AnanseScanpy_Jupyter_PBMC_vignette-including-optional-Maelstrom

December 16, 2022

1 AnanseScanpy vignette for multiomics PBMC dataset

PBMC multiomics datasets scanpy objects (anndata) generated from Seurat objects with Seurat-Disk. This vignette includes the optional functions for Maelstrom (GimmeMotifs) analysis.

[1]:
```
import scanpy as sc
from anansescanpy import *

sc.set_figure_params(figsize=(4, 4))
```

[2]:
```
sc.logging.print_versions()
```

```
-----
anndata     0.8.0
scanpy      1.9.1
-----
PIL                 9.2.0
anansescanpy        0.2.3
asttokens           NA
backcall            0.2.0
beta_ufunc          NA
binom_ufunc         NA
cffi                1.15.1
colorama            0.4.6
cycler              0.10.0
cython_runtime      NA
dateutil            2.8.2
debugpy             1.6.3
decorator           5.1.1
defusedxml          0.7.1
entrypoints         0.4
executing           1.2.0
h5py                3.7.0
hypergeom_ufunc     NA
ipykernel           6.17.1
ipython_genutils    0.2.0
jedi                0.18.1
```

```
joblib              1.2.0
jupyter_server      1.23.2
kiwisolver          1.4.4
llvmlite            0.39.1
matplotlib          3.6.2
matplotlib_inline   0.1.6
mpl_toolkits        NA
natsort             8.2.0
nbinom_ufunc        NA
ncf_ufunc           NA
numba               0.56.3
numpy               1.23.4
packaging           21.3
pandas              1.5.1
parso               0.8.3
pexpect             4.8.0
pickleshare         0.7.5
pkg_resources       NA
platformdirs        2.5.2
prompt_toolkit      3.0.32
psutil              5.9.4
ptyprocess          0.7.0
pure_eval           0.2.2
pydev_ipython       NA
pydevconsole        NA
pydevd              2.8.0
pydevd_file_utils   NA
pydevd_plugins      NA
pydevd_tracing      NA
pygments            2.13.0
pyparsing           3.0.9
pytz                2022.6
scipy               1.9.3
session_info        1.0.0
setuptools          65.5.1
six                 1.16.0
sklearn             1.1.3
stack_data          0.6.1
threadpoolctl       3.1.0
tornado             6.2
traitlets           5.5.0
typing_extensions   NA
wcwidth             0.2.5
zmq                 24.0.1
zoneinfo            NA
-----
IPython             8.6.0
jupyter_client      7.4.7
```

```
jupyter_core        5.0.0
jupyterlab          3.5.0
notebook            6.5.2
-----
Python 3.10.6 | packaged by conda-forge | (main, Aug 22 2022, 20:36:39) [GCC
10.4.0]
Linux-5.15.0-56-generic-x86_64-with-glibc2.31
-----
Session information updated at 2022-12-16 19:51
```

[3]:
```
# Fill in the directories where the h5ad rna and atac objects are located
atac_PBMC = sc.read("atac_PBMC.h5ad")
rna_PBMC= sc.read("rna_PBMC.h5ad")

# Notes: the default assays for atac_PBMC and rna_PBMC are "peaks" and "counts"␣
 ↪respectively

# Nessesary pre-processing from converted Seurat object
rna_PBMC.obs['predicted.id'] = rna_PBMC.obs['predicted.id'].str.replace(' ',␣
 ↪'-')
atac_PBMC.obs['predicted.id'] = atac_PBMC.obs['predicted.id'].str.replace(' ',␣
 ↪'-')
```

