Package: Connectome (via r-universe)

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Title Single Cell Connectomics

Description A toolkit for exploring cell-cell connectivity patterns based on ligand and receptor expression in heterogeneous single-cell datasets. Implements methods for constructing, filtering, and visualizing connectomic networks from single-cell RNA sequencing data. Supports multiple species via the FANTOM5 ligand-receptor database. Methods described in Raredon et al (2022) <doi:10.1038/s41598-022-07959-x>.

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LazyDataCompression xz

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VignetteBuilder knitr

URL https://zaoqu-liu.github.io/Connectome, https://github.com/Zaoqu-Liu/Connectome

BugReports https://github.com/Zaoqu-Liu/Connectome/issues

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Imports dplyr, ggrepel, igraph, scales, Seurat (>= 4.0.0), data.table, ggplot2, cowplot, Matrix, gtools, circlize, ComplexHeatmap, grid

Suggests knitr, rmarkdown, future, future.apply, parallel, ggthemes

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RemoteUrl https://github.com/Zaoqu-Liu/Connectome

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Contents

`CellCellScatter` *CellCellScatter*

Description

Scatter plot of a single cell-cell vector, involving features of interest. Returns a ggplot object, colored by mechanism. Points are labeled if above label.threshold on both x- and y-axes.

Usage

```
CellCellScatter(
  connectome,
  sources.include,
  targets.include,
  label.threshold = 1,
  weight.attribute = "weight_sc",
  ...
)
```

Arguments

connectome	A connectomic edgelist
sources.include	Source nodes of interest. Output will be limited to edges coming from these sources.
targets.include	Target nodes of interest. Output will be limited to edges landing on these targets.
label.threshold	Threshold for labeling of plot, applied to both x- and y- axes. Defaults to 1.
weight.attribute	Column to use to define edgeweights for network analysis. 'weight_sc or weight_norm'. Defaults to 'weight_sc'
...	Arguments passed to FilterConnectome

Centrality *Centrality*

Description

This function takes a connectomic edgelist and creates a source and a sink mode- and cell- organized dot plot. The y-axis is the discrete variable 'mode', and the x-axis is the sum of the weights of all edges for each mode made by each cell. Points are organized by cell type, with the size of the point correlating to the Kleinberg hub score (for source graph) and Kleinberg authority score (for sink graph). Network filtration is performed prior to network centrality calculations.

Usage

```
Centrality(
  connectome,
  cols.use = NULL,
  weight.attribute = "weight_sc",
  min.z = NULL,
  normalize = TRUE,
  group.by = "mode",
  ...
)
```

Arguments

connectome	A connectomic edgelist
cols.use	Desired colors for cell types, alphabetized. Defaults to standard ggplot colors.
weight.attribute	Column to use to define edgeweights for network analysis. 'weight_sc' or 'weight_norm'. Defaults to 'weight_sc'
min.z	Minimum z-score for ligand and receptor.

normalize	Default TRUE. Scales each mode to have equivalent x-axes.
group.by	Default 'mode'. Determines how to subdivide network for centrality analysis. Accepts 'mode','ligand','receptor', 'gene', or 'mechanism'.
...	Arguments passed to FilterConnectome

Value

A ggplot object showing centrality analysis

`CircosDiff` *CircosDiff*

Description

Currently in beta testing. A Circos Plot for a differential edgelist made using DifferentialConnectome. Note that negative log fold changes cannot be plotted; therefore, this plot uses the 'score' for the differential comparison, which is always positive in proportion to perturbation.

Usage

```
CircosDiff(
  differential.connectome,
  features = NULL,
  sources.include = NULL,
  targets.include = NULL,
  min.score = NULL,
  min.pct = NULL,
  verbose = TRUE,
  infinity.to.max = TRUE,
  edge.color.by.source = TRUE,
  cols.use = NULL,
  lab.cex = 1,
  title = NULL,
  small.gap = 1,
  big.gap = 10
)
```

Arguments

differential.connectome	A differential connectome, made with DifferentialConnectome. May be filtered as desired prior to plotting.
features	Gene of interest. Output will be limited to edges including these specific genes.
sources.include	Source nodes of interest. Output will be limited to edges coming from these sources.

targets.include
: Target nodes of interest. Output will be limited to edges landing on these targets.

min.score
: Default NULL. Will limit output to edges with a differential score greater than this value.

min.pct
: Default NULL. Threshold to return clusterwise observations for both ligand and receptor. Only needs to be satisfied in connect.1 OR in connect.2.

verbose
: Whether to output feedback to user

infinity.to.max
: Default TRUE. If TRUE, will create a pseudo value to replace values of "Inf"

edge.color.by.source
: Default TRUE - edges will be colored by their source cell type. If false, edges will be colored by receiving cell instead.

cols.use
: Optional. Colors for plotting nodes.

lab.cex
: Text size for gene names

title
: Character string for title of plot.

small.gap
: Default 1. Amount of distance between sectors. If the number of edges is very large, this will have to be reduced in size.

big.gap
: Default 10. Amount of distance between the source cells and the target cells (top and bottom arc of graph). If the number of edges is very large, this can be reduced in size in addition to 'small.gap'

CircosPlot *CircosPlot*

Description

Plotting function to make Circos plots using the circlize package, following the vignette by the Saeys Lab at: https://github.com/saeyslab/nichenetr/blob/master/vignettes/circos.md Note that this plotting type is incompatible with edges where the ligand and the receptor are the exact same gene.

Usage

```
CircosPlot(
  connectome,
  weight.attribute = "weight_sc",
  cols.use = NULL,
  min.z = NULL,
  lab.cex = 1,
  balanced.edges = TRUE,
  edge.color.by.source = TRUE,
  small.gap = 1,
  big.gap = 10,
  title = NULL,
  ...
)
```

Arguments

connectome	A connectomic object, ideally filtered to only edges of interest.
weight.attribute	Column to use to define edgeweights for network analysis. 'weight_sc' or 'weight_norm'. Defaults to 'weight_sc'. If 'weight_sc', function will automatically filter at min.z = 0 to remove negative source/sink values.
cols.use	Optional. Colors for plotting nodes.
min.z	Minimum z-score for ligand and receptor.
lab.cex	Text size for gene names
balanced.edges	Edges in this plot can change thickness along their length. This parameter decides whether to scale edges by a single edgeweight (chosen in weight.attribute) or by the separate cell-specific ligand and receptor values. Default balanced (TRUE). If FALSE, the edges will expand or contract to join ligand weight to receptor weight.
edge.color.by.source	Default TRUE - edges will be colored by their source cell type. If false, edges will be colored by receiving cell instead.
small.gap	Default 1. Amount of distance between sectors. If the number of edges is very large, this will have to be reduced in size.
big.gap	Default 10. Amount of distance between the source cells and the target cells (top and bottom arc of graph). If the number of edges is very large, this can be reduced in size in addition to 'small.gap'
title	Character string for title of plot.
...	Arguments passed to FilterConnectome

CompareCentrality *CompareCentrality*

Description

Takes a list of connectomes and will compare sending- and receiving- centrality, side-by-side, for the given networks, or requested network subsets.

Usage

```
CompareCentrality(
  connectome.list,
  weight.attribute = "weight_norm",
  min.z = NULL,
  cols.use = NULL,
  normalize = TRUE,
  ...
)
```

Arguments

connectome.list	A named list of connectomic edgelists. The output plot will be split by the names of the list.
weight.attribute	Column to use to define edgeweights for network analysis. 'weight_sc' or 'weight_norm'. Defaults to 'weight_norm'
min.z	Minimum z-score for ligand and receptor.
cols.use	Desired colors for cell types, alphabetized. Defaults to standard ggplot colors.
normalize	Default TRUE. Scales each mode to have equivalent x-axes.
...	Arguments passed to FilterConnectome. Will be applied to each connectome within the list.

Value

A ggplot object comparing centrality across connectomes

CreateConnectome *CreateConnectome*

Description

Creates a connectomic edgelist from a Seurat object. The connectome represents all possible ligand-receptor interactions between cell populations, weighted by expression levels and statistical significance.

Usage

```
CreateConnectome(
  object,
  LR.database = "fantom5",
  species = NULL,
  include.putative = TRUE,
  include.rejected = FALSE,
  p.values = TRUE,
  max.cells.per.ident = NULL,
  min.cells.per.ident = NULL,
  weight.definition.norm = "product",
  weight.definition.scale = "mean",
  custom.list = NULL,
  calculate.DOR = FALSE,
  assay = "RNA",
  parallel = FALSE,
  n.cores = NULL,
  ...
)
```

Arguments

object	A Seurat object with normalized and scaled data
LR.database	Ligand-receptor database to use. Either 'fantom5' (default) or 'custom'. If 'custom', provide a dataframe via custom.list parameter.
species	Species for FANTOM5 database. Required if LR.database = 'fantom5'. Options: 'human', 'mouse', 'rat', or 'pig'
include.putative	Include putative ligand-receptor pairs from FANTOM5. Default TRUE.
include.rejected	Include pairs labeled "EXCLUDED" in FANTOM5. Default FALSE.
p.values	Calculate Wilcoxon Rank Sum test p-values. Default TRUE. Set to FALSE for faster computation when statistical testing is not needed.
max.cells.per.ident	Maximum cells per identity for downsampling. Default NULL (no downsampling). Useful for large datasets.
min.cells.per.ident	Minimum cells required per identity. Default NULL. Clusters below this threshold are excluded from analysis.
weight.definition.norm	Method for edge weight from normalized data. Options: 'sum', 'mean', or 'product' (default).
weight.definition.scale	Method for edge weight from scaled data. Options: 'sum', 'mean' (default), or 'product'.
custom.list	Dataframe with custom ligand-receptor pairs. Required columns: ligand (col 1), receptor (col 2), mode/category (col 3).
calculate.DOR	Calculate log-normalized Diagnostic Odds Ratio. Default FALSE.
assay	Seurat assay to use. Default 'RNA'. Also accepts 'SCT'.
parallel	Use parallel processing for p-value calculations. Default FALSE.
n.cores	Number of cores for parallel processing. Default: detectCores() - 1
...	Additional arguments passed to internal functions

Details

The function calculates edge weights using the formula specified by weight.definition.norm and weight.definition.scale parameters:

- 'product': ligand * receptor (captures co-expression)
- 'sum': ligand + receptor (additive contribution)
- 'mean': (ligand + receptor) / 2 (average contribution)

Value

A data.frame containing the connectomic edgelist with columns: source, target, ligand, receptor, pair, mode, expression values, z-scores, percent expressing, edge weights, and optionally p-values and DOR.

Examples

```
## Not run:
# Basic usage
connectome <- CreateConnectome(seurat_obj, species = "human")

# Without p-value calculation (faster)
connectome <- CreateConnectome(seurat_obj, species = "human", p.values = FALSE)

# With custom ligand-receptor list
my_lr <- data.frame(ligand = c("IL6", "TNF"), receptor = c("IL6R", "TNFRSF1A"),
                    mode = c("cytokine", "cytokine"))
connectome <- CreateConnectome(seurat_obj, LR.database = "custom", custom.list = my_lr)

## End(Not run)
```

`DiffEdgeDotPlot` *DiffEdgeDotPlot*

Description

Plotting function to make a DotPlot of differential edges for a network comparison. This function first finds all edges meeting the desired thresholding criteria, and then plots complete information regarding all mechanisms and celltype vectors implicated.

Usage

```
DiffEdgeDotPlot(
  differential.connectome,
  features = NULL,
  sources.include = NULL,
  targets.include = NULL,
  min.score = NULL,
  min.pct = NULL,
  verbose = TRUE,
  infinity.to.max = TRUE,
  ...
)
```

Arguments

`differential.connectome`	A differential connectome object
`features`	Gene of interest. Output will be limited to edges including these specific genes.
`sources.include`	Source nodes of interest. Output will be limited to edges coming from these sources.

`targets.include`
: Target nodes of interest. Output will be limited to edges landing on these targets.

`min.score`
: Default NULL. Will limit output to edges with a differential score greater than this value.

`min.pct`
: Default NULL. Threshold to return clusterwise observations for both ligand and receptor. Only needs to be satisfied in connect.1 OR in connect.2.

`verbose`
: Whether to output feedback to user

`infinity.to.max`
: Default TRUE. If TRUE, will create a pseudo value to replace values of "Inf"

`...`
: Additional arguments (not currently used)

Value

A ggplot object

`DifferentialConnectome` *DifferentialConnectome*

Description

Currently in beta testing. Creates a fold-change connectome from two input connectomes, generally unfiltered. Must be node-aligned, from the same reference mapping, and unfiltered. ('edge' columns must contain identical entries, though not necessarily in the same order.)

Usage

```
DifferentialConnectome(connect.ref, connect.test, min.pct = NULL)
```

Arguments

`connect.ref`
: A connectome from a system

`connect.test`
: A connectome from a different system, to be compared to connect.ref

`min.pct`
: Default NULL. Threshold to return clusterwise observations for both ligand and receptor. Only needs to be satisfied in connect.ref OR in connect.test.

DifferentialScoringPlot
DifferentialScoringPlot

Description

Currently in beta testing. Creates x3 aligned heatmaps allowing visualization of ligand, receptor, and perturbation scores for a given cell-system of interest.

Usage

```
DifferentialScoringPlot(
  differential.connectome,
  features = NULL,
  sources.include = NULL,
  targets.include = NULL,
  min.score = NULL,
  min.pct = NULL,
  verbose = TRUE,
  infinity.to.max = TRUE,
  aligned = FALSE
)
```

Arguments

differential.connectome	A differential connectome, made with DifferentialConnectome. May be filtered as desired prior to plotting.
features	Gene of interest. Output will be limited to edges including these specific genes.
sources.include	Source nodes of interest. Output will be limited to edges coming from these sources.
targets.include	Target nodes of interest. Output will be limited to edges landing on these targets.
min.score	Default NULL. Will limit output to edges with a differential score greater than this value.
min.pct	Default NULL. Threshold to return clusterwise observations for both ligand and receptor. Only needs to be satisfied in connect.1 OR in connect.2.
verbose	Whether to output feedback to user
infinity.to.max	Default TRUE. If TRUE, will create a pseudo value to replace values of "Inf"
aligned	Default FALSE. If TRUE, will create edge-aligned heatmaps (duplicate rows and columns in first two plots, to dimension map all three plots)

EdgeDotPlot *EdgeDotPlot*

Description

Plotting function to make a DotPlot of edges within a specified network. This function first finds all edges meeting the desired thresholding criteria, and then plots complete information regarding all mechanisms and celltype vectors implicated.

Usage

```
EdgeDotPlot(connectome, ...)
```

Arguments

connectome	A connectomic object
...	Arguments passed to FilterConnectome

EvenSplit *EvenSplit*

Description

Splits a Seurat object using SplitObject, but makes sure that for each identity, an even number of cells are sampled.

Usage

```
EvenSplit(object, split.by)
```

Arguments

object	A Seurat object
split.by	Parameter by which to split the object (e.g., a metadata column name)

Value

A named list of Seurat objects, one for each level of split.by

FilterConnectome *FilterConnectome*

Description

Filters a connectomic edgelist output from CreateConnectome according to user inputs. Defaults are set to reasonable initial parameters for data clean-up and exploration.

Usage

```
FilterConnectome(
  connectome,
  min.pct = NULL,
  max.p = NULL,
  min.DOR = NULL,
  min.exp = NULL,
  min.z = NULL,
  modes.include = NULL,
  sources.include = NULL,
  targets.include = NULL,
  mechanisms.include = NULL,
  features = NULL,
  verbose = TRUE,
  remove.na = FALSE
)
```

Arguments

connectome	A connectomic edgelist output from CreateConnectome
min.pct	Minimum fraction of cells within a given cluster expressing the ligand or receptor.
max.p	Maximum p-value for ligand and receptor. Filtration on this column requires prior p-value calculation.
min.DOR	Minimum log-normalized Diagnostic Odds Ratio for the ligand or receptor for its cell type within an edge.
min.exp	Minimum normalized expression level of ligand and receptor.
min.z	Minimum z-score for ligand and receptor.
modes.include	String or vector signifying mode(s) of interest in include.
sources.include	Source nodes of interest. Output will be limited to edges coming from these sources.
targets.include	Target nodes of interest. Output will be limited to edges landing on these targets.
mechanisms.include	Ligand - Receptor pairs of interest. The character string should match entries in the 'pair' column of the connectome.

features	Genes of interest. Output will be limited to edges including these specific genes (as ligand or receptor).
verbose	Whether to output feedback to user
remove.na	Whether to remove edges containing 'NA' (no mapping to original object - only useful if investigating orphan ligands and receptors)

Value

A filtered connectome data.frame

ModalDotPlot *ModalDotPlot*

Description

This function takes a connectomic edgelist and creates a source and a sink mode- and cell- organized dot plot. The y-axis is the discrete variable 'mode', and the x-axis is the sum of the weights of all edges for each mode made by each cell. Points are organized by cell type, with the size of the point correlating to the Kleinberg hub score (for source graph) and Kleinberg authority score (for sink graph). Network filtration is performed prior to network centrality calculations.

Usage

```
ModalDotPlot(
  connectome,
  cols.use = NULL,
  weight.attribute = "weight_sc",
  min.z = NULL,
  normalize = TRUE,
  ...
)
```

Arguments

connectome	A connectomic edgelist
cols.use	Desired colors for cell types, alphabetized. Defaults to standard ggplot colors.
weight.attribute	Column to use to define edgeweights for network analysis. 'weight_sc' or 'weight_norm'. Defaults to 'weight_sc'
min.z	Minimum z-score for ligand and receptor.
normalize	Default TRUE. Scales each mode to have equivalent x-axes.
...	Arguments passed to FilterConnectome

Value

A ggplot object showing modal centrality analysis

ncomms8866 *FANTOM5 Ligand-Receptor Database (Original)*

Description

The original FANTOM5 ligand-receptor database before species conversion.

Usage

```
ncomms8866
```

Format

A data frame with the original ligand-receptor pairs from FANTOM5.

Source

Ramilowski, J.A. et al. Nat Commun 6, 7866 (2015). doi:10.1038/ncomms8866

ncomms8866_human *FANTOM5 Ligand-Receptor Database (Human)*

Description

A dataset containing ligand-receptor pairs curated from the FANTOM5 project, converted to human gene symbols.

Usage

```
ncomms8866_human
```

Format

A data frame with 2557 rows and 17 columns:

Pair.Name Unique identifier for the ligand-receptor pair

Ligand.ApprovedSymbol HGNC approved symbol for the ligand

Ligand.Name Full name of the ligand

Receptor.ApprovedSymbol HGNC approved symbol for the receptor

Receptor.Name Full name of the receptor

DLRP DLRP database reference

HPMR HPMR database reference

IUPHAR IUPHAR database reference

HPRD HPRD database reference

STRING.binding STRING binding evidence

STRING.experiment STRING experimental evidence

HPMR.Ligand HPMR ligand identifier

HPMR.Receptor HPMR receptor identifier

PMID.Manual PubMed ID for manual curation

Pair.Source Source classification: "known" or "novel"

Pair.Evidence Evidence level: "literature supported" or "putative"

mode Signaling mode/category of the interaction

Source

Ramilowski, J.A. et al. A draft network of ligand-receptor-mediated multicellular signalling in human. Nat Commun 6, 7866 (2015). doi:10.1038/ncomms8866

See Also

ncomms8866_mouse, ncomms8866_rat, ncomms8866_pig

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ncomms8866_mouse *FANTOM5 Ligand-Receptor Database (Mouse)*

Description

A dataset containing ligand-receptor pairs from the FANTOM5 project, converted to mouse gene symbols using ortholog mapping.

Usage

```
ncomms8866_mouse
```

Format

A data frame with ligand-receptor pairs. See ncomms8866_human for column descriptions.

Source

Ramilowski, J.A. et al. Nat Commun 6, 7866 (2015). doi:10.1038/ncomms8866

See Also

ncomms8866_human

ncomms8866_pig *FANTOM5 Ligand-Receptor Database (Pig)*

Description

A dataset containing ligand-receptor pairs from the FANTOM5 project, converted to pig gene symbols using ortholog mapping.

Usage

```
ncomms8866_pig
```

Format

A data frame with ligand-receptor pairs. See ncomms8866_human for column descriptions.

Source

Ramilowski, J.A. et al. Nat Commun 6, 7866 (2015). doi:10.1038/ncomms8866

See Also

ncomms8866_human

ncomms8866_rat *FANTOM5 Ligand-Receptor Database (Rat)*

Description

A dataset containing ligand-receptor pairs from the FANTOM5 project, converted to rat gene symbols using ortholog mapping.

Usage

```
ncomms8866_rat
```

Format

A data frame with ligand-receptor pairs. See ncomms8866_human for column descriptions.

Source

Ramilowski, J.A. et al. Nat Commun 6, 7866 (2015). doi:10.1038/ncomms8866

See Also

ncomms8866_human

NetworkPlot *NetworkPlot*

Description

Creates a network plot of a connectomic object. Wrapper for igraph functionality.

Usage

```
NetworkPlot(
  connectome,
  weight.attribute = "weight_sc",
  title = NULL,
  cols.use = NULL,
  include.all.nodes = FALSE,
  min.z = NULL,
  mar = 1.5,
  layout = "circle",
  edge.label.cex = 0.4,
  ...
)
```

Arguments

connectome	A connectomic object, ideally filtered to only edges of interest.
weight.attribute	Column to use to define edgeweights for network analysis. 'weight_sc' or 'weight_norm'. Defaults to 'weight_sc'
title	Description of the network being plotted
cols.use	Optional. Colors for plotting nodes.
include.all.nodes	If TRUE, include all nodes from original connectome even after filtering. Default FALSE.
min.z	Minimum z-score for ligand and receptor.
mar	Default 1.5. Symmetric margin around plot.
layout	Layout algorithm. Either "circle" (default) or "force.directed".
edge.label.cex	Text size for edge labels. Default 0.4.
...	Arguments passed to FilterConnectome

Value

A recorded plot object

`SignalScatter` *SignalScatter*

Description

Scatter plot of signaling vectors involving features of interest. Returns a ggplot object, colored by mechanism, labeled by cell-cell vector.

Usage

```
SignalScatter(
  connectome,
  features,
  label.threshold = 1,
  weight.attribute = "weight_sc",
  ...
)
```

Arguments

`connectome`	A connectomic edgelist
`features`	Features of interest
`label.threshold`	Threshold for labeling of plot. Sum of receptor score and ligand score must be greater than this number.
`weight.attribute`	Column to use to define edgeweights for network analysis. 'weight_sc or weight_norm'. Defaults to 'weight_sc'
`...`	Arguments passed to FilterConnectome

`SingleCellConnectome` *SingleCellConnectome*

Description

Takes a Seurat object and creates a single-cell connectome (rows are ligand-receptor mechanisms and columns are cell-cell vectors)

Usage

```
SingleCellConnectome(
  object,
  LR.database = "fantom5",
  species = NULL,
  include.putative = TRUE,
  include.rejected = FALSE,
  max.cells.per.ident = NULL,
  min.cells.per.ident = NULL,
  slot.use = "data",
  weight.definition = "product",
  custom.list = NULL,
  assay = "RNA",
  ...
)
```

Arguments

object	A Seurat object
LR.database	Accepts either 'fantom5' or 'custom'. If custom, a dataframe must be provided to argument custom.list with the first column equal to ligands, second column equal to associated receptors, and third column equal to desired modal categorizations.
species	The species of the object that is being processed. Only required if LR.database = 'fantom5', and allows 'human','mouse','rat', or 'pig'
include.putative	Default TRUE. Includes ligand-receptor pairs deemed putative in FANTOM5 database.
include.rejected	Default FALSE. If TRUE, includes gene pairs labeled "EXCLUDED" in FANTOM5 database.
max.cells.per.ident	Default NULL. If a value is input, input object will be downsampled to requested number of cells per identity.
min.cells.per.ident	Default NULL. If a value is input, only cell populations meeting this threshold will be included in network analysis.
slot.use	Which data slot to use. Default "data".
weight.definition	Method for combining ligand and receptor values. Options: "product" (default), "sum", "mean".
custom.list	Optional. A dataframe for custom mapping, with the first column equal to ligands, second column equal to associated receptors, and third column equal to desired modal categorizations.
assay	Assay to use. Default "RNA".
...	Additional arguments (not currently used)

Value

A sparse matrix with ligand-receptor pairs as rows and cell-cell combinations as columns

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