In [46]:
```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)
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\lib\site-packages (from pandas) (2.9.0.post0)
Requirement already satisfied: pytz>=2020.1 in c:\programdata\anaconda3\lib\site-
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[notice] A new release of pip is available: 25.1.1 -> 25.2
[notice] To update, run: python.exe -m pip install --upgrade pip
```

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Defaulting to user installation because normal site-packages is not writeable
Requirement already satisfied: matplotlib>=3.4 in c:\programdata\anaconda3\lib\si
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\site-packages (from matplotlib>=3.4) (4.55.3)
Requirement already satisfied: kiwisolver>=1.3.1 in c:\programdata\anaconda3\lib
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Requirement already satisfied: numpy>=1.23 in c:\users\hp\appdata\roaming\python
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Requirement already satisfied: six>=1.5 in c:\programdata\anaconda3\lib\site-pack
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Requirement already satisfied: numpy in c:\users\hp\appdata\roaming\python\python
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[notice] To update, run: python.exe -m pip install --upgrade pip
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```
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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
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Requirement already satisfied: pytz>=2020.1 in c:\programdata\anaconda3\lib\site-
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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
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```

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

[notice] A new release of pip is available: 25.1.1 -> 25.2
[notice] To update, run: python.exe -m pip install --upgrade pip
```

In [47]:
```python
import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
```

In [48]:
```python
from compass_analysis import cohens_d, wilcoxon_test, get_reaction_consistencies
```

In [49]:
```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 [50]: 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[50]: 0

```
In [51]: import os
         print(os.getcwd())
```

C:\Users\HP\Human datasets

```
In [52]: reaction_penalties = pd.read_csv("reactions.tsv", sep="\t", index_col=0)
```

```
In [53]: 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 [54]:  cell_metadata = pd.read_csv("cell_metadata.csv", sep=",")
          cell_metadata.set_index("cell_id", inplace=True)
```

```
In [55]:  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 [56]:  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 [57]:  print(cell_metadata.columns.tolist())
```

```
['cell_type']
```

```
In [58]:  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 [59]:  reaction_metadata = pd.read_csv("reaction_metadata.csv", index_col = 0)
```

```
In [60]:  reaction_metadata.loc[['r0281']]
```

Out[60]:

	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

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```
In [61]:  #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 [62]:
```
reaction_consistencies = get_reaction_consistencies(reaction_penalties)
```

In [63]:
```
common_cells = list(set(reaction_consistencies.columns).intersection(set(cell_me
reaction_consistencies = reaction_consistencies[common_cells]
```

In [64]:
```
wilcox_results = wilcoxon_test(reaction_consistencies, Tregs_cells, Th1_cells)
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 [65]:
```
print(reaction_consistencies.columns.tolist()[:10])
```

```
['Tregs_Don5_Ln1', 'Tregs_Don4_Ln2', 'exTregs_Don4_Ln1', 'exTregs_Don7_Ln1', 'Tre
gs_Don3_Ln2', 'Th1_Don6_Ln2', 'Tn_Don7_Ln2', 'Th1_Don3_Ln1', 'Th1_Don2_Ln2', 'Th1
_Don5_Ln2']
```

In [66]:
```
print(Treg_cells[:10])
```

```
Index([], dtype='object', name='cell_id')
```

In [67]:
```
# Run the test
wilcox_results = wilcoxon_test(reaction_consistencies, Tregs_cells, Th1_cells)

# 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 [68]:
```
# Run the test
wilcox_results = wilcoxon_test(reaction_consistencies, Tregs_cells, Th1_cells)

# 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 [69]:
```
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 [70]:
```
wilcox_results.loc[['r0281_pos']]
```

Out[70]:

	wilcox_stat	wilcox_pval	cohens_d	adjusted_pval	metadata_r_id
r0281_pos	98.0	1.0	0.07424	1.0	r0281

In [71]:
```
reaction_metadata.loc['r0281']['formula']
```

Out[71]:
```
'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 [142...]
```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(-8, 8)
    axs.set_ylim(0, 5)
    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('Th1_cells', xy=(0.25, -0.12), xycoords='axes fraction', fontsi

    # 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
            )
```
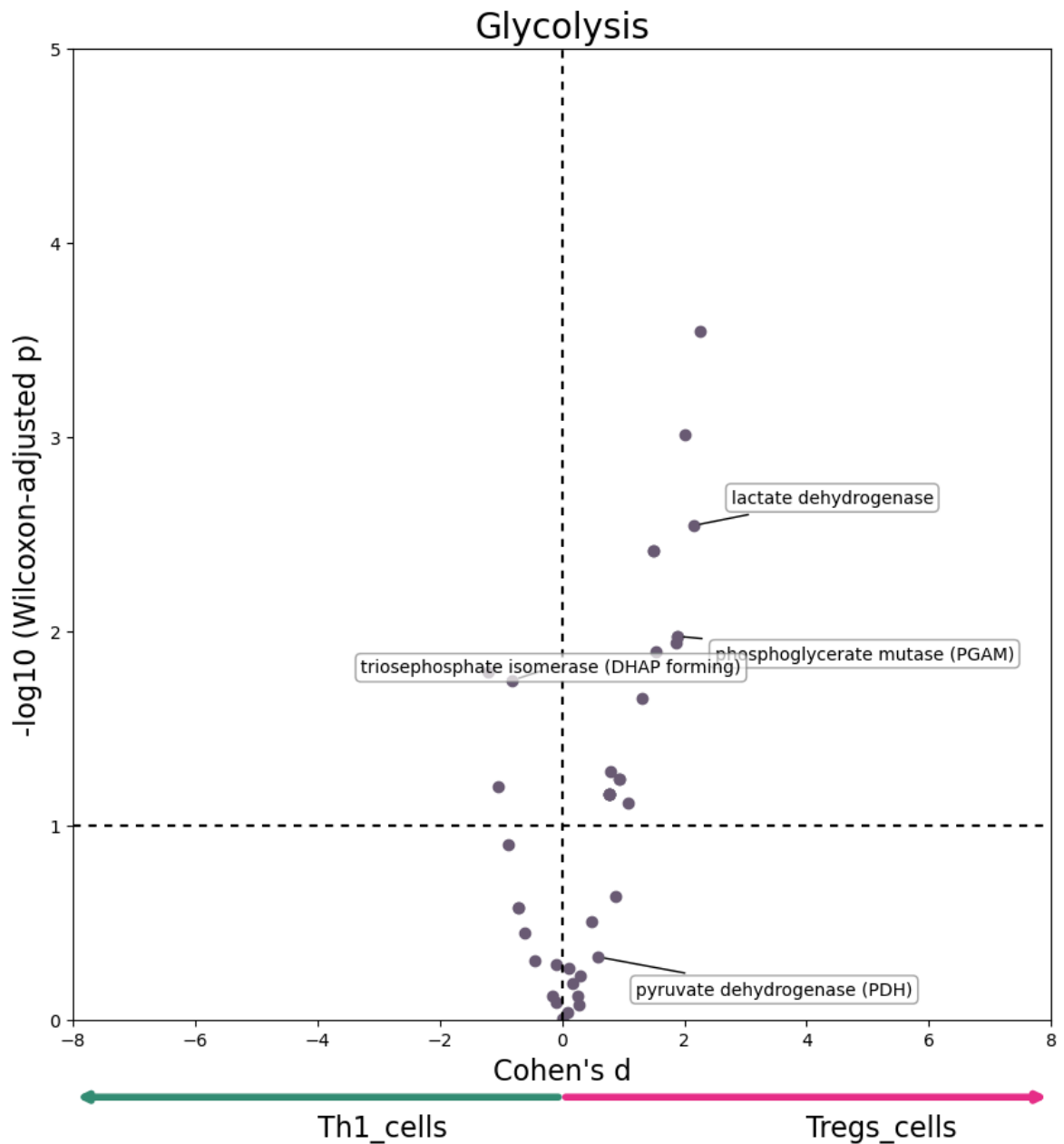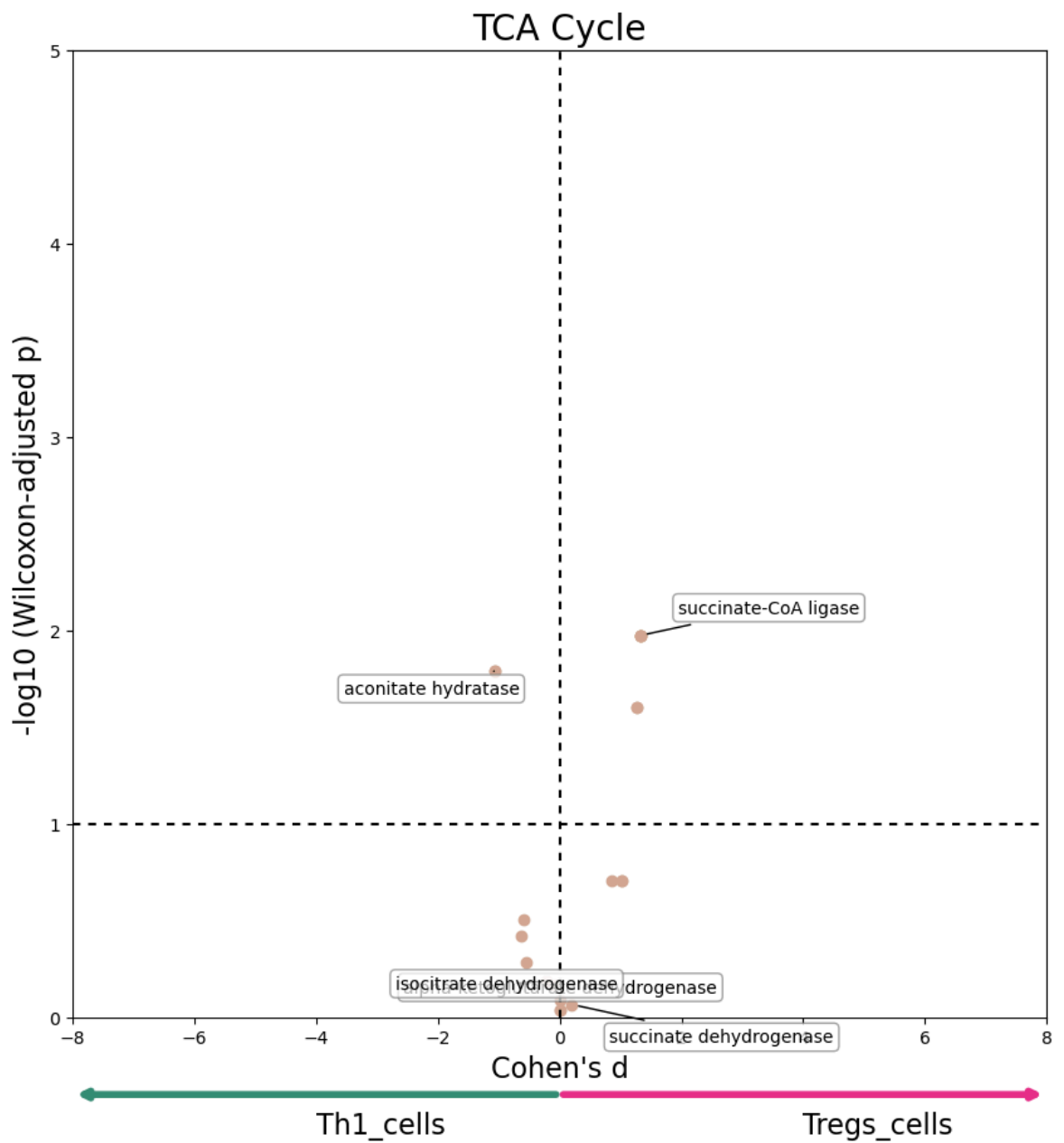
```
In [144...  filtered_data = pd.concat([W[W['subsystem'] == "Glycolysis/gluconeogenesis"],
                          W[W['subsystem'] == "Citric acid cycle"],
                          W[W['subsystem'].isin(amino_acid_metab)],
                          W[W['subsystem'] == "Fatty acid oxidation"]])
```

```
In [146...  data = W[W['subsystem'] == "Glycolysis/gluconeogenesis"]
           plot_differential_scores(data, title='Glycolysis', c="#695D73")
```



```
In [148...  data = W[W['subsystem'] == "Citric acid cycle"]
           plot_differential_scores(data, title="TCA Cycle", c="#D3A991")
```

TCA Cycle

- succinate-CoA ligase
- aconitate hydratase
- isocitrate dehydrogenase / drogenase
- succinate dehydrogenase

-log10 (Wilcoxon-adjusted p)

Cohen's d

Th1_cells   Tregs_cells

```
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
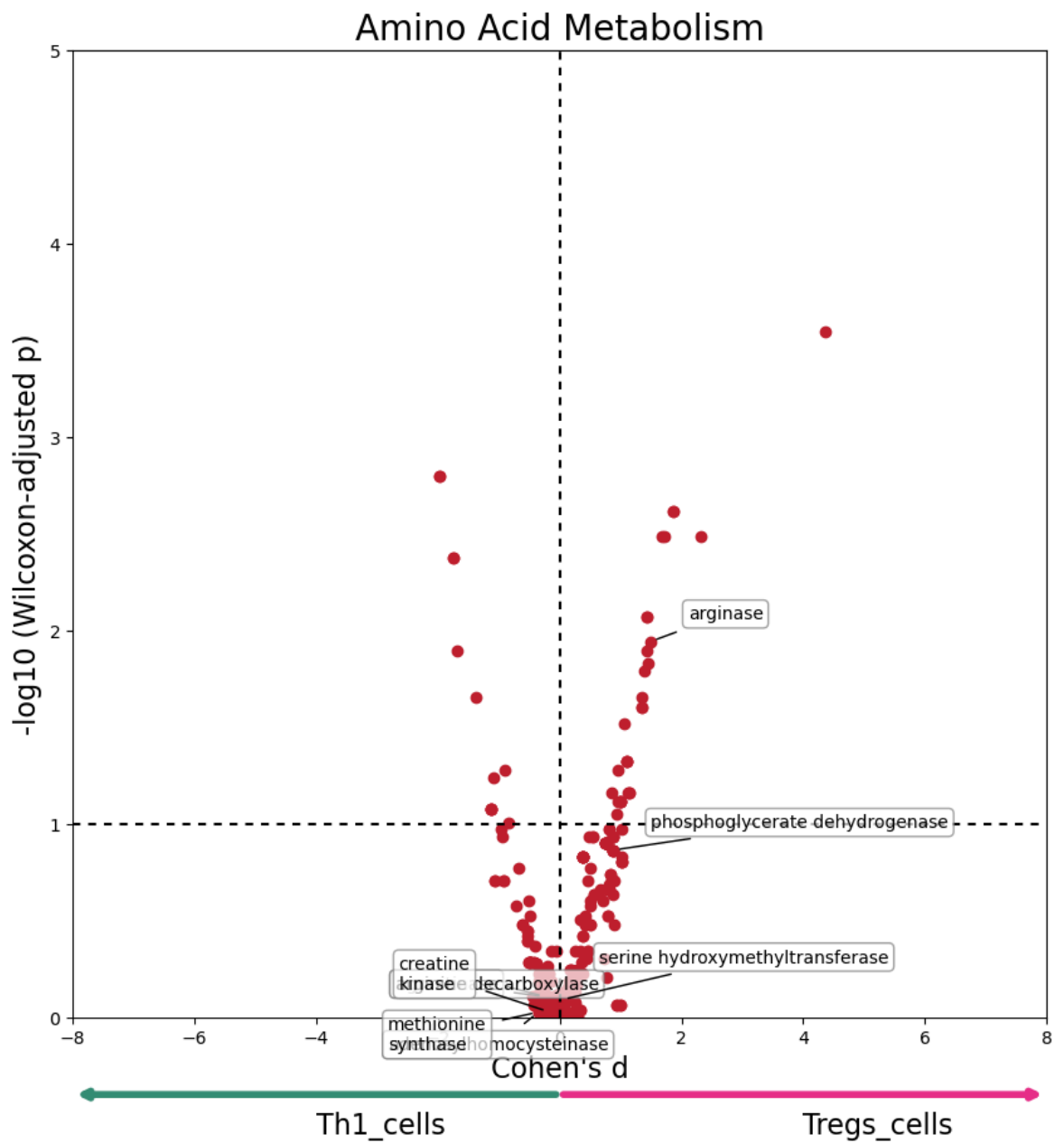

```
data = W[W['subsystem'] == "Fatty acid oxidation"]
plot_differential_scores(data, "Fatty Acid Oxidation", c="#040772")
```