[4]:
```
# Run the functions in python:
outputdir="AnanseScanpy_outs/"
contrasts=["B-naive_B-memory","B-memory_B-naive","B-naive_CD14-Mono"
           ,"CD14-Mono_B-naive"]
minimal=25
export_CPM_scANANSE(anndata=rna_PBMC,min_cells=minimal,outputdir=outputdir
                    ,cluster_id="predicted.id")
export_ATAC_scANANSE(anndata=atac_PBMC,min_cells=minimal,outputdir=outputdir
                     ,cluster_id="predicted.id")
config_scANANSE(anndata=rna_PBMC,min_cells=minimal,outputdir=outputdir,
                cluster_id="predicted.id",additional_contrasts=contrasts)
DEGS_scANANSE(anndata=rna_PBMC,min_cells=minimal,outputdir=outputdir,
              cluster_id="predicted.id",additional_contrasts=contrasts)
```

```
/vol/mbconda/julian/envs/AnanseScanpy/lib/python3.10/site-
packages/anndata/_core/raw.py:139: FutureWarning: X.dtype being converted to
np.float32 from float64. In the next version of anndata (0.9) conversion will
not be automatic. Pass dtype explicitly to avoid this warning. Pass `AnnData(X,
dtype=X.dtype, …)` to get the future behavour.
  return anndata.AnnData(
```

```
gather data from CD4-Naive with 1414 cells
gather data from CD4-TCM with 1592 cells
gather data from CD8-Naive with 1496 cells
gather data from CD16-Mono with 527 cells
gather data from NK with 492 cells
gather data from Treg with 160 cells
gather data from CD14-Mono with 3095 cells
gather data from CD8-TCM with 73 cells
gather data from B-intermediate with 351 cells
gather data from cDC2 with 168 cells
gather data from B-memory with 159 cells
gather data from CD4-TEM with 172 cells
gather data from MAIT with 121 cells
gather data from CD8-TEM with 664 cells
gather data from B-naive with 424 cells
gather data from gdT with 164 cells
gather data from pDC with 110 cells
gather data from HSPC with 26 cells
gather data from CD4-Naive with 1414 cells
gather data from CD4-TCM with 1592 cells
gather data from CD8-Naive with 1496 cells
gather data from CD16-Mono with 527 cells
gather data from NK with 492 cells
gather data from Treg with 160 cells
gather data from CD14-Mono with 3095 cells
gather data from CD8-TCM with 73 cells
gather data from B-intermediate with 351 cells
gather data from cDC2 with 168 cells
gather data from B-memory with 159 cells
gather data from CD4-TEM with 172 cells
gather data from MAIT with 121 cells
gather data from CD8-TEM with 664 cells
gather data from B-naive with 424 cells
gather data from gdT with 164 cells
gather data from pDC with 110 cells
gather data from HSPC with 26 cells
adding additional contrasts
anansesnake_CD4-Naive_average
anansesnake_CD4-TCM_average
anansesnake_CD8-Naive_average
anansesnake_CD16-Mono_average
anansesnake_NK_average
anansesnake_Treg_average
anansesnake_CD14-Mono_average
anansesnake_CD8-TCM_average
anansesnake_B-intermediate_average
anansesnake_cDC2_average
anansesnake_B-memory_average
anansesnake_CD4-TEM_average
anansesnake_MAIT_average
anansesnake_CD8-TEM_average
anansesnake_B-naive_average
```

```
anansesnake_gdT_average
anansesnake_pDC_average
anansesnake_HSPC_average
anansesnake_B-naive_B-memory
anansesnake_B-memory_B-naive
anansesnake_B-naive_CD14-Mono
anansesnake_CD14-Mono_B-naive
adding additional contrasts
calculating DEGS for contrast anansesnake_CD4-Naive_average
skip
calculating DEGS for contrast anansesnake_CD4-TCM_average
skip
calculating DEGS for contrast anansesnake_CD8-Naive_average
skip
calculating DEGS for contrast anansesnake_CD16-Mono_average
skip
calculating DEGS for contrast anansesnake_NK_average
skip
calculating DEGS for contrast anansesnake_Treg_average
skip
calculating DEGS for contrast anansesnake_CD14-Mono_average
skip
calculating DEGS for contrast anansesnake_CD8-TCM_average
skip
calculating DEGS for contrast anansesnake_B-intermediate_average
skip
calculating DEGS for contrast anansesnake_cDC2_average
skip
calculating DEGS for contrast anansesnake_B-memory_average
skip
calculating DEGS for contrast anansesnake_CD4-TEM_average
skip
calculating DEGS for contrast anansesnake_MAIT_average
skip
calculating DEGS for contrast anansesnake_CD8-TEM_average
skip
calculating DEGS for contrast anansesnake_B-naive_average
skip
calculating DEGS for contrast anansesnake_gdT_average
skip
calculating DEGS for contrast anansesnake_pDC_average
skip
calculating DEGS for contrast anansesnake_HSPC_average
skip
calculating DEGS for contrast anansesnake_B-naive_B-memory
skip
calculating DEGS for contrast anansesnake_B-memory_B-naive
skip
```

```
calculating DEGS for contrast anansesnake_B-naive_CD14-Mono
skip
calculating DEGS for contrast anansesnake_CD14-Mono_B-naive
skip
```

