Single cell analysis of conventional chondrosarcomas

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This script analyze single cell RNA-seq data (GSE184118) by selecting conventional chondrosarcomas. This permite us to confirm the presence of CHM type 1 on tumoral cell surfaces because at least it as necessary in the project

Table of contents

Load packages

In [ ]:
```
import scanpy as sc
import matplotlib as mpl
import matplotlib.pylab as plt
```

```
import anndata as ad
sc.settings.verbosity = 0
```

```
C:\Users\learogue\AppData\Local\Packages\PythonSoftwareFoundation.Python.3.1
1_qbz5n2kfra8p0\LocalCache\local-packages\Python311\site-packages\anndata\ut
ils.py:429: FutureWarning: Importing read_csv from `anndata` is deprecated.
Import anndata.io.read_csv instead.
  warnings.warn(msg, FutureWarning)
C:\Users\learogue\AppData\Local\Packages\PythonSoftwareFoundation.Python.3.1
1_qbz5n2kfra8p0\LocalCache\local-packages\Python311\site-packages\anndata\ut
ils.py:429: FutureWarning: Importing read_excel from `anndata` is deprecate
d. Import anndata.io.read_excel instead.
  warnings.warn(msg, FutureWarning)
C:\Users\learogue\AppData\Local\Packages\PythonSoftwareFoundation.Python.3.1
1_qbz5n2kfra8p0\LocalCache\local-packages\Python311\site-packages\anndata\ut
ils.py:429: FutureWarning: Importing read_hdf from `anndata` is deprecated.
Import anndata.io.read_hdf instead.
  warnings.warn(msg, FutureWarning)
C:\Users\learogue\AppData\Local\Packages\PythonSoftwareFoundation.Python.3.1
1_qbz5n2kfra8p0\LocalCache\local-packages\Python311\site-packages\anndata\ut
ils.py:429: FutureWarning: Importing read_loom from `anndata` is deprecated.
Import anndata.io.read_loom instead.
  warnings.warn(msg, FutureWarning)
C:\Users\learogue\AppData\Local\Packages\PythonSoftwareFoundation.Python.3.1
1_qbz5n2kfra8p0\LocalCache\local-packages\Python311\site-packages\anndata\ut
ils.py:429: FutureWarning: Importing read_mtx from `anndata` is deprecated.
Import anndata.io.read_mtx instead.
  warnings.warn(msg, FutureWarning)
C:\Users\learogue\AppData\Local\Packages\PythonSoftwareFoundation.Python.3.1
1_qbz5n2kfra8p0\LocalCache\local-packages\Python311\site-packages\anndata\ut
ils.py:429: FutureWarning: Importing read_text from `anndata` is deprecated.
Import anndata.io.read_text instead.
  warnings.warn(msg, FutureWarning)
C:\Users\learogue\AppData\Local\Packages\PythonSoftwareFoundation.Python.3.1
1_qbz5n2kfra8p0\LocalCache\local-packages\Python311\site-packages\anndata\ut
ils.py:429: FutureWarning: Importing read_umi_tools from `anndata` is deprec
ated. Import anndata.io.read_umi_tools instead.
  warnings.warn(msg, FutureWarning)
```

Process data

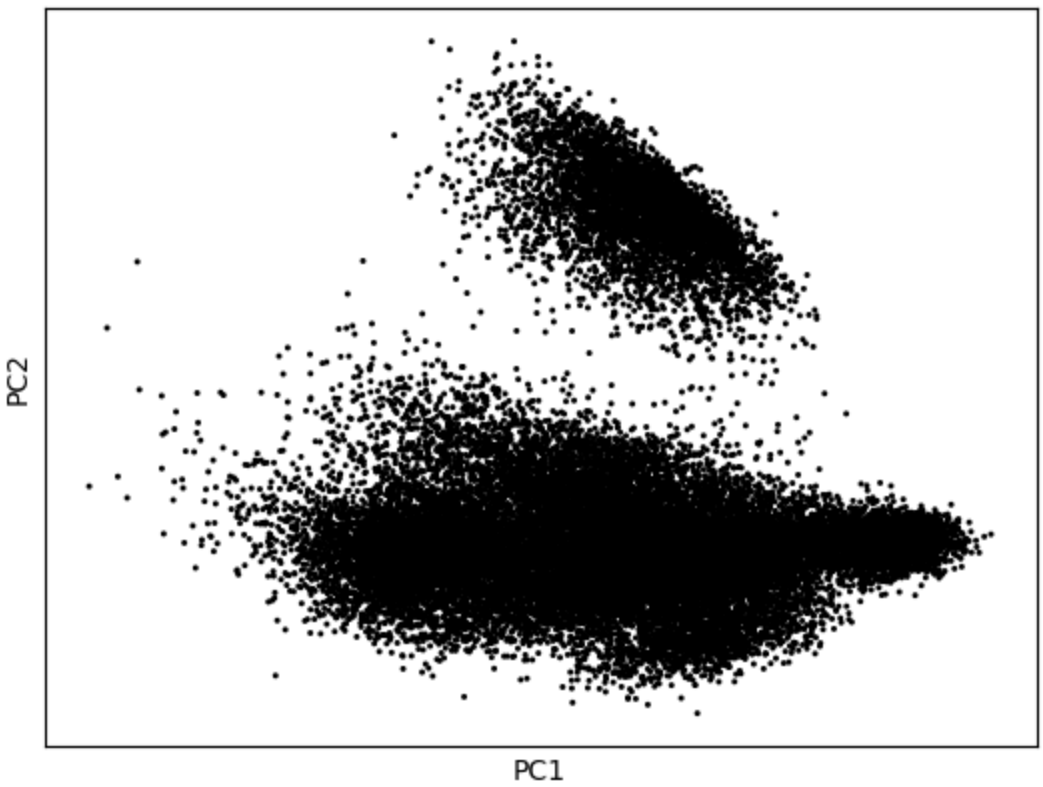
The object has been processed with a tool created during my M1 intership that we have adapted for this project. The samples were filtered with 1000 min genes per cell, 3 cells per genes and 5% max of mitochondrial genes. After, these sample filtered were merged.

In [2]:
```
# Read data
adata = ad.read_h5ad('../results/sc_results/chondro_conv_ben_analysis/Object
adata
```

```
C:\Users\learogue\AppData\Local\Packages\PythonSoftwareFoundation.Python.3.11_qbz5n2kfra8p0\LocalCache\local-packages\Python311\site-packages\anndata\_core\anndata.py:1756: UserWarning: Observation names are not unique. To make them unique, call `.obs_names_make_unique`.
  utils.warn_names_duplicates("obs")
```

Out[2]:
```
AnnData object with n_obs × n_vars = 30434 × 23175
    obs: 'n_genes_by_counts', 'total_counts', 'total_counts_MT', 'pct_counts_MT', 'dataset', 'n_genes'
```

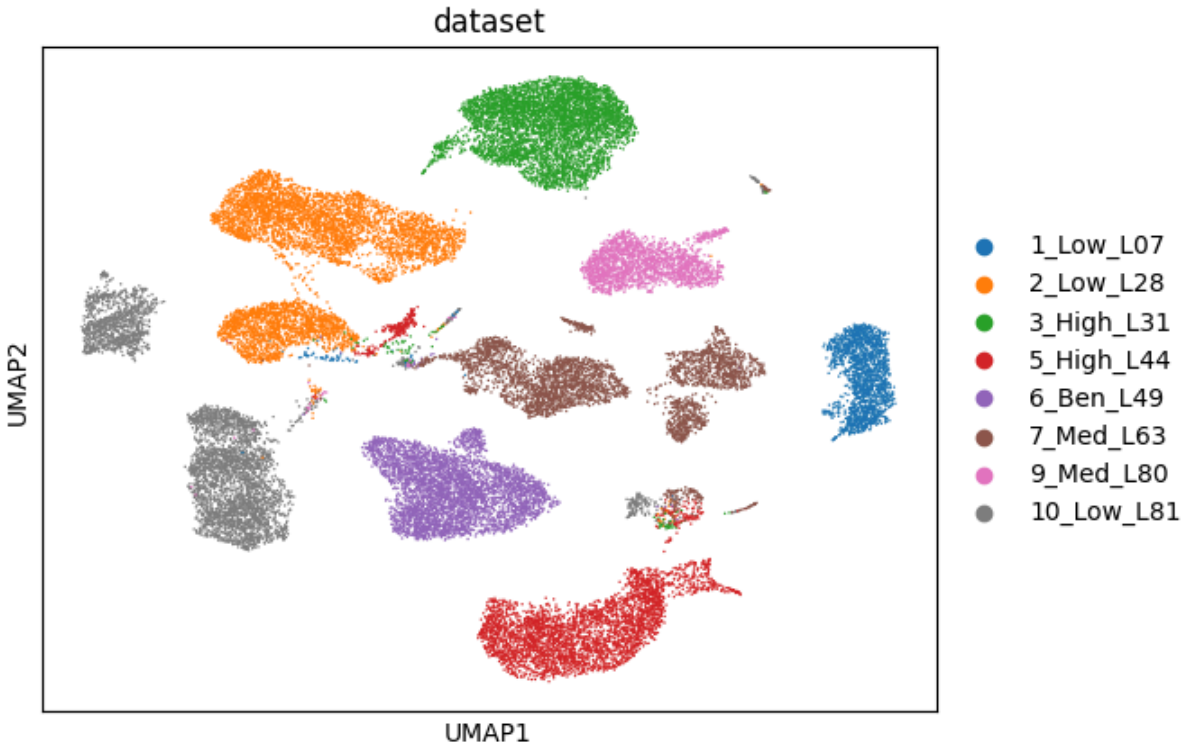
In [3]:
```
# Normalize the data
sc.pp.normalize_total(adata, target_sum = 1e4)

# Log-transform the data
sc.pp.log1p(adata)

# Store the raw data
adata.raw = adata

# Scale the data
sc.pp.scale(adata)

# Run PCA
sc.tl.pca(adata)

# Plot PCA
sc.pl.pca(adata, size = 18, na_color = 'black')
```



In [4]:
```
resolution = 0.1  # resolution for clustering, more high = more clusters, lo
n_neighbors = 15  # number of neighbors for compute the neighborhood graph
n_pcs = 20  # number of principal components analysis
```

```
# Compute the neighborhood graph
sc.pp.neighbors(adata, n_neighbors = n_neighbors)

# Cluster the cells using the leiden algorithm
sc.tl.leiden(adata, resolution = resolution)

# Compute UMAP
sc.tl.umap(adata)

# Plot the UMAP datasets Louvain plot
sc.pl.umap(adata, color = 'dataset')
```

```
C:\Users\learogue\AppData\Local\Temp\ipykernel_17160\2542342129.py:9: Future
Warning: In the future, the default backend for leiden will be igraph instea
d of leidenalg.

 To achieve the future defaults please pass: flavor="igraph" and n_iteration
s=2.  directed must also be False to work with igraph's implementation.
  sc.tl.leiden(adata, resolution = resolution)
```



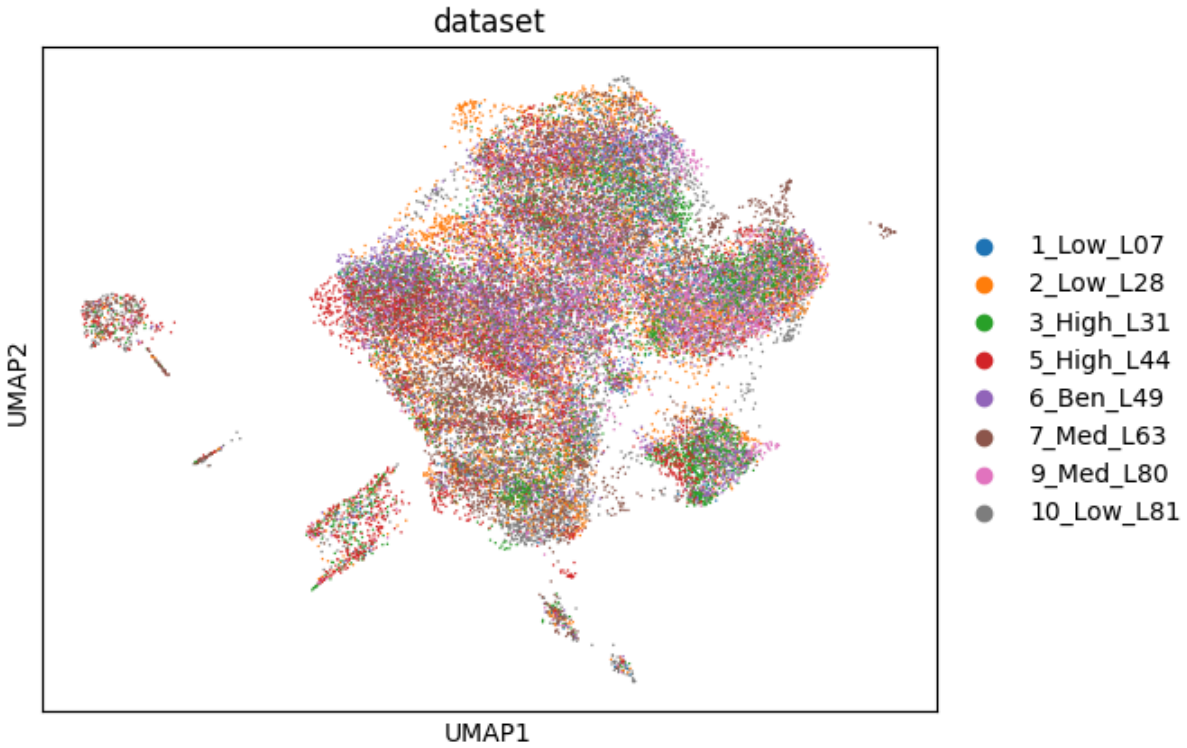
Here, we see that samples are not mixed because of batch effects, so we use harmony to integrate data and delete batch effects.

I. Integration

In [5]:
```
# Integrate data using Harmony
sc.external.pp.harmony_integrate(adata, 'dataset', theta = 2.5, max_iter_har

# Save the Harmony-corrected PCA results
adata.obsm['X_pca'] = adata.obsm['X_pca_harmony']
```

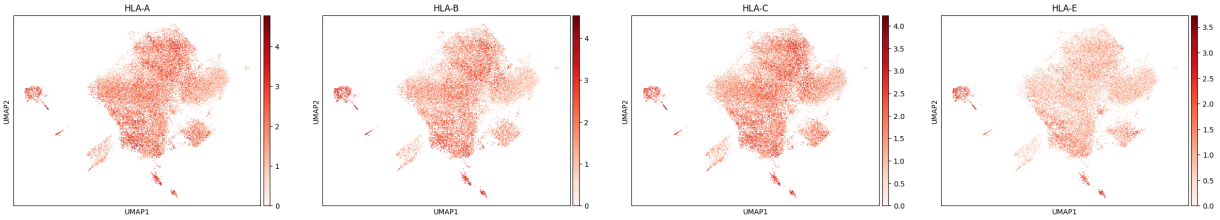
```
2025-04-10 16:10:32,144 - harmonypy - INFO - Computing initial centroids wit
h sklearn.KMeans...
2025-04-10 16:10:35,335 - harmonypy - INFO - sklearn.KMeans initialization c
omplete.
2025-04-10 16:10:35,496 - harmonypy - INFO - Iteration 1 of 10
2025-04-10 16:10:46,915 - harmonypy - INFO - Iteration 2 of 10
2025-04-10 16:10:57,954 - harmonypy - INFO - Iteration 3 of 10
2025-04-10 16:11:08,871 - harmonypy - INFO - Iteration 4 of 10
2025-04-10 16:11:19,715 - harmonypy - INFO - Iteration 5 of 10
2025-04-10 16:11:30,595 - harmonypy - INFO - Iteration 6 of 10
2025-04-10 16:11:40,020 - harmonypy - INFO - Iteration 7 of 10
2025-04-10 16:11:48,058 - harmonypy - INFO - Converged after 7 iterations
```

In [6]:
```
resolution = 0.3  # resolution for clustering, more high = more clusters, lo
n_neighbors = 15  # number of neighbors for compute the neighborhood graph,
n_pcs = 20  # number of principal components analysis

# Compute the neighborhood graph
sc.pp.neighbors(adata, n_neighbors = n_neighbors)

# Cluster the cells using the leiden algorithm
sc.tl.leiden(adata, resolution = resolution)

# Compute UMAP
sc.tl.umap(adata)

# Plot the UMAP datasets
sc.pl.umap(adata, color = 'dataset')
```



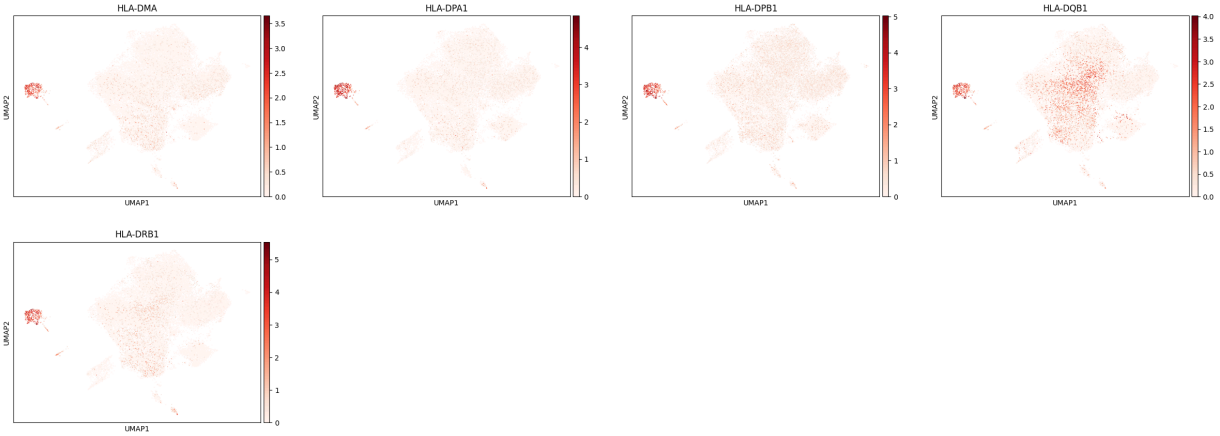
We see that all samples are now mixed so now we can determine cell types of each groups and genes of interest.

In [7]:
```
# MHC I
sc.pl.umap(adata, color = ['HLA-A', 'HLA-B', 'HLA-C', 'HLA-E'], legend_loc =
```



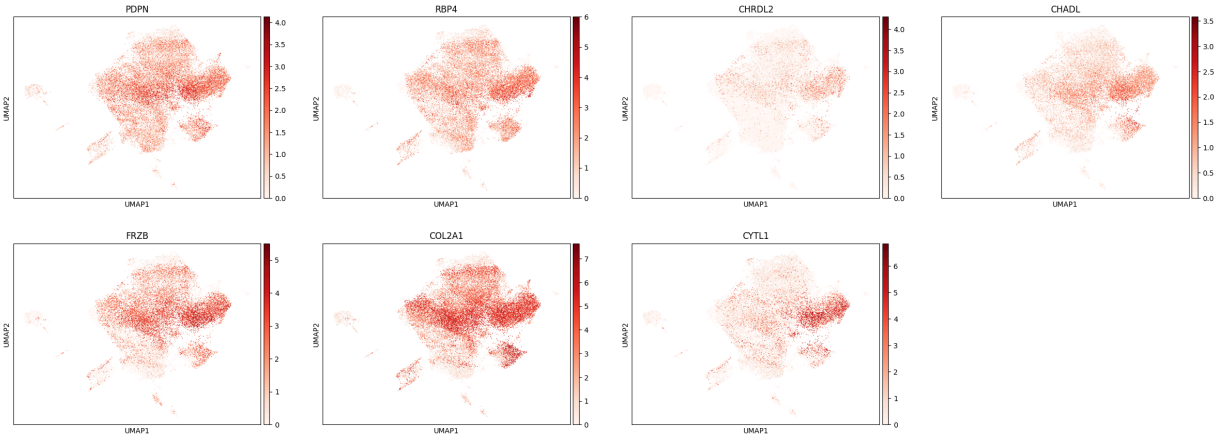
These UMAPs show that all cells expressed HLA type 1 genes. So we can deduce that cancer cells expressed HLA type 1 and potentially present peptides.

In [8]:
```
# MHC II
sc.pl.umap(adata, color = ['HLA-DMA', 'HLA-DPA1', 'HLA-DPB1', 'HLA-DQB1', 'H
```



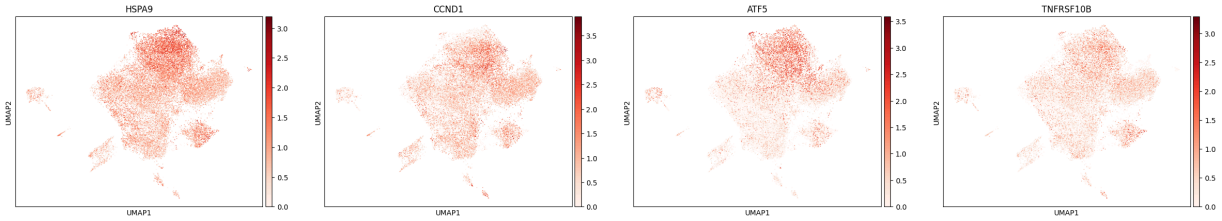
This shows the expression of HLA type 2 and it expressed in the left cluster, so we can suppose that this cluster contains immune cells. The next genes are from the data article, they use these genes to determine their clusters.

In [9]:
```
# Suggesting represents well differentiated neoplastic chondrocytes Chon1
genes = ['COL2A1', 'PDPN', 'CHADL', 'CYTL1', 'FRZB', 'CHRDL2', 'RBP4']

common_genes = list(set(genes).intersection(adata.var.index))

sc.pl.umap(adata, color = common_genes, legend_loc = 'on data', color_map =
```



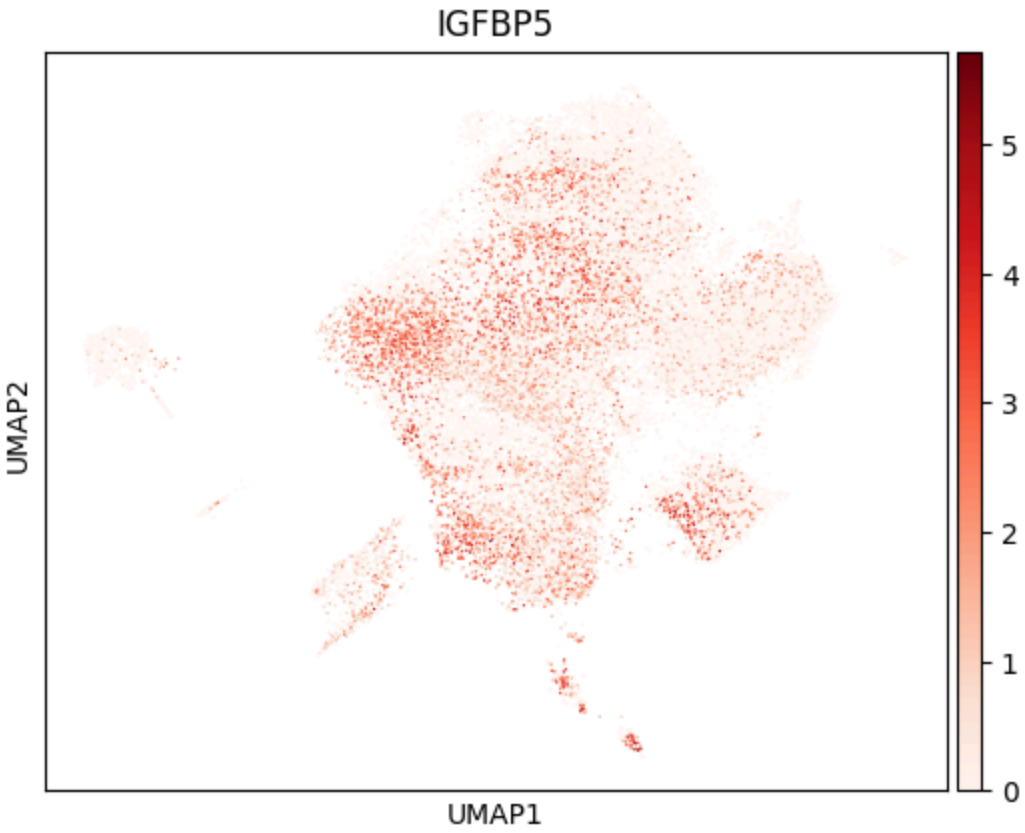
So, we see that chondrocytes are present in the big cluster, more in the middel and right.

In [10]:
```
# lower levels of cartilaginous matrix Chon2
genes = ['ATF5', 'HSPA9', 'CCND1', 'TNFRSF10B']

common_genes = list(set(genes).intersection(adata.var.index))

sc.pl.umap(adata, color = common_genes, legend_loc = 'on data', color_map =
```



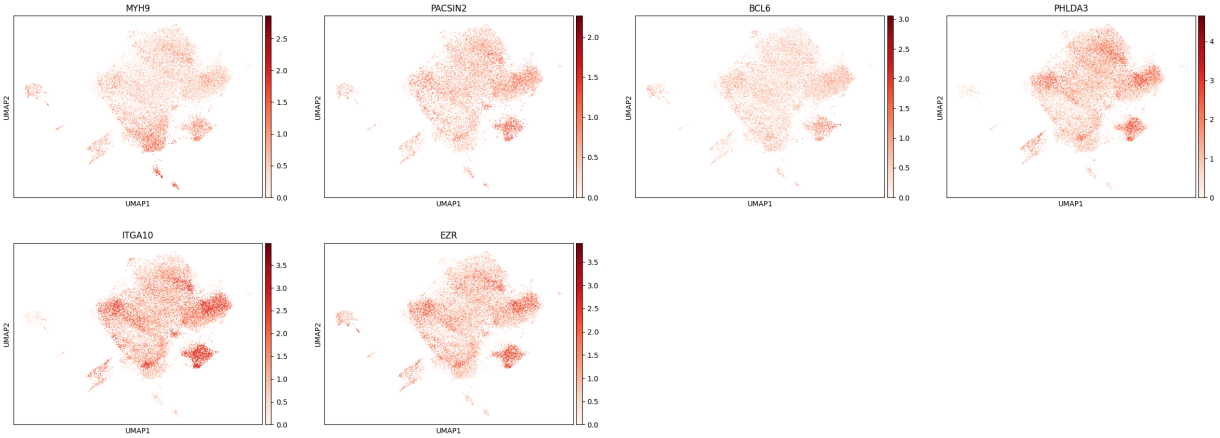
With these markers, we can deduce that there is also chondrocytes in the big cluster on the top.

