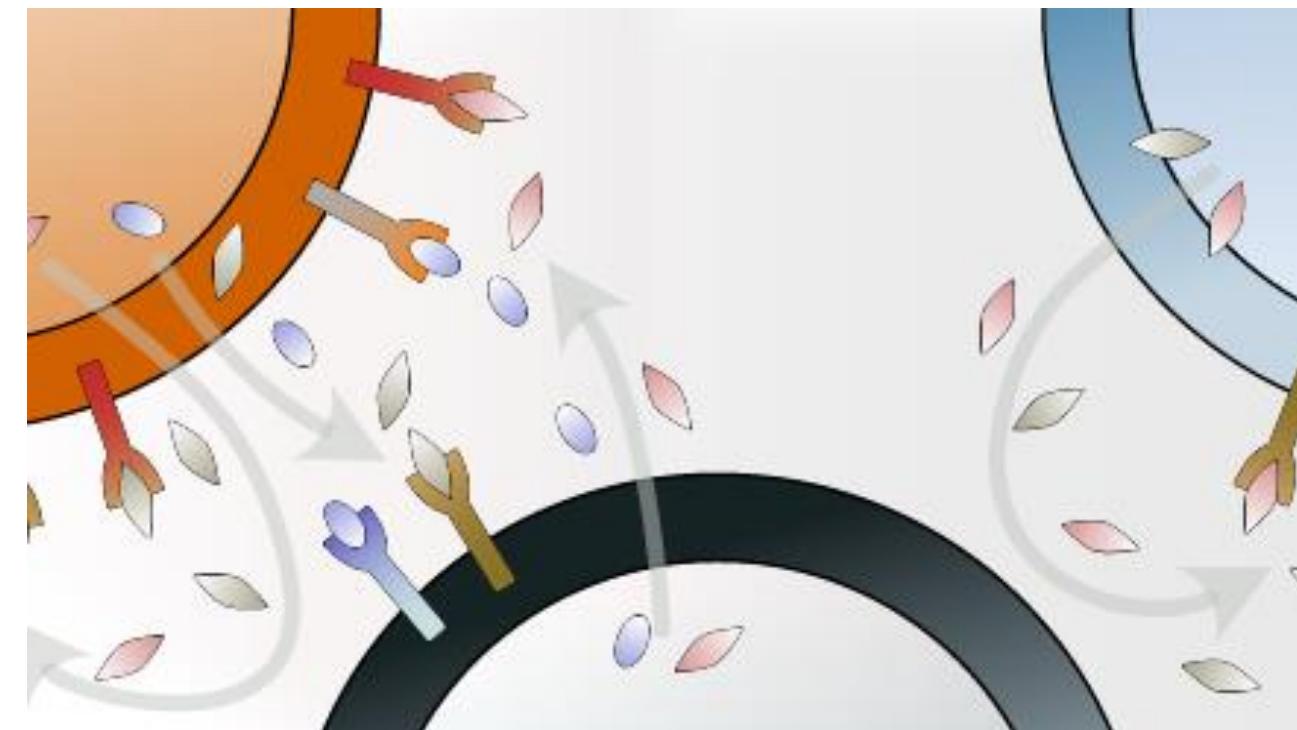


BESE394 Final

Exploring Cell-to-Cell Interactions (CCI) Within GABAergic Interneurons During Development

Ali Balubaid, Ana Cecilia, Azari Bantan, Daniel Soto



Cell2Cell interactions

nature

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Article | Published: 22 September 2021

Genetic and epigenetic coordination of cortical interneuron development

Kathryn C. Allaway, Mariano I. Gabbitto, Orly Wapinski, Giuseppe Saldi, Chen-Yu Wang, Rachel C. Bandler, Sherry Jingjing Wu, Richard Bonneau & Gord Fishell

[Nature](#) 597, 693–697 (2021) | [Cite this article](#)

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Questions ?

A

The interaction profiles between sub-types of GABAergic inhibitory neurons using single-cell transcriptomics

B

The behavior of interactions during development



Dataset

Source name: Cortex-V1

Organism: *Mus musculus*

Characteristics cell type: Cortical interneurons

Age: P10

Sample: GSM5014306

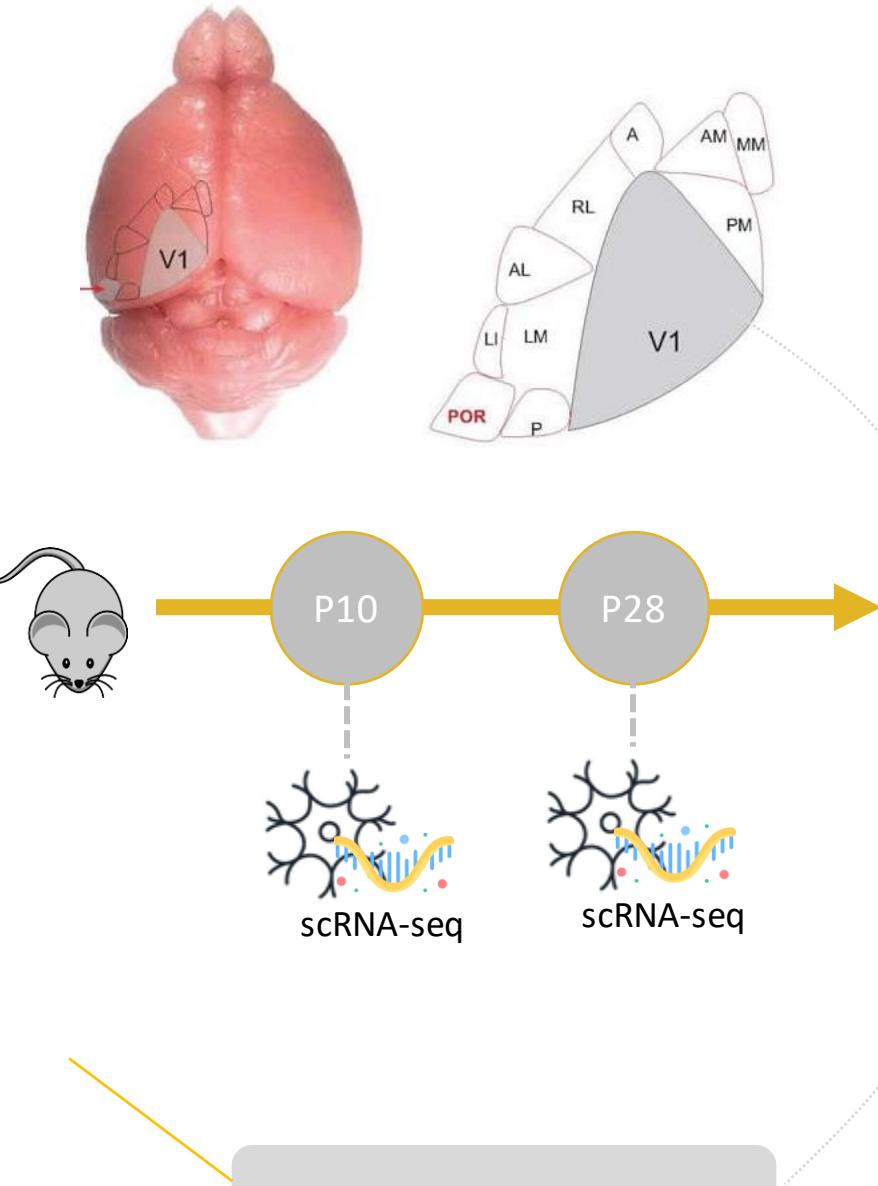
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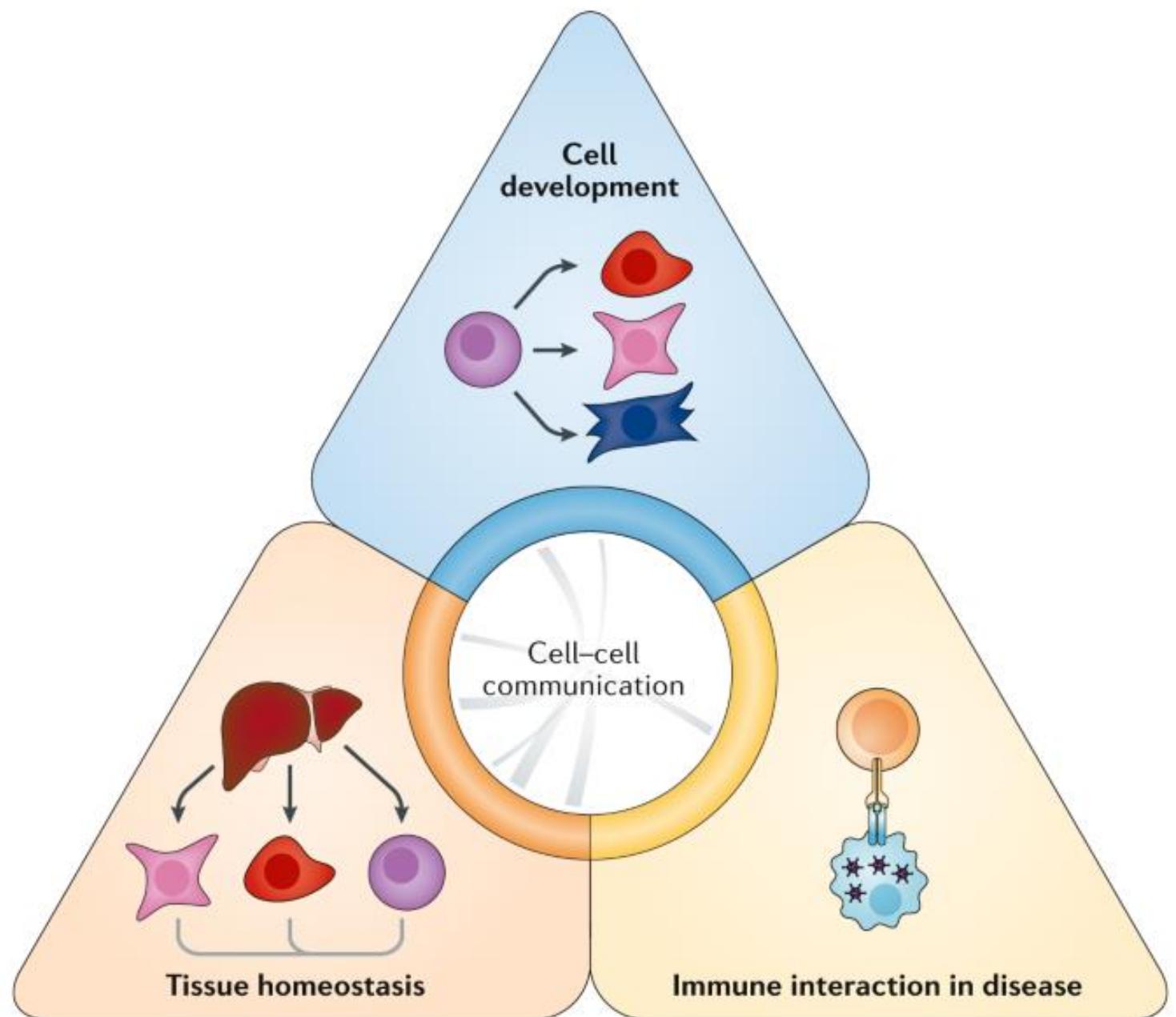
Age: P28

Sample: GSM5014308

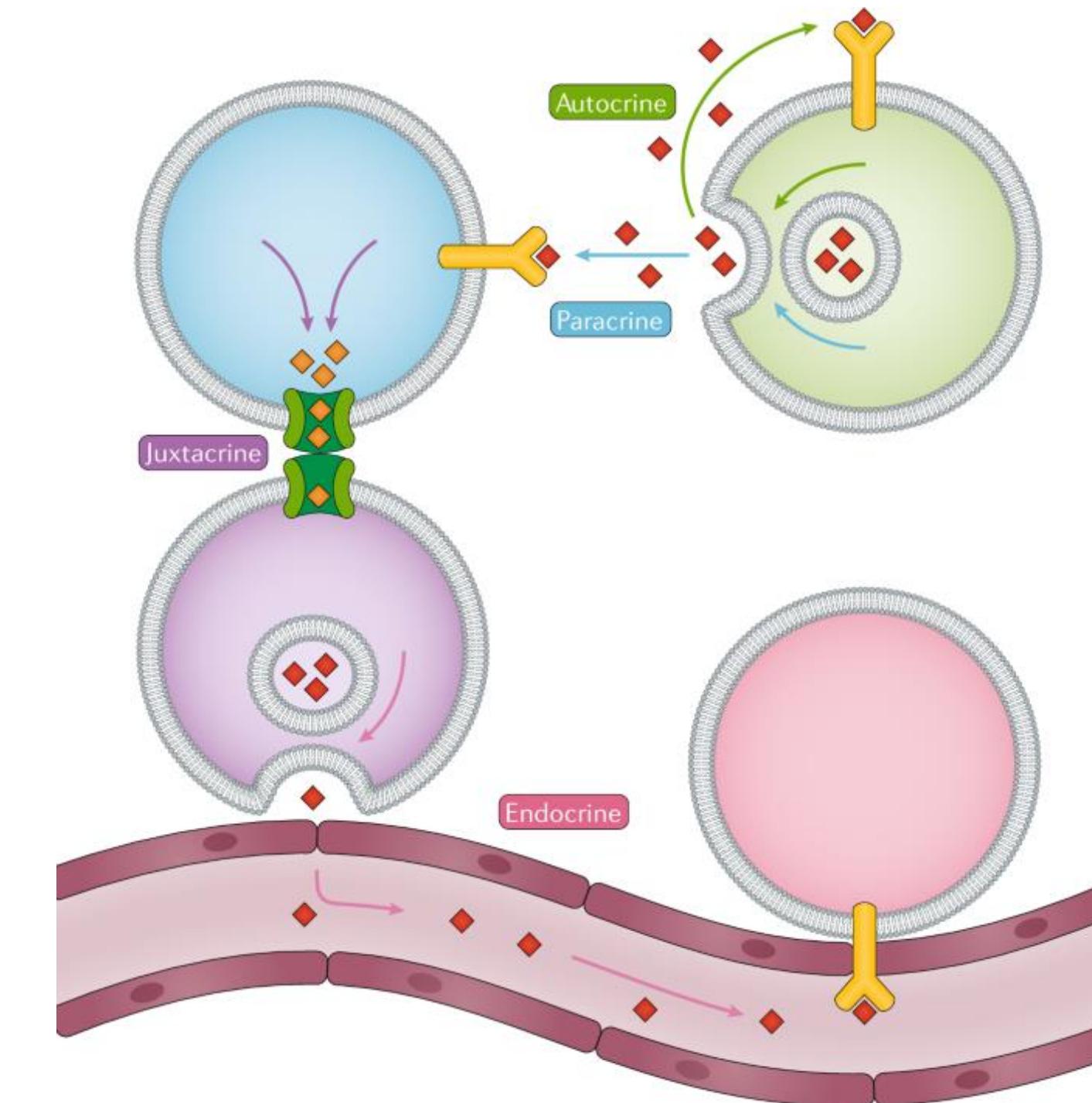
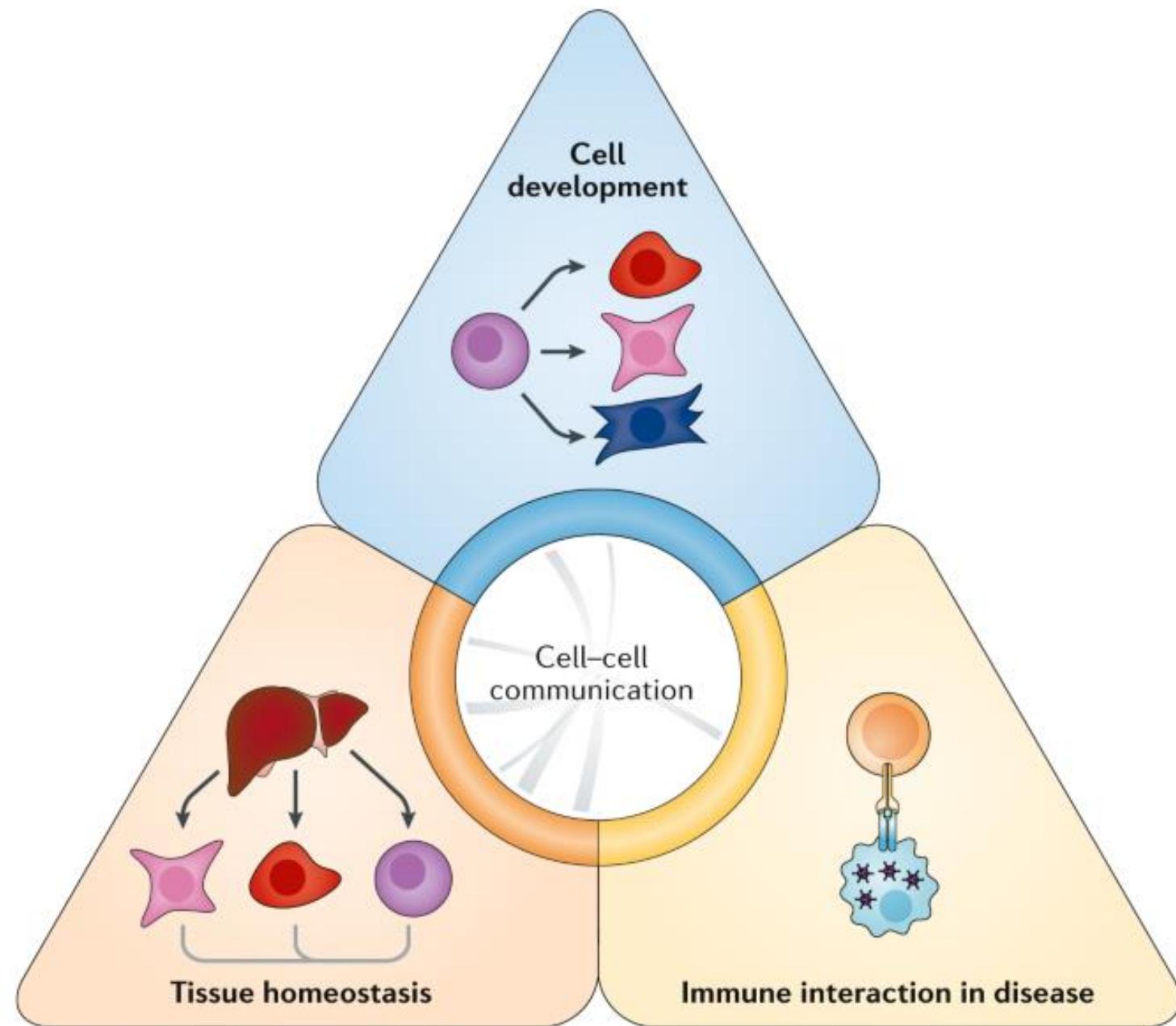


Why & how study cell2cell Communications (CCC)?

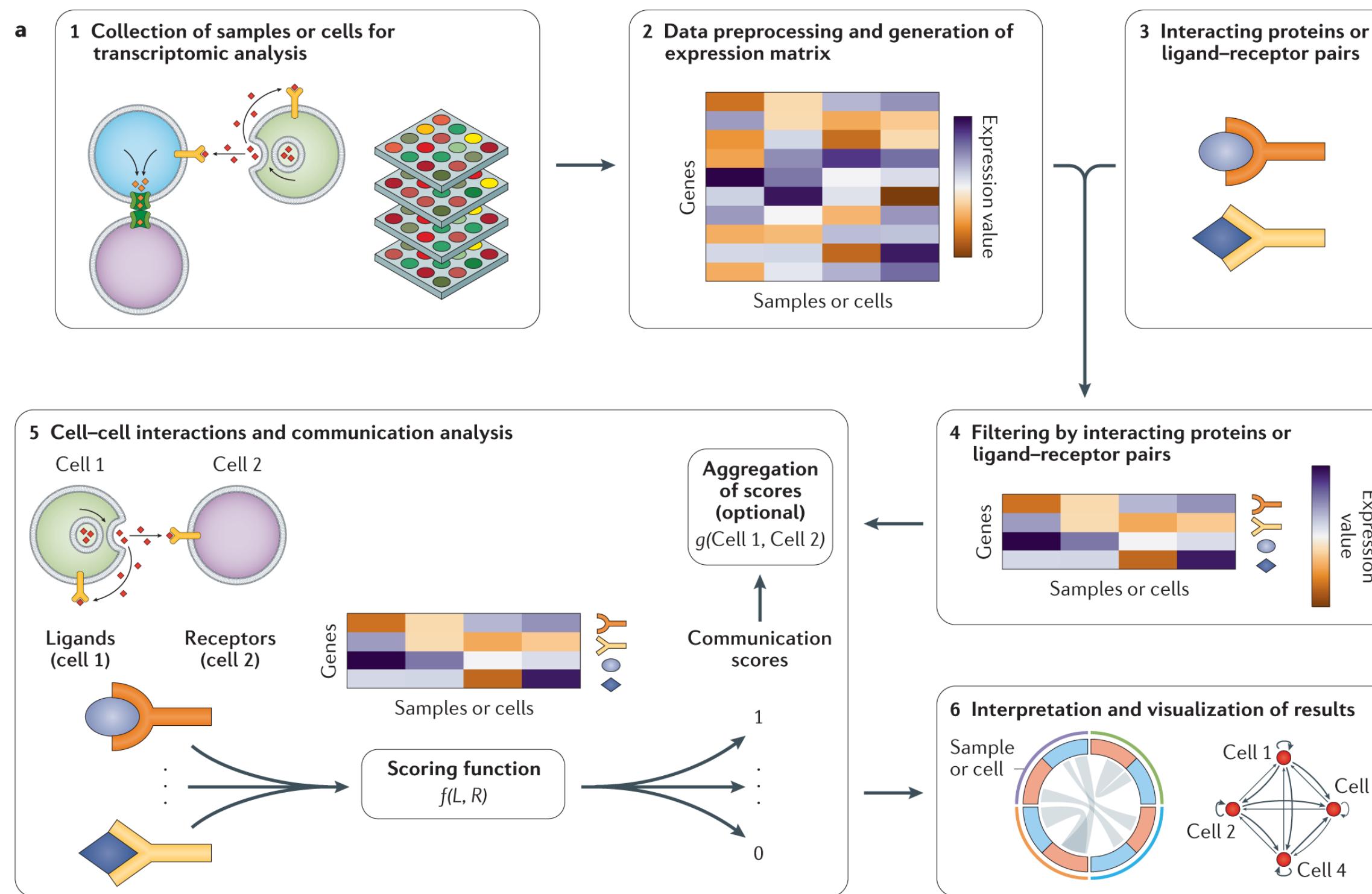
Why & how study cell2cell Communications (CCC)?



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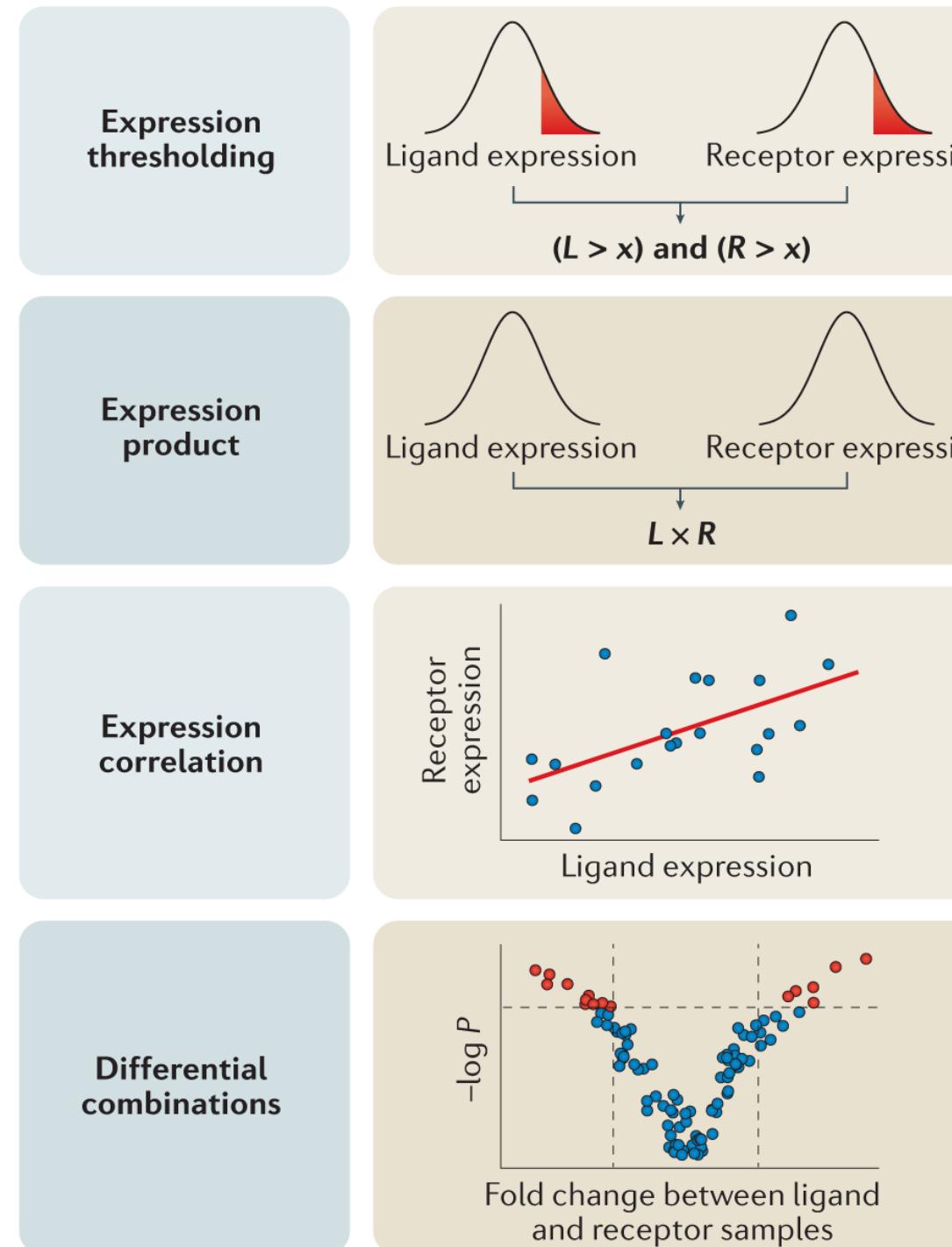


Why & how study cell2cell Communications (CCC)?

b

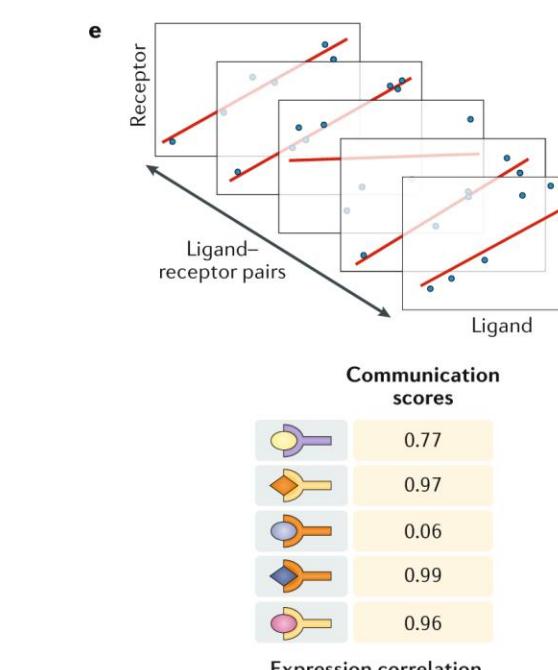
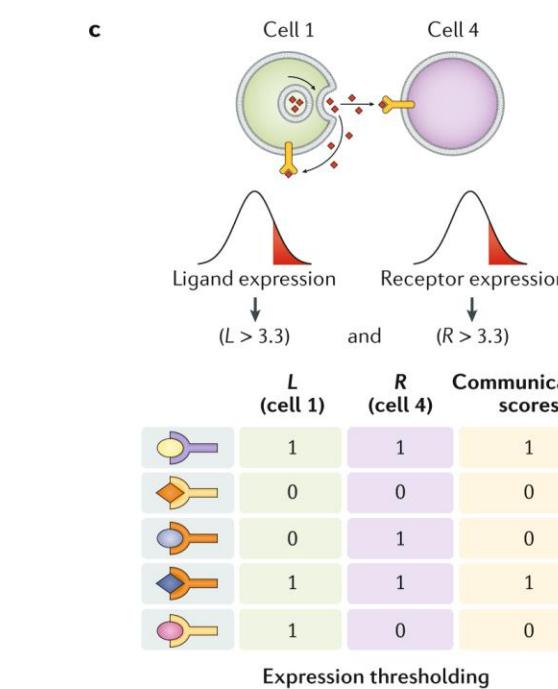
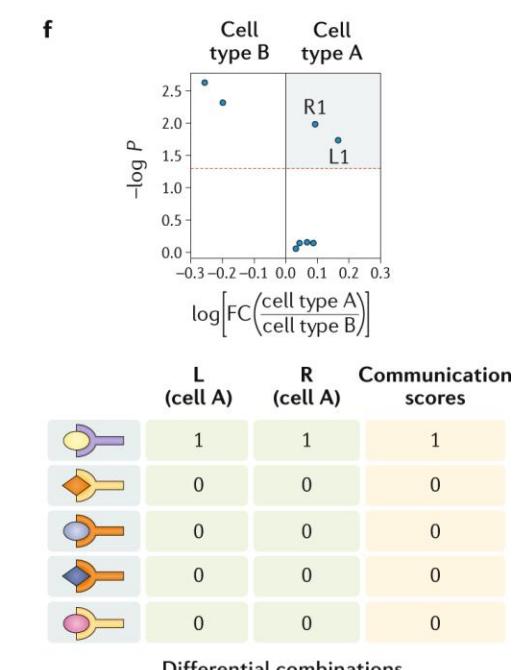
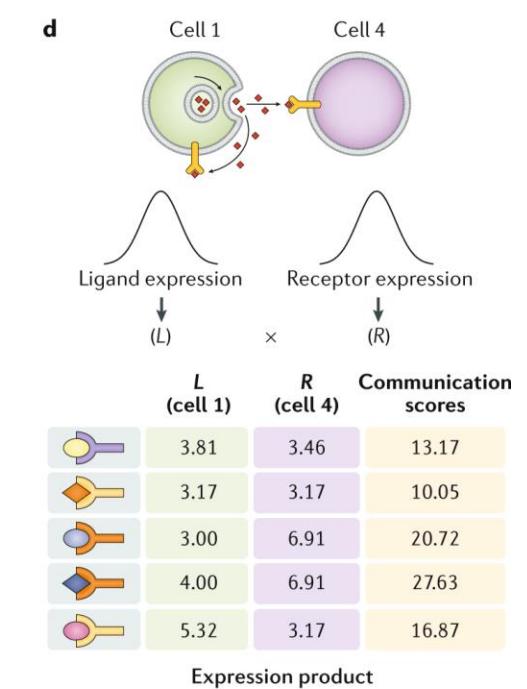
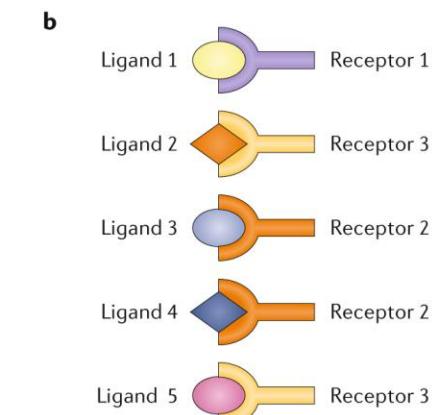
Method	Recommended data	Communication score
Expression thresholding	Bulk, single cell	Binary
Expression product	Single cell	Continuous
Expression correlation	Bulk, single cell	Continuous
Differential combinations	Bulk, single cell	Binary

Why & how study cell2cell Communications (CCC)?

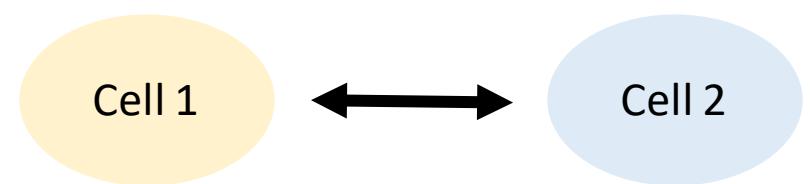
b**Recommended data****Communication score****Bulk, single cell****Binary****Single cell****Continuous****Bulk, single cell****Continuous****Bulk, single cell****Binary**

a

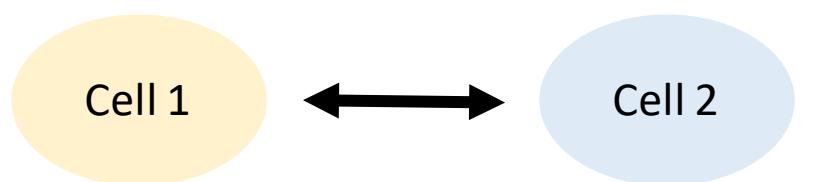
			Cell type A			Cell type B				
			Ligand 1	Ligand 2	Ligand 3	Ligand 4	Ligand 5	Receptor 1	Receptor 2	Receptor 3
Ligand 1	3.81	3.46	4.32	2.32	3.00	2.58				
Ligand 2	3.17	1.58	2.32	2.32	0.00	3.46				
Ligand 3	3.00	3.46	4.91	2.32	6.64	1.58				
Ligand 4	4.00	5.32	5.64	7.71	8.02	7.91				
Ligand 5	5.32	3.32	4.32	3.91	2.32	5.49				
Receptor 1	4.70	4.58	4.17	3.46	3.81	3.58				
Receptor 2	3.00	4.09	4.46	6.91	7.13	6.78				
Receptor 3	4.32	2.00	3.00	3.17	1.00	3.81				


**Expression correlation**

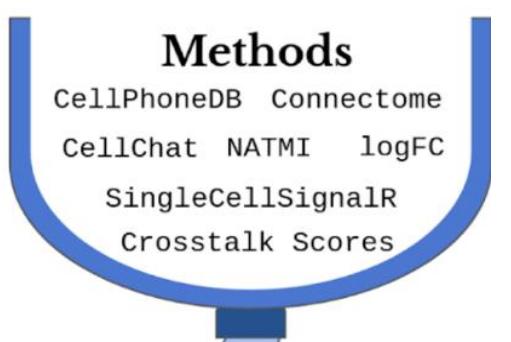
What are the possible interactions?



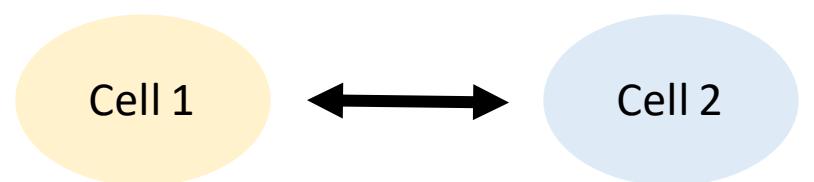
What are the possible interactions?



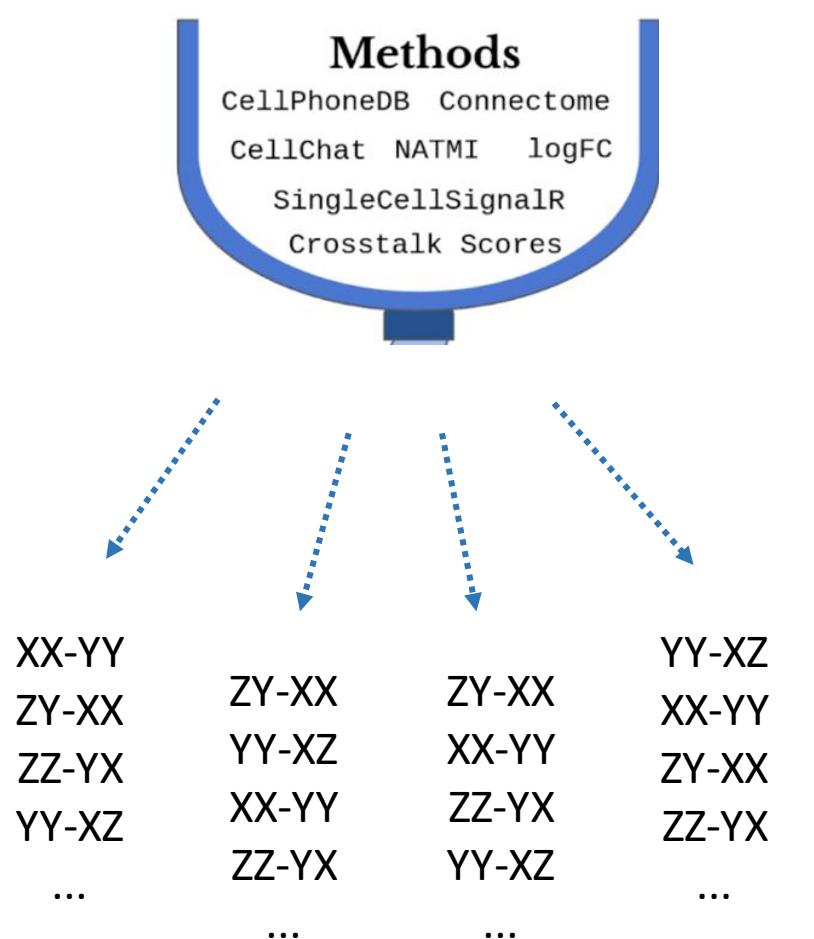
Can be answered by many existing methods ...



What are the possible interactions?

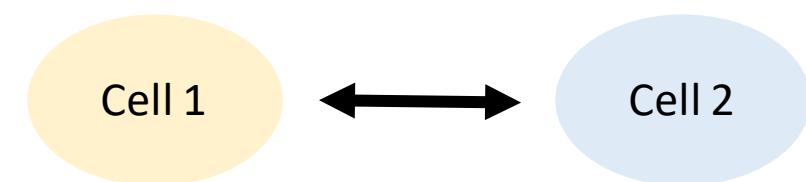


Can be answered by many existing methods ...

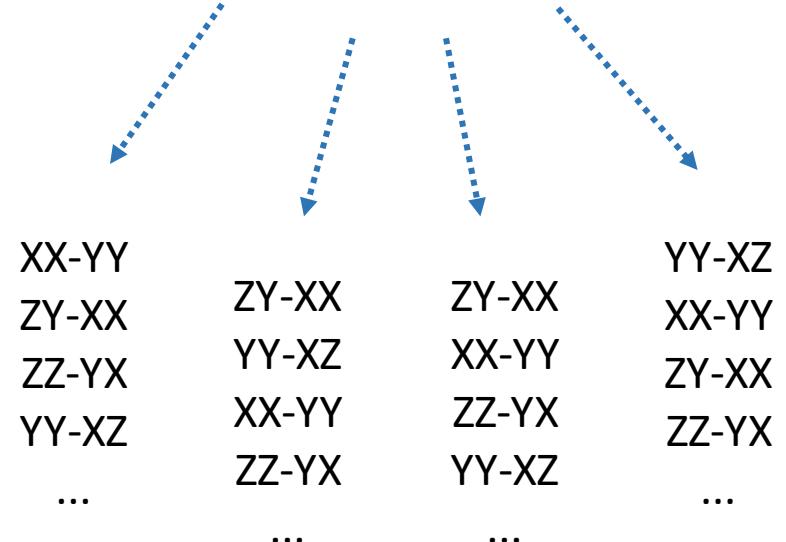
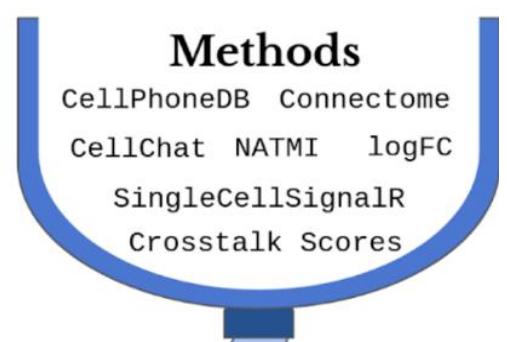


Individual ranked lists

What are the possible interactions?

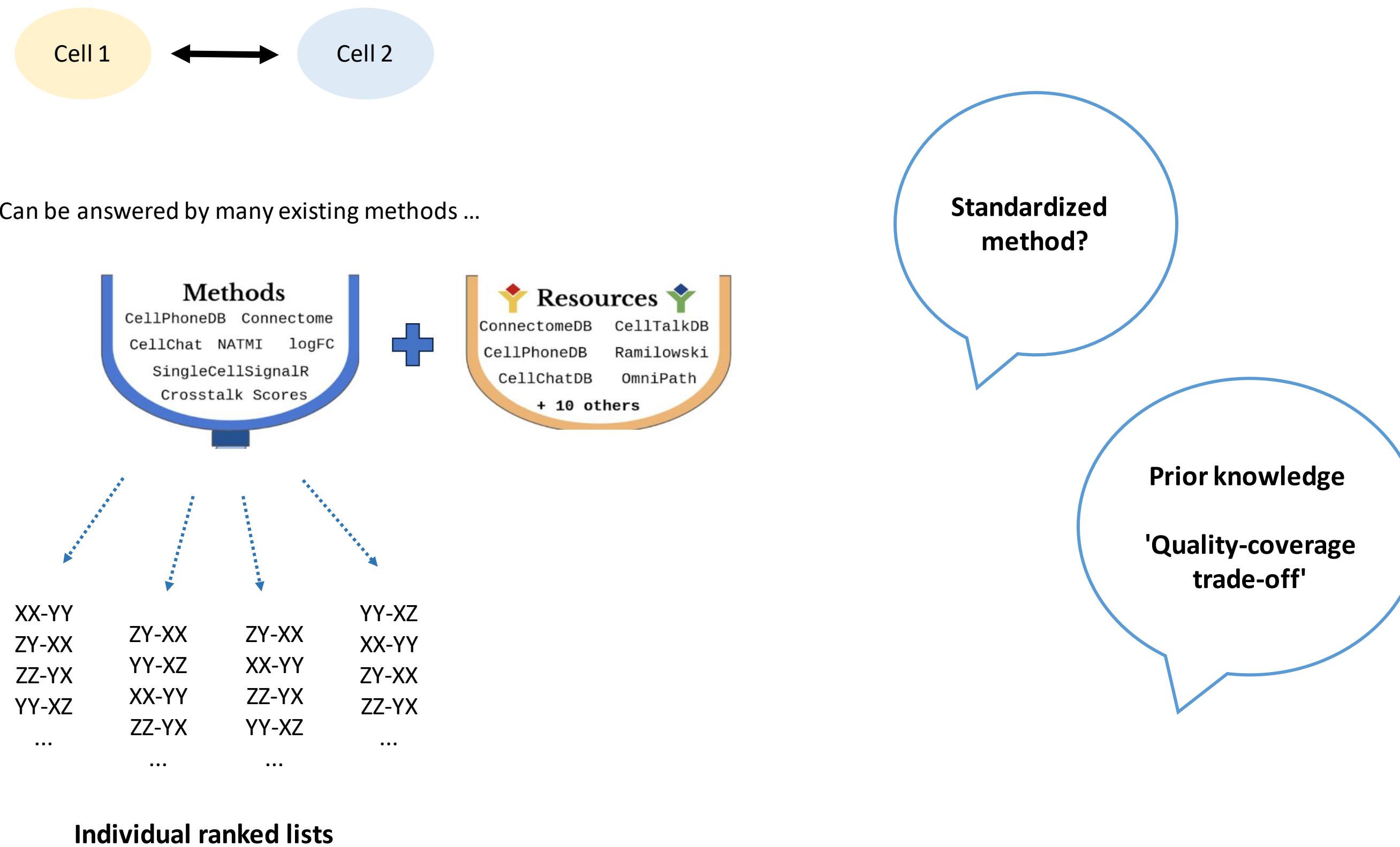


Can be answered by many existing methods ...

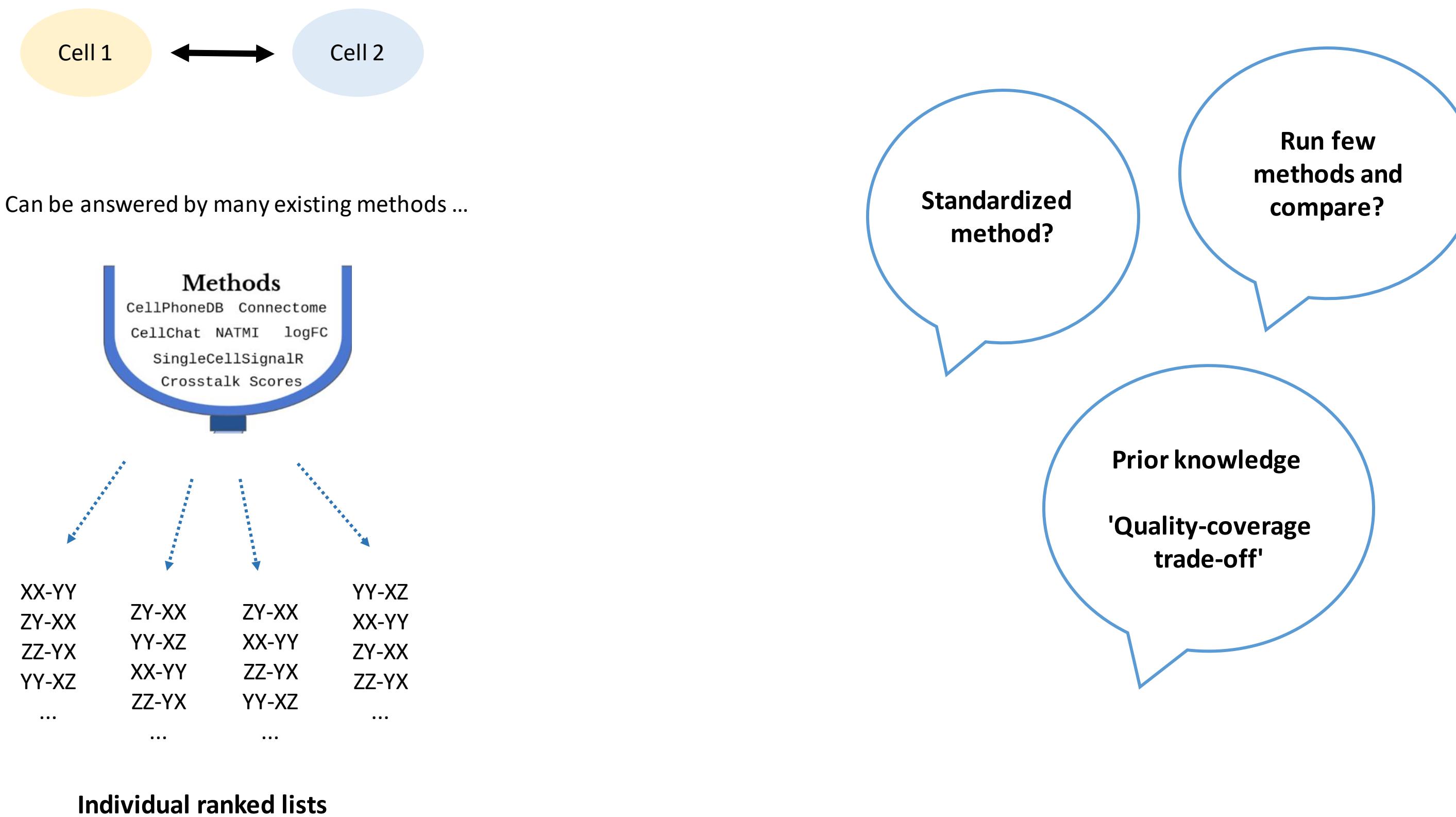


Individual ranked lists

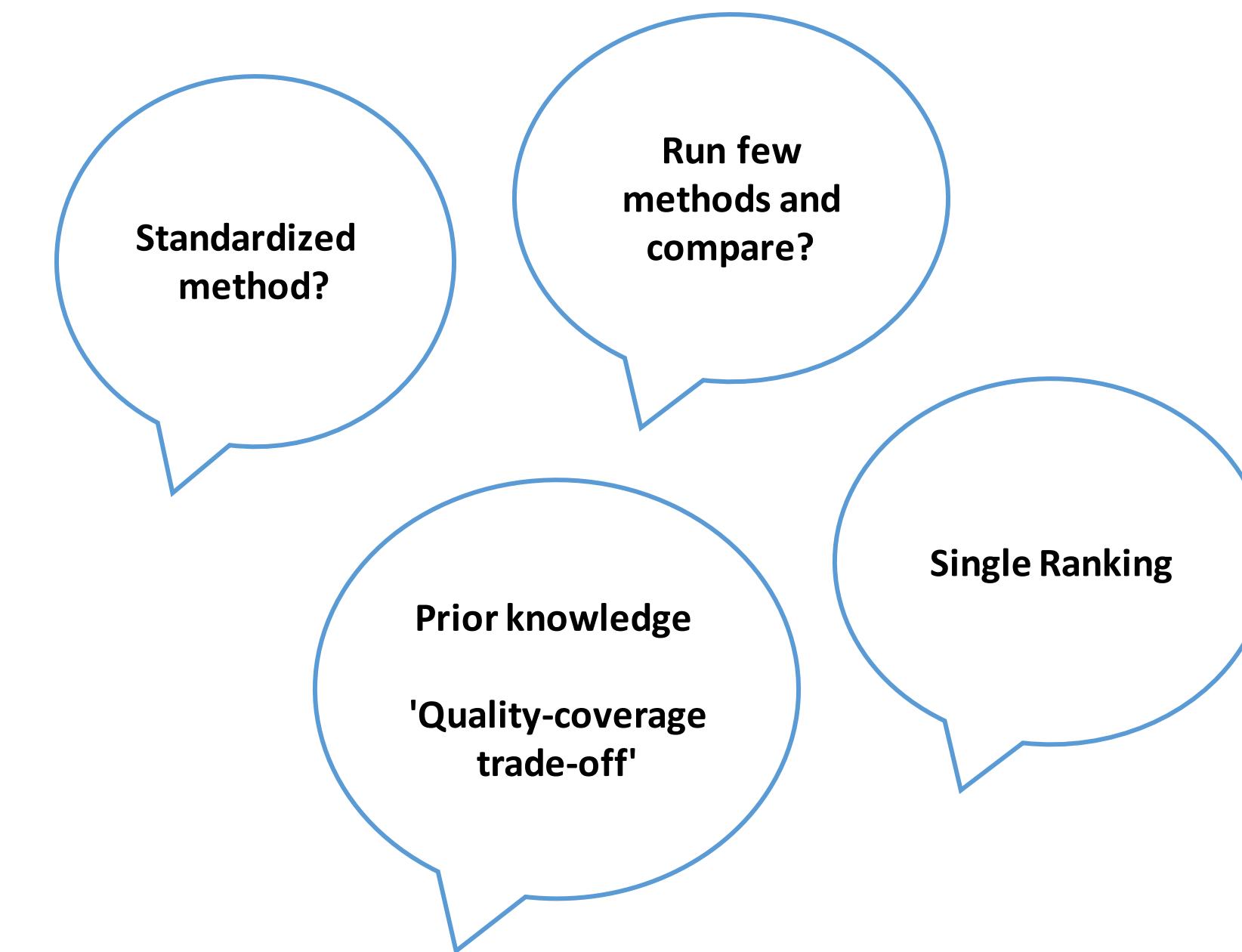
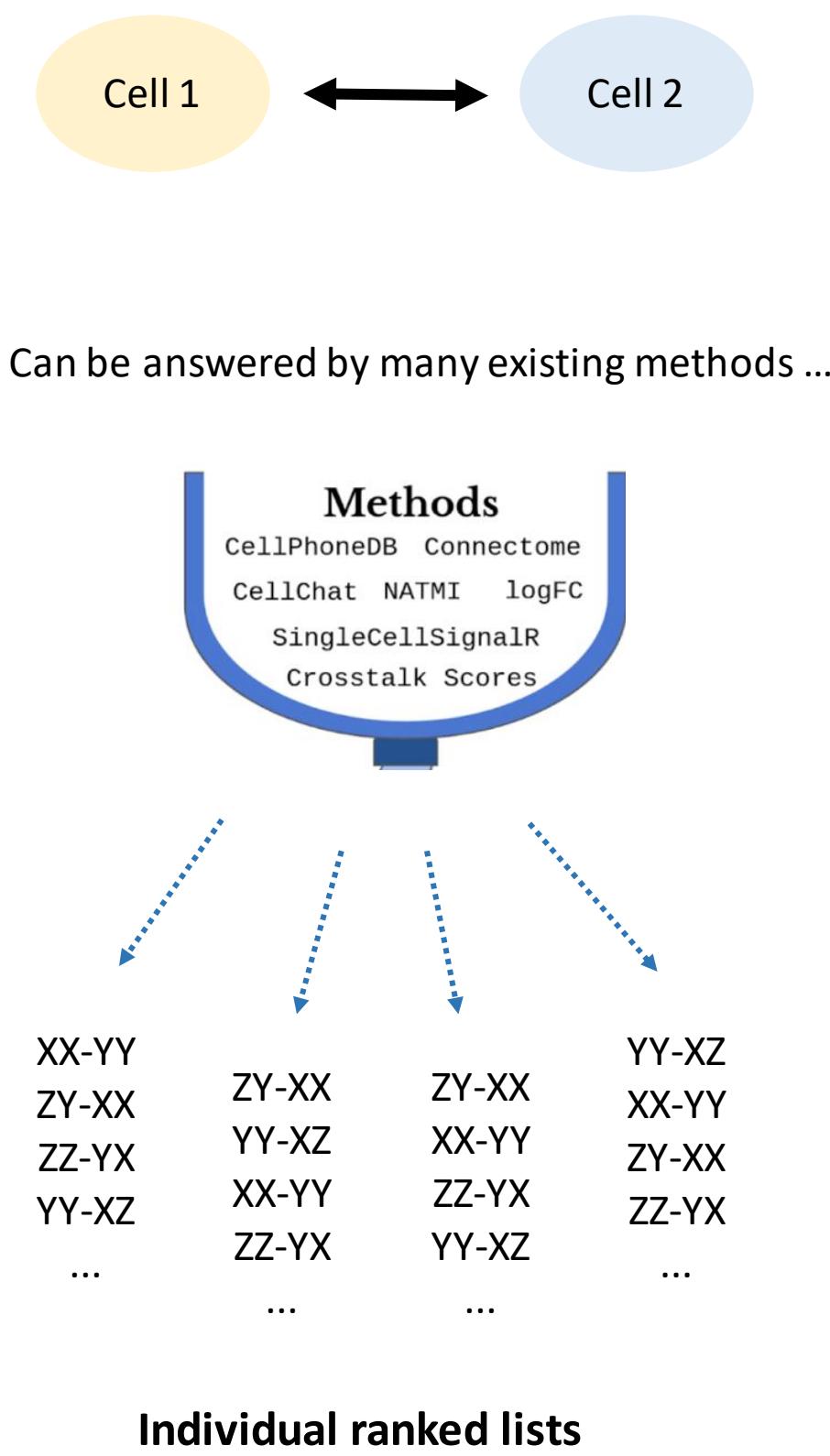
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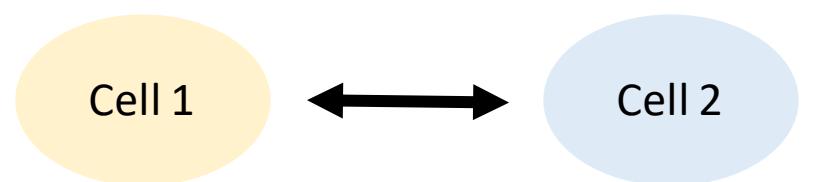
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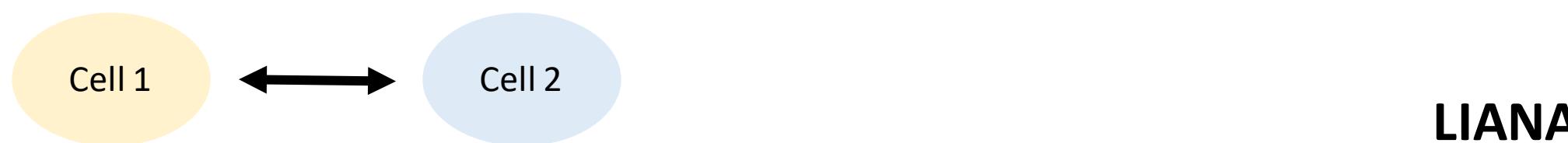
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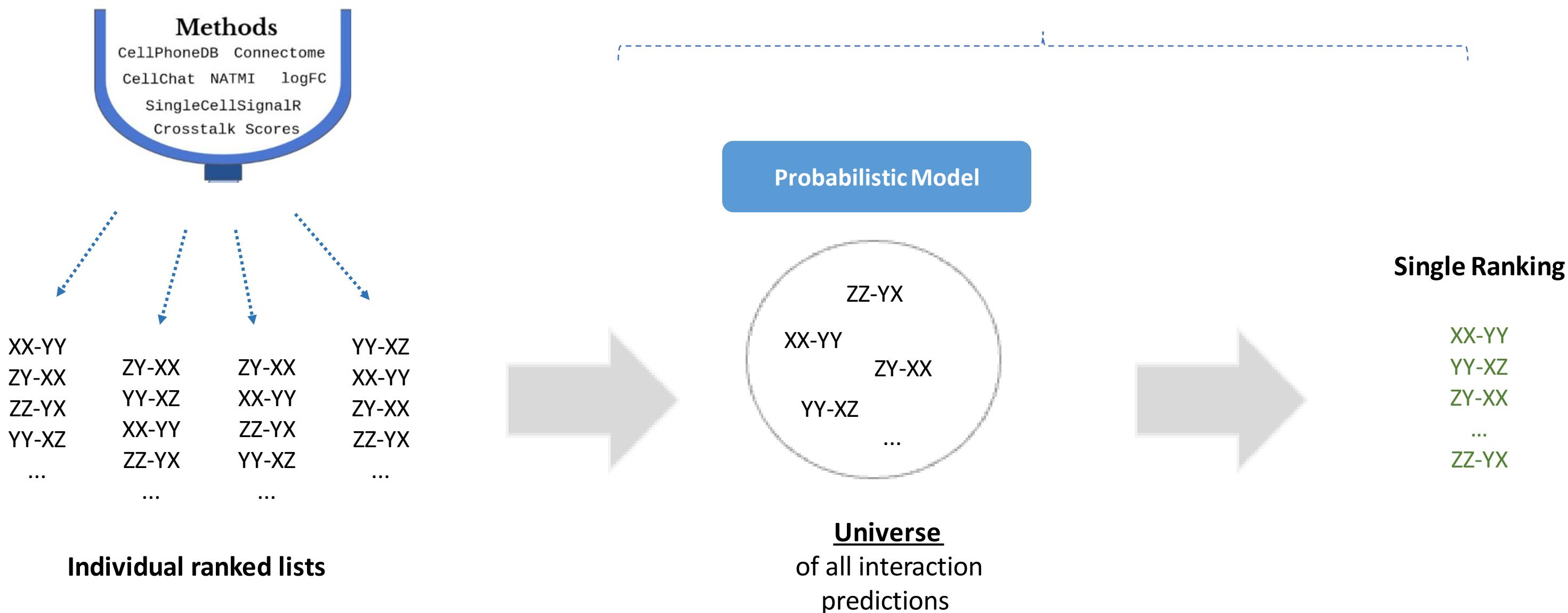


What are the possible interactions?



Can be answered by many existing methods ...

