In [1]:
```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
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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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Requirement already satisfied: kiwisolver>=1.3.1 in c:\programdata\anaconda3\lib
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```
Defaulting to user installation because normal site-packages is not writeable
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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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```
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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 [2]:
```python
import pandas as pd
import numpy as np
import matplotlib.pyplot as plt
```

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

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

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

C:\Users\HP\Human datasets

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

In [8]:
```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("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]:
```python
cell_metadata = pd.read_csv("cell_metadata.csv", sep=",")
cell_metadata.set_index("cell_id", inplace=True)
```

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

```
['cell_type']
```

In [13]:
```python
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]:
```python
reaction_metadata = pd.read_csv("reaction_metadata.csv", index_col = 0)
```

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

Out[15]:

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

In [16]:
```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 [21]:
```
reaction_consistencies = get_reaction_consistencies(reaction_penalties)
```

In [22]:
```
common_cells = list(set(reaction_consistencies.columns).intersection(set(cell_me
reaction_consistencies = reaction_consistencies[common_cells]
```

In [23]:
```
wilcox_results = wilcoxon_test(reaction_consistencies, Tn_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 [24]:
```
print(reaction_consistencies.columns.tolist()[:10])
```

```
['Tn_Don3_Ln2', 'Tregs_Don1_Ln1', 'Th1_Don7_Ln1', 'Tn_Don4_Ln2', 'Tn_Don6_Ln2',
'exTregs_Don1_Ln2', 'Tn_Don5_Ln1', 'Tregs_Don7_Ln2', 'Th1_Don3_Ln1', 'Tregs_Don2_
Ln2']
```

In [25]:
```
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 [40]:
```