In [11]:
```
# High1 marker
sc.pl.umap(adata, color = 'IGFBP5', legend_loc = 'on data', color_map = mpl.
```



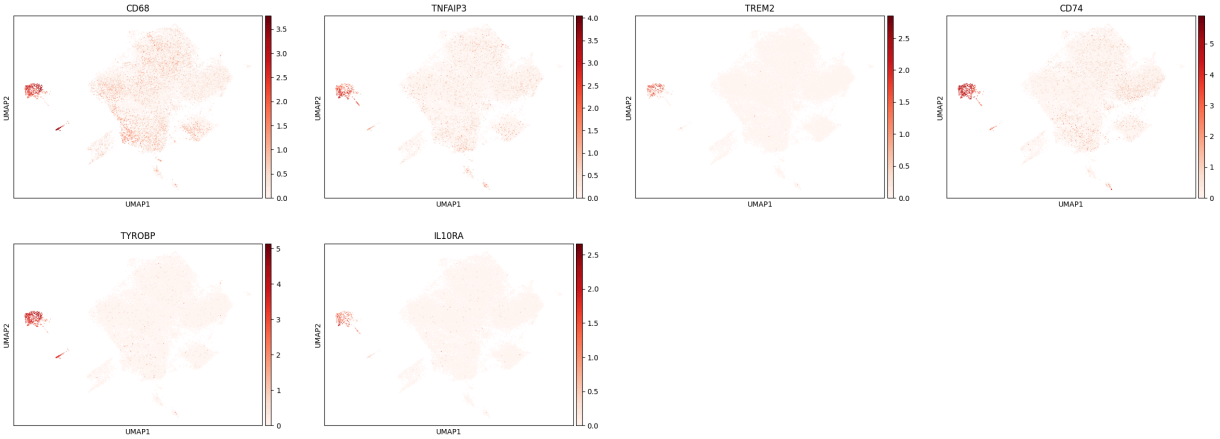
High1 markers are not very clear except IGFBP5, we see a diffuse expression, maybe more in the middle.

In [12]:
```
# High2 markers
genes = ['ITGA10', 'PHLDA3', 'EZR', 'PACSIN2', 'MYH9', 'BCL6']

common_genes = list(set(genes).intersection(adata.var.index))

sc.pl.umap(adata, color = common_genes, legend_loc = 'on data', color_map =
```



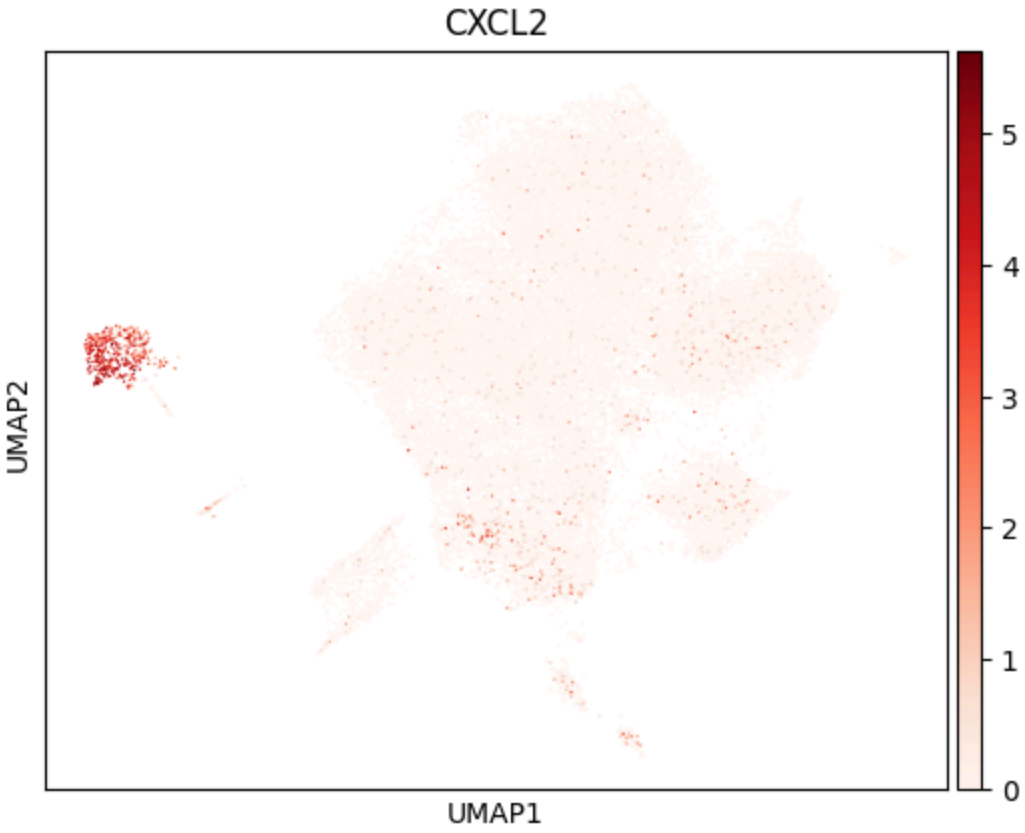
It is not very clear also, we can deduce that cluster on the bottom right and on the top of it correspond to High2 markers so may be contains tumoral cells.

In [13]:
```
# Leuk markers
genes = ['CD68', 'CD74', 'TREM2', 'IL10RA', 'TYROBP', 'TNFAIP3']

common_genes = list(set(genes).intersection(adata.var.index))

sc.pl.umap(adata, color = common_genes, legend_loc = 'on data', color_map =
```

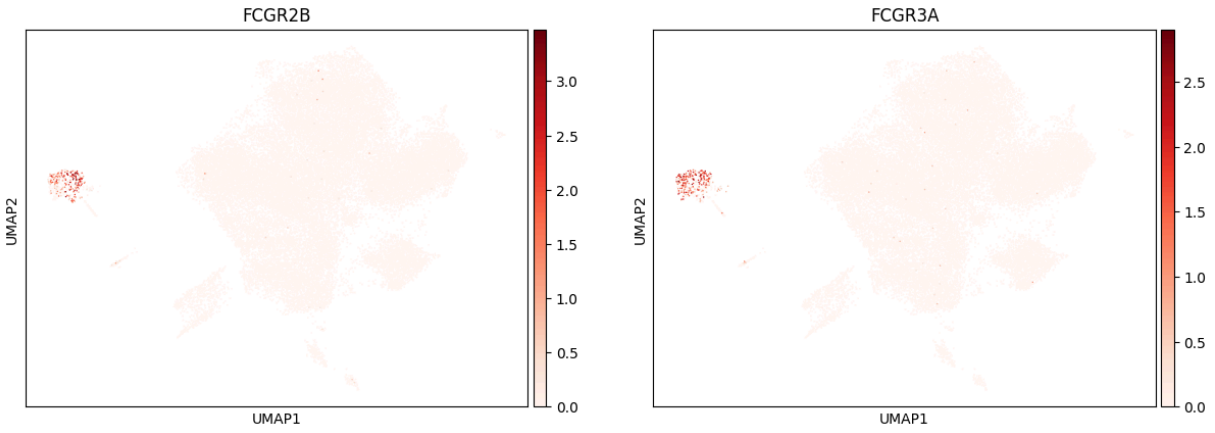


Here, we can confirm that the lef cluster is immune cells.

In [14]:
```
# M1
genes = ['CLL3L1', 'CLL3', 'CXCL2']

common_genes = list(set(genes).intersection(adata.var.index))

sc.pl.umap(adata, color = common_genes, legend_loc = 'on data', color_map =
```

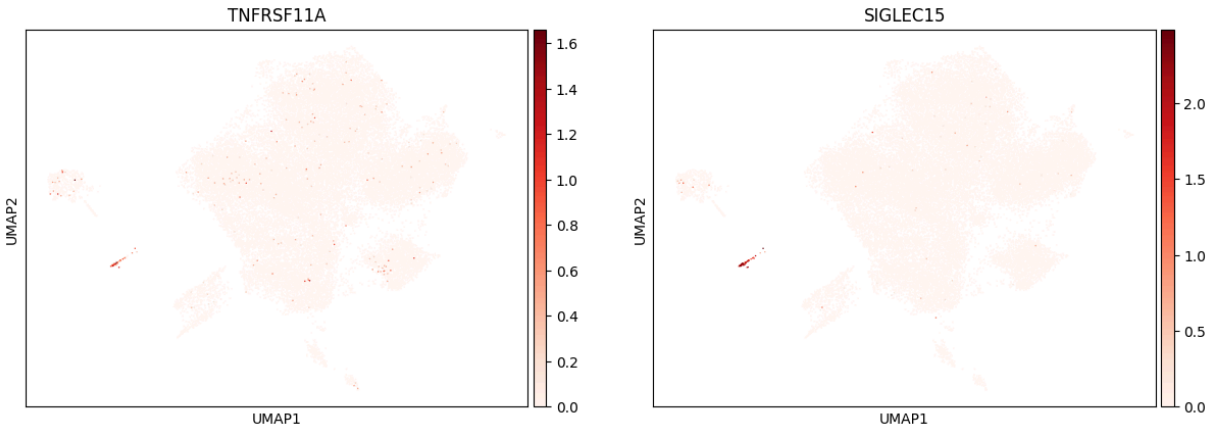


In [15]:
```
# M2
genes = ['FCGR2B', 'FCGR3A']

common_genes = list(set(genes).intersection(adata.var.index))

sc.pl.umap(adata, color = common_genes, legend_loc = 'on data', color_map =
```



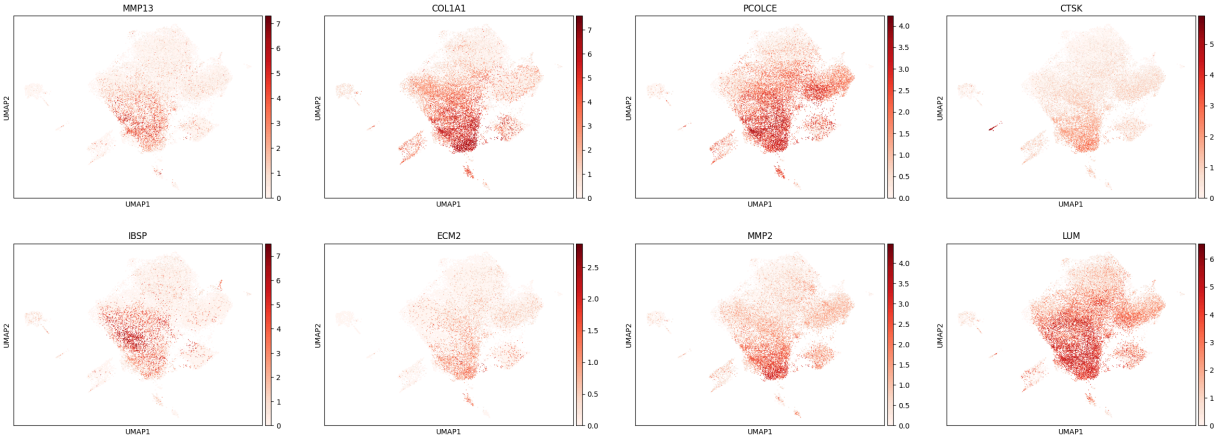
We see that M1 and M2 are mixed in the immune cells cluster.

In [16]:
```
# Osteoclasts
genes = ['TNFRSF11A', 'SIGLEC15']

common_genes = list(set(genes).intersection(adata.var.index))

sc.pl.umap(adata, color = common_genes, legend_loc = 'on data', color_map =
```



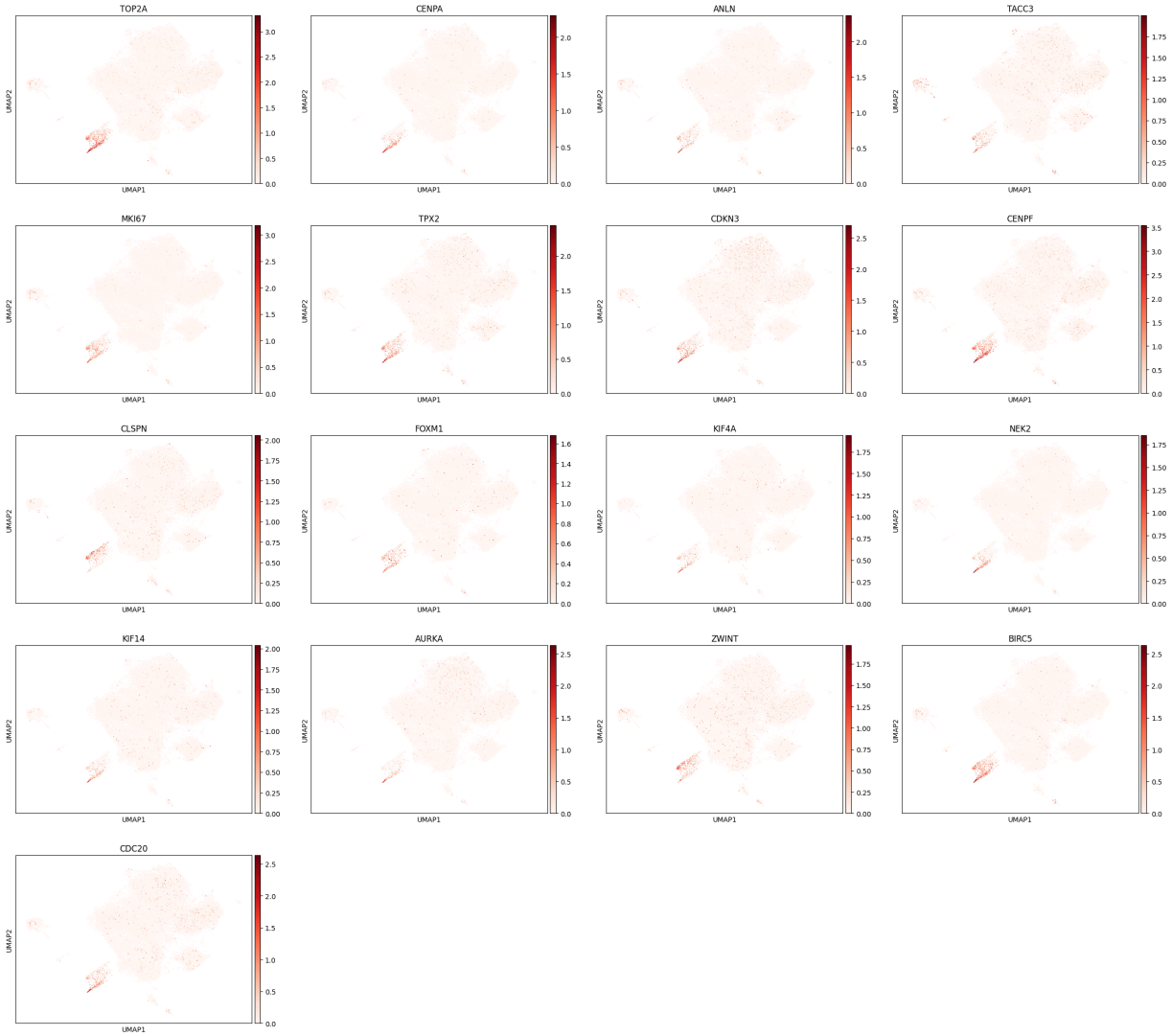
Finally the little cluster like a line is osteoclasts that are cells which degrade bones.

In [17]:
```
# Stromal markers
genes = ['COL1A1', 'LUM', 'PCOLCE', 'MMP13', 'CTSK', 'MMP2', 'ECM2', 'IBSP']

common_genes = list(set(genes).intersection(adata.var.index))

sc.pl.umap(adata, color = common_genes, legend_loc = 'on data', color_map =
```



Here, we can deduce that the middle bottom cluster is stromal cells.

In [ ]:
```
# Prolif markers
genes = ['TOP2A', 'BIRC5', 'MKI67', 'AURKA', 'TPX2', 'KIF4A', 'CLSPN', 'CDKN
         'ANLN', 'TACC3', 'FOXM1', 'CENPA', 'CDC20', 'NEK2', 'CENPF', 'KIF14

common_genes = list(set(genes).intersection(adata.var.index))

sc.pl.umap(adata, color = common_genes, legend_loc = 'on data', color_map =
```



Here, we see that the bottom left cluster are cells in proliferation.

II. Expression of interest CTAs

This section permite us to if CTAs are expressed and see were there are expressed.

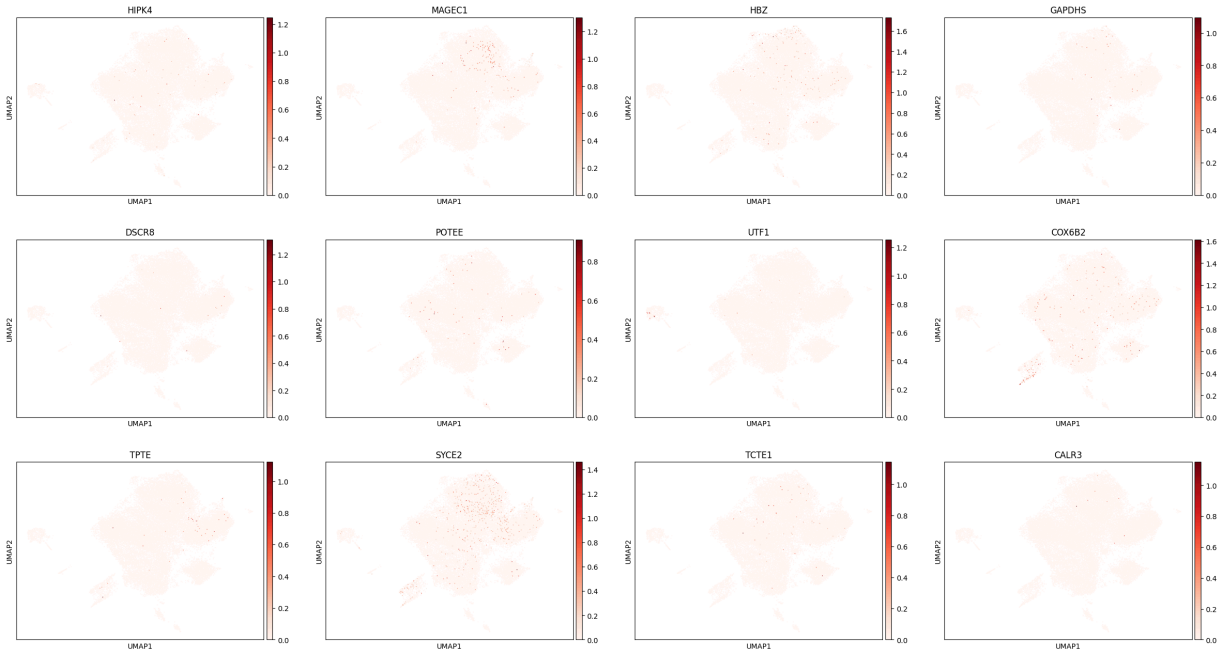
1) CTAs that impact survival

Here is the CTAs that impact survival in conventional chondrosarcomas with the clustering from heatmap of script 5 which analyze expression of genes in chondrosarcoma.

a- Cluster 1

In [ ]:
```
# Markers
# Cluster 1 conv
genes_clust_1 = ['HMGB4', 'CALR3', 'UTF1', 'HBZ', 'HES3', 'CCDC63', 'TSPY2',
    'TCTE1', 'KRT72', 'CTAG2', 'SPACA5', 'COX6B2', 'HIPK4', 'GAPDHS',
```

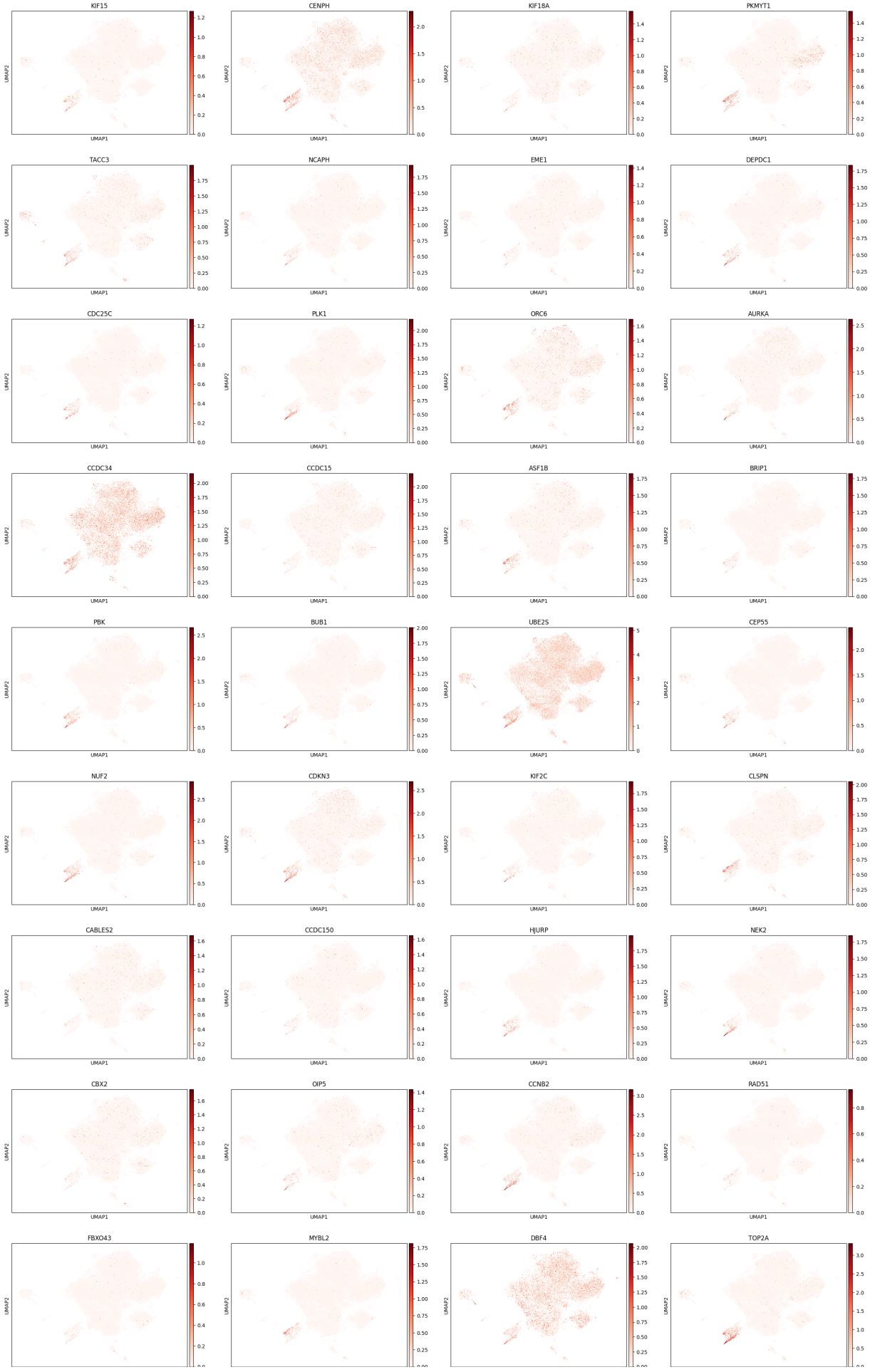
```
    'SCAND3', 'TPTE', 'SYCE2', 'DSCR8', 'MAGEC1', 'POTEE', 'POTEG', 'ARGFX']

genes_clust_1 = list(set(genes_clust_1).intersection(adata.var.index))
sc.pl.umap(adata, color = genes_clust_1 , color_map = plt.cm.Reds)
```

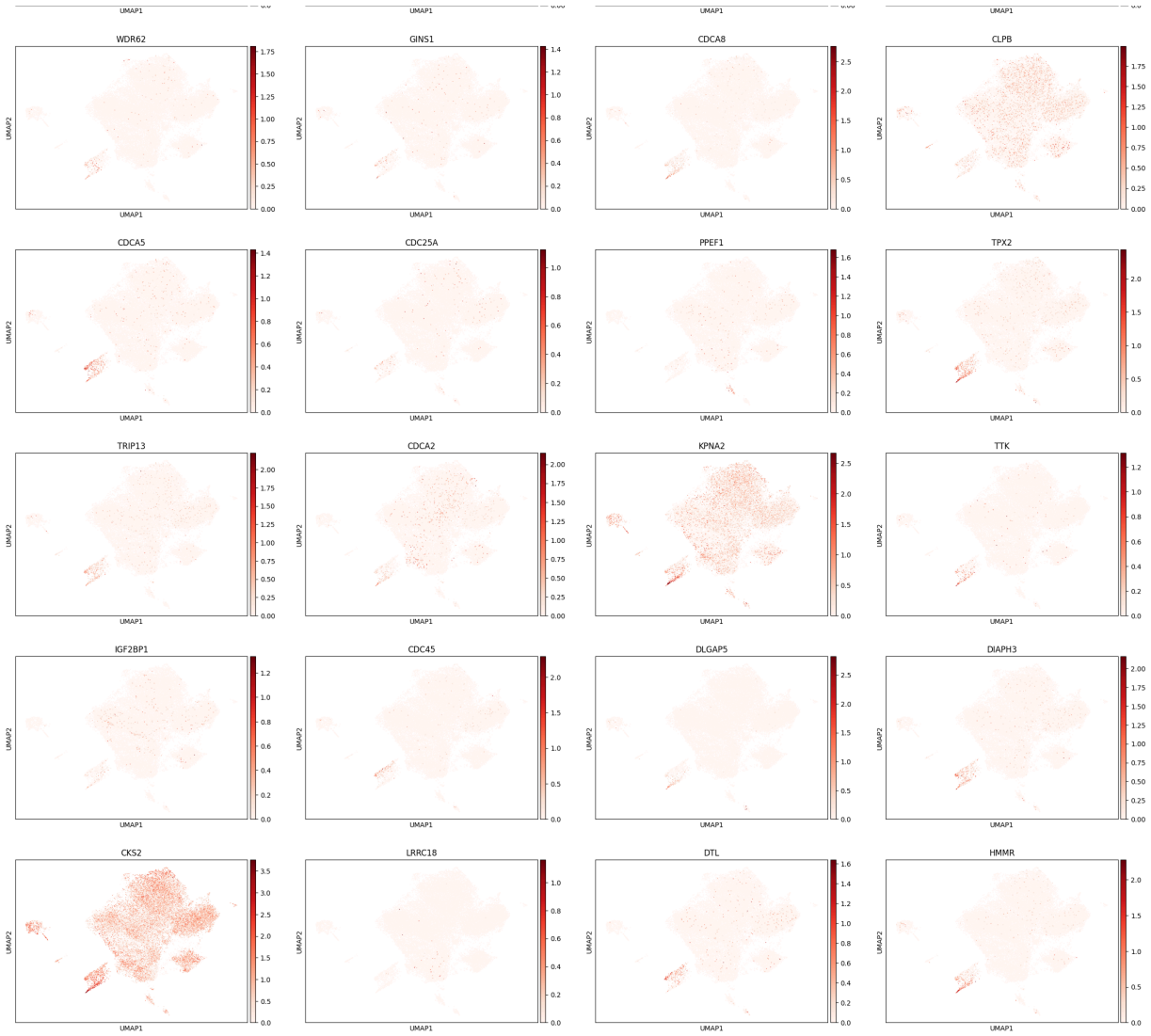


We see that the some CTA are expressed in some cells of the big cluster that probably contains chondrocytes, stromal cells and tumoral cells. We cannot conclude on the choice of one marker. Moreover, sc is not very sensitive so it is not suprising to see this.