Robust Rank Aggregate (RRA) method from *RobustRankAggreg* package



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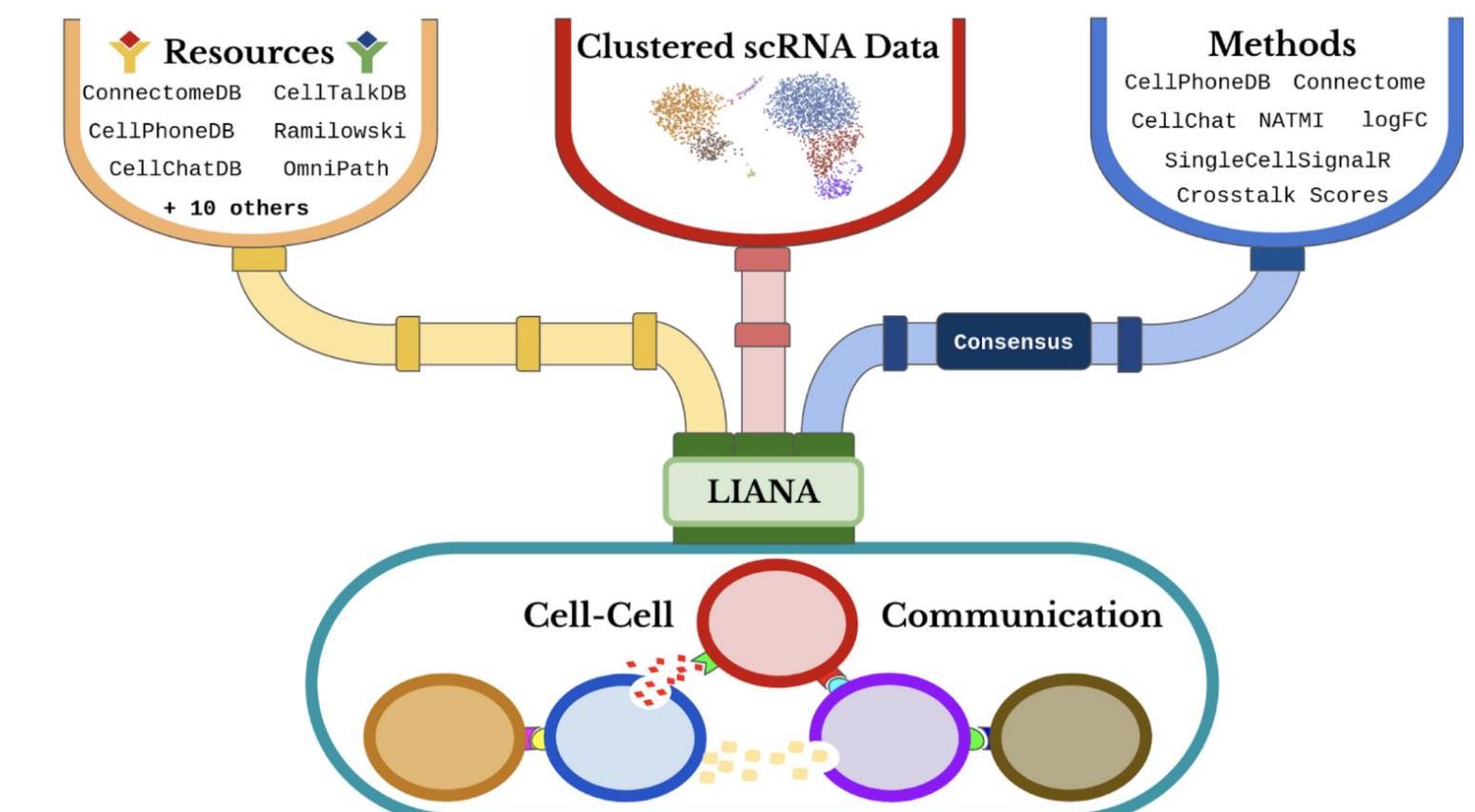
Article | [Open access](#) | Published: 09 June 2022

Comparison of methods and resources for cell-cell communication inference from single-cell RNA-Seq data

[Daniel Dimitrov](#), [Dénes Türei](#), [Martin Garrido-Rodriguez](#), [Paul L. Burmedi](#), [James S. Nagai](#), [Charlotte Boys](#), [Ricardo O. Ramirez Flores](#), [Hyojin Kim](#), [Bence Szalai](#), [Ivan G. Costa](#), [Alberto Valdeolivas](#), [Aurélien Dugourd](#) & [Julio Saez-Rodriguez](#)✉

[Nature Communications](#) **13**, Article number: 3224 (2022) | [Cite this article](#)

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LIANA

liana 0.1.12 Reference Articles ▾ Sea

LIANA: a Ligand-receptor ANalysis frAmework



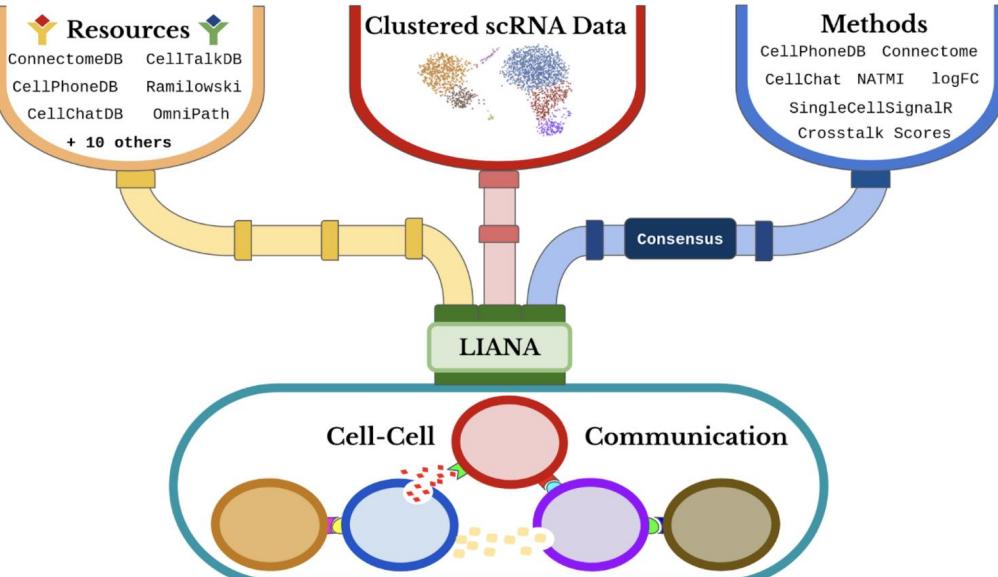
LIANA enables the use of any combination of ligand-receptor methods and resources, and their consensus. A faster and memory efficient Python implementation is available [here](#).

Install LIANA

```
if (!requireNamespace("BiocManager", quietly = TRUE))
  install.packages("BiocManager")

if (!requireNamespace("remotes", quietly = TRUE))
  install.packages("remotes")

remotes::install_github('saezlab/liana')
```



Resources

Cell-cell Communication resources

The following CCC resources are accessible via this pipeline:

- *Consensus*
 - CellCall
 - CellChatDB
 - CellPhoneDB
 - Ramilowski2015
 - Baccin2019
 - LRdb
 - Kiroauc2010
 - ICELLNET
 - iTALK
 - EMBRACE
 - HPMR
 - Guide2Pharma
 - ConnectomeDB2020
 - CellTalkDB
 - OmniPath [Deprecated]
- ## Tools
- The tools implemented in this repository are:
- [CellPhoneDBv2](#) (*, \$)
 - [CellChat](#)
 - [NATMI](#) (*, \$)
 - [Connectome](#) (edge_weights) (*, \$)
 - [SingleCellSignalR](#) (LRscores) (SCA) (*, \$)
 - [iTALK](#)-inspired 1-vs-rest LogFC score (logfc_comb) (*, \$)
 - [CytoTalk](#)-inspired cross-talk scores (*)
 - [consensus_rank](#) of the predictions is also provided using the [RobustRankAggregate](#) method

LIANA

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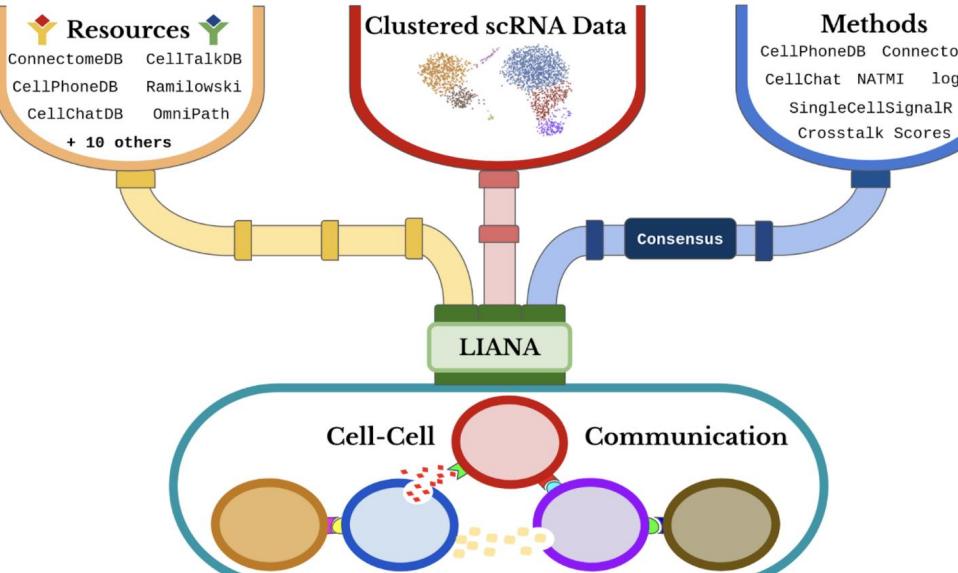
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> *Bioinformatics*. 2012 Feb 15;28(4):573-80. doi: 10.1093/bioinformatics/btr709. Epub 2012 Jan 12.

Robust rank aggregation for gene list integration and meta-analysis

Raivo Kolde ¹, Sven Laur, Priit Adler, Jaak Vilo

Affiliations + expand

PMID: 22247279 PMCID: PMC3278763 DOI: 10.1093/bioinformatics/btr709

LIANA

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LIANA: a Ligand-receptor ANalysis frAmework



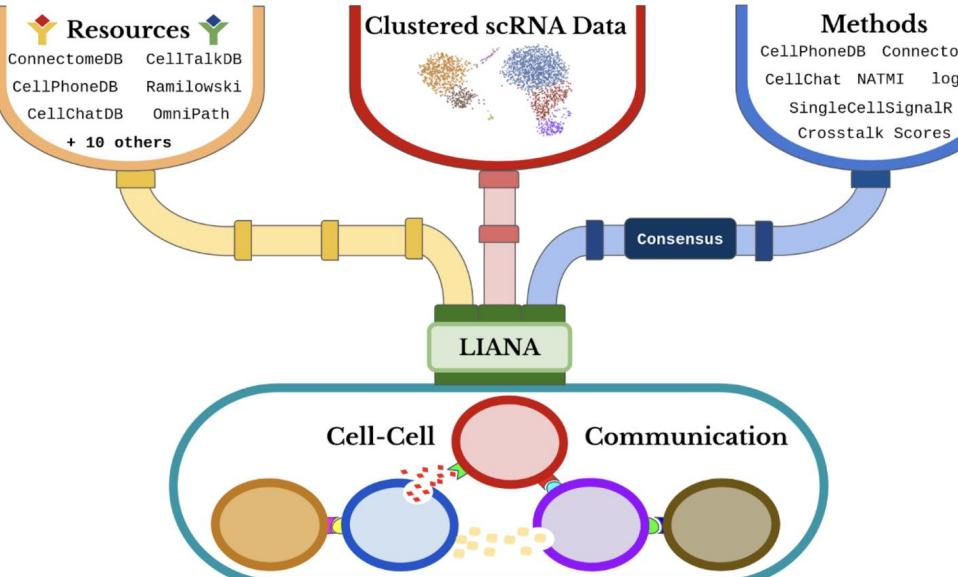
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<https://saezlab.github.io/liana/>



Table 1 Tools included in the framework.

From: [Comparison of methods and resources for cell-cell communication inference from single-cell RNA-Seq data](#)

Tool/Method	Resource	Methods' scoring systems
CellChat#¹⁴	CellChatDB	(1) Probability —based on the expression of differentially expressed transmitter and receiver genes and their mediators, calculated with the law of mass action (2) P-values† —significance identified via permutation of cell cluster labels and recalculating the probabilities for each cell pair and each transmitter-receiver interaction
CellPhoneDBv2#⁸	CellPhoneDB	(1) Truncated Mean —average expression of transmitter and receivers, the minimum expression (by default) of heteromeric complex of subunits (2) P-values† —significance identified via permutation of cell cluster labels to determine a null distribution of means for each receiver-transmitter interaction
Connectome¹⁰	Ramilowski	(1) weight_norm —derived via the product (by default) of the normalised expression of transmitter and receiver genes (2) weight_scale† —derived from a function (mean, by default) of the z-scores of the transmitter and the receiver, scaled according to cell cluster specificity
Crosstalk scores	-	(1) Crosstalk score† —Cytotalk-inspired ²² crosstalk scores were derived from the expression of transmitters and receivers, weighted by the likelihood of autocrine signalling between the source and target cell types.
logFC Mean	-	(1) logFC Mean† —iTALK-inspired ⁶ logFC means, derived using the mean of the logged one-versus-all fold change of receiver and transmitter gene expression
NATMI¹¹	ConnectomeDB	(1) Mean-expression edge weight —transmitter and receiver gene expression product (2) Specificity-based edge weight† —the mean expression of the transmitter and receiver are divided by the sum of the means of the same transmitters/receivers across all cell clusters
SingleCellSignalR#¹²	LRdb	(1) LRscore —a regularised score calculated using the squared expression of the transmitter and receiver (sqTRE) divided by sum of the mean of the count matrix and sqTRE.
Consensus	-	(1) Robust Rank Aggregate⁶⁵ —preferentially highly-ranked interactions are obtained from a distribution generated from the interaction rankings of other methods

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SingleCellSignalR#¹²	LRdb	(1) LRscore —a regularised score calculated using the squared expression of the transmitter and receiver (sqTRE) divided by sum of the mean of the count matrix and sqTRE.
Consensus	-	(1) Robust Rank Aggregate⁶⁵ —preferentially highly-ranked interactions are obtained from a distribution generated from the interaction rankings of other methods

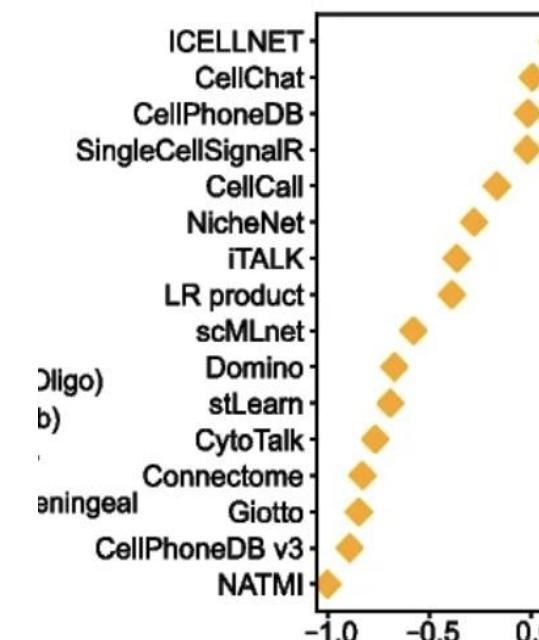
Research | [Open access](#) | Published: 17 October 2022

Evaluation of cell-cell interaction methods by integrating single-cell RNA sequencing data with spatial information

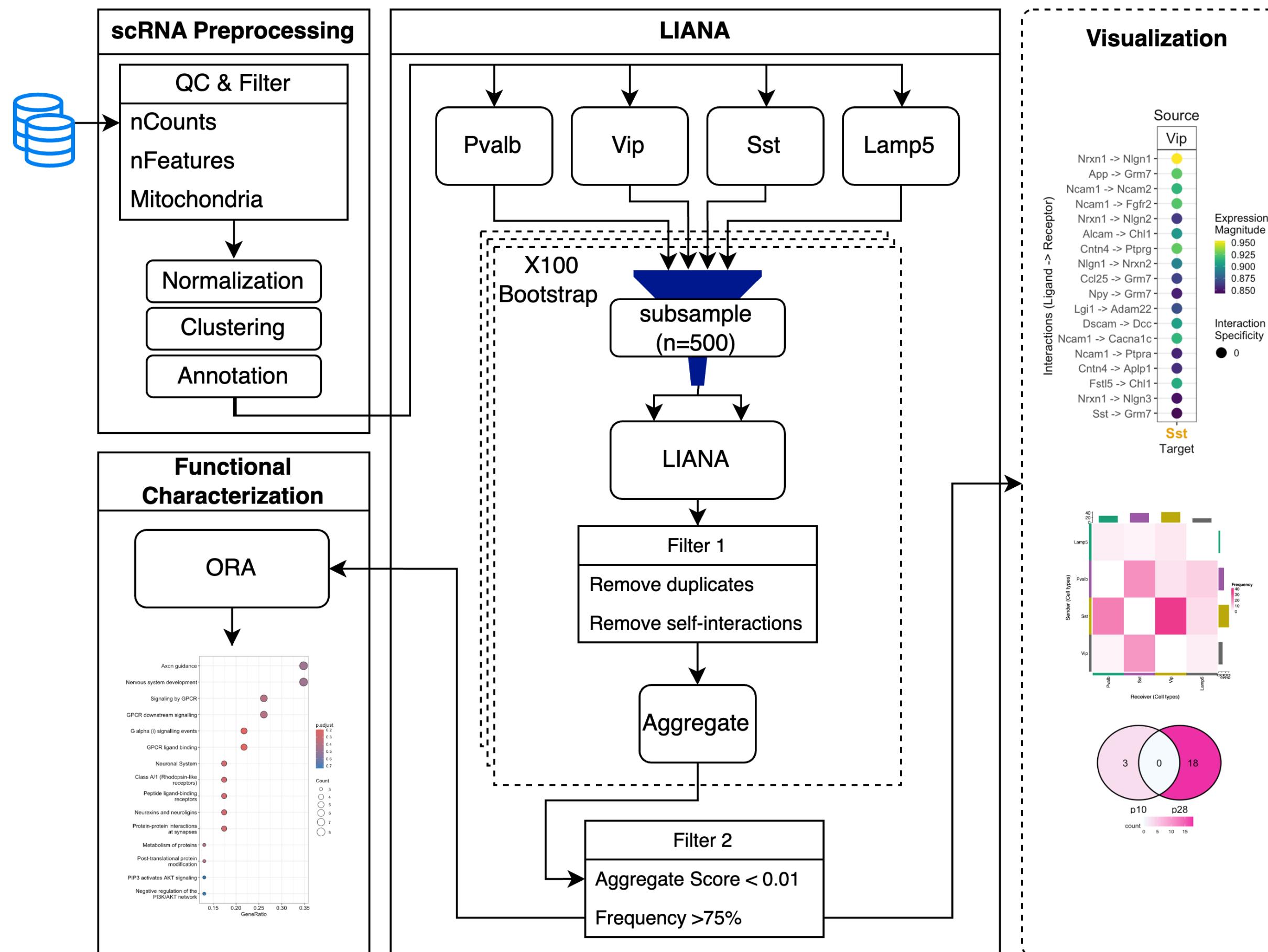
Zhaoyang Liu, Dongqing Sun & Chenfei Wang [✉](#)

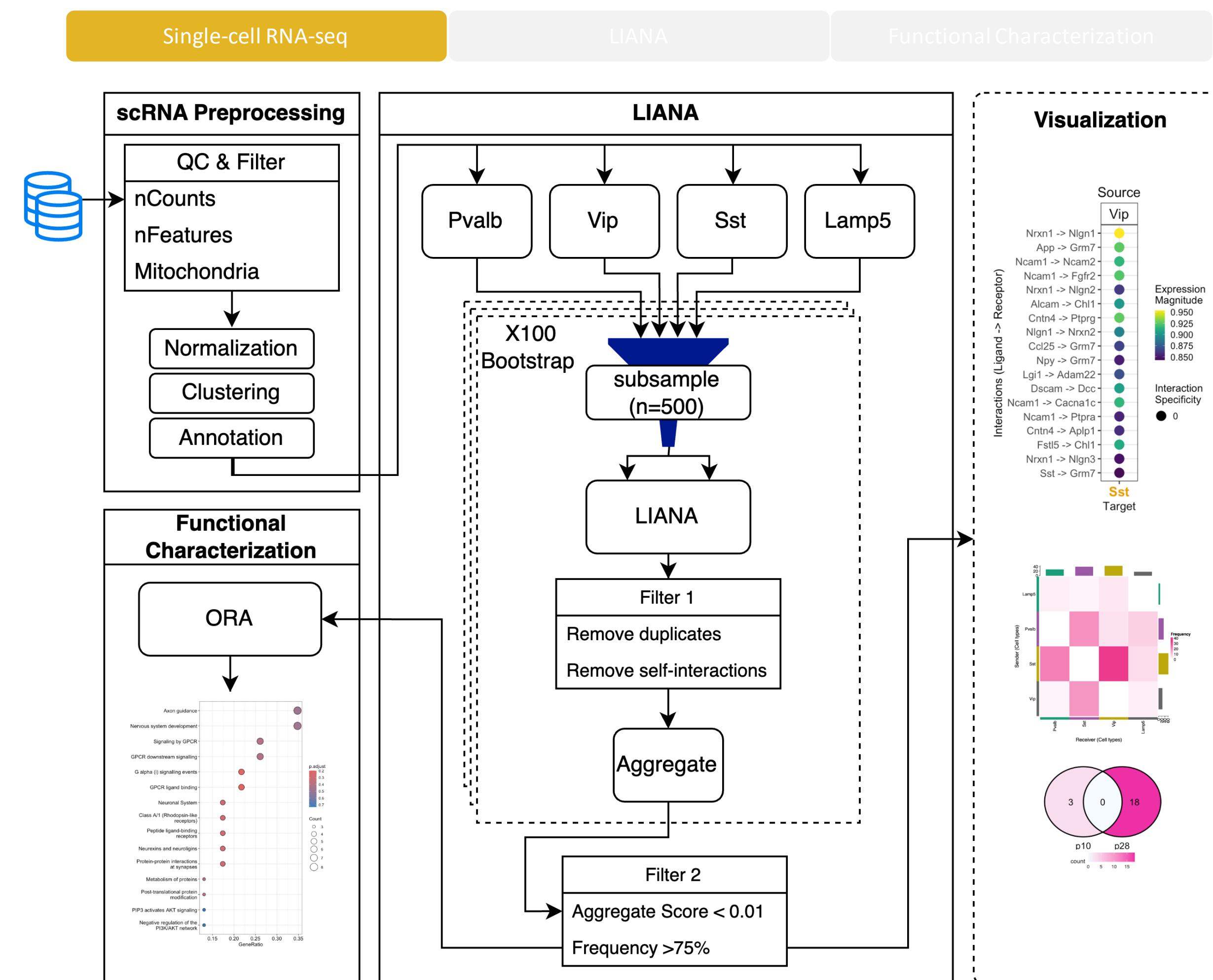
[Genome Biology](#) 23, Article number: 218 (2022) | [Cite this article](#)

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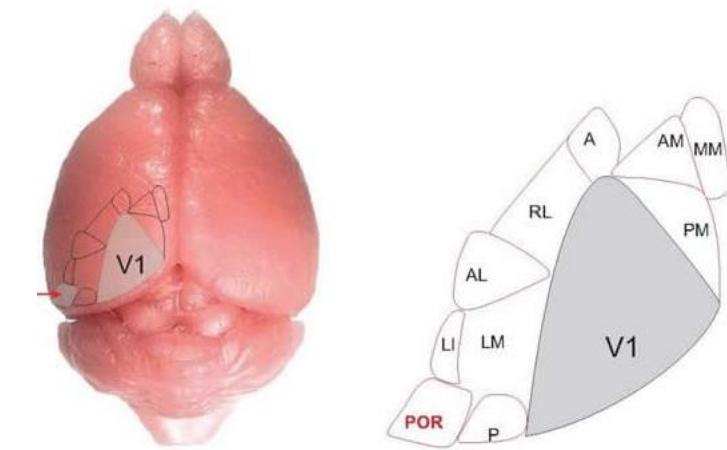
Pipeline workflow





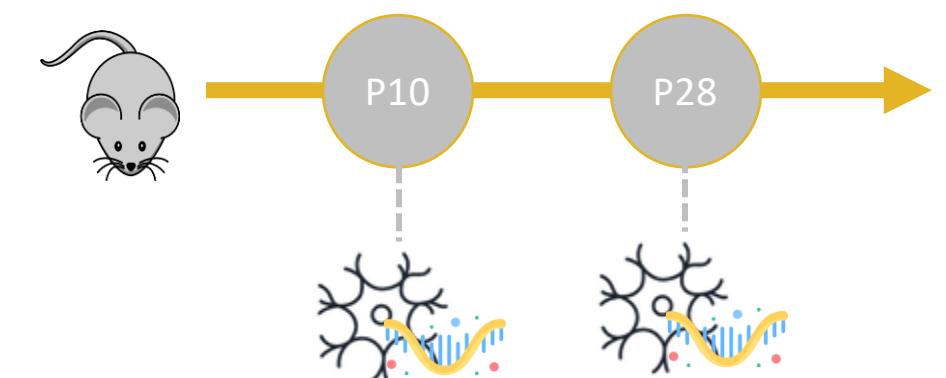
1 Loading data:

- p10: GSM5014306
- p28: GSM5014308



2 Creating Seurat objects

```
for (sample in dirs) {
  cts <- ReadMtx(mtx = paste0(path, sample, "/", sample, "_V1_Dlxpos_RNA_matrix.mtx"),
                  features = paste0(path, sample, "/", sample, "_V1_Dlxpos_RNA_features.tsv"),
                  cells = paste0(path, sample, "/", sample, "_V1_Dlxpos_RNA_barcodes.tsv"))
  assign(sample, CreateSeuratObject(counts = cts)) }
```



3 Merging data (p10 & p28)

```
merged_seurat <- merge(x = GSM5014306_P10, y = c(GSM5014308_P28), add.cell.id = c("GSM5014306_P10", "GSM5014308_P28"))
```

4 Adding QC matrices metadata

```
merged_seurat$log10GenesPerUMI_RNA <- log10(merged_seurat$nFeature_RNA) / log10(merged_seurat$nCount_RNA)
merged_seurat$mitoRatio <- PercentageFeatureSet(merged_seurat, pattern = "mt-")
merged_seurat$mitoRatio <- merged_seurat@meta.data$mitoRatio / 100
```

5

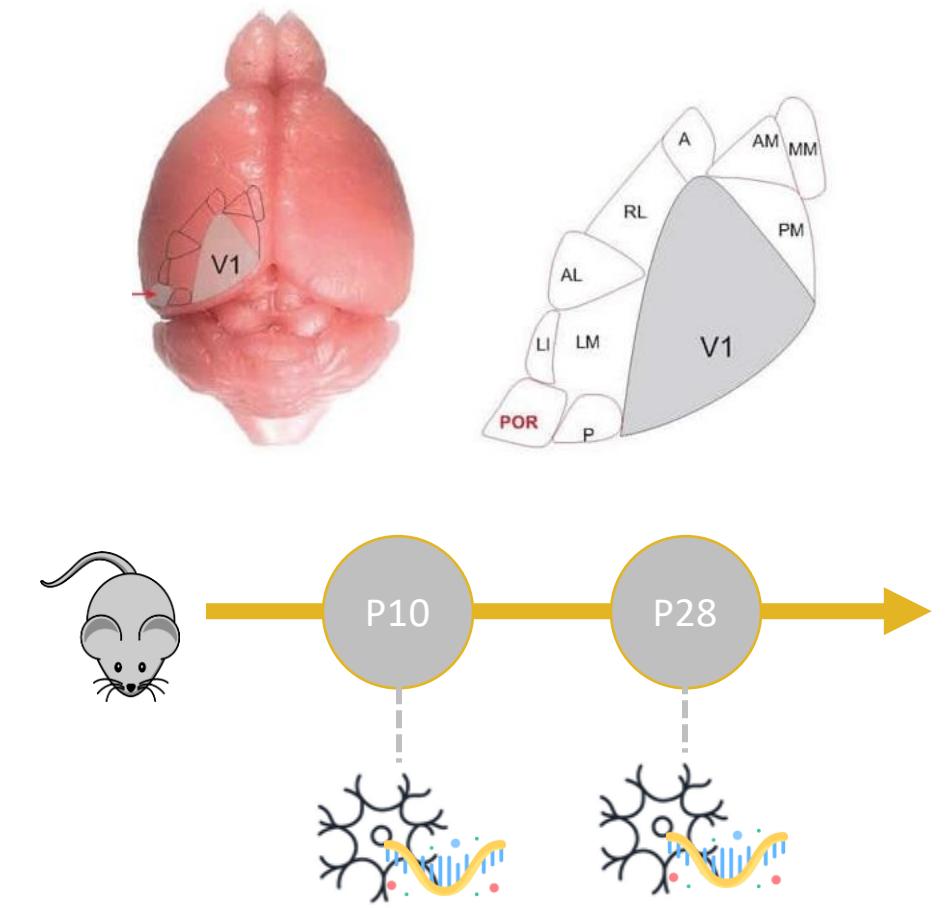
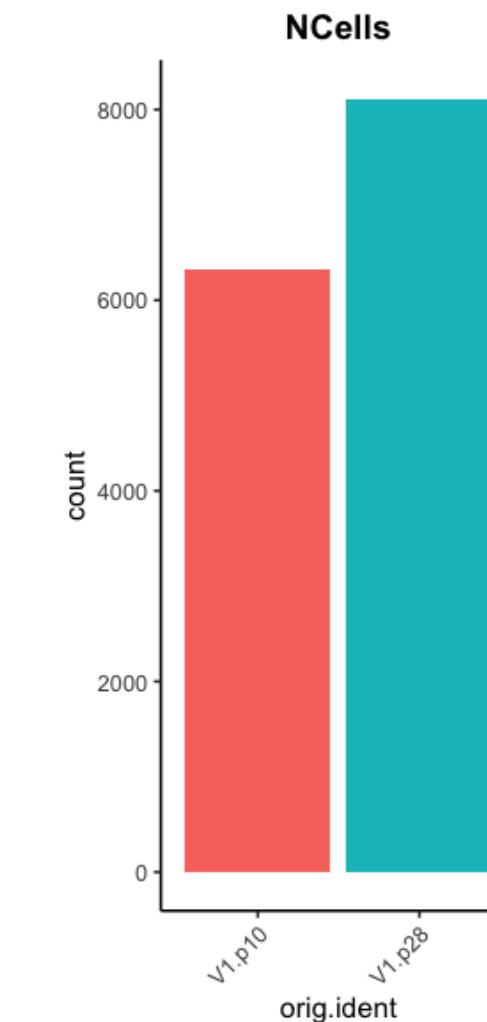
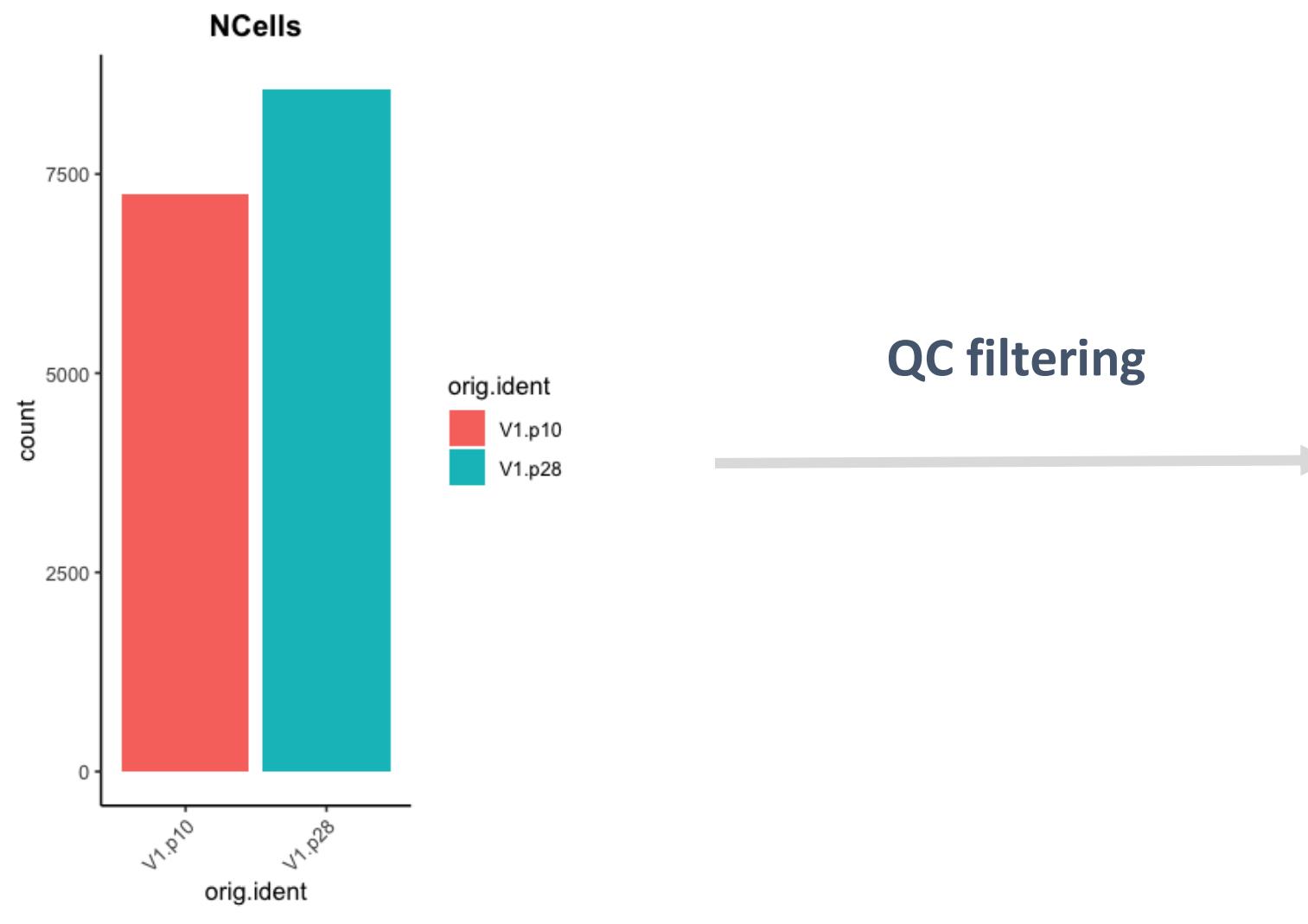
Filtering based on QC

```

nCount_min_filt <- 2000
nCount_max_filt <- 70000
nFeature_min_filt <- 1000
nFeature_max_filt <- 8000
mitoRatio_filt <- 0.1

filtered_seurat <- subset(merged_seurat, nCount_RNA >= nCount_min_filt & nCount_RNA <=
nCount_max_filt & nFeature_RNA >= nFeature_min_filt & nFeature_RNA <= nFeature_max_filt
& mitoRatio < mitoRatio_filt)