[5]:
```
# Export ATAC data for maelstrom analysis (see methods scANANSE paper)
export_ATAC_maelstrom(anndata=atac_PBMC,min_cells=minimal,outputdir=outputdir
                      ,cluster_id="predicted.id")
```

```
gather data from CD4-Naive with 1414 cells
gather data from CD4-TCM with 1592 cells
gather data from CD8-Naive with 1496 cells
gather data from CD16-Mono with 527 cells
gather data from NK with 492 cells
gather data from Treg with 160 cells
gather data from CD14-Mono with 3095 cells
gather data from CD8-TCM with 73 cells
gather data from B-intermediate with 351 cells
gather data from cDC2 with 168 cells
gather data from B-memory with 159 cells
gather data from CD4-TEM with 172 cells
gather data from MAIT with 121 cells
gather data from CD8-TEM with 664 cells
gather data from B-naive with 424 cells
gather data from gdT with 164 cells
gather data from pDC with 110 cells
gather data from HSPC with 26 cells
large dataframe detected, selecting top variable rows n = 100000
if entire dataframe is required, add select_top_rows = False as a parameter
or change ammount of rows via the n_top_rows parameter
```

[6]:
```
# Import the maelstrom results to a dataframe and into the scanpy object
df_mael = import_scanpy_maelstrom(anndata=rna_PBMC,cluster_id="predicted.id",
                        maelstrom_dir="AnanseScanpy_outs/maelstrom/",return_df =␣
 ↪True)
```

```
/vol/mbconda/julian/envs/AnanseScanpy/lib/python3.10/site-
packages/anndata/_core/anndata.py:798: UserWarning:
AnnData expects .obs.index to contain strings, but got values like:
    [0, 1, 2, 3, 4]

    Inferred to be: integer

  value_idx = self._prep_dim_index(value.index, attr)
```

[7]:
```
# Make a dataframe with the values per cluster from the scanpy object, like␣
 ↪df_mael above:
```

```
df_mael2 = per_cluster_df(anndata=rna_PBMC,assay="maelstrom",cluster_id =␣
↪"predicted.id")
```

[8]:
```
# Link motifs to transcription factors specified with "combine_motifs"␣
↪parameter.
# Here, the means of all motifs will be used (other options include: max_var␣
↪and max_cor; see help)

rna_PBMC=Maelstrom_Motif2TF(anndata=rna_PBMC,
                    cluster_id = 'predicted.id',
                    maelstrom_dir= "AnanseScanpy_outs/maelstrom/
↪",combine_motifs="max_cor")

# Note: if you already have a dataframe from maelstrom as input, the function␣
↪will run faster
```

```
loading maelstrom values from maelstrom assay using the cluster identifier
predicted.id
```

```
/vol/mbconda/julian/envs/AnanseScanpy/lib/python3.10/site-
packages/anndata/_core/raw.py:139: FutureWarning: X.dtype being converted to
np.float32 from float64. In the next version of anndata (0.9) conversion will
not be automatic. Pass dtype explicitly to avoid this warning. Pass `AnnData(X,
dtype=X.dtype, …)` to get the future behavour.
  return anndata.AnnData(
```

```
Seurat NormalizeData with default settings will be run on all the genes
Only keep motif-TF combinations with an R > 0.3
total length m2f_df_unique 279
Selecting correlating TFs
total m2f: 164
Motif best (absolute)correlated to expression is selected per TF
Selecting anticorrelating TFs
total m2f: 115
Motif best (absolute)correlated to expression is selected per TF
```

```
/vol/mbconda/julian/envs/AnanseScanpy/lib/python3.10/site-
packages/anndata/_core/anndata.py:798: UserWarning:
AnnData expects .obs.index to contain strings, but got values like:
    [0, 1, 2, 3, 4]

    Inferred to be: integer

  value_idx = self._prep_dim_index(value.index, attr)
/vol/mbconda/julian/envs/AnanseScanpy/lib/python3.10/site-
packages/anndata/_core/anndata.py:798: UserWarning:
AnnData expects .obs.index to contain strings, but got values like:
    [0, 1, 2, 3, 4]
    Inferred to be: integer

  value_idx = self._prep_dim_index(value.index, attr)
/vol/mbconda/julian/envs/AnanseScanpy/lib/python3.10/site-
packages/anndata/_core/anndata.py:798: UserWarning:
AnnData expects .obs.index to contain strings, but got values like:
    [0, 1, 2, 3, 4]

    Inferred to be: integer

  value_idx = self._prep_dim_index(value.index, attr)
/vol/mbconda/julian/envs/AnanseScanpy/lib/python3.10/site-
packages/anndata/_core/anndata.py:798: UserWarning:
AnnData expects .obs.index to contain strings, but got values like:
    [0, 1, 2, 3, 4]

    Inferred to be: integer

  value_idx = self._prep_dim_index(value.index, attr)
```

[9]:
```
# If you want to see which motif corresponds with which factor, you can extract␣
↪the metadata
# Here the anti-correlation assay is shown (TFanticor); other option includes␣
↪TFcor_means
# Based on your used "combine_motifs" parameter; you can change below to␣
↪rna_PBMC.uns["TFanticor_max_var"]
# Here, the max_cor is the GM.5.0.Ets.0015 motif used factor ETS1
rna_PBMC.uns["TFanticor_max_cor"]
```

[9]:
```
       abscor                         Motif  Factor       cor        var
0    0.301583              GM.5.0.IRF.0009    SPI1 -0.301583   7.027371
3    0.306479       GM.5.0.Paired_box.0013     AHR -0.306479   1.991885
5    0.310215             GM.5.0.bZIP.0079     FOS -0.310215   1.202970
7    0.312816              GM.5.0.IRF.0009   TCF12 -0.312816   7.027371
8    0.314340              GM.5.0.Rel.0009  HIVEP1 -0.314340   4.409965
..        …                            …       …         …          …
255  0.780393  GM.5.0.Nuclear_receptor.0011   NR3C1 -0.780393   1.321586
263  0.829559           GM.5.0.C2H2_ZF.0190     SP3 -0.829559   4.522588
264  0.833679           GM.5.0.C2H2_ZF.0190    PBX3 -0.833679   4.522588
268  0.841159           GM.5.0.C2H2_ZF.0195    ETS1 -0.841159   3.124414
272  0.882388              GM.5.0.Ets.0015    ETS1 -0.882388  32.697519

[115 rows x 5 columns]
```

[10]:
```
# Generate a UMAP if not performed already during pre-processing
adata=rna_PBMC
adata.raw = adata
```

```
sc.pp.normalize_total(adata, target_sum=1e4)
sc.pp.log1p(adata)
sc.pp.pca(adata)
sc.pp.neighbors(adata, n_neighbors=10, n_pcs=30)
sc.tl.umap(adata)
```

```
WARNING: adata.X seems to be already log-transformed.
```

```
/vol/mbconda/julian/envs/AnanseScanpy/lib/python3.10/site-
packages/tqdm/auto.py:22: TqdmWarning: IProgress not found. Please update
jupyter and ipywidgets. See
https://ipywidgets.readthedocs.io/en/stable/user_install.html
  from .autonotebook import tqdm as notebook_tqdm
```

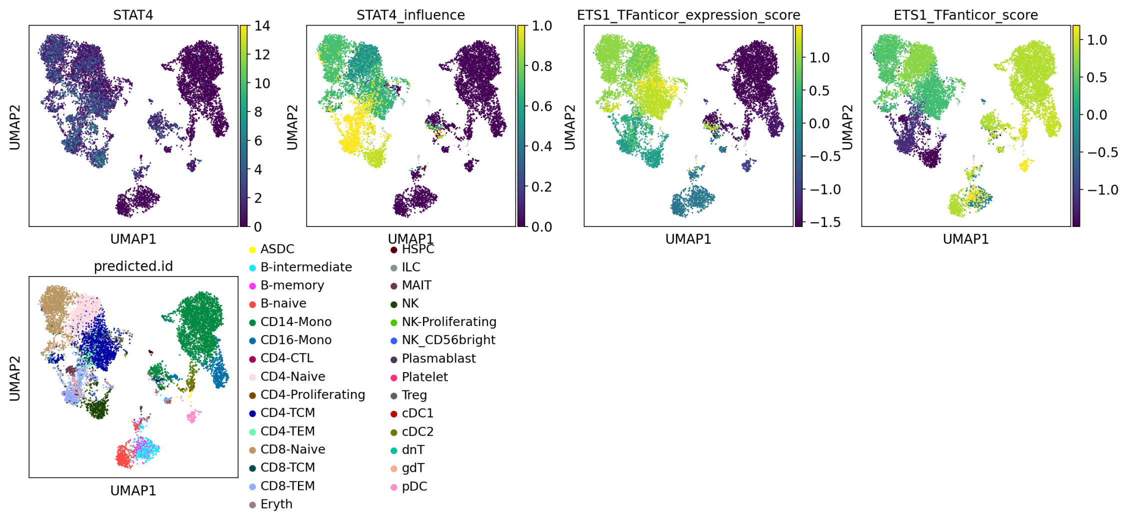
After running ANANSNAKE you can import back the results to the scanpy object and visualize a heatmap of the top factors with seaborn

[11]:
```
# Import the Ananse results to the scanpy object and a separate dataframe as␣
 ↪well
df_influence=import_scanpy_scANANSE(anndata=rna_PBMC,cluster_id="predicted.id",
                        anansnake_inf_dir="AnanseScanpy_outs/influence/")
```

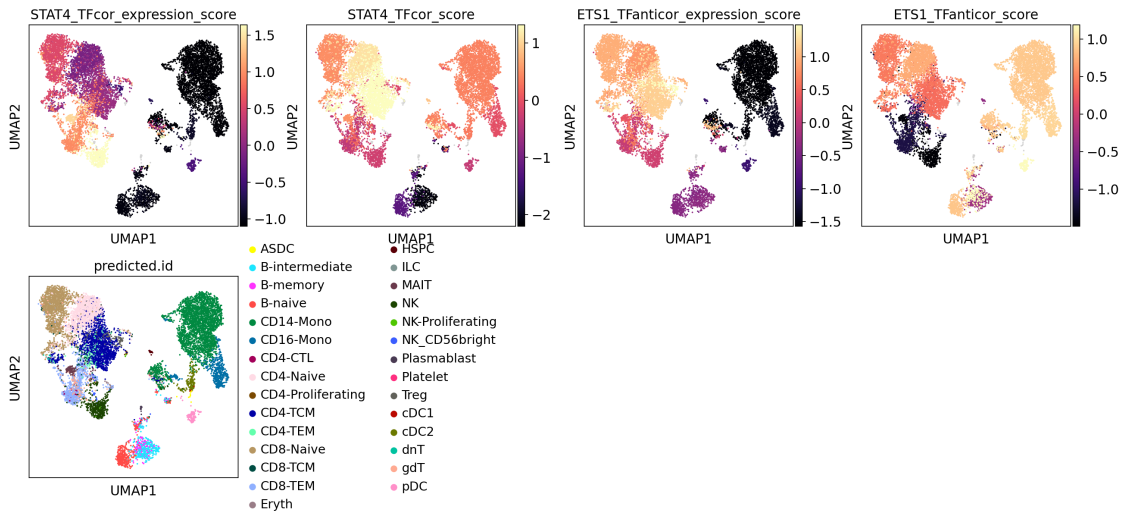
```
/vol/mbconda/julian/envs/AnanseScanpy/lib/python3.10/site-
packages/anndata/_core/anndata.py:798: UserWarning:
AnnData expects .obs.index to contain strings, but got values like:
    [0, 1, 2, 3, 4]

    Inferred to be: integer

  value_idx = self._prep_dim_index(value.index, attr)
```

[12]:
```
# Show absolute expression and influence values of transcription factors on the␣
 ↪UMAP
sc.pl.umap(adata,␣
 ↪color=["STAT4","STAT4_influence","ETS1_TFanticor_expression_score","ETS1_TFanticor_score","
 ↪id"], cmap="viridis")

# Optional: show relative expression and maelstrom values of transcription␣
 ↪factors on the UMAP
sc.pl.umap(adata,␣
 ↪color=["STAT4_TFcor_expression_score","STAT4_TFcor_score","ETS1_TFanticor_expression_score"
 ↪id"], cmap="magma")

# For STAT4, you can see that the relative expression corresponds with motif␣
 ↪enrichment
# For ETS1, a predicted repressive factor, you can see that higher relative␣
 ↪expression
# corresponds with a relative inaccessible motif
```

```
/vol/mbconda/julian/envs/AnanseScanpy/lib/python3.10/site-
packages/scanpy/plotting/_tools/scatterplots.py:392: UserWarning: No data for
colormapping provided via 'c'. Parameters 'cmap' will be ignored
  cax = scatter(
```



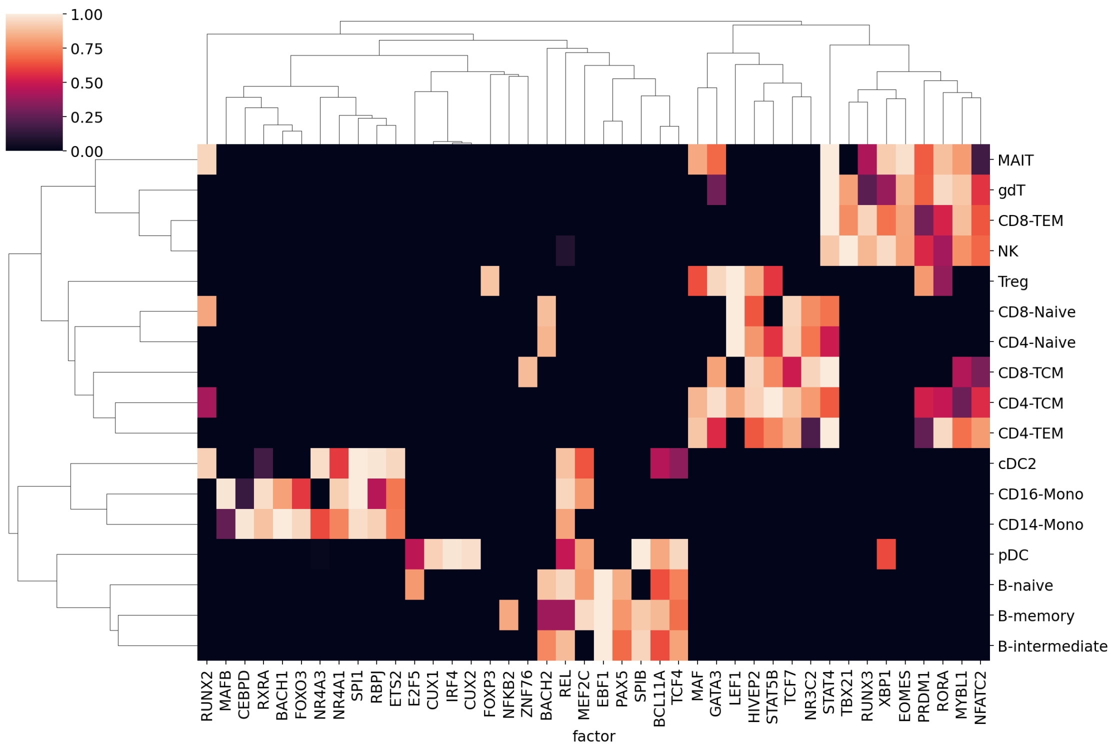
```
/vol/mbconda/julian/envs/AnanseScanpy/lib/python3.10/site-
packages/scanpy/plotting/_tools/scatterplots.py:392: UserWarning: No data for
colormapping provided via 'c'. Parameters 'cmap' will be ignored
  cax = scatter(
```