b- Cluster 2

In [ ]:
```
# Markers
# Cluster 2 conv
genes_clust_2 = ['CLSPN', 'PPEF1', 'CABLES2', 'LRRC18', 'CCDC150', 'FBXO43',
    'CCDC34', 'OIP5', 'CCDC15', 'ORC6', 'EME1', 'CDC25A', 'CENPH',
    'ASF1B', 'MYBL2', 'GINS1', 'TRIP13', 'BRIP1', 'DTL', 'RAD51',
    'CDCA5', 'CDC45', 'PKMYT1', 'IGF2BP1', 'CBX2', 'CLPB', 'UBE2S',
    'AURKA', 'CKS2', 'DBF4', 'KPNA2', 'CEP55', 'TACC3', 'KIF15',
    'CCNB2', 'TOP2A', 'TPX2', 'CDCA8', 'CDKN3', 'HMMR', 'KIF2C',
    'DLGAP5', 'KIF18A', 'PLK1', 'NCAPH', 'NEK2', 'DEPDC1', 'CDCA2',
    'BUB1', 'PBK', 'HJURP', 'TTK', 'NUF2', 'WDR62', 'CDC25C', 'DIAPH3']

genes_clust_2 = list(set(genes_clust_2).intersection(adata.var.index))
sc.pl.umap(adata, color = genes_clust_2 , color_map = plt.cm.Reds)
```

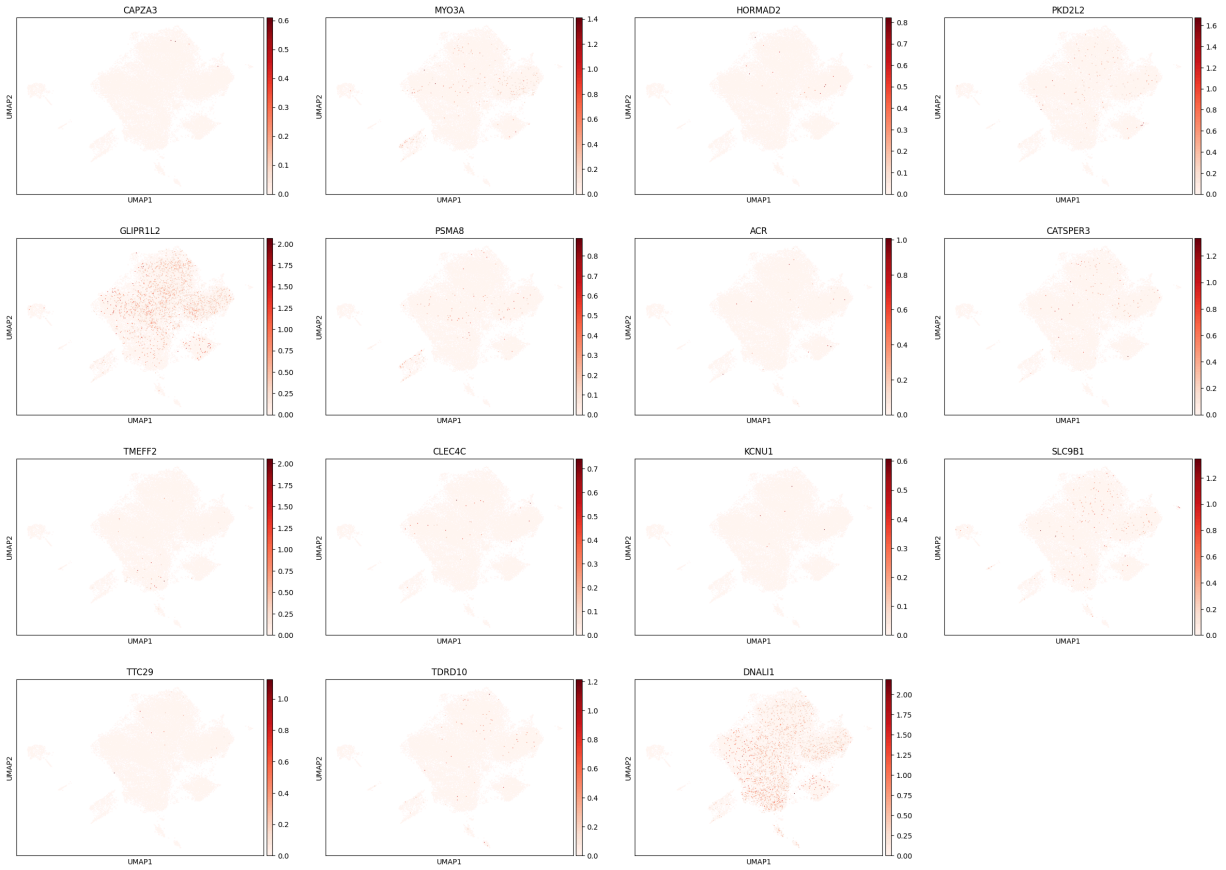

KIF15
CENPH
KIF18A
PKMYT1
TACC3
NCAPH
EME1
DEPDC1
CDC25C
PLK1
ORC6
AURKA
CCDC34
CCDC15
ASF1B
BRIP1
PBK
BUB1
UBE2S
CEP55
NUF2
CDKN3
KIF2C
CLSPN
CABLES2
CCDC150
HJURP
NEK2
CBX2
OIP5
CCNB2
RAD51
FBXO43
MYBL2
DBF4
TOP2A
UMAP1
UMAP2



Thanks to literature, we observe that genes from cluster 2 may be proliferation genes and this fact is confirmed here. Most of the genes are expressed in the proliferation cluster.

c- Cluster 3

In [ ]:
```
# Markers
# Cluster 3 conv
genes_clust_3 = ['ACR', 'DNALI1', 'GK2', 'TDRD10', 'PKD2L2', 'CATSPER3', 'TT
    'BOLL', 'DEFB126', 'TMEFF2', 'MYO3A', 'HORMAD2', 'PRSS37', 'SLCO6A1',
    'GLIPR1L2', 'CLEC4C', 'FSHR', 'ASB17', 'OR13C3', 'SLC9B1', 'SLC9C1',
    'GLIPR1L1', 'TMCO5A', 'ADAM7', 'SEPTIN14', 'PSMA8', 'OR56A3', 'TMEM202',
    'KCNU1', 'CAPZA3']

genes_clust_3 = list(set(genes_clust_3).intersection(adata.var.index))
sc.pl.umap(adata, color = genes_clust_3 , color_map = plt.cm.Reds)
```

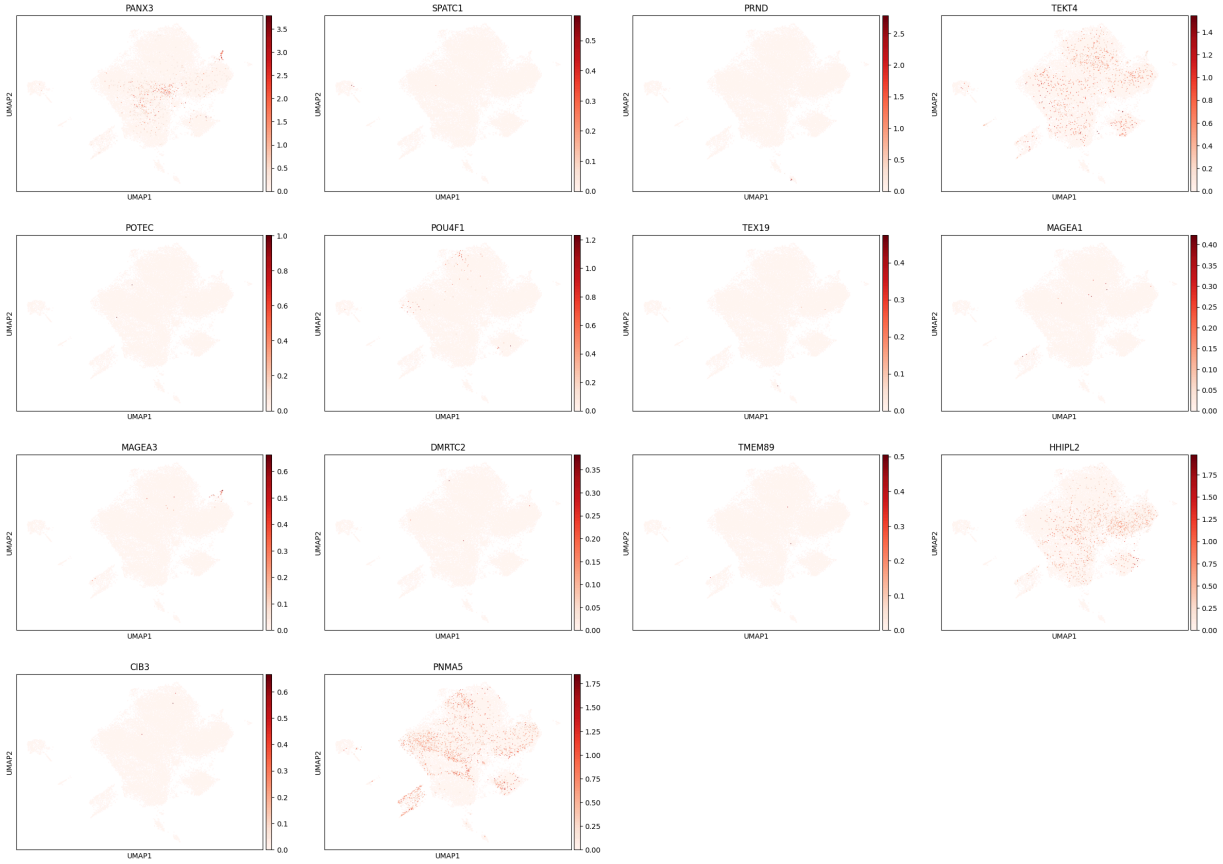


Like cluster 1, we have not a CTA very expressed, but some of it are expressed in the big cluster.

2) CTAs a bit expressed in normal tissues but expressed in chondrosarcoma

In this part, we observe CTA that are not very expressed in normal tissues and expressed in chondrosarcoma thanks to the scatter plot from script 8.

In [ ]:
```
# Markers of specific chondrosarcoma genes
genes_scatter = ['ZNF705D', 'MAGEB6', 'MAGEB16', 'CNGA2', 'POU4F1',
                 'DMRTC2', 'VCX2', 'CSH2', 'TMEM89', 'PRND', 'PNMA5',
                 'CT45A1', 'PANX3', 'HHIPL2', 'SPATC1', 'TEKT4', 'MAGEA1',
                 'POTEC', 'CT47B1', 'MTNR1B', 'FTHL17', 'C2orf78', 'CIB3',
                 'CCDC166', 'TEX19', 'DMBX1', 'C20orf141', 'PRDM14', 'BIRC8',
                 'MAGEA3', 'BSX']

genes_scatter = list(set(genes_scatter).intersection(adata.var.index))

sc.pl.umap(adata, color = genes_scatter, legend_loc = 'on data', color_map =
```

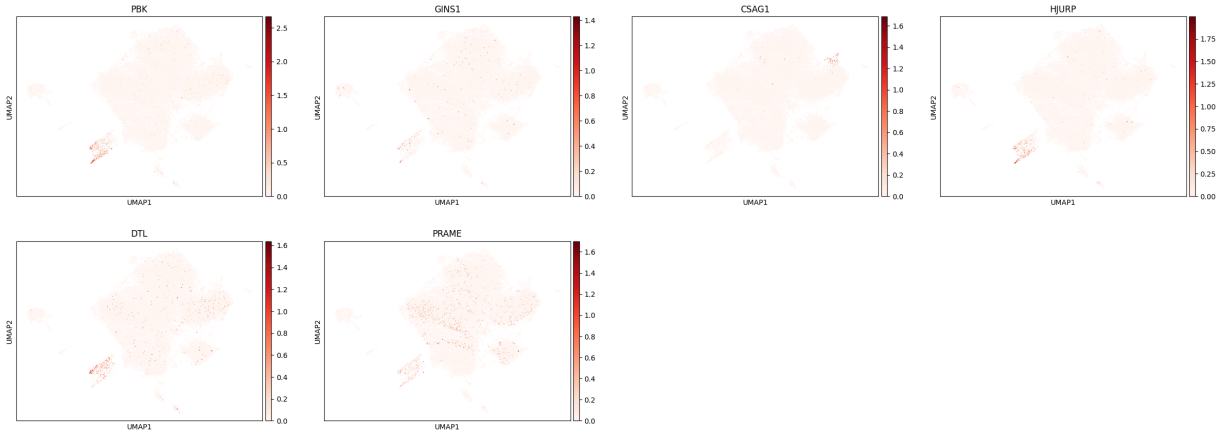


PNMA5, TEKT4, HHPL2 and PANX3 seems to be the most interested CTAs in this dataset.

3) CTAs of interest

We interested us in PRAME because it is a known CTA.

In [ ]:
```
genes = ['DTL', 'HJURP', 'GINS1', 'PBK', 'CTAG2', 'CT45A1', 'CSAG1', 'PRAME'

genes = list(set(genes).intersection(adata.var.index))

sc.pl.umap(adata, color = genes, legend_loc = 'on data', color_map = mpl.cm.
```

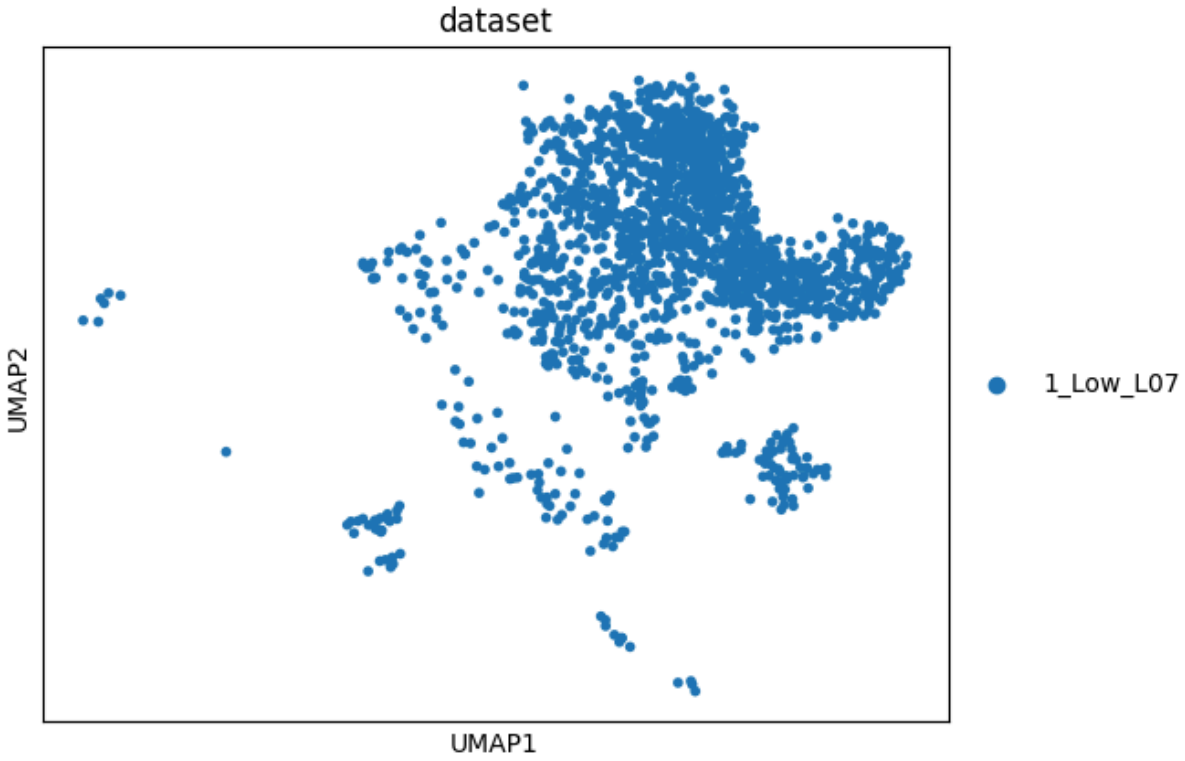


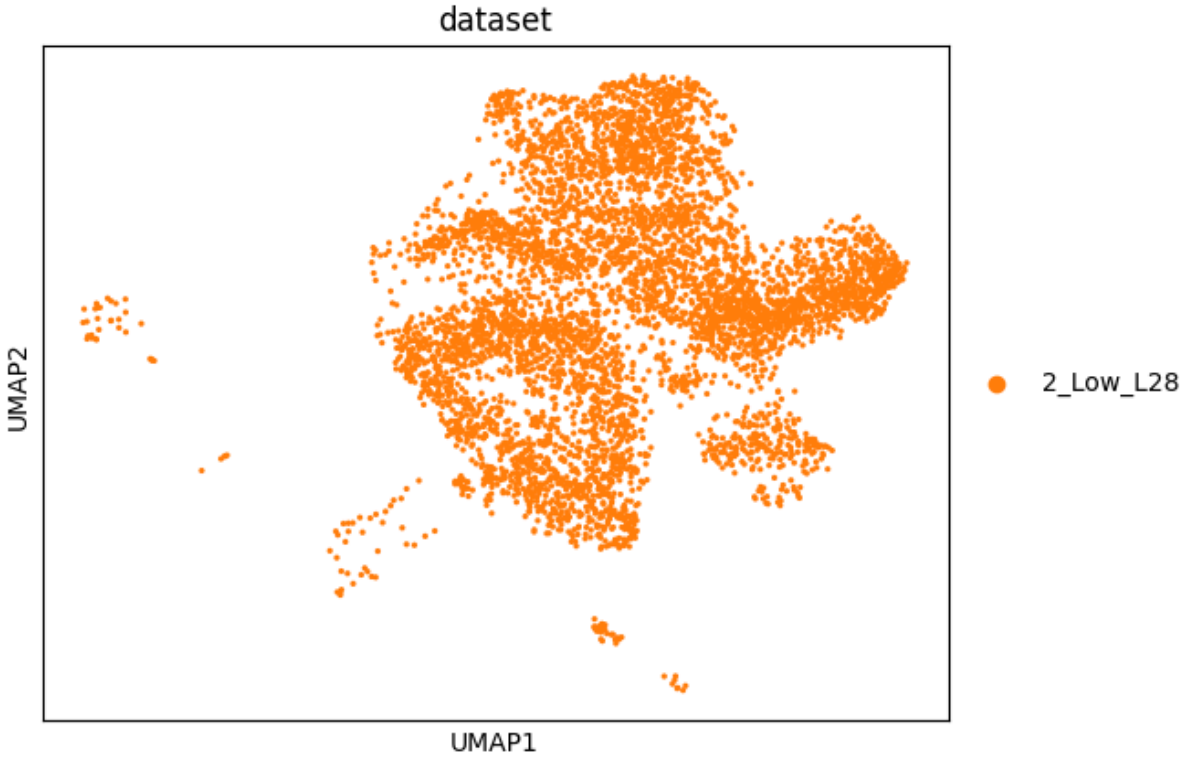
III. Expression of genes per patients

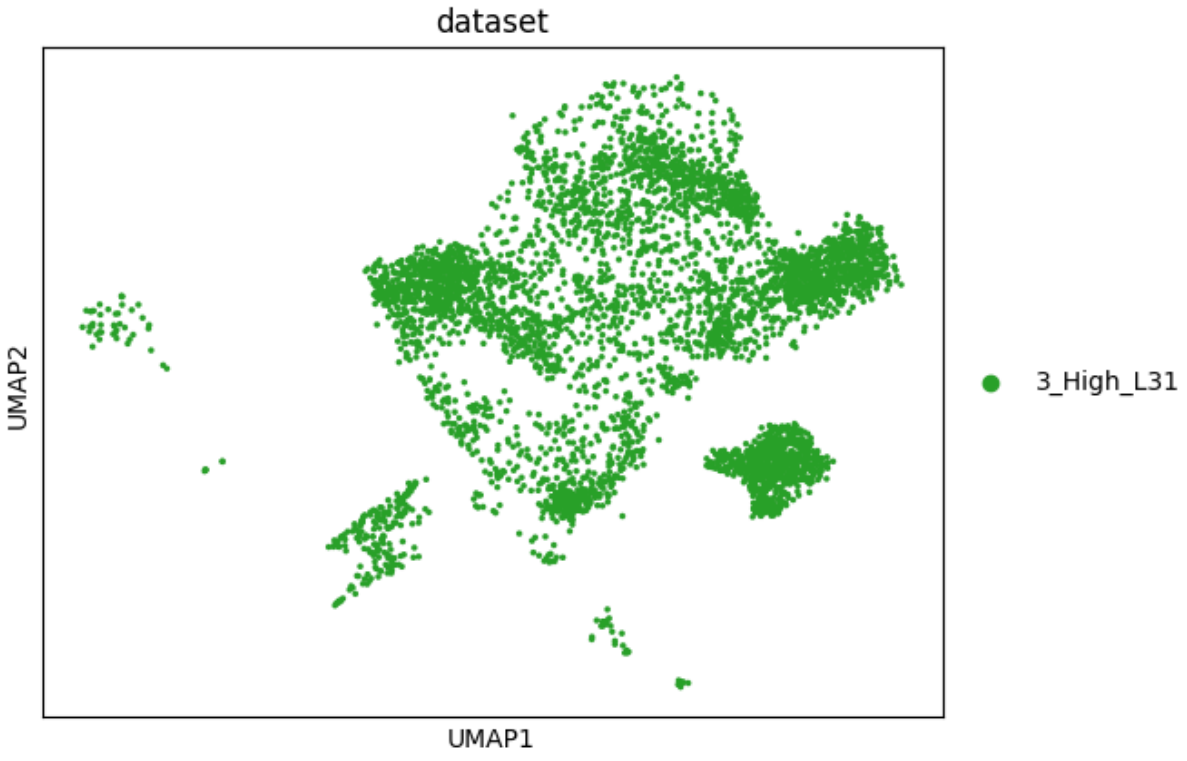
This section is about the expression of genes per patients to see if CTAs are more expressed in high grade or not and also the expression of MHC.

