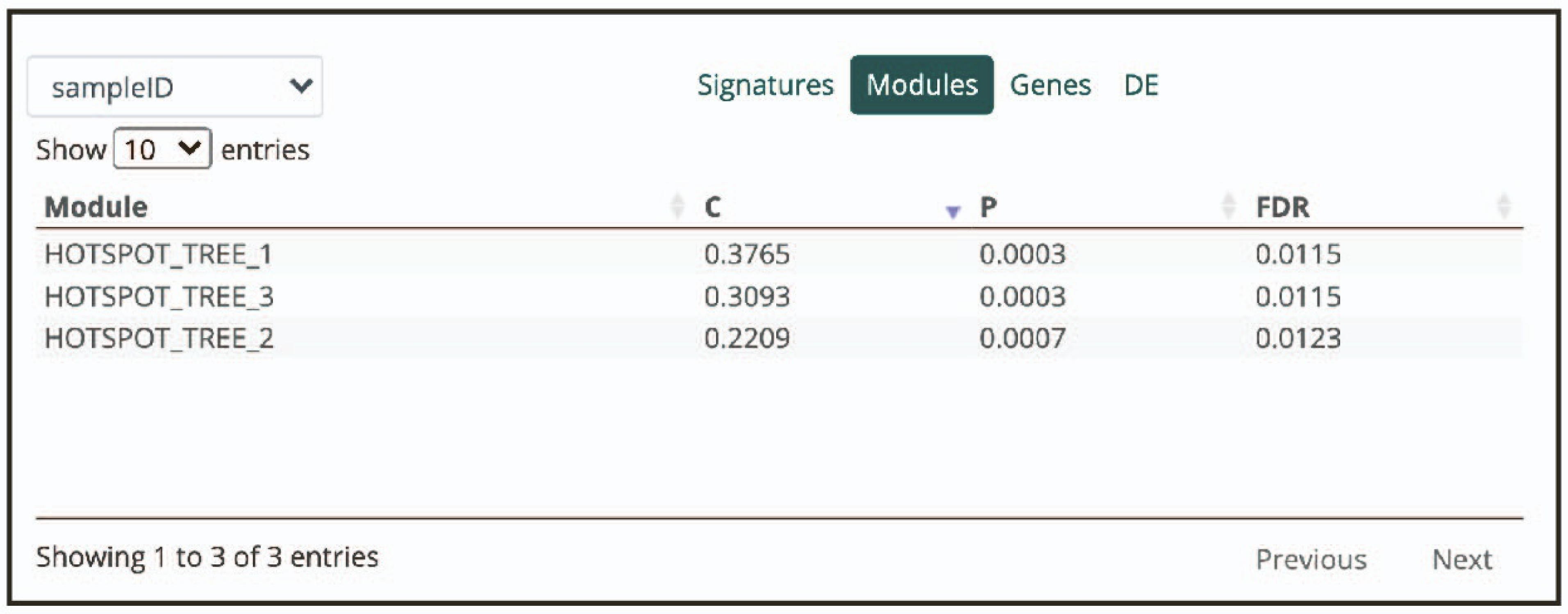


	Overlap	Liv	LL	M1	M2	RE	RW
▼ HOTSPOT_TREE_1	5.1		*	*	*	*	*
REACTOME_ENDOSO[illegible]UOLAR_PATHWAY_O[illegible]OTSPOT_TREE_1	[illegible]						*
YANG_MUC2_TARGETS_DUODENUM_6MO_UP_OVERLAP_HOTSPOT_TREE_1	4.5		*		*		*
SOUCEK_MYC_TARGETS_OVERLAP_HOTSPOT_TREE_1	4.2	*	*			*	*
GO_INNATE_IMMUNE_RESPONSE_IN_MUCOSA_OVERLAP_HOTSPOT_TREE_1	4.2	*	*	*	*	*	*
GO_PROTEIN_TRANSPORT_INTO_MEMBRANE_RAFT_OVERLAP_HOTSPOT_TREE_1	4.2					*	
RICKMAN_HEAD_AND_NECK_CANCER_D_OVERLAP_HOTSPOT_TREE_1	4.1	*	*		*		*
MALIK_REPRESSED_BY_ESTROGEN_OVERLAP_HOTSPOT_TREE_1	4.1	*	*			*	
GO_MAINTENANCE_OF_GASTROINTESTINAL_EPITHELIUM_OVERLAP_HOTSPOT_TREE_1	4.1	*	*		*		*
FUJIWARA_PARK2_HEPATOCYTE_PROLIFERATION_UP_OVERLAP_HOTSPOT_TREE_1	4.0		*	*	*	*	*
LUDWICZEK_TREATING_IRON_OVERLOAD_OVERLAP_HOTSPOT_TREE_1	4.0		*			*	*

Clicking on a row displays the signature score on the cell-projection and tree. In Hotspot mode, signature scores are computed on the overlapping genes.

Hovering over a cell dispalys its enrichment statistic and FDR-corrected p-value.

Additional columns show the result of 1 vs. All differential signature tests for the selected grouping variable.



