In [2]:
```python
def install_reqs():
    #This defines a function named install_reqs.

    !pip install pandas
    #Installs the pandas library, used for reading and manipulating tabular data (li

    !pip install "matplotlib>=3.4"
    #Installs matplotlib version 3.4 or higher, used for plotting graphs and visuali

    !pip install numpy
    #Installs numpy, a fundamental package for numerical computations and arrays.

    !pip install statsmodels
    #Installs statsmodels, a library used for statistical tests (e.g., t-tests, Wilc

    !pip install scipy
    #Installs scipy, which contains scientific computing tools, including statistica

install_reqs()
# to install all the above dependencies uncomment the above line.
```

```
Defaulting to user installation because normal site-packages is not writeable
Requirement already satisfied: pandas in c:\programdata\anaconda3\lib\site-packag
es (2.3.0)
Requirement already satisfied: numpy>=1.22.4 in c:\users\hp\appdata\roaming\pytho
n\python310\site-packages (from pandas) (1.26.4)
Requirement already satisfied: python-dateutil>=2.8.2 in c:\programdata\anaconda3
\lib\site-packages (from pandas) (2.9.0.post0)
Requirement already satisfied: pytz>=2020.1 in c:\programdata\anaconda3\lib\site-
packages (from pandas) (2025.2)
Requirement already satisfied: tzdata>=2022.7 in c:\programdata\anaconda3\lib\sit
e-packages (from pandas) (2025.2)
Requirement already satisfied: six>=1.5 in c:\programdata\anaconda3\lib\site-pack
ages (from python-dateutil>=2.8.2->pandas) (1.17.0)
```

```
WARNING: Ignoring invalid distribution -atplotlib (c:\programdata\anaconda3\lib\s
ite-packages)
WARNING: Ignoring invalid distribution -orch (c:\programdata\anaconda3\lib\site-p
ackages)
WARNING: Ignoring invalid distribution -atplotlib (c:\programdata\anaconda3\lib\s
ite-packages)
WARNING: Ignoring invalid distribution -orch (c:\programdata\anaconda3\lib\site-p
ackages)
```

```
Defaulting to user installation because normal site-packages is not writeable
Requirement already satisfied: matplotlib>=3.4 in c:\programdata\anaconda3\lib\si
te-packages (3.10.0)
Requirement already satisfied: contourpy>=1.0.1 in c:\programdata\anaconda3\lib\s
ite-packages (from matplotlib>=3.4) (1.3.1)
Requirement already satisfied: cycler>=0.10 in c:\programdata\anaconda3\lib\site-
packages (from matplotlib>=3.4) (0.11.0)
Requirement already satisfied: fonttools>=4.22.0 in c:\programdata\anaconda3\lib
\site-packages (from matplotlib>=3.4) (4.55.3)
Requirement already satisfied: kiwisolver>=1.3.1 in c:\programdata\anaconda3\lib
\site-packages (from matplotlib>=3.4) (1.4.8)
Requirement already satisfied: numpy>=1.23 in c:\users\hp\appdata\roaming\python
\python310\site-packages (from matplotlib>=3.4) (1.26.4)
Requirement already satisfied: packaging>=20.0 in c:\programdata\anaconda3\lib\si
te-packages (from matplotlib>=3.4) (25.0)
Requirement already satisfied: pillow>=8 in c:\programdata\anaconda3\lib\site-pac
kages (from matplotlib>=3.4) (11.1.0)
Requirement already satisfied: pyparsing>=2.3.1 in c:\programdata\anaconda3\lib\s
ite-packages (from matplotlib>=3.4) (3.2.0)
Requirement already satisfied: python-dateutil>=2.7 in c:\programdata\anaconda3\l
ib\site-packages (from matplotlib>=3.4) (2.9.0.post0)
Requirement already satisfied: six>=1.5 in c:\programdata\anaconda3\lib\site-pack
ages (from python-dateutil>=2.7->matplotlib>=3.4) (1.17.0)
```

```
WARNING: Ignoring invalid distribution -atplotlib (c:\programdata\anaconda3\lib\s
ite-packages)
WARNING: Ignoring invalid distribution -orch (c:\programdata\anaconda3\lib\site-p
ackages)
WARNING: Ignoring invalid distribution -atplotlib (c:\programdata\anaconda3\lib\s
ite-packages)
WARNING: Ignoring invalid distribution -orch (c:\programdata\anaconda3\lib\site-p
ackages)
```

```
Defaulting to user installation because normal site-packages is not writeable
Requirement already satisfied: numpy in c:\users\hp\appdata\roaming\python\python
310\site-packages (1.26.4)
```

```
WARNING: Ignoring invalid distribution -atplotlib (c:\programdata\anaconda3\lib\s
ite-packages)
WARNING: Ignoring invalid distribution -orch (c:\programdata\anaconda3\lib\site-p
ackages)
WARNING: Ignoring invalid distribution -atplotlib (c:\programdata\anaconda3\lib\s
ite-packages)
WARNING: Ignoring invalid distribution -orch (c:\programdata\anaconda3\lib\site-p
ackages)
```

```
Defaulting to user installation because normal site-packages is not writeable
Requirement already satisfied: statsmodels in c:\programdata\anaconda3\lib\site-p
ackages (0.14.4)
Requirement already satisfied: numpy<3,>=1.22.3 in c:\users\hp\appdata\roaming\py
thon\python310\site-packages (from statsmodels) (1.26.4)
Requirement already satisfied: scipy!=1.9.2,>=1.8 in c:\programdata\anaconda3\lib
\site-packages (from statsmodels) (1.15.3)
Requirement already satisfied: pandas!=2.1.0,>=1.4 in c:\programdata\anaconda3\li
b\site-packages (from statsmodels) (2.3.0)
Requirement already satisfied: patsy>=0.5.6 in c:\programdata\anaconda3\lib\site-
packages (from statsmodels) (1.0.1)
Requirement already satisfied: packaging>=21.3 in c:\programdata\anaconda3\lib\si
te-packages (from statsmodels) (25.0)
Requirement already satisfied: python-dateutil>=2.8.2 in c:\programdata\anaconda3
\lib\site-packages (from pandas!=2.1.0,>=1.4->statsmodels) (2.9.0.post0)
Requirement already satisfied: pytz>=2020.1 in c:\programdata\anaconda3\lib\site-
packages (from pandas!=2.1.0,>=1.4->statsmodels) (2025.2)
Requirement already satisfied: tzdata>=2022.7 in c:\programdata\anaconda3\lib\sit
e-packages (from pandas!=2.1.0,>=1.4->statsmodels) (2025.2)
Requirement already satisfied: six>=1.5 in c:\programdata\anaconda3\lib\site-pack
ages (from python-dateutil>=2.8.2->pandas!=2.1.0,>=1.4->statsmodels) (1.17.0)
```

```
WARNING: Ignoring invalid distribution -atplotlib (c:\programdata\anaconda3\lib\s
ite-packages)
WARNING: Ignoring invalid distribution -orch (c:\programdata\anaconda3\lib\site-p
ackages)
WARNING: Ignoring invalid distribution -atplotlib (c:\programdata\anaconda3\lib\s
ite-packages)
WARNING: Ignoring invalid distribution -orch (c:\programdata\anaconda3\lib\site-p
ackages)
```

```
Defaulting to user installation because normal site-packages is not writeable
Requirement already satisfied: scipy in c:\programdata\anaconda3\lib\site-package
s (1.15.3)
Requirement already satisfied: numpy<2.5,>=1.23.5 in c:\users\hp\appdata\roaming
\python\python310\site-packages (from scipy) (1.26.4)
```

```
WARNING: Ignoring invalid distribution -atplotlib (c:\programdata\anaconda3\lib\s
ite-packages)
WARNING: Ignoring invalid distribution -orch (c:\programdata\anaconda3\lib\site-p
ackages)
WARNING: Ignoring invalid distribution -atplotlib (c:\programdata\anaconda3\lib\s
ite-packages)
WARNING: Ignoring invalid distribution -orch (c:\programdata\anaconda3\lib\site-p
ackages)
```