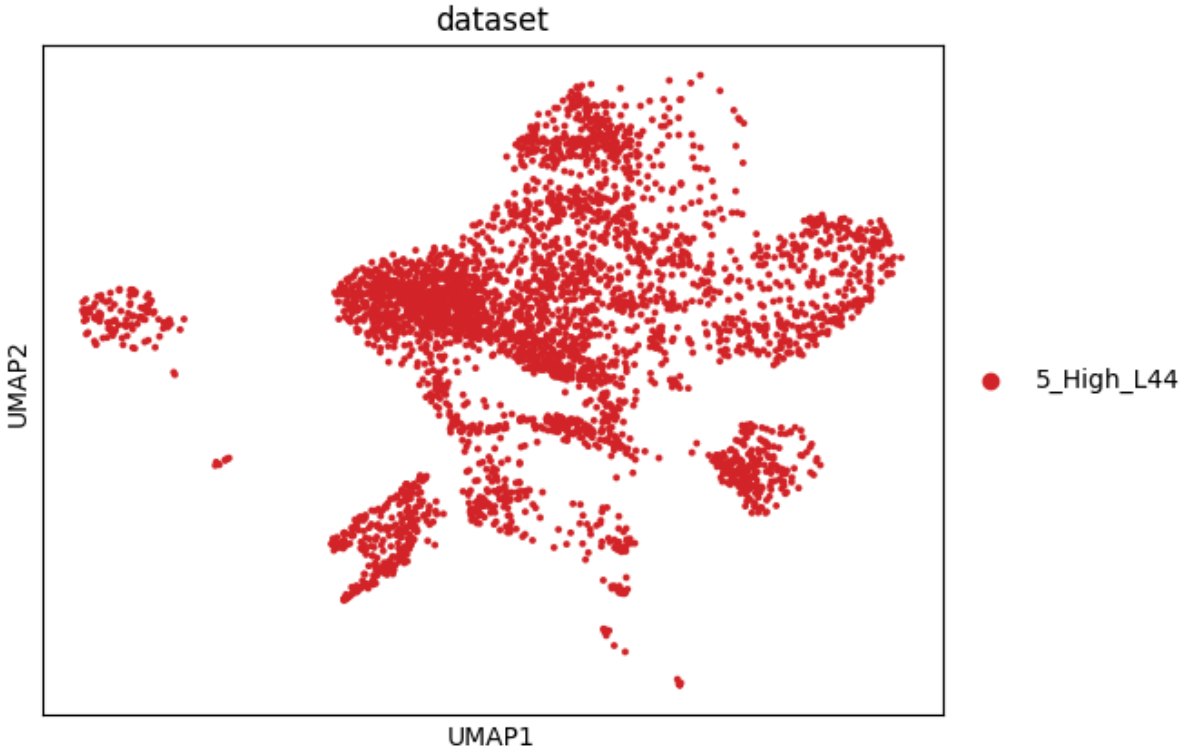
1) Colored by patients

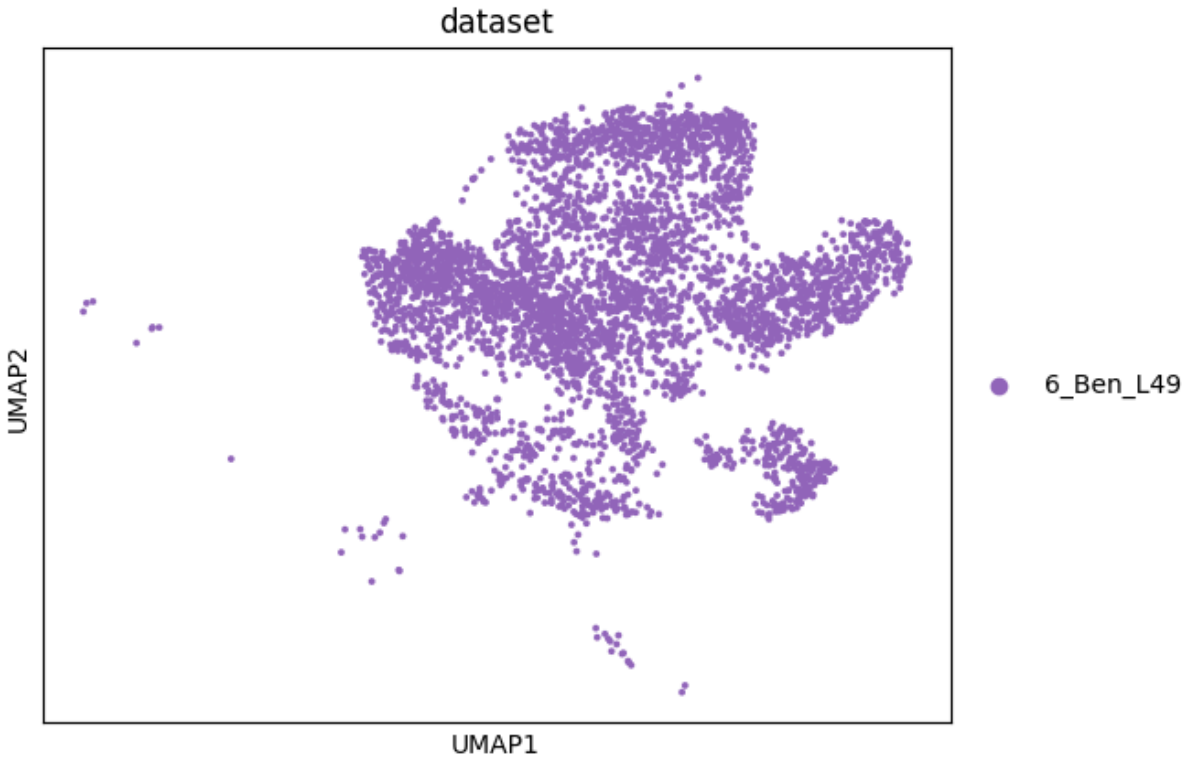
In [ ]:
```
l = ['1_Low_L07', '2_Low_L28', '3_High_L31', '5_High_L44', '6_Ben_L49', '7_M

for dataset in l:
    adata_filtered = adata[adata.obs['dataset'] == dataset, :]
    sc.pl.umap(adata_filtered, color = 'dataset', color_map = plt.cm.Reds)
```

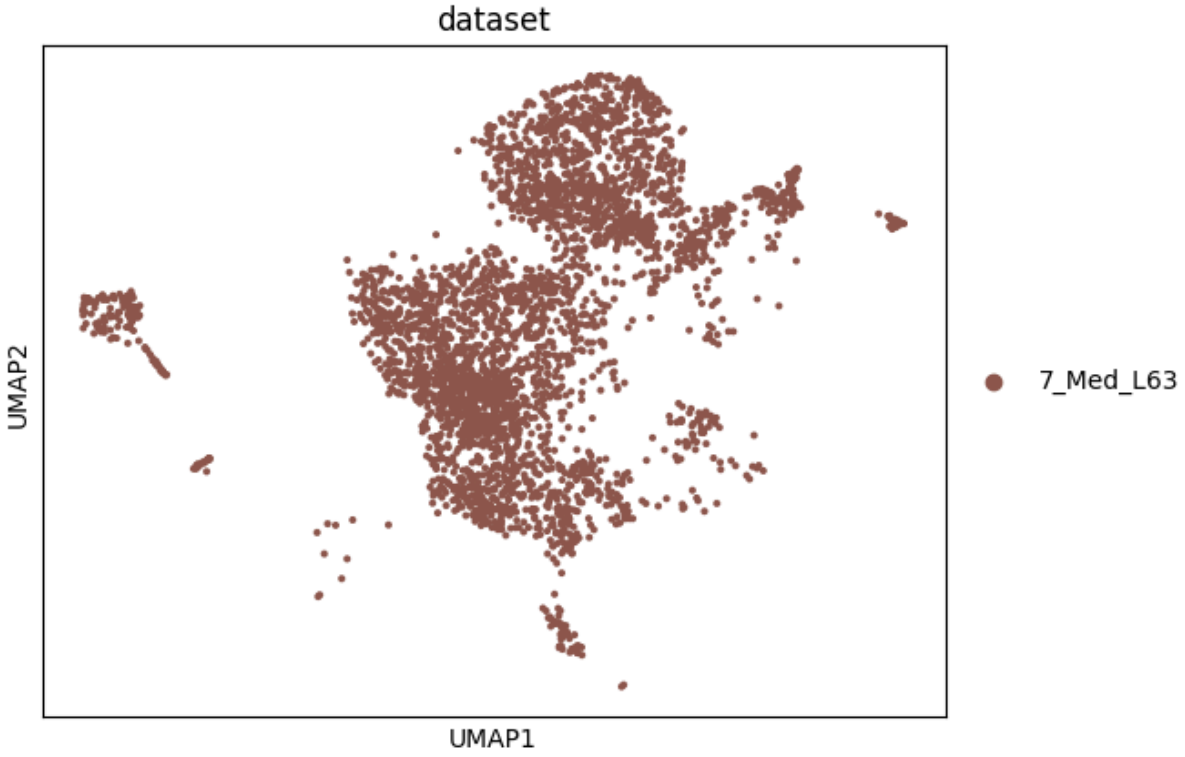


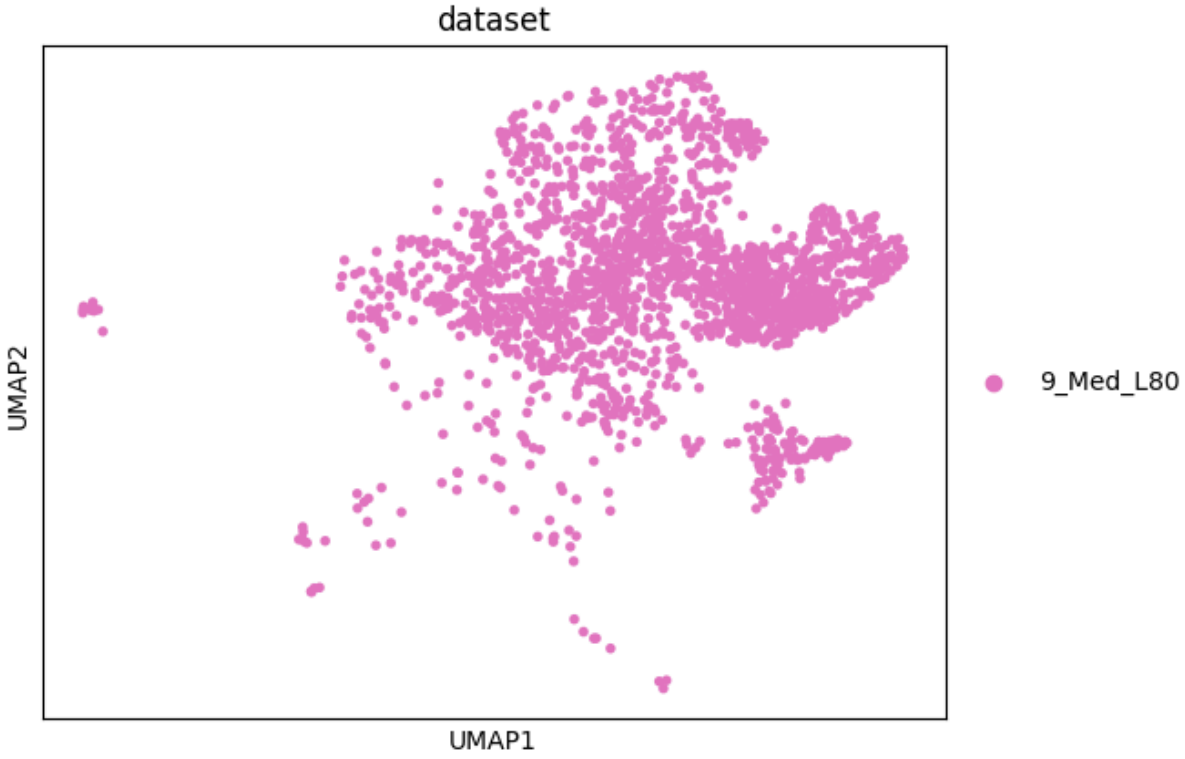

dataset
UMAP2
UMAP1
2_Low_L28

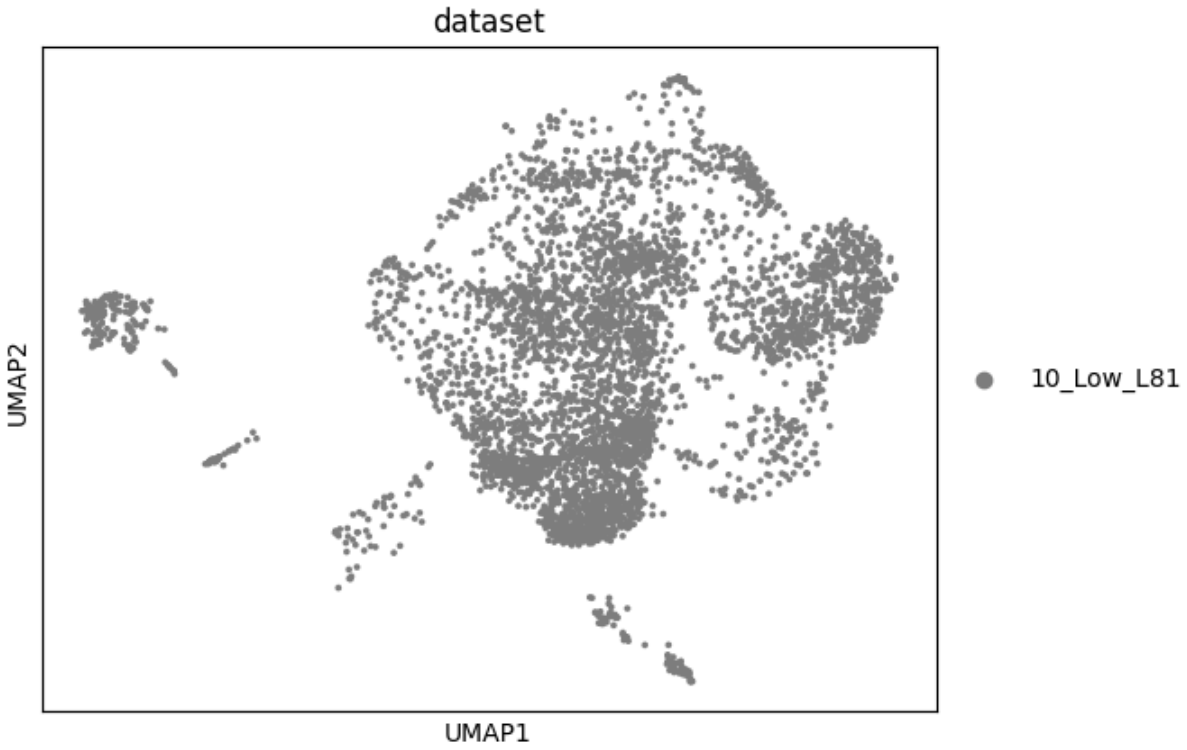

dataset
UMAP2
UMAP1
3_High_L31


dataset
UMAP2
UMAP1
5_High_L44


dataset
UMAP2
UMAP1
6_Ben_L49

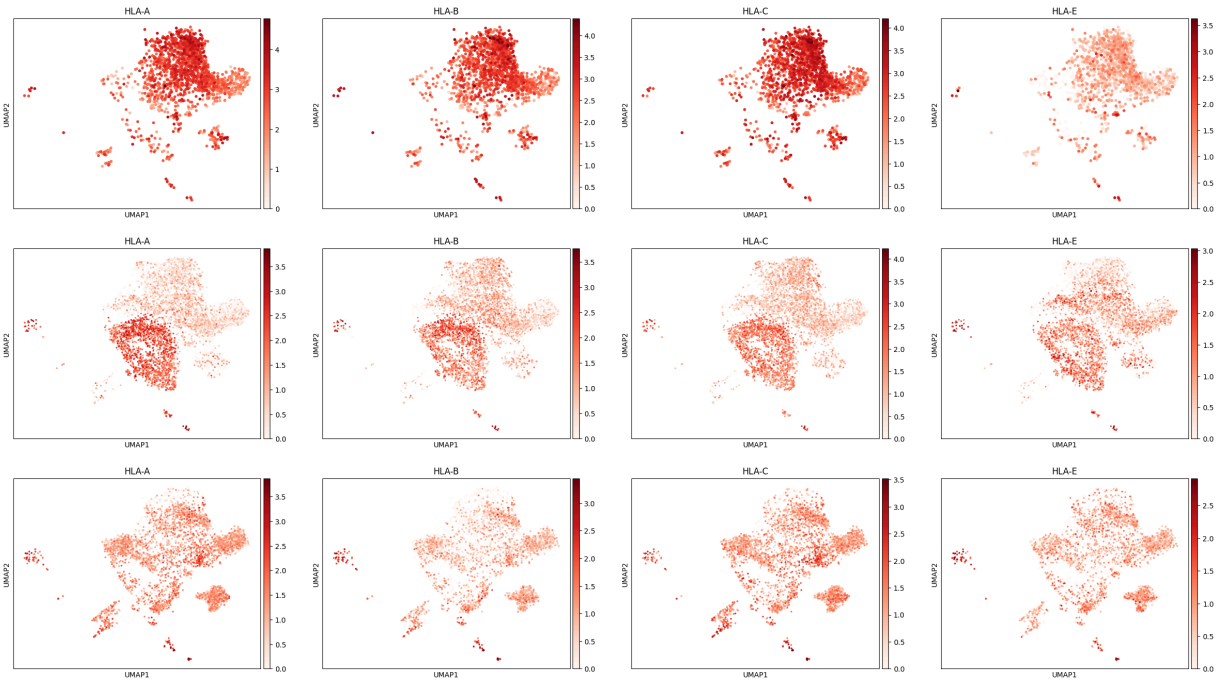

dataset
UMAP2
UMAP1
7_Med_L63

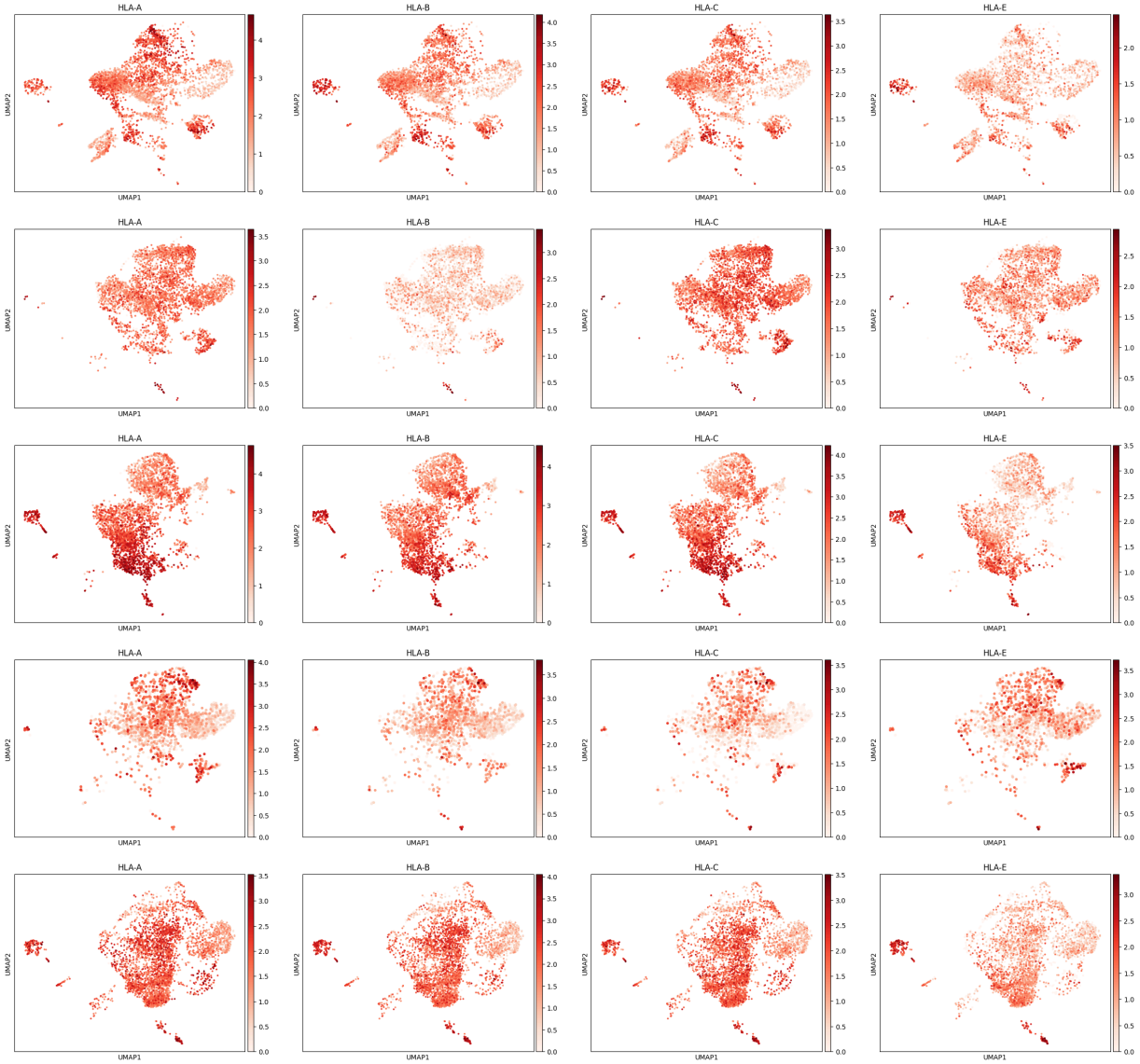

dataset
UMAP2
UMAP1
9_Med_L80



2) MHC 1 genes by samples

In [ ]:
```
for dataset in l:
    adata_filtered = adata[adata.obs['dataset'] == dataset, :]
    sc.pl.umap(adata_filtered, color =  ['HLA-A', 'HLA-B', 'HLA-C', 'HLA-E']
```



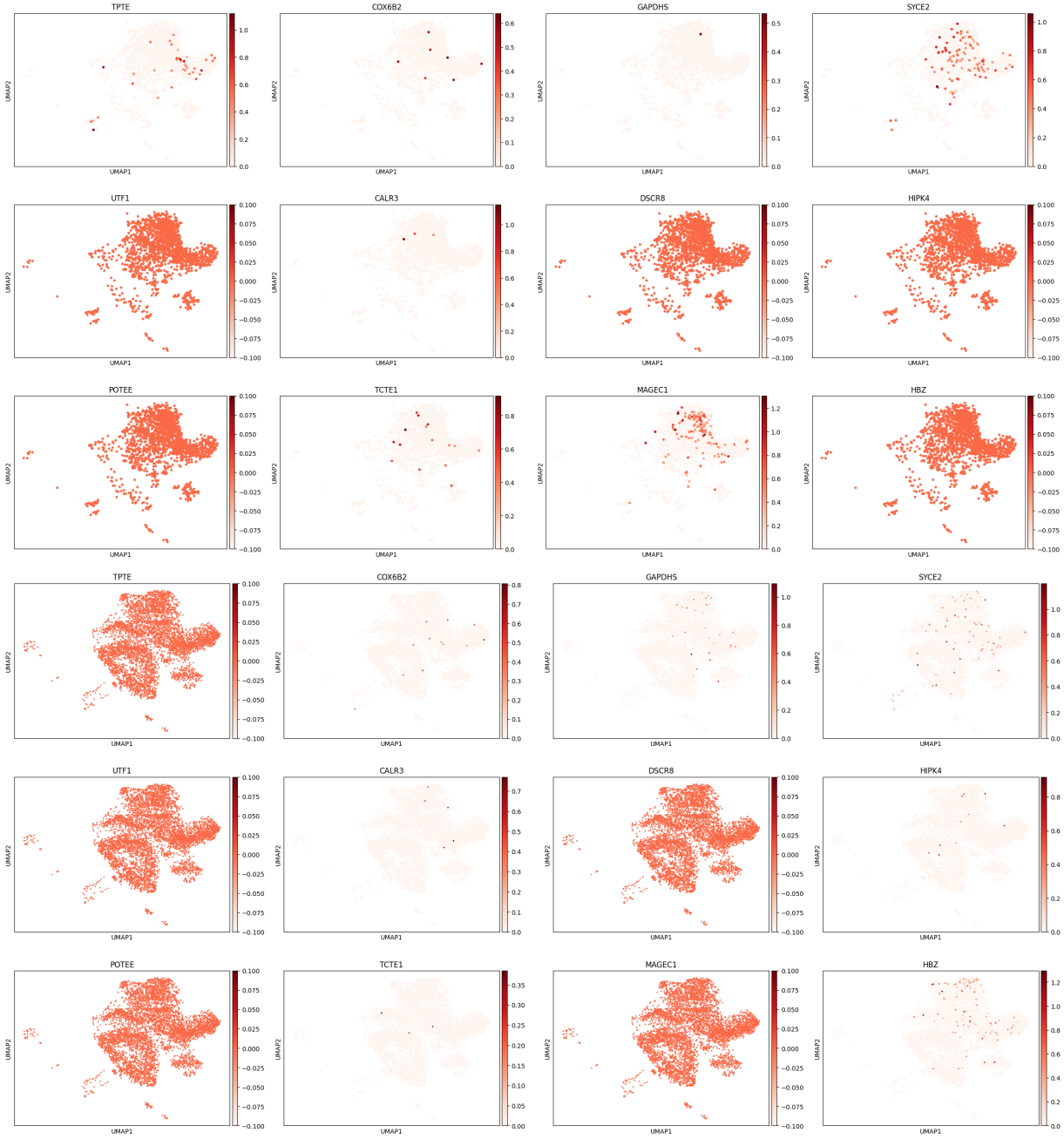


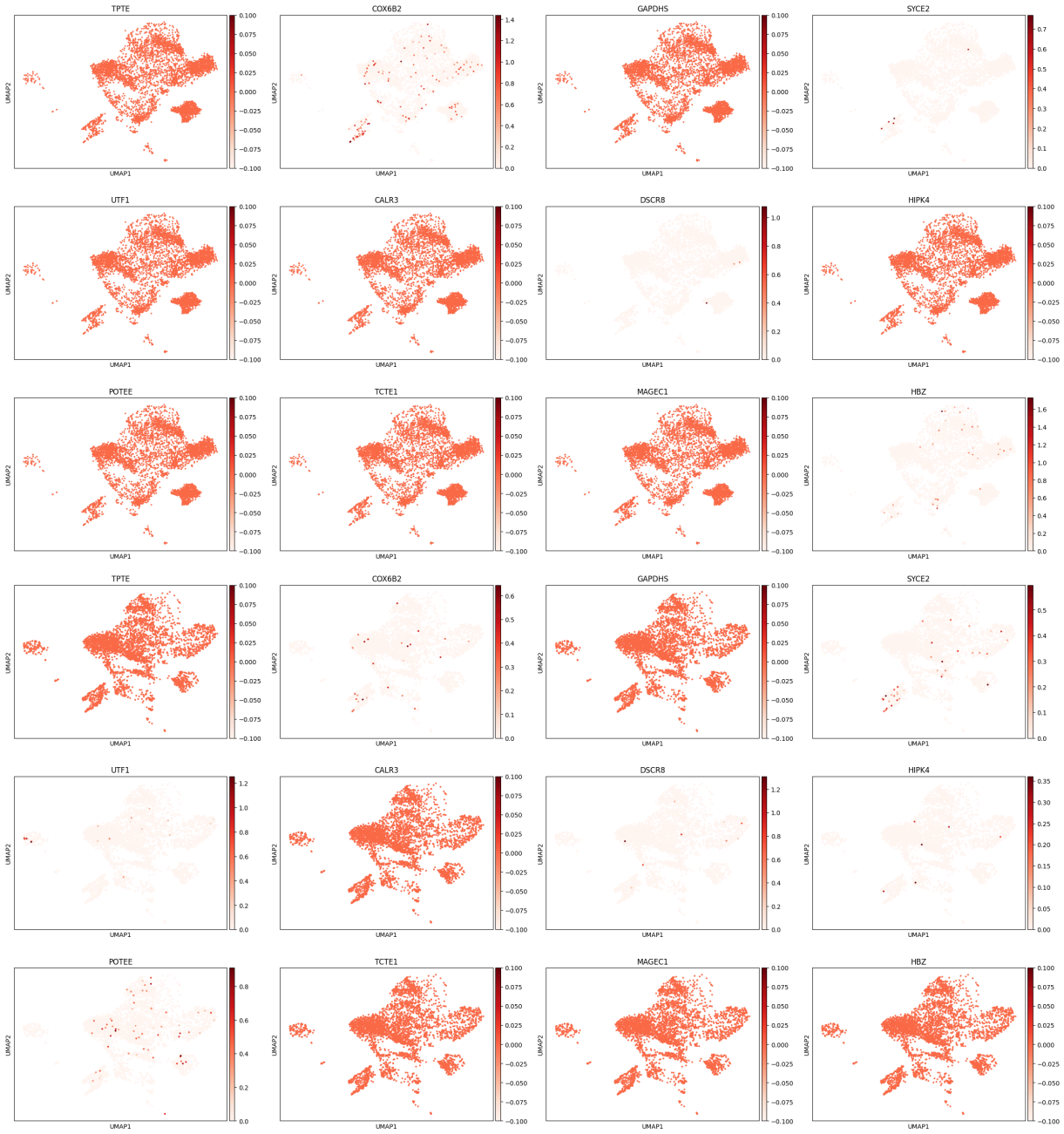
We see that MHC 1 is expressed everywhere more or less for some samples. We can deduce that tumoral cells expressed MHC 1 in chondrosarcoma, so a peptide is presented and could be targeted.

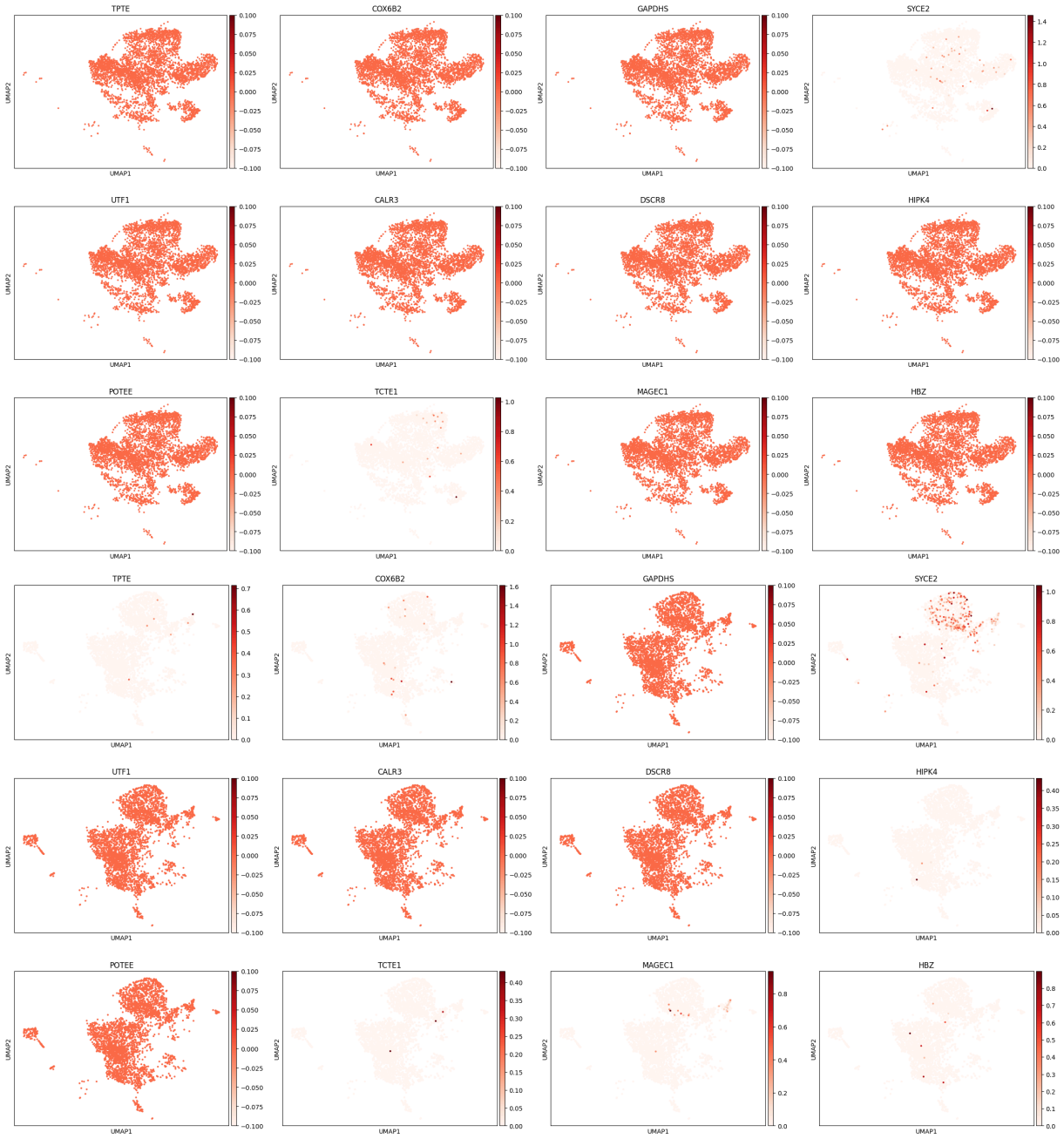
3) CTAs that impact survival

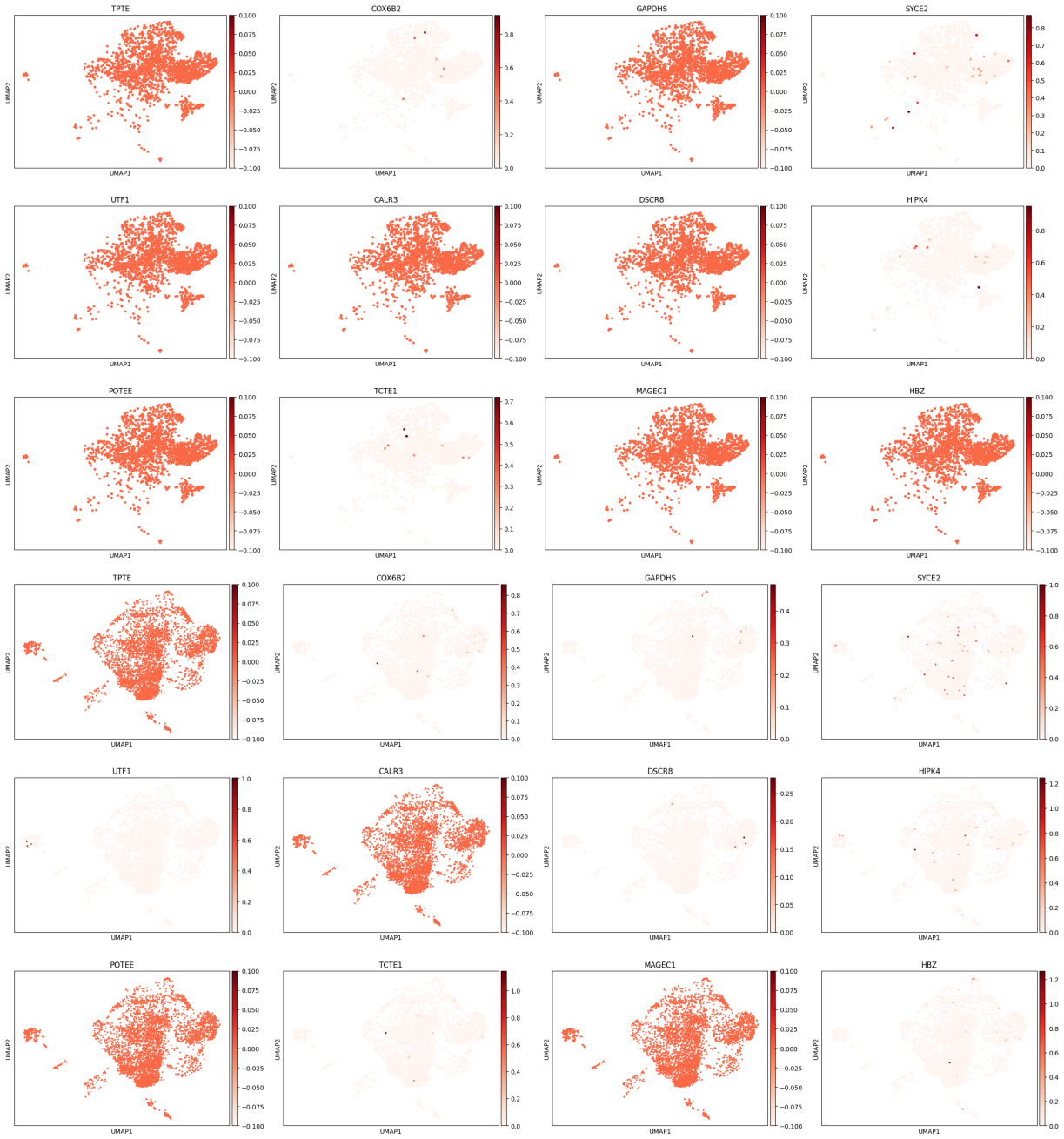
a- Cluster 1

In [ ]:
```
# Markers
# Cluster 1 conv
for dataset in l:
    adata_filtered = adata[adata.obs['dataset'] == dataset, :]
    sc.pl.umap(adata_filtered, color = genes_clust_1 , color_map = plt.cm.Re
```


TPTE
COX6B2
GAPDHS
SYCE2
UTF1
CALR3
DSCR8
HIPK4
POTEE
TCTE1
MAGEC1
HBZ
TPTE
COX6B2
GAPDHS
SYCE2
UTF1
CALR3
DSCR8
HIPK4
POTEE
TCTE1
MAGEC1
HBZ
UMAP1
UMAP2


TPTE
COX6B2
GAPDHS
SYCE2
UTF1
CALR3
DSCR8
HIPK4
POTEE
TCTE1
MAGEC1
HBZ
TPTE
COX6B2
GAPDHS
SYCE2
UTF1
CALR3
DSCR8
HIPK4
POTEE
TCTE1
MAGEC1
HBZ
UMAP1
UMAP2

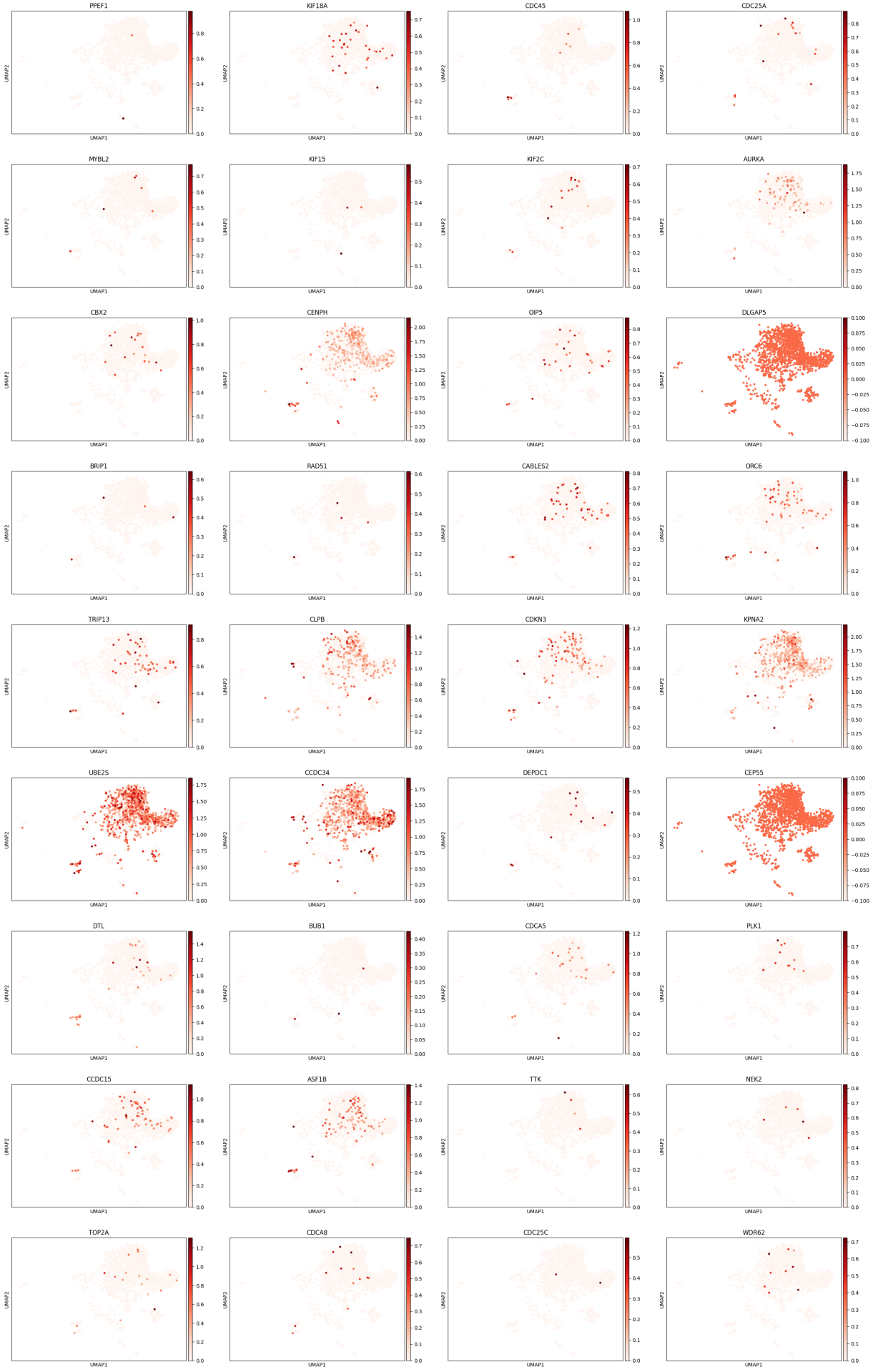

TPTE
COX6B2
GAPDHS
SYCE2
UTF1
CALR3
DSCR8
HIPK4
POTEE
TCTE1
MAGEC1
HBZ
TPTE
COX6B2
GAPDHS
SYCE2
UTF1
CALR3
DSCR8
HIPK4
POTEE
TCTE1
MAGEC1
HBZ
UMAP1
UMAP2

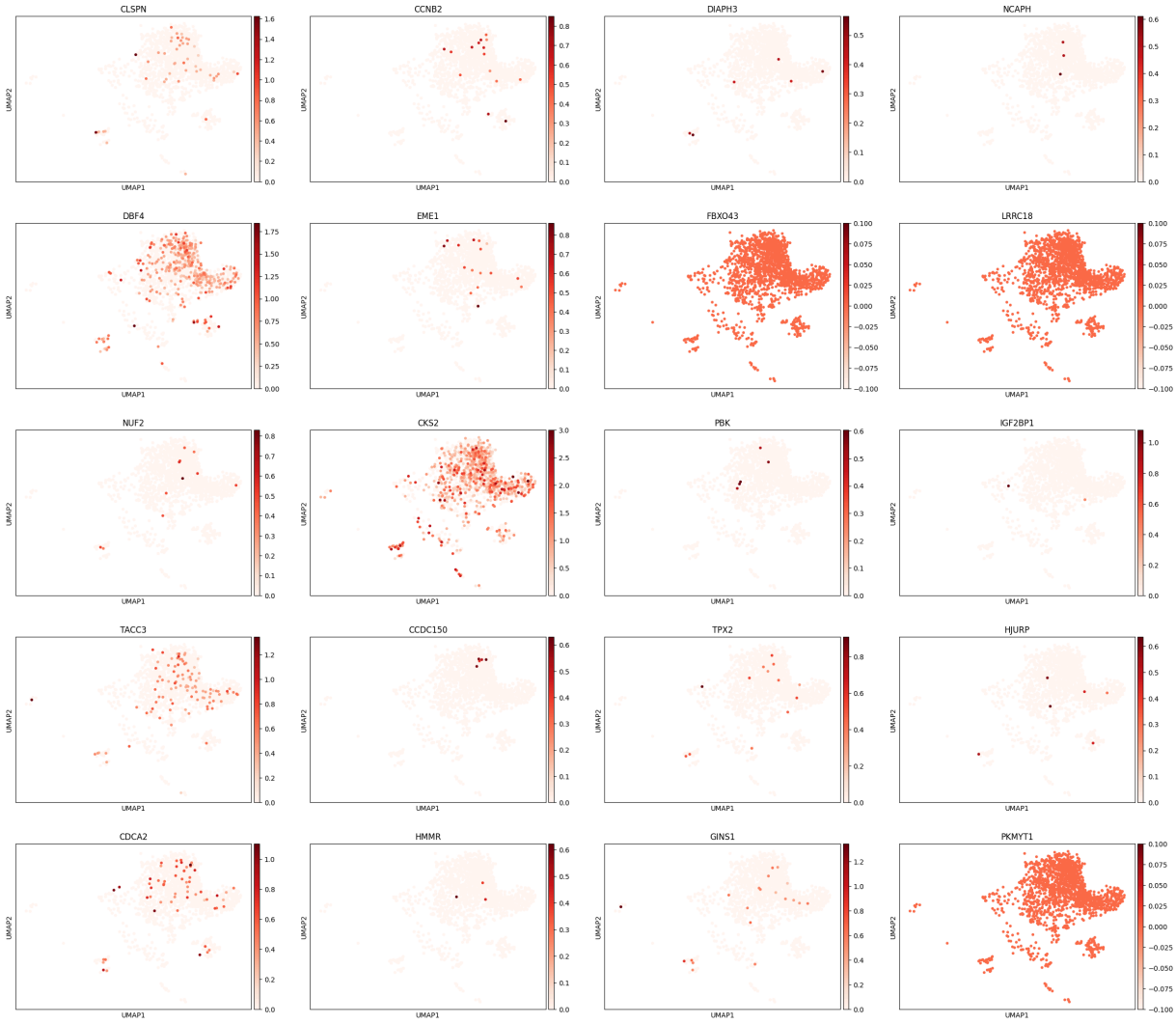


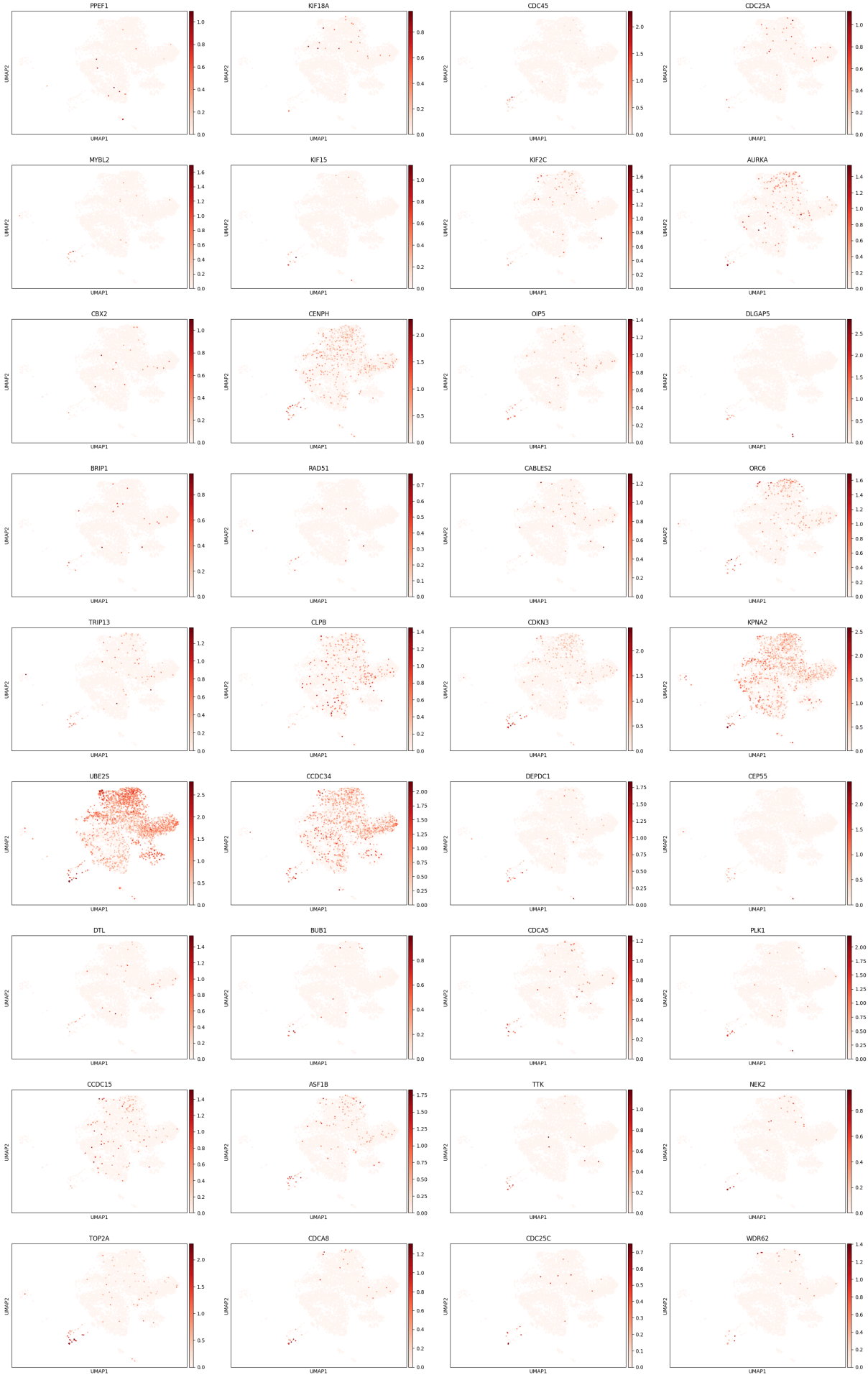
We can better see the expression of CTA per samples.

b- Cluster 2

In [ ]:
```
# Markers cluster 2
for dataset in l:
    adata_filtered = adata[adata.obs['dataset'] == dataset, :]
    sc.pl.umap(adata_filtered, color = genes_clust_2 , color_map = plt.cm.Re
```


PPEF1
KIF18A
CDC45
CDC25A
MYBL2
KIF15
KIF2C
AURKA
CBX2
CENPH
OIP5
DLGAP5
BRIP1
RAD51
CABLES2
ORC6
TRIP13
CLPB
CDKN3
KPNA2
UBE2S
CCDC34
DEPDC1
CEP55
DTL
BUB1
CDCA5
PLK1
CCDC15
ASF1B
TTK
NEK2
TOP2A
CDCA8
CDC25C
WDR62
UMAP1
UMAP2


CLSPN
CCNB2
DIAPH3
NCAPH
DBF4
EME1
FBXO43
LRRC18
NUF2
CKS2
PBK
IGF2BP1
TACC3
CCDC150
TPX2
HJURP
CDCA2
HMMR
GINS1
PKMYT1
UMAP1
UMAP2


PPEF1
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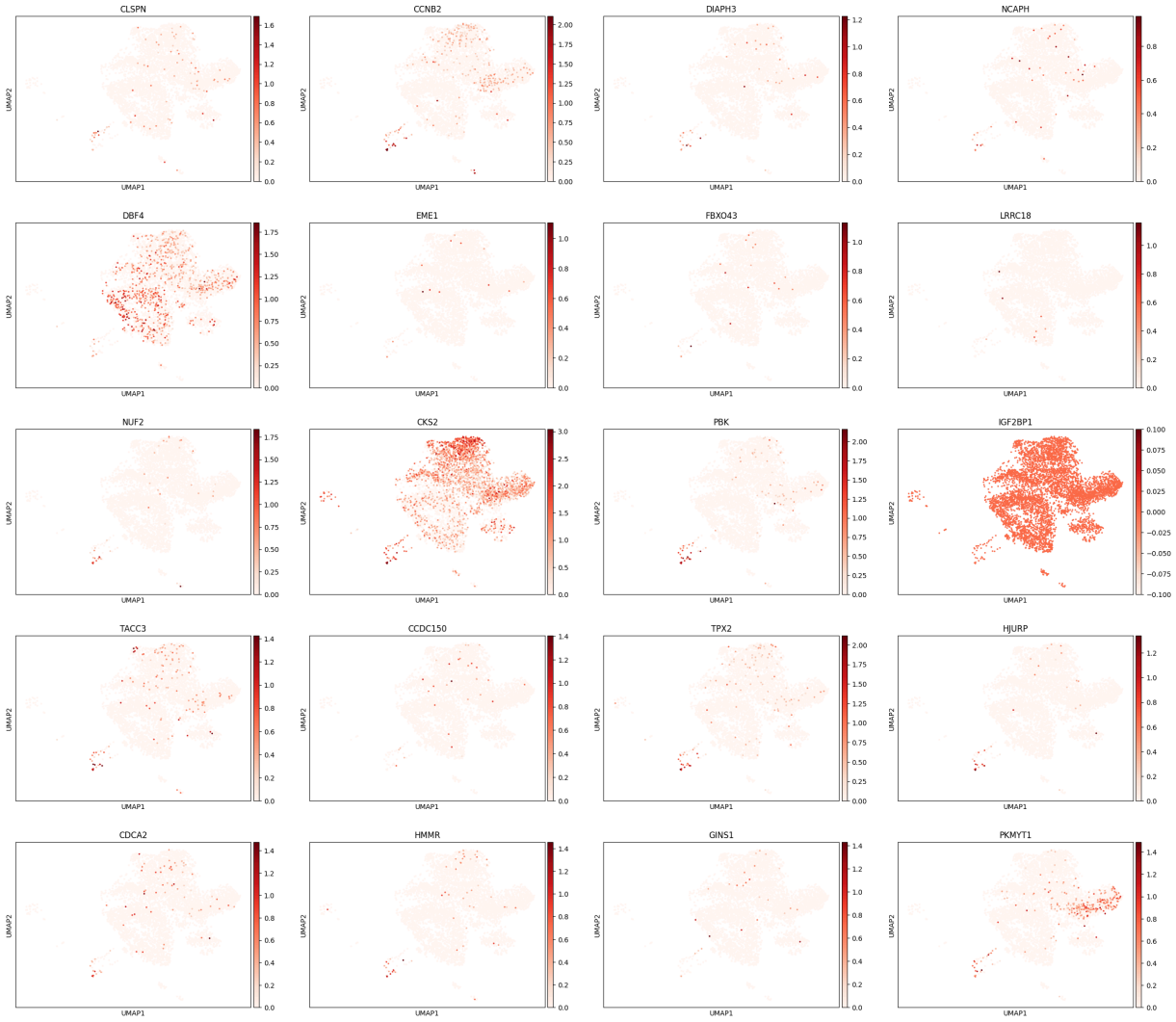
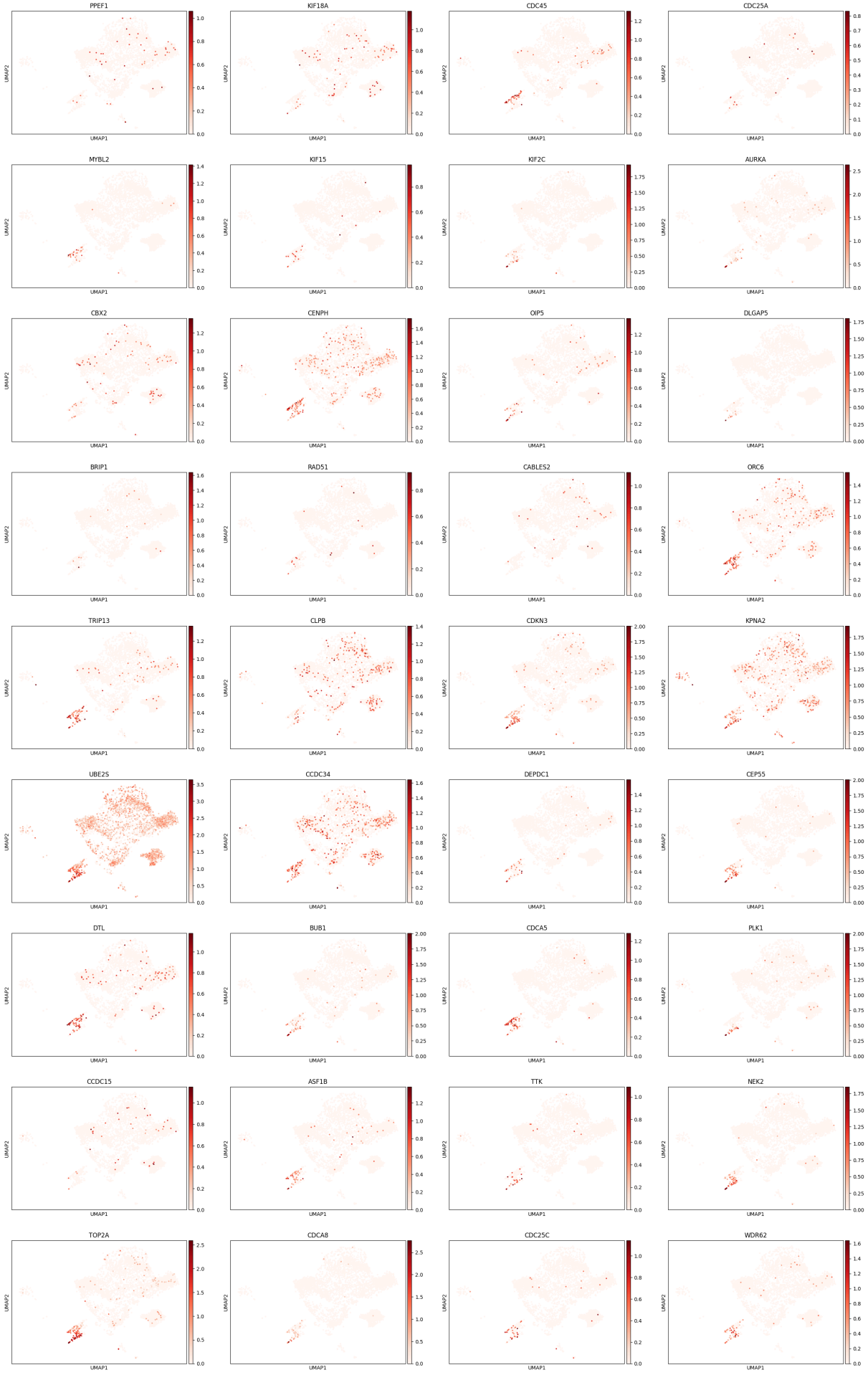
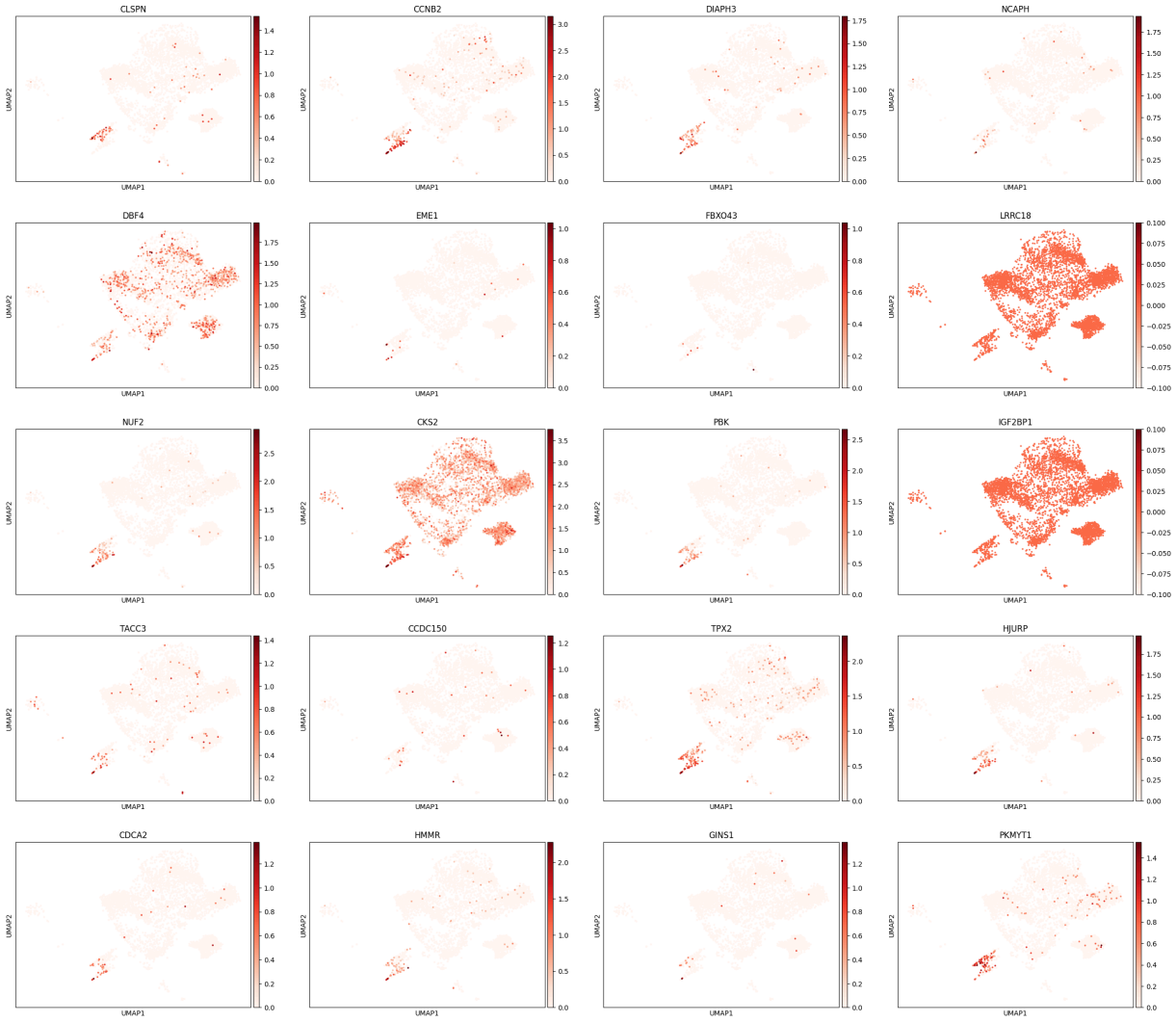
CDC45
CDC25A
MYBL2
KIF15
KIF2C
AURKA
CBX2
CENPH
OIP5
DLGAP5
BRIP1
RAD51
CABLES2
ORC6
TRIP13
CLPB
CDKN3
KPNA2
UBE2S
CCDC34
DEPDC1
CEP55
DTL
BUB1
CDCA5
PLK1
CCDC15
ASF1B
TTK
NEK2
TOP2A
CDCA8
CDC25C
WDR62
UMAP1
UMAP2


CLSPN
CCNB2
DIAPH3
NCAPH
DBF4
EME1
FBXO43
LRRC18
NUF2
CKS2
PBK
IGF2BP1
TACC3
CCDC150
TPX2
HJURP
CDCA2
HMMR
GINS1
PKMYT1
UMAP1
UMAP2


PPEF1
KIF18A
CDC45
CDC25A
MYBL2
KIF15
KIF2C
AURKA
CBX2
CENPH
OIP5
DLGAP5
BRIP1
RAD51
CABLES2
ORC6
TRIP13
CLPB
CDKN3
KPNA2
UBE2S
CCDC34
DEPDC1
CEP55
DTL
BUB1
CDCA5
PLK1
CCDC15
ASF1B
TTK
NEK2
TOP2A
CDCA8
CDC25C
WDR62
UMAP1
UMAP2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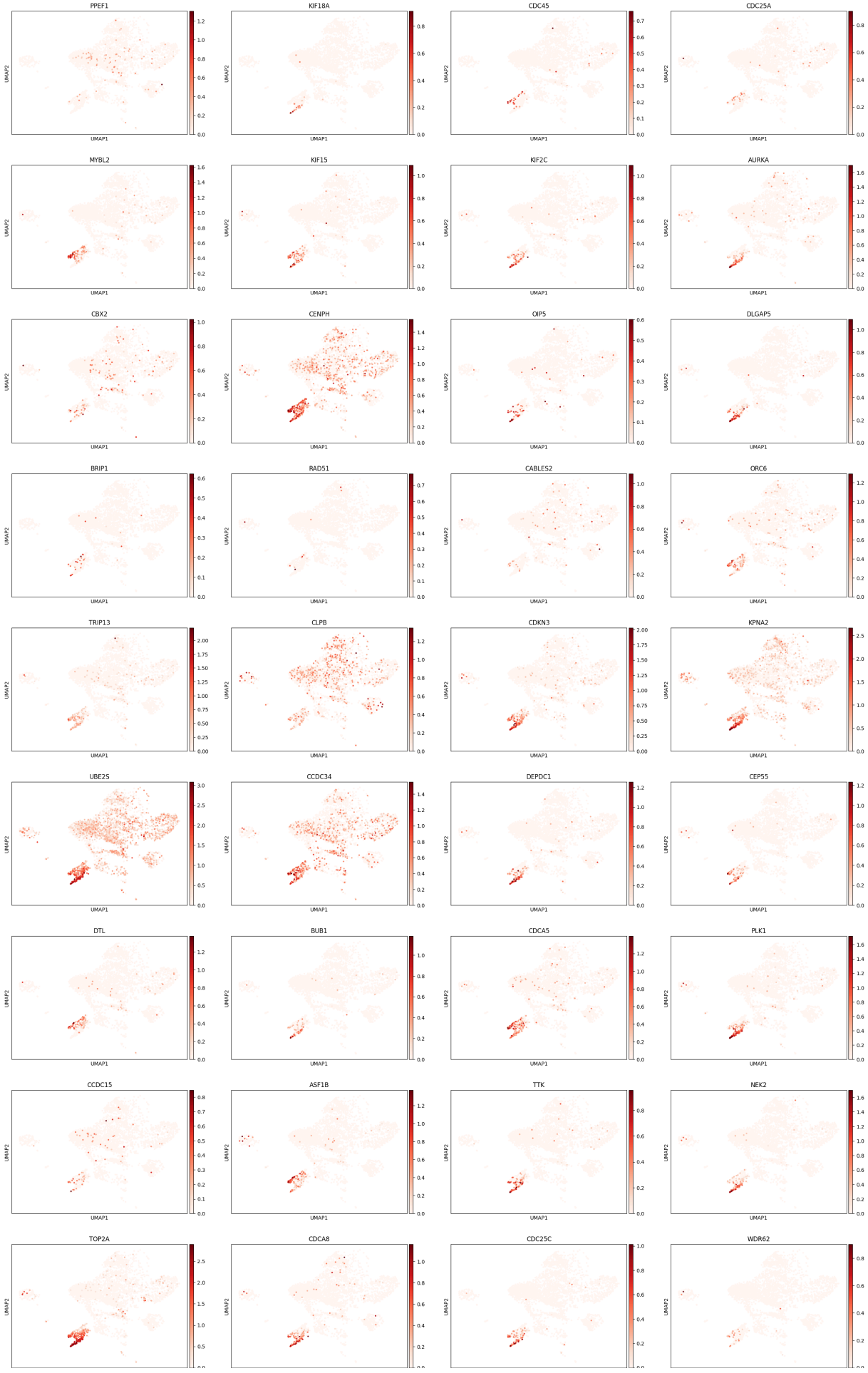
DBF4
EME1
FBXO43
LRRC18
NUF2
CKS2
PBK
IGF2BP1
TACC3
CCDC150
TPX2
HJURP
CDCA2
HMMR
GINS1
PKMYT1
UMAP1
UMAP2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CDC25A
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CBX2
CENPH
OIP5
DLGAP5
BRIP1
RAD51
CABLES2
ORC6
TRIP13
CLPB
CDKN3
KPNA2
UBE2S
CCDC34
DEPDC1
CEP55
DTL
BUB1
CDCA5
PLK1
CCDC15
ASF1B
TTK
NEK2
TOP2A
CDCA8
CDC25C
WDR62
UMAP1
UMAP2


CLSPN
CCNB2
DIAPH3
NCAPH
DBF4
EME1
FBXO43
LRRC18
NUF2
CKS2
PBK
IGF2BP1
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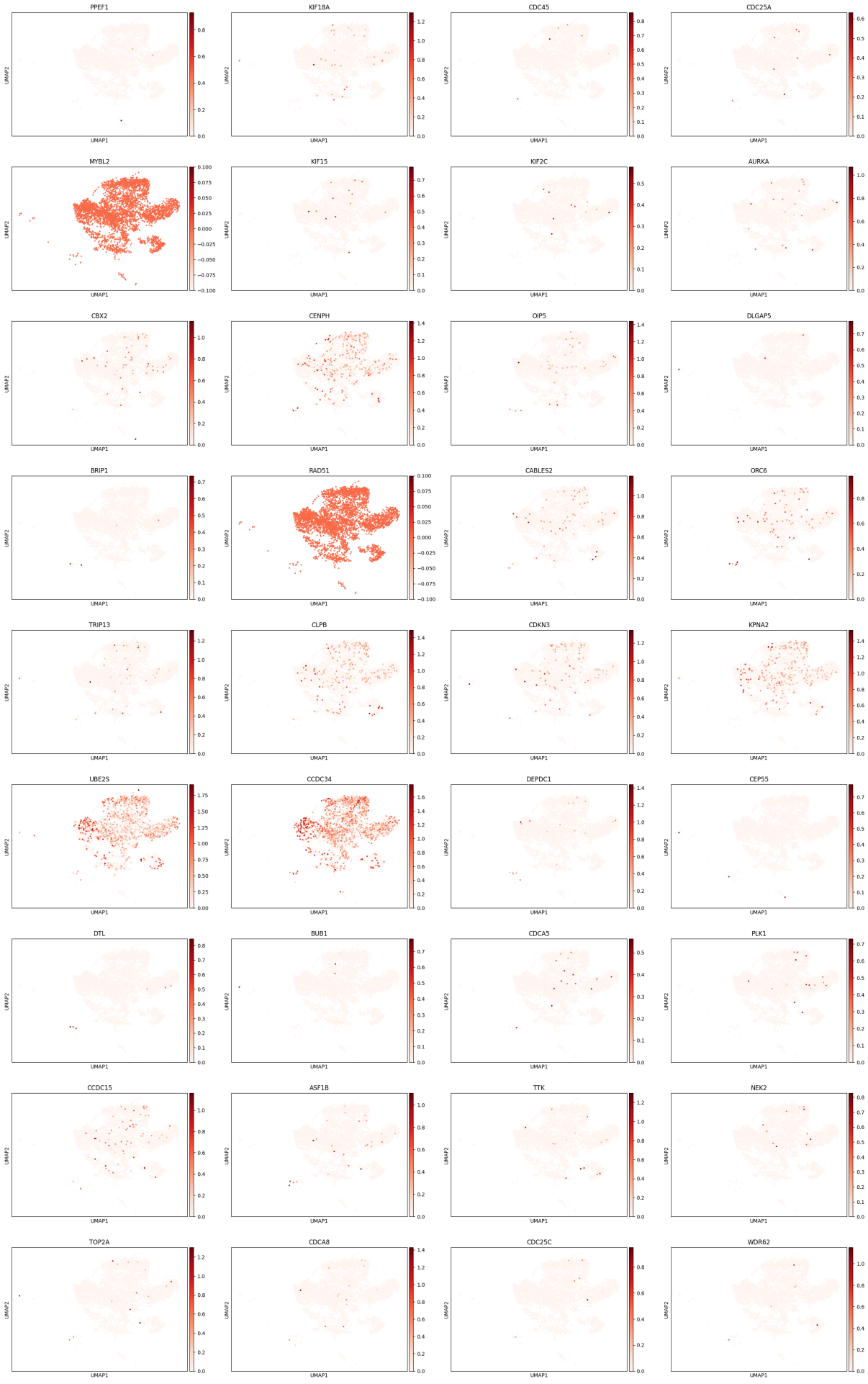
HMMR
GINS1
PKMYT1
UMAP1
UMAP2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CDC25A
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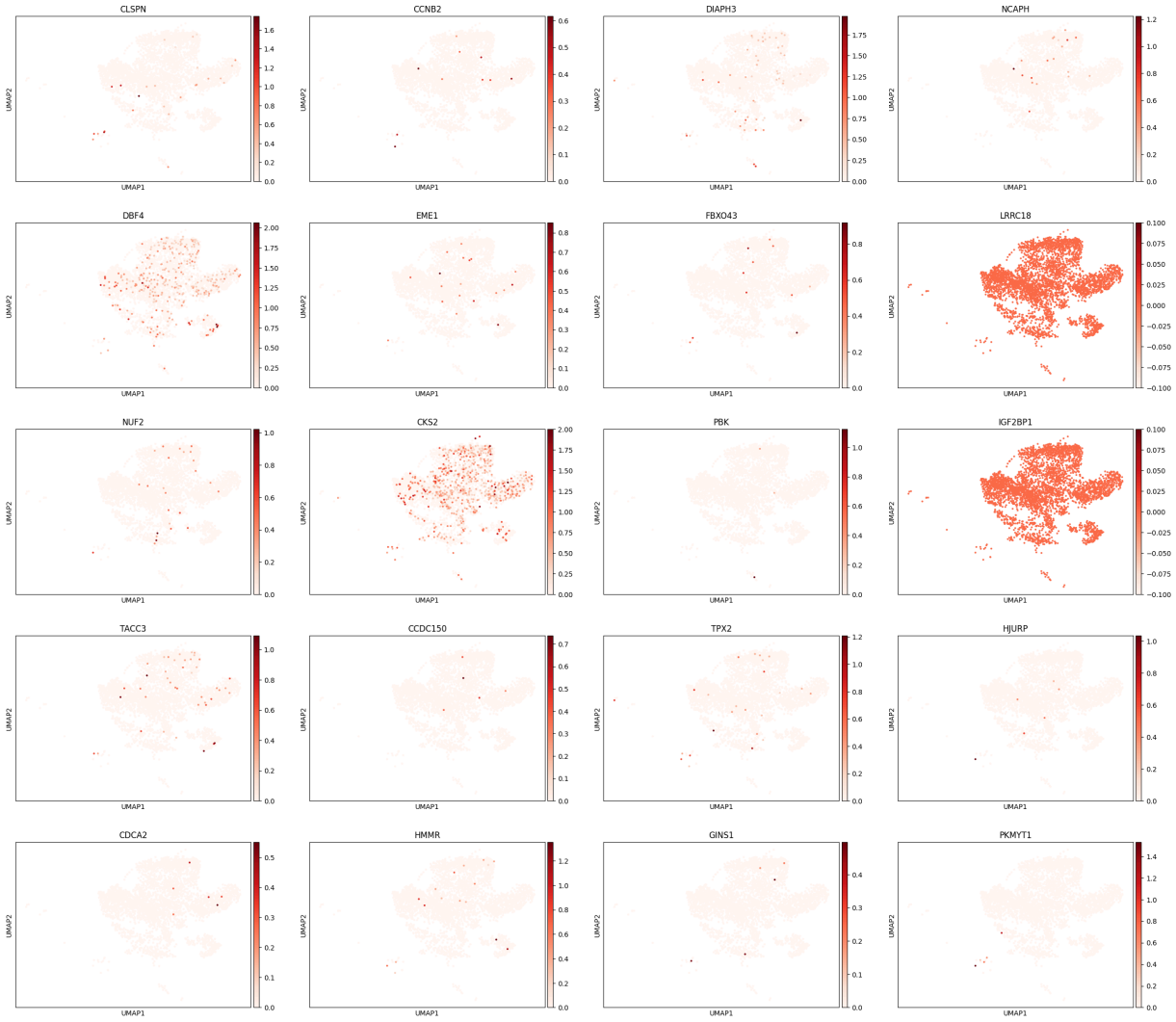
KIF15
KIF2C
AURKA
CBX2
CENPH
OIP5
DLGAP5
BRIP1
RAD51
CABLES2
ORC6
TRIP13
CLPB
CDKN3
KPNA2
UBE2S
CCDC34
DEPDC1
CEP55
DTL
BUB1
CDCA5
PLK1
CCDC15
ASF1B
TTK
NEK2
TOP2A
CDCA8
CDC25C
WDR62
UMAP1
UMAP2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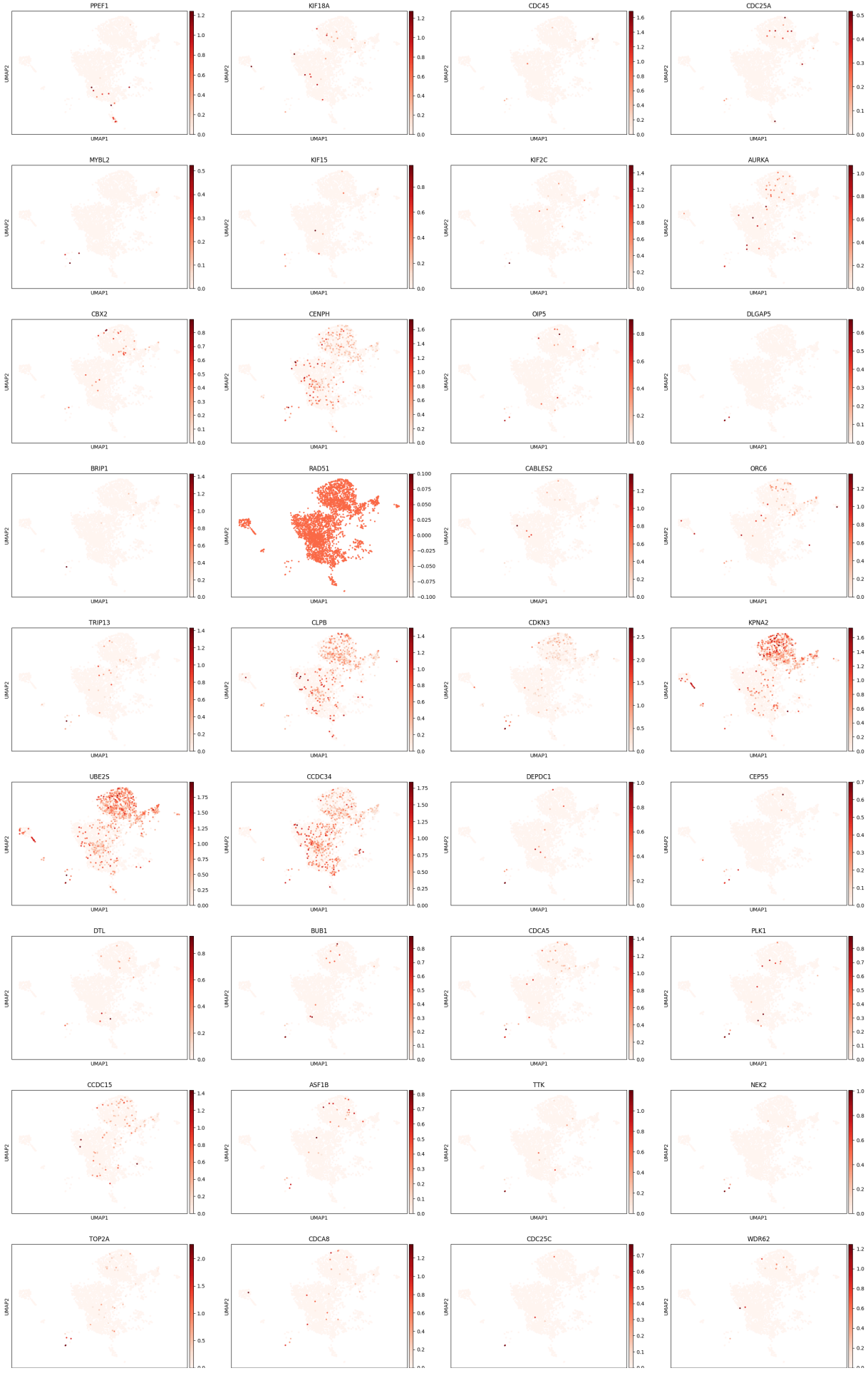
NCAPH
DBF4
EME1
FBXO43
LRRC18
NUF2
CKS2
PBK
IGF2BP1
TACC3
CCDC150
TPX2
HJURP
CDCA2
HMMR
GINS1
PKMYT1
UMAP1
UMAP2


PPEF1
KIF18A
CDC45
CDC25A
MYBL2
KIF15
KIF2C
AURKA
CBX2
CENPH
OIP5
DLGAP5
BRIP1
RAD51
CABLES2
ORC6
TRIP13
CLPB
CDKN3
KPNA2
UBE2S
CCDC34
DEPDC1
CEP55
DTL
BUB1
CDCA5
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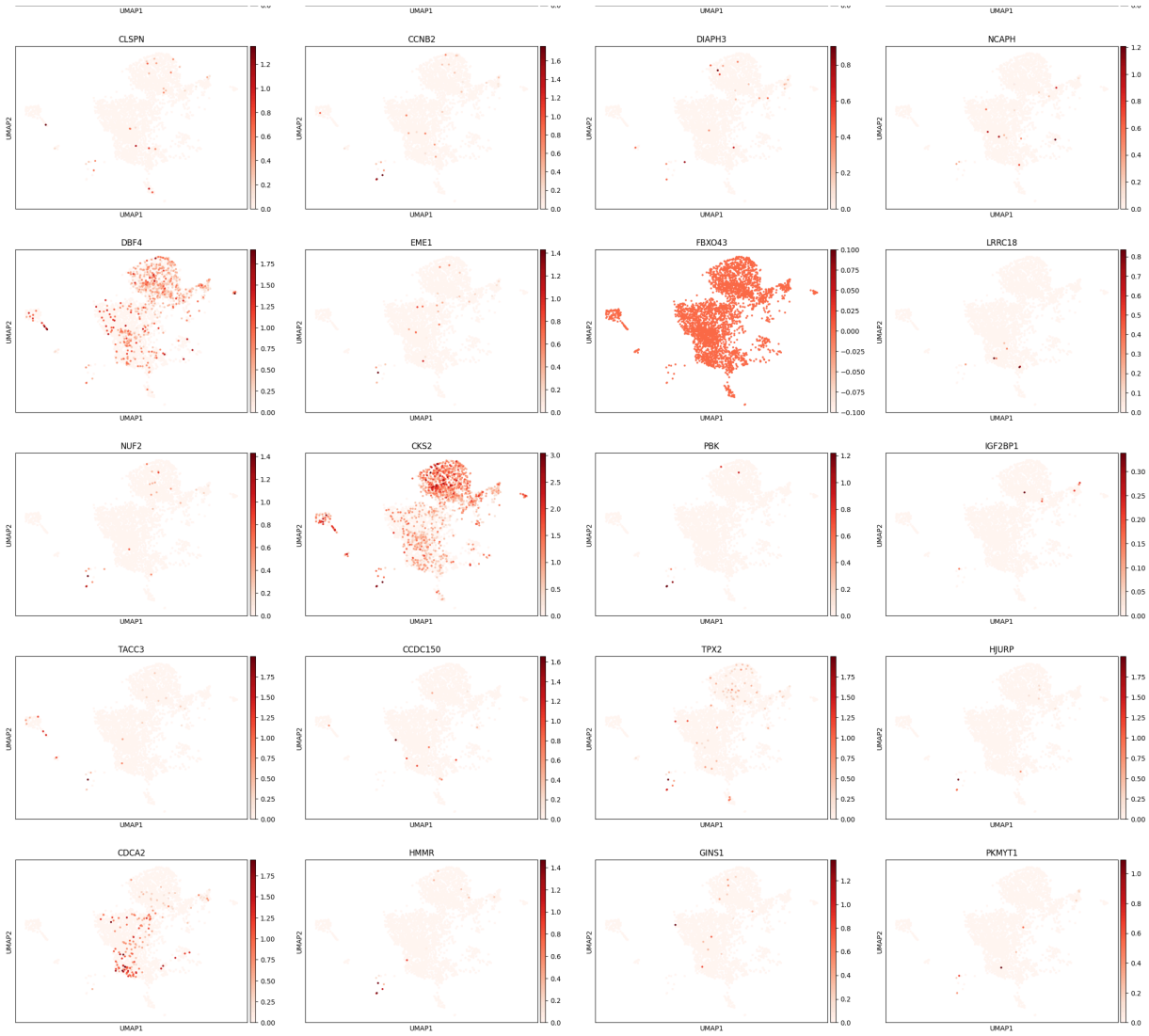
CCDC15
ASF1B
TTK
NEK2
TOP2A
CDCA8
CDC25C
WDR62
UMAP1
UMAP2


CLSPN
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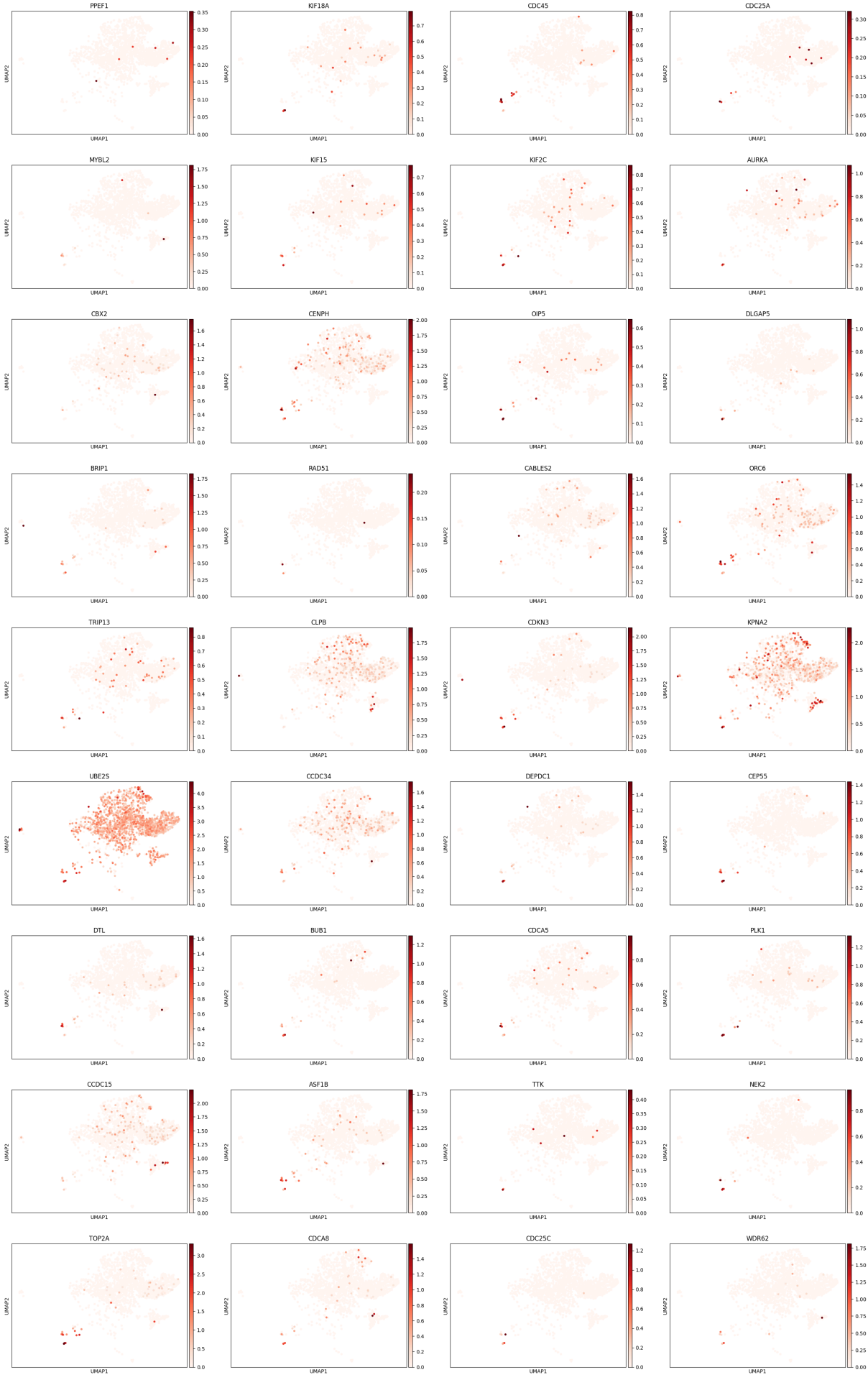
DIAPH3
NCAPH
DBF4
EME1
FBXO43
LRRC18
NUF2
CKS2
PBK
IGF2BP1
TACC3
CCDC150
TPX2
HJURP
CDCA2
HMMR
GINS1
PKMYT1
UMAP1
UMAP2


PPEF1
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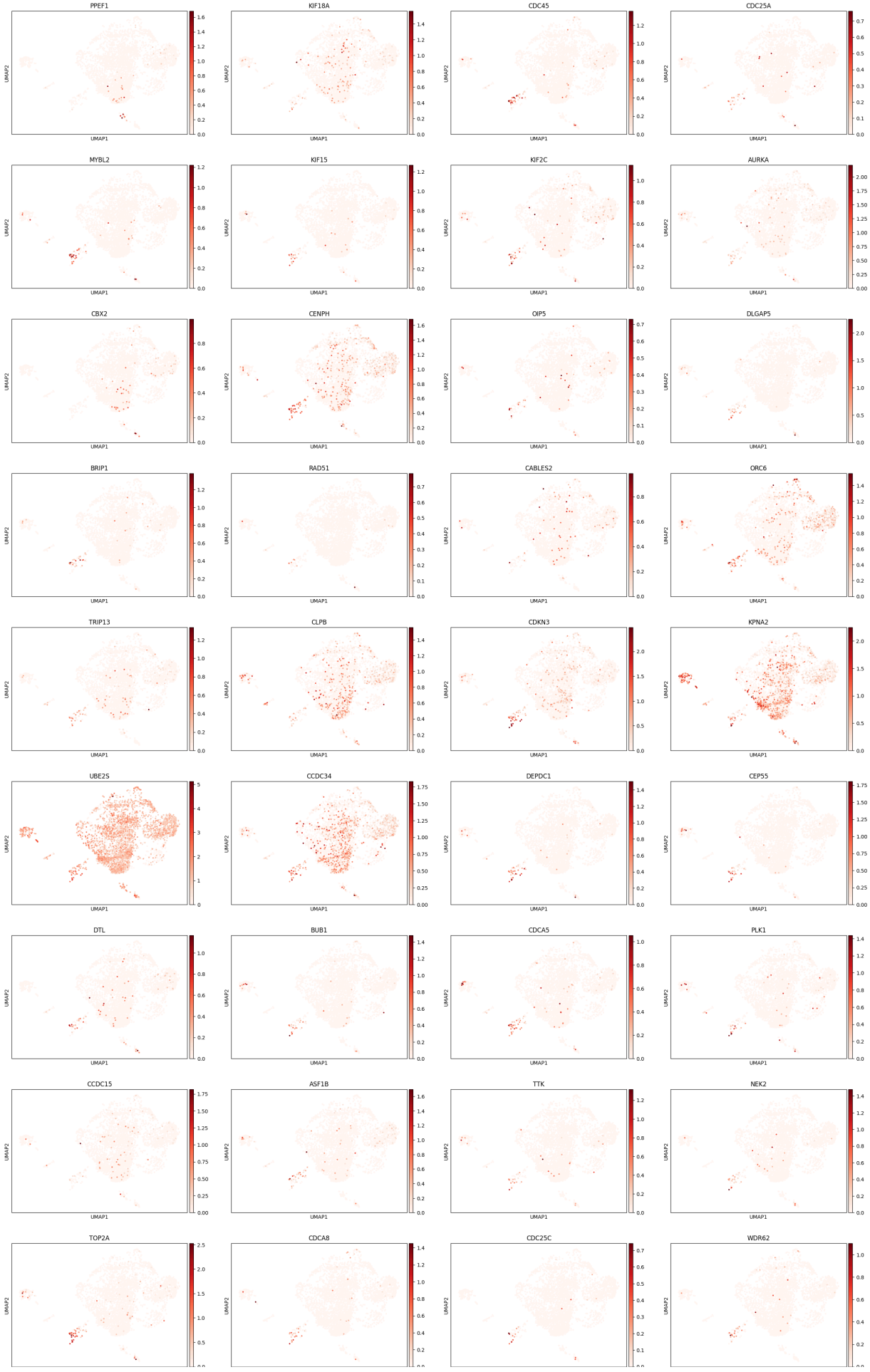
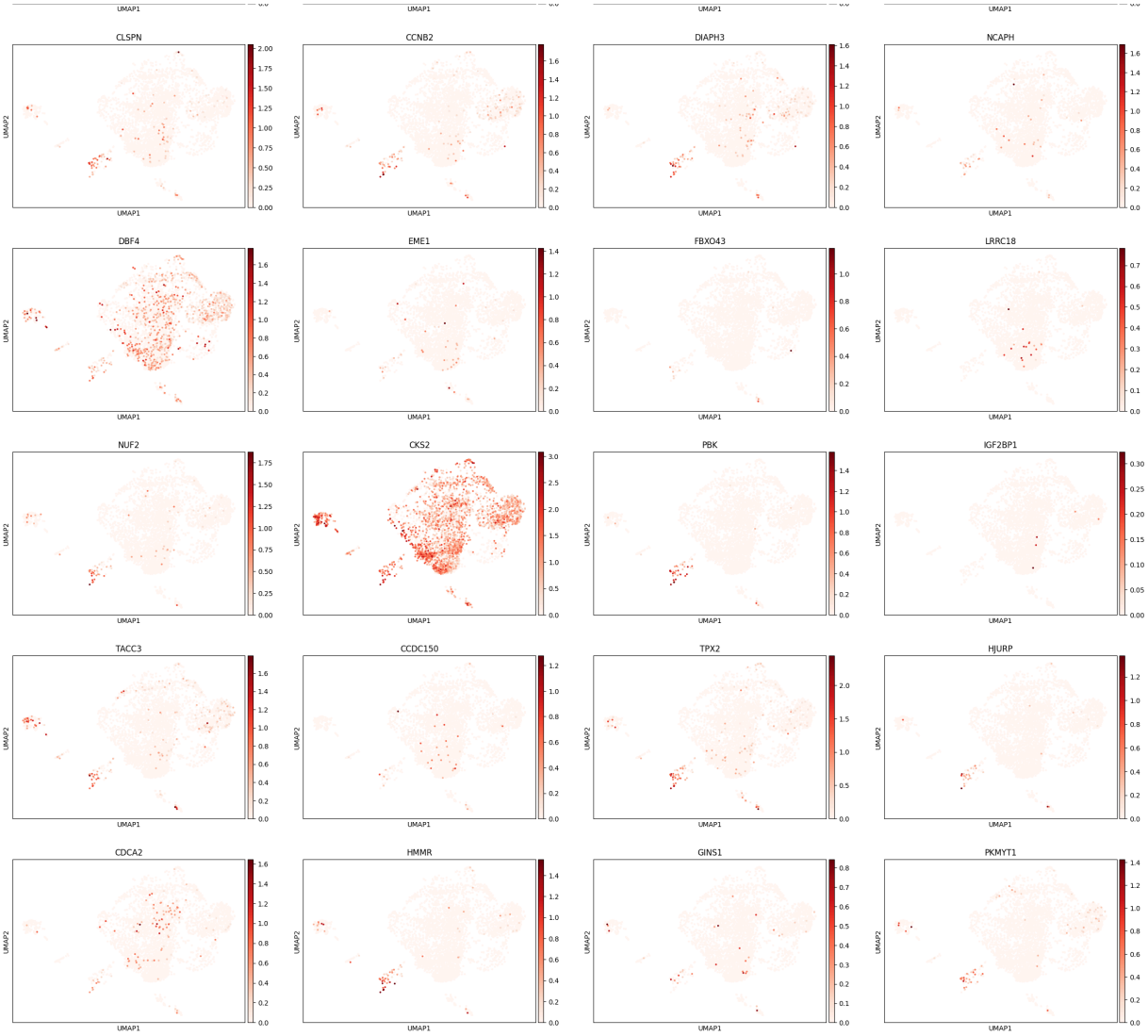
CDC45
CDC25A
MYBL2
KIF15
KIF2C
AURKA
CBX2
CENPH
OIP5
DLGAP5
BRIP1
RAD51
CABLES2
ORC6
TRIP13
CLPB
CDKN3
KPNA2
UBE2S
CCDC34
DEPDC1
CEP55
DTL
BUB1
CDCA5
PLK1
CCDC15
ASF1B
TTK
NEK2
TOP2A
CDCA8
CDC25C
WDR62
UMAP1
UMAP2


CLSPN
CCNB2
DIAPH3
NCAPH
DBF4
EME1
FBXO43
LRRC18
NUF2
CKS2
PBK
IGF2BP1
TACC3
CCDC150
TPX2
HJURP
CDCA2
HMMR
GINS1
PKMYT1
UMAP1
UMAP2


PPEF1
KIF18A
CDC45
CDC25A
MYBL2
KIF15
KIF2C
AURKA
CBX2
CENPH
OIP5
DLGAP5
BRIP1
RAD51
CABLES2
ORC6
TRIP13
CLPB
CDKN3
KPNA2
UBE2S
CCDC34
DEPDC1
CEP55
DTL
BUB1
CDCA5
PLK1
CCDC15
ASF1B
TTK
NEK2
TOP2A
CDCA8
CDC25C
WDR62
UMAP1
UMAP2

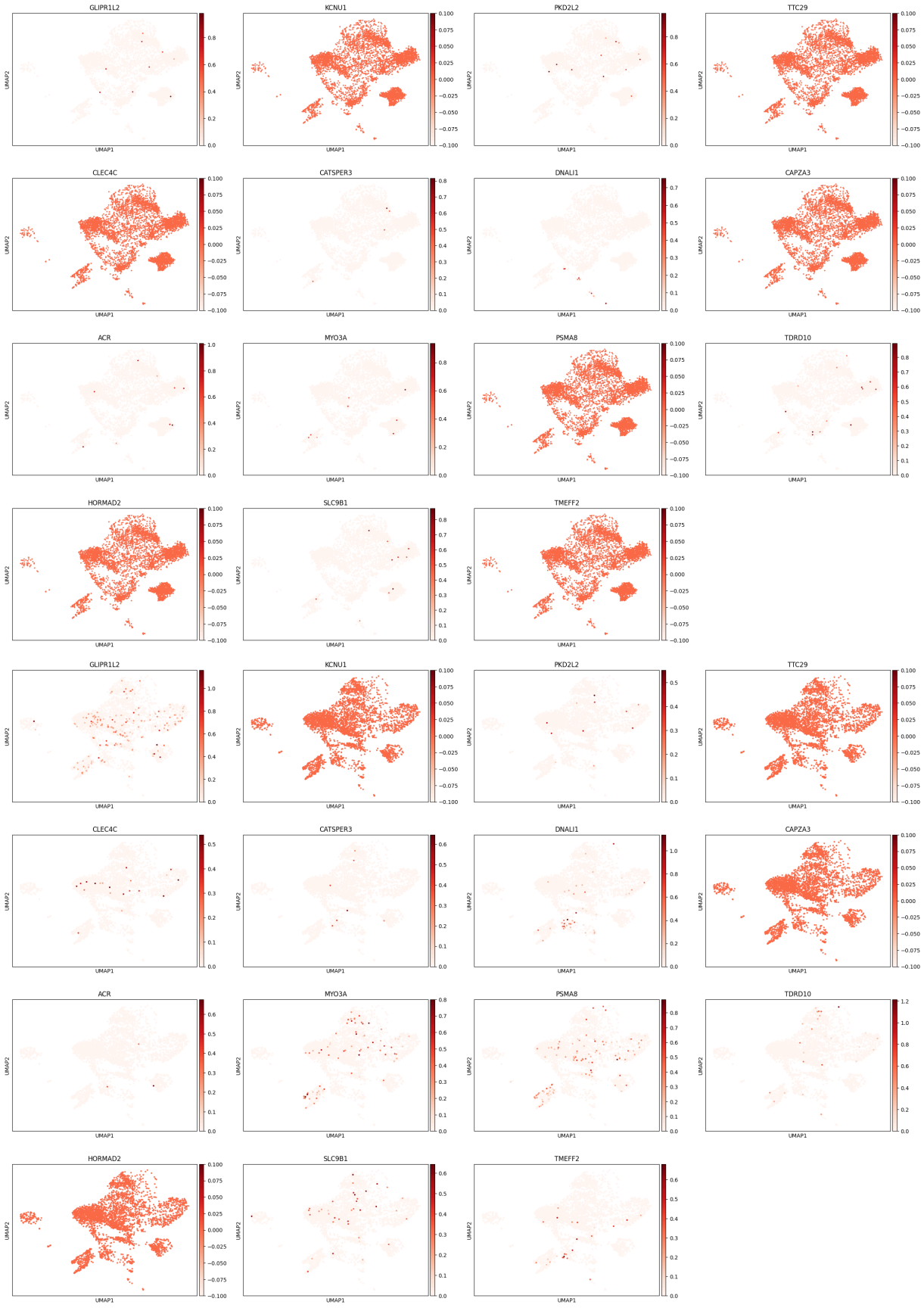


It seems that high grade patient have cells that expressed more CTA than low grade.

c- Cluster 3

In [ ]:
```
# Markers cluster 3
for dataset in l:
    adata_filtered = adata[adata.obs['dataset'] == dataset, :]
    sc.pl.umap(adata_filtered, color = genes_clust_3 , color_map = plt.cm.Re
```

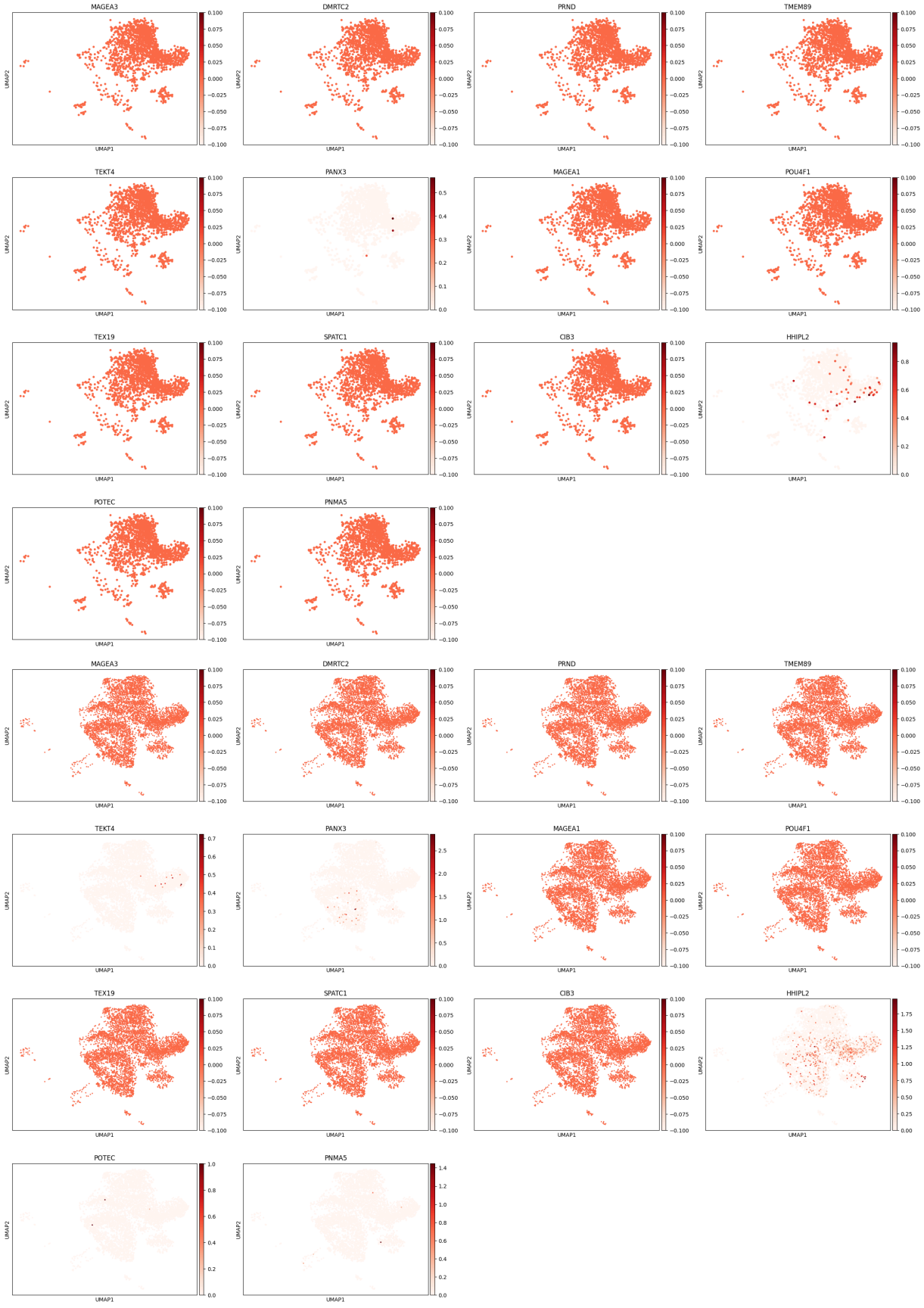

GLIPR1L2
KCNU1
PKD2L2
TTC29
CLEC4C
CATSPER3
DNALI1
CAPZA3
ACR
MYO3A
PSMA8
TDRD10
HORMAD2
SLC9B1
TMEFF2
GLIPR1L2
KCNU1
PKD2L2
TTC29
CLEC4C
CATSPER3
DNALI1
CAPZA3
ACR
MYO3A
PSMA8
TDRD10
HORMAD2
SLC9B1
TMEFF2
UMAP1
UMAP2