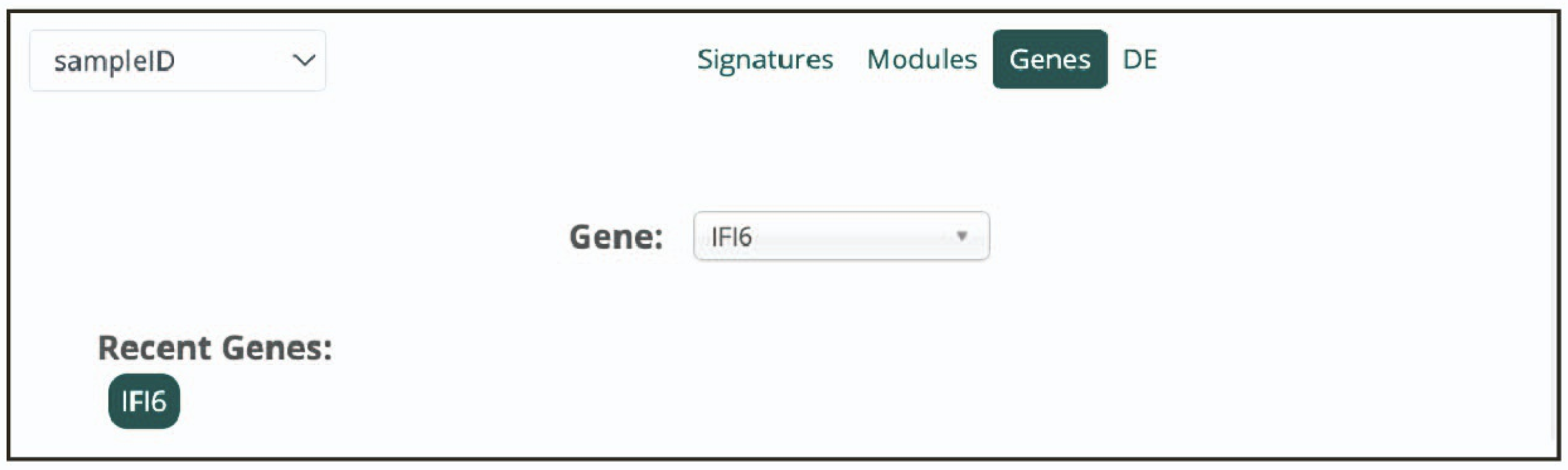
Module	C	P	FDR
HOTSPOT_TREE_1	0.3765	0.0003	0.0115
HOTSPOT_TREE_3	0.3093	0.0003	0.0115
HOTSPOT_TREE_2	0.2209	0.0007	0.0123

Showing 1 to 3 of 3 entries

Hotspot module statistics can be viewed under the “Modules” tab.

The Geary’s C, p-value, and FDR value are displayed for each module.

The number of modules per page can be selected.