In [3]:
```python
import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
```

In [4]:
```python
from compass_analysis import cohens_d, wilcoxon_test, get_reaction_consistencies
```

In [5]:
```python
from matplotlib import __version__ as matplotlibversion
if matplotlibversion < "3.4":
    print("Matplotlib versions older than 3.4 may not be able to generate figure
```

```
Matplotlib versions older than 3.4 may not be able to generate figure 2E, as they
do not support alpha arrays
```

In [6]:
```python
import os
os.system("compass --data expression.tsv \
        --model RECON2_mat --species mus_musculus --media default-media --lamb
```

```
                        --and-function mean --output-dir extdata/Th17 --penalty-diffusion knn
                        --isoform-summing legacy --num-processes 50")
```

Out[6]: 0

In [7]:
```python
import os
print(os.getcwd())
```

C:\Users\HP\Tcon vs Treg\extdata

In [8]:
```python
reaction_penalties = pd.read_csv("reactions.tsv", sep="\t", index_col=0)
```

In [9]:
```python
import pandas as pd

# Load reactions.tsv
reaction_penalties = pd.read_csv("reactions.tsv", sep="\t", index_col=0)

# Extract cell IDs from the columns
cell_ids = reaction_penalties.columns

# Infer cell type from prefix
def infer_cell_type(cell_id):
    if cell_id.startswith("Treg"):
        return "Treg"
    elif cell_id.startswith("Tcon"):
        return "Tcon"
    elif cell_id.startswith("exTreg"):
        return "exTreg"
    else:
        return "Unknown"

cell_types = [infer_cell_type(cell) for cell in cell_ids]

# Create metadata DataFrame
cell_metadata = pd.DataFrame({
    "cell_id": cell_ids,
    "cell_type": cell_types
}).set_index("cell_id")

# Save to CSV
cell_metadata.to_csv("cell_metadata.csv")

print("✅ Created cell_metadata.csv with the following cell types:")
print(cell_metadata["cell_type"].value_counts())
```

```
✅ Created cell_metadata.csv with the following cell types:
cell_type
exTreg    20
Tcon      20
Treg      16
Name: count, dtype: int64
```

In [10]:
```python
cell_metadata = pd.read_csv("cell_metadata.csv", sep=",")
cell_metadata.set_index("cell_id", inplace=True)
```

In [11]:
```python
print(cell_metadata.columns.tolist())
print(cell_metadata.head())
```

```
                                      ['cell_type']
                                                          cell_type
             cell_id
             exTreg_Lymph_exTreg2_L1          exTreg
             Tcon_Lymph_Tcon9_L2                Tcon
             exTreg_Spleen_exTreg8_L1         exTreg
             Tcon_Spleen_Tcon5_L2               Tcon
             Treg_Spleen_Treg5_L1               Treg
```

