# Welcome to spSAM's documentation!

## spSAM: 10X visium **sp**ot **S**plit **A**lign **M**ap

In [1]:
```
import spsam as ss
import matplotlib.pyplot as plt

import warnings
warnings.filterwarnings('ignore')

plt.rcParams['figure.figsize'] = (6, 6)
```

## Load Data

Load anndata object intergrating 10X Visium data with scRNA-seq reference of cell types through **cell2location** package.

In [2]:
```
adata = ss.read_h5ad('E:\geneplus\job_code\spsam\data\demo.h5ad')
```

In [3]:
```
adata.obs.keys()
```

Out[3]:
```
Index(['in_tissue', 'array_row', 'array_col', 'sample', 'n_genes_by_counts',
       'log1p_n_genes_by_counts', 'total_counts', 'log1p_total_counts',
       'pct_counts_in_top_50_genes', 'pct_counts_in_top_100_genes',
       'pct_counts_in_top_200_genes', 'pct_counts_in_top_500_genes',
       'total_counts_mt', 'log1p_total_counts_mt', 'pct_counts_mt', '_indices',
       '_scvi_batch', '_scvi_labels', 'B cells', 'B-cell lineage',
       'Cycling cells', 'DC', 'ILC', 'Macrophages', 'Monocytes',
       'Plasma cells', 'T cells', 'pDC', 'clusters'],
      dtype='object')
```

Through `adata.obs.keys()` , we can see total 10 cell types `['B cells', 'B-cell lineage', 'Cycling cells', 'DC', 'ILC', 'Macrophages', 'Monocytes', 'Plasma cells', 'T cells', 'pDC']` were mapped to 10X Visium data.

# Score Genes

This reproduces the approach in Seurat (**Satija**) and has been implemented for Scanpy by Davide Cittar.

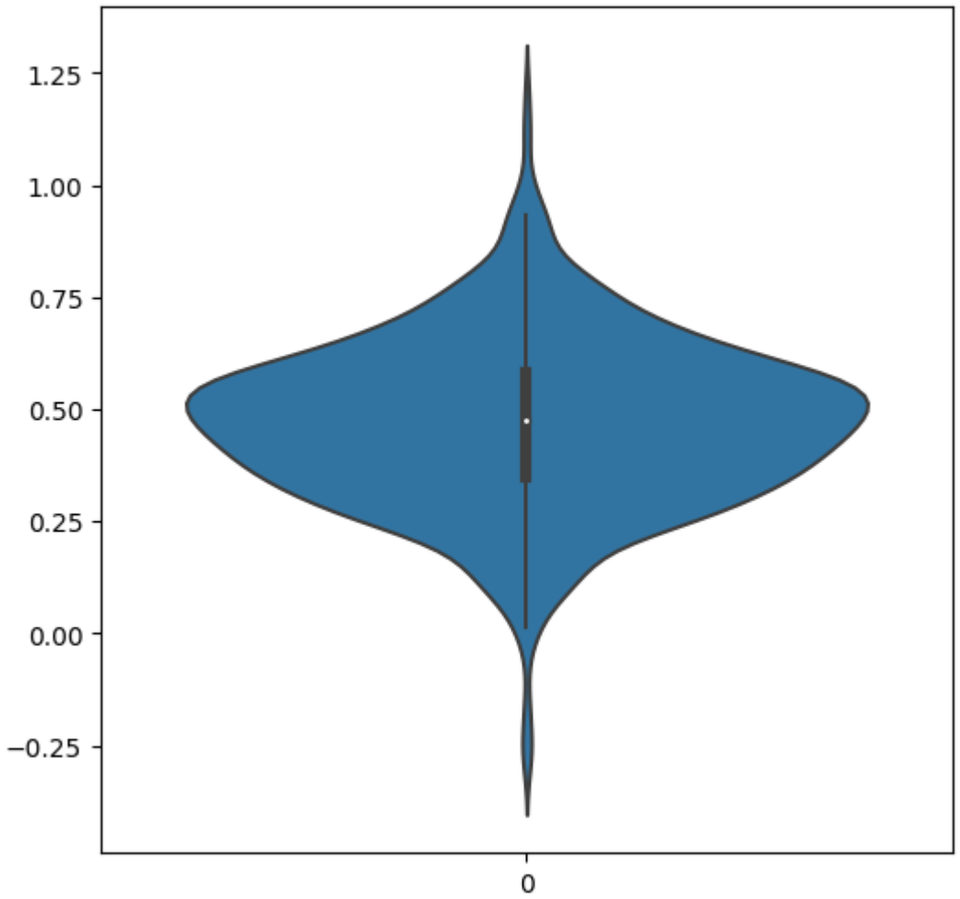
Here we use 15 top-ranked hypoxia-associated genes, `['VEGFA', 'SLC2A1', 'PGAM1', 'ENO1', 'LDHA', 'TP11', 'P4HA1', 'MRPS1', 'CDKN3', 'ADM', 'NDRG1', 'TUBB6', 'ALDOA', 'MIF', 'ACOT7']`, which are collectively considered to be hypoxia signature (**Buffa signature**) to assess hypoxia statu. Custom gene sets are also supported.

In [4]:
```
hypoxia_gene_lt = ['VEGFA', 'SLC2A1', 'PGAM1', 'ENO1', 'LDHA', 'TP11', 'P4HA1', 'MRPS1', 'CDKN3', 'ADM', 'NDRG1', 'TUBB6', 'ALDOA', 'MIF', 'ACOT7']
ss.tl.score_genes(adata, gene_list=hypoxia_gene_lt, ctrl_size=len(hypoxia_gene_lt), score_name='hypoxia_score')
```

```
WARNING:root:genes are not in var_names and ignored: ['VEGFA', 'LDHA', 'TP11', 'MRPS1', 'ALDOA']
computing score 'hypoxia_score'
finished
hypoxia_score, score of gene set (adata.obs).
104 total control genes are used.
```

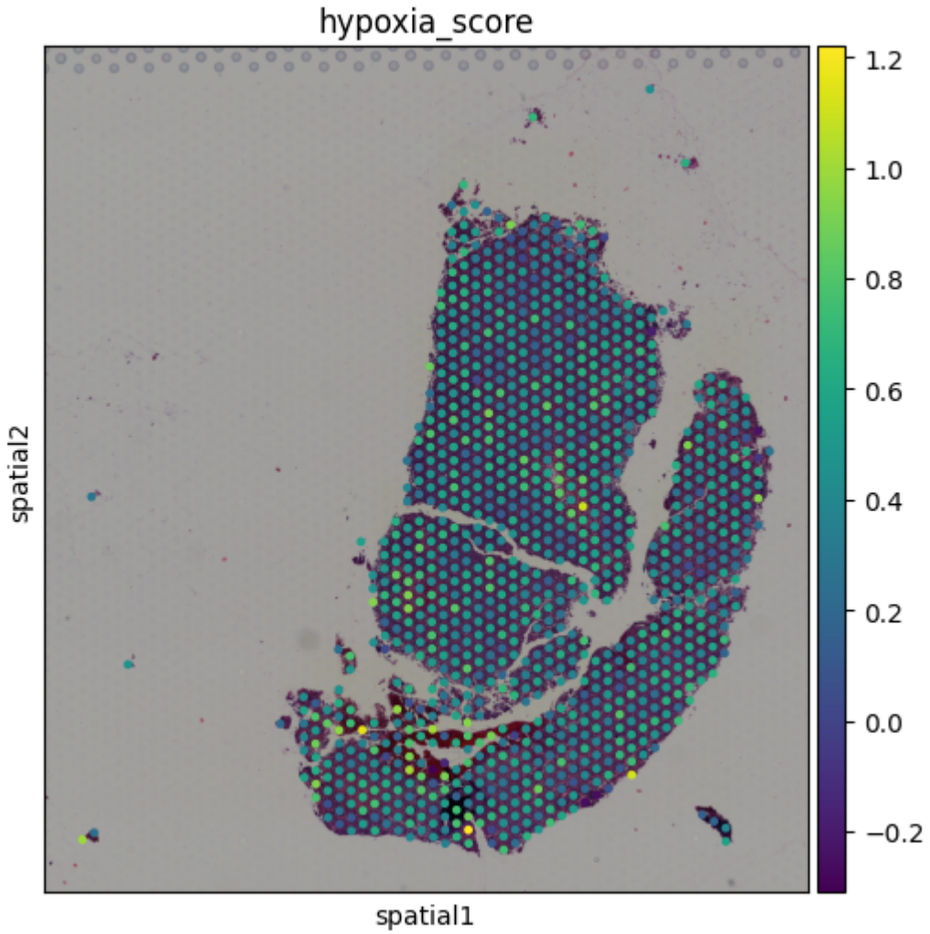
Plot hypoxia_score distribution

In [5]:
```
ss.pl.violin(adata, 'hypoxia_score')
```




Show score in visium image

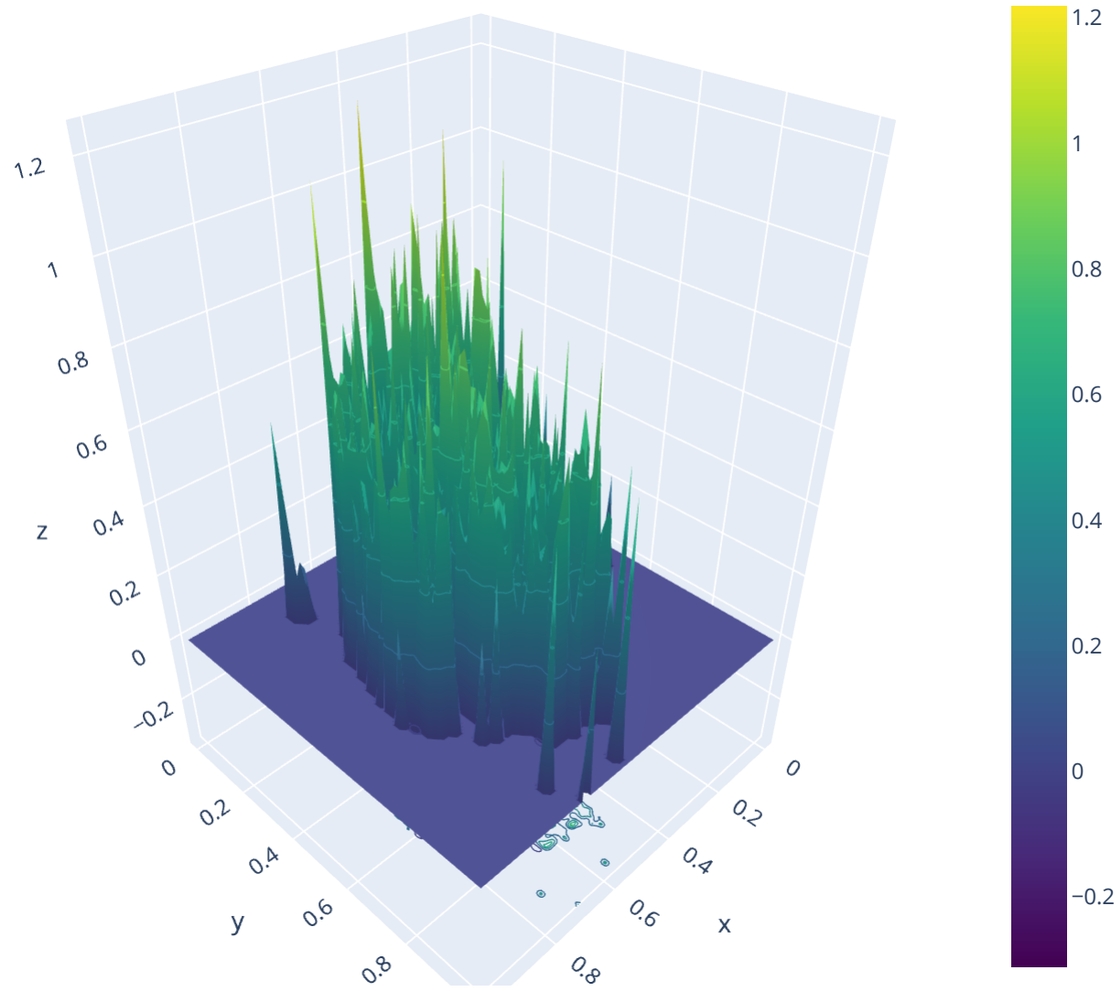
In [6]:
```
ss.pl.spatial(adata, img_key='hires', color=['hypoxia_score'])
```




Additionally, you can use `ss.pl.plot_3d_score()` function to plot hypoxia_score in 3D spatial dimension of 10x visium image

In [7]:
```
ss.pl.plot_3d_score(adata, 'hypoxia_score')
```

hypoxia_score



# Score Lever

Divide spot into three parts based on the distribution of the score values.
**lever1(default):** hypoxia_score >= 95% distribution of the score values.
**lever2(default):** hypoxia_score >= 50% distribution of the score values.
**background:** hypoxia_score < 50% distribution of the score values.
If using other thresholds to divide spot, pass two values through (lever1, lever2) parameter, like `ss.pp.score_lever(adata, 'hypoxia_score', lever1=0.8, lever2=0.6)`, then background part will set hypoxia_score < 80% automatically.

In [8]:
```
ss.pp.score_lever(adata, 'hypoxia_score')
```

```
hypoxia_score median:0.4765155938955453; mean:0.4701082144745993; std:0.18736050562146309
hypoxia_score lever1 value:0.7705623546013465; lever2 value:0.4765155938955453
score lever definition finished, score_type key is added to adata.obs, use adata.obs_keys() to check
```

In [9]:
```
adata.obs.head()
```

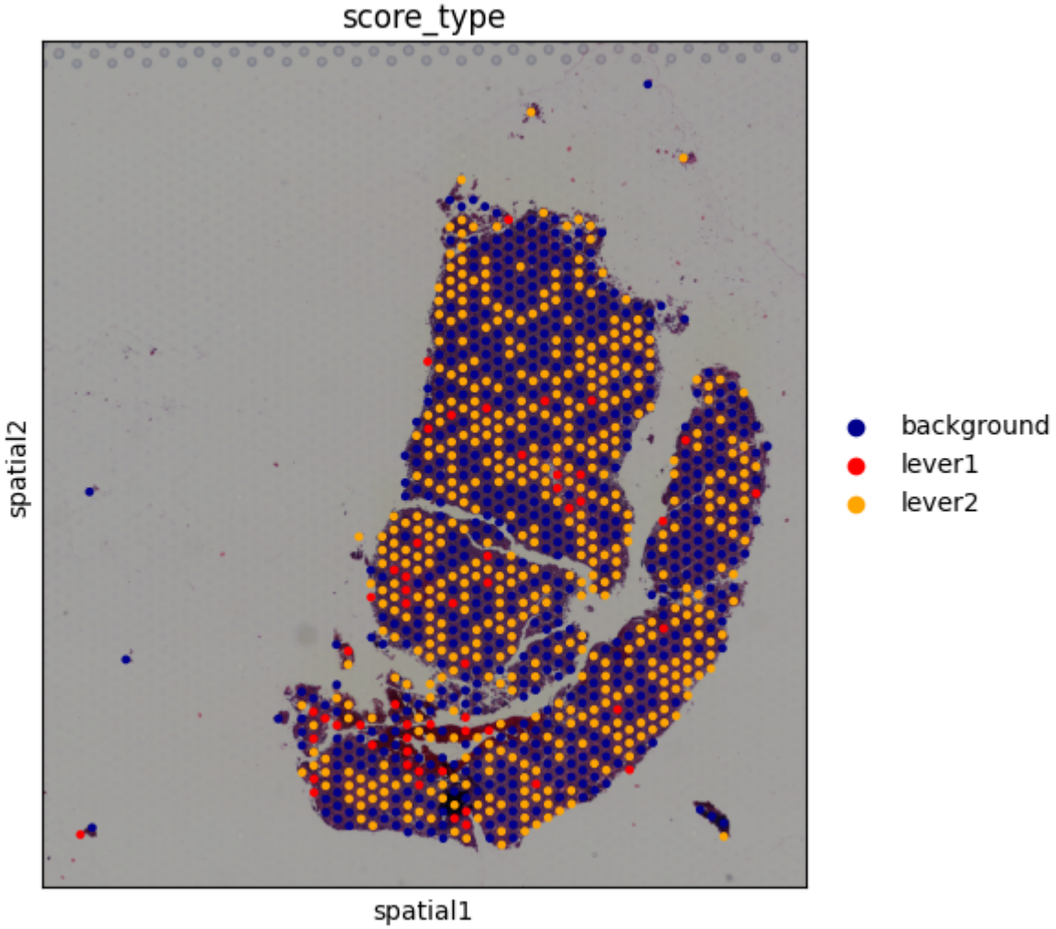
Out[9]:

| | in_tissue | array_row | array_col | sample | n_genes_by_counts | log1p_n_genes_by_counts | total_counts | log1p_total_counts | pct_counts_in_top_5 |
|---|---|---|---|---|---|---|---|---|---|
| **AAACCGGGTAGGTACC-1** | 1 | 42 | 28 | LD | 7324 | 8.899048 | 25961.0 | 10.164390 | 1 |
| **AAACCGTTCGTCCAGG-1** | 1 | 52 | 42 | LD | 7850 | 8.968396 | 37458.0 | 10.531002 | 1 |
| **AAACCTCATGAAGTTG-1** | 1 | 37 | 19 | LD | 3961 | 8.284504 | 7936.0 | 8.979291 | 1 |
| **AAACGAGACGGTTGAT-1** | 1 | 35 | 79 | LD | 7348 | 8.902320 | 29413.0 | 10.289227 | 1 |
| **AAACTGCTGGCTCCAA-1** | 1 | 45 | 67 | LD | 7902 | 8.974998 | 35196.0 | 10.468717 | 1 |

5 rows × 31 columns

Use a color_map directory to plot dividing three parts into visium image.

In [10]:
```
color_map_dt = {
    'lever1': 'red',
    'lever2': 'orange',
    'background': 'darkblue',
}
ss.pl.spatial(adata, img_key='hires', color='score_type', palette=color_map_dt)
```




## Split

In this step, we use the DFS algorithm starting from the red spot to expand outward, with the expansion condition being that the adjacent point is either a red spot or a yellow spot, until it cannot extend any further or reaches the edge of the image. After traversal, the red spots will be clustered into clusters. In a cluster, if there is only one cluster of red spots, it is called an 'independent' type; if there are more than two clusters of red spots in a cluster, the cluster will be further divided into smaller units based on the boundaries of each red spot cluster, known as 'adjacent' type. A new column 'class' will be created in the cluster_df.

In [11]:
```
cluster_df = ss.pp.find_lever_core(adata, 'hypoxia_score')
```

In [12]:
```
cluster_df
```

Out[12]:

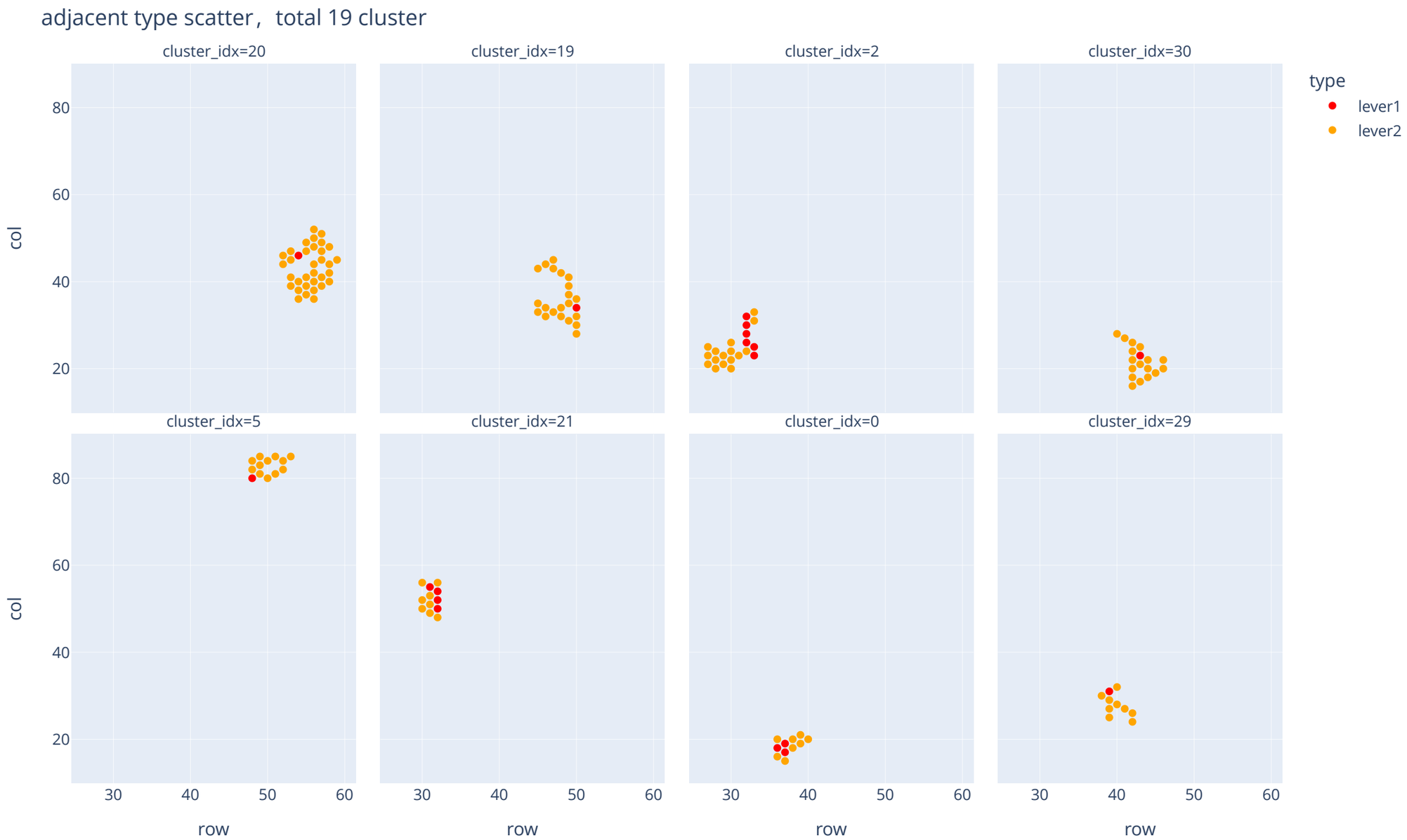
| | row | col | score | type | cluster_idx | class |
|---|---|---|---|---|---|---|
| **0** | 37 | 19 | 0.857315 | lever1 | 0 | adjacent |
| **1** | 37 | 17 | 1.219024 | lever1 | 0 | adjacent |
| **2** | 37 | 15 | 0.697438 | lever2 | 0 | adjacent |
| **3** | 36 | 16 | 0.661241 | lever2 | 0 | adjacent |
| **4** | 36 | 18 | 0.774277 | lever1 | 0 | adjacent |
| **...** | ... | ... | ... | ... | ... | ... |
| **272** | 59 | 65 | 0.535874 | lever2 | 6 | independent |
| **273** | 61 | 65 | 0.540075 | lever2 | 6 | independent |
| **274** | 60 | 60 | 0.602142 | lever2 | 6 | independent |
| **275** | 61 | 59 | 0.533772 | lever2 | 6 | independent |
| **276** | 29 | 29 | 0.781194 | lever1 | 7 | independent |

277 rows × 6 columns

Plot 'adjacent' type spot cluster, 8 means show top8 clusters.

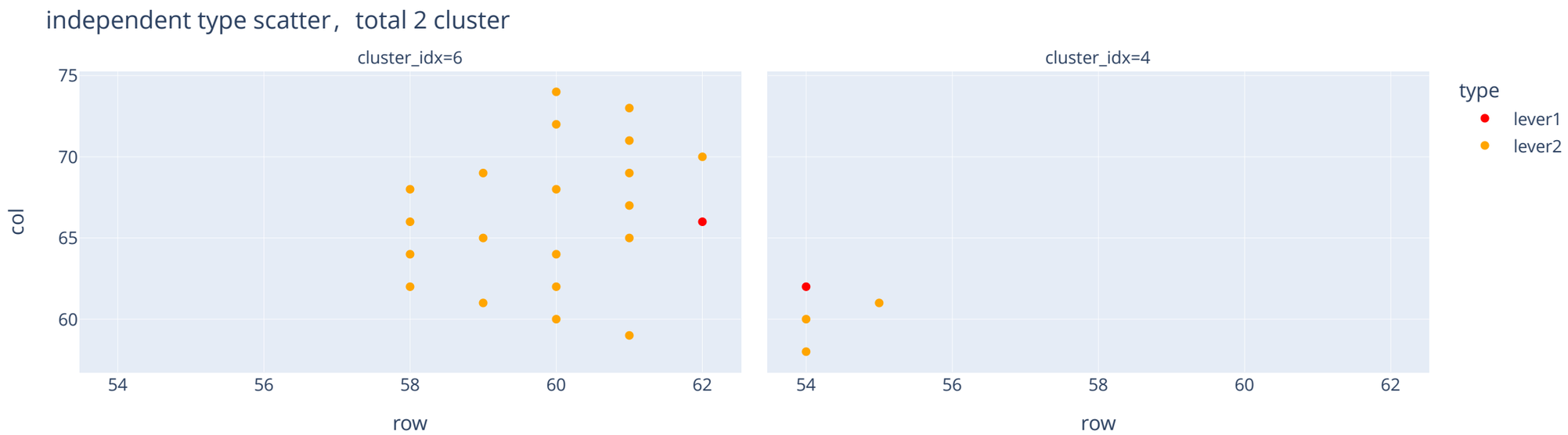
`ss.pl.plot_lever_core(cluster_df, 'adjacent', 8, col_wrap=4, fig_height=350)` : In order to display the points more clearly, you can use col_wrap(default: int=4) fig_height(default: int=350) to adjust the layout.

In [26]:
```
ss.pl.plot_lever_core(cluster_df, 'adjacent', 8)
```


adjacent type scatter， total 19 cluster
cluster_idx=20
cluster_idx=19
cluster_idx=2
cluster_idx=30
cluster_idx=5
cluster_idx=21
cluster_idx=0
cluster_idx=29
type
lever1
lever2
col
row
20
40
60
80
30
40
50
60

Plot 'independent' type spot cluster

In [14]:
```
ss.pl.plot_lever_core(cluster_df, 'independent')
```



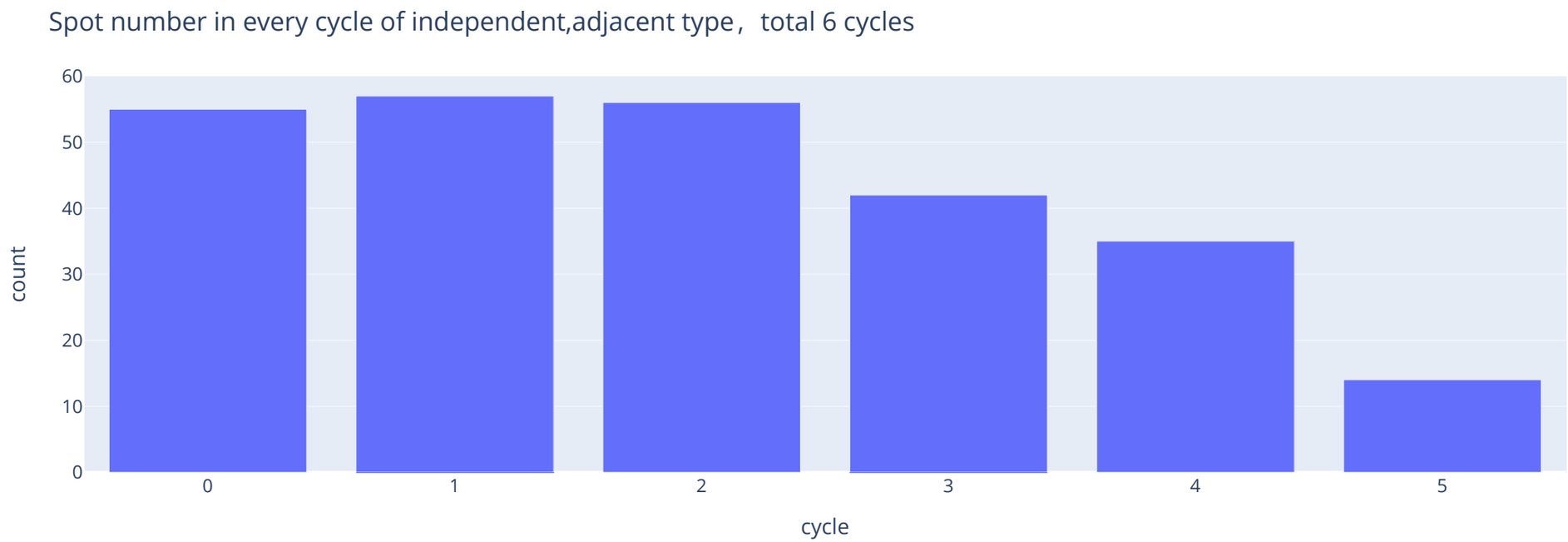

# Align

Aligning with the red spots as reference points, merge all clustered units partitioned based on their distances to the red spots into one cluster.

In [15]:
```
ss.pp.cycle_spot_count(cluster_df)
```

Next, show spot number in every cycle through `ss.pl.plot_cycle_bar()`, cycles containing less than 10 spots were filtered.

In [16]:
```
ss.pl.plot_cycle_bar(cluster_df)
```




Cycle 0 means core red cluster(lever1), it contains 55 spots.
Cycle 1 means yellow spot(lever2) that have a distance of 1 unit from cycle 0.
Cycle 2 means yellow spot(lever2) that have a distance of 2 units from cycle 0.
...
Cycle 5 means yellow spot(lever2) that have a distance of 5 units from cycle 0.

In [17]:
```
cluster_df
```

Out[17]:

| | row | col | score | type | cluster_idx | class | cycle |
|---|---|---|---|---|---|---|---|
| **0** | 37 | 19 | 0.857315 | lever1 | 0 | adjacent | 0.0 |
| **1** | 37 | 17 | 1.219024 | lever1 | 0 | adjacent | 0.0 |
| **2** | 37 | 15 | 0.697438 | lever2 | 0 | adjacent | 1.0 |
| **3** | 36 | 16 | 0.661241 | lever2 | 0 | adjacent | 1.0 |
| **4** | 36 | 18 | 0.774277 | lever1 | 0 | adjacent | 0.0 |
| **...** | ... | ... | ... | ... | ... | ... | ... |
| **272** | 59 | 65 | 0.535874 | lever2 | 6 | independent | 3.0 |
| **273** | 61 | 65 | 0.540075 | lever2 | 6 | independent | 1.0 |
| **274** | 60 | 60 | 0.602142 | lever2 | 6 | independent | 3.0 |
| **275** | 61 | 59 | 0.533772 | lever2 | 6 | independent | 4.0 |
| **276** | 29 | 29 | 0.781194 | lever1 | 7 | independent | 0.0 |

277 rows × 7 columns

# Map

Map the expression levels of specified cell types to the corresponding cycles.

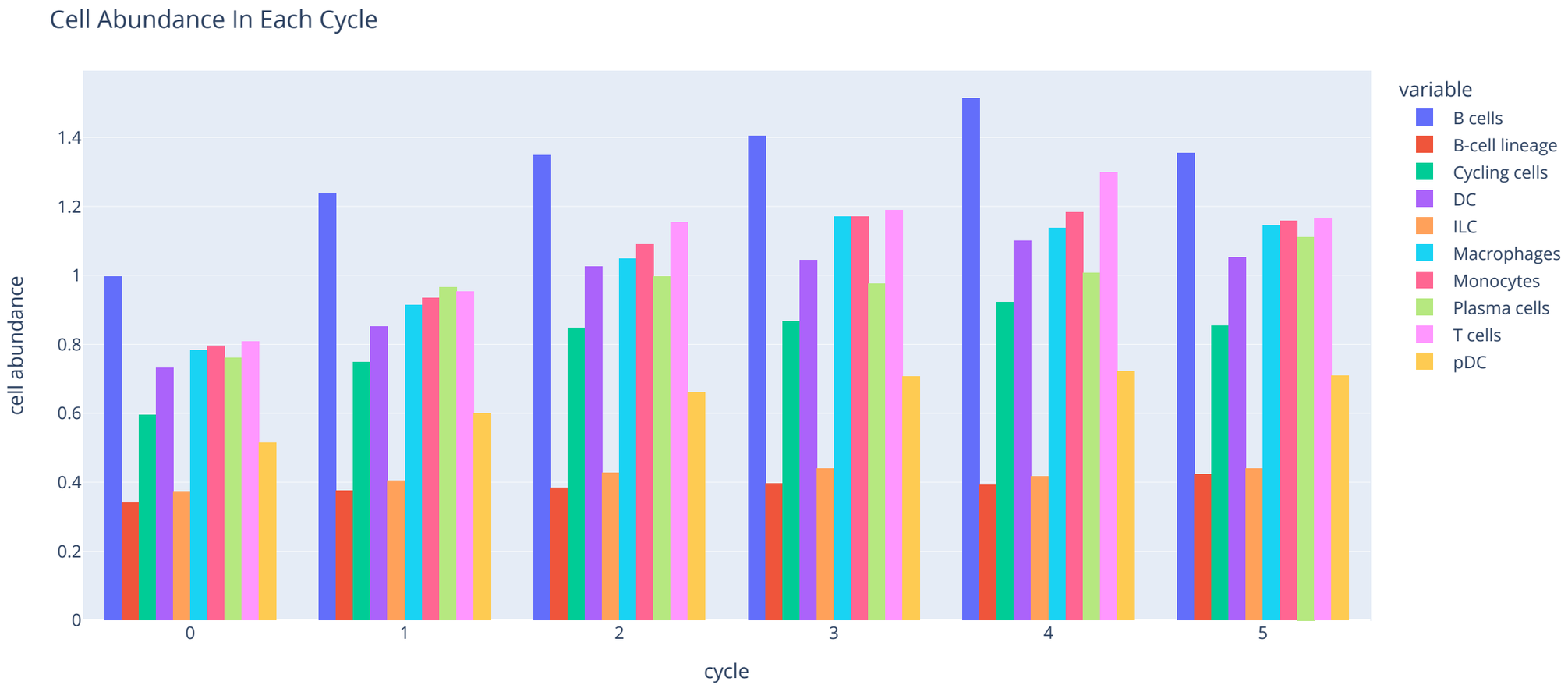
In [18]:
```
cell_type_lt = ['B cells', 'B-cell lineage', 'Cycling cells', 'DC', 'ILC', 'Macrophages', 'Monocytes', 'Plasma cells', 'T cells', 'pDC']
```

The `ss.pp.get_cell_abundance()` function requires three input parameters: adata, cluster, and cell_type_lt, where the cell_type_lt cell type list can be customized but must be included in the adata.obs object.

In [19]:
```
ss.pp.get_cell_abundance(adata, cluster_df, cell_type_lt)
```

Visualization of different cell abundance in different cycles.

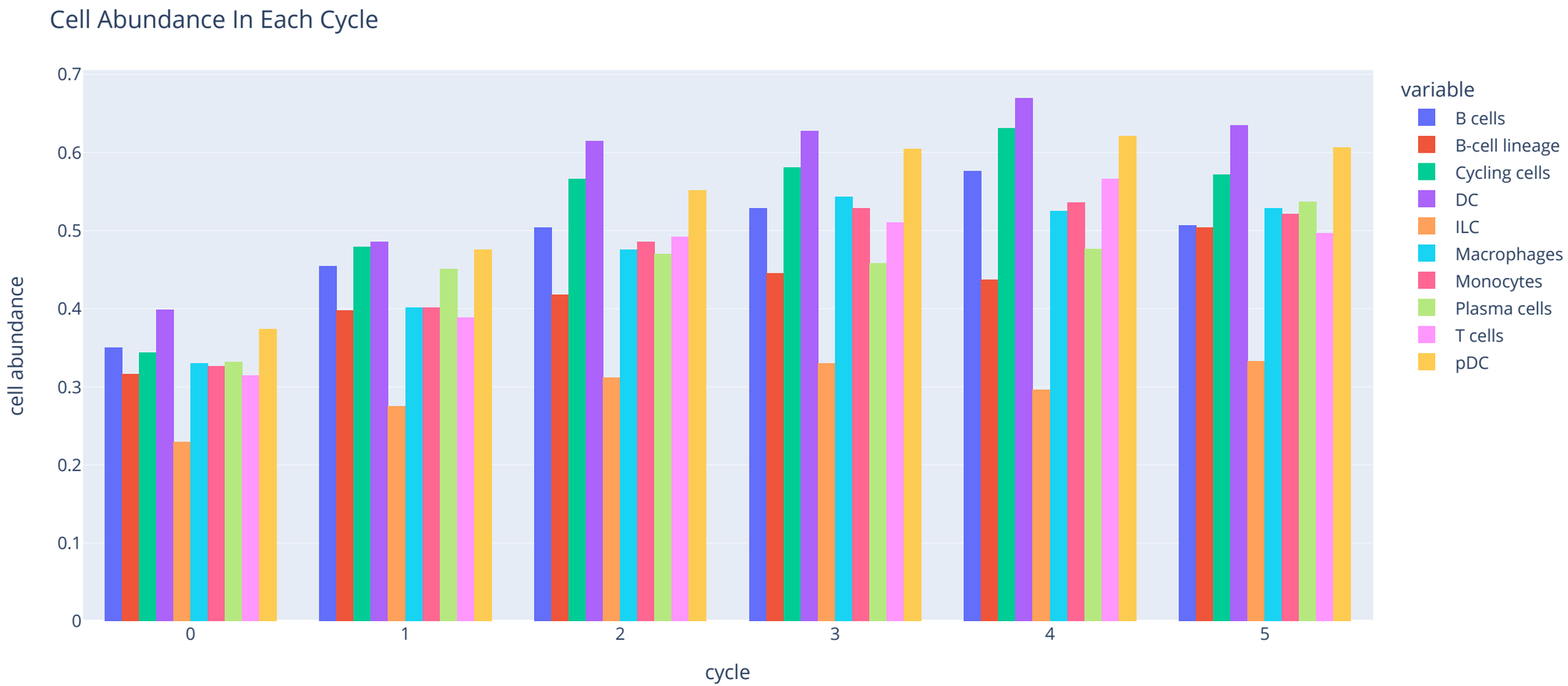
In [20]:
```
ss.pl.plot_cycle_abundance(cluster_df, cell_type_lt)
```




From the above bar graph, it can be observed that the expression levels of various immune cells increase with the number of cycles from 0 to 4, and slightly decrease at the 5th cycle. However, there are significant differences in expression levels among different cell types. For better comparison, the `ss.pp.minmax_scaler()` function can be used to map the expression levels of different cells to the range of 0-1.

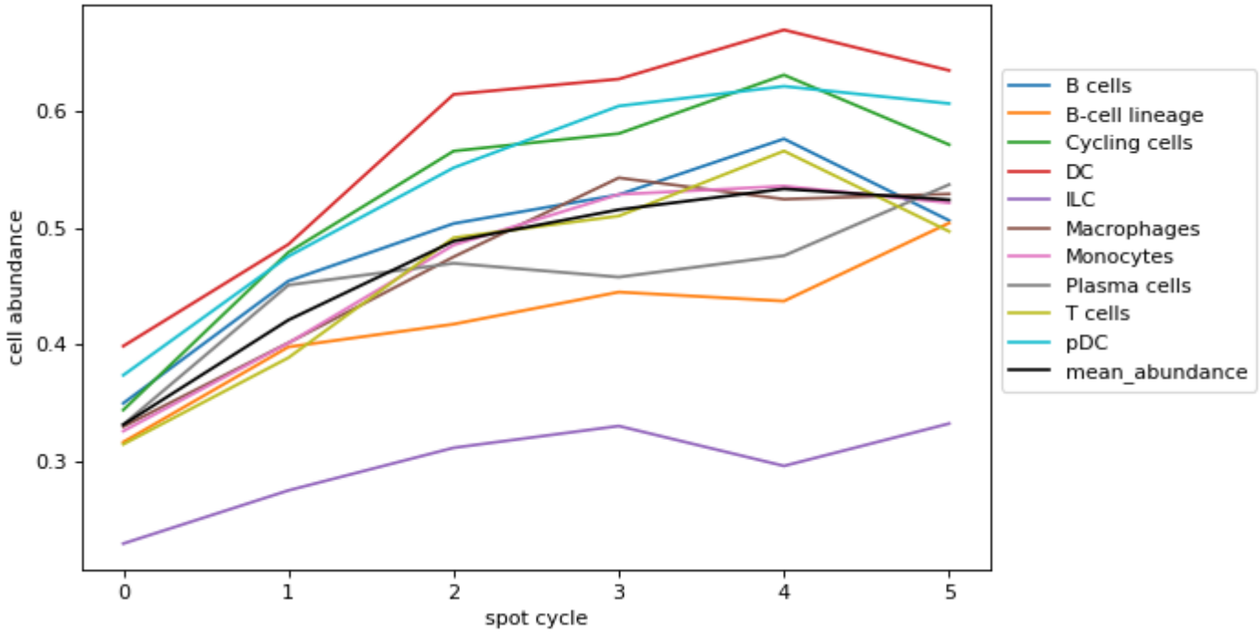
In [21]:
```
cluster_scale_df = ss.pp.minmax_scaler(cluster_df, cell_type_lt)
```

In [22]:
```
ss.pl.plot_cycle_abundance(cluster_scale_df, cell_type_lt)
```




To represent cell abundance using a line graph, the `ss.pl.plot_line_abundance()` function can be used.

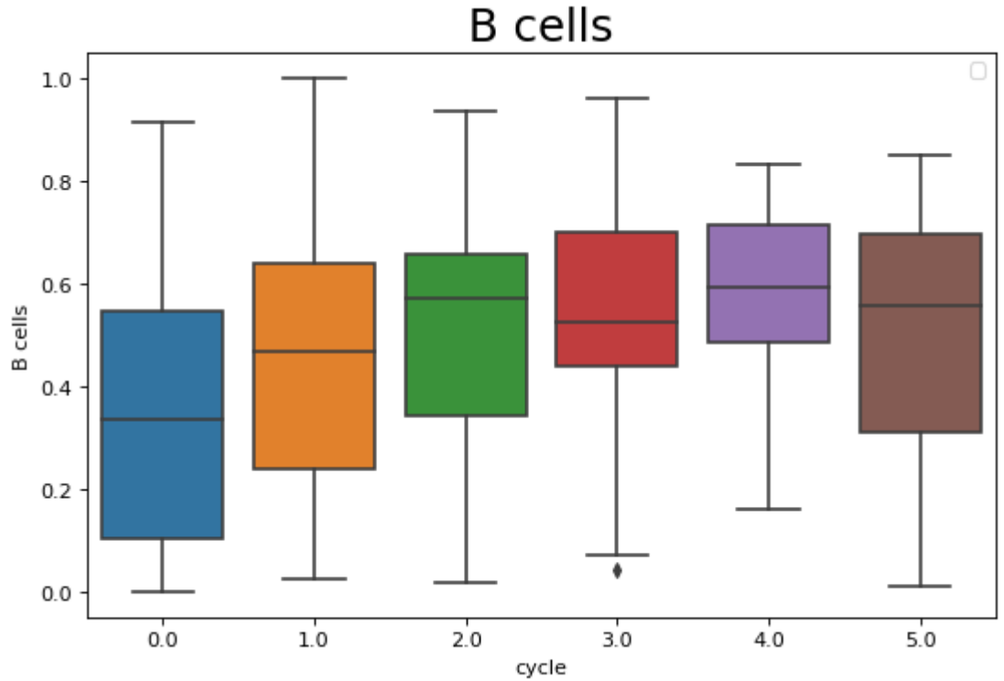
In [23]:
```
ss.pl.plot_line_abundance(cluster_scale_df, cell_type_lt)
```




To view the expression level of a specific cell type, the `ss.pl.plot_cell_abundance()` function can be used

In [24]:
```
ss.pl.plot_cell_abundance(cluster_scale_df, 'B cells')
```

```
WARNING:matplotlib.legend:No artists with labels found to put in legend.  Note that artists whose label start with an underscore are ignored when legend() is called with no argument.
```


B cells
1.0
0.8
0.6
0.4
0.2
0.0
B cells
0.0
1.0
2.0
3.0
4.0
5.0
cycle