```



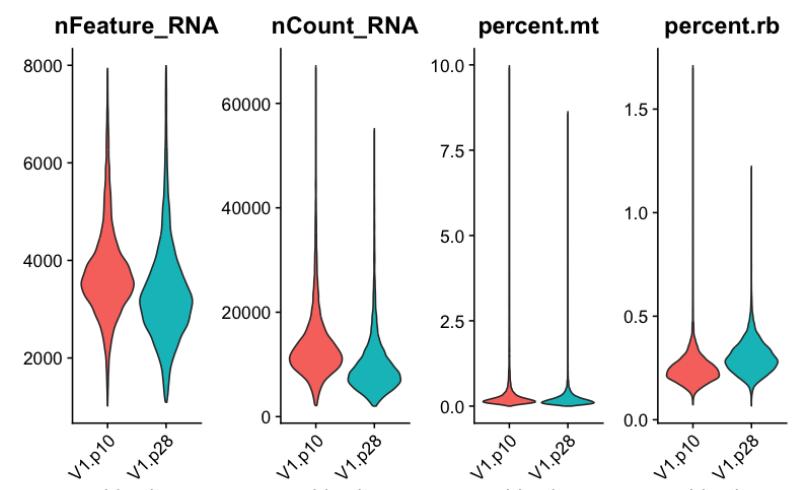
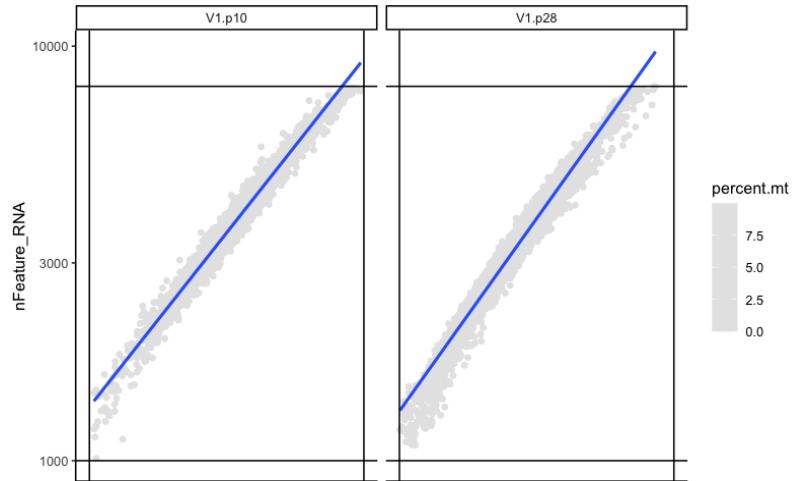
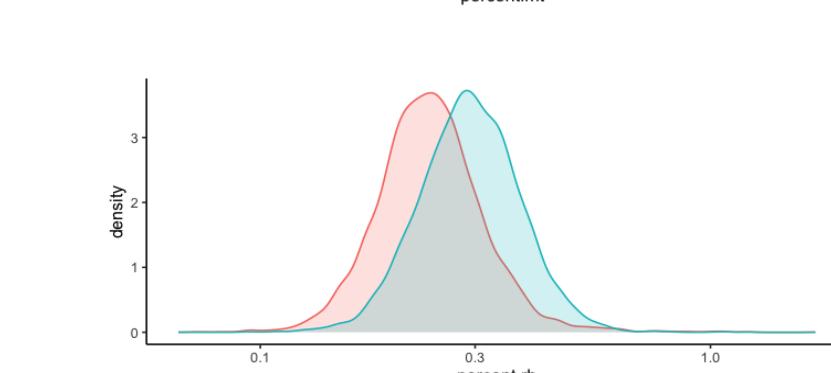
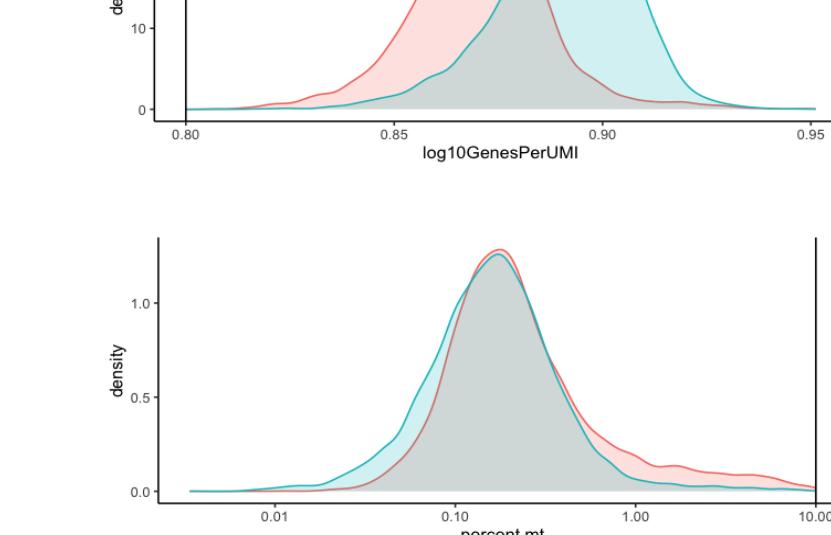
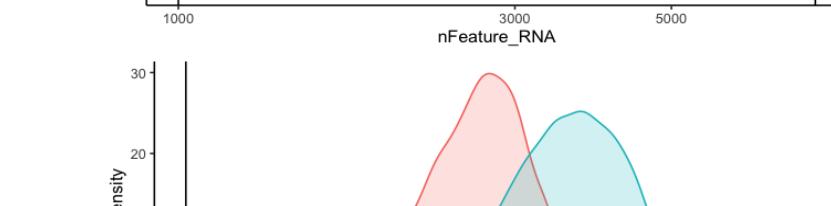
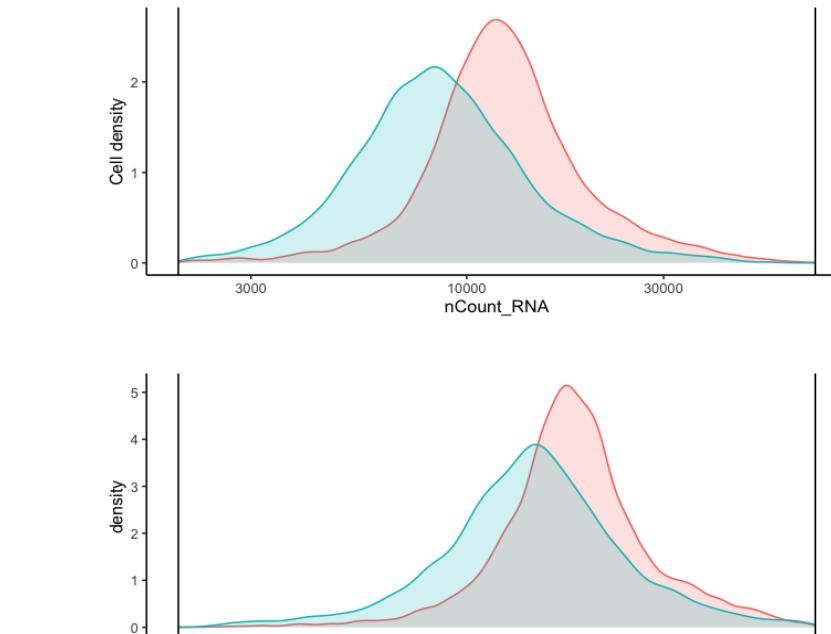
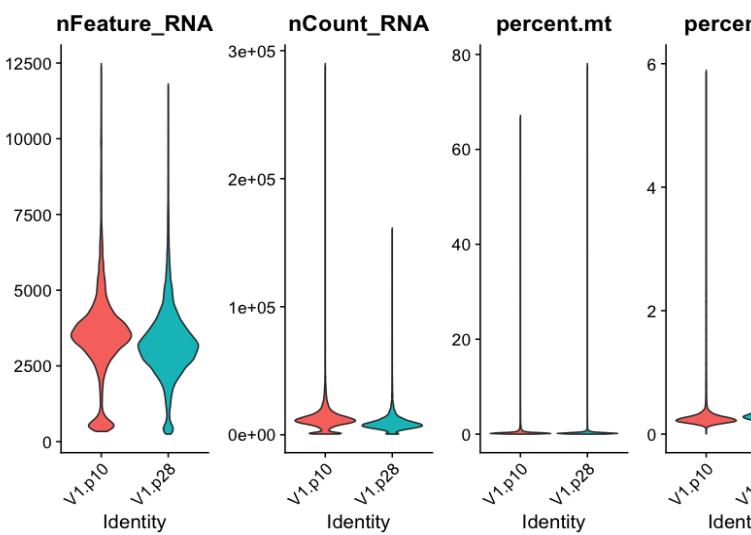
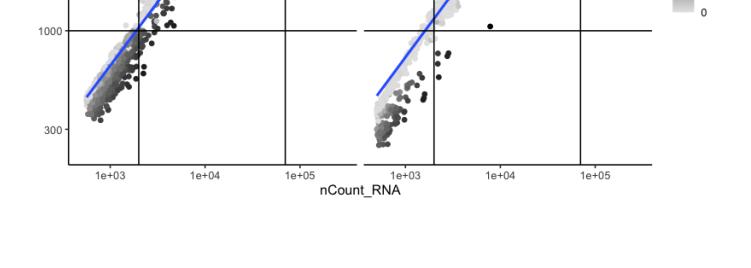
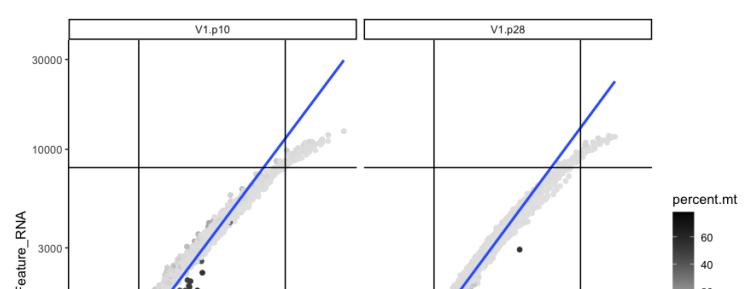
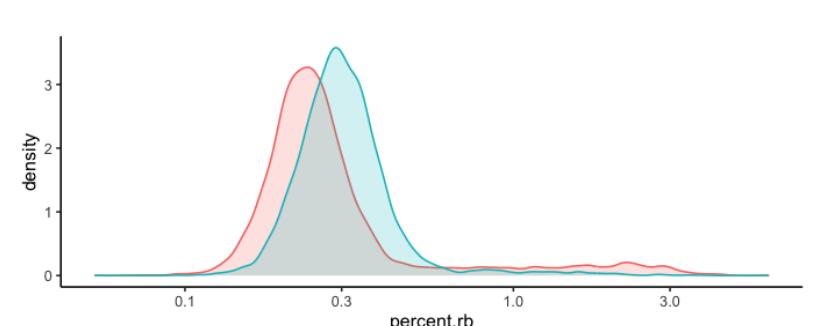
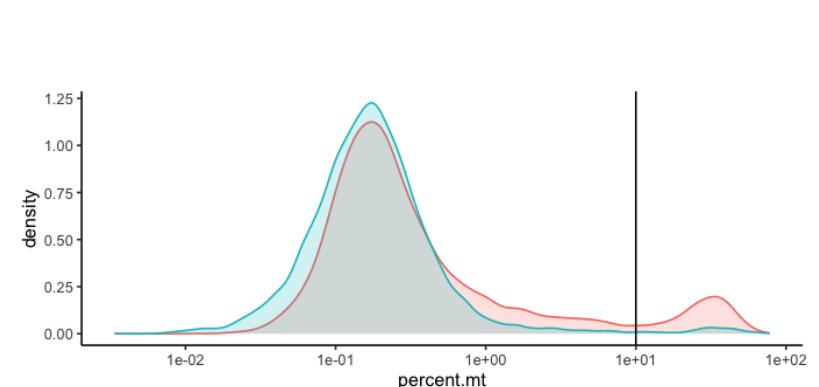
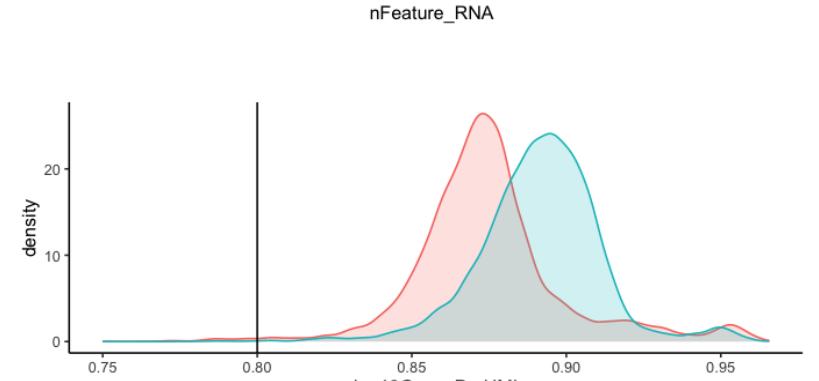
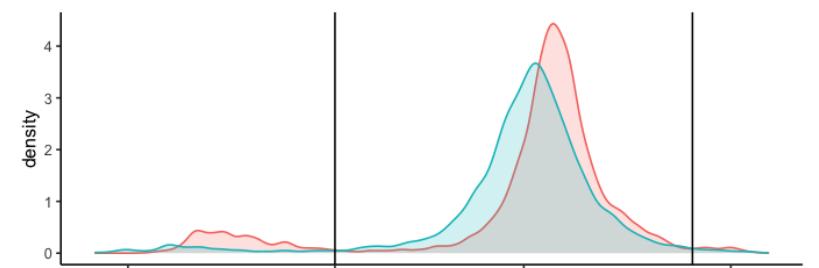
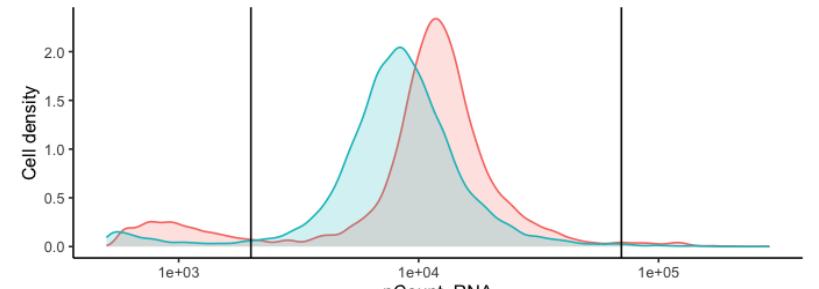
Single-cell RNA-seq

LIANA

Functional Characterization

5

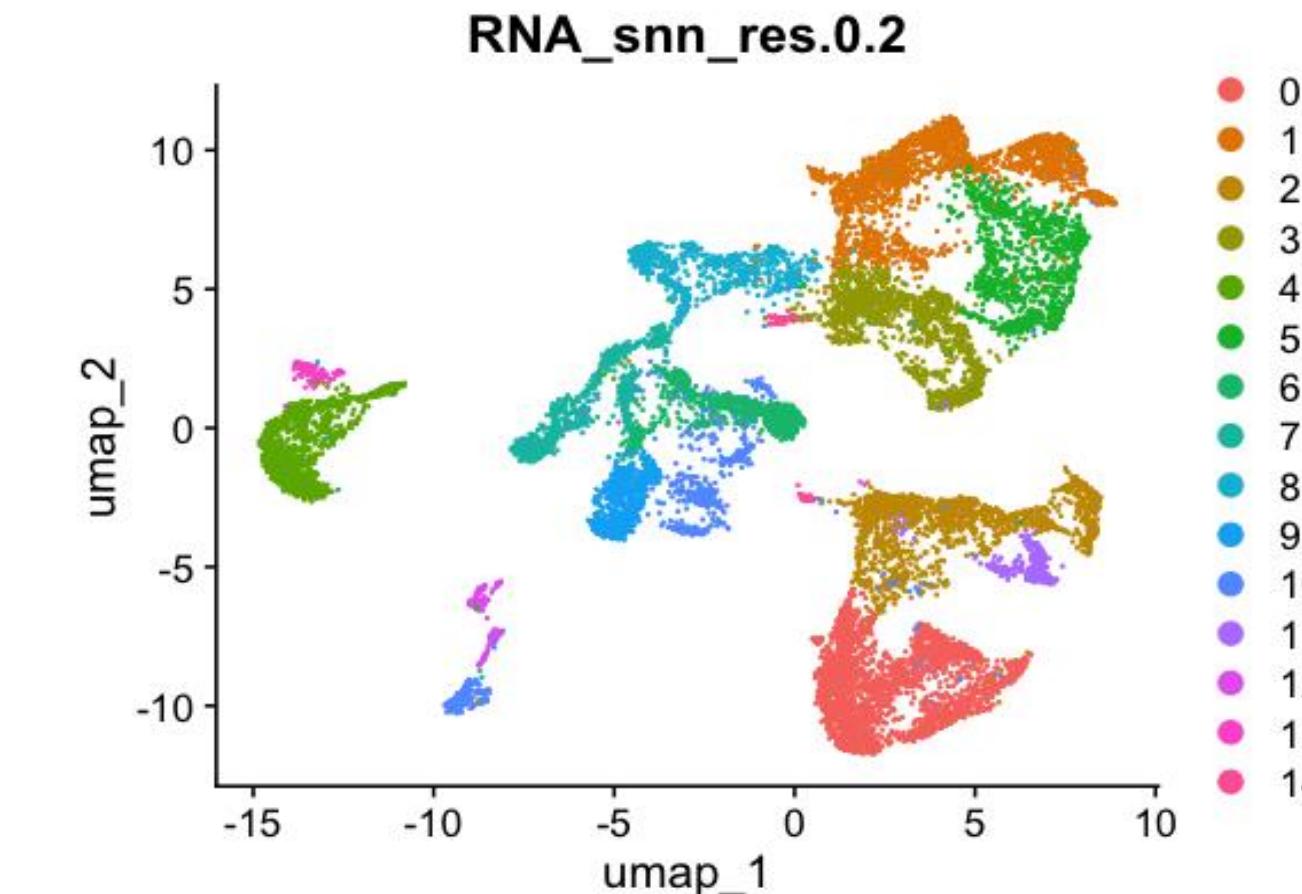
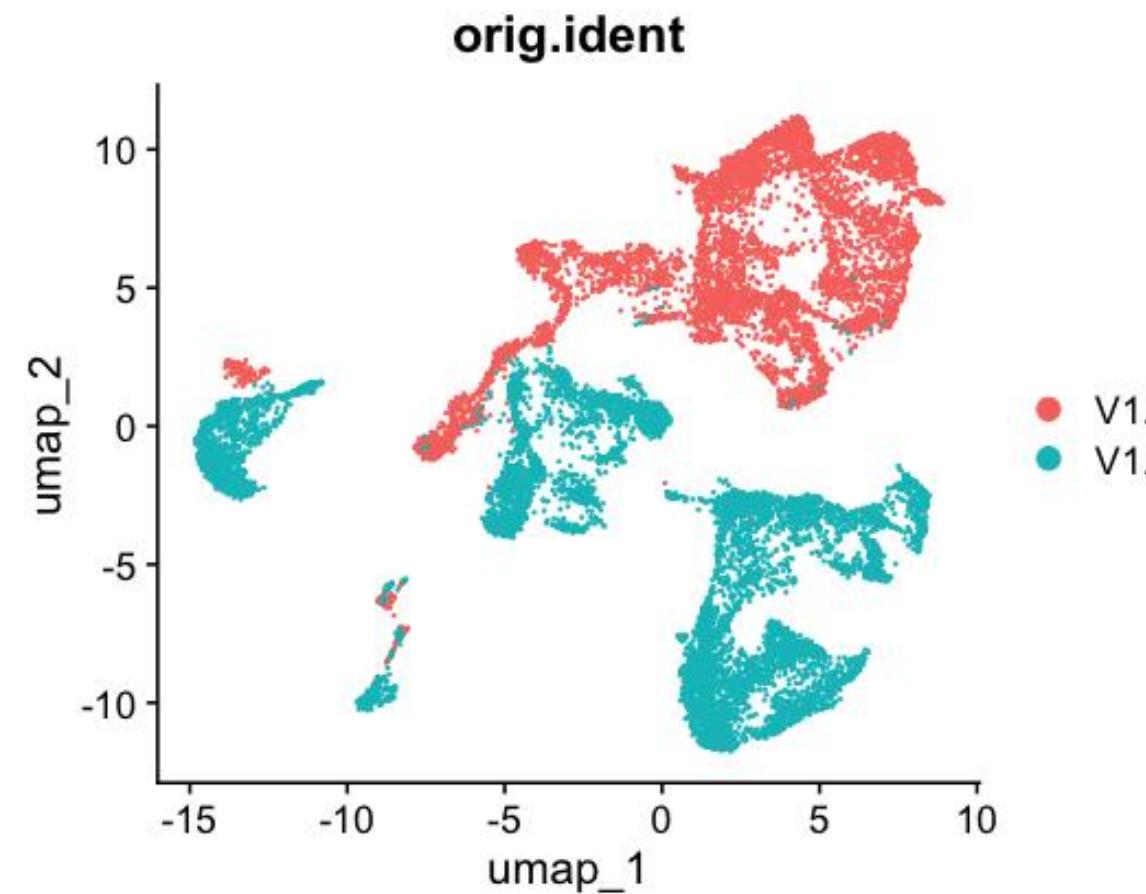
Filtering based on QC



6

Normalize, VairableFeatures, Scale data, PCA, Clustering, visualize ...

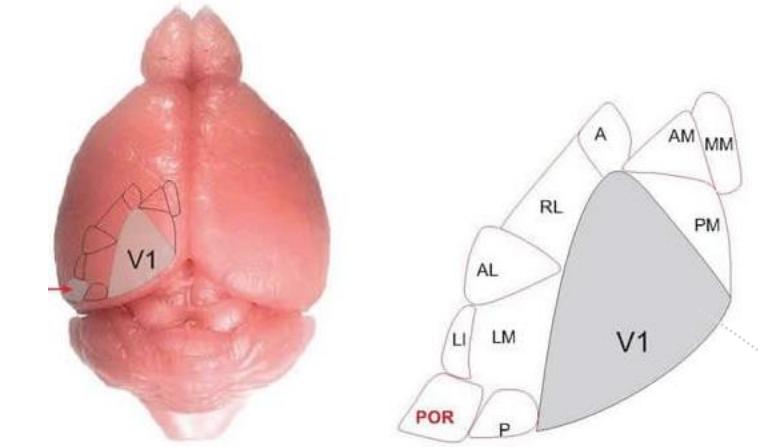
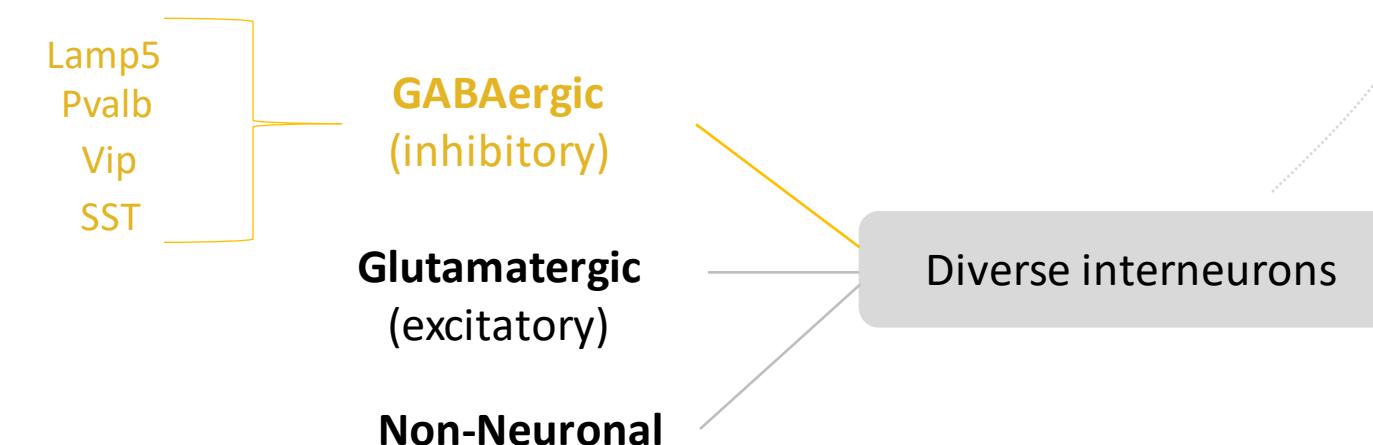
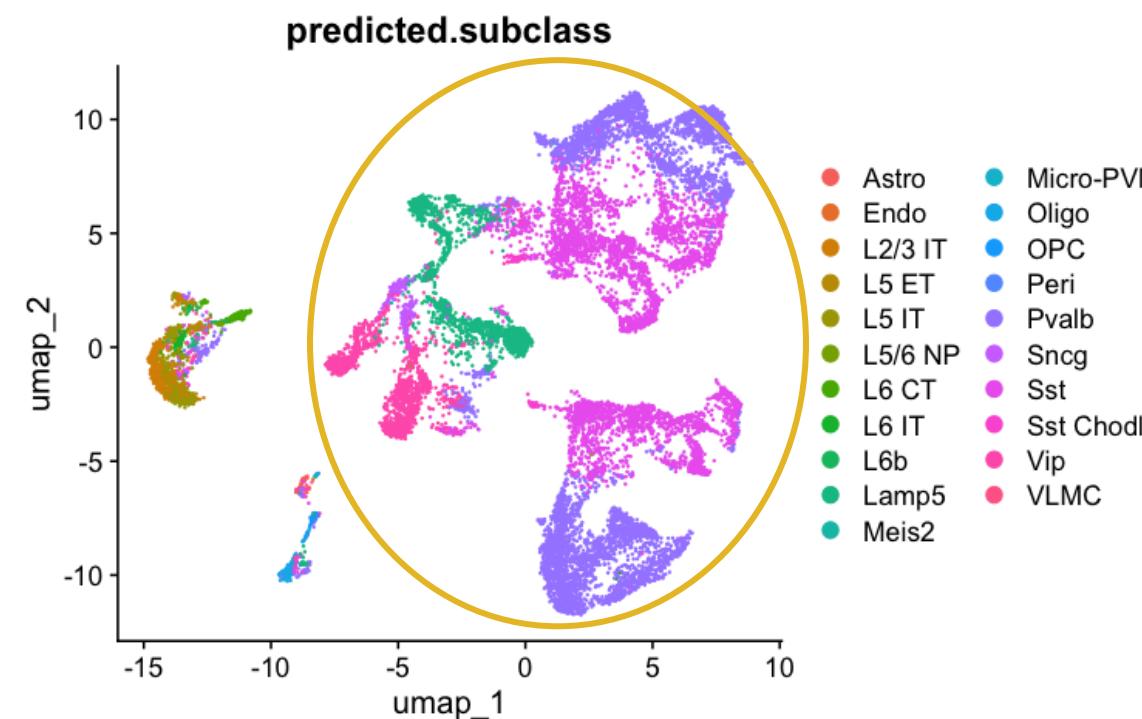
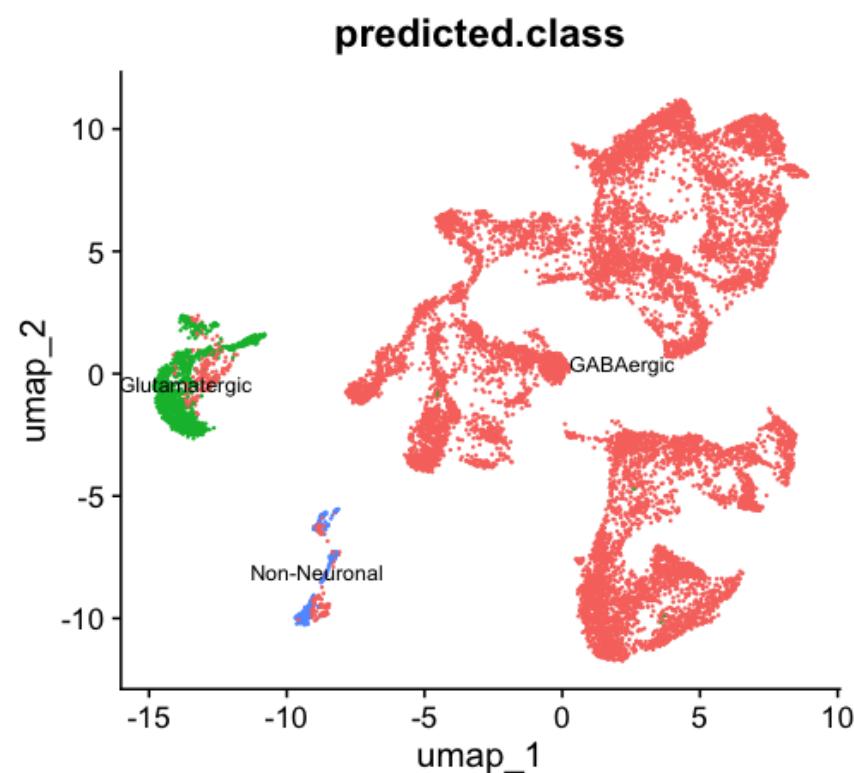
```
seurat_obj <- NormalizeData(seurat_obj)
seurat_obj <- FindVariableFeatures(seurat_obj, selection.method = "vst", nfeatures = 2000, verbose = FALSE)
seurat_obj <- ScaleData(seurat_obj)
seurat_obj <- RunPCA(seurat_obj)
seurat_obj <- FindNeighbors(seurat_obj, dims = 1:30, reduction='pca')
seurat_obj <- FindClusters(seurat_obj, resolution = c(0.2,0.4, 0.6, 0.8, 1.0, 1.2))
seurat_obj <- RunUMAP( seurat_obj, dims=1:30, min.dist=0.3, reduction.name = 'umap')
```

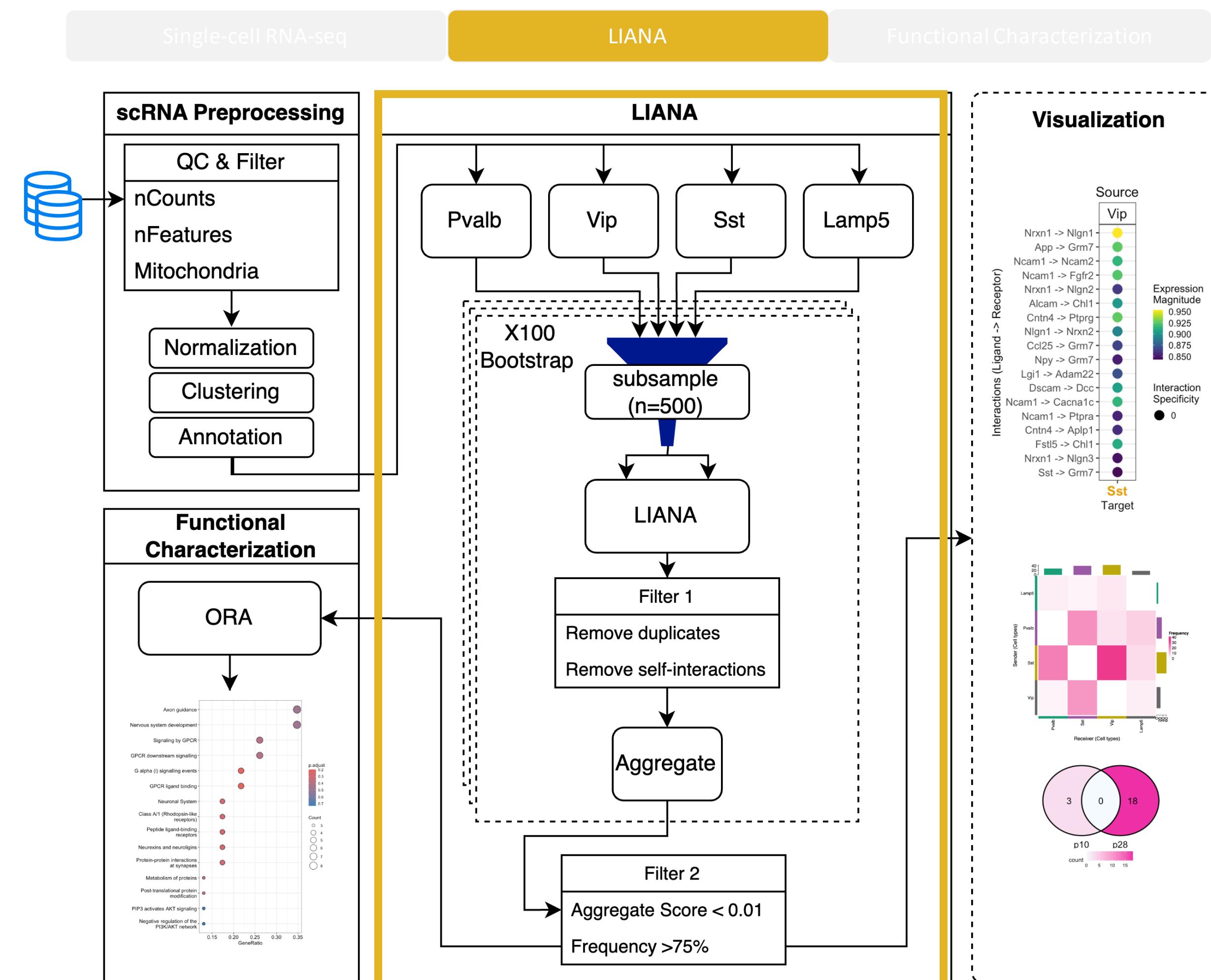


7

Cells Annotation

```
seurat_obj <- Azimuth::RunAzimuth(seurat_obj, reference = "mousecortexref")
```

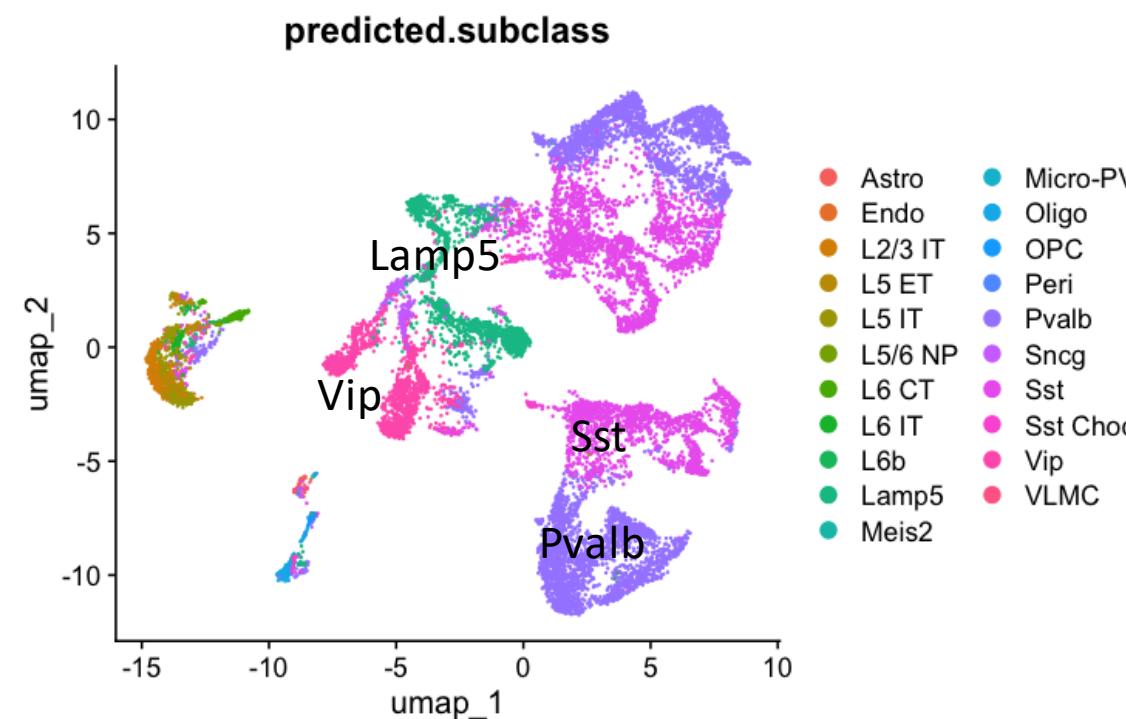




Single-cell RNA-seq

LIANA

Functional Characterization

INPUT to LIANA

orig.ident	nCount_RNA	nFeature_RNA	seurat_annotations
GSM5014306_P10_AAACCCAAGACGCCAA-1 SeuratProject	16777	4308	Pvalb
GSM5014306_P10_AAACCCAAGGCCTTGC-1 SeuratProject	6080	2634	Chodl
GSM5014306_P10_AAACCCACAGTGCCTG-1 SeuratProject	15234	4184	Sst
GSM5014306_P10_AAACCCATCAGCGCAC-1 SeuratProject	22537	5134	Sst
GSM5014306_P10_AAACCCATCTGCGGAC-1 SeuratProject	4147	2071	OPC
GSM5014306_P10_AAACGAAAGCTGACCC-1 SeuratProject	14185	4021	Sst

Seurat Object
Expression Data for Labelled cells

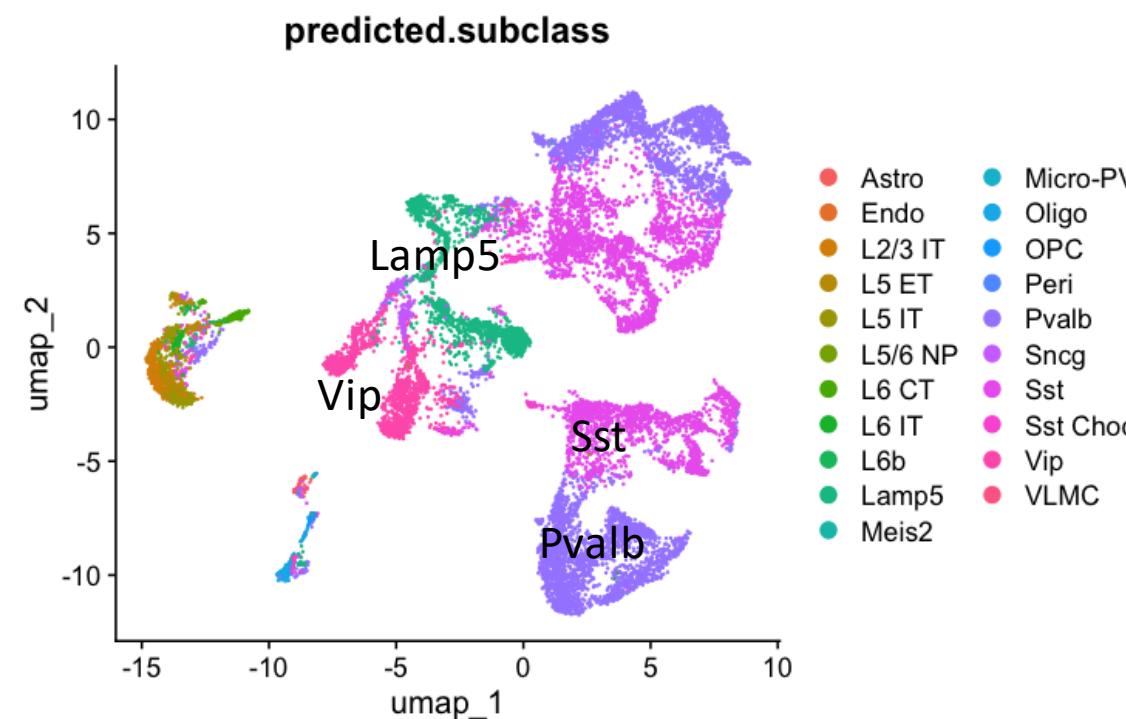
Liana functions:`liana_wrap()``liana_aggregate()`

Single-cell RNA-seq

LIANA

Functional Characterization

INPUT to LIANA



orig.ident	nCount_RNA	nFeature_RNA	seurat_annotations
GSM5014306_P10_AAACCCAAGACGCCAA-1 SeuratProject	16777	4308	Pvalb
GSM5014306_P10_AAACCCAAGGCCTTGC-1 SeuratProject	6080	2634	Chodl
GSM5014306_P10_AAACCCACAGTGCCTG-1 SeuratProject	15234	4184	Sst
GSM5014306_P10_AAACCCATCAGCGCAC-1 SeuratProject	22537	5134	Sst
GSM5014306_P10_AAACCCATCTGCGGAC-1 SeuratProject	4147	2071	OPC
GSM5014306_P10_AAACGAAAGCTGACCC-1 SeuratProject	14185	4021	Sst

Seurat Object
Expression Data for Labelled cells

Liana functions:
`liana_wrap()`
`liana_aggregate()`



In-house functions:
`SampleCells()`
`bootstrap_keep_dir()`
`bootstrap_results_agg()`
`filter_aggRank()`
`keep_specific_inter()`

Robust interactions

Single-cell RNA-seq

LIANA

Functional Characterization

We have
expression data
for our cells of
interest

Pvalb
2221

Vip
515

Sst
2501

Lamp5
647

1

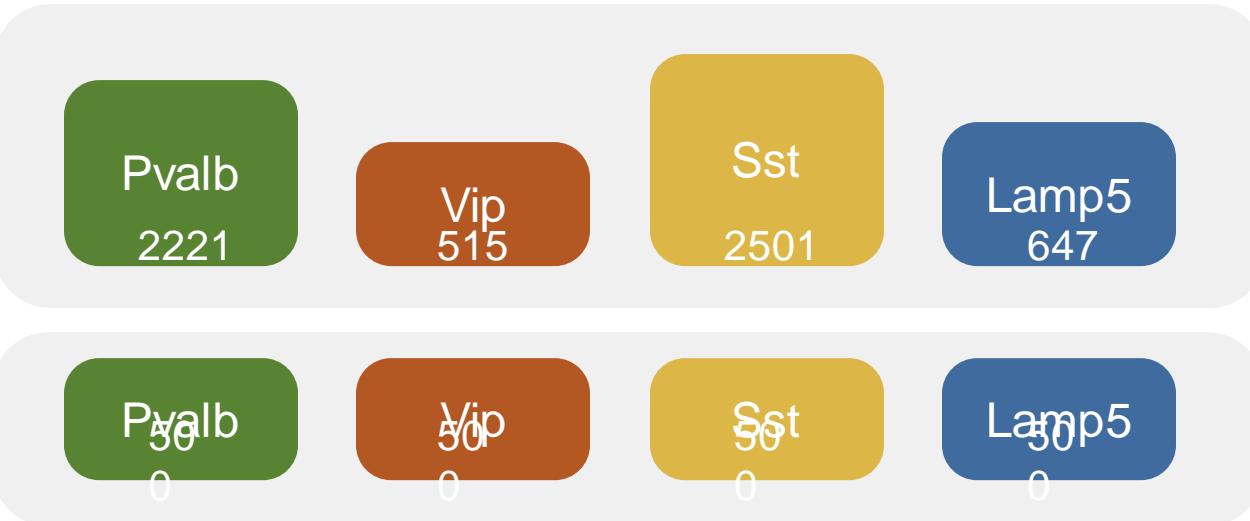
Bias: unequal cell
number in each
subtype

Single-cell RNA-seq

LIANA

Functional Characterization

We have
expression data
for our cells of
interest



1

Bias: unequal cell
number in each
subtype

Thus, **subsampling**

Single-cell RNA-seq

LIANA

Functional Characterization

We have
expression data
for our cells of
interest

Pvalb
2221

Vip
515

Sst
2501

Lamp5
647

Pvalb
0

Vip
0

Sst
0

Lamp5
0

Search in known
L-R databases and
get predictions for
3 methods used

LIANA

Cell_chat
Cellphone
Sca

Single-cell RNA-seq

LIANA

Functional Characterization

We have expression data for our cells of interest

Pvalb
2221

Vip
515

Sst
2501

Lamp5
647

Pvalb
0

Vip
0

Sst
0

Lamp5
0

Search in known L-R databases and get predictions for 3 methods used

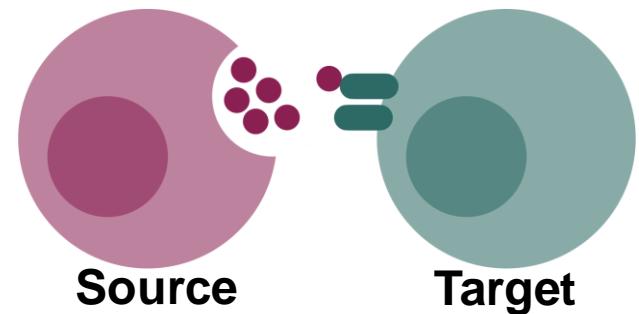
LIANA

Cell_chat
Cellphone
Sca

source	target	ligand	receptor	prob	pval
Lamp5	Lamp5	L1cam	Egfr	0.0134	0
Pvalb	Lamp5	L1cam	Egfr	0.0124	0
Sst	Lamp5	L1cam	Egfr	0.0283	0
Vip	Lamp5	L1cam	Egfr	0.0164	0

source	target	ligand	receptor	receptor.prop	ligand.p	ligand.e	receptor.expr	Irs.to.kep	lr.mean	pvalue
Lamp5	Lamp5	Adam10	Tspan12	0.188	0.722	0.681	0.0827	TRUE	0.382	0
Lamp5	Lamp5	Adam10	Met	0.174	0.722	0.681	0.0492	TRUE	0.365	0
Lamp5	Lamp5	Adam10	Epha3	0.978	0.722	0.681	1.29	TRUE	0.985	0.716
Lamp5	Lamp5	Adam10	Nrcam	0.922	0.722	0.681	0.0632	TRUE	0.372	0

source	target	ligand	receptor	receptor.prop	ligand.prop	ligand.expr	receptor.expr	global_mean	Irs.to.kEEP	LRscore
Lamp5	Lamp5	Adam10	Tspan12	0.188	0.722	0.681	0.0827	0.145	TRUE	0.621
Lamp5	Lamp5	Adam10	Met	0.174	0.722	0.681	0.0492	0.145	TRUE	0.558
Lamp5	Lamp5	Adam10	Epha3	0.978	0.722	0.681	1.29	0.145	TRUE	0.866
Lamp5	Lamp5	Adam10	Nrcam	0.922	0.722	0.681	0.0632	0.145	TRUE	0.589



Single-cell RNA-seq

LIANA

Functional Characterization

We have expression data for our cells of interest

Pvalb
2221

Vip
515

Sst
2501

Lamp5
647

Pvalb
0

Vip
0

Sst
0

Lamp5
0

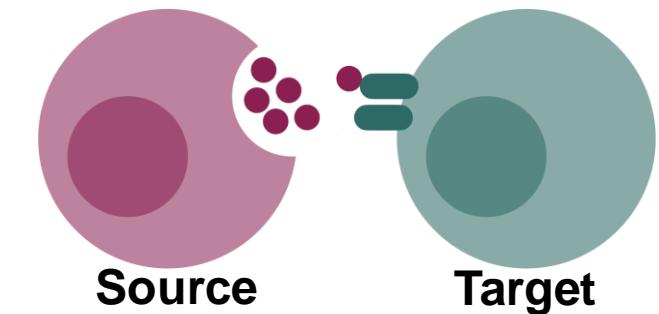
Search in known L-R databases and get predictions for 3 methods used

Cell_chat

Cellphone

Sca

LIANA



Source

Target

source	target	ligand	receptor	prob	pval
Lamp5	Lamp5	L1cam	Egfr	0.0134	0
Pvalb	Lamp5	L1cam	Egfr	0.0124	0
Sst	Lamp5	L1cam	Egfr	0.0283	0
Vip	Lamp5	L1cam	Egfr	0.0164	0

source	target	ligand	receptor	receptor.prop	ligand.p	ligand.e	receptor.expr	Irs.to.kep	lr.mean	pvalue
Lamp5	Lamp5	Adam10	Tspan12	0.188	0.722	0.681	0.0827	TRUE	0.382	0
Lamp5	Lamp5	Adam10	Met	0.174	0.722	0.681	0.0492	TRUE	0.365	0
Lamp5	Lamp5	Adam10	Epha3	0.978	0.722	0.681	1.29	TRUE	0.985	0.716
Lamp5	Lamp5	Adam10	Nrcam	0.922	0.722	0.681	0.0632	TRUE	0.372	0

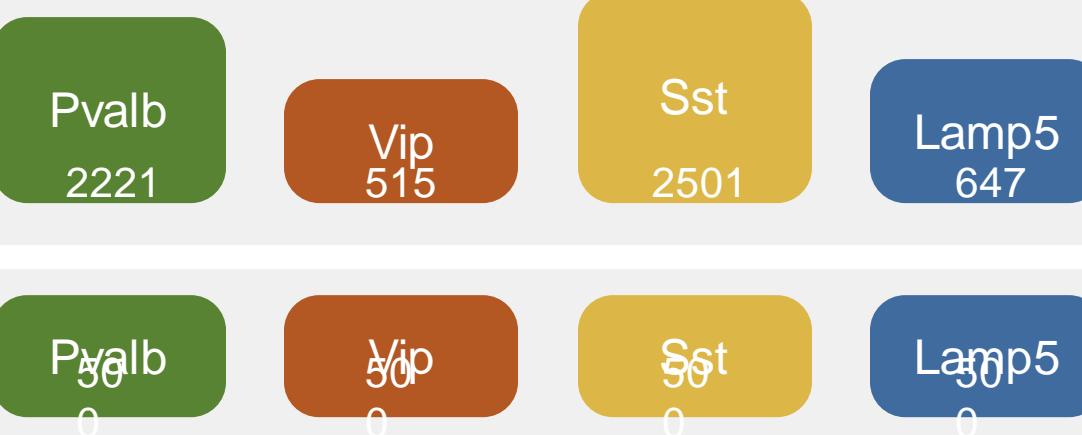
source	target	ligand	receptor	receptor.prop	ligand.prop	ligand.expr	receptor.expr	global_mean	Irs.to.k	LRscore
Lamp5	Lamp5	Adam10	Tspan12	0.188	0.722	0.681	0.0827	0.145	TRUE	0.621
Lamp5	Lamp5	Adam10	Met	0.174	0.722	0.681	0.0492	0.145	TRUE	0.558
Lamp5	Lamp5	Adam10	Epha3	0.978	0.722	0.681	1.29	0.145	TRUE	0.866
Lamp5	Lamp5	Adam10	Nrcam	0.922	0.722	0.681	0.0632	0.145	TRUE	0.589

Single-cell RNA-seq

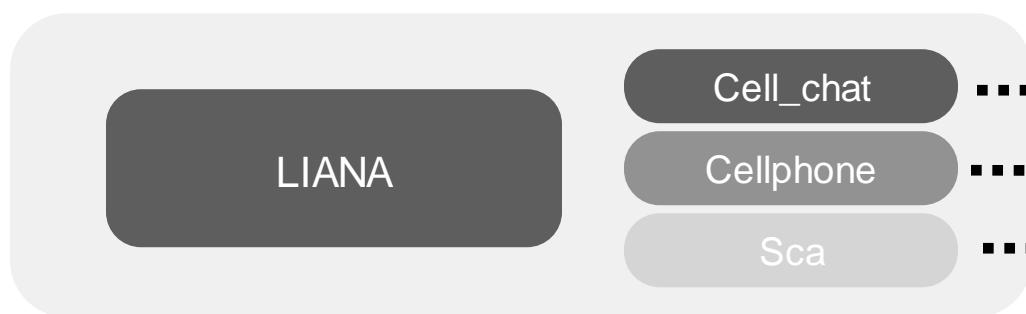
LIANA

Functional Characterization

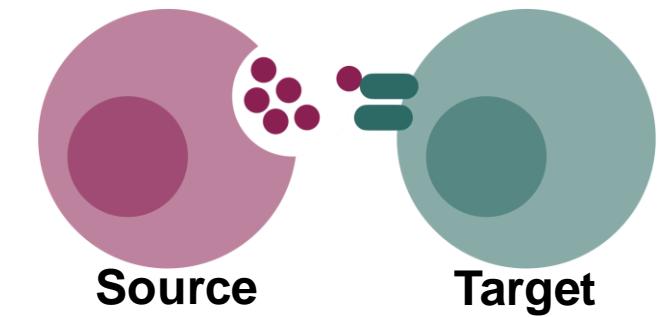
We have expression data for our cells of interest



Search in known L-R databases and get predictions for 3 methods used



source	target	ligand	receptor	prob	pval
Lamp5	Lamp5	L1cam	Egfr	0.0134	0
Pvalb	Lamp5	L1cam	Egfr	0.0124	0
Sst	Lamp5	L1cam	Egfr	0.0283	0
Vip	Lamp5	L1cam	Egfr	0.0164	0



source	target	ligand	receptor	receptor.prop	ligand.p	ligand.e	receptor.expr	Irs.to.keep	lr.mean	pvalue
Lamp5	Lamp5	Adam10	Tspan12	0.188	0.722	0.681	0.0827	TRUE	0.382	0
Lamp5	Lamp5	Adam10	Met	0.174	0.722	0.681	0.0492	TRUE	0.365	0
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Lamp5	Lamp5	Adam10	Nrcam	0.922	0.722	0.681	0.0632	TRUE	0.372	0

source	target	ligand	receptor	receptor.prop	ligand.prop	ligand.expr	receptor.expr	global_mean	Irs.to.keep	LRscore
Lamp5	Lamp5	Adam10	Tspan12	0.188	0.722	0.681	0.0827	0.145	TRUE	0.621
Lamp5	Lamp5	Adam10	Met	0.174	0.722	0.681	0.0492	0.145	TRUE	0.558
Lamp5	Lamp5	Adam10	Epha3	0.978	0.722	0.681	1.29	0.145	TRUE	0.866
Lamp5	Lamp5	Adam10	Nrcam	0.922	0.722	0.681	0.0632	0.145	TRUE	0.589

Single-cell RNA-seq

LIANA

Functional Characterization

We have
expression data
for our cells of
interest

Pvalb
2221

Vip
515

Sst
2501

Lamp5
647

Pvalb
0

Vip
0

Sst
0

Lamp5
0

Search in known
L-R databases and
get predictions for
3 methods used

LIANA

Cell_chat
Cellphone
Sca

LIANA aggregate

Single-cell RNA-seq

LIANA

Functional Characterization

We have expression data for our cells of interest

Pvalb
2221

Vip
515

Sst
2501

Lamp5
647

Pvalb
0

Vip
0

Sst
0

Lamp5
0

Search in known L-R databases and get predictions for 3 methods used

LIANA

Cell_chat
Cellphone
Sca

LIANA aggregate

Aggregate score that considers 3 methods

Cell_chat
Cellphone
Sca

source	target	ligand	receptor	aggregate_rank	mean_rank	call_ce_llchat.p_val	call_ce_llchat.rank	cellphonedb.p_value	cellphonedb.rank	sca.LR score	sca.rank
Lamp5	Sst	L1cam	Cntn1	0.0002	86.66667	0.43	17	0	122	0.853760711	121
Lamp5	Sst	Jam3	Itgb1	0.0038	153.8333	0	6.5	0	122	0.692454958	333
Lamp5	Sst	Vegfc	Itgb1	0.0042	157.5	0	6.5	0	122	0.685291037	344
Lamp5	Pvalb	L1cam	Cntn1	0.0019	139.8333	0.63	20	0.007	263.5	0.845365391	136
Lamp5	Pvalb	Lamc1	Itga9	0.0027	141.1667	0	9	0	117.5	0.718608224	297
Lamp5	Pvalb	Vegfc	Itgb1	0.0034	149.1667	0	9	0	117.5	0.698217147	321
Lamp5	Pvalb	Jam3	Itgb1	0.0036	151.8333	0	9	0	117.5	0.692983726	329
Lamp5	Pvalb	Vegfc	Itga9	0.0064	174.8333	0	9	0	117.5	0.645009063	398

Single-cell RNA-seq

LIANA

Functional Characterization

We have
expression data
for our cells of
interest

Pvalb
2221

Vip
515

Sst
2501

Lamp5
647

Pvalb
0

Vip
0

Sst
0

Lamp5
0

Search in known
L-R databases and
get predictions for
3 methods used

LIANA

Cell_chat
Cellphone
Sca

LIANA aggregate

Filter

Agg score < 0.01
Self interactions

Single-cell RNA-seq

LIANA

Functional Characterization

We have expression data for our cells of interest

Pvalb 2221
Vip 515
Sst 2501
Lamp5 647

Pvalb 0
Vip 0
Sst 0
Lamp5 0

Search in known L-R databases and get predictions for 3 methods used

LIANA

Cell_chat
Cellphone
Sca

LIANA aggregate

Filter

Agg score < 0.01
Self interactions

source	target	ligand	receptor	aggregate_rank	mean_rank	call_ce_llchat.p_val	call_ce_llchat.rank	cellpho_nedb.p_value	cellpho_nedb.rank	sca.LR_score	sca.rank
Lamp5	Sst	L1cam	Cntn1	0.0002	86.66667	0.43	17	0	122	0.853760711	121
Lamp5	Sst	Jam3	Itgb1	0.0038	153.8333	0	6.5	0	122	0.692454958	333
Lamp5	Sst	Vegfc	Itgb1	0.0042	157.5	0	6.5	0	122	0.685291037	344
Lamp5	Pvalb	L1cam	Cntn1	0.0019	139.8333	0.63	20	0.007	263.5	0.845365391	136
Lamp5	Pvalb	Lamc1	Itga9	0.0027	141.1667	0	9	0	117.5	0.718608224	297
Lamp5	Pvalb	Vegfc	Itgb1	0.0034	149.1667	0	9	0	117.5	0.698217147	321
Lamp5	Pvalb	Jam3	Itgb1	0.0036	151.8333	0	9	0	117.5	0.692983726	329
Lamp5	Pvalb	Vegfc	Itga9	0.0064	174.8333	0	9	0	117.5	0.645009063	398

2

Robust interactions?

✓ Filter by agg score < 0.01

Single-cell RNA-seq

LIANA

Functional Characterization

We have expression data for our cells of interest

Pvalb 2221
Vip 515
Sst 2501
Lamp5 647

Pvalb 0
Vip 0
Sst 0
Lamp5 0

Search in known L-R databases and get predictions for 3 methods used

LIANA

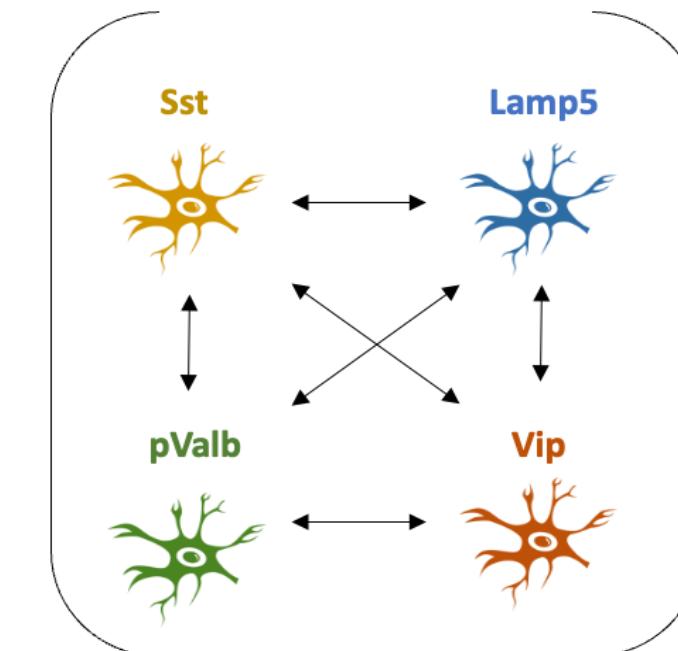
Cell_chat
Cellphone
Sca

LIANA aggregate

Filter

Agg score < 0.01
Self interactions

source	target	ligand	receptor	aggregate_rank	mean_rank	call_ce_llchat.p_val	call_ce_llchat.rank	cellpho_nedb.p_value	cellpho_nedb.rank	sca.LR_score	sca.rank
Lamp5	Sst	L1cam	Cntn1	0.0002	86.66667	0.43	17	0	122	0.853760711	121
Lamp5	Sst	Jam3	Itgb1	0.0038	153.8333	0	6.5	0	122	0.692454958	333
Lamp5	Sst	Vegfc	Itgb1	0.0042	157.5	0	6.5	0	122	0.685291037	344
Lamp5	Pvalb	L1cam	Cntn1	0.0019	139.8333	0.63	20	0.007	263.5	0.845365391	136
Lamp5	Pvalb	Lamc1	Itga9	0.0027	141.1667	0	9	0	117.5	0.718608224	297
Lamp5	Pvalb	Vegfc	Itgb1	0.0034	149.1667	0	9	0	117.5	0.698217147	321
Lamp5	Pvalb	Jam3	Itgb1	0.0036	151.8333	0	9	0	117.5	0.692983726	329
Lamp5	Pvalb	Vegfc	Itga9	0.0064	174.8333	0	9	0	117.5	0.645009063	398



Able to differentiate direction
Not interested in self interactions

Single-cell RNA-seq

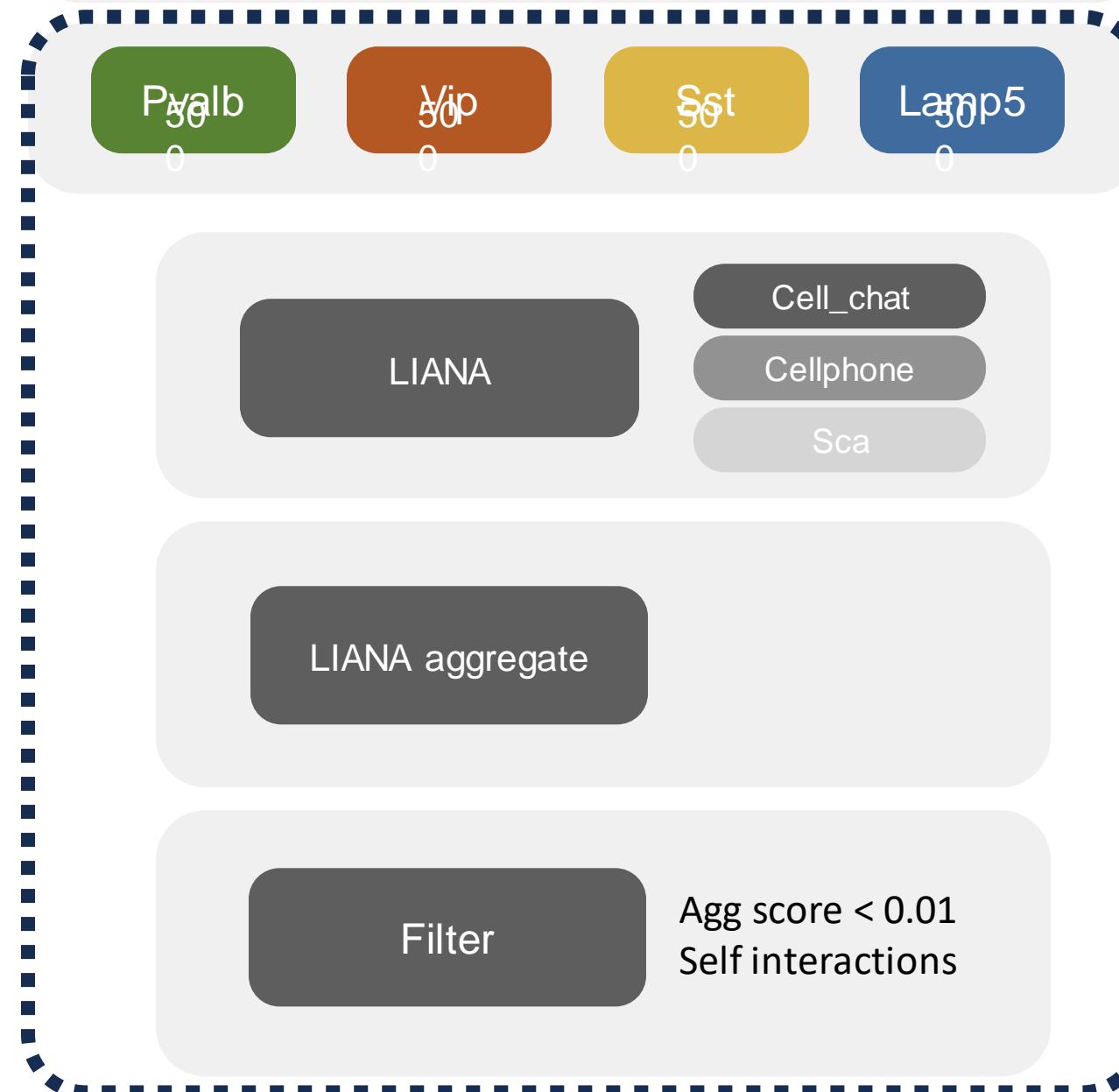
LIANA

Functional Characterization

We have expression data for our cells of interest

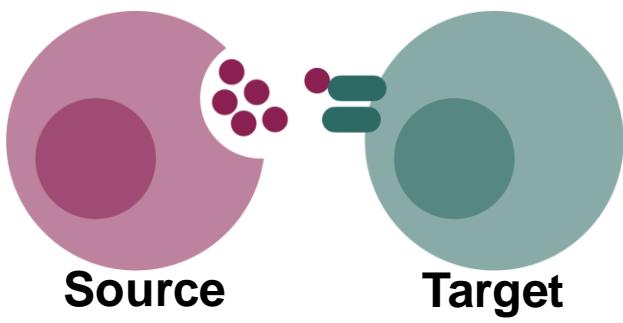


Search in known L-R databases and get predictions for 3 methods used



Dataframe with L-R interactions

Filter by agg score < 0.01



Single-cell RNA-seq

LIANA

Functional Characterization

We have expression data for our cells of interest

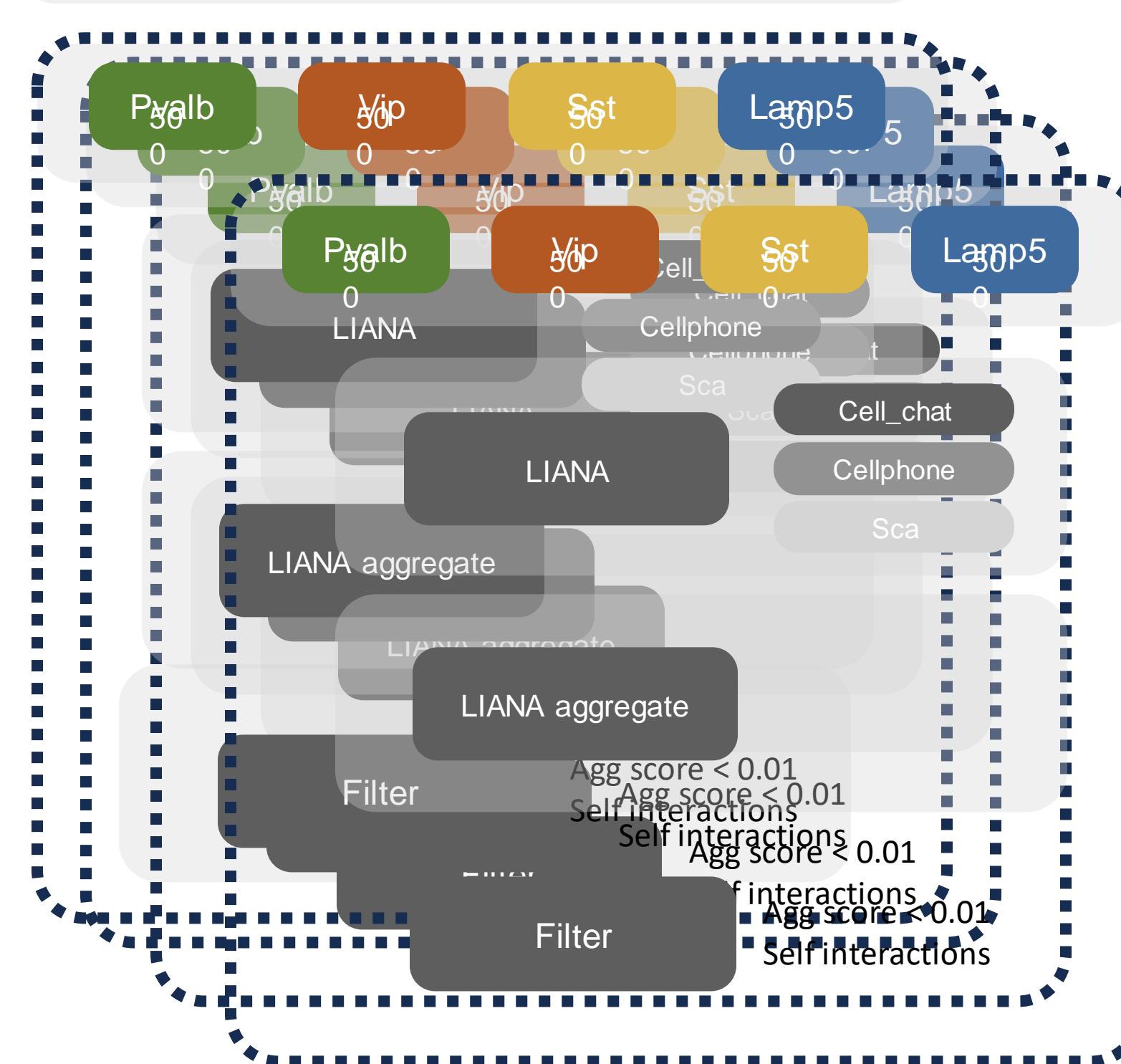
Pvalb
2221

Vip
515

Sst
2501

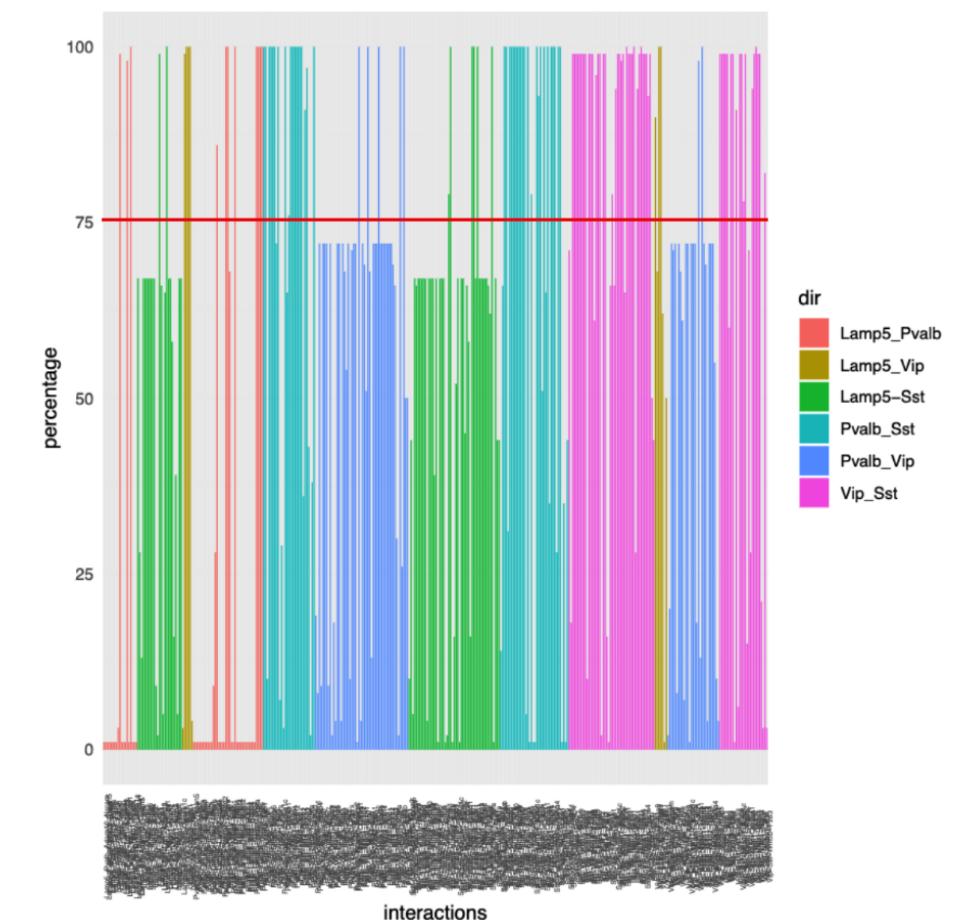
Lamp5
647

Search in known L-R databases and get predictions for 3 methods used



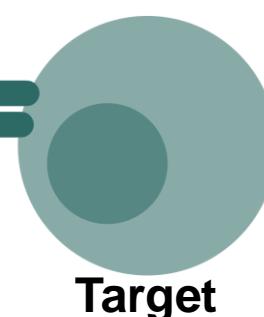
100 Dataframes with L-R interactions

Keep only the ones that appeared 75/100 bootstraps

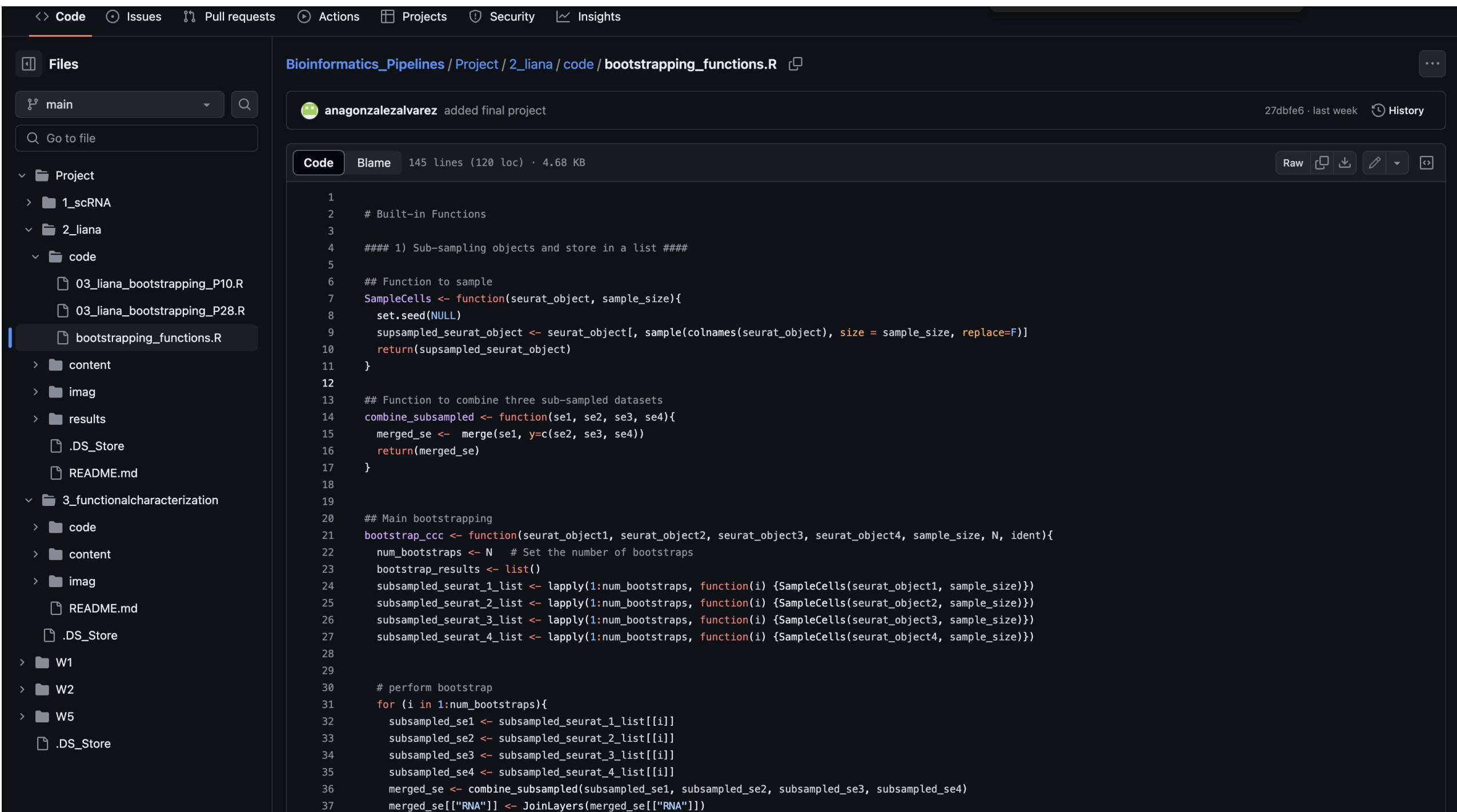


Robust interactions?

- ✓ Filter by agg score < 0.01
- ✓ Filter by %



Bootstrapping approach:



```

1  # Built-in Functions
2
3  ##### 1) Sub-sampling objects and store in a list #####
4
5
6  ## Function to sample
7  SampleCells <- function(seurat_object, sample_size){
8    set.seed(NULL)
9    subsampled_seurat_object <- seurat_object[, sample(colnames(seurat_object), size = sample_size, replace=F)]
10   return(subsampled_seurat_object)
11 }
12
13 ## Function to combine three sub-sampled datasets
14 combine_subsampled <- function(se1, se2, se3, se4){
15   merged_se <- merge(se1, y=c(se2, se3, se4))
16   return(merged_se)
17 }
18
19
20 ## Main bootstrapping
21 bootstrap_ccc <- function(seurat_object1, seurat_object2, seurat_object3, seurat_object4, sample_size, N, ident){
22   num_bootstraps <- N # Set the number of bootstraps
23   bootstrap_results <- list()
24   subsampled_seurat_1_list <- lapply(1:num_bootstraps, function(i) {SampleCells(seurat_object1, sample_size)})
25   subsampled_seurat_2_list <- lapply(1:num_bootstraps, function(i) {SampleCells(seurat_object2, sample_size)})
26   subsampled_seurat_3_list <- lapply(1:num_bootstraps, function(i) {SampleCells(seurat_object3, sample_size)})
27   subsampled_seurat_4_list <- lapply(1:num_bootstraps, function(i) {SampleCells(seurat_object4, sample_size)})
28
29
30   # perform bootstrap
31   for (i in 1:num_bootstraps){
32     subsampled_se1 <- subsampled_seurat_1_list[[i]]
33     subsampled_se2 <- subsampled_seurat_2_list[[i]]
34     subsampled_se3 <- subsampled_seurat_3_list[[i]]
35     subsampled_se4 <- subsampled_seurat_4_list[[i]]
36     merged_se <- combine_subsampled(subsampled_se1, subsampled_se2, subsampled_se3, subsampled_se4)
37     merged_se[["RNA"]] <- JoinLayers(merged_se[["RNA"]])
}

```

Liana functions:

`liana_wrap()`

`liana_aggregate()`

In-house functions:

`SampleCells()`

`bootstrap_keep_dir()`

`bootstrap_results_agg()`

`filter_aggRank()`

`keep_specific_inter()`

...

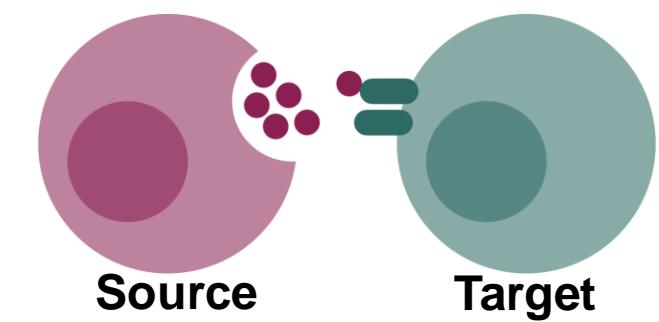
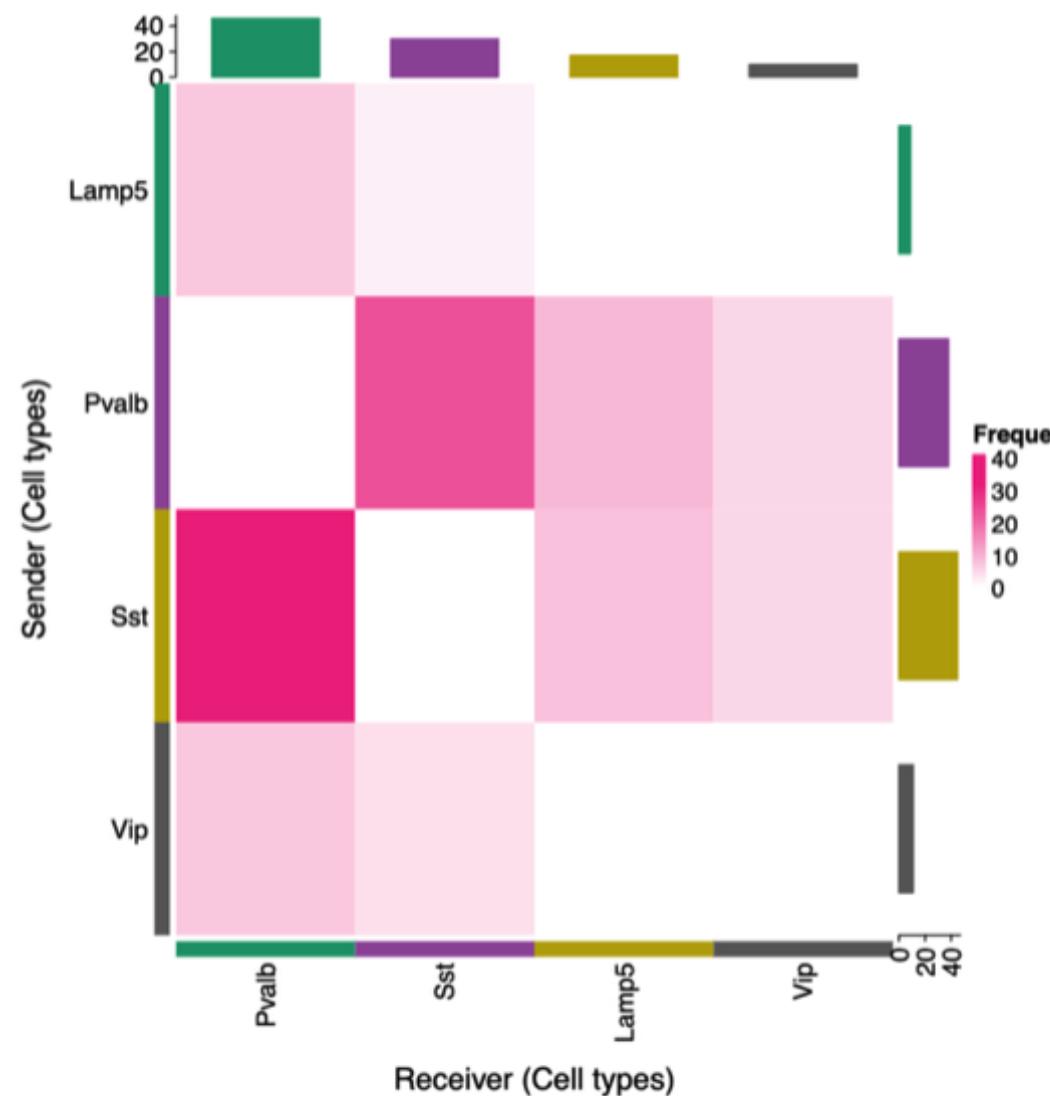
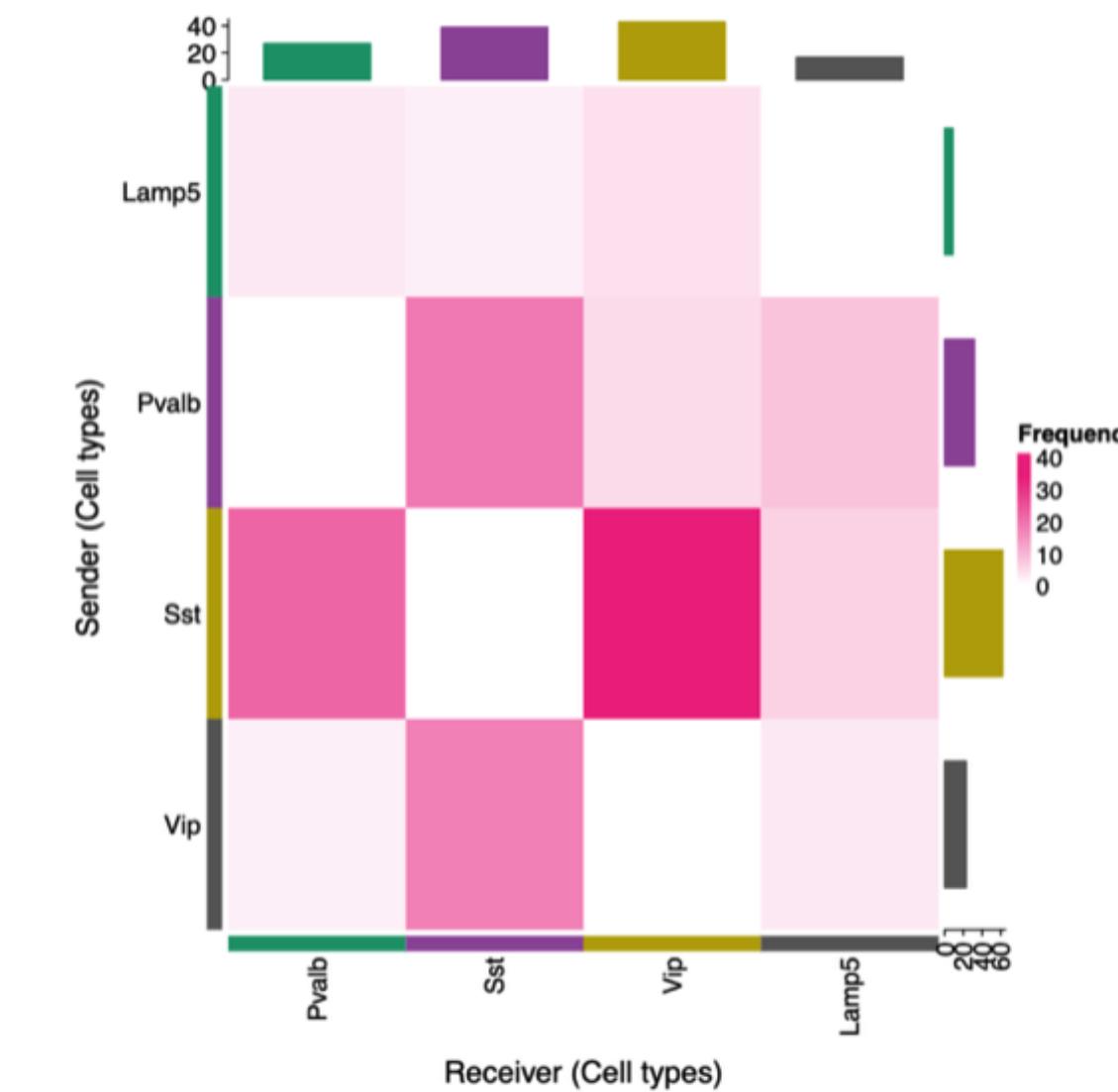
✓ **Handling multiple tibbles from the bootstrapping**

Available at: https://github.com/anagonzalezalvarez/Bioinformatics_Pipelines/blob/main/Project/2_liana/code/bootstrapping_functions.R

Single-cell RNA-seq

LIANA

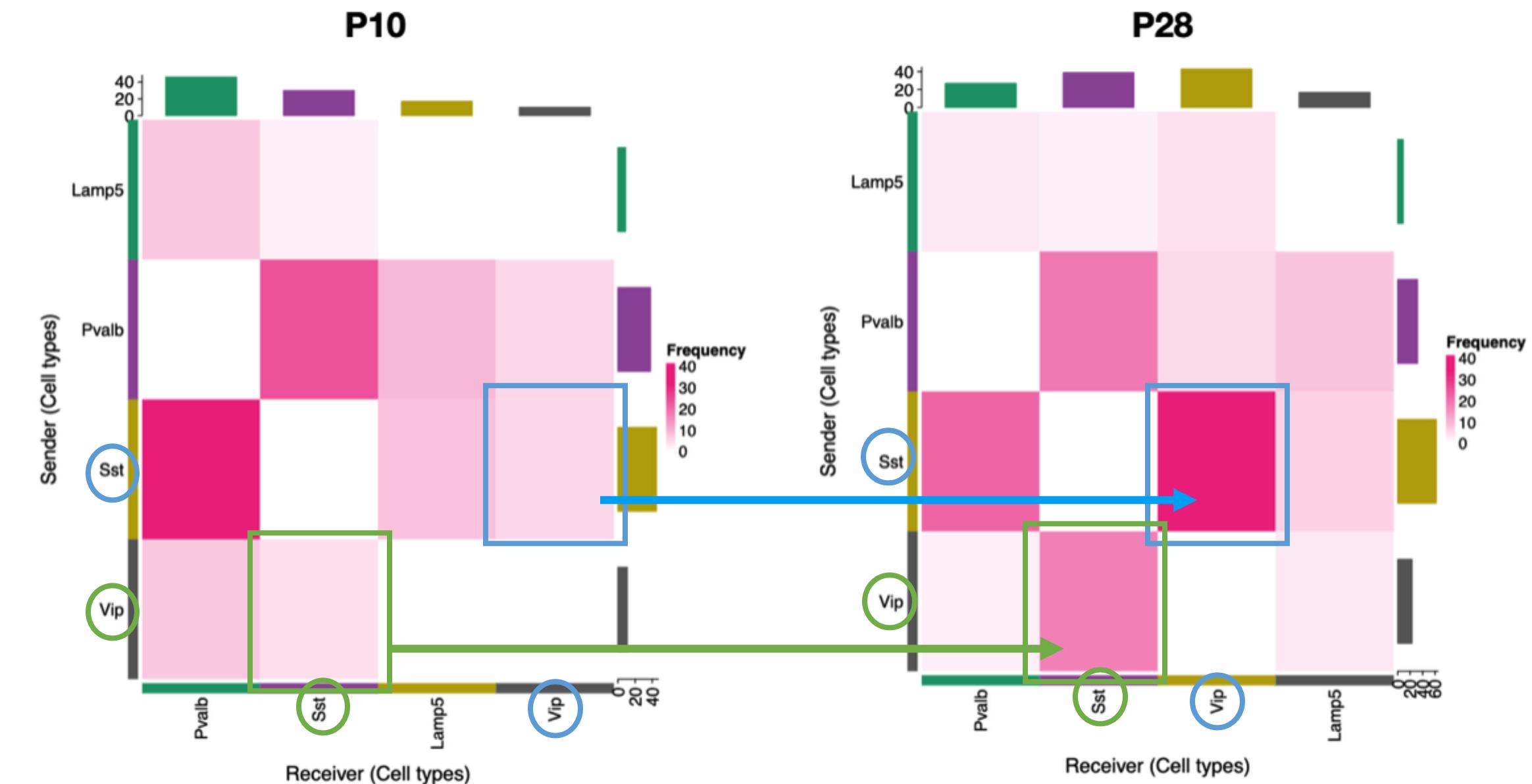
Functional Characterization

**P10****P28**

Interactions between Inhibitory GABAergic Cells

Two prevalent interactions by frequency of occurrence:

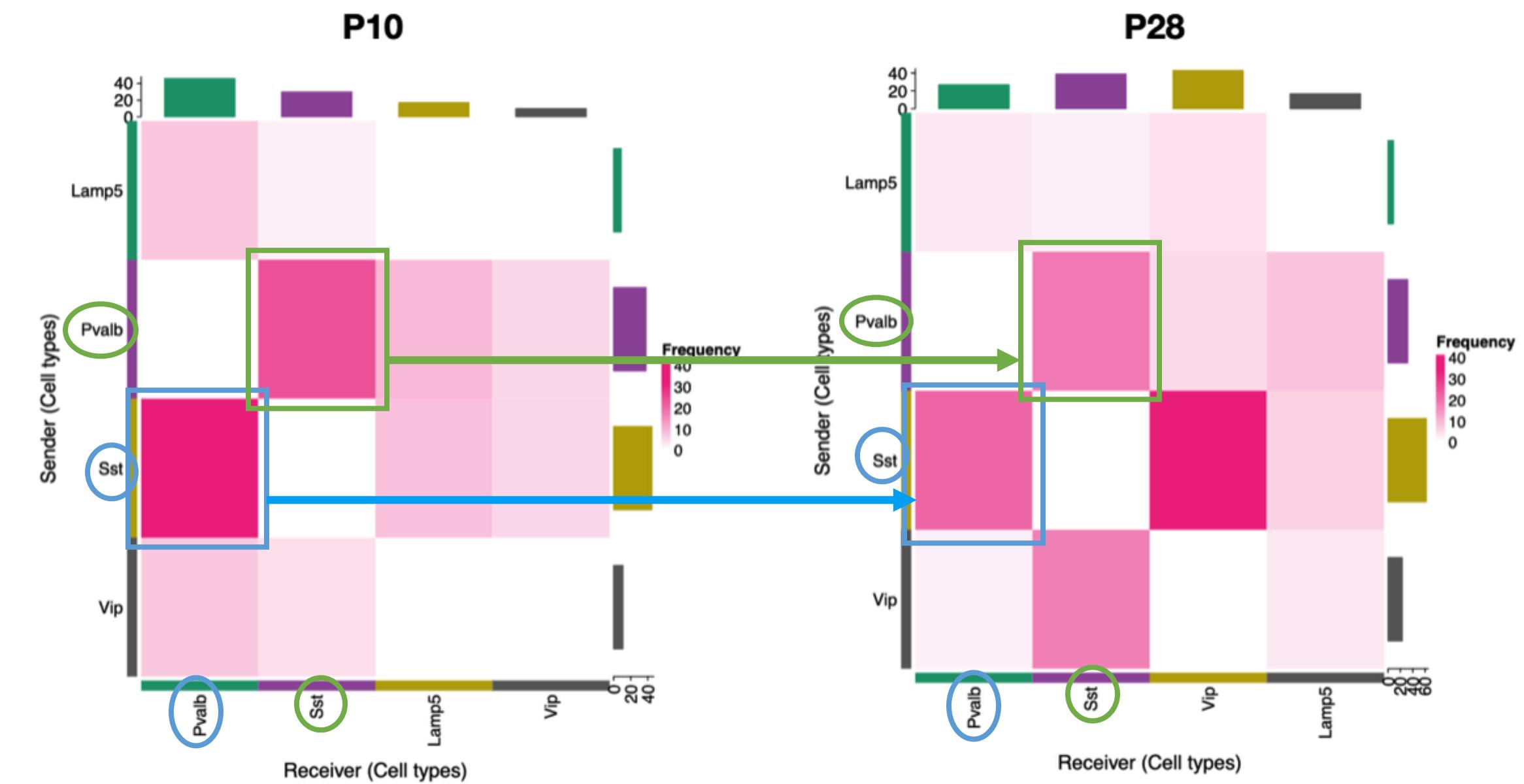
- $Sst \leftrightarrow Vip$



Interactions between Inhibitory GABAergic Cells

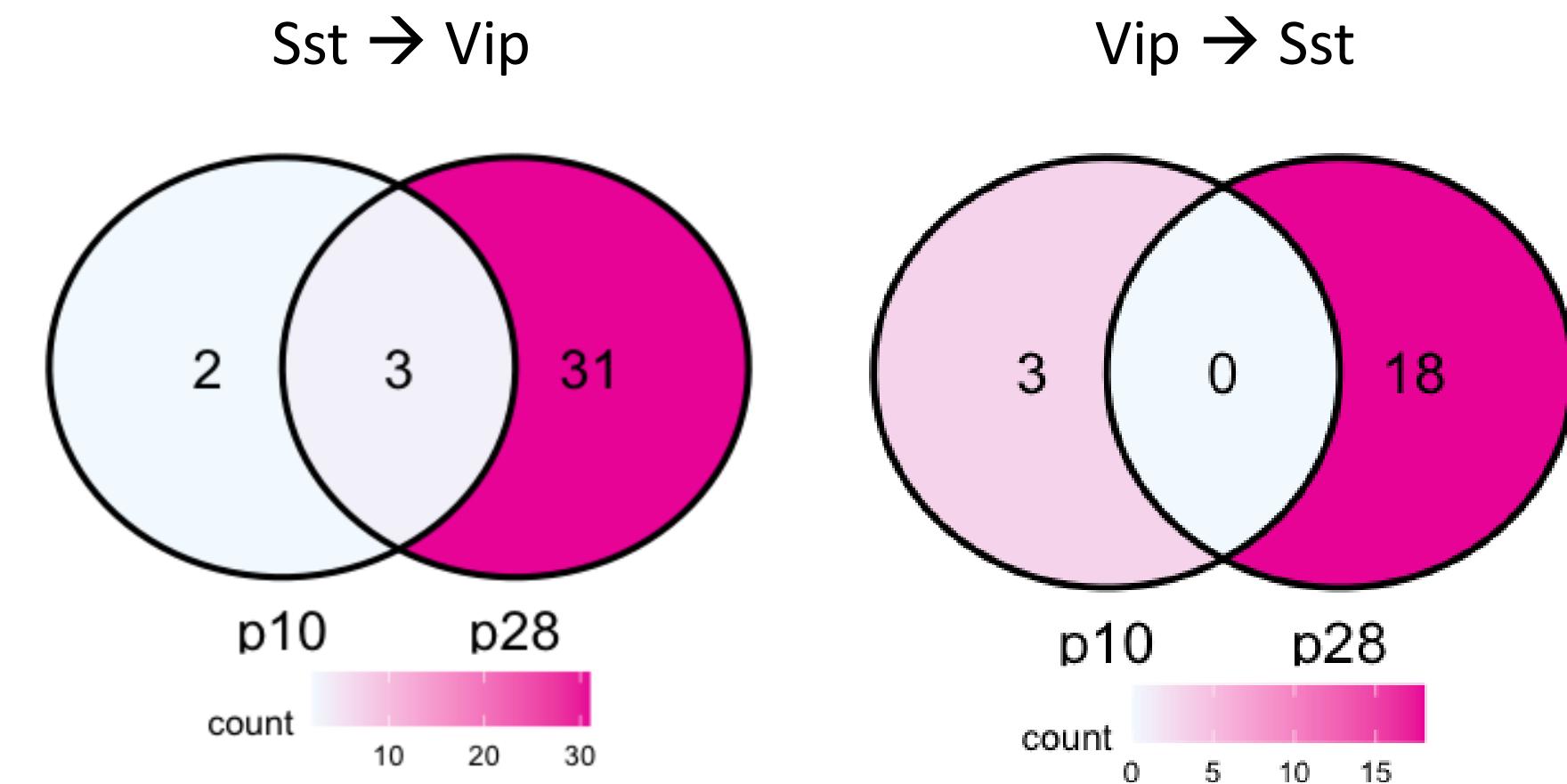
Two prevalent interactions by frequency of occurrence:

- $Sst \leftrightarrow Vip$
- $Sst \leftrightarrow Pvalb$



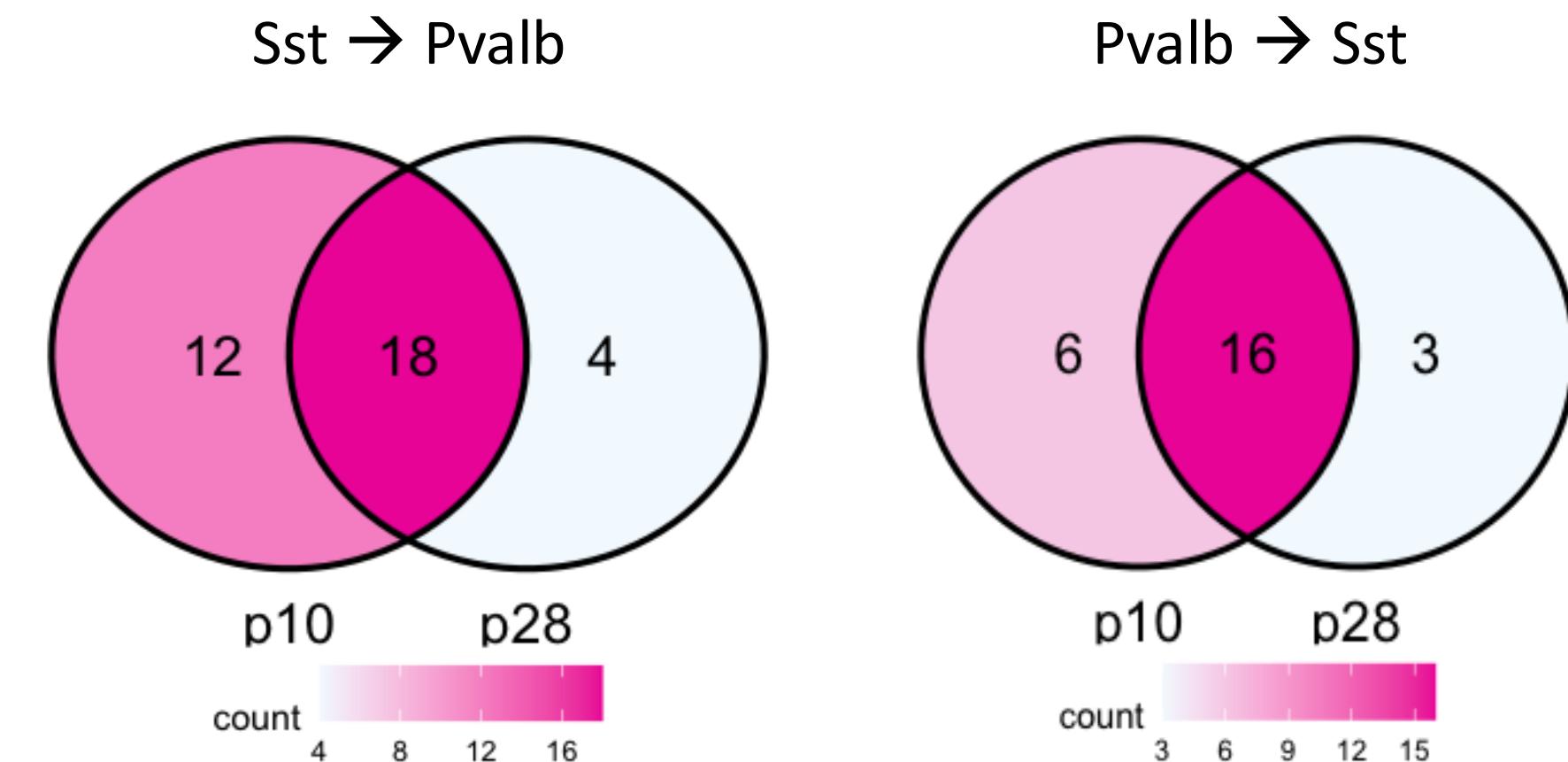
Interactions between Inhibitory GABAergic Cells

- Interactions between Sst and Vip cells increase at later stages (P28).

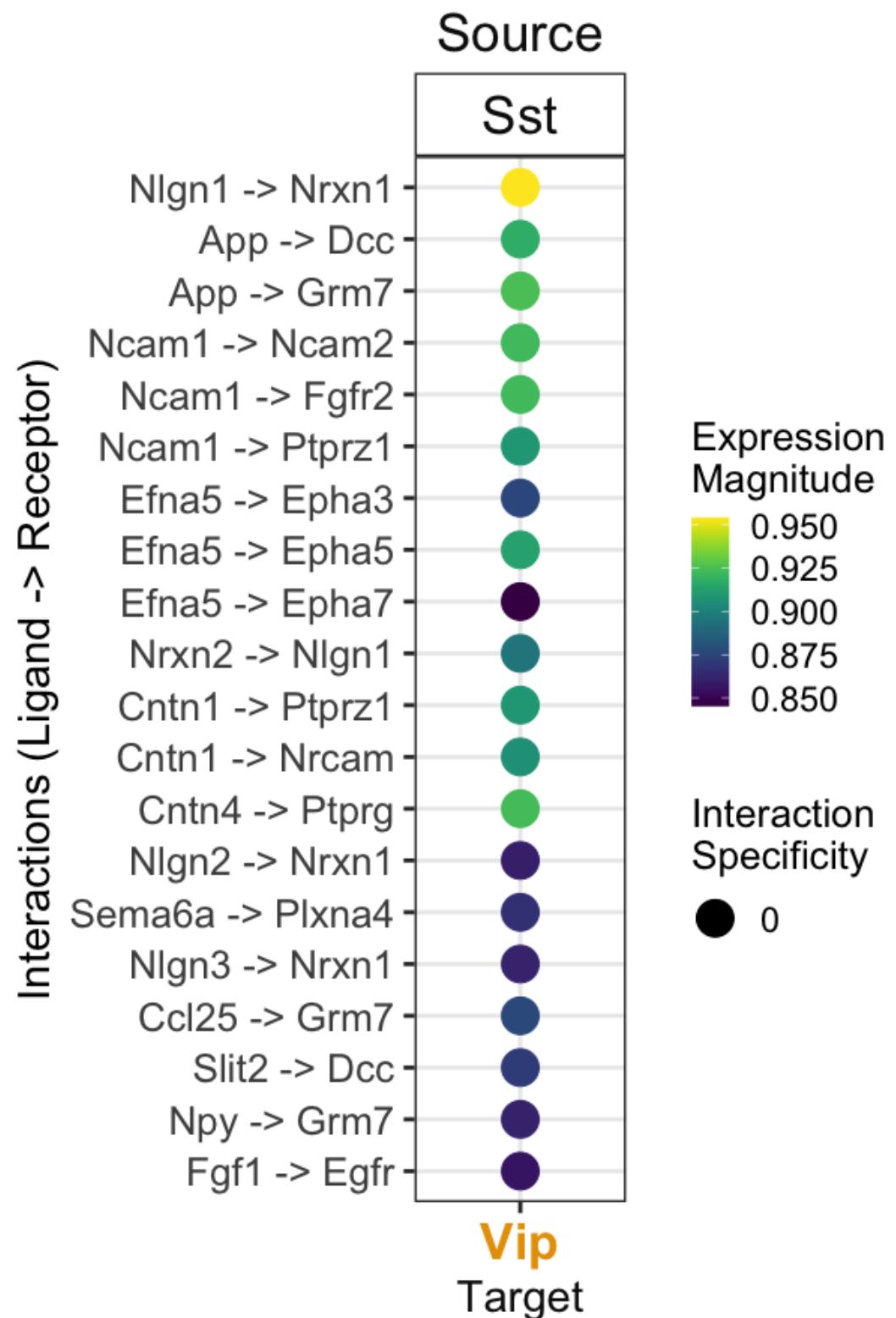


Interactions between Inhibitory GABAergic Cells

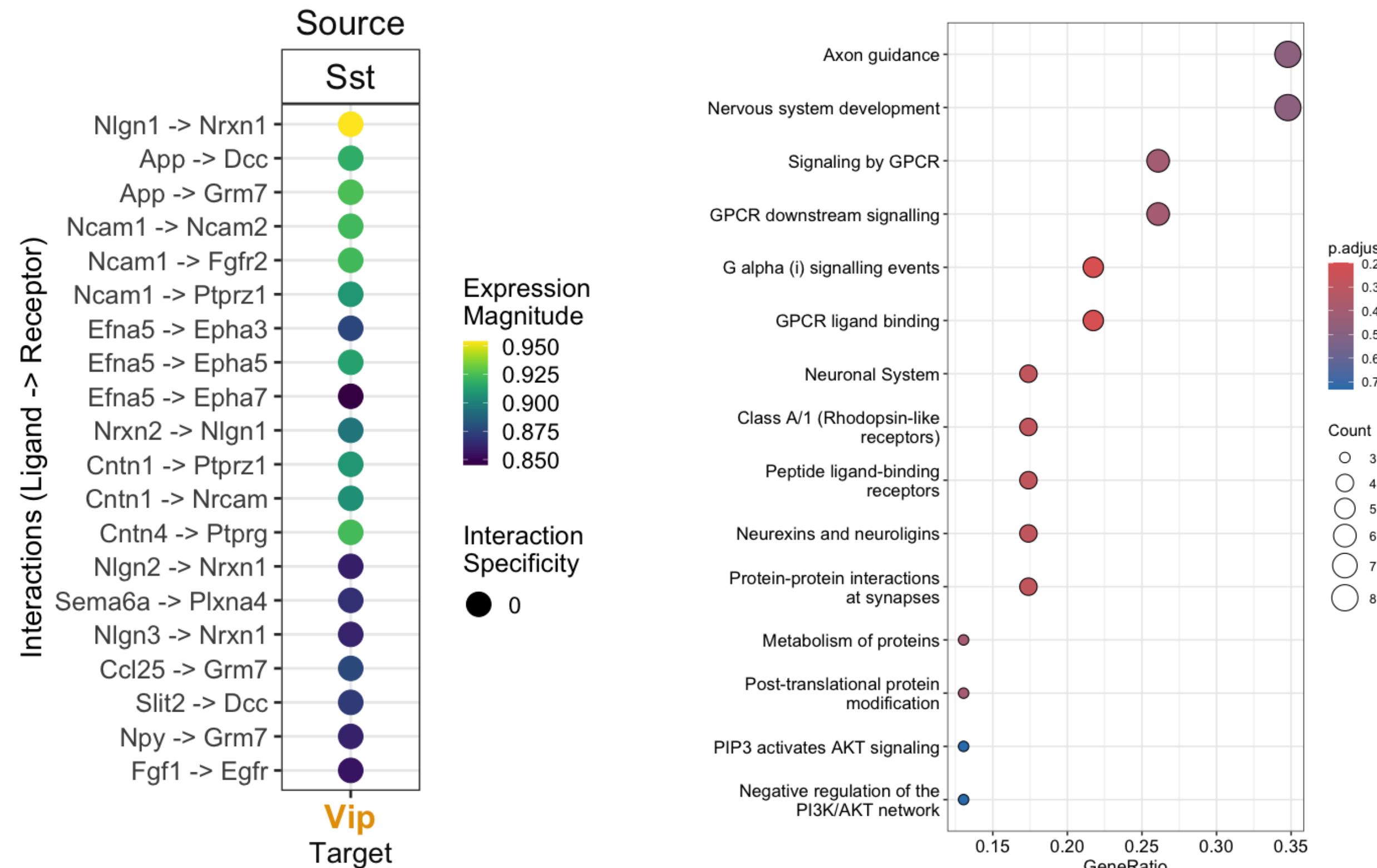
- Prevalence of *Sst* and *Pvalb* cells in early development reflected by higher frequency of interactions.
- Interactions between *Sst* and *Pvalb* cells persist but decrease slightly.



Functional Characterization

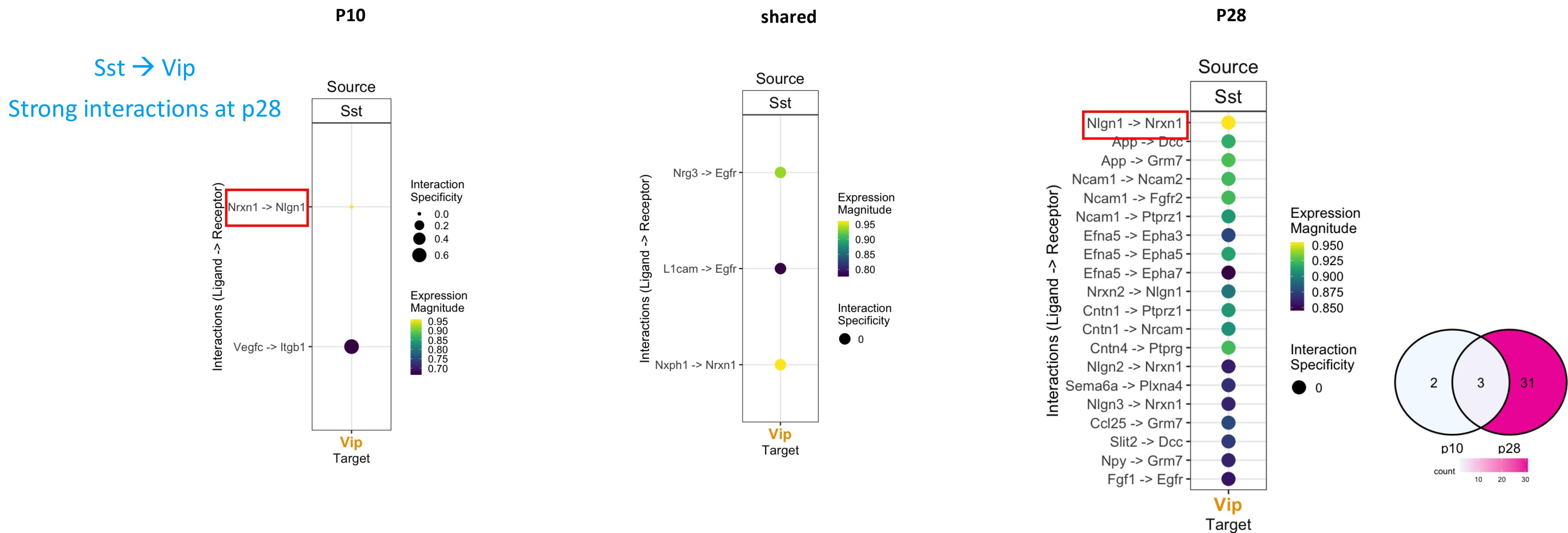


Functional Characterization



Neurexin and Neuroligin L-R

Neurexin (Nrnxn1) and Neuroligin (Nlgn1) interactions are observed between *Sst* → *Vip* cells at both stages P10 and P28 – but reversed.



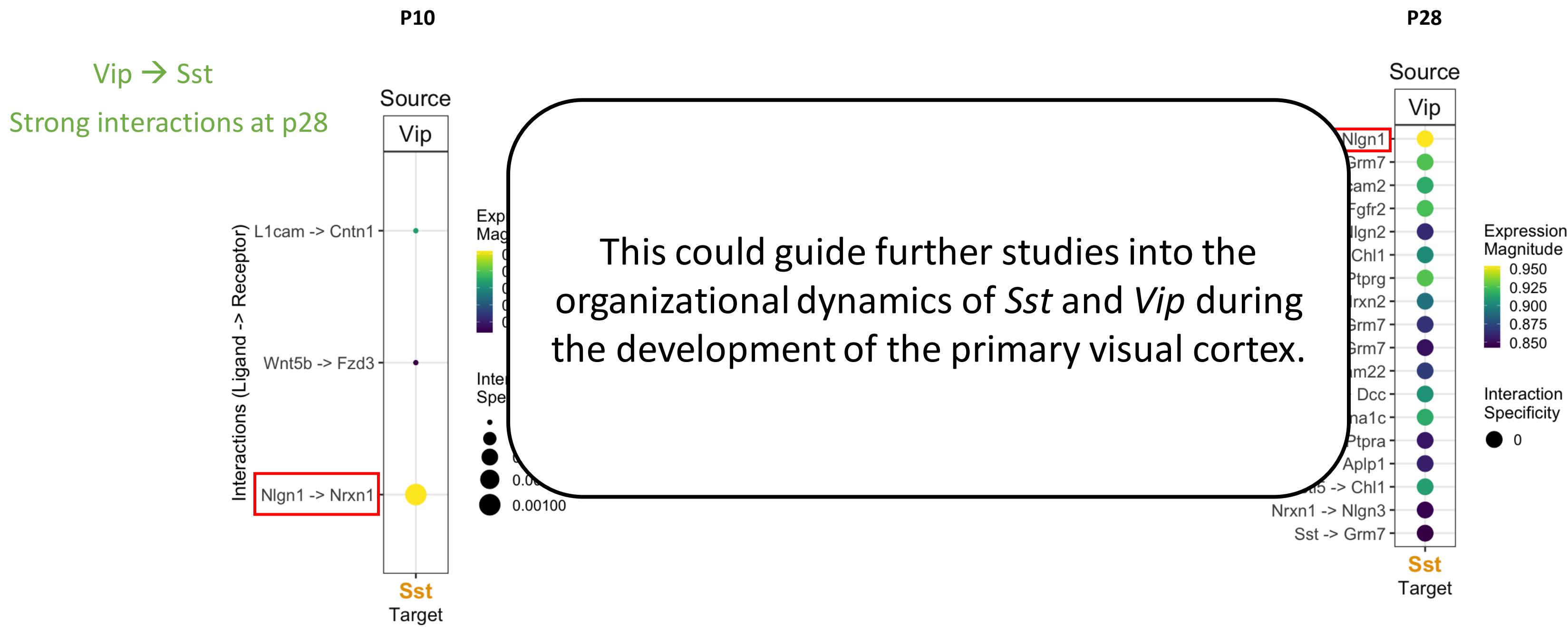
Neurexin and Neuroligin L-R

Neurexin (Nrnxn1) and Neuroligin (Nlgn1) signaling is bidirectional, mirror is observed in *Vip* → *Sst*.



Neurexin and Neuroligin L-R

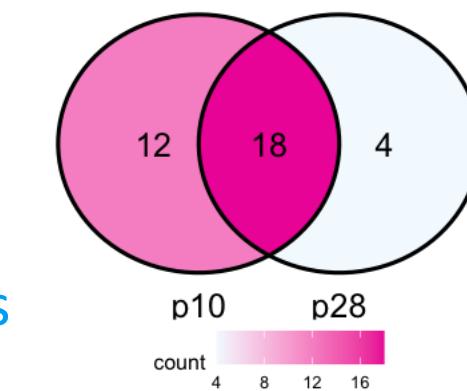
Neurexin (Nrnxn1) and Neuroligin (Nlgn1) signaling is bidirectional, mirror is observed in *Vip* → *Sst*.



Direction-Specific Functionality

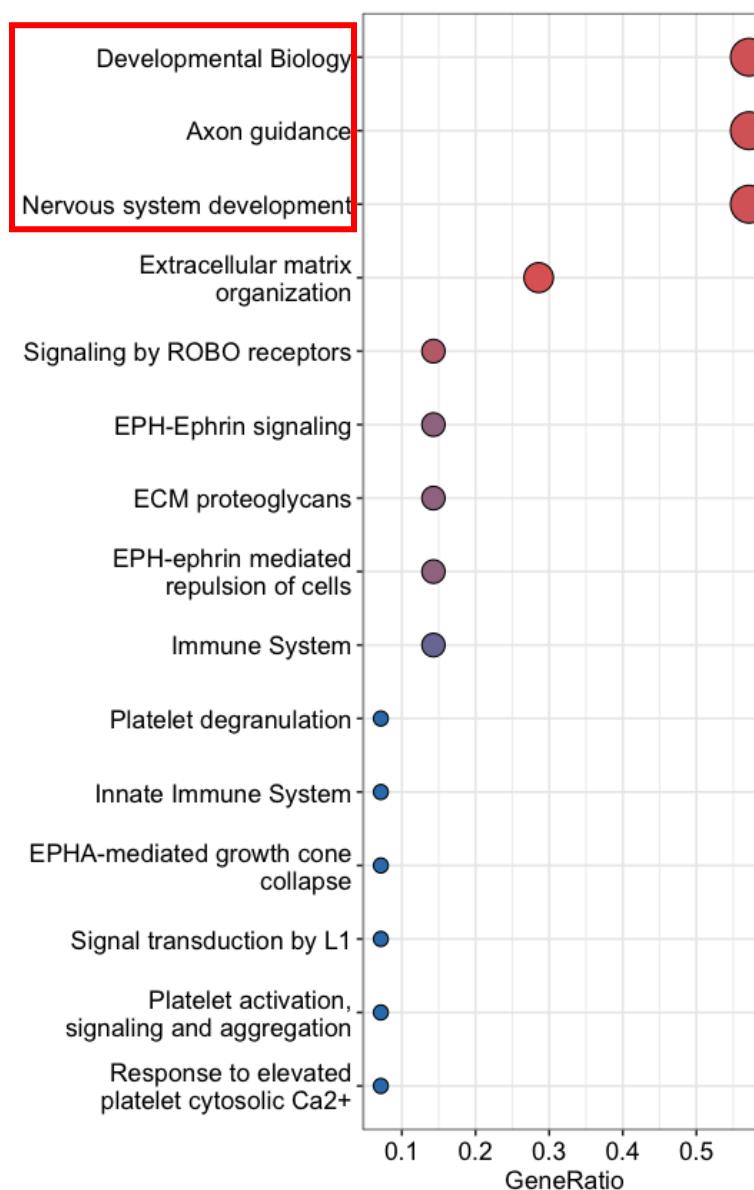
Sst → Pvalb

Interactions remains

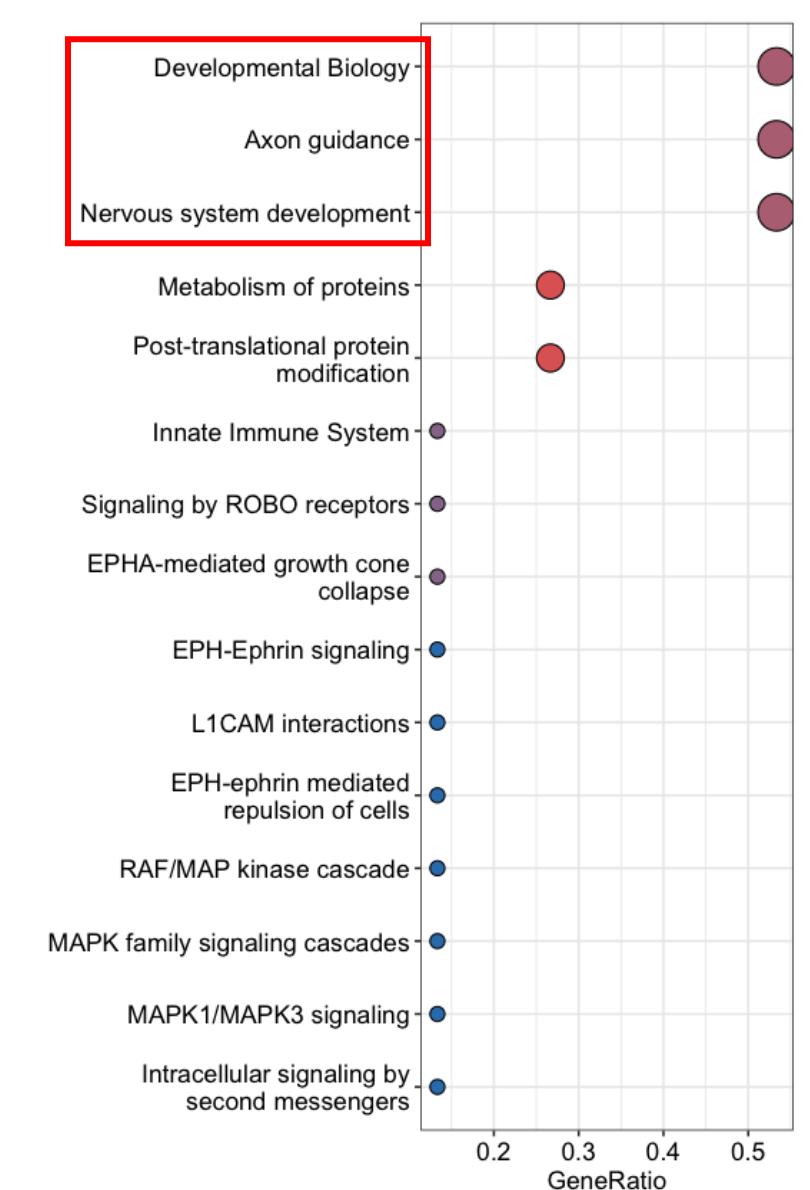


Sst → Pvalb L-R pairs are enriched with developmental terms.

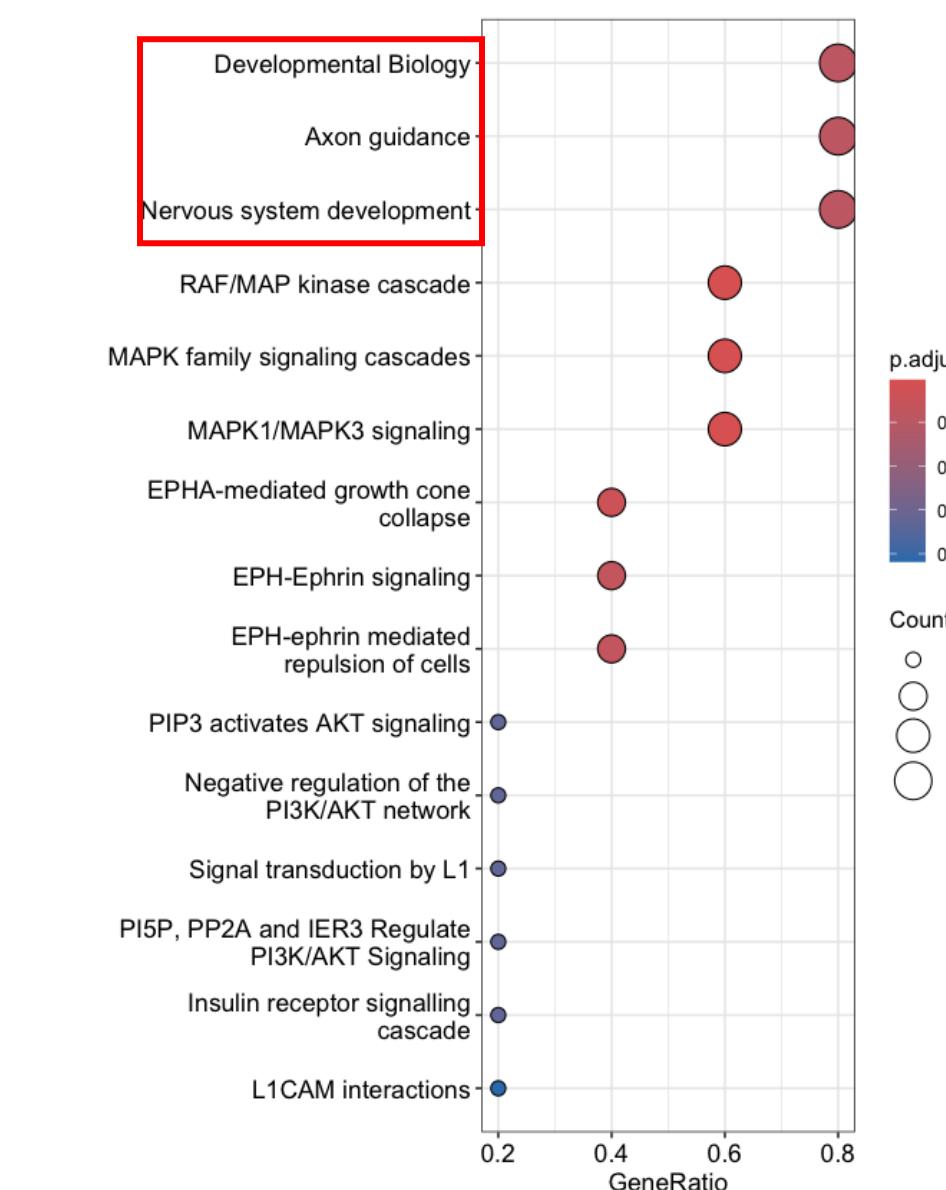
P10



shared



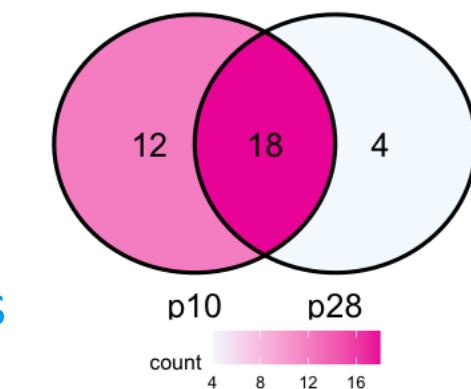
P28



Direction-Specific Functionality

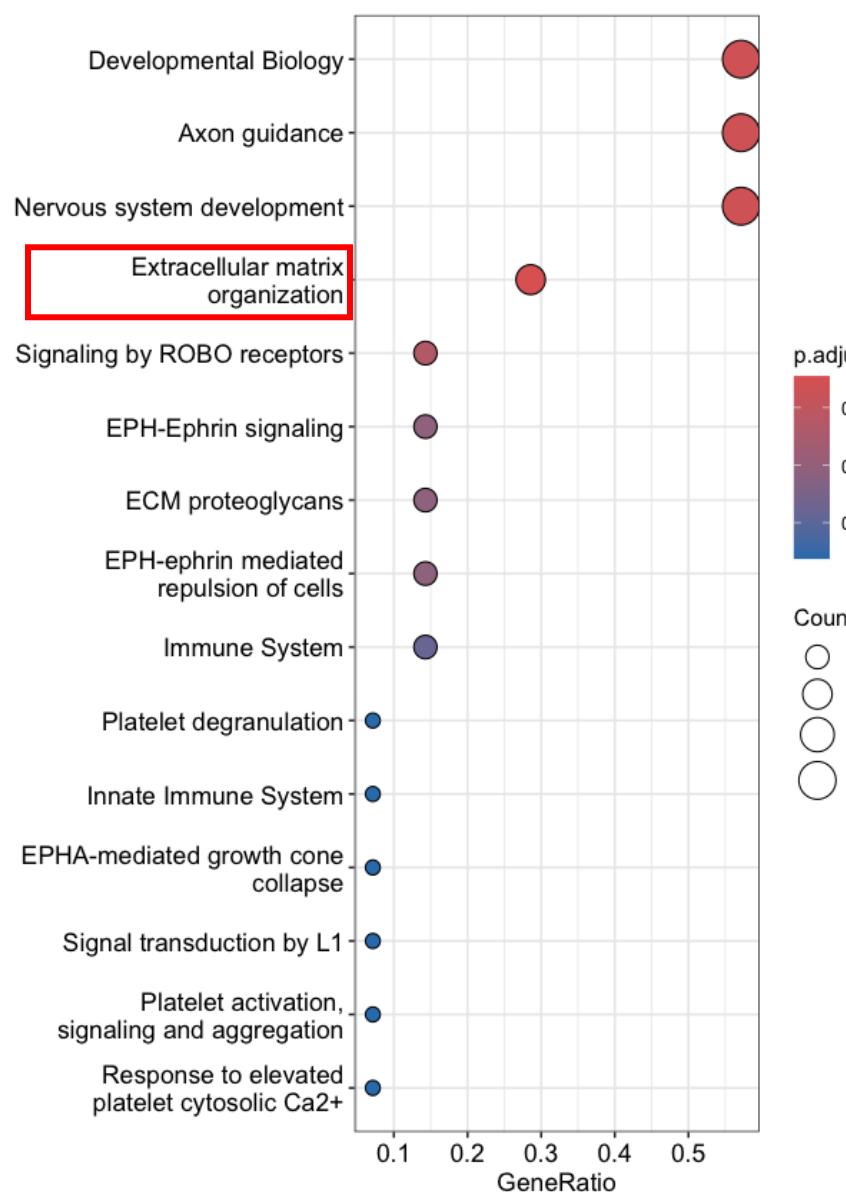
Sst → Pvalb

Interactions remains



Sst → Pvalb L-R pairs are enriched with developmental terms.

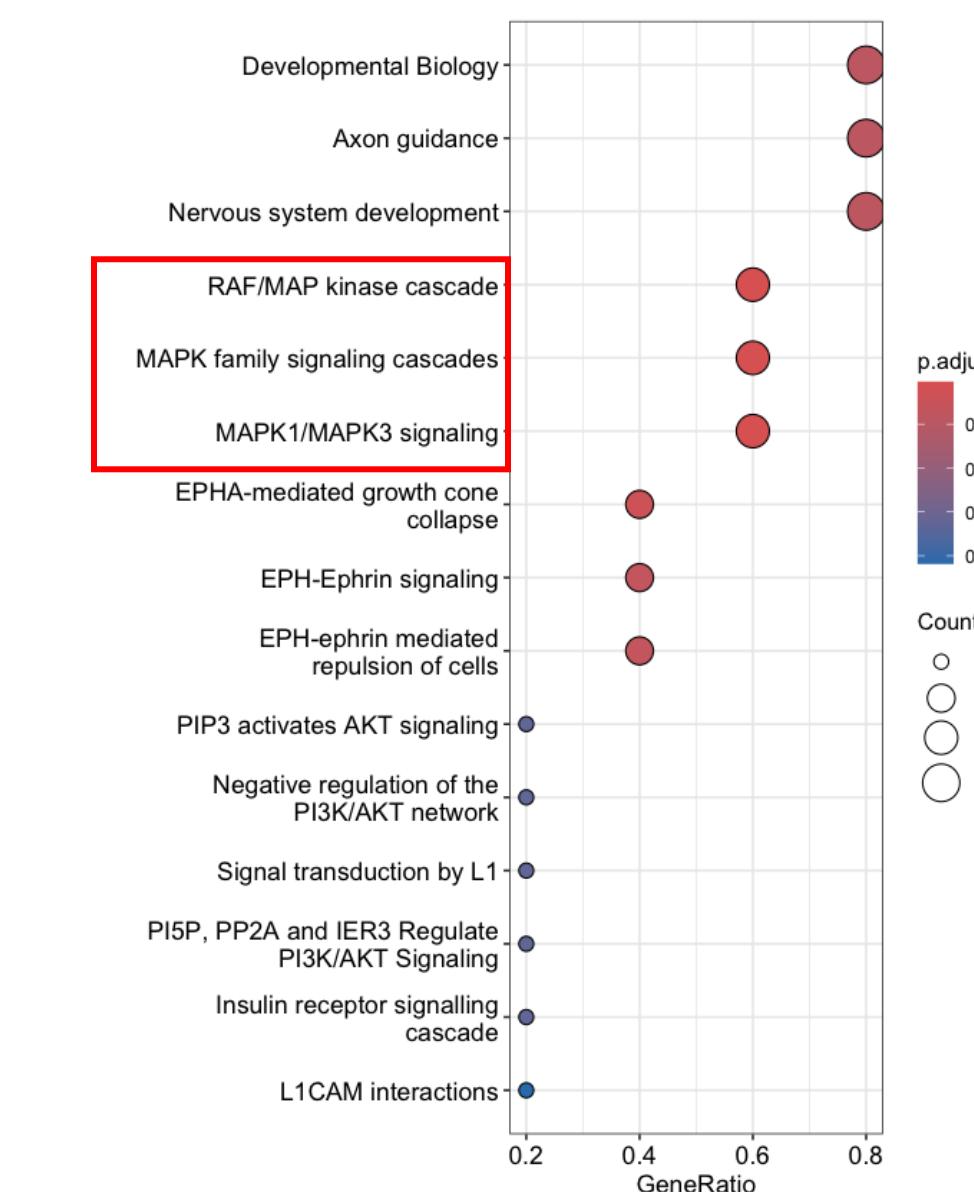
P10



shared



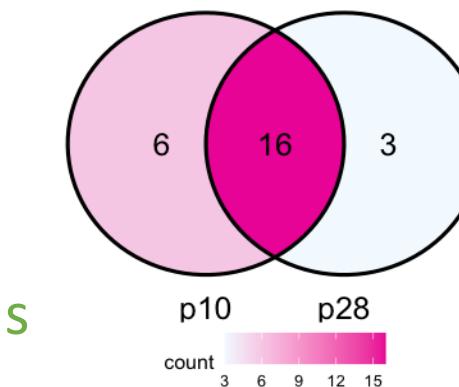
P28



Direction-Specific Functionality

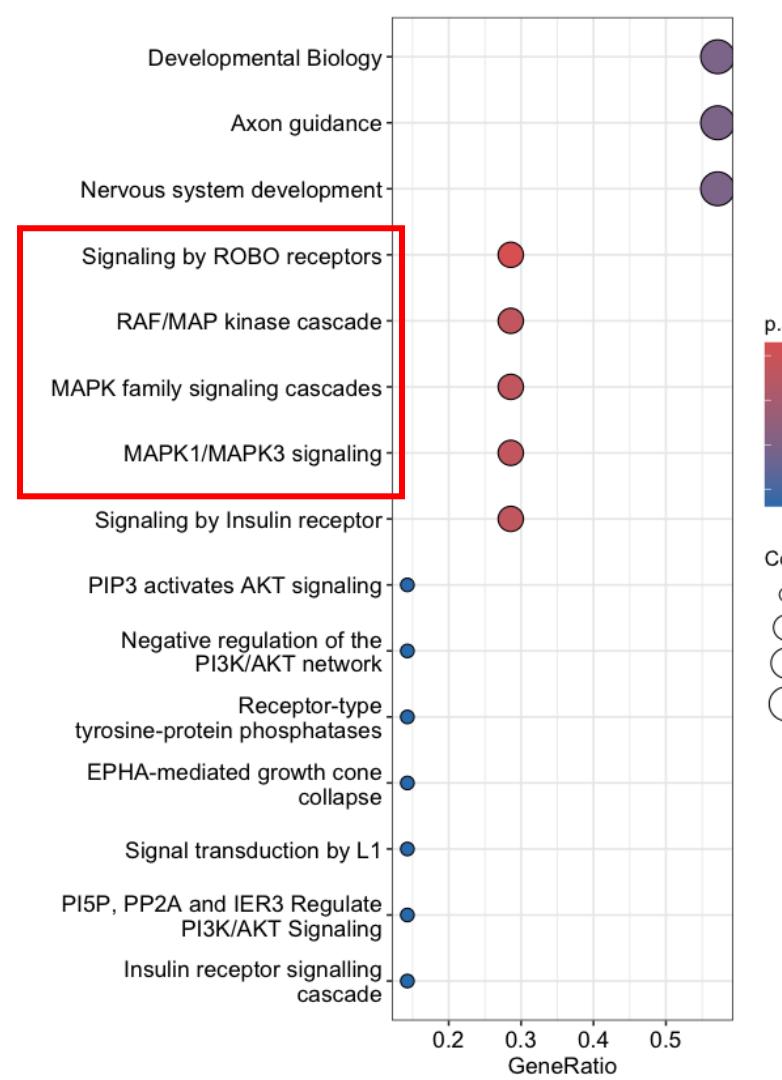
Pvalb → Sst

Interactions remains

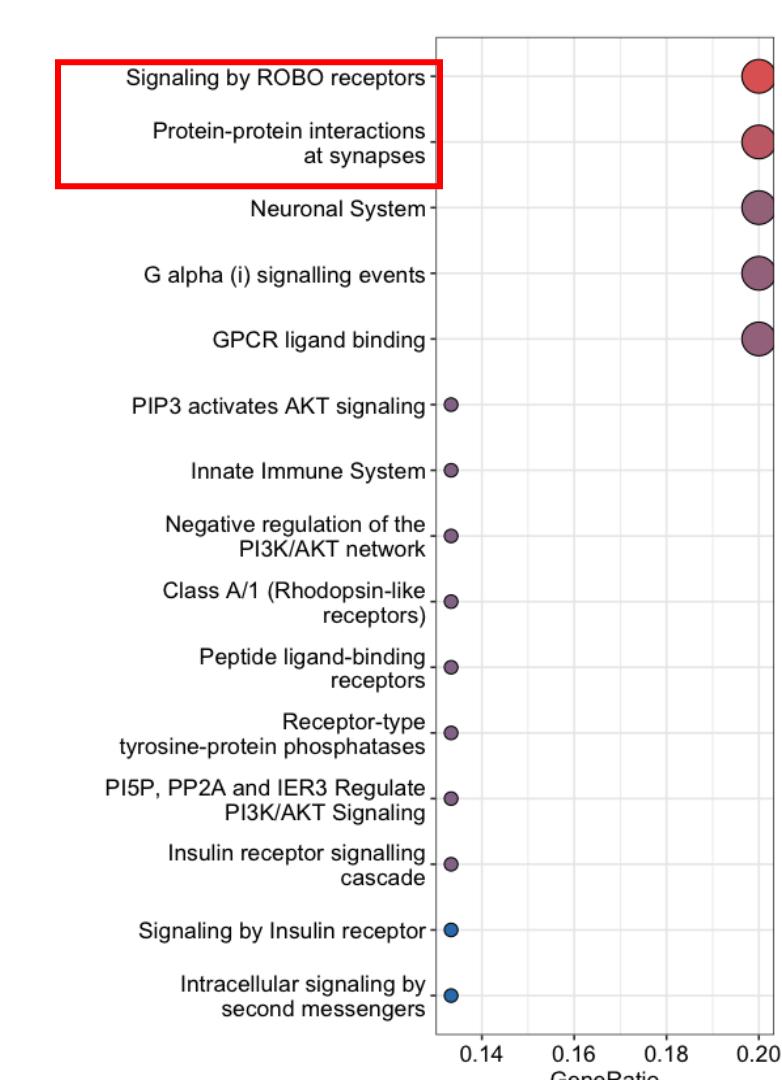


Pvalb → Sst directed signaling is variably enriched, involving various signaling pathways such as ROBO, MAPK, GPCR, and Tyrosine Kinase pathways.

P10



shared



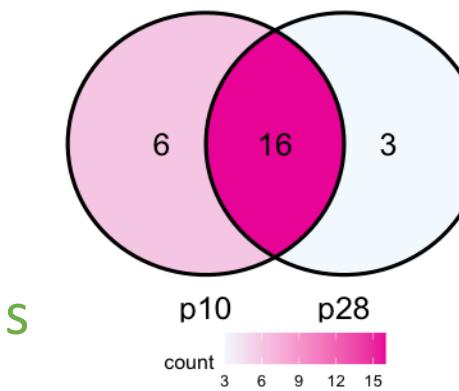
P28



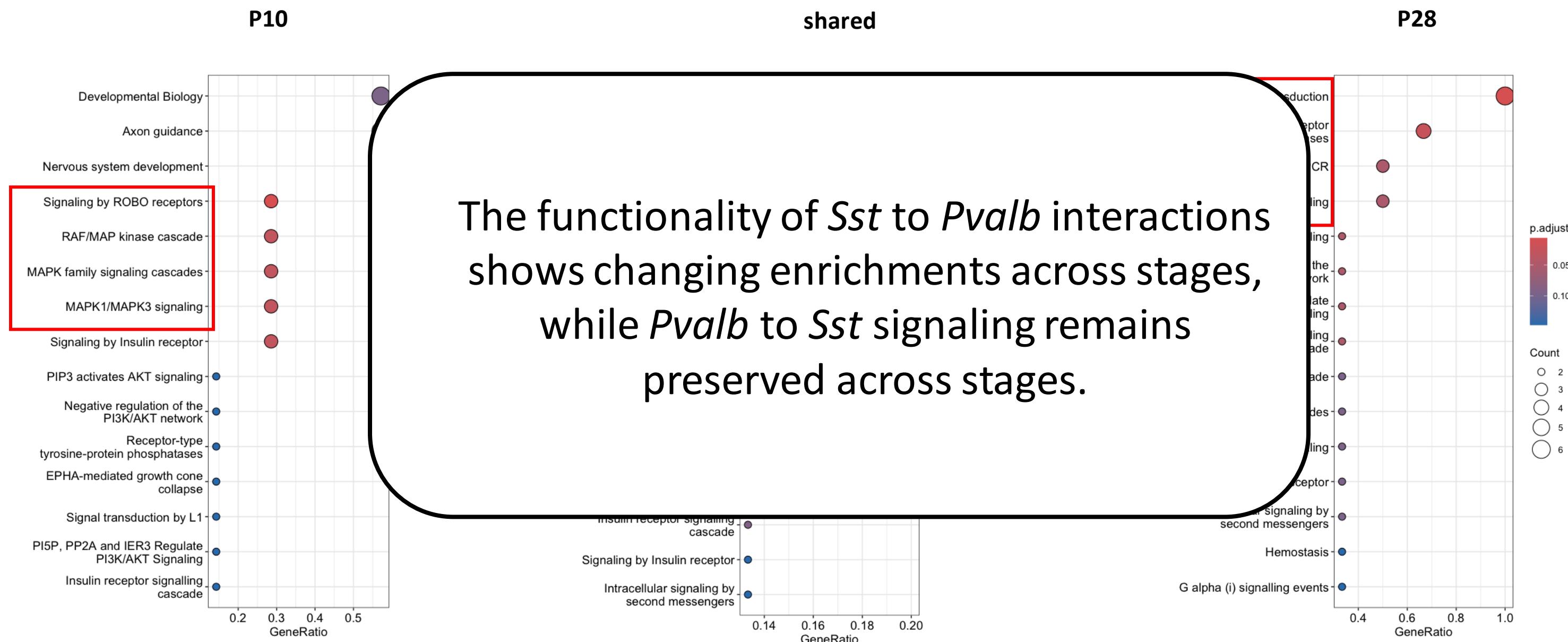
Direction-Specific Functionality

Pvalb → *Sst*

Interactions remains



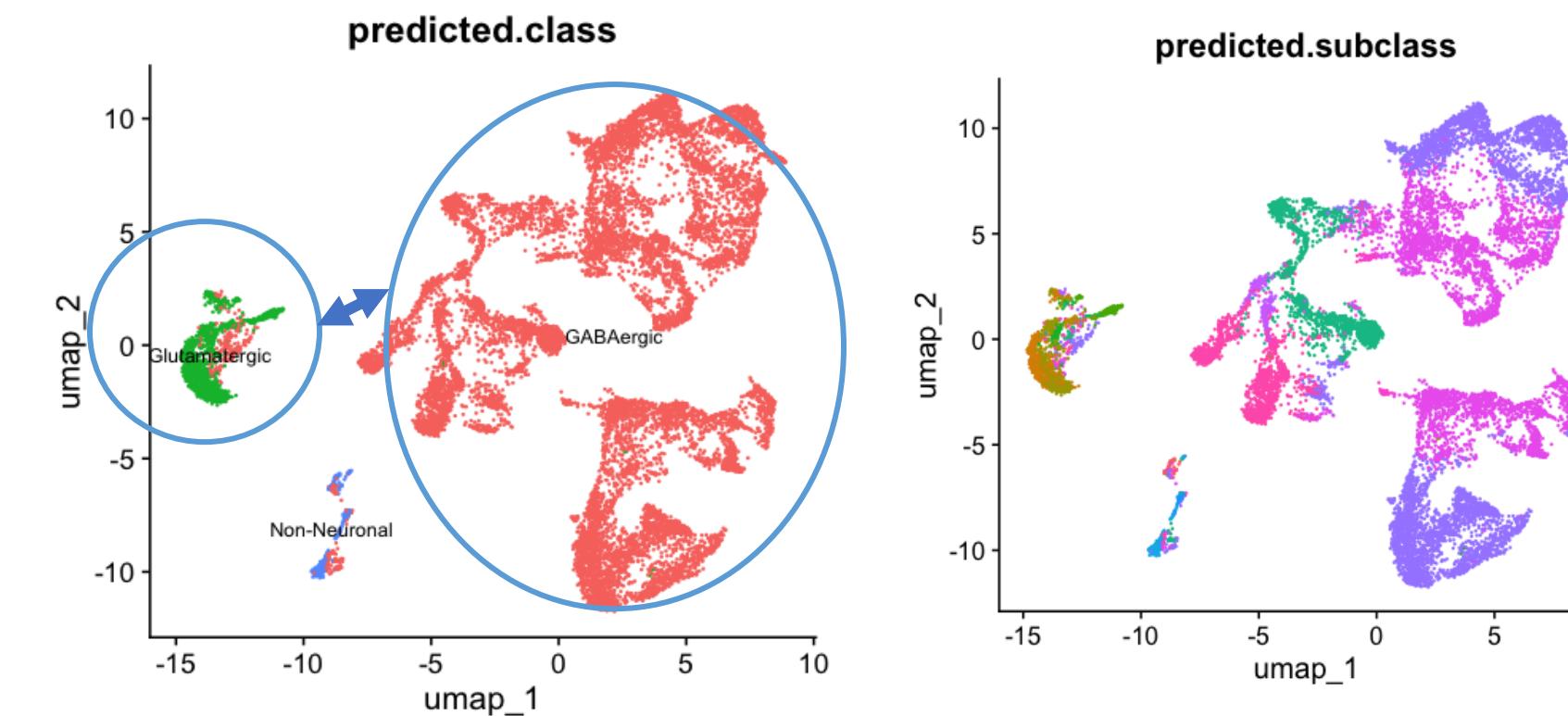
Pvalb → *Sst* directed signaling is variably enriched, involving various signaling pathways such as ROBO, MAPK, GPCR, and Tyrosine Kinase pathways.



Results summary

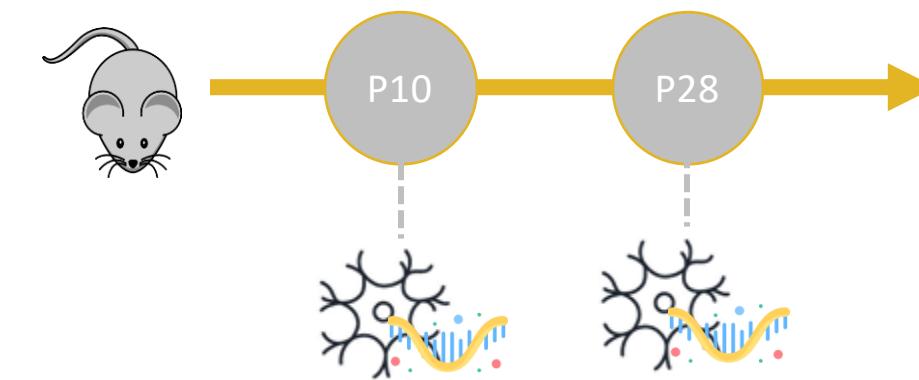
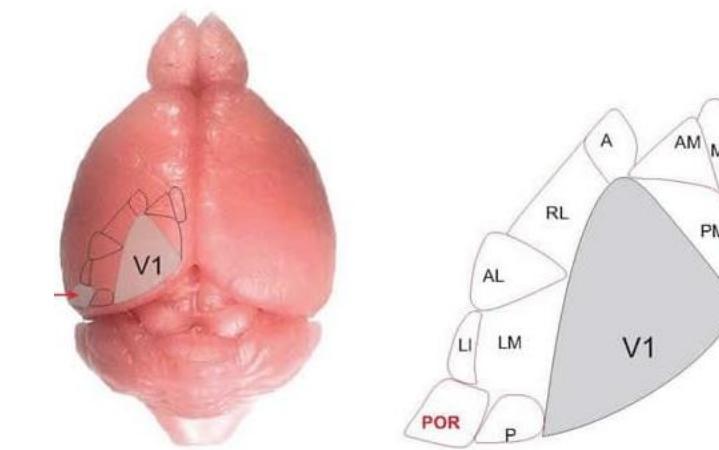
- Changing interactions between *Sst* and *Vip* over developmental stages.
- Persistent interactions between *Sst* and *Pvalb* over developmental stages.
- L-R pairs could reveal more specific information, such as those between neurexin and neuroligin pairs and their positional implications.
- GO enrichment reveals changes at the functional level, such as the direction specific interactions between *Sst* and *Pvalb*.
- Interactions between GABAergic neurons are functionally relevant for development and signaling.

- Investigate interactions between
Excitatory vs. Inhibitory interneurons

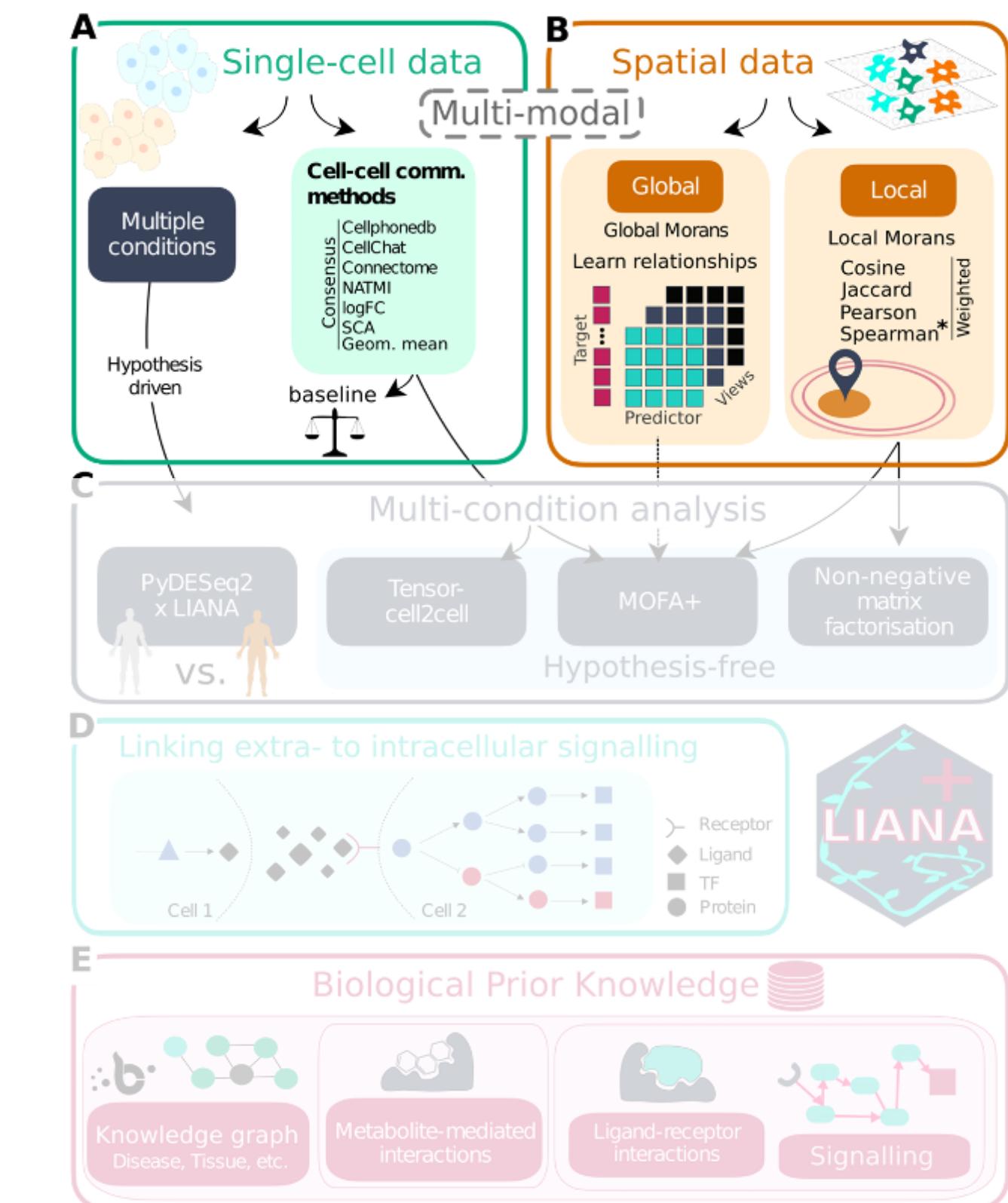


Astro	Micro-PVM
Endo	Oligo
L2/3 IT	OPC
L5 ET	Peri
L5 IT	Pvalb
L5/6 NP	Sncg
L6 CT	Sst
L6 IT	Sst Chodl
L6b	Vip
Lamp5	VLMC
Meis2	

- Investigate interactions between
Excitatory vs. Inhibitory interneurons
- **How about other regions?**
- **More time-points?**



- Investigate interactions between Excitatory vs. Inhibitory interneurons
- How about other regions?
- More time-points?
- Multi-omics? (LIANA+)**
 - Integrate spatial data



Questions???