In [12]:
```python
Treg_cells = cell_metadata.index[cell_metadata["cell_type"] == "Treg"]
Tcon_cells = cell_metadata.index[cell_metadata["cell_type"] == "Tcon"]
exTreg_cells = cell_metadata.index[cell_metadata["cell_type"] == "exTreg"]
```

In [13]:
```python
print(cell_metadata.columns.tolist())
```

```
['cell_type']
```

In [14]:
```python
print(cell_metadata.head())
print(cell_metadata.columns.tolist())
```

```
                                    cell_type
             cell_id
             exTreg_Lymph_exTreg2_L1          exTreg
             Tcon_Lymph_Tcon9_L2                Tcon
             exTreg_Spleen_exTreg8_L1         exTreg
             Tcon_Spleen_Tcon5_L2               Tcon
             Treg_Spleen_Treg5_L1               Treg
             ['cell_type']
```

In [15]:
```python
reaction_metadata = pd.read_csv("reaction_metadata.csv", index_col = 0)
```

In [16]:
```python
reaction_metadata.loc[['r0281']]
```

Out[16]:

	reaction_name	formula	associated_genes	subsystem	EC_num|
reaction_no_direction					
r0281	Putrescine:oxygen oxidoreductase (deaminating)...	1.00 * Water [e] + 1.00 * O2 [e] + 1.00 * Putr...	AOC1	Methionine and cysteine metabolism	1.4

In [17]:
```python
#This function is repeated here for clarity
def get_reaction_consistencies(compass_reaction_penalties, min_range=1e-3):
    """
        Converts the raw penalties outputs of compass into scores per reactions
    """
    df = -np.log(compass_reaction_penalties + 1)
    df = df[df.max(axis=1) - df.min(axis=1) >= min_range]
    df = df - df.min().min()
    return df
```

In [18]:
```python
reaction_consistencies = get_reaction_consistencies(reaction_penalties)
```

In [19]:
```python
common_cells = list(set(reaction_consistencies.columns).intersection(set(cell_me
reaction_consistencies = reaction_consistencies[common_cells]
```

In [20]:
```python
import pandas as pd

cell_metadata = pd.read_csv("cell_metadata.csv", index_col=0)

cell_metadata.columns = cell_metadata.columns.str.strip()
treg_sp_cells = cell_metadata[
    (cell_metadata["cell_type"] == "Treg") &
    (cell_metadata.index.str.contains("Spleen"))
].index

extreg_sp_cells = cell_metadata[
    (cell_metadata["cell_type"] == "exTreg") &
    (cell_metadata.index.str.contains("Spleen"))
].index
```

In [21]:
```python
wilcox_results = wilcoxon_test(reaction_consistencies, treg_sp_cells, extreg_sp_
wilcox_results['metadata_r_id'] = ""
for r in wilcox_results.index:
    if r in reaction_metadata.index:
        wilcox_results.loc[r, 'metadata_r_id'] = r
    elif r[:-4] in reaction_metadata.index:
        wilcox_results.loc[r, 'metadata_r_id'] = r[:-4]
    else:
        print("Should not occur")
```

In [22]:
```python
cell_metadata = pd.read_csv("cell_metadata.csv", index_col=0)

cell_metadata.columns = cell_metadata.columns.str.strip()

treg_sp_cells = cell_metadata[
    (cell_metadata["cell_type"] == "Treg") &
    (cell_metadata.index.str.contains("Spleen"))
].index

extreg_sp_cells = cell_metadata[
    (cell_metadata["cell_type"] == "exTreg") &
    (cell_metadata.index.str.contains("Spleen"))
].index
```

In [23]:
```python
print(reaction_consistencies.columns.tolist()[:10])
```

```
['Tcon_Spleen_Tcon5_L2', 'Tcon_Spleen_Tcon1_L1', 'Treg_Lymph_Treg7_L2', 'exTreg_S
pleen_exTreg10_L1', 'Treg_Lymph_Treg8_L1', 'exTreg_Lymph_exTreg5_L2', 'Tcon_Lymph
_Tcon8_L1', 'Tcon_Lymph_Tcon7_L1', 'exTreg_Lymph_exTreg3_L2', 'Treg_Spleen_Treg3_
L1']
```

In [24]:
```python
print(exTreg_cells[:10])
```

```
Index(['exTreg_Lymph_exTreg2_L1', 'exTreg_Spleen_exTreg8_L1',
       'exTreg_Spleen_exTreg7_L2', 'exTreg_Lymph_exTreg3_L2',
       'exTreg_Spleen_exTreg8_L2', 'exTreg_Lymph_exTreg2_L2',
       'exTreg_Lymph_exTreg3_L1', 'exTreg_Spleen_exTreg7_L1',
       'exTreg_Spleen_exTreg6_L2', 'exTreg_Lymph_exTreg4_L2'],
      dtype='object', name='cell_id')
```

In [25]:
```python
W = wilcox_results.merge(reaction_metadata, how='left',
                         left_on='metadata_r_id', right_index=True, validate='m:
W = W[W['confidence'].isin([0,4])]
W = W[~W['EC_number'].isna()]
W.loc[(W['formula'].map(lambda x: '[m]' not in x)) & (W['subsystem'] == "Citric
```

In [26]:
```python
wilcox_results.loc[['r0281_pos']]
```

Out[26]:

	wilcox_stat	wilcox_pval	cohens_d	adjusted_pval	metadata_r_id
r0281_pos	99.0	0.000246	2.663348	0.000531	r0281

In [27]:
```python
reaction_metadata.loc['r0281']['formula']
```

Out[27]:
```
'1.00 * Water [e] + 1.00 * O2 [e] + 1.00 * Putrescine [e] --> 1.00 * Ammonium
[e] + 1.00 * Hydrogen peroxide [e] + 1.00 * 4-Aminobutanal [e]\nAOC1'
```

In [97]:
```python
def plot_differential_scores(data, title, c):
    plt.figure(figsize=(10,10))
    axs = plt.gca()
    axs.scatter(data['cohens_d'], -np.log10(data['adjusted_pval']), c=c)
    axs.set_xlabel("Cohen's d", fontsize=16)
    axs.set_ylabel("-log10 (Wilcoxon-adjusted p)", fontsize=16)

    # Visual markers and title
    axs.set_xlim(-10, 10)
    axs.axvline(0, dashes=(3,3), c='black')
    axs.axhline(1, dashes=(3,3), c='black')
    axs.set_title(title, fontdict={'fontsize':20})

    # Arrows for sample identity
    axs.annotate('', xy=(0.5, -0.08), xycoords='axes fraction', xytext=(0, -0.08
                arrowprops=dict(arrowstyle="<-", color='#348C73', linewidth=4))
    axs.annotate('treg_ln_cells', xy=(0.75, -0.12), xycoords='axes fraction', fo
    axs.annotate('', xy=(0.5, -0.08), xycoords='axes fraction', xytext=(1, -0.08
                arrowprops=dict(arrowstyle="<-", color='#E92E87', linewidth=4))
    axs.annotate('extreg_ln_cells', xy=(0.25, -0.12), xycoords='axes fraction',

    # Dynamic annotation loop with variable offsets and labels
    for i, r in enumerate(data.index):
        if r in labeled_reactions:
            x = data.loc[r, 'cohens_d']
            y = -np.log10(data.loc[r, 'adjusted_pval'])

            dx = 30 if x >= 0 else -120   # horizontal offset
            dy = (i % 6) * 12 - 30        # vertical offset varies to reduce stac

            axs.annotate(
                labeled_reactions[r],
                (x, y),
                xytext=(dx, dy),
                textcoords='offset pixels',
                arrowprops=dict(arrowstyle="-", shrinkA=6, shrinkB=4),
                fontsize=10,
                zorder=5,   # make sure text is on top
                bbox=dict(boxstyle="round,pad=0.3", fc="white", ec="gray", alpha
            )
```
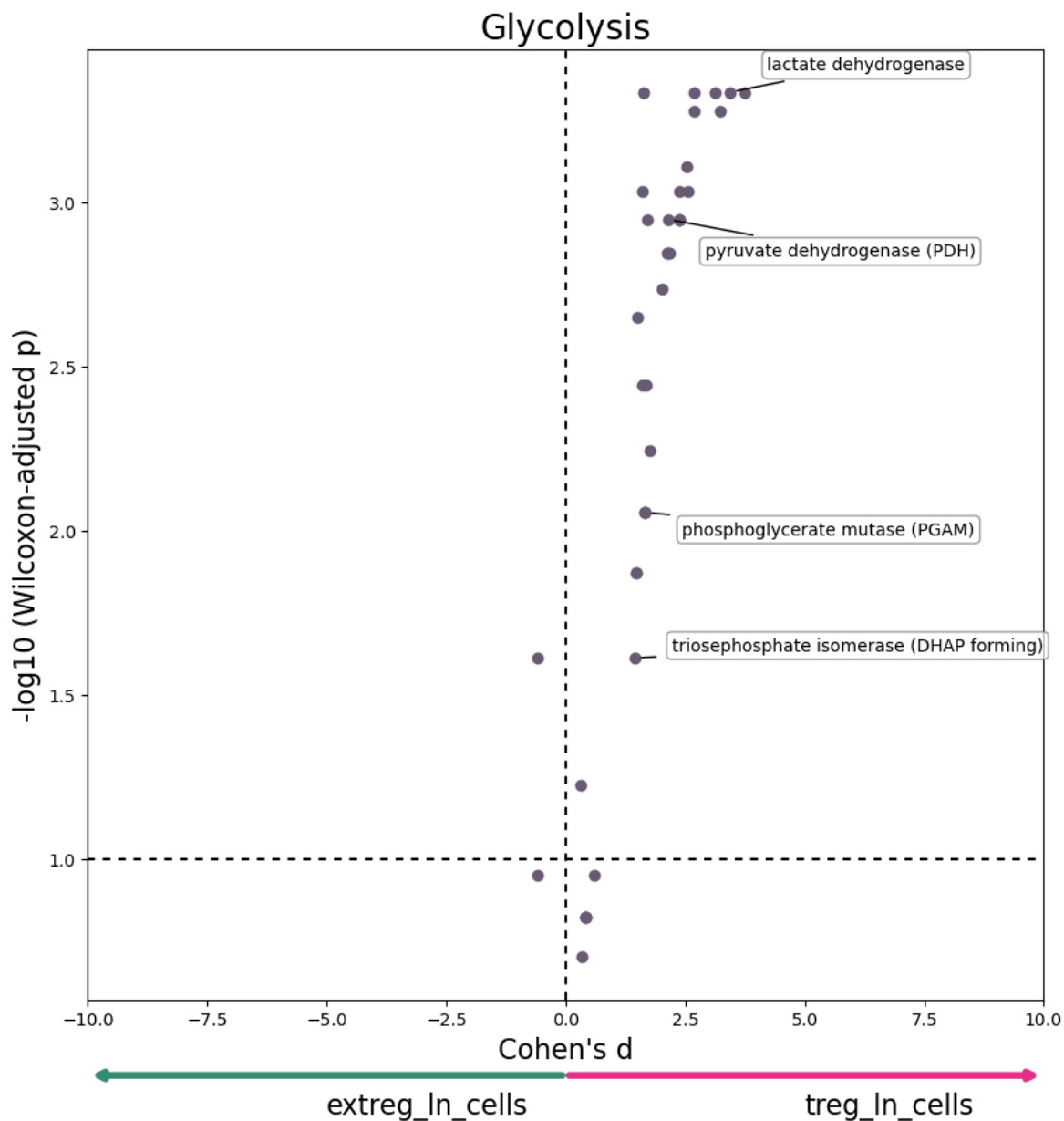
```
In [99]:  filtered_data = pd.concat([W[W['subsystem'] == "Glycolysis/gluconeogenesis"],
                        W[W['subsystem'] == "Citric acid cycle"],
                      W[W['subsystem'].isin(amino_acid_metab)],
                    W[W['subsystem'] == "Fatty acid oxidation"]])
```

```
In [101…  data = W[W['subsystem'] == "Glycolysis/gluconeogenesis"]
          plot_differential_scores(data, title='Glycolysis', c="#695D73")
```
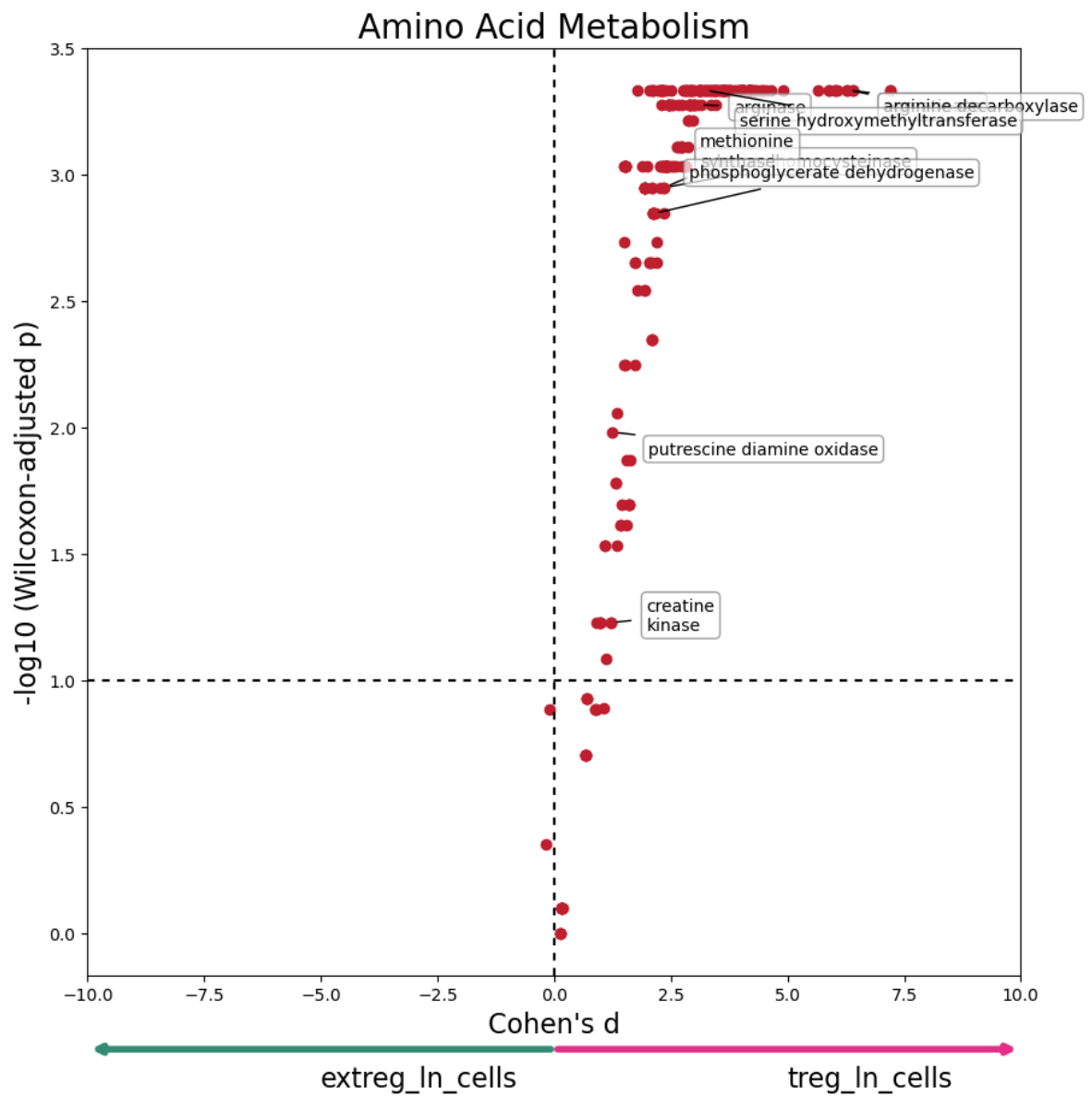


```
In [102…  data = W[W['subsystem'] == "Citric acid cycle"]
          plot_differential_scores(data, title="TCA Cycle", c="#D3A991")
```
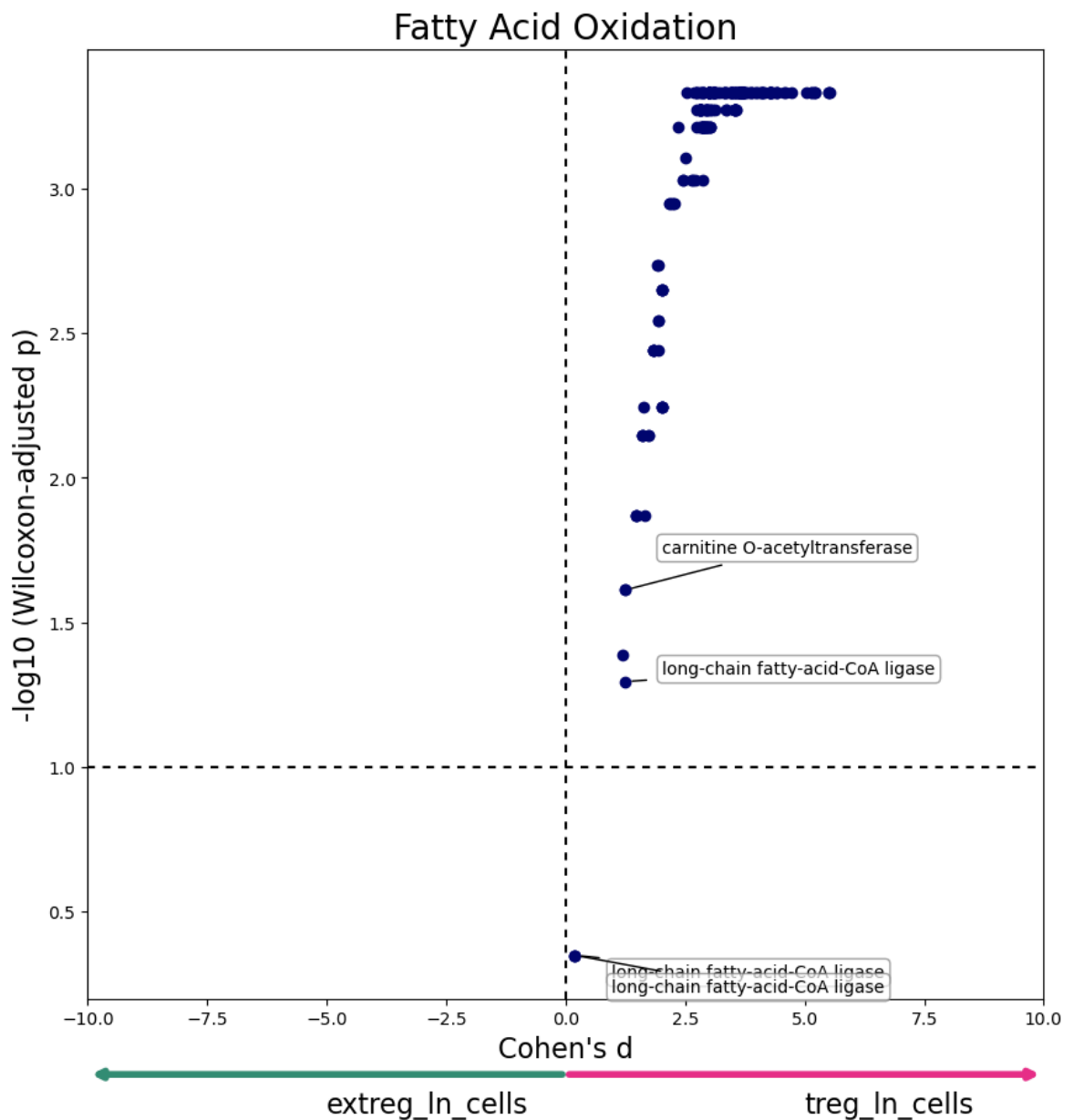
TCA Cycle
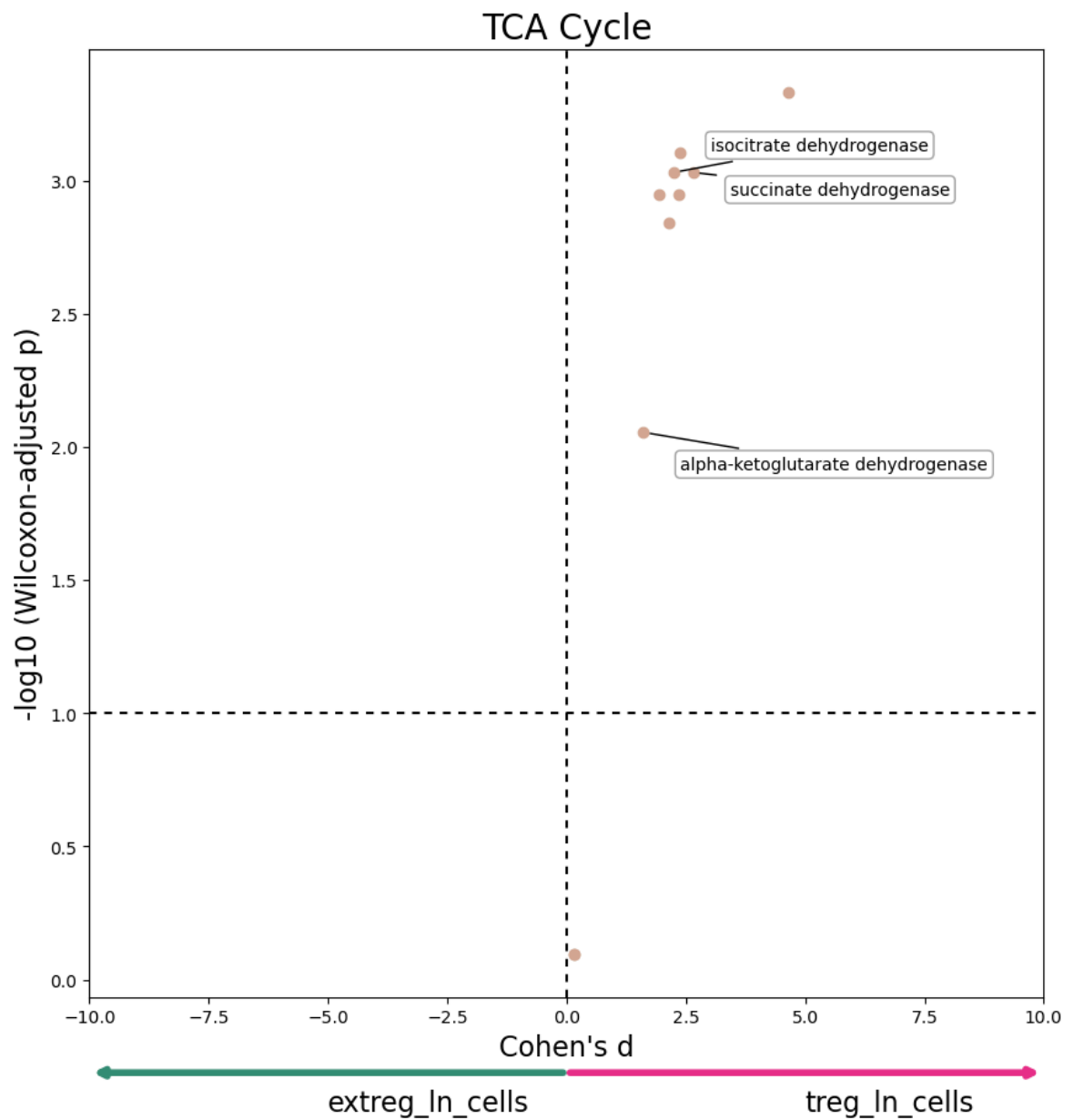
```
data = W[W['subsystem'].isin(amino_acid_metab)].copy()
data['adjusted_pval'] = data['adjusted_pval'].clip(1e-12)
plot_differential_scores(data, "Amino Acid Metabolism", c="#BF1E2E")
```

Amino Acid Metabolism

data = W[W['subsystem'] == "Fatty acid oxidation"]

```
data = W[W['subsystem'] == "Fatty acid oxidation"]
plot_differential_scores(data, "Fatty Acid Oxidation", c="#040772")
```

Fatty Acid Oxidation

```python
data = W[~W['subsystem'].isin(["Miscellaneous", "Unassigned"])]
data = data[~data['subsystem'].map(lambda x: "Transport" in x or "Exchange" in x
items, counts = np.unique(data['subsystem'], return_counts=True)
items = [items[i] for i in range(len(items)) if counts[i] > 5] #filter(n() > 5)
data = data[data['subsystem'].isin(items)]
```

```python
import matplotlib.pyplot as plt

plt.figure(figsize=(12, 12))
axs = plt.gca()

d = data[data['adjusted_pval'] < 0.1].groupby('subsystem')['cohens_d'].median().
d_sorted = d.sort_values()

axs.scatter(d_sorted, d_sorted.index, alpha=0)

color = data['cohens_d'].map(lambda x: 'r' if x >= 0 else 'b')
alpha = data['adjusted_pval'].map(lambda x: 1.0 if x < 0.1 else 0.25)

axs.scatter(data['cohens_d'], data['subsystem'], c=color, alpha=alpha)
```
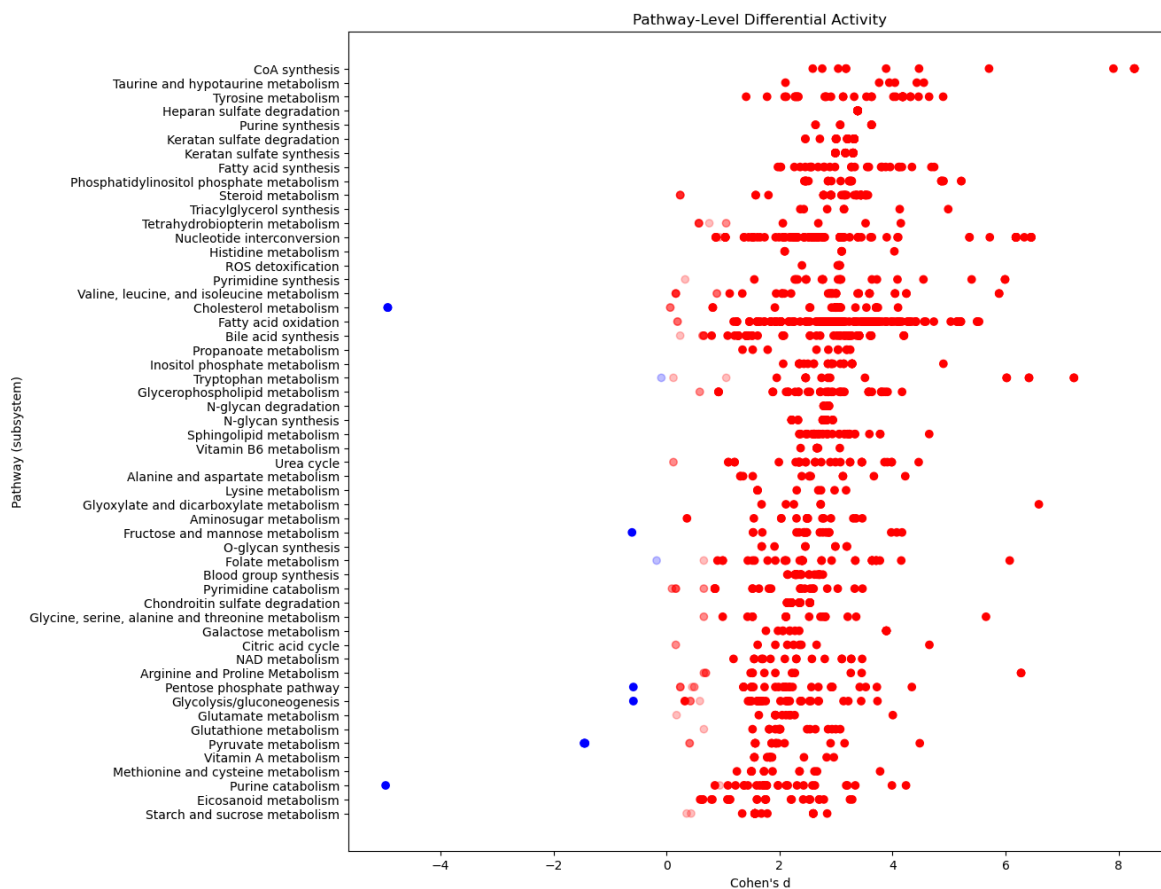
```
axs.set_xlabel("Cohen's d")
axs.set_ylabel("Pathway (subsystem)")
axs.set_title("Pathway-Level Differential Activity")
```

Out[35]: Text(0.5, 1.0, 'Pathway-Level Differential Activity')

Pathway-Level Differential Activity

In [36]:
```
reaction_penalties = pd.read_csv("reactions.tsv", sep="\t", index_col = 0)
reaction_penalties[reaction_penalties <= 1e-4] = 0
reaction_penalties = reaction_penalties[np.all(reaction_penalties != 0, axis=1)]
```

In [37]:
```
reaction_penalties = reaction_penalties[reaction_penalties.max(axis=1) - reactio
```

In [38]:
```
meta_rxns_map = get_metareactions(reaction_penalties)
meta_rxns = reaction_penalties.join(pd.DataFrame(meta_rxns_map, columns=["meta_r
```

In [39]:
```
meta_rxn_consistencies = get_reaction_consistencies(meta_rxns)
```

In [40]:
```
treg_cells = cell_metadata.index[cell_metadata["cell_type"] == "treg_sp_cells"]
tcon_cells = cell_metadata.index[cell_metadata["cell_type"] == "extreg_sp_cells"

wilcox_meta_rxn_results = wilcoxon_test(meta_rxn_consistencies, treg_sp_cells, e
```

In [41]:
```
wilcox_meta_rxn_results.iloc[0:1]
```

Out[41]:

meta_rxn_id	wilcox_stat	wilcox_pval	cohens_d	adjusted_pval
1	81.0	0.021134	1.26674	0.02496

```python
In [42]: wilcox_meta_rxn_expanded = pd.DataFrame(index=reaction_penalties.index, columns=
         for i in range(len(wilcox_meta_rxn_expanded.index)):
             if (meta_rxns_map[i] in wilcox_meta_rxn_results.index):
                 wilcox_meta_rxn_expanded.loc[wilcox_meta_rxn_expanded.index[i]] = wilcox
         wilcox_meta_rxn_expanded = wilcox_meta_rxn_expanded.dropna().astype('float64')
```

```python
In [43]: wilcox_meta_rxn_expanded['metadata_r_id'] = ""
         for r in wilcox_meta_rxn_expanded.index:
             if r in reaction_metadata.index:
                 wilcox_meta_rxn_expanded.loc[r, 'metadata_r_id'] = r
             elif r[:-4] in reaction_metadata.index:
                 wilcox_meta_rxn_expanded.loc[r, 'metadata_r_id'] = r[:-4]
             else:
                 print("Should not occur")
```

```python
In [44]: wilcox_meta_rxn_expanded.iloc[0:1]
```

Out[44]:

	wilcox_stat	wilcox_pval	cohens_d	adjusted_pval	metadata_r_id
10FTHF5GLUtl_pos	97.0	0.00044	2.656367	0.000821	10FTHF5GLUtl

```python
In [45]: outputs = {
             "wilcox_results.csv": wilcox_results,
             "reaction_consistencies.csv": reaction_consistencies,
             "reaction_metadata.csv": reaction_metadata,
             "wilcox_meta_rxn_results.csv": wilcox_meta_rxn_results,
             "wilcox_meta_rxn_expanded.csv": wilcox_meta_rxn_expanded,
             "final_stats_with_metadata.csv": W,
         }

         for name, df in outputs.items():
             df.to_csv(name)
```

```python
In [46]: import zipfile

         with zipfile.ZipFile("Treg SP vs exTreg SP.zip", "w") as zipf:
             for filename in outputs:
                 zipf.write(filename)
```

```python
In [47]: from IPython.display import FileLink
         FileLink("Treg SP vs exTreg SP.zip")
```

Out[47]: Treg SP vs exTreg SP.zip

```python
In [ ]:
```

```python
In [ ]:
```

```python
In [ ]:
```