CellPhoneDB notebook

Goal: Use CellPhoneDB to get the list of ligand-receptor pairs detected between different cell types of peripheral blood mononuclear cells (scRNA).

This notebook contains the code that generated the results used for module6 scRNA-lab, part 2, CellPhoneDB. It is starting from the 25K PMBC scRNA generated by Khag et al in 2018 (https://www.nature.com/articles/nbt.4042). It is the same dataset that is used in the scRNA-lab, part 1 of the module 6 lab (Seurat tutorial: https://satijalab.org/seurat/archive/v3.1/immune_alignment.html).

To use this notebook:

1. first install python
2. then install jupyter notebook
3. install the necessary python packages (see below)
4. example to install 1 package : pip3 install numpy
5. launch the notebook: open a terminal window; type jupyter notebook ; open a web-browser and go to http://localhost:8888/tree
6. open the notebook (mycellphobeDB.ipynb)

References:

- https://pypi.org/project/cellphonedb/
- https://www.sc-best-practices.org/mechanisms/cell_cell_communication.html
- https://zktuong.github.io/ktplots/articles/vignette.html

In [1]:
```
# import python libraries
import numpy as np
import pandas as pd
import matplotlib.pyplot as plt
import seaborn as sns

import scanpy as sc
import liana as li
import decoupler as dc

import session_info
```

In [2]:
```
# Setting up R dependencies
import anndata2ri
import rpy2
from rpy2.robjects import r
import random

anndata2ri.activate()
```

```
%load_ext rpy2.ipython
```

```
/var/folders/jn/drzgdc711z58q9wws29jm5t80000gn/T/ipykernel_14107/2969930097.
py:7: DeprecationWarning: The global conversion available with activate() is
deprecated and will be removed in the next major release. Use a local conver
ter.
```

In [3]:
```
%%R
suppressPackageStartupMessages({
    library(reticulate)
    library(ggplot2)
    library(tidyr)
    library(dplyr)
    library(purrr)
    library(tibble)
})
```

```
    WARNING: The R package "reticulate" only fixed recently
    an issue that caused a segfault when used with rpy2:
    https://github.com/rstudio/reticulate/pull/1188
    Make sure that you use a version of that package that includes
    the fix.

In addition: Warning message:
package ‘reticulate’ was built under R version 4.3.3
```

In [4]:
```
# figure settings (size and resolution)
sc.settings.set_figure_params(dpi=200, frameon=False)
sc.set_figure_params(dpi=200, facecolor="white")
sc.set_figure_params(figsize=(5, 5))
```

In [5]:
```
# Read in the PBMC scRNA data. Store it in an Annotated data (AnnData) objec
# AnnData is very efficient as storing big matrices from scRNA as it handles
adata = sc.read(
    "kang_counts_25k.h5ad", backup_url="https://figshare.com/ndownloader/fil
)
adata

# Extract and store the counts (gene count for each cell) for later use
adata.layers["counts"] = adata.X.copy()
```

In [6]:
```
##this describes the object: it contains 15706 cells (columns) and 24673 fea
## It has one layer which contains the count data
## the other features stored in obs can be named metadata and are additional
## we will use the cell_type annotate later on in this tutorial
adata
```

Out[6]:
```
AnnData object with n_obs × n_vars = 24673 × 15706
    obs: 'nCount_RNA', 'nFeature_RNA', 'tsne1', 'tsne2', 'label', 'cluste
r', 'cell_type', 'replicate', 'nCount_SCT', 'nFeature_SCT', 'integrated_snn
_res.0.4', 'seurat_clusters'
    var: 'name'
    obsm: 'X_pca', 'X_umap'
    layers: 'counts'
```

In [7]:
```
##this step remove bad quality cells that do not contain a minimum of 200 ce
## This is basic preprocessing of scRNA. This is just basic processing for t
sc.pp.filter_cells(adata, min_genes=200)
sc.pp.filter_genes(adata, min_cells=3)
```

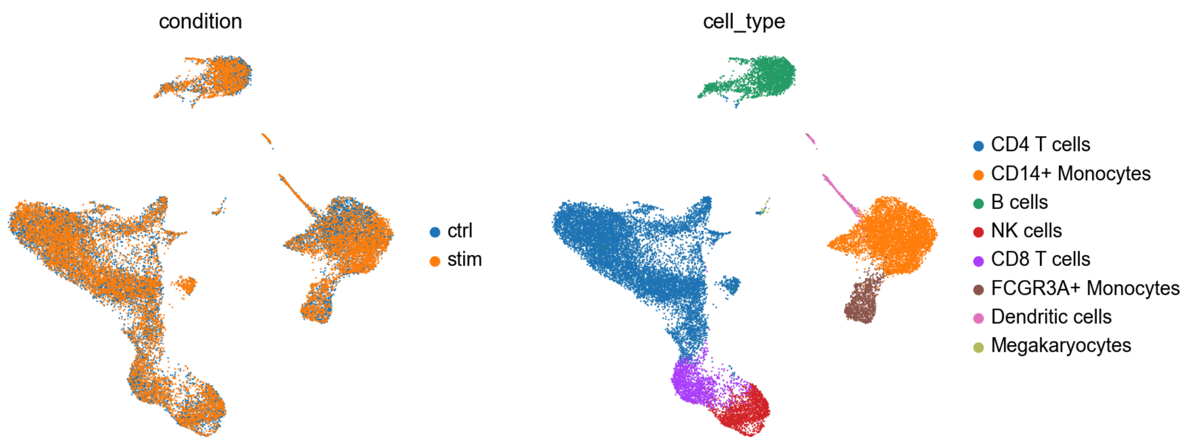
In [8]:
```
# Store the counts for later use
adata.layers["counts"] = adata.X.copy()
# Rename label to condition, replicate to patient
adata.obs = adata.obs.rename({"label": "condition", "replicate": "patient"},
# assign sample
adata.obs["sample"] = (
    adata.obs["condition"].astype("str") + "&" + adata.obs["patient"].astype
)
```

In [9]:
```
# log1p normalize the data
sc.pp.normalize_total(adata)
sc.pp.log1p(adata)
```

In [10]:
```
adata.obs["cell_type"].cat.categories
```

Out[10]:
```
Index(['CD4 T cells', 'CD14+ Monocytes', 'B cells', 'NK cells', 'CD8 T cell
s',
       'FCGR3A+ Monocytes', 'Dendritic cells', 'Megakaryocytes'],
      dtype='object')
```

In [11]:
```
sc.pl.umap(adata, color=["condition", "cell_type"], frameon=False)
```



In [12]:
```
## this stem only keep the cells that the stim phenotype: samples were stimu

adata_stim = adata[adata.obs["condition"] == "stim"].copy()
adata_stim
```

Out[12]:
```
AnnData object with n_obs × n_vars = 12301 × 15701
    obs: 'nCount_RNA', 'nFeature_RNA', 'tsne1', 'tsne2', 'condition', 'clus
ter', 'cell_type', 'patient', 'nCount_SCT', 'nFeature_SCT', 'integrated_snn
_res.0.4', 'seurat_clusters', 'n_genes', 'sample'
    var: 'name', 'n_cells'
    uns: 'log1p', 'condition_colors', 'cell_type_colors'
    obsm: 'X_pca', 'X_umap'
    layers: 'counts'
```

```
In [13]: # import cellphonedb method via liana
         from liana.method import cellphonedb

         cellphonedb(
             adata_stim, groupby="cell_type", use_raw=False, return_all_lrs=True, ver
         )

         ##This step calls cellphoneDB from liana : https://saezlab.github.io/liana/a
         ## Liana can run different methods for interrogating cell cell communicatior
         ## liana takes Seurat and SingleCellExperiment objects as input, containing
```

```
Using `.X`!
Converting mat to CSR format
227 features of mat are empty, they will be removed.
0.46 of entities in the resource are missing from the data.
/Library/Frameworks/Python.framework/Versions/3.12/lib/python3.12/site-packa
ges/anndata/_core/anndata.py:430: FutureWarning: The dtype argument is depre
cated and will be removed in late 2024.
/Library/Frameworks/Python.framework/Versions/3.12/lib/python3.12/site-packa
ges/pandas/core/indexing.py:1857: ImplicitModificationWarning: Trying to mod
ify attribute `.obs` of view, initializing view as actual.
/Library/Frameworks/Python.framework/Versions/3.12/lib/python3.12/site-packa
ges/liana/method/_pipe_utils/_pre.py:150: FutureWarning: The default of obse
rved=False is deprecated and will be changed to True in a future version of
pandas. Pass observed=False to retain current behavior or observed=True to a
dopt the future default and silence this warning.
Generating ligand-receptor stats for 12301 samples and 15474 features
```

```
/Library/Frameworks/Python.framework/Versions/3.12/lib/python3.12/site-packa
ges/liana/method/_pipe_utils/_reassemble_complexes.py:58: FutureWarning: A v
alue is trying to be set on a copy of a DataFrame or Series through chained
assignment using an inplace method.
The behavior will change in pandas 3.0. This inplace method will never work
because the intermediate object on which we are setting values always behave
s as a copy.

For example, when doing 'df[col].method(value, inplace=True)', try using 'd
f.method({col: value}, inplace=True)' or df[col] = df[col].method(value) ins
tead, to perform the operation inplace on the original object.


/Library/Frameworks/Python.framework/Versions/3.12/lib/python3.12/site-packa
ges/liana/method/_pipe_utils/_reassemble_complexes.py:58: FutureWarning: Dow
ncasting object dtype arrays on .fillna, .ffill, .bfill is deprecated and wi
ll change in a future version. Call result.infer_objects(copy=False) instea
d. To opt-in to the future behavior, set `pd.set_option('future.no_silent_do
wncasting', True)`
/Library/Frameworks/Python.framework/Versions/3.12/lib/python3.12/site-packa
ges/liana/method/_pipe_utils/_reassemble_complexes.py:59: FutureWarning: A v
alue is trying to be set on a copy of a DataFrame or Series through chained
assignment using an inplace method.
The behavior will change in pandas 3.0. This inplace method will never work
because the intermediate object on which we are setting values always behave
s as a copy.

For example, when doing 'df[col].method(value, inplace=True)', try using 'd
f.method({col: value}, inplace=True)' or df[col] = df[col].method(value) ins
tead, to perform the operation inplace on the original object.


100%|██████████████████████████████████████| 1000/1000 [00:02<00:00, 434.26i
t/s]
```

In [14]:
```
##exploring the results
adata_stim.uns["liana_res"].head()


## each row indicates a ligand-receptor pair with a different combination of
## pvalue indicates specificity
## lr_means indicates the magnitude, strenght of the interaction
## lr_means (ligand-receptor) means is the average of ligand mean and recept
## lrs_to_keep indicate rows (ligand-receptor pairs) to keep based on the pv
## props: represents the proportion of cells that express the entity
```

Out[14]:

	ligand	ligand_complex	ligand_means	ligand_props	receptor	receptor_comp
56717	B2M	B2M	4.431647	1.0	CD3D	CD
55149	B2M	B2M	4.359549	1.0	CD3D	CD
44181	B2M	B2M	4.431647	1.0	KLRD1	KLF
6541	B2M	B2M	4.431647	1.0	CD3D	CD
42613	B2M	B2M	4.359549	1.0	KLRD1	KLF

In [15]:
```
##filtering the results to only results the source (ligand) from CD8 T cells
## we will use this output to create a network in Cytoscape for it.
df = adata_stim.uns["liana_res"]
df2 = df.loc[(df['source'] == 'CD8 T cells') &  (df['cellphone_pvals'] <= 0.
df2.shape
df2.to_csv("cellphoneDB_final.csv")
```

In [16]:
```
##overview of the filtered results:
df2.head()
```

Out[16]:

	ligand	ligand_complex	ligand_means	ligand_props	receptor	receptor_comp
44181	B2M	B2M	4.431647	1.0	KLRD1	KLF
6541	B2M	B2M	4.431647	1.0	CD3D	CD
4973	B2M	B2M	4.359549	1.0	CD3D	CD
44175	B2M	B2M	4.431647	1.0	CD247	CD2
44176	B2M	B2M	4.431647	1.0	KLRC1	KLF

PART2

We have learned to run CellPhoneDB from the Liana package.

It has a lot of advantages as it is easy to run and the result of cellphoneDB can be aggregated with other methods as well.

However, the disadvantage of running it from Liana is that we can't have the choices of options or database version.

Therefore in this section, we will learn how to run it from CellPhoneDB directly.

In [17]:
```
##import necessary libraries for part2
import pandas as pd
import glob
import os
import sys
```

In [18]:
```
##look at different cell phone DB version for ligand-receptor database
from IPython.display import HTML, display
from cellphonedb.utils import db_releases_utils

display(HTML(db_releases_utils.get_remote_database_versions_html()['db_relea
```

Version	Release date
v5.0.0	2023-10-31
v4.1.0	2023-03-09

In [19]:
```
# -- Set version of the database
cpdb_version = 'v5.0.0'
```

In [20]:
```
# -- Path where the input files to generate the database are located
#cpdb_target_dir = os.path.join('/home/jovyan/cpdb_tutorial/db/test', cpdb_v
cpdb_target_dir = os. getcwd()
cpdb_target_dir
#os.listdir(cpdb_target_dir)
```

Out[20]:
```
'/Users/veronique'
```

In [21]:
```
from cellphonedb.utils import db_utils

db_utils.download_database(cpdb_target_dir, cpdb_version)
```

```
Downloaded cellphonedb.zip into /Users/veronique
Downloaded complex_input.csv into /Users/veronique
Downloaded gene_input.csv into /Users/veronique
Downloaded interaction_input.csv into /Users/veronique
Downloaded protein_input.csv into /Users/veronique
Downloaded uniprot_synonyms.tsv into /Users/veronique/sources
Downloaded transcription_factor_input.csv into /Users/veronique/sources
```

Note: The CellPhoneDB was downloaded in my current directory. For better organization, I moved all these files to a folder that will contain all my files necessary to run CellPhoneDB: /Users/veronique/Downloads/cellphonedb

In [22]:
```
## I set up the path to this folder here:
pd.set_option('display.max_columns', 100)
mypath = '/Users/veronique/Downloads/cellphonedb'
```

Preparing the input data

- CellPhoneDB takes 5 input:
- cpdb_file_path = path to the downloaded cellphoneDb database
- meta_file_path = path to the downloaded metadata that will tell the different cell types
- counts_file_path = path to the scRNA count data stored in a h5ad object
- microenvs_file_path = path to the microenvironment that will tell which cells are from the stimulated samples or not
- out_path = path where the cellphoneDB results will be stored

In [23]:
```
## prepare input data
#write the metadata to local computer (we will use later)

adata = sc.read(
    "kang_counts_25k.h5ad", backup_url="https://figshare.com/ndownloader/fil
)
adata


tosave = adata.obs[[ "cell_type"]]
tosave.to_csv("metadadata25KPBMC.tsv", sep="\t")

tosave = adata.obs[["cell_type", "label"]]
tosave.to_csv("microenvironment.tsv", sep="\t")
```

In [24]:
```
cpdb_file_path = '/Users/veronique/Downloads/cellphonedb/cellphonedb.zip'
meta_file_path = '/Users/veronique/Downloads/cellphonedb/metadadata25KPBMC.t
counts_file_path = '/Users/veronique/Downloads/cellphonedb/kang.h5ad'
microenvs_file_path = '/Users/veronique/Downloads/cellphonedb/microenvironme
out_path = '/Users/veronique/Downloads/cellphonedb/'
```

In [31]:
```
##run cellphoneDB method 1 (basic method)

from cellphonedb.src.core.methods import cpdb_analysis_method

cpdb_results = cpdb_analysis_method.call(
    cpdb_file_path = cpdb_file_path,            # mandatory: CellphoneDB data
    meta_file_path = meta_file_path,            # mandatory: tsv file definin
    counts_file_path = counts_file_path,        # mandatory: normalized count
    counts_data = 'hgnc_symbol',                # defines the gene annotation
    #microenvs_file_path = microenvs_file_path, # optional (default: None):
    score_interactions = True,                  # optional: whether to score
    output_path = mypath,                     # Path to save results    micro
    separator = '|',                            # Sets the string to employ t
    threads = 5,                                # number of threads to use in
    threshold = 0.1,                            # defines the min % of cells
    result_precision = 3,                       # Sets the rounding for the m
    debug = False,                              # Saves all intermediate tabl
    output_suffix = None                        # Replaces the timestamp in t
)
```

```
[ ][CORE][08/06/24-11:32:16][INFO] [Non Statistical Method] Threshold:0.1 Pr
ecision:3
Reading user files...
The following user files were loaded successfully:
/Users/veronique/Downloads/cellphonedb/kang.h5ad
/Users/veronique/Downloads/cellphonedb/metadadata25KPBMC.tsv
/Users/veronique/Downloads/cellphonedb/microenvironment.tsv
[ ][CORE][08/06/24-11:32:17][INFO] Running Basic Analysis
[ ][CORE][08/06/24-11:32:17][INFO] Limiting cluster combinations using micro
environments
[ ][CORE][08/06/24-11:32:17][INFO] Building results
[ ][CORE][08/06/24-11:32:17][INFO] Scoring interactions: Filtering genes per
cell type..
100%|██████████████████████████████████████████████| 8/8 [00:00<00:00, 83.67i
t/s]
[ ][CORE][08/06/24-11:32:17][INFO] Scoring interactions: Calculating mean ex
pression of each gene per group/cell type..
100%|█████████████████████████████████████████████| 8/8 [00:00<00:00, 277.35i
t/s]
/Library/Frameworks/Python.framework/Versions/3.12/lib/python3.12/site-packa
ges/cellphonedb/utils/scoring_utils.py:138: FutureWarning: A value is trying
to be set on a copy of a DataFrame or Series through chained assignment usin
g an inplace method.
The behavior will change in pandas 3.0. This inplace method will never work
because the intermediate object on which we are setting values always behave
s as a copy.

For example, when doing 'df[col].method(value, inplace=True)', try using 'd
f.method({col: value}, inplace=True)' or df[col] = df[col].method(value) ins
tead, to perform the operation inplace on the original object.


[ ][CORE][08/06/24-11:32:18][INFO] Scoring interactions: Calculating scores
for all interactions and cell types..
100%|████████████████████████████████████████████| 64/64 [00:02<00:00, 24.28i
t/s]
Saved means_result to /Users/veronique/Downloads/cellphonedb/simple_analysis
_means_result_06_08_2024_113220.txt
Saved deconvoluted to /Users/veronique/Downloads/cellphonedb/simple_analysis
_deconvoluted_06_08_2024_113220.txt
Saved deconvoluted_percents to /Users/veronique/Downloads/cellphonedb/simple
_analysis_deconvoluted_percents_06_08_2024_113220.txt
Saved interaction_scores to /Users/veronique/Downloads/cellphonedb/simple_an
alysis_interaction_scores_06_08_2024_113220.txt
```

Note: The CellPhoneDB output are saved on the local computer in the folder that is defined in the variable "mypath".

In [33]:
```
##run cellphoneDB method 2 (statistical method)
from cellphonedb.src.core.methods import cpdb_statistical_analysis_method

cpdb_results = cpdb_statistical_analysis_method.call(
        cpdb_file_path = cpdb_file_path,
        meta_file_path = meta_file_path,
        counts_file_path = counts_file_path,
        counts_data = 'hgnc_symbol',
        #active_tfs_file_path = active_tf.txt,
        #microenvs_file_path = microenvs_file_path
        score_interactions = True,
        threshold = 0.1,
        output_path = out_path,
        result_precision = 3,                    # Sets the rounding for t
        debug = False,                           # Saves all intermediate
         output_suffix = None)
```

```
Reading user files...
The following user files were loaded successfully:
/Users/veronique/Downloads/cellphonedb/kang.h5ad
/Users/veronique/Downloads/cellphonedb/metadadata25KPBMC.tsv
[ ][CORE][08/06/24-11:46:09][INFO] [Cluster Statistical Analysis] Threshold:
0.1 Iterations:1000 Debug-seed:-1 Threads:4 Precision:3
[ ][CORE][08/06/24-11:46:10][INFO] Running Real Analysis
[ ][CORE][08/06/24-11:46:10][INFO] Running Statistical Analysis
100%|███████████████████████████████████████| 1000/1000 [01:42<00:00,  9.72i
t/s]
[ ][CORE][08/06/24-11:47:53][INFO] Building Pvalues result
[ ][CORE][08/06/24-11:47:53][INFO] Building results
[ ][CORE][08/06/24-11:47:53][INFO] Scoring interactions: Filtering genes per
cell type..
100%|█████████████████████████████████████████████| 8/8 [00:00<00:00, 68.49i
t/s]
[ ][CORE][08/06/24-11:47:53][INFO] Scoring interactions: Calculating mean ex
pression of each gene per group/cell type..
100%|████████████████████████████████████████████| 8/8 [00:00<00:00, 217.69i
t/s]
[ ][CORE][08/06/24-11:47:53][INFO] Scoring interactions: Calculating scores
for all interactions and cell types..
100%|███████████████████████████████████████████| 64/64 [00:02<00:00, 22.39i
t/s]
Saved deconvoluted to /Users/veronique/Downloads/cellphonedb/statistical_ana
lysis_deconvoluted_06_08_2024_114756.txt
Saved deconvoluted_percents to /Users/veronique/Downloads/cellphonedb/statis
tical_analysis_deconvoluted_percents_06_08_2024_114756.txt
Saved means to /Users/veronique/Downloads/cellphonedb/statistical_analysis_m
eans_06_08_2024_114756.txt
Saved pvalues to /Users/veronique/Downloads/cellphonedb/statistical_analysis
_pvalues_06_08_2024_114756.txt
Saved significant_means to /Users/veronique/Downloads/cellphonedb/statistica
l_analysis_significant_means_06_08_2024_114756.txt
Saved interaction_scores to /Users/veronique/Downloads/cellphonedb/statistic
al_analysis_interaction_scores_06_08_2024_114756.txt
```

How to interpret the output results

- https://cellphonedb.readthedocs.io/en/latest/RESULTS-DOCUMENTATION.html#p-value-pvalues-txt-mean-means-txt-significant-mean-significant-means-txt-and-relevant-interactions-relevant-interactions-txt

- https://cellphonedb.readthedocs.io/en/latest/RESULTS-DOCUMENTATION.html#interpreting-the-outputs

Table guide from: https://github.com/Teichlab/cellphonedb/blob/master/Docs/RESULTS-DOCUMENTATION.md

P-value (pvalues.txt), Mean (means.txt), Significant mean (significant_means.txt)

- id_cp_interaction: Unique CellPhoneDB identifier for each interaction stored in the database.
- interacting_pair: Name of the interacting pairs separated by "|".
- partner A or B: Identifier for the first interacting partner (A) or the second (B). It could be: UniProt (prefix `simple:`) or complex (prefix `complex:`)
- gene A or B: Gene identifier for the first interacting partner (A) or the second (B). The identifier will depend on the input user list.
- secreted: True if one of the partners is secreted.
- Receptor A or B: True if the first interacting partner (A) or the second (B) is annotated as a receptor in our database.
- annotation_strategy: Curated if the interaction was annotated by the CellPhoneDB developers. Otherwise, the name of the database where the interaction has been downloaded from.
- is_integrin: True if one of the partners is integrin.
- rank: Total number of significant p-values for each interaction divided by the number of cell type-cell type comparisons. (Only in significant_means.txt)
- means: Mean values for all the interacting partners: mean value refers to the total mean of the individual partner average expression values in the corresponding interacting pairs of cell types. If one of the mean values is 0, then the total mean is set to 0. (Only in mean.txt)
- p.values: p-values for the all the interacting partners: p.value refers to the enrichment of the interacting ligand-receptor pair in each of the interacting pairs of cell types. (Only in pvalues.txt)
- significant_mean: Significant mean calculation for all the interacting partners. If p.value < 0.05, the value will be the mean. Alternatively, the value is set to 0. (Only in significant_means.txt)

Importantly, the interactions are not symmetric. Partner A expression is considered on the first cluster, and partner B expression is considered on the second cluster. In other words:

- clusterA_clusterB = clusterA expressing partner A and clusterB expressing partner B.
- clusterA_clusterB and clusterB_clusterA values will be different.

Deconvoluted (deconvoluted.txt)

- gene_name: Gene identifier for one of the subunits that are participating in the interaction defined in “means.csv” file. The identifier will depend on the input of the user list.
- uniprot: UniProt identifier for one of the subunits that are participating in the interaction defined in “means.csv” file.
- is_complex: True if the subunit is part of a complex. Single if it is not, complex if it is.
- protein_name: Protein name for one of the subunits that are participating in the interaction defined in “means.csv” file.
- complex_name: Complex name if the subunit is part of a complex. Empty if not.
- id_cp_interaction: Unique CellPhoneDB identifier for each of the interactions stored in the database.
- mean: Mean expression of the corresponding gene in each cluster.