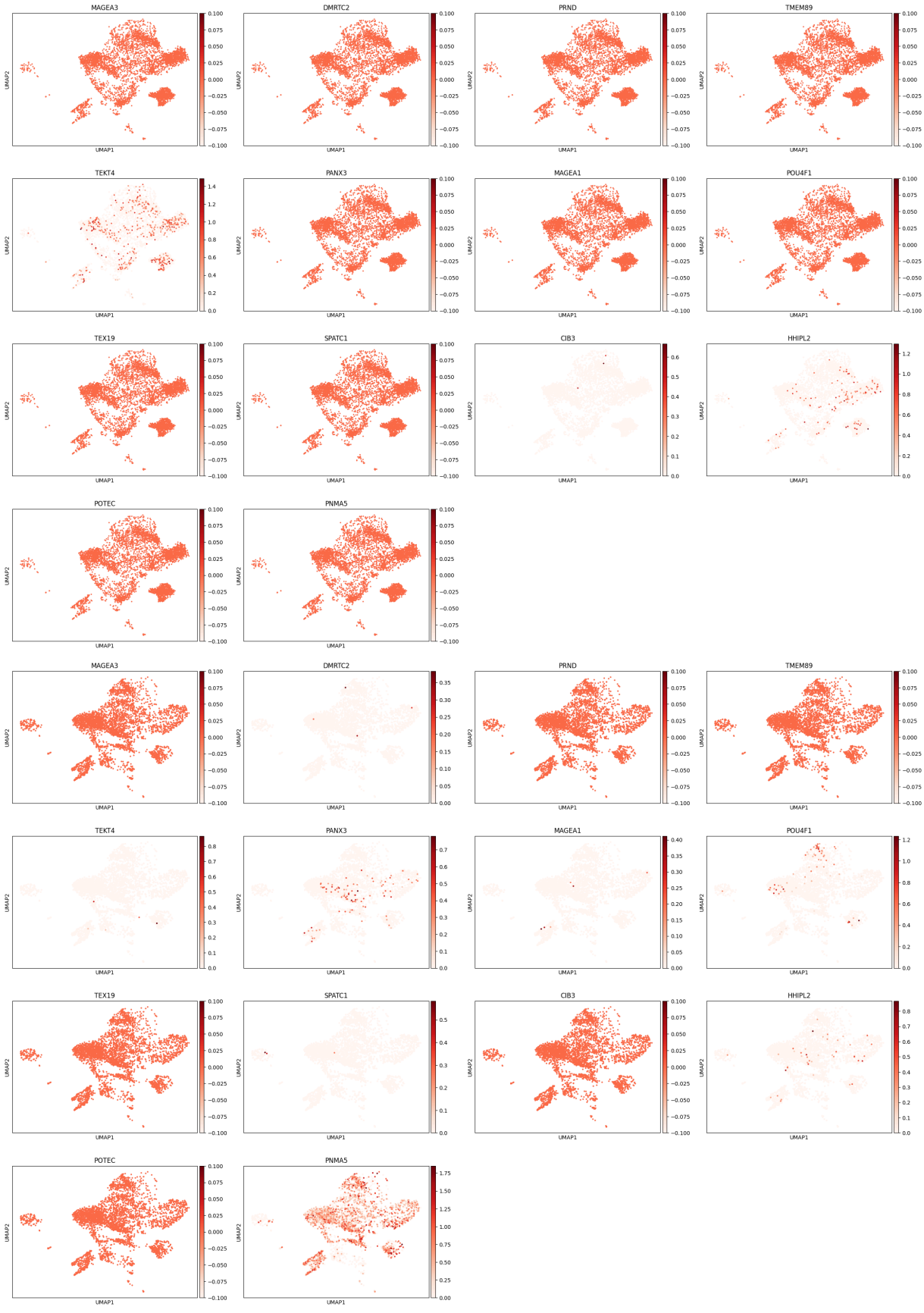

GLIPR1L2
KCNU1
PKD2L2
TTC29
CLEC4C
CATSPER3
DNALI1
CAPZA3
ACR
MYO3A
PSMA8
TDRD10
HORMAD2
SLC9B1
TMEFF2
GLIPR1L2
KCNU1
PKD2L2
TTC29
CLEC4C
CATSPER3
DNALI1
CAPZA3
ACR
MYO3A
PSMA8
TDRD10
HORMAD2
SLC9B1
TMEFF2
UMAP1
UMAP2

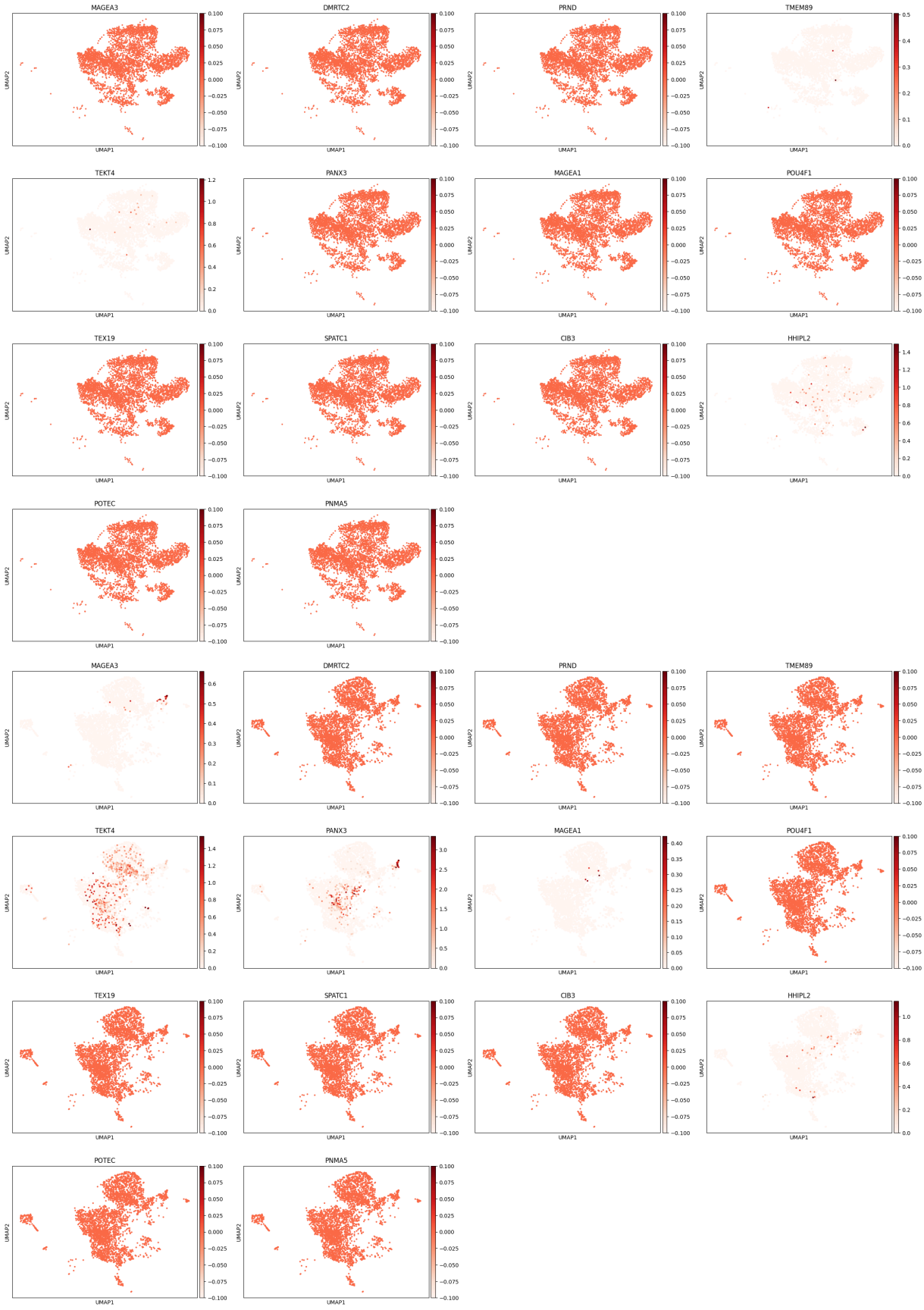

GLIPR1L2
KCNU1
PKD2L2
TTC29
CLEC4C
CATSPER3
DNALI1
CAPZA3
ACR
MYO3A
PSMA8
TDRD10
HORMAD2
SLC9B1
TMEFF2
GLIPR1L2
KCNU1
PKD2L2
TTC29
CLEC4C
CATSPER3
DNALI1
CAPZA3
ACR
MYO3A
PSMA8
TDRD10
HORMAD2
SLC9B1
TMEFF2
UMAP1
UMAP2

4) Low expressed CTAs in normal tissues and expressed in chondrosarcoma

In [ ]:
```
for dataset in l:
    adata_filtered = adata[adata.obs['dataset'] == dataset, :]
    sc.pl.umap(adata_filtered, color = genes_scatter, color_map = plt.cm.Rec
```



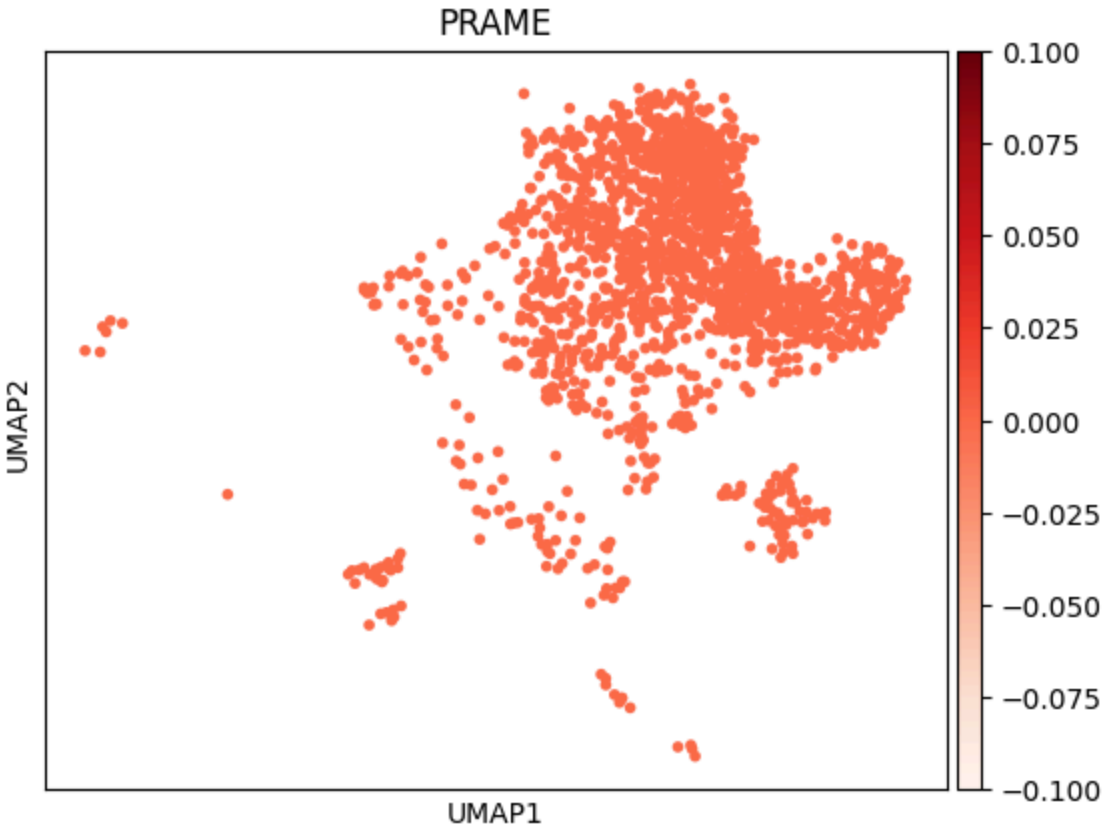

MAGEA3
DMRTC2
PRND
TMEM89
TEKT4
PANX3
MAGEA1
POU4F1
TEX19
SPATC1
CIB3
HHIPL2
POTEC
PNMA5
MAGEA3
DMRTC2
PRND
TMEM89
TEKT4
PANX3
MAGEA1
POU4F1
TEX19
SPATC1
CIB3
HHIPL2
POTEC
PNMA5
UMAP1
UMAP2

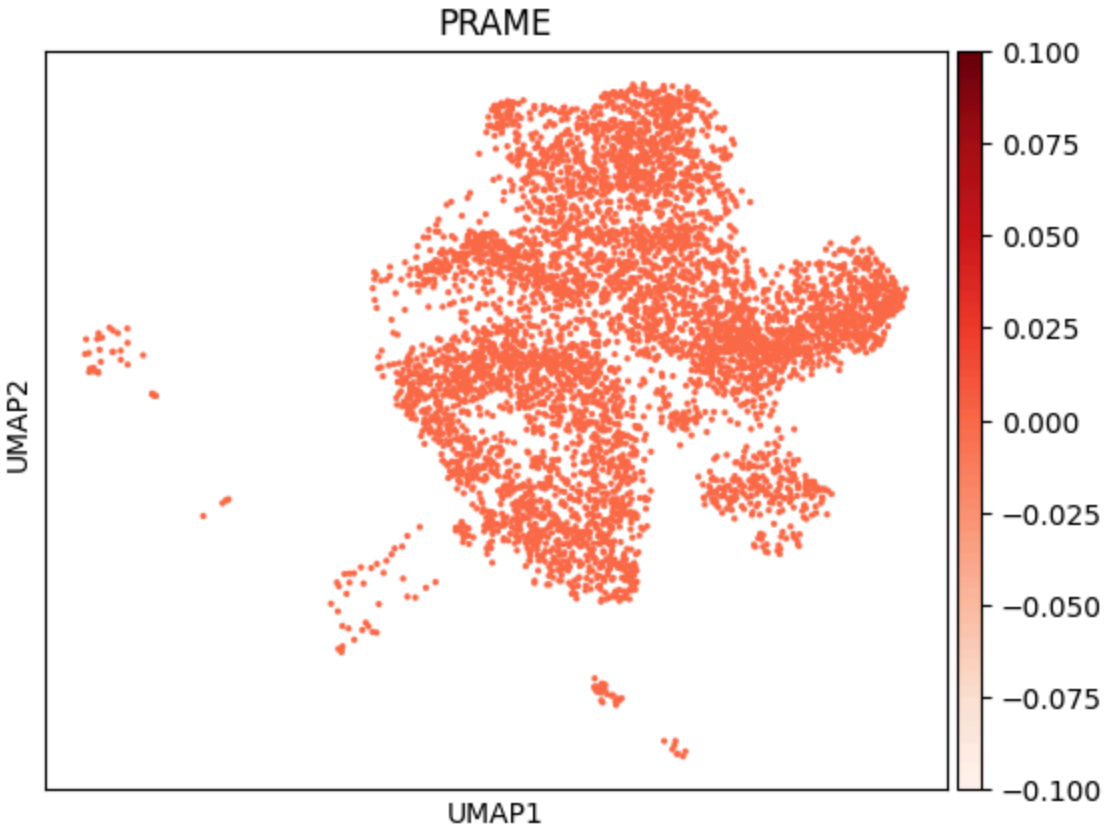

MAGEA3
DMRTC2
PRND
TMEM89
TEKT4
PANX3
MAGEA1
POU4F1
TEX19
SPATC1
CIB3
HHIPL2
POTEC
PNMA5
MAGEA3
DMRTC2
PRND
TMEM89
TEKT4
PANX3
MAGEA1
POU4F1
TEX19
SPATC1
CIB3
HHIPL2
POTEC
PNMA5
UMAP1
UMAP2

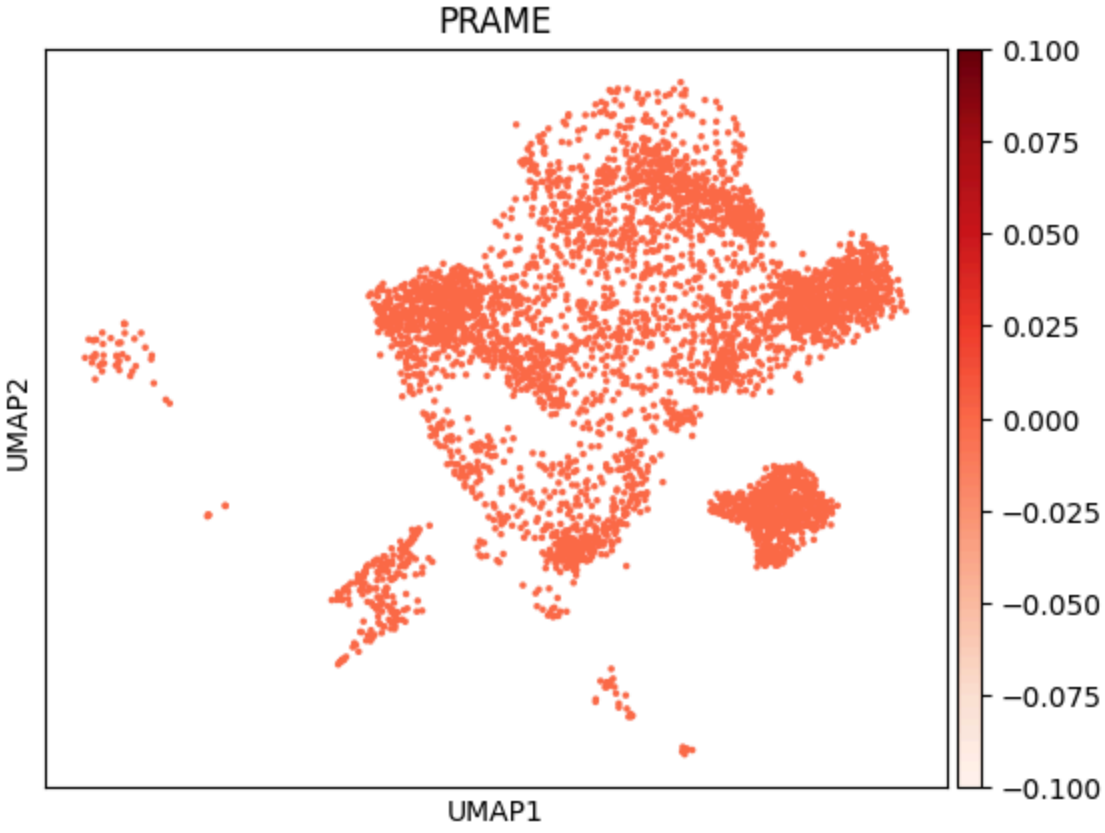
With this, PANX3 and TEKT4 seem to be the most expressed CTAs.

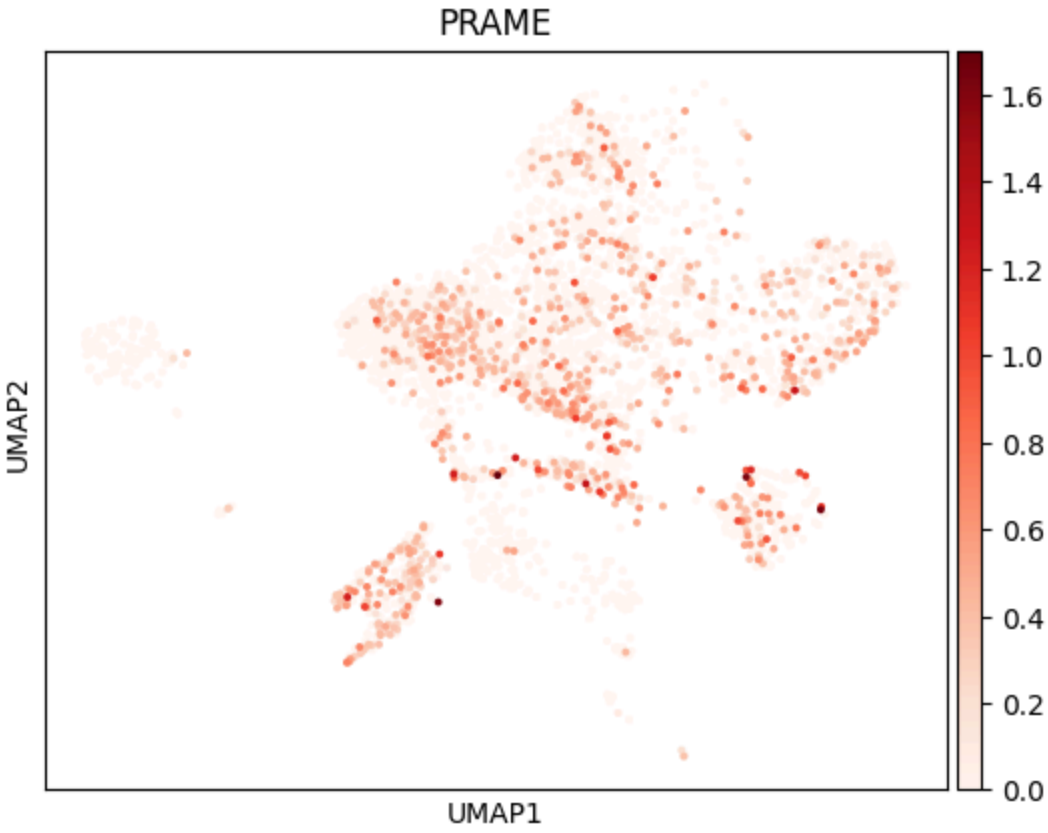
5) Selected CTA of interest

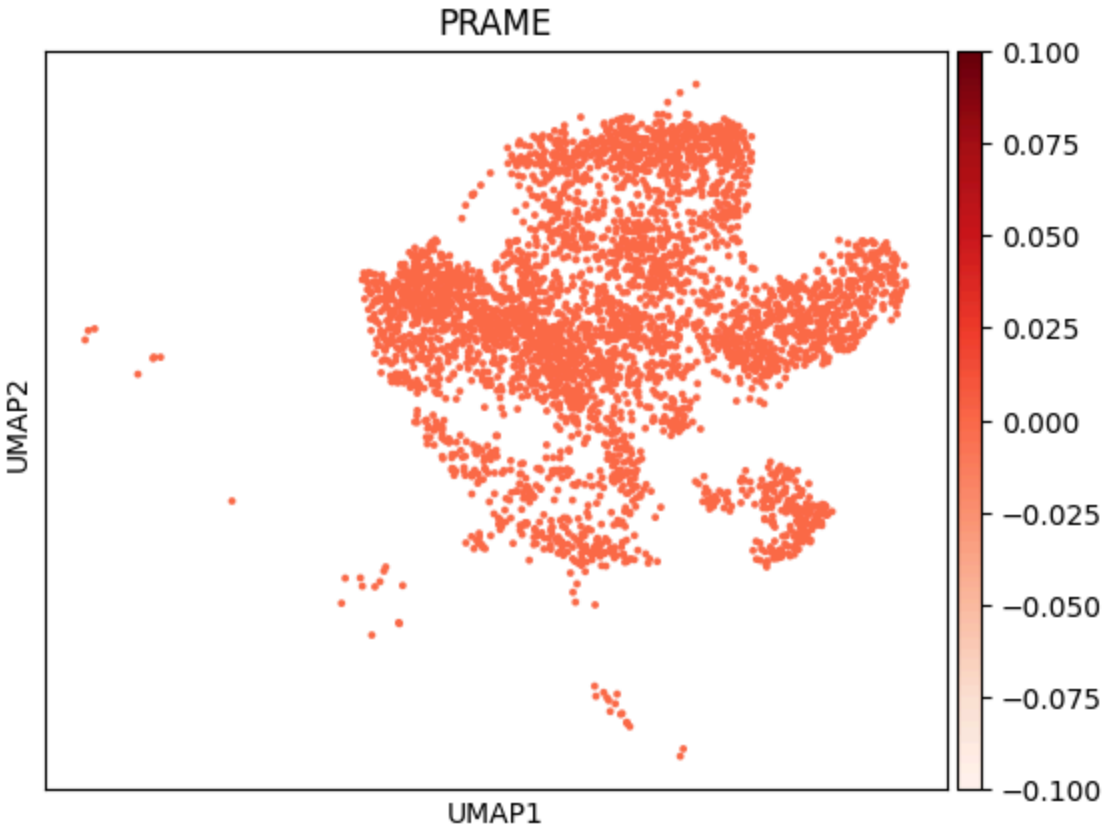
In [ ]:
```
for dataset in l:
    adata_filtered = adata[adata.obs['dataset'] == dataset, :]
    sc.pl.umap(adata_filtered, color = ['DTL', 'HJURP', 'GINS1', 'PBK', 'CT4
```

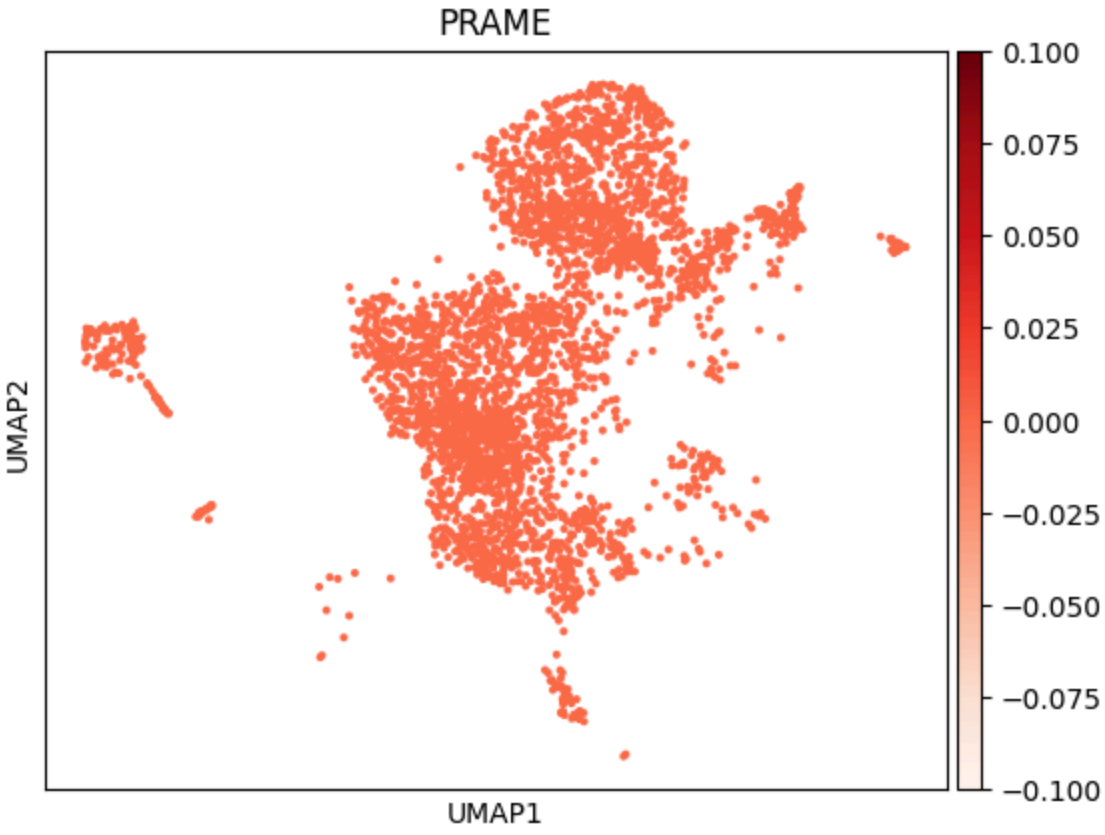





PRAME
UMAP2
UMAP1
0.100
0.075
0.050
0.025
0.000
−0.025
−0.050
−0.075
−0.100


PRAME
UMAP2
UMAP1
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0


PRAME
UMAP2
UMAP1
0.100
0.075
0.050
0.025
0.000
−0.025
−0.050
−0.075
−0.100


PRAME
UMAP2
UMAP1
0.100
0.075
0.050
0.025
0.000
−0.025
−0.050
−0.075
−0.100