[13]:
```python
# Make a heatmap of the top 5 transcription factors for each population
import seaborn as sns
top=5
```

```
df_t = df_influence.transpose()
factors_topn = []
for i in df_t:
    df_sub=df_t[i]
    test = df_sub.sort_values(ascending=False)
    factors_topn.append(list(test[0:top].index))

factors_topn=[j for i in factors_topn for j in i]
factors_topn=set(factors_topn)

selected_df = df_influence[list(factors_topn)]
sns.clustermap(selected_df, annot=False, figsize=(15, 10))
```

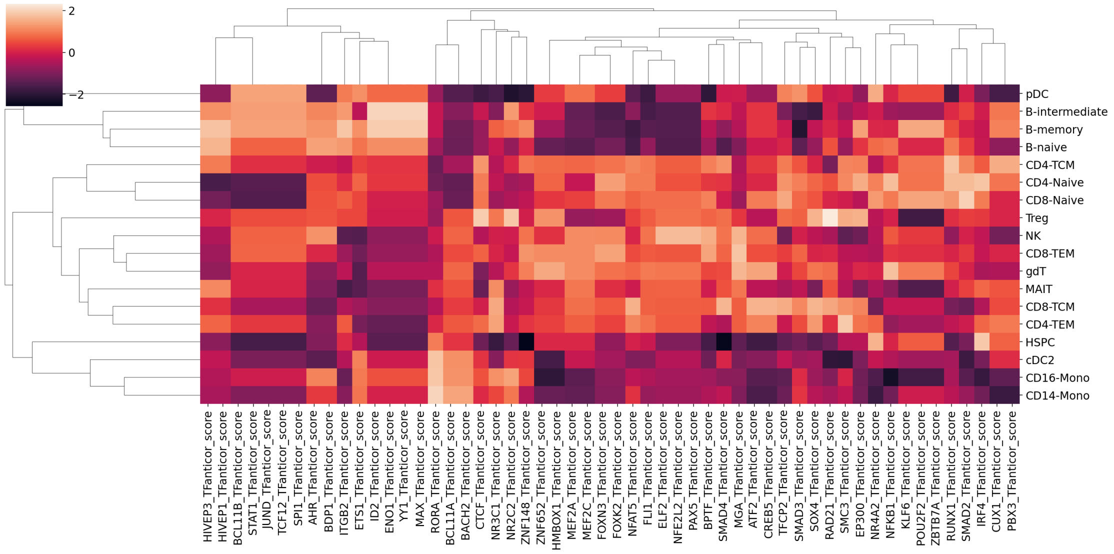
[13]:
```
<seaborn.matrix.ClusterGrid at 0x14cc63046470>
```



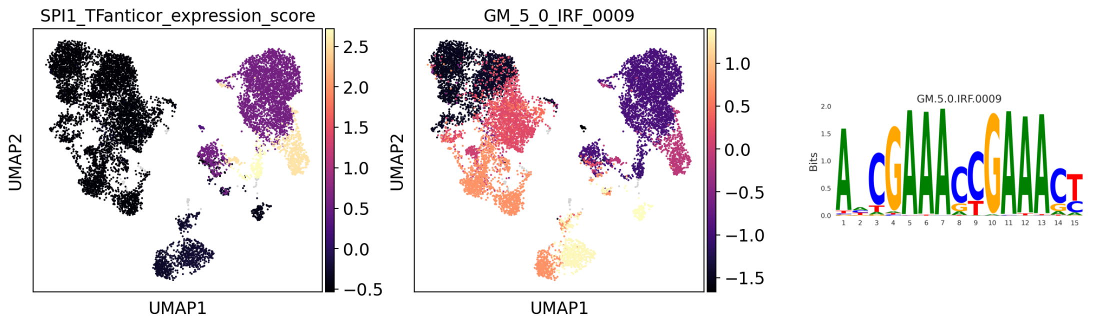
[14]:
```
# Make a dataframe with the values of the anti-correlation per cluster from the␣
↪scanpy object:
df_anticor = per_cluster_df(anndata=rna_PBMC,assay="TFanticor_score",cluster_id␣
↪= "predicted.id")

# Visualize the top 5 anticorrelating factors in a heatmap
top=5
```

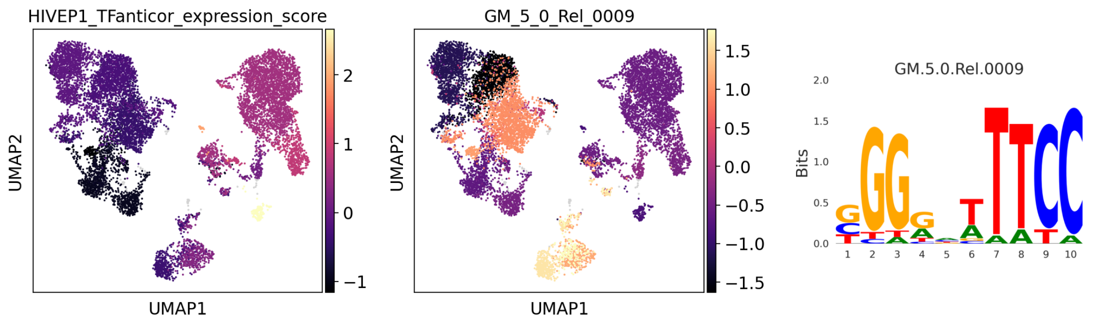
```
df_t = df_anticor.transpose()
factors_topn = []
for i in df_t:
    df_sub=df_t[i]
    test = df_sub.sort_values(ascending=True)
    factors_topn.append(list(test[0:top].index))

factors_topn=[j for i in factors_topn for j in i]
factors_topn=set(factors_topn)

selected_df = df_anticor[list(factors_topn)]
sns.clustermap(selected_df, annot=False, figsize=(20, 10))

# Note: the ETS1 motif shows high repression score for CD8 like cell types,␣
 ↪like on the UMAP above

# Note: PAX5 is an important repressive factor in B-cells, also indiciated in␣
 ↪our heatmap
# Source: Delogu A, Schebesta A, Sun Q, Aschenbrenner K, Perlot T, Busslinger M.
 ↪
# Gene repression by Pax5 in B cells is essential for blood cell homeostasis␣
 ↪and is reversed in plasma cells.
# Immunity. 2006 Mar;24(3):269-81. doi: 10.1016/j.immuni.2006.01.012. PMID:␣
 ↪16546096.
```

```
[14]: <seaborn.matrix.ClusterGrid at 0x14cbcf9fc7f0>
```



[15]:
```python
# Showing the negative correlating factors of interest with "max_cor" as the default "combine_motifs" parameter
factors = ["SPI1","HIVEP1"]
Factor_Motif_Plot(rna_PBMC,factor_list=factors,logo_dir='AnanseScanpy_outs/maelstrom/logos/',assay_maelstrom = 'TFanticor')
```


SPI1_TFanticor_expression_score
UMAP2
UMAP1
GM_5_0_IRF_0009
GM.5.0.IRF.0009
Bits


HIVEP1_TFanticor_expression_score
UMAP2
UMAP1
GM_5_0_Rel_0009
GM.5.0.Rel.0009
Bits