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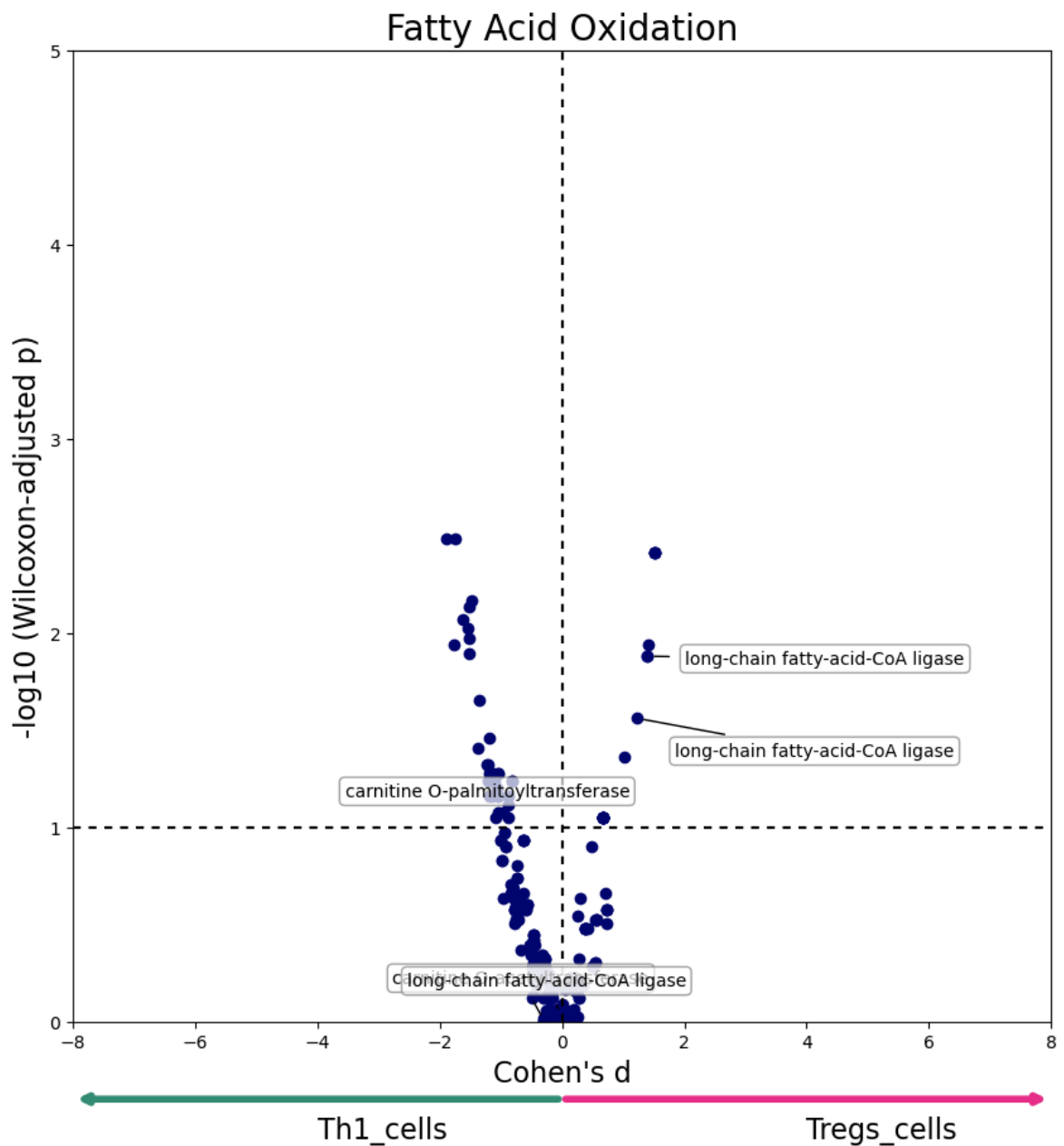
```
data = W[~W['subsystem'].isin(["Miscellaneous", "Unassigned"])]
data = data[~data['subsystem'].map(lambda x: "Transport" in x or "Exchange" in x
items, counts = np.unique(data['subsystem'], return_counts=True)
items = [items[i] for i in range(len(items)) if counts[i] > 5] #filter(n() > 5)
data =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

In [79]:
```
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)
```
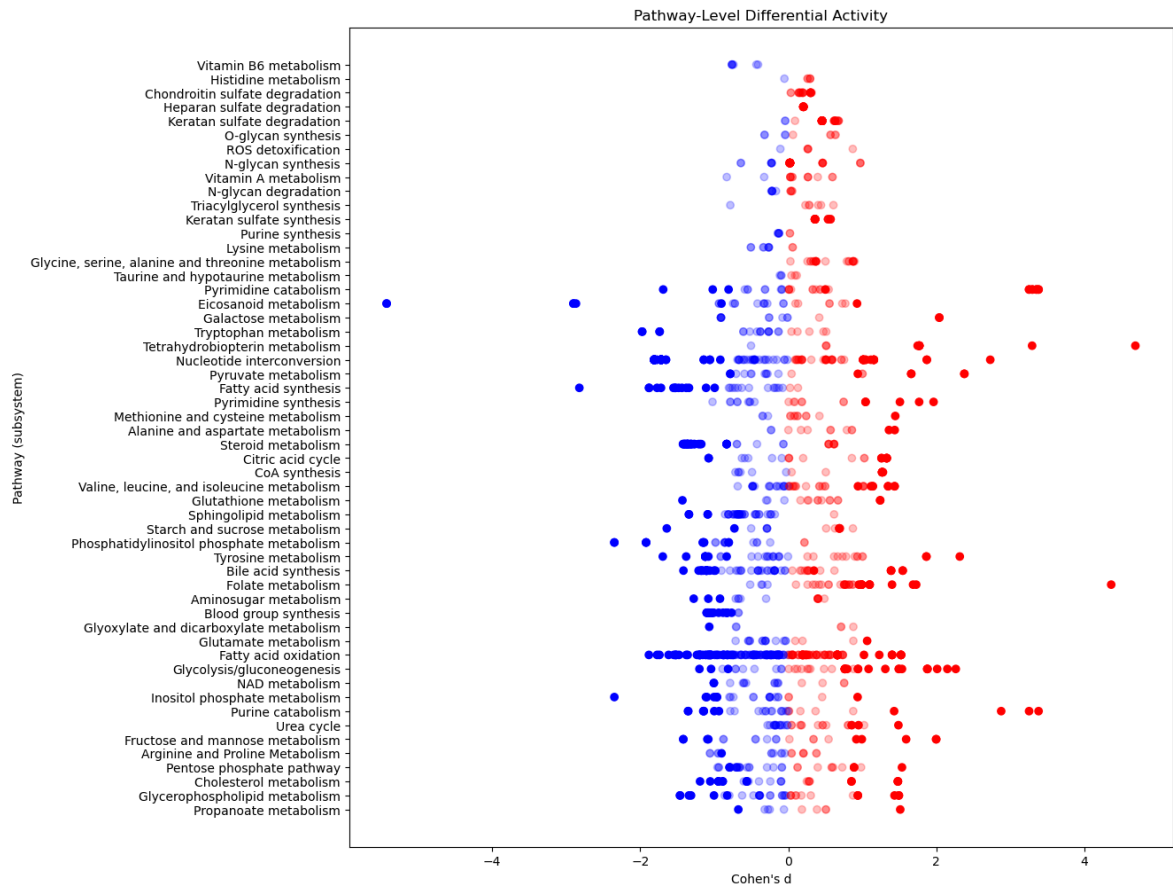
```python
axs.set_xlabel("Cohen's d")
axs.set_ylabel("Pathway (subsystem)")
axs.set_title("Pathway-Level Differential Activity")
```

Out[79]: Text(0.5, 1.0, 'Pathway-Level Differential Activity')

In [80]:
```python
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 [81]:
```python
reaction_penalties = reaction_penalties[reaction_penalties.max(axis=1) - reactio
```

In [82]:
```python
meta_rxns_map = get_metareactions(reaction_penalties)
meta_rxns = reaction_penalties.join(pd.DataFrame(meta_rxns_map, columns=["meta_r
```

In [83]:
```python
meta_rxn_consistencies = get_reaction_consistencies(meta_rxns)
```

In [84]:
```python
treg_cells = cell_metadata.index[cell_metadata["cell_type"] == "Tregs"]
tcon_cells = cell_metadata.index[cell_metadata["cell_type"] == "Th1"]

wilcox_meta_rxn_results = wilcoxon_test(meta_rxn_consistencies, Tregs_cells, Th1
```

In [85]:
```python
wilcox_meta_rxn_results.iloc[0:1]
```

Out[85]:

	wilcox_stat	wilcox_pval	cohens_d	adjusted_pval
meta_rxn_id				
1	95.0	0.908549	0.182117	0.962467

In [86]:
```python
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 [87]:
```python
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 [88]:
```python
wilcox_meta_rxn_expanded.iloc[0:1]
```

Out[88]:

	wilcox_stat	wilcox_pval	cohens_d	adjusted_pval	metadata_r_id
10FTHF5GLUtl_pos	143.0	0.040887	0.766317	0.143336	10FTHF5GLUtl

In [89]:
```python
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 [138…
```python
import zipfile

with zipfile.ZipFile("Tregs vs Th1.zip", "w") as zipf:
    for filename in outputs:
        zipf.write(filename)
```

In [140…
```python
from IPython.display import FileLink
FileLink("Tregs vs Th1.zip")
```

Out[140… Tregs vs Th1.zip

In [ ]: