
celloracle

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CellOracle is a python library for the analysis of Gene Regulatory Network with single cell data.

Source code is available at [celloracle GitHub repository](#)

For more information, please read our bioarxiv preprint: [CellOracle: Dissecting cell identity via network inference and in silico gene perturbation](#)

Note:

Documentation is also available as a pdf file.

[pdf documentation](#)

Warning: CellOracle is still under development. It is beta version and functions in this package may change in the future release.

**CHAPTER
ONE**

CONTENTS

1.1 Installation

`celloracle` uses several python libraries and R library. Please follow this guide below to install the dependent software of `celloracle`.

1.1.1 Docker image

- Not available now. Coming soon.

1.1.2 System Requirements

- Operating system: macOS or linux are highly recommended. `celloracle` was developed and tested in Linux and macOS.
- We found that the `celloracle` calculation may be EXTREMELY SLOW under an environment of Windows Subsystem for Linux (WSL). We do not recommend using WSL.
- While you can install `celloracle` in Windows OS, please do so at your own risk and responsibility. We DO NOT provide any support for the use in the Windows OS.
- Memory: 8 G byte or more. Memory usage also depends on your scRNA-seq data. Especially in silico perturbation requires large amount of memory.
- CPU: Core i5 or better processor. GRN inference supports multicore calculation. Higher number of CPU cores enables faster calculation.

1.1.3 Python Requirements

- `celloracle` was developed with python 3.6. We do not support python 2.7x or python <=3.5.
- Please install all dependent libraries before installing `celloracle` according to the instructions below.
- `celloracle` is still beta version and it is not available through PyPI or anaconda distribution yet. Please install `celloracle` from GitHub repository according to the instruction below.

0. (Optional) Make a new environment

This step is optional. Please make a new python environment for `celloracle` and install dependent libraries in it if you get some software conflicts.

```
conda create -n celloracle_env python=3.6
conda activate celloracle_env
```

1. Add conda channels

Installation of some libraries requires non-default anaconda channels. Please add the channels below. Instead, you can explicitly enter the channel when you install a library.

```
conda config --add channels defaults
conda config --add channels bioconda
conda config --add channels conda-forge
```

2. Install velocyto

Please install velocyto with the following commands or [the author's instruction](#).

```
conda install numpy scipy cython numba matplotlib scikit-learn h5py click pysam gcc
→ llvm
```

Then

```
pip install velocyto
```

It was reported that some compile errors might occur during the installation of velocyto on MacOS. Various errors were reported and you need to find the best solution depending on your error. You may find the solution with these links below.

- [Solution 1: Install Xcode](#). Please try this first.
- [Solution 2: Install macOS_SDK_headers](#). This solution is needed in addition to Solution-1 if your OS is MacOS Mojave.
- [Solution 3](#). This is the solution reported by a CellOracle user. Thank you very much!
- [Other solutions on Velocyto github issue page](#)

3. Install scanpy

Please install scanpy with the following commands or [the author's instruction](#).

```
conda install seaborn statsmodels numba pytables python-igraph louvain
```

Then

```
pip install scanpy
```

4. Install gimmemotifs

Please install gimmemotifs with the following commands or [the author's instruction](#).

```
conda install genomepy=0.5.5 gimmemotifs=0.13.1
```

5. Install other python libraries

Please install other python libraries below with the following commands.

```
conda install goatools pyarrow tqdm joblib jupyter
```

6. install celloracle from github

```
pip install git+https://github.com/morris-lab/CellOracle.git
```

1.1.4 R requirements

celloracle use R libraries for the network analysis and scATAC-seq analysis. Please install [R](#) (≥ 3.5) and R libraries below according to the author's instruction.

Seurat

Please install Seurat with the following r-script or [the author's instruction](#). celloracle is compatible with both Seurat V2 and V3. If you use only scanpy for the scRNA-seq preprocessing and do not use Seurat , you can skip installation of Seurat.

In R console,

```
install.packages('Seurat')
```

Cicero

Please install Cicero with the following r-script or [the author's instruction](#). If you do not have scATAC-seq data and plan to use celloracle's base GRN, you do not need to install Cicero.

In R console,

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

igraph

Please install igraph with the following r-script or [the author's instruction](#).

In R console,

```
install.packages("igraph")
```

linkcomm

Please install linkcomm with the following r-script or the author's instruction .

In R console,

```
install.packages("linkcomm")
```

rnetcarto

Please install `rnetcarto` with the following r-script or [the author's instruction](#).

In R console,

```
install.packages("rnetcarto")
```

Check installation

These R libraries above are necessary for the network analysis in celloracle. You can check installation using celloracle's function.

In python console,

```
import celloracle as co
co.network_analysis.test_R_libraries_installation()
```

Please make sure that all R libraries are installed. The following message will be shown when all R libraries are appropriately installed.

```
checking R library installation: igraph -> OK
checking R library installation: linkcomm -> OK
checking R library installation: rnetcarto -> OK
```

1.2 Tutorial

The analysis proceeds through multiple steps. Please run the notebooks sequentially. If you do not have ATAC-seq data and want to use the default TF binding information, you can skip the first and second step and start from the third step.

Please refer to the `celloracle` paper for scientific premise and the detail of the algorithm of celloracle.

The jupyter notebook files in this tutorial are available [here](#).

1.2.1 ATAC-seq data preprocessing

In this step, we process scATAC-seq data (or bulk ATAC-seq data) to obtain the accessible promoter/enhancer DNA sequence. We can get the active proximal promoter/enhancer genome sequences by picking up the ATAC-seq peaks that exist around the transcription starting site (TSS). Distal cis-regulatory elements can be picked up using [Cicero](#). Cicero analyzes scATAC-seq data to calculate a co-accessible score between peaks. We can identify cis-regulatory elements using Cicero's co-access score and TSS information.

If you have bulk ATAC-seq data instead of scATAC-data, we'll get only the proximal promoter/enhancer genome sequences.

A. Extract TF binding information from scATAC-seq data

If you have scATAC-seq data, you can get information on the distal cis-regulatory elements. This step uses Cicero and does not use celloracle. Please refer to [the documentation of Cicero](#) for the detailed usage.

R notebook

0. Import library

```
[2]: library(cicero)
```

1. Prepare data

In this tutorial we'll use acATAC-seq data from the 10x genomics database. You do not need to download these data if you analyze your own scATAC-seq data.

```
[4]: # Create folder to store data
dir.create("data")

# Download demo dataset from 10x genomics
system("wget -O data/matrix.tar.gz http://cf.10xgenomics.com/samples/cell-atac/1.1.0/
        ↪atac_v1_E18_brain_fresh_5k/atac_v1_E18_brain_fresh_5k_filtered_peak_bc_matrix.tar.gz
        ↪")

# Unzip data
system("tar -xvf data/matrix.tar.gz -C data")
```

```
[6]: # You can substitute the data path below with the data path of your scATAC data.
data_folder <- "data/filtered_peak_bc_matrix"

# Create a folder to save results
output_folder <- "cicero_output"
dir.create(output_folder)
```

2. Load data and make Cell Data Set (CDS) object

2.1. Process data to make CDS object

```
[7]: # Read in matrix data using the Matrix package
indata <- Matrix:::readMM(paste0(data_folder, "/matrix.mtx"))
# binarize the matrix
indata@x[indata@x > 0] <- 1

# Format cell info
cellinfo <- read.table(paste0(data_folder, "/barcodes.tsv"))
row.names(cellinfo) <- cellinfo$V1
names(cellinfo) <- "cells"

# Format peak info
peakinfo <- read.table(paste0(data_folder, "/peaks.bed"))
names(peakinfo) <- c("chr", "bp1", "bp2")
```

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```

peakinfo$site_name <- paste(peakinfo$chr, peakinfo$bp1, peakinfo$bp2, sep="_")
row.names(peakinfo) <- peakinfo$site_name

row.names(indata) <- row.names(peakinfo)
colnames(indata) <- row.names(cellinfo)

# Make CDS
input_cds <- suppressWarnings(newCellDataSet(indata,
                                              phenoData = methods::new("AnnotatedDataFrame", data =
cellinfo),
                                              featureData = methods::new("AnnotatedDataFrame", data =
peakinfo),
                                              expressionFamily=VGAM::binomialff(),
                                              lowerDetectionLimit=0))
input_cds@expressionFamily@vfamily <- "binomialff"
input_cds <- monocle::detectGenes(input_cds)

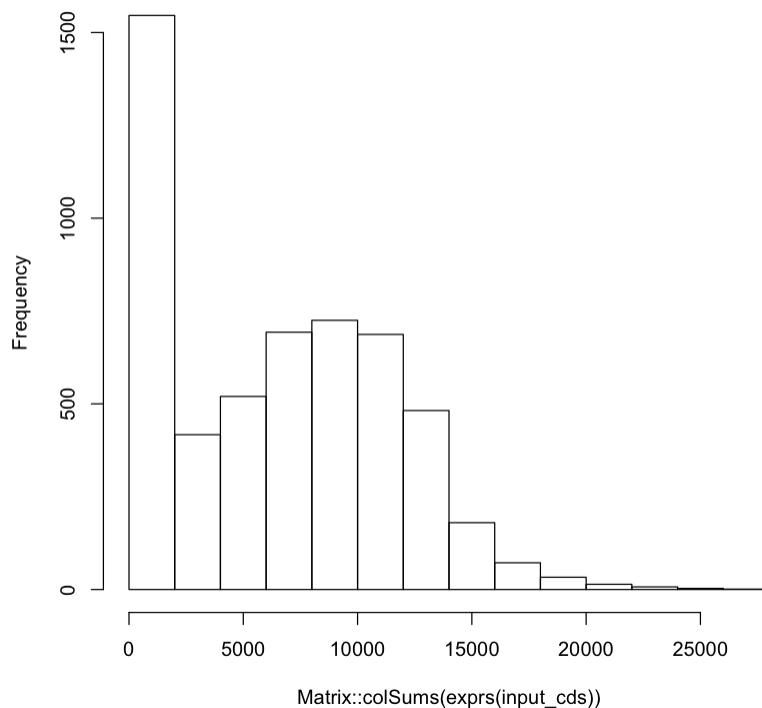
#Ensure there are no peaks included with zero reads
input_cds <- input_cds[Matrix::rowSums(exprs(input_cds)) >= 100,]

```

3. Quality check and Filtering

```
[8]: # Visualize peak_count_per_cell
hist(Matrix::colSums(exprs(input_cds)))
```

Histogram of Matrix::colSums(exprs(input_cds))



```
[9]: # Filter cells by peak_count
max_count <- 15000 # Please change the threshold value according to the distribution_
# of the peak_count of your data
min_count <- 2000 # Please change the threshold value according to the distribution_
# of the peak_count of your data
input_cds <- input_cds[, Matrix::colSums(exprs(input_cds)) >= min_count]
input_cds <- input_cds[, Matrix::colSums(exprs(input_cds)) <= max_count]
```

4. Process cicero-CDS object

```
[10]: # Run cicero to get cis-regulatory networks
set.seed(2017)
input_cds <- detectGenes(input_cds)
input_cds <- estimateSizeFactors(input_cds)

input_cds <- reduceDimension(input_cds, max_components = 2, verbose=T, scaling = FALSE,
#relative_expr=FALSE,
reduction_method = 'tSNE', norm_method = "none")

tsne_coords <- t(reducedDimA(input_cds))
row.names(tsne_coords) <- row.names(pData(input_cds))
cicero_cds <- make_cicero_cds(input_cds, reduced_coordinates = tsne_coords)

# Save cicero-CDS object if you want.
#saveRDS(cicero_cds, paste0(output_folder, "/cicero_cds.Rds"))
```

Remove noise by PCA ...

Reduce dimension by tSNE ...

Overlap QC metrics:

Cells per bin: 50
Maximum shared cells bin-bin: 44
Mean shared cells bin-bin: 0.76256263875674
Median shared cells bin-bin: 0

5. Run cicero to get cis-regulatory connection scores

```
[11]: # Import genome length, which is needed for the function, run_cicero
mm10_chromosome_length <- read.table("./mm10_chromosome_length.txt")

# Run the main function
conns <- run_cicero(cicero_cds, mm10_chromosome_length) # Takes a few minutes to run

# Check results
head(conns)

[1] "Starting Cicero"
[1] "Calculating distance_parameter value"
[1] "Running models"
[1] "Assembling connections"
[1] "Done"
```

	Peak1 <fct>	Peak2 <fct>	coaccess <dbl>
A data.frame: 6 × 3	2 chr1_3094484_3095479	chr1_3113499_3113979	-0.316289004
	3 chr1_3094484_3095479	chr1_3119478_3121690	-0.419240532
	4 chr1_3094484_3095479	chr1_3399730_3400368	-0.050867246
	5 chr1_3113499_3113979	chr1_3094484_3095479	-0.316289004
	7 chr1_3113499_3113979	chr1_3119478_3121690	0.370342744
	8 chr1_3113499_3113979	chr1_3399730_3400368	-0.009276026

6. Save results for next step

```
[ ]: all_peaks <- row.names(exprs(input_cds))
write.csv(x = all_peaks, file = paste0(output_folder, "/all_peaks.csv"))
write.csv(x = conns, file = paste0(output_folder, "/cicero_connections.csv"))
```

Next, the results of Cicero analysis will be processed to make TSS annotations.

Python notebook

In this notebook, we process the results of cicero analysis to get active promoter/enhancer DNA peaks. First, we pick up peaks around the transcription starting site (TSS). Second, we merge cicero data with the peaks around TSS. Then we remove peaks that have a weak connection to TSS peak so that the final product includes TSS peaks and peaks that have a strong connection with the TSS peaks. We use this information as an active promoter/enhancer elements.

0. Import libraries

```
[1]: import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
%matplotlib inline

import seaborn as sns

import os, sys, shutil, importlib, glob
from tqdm.notebook import tqdm

from celloracle import motif_analysis as ma
```

```
[2]: %config InlineBackend.figure_format = 'retina'

plt.rcParams['figure.figsize'] = [6, 4.5]
plt.rcParams["savefig.dpi"] = 300
```

1. Load data made with cicero

```
[3]: # Load all peaks
peaks = pd.read_csv("cicero_output/all_peaks.csv", index_col=0)
peaks = peaks.x.values
peaks
```

```
[3]: array(['chr1_3094484_3095479', 'chr1_3113499_3113979',
       'chr1_3119478_3121690', ..., 'chrY_90804622_90805450',
       'chrY_90808626_90809117', 'chrY_90810560_90811167'], dtype=object)
```

```
[4]: # Load cicero results
cicero_connections = pd.read_csv("cicero_output/cicero_connections.csv", index_col=0)
cicero_connections.head()

/home/k/anaconda3/envs/test/lib/python3.6/site-packages/numpy/lib/arraysetops.py:568:_
→FutureWarning: elementwise comparison failed; returning scalar instead, but in the_
→future will perform elementwise comparison
    mask |= (ar1 == a)

[4]:
```

	Peak1	Peak2	coaccess
2	chr1_3094484_3095479	chr1_3113499_3113979	-0.316289
3	chr1_3094484_3095479	chr1_3119478_3121690	-0.419241
4	chr1_3094484_3095479	chr1_3399730_3400368	-0.050867
5	chr1_3113499_3113979	chr1_3094484_3095479	-0.316289
7	chr1_3113499_3113979	chr1_3119478_3121690	0.370343

2. Make TSS annotation

IMPORTANT: Please make sure that you are setting correct reference genome.

```
[5]: tss_annotated = ma.get_tss_info(peak_str_list=peaks, ref_genome="mm10")

# Check results
tss_annotated.tail()

que bed peaks: 72402
tss peaks in que: 16987

[5]:
```

	chr	start	end	gene_short_name	strand
16982	chr1	55130650	55132118	Mob4	+
16983	chr6	94499875	94500767	S1c25a26	+
16984	chr19	45659222	45660823	Fbxw4	-
16985	chr12	100898848	100899597	Gpr68	-
16986	chr4	129491262	129492047	Fam229a	-

3. Integrate TSS info and cicero connections

The output file after the integration process has three columns; “peak_id”, “gene_short_name”, and “coaccess”. “peak_id” is either the TSS peak or the peaks that have a connection with the TSS peak. “gene_short_name” is the gene name that associated with the TSS site. “coaccess” is the co-access score between a peak and TSS peak. Note, the TSS peak is indicated by a score of 1.

```
[8]: integrated = ma.integrate_tss_peak_with_cicero(tss_peak=tss_annotated,
                                                 cicero_connections=cicero_connections)
print(integrated.shape)
integrated.head()

(263279, 3)

[8]:
```

	peak_id	gene_short_name	coaccess
0	chr10_100015291_100017830	Kitl	1.000000

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1	chr10_100018677_100020384	Kitl	0.086299
2	chr10_100050858_100051762	Kitl	0.034558
3	chr10_100052829_100053395	Kitl	0.167188
4	chr10_100128086_100128882	Tmtc3	0.022341

4. Filter peaks

Remove peaks that have weak coaccess score.

```
[9]: peak = integrated[integrated.coaccess >= 0.8]
peak = peak[["peak_id", "gene_short_name"]].reset_index(drop=True)
```

```
[10]: print(peak.shape)
peak.head()
(15680, 2)
```

```
[10]:          peak_id gene_short_name
0  chr10_100015291_100017830        Kitl
1  chr10_100486534_100488209        Tmtc3
2  chr10_100588641_100589556  4930430F08Rik
3  chr10_100741247_100742505        Gm35722
4  chr10_101681379_101682124        Mgat4c
```

5. Save data

Save the promoter/enhancer peak.

```
[11]: peak.to_parquet("peak_file.parquet")
```

-> go to next notebook

B. Extract TF binding information from bulk ATAC-seq data or Chip-seq data

Bulk DNA-seq data can be used to get the accessible promoter/enhancer sequences.

Python notebook

0. Import libraries

```
[1]: import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
%matplotlib inline

import seaborn as sns

import os, sys, shutil, importlib, glob
from tqdm import tqdm_notebook as tqdm

%config InlineBackend.figure_format = 'retina'
```

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```
plt.rcParams['figure.figsize'] = [6, 4.5]
plt.rcParams["savefig.dpi"] = 300
```

```
[2]: # Import celloracle function
from celloracle import motif_analysis as ma
```

1. Load bed file

Import ATAC-seq bed file. This script can also be used with DNase-seq or Chip-seq data.

```
[3]: file_path_of_bed_file = "data/all_peaks.bed"
```

```
[4]: # Load bed_file
bed = ma.read_bed(file_path_of_bed_file)
print(bed.shape)
bed.head()

(436206, 4)

[4]:   chrom      start        end      seqname
0  chr1  3002478  3002968  chr1_3002478_3002968
1  chr1  3084739  3085712  chr1_3084739_3085712
2  chr1  3103576  3104022  chr1_3103576_3104022
3  chr1  3106871  3107210  chr1_3106871_3107210
4  chr1  3108932  3109158  chr1_3108932_3109158
```

```
[6]: # Convert bed file into peak name list
peaks = ma.process_bed_file.df_to_list_peakstr(bed)
peaks
```

```
[6]: array(['chr1_3002478_3002968', 'chr1_3084739_3085712',
       'chr1_3103576_3104022', ..., 'chrY_631222_631480',
       'chrY_795887_796426', 'chrY_2397419_2397628'], dtype=object)
```

2. Make TSS annotation

IMPORTANT: Please make sure that you are setting the correct ref genome!

```
[7]: tss_annotated = ma.get_tss_info(peak_str_list=peaks, ref_genome="mm9")
```

```
# Check results
tss_annotated.tail()
```

```
que bed peaks: 436206
tss peaks in que: 24822
```

```
[7]:      chr      start        end gene_short_name strand
24817  chr2  60560211  60561602          Itgb6      -
24818  chr15  3975177  3978654          BC037032      -
24819  chr14  67690701  67692101          Ppp2r2a      -
24820  chr17  48455247  48455773  B430306N03Rik      +
24821  chr10  59861192  59861608          Gm17455      +
```

```
[9]: # Change format
peak_id_tss = ma.process_bed_file.df_to_list_peakstr(tss_annotated)
tss_annotated = pd.DataFrame({"peak_id": peak_id_tss,
                               "gene_short_name": tss_annotated.gene_short_name.values})
    ↵
tss_annotated = tss_annotated.reset_index(drop=True)
print(tss_annotated.shape)
tss_annotated.head()

(24822, 2)

[9]:          peak_id gene_short_name
0  chr7_50691730_50692032      Nkg7
1  chr7_50692077_50692785      Nkg7
2  chr13_93564413_93564836     Thbs4
3  chr13_14613429_14615645     Hecw1
4  chr3_99688753_99689665    Spag17
```

3. Save data

```
[10]: tss_annotated.to_parquet("peak_file.parquet")
```

-> go to next notebook

1.2.2 Transcription factor binding motif scan

We identified accessible Promoter/enhancer DNA regions using ATAC-seq data. Next, we will obtain a list of TFs for each target gene by scanning the regulatory genomic sequences for TF-binding motifs. In the later GRN inference process, this list will be used to define potential regulatory connections.

Python notebook

0. Import libraries

```
[2]: import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
%matplotlib inline

import seaborn as sns

import os, sys, shutil, importlib, glob
from tqdm.notebook import tqdm

%config InlineBackend.figure_format = 'retina'

plt.rcParams['figure.figsize'] = (15, 7)
plt.rcParams["savefig.dpi"] = 600
```

```
[3]: from celloracle import motif_analysis as ma
from celloracle.utility import save_as_pickled_object
```

1. Load data

```
[4]: # Load annotated peak data.
peaks = pd.read_parquet("../01_ATAC-seq_data_processing/option1_scATAC-seq_data_"
                       "analysis_with_cicero/peak_file.parquet")
peaks.head()

[4]:
```

	peak_id	gene_short_name
0	chr10_100015291_100017830	Kitl
1	chr10_100486534_100488209	Tmtc3
2	chr10_100588641_100589556	4930430F08Rik
3	chr10_100741247_100742505	Gm35722
4	chr10_101681379_101682124	Mgat4c

2. Check data

```
[5]: # Check data
print(f"number of peak: {len(peaks.peak_id.unique())} ")

def getLength(x):
    a, b, c = x["peak_id"].split("_")
    return int(c) - int(b)

df = peaks.apply(lambda x: getLength(x), axis=1)
print(f"mean peak length: {df.values.mean()}")

number of peak: 13919
mean peak length: 1756.1744260204082
```

2.1. Remove short peaks

Short DNA fragment that are less than 5 bases, cannot be used for motif scanning. Therefore, we will remove the short DNA fragments.

```
[6]: peaks = peaks[df>=5]
```

3. Instantiate TFinfo object and search for TF binding motifs

The motif analysis module has a custom class; TFinfo. The TFinfo object converts a peak data into a DNA sequences and scans the DNA sequences searching for TF binding motifs. Then, the results of motif scan will be filtered and converted into either a python dictionary or a depending on your preference. This TF information is necessary for GRN inference.

3.1 check reference genome installation

```
[7]: # PLEASE make sure that you are setting correct ref genome.
ref_genome = "mm10"

ma.is_genome_installed(ref_genome=ref_genome)
```

```
genome mm10 is not installed in this environment.  
Please install genome using genomepy.  
e.g.  
>>> import genomepy  
>>> genomepy.install_genome("mm9", "UCSC")
```

```
[7]: False
```

3.2. Install reference genome (if refgenome is not installed)

```
[9]: import genomepy  
genomepy.install_genome(ref_genome, "UCSC")  
  
downloading from http://hgdownload.soe.ucsc.edu/goldenPath/mm10/bigZips/chromFa.tar.  
→gz...  
done...  
name: mm10  
local name: mm10  
fasta: /home/k/.local/share/genomes/mm10/mm10.fa
```

```
[9]: # check again  
ma.is_genome_installed(ref_genome=ref_genome)
```

```
[9]: True
```

```
[14]: # Instantiate TFinfo object  
tfi = ma.TFinfo(peak_data_frame=peaks, # peak info calculated from ATAC-seq data  
                 ref_genome=ref_genome)
```

4. Scan motifs and save object

This step may take long time

```
[15]: %%time  
# Scan motifs  
tfi.scan(fpr=0.02, verbose=True)  
  
# Save tfinfo object  
tfi.to_hdf5(file_path="test.celloracle.tfinfo")  
  
initiating scanner ...  
  
2019-09-22 23:00:18,604 - INFO - Using background: genome mm10 with length 200  
2019-09-22 23:00:18,986 - INFO - Determining FPR-based threshold  
  
getting DNA sequences ...  
scanning motifs ...  
  
HBox(children=(IntProgress(value=1, bar_style='info', max=1), HTML(value='')))  
  
CPU times: user 52min 23s, sys: 36.8 s, total: 53min  
Wall time: 52min 58s
```

```
[16]: # Check motif scan results  
tfi.scanned_df.head()
```

```
[16]:
```

	seqname	motif_id	factors_direct	\
0	chr10_100015291_100017830	GM.5.0.Homeodomain.0001	TGIF1	
1	chr10_100015291_100017830	GM.5.0.Mixed.0001		
2	chr10_100015291_100017830	GM.5.0.Mixed.0001		
3	chr10_100015291_100017830	GM.5.0.Mixed.0001		
4	chr10_100015291_100017830	GM.5.0.Nuclear_receptor.0002	NR2C2	

	factors_indirect	score	pos	strand
0	ENSG00000234254, TGIF1	10.311002	1003	1
1	SRF, EGR1	7.925873	481	1
2	SRF, EGR1	7.321375	911	-1
3	SRF, EGR1	7.276585	811	-1
4	NR2C2, Nr2c2	9.067331	449	-1

We have the score for each sequence and motif_id pair. In the next step we will filter the motifs with low score.

5. Filtering motifs

```
[17]: # Reset filtering
tfi.reset_filtering()

# Do filtering
tfi.filter_motifs_by_score(threshold=10.5)

# Do post filtering process. Convert results into several file format.
tfi.make_TFinfo_dataframe_and_dictionary(verbose=True)

peaks were filtered: 12934005 -> 2285279
1. converting scanned results into one-hot encoded dataframe.

HBox(children=(IntProgress(value=0, max=13919), HTML(value='')))

2. converting results into dictionaries.
converting scan results into dictionaries...
HBox(children=(IntProgress(value=0, max=14804), HTML(value='')))

HBox(children=(IntProgress(value=0, max=1090), HTML(value='')))
```

6. Get Final results

6.1. Get results as a dictionary

```
[18]: td = tfi.to_dictionary(dictionary_type="targetgene2TFs")
```

6.2. Get results as a dataframe

```
[20]: df = tfi.to_dataframe()
df.head()
```

```
[20]:
```

	peak_id	gene_short_name	9430076c15rik	Ac002126.6	\
0	chr10_100015291_100017830	Kitl	0	0	
1	chr10_100486534_100488209	Tmtc3	0	0	
2	chr10_100588641_100589556	4930430F08Rik	0	0	
3	chr10_100741247_100742505	Gm35722	0	0	
4	chr10_101681379_101682124	Mgat4c	0	0	

	Ac012531.1	Ac226150.2	Afp	Ahr	Ahrr	Aire	...	Znf784	Znf8	Znf816	\
0	0	0	0	1	1	0	...	0	0	0	
1	0	0	0	0	0	0	...	1	0	0	
2	1	0	0	1	1	0	...	0	0	0	
3	0	0	0	0	0	0	...	0	0	0	
4	0	0	0	0	0	0	...	0	0	0	

	Znf85	Zscan10	Zscan16	Zscan22	Zscan26	Zscan31	Zscan4
0	0	0	0	0	0	1	0
1	0	0	0	1	0	0	0
2	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0
4	0	0	0	0	0	0	1

[5 rows x 1092 columns]

7. Save TFinfo as dictionary or dataframe

We'll use this information when making the GRNs. Save the results.

```
[21]:
```

```
folder = "TFinfo_outputs"
os.makedirs(folder, exist_ok=True)

# save TFinfo as a dictionary
td = tfi.to_dictionary(dictionary_type="targetgene2TFs")
save_as_pickled_object(td, os.path.join(folder, "TFinfo_targetgene2TFs.pickled"))

# save TFinfo as a dataframe
df = tfi.to_dataframe()
df.to_parquet(os.path.join(folder, "TFinfo_dataframe.parquet"))
```

1.2.3 Single-cell RNA-seq data preprocessing

Network analysis and simulation in celloracle will be performed using scRNA-seq data. The scRNA-seq data should include the components below.

- Gene expression matrix; mRNA counts before scaling and transformation.
- Clustering results.
- Dimensional reduction results.

In addition to these minimum requirements, we highly recommend doing these analyses below in the preprocessing step.

- Data quality check and cell/gene filtering.
- Normalization
- Identification of highly variable genes

We recommend processing scRNA-seq data using either Scipy or Seurat. If you are not familiar with the general workflow of scRNA-seq data processing, please go to [the documentation for Scipy](#) and [the documentation for Seurat](#) before celloracle analysis.

If you already have preprocessed scRNA-seq data, which includes the necessary information above, you can skip this part.

A. scRNA-seq data preprocessing with scanpy

scanpy is a python library for the analysis of scRNA-seq data.

In this tutorial, we introduce an example of scRNA-seq preprocessing for celloracle with `scanpy`. We wrote the notebook based on [one of `scanpy`'s tutorials](#) with some modifications.

Python notebook

0. Import libraries

```
[1]: import os
      import matplotlib.pyplot as plt
      import numpy as np
      import pandas as pd
      import scanpy as sc
```

```
[2]: %matplotlib inline
%config InlineBackend.figure_format = 'retina'
plt.rcParams["savefig.dpi"] = 300
plt.rcParams["figure.figsize"] = [6, 4.5]
```

1. Load data

In this notebook, we will show an example of how to process scRNA-seq data using a scRNA-seq data of hematopoiesis (Paul, F., Arkin, Y., Giladi, A., Jaitin, D. A., Kenigsberg, E., Keren-Shaul, H., et al. (2015). Transcriptional Heterogeneity and Lineage Commitment in Myeloid Progenitors. *Cell*, 163(7), 1663–1677. <http://doi.org/10.1016/j.cell.2015.11.013>). You can easily download this scRNA-seq data with a scanpy function.

Please change the code below if you want to use your data.

```
[3]: # Download dataset. You can change the code below if you use another data.
```

```
adata = sc.datasets.paul15()
```

WARNING: In Scanpy 0.*, this returned logarithmized data. Now it returns non-logarithmized data.

```
... storing 'paul15_clusters' as categorical  
Trying to set attribute `uns` of view, making a copy.
```

2. Filtering

```
[4]: # Only consider genes with more than 1 count  
sc.pp.filter_genes(adata, min_counts=1)
```

3. Normalization

```
[5]: # Normalize gene expression matrix with total UMI count per cell
sc.pp.normalize_per_cell(adata, key_n_counts='n_counts_all')
```

4. Identification of highly variable genes

Removing non-variable genes not only reduces the calculation time during the GRN reconstruction and simulation, but also improve the accuracy of GRN inference. We recommend using the top 2000~3000 variable genes.

```
[6]: # Select top 2000 highly-variable genes
filter_result = sc.pp.filter_genes_dispersion(adata.X,
                                              flavor='cell_ranger',
                                              n_top_genes=2000,
                                              log=False)

# Subset the genes
adata = adata[:, filter_result.gene_subset]

# Renormalize after filtering
sc.pp.normalize_per_cell(adata)
```

Trying to set attribute ` `.obs` of view, making a copy.

5. Log transformation

We will do log transformation scaling because these are necessary for PCA, clustering, and differential gene calculations. However, we also need non-transformed gene expression data in the celloracle analysis. Thus we keep raw count in anndata using the following command before the log transformation.

```
[7]: # keep raw cont data before log transformation
adata.raw = adata

# Log transformation and scaling
sc.pp.log1p(adata)
sc.pp.scale(adata)
```

6. Dimensional reduction

Dimensional reduction is one of the most important parts of the scRNA-seq analysis. Celloracle needs dimensional reduction embeddings to simulate cell transition.

Please choose a proper algorithm for dimensional reduction so that the embedding appropriately represents the data structure. We recommend using one of these dimensional reduction algorithms (or trajectory inference algorithms); UMAP, tSNE, diffusion map, force-directed graph drawing or PAGA.

In this example, we use a combination of four algorithms; diffusion map, force-directed graph drawing, and PAGA.

```
[9]: # PCA
sc.tl.pca(adata, svd_solver='arpack')
```

```
[10]: # Diffusion map
sc.pp.neighbors(adata, n_neighbors=4, n_pcs=20)

sc.tl.diffmap(adata)
# Calculate neighbors again based on diffusionmap
sc.pp.neighbors(adata, n_neighbors=10, use_rep='X_diffmap')
```

7. Clustering

```
[11]: sc.tl.louvain(adata, resolution=0.8)
```

(Optional) Re-calculate Dimensional reduction graph

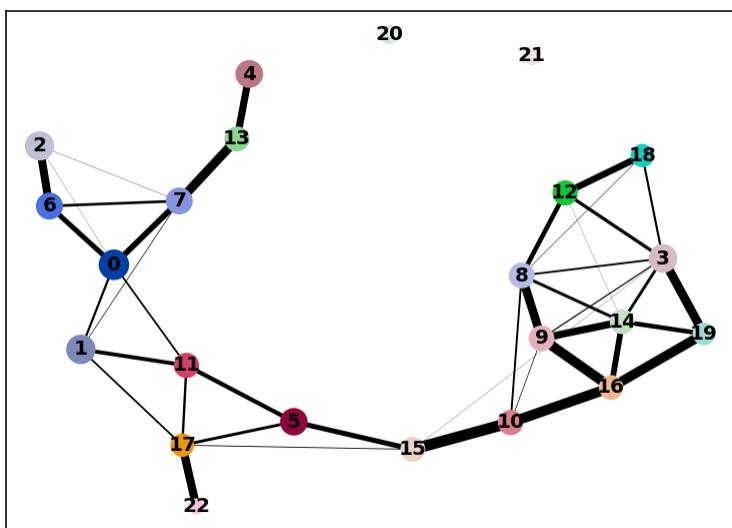
```
[12]: # PAGA graph construction
sc.tl.paga(adata, groups='louvain')
```

```
[13]: # Check current cluster name
cluster_list = adata.obs.louvain.unique()
cluster_list
```

```
[13]: [5, 2, 12, 13, 0, ..., 6, 20, 14, 15, 21]
Length: 23
Categories (23, object): [5, 2, 12, 13, ..., 20, 14, 15, 21]
```

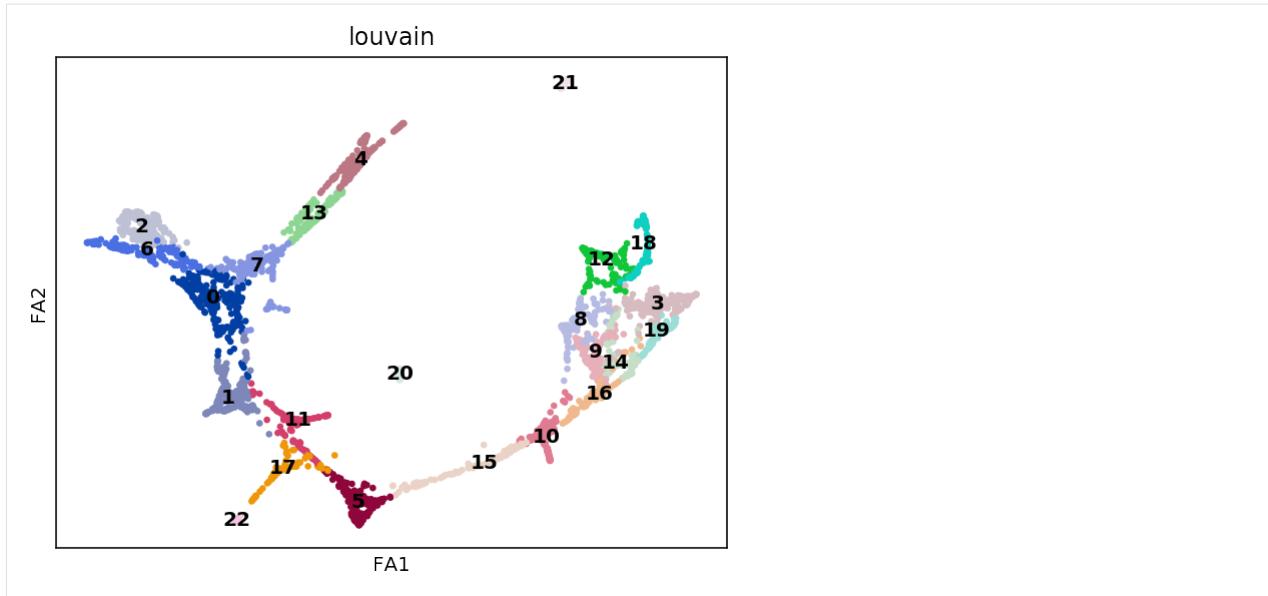
```
[14]: plt.rcParams["figure.figsize"] = [6, 4.5]
```

```
[15]: sc.pl.paga(adata)
```



```
[16]: sc.tl.draw_graph(adata, init_pos='paga', random_state=123)
```

```
[17]: sc.pl.draw_graph(adata, color='louvain', legend_loc='on data')
```



8. Check data

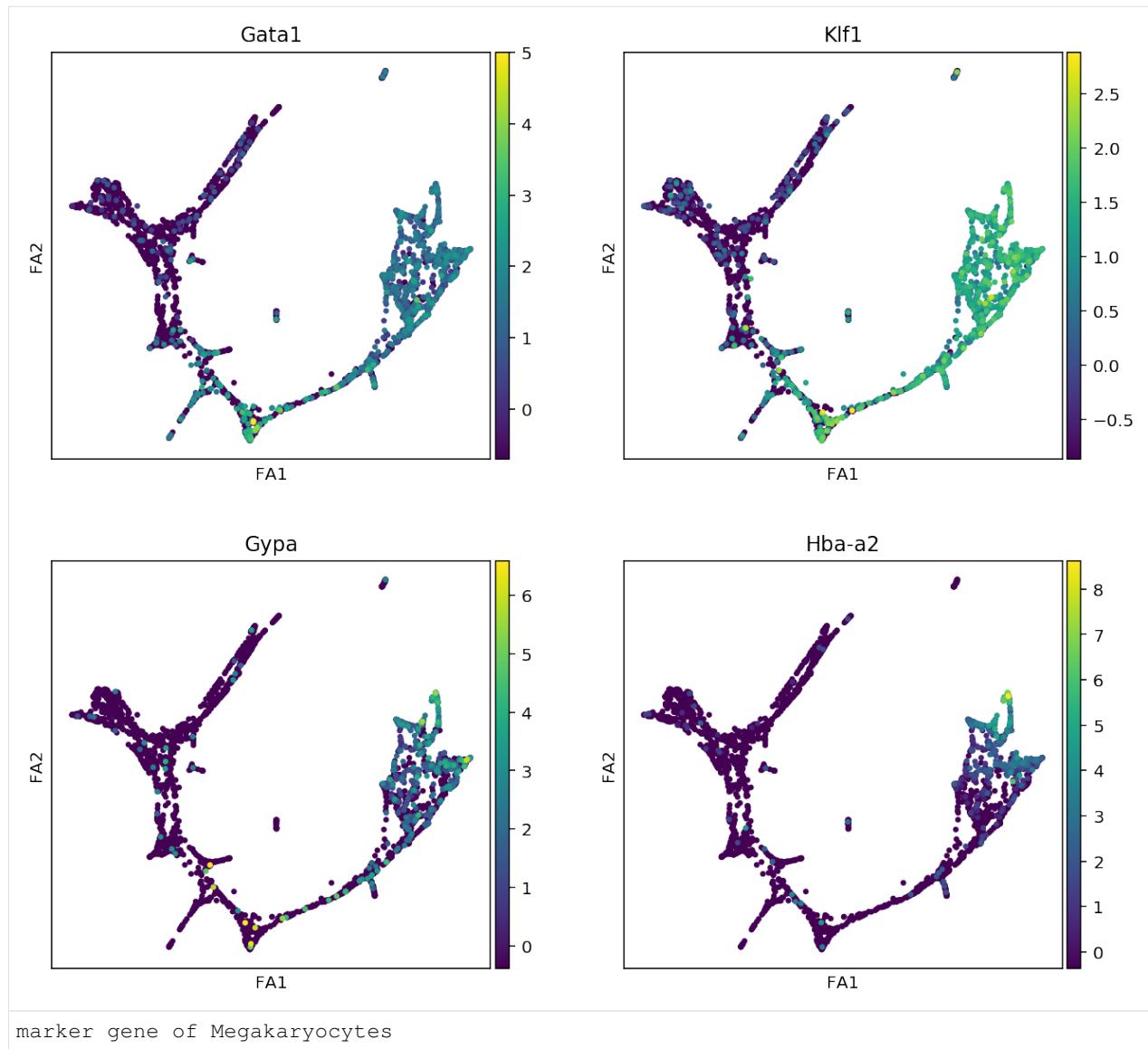
8.1. Visualize marker gene expression

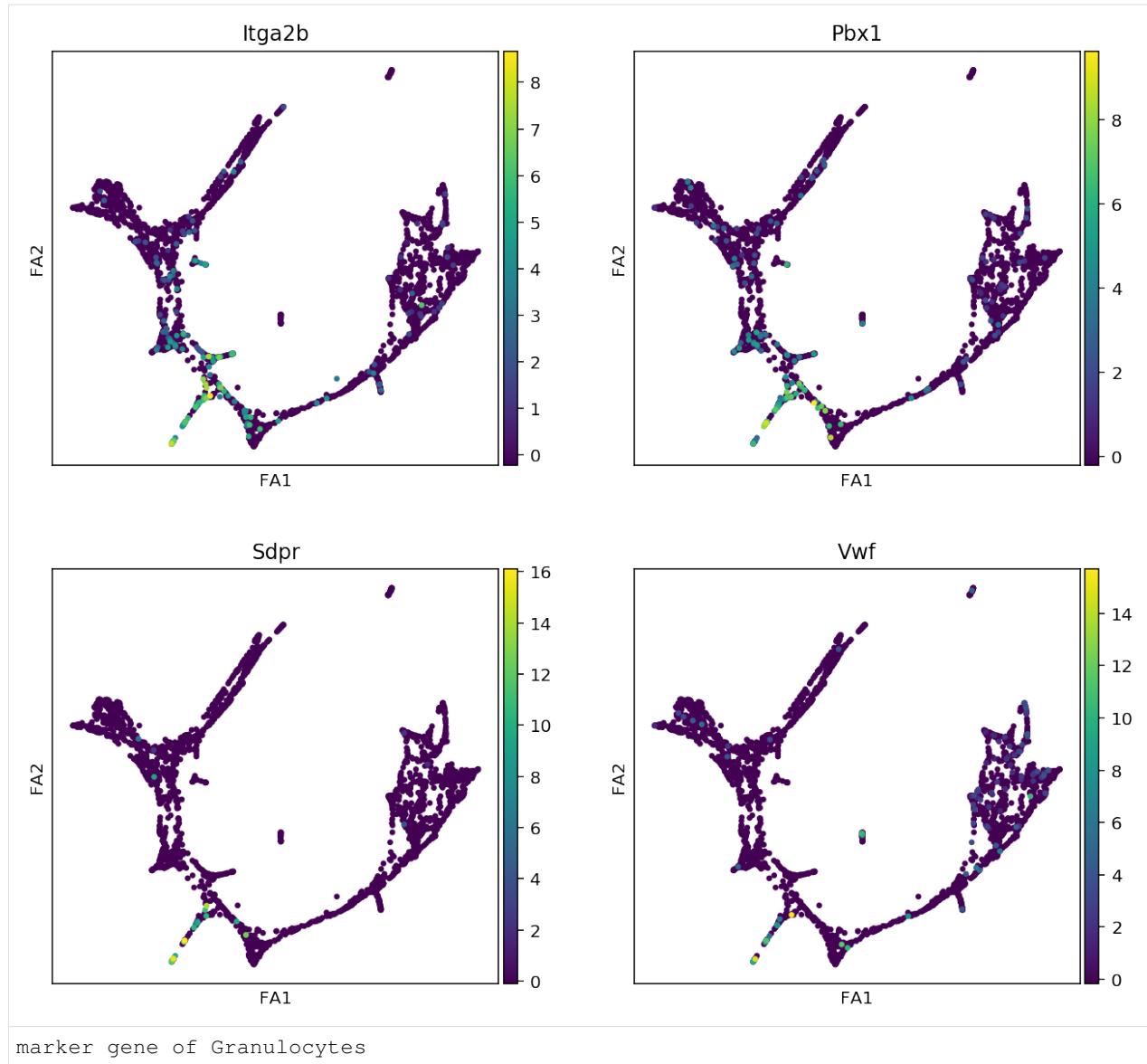
```
[18]: plt.rcParams["figure.figsize"] = [4.5, 4.5]

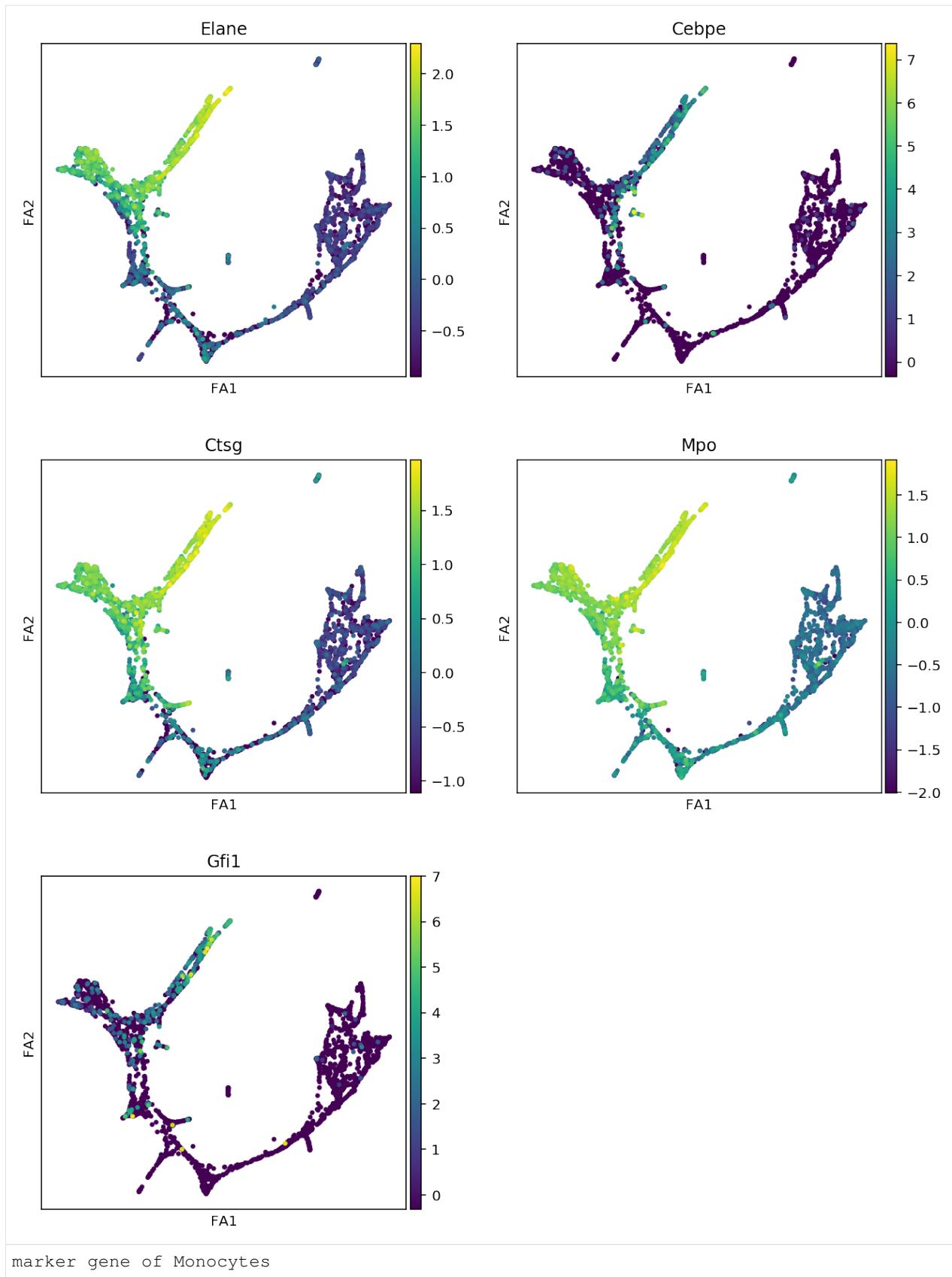
[19]: markers = {"Erythrocytes": ["Gata1", "Klf1", "Gypa", "Hba-a2"],
   "Megakaryocytes": ["Itga2b", "Pbx1", "Sdpr", "Vwf"],
   "Granulocytes": ["Elane", "Cebpe", "Ctsg", "Mpo", "Gfil1"],
   "Monocytes": ["Irf8", "Csflr", "Ctsg", "Mpo"],
   "Mast_cells": ["Cmal", "Gzmb", "Kit"],
   "Basophils": ["Mcpt8", "Prss34"]}
}

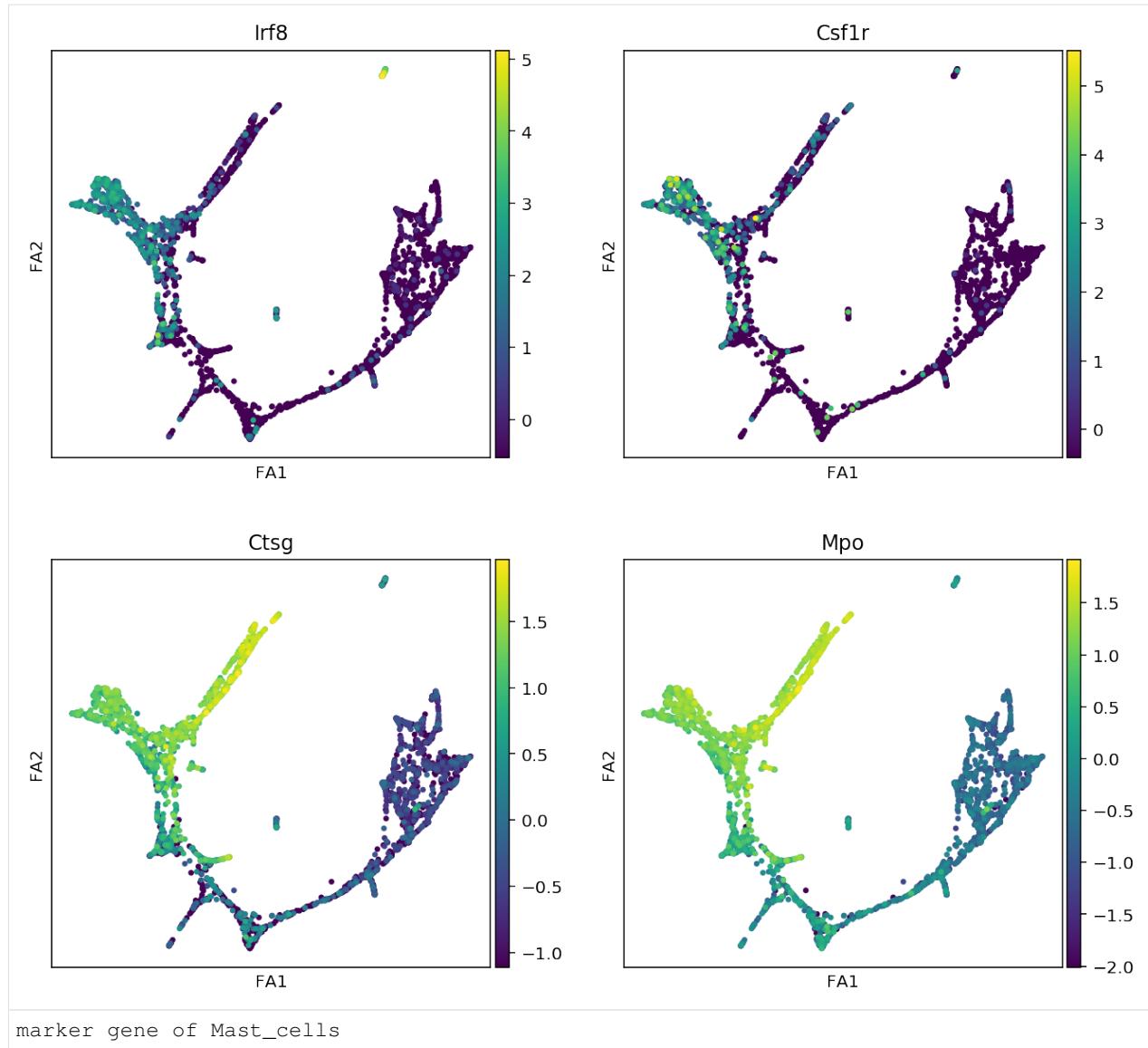
for cell_type, genes in markers.items():
    print(f"marker gene of {cell_type}")
    sc.pl.draw_graph(adata, color=genes, use_raw=False, ncols=2)
    plt.show()
```

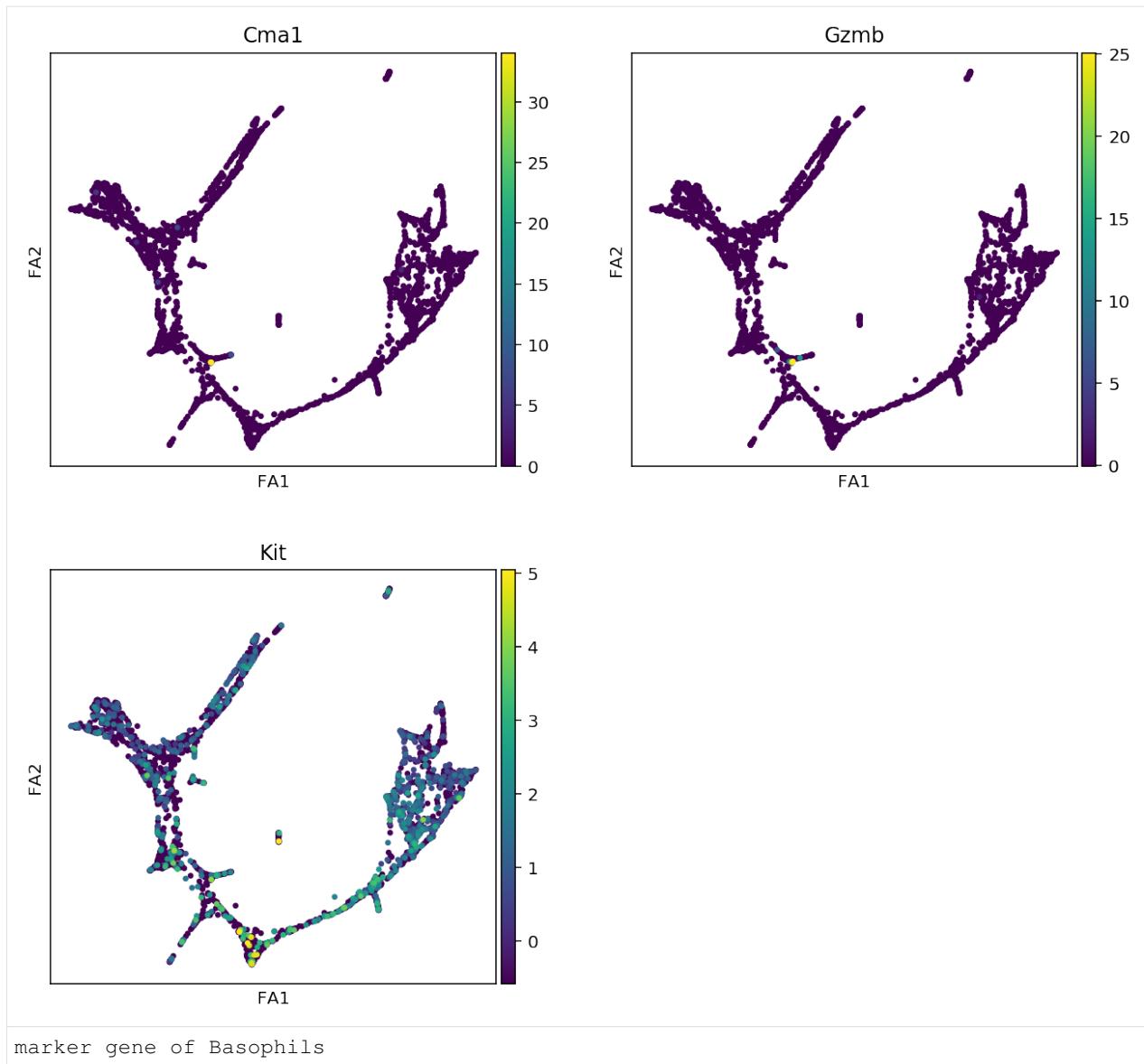
marker gene of Erythrocytes

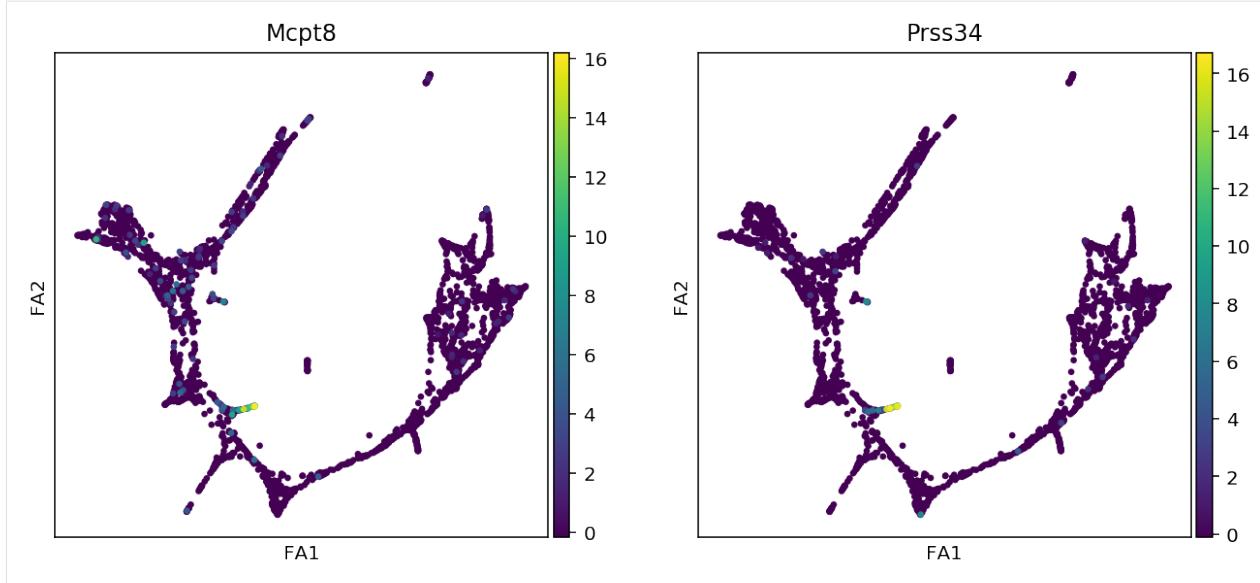










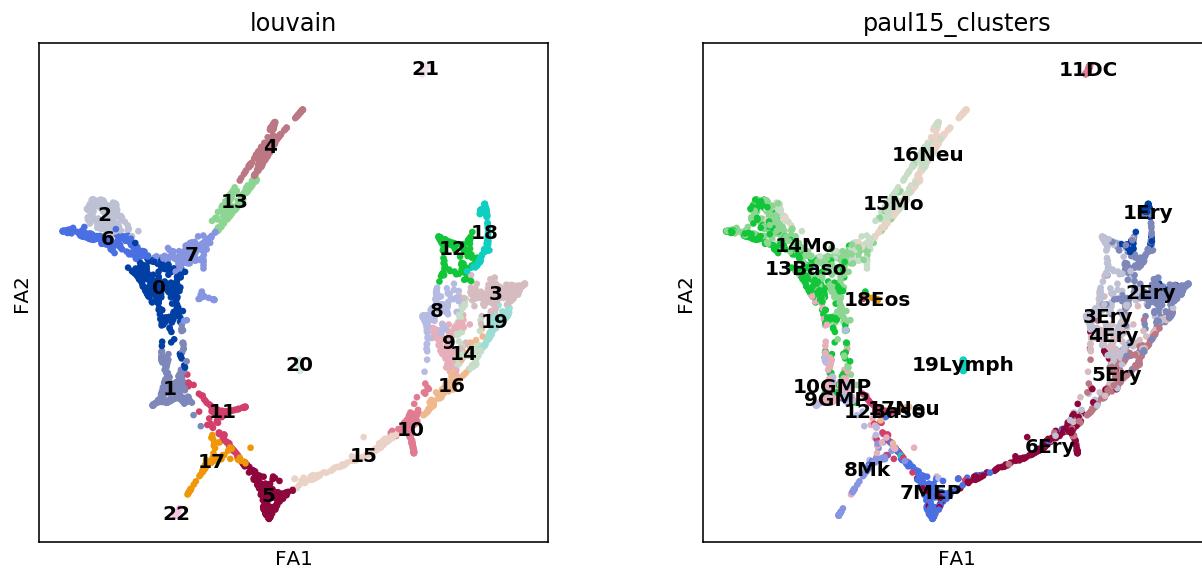


8. Make annotation for cluster

Based on the marker gene expression and previous reports, we will manually annotate each cluster. When using your own data, you will need to annotate the clusters appropriately.

8.1. Make annotation (1)

```
[20]: sc.pl.draw_graph(adata, color=['louvain', 'paul15_clusters'],
                      legend_loc='on data')
```



```
[21]: # Check current cluster name
cluster_list = adata.obs.louvain.unique()
cluster_list
```

```
[21]: [5, 2, 12, 13, 0, ..., 6, 20, 14, 15, 21]
Length: 23
Categories (23, object): [5, 2, 12, 13, ..., 20, 14, 15, 21]
```

!! Please change the dictionary below depending on the clustering results. The results may change depending on the execution environment.

```
[22]: # Make annotation dictionary
annotation = {"MEP": [5],
              "Erythroids": [15, 10, 16, 9, 8, 14, 19, 3, 12, 18],
              "Megakaryocytes": [17, 22],
              "GMP": [11, 1],
              "late_GMP": [0],
              "Granulocytes": [7, 13, 4],
              "Monocytes": [6, 2],
              "DC": [21],
              "Lymphoid": [20]}

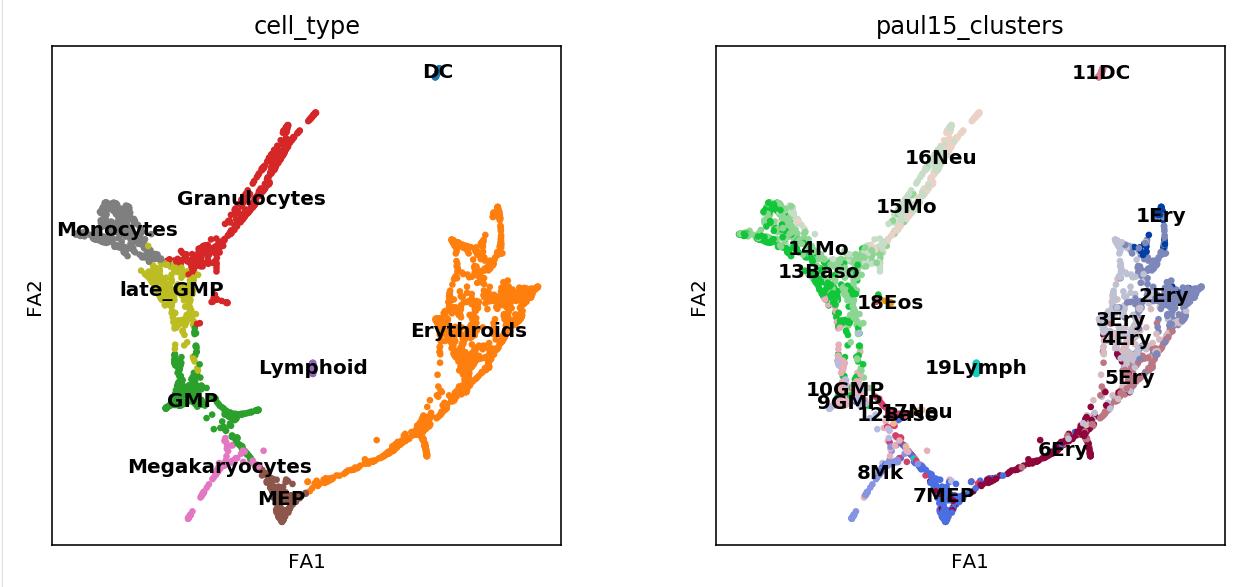
# change dictionary format
annotation_rev = {}
for i in cluster_list:
    for k in annotation:
        if int(i) in annotation[k]:
            annotation_rev[i] = k

# check dictionary
annotation_rev

[22]: {'5': 'MEP',
        '2': 'Monocytes',
        '12': 'Erythroids',
        '13': 'Granulocytes',
        '0': 'late_GMP',
        '10': 'Erythroids',
        '3': 'Erythroids',
        '18': 'Erythroids',
        '11': 'GMP',
        '7': 'Granulocytes',
        '8': 'Erythroids',
        '22': 'Megakaryocytes',
        '16': 'Erythroids',
        '1': 'GMP',
        '17': 'Megakaryocytes',
        '4': 'Granulocytes',
        '19': 'Erythroids',
        '9': 'Erythroids',
        '6': 'Monocytes',
        '20': 'Lymphoid',
        '14': 'Erythroids',
        '15': 'Erythroids',
        '21': 'DC'}
```

```
[23]: adata.obs["cell_type"] = [annotation_rev[i] for i in adata.obs.louvain]
```

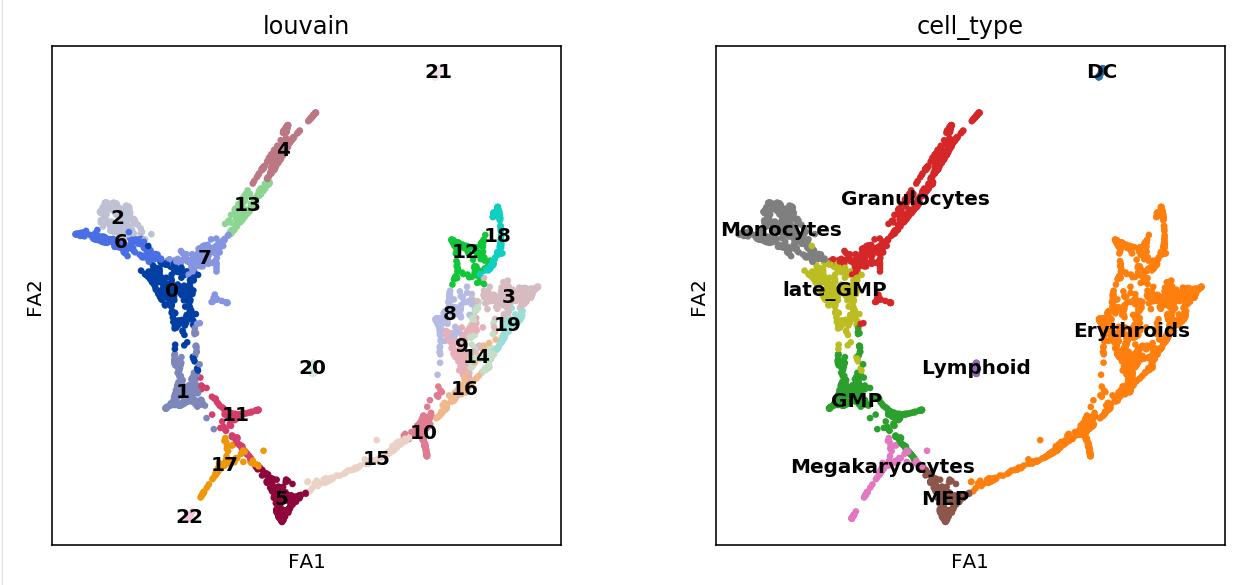
```
[24]: # check results
sc.pl.draw_graph(adata, color=['cell_type', 'paul15_clusters'],
                 legend_loc='on data')
... storing 'cell_type' as categorical
```



8.2. Make annotation (2)

We'll make another annotation manually for each Louvain clusters.

```
[25]: sc.pl.draw_graph(adata, color=['louvain', 'cell_type'],
                     legend_loc='on data')
```

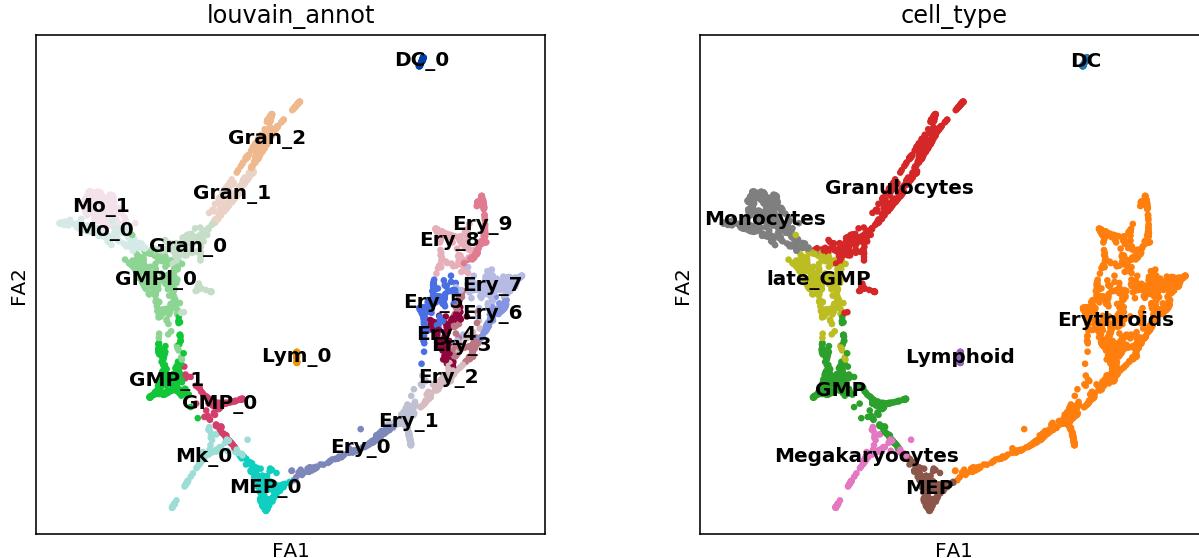


!! Please change the dictionary below depending on the clustering results. The results may change depending on the execution environment.

```
[26]: annotation_2 = { '5': 'MEP_0',
                     '15': 'Ery_0',
                     '10': 'Ery_1',
                     '16': 'Ery_2',
                     '14': 'Ery_3',
                     '9': 'Ery_4',
                     '8': 'Ery_5',
                     '19': 'Ery_6',
                     '3': 'Ery_7',
                     '12': 'Ery_8',
                     '18': 'Ery_9',
                     '17': 'Mk_0',
                     '22': 'Mk_0',
                     '11': 'GMP_0',
                     '1': 'GMP_1',
                     '0': 'GMP1_0',
                     '7': 'Gran_0',
                     '13': 'Gran_1',
                     '4': 'Gran_2',
                     '6': 'Mo_0',
                     '2': 'Mo_1',
                     '21': 'DC_0',
                     '20': 'Lym_0'}
```

```
[27]: adata.obs["louvain.annot"] = [annotation_2[i] for i in adata.obs.louvain]
```

```
[28]: # Check result
sc.pl.draw_graph(adata, color=['louvain.annot', 'cell_type'],
                 legend_loc='on data')
... storing 'louvain.annot' as categorical
```



We've done several scRNA-preprocessing steps; filtering, normalization, clustering, and dimensional reduction. In the next step, we'll do the GRN inference, network analysis, and in silico simulation based on this information.

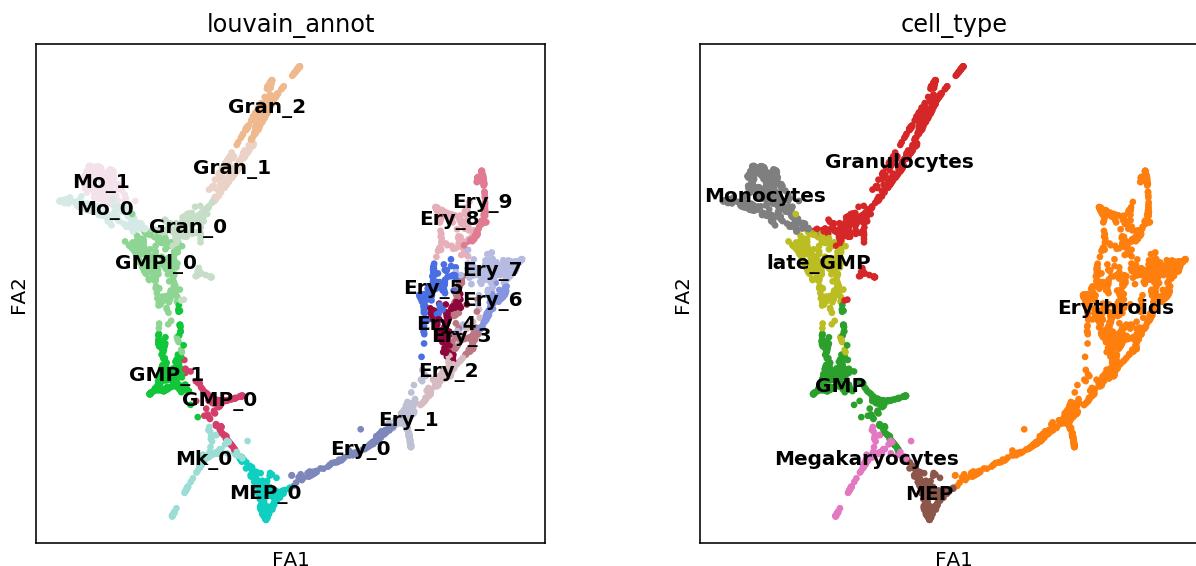
9. (Option) Subset cells

In this tutorial, we are using scRNA-seq data of hematopoiesis. In the latter part, we will focus on the cell fate decision in the myeloid lineage. So we will remove non-myeloid cell cluster; DC and Lymphoid cell cluster.

```
[29]: adata.obs.cell_type.unique()
[29]: [MEP, Monocytes, Erythroids, Granulocytes, late_GMP, GMP, Megakaryocytes, Lymphoid, DC]
Categories (9, object): [MEP, Monocytes, Erythroids, Granulocytes, ..., GMP, Megakaryocytes, Lymphoid, DC]
```

```
[30]: cell_of_interest = adata.obs.index[~adata.obs.cell_type.isin(["Lymphoid", "DC"])]
adata = adata[cell_of_interest, :]
```

```
[31]: # check result
sc.pl.draw_graph(adata, color=['louvain_annot', 'cell_type'],
                 legend_loc='on data')
```



10. Save data

```
[32]: adata.write_h5ad("data/Paul_et al_15.h5ad")
```

B. scRNA-seq data preprocessing with Seurat

R notebook ... comming in the future update.

Note: If you use Seurat for preprocessing, you need to convert the scRNA-seq data (Seurat object) into anndata to analyze the data with celloracle. celloracle has a python API and command-line API to convert a Seurat object into an anndata. Please go to the documentation of celloracle's API documentation for more information.

1.2.4 Network analysis

celloracle imports the scRNA-seq dataset and TF binding information to find active regulatory connections for all genes, generating sample-specific GRNs.

The inferred GRN is analyzed with several network algorithms to get various network scores. The network score is useful to identify key regulatory genes.

Celloracle reconstructs a GRN for each cluster, enabling us to compare GRNs to each other. It is also possible to analyze how the GRN changes over differentiation. The dynamics of the GRN structure can provide us insight into the context-dependent regulatory mechanisms.

Python notebook

0. Import libraries

```
[32]: # 0. Import

import os
import sys

import matplotlib.pyplot as plt
import numpy as np
import pandas as pd
import scanpy as sc
import seaborn as sns
```

```
[33]: import celloracle as co
```

```
[34]: # visualization settings
%config InlineBackend.figure_format = 'retina'
%matplotlib inline

plt.rcParams['figure.figsize'] = [6, 4.5]
plt.rcParams["savefig.dpi"] = 300
```

0.1. Check installation

Celloracle uses some R libraries in network analysis. Please make sure that all dependent R libraries are installed on your computer. You can test the installation with the following command.

```
[81]: co.network_analysis.test_R_libraries_installation()

checking R library installation: igraph -> OK
checking R library installation: linkcomm -> OK
checking R library installation: rnetcarto -> OK
```

0.2. Make a folder to save graph

```
[36]: save_folder = "figures"
os.makedirs(save_folder, exist_ok=True)
```

1. Load data

1.1. Load processed gene expression data (anndata)

Please refer to the previous notebook in the tutorial for an example of how to process scRNA-seq data.

```
[37]: # Load data. !!Replace the data path below when you use another data.
adata = sc.read_h5ad("../03_scRNA-seq_data_preprocessing/data/Paul_etal_15.h5ad")
```

1.2. Load TF data.

For the GRN inference, celloracle needs TF information, which contains lists of the regulatory candidate genes. There are several ways to make such TF information. We can generate TF information from scATAC-seq data or bulk ATAC-seq data. Please refer to the first step of the tutorial for the details of this process.

If you do not have your scATAC-seq data, you can use some built-in data in celloracle. The built-in TFinfo wqs made using various tissue/cell-types from the mouse ATAC-seq atlas dataset (<http://atlas.gs.washington.edu/mouse-atac/>).

You can load and use the data with the following command.

```
[38]: # Load TF info which was made from mouse cell atlas dataset.
TFinfo_df = co.data.load_TFinfo_df_mm9_mouse_atac_atlas()

# Check data
TFinfo_df.head()
```

	peak_id	gene_short_name	9430076c15rik	Ac002126.6	\
0	chr10_100050979_100052296	4930430F08Rik	0.0	0.0	
1	chr10_101006922_101007748	SNORA17	0.0	0.0	
2	chr10_101144061_101145000	Mgat4c	0.0	0.0	
3	chr10_10148873_10149183	9130014G24Rik	0.0	0.0	
4	chr10_10149425_10149815	9130014G24Rik	0.0	0.0	

	Ac012531.1	Ac226150.2	Afp	Ahr	Ahrr	Aire	...	Znf784	Znf8	Znf816	\
0	1.0	0.0	0.0	0.0	0.0	0.0	...	0.0	0.0	0.0	
1	0.0	0.0	0.0	0.0	0.0	0.0	...	0.0	0.0	0.0	
2	0.0	0.0	0.0	0.0	0.0	0.0	...	0.0	0.0	0.0	
3	0.0	0.0	0.0	0.0	0.0	0.0	...	0.0	0.0	0.0	
4	0.0	0.0	0.0	0.0	0.0	0.0	...	0.0	0.0	0.0	

	Znf85	Zscan10	Zscan16	Zscan22	Zscan26	Zscan31	Zscan4
0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1	0.0	0.0	0.0	0.0	0.0	1.0	0.0
2	0.0	0.0	0.0	0.0	0.0	0.0	1.0
3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
4	0.0	0.0	0.0	0.0	0.0	0.0	0.0

[5 rows x 1095 columns]

```
[ ]:
```

2. Initiate Oracle object

Celloracle has a custom called Oracle. We can use Oracle for the data preprocessing and GRN inference steps. The Oracle object stores all of necessary information and does the calculations with its internal functions. We instantiate

an Oracle object, then input the gene expression data (anndata) and a TFinfo into the Oracle object.

```
[39]: # Instantiate Oracle object
oracle = co.Oracle()
```

2.1. load gene expression data into oracle object.

When you load a scRNA-seq data, please enter the name of clustering data and dimensional reduction data. The clustering data should be to be stored in the attribute of “obs” in the anndata. Dimensional reduction data suppose to be stored in the attribute of “obsm” in the anndata. You can check these data by the following command.

If you are not familiar with anndata, please look at the documentation of annata (<https://anndata.readthedocs.io/en/stable/>) or Scanpy (<https://scanpy.readthedocs.io/en/stable/>).

For the celloracle analysis, the anndata shoud include (1) gene expression count, (2) clustering information, (3) trajectory (dimensional reduction embeddings) data. Please refer to another notebook for more information on anndata preprocessing.

```
[41]: # show data name in anndata
print("metadata columns : ", list(adata.obs.columns))
print("dimensional reduction: ", list(adata.obsm.keys()))

metadata columns : ['paul15_clusters', 'n_counts_all', 'n_counts', 'louvain', 'cell_
↪type', 'louvain_annot']
dimensional reduction: ['X_diffmap', 'X_draw_graph_fa', 'X_pca']
```

```
[44]: # In this notebook, we use raw mRNA count as an input of Oracle object.
adata.X = adata.raw.X.copy()

# Instantiate Oracle object.
oracle.import_anndata_as_raw_count(adata=adata,
                                    cluster_column_name="louvain_annot",
                                    embedding_name="X_draw_graph_fa")
```

2.2. Load TFinfo into oracle object

```
[46]: # You can load TF info dataframe with the following code.
oracle.import_TF_data(TF_info_matrix=TFinfo_df)

# Alternatively, if you saved the information as a dictionary, you can use the code
↪below.
# oracle.import_TF_data(TFdict=TFinfo_dictionary)
```

2.3. (Optional) Add TF info manually

While we mainly use TF info data made from scATAC-seq data, we can also add additional information about the TF-target gene pair manually.

For example, if there is a study or database that includes specific TF-target pairs, you can use such information in the following way.

2.3.1. Make TF info dictionary manually

Here, we will introduce how to add TF binding information.

We will start with TF binding data from supplemental table 4 in (<http://doi.org/10.1016/j.cell.2015.11.013>).

In order to import TF data into the Oracle object, we need to convert them into a python dictionary. The dictionary keys will be the target genes, and the values will be the regulatory candidate TFs.

```
[50]: # We have TF and its target gene information. This is from a supplemental Fig of Paul_ et. al, (2015).
Paul_15_data = pd.read_csv("TF_data_in_Paul15.csv")
Paul_15_data
```

```
[50]:      TF                  Target_genes
0   Cebpa    Abcb1b, Acot1, C3, Cnpy3, Dhrs7, Dtx4, Edem2, ...
1   Irf8     Abcd1, Aif1, BC017643, Cbl, Ccdc109b, Ccl6, d6...
2   Irf8  1100001G20Rik, 4732418C07Rik, 9230105E10Rik, A...
3   Klf1  2010011I20Rik, 5730469M10Rik, Acs16, Add2, Ank...
4   Sfpil  0910001L09Rik, 2310014H01Rik, 4632428N05Rik, A...
```

```
[51]: # Make dictionary: dictionary Key is TF, dictionary Value is list of target genes
TF_to_TG_dictionary = {}

for TF, TGs in zip(Paul_15_data.TF, Paul_15_data.Target_genes):
    # convert target gene to list
    TG_list = TGs.replace(" ", "").split(",")
    # store target gene list in a dictionary
    TF_to_TG_dictionary[TF] = TG_list

# We have to make a dictionary, in which a Key is Target gene and value is TF.
# We invert the dictionary above using a utility function in celloracle.
TG_to_TF_dictionary = co.utility.inverse_dictionary(TF_to_TG_dictionary)

HBox(children=(FloatProgress(value=0.0, max=178.0), HTML(value='')))
```

2.3.2. Add TF informatio dictionary into the oracle object

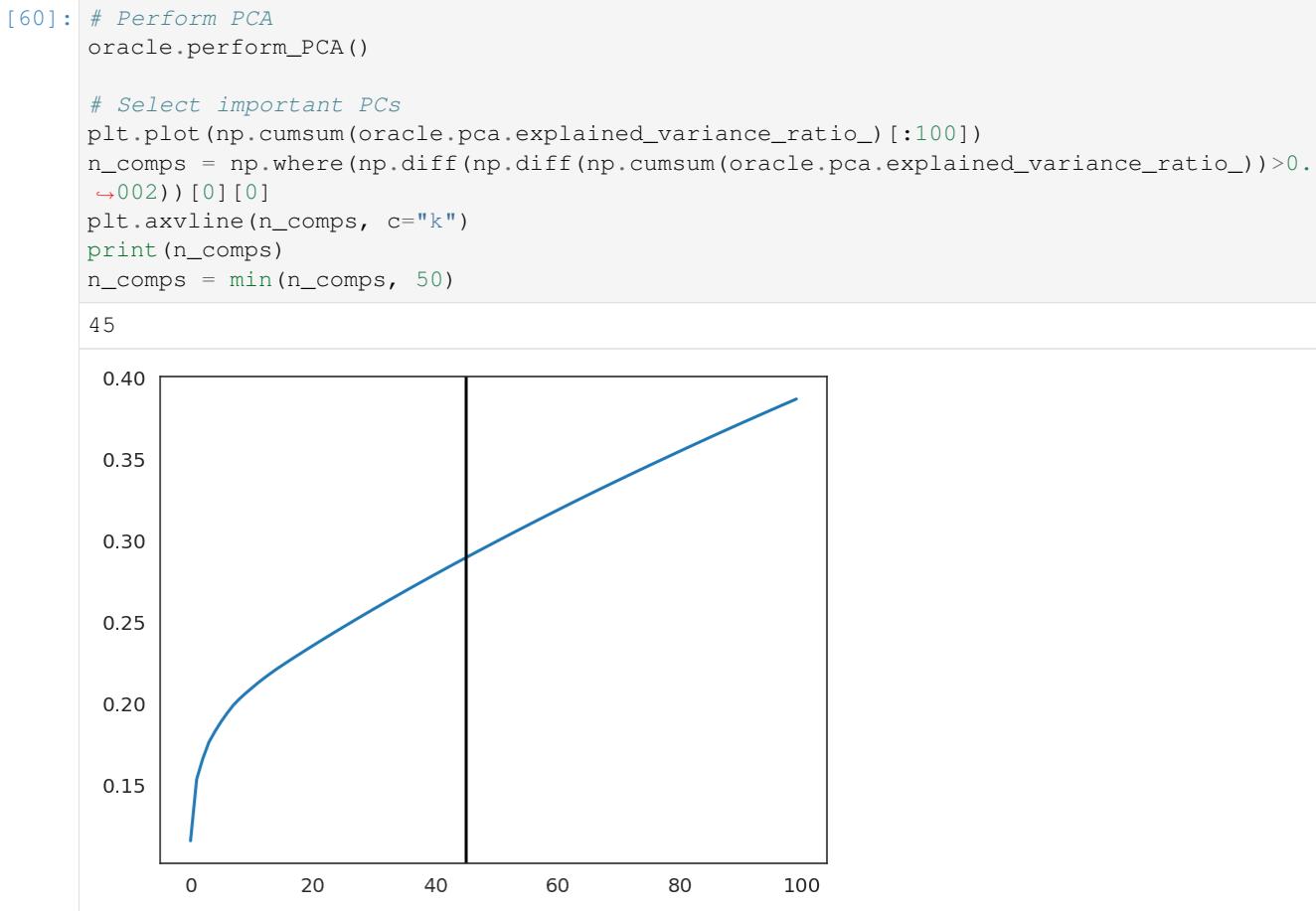
```
[53]: # Add TF information
oracle.addTFinfo_dictionary(TG_to_TF_dictionary)
```

3. Knn imputation

Celloracle uses almost the same strategy as velocyto for visualizing cell transitions. This process requires KNN imputation in advance.

For the KNN imputation, we need PCA and PC selection first.

3.1. PCA



3.2. KNN imputation

Estimate the optimal number of nearest neighbors for KNN imputation.

[63]:

```
n_cell = oracle.adata.shape[0]
print(f"cell number is :{n_cell}")

cell number is :2671
```

[64]:

```
k = int(0.025*n_cell)
print(f"Auto-selected k is :{k}")

Auto-selected k is :66
```

[65]:

```
oracle.knn_imputation(n_pca_dims=n_comps, k=k, balanced=True, b_sight=k*8,
                      b_maxl=k*4, n_jobs=4)
```

4. Save and Load.

Celloracle has some custom-classes: Links, Oracle and TFinfo. You can save such an object using “to_hdf5”.

Please use “load_hdf5” function to load the file.

```
[66]: # Save oracle object.  
oracle.to_hdf5("Paul_15_data.celloracle.oracle")
```

```
[19]: # Load file.  
#oracle = co.load_hdf5("Paul_15_data.celloracle.oracle")
```

4. GRN calculation

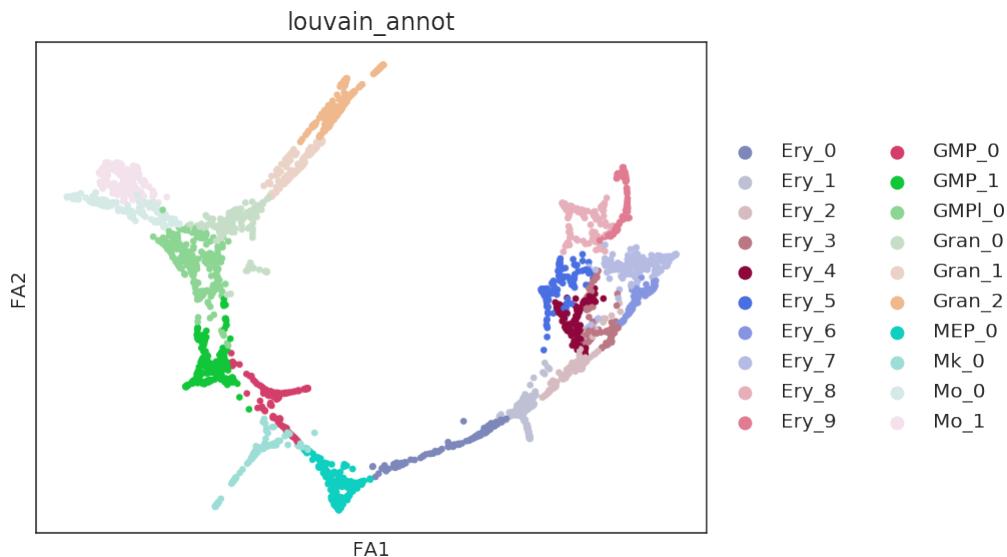
The next step is constructing a cluster-specific GRN for all clusters.

You can calculate GRNs with the “get_links” function, and the function returns GRNs as a Links object. The Links object stores inferred GRNs and the corresponding metadata. You can do network analysis with the Links object.

The GRN will be calculated for each cluster/sub-group. In the example below, we construct GRN for each unit of the “louvain_annot” clustering.

The GRNs can be calculated at any arbitrary unit as long as the clustering information is stored in anndata.

```
[67]: # check data  
sc.pl.draw_graph(oracle.adata, color="louvain_annot")
```



4.1. Get GRNs

```
[ ]: %%time  
# Calculate GRN for each population in "louvain_annot" clustering unit.  
# This step may take long time.  
links = oracle.get_links(cluster_name_for_GRN_unit="louvain_annot", alpha=10,  
                        verbose_level=10, test_mode=False)
```

4.2. (Optional) Export GRNs

Although celloracle has many functions for network analysis, you can analyze GRNs by hand if you choose. The raw GRN data is stored in the attribute of “links_dict”.

For example, you can get the GRN for the “Ery_0” cluster with the following commands.

```
[72]: links.links_dict.keys()
[72]: dict_keys(['Ery_0', 'Ery_1', 'Ery_2', 'Ery_3', 'Ery_4', 'Ery_5', 'Ery_6', 'Ery_7',
   ↪ 'Ery_8', 'Ery_9', 'GMP_0', 'GMP_1', 'GMP1_0', 'Gran_0', 'Gran_1', 'Gran_2', 'MEP_0',
   ↪ 'Mk_0', 'Mo_0', 'Mo_1'])

[73]: links.links_dict["Ery_0"]
[73]:      source      target  coef_mean  coef_abs      p    -logp
0       Stat3  0610007L01Rik -0.010275  0.010275 3.476931e-07  6.458804
1      Gata1  0610007L01Rik -0.000380  0.000380 7.598357e-01  0.119280
2     Zbtb1  0610007L01Rik  0.004452  0.004452 1.018526e-03  2.992028
3      Rara  0610007L01Rik -0.000669  0.000669 7.065405e-01  0.150863
4      Myc  0610007L01Rik -0.010705  0.010705 1.696471e-05  4.770454
...
...      ...      ...      ...      ...
74420  Smarcc2      Zyx -0.003475  0.003475 2.754236e-02  1.559999
74421   Nfe2      Zyx  0.031430  0.031430 1.461503e-11  10.835200
74422   Zbtb4      Zyx  0.001684  0.001684 1.915555e-01  0.717705
74423  Smarcc1      Zyx  0.011356  0.011356 1.843519e-04  3.734352
74424   Nfkbl      Zyx  0.010803  0.010803 1.805959e-06  5.743292
[74425 rows x 6 columns]
```

You can export the file as follows.

```
[ ]: # Set cluster name
cluster = "Ery_0"

# Save as csv
links.links_dict[cluster].to_csv(f"raw_GRN_for_{cluster}.csv")
```

4.3. (Optional) Change order

The links object has a color information in an attribute, “palette”. This information is used for the visualization

The sample will be visualized in that order. Here we can change the order.

```
[75]: # Show the contents of palette
links.palette
[75]:      palette
MEP_0  #0FCFC0
Mk_0   #9CDED6
Ery_0  #7D87B9
Ery_1  #BEC1D4
Ery_2  #D6BCC0
Ery_3  #BB7784
Ery_4  #8E063B
Ery_5  #4A6FE3
Ery_6  #8595E1
```

(continues on next page)

(continued from previous page)

Ery_7	#B5BBE3
Ery_8	#E6AFB9
Ery_9	#E07B91
GMP_0	#D33F6A
GMP_1	#11C638
GMP1_0	#8DD593
Mo_0	#D5EAE7
Mo_1	#F3E1EB
Gran_0	#C6DEC7
Gran_1	#EAD3C6
Gran_2	#F0B98D

```
[76]: # Change the order of palette
order = ['MEP_0', 'Mk_0', 'Ery_0', 'Ery_1', 'Ery_2', 'Ery_3', 'Ery_4', 'Ery_5',
         'Ery_6', 'Ery_7', 'Ery_8', 'Ery_9', 'GMP_0', 'GMP_1',
         'GMP1_0', 'Mo_0', 'Mo_1', 'Gran_0', 'Gran_1', 'Gran_2']
links.palette = links.palette.loc[order]
links.palette
```

```
[76]: palette
MEP_0    #0FCFC0
Mk_0     #9CDED6
Ery_0    #7D87B9
Ery_1    #BEC1D4
Ery_2    #D6BCC0
Ery_3    #BB7784
Ery_4    #8E063B
Ery_5    #4A6FE3
Ery_6    #8595E1
Ery_7    #B5BBE3
Ery_8    #E6AFB9
Ery_9    #E07B91
GMP_0    #D33F6A
GMP_1    #11C638
GMP1_0   #8DD593
Mo_0     #D5EAE7
Mo_1     #F3E1EB
Gran_0   #C6DEC7
Gran_1   #EAD3C6
Gran_2   #F0B98D
```

5. Network preprocessing

5.1. Filter network edges

Celloracle utilizes bagging ridge or Bayesian ridge regression to infer gene regulatory networks. These methods provide a network edge strength as a distribution rather than a point value. We can use the distribution to know the certainness of the connection.

We filter the network edges as follows.

- (1) Remove uncertain network edges based on the p-value.
- (2) Remove weak network edge. In this tutorial, we pick up the top 2000 edges in terms of network strength.

The raw network data is stored as an attribute, “links_dict,” while filtered network data is stored in “filtered_links.” Thus the filtering function keeps raw network information rather than overwriting the data. You can come back to the

filtering process to filter the data with different parameters if you want.

```
[78]: links.filter_links(p=0.001, weight="coef_abs", thread_number=2000)
```

5.2. Degree distribution

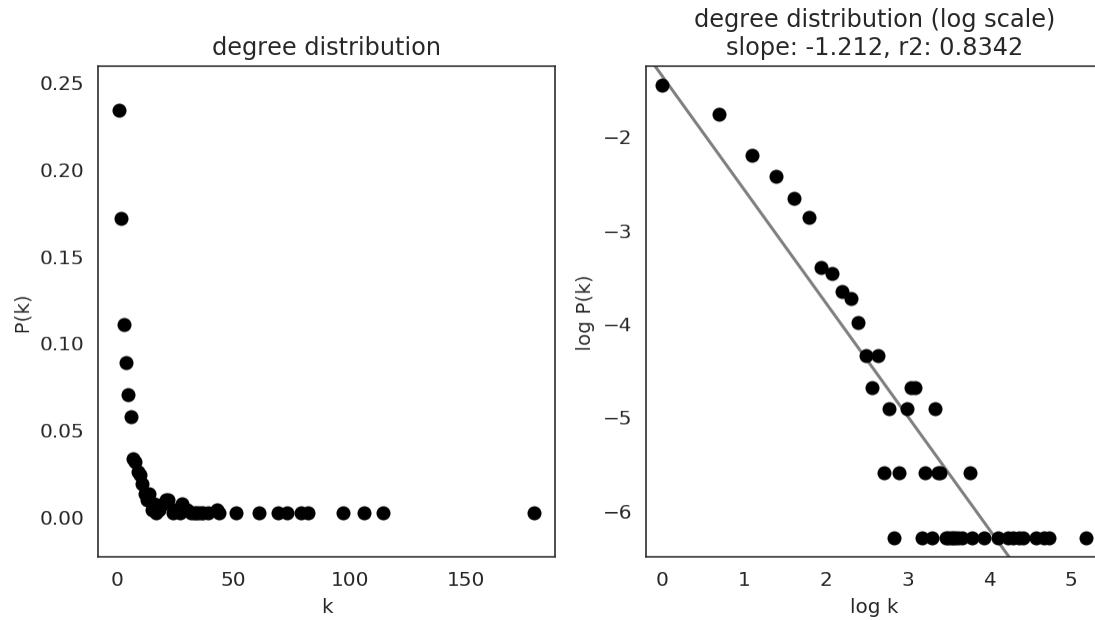
In the first step, we examine the network degree distribution. Network degree, which is the number of edges for each node, is one of the important metrics used to investigate the network structure (https://en.wikipedia.org/wiki/Degree_distribution).

Please keep in mind that the degree distribution may change depending on the filtering threshold.

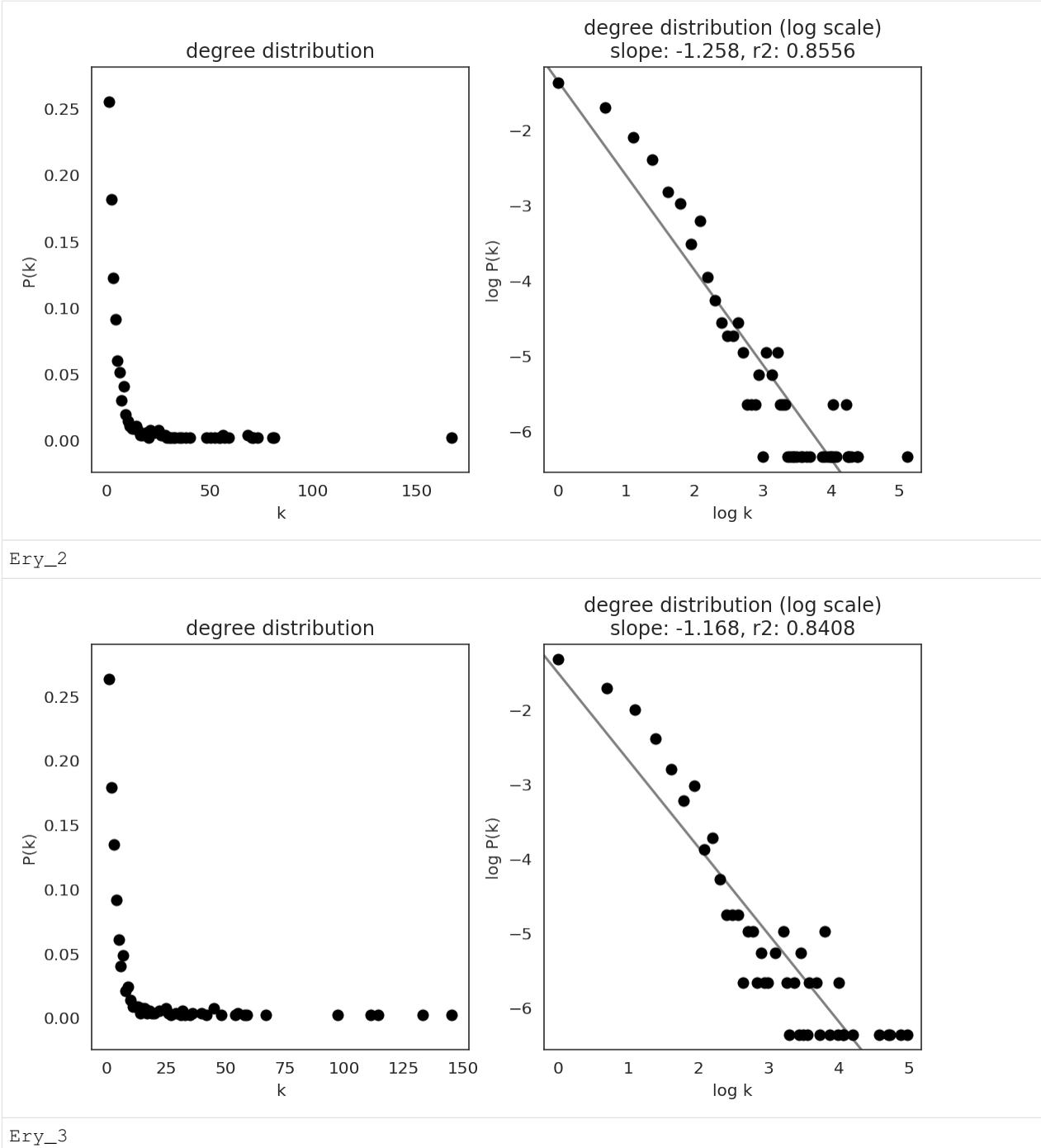
```
[79]: plt.rcParams["figure.figsize"] = [9, 4.5]
```

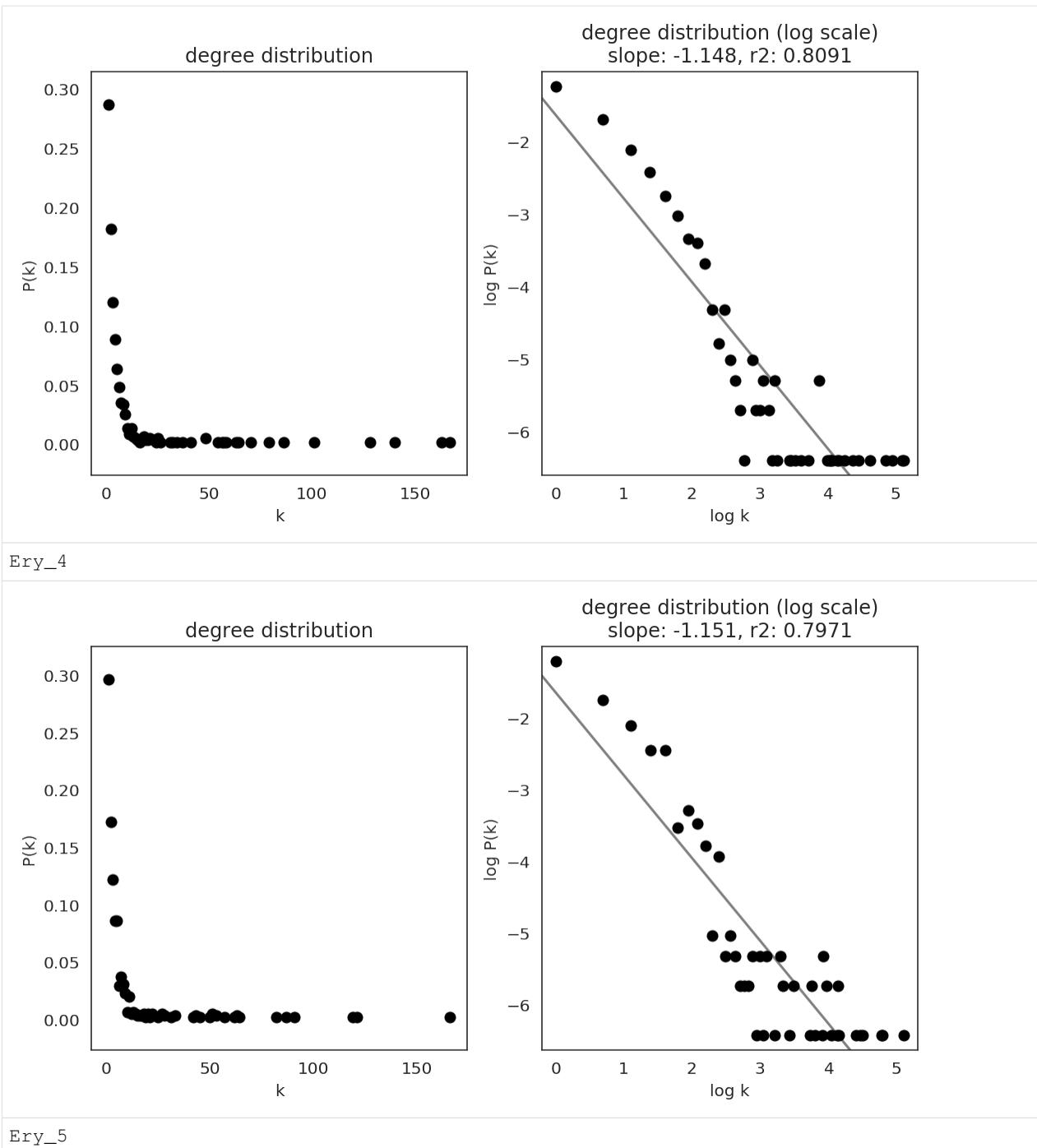
```
[80]: links.plot_degree_distributions(plot_model=True, save=f"{save_folder}/degree_distribution/")
```

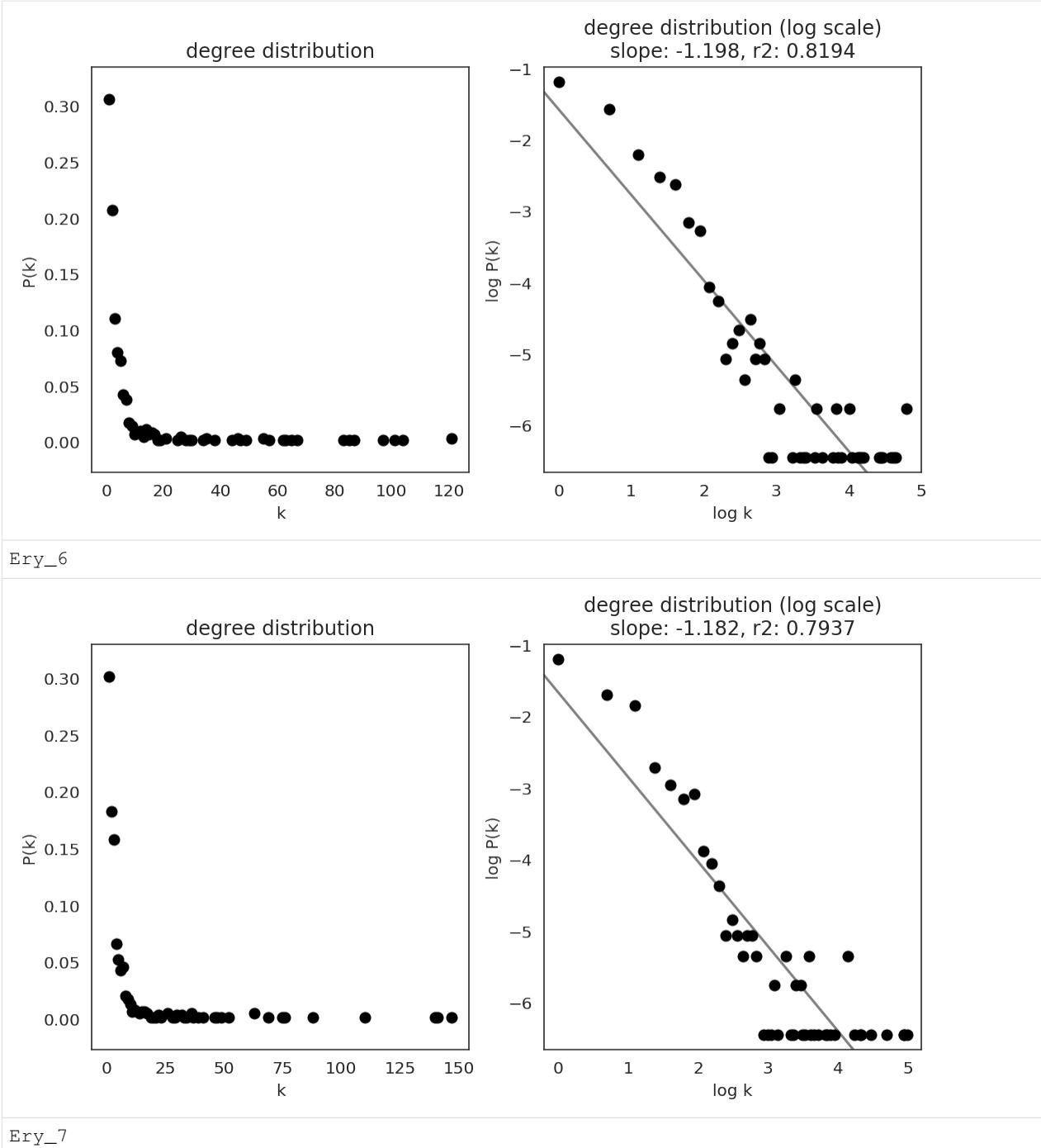
Ery_0

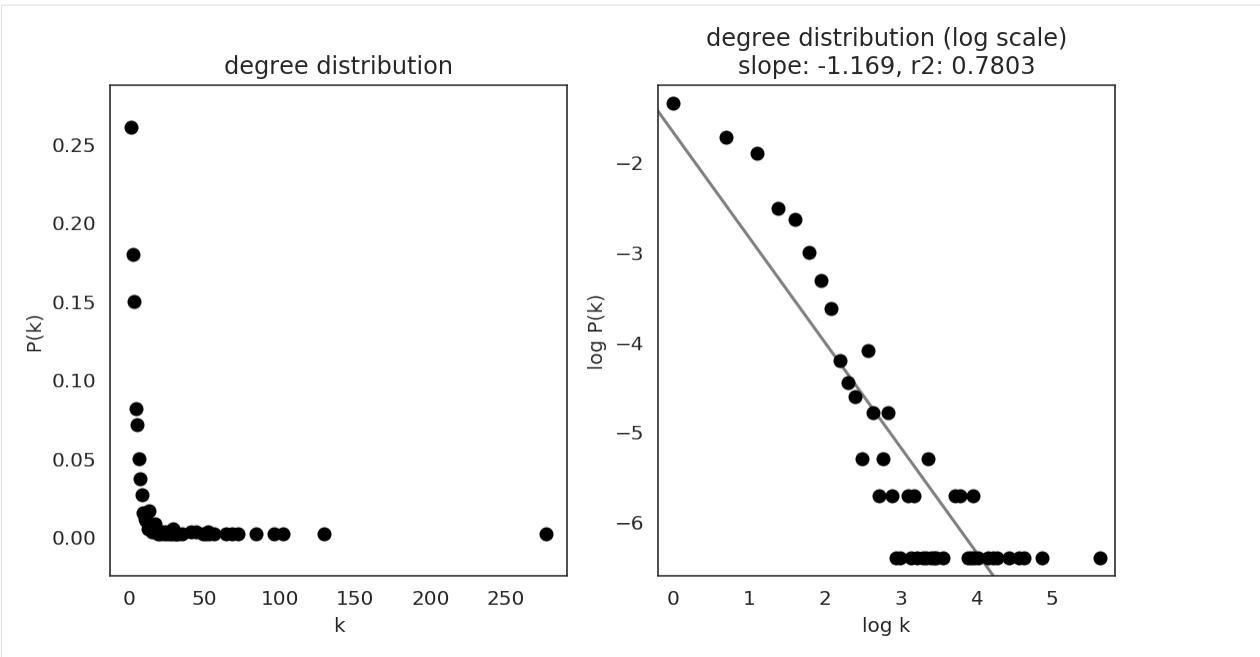


Ery_1

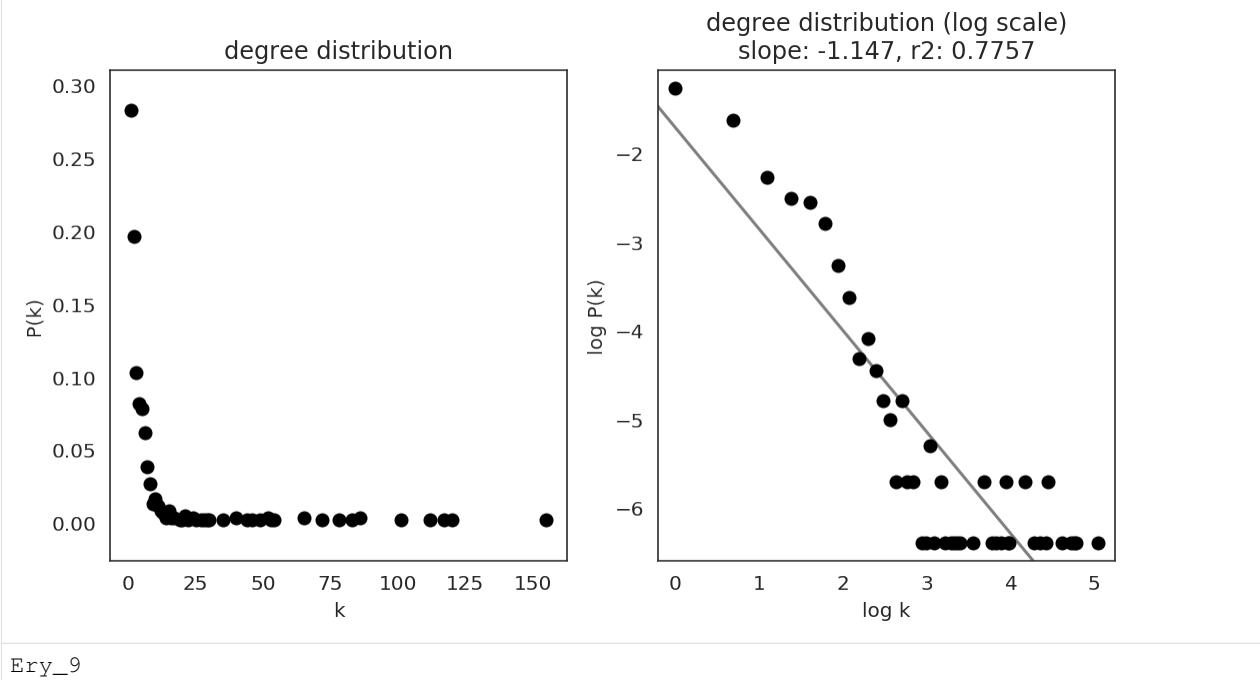




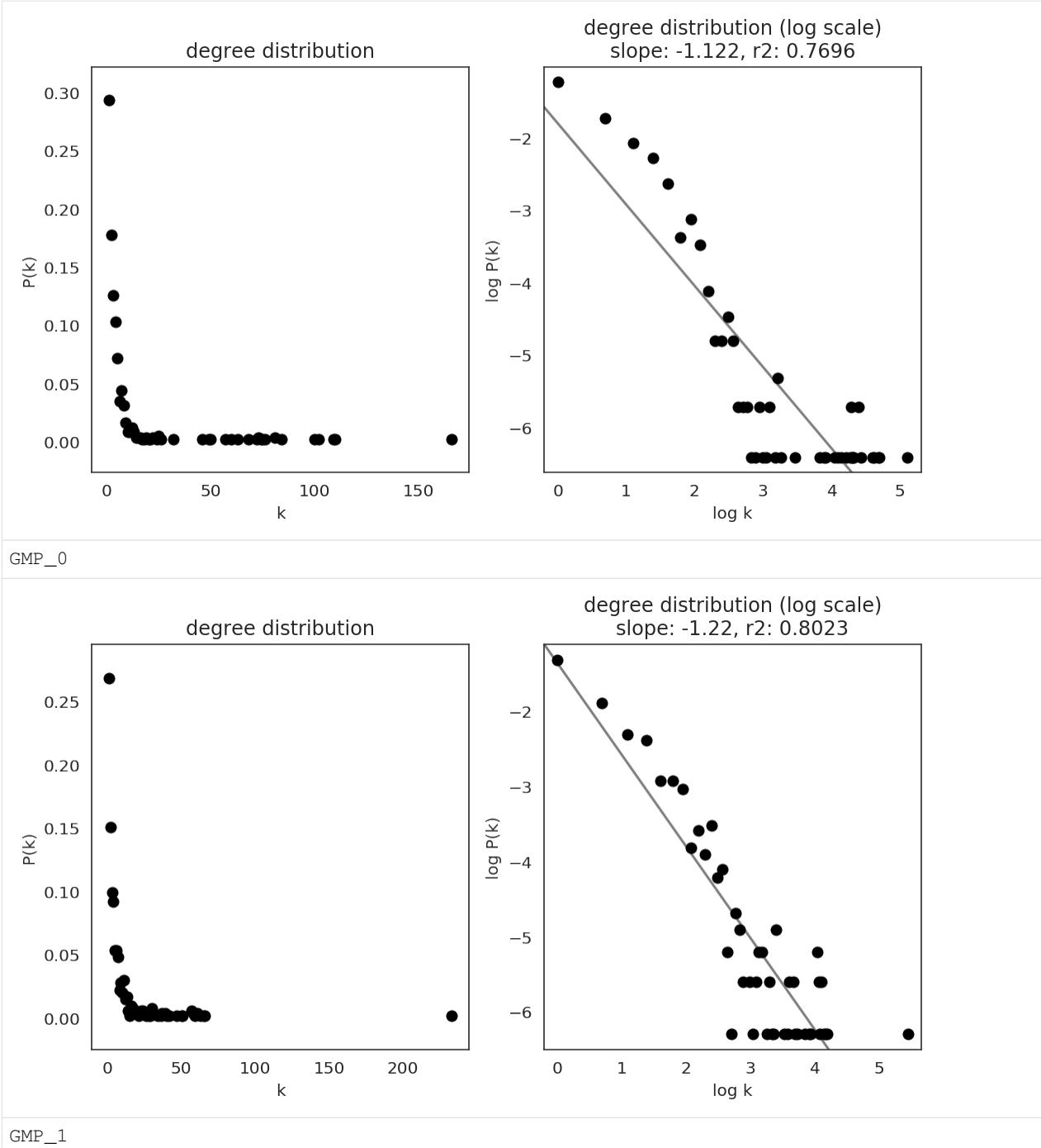


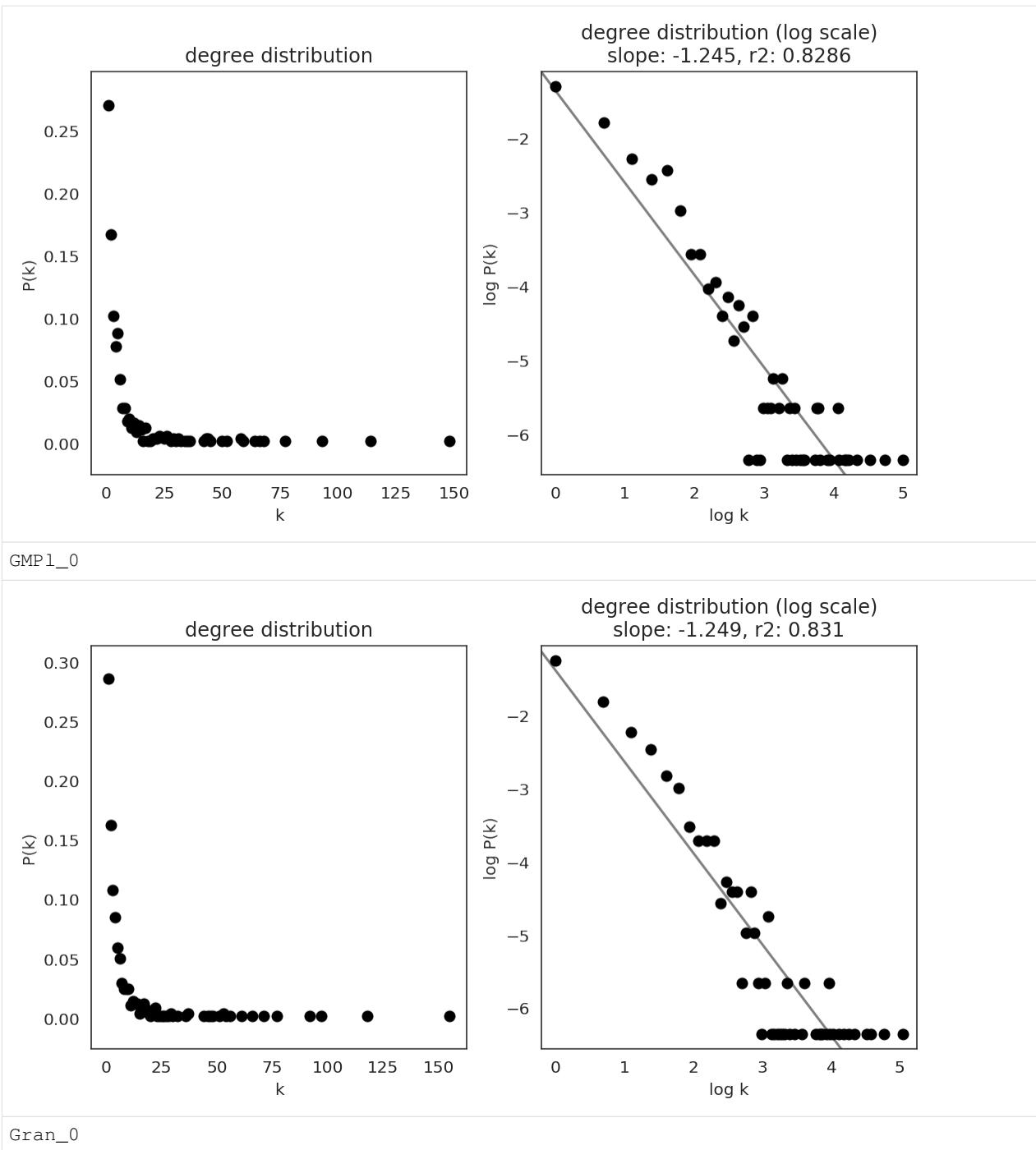


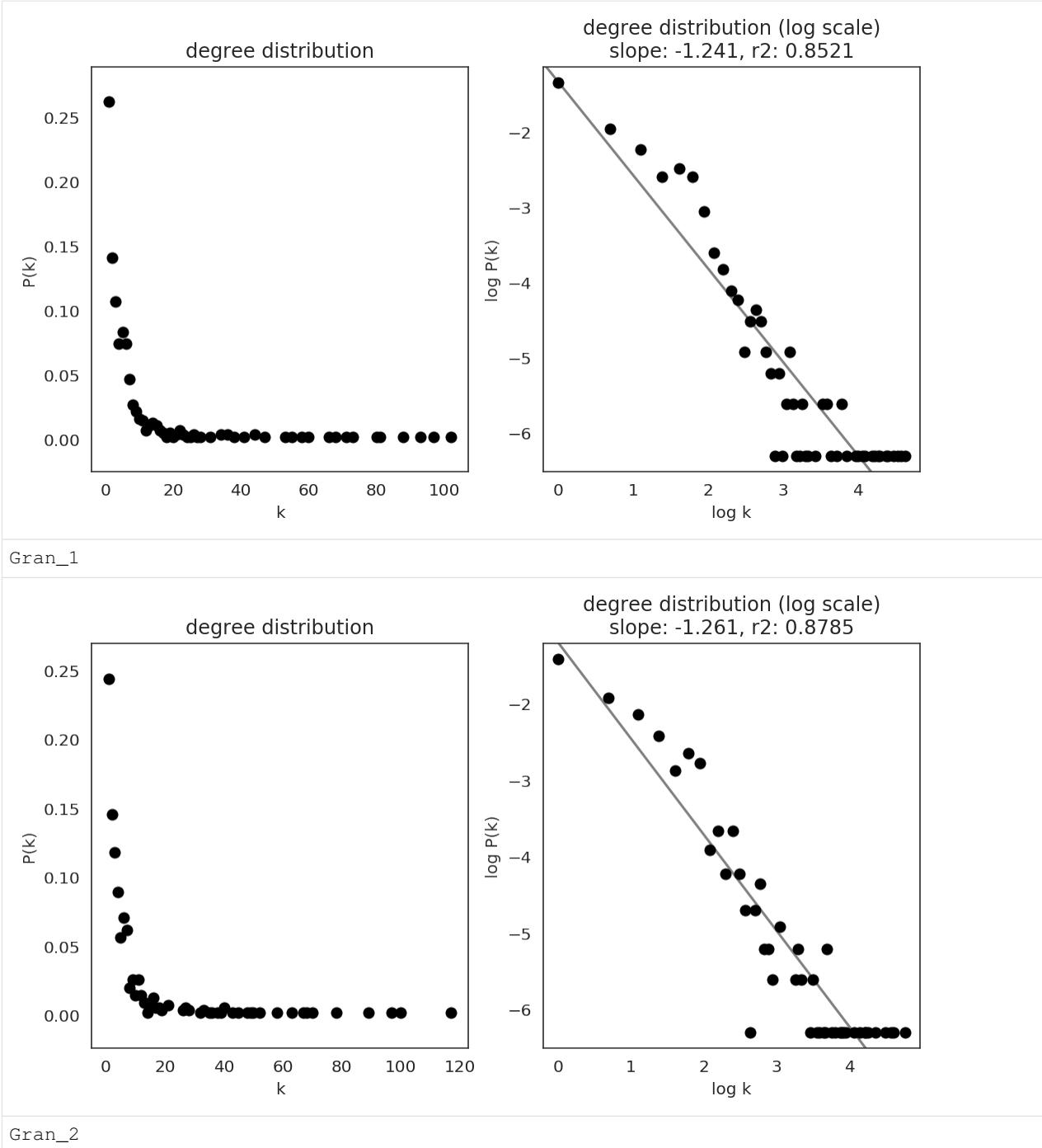
Ery_8

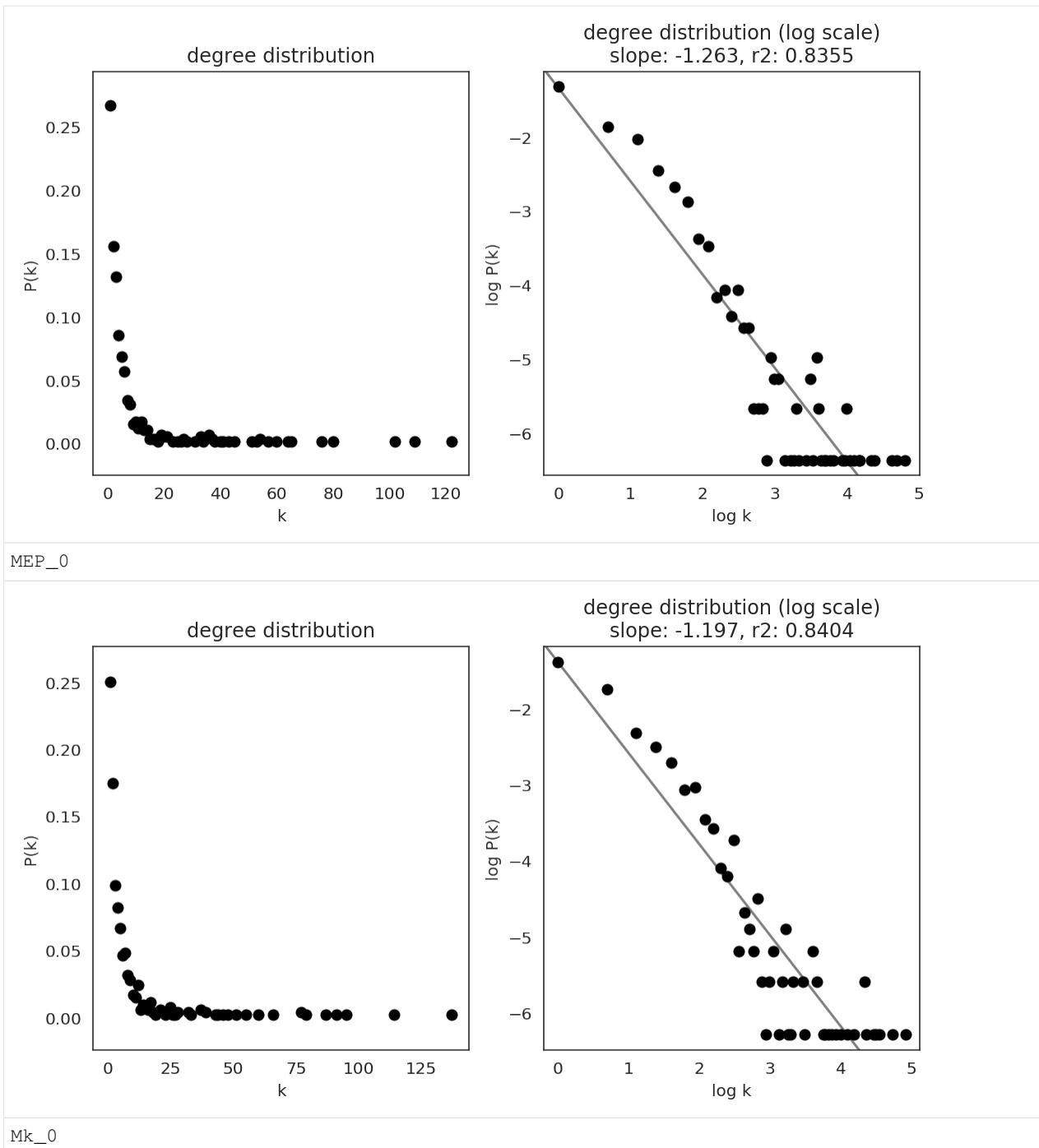


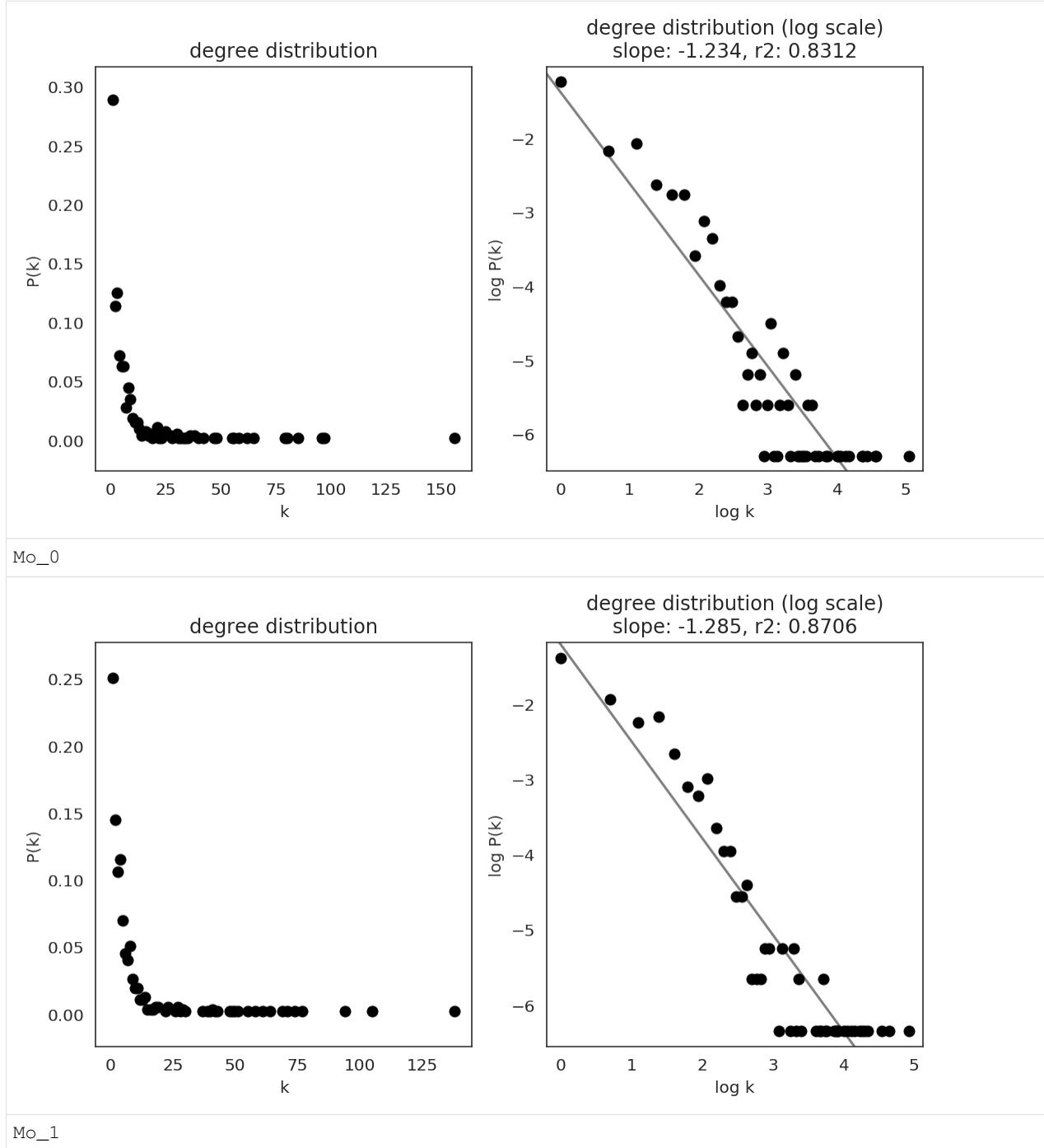
Ery_9

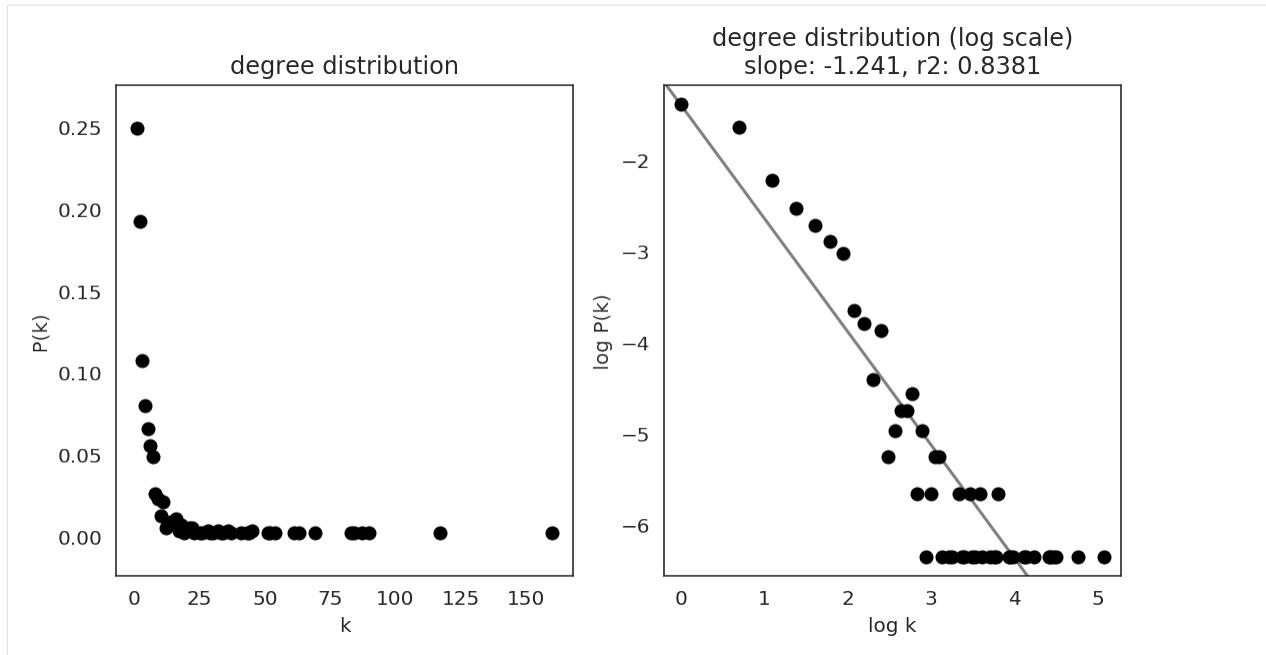












```
[28]: plt.rcParams["figure.figsize"] = [6, 4.5]
```

5.3. Calculate netowrk score

Next, we calculate several network score using some R libraries. Please make sure that R libraries are installed in your PC before running the command below.

```
[25]: # Calculate network scores. It takes several minutes.
links.get_score()

processing... batch 1/3
Ery_0: finished.
Ery_1: finished.
Ery_2: finished.
Ery_3: finished.
Ery_4: finished.
Ery_5: finished.
Ery_6: finished.
Ery_7: finished.
processing... batch 2/3
Ery_8: finished.
Ery_9: finished.
GMP_0: finished.
GMP_1: finished.
GMP_0: finished.
Gran_0: finished.
Gran_1: finished.
Gran_2: finished.
processing... batch 3/3
MEP_0: finished.
Mk_0: finished.
Mo_0: finished.
Mo_1: finished.
```

The score is stored as a attribute called “merged_score”, and the score will also be saved in a folder in your computer.

[82]:	links.merged_score.head()
[82]:	degree_all degree_in degree_out clustering_coefficient \\\nStat3 82 0 82 0.021981\\nMycn 30 0 30 0.011494\\nE2f4 181 2 179 0.009822\\nZbtb1 22 0 22 0.000000\\nYbx1 69 9 60 0.028133\\nclustering_coefficient_weighted degree_centrality_all \\\nStat3 0.022055 0.151292\\nMycn 0.009986 0.055351\\nE2f4 0.011874 0.333948\\nZbtb1 0.000000 0.040590\\nYbx1 0.027709 0.127306\\ndegree_centrality_in degree_centrality_out betweenness_centrality \\\nStat3 0.000000 0.151292 0\\nMycn 0.000000 0.055351 0\\nE2f4 0.003690 0.330258 3158\\nZbtb1 0.000000 0.040590 0\\nYbx1 0.016605 0.110701 1051\\ncloseness_centrality eigenvector_centrality page_rank \\\nStat3 0.000012 0.487978 0.001633\\nMycn 0.000010 0.245650 0.001633\\nE2f4 0.000010 1.000000 0.001724\\nZbtb1 0.000004 0.113727 0.001633\\nYbx1 0.000004 0.385376 0.002133\\nassortative_coefficient average_path_length community_random_walk \\\nStat3 -0.161693 2.621324 1\\nMycn -0.161693 2.621324 1\\nE2f4 -0.161693 2.621324 1\\nZbtb1 -0.161693 2.621324 18\\nYbx1 -0.161693 2.621324 2\\nmodule connectivity participation role cluster\\nStat3 0 3.573858 0.647531 Connector Hub Ery_0\\nMycn 2 1.767680 0.595556 Peripheral Ery_0\\nE2f4 0 8.575037 0.632252 Connector Hub Ery_0\\nZbtb1 2 1.317952 0.533058 Peripheral Ery_0\\nYbx1 3 5.306800 0.687251 Connector Hub Ery_0

5.4. Save

Save processed GRN. We use this file in the next notebook; “in silico perturbation with GRNs”.

[31]:	# Save Links object. links.to_hdf5(file_path="links.celloracle.links")
-------	---

[70]:	# You can load files with the following command. links = co.load_hdf5(file_path="links.celloracle.links")
-------	--

6. Network analysis; Network score for each gene

The Links class has many functions to visualize network score. See the documentation for the details of the functions.

6.1. Network score in each cluster

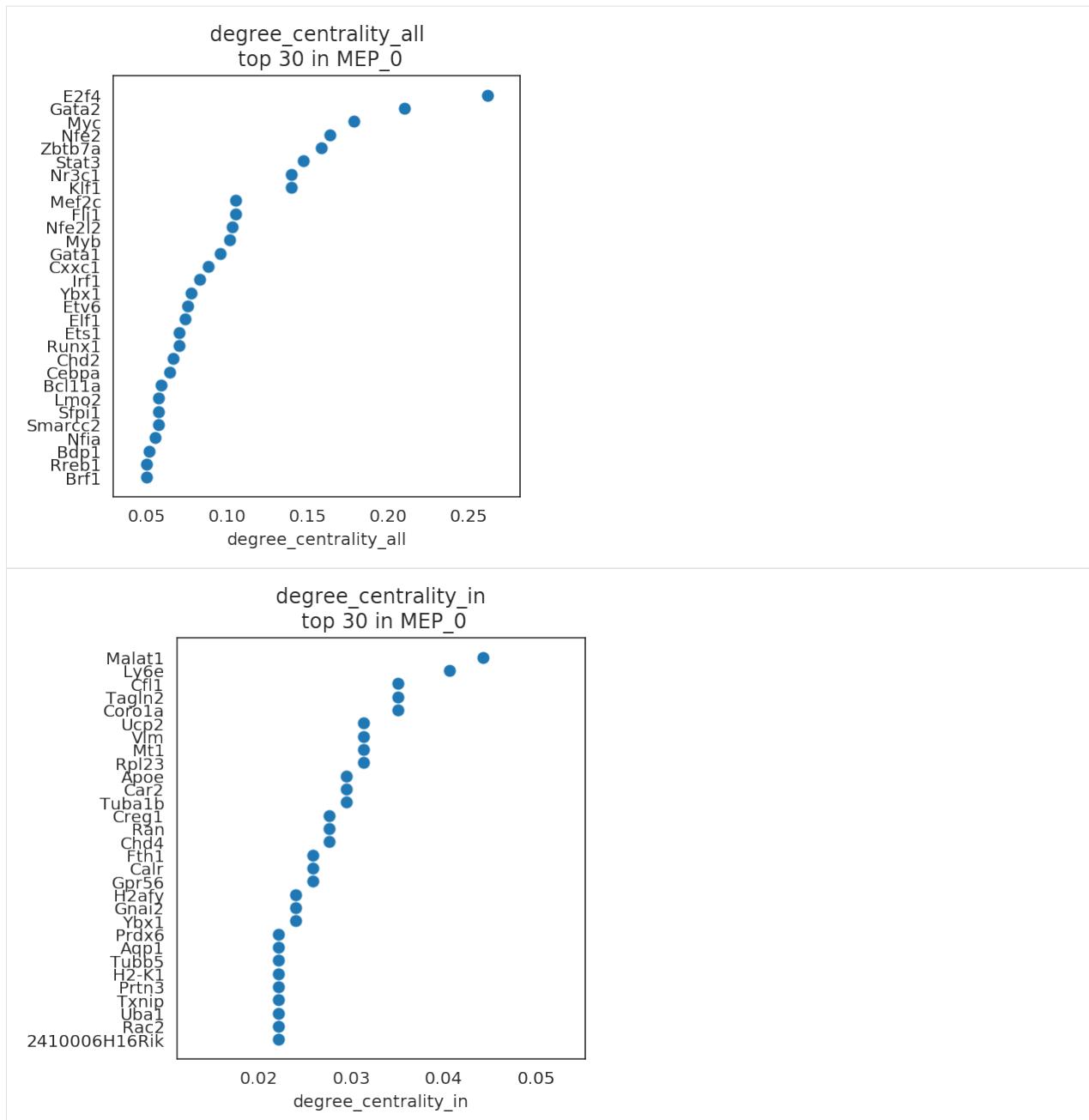
We have calculated several network scores using different centrality metrics. We can use the centrality score to identify key regulatory genes because centrality is one of the important indicators of network structure (<https://en.wikipedia.org/wiki/Centrality>).

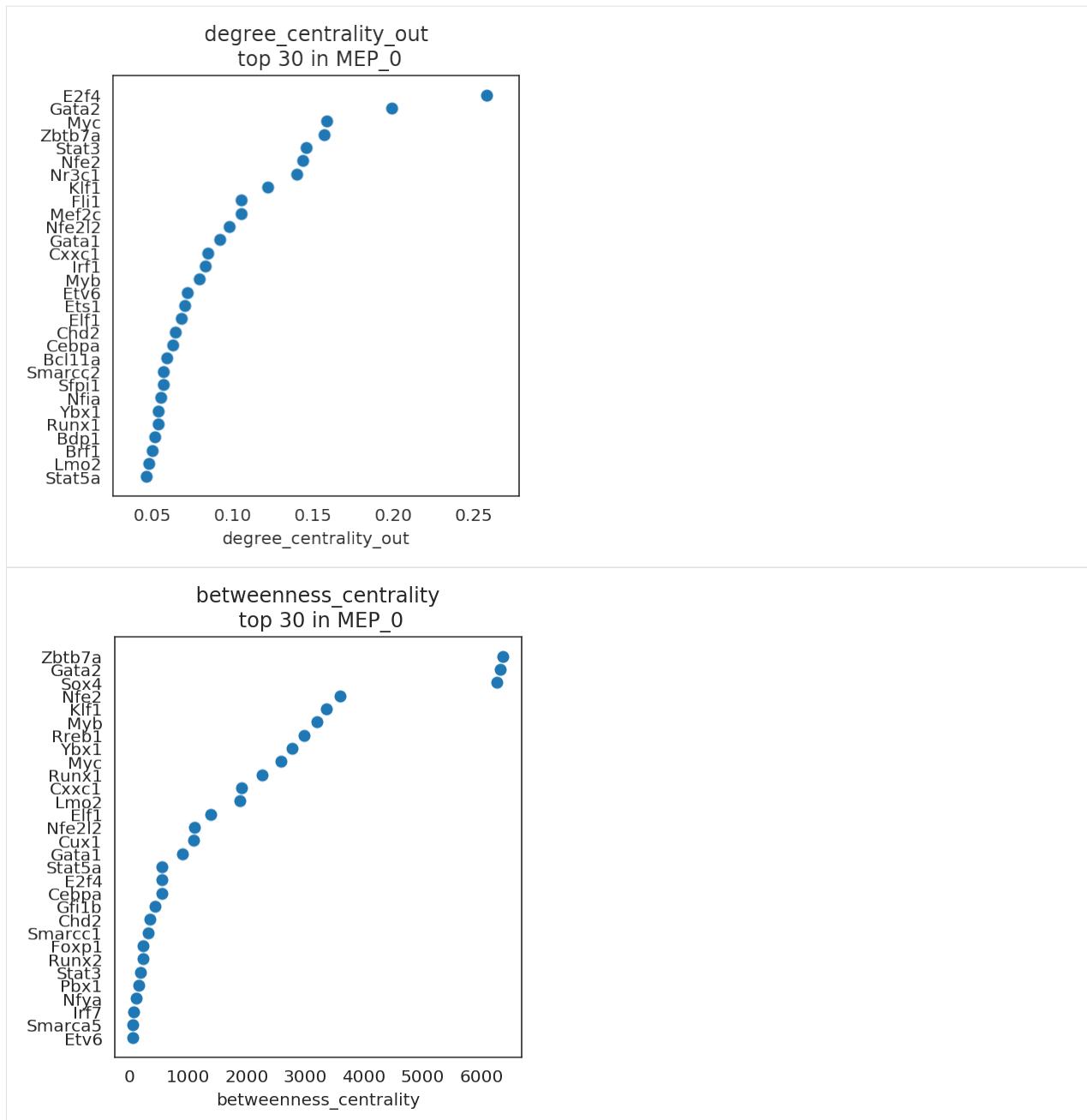
Let's visualize genes with high network centrality.

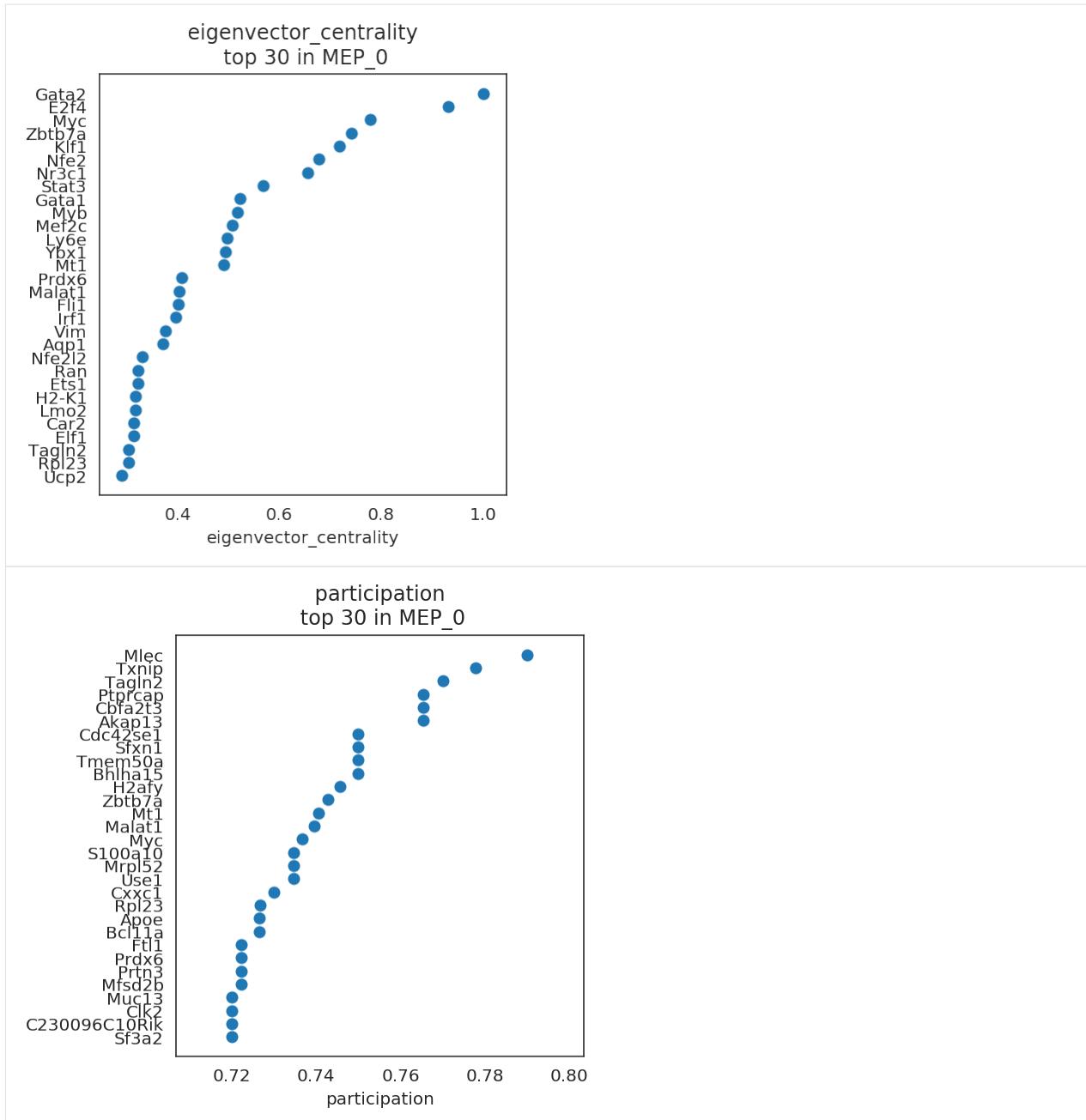
```
[83]: # Check cluster name
links.cluster
```

```
[83]: ['Ery_0',
'Ery_1',
'Ery_2',
'Ery_3',
'Ery_4',
'Ery_5',
'Ery_6',
'Ery_7',
'Ery_8',
'Ery_9',
'GMP_0',
'GMP_1',
'GMP1_0',
'Gran_0',
'Gran_1',
'Gran_2',
'MEP_0',
'Mk_0',
'Mo_0',
'Mo_1']
```

```
[53]: # Visualize top n-th genes that have high scores.
links.plot_scores_as_rank(cluster="MEP_0", n_gene=30, save=f"{save_folder}/ranked_
➥score")
```



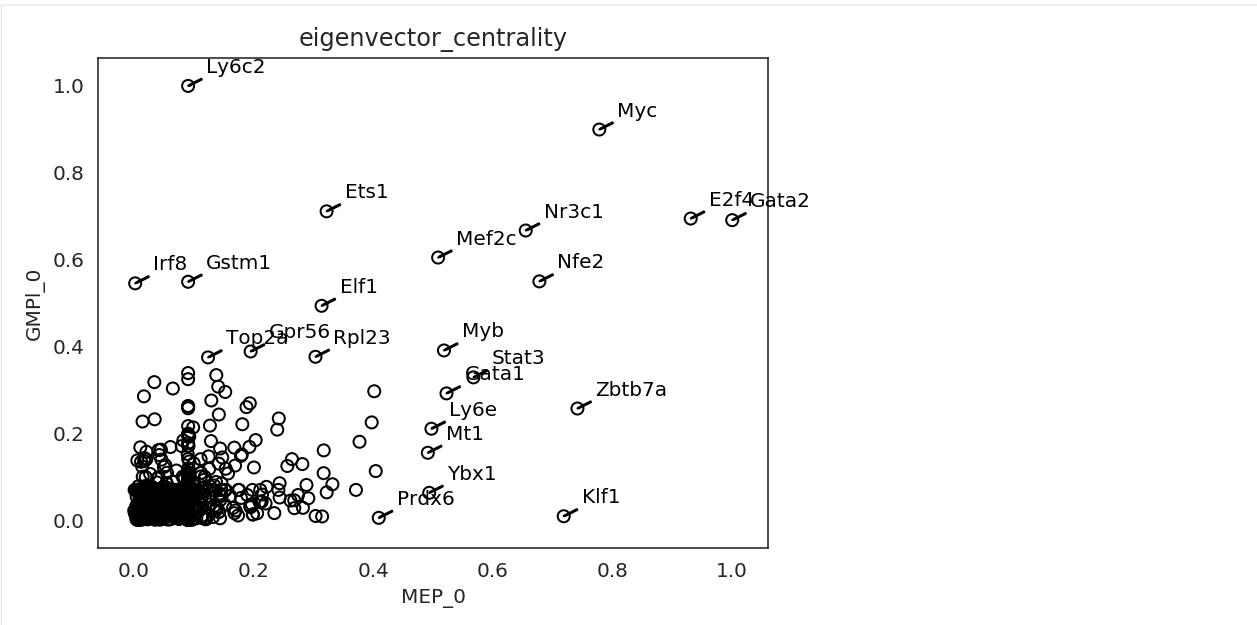




6.2. Network score comparison between two clusters

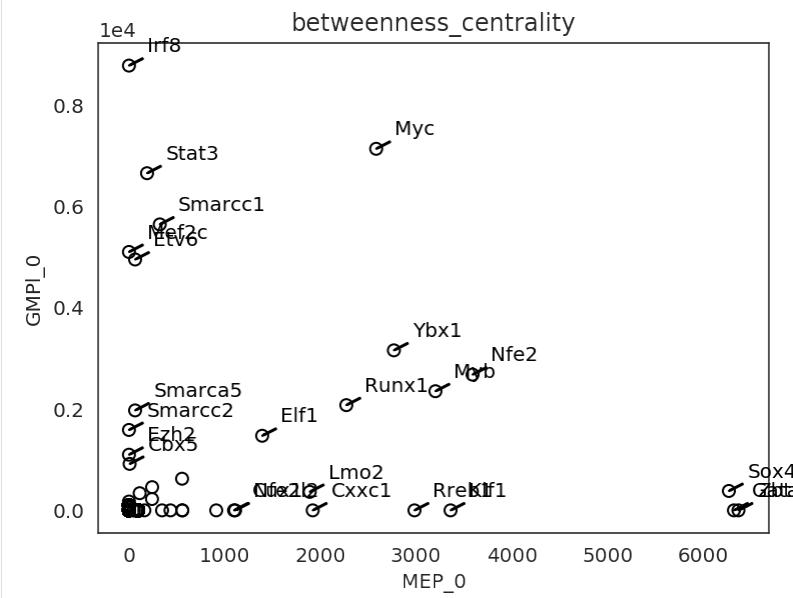
By comparing network scores between two clusters, we can analyze differences in GRN structure.

```
[54]: plt.ticklabel_format(style='sci',axis='y',scilimits=(0,0))
links.plot_score_comparison_2D(value="eigenvector_centrality",
                                cluster1="MEP_0", cluster2="GMP1_0",
                                percentile=98, save=f"{save_folder}/score_comparison")
```



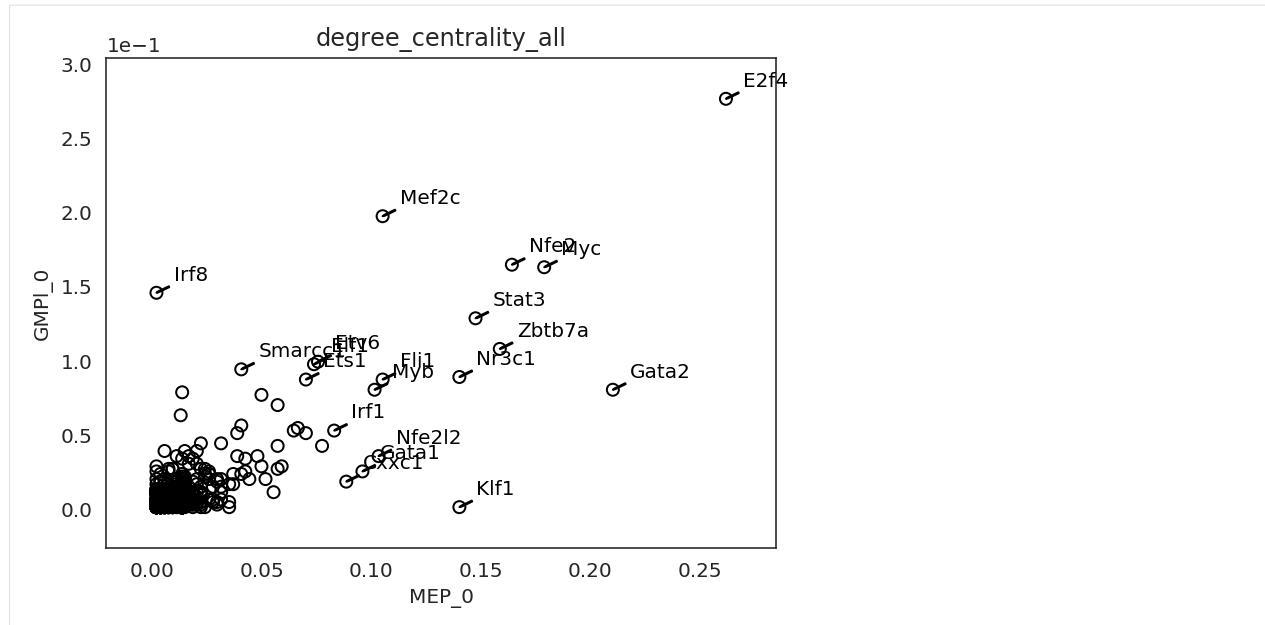
[55]:

```
plt.ticklabel_format(style='sci', axis='y', scilimits=(0,0))
links.plot_score_comparison_2D(value="betweenness_centrality",
                                cluster1="MEP_0", cluster2="GMP1_0",
                                percentile=98, save=f"{save_folder}/score_comparison")
```



[56]:

```
plt.ticklabel_format(style='sci', axis='y', scilimits=(0,0))
links.plot_score_comparison_2D(value="degree_centrality_all",
                                cluster1="MEP_0", cluster2="GMP1_0",
                                percentile=98, save=f"{save_folder}/score_comparison")
```

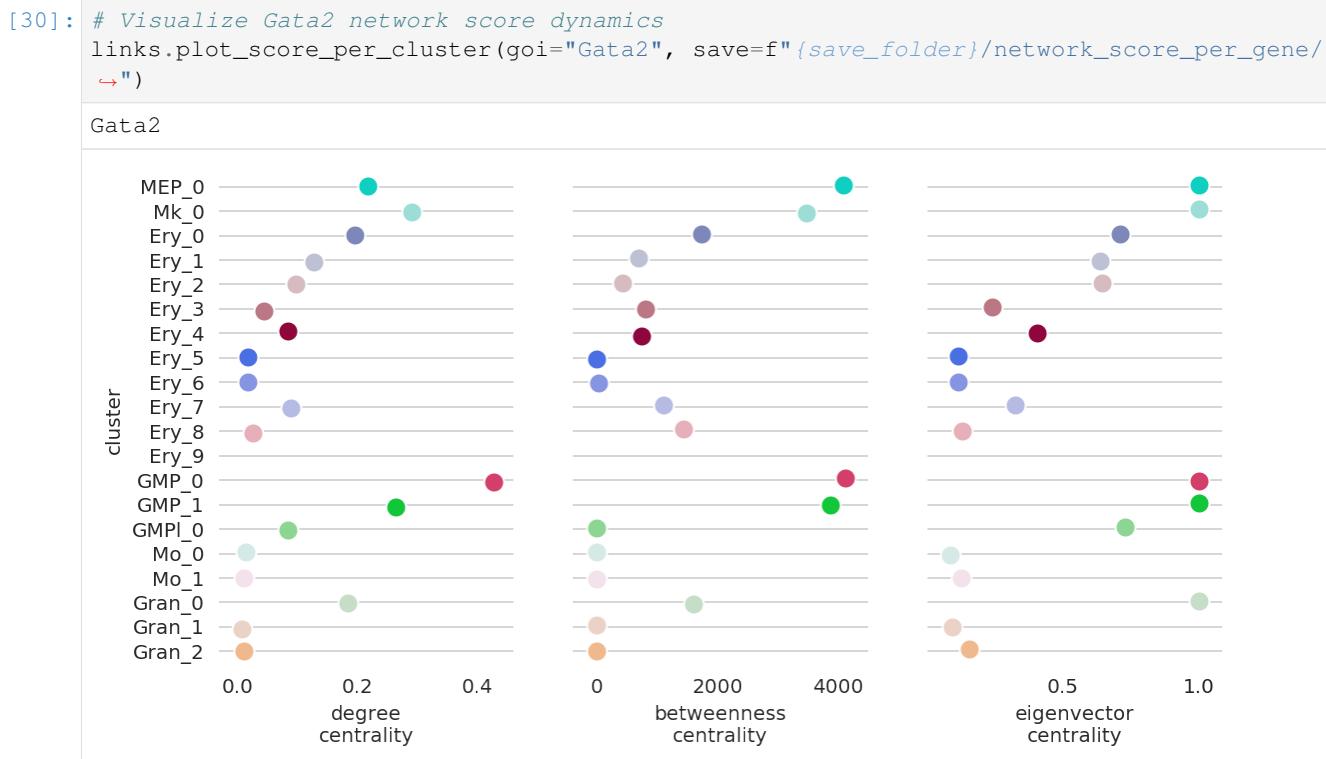


6.3. Network score dynamics

In the following session, we focus on how a gene's network score changes during the differentiation.

Using Gata2, we will demonstrate how you can visualize networks scores for a single gene.

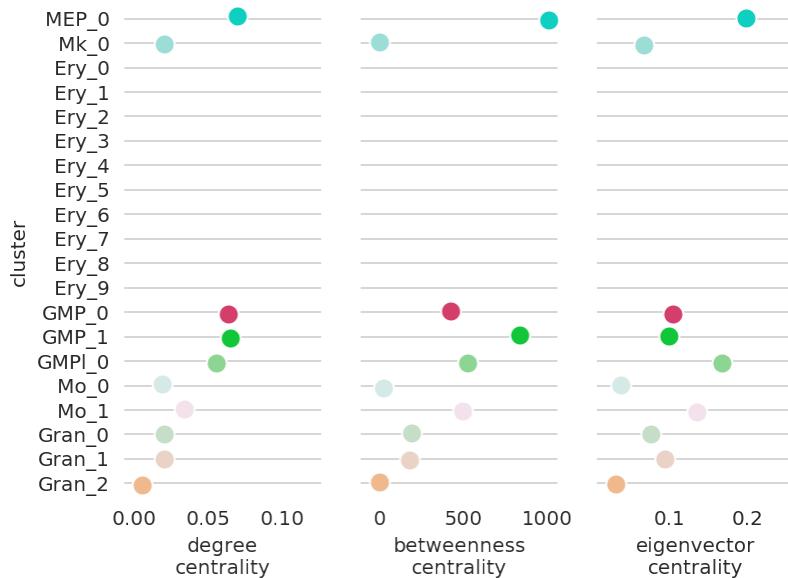
Gata2 is known to play an essential role in the early MEP and GMP populations. .



If a gene have no connections in a cluster, it is impossible to calculate network degree scores. Thus the scores will not be shown. For example, Cebpa have no connection in the erythroids clusters, and there is no degree scores for Cebpa in these clusters as follows.

```
[38]: links.plot_score_per_cluster(goi="Cebpa")
```

Cebpa



You can check filtered network edge as follows.

```
[39]: cluster_name = "Ery_0"
filtered_links_df = links.filtered_links[cluster_name]
filtered_links_df.head()
```

	source	target	coef_mean	coef_abs	p	-logp
68775	Stat3	Top2a	-0.107635	0.107635	1.976987e-14	13.703996
51655	Mycn	Prdx6	-0.096651	0.096651	8.076169e-11	10.092795
41345	Mycn	Mt1	-0.093897	0.093897	8.228218e-15	14.084694
5136	Ybx1	Anp32b	0.089403	0.089403	4.498303e-14	13.346951
41326	E2f4	Mt1	0.089261	0.089261	7.447929e-10	9.127964

You can confirm that there is no Cebpa connection in Ery_0 cluster.

```
[41]: filtered_links_df[filtered_links_df.source == "Cebpa"]
```

```
[41]: Empty DataFrame
Columns: [source, target, coef_mean, coef_abs, p, -logp]
Index: []
```

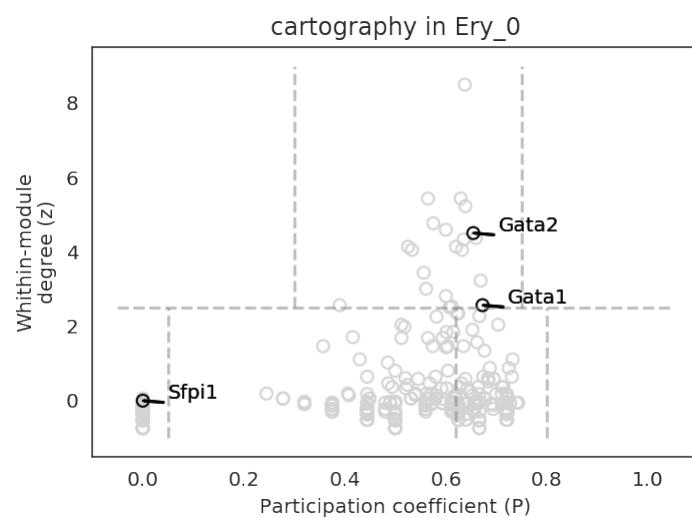
6.4. Gene cartography analysis

Gene cartography is a method for gene network analysis. The method classifies gene into several groups using the network module structure and connections. It provides us an insight about the role and regulatory mechanism for each gene. For more information on gene cartography, please refer to the following paper (<https://www.nature.com/articles/nature03288>).

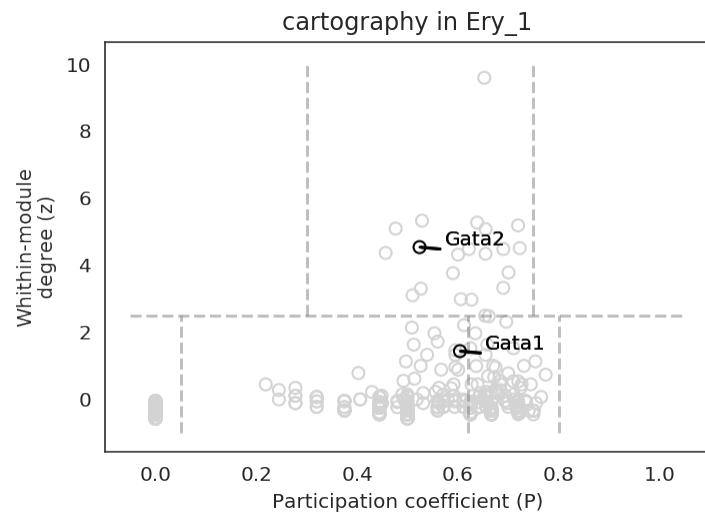
The gene cartography will be calculated for the GRN in each cluster. Thus we can know how the gene cartography change by comparing the the score between clusters.

```
[58]: # Plot cartography as a scatter plot
links.plot_cartography_scatter_per_cluster(scatter=True,
                                             kde=False,
                                             gois=["Gata1", "Gata2", "Sfpi1"],
                                             auto_gene_annot=False,
                                             args_dot={"n_levels": 105},
                                             args_line={"c": "gray"}, save=
                                             ↪folder)/cartography")
```

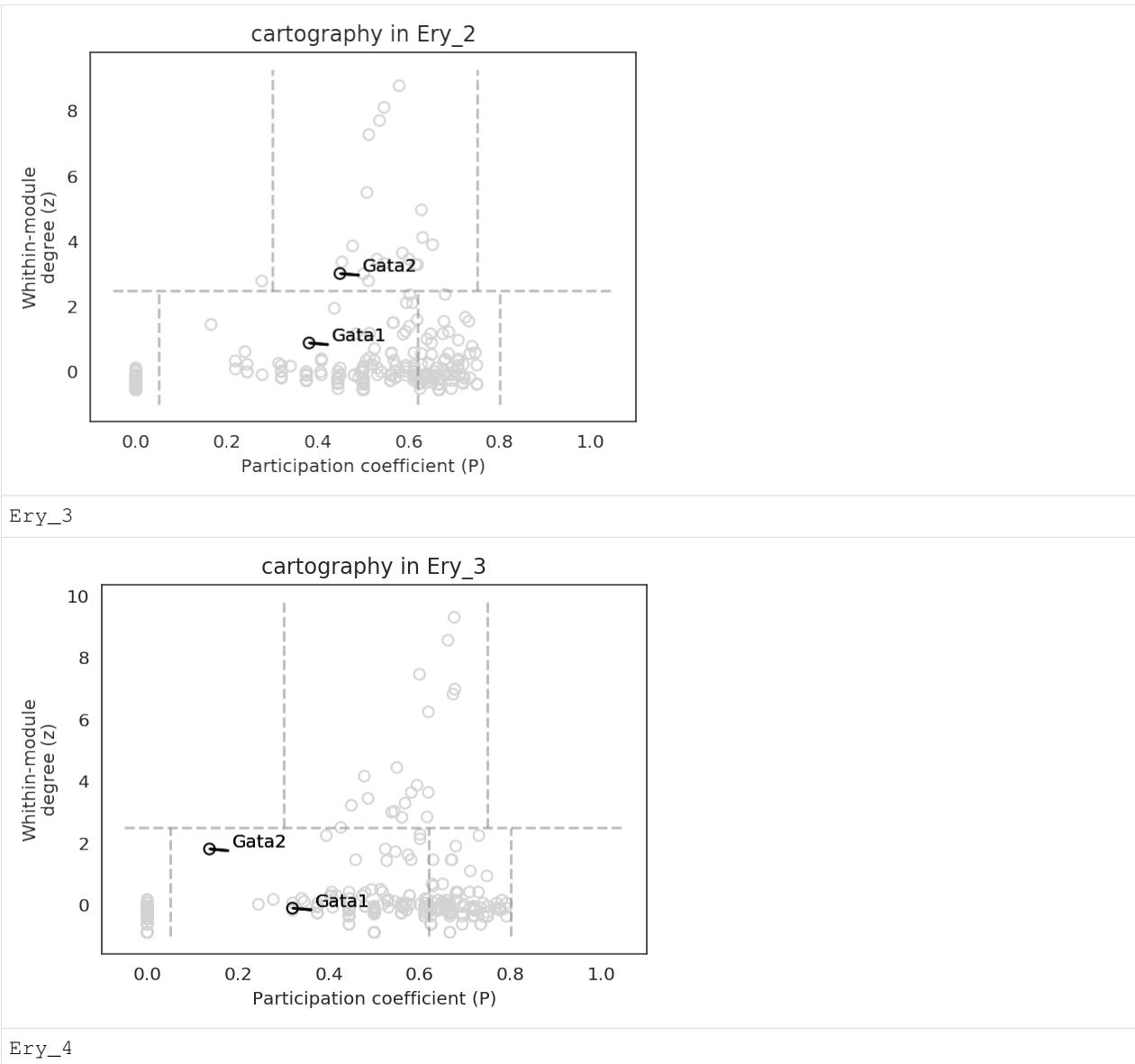
Ery_0

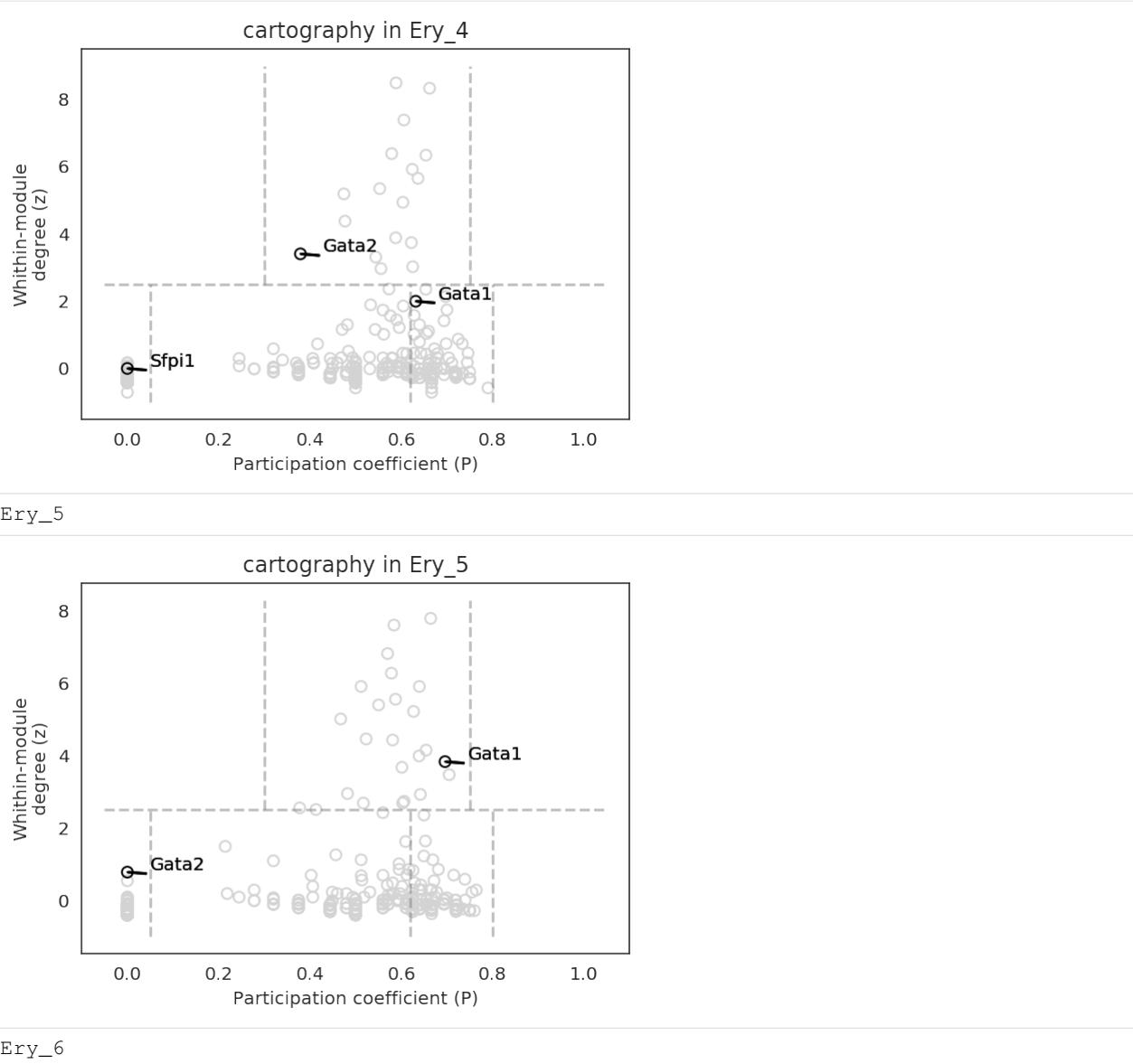


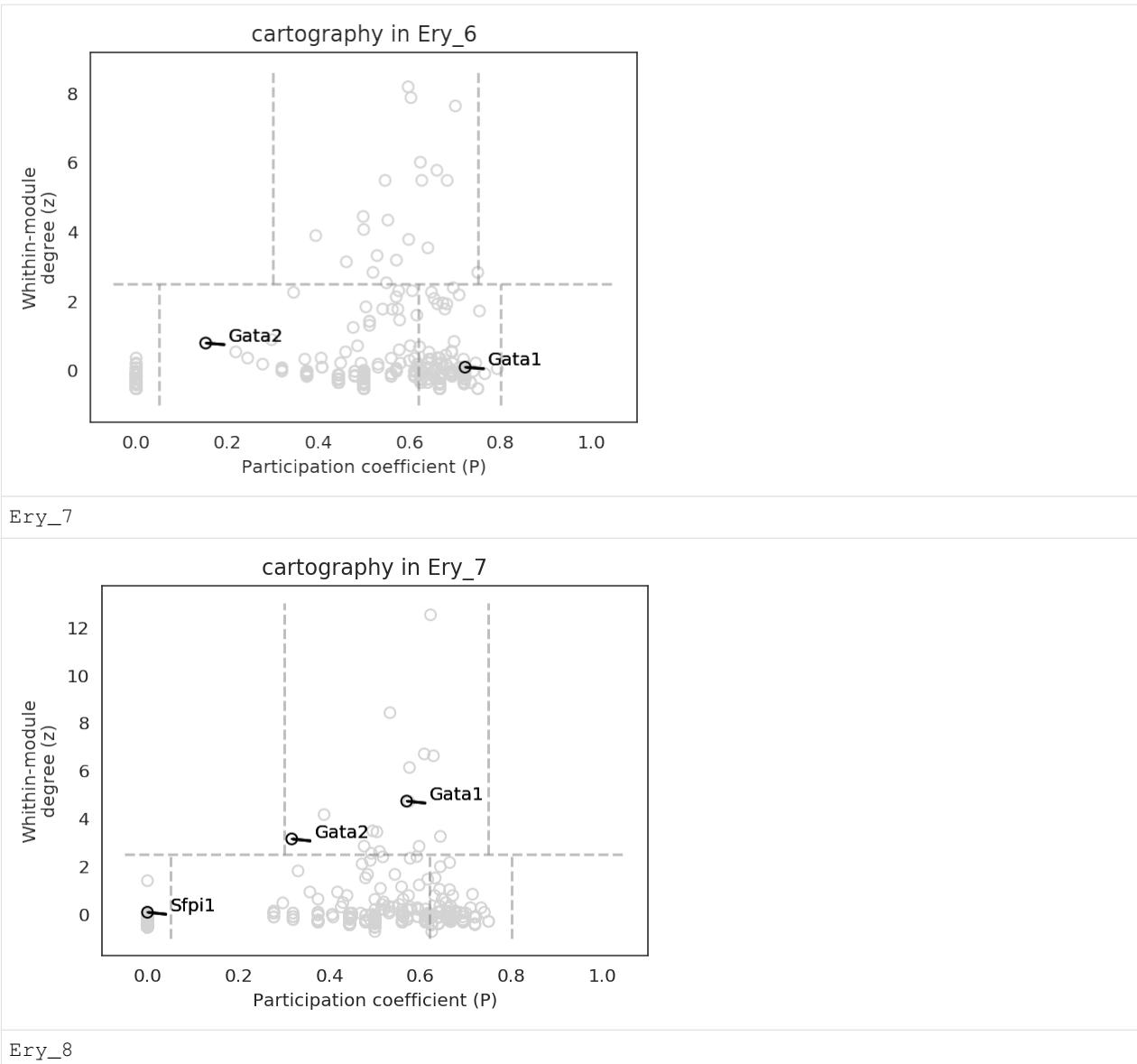
Ery_1

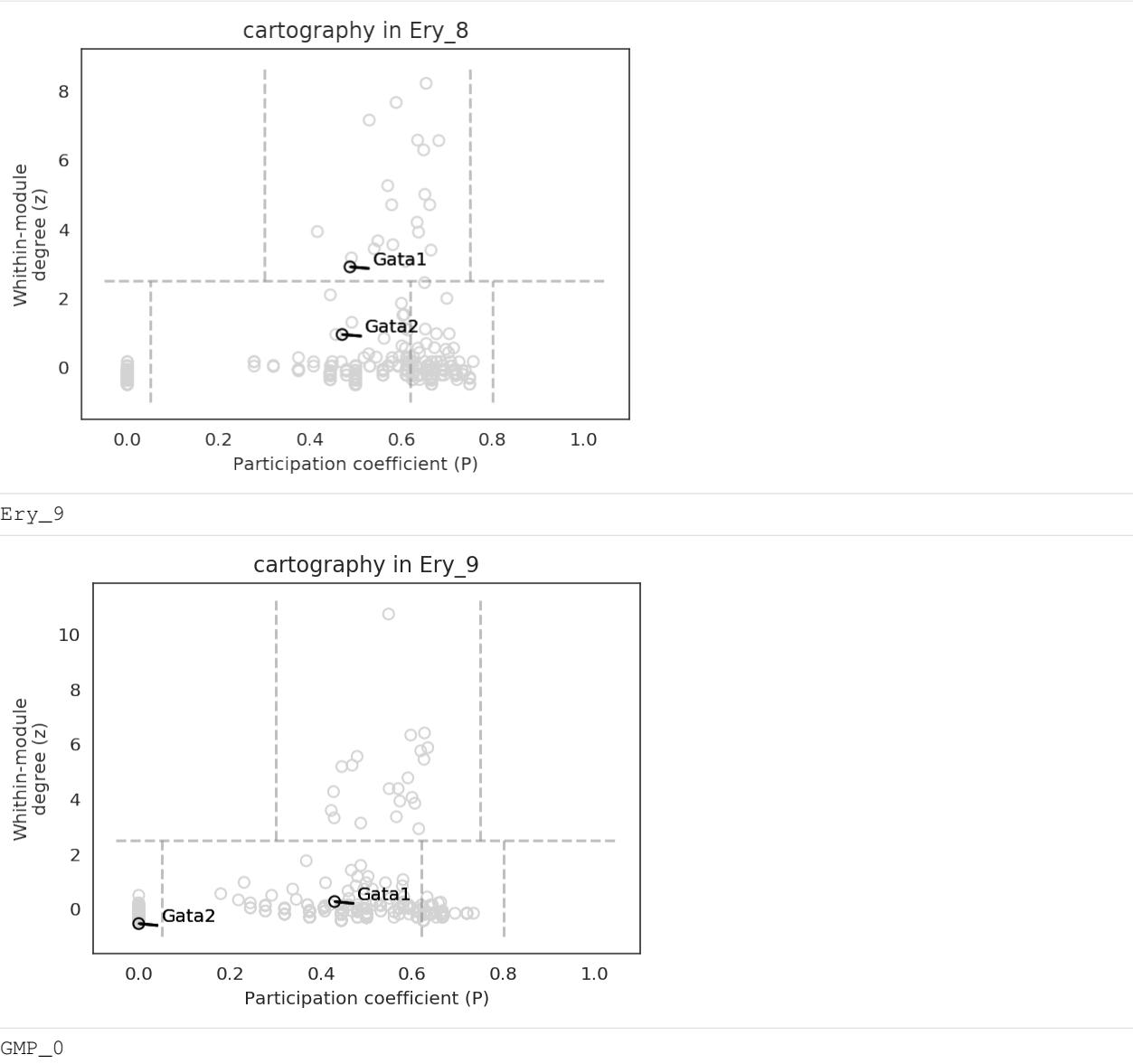


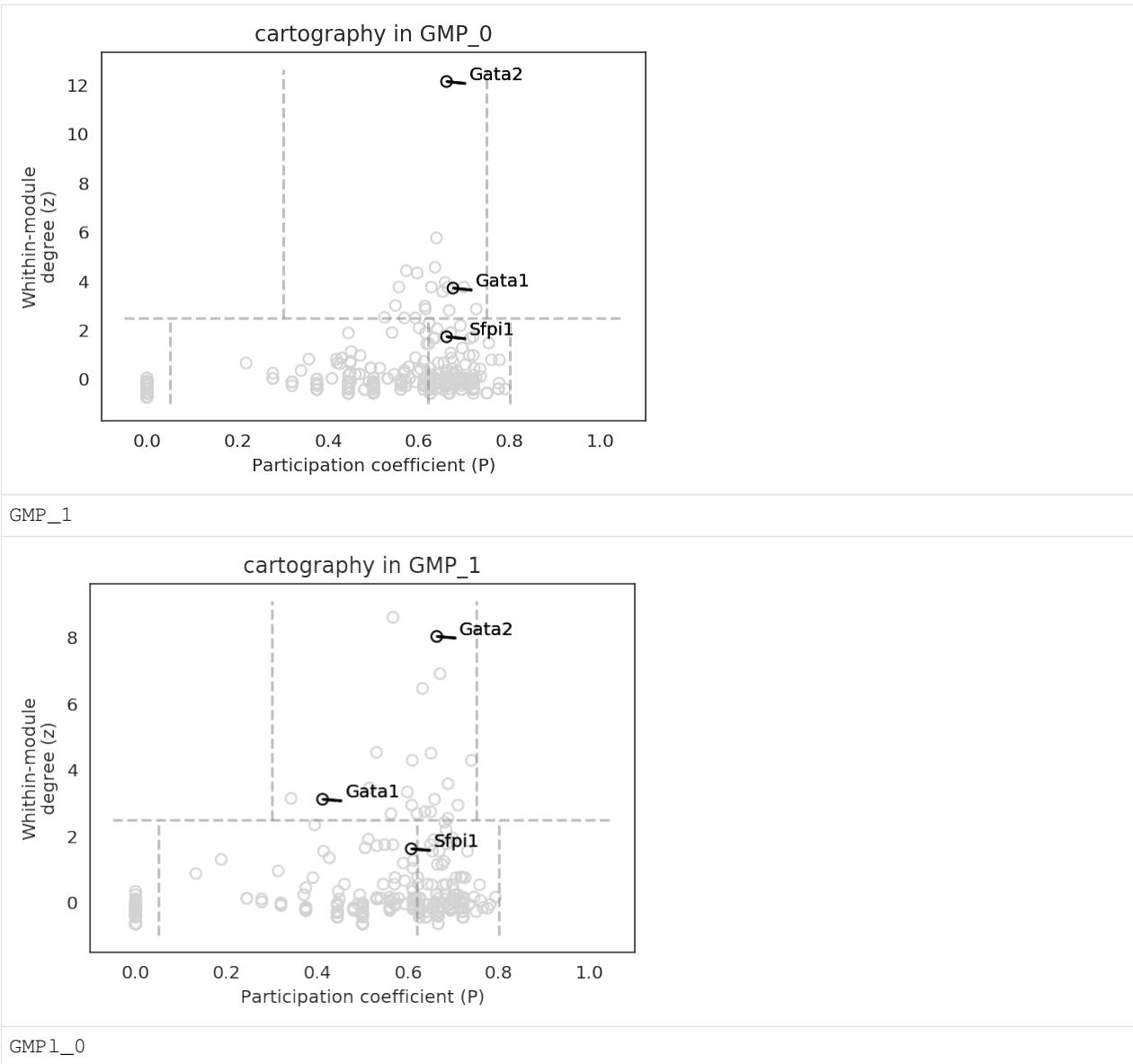
Ery_2

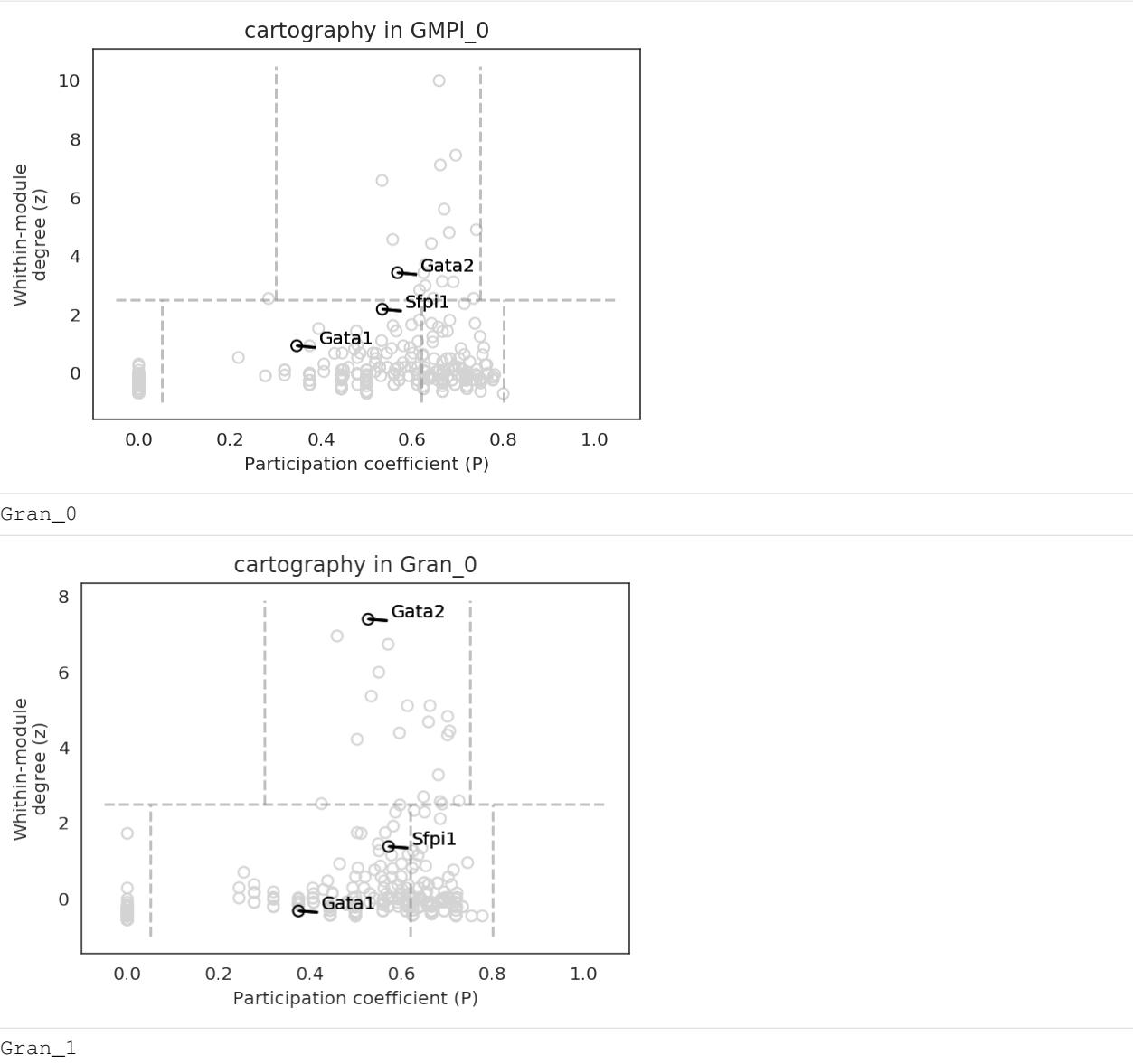


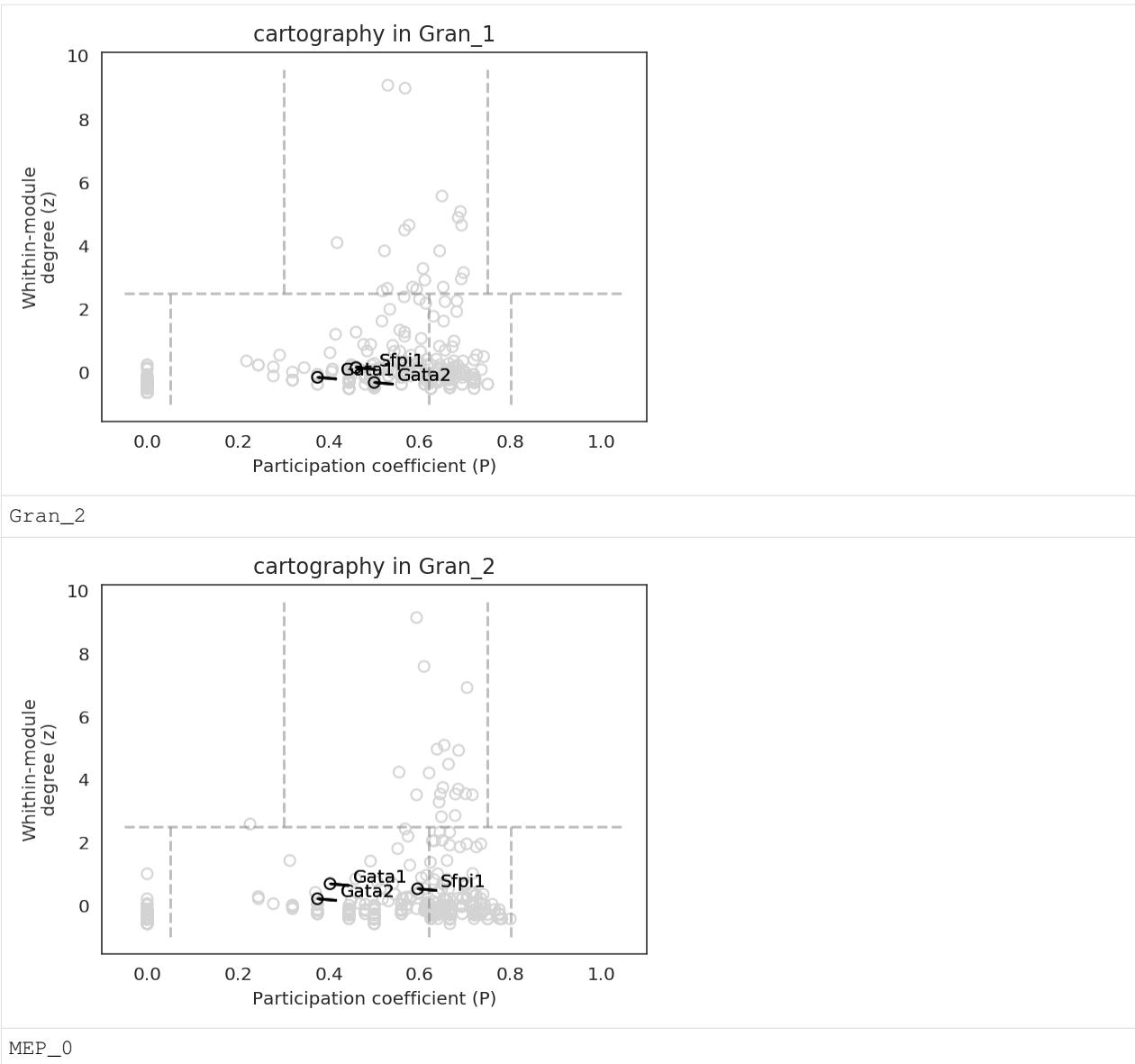


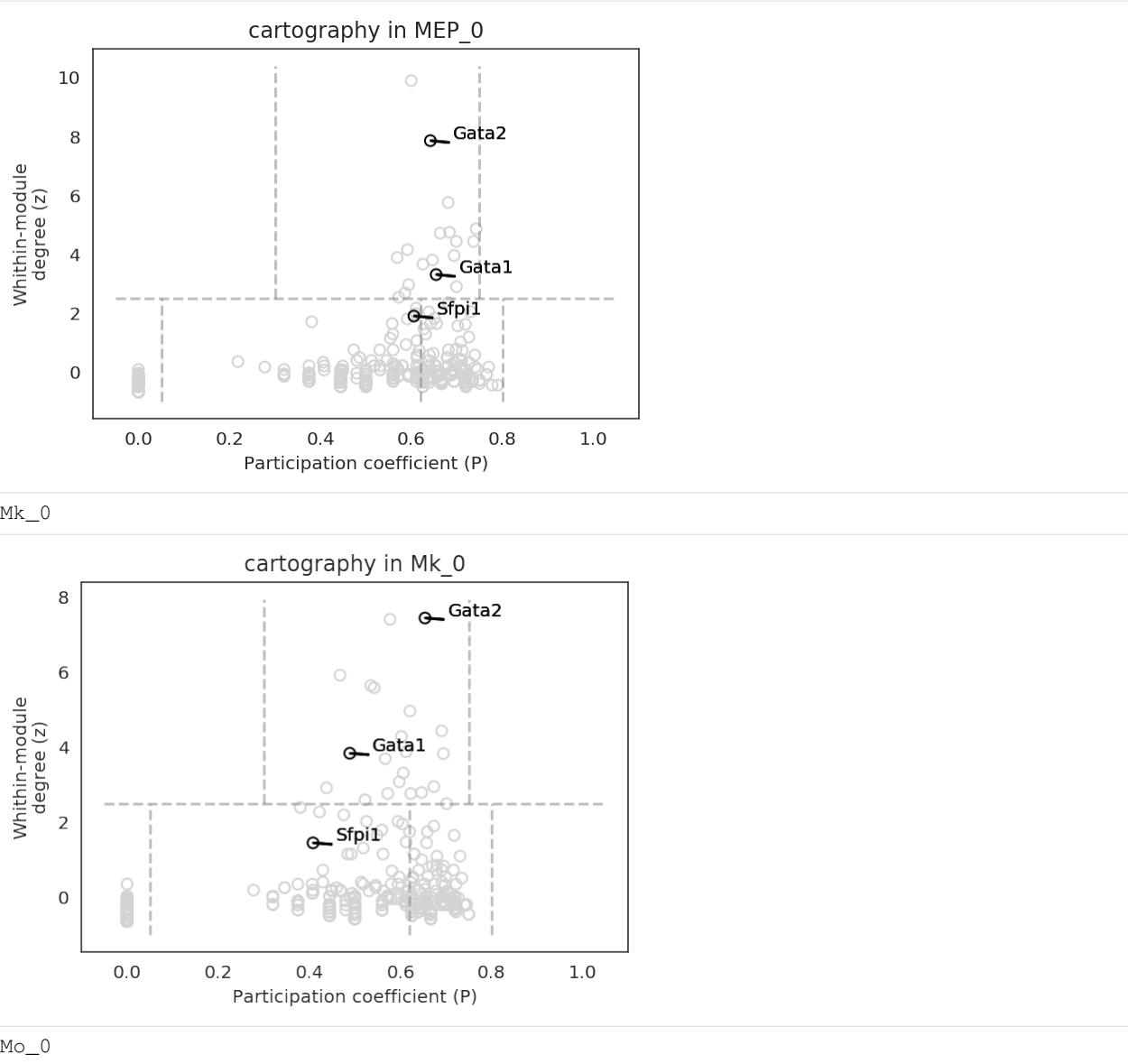


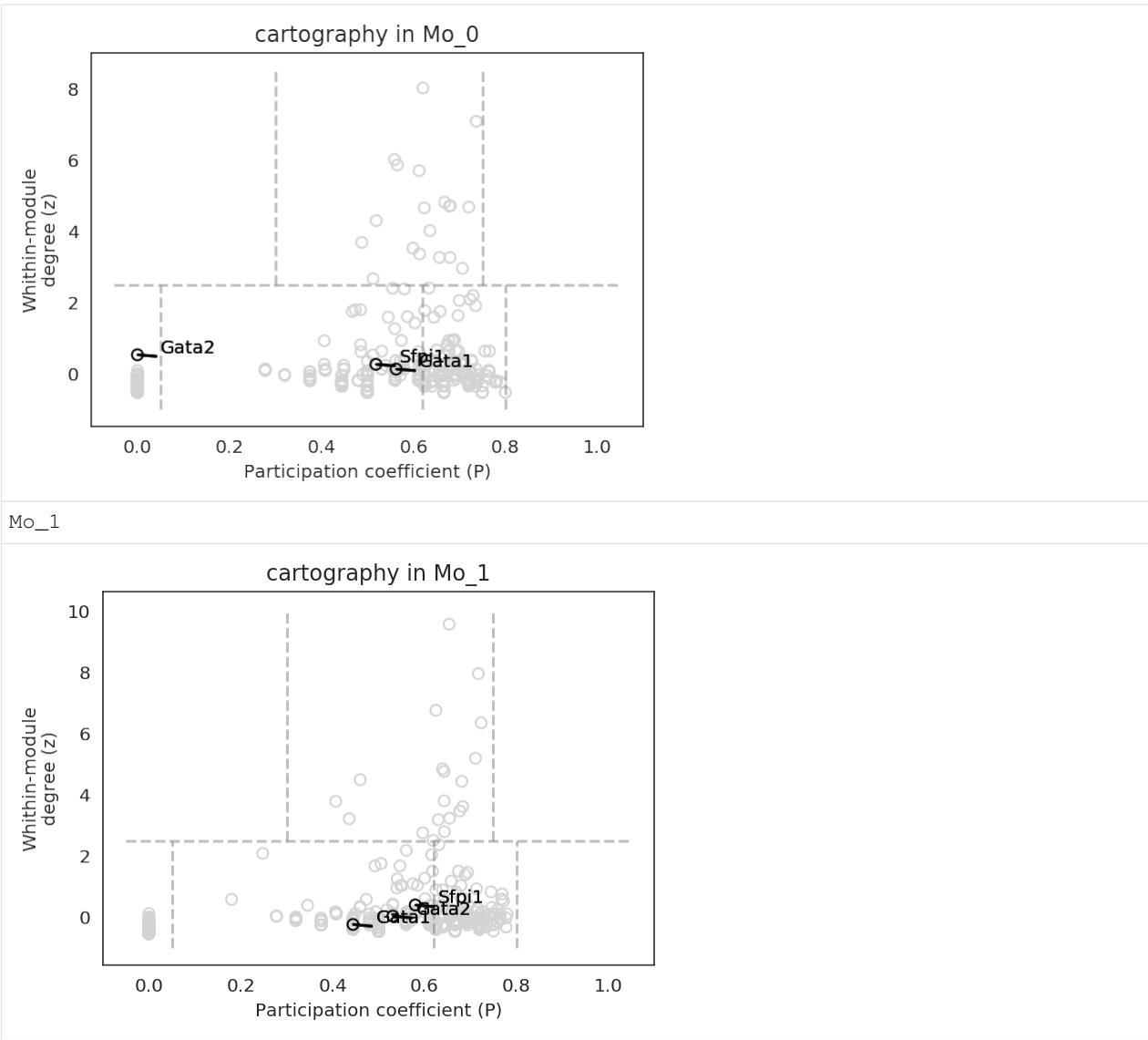








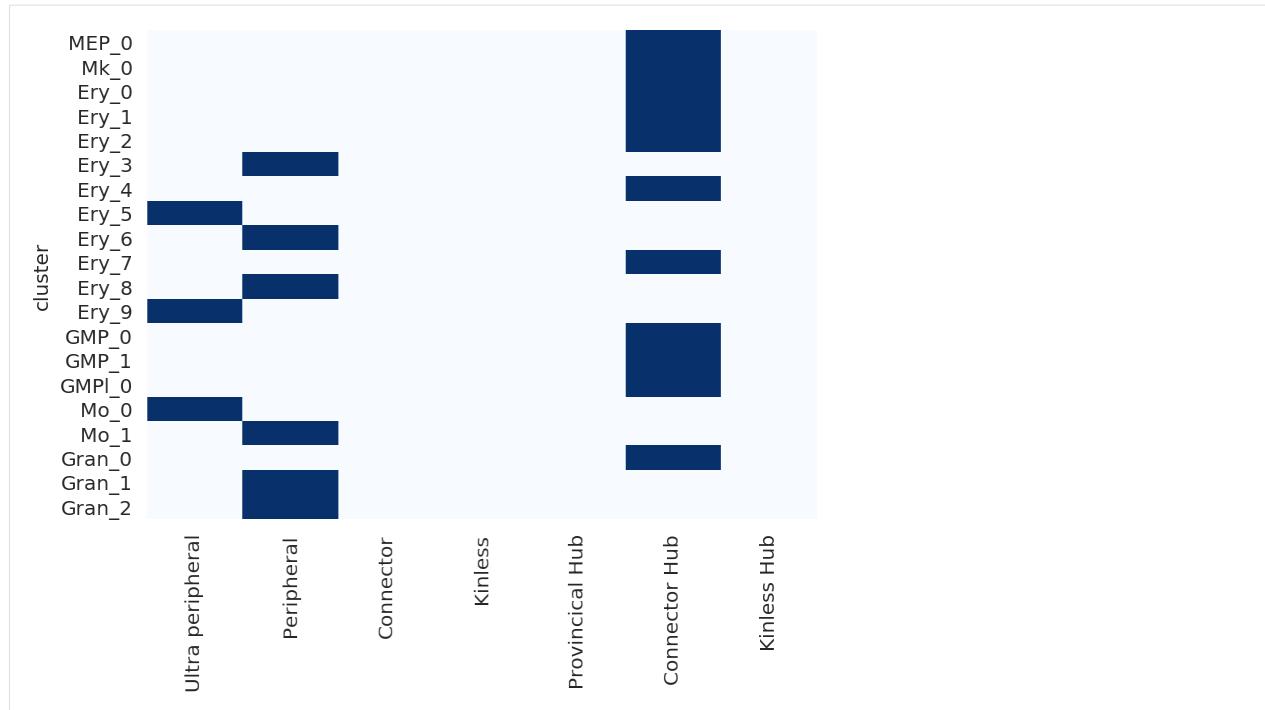




```
[66]: # Plot the summary of cartography analysis
```

```
links.plot_cartography_term(goi="Gata2", save=f"{save_folder}/cartography")
```

```
Gata2
```



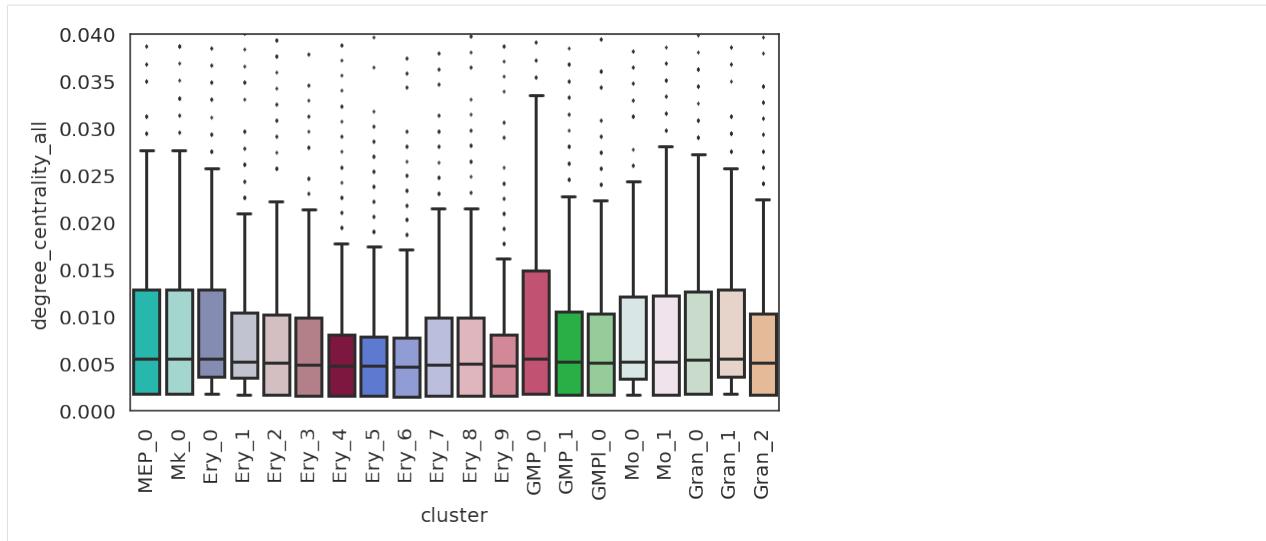
7. Network analysis; network score distribution

Next, we visualize the distribution of network score to get insight into the global trend of the GRNs.

7.1. Distribution of network degree

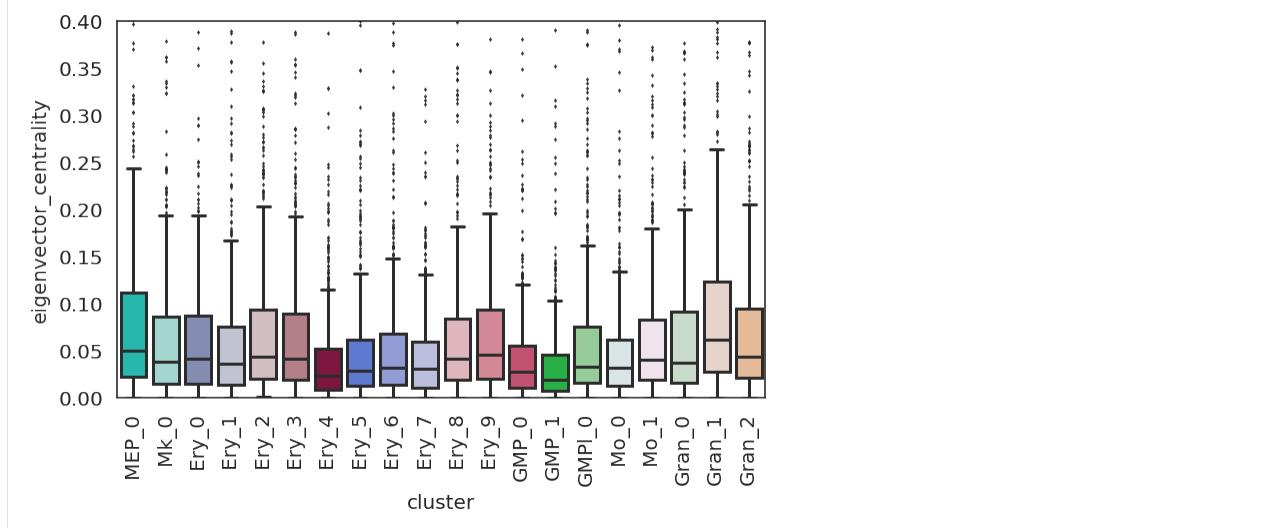
```
[60]: plt.subplots_adjust(left=0.15, bottom=0.3)
plt.ylim([0,0.040])
links.plot_score_distributions(values=["degree_centrality_all"], method="boxplot",
                                save=f"{save_folder}")
```

degree_centrality_all



```
[61]: plt.subplots_adjust(left=0.15, bottom=0.3)
plt.ylim([0, 0.40])
links.plot_score_distributions(values=["eigenvector_centrality"], method="boxplot", ↴
save=f"{save_folder}")
```

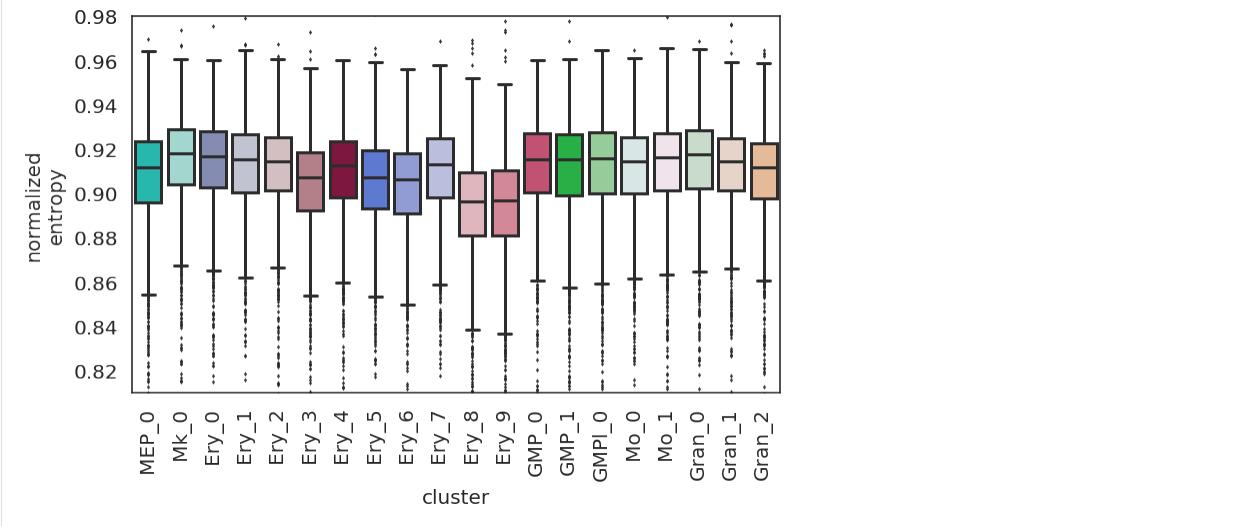
eigenvector_centrality



7.2. Distribution of network entropy

```
[62]: plt.subplots_adjust(left=0.15, bottom=0.3)
links.plot_network_entropy_distributions(save=f"{save_folder}")
```

```
/home/k/anaconda3/envs/test/lib/python3.6/site-packages/scipy/stats/_distn_
    ↪infrastructure.py:2614: RuntimeWarning: invalid value encountered in true_divide
      pk = 1.0*pk / np.sum(pk, axis=0)
/home/k/anaconda3/envs/test/lib/python3.6/site-packages/celloracle/network_analysis/
    ↪links_object.py:345: RuntimeWarning: divide by zero encountered in log
      ent_norm.append(en/np.log(k[i]))
/home/k/anaconda3/envs/test/lib/python3.6/site-packages/celloracle/network_analysis/
    ↪links_object.py:345: RuntimeWarning: invalid value encountered in double_scalars
      ent_norm.append(en/np.log(k[i]))
```



Using the network scores, we could pick up cluster-specific key TFs. Gata2, Gata1, Klf1, E2f1, for example, are known to play an essential role in MEP, and these TFs showed high network score in our GRN.

However, it is important to note that network analysis alone cannot shed light on the specific functions or roles these TFs play in cell fate determination.

In the next section, we will begin to investigate each TF's contribution to cell fate by running GRN simulations

[]:

1.2.5 Simulation with GRNs

celloracle leverage GRNs to simulate signal propagation inside a cell. We can estimate the effect of gene perturbation by the simulation with GRNs.

Additionally, we will combine the signal propagation simulation with a cell state transition simulation. The latter simulation is performed by a python library for RNA-velocity analysis, called *velocyto*. This analysis may provide an insight into a complex system how TF controls enormous target genes to determine cell fate.

Python notebook

0. Import libraries

0.1. Import public libraries

```
[1]: import os
import sys

import matplotlib.colors as colors
import matplotlib.pyplot as plt
import numpy as np
import pandas as pd
import scanpy as sc
import seaborn as sns
```

```
[2]: import celloracle as co
```

```
[3]: plt.rcParams["font.family"] = "arial"
plt.rcParams["figure.figsize"] = [9, 6]
%config InlineBackend.figure_format = 'retina'
plt.rcParams["savefig.dpi"] = 600

%matplotlib inline
```

0.1. Make a folder to save graph

```
[5]: # Make folder to save plots
save_folder = "figures"
os.makedirs(save_folder, exist_ok=True)
```

1. Load data

1.1. Load processed oracle object

Load the oracle object. See the previous notebook for the notes on how to prepare the oracle object.

```
[7]: oracle = co.load_hdf5("../04_Network_analysis/Paul_15_data.celloracle.oracle")
```

1.2. Load inferred GRNs

In the previous notebook, we calculated GRNs. Now, we will use these GRNs for simulation. We import GRNs which were saved in the Links object.

```
[8]: links = co.load_hdf5("../04_Network_analysis/links.celloracle.links")
```

3. Make predictive models for simulation

We will fit ridge regression models again. This process takes less time than the GRN inference in the previous notebook because we only use significant TFs to predict target gene instead of all regulatory candidate TFs.

```
[12]: links.filter_links()
oracle.get_cluster_specific_TFdict_from_Links(links_object=links)
oracle.fit_GRN_for_simulation(alpha=10, use_cluster_specific_TFdict=True)
```

```
calculating GRN using cluster specific TF dict...
calculating GRN in Ery_0
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1074 genes
calculating GRN in Ery_1
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1092 genes
calculating GRN in Ery_2
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1064 genes
calculating GRN in Ery_3
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1105 genes
calculating GRN in Ery_4
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1102 genes
calculating GRN in Ery_5
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1116 genes
calculating GRN in Ery_6
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1097 genes
calculating GRN in Ery_7
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1062 genes
calculating GRN in Ery_8
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))

genes_in_gem: 1999
models made for 1117 genes
calculating GRN in Ery_9
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1121 genes
calculating GRN in GMP_0
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1107 genes
calculating GRN in GMP_1
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1104 genes
calculating GRN in GMP1_0
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1089 genes
calculating GRN in Gran_0
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1067 genes
calculating GRN in Gran_1
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1076 genes
calculating GRN in Gran_2
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1105 genes
calculating GRN in MEP_0
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1152 genes
calculating GRN in Mk_0
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1114 genes
calculating GRN in Mo_0
```

```
HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1085 genes
calculating GRN in Mo_1

HBox(children=(IntProgress(value=0, max=1999), HTML(value='')))
```

```
genes_in_gem: 1999
models made for 1074 genes
```

4. in silico Perturbation-simulation

Next, we will simulate the effects of perturbing a single TF to investigate its function and regulatory mechanism. See the celloracle paper for the details and scientific premise on the algorithm.

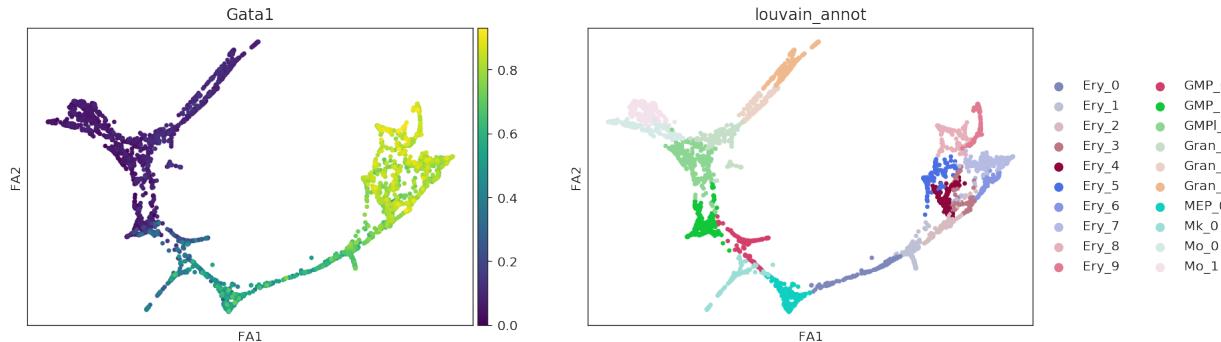
In this notebook, we'll show an example of the simulation; we'll simulate knock-out of Gata1 gene in the hematopoiesis.

Previous studies have shown that Gata1 is one of the TFs that regulates cell fate decisions in myeloid progenitors. Additionally, Gata1 has been shown to affect erythroid cell differentiation.

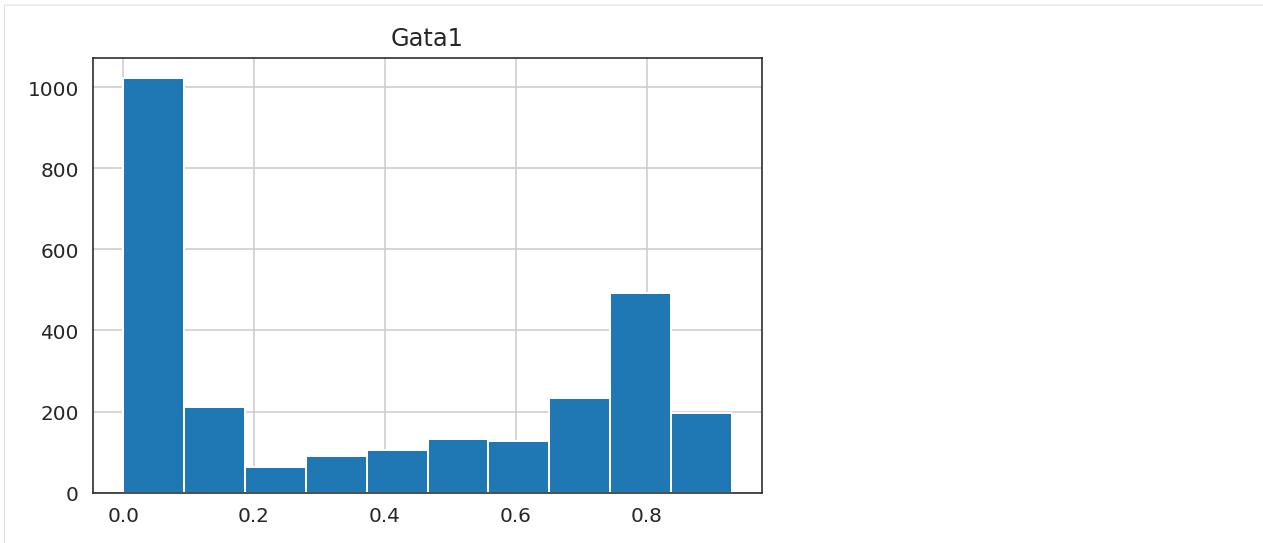
Here, we will analyze Gata1 for the demonstration of celloracle; Celloracle try to recapitulate the previous findings of Gata1 gene above.

4.1. Check gene expression pattern.

```
[26]: # Check gene expression
goi = "Gata1"
sc.pl.draw_graph(oracle.adata, color=[goi, oracle.cluster_column_name],
                 layer="imputed_count", use_raw=False, cmap="viridis")
```



```
[33]: # Plot gene expression in histogram
sc.get.obs_df(oracle.adata, keys=[goi], layer="imputed_count").hist()
plt.show()
```



4.1. calculate future gene expression after perturbation.

Although you can use any gene expression value for the input of in silico perturbation, we recommend avoiding extreme values which are far from natural gene expression ranges. If you set Gata1 gene expression to 100, for example, it may lead to biologically infeasible results.

Here we simulate Gata1 KO; we predict what happens to the cells if Gata1 gene expression changed into 0.

```
[34]: # Enter perturbation conditions to simulate signal propagation after the perturbation.
oracle.simulate_shift(perturb_condition={goi: 0.0},
                      n_propagation=3)
```

4.2. calculate transition probability between cells

In the step above, we simulated simulated future gene expression values after perturbation. This prediction is based on iterative calculations of signal propagations within the GRN.

Next step, we will calculate the probability of a cell state transition based on the simulated data. Using the transition probability between cells, we can predict how a cell changes after perturbation.

This transition probability will be used in two ways.

- (1) Visualization of directed trajectory graph.
- (2) Markov simulation.

In Step 4.2 and 4.3, we use functions imported from the `velocytoloom` class in `velocyto.py`. Please see the documentation of `VelocytoLoom` for more information. http://velocyto.org/velocyto.py/fullapi/api_analysis.html

```
[35]: # Get transition probability
oracle.estimate_transition_prob(n_neighbors=200, knn_random=True, sampled_fraction=0.
                                ↪5)

# Calculate embedding
oracle.calculate_embedding_shift(sigma_corr = 0.05)
```

(continues on next page)

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```
# Calculate global trend of cell transition
oracle.calculate_grid_arrows(smooth=0.8, steps=(40, 40), n_neighbors=300)

/home/k/anaconda3/envs/test/lib/python3.6/site-packages/IPython/core/interactiveshell.py:3326: FutureWarning: arrays to stack must be passed as a "sequence" type such as
list or tuple. Support for non-sequence iterables such as generators is deprecated
as of NumPy 1.16 and will raise an error in the future.
    exec(code_obj, self.user_global_ns, self.user_ns)
WARNING:root:Nans encountered in corrcoef and corrected to 1s. If not identical cells
were present it is probably a small isolated cluster converging after imputation.
```

4.3. Visualization

4.3.1. Detailed directed trajectory graph

```
[36]: plt.figure(None, (6, 6))
quiver_scale = 40

ix_choice = np.random.choice(oracle.adata.shape[0], size=int(oracle.adata.shape[0]/1.,
    ), replace=False)

embedding = oracle.adata.obsm[oracle.embedding_name]

plt.scatter(embedding[ix_choice, 0], embedding[ix_choice, 1],
            c="0.8", alpha=0.2, s=38, edgecolor=(0,0,0,1), lw=0.3, rasterized=True)

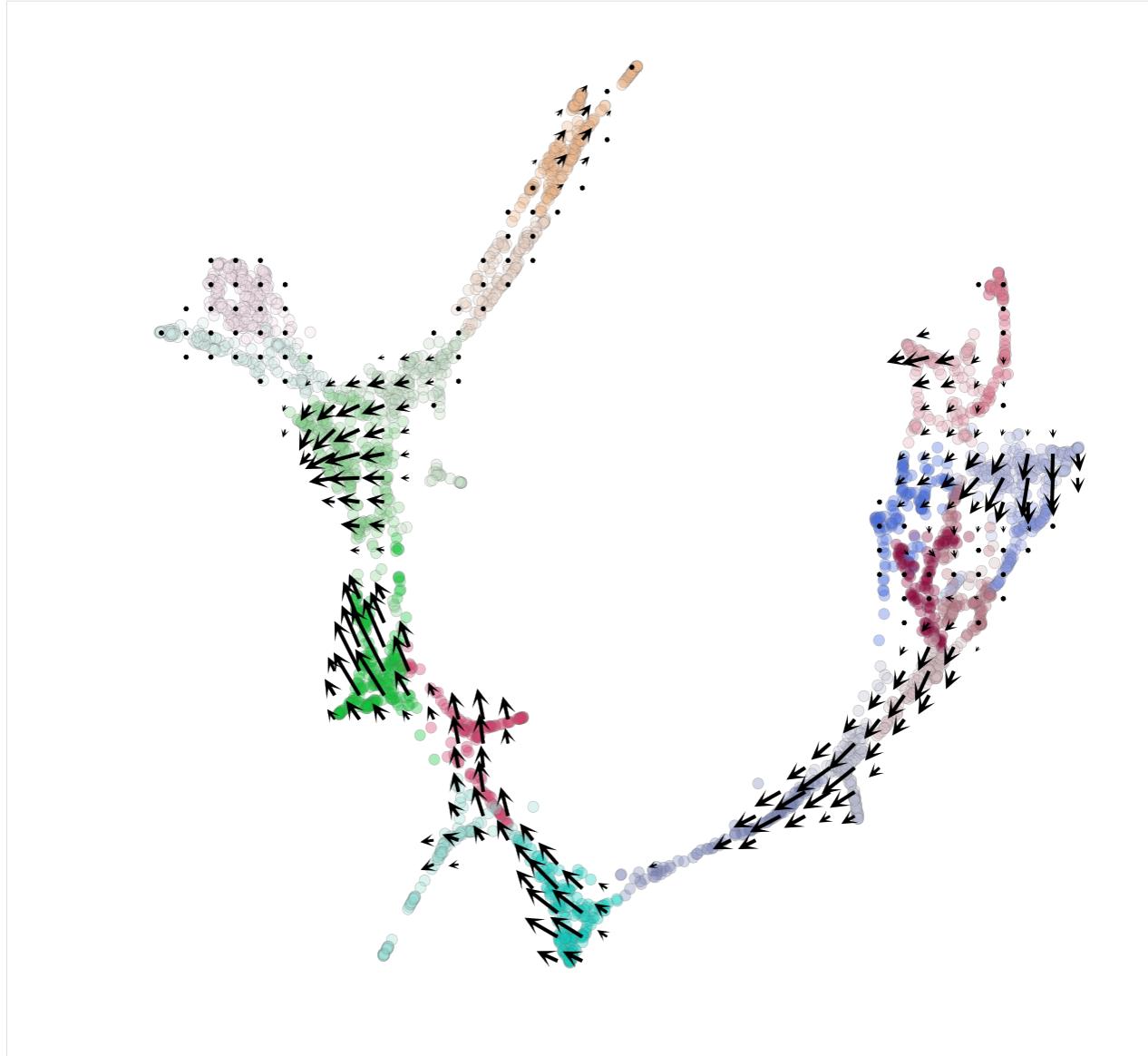
quiver_kw_args=dict(headaxislength=7, headlength=11, headwidth=8,
                     linewidths=0.35, width=0.0045, edgecolors="k",
                     color=oracle.colorandum[ix_choice], alpha=1)
plt.quiver(embedding[ix_choice, 0], embedding[ix_choice, 1],
            oracle.delta_embedding[ix_choice, 0], oracle.delta_embedding[ix_choice, 1],
            scale=quiver_scale, **quiver_kw_args)

plt.axis("off")
# plt.savefig(f"{save_folder}/full_arrows{goi}.png", transparent=True)
[36]: (-10815.27020913708, 10950.84121716522, -10711.36365432337, 10949.477199695968)
```



4.3.2. Grid graph

```
[37]: # Plot whole graph
plt.figure(None, (10,10))
oracle.plot_grid_arrows(quiver_scale=2.0,
                        scatter_kwarg_dict={"alpha":0.35, "lw":0.35,
                                            "edgecolor":"0.4", "s":38,
                                            "rasterized":True},
                        min_mass=0.015, angles='xy', scale_units='xy',
                        headaxislength=2.75,
                        headlength=5, headwidth=4.8, minlength=1.5,
                        plot_random=False, scale_type="relative")
# plt.savefig(f"{save_folder}/vectorfield_{goi}.png", transparent=True)
```



4.4. Markov simulation to analyze the effects of perturbation on cell fate transition

We can also simulate cell state transition using Markov simulation.

4.4.1. Do simulation

We will simulate using the parameters, “n_steps=200” and “n_duplication=5” in the following example.

To elaborate, this means:

- (1) We will do 200 times of iterative simulations to predict how the cell changes over time
- (2) We will repeat 5 rounds of simulations

```
[83]: %time
# n_steps is the number of steps in markov simulation.
# n_duplication is the number of technical duplication for the simulation
oracle.run_markov_chain_simulation(n_steps=200, n_duplication=5)

CPU times: user 1.33 s, sys: 0 ns, total: 1.33 s
Wall time: 1.33 s
```

4.4.2. Check the results of the simulation for specific cells

Check the results of simulation. Pick up some cells and visualize their transition trajectory.

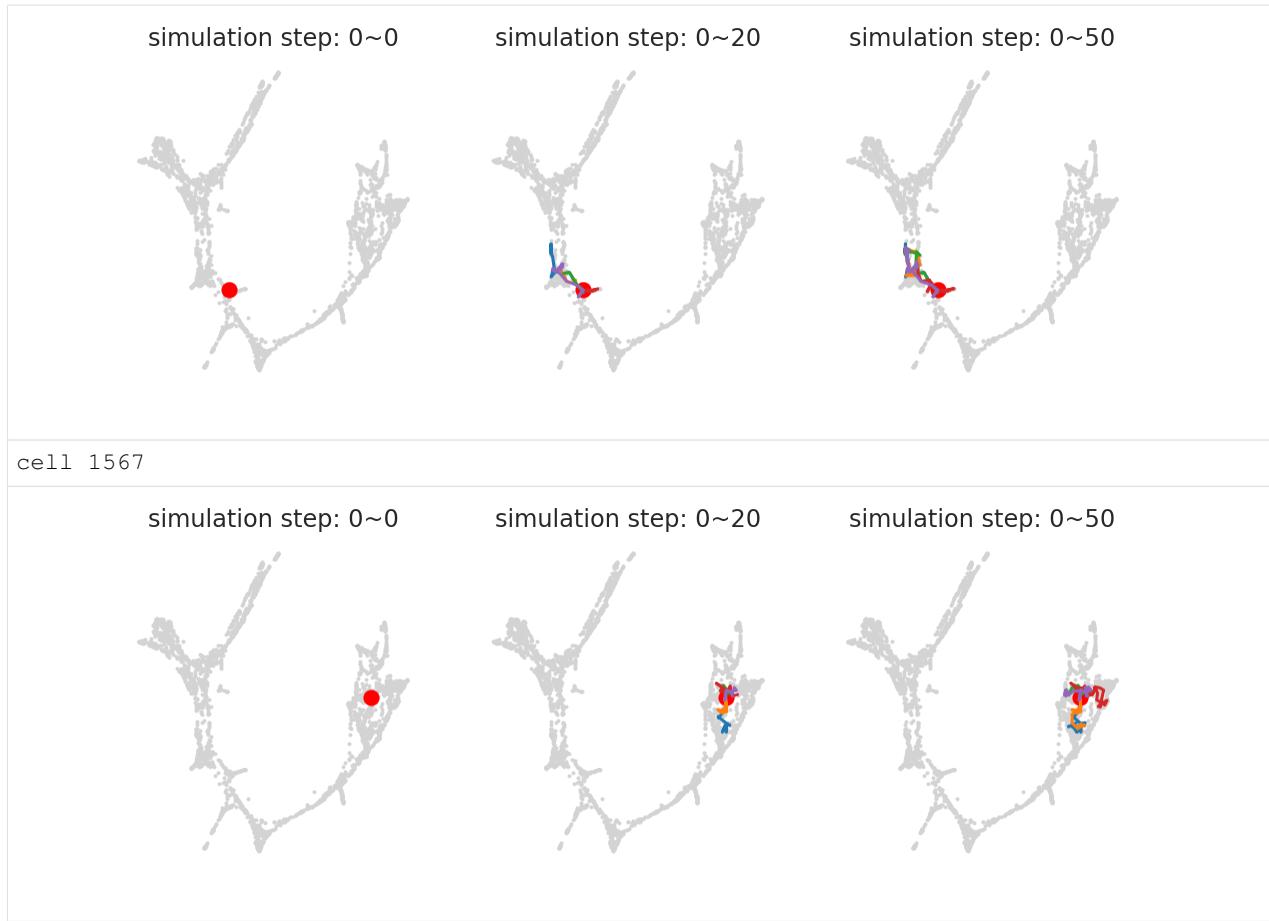
```
[88]: # Randomly pick up 3 cells
np.random.seed(12)
cells = oracle.adata.obs.index.values[np.random.choice(oracle.ixs_mcmc, 3)]

# Visualize the simulated results of cell transition after perturbation
for k in cells:
    print(f"cell {k}")
    plt.figure(figsize=[9, 3])
    for j, i in enumerate([0, 20, 50]): # time points
        plt.subplot(1, 3, (j+1))
        oracle.plot_mc_result_as_trajectory(k, range(0, i))
        plt.title(f"simulation step: 0~{i}")
        plt.axis("off")
    plt.show()
```

cell 1961



cell 43

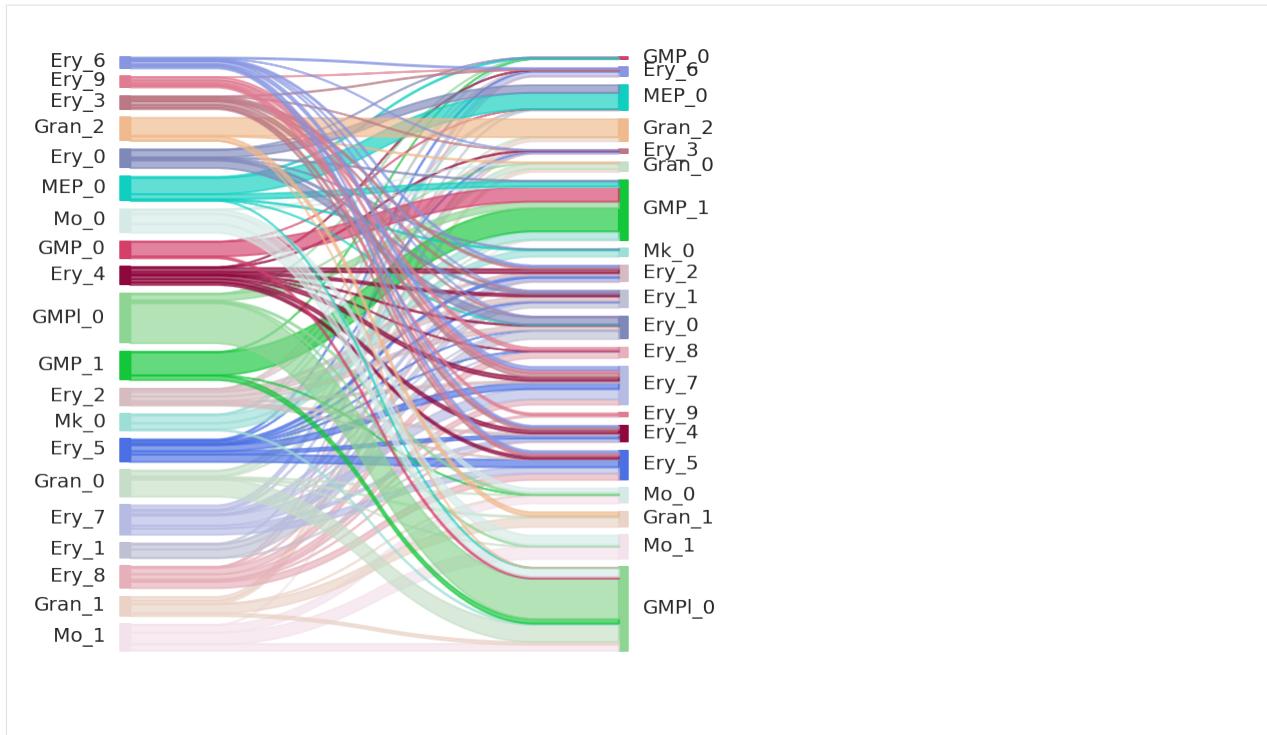


4.4.3. Summarize the results of simulation by plotting sankey diagram

Sankey diagrams are useful when you want to visualize proportional cell transitions between some groups.

For the grouping of cells, you can use arbitrary cluster unit.

```
[89]: # Plot sankey diagram
plt.figure(figsize=[5, 6])
cl = "louvain_annot"
oracle.plot_mc_results_as_sankey(cluster_use=cl, start=0, end=100)
```

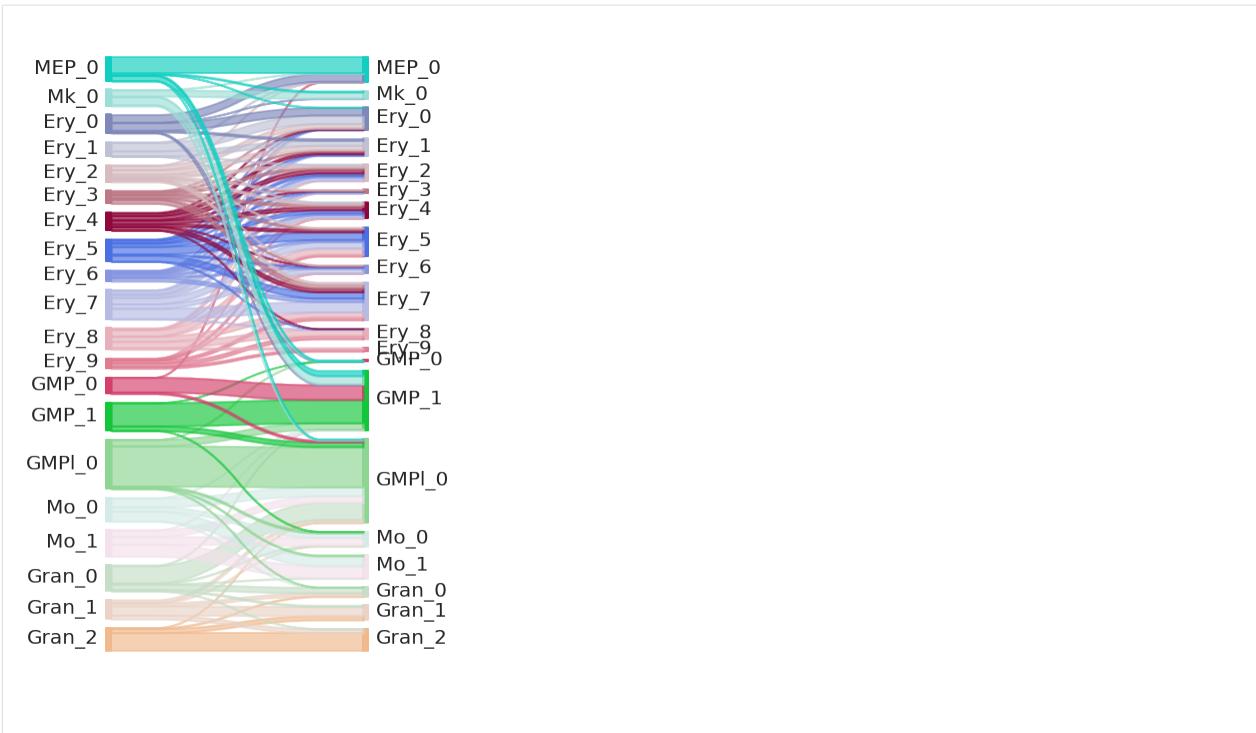


The Sankey diagram above looks messy because the cluster order is random.

Let's change the cluster order and make the plot again

```
[90]: cl = "louvain_annotation"
order = ['MEP_0', 'Mk_0', 'Ery_0', 'Ery_1', 'Ery_2', 'Ery_3', 'Ery_4',
         'Ery_5', 'Ery_6', 'Ery_7', 'Ery_8', 'Ery_9',
         'GMP_0', 'GMP_1', 'GMP_2', 'GMPI_0', 'GMPI_1',
         'Mo_0', 'Mo_1', 'Mo_2', 'Gran_0', 'Gran_1', 'Gran_2', 'Gran_3']

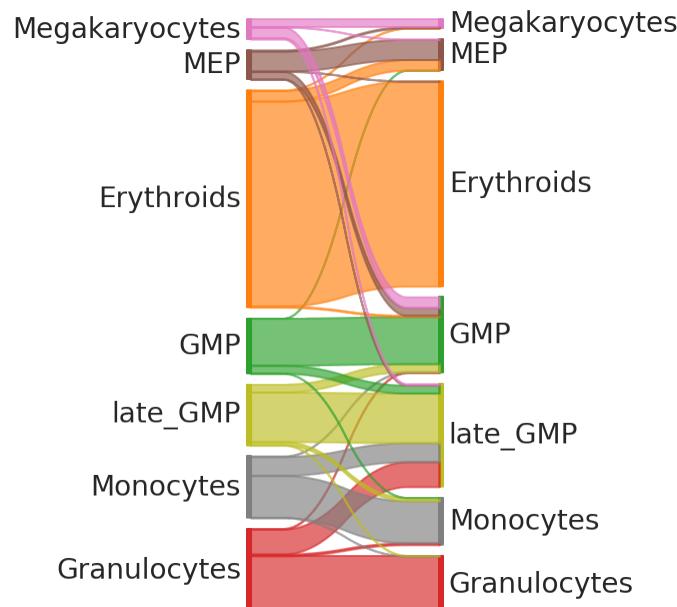
plt.figure(figsize=[5, 6])
plt.subplots_adjust(left=0.3, right=0.7)
oracle.plot_mc_results_as_sankey(cluster_use=cl, start=0, end=100, order=order)
# plt.savefig(f"{save_folder}/mcmc_{cl}.png")
```



Make another Saneky diagram with different cluster units.

```
[92]: order = ['Megakaryocytes', 'MEP', 'Erythroids', 'GMP', 'late_GMP', 'Monocytes',
   ↪'Granulocytes']
cl = "cell_type"

plt.figure(figsize=[5, 6])
plt.subplots_adjust(left=0.35, right=0.65)
oracle.plot_mc_results_as_sankey(cluster_use=cl, start=0, end=100, order=order, font_
   ↪size=14)
# plt.savefig(f"{save_folder}/mcmc_{cl}{goi}.png", transparent=True)
```



Based on the results, we may conclude several things as follows.

Gata1 KO induced both cell state transitions from Erythroblasts to MEP, and from MEP to GMP.

- (1) These results suggest that Gata1 may play a role in the progression of Erythroid differentiation and cell state determination between the MEP and GMP lineages.
- (2) Gata1 KO also induced cell state transitions from granulocytes to late GMP, suggesting Gata1's involvement in Granulocytes differentiation.

These results agree with previous reports about Gata1 and recapitulate Gata1's cell-type-specific function regarding the cell fate decisions in hematopoiesis.

1.3 API

1.3.1 Command Line API

CellOracle has a command line API. This command can be used to convert scRNA-seq data. If you have a scRNA-seq data which was processed with Seurat and saved as Rds file, you can use the following command to make anndata from Seurat object. The anndata object produced by this command can be used for input of celloracle.

```
seuratToAnndata YOUR_SEURAT_OBJECT.Rds OUTPUT_PATH
```

1.3.2 Python API

Custom class in celloracle

We define some custom classes in celloracle.

```
class celloracle.Oracle
Bases:                                     celloracle.trajectory.modified_VelocytoLoom_class.
modified_VelocytoLoom
```

Oracle is the main class in CellOracle. Oracle object imports scRNA-seq data (anndata) and TF information to infer cluster-specific GRNs. It can predict the future gene expression patterns and cell state transitions in response to the perturbation of TFs. Please see the CellOracle paper for details. The code of the Oracle class was made of the three components below.

- (1) Anndata: Gene expression matrix and metadata from single-cell RNA-seq are stored in the anndata object. Processed values, such as normalized counts and simulated values, are stored as layers of anndata. Metadata (i.e., Cluster info) are saved in anndata.obs. Refer to scanpy/anndata documentation for detail.
- (2) Net: Net is a custom class in celloracle. Net object processes several data to infer GRN. See the Net class documentation for details.
- (3) VelocytoLoom: Calculation of transition probability and visualization of directed trajectory graph will be performed in the same way as velocytoloom. VelocytoLoom is class from Velocyto, a python library for RNA-velocity analysis. In celloracle, we use some functions in velocytoloom for the visualization.

adata

Imported anndata object

Type anndata

cluster_column_name

The column name in adata.obs containing cluster info

Type str

embedding_name

The key name in adata.obsm containing dimensional reduction coordinates

Type str

addTFinfo_dictionary (TFdict)

Add new TF info to pre-existing TFdict. Values in the old TF dictionary will remain.

Parameters **TFdict** (*dictionary*) – Python dictionary of TF info.

copy()

Deepcopy itself.

fit_GRN_for_simulation (GRN_unit='cluster', alpha=1, use_cluster_specific_TFdict=False)

Do GRN inference. Please see the paper of CellOracle paper for details.

GRN can be constructed for the entire population or each clusters. If you want to infer cluster-specific GRN, please set [GRN_unit="cluster"]. You can select cluster information when you import data.

If you set [GRN_unit="whole"], GRN will be made using all cells.

Parameters

- **GRN_unit** (*str*) – Select “cluster” or “whole”
- **alpha** (*float or int*) – The strength of regularization. If you set a lower value, the sensitivity increases, and you can detect weaker network connections. However, there may be more noise. If you select a higher value, it will reduce the chance of overfitting.

get_cluster_specific_TFdict_from_Links (links_object)

Extract TF and its target gene information from Links object. This function can be used to reconstruct GRNs based on pre-existing GRNs saved in Links object.

Parameters **links_object** ([Links](#)) – Please see the explanation of Links class.

```
get_links(cluster_name_for_GRN_unit=None, alpha=10, bagging_number=20, verbose_level=1,
           test_mode=False)
```

Makes GRN for each cluster and returns results as a Links object. Several preprocessing should be done before using this function.

Parameters

- **cluster_name_for_GRN_unit** (*str*) – Cluster name for GRN calculation. The cluster information should be stored in Oracle.adata.obs.
- **alpha** (*float or int*) – The strength of regularization. If you set a lower value, the sensitivity increases, and you can detect weaker network connections. However, there may be more noise. If you select a higher value, it will reduce the chance of overfitting.
- **bagging_number** (*int*) – The number used in bagging calculation.
- **verbose_level** (*int*) – if [verbose_level>1], most detailed progress information will be shown. if [verbose_level > 0], one progress bar will be shown. if [verbose_level == 0], no progress bar will be shown.
- **test_mode** (*bool*) – If test_mode is True, GRN calculation will be done for only one cluster rather than all clusters.

```
import_TF_data(TF_info_matrix=None, TF_info_matrix_path=None, TFdict=None)
```

Load data about potential-regulatory TFs. You can import either TF_info_matrix or TFdict. For more information on how to make these files, please see the motif analysis module within the celloracle tutorial.

Parameters

- **TF_info_matrix** (*pandas.DataFrame*) – TF_info_matrix.
- **TF_info_matrix_path** (*str*) – File path for TF_info_matrix (*pandas.DataFrame*).
- **TFdict** (*dictionary*) – Python dictionary of TF info.

```
import_anndata_as_normalized_count(adata, cluster_column_name=None, embedding_name=None)
```

Load scRNA-seq data. scRNA-seq data should be prepared as an anndata object. Preprocessing (cell and gene filtering, dimensional reduction, clustering, etc.) should be done before loading data. The method will import NORMALIZED and LOG TRANSFORMED data but NOT SCALED and NOT CENTERED data. See the tutorial for more details on how to process scRNA-seq data.

Parameters

- **adata** (*anndata*) – anndata object containing scRNA-seq data.
- **cluster_column_name** (*str*) – the name of column containing cluster information in anndata.obs. Clustering data should be in anndata.obs.
- **embedding_name** (*str*) – the key name for dimensional reduction information in anndata.obsm. Dimensional reduction (or 2D trajectory graph) should be in anndata.obsm.
- **transform** (*str*) – The method for log-transformation. Chose one from “natural_log” or “log2”.

```
import_anndata_as_raw_count(adata, cluster_column_name=None, embedding_name=None,
                           transform='natural_log')
```

Load scRNA-seq data. scRNA-seq data should be prepared as an anndata object. Preprocessing (cell and gene filtering, dimensional reduction, clustering, etc.) should be done before loading data. The method imports RAW GENE COUNTS because unscaled and uncentered gene expression data are required for the GRN inference and simulation. See tutorial notebook for the details about how to process scRNA-seq data.

Parameters

- **adata** (*anndata*) – anndata object that stores scRNA-seq data.
- **cluster_column_name** (*str*) – the name of column containing cluster information in anndata.obs. Clustering data should be in anndata.obs.
- **embedding_name** (*str*) – the key name for dimensional reduction information in anndata.obsm. Dimensional reduction (or 2D trajectory graph) should be in anndata.obsm.
- **transform** (*str*) – The method for log-transformation. Chose one from “natural_log” or “log2”.

plot_mc_result_as_kde (*n_time*, *args={}*)

Pick up one timepoint in the cell state-transition simulation and plot as a kde plot.

Parameters

- **n_time** (*int*) – the number in Markov simulation
- **args** (*dictionary*) – An argument for seaborn.kdeplot. See seaborn documentation for details (<https://seaborn.pydata.org/generated/seaborn.kdeplot.html#seaborn.kdeplot>).

plot_mc_result_as_trajectory (*cell_name*, *time_range*, *args={}*)

Pick up several timepoints in the cell state-transition simulation and plot as a line plot. This function can be used to visualize how cell-state changes after perturbation focusing on a specific cell.

Parameters

- **cell_name** (*str*) – cell name. chose from adata.obs.index
- **time_range** (*list of int*) – the list of index in Markov simulation
- **args** (*dictionary*) – dictionary for the arguments for matplotlib.pyplot.plot. See matplotlib documentation for details (https://matplotlib.org/api/_as_gen/matplotlib.pyplot.plot.html#matplotlib.pyplot.plot).

plot_mc_results_as_sankey (*cluster_use*, *start=0*, *end=-1*, *order=None*, *font_size=10*)

Plot the simulated cell state-transition as a Sankey-diagram after groping by the cluster.

Parameters

- **cluster_use** (*str*) – cluster information name in anndata.obs. You can use any cluster information in anndata.obs.
- **start** (*int*) – The starting point of Sankey-diagram. Please select a step in the Markov simulation.
- **end** (*int*) – The end point of Sankey-diagram. Please select a step in the Markov simulation. if you set [end=-1], the final step of Markov simulation will be used.
- **order** (*list of str*) – The order of cluster name in the Sankey-diagram.
- **font_size** (*int*) – Font size for cluster name label in the Sankey diagram.

prepare_markov_simulation (*verbose=False*)

Pick up cells for Markov simulation.

Parameters verbose (*bool*) – If True, it plots selected cells.

run_markov_chain_simulation (*n_steps=500*, *n_duplication=5*, *seed=123*)

Do Markov simulations to predict cell transition after perturbation. The transition probability between cells has been calculated based on simulated gene expression values in the signal propagation process. The cell state transition will be simulated based on the probability. You can simulate the process multiple times to get a robust outcome.

Parameters

- **n_steps** (*int*) – steps for Markov simulation. This value is equivalent to the amount of time after perturbation.
- **n_duplication** (*int*) – the number for multiple calculations.

simulate_shift (*perturb_condition=None*, *GRN_unit='cluster'*, *n_propagation=3*, *ignore_warning=False*)

Simulate signal propagation with GRNs. Please see the CellOracle paper for details. This function simulates a gene expression pattern in the near future. Simulated values will be stored in anndata.layers: [“simulated_count”]

The simulation use three types of data. (1) GRN inference results (coef_matrix). (2) Perturb_condition: You can set arbitrary perturbation condition. (3) Gene expression matrix: The simulation starts from imputed gene expression data.

Parameters

- **perturb_condition** (*dictionary*) – condition for perturbation. if you want to simulate knockout for GeneX, please set [perturb_condition={“GeneX”: 0.0}] Although you can set any non-negative values for the gene condition, avoid setting biologically infeasible values for the perturb condition. It is strongly recommended to check gene expression values in your data before selecting the perturb condition.
- **GRN_unit** (*str*) – GRN type. Please select either “whole” or “cluster”. See the documentation of “fit_GRN_for_simulation” for the detailed explanation.
- **n_propagation** (*int*) – Calculation will be performed iteratively to simulate signal propagation in GRN. You can set the number of steps for this calculation. With a higher number, the results may recapitulate signal propagation for many genes. However, a higher number of propagation may cause more error/noise.

summarize_mc_results_by_cluster (*cluster_use*)

This function summarizes the simulated cell state-transition by groping the results into each cluster. It returns summarized results as a pandas.DataFrame.

Parameters **cluster_use** (*str*) – cluster information name in anndata.obs. You can use any arbitrary cluster information in anndata.obs.

to_hdf5 (*file_path*)

Save object as hdf5.

Parameters **file_path** (*str*) – file path to save file. Filename needs to end with ‘.celloracle’

updateTFinfo_dictionary (*TFdict*)

Update a TF dictionary. If a key in the new TF dictionary already exists in the old TF dictionary, old values will be replaced with a new one.

Parameters **TFdict** (*dictionary*) – Python dictionary of TF info.

class **celloracle.Links** (*name, links_dict={}*)

Bases: object

This is a class for the processing and visualization of GRNs. Links object stores cluster-specific GRNs and metadata. Please use “get_links” function in Oracle object to generate Links object.

links_dict

Dictionary that store unprocessed network data.

Type dictionary

filtered_links

Dictionary that store filtered network data.

Type dictionary

merged_score

Network scores.

Type pandas.dataframe

cluster

List of cluster name.

Type list of str

name

Name of clustering unit.

Type str

palette

DataFrame that store color information.

Type pandas.dataframe

filter_links (*p=0.001, weight='coef_abs', thread_number=10000, genelist_source=None, genelist_target=None*)

Filter network edges. In most cases, inferred GRN has non-significant random edges. We have to remove these edges before analyzing the network structure. You can do the filtering in any of the following ways.

- (1) Filter based on the p-value of the network edge. Please enter p-value for thresholding.
- (2) Filter based on network edge number. If you set the number, network edges will be filtered based on the order of a network score. The top n-th network edges with network weight will remain, and the other edges will be removed. The network data has several types of network weight, so you have to select which network weight do you want to use.
- (3) Filter based on an arbitrary gene list. You can set a gene list for source nodes or target nodes.

Parameters

- **p** (*float*) – threshold for p-value of the network edge.
- **weight** (*str*) – Please select network weight name for the filtering
- **genelist_source** (*list of str*) – gene list to remain in regulatory gene nodes. Default is None.
- **genelist_target** (*list of str*) – gene list to remain in target gene nodes. Default is None.

get_network_entropy (*value='coef_abs'*)

Calculate network entropy scores.

Parameters **value** (*str*) – Default is “coef_abs”.

get_score (*test_mode=False*)

Get several network scores using R libraries. Make sure all dependent R libraries are installed in your environment before running this function. You can check the installation for the R libraries by running `test_installation()` in `network_analysis` module.

plot_cartography_scatter_per_cluster (*gois=None, clusters=None, scatter=True, kde=False, auto_gene_annot=False, percentile=98, args_dot={'n_levels': 105}, args_line={'c': 'gray'}, args_annot={}, save=None*)

Make a gene network cartography plot. Please read the original paper describing gene network cartography

for more information. <https://www.nature.com/articles/nature03288>

Parameters

- **links** ([Links](#)) – See network_analysis.Links class for detail.
- **gois** (*list of str*) – List of gene name to highlight.
- **clusters** (*list of str*) – List of cluster name to analyze. If None, all clusters in Links object will be analyzed.
- **scatter** (*bool*) – Whether to make a scatter plot.
- **auto_gene_annot** (*bool*) – Whether to pick up genes to make an annotation.
- **percentile** (*float*) – Genes with a network score above the percentile will be shown with annotation. Default is 98.
- **args_dot** (*dictionary*) – Arguments for scatter plot.
- **args_line** (*dictionary*) – Arguments for lines in cartography plot.
- **args_annot** (*dictionary*) – Arguments for annotation in plots.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

`plot_cartography_term(goi, save=None)`

Plot the gene network cartography term like a heatmap. Please read the original paper of gene network cartography for the principle of gene network cartography. <https://www.nature.com/articles/nature03288>

Parameters

- **links** ([Links](#)) – See network_analysis.Links class for detail.
- **gois** (*list of str*) – List of gene name to highlight.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

`plot_degree_distributions(plot_model=False, save=None)`

Plot the network degree distributions (the number of edge per gene). The network degree will be visualized in both linear scale and log scale.

Parameters

- **links** ([Links](#)) – See network_analysis.Links class for detail.
- **plot_model** (*bool*) – Whether to plot linear approximation line.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

`plot_network_entropy_distributions(update_network_entropy=False, save=None)`

Plot the distribution for network entropy. See the CellOracle paper for more detail.

Parameters

- **links** (*Links object*) – See network_analysis.Links class for detail.
- **values** (*list of str*) – The list of score to visualize. If it is None, all network score (listed above) will be used.
- **update_network_entropy** (*bool*) – Whether to recalculate network entropy.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

plot_score_comparison_2D(*value*, *cluster1*, *cluster2*, *percentile*=99, *annot_shifts*=None, *save*=None)

Make a scatter plot that compares specific network scores in two groups.

Parameters

- **links** ([Links](#)) – See network_analysis.Links class for detail.
- **value** (*srt*) – The network score type.
- **cluster1** (*str*) – Cluster name. Network scores in cluster1 will be visualized in the x-axis.
- **cluster2** (*str*) – Cluster name. Network scores in cluster2 will be visualized in the y-axis.
- **percentile** (*float*) – Genes with a network score above the percentile will be shown with annotation. Default is 99.
- **annot_shifts** ((*float*, *float*)) – Annotation visualization setting.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

plot_score_distributions(*values*=None, *method*='boxplot', *save*=None)

Plot the distribution of network scores. An individual data point is a network edge (gene).

Parameters

- **links** ([Links](#)) – See Links class for details.
- **values** (*list of str*) – The list of score to visualize. If it is None, all of the network score will be used.
- **method** (*str*) – Plotting method. Select either “boxplot” or “barplot”.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

plot_score_per_cluster(*goi*, *save*=None)

Plot network score for a gene. This function visualizes the network score for a specific gene between clusters to get an insight into the dynamics of the gene.

Parameters

- **links** ([Links](#)) – See network_analysis.Links class for detail.
- **goi** (*srt*) – Gene name.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

plot_scores_as_rank(*cluster*, *n_gene*=50, *save*=None)

Pick up top n-th genes with high-network scores and make plots.

Parameters

- **links** ([Links](#)) – See network_analysis.Links class for detail.
- **cluster** (*str*) – Cluster name to analyze.
- **n_gene** (*int*) – Number of genes to plot. Default is 50.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

to_hdf5 (file_path)

Save object as hdf5.

Parameters `file_path` (`str`) – file path to save file. Filename needs to end with ‘.celloracle.links’

```
class celloracle.Net (gene_expression_matrix, gem_standerdized=None, TFinfo_matrix=None, cell-state=None, TFinfo_dic=None, annotation=None, verbose=True)
```

Bases: object

Net is a custom class for inferring sample-specific GRN from scRNA-seq data. This class is used inside the Oracle class for GRN inference. This class requires two types of information below.

- (1) Single-cell RNA-seq data: The Net class needs processed scRNA-seq data. Gene and cell filtering, quality check, normalization, log-transformation (but not scaling and centering) have to be done before starting the GRN calculation with this class. You can also use any arbitrary metadata (i.e., mRNA count, cell-cycle phase) for GRN input.
- (2) Potential regulatory connection (or base GRN): This method uses the list of potential regulatory TFs as input. This information can be calculated from ATAC-seq data using the motif-analysis module. If sample-specific ATAC-seq data is not available, you can use general TF-binding info derived from public ATAC-seq dataset of various tissue/cell type.

linkList

The results of the GRN inference.

Type pandas.DataFrame

all_genes

An array of all genes that exist in the input gene expression matrix

Type numpy.array

embedding_name

The key name name in adata.obsm containing dimensional reduction coordinates

Type str

annotation

Annotation. you can add custom annotation.

Type dictionary

coefs_dict

Coefs of linear regression.

Type dictionary

stats_dict

Statistic values about coefs.

Type dictionary

fitted_genes

List of genes where the regression model was successfully calculated.

Type list of str

failed_genes

List of genes that were not assigned coefs

Type list of str

cellstate

A metadata for GRN input

Type pandas.DataFrame

TFinfo

Information about potential regulatory TFs.

Type pandas.DataFrame

gem

Merged matrix made with gene_expression_matrix and cellstate matrix.

Type pandas.DataFrame

gem_standerdized

Almost the same as gem, but the gene_expression_matrix was standarized.

Type pandas.DataFrame

library_last_update_date

Last update date of this code. This info is for code development. It can be deprecated in the future

Type str

object_initiation_date

The date when this object was made.

Type str

addAnnotation (annotation_dictionary)

Add a new annotation.

Parameters **annotation_dictionary** (*dictionary*) – e.g. {“sample_name”: “NIH 3T3 cell”}

addTFinfo_dictionary (TFdict)

Add a new TF info to pre-existing TFdict.

Parameters **TFdict** (*dictionary*) – python dictionary of TF info.

addTFinfo_matrix (TFinfo_matrix)

Load TF info dataframe.

Parameters **TFinfo** (*pandas.DataFrame*) – information about potential regulatory TFs.

copy ()

Deepcopy itself

fit_All_genes (bagging_number=200, scaling=True, model_method='bagging_ridge', command_line_mode=False, log=None, alpha=1, verbose=True)

Make ML models for all genes. The calculation will be performed in parallel using scikit-learn bagging function. You can select a modeling method (bagging_ridge or bayesian_ridge). This calculation usually takes a long time.

Parameters

- **bagging_number** (*int*) – The number of estimators for bagging.
- **scaling** (*bool*) – Whether or not to scale regulatory gene expression values.
- **model_method** (*str*) – ML model name. Please select either “bagging_ridge” or “bayesian_ridge”
- **command_line_mode** (*bool*) – Please select False if the calculation is performed on jupyter notebook.
- **log** (*logging object*) – log object to output log
- **alpha** (*int*) – Strength of regularization.

- **verbose** (*bool*) – Whether or not to show a progress bar.

fit_All_genes_parallel (*bagging_number=200, scaling=True, log=None, verbose=10*)

IMPORTANT: this function being debugged and is currently unavailable.

Make ML models for all genes. The calculation will be performed in parallel using joblib parallel module.

Parameters

- **bagging_number** (*int*) – The number of estimators for bagging.
- **scaling** (*bool*) – Whether or not to scale regulatory gene expression values.
- **log** (*logging object*) – log object to output log
- **verbose** (*int*) – verbose for joblib parallel

fit_genes (*target_genes, bagging_number=200, scaling=True, model_method='bagging_ridge', save_coefs=False, command_line_mode=False, log=None, alpha=1, verbose=True*)

Make ML models for genes of interest. This calculation will be performed in parallel using scikit-learn's bagging function. You can select a modeling method; Please chose either bagging_ridge or bayesian_ridge.

Parameters

- **target_genes** (*list of str*) – gene list
- **bagging_number** (*int*) – The number of estimators for bagging.
- **scaling** (*bool*) – Whether or not to scale regulatory gene expression values.
- **model_method** (*str*) – ML model name. Please select either "bagging_ridge" or "bayesian_ridge"
- **save_coefs** (*bool*) – Whether or not to store details of coef values in bagging model.
- **command_line_mode** (*bool*) – Please select False if the calculation is performed on jupyter notebook.
- **log** (*logging object*) – log object to output log
- **alpha** (*int*) – Strength of regularization.
- **verbose** (*bool*) – Whether or not to show a progress bar.

plotCoefs (*target_gene, sort=True, threshold_p=None*)

Plot the distribution of Coef values (network edge weights).

Parameters

- **target_gene** (*str*) – gene name
- **sort** (*bool*) – Whether or not to sort genes by its strength
- **bagging_number** (*int*) – The number of estimators for bagging.
- **threshold_p** (*float*) – the threshold for p-values. TFs will be filtered based on the p-value. if None, no filtering is applied.

to_hdf5 (*file_path*)

Save object as hdf5.

Parameters **file_path** (*str*) – file path to save file. Filename needs to end with '.celloracle.net'

updateLinkList (*verbose=True*)

Update LinkList. LinkList is a data frame that store information about inferred GRNs.

Parameters **verbose** (*bool*) – Whether or not to show a progress bar

`updateTFinfo_dictionary (TFdict)`

Update TF info matrix

Parameters `TFdict` (*dictionary*) – A python dictionary in which a key is Target gene, value are potential regulatory genes for the target gene.

`celloracle.load_hdf5 (file_path, object_class_name=None)`

Load an object of celloracle's custom class that was saved as hdf5.

Parameters

- `file_path` (*str*) – file_path.
- `object_class_name` (*str*) – Types of object. If it is None, object class will be identified from the extension of file_name. Default is None.

Modules for ATAC-seq analysis

celloracle.motif_analysis module

The `motif_analysis` module implements transcription factor motif scan.

Genomic activity information (peak of ATAC-seq or Chip-seq) is extracted first. Then the peak DNA sequence will be subjected to TF motif scan. Finally we will get list of TFs that potentially binds to a specific gene.

`celloracle.motif_analysis.is_genome_installed (ref_genome)`

Celloracle motif_analysis module uses gimmemotifs and genomepy internally. Reference genome files should be installed in the PC to use gimmemotifs and genomepy. This function checks the installation status of the reference genome.

Parameters `ref_genome` (*str*) – names of reference genome. i.e., “mm10”, “hg19”

`celloracle.motif_analysis.peak2fasta (peak_ids, ref_genome)`

Convert peak_id into fasta object.

Parameters

- `peak_id` (*str or list of str*) – Peak_id. e.g. “chr5_0930303_9499409” or it can be a list of peak_id. e.g. [“chr5_0930303_9499409”, “chr11_123445555_123445577”]
- `ref_genome` (*str*) – Reference genome name. e.g. “mm9”, “mm10”, “hg19” etc

Returns DNA sequence in fasta format

Return type gimmemotifs.fasta object

`celloracle.motif_analysis.read_bed (bed_path)`

Load bed file and return as dataframe.

Parameters `bed_path` (*str*) – File path.

Returns bed file in dataframe.

Return type pandas.dataframe

`celloracle.motif_analysis.load_TFinfo_from_parquets (folder_path)`

Load TFinfo object which was saved with the function; “`save_as_parquet`”.

Parameters `folder_path` (*str*) – folder path

Returns Loaded TFinfo object.

Return type `TFinfo`

```
celloracle.motif_analysis.make_TFinfo_from_scanned_file(path_to_raw_bed,
                                                       path_to_scanned_result_bed,
                                                       ref_genome)
```

This function is currently an available.

```
class celloracle.motif_analysis.TFinfo(peak_data_frame, ref_genome)
Bases: object
```

This is a custom class for motif analysis in celloracle. TFinfo object performs motif scan using the TF motif database in gimmemotifs and several functions of genomepy. Analysis results can be exported as a python dictionary or dataframe. These files; python dictionary of dataframe of TF binding information, are needed during GRN inference.

peak_df

dataframe about DNA peak and target gene data.

Type pandas.DataFrame

all_target_gene

target genes.

Type array of str

ref_genome

reference genome name that was used in DNA peak generation.

Type str

scanned_df

Results of motif scan. Key is a peak name. Value is a dataframe of motif scan.

Type dictionary

dic_targetgene2TFs

Final product of motif scan. Key is a target gene. Value is a list of regulatory candidate genes.

Type dictionary

dic_peak2Targetgene

Dictionary. Key is a peak name. Value is a list of the target gene.

Type dictionary

dic_TF2targetgenes

Final product of motif scan. Key is a TF. Value is a list of potential target genes of the TF.

Type dictionary

copy()

Deepcopy itself.

filter_motifs_by_score(threshold, method='cumulative_score')

Remove motifs with low binding scores.

Parameters **method** (str) – thresholding method. Select either of [“individual_score”, “cumulative_score”]

filter_peaks(peaks_to_be_remainded)

Filter peaks.

Parameters **peaks_to_be_remainded**(array of str) – list of peaks. Peaks that are NOT in the list will be removed.

make_TFinfo_dataframe_and_dictionary(verbose=True)

This is the final step of motif_analysis. Convert scanned results into a data frame and dictionaries.

Parameters **verbose** (bool) – Whether to show a progress bar.

reset_dictionary_and_df()

Reset TF dictionary and TF dataframe. The following attributes will be erased: TF_onehot, dic_targetgene2TFs, dic_peak2Targetgene, dic_TF2targetgenes.

reset_filtering()

Reset filtering information. You can use this function to stat over the filtering step with new conditions. The following attributes will be erased: TF_onehot, dic_targetgene2TFs, dic_peak2Targetgene, dic_TF2targetgenes.

save_as_parquet(folder_path=None)

Save itself. Some attributes are saved as parquet file.

Parameters **folder_path** (*str*) – folder path

scan(background_length=200, fpr=0.02, n_cpus=-1, verbose=True)

Scan DNA sequences searching for TF binding motifs.

Parameters

- **background_length** (*int*) – background length. This is used for the calculation of the binding score.
- **fpr** (*float*) – False positive rate for motif identification.
- **n_cpus** (*int*) – number of CPUs for parallel calculation.
- **verbose** (*bool*) – Whether to show a progress bar.

to_dataframe(verbose=True)

Return results as a dataframe. Rows are peak_id, and columns are TFs.

Parameters **verbose** (*bool*) – Whether to show a progress bar.

Returns TFinfo matrix.

Return type pandas.dataframe

to_dictionary(dictionary_type='targetgene2TFs', verbose=True)

Return TF information as a python dictionary.

Parameters **dictionary_type** (*str*) – Type of dictionary. Select from [“targetgene2TFs”, “TF2targetgenes”]. If you chose “targetgene2TFs”, it returns a dictionary in which a key is a target gene, and a value is a list of regulatory candidate genes (TFs) of the target. If you chose “TF2targetgenes”, it returns a dictionary in which a key is a TF and a value is a list of potential target genes of the TF.

Returns dictionary.

Return type dictionary

to_hdf5(file_path)

Save object as hdf5.

Parameters **file_path** (*str*) – file path to save file. Filename needs to end with ‘.celloracle.tfinfo’

celloracle.motif_analysis.get_tss_info(peak_str_list, ref_genome, verbose=True)

Get annotation about Transcription Starting Site (TSS).

Parameters

- **peak_str_list** (*list of str*) – list of peak_id. e.g., [`chr5_0930303_9499409`, `chr11_123445555_123445577`]
- **ref_genome** (*str*) – reference genome name.
- **verbose** (*bool*) – verbosity.

celloracle.motif_analysis.integrate_tss_peak_with_cicero(tss_peak, cicero_connections)

Process output of cicero data and returns DNA peak information for motif analysis in celloracle.
Please see the celloracle tutorial for more information.

Parameters

- **tss_peak** (`pandas.DataFrame`) – dataframe about TSS information. Please use the function, “`get_tss_info`” to get this dataframe.
- **cicero_connections** (`DataFrame`) – dataframe that stores the results of cicero analysis.

Returns DNA peak about promoter/enhancer and its annotation about target gene.

Return type `pandas.DataFrame`

Modules for Network analysis

celloracle.network_analysis module

The `network_analysis` module implements Network analysis.

```
celloracle.network_analysis.get_links(oracle_object, cluster_name_for_GRN_unit=None, alpha=10, bagging_number=20, verbose_level=1, test_mode=False)
```

Make GRN for each cluster and returns results as a `Links` object. Several preprocessing should be done before using this function.

Parameters

- **oracle_object** (`Oracle`) – See Oracle module for detail.
- **cluster_name_for_GRN_unit** (`str`) – Cluster name for GRN calculation. The cluster information should be stored in `Oracle.adata.obs`.
- **alpha** (`float or int`) – The strength of regularization. If you set a lower value, the sensitivity increases, and you can detect weaker network connections. However, there may be more noise. If you select a higher value, it will reduce the chance of overfitting.
- **bagging_number** (`int`) – The number used in bagging calculation.
- **verbose_level** (`int`) – if [verbose_level>1], most detailed progress information will be shown. if [verbose_level > 0], one progress bar will be shown. if [verbose_level == 0], no progress bar will be shown.
- **test_mode** (`bool`) – If `test_mode` is True, GRN calculation will be done for only one cluster rather than all clusters.

`celloracle.network_analysis.test_R_libraries_installation()`

`CellOracle.network_analysis` use several R libraries for network analysis. This is a test function to check for instalation of the necessary R libraries.

`celloracle.network_analysis.load_links(file_path)`

Load links object saved as a hdf5 file.

Parameters `file_path` (`str`) – file path.

Returns loaded links object.

Return type `Links`

```
class celloracle.network_analysis.Links(name, links_dict={})  
Bases: object
```

This is a class for the processing and visualization of GRNs. Links object stores cluster-specific GRNs and metadata. Please use “get_links” function in Oracle object to generate Links object.

links_dict

Dictionary that store unprocessed network data.

Type dictionary

filtered_links

Dictionary that store filtered network data.

Type dictionary

merged_score

Network scores.

Type pandas.dataframe

cluster

List of cluster name.

Type list of str

name

Name of clustering unit.

Type str

palette

DataFrame that store color information.

Type pandas.dataframe

filter_links (*p=0.001, weight='coef_abs', thread_number=10000, genelist_source=None, genelist_target=None*)

Filter network edges. In most cases, inferred GRN has non-significant random edges. We have to remove these edges before analyzing the network structure. You can do the filtering in any of the following ways.

- (1) Filter based on the p-value of the network edge. Please enter p-value for thresholding.
- (2) Filter based on network edge number. If you set the number, network edges will be filtered based on the order of a network score. The top n-th network edges with network weight will remain, and the other edges will be removed. The network data has several types of network weight, so you have to select which network weight do you want to use.
- (3) Filter based on an arbitrary gene list. You can set a gene list for source nodes or target nodes.

Parameters

- **p** (*float*) – threshold for p-value of the network edge.
- **weight** (*str*) – Please select network weight name for the filtering
- **genelist_source** (*list of str*) – gene list to remain in regulatory gene nodes. Default is None.
- **genelist_target** (*list of str*) – gene list to remain in target gene nodes. Default is None.

get_network_entropy (*value='coef_abs'*)

Calculate network entropy scores.

Parameters **value** (*str*) – Default is “coef_abs”.

get_score (*test_mode=False*)

Get several network scores using R libraries. Make sure all dependent R libraries are installed in your environment before running this function. You can check the installation for the R libraries by running test_installation() in network_analysis module.

```
plot_cartography_scatter_per_cluster(gois=None, clusters=None,
                                         scatter=True, kde=False,
                                         auto_gene_annot=False, percentile=98, per-
                                         centile=98, args_dot={'n_levels': 105}, args_line={'c': 'gray'},
                                         args_annot={}, save=None)
```

Make a gene network cartography plot. Please read the original paper describing gene network cartography for more information. <https://www.nature.com/articles/nature03288>

Parameters

- **links** ([Links](#)) – See `network_analysis.Links` class for detail.
- **gois** (*list of str*) – List of gene name to highlight.
- **clusters** (*list of str*) – List of cluster name to analyze. If None, all clusters in `Links` object will be analyzed.
- **scatter** (*bool*) – Whether to make a scatter plot.
- **auto_gene_annot** (*bool*) – Whether to pick up genes to make an annotation.
- **percentile** (*float*) – Genes with a network score above the percentile will be shown with annotation. Default is 98.
- **args_dot** (*dictionary*) – Arguments for scatter plot.
- **args_line** (*dictionary*) – Arguments for lines in cartography plot.
- **args_annot** (*dictionary*) – Arguments for annotation in plots.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

```
plot_cartography_term(goi, save=None)
```

Plot the gene network cartography term like a heatmap. Please read the original paper of gene network cartography for the principle of gene network cartography. <https://www.nature.com/articles/nature03288>

Parameters

- **links** ([Links](#)) – See `network_analysis.Links` class for detail.
- **gois** (*list of str*) – List of gene name to highlight.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

```
plot_degree_distributions(plot_model=False, save=None)
```

Plot the network degree distributions (the number of edge per gene). The network degree will be visualized in both linear scale and log scale.

Parameters

- **links** ([Links](#)) – See `network_analysis.Links` class for detail.
- **plot_model** (*bool*) – Whether to plot linear approximation line.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is None.

```
plot_network_entropy_distributions(update_network_entropy=False, save=None)
```

Plot the distribution for network entropy. See the CellOracle paper for more detail.

Parameters

- **links** (*Links object*) – See `network_analysis.Links` class for detail.
- **values** (*list of str*) – The list of score to visualize. If it is None, all network score (listed above) will be used.
- **update_network_entropy** (*bool*) – Whether to recalculate network entropy.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [save=None]. Default is

None.

plot_score_comparison_2D(*value*, *cluster1*, *cluster2*, *percentile*=99, *annotation_shifts*=None, *save*=None)

Make a scatter plot that compares specific network scores in two groups.

Parameters

- **links** ([Links](#)) – See `network_analysis.Links` class for detail.
- **value** (*srt*) – The network score type.
- **cluster1** (*str*) – Cluster name. Network scores in cluster1 will be visualized in the x-axis.
- **cluster2** (*str*) – Cluster name. Network scores in cluster2 will be visualized in the y-axis.
- **percentile** (*float*) – Genes with a network score above the percentile will be shown with annotation. Default is 99.
- **annotation_shifts** ((*float*, *float*)) – Annotation visualization setting.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [*save*=None]. Default is None.

plot_score_distributions(*values*=None, *method*='boxplot', *save*=None)

Plot the distribution of network scores. An individual data point is a network edge (gene).

Parameters

- **links** ([Links](#)) – See `Links` class for details.
- **values** (*list of str*) – The list of score to visualize. If it is None, all of the network score will be used.
- **method** (*str*) – Plotting method. Select either “boxplot” or “barplot”.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [*save*=None]. Default is None.

plot_score_per_cluster(*goi*, *save*=None)

Plot network score for a gene. This function visualizes the network score for a specific gene between clusters to get an insight into the dynamics of the gene.

Parameters

- **links** ([Links](#)) – See `network_analysis.Links` class for detail.
- **goi** (*srt*) – Gene name.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [*save*=None]. Default is None.

plot_scores_as_rank(*cluster*, *n_gene*=50, *save*=None)

Pick up top n-th genes with high-network scores and make plots.

Parameters

- **links** ([Links](#)) – See `network_analysis.Links` class for detail.
- **cluster** (*str*) – Cluster name to analyze.
- **n_gene** (*int*) – Number of genes to plot. Default is 50.
- **save** (*str*) – Folder path to save plots. If the folder does not exist in the path, the function creates the folder. Plots will not be saved if [*save*=None]. Default is None.

to_hdf5(*file_path*)

Save object as hdf5.

Parameters **file_path** (*str*) – file path to save file. Filename needs to end with ‘.celloracle.links’

```
celloracle.network_analysis.transfer_scores_from_links_to_adata(adata,  
                                                               links,  
                                                               method='median')
```

Transfer the summary of network scores (median or mean) per group from Links object into adata.

Parameters

- **adata** (*anndata*) – anndata
- **links** ([Links](#)) – Likns object
- **method** (*str*) – The method to summarize data.

```
celloracle.network_analysis.linkList_to_networkgraph(filteredlinkList)
```

Convert linkList into Graph object in NetworkX.

Parameters **filteredlinkList** (*pandas.DataFrame*) – GRN saved as linkList.

Returns Network X graph objenct.

Return type Graph object

```
celloracle.network_analysis.draw_network(linkList, return_graph=False)
```

Plot network graph.

Parameters

- **linkList** (*pandas.DataFrame*) – GRN saved as linkList.
- **return_graph** (*bool*) – Whether to return graph object.

Returns Network X graph objenct.

Return type Graph object

Other modules

[celloracle.go_analysis module](#)

The [go_analysis](#) module implements Gene Ontology analysis. This module use goatools internally.

```
celloracle.go_analysis.geneSymbol2ID(symbols, species='mouse')
```

Convert gene symbol into Entrez gene id.

Parameters

- **symbols** (*array of str*) – gene symbol
- **species** (*str*) – Select species. Either “mouse” or “human”

Returns Entrez gene id

Return type list of str

```
celloracle.go_analysis.geneID2Symbol(IDs, species='mouse')
```

Convert Entrez gene id into gene symbol.

Parameters

- **IDs** (*array of str*) – Entrez gene id.
- **species** (*str*) – Select species. Either “mouse” or “human”.

Returns Gene symbol

Return type list of str

```
celloracle.go_analysis.get_GO(gene_query, species='mouse')
```

Get Gene Ontologies (GOs).

Parameters

- **gene_query** (*array of str*) – gene list.

- **species** (*str*) – Select species. Either “mouse” or “human”
- Returns** GO analysis results as dataframe.
Return type pandas.dataframe

celloracle.utility module

The `utility` module has several functions that support celloracle.

class celloracle.utility.**makelog** (*file_name=None, directory=None*)
Bases: object

This is a class for making log.

info (*comment*)

Add comment into the log file.

Parameters `comment` (*str*) – comment.

celloracle.utility.**save_as_pickled_object** (*obj, filepath*)

Save any object using pickle.

Parameters

- **obj** (*any python object*) – python object.
- **filepath** (*str*) – file path.

celloracle.utility.**load_pickled_object** (*filepath*)

Load pickled object.

Parameters `filepath` (*str*) – file path.

Returns loaded object.

Return type python object

celloracle.utility.**intersect** (*list1, list2*)

Intersect two list and get components that exists in both list.

Parameters

- **list1** (*list*) – input list.
- **list2** (*list*) – input list.

Returns intersected list.

Return type list

celloracle.utility.**exec_process** (*commands, message=True, wait_finished=True, return_process=True*)

Excute a command. This is a wrapper of “subprocess.Popen”

Parameters

- **commands** (*str*) – command.
- **message** (*bool*) – Whether to return a message or not.
- **wait_finished** (*bool*) – Whether or not to wait for the process to finish. If false, the process will be perfomed in background and the function will finish immediately
- **return_process** (*bool*) – Whether to return “process”.

celloracle.utility.**standard** (*df*)

Standardize value.

Parameters `df` (*pandas.DataFrame*) – dataframe.

Returns Data after standardization.

Return type pandas.DataFrame

celloracle.utility.**load_hdf5** (*file_path, object_class_name=None*)

Load an object of celloracle’s custom class that was saved as hdf5.

Parameters

- **file_path** (*str*) – file_path.
- **object_class_name** (*str*) – Types of object. If it is None, object class will be identified from the extension of file_name. Default is None.

`celloracle.utility.inverse_dictionary(dictionary, verbose=True, return_value_as_numpy=False)`

Make inverse dictionary. See examples below for detail.

Parameters

- **dictionary** (*dict*) – python dictionary
- **verbose** (*bool*) – Whether to show progress bar.
- **return_value_as_numpy** (*bool*) – Whether to convert values into numpy array.

Returns Python dictionary.

Return type dict

Examples

```
>>> dic = {"a": [1, 2, 3], "b": [2, 3, 4]}
>>> inverse_dictionary(dic)
{1: ['a'], 2: ['a', 'b'], 3: ['a', 'b'], 4: ['b']}
```

```
>>> dic = {"a": [1, 2, 3], "b": [2, 3, 4]}
>>> inverse_dictionary(dic, return_value_as_numpy=True)
{1: array(['a'], dtype='<U1'),
 2: array(['a', 'b'], dtype='<U1'),
 3: array(['a', 'b'], dtype='<U1'),
 4: array(['b'], dtype='<U1')}
```

celloracle.data module

The `data` module implements data download and loading.

`celloracle.data.load_TFinfo_df_mm9_mouse_atac_atlas()`

Load Transcription factor binding information made from mouse scATAC-seq atlas dataset. mm9 genome was used for the reference genome.

Args:

- Returns** TF binding info.
- Return type** pandas.dataframe

celloracle.data_conversion module

The `data_conversion` module implements data conversion between different platform.

`celloracle.data_conversion.seurat_object_to_anndata(file_path_seurat_object, delete_tmp_file=True)`

Convert seurat object into anndata.

Parameters

- **file_path_seurat_object** (*str*) – File path of seurat object. Seurat object should be saved as Rds format.
- **delete_tmp_file** (*bool*) – Whether to delete temporary file.

Returns anndata object.

Return type anndata

1.4 Changelog

- 0.3.1 <2020-03-23>
 - Fix an error when try to save file larger than 4GB file
- 0.3.0 <2020-2-17>
 - Release beta version

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1.6 Authors and citations

1.6.1 Cite celloracle

If you use celloracle please cite our bioarxiv preprint [CellOracle: Dissecting cell identity via network inference and in silico gene perturbation](#).

1.6.2 celloracle software development

celloracle is developed and maintained by Kenji Kamimoto and members of Samantha Morris Lab. Please post troubles or questions on the [Github repository](#).

**CHAPTER
TWO**

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