

```
In [1]: 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.
```

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[notice] To update, run: python.exe -m pip install --upgrade pip

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Requirement already satisfied: six>=1.5 in c:\programdata\anaconda3\lib\site-pack
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Defaulting to user installation because normal site-packages is not writeable
Requirement already satisfied: scipy in c:\programdata\anaconda3\lib\site-package
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Requirement already satisfied: numpy<2.5,>=1.23.5 in c:\users\hp\appdata\roaming
\python\python310\site-packages (from scipy) (1.26.4)

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WARNING: Ignoring invalid distribution -atplotlib (c:\programdata\anaconda3\lib\s
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```

```

In [2]: import pandas as pd
import numpy as np
import matplotlib.pyplot as plt

```

```

In [3]: from compass_analysis import cohens_d, wilcoxon_test, get_reaction_consistencies

```

```

In [4]: 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 [5]: 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[5]: 0

```
In [6]: import os
print(os.getcwd())
```

C:\Users\HP\Human datasets

```
In [7]: reaction_penalties = pd.read_csv("reactions.tsv", sep="\t", index_col=0)
```

```
In [8]: 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("Tregs"):
        return "Tregs"
    elif cell_id.startswith("Tn"):
        return "Tn"
    elif cell_id.startswith("Th1"):
        return "Th1"
    elif cell_id.startswith("exTregs"):
        return "exTregs"
    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
Tregs      14
Tn          14
Th1         14
exTregs    14
Name: count, dtype: int64
```

```
In [9]: cell_metadata = pd.read_csv("cell_metadata.csv", sep=",")
        cell_metadata.set_index("cell_id", inplace=True)
```

```
In [10]: print(cell_metadata.columns.tolist())
         print(cell_metadata.head())
```

```
['cell_type']
      cell_type
cell_id
Tregs_Don7_Ln2    Tregs
Tregs_Don7_Ln1    Tregs
Tregs_Don6_Ln1    Tregs
Tregs_Don6_Ln2    Tregs
Tregs_Don5_Ln2    Tregs
```

```
In [11]: Tregs_cells = cell_metadata.index[cell_metadata["cell_type"] == "Tregs"]
         Tn_cells = cell_metadata.index[cell_metadata["cell_type"] == "Tn"]
         Th1_cells = cell_metadata.index[cell_metadata["cell_type"] == "Th1"]
         exTregs_cells = cell_metadata.index[cell_metadata["cell_type"] == "exTregs"]
```

```
In [12]: print(cell_metadata.columns.tolist())
```

```
['cell_type']
```

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

```
      cell_type
cell_id
Tregs_Don7_Ln2    Tregs
Tregs_Don7_Ln1    Tregs
Tregs_Don6_Ln1    Tregs
Tregs_Don6_Ln2    Tregs
Tregs_Don5_Ln2    Tregs
['cell_type']
```

```
In [14]: reaction_metadata = pd.read_csv("reaction_metadata.csv", index_col = 0)
```

```
In [15]: reaction_metadata.loc[['r0281']]
```

```
Out[15]:
```

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

reaction_no_direction

1.00 *
Water
[e] +
1.00 *
O2 [e] +
1.00 *
Putr...

AOC1

Methionine and cysteine metabolism

1.4



```
In [16]: #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 [17]: reaction_consistencies = get_reaction_consistencies(reaction_penalties)
```

```
In [18]: common_cells = list(set(reaction_consistencies.columns).intersection(set(cell_me
reaction_consistencies = reaction_consistencies[common_cells])
```

```
In [20]: wilcox_results = wilcoxon_test(reaction_consistencies, Tregs_cells, exTregs_cell
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]: print(reaction_consistencies.columns.tolist()[:10])
```

```
['Tregs_Don6_Ln1', 'Th1_Don6_Ln1', 'Th1_Don3_Ln1', 'Tregs_Don2_Ln2', 'Th1_Don2_Ln
2', 'Th1_Don1_Ln2', 'Th1_Don4_Ln1', 'Tn_Don1_Ln2', 'Th1_Don7_Ln2', 'Tn_Don2_Ln1']
```

```
In [41]: print(Tregs_cells[:10])
```

```
Index(['Tregs_Don7_Ln2', 'Tregs_Don7_Ln1', 'Tregs_Don6_Ln1', 'Tregs_Don6_Ln2',
      'Tregs_Don5_Ln2', 'Tregs_Don5_Ln1', 'Tregs_Don4_Ln2', 'Tregs_Don4_Ln1',
      'Tregs_Don3_Ln2', 'Tregs_Don3_Ln1'],
      dtype='object', name='cell_id')
```

```
In [43]: # Run the test
wilcox_results = wilcoxon_test(reaction_consistencies, Tregs_cells, exTregs_cell

# Annotate metadata keys
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(f"Should not occur → {r}")
```

```
In [44]: # Run the test
wilcox_results = wilcoxon_test(reaction_consistencies, Tregs_cells, exTregs_cell

# Annotate metadata keys
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(f"Should not occur → {r}")
```

```
In [45]: 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 [46]: wilcox_results.loc[['r0281_pos']]
```

```
Out[46]:
```

	wilcox_stat	wilcox_pval	cohens_d	adjusted_pval	metadata_r_id
r0281_pos	196.0	0.000007	4.499246	0.000013	r0281

```
In [47]: reaction_metadata.loc['r0281']['formula']
```

```
Out[47]: '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 [105... 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(-8, 12)
    axs.set_ylim(0, 8)
    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('Tregs_cells', xy=(0.75, -0.12), xycoords='axes fraction', font
    axs.annotate('', xy=(0.5, -0.08), xycoords='axes fraction', xytext=(1, -0.08),
        arrowprops=dict(arrowstyle="<-", color='#E92E87', linewidth=4))
    axs.annotate('exTregs_cells', xy=(0.25, -0.12), xycoords='axes fraction', fo

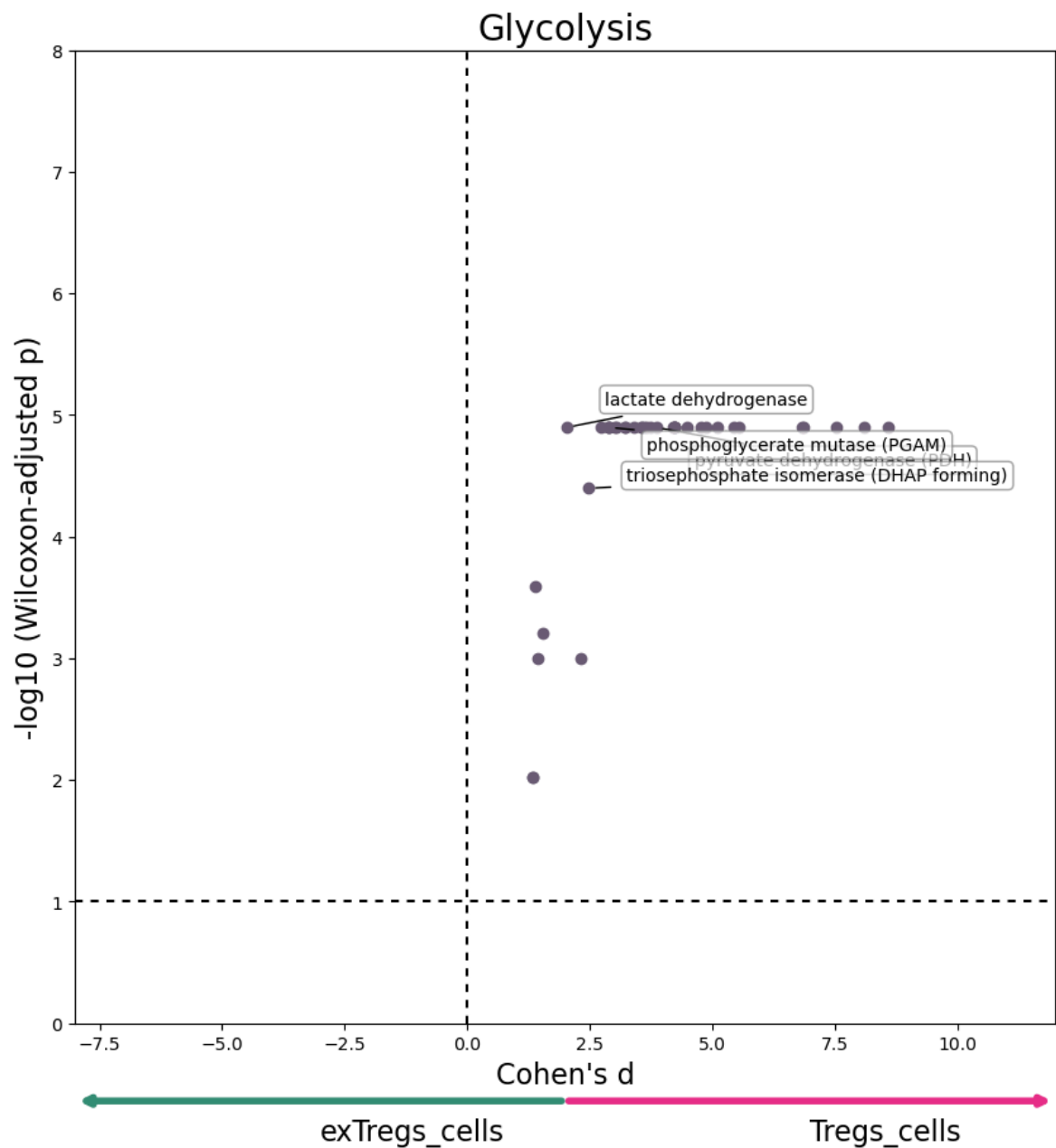
    # Dynamic annotation Loop with variable offsets and Labels
    for i, r in enumerate(data.index):
        if r in labeled_reactions:
            x = data.loc[r, 'cohens_d']
            y = -np.log10(data.loc[r, 'adjusted_pval'])

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

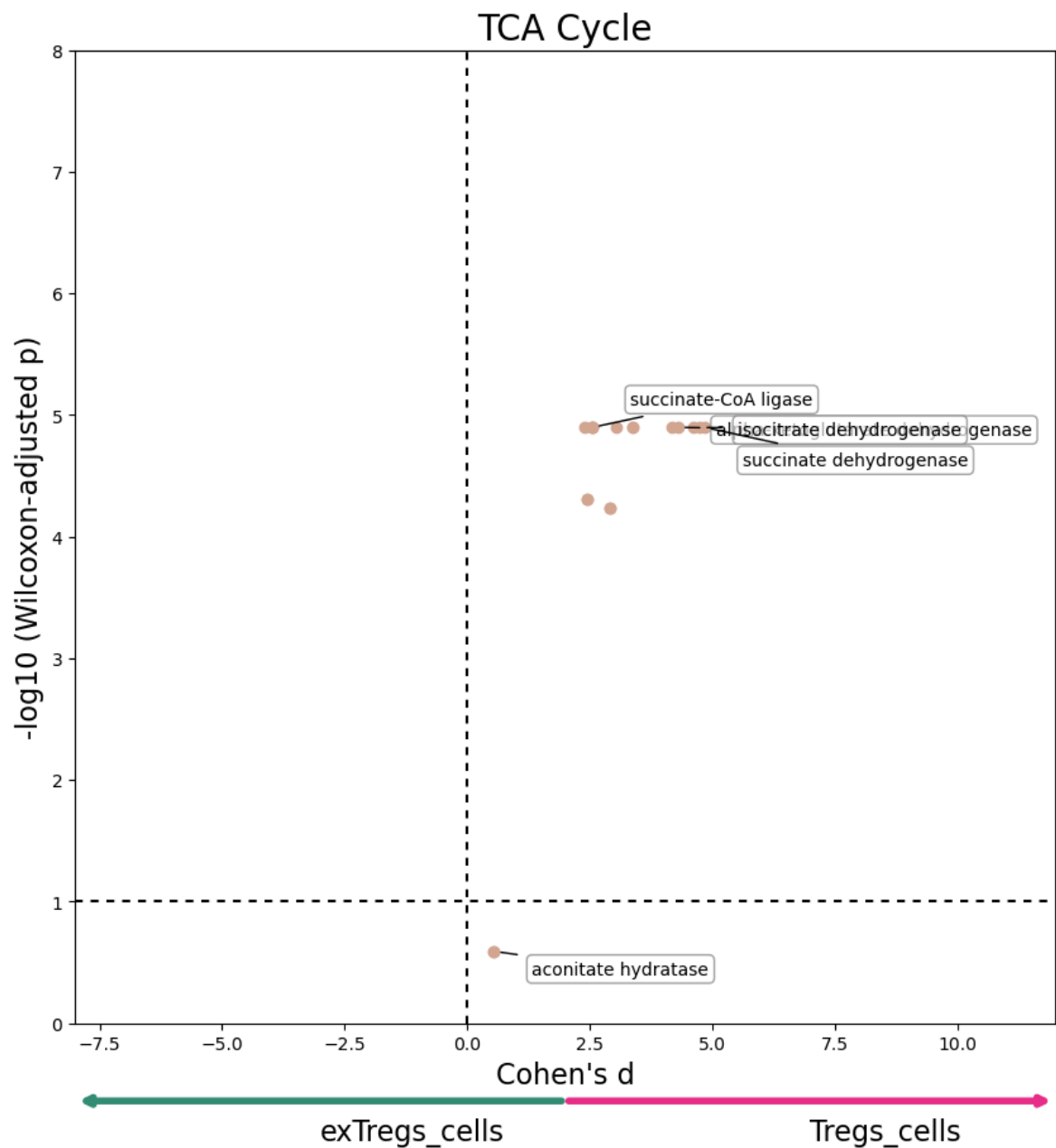
            axs.annotate(
                labeled_reactions[r],
                (x, y),
                xytext=(dx, dy),
                textcoords='offset pixels',
                arrowprops=dict(arrowstyle="-", shrinkA=6, shrinkB=4),
                fontsize=10,
                zorder=5, # make sure text is on top
                bbox=dict(boxstyle="round,pad=0.3", fc="white", ec="gray", alpha
            )
```

```
In [107... 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"]])
```

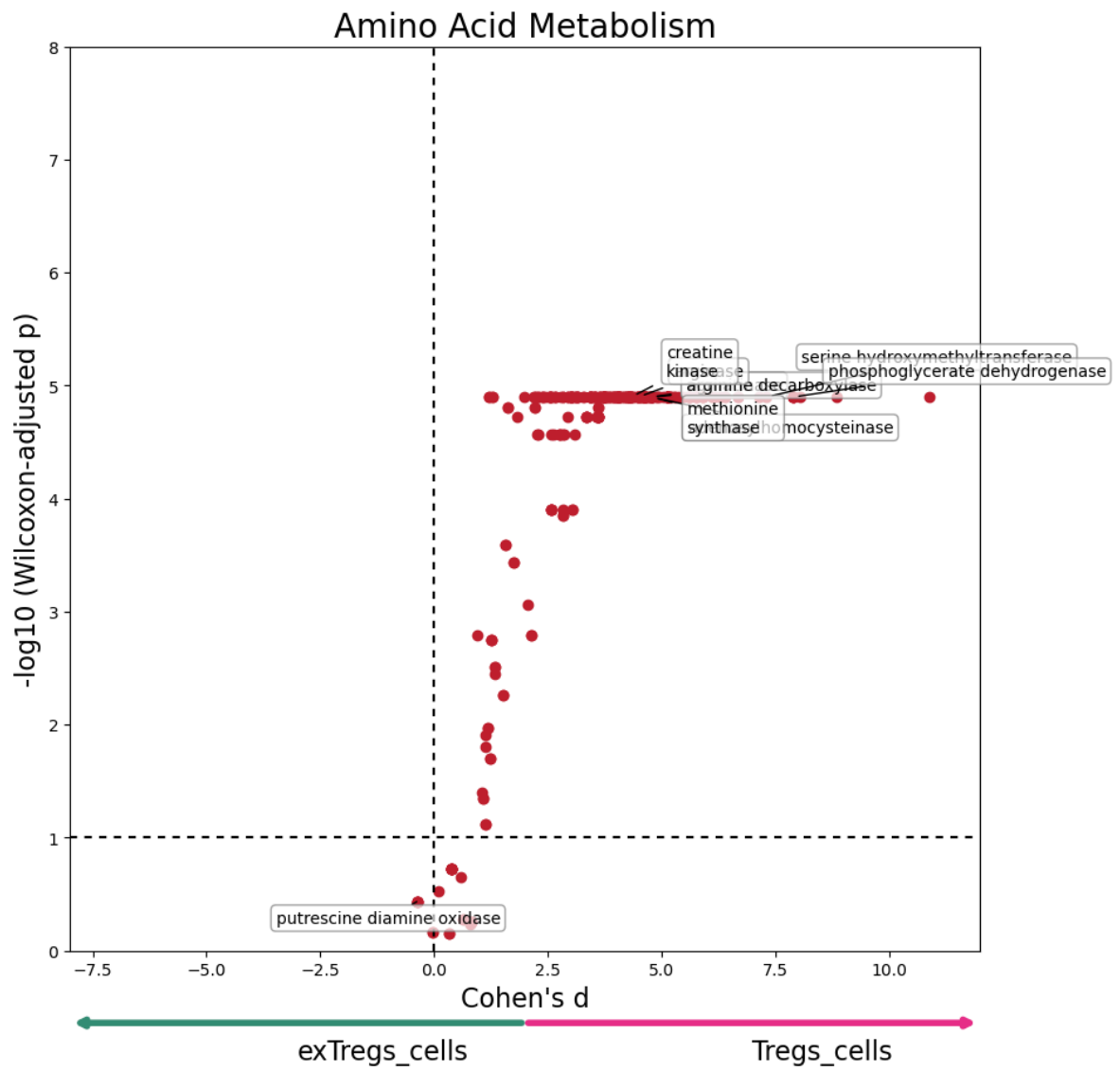
```
In [109... data = W[W['subsystem'] == "Glycolysis/gluconeogenesis"]
plot_differential_scores(data, title='Glycolysis', c="#695D73")
```

```
In [111... data = W[W['subsystem'] == "Citric acid cycle"]
plot_differential_scores(data, title="TCA Cycle", c="#D3A991")
```

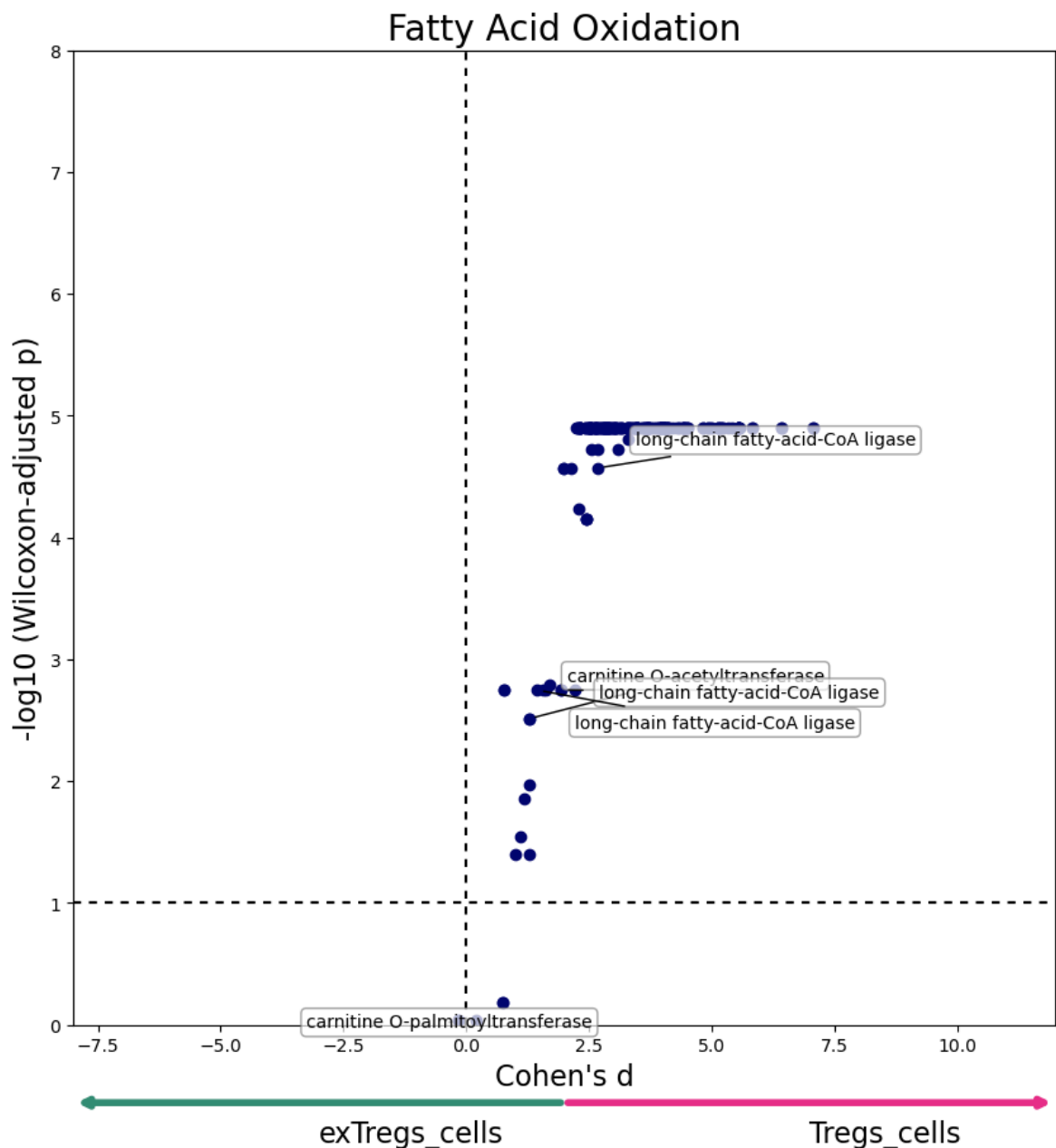


```
In [113... data = W[W['subsystem'].isin(amino_acid_metab)].copy()
data['adjusted_pval'] = data['adjusted_pval'].clip(1e-12)
plot_differential_scores(data, "Amino Acid Metabolism", c="#BF1E2E")
```



In [115...

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



```
In [117...] 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)]
```

```
In [119...] 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)

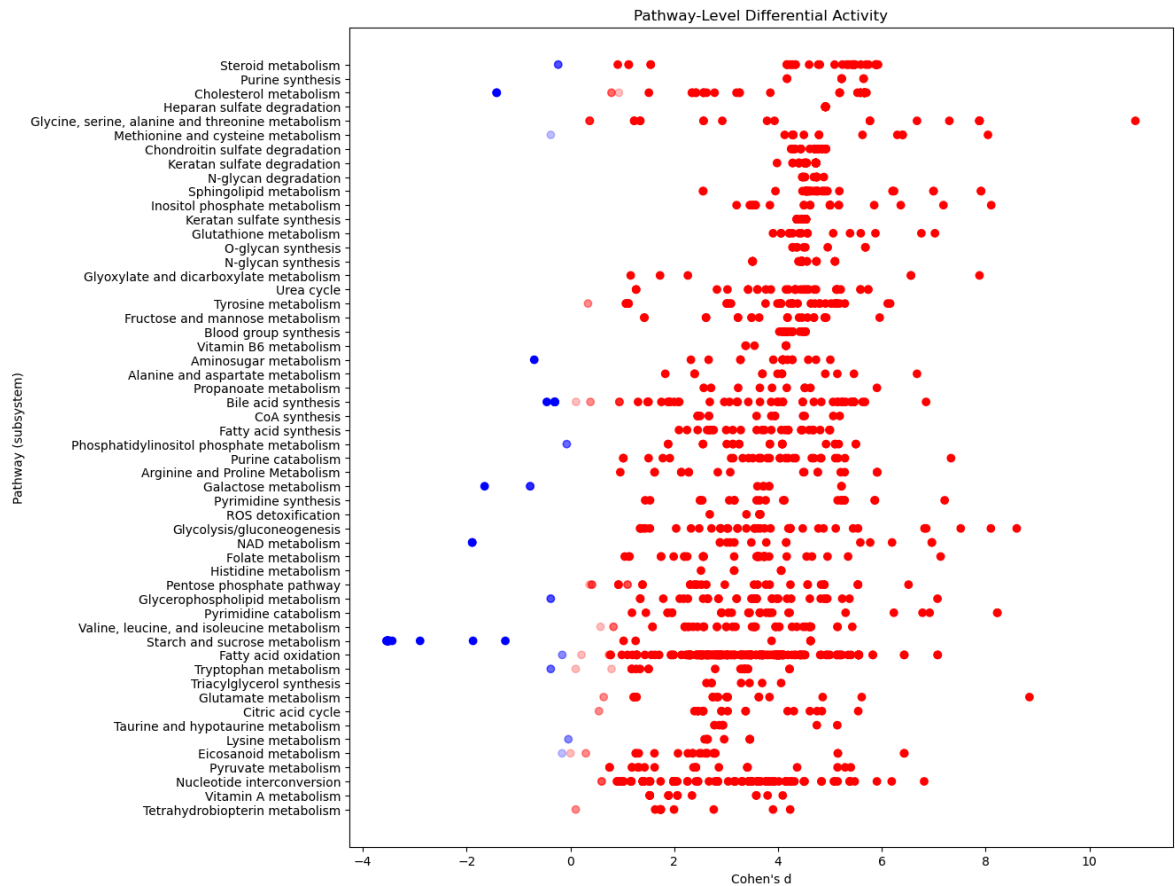
axs.scatter(data['cohens_d'], data['subsystem'], c=color, alpha=alpha)
```

```

axs.set_xlabel("Cohen's d")
axs.set_ylabel("Pathway (subsystem)")
axs.set_title("Pathway-Level Differential Activity")

```

Out[119...] Text(0.5, 1.0, 'Pathway-Level Differential Activity')



```

In [56]: 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 [57]: reaction_penalties = reaction_penalties[reaction_penalties.max(axis=1) - reaction_penalties.min(axis=1) > 0]

```

```

In [58]: meta_rxns_map = get_metareactions(reaction_penalties)
meta_rxns = reaction_penalties.join(pd.DataFrame(meta_rxns_map, columns=["meta_rxn_id", "meta_rxn_name"]))

```

```

In [59]: meta_rxn_consistencies = get_reaction_consistencies(meta_rxns)

```

```

In [61]: treg_cells = cell_metadata.index[cell_metadata["cell_type"] == "Tregs"]
tcon_cells = cell_metadata.index[cell_metadata["cell_type"] == "exTregs"]

wilcox_meta_rxn_results = wilcoxon_test(meta_rxn_consistencies, Tregs_cells, exTregs_cells)

```

```

In [62]: wilcox_meta_rxn_results.iloc[0:1]

```

```

Out[62]:
      wilcox_stat  wilcox_pval  cohens_d  adjusted_pval
meta_rxn_id
1             153.0     0.012275   0.965008     0.014344

```

```
In [63]: 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')
```

```
In [64]: 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")
```

```
In [65]: wilcox_meta_rxn_expanded.iloc[0:1]
```

```
Out[65]:
```

	wilcox_stat	wilcox_pval	cohens_d	adjusted_pval	metadata_r_id
10FTHF5GLUtl_pos	194.0	0.000011	3.603294	0.000019	10FTHF5GLUtl

```
In [66]: 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)
```

```
In [78]: import zipfile

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

```
In [82]: from IPython.display import FileLink
FileLink("Tregs vs exTregs.zip")
```

```
Out[82]: Tregs vs exTregs.zip
```

```
In [ ]:
```