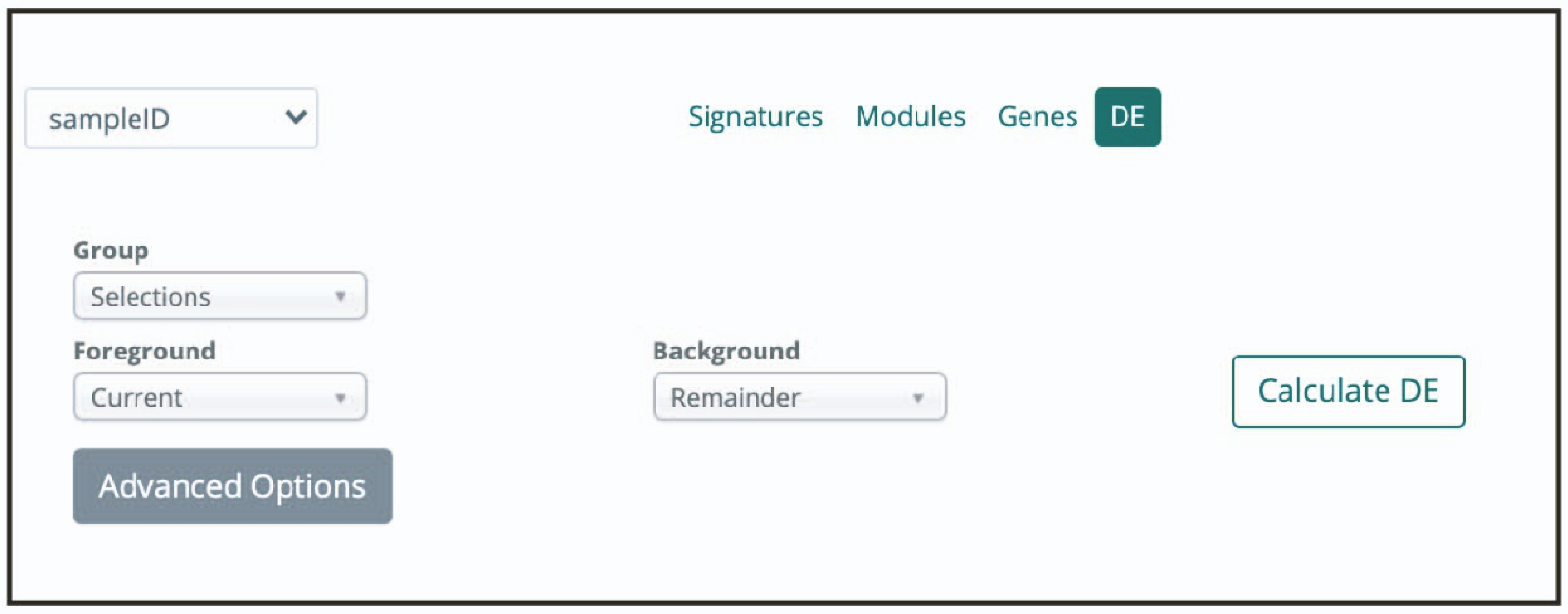
Gene: IFI6

Recent Genes: IFI6

Gene expression can be overlaid onto the tree and two-dimensional embedding.

Genes can be selected via the dropdown menu.

Recently used genes can be selected under the “Recent Genes” list.



Group: Selections

Foreground: Current

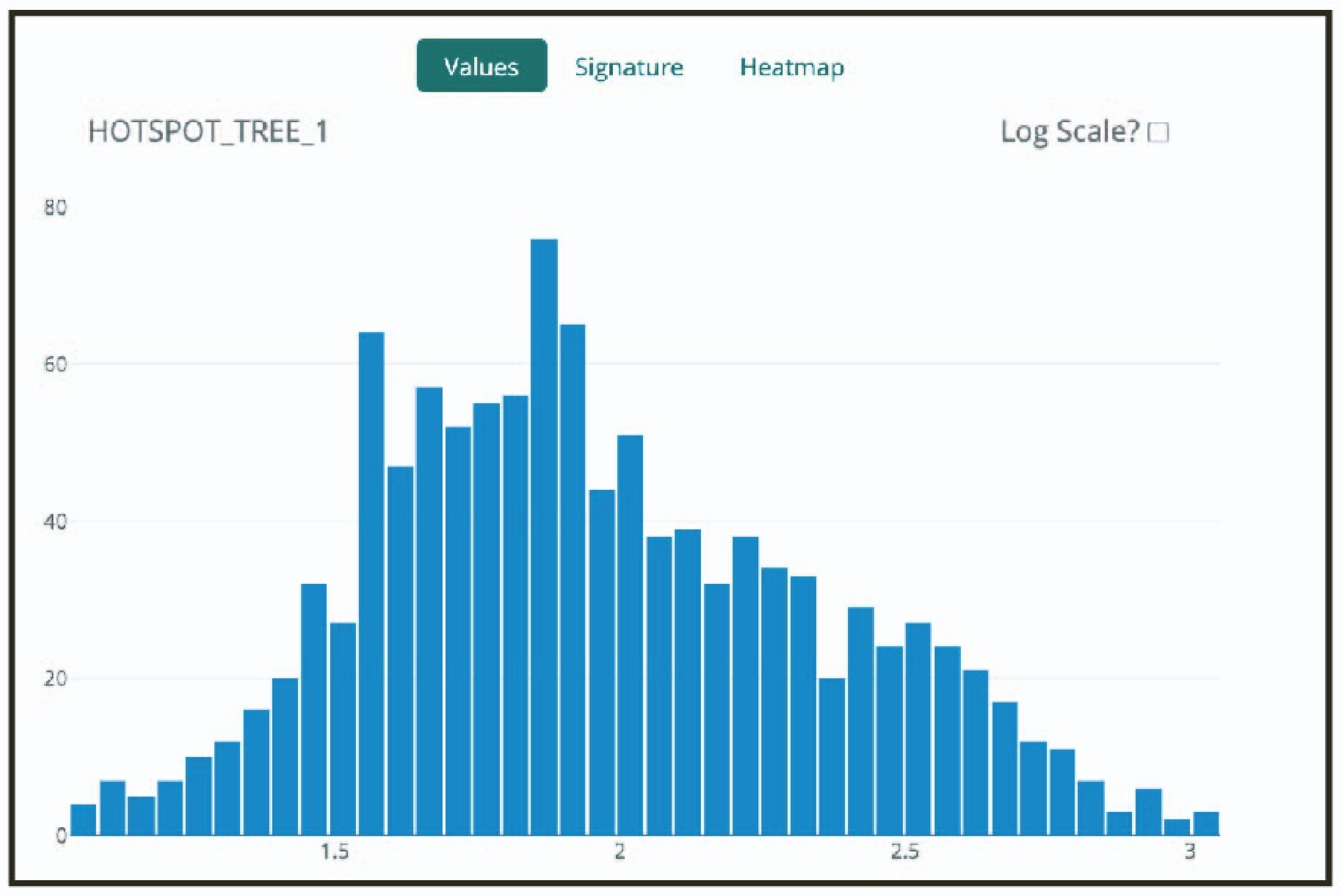
Background: Remainder

A differential expression anlaysis can be conducted under the “DE” tab. Various tests can be selected under “Advanced Options.”

A selected group can be compared against another group, or the remainder of cells if no group is specified.

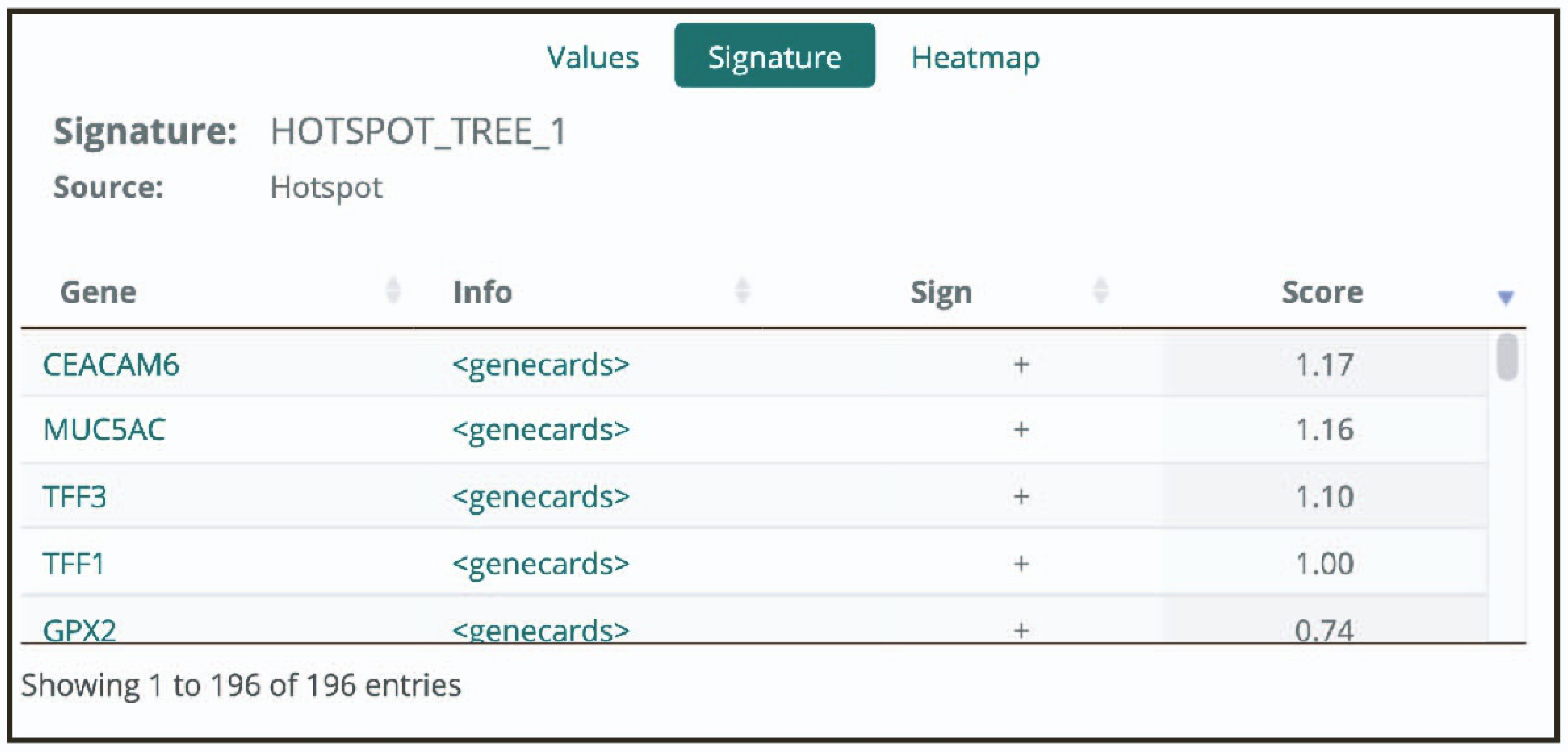
Figure S2: Elements of the *Hotspot* mode control panel, related to Figure 1. Users can toggle between signature autocorrelation and *Hotspot* modes in the top-right corner of the web-based report. In *Hotspot* mode, each module is presented in a collapsable table. Elements in the table are gene signatures that significantly overlap with the *Hotspot* module (see Methods). Users can also view the *Hotspot* summary statistics with the “Modules” tab, where the Geary’s C, p-values, and FDR of each module are displayed. Users can also select specific genes for displaying on the phylogeny and scatter plot, as well as perform differential expression tests. Multiple differential expression tests are supported under “Advanced Options”.

Display Legend



When selecting a Hotspot module, the exploration panel displays the distribution of scores.

Selecting the "log scale" radio button shows the logged distribution.



Signature: HOTSPOT_TREE_1
Source: Hotspot

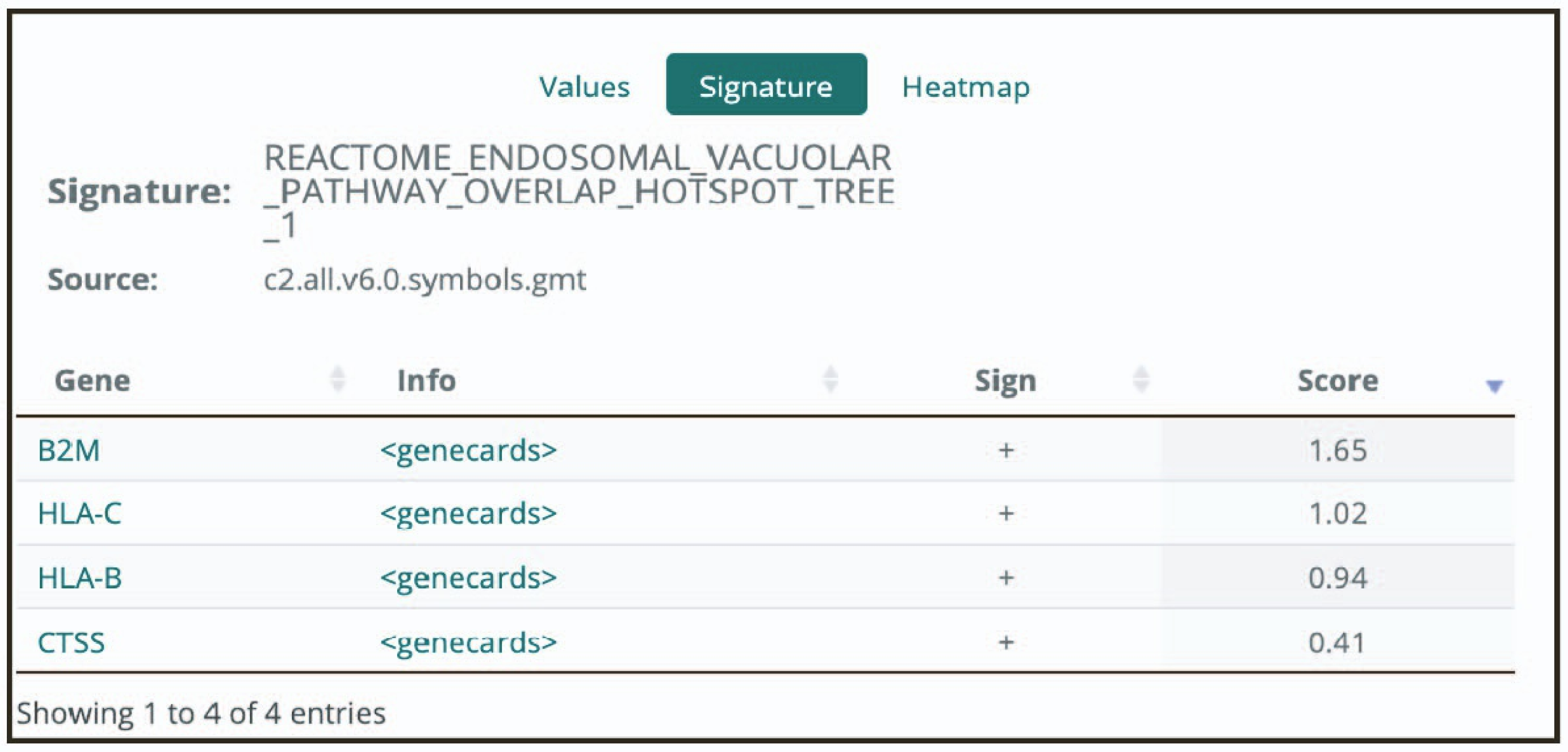
Gene	Info	Sign	Score
CEACAM6	<genecards>	+	1.17
MUC5AC	<genecards>	+	1.16
TFF3	<genecards>	+	1.10
TFF1	<genecards>	+	1.00
GPX2	<genecards>	+	0.74

Showing 1 to 196 of 196 entries

The "Signature" tab shows the genes in the Hotspot gene module.

Clicking on a gene name displays the counts on the cell-projection and tree.

Clicking on "genecards" directs the user to the gene-card entry for the gene of interest



Signature: REACTOME_ENDOSOMAL_VACUOLAR_PATHWAY_OVERLAP_HOTSPOT_TREE_1
Source: c2.all.v6.0.symbols.gmt

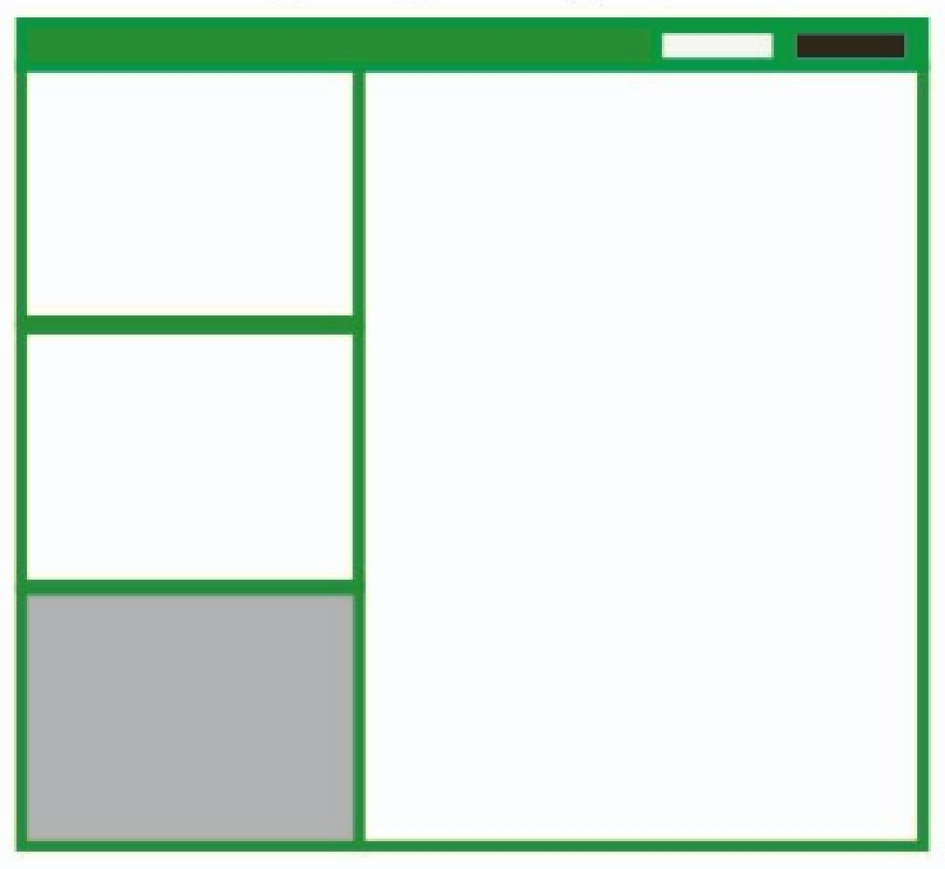
Gene	Info	Sign	Score
B2M	<genecards>	+	1.65
HLA-C	<genecards>	+	1.02
HLA-B	<genecards>	+	0.94
CTSS	<genecards>	+	0.41

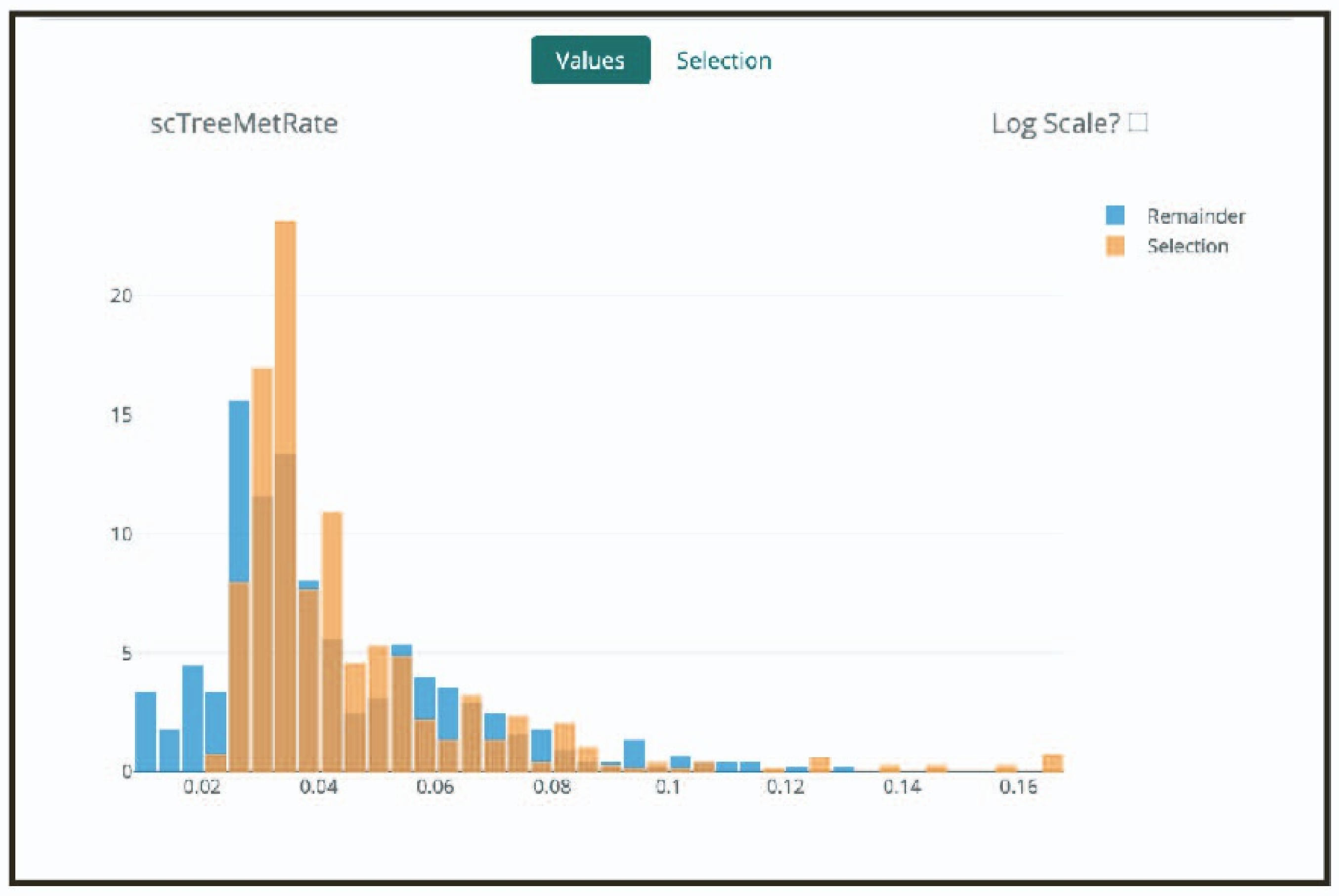
Showing 1 to 4 of 4 entries

If a signature is selected in the Hotspot mode, the overlapping genes are dispalyed in the "Signature" information tab.

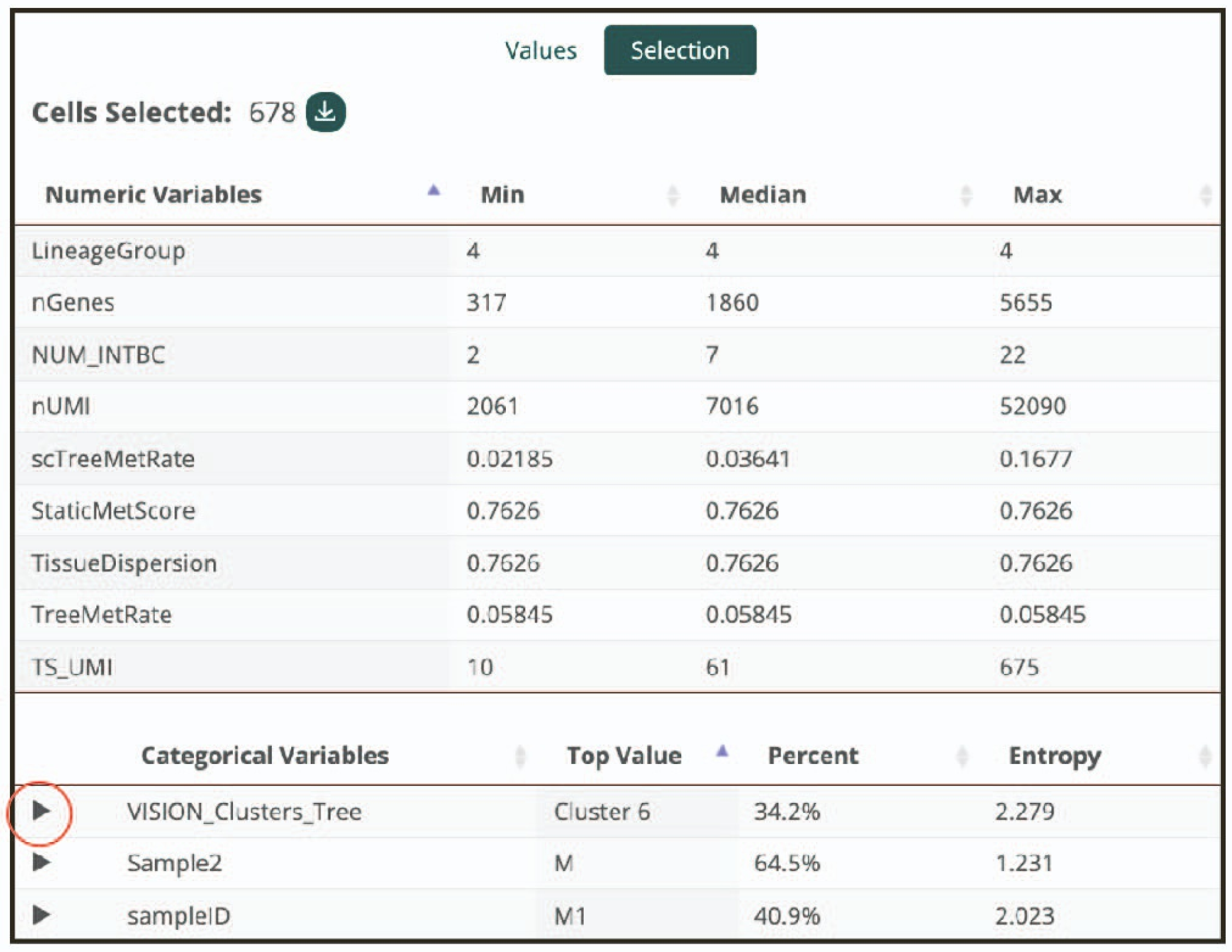
Figure S3: **Elements of the *Hotspot* mode signature exploration panel, related to Figure 1.** Users can view the distribution of scores in the *Hotspot* mode on both normal and log scales. If the *Hotspot* module is selected, scores are the *VISION*-computed score for the genes in the *Hotspot* module. If a signature is selected, the distribution displayed is the signature score of only the genes overlapping between the *Hotspot* module and the gene signature. Users can also view the genes in a *Hotspot* module, or in the overlap between a gene signature and *Hotspot* module.

Display Legend





If a cell selection is active, the “Values” distribution will show two distributions: one for the selected cells (orange) and one for the rest of the cells (blue).



Cells Selected: 678

Numeric Variables	Min	Median	Max
LineageGroup	4	4	4
nGenes	317	1860	5655
NUM_INTBC	2	7	22
nUMI	2061	7016	52090
scTreeMetRate	0.02185	0.03641	0.1677
StaticMetScore	0.7626	0.7626	0.7626
TissueDispersion	0.7626	0.7626	0.7626
TreeMetRate	0.05845	0.05845	0.05845
TS_UMI	10	61	675

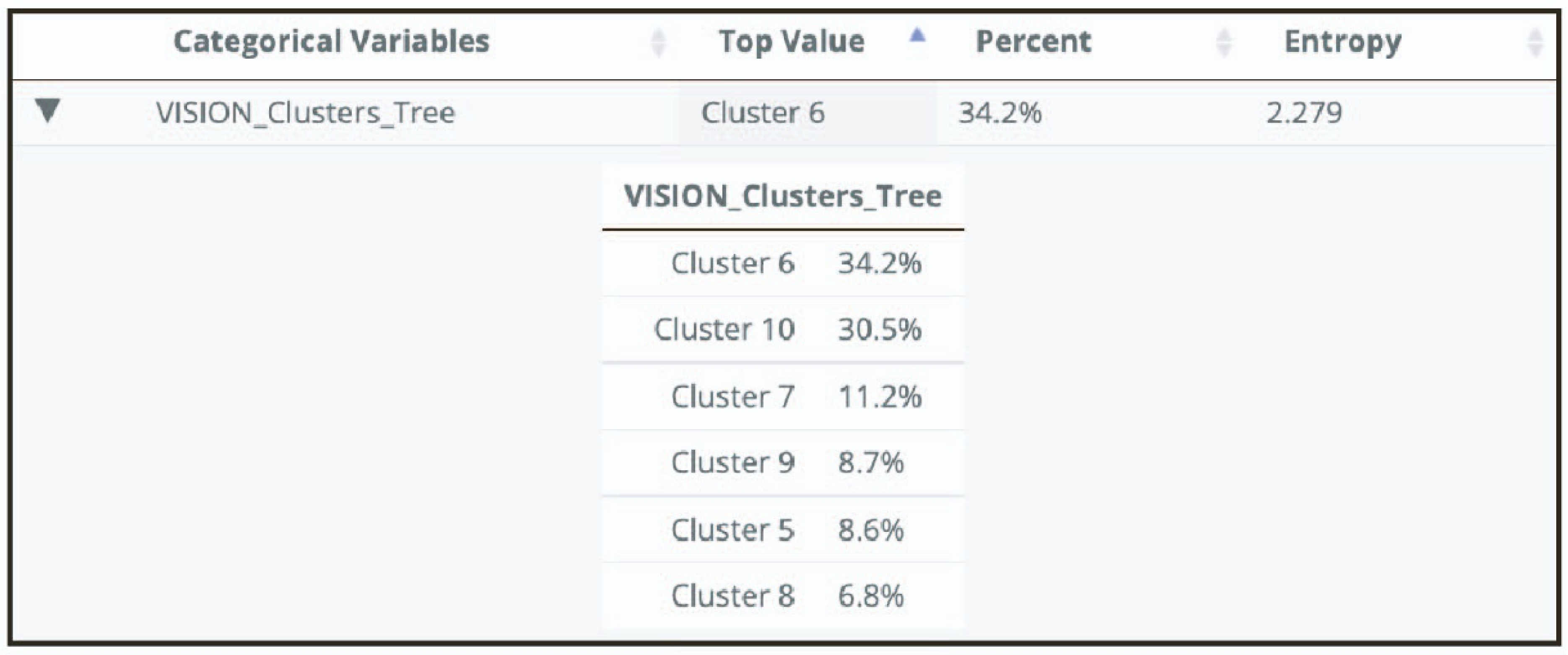
Categorical Variables	Top Value	Percent	Entropy
▶ VISION_Clusters_Tree	Cluster 6	34.2%	2.279
▶ Sample2	M	64.5%	1.231
▶ sampleID	M1	40.9%	2.023

The “Selection” tab will show a breakdown of meta data within the selected cell population.

Summary statistics are shown for numerical meta data.

The most frequent category, percentage of cells reporting this category, and entropy of the variable are shown for categorical meta data.

A list of selected calls can be downloaded with the arrow button next to “Cells Selected”.



Categorical Variables	Top Value	Percent	Entropy
▼ VISION_Clusters_Tree	Cluster 6	34.2%	2.279

VISION_Clusters_Tree	
Cluster 6	34.2%
Cluster 10	30.5%
Cluster 7	11.2%
Cluster 9	8.7%
Cluster 5	8.6%
Cluster 8	6.8%

Clicking on an arrow next to a categorical variable reveals a more granular breakdown of the composition of a given selection of cells.

Figure S4: **Investigating cell selections, related to Figure 1.** Users can investigate a selection with respect to its distribution of the plotted value as compared to the background (i.e., non-selected cells). *PhyloVision*'s UI also provides a breakdown of metadata within a given selection, including summary statistics over numerical metadata as well as measures of entropy and composition of categorical metadata.