# Run the test
wilcox_results = wilcoxon_test(reaction_consistencies, Tn_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 [41]:
```
# Run the test
wilcox_results = wilcoxon_test(reaction_consistencies, Tn_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 [42]:
```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 [43]:
```python
wilcox_results.loc[['r0281_pos']]
```

Out[43]:

	wilcox_stat	wilcox_pval	cohens_d	adjusted_pval	metadata_r_id
r0281_pos	9.0	0.000048	-2.042376	0.000178	r0281

In [44]:
```python
reaction_metadata.loc['r0281']['formula']
```

Out[44]:
```
'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 [93]:
```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, 10)
    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('Tn_cells', xy=(0.75, -0.12), xycoords='axes fraction', fontsiz
    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
            )
```
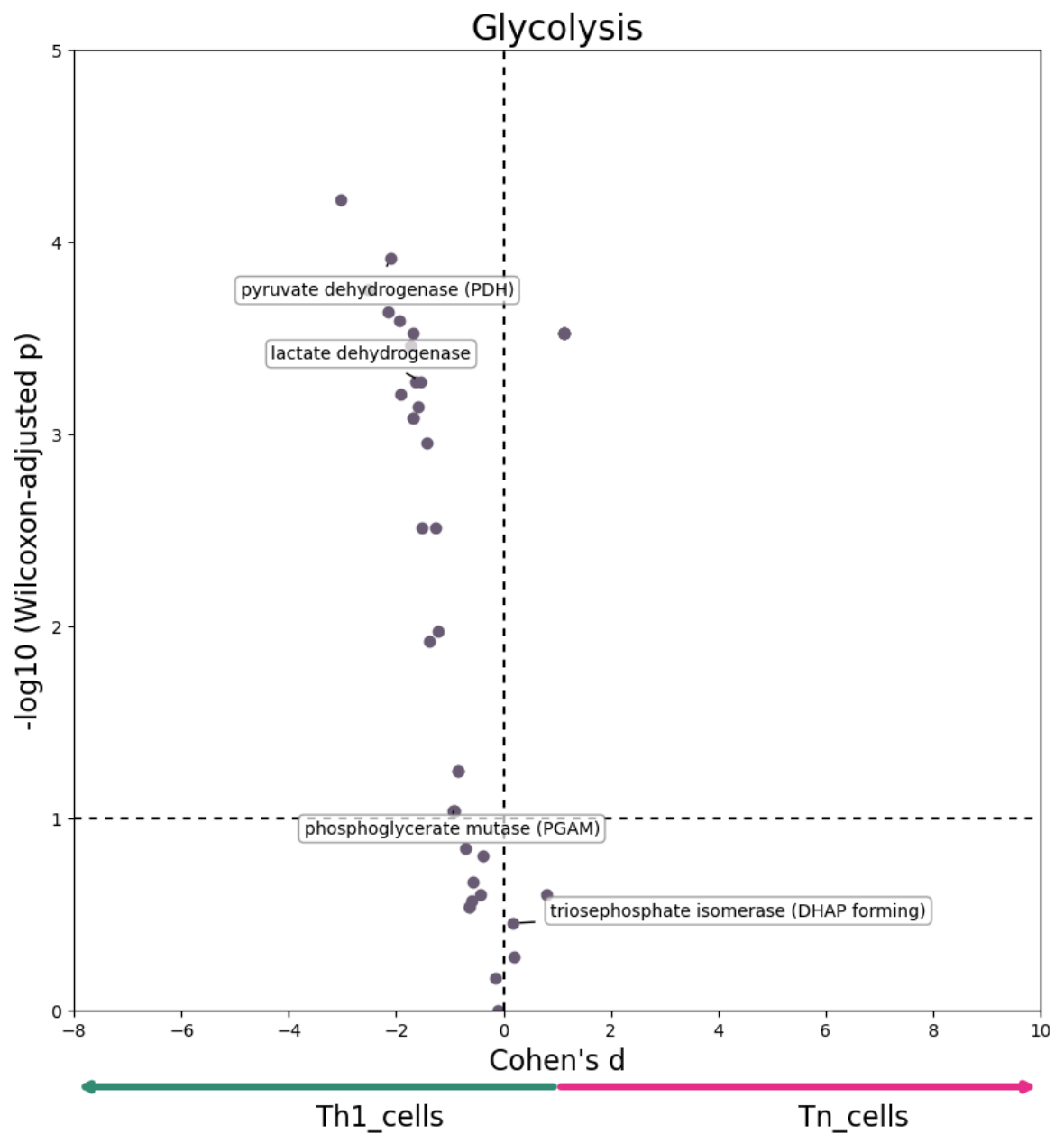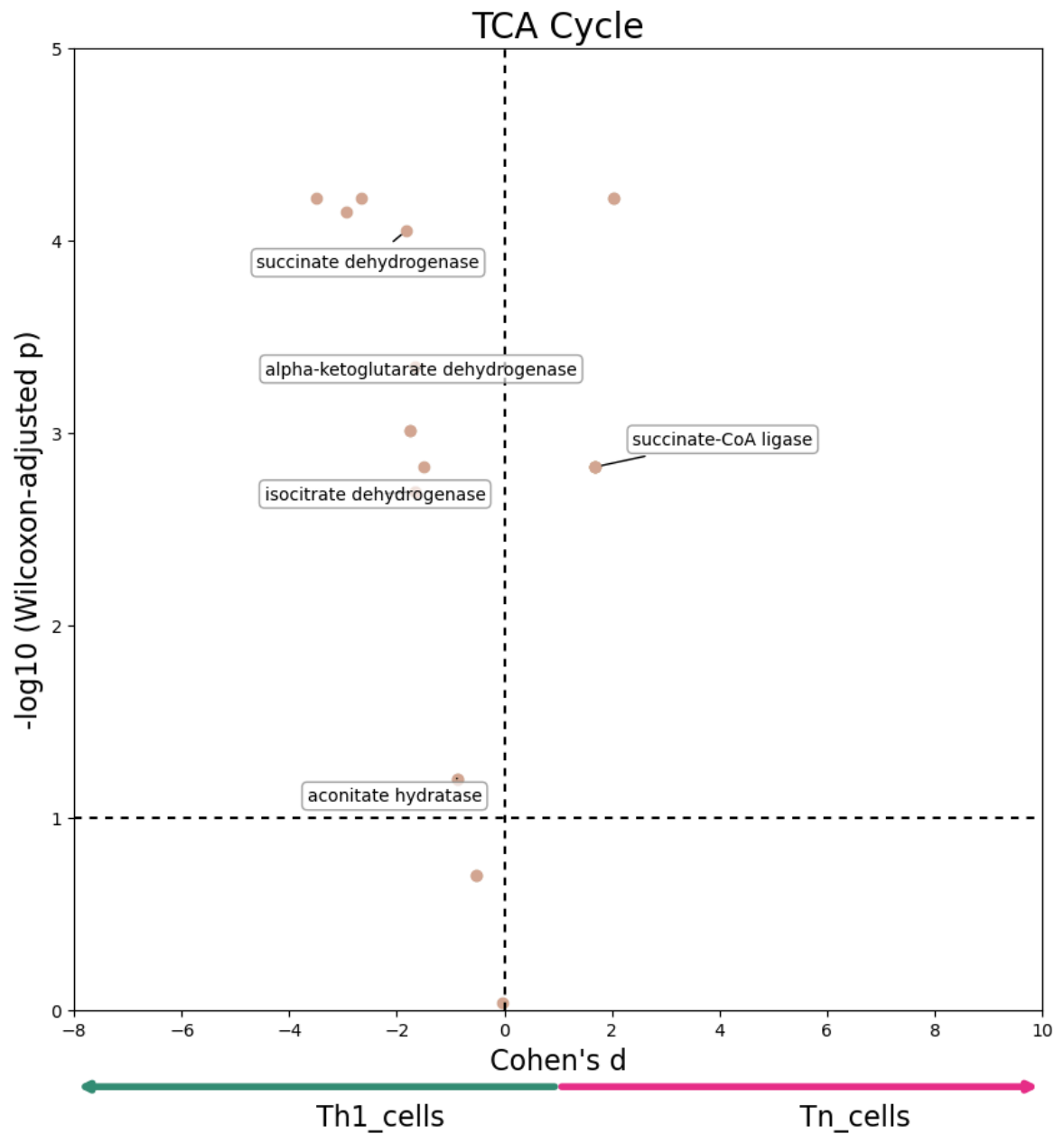
In [95]:
```python
filtered_data = pd.concat([W[W['subsystem'] == "Glycolysis/gluconeogenesis"],
                W[W['subsystem'] == "Citric acid cycle"],
               W[W['subsystem'].isin(amino_acid_metab)],
             W[W['subsystem'] == "Fatty acid oxidation"]])
```

In [97]:
```python
data = W[W['subsystem'] == "Glycolysis/gluconeogenesis"]
plot_differential_scores(data, title='Glycolysis', c="#695D73")
```

Glycolysis


```
In [99]: data = W[W['subsystem'] == "Citric acid cycle"]
         plot_differential_scores(data, title="TCA Cycle", c="#D3A991")
```

TCA Cycle

(Plot: x-axis "Cohen's d" from −8 to 10; y-axis "−log10 (Wilcoxon-adjusted p)" from 0 to 5. Labeled points: succinate dehydrogenase, alpha-ketoglutarate dehydrogenase, succinate-CoA ligase, isocitrate dehydrogenase, aconitate hydratase. Below the plot a green leftward arrow labeled "Th1_cells" and a pink rightward arrow labeled "Tn_cells".)

```
In [100…    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
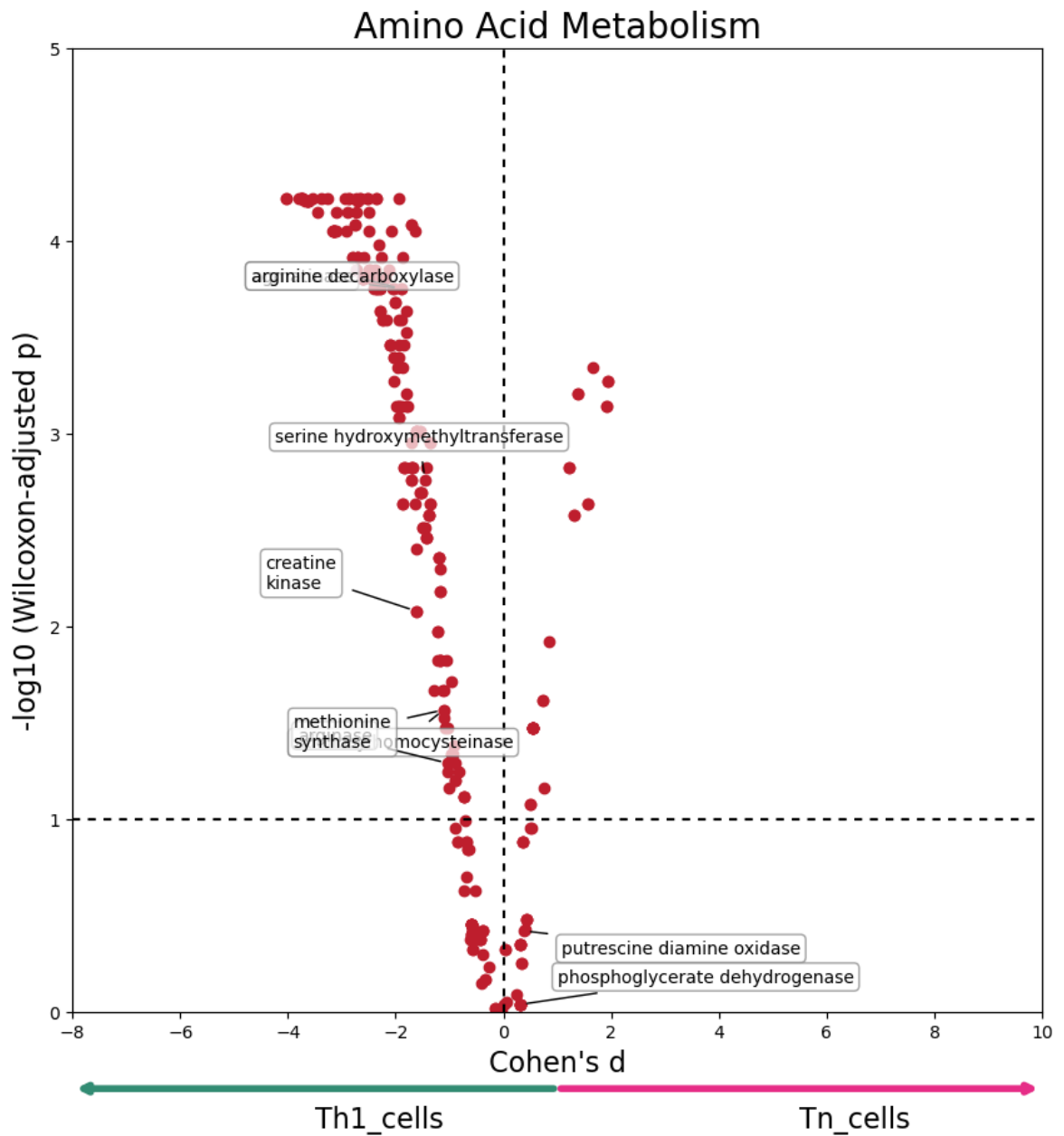
```
In [102…    data = W[W['subsystem'] == "Fatty acid oxidation"]
            plot_differential_scores(data, "Fatty Acid Oxidation", c="#040772")
```

Fatty Acid Oxidation
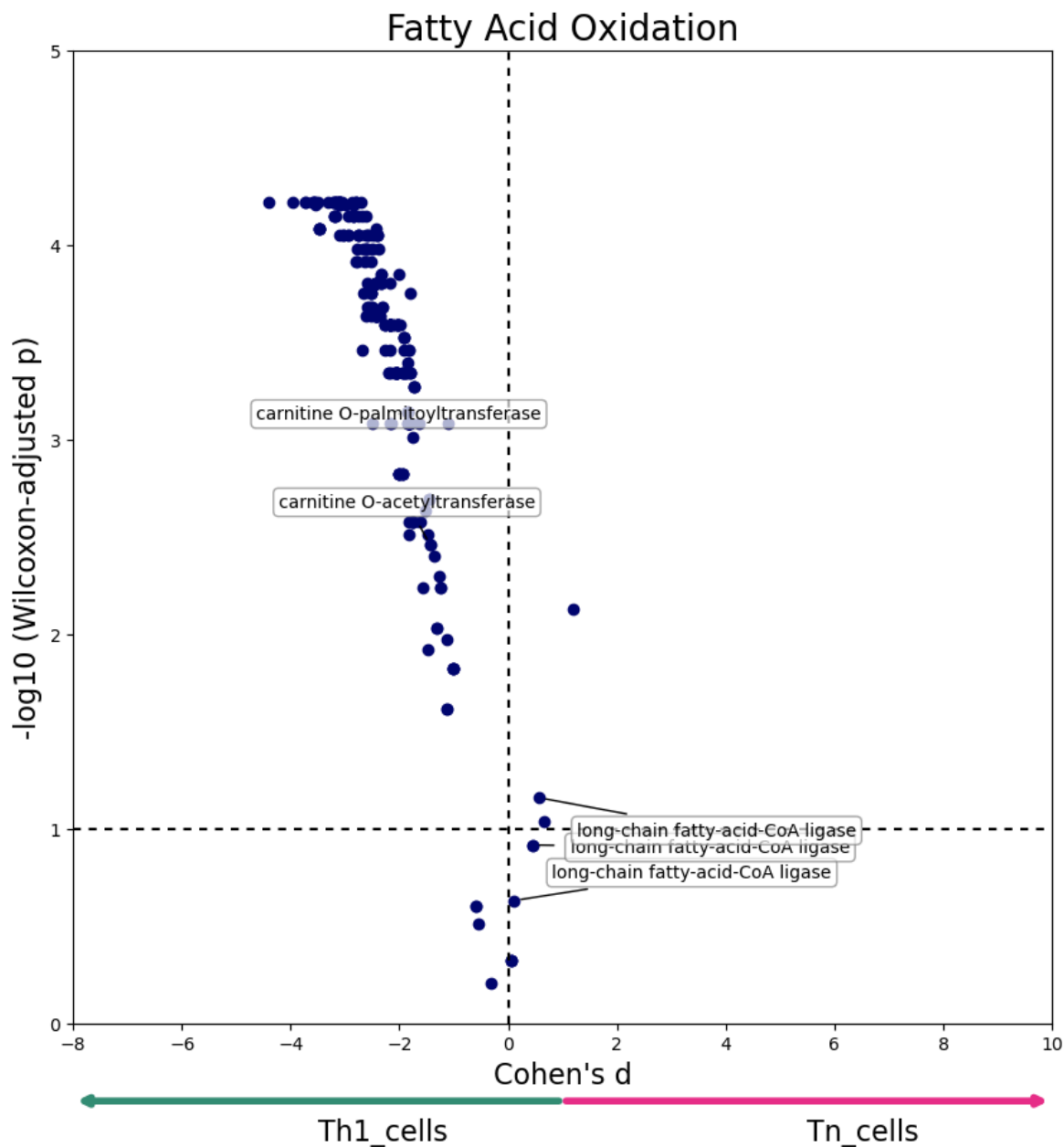

```
In [104…   data = W[~W['subsystem'].isin(["Miscellaneous", "Unassigned"])]
           data = data[~data['subsystem'].map(lambda x: "Transport" in x or "Exchange" in x
           items, counts = np.unique(data['subsystem'], return_counts=True)
           items = [items[i] for i in range(len(items)) if counts[i] > 5] #filter(n() > 5)
           data = data[data['subsystem'].isin(items)]
```

```
In [105…   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)
```
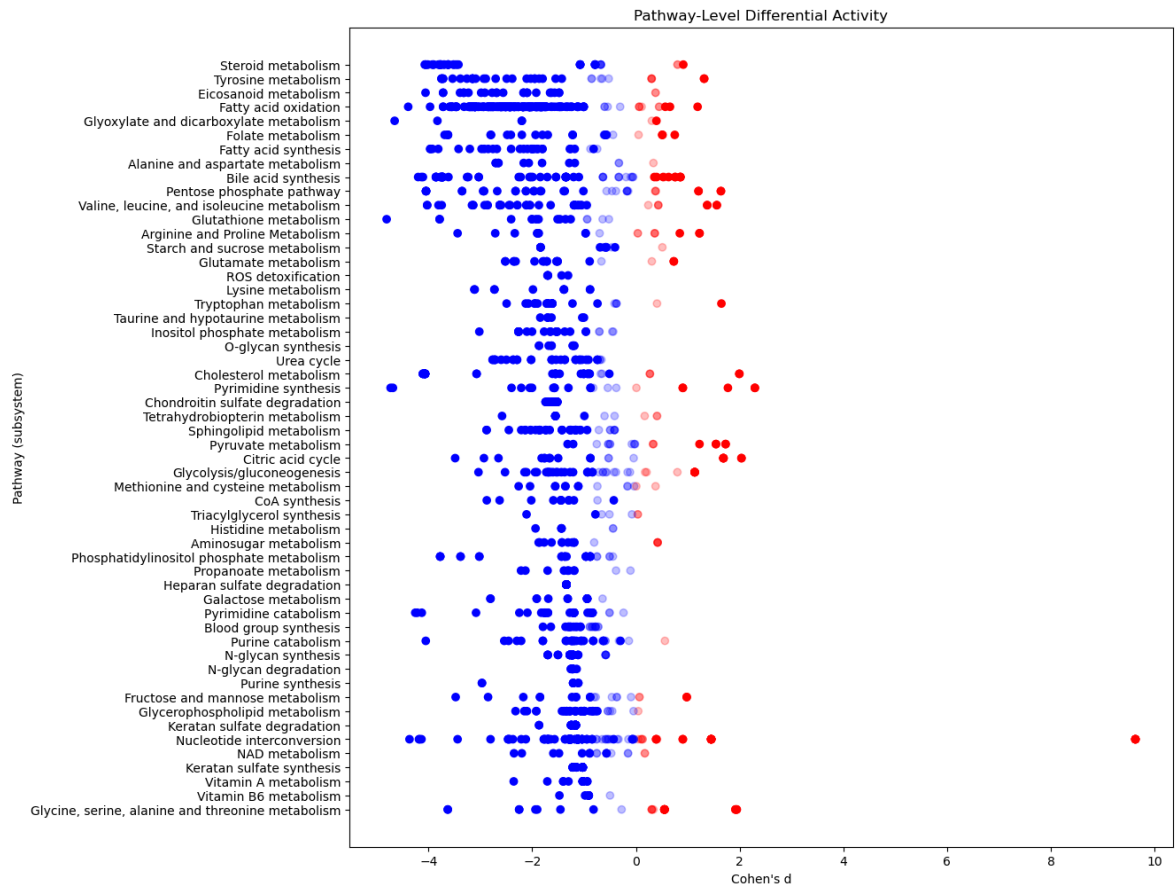
```
axs.set_xlabel("Cohen's d")
axs.set_ylabel("Pathway (subsystem)")
axs.set_title("Pathway-Level Differential Activity")
```

Out[105…   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) - reactio
```

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

In [59]:
```
meta_rxn_consistencies = get_reaction_consistencies(meta_rxns)
```

In [60]:
```
treg_cells = cell_metadata.index[cell_metadata["cell_type"] == "Tn"]
tcon_cells = cell_metadata.index[cell_metadata["cell_type"] == "Th1"]

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

In [61]:   `wilcox_meta_rxn_results.iloc[0:1]`

Out[61]:

	wilcox_stat	wilcox_pval	cohens_d	adjusted_pval
meta_rxn_id				
1	95.0	0.908549	0.182117	0.962467

In [62]:
```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 [63]:
```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 [64]:
```python
wilcox_meta_rxn_expanded.iloc[0:1]
```

Out[64]:

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

In [65]:
```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 [66]:
```python
import zipfile

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

In [68]:
```python
from IPython.display import FileLink
FileLink("Tn vs Th1.zip")
```

Out[68]: Tn vs Th1.zip

In [ ]: