MultiNicheNet output interpretation guidelines

Interaction validation guidelines

What are the properties of interactions that we recommend for follow-up experimental validation? In which downstream visualizations can MultiNicheNet users assess these properties?

Ligand-receptor validation guidelines

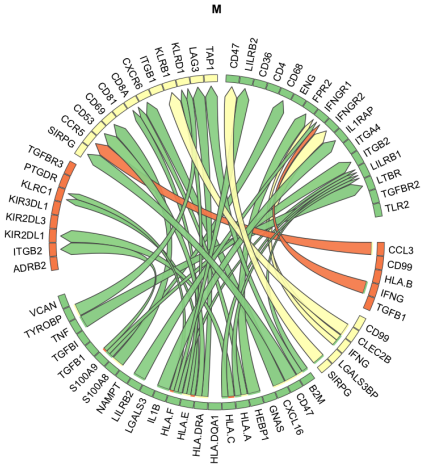
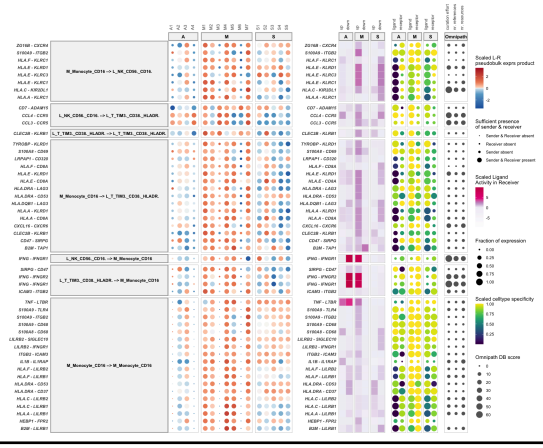
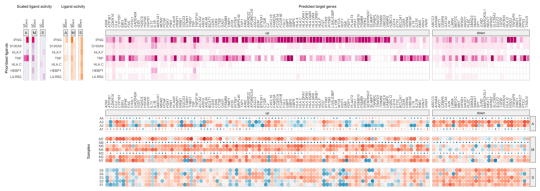
	Downstream visualizations			
Properties of ideal interactions for follow-up experimental validation	*Interpretable bubble plot*	*Ligand activity - target gene combination plot*	*Intercellular regulatory network*	*Ligand-receptor single-cell expression violin plot*
Ligand and receptor are upregulated in the condition of interest	✓			✓
The ligand has a strong scaled “upregulatory” ligand activity in the condition of interest	✓			
The predicted ligand activity seems to be the result of an enrichment of multiple and specific target genes in the condition of interest		✓		
The ligand and receptor are cell-type specifically expressed	✓			
The ligand and receptor are sufficiently expressed in the majority of samples in the group of interest	✓			
The ligand-receptor interaction is a trustworthy protein-protein interaction with downstream signaling potential as supported by several databases	✓			
The ligand and receptor are target genes regulated by another prioritized ligand-receptor interaction.			✓	
The prioritized interaction is concordant with additional data on the research question.	Not applicable			

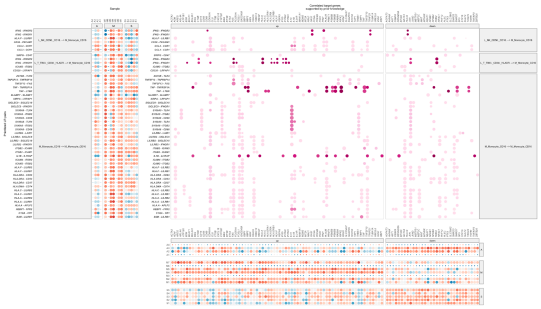
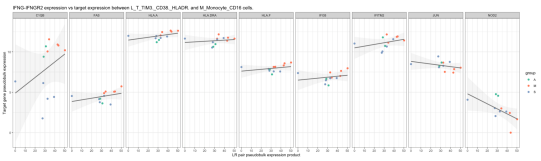
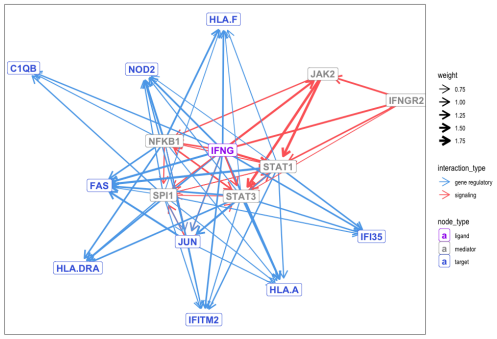
Ligand-target validation guidelines

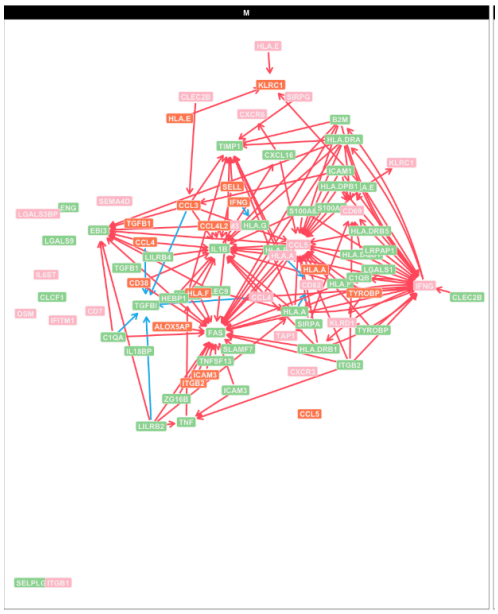
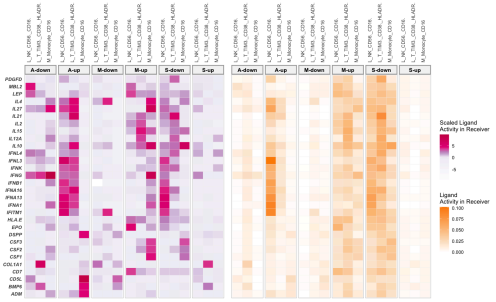
	Downstream visualizations				
Properties of ideal interactions for follow-up experimental validation	*Ligand activity - target gene - expression correlation combination plot*	*LR-target expression scatter plots*	*Ligand-to-target signaling path plot*	*DE gene pseudobulk and single-cell-level expression bubble plot*	*Ligand-target single-cell expression violin plot*
Target genes are strongly upregulated in the condition of interest.				✓	✓
Target genes show expression correlation with the upstream ligand-receptor pair.	✓	✓			
Target genes are linked to upstream ligands through a sensible signaling path. The individual interactions in this path are supported by trustworthy and complementary data sources.			✓		

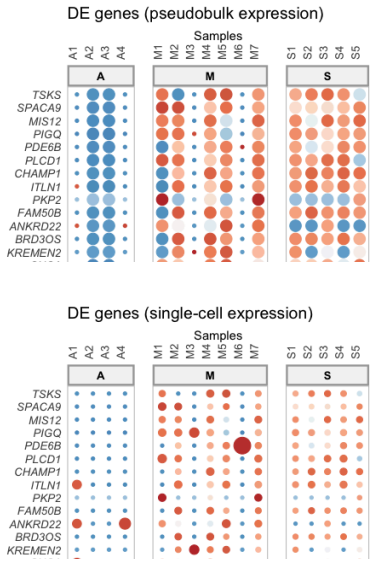
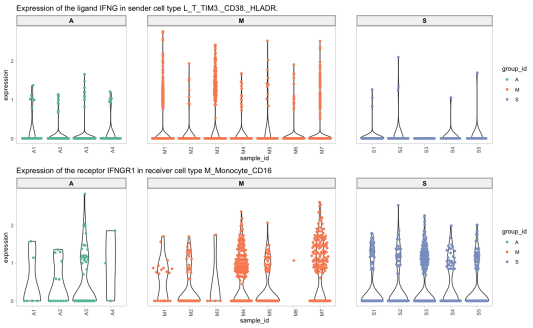
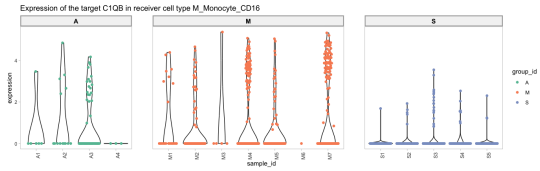
Visualization interpretation guidelines

The following table documents the goals and limitations of each downstream visualization of MultiNicheNet's output:

Visualization type	Aims of the visualization	Limitations of the visualization
ChordDiagram circos plot 	Summary of the top prioritized senderLigand-receiverReceptor interactions per condition (between all cell types or between cell type pairs of interest).	Does not visualize data underlying the prioritization of these interactions.
Interpretable bubble plot 	Interpret the prioritization: help users decide which interactions may be most interesting for follow-up experimental validation. This visualization shows differential expression, ligand activity, cell-type specific expression, fraction of expression, and Omnipath database metrics for a selected subset of senderLigand-receiverReceptor interactions.	Does not visualize the specific target genes downstream of the prioritized interactions. Hereby, the user cannot assess whether high activity values may be due to a reasonable number of specific target genes.
Ligand activity - target gene combination plot 	Inspect the predicted target genes behind ligand activity predictions. The genes shown are the top target genes of the ligand that have contributed to the ligand activity prediction of that interaction. Users can inspect the regulatory potential scores of each ligand-target link and the expression of each target gene in each sample.	Shows how well ligand-target links are supported by general prior knowledge, but not whether they are likely to be active in the system under study.

Ligand activity - target gene - expression correlation combination plot 	This visualization can help users assess whether ligand-target links that are supported by general prior knowledge, are also potentially active in the system under study: target genes that show across-sample expression correlation with their upstream ligand-receptor pairs may be more likely true target genes than target genes that don't show this pattern.	Indicates the strength of the correlation between ligand-receptor expression and target gene expression, but it's hard to assess the pattern of correlation.
LR-target expression scatter plots 	Visualize the pattern of correlation between ligand-receptor and target gene expression. This can help users evaluate whether high correlation values are not due to artifacts.	
Ligand-to-target signaling path plot 	Visualize potential signaling paths between ligands and target genes of interest. Hence, this plot shows the prior knowledge behind ligand-target regulatory potential scores. In addition to this visualization, the software returns a network table documenting the underlying data source(s) behind each of the links shown in this graph. This analysis can help users to assess the trustworthiness of ligand-target predictions.	

Intercellular regulatory network  	Visualize gene regulatory links between ligands and ligand- or receptor-encoding target genes. As a result, this visualization can give insights into potential intercellular cascade and feedback mechanisms. Ligands/receptors visualized in this network can be considered as additionally prioritized because they are not only a prioritized ligand/receptor but also a target gene of another prioritized ligand-receptor interaction.	It may be challenging to discern individual links when several interactions are shown. Therefore, inspection of the underlying data table may be necessary to discern individual interactions. It is also suggested to export this data table into more sophisticated network visualization tools (e.g., CytoScape) for better inspection of this network.
Sender-agnostic ligand activity heatmap  	Visualize the regular and scaled ligand activity values of ligands of interest. By default, the visualized ligands are the *n* ligands with the highest scaled activity for a certain receiver-contrast combination. This visualization can give users insights into potential signaling pathways and ligands that are not prioritized in the regular MultiNicheNet analysis because no differential expression was observed in the data. The benefits of this analysis are the possibility to infer the activity of ligands that are expressed by cell types that are not in your single-cell dataset or that are hard to pick up at the RNA level.	No indication of the source cell types of the predicted ligands.

DE gene pseudobulk and single-cell-level expression bubble plot 	Visualize scaled expression of genes of interest in a cell type of interest. In addition to pseudobulk expression, single-cell level expression is also visualized in a second plot. This may be informative for users to inspect the data behind DE results.	
Ligand-receptor single-cell expression violin plot 	Visualize the expression distribution of a ligand and receptor of interest in a sender and receiver cell type of interest, respectively. This may be informative for users to inspect the data behind DE results. This can help users evaluate whether DE results at pseudobulk level are not due to artifacts.	
Target gene single-cell expression violin plot 	Visualize the expression distribution of a gene of interest in a cell type of interest. This may be informative for users to inspect the data behind DE results. This can help users evaluate whether DE results at pseudobulk level are not due to artifacts.	